

Ligand-independent gp130-type receptor activation by forced homo- and heterodimerization

Dissertation

zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Christian-Albrechts-Universität
zu Kiel

vorgelegt von
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Kiel, Juli 2010

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Tag der mündlichen Prüfung: 21.09.2010

TABLE OF CONTENTS

1	INTRODUCTION	- 1 -
1.1	Class I cytokine receptors	- 1 -
1.2	The family of IL-6 cytokine receptors	- 3 -
1.2.1	Receptor utilization	- 3 -
1.2.2	Receptor complex formation and transmission of the signal	- 5 -
1.2.3	Signal transduction	- 7 -
1.2.4	Biological effects of IL-6-like cytokines	- 10 -
1.2.5	Ligand-dependent, constitutively active gp130 variants	- 12 -
1.3	IL-15 and the IL-15R α	- 13 -
2	AIM OF THE WORK	- 16 -
3	MATERIAL & METHODS	- 17 -
3.1	Material	- 17 -
3.1.1	Antibiotics	- 17 -
3.1.2	Antibodies	- 17 -
3.1.2.1	Primary antibodies	- 17 -
3.1.2.2	Secondary antibodies	- 19 -
3.1.3	Bacterial media	- 20 -
3.1.4	Buffers and Solutions	- 20 -
3.1.5	Cell culture media, solutions and material	- 23 -
3.1.6	Cell lines	- 24 -
3.1.7	Chemicals	- 25 -
3.1.8	Enzymes	- 25 -
3.1.9	Molecular weight markers	- 25 -
3.1.10	Kits	- 25 -
3.1.11	Oligonucleotides	- 26 -
3.1.12	Plasmids	- 27 -
3.1.13	Recombinant proteins	- 28 -
3.2	Methods	- 29 -
3.2.1	Molecular biology	- 29 -
3.2.1.1	Polymerase chain reaction (PCR)	- 29 -
3.2.1.2	Phosphorylation of PCR fragments	- 29 -
3.2.1.3	Digestion of DNA	- 30 -

3.2.1.4	DNA blunting	- 30 -
3.2.1.5	Dephosphorylation of Plasmid DNA	- 30 -
3.2.1.6	DNA Gel-electrophoresis	- 31 -
3.2.1.7	Extraction of DNA fragments from agarose gel	- 31 -
3.2.1.8	Determination of DNA or RNA concentration	- 31 -
3.2.1.9	Ligation of DNA fragments	- 32 -
3.2.1.10	Transformation of chemocompetent E.coli	- 32 -
3.2.1.11	Plasmid isolation from 2 ml bacteria suspension – plasmid mini preparation	- 32 -
3.2.1.12	Plasmid isolation from 100 ml bacteria suspension – plasmid midi preparation	- 33 -
3.2.1.13	DNA sequencing	- 33 -
3.2.1.14	RNA isolation	- 33 -
3.2.1.15	Reverse Transcription (RT)	- 33 -
3.2.2	Cell culture and cell based assays	- 34 -
3.2.2.1	Cell cultivation	- 34 -
3.2.2.2	Transfection of eucaryotic cells	- 34 -
3.2.2.3	Starvation of cells	- 34 -
3.2.2.4	Pervanadat treatment of COS-7 cells	- 35 -
3.2.2.5	Retroviral transduction of Ba/F3-gp130 cells	- 35 -
3.2.2.6	Cell viability assay	- 35 -
3.2.2.7	Co-culture experiments of Ba/F-gp130-GFP cells with Ba/F-gp130 cells stably transduced with fusion receptor genes	- 36 -
3.2.2.8	Co-culture assay of murine dendritic cells with Ba/F3-gp130 cells	- 36 -
3.2.2.9	Flow cytometry staining and analysis	- 36 -
3.2.3	Protein-biochemical methods	- 37 -
3.2.3.1	Cell lysis	- 37 -
3.2.3.2	Determination of protein concentration (BCA protein Assay)	- 37 -
3.2.3.3	SDS-polyacrylamid gel electrophoresis (SDS-PAGE)	- 37 -
3.2.3.4	Western blotting	- 38 -
3.2.3.5	Stripping of membranes	- 38 -
3.2.4	Computational biology	- 38 -
4	RESULTS	- 40 -
4.1	Generation of forced heterodimers of gp130 and WSX-1 using a leucine zipper based strategy.	- 40 -
4.2	Construction of Fos-gp130, Δcys-Fos-gp130 and Jun-WSX-1 expression plasmids	- 41 -
4.3	Specific Jun/Fos chimeric gp130-type receptor heterodimerization was not achieved due to unexpected homodimerization of the Fos-gp130 receptor chimera	- 44 -
4.4	Generation of forced heterodimers of gp130 and WSX-1, LIFR, OSMR or GPL and OSMR using a novel strategy comprising IL-15 and IL-15Rα-sushi.	- 49 -

4.5	Construction of IL-15-gp130, IL-15-WSX-1, IL-15-LIFR, IL-15-OSMR, IL-15-GPL, sushi-gp130, sushi-WSX-1, sushi-LIFR, sushi-OSMR and sushi-GPL expression plasmids	- 50 -
4.6	Heterodimerization of gp130 and WSX-1 mediated by IL-15 and IL-15R α -sushi was achieved and led to cytokine independent growth of Ba/F3-gp130 cells	- 55 -
4.7	Interaction analysis of IL-15 mediated homodimerization of gp130/gp130 and IL-15/IL-15R α -sushi mediated heterodimerization of gp130/WSX-1	- 58 -
4.8	Unexpected homodimerization of sushi-gp130 does not lead to a functional receptor complex	- 61 -
4.9	Phosphorylation of STAT1/3 and ERK1/2 proteins in Ba/F3-gp130 cells expressing IL-15 or IL-15/IL-15R α -sushi mediated homo- and heterodimers of gp130/gp130 and gp130/WSX-1	- 65 -
4.10	The IL-15/IL-15R α -sushi mediated heterodimerization of receptors can be adapted to other heterodimeric receptor complexes of the gp130-type family	- 66 -
4.11	Analysis of all possible combinations between all gp130-type receptors using the IL-15/IL-15R α -sushi based system.	- 70 -
4.12	Second generation of IL-15/IL-15R α -sushi based heterodimerization system – avoiding signaling capacity of IL-15 and expression of two open reading frames from one cDNA: FUSIO	- 74 -
5	DISCUSSION	- 78 -
5.1	Establishment of a novel system to generate ligand-independent, constitutively active, heterodimeric receptor complexes.	- 78 -
5.2	Dimerization and activation vs. dimerization and non-activation	- 80 -
5.3	Applications of the IL-15/IL-15R α -sushi based system	- 83 -
5.3.1	<i>In vitro</i> applications	- 83 -
5.3.2	Optimization of the IL-15/IL-15R α -sushi based system for future <i>in vivo</i> applications	- 84 -
6	SUMMARY	- 90 -
7	ZUSAMMENFASSUNG	- 91 -
8	REFERENCES	- 93 -

9 APPENDIX	- 109 -
9.1 Abbreviations	- 109 -
9.2 Amino acids	- 111 -
9.3 Sequences and plasmid maps	- 112 -
10 LIST OF PUBLICATIONS	- 133 -
11 ACKNOWLEDGEMENT	- 134 -
12 CURRICULUM VITAE	- 135 -
13 EIDESSTÄTTLICHE ERKLÄRUNG	- 136 -

1 Introduction

1.1 Class I cytokine receptors

Cytokines are secreted mediators (15-25 kDa) for intercellular communication. Cytokines elicit their broad spectrum of activities in development, differentiation, proliferation, immune responses, and apoptosis through binding to specific cell surface receptors. They can act in an autocrine as well as a paracrine fashion [1, 2].

Structural analysis was the basis to group cytokines into different structural classes, including the helical cytokines [3], the trimeric tumor necrosis factor (TNF) family [4], the cysteine knot growth factors [5], and the β -trefoil growth factors [6]. Another classification is based on receptor engagement by cytokines. On the basis of common structural features, the cytokine receptors are grouped into different superfamilies: class I cytokine receptors [7], class II cytokine receptors [8], tumor necrosis factor receptors [9], interleukin-1 (IL-1) receptors [10], tyrosine kinase receptors [11], serine/threonine kinase receptors [12], chemokine receptors [13], and IL-17 receptors [14].

Class I cytokine receptors bind helical cytokines. These polypeptides are synthesized and secreted by cells in response to many stimuli, and mediate their effects by binding to specific receptors on the surface of target cells [15].

Class I cytokine receptors share little primary sequence homology [16] but they share a modular architecture, which is characterized by a ~200 residue-long cytokine-binding homology domain (CHD) possessing the classical binding motif for cytokines [16]. The CHD consists of two tandem fibronectin type-III (FNIII) domains and it represents the signature recognition module for helical cytokines that is present on every type I cytokine receptor. The upper, N-terminal domain contains two pairs of conserved cysteines that form interstrand disulfide bonds [17]. The lower, C-terminal domain contains a conserved Trp-Ser-X-Trp-Ser (WSXWS) motif [16, 18, 19]. The basic CHD motif is present in every member of the class I cytokine receptors, and for some, such as the receptor for the growth hormone, a single CHD is sufficient to mediate ligand binding and receptor homodimerization [19]. Other receptors require additional domains like immunoglobulin-like (Ig-like) domain and additional membrane-proximal fibronectin type-III domains to function and respond to cytokines [20, 21].

cytokine	receptor chain(s)			
erythropoietin	erythropoietin receptor			
thrompoietin	thrombopoietin receptor			
growth hormone	growth hormone receptor			
prolactin	prolactin receptor			
leptin	leptin receptor			
granulocyte-colony stimulating factor	granulocyte-colony stimulating factor receptor			
viral interleukin-6	glycoprotein 130			
interleukin-6			interleukin 6 receptor α	
interleukin-11			interleukin-11 receptor α	
interleukin-27 (p28)			Epstein-Barr induced gene 3	interleukin-27 receptor α (WSX-1)
cardiotrophin-like cytokine			cytokine-like factor-1	leukemia inhibitory factor receptor
ciliary neurotrophic factor			ciliary neurotrophic factor receptor α	
neuropoetin				
leukemia inhibitory factor				
oncostatin M			oncostatin M receptor	
interleukin-31			glycoprotein 130-like receptor	
interleukin-12 (p35)	interleukin-12 (p40)	interleukin-12 receptor β 2	interleukin-12 receptor β 1	
interleukin-23 (p19)		interleukin-23 receptor		
interleukin-4	interleukin-4 receptor α		interleukin-13 receptor α 1	
Interleukin-13	interleukin-13 receptor α 2			
thymic stromal lymphopoietin	interleukin-7 receptor α		thymic stromal lymphopoietin receptor	
interleukin 7	interleukin-2 receptor β		interleukin-2 receptor γ c	
interleukin-2				interleukin-2 receptor α
interleukin-15				Interleukin-15 receptor α
interleukin-4				interleukin-4 receptor α
interleukin-9				interleukin-9 receptor α
interleukin-21	interleukin-21 receptor α		interleukin-3 receptor α	
interleukin-3	interleukin-3 receptor β c			interleukin-5 receptor α
interleukin-5				granulocyte-macrophage colony-stimulating factor receptor α
granulocyte-macrophage colony-stimulating factor				

Table 1.1 Overview of all class I cytokine receptor chain complexes and their utilization by cytokines. Group 1 receptor chains (purple) have an extracellular domain that consists solely of a CHD [7]. Group 2 receptors (blue) are structurally related to the archetypal glycoprotein 130 (gp130). Specific for this group are the N-terminally Ig-like domains and/or the three FNIII domains between their CHD and transmembrane domains [7, 22]. Group 3 receptor chains (yellow) also generally possess an N-terminal Ig-like domain in addition to the CHD, and are either soluble or have short intracellular regions [7]. These receptors belong to the α -receptors which do not participate in signaling but are important for initial ligand binding. Group 4 receptor chains (brown) are characterized by an extracellular CHD motif and a long intracellular domain [7]. Group 5 receptors (orange) often have extracellular, N-terminal Ig-like domains in addition to the CHD domain, and have short intracellular domains [7]. Additionally constituents of receptor complexes of the IL-2R functional family, the IL-2R α and IL-15R α receptor chains (both highlighted in green) are not members of the class I family receptors, but instead contain distinctive ‘sushi domain’ structures. This table has been adapted in modified form from Liongue and Ward [23].

The superfamily of class I cytokine receptors can be subdivided into five families based on sequence and structural homology of the receptor and their ligands which are listed in Table 1.1. Class I cytokine receptors lack intrinsic kinase activity. However, they are non-covalently associated with Janus kinases (JAKs – JAK1, JAK2, JAK3 and TYK2). These tyrosine kinases are autophosphorylated upon receptor engagement by cytokines, and subsequently activate downstream signaling events, e.g. the phosphorylation of signaling transducing and activators of transcription (STATs – STAT1-6) transcription factors [24, 25].

In the following chapters the IL-6 family of receptor complexes, which are of special interest for this work, should be introduced with focus on receptor utilization, receptor complex formation, signal transduction, and biological effects.

1.2 The family of IL-6 cytokine receptors

1.2.1 Receptor utilization

The IL-6 family of cytokines signaling through the common receptor subunit gp130 comprises IL-6, viral IL-6, IL-11, IL-27, CNTF, CT-1, LIF, cardiotrophin-like cytokine (CLC/NNT-1), neuropoetin (NP), and OSM [26-29]. NP is expressed in mice but not in humans due to a frameshift in its coding sequence caused by an 8-bp deletion [29]. Additionally, also IL-31 can be grouped to this family because of binding to the gp130-like protein (GPL or IL-31R) [30]. All these cytokines belong to the long chain four helix bundle class of cytokines, consisting of four long α -helixes termed A, B, C, and D, which are arranged in an up-up-down-down topology (Figure 1.2A) [3].

Gp130 can form homodimers (based on the usage of the signal transducing receptor chains) in the presence of IL-6, viral IL-6 or IL-11 [28, 31, 32], or heterodimerize with the LIFR, when recruited by LIF, OSM, CNTF, CT-1, CLC, and NP [29, 33-36]. Additionally OSM can bind the OSMR, which again, heterodimerizes with gp130 [37]. IL-27, which a composite cytokine consisting of the four helix bundle cytokine p28 and soluble cytokine receptor EBI3, binds to another related type I cytokine receptor, WSX-1, which heterodimerizes with gp130 [27]. IL-31 instead does not bind to gp130 but to the related GPL, which heterodimerizes with OSMR to trigger IL-31 mediated events [30] (Figure 1.1).

Some cytokines of the IL-6-like family depend on the initial binding to a specific α -receptor before a signaling competent complex of the β -receptors can be formed (Figure 1.1).

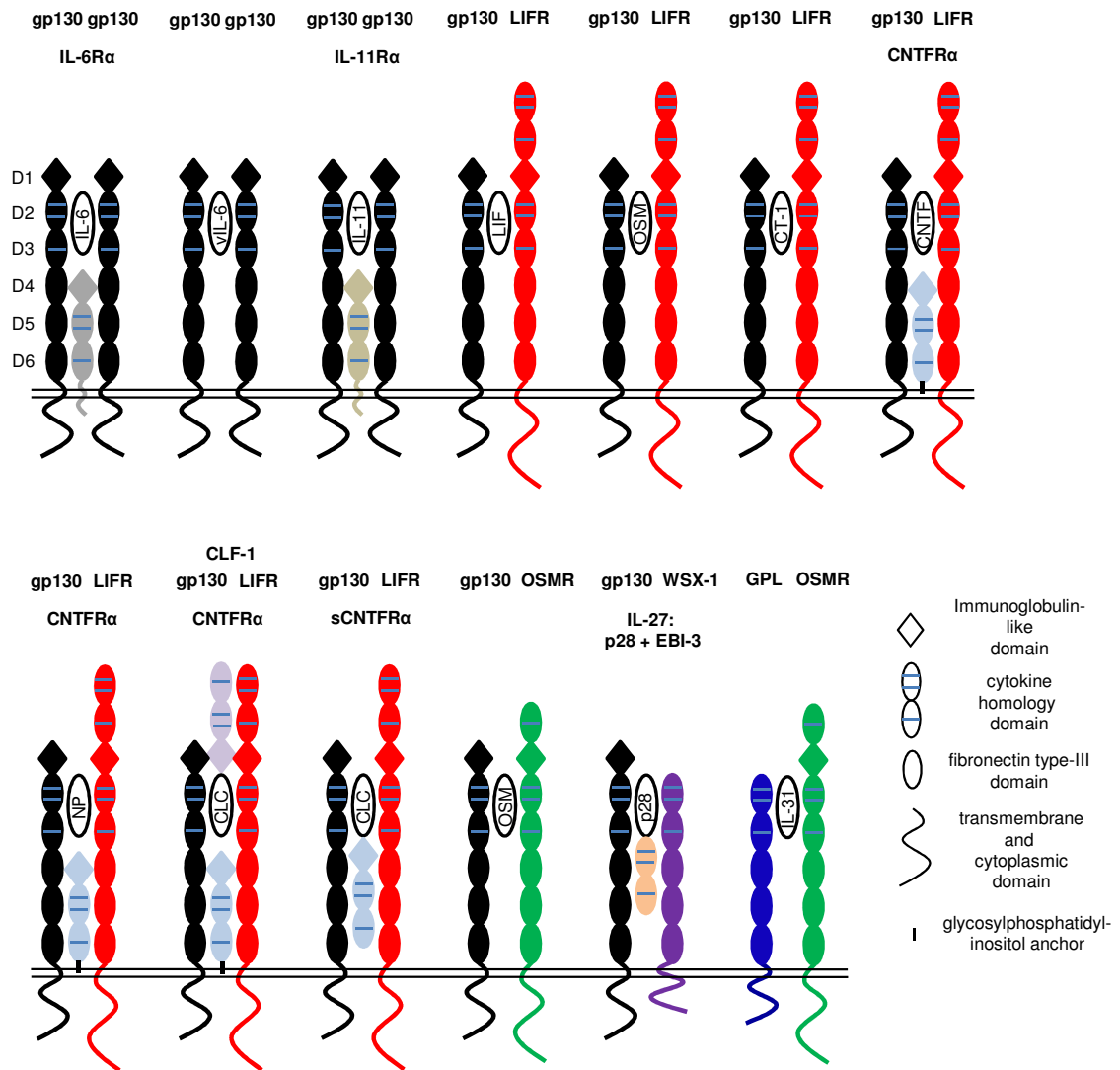


Figure 1.1 Gp130-type receptors and utilization by IL-6-like cytokines. Depicted are receptor complexes with IL-6-like cytokines and the domain structure of each receptor (e.g. gp130: D1-D6). The α -receptors consist of IL-6Ra (grey), IL-11Ra (yellow-brownish), CNTFRa (light blue) or soluble CNTFRa (sCNTFRa), CLF-1 (light purple) and EBI-3 (light orange). The latter two are part of the composite cytokines CLC/CLF-1 and IL-27, respectively. The scheme of the complex of CLC/membrane bound CNTFRa/LIFR/gp130 does not include CLF-1 [38]. It is shown to point out the differences in the delivery of CLC. The β -receptors are gp130 (black), LIFR (red), OSMR (green), WSX-1 (purple), and GPL (blue). In contrast to the α -receptors they are characterized by longer cytoplasmic domains, reflecting their involvement in signal transduction. See text for details.

IL-6 and IL-11 first bind to the IL-6 receptor α (IL-6Ra), and IL-11 receptor α (IL-11Ra), respectively [31, 32]. The IL-6Ra can also be expressed as a soluble form. It is generated by shedding or alternative splicing and acts agonistically [39]. CNTF, CLC, and NP were shown to bind initially to the CNTF receptor (CNTFR), and they share a conserved binding site [41]. The CNTFR carries a glycosyl-

phosphatidylinositol anchor to attach to the membrane. It has been shown that CLC forms a composite cytokine when it is associated with the soluble receptor cytokine-like factor (CLF-1) [36]. CLF-1 mainly behaves as a chaperone protein, allowing secretion of CLC. Furthermore, it has also been observed that CLC can form a second secreted composite cytokine when associated with the soluble form of CNTFR [40]. CT-1 binds directly to the LIFR, and induces gp130/LIFR heterodimer formation, but the presence of an additional glycosylphosphatidylinositol-anchored receptor specific for CT-1 has been hypothesized [35].

1.2.2 Receptor complex formation and transmission of the signal

A major feature of the gp130-type receptors is the presence of more domains than needed for ligand binding. Thus, one part is responsible for ligand binding (e.g. gp130: D1-D3) and the other is involved in transmitting the signal into cells (e.g. gp130: D4-D6) [41]. According to this gp130-type receptors are often called 'complex' or tall receptors.

A major feature of IL-6-like cytokines is the existence of three different sites for receptor subunit binding (Figure 1.2B) in contrast to other four helix bundle cytokines like the growth hormone which possess only two sites for receptor binding [19]. The crystal structure of the hexameric complex of IL-6/IL-6R α /gp130-D1D2D3 revealed that IL-6 use the canonical sites I and II to engage the α -receptor and gp130 CHD, respectively. Site III of IL-6 engages the Ig-like domain of gp130 so that each gp130 contacts two different IL-6 molecules in an antiparallel fashion [21]. Other reports strengthen the site III paradigm for this family of cytokines. The 4.0 Å structure of the D1-D5 domains of the LIFR in complex with LIF used a high-affinity site III interaction to engage the D3 Ig-like domain of LIFR [42]. Also modeling of the quaternary CNTF/CNTFR α /LIFR/gp130 using single-particle reconstruction of the entire complex [43], crystal structure of LIF/gp130 [44] and CNTF [45] revealed that site I of CNTF engages CNTFR α CHD, site II engages gp130 CHD and site III engages the Ig-like domain of the LIFR as presented in the work of Wang *et al.* [46]. It has also been hypothesized that WSX-1 which lacks the Ig-like domain engages site II of IL-27 (p28 subunit) via its CHD, while site III is free to interact with the second signaling receptor gp130 [43].

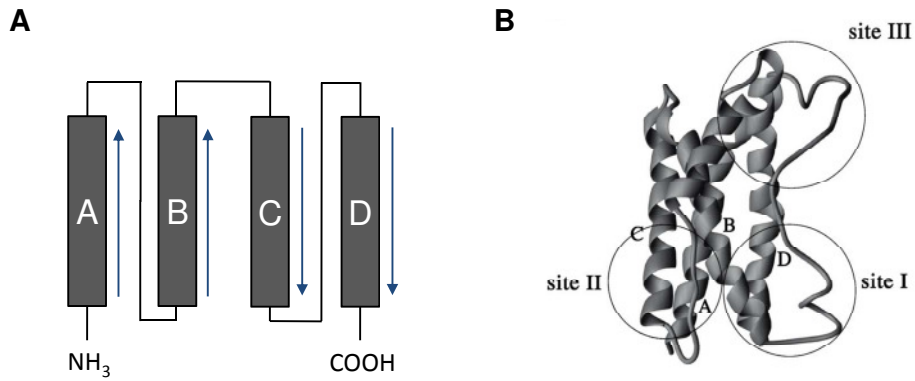


Figure 1.2 The four helix bundle cytokines. (A) Schematic representation of the four helix bundle cytokines with an up-up-down-down topology. The four helices are designated as A, B, C, D. (B) Ribbon representation of the IL-6 structure solved by NMR spectroscopy [47]. The interaction sites with the different receptor subunits are encircled and marked (site I: IL-6R α , site II: gp130 CHD, site III: gp130 Ig-like domain) [41].

The membrane proximal domains of gp130 (D4-D6) are involved in transmitting the signal to the cytoplasmic domain. Deletion mutants lacking D4, D5, or D6 showed no or impaired signal transduction activities [48]. Furthermore, a study using single-particle electron microscopy of the symmetric IL-6/IL-6R α /gp130-D1-D6 revealed that gp130 is bent such that the membrane proximal domains of gp130 are close together at the cell surface before entering the cell membrane, enabling activation of intracellular signaling [49]. This has also been observed by analyzing the hexameric IL-11/IL-11R α /gp130 complex by cryoelectron microscopic analysis [50]. A high-resolution crystal structure of gp130-D1-D6 could confirm this and furthermore revealed that the domains D4 and D5 are orientated such that they lie across each other at an angle of approximately 80°, resulting in an acute bend in the structure. Thus the ectodomain of gp130 adopts a wide-open 'C'-shape [51]. Moreover, the elucidation of the asymmetric full-length CNTF/CNTFR α /LIFR/gp130 complex displayed also a close apposition of gp130 D5 and LIFR D7 domains, which is a result of a bend between D4 and D5 of gp130 and D6 and D7 of the LIFR [43]. This is consistent with a functional study of the gp130/LIFR complex [52]. This study showed that the juxtamembrane and transmembrane regions of the full-length CNTF/CNTFR α /LIFR/gp130 complex join as a continuous, rigid unit, which indicates that they are closely associated at all times after ligand engagement and sensitively transduce ligand engagement to the membrane-proximal intracellular signaling regions [43]. This finding is consistent with a cellular study in which insertion of 1-4

alanine residues to the juxtamembrane intracellular region of gp130 lead to successive impairment but not abolishment of JAKs and receptor phosphorylation [53], so that the dimer needs to be positioned not only in close proximity to allow for full activation of the receptor, but also to be aligned in a specific manner [53]. However, the molecular mechanism how cytokine binding is coupled to JAK activation is not fully understood.

1.2.3 Signal transduction

As found for many cytokines, IL-6-like cytokines also induce activation of tyrosine kinases of the JAK family. The signal transducing chains gp130, LIFR and OSMR are associated with JAK1, JAK2 and TYK2 [54-56]. For WSX-1 only the association with JAK1 has been shown [57]. The same is true for GPL, but has not been tested for other JAK family members so far [58]. The association of JAKs to cytokine receptors is mediated by membrane-proximal box1/2 regions. Box1 is a proline-rich motif of eight amino acid residues essential for JAK association, whereas box2, a cluster of hydrophobic amino acid residues followed by positively charged amino acids, is necessary for JAK association only in some receptors. gp130, LIFR and OSMR contain box1/2 regions [33, 37, 59]. WSX-1 as well as GPL only exhibits a box1 motif [60, 61].

After ligand binding and close apposition of the cytoplasmic regions the JAKs become autophosphorylated at distinct tyrosine residues. Subsequently, the receptor is phosphorylated at specific tyrosines. For human gp130 it has been shown that at least five of six tyrosines are phosphorylated after ligand binding. The LIFR contains five [26], the OSMR nine [26], the WSX-1 two [60], and the GPL three [61] potential tyrosine-phosphorylation sites. It has been demonstrated that phosphorylation of the four membrane distal tyrosine residues in gp130 contribute to STAT binding and phosphorylation [62, 63]. The consensus sequences YXPQ and YXXQ are proposed for STAT1 and STAT3 binding [62, 63]. Therefore, every IL-6-like cytokine has the ability to induce STAT3 and to a minor extent STAT1 phosphorylation. This is also true for IL-31, even though it does not engage gp130, because the OSMR as well as GPL can activate STAT1 and STAT3 on their own [58, 64]. Phosphorylated WSX-1 can only bind STAT1 [57] even if the consensus sequence YXPQ is missing but IL-27 also induces STAT3 phosphorylation since it engages gp130 [27].

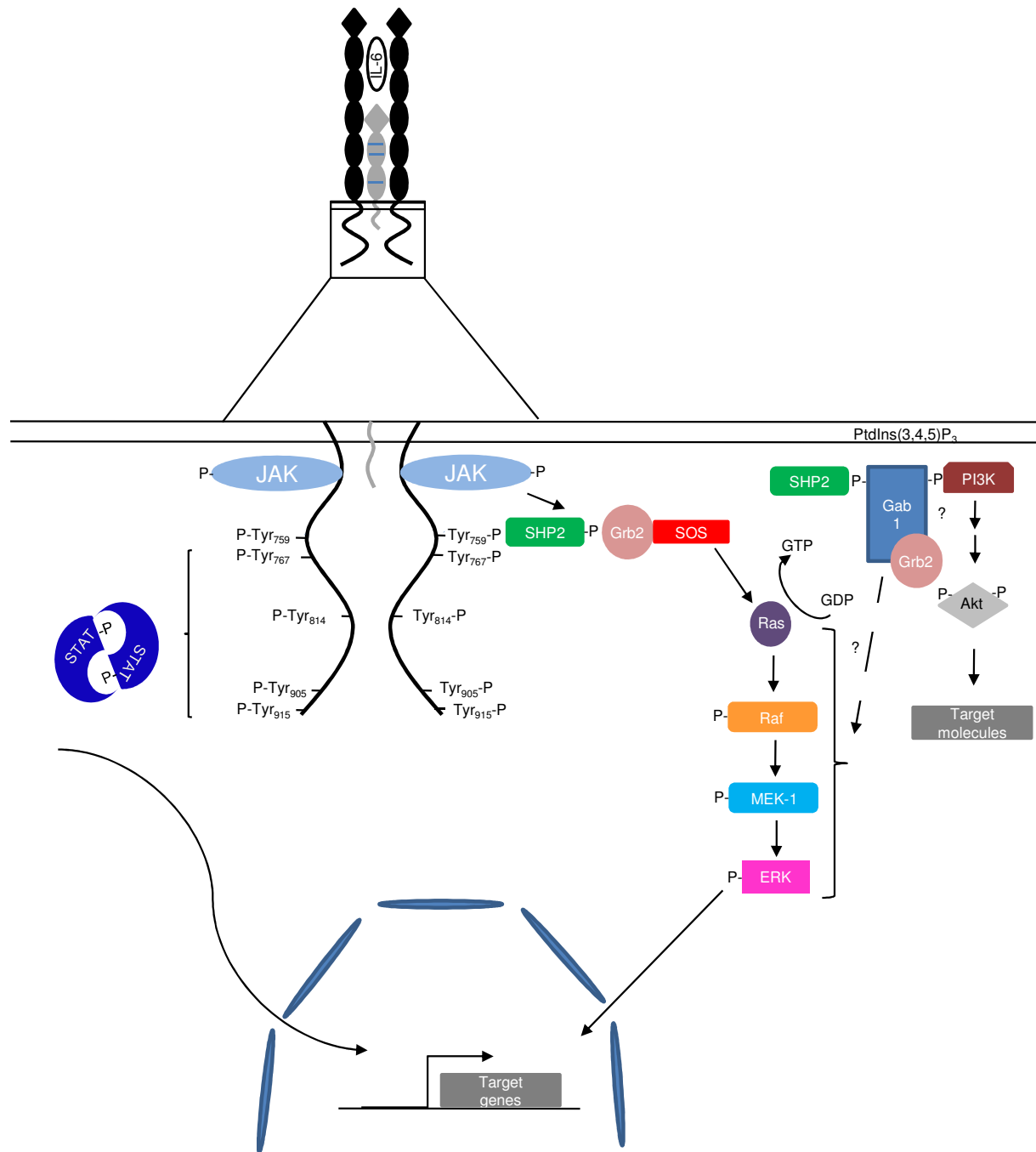


Figure 1.3 Signaling cascades activated by IL-6 with focus on STAT and MAPK/ERK and PI3K activation. See text for details.

The LIFR and the OSMR can also activate STAT5, with OSMR being the most potent activator [65, 66]. Interestingly, this activation may not require receptor phosphorylation due to direct interaction between STAT5 with JAKs [67], but as shown for human OSMR, STAT5 can also be activated by binding to a phosphorylated tyrosine motif (Tyr⁸³⁷/Tyr⁸³⁹) [68]. Upon STAT phosphorylation, the STAT members dimerize and translocate into the nucleus where they recognize DNA

binding sites in the promoter of their target genes [69]. STAT3 target genes are e.g. acute-phase proteins [70], the transcription factor c-myc [71] or the serine/threonine kinase Pim-1 [72].

Signaling via STATs is tightly regulated. There are different pathways for the inhibition of STATs. So called suppressor of cytokine signaling (SOCS) proteins represent negative-feedback inhibitors and their expression is induced by e.g. IL-6, LIF, IL-11 and OSM [73-75]. Those proteins exhibit their inhibitory potential by directly interacting with JAK proteins, thereby inhibiting the phosphorylation of gp130, STATs and JAKs themselves [73, 75]. Another mechanism has been proposed for SOCS3 which binds directly to Tyr⁷⁵⁹ of gp130 [76, 77]. It has been proposed that receptor recruitment of SOCS3 is a prerequisite for subsequent JAK binding. Another pathway of inhibition is dephosphorylation of phosphorylated STAT proteins by tyrosine-phosphatases, as shown for STAT1 [78, 79]. Cytokine signaling by STATs can also be inhibited by protein inhibitor of activated STATs (PIAS) [80, 81].

Besides the JAK/STAT pathway, IL-6-like cytokines can also activate the mitogen activated protein kinase (MAPK) pathway. The Src homology protein 2 tyrosine phosphatase-2 (SHP2) binds to Tyr⁷⁵⁹ of gp130 [63] or Tyr⁹⁷⁴ of LIFR [82] and becomes phosphorylated. The adaptor protein growth factor receptor bound protein 2 (Grb2) is recruited to further activate the Ras/Raf/MAPK pathway. The adaptor protein Grb2-associating binder 1 (Gab1) is also involved in the activation of the Ras/Raf/MAPK pathway [83]. It is known that upon cytokine treatment, Gab1 is recruited to the plasma membrane and binds to phosphatidylinositol-(3,4,5)-phosphate (PtdIns(3,4,5)P₃) and becomes phosphorylated by unknown tyrosine-kinases [84]. Thereafter, SHP2 and PI3K as well as other proteins are recruited to Gab1 [83, 85]. The OSMR also can activate the MAPK-pathway but instead of SHP2 utilizes the SH2- and collagen-homology-domain containing protein (Shc), which is recruited to the receptor via Tyr⁸⁶¹ [86]. Thus, all IL-6-like cytokines including IL-31 can activate the MAPK-pathway. Besides ERK1/2 activation, IL-6 or OSM can also activate stress-induced MAPK: p38 and c-Jun N-terminal kinase (JNK) [66, 87]. However, the molecular mechanism has not been elucidated so far.

Furthermore, it has been demonstrated that IL-6 as well as OSM can activate the phosphoinositol-3-kinase (PI3K) cascade. The molecular mechanisms that lead to the activation of PI3K are also not completely clarified. After stimulation with IL-6, the adaptor protein Grb2-associating binder 1 (Gab1) binds SHP-2 and to PI3K as

described above [83, 85]. In addition, the adaptor protein insulin receptor substrate-1 (IRS-1) is connected with PI3K following OSM stimulation [88]. One downstream target of PI3K is the serine/threonine kinase Akt, which can phosphorylate many other downstream targets [89]. A summary of the principle signaling pathways mediated by IL-6 is shown in Figure 1.3.

1.2.4 Biological effects of IL-6-like cytokines

All IL-6-like cytokines trigger a similar pattern of signal transduction pathways because they share gp130 as a signaling subunit. Hence, IL-6-like cytokines exhibit redundant functions. This has been shown by analyzing the potency of IL-6, IL-11, LIF, OSM, CNTF, and CT-1 to induce acute-phase proteins [90-92].

Despite this redundancy, specific biological effects of cytokines in this family exist. This can be explained by a different cellular expression of the respective receptor subunits. In the following text some important redundant and non-redundant biological effects with respect to physiological functions of IL-6-like cytokines are presented.

IL-6 is involved in haematopoiesis. IL-6 was initially described as a B-cell maturing factor that activates B-cells in a T-cell-independent manner to produce immunoglobulins [93, 94]. Furthermore, IL-6 controls the differentiation of monocytes into macrophages [95] and acts a promoter of maturation of megacaryocytes [96]. IL-6 also acts on T-cells. It has a helper function in the induction of cytotoxic CD8 T-cells [97] and is responsible for the differentiation of naïve CD4 T-cells into follicular T-cells [98] or B-cell helper T-cells (ThB cells), which do not show characteristics of Th1, Th2, Th17, or follicular T-cells [99]. Interestingly, IL-6 together with TGF β leads to the differentiation of naïve CD4 T-cells into Th17 cells [100]. IL-6 in combination with the soluble IL-6R α , which has been named trans-signaling, acts as a pyrogenic factor, thereby inducing leucocytosis which is beneficial for clearance of infection [101]. Another role of IL-6 trans-signaling in acute inflammation is the replacement of neutrophils by mononuclear cells/macrophages, which is an important step in the resolution of inflammation [102, 103]. Moreover, IL-6 trans-signaling has a beneficial impact on liver regeneration after liver injury [104].

IL-11 also acts on haematopoietic cells. It has been shown that IL-11 acts as a potentiator of megacaryopoiesis and thrombocytopoiesis [105, 106]. The IL-11R α is also expressed on a variety of epithelial cells including alveolar, bronchial and

gastrointestinal epithelial cells, suggesting that IL-11 may play an important role in pulmonary inflammation [107] and normal growth control of gastrointestinal epithelial cells [108]. Moreover, IL-11 plays a role in embryogenesis because IL-11R^{-/-} female mice are sterile due to a defect in the decidua formation and fetoplacental development [109].

LIF also is a factor which potentiates megakaryopoiesis [110]. Furthermore, LIF is important for embryogenesis because LIF^{-/-} female mice are sterile due to defect during blastocyst implantation [111]. It has also been demonstrated that LIF preserves the totipotentiality of murine embryonic stem cells [112]. LIF also displays effects in the nervous system where it enhances the survival of sensory and motor neurons [113] as well as oligodendrocytes [114].

OSM also exhibit thrombocytopoietic properties [115]. OSMR^{-/-} mice have reduced frequencies of erythroid and megakaryocyte progenitors in bone marrow [116]. Besides, those mice exhibit impaired regeneration after liver injury [117]. OSM also is involved in neuronal development since OSM^{-/-} mutants have a defect in the development of a subtype of nociceptive neurons [118]. Unlike IL-6 trans-signaling, OSM signaling via OSMR limits monocytic cell recruitment in acute inflammation [119].

CNTF exhibits its function by maintaining the survival of parasympathetic and motorneurons [120]. Thus CNTF^{-/-} mice develop mild progressive motorneuron degeneration [121]. CNTF also exhibits leptin-like abilities. It reduces adiposity, hyperphagia, and hyperinsulinemia of leptin^{-/-} or leptin-resistant (^{db/db}) mice [122]. However, CNTF does not inhibit adipogenesis [123].

CLC regulates immunity by stimulating B cell function and antibody production, with preference for Th2 over Th1 immunoglobuline types [124]. Furthermore, CLC is a circadian regulator of locomotor activity [125].

CT-1 seems to play an important role in neuronal development because CT^{-/-} mice show a lethal phenotype due to an increased loss of motorneurons [126]. Furthermore, CT-1, which is released from the heart *in vivo* in response to hypoxia, acts in an autocrine or paracrine manner to serve potentially critical cardioprotective roles [127, 128].

NP like CNTF also mediates motorneuron survival [29]. With respect to obesity, NP can inhibit adipogenesis but induces insulin-resistance [123].

IL-27 is a potent initiator of Th1-type immune responses [57, 129]. Moreover, IL-27 can inhibit the differentiation of Th17 cells [130]. Besides the T-cell developmental functions of IL-27, it has also been demonstrated that IL-27 exerts interferon-like functions in liver cells and that this can contribute to the antiviral response in these cells [131].

IL-31 also has an impact on haematopoiesis. $GPL^{-/-}$ mice display decreased absolute numbers and cycling status of immature subsets of hematopoietic progenitor cells in bone marrow and spleen [132]. Moreover, $GPL^{-/-}$ mice show negative regulatory abilities of limiting type 2 (Th2-mediated) inflammation [133, 134].

1.2.5 Ligand-dependent, constitutively active gp130 variants

Interestingly, a marked activation of the Interleukin-6 signaling pathway in inflammatory hepatocellular adenomas was directly caused by gain-of-function mutations within the gp130 receptor chain, which led to ligand-independent, constitutively active gp130 proteins [135]. Almost all of these gp130 variants display a somatic in-frame deletion mutation that targets the binding site for IL-6 (site II). The gain-of-function gp130 mutations in these human hepatocellular adenomas are linked to the activation of the acute inflammatory phase observed in tumourous hepatocytes [135].

Previously, a ligand-independent, constitutively active gp130 variant has been established in this laboratory. Stuhlmann-Laeisz *et al.* replaced the entire extracellular portion of gp130 with the c-jun leucine zipper region to create the chimeric receptor protein L-gp130 [136]. Upon retroviral transduction into a factor-dependent pre B-cell line, STAT3 as well as ERK1/2 phosphorylation was detected and the cells exhibited ligand-independent growth over several months [136]. Furthermore transfection of murine embryonal stem cells with L-gp130 led to up-regulation of the stem cell factor OCT-4 and thus suppression of differentiation [136]. The leucine zippers used to ligand independently activate gp130 are naturally found in transcription factors, such as Fos and Jun which form the AP-1 transcription factor complex. Naturally, Fos and Jun leucine zippers form heterodimers, but the Jun leucine zipper can also form stable homodimers albeit with significantly lower stability. The Fos leucine zipper forms an unstable homodimer, which provides a thermodynamic driving force for preferential heterodimer formation with Jun leucine zipper [137, 138]. Therefore, the leucine zipper based technology should be used to

generate ligand-independent, constitutively active receptor gp130-type complexes. During this work, it was shown that this approach was not suitable to generate ligand-independent, constitutively active heterodimeric receptor complexes (Results 4.1-4.3). Therefore a novel system comprising IL-15/IL-15R α should be used to achieve ligand-independent, constitutive activation of heterodimeric gp130-type receptor complexes.

1.3 IL-15 and the IL-15R α

IL-15 belong to the group of four helix bundle cytokines (Figure 1.4A) [3]. Interestingly, the α -receptor for IL-15 does not belong to the class I cytokine receptors like IL-6R α . The IL-15R α it is a type I transmembrane protein and is related to the IL-2R α [139]. The cytokine binding domain of the IL-15R α is called sushi domain (Figure 1.4A). Sushi domains are common motifs. They contain four cysteines forming two disulfide bonds in a 1–3 and 2–4 pattern and found in a number of proteins involved in the complement and coagulation cascades [140]. New insight in cytokine binding has been revealed by analyzing the complex of IL-15/IL-15R α . So far cytokine/cytokine receptor interaction was believed to occur in a ‘hot spot’ manner, meaning that the binding interfaces interact by hydrophobic forces (Figure 1.4 A)[141]. Another common mechanism was solved by analyzing the IL-4/IL-4R α complex which has been described to interact in an ‘avocado-like’ manner, with an outer mantle of hydrophobic side chain moieties and an inner core of polar groups (Figure 1.4 A) [142].

For the IL-15/IL-15R α complex instead, a novel mechanism of cytokine binding has been postulated. Most residues participating in the interaction are charged amino acids. The binding epitope of the IL-15R α -sushi domain exhibits almost basic residues, whereas the IL-15 epitope comprises acidic residues (Figure 1.4A and C) [143]. Hence, the IL-15/IL-15R α complex exhibits a very low dissociation rate (k_{off}). This results in a low equilibrium binding constant (k_D) in comparison to other cytokine/cytokine receptors as shown in Table 1.2.

The IL-15/IL-15R α complex binds to cognate β - and γ -receptors to initiate signaling. Interestingly, the IL-15/IL-15R α complex utilizes the same signaling complex like the IL-2/IL-2R α complex, the IL-2R β and IL-2R γ c [144]. The IL-2R β and IL-2R γ c belong to the group 4 and group 5 of class I cytokine receptors, respectively (Table 1.1). After receptor-ligand interaction associated JAKs are activated. The IL-2R β chain is

associated with JAK1, whereas IL-2R γ c is associated with JAK3, which in turn results in the phosphorylation of STAT3 and STAT5, respectively [145]. Additional signaling pathways triggered by IL-15 involve the phosphorylation of the Src family cytoplasmic tyrosine kinases Lck, Fyn, and Lyn, Syk kinase, PI3K and Akt as well as the Ras/Raf/MAPK pathway and NF- κ B activation [146-150].

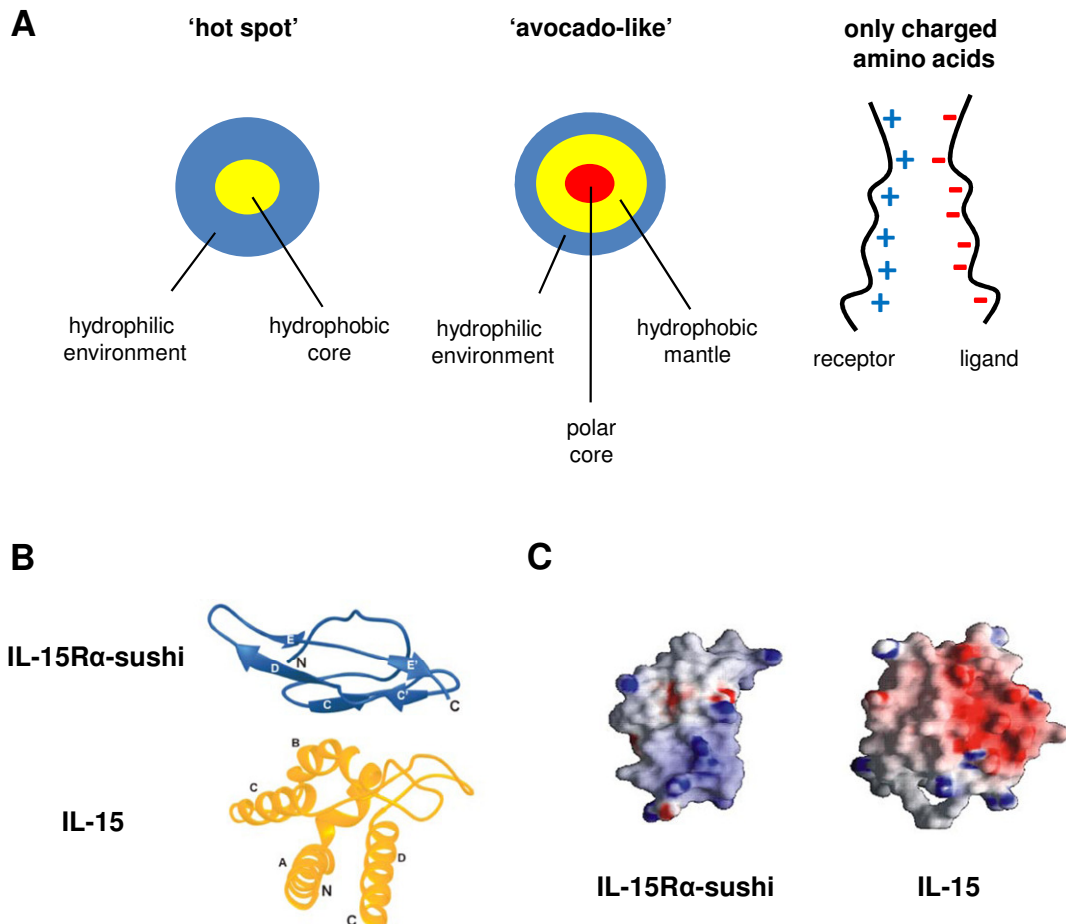


Figure 1.4 Biochemical mechanisms of ligand/receptor interaction, structure and electrostatic surface potential of IL-15 and IL-15R α -sushi domain. A) Schematic representations of different biochemical mechanisms of ligand/receptor interaction. Only the ligands and their biochemical properties of the binding interfaces are shown for the 'hot spot' and the 'avocado-like' models. (B) Model of the complex of the IL-15R α -sushi domain (blue) and IL-15 (yellow) as *ribbon* representation [143]. (C) Electrostatic surface potential of the IL-15R α -sushi domain and IL-15. Negative charges are displayed in red, positive charges in blue. The complex of the α -receptor and its ligand were rotated by 90° (the receptor left hand, the ligand right hand) [143].

complex	$k_{on} (M^{-1} s^{-1})$	$k_{off} (s^{-1})$	$k_D (pM)$	reference
IL-2/IL-2R α	1.06×10^7	0.298	28100	[151]
IL-4/IL-4R α	1.9×10^7	2×10^{-3}	105	[152]
IL-6/IL-6R α	1.9×10^7	6.4×10^{-3}	34000	[153]
IL-15/IL-15R α	3.7×10^5	1.4×10^{-5}	38	[154]
IL-15/IL-15R α -sushi	2.5×10^5	3.8×10^{-4}	1500	[154]

Table 1.2 Binding constants of cytokines and their cognate receptor.

Several reports have suggested that the simultaneous expression of IL-15R α in the same cell is necessary for the production and secretion of IL-15 under physiological conditions [155-159]. IL-15 complexes with the IL-15R α in the endoplasmic reticulum (ER) in a chaperone-like manner and is passaged through the classical secretion pathway involving ER and the Golgi apparatus [160, 161]. Thereafter, IL-15 in complex with its α -receptor is expressed at the cell surface and can stimulate neighbouring cells expressing IL-2R β and IL-2R γ c in a trans-presentation manner. For example, this has been demonstrated for dendritic cells (DC), which presented the IL-15/IL-15R α complex at the cell surface, supported natural killer cell (NK) activation [160].

IL-15 can be detected in sera of mice and cell culture supernatants in a heterocomplex with soluble IL-15R α [162]. Different sIL-15R α molecules can be generated by alternative splicing or proteolytic cleavage with different outcomes for signaling [162]. Whereas proteolysis might primarily be responsible for the generation of sIL-15R α capable of inhibiting IL-15 action [162, 163], the secreted sIL-15R α -sushi domain, which is generated by alternative splicing, exhibits agonistic qualities [162]. The latter has also been tested in a study using recombinant sIL-15R α -sushi domain [154]. A study using different sIL-15R α -sushi variants revealed that addition of 13 amino acids encoded by exon 3 (the sushi domain is encoded by exon 2) leads to a stabilization of the IL-15/IL-15R α complex by decreasing its dissociation rate but acted antagonistically in the presence of IL-15 [164].

Taken together, the complex of IL-15 and IL-15R α displays an exceptional low k_{off} and therefore a very low k_D . This finding should be used in this work to generate ligand-independent and constitutively active heterodimeric complexes of all gp130-type receptors.

2 Aim of the work

The aim of this work was to generate ligand-independent, constitutively active, heterodimeric receptor complexes of all gp130-type receptors. Besides the homodimeric gp130-mediated signaling, other receptor complexes such as gp130/LIFR, gp130/OSMR, gp130/WSX-1, and GPL/OSMR could be involved in inflammatory diseases and cancer. Furthermore, a new methodology to generate ligand-independent, constitutively active receptor complexes could be a useful tool to study the biochemical characteristics of several gp130-type heterodimeric receptor complexes with respect to signaling without any need of ligand treatment. Additionally, using this technology *in vivo* would be an advantage in comparison to ectopically expressed cytokines because expression of those ligand-independent, constitutively active, heterodimeric receptor complexes could be addressed at a cell autonomous level.

3 Material & methods

3.1 Material

3.1.1 Antibiotics

Ampicillin (Carl Roth GmbH, Karlsruhe, Germany)	f.c. 100 µg/ml
Kanamycin (Carl Roth GmbH, Karlsruhe, Germany)	f.c. 25 µg/ml
Puromycin (PAA Laboratories GmbH, Cölbe, Germany)	f.c. 1.5 µg/ml
Hygromycin B (PAA Laboratories GmbH, Cölbe, Germany)	f.c. 1 mg/ml

3.1.2 Antibodies

3.1.2.1 Primary antibodies

α -c-myc	mouse monoclonal antibody (9E10) recognizes human, mouse and rat c-myc protein as well as Myc-tag. Raised against a peptid corresponding to residues 408 – 439 of human c-myc (Santa Cruz Biotechnology, Inc., Heidelberg, Germany). → WB: 1:1000
α -FLAG	rabbit polyclonal antibodies recognize FLAG [®] -tag (DYKDDDDK) (Sigma-Aldrich Chemie, Taufkirchen, Germany). → WB: 1:1000
α -FLAG	mouse monoclonal antibody (M2) recognizes FLAG [®] -tag (DYKDDDDK) (Sigma-Aldrich Chemie, Taufkirchen, Germany). → WB: 1:1000; FC 1:1000 (1 µg)
α -GFP	mouse monoclonal antibody (7.1 and 13.1) recognizes the green fluorescent protein (GFP) (<i>Aequorea victoria</i>), GFP derivatives such as e.g. YFP, EYFP and CFP (Roche Applied Science, Mannheim, Germany). → WB: 1:1000; IP: 1:40 (2 µg)

- α -IL-15 mouse monoclonal antibody recognizes human IL-15 (PeproTech GmbH, Hamburg Germany).
→ FC: 1:100 (1 μ g)
- α -IL-15 rabbit polyclonal antibody recognizes human IL-15 (AbD Serotec, Düsseldorf, Germany).
→ WB: 1:5000; IP: 1:100 (2 μ g)
- α -gp130 rabbit polyclonal antibody (C-20) recognizes the intracellular domain of human, mouse and rat gp130 (Santa Cruz Biotechnology, Inc., Heidelberg, Germany).
→ WB: 1:1000; IP: 1:40 (1 μ g)
- α -Myc-tag rabbit monoclonal antibody (71D10) recognizes Myc-tag sequence (EQKLISEEDL) corresponding to residues 410 – 419 of human c-myc (Cell Signaling Technology, Inc., Danvers, MA, USA, (New England Biolabs GmbH, Frankfurt, Germany)).
→ WB: 1:1000; FC: 1:1000
- α -Phospho-p44/42 MAP kinase rabbit polyclonal antibody recognizes broad species p44/42 MAP kinase only if phosphorylated at residues Thr202 / Tyr204 (Cell Signaling Technology, Inc., Danvers, MA, USA, (New England Biolabs GmbH, Frankfurt, Germany)).
→ WB: 1:1000
- α -p44/42 MAP Kinase rabbit polyclonal antibody recognizes broad species p44/42 MAP kinase (Cell Signaling Technology, Inc., Danvers, MA, USA, (New England Biolabs GmbH, Frankfurt, Germany)).
→ WB: 1:1000
- α -Phospho-STAT1 rabbit monoclonal antibody (58D6) recognizes human, mouse and rat STAT1 only if phosphorylated at residue Tyr701 (Cell Signaling Technology, Inc., Danvers, MA, USA, (New England Biolabs GmbH, Frankfurt, Germany)).

