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Provided are novel IFN- α binding molecules of human origin, particularly human-derived anti-IFN- α antibodies as well as IFN- α binding fragments, derivatives and variants thereof. In addition, pharmaceutical compositions, kits and methods for use in diagnosis and therapy are described.



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Human anti-IFN-alpha antibodies

Field of the invention

The present invention generally relates to novel molecules binding IFN- α of mammal, preferably human origin, particularly human monoclonal antibodies as well as fragments, derivatives and variants thereof that recognize different subtypes of IFN- α . In particular, recombinant human patient-derived anti-IFN- α antibodies and methods of making the same are provided. In addition, compositions comprising such binding molecules, antibodies and mimics thereof useful in the treatment and diagnosis of disorders are described. Furthermore, the present invention relates to autoantibodies as agents for use in immunotherapy as well as targets in the therapeutic intervention of autoimmune and autoinflammatory disorders as well as malignancies, such as systemic lupus erythematosus (SLE) and type 1 diabetes mellitus (T1DM). More specifically, the present invention relates to monoclonal autoantibodies which have been isolated from B cells derived from subjects affected with an impaired central and/or peripheral tolerance or loss of self-tolerance typically due to a mutation in a gene involved in immune regulation.

Background of the invention

Inappropriate responses of the immune system may cause stressful symptoms to the involved organism. Exaggerated immune answers to foreign substances or physical states which usually do not have a significant effect on the health of an animal or human may lead to allergies with symptoms ranging from mild reactions, such as skin irritations to life-threatening situations such as an anaphylactic shock or various types of vasculitis. Immune answers to endogenous antigens may cause autoimmune disorders such as systemic lupus erythematosus (SLE), type 1 or insulin dependent diabetes mellitus (T1DM or IDDM) and different forms of arthritis.

Immune responses occur in a coordinated manner, involving several cells and requiring communication by signaling molecules such as cytokines between the cells involved. This communication may be influenced or inhibited by, *e.g.*, interception of the signals or block of the respective receptors.

Cytokines are secreted soluble proteins, peptides and glycoproteins acting as humoral regulators at nano- to picomolar concentrations behaving like classical hormones in that they act at a systemic level and which, either under normal or pathological conditions, modulate the

functional activities of individual cells and tissues. Cytokines differ from hormones in that they are not produced by specialized cells organized in specialized glands, *i.e.* there is not a single organ or cellular source for these mediators as they are expressed by virtually all cells involved in innate and adaptive immunity such as epithelial cells, macrophages, dendritic cells (DC),
5 natural killer (NK) cells and especially by T cells, prominent among which are T helper (Th) lymphocytes.

Depending on their respective functions, cytokines may be classified into three functional categories: regulating innate immune responses, regulating adaptive immune responses and
10 stimulating hematopoiesis. Due to their pleiotropic activities within said three categories, *e.g.*, concerning cell activation, proliferation, differentiation, recruitment, or other physiological responses, *e.g.*, secretion of proteins characteristic for inflammation by target cells, disturbances of the cell signaling mediated by aberrantly regulated cytokine production have been found as a cause of many disorders associated with defective immune response, for
15 example, inflammation and cancer.

Interferons (IFN), consisting from three known protein families, type I, II and III interferons constitute one of the most important classes of cytokines. All human type I interferons bind to a cell surface receptor (IFN alpha receptor, IFNAR) consisting of two transmembrane proteins,
20 IFNAR-1 and IFNAR-2 leading to JAK-STAT activation, the formation of ISGF3 and subsequent onset of gene expression (Platanias and Fish, *Exp. Hematol.* (1999), 1583-1592). The composition, receptors and signaling pathways of type I IFNs have been reviewed, *e.g.*, in Stark et al., *Annu. Rev. Biochem.* (1998), 227-64; Pestka S., *Biopolymers* (2000), 254-87. Type I interferons build a structurally related family (IFN- α (alpha), IFN- β (beta), IFN- κ (kappa),
25 IFN- δ (delta), IFN- ϵ (epsilon), IFN- τ (tau), IFN- ω (omega), and IFN- ζ (zeta)), of which IFN- δ and IFN- τ do not occur in humans. Human type I interferon (IFN) genes are clustered on human chromosome 9p21 and the mouse genes are located in the region of conserved synteny on mouse chromosome 4. So far, 14 IFN- α genes and 3 pseudogenes have been identified in the mouse. In humans 13 IFN- α (or IFNA) genes (IFNA1, IFNA2, IFNA4, IFNA5, IFNA6,
30 IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17 and IFNA21) and 1 pseudogene have been identified, wherein two human IFN- α genes (IFNA1/IFN- α 1 and IFNA13/IFN- α 13) encode for identical proteins (van Pesch *et al.*, *J. Virol.* (2004), 8219-8228).

IFN- γ is the sole Type II interferon. It is mainly involved in the induction of antimicrobial and antitumor mechanisms by macrophage stimulation. The IFN- γ receptor (IFNGR) is a heterodimeric receptor comprised of two ligand-binding IFNGR1 chains associated with two signal-transducing IFNGR2 chains (Schroder et al., *J. Leukoc. Biol.* 75 (2004), 163-189; Bach et al., *Annu. Rev. Immunol.* 15 (1997), 563-591). Type III interferons consist of three subtypes and are also termed IFN λ (IFN λ 1 or IL-29, IFN λ 2 or IL-28A and IFN λ 3 or IL-28B) and have antiviral, antitumor, and immunoregulatory activity. The IFN- λ receptor is also a heterodimeric complex consisting of a unique ligand-binding chain, IFN- λ R1 (also designated IL-28R α), and an accessory chain IL-10R2, which is shared with receptors for IL-10-related cytokines (Li et al., *J. Leukoc. Biol.* 86 (2009), 23-32)

Type I interferons are pleiotropic cytokines with antiviral, antitumor and immunoregulatory functions. Depending on context, they can be anti-inflammatory and tissue protective or proinflammatory and promote autoimmunity. IFN- β 1a or 1b is used for the treatment of multiple sclerosis and IFN- α 2b therapy for many cancers (melanoma, hemat. malign.). Elevated IFN- α activity has been frequently detected in the sera of patients with systemic lupus erythematosus (SLE) indicating that IFN- α plays a central role in SLE development (Rönnblom and Alm, *J. Exp. Med.* (2001), F59-F63; Crow MK, *Arthritis Rheum.* (2003), 2396-2401; Crow MK., *Curr Top Microbiol. Immunol.* (2007), 359-386; Crow MK. *Rheum Dis Clin North Am.* (2010), 173-186).

On the other side, a specific expression pattern of interferon-dependent genes (termed the "interferon signature") is displayed in the leukocytes of patients with various autoimmune disorders such as SLE, T1DM, Sjögren's syndrome, Dermatomyositis, Multiple Sclerosis (MS), Psoriasis and rheumatic arthritis (RA) patients. In addition, development of inflammatory arthritis, MS and T1DM has been repeatedly observed during IFN- α therapy indicating that IFN- α at least promotes those diseases (Crow MK., *Arthritis Res Ther.* (2010), Suppl 1:S5). Further data suggest an involvement of IFN- α in myositis, systemic scleroderma, chronic psoriasis (Higgs et al., *Eur Musc Rev* (2012), 22-28; Bissonnette et al., *J Am Acad Dermatol* (2009), 427-436; Greenberg SA, *Arth Res Ther* (2010): S4;) and autoimmune thyroiditis (Prummel and Laurberg, *Thyroid* (2003), 547-551).

Accordingly, wherein depending on the context situation, treatment with Type I interferons, as in RA, MS and different leukemia or treatment with antibodies neutralizing Type I interferons,

e.g., in SLE may be indicated, the very same treatment may be detrimental to the patient by promoting autoimmunity, inflammation and interferon-treatment related toxicities or even leading to the development of diseases such as MS and T1DM. One factor in these different effects may arise from the fact that, despite that different IFN subtypes activate the same cell surface receptor complex, they mediate variable responses which are also cell type dependent (van Pesch et al., *J Virol* 78 (2004), 8219-8228; Antonelli G., *New Microbiol.* 31 (2008), 305-318; Gibbert et al., *PLoS Pathog.* 8 (2012), e1002868). The treatment therefore, should preferably be performed in a selective manner, wherein only particular IFN- α subtypes are administered to a patient, or particular IFN- α subtypes are neutralized, which are associated with a given pathological condition. Regarding IFN- α treatment such selectivity may be obtained by usage of highly purified IFN- α preparations for therapeutic purposes (Antonelli G., *New Microbiol.* 31 (2008), 305-318). However, regarding the use of IFN- α antibodies it is more difficult to obtain selectivity in respect of specific IFN- α subtypes, because there is a high degree of homology at the amino acid level with 80–95% homology between the IFN- α subtypes and 50% homology with IFN- β . It would be desirable therefore to provide a pool of IFN- α antibodies tolerable in humans of varying specificity towards all, selected or particular human IFNA subtypes, which might be selectively used depending on the therapeutic and/or diagnostic indication.

First attempts to achieve this aim have already been made. For example, patent application US 2009/0214565 A1 describes isolation of several mouse anti-human IFN- α antibodies which neutralize between three and thirteen different subtypes of human IFN- α and US patent No. 7,087,726 B2 describes a murine anti-human IFN- α antibody and its humanized version which recognize seven subtypes of human IFN- α . However, most if not all anti-IFN-antibodies provided so far are of murine origin and thus prone to an adverse reaction in humans.

Due to immunological responses to foreign antibodies, as mouse antibodies in humans (HAMA-response; Schroff et al., *Cancer Res.* 45 (1985), 879-885; Shawler et al., *J. Immunol.* 135 (1985), 1530-1535), mostly humanized versions of antibodies are used in present therapeutic approaches (Chan and Carter, *Nature Reviews Immunology* 10 (2010), 301-316; Nelson et al., *Nature Reviews Drug Discovery* 9 (2010), 767-774). One approach to gain such antibodies was to transplant the complementarity determining regions (CDR) into a completely human framework, a process known as antibody humanization (Jones et al., *Nature* 321 (1986), 522–525). This approach is often complicated by the fact that mouse CDR do not easily transfer

to a human variable domain framework, resulting in lower affinity of the humanized antibody over their parental murine antibody. Therefore, additional and elaborate mutagenesis experiments are often required, to increase the affinity of the so engineered antibodies. Another approach for achieving humanized antibodies is to immunize mice which have had their innate
5 antibody genes replaced with human antibody genes and to isolate the antibodies produced by these animals. However, this method still requires immunization with an antigen, which is not possible with all antigens because of the toxicity of some of them. Furthermore, this method is limited to the production of transgenic mice of a specific strain.

10 Another method is to use libraries of human antibodies, such as phage display, as described, for example, for the generation of IL-13 specific antibodies in international application WO 2005/007699. Here, bacteriophages are engineered to display human scFv/Fab fragments on their surface by inserting a human antibody gene into the phage population. Unfortunately, there is a number of disadvantages of this method as well, including size limitation of the protein
15 sequence for polyvalent display, the requirement of secretion of the proteins, *i.e.* antibody scFv/Fab fragments, from bacteria, the size limits of the library, limited number of possible antibodies produced and tested, a reduced proportion of antibodies with somatic hypermutations produced by natural immunization and that all phage-encoded proteins are fusion proteins, which may limit the activity or accessibility for the binding of some proteins.
20 Similarly, European patent application EP 0 616 640 A1 describes the production of auto-antibodies from antibody segment repertoires displayed on phage. Phage libraries are generated from unimmunized humans in this respect (see, *e.g.*, Example 1; page 16, lines 43-51; Example 2, at page 17, paragraph [0158], lines 57-58). However, also the methods described in this patent application suffer from above mentioned general disadvantages of antibodies generated from
25 phage libraries, in comparison to antibodies produced and matured in a mammalian, *i.e.* human body.

The same applies to the most prominent anti-IFN α monoclonal antibody Sifalimumab (formerly, MEDI-545) that binds to and specifically neutralizes most IFN- α subtypes,
30 preventing signaling through the type I IFN receptor. Sifalimumab is said to be a "human" anti-IFN α monoclonal antibody but actually has been derived from humanized mice, *i.e.* from the former company Medarex' UltiMab platform which is based on transgenic mice in which the largest fraction of the human germline repertoire was introduced.

Nevertheless, though the amino acid sequences of the antibodies derived from humanized mice are of human origin these antibodies are artificial and not truly human as they have not undergone immunization, recombination, selection and affinity maturation in a human being for which reason there is still the risk of their being immunogenic and less effective, in particular compared to human-derived antibodies.

In view of the above, there is still a need for additional and new compounds like binding molecules of high specificity for particular human IFN- α subtypes, specific for a selected range or for all IFN- α subtypes which are tolerable in humans either for monotherapy or combinatorial approaches.

The solution to this problem is provided by the embodiments of the present invention as characterized in the claims and disclosed in the description and illustrated in the Examples and Figures further below.

Summary of the invention

The present invention relates to IFN- α specific human monoclonal antibodies and IFN- α binding fragments thereof. In particular, human monoclonal anti-IFN- α antibodies are provided with a selective binding profile towards IFN- α subtypes and displaying binding and neutralizing activity as shown in the Examples and the Figures. Due to their neutralizing property, the antibodies of the present invention have therapeutic, prognostic and diagnostic utility, which make them in particular valuable for applications in relationship with diverse autoimmune or autoinflammatory disorders and conditions associated with/ involving IFN- α activity in initiation and/or maintenance of undesired immune responses, such as systemic lupus erythematosus (SLE), various forms of arthritis including but not limited to rheumatoid arthritis (RA), type 1 or insulin dependent diabetes mellitus (T1DM or IDDM), Sjögren's syndrome, Dermatomyositis, Multiple Sclerosis (MS), psoriasis, chronic psoriasis, myositis, systemic scleroderma, autoimmune thyroiditis and cancer including leukemia (ALL; Einav et al., Oncogene 24 (2005), 6367–6375); see as well the section "Background of the invention" above, describing the IFN- α subtypes and their possible involvement disorders and implications towards possible indications of the antibodies of the present invention in related therapeutic, diagnostic and/or prognostic applications.

The antibodies of the present invention are preferably isolated from mammals, in particular humans, which are affected with an impaired central and/or peripheral tolerance or loss of self-tolerance which may be due to or associated with a disrupted or deregulated genesis of self-tolerance, preferably caused by a monogenic autoimmune disorder. Examples of mammals
5 which provide a particularly suitable source for autoantibodies in accordance with the present invention are mammals, *e.g.*, humans having a disorder associated with a mutation in the *AIRE* (Autoimmune Regulator) gene such as Autoimmune polyendocrinopathy syndrome type 1 (APS1) (Peterson *et al.*, *Nat. Rev. Immunol.* 8 (2008), 948-957), Autoimmune polyendocrinopathy syndrome type 2 (APS2) (Baker *et al.*, *J. Clin. Endocrinol. Metab.* 95
10 (2010), E263-E270) and immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) (Powell *et al.*, *J. Pediatr.* 100 (1982), 731-737; Ochs *et al.*, *Immunol. Rev.* 203 (2005), 156-164). Preferably, the patients from whom the antibodies were isolated are APS1 patients characterized by being symptom-free for Lupus erythematoses (SLE) and displaying seroreactivity against dsDNA and at least one at least one of the human IFN- α
15 subtypes.

In particular, in accordance with the present invention for the first time human and human patient derived anti-IFN- α antibodies are provided with different IFN- α binding profiles and IFN- α neutralizing activity, thereby alone or in combination covering substantially all IFN- α
20 subtypes.

Therefore, in one aspect the present invention generally relates to high affinity neutralizing monoclonal antibodies to several IFN- α subtypes. In a further aspect, the present invention relates to human monoclonal antibodies (mAbs, or MABs) against several IFN- α subtypes, *i.e.*
25 at least one of the human IFN- α (IFNA) subtypes IFN- α 1/13 (IFNA1/13; IFNA1b), IFN- α 2 (IFNA2), IFN- α 4 (IFNA4), IFN- α 5 (IFNA5), IFN- α 6 (IFNA6), IFN- α 8 (IFNA8), IFN- α 10 (IFNA10), IFN- α 14 (IFNA14) or IFN- α 21 (IFNA21), described in detail below, which are considered to be safe and effective therapeutics for disorders in which those cytokines are involved. In one embodiment, the IFN- α binding molecule of the present invention does not
30 bind and/or neutralize to any significant extent IFN- β (beta interferon, IFNB), IFN- γ (gamma interferon, IFNG) or IFN- ω (interferon omega, IFNW).

In another embodiment, in accordance with the present invention anti-IFN- α antibodies are provided, which in addition bind to and neutralize the activity of IFN- ω , respectively, hitherto

described to be unrelated to IFN- α in its antigenic properties, as it usually does not cross-react with antisera or monoclonal antibodies in immunoassays or antiviral neutralization bioassays. Thus, in one aspect the present invention relates to novel IFN binding molecules, preferably human-derived monoclonal antibodies as well as fragments and biotechnological derivatives thereof which are capable of binding to/and or neutralizing the activity of at least one human IFN- α subtype and of human IFN- ω . Preferably, the binding and neutralizing activity, respectively, of the IFN-binding molecule is essentially the same or at least in the same order of magnitude for IFN- α subtype(s) and IFN- ω .

Naturally, the present invention extends to nucleic acids, in particular cDNA encoding at least one variable, constant and/or complementarity determining region of the antibodies of the present invention, vectors comprising such nucleic acids, antibody producing cell lines and recombinant cells. The present invention further relates to pharmaceutical compositions, diagnostic assays and kits that comprise the binding molecules or peptides recognized by the antibodies isolated in accordance with the present invention and to therapeutic methods based thereon.

In addition, the present invention relates to a process for the manufacture of a human-derived anti-IFNA and anti-IFNA/IFNW monoclonal antibody, respectively, or an IFN-binding fragment or biotechnological derivative thereof or of a composition comprising the anti-IFNA and anti-IFNA/IFNW monoclonal antibody, respectively, or an IFN-binding fragment or biotechnological derivative thereof, which manufacture comprises the step of preparation of the antibody, IFN-binding fragment or biotechnological derivative thereof by expression in a recombinant host organism of transforming DNA encoding the antibody, IFN-binding fragment or biotechnological derivative thereof. In one embodiment, the composition is a pharmaceutical composition, wherein the step of preparation of the antibody, IFN-binding fragment or biotechnological derivative thereof is followed by admixing the antibody, IFN-binding fragment or biotechnological derivative thereof with a pharmaceutically acceptable carrier in the manufacture of a pharmaceutical composition.

While the invention is illustrated and described by way of reference to the human-derived antibody originally obtained in the experiments performed in accordance with the present invention and described in the Examples it is to be understood that the antibody or antibody fragment of the present invention include synthetic and biotechnological derivatives of an

antibody which means any engineered antibody or antibody-like IFN binding molecule, synthesized by chemical or recombinant techniques, which retains one or more of the functional properties of the subject antibody, in particular its neutralizing activity towards IFNA and IFNW. Thus, while the present invention may be described for the sake of conciseness by way of reference to an antibody, unless stated otherwise synthetic and biotechnological derivatives thereof as well as equivalent IFN binding molecules are meant and included with the meaning of the term antibody.

Further embodiments of the present invention will be apparent from the description and Examples that follow.

Brief description of the drawings

Fig. 1: Amino acid sequences of the variable region, *i.e.* heavy chain and kappa/lambda light chain (VH, VL) of anti-IFN- α specific human antibodies of the present invention. IgG1, kappa, anti-IFN- α specific antibodies **A:** 5D1; **B:** 13B11; **C:** 19D11; **D:** 25C3; **E:** 26B9; **F:** 31B4, **G:** 8H1, **H:** 12H5 and **I:** 50E11. Framework (FR) and complementarity determining regions (CDRs) are indicated with the CDRs being underlined. Italic amino acids indicate sequences which have not been sequenced but *obtained from database*. Due to the cloning strategy the amino acid sequence at the N-terminus of the heavy chain and light chain may potentially contain primer-induced alterations in FR1, which however do not substantially affect the biological activity of the antibody.

Fig. 2: Cross-competition - Epitope mapping. Differential binding of exemplary anti-IFNA MABs of the present invention to distinct binding sites was investigated in cross-competition experiments on **A:** IFNA2, **B:** IFNA4 and **C:** IFNA14. As an IFN- α non-binding control (hIgG1) a human antibody binding to an unrelated antigen has been used.

Fig. 3: EC50 ELISA determination. EC50 binding of hMABs 5D1, 13B11, 25C3 and 26B9 to **A:** IFNA2, **B:** IFNA4 and **C:** IFNA14. EC50 binding to IFNA2/-4/-14 of hMABs **D:** 19D11 and **E:** 31B4.

Fig. 4: Correlating the presence of known disease-associated and protective autoantibodies in APS1 patients indicates that IFN- α autoantibodies prevent the onset of lupus erythematosus in APS1 patients (columns dsDNA to IFN α 14). Target: Coding number of the particular APS1-patient examined in the correlation study. Anti-dsDNA antibodies are highly specific for SLE and used in the diagnosis of the disease. No APS1 patients have lupus despite the frequent presence of anti-dsDNA antibodies as assessed by ProtoArray analysis (Life Technologies). APS1 patients display pronounced seroreactivity against several IFN- α subtypes which are clinically-relevant drug targets involved in many lupus-implicated molecular mechanisms. Circles indicate the presence, empty cells the absence of particular antibodies. Patients 2, 4, 13 and 21 (black arrows) are T1DMs. Patients 10, 14, 16, 17 and 18 (white arrows) have characteristics of being T1DM but are not. The results suggest differential neutralizing activity in serum of APS1 patients suffering from T1DM and these who are not. As can be inferred from Fig. 31 and 32 this difference observed here seems to be due to differences in titers of anti-IFN antibodies in both patient classes, with much higher antibody titers in APS1 patients not suffering from T1DM, rather than differences in the neutralizing activity of individual anti-IFN antibodies.

Fig. 5: Human-derived anti-IFN- α monoclonal antibodies neutralize rhIFNA-mediated STAT1 activation in HEK 293T cells. HEK 293T cells were either left untreated (-) or stimulated (+) with recombinant human rhIFNAs or IFN-Gaussia luciferase fusion proteins (g1 IFNs), in the absence of antibodies or in the presence of human-derived IFNA monoclonal antibodies or a human control IgG as indicated (Ctrl). Cell lysates were subjected to SDS-PAGE and phosphorylated STAT1 levels (pSTAT1) were visualized in Western blots. Total STAT1 levels or tubulin levels serve as loading control. Antibody concentration: 5 μ g/ml. rhIFNA concentration: 10 ng/ml (rhIFNA1), 2 ng/ml (all other rhIFNs); g1 IFNA-containing supernatants of 293T cells transiently expressing IFN-Gaussia luciferase fusion proteins were used at their respective EC80 dilutions. A1: Control: detection of total and phosphorylated STAT1 (pSTAT1) by Western Blots (WB) in 293T cells after 100 ng/ml IFNA2/-4/-14 stimulation. 293T-cells stimulation by IFNA2/-4/-14. (i): phosphorylated STAT1, (ii): total STAT1, (iii): Tubulin loading control. Time: indicates treatment duration of the cells with the respective IFN. After stimulation

phosphorylated STAT1 can be observed for all three IFNA subtypes. Positions of respective molecular weight (kDa) standard bands are indicated on the left of the blot for comparison. **A2:** Control: detection of total and phosphorylated STAT1 (pSTAT1) by Western Blots (WB) in 293T cells after stimulation with different doses of rhIFNA1, rhIFNA2 and rhIFNA16. **B:** IFNA1, IFNA2, IFNA4, g1 IFNA5 and IFNA6 stimulation. All exemplary antibodies except 5D1 neutralize IFNA1 efficiently, and all exemplary antibodies neutralize IFNA2, IFNA4 and g1 IFNA5. Exemplary antibodies 25C3, 5D1 and 13B11 display weaker neutralization of IFNA6. **C:** IFNA7, g1 IFNA8, IFNA10, IFNA14 and IFNA16 stimulation. All exemplary antibodies neutralize IFNA7. All exemplary antibodies except 25C3 and 13B11 neutralize g1 IFNA8 efficiently. IFNA10 is efficiently neutralized by all exemplary antibodies except 25C3, while IFNA16 is efficiently neutralized only by exemplary antibodies 19D11, 5D1 and 13B11. **D:** IFNA17, IFNA21, IFNW, IFNB and g1 IFNG stimulation. All exemplary antibodies neutralize IFNA17, and all exemplary antibodies except 13B11 efficiently neutralize IFNA21. IFNW is apparently only efficiently neutralized by exemplary antibodies 26B9 and 31B4. None of the exemplary antibodies neutralizes IFNB or g1 IFNG.

Fig. 6: Schematic representation of the Firefly-Luciferase reporter construct and the Renilla-Luciferase construct used as internal normalization control. TRE-Transcription response element; CMV – cytomegalovirus.

Fig. 7: Human-derived anti-IFN- α monoclonal antibodies neutralize rhIFNA-induced ISRE-Luciferase reporter gene activation in HEK 293T cells. **A** and **B:** Testing of exemplary anti-IFN- α antibodies 19D11, 25C3, 26B9 and 31B4. HEK 293T cells transiently expressing ISRE dual luciferase reporter constructs were either left untreated (-) or stimulated with rhIFNs (+), in the absence of antibodies or in the presence of human-derived IFNA monoclonal antibodies or a human control IgG as indicated. rhIFN concentration: 2 ng/ml; antibody concentration: 5 μ g/ml, **C** and **D:** Testing of exemplary anti-IFN- α antibodies 5D1 and 13B11. HEK 293T cells transiently expressing ISRE dual luciferase reporter constructs were treated as in (**A/B**) and ISRE reporter activity was analyzed after 24 hours. rhIFNA concentration: 1 ng/ml; antibody concentration: 5 μ g/ml.) **A/C:** measurements of the relative luciferase units, **B/D:** calculation of the expressional fold change. **E:**

Neutralization of rhIFNA1, A2, A4, A5, A6, A7, A8, A10, A14, A16, A17, A21, rhIFNW and rhIFNB by exemplary human-derived monoclonal antibodies of the present invention 8H1 and 12H5. Exemplary antibody 8H1 fully neutralizes IFNW, together with IFNA1, A4, A5, A6, A7, A10, A16, A17 and A21, while displaying slightly weaker neutralization of IFNA2, A8 and A14. Exemplary antibody 12H5 neutralizes all IFNA subtypes and not IFNW. Neither exemplary antibody 8H1 nor 12H5 neutralizes IFNB. HEK 293T MSR were treated as in **A** and ISRE reporter activity was analyzed after 24 hours. rhIFN concentration: 10 ng/ml (IFNA1), 1.3 ng/ml (IFNA16), 4 ng/ml (IFNA21), 1 ng/ml (IFNB) and 2 ng/ml (all other IFNs). Antibody concentration: 5 µg/ml.

Fig. 8: IC 50 analysis of exemplary human-derived IFNA mAb 26B9 by ISRE-Luciferase reporter neutralization assay. IC 50 neutralization graphs by exemplary antibody 26B9 of **A:** IFNA2; **B:** IFNA4; **C:** IFNA5; **D:** IFNA8; **E:** IFNA14. IC 50 analysis results of a confirmative round of neutralization assays for the exemplary antibody 26B9 are shown of **F:** IFNA1, **G:** IFNA2, **H:** IFNA4, **I:** IFNA5, **J:** IFNA6, **K:** IFNA7, **L:** IFNA8, **M:** IFNA10, **N:** IFNA14, **O:** IFNA16, **P:** IFNA17, **Q:** IFNA21 and **R:** IFNW. IC 50 data is summarized in Table 4. RLU at the Y-axis = relative light units, as in foregoing figures.

Fig. 9: IC50 analysis of exemplary human-derived anti-IFN-α mAb 25C3 by ISRE Luciferase reporter neutralization assay. Assay performed as described in Fig.8. IC50 neutralization graphs by exemplary antibody 26B9 of: **A:** IFNA2; **B:** IFNA4; **C:** IFNA5; **D:** IFNA8 and **E:** IFNA14.

Fig. 10: IC50 analysis of exemplary human-derived anti-IFN-α mAb 19D11 by ISRE Luciferase reporter neutralization assay. Assay performed as described in Fig.8. IC50 graphs of neutralization by exemplary antibody 19D11 of: **A:** IFNA2; **B:** IFNA4; **C:** IFNA5; **D:** IFNA8; **E:** IFNA14. IC 50 neutralization graphs of a confirmative experimental round by exemplary antibody 19D11 of **F:** IFNA1, **G:** IFNA2, **H:** IFNA4, **I:** IFNA5, **J:** IFNA6, **K:** IFNA7, **L:** IFNA8, **M:** IFNA10, **N:** IFNA14, **O:** IFNA16, **P:** IFNA17 and **Q:** IFNA21. IC 50 data is summarized in Table 4.

- Fig. 11:** **A:** Determination and comparison of binding of exemplary MABs 19D11, 25C3, 26B9, 5D1 and 13B11 of the present invention to IFNA1 and IFNA2 (ImmunoTools) by ELISA. Exemplary anti-IFNA- α antibody 5D1 does not cross-react with IFNA1. **B:** Determination and comparison of binding of exemplary MABs 5D1, 13B11, 19D11, 25C3, 26B9 and 31B4 of the present invention to IFNA8 and IFNA14 (IFN-Gaussia luciferase fusion proteins) by LIPS. Exemplary MAB 13B11 does not have cross-reactivity with IFNA8 (g1IFNA8). **C:** Determination and comparison of binding of exemplary MABs 5D1, 13B11 19D11, 25C3, 26B9 and 31B4 of the present invention to IFNA5, IFNA6, IFNA8, IFNA21 (all from PBL) and IFNA14 (ATGen). Exemplary anti-IFNA- α antibody 13B1 does not cross-react with IFNA8 and IFNA21. Antibody 19D11 cross-reacts with a lower affinity with IFNA21 than with the other IFNA subtypes. MABs were tested at 1 μ g/ml in (C).
- Fig. 12:** LIPS assay – determination of binding of the antibodies of the present invention to different IFNA subtypes (IFN-Gaussia luciferase fusion proteins). Binding of the exemplary antibodies of the present invention to **A:** IFNA5; **B:** IFNA6 and **C:** IFNA8. Exemplary anti-IFN- α antibody 13B11 shows no substantial cross-reactivity with IFNA8. As an IFN- α non-binding control (hIgG1) an human antibody binding to an unrelated antigen was used.
- Fig. 13:** LIPS assay – determination of binding characteristics of the exemplary antibodies 5D1, 13B11, 19D11, 25C3, 26B9 and 31B4 of the present invention towards IFNA1, IFNA2, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14, IFNA16 and IFNA 21 (IFN-Gaussia luciferase fusion proteins). As an IFN- α non-binding control (hIgG1) a human antibody binding to an unrelated antigen was used. All antibodies were tested at 0.5 μ g/ml. All IFN- α subtypes used in Figs. 12 and 13 are IFN- α -Gaussia luciferase fusion proteins (g1IFNAs).
- Fig. 14:** Ear inflammation assay – test of the proinflammatory effect of human IFN subtypes in mice. **A:** Exemplary 6-day experimental timeline. **B:** CytoEar ear thickness measurements calculated as fold change relative to day 0 measurements than normalized to relevant PBS controls, for each cohort. **B1:** Overview of the effect of all normalized measurements. **B2:** Effects of the IFNa2a and IFNa2b injections.

B3: Effects of the IFNa4 and IFNa14 injections. All four human IFNA subtypes tested were able to significantly induce ear swelling following ID. All ears were markedly thicker than PBS treated ears. IFNa14 was the most potent proinflammatory agent. Mean +/- SEM, I1-3 or ID = intradermal cytokine injections, M = Measurements – ear thickness and animal weight, S = Sacrifice of the animals; short arrows – cytokine injections; long arrows – exemplary days of anti-IFN- α antibody injections.

Fig. 15: Ear inflammation assay – test of the proinflammatory effect of human IFN subtypes in mice. CytoEar ear thickness measurements are shown as absolute values (mm) for each cohort. **A:** Overview of the effect of all measurements. **B:** Effects of the IFNa2a and IFNa2b injections. **C:** Effects of the IFNa4 and IFNa14 injections. All indications as in Fig. 14.

Fig. 16: Ear inflammation assay – test of the proinflammatory effect of human IFN subtypes in mice. CytoEar ear thickness measurements are shown fold change from Day 0 for each cohort. The thickness on Day 0 has been set as 1. **A:** Overview of the effect of all measurements. **B:** Effects of the IFNa2a and IFNa2b injections. **C:** Effects of the IFNa4 and IFNa14 injections. All indications as in Fig. 14.

Fig. 17: Summary of the ear inflammation assay in respect of human IFN- α subtype capabilities for induction of ear inflammation. P values obtained by 2-way ANOVA testing, ns (not significant) = $P > 0.05$; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P < 0.001$, **** = $p < 0.0001$. All ears were markedly thicker than PBS treated ears; this was significant in all groups after the 2nd ID, from Day 3 until the end of the experiment.

Fig. 18: LIPS assay – determination and comparison of binding characteristics of the exemplary anti-IFN- α antibodies 5D1, 13B11, 19D11, 25C3, 26B9 and 31B4 of the present invention towards IFN γ (IFNG), IFN- β 1 (IFNB1), IFN ϵ (IFNE), IFN- ω (IFNW), three IFN λ (IL28A, IL28B and IL29) and IFNA10 (all IFN-Gaussia luciferase fusion proteins). **A:** Binding of the antibodies towards the different IFN-types. **B:** Reciprocal diagram, binding of the IFNs by the antibodies. Exemplary antibodies 26B9 and 31B4 show binding of IFN- ω (IFNW) besides their affinity

towards IFN α 10. No substantial binding of the exemplary antibodies of IFN- β 1 (IFNB1), IFN ϵ (IFNE), the three IFN λ and IFN γ (IFNG) could be observed.

Fig. 19: Cross-reactivity of MABs towards mouse IFNAs (ELISA). hIgG1= antibody of non-IFNA related specificity (negative control). **A:** Test of cross-reactivity of the exemplary antibodies 5D1, 13B11, 19D11, 25C3, 26B9 and 31B4 against human and murine IFNA2, IFNA4 and IFNA14 in an ELISA assay. Except antibody 25C3 showing cross-reactivity towards murine IFNA2, the other antibodies show no or only residual binding specificity towards the murine IFNA subtypes tested. **B:** Cross-reactivity of MABs on mouse IFNAs (LIPS-assay). Antibodies 5D1 and 19D11 show cross-reactivity towards murine g1mIFNA1, wherein the other antibodies only show reactivity below the level observed for the negative control. None of the antibodies shows cross-reactivity towards murine IFN- α subtype g1mIFNA9.

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Fig. 20: IC 50 analysis of exemplary human-derived anti-IFN- α mAb 8H1 by ISRE Luciferase reporter neutralization assay. IC 50 neutralization graphs of exemplary antibody 8H1. **A:** IFNA1, **B:** IFNA2, **C:** IFNA4, **D:** IFNA5, **E:** IFNA6, **F:** IFNA7, **G:** IFNA8, **H:** IFNA10, **I:** IFNA14, **J:** IFNA16, **K:** IFNA17, **L:** IFNA21 and **M:** IFNW. IC 50 data is summarized in Table 4.

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Fig. 21: IC 50 analysis of exemplary human-derived anti-IFN- α mAb 12H5 by ISRE Luciferase reporter neutralization assay. IC 50 neutralization graphs of exemplary antibody 12H5. **A:** IFNA1, **B:** IFNA2, **C:** IFNA4, **D:** IFNA5, **E:** IFNA6, **F:** IFNA7, **G:** IFNA8, **H:** IFNA10, **I:** IFNA14, **J:** IFNA16, **K:** IFNA17 and **L:** IFNA21. IC 50 data is summarized in Table 4.

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Fig. 22: IC 50 analysis of exemplary human-derived anti-IFN- α mAb 50E11 by ISRE Luciferase reporter neutralization assay. IC 50 neutralization graphs of exemplary antibody 50E11. **A:** IFNA1, **B:** IFNA2, **C:** IFNA4, **D:** IFNA5, **E:** IFNA6, **F:** IFNA7, **G:** IFNA8, **H:** IFNA10, **I:** IFNA14, **J:** IFNA16, **K:** IFNA17, **L:** IFNA21 and **M:** IFNW. IC 50 data is summarized in Table 4.

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Fig. 23: Human-derived anti-IFN- α monoclonal antibodies neutralize binding of IFN-Gaussia luciferase fusion proteins to HEK 293T MSR cells endogenously expressing IFN receptors in the luminescent cellular binding assay. Cells were incubated with supernatants of HEK 293T cells expressing Interferon-Gaussia luciferase fusion proteins in the absence of inhibitors (-) or in the presence of competitive inhibitors as indicated. **A:** Schematic representation of the luciferase-based chemiluminescent cellular binding assay. A ligand of interest (3) is fused to Gaussia luciferase (4). The fusion protein is bound to cells (1) expressing receptors for the ligand of interest (2). After removal of unbound fusion proteins a luciferase substrate is added (5) and light emission is recorded (6). The light output is proportional to the amount of bound fusion protein. Anti-ligand antibodies that compete with the receptors for binding of the ligand of interest (7) lead to a decrease in bound ligand and reduced light output. **B:** Control: Human IFNA-Gaussia luciferase fusion proteins bind specifically to HEK 293T MSR cells. Binding of IFNA5-Gaussia luciferase fusion proteins (g1 IFNA5) to HEK 293T MSR cells is inhibited by unlabeled rhIFNA2 (3 $\mu\text{g/ml}$) and by exemplary human-derived monoclonal IFNA antibody 19D11 (1.7 $\mu\text{g/ml}$). A human control antibody (huIgG, 15 $\mu\text{g/ml}$) shows no effect. **C:** Binding of g1 IFNA2, A4, A5, A6, A7, A8, A10, A14, A16, A17 and A21 fusion proteins to HEK 293T MSR cells is inhibited by exemplary human-derived monoclonal IFN antibody 19D11. Binding of g1 IFNB and IFNW is unaffected by 19D11. Binding of all g1 IFNs is unaffected by a control human antibody (huIgG). Antibody concentration: 5 $\mu\text{g/ml}$. **D:** Binding neutralization of g1 IFNA16 and g1 IFNW by exemplary antibodies 19D11 and 26B9. Cells were treated with g1 IFNA16 or g1 IFNW as indicated in the absence of antibodies (-) or in the presence of exemplary antibodies 19D11, 26B9 or of a control human antibody (huIgG). Antibody concentration: 10 $\mu\text{g/ml}$. Exemplary antibody 19D11 is more potent than 26B9 at neutralizing g1 IFNA16. Exemplary antibody 26B9 efficiently blocks binding of g1 IFNW to its receptors on 293T MSR cells, while exemplary antibody 19D11 shows no apparent effect against this ligand.

Fig. 24: Human IFNW-Gaussia luciferase fusion proteins bind specifically to HEK 293T MSR cells expressing a transmembrane anti-IFNW mAb. HEK 293T MSR cells were reverse-transfected with the indicated amounts of cDNA encoding a membrane-bound version of anti-IFNW mAb 26B9 (26B9-TM) or empty vector

(Mock). Forty-eight hours after transfection, IFNW-Gaussia luciferase was added (g1 IFNW) and binding was analysed in the chemiluminescent cellular binding assay. **A:** Control: 26B9-TM is expressed at the cell surface of transfected HEK 293T MSR cells. Surface antibody expression was analysed 48 hours after transfection in a cell-based ELISA. **B:** g1 IFNW specifically binds to cells expressing 26B9-TM in the luminescent cellular binding assay.

Fig.25: Crosscompetition assay of anti-IFNW antibodies. Binding of g1 IFNW to 26B9-TM is competed dose-dependently by soluble 26B9 and by the clonally related 31B4 antibody. In contrast, binding is not affected by a control IgG or by exemplary anti-IFNW antibody 8H1.

Fig. 26: SPR analysis. **A:** Detailed analysis of the sensograms concerning of the binding of human **A1/B1:** IFNA2b, **A2/B2:** IFNA4, **A3/B3:** IFN14 and **A4/B4:** IFN ω to exemplary antibodies 19D11 (**A1-A4**) and 26B9 (**B1-B4**) of the present invention. A 1:1 binding kinetic was observed. The antigens were injected in concentrations of 1 nM, 2.5 nM, 5 nM, 10 nM, 15 nM, 25 nM, 50 nM and 100 nM. Calculated Affinities (KD values [M]) are indicated in the diagrams. **C:** The plot shows the kinetic parameters derived from the fitted curves for the association (on-rate k_a) and dissociation (off-rate k_d) of all tested antibodies. Dashed diagonals indicate affinities (KD). **D:** KD values of exemplary antibodies of this invention in comparison with SPR literature value of Biotin-Straptavidin binding. The affinities towards human IFNA4 and IFNA14 are in the sub-picomolar range and in sub-nanomolar range for IFN2b, respectively. 26B9 also binds human IFN ω with a sub-picomolar affinity.

Fig. 27: Epitope mapping. **A:** Binding of antibodies of the present invention to full length antigens coupled to the microarray. The Y-axis indicated the fluorescence intensity (RFU) upon detection with a Cy5-conjugated secondary antibody. **B:** Primary peptide array of 18mer peptides of human IFNA2 against the antibody 19D11 of the present invention. In the lower panel the peptides covering the sequence from Asparagine 65 to Lysine 98 and from Lysine 117 to Serine 150 are depicted. The antibody 19D11 binds specifically to peptides 19 and 32. **C:** Primary peptide array of 18mer peptides of human IFNA2 against the antibody 26B9 of the present

invention. In the lower panel the peptides covering the sequence from Aspartic acid 77 to Lysine 110 are depicted. The antibody 26B9 binds specifically to peptide 22.

D: Primary peptide array of 18mer peptides of human IFNAW against the antibody 26B9 of the present invention. In the lower panel the peptides covering the sequence from Methionine 102 to Alanine 135 are depicted. The antibody 26B9 binds specifically to peptide 23.

Fig. 28: Ear inflammation assay CytoEar IFNA14. Testing the effect of different IFNA blocking antibodies of the present invention following hIFNA14 induced inflammation. To induce inflammation 20µl IFNa14 was injected per ear at a concentration of 25µg/ml. Measurement of thickness was also performed before giving the injection, and with two measurements per ear. **A:** Exemplary 10-day experimental timeline. **B:** Overview of the experimental treatment of the experimental animal groups A to I. Ref. A – IFNA-specific reference antibody. CytoEar ear thickness measurements calculated as fold change relative to day 0 measurements than normalized to relevant PBS controls, for each cohort. **C:** Overview of the effect of all normalized measurements. **D:** Effects of 26B9 treatment following IFNa14 injections. **E:** Effects of 19D11 treatment following IFNa14 injections. **F:** Effect of the treatment with reference anti-IFN-α antibody Ref. A following IFNa14 injections. Treatment with antibodies 26B9 and 19D11 (significant reduction of ear thickness at days 7, 9, 10, respective at days 4, 7-10 for 19D11) of the present invention leads to pronounced reduction of the ear thickness resulting from IFNa14 injections compared to the control treatment with IgG (of IFN-α non-related binding specificity) and of treatment with Ref. A. Mean +/- SEM, ID = intradermal cytokine injections, M = Measurements – ear thickness, S = Sacrifice of the animals; ID – cytokine injections; tested antibodies 26B9, 19D11, Ref.A and the control IgG were injected at day 0 (IP).

Fig. 29: Ear inflammation assay CytoEar IFNA5. Testing the effect of different IFNA blocking antibodies of the present invention following hIFNA5 induced inflammation. To induce inflammation 20µl IFNa5 was injected per ear at a concentration of 25µg/ml. Measurement of thickness was also performed before giving the injection, and with two measurements per ear. **A:** Exemplary 10-day experimental timeline. **B:** Overview of the experimental treatment of the

experimental animal groups A to I. Ref. A – reference IFN α -specific antibody. CytoEar ear thickness measurements calculated as fold change relative to day 0 measurements than normalized to relevant PBS controls, for each cohort. **C**: Overview of the effect of all normalized measurements. **D**: Effects of 26B9 treatment following IFN α 5 injections. **E**: Effects of 19D11 treatment following IFN α 5 injections. **F**: Effect of the treatment with reference anti-IFN- α antibody Ref. A following IFN α 5 injections. Treatment with antibodies 26B9 and 19D11 of the present invention leads to a reduction of the ear thickness resulting from IFN α 5 injections (significant reduction for 26B9 at days 4, 6, 7, 8 and 9; for 19D11 at days 7-9). See description of Fig. 26 for further details. Tested antibodies 26B9, 19D11, Ref.A and the control IgG were injected at day 0 (IP).

Fig. 30: Ear inflammation assay CytoEar IFN ω . Testing the effect of different IFN α blocking antibodies of the present invention following hIFN ω (IFN ω) induced inflammation To induce inflammation 20 μ l IFN ω was injected per ear at a concentration of 6,25 μ g/ml, 125 ng/ear. Measurement of thickness was also performed before giving the injection, and with two measurements per ear. **A**: Exemplary 10-day experimental timeline. **B**: Overview of the experimental treatment of the experimental animal groups A to I. Ref. A – reference IFN α -specific antibody. CytoEar ear thickness measurements calculated as fold change relative to day 0 measurements than normalized to relevant PBS controls, for each cohort. **C**: Overview of the effect of all normalized measurements. **D**: Effects of 26B9 treatment following IFN ω injections. **E**: Effects of 19D11 treatment following IFN ω injections. **F**: Effect of the treatment with reference anti-IFN- α antibody Ref. A following IFN ω injections. Treatment with antibody 26B9 of the present invention leads to a significant reduction of the ear thickness resulting from IFN ω injections at experimental Day 9. Treatment with 19D11 or Ref.A leads to apparently none or very slight reduction of the ear thickness (not significant (ns) compared to control IgG injections at all days). See description of Fig. 26 for further details. Tested antibodies 26B9, 19D11, Ref.A and the control IgG were injected at day 0 (IP).

Fig. 31: Comparison of IFN neutralizing activity in serum of APS1/APECED patients with type 1 diabetes (T1D) or without (N). **A:** IFNA1, **B:** IFNA2a, **C:** IFNA4, **D:** IFNA5, **E:** IFNA6, **F:** IFNA7, **G:** IFNA16, **H:** IFNA17, **I:** IFNA21 and **J:** IFNW.

5 **Fig. 32:** Comparison of IFN neutralizing activity in serum of APS1/APECED patients with type 1 diabetes (T1D) or without (N). As in Fig. 31 however with logarithmic scale at the Y-axis for a better visualization of the neutralizing activity measured in serum of APS1/APECED patients with T1D. **A:** IFNA1, **B:** IFNA2a, **C:** IFNA4, **D:** IFNA5, **E:** IFNA6, **F:** IFNA7, **G:** IFNA8, **H:** IFNA10, **I:** IFNA14, **J:** IFNA16, **K:** IFNA17, and **L:** IFNA21. APS1 patients not suffering from T1D (N) show for all antibodies a higher titer in their serum than T1D-APS1-patients. This titer difference may indicate differential therapeutic requirements in both patient groups.

15 **Detailed description of the invention**

The present invention generally relates to novel molecules binding IFN- α of mammal, preferably human origin, particularly patient-derived human monoclonal antibodies as well as fragments, derivatives and variants thereof that recognize and more importantly are capable of neutralizing the activity of different subtypes of IFN- α ; see also the background section supra
20 for description of the IFNA subtypes, including IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17 and IFNA21, wherein the IFNA1 and IFNA13 genes encode for the same IFNA1/13 subtype.

As described in the Examples, the exemplary antibodies of the present invention have been
25 isolated by a method subject of and disclosed in applicant's international application WO2013/098419 A1, based on screening the sera of patients with an impaired central and/or peripheral tolerance or loss of self-tolerance, such as APECED/APS1 patients for autoantibodies against IFN- α proteins as well as using a novel method of isolating antibodies from B cells of subject suffering from an autoimmune disorder or auto-inflammatory disease,
30 which is subject of and disclosed in applicant's international application WO2013/098420 A1.

Experiments performed in accordance with the present invention were directed towards the provision of IFN- α binding molecules of selective IFN- α subtype specificities, *i.e.* antibodies and IFN- α binding fragments thereof which immunoreactivity has been shown in

APECED/APS1 patients as protective against the onset of, *e.g.*, SLE or at least reducing manifestation of SLE symptoms.

Accordingly, in a first general aspect the present invention provides a human-derived anti-interferon-alpha (IFN- α) antibody or an IFN- α binding fragment, synthetic or biotechnological derivative thereof showing a binding specificity and preferably neutralizing activity towards all or only a sub-range or particular IFN- α subtypes. In a preferred embodiment of the present invention the anti-IFN- α antibody or IFN- α binding fragment

- (i) binds to human IFN- α subtypes IFNA2, IFNA4, IFNA5, IFNA6, IFNA10 and IFNA14;
 - 10 (ii) binds to at least one human IFN- α subtype IFNA1/13 (IFNA1b), IFNA8, IFNA16 and/or IFNA21; and/or
 - (iii) is capable of neutralizing a biological activity of at least one of the human IFN- α subtypes.
- Accordingly, the present invention provides a pool of antibodies of different IFN α subtype specificity different from and providing a broader application profile than antibodies known in
- 15 the art.

All IFN α subtypes utilize the heterodimeric IFN α / β receptor (IFNAR) and generate a signal in the receiving cell via cytoplasmic tyrosine kinases Tyk2 and Jak1 performed phosphorylation and concomitant activation of STAT1 and STAT2 (signal transducers and activators of

20 transcription). The STAT proteins translocate to the nucleus and activate the expression of genes that have either GAS or ISRE sites, or both, in their promoters (Borden et al., Nat. Rev. Drug Discov. 6 (2007), 975-990; Hu et al., Immunol. Rev. 226 (2008), 41-56; van Boxel-Dezaire et al., Immunity 25 (2006), 361-372). This activation mechanism has been used within the present invention as well as for designing IFN-activity measurement in vitro methods, such

25 as the cell based STAT (signal transducers and activators of transcription) activation assay and the ISRE (Interferon stimulated response element) reporter gene assay as described and used herein (Cignal Reporter Assay, Qiagen), *e.g.*, in Example 3 and in Figs. 5 to 7 to monitor the neutralizing abilities of the antibodies of the present invention. As described in detail therein, the antibodies of the present invention have been found to have a potent neutralizing activity

30 towards several IFNA subtypes as specified in detail further below.

Therefore, in one embodiment of the present invention, the biological activity neutralized by the antibody or IFN- α binding fragment of the present invention is IFN- α signaling in a cell based STAT (signal transducers and activators of transcription) activation assay, in an ISRE

(Interferon stimulated response element) reporter gene assay, and/or in an cellular binding assay (see Example 9).

Furthermore, the binding affinities of the antibodies of the present invention have been tested by ELISA, LIPS and cellular binding assays as described herein, *e.g.*, in Examples 1, 2, 6 and 9 and shown in Figs. 3, 7 to 13, and 18 to 27. In accordance with the results of these experiments, the present invention provides several exemplary anti-IFN- α antibodies showing differential binding affinities towards distinct IFNA subtypes, which exemplify the binding and neutralization characteristics of the IFN- α binding molecules provided herein.

For instance, the exemplary anti-IFN- α antibody 19D11 of the present invention binds to at least human IFN- α subtypes IFNA1/13, IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14, IFNA16 and IFNA21 (see, *e.g.*, Figs. 13 and 18 to 27) and has neutralization properties as described in the Examples and in Figs. 5 to 7. Accordingly, in one embodiment, the antibody or IFN- α binding fragment of the present invention in addition to the IFN- α subtypes defined in section (i), *supra*, binds to at least human IFN- α subtypes IFNA1/13, IFNA8, IFNA16 and IFNA21. Thus, in one preferred embodiment, the anti-IFN- α antibody or an IFN- α binding fragment, synthetic or biotechnological derivative thereof has the immunological characteristics and/or biological properties of antibody 19D11 as illustrated in the appended Examples and Figures, in particular with respect to its neutralizing activity towards the different IFN- α subtypes.

Exemplary anti-IFN- α antibodies 26B9 and 31B4 of the present invention bind to at least human IFN- α subtypes IFNA1/13, IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14 and IFNA21 (see, *e.g.*, Figs. 13 and 18 to 27) and have neutralization properties as described in the Examples and in Figs. 5 to 7. Therefore, in one embodiment, the antibody or IFN- α binding fragment of the present invention in addition to the IFN- α subtypes defined in section (i), *supra*, binds to at least human IFN- α subtypes IFNA1/13, IFNA8 and IFNA21. Thus, in another preferred embodiment, the anti-IFN- α antibody or an IFN- α binding fragment, synthetic or biotechnological derivative thereof has the immunological characteristics and/or biological properties of antibody 26B9 or 31B4 as illustrated in the appended Examples and Figures, in particular with respect to its neutralizing activity towards the different IFN- α subtypes.

Exemplary anti-IFN- α antibody 25C3 of the present invention binds to at least human IFN- α subtypes IFNA1/13, IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14, IFNA16 and IFNA21 (see, *e.g.*, Figs. 13 and 18 to 19) and has neutralization properties as described in the Examples and in Figs. 5 to 7, showing in particular a reduced neutralization capability towards IFNA6, IFNA8, IFNA10 and IFNA16. In one embodiment thus, the antibody or IFN- α binding fragment of the present invention in addition to the IFN- α subtypes defined in section (i), *supra*, binds to at least human IFN- α subtypes IFNA1/13, IFNA8, IFNA16 and IFNA21 but shows only weak neutralization of IFNA16. In a further embodiment, the antibody or IFN- α binding fragment of the present invention shows in addition only weak neutralization of IFNA6, IFNA8 and IFNA10. Thus, in another preferred embodiment, the anti-IFN- α antibody or an IFN- α binding fragment, synthetic or biotechnological derivative thereof has the immunological characteristics and/or biological properties of antibody 25C3 as illustrated in the appended Examples and Figures, in particular with respect to its neutralizing activity towards the different IFN- α subtypes.

Exemplary anti-IFN- α antibody 5D1 of the present invention binds to at least human IFN- α subtypes IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14, IFNA16 and IFNA21 but not to IFNA1/13 and has neutralization properties as described in the Examples and in Figs. 5 to 7. Accordingly, in one embodiment, the antibody or IFN- α binding fragment of the present invention in addition to the IFN- α subtypes defined in section (i), *supra*, binds to at least human IFN- α subtypes IFNA8, IFNA16 and IFNA21 but not to IFNA1/13. Thus, in another preferred embodiment, the anti-IFN- α antibody or an IFN- α binding fragment, synthetic or biotechnological derivative thereof has the immunological characteristics and/or biological properties of antibody 5D1 as illustrated in the appended Examples and Figures, in particular with respect to its neutralizing activity towards the different IFN- α subtypes.

Exemplary anti-IFN- α antibody 13B11 of the present invention binds to at least human IFN- α subtypes IFNA1/13, IFNA2, IFNA4, IFNA5, IFNA6, IFNA10, IFNA14 and IFNA16 but not to IFNA8 and has neutralization properties as described in the Examples and in Figs. 5 to 7. Therefore, in one embodiment, the antibody or IFN- α binding fragment of the present invention in addition to the IFN- α subtypes defined in section (i), *supra*, binds to at least human IFN- α subtypes IFNA1/13 and IFNA16 but not to IFNA8. Thus, in a further preferred embodiment, the anti-IFN- α antibody or an IFN- α binding fragment, synthetic or biotechnological derivative

thereof has the immunological characteristics and/or biological properties of antibody 13B11 as illustrated in the appended Examples and Figures, in particular with respect to its neutralizing activity towards the different IFN- α subtypes.

5 Exemplary anti-IFN- α antibody 8H1 of the present invention binds to at least human IFN- α subtypes IFNA1, IFNA4, IFNA5, IFNA6, IFNA7, IFNA10, IFNA16, IFNA17 and IFNA21 while showing weaker neutralization of IFNA2, IFNA8 and IFNA14 and has neutralization properties as described in the Examples and in Fig. 7. Therefore, in one embodiment, the antibody or IFN- α binding fragment of the present invention binds in addition
10 to the IFN- α subtypes defined in section (i), supra, to at least human IFN- α subtypes IFNA1/13, IFNA17 and IFNA21. Thus, in a still further preferred embodiment, the anti-IFN- α antibody or an IFN- α binding fragment, synthetic or biotechnological derivative thereof has the immunological characteristics and/or biological properties of antibody 8H1 as illustrated in the appended Examples and Figures, in particular with respect to its neutralizing activity towards
15 the different IFN- α subtypes.

Exemplary anti-IFN- α antibody 12H5 of the present invention binds to and neutralizes all human IFN- α subtypes, namely IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA14, IFNA16, IFNA17 and IFNA21 and has neutralization properties as
20 described in the Examples and in Fig. 7. Therefore, in one embodiment, the antibody or IFN- α binding fragment of the present invention binds to all human IFN- α subtypes. Thus, in a still further preferred embodiment, the anti-IFN- α antibody or an IFN- α binding fragment, synthetic or biotechnological derivative thereof has the immunological characteristics and/or biological properties of antibody 12H5 as illustrated in the appended Examples and Figures, in particular
25 with respect to its neutralizing activity towards the different IFN- α subtypes.

In one embodiment, the anti-IFN- α antibody and IFN- α binding fragment of the present invention recognize and/or neutralize IFNA21, preferably with at least substantially the same binding preference/neutralizing activity as for any other IFNA recognized and/or neutralized
30 by the antibody or fragment thereof.

Furthermore, after preliminary results obtained in the experiments performed within the scope of the present invention already indicated that exemplary antibodies provided by the present invention bind in addition to at least one IFNA subtype to another type I interferon, i.e. IFN-

omega (also indicated as IFNW or as IFN- ω herein) as exemplarily shown for antibodies 26B9 and 31B4 in Fig. 18, further experiments confirmed the surprising neutralizing properties of some of the subject antibodies towards IFNW, i.e. for antibodies 26B9 and 31B4 in Fig. 5 and for antibody 26B9 in comparison to antibody 19D11 in Fig. 24, for antibody 26B9 in Figs. 25-26, for antibody 31B4 in Fig. 25, for antibody 8H1 in Fig. 7 and 20 and for antibody 50E11 in Fig. 22. Accordingly, in one embodiment the present invention also relates to anti-IFN- α antibodies and IFN- α binding fragments thereof which besides at least one IFNA subtype bind in addition at least one other type I interferon, wherein preferably the other type I interferon is human IFN- ω (IFNW). The corresponding IFN-binding molecules may also be denoted IFNA/IFNW binding molecule or anti-IFNA/IFNW antibody. Preferably, the anti-IFN- α antibody or IFN- α binding fragment of the present invention, in addition to the at least one IFN- α subtype is capable of neutralizing a biological activity of human IFN- ω (IFNW). Such an antibody is of particular value for use in the prevention, treatment or diagnosis of patients having a higher titer of IFNW and/or for patients showing both, an increased titer of an IFNA subtype and of IFNW. Thus, in a still further preferred embodiment, the anti-IFN- α antibody or an IFN- α binding fragment, synthetic or biotechnological derivative thereof has the immunological characteristics and/or biological properties of antibody 26B9, 31B4, 8H1 or 50E11 as illustrated in the appended Examples and Figures, in particular with respect to its neutralizing activity towards IFN- ω .

The IFNA/IFNW binding molecules of the present invention are particularly useful in the prevention, therapy and/or diagnosis of diseases associated with an increased expression or level of activity of IFNW in a subject, in particular in case the expression or level of activity of IFNA is increased as well. For example, IFNW is found in the serum of patients with systemic lupus erythematosus (SLE) and renal disease in addition to elevated levels of IFNA subtypes; see, e.g., M C Dall'Era et al., *Ann. Rheum. Dis.* 64 (2005), 1692–1697 and Han et al., *Genes and Immunity* 4 (2003), 177-186. Accordingly, the IFNA/IFNW binding molecules of the present invention broadly blocking multiple type I IFNs may be more advantageous as a potential therapeutic agent than an antibody specific for IFNA only. In addition, it has been reported that sera of patients with rheumatoid arthritis besides IFN- β have an elevated level of IFN- ω relative to normal controls, but not of IFN- α ; see Lavoie et al., *J. Immunol.* 186 (2011), 186, meeting abstract 101.37. Thus, the IFNA/IFNW binding molecules of the present invention may also prove useful in the treatment of autoimmune disorders which do not or not significantly involve IFNA but IFNW.

