

MASKED INTERFERON FUSION PROTEINS AND USES THEREOF**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of PCT Application No. PCT/CN2023/109070, filed July 25, 2023, the content of which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] The type I interferon (IFN) system involves a single form of IFN β , several variants of IFN α and other less well-characterized IFNs, all of which signal via a heterodimeric IFN α/β receptor 1 (IFNAR1)-IFNAR2 receptor to transactivate IFN-stimulated genes. Type I IFNs regulate many important physiological processes such as maintaining the hematopoietic stem cell niche, maintaining synaptic plasticity and cognitive function, sustaining microbiota-driven optimal antiviral immunity, and regulating bone remodeling. Another important regulatory function of the type I IFNs is the induction of antiviral state, such as inhibiting viral protein synthesis, degrading viral RNA and trapping viral components. Type I IFNs are expressed rapidly after infection and participate in and function as a linker between the innate immune response and the adaptive immune responses in defense against pathogens.

[0003] The interferon-alpha (IFN-alpha) family of proteins has proven to be useful in treatment of a variety of diseases. For example, interferons alpha 2a and 2b (trade names Roferon and Intron A, respectively) have been used in the treatment of chronic hepatitis B, C and D (life-threatening viral diseases of the liver), condylomata acuminata (genital warts), AIDS-related Kaposi's sarcoma, hairy cell leukemia, malignant melanoma, basal cell carcinoma, multiple myeloma, renal cell carcinoma, herpes I and II, varicella herpes zoster, and mycosis fungoides. The efficacy of treatment regimes containing interferon-alpha prostate cancer and chronic myelogenous leukemia have also been studied. It is reported that the IFN-alpha induces malignant cells apoptosis via the caspase pathways. There is a need for potential cancer targeting IFN therapy.

SUMMARY

[0004] The present disclosure provides masked interferon fusion proteins comprising an interferon masking moiety that binds to type I interferon (IFN), a IFN polypeptide that retains type I interferon activity when not engaged by said masking moiety, an immunoglobulin (Ig) Fc region, and a cleavable linker connecting the masking moiety and the IFN polypeptide,

wherein the IFN polypeptide is linked to said Ig Fc region. These masked interferon fusion proteins are useful in the treatment of diseases and conditions such as cancers, autoimmune disease or inflammatory conditions.

[0005] In one aspect, the present disclosure provides a fusion protein comprising (a) an interferon alpha and beta receptor subunit 2 domain 1 (IFNAR2-D1) or a masking peptide having at least 55% sequence identity to the IFNAR2-D1, (b) a type I interferon (IFN), and (c) a cleavable linker between (a) and (b), wherein the peptide is capable of binding the type I IFN, and wherein the fusion protein does not include the entire sequence of IFNAR2 domain 2 (IFNAR2-D2).

[0006] In certain embodiments, the cleavable linker is fused to the C-terminus of (a) and N-terminus of (b), or vice versa. In certain embodiments, the fusion protein further comprising an immunoglobulin (Ig) Fc region fused to the C-terminus of the IFN. In certain embodiments, the fusion protein further comprising an immunoglobulin (Ig) Fc region fused to the N-terminus of (a).

[0007] In certain embodiments, the fusion protein does not include at least 50% of IFNAR2-D2, or does not include at least 80% of IFNAR2-D2. In certain embodiments, the IFNAR2-D1 comprises the amino acid sequence of SEQ ID NO: 8 or 3.

[0008] In certain embodiments, the masking peptide comprises an IFN binding loop 1 comprising the amino acid sequence of YTIMSKPEDLK (SEQ ID NO: 74), an IFN binding loop 2 comprising the amino acid sequence of STHEAYVTVL (SEQ ID NO: 75), STQEIVTVL (SEQ ID NO: 77) or STDEAYVTVL (SEQ ID NO: 78), and an IFN binding loop 3 comprising the amino acid sequence of SHNFWLAID (SEQ ID NO: 76) or SHEFWLAID (SEQ ID NO: 79). In certain embodiments, the masking peptide has at least 85% sequence identity to any of SEQ ID NOS: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40. In certain embodiments, the masking peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40.

[0009] In certain embodiments, the masking peptide has at least 85% sequence identity to SEQ ID NO: 8 or 3.

[0010] In certain embodiments, the masking peptide comprises at least one disulfide bond. In certain embodiments, the masking peptide comprises substitutions at R9C and E98C relative to

SEQ ID NO:8. In certain embodiments, the masking peptide comprises the amino acid sequence of SEQ ID NO:12 or 42.

[0011] In certain embodiments, the masking peptide comprises the amino acid sequence of SEQ ID NO: 42 with one or more substitutions selected from the group consisting of:

- (1) L19: S, Q, E, or R;
- (2) F22: G, N, or D;
- (3) I46R;
- (4) M47: I or F;
- (5) S48: N, Y, or Q;
- (6) K49Q;
- (7) H77: Y, Q or D;
- (8) A79: D, I, V, E or N;
- (9) V81: I or F;
- (10) V83: F, I or K;
- (11) N99: E, D or W;
- (12) W101: M or F;
- (13) L102: M or I;
- (14) I104: F or W;
- (15) M106: A, T, L, or E;
- (16) F108: N, Y, T, or E;
- (17) P110: S or E;
- (18) P111: S or E; and
- (19) the combination thereof.

[0012] In certain embodiments, the masking peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 42, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 or 73.

[0013] In certain embodiments, the fusion protein does not bind to IFNAR2.

[0014] In certain embodiments, the cleavable linker contains a protease cleavage site. In certain embodiments, the protease is selected from the group consisting of a thrombin, a neutrophil elastase, a cysteine protease, FAPa, Cathepsin B, legumain, a serine protease, such as a matriptase or a urokinase (uPA), and matrix metalloproteinases (MMPs), such as MMP1, MMP2, MMP3, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, and MMP17.

[0015] In certain embodiments, the protease cleavage site comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 45 and 10.

[0016] In certain embodiments, the cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 13 and 44.

[0017] In certain embodiments, the type 1 IFN is selected from the group consisting of IFN- α 1, - α 2, - α 4, - α 5, - α 6, - α 7, - α 8, - α 10, - α 13, - α 14, - α 16, - α 17 and - α 21, IFN- β , IFN- κ , IFN- ϵ , or IFN- ω and any an amino acid sequence having at least 75% sequence identity thereof. In certain embodiments, the IFN comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence having at least 75% sequence identity to SEQ ID NO: 4.

[0018] In certain embodiments, the Fc is of an isotype of IgG1, IgG2, IgG3, or IgG4. In certain embodiments, the Fc region comprises the amino acid sequence of SEQ ID NO: 6.

[0019] In certain embodiments, the fusion protein forms a dimer.

[0020] In certain embodiments, the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 41, 43, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, and 39.

[0021] In one aspect, the present disclosure provides a composition comprising the fusion protein, and a pharmaceutically acceptable carrier.

[0022] In one aspect, the present disclosure provides an isolated cell comprising one or more polynucleotide encoding the fusion protein provided herein.

[0023] In one aspect, the present disclosure provides a polynucleotide encoding the fusion protein provided herein.

[0024] In one aspect, the present disclosure provides a method of treating a cancer in a patient in need thereof, comprising administering to the patient the fusion protein provided herein, the

composition provided herein, the cell provided herein, or the polynucleotide provided herein. In certain embodiments, the cancer is selected from the group consisting of bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer.

[0025] In certain embodiments, the cleavable linker of the fusion protein is cleaved by an enzyme within a tumor microenvironment in the patient.

[0026] In one aspect, the present disclosure provides a method for treating an autoimmune disease or inflammatory condition in a patient in need thereof, comprising administering to the patient the fusion protein provided herein, the composition provided herein, the cell provided herein, or the polynucleotide provided herein.

[0027] In one aspect, the present disclosure provides a polypeptide comprising an interferon alpha and beta receptor subunit 2 domain 1 (IFNAR2-D1) or a peptide having at least 55% sequence identity to the IFNAR2-D1, wherein the polypeptide is capable of binding the type 1 IFN, and wherein the polypeptide does not include the entire sequence of IFNAR2 domain 2 (IFNAR2-D2).

[0028] In certain embodiments, the polypeptide does not include at least 50% of IFNAR2-D2, or does not include at least 80% of IFNAR2-D2.

[0029] In certain embodiments, the IFNAR2-D1 comprises the amino acid sequence of SEQ ID NO: 8 or 3.

[0030] In certain embodiments, the polypeptide comprises an IFN binding loop 1 comprising the amino acid sequence of YTIMSKPEDLK (SEQ ID NO: 74), an IFN binding loop 2 comprising the amino acid sequence of STHEAYVTVL (SEQ ID NO: 75), STQEIVTVL (SEQ ID NO: 77) or STDEAYVTVL (SEQ ID NO: 78), and an IFN binding loop 3 comprising the amino acid sequence of SHNFWLAID (SEQ ID NO: 76) or SHEFWLAID (SEQ ID NO: 79).

[0031] In certain embodiments, the polypeptide has at least 85% sequence identity to any of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40. In certain embodiments, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40.

[0032] In certain embodiments, the polypeptide has at least 85% sequence identity to SEQ ID NO: 8 or 3.

[0033] In certain embodiments, the polypeptide comprises at least one disulfide bond.

[0034] In certain embodiments, the polypeptide comprises substitutions at R9C and E98C relative to SEQ ID NO:8. In certain embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:12 or 42.

[0035] In certain embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 42 with one or more substitutions selected from the group consisting of:

- (1) L19: S, Q, E, or R;
- (2) F22: G, N, or D;
- (3) I46R;
- (4) M47: I or F;
- (5) S48: N, Y, or Q;
- (6) K49Q;
- (7) H77: Y, Q or D;
- (8) A79: D, I, V, E or N;
- (9) V81: I or F;
- (10) V83: F, I or K;
- (11) N99: E, D or W;
- (12) W101: M or F;
- (13) L102: M or I;
- (14) I104: F or W;
- (15) M106: A, T, L, or E;
- (16) F108: N, Y, T, or E;
- (17) P110: S or E;
- (18) P111: S or E; and
- (19) the combination thereof.

[0036] In certain embodiments, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 42, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 or 73. In one aspect, the present disclosure provides a polynucleotide encoding the polypeptide provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] FIG. 1 shows the exemplary format of the masked IFN fusion protein.

[0038] FIG. 2 shows the human IFNAR2 binding efficiency of I-09 via ELISA (A) and inhibition activity to Daudi cell proliferation of I-09 and I-09 cut (MMP-14 incubated masked IFN-Fc was labeled as “I-XX cut”) (B).

[0039] FIG. 3A-C show the human IFNAR2 binding affinity of the *de novo* designed masking IFN-Fc fusion proteins.

[0040] FIG. 4A-C show the Daudi cell proliferation inhibition activity of the *de novo* designed masking IFN-Fc fusion proteins with or without masking moiety (MMP-14 incubated masked IFN-Fc was labeled as “I-XX cut”).

[0041] FIG. 5 shows the sequence alignment of M-09 and M-140 (A) and the comparison of I-09 and I-40 protein expression and purity by SDS-PAGE and size exclusion chromatography (SEC) thereof (B).

[0042] FIG. 6 shows the human IFNAR2 binding affinity (ELISA) (A) and the Daudi cell proliferation inhibition activity (B) of I-140 and I-140 cut (MMP-14 incubated I-140 was labeled as “I-140 cut”).

[0043] FIG. 7A-C show the human IFNAR2 binding affinity of the I-140 derived site mutated masked IFN-Fc fusion proteins as measured by ELISA.

[0044] FIG. 8A-C show the Daudi cell proliferation inhibition activity of the I-140 derived site mutated masked IFN-Fc fusion proteins.

DETAILED DESCRIPTION

Definitions

[0045] It is to be noted that the term “a” or “an” entity refers to one or more of that entity; for example, “a polypeptide,” is understood to represent one or more polypeptides. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

[0046] The term “CH2 region” or “CH2 domain” as used herein is intended to refer the CH2 region of an immunoglobulin, including the portion of a heavy chain molecule that extends, *e.g.*, from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; *see* Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983). CH2 region of a human IgG1 antibody, for example, corresponds to amino acids 228-340 according to the EU numbering system. However, the CH2 region may also be any of the other subtypes as described herein.