→ WB: 1:1000

α - STAT1 rabbit polyclonal antibodies recognize human, mouse, rat and monkey STAT1 (Cell Signaling Technology, Inc., Danvers, MA, USA, (New England Biolabs GmbH, Frankfurt, Germany)).
→ WB: 1:1000

α -Phospho-STAT3 rabbit monoclonal antibody (D3A7) recognizes human, mouse and rat STAT3 only if phosphorylated at residue Tyr705 (Cell Signaling Technology, Inc., Danvers, MA, USA, (New England Biolabs GmbH, Frankfurt, Germany)).
→ WB: 1:1000

α - STAT3 mouse monoclonal antibody (124H6) recognizes human, mouse, rat and monkey STAT3 (Cell Signaling Technology, Inc., Danvers, MA, USA, (New England Biolabs GmbH, Frankfurt, Germany)).
→ WB: 1:1000

α -Phospho-Tyrosine mouse monoclonal antibody (P-Tyr-100) recognizes tyrosine-phosphorylated residues (Cell Signaling Technology, Inc., Danvers, MA, USA, (New England Biolabs GmbH, Frankfurt, Germany)).
→ WB: 1:2000

3.1.2.2 Secondary antibodies

ImmunoPure peroxidase conjugated goat anti-mouse IgG (H+L) (Thermo Fisher Scientific, p/a Perbio Science Deutschland, Bonn, Germany)

→ WB: 1:10,000

ImmunoPure peroxidase conjugated goat anti-rabbit IgG (H+L) (Thermo Fisher Scientific, p/a Perbio Science Deutschland, Bonn, Germany)

→ WB: 1:10,000

Allophycocyanin conjugated AffiniPure F(ab')₂ fragment goat anti-mouse IgG (H+L) (Dianova GmbH, Hamburg, Germany)

→ FC: 1:100

AlexaFluor® 488 conjugated F(ab')₂ fragment goat anti-rabbit IgG (H+L) (Invitrogen GmbH, Karlsruhe, Germany)

→ FC: 1:100

3.1.3 Bacterial media

Luria Bertani (LB) medium	10 g NaCl 10 g peptone 5 g yeast extract ad 1 l (pH 7.5)
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Luria Bertani (LB) plates	10 g NaCl 10 g peptone 5 g yeast extract 20 g agar ad 1 l (pH 7.5)
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3.1.4 Buffers and Solutions

Blocking buffer (<i>Western blot</i>)	1x TBS-T 5% nonfat dry milk
DNA loading dye (6x) (agarose gel)	0.1% bromophenol blue 0.1% xylene cyanol 10 mM EDTA 40% glycerol

FACS buffer (<i>flow cytometry</i>)	1x PBS 1% BSA
Mild lysis buffer (<i>cell lysis</i>)	50 mM Tris-HCl (pH 7.5) 150 mM NaCl 1% Triton X-100 1 x protease inhibitor cocktail
Laemmli buffer (5x) (<i>SDS-PAGE</i>)	185 mM Tris-HCl (pH 6.8) 50% glycerol 10% sodium dodecyl sulfate 5% β -mercaptoethanol 0.05% bromphenol blue
Lysis buffer (<i>cell lysis</i>)	50 mM Tris-HCl (pH 7.5) 150 mM NaCl 2 mM EDTA 1 mM NaF 1 mM Na_3VO_4 1% IGEPAL (NP-40) 1% Triton-X-100
Immunoprecipitation buffer (<i>cell lysis</i>)	20 mM Tris-HCl (pH 7.5) 150 mM NaCl 1 mM EDTA 1 mM EGTA 1% Triton X-100 2.5 mM $\text{Na}_4\text{P}_2\text{O}_7$ 1 mM β -glycerophosphate 1 mM Na_3VO_4 1 x protease inhibitor cocktail

Phosphate buffered saline - PBS (1x) (<i>cell culture</i>)		137 mM NaCl 2.7 mM KCl 4.3 mM Na ₂ HPO ₄ 1.47 mM KH ₂ PO ₄ (pH 7.6)
Running gel (10%) (<i>SDS-PAGE</i>)	3.87 ml 2.55 ml 0.1 ml 3.33 ml 100 µl 10 µl	ddH ₂ O 1.5 M Tris-HCl (pH 8.8) 10% sodium dodecyl sulfate 30% Acrylamide-Bis 29:1 10% Ammonium persulfate TEMED
S1 Buffer (<i>Miniprep</i>)		50 mM Tris-HCl (pH 8.0) 10 mM EDTA 100 µg/ml RnaseA
S2 Buffer (<i>Miniprep</i>)		200 mM NaOH 1% sodium dodecyl sulfate
S3 Buffer (<i>Miniprep</i>)		2.8 M KAc, pH 5.1
SDS Running buffer (<i>SDS-PAGE</i>)		25 mM Tris base 192 mM glycine 0.1% sodium dodecyl sulfate
Stacking gel (4 %) (<i>SDS-PAGE</i>)	3.725 ml 0.625 ml 0.05 ml 0.67 ml 100 µl 10 µl	ddH ₂ O 0.5 M Tris-HCl (pH 6.8) 10% sodium dodecyl sulfate 30% Acrylamide-Bis 29:1 10% Ammonium persulfate TEMED

Stripping buffer (<i>Western blot</i>)	62.5 mM Tris-HCl (pH 6.8) 2% sodium dodecyl sulfate 0.1% β -mercaptoethanol
TBE (0.5x) (<i>agarose-gels</i>)	44.5 mM boric-acid 10 mM EDTA, pH 8.0 44.5 mM Tris-HCl
Transfer Buffer (<i>Western blot</i>)	25 mM Tris base 0.2 M glycine 20% methanol (pH 8.5)
Tris buffered saline with Tween 20 (1xTBS-T) (<i>Western blot</i>)	10 mM Tris base 150 mM NaCl 0.1 % Tween 20 (pH 7.6)

3.1.5 Cell culture media, solutions and material

DMEM or DMEM -/-	Dulbecco's Modified Eagle Medium (high glucose 4.5 g/l and stable glutamin) (PAA Laboratories GmbH, Cölbe, Germany)
DMEM +/-	Dulbecco's Modified Eagle Medium (high glucose 4.5 g/l and stable glutamin) (PAA Laboratories GmbH, Cölbe, Germany) supplemented with 10% fetal bovine serum (PAA Laboratories GmbH, Cölbe, Germany) and 1% penicillin / streptomycin (PAA Laboratories GmbH, Cölbe, Germany)

IMDM Iscove's modified DMEM (with L-Glutamine) (PAA Laboratories GmbH, Cölbe, Germany)

Trypsin EDTA (PAA Laboratories GmbH, Cölbe, Germany)

Cell culture material was from Sarstedt AG & Co (Nürnbrecht, Germany).

3.1.6 Cell lines

Ba/F3-gp130 murine pre B-cell line stably transfected with a cDNA coding for human gp130. These cells grow in dependence of IL-6 and sIL-6R. Ba/F3-gp130 cells were from Immunex (Seattle, WA, USA).

COS-7 African green monkey (*Cercopithecus aethiops*) kidney fibroblast-like cell line suitable for transient transfections (ATCC: CRL-1651).

Phoenix-Eco packaging cell line (HEK293T) which is stably transfected with cDNAs expressing group-specific antigen, polymerase and envelope proteins for ecotropic viruses. This cell line was a kind gift of Ursula Klingmüller (DKFZ, Heidelberg, Germany).

3.1.7 Chemicals

All chemicals were from Carl Roth GmbH (Karlsruhe, Germany), Merck KGAA (Darmstadt, Germany) or Sigma-Aldrich Chemie, Taufkirchen, Germany).

3.1.8 Enzymes

All enzymes, including restriction enzymes, polymerases and DNA modifying enzymes are from Fermentas GmbH (St. Leon-Rot, Germany).

3.1.9 Molecular weight markers

For DNA analysis, GeneRuler™ 1 kb DNA ladder (250 – 10000 bp) was used. For protein analysis prestained molecular weight marker (25 – 120 kDa) was used. Both markers were obtained from Fermentas GmbH (St. Leon-Rot, Germany)

3.1.10 Kits

Cell viability assay: CellTiter-Blue® Cell Viability Assay (Promega GmbH, Mannheim, Germany)

ECL detektion: ECL Plus™ (GE Healthcare, Munich, Germany)

PCR clean up and gel extraction: Nucleospin® Extract II (Macherey-Nagel GmbH & Co. KG, Düren, Germany)

Plasmid midi preparation: Nucleobond® Xtra Midi (Macherey-Nagel GmbH & Co. KG, Düren, Germany)

Protein concentration determination: BCA protein assay (Thermo Fisher Scientific, p/a Perbio Science Deutschland, Bonn, Germany)

RNA preparation: Nucleospin® RNA II (Macherey-Nagel GmbH & Co. KG, Düren, Germany)

3.1.11 Oligonucleotides

All oligonucleotides were purchased from Metabion GmbH (Munich, Germany). All oligonucleotides used are listed in Table 3.1.

Name	Sequence 5'-3'
5'Fos	GAATTGTGCGGCGGCTTAACTGATACACTCCAA
5' Δ cysFos	GAATTGGGCGGCGGCTTAACTGATACACTCCAA
3'Fos	AGTCGAATTCAGCTGCCAGGATGAACTC
5'SPg130FLAG	GATCCTCGAGTCTAGACCCCGCAAG
3'SPg130FLAG for Fos-gp130	GCCGCCGCACAATTCTTTATC
3'SPg130FLAG for Δ cysFos-gp130	GCCGCCGCCCAATTCTTTATC
5'mWSX-1	GACTGAATTCTCACTTCACCTACCAGATAATAGG
3'mWSX-1	GACTGGATCCTCAGACTAGAAGGCCAGCTCCTC
5'PPL-IL-15	GATCCTCGAGCCACCATGGACAGCAAAGGTTCCG
3'PPL-IL-15	GATCGAATTCAGAAGTGTGATGAACATTTG
5'mycsushi	AGCGAGGAGGACCTGATCACATGCCCTCCCCC
3'sushi	GATCGAATTCGTCTCTAATGCATTTGAG
5'SPg130myc	GATCCTCGAGCCACCATGTTGACGTTGCAGACTTG
3'SPg130myc	CAGGTCCTCCTCGCTGATCAGCTTCTGCTCACCTGTAGATTCAGTGG
5'hygromycin B resistance	GATCAAGCTTCCACCATGAAAAGCCTGAACTCACC
3'hygromycin B resistance	GATCGTCGACTCAGTTAGCCTCCCCCATC
5'hLIFR	GAATTCCCGGAGAAGAGTATGTATGTG
3'hLIFR	GGATCCTTAATCGTTTGGTTTGTCTG
5'hOSMR	GAATTCCCCAGTGCTACGTTCCAG
3'hOSMR	GGATCCTTAGCAGTAGTGTT CACC
5'hGPL	GAATTCGGGACCAGCATAAATTC
3'hGPL	GGATCCTTAGACTTCTCCCTTGG
oligo-dT	TTTTTTTTTTTTTTTTTT
5'IL-15check	CCACCATGGACAGCAAAG
5'SPg130check	CCACCATGTTGACGTTGC
3'gp130check	TCACTGAGGCATGTAGCC
3'WSX-1check	TCAGACTAGAAGGCCAG
3'LIFRcheck	ATCGTTTGGTTTGTCTG
3'OSMRcheck	TTAGCAGTAGTGTTCCACC
3'GPLcheck	TTAGACTTCTCCCTTGG
5'mc-myc	TTCTCAGCCGCTGCCAAGCTGGTC
3'mc-myc	GGTTTGCTGTGGCCTCGGGATGGA
5'mPIM-1	GATCATCAAGGGCCAAGTGT
3'mPim-1	CCATCTTGGTGACCCAGTCT
5'beta-actin	GTGGGGCGCCCCAGGCACCA
3'beta-actin	CTCCTTAATGTCACGCACGATTC
T7 promotor	TAATACGACTCACTATAGGG
M13 rev	GGAAACAGCTATGACCATG
5'pMOWS	AGCCCTTTGTACACCCTAAGC
3'pMOWS	AGCAATAGCATGATACAAAGG

Table 3.1 List of all primers used in this work.

3.1.12 Plasmids

p409	mammalian expression vector for transient transfection; ampicillin-resistance
p409-hLIFR	mammalian expression vector for transient transfection; encodes human full-length human LIFR; ampicillin-resistance [165]
p409-hOSMR	mammalian expression vector for transient transfection; encodes human full-length human OSMR; ampicillin resistance [166]
pBSK	cloning vector; ampicillin-resistance
pBSK-L-gp130	cloning vector; it encodes constitutively active L-gp130; ampicillin resistance [136]
pBSK-PPL-IL-15	cloning vector; it encodes human IL-15 which has an alternative signal peptide of bovine preprolactin; ampicillin-resistance; was a kind gift of Yannick Jacques (Nantes, France) [167]
pcDNA3.1-hGPL	mammalian expression vector for transfection; encodes human full-length GPL (IL-31R); ampicillin-resistance; was a kind gift of Hugues Gascan (Angers, France)
pCEP4	an episomal mammalian expression vector; encodes hygromycin B resistance gene; ampicillin-resistance (Invitrogen GmbH, Karlsruhe, Germany).
pEYFP-hgp130	Mammalian expression vector for transient transfection; it encodes EYFP-tagged full-length human gp130; kanamycin-resistance [168]
pMOWS	Mammalian expression vector for retroviral transduction, ampicillin resistance and puromycin-resistance [169]

pPCR-script-HIL-6 cloning vector; encodes Hyper-IL-6; ampicillin-resistance

pQE30-Fos bacterial expression vector; it encodes codon optimized Fos peptide (Geneart); ampicillin-resistance

pQE30-IL-15R α -sushi bacterial expression vector, it encodes the sushi domain of human IL-15R α ; ampicillin resistance [143]

3.1.13 Recombinant proteins

Hyper-IL-6 (HIL-6), a fusion protein of human IL-6 and human sIL-6R was produced in our working group as described [170]. Human IL-27 was a kind gift of Christoph Hölscher (Borstel, Germany). Human IL-15, human IL-15R α -sushi domain was produced in our institute by Inken Lorenzen [143].

3.2 Methods

3.2.1 Molecular biology

3.2.1.1 Polymerase chain reaction (PCR)

To amplify DNA sequences for cloning or detection, PCR was used. Pfu polymerase (Fermentas GmbH, St. Leon-Rot, Germany) was used for amplification of DNA for subsequent cloning, while Taq or DreamTaq™ DNA polymerases (Fermentas GmbH, St. Leon-Rot, Germany) were used for detection of specific DNA sequences.

The following reaction mixture was used for the different applications:

Template	10 – 100 ng plasmid or 1 µl of cDNA or bacterial colonies
Forward primer	10 µM
Reverse primer	10 µM
dNTPs	10 mM each
PCR buffer (10x)	5 µl
Polymerase	1 U
ddH ₂ O	ad 50 µl

The PCR was performed with the following step gradient:

95 °C	2 min	} 30 - 35 cycles
95 °C	45 s	
55 °C	45 s	
72 °C	60-90 s	
72 °C	5 min	

A splicing by overlapping extension PCR (SOE-PCR) approach was used to ligate two PCR fragments. The mixture, as described above, was used and 1 µl of each PCR reaction was used as a template.

3.2.1.2 Phosphorylation of PCR fragments

For a subsequent blunt end ligation, PCR fragments obtained from PCR with *Pfu* polymerase were phosphorylated with T4 polynucleotid kinase (Fermentas GmbH, St. Leon-Rot, Germany). In detail, 8 µl of PCR product were mixed with 1 µl ligase buffer

and 1 μ l (10 U) of T4 polynucleotid kinase and incubated for 30 min at 37°C. Subsequently, the mixture was incubated for 20 min at 65°C to inactivate the enzyme. 5 μ l thereof was used for ligation.

3.2.1.3 Digestion of DNA

The digestion of plasmids or PCR fragments was performed with restriction enzymes. The DNA was incubated with an appropriate amount of restriction enzymes in the recommended buffer for 1-3 h or overnight at 37°C. The digestion was terminated by addition of DNA sample buffer and the digested DNA was applied to an agarose gel. If a digestion with two enzymes was performed, the guide webpage of Fermentas GmbH was consulted (http://www.Fermentas_GmbH.com/en/tools/doubledigest). If DNA blunting by fill-in 5'-overhangs was performed after digestion with the first enzyme, the DNA was purified using Nucleospin[®] Extract II (Macherey-Nagel GmbH & Co. KG, Düren, Germany) following the manufacturer's instructions before the second digestion step was prepared. For a preparative digestion 10 μ g of plasmid DNA and 10 U of each restriction enzyme were used in a total volume of 50 μ l. For an analytical digestion 1 μ g of plasmid DNA or 5 μ l of a plasmid mini preparation and at least 3 U of each restriction enzyme were used in a total volume of 20 μ l and incubated for 1 h at 37°C

3.2.1.4 DNA blunting

Klenow fragment (Fermentas GmbH, St. Leon-Rot, Germany) was used for DNA blunting by fill-in 5'-overhangs. After digestion with the appropriate restriction enzyme 2 μ l of dNTPs (10 mM) and 10 U of Klenow fragment were added (in all cases of DNA blunting the buffer used for digestion was 100% compatible with Klenow fragment). The mixture was incubated for 1 h at 37°C and the DNA was purified using Nucleospin[®] Extract II (Macherey-Nagel GmbH & Co. KG, Düren, Germany) following the manufacturer's instructions.

3.2.1.5 Dephosphorylation of Plasmid DNA

After enzymatic restriction, the plasmid DNA was dephosphorylated by direct addition of 1 U Calf Intestinal Alkaline Phosphatase (CIAP) (Fermentas GmbH, St. Leon-Rot,

Germany) to the restriction reaction (the buffer used for digestion was 100% compatible with CIAP). The reaction was carried out at 37°C for 1-2 h.

3.2.1.6 DNA Gel-electrophoresis

DNA fragments were separated with horizontal electrophoresis chambers (Bio-Rad Laboratories GmbH, Munich, Germany) using agarose gels. Agarose gels were prepared by heating 1-2% (w/v) agarose (Biozym Scientific GmbH, Hess. Oldendorf, Germany) in 0.5x TBE buffer, depending on the size of the DNA fragments and the gels were supplemented with 0.002% ethidium bromide. The samples were mixed with an appropriate amount of 6x DNA sample buffer and loaded on the agarose gel. The gels were run at constant voltage (100 V) and finally documented using the Gel Doc 2000 UV-light documentation system (Bio-Rad Laboratories GmbH, Munich, Germany).

3.2.1.7 Extraction of DNA fragments from agarose gel

For isolation and purification of DNA fragments from agarose gels, ethidium bromide-stained gels were illuminated with UV-light and the appropriate DNA band was excised from the gel with a clean scalpel and transferred into a reaction tube. The fragment was isolated by using Nucleospin[®] Extract II (Macherey-Nagel GmbH & Co. KG, Düren, Germany) following the manufacturer's instructions.

3.2.1.8 Determination of DNA or RNA concentration

DNA concentration was determined spectroscopically using an Ultrospec 3000 spectrophotometer (Pharmacia Biotech) or NanoDrop[®] ND-1000 (Peqlab Biotechnologie GmbH, Erlangen, Germany). For the Ultrospec 3000 spectrophotometer the DNA or RNA were diluted 1:100 and the absorbance of 260 nm, 280 nm and 320 nm were measured. The NanoDrop[®] ND-1000 can detect concentrations between 2 and 3700 ng/ul for dsDNA; 2 and 3000 ng/ul RNA and 2 and 2400 ng/ul ssDNA, without dilution of samples. The ratio of A_{260}/A_{280} of 1.8 or higher for DNA and 2.0 or higher for RNA indicated the purity of nucleic acid samples.

3.2.1.9 Ligation of DNA fragments

For ligation cleaved and dephosphoylated vector and cleaved or phosphorylated insert were mixed in a ratio of 1:3 – 1:5 supplemented with 2 µl of ligation buffer and 1 µl of T4 DNA ligase (Fermentas GmbH, St. Leon-Rot, Germany) in a total volume of 20 µl. The ligation approach was incubated for 2 h at room temperature and/or at 4 °C overnight.

3.2.1.10 Transformation of chemocompetent *E.coli*

One aliquot of chemocompetent XL-1-Blue *E.coli* were mixed with 10 – 100 ng of plasmid DNA or 20 µl of a ligation mixture and incubated for 10 min on ice. After a heat shock of 90 s at 42 °C the cells were incubated for 10 min on ice. Subsequently, 1 ml of LB-medium was added and the bacteria were incubated for 1 h at 37 °C. Afterwards the bacteria were centrifuged (6000 x g, 1 min), the supernatant was removed, the pellet was resuspended in 100 µl LB-medium, and plated on LB-agar plates containing the appropriate antibiotics. Plates were incubated overnight at 37 °C.

3.2.1.11 Plasmid isolation from 2 ml bacteria suspension – plasmid mini preparation

2 ml LB-medium supplemented with the appropriate antibiotic was inoculated with a single colony and incubated overnight at 37 °C and 600 rpm. Cultures were pelleted by centrifugation (6,000 x g, 1 min) and resuspended in 100 µl Buffer S1. For bacterial lysis the suspension was supplemented with 100 µl Buffer S2 and incubated for 2-3 min at room temperature. Subsequent 100 µl of Buffer S3 was added and the mixture was inverted until a homogenous suspension containing a white precipitate was formed. The bacterial lysate was cleared by centrifugation (16,000 x g, 10 min) and the resulting supernatant was transferred to a new reaction tube and supplemented with 600 µl ice cold 100% ethanol. The plasmid DNA was pelleted by centrifugation (16,000 x g, 10 min at 4 °C) and washed once with ice cold 70% ethanol. The DNA pellet was dried in a speedvac and dissolved in 50 µl ddH₂O.

3.2.1.12 Plasmid isolation from 100 ml bacteria suspension – plasmid midi preparation

For preparation of large quantities of DNA, the Nucleobond® Xtra Midi kit (Macherey-Nagel GmbH & Co. KG Düren, Germany) was used. A single colony was inoculated in 100 ml LB-medium supplemented with the appropriate antibiotic and incubated overnight at 37°C and 180 rpm. The culture was transferred into two 50 ml tubes and the cells were pelleted by centrifugation (4,000 x g, 25 min, 4°C). The plasmid was isolated from the bacteria according to the manufacturer's protocol. Finally the DNA pellet was resuspended in 120 µl ddH₂O and the DNA concentration was determined.

3.2.1.13 DNA sequencing

DNA sequencing was performed by the company SeqLab GmbH, (Göttingen, Germany). For sequencing 0.7 µg of DNA and 20 µM sequencing primer were diluted in 7 µl ddH₂O.

3.2.1.14 RNA isolation

Total RNA from Ba/F3-gp130 cells (~10 x 10⁶ cells) was isolated with the Nucleospin® Extract II (Macherey-Nagel GmbH & Co. KG, Düren, Germany) following the manufacturer's instructions and the RNA concentration was determined by UV-spectrophotometry.

3.2.1.15 Reverse Transcription (RT)

2 µg of total RNA was used for reverse transcription reaction. In detail, 2 µg of total RNA were mixed with 100 µM oligo-dT primer in a total volume of 13 µl. This mixture was incubated for 10 min at 70°C and subsequently cooled to room temperature to achieve primer annealing. 200 U (1 µl) of RevertAid™ M-MuLV Reverse Transcriptase (Fermentas GmbH, St. Leon-Rot, Germany), 4 µl of the appropriate buffer and 2 µl of dNTPs (10 mM) were added to the mixture and incubated for 1.5 h at 37°C. Afterwards the mixture was again incubated for 10 min at 70°C. 1 µl of this RT-reaction was used for the subsequent PCR.

3.2.2 Cell culture and cell based assays

3.2.2.1 Cell cultivation

All cells (Ba/F3-gp130, COS-7 and Phoenix-Eco) were cultured in DMEM + 10% FBS + 1% Pen/Strep (DMEM +/+). The medium for Ba/F3-gp130 cells was supplemented with 10 ng/ml Hyper-IL-6. The cells were maintained at 37 °C with 5% CO₂ in a water saturated atmosphere. All cells were cultured in 10 cm dishes. For passaging COS-7 cells were washed with 10 ml sterile PBS and detached with 2 ml trypsin EDTA (PAA Laboratories GmbH, Cölbe, Germany). The cells were passaged in a ratio of 1:10 to a new 10 cm dish containing 10 ml fresh medium. Phoenix-Eco cells were directly trypsinised with 2 ml of trypsin EDTA and passaged in a ratio of 1:20. Ba/F3-gp130 cells were passaged in a ratio of 1:100. Stably transduced Ba/F3-gp130 cells which exhibited cytokine independent growth were maintained in DMEM +/+ lacking Hyper-IL-6.

3.2.2.2 Transfection of eucaryotic cells

For transfection of Phoenix-Eco cells either Lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany) or Turbofect (Fermentas GmbH, St. Leon-Rot, Germany) was used. For transfection of COS-7 cells only Turbofect was used. In principle the manufacturer's instructions were followed but the amount of DNA and transfection reaction were changed. In detail, the day before transfection 8×10^5 Phoenix-Eco cells (COS-7: 5×10^5) were seeded on a well of a 6-well plate (COS-7: 10 cm dish). The day after seeding Phoenix-Eco cells were transfected with 1 µg of DNA (COS-7: 5 µg) and 3 µl of Lipofectamine 2000 or 2 µl of Turbofect (COS-7: 10 µl). After 6 h of incubation the medium was exchanged by fresh DMEM +/+. In case of Phoenix-Eco cells IMEM (DMEM) + 30% FBS, 50 µM β-mercaptoethanol + 1% pen/strep was used. 24 h later this supernatant was used for retroviral transduction of Ba/F3-gp130 cells. In the case of COS-7 cells normal DMEM +/+ was used for medium change and the cells were lysed 48 h after transfection.

3.2.2.3 Starvation of cells

Ba/F3-gp130 cells ($\sim 10 \times 10^6$ cells) were collected in a 15 ml tube and pelleted (1000 x g, 5 min). The medium was removed and the cells were washed three times with

PBS. The cells were resuspended in DMEM -/- containing no FBS. The cells were starved for 6 h, again collected in a 15 ml tube and pelleted (1000 x g, 5 min). After removal of the medium, RNA isolation or cell lysis for protein-biochemical analysis was performed.

Transiently transfected COS-7 cells were also washed three times with PBS and the cells were starved in DMEM -/- for at least 20 h prior lysis.

3.2.2.4 Pervanadat treatment of COS-7 cells

500 μ l of 100 mM sodium orthovanadate solution was incubated with 2.8 μ l of 30% H₂O₂ to obtain pervanadate. Immediately after preparation of pervanadate COS-7 cells were treated with 1 mM pervanadate for 15 min at 37°C prior lysis to block tyrosine-dephosphorylation.

3.2.2.5 Retroviral transduction of Ba/F3-gp130 cells

Ba/F3-gp130 were retrovirally transduced with supernatants of pMOWS transfected Phoenix-Eco cells [169]. In detail, 1 x 10⁵ Ba/F3-gp130 in 50 μ l of DMEM +/+ were mixed with 250 μ l filtrated (pore size 0.45 μ m, Carl Roth GmbH, Karlsruhe, Germany) Phoenix-Eco supernatants. Additionally 3 μ l of polybrene (f.c. 8 μ g/ml) were added. The cells were centrifuged for 2 h (300 x g, 25°C). Afterwards the cells were cultivated for 48 h in wells of 6-wells plates with 5 ml DMEM +/+ supplemented with 10 ng/ml Hyper-IL-6. Thereafter, the cells were treated with the appropriate antibiotics (puromycin: 1.5 μ g/ml and/or hygromycin B: 1 mg/ml or both) for at least 2 weeks.

3.2.2.6 Cell viability assay

Retrovirally transduced Ba/F3-gp130 cells were washed three times with sterile PBS and resuspended in DMEM +/+ at 5 x 10³ cells per well of a 96-well plate. The cells were cultured for three days in a final volume of 100 μ l with or without additional cytokines as indicated. For inhibition studies with soluble IL-15R α -sushi domain the recombinant protein was added as indicated. The CellTiter-Blue[®] Cell Viability Assay (Promega GmbH, Mannheim, Germany) was used to determine the proliferation following the manufacturer's instructions and measured on a Lambda Fluoro 320 Fluorimeter (ex-filter 530/25, em-filter 590/35, sensitivity 75, Software KC4). Relative

light units (RLU) values were normalized by subtraction of negative control values (unstimulated Ba/F3-gp130 cells or transduced Ba/F3-gp130 cells which exhibited no cytokine independent growth) from all other values. All values were measured in quadruplicates.

3.2.2.7 Co-culture experiments of Ba/F-gp130-GFP cells with Ba/F-gp130 cells stably transduced with fusion receptor genes

Ba/F3-gp130 cells expressing GFP (2×10^5 cells) were co-cultured in a 12-well plate with Ba/F3-gp130 cells expressing either IL-15-gp130, IL-15-WSX-1 + sushi-gp130 or Fos-gp130 (1×10^4 cells) at a ratio of 20:1. After 24h, 48h, 72h and 96h the green fluorescent cells were quantified by flow cytometry.

3.2.2.8 Co-culture assay of murine dendritic cells with Ba/F3-gp130 cells

Murine bone marrow derived dendritic cells were prepared as described [171]. Murine dendritic cells were plated (2×10^5 cells) into a 12-well plate and stimulated with LPS (10 ng/ml) overnight. The cells were washed three times with PBS to wash away LPS and Ba/F3-gp130 cells expressing fusion receptors were co-cultured (2×10^6 cells) at a ratio of 1:10. After 24 h of co-culturing the supernatants were analyzed for mIL-6 secretion. Murine IL-6 was measured by ELISA (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany) and normalized to different Ba/F3-gp130 cell numbers obtained after 24 h. (*this experiment was performed by Elena Bulanova, Borstel, Germany*)

3.2.2.9 Flow cytometry staining and analysis

To detect the surface expression of the fusion receptors, 3×10^5 cells were washed with 1 ml FACS buffer, centrifuged (300 x g, 5 min, 4°C) and incubated with the appropriate antibody in 100 µl FACS buffer for 1 h at 4°C, respectively. After a single wash with 1 ml of FACS buffer and centrifugation (300 x g, 5 min, 4°C), cells were incubated with the appropriate secondary antibody in 100 µl FACS buffer for 1 h at 4°C. Cells were washed once with 1 ml FACS buffer, centrifuged (300 x g, 5 min, 4°C), resuspended in 500 µl FACS buffer and analyzed by flow cytometry (BD – FACS-Canto and FACS DIVA software, Heidelberg, Germany).

3.2.3 Protein-biochemical methods

3.2.3.1 Cell lysis

The medium of COS-7 cells was removed and the cells were once washed with 10 ml ice cold PBS. Cells were scraped from the dishes with 1 ml ice cold PBS, the suspension was transferred to a 1.5 ml reaction tube and the cells were pelleted (16,000 x g, 15 s, 4°C). The cells were lysed in 250 µl of the appropriate lysis buffer, vortexed and incubated for at least 30 min at 4°C under gentle agitation.

Afterwards the lysates were centrifuged (16,000 x g, 20 min, 4°C) to remove debris. The lysate was used directly for protein concentration determination or immunoprecipitation and/or subsequent Western blot analysis.

Starved Ba/F3-gp130 cells were pelleted (16,000 x g, 15 s, 4°C) and lysed in 300 µl of lysis buffer, vortexed and incubated for at least 30 min at 4°C under gentle agitation. Afterwards the lysates were transferred to a 1.5 ml reaction tube and centrifuged (16,000 x g, 20 min, 4°C) to remove debris. The lysates were directly used for protein concentration determination and subsequent western blot analysis. All lysates were stored at -20°C.

3.2.3.2 Determination of protein concentration (BCA protein Assay)

To analyse the protein concentration of lysates the BCA protein assay (Thermo Fisher Scientific, p/a Perbio Science Deutschland, Bonn, Germany) was used following the manufacturer's instructions. The lysates of COS-7 cells were diluted 1:2 in lysis buffer, Ba/F3-gp130 lysates were diluted 1:5 in lysis buffer. The assay was performed in a 96-well plate, incubated for 15 min at 37°C and subsequently analyzed at 562 nm in a microtiter plate reader (Tecan Germany GmbH, Crailsheim, Germany).

3.2.3.3 SDS-polyacrylamid gel electrophoresis (SDS-PAGE)

Separation of proteins was performed with a discontinuous SDS-PAGE using the mini Trans-Blot[®] cell gadgetry (Bio-Rad Laboratories GmbH, Munich, Germany). First the running gel was poured (1 mm thickness; 5.5 cm height) and covered with isopropanol to avoid air bubbles. After polymerization the isopropanol was removed and the stacking gel was poured (1 mm thickness; 1.2 cm height) and a comb with 10

wells was added. After polymerization the mini Trans-Blot[®] cell gadgetry was assembled and SDS running buffer was added. Protein lysates were supplemented with Laemmli buffer and heated to 95°C for 5 min prior loading to the gel. The gel was run at 100 -180 V until the bromphenol blue line reached the end of the gel.

3.2.3.4 Western blotting

Proteins were transferred from the SDS-gel on a polyvinylidene difluoride (PVDF) membrane (GE Healthcare) using Trans-Blot[®] SD semi-dry transfer cell (Bio-Rad Laboratories GmbH, Munich, Germany). First the PVDF membrane was activated by incubation with methanol for 3 min and subsequently incubated with ddH₂O for additional 3 min. The ddH₂O was removed and the membrane and the the Whatman[®] papers were soaked in blotting buffer.

The blotting sandwich was assembled as described in the manufacturer's protocol. Proteins were transferred electrophoretically semidry at constant voltage (15 V, 60 min). Afterwards the membrane was blocked in 5% milk powder in TBS-T for 1 h at room temperature. The membrane was probed with the primary antibody overnight at 4°C. The membrane was washed three times with TBS-T for 5 min and the appropriate secondary antibody was probed for 1 h at room temperature. After three washing steps, the membrane was incubated with ECL Plus[™] (GE Healthcare, Munich, Germany) following the manufacturer's protocol. The chemiluminescence was measured with the CCD camera systems LAS-1000 (Fujifilm, Düsseldorf, Germany) or FluorChem[®] Q (Alpha Innotech, Cell Biosciences, Inc., Santa Clara, CA, USA).

3.2.3.5 Stripping of membranes

After detection, the membranes were stripped in stripping buffer for 30 min at 60°C, washed three times in TBS-T, blocked again and probed with a new primary antibody.

3.2.4 Computational biology

To determine the transmembrane region of WSX-1 the program DAS (Dense Alignment Surface) [172] was used. To determine the transmembrane regions of

LIFR, OSMR and GPL the program TMHMM (Tied Mixture Hidden Markov Model), [173] was used.

4 Results

4.1 Generation of forced heterodimers of gp130 and WSX-1 using a leucine zipper based strategy.

To achieve forced, ligand-independent heterodimerization of gp130 and WSX-1 based on leucine zipper heterodimerization, the entire extracellular portion of gp130 except of 15 membrane proximal amino acids was substituted by the 40 amino acid Fos leucine zipper sequence of the transcription factor c-Fos [137] and the entire extracellular portion of WSX-1 except of 15 amino acids by the 39 amino acid Jun leucine zipper sequence of the transcription factor c-Jun [137] as schematically illustrated in Figure 4.1. The chimeric proteins were named Fos-gp130 and Jun-WSX-1.

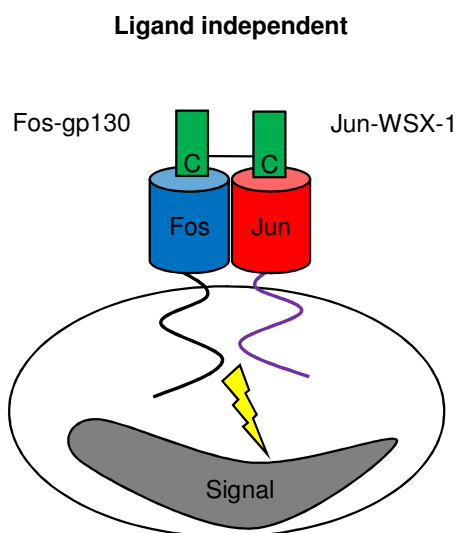


Figure 4.1 Forced heterodimerization strategy scheme of gp130 and WSX-1 based on leucine zipper heterodimerization. Gp130 and WSX-1 were truncated 15 amino acids above the transmembrane domain and replaced by the leucine zipper region of the human c-Jun or c-Fos gene. The chimeric proteins are stabilized with an additional cysteine and a FLAG-tag was introduced to ease detection (green rectangle).

NH₂-terminally of the leucine zipper a linker was introduced, which consists of a cysteine residue to stabilize the leucine mediated heterodimerization of gp130 and WSX-1. Additionally, we constructed the chimeric protein Δ cys-Fos-gp130, which lacks the cysteine residue to prevent unwanted stabilization of a Fos-gp130 homodimer by a disulfide-bridge. To ease detection of the chimeric proteins, a FLAG epitope tag was placed NH₂-terminally of the linker. In the following text the cloning strategy of Fos-gp130, Δ cys-Fos-gp130 and Jun-WSX-1 will be explained in detail.

4.2 Construction of Fos-gp130, Δ cys-Fos-gp130 and Jun-WSX-1 expression plasmids

The Fos-gp130 fusion receptor has the following assembly: gp130 signal peptide (MLTLQTWLVQALFIFLTTESTG), FLAG sequence (DYKDDDDK), linker (for Fos-gp130:ELCGG, for Δ cys-Fos-gp130: ELGGG), 39 amino acid human Fos protein fragment (LTDTLQAETDQLEDEKSAEQTEIANLLKEKEKLEFILAA) [137] and a N-terminally truncated gp130-protein (15 aa of the extracellular domain, transmembrane domain and cytoplasmic domain – amino acids 606-918). In detail, we used pQE30-Fos, which encodes a codon optimized Fos protein (Geneart, Regensburg, Germany), as a template for PCR to amplify the Fos-coding sequence (5'Fos [Fos-gp130] or 5' Δ cysFos [Δ cys-Fos-gp130] and 3'Fos [for both constructs]). The coding sequence of the gp130 signal peptide, FLAG tag and linker was amplified by PCR, using the plasmid pBSK-Jun-gp130 as a template (5'SPgp130FLAG and 3'SPgp130FLAG [Fos-gp130] or 3'SPgp130FLAG Δ cys [Δ cys-Fos-gp130]). Both PCR products were combined in subsequent SOE-PCR, because the coding sequence of Fos contains a 5' overhang, which primes the coding sequence of the linker, using the 5' and 3' primers of the initial PCRs. The purified PCR products [SP_FLAG_Fos or SP_FLAG_ Δ cys-Fos] were digested with *Xho*I and *Eco*RI and subcloned into pBSK-gp130 (Δ N-term). This vector was obtained by digestion of pBSK-Jun-gp130 with *Xho*I and *Eco*RI. The resulting plasmids were named pBSK-Fos-gp130 or pBSK- Δ cys-Fos-gp130 (Figure 4.1). All constructs were subcloned into pMOWS [169]. In detail, the pMOWS plasmid was digested with *Xag*I and blunt ends were generated by Klenow fragment reaction. The pBSK plasmids encoding Fos-gp130 and Δ cys-Fos-gp130 were digested with *Xho*I and blunt ends were generated by Klenow fragment reaction. In a second digestion step, the target plasmid and the insert encoding plasmids were digested with *Bam*HI, purified and subcloned. The resulting plasmids were named pMOWS-Fos-gp130 and pMOWS- Δ cys-Fos-gp130 (Figure 4.2). The constructs Fos-gp130 and Δ cys-Fos-gp130 were additionally subcloned into p409. In brief, the plasmid p409 was digested with *Sal*I and *Not*I. The pBSK plasmids encoding Fos-gp130 and Δ cys-Fos-gp130 were digested with *Xho*I and *Not*I, purified and subcloned. The resulting plasmids were named p409-Fos-gp130 and p409- Δ cys-Fos-gp130 (Figure 4.1). The cDNA coding for Δ cys-Fos-gp130 was additionally subcloned into pEYFP-gp130 [168]. In detail, the plasmid pEYFP-gp130 was

digested with *Xho*I and *Acc*I as well as the plasmid pBSK- Δ cys-Fos-gp130. Both were purified and subcloned. The resulting plasmid was named pEYFP- Δ cys-Fos-gp130 (Figure 4.2).

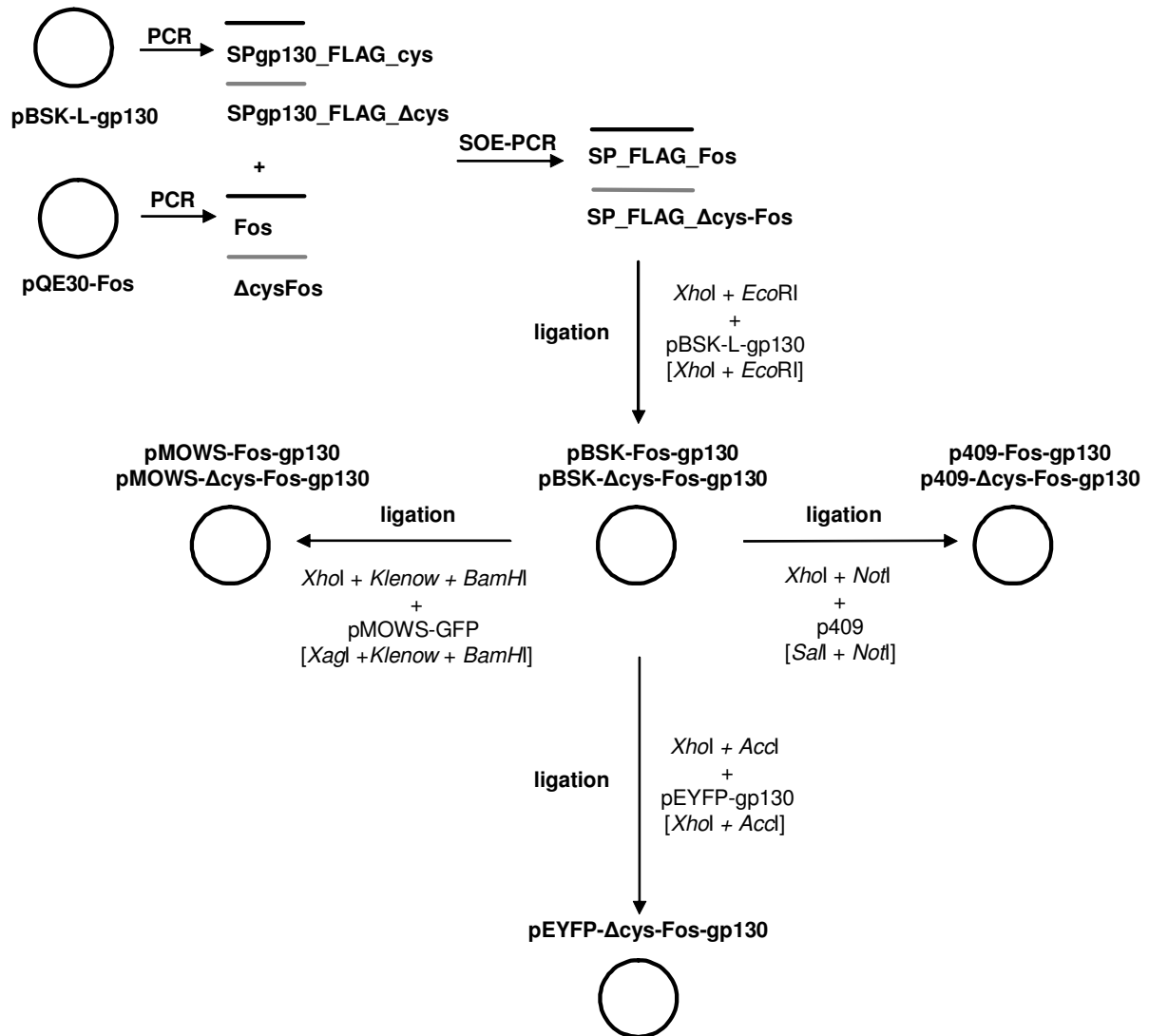


Figure 4.2 Schematic overview of cloning of Fos-gp130 and Δ cys-Fos-gp130 into different plasmids. See text for details.

The Jun-WSX-1 receptor has the following assembly: gp130 signal peptide (MLTLQTWLVQALFIFLTTESTG), FLAG sequence (DYKDDDDK), linker (ELCGG), 39 amino acid human Jun protein fragment (RIARLEEKVKTLKAQNSELASTANMLREQVAQLKQKVMN) [137] and N-terminally truncated WSX-1-protein (15 amino acids of the extracellular domain, transmembrane domain and cytoplasmic domain – amino acids 500-623). In detail, the

truncated murine WSX-1 coding sequence was amplified by PCR using cDNA from Ba/F3-gp130 cells as a template (5'mWSX-1 and 3'mWSX-1). The purified PCR product was digested with *EcoRI* and *BamHI* and subcloned in the vector pBSK-Jun, which was obtained by digestion of pBSK-Jun-gp130 with *EcoRI* and *BamHI*. The resulting plasmid was named pBSK-Jun-WSX-1 (Figure 4.3). The cDNA coding for Jun-WSX-1 was subcloned to the plasmid pMOWS as described above. The plasmid was named pMOWS-Jun-WSX-1 (Figure 4.3).

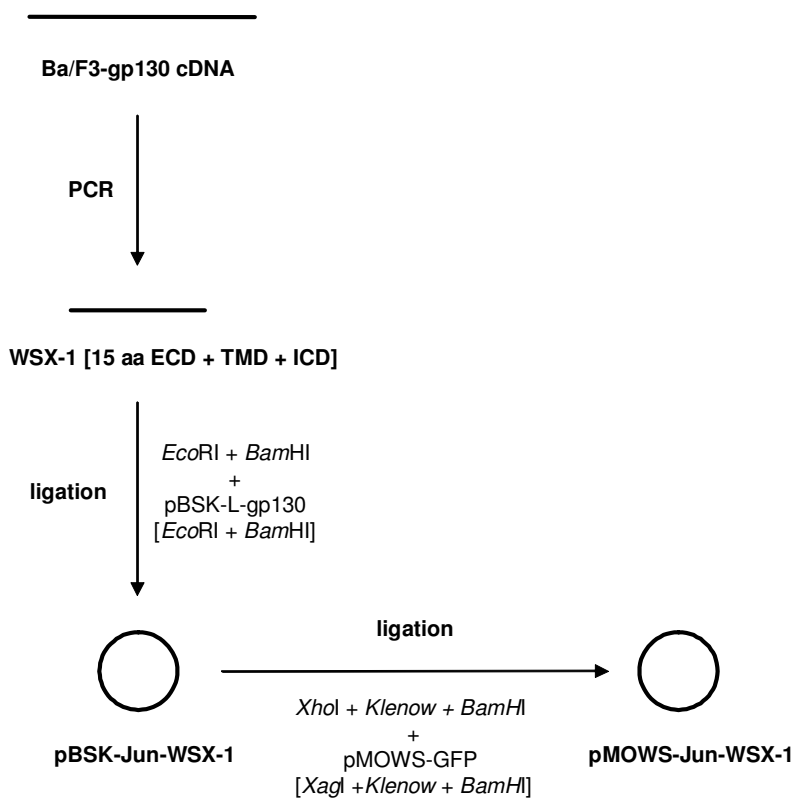


Figure 4.3 Schematic overview of cloning of Jun-WSX-1 into different expression plasmids. See text for details.

4.3 Specific Jun/Fos chimeric gp130-type receptor heterodimerization was not achieved due to unexpected homodimerization of the Fos-gp130 receptor chimera

First, it was asked whether the chimeric receptors Fos-gp130, Δ cys-Fos-gp130 and Jun-WSX-1 induced ligand-independent cell growth in Ba/F3-gp130 cells if expressed alone. Ba/F3-gp130 cells are factor-dependent and grow in the presence of Hyper-IL-6, a fusion protein of IL-6 and the soluble IL-6R connected by a flexible linker [170], or IL-3. We selected Ba/F3-gp130 cells as a cellular system to assess the long-term activity of the chimeric heterodimeric receptor complexes, because these cells were successfully used to show ligand-independent and constitutive activation of the homodimeric Jun-gp130 receptor fusion protein (L-gp130) [136]. Ba/F3-gp130 cells were retrovirally transduced with the cDNA encoding Fos-gp130, Δ cys-Fos-gp130 or Jun-WSX-1. After selection, surface expression of the chimeric receptors was measured by flow cytometry (Figure 4.4).

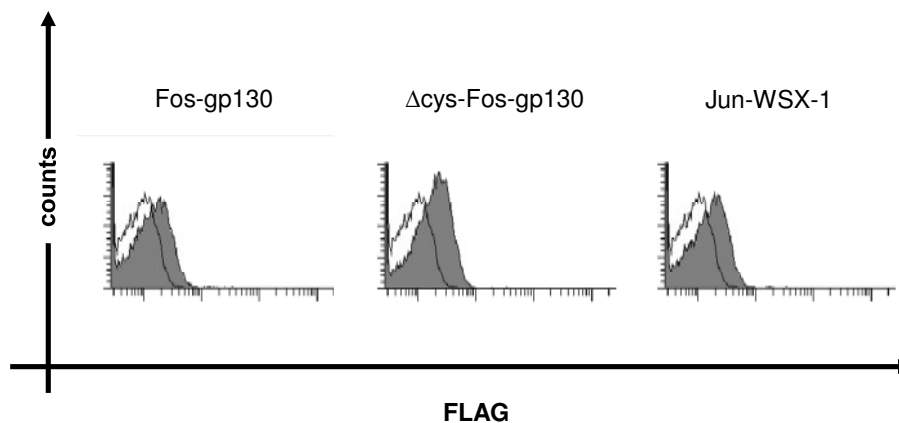


Figure 4.4 Cell surface expression of Fos-gp130, Δ cys-Fos-gp130 and Jun-WSX-1 in stably transduced Ba/F3-gp130 cell lines analyzed by flow cytometry. The cell lines were labeled as described in material & methods. Untransfected Ba/F3-gp130 cells were used as negative control (unfilled histograms).

To address the question whether the stably transfected Ba/F3-gp130 cell lines grow cytokine independently, a proliferation assay in medium lacking Hyper-IL-6 was performed. Previously it has been shown that cytokine withdrawal led to induction of the intrinsic apoptotic pathway in Ba/F3-gp130 cells [103]. In addition we used the previously described cell line Ba/F3-gp130-L-gp130 as a positive control for cytokine

independent growth [136]. Surprisingly, Ba/F3-gp130-Fos-gp130 cells and Ba/F3-gp130- Δ cys-Fos-gp130 cells proliferated in the absence of Hyper-IL-6, indicating that the Fos peptide alone was able to mediate homodimerization of gp130. On the other hand, Ba/F3-gp130-Jun-WSX-1 cells were not able to grow cytokine independently (Figure 4.5).

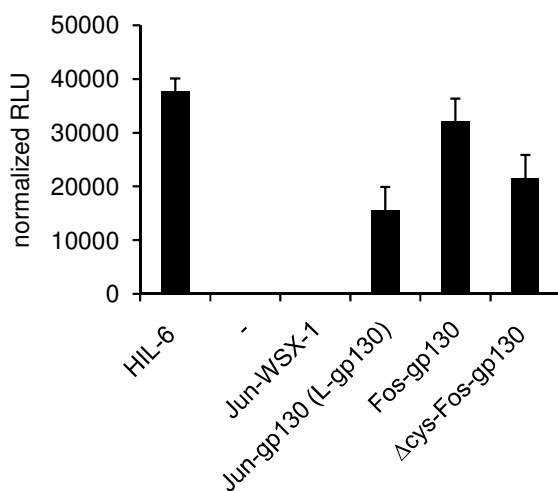


Figure 4.5 Functionality of Fos-gp130 chimeric receptor proteins in stable transfected Ba/F3-gp130 cell lines. Equal numbers of Ba/F3-gp130 cells stably transfected with Jun-WSX-1, L-gp130, Fos-gp130 or Δ cys-Fos-gp130 were cultured for three days in the absence of Hyper-IL-6. As a control Ba/F3-gp130 were cultured in the presence or absence of 10 ng/ml Hyper-IL-6. Proliferation was measured as indicated in material & methods.

To demonstrate that Fos-gp130 homodimers were stabilized by intermolecular disulphide bridges, a Western blot analysis of COS-7 cell lysates containing Fos-gp130 and Δ cys-Fos-gp130 under reducing and non-reducing conditions was performed.

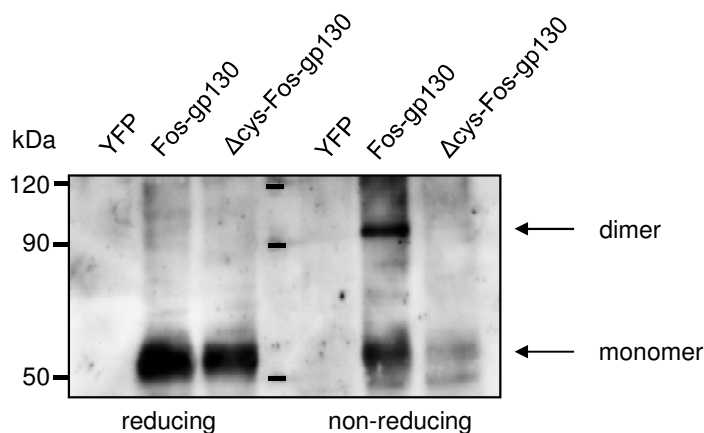


Figure 4.6 Detection of Fos-gp130 homodimers under non-reducing conditions. COS-7 cells were transiently transfected with p409-YFP, p409-Fos-gp130 or p409- Δ cys-Fos-gp130. 48 h after transfection, cells were lysed and 50 μ g of protein lysates were separated by SDS-PAGE under reducing or non-reducing conditions. Proteins were detected with a anti-FLAG-tag antibodies and visualized by ECL detection.

Monomeric Fos-gp130 could be detected under reducing conditions and a dimeric form of Fos-gp130 under non-reducing conditions, as depicted in Figure 4.6.