Hence, in a further aspect the present invention relates to IFNW binding molecules which are synthetic or biotechnological derivatives of the human-derived anti-IFN- α antibody 26B9, 31B4, 8H1 or 50E11 which substantially retain their neutralizing activity towards IFNW, but lost their binding specificity to one or more IFN- α subtypes or the capability of binding IFN- α altogether. In addition, the present invention relates to any IFNW binding molecule, preferably antibody or antibody fragment which competes with any of the human-derived anti-IFN- α antibodies 26B9, 31B4, 8H1 or 50E11 for binding and/or neutralizing IFNW in any of the assays described in the Examples but not necessarily with respect to binding and/or neutralizing IFN- α .

Furthermore, as shown in Fig. 18, the exemplary anti-IFN- α antibodies of the present invention display a much weaker or no affinity towards type I interferon IFN- ϵ , IFN- β , towards IFN- γ as a type II interferon and/or towards IFN- λ (IL-29, IL-28A and IL-28B in Fig. 18) as type III interferons. Accordingly, in one embodiment the present invention relates to anti-IFN- α antibodies and IFN- α binding fragments thereof which preferably bind type I over type II and/or type III interferons, more preferably anti-IFN- α antibodies and IFN- α binding fragments of the present invention do not substantially recognize type II and type III interferons.

The present invention exemplifies IFN- α binding molecules, *i.e.* antibodies and IFN- α binding fragments thereof, which may be generally characterized by comprising in their variable region, *i.e.* binding domain at least one complementarity determining region (CDR) of the V_H and/or V_L of the variable region comprising the amino acid sequence depicted in Figure 1 of (V_H) (SEQ ID NOs: 2, 10, 18, 22, 30, 38, 76, 84 and 92) and (V_L) (SEQ ID NOs: 4, 12, 20, 24, 32, 40, 78, 86 and 94) – see the exemplary CDR sequences underlined in Fig. 1 and identified in Table 1. However, as discussed in the following, the person skilled in the art is well aware of the fact that in addition or alternatively CDRs may be used, which differ in their amino acid sequence from those indicated in Fig. 1 by one, two, three or even more amino acids, in particular in case of CDR2 and CDR3.

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In respect of the particular binding preferences and neutralization abilities towards specific IFN- α subtypes and IFNW as shown in the Examples and Figures, the general characterization provided above may be subdivided into the following groups of IFN- α binding molecules, *i.e.* antibodies and binding fragments thereof of the present invention.

In one embodiment, the antibody or IFN- α binding fragment of the present invention is a human-derived antibody and binds to at least one IFN- α subtype selected from the group consisting of IFNA1/13, IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14, IFNA16 and IFNA21, comprising in its variable region:

- (a) at least one complementarity determining region (CDR) of the V_H and/or V_L variable region amino acid sequences depicted in Figure 1 (V_H) (SEQ ID NO: 18); and Figure 1 (V_L) (SEQ ID NO: 20);
- (b) an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1;
- 10 (c) at least one CDR consisting of an amino acid sequence resulted from a partial alteration of any one of the amino acid sequences of (a); and/or
- (d) a heavy chain and/or light variable region comprising an amino acid sequence resulted from a partial alteration of the amino acid sequence of (b).

In addition, or alternatively, the antibody or IFN- α binding fragment of the present invention is characterized by displaying the binding characteristics of exemplary antibody 19D11.

As illustrated in Example 5 and shown in Figure 27A/B, epitopes in IFNA2 specifically recognized by exemplary antibody 19D11 have been identified. Accordingly, in one embodiment, the antibody or IFN- α binding fragment, synthetic or biotechnological derivative of the present invention is capable of binding an epitope in IFNA2 consisting of the amino acid sequence SAAWDETLLDKFYTELYQ (SEQ ID NO: 99) and/or RITLYLKEKKYSPCAWEV (SEQ ID NO: 100).

In another embodiment, the antibody or IFN- α binding fragment of the present invention is a human-derived antibody and binds to at least one IFN- α subtype selected from the group consisting of IFNA1/13, IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14 and IFNA21, comprising in its variable region:

- (a) at least one complementarity determining region (CDR) of the V_H and/or V_L variable region amino acid sequences depicted in Figure 1 (V_H) (SEQ ID NO: 30 or SEQ ID NO: 38); and Figure 1 (V_L) (SEQ ID NO: 32 or SEQ ID NO: 40);
- 30 (b) an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1;
- (c) at least one CDR consisting of an amino acid sequence resulted from a partial alteration of any one of the amino acid sequences of (a); and/or

- (d) a heavy chain and/or light variable region comprising an amino acid sequence resulted from a partial alteration of the amino acid sequence of (b).

In addition, or alternatively, the antibody or IFN- α binding fragment of the present invention is characterized by displaying the binding characteristics of exemplary antibody 26B9 or 31B4.

- 5 Thus, in this embodiment the anti-IFN- α antibody or IFN- α binding fragment preferably also recognizes and neutralizes IFNW; see also supra.

As illustrated in Example 5 and shown in Figure 27C/D, epitopes in IFNA2 and IFNW specifically recognized by exemplary antibody 26B9 have been identified. Accordingly, in one
10 embodiment, the antibody or IFN- α binding fragment, synthetic or biotechnological derivative of the present invention is capable of binding an epitope of IFNA2 consisting of the amino acid sequence YTELYQQQLNDLEACVIQG (SEQ ID NO: 101) and/or an epitope of IFNAW consisting of the amino acid sequence TGLHQQLQHLETCLLQVV (SEQ ID NO: 102).

- 15 In another embodiment, the antibody or IFN- α binding fragment of the present invention is a human-derived antibody and binds to at least one IFN- α subtype selected from the group consisting of subtypes IFNA1/13, IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14, IFNA16 and IFNA21, comprising in its variable region:

- (a) at least one complementarity determining region (CDR) of the V_H and/or V_L variable
20 region amino acid sequences depicted in Figure 1 (V_H) (SEQ ID NO: 22); and Figure 1 (V_L) (SEQ ID NO: 24);
- (b) an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1;
- (c) at least one CDR consisting of an amino acid sequence resulted from a partial alteration of any one of the amino acid sequences of (a); and/or
- 25 (d) a heavy chain and/or light variable region comprising an amino acid sequence resulted from a partial alteration of the amino acid sequence of (b).

In addition, or alternatively, the antibody or IFN- α binding fragment of the present invention is characterized by displaying the binding characteristics of exemplary antibody 25C3.

- 30 In another embodiment, the antibody or IFN- α binding fragment of the present invention is a human-derived antibody and binds to at least one IFN- α subtype selected from the group consisting of subtypes IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14, IFNA16 and IFNA21 but not to IFNA1/13, comprising in its variable region:

- (a) at least one complementarity determining region (CDR) of the V_H and/or V_L variable region amino acid sequences depicted in Figure 1 (V_H) (SEQ ID NO: 2); and Figure 1 (V_L) (SEQ ID NO: 4);
- (b) an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1;
- 5 (c) at least one CDR consisting of an amino acid sequence resulted from a partial alteration of any one of the amino acid sequences of (a); and/or
- (d) a heavy chain and/or light variable region comprising an amino acid sequence resulted from a partial alteration of the amino acid sequence of (b).

In addition, or alternatively, the antibody or IFN- α binding fragment of the present invention
 10 is characterized by displaying the binding characteristics of exemplary antibody 5D1.

In another embodiment, the antibody or IFN- α binding fragment of the present invention is a human-derived antibody and binds to at least one IFN- α subtype selected from the group consisting of subtypes IFNA1/13, IFNA2, IFNA4, IFNA5, IFNA6, IFNA10, IFNA14 and
 15 IFNA16, comprising in its variable region:

- (a) at least one complementarity determining region (CDR) of the V_H and/or V_L variable region amino acid sequences depicted in Figure 1 (V_H) (SEQ ID NO: 10); and Figure 1 (V_L) (SEQ ID NO: 12);
- (b) an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1;
- 20 (c) at least one CDR consisting of an amino acid sequence resulted from a partial alteration of any one of the amino acid sequences of (a); and/or
- (d) a heavy chain and/or light variable region comprising an amino acid sequence resulted from a partial alteration of the amino acid sequence of (b).

In addition, or alternatively, the antibody or IFN- α binding fragment of the present invention
 25 is characterized by displaying the binding characteristics of exemplary antibody 13B11.

In another embodiment, the antibody or IFN- α binding fragment of the present invention is a human-derived antibody and binds to at least one IFN- α subtype selected from the group consisting of IFNA1, IFNA4, IFNA5, IFNA6, IFNA7, IFNA10, IFNA16, IFNA17 and
 30 IFNA21, comprising in its variable region:

- (a) at least one complementarity determining region (CDR) of the V_H and/or V_L variable region amino acid sequences depicted in Figure 1 (V_H) (SEQ ID NO: 76); and Figure 1 (V_L) (SEQ ID NO: 78);
- (b) an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1;

- (c) at least one CDR consisting of an amino acid sequence resulted from a partial alteration of any one of the amino acid sequences of (a); and/or
- (d) a heavy chain and/or light variable region comprising an amino acid sequence resulted from a partial alteration of the amino acid sequence of (b).

5 In addition, or alternatively, the antibody or IFN- α binding fragment of the present invention is characterized by displaying the binding characteristics of exemplary antibody 8H1. Thus, in this embodiment the anti-IFN- α antibody or IFN- α binding fragment preferably also recognizes and neutralizes IFNW; see also supra.

10 In another embodiment, the antibody or IFN- α binding fragment of the present invention is a human-derived antibody and binds to at least one IFN- α subtype selected from the group consisting of IFNA1/13, IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14 and IFNA21, comprising in its variable region:

- (a) at least one complementarity determining region (CDR) of the V_H and/or V_L variable
15 region amino acid sequences depicted in Figure 1 (V_H) (SEQ ID NO: 84); and Figure 1 (V_L) (SEQ ID NO: 86);
- (b) an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1;
- (c) at least one CDR consisting of an amino acid sequence resulted from a partial alteration of any one of the amino acid sequences of (a); and/or
- 20 (d) a heavy chain and/or light variable region comprising an amino acid sequence resulted from a partial alteration of the amino acid sequence of (b).

In addition, or alternatively, the antibody or IFN- α binding fragment of the present invention is characterized by displaying the binding characteristics of exemplary antibody 12H5.

25 In another embodiment, the antibody or IFN- α binding fragment of the present invention is a human-derived antibody and binds to at least one IFN- α subtype selected from the group consisting of IFNA1/13, IFNA2, IFNA4, IFNA5, IFNA6, IFNA8, IFNA10, IFNA14 and IFNA21, comprising in its variable region:

- (a) at least one complementarity determining region (CDR) of the V_H and/or V_L variable
30 region amino acid sequences depicted in Figure 1 (V_H) (SEQ ID NO: 92); and Figure 1 (V_L) (SEQ ID NO: 94);
- (b) an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1;
- (c) at least one CDR consisting of an amino acid sequence resulted from a partial alteration of any one of the amino acid sequences of (a); and/or

- (d) a heavy chain and/or light variable region comprising an amino acid sequence resulted from a partial alteration of the amino acid sequence of (b).

In addition, or alternatively, the antibody or IFN- α binding fragment of the present invention is characterized by displaying the binding characteristics of exemplary antibody 50E11. Thus, in this embodiment the anti-IFN- α antibody or IFN- α binding fragment preferably also recognizes and neutralizes IFNW at least to some extent; see also supra.

In summary, in one aspect the present invention relates to human-derived monoclonal antibodies and IFN- α binding fragments as well as synthetic and biotechnological derivatives of the subject antibodies exemplified herein comprising in their variable regions:

- (a) at least one complementarity determining region (CDR) of the V_H and/or V_L variable region amino acid sequences depicted in
- (i) Figure 1 (V_H) (SEQ ID NOs: 2, 10, 18, 22, 30, 38, 76, 84 and 92); and
 - (ii) Figure 1 (V_L) (SEQ ID NOs: 4, 12, 20, 24, 32, 40, 78, 86 and 94);
- (b) an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1;
- (c) at least one CDR consisting of an amino acid sequence resulted from a partial alteration of any one of the amino acid sequences of (a); and/or
- (d) a heavy chain and/or light variable region comprising an amino acid sequence resulted from a partial alteration of the amino acid sequence of (b);

preferably wherein the antibody, IFN- α binding fragment or synthetic and biotechnological derivative thereof retains at least one of the immunological characteristics and/or biological activities of any of the subject antibodies described in the Examples, e.g. neutralizing activity towards different IFNA subtypes and/or IFNW.

In addition, in one aspect the present invention relates to an IFN- α neutralizing antibody or IFN- α binding fragment thereof which competes with said antibody or fragment thereof as defined hereinabove for binding to and/neutralizing of at least one IFN- α subtype and/or IFNW. Furthermore, in one embodiment the antibody of the present invention or the IFN- α binding fragment thereof contains at least CDR3 of the V_H and/or V_L variable region depicted in

- (i) Figure 1 (V_H) (SEQ ID NOs: 2, 10, 18, 22, 30, 38, 76, 84 and 92); and
 - (ii) Figure 1 (V_L) (SEQ ID NOs: 4, 12, 20, 24, 32, 40, 78, 86 and 94);
- or a corresponding CDR3 which differs in its amino acid sequence by substitution, deletion and/or addition of 6, 5 or 4, preferably not more than 3 and most preferably red no more than 2 or 1 amino acids.

In order to provide antibodies particularly suitable for therapeutic applications, *i.e.* to avoid immunological responses to the antibodies of the present invention as observed for foreign antibodies such as mouse antibodies in humans (HAMA-response) the present invention preferably relates to fully human or human-derived antibodies since the exemplary IFN- α antibodies, which are described in the Examples illustrating the present invention, have been derived from a human patient.

In this context, contrary to humanized antibodies and otherwise human-like antibodies, see also the discussion *infra*, the human-derived antibodies of the present invention are characterized by comprising CDRs which have been seen by the human body and therefore are substantially devoid of the risk of being immunogenic. Therefore, the antibody of the present invention may still be denoted human-derived if at least one, preferably two and most preferably all three CDRs of one or both the variable light and heavy chain of the antibody are derived from the human antibodies illustrated herein.

The human-derived antibodies of the present invention may also be called "human auto-antibodies" in order to emphasize that those antibodies were indeed expressed initially by a human subject and are not *in vitro* selected constructs generated, for example, by means of human immunoglobulin expressing phage libraries or xenogeneic antibodies generated in a transgenic animal expressing part of the human immunoglobulin repertoire, which hitherto represented the most common method for trying to provide human-like antibodies. On the other hand, the human-derived antibody of the present invention may be denoted synthetic, recombinant, and/or biotechnological in order to distinguish it from human serum antibodies *per se*, which may be purified via protein A or affinity column.

However, the present invention uses and envisages further studies of the antibodies of the present invention in animal models, *e.g.*, in transgenic mice expressing human IFN- α . To avoid immunogenic effects in the experimental animals analogous to the HAMA-response in humans, in one aspect, the antibody of the present invention may be a humanized, xenogeneic, or chimeric human-murine antibody, preferably a chimeric rodent-human or a rodentized antibody, most preferred a chimeric murine-human or murinized antibody.

As described herein below in more detail, the antibody or antigen-binding fragment thereof of the present invention can be of or derived from any type, class or subclass of an immunoglobulin molecule. However, in a preferred embodiment, the antibody of the present invention is provided, which is of the IgG isotype, most preferably of the IgG1 subclass.

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In order to provide such humanized, chimeric and in particular fully human antibodies, fragments and/or native Fab fragments thereof, the antibody of the present invention preferably further comprises a C_H and/or C_L constant region comprising an amino acid sequence selected from the C_H and C_L amino acid sequences set forth in Table 1 (SEQ ID NOs.: 6, 8, 14, 16, 26, 28, 34, 36, 42, 44, 72, 74, 80, 82, 88, 90, 96 and 98) or an amino acid sequence with at least 60 % identity, preferably 70% identity, more preferably 80% identity, still more preferably 90% identity, and particularly preferred at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, identity to the mentioned reference sequences.

15 In addition, or alternatively, the framework region of the antibody of the present invention comprises an amino acid sequence selected from Figure 1 (V_H) (SEQ ID NOs: 2, 10, 18, 22, 30, 38, 76, 84 and 92); and Figure 1 (V_L) (SEQ ID NOs.: 4, 12, 20, 24, 32, 40, 78, 86 and 94) or an amino acid sequence with at least 60 % identity, preferably 70% identity, more preferably 80% identity, still more preferably 90% identity, and particularly preferred at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, identity to the mentioned reference sequences.

As mentioned above, the antibodies of the present invention have been isolated from APECED/APS1 patients. In this context, experiments disclosed in applicant's co-pending international application WO2013/098419 surprisingly revealed that APECED/APS1 patients display an auto-immunosome, *i.e.* an autoantibody profile comprising as well a broad spectrum of binding molecules specific for different IFN α subtypes. APS1 is a rare autoimmune disease caused by mutations in the Autoimmune Regulator (*AIRE*) gene. The AIRE protein governs the expression in medullary thymic epithelium of many peripheral self-antigens (*e.g.*, insulin) that are presented by MHC to tolerate developing thymocytes. In APS1, *AIRE* mutations cause aberrant negative selection, which enables autoreactive T cells to escape to the periphery. Accordingly, the patients show an extremely variable spectrum of clinical features in APS1, but usually with several autoimmune disorders of endocrine tissues. The defining APS1 triad comprises chronic mucocutaneous candidiasis, hypoparathyroidism and adrenal failure (Perheentupa, Endocrinol. Metab. Clin. North Am. 31 (2002), 295-320). Other clinical

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conditions seen in APECED patients include thyroid autoimmune diseases, diabetes mellitus, gonadal failure, vitiligo, alopecia, chronic hepatitis, chronic gastritis and pernicious anemia and different forms other gastrointestinal symptoms. For further details concerning APECED/APS1 patients and the screening of their auto-immunosome see the description of international application WO2013/098419 and the Examples described therein, in particular the Material and Methods section on pages 112-117; Example 1 on pages 117-118 and Example 7 on page 128 and the following Tables 1 to 14; and Example 17 on pages 168-171.

As described in detail above and indicated in Example 1, in one preferred embodiment the antibody of the present invention is obtained from a sample of a human subject affected with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED/APS1) or from a patient affected with a similar autoimmune disease as described in international application WO2013/098419 and the Examples therein, in particular the Materials and Methods section on pages 112-117; Example 1 on pages 117-118; in Example 10 on pages 156-161, specifically in section "Patients and controls" on page 156 therein; and Example 17 on pages 168-171.

In this context it is noted that the subject anti-IFN- α antibodies of the present invention have been cloned by a novel and proprietary method of isolating human antibodies, which is disclosed in applicant's co-pending international application WO2013/098420.

Briefly, the sample for isolating the antibody of interest comprises or consists of peripheral blood mononuclear cells (PBMC) and serum for the detection of possible antibody reactivities. The sample derived from the subject may either be directly used for, *e.g.*, testing seroreactivity against one or more of the desired antigen(s) or may be further processed, for example enriched for B lymphocytes. In particular, it is preferred that the sample comprises or is derived from B cells that produce the antibody of interest, most preferably memory B-cells. The memory B cells are cultured under conditions allowing only a definite life span of the B cells, typically no more than 1 to 2 weeks until singling out the cells from B cell cultures which are reactive against the desired antigen subsequently followed by RT-PCR of single sorted cells for obtaining the immunoglobulin gene repertoire; see for detailed description Examples 1 and 2 on pages 118 to 120 of WO2013/098419 and in particular Examples 1 to 4 on pages 27 to 31 of

WO2013/098420. Naturally, the present invention extends to the immortalized human B memory lymphocyte and B cell, respectively, that produces the antibody having the distinct and unique characteristics as defined herein above and below.

- 5 Thus, besides using a selected patient pool, the anti-IFN- α antibodies have been provided by employing a particular method specifically developed and adapted for isolating human monoclonal antibodies from B cells of patients with an autoimmune disease such as APECED/APS1 patients.
- 10 In one embodiment, the antibody or IFN- α binding molecule of the present invention comprises an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1 or as encoded by the corresponding nucleic acids as indicated in Table 1. In addition, in another embodiment the present invention relates to an anti-IFN- α antibody or IFN- α binding molecule, which competes with an antibody of the present invention as defined hereinabove for specific binding to at least
- 15 one human IFN- α subtype and/or human IFN- ω .

In particular, anti-IFN- α antibodies are provided which demonstrate the immunological binding characteristics and/or biological properties as outlined for the antibodies illustrated in the Examples and in the Figures. Where present, the term "immunological binding characteristics,"

- 20 or other binding characteristics of an antibody with an antigen, in all of its grammatical forms, refers to the specificity, affinity, cross-reactivity, and other binding characteristics of an antibody.

As demonstrated in the Examples, the antibodies of the present invention are particularly

- 25 characterized by their high neutralizing activity towards the majority of the IFNA subtypes as well as in case of the 26B9, 31B4 and 8H1 antibody against IFNW in the sub-nanomolar range. Preferably, the human-derived monoclonal antibodies and IFN- α binding fragments as well as synthetic and biotechnological derivatives thereof display the IC₅₀ values as determined in an ISRE (Interferon stimulated response element) reporter gene assay of the subject antibodies
- 30 exemplified herein for at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or almost all IFNA subtypes and optionally for IFNW or their IC₅₀ values differ no more than 50%, preferably less than 40%, more preferably less than 30%, still more preferably less than 20% and particularly preferred less than 10% from the IC₅₀ values determined for the

subject antibodies illustrated in the Examples and Figures for any of the IFNA subtypes and optionally for IFNW. In a preferred embodiment, the antibody or like IFN-binding molecule of the present invention has an IC 50 value of ≤ 10 ng for at least 5, preferably 6, more preferably 7, still more preferably 8 or advantageously 9 or 10 human IFN- α subtypes and/or human IFN-

5 ω .

In a further embodiment, the antibody of the present invention is an antibody fragment. For example, the antibody or antibody fragment of the present invention may be elected from the group consisting of a single chain Fv fragment (scFv), an F(ab') fragment, an F(ab) fragment, 10 an F(ab')₂ fragment and a single domain antibody fragment (sdAB).

A further advantage of the antibodies of the present invention is that due to the fact that the humoral immune response has been elicited against the native antigen in its physiologic and cellular environment, typically autoantibodies are produced and can be isolated which 15 recognize a conformational epitope of the antigen due to its presentation in context for example with other cellular components, presentation on a cell surface membrane and/or binding to a receptor. In contrast, conventional methods of generating monoclonal antibodies such as mouse monoclonals, humanized versions thereof or antibodies obtained from phage display typically employ an antigenic fragment of the target protein for immunizing a non-human mammal and 20 detection, respectively, upon which usually antibodies are obtained which recognize linear epitopes or conformational epitopes limited to a two-dimensional structure of the immunogen rather than the presence of the native protein in its physiological and cellular context. Accordingly, it is prudent to expect that the autoantibodies of the present invention are unique in terms of their epitope specificity. Therefore, the present invention also relates to antibodies 25 and like-binding molecules which display substantially the same binding specificity as the autoantibodies isolated in accordance with the method of the present invention. Such antibodies can be easily tested by for example competitive ELISA or more appropriately in a cell based neutralization assay using an autoantibody and a monoclonal derivative, respectively, thereof of the present invention as a reference antibody and the immunological tests described in the 30 Examples or otherwise known to the person skilled in the art.

As further illustrated in the Examples and in the Figures, *e.g.*, in Fig.19, the antibodies of the present invention are isolated preferably from human donors and thus bind human IFNA subtypes. Therefore, in one embodiment the anti-IFN- α antibody and IFN- α binding fragment

of the present invention recognize only the human antigen or at least preferentially over the corresponding antigen from other species such as mice. In another embodiment the anti-IFN- α antibody and IFN- α binding fragment of the present invention bind at least one IFNA subtypes of other species, preferably wherein the other species is mice. Binding characteristics such as specificity and affinity of the antibodies of the present invention have been tested in several experimental assays as described and shown herein, *e.g.*, in Examples 2, 5 and 6 and in Figs. 2-3 and 8-13, and 18 to 27.

As has been further demonstrated for the antibodies of the present invention, they are capable of neutralizing the biological activity of their target protein; see, *e.g.*, the results of the STAT1 phosphorylation assay and the interferon specific response element reporter-gene assay described in Example 3 and Figs. 5 to 7. In this context, the term "neutralizing" means that the antibody of the present invention is capable of intervening with the biological activity of its target protein in a biochemical or cell-based assay as can be evaluated by performing the respective assay in the presence of the subject antibody of the present invention, wherein the biological activity of the target protein is reduced concomitantly with increasing level of the antibody of the present invention subjected to the assay compared to the biological activity of the protein without the presence of the antibody of the present invention and in the presence of a compound for example a control antibody which is known to leave the biological activity of the target protein unaffected in kind. Such biochemical and in vitro based assay can also be performed using a reference antibody known to be capable of neutralizing the biological activity of the target protein such as has been shown for the anti-IFN- α antibodies of the present invention and subjecting the candidate antibody to the test sample, wherein either an additive neutralizing effect may be observed resulting from the combined activity of the reference and candidate antibody or a competition of the candidate antibody and reference antibody is observed which may be determined by labelling either antibody. Thus, in a preferred embodiment of the present invention, the antibody obtained by the method of the present invention is capable of neutralizing the biological activity of its antigen, *e.g.*, at least one human IFN- α subtype.

The antibodies or antigen-binding fragments, *e.g.*, peptides, polypeptides or fusion proteins of the present invention may be provided, as indicated above, by expression in a host cell or in an in vitro cell-free translation system, for example. To express the peptide, polypeptide or fusion protein in a host cell, the nucleic acid molecule encoding said peptide, polypeptide or fusion

protein may be inserted into appropriate expression vector, *i.e.* a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook et al., *Molecular Cloning, A Laboratory Manual* (1989), and Ausubel et al., *Current Protocols in Molecular Biology* (1989); see also the sections "Polynucleotides" and "Expressions" further below and literature cited in the Examples section for further details in this respect.

A suitable host cell for expression of the product may be any prokaryotic or eukaryotic cell; *e.g.*, bacterial cells such as *E. coli* or *B. subtilis*, insect cells (baculovirus), yeast cells, plant cell or an animal cell. For efficient processing, however, mammalian cells are preferred. Typical mammalian cell lines useful for this purpose include CHO cells, HEK 293 cells, COS cells and NSO cells.

The isolated antibodies of the present invention may of course not be applied as such to a patient, but usually have to be pharmaceutically formulated to ensure, *e.g.*, their stability, acceptability and bioavailability in the patient. Therefore, in one embodiment, the method of the present invention is provided, further comprising the step of admixing the isolated monoclonal antibody or a fragment thereof with a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers will be described in detail further below.

As a measure to obtain a stable and permanent source of binding molecules of the present invention, in particular for pharmaceutical use heterologous genes encoding these binding molecules may be isolated by direct cloning, PCR amplification, or artificial synthesis and introduced and expressed in suitable host cells or organisms. Therefore, it is also an object of the present invention to provide a method for preparing a recombinant cell useful for the production of a recombinant human anti-IFN- α antibody or IFN- α binding fragment thereof, comprising the steps of:

- (a) preparing a B cell by a method as described above;
- (b) sequencing a nucleic acid and/or obtaining from the B cell a nucleic acid that encodes;

- (i) at least one of the C_H and C_L amino acid sequences set forth in Table 1 (SEQ ID NOs.: 6, 8, 14, 16, 26, 28, 34, 36, 42, 44, 72, 74, 80, 82, 88, 92, 96 and 98) or an amino acid sequence with at least 60 % identity;
- (ii) at least one complementarity determining region (CDR) of the V_H and/or V_L variable region amino acid sequences depicted in
 - Figure 1 (V_H) (SEQ ID NOs: 2, 10, 18, 22, 30, 38, 76, 84 and 92); and
 - Figure 1 (V_L) (SEQ ID NOs: 4, 12, 20, 24, 32, 40, 78, 86 and 94);
- (iii) an amino acid sequence of the V_H and/or V_L region as depicted in Figure 1;
- (iv) at least one CDR consisting of an amino acid sequence resulted from a partial alteration of any one of the amino acid sequences of (a);
- (v) a heavy chain and/or light variable region comprising an amino acid sequence resulted from a partial alteration of the amino acid sequence of (ii); and/or
- (c) inserting the nucleic acid into an expression host in order to permit expression of the antibody of interest in that host.

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Host cells as described herein may be used as well in the preceding method and as described in detail in the "Host" section of this specification. In this respect, in one embodiment the above method is provided, where the expression host is a yeast cell, a plant cell or an animal cell.

20 Furthermore, in one embodiment a method is provided for preparing a pharmaceutical composition for use in the treatment of a disorder associated with the expression and activity of IFN- α and/or IFN- ω , the method comprising:

- (a) culturing host cells as defined above;
- (b) purifying the antibody, IFN- α binding fragment thereof, biotechnological derivative or immunoglobulin chain(s) thereof of the present invention from the culture to pharmaceutical grade; and
- (c) admixing the antibody, IFN- α binding fragment thereof or biotechnological derivative thereof of the present invention with a pharmaceutically acceptable carrier.

30 In respect of the above described methods for production of the respective antibody of interest, in one embodiment the present invention provides a method, wherein the nucleic acid is manipulated between above steps (b) and (c) to introduce restriction sites, to change codon usage, and/or to add or optimize transcription and/or translation regulatory sequences.

As demonstrated in appended Examples 2 and 3 and summarized in Table 4, binding molecules, *i.e.* antibodies have been identified and cloned, which display a particularly high apparent binding affinity (EC50/ED50) and/or a particularly high in vitro neutralizing activity with low inhibitory concentrations (IC50) for at least one human IFN- α subtype. In this respect, in one embodiment, the anti-IFN- α antibody and IFN- α binding fragment of the present invention thereof have a high affinity for its respective target molecule, *e.g.*, human IFN- α subtypes as defined hereinabove, showing an EC50 at concentrations below 100 ng/ml, preferably below 20 ng/ml and more preferably below 10 ng/ml. Alternatively or in addition, in one embodiment the anti-IFN- α antibody and IFN- α binding fragment thereof have high neutralizing ability for at least one human IFN- α subtype, showing IC50 at concentrations below 500, 400, 300 or 100 ng/ml, preferably below 20 ng/ml, more preferably below 10 ng/ml and most preferred below 5 ng/ml. For more details in respect of the binding affinity of the antibodies of the present invention see, *e.g.*, section "Binding characteristics" further below.

The present invention also relates to polynucleotides encoding at least a variable region of an immunoglobulin chain of the antibody or antigen-binding fragment of the invention. Preferably, said variable region comprises at least one complementarity determining region (CDR) of the V_H and/or V_L of the variable region as set forth in Figure 1.

In case of a derived sequence, said sequence shows at least 60% identity, more preferably (in the following order) at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and most preferably 95%, at least 96-99%, or even 100% identity to a sequence of the group consisting of those sequences referred to above and identified in the Sequence Listing. The percent identity between two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, which is well known to those skilled in the art. The identities referred to herein are to be determined by using the BLAST programs as further referred to herein *infra*.

As mentioned above, in a preferred embodiment, the present invention relates to substantially fully human antibodies, preferably IgG including at least the constant heavy chain I (C_{H1}) and the corresponding light chain of the constant region, *i.e.* γ -1, γ -2, γ -3 or γ -4 in combination with lambda or kappa. In a particularly preferred embodiment, the nucleotide and amino acid

sequences of those constant regions isolated for the subject antibodies illustrated in the Examples are used as depicted in Table 1 below and in SEQ ID NOs: 5, 7, 13, 15, 25, 27, 33, 35, 41, 43, 71, 73, 79, 81, 87, 89, 95 and 97 in respect of the nucleotide sequences and/or SEQ ID NOs: 6, 8, 14, 16, 26, 28, 34, 36, 42, 44, 72, 74, 80, 82, 88, 90, 96 and 98 in respect of the amino acid sequences or amino acid sequences with at least 60 % identity to these referenced before.

In accordance with the above, the present invention also relates to a polynucleotide, in particular recombinant polynucleotide encoding at least the variable region of one immunoglobulin chain of the anti-IFN- α antibody or IFN- α binding fragment of the present invention. Typically, said variable region encoded by the polynucleotide comprises at least one complementarity determining region (CDR) of the V_H and/or V_L of the variable region of the said antibody. Variable and constant regions of antibodies are described in more detail in the section "IgG structure" below. In a preferred embodiment of the present invention, the polynucleotide comprises, consists essentially of, or consists of a nucleic acid having a polynucleotide sequence encoding the V_H or V_L region of an antibody of the present invention as depicted in Table 1 below. In this respect, the person skilled in the art will readily appreciate that the polynucleotides encoding at least the variable domain of the light and/or heavy chain may encode the variable domain of either immunoglobulin chains or only one of them. In a preferred embodiment, the polynucleotide encodes the anti-IFN- α antibody or IFN- α binding fragment as defined hereinabove.

Table 1: Nucleotide sequences of the variable and constant regions (V_H, V_L, C_H, C_L) regions of IgG1, kappa, IFN- α specific 5D1, 13B11, 19D11, 25C3, 26B9, 31B4, 8H1, 12H5 and 50E11 antibodies of the present invention. Underlined, bold nucleotides or amino acids indicate the **CDR** coding regions in the variable chain sequence. Underlined, italic nucleotides or amino acids indicate sequences which have not been sequenced but *obtained from database*. In the constant chains, such regions are aligned with and tuned in accordance with the pertinent human germ line variable region sequences in the database; see, e.g., Vbase (<http://vbase.mrc-cpe.cam.ac.uk>) hosted by the MRC Centre for Protein Engineering (Cambridge, UK).

Antibody	Nucleotide and amino acid sequences of variable heavy (V _H) and variable light (V _L), constant heavy (C _H) and constant light (C _L) chains.
5D1-V _H	gaagtgcactggtgcaggccggcgagaggtgaaagcgcccgaggagtctctg aggatctcctgtaagggtgtctggatacacctttaca <u>agttattggatcagttgg</u> gtgcgccagattcccggaagggcctggagtggatgggtg <u>aaaattgatcctaga</u> <u>gactcttataccatctacaacccgtccttccaaggc</u> caagtcctccatctcagtt gacaagtcctaccactgtctacctgcagtgagcagcctgcaggcctcgac accgccatttattattgtgtgaga <u>cattatcttacacagtcattggtggactac</u> <u>tttgaccac</u> tggggccagggaacgctggtcgccgtctcctct SEQ ID NO:1

5D1-V _H	EVQLVQAGAEVKAPGESLRISCKVSGYTFT SYWIS WVRQIPGKGLEWMV KIDPR DSYTIYNPSFQG HVSIISVDKSIITTVYLQWSSLQASDTAIYYCVR HYLTQSLVDY FDHWGQGT LVAVSS SEQ ID NO: 2
5D1-V _L kappa-type	gacattcagatgacccagtcctccatcctccctgtctgcatctgtgggagacagt gtcaccatcacttgc cgggcaagtcagagcgtatccaactacttccat tgggtat cgacagaagcccgggaaagccctgaactcctgatctat tctgcatccaatttg caaact gggggtcccatcaagattcactggcagtggtctgggacagaatgcact ctcaccatcaccagtcctgcagcctgatgatttcgcaacttactactgt caacag actcacggttaccggttact tttggccaggggaccaagctggacgtcaga SEQ ID NO: 3
5D1-V _L kappa-type	DIQMTQSPSSLSASVGDSTITTC RASQSVSNYFH WYRQKPGKAPELLIY SASNL QT GVPSRFTGSGSGTECTLTITSLQPDFATYYC QQTHGYPFT FGQGTKLDVR SEQ ID NO: 4
5D1-C _H	gcctccaccaagggcccatcggtcttccccctggcaccctcctccaagagcacc tctgggggacagcgccctgggctgctgggtcaaggactacttccccgaaccg gtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccg gctgtcctacagtcctcaggactctactccctcagcagcgtgggtgacctgccc tccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagc aacaccaaggtggacaagaaagttgagccaaatcttgtgacaaaactcacaca tgcccaccgtgcccagcacctgaactcctggggggaccgtcagtcctcctcttc cccccaaaacccaaggacacctcatgatctcccggacccctgaggtcacatgc gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtg gacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaac agcacgtaccgtgtgggtcagcgtcctcaccgtcctgcaccaggactggctgaat ggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg cccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtc aaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccg gagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttc ctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaaagctcttc tcatgtcctgtgatgcatgaggtctctgcacaaccactacacgcagaagagcctc tccctgtctccgggtaaatga SEQ ID NO: 5
5D1-C _H	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSVHTFP AVLQSSGLYSLSSVTVPPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSL SLSPGK SEQ ID NO: 6
5D1-C _L kappa-type	cgaactgtggctgcaccatctgtcttcatcttcccgcctctgatgagcagttg aaatctggaactgcctctgttggctgctgtaataacttctatcccagagag gccaaagtacagtgggaaggtggataaacgcctccaatcgggtaactcccaggag agtgtcacagagcaggacagcaaggacacacctacagcctcagcagcaccctg acgctgagcaaagcagactacgagaaacacaaaagtctacgcctgcgaagt cacc catcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag SEQ ID NO: 7
5D1-C _L kappa-type	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNFFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEV THQGLSSPVTKSFNRGEC SEQ ID NO: 8
13B11-V _H	Gacgtacagctgttgacgtctgggggagggcttgatacagccgggggggtccctg agactctcctgtgcagcctctggctttacttttaag gactatgccatgagt tgg gtccgccaggctccagggaaggcctggagtgggtctcag gtaataagtcgtagt ggtaataattgtagactatgtcgactccggtgaagggc cggttcaccgtctccaga gacaattccaacaacacactctttctgcaaatggacggcctgagagccgacgac acggccatttattactgtgcgaaa cccaaggatatgattgtcgtggctccctgcg ggctttgactcc tggggccagggaacccttgtctccgtctcctca

	SEQ ID NO:9
13B11-V _H	DVQLLQSGGGLIQPGGSLRLSCAASGFTFK DYAMS WVRQAPGKGLEWVS VISRS GNIVDYVDSVKGR FTVSRDNSNNTLFLQMDGLRADDTAIYYCAK PKDMI VV VPA GFDS WGQGTLSVSS SEQ ID NO:10
13B11-V _L kappa-type	gacatccagatgacccagtttccatccaccctgtctgcatctgttgagacagc gtcaccatcacttgc cgggccagtcagagcattagtgccctgggtggcc tggtat cagcagaaaccagggaaagcccctaaactcctgatctat aaggggtctagatta gaaaac gggggtcccacgaggttcagcggcagtggtatctgggacagaattcact ctcaccatcggcagcctgcagcctgatgattttgcaacttattactgc caacaa tataagacttggacg ttcggccaagggaaccaaggtggaaatcaaa SEQ ID NO:11
13B11-V _L kappa-type	DIQMTQFPSTLSASVGDSTITC RASQSI SAWLAWYQQKPGKAPKLLIY KGSRL ENG VPSRFSGSGSGTEFTLTIGSLQPDFATYYC QYKWT FGQGTKVEIK SEQ ID NO:12
13B11-C _H	gcctccaccaagggcccacatcggtcttccccctggcaccctcctccaagagcacc tctgggggacagcggccctgggctgctggtcaaggactacttccccgaaccg gtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttccc gctgtcctacagtctcaggactctactccctcagcagcgtgggtgacgtgccc tccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagc aacaccaaggtggacaagaaagttgagccaaatcttgtgacaaaactcacaca tgcccaccgtgcccagcacctgaactcctggggggaccgtcagtccttctcttc cccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtcacatgc gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtg gacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaac agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat ggcaaggagtacaagtgaaggtctccaacaaagccctcccagcccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg cccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtc aaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccg gagaacaactacaagaccagcctcccgtgctggactccgacggctccttcttc ctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttc tcatgtcctgtagtgcaggtcctgcacaaccactacacgcagaagagcctc tccctgtctccgggtaaatga SEQ ID NO:13
13B11-C _H	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSVHTFP AVLQSSGLYSLSSVTVPPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSL SLSPGK SEQ ID NO:14
13B11-C _L kappa-type	cgaactgtggctgcaccatctgtcttcatcttcccgcacatctgatgagcagttg aaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagag gccaaagtacagtggaggtggataacgcctccaatcgggtaactcccaggag agtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctg acgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacc catcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgtag SEQ ID NO:15
13B11-C _L kappa-type	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNFFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO:16
19D11-V _H	gaggtgcagctgttggagt ctggggctgaggtgaagaggcctgggtcgtcggtg agggtctcctgcagggtcttggagacaccttcagc agttaccctatcagt tgg gtgcgacaggccccctggacaaggccttgagtggatggga aggatcctccctgcc cttgggtgtcacaactacgctcagaacttccggggc agaatcacgattaccgcg gacaagtcgccccctcacagcctacttggaaactgagtagcctcagatttgaggac

	acggccgtgtattactgtgcgagt <u>cccagtgcggaacataattccttcgattttg</u> <u>gggacgaccctctttgccttc</u> tggggccaggggaagc <u>ctgggtcaccgtctcctca</u> SEQ ID NO:17
19D11-V _H	<u>EVQLLES</u> GAEVKRPGSSVRVSCRASGDTFSS <u>SYPI</u> SWVRQAPGQGLEWMGRILPA <u>LGVTNYAQNFRG</u> RITITADKSPLTAYLELSSLRFEDTAVYYCAS <u>PSADIIPSIL</u> <u>GTTLF</u> AFWGQGS <u>LVT</u> VSS SEQ ID NO: 18
19D11-V _L kappa-type	<u>gaaattgtgttgacgcag</u> tctccaggcacccctgtctctgtctccgggggaaggg gccaccctctcctgc <u>agggccagtcagaatgttagcagacactacttaacc</u> tgg taccagcagaaacctggccagtcctccccggctcctcatctat <u>gggtggctccagc</u> <u>agggccact</u> ggcgtcccgacagaggttcagtggcggtgggtctgggacagacttc actctcaccatcagcaggtctggagcctgaagactttgcagtgttttactgc <u>cag</u> <u>agctatcatagcccacctcctgtgtacact</u> ttcggccagggg <u>accaaggtggag</u> <u>atcaaa</u> SEQ ID NO:19
19D11-V _L kappa-type	<u>EIVLTQ</u> SPGTLSPGEGATLSC <u>RASQNVSRHYLT</u> WYQQKPGQSPRLIY <u>GGSS</u> <u>RAT</u> GVPDRFSGGGSGTDFTLTISRLEPEDFAVFYCY <u>QSYHSPPPVYTF</u> GQGT <u>KVE</u> <u>IK</u> SEQ ID NO:20
19D11-C _H	<u>gcctccaccaagggcccatcggtcttccccctggc</u> accctcctccaagagcacc tctgggggcacagcgccctgggctgctgggtcaaggactacttccccgaaccg gtgacggtgtcgtggaactcaggcgccttgaccagcggcgtgcacaccttccc gctgtcctacagtcctcaggactctactccctcagcagcgtgggtgaccgtgccc tccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagc aacaccaaggtggacaagagagttgagcccaaatcttgtgacaaaactcacaca tgcccaccgtgcccagcacctgaactcctggggggaccgtcagtccttctcttc ccccaaaacccaaggacacctcatgatctcccggaacctgaggtcacatgc gtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtg gacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaac agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat ggcaaggagtacaagtgcagggtctccaacaaagccctcccagcccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg cccccatcccgggaggagatgaccaagaaccagggtcagcctgacctgctggtc aaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccg gagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttc ctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttc tcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaaanancctc tccctgtccccgggtaaatga SEQ ID NO: 71
19D11-C _H	<u>ASTKGPSVF</u> FLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSVHTFP AVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQXXL SLSPGK SEQ ID NO: 72
19D11-C _L kappa-type	cgaactgtggctgcaccatctgtcttcatcttcccgcctctgatgagcagttg aaatctggaactgcctctgttgtgtgctgctgaataacttctatcccagagag gccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggag agtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccttg acgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacc catcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgtag SEQ ID NO: 73
19D11-C _L kappa-type	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO: 74
25C3-V _H	gagatgcagctgatggagtctgggggaggtttggtacaacccgggggggtccctg agactctcctgtgtagcctctggtttcacctttaaaa <u>agttttgcgatgagt</u> tgg gtccgccaggctccaggaaggggctggagtgggtcgct <u>agtgctcggtctcag</u>

	<u>ggtggcagcaaatactatgcaccctccgtgaagggc</u>cggttctccatctccaga gacaattccaacaacactctctatgtgcaaatgaacagcctgggagtcgaggac acggccttttattattgtgttaaa<u>gagaccgatgcagtggcgacgatggacgct</u> <u>cttgacatg</u>tggggccaagggaccctggtcatcgtctctacc SEQ ID NO: 21
25C3-V _H	EMQIMESGGGLVQPGGSLRLSCVASGFTFK<u>SFAMSWVRQAPGKGLEWVASVGSQ</u> <u>GGSKYYAPSVKGR</u>FSISRDNSTNTLYVQMNSLGVEDTAFYYCVK<u>ETDAVATMDA</u> <u>LDM</u>WGQGLVIVST SEQ ID NO: 22
25C3-V _L kappa-type	gacatccgggtgacccagtcctccatcctccctgtctgcatctgtcggagacagg gtctccatctcttg<u>cagacaagtcagagtgttaacatatataa</u>tggtat caacagagaccagggaaagggcctcagtcctgatctct<u>gctgcttccactttg</u> <u>cagagt</u>gggggtcccatcaaggttcagtggcagtggtctgggacagacttcac ctcaccatcatcagtcctacaacctgaagattctgcacccactactactgt<u>caacag</u> <u>ggttacattaccccgctacact</u>tttgccaggggaccaaggtggagatcaaa SEQ ID NO: 23
25C3-V _L kappa-type	DIRVTQSPSSLSASVGDRVSI<u>SCQTSQSVNIYLN</u>WYQQRPGKGPQLLIS<u>AASTL</u> <u>QS</u>GVPSRFSGSGSGTDFILTIISLQPEDSASY<u>YCQQGYITPYT</u>FGQGTKVEIK SEQ ID NO: 24
25C3-C _H	gcctccaccaagggcccatcggtcttccccctggcaccctcctccaagagcacc tctgggggcacagcggccctgggctgctggtcaaggactacttccccgaaccg gtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccg gctgtcctacagtcctcaggactctactccctcagcagcgtgggtgaccgtgcc tccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagc aacaccaaggtggacaagagagttgagcccaaattctgtgacaaaactcacaca tgcccaccgtgccagcacctgaactcctggggggaccgtcagtccttctcttc cccccaaaacccaaggacacctcatgatctcccggacccctgaggtcacatgc gtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtg gacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaac agcacgtaccgtgtggtcagcgtcctcacctcctgcaccaggactggctgaat ggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg cccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgctggtc aaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccg gagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttc ctctatagcaagctcacctgggacaagagcaggtggcagcaggggaaagctcttc tcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc tccctgtccccgggtaaatga SEQ ID NO: 25
25C3-C _H	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL SLSPGK SEQ ID NO: 26
25C3-C _L kappa-type	cgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttg aaatctggaactgcctctgtttgtgtgctgctgaataacctctatcccagagag gccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggag agtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccttg acgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtc<u>acc</u> <u>catcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgtag</u> SEQ ID NO: 27
25C3-C _L kappa-type	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNLYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEV<u>THQGLSSPVTKSFNRGEC</u> SEQ ID NO: 28

26B9-V _H	cagatactactgcaggagtcgggcccaggactggtgaagcccacggagaccctg tccctcacctgtagtgtctctggtgactccatcagt <u>gatagtagtcactactgg</u> <u>gcc</u> tggattcgccagccccagggaaaggaccagagtggttggc <u>agtgtctat</u> <u>tttagttcgatgaccactacaacccgtccctcaaaagt</u> cgcgtcagcatctcc gttgacaagcccagaaccagttctccttaaaagtgaacctctgtgactgtcgcc gacacggccacatattactgtgcgaga <u>caagcccttgcccagagtcggagccatg</u> <u>aattggttcgacccc</u> tggggccagggatctctggtcacagtctcctca SEQ ID NO:29
26B9-V _H	QILLQESGPGLVKPTETLSLTCVSGDSIS <u>DSSHYWAWIRQPPGKGPEWIGSVY</u> <u>FSSMTHYNPSLKS</u> RVSISVDKPKNQFSLKVTSTVTADTATYYCAR <u>QALARVGAM</u> <u>NWEDP</u> WGQGS�TVSS SEQ ID NO: 30
26B9-V _L kappa-type	gacatcataatgaccagtcctccagactccctgcctgtgtctctggtggcgagggg gtcaccatcaactgc <u>aagtccagccagagcgtctttttcacctccagtaataag</u> <u>agttgttttagct</u> tggatcagcagaagccaggaaagtctccaaattgtcatt tact <u>tgggcatcaaccgcgaatcc</u> gggtccctgaccgattcagaggcagcggg tctgggacagatttctctctcaccatcaccagtcctgcaggtgaagatgtggct gtttatttctgt <u>cagcagtgtcagacatccccctcccact</u> ttcggcggaggacc aggttgagatcaaa SEQ ID NO:31
26B9-V _L kappa-type	DIIMTQSPDSLPLVSLGEGVTINC <u>KSSQSVFFTSSNKSLAWY</u> QQKPGKSPKLLI <u>YWASTRQS</u> GVPDRFRGSGSGTDFSLTITSLQAEDVAVYFC <u>QQCQTSPT</u> FGGGT RLKIK SEQ ID NO:32
26B9-C _H	gcctccaccaagggcccatcggtcttccccctggcaccctcctccaagagcacc tctgggggcacagcggccctgggctgctggtcaaggactacttccccgaaccg gtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccg gctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgcc tccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagc aacaccaaggtggacaagagagttgagcccaatcttgtgacaaaactcacaca tgcccaccgtgcccagcactgaaactcctggggggaccgtcagttctctcttc cccccaaaacccaaggacacctcatgatctcccggaacctgaggtcacatgc gtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtg gacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaac agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat ggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg ccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtc aaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccg gagaacaactacaagaccacgcctcccgctgctggactccgacggctccttcttc ctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttc tcatgctccgtgatgcattgaggtctgcacaaccactacacgcagaagagcctc tccctgtccccgggtaaatga SEQ ID NO:33
26B9-C _H	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPFPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL SLSPGK SEQ ID NO:34
26B9-C _L kappa-type	cgaactgtggctgcaccatctgtcttcatcttcccgcctctgatgagcagttg aaatctggaactgcctctgttgtgtgctgctgaataacttctatcccagagag gccaaagtacagtgggaaggtggataacgccctccaatcgggtaactcccaggag agtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcacctg acgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacc catcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgtag SEQ ID NO:35
26B9-C _L	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

kappa-type	SEQ ID NO:36
31B4-V _H	cagatacagctgcaggagtcgggcccaggactggtgagggccacggagaccctg tccctcacttgtagtgtctctggtgactccatcagt <u>cagagtagtcattactgg</u> <u>gcc</u> tggattcgccagccccaggggaaggaccagaatggattggc <u>agtgctctat</u> <u>tttagctcgatgacccactacaacccgtccctcacaagt</u> cgcgtcagcatctcc attgacaaggccatgaataagttctccttaaaagtgcctctgtgactgtcgcc gacacggccacatattactgtgcgaga <u>caggcccttgcccagagtcggagccatg</u> <u>aattggttcgacccc</u> tggggccagggatctctggtcacagtctcctca SEQ ID NO:37
31B4-V _H	QIQIQESGPGGLVRPTETLSLTCSVSGDSIS <u>QSSHYWAWIRQPPGKGP</u> EWIGSVY FSSMTHYNPSLTISRVSISIDKAMNKFSLKVTSTVADTATYYCAR <u>QALARVGAM</u> NWEDPWGQGS�TVSS SEQ ID NO:38
31B4-V _L kappa-type	gacatcataatgacccagtcctccagagtcctctgctgtgtctctgggagggg gtcaccatcaactgc <u>aagtccagccagagcgtctttttcacctccagtaatagg</u> <u>agttgttttagct</u> tggatcagcagaagccaggacagtcctccaaattgtcatt tac <u>tgggcatcaacccgccaatcc</u> gggtccctgaccgattcacaggcagcggg tctgggacagatttctctctcaccatcgccggtctgcaggttgaagatgtggct gtttatttctgt <u>cagcagtgacgcacatcccctcccact</u> ttcggcggcgggacc aggttggagctcaga SEQ ID NO:39
31B4-V _L kappa-type	DIIMTQSPESLPVSLGEGVTINC <u>KSSQSVFFTSNRSCLAWY</u> QQKPGQSPKLLI Y <u>WASTRQS</u> GVPDRFTGSGSGTDFSLTIAGLQVEDVAVYFC <u>QQCHASPT</u> FGGGT RLRLR SEQ ID NO:40
31B4-C _H	gcctccaccaagggcccatcggtcttccccctggcaccctcctccaagagcacc tctgggggacagcggccctgggctgctggtcaaggactacttccccgaaccg gtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccg gctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccc tccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagc aacaccaaggtggacaagagagttgagcccaatcttgtgacaaaactcacaca tgcccaccgtgcccagcacctgaactcctggggggaccgtcagtccttctcttc cccccaaaacccaaggacacctcatgatctcccggaacctgaggtcacatgc gtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtg gacggcgtggaggtgcataatgccaaagacaaagccgaggaggagcagtaaac agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat ggcaaggagtacaagtgaaggtctccaacaaagccctcccagcccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg cccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgctggtc aaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccg gagaacaactacaagaccagcctcccgtgctggactccgacggctccttcttc ctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttc tcatgctccgtgatgcagtgaggtctgcacaaccactacacgcagaagagcctc tccctgtccccgggtaaatga SEQ ID NO:41
31B4-C _H	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVSVLTVHLQDNLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL SLSPGK SEQ ID NO:42
31B4-C _L kappa-type	cgaactgtggctgcaccatctgtcttcatcttcccgcctctgatgagcagttg aaatctggaactgcctctgttgtgtgctgctgaataacttctatcccagagag gccaaagtacagtgggaaggtggataacgccctccaatcgggtaactcccaggag agtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcacctg acgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagt <u>cacc</u> <u>catcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag</u> SEQ ID NO:43

31B4-C _L kappa-type	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEV <u>THQGLSSPVTKSFNRGEC</u> SEQ ID NO:44
8H1 V _H	caggtgcagctggtgcagctctggggctgaggtgaagaagcctggggcctcagtg aaggtctcctgcaaggcttctggacagaccttcacc <u>agtgatgatatcaact</u> gg gtgcgacaggcccttgacaggggctagagtggatggga <u>tgagggaaccctaac</u> <u>actcaggacacgggctatgcacagaagtccacggc</u> agactcaccttgaccagc aacagttccataagtacatcctatctggagttgagcggcctgagatctgaggac acggccgtgtattactgtgcgaga <u>gcggggacttcgaccttgaccggccactac</u> <u>ttcgctttgggggtc</u> tggggccaggggaccacgggtcatcgctctcctca SEQ ID NO:75
8H1 V _H	QVQLVQSGAEVKKPGASVKVSKASGQTFT <u>SDDINWVRQAPGQGLEWMGWRNPN</u> <u>TQDTGYAQKFHGRLTLTSNSSISTSYLELSGLRSEDTAVYYCARAGTSTLTGHY</u> <u>FALGVWGQTTIVSS</u> SEQ ID NO:76
8H1 V _L kappa-type	gacatccagctgacccagctctccatcctcctgtctgcatctgtaggagacaga gtcaccatcacttgt <u>caggcgactcaggatattagcaaatatttaaat</u> tgggtat cagcagaaaccagggaagtccctaaactcctgatctac <u>gaaacatccaatttg</u> <u>gaagta</u> gggggtcccatcaaggttcagtggaagtgggtctgggacacattttact ctcaccatcagcagcctgcaggctgaagattttgcaacatattactgt <u>caacag</u> <u>tatgagaatttcccggtcact</u> ttcgcgaggaggaccaaggtggagatcaaa SEQ ID NO:77
8H1 V _L kappa-type	DIQLTQSPSSLSASVGRVTITC <u>QATQDISKYL</u> NWYQQKPKGKPKLLIY <u>ETSNL</u> <u>EV</u> GVPSRFSGSGSGTHFTLTISSLQAE ~~DF~~ ATYYC <u>QQYENFPFT</u> FGGGTKVEIK SEQ ID NO: 78
8H1 C _H	gcctccaccaagggcccatcggtcttccccctggcaccctcctccaagagcacc tctgggggcacagcggccctgggtgctgctggtcaaggactacttccccgaaccg gtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccg gctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgcc tcagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagc aacaccaaggtggacaagagagttgagcccaaatcttgtgacaaaactcacaca tgcccaccgtgcccagcacctgaactcctggggggaccgtcagttctctcttc cccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtcacatgc gtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtg gacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtacaac agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat ggcaaggagtacaagtgaaggtctccaacaaagccctcccagcccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg cccccatcccgaggagatgaccaagaaccaggtcagcctgacctgcctggctc aaaggcttctatcccagcgacatcgccgtggagtgaggagagcaatgggcagccg gagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttc ctctatagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttc tcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc tcctgtccccgggtaaa SEQ ID NO:79
8H1 C _H	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL SLSPGK SEQ ID NO: 80
8H1 C _L kappa-type	cgaactgtggctgcaccatctgtcttcatcttcccgcctctgatgagcagttg aatctggaactgcctctgttgtgtgctgctgaataacttctatcccagagag gccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggag agtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcacctg acgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacc catcagggcctgagctcgccgtcacaagagcttcaacaggggagagtgtag

	SEQ ID NO: 81
8H1 C _L kappa-type	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	SEQ ID NO: 82
12H5 V _H	caagtgcaactgatacagtcctgggcctgaggtgaagaggcctggggcctcagtg aaggtctcctgcaaggcgtctgaaaacaccttcgac <u>actcattatattaat</u> tgg gtgcgacagggccctggacaagggttaacttggtggga <u>tggtgaaccctacc</u> <u>actggtaaaacaggctttccacaaaagtttaagggc</u> agagtcattctgaccagc gacacctccctaaatactgcctatatggaagtgagccgctgacatctgaggac acggccgtttatctgtgcccagag <u>gttttgaagttgtctgatgagtacaactat</u> <u>ggtttcgacgtc</u> tggggccaagggaaccacggtcatcgtctcctca
	SEQ ID NO: 83
12H5 V _H	QVQLIQSGPEVKRPGASVKVSKASENTFD <u>THYIN</u> WVRQAPGQGLTWLG <u>WLNPT</u> <u>TGKTGFPOKFKGR</u> VILTSDTSLNTAYMEVSRLTSEDTAVYFCAR <u>VLKLSDEYNY</u> <u>GFDV</u> WGQGTTIVVSS
	SEQ ID NO: 84
12H5 V _L kappa-type	gacatccaggtgaccagtcctccatcctccctgtctgcatctattggggacaga gtcaccatcacgtgc <u>cgggcaagtcagaacattctcacctttataaat</u> tgggtat cagcacaaccagggaagcccctaaactcctgatctat <u>gctgcatccggttta</u> <u>caaaat</u> gaagtcctcatcaaggttcagtggcagtggtctgggacagatttact ctcaccatcaccagtcctgcaacctgacgattttggaacttactactgt <u>cagcag</u> <u>acttaccttaccctcaatgcagt</u> tttggccaggggaccaaggtggagatcaaa
	SEQ ID NO: 85
12H5 V _L kappa-type	DIQVTSPPSSLSASIGDRVITITCR <u>ASQNILTFIN</u> WYQHKPGKAPKLLIY <u>AASVL</u> <u>QNEV</u> PSRFSGSGSGTDFTLTITSLQPDFGTYYC <u>QQTYLTPQCS</u> FGQGTKVEIK
	SEQ ID NO: 86
12H5 C _H	gcctccaccaagggcccatcggtcttccccctggcaccctcctccaagagcacc tctgggggcacagcgccctgggctgcctgggtcaaggactacttccccgaaccg gtgacggtgtcgtggaactcaggcgccctgaccagcgcggtgcacaccttcccg gctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccc tccagcagcttggggcaccagacctacatctgcaacgtgaatcacaagccagc aacaccaaggtggacaagaaagttgagcccaaactctgtgacaaaactcacaca tgcccaacgtgcccagcacctgaactcctggggggaccgtcagtccttctcttc ccccaaaacccaaggacacctcatgatctcccggaacctgaggtcacatgc gtggtggtggagctgagccacgaagacctgaggtcaagttcaactggtacgtg gacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtaaac agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat ggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg ccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgctggtc aaaggcttctatcccagcgacatcgccgtggagtgaggagcaatgggcagccg gagaacaactacaagaccacgcctcccgtgctggactccgacggtccttcttc ctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttc tcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctc tccctgtctccgggtaaatga
	SEQ ID NO: 87
12H5 C _H	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVHLQDNLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK
	SEQ ID NO: 88
12H5 C _L kappa-type	cgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttg aatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagag gccaaagtacagtggaaaggtggataacgcctccaatcgggtaactcccaggag agtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcacctcgt

	acgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacc catcagggcctgagctcgcccgctcaciaaagagcttcaacaggggagagtgttag SEQ ID NO: 89
12H5 C _L kappa-type	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO: 90
50E11 V _H	caggtgcagctggtgcagctctggggcagagatgaagaagcctgggtcctcggtg aaggtctcctgcaaggattttggaggcaccttcagc gtctatgggtgcaact gg gtgacgacagggccctggacaaggccttgagtgatgggg gggctcatccctgtc attggggccagctaactacgcacagaagtccaggggc agaatcaccattactgcg gacgaatccacgagcagacgctatatggagttgagcagcctgagatttgacgac acggccatttattattgtgtgagag gacgacaacgaatat tggggccagggaacc ctggtcaccgtctcctcg SEQ ID NO: 91
50E11 V _H	QVQLVQSGAEMKKPGSSVKVSCDKFGGTFV VYGVN WVRQAPGQGLEWMG GLIPV IGPANYAQKFQG RITITADESTSTAYMELSSLRFDDTAIYYCVR DDNEY WGQGT LTVVSS SEQ ID NO: 92
50E11 V _L kappa-type	gaaatggtgctgacacagctctccagccaccctgtctttgtctccaggagaaaga gccaccctctcctgt agggccagtcagactgttagcaccttcttagcct tggtac caacagaaacctggccaggttcccaggctcctcgtctac gatctctcctccagg gccaat ggcactccagccaggttcagtgccggtgggtctgggacagacttcaact ctcaccatcagcagcctagaacttgaagattttgcggtttattactgt cagtg cgtagcaactggcctccctcgctcact ttcggcgaggaggaccaggggtggagatc aaa SEQ ID NO: 93
50E11 V _L kappa-type	EMVLTQSPATLSLSPGERATLSC RASQTVSTFLA WYQQKPGQVPRLLVY DISSR ANG TPARFSGGGSGTDFTLTISSELEDFAVYYC QWRSNWPPSLT FGGGTRVEI K SEQ ID NO: 94
50E11 C _H	gcctccaccaagggcccatcggtcttccccctggcaccctcctccaagagcacc tctgggggacacagcgccctgggctgcctgggtcaaggactacttccccgaaccg gtgacggtgtcgtggaactcaggcgccctgaccagcggtgcacaccttcccg gctgtcctacagtcctcaggactctactccctcagcagcgtgggtgaccgtgcc tccagcagcttggggcaccagacctacatctgcaacgtgaatcacaagccagc aacaccaaggtggacaagaaagttgagccaaatcttgtgacaaaactcacaca tgcccacggtgccagcacctgaactcctggggggaccgtcagttcttctcttc cccccaaaacccaaggacacctcatgatctcccgaccctgaggtcacatgc gtggtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtg gacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtaaac agcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaat ggcaaggagtacaagtgaaggtctccaacaaagccctcccagcccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctg cccccatcccggtatgagctgaccaagaaccaggtcagcctgacctgctggtc aaaggcttctatccagcgacatcgccgtggagtgggagagcaatgggcagccg gagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttc ctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttc tcatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctc tccctgtctccgggtaaatga SEQ ID NO: 95
50E11 C _H	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVTLHQDLNKGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEGLHNHYTQKSL SLSPGK SEQ ID NO: 96
50E11 C _L kappa-type	cgaactgtggtgcaccatctgtcttcatcttcccgcctctgatgagcagttg aaatctggaactgcctctgttggtgctgctgaataacttctatcccagagag gcaaagtacagtgggaaggtggataacgcctccaatcgggttaactcccaggag agtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcacctg

	acgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacc catcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag SEQ ID NO: 97
50E11 C _L kappa-type	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC SEQ ID NO: 98

The person skilled in the art will readily appreciate that the variable domain of the antibody having the above-described variable domain can be used for the construction of other polypeptides or antibodies of desired specificity and biological function. Thus, the present invention also encompasses polypeptides and antibodies comprising at least one CDR of the above-described variable domain and which advantageously have substantially the same or similar binding properties as the antibody described in the appended examples. The person skilled in the art will readily appreciate that using the variable domains or CDRs described herein antibodies can be constructed according to methods known in the art, *e.g.*, as described in European patent applications EP 0 451 216 A1 and EP 0 549 581 A1. Furthermore, the person skilled in the art knows that binding affinity may be enhanced by making amino acid substitutions within the CDRs or within the hypervariable loops (Chothia and Lesk, *J. Mol. Biol.* 196 (1987), 901-917) which partially overlap with the CDRs as defined by Kabat. Thus, the present invention also relates to antibodies wherein one or more of the mentioned CDRs comprise one or more, preferably not more than two amino acid substitutions. Preferably, the antibody of the invention comprises in one or both of its immunoglobulin chains two or all three CDRs of the variable regions as set forth for V_H regions in SEQ ID NOs: 2, 10, 18, 22, 30, 38, 76, 84 and 92, for V_L regions and SEQ ID NOs: 4, 12, 20, 24, 32, 40, 78, 86 and 94 or as indicated in Fig. 1.