[0047] The term “CH3 region” or “CH3 domain” as used herein is intended to refer the CH3 region of an immunoglobulin. Thus, for example the CH3 region of a human IgG1 antibody corresponds to amino acids 341-447 according to the EU numbering system. However, the CH3 region may also be any of the other subtypes as described herein.

[0048] As used herein, the term “hinge region” includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux *et al.*, *J. Immunol* 161:4083 (1998)).

[0049] The term “immunoglobulin” or “Ig” refers to a class of structurally related glycoproteins consisting of two pairs of polypeptide chains, one pair of light (L) low molecular weight chains and one pair of heavy (H) chains, all four potentially inter-connected by disulfide bonds. The structure of immunoglobulins has been well characterized. See for instance Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). Briefly, each heavy chain typically is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region typically is comprised of three domains, CH1, CH2, and CH3. The heavy chains are inter-connected via disulfide bonds in the so-called “hinge region”. Each light chain typically is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region typically is comprised of one domain, CL. The VH and VL regions may be further subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form of structurally defined loops), also termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each VH and VL is typically composed of three CDRs and four FRs, arranged from

amino-terminal to carboxy-terminal in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (see also Chothia and Lesk J. Mol. Biol. 196, 901 917 (1987)). Unless otherwise stated or contradicted by context, the amino acids of the constant region sequences are herein numbered according to the EU-index (described in Kabat, E.A. et al., Sequences of proteins of immunological interest. 5th Edition – US Department of Health and Human Services, NIH publication No. 91-3242, pp 662,680,689 (1991)).

[0050] The terms “inhibit” or “inhibition of” as used herein means to reduce by a measurable amount, or to prevent entirely.

[0051] The term “interferon” as used herein means a group of signaling proteins made and released by host cells in response to the presence of several viruses. In a typical scenario, a virus- infected cell will release interferons causing nearby cells to heighten their anti-viral defenses. IFNs belong to the large class of proteins known as cytokines, molecules used for communication between cells to trigger the protective defenses of the immune system that help eradicate pathogens.

[0052] The term “mask” or “masking” in referring to a masking moiety of IFN means for purposes of the present disclosure, any polypeptide or protein that blocks cytokine interaction and/or activation between the IFN(s) and the IFNARs. It is within the scope of the invention that “mask” can be modified by recombinant means. The modification in amino acids includes deletions, additions, and substitutions of amino acids.

[0053] The term “masked IFN” or “engaged IFN” as used herein means a type I interferon in which a polypeptide is attached or engaged to the IFN thereby reducing IFN’s ability to bind the IFNARs.

[0054] As used herein, the term “affinity” is the strength of binding of one molecule, e.g. a polypeptide, to another, e.g. a target or ligand, at a single site, such as the monovalent binding of the individual target site of a masking moiety to the IFN polypeptide.

[0055] As used herein, the terms “treat” or “treatment” refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease

progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[0056] By “subject” or “individual” or “animal” or “patient” or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sport, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on.

[0057] As used herein, phrases such as “to a patient in need of treatment” or “a subject in need of treatment” includes subjects, such as mammalian subjects, that would benefit from administration of a polypeptide or composition of the present disclosure used, *e.g.*, for detection, for a diagnostic procedure and/or for treatment.

[0058] “Pharmaceutically acceptable” refers to a non-toxic, inert, and/or composition that is physiologically compatible with humans or other mammals.

Type 1 interferons and IFN- α/β receptor (IFNAR)

[0059] The term “Type 1 interferon” or “Type I interferon” as used herein means a large subgroup of interferon proteins that help regulate the activity of the immune system. Human type I IFNs belong to a multigene family consisting of 13 IFN- α subtypes (IFN- α 1 (Uniprot No. P01562), - α 2 (Uniprot No. P01563), - α 4 (Uniprot No. P05014), - α 5 (Uniprot No. P01569), - α 6 (Uniprot No. P05013), - α 7 (Uniprot No. P01567), - α 8 (Uniprot No. P32881), - α 10 (Uniprot No. P01566), - α 13 (Uniprot No. P01562), - α 14 (Uniprot No. P01570), - α 16 (Uniprot No. P05015), - α 17 (Uniprot No. P05017) and - α 21 (Uniprot No. P01568)) but only one IFN- β (Uniprot No. P01574), IFN- ϵ (Uniprot No. Q86WN2), IFN- κ (Uniprot No. Q9P0W0), and IFN- ω (Uniprot No. P05000). All type I IFNs have similarities in structure, and their protein sequences are highly conserved (75–99% amino acid sequence identity). All type I IFNs bind to a specific cell surface receptor complex known as the IFN- α receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains. It is within the scope of the present disclosure that type 1 IFN(s) can be modified using recombinant means. The modification in amino acids includes deletions, additions, and substitutions of amino acids.

[0060] IFN- α and IFN- β are secreted by many cell types including lymphocytes (NK cells, B-cells and T-cells), macrophages, fibroblasts, endothelial cells, osteoblasts and others. They stimulate both macrophages and NK cells to elicit an anti-viral response, and are also active against tumors. Plasmacytoid dendritic cells have been identified as the most potent producers of type I IFNs in response to antigen, and have thus been coined natural IFN producing cells. Current study findings suggest that by forcing IFN- α expression in tumor-infiltrating macrophages, it is possible to elicit a more effective dendritic cell activation and immune effector cell cytotoxicity.

[0061] In certain embodiments, a type 1 IFN(s) of the present disclosure may be the native IFN proteins from humans or animals, or may be recombinant IFN from transformed cells. Preferably, a recombinant human IFN is prepared using transformed *E. coli*. Unless their biological activity significantly deviates from that of the wild-type, mutants formed by substitution, deletion or insertion of amino acids are also included within the scope of the interferon alpha.

[0062] In certain embodiments, the human type 1 IFN comprises IFN- α subtypes, IFN- β , IFN- ϵ , IFN- κ , or IFN- ω or any an amino acid sequence having at least 75% (or at least 80%, at least 85%, at least 90%, at least 95% or at least 99%) sequence identity thereof. In certain embodiments, the type 1 IFN comprises IFN- $\alpha 2\beta$ or any an amino acid sequence having at least 75% (or at least 80%, at least 85%, at least 90%, at least 95% or at least 99%) sequence identity thereof. In certain embodiments, the type 1 IFN of the present disclosure comprises an amino acid sequence of SEQ ID NO: 5 or any an amino acid sequence having at least 75% (or at least 80%, at least 85%, at least 90%, at least 95% or at least 99%) sequence identity thereof.

[0063] The interferon- α/β receptor (IFNAR) is a receptor which binds type I interferons. It is a heteromeric cell surface receptor composed of one chain with two subunits referred to as IFNAR1 (*Homo sapiens*, Uniprot No. P17181) and IFNAR2 (*Homo sapiens*, Uniprot No. P48551). Upon binding of type I IFNs, IFNAR activates the JAK-STAT signaling pathway. Interferon stimulation classically results in an anti-viral immune response.

[0064] A model of the human IFNAR2 expressed in *E. coli* reveals a predominantly hydrophobic patch on the receptor that interacts with a matching hydrophobic surface on IFN- α . An adjacent motif of charged side chains then guides the proteins into a tight complex. The binding interface may account for cross reactivity and ligand specificity of the receptor. The

high affinity IFNAR2 chain adopts a two-domain D1/D2 receptor structure, whereas D1 contributes to most of the binding affinity.

Masked interferon fusion protein

[0065] As demonstrated in the experimental examples, numerous masked IFN fusion proteins designed and prepared herein showed greatly reduced, often hundreds or even thousands of times less, binding affinity to the human IFNAR. Upon removal of the masking moiety, however, the released IFN-Fc protein retained excellent binding affinity to the human IFNAR and efficiently blocked the Daudi cell proliferation.

[0066] In accordance with one embodiment of the present disclosure, provided are masked IFN fusion proteins that include a masking moiety fused, through a cleavable linker, to the N-terminus of a type I IFN, which is optionally fused to an immunoglobulin Fc region.

[0067] In certain embodiments, the masked IFN fusion protein of the present disclosure comprises, from the N-terminus to the C-terminus, a masking moiety, a cleavable linker, a type I IFN, and an immunoglobulin Fc region. In certain embodiments, the masked IFN fusion protein of the present disclosure comprises, from the N-terminus to the C-terminus, a masking moiety, a cleavable linker, a type I IFN, a second linker and an immunoglobulin Fc region.

Type I IFN masking moiety

[0068] The present disclosure provides masking moieties that have binding specificity to the human type I IFN and efficiently block the binding between the human type I IFN and IFNAR. In certain embodiments, the IFNAR includes IFNAR1 and IFNAR2.

[0069] Also provided are polypeptides that include the masking moiety as disclosed herein, optionally in combination with other protein elements.

[0070] In order to be conferred with a “masking” function to the type 1 IFN, the IFN masking moiety need to maintain high binding affinity to the type 1 IFN. The IFNAR2 Domain 1 (IFNAR2-D1), which interacts with type 1 IFN via its three binding loops, is observed to contribute to most of the binding affinity with type 1 IFN. Accordingly, in some embodiments, a masking moiety of the present disclosure includes the IFNAR2-D1 or a derivative (such as the mutants described below) and does not include some or all of the remaining sequence of IFNAR2. In one embodiment, the masking moiety does not include all of IFNAR2-D2 (SEQ

ID NO: 16), or does not include at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of IFNAR2-D2 (SEQ ID NO: 16).

[0071] In some embodiments, the IFNAR2-D1 is mutated to provide better masking activity or to assist with expression or stability. With mutations in the binding loops, in some embodiments, the binding affinity of IFNAR2-D1 can be improved, thus enhancing the masking activity. The amino acid sequences of the three binding loops within the IFNAR2-D1 that form the binding interfaces with the type 1 IFNs are summarized as in Table 1.

Table 1. Sequences of the IFN binding loop

IFN Binding Loop	Sequence	SEQ ID NO:
IFN Binding loop 1	YTIMSKPEDLK	74
IFN Binding loop 2	STHEAYVTVL	75
IFN Binding loop 3	SHNFWLAID	76

[0072] In some embodiments, the IFN binding loops 1-3 are selected from any set of IFN binding loops 1-3 shown in Table 1. In some embodiments, the IFN binding loops 1-3 are selected from those derived from the same IFN masking moiety in the examples.

[0073] In some embodiments, at least one, or two, or three of the IFN binding loops 1-3 of the above are modified by one, two or three amino acid additions, deletions, substitutions, or the combinations thereof.

[0074] In certain embodiments, the type 1 IFN masking moiety comprises IFN binding loop 1: YTIMSKPEDLK (SEQ ID NO: 74), IFN binding loop 2: STHEAYVTVL (SEQ ID NO: 75), and IFN binding loop 3: SHNFWLAID (SEQ ID NO: 76).

[0075] The tool for redesigning the polypeptide can be a Machine Learning (ML) tool or other artificial intelligence (AI)-based computational tools in mutagenesis-based protein engineering, enabling the *de novo* design of a variant library containing proteins with a desired function that never exist before in nature ((Saito et al., ACS Catal., 11, 14615–14624 (2021); Eisenstein, Nature biotechnology., 41, 303–305 (2023)).

[0076] With the assistance of the ML or AI-based computational tools, mutations can be made within the binding loops to improve the IFN-IFNAR2-D1 binding affinity and subsequently enhance the masking efficiency and/or peptide expression. Some redesigned sequences of the binding loops are listed in Table 2.

Table 2. Sequences of the mutated IFN binding loop

IFN Binding Loop	Sequence	SEQ ID NO:
IFN Binding loop 2	STQ Q EIYVTVL	77
	STD E AYVTVL	78
IFN Binding loop 3	SH E FWLAID	79

[0077] In certain embodiments, the type 1 IFN masking moiety comprises IFN binding loop 1: YTIMSKPEDLK (SEQ ID NO: 74), IFN binding loop 2: STQ**Q**EIYVTVL (SEQ ID NO: 77), and IFN binding loop 3: SHN**F**WLAID (SEQ ID NO: 76).

[0078] In certain embodiments, the type 1 IFN masking moiety comprises IFN binding loop 1: YTIMSKPEDLK (SEQ ID NO: 74), IFN binding loop 2: STQ**Q**EIYVTVL (SEQ ID NO: 77), and IFN binding loop 3: SH**E**FWLAID (SEQ ID NO: 79).

