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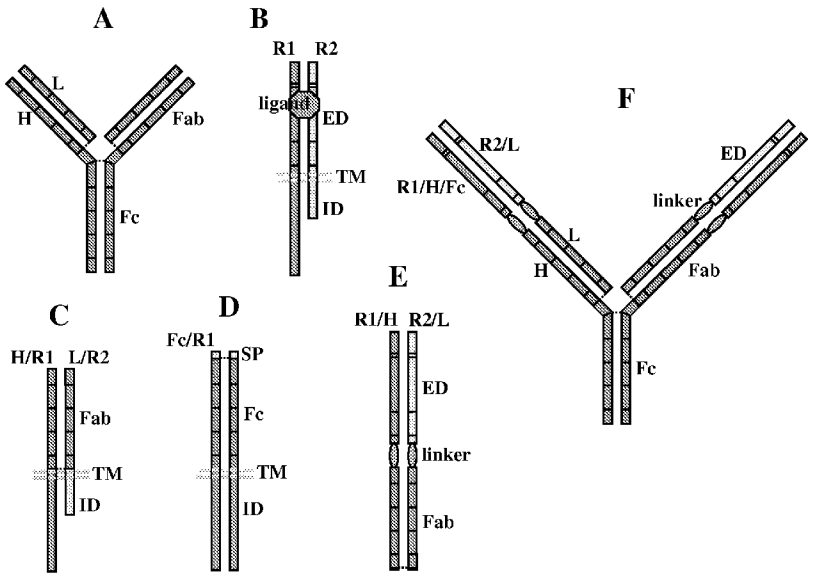
(54) **Title:** A STRATEGY FOR HOMO- OR HETERO-DIMERIZATION OF VARIOUS PROTEINS IN SOLUTIONS AND IN CELLS



(57) **Abstract:** The present invention describes a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain. The present invention also describes a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region. Polynucleotides encoding the chimeric polypeptides and methods of use of the chimeric polypeptides are also described.

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A Strategy for Homo- or Hetero-Dimerization of Various Proteins in Solutions and in Cells

Field of the Invention

This invention relates to a novel method for the generation of homo- and hetero-dimeric protein complexes which include heterodimeric chimeric soluble receptor complexes and constitutively active ligand-independent homo- or heterodimeric membrane-bound chimeric receptor complexes. These chimeric complexes may be used for the treatment of various diseases or as a research tool.

Background of the Invention

Development of cancer is a multi-step process. It is likely to be started with one point mutation which gives a single cell a minor advantage - either increased rate of uncontrolled proliferation, or unresponsiveness to external signals commanding the cell to commence apoptosis. The immune system also reacts to and destroys the mutated cell and its progeny. However, in rare events, one or more of the cell's offspring, in addition to the initial advantageous mutation, gain other mutations to protect themselves from the immune surveillance. Thus, the immune system is engaged in all steps of tumor development and progression, and the failure of the immune system to recognize and eliminate cancerous cells is a must for tumor survival and progression.

Cytokines regulate a broad array of cellular and immunological functions. They exert their biological activities through the binding to cell surface receptors leading to

induction of specific signal transduction events. Cytokines are powerful weapons which are utilized by both cancer cells and the immune system for their own advantages. For example, tumors can benefit from immunosuppressive properties of IL-10. By expressing IL-10, tumors can downregulate antigen presentation and inhibit immune response directed against tumor cells. In addition, several cytokines can stimulate proliferation of tumor cells. If such cytokines are produced by tumors, they may serve as autocrine growth factors for tumor cells. On the other hand, the immune system can inhibit tumor growth through the expression of IFNs, cytokines with strong antiproliferative activity. In addition, IFNs and other cytokines can mobilize and activate various aspects of the immune system to fight cancer.

Many drugs and methods targeting tumors for destruction are being developed and are in trials for their effectiveness. However, it is unlikely that a single drug equally effective in fighting different cancers can ultimately be created, particularly since even a single tumor often represents a pool of cells which have accumulated different multiple mutations. The immune system, on the other hand, is the army of "universal soldiers" which ideally should be able to deal with any type of cancer. In reality, however, the immune system, in the case of cancers, is not able to handle them on its own and several strategies have been developed to help the immune system combat the disease. Such strategies include: the enhancement of the immune system with the use of immunostimulating cytokines, antibodies (Ab) specific for cancer cells (tumor antigens), Ab-cytokine fusion proteins to attract the immune cells to and/or stimulate them at the cancer site, and the boosting of the immune system with in-vitro stimulated immune cells or in-vitro modified cancer cells. The present invention is directed, in part, to the latter

strategy and has several advantages including a new method of modifying cancer cells to make them highly immunostimulatory.

In one possible approach to treating cancer using the methods and compositions of the present invention, solid tumors can be transfected with and forced to express constitutively active chimeric IFN receptors (tumor cells can be transfected with expression plasmids or infected with adenovirus harboring the receptor). Infected tumor cells can become highly immunogenic and stimulate an anti-tumor immune response. After stimulating an anti-tumor immune response these modified tumor cells will undergo apoptosis.

This approach will also work for blood cell tumors. Malignant cells can be collected, modified as described for solid tumors and delivered back to the patient. When a primary solid tumor is removed, tumor cells are modified as described above and delivered back to the patient in the case of metastasis or to prevent metastasis. Importantly, targeted delivery of modified tumor cells to sites of residual tumors or metastases can be achieved by using an Ab that is specific for tumor antigens. The extracellular part of the Ab-receptor fusion complex, or the Ab part which is expressed on the cell surface can be specific for tumor antigens. Therefore, modified tumor cells injected into the blood stream may be able to accumulate in tumor sites. This will provide recruitment of immune cells and activation of an antitumor immune response specifically in sites of residual tumors or metastases.

In addition, the present invention can be used to constitutively express active IFN receptors in pathogen-infected cells to stimulate intracellular anti-viral or anti-bacterial

protection, and/or make pathogen-infected cells more immunogenic, and/or stimulate apoptosis of infected cells.

Summary of the Invention

In one aspect the present invention is directed to a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain. In certain embodiments the first portion consists of the receptor domain and the second portion consists of the dimerization domain. In another embodiment the chimeric polypeptide comprises a linker sequence between the first portion and the second portion.

In another embodiment, the dimerization domain comprises an amino acid sequence derived from an antibody. In certain embodiments the dimerization domain comprises an antibody Fc region or a fragment thereof, an antibody heavy chain region of a Fab region or a fragment thereof, or an antibody light chain region or a fragment thereof. In certain embodiments, the dimerization domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 16 to 25.

In another embodiment, the receptor domain comprises an amino acid sequence of a receptor selected from the group consisting of an interleukin receptor, a cytokine receptor, an interferon receptor and a growth factor receptor. In certain embodiments, the receptor domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 41 to 50.

In another aspect the present invention is directed to a composition comprising a first chimeric polypeptide wherein the first a chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an

intracellular region and a transmembrane region; and a second portion comprising a dimerization domain; and a second chimeric polypeptide wherein the second chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain; receptor domain of the second chimeric polypeptide is derived from a receptor the same as the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

In another aspect the present invention is directed to a composition comprising a first chimeric polypeptide wherein the first a chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain; and a second chimeric polypeptide wherein the second chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain; receptor domain of the second chimeric polypeptide is derived from a receptor different from the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

In another aspect the present invention is directed to a composition comprising a first chimeric polypeptide wherein the first chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain comprising an antibody Fc region or a fragment thereof; and a second chimeric polypeptide wherein the second chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain comprising an antibody Fc region or a fragment thereof, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor the same as the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

In another aspect the present invention is directed to composition comprising a first chimeric polypeptide wherein the first chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain comprising an antibody heavy chain region of a Fab region or a fragment thereof; and a second chimeric polypeptide wherein the second chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain comprising an antibody light chain region or a fragment thereof, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor

different from the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

In another aspect the present invention is directed to a method of activating a signaling pathway in a cell comprising administering to the cell a composition of the present invention. In one embodiment the signaling pathway is a pathway activated by a receptor selected from the group consisting of an interleukin receptor, a cytokine receptor, an interferon receptor and a growth factor receptor. In another embodiment the signaling pathway is a JAK/STAT signaling pathway or a MAP kinase signaling pathway.

In another aspect the present invention is directed to a method of preventing, treating, or ameliorating a disease related to an increased or extended signaling pathway induced by cytokines or growth factors in a subject comprising administering to the subject a composition according of the present invention wherein the composition activates a signaling pathway that counterbalances, suppresses or alters the increased or extended signaling pathway in the subject. In one embodiment the disease is selected from the group consisting of cancer, malignant conditions, chronic infections with various pathogens such as viruses and bacteria, chronic inflammatory conditions and autoimmune diseases. For example, the Type I, II, and III interferons have antiproliferative/ pro-apoptotic activity. Membrane-bound chimeric polypeptides comprising the intracellular domains of the receptors for these interferons would be useful to treat proliferative disorders such as cancer. Proliferation and cell survival can be induced by various ligands including EGF, IL-20, and IL-22 or oncoproteins such as v-Abl or v-Src. Soluble

chimeric polypeptides (described in more detail below) comprising the extracellular domains of the receptors for these ligands would be useful to treat proliferative disorders such as cancer.

Similarly, IL-10 has an anti-inflammatory activity. Membrane-bound chimeric polypeptides comprising the intracellular domains of the IL-10 receptor would be useful to treat chronic inflammatory conditions. Conversely, IL-12; IL-23; IFN-gamma (type II IFN), IL-17, IL-22 have proinflammatory activity. Soluble chimeric polypeptides (described in more detail below) comprising the extracellular domains of the receptors for these ligand would be useful to treat chronic inflammatory conditions.

In another aspect the present invention is directed to a method of preventing, treating, or ameliorating a disease related to increased or extended STAT3 and/or STAT5 activity in a subject comprising administering to the subject a composition according to the present invention wherein the composition activates a STAT1 signaling pathway in the subject.

In another aspect the present invention is directed to a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region. In one embodiment, the first portion consists of the receptor domain and the second portion consists of the dimerization domain. In another embodiment, the chimeric polypeptide comprises a linker sequence between the first portion and the second portion. In another embodiment, the receptor

domain comprises amino acid sequence from a receptor selected from the group consisting of an interleukin receptor, a cytokine receptor, an interferon receptor and a growth factor receptor. In another embodiment, the receptor domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 51 to 55. In another embodiment, the dimerization domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 26 to 30.

In another aspect the present invention is direct to a composition comprising first chimeric polypeptide wherein the first chimeric polypeptide is a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region; and a second chimeric polypeptide wherein the second chimeric polypeptide is a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor different from the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

In another aspect the present invention is direct to a method of inhibiting or reducing activation of a signaling pathway in a cell comprising administering to the cell a composition comprising first chimeric polypeptide wherein the first chimeric polypeptide is a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region; and a second chimeric polypeptide wherein the second chimeric polypeptide is a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor different from the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide. In one embodiment the signaling pathway is activated by a cytokine. In another embodiment the cytokine is selected from the group consisting of type I IFN, type II IFN, type III IFN, IL-10, IL-19, IL-20, IL-22, IL-24, IL-26 and Epidermal Growth Factor (EGF)

In another aspect the present invention is directed to a polynucleotide encoding a chimeric polypeptide according to the present invention. In one embodiment the polynucleotide encodes a receptor domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 41 to 50 or a receptor domain comprising an

amino acid sequence selected from the group consisting of SEQ ID NO: 51 to 55. In another embodiment the polynucleotide encodes a dimerization domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16 to 25 or a dimerization domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 26 to 30. In another embodiment the polynucleotide the polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 86 to 95 and 111 to 120 or a sequence selected from the group consisting of SEQ ID NO: 96 to 100 and 121 to 125.

In another aspect the present invention is directed to a vector comprising a polynucleotide sequence of the present invention. In one embodiment the vector comprises a polynucleotide wherein the polynucleotide sequence is operably linked to a promoter selected from the group consisting of HSV, TK, RSV, SV40, CMV, elongation factor 1α (EF-1α), and β-actin promoters.

In another aspect the present invention is directed to a cell comprising a chimeric polypeptide, a polynucleotide or a vector of the present invention.

In another aspect the present invention is directed to a composition comprising a chimeric polypeptide, a polynucleotide or a vector of the present invention.

Brief Description of the Drawings

Figure 1. Schematic models of various natural and fusion protein complexes.

Figure 2. Interleukins and Growth Hormones as Oncoproteins.

Figure 3. Role of Cytokines in Cancer.

Figure 4. Class II cytokine receptor family and its associated cytokines.

Figure 5. Ligand-independent constitutively active IFN-γ signaling.

Figure 6. Balancing STAT1 and STAT3 Activation.

Figure 7. IL-10R-Ab chimeric complex neutralizes IL-10 signaling.

Figure 8. Nucleotide and amino acid sequences of chimeric antibody-receptor constructs.

Figure 9. Schematic of relative position of primers used to amplify various parts of Ab molecules.

Figure 10. Constitutive ligand-independent STAT activation of receptors transiently expressed in COS cells.

Detailed Description of the Invention

In one aspect the present invention is directed to a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain.

A chimeric polypeptide is a human-engineered or in vivo mutated protein that is encoded by a nucleotide sequence made by a splicing together of two or more complete or partial genes or cDNA. The pieces used may be from different species.

A receptor is a protein on the cell membrane or within the cytoplasm or cell nucleus that binds to a specific molecule (a ligand). A receptor domain is a portion or fragment of a receptor amino acid sequence or an amino acid sequence derived from the amino acid sequence of a receptor. Different receptor domains can be characterized based on where they are located in relation to a cellular membrane. Receptors generally have an intracellular region, a transmembrane region, and an extracellular region. The transmembrane region of a receptor is that portion of a receptor that is embedded within the cell membrane. The transmembrane region is generally a hydrophobic region. The intracellular region is in the cytoplasm of the cell. This region interacts with cytoplamsic molecules and activates signaling pathways. As used herein, the term "intracellular domain" means the intracellular domain of the receptor or a portion of the intracellular domain that is necessary for activation of the receptor signaling pathways. The extracellular region is outside the cell. This region interacts with the receptor ligands. As used herein, the term "extracellular domain" means the extracellular domain of the receptor or a portion of the extracellular domain that is necessary for binding to its ligand.

A dimerization domain is an amino acid sequence capable of associating with or binding to another dimerization domain. The association or binding may be covalent or non-covalent. The association or binding may be facilitated by the binding of non-amino acid molecules to the dimerization domain. For example, an avidin molecule attached to one dimerization domain and a biotin molecule attached to another dimerization domain

would result in formation of an avidin-biotin bridge, thus promoting dimerization of the two domains. In another embodiment, the association or binding is through protein-protein interactions of the dimerization domains, for example, two leucine zipper domains. In one embodiment the dimerization domain is derived from an antibody.

In certain embodiments the first portion consists of the receptor domain and the second portion consists of the dimerization domain. In another embodiment the chimeric polypeptide comprises a linker sequence between the first portion and the second portion. A linker is a molecule that acts as a bridge between different domains of the chimeric polypeptide. A linker sequence is a polypeptide sequence that acts as a bridge. Linker sequences can be of any length. In one embodiment the linker sequence is less than 50 amino acids. In other embodiments the linker sequence is less than 40, less than 30 or less than 20 amino acids in length. In certain embodiments, the linker sequence is between 21 and 25 amino acids in length. In other embodiments the linker sequence comprises an amino acid sequence comprising a sequence selected from the group consisting of amino acids 221-245 of SEQ ID NO: 11; amino acids 464-470 of SEQ ID NO: 11; amino acids 236-260 of SEQ ID NO: 12; amino acids 479-490 of SEQ ID NO: 12; amino acids 236-260 of SEQ ID NO: 13; amino acids 227-241 of SEQ ID NO: 14; and amino acids 229-253 of SEQ ID NO: 15.

In another embodiment, the dimerization domain comprises an amino acid sequence derived from an antibody. Generally, an antibody (or immunoglobulin) is a large Y-shaped protein used by the immune system to identify and neutralize foreign objects like bacteria and viruses. Each antibody recognizes a specific antigen unique to its target. The basic unit of each antibody is a monomer (one Ig unit). The monomer is a "Y"-

shaped molecule that consists of four polypeptide chains; two identical heavy chains and two identical light chains connected by disulfide bonds or through protein-protein interaction (Fig. 1A). The Fc region is derived from the stem of the "Y," and is composed of two heavy chains that each contribute two to three constant domains (depending on the class of the antibody). Fc regions are capable of binding to Fc regions with identical or similar amino acid sequences. Such a dimer is referred to a homodimer.

Each end of the forked portion of the "Y" on the antibody is called the Fab region (Fragment, antigen binding). It is composed of a portion of the heavy chain and one light chain. These domains shape the antigen binding site at the amino terminal end of the monomer. Fc regions are capable of binding to Fc regions with identical or similar amino acid sequences. The heavy chain of the Fab fragment binds to and forms a dimer with the light chain. Because the heavy and light chains are different amino acid sequences, such a dimer is referred to a heterodimer.

The following tables refer to chimeric polypeptides, and fragments thereof, and polynucleotides that encode the chimeric polypeptides, and fragments thereof, that are disclosed in the Examples below. The present invention is directed to the use of any of these amino acid of polynucleotide sequences to generate a chimeric polypeptide or polynucleotide of the present invention. Accordingly, the present invention is directed to a chimeric polypeptide comprising and a polynucleotide encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 55, and a polynucleotide selected from the group consisting of SEQ ID NO: 56 to 125.