20

The polynucleotide of the invention encoding the above described antibody may be, *e.g.*, DNA, cDNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric nucleic acid molecule comprising any of those polynucleotides either alone or in combination. In one embodiment the polynucleotide is a cDNA encoding the variable region and at least part of the constant domain. In a preferred embodiment a vector comprising the above polynucleotide is provided, optionally in combination with said polynucleotide which encodes the variable region of the other immunoglobulin chain of said antibody. Such vectors may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions.

30

Preferably, the polynucleotide of the invention is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells. Expression of said polynucleotide comprises transcription of the polynucleotide into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known to those skilled in the art. They usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally associated or heterologous promoter regions.

10 In this respect, the person skilled in the art will readily appreciate that the polynucleotides encoding at least the variable domain of the light and/or heavy chain may encode the variable domains of both immunoglobulin chains or one chain only.

Likewise, said polynucleotides may be under the control of the same promoter or may be separately controlled for expression. Possible regulatory elements permitting expression in prokaryotic host cells comprise, *e.g.*, the P_L, lac, trp or tac promoter in *E. coli*, and examples for regulatory elements permitting expression in eukaryotic host cells are the AOX1 or GAL1 promoter in yeast or the CMV-, SV40-, RSV-promoter, CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells.

20 Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system used leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the polynucleotide of the invention and are well known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a portion thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including a C- or N-terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogen), or pSPORT1 (GIBCO BRL).

Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate
5 host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the immunoglobulin light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow; see, Beychok, *Cells of Immunoglobulin Synthesis*, Academic Press, N.Y., (1979).

10

Furthermore, the present invention relates to vectors, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that comprise a polynucleotide encoding the antigen or preferably a variable domain of an immunoglobulin chain of an antibody of the invention; optionally in combination with a polynucleotide of the invention that
15 encodes the variable domain of the other immunoglobulin chain of the antibody of the invention. Preferably, said vector is an expression vector and/or a gene transfer or targeting vector.

20

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector of the invention into targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors; see, for example, the techniques described in Sambrook, *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, *Current Protocols in Molecular Biology*, Green
25 Publishing Associates and Wiley Interscience, N.Y. (1994). Alternatively, the polynucleotides and vectors of the invention can be reconstituted into liposomes for delivery to target cells. The vectors containing the polynucleotides of the invention (*e.g.*, the heavy and/or light variable domain(s) of the immunoglobulin chains encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending
30 on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for the transformation of other cellular hosts; see Sambrook, *supra*.

In respect to the above, the present invention furthermore relates to a host cell comprising said polynucleotide or vector. Said host cell may be a prokaryotic or eukaryotic cell. The polynucleotide or vector of the invention which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained extrachromosomally. The host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell; suitable host cells and methods for production of the antibodies of the present invention are described in more detail in the section "Host cells" below.

Using the above-mentioned host cells it is possible to produce and prepare an antibody of the present invention for, *e.g.*, a pharmaceutical use or as a target for therapeutic intervention. Therefore, in one embodiment, it is also an object of the present invention to provide a method for preparing an anti-IFN- α antibody or IFN- α binding fragment thereof, said method comprising

- (a) culturing the cell as defined hereinabove; and
- (b) isolating said antibody or IFN- α binding fragment thereof from the culture.

Accordingly, the present invention relates to recombinant antibody or IFN- α binding fragment thereof immunoglobulin chain(s) thereof encoded by the polynucleotide of the present invention or obtainable by the above-mentioned method for preparing an anti-IFN- α antibody or immunoglobulin chain(s) thereof. Means and methods for the recombinant production of antibodies and mimics thereof as well as methods of screening for competing binding molecules, which may or may not be antibodies, are known in the art. However, as described herein, in particular with respect to therapeutic applications in human the antibody of the present invention is a recombinant human antibody in the sense that application of said antibody is substantially free of an immune response directed against such antibody otherwise observed for chimeric and even humanized antibodies.

The binding molecules, antibodies or fragments thereof may be directly used as a therapeutic. However, in one embodiment the antibody or antigen-binding fragment which is provided by the present invention, is detectably labeled or attached to a drug, preferably wherein the detectable label is selected from the group consisting of an enzyme, a radioisotope, a fluorophore, a peptide and a heavy metal. Labeled antibodies or antigen-binding fragments of the present invention may be used to detect specific targets *in vivo* or *in vitro* including "immunochemistry/immunolabelling" like assays *in vitro*. *In vivo* they may be used in a manner

similar to nuclear medicine imaging techniques to detect tissues, cells, or other material expressing the antigen of interest. Labels, their use in diagnostics and their coupling to the binding molecules of the present invention are described in more detail in section "labels and diagnostics" further below.

5

The antibodies of the present invention are isolated from animals or humans affected by an autoimmune disorder. On the other hand, IFN- α specific antibodies identified in the present invention may be involved in severely impairing the immune system of the affected individual, which is associated with, *e.g.*, symptoms observed in APECED patients. Therefore, it is a further aspect of the present invention, to extinguish or at least relieve the pathological reactions of subjects suffering from autoimmune disorders by providing means and measures to minimize the number of auto-antibodies and/or their effects in a diseased human patient or animal. Thus, in one embodiment the present invention also relates to a peptide or peptide-based compound comprising an epitope specifically recognized by an autoantibody of the present invention. A similar effect as by application of competitive antigens, sequestering and preventing thereby the binding of the autoantibodies to their respective targets may be obtained by anti-idiotypic antibodies, as described in detail further below. Therefore, in one embodiment the present invention also provides an anti-idiotypic antibody of an autoantibody of the present invention.

As already indicated above, the present invention also relates to the anti-idiotypic antibody or the peptide or peptide-based compound of the present invention for use in the treatment of a disorder as defined above, *i.e.* a disorder associated with a disrupted or deregulated genesis of self-tolerance. These isolated antibodies or fragments thereof of the present invention can be used as immunogenes to generate a panel of monoclonal anti-idiotypes. For suitable methods for the generation of anti-idiotypic antibodies see Raychadhuri et al., *J. Immunol.* 137 (1986), 1743 and for T-cells see Ertl et al., *J. Exp. Med.* 159 (1985), 1776. The anti-idiotypic antibodies will be characterized with respect to the expression of internal image and non-internal image idiotypes using standard assays routinely practiced in the art as described in detail by Raychaudhuri et al., *J. Immunol.* 137 (1986), 1743. If an anti-idiotypic antibody structurally mimics the antigen of the antibody it is binding to or bound by, it is called the "internal image" of the antigen.

Methods of providing molecules which mimic an idiotypic of an autoimmune disease-associated auto-antibody (autoantibodies) are described in the art; see, *e.g.*, international application

WO03/099868. For example, such method may comprise the following steps: (a) providing autoantibodies in accordance with the method of the present invention; (b) binding the autoantibodies to a solid phase to form an affinity matrix; (c) contacting pooled plasma or B cells comprising immunoglobulins with the affinity matrix followed by removal of unbound plasma components; (d) eluting bound immunoglobulins, being anti-Idiotypic antibodies (anti-Id) to autoantibodies, from the matrix; (e) providing a molecular library comprising a plurality of molecule members; and (e) contacting the anti-Id with the molecular library and isolating those bound molecules which are bound by the anti-Id, the bound molecules being molecules which mimic an idiotype of autoantibodies. A method of isolating idiotypic autoantibodies in disclosed in international application WO2010/136196, which describes immunoglobulin preparations containing natural polyclonal IgG-reactive antibodies (Abs) isolated from normal human serum (NHS), for the treatment of autoimmune diseases and immune system disorders. The IgG-reactive Abs potently neutralize disease-associated or pathogenic autoantibodies present in sera of patients suffering from autoimmune diseases, by binding to their antigenic determinants located either within or near (*e.g.* overlapping with) the antigen combining sites.

The present invention also relates to compositions comprising any one of the aforementioned anti-IFN- α antibodies and/or IFN- α binding fragments, the polynucleotide, the vector, the cell, the peptide or peptide-based compound of the present invention and/or a cocktail of antibodies or IFN- α binding fragments thereof which in combination display the features of an antibody or IFN- α binding fragment thereof of the present invention. In addition or alternatively in one embodiment the composition or the kit of the present invention comprises the anti-idiotypic antibody of the present invention. In one embodiment the composition is a pharmaceutical composition and further comprises a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers, administration routes and dosage regimen can be taken from corresponding literature known to the person skilled in the art and are described as well in more detail in sections "Pharmaceutical carriers" and "Dosage regimen" further below.

Besides biochemical and cell based in vitro assays therapeutic utility of the antibodies of the present invention can be validated in appropriate animal models such as for RA, psoriasis, SLE or T1DM; see the Examples below, *e.g.*, Example 4.

In one embodiment the pharmaceutical composition further comprises an additional agent useful for treating an inflammation or an autoimmune disorder, preferably wherein said agent is selected from the group consisting of Non-Steroidal Antiinflammatory Drugs (NSAIDs), Corticosteroids, Anti-Histamines and combinations thereof. In addition or alternatively, in a
5 further embodiment the pharmaceutical composition further comprises an additional agent useful for treating an inflammation related disease, selected from the group consisting of immunosuppressive and anti-inflammatory or "anti-rheumatic" drugs.

10 In another embodiment, the composition is a diagnostic composition or kit and further comprises reagents conventionally used in immuno- or nucleic acid based diagnostic methods.

Furthermore, the present invention relates to any one of the aforementioned anti-IFN- α antibodies and IFN- α binding fragment thereof, or the composition as defined hereinabove for use in a method of:

- 15 (a) treating or preventing the progression of an immune mediated or autoimmune disease or condition;
(b) amelioration of symptoms associated with an immune mediated or autoimmune disease or condition; and/or
(c) diagnosing or screening a subject for the presence or for determining a subject's risk for
20 developing an immune mediated or autoimmune disease or condition; wherein the disease or condition associated with the expression of IFN- α and/or IFN- ω in a patient

In this respect several application routes may be used. In one embodiment of the present invention the aforementioned antibody or antigen-binding fragment, the anti-idiotypic antibody
25 or peptide or peptide-based compound and/or a cocktail of antibodies which in combination display the features of an antibody of the present invention is provided, which is designed to be administered intravenously, intramuscularly, subcutaneously, intraperitoneally, intranasally, parenterally or as an aerosol.

30 As indicated above, due to their binding specificity, the molecules of the present invention such as antibodies and fragments thereof may preferably be used in the above defined method of treatment, amelioration, diagnosing and/or screening of an immune mediated or autoimmune disorder or condition. For example, elevated IFN- α activity has been frequently detected in the sera of patients with SLE (Rönnblom *et al.*, Sem. Immun. 23 (2011), 113-121). A specific

expression pattern of interferon-dependent genes (termed the "interferon signature") is further displayed in the leukocytes of patients with various autoimmune disorders such as Sjögren's syndrome, Dermatomyositis, Multiple Sclerosis (MS), Psoriasis, type 1 or insulin dependent diabetes mellitus (T1DM or IDDM) and a fraction of RA patients (see, *e.g.*, Higgs et al., *Eur Musc Rev* (2012), 22-28). In addition, development of inflammatory arthritis, MS and T1DM has been repeatedly observed during IFN- α therapy indicating that IFN- α at least promotes those diseases (Crow MK., *Arthritis Res Ther.* (2010), Suppl 1:S5). Further data suggest an involvement of IFN- α in myositis, systemic scleroderma, chronic psoriasis (Higgs et al., *Eur Musc Rev* (2012), 22-28; Bissonnette et al., *J Am Acad Dermatol* (2009), 427-436; Greenberg SA, *Arth Res Ther* (2010): S4;) and autoimmune thyroiditis (Prummel and Laurberg, *Thyroid* (2003), 547-551). Therefore, in one embodiment the antibody or IFN- α binding fragment thereof or the composition as defined hereinabove for use in the above-mentioned method is provided, wherein said disease is an autoimmune disease, preferably selected from the group consisting of Systemic lupus erythematosus (SLE), cutaneous lupus erythematosus (CLE), discoid lupus erythematosus (DLE), type 1 diabetes mellitus (T1DM) Sjögren's syndrome, dermatomyositis, psoriasis, autoimmune thyroiditis, rheumatoid arthritis, spondyloarthritis, scleroderma and different cancer forms including leukemia, such as breast and ovarian cancer and childhood lymphoblastic leukemia (ALL; Einav et al., *Oncogene* 24 (2005), 6367-6375). Preliminary results suggested a difference in neutralizing activity of antibodies obtained from APS1-patients suffering or not suffering in addition from T1DM (Fig. 4). However, a more detailed analysis of the patient's sera revealed that the neutralizing activity of individual anti-IFNA antibodies in both kind of patients may be substantially the same, while the antibody titers and total level of IFN neutralizing activity significantly differ with a very low titer in T1DM patients; see Figs. 31 and 32. Accordingly, in one embodiment the present invention also relates to the IFN- α and IFN- α/ω binding molecules described herein as well as other IFN- α binding molecules for use in the treatment of T1DM in a patient, from whom a sample of sera has been determined to display a lower neutralizing activity against cytokines of at least one IFN- α subtype compared to a control sample, *i.e.* sera from a healthy subject or from a T1DM patient which is free of disease symptoms.

Due to the multitude of molecules suitable in treatment of, *e.g.*, disorders associated with inflammation presented herein, the present invention also relates to methods of treatment, diagnosing and/or prognosticate the probable course and outcome of such disorders, preferably wherein the immune mediated or autoimmune disease or condition is associated with the

expression of IFN- α and/or IFN- ω to the use of the molecules of the present invention. In one embodiment a method for treating of such a disorder is provided, which method comprises administering to a subject in need thereof a therapeutically effective amount of the aforementioned antibody or antigen-binding fragment, the cocktail of antibodies which in
5 combination display the features of an antibody of the present invention, the anti-idiotypic antibody or the peptide or peptide-based compound.

Furthermore, in one embodiment the present invention relates to a method of treating an immune mediated or autoimmune disease or condition associated with the expression of IFN-
10 α and/or IFN- ω comprising administering to a subject a therapeutically effective amount of a ligand binding molecule comprising:

- (i) at least one CDR of the anti-IFN- α antibodies and IFN- α binding fragment of the present invention; or
- (ii) at least one anti-idiotypic antibody and/or peptide or peptide-based compound as defined
15 hereinabove.

Treatment methods based on the use of only one monoclonal antibody specific for an epitope of a particular antigen, which is related or causing a disease may suffer from several shortcomings. For example, difficulties and probably inefficiency of treatment may stem from
20 the multiplicity of the pathogenic mechanisms causing a specific disorder requiring targeting of several antigens simultaneously. Furthermore, the inherent diversity of the patient population has to be taken into account concerning, *e.g.*, polymorphism, heterogeneity of glycosylation or slight denaturation of a given antigen, either in different or in one patient which may lead to a decreased binding efficiency of the monoclonal antibody used at least. Some of these
25 shortcomings may be circumvented by, *e.g.*, pretreatment screenings to determine whether the antigen is immunologically relevant to the patients intended to be treated and whether there are any epitope changes in the particular patients. However, such screenings are often omitted either due to treatment urgency or to cost restraints. Therefore, the present invention further relates to methods based on the application of more than one type of a binding molecule at once
30 to a patient, *i.e.* to the application of a cocktail of binding molecules. These binding molecules may specifically bind to one IFNA subtype at different epitopes and/or of an epitope of IFN- ω , each of the binding molecules applied may bind specifically another IFNA subtype or several binding molecules are used binding to several epitopes of more than one IFNA subtype and/or IFN- ω . In case the binding molecules of the present invention are directed (bind specifically)

towards one IFN α subtype as antigen, their binding specificity is directed towards distinct epitopes of said antigen. The use of such cocktails is in particular envisaged for the treatment of patients suffering from autoimmune disorders such as APS1, who in view of the presence of autoantibodies against about 3000 endogenous antigens are often not amenable to monotherapy with one particular antibody. In such cases, combination therapy with two or more monoclonal antibodies and/or peptides and peptide-based compounds of the present invention with the same or different antigen specificity are expected to achieve at least some relief of the symptoms.

Therefore, in one embodiment a further method of treating a disorder is provided comprising administering to a subject a therapeutically effective amount of a cocktail consisting essentially of at least two, three, four, five and more components selected from the groups consisting of:

- an anti-IFN- α antibody and IFN- α binding fragment of the present invention specifically binding the IFN α subtypes and/or IFN- ω as defined hereinabove; and/or
- an anti-idiotypic antibody of the present invention, and/or from a peptide or peptide-based compound of the present invention, which peptide or peptide-based compound comprises an epitope specifically recognized by an anti-IFN- α antibodies and IFN- α binding fragment of the present invention.

The present invention naturally extends also to diagnostic and prognostic methods directed towards diagnosing immune mediated or autoimmune conditions and disorders associated with expression of one or more subtypes of IFN- α and/or IFN- ω and/or prognosis of the development of the disease, *i.e.* its progression, response to treatment or recovery. Therefore, in one embodiment the present invention relates to a method for diagnosing an immune mediated or autoimmune disease or condition in a subject associated with the expression of IFN- α comprising contacting a biological sample of the subject with anti-IFN- α antibody and IFN- α binding fragment of the present invention, and detecting the presence of IFN- α and/or IFN- ω . Furthermore, in one embodiment the present invention relates to a method of detecting or determining IFN- α and/or IFN- ω in an isolated biological sample comprising admixing the sample with an anti-IFN- α antibody of the present invention, allowing the antibody to form a complex with any IFN- α subtype and/or IFN- ω present in the mixture, and detecting the complex present in the mixture.

As already mentioned above, in one embodiment the present invention relates to a kit for the diagnosis of an immune mediated or autoimmune disease or condition associated with the

expression of IFN- α and/or IFN- ω , said kit comprising the aforementioned anti-IFN- α antibody and IFN- α binding fragment, the anti-idiotypic antibody or the peptide or peptide-based compound, the polynucleotide, the vector or the cell, optionally with reagents and/or instructions for use. Associated with the kits of the present invention, *e.g.*, within a container

5 comprising the kit can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition or alternatively the kit comprises reagents and/or instructions for use in appropriate diagnostic assays. The compositions, *i.e.* kits of the present invention are of course particularly suitable

10 for the diagnosis, prevention and treatment of a disorder which is accompanied with the expression of IFN- α , in particular applicable for the treatment of diseases as mentioned above. In a particularly preferred embodiment the disorder is associated with expression of one or more of IFN- α subtypes and/or IFN- ω .

15 In another embodiment the present invention relates to a diagnostic composition comprising any one of the above described binding molecules, antibodies, antigen-binding fragments, peptides or peptide-based compounds, polynucleotides, vectors or cells of the invention and optionally suitable means for detection such as reagents conventionally used in immune- or nucleic acid based diagnostic methods. The antibodies of the invention are, for example, suited

20 for use in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. Examples of immunoassays which can utilize the antibody of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA), the sandwich (immunometric assay), flow cytometry and the Western blot assay. The antigens and antibodies of the invention can

25 be bound to many different carriers and used to isolate cells specifically bound thereto. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. There are many different labels and methods of labeling

30 known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, colloidal metals, fluorescent compounds, chemiluminescent compounds, and bioluminescent compounds; see also the embodiments discussed hereinabove.

In this context, the present invention also relates to means specifically designed for this purpose. For example, a protein- or antibody-based array may be used, which is for example loaded with either antigens derived from one or more IFN- α subtypes and containing the disease-associated antigen in order to detect autoantibodies which may be present in patients suffering from an autoimmune diseases, in particular SLE or APECED/APS1, or with antibodies or equivalent antigen-binding molecules of the present invention which specifically recognize any one of those inflammation-associated antigens. Design of microarray immunoassays is summarized in Kusnezow et al., *Mol. Cell Proteomics* 5 (2006), 1681-1696. Accordingly, the present invention also relates to microarrays loaded with binding molecules, in particular anti-IFN- α antibody and IFN- α binding fragment of the present invention or antigens identified in accordance with the present invention.

Definitions and Embodiments

Unless otherwise stated, a term and an embodiment as used herein is given the definition as provided and used in international application WO2013/098419 and international application WO2013/098420. Supplementary, a common term as used herein is given the definition as provided in the *Oxford Dictionary of Biochemistry and Molecular Biology*, Oxford University Press, 1997, revised 2000 and reprinted 2003, ISBN 0 19 850673 2.

It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "an antibody," is understood to represent one or more antibodies. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

The term "neutralizing" and "neutralizing antibody", respectively, is used as common in the art in that an antibody is meant that reduces or abolishes at least some biological activity of an antigen or of a living microorganism. For example, a subtype-specific anti-IFN- α antibody of the present invention is a neutralizing antibody, if, in adequate amounts, it abolishes or reduces the activity of the respective IFN- α subtype(s) for example in an assay as described in the Examples. Neutralization is commonly defined by 50 % inhibitory concentrations (IC 50) and can be statistically assessed based on the area under the neutralization titration curves (AUC). IC 50 values of exemplary anti-IFN- α antibodies of the present invention are described and shown herein, e.g., in Figs. 8-10 and in Table 4.

Central and Peripheral Tolerance

Central and peripheral tolerance are described in more detail in the respective chapter of the international application WO2013/098419 on pages 62-63.

Peptides and polypeptides:

5 The term "peptide" is understood to include the terms "polypeptide" and "protein" (which, at times, may be used interchangeably herein) and any amino acid sequence such as those of the heavy and light chain variable region as well as constant region of the present invention within its meaning. Similarly, fragments of proteins and polypeptides are also contemplated and may be referred to herein as "peptides". Nevertheless, the term "peptide" preferably denotes an
10 amino acid polymer including at least 5 contiguous amino acids, preferably at least 10 contiguous amino acids, more preferably at least 15 contiguous amino acids, still more preferably at least 20 contiguous amino acids, and particularly preferred at least 25 contiguous amino acids. In addition, the peptide in accordance with present invention typically has no more than 100 contiguous amino acids, preferably less than 80 contiguous amino acids and more
15 preferably less than 50 contiguous amino acids.

As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides" such as antibodies of the present invention, and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as
20 peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, "peptides," "dipeptides," "tripeptides," "oligopeptides," "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" may be used instead of, or interchangeably with any of these terms.

25 The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide may be
30 derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

A polypeptide of the invention may be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Nevertheless, the term "polypeptide" preferably denotes an amino acid polymer including at least 100 amino acids. Polypeptides may have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded. As used herein, the term glycoprotein refers to a protein coupled to at least one carbohydrate moiety that is attached to the protein via an oxygen-containing or a nitrogen-containing side chain of an amino acid residue, *e.g.*, a serine residue or an asparagine residue.

By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for purposes of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

"Recombinant peptides, polypeptides or proteins" refer to peptides, polypeptides or proteins produced by recombinant DNA techniques, *i.e.* produced from cells, microbial or mammalian, transformed by an exogenous recombinant DNA expression construct encoding the fusion protein including the desired peptide. Proteins or peptides expressed in most bacterial cultures will typically be free of glycan. Proteins or polypeptides expressed in yeast may have a glycosylation pattern different from that expressed in mammalian cells.

Also included as polypeptides of the present invention are fragments, derivatives, analogs and variants of the foregoing polypeptides and any combination thereof. The terms "fragment," "variant," "derivative" and "analog" include peptides and polypeptides having an amino acid sequence sufficiently similar to the amino acid sequence of the natural peptide. The term "sufficiently similar" means a first amino acid sequence that contains a sufficient or minimum number of identical or equivalent amino acid residues relative to a second amino acid sequence such that the first and second amino acid sequences have a common structural domain and/or common functional activity. For example, amino acid sequences that comprise a common

structural domain that is at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100%, identical are defined herein as sufficiently similar. Preferably, variants will be sufficiently similar to the amino acid sequence of the preferred peptides of the present invention, in particular to antibodies or antibody fragments, or to synthetic peptide or peptide-based compound comprising epitopes recognized by the antibodies of the present invention or fragments, variants, derivatives or analogs of either of them. Such variants generally retain the functional activity of the peptides of the present invention, *i.e.* are bound by the antibodies of the present invention. Variants include peptides that differ in amino acid sequence from the native and wt peptide, respectively, by way of one or more amino acid deletion(s), addition(s), and/or substitution(s). These may be naturally occurring variants as well as artificially designed ones.

The terms "fragment," "variant," "derivative" and "analog" when referring to antibodies or antibody polypeptides of the present invention include any polypeptides which retain at least some of the antigen-binding properties of the corresponding native binding molecule, antibody, or polypeptide. Fragments of polypeptides of the present invention include proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein. Variants of antibodies and antibody polypeptides of the present invention include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants may occur naturally or be non-naturally occurring. Non-naturally occurring variants may be produced using art-known mutagenesis techniques. Variant polypeptides may comprise conservative or non-conservative amino acid substitutions, deletions or additions. Derivatives of binding molecules of the present invention, *e.g.*, antibodies and antibody polypeptides of the present invention, are polypeptides which have been altered so as to exhibit additional features not found on the native polypeptide. Examples include fusion proteins. Variant polypeptides may also be referred to herein as "polypeptide analogs". As used herein a "derivative" of a binding molecule or fragment thereof, an antibody, or an antibody polypeptide refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Also included as "derivatives" are peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For example, 4-hydroxyproline may be substituted for

proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine.

5 Anti-idiotypic antibodies:

The term "anti-idiotypic antibodies" when referring to antibodies or other binding molecules includes molecules which bind to the unique antigenic peptide sequence located on an antibody's variable region near or at the antigen binding site, inhibiting by this a specific immune response by otherwise caused by the given auto-antibody. In an analogous manner
10 synthetic peptide or peptide-based compound comprising an epitope specifically recognized by an antibody of the present invention may be used.

Anti-idiotypic antibodies may be obtained in a similar fashion as other antibodies. The particular anti-idiotypic antibody is detected by any sort of cross-linking, either by agglutination
15 (in turbidimetric or nephelometric assays), precipitation (radial immunodiffusion), or sandwich immunoassays such as ELISAs. U.S. patent application No. 20020142356 provides a method for obtaining anti-idiotypic monoclonal antibody populations directed to an antibody that is specific for a high-concentration, high-molecular-weight target antigen wherein said anti-idiotypic antibody populations have a wide range of binding affinities for the selected antibody
20 specific to said target antigen and wherein a subset of said anti-idiotypic antibody populations can be selected having the required affinity for a particular application.

U.S. patent application No. 20020142356 describes a competitive immunoassay of an antigen using an antibody as coat and an anti-idiotypic antibody as detection or vice-versa. Other
25 references disclosing use of an anti-idiotypic antibody as a surrogate antigen include Losman et al., *Cancer Research*, 55 (1995) (23 suppl. S):S5978-S5982; Becker et al., *J. of Immunol. Methods* 192 (1996), 73-85; Baral et al., *International J. of Cancer*, 92 (2001), 88-95; and Kohen et al., *Food and Agriculture Immunology*, 12 (2000), 193-201. Use of anti-idiotypic antibodies in treatment of diseases or against parasites is known in the art; see, e.g., in Sacks et
30 al., *J. Exper. Medicine*, 155 (1982), 1108-1119.

Determination of similarity and/or identity of molecules:

"Similarity" between two peptides is determined by comparing the amino acid sequence of one peptide to the sequence of a second peptide. An amino acid of one peptide is similar to the

corresponding amino acid of a second peptide if it is identical or a conservative amino acid substitution. Conservative substitutions include those described in Dayhoff, M.O., ed., *The Atlas of Protein Sequence and Structure 5*, National Biomedical Research Foundation, Washington, D.C. (1978), and in Argos, *EMBO J.* 8 (1989), 779-785. For example, amino acids
 5 belonging to one of the following groups represent conservative changes or substitutions: -Ala, Pro, Gly, Gln, Asn, Ser, Thr; -Cys, Ser, Tyr, Thr; -Val, Ile, Leu, Met, Ala, Phe; -Lys, Arg, His; -Phe, Tyr, Trp, His; and -Asp, Glu.

The determination of percent identity or similarity between two sequences is preferably
 10 accomplished using the mathematical algorithm of Karlin and Altschul (1993) *Proc. Natl. Acad. Sci USA* 90: 5873-5877. Such an algorithm is incorporated into the BLASTn and BLASTp programs of Altschul et al. (1990) *J. Mol. Biol.* 215: 403-410 available at NCBI (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>).

15 The determination of percent identity or similarity is performed with the standard parameters of the BLASTn and BLASTp programs.

BLAST polynucleotide searches are performed with the BLASTn program.

For the general parameters, the "Max Target Sequences" box may be set to 100, the "Short
 20 queries" box may be ticked, the "Expect threshold" box may be set to 10 and the "Word Size" box may be set to 28. For the scoring parameters the "Match/mismatch Scores" may be set to 1,-2 and the "Gap Costs" box may be set to linear. For the Filters and Masking parameters, the "Low complexity regions" box may not be ticked, the "Species-specific repeats" box may not be ticked, the "Mask for lookup table only" box may be ticked, and the "Mask lower case letters"
 25 box may not be ticked.

BLAST protein searches are performed with the BLASTp program. For the general parameters, the "Max Target Sequences" box may be set to 100, the "Short queries" box may be ticked, the "Expect threshold" box may be set to 10 and the "Word Size" box may be set to "3". For the scoring parameters the "Matrix" box may be set to "BLOSUM62", the "Gap Costs" Box may
 30 be set to "Existence: 11 Extension: 1", the "Compositional adjustments" box may be set to "Conditional compositional score matrix adjustment". For the Filters and Masking parameters the "Low complexity regions" box may not be ticked, the "Mask for lookup table only" box may not be ticked and the "Mask lower case letters" box may not be ticked.

Polynucleotides:

The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA) or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid" refers to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding an antibody contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides of the present invention. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. Two or more coding regions of the present invention can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, *e.g.*, a single vector may separately encode an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or not fused to a nucleic acid encoding a binding molecule, an antibody, or fragment, variant, or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid which encodes a polypeptide normally may include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" or "operably linked" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein.

A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit β -globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product.

5 Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain
10 across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full-length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.*, an immunoglobulin heavy chain or light chain signal peptide is used, or a
15 functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β -glucuronidase. However, intracellular production of the polypeptides, in
20 particular of the immunoglobulins and fragments thereof of the present invention is also possible.

Expression:

The term "expression" as used herein refers to a process by which a gene produces a
25 biochemical, for example, an RNA or polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product, and the
30 translation of such mRNA into polypeptide(s). If the final desired product is a biochemical, expression includes the creation of that biochemical and any precursors. Expression of a gene produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, small interfering RNA (siRNA), a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further

include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

- 5 A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transformed with virus expression
10 vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids); or animal cell systems.

- To express the peptide, polypeptide or fusion protein (hereinafter referred to as "product") in a host cell, a procedure such as the following can be used. A restriction fragment containing a
15 DNA sequence that encodes said product may be cloned into an appropriate recombinant plasmid containing an origin of replication that functions in the host cell and an appropriate selectable marker. The plasmid may include a promoter for inducible expression of the product (*e.g.*, pTrc (Amann et al, *Gene* 69 (1988), 301 315) and pET1 Id (Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, Calif. (1990), 60 89).
20 The recombinant plasmid may be introduced into the host cell by, for example, electroporation and cells containing the recombinant plasmid may be identified by selection for the marker on the plasmid. Expression of the product may be induced and detected in the host cell using an assay specific for the product.

- 25 In some embodiments, the DNA that encodes the product/peptide may be optimized for expression in the host cell. For example, the DNA may include codons for one or more amino acids that are predominant in the host cell relative to other codons for the same amino acid.

- Alternatively, the expression of the product may be performed by *in vitro* synthesis of the
30 protein in cell-free extracts which are also particularly suited for the incorporation of modified or unnatural amino acids for functional studies; see also *infra*. The use of *in vitro* translation systems can have advantages over *in vivo* gene expression when the over-expressed product is toxic to the host cell, when the product is insoluble or forms inclusion bodies, or when the protein undergoes rapid proteolytic degradation by intracellular proteases. The most frequently

used cell-free translation systems consist of extracts from rabbit reticulocytes, wheat germ and *Escherichia coli*. All are prepared as crude extracts containing all the macromolecular components (70S or 80S ribosomes, tRNAs, aminoacyl-tRNA synthetases, initiation, elongation and termination factors, etc.) required for translation of exogenous RNA. To ensure efficient translation, each extract must be supplemented with amino acids, energy sources (ATP, GTP), energy regenerating systems (creatine phosphate and creatine phosphokinase for eukaryotic systems, and phosphoenol pyruvate and pyruvate kinase for the *E. coli* lysate), and other co-factors known in the art (Mg^{2+} , K^{+} , etc.). Appropriate transcription/translation systems are commercially available, for example from Promega Corporation, Roche Diagnostics, and Ambion, *i.e.* Applied Biosystems (Anderson, C. et al., *Meth. Enzymol.* 101 (1983), 635–644; Arduengo, M. et al. (2007), *The Role of Cell-Free Rabbit Reticulocyte Expression Systems in Functional Proteomics* in, Kudlicki, Katzen and Bennett eds., *Cell-Free Expression* Vol. 2007. Austin, Tx: Landes Bioscience, pp. 1-18; Chen and Zubay, *Meth. Enzymol.* 101 (1983), 674–90; Ezure et al., *Biotechnol. Prog.* 22 (2006), 1570–1577).

Host cells:

In respect of the present invention, host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell. Preferred fungal cells are, for example, those of the genus *Saccharomyces*, in particular those of the species *S. cerevisiae*. The term "prokaryotic" is meant to include all bacteria which can be transformed or transfected with a DNA or RNA molecules for the expression of an antibody of the invention or the corresponding immunoglobulin chains. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. The term "eukaryotic" is meant to include yeast, higher plant, insect and preferably mammalian cells, most preferably HEK 293, NSO, CSO and CHO cells. Depending upon the host employed in a recombinant production procedure, the antibodies or immunoglobulin chains encoded by the polynucleotide of the present invention may be glycosylated or may be non-glycosylated. Antibodies of the invention or the corresponding immunoglobulin chains may also include an initial methionine amino acid residue. A polynucleotide of the invention can be used to transform or transfect the host using any of the techniques commonly known to those of ordinary skill in the art. Furthermore, methods for preparing fused, operably linked genes and expressing them in, *e.g.*, mammalian cells and bacteria are well-known in the art (Sambrook, Molecular Cloning: *A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989). The genetic constructs and methods described therein can be

utilized for expression of the antibody of the invention or the corresponding immunoglobulin chains in eukaryotic or prokaryotic hosts. In general, expression vectors containing promoter sequences which facilitate the efficient transcription of the inserted polynucleotide are used in connection with the host. The expression vector typically contains an origin of replication, a promoter, and a terminator, as well as specific genes which are capable of providing phenotypic selection of the transformed cells. Suitable source cells for the DNA sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A.). Furthermore, transgenic animals, preferably mammals, comprising cells of the invention may be used for the large scale production of the antibody of the invention.

The transformed hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like; see, Scopes, "*Protein Purification*", Springer Verlag, N.Y. (1982). The antibody or its corresponding immunoglobulin chain(s) of the invention can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the, *e.g.*, recombinantly expressed antibodies or immunoglobulin chains of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies directed, *e.g.*, against the constant region of the antibody of the invention. It will be apparent to those skilled in the art that the antibodies of the invention can be further coupled to other moieties for, *e.g.*, drug targeting and imaging applications. Such coupling may be conducted chemically after expression of the antibody or antigen to site of attachment or the coupling product may be engineered into the antibody or antigen of the invention at the DNA level. The DNAs are then expressed in a suitable host system, and the expressed proteins are collected and renatured, if necessary.

Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified,

partially or to homogeneity as desired, the antibodies may then be used therapeutically (including extracorporally) or in developing and performing assay procedures.

The present invention also involves a method for producing cells capable of expressing an antibody of the invention or its corresponding immunoglobulin chain(s) comprising genetically engineering cells with the polynucleotide or with the vector of the invention. The cells obtainable by the method of the invention can be used, for example, to test the interaction of the antibody of the invention with its antigen.

10 ELISA-assays:

Enzyme-linked immunosorbent assays (ELISAs) for various antigens include those based on colorimetry, chemiluminescence, and fluorometry. ELISAs have been successfully applied in the determination of low amounts of drugs and other antigenic components in plasma and urine samples, involve no extraction steps, and are simple to carry out. ELISAs for the detection of antibodies to protein antigens often use direct binding of short synthetic peptides to the plastic surface of a microtitre plate. The peptides are, in general, very pure due to their synthetic nature and efficient purification methods using high-performance liquid chromatography. A drawback of short peptides is that they usually represent linear, but not conformational or discontinuous epitopes. To present conformational epitopes, either long peptides or the complete native protein is used. Direct binding of the protein antigens to the hydrophobic polystyrene support of the plate can result in partial or total denaturation of the bound protein and loss of conformational epitopes. Coating the plate with an antibody, which mediates the immobilization (capture ELISA) of the antigens, can avoid this effect.

25 However, frequently, overexpressed recombinant proteins are insoluble and require purification under denaturing conditions and renaturation, when antibodies to conformational epitopes are to be analyzed. See, for example, U.S. patent application No. 20030044870 for a generic ELISA using recombinant fusion proteins as coat proteins.

30 Binding molecules:

A "binding molecule" as used in the context of the present invention relates primarily to antibodies, and fragments thereof, but may also refer to other non-antibody molecules that bind to the "molecules of interest" of the present invention, wherein the molecules of interest are proteins of the class of glycoproteins known as cytokines, in particular interferons selected from

the group of different IFN- α subtypes. The molecules of interest of the present invention are defined in further detail within the description of the particular embodiments of the present invention above and below. The binding molecules of the present invention include but are not limited to hormones, receptors, ligands, major histocompatibility complex (MHC) molecules, 5 chaperones such as heat shock proteins (HSPs) as well as cell-cell adhesion molecules such as members of the cadherin, integrin, C-type lectin and immunoglobulin (Ig) superfamilies. Thus, for the sake of clarity only and without restricting the scope of the present invention most of the following embodiments are discussed with respect to antibodies and antibody-like molecules which represent the preferred binding molecules for the development of therapeutic 10 and diagnostic agents.

Antibodies:

The terms "antibody" and "immunoglobulin" are used interchangeably herein. An antibody or immunoglobulin is a molecule binding to a molecule of interest of the present invention as 15 defined hereinabove and below, which comprises at least the variable domain of a heavy chain, and normally comprises at least the variable domains of a heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are relatively well understood; see, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, (*Cold Spring Harbor Laboratory Press*, 2nd ed. 1988). The terms "binds" and "recognizes" are used interchangeably in respect of the 20 binding affinity of the binding molecules of the present invention, *e.g.*, antibodies.

Any antibody or immunoglobulin fragment which contains sufficient structure to specifically bind to the molecules of interest, as defined hereinabove and below, is denoted herein interchangeably as a "binding molecule", "binding fragment" or an "immunospecific fragment." 25

Antibodies or antigen-binding fragments, immunospecific fragments, variants, or derivatives thereof of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, murinized or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), single-chain 30 antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a V_L or V_H domain, fragments produced by a Fab expression library, and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies disclosed herein). ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019. In this respect, antigen-binding fragment of the antibody can be as well domain antibodies (dAb) also known as single domain

antibodies (sdAB) or nanobodies™ (Ablynx, Gent, Belgium), see, *e.g.*, De Haard et al., *J. Bacteriol.* 187 (2005), 4531-4541; Holt et al., *Trends Biotechnol.* 21 (2003), 484-490. As will be discussed in more detail below, the term "immunoglobulin" comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ , μ , α , δ , ϵ) with some subclasses among them (*e.g.*, $\gamma 1$ - $\gamma 4$). It is the nature of this chain that determines the "class" of the antibody as IgG, IgE, IgM, IgD, IgA, and IgY, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, etc. are well characterized and are known to confer functional specialization. Immunoglobulin or antibody molecules of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, etc.) or subclass of immunoglobulin molecule. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant invention. Although all immunoglobulin classes are clearly within the scope of the present invention, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

As evident from the classification of the exemplary anti-IFN α antibodies of the present invention enlisted in Table 1 above, the exemplary antibodies of the present invention are of the IgG1 class, possibly implicating regulatory T-cell responses and/or epithelia in their initiation in these AIRE-deficiency states. These findings are confirmed by the classification of corresponding autoantibodies found in the AIRE-deficient mice described by Kärner et al., in *Clin. Exp. Immunol.* (2012); doi: 10.1111/cei.12024. Accordingly, in a preferred embodiment of the present invention, the antibodies of the present invention are of the IgG type, even more preferred IgG1.

IgG structure:

Light chains are classified as either kappa or lambda (κ , λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to

each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

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Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (V_L) and heavy (V_H) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CL domains actually

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As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the V_L domain and V_H domain, or subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the V_H and V_L chains. Any antibody or immunoglobulin fragment which contains sufficient structure to specifically bind to a molecule of interest of the present invention is denoted herein interchangeably as a "binding

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fragment" or an "immunospecific fragment."

In naturally occurring antibodies, an antibody comprises six hypervariable regions, sometimes called "complementarity determining regions" or "CDRs" present in each antigen-binding domain, which are short, non-contiguous sequences of amino acids that are specifically

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positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The "CDRs" are flanked by four relatively conserved "framework" regions or "FRs" which show less inter-molecular variability. The framework regions largely adopt a β -sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold

that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined; see, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, *U.S. Department of Health and Human Services*, (1983); and Chothia and Lesk, *J. Mol. Biol.* 196 (1987), 901-917.

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In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, *U.S. Dept. of Health and Human Services*, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia and Lesk, *J. Mol. Biol.* 196 (1987), 901-917 where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 2 as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular hypervariable region or CDR of the human IgG subtype of antibody given the variable region amino acid sequence of the antibody.

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Table 2: CDR Definitions¹

	Kabat	Chothia
VH CDR1	31-35	26-32
VH CDR2	50-65	52-58
VH CDR3	95-102	95-102
VL CDR1	24-34	26-32
VL CDR2	50-56	50-52
VL CDR3	89-97	91-96

¹Numbering of all CDR definitions in Table 2 is according to the numbering conventions set forth by Kabat *et al.* (see below).

5 Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, *U.S. Dept. of Health and Human Services*, "Sequence of Proteins of
10 Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody or antigen-binding fragment, variant, or derivative thereof of the present invention are according to the Kabat numbering system, which however is theoretical and may not equally apply to every antibody of the present invention. For example, depending on the position of the first CDR the following CDRs might be shifted
15 in either direction.

In one embodiment, the antibody of the present invention is not IgM or a derivative thereof with a pentavalent structure. Particular, in specific applications of the present invention, especially therapeutic use, IgMs are less useful than IgG and other bivalent antibodies or
20 corresponding binding molecules since IgMs due to their pentavalent structure and lack of affinity maturation often show unspecific cross-reactivities and very low affinity.

In a particularly preferred embodiment, the antibody of the present invention is not a polyclonal antibody, *i.e.* it substantially consists of one particular antibody species rather than being a
25 mixture obtained from a plasma immunoglobulin sample.

Antibody fragments, animalization:

Antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2,
30 and CH3 domains. Also included in the invention are fragments binding to a molecule of

interest of the present invention, said fragments comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. Antibodies or immunospecific fragments thereof of the present invention equivalent to the monoclonal antibodies isolated in accordance with the method of the present invention, in particular to the human monoclonal antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be chondrichthoid in origin (*e.g.*, from sharks).

In a particularly preferred embodiment of the present invention, the antibodies are naturally occurring human monoclonal antibodies or binding fragments, derivatives and variants thereof cloned from human subjects, which bind specifically to specific IFN α subtypes of the present invention, as defined in detail above and below, *e.g.*, in Table 1, the Figures, in particular Figures 1 to 4 and in the Examples, *e.g.*, in Examples 2 and 6.

Optionally, the framework region of the human antibody is aligned and adopted in accordance with the pertinent human germ line variable region sequences in the database; see, *e.g.*, Vbase (<http://vbase.mrc-cpe.cam.ac.uk/>) hosted by the MRC Centre for Protein Engineering (Cambridge, UK). For example, amino acids considered to potentially deviate from the true germ line sequence could be due to the PCR primer sequences incorporated during the cloning process. Compared to artificially generated human-like antibodies such as single chain antibody fragments (scFvs) from a phage displayed antibody library or xenogeneic mice the human monoclonal antibody of the present invention is characterized by (i) being obtained using the human immune response rather than that of animal surrogates, *i.e.* the antibody has been generated in response to natural IFN α subtypes in their relevant conformation in the human body, (ii) having protected the individual from or minimized at least significant the presence of symptoms of a disease, *e.g.*, SLE, and (iii) since the antibody is of human origin the risks of cross-reactivity against self-antigens is minimized. Thus, in accordance with the present invention the terms "human monoclonal antibody", "human monoclonal autoantibody", "human antibody" and the like are used to denote a IFN- α binding molecule of a particular IFN- α subtype specificity which is of human origin, *i.e.* which has been isolated from a human cell such as a B cell or hybridoma thereof or the cDNA of which has been directly cloned from mRNA of a human cell, for example a human memory B cell. A human antibody is still

considered as "human" even if amino acid substitutions are made in the antibody, *e.g.*, to improve its binding characteristics.

Antibodies derived from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described *infra* and, for example in, US patent no 5,939,598 by Kucherlapati *et al.*, are denoted human-like antibodies in order distinguish them from truly human antibodies of the present invention.

For example, the paring of heavy and light chains of human-like antibodies such as synthetic and semi-synthetic antibodies typically isolated from phage display do not necessarily reflect the original paring as it occurred in the original human B cell. Accordingly Fab and scFv fragments obtained from recombinant expression libraries as commonly used in the prior art can be considered as being artificial with all possible associated effects on immunogenicity and stability.

In contrast, the present invention provides isolated affinity-matured antibodies from selected human subjects, which are characterized by their therapeutic utility.

Grafted antibodies (equivalents)

The invention also relates to grafted antibodies (interchangeably referred to as equivalents) containing CDRs derived from the antibodies of the present invention, such as anti-IFN- α antibodies, respectively. Such grafted CDRs include animalized antibodies, in which CDRs from the antibodies of the present invention have been grafted or in which a CDR containing one or more amino acid substitutions is grafted. The CDRs can be grafted directly into a human framework or an antibody framework from animal origin as indicated above. If desired, framework changes can also be incorporated by generating framework libraries. The optimization of CDRs and/or framework sequences can be performed independently and sequentially combined or can be performed simultaneously, as described in more detail below.

To generate grafted antibodies donor CDRs of the antibodies of the present invention are grafted onto an antibody acceptor variable region framework. Methods for grafting antibodies and generating CDR variants to optimize activity have been described previously (see, *e.g.*, international patent applications WO 98/33919; WO 00/78815; WO 01/27160). The procedure

can be performed to achieve grafting of donor CDRs and affinity reacquisition in a simultaneous process. The methods similarly can be used, either alone or in combination with CDR grafting, to modify or optimize the binding affinity of a variable region. The methods for conferring donor CDR binding affinity onto an acceptor variable region are applicable to both heavy and light chain variable regions and as such can be used to simultaneously graft and optimize the binding affinity of an antibody variable region.

The donor CDRs can be altered to contain a plurality of different amino acid residue changes at all or selected positions within the donor CDRs. For example, random or biased incorporation of the twenty naturally occurring amino acid residues, or preselected subsets, can be introduced into the donor CDRs to produce a diverse population of CDR species. Inclusion of CDR variant species into the diverse population of variable regions allows for the generation of variant species that exhibit optimized binding affinity for a predetermined antigen. A range of possible changes can be made in the donor CDR positions. Some or all of the possible changes that can be selected for change can be introduced into the population of grafted donor CDRs. A single position in a CDR can be selected to introduce changes or a variety of positions having altered amino acids can be combined and screened for activity.

One approach is to change all amino acid positions along a CDR by replacement at each position with, for example, all twenty naturally occurring amino acids. The replacement of each position can occur in the context of other donor CDR amino acid positions so that a significant portion of the CDR maintains the authentic donor CDR sequence, and therefore, the binding affinity of the donor CDR. For example, an acceptor variable region framework, either a native or altered framework, can be grafted with a population of CDRs containing single position replacements at each position within the CDRs. Similarly, an acceptor variable region framework can be targeted for grafting with a population of CDRs containing more than one position changed to incorporate all twenty amino acid residues, or a subset of amino acids. One or more amino acid positions within a CDR, or within a group of CDRs to be grafted, can be altered and grafted into an acceptor variable region framework to generate a population of grafted antibodies. It is understood that a CDR having one or more altered positions can be combined with one or more other CDRs having one or more altered positions, if desired.

A population of CDR variant species having one or more altered positions can be combined with any or all of the CDRs which constitute the binding pocket of a variable region. Therefore,

an acceptor variable region framework can be targeted for the simultaneous incorporation of donor CDR variant populations at one, two or all three recipient CDR locations in a heavy or light chain. The choice of which CDR or the number of CDRs to target with amino acid position changes will depend on, for example, if a full CDR grafting into an acceptor is desired or whether the method is being performed for optimization of binding affinity.

Another approach for selecting donor CDR amino acids to change for conferring donor CDR binding affinity onto an antibody acceptor variable region framework is to select known or readily identifiable CDR positions that are highly variable. For example, the variable region CDR3 is generally highly variable. This region therefore can be selectively targeted for amino acid position changes during grafting procedures to ensure binding affinity reacquisition or augmentation, either alone or together with relevant acceptor variable framework changes.

Murinized antibodies:

An example of antibodies generated by grafting, as described above, are murinized antibodies. As used herein, the term "murinized antibody" or "murinized immunoglobulin" refers to an antibody comprising one or more CDRs from a human antibody of the present invention; and a human framework region that contains amino acid substitutions and/or deletions and/or insertions that are based on a mouse antibody sequence. The human immunoglobulin providing the CDRs is called the "parent" or "acceptor" and the mouse antibody providing the framework changes is called the "donor". Constant regions need not be present, but if they are, they are usually substantially identical to mouse antibody constant regions, *i.e.* at least about 85- 90%, preferably about 95% or more identical. Hence, in some embodiments, a full-length murinized human heavy or light chain immunoglobulin contains a mouse constant region, human CDRs, and a substantially human framework that has a number of "murinizing" amino acid substitutions. Typically, a "murinized antibody" is an antibody comprising a murinized variable light chain and/or a murinized variable heavy chain. For example, a murinized antibody would not encompass a typical chimeric antibody, *e.g.*, because the entire variable region of a chimeric antibody is non-mouse. A modified antibody that has been "murinized" by the process of "murinization" binds to the same antigen as the parent antibody that provides the CDRs and is usually less immunogenic in mice, as compared to the parent antibody.

Antibody fragments:

As used herein, the term "heavy chain portion" includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain portion comprises at least one of: a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, a binding polypeptide for use in the invention may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the invention comprises a polypeptide chain comprising a CH3 domain. Further, a binding polypeptide for use in the invention may lack at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain portions) may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

In certain antibodies, or antigen-binding fragments, variants, or derivatives thereof disclosed herein, the heavy chain portions of one polypeptide chain of a multimer are identical to those on a second polypeptide chain of the multimer. Alternatively, heavy chain portion-containing monomers of the invention are not identical. For example, each monomer may comprise a different target binding site, forming, for example, a bispecific antibody or diabody.

In another embodiment, the antibodies, or antigen-binding fragments, variants, or derivatives thereof disclosed herein are composed of a single polypeptide chain such as scFvs and are to be expressed intracellularly (intrabodies) for potential *in vivo* therapeutic and diagnostic applications.

The heavy chain portions of a binding polypeptide for use in the diagnostic and treatment methods disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain portion of a polypeptide may comprise a CH1 domain derived from an IgG1 molecule and a hinge region derived from an IgG3 molecule. In another example, a heavy chain portion can comprise a hinge region derived, in part, from an IgG1 molecule and, in part,

from an IgG3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG1 molecule and, in part, from an IgG4 molecule.

Thus, as also exemplified in the Examples, in one embodiment the constant region of the antibody of the present invention or part thereof, in particular the CH2 and/or CH3 domain but optionally also the CH1 domain is heterologous to the variable region of the native human monoclonal antibody isolated in accordance with the method of the present invention. In this context, the heterologous constant region(s) are preferably of human origin in case of therapeutic applications of the antibody of the present invention but could also be of for example rodent origin in case of animal studies; see also the Examples.

As used herein, the term "light chain portion" includes amino acid sequences derived from an immunoglobulin light chain. Preferably, the light chain portion comprises at least one of a V_L or C_L domain.

As previously indicated, the subunit structures and three dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term "V_H domain" includes the amino terminal variable domain of an immunoglobulin heavy chain and the term "CH1 domain" includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the V_H domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

As used herein the term "CH2 domain" includes 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 EA *et al. op. cit.*). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

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; see Roux *et al.*, *J. Immunol.* 161 (1998), 4083.

As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CL regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

As used herein, the terms "linked", "fused" or "fusion" are used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more polynucleotide open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the correct translational reading frame of the original ORFs. Thus, a recombinant fusion protein is a single protein containing two or more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature). Although the reading frame is thus made continuous throughout the fused segments, the segments may be physically or spatially separated by, for example, in-frame linker sequence. For example, polynucleotides encoding the CDRs of an immunoglobulin variable region may be fused, in-frame, but be separated by a polynucleotide encoding at least one immunoglobulin framework region or additional CDR regions, as long as the "fused" CDRs are co-translated as part of a continuous polypeptide. Accordingly, in one embodiment the polynucleotide is a cDNA encoding the variable region and at least part of the constant domain. In one embodiment the polynucleotide is a cDNA encoding the variable region and the constant domain of an antibody of the present invention as defined herein.