[0079] In certain embodiments, the type 1 IFN masking moiety comprises IFN binding loop 1: YTIMSKPEDLK (SEQ ID NO: 74), IFN binding loop 2: STD**E**AYVTVL (SEQ ID NO: 78), and IFN binding loop3: SHN**F**WLAID (SEQ ID NO: 76).

[0080] In certain embodiments, the type 1 IFN masking moiety comprises IFN binding loop 1: YTIMSKPEDLK (SEQ ID NO: 74), IFN binding loop 2: ST**H**EAYVTVL (SEQ ID NO: 75), and IFN binding loop 3: SH**E**FWLAID (SEQ ID NO: 79).

[0081] The IFNAR2-D1 framework region can be redesigned by ML or AI-based computational tools to improve the spatial structure stability of the polypeptide. The “IFNAR2-D1 framework” as used herein refers to the structure supporting regions of IFNAR2-D1 within which the IFN binding loops are interspersed, and the IFNAR2-D1 framework regions are not engaged in the binding with the type 1 IFNs. In one aspect of the present disclosure, the type 1 IFN masking moiety provided herein are IFNAR2-D1 framework redesigned based on the structure of IFNAR2-D1 comprising an amino acid sequence of SEQ ID NO: 8.

[0082] In certain embodiments, the redesigned type 1 IFN masking moiety comprises an amino acid sequence having at least 55% (or at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%) sequence identity to SEQ ID NO: 8 and is still capable of binding to type 1 IFN.

In certain embodiments, the type 1 IFN masking moiety comprises an amino acid sequence of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, or 40. In some embodiments, the masking moiety includes a peptide having at least 55% (or at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%) sequence identity to any one of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, or 40. In some embodiments, such an amino acid sequence retains one or more of the original or mutated IFN binding loops 1, 2 and/or 3.

[0083] In another aspect of the present disclosure, the type 1 IFN masking moiety provided herein are derived from sequence optimization based on a N-terminal extended IFNAR2-D1 comprising an amino acid sequence of SEQ ID NO: 3. One or more further substitutions can be made to enhance the properties of the type 1 IFN masking moiety polypeptide, such as protein expression and masking efficiency.

[0084] In certain embodiments, one or more disulfide bonds are introduced into the type 1 IFN masking moiety polypeptide to stabilize the polypeptide. As used herein the term “disulfide bond” includes the covalent bond formed between two sulfur atoms with the structure R-S-S-R. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group from another cysteine residue. Incorporation of disulfide bonds into proteins is one of nature's ways of improving protein stability; a correlation between the abundance of disulfide bonds and the maximum growth temperature among thermophilic organisms has been found, implicating the importance of disulfide bonds in protein stabilization in high temperature environments (Mallick P, et al, 2002, Proc. Natl. Acad. Sci. USA, 99, 9679-9684.; Ladenstein R, et al, 2006, FEBS J., 273, 4170-4185).

[0085] The number of disulfide bonds in a protein can be readily determined by accurate intact mass measurements. The disulfide bonds connectivity can be verified (determined) by standard techniques known in the art, such as peptide mapping.

[0086] In certain embodiments of the present disclosure, a cystine pair is introduced with amino acid substitutions at R20C and E109C corresponding to the positions in SEQ ID NO: 3 to form a disulfide bond. In certain embodiments, type 1 IFN masking moiety comprises an amino acid sequence of SEQ ID NO: 42.

[0087] In certain embodiments, one or more substitutions are introduced to the type 1 IFN masking moiety and the type 1 IFN masking moiety comprises an amino acid sequence having at least 90% (or at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO: 3 and is still capable of binding to type 1 IFN.

[0088] In certain embodiments, type 1 IFN masking moiety comprises an amino acid sequence of SEQ ID NO: 42 with one or more amino acid substitutions selected from the group consisting of

- (1) L19: S, Q, E, or R;
- (2) F22: G, N, or D;
- (3) I46R;
- (4) M47: I or F;
- (5) S48: N, Y, or Q;
- (6) K49Q;
- (7) H77: Y, Q or D;
- (8) A79: D, I, V, E or N;
- (9) V81: I or F;
- (10) V83: F, I or K;
- (11) N99: E, D or W;
- (12) W101: M or F;
- (13) L102: M or I;
- (14) I104: F or W;
- (15) M106: A, T, L, or E;
- (16) F108: N, Y, T, or E;
- (17) P110: S or E;
- (18) P111: S or E; and
- (19) the combination thereof.

[0089] In certain embodiments, type 1 IFN masking moiety comprises an amino acid sequence of SEQ ID NO: 42 with amino acid substitutions of:

- (1) R20C, M47I, H77Q, A79I and E109C;
- (2) R20C, H77D, A79I, N99E and E109C;
- (3) R20C, H77D and E109C;
- (4) R20C, N99E and E109C;
- (5) L19S, R20C, F22G, M106A, F108N, E109C, P110S and P111S;
- (6) L19Q, R20C, F22N, M106T, F108Y and E109C;
- (7) L19E, R20C, F22G, M106L, F108T, E109C, P110S and P111S;
- (8) L19R, R20C, F22D, F108E and E109C;
- (9) L19R, R20C, F22N, M106E, E109C, P110S and P111S;
- (10) R20C, F22G, M106T, E109C, P110S and P111S;
- (11) L19S, R20C, F22N, M106L, F108E and E109C;
- (12) L19E, R20C, F22G and E109C;
- (13) R20C, F22N, F108T, E109C, P110S and P111S; or
- (14) R20C, F22G, F108N and E109C.

[0090] In certain embodiments, type 1 IFN masking moiety comprises an amino acid sequence of SEQ ID NO: 42, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 or 73. In some embodiments, the masking moiety includes a peptide having at least 55% (or at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%) sequence identity to any one of SEQ ID NOs: 42, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 or 73. In some embodiments, such an amino acid sequence retains one or more of the mutations as provided above.

[0091] In certain embodiments, the IFN masking moiety of the present disclosure is fused to an immunoglobulin Fc region. In certain embodiments, the masking moiety is fused to the Ig Fc region directly or via a linker. In certain embodiments, the C-terminus or N-terminus of the IFN masking moiety is fused to the N-terminus of the Ig Fc region. In certain embodiments, the C-terminus or N-terminus of the IFN masking moiety is fused to the C-terminus of the Ig Fc region.

Linker

[0092] Linkers within the scope of the present disclosure are characterized in terms of amino acid content, length, rigidity and secondary structure. Linkers within the scope of the present disclosure separate the type 1 interferon and another functional polypeptide (such as a masking moiety or an Ig Fc region) and allow proper folding and functioning of each domain. In this manner, a linker can be tailored to the particular type 1 interferon and the other functional polypeptide. According to one aspect, functional independence of the structural (i.e., type 1 interferon) and fused (heterologous) domains is maximized by a suitable linker to limit steric interference between domains during the export and assembly processes of the bacterial cell. According to one aspect, longer and more flexible linkers of the type (GGGGS)_n, wherein n is an integer from 1 to 20 (herein after the “GS repeat”, SEQ ID NO:1) are exemplary. According to an additional aspect, cell stress is minimized by limiting the overall length of the fusion protein. Longer linker sequences and higher induction levels stress the biosynthetic machinery of the cells, inhibiting cell growth and leading to cell lysis in extreme cases.

[0093] Linkers within the scope of the present disclosure facilitate functioning of the type 1 interferon domain and the other functional polypeptide. Linkers within the scope of the present disclosure allow efficient protein processing and export through the bacterial curli secretion machinery as well as provide the proper spatial and physicochemical separation of the type 1 interferon and the other functional polypeptide to retain their respective functions.

[0094] Linkers within the scope of the present disclosure include amino acid residues. The amino acid residues may be any of the naturally occurring amino acid residues. Amino acid residues may also be synthetic amino acids known to those of skill in the art. Representative amino acids which may be used in linkers include Glycine, Alanine, Valine, Leucine, Isoleucine, Serine, Cysteine, Selenocysteine, Threonine, Methionine, Proline, Phenylalanine, Tyrosine, Tryptophan, Histidine, Lysine, Arginine, Aspartate, Glutamate, Asparagine, and Glutamine.

[0095] Linkers within the scope of the present disclosure include a cleavage site. Cleavage sites and enzymes for cleavage are known to those of skill in the art. According to this embodiment, the cleavable linker is cleaved by the enzyme within a tumor microenvironment in the patient. A “tumor microenvironment” as used herein refers to a “complex network” of different cell types, soluble factors, signaling molecules and extracellular matrix components, which orchestrate the fate of tumor progression. The proteases that are expressed by cell in

the tumor microenvironment (e.g., tumor cells and tumor-associated macrophages) and thus are tumor-abundant cleave the linkers on the cleavage sites and release the functional polypeptide from the linker into the surrounding environment, for example for therapeutic or diagnostic purposes.

[0096] Proteinases are a class of enzymes that are involved in the cleavage or hydrolysis of a variety of proteins. The proteinase is tumor-abundant proteinase, such as matrix cathepsins, caspases, and metalloproteinases. Exemplary enzymes include those from the family of matrix metalloproteinases (MMPs), which have their own recognition sequences, a thrombin, a neutrophil elastase, a cysteine protease, FAPa, Cathepsin B, legumain, a serine protease, such as a matriptase or a urokinase (uPA), or matrix metalloproteinases (MMPs), such as MMP1, MMP2, MMP3, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, or MMP17. Other enzymes are proteases secreted by pathogens such as CD2830 from *C. difficile*.

[0097] The cleavage site sequence(s) of tumor-abundant MMPs include, among others: SGVFSIPLTA (SEQ ID NO: 143), SGIKYHSLTA (SEQ ID NO: 144), VPQSLTMG (SEQ ID NO: 145), VPRSLTMG (SEQ ID NO: 146), VPLGLTMG (SEQ ID NO: 80), VPLSITMG (SEQ ID NO: 81), VPLSLAMG (SEQ ID NO: 82), VPLSLDMG (SEQ ID NO: 83), VPLSLTGG (SEQ ID NO: 84), GPQGIAGQ (SEQ ID NO: 85), GPQGIAGQ (SEQ ID NO: 86), VPMSMRGG (SEQ ID NO: 87), IPVSLRSG (SEQ ID NO: 88), RPFISMIMG (SEQ ID NO: 89), VPLSLTMG (SEQ ID NO: 90), VPLSLYSG (SEQ ID NO: 91), IPESLRAG (SEQ ID NO: 92), IPENFFGV (SEQ ID NO: 93), VPYGLGSP (SEQ ID NO: 94), HPSAFSEA (SEQ ID NO: 95), GPQGIAGQ (SEQ ID NO: 96), GPQGILLGA (SEQ ID NO: 97), GPAGLSVL (SEQ ID NO: 98), GPAGIVTK (SEQ ID NO: 99), DAASLLGL (SEQ ID NO: 100), RPAVMTSP (SEQ ID NO: 101), PPGAYHGA (SEQ ID NO: 102), LRAYLLPA (SEQ ID NO: 103), GPYELKAL (SEQ ID NO: 104), TAAALTSC (SEQ ID NO: 105), GPEGLRVG (SEQ ID NO: 106), GHARLVHV (SEQ ID NO: 107), QPVGINTS (SEQ ID NO: 108), ELGTYNVI (SEQ ID NO: 109), DVAQFVLY (SEQ ID NO: 110), DVANYNFF (SEQ ID NO: 111), HPVGLLAR (SEQ ID NO: 112), KPQQFFGL (SEQ ID NO: 113), IPVSLRSG (SEQ ID NO: 114), HPVGLLAR (SEQ ID NO: 115), QPVGINTS (SEQ ID NO: 116), RPAVMTSP (SEQ ID NO: 117), GPQGIAGQ (SEQ ID NO: 118), HVLNLRST (SEQ ID NO: 119), DPESIRSE (SEQ ID NO: 120), DPLEFKSH (SEQ ID NO: 121), RPIPTAS (SEQ ID NO: 122), RVLGLKAH (SEQ ID NO: 123), KVLNLTDN (SEQ ID NO: 124), PPEALRGI (SEQ ID NO: 125), IVAMLRAP (SEQ ID NO: 126), TAAAITGA (SEQ ID NO: 127), SGRIGFLRTA (SEQ

ID NO: 128), SGAIGFLRTA (SEQ ID NO: 129), SGRAMHMYTA (SEQ ID NO: 130), SGAAMHMYTA (SEQ ID NO: 131), SGRSENIRTA (SEQ ID NO: 10), SGASENIRTA (SEQ ID NO: 132), SGRPENIRTA (SEQ ID NO: 133), SGAPENIRTA (SEQ ID NO: 134), SGARYRWLTA (SEQ ID NO: 135), SGLISHSITA (SEQ ID NO: 136), SGNLRSKLT (SEQ ID NO: 137), VPLSLTMG (SEQ ID NO: 138), GPLSLTMG (SEQ ID NO: 139), GVLSLTMG (SEQ ID NO: 140), GGLSLTMG (SEQ ID NO: 141), and VHLSLTMG (SEQ ID NO: 142).