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-484	1	Full Length	1-1452	56
			Full Length + Stop Codon	1-1455	71
Ab portion	1-241	16	Ab portion	1-723	86
Trans-membrane	242-265	31	Trans-membrane	724-795	101
Intracellular	266-484	41	Intracellular	796-1452	111

mL2/γR2

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-327	2	Full Length	1-981	57
			Full Length + Stop Codon	1-984	72
Ab portion	1-236	17	Ab portion	1-711	87
Trans-membrane	237-231	32	Trans-membrane	712-783	102
Intracellular	262-327	41	Intracellular	784-981	112

SPd6xHis-hH2/γR1

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-20	3	Full Length	1-1485	58
			Full Length + Stop Codon	1-1488	73
Ab portion	27-252	18	Ab portion	79-756	88
Trans-membrane	253-276	33	Trans-membrane	757-828	103
Intracellular	277-495	43	Intracellular	829-1485	113

SPFL-hL2/γR2

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-342	4	Full Length	1-1026	59
			Full Length + Stop Codon	1-1029	74
Ab portion	28-252	19	Ab portion	82-756	89
Trans-membrane	253-276	34	Trans-membrane	757-828	104
Intracellular	277-342	44	Intracellular	829-1026	114

SPFL-mFc/γR1

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-597	5	Full Length	1-1491	60
			Full Length + Stop Codon	1-1494	75
Ab portion	28-254	20	Ab portion	82-762	90
Trans-membrane	255-278	35	Trans-membrane	763-834	105
Intracellular	279-597	45	Intracellular	835-1491	115

SPFL-mFc/IFN-λR1

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-548	6	Full Length	1-1637	61
			Full Length + Stop Codon	1-1640	76
Ab portion	30-254	21	Ab portion	88-762	91
Trans-membrane	255-277	36	Trans-membrane	763-831	106
Intracellular	278-548	46	Intracellular	832-1637	116

SPFL-mFc/IFN-αR2

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-526	7	Full Length	1-1577	62
			Full Length + Stop Codon	1-1560	77
Ab portion	30-254	22	Ab portion	88-762	92
Trans-membrane	255-275	37	Trans-membrane	763-825	107
Intracellular	276-526	47	Intracellular	826-1577	117

SPFL-mFc/IL-20R1

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-557	8	Full Length	1-1667	63
			Full Length + Stop Codon	1-1670	78
Ab portion	30-254	23	Ab portion	88-762	93
Trans-membrane	255-278	38	Trans-membrane	763-836	108
Intracellular	279-557	48	Intracellular	837-1667	118

SPFL-mFc/IL-20R1

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-600	9	Full Length	1-1800	65
			Full Length + Stop Codon	1-1803	79
Ab portion	30-254	24	Ab portion	88-762	94
Trans-membrane	255-277	38	Trans-membrane	763-831	109
Intracellular	278-600	49	Intracellular	832-1800	119

SPFL-mFc/EGFR

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-818	10	Full Length	1-2447	65
			Full Length + Stop Codon	1-	80
Ab portion	30-254	25	Ab portion	88-762	95
Trans-membrane	255-276	40	Trans-membrane	763-828	110
Intracellular	277-818	50	Intracellular	829-2447	120

IL-10R2-2xlinker-L2-linker-FL

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-479	11	Full Length	1-1437	66
			Full Length + Stop Codon	1-1440	81
Ab portion	246-463	26	Ab portion	736-1398	96
Extracellular	1-220	51	Extracellular	1-660	121

IL-10R1-2xlinker-H2-linker-6xHis

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-496	12	Full Length	1-1491	67
			Full Length + Stop Codon	1-1494	82
Ab portion	261-478	27	Ab portion	781-1434	97
Extracellular	1-235	52	Extracellular	1-705	122

IL-10R1-2xlinker-H2-Fc

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO

Full Length	1-705	13	Full Length	1-2115	68
			Full Length + Stop Codon	1-2118	83
Ab portion	261-705	28	Ab portion	781-2115	98
Extracellular	1-235	53	Extracellular	1-705	123

IFN-λR1-linker-H2-Fc

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-386	14	Full Length	1-2058	69
			Full Length + Stop Codon	1-2061	84
Ab portion	242-386	29	Ab portion	724-2058	99
Extracellular	1-226	54	Extracellular	1-678	124

IL-22R1-2xlinker-H2-Fc

Amino Acid			Polynucleotide		
Description	Residues	SEQ ID NO	Description	Nucleotides	SEQ ID NO
Full Length	1-699	15	Full Length	1-2097	70
			Full Length + Stop Codon	1-2100	85
Ab portion	254-699	30	Ab portion	760-2097	100
Extracellular	1-228	55	Extracellular	1-684	125

In certain embodiments the dimerization domain comprises an antibody Fc region or a fragment thereof, an antibody heavy chain region of a Fab region or a fragment thereof, or an antibody light chain region or a fragment thereof. As used herein, the term "Fc region or a fragment thereof" means the Fc region or a fragment thereof that is necessary for homodimer formation. As used herein, the term "an antibody heavy chain region of a Fab region or a fragment thereof" means the antibody heavy chain region of a Fab region or a fragment thereof that is necessary for heterodimer formation with a light chain or a fragment thereof. As used herein, the term "an antibody light chain region or a fragment thereof" means the antibody light chain region or a fragment thereof that is necessary for heterodimer formation with an antibody heavy chain region of a Fab region or a fragment thereof. In certain embodiments, the dimerization domain comprises an

amino acid sequence selected from the group consisting of SEQ ID NO: 16 to 25 or an amino acid sequence selected from the group consisting of SEQ ID NO: 26 to 30.

The unique property of antibody molecules can be utilized to design a universal approach of creating various homo- or hetero-dimeric fusion proteins. The overall design is shown in Fig. 1. Generalized structures of natural and designed fusion (or chimeric) protein complexes are schematically presented on Fig. 1. Whole Fc and Fab Ab portions or their fragments that are sufficient for dimerization are utilized to create membrane bound constitutively active receptor complexes. Such receptor complexes do not require a ligand. Fab parts of heavy and light chains of Ab serve as the extracellular domains of the Fab/receptor chimeric complex, heterodimerize on the cell surface without any stimulus and cause heterodimerization of the intracellular domains (ID) of receptor subunits that triggers signal transduction cascade specific for the given receptor subunits. Very often ID of only one receptor subunit determines the specificity of signaling (Kotenko et al., 1996, 1999, 2000, 2001, 2002, 2004). In such cases homodimerization of the R1 type of the receptor subunit is sufficient (Kotenko et al., 1996, 1999, 2000) and Fc/R1 fusion proteins can be utilized to create constitutively active cytokine-specific receptor complex (see Fig.1 B and C).

In addition, whole Ab, Fab Ab portions or their fragments that are sufficient for dimerization are utilized to create heterodimeric soluble cytokine-specific receptor complexes as schematically shown on Fig. 1 E and F.

In Fig. 1, R1 can be IFN-αR2c, IFN-γR1, IL-10R1, IL-22R1, IL-20R1 or IFN-λR1; whereas R2 can be IFN-αR1, IFN-γR2, IL-20R2 or IL-10R2. Receptor activation by

various IFNs is shown in Fig. 4. The two subunits of the IFN-γ and the type I IFN receptor complexes are indicated, respectively, as "γR1" and "γR2", and "αR1" and "αR2". Tissue factor (TF) is a receptor for coagulation factor VIIa (FVIIa). IL-10R2 can be combined with either IL-10R1, IL-22R1, IFN-λR1 or IL-20R1 to assemble the IL-10, IL-22, IFN-λ or IL-26 receptor complexes, respectively. IL-22R1 can also function with IL-20R2, generating the receptor complexes for IL-20 and IL-24. In turn, IL-20R2 can also join IL-20R1 to form the receptor complex for IL-19, IL-20 and IL-24. IL-22BP is the IL-22 binding protein, the only soluble receptor from this family.

In another embodiment, the receptor domain comprises an amino acid sequence of a receptor selected from the group consisting of an interleukin receptor, a cytokine receptor, an interferon receptor and a growth factor receptor. In certain embodiments, the receptor is a receptor shown in Figure 4. In another embodiment, the receptor is IFNγR, IFNαR, IL-λR, IL-10R, IL-20R, IL-22R or EGFR. In another embodiment, the receptor domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 41 to 50.

Further provided by the present invention are isolated polypeptides having an amino acid sequence corresponding to SEQ ID NO.: 1 to 55, or the amino acid sequence of a functionally equivalent fusion protein product, fragment or bioprecursor of the protein. A protein of the invention can be in a substantially purified form, in which case it will generally comprise the polypeptide in a preparation in which more than 90%, e.g. 95%, 98%, or 99% of the polypeptide in the preparation is a polypeptide of the invention. Proteins of the invention can be modified, for example by the addition of histidine residues to assist their purification or by the addition of a signal sequence to promote their secretion

from a cell. Proteins having at least 90% sequence identity, for example at least 95%, 98% or 99% sequence identity to the polypeptide protein depicted in SEQ ID NO.: 1 to 55 may be proteins which are amino acid sequence variants, alleles, derivatives, or mutants of the protein depicted in SEQ ID NO.: 1 to 55, and are also provided by the present invention.

In certain embodiments the chimeric polypeptides of the present invention comprise an epitope tag. Common epitopes tags used for this purpose are c-myc, HA, FLAG, V5, and His 6X. In other embodiments the epitope tag sequence comprises an amino acid sequence comprising a sequence selected from the group consisting of amino acids 21-26 of SEQ ID NO: 3; amino acids 22-27 of SEQ ID NOS: 4-10; amino acids 471-478 of SEQ ID NO: 11; and amino acids 491-496 of SEQ ID NO: 12. In certain embodiments a signal peptide is introduced in the beginning of the chimeric polypeptides to target the polypeptide to the membrane.

In another aspect the present invention is directed to a composition comprising a first chimeric polypeptide wherein the first a chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain; and a second chimeric polypeptide wherein the second chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain; receptor domain of the second chimeric polypeptide is derived from a receptor the same as the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the

receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

In another aspect the present invention is directed to a composition comprising a first chimeric polypeptide wherein the first a chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain; and a second chimeric polypeptide wherein the second chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain; receptor domain of the second chimeric polypeptide is derived from a receptor different from the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

In another aspect the present invention is directed to a composition comprising a first chimeric polypeptide wherein the first chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain comprising an antibody Fc region or a fragment thereof; and a second chimeric polypeptide wherein the second chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain

comprising an antibody Fc region or a fragment thereof, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor the same as the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

In another aspect the present invention is directed to composition comprising a first chimeric polypeptide wherein the first chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain comprising an antibody heavy chain region of a Fab region or a fragment thereof; and a second chimeric polypeptide wherein the second chimeric polypeptide comprises a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and a second portion comprising a dimerization domain comprising an antibody light chain region or a fragment thereof, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor different from the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

In another aspect the present invention is directed to a method of activating a signaling pathway in a cell comprising administering to the cell a composition of the present invention. In one embodiment the signaling pathway is a pathway activated by a

receptor selected from the group consisting of an interleukin receptor, a cytokine receptor, an interferon receptor and a growth factor receptor. In another embodiment the signaling pathway is a JAK/STAT signaling pathway or a MAP kinase signaling pathway.

Cytokines secreted by tumor cells can have several effects on tumor growth. Several cytokines such as IL-10 or TGF-β can suppress anti-tumor immune response leading to the establishment of immunological tolerance to tumor antigens. Tumor cells may also express functional receptor complex for a cytokine secreted by the same tumor cells. If this cytokine can support proliferation of tumor cells, for example through the activation of STAT3 and/or STAT5, then this cytokine may function as an autocrine growth factor for tumor cells.

Many interleukins (ILs) and growth factors (GFs) as well as several oncoproteins are able to predominantly activate STAT3 during signal transduction events. Constitutive signaling through STAT3 leads to malignant transformation (Fig. 2). Cytokine milieu can shift the balance between STAT1 and STAT3 activation inside of tumor cells. IFNs are well known for their ability to signal predominantly through STAT1 activation that leads to the induction of anti-proliferative response, cell cycle arrest and apoptosis.

To circumvent immune surveillance, tumors must develop means of protecting themselves from being recognized by immune cells as an abnormal cell type thus avoiding destruction by the immune cells. Often this leads to general suppression of the immune system. In addition, tumor cells constitutively proliferate. Agents inducing constitutive proliferation are often driving forces in cancer development. Autocrine growth factors or mutations leading to activation of one or more signal transduction pathways (e.g., Ras-

MAP kinase or Jak-STAT pathways) induced by growth factors are examples of such agents. The present invention includes a method to deliver anti-proliferative signals to cancer cells forcing them to stop or at least to slow down their proliferation. For example, expression of constitutively active STAT3 was shown to drive cells to malignant transformation (Bromberg et al., 1999). Studies also demonstrated that STAT3 is maintained in activated form in many tumors. In contrast, activation of STAT1 is linked to anti-proliferative effect, cell cycle arrest and induction of apoptosis (Fig. 3 and 6). Therefore, a method was designed to force tumor cells to express constitutively active STAT1. STAT1 is predominantly activated by interferons (IFNs) (Fig. 3). IFNs are also well known for their strong anti-proliferative activities. Therefore, a constitutively active IFN signaling was designed. The chimeric receptors shown in Fig. 1 C and D are examples of such IFN signaling constructs.

In another aspect the present invention is directed to a method of preventing, treating, or ameliorating a disease related to an increased or extended signaling pathway induced by cytokines or growth factors in a subject comprising administering to the subject a composition according of the present invention wherein the composition activates a signaling pathway that counterbalances, suppresses or alters the increased or extended signaling pathway in the subject. In one embodiment the disease is selected from the group consisting of cancer, malignant conditions, chronic infections with various pathogens such as viruses and bacteria, chronic inflammatory conditions and autoimmune diseases. In another aspect the present invention is directed to a method of preventing, treating, or ameliorating a disease related to increased or extended STAT3 activity in a subject comprising administering to the subject a composition according to the present invention wherein the composition activates a STAT1 signaling pathway in the subject.

Many cancer cells demonstrate constitutively active STAT3 signaling that can be induced through several mechanisms (Fig. 3) including response to various interleukins (ILs) present in the local environment (cytokine milieu). By forcing cells to undergo constitutive IFN signaling (Fig. 5) resulting in STAT1 activation, the balance between activated STAT3 and STAT1 is shifted toward STAT1. This event leads to the induction of various biological activities (some are listed on the figure) that are beneficial for immune system and disadvantageous for cancer progression.

In another aspect the present invention is directed to a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region. In one embodiment, the first portion consists of the receptor domain and the second portion consists of the dimerization domain. In another embodiment, the chimeric polypeptide comprises a linker sequence between the first portion and the second portion. In another embodiment, the receptor domain comprises amino acid sequence from a receptor selected from the group consisting of an interleukin receptor, a cytokine receptor, an interferon receptor and a growth factor receptor. In another embodiment, the receptor domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 51 to 55. In another embodiment, the dimerization domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 26 to 30.

In another aspect the present invention is direct to a composition comprising first chimeric polypeptide wherein the first chimeric polypeptide is a chimeric polypeptide

comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region; and a second chimeric polypeptide wherein the second chimeric polypeptide is a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor different from the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

Most cytokines signal through heterodimeric receptor complexes. In general, a homodimeric receptor has lower affinity for the cytokine then the combination of two different receptor subunits. Therefore, soluble receptors representing just one receptor subunit are very inefficient in neutralizing cytokine functions. The use of Ab-receptor chimeric polypeptides as presented on Fig. 1 E and F allows the generation of heterodimeric soluble receptors. This technique has been successfully applied to the IL-10 ligand-receptor system. IL-10R-Ab fusion molecule (IL-10R1/H/Fc+IL-10R2/L) is very efficient in specifically inhibiting IL-10 signaling (Fig. 7A). Both IL-10R1/H/Fc and IL-10R2/L chimeric chains are coexpressed in a cell to allow proper assembling of the IL-10R-Ab complex.

In another aspect the present invention is direct to a method of inhibiting or reducing activation of a signaling pathway in a cell comprising administering to the cell a composition comprising first chimeric polypeptide wherein the first chimeric polypeptide is a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region; and a second chimeric polypeptide wherein the second chimeric polypeptide is a chimeric polypeptide comprising a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor different from the receptor domain of the first chimeric polypeptide; wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide. In one embodiment the signaling pathway is activated by a cytokine. In another embodiment the cytokine is selected from the group consisting of type I IFN, type II IFN, type III IFN, IL-10, IL-19, IL-20, IL-22, IL-24, IL-26 and Epidermal Growth Factor (EGF)

Chimeric polypeptides and compositions of the present invention can be used for the treatment of autoimmune diseases, cancers, immunomodulation, and any antibody-mediated pathologies. Administration of chimeric polypeptides and compositions of the present invention to a subject can be intravenous, intraarterial, intraperitoneal,

intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses.

Additional routes of administration include oral, mucosal-membrane, pulmonary, and transcutaneous. A pharmaceutical composition comprising a chimeric polypeptides and compositions of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the chimeric polypeptides and compositions of the present invention are combined in a mixture with a pharmaceutically acceptable carrier. A composition is to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art.

For purposes of therapy, chimeric polypeptides and compositions of the present invention and a pharmaceutically acceptable carrier are administered to a patient in a therapeutically effective amount. A combination of a chimeric polypeptides and compositions of the present invention and a pharmaceutically acceptable carrier is to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response. As another example, an agent used to inhibit the growth of tumor cells is physiologically significant if the administration of the agent results in a decrease in the

number of tumor cells, decreased metastasis, a decrease in the size of a solid tumor, or increased necrosis of a tumor.

A pharmaceutical composition comprising chimeric polypeptides and compositions of the present invention can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms.

Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments. Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 μm to greater than 10 μm. A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

Liposomes can also be prepared to target particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the liposomes. For example, liposomes, prepared with a high content of a nonionic surfactant, have been used

to target the liver (Hayakawa et al., Japanese Patent 04-244,018; Kato et al., Biol. Pharm. Bull. 16:960 (1993)). These formulations were prepared by mixing soybean phospatidylcholine, α-tocopherol, and ethoxylated hydrogenated castor oil (HCO-60) in methanol, concentrating the mixture under vacuum, and then reconstituting the mixture with water. A liposomal formulation of dipalmitoylphosphatidylcholine (DPPC) with a soybean-derived sterylglucoside mixture (SG) and cholesterol (Ch) has also been shown to target the liver (Shimizu et al., Biol. Pharm. Bull. 20:881 (1997)).

Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies, antibody fragments, carbohydrates, vitamins, and transport proteins. In one embodiment of the present invention a first chimeric polypeptide comprising a heavy chain of a Fab fragment associates with a second chimeric polypeptide comprising a light of a Fab fragment such that upon dimerization the heavy and light chains can bind to a target molecule. In one embodiment the target molecule is found on the surface of a target cell.

In another aspect the present invention is directed to a polynucleotide encoding a chimeric polypeptide according to the present invention. In one embodiment the polynucleotide encodes a receptor domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 41 to 50 or a receptor domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 51 to 55. In another embodiment the polynucleotide encodes a dimerization domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16 to 25 or a dimerization domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 26 to 30. In another embodiment the polynucleotide the

polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 86 to 95 and 111 to 120 or a sequence selected from the group consisting of SEQ ID NO: 96 to 100 and 121 to 125.

In another aspect the present invention is directed to a vector comprising a polynucleotide sequence of the present invention. In one embodiment the vector comprises a polynucleotide wherein the polynucleotide sequence is operably linked to a promoter selected from the group consisting of HSV, TK, RSV, SV40, CMV, elongation factor 1α (EF-1α), and β-actin promoters. Expression vectors can also contain other nucleotide sequences, such as IRES elements, polyadenylation signals, splice donor/splice acceptor signals, and the like.

In another aspect the present invention is directed to a cell comprising a chimeric polypeptide, a polynucleotide or a vector of the present invention.

In another aspect the present invention is directed to a composition comprising a chimeric polypeptide, a polynucleotide or a vector of the present invention.

The present invention includes the use of polynucleotides of the present invention to provide the chimeric polypeptides of the present invention to a subject in need of treatment with the chimeric polypeptide. The present nucleic acids can be incorporated into an expression vector and subsequently used to transform, transfect or infect a suitable host cell. In such an expression vector the nucleic acid according to the invention is operably linked to a control sequence, such as a suitable promoter or the like, ensuring expression of the proteins according to the invention in a suitable host cell. The expression vector can be a plasmid, cosmid, virus or other suitable vector. The expression vector and

the host cell transfected, transformed or infected with the vector also form part of the present invention. Preferably, the host cell is a eukaryotic cell or a bacterial cell and even more preferably a mammalian cell or insect cell. Mammalian host cells are particularly advantageous because they provide the necessary post-translational modifications to the expressed proteins according to the invention, such as glycosylation or the like, which modifications confer optimal biological activity of the proteins, which when isolated can advantageously be used in diagnostic kits or the like.

The recombinant vectors of the invention generally comprise a polynucleotide of the present invention operatively positioned downstream from a promoter. The promoter is capable of directing expression of the polynucleotide of the present invention in a mammalian, e.g. human cell. Such promoters are thus "operative" in mammalian cells, e.g. human cells.

Expression vectors and plasmids embodying the present invention comprise one or more constitutive promoters, such as viral promoters or promoters from mammalian genes that are generally active in promoting transcription. Examples of constitutive promoters include the HSV, TK, RSV, SV40, CMV, elongation factor 1α (EF-1α), and β-actin promoters.

Inducible promoters and/or regulatory elements are also contemplated for use with the expression vectors of the invention. Examples of suitable inducible promoters include promoters from genes such as cytochrome P450 genes, heat shock protein genes, metallothionein genes, hormone-inducible genes, such as the estrogen gene promoter, and such like. Promoters that are activated in response to exposure to ionizing radiation, such

as fos, jun and erg-1, are also contemplated. The tetVP16 promoter that is responsive to tetracycline is a currently preferred example.