Epitopes:

The minimum size of a peptide or polypeptide epitope for an antibody is thought to be about four to five amino acids. Peptide or polypeptide epitopes preferably contain at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids. Since a CDR can recognize an antigenic peptide or polypeptide in its tertiary form, the amino acids comprising an epitope need not be contiguous, and in some cases, may not even be on the same peptide chain. In the present invention, a peptide or polypeptide epitope recognized by

antibodies of the present invention contains a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, between about 15 to about 30 or between about 30 to about 50 contiguous or non-contiguous amino acids of a molecule of interest of the present invention, *i.e.* at least one IFNA subtype, or the homologous sequences of the other IFNA subtypes, in case the antibody recognizes more than one subtype. For mapping of epitopes of IFNA2 for the exemplary antibodies 19D11 and 26B9 and of epitope of IFNW for the exemplary antibody 26B9 of the present invention see Fig. 27.

Binding characteristics:

By "binding" or "recognizing", used interchangeably herein, it is generally meant that a binding molecule, *e.g.*, an antibody binds to a predetermined epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" may be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D". Unrelated epitopes are usually part of a nonspecific antigen (*e.g.*, BSA, casein, or any other specified polypeptide), which may be used for the estimation of the binding specificity of a given binding molecule. In this respect, term "specific binding" refers to antibody binding to a predetermined antigen with a K_D that is at least twofold less than its K_D for binding to a nonspecific antigen. The term "highly specific" binding as used herein means that the relative K_D of the antibody for the specific target epitope is at least 10-fold less than the K_D for binding that antibody to other ligands.

Where present, the term "immunological binding characteristics," or other binding characteristics of an antibody with an antigen, in all of its grammatical forms, refers to the specificity, affinity, cross-reactivity, and other binding characteristics of an antibody.

By "preferentially binding", it is meant that the binding molecule, *e.g.*, antibody specifically binds to an epitope more readily than it would bind to a related, similar, homologous, or analogous epitope. Thus, an antibody which "preferentially binds" to a given epitope would more likely bind to that epitope than to a related epitope, even though such an antibody may

cross-react with the related epitope. In respect of particular antigens, such as specific IFNA subtypes the term "preferentially binding" means that the binding molecule, *e.g.*, antibody specifically binds to an IFNA subtype more readily than it would bind to a related, similar, homologous, or analogous IFNA subtype.

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By way of non-limiting example, a binding molecule, *e.g.*, an antibody may be considered to bind a first epitope preferentially if it binds said first epitope with dissociation constant (K_D) that is less than the antibody's K_D for the second epitope. In another non-limiting example, an antibody may be considered to bind a first antigen preferentially if it binds the first epitope with an affinity that is at least one order of magnitude less than the antibody's K_D for the second epitope. In another non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least two orders of magnitude less than the antibody's K_D for the second epitope.

15 In another non-limiting example, a binding molecule, *e.g.*, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an off rate ($k(\text{off})$) that is less than the antibody's $k(\text{off})$ for the second epitope. In another non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least one order of magnitude less than the antibody's $k(\text{off})$ for the second epitope. In another non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least two orders of magnitude less than the antibody's $k(\text{off})$ for the second epitope.

25 A binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative disclosed herein may be said to bind a molecule of interest of the present invention, a fragment or variant thereof with an off rate ($k(\text{off})$) of less than or equal to $5 \times 10^{-2} \text{ sec}^{-1}$, 10^{-2} sec^{-1} , $5 \times 10^{-3} \text{ sec}^{-1}$ or 10^{-3} sec^{-1} . More preferably, an antibody of the invention may be said to bind a molecule of interest of the present invention or a fragment or variant thereof with an off rate ($k(\text{off})$) less than or equal to $5 \times 10^{-4} \text{ sec}^{-1}$, 10^{-4} sec^{-1} , $5 \times 10^{-5} \text{ sec}^{-1}$, or 10^{-5} sec^{-1} $5 \times 10^{-6} \text{ sec}^{-1}$, 10^{-6} sec^{-1} , $5 \times 10^{-7} \text{ sec}^{-1}$ or 10^{-7} sec^{-1} .

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A binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative disclosed herein may be said to bind a molecule of interest of the present invention or a fragment or variant thereof with an on rate ($k(\text{on})$) of greater than or equal to $10^3 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^3 \text{ M}^{-1}$

sec⁻¹, 10⁴ M⁻¹ sec⁻¹ or 5 x 10⁴ M⁻¹ sec⁻¹. More preferably, an antibody of the invention may be said to bind a molecule of interest of the present invention or a fragment or variant thereof with an on rate (k(on)) greater than or equal to 10⁵ M⁻¹ sec⁻¹, 5 x 10⁵ M⁻¹ sec⁻¹, 10⁶ M⁻¹ sec⁻¹, or 5 x 10⁶ M⁻¹ sec⁻¹ or 10⁷ M⁻¹ sec⁻¹.

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A binding molecule, *e.g.*, an antibody is said to competitively inhibit binding of a reference antibody to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody to the epitope. Competitive inhibition may be determined by any method known in the art, for example, competition ELISA assays. An antibody may be said to competitively inhibit binding of the reference antibody to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with the CDR of a binding molecule, *e.g.*, an immunoglobulin molecule; *see, e.g.*, Harlow *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 2nd ed. (1988) at pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a population of immunoglobulins and an antigen, that is, the functional combining strength of an immunoglobulin mixture with the antigen; *see, e.g.*, Harlow at pages 29-34. Avidity is related to both the affinity of individual immunoglobulin molecules in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity. The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method; *see, for example*, Berzofsky *et al.*, "Antibody-Antigen Interactions" In *Fundamental Immunology*, Paul, W. E., Ed., Raven Press New York, N Y (1984), Kuby, Janis *Immunology*, W. H. Freeman and Company New York, N Y (1992), and methods described therein. General techniques for measuring the affinity of an antibody for an antigen include ELISA, RIA, and surface plasmon resonance. The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions, *e.g.*, salt concentration, pH. Thus, measurements of affinity and other antigen-binding parameters, *e.g.*, K_D, IC₅₀, are preferably made with standardized solutions of antibody and antigen, and a standardized buffer.

Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants or derivatives thereof of the invention may also be described or specified in terms of their cross-reactivity. As

used herein, the term "cross-reactivity" refers to the ability of an antibody, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, an antibody is cross reactive if it binds to an epitope other than the one that induced its formation. The cross reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, may actually fit better than the original.

For example, certain antibodies have some degree of cross-reactivity, in that they bind related, but non-identical epitopes, *e.g.*, epitopes with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a reference epitope. An antibody may be said to have little or no cross-reactivity if it does not bind epitopes with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a reference epitope. An antibody may be deemed "highly specific" for a certain epitope, if it does not bind any other analog, ortholog, or homolog of that epitope.

Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants or derivatives thereof of the invention may also be described or specified in terms of their binding affinity to a molecule of interest of the present invention. Preferred binding affinities include those with a dissociation constant or K_D less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M. Typically, the antibody binds with a dissociation constant (K_D) of 10^{-7} M or less to its predetermined antigen. Preferably, the antibody binds its cognate antigen with a dissociation constant (K_D) of 10^{-9} M or less and still more preferably with a dissociation constant (K_D) of 10^{-11} M or less.

Modifications of antibodies:

The immunoglobulin or its encoding cDNAs may be further modified. Thus, in a further embodiment the method of the present invention comprises any one of the step(s) of producing a chimeric antibody, humanized antibody, single-chain antibody, Fab-fragment, bi-specific antibody, fusion antibody, labeled antibody or an analog of any one of those. Corresponding

methods are known to the person skilled in the art and are described, *e.g.*, in Harlow and Lane "Antibodies, A Laboratory Manual", *CSH Press, Cold Spring Harbor*, 1988. When derivatives of said antibodies are obtained by the phage display technique, surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies which bind to the same epitope as that of any one of the antibodies provided by the present invention (Schier, *Human Antibodies Hybridomas* 7 (1996), 97-105; Malmborg, *J. Immunol. Methods* 183 (1995), 7-13). The production of chimeric antibodies is described, for example, in international application WO89/09622. Methods for the production of humanized antibodies are described in, *e.g.*, European application EP-A1 0 239 400 and international application WO90/07861. Further sources of antibodies to be utilized in accordance with the present invention are so-called xenogeneic antibodies. The general principle for the production of xenogeneic antibodies such as human antibodies in mice is described in, *e.g.*, international applications WO91/10741, WO94/02602, WO96/34096 and WO 96/33735. As discussed above, the antibody of the invention may exist in a variety of forms besides complete antibodies; including, for example, Fv, Fab and F(ab)₂, as well as in single chains; see *e.g.* international application WO88/09344.

The antibodies of the present invention or their corresponding immunoglobulin chain(s) can be further modified using conventional techniques known in the art, for example, by using amino acid deletion(s), insertion(s), substitution(s), addition(s), and/or recombination(s) and/or any other modification(s) known in the art either alone or in combination. Methods for introducing such modifications in the DNA sequence underlying the amino acid sequence of an immunoglobulin chain are well known to the person skilled in the art; see, *e.g.*, Sambrook, *Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory* (1989) N.Y. and Ausubel, *Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience*, N.Y. (1994). Modifications of the antibody of the invention include chemical and/or enzymatic derivatizations at one or more constituent amino acids, including side chain modifications, backbone modifications, and N- and C-terminal modifications including acetylation, hydroxylation, methylation, amidation, and the attachment of carbohydrate or lipid moieties, cofactors, and the like. Likewise, the present invention encompasses the production of chimeric proteins which comprise the described antibody or some fragment thereof at the amino terminus fused to heterologous molecule such as a label or a drug. Antigen binding molecules generated this way may be used for drug localization to cells expressing the appropriate surface structures of the diseased cell and tissue, respectively. This targeting and

binding to cells could be useful for the delivery of therapeutically or diagnostically active agents and gene therapy/gene delivery. Molecules/particles with an antibody of the invention would bind specifically to cells/tissues expressing the particular antigen of interest, and therefore could have diagnostic and therapeutic use.

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Samples:

As used herein, the term "sample" or "biological sample" refers to any biological material obtained from a subject or patient. In one aspect, a sample can comprise blood, cerebrospinal fluid ("CSF"), or urine. In other aspects, a sample can comprise whole blood, plasma,
 10 mononuclear cells enriched from peripheral blood (PBMC) such as lymphocytes (*i.e.* T-cells, NK-cell or B-cells), monocytes, macrophages, dendritic cells and basophils; and cultured cells (*e.g.*, B-cells from a subject). A sample can also include a biopsy or tissue sample including tumor tissue. In still other aspects, a sample can comprise whole cells and/or a lysate of the cells. In one embodiment a sample comprises peripheral blood mononuclear cells (PBMC).
 15 Samples can be collected by methods known in the art.

Identification of anti-IFN- α antibodies, isolation of corresponding B cells and recombinant expression of anti-IFN- α antibodies:

Identification of B-cells specific for the anti-IFN- α antibodies of the present invention, as
 20 enlisted in Table 1, and as exemplary shown for several IFN- α subtypes and molecular cloning of antibodies displaying specificity of interest as well as their recombinant expression and functional characterization can be generally performed as described in the international applications WO2013/098419 and WO2013/098420; see Examples sections therein, in particular Examples 1 and 2 on pages 118 to 120 of WO2013/098419 and Examples 1 to 4 on
 25 pages 27 to 31 of WO2013/098420.

Briefly, in one embodiment of the present invention cultures of single or oligoclonal B-cells were cultured and the supernatant of the culture, which contains antibodies produced by said B-cells was screened for presence and affinity of antibodies specific for one or more of the IFN-
 30 α subtypes, as described in the Examples. In another embodiment, patient sera were first screened for the presence of autoantibodies against IFN- α subtypes and then those with high titer were selected for peripheral blood mononuclear cells isolation; see Example 2 on pages 118-120 of WO2013/098419. The screening process comprises screening for binding on fragments, peptides or derivatives of IFN- α subtypes. Subsequently, the antibody for which

binding is detected or the cell producing said antibody were isolated; see Example 3 on page 120 of WO2013/098419. Thus, a preliminary screen can be done on a panel of candidate donors, using samples containing antibody secreting cells (such as total peripheral blood or serum). In particular, mononuclear cells can be isolated from blood or lymphatic tissues using standard separation techniques for isolating peripheral blood mononuclear cells (PBMCs), such as gradient centrifugation. After and/or before this separation step, the samples of sera (or plasma), cell culture supernatants, or cells (obtained from different patients, from different tissues, and/or at different time points) can be prescreened using standard technologies for detecting the presence of antibodies and antibody-secreting cells (*e.g.* ELISA, BIACORE, Western blot, FACS, SERPA, antigen arrays, neutralization of viral infection in a cell culture system, or ELISPOT assays). The literature provides several examples of these technologies showing, for example, the use of ELISPOT for characterizing the immune response in vaccinated donors (Crotty et al., *Immunol Meth.* 286 (2004), 111-122), the use of antigen microarrays as diagnostic tools for newly infected patients (Mezzasoma et al., *Clin Chem.* 48 (2002), 121-130, and other technologies for measuring antigen-specific immune responses (Kern et al., *Trends Immunol.* 26 (2005), 477-484).

After identification of candidate anti-IFN- α antibodies and B cells secreting them, respectively, the nucleic acid sequence that encodes the antibody of interest is obtained, comprising the steps of preparing a B cell and obtaining/sequencing nucleic acid from the B cell that encodes the antibody of interest and further inserting the nucleic acid into or using the nucleic acid to prepare an expression host that can express the antibody of interest, culturing or sub-culturing the expression host under conditions where the antibody of interest is expressed and, optionally, purifying the antibody of interest. It goes without saying that the nucleic acid may be manipulated in between to introduce restriction sites, to change codon usage, and/or to add or optimize transcription and/or translation regulatory sequences. These techniques are state of the art and can be performed by the person skilled in the art without undue burden. For example, the heavy chain constant region can be exchanged for that of a different isotype or eliminated altogether. The variable regions can be linked to encode single chain Fv regions. Multiple Fv regions can be linked to confer binding ability to more than one target or chimeric heavy and light chain combinations can be employed. Once the genetic material is available, design of analogs as described above which retain both their ability to bind the desired target is straightforward. Methods for the cloning of antibody variable regions and generation of recombinant antibodies are known to the person skilled in the art and are described, for

example, in Gilliland *et al.*, *Tissue Antigens* 47 (1996), 1-20; Doenecke *et al.*, *Leukemia* 11 (1997), 1787-1792. In a preferred embodiment of the present invention however, B cells are obtained and the corresponding antibody is expressed by the methods described in international application WO2013/098420, in particular in Example 3, on pages 28-30 therein.

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Diseases and disorders:

Unless stated otherwise, the terms "disorder" and "disease" are used interchangeably herein.

The term "autoimmune disorder" as used herein is a disease or disorder arising from and directed against an individual's own tissues or organs or a co-segregate or manifestation thereof or resulting condition therefrom. Autoimmune diseases are primarily caused by dysregulation of adaptive immune responses and autoantibodies or autoreactive T cells against self-structures are formed. Nearly all autoimmune diseases have an inflammatory component, too. Autoinflammatory diseases are primarily inflammatory, and some classic autoinflammatory diseases are caused by genetic defects in innate inflammatory pathways. In autoinflammatory diseases, no autoreactive T cells or autoantibodies are found. In many of these autoimmune and autoinflammatory disorders, a number of clinical and laboratory markers may exist, including, but not limited to, hypergammaglobulinemia, high levels of autoantibodies, antigen-antibody complex deposits in tissues, benefit from corticosteroid or immunosuppressive treatments, and lymphoid cell aggregates in affected tissues. Without being limited to a theory regarding B-cell mediated autoimmune disorder, it is believed that B cells demonstrate a pathogenic effect in human autoimmune diseases through a multitude of mechanistic pathways, including autoantibody production, immune complex formation, dendritic and T-cell activation, cytokine synthesis, direct chemokine release, and providing a nidus for ectopic neo-lymphogenesis. Each of these pathways may participate to different degrees in the pathology of autoimmune diseases.

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As used herein, an "autoimmune disorder" can be an organ-specific disease (*i.e.*, the immune response is specifically directed against an organ system such as the endocrine system, the hematopoietic system, the skin, the cardiopulmonary system, the gastrointestinal and liver systems, the renal system, the thyroid, the ears, the neuromuscular system, the central nervous system, etc.) or a systemic disease that can affect multiple organ systems (for example, systemic lupus erythematosus (SLE), rheumatoid arthritis, polymyositis, autoimmune

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polyendocrinopathy syndrome type 1 (APS1)/autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) etc. Preferred such diseases include autoimmune rheumatologic disorders (such as, for example, rheumatoid arthritis, Sjogren's syndrome, scleroderma, lupus such as SLE and lupus nephritis, polymyositis/dermatomyositis, and psoriatic arthritis), autoimmune dermatologic disorders (such as, for example, psoriasis, pemphigus group diseases, bullous pemphigoid diseases, and cutaneous lupus erythematosus), and autoimmune endocrine disorders (such as, for example, diabetic-related autoimmune diseases such as type 1 or insulin dependent diabetes mellitus (T1DM or IDDM), autoimmune thyroid disease (e.g., Graves' disease and thyroiditis)) and diseases affecting the generation of autoimmunity such as autoimmune polyendocrinopathy syndrome type 1 (APS1)/autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) Myasthenia Gravis (MG/Thymoma).

Preferred diseases include, for example, SLE, RA, T1DM, MS, Sjogren's syndrome, Graves' disease, thyroiditis, and glomerulonephritis, and APS1. Still more preferred are RA, SLE, and MS, and mostly preferred SLE.

Labels and diagnostics:

Labeling agents can be coupled either directly or indirectly to the antibodies or antigens of the invention. One example of indirect coupling is by use of a spacer moiety. Furthermore, the antibodies of the present invention can comprise a further domain, said domain being linked by covalent or non-covalent bonds. The linkage can be based on genetic fusion according to the methods known in the art and described above or can be performed by, e.g., chemical cross-linking as described in, e.g., international application WO94/04686. The additional domain present in the fusion protein comprising the antibody of the invention may preferably be linked by a flexible linker, advantageously a polypeptide linker, wherein said polypeptide linker comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of said further domain and the N-terminal end of the antibody of the invention or vice versa. The therapeutically or diagnostically active agent can be coupled to the antibody of the invention or an antigen-binding fragment thereof by various means. This includes, for example, single-chain fusion proteins comprising the variable regions of the antibody of the invention coupled by covalent methods, such as peptide linkages, to the therapeutically or diagnostically active agent. Further examples include molecules which comprise at least an antigen-binding fragment coupled to additional molecules covalently or

non-covalently include those in the following non-limiting illustrative list. Traunecker, *Int. J. Cancer Surp. SuDP* 7 (1992), 51-52, describe the bispecific reagent janusin in which the Fv region directed to CD3 is coupled to soluble CD4 or to other ligands such as OVCA and IL-7. Similarly, the variable regions of the antibody of the invention can be constructed into Fv molecules and coupled to alternative ligands such as those illustrated in the cited article. Higgins, *J. Infect. Disease* 166 (1992), 198-202, described a hetero-conjugate antibody composed of OKT3 cross-linked to an antibody directed to a specific sequence in the V3 region of GP120. Such hetero-conjugate antibodies can also be constructed using at least the variable regions contained in the antibody of the invention methods. Additional examples of specific antibodies include those described by Fanger, *Cancer Treat. Res.* 68 (1993), 181-194 and by Fanger, *Crit. Rev. Immunol.* 12 (1992), 101-124. Conjugates that are immunotoxins including conventional antibodies have been widely described in the art. The toxins may be coupled to the antibodies by conventional coupling techniques or immunotoxins containing protein toxin portions can be produced as fusion proteins. The antibodies of the present invention can be used in a corresponding way to obtain such immunotoxins. Illustrative of such immunotoxins are those described by Byers, *Seminars Cell. Biol.* 2 (1991), 59-70 and by Fanger, *Immunol. Today* 12 (1991), 51-54.

The above described fusion protein may further comprise a cleavable linker or cleavage site for proteinases. These spacer moieties, in turn, can be either insoluble or soluble (Diener *et al.*, *Science* 231 (1986), 148) and can be selected to enable drug release from the antigen at the target site. Examples of therapeutic agents which can be coupled to the antibodies and antigens of the present invention for immunotherapy are chemokines, homing molecules, drugs, radioisotopes, lectins, and toxins. The drugs with which can be conjugated to the antibodies and antigens of the present invention depend on the disease context in which the conjugated molecules are intended to be used. For example, antibodies specific for targets useful in treatment of tumor diseases can be conjugated to compounds which are classically referred to as anti-neoplastic drugs such as mitomycin C, daunorubicin, and vinblastine. In using radioisotopically conjugated antibodies or antigens of the invention for, *e.g.*, tumor immunotherapy, certain isotopes may be more preferable than others depending on such factors as leukocyte distribution as well as stability and emission. Depending on the autoimmune response, some emitters may be preferable to others. In general, α and β particle emitting radioisotopes are preferred in immunotherapy. Preferred are short range, high energy α emitters such as ^{212}Bi . Examples of radioisotopes which can be bound to the antibodies or antigens of

the invention for therapeutic purposes are ^{125}I , ^{131}I , ^{90}Y , ^{67}Cu , ^{212}Bi , ^{212}At , ^{211}Pb , ^{47}Sc , ^{109}Pd and ^{188}Re . Other therapeutic agents which can be coupled to the antibody or antigen of the invention, as well as ex vivo and in vivo therapeutic protocols, are known, or can be easily ascertained, by those of ordinary skill in the art. Non-limiting examples of suitable radionuclides for labeling
5 are ^{198}Au , ^{212}Bi , ^{11}C , ^{14}C , ^{57}Co , ^{67}Cu , ^{18}F , ^{67}Ga , ^{68}Ga , ^3H , ^{197}Hg , ^{166}Ho , ^{111}In , $^{113\text{m}}\text{In}$, ^{123}I , ^{125}I , ^{127}I , ^{131}I , ^{111}In , ^{177}Lu , ^{15}O , ^{13}N , ^{32}P , ^{33}P , ^{203}Pb , ^{186}Re , ^{188}Re , ^{105}Rh , ^{97}Ru , ^{35}S , ^{153}Sm and $^{99\text{m}}\text{Tc}$. Other molecules suitable for labeling are a fluorescent or luminescent dye, a magnetic particle, a metal, and a molecule which may be detected through a secondary enzymatic or binding step such as an enzyme or peptide tag. Commercial fluorescent probes suitable for use as labels in
10 the present invention are listed in the Handbook of Fluorescent Probes and Research Products, 8th Edition. Magnetic particles suitable for use in magnetic particle-based assays (MPAs) may be selected from paramagnetic, diamagnetic, ferromagnetic, ferromagnetic and superpara-magnetic materials.

15 General methods in molecular and cellular biochemistry useful for diagnostic purposes can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook *et al.*, Harbor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel *et al.* eds., John Wiley & Sons 1999); Protein Methods (Bollag *et al.*, John Wiley & Sons 1996). Reagents, detection means and kits for diagnostic purposes are available from
20 commercial vendors such as Pharmacia Diagnostics, Amersham, BioRad, Stratagene, Invitrogen, and Sigma-Aldrich as well as from the sources given any one of the references cited herein, in particular patent literature.

Treatment and drugs:

25 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 development of an autoimmune and/or autoinflammatory disease. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not
30 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 manifestation of the condition or disorder is to be prevented.

If not stated otherwise the term "drug," "medicine," or "medicament" are used interchangeably herein and shall include but are not limited to all (A) articles, medicines and preparations for internal or external use, and any substance or mixture of substances intended to be used for diagnosis, cure, mitigation, treatment, or prevention of disease of either man or other animals; and (B) articles, medicines and preparations (other than food) intended to affect the structure or any function of the body of man or other animals; and (C) articles intended for use as a component of any article specified in clause (A) and (B). The term "drug," "medicine," or "medicament" shall include the complete formula of the preparation intended for use in either man or other animals containing one or more "agents," "compounds", "substances" or "(chemical) compositions" as and in some other context also other pharmaceutically inactive excipients as fillers, disintegrants, lubricants, glidants, binders or ensuring easy transport, disintegration, disaggregation, dissolution and biological availability of the "drug," "medicine," or "medicament" at an intended target location within the body of man or other animals, *e.g.*, at the skin, in the stomach or the intestine. The terms "agent," "compound" or "substance" are used interchangeably herein and shall include, in a more particular context, but are not limited to all pharmacologically active agents, *i.e.* agents that induce a desired biological or pharmacological effect or are investigated or tested for the capability of inducing such a possible pharmacological effect by the methods of the present invention.

Examples of "anti-rheumatic drugs" and immunosuppressive drugs include chloroquine, hydroxychloroquine, myocrisin, auranofm, sulfasalazine, methotrexate, leflunomide, etanercept, infliximab (plus oral and subcutaneous methotrexate), adalimumab etc., azathioprine, D-penicilamine, gold salts (oral), gold salts (intramuscular), minocycline, cyclosporine including cyclosporine A and topical cyclosporine, tacrolimus, mycophenolate mofetil, cyclophosphamide, staphylococcal protein A (Goodyear and Silverman, J. Exp. Med., 197 (2003), 125-39), including salts and derivatives thereof, etc.

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Examples of "non-steroidal anti-inflammatory drugs" or "NSAIDs" include aspirin, acetylsalicylic acid, ibuprofen and ibuprofen retard, fenoprofen, piroxicam, flurbiprofen, naproxen, ketoprofen, naproxen, tenoxicam, benorylate, diclofenac, naproxen, nabumetone, indomethacin, ketoprofen, mefenamic acid, diclofenac, fenbufen, azapropazone, acemetacin,

tiaprofenic acid, indomethacin, sulindac, tolmetin, phenylbutazone, diclofenac and diclofenac retard, cyclooxygenase (COX)-2 inhibitors such as GR 253035, MK966, celecoxib (CELEBREX®; 4-(5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl), benzenesulfonamide and valdecoxib (BEXTRA®), and meloxicam (MOBIC®), including salts and
5 derivatives thereof, etc. Preferably, they are aspirin, naproxen, ibuprofen, indomethacin, or tolmetin. Such NSAIDs are optionally used with an analgesic such as codenine, tramadol, and/or dihydrocodinine or narcotic such as morphine.

By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject,
10 particularly a mammalian subject, *e.g.*, a human patient, for whom diagnosis, prognosis, prevention, or therapy is desired.

Pharmaceutical carriers:

Pharmaceutically acceptable carriers and administration routes can be taken from corresponding literature known to the person skilled in the art. The pharmaceutical
15 compositions of the present invention can be formulated according to methods well known in the art; see for example Remington: *The Science and Practice of Pharmacy* (2000) by the University of Sciences in Philadelphia, ISBN 0-683-306472, *Vaccine Protocols*. 2nd Edition by Robinson *et al.*, Humana Press, Totowa, New Jersey, USA, 2003; Banga, *Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems*. 2nd Edition by Taylor
20 and Francis. (2006), ISBN: 0-8493-1630-8. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well-known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose.
25 Administration of the suitable compositions may be effected by different ways. Examples include administering a composition containing a pharmaceutically acceptable carrier via oral, intranasal, rectal, topical, intraperitoneal, intravenous, intramuscular, subcutaneous, subdermal, transdermal, intrathecal, and intracranial methods. Aerosol formulations such as nasal spray formulations include purified aqueous or other solutions of the active agent with preservative
30 agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Pharmaceutical compositions for oral administration, such as single domain antibody molecules (*e.g.*, "nanobodies™") etc are also envisaged in the present invention. Such oral formulations may be in tablet, capsule, powder, liquid or semi-solid form. A tablet may comprise a solid carrier, such as gelatin or an adjuvant.

Formulations for rectal or vaginal administration may be presented as a suppository with a suitable carrier; see also O'Hagan *et al.*, *Nature Reviews, Drug Discovery* 2(9) (2003), 727-735. Further guidance regarding formulations that are suitable for various types of administration can be found in *Remington's Pharmaceutical Sciences*, Mace Publishing
5 Company, Philadelphia, PA, 17th ed. (1985) and corresponding updates. For a brief review of methods for drug delivery see Langer, *Science* 249 (1990), 1527-1533.

Dosage regimen:

The dosage regimen will be determined by the attending physician and clinical factors. As is
10 well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. A typical dose can be, for example, in the range of 0.001 to 1000 μg (or of nucleic acid for expression or for inhibition of expression in this range); however, doses below or above
15 this exemplary range are envisioned, especially considering the aforementioned factors. Generally, the regimen as a regular administration of the pharmaceutical composition should be in the range of 1 μg to 10 mg units per day. If the regimen is a continuous infusion, it should also be in the range of 1 μg to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. Preparations for parenteral administration
20 include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient
25 replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents such as anti-tumor agents and
30 cytotoxic drugs, depending on the intended use of the pharmaceutical composition.

In addition, co-administration or sequential administration of other agents may be desirable. A therapeutically effective dose or amount refers to that amount of the active ingredient sufficient to ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds

can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50.

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Preferably, the therapeutic agent in the composition is present in an amount sufficient for preventing inflammation or suppression of the immune response.

10 These and other embodiments are disclosed and encompassed by the description and examples of the present invention. Further literature concerning any one of the materials, methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries and databases, using for example electronic devices. For example the public database "Medline" may be utilized, which is hosted by the National Center for Biotechnology Information and/or the National Library of Medicine at the National Institutes of Health. Further
15 databases and web addresses, such as those of the European Bioinformatics Institute (EBI), which is part of the European Molecular Biology Laboratory (EMBL) are known to the person skilled in the art and can also be obtained using internet search engines. An overview of patent information in biotechnology and a survey of relevant sources of patent information useful for retrospective searching and for current awareness is given in Berks, *TIBTECH 12* (1994), 352-
20 364.

The above disclosure generally describes the present invention. Several documents are cited throughout the text of this specification. Full bibliographic citations may be found at the end of the specification immediately preceding the claims. A more complete understanding can be
25 obtained by reference to the following specific examples which are provided herein for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLES

The Examples 1 to 9 which follow and corresponding Figures 1 to 32 further illustrate the invention, but should not be construed to limit the scope of the invention in any way. Detailed descriptions of conventional methods, such as those employed herein can be found in the cited literature; see also *"The Merck Manual of Diagnosis and Therapy"* Seventeenth Ed. ed. by Beers and Berkow (Merck & Co., Inc., 2003).

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Methods in molecular genetics and genetic engineering are described generally in the current editions of *Molecular Cloning: A Laboratory Manual*, (Sambrook *et al.*, (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press); *DNA Cloning*, Volumes I and II (Glover ed., 1985); *Oligonucleotide Synthesis* (Gait ed., 1984); *Nucleic Acid Hybridization* (Hames and Higgins eds. 1984); *Transcription And Translation* (Hames and Higgins eds. 1984); *Culture Of Animal Cells* (Freshney and Alan, Liss, Inc., 1987); *Gene Transfer Vectors for Mammalian Cells* (Miller and Calos, eds.); *Current Protocols in Molecular Biology and Short Protocols in Molecular Biology*, 3rd Edition (Ausubel *et al.*, eds.); and *Recombinant DNA Methodology* (Wu, ed., Academic Press). *Gene Transfer Vectors For Mammalian Cells* (Miller and Calos, eds., 1987, Cold Spring Harbor Laboratory); *Methods In Enzymology*, Vols. 154 and 155 (Wu *et al.*, eds.); *Immobilized Cells And Enzymes* (IRL Press, 1986); *Perbal, A Practical Guide To Molecular Cloning* (1984); *the treatise, Methods In Enzymology* (Academic Press, Inc., N.Y.); *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (Weir and Blackwell, eds., 1986). Reagents, cloning vectors, and kits for genetic manipulation referred to in this disclosure are available from commercial vendors such as BioRad, Stratagene, Invitrogen, and Clontech. General techniques in cell culture and media collection are outlined in *Large Scale Mammalian Cell Culture* (Hu *et al.*, *Curr. Opin. Biotechnol.* 8 (1997), 148); *Serum-free Media* (Kitano, *Biotechnology* 17 (1991), 73); *Large Scale Mammalian Cell Culture* (*Curr. Opin. Biotechnol.* 2 (1991), 375); and *Suspension Culture of Mammalian Cells* (Birch *et al.*, *Bioprocess Technol.* 19 (1990), 251).

Material and Methods

Patients selection, peripheral blood mononuclear cells (PBMC) isolation from APECED/APS1 patients memory B cell culture and antibody isolation were carried out as described in the international applications WO2013/098419 and WO2013/098420 with the difference that
 5 specificity of the antibodies isolated and analyzed was directed towards IFNA subtypes as defined hereinabove and below instead of IL-17 and IL-22, which were specifically used in the mentioned PCT applications; see Examples sections therein, in particular Examples 1 and 2 on pages 117 to 120 and Example 17 on pages 168-171 of WO2013/098419 and Examples 1 to 4 on pages 27 to 31 of WO2013/098420.

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The molecular cloning of human antibodies of the present invention and subsequent antibody production and purification were performed as described in the international application WO2013/098419, see the Examples section of the application and in particular Examples 1 to 3 on pages 117-120 therein. Mutation analysis of the AIRE gene was performed as described
 15 in the international application WO2013/098419; see the Examples therein, in particular the "Mutation analysis of the AIRE gene" section in Materials and Methods of the Examples, on pages 115-116, with particular steps performed as described in WO99/15559. In this concern, genotyping of the respective mutations in the AIRE (APECED) gene is performed as described in international application WO99/15559 in Example 2 at pages 12 to 13; a confirmation of the
 20 mutations in exons 2 and 6 of the AIRE gene as described in Example 3 of international application WO99/15559 at page 13, line 5 bridging to page 14, line 13. In particular, for the mutation analysis the DNA samples are purified from peripheral blood mononuclear cells from patients with APECED and from suspected carriers of APECED and from normal healthy controls (according to Sambrook *et al.* 1989, *Molecular Cloning. A Laboratory Manual*. CSH
 25 Press) and subjected to PCR using primers specific for all identified exons.

Example 1: Detection of cytokine specific antibodies in the serum of patients

The general presence of various cytokine and disease specific antibodies in the sera of the patients suffering from the genetic condition APECED (Autoimmune polyendocrinopathy
 30 candidiasis epidermal dysplasia, also called Autoimmune polyendocrinopathy type 1 (APS1)) has been determined by protoarray analysis as described in Example 7 on page 128 and indicated Tables 1 and 2 on pages 128-130 of applicant's international application WO2013/098419. Altogether sera from 23 patients, presented by codes from APS1-1 to APS1-23 were used in the assays. Eight control sera were obtained from healthy laboratory personnel,

age matched with the patients and coded as C1-C8. See Fig. 4 indicating the presence of IFNA subtype specific and dsDNA-specific antibodies in the 23 patients examined. In addition to the seroreactivities shown in Fig. 4, patients APS1-9 and APS1-2 have shown seroreactivity towards IFNA1/13, IFNA5, IFNA6, IFNA10, IFNA14, IFNA16 and IFNA21.

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Anti-dsDNA antibodies are highly specific for SLE and used in the diagnosis of the disease. Surprisingly, no APS1 patients have lupus despite the frequent presence of anti-dsDNA antibodies. However, APS1 patients display pronounced seroreactivity against several interferon- α subtypes which are clinically-relevant drug targets involved in many lupus-implicated molecular mechanisms, implicating the possible suitability of these antibodies in SLE treatment.

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ELISA - IFN- α

96 well microplates (Costar, USA) were coated with human IFNA1, IFNA2 (ImmunoTools), IFNA4 (SinoBiological), IFNA5, IFNA6, IFNA8, IFNA21 (all from PBL) and IFNA14 (ATGen) or IFNA8 (Novus Biologicals). Plates were washed with PBS-T and blocked 1h at room temperature with PBS containing 2% BSA (Sigma, *Buchs*, Switzerland). Patient sera, B cell conditioned medium, or recombinant antibody preparations were incubated for 2h at room temperature. Binding of human IgG to the antigen of interest was determined using a horseradish peroxidase conjugated goat anti human Fc-gamma-specific antibody (Jackson ImmunoResearch, Europe Ltd., Cambridgeshire, UK) followed by measurement of the HRP activity using a TMB substrate solution (TMB, Sigma, *Buchs*, Switzerland). Binding to human Fc fusion mouse IFNA2, IFNA4, IFNA14 (SinoBiological) was detected with horseradish peroxidase conjugated anti-F(ab')₂-specific antibody (see Fig. 19A).

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Example 2: EC50 ELISA determination of the antibodies of the present invention

EC50 binding of the hMABs of the present invention to IFNA2 (ImmunoTools), IFNA4 (SinoBiological), IFNA14 (ATGen) was determined by ELISA (see also Table 3 below for details concerning the recombinant proteins used). Serial dilutions of MABs (from 1000 ng/ml down to 0.0169 ng/ml) were incubated for 2 hours with antigen-coated plates (coating overnight

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at 1 µg/ml in PBS, followed by wash out and blocking with 2% BSA in PBS). The plates were subsequently washed and binding of MABs was detected with anti-human HRP-conjugated secondary antibody. Concentrations of MAB resulting in half of maximal binding to respective antigens (EC₅₀, ng/ml) were calculated using Prism 4 GraphPad software on sigmoidal dose-response curves (variable slope, 4 parameters) obtained by plotting the log of the concentration versus OD 450 nm measurements; see Fig. 3 and Table 4 below.

Target	Provider	Catalog number
IFN-alpha 1beta (IFNA1/13)	Immunotools	11343596
IFNA2	ImmunoTools	11343516
IFNA4	Sino Biological	10336-H08B
IFNA5	PBL	11135
IFNA6	PBL	11165
IFNA8	PBL	11115
IFNA21	PBL	11130
IFNA14	ATGen	ATGP1500
IFN-omega 1	ProSpec	CYT-040
IFN.gamma	Immunotools	11343536
gIFNA1, 2, 4, 5, 6, 7, 8, 10, 14, 16, 17, 21, B, W	In-house production, Fusion construct with Gaussia luciferase	

Table 3: List of recombinant proteins used in the Elisa assays.

Antigen	IC 50 (ng/ml)				
	19D11	26B9	8H1	12H5	50E11
IFNA1	3.80	8.60	28.98	51.32	97.38
IFNA2	1.62	2.83	1039.0	10.66	158.6
IFNA4	0.95	2.07	5.43	0.48	20.25
IFNA5	0.85	3.74	1.78	22.61	0.91
IFNA6	0.79	3.16	77.70	3.55	39.30
IFNA7	0.37	1.57	0.90	0.64	1.90
IFNA8	27.69	205.0	571.0	0.86	4.02

IFNA10	0.72	1.84	2.69	0.66	3.63
IFNA14	0.31	2.01	784.6	2.15	9.20
IFNA16	1.86	4013.0	1.86	32.99	2.35
IFNA17	0.75	2.22	2.11	0.75	2.31
IFNA21	2.22	4.65	2.78	13.02	3.05
IFNW	-	0.50	2.13	-	113.2

Table 4: Summary of IC₅₀ neutralization values of exemplary anti-IFN- α antibodies 19D11, 26B9, 8H1, 12H5 and 50E11 of the present invention as obtained in the ISRE dual luciferase reporter assay.

- 5 Previous EC₅₀ binding versus IC₅₀ neutralization assays performed in accordance with the present invention of the exemplary anti-IFN- α antibodies using IFNA-proteins obtained from commercial suppliers as indicated in Table 3 and IFNA-proteins gIFNA2/-4 and -14 generated in accordance with the present invention gave similar results.
- 10 In addition, binding of exemplary MABs 19D11, 25C3, 26B9, 5D1 and 13B11 of the present invention to IFNA1 (ImmunoTools) was compared to the binding of these antibodies to IFNA2 (ImmunoTools) by ELISA. In this experiment, examined MABs (300 ng/ml) were incubated for 2 hours with antigen-coated plates (coating overnight at 1 μ g/ml in PBS, followed by wash out and blocking with 2% BSA in PBS). The plates were subsequently washed and binding of
- 15 MABs was detected with anti-human HRP-conjugated secondary antibody. Exemplary MABs 19D11, 25C3, 26B9 and 13B11 have shown a comparable binding affinity to IFNA1 and IFNA2 (see Fig. 11A). However, exemplary anti-IFN- α MAB 5D1 binds IFNA2 and does not cross-react with IFNA1 (see Fig. 11A). In a further experiment binding of exemplary MABs 19D11, 25C3, 26B9, 5D1 and 13B11 of the present invention to IFNA8 was compared by LIPS to the
- 20 binding of these antibodies to IFNA14 (both IFN-Gaussia luciferase fusion proteins). Herein, exemplary MAB 13B11 did not show cross-reactivity with IFNA8. The other exemplary antibodies 19D11, 25C3, 26B9 and 5D1 have shown stronger binding of IFNA8 than of IFNA14 (see Fig. 11B). In an additional experiment binding of exemplary MABs 5D1, 13B11 19D11, 25C3, 26B9 and 31B4 of the present invention to IFNA5, IFNA6, IFNA8, IFNA21 (all from
- 25 PBL) and IFNA14 (ATGen) was determined and compared. Exemplary anti-IFN- α antibody 13B1 did not cross-react with IFNA8 and IFNA21 and antibody 19D11 cross-reacted with a lower affinity with IFNA21 than with the other IFNA subtypes (see Fig. 11C).

Example 3: Neutralization assays

The neutralizing assays are carried out on cell lines that respond to the studied cytokine, *i.e.* carry the necessary receptor. The ligand binding to receptor activates a corresponding signaling pathway, translocation of transcription factors to the nucleus and upregulate responder gene transcription, translation and if applicable product secretion. The cytokine concentration used is selected from the beginning of the linear part of the dose-response curve to maximize the sensitivity of the assay. To test the neutralizing capacity of antibodies the optimal concentration of the target cytokine is preincubated with serial dilutions of serum, supernatant or purified antibody samples. The results are expressed as titer or concentration of antibody that show the value half-way between the positive and negative controls.

Phospho-STAT1 assay

30,000 HEK 293T or HEK 293T MSR cells were seeded into Poly-L-Lysine-coated 96-well plates (BD Biocoat, Bedford, MA, USA) or into regular tissue culture-treated 96-well plates (Cat. No. 3598, Corning Inc., Corning, NY, USA), respectively. The following day, rhIFNAs or supernatants of HEK 293T cells transiently expressing IFN-Gaussia luciferase fusion proteins (g1 IFNs) were mixed with anti-IFNA mAbs or control IgG (5 µg/ml) and preincubated for one hour at 37°C. After preincubation, the mixtures were used to stimulate HEK 293T or HEK 293T MSR cells for 10 min at 37°C. Following stimulation, cells were lysed with CelLytic™ M lysis buffer supplemented with protease and phosphatase inhibitors (Cat. No. C2978, P5726, P0044, P8340, SIGMA-ALDRICH, St. Louis, MO, USA) and the collected lysates were cleared at 13,000 RPM, 4°C in a tabletop centrifuge. Lysates were subjected to reducing SDS-PAGE and blotted onto nitrocellulose membranes. Membranes were blocked with a buffer containing 0.25% bovine gelatin, 150 mM NaCl, 5 mM EDTA, 50 mM Tris/HCl pH 7.5, 0.05% Triton X-100 for one hour at room temperature, followed by incubation with rabbit monoclonal antibodies against phosphorylated STAT1 (Tyr701, diluted 1:1000 in blocking buffer, Cat. No. 9167, Cell Signaling Technology, Danvers, MA, USA) at 4°C over night. On the next day, blots were washed three times with blocking buffer followed by incubation with horseradish peroxidase-linked secondary antibodies against rabbit IgG (diluted 1:20,000 in blocking buffer, Cat. No. 111-035-144, Jackson ImmunoResearch, West Grove, PA, USA). After three additional washing steps, an ECL substrate was added (Cat. No. 34087, Thermo Fisher Scientific, Rockford, IL, USA) and reactive bands were visualized via autoradiography. Bound antibodies were removed by incubation in Restore Western Blot Stripping Buffer (Cat. No. 21059, Thermo Fisher Scientific) and a rabbit polyclonal anti-

STAT1 serum was used to visualize total STAT1 levels (diluted 1:1000 in blocking buffer, Cat. No. 9172, Cell Signaling Technology).

In the following the results of two Phospho-STAT1 assays performed with exemplary anti-IFN- α specific antibodies 5D1, 19D11, 25C3, 26B9, 31B4 and 13B11 are discussed. As may be seen in Fig. 5B and 5C, addition of exemplary anti-IFN- α specific antibodies 19D11, 25C3, 26B9, 31B4 and 13B11 prohibits the IFNA2, IFNA4 and IFNA14 dependent phosphorylation of STAT1 indicating the IFN- α neutralizing capacity of the antibodies of the present invention. In addition, exemplary anti-IFN- α specific antibodies 19D11, 26B9 and 31B4 also prohibit the IFNA1, g1 IFNA5 and IFNA6 dependent phosphorylation of STAT1, with the neutralization capacity of antibody 26B9 slightly reduced against IFNA5. Antibody 25C3 showed a slightly weaker neutralization capacity concerning IFNA6 and IFNA14 activity in comparison to its neutralizing capacity towards IFNA2 and IFNA4. Addition of exemplary anti-IFN- α specific antibody 5D1 of the present invention showed neutralizing activity of this antibody against IFNA2 and IFNA4, wherein no or very weak neutralization could be observed in respect of IFNA14 dependent STAT1-phosphorylation (Fig. 5C, right panel) and a slightly weaker neutralization of IFNA6.

After IFNA1b or IFNA16 stimulation exemplary antibodies 19D11, 25C3, 26B9, 31B4 and 13B11 have shown neutralization capacity towards IFNA1b (IFNA1/13) activity, wherein no neutralization could be observed in respect of antibody 5D1 (Fig. 5B). Concerning IFNA16, only antibodies 19D11, 5D1 and 13B11 have shown neutralization capacity, wherein exemplary antibodies 25C3, 26B9 and 31B4 indicated at least severely reduced or even a lack of neutralizing capacity towards IFNA16 (Fig. 5C). Exemplary antibody 13B11 potently neutralized IFNA1, IFNA2, IFNA4 and g1 IFNA5, whereas very weak neutralization could be observed in respect of IFNA6 dependent STAT1 phosphorylation. rhIFNA concentration as used was: 10 ng/ml (IFNA1b), 2 ng/ml (IFNA2, IFNA4, IFNA6, IFNA16). g1 IFNA5 supernatant was used at its EC 80 dilution.

While all exemplary antibodies except 13B11 neutralized IFNA21 in this functional assay (Fig. 5D), exemplary antibodies 25C3 and 13B11 did not neutralize IFNA6 (Fig. 5B). MABs concentration was at 5 μ g/ml, rhIFNAs (PBL) at 2 ng/ml.

All antibodies were neutralizing IFNA5 (g1IFNA5) (Fig. 5B). Antibodies 25C3 and 13B11 did not neutralize IFNA8 (g1IFNA8). None of the exemplary anti-IFN- α antibodies of the present invention antibodies neutralized IFN- γ (IFN-gamma/IFNG) (Fig. 5D). Other than in the above general experimental description, here HEK 293T cells were either left untreated (-) or stimulated with supernatants of HEK 293T cells transiently expressing human IFN-Gaussia luciferase fusion proteins (+) in the absence of antibodies or in the presence of 5 μ g/ml human-derived exemplary human MABs or a human control IgG (huIgG) as indicated in Fig. 5D.

In a second experimental round, additional IFNA subtypes have been tested. The results of these two experimental rounds have been combined in Fig. 5. After IFNA7, g1 IFNA8, IFNA10, IFNA14 or IFNA16 stimulation exemplary antibody 19D11 has shown neutralization capacity towards all tested IFNs, wherein exemplary antibodies 26B9 and 31B4 could not neutralize IFNA16 (Fig. 5C). Exemplary antibody 25C3 fully neutralized IFNA7, but showed weaker neutralization of g1 IFNA8, IFNA10, IFNA14 and IFNA16. Exemplary antibody 5D1 neutralized IFNA7, g1 IFNA8, IFNA10 and IFNA16 but no neutralization of IFNA14. Exemplary antibody 13B11 showed neutralization of IFNA7, IFNA10, IFNA14 and IFNA16, but no neutralization of g1 IFNA8. rhIFNA concentration as used was 2 ng/ml (IFNA7, IFNA10, IFNA14, IFNA16). g1 IFNA8 supernatant was used at its EC 80 dilution.

All exemplary antibodies neutralized IFNA17 in this functional assay (Fig. 5D). All exemplary antibodies except 13B11 neutralized IFNA21. Only two exemplary antibodies, 26B9 and 31B4, neutralized IFNW in this functional assay, while none of the exemplary antibodies neutralized IFNB or g1 IFNG. rhIFNA concentration was 2 ng/ml, g1 IFNG supernatant was used at its EC 80 dilution.

ISRE-Luciferase reporter assay

20,000 HEK 293T or 10,000 HEK 293T MSR cells were seeded in Poly-L-Lysine-coated 96-well plates (BD Biocoat) (in white half-area 96-well plates (Cat. No. 3688, Corning Inc.) – values in brackets indicate the differences in the second experimental option with HEK 293 T MSR cells) and reverse-transfected with 100 ng (50 ng) of premixed ISRE-Firefly luciferase reporter and Renilla luciferase constructs (Cat. No. CCS-008L, Qiagen, Hilden, Germany) (see scheme of the constructs in Fig. 6A) using Fugene HD according to the manufacturer's instructions (Promega, Madison, WI, USA). The Renilla luciferase-expressing construct served as an internal normalization control. Cells were incubated overnight in Opti-MEM® I Reduced

Serum Medium supplemented with 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 10% (0,5%) fetal bovine serum (Life Technologies, Carlsbad, CA, USA) at 37°C, 5% CO₂ in a humidified atmosphere. Following overnight incubation, cells were stimulated for 24 hours with medium containing mixtures of rhIFNAs with or without anti-IFNA mAbs or control IgG that had been preincubated for one hour at 37°C. After 24 hours of stimulation, dual luciferase reporter assays were performed according to the manufacturer's instructions (Promega).

As may be seen in Fig. 7, addition of exemplary anti-IFN- α specific antibodies 19D11, 25C3, 26B9, 31B4 (Fig. 7A and B) and 13B11 (Fig. 7C and D) prohibits the IFNA2, IFNA4 and IFNA14 dependent ISRE-Luciferase reporter activation indicating the IFN- α neutralizing capacity of the antibodies of the present invention. Addition of exemplary anti-IFN- α specific antibody 5D1 of the present invention showed neutralizing activity of this antibody against IFNA2 and IFNA4, wherein no or very weak neutralization could be observed in respect of IFNA14 dependent ISRE-Luciferase activation (Fig. 7C and D). As already seen in the phospho-STAT1-assay, antibody 25C3 showed a slightly weaker neutralization capacity concerning IFNA14 activity in comparison to its neutralizing capacity towards IFNA2 and IFNA4. Similarly, exemplary anti-IFN- α specific antibody 13B11 has shown a slightly weaker neutralization capacity towards IFNA14 (Fig. 7C and D). Exemplary antibody 8H1 neutralizes IFNW, together with IFNA1, A4, A5, A6, A7, A10, A16, A17 and A21, while showing weaker neutralization of IFNA2, A8 and A14 (Fig. 7E). Exemplary antibody 12H5 strongly inhibits ISRE-Luciferase reporter induction by all alpha interferons, namely IFNA1, A2, A4, A5, A6, A7, A8, A10, A14, A16, A17 and A21, while virtually not affecting IFNW-mediated reporter induction. Neither exemplary antibody 8H1 nor 12H5 interfere with IFNB-mediated reporter induction (Fig. 7E).

As shown in Fig. 8, exemplary antibody 26B9 potently neutralizes rhIFNA1, A2, A4, A5, A6, A8, A10, A14, A17, A21 and rhIFNW, while weakly neutralizing rhIFNA16. rhIFN concentration was 10 ng/ml (IFNA1), 1.3 ng/ml (IFNA16) and 2 ng/ml (all other rhIFNs). A summary of the IC₅₀ values as determined in the ISRE-Luciferase reporter assay is shown in Table 4.

As may be seen in Fig. 9, exemplary antibody 19D11 neutralizes all rhIFNA molecules, namely IFNA1, A2, A4, A5, A6, A7, A8, A10, A14, A16, A17 and A21. rhIFN concentration was 10

ng/ml (IFNA1), 1.3 ng/ml (IFNA16) and 2 ng/ml (all other rhIFNs). A summary of the IC 50 values as determined in the ISRE-Luciferase reporter assay is shown in Table 4.

Chemiluminescent cellular binding assay

5 30,000 HEK 293T MSR cells were seeded in white half-area 96-well tissue culture plates (Cat. No. 3688, Corning Inc.). The following day, supernatants of HEK 293T cells transiently expressing human IFN-Gaussia luciferase fusion proteins were mixed with anti-IFN mAbs, control IgG or excess concentrations of unlabeled recombinant IFNA2 and preincubated for one hour at 37°C. After preincubation, the mixtures were used to stimulate HEK 293T MSR
10 cells for 40 minutes at 37°C. Upon binding, cells were washed three times with PBS, and the Gaussia luciferase assay was developed using the Gaussia Flash Assay Kit according to the manufacturer's instructions (Cat. No. 16159, Thermo Fisher Scientific).

As shown in Fig. 10C, exemplary antibody 19D11 efficiently neutralizes binding of g1 IFNA2,
15 A4, A5, A6, A7, A8, A10, A14, A16, A17 and A21 to HEK 293T MSR cells endogenously expressing IFN receptors. In contrast, exemplary antibody 19D11 does virtually not interfere with binding of g1 IFNB and g1 IFNW. Binding of all g1 IFNs showed herein is apparently not affected by a control human antibody (huIgG). Antibody concentration: 5 µg/ml.

20 As may be seen in Fig. 10D, binding of g1 IFNA16 to HEK 293T MSR cells is more efficiently neutralized by exemplary antibody 19D11 as compared to exemplary antibody 26B9. However, exemplary antibody 26B9 strongly neutralizes binding of g1 IFNW to HEK 293T MSR cells, whereas exemplary antibody 19D11 shows no apparent neutralization capacity towards this ligand. Binding of both g1 IFNA16 and g1 IFNW remains unaffected by a control human
25 antibody (huIgG). Antibody concentration: 10 µg/ml.

Example 4: Validation of subject antibodies

Antibodies provided by the present invention are tested in concern of their neutralizing activity towards human IFN- α in animal disease models. When performing such experiments it has to
30 be ensured that human IFN- α subtypes induce diseased phenotypes in mice and that no cross-reaction occurs between the tested IFN- α antibodies of the present invention and the murine IFN- α homologues. Since no adequate model system for IFN-alpha was available in the prior art, the present Example describes and provides such a system to test IFN-alpha neutralizing antibodies that do not cross react with mouse IFNs. First, the effect of different IFN- α subtypes

was tested *in vivo* for the induction of ear inflammation. In particular the proinflammatory activity of human subtypes IFNA2a, IFNA2b, IFNA4 and IFNA14 have been tested. IFNA2a differs from IFNA2b by amino acid 23 (Lys in IFNA2a and Arg in IFNA2b)

5 *Ear inflammation assay*

Ear inflammation phenotype was induced in 8 weeks old C57BL/6J (WT; from Charles River) mice by intradermal injection of human IFNA2a, IFNA2b, IFNA4 and IFNA14 in 20µl of PBS, or PBS control into each ear given on alternate days at Day 0, Day 2 and Day 4 (20µl/ear, 500ng/ear, 1µg total/mouse/day) using a 30-gauge needle. The mice were sacrificed at day 6; see Table 5 below and Fig. 14A for the experimental timeline.

Group	n	Cytokine	ng/20µl
A	5	PBS	Na
B	5	IFNA2a	500
C	5	IFNA2b	500
D	5	IFNA4	500
E	5	IFNA14	500

Table 5: Group allocation of animals to the different cytokines tested. n – number of animals in the group, ng/20µl – amount of cytokine injected per ear.

15 To test the proinflammatory effect of the injected IFNA subtypes ear thickness measurements of the animals were taken with a Mitutoyo digital micrometer during the cytokine administration by 2 measurements per ear prior to cytokine injection at Day 0 and at alternate days at Day 1, Day 3, Day 5 (indicated by letter M in Fig. 14A) and alternatively or in addition at Day 6 after sacrifice of the animal.

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Furthermore, body weight is monitored during the treatment, to observe any possible weight changes due to the inflammation induction or its respective reduction due to the treatment applied. In addition, after sacrifice of the animals H&E ((hematoxylin and eosin; see Harris, H.F., J. Appl. Microscopy III (1900), 777-781 and Mallory, F.B.: Pathological technique. Philadelphia, Saunders, 1938.) histology stainings of the ears are performed.

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All four human IFNA subtypes tested were able to significantly induce ear swelling following cytokine injection; see Figs. 14-16 and results of the experiment summarized in the table in Fig. 17. All ears were markedly thicker than PBS treated ears, this was significant in all groups after the 2nd intradermal injection, from Day 3 until the end of the experiment. IFNA14 was the most potent especially at Day 5 in this experiment. IFNA2a and IFNA4 induced similar levels of ear

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thickening. IFNA2b was most similar to both IFNA4 and IFNA14 induced swelling. IFNA2b induced swelling more than the IFNA2a isoform in this experiment; see Figs. 14-16 and the experimental results summary in the table in Fig.17.

5 The results of this experiment show the applicability of the ear inflammation assay for tests of the therapeutic applicability of the antibodies of the present invention. Since the exemplary anti-IFN- α antibodies 19D11, 26B9, 31B4, 5D1 and 13B11 of the present invention did not show any apparent cross-reaction with at least murine IFN- α subtypes 2, 4 and 14 (see Fig. 19A), they are tested in the above indicated assay in respect of their neutralization properties
10 towards human IFN- α used for induction of inflammation. Apparent binding affinity of exemplary anti-IFN- α antibody 25C3 towards murine IFNA2 and the affinity of exemplary anti-IFN- α antibodies 5D1 and 19D11 towards murine IFNA1 is taken into consideration when designing the *in vivo* CytoEar neutralization experiments described herein.

15 Such treatment tests are performed with the exemplary anti-IFN- α antibodies of the present invention by injection of the antibodies at different time points during the above drafted experimental timeline (see also Fig. 14A) for testing induction of inflammation by human IFN- α . For example, for testing the preventive and/or therapeutic effect one or more of the exemplary antibodies of the present invention are injected together with or separately to the
20 IFNA subtype or subtypes at Day 0 of the experiment. In addition or alternatively, one or more of the antibodies of the present invention or IFN- α binding fragments thereof are injected on alternate days with the IFNA subtype or subtypes. For example, if the IFNA subtype or subtypes are injected as indicated above at Days 0, 2 and 4 (short arrows in Fig. 14A), the antibodies are injected on the alternate Days 1, 3 and/or 5 (long arrows in Fig. 14A).

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The neutralizing potential of the antibodies of the present invention or IFN- α binding fragments thereof to reduce the induced ear inflammation phenotype and/or to prevent such an induction is examined by comparison of ear swelling (thickness) observed in animals obtaining the anti-IFN- α antibody treatment and the control groups obtaining either PBS or human IgGs of a
30 binding specificity directed towards other molecules than human IFNA subtypes (of IFN- α non-related binding specificity).

Furthermore, or alternatively body weight is monitored during the treatment, to observe any possible weight changes due to the inflammation induction or its respective reduction due to

the treatment applied. In addition, after sacrifice of the animals H&E (hematoxylin and eosin; see supra) histology stainings of the ears are performed. This assay is used preferably as a surrogate model for psoriasis.