[0098] According to one aspect, the linker length can be any length which may be expressed from a cell, such as a bacterial cell when linking a type 1 interferon protein and a functional polypeptide. According to one aspect, the functional polypeptide length can be any length which may be expressed from a cell, such as a bacterial cell when linked to a type 1 interferon protein by a linker.

[0099] According to one aspect, a linker sequence is a polypeptide sequence of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 48 or more amino acids. In some embodiments, the linker sequence comprises from about 3 amino acids to about 50 amino acids. In some embodiments, the linker sequence comprises from about 3 amino acids to about 40 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 30 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 25 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 24 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 23 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 22 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 21 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 20 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 19 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 18 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 17 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 16 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 15 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 14 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 13 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 12 amino acids. In some embodiments, the linker sequence comprises

from about 5 amino acids to about 11 amino acids. In some embodiments, the linker sequence comprises from about 5 amino acids to about 10 amino acids.

[0100] In some embodiments, the linker sequence comprises a flexible polypeptide, e.g. a polypeptide not having a rigid secondary and/or tertiary structure. In some embodiments, the linker sequence comprises glycine and serine residues. In some embodiments at least 50% of the amino acids comprised by the linker sequence are glycine or serine residues, e.g. at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or more are glycine or serine residues. In some embodiments, the linker sequence consists of glycine and serine residues.

[0101] In certain embodiments, the linker that connects the type 1 IFN and the masking moiety is a cleavable linker that contains one or more protease cleavage sites interspersed within the linker peptide sequence. The more than one protease cleavage sites can be the same or different. In certain embodiments, the cleavable linker contains one protease cleavage site. In certain embodiments of the present disclosure, the linker contains a single GS repeat and the protease cleavage site is located within the single GS repeat. In certain embodiments of the present disclosure, the linker contains more than one GS repeats and the protease cleavage site(s) are located after at least one GS repeat. Following the one or more GS repeats, a single protease cleavage site can be located within a GS repeat sequence, or by replacing one, two, three or four amino acid residues of the GS repeat. In certain embodiments, the starting and/or ending GS repeat of a linker can be a shortened GS repeat with one, two, three or four amino acid residues.

[0102] In certain embodiments of the present disclosure, the protease is MMP-14 and the cleavage site comprises an amino acid sequence of SGQLLGFLTA (SEQ ID NO: 45) and/or SGRSENIRTA (SEQ ID NO: 10). In certain embodiments, the sequences of the cleavable linker provided in the present disclosure are provided as in Table 3.

Table 3. Cleavable linkers containing protease cleavage site:

Cleavable linker	SEQ ID NOs:
GG <u>SGRSENIRTA</u> GGG	9
GGGGSGGGG <u>SGRSENIRTA</u> GGG	13
GGSGGGGS <u>SGQLLGFLTA</u> GGGG	44

[0103] In certain embodiments, the second linker that connects the type 1 IFN and the Ig Fc region comprises 3-50 amino acids. In certain embodiments, the second linker comprises 3-30 amino acids. In certain embodiments, the second linker comprises 3-20 amino acids. In certain embodiments, the second linker comprises 3-10 amino acids. In certain embodiments, the second linker comprises 3, 4, 5, 6, 7, 8, 9 or 10 amino acids. In certain embodiments, the second linker comprises 3 amino acids. In certain embodiments, the second linker comprises three Glycine residues.

Immunoglobulin Fc region

[0104] The masked IFN fusion protein of the present disclosure contains an immunoglobulin Fc region. As used herein, the term “immunoglobulin Fc region” refers to an immunoglobulin fragment that is devoid of the variable regions of light and heavy chains, the constant region 1 of the heavy chain (CH1), and the constant region 1 of the light chain (CL1), that is, a fragment comprised of the constant regions 2 and 3 of the heavy chain (CH2 and CH3). Optionally, the immunoglobulin Fc region may further comprise a hinge region. Also, the immunoglobulin Fc region of the present disclosure may be an extended Fc region which comprises a part of or the entirety of the constant region 1 of the heavy chain (CH1) and/or the constant region 1 of the light chain (CL1) in addition to the constant regions 2 and 3 of the heavy chain (CH2 and CH3) so long as it shows effects substantially identical or superior to those of the classical Fc region. Further, the immunoglobulin Fc region of the present disclosure may be comprised of CH2 and/or CH3 that lacks a significant part of the amino acid sequence.

[0105] Consequently, the immunoglobulin Fc region of the present disclosure may comprise 1) CH1 domain, CH2 domain, CH3 domain and CH4 domain, 2) CH1 domain and CH2 domain, 3) CH1 domain and CH3 domain, 4) CH2 domain and CH3 domain, 5) a combination of one or more constant domains and an immunoglobulin hinge region (or a partial hinge region), or 6) a dimer of each constant domain of the heavy chain and the constant region of the light chain. In certain embodiments, the immunoglobulin Fc region comprises CH2 domain and CH3 domain and an immunoglobulin hinge region (or a partial hinge region). In certain embodiments, the immunoglobulin Fc region comprises a dimer of the CH2 domain and CH3 domain and the immunoglobulin hinge region (or a partial hinge region).

[0106] Further, an amino acid sequence mutant of the wild-type Fc may be included within the scope of the immunoglobulin Fc region of the present disclosure. The term “amino acid

sequence mutant,” as used herein, refers to an amino acid sequence that is different from the wild-type as a result of deletion, insertion, conserved or non-conserved substitution of one or more amino acid residues, or a combination thereof. For instance, amino acid residues at positions 214 to 238, 297 to 299, 318 to 322, or 327 to 331 in IgG Fc, known to be important for linkage, may be used as the sites suitable for modification.

[0107] Various derivatives, such as those prepared by removing the sites of disulfide bonds, removing several N-terminal amino acids from native Fc, or adding methionine to the N-terminus of native Fc, may be used in the present disclosure. In addition, complement fixation sites, e.g., C1q fixation sites, or ADCC sites may be eliminated to remove the effector function from the native Fc region. The techniques of preparing amino acid sequence mutants of the immunoglobulin Fc region are disclosed in International Patent Publication Nos. WO 97/34631 and WO 96/32478.

[0108] The above-described Fc derivatives exhibit the same biological activity as that of the wild-type, but are improved in structural stability to heat and pH.

[0109] The type 1 interferon of the present disclosure is fused to the Fc region to improve its biological properties, such as increased solubility, prolonged serum half-life and increased binding affinity to the target cells (when not engaged with a masking moiety), such as cells of target organs, and receptors such as IFNAR1 and IFNAR2, over the non-fusion protein. In certain embodiments, the type 1 IFN is fused to the Ig Fc region directly or via a linker as described above. In certain embodiments of the present disclosure, the C-terminus of the type 1 IFN is fused to the N-terminus of the Fc region.

[0110] The masked IFN fusion protein of the present disclosure has increased solubility and prolonged serum half-life over the masked IFN that is not fused to a Fc region. When the masking moiety is cleaved, the fused interferon and Fc region may have increased binding affinity to the target cells, such as cells of target organs, and receptors such as IFNAR1 and IFNAR2, over the non-fused interferon.

[0111] In certain embodiments of the present disclosure, the IFN masking moiety is fused to the Fc region. The fused IFN masking moiety has improved biological properties, such as increased solubility, prolonged serum half-life and increased binding affinity to its ligand, such as the interferons. In certain embodiments, the interferon comprises human type 1 IFN.

[0112] Particularized modification of amino acids in the Fc region of human IgG is an active area of study yielding structure-function relationship information relevant to development of therapeutic proteins, particularly monoclonal antibodies (see, e.g., U.S. Pat. No. 6,165,745 and PCT Publication No. WO2004/035752 regarding alteration of serum half-life of a polypeptide operably linked to an Fc region and U.S. Pat. No. 6,737,056 and PCT Publication No. WO2004/029207 regarding alteration of an effector function of a monoclonal antibody comprising a modified Fc region).

[0113] The immunoglobulin Fc region useful in the present disclosure may be glycosylated to the same extent as or to a higher or lesser extent than the native form or may be deglycosylated or aglycosylated. Increased or decreased glycosylation or deglycosylation of the immunoglobulin region may be achieved by typical methods, for example, by using a chemical method, an enzymatic method, or a genetic engineering method. Herein, when deglycosylated, an immunoglobulin Fc region is significantly decreased in complement (C1q) binding force and has reduced or no antibody-dependent cytotoxicity or complement-dependent cytotoxicity, so that it does not induce unnecessary immune responses *in vivo*.

[0114] The term “deglycosylation,” as used herein, is intended to mean the enzymatic removal of sugars from an Fc region. The term “aglycosylation,” when used in conjunction with an Fc region, means an Fc region free of sugars, expressed from prokaryotes, preferably from *E. coli*.

[0115] For use in the present disclosure, the immunoglobulin Fc region has an amino acid sequence of human immunoglobulin Fc regions or their closely related analogues. The Fc regions may be obtained from native forms isolated from animals including cows, goats, swine, mice, rabbits, hamsters, rats and guinea pigs. In addition, the immunoglobulin Fc region may be an Fc region that is derived from IgG, IgA, IgD, IgE and IgM, or that is made by combinations thereof or hybrids thereof. Preferably, it is derived from IgG or IgM, which is among the proteins that are the most abundant in human blood, and most preferably from IgG, which is known to enhance the serum half-life of the ligand-binding proteins. Herein, the immunoglobulin Fc may be obtained from a native immunoglobulin by isolating whole immunoglobulin from human or animal organisms and treating them with a proteolytic enzyme or it may be recombinants or derivatives thereof, obtained from transformed animal cells or microorganisms. Preferable is recombinant human immunoglobulin Fc produced by *E. coli* transformants.

[0116] Among the four IgG subclasses, IgG1 and IgG3 induce the strongest Fc-effector functions. However, since IgG1 has the longest half-life and is more stable than IgG3, most therapeutic antibodies with Fc-mediated functions are of IgG1 isotype. IgG2 and IgG4 isotypes have significantly lower binding affinity to FcγRs. Recent evidence suggests that the IgG2 isotype is not completely devoid of effector function, whereas the IgG4 isotype can undergo *in vivo* Fab arm exchange leading to bispecific antibody and off-target effects.

[0117] In certain embodiments, the Fc region is of an IgG1 Fc region. In certain embodiments, the IgG1 Fc region comprises an amino acid sequence with a mutations C5A, as compared with a wild type human IgG1 Fc region, which prevents CH1 and CL interaction.

[0118] In certain embodiments, the IgG1 Fc region comprises an amino acid sequence of SEQ ID NO: 6 or any an amino acid sequence having at least 75% (or at least 80%, at least 85%, at least 90%, at least 95% or at least 99%) sequence identity thereof.

[0119] In certain embodiments, the immunoglobulin Fc regions form a dimer that contains two identical Fc polypeptides. In certain embodiments, the immunoglobulin Fc regions form a dimer that contain two Fc polypeptides with different modifications. The term “dimer” as used herein refers to an associated structure formed by two molecules, such as polypeptides or proteins, via covalent or non-covalent interactions. A homodimer or homodimerization is formed by two identical molecules, and a heterodimer or heterodimerization is formed by two different molecules.

The masked IFN fusion protein

[0120] The masked IFN fusion protein of the present disclosure comprises in an N- to C-terminal direction: a masking moiety-a cleavable linker-a type I IFN-an immunoglobulin Fc region, such as the format shown in **FIG. 1**. Alternatively, the masked IFN fusion protein of the present disclosure comprises in an N- to C-terminal direction of a masking moiety-a cleavable linker-a type I IFN-a second linker-an immunoglobulin Fc region.

[0121] In another embodiment, the masked IFN fusion protein of the present disclosure comprises in an N- to C-terminal direction of an immunoglobulin Fc region-a masking moiety-a cleavable linker-a type I IFN. In another embodiment, the masked IFN fusion protein of the present disclosure comprises in an N- to C-terminal direction of an immunoglobulin Fc region-a second linker-a masking moiety-a cleavable linker-a type I IFN.