Tissue-specific promoters and/or regulatory elements will be useful in certain embodiments. Examples of such promoters that can be used with the expression vectors of the invention include promoters from the liver fatty acid binding (FAB) protein gene, specific for colon epithelial cells; the insulin gene, specific for pancreatic cells; the transphyretin, α-1-antitrypsin, plasminogen activator inhibitor type 1 (PAI-1), apolipoprotein AI and LDL receptor genes, specific for liver cells; the myelin basic protein (MBP) gene, specific for oligodendrocytes; the glial fibrillary acidic protein (GFAP) gene, specific for glial cells; OPSIN, specific for targeting to the eye; and the neural-specific enolase (NSE) promoter that is specific for nerve cells.

The construction and use of expression vectors and plasmids is well known to those of skill in the art. Virtually any mammalian cell expression vector can thus be used in connection with the genes disclosed herein.

Preferred vectors and plasmids are constructed with at least one multiple cloning site. In certain embodiments, the expression vector will comprise a multiple cloning site that is operatively positioned between a promoter and a human Mus81 or murine Mus81 encoding gene sequence. Such vectors can be used, in addition to uses in other embodiments, to create N-terminal or C-terminal fusion proteins by cloning a second protein-encoding DNA segment into the multiple cloning site so that it is contiguous and in-frame with the mammalian Mus81 encoding nucleotide sequence.

There are numerous approaches to introduce a gene to a subject, including the use of recombinant host cells that express, delivery of naked nucleic acid, use of a cationic lipid carrier with a nucleic acid molecule, and the use of viruses, such as recombinant retroviruses, recombinant adeno-associated viruses, recombinant adenoviruses, and recombinant Herpes simplex viruses. In an ex vivo approach, for example, cells are isolated from a subject, transfected with a vector that expresses the chimeric polypeptides of the present invention, and then transplanted into the subject.

In order to effect expression of a chimeric polypeptide of the present invention, an expression vector is constructed in which a nucleotide sequence encoding a chimeric polypeptide of the present invention is operably linked to a core promoter, and optionally a regulatory element, to control gene transcription.

Alternatively, a polynucleotide encoding a chimeric polypeptide of the present invention can be delivered using recombinant viral vectors, including for example, adenoviral vectors, adenovirus-associated viral vectors, alphaviruses such as Semliki Forest Virus and Sindbis Virus, herpes viral vectors, parvovirus vectors, pox virus vectors, pox viruses, such as canary pox virus or vaccinia virus, and retroviruses. Within various embodiments, either the viral vector itself, or a viral particle which contains the viral vector may be utilized.

Alternatively, an expression vector encoding a chimeric polypeptide of the present invention can be introduced into a subject's cells by lipofection in vivo using liposomes. Synthetic cationic lipids can be used to prepare liposomes for in vivo transfection of a gene encoding a marker. The use of lipofection to introduce exogenous genes into specific

organs in vivo has certain practical advantages. Liposomes can be used to direct transfection to particular cell types, which is particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

Electroporation is another alternative mode of administration. For example, Aihara and Miyazaki, Nature Biotechnology 16:867 (1998), have demonstrated the use of in vivo electroporation for gene transfer into muscle.

In general, the dosage of a composition comprising a therapeutic vector encoding a chimeric polypeptide of the present invention, such as a recombinant virus, will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. Suitable routes of administration of therapeutic vectors include intravenous injection, intraarterial injection, intraperitoneal injection, intramuscular injection, intratumoral injection, and injection into a cavity that contains a tumor.

It is apparent that many modifications and variations of this invention as hereinabove set forth may be made without departing from the spirit and scope thereof. The specific embodiments are given by way of example only and the invention is limited only by the terms of the appended claims. All publications cited herein are incorporated by reference in their entireties.

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What is claimed is:

1. A chimeric polypeptide comprising:

a first portion comprising a receptor domain, wherein the receptor domain comprises an intracellular region and a transmembrane region; and

a second portion comprising a dimerization domain.

2. The chimeric polypeptide according to claim 1, wherein the first portion consists of the receptor domain and the second portion consists of the dimerization domain.

3. The chimeric polypeptide according to any one of claims 1 and 2, wherein the chimeric polypeptide comprises a linker sequence between the first portion and the second portion.

4. The chimeric polypeptide according to any one of claims 1 to 3, wherein the dimerization domain comprises an amino acid sequence derived from an antibody.

5. The chimeric polypeptide according to claim 4, wherein the dimerization domain comprises an antibody Fc region or a fragment thereof.

6. The chimeric polypeptide according to claim 4, wherein the dimerization domain comprises an antibody heavy chain region of a Fab region or a fragment thereof.

7. The chimeric polypeptide according to claim 4 wherein the dimerization domain comprises an antibody light chain region or a fragment thereof.

8. The chimeric polypeptide according to any one of claims 1 to 7, wherein the receptor domain comprises an amino acid sequence from a receptor selected from the group consisting of an interleukin receptor, a cytokine receptor, an interferon receptor and a growth factor receptor.

9. The chimeric polypeptide according to any one of claims 1 to 8, wherein the receptor domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 41 to 50.

10. The chimeric polypeptide according to any one of claims 1 to 8, wherein the dimerization domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 16 to 25.

11. A cell comprising the chimeric polypeptide according to any one of claims 1 to 10.

12. A composition comprising a chimeric polypeptide according to any one of claims 1 to 10.

13. A composition comprising:

a first chimeric polypeptide wherein the first chimeric polypeptide is a chimeric polypeptide according to claim 1; and

a second chimeric polypeptide wherein the second chimeric polypeptide is a chimeric polypeptide according to claim 1, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor the same as the receptor domain of the first chimeric polypeptide;

wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

14. A composition comprising:

a first chimeric polypeptide wherein the first chimeric polypeptide is a chimeric polypeptide according to claim 1; and

a second chimeric polypeptide wherein the second chimeric polypeptide is a chimeric polypeptide according to claim 1, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor different from the receptor domain of the first chimeric polypeptide;

wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

15. A composition comprising:

a first chimeric polypeptide wherein the first chimeric polypeptide is a chimeric polypeptide according to claim 5; and

a second chimeric polypeptide wherein the second chimeric polypeptide is a chimeric polypeptide according to claim 5, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor the same as the receptor domain of the first chimeric polypeptide;

wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

16. A composition comprising:

a first chimeric polypeptide wherein the first chimeric polypeptide is a chimeric polypeptide according to claim 6; and

a second chimeric polypeptide wherein the second chimeric polypeptide is a chimeric polypeptide according to claim 7, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor different from the receptor domain of the first chimeric polypeptide;

wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

17. A polynucleotide encoding a chimeric polypeptide according to any one of claims 1 to 10:

18. The polynucleotide according to claim 17, wherein the receptor domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 41 to 50.

19. The polynucleotide according to claim 17, wherein the dimerization domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 16 to 25.

20. The polynucleotide according to claim 17, wherein the polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 86 to 95 and 111 to 120.

21. A vector comprising the polynucleotide sequence according to any one of claims 18 to 20.

22. The vector according to claim 21, wherein the polynucleotide sequence is operably linked to a promoter selected from the group consisting of HSV, TK, RSV, SV40, CMV, elongation factor 1α (EF-1α), and β-actin promoters.

23. A cell comprising the polynucleotide according to any one of claims 18 to 20.

24. A cell comprising the vector according to any one of claims 21 and 22.

25. A composition comprising the polynucleotide according to any one of claims 18 to 20.

26. A composition comprising the vector according to any one of claims 21 and 22.

27. A method of activating a signaling pathway in a cell comprising administering to the cell a composition according to any one of claims 13 to 16.

28. The method of claim 27 wherein the signaling pathway is a pathway activated by a receptor selected from the group consisting of an interleukin receptor, a cytokine receptor, an interferon receptor and a growth factor receptor. .

29. The method of claim 28 wherein the signaling pathway is a JAK/STAT signaling pathway.

30. The method of claim 26 wherein the signaling pathway is a MAP kinase signaling pathway.

31. A method of preventing, treating, or ameliorating a disease related to increased or extended STAT3 activity in a subject comprising

administering to the subject a composition according to any one of claims 13 to 16 wherein the composition activates a STAT1 signaling pathway in the subject.

32. A method of preventing, treating, or ameliorating a disease related to increased or extended signaling pathway induced by cytokines or growth factors in a subject comprising

administering to the subject a composition according to any one of claims 13 to 16 wherein the composition activates a signaling pathway that counterbalances, suppresses or alters the increased or extended signaling pathway in the subject.

33. The method according to claim 32 wherein the disease is selected from the group consisting of cancer, malignant conditions, chronic infections with various pathogens such as viruses and bacteria, chronic inflammatory conditions and autoimmune diseases.

34. A chimeric polypeptide comprising:

a first portion comprising a receptor domain, wherein the receptor domain comprises a receptor extracellular region; and

a second portion comprising a dimerization domain, wherein the dimerization domain comprises an antibody heavy chain region of a Fab fragment or an antibody light chain region.

35. The chimeric polypeptide according to claim 34, wherein the first portion consists of the receptor domain and the second portion consists of the dimerization domain.

36. The chimeric polypeptide according to any one of claims 34 and 35, wherein the chimeric polypeptide comprises a linker sequence between the first portion and the second portion.

37. The chimeric polypeptide according to any one of claims 34 to 36, wherein the receptor domain comprises amino acid sequence from a receptor selected from the group

consisting of an interleukin receptor, a cytokine receptor, an interferon receptor and a growth factor receptor.

38. The chimeric polypeptide according to any one of claims 34 to 37, wherein the receptor domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 51 to 55.

39. The chimeric polypeptide according to any one of claims 34 to 37, wherein the dimerization domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 26 to 30.

40. A cell comprising the chimeric polypeptide according to any one of claims 34 to 39.

41. A composition comprising a chimeric polypeptide according to any one of claims 34 to 39.

42. A composition comprising:

a first chimeric polypeptide wherein the first chimeric polypeptide is the chimeric polypeptide according to claim 34; and

a second chimeric polypeptide wherein the second chimeric polypeptide is the chimeric polypeptide according to claim 34, wherein the receptor domain of the second chimeric polypeptide is derived from a receptor different from the receptor domain of the first chimeric polypeptide;

wherein the dimerization domain of the first chimeric polypeptide binds to the dimerization domain of the second chimeric polypeptide resulting in the receptor domain of the first chimeric polypeptide associating with the receptor domain of the second chimeric polypeptide.

43. A polynucleotide encoding a chimeric polypeptide according to any one of claims 34 to 39:

44. The polynucleotide according to claim 43, wherein the receptor domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 51 to 55.

45. The polynucleotide according to claim 43, wherein the dimerization domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 26 to 30.

46. The polynucleotide according to claim 43, wherein the polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO: 96 to 100 and 121 to 125.

47. A vector comprising the polynucleotide sequence according to any one of claims 43 to 46.

48. The vector according to claim 47, wherein the polynucleotide sequence is operably linked to a promoter selected from the group consisting of HSV, TK, RSV, SV40, CMV, elongation factor 1α (EF-1α), and β-actin promoters.

49. A cell comprising the polynucleotide according to any one of claims 43 to 46.

50. A cell comprising the vector according to any one of claims 47 and 48.

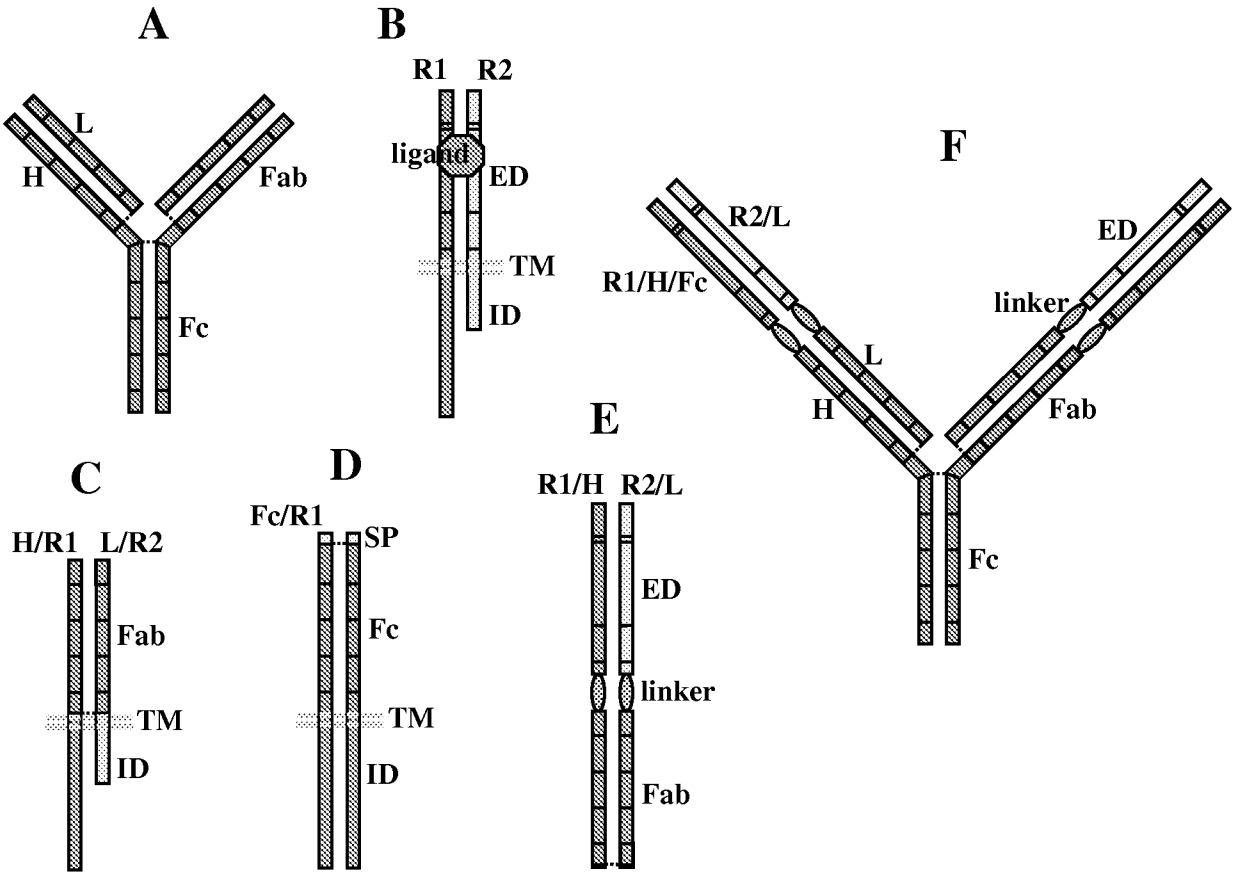
51. A composition comprising the polynucleotide according to any one of claims 43 to 46.

52. A composition comprising the vector according to any one of claims 47 and 48.

53. A method of inhibiting or reducing activation of a signaling pathway in a cell comprising administering to the cell a composition of claim 42.

54. The method according to claim 53 wherein the signaling pathway is activated by a cytokine.

55. The method according to claim 54 wherein the cytokine is selected from the group consisting of type I IFN, type II IFN, type III IFN, IL-10, IL-19, IL-20, IL-22, IL-24, IL-26 and Epidermal Growth Factor (EGF).


A
L
H
Fab
Fc
B
R1
R2
ligand
ED
TM
ID
C
H/R1
L/R2
Fab
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ID
D
Fc/R1
SP
Fc
TM
ID
E
R1/H
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ED
linker
L
H
Fab
Fc

Fig. 1.

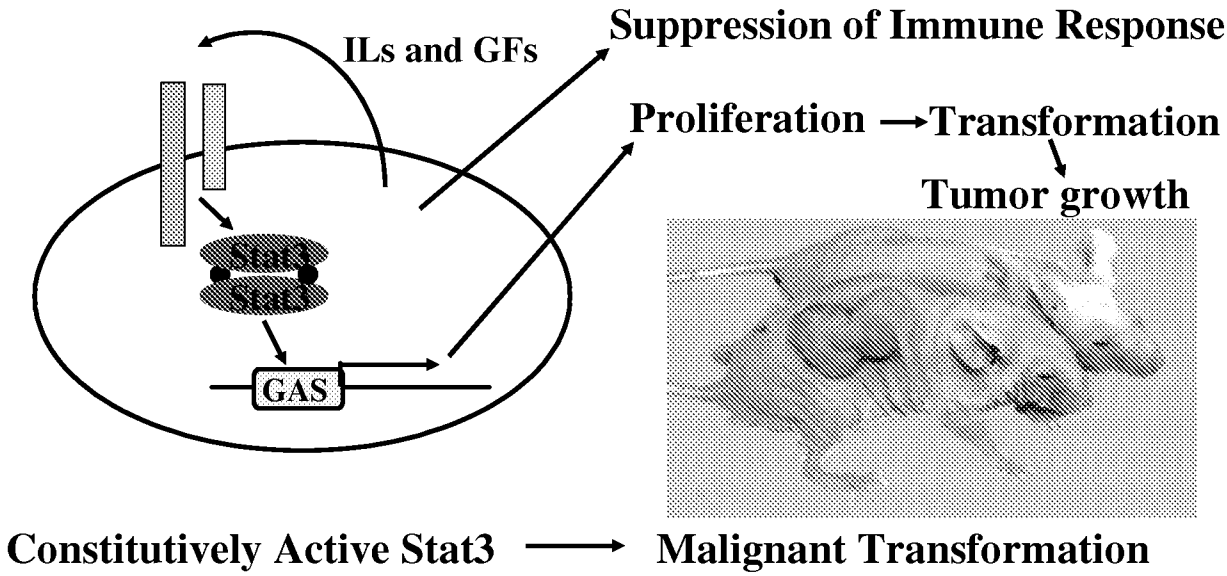
ILs and GFs
Suppression of Immune Response
Proliferation
Transformation
Tumor growth
Stat3
Stat3
GAS
Constitutively Active Stat3
Malignant Transformation

Fig. 2.

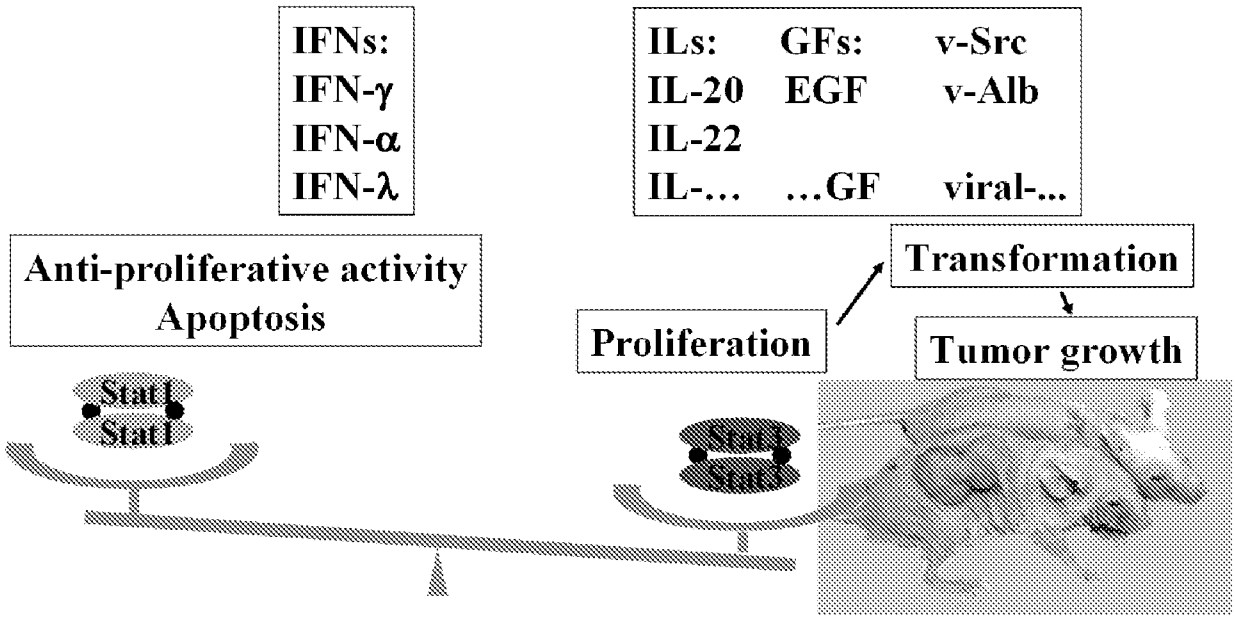
IFNs:
IFN-γ
IFN-α
IFN-λ
ILs: GFs: v-Src
IL-20 EGF v-Alb
IL-22
IL-… …GF viral-...
Anti-proliferative activity
Apoptosis
Proliferation
Transformation
Tumor growth
Stat1
Stat1
Stat3
Stat3

Fig. 3.