5 In a second experimental round, the above indicated assay has been used with some modifications to test neutralization properties of exemplary antibodies 26B9 and 19D11 of the present invention towards inflammation induced in mice ears by injections of human IFNA14 (Fig. 28), IFNA5 (Fig. 29) and IFNW (Fig. 30). As may be seen from the time schemes shown in Figs. 28A, 29A and 30A the experimental time line has been prolonged here to 10 days with
10 tested antibodies and controls injected at experimental day 0 (IP), intradermal cytokine injections at days 1, 3, 6 and 8 and a sacrifice of the test animals at day 10. Group allocations of animals to the different cytokines tested and the respective concentrations and amounts of the cytokines, respective antibodies tested are indicated in tables in panels B of the respective figures. Both antibodies, 26B9 and 19D11 have shown pronounced preventive and/or
15 therapeutic potential due to a significant reduction of the ear thickness on several experimental days after IFNA14 (Figs. 28 D and E) and IFNA5 (Figs. 29 D and E) induced ear inflammation in comparison to IgG controls. Treatment with the reference IFN- α specific antibody (Ref. A in Figs. 28, 29, in particular Figs. 28F and 29F) led also to a significant reduction of ear swelling after IFNA14 treatment on several experimental days, however with a slightly lower reduction
20 at day 10 compared with antibodies 26B9 and 19D11 of the present invention (compare curves F for 26B9 in Fig. 28D and G for 19D11 in Fig. 28E with curve H for Ref.A in Fig. 28F and with curve E in each Fig. for the IgG-control). Furthermore, exemplary antibody 26B9 has shown a significant reduction of IFNW induced ear swelling at experimental day 9 (Fig. 30D), wherein injections of antibody 19D11 and Ref.A did not show any significant reduction of the
25 ear swelling compared to non-specific IgG treatment. Accordingly, antibodies provided with the present invention have a high potential for use in prevention and/or treatment of diseases associated with enhanced IFNA and/or IFNW activity.

CytoAnkle assay:

30 In this assay mice cohorts (c57/bl6, 7-8weeks) are intraarticular (IA) injected with 62.5-1000ng cytokine, e.g., at least one IFNA subtype such as IFNA2a, IFNA2b, IFNA4 or IFNA14 or mixtures of several IFNA subtypes in 10ul of PBS (or PBS control) into ankles every 48-72 hours. Axial ankle thickness measurements are then taken with a Mitutoyo digital micrometer. Animals are weighed each day and respective IFNA subtype or subtypes are administered while

the mice are anaesthetized with isoflurane. The experimental time frame is designed as indicated above for the ear inflammation assay, with injections of the anti-IFN- α antibody or antibodies of the present invention, respective the control groups obtaining either PBS or human IgGs of IFN- α non-related binding specificity as indicated above. Reduction of the ankle swelling is used as readout of the therapeutic effect of the antibodies of the present invention. This assay is used preferably as a surrogate model for arthritis, *e.g.*, rheumatoid arthritis.

Example 5: Epitope mapping of exemplary IFN- α antibodies

As a first step of mapping, differential binding of anti-IFN- α MABs of the present invention to distinct antigen binding sites was examined to determine the number of different binding sites.

For this purpose, MABs were expressed either with human (hMAB) or mouse (hmMAB) Fc and cross-competition experiments were carried out by coating antigen on plates and by detecting binding of hmMABs in the presence of large excess of human MABs. Detection of hmMABs bound to the ligand was performed by a HRP-conjugated secondary antibody directed against the Fc portion of the primary antibody.

As may be seen from Fig. 2A and Table 6A below, exemplary anti-IFN- α antibodies 19D11, 26B9, 31B4 and 13B11 of the present invention compete each other for binding of IFNA2 but not with antibodies 5D1 and 25C3, indicating that 5D1 and 25C3 bind other site(s) of IFNA2 than 19D11, 26B9, 31B4 and 13B11. The same competition pattern may be seen for IFNA4 and IFNA14 with the difference however that 13B1 does not compete for binding of IFNA4 and only weakly competes for binding of IFNA14 with 19D11, 26B9, 31B4 indicating that the epitope the antibodies are binding to may be not conserved in this IFN- α subtypes and in particular divergent in IFNA4. The results of the first approach also indicate a weak competition of each other of anti-IFN- α antibodies 5D1 and 25C3 of the present invention for binding of IFNA2 and IFNA4 indicating a possible partially overlapping epitope of these antibodies; see Figs. 2A, 2B and Tables 5A, 5B. Furthermore, a diverging competition pattern on IFNA14 may be observed, where hMAB 25C3 shows strong competition with hmMAB 5D1 but only a weak competition in the reverse situation (hMAB 5D1 against hmMAB 25C3) may be observed, indicating a possible preference of the 25C3 antibody towards an IFNA14-specific epitope; see Fig. 2C and Table 6C below.

Table 6: Results of the cross-competition experiments of exemplary antibodies of the present invention. Human MAB (hMAB) were added in large excess to plates coated with the respective antigens before addition of MABs with mouse Fc (hmMAB).

A Human IFNA2		Human MAB competitor					
		19D11	25C3	26B9	31B4	5D1	13B11
Competition of binding of hmMAB	19D11	+++++	-	+++++	+++++	-	++++
	25C3	-	+++++	-	-	+	-
	31B4	+++++	-	+++++	+++++	(+)	++++
	5D1	-	(+)	-	-	+++(+)	-
	13B11	-	-	-	-	-	+++(+)

5

B Human IFNA4		Human MAB competitor					
		19D11	25C3	26B9	31B4	5D1	13B11
Competition of binding of hmMAB	19D11	+++++	-	++++	+++(+)	-	-
	25C3	-	++++	-	-	(+)	-
	31B4	+++++	-	+++++	+++++	-	+++
	5D1	-	(+)	-	-	+++(+)	-
	13B11	-	-	-	-	-	+(+)

C Human IFNA14		Human MAB competitor					
		19D11	25C3	26B9	31B4	5D1	13B11
Competition of binding of hmMAB	19D11	+++++	-	++++	+++(+)	-	++
	25C3	(+)	+++++	(+)	-	+++	(+)
	31B4	+++++	-	+++++	+++++	++	++(+)
	5D1	+++++	+++++	+++++	+++++	+++++	(+)
	13B11	+++(+)	-	+++(+)	+++	-	++++

Binding regions of MABs to their respective antigens can be mapped by, *e.g.*, PepStar™ analysis. Therefore, overlapping 20mer peptides (15 amino acid overlap) are designed to cover the IFNA- α subtypes of interest, *e.g.*, IFNA2, IFNA4 and IFNA14 including all known variants. The peptides and full length antigen (as positive control) are spotted on microarray and the peptide microarray is incubated with the primary antibody followed by a fluorescently labelled secondary antibody directed against the Fc portion of the primary antibody. To avoid false negatives caused by steric hindrance, an optimized hydrophilic linker moiety is inserted between the glass surface and the antigen derived peptide sequence.

10

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Such peptide mapping has been performed for exemplary antibodies 19D11 and 26B9 of the present invention on peptide arrays of 18mer peptides of human IFNA2, respective human IFNAW. The results of the assay are shown in Fig. 27. Exemplary antibody 19D11 binds specifically to peptides 19 (SAAWDETLLDKFYTELYQ SEQ ID NO: 99) and 32 (RITLYLKEKKYSPCAWEV SEQ ID NO: 100) of IFNA2 (Fig. 27B). The antibody 26B9 binds specifically to peptide 22 (YTELYQQQLNDLEACVIQG SEQ ID NO: 101) of IFNA2 (Fig. 27C) and to peptide 23 (TGLHQQLQHLETCLLQVV SEQ ID NO: 102) of IFNAW (Fig. 27D).

Example 6: Determination of MABs binding to IFNA subtypes by LIPS Assay.

In addition to the ELISA assay, binding of MABs to the different IFNA subtypes was determined by the LIPS assay. IFNA5-, IFNA6- and IFNA8-Gaussia fusion proteins were produced by cloning IFNA5, IFNA6 and IFNA8 each fused with Gaussia luciferase at N-terminus and expressing them individually by transient transfection of HEK293 cells (harvest of the supernatant after 2 days) as described in Example 10 on page 158 and Example 15 on pages 165-167 in applicant's international application WO2013/098419, using primers indicated in Table 8 below. MABs (4 µg/ml) were diluted in Buffer A (50 mM Tris, pH 7.5, 100 mM NaCl, 5 mM MgCl₂, 1% Triton X-100) and incubated for 1 hour with an equal Protein A agarose beads (Exalpha) in wells of MultiScreen HTS Filter Plates (Millipore) on rotary shaker. Two volumes of IFNA5- or IFNA6- or IFNA8-Gaussia fusion protein (1 million LU) were added and plates incubated for 1 hour. Plates were washed 5 times with Buffer A and 2 additional times with PBS before addition of the substrate (Gaussia Luciferase Flash Assay Kit, Pierce). Luminescence (CPS) was read using EnSpire (Perkin Elmer) (see Figs. 11B, 12A-C and 13). A human antibody binding to an unrelated antigen human MAB has been used as negative control.

As can be seen from the results shown in Figs. 11 and 12, exemplary MAB 13B11 binds to: IFNA2, 4, 5, 6, 10 and 14 but not, or weaker to IFNA1/13 and not to IFNA8. Furthermore, the results provided in experiments described herein, *e.g.*, in Examples 2 and 3 show that exemplary MAB 13B11 neutralizes: IFNA2, 4, 5, 14 but not IFNA6, 8 and IFNA21. All results concerning the binding and neutralization properties of the exemplary antibodies of the present invention as provided by the experiments described in Examples 2, 3 and 6 are summarized in Table 7 below.

Antigen	19D11		25C3		26B9		31B4		5D1		13B11	
	B	N	B	N	B	N	B	N	B	N	B	N
IFNA1	+++	+++	+++	+++	+++	+++	+++	+++	-	-	+++	+++
IFNA2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IFNA4	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IFNA5	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IFNA6	+++	+++	+++	+	+++	+++	+++	+++	+++	++	+++	+
IFNA7	nd	+++	nd	+++	nd	+++	nd	+++	nd	+++	nd	+++
IFNA8	+++	+++	+++	+	+++	+++	+++	+++	+++	+++	-	-
IFNA10	+++	+++	+++	+	+++	+++	+++	+++	+++	+++	+++	+++
IFNA14	+++	+++	+++	++	+++	+++	+++	+++	+++	-	+++	+++
IFNA16	+++	+++	+++	+	+++	-	+++	-	+++	+++	+++	+++
IFNA17	nd	+++	nd	+++	nd	+++	nd	+++	nd	+++	nd	+++
IFNA21	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+
IFNW	-	-	nd	-	+++	+++	nd	+++	nd	-	nd	-

Table 7: Binding (B) and neutralization (N) of different IFN subtypes by exemplary antibodies of the present invention as obtained in ELISA, LIPS or neutralization assays described herein.

Exemplary human anti-IFN- α antibody of the present invention 19D11 neutralizes all IFNA subtypes and not IFNW. Exemplary antibody 26B9 neutralizes IFNW and all IFNA subtypes except IFNA16. +/+/+++ = binding, respective neutralisation; - = lack of binding/neutralization. nd = not determined; B = binding, determined by ELISA with commercial recombinant proteins or by LIPS with self-produced IFN-Gaussia luciferase fusion proteins; N= neutralization, determined by ISRE-Luciferase reporter assay or by phospho-STAT1 assay.

Previous experiments on binding and neutralization of different IFNA subtypes by the exemplary antibodies of the present invention in ELISA, LIPS and neutralization assays gave similar results.

Gene	AA	Primer Name	Sequence and SEQ ID NO:
IFNA1	24-189	IFNA1F	TTTGGATCCTATGTGATCTCCCTGAGACCCACAGCCTGGA SEQ ID NO:45
		IFNA1R	TTTGCGGCCGCGACCAGATGTTATTCCTTCCTCCTTAATCTTTC SEQ ID NO:46
IFNA2	24-188	IFNA2F	TTTGGGATCCTCTGTGATCTGCCTCAAACCCACA SEQ ID NO:47
		IFNA2R	TTTGCGGCCGCTTACTTCTTAAACTTTCTTGCA SEQ ID NO:48
IFNA4	24-189	IFNA4F	TTTGGATCCTATGTGATCTGCCTCAGACCCACAGCCTGG SEQ ID NO:49
		IFNA4R	TTTGCGGCCGCTCAATCCTTCCTCCTTAATCTTTTTTGCAAGTTTGT TGAAAAC SEQ ID NO:50
IFNA5	22-189	IFNA5F	TTTGGATCCTACTGGGCTGTGATCTGCCTCAGACCCACAGCCTGAG SEQ ID NO:51
		IFNA5R	TTTGCGGCCGCTCATTCCTTCCTCCTTAATCTTCTTGCAAGTTTGC SEQ ID NO:52
IFNA6	21-189	IFNA6F	TTTGGATCCTATCTCTGGACTGTGATCTGCCTCAGACCCACAGCCTG GGTC SEQ ID NO:53
		IFNA6R	TTTGCGGCCGCTTATTCCTTCCTCCTTAACCTTCTTGCAAGTTTC SEQ ID NO:54
IFNA7	24-189	IFNA7F	TTTGGATCCTATGTGATCTGCCTCAGACCCACAGCCTGC SEQ ID NO:55
		IFNA7R	TTTGCGGCCGCGAACCAGTTTTCAATCCTTCCTCCTTAATCCTTTTT T SEQ ID NO:56
IFNA8	23-189	IFNA8F	TTTGGGATCCTCTGTGATCTGCCTCAGACTCACA SEQ ID NO:57
		IFNA8R	TTTGCGGCCGCTCATTCCTTACTCTTCAATCTT SEQ ID NO:58
IFNA10	24-189	IFNA17F	TTTGGATCCTATGTGATCTGCCTCAGACCCACAGCCTGGG SEQ ID NO:59
		IFNA4R	TTTGCGGCCGCTCAATCCTTCCTCCTTAATCTTTTTTGCAAGTTTGT TGAAAAC SEQ ID NO:50
IFNA14	24-189	IFNA14F	TTTGGATCCTATGTAATCTGTCTCAAACCCACAGCCTGAA SEQ ID NO:60
		IFNA14R	TTTGCGGCCGCTCAATCCTTCCTCCTTAATCTTTTTTGCAAGTTTGT SEQ ID NO:61
IFNA16	24-189	IFNA16F	TTTGGATCCTATGTGATCTGCCTCAGACT SEQ ID NO:62
		IFNA16R	TTTGCGGCCGCTCAATCCTTCCTTCTTAATCC SEQ ID NO:63
IFNA17	24-189	IFNA17F	TTTGGATCCTATGTGATCTGCCTCAGACCCACAGCCTGGG SEQ ID NO:59
		IFNA17R	TTTGCGGCCGCGTTGAACCAGTTTTCAATCCTTCCTCCTTAATA SEQ ID NO:64
IFNA21	24-189	IFNA21F	TTTGGATCCTATGTGATCTGCCTCAGACCCACAGCCT SEQ ID NO:65
		IFNA21R	TTTGCGGCCGCTCATTCCTTCCTCCTTAATCTTCTTGAAAAA SEQ ID NO:66
IFNB	22-187	IFNB1F	TTTGGATCCTAATGAGCTACAACCTTGCTTGGATTCTAC SEQ ID NO:67
		IFNB1R	TTTGCGGCCGCTCAGTTTCGGAGGTAACCTGTAAGTCT SEQ ID NO:68
IFNG		IFNGF	TTTGGATCCTACAGGACCCATATGTAAAAGAAGCAGAAAAC SEQ ID NO:69

	24- 166	IFNGR	TTTGCGGCCGCCATTACTGGGATGCTCTTCGACCT SEQ ID NO:70
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Table 8: Overview of all IFN proteins and used primer sequences used for cloning the Gaussia-luciferase-IFN-fusion constructs.

5 Cloning human IFN subtypes in fusion to Gaussia luciferase

Coding sequences of IFNA1, IFNA6, IFNA7, IFNA8, IFNA10, IFNA14, IFNA16, IFNA17, IFNA21, IFNW, IFNB, IFNG, IFNE and IFNK without signal peptides were cloned into modified pPK-CMV-F4 fusion vector (PromoCell GmbH, Heidelberg; Germany) downstream of naturally secreted Gaussia luciferase (Gluc) that was replaced to the plasmid instead of
10 Firefly luciferase.

Example 7: IC 50 analysis of exemplary human-derived IFNA mAbs by ISRE Luciferase reporter neutralization assay.

HEK 293T MSR cells (Cat. No. R79507, Invitrogen, Carlsbad, CA, USA) transiently
15 expressing ISRE-Firefly luciferase reporter and Renilla luciferase constructs (Cat. No. CCS-008L, Quiagen, Hilden, Germany) were stimulated with 2 ng/ml rhIFNA2, rhIFNA4, rhIFNA14 or with supernatants of HEK 293T cells transiently expressing human IFN-Gaussia luciferase fusion proteins (IFNA5, IFNA8), in the presence of human-derived IFNA mAbs 26B9 (Fig. 8A-E), 25C3 (Fig. 9A-E) or 19D11 (Fig. 10A-E) as indicated. After 24 hours of
20 stimulation, dual luciferase reporter assays were performed according to the manufacturer's instructions (Promega, Madison, WI, USA).

In a confirmatory experimental round HEK 293T MSR cells transiently expressing ISRE-Firefly luciferase reporter and Renilla luciferase constructs as described above were stimulated
25 with 10 ng/ml rhIFNA1, 2 ng/ml rhIFNA2, rhIFNA4, rhIFNA5, rhIFNA6, rhIFNA8, rhIFNA10, rhIFNA14, rhIFNA17, rhIFNA21, 1.3 ng/ml rhIFNA16, in the presence of human-derived IFNA mAbs 26B9 (Fig. 8F-R) or 19D11 (Fig. 10F-Q) as indicated. After 24 hours of stimulation, dual luciferase reporter assays were performed according to the manufacturer's instructions (Promega, Madison, WI, USA). The same experimental setup has been further used
30 to perform the IC 50 analysis of human-derived mAbs 8H1 (Fig. 20), 12H5 (Fig.21) and 50E11 (Fig.22). The results of the assays are summarized in Table 4 above.

Example 8: Antibody affinity measurements using surface plasmon resonance (SPR) technology

For affinity determination of the antibodies of the present invention SPR measurements are performed using a ProteOn™ XPR36 instrument, according to the instructions of the manufacturer (BIO-RAD; Hercules CA, USA) using the molecules of interest of the present invention in an analogous experimental setup as described in Example 14 of international application WO2013/098419 on pages 163-165. Alternatively or in addition a similar analysis is made using Biacore SPR instruments according to the manufacturer's instructions.

Results for the SPR measurements performed on exemplary antibodies 19D11 and 26B9 of the present invention are shown in Fig. 26, with a 1:1 binding kinetic observed for the antibodies towards IFNA2b, IFNA4, IFNA14 and in respect of antibody 26B9 also towards IFNW. The affinities towards human IFNA4 and IFNA14 are in the sub-picomolar range and in sub-nanomolar range for IFNA2b. 26B9 also binds human IFNW with a sub-picomolar affinity.

Example 9: Chemiluminescent cellular binding assays

Interferons-Gaussia luciferase

30,000 HEK 293T MSR cells were seeded in white half area 96-well tissue culture plates (Cat. No. 3688, Corning Inc.). The following day, supernatants of HEK 293T cells transiently expressing human IFN-Gaussia luciferase fusion proteins were mixed with anti-IFN mAbs, control IgG or excess concentrations of unlabeled recombinant IFNA2 and preincubated for one hour at 37°C. After preincubation, the mixtures were used to stimulate HEK 293T MSR cells for 40 minutes at 37°C. Upon binding, cells were washed three times with PBS, and the Gaussia luciferase assay was developed using the Gaussia Flash Assay Kit according to the manufacturer's instructions (Cat. No. 16159, Thermo Fisher Scientific).

Binding to transmembrane antibodies

30,000 HEK 293T MSR cells were seeded in white half area 96-well tissue culture plates (Cat. No. 3688, Corning Inc.). During seeding, cells were transfected with 100 ng cDNA encoding a transmembrane version of anti-IFN mAb 26B9 (26B9-TM) using Fugene HD (Cat. No. E2311, Promega, Madison, WI, USA). Surface antibody (26B9-TM) expression was analyzed 48 hours after transfection in a cell-based ELISA (Fig. 24A). Forty-eight hours following transfection, supernatants of HEK 293T cells transiently expressing human IFNW-Gaussia luciferase fusion

proteins (g1 IFNW) were used to stimulate the previously transfected HEK 293T MSR cells for 40 minutes at 37°C. Alternatively, the g1 IFNW supernatants were mixed with anti-IFN mAbs or control IgG and preincubated for one hour at 37°C. After preincubation, the mixtures were used to stimulate HEK 293T MSR cells transiently expressing 26B9-TM for 40 minutes at 37°C. Upon binding, cells were washed three times with PBS, and the Gaussia luciferase assay was developed using the Gaussia Flash Assay Kit according to the manufacturer's instructions (Cat. No. 16159, Thermo Fisher Scientific) showing that g1 IFNW was specifically binding to cells expressing 26B9-TM (Fig. 24B).

10 *Crosscompetition assay of anti-IFNW antibodies.*

Above experimental setup has been also used to test crosscompetition between exemplary antibodies 26B9, 31B4 and 8H1 of the present invention (see Fig. 25). HEK 293T MSR cells were reverse-transfected with cDNA encoding 26B9-TM. Forty-eight hours after transfection, g1 IFNW was mixed and preincubated for one hour with soluble anti-IFNW antibodies 26B9, 31B4, 8H1 or a control IgG (huIgG). Following incubation, the mixtures were added to the transfected cells and binding was analysed in the chemiluminescent cellular binding assay. Binding of g1 IFNW to 26B9-TM is competed dose-dependently by soluble 26B9 and by the clonally related 31B4 antibody. In contrast, binding is not affected by a control IgG or by exemplary anti-IFNW antibody 8H1. These results indicate that exemplary antibodies 26B9 and 31B4 share similar epitopes, while 8H1 appears to bind to a distinct epitope.

Claims

1. A human anti-interferon-alpha (IFN- α) antibody or an IFN- α binding fragment thereof which
 - (i) binds to human IFN- α subtypes IFNA2, IFNA4, IFNA5, IFNA6, IFNA10 and IFNA14;
 - (ii) binds to at least one human IFN- α subtype IFNA1/13 (IFNA1b), IFNA8, IFNA16 and/or IFNA21; and
 - (iii) is capable of neutralizing a biological activity of at least one of the human IFN- α subtypes, and which comprises in its variable region the complementarity determining regions (CDRs) VH-CDR1, VH-CDR2, VH-CDR3 and VL-CDR1, VL-CDR2 and VL-CDR3 selected from:
 - (a) VH-CDR1: amino acids 31-35 of SEQ ID NO: 2
 VH-CDR2: amino acids 50-66 of SEQ ID NO: 2
 VH-CDR3: amino acids 99-111 of SEQ ID NO: 2
 VL-CDR1: amino acids 24-34 of SEQ ID NO: 4
 VL-CDR2: amino acids 50-56 of SEQ ID NO: 4,
 VL-CDR3: amino acids 89-97 of SEQ ID NO: 4;
 - (b) VH-CDR1: amino acids 31-35 of SEQ ID NO: 10
 VH-CDR2: amino acids 50-66 of SEQ ID NO: 10
 VH-CDR3: amino acids 99-112 of SEQ ID NO: 10
 VL-CDR1: amino acids 24-34 of SEQ ID NO: 12
 VL-CDR2: amino acids 50-56 of SEQ ID NO: 12,
 VL-CDR3: amino acids 89-95 of SEQ ID NO: 12;
 - (c) VH-CDR1: amino acids 31-35 of SEQ ID NO: 18
 VH-CDR2: amino acids 50-66 of SEQ ID NO: 18
 VH-CDR3: amino acids 99-115 of SEQ ID NO: 18
 VL-CDR1: amino acids 24-35 of SEQ ID NO: 20
 VL-CDR2: amino acids 51-57 of SEQ ID NO: 20
 VL-CDR3: amino acids 90-100 of SEQ ID NO: 20;
 - (d) VH-CDR1: amino acids 31-35 of SEQ ID NO: 22
 VH-CDR2: amino acids 50-66 of SEQ ID NO: 22
 VH-CDR3: amino acids 99-111 of SEQ ID NO: 22
 VL-CDR1: amino acids 24-34 of SEQ ID NO: 24
 VL-CDR2: amino acids 50-56 of SEQ ID NO: 24
 VL-CDR3: amino acids 89-97 of SEQ ID NO: 24;
 - (e) VH-CDR1: amino acids 31-37 of SEQ ID NO: 30
 VH-CDR2: amino acids 52-67 of SEQ ID NO: 30
 VH-CDR3: amino acids 100-113 of SEQ ID NO: 30
 VL-CDR1: amino acids 24-40 of SEQ ID NO: 32

- VL-CDR2: amino acids 56-62 of SEQ ID NO: 32
 - VL-CDR3: amino acids 95-103 of SEQ ID NO: 32;
 - (f) VH-CDR1: amino acids 31-37 of SEQ ID NO: 38
 - VH-CDR2: amino acids 52-67 of SEQ ID NO: 38
 - VH-CDR3: amino acids 100-113 of SEQ ID NO: 38
 - VL-CDR1: amino acids 24-40 of SEQ ID NO: 40
 - VL-CDR2: amino acids 56-62 of SEQ ID NO: 40
 - VL-CDR3: amino acids 95-103 of SEQ ID NO: 40;
 - (g) VH-CDR1: amino acids 31-35 of SEQ ID NO: 76
 - VH-CDR2: amino acids 50-66 of SEQ ID NO: 76
 - VH-CDR3: amino acids 99-113 of SEQ ID NO: 76
 - VL-CDR1: amino acids 24-34 of SEQ ID NO: 78
 - VL-CDR2: amino acids 50-56 of SEQ ID NO: 78
 - VL-CDR3: amino acids 89-97 of SEQ ID NO: 78;
 - (h) VH-CDR1: amino acids 31-35 of SEQ ID NO: 84
 - VH-CDR2: amino acids 50-66 of SEQ ID NO: 84
 - VH-CDR3: amino acids 99-112 of SEQ ID NO: 84
 - VL-CDR1: amino acids 24-34 of SEQ ID NO: 86
 - VL-CDR2: amino acids 50-56 of SEQ ID NO: 86
 - VL-CDR3: amino acids 89-98 of SEQ ID NO: 86; or
 - (i) VH-CDR1: amino acids 31-35 of SEQ ID NO: 92
 - VH-CDR2: amino acids 50-66 of SEQ ID NO: 92
 - VH-CDR3: amino acids 99-103 of SEQ ID NO: 92
 - VL-CDR1: amino acids 24-34 of SEQ ID NO: 94
 - VL-CDR2: amino acids 50-56 of SEQ ID NO: 94
 - VL-CDR3: amino acids 89-99 of SEQ ID NO: 94.
2. The anti-IFN- α antibody or IFN- α binding molecule of claim 1, which is capable of binding to and/or neutralizing the activity of IFN- ω .
3. The antibody or IFN- α binding fragment of claim 1 or 2 comprising in its variable region an amino acid sequence of the VH and VL region selected from:
- (i) SEQ ID NO: 2 and SEQ ID NO: 4,
 - (ii) SEQ ID NO: 10 and SEQ ID NO: 12,
 - (iii) SEQ ID NO: 18 and SEQ ID NO: 20,
 - (iv) SEQ ID NO: 22 and SEQ ID NO: 24,
 - (v) SEQ ID NO: 30 and SEQ ID NO: 32,
 - (vi) SEQ ID NO: 38 and SEQ ID NO: 40,
 - (vii) SEQ ID NO: 76 and SEQ ID NO: 78,

- (viii) SEQ ID NO: 84 and SEQ ID NO: 86, or
- (ix) SEQ ID NO: 92 and SEQ ID NO: 94.

4. The antibody or IFN- α binding fragment of claim 3, which is capable of binding an epitope of IFNA2 consisting of the amino acid sequence SAAWDETLLDKFYTELYQ (SEQ ID NO: 99), RITLYLKEKKYSPCAWEV (SEQ ID NO: 100), YTELYQQLNDLEACVIQG (SEQ ID NO: 101) and/or an epitope of IFNAW consisting of the amino acid sequence TGLHQQLQHLETCLLQVV (SEQ ID NO: 102).
5. The antibody or IFN- α binding fragment of any one of claims 1 to 4, which is IgG1.
6. The antibody or IFN- α binding fragment of any one of claims 1 to 5 comprising a CH and/or CL constant region comprising an amino acid sequence selected from the CH and CL amino acid sequences set forth in SEQ ID NOs.: 6, 8, 14, 16, 26, 28, 34, 36, 42, 44, 72, 74, 80, 82, 88, 90, 96 and 98, or an amino acid sequence with at least 60 % identity.
7. The antibody or IFN α -binding fragment of any one of claims 1 to 6 which has an IC₅₀ value of ≤ 10 ng for at least 5 human IFN- α subtypes and/or human IFN- ω .
8. The antibody or IFN- α binding fragment of any one of claims 1 to 7, which is selected from the group consisting of a single chain Fv fragment (scFv), an F(ab') fragment, an F(ab) fragment and an F(ab')₂ fragment.
9. A polynucleotide encoding the antibody or IFN- α binding fragment of any one of claims 1 to 8, wherein the polynucleotide is a cDNA encoding the variable region and at least part of the constant domain.
10. A vector comprising the polynucleotide of claim 9.
11. A host cell comprising a polynucleotide of claim 9 or a vector of claim 10.
12. The antibody of any one of claims 1 to 8, which is detectably labeled or attached to a drug, wherein the detectable label is selected from the group consisting of an enzyme, a radioisotope, a fluorophore, a peptide and a heavy metal.

13. A composition or kit comprising the antibody or IFN- α binding fragment thereof of any one of claims 1 to 8 or 12, the polynucleotide of claim 9, the vector of claim 10 and/or the cell of claim 11, wherein the composition is
 - (i) a pharmaceutical composition and further comprises a pharmaceutically acceptable carrier and optionally further comprises an additional agent useful in treating an immune mediated or autoimmune disease or condition, associated with the expression of IFN- α and/or IFN- ω in a patient; or
 - (ii) a diagnostic composition or kit and further comprises reagents conventionally used in immuno- or nucleic acid based diagnostic methods.
14. An antibody or IFN- α binding fragment thereof of any one of claims 1 to 8 or 12, or the composition of claim 13 for use in:
 - (a) treating or preventing the progression of an immune mediated or autoimmune disease or condition;
 - (b) amelioration of symptoms associated with an immune mediated or autoimmune disease or condition; and/or
 - (c) diagnosing or screening a subject for the presence or for determining a subject's risk for developing an immune mediated or autoimmune disease or condition; associated with the expression of IFN- α and/or IFN- ω in a patient.
15. The antibody or IFN- α binding fragment thereof of claim 14 or the composition of claim 13 or 14, wherein said autoimmune disease is selected from the group consisting of systemic lupus erythematosus (SLE), cutaneous lupus erythematosus (CLE), discoid lupus erythematosus (DLE), type 1 diabetes mellitus (T1DM), Sjogren's syndrome, Graves' disease, dermatomyositis, multiple sclerosis (MS), psoriasis, autoimmune thyroiditis, rheumatoid arthritis (RA), spondyloarthritis and scleroderma, glomerulonephritis and APS1.
16. A method for diagnosing an immune mediated or autoimmune disease or condition in a subject associated with the expression of IFN- α and/or IFN- ω comprising contacting a biological sample of the subject with an anti-IFN- α antibody according to any one of claims 1 to 8 or 12, and detecting the presence of IFN- α and/or IFN- ω .
17. The method of claim 16, wherein said autoimmune disease is selected from the group consisting of systemic lupus erythematosus (SLE), cutaneous lupus erythematosus (CLE), discoid lupus erythematosus (DLE), type 1 diabetes mellitus (T1DM), Sjogren's syndrome, Graves' disease, dermatomyositis, multiple sclerosis (MS), psoriasis, autoimmune thyroiditis, rheumatoid arthritis (RA), spondyloarthritis and scleroderma, glomerulonephritis and APS1.

18. A method of detecting or determining IFN- α and/or IFN- ω in an isolated biological sample comprising admixing the sample with an anti-IFN- α antibody according to any one of claims 1 to 8 or 12, allowing the antibody to form a complex with any IFN- α subtype and/or IFN- ω present in the mixture, and detecting the complex present in the mixture.
19. A process for the manufacture of a human-derived anti-IFN α or anti-IFN α /IFN ω monoclonal antibody, respectively, or a IFN α -binding fragment or of a composition comprising the anti-IFN α or anti-IFN α /IFN ω monoclonal antibody, or a IFN α -binding fragment, which manufacture comprises the step of preparation of the antibody or IFN α -binding fragment thereof by expression in a recombinant host organism of transforming DNA encoding the antibody or the IFN α -binding fragment thereof of any one of claims 1 to 8 or 12.
20. The process of claim 19, wherein the composition is a pharmaceutical composition, and wherein the step of preparation of the antibody or IFN α -binding fragment thereof is followed by admixing the antibody or IFN α -binding fragment thereof with a pharmaceutically acceptable carrier in the manufacture of a pharmaceutical composition.

1/63

A 5D1 (variable heavy chain sequence VH) – SEQ ID NO: 2

FR1-----CDR1--FR2-----CDR2-----
 EVQLVQAGAEVKAPGESLRISCKVSGYTFTSYWISWVRQIPGKGLEWMVKIDPRDSYTIYNPSFQG

FR3-----CDR3-----FR4-----
 HVSISVDKSITTVYLQWSSLQASDTAIYYCVRHYLTQSLVDYFDHWGQGTLLVAVSS

5D1 (variable kappa chain sequence VL) – SEQ ID NO: 4

FR1-----CDR1-----FR2-----CDR2---
 DIQMTQSPSSLSASVGDSVTITCRASQSVSNYFHWYRQKPGKAPELLIYSASNLOQT

FR3-----CDR3-----FR4-----
 GVPSRFTGSGSGTECTLTITSLQPDDEFATYYCQQTHGYPFTFGQGTKLDVR

B 13B11 (variable heavy chain sequence VH) – SEQ ID NO: 10

FR1-----CDR1--FR2-----CDR2-----
 DVQLLQSGGGGLIQPGGSLRLSCAASGFTFKDYAMSWVRQAPGKGLEWVSVLSRSGNIVDYVDSVKG

FR3-----CDR3-----FR4-----
 RFTVSRDNSNNTLFLQMDGLRADDTAIYYCAKPKDMIVVVPAGFDSWGQGTLLVSVSS

13B11 (variable kappa chain sequence VL) – SEQ ID NO: 12

FR1-----CDR1-----FR2-----CDR2---
 DIQMTQFPSTLSASVGDSVTITCRASQSISAWLAWYQQKPGKAPKLLIYKGSRLEN

FR3-----CDR3---FR4-----
 GVPSRFSGSGSGTEFTLTIGSLQPDDEFATYYCQQYKTWTFGQGTKVEIK

C 19D11 (variable heavy chain sequence VH) – SEQ ID NO: 18

FR1-----CDR1--FR2-----CDR2-----
 EVQLLESGAEVKRPGSSVRVSCRASGDTFSSYPISWVRQAPGQGLEWMGRILPALGVTNYAQNFRG

FR3-----CDR3-----FR4-----
 RITITADKSPLTAYLELSSLRFDFTAVYYCASPSADIIPSILGTTLFAFWGQGSLLVTVSS

19D11 (variable kappa chain sequence VL) – SEQ ID NO: 20

FR1-----CDR1-----FR2-----CDR2---
 EIVLTQSPGTLSSLSPGEGATLSCRASQNVSRHYLTWYQQKPGQSPRLLIYGGSSRAT

FR3-----CDR3-----FR4-----
 GVPDRFSGGGSGTDFTLTISRLEPEDFAVYFCQSYHSPPPVYTFGQGTKVEIK

Fig. 1

2/63

D 25C3 (variable heavy chain sequence VH) – SEQ ID NO: 22

FR1-----CDR1-----FR2-----CDR2-----
 EMQLMESGGGLVQPGGSLRLSCVASGFTFKSFAMSWVRQAPGKGLEWVASVGSQGGSKYYAPSVKG

FR3-----CDR3-----FR4-----
 RFSISRDNNSNTLYVQMNSLGVEDTAFYYCVKETDAVATMDALDMWGQGTLLIVST

25C3 (variable kappa chain sequence VL) – SEQ ID NO: 24

FR1-----CDR1-----FR2-----CDR2---
 DIRVTQSPSSLSASVGDRVSISCQTSQSVNIYLNWYQRPKGKGPQLLISAASTLQS

FR3-----CDR3-----FR4-----
 GVPSRFSGSGSGTDFILTIISLQPEDSASYCQQGYITPYTFGQGTKVEIK

E 26B9 (variable heavy chain sequence VH) – SEQ ID NO: 30

FR1-----CDR1---FR2-----CDR2-----
 QIILLQESGPGLVKPTETLSLTCSVSGDSISDSSHYWAWIRQPPGKGPEWIGSVYFSSMTHYNPSLKS

FR3-----CDR3-----FR4-----
 RVSISVDKPKNQFSLKVTSVTVADTATYYCARQALARVGAMNWFDPWGQGS�TVSS

26B9 (variable lambda chain sequence VL) – SEQ ID NO: 32

FR1-----CDR1-----FR2-----CDR2---
 DIIMTQSPDSLPLVSLGEGVTINCKSSQSVFFTSSNKSCLAWYQKPGKSPKLLIYWASTRQS

FR3-----CDR3-----FR4-----
 GVPDRFRGSGSGTDFSLTITSLQAEDVAVYFCQQCQTSPTFGGGTRLEIK

F 31B4 (variable heavy chain sequence VH) – SEQ ID NO: 38

FR1-----CDR1---FR2-----CDR2-----
 QIQLQESGPGLVRPTETLSLTCSVSGDSISQSSHYWAWIRQPPGKGPEWIGSVYFSSMTHYNPSLTS

FR3-----CDR3-----FR4-----
 RVSISIDKAMNKFSLKVTSVTVADTATYYCARQALARVGAMNWFDPWGQGS�TVSS

31B4 (variable lambda chain sequence VL) – SEQ ID NO: 40

FR1-----CDR1-----FR2-----CDR2---
 DIIMTQSPESLPLVSLGEGVTINCKSSQSVFFTSSNRSCLAWYQKPGQSPKLLIYWASTRQS

FR3-----CDR3-----FR4-----
 GVPDRFTGSGSGTDFSLTIAGLQVEDVAVYFCQQCHASPPTFGGGTRLELR

Fig. 1 (continued)

3/63

G 8H1 (variable heavy chain sequence VH) – SEQ ID NO: 76

FR1-----CDR1-FR2-----CDR2-----
 QVQLVQSGAEVKKPGASVKVSCKASGQTFTSDDINWVRQAPGQGLEWMGWRNPNTQDTGYAQKFHG

FR3-----CDR3-----FR4-----
 RLTLTSNSSISTSYLELSGLRSED TAVYYCARAGTSTLTGHYFALGVWGQGTIVVSS

8H1 (variable kappa chain sequence VL) – SEQ ID NO: 78

FR1-----CDR1-----FR2-----CDR2---
 DIQLTQSPSSLSASVGDRVTITCQATQDISKYLNWYQQKPGKVPKLLIYETSNLEV

FR3-----CDR3-----FR4-----
 GVPSRFSGSGSGTHFTLTISSLQAEDEFATYYCQQYENFPFTFGGGTKVEIK

H 12H5 (variable heavy chain sequence VH) – SEQ ID NO: 84

FR1-----CDR1-FR2-----CDR2-----
 QVQLIQSGPEVKRPGASVKVSCKASENTFDTHYINWVRQAPGQGLTWLGWLNPTTGKTGFPQKFQK

FR3-----CDR3-----FR4-----
 RVILTSDTSLNTAYMEVSRLTSED TAVYFCARVLKLSDEYNYGFDVWGQGTIVVSS

12H5 (variable kappa chain sequence VL) – SEQ ID NO: 86

FR1-----CDR1-----FR2-----CDR2---
 DIQVTQSPSSLSASIGDRVTITCRASQNILTFINWYQHKGKAPKLLIYAASVLQN

FR3-----CDR3-----FR4-----
 EVPSRFSGSGSGTDFTLTITSLQPD DFGTYYCQOTYLTPQCSFGGQGTKVEIK

I 50E11 (variable heavy chain sequence VH) – SEQ ID NO: 92

FR1-----CDR1-FR2-----CDR2-----
 QVQLVQSGAEMKKPGSSVKVSCKDFGGTFSVYGVNWVRQAPGQGLEWMGGLIPVIGPANYAQKFQG

FR3-----CDR3-FR4-----
 RITITADESTSTAYMELSSLRFD DTAIYYCVRDDNEYWGQGTIVTVSS

50E11 (variable kappa chain sequence VL) – SEQ ID NO: 94

FR1-----CDR1-----FR2-----CDR2---
 EMVLTQSPATLSLSPGERATLSCRASQTVSTFLAWYQQKPGQVPRLLIYDISSRAN

FR3-----CDR3-----FR4-----
 GTPARFSGGGSGTDFTLTISSELEDFAVYYCQWRSNWPPSLTFGGGTRVEIK

Fig. 1 (continued)

4/63

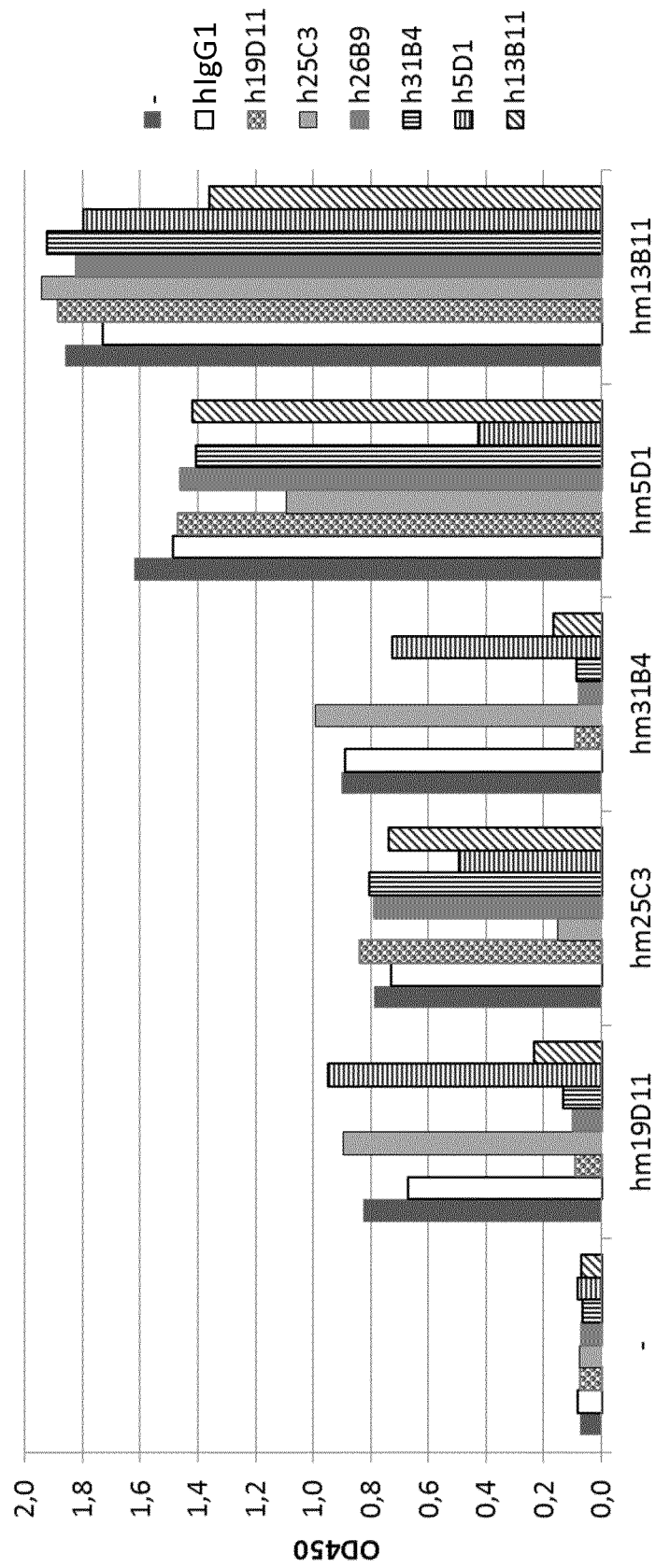
A**IFNA2**

Fig. 2

IFNA4

B

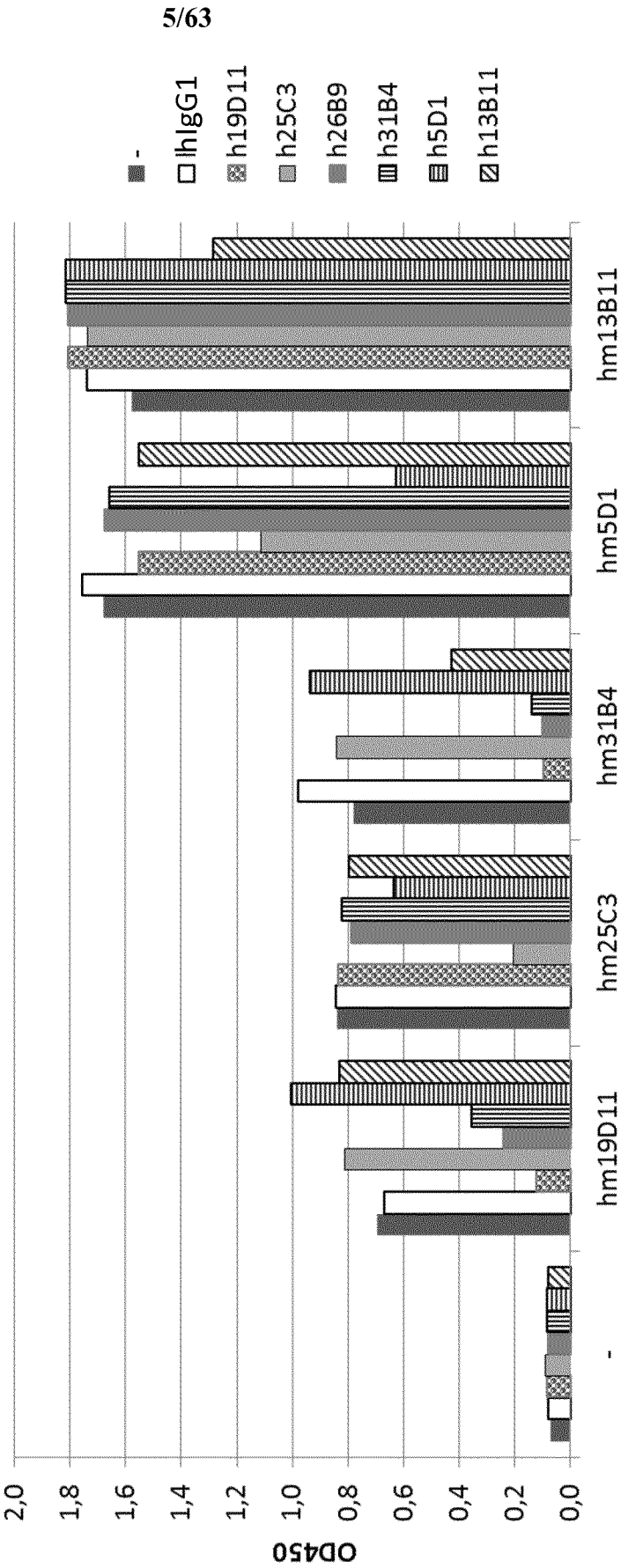


Fig. 2 (continued)

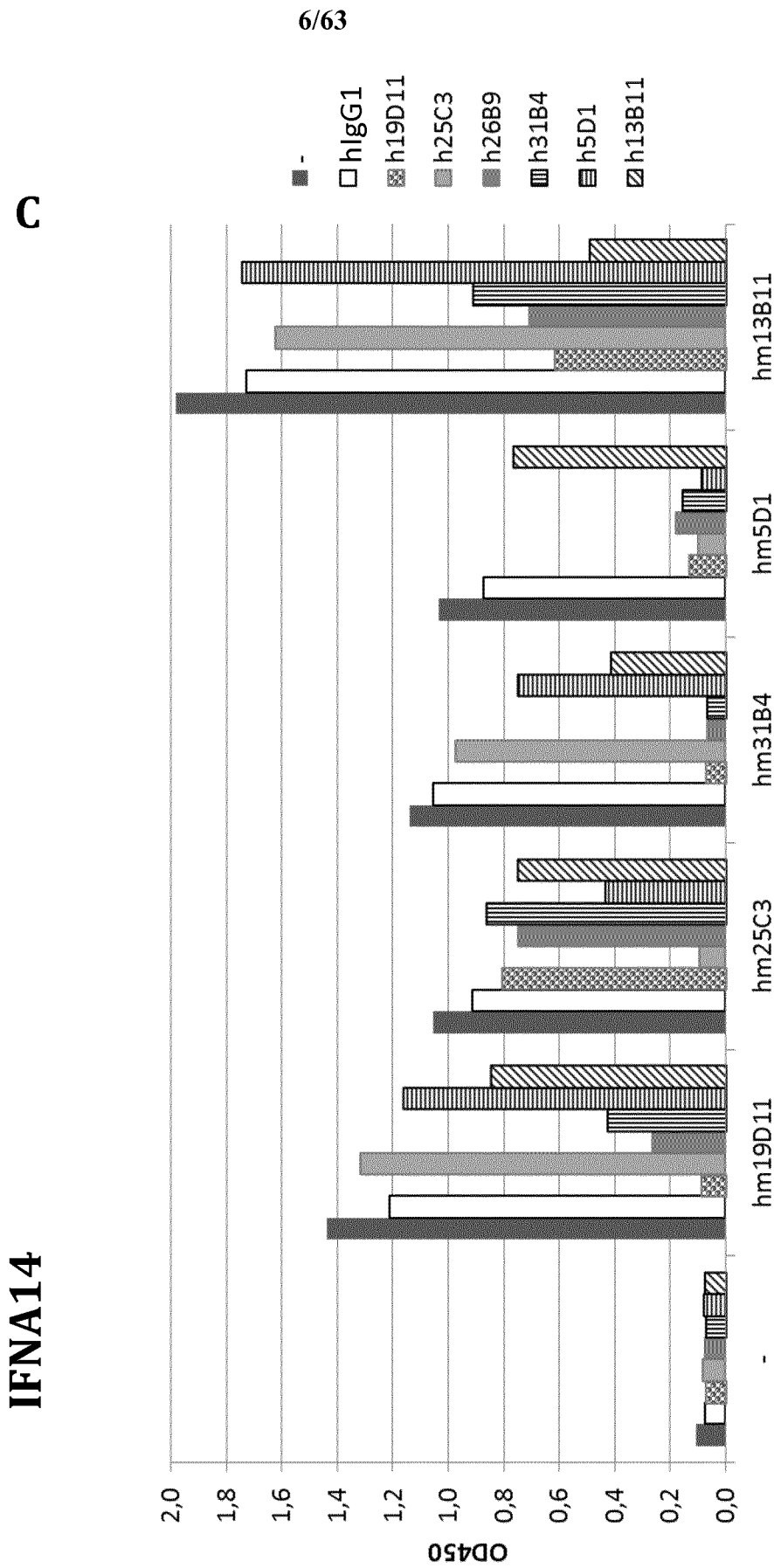


Fig. 2 (continued)

7/63

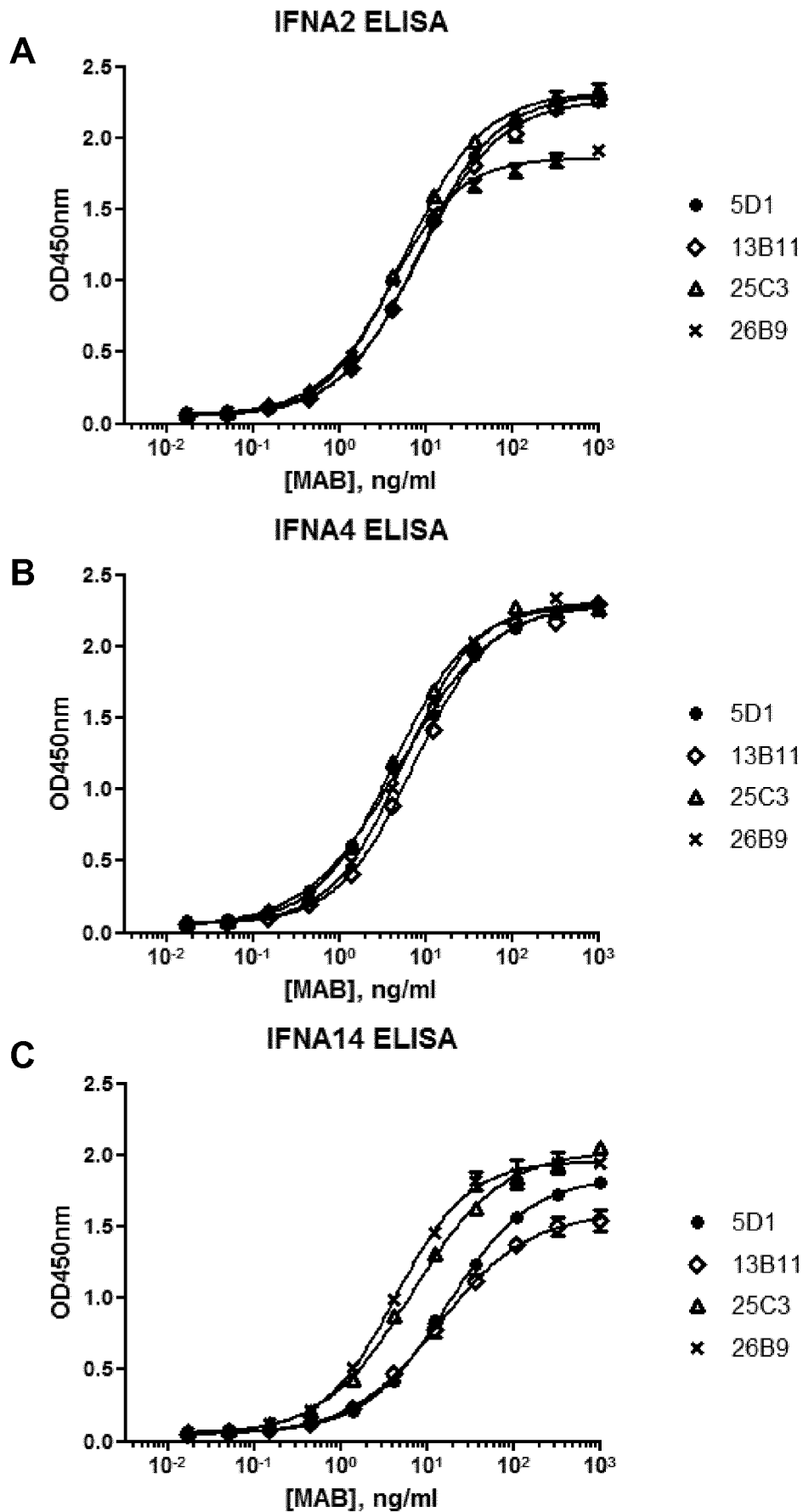


Fig. 3

8/63

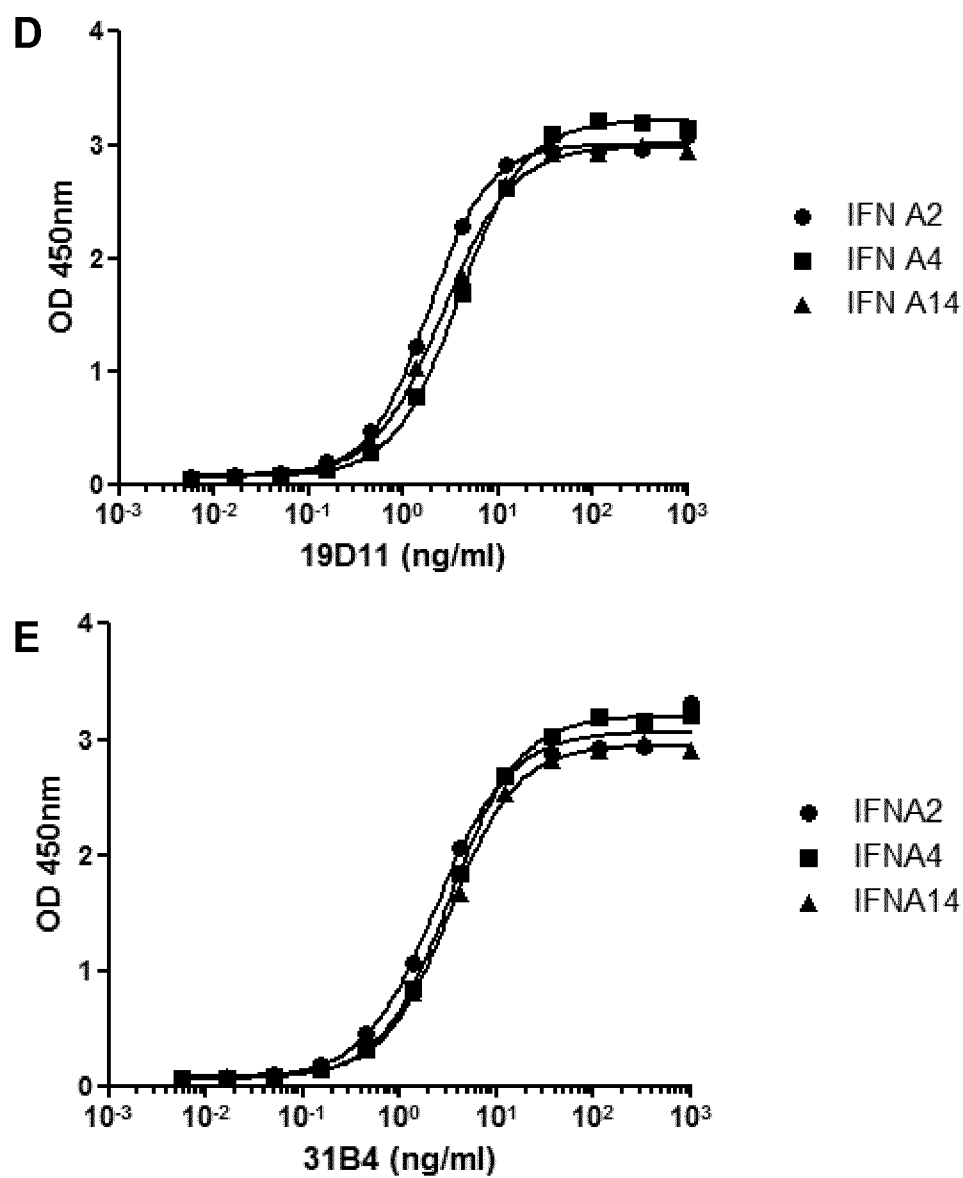


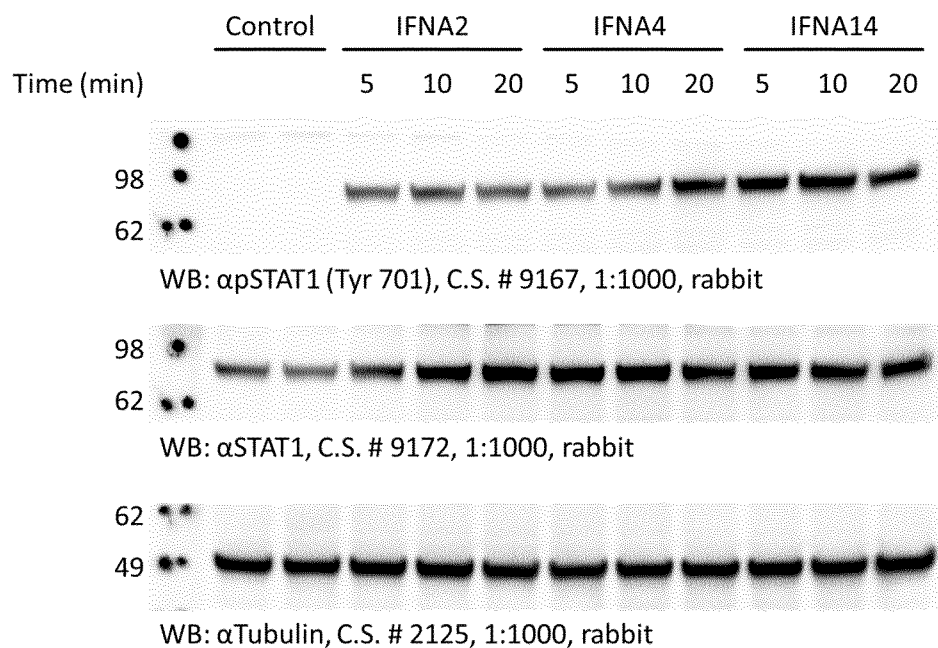
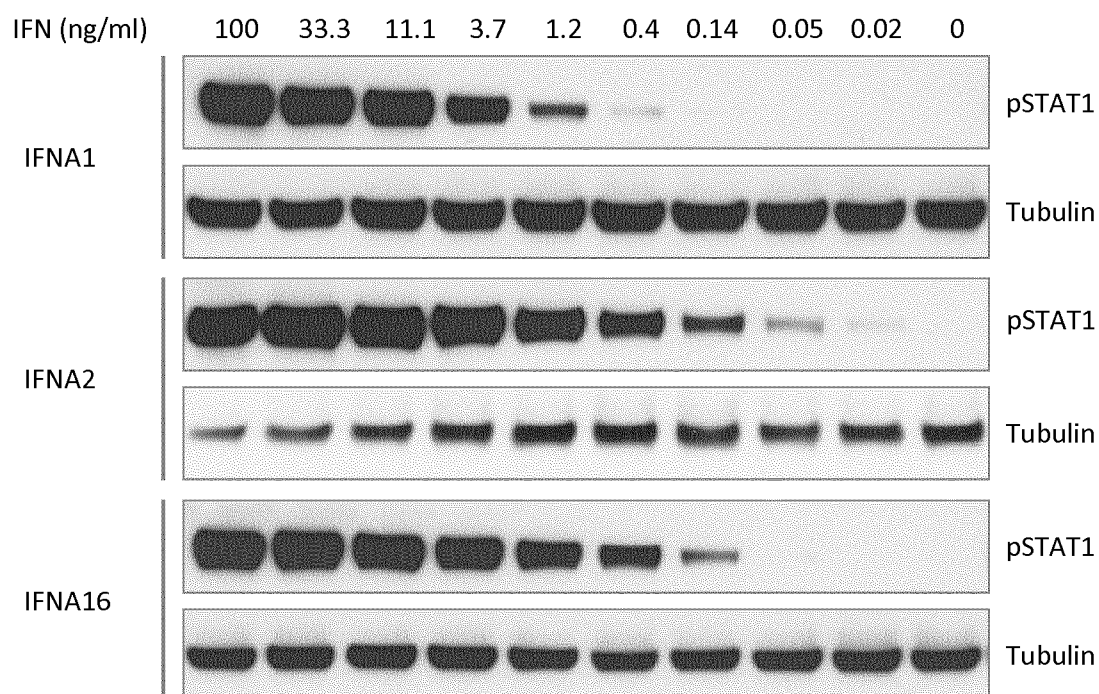
Fig. 3 (continued)