Importantly, dimeric Δ cys-Fos-gp130 was not detected under non-reducing conditions.

Co-immunoprecipitation analysis was performed to show the physical interaction between two Δ cys-Fos-gp130 proteins. Lysates of COS-7 cells containing Δ cys-Fos-gp130 alone or Δ cys-Fos-gp130 and Δ cys-Fos-gp130-EYFP were incubated with anti-GFP antibodies for immunoprecipitation. EYFP is a derivative of GFP and therefore can be precipitated by anti-GFP antibodies. As indicated in the left panel of Figure 4.7, Δ cys-Fos-gp130 could only be detected in the lysates (input) but not after anti-GFP immunoprecipitation in single transfected cells, showing that Δ cys-Fos-gp130 does not unspecifically interact with anti-GFP antibodies or protein G agarose. However, Δ cys-Fos-gp130 could be specifically co-immunoprecipitated with Δ cys-Fos-gp130-EYFP as indicated in the right panel of Figure 4.7. Consequently, it can be concluded that Fos-gp130 homodimers can be formed even in the absence of a stabilizing intermolecular disulfide bridge using Δ cys-Fos-gp130.

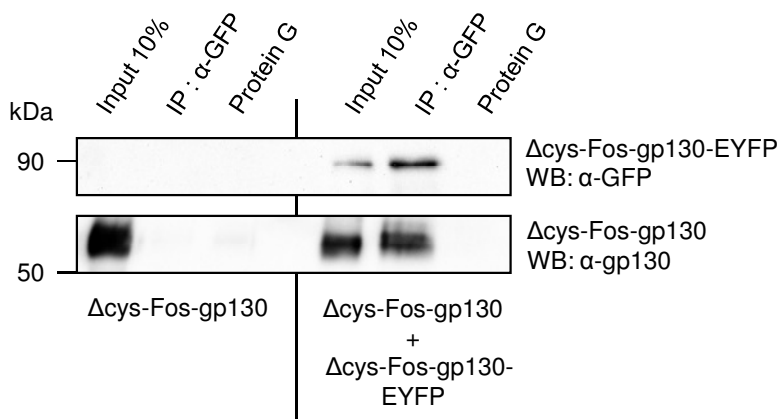


Figure 4.7 Detection of Δ cys-Fos-gp130 homodimers by co-immunoprecipitation. COS-7 cells were transiently transfected with p409- Δ cys-Fos-gp130 or p409- Δ cys-Fos-gp130 and pEYFP- Δ cys-Fos-gp130. 48 h after transfection cells were lysed and Δ cys-Fos-gp130-EYFP was immunoprecipitated with anti-GFP antibodies. As a control,

lysates were incubated only with protein G agarose. Input, immunoprecipitation supernatant and protein G agarose control supernatant were separated by SDS-PAGE. Proteins were detected with anti-GFP specific antibodies and an anti-gp130 specific antibody and visualized by ECL detection.

Upon binding of IL-6/IL-6R to gp130, JAK proteins are activated and gp130 is subsequently phosphorylated at distinct tyrosine residues [54, 174]. To demonstrate that Δ cys-Fos-gp130 is also phosphorylated at tyrosine residues after forced dimerization via the Fos peptide, an immunoprecipitation assay with COS-7 cell lysate containing Δ cys-Fos-gp130 was performed. COS-7 cell lysate containing Δ cys-Fos-gp130 was incubated with anti-gp130 specific antibodies and Δ cys-Fos-gp130

was immunoprecipitated. Western blot analysis with an anti-phospho-tyrosine specific antibody revealed that precipitated Δ cys-Fos-gp130 was phosphorylated at tyrosine residues (Figure 4.8).

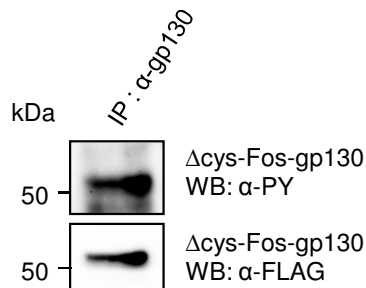


Figure 4.8 Tyrosine-phosphorylation of Δ cys-Fos-gp130. COS-7 cells were transiently transfected with p409- Δ cys-Fos-gp130 and starved 20 h before lysis. 48 h after transfection, COS-7 cells were lysed and Δ cys-Fos-gp130 was immunoprecipitated with anti-gp130 antibodies. Immunoprecipitation supernatant was separated by SDS-PAGE. Proteins were transferred onto PVDF membrane

and were detected with anti-phospho-tyrosine (α -PY) antibodies and visualized by ECL detection. To confirm immunoprecipitation of Δ cys-Fos-gp130 the membrane was stripped and probed with anti-FLAG antibodies.

These results were verified by analysis of the STAT3 phosphorylation status of the retrovirally transduced cells. Here, Ba/F3-gp130-Fos-gp130 cells and Ba/F3-gp130- Δ cys-Fos-gp130 cells as well as Ba/F3-gp130-L-gp130 cells but not Ba/F3-gp130-Jun-WSX-1 cells cells showed a cytokine independent phosphorylation of STAT3 (Figure 4.9).

These results indicated that the heterodimerization strategy comprising heterodimers of Jun and Fos will not exclusively lead to gp130 heterodimerization with WSX-1 or other members of the gp130-like cytokine receptor family. Although it has been reported that Fos leucine zippers form unstable homodimers [137], the Fos mediated homodimerization of Δ cys-Fos-gp130 was strong enough to induce ligand-independent growth in Ba/F3-gp130- Δ cys-Fos-gp130 cells (Figure 4.5 and 4.10).

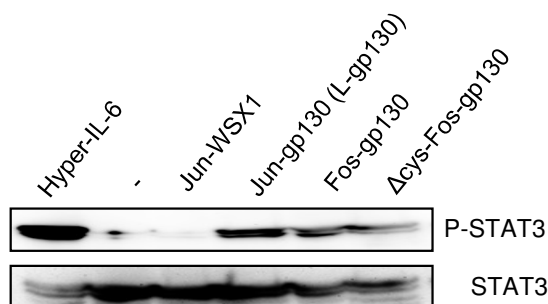


Figure 4.9 Ligand-independent phosphorylation of STAT3 proteins in Ba/F3-gp130 cells. After 6 h of serum starvation Ba/F3-gp130 cells stably transfected with Jun-WSX-1, L-gp130, Fos-gp130 or Δ cys-Fos-gp130 were left untreated, whereas untransfected Ba/F3-gp130 were stimulated for 10 min with 10 ng/ml Hyper-IL-6 or were left untreated. Subsequently, cells were lysed and 50 μ g were

separated by SDS-PAGE. Proteins were transferred onto PVDF membranes and were detected with an anti-P-STAT3 specific antibody and visualized by ECL detection. After detection the membrane was stripped and probed with an anti-STAT3 specific antibody and proteins were visualized by ECL detection.

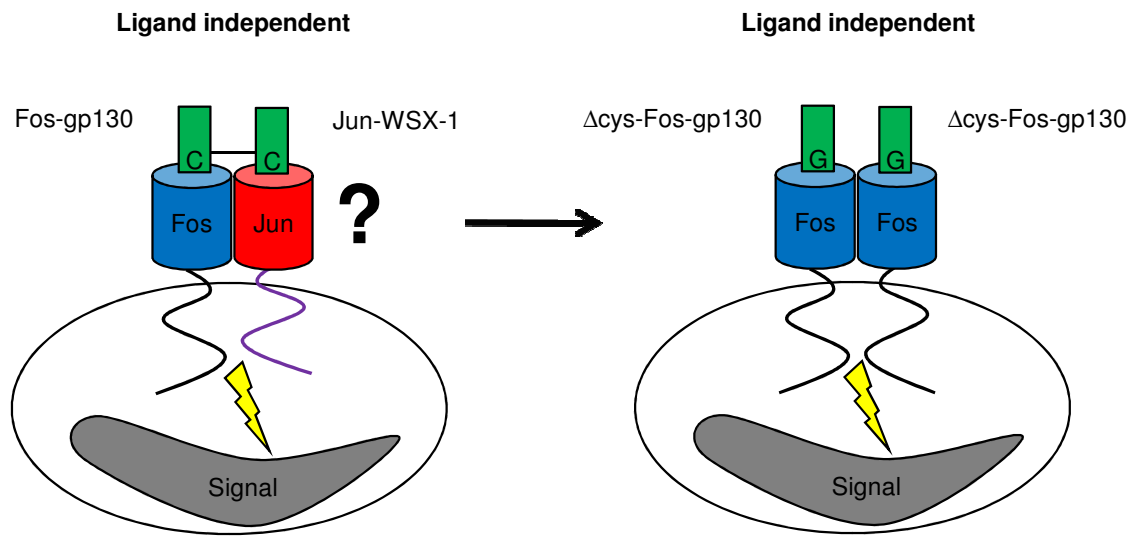


Figure 4.10 Attempt to establish Jun-Fos heterodimeric chimeric receptors failed upon Fos homodimerization of Δ cys-Fos-gp130 as schematically illustrated.

4.4 Generation of forced heterodimers of gp130 and WSX-1, LIFR, OSMR or GPL and OSMR using a novel strategy comprising IL-15 and IL-15R α -sushi.

Due to the disappointing results in terms of leucine zipper-based strategy, a complete novel strategy to achieve forced receptor heterodimerization was used. Because of the high affinity of IL-15 to the sushi domain of IL-15R α (sushi) [154, 164] it was decided to use these proteins or domains to investigate the potency of their ability to mediate forced heterodimerization of gp130 with WSX-1, LIFR, OSMR or GPL and OSMR.

The complete extracellular portions of gp130, WSX-1, LIFR, OSMR and GPL except of 15 amino acids were exchanged to either the 148-amino acid sequence of IL-15 (PPL-IL-15) or the 66-amino acid sequence of IL-15R α -sushi-domain. The transport of the IL-15 fused chimeric receptors to the plasma membrane was driven by the signal peptide of bovine preprolactin (PPL) and of the IL-15R α -sushi domain fused receptors by the signal peptide of human gp130. To ease detection of IL-15R α -sushi-domain fused receptors, a Myc-tag was introduced NH₂-terminally of the sushi domain. The resulting fusion receptors were named IL-15-gp130, IL-15-WSX-1, IL-15-LIFR, IL-15-OSMR and IL-15-GPL as well as sushi-gp130, sushi-WSX-1, sushi-LIFR, sushi-OSMR and sushi-GPL (Figure 4.11). In the following text the cloning strategy of the new chimeric receptors will be explained in detail.

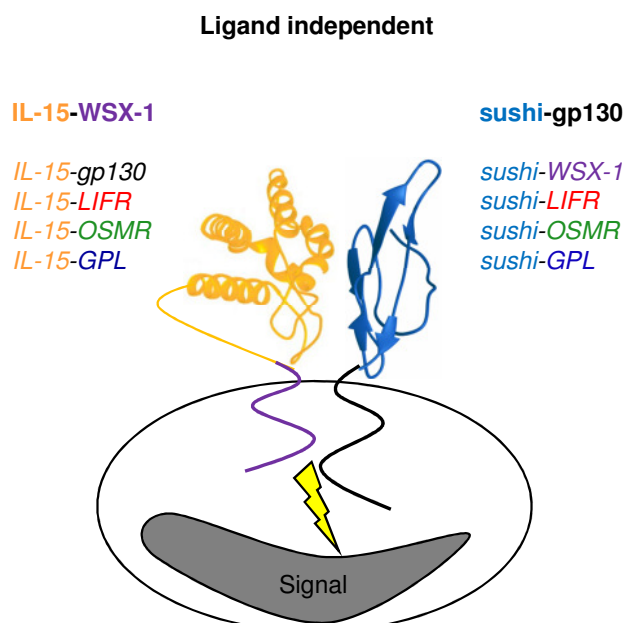


Figure 4.11 Forced heterodimerization strategy scheme of gp130 and WSX-1, LIFR, OSMR or GPL and OSMR based on IL-15/IL-15R α -sushi heterodimerization. Gp130, WSX-1, LIFR, OSMR and GPL were truncated 15 amino acids above the transmembrane domain and replaced by IL-15 or IL-15R α -sushi domain. All IL-15R α -sushi domain chimeras were N-terminally tagged with a Myc-tag to ease detection (not shown).

4.5 Construction of IL-15-gp130, IL-15-WSX-1, IL-15-LIFR, IL-15-OSMR, IL-15-GPL, sushi-gp130, sushi-WSX-1, sushi-LIFR, sushi-OSMR and sushi-GPL expression plasmids

The IL-15-fusion receptors pBSK-IL-15-gp130, pBSK-IL-15-WSX-1, pBSK-IL-15-LIFR, pBSK-IL-15-OSMR and pBSK-IL-15-GPL were assembled as followed: bovine preprolactin (PPL) signal peptide (MDSKGSSQKAGSRLLLLLVVSNLLLCQGVVSTTR), human IL-15 protein fragment [167] and the N-terminally truncated receptor proteins (15 amino acids of the extracellular domain, transmembrane domain and cytoplasmic domain). For construction of IL-15 fusion receptors, pBSK-PPL-IL-15 was used [167]. The plasmid pBSK-PPL-IL-15 encodes human IL-15 which consists of an alternative signal peptide from the bovine preprolactin and was used as a template to amplify the coding sequence by PCR (5'PPL-IL-15 and 3'PPL-IL-15). The purified PCR product was digested with *XhoI* and *EcoRI* and subcloned into pBSK-gp130 (Δ N-term – amino acids 606-918), which was obtained by digestion of pBSK-Jun-gp130 with *XhoI* and *EcoRI*. The resulting plasmid was named pBSK-IL-15-gp130 (Figure 4.11).

The IL-15R α -sushi (sushi) fusion receptors IL-15R α -sushi-gp130 (sushi-gp130), sushi-WSX-1, sushi-LIFR, sushi-OSMR and sushi-GPL were assembled as followed: gp130 signal peptide (MLTLQTWLVQALFIFLTTESTG), Myc-tag (EQKLISEEDL), human IL-15R α -sushi protein fragment [143] and the N-terminally truncated receptor proteins. pQE30-IL-15R α -sushi [143] was used as a template for the PCR-amplification of the cDNA coding for IL-15R α -sushi (5'mycsushi and 3'sushi). The coding sequence of the gp130 signal peptide and Myc-tag was amplified by using the plasmid pBSK-Fos-gp130 as a template (5'SPgp130myc and 3'SPgp130myc). Both PCR products were used for a SOE-PCR, because the coding sequence for IL-15R α -sushi contains a 5' overhang, which primes the coding sequence of the Myc-tag (5'SPgp130myc and 3'sushi). The resulting PCR product was purified, digested with *XhoI* and *EcoRI* and ligated into pBSK-gp130 (Δ N-term – amino acids 606-918) also digested with *XhoI* and *EcoRI*. The resulting plasmid was named pBSK-IL-15R α -sushi-gp130 (Figure 4.12).

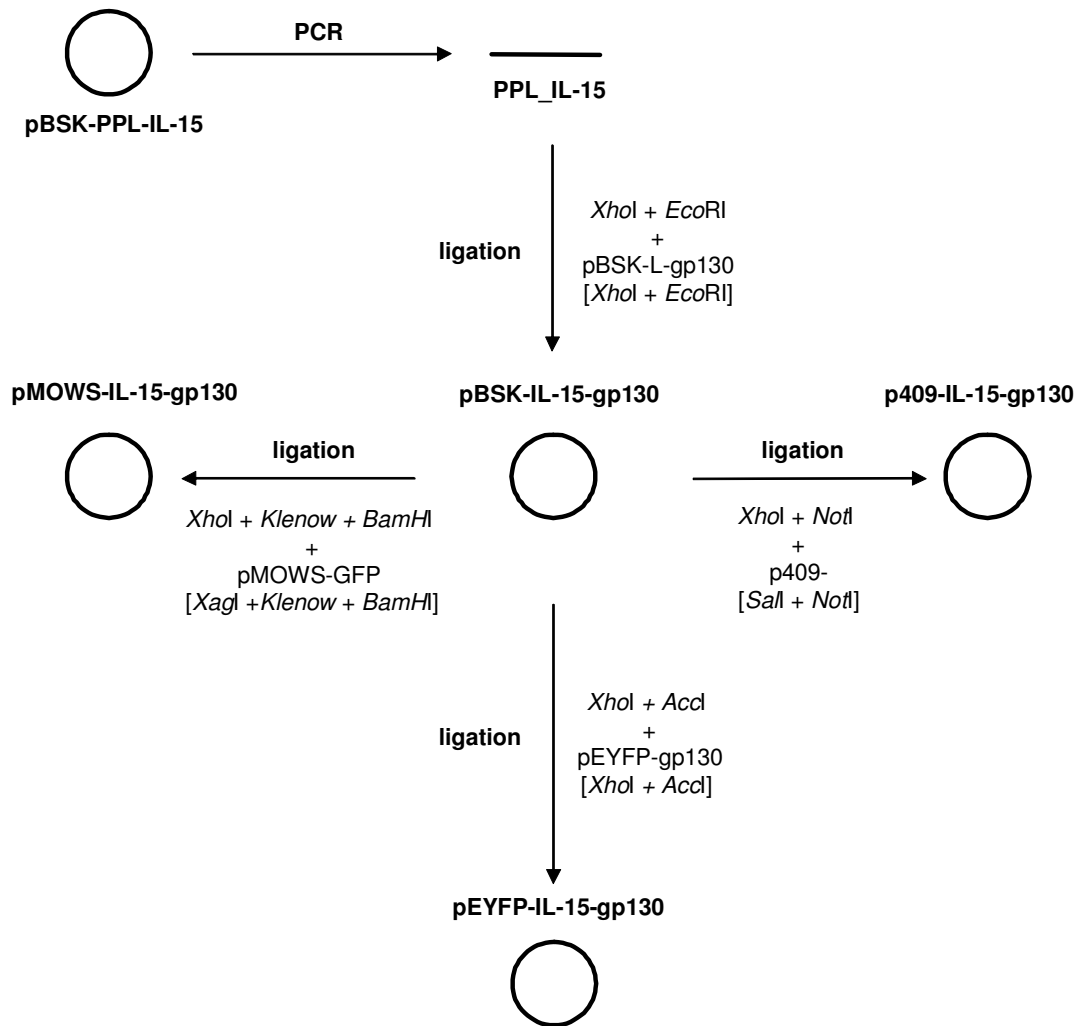


Figure 4.11 Schematic overview of cloning of IL-15-gp130 into different plasmids. See text for details

pBSK-IL-15-gp130 was used to generate the remaining IL-15 fusion receptors by digestion of pBSK-IL-15-gp130 with *EcoRI* and *BamHI* and subcloning of truncated WSX-1 (*EcoRI* and *BamHI* digested pBSK-Jun-WSX-1 – amino acids 500-623), or PCR produced truncated LIFR (template: p409-hLIFR [165] – 5'hLIFR and 3'hLIFR – amino acids 820-1097), truncated OSMR (template: p409-hOSMR [166] – 5'hOSMR: and 3'hOSMR – amino acids 723-979) or truncated GPL (template: pcDNA3.1-GPL – 5'hGPL and 3'hGPL – amino acid 500-727), respectively.

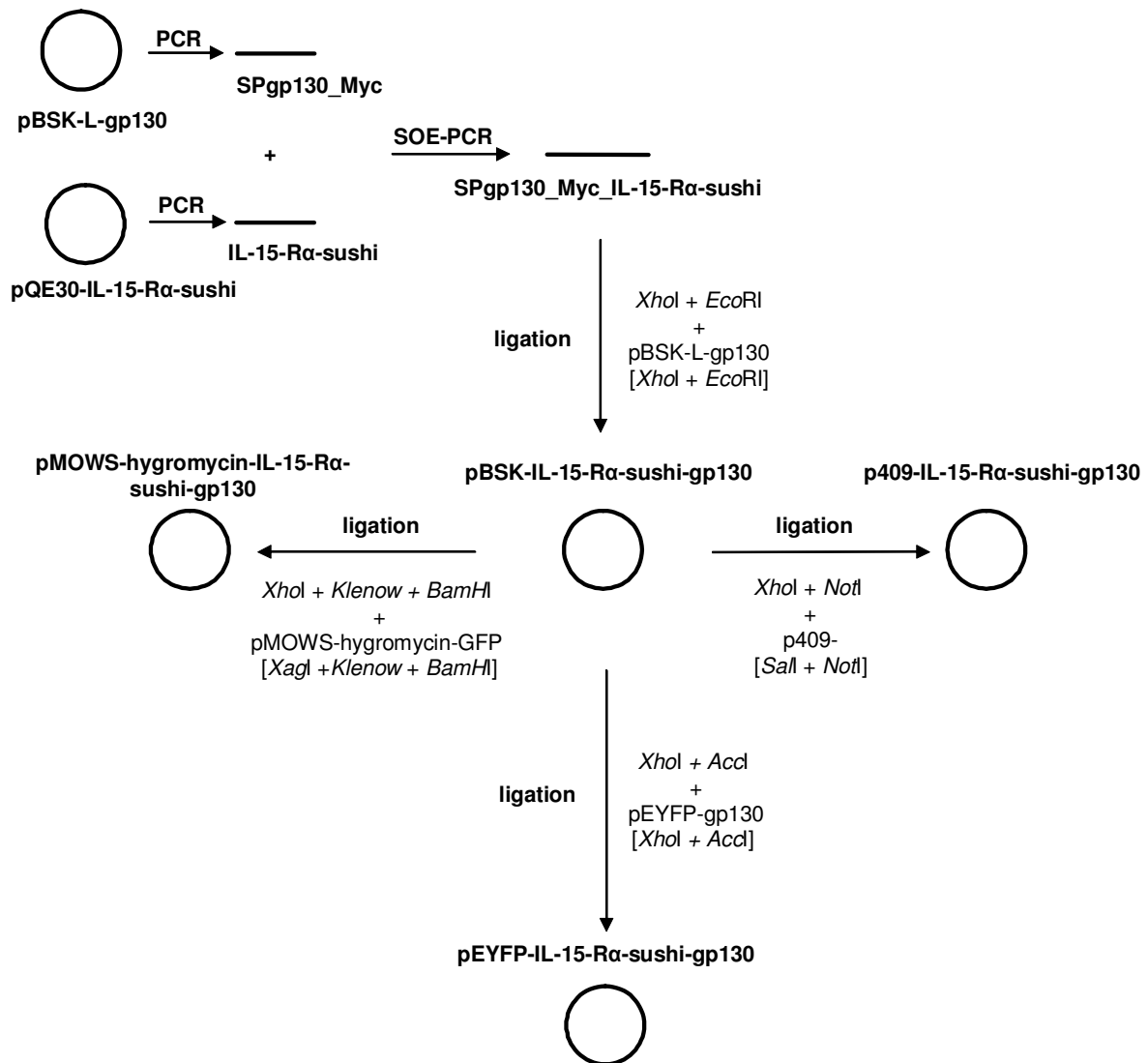


Figure 4.12 Schematic overview of cloning of IL-15R α -sushi-gp130 into different plasmids. See text for details.

The resulting plasmids were named pBSK-IL-15-WSX-1, pBSK-IL-15-LIFR, pBSK-IL-15-OSMR or pBSK-IL-15-GPL (Figure 4.13).

To generate the plasmids pBSK-IL-15R α -sushi-WSX-1, pBSK-IL-15R α -sushi-LIFR, pBSK-IL-15R α -sushi-OSMR and pBSK-IL-15R α -sushi-GPL, the gp130-receptor portion was exchanged by digestion of the plasmid pBSK-IL-15R α -sushi-gp130 with *EcoRI* and *BamHI* and subcloning of the fragments coding for N-terminally truncated WSX-1, LIFR, OSMR or GPL as described above (Figure 4.13).

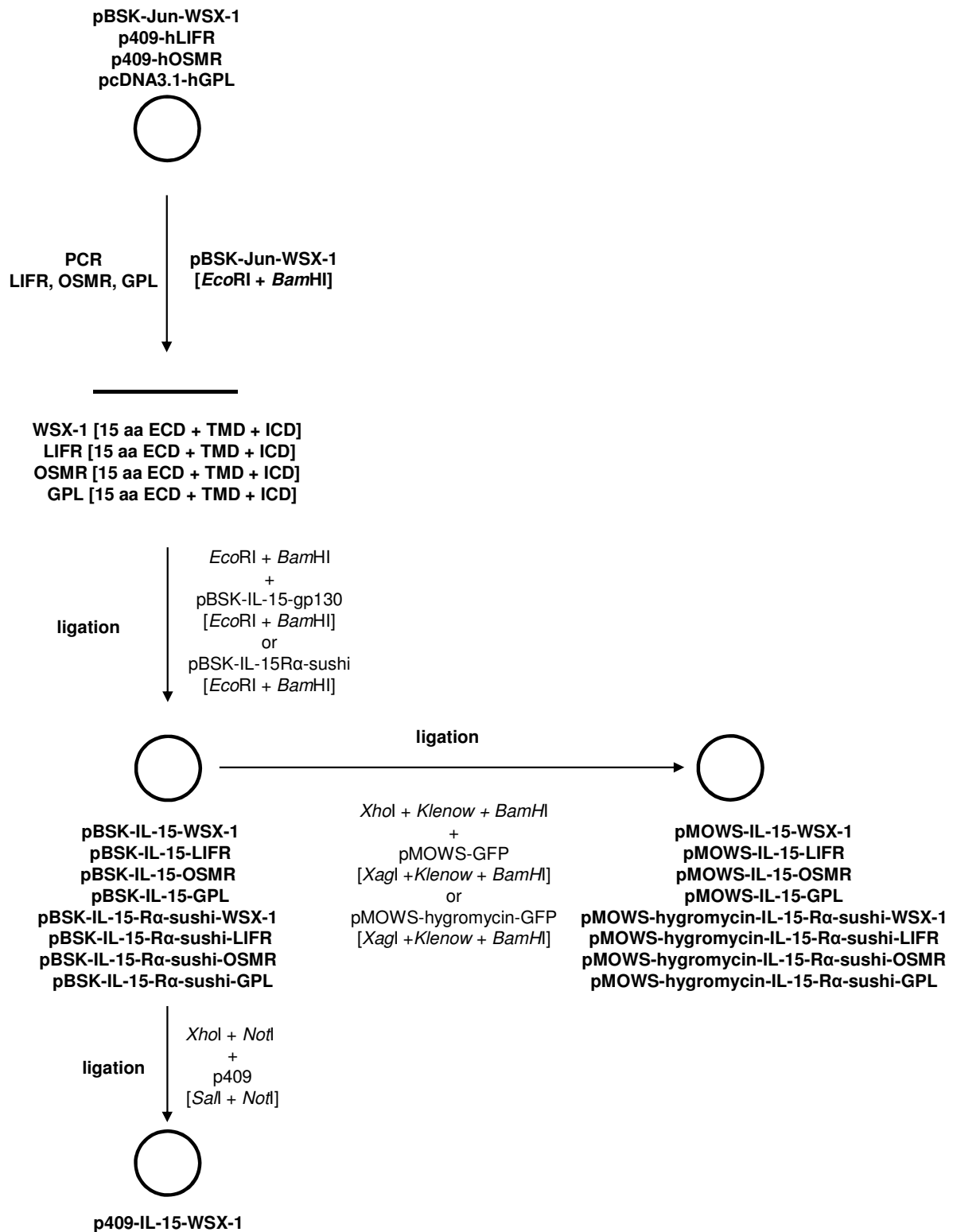


Figure 4.13 Schematic overview of cloning of IL-15-WSX-1, IL-15-LIFR, IL-15-OSMR, IL-15-GPL and IL-15R α -sushi-WSX-1, IL-15R α -sushi-LIFR, IL-15R α -sushi-OSMR and IL-15-sushi-GPL into different plasmids. See text for details.

The retroviral plasmid pMOWS encodes the puromycin resistance gene [169]. For this study we modified the plasmid pMOWS-GFP by replacing the puromycin

resistance gene with a hygromycin resistance gene. The hygromycin gene from the plasmid pCEP4 was amplified by PCR (5'hygromycinBresistance and 3'hygromycinBresistance). The purified PCR product was digested with *HindIII* and *SalI* and subcloned into pMOWS. The resulting plasmid was named pMOWS-GFP-hygromycin (Figure 4.14).

The IL-15 fusion receptors and IL-15R α -sushi fusion receptors were subcloned into pMOWS and pMOWS-hygromycin as described above. The resulting plasmids were named pMOWS-IL-15-gp130, pMOWS-IL-15-WSX-1, pMOWS-IL-15-LIFR, pMOWS-IL-15-OSMR, pMOWS-IL-15-GPL as well as pMOWS-hygromycin-IL-15R α -sushi-gp130, pMOWS-hygromycin-IL-15R α -sushi-WSX-1, pMOWS-hygromycin-IL-15R α -sushi-LIFR, pMOWS-hygromycin-IL-15R α -sushi-OSMR and pMOWS-hygromycin-IL-15R α -sushi-GPL (Figure 4.13).

The cDNAs coding for IL-15-gp130, sushi-gp130 and IL-15-WSX-1 were subcloned into p409 as described above. The resulting plasmids were named p409-IL-15-gp130, p409-IL-15R α -sushi-gp130 and p409-IL-15-WSX-1 (Figures 4.11, 4.12 and 4.13). The cDNAs coding for IL-15-gp130 and sushi-gp130 were additionally subcloned into pEYFP-gp130 as described above. The resulting plasmids were named pEYFP-IL-15-gp130 and pEYFP-IL-15R α -sushi-gp130 (Figures 4.11, 4.12).

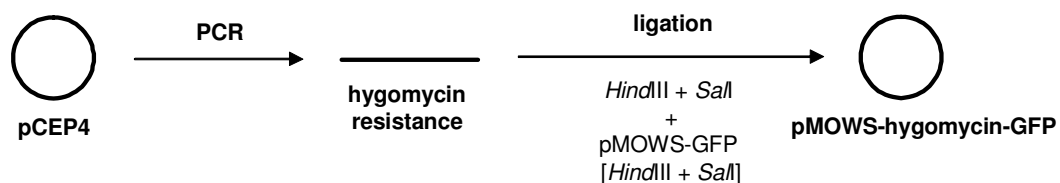


Figure 4.14 Schematic overview of cloning of hygromycin B resistance into pMOWS-GFP. See text for details.

4.6 Heterodimerization of gp130 and WSX-1 mediated by IL-15 and IL-15R α -sushi was achieved and led to cytokine independent growth of Ba/F3-gp130 cells

To address the question whether expression of the new chimeric receptors led to constitutively active heterodimers and induction of cytokine independent proliferation, Ba/F3-gp130 cells were used.

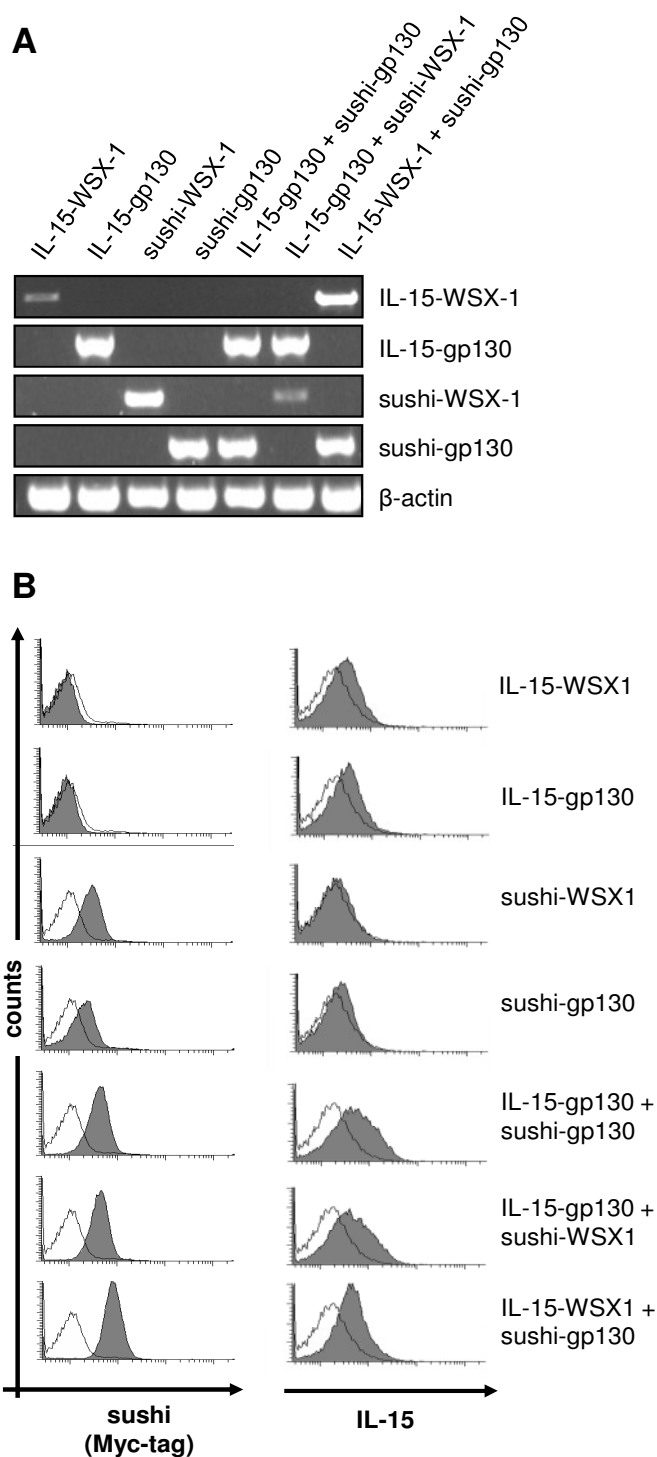


Figure 4.15 Analysis of gp130 and WSX-1 chimeric receptor expression in various stably transduced Ba/F3-gp130 cell lines. (A) The mRNAs of the respective cell lines expressing IL-15-WSX-1, IL-15-gp130, sushi-WSX-1, sushi-gp130, IL-15-gp130 + sushi-gp130, IL-15-gp130 + sushi-WSX-1 and IL-15-WSX-1 + sushi-gp130 were reversely transcribed into cDNA and chimeric receptors were detected with specific primers (see material & methods) by PCR. (B) Cell surface expression of gp130 and WSX-1 chimeric receptors analyzed by flow cytometry. The cell lines were labelled as described in material & methods. Untransduced Ba/F3-gp130 cells were used as negative controls (unfilled histograms).

These cells lack IL-2R β expression [147, 175], which is part of the signaling receptor complex of IL-15 [144].

It has been proposed that IL-15R α is necessary for IL-15 secretion and that the complex of IL-15/IL-15R α is presented on the cell surface to cells expressing IL-2R β and IL-2R γ c. This signaling mode has been named trans-presentation [160, 161]. Recombinantly expressed IL-15 can also induce the proliferation of cells expressing IL-15R α /IL-2R β /IL-2R γ c which is increased by addition of agonistically acting soluble IL-15R α -sushi domain [154].

Ba/F3-gp130 cells were retrovirally transduced with IL-15-WSX-1, IL-15-gp130, sushi-WSX-1 and sushi-gp130. Additionally double transduced Ba/F3-gp130 cells were generated for IL-15-gp130 + sushi-gp130, IL-15-gp130 + sushi-WSX-1 or IL-15-WSX-1 + sushi-gp130 expression plasmids. The expression of all designated receptors and combinations thereof was verified by RT-PCR (Figure 4.15A) and flow cytometry (Figure 4.15B) indicating expression of the respective mRNAs and surface expression of all chimeric receptors.

To verify that Ba/F3-gp130 cells do not respond to IL-15 either if presented at the cells surface or added to the cell culture medium alone or in combination with soluble IL-15R α -sushi domain the following experiments were performed. On the one hand Ba/F3-gp130 cells expressing GFP were cocultured with Ba/F3-gp130 cells expressing IL-15-gp130, IL-15-WSX-1 + sushi-gp130, or Fos-gp130 and GFP expression was monitored by flow cytometry for a time period of 96h (Figure 4.16A). On the other hand Ba/F3-gp130 cells were stimulated with IL-15 alone or in combination with soluble IL-15R α -sushi domain. As a positive control cells were stimulated with Hyper-IL-6 (Figure 4.16B). As depicted in Figure 4.16 neither surface presented IL-15 alone or in combination with IL-15R α -sushi domain nor recombinant IL-15 alone or in combination with soluble IL-15R α -sushi domain induced proliferation of Ba/F3-gp130 cells. It can be concluded that Ba/F3-gp130 are suitable for further experiments.

Next, to investigate whether IL-15/IL-15R α -sushi heterodimerization of chimeric receptors was sufficient to induce ligand-independent cell growth, the retrovirally transduced Ba/F3-gp130 cell lines were cultured in medium lacking Hyper-IL-6. Surprisingly, cells transduced with IL-15-gp130 alone proliferated cytokine independently, indicating that this chimeric receptor forms active IL-15-gp130 homodimeric complexes (Figure 4.17). However, expression of neither IL-15-WSX-1

nor sushi-WSX-1 nor sushi-gp130 alone led to cytokine independent growth in Ba/F3-gp130 cells, respectively (Figure 4.17).

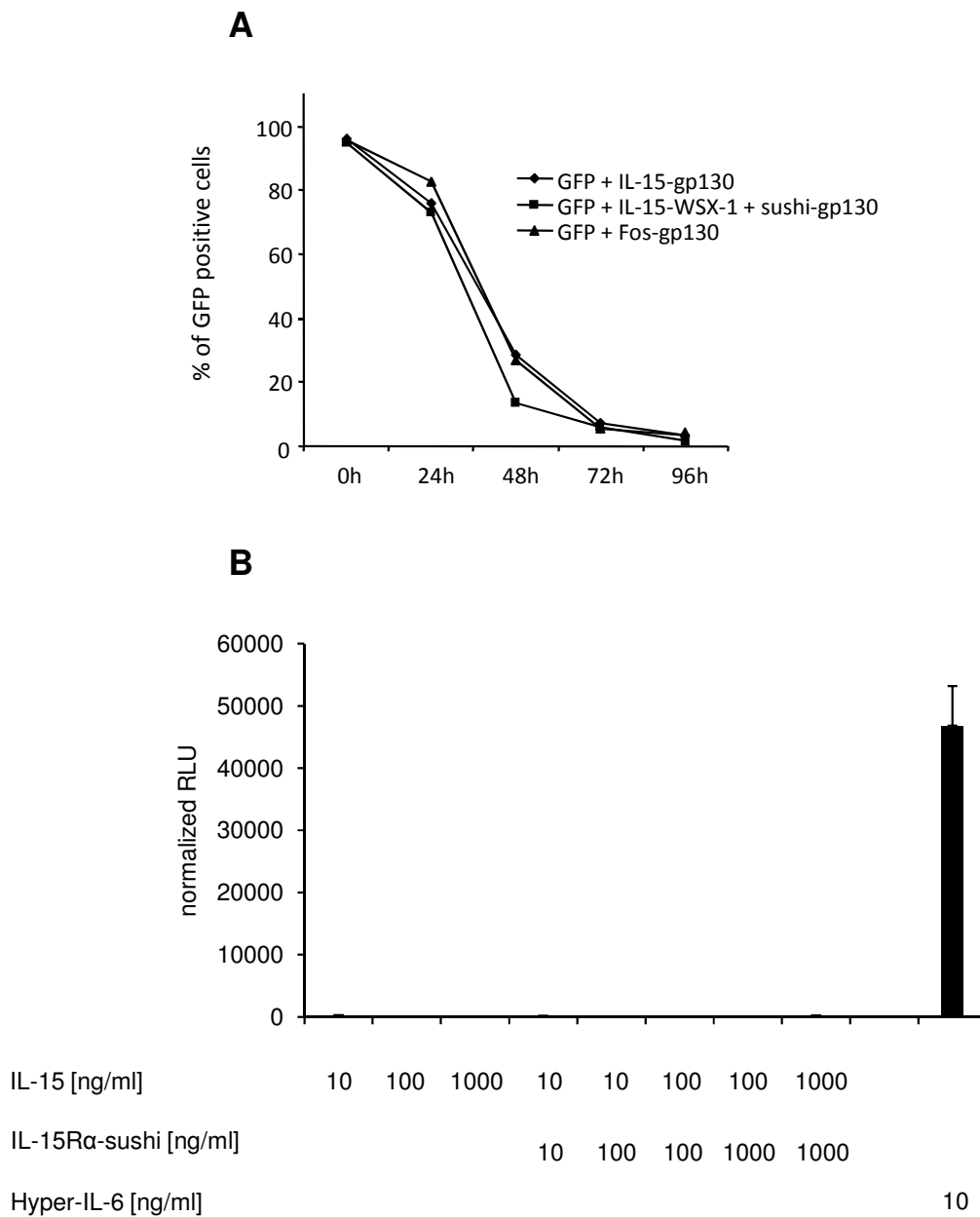


Figure 4.16 Unresponsiveness of Ba/F3-gp130 cells to IL-15. (A) Co-culture experiment of Ba/F3-gp130 expressing GFP with Ba/F3-gp130 cells expressing IL-15-gp130, IL-15-WSX-1 + sushi-gp130 or Fos-gp130 at a ratio of 20:1. Decrease/death of GFP expressing cells was measured by flow cytometry during a time period of 96 h. (B) Equal numbers of Ba/F3-gp130 cells were cultured for 3 days in the presence of increasing amounts of recombinant IL-15 or recombinant IL-15 and soluble IL-15R α -sushi domain. As a control cells were left untreated or cultured in the presence of Hyper-IL-6 (10 ng/ml). Proliferation was measured as indicated in material & methods.

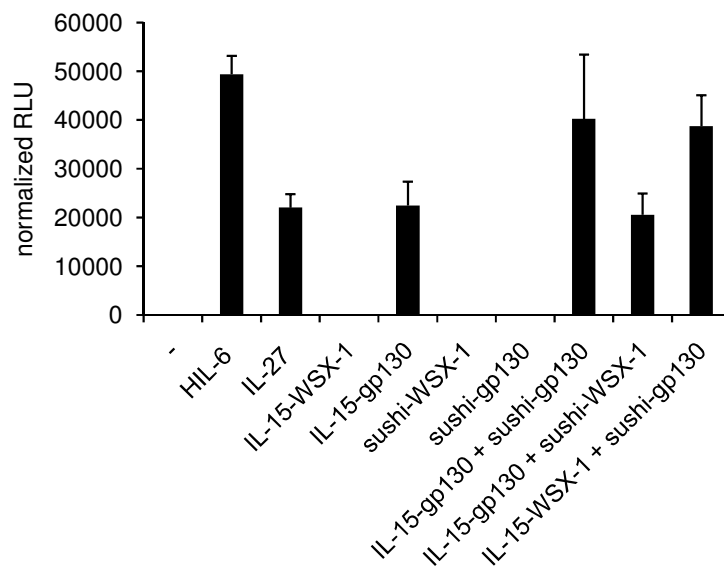


Figure 4.17 Functionality of IL-15 and IL-15R α mediated heterodimerization of gp130 and WSX-1. Equal numbers of Ba/F3-gp130 cells stably transduced with IL-15-gp130, IL-15-WSX-1, sushi-gp130, sushi-WSX-1, IL-15-gp130 + sushi-gp130, IL-15-gp130 + sushi-WSX-1 and IL-15-WSX-1 + sushi-gp130 were cultured for 3 days in the absence of Hyper-IL-6. As a control Ba/F3-gp130 were cultured in the presence or absence of 10

ng/ml Hyper-IL-6 or IL-27. Ba/F3-gp130 cells can also respond to IL-27 because they endogenously express WSX-1. Proliferation was measured as indicated in material & methods.

However, co-transduction of IL-15-WSX-1 with sushi-gp130 led to cytokine independent growth in Ba/F3-gp130 cells, indicating that the IL-15/IL-15R α -sushi mediated heterodimerization of these receptors induced a biologically functional receptor complex (Figure 4.17). Moreover, Ba/F3-gp130 cells expressing IL-15-gp130 + sushi-gp130 or IL-15-gp130 + sushi-WSX-1 also displayed cytokine independent proliferation (Figure 4.17). Since the IL-15-gp130 receptor chimera already induced cytokine independent proliferation in Ba/F3-gp130 cells, it is not clear whether the proliferation was induced by IL-15 mediated homodimerization of IL-15-gp130 or IL-15/IL-15R α -sushi mediated homo-/heterodimerization of IL-15-gp130/sushi-gp130 or IL-15-gp130/sushi-WSX-1 or a mixed type of both. Nevertheless it can be concluded that IL-15/IL-15R α -sushi mediated heterodimerization of gp130/WSX-1 led to functional receptor complexes.

4.7 Interaction analysis of IL-15 mediated homodimerization of gp130/gp130 and IL-15/IL-15R α -sushi mediated heterodimerization of gp130/WSX-1

To demonstrate the physical interaction of two IL-15-gp130 proteins, co-immunoprecipitation analysis was performed with COS-7 lysates containing IL-15-gp130 alone or in combination with IL-15-gp130-EYFP. Immunoprecipitation was performed with anti-GFP antibodies.

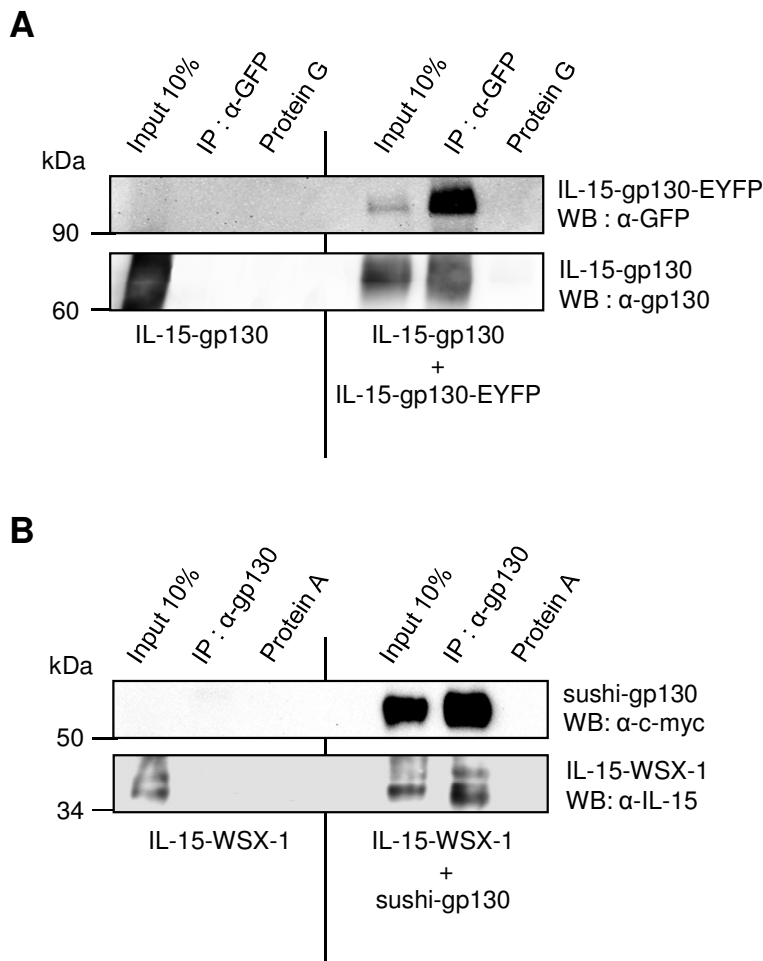


Figure 4.18 Detection of IL-15-gp130 homodimers and IL-15-WSX-1 / sushi-gp130 heterodimers by co-immunoprecipitation. (A) COS-7 cells were transiently transfected with p409-IL-15-gp130 or p409-IL-15-gp130 and pEYFP-IL-15-gp130. 48 h after transfection cells were lysed and IL-15-gp130-EYFP was immunoprecipitated with anti-GFP antibodies. As a control lysates were incubated only with protein G agarose. Input, immunoprecipitation supernatant and protein G agarose control supernatant were separated by SDS-PAGE. Proteins were detected with anti-GFP antibodies and visualized by ECL detection. The membrane was stripped, probed with anti-

gp130 antibodies and proteins were visualized by ECL detection. (B) COS-7 cells were transiently transfected with p409-IL-15-WSX-1 or p409-IL-15-WSX-1 and p409-sushi-gp130. 48 h after transfection cells were lysed and sushi-gp130 was immunoprecipitated with anti-gp130 antibodies. As a control lysates were incubated only with protein A agarose. Input, immunoprecipitation supernatant and protein A agarose control supernatant were separated by SDS-PAGE. Proteins were detected with anti-c-myc antibodies and visualized by ECL detection. The membrane was stripped, probed with IL-15 antibodies and proteins were visualized by ECL detection.

As depicted in the left panel of Figure 4.18A, IL-15-gp130 alone was not precipitated by anti-GFP antibodies, indicating that IL-15-gp130 does not bind unspecifically to the anti-GFP antibodies or to protein G agarose. However, IL-15-gp130 was co-immunoprecipitated if IL-15-gp130-EYFP was immunoprecipitated, as indicated in the right panel of Figure 4.18 A. Previously, it has been shown that recombinant IL-15 protein, produced in the yeast strain *Pichia pastoris* could not be purified as a monomer but always as a dimer or multimer (Inken Lorenzen, personal communication). This observation was also reported by Hanick *et al.* [176].

Furthermore, the physical interaction of sushi-gp130 and IL-15-WSX-1 proteins was demonstrated. Again, co-immunoprecipitation analysis was performed with COS-7 lysates containing either IL-15-WSX-1 alone or in combination with sushi-gp130. Immunoprecipitation was performed with anti-gp130 antibodies. As shown in the left panel of Figure 4.18B, IL-15-WSX-1 alone was not precipitated, indicating that IL-15-WSX-1 does not bind unspecifically to anti-gp130 antibodies or to protein A agarose. Nonetheless, IL-15-WSX-1 was co-immunoprecipitated if sushi-gp130 was immunoprecipitated, as indicated in the right panel of Figure 4.18B.

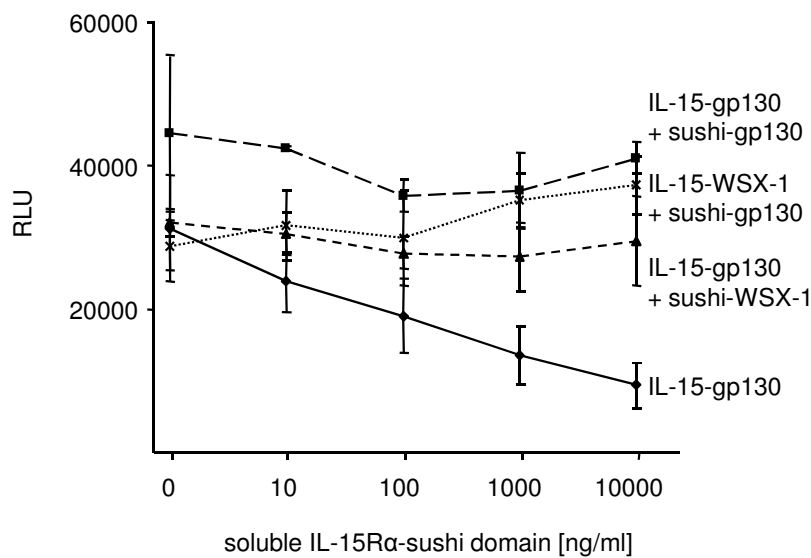


Figure 4.19 Inhibition of IL-15 mediated homodimerization of IL-15-gp130 by soluble IL-15R α -sushi domain. Equal numbers of Ba/F3-gp130 cells stably transduced with IL-15-gp130, IL-15-gp130 + sushi-gp130, IL-15-gp130 + sushi-WSX-1 and IL-15-WSX-1 + sushi-gp130 were cultured for 3 days in the absence of Hyper-IL-6 and increasing amounts of soluble IL-15R α -sushi domain.

Proliferation was measured as indicated in material & methods.

To test whether soluble IL-15R α -sushi domain was able to inhibit the IL-15 mediated homodimerization of gp130 or the IL-15/IL-15R α -sushi mediated homo-/heterodimerization of gp30/gp130 or gp130/WSX-1, respectively, a proliferation assay with increasing amounts of soluble IL-15R α -sushi was performed. As shown in Figure 4.19 the proliferation of Ba/F3-gp130 cells expressing IL-15-gp130 was inhibited by soluble IL-15R α -sushi domain in a concentration dependent manner. These data indicated that the proliferation of these cells is mediated by IL-15 homodimerization and is competed by soluble IL-15R α -sushi to abrogate gp130 signaling. Interestingly, the proliferation of the double transduced Ba/F3-gp130 cells expressing IL-15- and IL-15R α -sushi-receptor chimeras (gp130/gp130, gp130/WSX-1) was not inhibited by the treatment of soluble IL-15R α -sushi domain (Figure 4.19). Duitman *et al.* showed that IL-15R α is essential for the secretion of IL-15 via the classical secretion pathway, involving the ER and Golgi apparatus [161]. This might

imply that IL-15 and IL-15R α form a complex before they are presented at the cell surface. The high affinity constant of an IL-15/IL-15R α complex ($K_D = 114$ pM) is achieved by a large network of ionic interactions characterized by a low dissociation rate ($1.56 \times 10^{-5} \text{ min}^{-1}$) [164]. Hence, it can be assumed that the already formed complexes between IL-15 and IL-15R α -sushi chimeric receptors cannot be dissociated by high molecular excess of soluble IL-15R α -sushi domain.

4.8 Unexpected homodimerization of sushi-gp130 does not lead to a functional receptor complex

Since it was indeed shown that IL-15-gp130 forms a functional homodimer in terms of cytokine independent proliferation of stably transduced Ba/F3-gp130 cells, also sushi-gp130, which is not functional alone, was analyzed with respect to the ability of homodimerization. Co-immunoprecipitation analysis was performed with COS-7 lysates containing sushi-gp130 alone or in combination with sushi-gp130-EYFP. Immunoprecipitation was performed with anti-GFP antibodies.