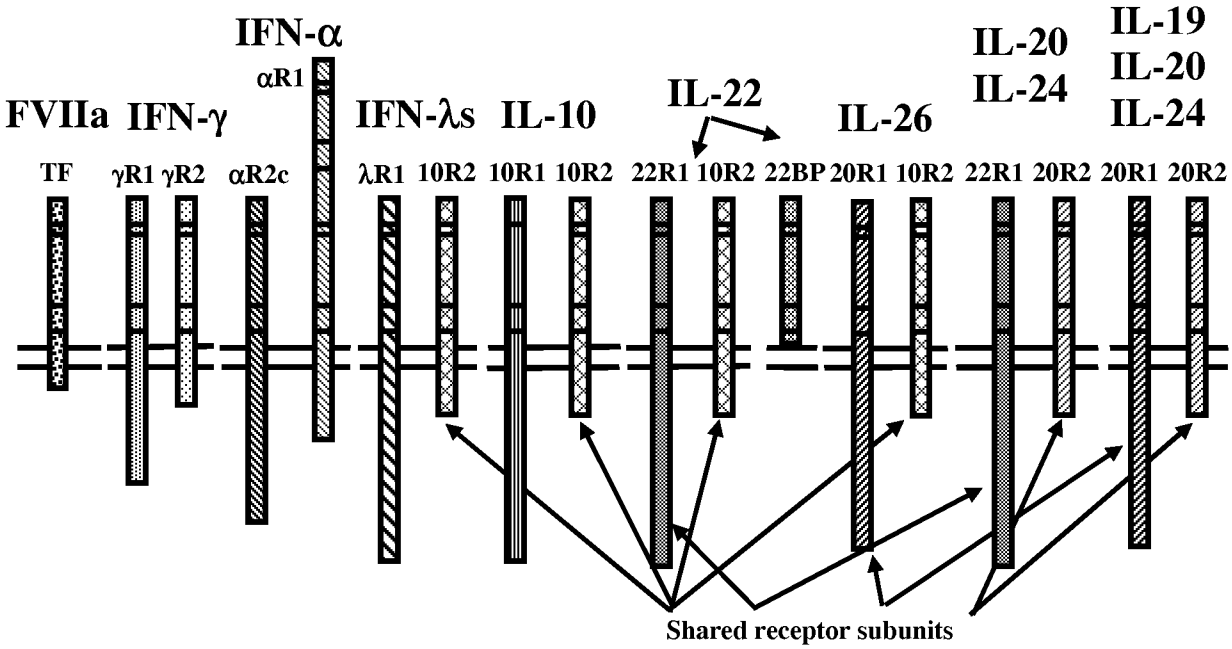
IFN-α
αR1
FVIIa
IFN-γ
IFN-λs
IL-10
IL-22
IL-26
IL-20
IL-24
IL-19
IL-20
IL-24
TF
γR1
γR2
αR2c
λR1
10R2
10R1
10R2
22R1
10R2
22BP
20R1
10R2
22R1
20R2
20R1
20R2
Shared receptor subunits

Fig. 4.

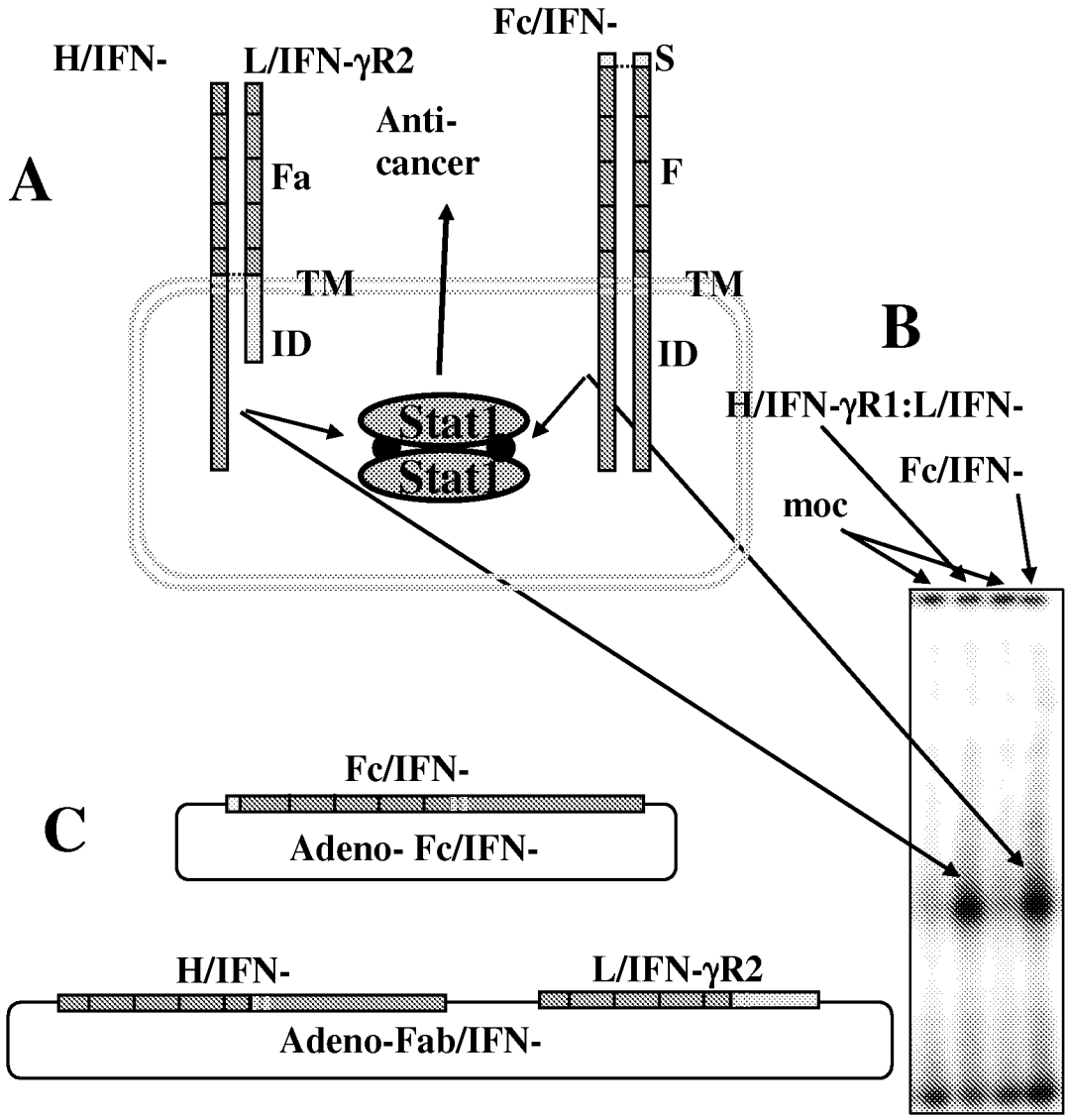
A
H/IFN-
L/IFN-γR2
Fa
Anti-
cancer
Fc/IFN-
S
F
TM
TM
ID
ID
Stat1
Stat1
B
H/IFN-γR1:L/IFN-
Fc/IFN-
moc
C
Fc/IFN-
Adeno- Fc/IFN-
H/IFN-
L/IFN-γR2
Adeno-Fab/IFN-

Fig. 5.

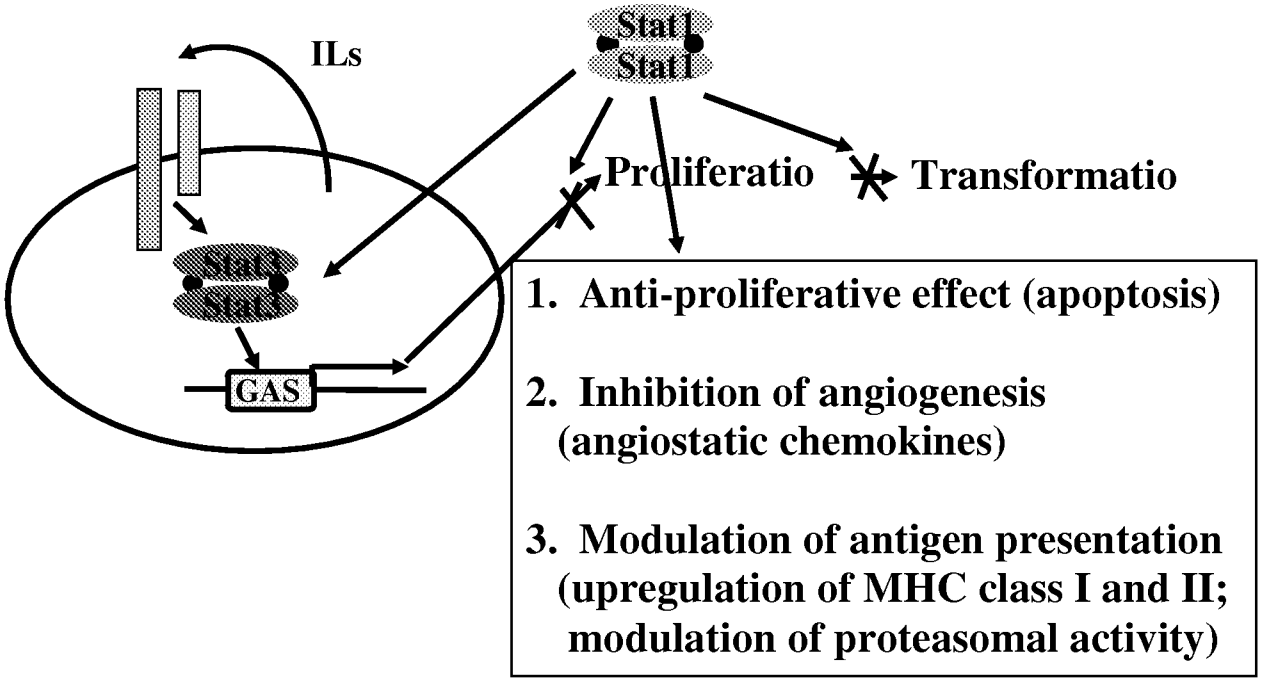
ILs
Stat1
Stat1
Proliferatio
Transformatio
Stat3
Stat3
GAS
1. Anti-proliferative effect (apoptosis)
2. Inhibition of angiogenesis (angiostatic chemokines)
3. Modulation of antigen presentation (upregulation of MHC class I and II; modulation of proteasomal activity)

Fig. 6.

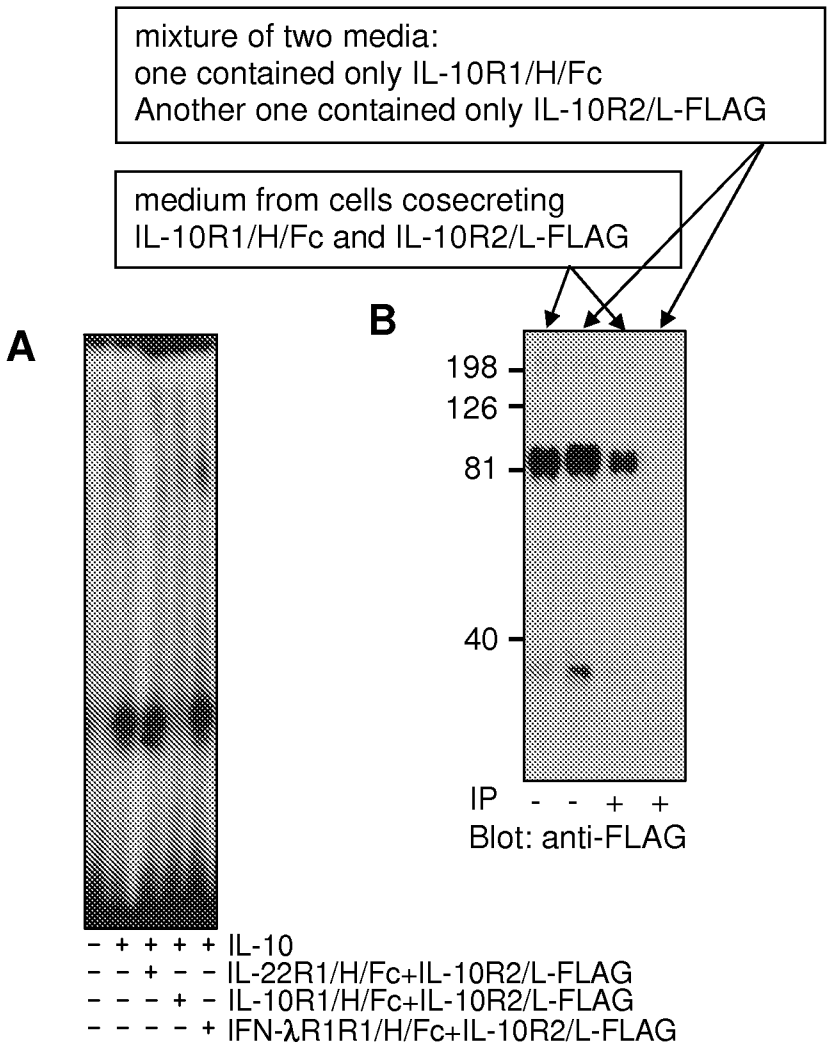
mixture of two media:
one contained only IL-10R1/H/Fc
Another one contained only IL-10R2/L-FLAG
medium from cells cosecreting
IL-10R1/H/Fc and IL-10R2/L-FLAG
A
B
198
126
81
40
IP - - + +
Blot: anti-FLAG
- + + + + IL-10
- - + - - IL-22R1/H/Fc+IL-10R2/L-FLAG
- - - + - IL-10R1/H/Fc+IL-10R2/L-FLAG
- - - - + IFN-λR1R1/H/Fc+IL-10R2/L-FLAG

Fig. 7.

Fig. 8A mH2/γR1

```
ATGGAATGGAGCTGGGTCTTTCTCTTCATCCTCTCAGGAACTGCAAGTGTCCACTCCCAG  60
M  E  W  S  W  V  F  L  F  I  L  S  G  T  A  S  V  H  S  Q

GTTCAGCTGCAGCAGCCTGGAGCTGAGCTGGCGAGGCCTGGGGCTTCAGTGAAGATGTCC  120
V  Q  L  Q  Q  P  G  A  E  L  A  R  P  G  A  S  V  K  M  S

TGCAAGGCTTCTGGTTACTCATTCACAAGCTATGGTATAAGCTGGGTGAAGCAGAAAACT  180
C  K  A  S  G  Y  S  F  T  S  Y  G  I  S  W  V  K  Q  K  T

AGACAGGGCCTTGAGTGGATTGGAGAGATTTATCCTGGAAGTGGTAACACTTACTACAAT  240
R  Q  G  L  E  W  I  G  E  I  Y  P  G  S  G  N  T  Y  Y  N

GAAAAGTTCAAGGGCAAGGCCACACTGACTGCAGACAAATCCTCCAGCACAGCCTACATG  300
E  K  F  K  G  K  A  T  L  T  A  D  K  S  S  S  T  A  Y  M

CAGCTCAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTTCTGTGCAAGATGGGGGGAG  360
Q  L  S  S  L  T  S  E  D  S  A  V  Y  F  C  A  R  W  G  E

CCCTGGGACGTGGACTACTGGGGCCAAGGCACCACTATCACAGTCTCCTCAGCCAAAACG  420
P  W  D  V  D  Y  W  G  Q  G  T  T  I  T  V  S  S  A  K  T

ACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTG  480
T  P  P  S  V  Y  P  L  A  P  G  S  A  A  Q  T  N  S  M  V

ACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCT  540
T  L  G  C  L  V  K  G  Y  F  P  E  P  V  T  V  T  W  N  S

GGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACT  600
G  S  L  S  S  G  V  H  T  F  P  A  V  L  Q  S  D  L  Y  T

CTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCCAGACCGTCACCTGCAAC  660
L  S  S  S  V  T  V  P  S  S  T  W  P  S  Q  T  V  T  C  N

GTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGT  720
V  A  H  P  A  S  S  T  K  V  D  K  K  I  V  P  R  D  C  G

AAGTGGCTAGCGCCAGTTGTTGCTGCTTTACTACTCTTTCTAGTGCTTAGCCTGGTATTC  780
K  W  L  A  P  V  V  A  A  L  L  L  F  L  V  L  S  L  V  F

ATCTGTTTTTATATTAAGAAAATTAATCCATTGAAGGAAAAAAGCATAATATTACCCAAG  840
I  C  F  Y  I  K  K  I  N  P  L  K  E  K  S  I  I  L  P  K

TCCTTGATCTCTGTGGTAAGAAGTGCTACTTTAGAGACAAAACCTGAATCAAAATATGTA  900
S  L  I  S  V  V  R  S  A  T  L  E  T  K  P  E  S  K  Y  V

TCACTCATCACGTCATACCAGCCATTTTCCTTAGAAAAGGAGGTGGTCTGTGAAGAGCCG  960
S  L  I  T  S  Y  Q  P  F  S  L  E  K  E  V  V  C  E  E  P

TTGTCTCCAGCAACAGTTCCAGGCATGCATACCGAAGACAATCCAGGAAAAGTGGAACAT
1020
```

```
L  S  P  A  T  V  P  G  M  H  T  E  D  N  P  G  K  V  E  H

ACAGAAGAACTTTCTAGTATAACAGAAGTGGTGACTACTGAAGAAAATATTCCTGACGTG
1080
T  E  E  L  S  S  I  T  E  V  V  T  T  E  E  N  I  P  D  V

GTCCCGGGCAGCCATCTGACTCCAATAGAGAGAGAGAGTTCTTCACCTTTAAGTAGTAAC
1140
V  P  G  S  H  L  T  P  I  E  R  E  S  S  S  P  L  S  S  N

CAGTCTGAACCTGGCAGCATCGCTTTAAACTCGTATCACTCCAGAAATTGTTCTGAGAGT
1200
Q  S  E  P  G  S  I  A  L  N  S  Y  H  S  R  N  C  S  E  S

GATCACTCCAGAAATGGTTTTGATACTGATTCCAGCTGTCTGGAATCACATAGCTCCTTA
1260
D  H  S  R  N  G  F  D  T  D  S  S  C  L  E  S  H  S  S  L

TCTGACTCAGAATTTCCCCCAAATAATAAAGGTGAAATAAAAACAGAAGGACAAGAGCTC
1320
S  D  S  E  F  P  P  N  N  K  G  E  I  K  T  E  G  Q  E  L

ATAACCGTAATAAAAGCCCCCACCTCCTTTGGTTATGATAAACCACATGTGCTAGTGGAT
1380
I  T  V  I  K  A  P  T  S  F  G  Y  D  K  P  H  V  L  V  D

CTACTTGTGGATGATAGCGGTAAAGAGTCCTTGATTGGTTATAGACCAACAGAAGATTCC
1440
L  L  V  D  D  S  G  K  E  S  L  I  G  Y  R  P  T  E  D  S

AAAGAATTTTCATGA  1455
K  E  F  S  *
```