[0122] The cleavable linker and the second linker can be identical or different. In certain embodiments, the second linker comprises a cleavable site.

[0123] In certain embodiments, the second linker is not cleavable. In certain embodiments, the second linker comprises 3 amino acids of Glycine.

[0124] In some embodiments, there are no more than 100 amino acid residues between the C-terminus of the masking moiety and the N-terminus of the type I IFN. In some embodiments, there are no more than 90, 80, 70, 60, 50, 40, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 amino acid residues between the C-terminus of the masking moiety and the N-terminus of the type I IFN.

[0125] In one embodiment, two fusion proteins may associate, either covalently, for example, by a disulfide bond, a polypeptide bond or a crosslinking agent, or non-covalently, to produce a dimeric protein. In a preferred embodiment, the two fusion proteins are associated covalently by means of at least one and more preferably two interchain disulfide bonds via cysteine residues, preferably located within immunoglobulin hinge regions disposed within the immunoglobulin Fc regions of each chain.

[0126] In one aspect, the masked IFN fusion protein of the present disclosure may be prepared using commonly known genetic engineering techniques in the art and the resulting fusion protein preferably is synthesized in a cell that glycosylates the Fc region at normal glycosylation sites, i.e., which usually exist in template antibodies. In another aspect, the present disclosure provides methods of producing a masked IFN fusion protein comprising a masking moiety, a cleavable linker, a type I IFN, an immunoglobulin Fc region and optionally a second linker interposed between the type I IFN and the immunoglobulin Fc region. The method comprises the steps of (a) providing a mammalian cell containing a DNA molecule encoding such a fusion protein, either with or without a signal sequence, and (b) culturing the mammalian cell to produce the fusion protein. The resulting fusion protein can then be harvested, refolded, if necessary, and purified using conventional purification techniques well known and used in the art.

[0127] The amino acid sequence of the masked IFN fusion protein can be substituted at one or more residues. Such substitutions, in some embodiments, are conservative substitutions. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having

similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an immunoglobulin polypeptide is preferably replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0128] Non-limiting examples of conservative amino acid substitutions are provided in the table below, where a similarity score of 0 or higher indicates conservative substitution between the two amino acids.

Amino Acid Similarity Matrix

	C	G	P	S	A	T	D	E	N	Q	H	K	R	V	M	I	L	F	Y	W
W	-8	-7	-6	-2	-6	-5	-7	-7	-4	-5	-3	-3	2	-6	-4	-5	-2	0	0	17
Y	0	-5	-5	-3	-3	-3	-4	-4	-2	-4	0	-4	-5	-2	-2	-1	-1	7	10	
F	-4	-5	-5	-3	-4	-3	-6	-5	-4	-5	-2	-5	-4	-1	0	1	2	9		
L	-6	-4	-3	-3	-2	-2	-4	-3	-3	-2	-2	-3	-3	2	4	2	6			
I	-2	-3	-2	-1	-1	0	-2	-2	-2	-2	-2	-2	-2	4	2	5				
M	-5	-3	-2	-2	-1	-1	-3	-2	0	-1	-2	0	0	2	6					
V	-2	-1	-1	-1	0	0	-2	-2	-2	-2	-2	-2	-2	4						
R	-4	-3	0	0	-2	-1	-1	-1	0	1	2	3	6							
K	-5	-2	-1	0	-1	0	0	0	1	1	0	5								
H	-3	-2	0	-1	-1	-1	1	1	2	3	6									
Q	-5	-1	0	-1	0	-1	2	2	1	4										
N	-4	0	-1	1	0	0	2	1	2											
E	-5	0	-1	0	0	0	3	4												
D	-5	1	-1	0	0	0	4													
T	-2	0	0	1	1	3														
A	-2	1	1	1	2															
S	0	1	1	1																
P	-3	-1	6																	
G	-3	5																		
C	12																			

Conservative Amino Acid Substitutions

For Amino Acid	Substitution With
Alanine	D-Ala, Gly, Aib, β -Ala, L-Cys, D-Cys
Arginine	D-Arg, Lys, D-Lys, Orn D-Orn
Asparagine	D-Asn, Asp, D-Asp, Glu, D-Glu Gln, D-Gln
Aspartic Acid	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr, L-Ser, D-Ser
Glutamine	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	Ala, D-Ala, Pro, D-Pro, Aib, β -Ala
Isoleucine	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	Val, D-Val, Met, D-Met, D-Ile, D-Leu, Ile
Lysine	D-Lys, Arg, D-Arg, Orn, D-Orn
Methionine	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	D-Phe, Tyr, D-Tyr, His, D-His, Trp, D-Trp
Proline	D-Pro
Serine	D-Ser, Thr, D-Thr, allo-Thr, L-Cys, D-Cys
Threonine	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Val, D-Val
Tyrosine	D-Tyr, Phe, D-Phe, His, D-His, Trp, D-Trp
Valine	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

[0129] It will also be understood by one of ordinary skill in the art that the masked IFN fusion proteins as disclosed herein may be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein may be similar, *e.g.*, have a certain percent identity to the starting sequence, *e.g.*, it may be 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the starting sequence.

[0130] In certain embodiments, the masked IFN fusion protein comprises an amino acid sequence or one or more moieties. For example, the masked IFN fusion proteins of the present disclosure may comprise a further flexible linker sequence, or may be modified to add an extra functional moiety (*e.g.*, PEG, a drug, a toxin, or a label). In some embodiments, the masked IFN fusion protein or the IFN masking moiety may be conjugated to therapeutic agents, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents, or PEG.

[0131] The masked IFN fusion proteins or the IFN masking moieties, variants, or derivatives thereof of the present disclosure include derivatives that are modified, *i.e.*, by the covalent

attachment of any type of molecule to the polypeptide such that covalent attachment does not prevent the polypeptide's function. For example, but not by way of limitation, the masked IFN fusion protein or the IFN masking moiety can be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the masked IFN fusion protein or the IFN masking moiety may contain one or more non-classical amino acids.

[0132] The masked IFN fusion protein or the IFN masking moiety of the present disclosure may be conjugated or fused to a therapeutic agent, which may include detectable labels such as radioactive labels, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic or diagnostic agent, a cytotoxic agent, which may be a drug or a toxin, an ultrasound enhancing agent, a non-radioactive label, a combination thereof and other such agents known in the art.

Polynucleotides Encoding the polypeptide and Methods of Preparing the polypeptide

[0133] The present disclosure also provides isolated polynucleotides or nucleic acid molecules encoding the polypeptide (such as the masked IFN fusion protein or the IFN masking moiety of the present disclosure), variants or derivatives thereof of the disclosure.

[0134] DNA encoding the polypeptide is readily isolated and sequenced using conventional procedures (*e.g.* by using oligonucleotide probes that are capable of binding specifically to genes encoding the polypeptide). The encoding DNA may also be obtained by synthetic methods.

[0135] The present disclosure provides vectors (*e.g.* expression vectors) comprising the isolated polynucleotide provided herein. A vector may be used to transform, transduce, or transfect a host cell so as to bring about expression of the genetic element it carries within the host cell. A vector may contain a variety of elements for controlling expression, including promoter sequences, transcription initiation sequences, enhancer sequences, selectable elements, and reporter genes. In addition, the vector may contain an origin of replication. A vector may also include materials to aid in its entry into the cell, including but not limited to a

viral particle, a liposome, or a protein coating. A vector can be an expression vector or a cloning vector.

[0136] In certain embodiments, an expression vector provided herein comprises the polynucleotide encoding the polypeptide provided herein, at least one promoter (e.g. SV40, CMV, EF-1 α) operably linked to the polynucleotide sequence, and at least one selection marker.

[0137] Vectors comprising the polynucleotide sequence encoding the masked IFN fusion protein or the IFN masking moiety can be introduced to a host cell for cloning or gene expression. Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Examples of useful mammalian host cell lines are CHO, BHK, NS0, 293 and their derivatives.

[0138] Host cells are transformed with the above-described expression or cloning vectors for polypeptide production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. In another embodiment, the polypeptide may be produced by homologous recombination known in the art.

[0139] The host cells used to produce the polypeptides provided herein may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium (MEM), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM), (Sigma) are suitable for culturing the host cells with addition of necessary hormones and/or other growth factors, salts, buffers, nucleotides, antibiotics, trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[0140] When using recombinant techniques, the polypeptides can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the polypeptide is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. The polypeptide prepared from the cells can then be purified using any suitable purification technique known to the art.

Treatment and Diagnostic Methods

[0141] As described herein, the masked IFN fusion protein or the IFN masking moiety, variants or derivatives of the present disclosure may be used in certain treatment and diagnostic methods.

[0142] The present disclosure is further directed to polypeptide-based therapies which involve administering the masked IFN fusion protein of the present disclosure to a patient such as an animal, a mammal, and a human for treating one or more of the disorders or conditions described herein. Therapeutic compounds of the disclosure include, but are not limited to, the masked IFN fusion protein of the disclosure (including variants and derivatives thereof as described herein) and nucleic acids or polynucleotides encoding the masked IFN fusion protein of the present disclosure (including variants and derivatives thereof as described herein).

[0143] The present disclosure is further directed to polypeptide-based therapies which involve administering the IFN masking moiety of the present disclosure to a patient such as an animal, a mammal, and a human for treating one or more of the disorders or conditions described herein. Therapeutic compounds of the disclosure include, but are not limited to, the IFN masking moiety of the disclosure (including variants and derivatives thereof as described herein) and nucleic acids or polynucleotides encoding the IFN masking moiety of the present disclosure (including variants and derivatives thereof as described herein).

[0144] In some embodiments, provided are methods for treating a cancer in a patient in need thereof. The method, in one embodiment, entails administering to the patient an effective amount of the masked IFN fusion protein of the present disclosure.

[0145] In some embodiments, provided are uses of the masked IFN fusion protein of the present disclosure in the manufacture of a medicament for treating a cancer in a patient in need thereof.

[0146] In some embodiments, provided are the masked IFN fusion protein of the present disclosure for use in the treatment of a cancer in a patient in need thereof.

[0147] Non-limiting examples of cancers include bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer.

[0148] Additional diseases or conditions associated with increased cell survival, that may be treated, prevented, diagnosed and/or prognosed with the polypeptides or variants, or derivatives thereof of the disclosure include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (*e.g.*, acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (*e.g.*, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (*e.g.*, Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma and retinoblastoma.

[0149] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular polypeptide, variant or derivative thereof used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

[0150] Methods of administration of the masked IFN fusion protein or the IFN masking moiety of the present disclosure include but are not limited to intradermal, intramuscular,

intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The antigen-binding polypeptides or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides of the disclosure may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray.

[0151] The term “parenteral” as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

[0152] Administration can be systemic or local. In addition, it may be desirable to introduce the polypeptides or compositions of the present disclosure into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0153] It may be desirable to administer the masked IFN fusion protein or the IFN masking moiety or compositions of the present disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction, with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an masked IFN fusion protein or the IFN masking moiety, of the disclosure, care must be taken to use materials to which the protein does not absorb.

[0154] Methods of *in vivo/ex vivo* detecting expression of a human type 1 IFN target cells, such as cells of target organs, and receptors such as IFNAR1 and IFNAR2 in a subject are also provided, in some embodiments, comprising administering the IFN masking moiety of the present disclosure to the subject or contacting the *ex vivo* sample with the IFN masking moiety

of the present disclosure, and detecting the binding which indicates expression of type 1 IFN target cells or receptors thereof in the sample.

[0155] Methods of detecting expression of a human type 1 IFN protein in a sample are also provided, in some embodiments, comprising contacting the sample with the IFN masking moiety of the present disclosure, and detecting the binding which indicates expression of type 1 IFN in the sample.

[0156] In certain embodiments, provided are uses of the IFN masking moiety of the present disclosure in the manufacture of a kit for detecting expression of a human type 1 IFN protein in a sample.

Compositions

[0157] The present disclosure also provides pharmaceutical compositions. Such compositions comprise an effective amount of masked IFN fusion protein or the IFN masking moiety, and an acceptable carrier. In some embodiments, the composition further includes a second anticancer agent (*e.g.*, an immune checkpoint inhibitor).