Unexpectedly, sushi-gp130 was co-immunoprecipitated with sushi-gp130-EYFP, as indicated in the right panel of Figure 4.20. No unspecific binding to the anti-GFP antibodies or protein G agarose of sushi-gp130 could be shown as depicted in the left panel of Figure 4.20. Additionally, a series of co-immunoprecipitation experiments were performed with sushi-gp130 + Δ cys-Fos-gp130 or IL-15-gp130 + Δ cys-Fos-gp130. In those combinations, the proteins were not expected to interact with each other. In each experiment only one of the gp130 chimeric receptors was tagged with EYFP. All co-immunoprecipitation experiments were also performed *vice versa*. Interestingly, independent of the extracellularly fused domain or protein (Fos leucine zipper, IL-15 or IL-15R α -sushi), each chimeric gp130 receptor could be co-immunoprecipitated by every other chimeric gp130 receptor in all applied combinations (Figure 4.21A-D). To show that this effect was not mediated by overexpression or any other experimental cause, the transmembrane protease BACE, which does not belong to any cytokine receptor family, was chosen for co-immunoprecipitation analysis. As depicted in Figure 4.20E, sushi-gp130-EYFP does not interact with BACE.

Taken together all results of the co-immunoprecipitation experiments implicate that the interaction of all gp130 chimeric receptors were specific, even if they are not only mediated by IL-15 or IL-15 and IL-15R α -sushi domain.

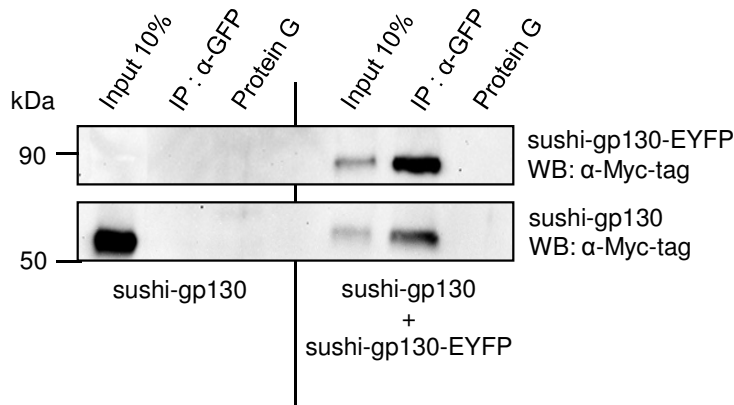


Figure 4.20 Detection of sushi-gp130 homodimers by co-immunoprecipitation. COS-7 cells were transiently transfected with p409-sushi-gp130 or p409-sushi-gp130 and pEYFP-sushi-gp130. 48 h after transfection cells were lysed and sushi-gp130-EYFP was immunoprecipitated with anti-GFP antibodies. As a control lysates were incubated with protein G

agarose alone. Input, immunoprecipitation supernatant and protein G agarose control supernatant were separated by SDS-PAGE. Proteins were detected with anti-Myc-tag antibodies and visualized by ECL detection.

The possibility that the sushi domain of IL-15R α is able to mediate homodimerization cannot be excluded completely, but it seems likely that the interaction of those chimeric receptors is mediated by the gp130 transmembrane or intracellular domain, independent of the N-terminally fused domain. A possible conclusion for those experiments is the finding that gp130 forms preformed non-active homodimers as well as heterodimers with LIFR in the absence of a ligand [168, 177].

To analyze the difference between the dimerization of 'non-active' sushi-gp130 and 'active' IL-15-gp130, the phosphorylation status of the gp130 receptor chimeras was analyzed. An 'active' chimeric receptor, in this particular case, is characterized by the ability to mediate ligand-independent growth in Ba/F3-gp130 cells. In detail, the gp130 chimeric receptors were immunoprecipitated with anti-gp130 antibodies and were analyzed for tyrosine-phosphorylation. Interestingly, no tyrosine-phosphorylation was observed after precipitation of the IL-15-/sushi-gp130 receptor chimeras from transiently transfected and starved COS-7 cells. Pervanadate blocks tyrosine-phosphatases very efficiently [178, 179]. Precipitated IL-15-gp130 from transiently transfected, starved and pervanadate treated COS-7 cells displayed an intensive tyrosine-phosphorylation signal if transfected alone or in combination with sushi-gp130. In contrast, sushi-gp130 displayed only a faint tyrosine-phosphorylation signal when transfected alone, which increased after the co-transfection of IL-15-gp130 (Figure 4.22).

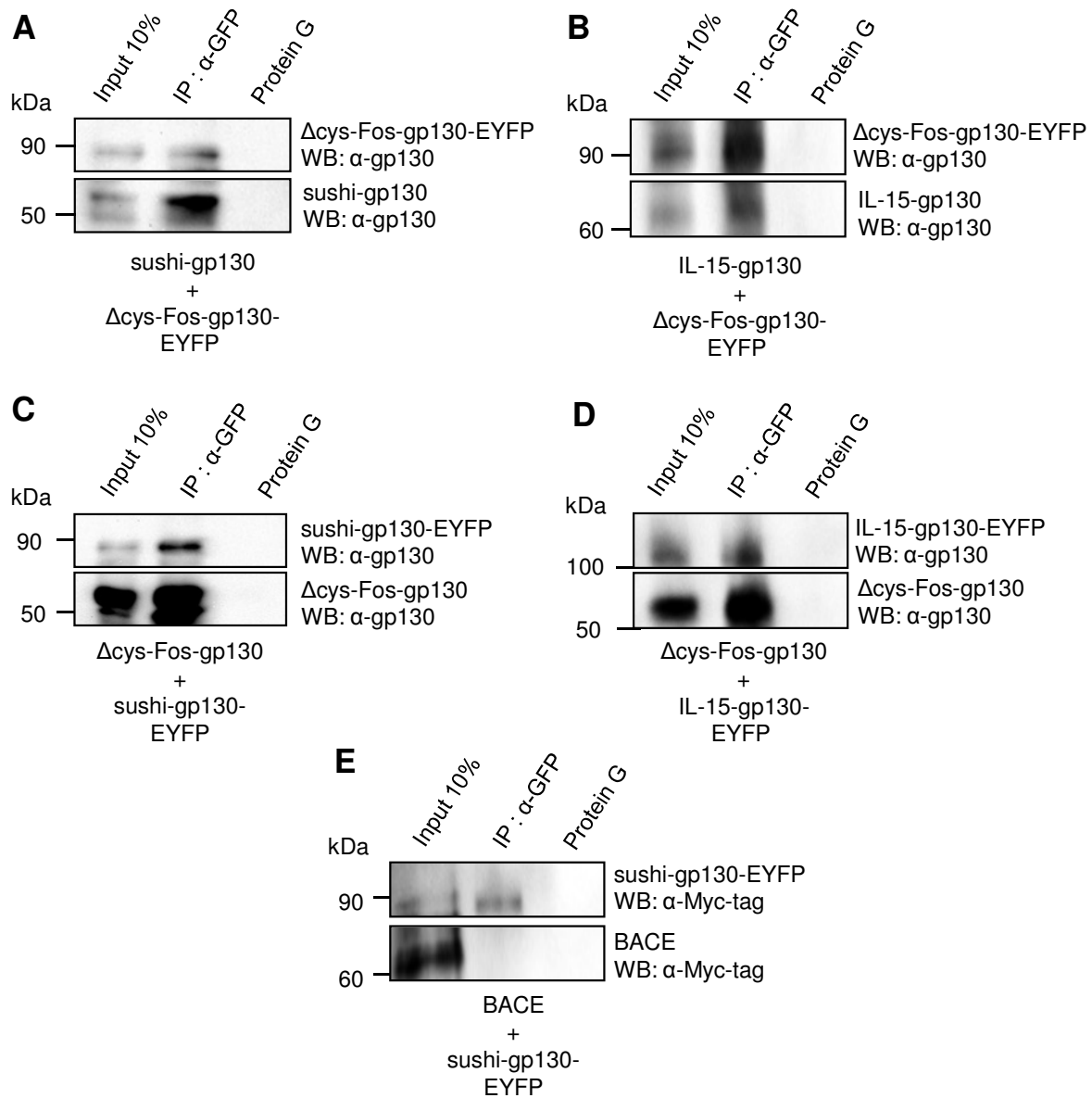


Figure 4.21 Dimerization of sushi-gp130 and IL-15-gp130 with Δ cys-Fos-gp130. (A) Co-immunoprecipitation of Δ cys-Fos-gp130-EYFP and sushi-gp130. COS-7 cells were transiently transfected with p409-sushi-gp130 and pEYFP- Δ cys-Fos-gp130. 48 h after transfection cells were lysed and Δ cys-Fos-gp130-EYFP was immunoprecipitated with anti-GFP antibodies. As a control lysates were incubated only with protein G agarose. Input, immunoprecipitation supernatant and protein G agarose control supernatant were separated by SDS-PAGE. Proteins were detected with anti-gp130 antibodies and visualized by ECL detection. (B) Co-immunoprecipitation of Δ cys-Fos-gp130-EYFP and IL-15-gp130. The experiment was performed as described in (A). (C) Co-immunoprecipitation of sushi-gp130-EYFP and Δ cys-Fos-gp130. The experiment was performed as described in (A). (D) Co-immunoprecipitation of IL-15-gp130-EYFP and Δ cys-Fos-gp130. The experiment was performed as described in (A). No co-immunoprecipitation of sushi-gp130-EYFP and BACE. The experiment was performed as described in (A) despite the fact that both proteins were detected with anti-Myc-tag antibodies.

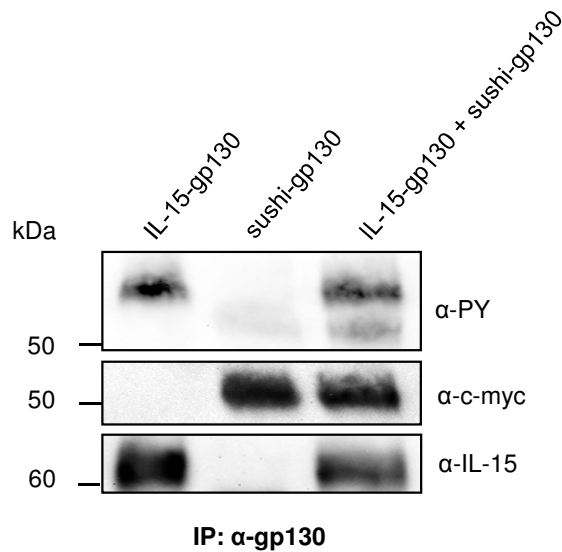


Figure 4.22 Tyrosine-phosphorylation of IL-15-gp130 and sushi-gp130. COS-7 cells were transiently transfected with p409-IL-15-gp130 or p409-sushi-gp130 or p409-IL-15-gp130 and p409-sushi-gp130 and starved for the last 20 h before lysis. 48 h after transfection, COS-7 cells were treated with pervanadate as described in Material & methods and lysed rapidly. The gp130 chimeric proteins were immunoprecipitated with anti-gp130 antibodies. Immunoprecipitation supernatant was separated by SDS-PAGE. Proteins were transferred onto PVDF membrane and were detected with anti-phosphotyrosine (PY) antibodies and visualized by ECL detection. To confirm immunoprecipitation of IL-

15-gp130 and sushi-gp130 Western blots against IL-15 or c-myc were performed additionally.

It can be concluded that dimerized sushi-gp130 proteins do not have the capacity to induce receptor phosphorylation and ligand-independent growth in Ba/F3-gp130 cells because sushi-gp130 only appeared to be slightly tyrosine-phosphorylated which might be background signal. In contrast to that, IL-15-gp130 dimers were able to induce receptor phosphorylation and ligand-independent growth of Ba/F3-gp130 cells. Co-expression of IL-15-gp130 and sushi-gp130 led to an increase in the phosphorylation of sushi-gp130 chain. However, in Ba/F3-gp130 cells expressing IL-15-gp130 + sushi-gp130 it cannot be dissected which receptor chain contributes to ligand-independent growth. This is different to Ba/F3-gp130 cells expressing IL-15-WSX-1 + sushi-gp130. In those cells expression of both receptor chains is essential for ligand-independent growth.

4.9 Phosphorylation of STAT1/3 and ERK1/2 proteins in Ba/F3-gp130 cells expressing IL-15 or IL-15/IL-15R α -sushi mediated homo- and heterodimers of gp130/gp130 and gp130/WSX-1

As shown previously, Δ cys-Fos-gp130 induced ligand-independent STAT3 phosphorylation and growth of Ba/F3-gp130 cells. So it was asked whether the chimeric receptors comprising IL-15/IL-15R α -sushi, which induced cytokine independent growth in Ba/F3-gp130 cells, also induced phosphorylation of signaling proteins. Therefore lysates of Ba/F3-gp130 cells expressing the different IL-15/IL-15R α -sushi based receptor chimeras were analyzed by Western blot for phosphorylated STAT1/3 as well as phosphorylated ERK1/2 with specific antibodies. As depicted in Figure 4.23A, only those cells expressing IL-15-gp130 alone or in combination with sushi-gp130 or sushi-WSX-1 as well as the cells expressing IL-15-WSX-1 + sushi-gp130 showed phosphorylation of STAT1/3 and ERK1/2. These cell lines also induced cytokine independent growth of Ba/F3-gp130 cells. None of the cell lines expressing IL-15-WSX-1, sushi-gp130 or sushi-WSX-1 alone, which failed to induce cytokine independent growth, showed any phosphorylation of STAT1/3 and ERK1/2 molecules.

The transcription of the genes c-myc and Pim-1 has been shown to be dependent on IL-6- and IL-27-induced STAT3 activation [72, 180]. Therefore it was asked whether the expression of the functional IL-15 or IL-15/IL-15R α -sushi homo- or heterodimers, which already led to proliferation in a cytokine independent manner and to phosphorylation of STAT3 in Ba/F3-gp130 cells, led to an increased expression of such STAT3 target genes. As shown in Figure 4.23B, all functional chimeric receptors showed an up-regulation of the transcription factor c-myc and the serine/threonine kinase Pim-1 with respect to mRNA levels.

It can be concluded that forced homo- and heterodimeric receptor complexes comprising gp130/gp130 and gp130/WSX-1 are fully functional in terms of STAT3 phosphorylation and triggering the expression of c-myc and Pim-1 thus leading to cell growth and proliferation. Cell growth and proliferation can also contribute to ERK1/2 mediated signaling [181]. ERK1/2 phosphorylation was also mediated by the forced homo- and heterodimerized gp130/gp130 and gp130/WSX-1 signaling complexes.

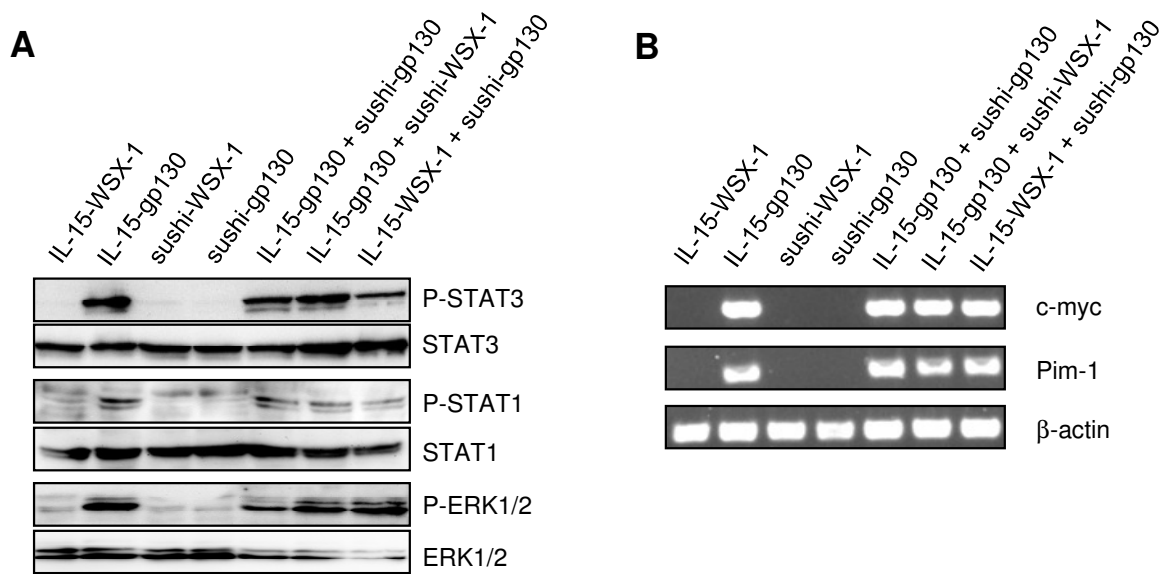


Figure 4.23 Activation of STAT and ERK proteins and transcription of the STAT3 target genes c-myc and Pim-1 in BaF3-gp130 cells stably transduced with chimeric receptors comprising IL-15/IL-15R α . (A) After 6 h of serum starvation Ba/F3-gp130 cells stably transduced with IL-15-WSX-1, IL-15-gp130, sushi-WSX-1, sushi-gp130, sushi-gp130 + IL-15-gp130, sushi-WSX-1 + IL-15-gp130, IL-15-WSX-1 + sushi-gp130 were left untreated. Subsequently, cells were lysed and separated by SDS-PAGE. Proteins were transferred onto PVDF membranes and were detected with anti-P-STAT1, anti-P-STAT3 or anti-P-ERK1/2 antibodies and visualized by ECL detection. The membranes were stripped, probed with anti-STAT1, anti-STAT3 or anti-ERK1/2 antibodies and proteins were visualized by ECL detection. (B) Analysis of STAT3 target gene expression of c-myc and Pim-1 in Ba/F3-gp130 cells stably transduced with the respective chimeric receptor after 6 h of serum starvation by RT-PCR.

4.10 The IL-15/IL-15R α -sushi mediated heterodimerization of receptors can be adapted to other heterodimeric receptor complexes of the gp130-type family

As described previously, the generation of IL-15/IL-15R α -sushi chimeric receptors was applied to all gp130-type receptors comprising gp130, WSX-1, LIFR, OSMR and GPL. In this family, also heterodimeric receptor complexes beside gp130/WSX-1 can be formed comprising gp130/LIFR, gp130/OSMR and OSMR/GPL [26, 30].

To prove the applicability of the IL-15/IL-15R α -sushi mediated heterodimerization system, Ba/F3-gp130 cells were stably transduced with IL-15-LIFR, IL-15-LIFR + sushi-gp130, IL-15-OSMR, IL-15-OSMR + sushi-gp130, sushi-GPL and IL-15-OSMR + sushi-GPL. Again, the expression of all designated receptors and combinations

thereof were verified by RT-PCR (Figure 4.24A) and flow cytometry (Figure 4.24B) indicating expression of the respective mRNAs and surface expression of all new chimeric receptors.

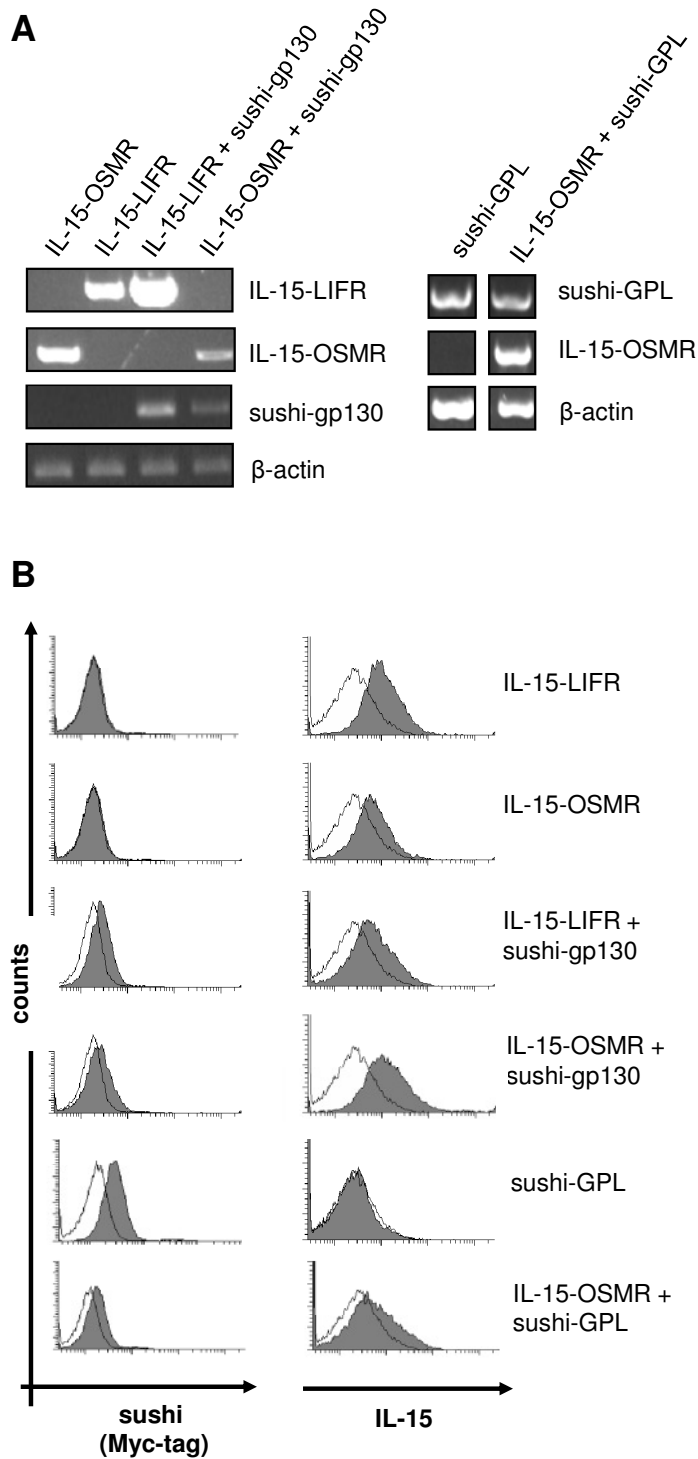


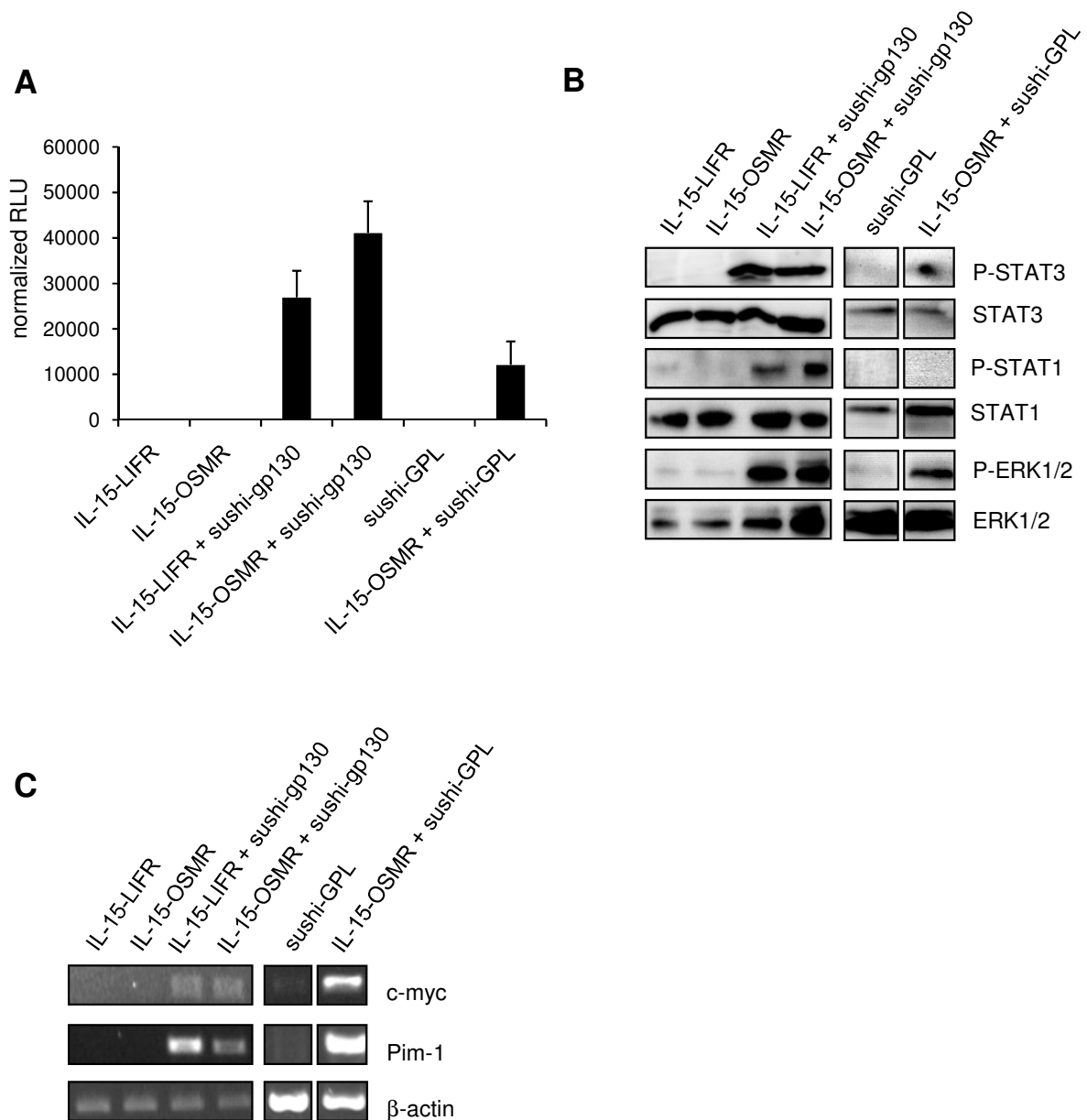
Figure 4.24 Analysis of LIFR, OSMR and GPL chimeric receptor expression in various stably transduced Ba/F3-gp130 cell lines.

(A) The mRNAs of the respective cell lines expressing IL-15-OSMR, IL-15-LIFR, IL-15-LIFR + sushi-gp130, IL-15-OSMR + sushi-gp130, sushi-GPL or IL-15-OSMR + sushi-GPL were reversely transcribed into cDNA and chimeric receptors were detected with specific primers (see material & methods) by PCR. (B) Cell surface expression of LIFR, OSMR, gp130 and GPL chimeric receptors analyzed by flow cytometry. The cell lines were labeled as described in material & methods. Untransduced Ba/F3-gp130 cells were used as negative controls (unfilled histograms). This figure is adapted from the diploma thesis of Anna Tillmann [182], who was supervised by the author of this work.

After expression analysis, it was asked whether the chimeric receptors alone or in combination induced ligand-independent growth in Ba/F3-gp130 cells. A proliferation assay was performed in medium lacking Hyper-IL-6. Like IL-15-WSX-1, IL-15-LIFR and IL-15-OSMR alone did not induce cytokine independent growth of the respective cell line. Otherwise, if sushi-gp130 was co-transduced with either IL-15-LIFR or IL-15-OSMR cytokine independent growth was achieved as depicted in Figure 4.25A. Also sushi-GPL alone was not able to induce cytokine independent growth, but co-transduction of sushi-GPL and IL-15-OSMR lead to proliferation of the respective Ba/F3-gp130 cell line (Figure 4.25A). So it was asked again whether the chimeric receptors comprising IL-15/IL-15R α -sushi, which induced cytokine independent growth in Ba/F3-gp130 cells, also induce phosphorylation of signaling proteins. Therefore lysates of starved Ba/F3-gp130 cells expressing the different IL-15/IL-15R α -sushi based receptor chimeras (gp130/LIFR, gp130/OSMR and OSMR/GPL) were analyzed by Western blot for phosphorylated STAT1/3 as well as phosphorylated ERK1/2 with specific antibodies. As shown in Figure 4.25B the cell lines expressing IL-15-LIFR + sushi-gp130 and IL-15-OSMR + sushi-gp130 but not IL-15-LIFR, IL-15-OSMR or sushi-GPL alone exhibited phosphorylation of STAT1/3 as well as ERK1/2. The cell line expressing IL-15-OSMR + sushi-GPL exhibited phosphorylation of STAT3 and ERK1/2 but not STAT1. It has been shown previously that IL-31 only show low level phosphorylation of STAT1 [183].

To investigate whether the phosphorylation of STAT3 lead to an up-regulation of c-myc and Pim-1 in the respective Ba/F3-gp30 cell lines, a RT-PCR study was performed. As shown in Figure 4.26C the Ba/F3-gp130 cell lines expressing IL-15-LIFR + sushi-gp130, IL-15-OSMR + sushi-gp130 and IL-15-OSMR + sushi-GPL exhibited an up-regulation of c-myc and Pim-1 expression at mRNA levels. No up-regulation of the respective STAT3 target genes was observed in cells expressing IL-15-LIFR, IL-15-OSMR and sushi-GPL.

Taken together, it can be concluded that the IL-15/IL-15R α -sushi heterodimerization system can be adapted to all gp130-type receptor complexes comprising gp130/gp130, gp130/WSX-1, gp130/LIFR, gp130/OSMR and OSMR/GPL.



4.26 Functionality of IL-15/IL-15R α -sushi mediated heterodimerization of gp130/LIFR, gp130/OSMR and OSMR/GPL. (A) Equal numbers of Ba/F3-gp130 cells stably transduced with IL-15-LIFR, IL-15-LIFR + sushi-gp130, IL-15-OSMR, IL-15-OSMR + sushi-gp130, sushi-GPL or IL-15-OSMR + sushi-GPL were cultured for 3 days in the absence of Hyper-IL-6. Proliferation was measured as indicated in Material & methods. (B) After 6 h of serum starvation Ba/F3-gp130 cells expressing the respective chimeric receptors were left untreated. Subsequently, cells were lysed and separated by SDS-PAGE. Proteins were transferred onto PVDF membranes and were detected with anti-P-STAT1, anti-P-STAT3 or anti-P-ERK1/2 antibodies and visualized by ECL detection. The membranes were stripped, probed with anti-STAT1, anti-STAT3 or anti-ERK1/2 antibodies and proteins were visualized by ECL detection. (C) Analysis of STAT3 target gene expression of c-myc and Pim-1 in Ba/F3-gp130 cells stably transduced with the respective chimeric receptors after 6 h of serum starvation by RT-PCR. This figure is adapted from the diploma thesis of Anna Tillmann [182], who was supervised by the author of this work.

4.11 Analysis of all possible combinations between all gp130-type receptors using the IL-15/IL-15R α -sushi based system.

As shown before, the IL-15/IL-15R α -sushi based system was used to generate ligand-independent, constitutively active heterodimeric receptor complexes of all known gp130-type receptors. The question was raised whether new combinations of receptors of the gp130-type family such as LIFR/OSMR or GPL/WSX-1 are functional in terms of cytokine independent growth induction of Ba/F3-gp130 cells. To analyze this, all cloned IL-15 and IL-15R α -sushi chimeras comprising WSX-1, LIFR, OSMR, GPL and gp130 (only sushi-gp130) were stably transduced into Ba/F3-gp130 cells. To ensure that every cell line expressed their respective receptors a RT-PCR analysis was performed as depicted in Figure 4.27.

Furthermore, all cell lines were screened for cytokine independent growth as depicted in Figure 4.28. This assay revealed that combinations beside the known naturally occurring heterodimers gp130/LIFR, gp130/OSMR, gp130/WSX-1 and OSMR/GPL are also functional in terms of cytokine independent growth of Ba/F3-gp130 cells. These constitutively active combinations, mediated by IL-15/IL-15R α -sushi, comprised of LIFR/OSMR (Figure 4.28B+C), LIFR/GPL (Figure 4.28B+D) and GPL/gp130 (Figure 4.28D), which have already been described using artificial ligand induced dimerization methods [58, 64]. In those works the extracellular domains of gp130, LIFR, OSMR, and GPL were exchanged to the extracellular domains of either IL-5R α or IL-3 β c. Heterodimerization, which led to phosphorylation of STAT proteins, of LIFR/OSMR, LIFR/GPL and GPL/gp130 could be achieved by triggering these chimeric receptors with IL-5.

The observation of ligand-independent heterodimerization of LIFR/OSMR and LIFR/GPL was not dependent on the choice of the fusion protein, meaning that e.g. either expression of IL-15-LIFR + sushi-OSMR or IL-15-OSMR + sushi-LIFR led to cytokine independent growth of the respective Ba/F3-gp130 cell line (Figure 4.28B+C). For the heterodimer of GPL/gp130 only IL-15-GPL + sushi-gp130 was used (Figure 4.28D) because IL-15-gp130 is active on its own as shown before. Remarkably, as shown before IL-15-LIFR alone did not induce cytokine independent growth but co-transduction of sushi-LIFR led to proliferation in a ligand-independent manner (Figure 4.28B). The same effect was observed monitoring ligand-independent proliferation of IL-15-GPL + sushi-GPL, whereas expression of IL-15-GPL alone did not induced cytokine independent proliferation of Ba/F3-gp130 cells

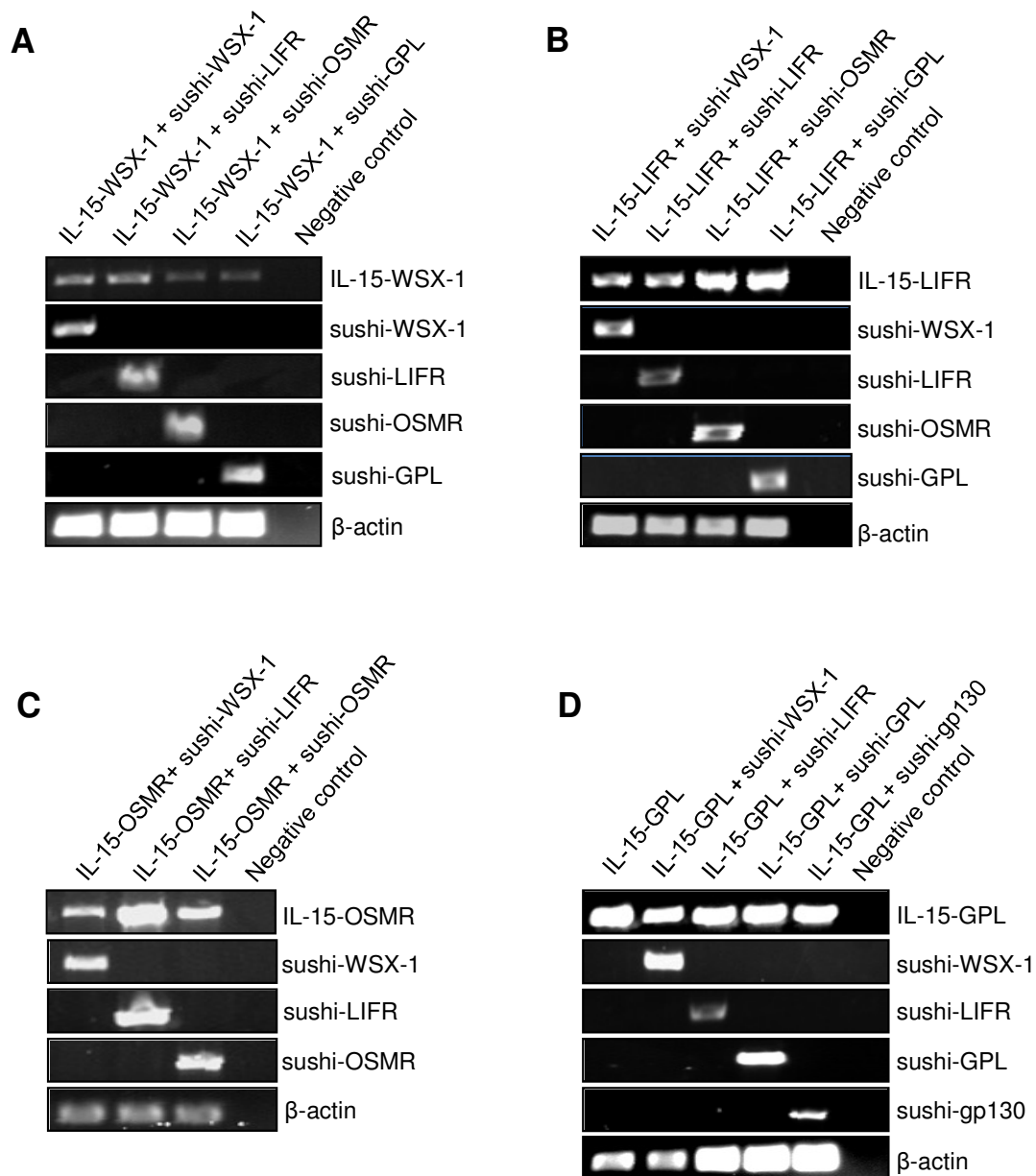


Figure 4.27 Analysis of WSX-1, LIFR, OSMR, GPL and gp130 chimeric receptor expression in various stably transduced Ba/F3-gp130 cell lines. mRNAs of the respective cell lines expressing (A) IL-15-WSX-1 + sushi-WSX-1, IL-15-WSX-1 + sushi-LIFR, IL-15-WSX-1 + sushi-OSMR, IL-15-WSX-1 + sushi-GPL or (B) IL-15-LIFR + sushi-WSX-, IL-15-LIFR + sushi-LIFR, IL-15-LIFR + sushi-OSMR, IL-15-LIFR + sushi-GPL or (C) IL-15-OSMR + sushi-WSX-1, IL-15-OSMR + sushi-LIFR, IL-15-OSMR + sushi-OSMR or (D) IL-15-GPL, IL-15-GPL + sushi-WSX-1, IL-15-GPL + sushi-LIFR, IL-15-GPL + sushi-GPL and IL-15-GPL + sushi-gp130 were reversely transcribed into cDNA and chimeric receptors were detected with specific primers (see material & methods) by PCR. As a negative control ddH₂O was used.

(Figure 4.28D). Homodimers of LIFR and GPL has been analyzed previously with the same artificial ligand induced system as mentioned above [58, 64].

This is an interesting finding with respect to the ability of IL-15 to dimerize or multimerize. As shown before, IL-15-gp130 was the only chimeric receptor which induced cytokine independent growth on its own. So, dimerization of IL-15-WSX-1, IL-15-LIFR, IL-15-OSMR or IL-15-GPL by IL-15 had no impact on the onset of signaling of those receptors. But co-expression of IL-15-LIFR + sushi-LIFR or IL-15-GPL + sushi-GPL induced signaling, leading to the assumption that the conformation of the transmembrane and cytoplasmic domains achieved by chimeric IL-15-LIFR/IL-15-LIFR or IL-15-GPL/IL-15-GPL homodimers are different to IL-15-LIFR/sushi-LIFR or IL-15-GPL/sushi-GPL homodimers.

The co-expression of IL-15-OSMR + sushi-OSMR instead did not lead to cytokine independent growth of the respective Ba/F3-gp130 cell line (Figure 4.28 C). However, OSMR homodimers using artificial ligand induced dimerization have been described previously [64] but the authors of this work announced that the signaling ability of OSMR homodimers seems to depend on the cellular context. It could also be true, that the IL-15/IL-15R α -sushi based system is not suitable to induce OSMR homodimerization.

Interestingly, WSX-1 only led to a functional heterodimer with gp130 (Figure 4.17) but not with LIFR, OSMR, GPL or a homodimer with itself (Figure 4.28A-D). There were also no detectable amounts of phosphorylated STAT1 measured for all combinations applied here with IL-15-WSX-1 by Western blot analysis (data not shown). To date there is no report in literature which showed the ability of WSX-1 to homodimerize or heterodimerize with any other member of the gp130-type or IL-12R-type family except gp130.

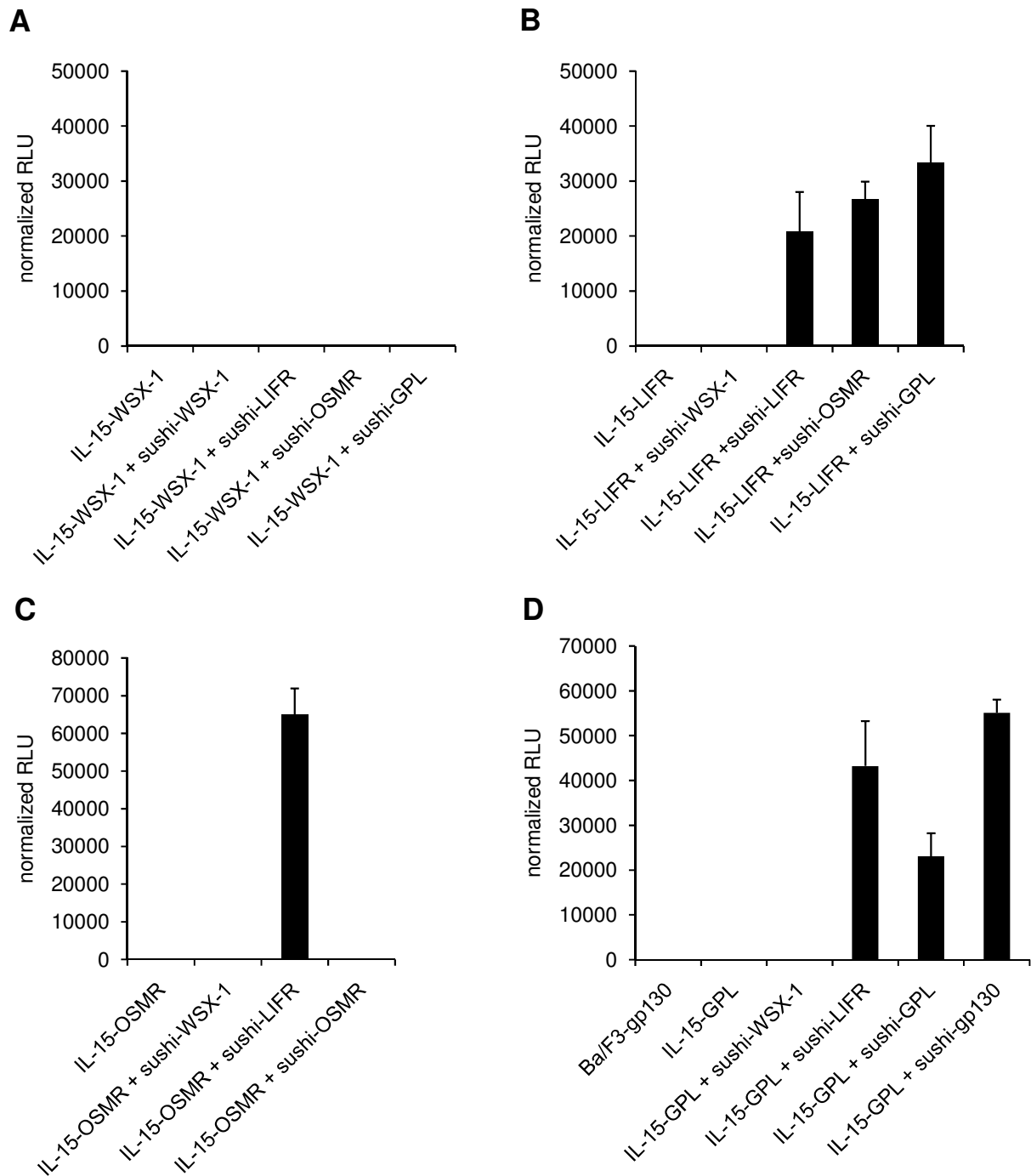
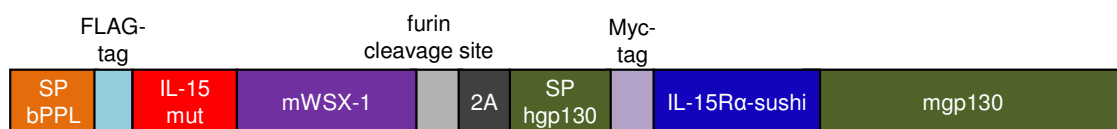


Figure 4.28 Functionality of IL-15/IL-15 α -sushi mediated homo-/heterodimerization of LIFR/LIFR, LIFR/OSMR and LIFR/GPL and GPL/GPL. Equal numbers of Ba/F3-gp130 cells stably transduced with (A) IL-15-WSX-1, IL-15-WSX-1 + sushi-WSX-1, IL-15-WSX-1 + sushi-LIFR, IL-15-WSX-1 + sushi-OSMR, IL-15-WSX-1 + sushi-GPL or (B) IL-15-LIFR, IL-15-LIFR + sushi-WSX-, IL-15-LIFR + sushi-LIFR, IL-15-LIFR + sushi-OSMR, IL-15-LIFR + sushi-GPL or (C) IL-15-OSMR, IL-15-OSMR + sushi-WSX-1, IL-15-OSMR + sushi-LIFR, IL-15-OSMR + sushi-OSMR or (D) IL-15-GPL, IL-15-GPL + sushi-WSX-1, IL-15-GPL + sushi-LIFR, IL-15-GPL + sushi-GPL and IL-15-GPL + sushi-gp130 were cultured for 3 days in the absence of Hyper-IL-6. Proliferation was measured as indicated in material & methods.

4.12 Second generation of IL-15/IL-15R α -sushi based heterodimerization system – avoiding signaling capacity of IL-15 and expression of two open reading frames from one cDNA: FUSIO

In order to improve the IL-15/IL-15R α -sushi based heterodimerization system a new cDNA coding for human IL-15/murine WSX-1 and human IL-15R α -sushi/murine gp130 was generated. The cDNA sequence of human IL-15 was mutated at two distinct sites, which lead to two amino acid changes at D8S and Q108S resulting in a IL-2R β and IL-2R γ c binding deficiency, respectively [184]. Moreover, both cDNAs coding for mutated IL-15-WSX-1 and sushi-gp130 were combined in one transcript, separated by a furin cleavage site and a 2A peptide. The 2A peptide from the foot-and-mouth disease virus and other members of the picornavirus family is a self processing sequence (D(V/I)EXNPGP) to achieve expression of at least two separate proteins from one single open reading frame [185, 186]. It is believed that the reaction driven by 2A is a translational recoding event in which a peptide bond is 'skipped' during elongation [187]. The 2A peptide reaction occurs in the peptidyltransferase center of the ribosome. Ribosomes pause at the end of the 2A coding sequence, at the glycine and proline codons, and the nascent chain up to and including this glycine is released. Thereafter the translation continues leading to generation of the separate downstream product [188]. The 2A peptide technology has been used for expression of two proteins from one open reading frame [189-193].

A



B

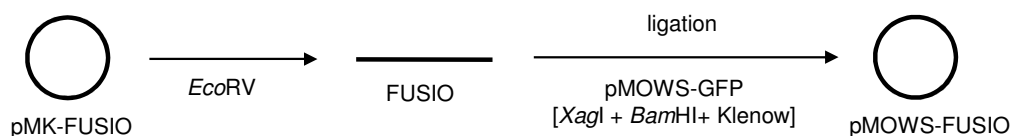


Figure 4.29 Schematic overview of FUSIO cDNA (A) and cloning into pMOWS plasmid (B). See text for details.

The resulting cDNA was named FUSIO. As depicted in Figure 4.29A FUSIO cDNA consist of the signal peptide from bovine preprolactin, a FLAG-tagged human IL-15 mutant, N-terminally truncated murine WSX-1, furin cleavage site, 2A peptid, signal peptide of human gp130 Myc-tagged human IL-15R α -sushi and N-terminally truncated murine gp130. The cDNA was synthesized by Geneart (Regensburg, Germany) and delivered in a pMk plasmid. The pMk-FUSIO plasmid was digested with *EcoRV*. The target plasmid pMOWS was digested with *XagI* and *BamHI* and blunt ends were generated by Klenow fragment reaction. Plasmid and FUSIO were purified and subcloned. The resulting plasmid was named pMOWS-FUSIO (Figure 4.29 B).

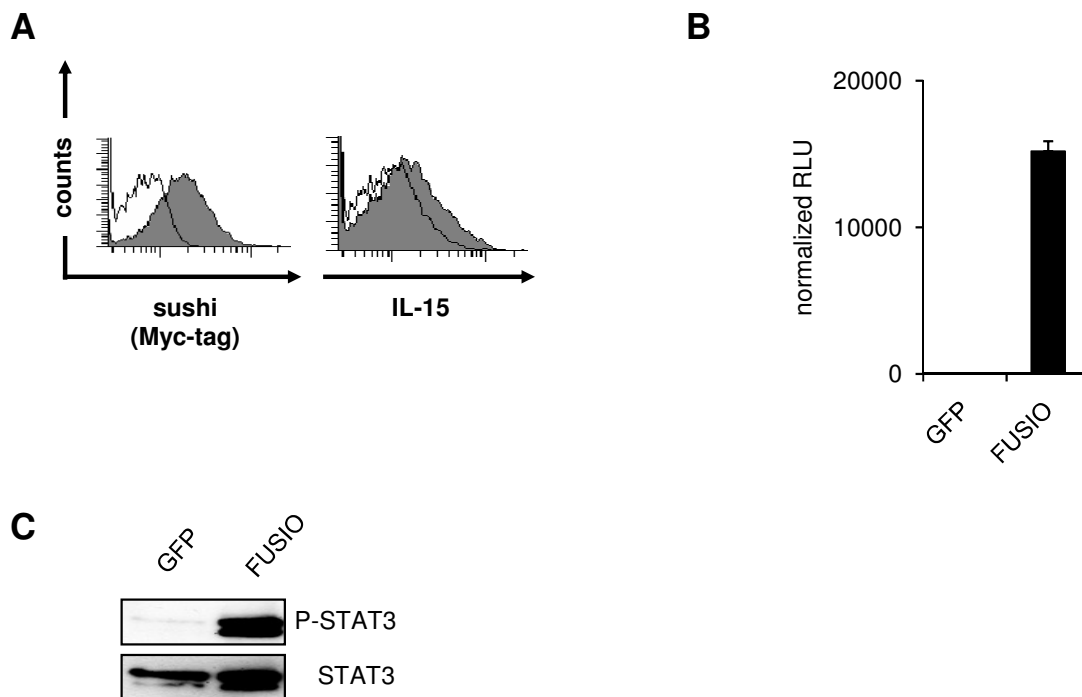


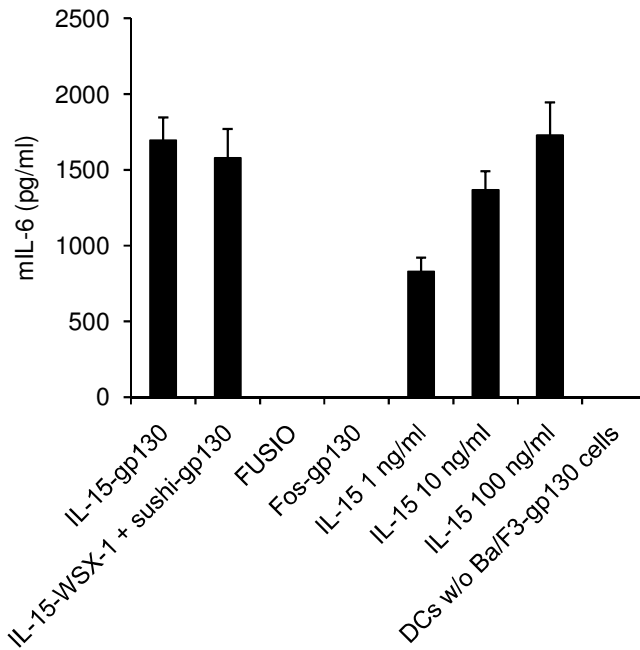
Figure 4.30 Functionality of FUSIO. (A) Cell surface expression of gp130 and WSX-1 chimeric receptor encoded by FUSIO in stably transduced Ba/F3-gp130 cells analyzed by flow cytometry. The cell lines were labeled as described in material & methods. Untransduced Ba/F3-gp130 cells were used as negative controls (unfilled histograms). (B) Equal numbers of Ba/F3-gp130 cells stably transduced with GFP or FUSIO were cultured for 3 days in the absence of Hyper-IL-6. Proliferation was measured as indicated in material & methods. (D) Activation of STAT-3 proteins in Ba/F3-gp130 cells stably transduced with GFP or FUSIO. After 6 h of serum starvation Ba/F3-gp130 cells stably transduced with GFP or FUSIO were left untreated. Subsequently, cells were lysed and 50 μ g were separated by SDS/PAGE. Proteins were transferred onto a PVDF membrane and were detected with anti-P-STAT3 antibodies and visualized by ECL detection. The membrane was stripped and probed with anti-STAT3 antibodies and proteins were visualized by ECL detection.

Ba/F3-gp130 cells were stably transduced with the cDNA encoding FUSIO or GFP as a control. As shown in Figure 4.30A both chimeric receptor chains were expressed at the cell surface of stably transduced Ba/F3-gp130 cells. Furthermore, Ba/F3-gp130 cells expressing FUSIO also exhibited cytokine independent growth and phosphorylation of STAT3, whereas control cells expressing GFP were not able to proliferate in a cytokine independent manner and showed no phosphorylation of STAT3 (Figure 4.30B and C), demonstrating that the 2A peptide technology is applicable for our approach.

Since it was not known whether the presentation of IL-15 chimeric receptors at the cell surface led to an induction of IL-15-mediated signaling in neighbouring cells, a co-culture experiment with Ba/F3-gp130 cells expressing IL-15-gp130, IL-15-WSX-1 + sushi-gp130, FUSIO or Fos-gp130 and primary murine dendritic cells was performed. Primary murine dendritic cells were described to produce IL-6 after stimulation with IL-15 [194]. This approach was used to prove if the two point mutations of IL-15 used for the FUSIO construct lead to an abrogation of IL-15 mediated signaling. The co-culture experiment revealed that Ba/F3-gp130 cells expressing IL-15-gp130 or IL-15-WSX-1 + sushi-gp130 but not FUSIO or Fos-gp130 induced a strong induction of IL-6 expression, indicating that the membrane located wildtype IL-15 protein was biologically active and induced IL-15 trans-presentation mediated signaling. Remarkably, Ba/F3-gp130-FUSIO cells, which expressed the IL-15 mutant, which cannot bind to the β - and γ -receptor subunits of IL-15 signaling receptor complex, failed to induce IL-6 protein expression by trans-presentation of IL-15 (Figure 4.31).

This further development of the IL-15/IL-15R α -sushi based heterodimerization system is the first step towards a potential analysis of *in vivo* consequences of cytokine independent, constitutively active heterodimeric receptor complexes of the gp130-type receptor family.