9/63

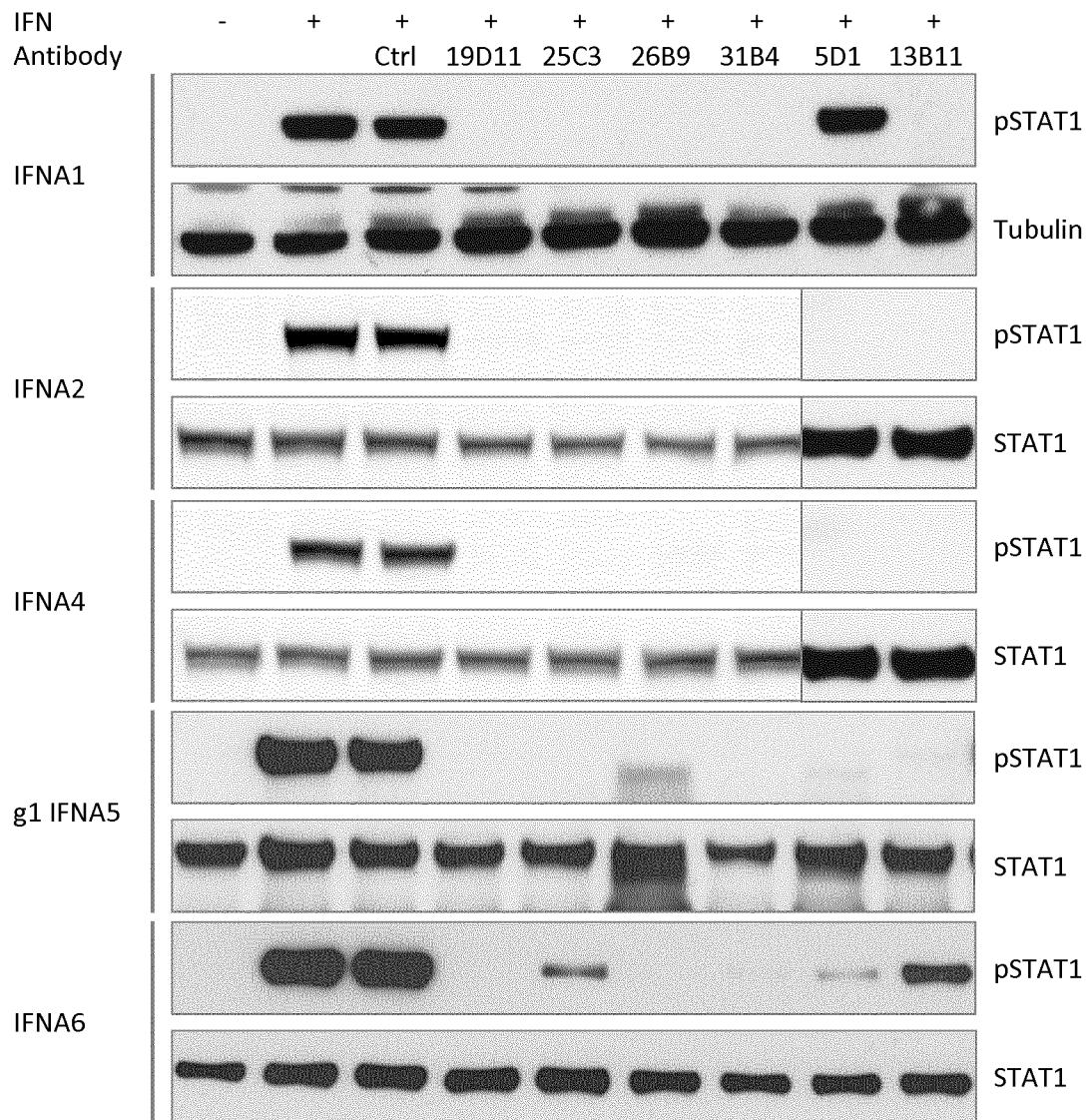
Target	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
dsDNA	○	○	○	○	○	○	○	○	○	○	○		○	○	○	○	○	○	○			○	○
IFN- α 8	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
IFN- α 2	○	○	○	○	○	○	○	○	○	○		○	○	○	○	○	○	○	○	○	○	○	○
IFN- α 4	○	○	○	○	○	○	○	○	○	○		○	○	○	○	○	○	○	○	○	○	○	○
IFN- α 14	○	○	○	○	○	○	○	○	○	○		○	○	○	○	○	○	○	○	○	○	○	○
GAD1 brain		○		○						○				○		○	○	○			○		
GAD2		○	○	○			○			○				○		○	○	○			○		
Insulin		○																					
IFN- α 5	○		○		○	○	○	○	○	○				○	○	○	○	○	○	○		○	○
IFN- α 6	○		○		○	○	○	○	○	○				○	○	○	○	○	○	○		○	○
IFN- α 21	○		○		○	○	○	○	○	○				○	○	○	○	○	○	○			○

Fig. 4

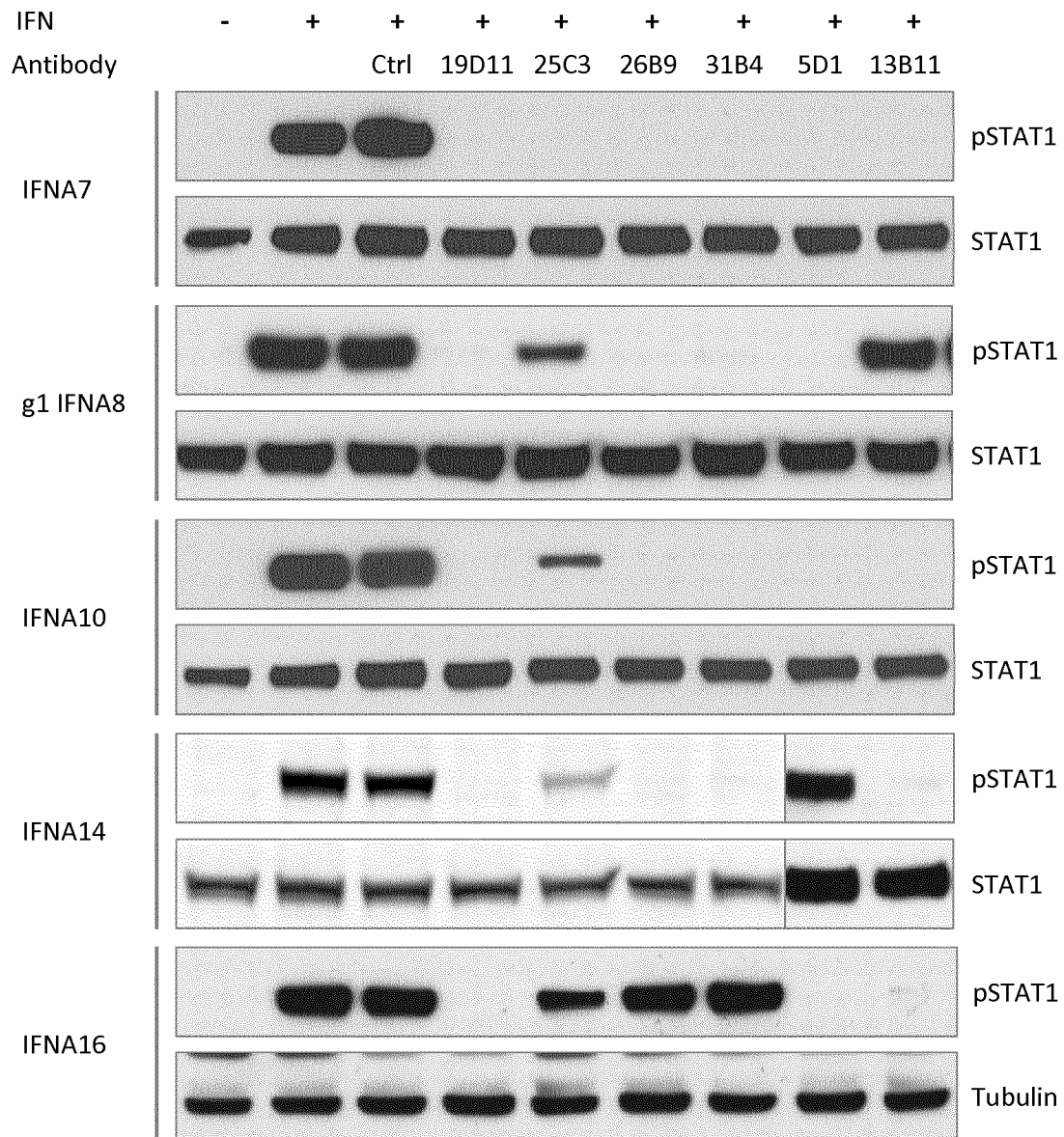
10/63

A1**A2****Fig. 5**

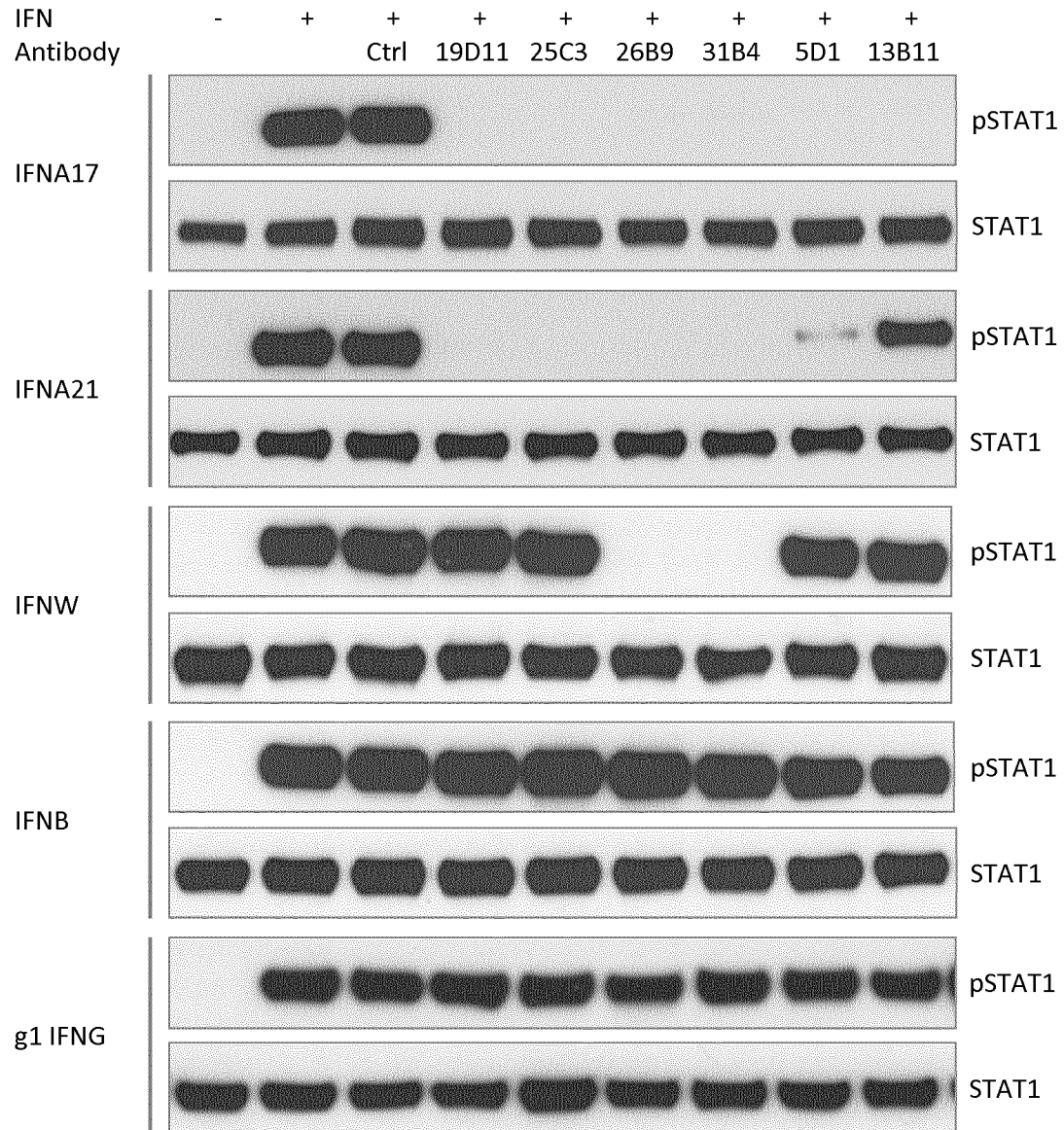
11/63

B**Fig. 5 (continued)**

12/63

C**Fig. 5 (continued)**

13/63

D**Fig. 5 (continued)**

14/63

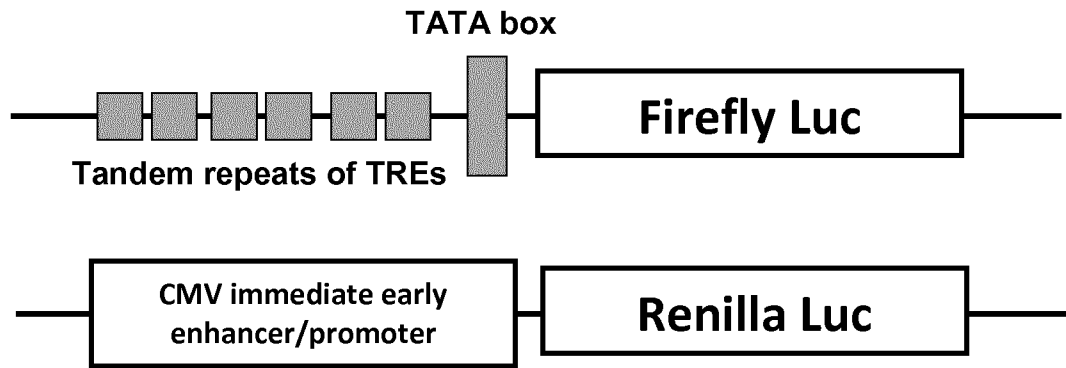


Fig. 6

15/63

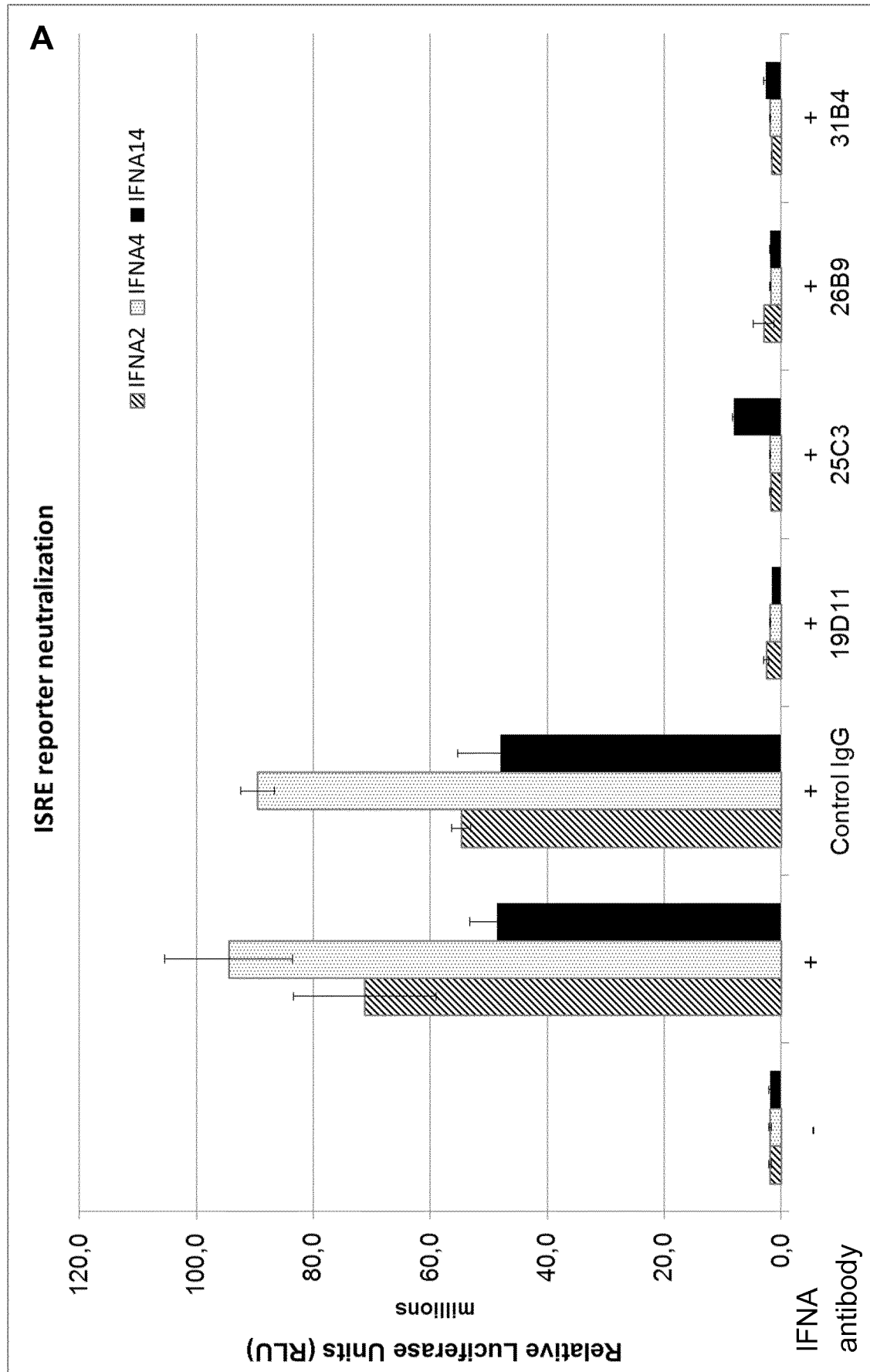


Fig. 7

16/63

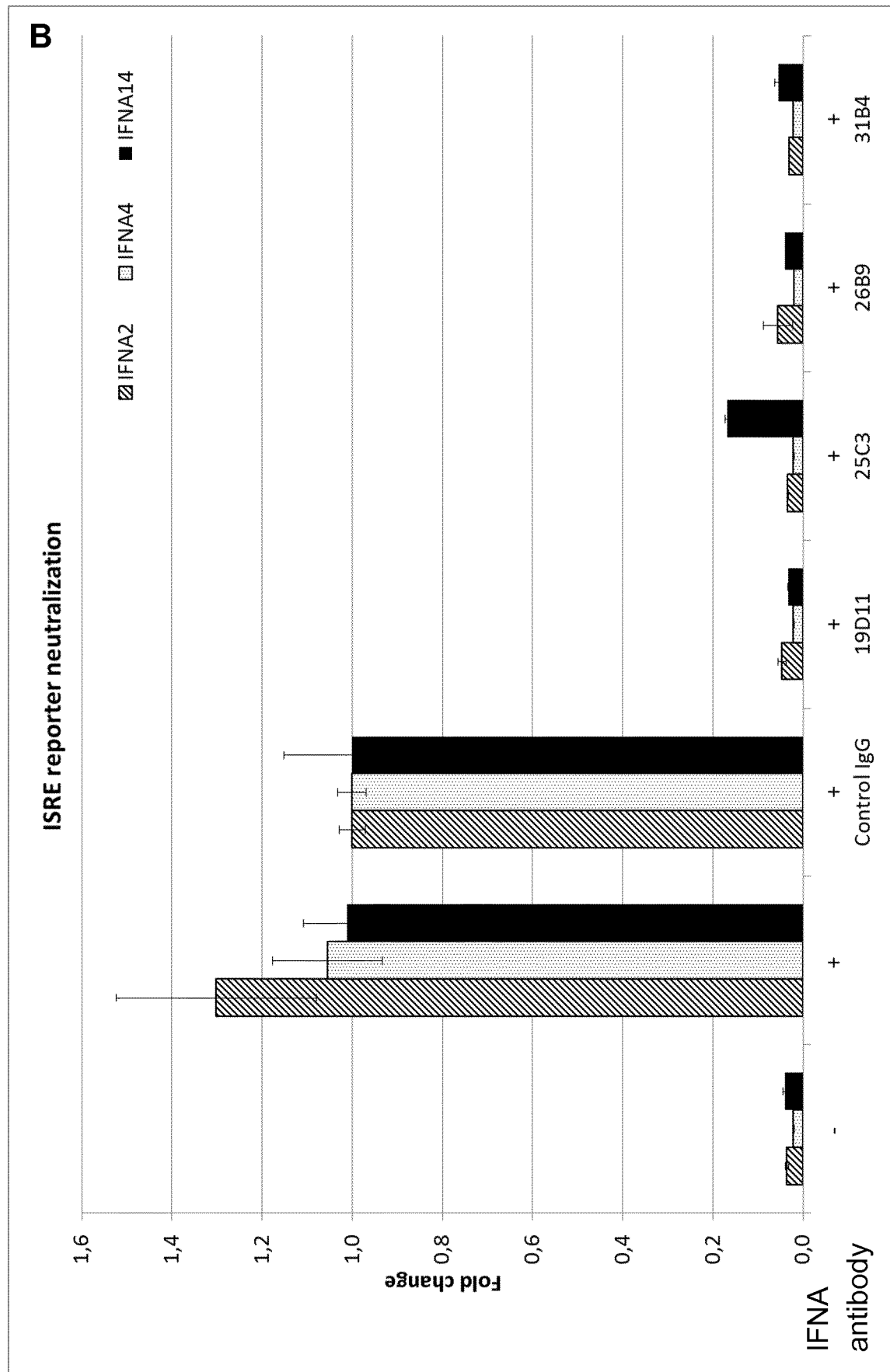


Fig. 7 (continued)

17/63

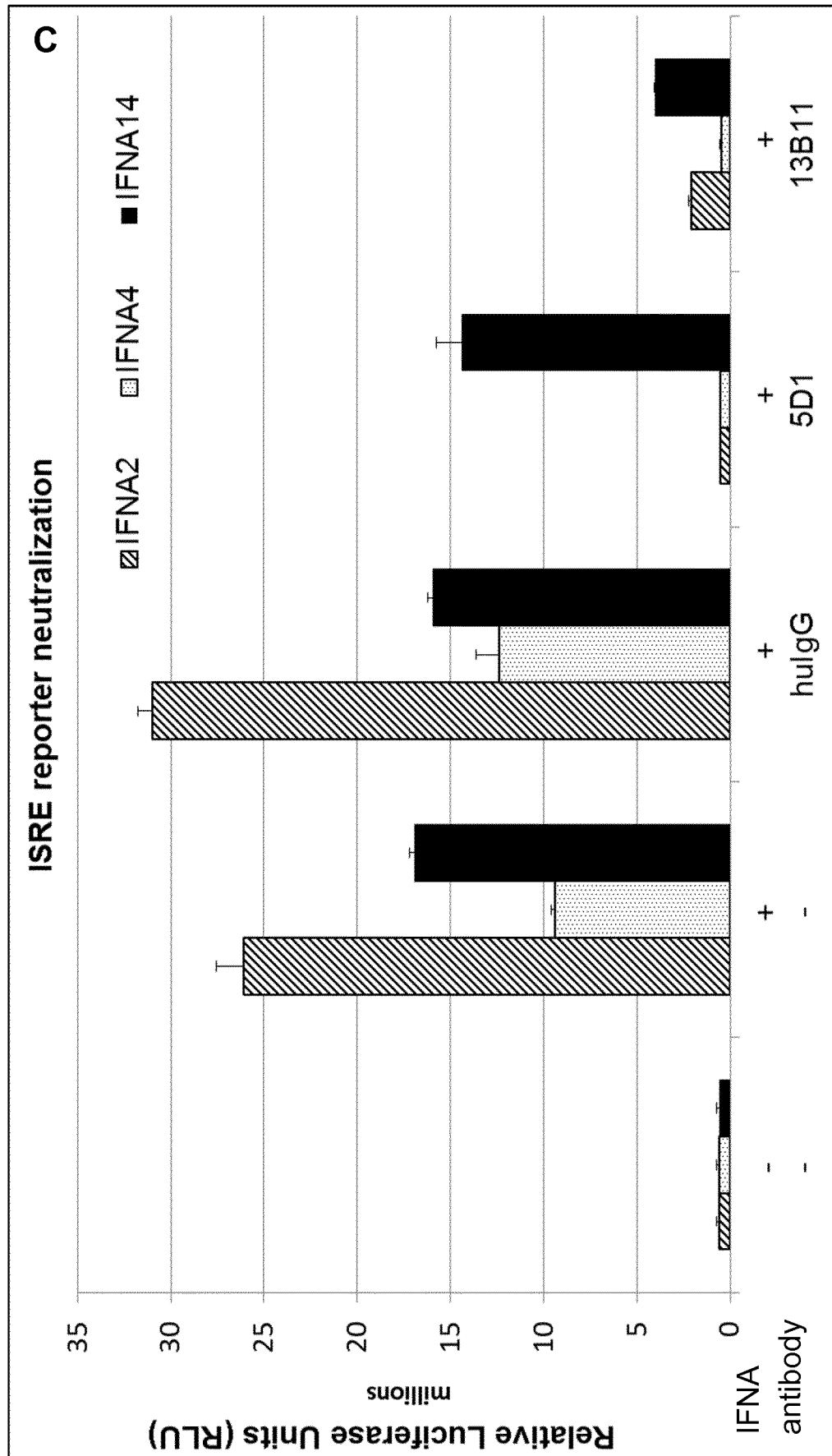


Fig. 7 (continued)

18/63

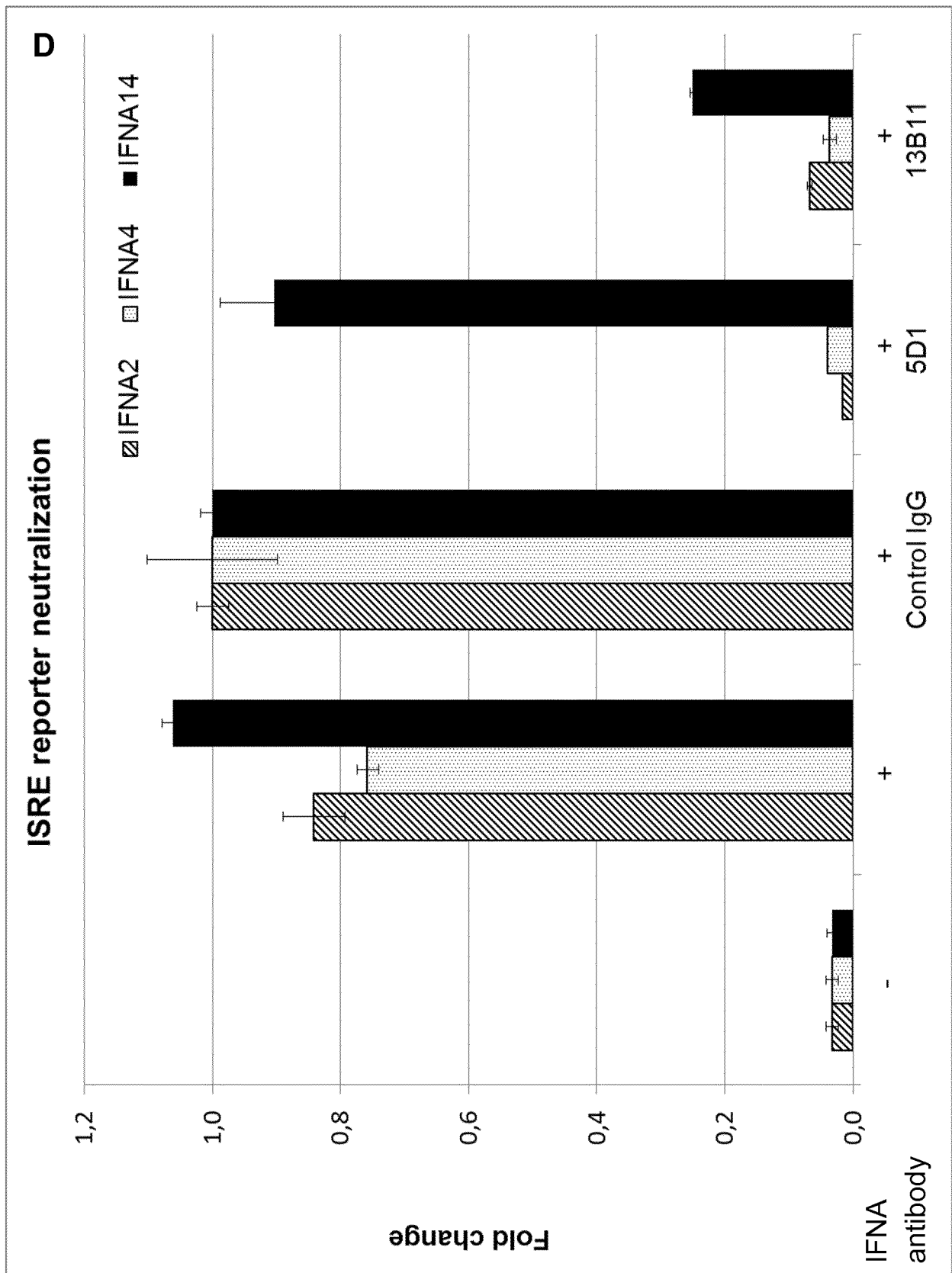


Fig. 7 (continued)

19/63

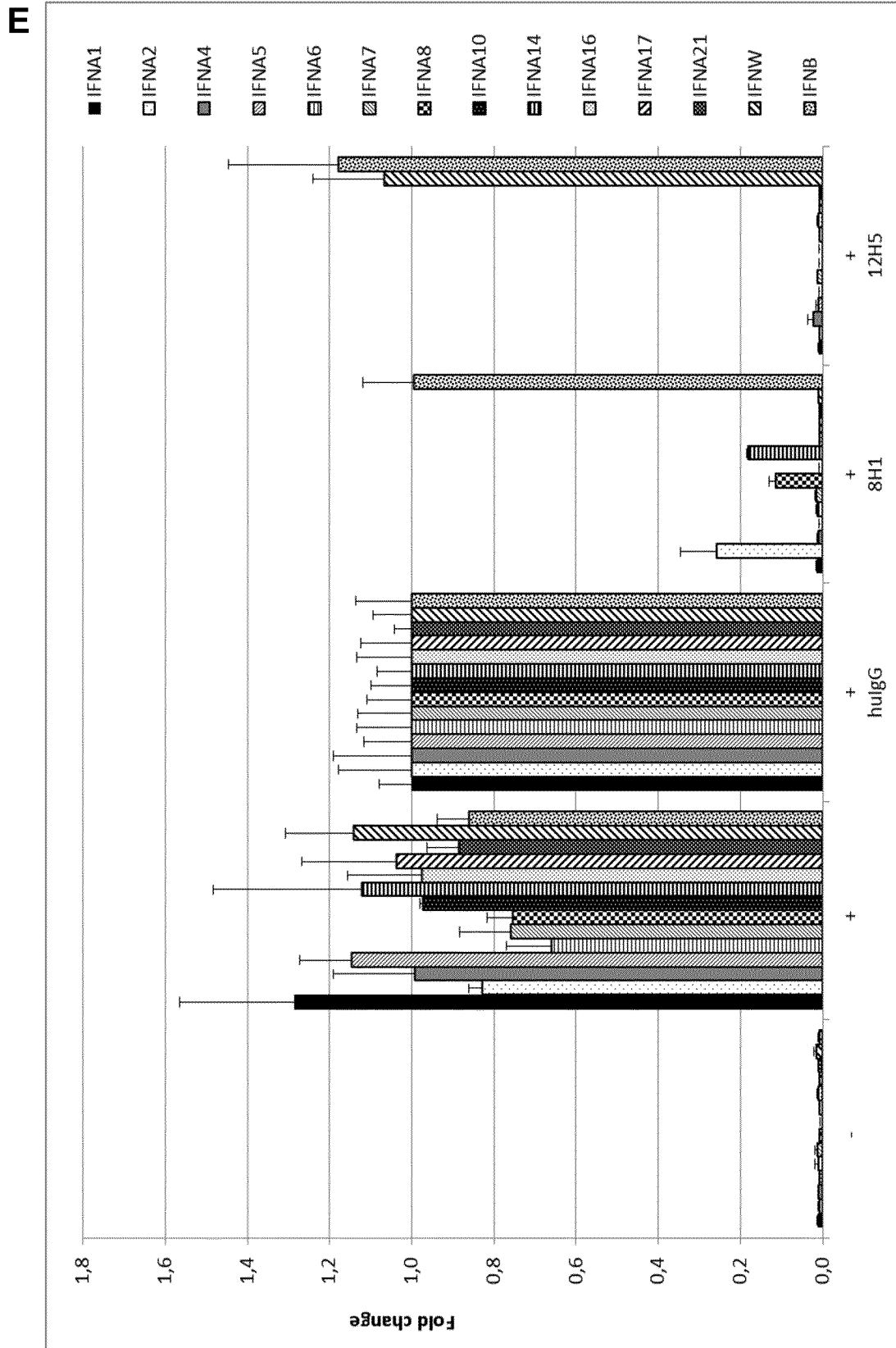


Fig. 7 (continued)

20/63

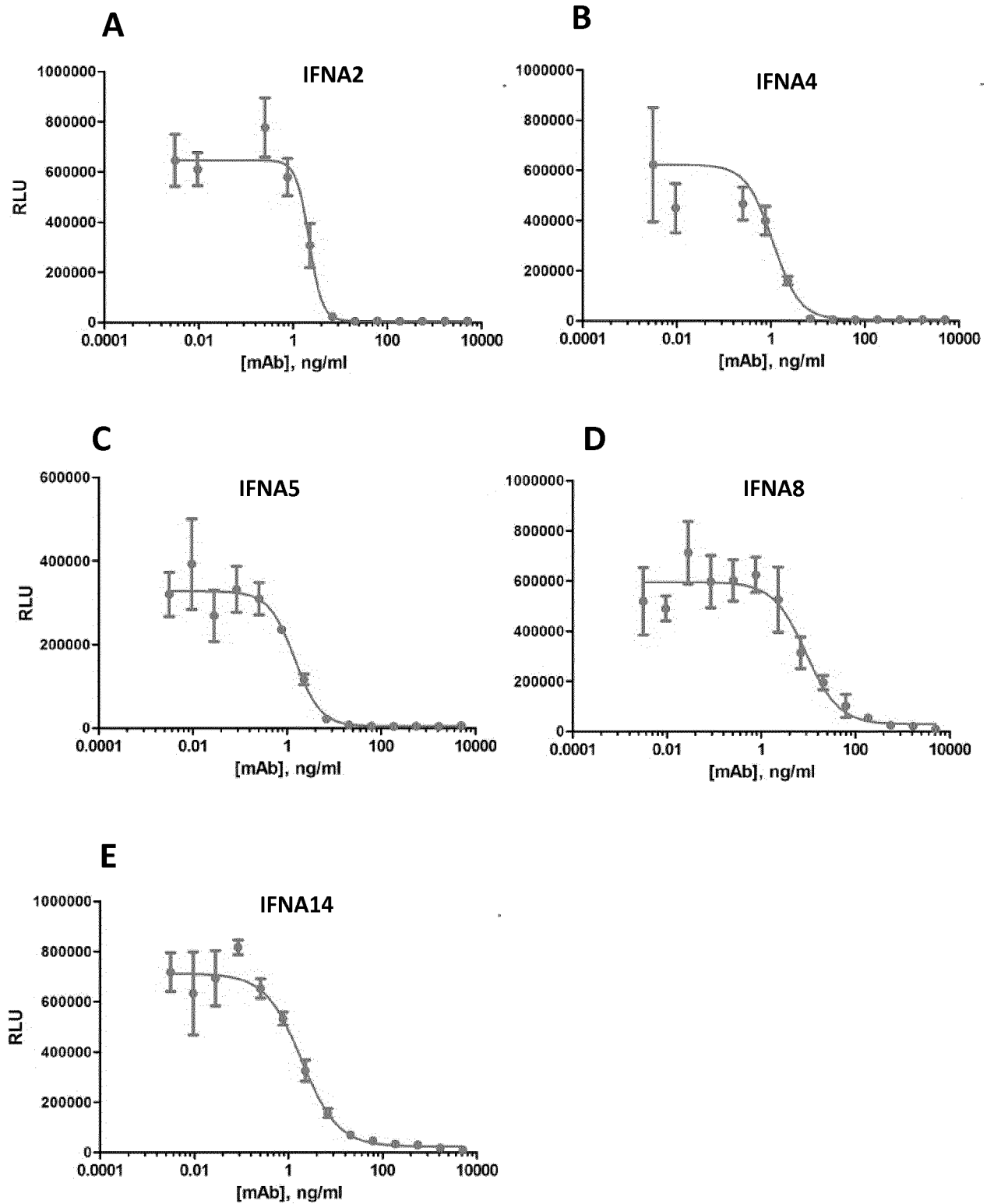


Fig. 8

21/63

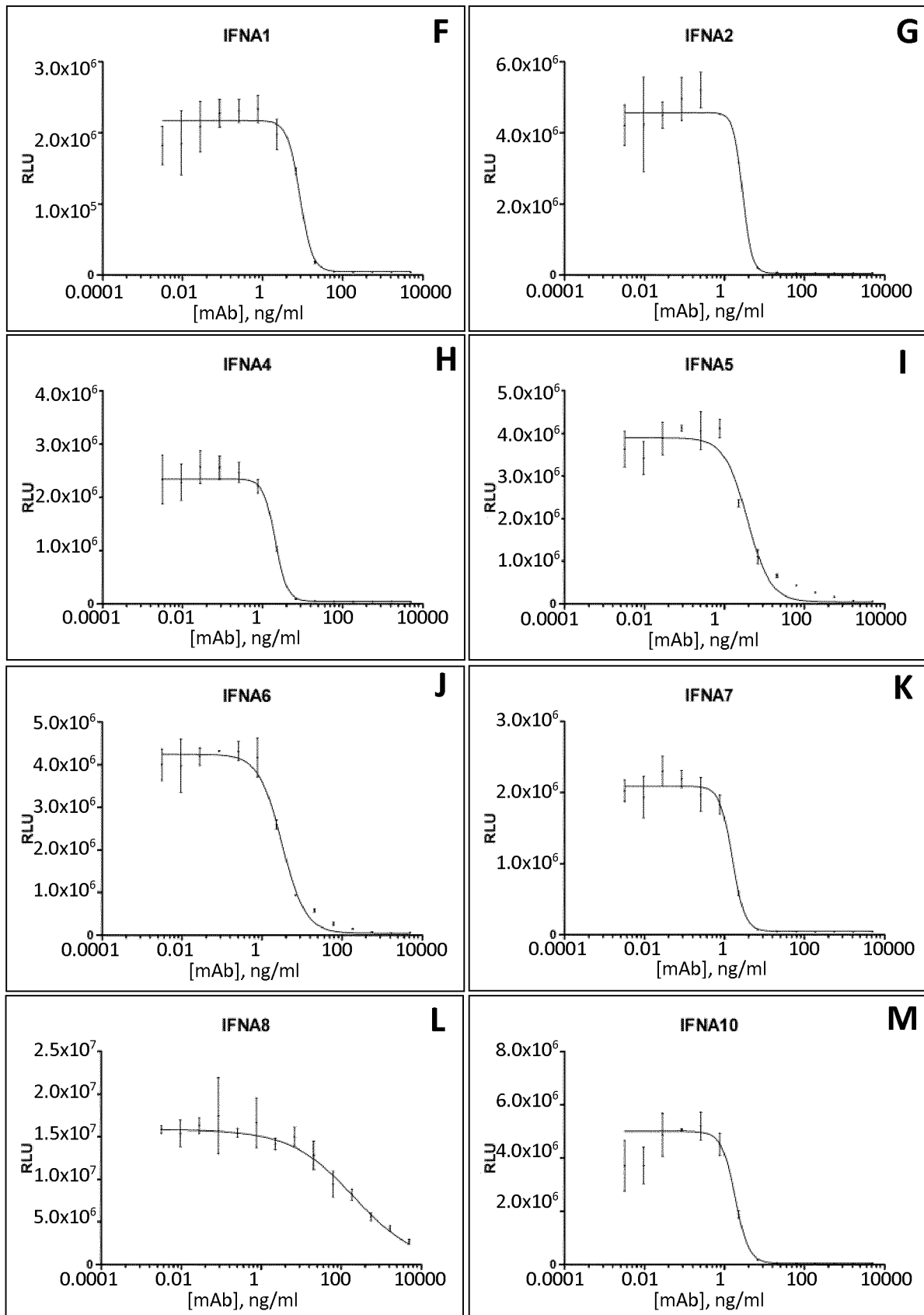


Fig. 8 (continued)

22/63

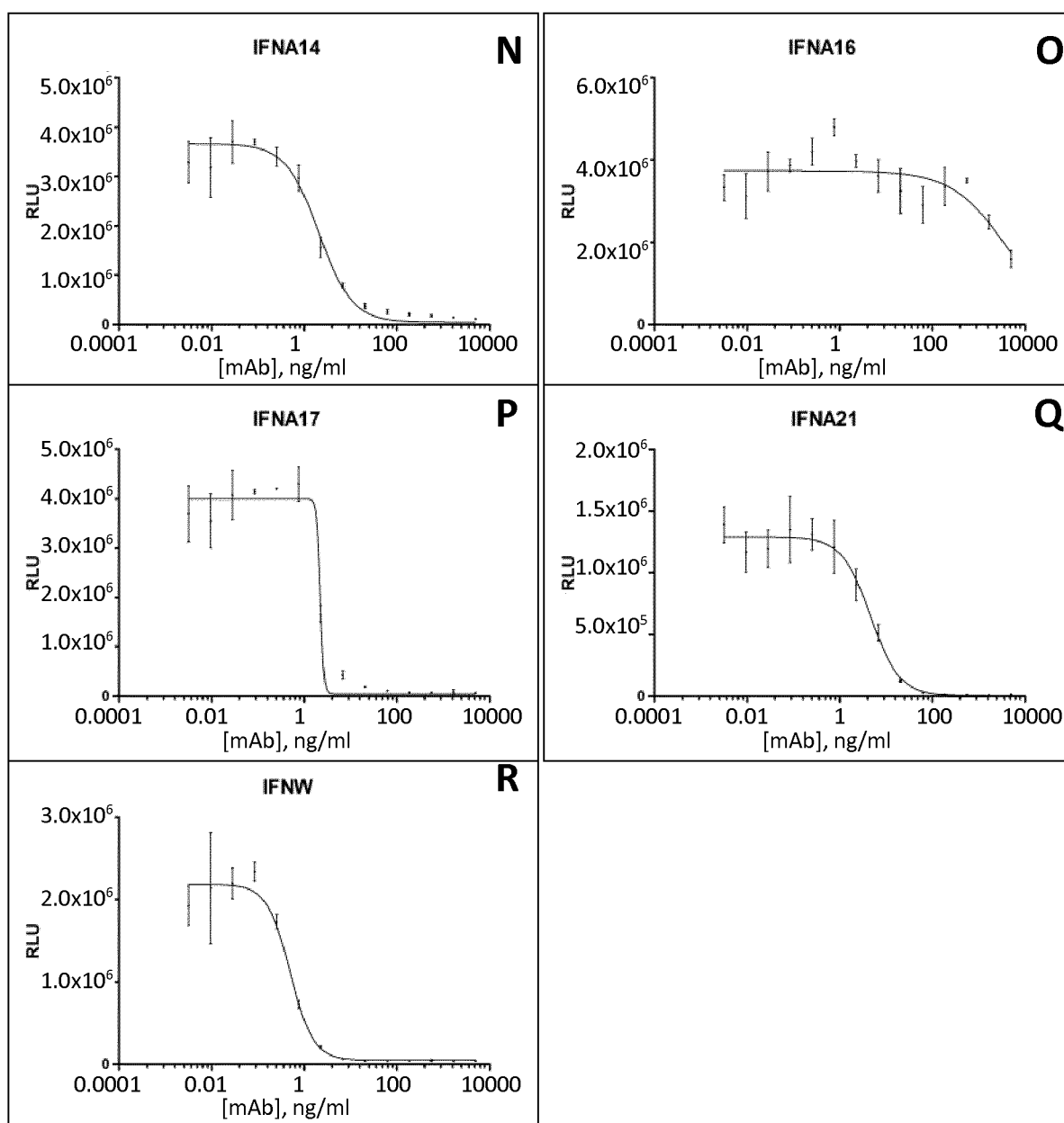


Fig. 8 (continued)

23/63

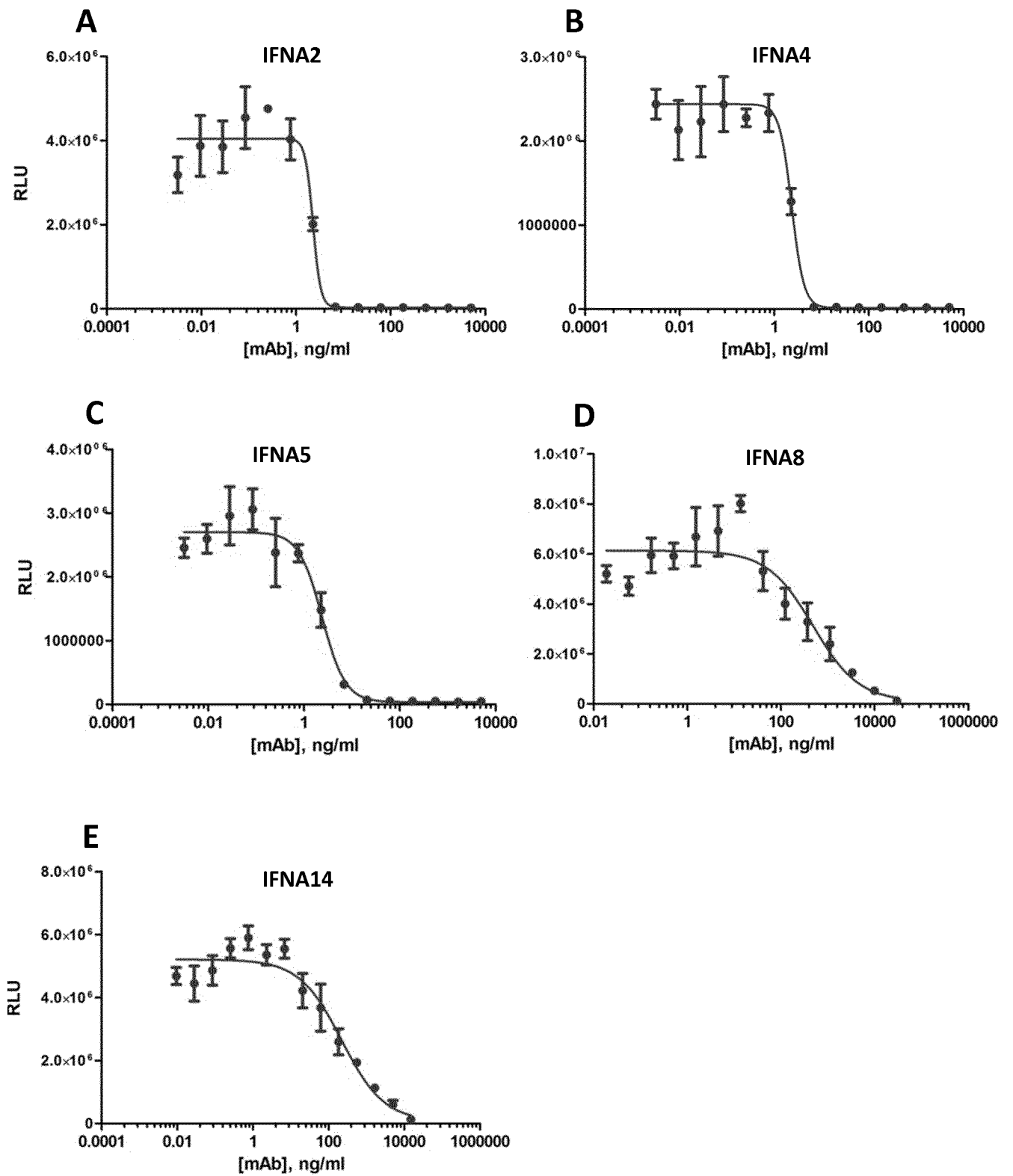


Fig. 9

24/63

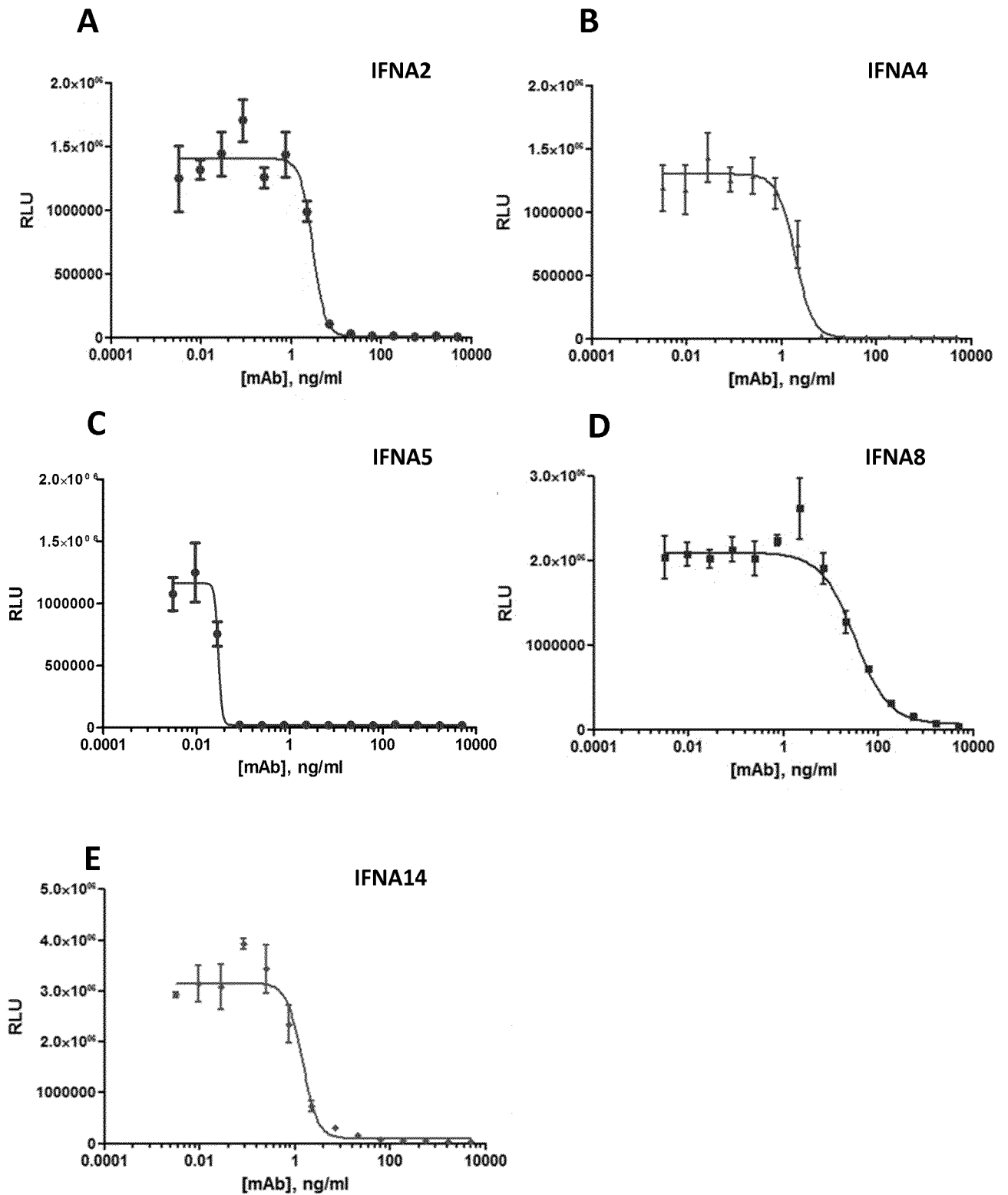


Fig. 10

25/63

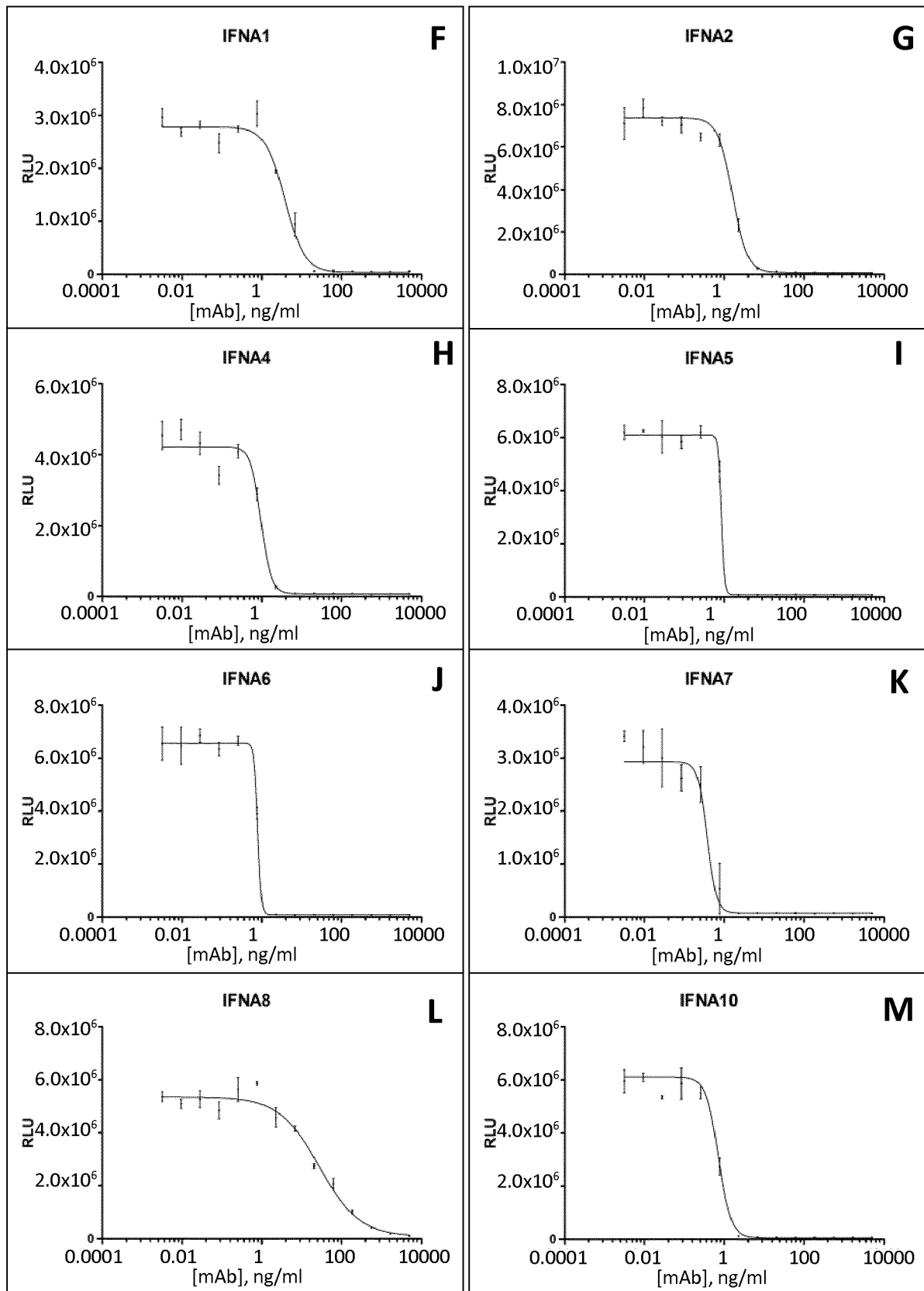


Fig. 10 (continued)

26/63

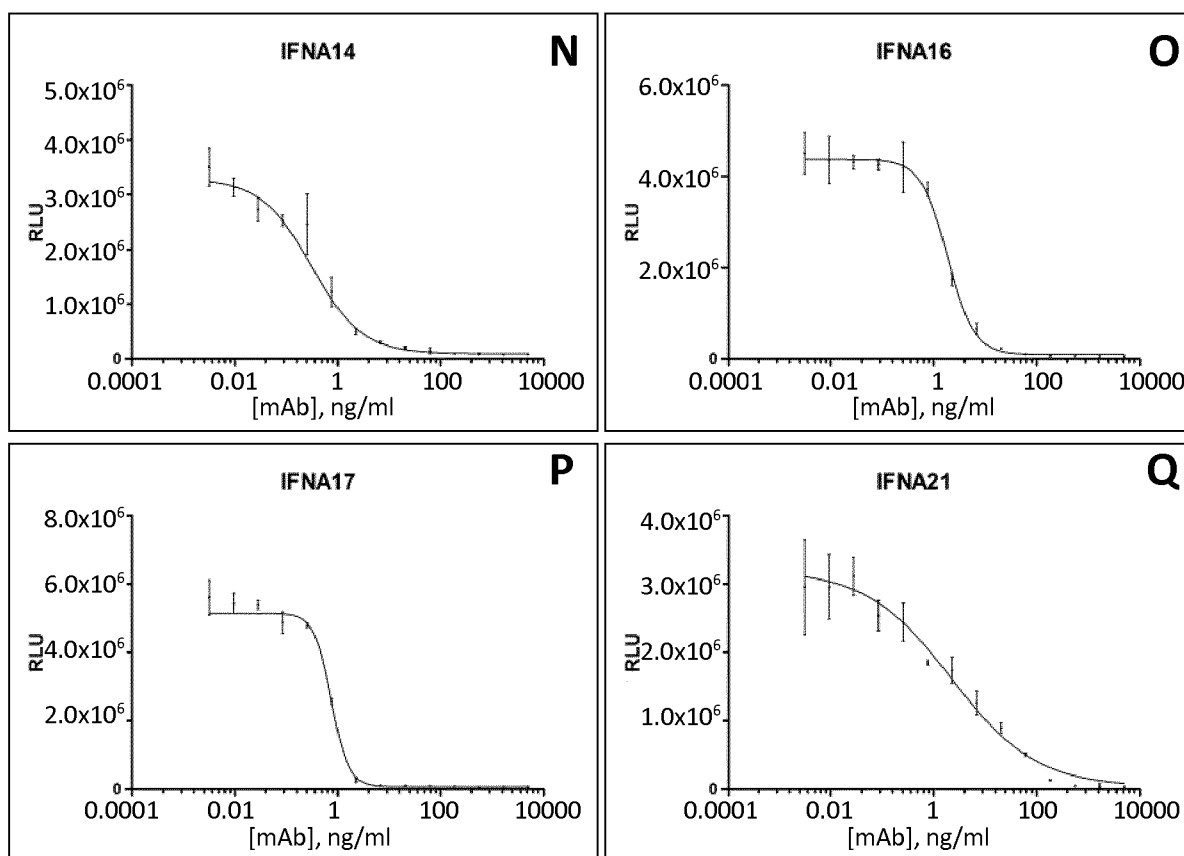


Fig. 10 (continued)