[0158] The term “pharmaceutically effective amount,” as used herein, is intended to refer to a sufficient amount of the pharmaceutical composition to treat a disease, at a reasonable benefit/risk ratio applicable to any medical treatment. The effective amount may vary depending on various factors including the severity and type of the disease being treated, the patient’s age and sex, drug activity, sensitivity to drugs, the time of administration, the route of administration, the rate of excretion, the length of the treatment period, the co-administration with other drugs, and other parameters well known in medicinal and pharmaceutical fields.

[0159] In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Further, a “pharmaceutically acceptable carrier” will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

[0160] The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil,

soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E. W. Martin, incorporated herein by reference. Such compositions will contain a therapeutically effective amount of the antigen-binding polypeptide, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0161] In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an

ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0162] The compounds of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

EXAMPLES

Example 1. AI-guided IFNAR2 Domain 1 based masking moiety design

[0163] This example shows the design, preparation and testing of IFNAR2 Domain 1-based masking moieties.

[0164] Three masking moieties were tested, including (1) wild type IFNAR2-D1 core sequence (“M-09”); (2) IFNAR2-D1 core sequence with an introduced disulfide bond at R20C and E109C corresponding to the IFNAR2-D1 peptide (SEQ ID NO: 3) (“M-10”); and (3) wild type IFNAR2-D1 core sequence with an C-terminal extended domain 2 loop (“M-11”), each of which was fused with human type 1 IFN α 2 β -Fc via a cleavable linker (SEQ ID NO:9). The Fc fragment used included C5A mutation in hinge region.

[0165] The corresponding fusion proteins are referred to as I-09 (M-09 + linker + IFN α 2 β -Fc), I-10 (M-10 + linker + IFN α 2 β -Fc), I-11 (M-11 + linker + IFN α 2 β -Fc), and I-31 (M-10 + extended linker + IFN α 2 β -Fc). The sequences of these fusion proteins and each part of the fusion proteins are shown below in Table 4-1.

Table 4-1. Sequences of each part of the masked IFN fusion proteins

Name	Sequence	SEQ ID NO:
Human IFNAR2	ISYDSPDYTDSECTFKISLRNFRSILSWELKNHSIVPHTYLLYTIMSKPEDLKVV KNCANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPPE FEIVGFTNHINVMVKFPSIVEEELQFDLSLVIEEQSEGIVKKHKPEIKGNMNGFT YIIDKLIPNTNYCVSVYLEHSDEQAVIKSPLKCTLLPPGQESAEASAKIGGIITV FLIALVLTSTIVTLKWIGYICLRNSLPKVLNFHNFRAWPFNLPPEAMDMVEVIY INRKKVWDYNYDDESDDTEAAPRTSGGGYTMHGLTVRPLGQASATSTESQLIDP ESEEEPDLPVDVELPTMPKDSPQQLLELLSGPCERRKSPLQDPFPEEDYSSTEGSG GRITFNVDLNSVFLRVLDDESDDEAPMLLSSHLEEMVDPEDPDNVQSNHLLASG EGTQPTFPSPSSEGLWSEDAPSDQSDTSESVDLGDGYIMR	2

Human IFNAR2-D1	ISYDSPDYTDSECTFKISLRNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVV KNCANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPP	3
Human type 1 IFNα2β	CDLPQTHSLGSRRTLMLLAQMRRI SLFSCCLKDRHDFGFPQEEFGNQFQKAETIPVL HEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDEACVIQGVGVTEPTPLMK EDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRSKE	4
IFN-Fc (I-01)	CDLPQTHSLGSRRTLMLLAQMRRI SLFSCCLKDRHDFGFPQEEFGNQFQKAETIPVL HEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDEACVIQGVGVTEPTPLMK EDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRSKEGGG EPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFCSVMHEALHNHYTQK SLSLSPGK	5
Fc region C5A	EPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFCSVMHEALHNHYTQK SLSLSPGK	6
I-09	SCTFKISLRNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCANTTRSFC DLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPPGGSGRSENIRTA GGSCDLPQTHSLGSRRTLMLLAQMRRI SLFSCCLKDRHDFGFPQEEFGNQFQKAETI PVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDEACVIQGVGVTEPT LMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRSKE GGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFCSVMHEALHNHY TQKSLSLSPGK	7
M-09 (human IFNAR2-D1 core)	SCTFKISLRNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCANTTRSFC DLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPP	8
cleavable linker	GGSGRSENIRTAGGS	9
Protease cleavage site	SGRSENIRTA	10
I-10	SCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCANTTRSFC DLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPPGGSGRSENIRTA GGSCDLPQTHSLGSRRTLMLLAQMRRI SLFSCCLKDRHDFGFPQEEFGNQFQKAETI PVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDEACVIQGVGVTEPT LMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRSKE GGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFCSVMHEALHNHY TQKSLSLSPGK	11
M-10	SCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCANTTRSFC DLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPP	12
cleavable linker	GGSGRSENIRTAGGS	9
Protease cleavage site	SGRSENIRTA	10
I-11	SCTFKISLRNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCANTTRSFC DLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPPGFP SIVEEELQF DLGHSDEQAVIGGSGRSENIRTAGGSCDLPQTHSLGSRRTLMLLAQMRRI SLFSC CLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFY TELYQQLNDEACVIQGVGVTEPTPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEV VRAEIMRSFSLSTNLQESLSRSKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSFCSVMHEALHNHYTQKSLSLSPGK	148

M-11	SCTFKISLRNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCAANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPFGFPFIVEEELQFDLGHSDEQAVI	14
cleavable linker	<u>GGSGRSENIRTAGGS</u>	9
Protease cleavage site	SGRSENIRTA	10
I-31	SCTFKISLNCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCAANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPFGGGGGGGGGSGRSENIRTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSFLSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDEACVIQGVGTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVTLHQDWLNCKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMHEALHNHYTQKSLSLSPGK	15
M-31	SCTFKISLNCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCAANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFECPP	12
Extended cleavable linker	<u>GGGGSGGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-03	ISYDSPDYTDESCTFKISLRNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCAANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPPEFEIVGFTNHINVMVKFPSIVEEELQFDLSLVIEEQSEGIVKKHKPEIKGNMMSGNFTYIIDKLI PNTNYCVSVYLEHSDEQAVIKSPLKCTLLPPGQSESAESA KGGGSSGQLLGFLTAGGGGSCDLPQTHSLGSRRTLMLLAQMRRLSFLSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDEACVIQGVGTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVTLHQDWLNCKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMHEALHNHYTQKSLSLSPGK	147
Human IFNAR2-D2	PEFEIVGFTNHINVMVKFPSIVEEELQFDLSLVIEEQSEGIVKKHKPEIKGNMMSGNFTYIIDKLI PNTNYCVSVYLEHSDEQAVIKSPLKCTLLP	16

[0166] For using IFNAR2 D1 as masking moiety, the D1 core sequence masked IFN-Fc fusion protein I-09 was evaluated. In an IFNAR2 binding assay, I-09 showed markedly decreased binding affinity to the human IFNAR2 as compared with the unmasked IFN α 2 β -Fc protein (or simply “IFN-Fc”, I-01) (EC₅₀ ~4.4 folds higher than IFN-Fc, see FIG. 2A). Treatment of Daudi cells with human interferons for 3 days progressively inhibited cell proliferation and induced Daudi cell apoptosis. In the Daudi proliferation inhibition assay, I-09 exhibited ~400 folds lower *in vitro* activity than IFN-Fc, and showed greatly recovered activity after removal of the masking moiety (see FIG. 2B).

[0167] . Masking efficiency of the other three fusion proteins I-10, I-11 and I-31 were also tested. As shown in the FIG. 3A&4A, like I-09, I-10, I-11, and I-31 also exhibited significantly reduced binding affinity to IFNAR2 and greatly reduced activity in Daudi anti-proliferation assay in their fusion form, and the activity was restored once the masking moiety was removed.

De novo design

[0168] Novel masking moiety sequences were designed using an AI sequence design tool on the IFNAR2-D1 framework region, while maintaining the residues of IFN-binding interface. The wild-type human IFNAR2 domain 1 (D1) is poorly expressed since domain 1 and domain 2 are originally expressed together and closely interact with each other through the hydrophobic interface. Elimination of domain 2 exposes the buried hydrophobic surface of domain 1 and causes stability issue in domain 1.

[0169] In order to stabilize domain 1 and keep the masking function, the IFN-binding loops of IFNAR2 domain 1 were maintained and an AI sequence design method was used to redesign the sequence of the domain 1 except the IFN binding loops. In this method, a neural network was employed to process large amount of available protein structure data and sequence-structure patterns were identified. With the trained model, new sequences for domain 1 with desired properties were created. The sequence identity between AI design sequences and the wildtype domain 1 is ~55-60% including the fixed IFN-binding loops. Among the 50 designs, 12 sequences M-40, M-41, M-55, M-60, M-62, M-65, M-66, M-67, M-82, M-83, M-87, and M-89, can be expressed at high levels.

[0170] All resulting masking moiety were fused with human type 1 IFN α 2 β -Fc via an extended cleavable linker (SEQ ID NO:13), respectively. The sequences of each part of the fusion proteins are shown below in Table 4-2.

Table 4-2. Sequences of each part of the masked IFN fusion proteins

Name	Sequence	SEQ ID Nos:
I-40	GCNFSLYLKNNKLILSWTPTGHSVTPDKYNLYYTIMSKPEDLKLVSQCNSSTSCDI TSELKSTHEAYVTVLKGYKGNTYLYSCSHNFWLAIDTKILPPGGGGSGGGG SGRSENI RTAGGSCDLPQTHSLGSRRTLMLLAQMRRI SLFSLCKDRHDFGFPQEEFGNQFQKAET IPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQGVGTETPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRKEGGG EPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPEVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	17
M-40 (D1 sequence redesign)	GCNFSLYLKNNKLILSWTPTGHSVTPDKYNLYYTIMSKPEDLKLVSQCNSSTSCDI TSELKSTHEAYVTVLKGYKGNTYLYSCSHNFWLAIDTKILPP	18
cleavable linker	GGGGSGGGG SGRSENI RTAGGS	13
Protease cleavage site	SGRSENI	10
I-41	CCNFSLYLKNNKIILSWTEVGHSITPDKYNLYYTIMSKPEDLKLKNCQNSSTKYCDL TSELKSTHEAYVTVLKGYKGNEFLYSCSHNFWLAIDKLVLPPGGGGSGGGG SGRSENI RTAGGSCDLPQTHSLGSRRTLMLLAQMRRI SLFSLCKDRHDFGFPQEEFGNQFQKAET IPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQGVGTETPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRKEGGG EPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV	19

	KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK	
M-41 (D1 sequence redesign)	CCNFSLYLKNNKIILSWTEVGHSTPDYKNLYYTIMSKPEDLKLVRNCQNSSKTYCDL TSELKSTHEAYVTVLKGYKGNFLYSCSHNFWLAIDLKVLPP	20
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-55	GCNFSLYLKDGKYILSWTEVGHSVTPDTYNLYYTIMSKPEDLKLLESCKNSSKTYCDI TSSSLKSTHEAYVTVLEGYKGNTFLYSCSHNFWLAIDLKVLPPGGGGSGGGGSGRSENI <u>RTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEFGNQFQKAET</u> IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQNLNDEACVIQGVGVTEPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKEGGG EPKSADKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK	21
M-55 (D1 sequence redesign)	GCNFSLYLKDGKYILSWTEVGHSVTPDTYNLYYTIMSKPEDLKLLESCKNSSKTYCDI TSSSLKSTHEAYVTVLEGYKGNTFLYSCSHNFWLAIDLKVLPP	22
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-60	CCNFSLYKNNKIILSWTLTGHSITPDYKNLYYTIMSKPEDLKLLENCKNSSKTSCTDI TSELKSTHEAYVTVLKGYSGNTFLYSCSHNFWLAIDLTVLPGGGGSGGGGSGRSENI <u>RTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEFGNQFQKAET</u> IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQNLNDEACVIQGVGVTEPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKEGGG EPKSADKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK	23
M-60 (D1 sequence redesign)	CCNFSLYKNNKIILSWTLTGHSITPDYKNLYYTIMSKPEDLKLLENCKNSSKTSCTDI TSELKSTHEAYVTVLKGYSGNTFLYSCSHNFWLAIDLTVLP	24
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-62	GCEFSLYLKNNKIILSWKLKGQKTPDKYTLKYTIMSKPEDLKTVESCKNSSKTYCDI TSEFKSTHEAYVTVLEGYKGNTLYLYSCSHNFWLAIDLKVLPPGGGGSGGGGSGRSENI <u>RTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEFGNQFQKAET</u> IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQNLNDEACVIQGVGVTEPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKEGGG EPKSADKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK	25
M-62 (D1 sequence redesign)	GCEFSLYLKNNKIILSWKLKGQKTPDKYTLKYTIMSKPEDLKTVESCKNSSKTYCDI TSEFKSTHEAYVTVLEGYKGNTLYLYSCSHNFWLAIDLKVLPP	26
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-65	GCNFKLYKNGKIILSWTLVGQSTTPDKYNLYYTIMSKPEDLKLVRNCQNSSKTYCDI TSELKSTHEAYVTVLEGWKGNTLYLYSCSHNFWLAIDLEVLPPGGGGSGGGGSGRSENI <u>RTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEFGNQFQKAET</u> IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQNLNDEACVIQGVGVTEPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKEGGG EPKSADKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP	27

	IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	
M-65 (D1 sequence redesign)	GCNFKLYKKNKGIILSWTLVGQSTTPDKYNLYYTIMSKPEDLKLKVNQCNSKTYCDI TSELKSTHEAYVTVLEGGWKNTYLYSCSHNFWLAIDLEVLPP	28
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-66	CCNFKLYLENNKVILSWTLVGQSIIPDKYNLYYTIMSKPEDLKLVPNCQNKSTTYCDI TSSLKSTHEAYVTVLEGYKGNEYLYSCSHNFWLAIDLEVLPPGGGGSGGGGSGRSENI RTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEFGNQFQKAET IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQNLNDEACVIQGVGTETPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRKEGGG EPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	29
M-66 (D1 sequence redesign)	CCNFKLYLENNKVILSWTLVGQSIIPDKYNLYYTIMSKPEDLKLVPNCQNKSTTYCDI TSSLKSTHEAYVTVLEGYKGNEYLYSCSHNFWLAIDLEVLPP	30
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-67	GAKFSLYLKDGVILSWTEEGHSITPDKYNLYYTIMSKPEDLKEVPNCNKNSKTYCDI TSVLKSTHEAYVTVLEGGWKNEFLYSSSHNFWLAIDLKVLPPGGGGSGGGGSGRSENI RTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEFGNQFQKAET IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQNLNDEACVIQGVGTETPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRKEGGG EPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	31
M-67 (D1 sequence redesign)	GAKFSLYLKDGVILSWTEEGHSITPDKYNLYYTIMSKPEDLKEVPNCNKNSKTYCDI TSVLKSTHEAYVTVLEGGWKNEFLYSSSHNFWLAIDLKVLPP	32
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-82	CCNFSLYKKNKGIILSWTLVGQSVTPDKYNLYYTIMSKPEDLKEVPNCNKNSKTYCDI TSVLKSTHEAYVTVLKGYKGNTYLYSCSHNFWLAIDLKVLPPGGGGSGGGGSGRSENI RTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEFGNQFQKAET IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQNLNDEACVIQGVGTETPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRKEGGG EPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	33
M-82 (D1 sequence redesign)	CCNFSLYKKNKGIILSWTLVGQSVTPDKYNLYYTIMSKPEDLKEVPNCNKNSKTYCDI TSVLKSTHEAYVTVLKGYKGNTYLYSCSHNFWLAIDLKVLPP	34
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-83	CCKFSIYLENNKGIILSWTLEGHSIIPDKYDLYYTIMSKPEDLKLKVNQCNSKTYCDI TSELKSTHEAYVTVLKGYKGNEFLYSCSHNFWLAIDLKVLPPGGGGSGGGGSGRSENI RTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEFGNQFQKAET IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQNLNDEACVIQGVGTETPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLSRKEGGG EPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	35

M-83 (D1 sequence redesign)	CCKFSIYLENNKIILSWTLEGHSIIPDKYDLYYTIMSKPEDLKLVKNCQNSSKTYCDI TSELKSTHEAYVTVLKGYKGNEFLYSCSHNFWLAIDLKVLPP	36
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-87	GCNFSLYLKDGKIVLSWTLSGHSTTPDTYDLYYTIMSKPEDLKLVPSCNNSKTYCDI TSSLTSTHEAYVTVLKGYKGNFTLYSCSHNFWLAIDLTVLPPGGGGSGGGGSGRSENI <u>RTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLKDRHDFGFPQEEFGNQFQKAET</u> IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGTETPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKEGGG EPKSADKTHTCPPEPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	37
M-87 (D1 sequence redesign)	GCNFSLYLKDGKIVLSWTLSGHSTTPDTYDLYYTIMSKPEDLKLVPSCNNSKTYCDI TSSLTSTHEAYVTVLKGYKGNFTLYSCSHNFWLAIDLTVLPP	38
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10
I-89	CCKFSLYLENGKYILSWTPEGQSITPDYTNLYYTIMSKPEDLKEVPNCNNSKTYCDI TSSLKSTHEAYVTVLEGYKGNFTLYSCSHNFWLAIDLTVLPPGGGGSGGGGSGRSENI <u>RTAGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLKDRHDFGFPQEEFGNQFQKAET</u> IPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGTETPL MKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKEGGG EPKSADKTHTCPPEPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTIKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK	39
M-89 (D1 sequence redesign)	CCKFSLYLENGKYILSWTPEGQSITPDYTNLYYTIMSKPEDLKEVPNCNNSKTYCDI TSSLKSTHEAYVTVLEGYKGNFTLYSCSHNFWLAIDLTVLPP	40
cleavable linker	<u>GGGGSGGGGSGRSENIRTAGGS</u>	13
Protease cleavage site	SGRSENIRTA	10

[0171] All of the *de novo* redesigned masked IFN-Fc fusion proteins, like I-09, I-10, I-11 and I-31, showed decreased binding to human IFNAR2 (see **FIG. 3A-C**). Except for I-87, the other 11 *de novo* redesigned sequences showed minimal binding signal at up to 1000 nM. In the Daudi proliferation inhibition assay, all the above masked IFN-Fc fusion proteins showed greatly decreased activity. After masking moiety removal by MMP-14 incubation which cleaves the linker and get rid of the masking moiety, most of the molecules showed fully or near fully restored activity (see **FIG. 4A-C**).

Site mutation design

[0172] In this experiment, an N-terminal extended (11 amino acids) IFNAR2-D1 sequence was used as the starting sequence (SEQ ID NO: 3). A disulfide bond was introduced to the sequence to improve expression yield and purity (SEQ ID NO: 42, see **FIG. 5A**).

[0173] After the N-terminal 11 amino acid extension and a disulfide bond introduction, the protein expression yield and purity of I-140 was much higher than that of I-09 (see FIG. 5B). I-140 showed no binding signal to hIFNAR2 at up to 1000 nM (see FIG. 6A) and ~7500 folds lower *in vitro* activity in inhibiting the Daudi proliferation than that of IFN-Fc, which was greatly recovered after the masking moiety removal (see FIG. 6A&B).

[0174] Further mutations were made into the masking moiety sequence to improve the protein expression profile and enhance masking efficiency. The masking moiety surface patches sequences and IFN/IFNAR2-D1 interaction interface were optimized by computing the mutation DDG of 20 amino acids using AI trained DDG predictor neoFormer/neoDDG/neoPPI. Fourteen sequences M-148, M-150, M-151, M-152, M-153, M-155, M-156, M-157, M-158, M-159, M-160, M-161, M-162, and M-163 were obtained.

[0175] All resulting masking moiety were fused with human type 1 IFN α 2 β -Fc via a cleavable linker, respectively. The sequences of each part of the fusion proteins are shown below in Table 4-3.

Table 4-3. Sequences of each part of the masked IFN fusion proteins

Name	Sequence	SEQ ID Nos:
I-140	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPPGGGGSGG <u>GGSGRSENIRTAGG</u> SCDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEFG NQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDEACVLIQGV GVTEETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLR SKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYTQKSLSLSP GK	41
M-140 (R20C+E109C)	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPP	42
cleavable linker	GGGGSGGGSGRSENIRTAGGS	13
Protease cleavage site	SGRSENIRTA	10
I-148	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPPGGGGG <u>SSGQLLGFLTAGG</u> GGGSCDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDEACVLIQGV GVTEETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYTQKSLSLS PGK	43
M-148 (R20C+E109C)	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPP	42

cleavable linker	<u>GGSGGGGSSGQLLGFLTAGGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45
I-150	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIISKPEDLKVVKNC ANTTRSFCDLTDEWRSTQEIYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPPGGSGGGG <u>SSGQLLGFLTAGGGGS</u> CDLPQTHSLGSRRTLMMLAQMRRLSLFSCDKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTEETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHAEALHNHYTQKSLSLS PGK	46
M-150 (R20C+M47I+ H77Q+A79I+E 109C)	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIISKPEDLKVVKNC ANTTRSFCDLTDEWRSTQEIYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPP	47
cleavable linker	<u>GGSGGGGSSGQLLGFLTAGGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45
I-151	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTDEIYVTVLEGFSGNTTLFSCSHEFWLAIDMSFCPPGGSGGGG <u>SSGQLLGFLTAGGGGS</u> CDLPQTHSLGSRRTLMMLAQMRRLSLFSCDKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTEETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHAEALHNHYTQKSLSLS PGK	48
M-151 (R20C+H77D+ A79I+N99E+E 109C)	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTDEIYVTVLEGFSGNTTLFSCSHEFWLAIDMSFCPP	49
cleavable linker	<u>GGSGGGGSSGQLLGFLTAGGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45
I-152	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTDEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPPGGSGGGG <u>SSGQLLGFLTAGGGGS</u> CDLPQTHSLGSRRTLMMLAQMRRLSLFSCDKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTEETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHAEALHNHYTQKSLSLS PGK	50
M-152 (R20C+H77D+ E109C)	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTDEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPP	51
cleavable linker	<u>GGSGGGGSSGQLLGFLTAGGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45
I-153	ISYDSPDYTDESCTFKISLCNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHEFWLAIDMSFCPPGGSGGGG <u>SSGQLLGFLTAGGGGS</u> CDLPQTHSLGSRRTLMMLAQMRRLSLFSCDKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTEETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL	52

	RSKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVMHEALHNHYTQKSLSLS PGK	
M-153 (R20C+N99E+ E109C)	ISYDSPDYTDESCTFKISL ^C NFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHFWLAIDMSFCPP	53
cleavable linker	<u>GGSGGGGS</u> SGQLLGFLTA <u>GGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45
I-154	ISYDSPDYTDESCTFKISSCNGRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHFWLAIDASNCSSGGSGGGG <u>S</u> SGQLLGFLTA <u>GGGGS</u> CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVMHEALHNHYTQKSLSLS PGK	54
M-154 (L19S+R20C+F 22G+M106A+F 108N +E109C +P110S+P111S)	ISYDSPDYTDESCTFKISS ^C NGRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHFWLAIDASNCSS	55
cleavable linker	<u>GGSGGGGS</u> SGQLLGFLTA <u>GGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45
I-155	ISYDSPDYTDESCTFKISQCNNRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHFWLAIDTSYCPGGSGGGG <u>S</u> SGQLLGFLTA <u>GGGGS</u> CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVMHEALHNHYTQKSLSLS PGK	56
M-155 (L19Q+R20C+ F22N+M106T+ F108Y+E109C)	ISYDSPDYTDESCTFKISQ ^C NNRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHFWLAIDTSYCPP	57
cleavable linker	<u>GGSGGGGS</u> SGQLLGFLTA <u>GGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45
I-156	ISYDSPDYTDESCTFKISECNGRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHFWLAIDLSTCSSGGSGGGG <u>S</u> SGQLLGFLTA <u>GGGGS</u> CDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSVMHEALHNHYTQKSLSLS PGK	58