It can be concluded from all experiments that the application of the IL-15/IL-15R α -sushi based system is useful for the generation of ligand-independent, constitutively active heterodimers of all known gp130-type receptor complexes leading to long-term activation of associated intracellular gp130-type receptor signaling pathways and target gene activation.



4.31 Co-culture experiment of primary murine dendritic cells with Ba/F3-gp130 cell lines expressing different chimeric receptors and quantification of IL-15 mediated murine IL-6 secretion. Primary murine dendritic cells were stimulated with LPS (10 ng/ml) overnight. The day after murine DCs were washed and co-cultured with Ba/F-gp130 cells expressing either IL-15-gp130, IL-15-WSX-1 + sushi-gp130, FUSIO or Fos-gp130 in a ratio of 1:10 for 24 h. As a control murine dendritic cells were stimulated with 1, 10 and 100 ng/ml recombinant IL-15 or left untreated. After 24 h supernatants were analyzed for mIL-6 secretion. This experiment was performed by Elena Bulanova (Borstel, Germany).

5 Discussion

5.1 Establishment of a novel system to generate ligand-independent, constitutively active, heterodimeric receptor complexes.

In this work, a novel strategy to generate ligand-independent, constitutively active heterodimeric receptor complexes was described. First, replacing the extracellular domains of gp130, LIFR, OSMR, WSX-1, and GPL by either IL-15 or IL-15R α -sushi domain is sufficient to heterodimerize gp130 with LIFR, OSMR, and WSX-1 as well as OSMR with GPL. As a consequence of forced dimerization leading to sustained STAT1 and/or STAT3 and ERK1/2 phosphorylation and transcriptional activation of the STAT3 target genes *c-myc* and *Pim-1*. Those functional combinations of the chimeric receptors reflected ligand-independent activation of the full-length receptor complexes. gp130/LIFR is engaged by LIF, OSM, CNTF, CT-1, CLC, and NP, gp130/OSMR is engaged by OSM, gp130/WSX-1 is engaged by IL-27, OSMR/GPL is engaged by IL-31. Furthermore, additional combinations of gp130-type receptor chimeras were obtained. These included LIFR/OSMR, gp130/GPL, LIFR/GPL heterodimers as well as LIFR/LIFR and GPL/GPL homodimers which have not been described so far to occur naturally. Introduction of functional combinations of the chimeric receptor proteins into Hyper-IL-6-dependent Ba/F3-gp130 pre B-cells confers sustained and long-term ligand-independent growth of these cells suggesting permanent activation of the heterodimeric receptor complexes.

Exploiting the higher stability of an IL-15/IL-15R α -sushi domain heterodimer when compared to IL-15 homodimers alone, we demonstrate that soluble IL-15R α -sushi domain cannot be used to destabilize heterodimeric IL-15/IL-15R α -sushi domain complexes. This was not the case for homodimeric IL-15-gp130 complexes. Soluble IL-15R α -sushi domain interfered with the constitutive signaling activity of IL-15-gp130 but not with IL-15/IL-15R α -sushi domain complexes, again demonstrating the exceptionally high affinity between the IL-15 receptor chimera and the IL-15R α -sushi receptor chimera. These findings indicate that once formed IL-15/IL-15R α -sushi chimeric receptor complexes could not be dissociated by soluble IL-15R α -sushi domain. Interestingly, IL-15 was recently described as a cytokine that can only leave the cell in complex with its α -receptor [161].

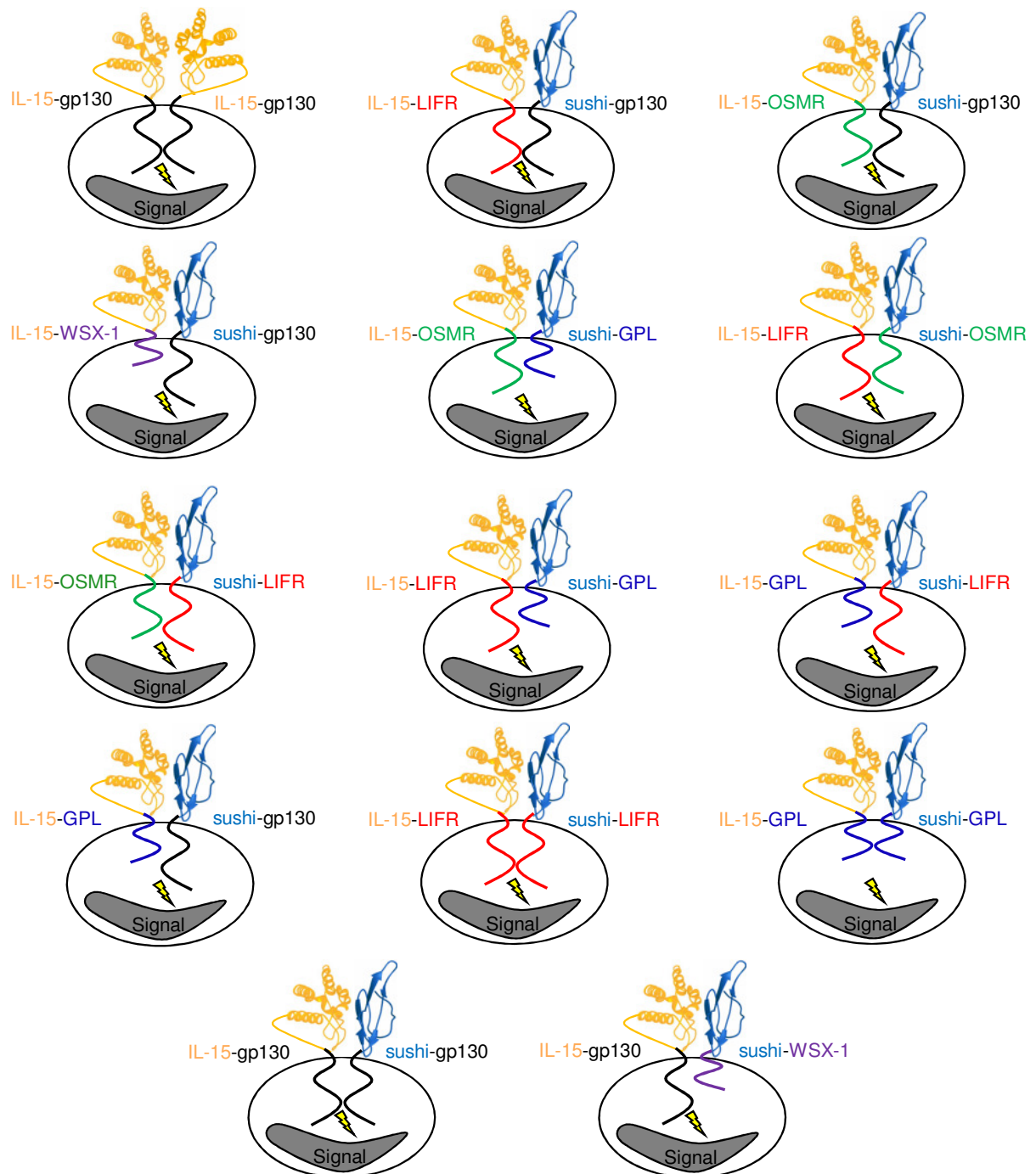


Figure 5.1 Scheme of all functional combinations of ligand-independent gp130-type receptor complexes in Ba/F3-gp130 cells achieved by the IL-15/IL-15R α -sushi based system. These combinations comprise gp130/gp130, gp130/LIFR, gp130/OSMR, gp130/WSX-1, OSMR/GPL, LIFR/OSMR, LIFR/GPL, gp130/GPL, LIFR/LIFR, and GPL/GPL. The combinations of IL-15-gp130 + sushi-gp130 and IL-15-gp130 + sushi-WSX-1 are also displayed here because it could be shown that high molecular access of soluble IL-15R α -sushi did not interfere with the complex of those receptors and the proliferation of Ba/F3-gp130 cells expressing those combinations did not change (Figure 4.19).

	IL-15-gp130	IL-15-LIFR	IL-15-OSMR	IL-15-WSX-1	IL-15-GPL
none	active	inactive	inactive	inactive	inactive
sushi-gp130	active	active	active	active	active
sushi-LIFR	n.d.	active	active	inactive	active
sushi-OSMR	n.d.	active	inactive	inactive	n.d. [†]
sushi-WSX-1	active	inactive	inactive	inactive	no
sushi-GPL	n.d.	active	active	no	active

Table 1.2 List of all functional combinations of ligand-independent gp130-type receptor complexes in Ba/F3-gp130 cells achieved by the IL-15/IL-15R α -sushi based system. These combinations comprises gp130/gp130, gp130/LIFR, gp130/OSMR, gp130/WSX-1, OSMR/GPL, LIFR/OSMR, LIFR/GPL, gp130/GPL, LIFR/LIFR, and GPL/GPL. abbreviations: n.d.: not determined; *: all combinations with IL-15-gp130 are supposed to grow ligand-independent because IL-15-gp130 is active on its own and all active combinations were tested with the sushi-gp130 variant; †: the combination of IL-15-GPL + sushi-OSMR was not achieved because IL-15-OSMR + sushi-GPL has been shown to be functionally active.

Here, surface expression of IL-15 was detected in the absence of IL-15R α (e.g. IL-15-gp130), demonstrating that IL-15 fused to a truncated gp130 can also be transported to the cell surface, and that the driving force in our experimental setting of IL-15 secretion is not its specific association with IL-15R α but its fusion or association with a membrane receptor.

5.2 Dimerization and activation vs. dimerization and non-activation

The approach to heterodimerize gp130-type receptor complexes with the help of the IL-15/IL-15R α -sushi domain is the first successful strategy to confer constitutive heterodimeric gp130-type receptor activation in the complete absence of any extracellular stimulation. Our findings add support to the current view that all IL-6-type cytokines induce conformational changes within the extracellular domains of heterodimeric gp130 receptor complexes that are necessary for optimal alignment and binding of the amino acids in the heterodimerization domain. Finally, this rearrangement stabilizes the heterodimerized receptor in a conformation that holds gp130-type receptor associated JAK kinases in close proximity thereby facilitating signaling.

In this work it was shown that the fusion of IL-15 to N-terminally truncated gp130 was sufficient to induce ligand-independent signaling. Importantly, fusion of IL-15R α -sushi domain to gp130 did not induced ligand-independent signaling even when either IL-

15-gp130 or sushi-gp130 could be detected as homodimers by co-immunoprecipitation. Furthermore, either IL-15-gp130 or sushi-gp130 showed dimerization with Δ cys-Fos-gp130, which was unexpected. There is increasing evidence that many cytokine receptors appear to exist as inactive, preassembled complexes on the cellular surface. This has been demonstrated for erythropoietin receptor [195-198], growth hormone receptor [199] interferon γ receptor [200], IL-3R β_c [201], IL-17R [202], IL-6R [203], and also for gp130 as well as the gp130/LIFR heterodimer [168, 177]. These data implied that ligand binding induces a local reorganization within the receptor complex, thereby triggering intracellular signaling. In the light of these data it seems likely that sushi-gp130 dimerizes without activation in contrast to IL-15-gp130 because IL-15 has a tendency to dimerize or oligomerize ([176] and Inken Lorenzen, personal communication). However, the current model of preformed dimerization of gp130 is that the extracellular domain D1 of one gp130 molecule interacts with the D2 and D3 domain of the other gp130 molecule. This model has been evolved using data obtained by purification of soluble gp130 D1-D6 which only could be purified as a dimer. Interestingly, deletion of the D1 domain resulted in the purification of monomeric gp130 D2-D6 (Stefan Rose-John, personal communication). It is known that the D1 domain (Ig-like domain) plays an important role in ligand recognition. IL-6 binds initially the CHD of its α -receptor via site I, the CHD (D2 and D3) of gp130 via its site II and the D1 (Ig-like domain) of the second gp130 to form a hexameric signaling complex consisting of two IL-6, two IL-6R α , and two gp130 molecules [21]. Interestingly, another report from our laboratory regarding the naturally occurring, ligand-independent, and constitutively active gp130 variants identified by Rebouissou *et al.* [135] showed also an important role for the D1 domain of gp130. Deletion of the D1 domain resulted in an abrogation of ligand-independent gp130-mediated signaling, suggesting that this domain is involved in dimerization, even if no physical interaction analysis has proved this so far (Timo Effenberger and Jan Sommer, personal communication). However, there were no extracellular domains of gp130 which could be involved in this preformed dimerization of sushi-gp130 in our experimental setting which formally did not exclude that preformed dimer formation is mediated by the extracellular domains *in vivo*. Here, one might suggest that preformed dimerization occurs via the transmembrane domain or intracellular domain. This has been assumed by studying the physical interaction between sushi-gp130 and Δ cys-Fos-gp130 as well as IL-15-gp130 and Δ cys-Fos-

gp130. It was not expected that these chimeric proteins interacted with each other. In this study, the interaction between gp130 molecules lacking the complete extracellular portion could not be addressed directly, since a completely extracellular truncated gp130 molecule was not transported to the cell surface [136]. It is a preferred option that gp130 forms preformed dimerization via its transmembrane domain. This has been assumed because of two studies showing preformed dimerization of the erythropoietin receptor which was mediated by the transmembrane domain [195, 198]. This view is supported by the work of Sternberg and Gullick which shows that a sequence motif in the transmembrane domain of certain growth factor receptors with tyrosine activity mediated dimerization [204]. This sequence motif encompasses a five residue segment in which position 0 (P0) requires a small side chain (Gly, Ala, Ser, Thr or Pro), P3 an aliphatic side chain (Ala, Val, Leu, Ile) and P4 only the smallest side chains (Gly or Ala) [204]. Interestingly, gp130 matches those criteria, even though gp130 does not belong to the group of receptor tyrosine kinases. The results regarding sushi-gp130 imply that a functional active gp130-type receptor dimer needs to be positioned not only in close proximity to allow for full activation of the receptor, but also to be aligned in a specific manner [53].

NH₃-AIVV*PVCL*AFLLTLLGVLF^{CF}-COOH

Figure 5.2 Assumed dimerization motif of the transmembrane domain of gp130 using the sequence motif found in many growth factor receptors with tyrosine activity by Sternberg and Gullick [204]. The sequence motif encompasses a five residue segment (italic) which starts with position P0 (red - small side chain), P3 (green - aliphatic side chain) and P3 (blue - smallest side chain).

Another interesting finding of this study is that gp130 was the only molecule which could be activated by IL-15 alone with respect to the onset of signaling. IL-15-WSX-1, IL-15-LIFR, IL-15-OSMR, and IL-15-GPL did not show any activation, although homodimerization of LIFR or GPL could be achieved by co-expression of e.g. IL-15-LIFR + sushi-LIFR. Because of this, functional dimerization of gp130 achieved by IL-15 must be considered as special. If a receptor has the capability to form homodimers, even if they can only be achieved by artificial dimerization, it is not clear why functional homodimerization of e.g. LIFR cannot be achieved by IL-15. A

possible explanation for this is the principle that gp130 is the only member of the gp130-type receptors which exhibits homodimers *in vivo*. This might explain why IL-15-gp130 does show activation of the receptor chains and the other IL-15 chimeric receptors do not. Contrary to this, another study from our laboratory revealed that IL-23R which is one component of the IL-23 signaling complex and belongs to the group two of class I cytokine receptor like gp130 also showed ligand-independent and constitutive activation if fused to IL-15 (Doreen Floss, personal communication). It is not known if IL-23R homodimerizes *in vivo*. Also, IL-23R does not belong to the cytokine receptors which are shared by many cytokines like IL-2R γ_c , IL-3R β_c or gp130 [46]. IL-23R and the IL-12R β_1 form the receptor complex for IL-23 (Table 1.1). Furthermore the IL-12R β_1 is also utilized by IL-12 to form the receptor complex of IL-12 with the IL-12R β_2 (Table 1.1). Interestingly, fusion of IL-15 to IL-12R β_1 did not lead to functional activation of this chimeric receptor (Doreen Floss, personal communication). It remains an open question which receptors are capable to induce ligand-independent and constitutive activation after fusion to IL-15 and how functional dimerization is achieved by IL-15 in contrast to IL-15 and IL-15R α -sushi.

5.3 Applications of the IL-15/IL-15R α -sushi based system

5.3.1 *In vitro* applications

It was successfully shown that the IL-15/IL-15R α -sushi based system was applicable to all gp130-type receptor complexes. It would be interesting to investigate the application of this system to other heterodimeric receptor complexes of the class I cytokine receptor family comprising all complexes listed in Table 1.1 to see if ligand-independent and constitutive activation is also achieved. Furthermore, heterodimeric receptor complexes from other superfamilies such as class II cytokine receptors [8], interleukin-1 (IL-1) receptors [10], tyrosine kinase receptors [11], and IL-17 receptors [14] might be targets for the application of the IL-15/IL-15R α -sushi based system.

The IL-15/IL-15R α -sushi based system can be used to study differences of ligand-dependent and ligand-independent signaling over a long period of time. Prolonged ligand-dependent signaling by IL-6-like cytokine is highly regulated and leads to attenuation of the phosphorylation of e.g. STAT3. This is achieved for instance by a negative-feedback loop inhibitors like SOCS3 [73]. One mechanism is the internalization of gp130-type receptor complexes after ligand binding [205]. In gp130 and LIFR dileucine-like motifs in the intracellular region have been identified to be

required for receptor mediated internalization [206, 207]. It would be interesting to investigate if the ligand-independent and constitutively active heterodimeric receptor complexes are also internalized and how this is regulated. Furthermore, another question to solve is if the constitutively active heterodimeric receptor complexes display also a constant STAT activation over time. Upregulation of SOCS3 as a direct target of STAT3 would also be an approach to analyze. A useful tool to study this is the usage of inducible expression system like the tetracycline-based systems [208].

5.3.2 Optimization of the IL-15/IL-15R α -sushi based system for future *in vivo* applications

Naturally occurring and constitutively active gp130-variants were described to play a role in inflammatory hepatocellular adenomas, which was directly caused by gain-of-function somatic mutations within the gp130 receptor chain [135]. The gain-of-function gp130 variants were active in a ligand-independent manner and led to marked activation of the IL-6 signaling pathway [135], which is highly comparable to our homo- and heterodimeric receptor complexes. Therefore, it would be of high interest to study constitutive activation of heterodimeric gp130-type receptors *in vivo*. Constitutive activation of STAT3 has been implicated in many human neoplastic malignancies including multiple myeloma [209, 210], prostate cancer, melanoma, ovarian cancer, pancreatic cancer, renal carcinoma [211], colon cancer [212, 213], breast cancer [214, 215] and gastric cancer [216-221]. Artificially dimerized, constitutively active STAT3 was shown to possess oncogenic potential and STAT3 was therefore designated as an oncogene [222]. In some of these malignancies, involvement of IL-6 or IL-11 has been shown [212, 213, 216-221]. ERK1/2 activities also contribute to different cancer diseases, in which Ras mutations have been found or not [223-228]. It is not unlikely that in such malignancies, constitutive activation of homodimeric gp130 receptor complex or a heterodimeric gp130/WSX-1, gp130/LIFR, gp130/OSMR or GPL/OSMR receptor complex lead to the activation of STAT3 and ERK1/2. This study might be the starting point to study the oncogenic potential of permanently activated heterodimeric gp130 receptor complexes resulting in constitutively activated STATs and ERK1/2 *in vivo*. Moreover, constitutively active heterodimeric gp130-type receptor complexes would provide novel molecular tools to establish animal models of diseases in which activated STATs and/or ERK1/2 are believed to play a role. There are two potential advantages of using models based on

ligand-independent, constitutive heterodimeric gp130-type receptor complexes rather than to rely on a constitutively active STAT3 mutant or to transgenic mice over-expressing the respective cytokines. First, activation of STAT3 does not reflect the activation pattern after heterodimeric gp130-type receptor activation since the Ras/Raf/MAPK pathway and the PI3K pathway is not activated. Secondly, systemic cytokine supply does not reflect situations in which chronic activation of the receptors is achieved only in certain tissues or areas of the body. Our receptor chimeras could now be used to induce gp130-type receptor signaling in a cell autonomous manner and if combined with tissue specific promoters in tissue specific transgenic mouse models. The use of the receptor chimeras therefore will provide novel insights into the consequences of uncontrolled activation of heterodimeric gp130-type receptor complexes and the resulting signaling cascades *in vivo*.

To avoid IL-15 mediated-responses of our receptor chimeras *in vivo*, we have used an IL-15 mutant, which can not bind to the IL-2R β - and IL-2R γ_c -receptor chains of the IL-15 receptor signaling complex, but was still able to bind the IL-15R α -sushi domain to induce cytokine independent dimerization of the chimeric receptor proteins [184]. Therefore, our constitutively active heterodimeric receptor molecules in combination with the 2A peptide technology [189-193] may be used to generate animal models to assess the consequences of constitutive pathophysiologic activation of gp130-type heterodimeric receptor complexes at a cell autonomous level.

To achieve this, the IL-15/IL-15R α -sushi based system needs to be further improved. IL-15 and the IL-15R α are expressed by many cell types like monocytes [229], macrophages [230], dendritic cells [160, 231], mast cells [232] other undefined hematopoietic cells, T-cells [233], parenchymal cells [158, 234], fibroblasts [235, 236], renal tubular epithelial cells [237, 238], endothelial cells [239], hepatocytes [240], and adipocytes [241]. Furthermore IL-15 and IL-15R α are widely expressed in the central nervous system of mice and human, including the frontal and parietal cortex, hippocampus, medulla oblongata, thalamus and cerebellum [242, 243].

It has been shown that human IL-15 can interact with murine IL-15R α and murine IL-15 with human IL-15R α with similar binding kinetics [244]. Expression of the chimeric receptors in cells, expressing endogenous IL-15 and IL-15R α , could interfere with normal signaling of IL-15 and complex formation of the chimeric heterodimeric receptors when meeting in the ER or Golgi apparatus. Since the binding affinities of IL-15 to its receptor is approximately 40-times higher than to the sushi domain of the

IL-15R α (Table 1.2), binding of e.g. IL-15-WSX-1 to murine IL-15R α would be preferred. Otherwise, as tested with increasing amounts of soluble IL-15R α -sushi domain, once formed complexes of e.g. IL-15-WSX-1 + sushi-gp130 could not be destroyed. This effect could be explained by the assumption that receptor fused IL-15 displays a higher affinity to its receptor fused IL-15R α -sushi domain and *vice versa*. It remains as an open question to what extent the affinity of IL-15 to its receptor is increased by fusion of IL-15 to a receptor (e.g. IL-15-WSX-1) and if binding of IL-15-WSX-1 to sushi-gp130 or endogenous IL-15R α is preferred. Besides, endogenous IL-15R α and sushi-gp130 would compete, in turn, for the binding of endogenous IL-15. Therefore, there are four major problems of *in vivo* usage of chimeric receptors in cells expressing endogenous IL-15 and IL-15R α .

First, endogenous murine IL-15 might bind to sushi-gp130. This endogenous murine IL-15 would have the capacity to mediate signaling because it would be presented at the cell surface, the negative regulation of IL-15 mediated signaling could be impaired. One regulatory mechanism to circumvent increased signaling by the endogenous IL-15/IL-15R α complex is shedding of the IL-15R α by the protease ADAM17 [162, 163]. The complex of IL-15/sIL-15R α is generated by cleavage exhibits antagonistic properties [162, 163].

Second, IL-15-WSX-1 might bind to the murine IL-15R α and as a consequence lead to a decreased endogenous IL-15 signaling because endogenous IL-15 cannot bind to its receptor and it is not presented the cell surface. As written above, IL-15 might bind to sushi-gp130 but in this context, it needs to be clarified whether expression levels of IL-15-WSX-1 and sushi-gp130, encoded by the FUSIO construct, are comparable. If IL-15-WSX-1 is expressed e.g. 1.1-fold higher than sushi-gp130 would lead to free IL-15-WSX-1 proteins which in turn would interact with the endogenous IL-15R α . Therefore, endogenous IL-15 and IL-15-WSX-1 would compete for the binding to sushi-gp130 and endogenous IL-15 biology would be impaired.

Third, the endogenous IL-15 and IL-15R α can interfere with the complex formation of chimeric heterodimers. Especially, this effect would take place in a situation of increased IL-15/IL-15R α expression leading to an abrogation of signaling by e.g. IL-15-WSX-1 + sushi-gp130.

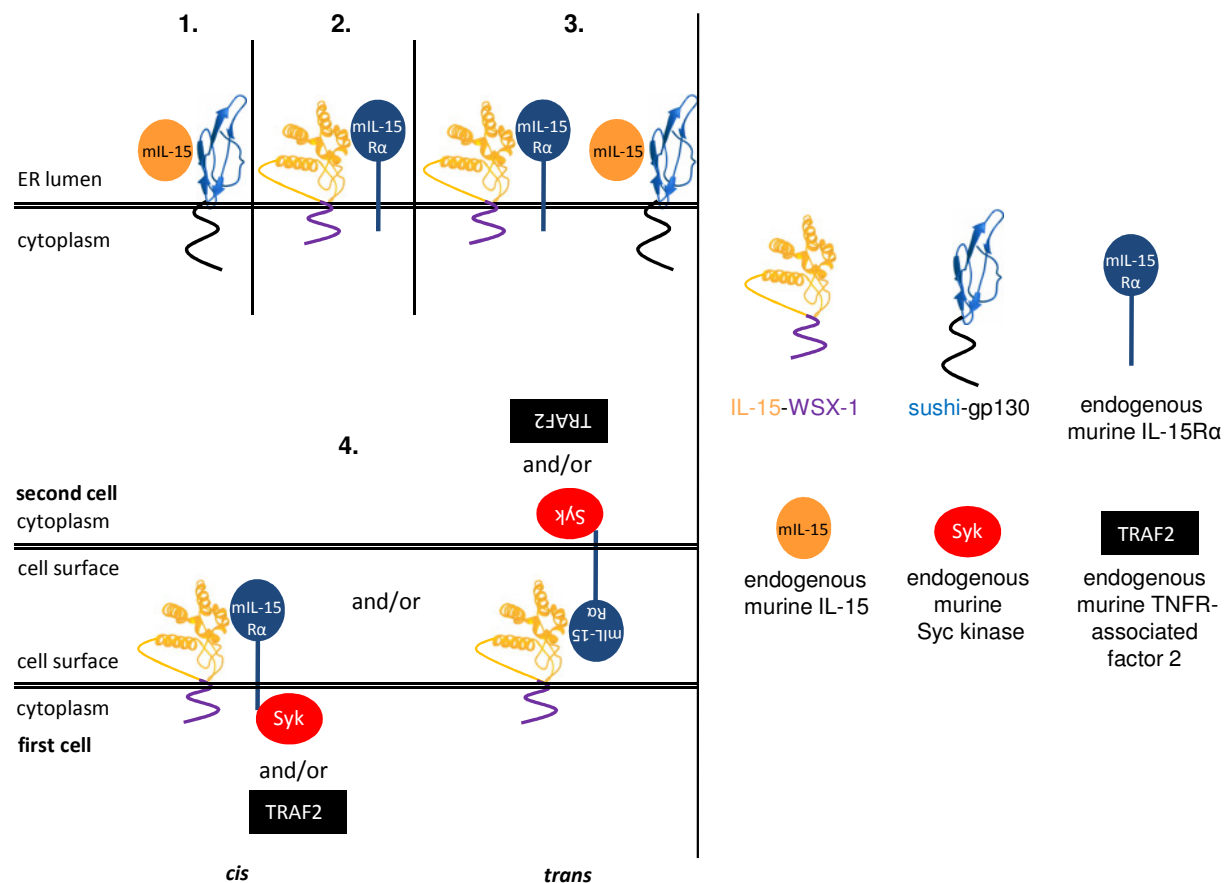


Figure 5.3 Problems of *in vivo* usage of chimeric receptors in cells expressing endogenous IL-15 and IL-15R α . (1.) No negative regulation of IL-15 signaling by shedding. (2.) Impairment of IL-15 signaling. (3.) Interference with chimeric heterodimer complex formation. (4.) Unwanted signaling mediated by IL-15R α in *cis* or *trans*. See text for further details.

Fourth, a study of Bulanova and coworkers suggested that the IL-15R α chain alone is capable of mediating a signal upon activation by IL-15 through selective association with Syk kinase in human B cells [149]. This could also be true for murine B cells. Furthermore, other data demonstrated that IL-15 is capable of rescuing murine L929 fibroblasts from TNF α -induced apoptosis through recruitment of TNFR-associated factor 2 (TRAF2) to the IL-15R α chain, and NF- κ B activation [245]. Expression of chimeric receptors (e.g. IL-15-WSX-1) in those or neighboring cells would lead to unwanted signaling events mediated by IL-15R α .

These suboptimal requirements for an *in vivo* model of constitutively active heterodimeric gp130-type receptor complexes can be avoided by rational redesign of the IL-15/IL-15R α -sushi complex. The binding interface of IL-15 could be transferred to IL-15R α -sushi and *vice versa*. The transfer of functional sites has been shown in

our group previously [165]. Transfer of the LIFR binding site from CNTF to IL-6 resulted in an IL-6/CNTF chimera which can signal via a complex of IL-6R α /gp130/LIFR [165]. Using molecular modeling techniques it was also possible to identify the residues of vIL-6 which are essential for direct binding to gp130. This has been shown using IL-6/vIL-6 chimeras [246]. Furthermore, transfer of the CNTFR α binding site of CLC to CNTF, which exhibits also binding to the IL-6R α at high concentrations, improved the specificity of CNTF with respect to CNTFR α binding [247].

A minor problem could be the aggregation potential of IL-15. Since nobody knows which residues are essential for this effect and if this effect is caused by over-expression or has a physiological role, only random mutagenesis of IL-15 could lead to a non-aggregating IL-15 molecule. However, in our system aggregation of IL-15 led to functional dimerization of the respective gp130 chimera. This effect could be diminished by addition of soluble IL-15R α -sushi domain. *In vivo* this could mean that even aggregated IL-15 is recruited to IL-15R α as a monomer. Solving the crystal structure of the IL-15/IL-15R α complex revealed that IL-15 binds to its α -receptor in a 1:1 stoichiometry resulting in a quaternary complex of IL-15/IL-15R β /IL-2R β /IL-2R γ_c [248].

To transfer these *in vitro* data into an *in vivo* situation, a useful tool to generate mouse models is the generation of conditional knock-in mice. This can be achieved by homologous recombination of the transgene into the *ROSA26* locus of mice. Knockout of the *ROSA26* locus has been shown to have no impact on development or physiology of the mice and lead to ubiquitous expression of an introduced β -galactosidase [249]. Combining the integration into the *ROSA26* locus with tissue-specific Cre recombinase/loxP inducible systems has been used for the analysis of skeletal-specific expression of vascular endothelial growth factor (VEGF) [250] or dendritic cell-specific expression of the diphtheria toxin [251].

Several reports suggested that LIF signaling promotes proliferation in different cancers such as prostate [252, 253] or skin [254]. Furthermore, the expression of LIFR, OSMR, and gp130 was highly increased in infiltrative breast carcinomas suggesting a highly active autocrine effect for LIF and OSM in the epithelial compartment of infiltrative breast carcinoma [255]. A Genome-wide gene copy number and expression analysis of primary gastric tumors and gastric cancer cell lines revealed that OSMR expression can be used as a prognostic marker [256].

Further studies are however required to decipher its biological significance in gastric cancer initiation and progression. Interestingly, IL-27 possesses a potent anti-tumor activity which has been shown by analysis of a murine tumor models comprising colon carcinoma [257], neuroblastoma [258] or melanoma [259]. The anti-tumor activity of IL-27 was mediated through CD8 T-cells, NK cells, or its own anti-angiogenic activity [257-259]. Especially in this context it would be interesting to analyze a constitutively active gp130/WSX-1 complex expressed in healthy tissues to clarify whether gp130/WSX-1 mediated signaling would never be involved in the onset of cancer.

In summary, the usage of tissue-specific expression of ligand-independent, constitutively active gp130/LIFR, gp130/OSMR, or gp130/WSX-1 complexes would give insight into the role of their signaling in initiation and progression of cancerogenesis or other hyperplastic disorders.

6 Summary

The IL-6 family of cytokines consists of IL-6, vIL-6, IL-11, IL-27, CNTF, CT-1, LIF, CLC, NP, OSM, and IL-31. IL-6, vIL-6, IL-11 induce the formation of a gp130-homodimer, whereas signaling by CNTF, CT-1, LIF, CLC, and NP results in the formation of a gp130/LIFR heterodimer. OSM can induce the formation of a dimer of gp130 with LIFR or the related protein OSMR. IL-27 exclusively signals via a heterodimer comprising gp130 and WSX-1, whereas IL-31 induces the formation of an OSMR/GPL heterodimer. Interestingly, naturally occurring, ligand-independent, and constitutively active gp130 variants were described to play a role in the onset of inflammatory hepatocellular adenomas. In the past, constitutively active gp130 variants based on homodimerization of Jun leucine zippers were generated in this laboratory. However, using a Fos leucine zipper-gp130 (Fos-gp130) chimera and a Jun leucine zipper-WSX-1 chimera (Jun-WSX-1), Fos and Jun form the AP-1 transcription factor complex, revealed that also Fos-gp130 alone was able to induce ligand-independent STAT3 phosphorylation and ligand-independent growth of cytokine-dependent cells indicating that this approach was not suitable for the generation of ligand-independent, constitutive activation of heterodimeric receptors. Therefore a novel IL-15/IL-15R α based system to generate ligand-independent, constitutively active heterodimeric complexes for all known gp130-receptor members was developed. In this system, IL-15 was genetically fused to one receptor component whereas IL-15R α was fused to the second receptor component. Ligand-independent heterodimerization of gp130 with WSX-1, LIFR and OSMR and of OSMR with GPL led to constitutive, ligand-independent STAT1 and/or STAT3 and ERK1/2 phosphorylation. Moreover, these receptor combinations induced transcription of the STAT3 target genes c-myc and Pim-1 upon stable transfection and conferred to independent growth of cytokine-dependent cells. The IL-15/IL-15R α system is established as a novel system to mimic constitutive and ligand-independent activation of homo- and heterodimeric receptor complexes, which could also be applicable to other heterodimeric cytokine receptor and growth factor receptor families. Additionally, in an improved approach a mutated IL-15 protein was used, which was still able to bind the IL-15R α -sushi domain, but not to IL-2R β and IL-2R γ_c receptor chains. In combination with the 2A peptide technology, this is the first step towards to translation of the *in vitro* data to analyse the tumorigenic potential of gp130-heterodimeric receptor complexes *in vivo*.

7 Zusammenfassung

Die IL-6 Zytokinfamilie besteht aus IL-6, vIL-6, IL-11, IL-27, CNTF, CT-1, LIF, CLC, NP, OSM und IL-31. IL-6, vIL-6 und IL-11 induzieren die Bildung eines gp130 Homodimers, wohingegen die Signalwirkung von CNTF, CT-1, LIF, CLC und NP zur Bildung eines gp130/LIFR Heterodimers führen. OSM induziert die Bildung eines Dimers aus gp130 und LIFR oder dem verwandten Protein OSMR. IL-27 betreibt ausschließlich Signalwirkung durch ein Heterodimer bestehend aus gp130 und WSX-1, wohingegen IL-31 die Bildung eines OSMR/GPL Heterodimers induziert. Interessanterweise wurden natürlich vorkommende, Liganden-unabhängige und konstitutiv aktive gp130 Varianten beschrieben, welche eine Rolle bei der Entstehung von entzündlichen hepatozellulären Adenomen spielen. In der Vergangenheit wurde in dieser Arbeitsgruppe eine Liganden-unabhängige und konstitutiv aktive gp130 Variante, auf der Homodimerisierung von Jun „Leucinzippern“ basierend, hergestellt. Allerdings, unter Verwendung einer Fos „Leucinzipper“ gp130 Chimäre (Fos-gp130) und einer Jun „Leucinzipper“ WSX-1 Chimäre (Jun-WSX-1), Fos und Jun bilden den AP-1 Transkriptionsfaktorkomplex, stellte sich heraus, dass Fos-gp130 bereits alleine in der Lage ist eine Liganden-unabhängige STAT3-Phosphorylierung und unabhängiges Wachstum von Zytokin-abhängigen Zellen zu induzieren. Dieser Ansatz konnte daher nicht für die Herstellung von Liganden-unabhängigen, konstitutiv aktiven heterodimeren Rezeptorkomplexen verwendet werden. Daher wurde ein neues auf IL-15/IL-15R α -sushi basierendes System zur Herstellung von Liganden-unabhängigen und konstitutiv aktiven Heterodimeren aller gp130 ähnlichen Rezeptoren entwickelt. In diesem System wurde eine Rezeptorkomponente mit IL-15, die andere mit der sushi Domäne des IL-15R α , fusioniert. Liganden-unabhängige Heterodimerisierung von gp130 mit WSX-1, LIFR und OSMR bzw. OSMR mit GPL führte zur konstitutiven, liganden-unabhängigen STAT1- und/oder STAT3- und ERK1/2-Phosphorylierung. Darüber hinaus führten diese Rezeptorkombinationen zur Induktion der Transkription der STAT3 Zielgene c-myc und Pim-1 und zu unabhängigem Wachstum von Zytokin-abhängigen Zellen. Die Etablierung des neuartigen IL-15/IL-15R α -sushi basierenden Systems, zur Nachahmung von konstitutiver, Liganden-unabhängiger Aktivierung homo- oder heterodimerer Rezeptorkomplexe, kann wohlmöglich auch auf andere heterodimere Zytokinrezeptor- oder Wachstumsfaktorrezeptor-Familien anwendbar sein. Zusätzlich wurde in einem verbesserten Ansatz ein mutiertes IL-15 Protein verwendet, welches zwar noch an die IL-15R α -sushi Domäne, allerdings nicht mehr an die IL-2R β und IL-2R γ_c Rezeptoren, binden kann. Kombiniert mit der 2A Peptid Technology, ist dies der

erste Schritt die *in vitro* Daten anzuwenden, um das tumorigene Potential von gp130 heterodimeren Rezeptorkomplexen *in vivo* zu analysieren.

8 References

1. Paul, W.E. and R.A. Seder, *Lymphocyte responses and cytokines*. Cell, 1994. **76**(2): p. 241-51.
2. Thomson, A.W. and M.T. Lotze, *The cytokine handbook*. 4th ed. 2003, Amsterdam ; Boston: Academic Press.
3. Nicola, N.A. and D.J. Hilton, *General classes and functions of four-helix bundle cytokines*. Adv Protein Chem, 1998. **52**: p. 1-65.
4. Idriss, H.T. and J.H. Naismith, *TNF alpha and the TNF receptor superfamily: structure-function relationship(s)*. Microsc Res Tech, 2000. **50**(3): p. 184-95.
5. Sun, P.D. and D.R. Davies, *The cystine-knot growth-factor superfamily*. Annu Rev Biophys Biomol Struct, 1995. **24**: p. 269-91.
6. Murzin, A.G., A.M. Lesk, and C. Chothia, *beta-Trefoil fold. Patterns of structure and sequence in the Kunitz inhibitors interleukins-1 beta and 1 alpha and fibroblast growth factors*. J Mol Biol, 1992. **223**(2): p. 531-43.
7. Boulay, J.L., J.J. O'Shea, and W.E. Paul, *Molecular phylogeny within type I cytokines and their cognate receptors*. Immunity, 2003. **19**(2): p. 159-63.
8. Langer, J.A., E.C. Cutrone, and S. Kotenko, *The Class II cytokine receptor (CRF2) family: overview and patterns of receptor-ligand interactions*. Cytokine Growth Factor Rev, 2004. **15**(1): p. 33-48.
9. Zhang, G., *Tumor necrosis factor family ligand-receptor binding*. Curr Opin Struct Biol, 2004. **14**(2): p. 154-60.
10. Daun, J.M. and M.J. Fenton, *Interleukin-1/Toll receptor family members: receptor structure and signal transduction pathways*. J Interferon Cytokine Res, 2000. **20**(10): p. 843-55.
11. Hubbard, S.R., *Structural analysis of receptor tyrosine kinases*. Prog Biophys Mol Biol, 1999. **71**(3-4): p. 343-58.
12. Massague, J., *Receptors for the TGF-beta family*. Cell, 1992. **69**(7): p. 1067-70.
13. Allen, S.J., S.E. Crown, and T.M. Handel, *Chemokine: receptor structure, interactions, and antagonism*. Annu Rev Immunol, 2007. **25**: p. 787-820.
14. Gaffen, S.L., *Structure and signalling in the IL-17 receptor family*. Nat Rev Immunol, 2009. **9**(8): p. 556-67.
15. Ihle, J.N., *Cytokine receptor signalling*. Nature, 1995. **377**(6550): p. 591-4.
16. Bazan, J.F., *Structural design and molecular evolution of a cytokine receptor superfamily*. Proc Natl Acad Sci U S A, 1990. **87**(18): p. 6934-8.
17. Mackiewicz, A., M. Wiznerowicz, E. Roeb, J. Nowak, T. Pawlowski, H. Baumann, P.C. Heinrich, and S. Rose-John, *Interleukin-6-type cytokines and their receptors for gene therapy of melanoma*. Ann N Y Acad Sci, 1995. **762**: p. 361-73; discussion 373-4.
18. Bagley, C.J., J.M. Woodcock, F.C. Stomski, and A.F. Lopez, *The structural and functional basis of cytokine receptor activation: lessons from the common beta subunit of the granulocyte-macrophage colony-stimulating factor, interleukin-3 (IL-3), and IL-5 receptors*. Blood, 1997. **89**(5): p. 1471-82.
19. de Vos, A.M., M. Ultsch, and A.A. Kossiakoff, *Human growth hormone and extracellular domain of its receptor: crystal structure of the complex*. Science, 1992. **255**(5042): p. 306-12.
20. Chow, D., X. He, A.L. Snow, S. Rose-John, and K.C. Garcia, *Structure of an extracellular gp130 cytokine receptor signaling complex*. Science, 2001. **291**(5511): p. 2150-5.
21. Boulanger, M.J., D.C. Chow, E.E. Brevnova, and K.C. Garcia, *Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex*. Science, 2003. **300**(5628): p. 2101-4.
22. Taga, T. and T. Kishimoto, *Gp130 and the interleukin-6 family of cytokines*. Annu Rev Immunol, 1997. **15**: p. 797-819.

23. Liongue, C. and A.C. Ward, *Evolution of Class I cytokine receptors*. BMC Evol Biol, 2007. **7**: p. 120.
24. Haan, C., S. Kreis, C. Margue, and I. Behrmann, *Jaks and cytokine receptors--an intimate relationship*. Biochem Pharmacol, 2006. **72**(11): p. 1538-46.
25. Levy, D.E. and J.E. Darnell, Jr., *Stats: transcriptional control and biological impact*. Nat Rev Mol Cell Biol, 2002. **3**(9): p. 651-62.
26. Heinrich, P.C., I. Behrmann, S. Haan, H.M. Hermanns, G. Muller-Newen, and F. Schaper, *Principles of interleukin (IL)-6-type cytokine signalling and its regulation*. Biochem J, 2003. **374**(Pt 1): p. 1-20.
27. Pflanz, S., L. Hibbert, J. Mattson, R. Rosales, E. Vaisberg, J.F. Bazan, J.H. Phillips, T.K. McClanahan, R. de Waal Malefyt, and R.A. Kastelein, *WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27*. J Immunol, 2004. **172**(4): p. 2225-31.
28. Molden, J., Y. Chang, Y. You, P.S. Moore, and M.A. Goldsmith, *A Kaposi's sarcoma-associated herpesvirus-encoded cytokine homolog (vIL-6) activates signaling through the shared gp130 receptor subunit*. J Biol Chem, 1997. **272**(31): p. 19625-31.
29. Derouet, D., F. Rousseau, F. Alfonsi, J. Froger, J. Hermann, F. Barbier, D. Perret, C. Diveu, C. Guillet, L. Preisser, A. Dumont, M. Barbado, A. Morel, O. deLapeyriere, H. Gascan, and S. Chevalier, *Neuropoietin, a new IL-6-related cytokine signaling through the ciliary neurotrophic factor receptor*. Proc Natl Acad Sci U S A, 2004. **101**(14): p. 4827-32.
30. Dillon, S.R., C. Sprecher, A. Hammond, J. Bilsborough, M. Rosenfeld-Franklin, S.R. Presnell, H.S. Haugen, M. Maurer, B. Harder, J. Johnston, S. Bort, S. Mudri, J.L. Kuijper, T. Bukowski, P. Shea, D.L. Dong, M. Dasovich, F.J. Grant, L. Lockwood, S.D. Levin, C. LeCiel, K. Waggle, H. Day, S. Topouzis, J. Kramer, R. Kuestner, Z. Chen, D. Foster, J. Parrish-Novak, and J.A. Gross, *Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice*. Nat Immunol, 2004. **5**(7): p. 752-60.
31. Taga, T., M. Hibi, Y. Hirata, K. Yamasaki, K. Yasukawa, T. Matsuda, T. Hirano, and T. Kishimoto, *Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130*. Cell, 1989. **58**(3): p. 573-81.
32. Hilton, D.J., A.A. Hilton, A. Raicevic, S. Rakar, M. Harrison-Smith, N.M. Gough, C.G. Begley, D. Metcalf, N.A. Nicola, and T.A. Willson, *Cloning of a murine IL-11 receptor alpha-chain; requirement for gp130 for high affinity binding and signal transduction*. Embo J, 1994. **13**(20): p. 4765-75.
33. Gearing, D.P., M.R. Comeau, D.J. Friend, S.D. Gimpel, C.J. Thut, J. McGourty, K.K. Brasher, J.A. King, S. Gillis, B. Mosley, and et al., *The IL-6 signal transducer, gp130: an oncostatin M receptor and affinity converter for the LIF receptor*. Science, 1992. **255**(5050): p. 1434-7.
34. Davis, S., T.H. Aldrich, D.M. Valenzuela, V.V. Wong, M.E. Furth, S.P. Squinto, and G.D. Yancopoulos, *The receptor for ciliary neurotrophic factor*. Science, 1991. **253**(5015): p. 59-63.
35. Pennica, D., K.L. King, K.J. Shaw, E. Luis, J. Rullamas, S.M. Luoh, W.C. Darbonne, D.S. Knutzon, R. Yen, K.R. Chien, and et al., *Expression cloning of cardiotrophin 1, a cytokine that induces cardiac myocyte hypertrophy*. Proc Natl Acad Sci U S A, 1995. **92**(4): p. 1142-6.
36. Elson, G.C., E. Lelievre, C. Guillet, S. Chevalier, H. Plun-Favreau, J. Froger, I. Suard, A.B. de Coignac, Y. Delneste, J.Y. Bonnefoy, J.F. Gauchat, and H. Gascan, *CLF associates with CLC to form a functional heteromeric ligand for the CNTF receptor complex*. Nat Neurosci, 2000. **3**(9): p. 867-72.
37. Mosley, B., C. De Imus, D. Friend, N. Boiani, B. Thoma, L.S. Park, and D. Cosman, *Dual oncostatin M (OSM) receptors. Cloning and characterization of an alternative signaling subunit conferring OSM-specific receptor activation*. J Biol Chem, 1996. **271**(51): p. 32635-43.
38. Perret, D., C. Guillet, G. Elson, J. Froger, H. Plun-Favreau, F. Rousseau, M. Chabbert, J.F. Gauchat, and H. Gascan, *Two different contact sites are recruited by*

- cardiotrophin-like cytokine (CLC) to generate the CLC/CLF and CLC/sCNTFRalpha composite cytokines.* J Biol Chem, 2004. **279**(42): p. 43961-70.
39. Rose-John, S., M. Ehlers, J. Grotzinger, and J. Mullberg, *The soluble interleukin-6 receptor.* Ann N Y Acad Sci, 1995. **762**: p. 207-20; discussion 220-1.
 40. Plun-Favreau, H., G. Elson, M. Chabbert, J. Froger, O. deLapeyriere, E. Lelievre, C. Guillet, J. Hermann, J.F. Gauchat, H. Gascan, and S. Chevalier, *The ciliary neurotrophic factor receptor alpha component induces the secretion of and is required for functional responses to cardiotrophin-like cytokine.* Embo J, 2001. **20**(7): p. 1692-703.
 41. Grotzinger, J., *Molecular mechanisms of cytokine receptor activation.* Biochim Biophys Acta, 2002. **1592**(3): p. 215-23.
 42. Huyton, T., J.G. Zhang, C.S. Luo, M.Z. Lou, D.J. Hilton, N.A. Nicola, and T.P. Garrett, *An unusual cytokine:Ig-domain interaction revealed in the crystal structure of leukemia inhibitory factor (LIF) in complex with the LIF receptor.* Proc Natl Acad Sci U S A, 2007. **104**(31): p. 12737-42.
 43. Skiniotis, G., P.J. Lupardus, M. Martick, T. Walz, and K.C. Garcia, *Structural organization of a full-length gp130/LIF-R cytokine receptor transmembrane complex.* Mol Cell, 2008. **31**(5): p. 737-48.
 44. Boulanger, M.J., A.J. Bankovich, T. Kortemme, D. Baker, and K.C. Garcia, *Convergent mechanisms for recognition of divergent cytokines by the shared signaling receptor gp130.* Mol Cell, 2003. **12**(3): p. 577-89.
 45. McDonald, N.Q., N. Panayotatos, and W.A. Hendrickson, *Crystal structure of dimeric human ciliary neurotrophic factor determined by MAD phasing.* Embo J, 1995. **14**(12): p. 2689-99.
 46. Wang, X., P. Lupardus, S.L. Laporte, and K.C. Garcia, *Structural biology of shared cytokine receptors.* Annu Rev Immunol, 2009. **27**: p. 29-60.
 47. Xu, G.Y., H.A. Yu, J. Hong, M. Stahl, T. McDonagh, L.E. Kay, and D.A. Cumming, *Solution structure of recombinant human interleukin-6.* J Mol Biol, 1997. **268**(2): p. 468-81.
 48. Kurth, I., U. Horsten, S. Pflanz, A. Timmermann, A. Kuster, H. Dahmen, I. Tacke, P.C. Heinrich, and G. Muller-Newen, *Importance of the membrane-proximal extracellular domains for activation of the signal transducer glycoprotein 130.* J Immunol, 2000. **164**(1): p. 273-82.
 49. Skiniotis, G., M.J. Boulanger, K.C. Garcia, and T. Walz, *Signaling conformations of the tall cytokine receptor gp130 when in complex with IL-6 and IL-6 receptor.* Nat Struct Mol Biol, 2005. **12**(6): p. 545-51.
 50. Matadeen, R., W.C. Hon, J.K. Heath, E.Y. Jones, and S. Fuller, *The dynamics of signal triggering in a gp130-receptor complex.* Structure, 2007. **15**(4): p. 441-8.
 51. Xu, Y., N.J. Kershaw, C.S. Luo, P. Soo, M.J. Pocock, P.E. Czabotar, D.J. Hilton, N.A. Nicola, T.P. Garrett, and J.G. Zhang, *Crystal structure of the entire ectodomain of GP130: insights into the molecular assembly of the tall cytokine receptor complexes.* J Biol Chem.
 52. Timmermann, A., A. Kuster, I. Kurth, P.C. Heinrich, and G. Muller-Newen, *A functional role of the membrane-proximal extracellular domains of the signal transducer gp130 in heterodimerization with the leukemia inhibitory factor receptor.* Eur J Biochem, 2002. **269**(11): p. 2716-26.
 53. Greiser, J.S., C. Stross, P.C. Heinrich, I. Behrmann, and H.M. Hermanns, *Orientational constraints of the gp130 intracellular juxtamembrane domain for signaling.* J Biol Chem, 2002. **277**(30): p. 26959-65.
 54. Luttkien, C., U.M. Wegenka, J. Yuan, J. Buschmann, C. Schindler, A. Ziemiecki, A.G. Harpur, A.F. Wilks, K. Yasukawa, T. Taga, and et al., *Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130.* Science, 1994. **263**(5143): p. 89-92.
 55. Stahl, N., T.G. Boulton, T. Farruggella, N.Y. Ip, S. Davis, B.A. Witthuhn, F.W. Quelle, O. Silvennoinen, G. Barbieri, S. Pellegrini, and et al., *Association and activation of*

- Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components.* Science, 1994. **263**(5143): p. 92-5.
56. Radtke, S., H.M. Hermanns, C. Haan, H. Schmitz-Van De Leur, H. Gascan, P.C. Heinrich, and I. Behrmann, *Novel role of Janus kinase 1 in the regulation of oncostatin M receptor surface expression.* J Biol Chem, 2002. **277**(13): p. 11297-305.
 57. Takeda, A., S. Hamano, A. Yamanaka, T. Hanada, T. Ishibashi, T.W. Mak, A. Yoshimura, and H. Yoshida, *Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment.* J Immunol, 2003. **170**(10): p. 4886-90.
 58. Dreuw, A., S. Radtke, S. Pflanz, B.E. Lippok, P.C. Heinrich, and H.M. Hermanns, *Characterization of the signaling capacities of the novel gp130-like cytokine receptor.* J Biol Chem, 2004. **279**(34): p. 36112-20.
 59. Murakami, M., M. Narazaki, M. Hibi, H. Yawata, K. Yasukawa, M. Hamaguchi, T. Taga, and T. Kishimoto, *Critical cytoplasmic region of the interleukin 6 signal transducer gp130 is conserved in the cytokine receptor family.* Proc Natl Acad Sci U S A, 1991. **88**(24): p. 11349-53.
 60. Sprecher, C.A., F.J. Grant, J.W. Baumgartner, S.R. Presnell, S.K. Schrader, T. Yamagiwa, T.E. Whitmore, P.J. O'Hara, and D.F. Foster, *Cloning and characterization of a novel class I cytokine receptor.* Biochem Biophys Res Commun, 1998. **246**(1): p. 82-90.
 61. Diveu, C., E. Lelievre, D. Perret, A.H. Lak-Hal, J. Froger, C. Guillet, S. Chevalier, F. Rousseau, A. Wesa, L. Preisser, M. Chabbert, J.F. Gauchat, A. Galy, H. Gascan, and A. Morel, *GPL, a novel cytokine receptor related to GP130 and leukemia inhibitory factor receptor.* J Biol Chem, 2003. **278**(50): p. 49850-9.
 62. Gerhartz, C., B. Heesel, J. Sasse, U. Hemmann, C. Landgraf, J. Schneider-Mergener, F. Horn, P.C. Heinrich, and L. Graeve, *Differential activation of acute phase response factor/STAT3 and STAT1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130. I. Definition of a novel phosphotyrosine motif mediating STAT1 activation.* J Biol Chem, 1996. **271**(22): p. 12991-8.
 63. Stahl, N., T.J. Farruggella, T.G. Boulton, Z. Zhong, J.E. Darnell, Jr., and G.D. Yancopoulos, *Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors.* Science, 1995. **267**(5202): p. 1349-53.
 64. Hermanns, H.M., S. Radtke, C. Haan, H. Schmitz-Van de Leur, J. Tavernier, P.C. Heinrich, and I. Behrmann, *Contributions of leukemia inhibitory factor receptor and oncostatin M receptor to signal transduction in heterodimeric complexes with glycoprotein 130.* J Immunol, 1999. **163**(12): p. 6651-8.
 65. Kuropatwinski, K.K., C. De Imus, D. Gearing, H. Baumann, and B. Mosley, *Influence of subunit combinations on signaling by receptors for oncostatin M, leukemia inhibitory factor, and interleukin-6.* J Biol Chem, 1997. **272**(24): p. 15135-44.
 66. Wang, Y., O. Robledo, E. Kinzie, F. Blanchard, C. Richards, A. Miyajima, and H. Baumann, *Receptor subunit-specific action of oncostatin M in hepatic cells and its modulation by leukemia inhibitory factor.* J Biol Chem, 2000. **275**(33): p. 25273-85.
 67. Fujitani, Y., M. Hibi, T. Fukada, M. Takahashi-Tezuka, H. Yoshida, T. Yamaguchi, K. Sugiyama, Y. Yamanaka, K. Nakajima, and T. Hirano, *An alternative pathway for STAT activation that is mediated by the direct interaction between JAK and STAT.* Oncogene, 1997. **14**(7): p. 751-61.
 68. Hintzen, C., C. Evers, B.E. Lippok, R. Volkmer, P.C. Heinrich, S. Radtke, and H.M. Hermanns, *Box 2 region of the oncostatin M receptor determines specificity for recruitment of Janus kinases and STAT5 activation.* J Biol Chem, 2008. **283**(28): p. 19465-77.
 69. Sekimoto, T., N. Imamoto, K. Nakajima, T. Hirano, and Y. Yoneda, *Extracellular signal-dependent nuclear import of Stat1 is mediated by nuclear pore-targeting complex formation with NPI-1, but not Rch1.* Embo J, 1997. **16**(23): p. 7067-77.
 70. Wegenka, U.M., J. Buschmann, C. Luttkien, P.C. Heinrich, and F. Horn, *Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is*

- rapidly activated by interleukin-6 at the posttranslational level.* Mol Cell Biol, 1993. **13**(1): p. 276-88.
71. Kiuchi, N., K. Nakajima, M. Ichiba, T. Fukada, M. Narimatsu, K. Mizuno, M. Hibi, and T. Hirano, *STAT3 is required for the gp130-mediated full activation of the c-myc gene.* J Exp Med, 1999. **189**(1): p. 63-73.
72. Shirogane, T., T. Fukada, J.M. Muller, D.T. Shima, M. Hibi, and T. Hirano, *Synergistic roles for Pim-1 and c-Myc in STAT3-mediated cell cycle progression and antiapoptosis.* Immunity, 1999. **11**(6): p. 709-19.
73. Naka, T., M. Narazaki, M. Hirata, T. Matsumoto, S. Minamoto, A. Aono, N. Nishimoto, T. Kajita, T. Taga, K. Yoshizaki, S. Akira, and T. Kishimoto, *Structure and function of a new STAT-induced STAT inhibitor.* Nature, 1997. **387**(6636): p. 924-9.
74. Blanchard, F., Y. Wang, E. Kinzie, L. Duplomb, A. Godard, and H. Baumann, *Oncostatin M regulates the synthesis and turnover of gp130, leukemia inhibitory factor receptor alpha, and oncostatin M receptor beta by distinct mechanisms.* J Biol Chem, 2001. **276**(50): p. 47038-45.
75. Magrangeas, F., O. Boisteau, S. Denis, Y. Jacques, and S. Minvielle, *Negative cross-talk between interleukin-3 and interleukin-11 is mediated by suppressor of cytokine signalling-3 (SOCS-3).* Biochem J, 2001. **353**(Pt 2): p. 223-30.
76. Schmitz, J., M. Weissenbach, S. Haan, P.C. Heinrich, and F. Schaper, *SOCS3 exerts its inhibitory function on interleukin-6 signal transduction through the SHP2 recruitment site of gp130.* J Biol Chem, 2000. **275**(17): p. 12848-56.
77. Nicholson, S.E., D. De Souza, L.J. Fabri, J. Corbin, T.A. Willson, J.G. Zhang, A. Silva, M. Asimakis, A. Farley, A.D. Nash, D. Metcalf, D.J. Hilton, N.A. Nicola, and M. Baca, *Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130.* Proc Natl Acad Sci U S A, 2000. **97**(12): p. 6493-8.
78. Haspel, R.L., M. Salditt-Georgieff, and J.E. Darnell, Jr., *The rapid inactivation of nuclear tyrosine phosphorylated Stat1 depends upon a protein tyrosine phosphatase.* Embo J, 1996. **15**(22): p. 6262-8.
79. Haspel, R.L. and J.E. Darnell, Jr., *A nuclear protein tyrosine phosphatase is required for the inactivation of Stat1.* Proc Natl Acad Sci U S A, 1999. **96**(18): p. 10188-93.
80. Liu, B., J. Liao, X. Rao, S.A. Kushner, C.D. Chung, D.D. Chang, and K. Shuai, *Inhibition of Stat1-mediated gene activation by PIAS1.* Proc Natl Acad Sci U S A, 1998. **95**(18): p. 10626-31.
81. Chung, C.D., J. Liao, B. Liu, X. Rao, P. Jay, P. Berta, and K. Shuai, *Specific inhibition of Stat3 signal transduction by PIAS3.* Science, 1997. **278**(5344): p. 1803-5.
82. Schiemann, W.P., J.L. Bartoe, and N.M. Nathanson, *Box 3-independent signaling mechanisms are involved in leukemia inhibitory factor receptor alpha- and gp130-mediated stimulation of mitogen-activated protein kinase. Evidence for participation of multiple signaling pathways which converge at Ras.* J Biol Chem, 1997. **272**(26): p. 16631-6.
83. Takahashi-Tezuka, M., Y. Yoshida, T. Fukada, T. Ohtani, Y. Yamanaka, K. Nishida, K. Nakajima, M. Hibi, and T. Hirano, *Gab1 acts as an adapter molecule linking the cytokine receptor gp130 to ERK mitogen-activated protein kinase.* Mol Cell Biol, 1998. **18**(7): p. 4109-17.
84. Eulenfeld, R. and F. Schaper, *A new mechanism for the regulation of Gab1 recruitment to the plasma membrane.* J Cell Sci, 2009. **122**(Pt 1): p. 55-64.
85. Hideshima, T., N. Nakamura, D. Chauhan, and K.C. Anderson, *Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma.* Oncogene, 2001. **20**(42): p. 5991-6000.
86. Hermanns, H.M., S. Radtke, F. Schaper, P.C. Heinrich, and I. Behrmann, *Non-redundant signal transduction of interleukin-6-type cytokines. The adapter protein Shc is specifically recruited to the oncostatin M receptor.* J Biol Chem, 2000. **275**(52): p. 40742-8.
87. Bode, J.G., S. Ludwig, C.A. Freitas, F. Schaper, M. Ruhl, S. Melmed, P.C. Heinrich, and D. Haussinger, *The MKK6/p38 mitogen-activated protein kinase pathway is*

- capable of inducing SOCS3 gene expression and inhibits IL-6-induced transcription. *Biol Chem*, 2001. **382**(10): p. 1447-53.
88. Burfoot, M.S., N.C. Rogers, D. Watling, J.M. Smith, S. Pons, G. Paonessaw, S. Pellegrini, M.F. White, and I.M. Kerr, *Janus kinase-dependent activation of insulin receptor substrate 1 in response to interleukin-4, oncostatin M, and the interferons*. *J Biol Chem*, 1997. **272**(39): p. 24183-90.
89. Kohn, A.D., F. Takeuchi, and R.A. Roth, *Akt, a pleckstrin homology domain containing kinase, is activated primarily by phosphorylation*. *J Biol Chem*, 1996. **271**(36): p. 21920-6.
90. Benigni, F., G. Fantuzzi, S. Sacco, M. Sironi, P. Pozzi, C.A. Dinarello, J.D. Sipe, V. Poli, M. Cappelletti, G. Paonessa, D. Pennica, N. Panayotatos, and P. Ghezzi, *Six different cytokines that share GP130 as a receptor subunit, induce serum amyloid A and potentiate the induction of interleukin-6 and the activation of the hypothalamus-pituitary-adrenal axis by interleukin-1*. *Blood*, 1996. **87**(5): p. 1851-4.
91. Moshage, H., *Cytokines and the hepatic acute phase response*. *J Pathol*, 1997. **181**(3): p. 257-66.
92. Ramadori, G. and B. Christ, *Cytokines and the hepatic acute-phase response*. *Semin Liver Dis*, 1999. **19**(2): p. 141-55.
93. Kishimoto, T., T. Hirano, H. Kikutani, and A. Muraguchi, *Delineation of human B cell differentiation: immunological and molecular characterization of human B cell differentiation factor (BSF-2)*. *Adv Exp Med Biol*, 1987. **213**: p. 177-88.
94. Muraguchi, A., T. Hirano, B. Tang, T. Matsuda, Y. Horii, K. Nakajima, and T. Kishimoto, *The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells*. *J Exp Med*, 1988. **167**(2): p. 332-44.
95. Chomarat, P., J. Banchereau, J. Davoust, and A.K. Palucka, *IL-6 switches the differentiation of monocytes from dendritic cells to macrophages*. *Nat Immunol*, 2000. **1**(6): p. 510-4.
96. Ishibashi, T., H. Kimura, T. Uchida, S. Kariyone, P. Friese, and S.A. Burstein, *Human interleukin 6 is a direct promoter of maturation of megakaryocytes in vitro*. *Proc Natl Acad Sci U S A*, 1989. **86**(15): p. 5953-7.
97. Okada, M., M. Kitahara, S. Kishimoto, T. Matsuda, T. Hirano, and T. Kishimoto, *IL-6/BSF-2 functions as a killer helper factor in the in vitro induction of cytotoxic T cells*. *J Immunol*, 1988. **141**(5): p. 1543-9.
98. Nurieva, R.I., Y. Chung, D. Hwang, X.O. Yang, H.S. Kang, L. Ma, Y.H. Wang, S.S. Watowich, A.M. Jetten, Q. Tian, and C. Dong, *Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages*. *Immunity*, 2008. **29**(1): p. 138-49.
99. Eddahri, F., S. Denanglaire, F. Bureau, R. Spolski, W.J. Leonard, O. Leo, and F. Andris, *Interleukin-6/STAT3 signaling regulates the ability of naive T cells to acquire B-cell help capacities*. *Blood*, 2009. **113**(11): p. 2426-33.
100. Hirota, K., B. Martin, and M. Veldhoen, *Development, regulation and functional capacities of Th17 cells*. *Semin Immunopathol*. **32**(1): p. 3-16.
101. Chen, Q., D.T. Fisher, K.A. Clancy, J.M. Gauguet, W.C. Wang, E. Unger, S. Rose-John, U.H. von Andrian, H. Baumann, and S.S. Evans, *Fever-range thermal stress promotes lymphocyte trafficking across high endothelial venules via an interleukin 6 trans-signaling mechanism*. *Nat Immunol*, 2006. **7**(12): p. 1299-308.
102. Rabe, B., A. Chalaris, U. May, G.H. Waetzig, D. Seegert, A.S. Williams, S.A. Jones, S. Rose-John, and J. Scheller, *Transgenic blockade of interleukin 6 transsignaling abrogates inflammation*. *Blood*, 2008. **111**(3): p. 1021-8.
103. Chalaris, A., B. Rabe, K. Paliga, H. Lange, T. Laskay, C.A. Fielding, S.A. Jones, S. Rose-John, and J. Scheller, *Apoptosis is a natural stimulus of IL6R shedding and contributes to the proinflammatory trans-signaling function of neutrophils*. *Blood*, 2007. **110**(6): p. 1748-55.
104. Drucker, C., J. Gewiese, S. Malchow, J. Scheller, and S. Rose-John, *Impact of interleukin-6 classic- and trans-signaling on liver damage and regeneration*. *J Autoimmun*. **34**(1): p. 29-37.

105. Teramura, M., S. Kobayashi, S. Hoshino, K. Oshimi, and H. Mizoguchi, *Interleukin-11 enhances human megakaryocytopoiesis in vitro*. *Blood*, 1992. **79**(2): p. 327-31.
106. Broudy, V.C., N.L. Lin, and K. Kaushansky, *Thrombopoietin (c-mpl ligand) acts synergistically with erythropoietin, stem cell factor, and interleukin-11 to enhance murine megakaryocyte colony growth and increases megakaryocyte ploidy in vitro*. *Blood*, 1995. **85**(7): p. 1719-26.
107. Elias, J.A., T. Zheng, O. Einarsson, M. Landry, T. Trow, N. Rebert, and J. Panuska, *Epithelial interleukin-11. Regulation by cytokines, respiratory syncytial virus, and retinoic acid*. *J Biol Chem*, 1994. **269**(35): p. 22261-8.
108. Booth, C. and C.S. Potten, *Effects of IL-11 on the growth of intestinal epithelial cells in vitro*. *Cell Prolif*, 1995. **28**(11): p. 581-94.
109. Bilinski, P., D. Roopenian, and A. Gossler, *Maternal IL-11R α function is required for normal decidua and fetoplacental development in mice*. *Genes Dev*, 1998. **12**(14): p. 2234-43.
110. Metcalf, D., D. Hilton, and N.A. Nicola, *Leukemia inhibitory factor can potentiate murine megakaryocyte production in vitro*. *Blood*, 1991. **77**(10): p. 2150-3.
111. Stewart, C.L., P. Kaspar, L.J. Brunet, H. Bhatt, I. Gadi, F. Kontgen, and S.J. Abbondanzo, *Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor*. *Nature*, 1992. **359**(6390): p. 76-9.
112. Williams, R.L., D.J. Hilton, S. Pease, T.A. Willson, C.L. Stewart, D.P. Gearing, E.F. Wagner, D. Metcalf, N.A. Nicola, and N.M. Gough, *Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells*. *Nature*, 1988. **336**(6200): p. 684-7.
113. Murphy, M., K. Reid, D.J. Hilton, and P.F. Bartlett, *Generation of sensory neurons is stimulated by leukemia inhibitory factor*. *Proc Natl Acad Sci U S A*, 1991. **88**(8): p. 3498-501.
114. Butzkueven, H., J.G. Zhang, M. Soilu-Hanninen, H. Hochrein, F. Chionh, K.A. Shipham, B. Emery, A.M. Turnley, S. Petratos, M. Ernst, P.F. Bartlett, and T.J. Kilpatrick, *LIF receptor signaling limits immune-mediated demyelination by enhancing oligodendrocyte survival*. *Nat Med*, 2002. **8**(6): p. 613-9.
115. Wallace, P.M., J.F. MacMaster, J.R. Rillema, J. Peng, S.A. Burstein, and M. Shoyab, *Thrombocytopoietic properties of oncostatin M*. *Blood*, 1995. **86**(4): p. 1310-5.
116. Tanaka, M., Y. Hirabayashi, T. Sekiguchi, T. Inoue, M. Katsuki, and A. Miyajima, *Targeted disruption of oncostatin M receptor results in altered hematopoiesis*. *Blood*, 2003. **102**(9): p. 3154-62.
117. Nakamura, K., H. Nonaka, H. Saito, M. Tanaka, and A. Miyajima, *Hepatocyte proliferation and tissue remodeling is impaired after liver injury in oncostatin M receptor knockout mice*. *Hepatology*, 2004. **39**(3): p. 635-44.
118. Morikawa, Y., S. Tamura, K. Minehata, P.J. Donovan, A. Miyajima, and E. Senba, *Essential function of oncostatin m in nociceptive neurons of dorsal root ganglia*. *J Neurosci*, 2004. **24**(8): p. 1941-7.
119. Hams, E., C.S. Colmont, V. Dioszeghy, V.J. Hammond, C.A. Fielding, A.S. Williams, M. Tanaka, A. Miyajima, P.R. Taylor, N. Topley, and S.A. Jones, *Oncostatin M receptor-beta signaling limits monocytic cell recruitment in acute inflammation*. *J Immunol*, 2008. **181**(3): p. 2174-80.
120. Sleeman, M.W., K.D. Anderson, P.D. Lambert, G.D. Yancopoulos, and S.J. Wiegand, *The ciliary neurotrophic factor and its receptor, CNTFR α* . *Pharm Acta Helv*, 2000. **74**(2-3): p. 265-72.
121. Masu, Y., E. Wolf, B. Holtmann, M. Sendtner, G. Brem, and H. Thoenen, *Disruption of the CNTF gene results in motor neuron degeneration*. *Nature*, 1993. **365**(6441): p. 27-32.
122. Gloaguen, I., P. Costa, A. Demartis, D. Lazzaro, A. Di Marco, R. Graziani, G. Paonessa, F. Chen, C.I. Rosenblum, L.H. Van der Ploeg, R. Cortese, G. Ciliberto, and R. Laufer, *Ciliary neurotrophic factor corrects obesity and diabetes associated with leptin deficiency and resistance*. *Proc Natl Acad Sci U S A*, 1997. **94**(12): p. 6456-61.

123. White, U.A., W.C. Stewart, R.L. Mynatt, and J.M. Stephens, *Neuropoietin attenuates adipogenesis and induces insulin resistance in adipocytes*. J Biol Chem, 2008. **283**(33): p. 22505-12.
124. Senaldi, G., M. Stolina, J. Guo, R. Faggioni, S. McCabe, S.A. Kaufman, G. Van, W. Xu, F.A. Fletcher, T. Boone, M.S. Chang, U. Sarmiento, and R.C. Cattley, *Regulatory effects of novel neurotrophin-1/b cell-stimulating factor-3 (cardiotrophin-like cytokine) on B cell function*. J Immunol, 2002. **168**(11): p. 5690-8.
125. Kraves, S. and C.J. Weitz, *A role for cardiotrophin-like cytokine in the circadian control of mammalian locomotor activity*. Nat Neurosci, 2006. **9**(2): p. 212-9.
126. Oppenheim, R.W., S. Wiese, D. Prevette, M. Armanini, S. Wang, L.J. Houenou, B. Holtmann, R. Gotz, D. Pennica, and M. Sendtner, *Cardiotrophin-1, a muscle-derived cytokine, is required for the survival of subpopulations of developing motoneurons*. J Neurosci, 2001. **21**(4): p. 1283-91.
127. Hamanaka, I., Y. Saito, T. Nishikimi, T. Magaribuchi, S. Kamitani, K. Kuwahara, M. Ishikawa, Y. Miyamoto, M. Harada, E. Ogawa, N. Kajiyama, N. Takahashi, T. Izumi, G. Shirakami, K. Mori, Y. Inobe, I. Kishimoto, I. Masuda, K. Fukuda, and K. Nakao, *Effects of cardiotrophin-1 on hemodynamics and endocrine function of the heart*. Am J Physiol Heart Circ Physiol, 2000. **279**(1): p. H388-96.
128. Kuwahara, K., Y. Saito, M. Harada, M. Ishikawa, E. Ogawa, Y. Miyamoto, I. Hamanaka, S. Kamitani, N. Kajiyama, N. Takahashi, O. Nakagawa, I. Masuda, and K. Nakao, *Involvement of cardiotrophin-1 in cardiac myocyte-nonmyocyte interactions during hypertrophy of rat cardiac myocytes in vitro*. Circulation, 1999. **100**(10): p. 1116-24.
129. Chen, Q., N. Ghilardi, H. Wang, T. Baker, M.H. Xie, A. Gurney, I.S. Grewal, and F.J. de Sauvage, *Development of Th1-type immune responses requires the type I cytokine receptor TCCR*. Nature, 2000. **407**(6806): p. 916-20.
130. Stumhofer, J.S., A. Laurence, E.H. Wilson, E. Huang, C.M. Tato, L.M. Johnson, A.V. Villarino, Q. Huang, A. Yoshimura, D. Sehy, C.J. Saris, J.J. O'Shea, L. Hennighausen, M. Ernst, and C.A. Hunter, *Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system*. Nat Immunol, 2006. **7**(9): p. 937-45.
131. Bender, H., M.Y. Wiesinger, C. Nordhoff, C. Schoenherr, C. Haan, S. Ludwig, R. Weiskirchen, N. Kato, P.C. Heinrich, and S. Haan, *Interleukin-27 displays interferon-gamma-like functions in human hepatoma cells and hepatocytes*. Hepatology, 2009. **50**(2): p. 585-91.
132. Broxmeyer, H.E., J. Li, G. Hangoc, S. Cooper, W. Tao, C. Mantel, B. Graham-Evans, N. Ghilardi, and F.J. de Sauvage, *Regulation of myeloid progenitor cell proliferation/survival by IL-31 receptor and IL-31*. Exp Hematol, 2007. **35**(4 Suppl 1): p. 78-86.
133. Perrigoue, J.G., J. Li, C. Zaph, M. Goldschmidt, P. Scott, F.J. de Sauvage, E.J. Pearce, N. Ghilardi, and D. Artis, *IL-31-IL-31R interactions negatively regulate type 2 inflammation in the lung*. J Exp Med, 2007. **204**(3): p. 481-7.
134. Perrigoue, J.G., C. Zaph, K. Guild, Y. Du, and D. Artis, *IL-31-IL-31R interactions limit the magnitude of Th2 cytokine-dependent immunity and inflammation following intestinal helminth infection*. J Immunol, 2009. **182**(10): p. 6088-94.
135. Rebouissou, S., M. Amessou, G. Couchy, K. Poussin, S. Imbeaud, C. Pilati, T. Izard, C. Balabaud, P. Bioulac-Sage, and J. Zucman-Rossi, *Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours*. Nature, 2009. **457**(7226): p. 200-4.
136. Stuhlmann-Laeisz, C., S. Lang, A. Chalaris, P. Krzysztof, S. Enge, J. Eichler, U. Klingmuller, M. Samuel, M. Ernst, S. Rose-John, and J. Scheller, *Forced dimerization of gp130 leads to constitutive STAT3 activation, cytokine-independent growth, and blockade of differentiation of embryonic stem cells*. Mol Biol Cell, 2006. **17**(7): p. 2986-95.

137. O'Shea, E.K., R. Rutkowski, W.F. Stafford, 3rd, and P.S. Kim, *Preferential heterodimer formation by isolated leucine zippers from fos and jun*. *Science*, 1989. **245**(4918): p. 646-8.
138. Patel, N., J.M. Herrman, J.C. Timans, and R.A. Kastelein, *Functional replacement of cytokine receptor extracellular domains by leucine zippers*. *J Biol Chem*, 1996. **271**(48): p. 30386-91.
139. Giri, J.G., S. Kumaki, M. Ahdieh, D.J. Friend, A. Loomis, K. Shanebeck, R. DuBose, D. Cosman, L.S. Park, and D.M. Anderson, *Identification and cloning of a novel IL-15 binding protein that is structurally related to the alpha chain of the IL-2 receptor*. *Embo J*, 1995. **14**(15): p. 3654-63.
140. Perkins, S.J., P.I. Haris, R.B. Sim, and D. Chapman, *A study of the structure of human complement component factor H by Fourier transform infrared spectroscopy and secondary structure averaging methods*. *Biochemistry*, 1988. **27**(11): p. 4004-12.
141. Clackson, T. and J.A. Wells, *A hot spot of binding energy in a hormone-receptor interface*. *Science*, 1995. **267**(5196): p. 383-6.
142. Hage, T., W. Sebald, and P. Reinemer, *Crystal structure of the interleukin-4/receptor alpha chain complex reveals a mosaic binding interface*. *Cell*, 1999. **97**(2): p. 271-81.
143. Lorenzen, I., A.J. Dingley, Y. Jacques, and J. Grotzinger, *The structure of the interleukin-15 alpha receptor and its implications for ligand binding*. *J Biol Chem*, 2006. **281**(10): p. 6642-7.
144. Giri, J.G., M. Ahdieh, J. Eisenman, K. Shanebeck, K. Grabstein, S. Kumaki, A. Namen, L.S. Park, D. Cosman, and D. Anderson, *Utilization of the beta and gamma chains of the IL-2 receptor by the novel cytokine IL-15*. *Embo J*, 1994. **13**(12): p. 2822-30.
145. Johnston, J.A., C.M. Bacon, D.S. Finbloom, R.C. Rees, D. Kaplan, K. Shibuya, J.R. Ortaldo, S. Gupta, Y.Q. Chen, J.D. Giri, and et al., *Tyrosine phosphorylation and activation of STAT5, STAT3, and Janus kinases by interleukins 2 and 15*. *Proc Natl Acad Sci U S A*, 1995. **92**(19): p. 8705-9.
146. Zhu, X., K.L. Suen, M. Barbacid, J.B. Bolen, and J. Fargnoli, *Interleukin-2-induced tyrosine phosphorylation of Shc proteins correlates with factor-dependent T cell proliferation*. *J Biol Chem*, 1994. **269**(8): p. 5518-22.
147. Miyazaki, T., Z.J. Liu, A. Kawahara, Y. Minami, K. Yamada, Y. Tsujimoto, E.L. Barsoumian, R.M. Permuter, and T. Taniguchi, *Three distinct IL-2 signaling pathways mediated by bcl-2, c-myc, and lck cooperate in hematopoietic cell proliferation*. *Cell*, 1995. **81**(2): p. 223-31.
148. Gu, H., H. Maeda, J.J. Moon, J.D. Lord, M. Yoakim, B.H. Nelson, and B.G. Neel, *New role for Shc in activation of the phosphatidylinositol 3-kinase/Akt pathway*. *Mol Cell Biol*, 2000. **20**(19): p. 7109-20.
149. Bulanova, E., V. Budagian, T. Pohl, H. Krause, H. Durkop, R. Paus, and S. Bulfone-Paus, *The IL-15R alpha chain signals through association with Syk in human B cells*. *J Immunol*, 2001. **167**(11): p. 6292-302.
150. McDonald, P.P., M.P. Russo, S. Ferrini, and M.A. Cassatella, *Interleukin-15 (IL-15) induces NF-kappaB activation and IL-8 production in human neutrophils*. *Blood*, 1998. **92**(12): p. 4828-35.
151. Liparoto, S.F., D.G. Myszka, Z. Wu, B. Goldstein, T.M. Laue, and T.L. Ciardelli, *Analysis of the role of the interleukin-2 receptor gamma chain in ligand binding*. *Biochemistry*, 2002. **41**(8): p. 2543-51.
152. Shen, B.J., T. Hage, and W. Sebald, *Global and local determinants for the kinetics of interleukin-4/interleukin-4 receptor alpha chain interaction. A biosensor study employing recombinant interleukin-4-binding protein*. *Eur J Biochem*, 1996. **240**(1): p. 252-61.
153. Weiergraber, O., U. Hemmann, A. Kuster, G. Muller-Newen, J. Schneider, S. Rose-John, P. Kurschat, J.P. Brakenhoff, M.H. Hart, S. Stabel, and et al., *Soluble human interleukin-6 receptor. Expression in insect cells, purification and characterization*. *Eur J Biochem*, 1995. **234**(2): p. 661-9.

154. Mortier, E., A. Quemener, P. Vusio, I. Lorenzen, Y. Boublik, J. Grotzinger, A. Plet, and Y. Jacques, *Soluble interleukin-15 receptor alpha (IL-15R alpha)-sushi as a selective and potent agonist of IL-15 action through IL-15R beta/gamma. Hyperagonist IL-15 x IL-15R alpha fusion proteins.* J Biol Chem, 2006. **281**(3): p. 1612-9.
155. Dubois, S., J. Mariner, T.A. Waldmann, and Y. Tagaya, *IL-15Ralpha recycles and presents IL-15 In trans to neighboring cells.* Immunity, 2002. **17**(5): p. 537-47.
156. Burkett, P.R., R. Koka, M. Chien, S. Chai, D.L. Boone, and A. Ma, *Coordinate expression and trans presentation of interleukin (IL)-15Ralpha and IL-15 supports natural killer cell and memory CD8+ T cell homeostasis.* J Exp Med, 2004. **200**(7): p. 825-34.
157. Sandau, M.M., K.S. Schluns, L. Lefrancois, and S.C. Jameson, *Cutting edge: transpresentation of IL-15 by bone marrow-derived cells necessitates expression of IL-15 and IL-15R alpha by the same cells.* J Immunol, 2004. **173**(11): p. 6537-41.
158. Schluns, K.S., E.C. Nowak, A. Cabrera-Hernandez, L. Puddington, L. Lefrancois, and H.L. Aguila, *Distinct cell types control lymphoid subset development by means of IL-15 and IL-15 receptor alpha expression.* Proc Natl Acad Sci U S A, 2004. **101**(15): p. 5616-21.
159. Giron-Michel, J., M. Giuliani, M. Fogli, D. Brouty-Boye, S. Ferrini, F. Baychelier, P. Eid, C. Lebousse-Kerdiles, D. Durali, R. Biassoni, B. Charpentier, A. Vasquez, S. Chouaib, A. Caignard, L. Moretta, and B. Azzarone, *Membrane-bound and soluble IL-15/IL-15Ralpha complexes display differential signaling and functions on human hematopoietic progenitors.* Blood, 2005. **106**(7): p. 2302-10.
160. Mortier, E., T. Woo, R. Advincula, S. Gozalo, and A. Ma, *IL-15Ralpha chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation.* J Exp Med, 2008. **205**(5): p. 1213-25.
161. Duitman, E.H., Z. Orinska, E. Bulanova, R. Paus, and S. Bulfone-Paus, *How a cytokine is chaperoned through the secretory pathway by complexing with its own receptor: lessons from interleukin-15 (IL-15)/IL-15 receptor alpha.* Mol Cell Biol, 2008. **28**(15): p. 4851-61.
162. Bulanova, E., V. Budagian, E. Duitman, Z. Orinska, H. Krause, R. Ruckert, N. Reiling, and S. Bulfone-Paus, *Soluble Interleukin IL-15Ralpha is generated by alternative splicing or proteolytic cleavage and forms functional complexes with IL-15.* J Biol Chem, 2007. **282**(18): p. 13167-79.
163. Budagian, V., E. Bulanova, Z. Orinska, A. Ludwig, S. Rose-John, P. Saftig, E.C. Borden, and S. Bulfone-Paus, *Natural soluble interleukin-15Ralpha is generated by cleavage that involves the tumor necrosis factor-alpha-converting enzyme (TACE/ADAM17).* J Biol Chem, 2004. **279**(39): p. 40368-75.
164. Bouchaud, G., L. Garrigue-Antar, V. Sole, A. Quemener, Y. Boublik, E. Mortier, H. Perdreau, Y. Jacques, and A. Plet, *The exon-3-encoded domain of IL-15Ralpha contributes to IL-15 high-affinity binding and is crucial for the IL-15 antagonistic effect of soluble IL-15Ralpha.* J Mol Biol, 2008. **382**(1): p. 1-12.
165. Kallen, K.J., J. Grotzinger, E. Lelievre, P. Vollmer, D. Aasland, C. Renne, J. Mullberg, K.H. Myer zum Buschenfelde, H. Gascan, and S. Rose-John, *Receptor recognition sites of cytokines are organized as exchangeable modules. Transfer of the leukemia inhibitory factor receptor-binding site from ciliary neurotrophic factor to interleukin-6.* J Biol Chem, 1999. **274**(17): p. 11859-67.
166. Cichy, J., S. Rose-John, and E. Pure, *Regulation of the type II oncostatin M receptor expression in lung-derived epithelial cells.* FEBS Lett, 1998. **429**(3): p. 412-6.
167. Bernard, J., C. Harb, E. Mortier, A. Quemener, R.H. Meloen, C. Vermot-Desroches, J. Wijdeness, P. van Dijken, J. Grotzinger, J.W. Slootstra, A. Plet, and Y. Jacques, *Identification of an interleukin-15alpha receptor-binding site on human interleukin-15.* J Biol Chem, 2004. **279**(23): p. 24313-22.
168. Tenhumberg, S., B. Schuster, L. Zhu, M. Kovaleva, J. Scheller, K.J. Kallen, and S. Rose-John, *gp130 dimerization in the absence of ligand: preformed cytokine receptor complexes.* Biochem Biophys Res Commun, 2006. **346**(3): p. 649-57.

169. Ketteler, R., S. Glaser, O. Sandra, U.M. Martens, and U. Klingmuller, *Enhanced transgene expression in primitive hematopoietic progenitor cells and embryonic stem cells efficiently transduced by optimized retroviral hybrid vectors*. Gene Ther, 2002. **9**(8): p. 477-87.
170. Fischer, M., J. Goldschmitt, C. Peschel, J.P. Brakenhoff, K.J. Kallen, A. Wollmer, J. Grotzinger, and S. Rose-John, *I. A bioactive designer cytokine for human hematopoietic progenitor cell expansion*. Nat Biotechnol, 1997. **15**(2): p. 142-5.
171. Lutz, M.B., N. Kukutsch, A.L. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler, *An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow*. J Immunol Methods, 1999. **223**(1): p. 77-92.
172. Cserzo, M., E. Wallin, I. Simon, G. von Heijne, and A. Elofsson, *Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: the dense alignment surface method*. Protein Eng, 1997. **10**(6): p. 673-6.
173. Krogh, A., B. Larsson, G. von Heijne, and E.L. Sonnhammer, *Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes*. J Mol Biol, 2001. **305**(3): p. 567-80.
174. Narazaki, M., B.A. Witthuhn, K. Yoshida, O. Silvennoinen, K. Yasukawa, J.N. Ihle, T. Kishimoto, and T. Taga, *Activation of JAK2 kinase mediated by the interleukin 6 signal transducer gp130*. Proc Natl Acad Sci U S A, 1994. **91**(6): p. 2285-9.
175. Chiba, T., Y. Nagata, M. Machide, A. Kishi, H. Amanuma, M. Sugiyama, and K. Todokoro, *Tyrosine kinase activation through the extracellular domains of cytokine receptors*. Nature, 1993. **362**(6421): p. 646-8.
176. Hanick, N.A., M. Rickert, L. Varani, A.J. Bankovich, J.R. Cochran, D.M. Kim, C.D. Surh, and K.C. Garcia, *Elucidation of the interleukin-15 binding site on its alpha receptor by NMR*. Biochemistry, 2007. **46**(33): p. 9453-61.
177. Giese, B., C. Roderburg, M. Sommerauer, S.B. Wortmann, S. Metz, P.C. Heinrich, and G. Muller-Newen, *Dimerization of the cytokine receptors gp130 and LIFR analysed in single cells*. J Cell Sci, 2005. **118**(Pt 21): p. 5129-40.
178. Fantus, I.G., S. Kadota, G. Deragon, B. Foster, and B.I. Posner, *Pervanadate [peroxide(s) of vanadate] mimics insulin action in rat adipocytes via activation of the insulin receptor tyrosine kinase*. Biochemistry, 1989. **28**(22): p. 8864-71.
179. Heffetz, D., I. Bushkin, R. Dror, and Y. Zick, *The insulinomimetic agents H2O2 and vanadate stimulate protein tyrosine phosphorylation in intact cells*. J Biol Chem, 1990. **265**(5): p. 2896-902.
180. Owaki, T., M. Asakawa, N. Morishima, I. Mizoguchi, F. Fukai, K. Takeda, J. Mizuguchi, and T. Yoshimoto, *STAT3 is indispensable to IL-27-mediated cell proliferation but not to IL-27-induced Th1 differentiation and suppression of proinflammatory cytokine production*. J Immunol, 2008. **180**(5): p. 2903-11.
181. Johnson, G.L. and R. Lapadat, *Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases*. Science, 2002. **298**(5600): p. 1911-2.
182. Tillmann, A.T., *Untersuchungen zur Aktivierung von gp130-Rezeptorheterodimeren*, in *Department of Biochemistry 2009*, Christian-Albrechts-University: Kiel. p. 135.
183. Chattopadhyay, S., E. Tracy, P. Liang, O. Robledo, S. Rose-John, and H. Baumann, *Interleukin-31 and oncostatin-M mediate distinct signaling reactions and response patterns in lung epithelial cells*. J Biol Chem, 2007. **282**(5): p. 3014-26.
184. Pettit, D.K., T.P. Bonnert, J. Eisenman, S. Srinivasan, R. Paxton, C. Beers, D. Lynch, B. Miller, J. Yost, K.H. Grabstein, and W.R. Gombotz, *Structure-function studies of interleukin 15 using site-specific mutagenesis, polyethylene glycol conjugation, and homology modeling*. J Biol Chem, 1997. **272**(4): p. 2312-8.
185. Ryan, M.D. and J. Drew, *Foot-and-mouth disease virus 2A oligopeptide mediated cleavage of an artificial polyprotein*. Embo J, 1994. **13**(4): p. 928-33.
186. Donnelly, M.L., D. Gani, M. Flint, S. Monaghan, and M.D. Ryan, *The cleavage activities of aphthovirus and cardiovirus 2A proteins*. J Gen Virol, 1997. **78** (Pt 1): p. 13-21.
187. Donnelly, M.L., G. Luke, A. Mehrotra, X. Li, L.E. Hughes, D. Gani, and M.D. Ryan, *Analysis of the aphthovirus 2A/2B polyprotein 'cleavage' mechanism indicates not a*

- proteolytic reaction, but a novel translational effect: a putative ribosomal 'skip'*. J Gen Virol, 2001. **82**(Pt 5): p. 1013-25.
188. Doronina, V.A., C. Wu, P. de Felipe, M.S. Sachs, M.D. Ryan, and J.D. Brown, *Site-specific release of nascent chains from ribosomes at a sense codon*. Mol Cell Biol, 2008. **28**(13): p. 4227-39.
189. Fang, J., J.J. Qian, S. Yi, T.C. Harding, G.H. Tu, M. VanRoey, and K. Jooss, *Stable antibody expression at therapeutic levels using the 2A peptide*. Nat Biotechnol, 2005. **23**(5): p. 584-90.
190. Moisini, I., P. Nguyen, L. Fugger, and T.L. Geiger, *Redirecting therapeutic T cells against myelin-specific T lymphocytes using a humanized myelin basic protein-HLA-DR2-zeta chimeric receptor*. J Immunol, 2008. **180**(5): p. 3601-11.
191. Okita, K., M. Nakagawa, H. Hyenjong, T. Ichisaka, and S. Yamanaka, *Generation of mouse induced pluripotent stem cells without viral vectors*. Science, 2008. **322**(5903): p. 949-53.
192. Chhabra, A., L. Yang, P. Wang, B. Comin-Anduix, R. Das, N.G. Chakraborty, S. Ray, S. Mehrotra, H. Yang, C.L. Hardee, R. Hollis, D.I. Dorsky, R. Koya, D.B. Kohn, A. Ribas, J.S. Economou, D. Baltimore, and B. Mukherji, *CD4+CD25- T cells transduced to express MHC class I-restricted epitope-specific TCR synthesize Th1 cytokines and exhibit MHC class I-restricted cytolytic effector function in a human melanoma model*. J Immunol, 2008. **181**(2): p. 1063-70.
193. Kaji, K., K. Norrby, A. Paca, M. Mileikovsky, P. Mohseni, and K. Woltjen, *Virus-free induction of pluripotency and subsequent excision of reprogramming factors*. Nature, 2009. **458**(7239): p. 771-5.
194. Brandt, K., S. Bulfone-Paus, D.C. Foster, and R. Ruckert, *Interleukin-21 inhibits dendritic cell activation and maturation*. Blood, 2003. **102**(12): p. 4090-8.
195. Constantinescu, S.N., T. Keren, M. Socolovsky, H. Nam, Y.I. Henis, and H.F. Lodish, *Ligand-independent oligomerization of cell-surface erythropoietin receptor is mediated by the transmembrane domain*. Proc Natl Acad Sci U S A, 2001. **98**(8): p. 4379-84.
196. Livnah, O., E.A. Stura, S.A. Middleton, D.L. Johnson, L.K. Jolliffe, and I.A. Wilson, *Crystallographic evidence for preformed dimers of erythropoietin receptor before ligand activation*. Science, 1999. **283**(5404): p. 987-90.
197. Remy, I., I.A. Wilson, and S.W. Michnick, *Erythropoietin receptor activation by a ligand-induced conformation change*. Science, 1999. **283**(5404): p. 990-3.
198. Kubatzky, K.F., W. Ruan, R. Gurezka, J. Cohen, R. Ketteler, S.S. Watowich, D. Neumann, D. Langosch, and U. Klingmuller, *Self assembly of the transmembrane domain promotes signal transduction through the erythropoietin receptor*. Curr Biol, 2001. **11**(2): p. 110-5.
199. Frank, S.J., *Receptor dimerization in GH and erythropoietin action--it takes two to tango, but how?* Endocrinology, 2002. **143**(1): p. 2-10.
200. Krause, C.D., E. Mei, J. Xie, Y. Jia, M.A. Bopp, R.M. Hochstrasser, and S. Pestka, *Seeing the light: preassembly and ligand-induced changes of the interferon gamma receptor complex in cells*. Mol Cell Proteomics, 2002. **1**(10): p. 805-15.
201. Carr, P.D., S.E. Gustin, A.P. Church, J.M. Murphy, S.C. Ford, D.A. Mann, D.M. Woltring, I. Walker, D.L. Ollis, and I.G. Young, *Structure of the complete extracellular domain of the common beta subunit of the human GM-CSF, IL-3, and IL-5 receptors reveals a novel dimer configuration*. Cell, 2001. **104**(2): p. 291-300.
202. Kramer, J.M., L. Yi, F. Shen, A. Maitra, X. Jiao, T. Jin, and S.L. Gaffen, *Evidence for ligand-independent multimerization of the IL-17 receptor*. J Immunol, 2006. **176**(2): p. 711-5.
203. Schuster, B., W. Meinert, S. Rose-John, and K.J. Kallen, *The human interleukin-6 (IL-6) receptor exists as a preformed dimer in the plasma membrane*. FEBS Lett, 2003. **538**(1-3): p. 113-6.
204. Sternberg, M.J. and W.J. Gullick, *A sequence motif in the transmembrane region of growth factor receptors with tyrosine kinase activity mediates dimerization*. Protein Eng, 1990. **3**(4): p. 245-8.

205. Wang, Y. and G.M. Fuller, *Phosphorylation and internalization of gp130 occur after IL-6 activation of Jak2 kinase in hepatocytes*. Mol Biol Cell, 1994. **5**(7): p. 819-28.
206. Dittrich, E., C.R. Haft, L. Muys, P.C. Heinrich, and L. Graeve, *A di-leucine motif and an upstream serine in the interleukin-6 (IL-6) signal transducer gp130 mediate ligand-induced endocytosis and down-regulation of the IL-6 receptor*. J Biol Chem, 1996. **271**(10): p. 5487-94.
207. Thiel, S., I. Behrmann, A. Timmermann, H. Dahmen, G. Muller-Newen, F. Schaper, J. Tavernier, V. Pitard, P.C. Heinrich, and L. Graeve, *Identification of a Leu-Ile internalization motif within the cytoplasmic domain of the leukaemia inhibitory factor receptor*. Biochem J, 1999. **339** (Pt 1): p. 15-9.
208. Stieger, K., B. Belbellaa, C. Le Guiner, P. Moullier, and F. Rolling, *In vivo gene regulation using tetracycline-regulatable systems*. Adv Drug Deliv Rev, 2009. **61**(7-8): p. 527-41.
209. Catlett-Falcone, R., T.H. Landowski, M.M. Oshiro, J. Turkson, A. Levitzki, R. Savino, G. Ciliberto, L. Moscinski, J.L. Fernandez-Luna, G. Nunez, W.S. Dalton, and R. Jove, *Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells*. Immunity, 1999. **10**: p. 105-15.
210. Rawat, R., G.J. Rainey, C.D. Thompson, M.R. Frazier-Jessen, R.T. Brown, and R.P. Nordan, *Constitutive activation of STAT3 is associated with the acquisition of an interleukin 6-independent phenotype by murine plasmacytomas and hybridomas*. Blood, 2000. **96**(10): p. 3514-21.
211. Bromberg, J., *Stat proteins and oncogenesis*. J Clin Invest, 2002. **109**: p. 1139-1142.
212. Bollrath, J., T.J. Phesse, V.A. von Burstin, T. Putoczki, M. Bennecke, T. Bateman, T. Nebelsiek, T. Lundgren-May, O. Canli, S. Schwitalla, V. Matthews, R.M. Schmid, T. Kirchner, M.C. Arkan, M. Ernst, and F.R. Greten, *gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis*. Cancer Cell, 2009. **15**(2): p. 91-102.
213. Grivnikov, S., E. Karin, J. Terzic, D. Mucida, G.Y. Yu, S. Vallabhapurapu, J. Scheller, S. Rose-John, H. Cheroutre, L. Eckmann, and M. Karin, *IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer*. Cancer Cell, 2009. **15**(2): p. 103-13.
214. Bromberg, J., *Stat proteins and oncogenesis*. J Clin Invest, 2002. **109**(9): p. 1139-42.
215. Selander, K.S., L. Li, L. Watson, M. Merrell, H. Dahmen, P.C. Heinrich, G. Muller-Newen, and K.W. Harris, *Inhibition of gp130 signaling in breast cancer blocks constitutive activation of Stat3 and inhibits in vivo malignancy*. Cancer Res, 2004. **64**(19): p. 6924-33.
216. Jenkins, B.J., D. Grail, T. Nheu, M. Najdovska, B. Wang, P. Waring, M. Inglese, R.M. McLoughlin, S.A. Jones, N. Topley, H. Baumann, L.M. Judd, A.S. Giraud, A. Boussioutas, H.J. Zhu, and M. Ernst, *Hyperactivation of Stat3 in gp130 mutant mice promotes gastric hyperproliferation and desensitizes TGF-beta signaling*. Nat Med, 2005. **11**: p. 845-852.
217. Ernst, M., M. Najdovska, D. Grail, T. Lundgren-May, M. Buchert, H. Tye, V.B. Matthews, J. Armes, P.S. Bhathal, N.R. Hughes, E.G. Marcusson, J.G. Karras, S. Na, J.D. Sedgwick, P.J. Hertzog, and B.J. Jenkins, *STAT3 and STAT1 mediate IL-11-dependent and inflammation-associated gastric tumorigenesis in gp130 receptor mutant mice*. J Clin Invest, 2008. **118**(5): p. 1727-38.
218. Howlett, M., A.S. Giraud, H. Lescesen, C.B. Jackson, A. Kalantzis, I.R. Van Driel, L. Robb, M. Van der Hoek, M. Ernst, T. Minamoto, A. Boussioutas, H. Oshima, M. Oshima, and L.M. Judd, *The interleukin-6 family cytokine interleukin-11 regulates homeostatic epithelial cell turnover and promotes gastric tumor development*. Gastroenterology, 2009. **136**(3): p. 967-77.
219. Jenkins, B.J., D. Grail, T. Nheu, M. Najdovska, B. Wang, P. Waring, M. Inglese, R.M. McLoughlin, S.A. Jones, N. Topley, H. Baumann, L.M. Judd, A.S. Giraud, A. Boussioutas, H.J. Zhu, and M. Ernst, *Hyperactivation of Stat3 in gp130 mutant mice promotes gastric hyperproliferation and desensitizes TGF-beta signaling*. Nat Med, 2005. **11**(8): p. 845-52.

220. Judd, L.M., K. Bredin, A. Kalantzis, B.J. Jenkins, M. Ernst, and A.S. Giraud, *STAT3 activation regulates growth, inflammation, and vascularization in a mouse model of gastric tumorigenesis*. *Gastroenterology*, 2006. **131**(4): p. 1073-85.
221. Merchant, J.L., *What lurks beneath: IL-11, via Stat3, promotes inflammation-associated gastric tumorigenesis*. *J Clin Invest*, 2008. **118**(5): p. 1628-31.
222. Bromberg, J.F., M.H. Wrzeszczynska, G. Devgan, Y. Zhao, R.G. Pestell, C. Albanese, and J.E. Darnell, *Stat3 as an oncogene*. *Cell*, 1999. **98**: p. 295-303.
223. Roberts, P.J. and C.J. Der, *Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer*. *Oncogene*, 2007. **26**(22): p. 3291-310.
224. White, M.A., C. Nicolette, A. Minden, A. Polverino, L. Van Aelst, M. Karin, and M.H. Wigler, *Multiple Ras functions can contribute to mammalian cell transformation*. *Cell*, 1995. **80**(4): p. 533-41.
225. Zuber, J., O.I. Tchernitsa, B. Hinzmann, A.C. Schmitz, M. Grips, M. Hellriegel, C. Sers, A. Rosenthal, and R. Schafer, *A genome-wide survey of RAS transformation targets*. *Nat Genet*, 2000. **24**(2): p. 144-52.
226. Mansour, S.J., W.T. Matten, A.S. Hermann, J.M. Candia, S. Rong, K. Fukasawa, G.F. Vande Woude, and N.G. Ahn, *Transformation of mammalian cells by constitutively active MAP kinase kinase*. *Science*, 1994. **265**(5174): p. 966-70.
227. Ward, Y., W. Wang, E. Woodhouse, I. Linnoila, L. Liotta, and K. Kelly, *Signal pathways which promote invasion and metastasis: critical and distinct contributions of extracellular signal-regulated kinase and Ral-specific guanine exchange factor pathways*. *Mol Cell Biol*, 2001. **21**(17): p. 5958-69.
228. Zebisch, A., A.P. Czernilofsky, G. Keri, J. Smigelskaite, H. Sill, and J. Troppmair, *Signaling through RAS-RAF-MEK-ERK: from basics to bedside*. *Curr Med Chem*, 2007. **14**(5): p. 601-23.
229. Musso, T., L. Calosso, M. Zucca, M. Millesimo, D. Ravarino, M. Giovarelli, F. Malavasi, A.N. Ponzi, R. Paus, and S. Bulfone-Paus, *Human monocytes constitutively express membrane-bound, biologically active, and interferon-gamma-upregulated interleukin-15*. *Blood*, 1999. **93**(10): p. 3531-9.
230. Doherty, T.M., R.A. Seder, and A. Sher, *Induction and regulation of IL-15 expression in murine macrophages*. *J Immunol*, 1996. **156**(2): p. 735-41.
231. Mattei, F., G. Schiavoni, F. Belardelli, and D.F. Tough, *IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation*. *J Immunol*, 2001. **167**(3): p. 1179-87.
232. Bulanova, E., V. Budagian, Z. Orinska, H. Krause, R. Paus, and S. Bulfone-Paus, *Mast cells express novel functional IL-15 receptor alpha isoforms*. *J Immunol*, 2003. **170**(10): p. 5045-55.
233. Burkett, P.R., R. Koka, M. Chien, S. Chai, F. Chan, A. Ma, and D.L. Boone, *IL-15R alpha expression on CD8+ T cells is dispensable for T cell memory*. *Proc Natl Acad Sci U S A*, 2003. **100**(8): p. 4724-9.
234. Schluns, K.S., K.D. Klonowski, and L. Lefrancois, *Transregulation of memory CD8 T-cell proliferation by IL-15Ralpha+ bone marrow-derived cells*. *Blood*, 2004. **103**(3): p. 988-94.
235. Rappl, G., A. Kapsokefalou, C. Heuser, M. Rossler, S. Ugurel, W. Tilgen, U. Reinhold, and H. Abken, *Dermal fibroblasts sustain proliferation of activated T cells via membrane-bound interleukin-15 upon long-term stimulation with tumor necrosis factor-alpha*. *J Invest Dermatol*, 2001. **116**(1): p. 102-9.
236. Briard, D., D. Brouty-Boye, B. Azzarone, and C. Jasmin, *Fibroblasts from human spleen regulate NK cell differentiation from blood CD34(+) progenitors via cell surface IL-15*. *J Immunol*, 2002. **168**(9): p. 4326-32.
237. Weiler, M., B. Rogashev, T. Einbinder, M.J. Hausmann, J. Kaneti, C. Chaimovitz, and A. Douvdevani, *Interleukin-15, a leukocyte activator and growth factor, is produced by cortical tubular epithelial cells*. *J Am Soc Nephrol*, 1998. **9**(7): p. 1194-201.
238. Tejman-Yarden, N., M. Zlotnik, E. Lewis, O. Etzion, C. Chaimovitz, and A. Douvdevani, *Renal cells express a functional interleukin-15 receptor*. *Nephrol Dial Transplant*, 2005. **20**(3): p. 516-23.

