



(51) International Patent Classification:

C07K 14/54 (2006.01) A61K 38/20 (2006.01)

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(21) International Application Number:

PCT/EP2024/068967

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))
- with sequence listing part of description (Rule 5.2(a))

(22) International Filing Date:

05 July 2024 (05.07.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

23184134.7 07 July 2023 (07.07.2023) EP

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE,

(54) Title: MUTEINS OF HUMAN INTERLEUKIN 12

(57) Abstract: The present invention refers, inter alia, to i) a mutein of the α -subunit of human Interleukin 12 (hIL-12), wherein at least one of the amino acid residue(s) of said α -subunit of hIL-12 selected from the group consisting of sequence position(s) 70, 71, 72, 185, 187 and 189 is/are mutated, ii) a mutein of the β -subunit of hIL-12, wherein at least one of the amino acid residue(s) of said β -subunit of hIL-12 selected from the group consisting of sequence position(s) 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232 and 312 is/are mutated, iii) a mutein of hIL-12, comprising an α -subunit (p35) and a β -subunit (p40), wherein the α -subunit is a mutein of the α -subunit of hIL-12 according to the present invention, and/or wherein the β -subunit is a mutein of the β -subunit of hIL-12 according to the present invention, and iv) uses thereof.



MUTEINS OF HUMAN INTERLEUKIN 12

FIELD OF THE INVENTION

[001] The present invention relates to muteins of the human Interleukin 12 (hIL-12). More specifically, in a first aspect, the present invention relates to a mutein of the α -subunit of hIL-12 (SEQ ID NO: 1), wherein at least one of the amino acid residue(s) of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 is/are mutated. In a further aspect, the present invention relates to a mutein of the β -subunit of hIL-12 (SEQ ID NO: 2), wherein at least one of the amino acid residue(s) of said β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312 is/are mutated. In yet another aspect, the present invention relates to a mutein of hIL-12, comprising an α -subunit (p35) and a β -subunit (p40), wherein the α -subunit is a mutein of the α -subunit of hIL-12 (SEQ ID NO: 1) according to the present invention and/or wherein the β -subunit is a mutein of the β -subunit of hIL-12 (SEQ ID NO: 2) according to the present invention. In yet another embodiment, the present invention relates to a nucleic acid molecule comprising i) a nucleotide sequence encoding the mutein of hIL-12 according to the present invention or ii) a nucleotide sequence encoding a) the mutein of the α -subunit of hIL-12 according to the present invention and/or b) the mutein of the β -subunit of hIL-12 according to the present invention, a vector comprising said nucleic acid molecule, and a host cell comprising said nucleic acid molecule and/or said vector, respectively. In yet another aspect, the present invention relates to an immune modulator comprising a mutein according to the present invention, or a pharmaceutical composition comprising a mutein according to the present invention and/or an immune modulator according to the present invention, preferably further comprising a pharmaceutically acceptable carrier. In yet another embodiment, the present invention relates to the use of a mutein according to the present invention for the manufacture of a medicament for treating a disease in a mammal. In yet another embodiment, the present invention relates to a mutein according to the present invention for use in the treatment of a

disease. In yet another embodiment, the present invention relates to a method of treating an Interleukin 12-mediated disease in a mammal, comprising the step of administering a composition comprising a mutein according to the present invention, a pharmaceutical composition according to the present invention and/or an immune modulator according to the present invention to said mammal in need thereof. In yet another aspect, the present invention relates to a method for producing a mutein according to the present invention comprising the steps of: (a) introducing into a nucleic acid molecule encoding a polypeptide, said polypeptide being (i) the hIL-12 α -subunit polypeptide (SEQ ID NO: 1), or (ii) a polypeptide comprising at least 90% sequence identity to the hIL-12 α -subunit polypeptide (SEQ ID NO: 1), or (iii) the hIL-12 β -subunit polypeptide (SEQ ID NO: 2), or (iv) a polypeptide comprising at least 90% sequence identity to the hIL-12 β -subunit polypeptide (SEQ ID NO: 2), or (v) the hIL-12 polypeptide comprising an α -subunit (p35), and a β -subunit (p40), a nucleotide sequence mutating at least one amino acid residue of said polypeptide in case of said α -subunit selected from the group consisting of sequence positions selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 and/or in case of said β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312, and (b) introducing the obtained nucleic acid molecule for expression into a host cell or into a cell extract or into a cell lysate.