M-156 (L19E+R20C+ F22G+M106L+ F108T+E109C +P110S+P111S)	ISYDSPDYTDESCTFKISECNGRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDLSTCSS	59
cleavable linker	GGSGGGGS SGQLLGFLTA GGGGS	44
Protease cleavage site	SGQLLGFLTA	45
I-157	ISYDSPDYTDESCTFKISRCNDRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSECPPGGSGGGG SSGQLLGFLTA GGGGSCDLPQTHSLGSRRTLMLLAQMRRISLFSCCLKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTEPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPELGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	60
M-157 (L19R+R20C+ F22D+F108E+ E109C)	ISYDSPDYTDESCTFKISRCNDRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSECPP	61
cleavable linker	GGSGGGGS SGQLLGFLTA GGGGS	44
Protease cleavage site	SGQLLGFLTA	45
I-158	ISYDSPDYTDESCTFKISRCNNRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDESFCSSGGSGGGG SSGQLLGFLTA GGGGSCDLPQTHSLGSRRTLMLLAQMRRISLFSCCLKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTEPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPELGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	62
M-158 (L19R+R20C+ F22N+M106E+ E109C+P110S+ P111S)	ISYDSPDYTDESCTFKISRCNNRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDESFCSS	63
cleavable linker	GGSGGGGS SGQLLGFLTA GGGGS	44
Protease cleavage site	SGQLLGFLTA	45
I-159	ISYDSPDYTDESCTFKISLCNRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDTSFCSSGGSGGGG SSGQLLGFLTA GGGGSCDLPQTHSLGSRRTLMLLAQMRRISLFSCCLKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTEPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPELGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	64
M-159 (R20C+F22G+ M106T+E109C)	ISYDSPDYTDESCTFKISLCNRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDTSFCSS	65

+P110S+P111S)		
cleavable linker	<u>GGSGGGGSSGQLLGFLTAGGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45
I-160	ISYDSPDYTDESCTFKISSCNRNRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCA ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDLSECPGGSGGGG <u>SSGQLLGFLTAGGGGS</u> CDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	66
M-160 (L19S+R20C+F22N+M106L+F108E+E109C)	ISYDSPDYTDESCTFKISSCNRNRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCA ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDLSECPP	67
cleavable linker	<u>GGSGGGGSSGQLLGFLTAGGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45
I-161	ISYDSPDYTDESCTFKISECNGRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCA ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPPGGSGGGG <u>SSGQLLGFLTAGGGGS</u> CDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	68
M-161 (L19E+R20C+F22G+E109C)	ISYDSPDYTDESCTFKISECNGRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCA ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFCPP	69
cleavable linker	<u>GGSGGGGSSGQLLGFLTAGGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45
I-162	ISYDSPDYTDESCTFKISLCNNRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCA ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSTCSSGGSGGGG <u>SSGQLLGFLTAGGGGS</u> CDLPQTHSLGSRRTLMLLAQMRRLSLFSLCKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK	70
M-162 (R20C+F22N+F108T+E109C+P110S+P111S)	ISYDSPDYTDESCTFKISLCNNRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCA ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSTCSS	71
cleavable linker	<u>GGSGGGGSSGQLLGFLTAGGGGS</u>	44
Protease cleavage site	SGQLLGFLTA	45

I-163	ISYDSPDYTDESCTFKISLCNGRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSNCPGGSGGGG <u>SSGQLLGFLTA</u> GGGGSCDLPQTHSLGSRRTLMLLAQMRRISLFSCLKDRHDFGFPQEEF GNQFQKAETIPVLHEMIQQIFNLFSTKDSAAWDETLLDKFYTELYQQLNDEACVIQG VGVTEETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESL RSKEGGGEPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLS PGK	72
M-163 (R20C+F22G+ F108N+E109C)	ISYDSPDYTDESCTFKISLCNGRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNC ANTTRSFCDLTDEWRSTHEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSNCP	73
cleavable linker	GGSGGGGS <u>SGQLLGFLTA</u> GGGGG	44
Protease cleavage site	SGQLLGFLTA	45

[0176] All the fourteen I-140 derived site mutation designed masked IFN-Fc fusion proteins showed minimal binding to human IFNAR2 at up to 1000 nM (see FIG. 7A-C) and greatly decreased activity in inhibiting the Daudi proliferation than that of IFN-Fc, and after the masking moiety removal (by MMP-14 incubation which cleaves the masking moiety), most of the molecules showed fully restored activity (see FIG. 8A-C).

* * *

[0177] The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

[0178] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. A fusion protein comprising (a) an interferon alpha and beta receptor subunit 2 domain 1 (IFNAR2-D1) or a masking peptide having at least 55% sequence identity to the IFNAR2-D1, (b) a type I interferon (IFN), and (c) a cleavable linker between (a) and (b), wherein the peptide is capable of binding the type I IFN, and wherein the fusion protein does not include the entire sequence of IFNAR2 domain 2 (IFNAR2-D2).
2. The fusion protein of claim 1, wherein the cleavable linker is fused to the C-terminus of (a) and N-terminus of (b), or vice versa.
3. The fusion protein of claim 1 or 2, further comprising an immunoglobulin (Ig) Fc region fused to the C-terminus of the IFN or the N-terminus of the IFNAR2-D1 or the masking peptide of (a).
4. The fusion protein of any preceding claim, which does not include at least 50% of IFNAR2-D2, or does not include at least 80% of IFNAR2-D2.
5. The fusion protein of any preceding claim, wherein the IFNAR2-D1 comprises the amino acid sequence of SEQ ID NO: 8 or 3.
6. The fusion protein of any preceding claim, wherein the masking peptide comprises an IFN binding loop 1 comprising the amino acid sequence of YTIMSKPEDLK (SEQ ID NO: 74), an IFN binding loop 2 comprising the amino acid sequence of STHEAYVTVL (SEQ ID NO: 75), STQEIYVTVL (SEQ ID NO: 77) or STDEAYVTVL (SEQ ID NO: 78), and an IFN binding loop 3 comprising the amino acid sequence of SHNFWLAID (SEQ ID NO: 76) or SHEFWLAID (SEQ ID NO: 79).
7. The fusion protein of claim 6, wherein the masking peptide has at least 85% sequence identity to any of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40.

8. The fusion protein of any preceding claim, wherein the masking peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40.
9. The fusion protein of any one of claims 1-6, wherein the masking peptide has at least 85% sequence identity to SEQ ID NO: 8 or 3.
10. The fusion protein of claim 9, wherein the masking peptide comprises at least one disulfide bond.
11. The fusion protein of claim 10, wherein the masking peptide comprises substitutions at R9C and E98C relative to SEQ ID NO:8.
12. The fusion protein of claim 10, wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:12 or 42.
13. The fusion protein of claim 10, wherein the masking peptide comprises the amino acid sequence of SEQ ID NO: 42 with one or more substitutions selected from the group consisting of:
- (1) L19: S, Q, E, or R;
 - (2) F22: G, N, or D;
 - (3) I46R;
 - (4) M47: I or F;
 - (5) S48: N, Y, or Q;
 - (6) K49Q;
 - (7) H77: Y, Q or D;
 - (8) A79: D, I, V, E or N;
 - (9) V81: I or F;
 - (10) V83: F, I or K;
 - (11) N99: E, D or W;
 - (12) W101: M or F;

- (13) L102: M or I;
- (14) I104: F or W;
- (15) M106: A, T, L, or E;
- (16) F108: N, Y, T, or E;
- (17) P110: S or E;
- (18) P111: S or E; and
- (19) the combination thereof.

14. The fusion protein of claim 13, wherein the masking peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 42, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 or 73.

15. The fusion protein of any preceding claim, wherein the fusion protein does not bind to IFNAR2.

16. The fusion protein of any preceding claim, wherein the cleavable linker contains a protease cleavage site.

17. The fusion protein of claim 11, wherein the protease is selected from the group consisting of a thrombin, a neutrophil elastase, a cysteine protease, FAPa, Cathepsin B, legumain, a serine protease, such as a matriptase or a urokinase (uPA), and matrix metalloproteinases (MMPs), such as MMP1, MMP2, MMP3, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, and MMP17.

18. The fusion protein of claim 16, wherein the protease cleavage site comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 45 and 10.

19. The fusion protein of claim 11, wherein the cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 9, 13 and 44.

20. The fusion protein of claim 1, wherein the type 1 IFN is selected from the group consisting of IFN- α 1, - α 2, - α 4, - α 5, - α 6, - α 7, - α 8, - α 10, - α 13, - α 14, - α 16, - α 17 and - α 21, IFN-

β , IFN- κ , IFN- ϵ , or IFN- ω and any an amino acid sequence having at least 75% sequence identity thereof.

21. The fusion protein of claim 20, wherein the IFN comprises the amino acid sequence of SEQ ID NO: 4 or an amino acid sequence having at least 75% sequence identity to SEQ ID NO: 4.

22. The fusion protein of any one of claims 3-21, wherein the Fc is of an isotype of IgG1, IgG2, IgG3, or IgG4.

23. The fusion protein of claim 22, wherein the Fc region comprises the amino acid sequence of SEQ ID NO: 6.

24. The fusion protein of any preceding claim, which forms a dimer.

25. The fusion protein of claim 1, which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 41, 43, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, and 39.

26. A composition comprising the fusion protein of any one of claims 1-25, and a pharmaceutically acceptable carrier.

27. An isolated cell comprising one or more polynucleotide encoding the fusion protein of any one of claims 1-25.

28. A polynucleotide encoding the fusion protein of any one of claims 1-25.

29. A method of treating a cancer in a patient in need thereof, comprising administering to the patient the fusion protein of any one of claims 1-25, the composition of claim 26, the cell of claim 27, or the polynucleotide of claim 28.

30. The method of claim 29, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head

and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer.

31. The method of claim 30, wherein the cleavable linker of the fusion protein is cleaved by an enzyme within a tumor microenvironment in the patient.

32. A method for treating an autoimmune disease or inflammatory condition in a patient in need thereof, comprising administering to the patient the fusion protein of any one of claims 1-25, the composition of claim 26, the cell of claim 27, or the polynucleotide of claim 28.

33. A polypeptide comprising an interferon alpha and beta receptor subunit 2 domain 1 (IFNAR2-D1) or a peptide having at least 55% sequence identity to the IFNAR2-D1, wherein the polypeptide is capable of binding the type 1 IFN, and wherein the polypeptide does not include the entire sequence of IFNAR2 domain 2 (IFNAR2-D2).

34. The polypeptide of claim 33, which does not include at least 50% of IFNAR2-D2, or does not include at least 80% of IFNAR2-D2.

35. The polypeptide of claim 33 or 34, wherein the IFNAR2-D1 comprises the amino acid sequence of SEQ ID NO: 8 or 3.

36. The polypeptide of any one of claims 33-35, which comprises an IFN binding loop 1 comprising the amino acid sequence of YTIMSKPEDLK (SEQ ID NO: 74), an IFN binding loop 2 comprising the amino acid sequence of STHEAYVTVL (SEQ ID NO: 75), STQEIYVTVL (SEQ ID NO: 77) or STDEAYVTVL (SEQ ID NO: 78), and an IFN binding loop 3 comprising the amino acid sequence of SHNFWLAID (SEQ ID NO: 76) or SHEFWLAID (SEQ ID NO: 79).

37. The polypeptide of claim 36, which has at least 85% sequence identity to any of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40.

38. The polypeptide of any one of claims 33-37, which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40.

39. The polypeptide of any one of claims 33-35, which has at least 85% sequence identity to SEQ ID NO: 8 or 3.

40. The polypeptide of claim 33, which comprises at least one disulfide bond.

41. The polypeptide of claim 40, which comprises substitutions at R9C and E98C relative to SEQ ID NO:8.

42. The polypeptide of claim 40, which comprises the amino acid sequence of SEQ ID NO:12 or 42.

43. The polypeptide of claim 40, which comprises the amino acid sequence of SEQ ID NO: 42 with one or more substitutions selected from the group consisting of:

- (1) L19: S, Q, E, or R;
- (2) F22: G, N, or D;
- (3) I46R;
- (4) M47: I or F;
- (5) S48: N, Y, or Q;
- (6) K49Q;
- (7) H77: Y, Q or D;
- (8) A79: D, I, V, E or N;
- (9) V81: I or F;
- (10) V83: F, I or K;
- (11) N99: E, D or W;
- (12) W101: M or F;
- (13) L102: M or I;
- (14) I104: F or W;

- (15) M106: A, T, L, or E;
- (16) F108: N, Y, T, or E;
- (17) P110: S or E;
- (18) P111: S or E; and
- (19) the combination thereof.

44. The polypeptide of claim 43, which an amino acid sequence selected from the group consisting of SEQ ID NO: 42, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71 or 73.

45. A polynucleotide encoding the polypeptide of any one of claims 33-44.