239. Oppenheimer-Marks, N., R.I. Brezinschek, M. Mohamadzadeh, R. Vita, and P.E. Lipsky, *Interleukin 15 is produced by endothelial cells and increases the transendothelial migration of T cells In vitro and in the SCID mouse-human rheumatoid arthritis model In vivo*. J Clin Invest, 1998. **101**(6): p. 1261-72.
240. Suzuki, A., S. McCall, S.S. Choi, J.K. Sicklick, J. Huang, Y. Qi, M. Zdanowicz, T. Camp, Y.X. Li, and A.M. Diehl, *Interleukin-15 increases hepatic regenerative activity*. J Hepatol, 2006. **45**(3): p. 410-8.
241. Alvarez, B., N. Carbo, J. Lopez-Soriano, R.H. Drivdahl, S. Busquets, F.J. Lopez-Soriano, J.M. Argiles, and L.S. Quinn, *Effects of interleukin-15 (IL-15) on adipose tissue mass in rodent obesity models: evidence for direct IL-15 action on adipose tissue*. Biochim Biophys Acta, 2002. **1570**(1): p. 33-7.
242. Hanisch, U.K., S.A. Lyons, M. Prinz, C. Nolte, J.R. Weber, H. Kettenmann, and F. Kirchhoff, *Mouse brain microglia express interleukin-15 and its multimeric receptor complex functionally coupled to Janus kinase activity*. J Biol Chem, 1997. **272**(46): p. 28853-60.
243. Kurowska, M., W. Rudnicka, D. Maslinska, and W. Maslinski, *Expression of IL-15 and IL-15 receptor isoforms in select structures of human fetal brain*. Ann N Y Acad Sci, 2002. **966**: p. 441-5.
244. Eisenman, J., M. Ahdieh, C. Beers, K. Brasel, M.K. Kennedy, T. Le, T.P. Bonnert, R.J. Paxton, and L.S. Park, *Interleukin-15 interactions with interleukin-15 receptor complexes: characterization and species specificity*. Cytokine, 2002. **20**(3): p. 121-9.
245. Bulfone-Pau, S.S., E. Bulanova, T. Pohl, V. Budagian, H. Durkop, R. Ruckert, U. Kunzendorf, R. Paus, and H. Krause, *Death deflected: IL-15 inhibits TNF-alpha-mediated apoptosis in fibroblasts by TRAF2 recruitment to the IL-15Ralpha chain*. Faseb J, 1999. **13**(12): p. 1575-85.
246. Adam, N., B. Rabe, J. Suthaus, J. Grotzinger, S. Rose-John, and J. Scheller, *Unraveling viral interleukin-6 binding to gp130 and activation of STAT-signaling pathways independently of the interleukin-6 receptor*. J Virol, 2009. **83**(10): p. 5117-26.
247. Aurich, M., *Ciliary Neurotrophic Factor Plus: Design, Expression und Testung von rezeptor-selektiven Zytokin-Chimären*. Department of Biochemistry. 2010, Christian-Albrechts-University: Kiel. p. 93.
248. Chirifu, M., C. Hayashi, T. Nakamura, S. Toma, T. Shuto, H. Kai, Y. Yamagata, S.J. Davis, and S. Ikemizu, *Crystal structure of the IL-15-IL-15Ralpha complex, a cytokine-receptor unit presented in trans*. Nat Immunol, 2007. **8**(9): p. 1001-7.
249. Zambrowicz, B.P., A. Imamoto, S. Fiering, L.A. Herzenberg, W.G. Kerr, and P. Soriano, *Disruption of overlapping transcripts in the ROSA beta geo 26 gene trap strain leads to widespread expression of beta-galactosidase in mouse embryos and hematopoietic cells*. Proc Natl Acad Sci U S A, 1997. **94**(8): p. 3789-94.
250. Maes, C., S. Goossens, S. Bartunkova, B. Drogat, L. Coenegrachts, I. Stockmans, K. Moermans, O. Nyabi, K. Haigh, M. Naessens, L. Haenebalcke, J.P. Tuckermann, M. Tjwa, P. Carmeliet, V. Mandic, J.P. David, A. Behrens, A. Nagy, G. Carmeliet, and J.J. Haigh, *Increased skeletal VEGF enhances beta-catenin activity and results in excessively ossified bones*. Embo J. **29**(2): p. 424-41.
251. Ohnmacht, C., A. Pullner, S.B. King, I. Drexler, S. Meier, T. Brocker, and D. Voehringer, *Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity*. J Exp Med, 2009. **206**(3): p. 549-59.
252. Chung, T.D., J.J. Yu, M.T. Spiotto, M. Bartkowski, and J.W. Simons, *Characterization of the role of IL-6 in the progression of prostate cancer*. Prostate, 1999. **38**(3): p. 199-207.
253. Royuela, M., M. Ricote, M.S. Parsons, I. Garcia-Tunon, R. Paniagua, and M.P. de Miguel, *Immunohistochemical analysis of the IL-6 family of cytokines and their receptors in benign, hyperplastic, and malignant human prostate*. J Pathol, 2004. **202**(1): p. 41-9.

254. McKenzie, R.C. and J. Szepietowski, *Cutaneous leukemia inhibitory factor and its potential role in the development of skin tumors*. *Dermatol Surg*, 2004. **30**(2 Pt 2): p. 279-90.
255. Garcia-Tunon, I., M. Ricote, A. Ruiz, B. Fraile, R. Paniagua, and M. Royuela, *OSM, LIF, its receptors, and its relationship with the malignance in human breast carcinoma (in situ and in infiltrative)*. *Cancer Invest*, 2008. **26**(3): p. 222-9.
256. Junnila, S., A. Kokkola, M.L. Karjalainen-Lindsberg, P. Puolakkainen, and O. Monni, *Genome-wide gene copy number and expression analysis of primary gastric tumors and gastric cancer cell lines*. *BMC Cancer*. **10**: p. 73.
257. Hisada, M., S. Kamiya, K. Fujita, M.L. Belladonna, T. Aoki, Y. Koyanagi, J. Mizuguchi, and T. Yoshimoto, *Potent antitumor activity of interleukin-27*. *Cancer Res*, 2004. **64**(3): p. 1152-6.
258. Salcedo, R., J.K. Stauffer, E. Lincoln, T.C. Back, J.A. Hixon, C. Hahn, K. Shafer-Weaver, A. Malyguine, R. Kastelein, and J.M. Wigginton, *IL-27 mediates complete regression of orthotopic primary and metastatic murine neuroblastoma tumors: role for CD8+ T cells*. *J Immunol*, 2004. **173**(12): p. 7170-82.
259. Yoshimoto, T., N. Morishima, I. Mizoguchi, M. Shimizu, H. Nagai, S. Oniki, M. Oka, C. Nishigori, and J. Mizuguchi, *Antiproliferative activity of IL-27 on melanoma*. *J Immunol*, 2008. **180**(10): p. 6527-35.

9 Appendix

9.1 Abbreviations

A	adenine
aa	amino acid
ADAM	a disintegrin and metalloproteinase
Amp	ampicillin
APC	allophycocyanine
C	celsius or cytosine
cDNA	complementary DNA
CD	cluster of differentiation
CLC	cardiotrophin-like cytokine
CLF-1	cytokine-like factor 1
CPD	cytoplasmic domain
CNTF	ciliary neurotrophic factor
CNTFR	ciliary neurotrophic factor receptor
DC	dendritic cells
DNA	deoxyribonucleic acid
dNTP	desoxynucleotide triphosphate
<i>E.coli</i>	<i>Escherichia coli</i>
ECD	extracellular domain
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
EYFP	enhanced yellow fluorescent protein
FBS	fetal bovine serum
FC	flow cytometry
g	gram or gravity
G	guanine
GFP	green fluorescent protein
gp130	glycoprotein 130
GPL	gp130-like receptor
Gab-1	Grb2-associating binder 1
GRB-2	growth factor receptor bound protein 2
h	human
HIL-6	Hyper-IL-6
IL	interleukin

IL-xR	interleukin-x receptor
IP	immunoprecipitation
JAK	Janus kinase
Kan	kanamycin
kb	kilobase
kDa	kilo Dalton
l	liter
LB	Luria Bertani
LIF	leukemia inhibitory factor
LIFR	leukemia inhibitory factor receptor
M	molar
m	murine, milli or meter
min	minute
μ	micro
MAPK	mitogen activated protein kinase
MEK-1	MAPK/ERK kinase 1
mRNA	messenger RNA
n	nano
n.d.	not determined
NP	neuropoeitin
P-x	phosphorylated x
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PtdIns(3,4,5)P ₃	phosphatidylinositol-(3,4,5)-phosphate
Puro	puromycin
OSM	oncostatin M
OSMR	oncostatin M receptor
rpm	rounds per minute
RT	room temperature
RT-PCR	reverse transcribition followed by a polymerase chain reaction
PIAS	protein inhibitor of STATs
s	second
sIL-6R	soluble interleukin-6 receptor
SOCS	suppressor of cytokine signaling
SP	signal peptide
STAT	signal transducer and activator of transcription
T	thymidine

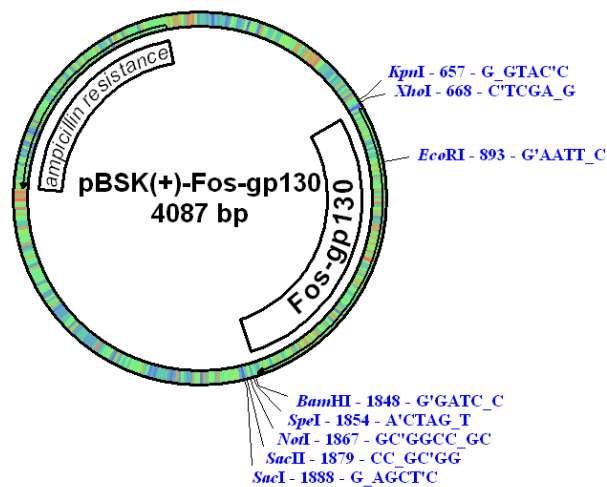
<i>Taq</i>	<i>thermophilus aquaticus</i>
Th	T helper cell
TBS	Tris buffered saline
TMD	transmembrane domain
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor
TRAF	TNFR-associated factor
U	unit(s)
V	volt
WB	Western Blot
WSX-1	interleukin-27 receptor alpha

9.2 Amino acids

A (Ala)	alanine
C (Cys)	cysteine
D (Asp)	aspartic acid
E (Glu)	glutamic acid
F (Phe)	phenylalanine
G (Gly)	glycine
H (His)	histidine
I (Ile)	isoleucine
K (Lys)	lysine
L (Leu)	leucine
M (Met)	methionine
N (Asn)	asparagine
P (Pro)	proline
Q (Gln)	glutamine
R (Arg)	arginine
S (Ser)	serine
T (Thr)	threonine
V (Val)	valine
W (Trp)	tryptophan
Y (Tyr)	tyrosine

9.3 Sequences and plasmid maps

1. pBSK(+)-Fos-gp130



Nucleotide sequence of Fos-gp130 including relevant restriction sites:

```

      GGTACCGG  GCCCCCCCTC  GAGTCTAGAC  CCCGCAAGAT  GTTGACGTTG
701  CAGACTTGGC  TAGTGCAAGC  CTTGTTTATT  TTCCTCACCA  CTGAATCTAC
751  AGGTGACTAC  AAAGATGACG  ATGATAAAGA  ATTGTGCGGC  GGCTTAACCG
801  ACACCTGCA   GGCTGAAACC  GACCAGCTGG  AAGACGAGAA  AAGCGCGCTC
851  CAGACCGAAA  TCGCTAACCT  GCTTAAGGAA  AAGGAGAAAC  TGAATTACAC
901  TTTTACTACC  CCAAAGTTTG  CTCAAGGAGA  AATTGAAGCC  ATAGTCGTGC
951  CTGTTTGCTT  AGCATTTCCTA  TTGACAACCTC  TTCTGGGAGT  GCTGTTCTGC
1001 TTTAATAAGC  GAGACCTAAT  TAAAAACAC  ATCTGGCCTA  ATGTTCCAGA
1051 TCCTCAAAG  AGTCATATTG  CCCAGTGGTC  ACCTCACACT  CCTCCAAGGC
1101 ACAATTTTAA  TTCAAAGAT  CAAATGTATT  CAGATGGCAA  TTCACTGAT
1151 GTAAGTGTG  TGGAAATAGA  AGCAAATGAC  AAAAAGCCTT  TTCCAGAAGA
1201 TCTGAAATTA  TTGGACCTGT  TCAAAAAGGA  AAAAATTAAT  ACTGAAGGAC
1251 ACAGCAGTGG  TATTGGGGGG  TCTTCATGCA  TGTCATCTTC  TAGGCCAAGC
1301 ATTTCTAGCA  GTGATGAAAA  TGAATCTTCA  CAAAACACTT  CGAGCACTGT
1351 CCAGTATTCT  ACCGTGGTAC  ACAGTGGCTA  CAGACACCAA  GTTCCGTCAG
1401 TCCAAGTCTT  CTCAAGATCC  GAGTCTACCC  AGCCCTTGTT  AGATTCAGAG
1451 GAGCGGCCAG  AAGATCTACA  ATTAGTAGAT  CATGTAGATG  GCGGTGATGG
1501 TATTTGCCC  AGGCAACAGT  ACTTCAAACA  GAACTGCAGT  CAGCATGAAT
1551 CCAGTCCAGA  TATTTACAT  TTTGAAAGGT  CAAAGCAAGT  TTCATCAGTC
1601 AATGAGGAAG  ATTTTGTTAG  ACTTAAACAG  CAGATTTTCA  ATCATATTTT
1651 ACAATCCTGT  GGATCTGGGC  AAATGAAAAT  GTTTCAGGAA  GTTTCTGCAG
1701 CAGATGCTTT  TGGTCCAGGT  ACTGAGGGAC  AAGTAGAAAG  ATTTGAAACA
1751 GTTGGCATGG  AGGCTGCGAC  TGATGAAGGC  ATGCCTAAAA  GTTACTTACC
1801 ACAGACTGTA  CGGCAAGGCG  GCTACATGCC  TCAGTCAAGG  ACTAGGGGGA
1851 TCCACTAGTT  CTAGAGCGGC  CGCCACCGCG  GTGGAGCTC

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Bold: start and stop codons

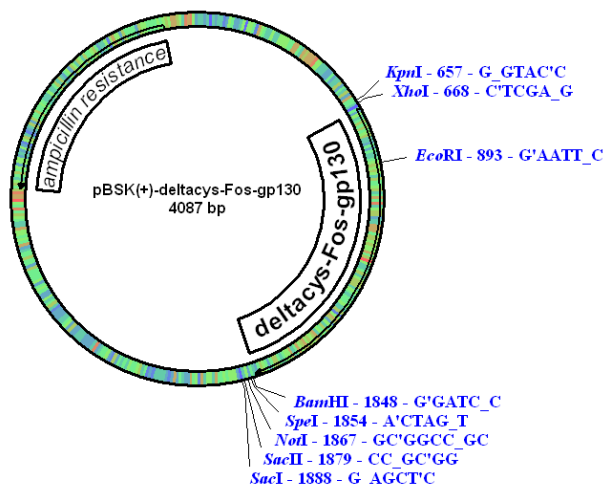
Amino acid sequence:

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MLTLQTLVQLFIFLTTTESTGDYKDDDDKELCGGLDLDLQAETDQLEDE 50
KSALQTEIANLLKEKEKLEFFTTTPKFAQGEIEAIVVPVCLAFLLTLLG 100
VLFNCFNKRDLIKKHIWPNVDPSPKSHIAQWSPHTPPRHNFNNSKQDMYSDG 150
NFTDVSVEIEANDKKPFPELKLKLLDLFKKEKINTEGHSSGIGGSSCMSS 200
SRPSISSDENESSQNTSSTVQYSTVHSGYRHQVPSVQVFSRSESTQPL 250
LDSEERPEDLQLVDHVDGGDGLPRQYFKQNCQHESSPDISHFERSKQ 300
VSSVNEEDFVRLKQQLSDHISQSCGSGQMKMFQEVSAADAFPGTGTEQVE 350
RFETVGMEEAATDEGMPKSYLPQTVRQGGYMPQ*

```

Black: human gp130 (SP and 15aa ECD+TMD+CPD); green: FLAG tag; italic: linker (with cysteine); blue: Fos leucine zipper; brown: *EcoRI* site

2. pBSK(+)- Δ cys-Fos-gp130

Nucleotide sequence of Δ cys-Fos-gp130 including relevant restriction sites :

```

GGTACCGG GCCCCCCCTC GAGTCTAGAC CCCGCAAGAT GTTGACGTTG
701 CAGACTTGGC TAGTGCAAGC CTTGTTTATT TTCCTCACCA CTGAATCTAC
751 AGGTGACTAC AAAGATGACG ATGATAAAGA ATTGGGCGGC GGCTTAACCG
801 ACACCCTGCA GGCTGAAACC GACCAGCTGG AAGACGAGAA AAGCGCGCTC
851 CAGACCGAAA TCGCTAACCT GCTTAAGGAA AAGGAGAAAC TGGAAATCAC
901 TTTTACTACC CCAAAGTTTG CTCAAGGAGA AATTGAAGCC ATAGTCGTGC
951 CTGTTTGCTT AGCATTCTTA TTGACAACCT TTCTGGGAGT GCTGTTCTGC
1001 TTTAATAAGC GAGACCTAAT TAAAAAACAC ATCTGGCCTA ATGTTCCAGA
1051 TCCTTCAAAG AGTCATATTG CCCAGTGGTC ACCTCACACT CCTCCAAGGC
1101 ACAATTTTAA TTCAAAGAT CAAATGTATT CAGATGGCAA TTTCCTGAT
1151 GTAAGTGTTG TGGAAATAGA AGCAAATGAC AAAAAAGCCTT TTCCAGAAGA
1201 TCTGAAATTA TTGGACCTGT TCAAAAAGGA AAAAAATTAAT ACTGAAGGAC
1251 ACAGCAGTGG TATTGGGGGG TCTTCATGCA TGTCATCTTC TAGGCCAAGC
1301 ATTTCTAGCA GTGATGAAAA TGAATCTTCA CAAAACACTT CGAGCACTGT
1351 CCAGTATTCT ACCGTGGTAC ACAGTGGCTA CAGACACCAA GTTCCGTCAG
1401 TCCAAGTCTT CTCAAGATCC GAGTCTACCC AGCCCTTGTT AGATTCAGAG
1451 GAGCGGCCAG AAGACTTACA ATTAGTAGAT CATGTAGATG GCGGTGATGG
1501 TATTTTGCCC AGGCAACAGT ACTTCAAACA GAACTGCAGT CAGCATGAAT
1551 CCAGTCCAGA TATTTACAT TTTGAAAGGT CAAAGCAAGT TTCATCAGTC
1601 AATGAGGAAG ATTTTGTTAG ACTTAAACAG CAGATTTTCA ATCATATTTT
1651 ACAATCCTGT GGATCTGGGC AAATGAAAAT GTTTCAGGAA GTTTCTGCAG
1701 CAGATGCTTT TGGTCCAGGT ACTGAGGGAC AAGTAGAAAG ATTTGAAACA
1751 GTTGGCATGG AGGCTGCGAC TGATGAAGGC ATGCCTAAAA GTTACTTACC
1801 ACAGACTGTA CGGCAAGGCG GCTACATGCC TCAGTGAAGG ACTAGGGGGA
1851 TCCACTAGTT CTAGAGCGGC CGCCACCGCG GTGGAGCTC

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Bold: start and stop codons

Amino acid sequence :

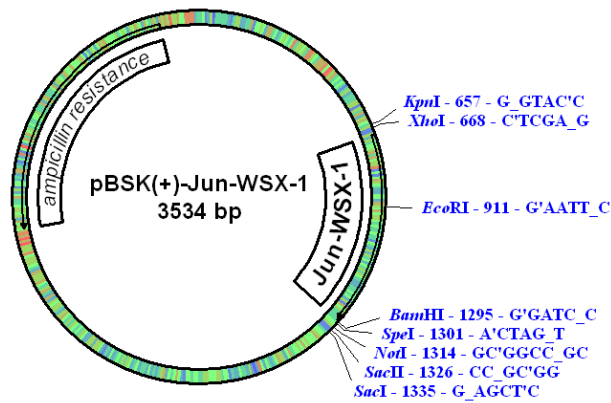
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VLFNCFNKRDLIKKHIWPNVDPSPKSHIAQWSPHTPPRHNFNSKDQMYSDG 150
NFTDVSVEIEANDKKFPEDLKLKLLDLFKKKEKINTEGHSSGIGSSCMSS 200
SRPSSSDENESSQNTSSTVQYSTVVHSGYRHQVPSVQVFSRSESTQPL 250
LDSEERPEDLQLVDHVDGGDGLPRQYFKQNCQHESSPDISHFERSKQ 300
VSSVNEEDFVRLKQQISDHISQSCGSGQMKMFQEVSAADAFPGTGEQVE 350
RFETVGMEAAATDEGMPKSYLPQTVRQGGYMPQ*

```

Black: human gp130 (SP and 15aa ECD+TMD+CPD); green: FLAG tag; italic: linker (w/o cysteine); blue: Fos leucine zipper; brown: *EcoRI* site

3. pBSK(+)-Jun-WSX-1



Nucleotide sequence of Jun-WSX-1 including relevant restriction sites:

```

      GGTACCGG  GCCCCCCCTC  GAGTCTAGAC  CCCGCAAGAT  GTTGACGTTG
701  CAGACTTGGC  TAGTGCAAGC  CTTGTTTATT  TTCCTCACCA  CTGAATCTAC
751  AGGTGACTAC  AAAGATGACG  ATGATAAAGA  ATTGTGCGGC  GGCAGAATCG
801  CCCGGCTGGA  GGAAAAAGTG  AAAACCTTGA  AAGCTCAGAA  CTCGGAGCTG
851  GCGTCCACGG  CCAACATGCT  CAGGGAACAG  GTGGCACAGC  TTAAACAGAA
901  AGTCATGAAC  GAATTCTCAC  TTCACCTACC  AGATAATAGG  ATCAGGTGGA
951  AAGCTCTGCC  CTGGTTTCTG  TCCCTGTGGG  GTTTGCTTCT  GATGGGCTGT
1001 GGCTGAGCC  TGGCCAGTAC  CAGGTGCCTA  CAGGCCAGGT  GCTTACTACTG
1051 GCGACACAAG  TTGCTTCCCC  AGTGGATCTG  GGAGAGGGTT  CCTGATCCTG
1101 CCAACAGCAA  TTCTGGGCAA  CTTACATCA  AGGAGGTGAG  CCTGCCCCAA
1151 CCGCCAAGG  ACGGACCCAT  CCTGGAGGTG  GAGGAAGTGG  AGCTACAGCC
1201 TGTTGTGGAG  TCCCCTAAAG  CCTCTGCCCC  GATTTACTCT  GGGTATGAGA
1251 AACACTTCCT  GCCCACACCA  GAGGAGCTGG  GCCTTCTAGT  CTGAGGATCC
1301 ACTAGTTCTA  GAGCGGCCGC  CACCGCGGTG  GAGCT

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Bold: start and stop codons

Amino acid sequence:

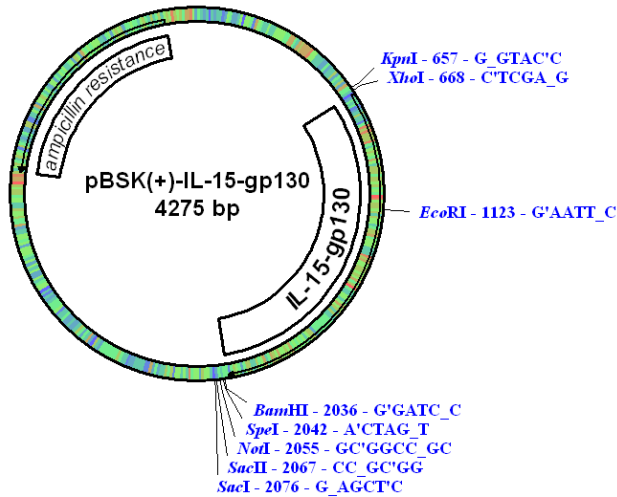
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MLTLQTLWLVLQALFIFLTTESTGDYKDDDDKELCGGRRIARLEEKVKTLKAQ  50
NSELASTANMLREQVAQLKQKVMNEFSLHLPDNRIRWKALPWFLSLWGLL  100
LMGCGLSLASTRCLQARCLHWRHKLLPQWIWERVDPANSNSGQPYIKEV  150
SLPQPPKDPILEVEEVELQPVVESPKASAPIYSGYEKHFLPTPEELLGLL  200
V*

```

Black: human gp130 SP; green: FLAG tag; italic: linker (w/o cysteine); red: Jun leucine zipper; brown: *EcoRI* site; purple: murine WSX-1 (15aa ECD+TMD+CPD)

4. pBSK(+)-IL-15-gp130



Nucleotide sequence of IL-15-gp130 including relevant restriction sites:

```

GGTACCGG GCCCCCCCTC GAGCCACCAT GGACAGCAAA GGTCGTCGC
701 AGAAAGCAGG GTCCCGCCTG CTCCTGCTGC TGGTGGTGTC AAATCTACTC
751 TTGTGCCAGG GTGTGGTCTC CACCACGCGT AACTGGGTGA ATGTAATAAG
801 TGATTGAAA AAAATTGAAG ATCTTATTCA ATCTATGCAT ATTGATGCTA
851 CTTTATATAC GGAAAGTGAT GTTCACCCCA GTTGCAAAGT AACAGCAATG
901 AAGTGCTTTC TCTTGGAGTT ACAAGTTATT TCACTTGAGT CCGGAGATGC
951 AAGTATTCAT GATACAGTAG AAAATCTGAT CATCCTAGCA AACAAACAGTT
1001 TGCTTCTAA TGGGAATGTA ACAGAATCTG GATGCAAAGA ATGTGAGGAA
1051 CTGGAGGAAA AAAATATTA AGAATTTTGG CAGAGTTTGG TACATATTGT
1101 CCAAATGTTC ATCAACACTT CTGAATTCAC TTTTACTACC CCAAAGTTTGG
1151 CTCAAGGAGA AATTGAAGCC ATAGTCGTGC CTGTTTGCTT AGCATTCCCTA
1201 TTGACAACTC TTCTGGGAGT GCTGTTCTGC TTTAATAAGC GAGACCTAAT
1251 TAAAAAACAC ATCTGGCCTA ATGTTCCAGA TCCTTCAAAG AGTCATATTG
1301 CCCAGTGGTC ACCTCACACT CCTCCAAGGC ACAATTTTAA TTCAAAAGAT
1351 CAAATGTATT CAGATGGCAA TTTCAGTAT GTAAGTGTG TGGAAATAGA
1401 AGCAAATGAC AAAAAGCCTT TTCCAGAAGA TCTGAAATTA TTGGACCTGT
1451 TCAAAAAGGA AAAAATTAAT ACTGAAGGAC ACAGCAGTGG TATTGGGGGG
1501 TCTTCATGCA TGTCATCTTC TAGGCCAAGC ATTTCTAGCA GTGATGAAAA
1551 TGAATCTTCA CAAAACACTT CGAGCACTGT CCAGTATCT ACCGTGGTAC
1601 ACAGTGGCTA CAGACACCAA GTTCCGTCAG TCCAAGTCTT CTCAAGATCC
1651 GAGTCTACCC AGCCCTTGTT AGATTCAGAG GAGCGGCCAG AAGATCTACA
1701 ATTAGTAGAT CATGTAGATG GCGGTGATGG TATTTTGCCC AGGCAACAGT
1751 ACTTCAAACA GAAGTGCAGT CAGCATGAAT CCAGTCCAGA TATTCACAT
1801 TTTGAAAGGT CAAAGCAAGT TTCATCAGTC AATGAGGAAG ATTTTGTTAG
1851 ACTTAAACAG CAGATTTTCA ATCATATTTT ACAATCTGTG GGATCTGGGC
1901 AAATGAAAAT GTTTCAGGAA GTTTCAGGAA GATTCAGGAA CAGATGCTTT TGGTCCAGGT
1951 ACTGAGGGAC AAGTAGAAAG ATTTGAAACA GTTGGCATGG AGGCTGCGAC
2001 TGATGAAGGC ATGCCTAAAA GTTACTTACC ACAGACTGTA CGGCAAGGCG
2051 GCTACATGCC TCAGTCAAGG ACTAGGGGGA TCCACTAGT CTAGAGCGGG
2101 CGCCACCGCG GTGGAGCTC
    
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Bold: start and stop codons

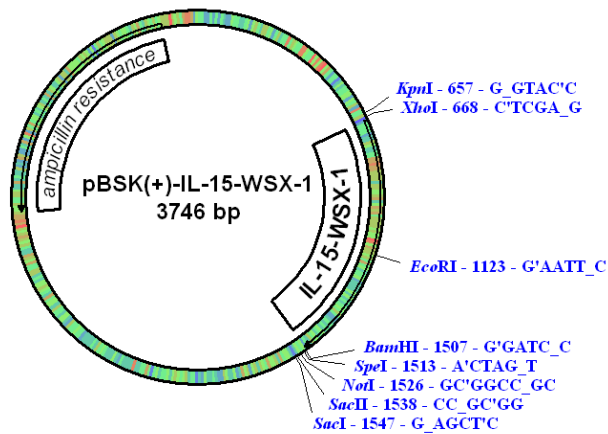
Amino acid sequence:

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IILANNSLSSNGNVTESGCKECELEEKNIKEFLQSFVHIVQMFINTSEF 150
FTTPKFAQGEIEAIVVPVCLAFLLTLLGLVLFCFNKRDLIKKHWPVNP 200
DPSKSHIAQWSPHTPPRHFNFSKDQMYSDGNFTDVSVEIEANDKKPFPE 250
DLKLLDLFKKEKINTEGHSSGIGSSCMSSSRPSISSSDENESSQNTSST 300
VQYSTVVHSGYRHRQVPSVQVFSRSESTQPLLDSEERPEDLQLVDHVDGGD 350
GILPRQQYFKQNCQSHESPDISHFERSKQVSSVNEEDFVRLKQQISDHI 400
SQSCGSGQMKMFQEVSAADAFPGTTEGQVERFETVGMEEATDEGMFKSYL 450
PQTVRQGGYMPQ*
    
```

Grey: bovine preprolactin SP; italic: one additional amino acid and MluI site orange: human IL-15; brown: EcoRI site; black: human gp130 (15aa ECD+TMD+CPD)

5. pBSK(+)-IL-15-WSX-1



Nucleotide sequence of IL-15-WSX-1 including relevant restriction sites

```

          GTACCGG  GCCCCCCTC  GAGCCACCAT  GGACAGCAAA  GGTCGTCGC
701  AGAAAGCAGG  GTCCCGCCTG  CTCCTGCTGC  TGGTGGTGTC  AAATCTACTC
751  TTGTGCCAGG  GTGTGGTCTC  CACCACGCGT  AACTGGGTGA  ATGTAATAAG
801  TGATTGAAA   AAAATTGAAG  ATCTTATTC  ATCTATGCAT  ATTGATGCTA
851  CTTTATATAC  GGAAAGTGAT  GTTCACCCCA  GTTGCAAAGT  AACAGCAATG
901  AAGTGCTTTC  TCTTGGAGTT  ACAAGTTATT  TCACTTGAGT  CCGGAGATGC
951  AAGTATTCAT  GATACAGTAG  AAAATCTGAT  CATCCTAGCA  AACAAACAGT
1001 TGTCTTCTAA  TGGGAATGTA  ACAGAATCTG  GATGCAAAGA  ATGTGAGGAA
1051 CTGGAGGAAA  AAAATATTA  AGAATTTT  CAGAGTTTTG  TACATATTGT
1101 CCAAATGTTC  ATCAACACTT  CTGAATCTC  ACTTCACCTA  CCAGATAATA
1151 GGATCAGGTG  GAAAGCTCTG  CCCTGGTTTC  TGTCCTGTG  GGGTTTGCTT
1201 CTGATGGGCT  GTGGCCTGAG  CCTGGCCAGT  ACCAGGTGCC  TACAGGCCAG
1251 GTGCTTACAC  TGGCGACACA  AGTTGCTTCC  CCAGTGGATC  TGGGAGAGGG
1301 TTCCTGATCC  TGCCAACAGC  AATTCTGGGC  AACCTTACAT  CAAGGAGGTG
1351 AGCCTGCCCC  AACCGCCCAA  GGACGGACCC  ATCCTGGAGG  TGGAGGAAGT
1401 GGAGCTACAG  CCTGTTGTGG  AGTCCCCTAA  AGCCTCTGCC  CCGATTTACT
1451 CTGGGTATGA  GAAACACTTC  CTGCCACAC  CAGAGGAGCT  GGGCCTTCTA
1501 GTCTGAGGAT  CCACTAGTTC  TAGAGCGGCC  GCCACCGCGG  TGGAGCTC

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Bold: start and stop codons

Amino acid sequence:

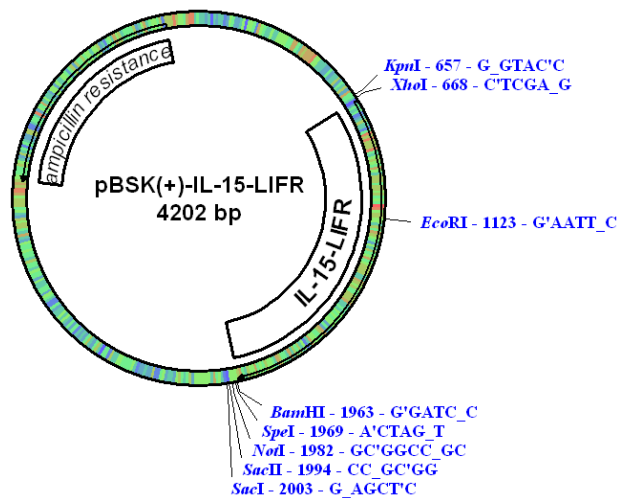
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IILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSEF 150
SLHLPDNRIRWKALPWFSLWGLLLMGCGLSLASTRCLQARCLHWRHLL 200
PQWIWERVPDPANSNSGQPYIKEVSLPQPPKDGPILEVVEVELQPVVESP 250
KASAPIYSGYEKHFLPTPEELGLLV*

```

Grey: bovine preprolactin SP; italic: one additional amino acid and MluI site
orange: human IL-15; brown: EcoRI site; purple: murine WSX-1 (15aa ECD+TMD+CPD)

6. pBSK(+)-IL-15-LIFR



Nucleotide sequence of IL-15-GPL including relevant restriction sites

```

GGTACCGG GCCCCCCCTC GAGCCACCAT GGACAGCAAA GGTTCGTCGC
701 AGAAAGCAGG GTCCCGCCTG CTCCTGCTGC TGGTGGTGTC AAATCTACTC
751 TTGTGCCAGG GTGTGGTCTC CACCACGCGT AACTGGGTGA ATGTAATAAG
801 TGATTTGAAA AAAATTGAAG ATCTTATTCA ATCTATGCAT ATTGATGCTA
851 CTTTATATAC GGAAAGTGAT GTTCACCCCA GTTGCAAAGT AACAGCAATG
901 AAGTGCTTTC TCTTGGAGTT ACAAGTTATT TCACTTGAGT CCGGAGATGC
951 AAGTATTCAT GATACAGTAG AAAATCTGAT CATCCTAGCA AACAACAGTT
1001 TGTCTCTAA TGGGAATGTA ACAGAATCTG GATGCAAAGA ATGTGAGGAA
1051 CTGGAGGAAA AAAATATTA AGAATTTTGG CAGAGTTTGG TACATATTGT
1101 CCAAATGTTT ATCAACACTT CTGAATTCCC GGAGAAGAGT ATGTATGTGG
1151 TGACAAAGGA AAATCTGTG GGATTAATTA TTGCCATTCT CATCCCAGTG
1201 GCAGTGGCTG TCATTGTGTT AGTGGTGACA AGTATCCTTT GCTATCGGAA
1251 ACGAGAATGG ATTAAGAAA CCTTCTACCC TGATATCCA AATCCAGAAA
1301 ACTGTAAAGC ATTACAGTTT CAAAAGAGTG TCTGTGAGGG AAGCAGTGCT
1351 CTTAAAACAT TGGAAATGAA TCCTTGTAAC CCAAATAATG TTGAGGTTCT
1401 GGAAACTCGA TCAGCATTTC CTAAAATAGA AGATACAGAA ATAATTTCCC
1451 CAGTAGCTGA GCGTCCTGAA GATCGCTCTG ATGCAGAGCC TGAACCAT
1501 GTGGTTGTGT CCTATTGTCC ACCCATCATT GAGGAAGAAA TACCAAACCC
1551 AGCCGCAGAT GAAGCTGGAG GGACTGCACA GGTTATTTAC ATTGATGTTC
1601 AGTCGATGTA TCAGCCTCAA GCAAACCAG AAGAAGAACA AGAAAATGAC
1651 CCGTGTAGGAG GGGCAGGCTA TAAGCCACAG ATGCACCTCC CCATTAATTC
1701 TACTGTGGAA GATATAGCTG CAGAAGAGGA CTTAGATAAA ACTGCGGGTT
1751 ACAGACCTCA GGCCAATGTA AATACATGGA ATTTAGTGTC TCCAGACTCT
1801 CCTAGATCCA TAGACAGCAA CAGTGAGATT GTCTCATTG GAAGTCCATG
1851 CTCCATTAAT TCCCAGCAAT TTTTGATTCC TCCTAAAGAT GAAGACTCTC
1901 CTAATCTAA TGGAGGAGGG TGGTCTTTA CAAACTTTT TCAGAACAAA
1951 CCAAACGATT AAGGATCCAC TAGTTCTAGA GCGGCCGCCA CCGCGGTGGA
2001 GCTC

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Bold: start and stop codons

Amino acid sequence:

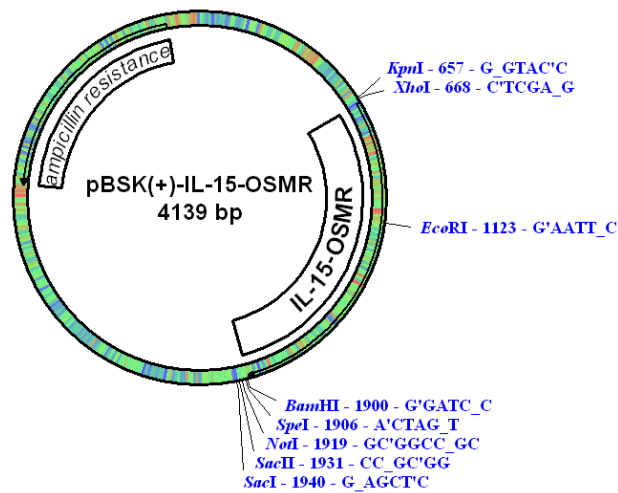
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IILANNSLSSNGNVTESGKCECELEEKNIKEFLQSFVHIVQMFINTSEF 150
PEKSMYVVTKENSVGLIIAIIIPVAVAVIVGVVTSILCYRKREWIKETFY 200
PDIPNPENCKALQFQKSVCEGSSALKTLEMNPCTPNNVVEVLETRSAFPKI 250
EDTEIISPVAERPEDRSDAEPENHVVVSYCPPIIIEEIPNPAADEAGGTA 300
QVIYIDVQSMYQPQAKPEEEQENDPVGGAGYKPMHLPINSTVEDIAAEE 350
DLDKTAGYRQPANVNTWNLVSPDPSRSDSNSEIVSFGSPCSINSRQFLI 400
PPKDEDSPKSNNGGWSFTNFFQNKPNPND*

```

Grey: bovine prolactin SP; italic: one additional amino acid and *Mlu*I site orange: human IL-15; brown: *Eco*RI site; red: human LIFR (15aa ECD+TMD+CPD)

7. pBSK(+)-IL-15-OSMR



Nucleotide sequence of IL-15-OSMR including relevant restriction sites

```

      GGTACCGG  GCCCCCCTC  GAGCCACCAT  GGACAGCAAA  GGTTCGTCCG
701  AGAAAGCAGG  GTCCCGCCTG  CTCCTGCTGC  TGGTGGTGTC  AAATCTACTC
751  TTGTGCCAGG  GTGTGGTCTC  CACCACGCGT  AACTGGGTGA  ATGTAATAAG
801  TGATTTGAAA  AAAATTGAAG  ATCTTATTC  ATCTATGCAT  ATTGATGCTA
851  CTTTATATAC  GGAAAGTGAT  GTTCACCCA  GTTGCAAAGT  AACAGCAATG
901  AAGTGCTTTC  TCTTGGAGTT  ACAAGTATT  TCACTTGAGT  CCGGAGATGC
951  AAGTATTCAT  GATACAGTAG  AAAATCTGAT  CATCCTAGCA  AACAAACAGT
1001  TGCTTCTAA  TGGGAATGTA  ACAGAATCTG  GATGCAAAGA  ATGTGAGGAA
1051  CTGGAGGAAA  AAAATATTTA  AGAATTTT  CAGAGTTTTG  TACATATTGT
1101  CCAATGTTT  ATCAACACTT  CTGAATCCC  CAGTGCTACG  TTCACGAAGG
1151  TCACGACTCC  GGATGAACAC  TCCTCGATG  TGATTCATAT  CCTACTGCC
1201  ATGGTTTTCT  GCGTCTTGCT  CATCATGGTC  ATGTGCTACT  TGAAAAGTCA
1251  GTGGATCAAG  GAGACCTGTT  ATCCTGACAT  CCCTGACCCT  TACAAGAGCA
1301  GCATCTGTC  ATTAATAAAA  TTCAAGGAGA  ACCCTCACCT  AATAATAATG
1351  AATGTCAGTG  ACTGTATCCC  AGATGCTATT  GAAGTTGTAA  GCAAGCCAGA
1401  AGGGACAAA  ATACAGTTCC  TAGGCACTAG  GAAGTCACTC  ACAGAAACCG
1451  AGTTGACTAA  GCCTAACTAC  CTTTATCTCC  TTCCAACAGA  AAAGAATCAC
1501  TCTGGCCCTG  GCCCCTGCAT  CTGTTTTGAG  AACTTGACCT  ATAACCAGGC
1551  AGCTTCTGAC  TCTGGCTCTT  GTGGCCATGT  TCCAGTATCC  CCAAAGCCC
1601  CAAGTATGCT  GGGACTAATG  ACCTCACCTG  AAAATGTACT  AAAGGCACTA
1651  GAAAAAACT  ACATGAACTC  CCTGGGAGAA  ATCCAGCTG  GAGAAACAAG
1701  TTTGAATTAT  GTGTCCCAGT  TGGCTTCACC  CATGTTTGA  GACAAGGACA
1751  GTCTCCAAC  AAACCCAGTA  GAGGCACCAC  ACTGTTTCA  GTATAAAATG
1801  CAAATGGCAG  TCTCCCTGCG  TCTTGCCTG  CCTCCCCGA  CCGAGAATAG
1851  CAGCCTCTCC  TCAATTACCC  TTTTAGATCC  AGGTGAACAC  TACTGCTAAG
1901  GATCCACTAG  TTCTAGAGCG  GCCGCCACCG  CGGTGGAGCT  C

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Amino acid sequence:

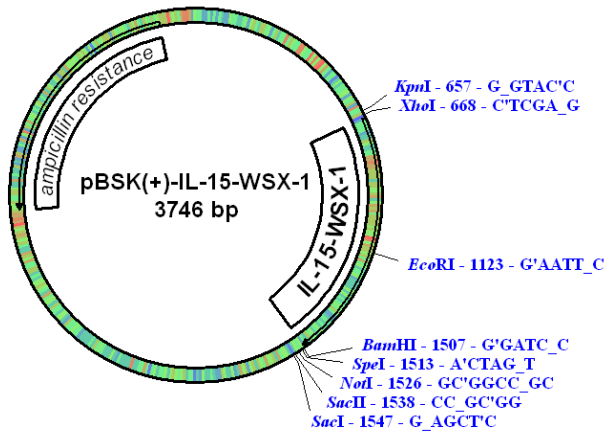
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MDSKGSSQKAGSRLLLLLVSNLLLCQGVVSTTRNWVNVISDLKKIEDLI 50
QSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESGDASIHDTVENL 100
IILANNLSSSNGNVTESGKECEEELEEKNIKEFLQSFVHIVQMFINTSEF 150
PSATFTKVTTPDEHSSMLIHILLPMVFCVLLIMVMCYLKSQWIKETCYPD 200
IPDPYKSSILSLIKFKENPHLIIMNVSDCIPDAIEVVSKPEGTKIQFLGT 250
RKSLTETELTKPNYLYLLPTEKNHSGPGPCICFENLTYNQAASDSGSCGH 300
VPVSPKAPSMLGLMTSPENVLKALEKNYMNSLGEIPAGETSLNYVSQLAS 350
PMFGDKDSLPTNPVEAPHCSEYKMQMAVSLRLALPPPTENSSLSSITLLD 400
PGEHYC*

```

Grey: bovine prolactin SP; italic: one additional amino acid and *MluI* site
orange: human IL-15; brown: *EcoRI* site; green: human OSMR (15aa ECD+TMD+CPD)

8. pBSK(+)-IL-15-GPL



Nucleotide sequence of IL-15-GPL including relevant restriction sites

```

      GGTACCGG  GCCCCCCTC  GAGCCACCAT  GGACAGCAAA  GGTTCGTCCG
701  AGAAAGCAGG  GTCCCGCCTG  CTCCTGCTGC  TGGTGGTGTC  AAATCTACTC
751  TTGTGCCAGG  GTGTGGTCTC  CACCACGCGT  AACTGGGTGA  ATGTAATAAG
801  TGATTGAAA  AAAATTGAAG  ATCTTATTC  ATCTATGCAT  ATTGATGCTA
851  CTTTATATAC  GGAAAGTGAT  GTTCACCCCA  GTTGCAAAGT  AACAGCAATG
901  AAGTGCTTTC  TCTTGGAGTT  ACAAGTTATT  TCACTTGAGT  CCGGAGATGC
951  AAGTATTCAT  GATACAGTAG  AAAATCTGAT  CATCCTAGCA  AACAAACAGTT
1001  TGTCTTCTAA  TGGGAATGTA  ACAGAATCTG  GATGCAAAGA  ATGTGAGGAA
1051  CTGGAGGAAA  AAAATATTAA  AGAATTTTGT  CAGAGTTTTG  TACATATTGT
1101  CCAAATGTTC  ATCAACACTT  CTGAATCAC  CAGCATAAAT  TTCAAGACAT
1151  TGTCATTAG  TGTCTTTGAG  ATTATCCTCA  TAACTTCTCT  GATTGGTGGA
1201  GGCCTTCTTA  TTCTCATTAT  CCTGACAGTG  GCATATGGTC  TCAAAAAACC
1251  CAACAAATTG  ACTCATCTGT  GTTGGCCAC  CGTTCCCAAC  CCTGCTGAAA
1301  TAGTATAGC  CACATGGCAT  GGAGATGATT  TCAAGGATAA  GCTAAACCTG
1351  AAGGAGTCTG  ATGACTCTGT  GAACACAGAA  GACAGGATCT  TAAAACCATG
1401  TTCCACCCCC  AGTGACAAGT  TGGTGATTGA  CAAGTTGGTG  GTGAACTTGT
1451  GGAATGTTCT  GCAAGAAATT  TTCACAGATG  AAGCCAGAAC  GGGTCAGGAA
1501  ACAATTTAG  GAGGGGAAAA  GAATGGGTAT  GTGACCTGCC  CCTTCAGGCC
1551  TGATTGTCCC  CTGGGGAAAA  GTTTTGAGGA  GCTCCAGTT  TCACCTGAGA
1601  TTCCGCCCAG  AAAATCCCAA  TACCTACGTT  CGAGGATGCC  AGAGGGGACC
1651  CGCCAGAAAG  CCAAAGAGCA  GCTTCTCTTT  TCTGGTCAAA  GTTTAGTACC
1701  AGATCATCTG  TGTGAGGAAG  GAGCCCCAAA  TCCATATTTG  AAAAATTCAG
1751  TGACAGCCAG  GGAATTTCTT  GTGTCTGAAA  AACTTCCAGA  GCACACCAAG
1801  GGAGAAGTCTT  AAGGATCCAC  TAGTCTAGA  GCGGCCGCCA  CCGCGG

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Bold: start and stop codons

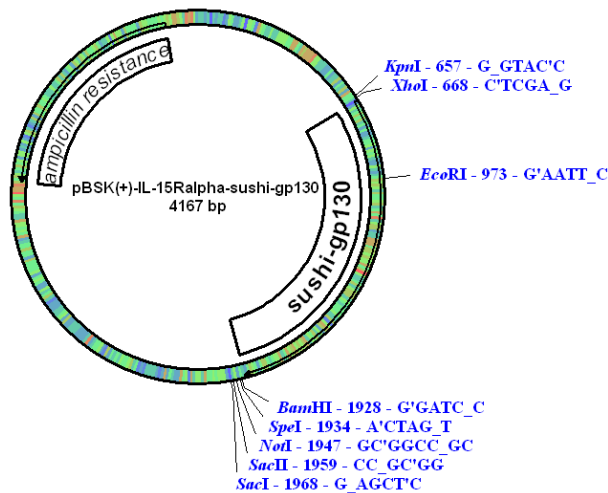
Amino acid sequence:

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MDSKGSSQKAGSRLLLLLVSNLLLCQGVVS TTRNWVNVISDLKKIEDLI 50
QSMHIDATLYTESDVHPCKVMTAMKCFLELQVISLESGDASIHDTVENL 100
IILANNSLSSNGNVTESGKKECEELEEKNIKEFLQSFVHIVQMFINTSEF 150
TSINFKTLFSVFEIILITSLIGGLLILIIITVAYGLKKNLTHLCWP 200
TVPNPAESSIATWHGDDFDKDLNLKESDDSVNTEDRILKPCSTPSDKLVI 250
DKLVVNFQVNLQEIFTDEARTQENNLGGEKNGYVTCFPRPDCPLGKSFE 300
ELPVSPEIPPRKSQYLRSRMPGTRPEAKEQLLFSGQSLVPDHLCEEGAP 350
NPYLKNSVTAREFLVSEKLPHEHTKGEV*

```

Grey: bovine preprolactin SP; italic: one additional amino acid and *Mlu*I site
orange: human IL-15; brown: *Eco*RI site; blue: human GPL (15aa ECD+TMD+CPD)

9. pBSK(+)-IL-15R α -sushi-gp130

Nucleotide sequence of sushi-gp130 including relevant restriction sites:

```

GGTACCGG GCCCCCCCTC GAGCCACCAT GTTGACGTTG CAGACTTGGC
701 TAGTGCAAGC CTTGTTTATT TTCCTCACCA CTGAATCTAC AGGTGAGCAG
751 AAGCTGATCA GCGAGGAGGA CCTGATCACA TGCCCTCCCC CCATGTCCGT
801 GGAACACGCA GACATCTGGG TCAAGAGCTA CAGCTTG↓TAC TCCAGGGAGC
851 GGTACATTTG TAAC↓TCTGGT TTCAAGCGTA AAGCCGGCAC GTCCAGCCTG
901 ACGGAGTGCG TGTTGAACAA GGCCACGAAT GTCGCCCACT GGACAACCCC
951 CAGTCTCAA TGCATTAGAG ACGAATTCAC TTTTACTACC CCAAAGTTTG
1001 CTCAAGGAGA AATTGAAGCC ATAGTCGTGC CTGTTTGCTT AGCATTCCCTA
1051 TTGACAAC↓TTCTGGGAGT GCTGTTCTGC TTTAATAAGC GAGACCTAAT
1101 TAAAAACAC ATCTGGCCTA ATGTTCAGAG TCCTTCAAAG AGTCATATTG
1151 CCCAGTGGTC ACCTCACACT CCTCCAAGGC ACAATTTTAA TTCAAAAGAT
1201 CAAATGTATT CAGATGGCAA TTTCAGTAT GTAAGTGTG TGGAAATAGA
1251 AGCAAATGAC AAAAAGCCTT TTCCAGAAGA TCTGAAATTA TTGGACCTGT
1301 TCAAAAAGGA AAAAATTAAT ACTGAAGGAC ACAGCAGTGG TATTGGGGGG
1351 TCTTCATGCA TGTCATCTC TAGGCCAAGC ATTTCTAGCA GTGATGAAAA
1401 TGAATCTTCA CAAAACACTT CGAGCACTGT CCAGTATTCT ACCGTGGTAC
1451 ACAGTGGCTA CAGACACCAA GTTCCGTCAG TCCAAGTCTT CTCAAGATCC
1501 GAGTCTACCC AGCCCTTGTT AGATTCAGAG GAGCGGCCAG AAGATCTACA
1551 ATTAGTAGAT CATGTAGATG GCGGTGATGG TATTTTGCCC AGGCAACAGT
1601 ACTTCAAACA GAAGTGCAGT CAGCATGAAT CCAGTCCAGA TATTTACAT
1651 TTTGAAAGGT CAAAGCAAGT TTCATCAGTC AATGAGGAAG ATTTTGTTAG
1701 ACTTAAACAG CAGATTTTCA ATCATATTTT ACAATCCTGT GGATCTGGGC
1751 AAATGAAAAA GTTTCAGGAA GTTCTGCAG CAGATGCTTT TGGTCCAGGT
1801 ACTGAGGGAC AAGTAGAAAG ATTTGAAACA GTTGGCATGG AGGCTGCGAC
1851 TGATGAAGGC ATGCCTAAA GTTACTTACC ACAGACTGTA CGGCAAGGCG
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1951 CGCCACCGCG GTGGAGCTC

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Bold: start and stop codons

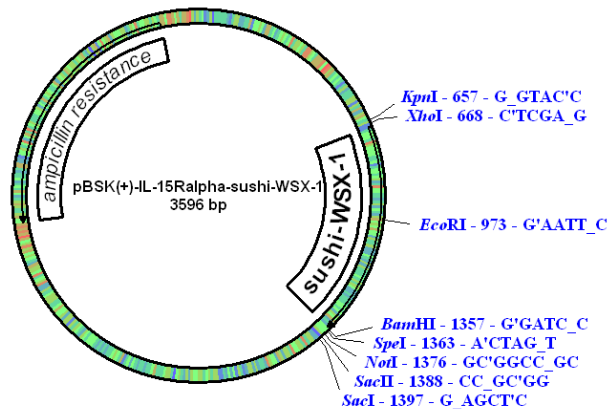
Amino acid sequence:

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FTTPPKFAQGEIEAIVVPVCLAFLLTLLGLVLFVCFNKRDLIKKHIWPNVP 150
DPSKSHIAQWSPHTPPRHNFNSKQMYSDGNFTDVSVVEIEANDKKPFPE 200
DLKLLDLFKKEKINTEGHSSGIGSSCMSSSRPSISSSDENESSQNTSST 250
VQYSTVVHSGYRHOVPSVQVFSRSESTQPLLDSEERPEDLQLVDHVDGGD 300
GILPRQYFYKQNCQSHESPDIHFERSKQVSSVNEEDFVRLKQQISDHI 350
SQSCGSGQMKMFQVSAADAFPGTGEQVERFETVGMEEATDEGMPKSYL 400
PQTVRQGGYMPQ*