27/63

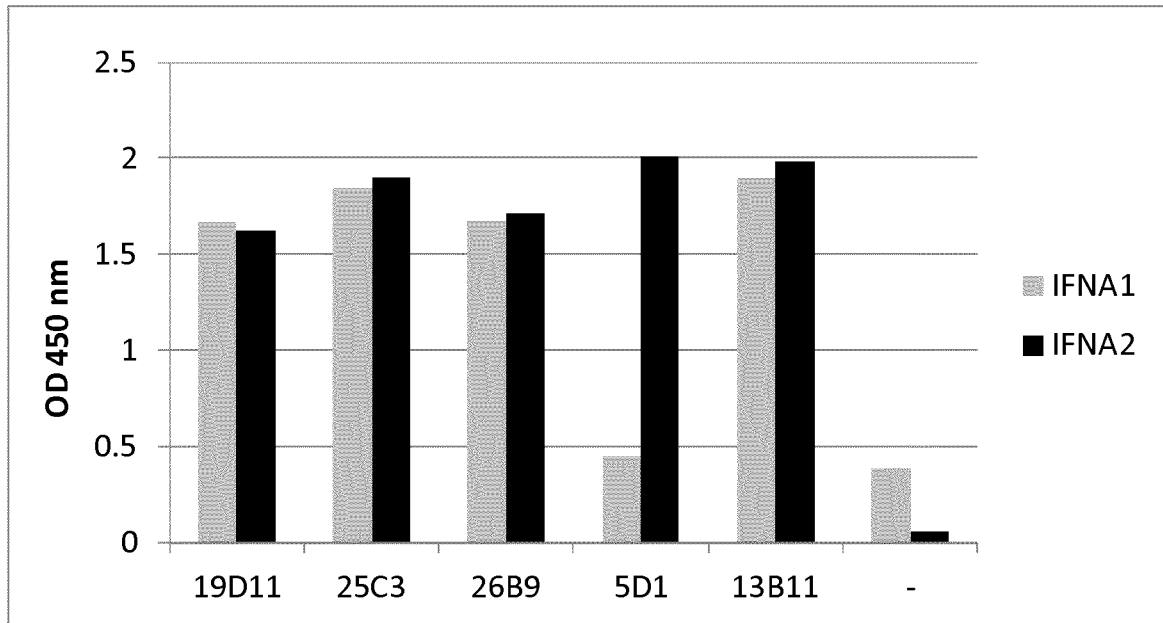
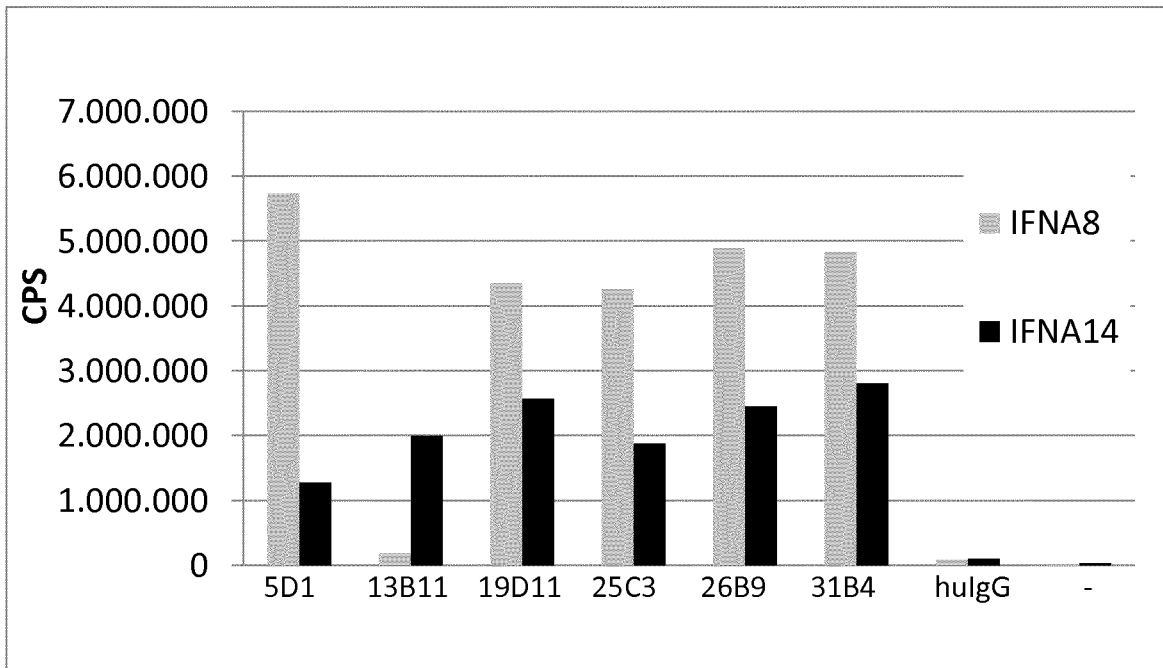
A**B**

Fig. 11

28/63

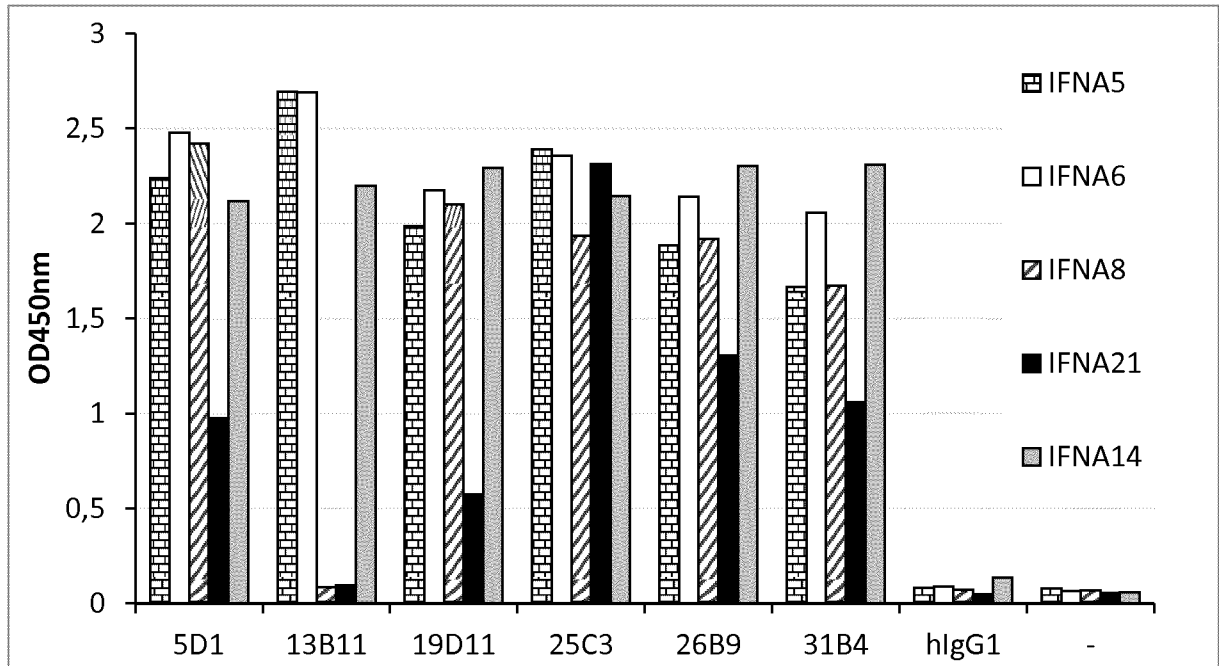
C

Fig. 11 (continued)

29/63

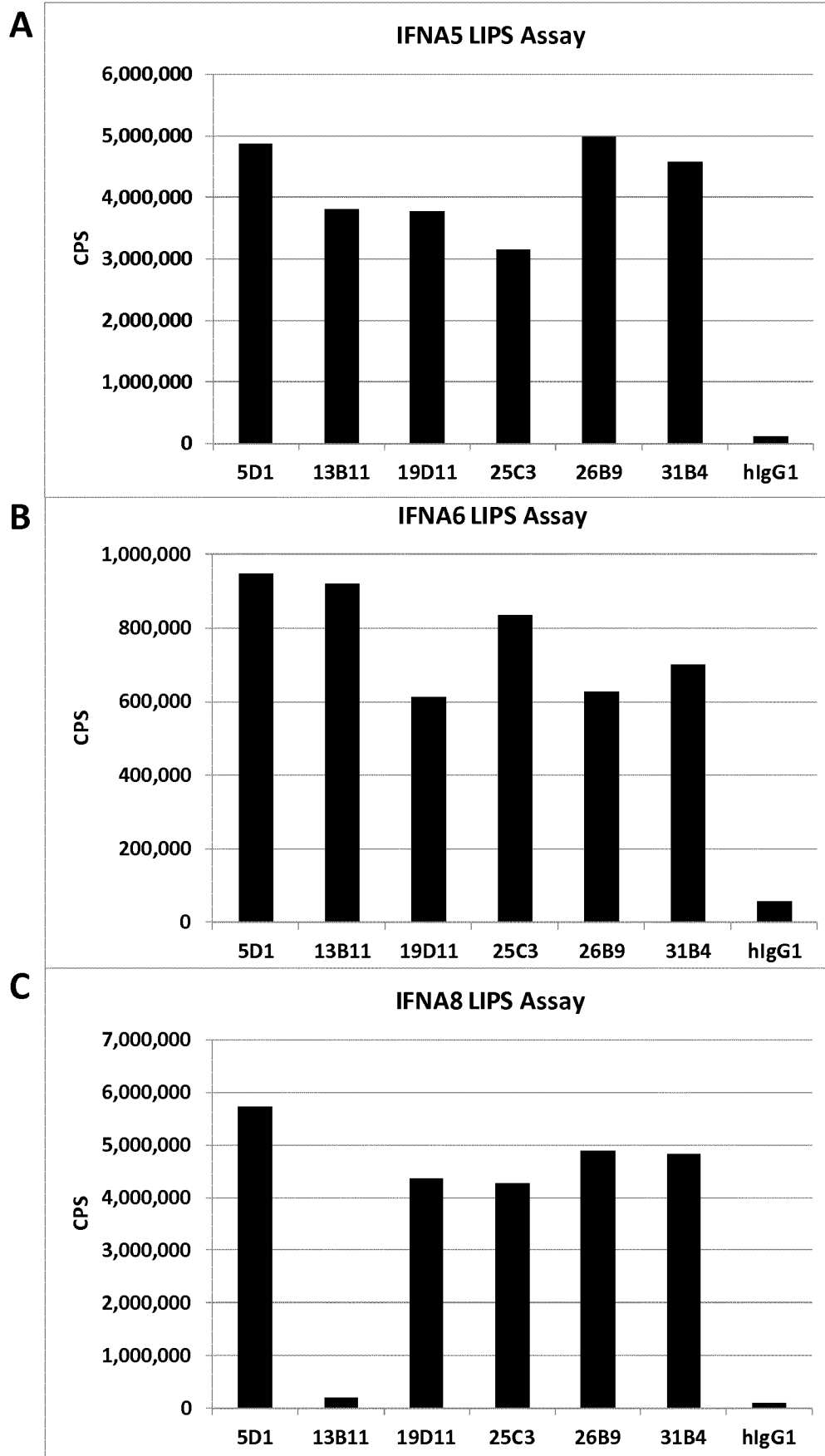


Fig. 12

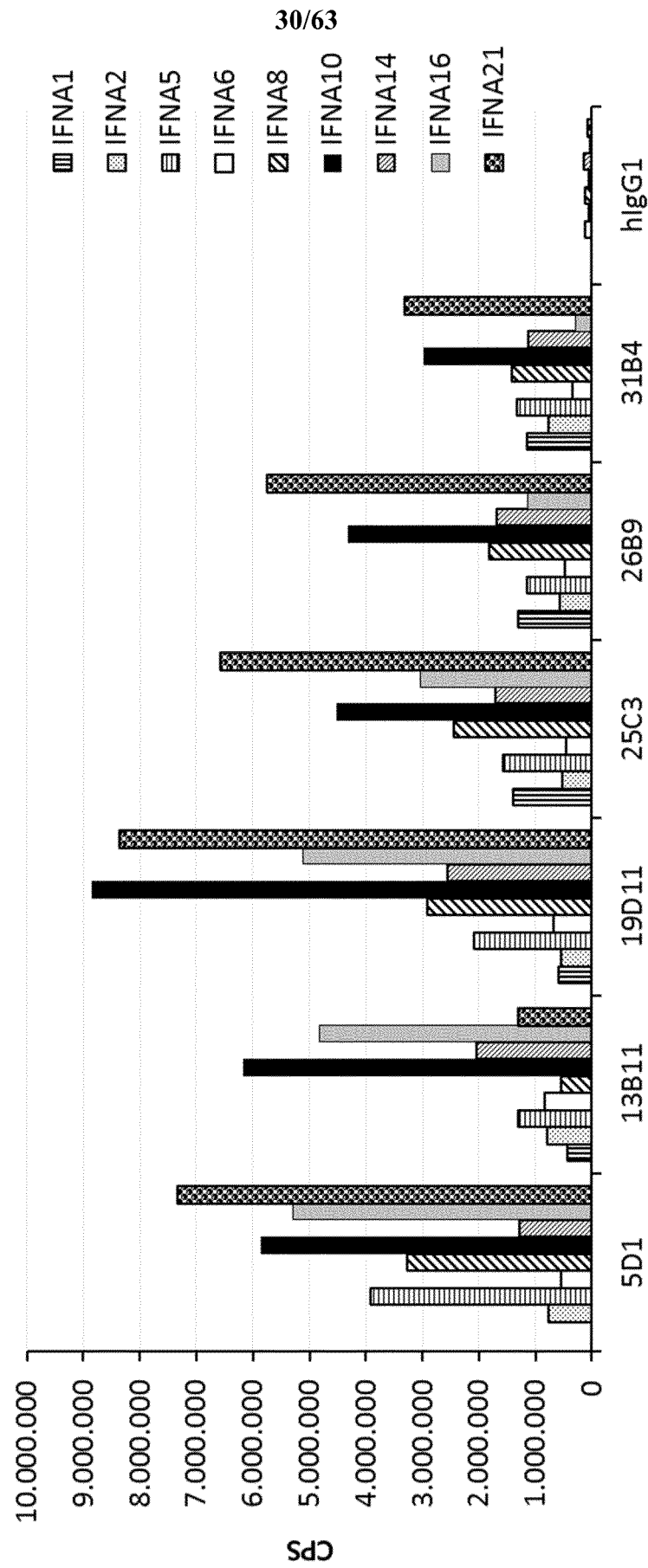


Fig. 13

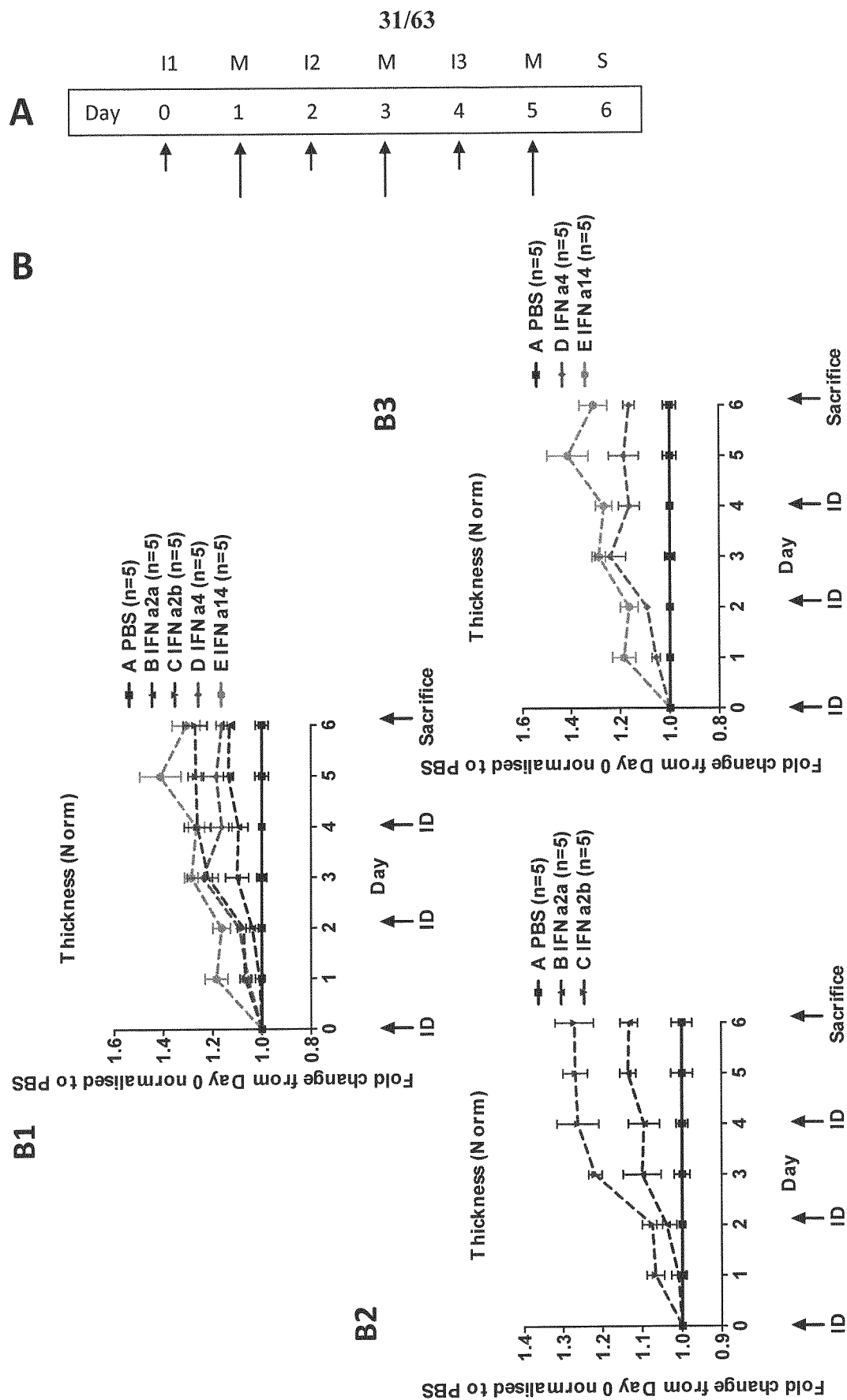


Fig. 14

32/63

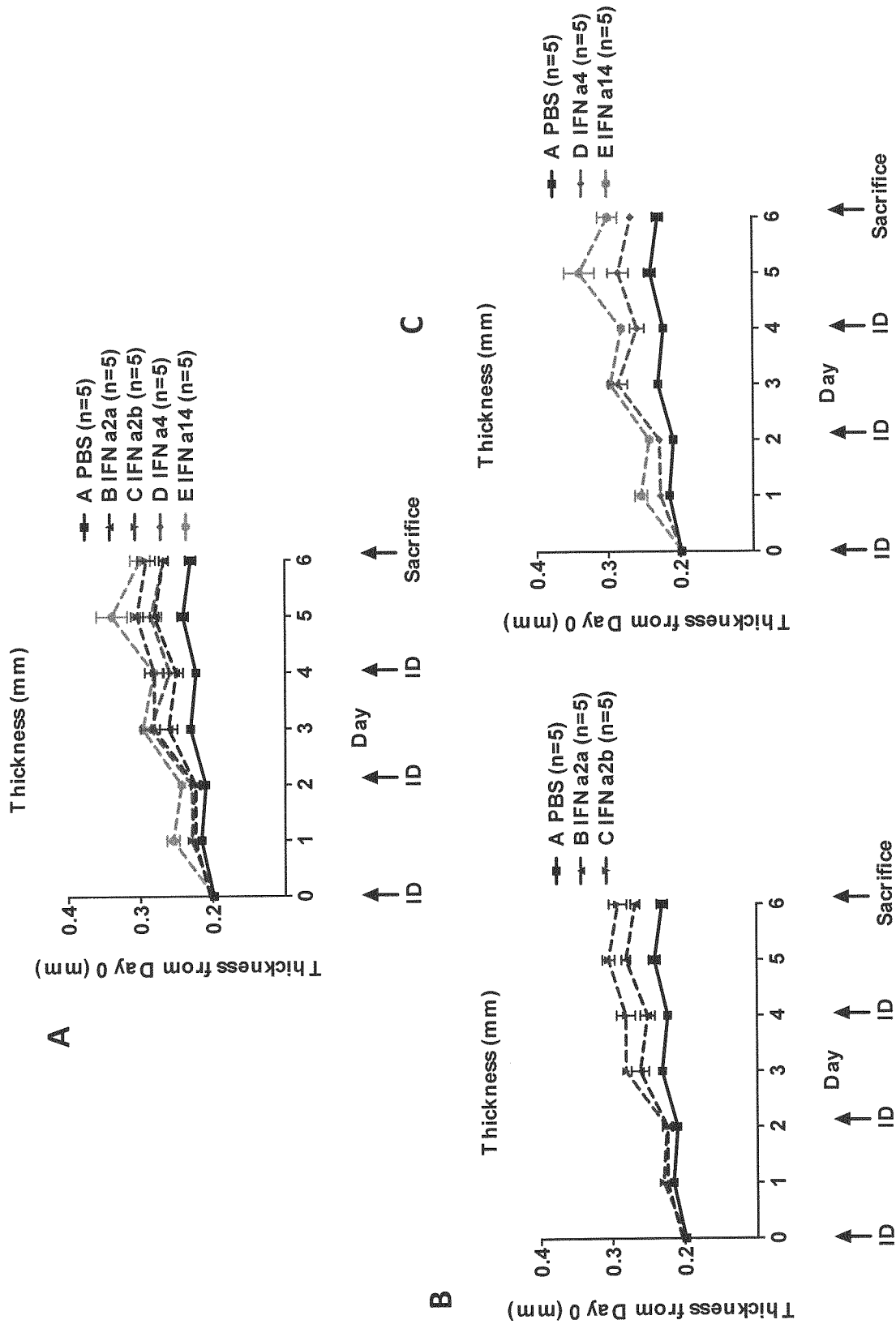


Fig. 15

33/63

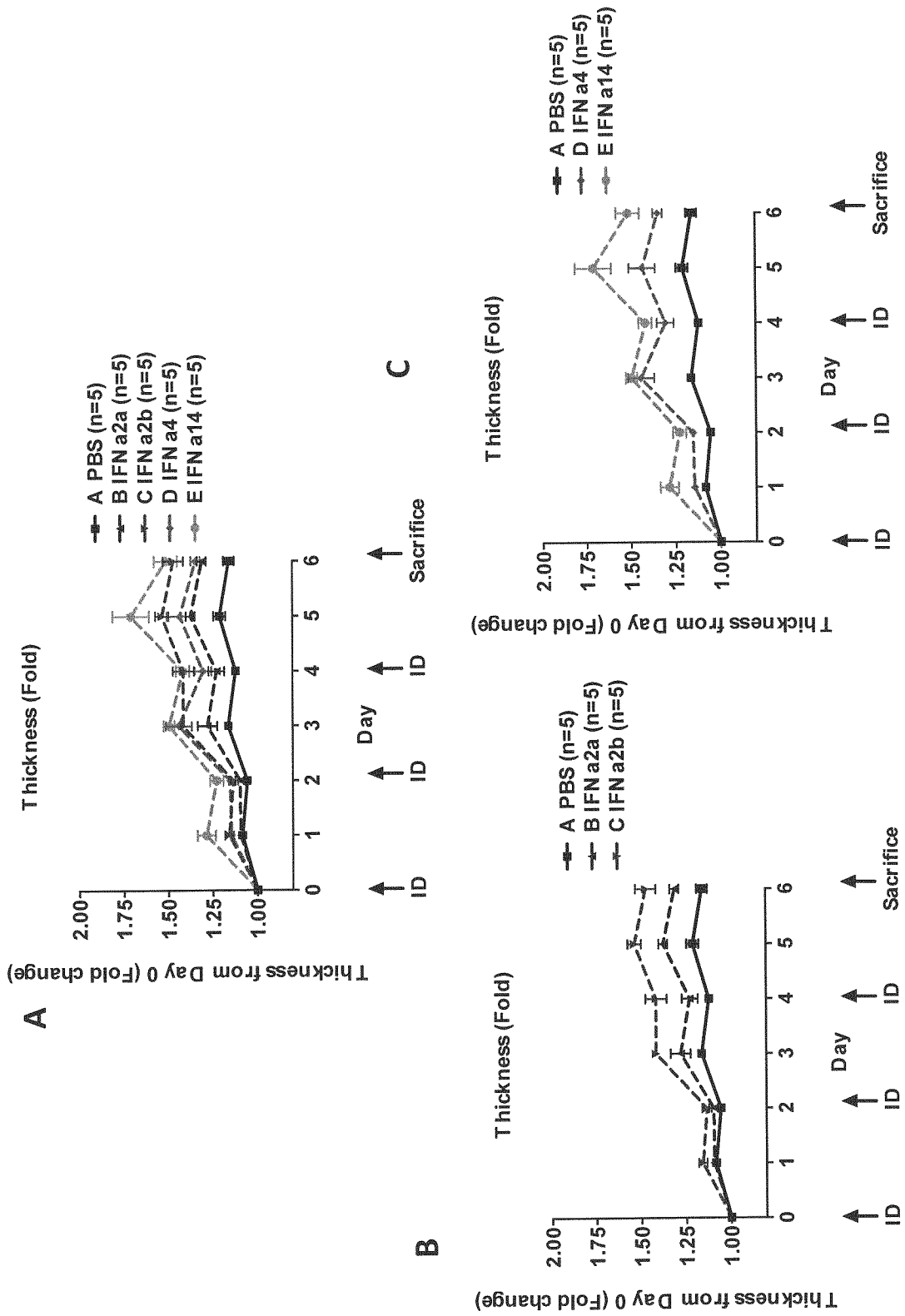


Fig. 16

34/63

Normalisation		0	1	2	3	4	5	6
A PBS	B v a2a	ns	ns	ns	*	*	**	**
A PBS	C v a2b	ns	ns	ns	****	****	****	****
A PBS	D v a4	ns	ns	ns	****	**	***	**
A PBS	E v a14	ns	**	*	****	****	****	****
B a2a	C v a2b	ns	ns	ns	ns	**	*	*
B a2a	D v a4	ns	ns	ns	*	ns	ns	ns
B a2a	E v a14	ns	*	ns	**	*	****	*
C a2b	D v a4	ns	ns	ns	ns	ns	ns	ns
C a2b	E v a14	ns	ns	ns	ns	ns	ns	ns
D a4	E v a14	ns	ns	ns	ns	ns	**	ns

Fig. 17

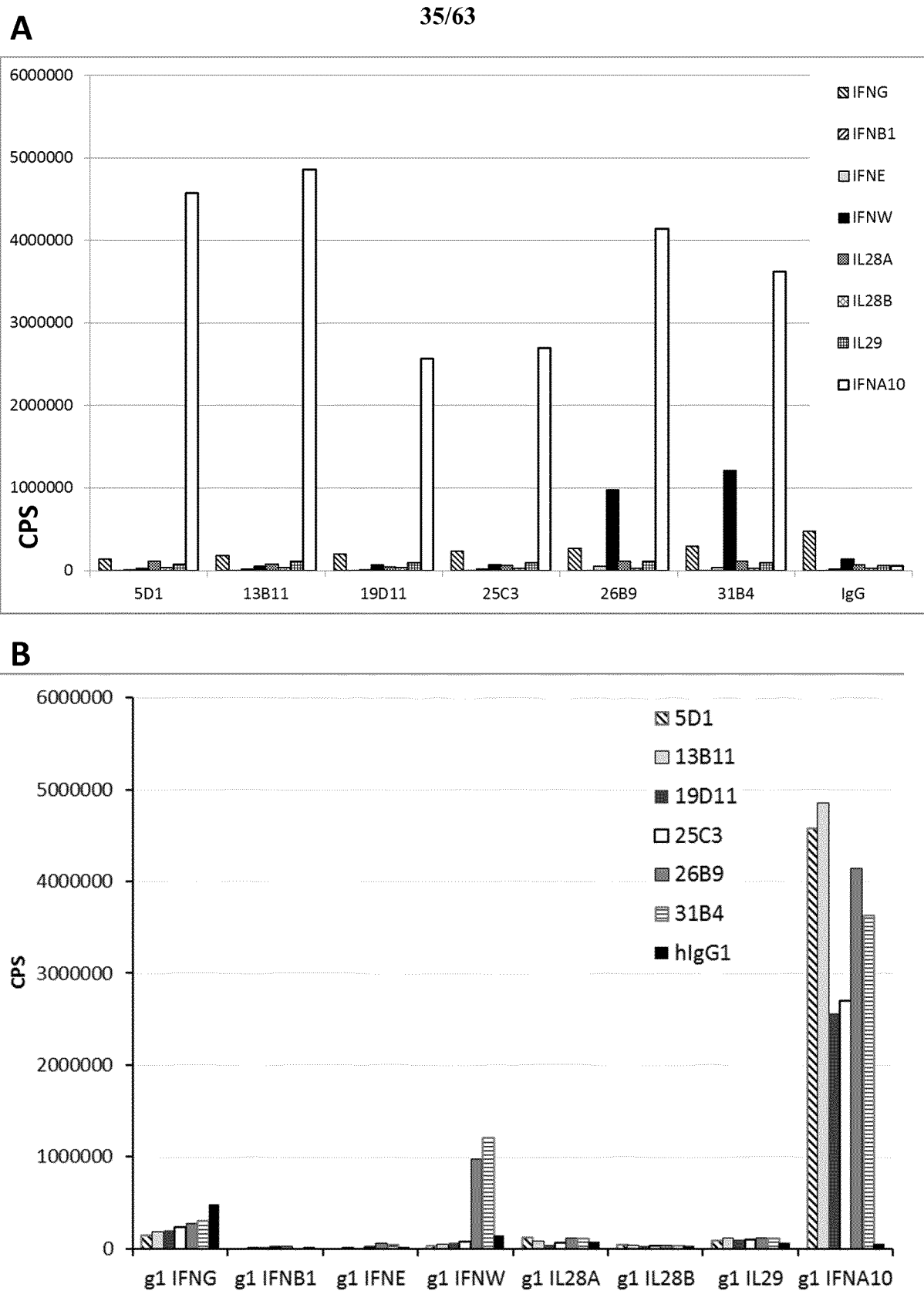


Fig. 18

36/63

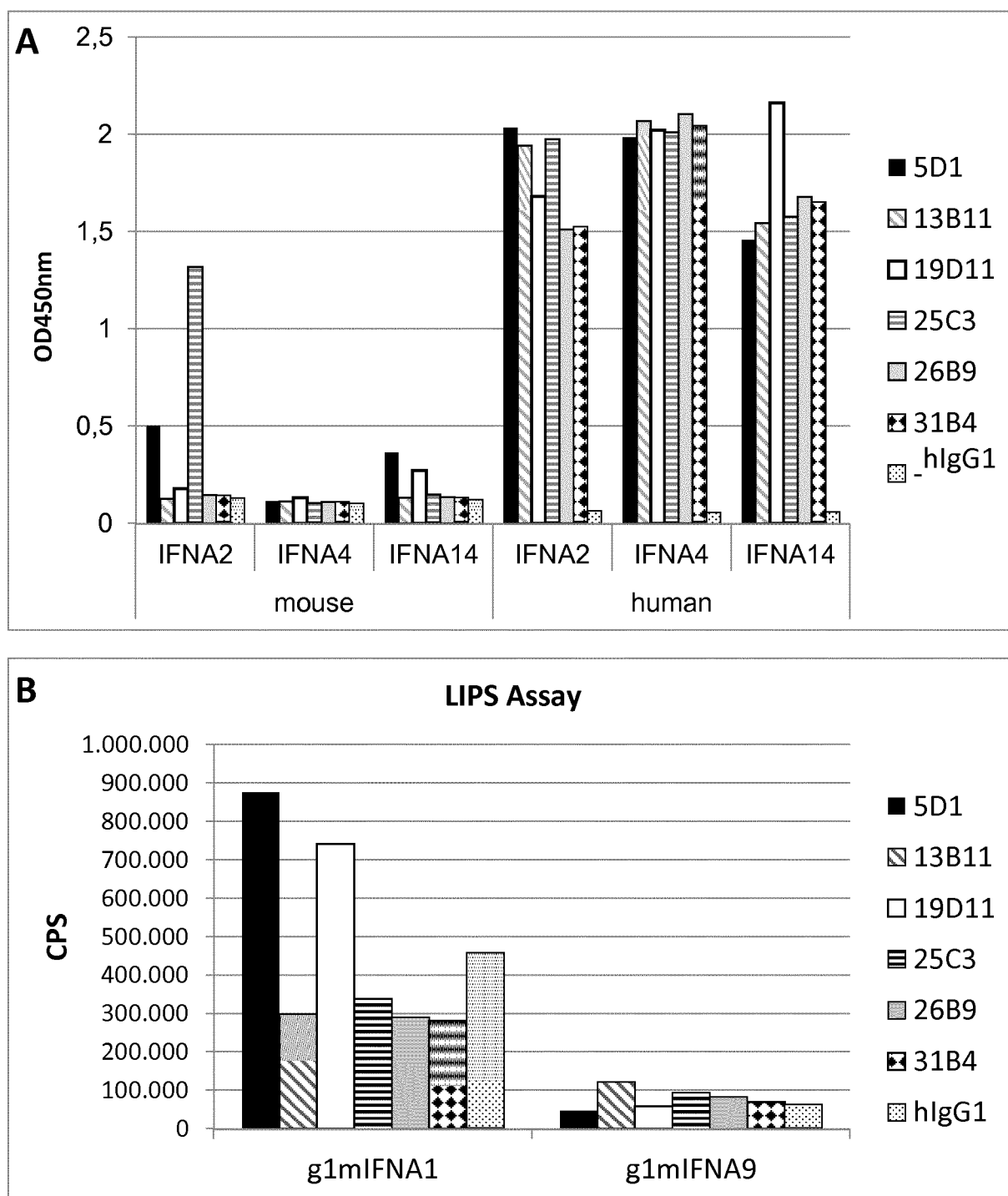


Fig. 19

37/63

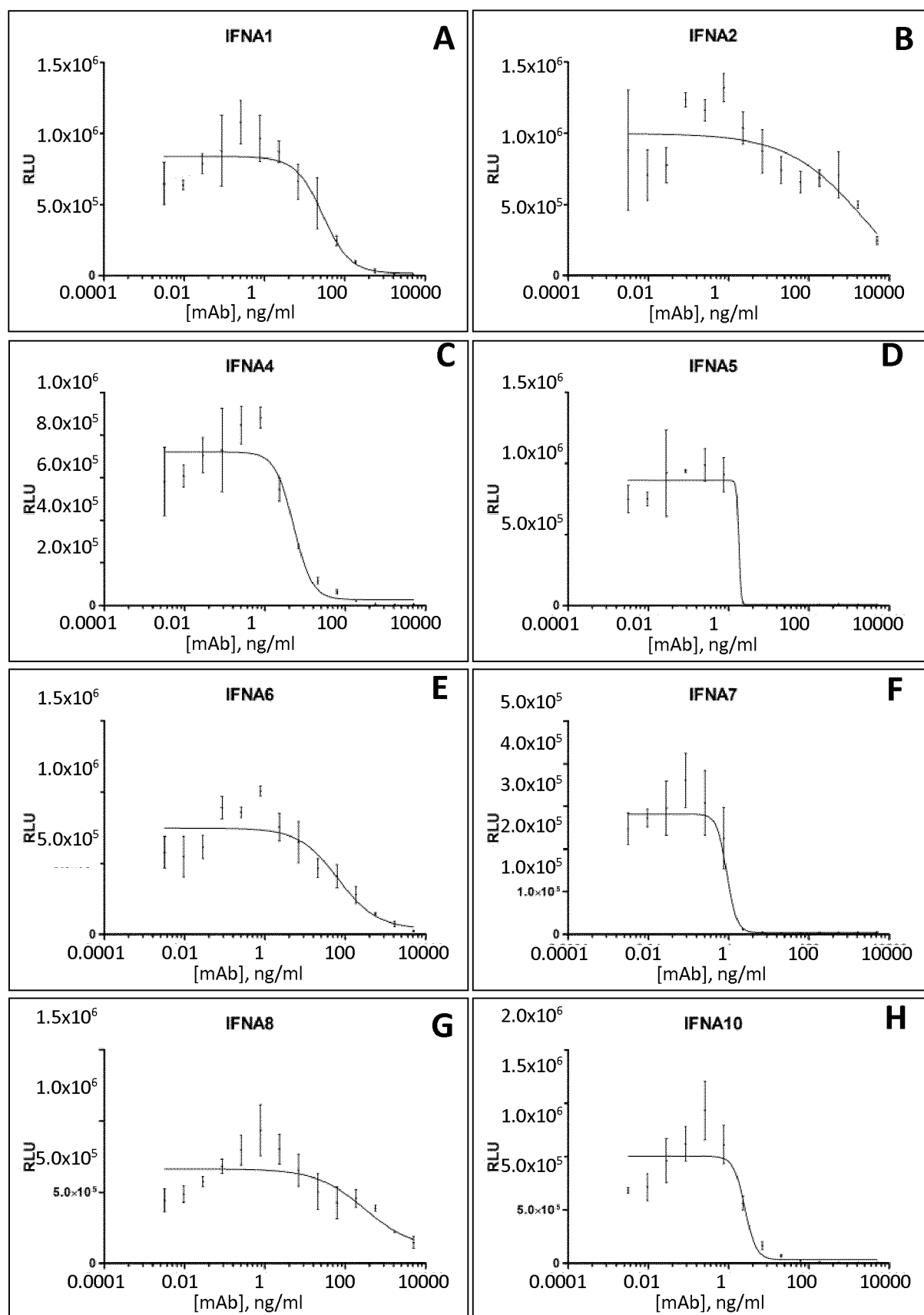


Fig. 20

38/63

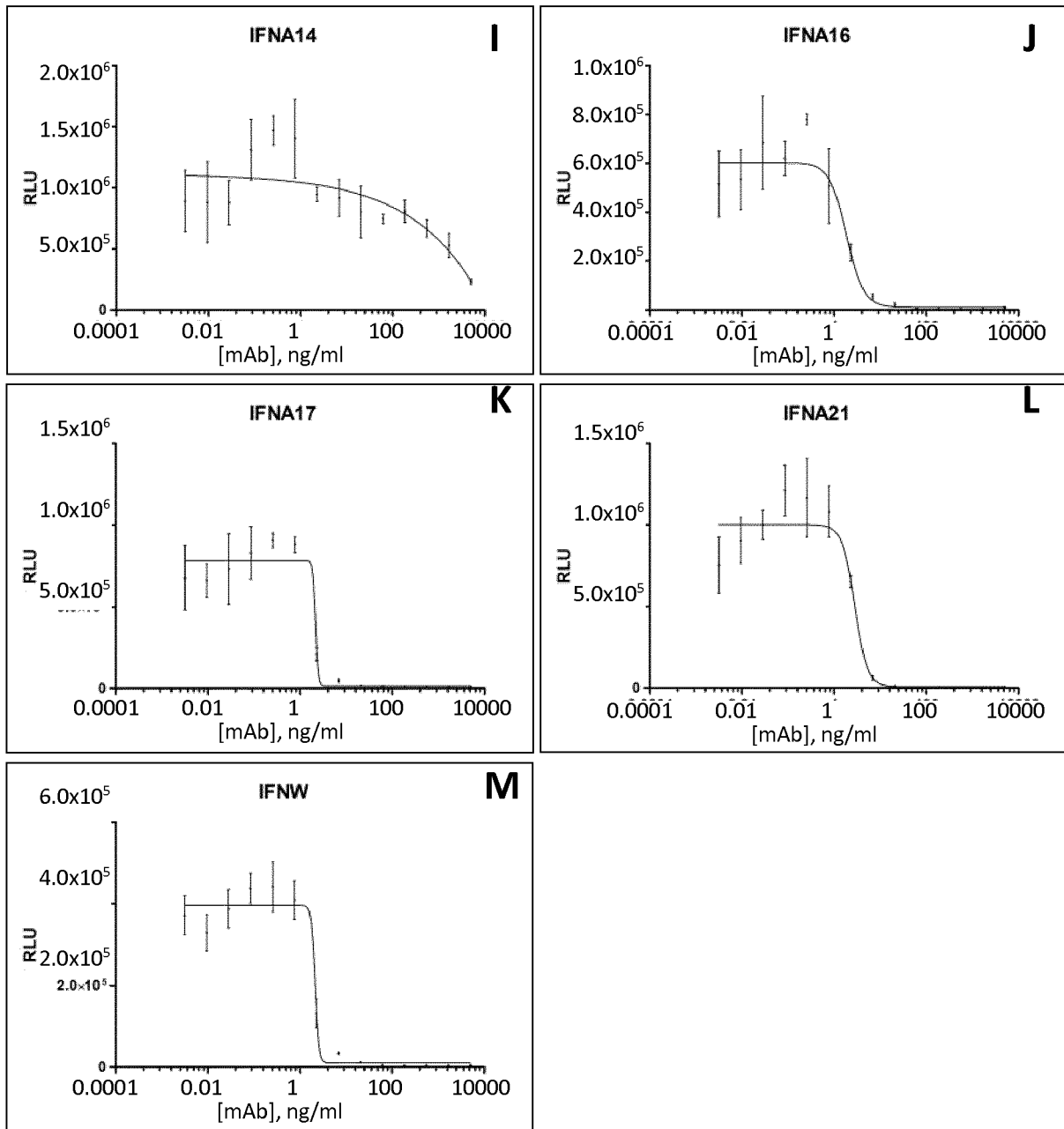


Fig. 20 (continued)

39/63

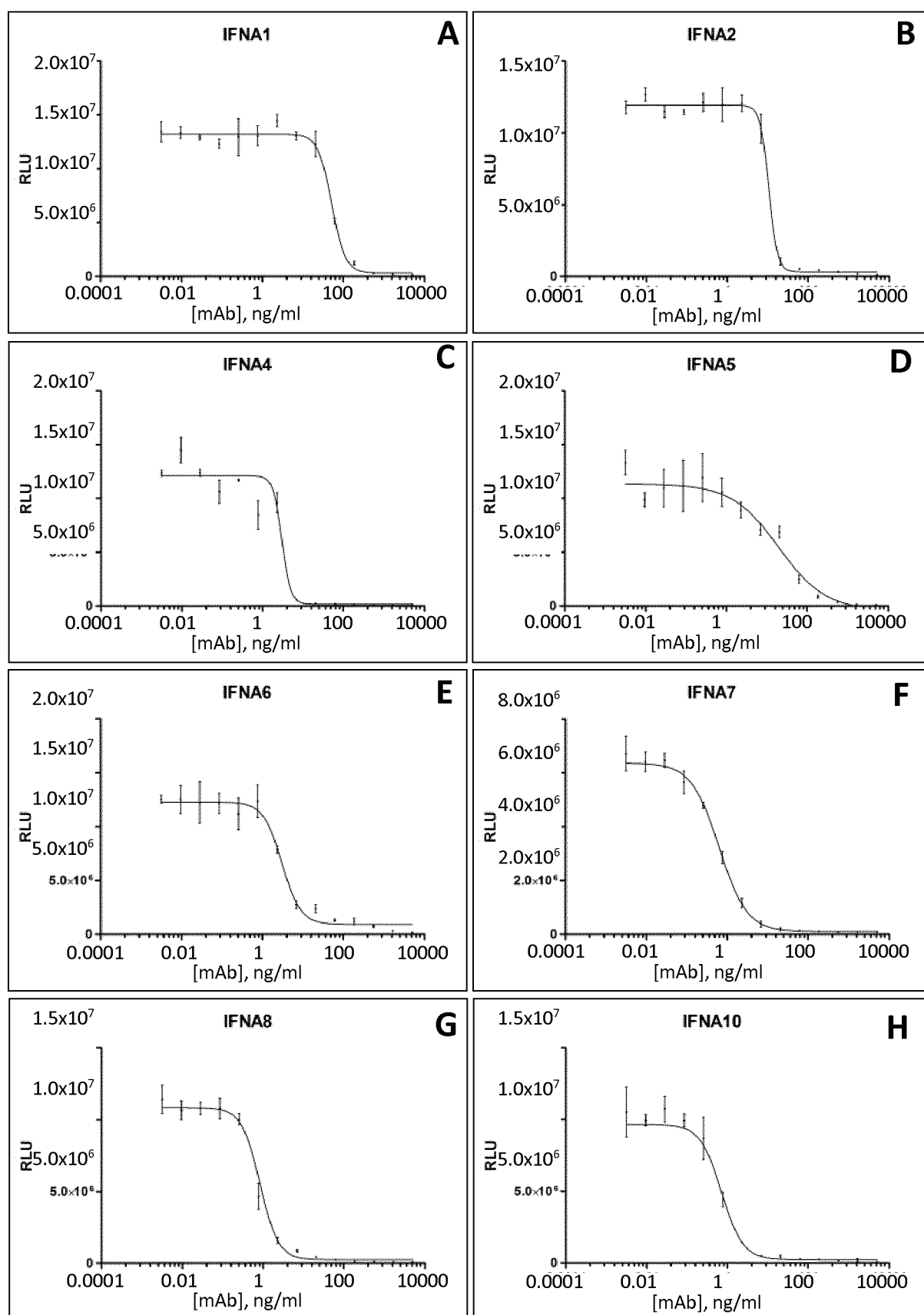


Fig. 21

40/63

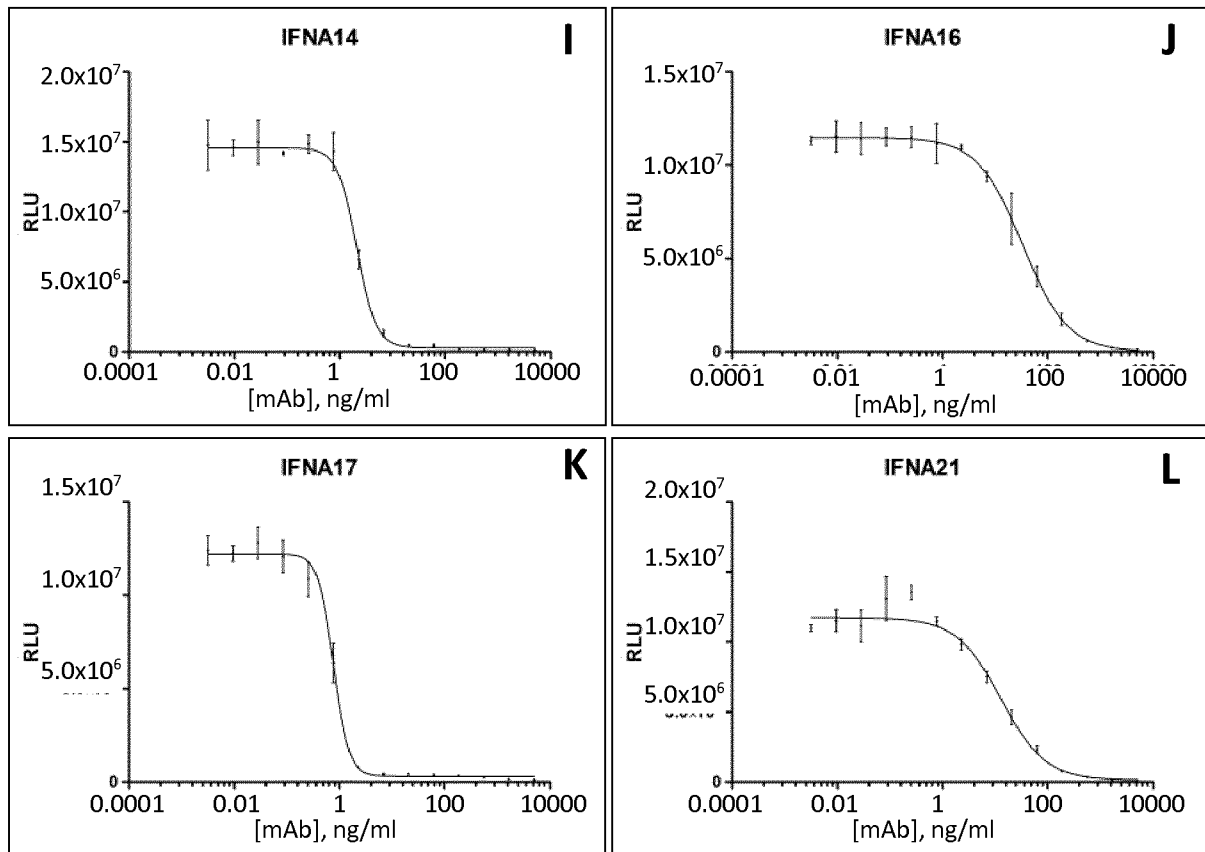


Fig. 21 (continued)

41/63

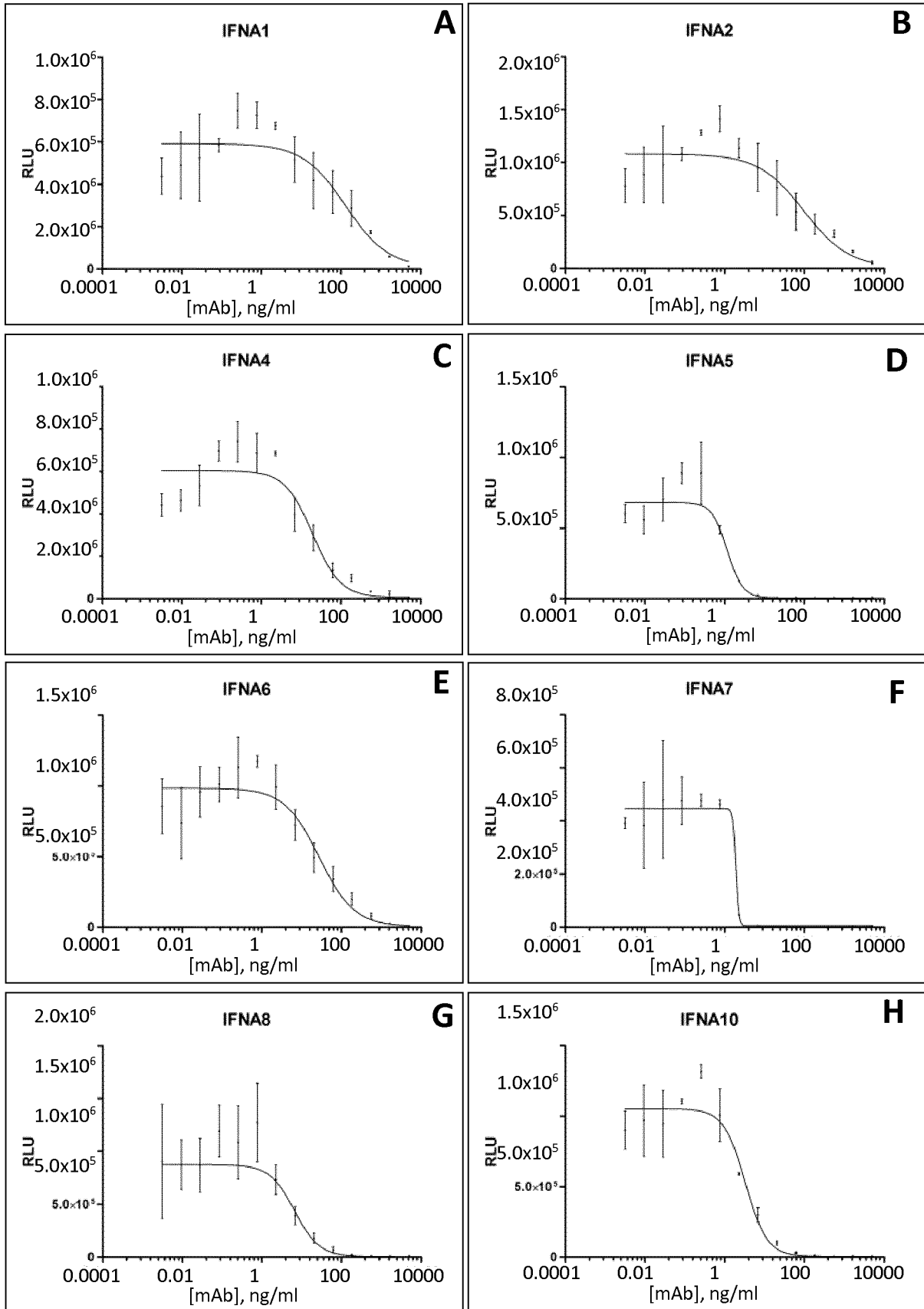


Fig. 22

42/63

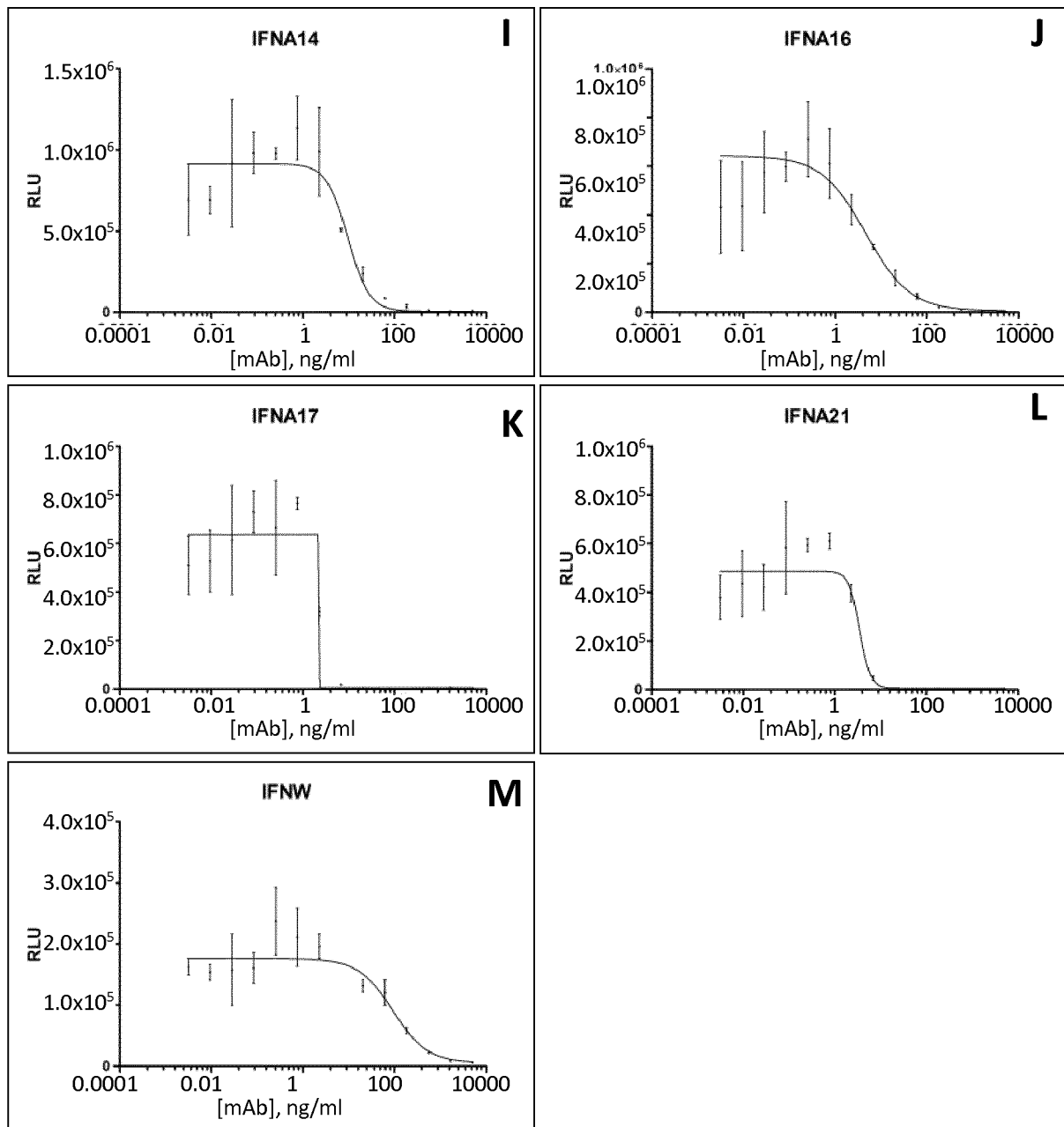


Fig. 22 (continued)