Fig. 8B mL2/γR2

```
ATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCCTCAGTCATAATATCC  60
M  D  F  Q  V  Q  I  F  S  F  L  L  I  S  A  S  V  I  I  S

AGAGGACAAATTGTTCTCACCCAGTCTCCGGCAATCATGTCTGCATCTCCAGGGGAGAGG  120
R  G  Q  I  V  L  T  Q  S  P  A  I  M  S  A  S  P  G  E  R

GTCACCATGACCTGCAGTGCCAGCTCAAGTGTAAGTCACATGCACTGGTACCAGCAGAAG  180
V  T  M  T  C  S  A  S  S  S  V  S  H  M  H  W  Y  Q  Q  K

TCAGGCACCTCCCCCAAAAGATGGATTTATGACACATTCAAGTTGACTTCTGGAGTCCCT  240
S  G  T  S  P  K  R  W  I  Y  D  T  F  K  L  T  S  G  V  P

GATCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAACATGGAG  300
D  R  F  S  G  S  G  S  G  T  S  Y  S  L  T  I  S  N  M  E

GCTGAAGATGTTGCCACTTATTACTGCCAGCAGTGGAGTAGAAACCCACCCACGTTCGGT  360
A  E  D  V  A  T  Y  Y  C  Q  Q  W  S  R  N  P  P  T  F  G

GTTGGGACCAAGCTGGAGCTGAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCA  420
V  G  T  K  L  E  L  K  R  A  D  A  A  P  T  V  S  I  F  P

CCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTC  480
P  S  S  E  Q  L  T  S  G  G  A  S  V  V  C  F  L  N  N  F

TACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTC  540
Y  P  K  D  I  N  V  K  W  K  I  D  G  S  E  R  Q  N  G  V

CTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTC  600
L  N  S  W  T  D  Q  D  S  K  D  S  T  Y  S  M  S  S  T  L

ACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAG  660
T  L  T  K  D  E  Y  E  R  H  N  S  Y  T  C  E  A  T  H  K

ACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAGTGTCAGCAGTGGCTAGCG  720
T  S  T  S  P  I  V  K  S  F  N  R  N  E  C  Q  Q  W  L  A

ATCTCCGTGGGAACATTTTCGTTGCTGTCGGTGCTGGCAGGAGCCTGTTTCTTCCTGGTC  780
I  S  V  G  T  F  S  L  L  S  V  L  A  G  A  C  F  F  L  V

CTGAAATATAGAGGCCTGATTAAATACTGGTTTCACACTCCACCAAGCATCCCATTACAG  840
L  K  Y  R  G  L  I  K  Y  W  F  H  T  P  P  S  I  P  L  Q

ATAGAAGAGTATTTAAAAGACCCAACTCAGCCCATCTTAGAGGCCTTGGACAAGGACAGC  900
I  E  E  Y  L  K  D  P  T  Q  P  I  L  E  A  L  D  K  D  S

TCACCAAAGGATGACGTCTGGGACTCTGTGTCCATTATCTCGTTTCCGGAAAAGGAGCAA  960
S  P  K  D  D  V  W  D  S  V  S  I  I  S  F  P  E  K  E  Q

GAAGATGTTCTCCAAACGCTTTGA  984
E  D  V  L  Q  T  L  *
```

Fig. 8C SPd6xHis-hH2/γR1

```
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CATCATCACCATCACCATCCGGATCCCCAGGTGCAGCTGGTGCAGTCTGGGGCAGAGGTG  120
H  H  H  H  H  H  P  D  P  Q  V  Q  L  V  Q  S  G  A  E  V

AAAAAGCCCGGGGAGTCTCTGAAGATCTCCTGTCAGGGTTCTGGCTACAAGTTTACCAGT  180
K  K  P  G  E  S  L  K  I  S  C  Q  G  S  G  Y  K  F  T  S

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TATCCTTCTGACTCTGATGCCAGATATAGCCCGTCCTTCCAAGGCCGGGTCACCATCTCA  300
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GCCGACAAGTCCACCAGCACCGCCTACATGCAGTGGAGCGCCCTGAATACCTCGGACACC  360
A  D  K  S  T  S  T  A  Y  M  Q  W  S  A  L  N  T  S  D  T

GCCATTTATTTCTGTGCGCGAGGCGGCTGGCGTGACGCTTTTGATGTCTGGGGCCAAGGG  420
A  I  Y  F  C  A  R  G  G  W  R  D  A  F  D  V  W  G  Q  G

ACAATTGTCACCGTCTCTTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCC  480
T  I  V  T  V  S  S  A  S  T  K  G  P  S  V  F  P  L  A  P

TCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC  540
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CCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTC  600
P  E  P  V  T  V  S  W  N  S  G  A  L  T  S  G  V  H  T  F

CCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC  660
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GTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAATGGCTAGCGCCAGTTGTTGCTGCT  780
V  D  K  K  V  E  P  K  S  C  D  K  W  L  A  P  V  V  A  A

TTACTACTCTTTCTAGTGCTTAGCCTGGTATTCATCTGTTTTTATATTAAGAAAATTAAT  840
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CCATTGAAGGAAAAAAGCATAATATTACCCAAGTCCTTGATCTCTGTGGTAAGAAGTGCT  900
P  L  K  E  K  S  I  I  L  P  K  S  L  I  S  V  V  R  S  A

ACTTTAGAGACAAAACCTGAATCAAAATATGTATCACTCATCACGTCATACCAGCCATTT  960
T  L  E  T  K  P  E  S  K  Y  V  S  L  I  T  S  Y  Q  P  F

TCCTTAGAAAAGGAGGTGGTCTGTGAAGAGCCGTTGTCTCCAGCAACAGTTCCAGGCATG
1020
```

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1080
H  T  E  D  N  P  G  K  V  E  H  T  E  E  L  S  S  I  T  E

GTGGTGACTACTGAAGAAAATATTCCTGACGTGGTCCCGGGCAGCCATCTGACTCCAATA
1140
V  V  T  T  E  E  N  I  P  D  V  V  P  G  S  H  L  T  P  I

GAGAGAGAGAGTTCTTCACCTTTAAGTAGTAACCAGTCTGAACCTGGCAGCATCGCTTTG
1200
E  R  E  S  S  S  P  L  S  S  N  Q  S  E  P  G  S  I  A  L

AACTCGTATCACTCCAGAAATTGTTCTGAGAGTGATCACTCCAGAAATGGTTTTGATACT
1260
N  S  Y  H  S  R  N  C  S  E  S  D  H  S  R  N  G  F  D  T

GATTCCAGCTGTCTGGAATCACATAGCTCCTTATCTGACTCAGAATTTCCCCCAAATAAT
1320
D  S  S  C  L  E  S  H  S  S  L  S  D  S  E  F  P  P  N  N

AAAGGTGAAATAAAAACAGAAGGACAAGAGCTCATAACCGTAATAAAAGCCCCCACCTCC
1380
K  G  E  I  K  T  E  G  Q  E  L  I  T  V  I  K  A  P  T  S

TTTGGTTATGATAAACCACATGTGCTAGTGGATCTACTTGTGGATGATAGCGGTAAAGAG
1440
F  G  Y  D  K  P  H  V  L  V  D  L  L  V  D  D  S  G  K  E

TCCTTGATTGGTTATAGACCAACAGAAGATTCCAAAGAATTTTCATGA  1488
S  L  I  G  Y  R  P  T  E  D  S  K  E  F  S  *
```

Fig. 8D SPFL-hL2/γR2

```
ATGCGACCGACGCTGCTGTGGTCGCTGCTGCTGCTGCTCGGAGTCTTCGCCGCCGCCGCC  60
M  R  P  T  L  L  W  S  L  L  L  L  L  G  V  F  A  A  A  A

GCCGACTACAAGGACGACGATGACAAGGCGGATCCCGTGATGACCCAGTCTCCAGACTCC  120
A  D  Y  K  D  D  D  D  K  A  D  P  V  M  T  Q  S  P  D  S

CTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTCCAGCCAGAGTGTTGTA  180
L  A  V  S  L  G  E  R  A  T  I  N  C  K  S  S  Q  S  V  V

TCCAGCTCCGACAATAGGTACTACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCTCCT  240
S  S  S  D  N  R  Y  Y  L  A  W  Y  Q  Q  K  P  G  Q  P  P

AAGCTGCTCATTTACTGGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATTCAGTGGC  300
K  L  L  I  Y  W  A  S  T  R  E  S  G  V  P  D  R  F  S  G

AGCGGGTCTGGGACAGATTTCACTCTCACCATCAGCAGCCTACAGGCTGAAGATATGGCA  360
S  G  S  G  T  D  F  T  L  T  I  S  S  L  Q  A  E  D  M  A

GTTTATTACTGTCAGCAATATTATAGTCCTCCGTGGACGTTCGGCCAAGGGACCAAGGTG  420
V  Y  Y  C  Q  Q  Y  Y  S  P  P  W  T  F  G  Q  G  T  K  V

GAAATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAG  480
E  I  K  R  T  V  A  A  P  S  V  F  I  F  P  P  S  D  E  Q

TTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC  540
L  K  S  G  T  A  S  V  V  C  L  L  N  N  F  Y  P  R  E  A

AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACA  600
K  V  Q  W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S  V  T

GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCA  660
E  Q  D  S  K  D  S  T  Y  S  L  S  S  T  L  T  L  S  K  A

GACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCC  720
D  Y  E  K  H  K  V  Y  A  C  E  V  T  H  Q  G  L  S  S  P

GTCACAAAGAGCTTCAACAGGGGAGAGTGTCAGCAGTGGCTAGCGATCTCCGTGGGAACA  780
V  T  K  S  F  N  R  G  E  C  Q  Q  W  L  A  I  S  V  G  T

TTTTCGTTGCTGTCGGTGCTGGCAGGAGCCTGTTTCTTCCTGGTCCTGAAATATAGAGGC  840
F  S  L  L  S  V  L  A  G  A  C  F  F  L  V  L  K  Y  R  G

CTGATTAAATACTGGTTTCACACTCCACCAAGCATCCCATTACAGATAGAAGAGTATTTA  900
L  I  K  Y  W  F  H  T  P  P  S  I  P  L  Q  I  E  E  Y  L

AAAGACCCAACTCAGCCCATCTTAGAGGCCTTGGACAAGGACAGCTCACCAAAGGATGAC  960
K  D  P  T  Q  P  I  L  E  A  L  D  K  D  S  S  P  K  D  D

GTCTGGGACTCTGTGTCCATTATCTCGTTTCCGGAAAAGGAGCAAGAAGATGTTCTCCAA
1020
```

V W D S V S I I S F P E K E Q E D V L Q

ACGCTTTGA 1029
T L *

Fig. 8E SPFL-mFc/γR1

```
ATGCGACCGACGCTGCTGTGGTCGCTGCTGCTGCTGCTCGGAGTCTTCGCCGCCGCCGCC  60
M  R  P  T  L  L  W  S  L  L  L  L  L  G  V  F  A  A  A  A

GCCGACTACAAGGACGACGATGACAAGGCGGATCCTGGTTGTAAGCCTTGCATATGTACA  120
A  D  Y  K  D  D  D  D  K  A  D  P  G  C  K  P  C  I  C  T

GTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATT  180
V  P  E  V  S  S  V  F  I  F  P  P  K  P  K  D  V  L  T  I

ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTC  240
T  L  T  P  K  V  T  C  V  V  V  D  I  S  K  D  D  P  E  V

CAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAG  300
Q  F  S  W  F  V  D  D  V  E  V  H  T  A  Q  T  Q  P  R  E

GAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACTGG  360
E  Q  F  N  S  T  F  R  S  V  S  E  L  P  I  M  H  Q  D  W

CTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAG  420
L  N  G  K  E  F  K  C  R  V  N  S  A  A  F  P  A  P  I  E

AAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCT  480
K  T  I  S  K  T  K  G  R  P  K  A  P  Q  V  Y  T  I  P  P

CCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTC  540
P  K  E  Q  M  A  K  D  K  V  S  L  T  C  M  I  T  D  F  F

CCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAAC  600
P  E  D  I  T  V  E  W  Q  W  N  G  Q  P  A  E  N  Y  K  N

ACTCAGCCCATCATGGACACAGATGGCTCTTACTTCATCTACAGCAAGCTCAATGTGCAG  660
T  Q  P  I  M  D  T  D  G  S  Y  F  I  Y  S  K  L  N  V  Q

AAGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCAC  720
K  S  N  W  E  A  G  N  T  F  T  C  S  V  L  H  E  G  L  H

AACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAATGGCTAGCGCCAGTTGTT  780
N  H  H  T  E  K  S  L  S  H  S  P  G  K  W  L  A  P  V  V

GCTGCTTTACTACTCTTTCTAGTGCTTAGCCTGGTATTCATCTGTTTTTATATTAAGAAA  840
A  A  L  L  L  F  L  V  L  S  L  V  F  I  C  F  Y  I  K  K

ATTAATCCATTGAAGGAAAAAAGCATAATATTACCCAAGTCCTTGATCTCTGTGGTAAGA  900
I  N  P  L  K  E  K  S  I  I  L  P  K  S  L  I  S  V  V  R

AGTGCTACTTTAGAGACAAAACCTGAATCAAAATATGTATCACTCATCACGTCATACCAG  960
S  A  T  L  E  T  K  P  E  S  K  Y  V  S  L  I  T  S  Y  Q

CCATTTTCCTTAGAAAAGGAGGTGGTCTGTGAAGAGCCGTTGTCTCCAGCAACAGTTCCA
1020
```

```
P  F  S  L  E  K  E  V  V  C  E  E  P  L  S  P  A  T  V  P

GGCATGCATACCGAAGACAATCCAGGAAAAGTGGAACATACAGAAGAACTTTCTAGTATA
1080
G  M  H  T  E  D  N  P  G  K  V  E  H  T  E  E  L  S  S  I

ACAGAAGTGGTGACTACTGAAGAAAATATTCCTGACGTGGTCCCGGGCAGCCATCTGACT
1140
T  E  V  V  T  T  E  E  N  I  P  D  V  V  P  G  S  H  L  T

CCAATAGAGAGAGAGAGTTCTTCACCTTTAAGTAGTAACCAGTCTGAACCTGGCAGCATC
1200
P  I  E  R  E  S  S  S  P  L  S  S  N  Q  S  E  P  G  S  I

GCTTTAAACTCGTATCACTCCAGAAATTGTTCTGAGAGTGATCACTCCAGAAATGGTTTT
1260
A  L  N  S  Y  H  S  R  N  C  S  E  S  D  H  S  R  N  G  F

GATACTGATTCCAGCTGTCTGGAATCACATAGCTCCTTATCTGACTCAGAATTTCCCCCA
1320
D  T  D  S  S  C  L  E  S  H  S  S  L  S  D  S  E  F  P  P

AATAATAAAGGTGAAATAAAAACAGAAGGACAAGAGCTCATAACCGTAATAAAAGCCCCC
1380
N  N  K  G  E  I  K  T  E  G  Q  E  L  I  T  V  I  K  A  P

ACCTCCTTTGGTTATGATAAACCACATGTGCTAGTGGATCTACTTGTGGATGATAGCGGT
1440
T  S  F  G  Y  D  K  P  H  V  L  V  D  L  L  V  D  D  S  G

AAAGAGTCCTTGATTGGTTATAGACCAACAGAAGATTCCAAAGAATTTTCATGA  1494
K  E  S  L  I  G  Y  R  P  T  E  D  S  K  E  F  S  *
```

Fig. 8F IL-10R2-2xlinker-L2-linker-FL

```
ATGGCGTGGAGCCTTGGGAGCTGGCTGGGTGGCTGCCTGCTGGTGTCAGCATTGGGAATG  60
M  A  W  S  L  G  S  W  L  G  G  C  L  L  V  S  A  L  G  M

GTGCCACCTCCCGAAAATGTCAGAATGAATTCTGTTAATTTCAAGAACATTCTACAGTGG  120
V  P  P  P  E  N  V  R  M  N  S  V  N  F  K  N  I  L  Q  W

GAGTCACCTGCTTTTGCCGAAGGGAACCTGACTTTCACAGCTCAGTACCTAAGTTATAGG  180
E  S  P  A  F  A  E  G  N  L  T  F  T  A  Q  Y  L  S  Y  R

ATATTCCAAGATAAATGCATGAATACTACCTTGACGGAATGTGATTTCTCAAGTCTTTCC  240
I  F  Q  D  K  C  M  N  T  T  L  T  E  C  D  F  S  S  L  S

AAGTATGGTGACCACACCTTGAGAGTCAGGGCTGAATTTGCAGATGAGCATTCAGACTGG  300
K  Y  G  D  H  T  L  R  V  R  A  E  F  A  D  E  H  S  D  W

GTAAACATCACCTTCTGTCCTGTGGATGACACCATTATTGGACCCCCTGGAATGCAAGTA  360
V  N  I  T  F  C  P  V  D  D  T  I  I  G  P  P  G  M  Q  V

GAAGTACTTGCTGATTCTTTACATATGCGTTTCTTAGCCCCTAAAATTGAGAATGAATAC  420
E  V  L  A  D  S  L  H  M  R  F  L  A  P  K  I  E  N  E  Y

GAAACTTGGACTATGAAGAATGTGTATAACTCATGGACTTATAATGTGCAATACTGGAAA  480
E  T  W  T  M  K  N  V  Y  N  S  W  T  Y  N  V  Q  Y  W  K

AACGGTACTGATGAAAAGTTTCAAATTACTCCCCAGTATGACTTTGAGGTCCTCAGAAAC  540
N  G  T  D  E  K  F  Q  I  T  P  Q  Y  D  F  E  V  L  R  N

CTGGAGCCATGGACAACTTATTGTGTTCAAGTTCGAGGGTTTCTTCCTGATCGGAACAAA  600
L  E  P  W  T  T  Y  C  V  Q  V  R  G  F  L  P  D  R  N  K

GCTGGGGAATGGAGTGAGCCTGTCTGTGAGCAAACAACCCATGACGAAACGGTCCCCTCC  660
A  G  E  W  S  E  P  V  C  E  Q  T  T  H  D  E  T  V  P  S

GCTAGCGGGAGTTCTGGTGGTAGCTCTGGGACTAGCGGGAGTTCTGGTGGTAGCTCTGGG  720
A  S  G  S  S  G  G  S  S  G  T  S  G  S  S  G  G  S  S  G

ACTAGTACGGATCCTGTGATGTCACAGTCTCCATCCTCCCTACCTGTGTCAGTTGGCGAG  780
T  S  T  D  P  V  M  S  Q  S  P  S  S  L  P  V  S  V  G  E

AAGGTTACTTTGAGCTGCAAGTCCAGTCAGAGCCTTTTATATAGTGGTAATCAAAAGAAC  840
K  V  T  L  S  C  K  S  S  Q  S  L  L  Y  S  G  N  Q  K  N

TACTTGGCCTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCTGATTTACTGGGCA  900
Y  L  A  W  Y  Q  Q  K  P  G  Q  S  P  K  L  L  I  Y  W  A

TCCGCTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTC  960
S  A  R  E  S  G  V  P  D  R  F  T  G  S  G  S  G  T  D  F

ACTCTCTCCATCAGCAGTGTGAAGACTGAAGACCTGGCAGTTTATTACTGTCAGCAGTAT
1020
```

```
T  L  S  I  S  S  V  K  T  E  D  L  A  V  Y  Y  C  Q  Q  Y

TATAGCTATCCCCTCACGTTCGGTGCTGGGACCAAGCTGGTGCTGAAACGGGCCGCGGCT
1080
Y  S  Y  P  L  T  F  G  A  G  T  K  L  V  L  K  R  A  A  A

GCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCT
1140
A  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S  G  T  A  S

GTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGAT
1200
V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q  W  K  V  D

AACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGC
1260
N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D  S  K  D  S

ACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTC
1320
T  Y  S  L  S  S  T  L  T  L  S  K  A  D  Y  E  K  H  K  V

TACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGG
1380
Y  A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K  S  F  N  R

GGAGAGTGTTCGTCGGGGGGAACTAGCCCTGACTACAAGGACGACGATGACAAGCTCTAG
1440
G  E  C  S  S  G  G  T  S  P  D  Y  K  D  D  D  D  K  L  *
```

Fig. 8G IL-10R1-2xlinker-H2-linker-6xHis

```
ATGCTGCCGTGCCTCGTAGTGCTGCTGGCGGCGCTCCTCAGCCTCCGTCTTGGCTCAGAC  60
M  L  P  C  L  V  V  L  L  A  A  L  L  S  L  R  L  G  S  D

GCTCATGGGACAGAGCTGCCCAGCCCTCCGTCTGTGTGGTTTGAAGCAGAATTTTTCCAC  120
A  H  G  T  E  L  P  S  P  P  S  V  W  F  E  A  E  F  F  H

CACATCCTCCACTGGACACCCATCCCAAATCAGTCTGAAAGTACCTGCTATGAAGTGGCG  180
H  I  L  H  W  T  P  I  P  N  Q  S  E  S  T  C  Y  E  V  A

CTCCTGAGGTATGGAATAGAGTCCTGGAACTCCATCTCCAACTGTAGCCAGACCCTGTCC  240
L  L  R  Y  G  I  E  S  W  N  S  I  S  N  C  S  Q  T  L  S

TATGACCTTACCGCAGTGACCTTGGACCTGTACCACAGCAATGGCTACCGGGCCAGAGTG  300
Y  D  L  T  A  V  T  L  D  L  Y  H  S  N  G  Y  R  A  R  V

CGGGCTGTGGACGGCAGCCGGCACTCCAACTGGACCGTCACCAACACCCGCTTCTCTGTG  360
R  A  V  D  G  S  R  H  S  N  W  T  V  T  N  T  R  F  S  V

GATGAAGTGACTCTGACAGTTGGCAGTGTGAACCTAGAGATCCACAATGGCTTCATCCTC  420
D  E  V  T  L  T  V  G  S  V  N  L  E  I  H  N  G  F  I  L

GGGAAGATTCAGCTACCCAGGCCCAAGATGGCCCCCGCGAATGACACATATGAAAGCATC  480
G  K  I  Q  L  P  R  P  K  M  A  P  A  N  D  T  Y  E  S  I

TTCAGTCACTTCCGAGAGTATGAGATTGCCATTCGCAAGGTGCCGGGAAACTTCACGTTC  540
F  S  H  F  R  E  Y  E  I  A  I  R  K  V  P  G  N  F  T  F

ACACACAAGAAAGTAAAACATGAAAACTTCAGCCTCCTAACCTCTGGAGAAGTGGGAGAG  600
T  H  K  K  V  K  H  E  N  F  S  L  L  T  S  G  E  V  G  E

TTCTGTGTCCAGGTGAAACCATCTGTCGCTTCCCGAAGTAACAAGGGGATGTGGTCTAAA  660
F  C  V  Q  V  K  P  S  V  A  S  R  S  N  K  G  M  W  S  K

GAGGAGTGCATCTCCCTCACCAGGCAGTATTTCACCGTGACCAACGCTAGCGGGAGTTCT  720
E  E  C  I  S  L  T  R  Q  Y  F  T  V  T  N  A  S  G  S  S

GGTGGTAGCTCTGGGACTAGCGGGAGTTCTGGTGGTAGCTCTGGGACTAGTACGGATCCC  780
G  G  S  S  G  T  S  G  S  S  G  G  S  S  G  T  S  T  D  P

CAGGTTCAGTTGCAGCAGTCTGACGCTGAGTTGGTGAAACCTGGGGCTTCAGTGAAGATT  840
Q  V  Q  L  Q  Q  S  D  A  E  L  V  K  P  G  A  S  V  K  I

TCCTGCAAGGCTTCTGGCTACACCTTCACTGACCATGCAATTCACTGGGTGAAACAGAAC  900
S  C  K  A  S  G  Y  T  F  T  D  H  A  I  H  W  V  K  Q  N

CCTGAACAGGGCCTGGAATGGATTGGATATTTTTCTCCCGGAAATGATGATTTTAAATAC 960
P  E  Q  G  L  E  W  I  G  Y  F  S  P  G  N  D  D  F  K  Y

AATGAGAGGTTCAAGGGCAAGGCCACACTGACTGCAGACAAATCCTCCAGCACTGCCTAC
1020
```

```
N  E  R  F  K  G  K  A  T  L  T  A  D  K  S  S  S  T  A  Y

GTGCAGCTCAACAGCCTGACATCTGAGGATTCTGCAGTGTATTTCTGTACAAGATCCCTG
1080
V  Q  L  N  S  L  T  S  E  D  S  A  V  Y  F  C  T  R  S  L

AATATGGCCTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAACGACGGGC
1140
N  M  A  Y  W  G  Q  G  T  S  V  T  V  S  S  A  K  T  T  G

CCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTG
1200
P  S  V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L

GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC
1260
G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A

CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC
1320
L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L

AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTG
1380
S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V

AATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTACTAGC
1440
N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  T  S

GGGAGTTCTGGTGGTAGCTCTGGGACTAGCCATCATCACCATCACCATTGA  1491
G  S  S  G  G  S  S  G  T  S  H  H  H  H  H  H  *
```

Fig. 8H IL-10R1-2xlinker-H2-Fc

```
ATGCTGCCGTGCCTCGTAGTGCTGCTGGCGGCGCTCCTCAGCCTCCGTCTTGGCTCAGAC  60
M  L  P  C  L  V  V  L  L  A  A  L  L  S  L  R  L  G  S  D

GCTCATGGGACAGAGCTGCCCAGCCCTCCGTCTGTGTGGTTTGAAGCAGAATTTTTCCAC  120
A  H  G  T  E  L  P  S  P  P  S  V  W  F  E  A  E  F  F  H

CACATCCTCCACTGGACACCCATCCCAAATCAGTCTGAAAGTACCTGCTATGAAGTGGCG  180
H  I  L  H  W  T  P  I  P  N  Q  S  E  S  T  C  Y  E  V  A

CTCCTGAGGTATGGAATAGAGTCCTGGAACTCCATCTCCAACTGTAGCCAGACCCTGTCC  240
L  L  R  Y  G  I  E  S  W  N  S  I  S  N  C  S  Q  T  L  S

TATGACCTTACCGCAGTGACCTTGGACCTGTACCACAGCAATGGCTACCGGGCCAGAGTG  300
Y  D  L  T  A  V  T  L  D  L  Y  H  S  N  G  Y  R  A  R  V

CGGGCTGTGGACGGCAGCCGGCACTCCAACTGGACCGTCACCAACACCCGCTTCTCTGTG  360
R  A  V  D  G  S  R  H  S  N  W  T  V  T  N  T  R  F  S  V

GATGAAGTGACTCTGACAGTTGGCAGTGTGAACCTAGAGATCCACAATGGCTTCATCCTC  420
D  E  V  T  L  T  V  G  S  V  N  L  E  I  H  N  G  F  I  L

GGGAAGATTCAGCTACCCAGGCCCAAGATGGCCCCCGCGAATGACACATATGAAAGCATC  480
G  K  I  Q  L  P  R  P  K  M  A  P  A  N  D  T  Y  E  S  I

TTCAGTCACTTCCGAGAGTATGAGATTGCCATTCGCAAGGTGCCGGGAAACTTCACGTTC  540
F  S  H  F  R  E  Y  E  I  A  I  R  K  V  P  G  N  F  T  F

ACACACAAGAAAGTAAAACATGAAAACTTCAGCCTCCTAACCTCTGGAGAAGTGGGAGAG  600
T  H  K  K  V  K  H  E  N  F  S  L  L  T  S  G  E  V  G  E

TTCTGTGTCCAGGTGAAACCATCTGTCGCTTCCCGAAGTAACAAGGGGATGTGGTCTAAA  660
F  C  V  Q  V  K  P  S  V  A  S  R  S  N  K  G  M  W  S  K

GAGGAGTGCATCTCCCTCACCAGGCAGTATTTCACCGTGACCAACGCTAGCGGGAGTTCT  720
E  E  C  I  S  L  T  R  Q  Y  F  T  V  T  N  A  S  G  S  S

GGTGGTAGCTCTGGGACTAGCGGGAGTTCTGGTGGTAGCTCTGGGACTAGTACGGATCCC  780
G  G  S  S  G  T  S  G  S  S  G  G  S  S  G  T  S  T  D  P

CAGGTTCAGTTGCAGCAGTCTGACGCTGAGTTGGTGAAACCTGGGGCTTCAGTGAAGATT  840
Q  V  Q  L  Q  Q  S  D  A  E  L  V  K  P  G  A  S  V  K  I

TCCTGCAAGGCTTCTGGCTACACCTTCACTGACCATGCAATTCACTGGGTGAAACAGAAC  900
S  C  K  A  S  G  Y  T  F  T  D  H  A  I  H  W  V  K  Q  N

CCTGAACAGGGCCTGGAATGGATTGGATATTTTTCTCCCGGAAATGATGATTTTAAATAC  960
P  E  Q  G  L  E  W  I  G  Y  F  S  P  G  N  D  D  F  K  Y

AATGAGAGGTTCAAGGGCAAGGCCACACTGACTGCAGACAAATCCTCCAGCACTGCCTAC
1020
```

N E R F K G K A T L T A D K S S S T A Y

GTGCAGCTCAACAGCCTGACATCTGAGGATTCTGCAGTGTATTTCTGTACAAGATCCCTG
1080
V Q L N S L T S E D S A V Y F C T R S L

AATATGGCCTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAACGACGGGC
1140
N M A Y W G Q G T S V T V S S A K T T G

CCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTG
1200
P S V F P L A P S S K S T S G G T A A L

GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC
1260
G C L V K D Y F P E P V T V S W N S G A

CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC
1320
L T S G V H T F P A V L Q S S G L Y S L

AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTG
1380
S S V V T V P S S S L G T Q T Y I C N V

AATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAA
1440
N H K P S N T K V D K K V E P K S C D K

ACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
1500
T H T C P P C P A P E L L G G P S V F L

TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTG
1560
F P P K P K D T L M I S R T P E V T C V

GTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG
1620
V V D V S H E D P E V K F N W Y V D G V

GAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTG
1680
E V H N A K T K P R E E Q Y N S T Y R V

GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
1740
V S V L T V L H Q D W L N G K E Y K C K

GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG
1800

```
V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  G  Q

CCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAG
1860
P  R  E  P  Q  V  Y  T  L  P  P  S  R  D  E  L  T  K  N  Q

GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAG
1920
V  S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E

AGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGC
1980
S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G

TCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTC
2040
S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V

TTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCC
2100
F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S

CTGTCTCCGGGTAAATGA  2118
L  S  P  G  K  *
```

Fig. 8I IFN-λR1-linker-H2-Fc

```
ATGGCGGGGCCCGAGCGCTGGGGCCCCCTGCTCCTGTGCCTGCTGCAGGCCGCTCCAGGG  60
M  A  G  P  E  R  W  G  P  L  L  L  C  L  L  Q  A  A  P  G

AGGCCCCGTCTGGCCCCTCCCCAGAATGTGACGCTGCTCTCCCAGAACTTCAGCGTGTAC  120
R  P  R  L  A  P  P  Q  N  V  T  L  L  S  Q  N  F  S  V  Y

CTGACATGGCTCCCAGGGCTTGGCAACCCCCAGGATGTGACCTATTTTGTGGCCTATCAG  180
L  T  W  L  P  G  L  G  N  P  Q  D  V  T  Y  F  V  A  Y  Q

AGCTCTCCTACCCGTAGACGGTGGCGCGAAGTGGAAGAGTGTGCGGGAACCAAGGAGCTG  240
S  S  P  T  R  R  R  W  R  E  V  E  E  C  A  G  T  K  E  L

CTATGTTCTATGATGTGCCTGAAGAAACAGGACCTGTACAACAAGTTCAAGGGACGCGTG  300
L  C  S  M  M  C  L  K  K  Q  D  L  Y  N  K  F  K  G  R  V

CGGACGGTTTCTCCCAGCTCCAAGTCCCCCTGGGTGGAGTCCGAATACCTGGATTACCTT  360
R  T  V  S  P  S  S  K  S  P  W  V  E  S  E  Y  L  D  Y  L

TTTGAAGTGGAGCCGGCCCCACCTGTCCTGGTGCTCACCCAGACGGAGGAGATCCTGAGT  420
F  E  V  E  P  A  P  P  V  L  V  L  T  Q  T  E  E  I  L  S

GCCAATGCCACGTACCAGCTGCCCCCCTGCATGCCCCCACTGGATCTGAAGTATGAGGTG  480
A  N  A  T  Y  Q  L  P  P  C  M  P  P  L  D  L  K  Y  E  V

GCATTCTGGAAGGAGGGGGCCGGAAACAAGACCCTATTTCCAGTCACTCCCCATGGCCAG  540
A  F  W  K  E  G  A  G  N  K  T  L  F  P  V  T  P  H  G  Q

CCAGTCCAGATCACTCTCCAGCCAGCTGCCAGCGAACACCACTGCCTCAGTGCCAGAACC  600
P  V  Q  I  T  L  Q  P  A  A  S  E  H  H  C  L  S  A  R  T

ATCTACACGTTCAGTGTCCCGAAATACAGCAAGTTCTCTAAGCCCACCTGCTTCTTGCTG  660
I  Y  T  F  S  V  P  K  Y  S  K  F  S  K  P  T  C  F  L  L

GAGGTCCCAGAAGCCAACGCTAGCGGGAGTTCTGGTGGTAGCTCTGGGACTAGTACGGAT  720
E  V  P  E  A  N  A  S  G  S  S  G  G  S  S  G  T  S  T  D

CCCCAGGTTCAGTTGCAGCAGTCTGACGCTGAGTTGGTGAAACCTGGGGCTTCAGTGAAG  780
P  Q  V  Q  L  Q  Q  S  D  A  E  L  V  K  P  G  A  S  V  K

ATTTCCTGCAAGGCTTCTGGCTACACCTTCACTGACCATGCAATTCACTGGGTGAAACAG  840
I  S  C  K  A  S  G  Y  T  F  T  D  H  A  I  H  W  V  K  Q

AACCCTGAACAGGGCCTGGAATGGATTGGATATTTTTCTCCCGGAAATGATGATTTTAAA  900
N  P  E  Q  G  L  E  W  I  G  Y  F  S  P  G  N  D  D  F  K

TACAATGAGAGGTTCAAGGGCAAGGCCACACTGACTGCAGACAAATCCTCCAGCACTGCC  960
Y  N  E  R  F  K  G  K  A  T  L  T  A  D  K  S  S  S  T  A

TACGTGCAGCTCAACAGCCTGACATCTGAGGATTCTGCAGTGTATTTCTGTACAAGATCC
1020
```

```
Y  V  Q  L  N  S  L  T  S  E  D  S  A  V  Y  F  C  T  R  S

CTGAATATGGCCTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAACGACG
1080
L  N  M  A  Y  W  G  Q  G  T  S  V  T  V  S  S  A  K  T  T

GGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCC
1140
G  P  S  V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A

CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGC
1200
L  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G

GCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
1260
A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S

CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
1320
L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N

GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGAC
1380
V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D

AAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGGACCGTCAGTCTTC
1440
K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F

CTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC
1500
L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C

GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
1560
V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G

GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGT
1620
V  E  V  H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R

GTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
1680
V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C

AAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGG
1740
K  V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  G

CAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAAC
1800
```

```
Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  D  E  L  T  K  N

CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGG
1860
Q  V  S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W

GAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGAC
1920
E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D

GGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC
1980
G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N

GTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
2040
V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L

TCCCTGTCTCCGGGTAAATGA 2061
S  L  S  P  G  K  *
```

Fig. 8J IL-22R1-2xlinker-H2-Fc

```
ATGAGGACGCTGCTGACCATCTTGACTGTGGGATCCCTGGCTGCTCACGCCCCTGAGGAC  60
M  R  T  L  L  T  I  L  T  V  G  S  L  A  A  H  A  P  E  D

CCCTCGGATCTGCTCCAGCACGTGAAATTCCAGTCCAGCAACTTTGAAAACATCCTGACG  120
P  S  D  L  L  Q  H  V  K  F  Q  S  S  N  F  E  N  I  L  T

TGGGACAGCGGGCCAGAGGGCACCCCAGACACGGTCTACAGCATCGAGTATAAGACGTAC  180
W  D  S  G  P  E  G  T  P  D  T  V  Y  S  I  E  Y  K  T  Y

GGAGAGAGGGACTGGGTGGCAAAGAAGGGCTGTCAGCGGATCACCCGGAAGTCCTGCAAC  240
G  E  R  D  W  V  A  K  K  G  C  Q  R  I  T  R  K  S  C  N

CTGACGGTGGAGACGGGCAACCTCACGGAGCTCTACTATGCCAGGGTCACCGCTGTCAGT  300
L  T  V  E  T  G  N  L  T  E  L  Y  Y  A  R  V  T  A  V  S

GCGGGAGGCCGGTCAGCCACCAAGATGACTGACAGGTTCAGCTCTCTGCAGCACACTACC  360
A  G  G  R  S  A  T  K  M  T  D  R  F  S  S  L  Q  H  T  T

CTCAAGCCACCTGATGTGACCTGTATCTCCAAAGTGAGATCGATTCAGATGATTGTTCAT  420
L  K  P  P  D  V  T  C  I  S  K  V  R  S  I  Q  M  I  V  H

CCTACCCCCACGCCAATCCGTGCAGGCGATGGCCACCGGCTAACCCTGGAAGACATCTTC  480
P  T  P  T  P  I  R  A  G  D  G  H  R  L  T  L  E  D  I  F

CATGACCTGTTCTACCACTTAGAGCTCCAGGTCAACCGCACCTACCAAATGCACCTTGGA  540
H  D  L  F  Y  H  L  E  L  Q  V  N  R  T  Y  Q  M  H  L  G

GGGAAGCAGAGAGAATATGAGTTCTTCGGCCTGACCCCTGACACAGAGTTCCTTGGCACC  600
G  K  Q  R  E  Y  E  F  F  G  L  T  P  D  T  E  F  L  G  T

ATCATGATTTGCGTTCCCACCTGGGCCAAGGAGAGTGCCCCCTACATGTGCCGAGTGAAG  660
I  M  I  C  V  P  T  W  A  K  E  S  A  P  Y  M  C  R  V  K

ACACTGCCAGACCGGACATGGACCGCTAGCGGGAGTTCTGGTGGTAGCTCTGGGACTAGC  720
T  L  P  D  R  T  W  T  A  S  G  S  S  G  G  S  S  G  T  S

GGGAGTTCTGGTGGTAGCTCTGGGACTAGTACGGATCCCCAGGTTCAGTTGCAGCAGTCT  780
G  S  S  G  G  S  S  G  T  S  T  D  P  Q  V  Q  L  Q  Q  S

GACGCTGAGTTGGTGAAACCTGGGGCTTCAGTGAAGATTTCCTGCAAGGCTTCTGGCTAC  840
D  A  E  L  V  K  P  G  A  S  V  K  I  S  C  K  A  S  G  Y

ACCTTCACTGACCATGCAATTCACTGGGTGAAACAGAACCCTGAACAGGGCCTGGAATGG  900
T  F  T  D  H  A  I  H  W  V  K  Q  N  P  E  Q  G  L  E  W

ATTGGATATTTTTCTCCCGGAAATGATGATTTTAAATACAATGAGAGGTTCAAGGGCAAG  960
I  G  Y  F  S  P  G  N  D  D  F  K  Y  N  E  R  F  K  G  K