```

Black: human gp130 (SP and 15aa ECD+TMD+CPD); pink: Myc-tag; light blue: human IL-15R α -sushi domain; brown: *EcoRI* site

10. pBSK(+)-IL-15R α -sushi-WSX-1

Nucleotide sequence of sushi-WSX-1 including relevant restriction sites

```

      GGTACCGG  GCCCCCCTC  GAGCCACCAT  GTTGACGTTG  CAGACTTGCC
701 TAGTGCAAGC  CTTGTTTATT  TTCCTACCA  CTGAATCTAC  AGGTGAGCAG
751 AAGCTGATCA  GCGAGGAGGA  CCTGATCACA  TGCCCTCCCC  CCATGTCCGT
801 GGAACACGCA  GACATCTGGG  TCAAGAGCTA  CAGCTTGATC  TCCAGGGAGC
851 GGTACATTTG  TAACTCTGGT  TTCAAGCGTA  AAGCCGGCAC  GTCCAGCCTG
901 ACGGAGTGCG  TGTTGAACAA  GGCCACGAAT  GTCGCCCACT  GGACAACCCC
951 CAGTCTCAAA  TGCATTAGAG  ACGAATTCTC  ACTTCACCTA  CCAGATAATA
1001 GGATCAGGTG  GAAAGCTCTG  CCCTGGTTTC  TGTCCCTGTG  GGGTTTGCTT
1051 CTGATGGGCT  GTGGCCTGAG  CCTGGCCAGT  ACCAGGTGCC  TACAGGCCAG
1101 GTGCTTACAC  TGGCGACACA  AGTTGCTTCC  CCAGTGGATC  TGGGAGAGGG
1151 TTCCTGATCC  TGCCAACAGC  AATTCTGGGC  AACCTTACAT  CAAGGAGGTG
1201 AGCCTGCCCC  AACCGCCCAA  GGACGGACCC  ATCCTGGAGG  TGGAGGAAGT
1251 GGAGCTACAG  CCTGTTGTGG  AGTCCCCTAA  AGCCTCTGCC  CCGATTTACT
1301 CTGGGTATGA  GAAACACTTC  CTGCCACAC  CAGAGGAGCT  GGGCCTTCTA
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Bold: start and stop codons

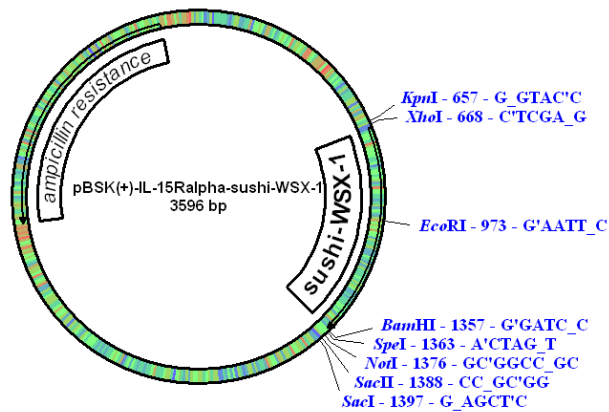
Amino acid sequence:

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MLTLQTWLVQALFIFLTTTESTGEQKLISEEDLITCPPPMSVEHADIWVKS  50
YSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTPSLKCIRDEF  100
SLHLPDNRIRWKALPWFLSLWGLLLMGCGLSLASTRCLQARCLHWRHKLL  200
PQWIWERPDPANSNSGQPYIKEVSLPQPPKDGPILEVEEVELQPVVESP  250
KASAPIYSGYEKHFLPTPEELLGLLV*

```

Black: human gp130 SP; pink: Myc-tag light blue: human IL-15R α -sushi domain;
brown: *EcoRI* site; purple: murine WSX-1 (15aa ECD+TMD+CPD)

11. pBSK(+)-IL-15R α -sushi-LIFR

Nucleotide sequence of sushi-LIFR including relevant restriction sites

```

GGTACCGG  GCCCCCCTC  GAGCCACCAT  GTTGACGTTG  CAGACTTGGC
701  TAGTGCAAGC  CTTGTTTTATT  TTCTCACCA  CTGAATCTAC  AGGTGAGCAG
751  AAGCTGATCA  GCGAGGAGGA  CCTGATCACA  TGCCCTCCCC  CCATGTCCGT
801  GGAACACGCA  GACATCTGGG  TCAAGAGCTA  CAGCTTGTA  TCCAGGGAGC
851  GGTACATTG  TAACTCTGGT  TTCAAGCGTA  AAGCCGGCAC  GTCCAGCCTG
901  ACGGAGTGCG  TGTTGAACAA  GGCCACGAAT  GTCGCCACT  GGACAACCCC
951  CAGTCTCAA  TGCATTAGAG  ACGAATTCCC  GGAGAAGAGT  ATGTATGTGG
1001  TGACAAAGGA  AAATTCTGTG  GGATTAATTA  TTGCCATTCT  CATCCCAGTG
1051  GCAGTGGCTG  TCATTGTTGG  AGTGGTGACA  AGTATCCTTT  GCTATCGGAA
1101  ACGAGAATGG  ATTAAGAAA  CCTTCTACCC  TGATATTCCA  AATCCAGAAA
1151  ACTGTAAAGC  ATTACAGTTT  CAAAAGAGTG  TCTGTGAGGG  AAGCAGTGCT
1201  CTTAAAACAT  TGGAAATGAA  TCCTTGTA  CCAAATAATG  TTGAGGTTCT
1251  GGAAACTCGA  TCAGCATTTC  CTAATAAG  AGATACAGAA  ATAATTTCCC
1301  CAGTAGCTGA  GCGTCCTGAA  GATCGCTCTG  ATGCAGAGCC  TGAAAACCAT
1351  GTGGTTGTGT  CCTATTGTCC  ACCCATCATT  GAGGAAGAAA  TACCAAACCC
1401  AGCCGCAGAT  GAAGCTGGAG  GGACTGCACA  GGTTATTTAC  ATTGATGTTC
1451  AGTCGATGTA  TCAGCCTCAA  GCAAACCCAG  AAGAAGAACA  AGAAAATGAC
1501  CCTGTAGGAG  GGGCAGGCTA  TAAGCCACAG  ATGCACCTCC  CCATTAATTC
1551  TACTGTGGAA  GATATAGCTG  CAGAAGAGGA  CTTAGATAAA  ACTGCGGGTT
1601  ACAGACCTCA  GGCCAATGTA  AATACATGGA  ATTTAGTGTC  TCCAGACTCT
1651  CCTAGATCCA  TAGACAGCAA  CAGTGAGATT  GTCTCATTG  GAAGTCCATG
1701  CTCCATTAAT  TCCCAGCAAT  TTTTGATTCC  TCCTAAAGAT  GAAGACTCTC
1751  CTAAATCTAA  TGGAGGAGGG  TGGTCCTTTA  CAAACTTTTT  TCAGAACAAA
1801  CCAAACGATT  AAGGATCCAC  TAGTTCTAGA  GCGGCCGCCA  CCGCGGTGGA
1851  GCTC

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Bold: start and stop codons

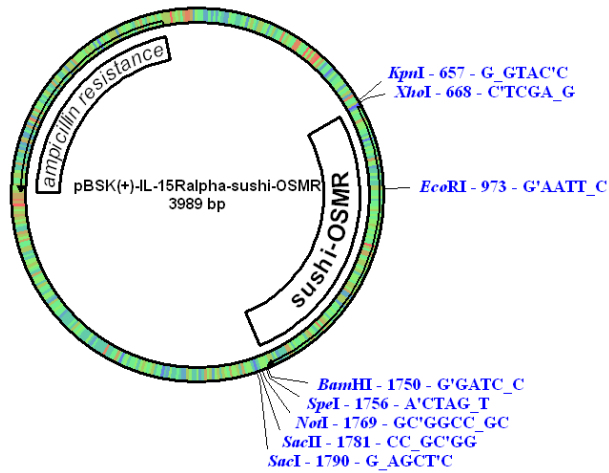
Amino acid sequence:

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MLTLQTLVQALFIFLTTESTGEQKLISEEDLITCPPPMSVEHADIWVKS 50
YSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDEF 100
PEKSMYVVTKENSVGLIIAILIPVAVAVIVGVVTSILCYRKREWIKETFY 200
PDIPNPENCKALQFQKSVCEGSSALKTLEMNPTPNNVEVLETRSAFPKI 250
EDTEIISPAERPEDRSDAEPENHVVSYCPPIIEEEIPNPAADEAGGTA 300
QVIYIDVQSMYQPQAKPEEEQENDPVGGAGYKPMHLPINSTVEDIAAEE 350
DLDKTAGYRPQANVNTWNLVSPDSPRSIDNSEIVSFGSPCSINSRQFLI 400
PKDEDSPKSNGGWSFTNFFQNKPND*

```

Black: human gp130 SP; pink: Myc-tag light blue: human IL-15R α -sushi domain;
brown: EcoRI site; red: human LIFR (15aa ECD+TMD+CPD)

12. pBSK(+)-IL-15R α -sushi-OSMR

Nucleotide sequence of sushi-OSMR including relevant restriction sites:

```

      GGTACCGG  GCCCCCCTC  GAGCCACCAT  GTTGACGTTG  CAGACTTGGC
701  TAGTGCAAGC  CTTGTTTATT  TTCCTCACCA  CTGAATCTAC  AGGTGAGCAG
751  AAGCTGATCA  GCGAGGAGGA  CCTGATCACA  TGCCCTCCCC  CCATGTCCGT
801  GGAACACGCA  GACATCTGGG  TCAAGAGCTA  CAGCTTGTAC  TCCAGGGAGC
851  GGTACATTTG  TAACTCTGGT  TTCAAGCGTA  AAGCCGGCAC  GTCCAGCCTG
901  ACGGAGTGCG  TGTTGAACAA  GGCCACGAAT  GTCGCCCACT  GGACAACCCC
951  CAGTCTCAAA  TGCATTAGAG  ACGAATTCCC  CAGTGCTACG  TTCACGAAGG
1001 TCACGACTCC  GGATGAACAC  TCCTCGATGC  TGATTCATAT  CCTACTGCCC
1051 ATGGTTTTCT  GCGTCTTGCT  CATCATGGTC  ATGTGCTACT  TGAAAAGTCA
1101 GTGGATCAAG  GAGACCTGTT  ATCCTGACAT  CCCTGACCCT  TACAAGAGCA
1151 GCATCCTGTC  ATTAATAAAA  TTCAAGGAGA  ACCCTCACCT  AATAATAATG
1201 AATGTCAGTG  ACTGTATCCC  AGATGCTATT  GAAGTTGTAA  GCAAGCCAGA
1251 AGGGACAAAG  ATACAGTTCC  TAGGCACTAG  GAAGTCACTC  ACAGAAACCG
1301 AGTTGACTAA  GCCTAACTAC  CTTTATCTCC  TTCCAACAGA  AAAGAATCAC
1351 TCTGGCCCTG  GCCCCTGCAT  CTGTTTGTAG  AACTTGACCT  ATAACCAGGC
1401 AGCTTCTGAC  TCTGGCTCTT  GTGGCCATGT  TCCAGTATCC  CAAAAGCCC
1451 CAAGTATGCT  GGGACTAATG  ACCTCACCTG  AAAATGTACT  AAAGGCACTA
1501 GAAAAAAACT  ACATGAACTC  CCTGGGAGAA  ATCCCAGCTG  GAGAAACAAG
1551 TTTGAATTAT  GTGTCCCAGT  TGGCTTACC  CATGTTTGA  GACAAGGACA
1601 GTCTCCAAC  AAACCCAGTA  GAGGCACCAC  ACTGTTTGA  GTATAAAATG
1651 CAAATGGCAG  TCTCCCTGCG  TCTTGCCCTG  CCTCCCCGA  CCGAGAATAG
1701 CAGCCTCTCC  TCAATTACCC  TTTTAGATCC  AGGTGAACAC  TACTGCTTAAG
1751 GATCCACTAG  TTCTAGAGCG  GCCGCCACCG  CGGTGGAGCT  C

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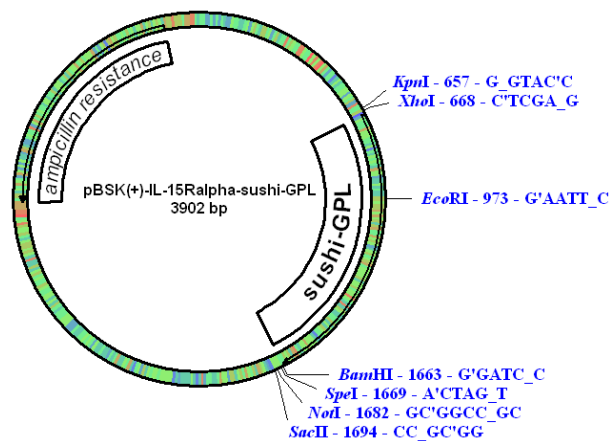
Amino acid sequence:

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MLTLQTLVLVQALFIFLTTTESTGEQKLISEEDLITCPPPMSVEHADIWVKS  50
YSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTPSLKCIRDEF  100
PSATFTKVITPDEHSSMLIHILLPMVFCVLLIMVMCYLKSQWIKETCYPD  200
IPDPYKSSILSLIKFKENPHLIIMNVSDCIPDAIEVVSKEPGTKIQFLGT  250
RKSLTETELTKPNYLYLLPTEKNHSGPGPCICFENLTYNQAASDSGSCGH  300
VPVSPKAPSMGLMTSPENVLKALEKNYMNSLGEIPAGETSLNYVSQLAS  350
PMFGDKDSLPTNPVEAPHCSEYKMQMAVSLRLALPPPTENSSLSSITLLD  400
PGEHYC*

```

Black: human gp130 SP; pink: Myc-tag light blue: human IL-15R α -sushi domain;
brown: *EcoRI* site; green: human OSMR (15aa ECD+TMD+CPD)

13. pBSK(+)-IL-15R α -sushi-GPL

Nucleotide sequence of sushi-OSMR including relevant restriction sites:

```

GGTACCGG  GCCCCCCTC  GAGCCACCAT  GTTGACGTTG  CAGACTTGGC
701 TAGTGCAAGC  CTTGTTTTATT  TTCCTCACCA  CTGAATCTAC  AGGTGAGCAG
751 AAGCTGATCA  GCGAGGAGGA  CCTGATCACA  TGCCCTCCCC  CCATGTCCGT
801 GGAACACGCA  GACATCTGGG  TCAAGAGCTA  CAGCTTGTAC  TCCAGGGAGC
851 GGTACATTTG  TAACCTCTGGT  TTCAAGCGTA  AAGCCGGCAC  GTCCAGCCTG
901 ACGGAGTGCG  TGTTGAACAA  GGCCACGAAT  GTCGCCCACT  GGACAACCCC
951 CAGTCTCAAA  TGCATTAGAG  ACGAATTCAC  CAGCATAAAT  TTCAAGACAT
1001 TGTCATTAG  TGTCTTTGAG  ATTATCCTCA  TAACTTCTCT  GATTGGTGGA
1051 GGCCTTCTTA  TTCTCATTAT  CCTGACAGTG  GCATATGGTC  TCAAAAAACC
1101 CAACAAATTG  ACTCATCTGT  GTTGGCCAC  CGTTCCCAAC  CCTGCTGAAA
1151 GTAGTATAGC  CACATGGCAT  GGAGATGATT  TCAAGGATAA  GCTAAACCTG
1201 AAGGAGTCTG  ATGACTCTGT  GAACACAGAA  GACAGGATCT  TAAAACCATG
1251 TTCCACCCCC  AGTGACAAGT  TGGTGATTGA  CAAGTTGGTG  GTGAACTTTG
1301 GGAATGTTCT  GCAAGAAATT  TTCACAGATG  AAGCCAGAAC  GGGTCAGGAA
1351 AACAAATTTAG  GAGGGGAAAA  GAATGGGTAT  GTGACCTGCC  CCTTCAGGCC
1401 TGATTGTCCC  CTGGGGAAAA  GTTTTGAGGA  GCTCCCAGTT  TCACCTGAGA
1451 TTCCGCCCAG  AAAATCCCAA  TACCTACGTT  CGAGGATGCC  AGAGGGGACC
1501 CGCCAGAAG  CCAAAGAGCA  GCTTCTCTT  TCTGGTCAAA  GTTTAGTACC
1551 AGATCATCTG  TGTGAGGAAG  GAGCCCCAAA  TCCATATTTG  AAAAAATCAG
1601 TGACAGCCAG  GGAATTTCTT  GTGTCTGAAA  AACTTCCAGA  GCACACCAAG
1651 GGAGAAGTCT  AAGGATCCAC  TAGTTCTAGA  GCGGCCGCCA  CCGCGG

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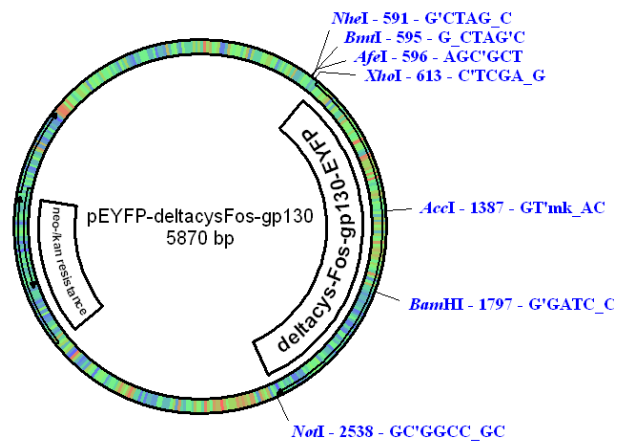
Amino acid sequence:

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TSINFKTLSFSVFEIILITSLIGGGLLILIIILTVAYGLKKPNKLTHLCWP 200
TVPNPAESSIATWHGDDFKDKLNLKESDDSVNTEDRILKPCSTPSDKLVI 250
DKLVVNFNGVLQEIFTDEARTQENNLGGEKNGYVTCFPRPCPLGKSFE 300
ELPVSPEIPPRKSQYLRSRMPEGTRPEAKEQLLFSGQSLVPDHLCEEGAP 350
NPYLKNSVTAREFLVSEKLPEHTKGEV*

```

Black: human gp130 SP; pink: Myc-tag light blue: human IL-15R α -sushi domain;
brown: *EcoRI* site; blue: human GPL (15aa ECD+TMD+CPD)

14. pEYFP- Δ cys-Fos-gp130

Nucleotide sequence of Δ cys-Fos-gp130-EYFP including relevant restriction sites:

```

GCTA
601 CCGGACTCAG ATCTCGAGTC TAGACCCCGC AAGATGTTGA CGTTGCAGAC
651 TTGGCTAGTG CAAGCCTTGT TTATTTTCCT CACCACTGAA TCTACAGGTG
701 ACTACAAAAGA TGACGATGAT AAAGAATTGG GCGGCGGCTT AACTGATACA
751 CTCCAAGCGG AGACAGACCA ACTAGAAGAT GAGAAGTCTG CTTTGCAGAC
801 CGAGATTGCC AACCTGCTGA AGGAGAAGGA AAAACTAGAG TTCATCCTGG
851 CAGCTGAATT CACTTTTACT ACCCCAAAGT TTGCTCAAGG AGAAATTGAA
901 GCCATAGTCG TGCCTGTTTG CTTAGCATTC CTATTGACAA CTCTTCTGGG
951 AGTGCTGTTC TGCTTTAATA AGCGAGACCT AATTAATAAAA CACATCTGGC
1001 CTAATGTTC AGATCCTTCA AAGAGTCATA TTGCCAGTG GTCACCTCAC
1051 ACTCCTCCAA GGCACAATTT TAATTCAAAA GATCAAATGT ATTCAGATGG
1101 CAATTTCACT GATGTAAGTG TTGTGGAAAT AGAAGCAAAT GACAAAAAGC
1151 CTTTCCAGA AGATCTGAAA TTATTGGACC TGTTCAAAAA GGAAAAAATT
1201 AATACTGAAG GACACAGCAG TGGTATTGGG GGGTCTTCAT GCATGTCATC
1251 TTCTAGGCCA AGCATTCTA GCAGTGATGA AAATGAATCT TCACAAAACA
1301 CTTGAGCAC TGTCCAGTAT TCTACCGTGG TACACAGTGG CTACAGACAC
1351 CAAGTCCGT CAGTCCAAGT CTTCTCAAGA TCCGAGTCTA CCCAGCCCTT
1401 GTTAGATTCA GAGGAGCGGC CAGAAGATCT ACAATTAGTA GATCATGTAG
1451 ATGGCGGTG TGGTATTTTG CCCAGGCAAC AGTACTTCAA ACAGAACTGC
1501 AGTCAGCATG AATCCAGTCC AGATATTTC AATTTGAAA GGTCAAAGCA
1551 AGTTTCATCA GTCAATGAGG AAGATTTTGT TAGACTTAAA CAGCAGATTT
1601 CAGATCATAT TTCACAATCC TGTGGATCTG GGCAAATGAA AATGTTTCAG
1651 GAAGTTTCTG CAGCAGATGC TTTTGGTCCA GGTACTGAGG GACAAGTAGA
1701 AAGATTGAA ACAGTTGGCA TGGAGGCTGC GACTGATGAA GGCATGCCTA
1751 AAAGTTACTT ACCACAGACT GTACGGCAAG GCGGCTACAT GCCTCAGGAT
1801 CCACCGGTCG CCACCATGGT GAGCAAGGGC GAGGAGCTGT TCACCGGGGT
1851 GGTGCCCATC CTGGTCGAGC TGGACGGCGA CGTAAACGGC CACAAGTTCA
1901 GCGTGTCGGG CGAGGGCGAG GCGGATGCCA CCTACGGCAA GCTGACCCTG
1951 AAGTTCATCT GCACCACCGG CAAGCTGCCG GTGCCCTGGC CCACCTCGT
2001 GACCACCTTC GGCTACGGCC TGCAGTGCTT CGCCCGCTAC CCCGACCACA
2051 TGAAGCAGCA CGACTTCTTC AAGTCCGCCA TGCCCGAAGG CTACGTCCAG
2101 GAGCGCACCA TCTTCTTCAA GGACGACGGC AACTACAAGA CCCGCGCCGA
2151 GGTGAAGTTC GAGGGCGACA CCCTGGTGAA CCGCATCGAG CTGAAGGGCA
2201 TCGACTCAA GGAGGACGGC AACATCCTGG GGCACAAGCT GGAGTACAAC
2251 TACAACAGCC ACAACGTCTA TATCATGGCC GACAAGCAGA AGAACGGCAT
2301 CAAGGTGAAC TTCAAGATCC GCCACAACAT CGAGGACGGC AGCGTGCAGC
2351 TCGCCGACCA CTACCAGCAG AACACCCCA TCGGCGACGG CCCCCTGCTG
2401 CTGCCCGACA ACCACTACCT GAGCTACCAG TCCGCCCTGA GCAAAGACCC
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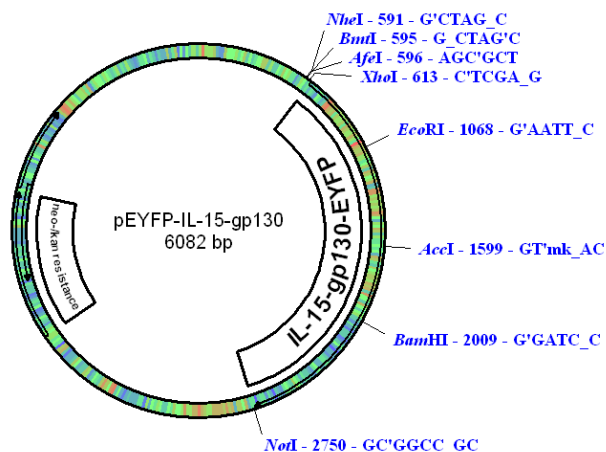
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Amino acid sequence:

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KSALQTEIANLLKEKEKL**EF**ILAAEFTFTTPKFAQGEIEAIVVPVCLAF 100
LTTLLGVLFVCFNKRDLIKKHIWPNVPDPSKSHIAQWSPHTPPRHNFNSKD 150
QMYSDGNFTDVSVVEIEANDKKPFPELKLDDLFKKEKINTEGHSSGIGG 200
SSCMSSSRPSISSDENESSQNTSSSTVQYSTVVHSGYRHQVPSVQVFSRS 250
ESTQPLLDSEERPDLQLVDHVDGGDILPRQQYFKQNCSEQHSSPDISH 300
FERSKQVSSVNEEDFVRLKQQISDHISQSCGSGQMKMFQEVSAADAFGPG 350
TEGQVERFETVGMEEATDEGMPKSYLPQTVRQGGYMPQ**DPPVATMVSKGE** 400
ELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTTLKFICTTGKLPV 450
PWPTLVTTFGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGN 500
YKTRAEVKFEQDTLVNRIELKGIKDFKEDGNILGHKLEYNYNSHNVYIMAD 550
KQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHLYSYQS 600
ALSKDPNEKRDHMLLEFVTAAGITLGMDELYK*

Black: human gp130 (SP and 15aa ECD+TMD+CPD); green: FLAG tag; italic: linker (w/o cysteine); blue: Fos leucine zipper; brown: *EcoRI* site, bold: linker; bold and yellow framed: EYFP

15. pEYFP-IL-15-gp130



Nucleotide sequence of IL-15-gp130 including relevant restriction sites:

```

GCTA
601 CCGGACTCAG ATCTCGAGCC ACCATGGACA GCAAAGTTC GTCGCAGAAA
651 GCAGGGTCCC GCCTGCTCCT GCTGCTGGTG GTGTCAAATC TACTCTTGTTG
701 CCAGGGTGTG GTCTCCACCA CGCGTAACTG GGTGAATGTA ATAAGTGATT
751 TGAAAAAAAT TGAAGATCTT ATTCAATCTA TGCATATGTA TGCTACTTTA
801 TATACGGAAA GTGATGTTCA CCCCAGTTGC AAAGTAACAG CAATGAAAGTG
851 CTTTCTCTTG GAGTTACAAG TTATTTCACT TGAGTCCGGA GATGCAAGTA
901 TTCATGATAC AGTAGAAAAT CTGATCATCC TAGCAAACAA CAGTTTGTCT
951 TCTAATGGGA ATGTAACAGA ATCTGGATGC AAAGAATGTG AGGAACTGGA
1001 GGAAAAAAAT ATTAAAGAAT TTTTGCAGAG TTTTGTACAT ATTGTCCAAA
1051 TGTTTCATCAA CACTTCTGAA TTCACTTTTA CTACCCAAA GTTTGCTCAA
1101 GGAGAAATTG AAGCCATAGT CGTGCCTGTT TGCTTAGCAT TCCTATTGAC
1151 AACTCTTCTG GGAGTGCTGT TCTGCTTTAA TAAGCGAGAC CTAATTAATA
1201 AACACATCTG GCCTAATGTT CCAGATCCTT CAAAGAGTCA TATTGCCAG
1251 TGGTCACCTC ACACTCCTCC AAGGCACAAT TTTAATTCAA AAGATCAAAT
1301 GTATTCAGAT GGCAATTTCA CTGATGTAAG TGTGTGGAA ATAGAAGCAA
1351 ATGACAAAAA GCCTTTTCCA GAAGATCTGA AATTATTGGA CCTGTTCAA
1401 AAGGAAAAAA TTAATACTGA AGGACACAGC AGTGGTATTG GGGGTCTTC
1451 ATGCATGTCA TCTTCTAGGC CAAGCATTTC TAGCAGTGAT GAAAAATGAAT
1501 CTTACAAAAA CACTTCGAGC ACTGTCCAGT ATTCTACCGT GGTACACAGT
1551 GGCTACAGAC ACCAAGTTCC GTCAGTCCAA GTCTTCTCAA GATCCGAGTC
1601 TACCCAGCCC TTGTTAGATT CAGAGGAGCG GCCAGAAGAT CTACAATTAG
1651 TAGATCATGT AGATGGCGGT GATGGTATTT TGCCAGGCA ACAGTACTTC
1701 AAACAGAACT CGAGTCAGCA TGAATCCAGT CCAGATATTT CACATTTTGA
1751 AAGTCAAAG CAAGTTTCAT CAGTCAATGA GGAAGATTTT GTTAGACTTA
1801 AACAGCAGAT TTCAGATCAT ATTTACAAT CCTGTGGATC TGGCAAATG
1851 AAAATGTTTC AGGAAGTTTC TGCAGCAGAT GCTTTTGGTC CAGGACTGA
1901 GGGACAAGTA GAAAGATTG AAACAGTTGG CATGGAGGCT GCGACTGATG
1951 AAGGCATGCC TAAAAGTTAC TTACCACAGA CTGTACGGCA AGGCGGCTAC
2001 ATGCCTCAGG ATCCACCGGT CGCCACCATG GTGAGCAAG GCGAGGAGCT
2051 GTTACCGGG GTGGTGCCCA TCCTGGTCTGA GCTGGACGGC GACGTAAACG
2101 GCCACAAGTT CAGCGTGTC GCGAGGGCG AGGGCGATGC CACCTACGGC
2151 AAGCTGACCC TGAAGTTCAT CTGACCACC GCAAGCTGC CCGTGCCCTG
2201 GCCCACCTC GTGACCACCT TCGGCTACGG CCTGCAGTGC TTCGCCCCT
2251 ACCCGACCA CATGAAGCAG CACGACTTCT TCAAGTCCGC CATGCCGAA
2301 GGCTACGTCC AGGAGCGCAC CATCTTCTTC AAGGACGACG GCAACTACAA
2351 GACCCGCGCC GAGGTGAAGT TCGAGGGCGA CACCCTGGTG AACCGCATCG
2401 AGCTGAAGGG CATCGACTTC AAGGAGGACG GCAACATCCT GGGCACAAG
2451 CTGGAGTACA ACTACAACAG CCACAACGTC TATATCATGG CCGACAAGCA
2501 GAAGAACGGC ATCAAGGTGA ACTTCAAGAT CCGCCACAAC ATCGAGGACG
2551 GCAGCGTGCA GTCGCGGAC CACTACCAGC AGAACACCCC CATCGGCGAC
2601 GGCCCGTGC TGCTGCCCGA CAACCACTAC CTGAGCTACC AGTCCGCCCT
2651 GAGCAAAGAC CCCAACGAGA AGCGCGATCA CATGGTCTTG CTGGAGTTCTG
2701 TGACCGCCG CCGGATCACT CTCGGCATGG ACGAGCTGTA CAAGTAAAGC
2751 GGCCGC

```

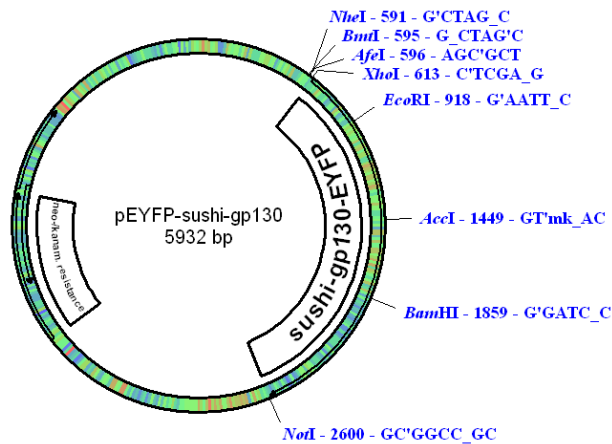
Bold: start and stop codons

Amino acid sequence:

MDSKGSSQKAGSRLLLLLLVSNLLLCQGVVS *TTR*NWVNI **SDLKKIEDLI** 50
QSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENL 100
IILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQMFINTSEF 150
TFTTPKFAQGEIEAIVVPVCLAFLLTLLGVLCFNKRDLIKKHIWPNVP 200
DPSKSHIAQWSPHTPPRHNFNSKDQMYSDGNFTDVSVVEIEANDKKPFPE 250
DLKLLDLFKKEKINTEGHSSGIGSSCMSSSRPSISSSDENESSQNTSST 300
VQYSTVHSGYRHOVPSVQVFSRSESTQPLLDSEERPEDLQLVDHVDGGD 350
GILPRQQYFKQNCSEQHSSPDISHFERSKQVSSVNEEDFVRLKQQISDHI 400
SQSCGSGQMKMFQEVSAADAFPGTEGQVERFETVGMEEAATDEGMPKSYL 450
PQTVRQGGYMPQ **DPPVATMVSKGEELFTGVVPILVELDGDVNGHKFSVSG** 500
EGEGDATYGKLT LKFICTTGKLPVPWPTLVTTFGYGLQCFARYPDHMKQH 550
DFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK 600
EDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGQVQLADH 650
YQQNTPIGDGPVLLPDNHLYSYQSALS KDPNEKRDMVLEFVTAAGITL 700
GMDELYK*

Grey: bovine preprolactin SP; italic: one additional amino acid and *Mlu*I site orange: human IL-15; brown: *Eco*RI site; black: human gp130 (15aa ECD+TMD+CPD); bold: linker; bold and yellow framed: EYFP

16. pEYFP-IL-15Ra-sushi-gp130



Nucleotide sequence of IL-15-gp130 including relevant restriction sites:

```

GCTA
601 CCGGACTCAG ATCTCGAGCC ACCATGTTGA CGTTGCAGAC TTGGCTAGTG
651 CAAGCCTTGT TTATTTTTCCT CACCACTGAA TCTACAGGTG AGCAGAAGCT
701 GATCAGCGAG GAGGACCTGA TCACATGCC TCCCCCATG TCCGTGGAAC
751 ACGCAGACAT CTGGGTCAAG AGCTACAGCT TGTACTCCAG GGAGCGGTAC
801 ATTTGTAAC CTGGTTTCAA GCGTAAAGCC GGCACGTCCA GCCTGACGGA
851 GTGCGTGTG AACAAAGGCCA CGAATGTCGC CCACTGGACA ACCCCAGTC
901 TCAAATGCAT TAGAGACGAA TTCACTTTTA CTACCCAAA GTTTGCTCAA
951 GGAGAAATTG AAGCCATAGT CGTGCCTGTT TGCTTAGCAT TCCTATTGAC
1001 AACTCTTCTG GGAGTGCTGT TCTGCTTTAA TAAGCGAGAC CTAATTAATA
1051 AACACATCTG GCCTAATGTT CCAGATCCCT CAAAGAGTCA TATTGCCAG
1101 TGGTCACCTC ACACCTCTCC AAGGCACAAT TTTAATTCAA AAGATCAAAT
1151 GTATTCATG GGCATTTCA CTGATGTAAG TGTGTGGAA ATAGAAGCAA
1201 ATGACAAAAA GCCTTTTCCA GAAGATCTGA AATTATTGGA CCTGTTCAA
1251 AAGGAAAAAA TTAATACTGA AGGACACAGC AGTGGTATTG GGGGGTCTTC
1301 ATGCATGTCA TCTTCTAGGC CAAGCATTTC TAGCAGTGAT GAAAATGAAT
1351 CTTACAAAAA CACTTCGAGC ACTGTCCAGT ATTCTACCGT GGTACACAGT
1401 GGCTACAGAC ACCAAGTTCC GTCAGTCCAA GTCTTCTCAA GATCCGAGTC
1451 TACCCAGCCC TTGTTAGATT CAGAGGAGCG GCCAGAAGT CTACAATTAG
1501 TAGATCATGT AGATGGCGGT GATGGTATT TGCCAGGCA ACAGTACTTC
1551 AAACAGAACT GCAGTCAGCA TGAATCCAGT CCAGATATTT CACATTTTGA
1601 AAGGTCAAAG CAAGTTTCAT CAGTCAATGA GGAAGATTTT GTTAGACTTA
1651 AACAGCAGAT TTCAGATCAT ATTTACAAT CCTGTGGATC TGGGCAAATG
1701 AAAATGTTTC AGGAAGTTTC TGCAGCAGAT GCTTTTGGTC CAGGTAAGTGA
1751 GGGACAAGTA GAAAGATTTG AAACAGTTGG CATGGAGGCT GCGACTGATG
1801 AAGCGATGCC TAAAAGTTAC TTACCACAGA CTGTACGGCA AGGCGGCTAC
1851 ATGCCTCAGG ATCCACCGGT CGCCACCATG GTGAGCAAGG GCGAGGAGCT
1901 GTTACCCGGG GTGGTGCCCA TCCTGGTCGA GCTGGACGGC GACGTAAACG
1951 GCCACAAGTT CAGCGTGTCC GCGGAGGGCG AGGGCGATGC CACCTACGGC
2001 AAGCTGACCC TGAAGTTCAT CTGCACCACC GGCAAGCTGC CCGTGCCCTG
2051 GCCCACCCCT GTGACCACCT TCGGCTACGG CCTGCAGTGC TTCGCCCGCT
2101 ACCCCGACCA CATGAAGCAG CACGACTTCT TCAAGTCCGC CATGCCCGAA
2151 GGCTACGTCC AGGAGCGCAC CATCTTCTTC AAGGACGACG GCAACTACAA
2201 GACCCGCGCC GAGGTGAAGT TCGAGGGCGA CACCCTGGTG AACCGCATCG
2251 AGCTGAAGGG CATCGACTTC AAGGAGGACG GCAACATCCT GGGGCACAAG
2301 CTGGAGTACA ACTACAACAG CCACAACGTC TATATCATGG CCGACAAGCA
2351 GAAGAACGGC ATCAAGGTGA ACTTCAAGAT CCGCCACAAC ATCGAGGACG
2401 GCAGCGTGCA GCTCGCCGAC CACTACCAGC AGAACACCCC CATCGGCGAC
2451 GGCCCCGTGC TGTGCCCCGA CAACCACTAC CTGAGCTACC AGTCCGCCCT
2501 GAGCAAAGAC CCCAACGAGA AGCGCGATCA CATGGTCCTG CTGGAGTTCC
2551 TGACCGCCGC CGGGATCACT CTCGGCATGG ACGAGCTGTA CAAGTAAAGC
2601 GGCCGC

```

Bold: start and stop codons

Amino acid sequence:

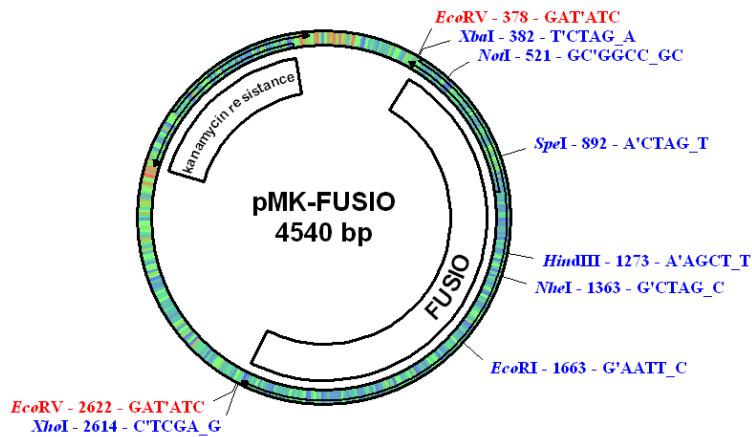
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MTTLQTWLVQALFIFLTTESTGEQKLISEEDLITCPPPMVSVEHADIWVKS 50
YSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTPSLKCIREF 100
TFTTPKFAQGEIEAIVVPVCLAFLLTLLGVLCFNKRDLIKKHIWPNVP 150
DPSKSHIAQWSPHTPPRHNFNSKDQMYSDGNFTDVSVVEIEANDKKPFPE 200
DLKLLDLFKKEKINTEGHSSGIGSSCMSSSRPSISSSDENESSQNTSST 250
VQYSTVHSGYRHQVPSVQVFSRSESTQPLLDSEERPEDLQLVDHVDGGD 300
GILPRQQYFKQNCSEQHSSPDISHFERSKQVSSVNEEDFVRLKQQISDHI 350
SQSCGSGQMKMFQEVSAADAFPGTEGQVERFETVGMEEATDEGMPKSYL 400
PQTVRQGGYMPQDPPVATMVSKGEELFTGVVPIVVELDGDVNGHKFSVSG 450
EGEGDATYGKLTCLKFICTTGKLPVPWPTLVTFYGLQCFARYPDHMKQH 500
DFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK 550
EDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGGSVQLADH 600
YQQNTPIGDGPVLLPDNHLYLSYQSALS KDPNEKRDHMLLEFVTAAGITL 650
GMDELYK*

```

Black: human gp130 (SP and 15aa ECD+TMD+CPD); pink: Myc-tag; light blue: human IL-15R α -sushi domain; brown: *Eco*RI site; bold: linker; bold and yellow framed: EYFP

17. pMK-FUSIO



Nucleotide sequence of IL-15-gp130 including relevant restriction sites:

```

                                     GATAT CTCTAGAGCC ACCATGGACA
401 GCAAGGGCTC TAGCCAGAAG GCCGGCAGCA GACTGCTCCT GCTCCTGGTC
451 GTGTCCAACC TGCTGCTGTG CCAGGGCGTG GTGTCCACCA CCAGAGACTA
501 CAAGGACGAC GACGACAAGG CGGCCGCCGG CCTGCCCAAG ACCGAGGCCA
551 ACTGGGTGAA CGTGATCAGC AGCCTGAAGA AGATCGAGGA CCTGATCCAG
601 AGCATGCACA TCGACGCCAC CCTGTACACA GAGAGCGACG TCCACCCAG
651 CTGCAAGGTG ACCGCCATGA AGTGCTTTCT GCTGGAGCTG CAGGTCATCA
701 GCCTGGAGAG CGGCCAGGCC AGCATCCACG ACACCGTGGA GAACCTGATC
751 ATCCTGGCCA ACAACAGCCT GAGCAGCAAC GGCAACGTGA CCGAGAGCGG
801 CTGCAAGGAG TGCAGGAGC TGGAGGAGAA GAACATCAAG GAGTTTCTGC
851 AGAGCTTCGT GCACATCGTG TCCATGTTCA TCAACACAAG CACTAGTAGC
901 CTGCATCTGC CCGACAACAG AATCAGATGG AAGGCCCTGC CCTGGTTCCT
951 GAGCCTGTGG GGCCGTGCTG TGATGGGCTG TGGCCTGAGC CTGGCCAGCA
1001 CCCGGTGTCT GCAGGCCAGA TGCCTGCATT GGCGGCACAA GCTGCTGCCC
1051 CAGTGGATCT GGGAGAGAGT GCCCAGCCCC GCCAACAGCA ACAGCGGCCA
1101 GCCCTACATC AAGGAGGTGT CCCTGCCCCA GCCCCCCAAG GACGGCCCCA
1151 TCCTGGAGGT GGAAGAGGTG GAGCTGCAGC CCGTGGTGGA GAGCCCTAAG
1201 GCCAGCGCCC CCATCTACAG CGGCTACGAG AAGCACTCC TGCCCACCCC
1251 TGAGGAGCTG GGACTGCTGG TGAAGCTTAG AGCCAAGAGG GCCCCTGTGA
1301 AGCAGACCCT GAACCTCGAC CTGCTGAAGC TGGCCGCGCA CGTGGAGAGC
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1401 CCTGTTTCATC TTCCTGACCA CCGAGAGCAC CGGCGAGCAG AAGCTGATCA
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1501 GACATCTGGG TGAAGTCCTA CTCTCTGTAT TCCAGAGAGC GGTACATCTG
1551 CAACTCCGGC TTCAAGAGAA AGGCCGGCAC CAGCTCCCTG ACCGAGTGCG
1601 TGCTGAACAA GGCCACCAAC GTGGCCCACT GGACCACCC CAGCCTGAAG
1651 TGCATCAGGG ACGAATTCAC CTTACCACC CCCAAGTTCG CTCAGGGCGA
1701 GATTGAGGCC ATCGTGGTGC CCGTGTGCC TGGCCTTCCTG CTGACAACCC
1751 TGCTGGGCGT GCTGTTCTGC TTCAACAAGC GGGACCTGAT CAAGAAGCAC
1801 ATCTGGCCCA ACGTGCCTGA CCCCAGCAAG AGCCACATCG CCCAGTGGAG
1851 CCCCCACACC CCCCCAGAC ACAACTTCAA CAGCAAGGAC CAGATGTACA
1901 GCGACGGCAA CTTACCAGAC GTGTCTGTGG TGGAGATCGA GGCTAACAAC
1951 AAGAAGCCCT GCCCCGACGA CCTGAAGTCC GTGGACCTGT TTAAGAAGGA
2001 GAAGGTCTCC ACCGAGGGCC ACAGCAGCGG CATCGGCGGC AGCAGCTGCA
2051 TGAGCAGCAG CAGACCCAGC ATCAGCAGCA ACGAGGAGAA CGAGAGCGCC
2101 CAGAGCACCG CCAGCACCGT GCAGTACAGC ACCGTGGTGC ACTCCGGCTA
2151 CAGACACCGG GTGCCAGCG TGCAAGTGT CAGCAGAAGC GAGAGCACCC
2201 AGCCCCTGCT GGACAGCGAG GAGAGGCCCG AGGACCTGCA GCTGGTCGAC
2251 AGCGTGGACG GCGGCGACGA GATCCTGCC AGACAGAGCT ACTTCAAGCA
2301 GAACTGCAGC CAGCCCGAGG CCTGCCCGA GATCAGCCAC TTCGAGAGAA
2351 GCAACCAGGT GCTGTCCGGC AATGAGGAG ACTTCGTCAG ACTGAAGCAG
2401 CAGCAGGTGT CCGACCACAT CAGCCAGCCT TACGGCAGCG AGCAGAGAAG
2451 GCTGTTCCAG GAGGACGCA CCGCCGACG TCTCGGAACA GGCGCCGACG
2501 GCCAGATGGA GAGATTGAG AGCGTGGGCA TGGAGACAAC CATCGACGAG
2551 GAGATCCCCA AGAGCTACCT GCCCAGACC GTGCGGCAGG GCGGCTACAT
2601 GCCCCAGTGA TGACTCGAGG ATATC
    
```

Bold: start and stop codons

Amino acid sequence (both proteins are separated by 2A peptide):

```

MDSKGSSQKAGSRLLLLLVVSNLLLCQGVVS TTRDYKDDDDKAAAGLPKT 50
EANWVNVISSLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQ 100
VISLESGDASIHDTVENLIILANNSSLSSNGNVTESGCKECEELEEKNIKE 150
FLQSFVHIVSMFINTS TSSLHLPDNRIRWKALPWFLSLWGLLLMGCGLSL 200
ASTRCLQARCLHWRHKLQWIWVDPANSSGQPYIKEVSLPQPPKD 250
GPILVEVEVELQPVVESPASAPIYSGYEKHF LPTPEELGLLVKL RAKRA 300
PVKQTLNFDLLKLAGDVESNPGPASMLTLQTWLVQALFIFLTTESTGEQK 350
LISEEDLITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSLT 400
ECVLNKATNVAHWTPSLKCI DEFTFTPKF AQGEIEAIVVPVCLAFLL 450
TLLGVLFCFNKRDLIKKHIWPNVDPSPKSHIAQWSPHTPPRHNFNSKDQ 500
MYSDGNFTDVSVVEIEANNKKPCPDDLKSVDLFKKEKVSTEGHSSGIGGS 550
SCMSSSRPSSISNEENESAQSTASTVQYSTVVHSGYRHQVPSVQVFSRSE 600
STQPLLDSEERPEDLQLVDSVDGGDEILPRQSYFKQNC SQPEACPEISHF 650
ERSNQVLSGNEEDFVRLKQQQVSDHISQPYGSEQRRLFQEGSTADALGTG 700
ADGQMERFESVGMETTIDEEIPKSYLPQTVRQGGYMPQ*

```

Grey: bovine preprolactin SP; italic and yellow framed: one additional amino acid and *Mlu*I site; green: FLAG-tag; brown: different restriction sites in chronological order *Not*I, *Spe*I, *Hind*III, *Nhe*I *Eco*RI; italic: linker; orange: human IL-15 mutated D8S + Q108S; purple: murine WSX-1 (15aa ECD+TMD+CPD); bold and green framed: furin cleavage site; bold and red framed: 2A peptide; pink: Myc-tag; light blue: human IL-15 α -sushi domain; black: human gp130 SP; black framed: murine gp130(15aa ECD+TMD+CPD)

Please contact the author of this work (Jan.Suthaus@web.de) if complete plasmid sequences and additional plasmid sequences (pMOWS and p409) containing the chimeric receptors ORFs are needed.

10 List of publications

Suthaus, J., A. Tillmann, I. Lorenzen, E. Bulanova, S. Rose-John, and J. Scheller, *Forced Homo- and Heterodimerization of all gp130-type Receptor Complexes Leads to Constitutive Ligand-independent Signaling, and Cytokine-independent Growth*. Mol Biol Cell, 2010. Mol Biol Cell, 2010. 21(15): p. 2797-807.

Waetzig, G.H., A. Chalaris, P. Rosenstiel, **J. Suthaus**, C. Holland, N. Karl, L. Valles Uriarte, A. Till, J. Scheller, J. Grotzinger, S. Schreiber, S. Rose-John, and D. Seegert, *N-linked glycosylation is essential for the stability but not the signaling function of the interleukin-6 signal transducer glycoprotein 130*. J Biol Chem. 285(3): p. 1781-9.

Adam, N., B. Rabe, **J. Suthaus**, J. Grotzinger, S. Rose-John, and J. Scheller, *Unraveling viral interleukin-6 binding to gp130 and activation of STAT-signaling pathways independently of the interleukin-6 receptor*. J Virol, 2009. 83(10): p. 5117-26.

Schreiner, A., M. Ruonala, V. Jakob, **J. Suthaus**, E. Boles, F. Wouters, and A. Starzinski-Powitz, *Junction protein shrew-1 influences cell invasion and interacts with invasion-promoting protein CD147*. Mol Biol Cell, 2007. 18(4): p. 1272-81.

11 Acknowledgement

Ich möchte mich sehr herzlich bei Herrn Prof. Dr. Stefan Rose-John und Herrn Prof. Dr. Jürgen Scheller, die mich während meiner Zeit im Institut für Biochemie intensiv betreut haben, für die schöne Zeit bedanken. Des Weiteren bedanke ich mich bei beiden für die schnelle Korrektur der vorliegenden Arbeit. Außerdem gilt beiden meinen Dank für die Möglichkeit, die Arbeit nach meinen experimentellen Vorstellungen anzufertigen.

Besonders möchte ich mich auch bei Herrn Professor Roeder für die Übernahme des Zweitgutachtens dieser externen Promotion bedanken.

Bei Frau Dr. Elena Bulanova und Frau Anna Tillmann möchte ich mich ganz herzlich für die gute Zusammenarbeit bedanken.

Meinen Kollegen bzw. Ex-Kollegen Athena Chalaris, Doreen Floss, Jessica Rabsch (geb. Gewiese), Björn Rabe und Inken Lorenzen sei ein besonderer Dank für die hilfreichen Diskussionen und die lustige Arbeitsatmosphäre, ohne die vieles schwerer zu ertragen gewesen wäre, ausgesprochen. Vielen Dank liebe Leute.

Liebe Eltern, auch an euch sei ein großes Dankeschön zu entrichten, ihr habt mich immer ermutigt weiterzumachen, selbst wenn euch meine Thematik mehr als fremd erscheint. Danke für alles und besonders für die tollen Süßigkeiten-Pakete aus der Heimat.

Zum Schluss möchte ich einer ganz besonderen Person danken. Worte können meinen Dank zwar nur sehr spärlich ausdrücken, aber ich versuche es mal: Liebe Ulrike, ohne dich würde mir wirklich etwas fehlen. Du bist die Muse, die mich geküsst hat. Ich verdanke dir wirklich die Inspiration das IL-15/IL-15R α -System oder das Heterodimerisierungssystem nach Suthaus, wie du es aus Spaß gerne nennst, zu entwickeln. Dank deiner aufmunternden Art hast du mir immer wieder Mut gemacht die Promotionszeit durchzustehen. Durch dich habe ich auch mich besser kennengelernt. Vielen, vielen Dank. Du bist die Beste.

12 Curriculum vitae

Name: Jan Suthaus
Geburtstag: 02.05.1980
Geburtsort: Bad Homburg v.d.H.
Staatsangehörigkeit: deutsch

- 2005 – 2010 Anfertigung der Promotionsarbeit bei Herrn Prof. Dr. Rose-John am Institut für Biochemie der Christian-Albrechts-Universität zu Kiel mit dem Titel: "Ligand-independent gp130-type Receptor Activation by Forced Homo- and Heterodimerization"
- 2004 – 2005 Anfertigung der Diplomarbeit bei Frau Prof. Dr. Starzinski-Powitz am Institut für Zellbiologie & Neurowissenschaft der Johann Wolfgang Goethe-Universität in Frankfurt am Main mit dem Titel: "Untersuchungen zur Invasivität von Tumorzellen in Abhängigkeit des Proteins shrew-1"
- 2004 Diplomprüfungen in den Fächern Zellbiologie (Hauptfach), Genetik sowie Physiologie & Biochemie der Pflanzen
- 2000 – 2005 Studium der Biologie an der Johann Wolfgang Goethe-Universität in Frankfurt am Main mit dem Abschluss Diplom-Biologe
- 1999 – 2000 Grundwehrdienst im Sanitätsdienst
- 1992 – 1999 Besuch des Kaiserin-Friedrichs-Gymnasiums in Bad Homburg v.d.H. mit Abschluss Abitur
- 1990 – 1992 Besuch der Förderstufe der Gesamtschule am Gluckenstein in Bad Homburg v.d.H.
- 1986 – 1990 Besuch der Grundschule Obereschbach in Bad Homburg v.d.H.

13 Eidesstattliche Erklärung

Hiermit versichere ich, Jan Suthaus, an Eides statt, dass ich die vorliegende Arbeit selbständig und nur mit Hilfe der angegebenen Hilfsmittel und Quellen unter Anleitung meiner akademischen Lehrer unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft angefertigt habe.

Diese Dissertation wurde bisher an keiner anderen Fakultät vorgelegt.

Ich erkläre, kein anderes Promotionsverfahren ohne Erfolg beendet zu haben und dass keine Aberkennung eines bereits erworbenen Doktorgrades vorliegt.

Kiel, den 27.07.2010

Jan Suthaus