43/63

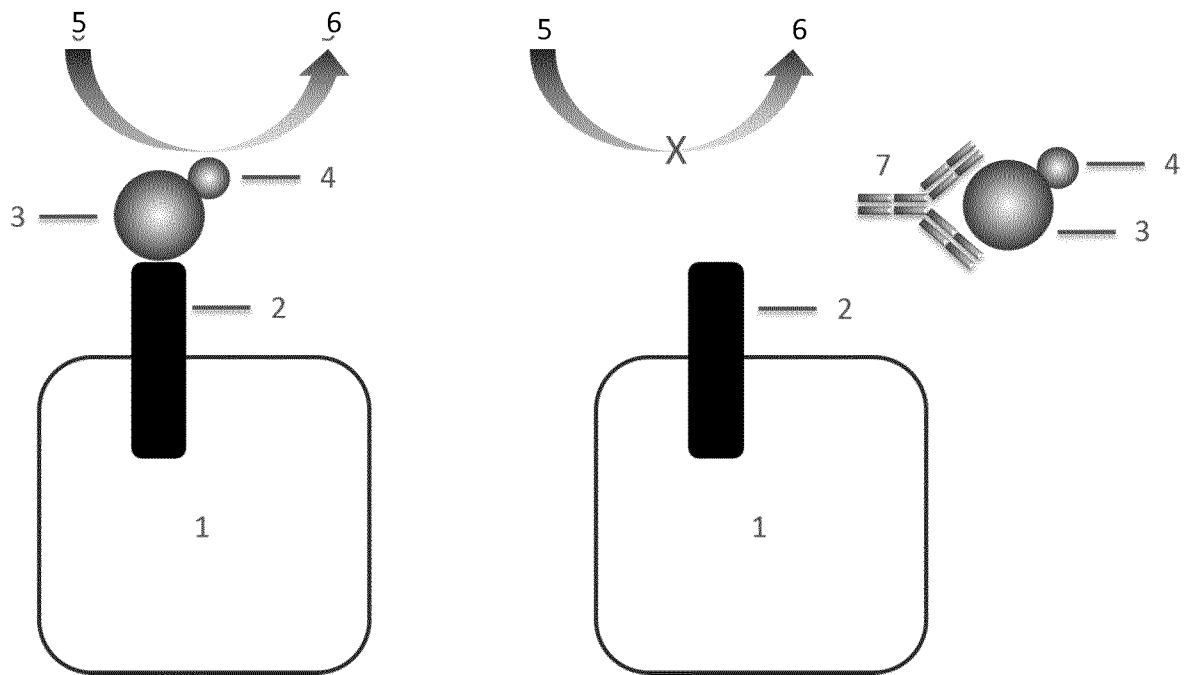
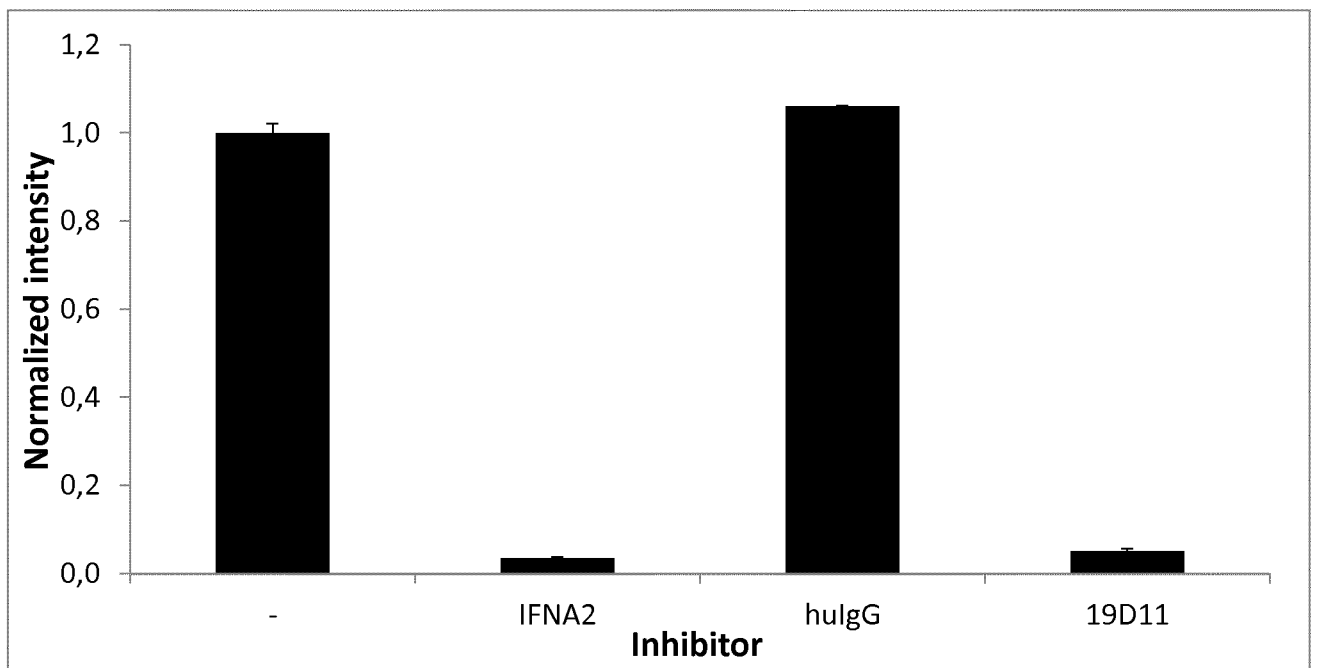
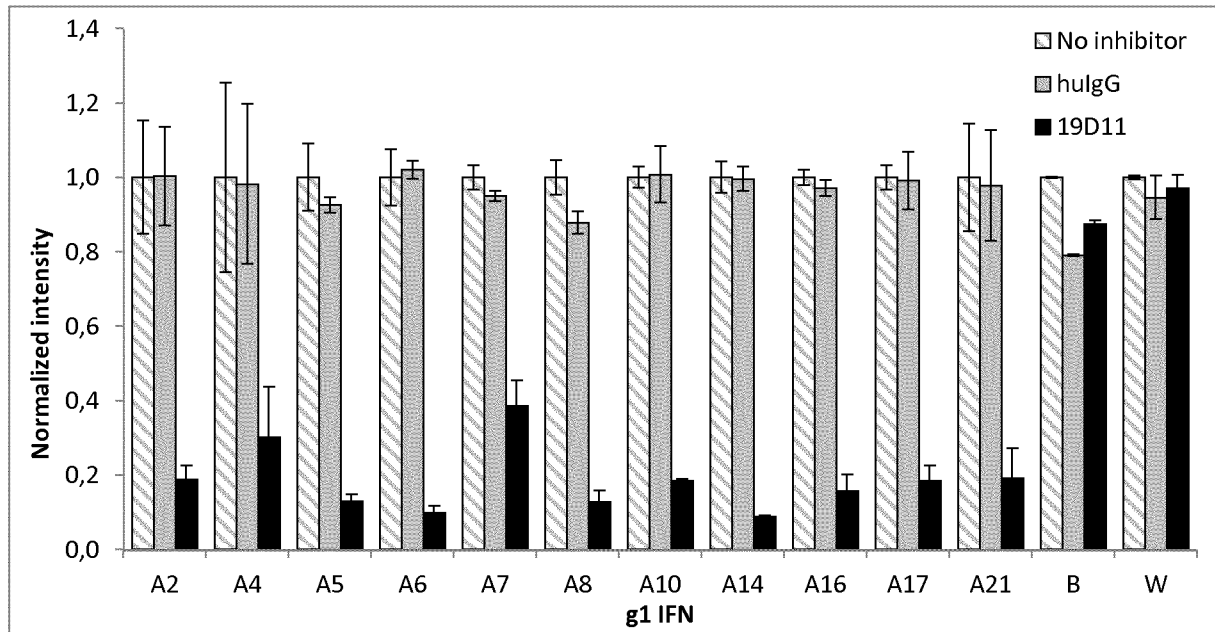
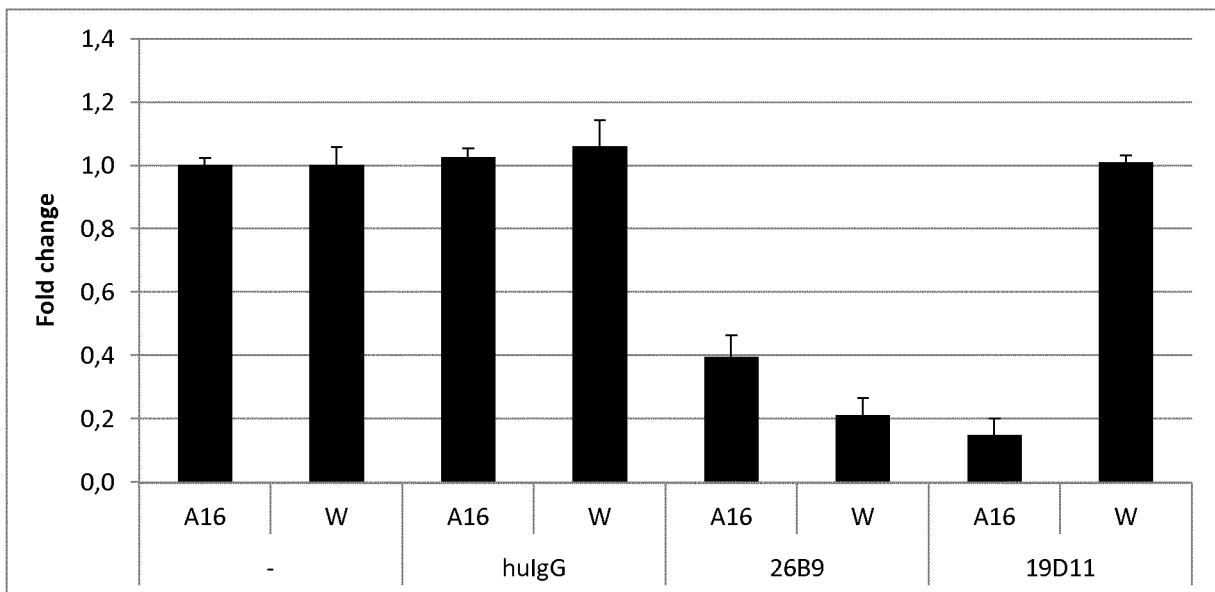
A**B**

Fig. 23

44/63

C**D****Fig. 23 (continued)**

45/63

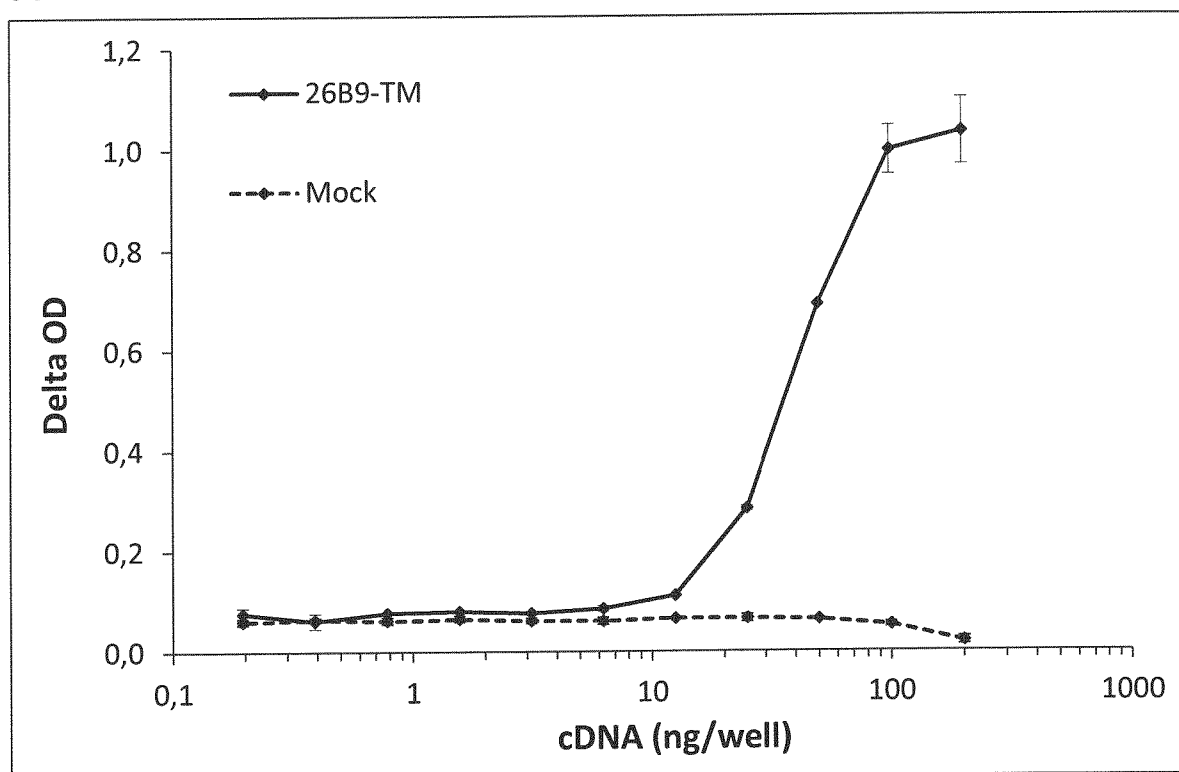
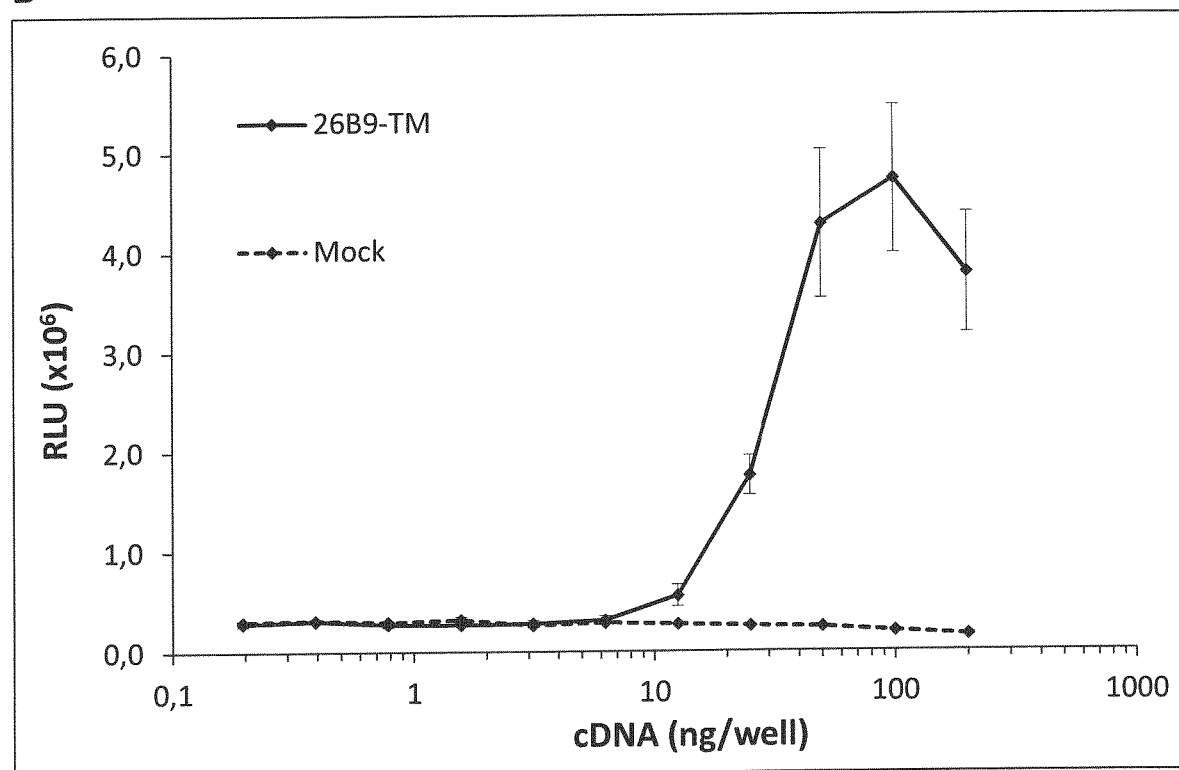
A**B**

Fig. 24

46/63

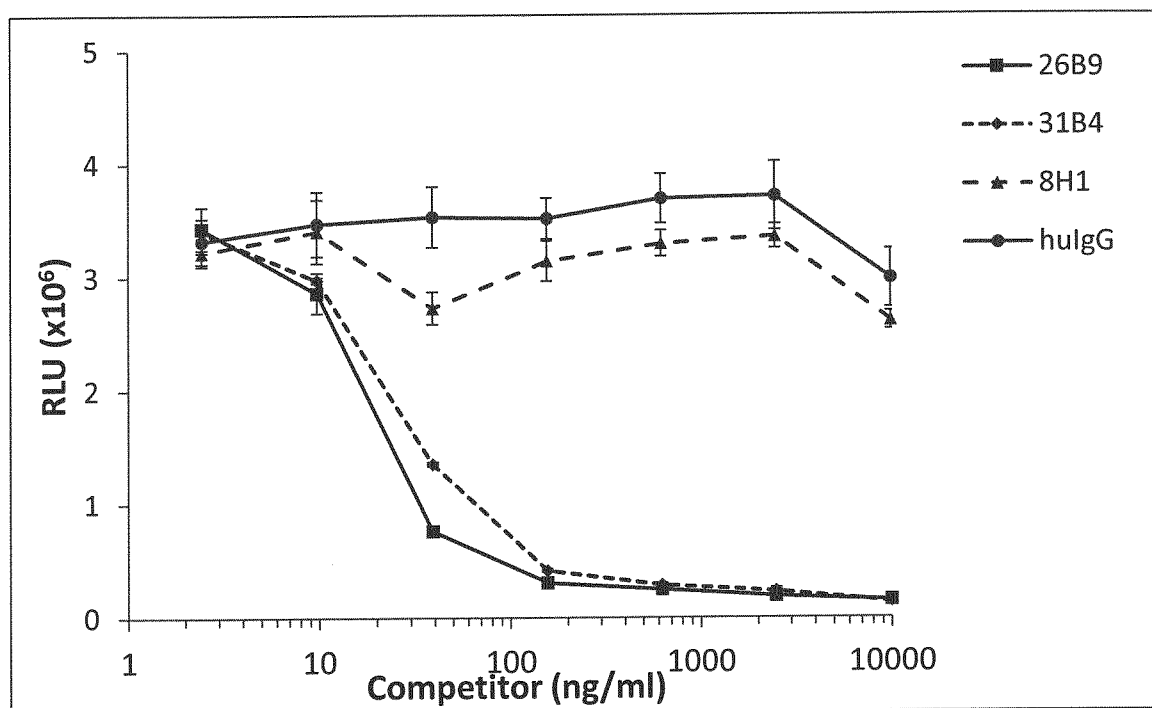
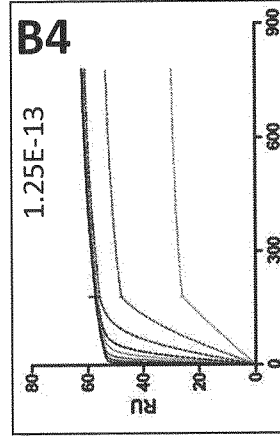
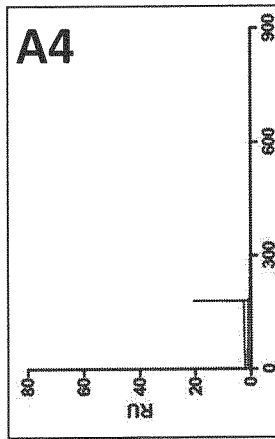


Fig. 25

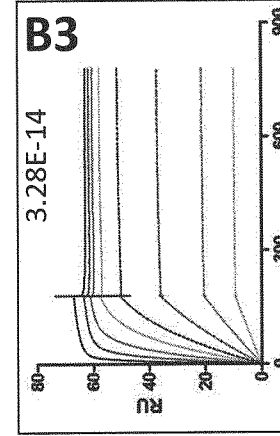
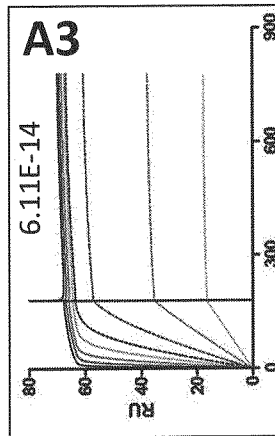
47/63

19D11

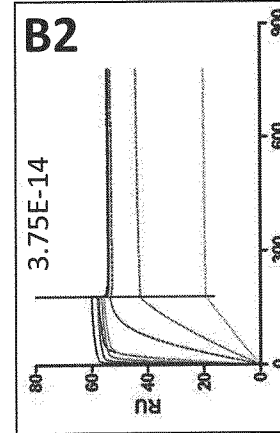
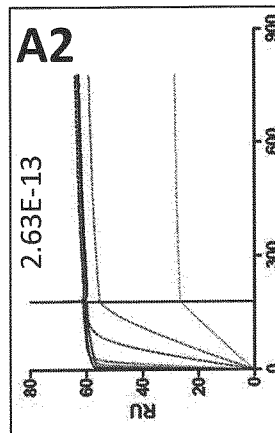
IFN ω 1

26B9

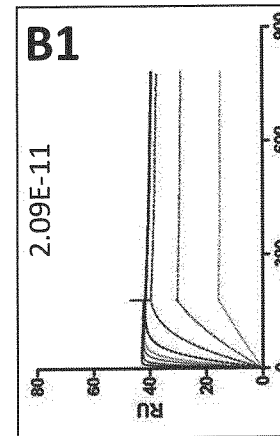
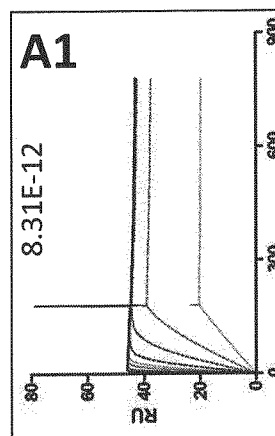
IFNA14



IFNA4



IFNA2b



antigen concentrations

— 100 nM 10 nM

— 50 nM 5 nM

— 25 nM 2.5 nM

— 15 nM 1 nM

Fig. 26

48/63

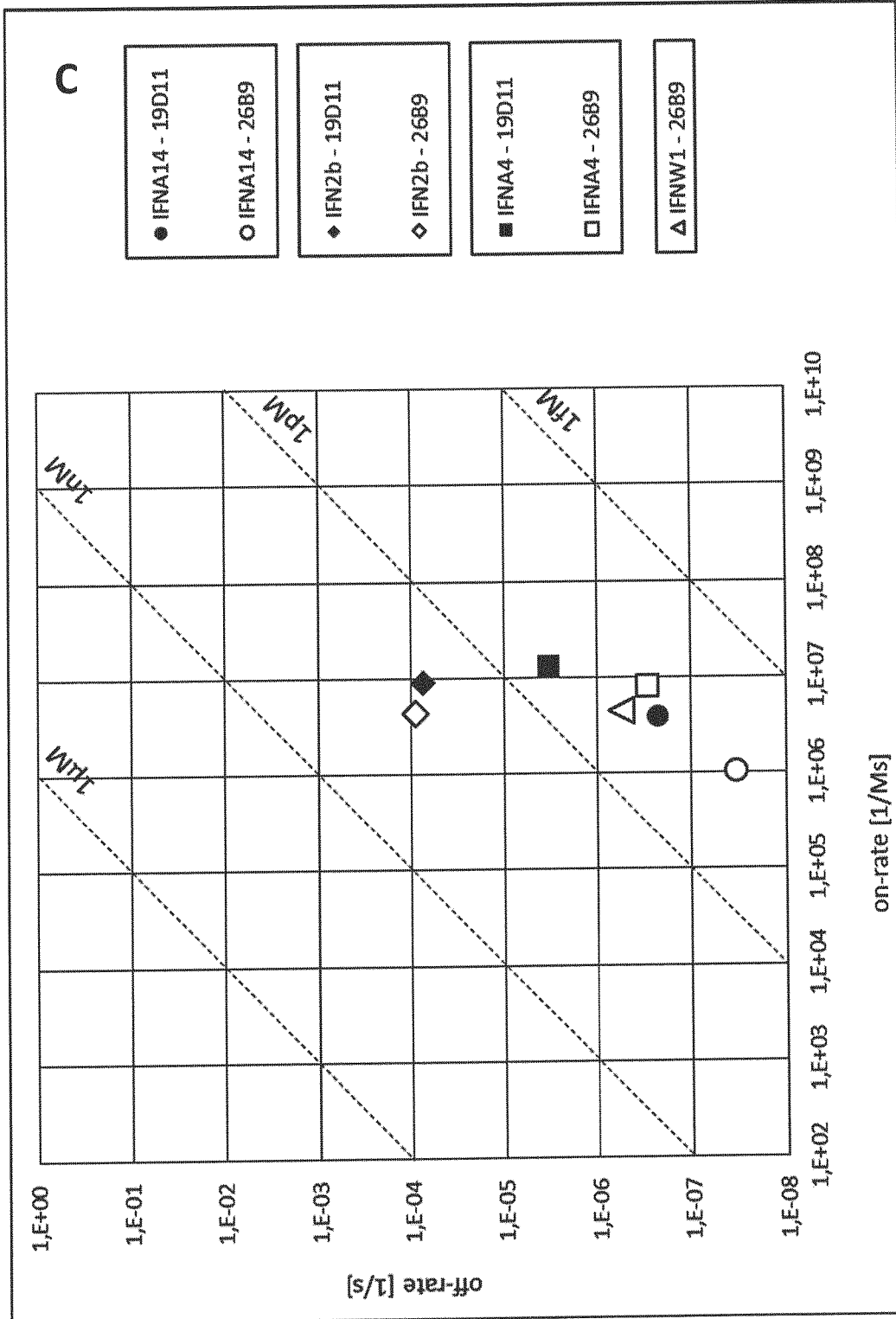


Fig. 26 (continued)

49/63

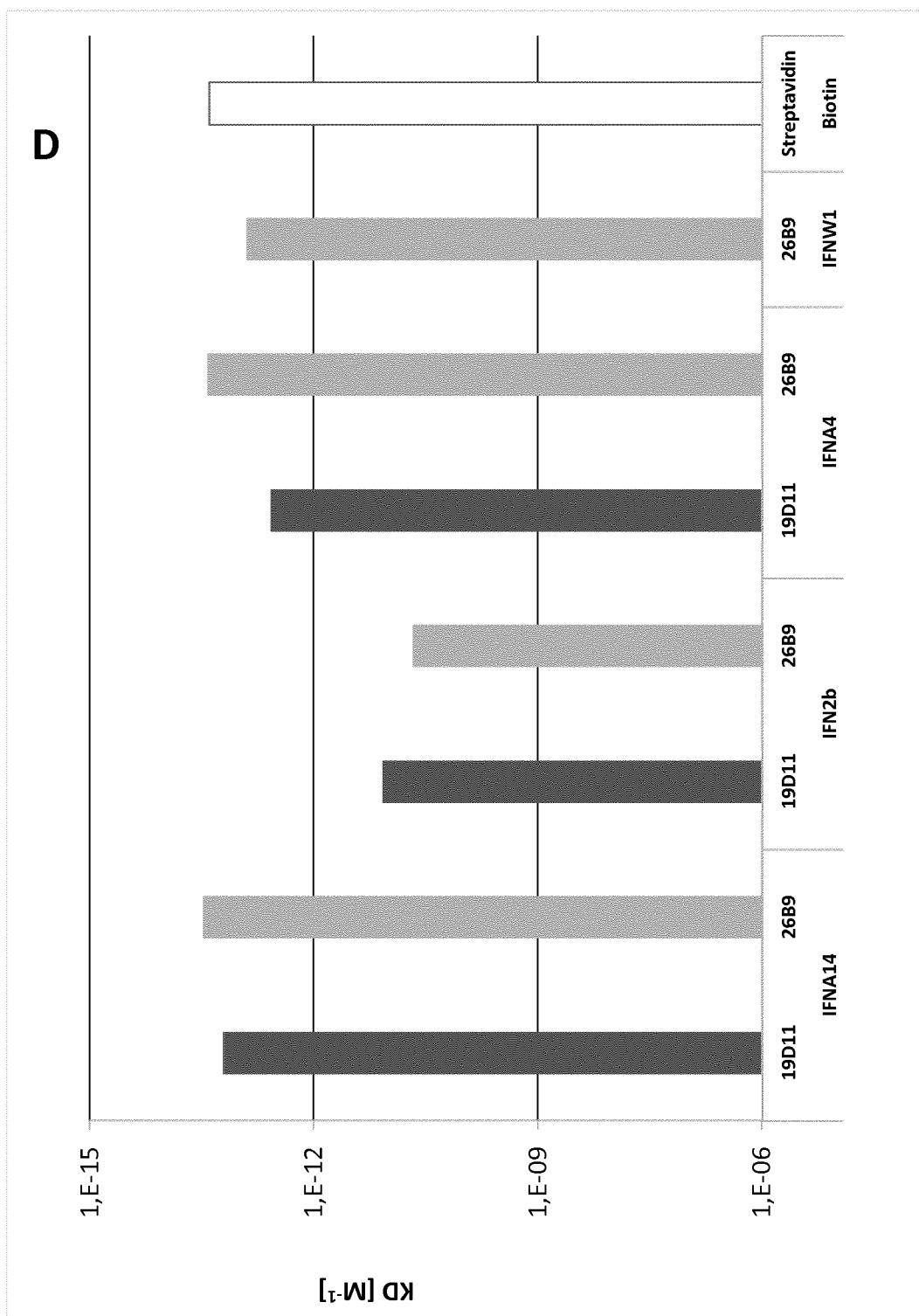
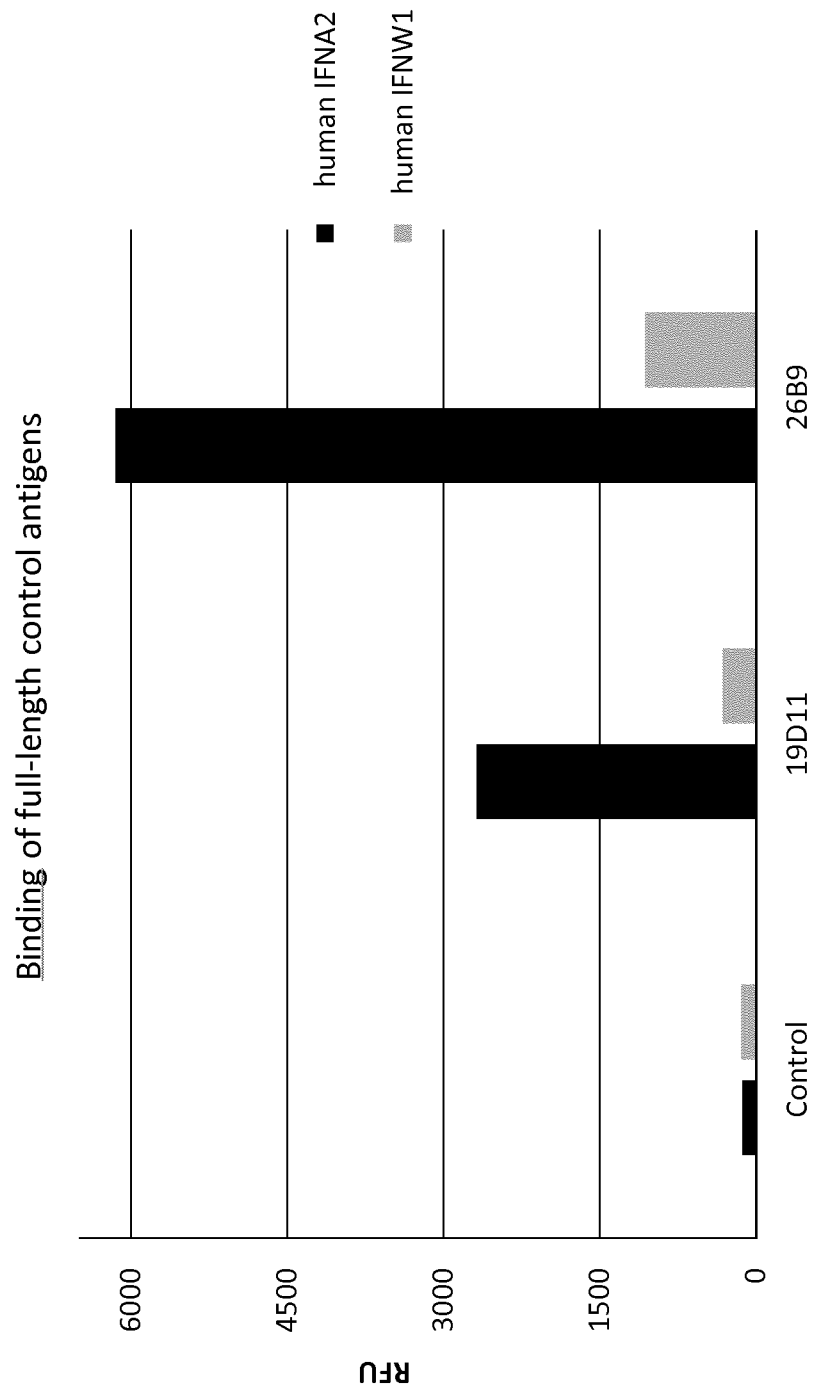


Fig. 26 (continued)

50/63

A**Fig. 27**

B 51/63

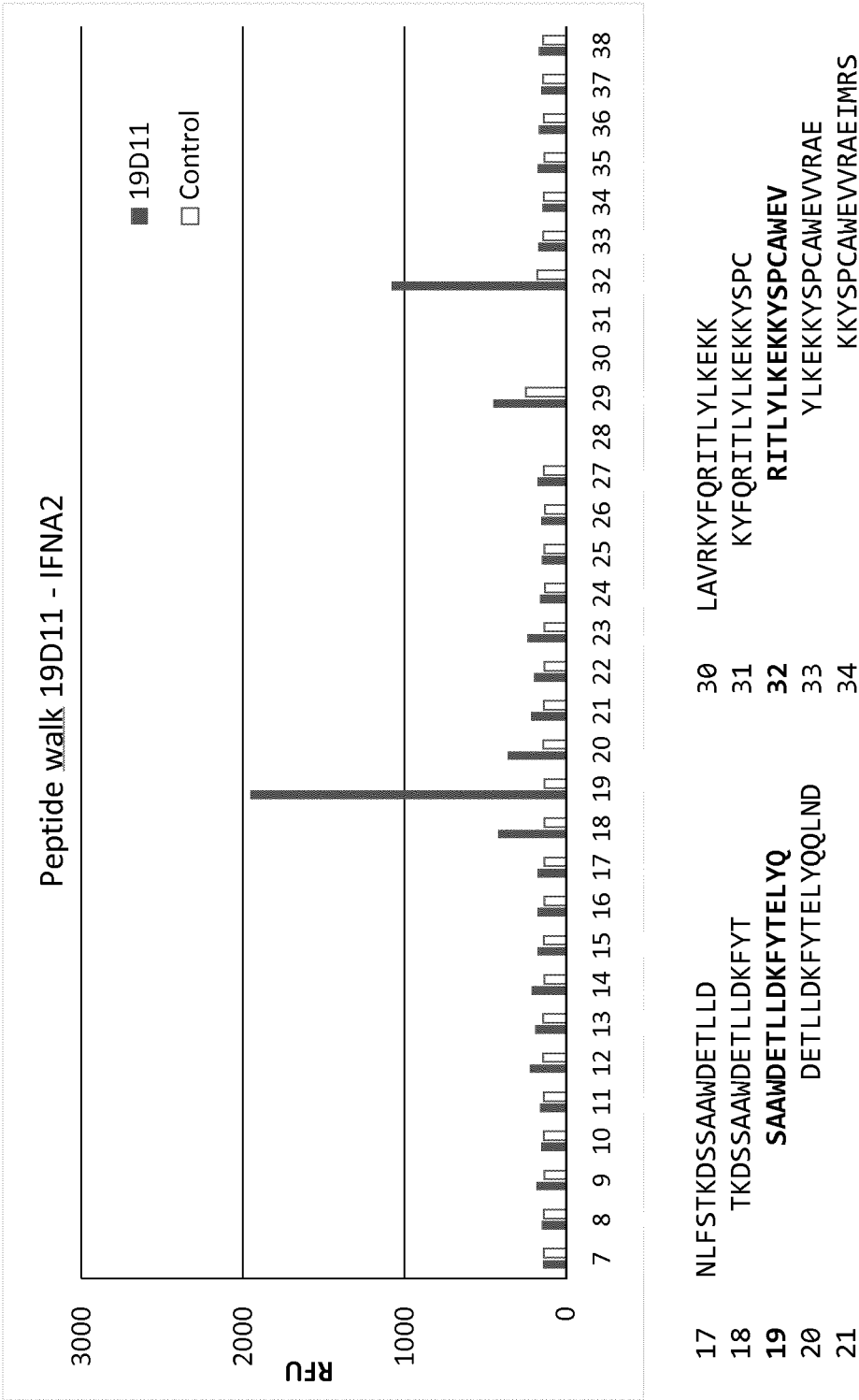
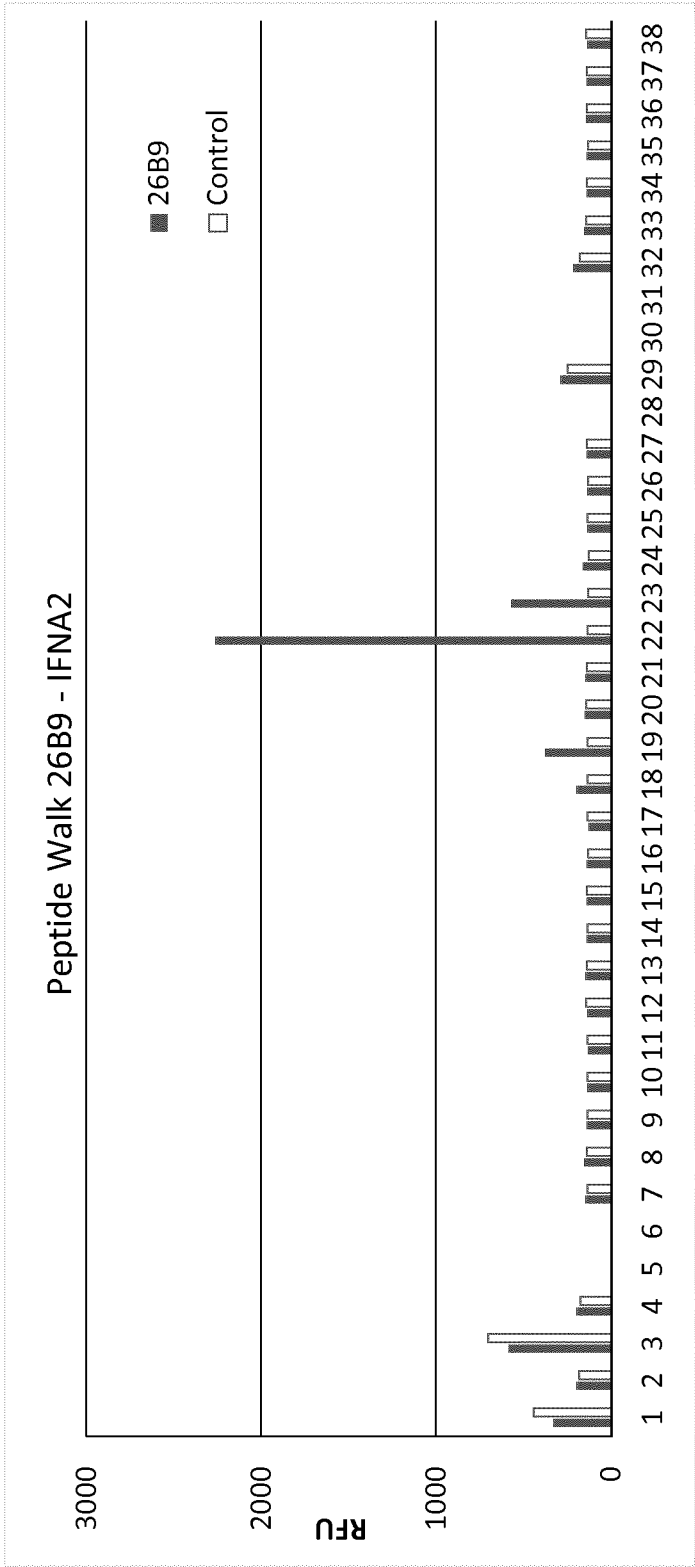


Fig. 27 (continued)

C



20 DETLLDKFYTELYQQQLND
21 LDKFYTELYQQQLNDLEAC
22 YTELYQQQLNDLEACVIQGG
23 YQQQLNDLEACVIQGGVGT
24 NDLEACVIQGGVGTETPL

Fig. 27 (continued)

D

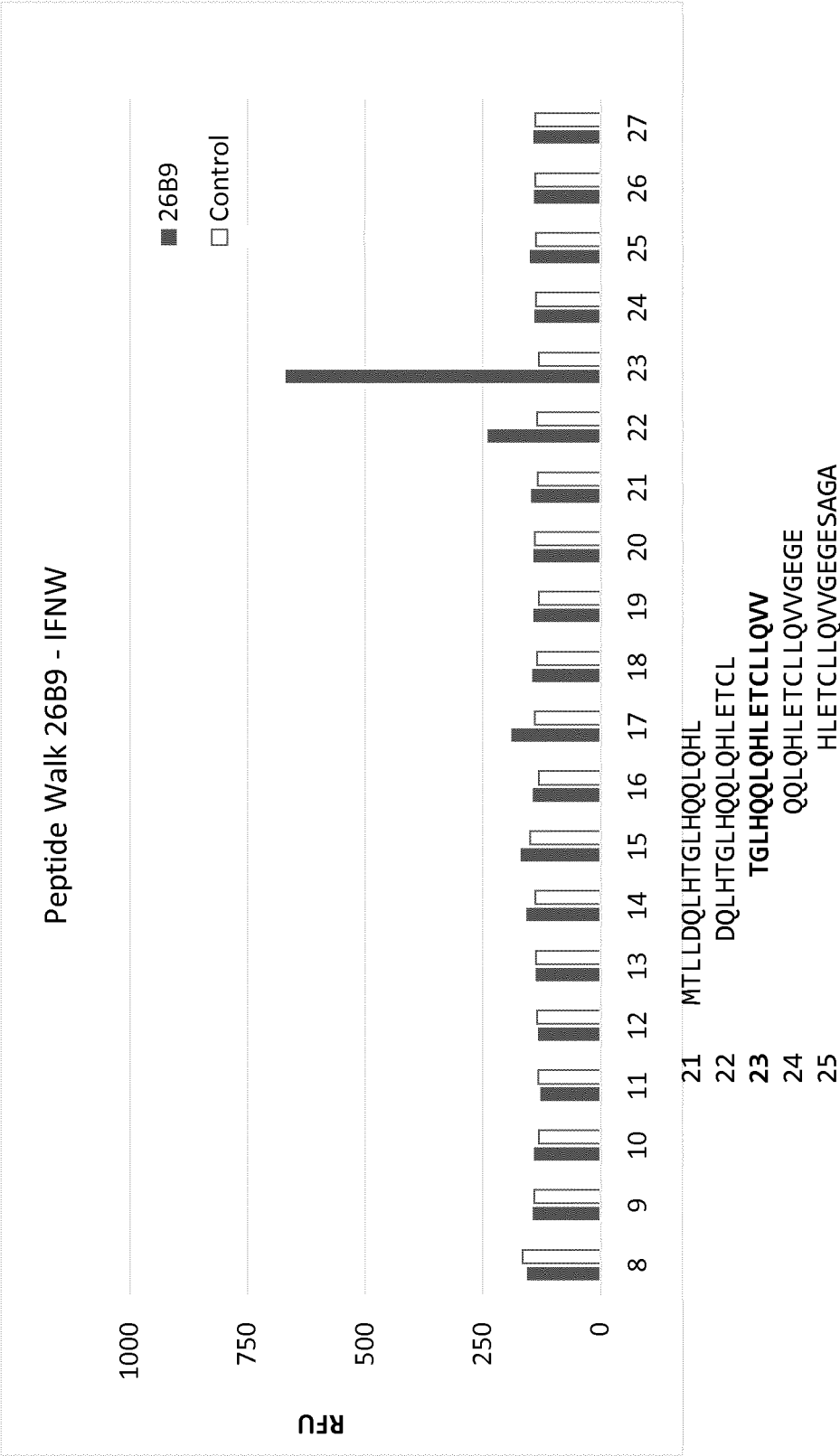


Fig. 27 (continued)

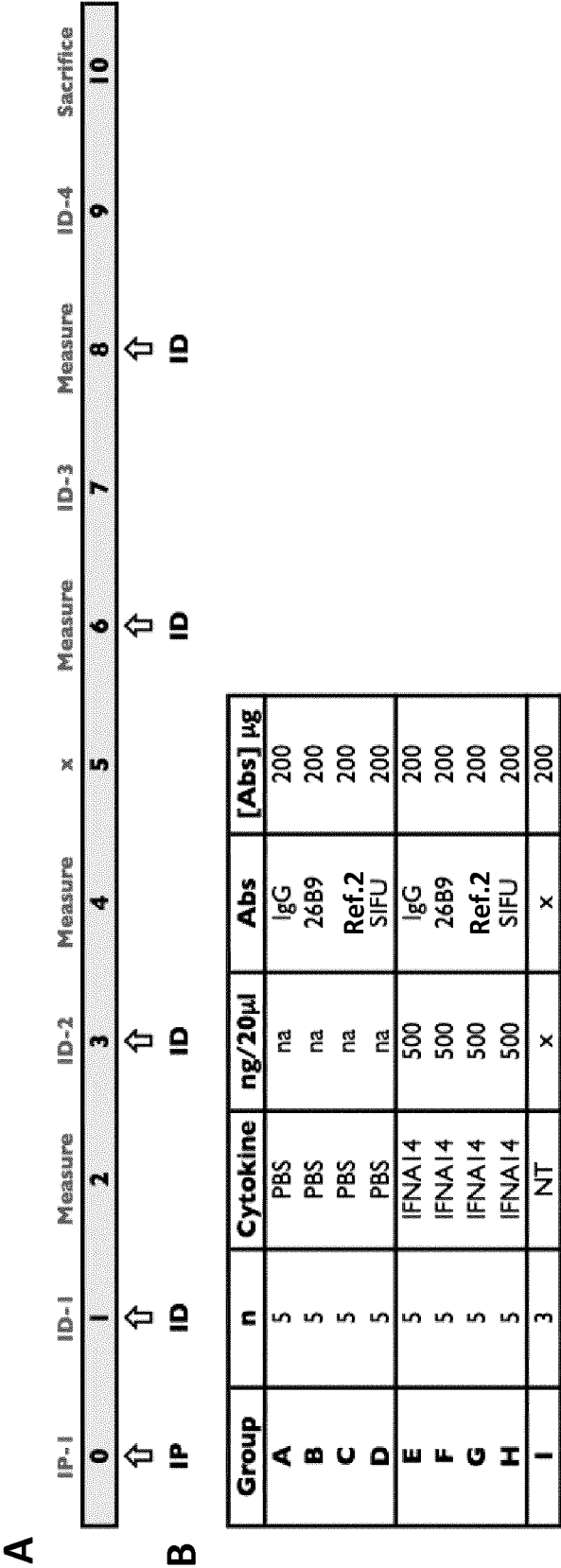


Fig. 28



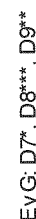
A

IP-1	ID-1	Measure	ID-2	Measure	x	Measure	ID-3	Measure	ID-4	Sacrifice
0	1	2	3	4	5	6	7	8	9	10
↑	↑		↑			↑		↑		
IP	ID		ID			ID		..		

B

Group	n	Cytokine	ng/20µl	Abs	[Abs] µg
A	5	PBS	na	IgG	200
B	5	PBS	na	26B9	200
C	5	PBS	na	Ref.2	200
D	5	PBS	na	SIFU	200
E	5	IFNA5	500	IgG	200
F	5	IFNA5	500	26B9	200
G	5	IFNA5	500	Ref.2	200
H	5	IFNA5	500	SIFU	200
I	5	NT	x	x	200

Fig. 29



SUBSTITUTE SHEET (RULE 26)

A

IP-1	ID-1	Measure	ID-2	Measure	x	Measure	ID-3	Measure	ID-4	Sacrifice
0	1	2	3	4	5	6	7	8	9	10
↑	↑		↑			↑		↑		
IP	ID		ID			ID		ID		

B

Group	n	Cytokine	ng/20μl	Abs	[Abs] μg
A	5	PBS	na	IgG	200
B	5	PBS	na	26B9	200
C	5	PBS	na	Ref.1	200
D	5	PBS	na	Ref.2	200
E	5	IFNw	125	IgG	200
F	5	IFNw	125	26B9	200
G	5	IFNw	125	Ref.1	200
H	5	IFNw	125	Ref.2	200
I	5	NT	x	x	x

Fig. 30

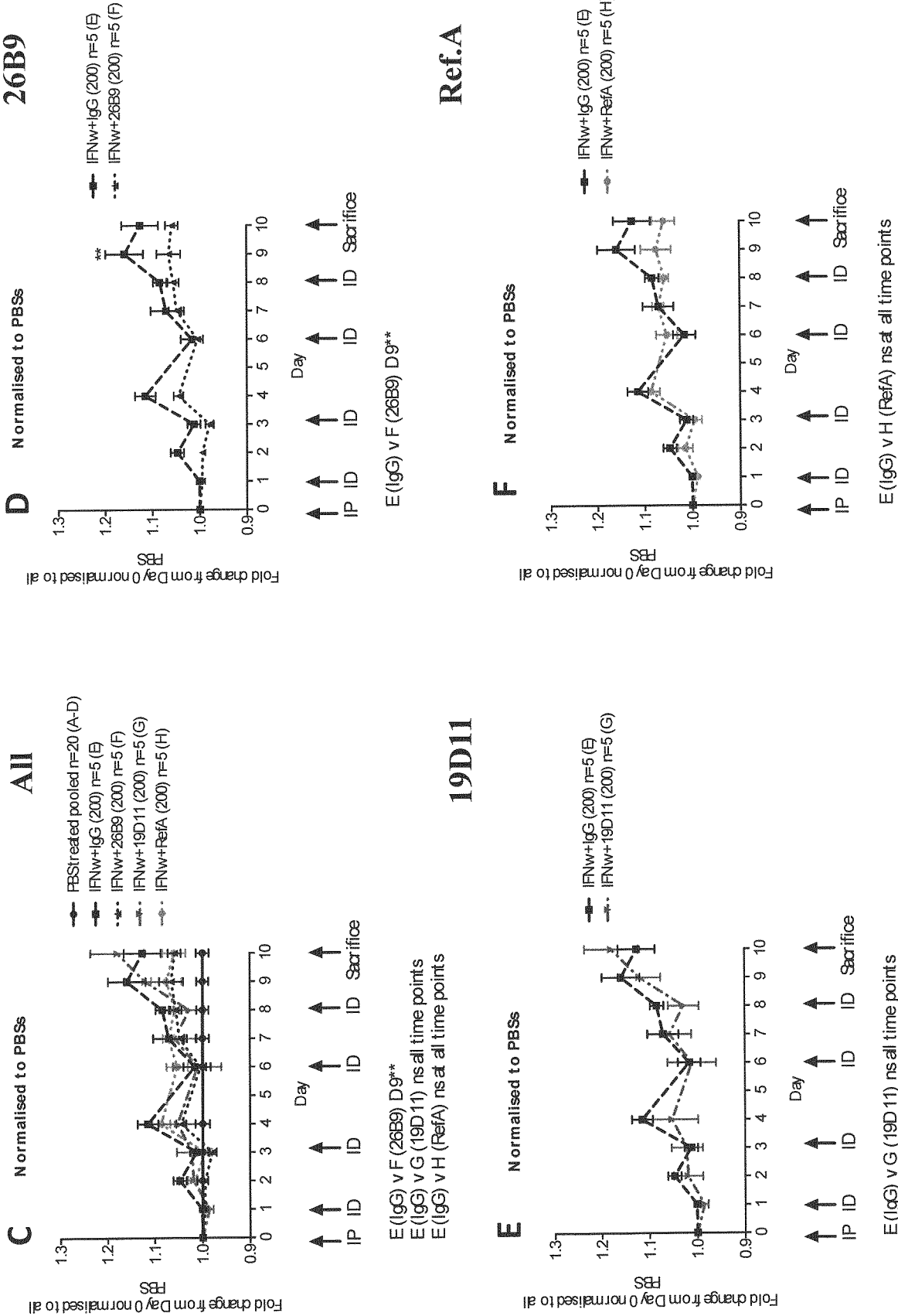
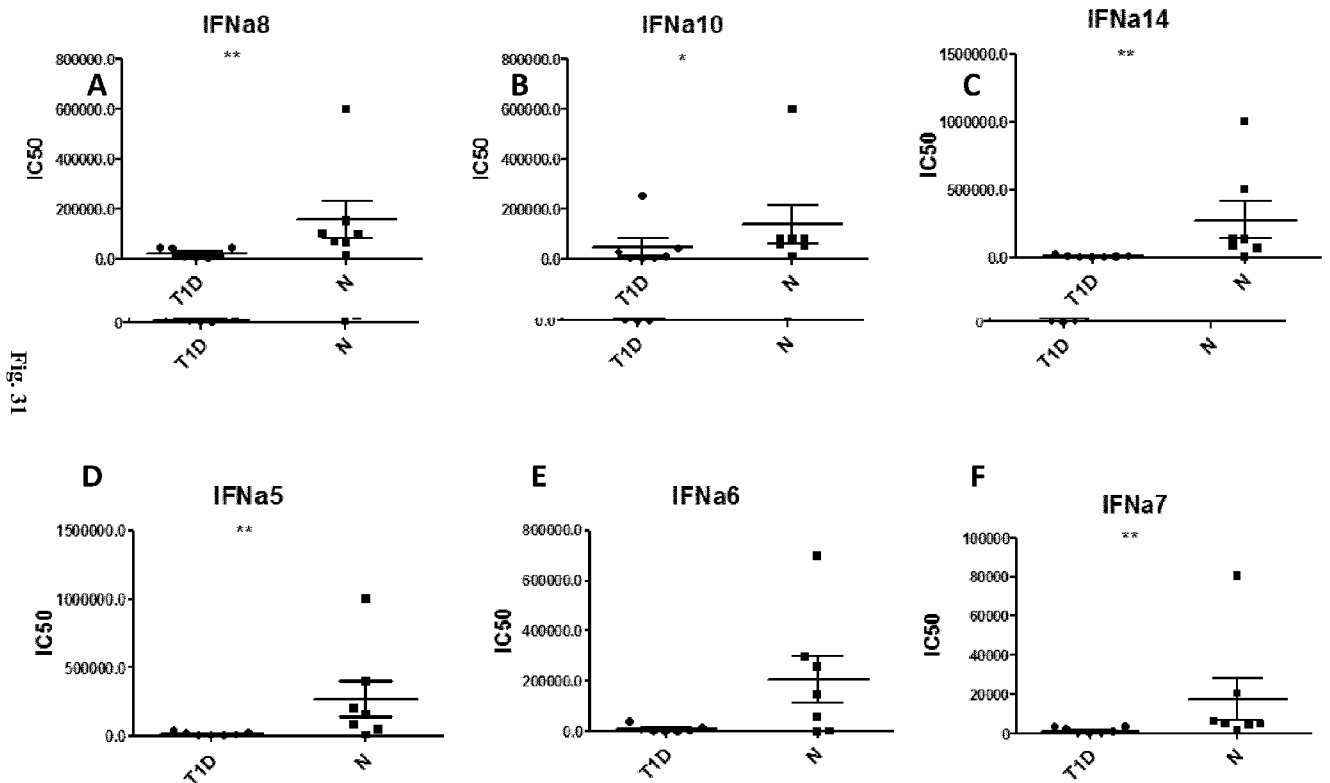
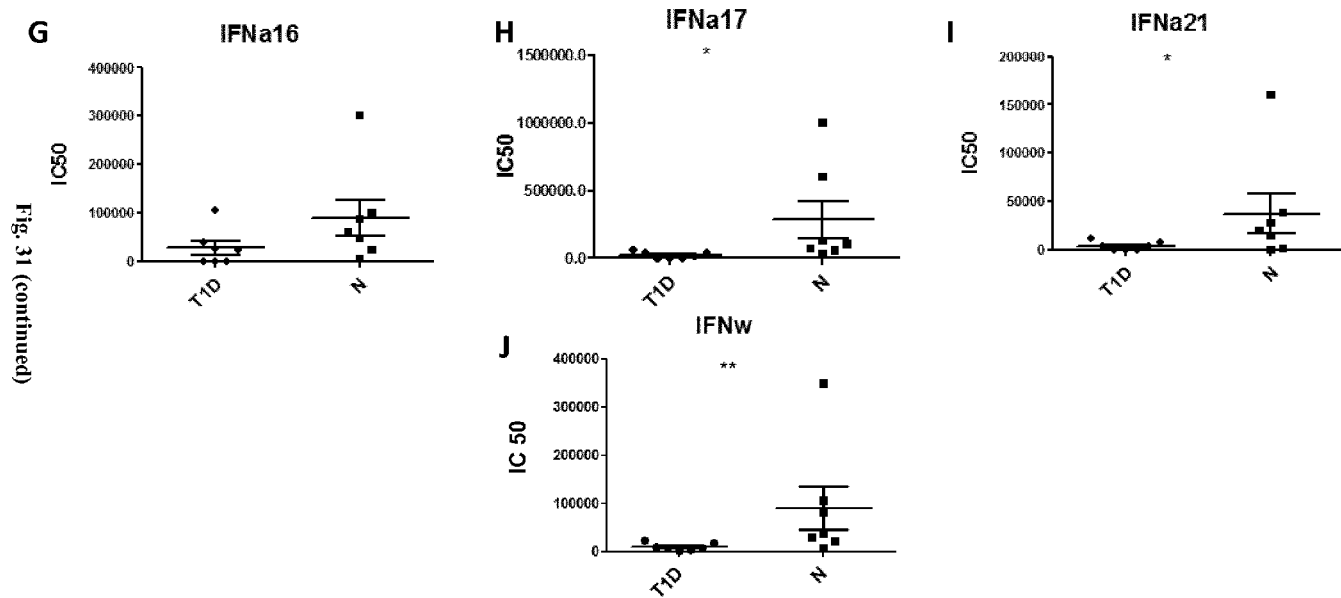


Fig. 30 (continued)





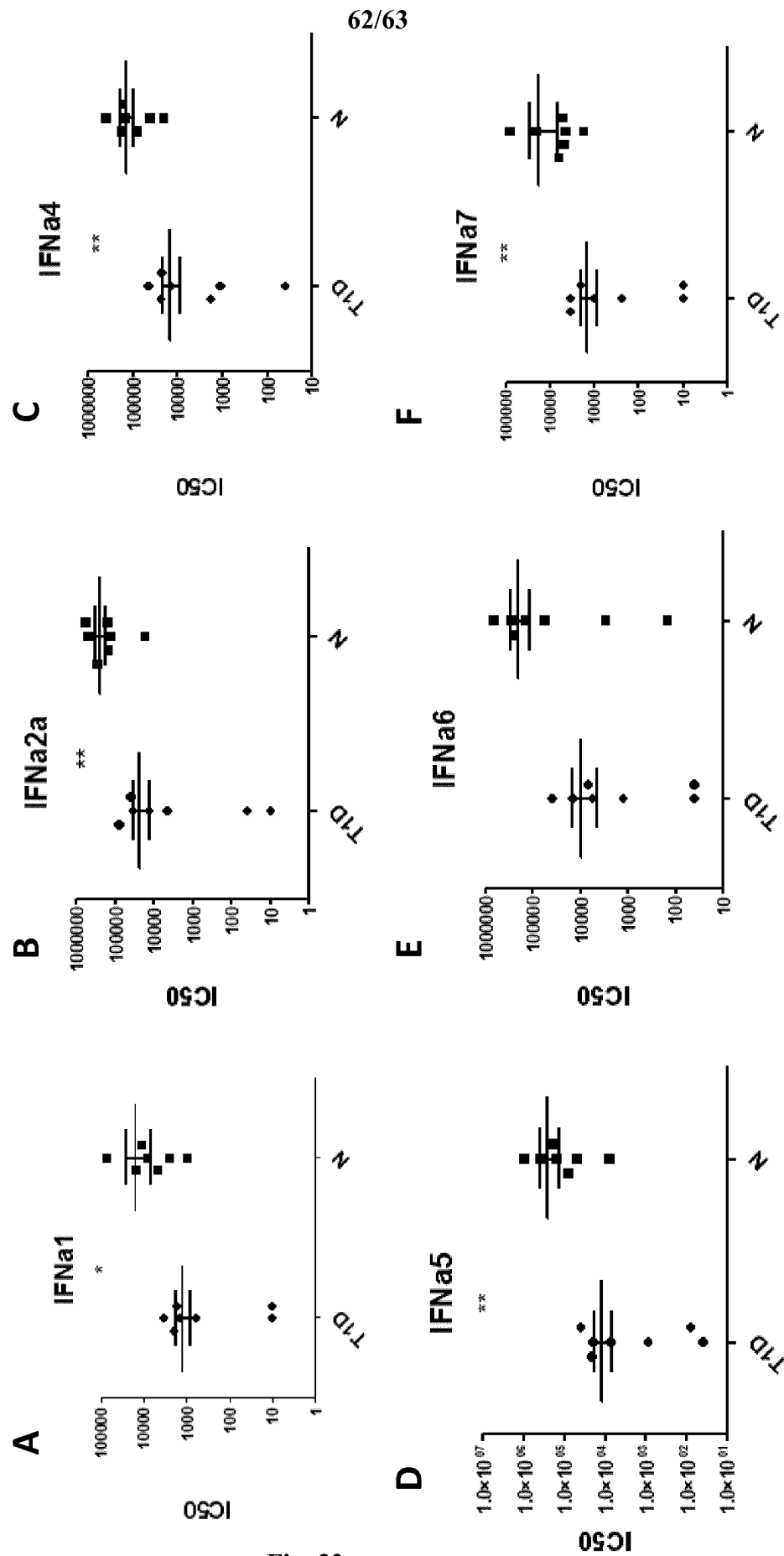


Fig. 32