GCCACACTGACTGCAGACAAATCCTCCAGCACTGCCTACGTGCAGCTCAACAGCCTGACA
1020
```

```
A  T  L  T  A  D  K  S  S  S  T  A  Y  V  Q  L  N  S  L  T

TCTGAGGATTCTGCAGTGTATTTCTGTACAAGATCCCTGAATATGGCCTACTGGGGTCAA
1080
S  E  D  S  A  V  Y  F  C  T  R  S  L  N  M  A  Y  W  G  Q

GGAACCTCAGTCACCGTCTCCTCAGCCAAAACGACGGGCCCATCGGTCTTCCCCCTGGCA
1140
G  T  S  V  T  V  S  S  A  K  T  T  G  P  S  V  F  P  L  A

CCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTAC
1200
P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D  Y

TTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACC
1260
F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S  G  V  H  T

TTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
1320
F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T  V  P

TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC
1380
S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K  P  S  N  T

AAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGC
1440
K  V  D  K  K  V  E  P  K  S  C  D  K  T  H  T  C  P  P  C

CCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGAC
1500
P  A  P  E  L  L  G  G  P  S  V  F  L  F  P  P  K  P  K  D

ACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAA 1560
T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  E

GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA
1620
D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  T

AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG
1680
K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L

CACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA
1740
H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P

GCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC
1800
A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y
```

```
ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
1860
T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V

AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC
1920
K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N

AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAG
1980
N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K

CTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT
2040
L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H

GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAATT
2100
E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G  K  *
```

Fig. 8K SPFL-mFc/IFN-λR1

```
ATGCGACCGACGCTGCTGTGGTCGCTGCTGCTGCTGCTCGGAGTCTTCGCCGCCGCCGCC  60
M  R  P  T  L  L  W  S  L  L  L  L  L  G  V  F  A  A  A  A

GCCGACTACAAGGACGACGATGACAAGGCGGATCCTGGTTGTAAGCCTTGCATATGTACA  120
A  D  Y  K  D  D  D  D  K  A  D  P  G  C  K  P  C  I  C  T

GTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATT  180
V  P  E  V  S  S  V  F  I  F  P  P  K  P  K  D  V  L  T  I

ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTC  240
T  L  T  P  K  V  T  C  V  V  V  D  I  S  K  D  D  P  E  V

CAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAG  300
Q  F  S  W  F  V  D  D  V  E  V  H  T  A  Q  T  Q  P  R  E

GAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACTGG  360
E  Q  F  N  S  T  F  R  S  V  S  E  L  P  I  M  H  Q  D  W

CTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAG  420
L  N  G  K  E  F  K  C  R  V  N  S  A  A  F  P  A  P  I  E

AAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCT  480
K  T  I  S  K  T  K  G  R  P  K  A  P  Q  V  Y  T  I  P  P

CCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTC  540
P  K  E  Q  M  A  K  D  K  V  S  L  T  C  M  I  T  D  F  F

CCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAAC  600
P  E  D  I  T  V  E  W  Q  W  N  G  Q  P  A  E  N  Y  K  N

ACTCAGCCCATCATGGACACAGATGGCTCTTACTTCATCTACAGCAAGCTCAATGTGCAG  660
T  Q  P  I  M  D  T  D  G  S  Y  F  I  Y  S  K  L  N  V  Q

AAGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCAC  720
K  S  N  W  E  A  G  N  T  F  T  C  S  V  L  H  E  G  L  H

AACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAATGGCTAGCCCTGGTGCTG  780
N  H  H  T  E  K  S  L  S  H  S  P  G  K  W  L  A  L  V  L

CCATCGCTTCTGATACTGCTGTTAGTAATTGCCGCAGGGGGTGTGATCTGGAAGACCCTC  840
P  S  L  L  I  L  L  L  V  I  A  A  G  G  V  I  W  K  T  L

ATGGGGAACCCCTGGTTTCAGCGGGCAAAGATGCCACGGGCCCTGGACTTTTCTGGACAC  900
M  G  N  P  W  F  Q  R  A  K  M  P  R  A  L  D  F  S  G  H

ACACACCCTGTGGCAACCTTTCAGCCCAGCAGACCAGAGTCCGTGAATGACTTGTTCCTC  960
T  H  P  V  A  T  F  Q  P  S  R  P  E  S  V  N  D  L  F  L

TGTCCCCAAAAGGAACTGACCAGAGGGGTCAGGCCGACGCCTCGAGTCAGGGCCCCAGCC
1020
```

```
C  P  Q  K  E  L  T  R  G  V  R  P  T  P  R  V  R  A  P  A

ACCCAACAGACAAGATGGAAGAAGGACCTTGCAGAGGACGAAGAGGAGGAGGATGAGGAG
1080
T  Q  Q  T  R  W  K  K  D  L  A  E  D  E  E  E  E  D  E  E

GACACAGAAGATGGCGTCAGCTTCCAGCCCTACATTGAACCACCTTCTTTCCTGGGGCAA
1140
D  T  E  D  G  V  S  F  Q  P  Y  I  E  P  P  S  F  L  G  Q

GAGCACCAGGCTCCAGGGCACTCGGAGGCTGGTGGGGTGGACTCAGGGAGGCCCAGGGCT
1200
E  H  Q  A  P  G  H  S  E  A  G  G  V  D  S  G  R  P  R  A

CCTCTGGTCCCAAGCGAAGGCTCCTCTGCTTGGGATTCTTCAGACAGAAGCTGGGCCAGC
1260
P  L  V  P  S  E  G  S  S  A  W  D  S  S  D  R  S  W  A  S

ACTGTGGACTCCTCCTGGGACAGGGCTGGGTCCTCTGGCTATTTGGCTGAGAAGGGGCCA
1320
T  V  D  S  S  W  D  R  A  G  S  S  G  Y  L  A  E  K  G  P

GGCCAAGGGCCGGGTGGGGATGGGCACCAAGAATCTCTCCCACCACCTGAATTCTCCAAG
1380
G  Q  G  P  G  G  D  G  H  Q  E  S  L  P  P  P  E  F  S  K

GACTCGGGTTTCCTGGAAGAGCTCCCAGAAGATAACCTCTCCTCCTGGGCCACCTGGGGC
1440
D  S  G  F  L  E  E  L  P  E  D  N  L  S  S  W  A  T  W  G

ACCTTACCACCGGAGCCGAATCTGGTCCCTGGGGGACCCCCAGTTTCTCTTCAGACACTG
1500
T  L  P  P  E  P  N  L  V  P  G  G  P  P  V  S  L  Q  T  L

ACCTTCTGCTGGGAAAGCAGCCCTGAGGAGGAAGAGGAGGCGAGGGAATCAGAAATTGAG
1560
T  F  C  W  E  S  S  P  E  E  E  E  E  A  R  E  S  E  I  E

GACAGCGATGCGGGCAGCTGGGGGGCTGAGAGCACCCAGAGGACCGAGGACAGGGGCCGG
1620
D  S  D  A  G  S  W  G  A  E  S  T  Q  R  T  E  D  R  G  R

ACATTGGGGCATTACATGGCCAGGTGA  1640
T  L  G  H  Y  M  A  R  *
```

Fig. 8L SPFL-mFc/IFN-αR2

```
ATGCGACCGACGCTGCTGTGGTCGCTGCTGCTGCTGCTCGGAGTCTTCGCCGCCGCCGCC  60
M  R  P  T  L  L  W  S  L  L  L  L  L  G  V  F  A  A  A  A

GCCGACTACAAGGACGACGATGACAAGGCGGATCCTGGTTGTAAGCCTTGCATATGTACA  120
A  D  Y  K  D  D  D  D  K  A  D  P  G  C  K  P  C  I  C  T

GTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATT  180
V  P  E  V  S  S  V  F  I  F  P  P  K  P  K  D  V  L  T  I

ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTC  240
T  L  T  P  K  V  T  C  V  V  V  D  I  S  K  D  D  P  E  V

CAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAG  300
Q  F  S  W  F  V  D  D  V  E  V  H  T  A  Q  T  Q  P  R  E

GAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACTGG  360
E  Q  F  N  S  T  F  R  S  V  S  E  L  P  I  M  H  Q  D  W

CTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAG  420
L  N  G  K  E  F  K  C  R  V  N  S  A  A  F  P  A  P  I  E

AAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCT  480
K  T  I  S  K  T  K  G  R  P  K  A  P  Q  V  Y  T  I  P  P

CCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTC  540
P  K  E  Q  M  A  K  D  K  V  S  L  T  C  M  I  T  D  F  F

CCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAAC  600
P  E  D  I  T  V  E  W  Q  W  N  G  Q  P  A  E  N  Y  K  N

ACTCAGCCCATCATGGACACAGATGGCTCTTACTTCATCTACAGCAAGCTCAATGTGCAG  660
T  Q  P  I  M  D  T  D  G  S  Y  F  I  Y  S  K  L  N  V  Q

AAGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCAC  720
K  S  N  W  E  A  G  N  T  F  T  C  S  V  L  H  E  G  L  H

AACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAATGGCTAGCAATAATTACT  780
N  H  H  T  E  K  S  L  S  H  S  P  G  K  W  L  A  I  I  T

GTGTTTTTGATAGCATTGGTCTTGACAAGCACCATAGTGACACTGAAATGGATTGGTTAT  840
V  F  L  I  A  L  V  L  T  S  T  I  V  T  L  K  W  I  G  Y

ATATGCTTAAGAAATAGCCTCCCCAAAGTCTTGAATTTTCATAACTTTTTAGCCTGGCCA  900
I  C  L  R  N  S  L  P  K  V  L  N  F  H  N  F  L  A  W  P

TTTCCTAACCTGCCACCGTTGGAAGCCATGGATATGGTGGAGGTCATTTACATCAACAGA  960
F  P  N  L  P  P  L  E  A  M  D  M  V  E  V  I  Y  I  N  R

AAGAAGAAAGTGTGGGATTATAATTATGATGATGAAAGTGATAGCGATACTGAGGCAGCG
1020
```

```
K  K  K  V  W  D  Y  N  Y  D  D  E  S  D  S  D  T  E  A  A

CCCAGGACAAGTGGCGGTGGCTATACCATGCATGGACTGACTGTCAGGCCTCTGGGTCAG
1080
P  R  T  S  G  G  G  Y  T  M  H  G  L  T  V  R  P  L  G  Q

GCCTCTGCCACCTCTACAGAATCCCAGTTGATAGACCCGGAGTCCGAGGAGGAGCCTGAC
1140
A  S  A  T  S  T  E  S  Q  L  I  D  P  E  S  E  E  E  P  D

CTGCCTGAGGTTGATGTGGAGCTCCCCACGATGCCAAAGGACAGCCCTCAGCAGTTGGAA
1200
L  P  E  V  D  V  E  L  P  T  M  P  K  D  S  P  Q  Q  L  E

CTCTTGAGTGGGCCCTGTGAGAGGAGAAAGAGTCCACTCCAGGACCCTTTTCCCGAAGAG
1260
L  L  S  G  P  C  E  R  R  K  S  P  L  Q  D  P  F  P  E  E

GACTACAGCTCCACGGAGGGGTCTGGGGGCAGAATTACCTTCAATGTGGACTTAAACTCT
1320
D  Y  S  S  T  E  G  S  G  G  R  I  T  F  N  V  D  L  N  S

GTGTTTTTGAGAGTTCTTGATGACGAGGACAGTGACGACTTAGAAGCCCCTCTGATGCTA
1380
V  F  L  R  V  L  D  D  E  D  S  D  D  L  E  A  P  L  M  L

TCGTCTCATCTGGAAGAGATGGTTGACCCAGAGGATCCTGATAATGTGCAATCAAACCAT
1440
S  S  H  L  E  E  M  V  D  P  E  D  P  D  N  V  Q  S  N  H

TTGCTGGCCAGCGGGGAAGGGACACAGCCAACCTTTCCCAGCCCCTCTTCAGAGGGCCTG
1500
L  L  A  S  G  E  G  T  Q  P  T  F  P  S  P  S  S  E  G  L

TGGTCCGAAGATGCTCCATCTGATCAAAGTGACACTTCTGAGTCAGATGTTGACCTTGGG
1560
W  S  E  D  A  P  S  D  Q  S  D  T  S  E  S  D  V  D  L  G

GATGGTTATATAATGAGATGA  1580
D  G  Y  I  M  R  *
```

Fig. 8M SPFL-mFc/IL-20R1

```
ATGCGACCGACGCTGCTGTGGTCGCTGCTGCTGCTGCTCGGAGTCTTCGCCGCCGCCGCC  60
M  R  P  T  L  L  W  S  L  L  L  L  L  G  V  F  A  A  A  A

GCCGACTACAAGGACGACGATGACAAGGCGGATCCTGGTTGTAAGCCTTGCATATGTACA  120
A  D  Y  K  D  D  D  D  K  A  D  P  G  C  K  P  C  I  C  T

GTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATT  180
V  P  E  V  S  S  V  F  I  F  P  P  K  P  K  D  V  L  T  I

ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTC  240
T  L  T  P  K  V  T  C  V  V  V  D  I  S  K  D  D  P  E  V

CAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAG  300
Q  F  S  W  F  V  D  D  V  E  V  H  T  A  Q  T  Q  P  R  E

GAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACTGG  360
E  Q  F  N  S  T  F  R  S  V  S  E  L  P  I  M  H  Q  D  W

CTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAG  420
L  N  G  K  E  F  K  C  R  V  N  S  A  A  F  P  A  P  I  E

AAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCT  480
K  T  I  S  K  T  K  G  R  P  K  A  P  Q  V  Y  T  I  P  P

CCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTC  540
P  K  E  Q  M  A  K  D  K  V  S  L  T  C  M  I  T  D  F  F

CCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAAC  600
P  E  D  I  T  V  E  W  Q  W  N  G  Q  P  A  E  N  Y  K  N

ACTCAGCCCATCATGGACACAGATGGCTCTTACTTCATCTACAGCAAGCTCAATGTGCAG  660
T  Q  P  I  M  D  T  D  G  S  Y  F  I  Y  S  K  L  N  V  Q

AAGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCAC  720
K  S  N  W  E  A  G  N  T  F  T  C  S  V  L  H  E  G  L  H

AACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAATGGCTAGCCTGGTATGTT  780
N  H  H  T  E  K  S  L  S  H  S  P  G  K  W  L  A  W  Y  V

TTGCCCATATCTATTACCGTGTTTCTTTTTTCTGTGATGGGCTATTCCATCTACCGATAT  840
L  P  I  S  I  T  V  F  L  F  S  V  M  G  Y  S  I  Y  R  Y

ATCCACGTTGGCAAAGAGAAACACCCAGCAAATTTGATTTTGATTTATGGAAATGAATTT  900
I  H  V  G  K  E  K  H  P  A  N  L  I  L  I  Y  G  N  E  F

GACAAAAGATTCTTTGTGCCTGCTGAAAAAATCGTGATTAACTTTATCACCCTCAATATC  960
D  K  R  F  F  V  P  A  E  K  I  V  I  N  F  I  T  L  N  I

TCGGATGATTCTAAAATTTCTCACCAGGATATGAGTTTACTGGGAAAAAGCAGTGATGTA
1020
```

```
S  D  D  S  K  I  S  H  Q  D  M  S  L  L  G  K  S  S  D  V

TCCAGCCTTAATGATCCTCAGCCCAGCGGGAACCTGAGGCCCCCTCAGGAGGAAGAGGAG
1080
S  S  L  N  D  P  Q  P  S  G  N  L  R  P  P  Q  E  E  E  E

GTGAAACATTTAGGGTATGCTTCGCATTTGATGGAAATTTTTTGTGACTCTGAAGAAAAC
1140
V  K  H  L  G  Y  A  S  H  L  M  E  I  F  C  D  S  E  E  N

ACGGAAGGTACTTCTCTCACCCAGCAAGAGTCCCTCAGCAGAACAATACCCCCGGATAAA
1200
T  E  G  T  S  L  T  Q  Q  E  S  L  S  R  T  I  P  P  D  K

ACAGTCATTGAATATGAATATGATGTCAGAACCACTGACATTTGTGCGGGGCCTGAAGAG
1260
T  V  I  E  Y  E  Y  D  V  R  T  T  D  I  C  A  G  P  E  E

CAGGAGCTCAGTTTGCAGGAGGAGGTGTCCACACAAGGAACATTATTGGAGTCGCAGGCA
1320
Q  E  L  S  L  Q  E  E  V  S  T  Q  G  T  L  L  E  S  Q  A

GCGTTGGCAGTCTTGGGCCCGCAAACGTTACAGTACTCATACACCCCTCAGCTCCAAGAC
1380
A  L  A  V  L  G  P  Q  T  L  Q  Y  S  Y  T  P  Q  L  Q  D

TTAGACCCCCTGGCGCAGGAGCACACAGACTCGGAGGAGGGGCCGGAGGAAGAGCCATCG
1440
L  D  P  L  A  Q  E  H  T  D  S  E  E  G  P  E  E  E  P  S

ACGACCCTGGTCGACTGGGATCCCCAAACTGGCAGGCTGTGTATTCCTTCGCTGTCCAGC
1500
T  T  L  V  D  W  D  P  Q  T  G  R  L  C  I  P  S  L  S  S

TTCGACCAGGATTCAGAGGGCTGCGAGCCTTCTGAGGGGGATGGGCTCGGAGAGGAGGGT
1560
F  D  Q  D  S  E  G  C  E  P  S  E  G  D  G  L  G  E  E  G

CTTCTATCTAGACTCTATGAGGAGCCGGCTCCAGACAGGCCACCAGGAGAAAATGAAACC
1620
L  L  S  R  L  Y  E  E  P  A  P  D  R  P  P  G  E  N  E  T

TATCTCATGCAATTCATGGAGGAATGGGGGTTATATGTGCAGATGGAAAACTGA 1670
Y  L  M  Q  F  M  E  E  W  G  L  Y  V  Q  M  E  N  *
```

Fig. 8N SPFL-mFc/IL-20R1

```
ATGCGACCGACGCTGCTGTGGTCGCTGCTGCTGCTGCTCGGAGTCTTCGCCGCCGCCGCC  60
M  R  P  T  L  L  W  S  L  L  L  L  L  G  V  F  A  A  A  A

GCCGACTACAAGGACGACGATGACAAGGCGGATCCTGGTTGTAAGCCTTGCATATGTACA  120
A  D  Y  K  D  D  D  D  K  A  D  P  G  C  K  P  C  I  C  T

GTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATT  180
V  P  E  V  S  S  V  F  I  F  P  P  K  P  K  D  V  L  T  I

ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTC  240
T  L  T  P  K  V  T  C  V  V  V  D  I  S  K  D  D  P  E  V

CAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAG  300
Q  F  S  W  F  V  D  D  V  E  V  H  T  A  Q  T  Q  P  R  E

GAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACTGG  360
E  Q  F  N  S  T  F  R  S  V  S  E  L  P  I  M  H  Q  D  W

CTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAG  420
L  N  G  K  E  F  K  C  R  V  N  S  A  A  F  P  A  P  I  E

AAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCT  480
K  T  I  S  K  T  K  G  R  P  K  A  P  Q  V  Y  T  I  P  P

CCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTC  540
P  K  E  Q  M  A  K  D  K  V  S  L  T  C  M  I  T  D  F  F

CCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAAC  600
P  E  D  I  T  V  E  W  Q  W  N  G  Q  P  A  E  N  Y  K  N

ACTCAGCCCATCATGGACACAGATGGCTCTTACTTCATCTACAGCAAGCTCAATGTGCAG  660
T  Q  P  I  M  D  T  D  G  S  Y  F  I  Y  S  K  L  N  V  Q

AAGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCAC  720
K  S  N  W  E  A  G  N  T  F  T  C  S  V  L  H  E  G  L  H

AACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAATGGCTAGCCTCCGGAGCC  780
N  H  H  T  E  K  S  L  S  H  S  P  G  K  W  L  A  S  G  A

TTCCTGTTCTCCATGGGCTTCCTCGTCGCAGTACTCTGCTACCTGAGCTACAGATATGTC  840
F  L  F  S  M  G  F  L  V  A  V  L  C  Y  L  S  Y  R  Y  V

ACCAAGCCGCCTGCACCTCCCAACTCCCTGAACGTCCAGCGAGTCCTGACTTTCCAGCCG  900
T  K  P  P  A  P  P  N  S  L  N  V  Q  R  V  L  T  F  Q  P

CTGCGCTTCATCCAGGAGCACGTCCTGATCCCTGTCTTTGACCTCAGCGGCCCCAGCAGT  960
L  R  F  I  Q  E  H  V  L  I  P  V  F  D  L  S  G  P  S  S

CTGGCCCAGCCTGTCCAGTACTCCCAGATCAGGGTGTCTGGACCCAGGGAGCCCGCAGGA
1020
```

```
L  A  Q  P  V  Q  Y  S  Q  I  R  V  S  G  P  R  E  P  A  G

GCTCCACAGCGGCATAGCCTGTCCGAGATCACCTACTTAGGGCAGCCAGACATCTCCATC
1080
A  P  Q  R  H  S  L  S  E  I  T  Y  L  G  Q  P  D  I  S  I

CTCCAGCCCTCCAACGTGCCACCTCCCCAGATCCTCTCCCCACTGTCCTATGCCCCAAAC
1140
L  Q  P  S  N  V  P  P  P  Q  I  L  S  P  L  S  Y  A  P  N

GCTGCCCCTGAGGTCGGGCCCCCATCCTATGCACCTCAGGTGACCCCCGAAGCTCAATTC
1200
A  A  P  E  V  G  P  P  S  Y  A  P  Q  V  T  P  E  A  Q  F

CCATTCTACGCCCCACAGGCCATCTCTAAGGTCCAGCCTTCCTCCTATGCCCCTCAAGCC
1260
P  F  Y  A  P  Q  A  I  S  K  V  Q  P  S  S  Y  A  P  Q  A

ACTCCGGACAGCTGGCCTCCCTCCTATGGGGTATGCATGGAAGGTTCTGGCAAAGACTCC
1320
T  P  D  S  W  P  P  S  Y  G  V  C  M  E  G  S  G  K  D  S

CCCACTGGGACACTTTCTAGTCCTAAACACCTTAGGCCTAAAGGTCAGCTTCAGAAAGAG
1380
P  T  G  T  L  S  S  P  K  H  L  R  P  K  G  Q  L  Q  K  E

CCACCAGCTGGAAGCTGCATGTTAGGTGGCCTTTCTCTGCAGGAGGTGACCTCCTTGGCT
1440
P  P  A  G  S  C  M  L  G  G  L  S  L  Q  E  V  T  S  L  A

ATGGAGGAATCCCAAGAAGCAAAATCATTGCACCAGCCCCTGGGGATTTGCACAGACAGA
1500
M  E  E  S  Q  E  A  K  S  L  H  Q  P  L  G  I  C  T  D  R

ACATCTGACCCAAATGTGCTACACAGTGGGGAGGAAGGGACACCACAGTACCTAAAGGGC1560
T  S  D  P  N  V  L  H  S  G  E  E  G  T  P  Q  Y  L  K  G

CAGCTCCCCCTCCTCTCCTCAGTCCAGATCGAGGGCCACCCCATGTCCCTCCCTTTGCAA
1620
Q  L  P  L  L  S  S  V  Q  I  E  G  H  P  M  S  L  P  L  Q

CCTCCTTCCGGTCCATGTTCCCCCTCGGACCAAGGTCCAAGTCCCTGGGGCCTGCTGGAG
1680
P  P  S  G  P  C  S  P  S  D  Q  G  P  S  P  W  G  L  L  E

TCCCTTGTGTGTCCCAAGGATGAAGCCAAGAGCCCAGCCCCTGAGACCTCAGACCTGGAG
1740
S  L  V  C  P  K  D  E  A  K  S  P  A  P  E  T  S  D  L  E

CAGCCCACAGAACTGGATTCTCTTTTCAGAGGCCTGGCCCTGACTGTGCAGTGGGAGTCC
1800
Q  P  T  E  L  D  S  L  F  R  G  L  A  L  T  V  Q  W  E  S
```

TGA 1803
*

Fig. 8O SPFL-mFc/EGFR

```
ATGCGACCGACGCTGCTGTGGTCGCTGCTGCTGCTGCTCGGAGTCTTCGCCGCCGCCGCC  60
M  R  P  T  L  L  W  S  L  L  L  L  L  G  V  F  A  A  A  A

GCCGACTACAAGGACGACGATGACAAGGCGGATCCTGGTTGTAAGCCTTGCATATGTACA  120
A  D  Y  K  D  D  D  D  K  A  D  P  G  C  K  P  C  I  C  T

GTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTCACCATT  180
V  P  E  V  S  S  V  F  I  F  P  P  K  P  K  D  V  L  T  I

ACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTC  240
T  L  T  P  K  V  T  C  V  V  V  D  I  S  K  D  D  P  E  V

CAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAG  300
Q  F  S  W  F  V  D  D  V  E  V  H  T  A  Q  T  Q  P  R  E

GAGCAGTTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACTGG  360
E  Q  F  N  S  T  F  R  S  V  S  E  L  P  I  M  H  Q  D  W

CTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAG  420
L  N  G  K  E  F  K  C  R  V  N  S  A  A  F  P  A  P  I  E

AAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCT  480
K  T  I  S  K  T  K  G  R  P  K  A  P  Q  V  Y  T  I  P  P

CCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTC  540
P  K  E  Q  M  A  K  D  K  V  S  L  T  C  M  I  T  D  F  F

CCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAAC  600
P  E  D  I  T  V  E  W  Q  W  N  G  Q  P  A  E  N  Y  K  N

ACTCAGCCCATCATGGACACAGATGGCTCTTACTTCATCTACAGCAAGCTCAATGTGCAG  660
T  Q  P  I  M  D  T  D  G  S  Y  F  I  Y  S  K  L  N  V  Q

AAGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCAC  720
K  S  N  W  E  A  G  N  T  F  T  C  S  V  L  H  E  G  L  H

AACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTAAATGGCTAGCGATGGTGGGG  780
N  H  H  T  E  K  S  L  S  H  S  P  G  K  W  L  A  M  V  G

GCCCTCCTCTTGCTGCTGGTGGTGGCCCTGGGGATCGGCCTCTTCATGCGAAGGCGCCAC  840
A  L  L  L  L  L  V  V  A  L  G  I  G  L  F  M  R  R  R  H

ATCGTTCGGAAGCGCACGCTGCGGAGGCTGCTGCAGGAGAGGGAGCTTGTGGAGCCTCTT  900
I  V  R  K  R  T  L  R  R  L  L  Q  E  R  E  L  V  E  P  L

ACACCCAGTGGAGAAGCTCCCAACCAAGCTCTCTTGAGGATCTTGAAGGAAACTGAATTC  960
T  P  S  G  E  A  P  N  Q  A  L  L  R  I  L  K  E  T  E  F

AAAAGATCAAAGTGCTGGGCTCCGGTGCGTTCGGCACGGTGTATAAGGGACTCTGGATC  1020
K  K  I  K  V  L  G  S  G  A  F  G  T  V  Y  K  G  L  W  I
```

```
CCAGAAGGTGAGAAAGTTAAAATTCCCGTCGCTATCAAGGAATTAAGAGAAGCAACATCT
1080
P  E  G  E  K  V  K  I  P  V  A  I  K  E  L  R  E  A  T  S

CCGAAAGCCAACAAGGAAATCCTCGATGAAGCCTACGTGATGGCCAGCGTGGACAACCCC
1140
P  K  A  N  K  E  I  L  D  E  A  Y  V  M  A  S  V  D  N  P

CACGTGTGCCGCCTGCTGGGCATCTGCCTCACCTCCACCGTGCAACTCATCACGCAGCTC
1200
H  V  C  R  L  L  G  I  C  L  T  S  T  V  Q  L  I  T  Q  L

ATGCCCTTCGGCTGCCTCCTGGACTATGTCCGGGAACACAAAGACAATATTGGCTCCCAG
1260
M  P  F  G  C  L  L  D  Y  V  R  E  H  K  D  N  I  G  S  Q

TACCTGCTCAACTGGTGTGTGCAGATCGCAAAGGGCATGAACTACTTGGAGGACCGTCGC
1320
Y  L  L  N  W  C  V  Q  I  A  K  G  M  N  Y  L  E  D  R  R

TTGGTGCACCGCGACCTGGCAGCCAGGAACGTACTGGTGAAAACACCGCAGCATGTCAAG
1380
L  V  H  R  D  L  A  A  R  N  V  L  V  K  T  P  Q  H  V  K

ATCACAGATTTTGGGCTGGCCAAACTGCTGGGTGCGGAAGAGAAAGAATACCATGCAGAA
1440
I  T  D  F  G  L  A  K  L  L  G  A  E  E  K  E  Y  H  A  E

GGAGGCAAAGTGCCTATCAAGTGGATGGCATTGGAATCAATTTTACACAGAATCTATACC
1500
G  G  K  V  P  I  K  W  M  A  L  E  S  I  L  H  R  I  Y  T

CACCAGAGTGATGTCTGGAGCTACGGGGTGACCGTTTGGGAGTTGATGACCTTTGGATCC
1560
H  Q  S  D  V  W  S  Y  G  V  T  V  W  E  L  M  T  F  G  S

AAGCCATATGACGGAATCCCTGCCAGCGAGATCTCCTCCATCCTGGAGAAAGGAGAACGC
1620
K  P  Y  D  G  I  P  A  S  E  I  S  S  I  L  E  K  G  E  R

CTCCCTCAGCCACCCATATGTACCATCGATGTCTACATGATCATGGTCAAGTGCTGGATG
1680
L  P  Q  P  P  I  C  T  I  D  V  Y  M  I  M  V  K  C  W  M

ATAGACGCAGATAGTCGCCCAAAGTTCCGTGAGTTGATCATCGAATTCTCCAAAATGGCC
1740
I  D  A  D  S  R  P  K  F  R  E  L  I  I  E  F  S  K  M  A

CGAGACCCCCAGCGCTACCTTGTCATTCAGGGGGATGAAAGAATGCATTTGCCAAGTCCT
1800
R  D  P  Q  R  Y  L  V  I  Q  G  D  E  R  M  H  L  P  S  P
```

```
ACAGACTCCAACTTCTACCGTGCCCTGATGGATGAAGAAGACATGGACGACGTGGTGGAT
1860
T  D  S  N  F  Y  R  A  L  M  D  E  E  D  M  D  D  V  V  D

GCCGACGAGTACCTCATCCCACAGCAGGGCTTCTTCAGCAGCCCCTCCACGTCACGGACT
1920
A  D  E  Y  L  I  P  Q  Q  G  F  F  S  S  P  S  T  S  R  T

CCCCTCCTGAGCTCTCTGAGTGCAACCAGCAACAATTCCACCGTGGCTTGCATTGATAGA
1980
P  L  L  S  S  L  S  A  T  S  N  N  S  T  V  A  C  I  D  R

AATGGGCTGCAAAGCTGTCCCATCAAGGAAGACAGCTTCTTGCAGCGATACAGCTCAGAC
2040
N  G  L  Q  S  C  P  I  K  E  D  S  F  L  Q  R  Y  S  S  D

CCCACAGGCGCCTTGACTGAGGACAGCATAGACGACACCTTCCTCCCAGTGCCTGAATAC
2100
P  T  G  A  L  T  E  D  S  I  D  D  T  F  L  P  V  P  E  Y

ATAAACCAGTCCGTTCCCAAAAGGCCCGCTGGCTCTGTGCAGAATCCTGTCTATCACAAT
2160
I  N  Q  S  V  P  K  R  P  A  G  S  V  Q  N  P  V  Y  H  N

CAGCCTCTGAACCCCGCGCCCAGCAGAGACCCACACTACCAGGACCCCCACAGCACTGCA
2220
Q  P  L  N  P  A  P  S  R  D  P  H  Y  Q  D  P  H  S  T  A

GTGGGCAACCCCGAGTATCTCAACACTGTCCAGCCCACCTGTGTCAACAGCACATTCGAC
2280
V  G  N  P  E  Y  L  N  T  V  Q  P  T  C  V  N  S  T  F  D

AGCCCTGCCCACTGGGCCCAGAAAGGCAGCCACCAAATTAGCCTGGACAACCCTGACTAC
2340
S  P  A  H  W  A  Q  K  G  S  H  Q  I  S  L  D  N  P  D  Y

CAGCAGGACTTCTTTCCCAAGGAAGCCAAGCCAAATGGCATCTTTAAGGGCTCCACAGCT
2400
Q  Q  D  F  F  P  K  E  A  K  P  N  G  I  F  K  G  S  T  A

GAAAATGCAGAATACCTAAGGGTCGCGCCACAAAGCAGTGAATTTATTGGAGCATGA  2450
E  N  A  E  Y  L  R  V  A  P  Q  S  S  E  F  I  G  A  *
```

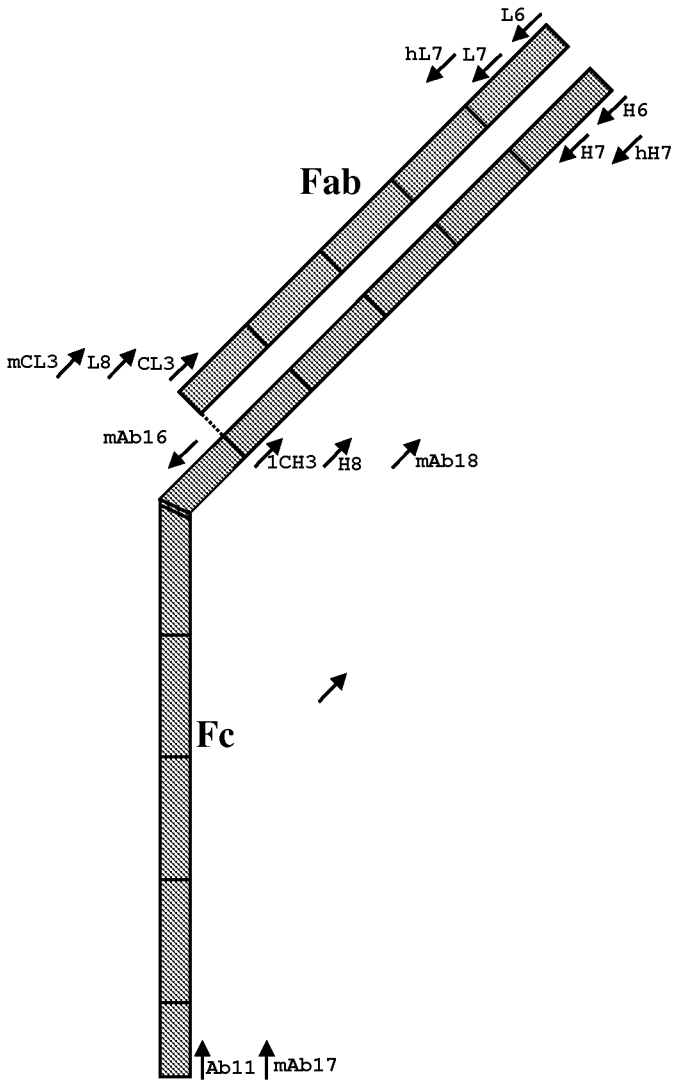
L6
hL7
L7
H6
H7
hH7
Fab
mCL3
L8
CL3
mAb16
1CH3
H8
mAb18
Fc
Ab11
mAb17

Fig. 9.

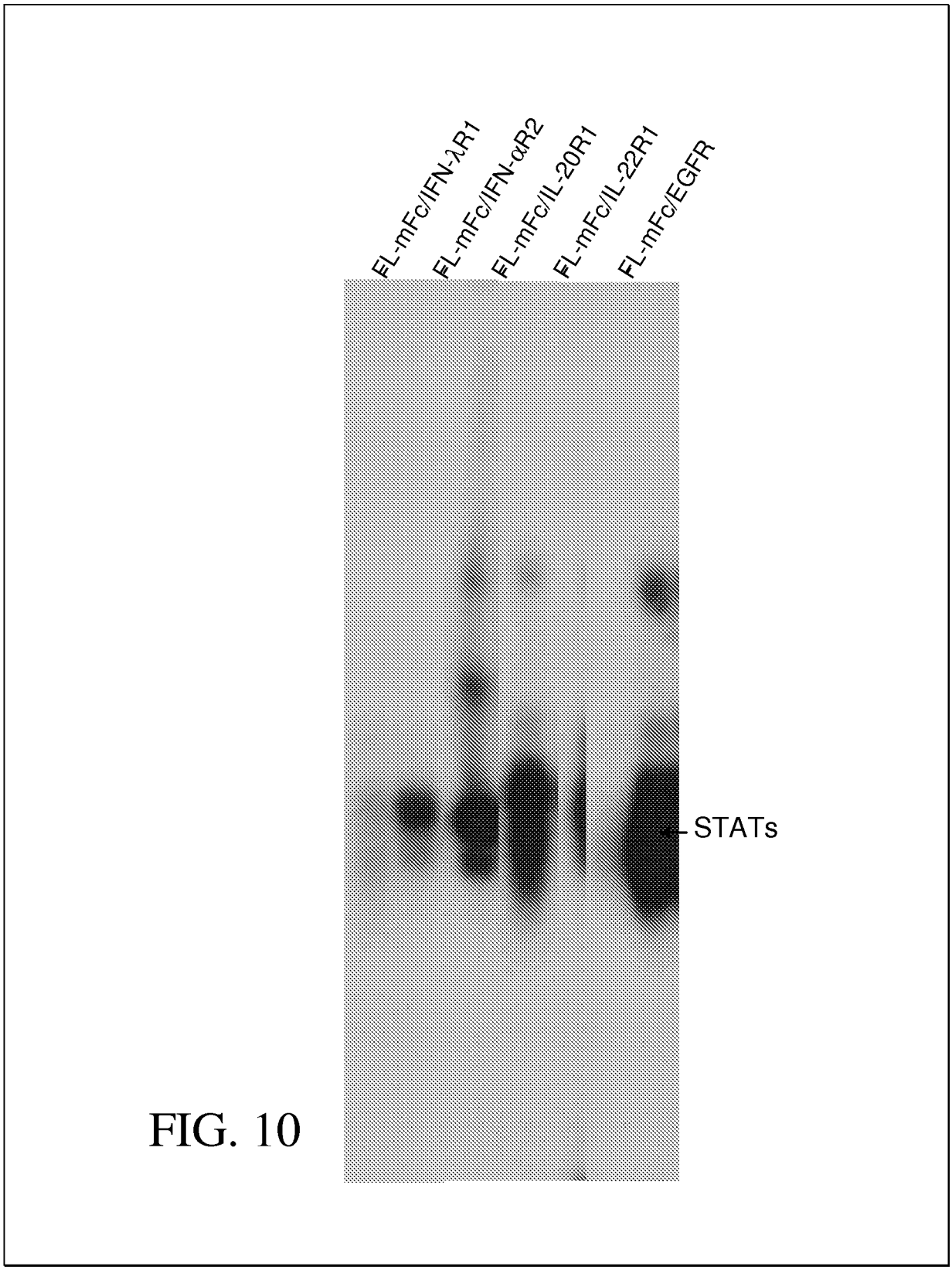
FL-mFc/IFN-λR1
FL-mFc/IFN-αR2
FL-mFc/IL-20R1
FL-mFc/IL-22R1
FL-mFc/EGFR
STATs

FIG. 10