BACKGROUND OF THE INVENTION

[002] Interleukins are key signaling molecules of the immune system that are classified into families based on structural similarities. The interleukin 12 (IL-12) family consists of at least four members (IL-12, IL-23, IL-27, and IL-35) that are assigned to one family due to their unique heterodimeric character that separate these members from other ILs. Each member is composed of an α -subunit that shows a cytokine-characteristic four-helix bundle fold (IL-12 α , IL-23 α , IL-27 α) and a β -subunit composed of two fibronectin (Fn) III domains (EBI3) with an additional immunoglobulin (Ig) domain in case of IL-12 β . A remarkable feature of the IL-12 family is that nature uses extensive sharing of only five subunits to build the four heterodimers. Likewise, this combinatorial complexity applies to the IL-12 family receptors, which are also heterodimers formed by five different chains (IL-12R β 1, IL-

12R β 2, IL-23R, IL-27R α , gp130). Binding of the suitable interleukin induces receptor chain dimerization thereby activating Jak STAT signaling pathways. Binding of interleukins to their respective receptors has been studied for example by Esch *et al.*, 2020, and Georgy *et al.*, 2021, and, for example, Glassman *et al.*, 2021 investigated the structural mechanism of receptor sharing used by IL-12 and IL-23 cytokines.

[003] Subunit sharing on the level of cytokines and receptors may suggest closely related functions of the IL-12 family members, but the opposite holds true. The effects of the four family members are surprisingly diverse and even opposing. The mostly pro-inflammatory IL-12 and IL-23 are drivers of inflammation via Th1 differentiation and Th17 development, respectively. IL-27 is an immunomodulatory cytokine, which on the one hand is able to promote Th1 differentiation and on the other hand suppresses pro-inflammatory Th17 cells and induces anti-inflammatory IL-10 producing T regulatory 1 cells. In contrast, IL-35 is the only strictly inhibitory family member and acts by suppression of conventional T cells.

[004] Interleukins are secreted proteins that regulate immune cell functions. As such, they are of great interest to apply or inhibit in the clinics to modulate immune responses. Whereas antibody-based inhibition is well-established for certain interleukins, including IL-12 and IL-23 (Gaffen *et al.*, 2014; Moschen *et al.*, 2019), use of ILs as medicaments is still in its infancy. A major reason for this relates to the fact that ILs often have pleiotropic functions. Thus, potentially occurring organism-wide side effects limit their usability as medicaments. A prime example for this is IL-12, a heterodimeric cytokine composed of an α - and a β -subunit. IL-12 induces interferon gamma (IFN γ) production and promotes the development of T cells into T cells with T helper 1 (Th1) phenotype, and as such IL-12 is considered an attractive molecule to re-activate or support immune responses in a generally immunosuppressive microenvironment as in case of solid tumors (Briukhovetska *et al.*, 2021). Although IL-12 based approaches showed promising effects in various (pre-)clinical settings, strong immune activation and systemic toxicity has been limiting the use of IL-12 as a therapeutic so far (Lasek *et al.*, 2014). Different approaches are under development to overcome these limitations and make advantageous properties of IL-12 clinically accessible. These include antibody or Fc

fusions (see e.g. US 2020/0216509 A1), techniques for on-site activation including protease- or pH-control, but also local release from engineered immune cells (see e.g. Cirella *et al.*, 2022), as well as agonism-based approaches (see e.g. WO 2023/023503 A1).

[005] However, while IL-12 plays major roles in immune defense against intracellular pathogens by activating T cells and increasing antigen presentation and is moreover considered a potent anti-tumor molecule, there remains a demand for further studies. In particular, there is still a need to have at hand alternative solutions for leveraging desired properties of IL-12 in the absence of undesired side-effects, in particular since the biological functions of IL-12 qualify it as a molecule of potentially very high medical relevance.

SUMMARY OF THE INVENTION

[006] The present application addresses the need for a solution making the human Interleukin 12 (hIL-12) amenable to (medical) uses by providing the embodiments as recited in the claims.

[007] In a first aspect, the present invention provides a mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1), wherein at least one of the amino acid residue(s) of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 is/are mutated.

[008] It is preferred that said mutein comprises at least 90% sequence identity to the α -subunit of human Interleukin 12 (SEQ ID NO: 1).

[009] Additionally or alternatively, it is preferred that at least one of the amino acid residue(s) of the α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, and 187 is/are mutated, preferably from the group consisting of sequence positions 70, 71, 72, and 187.

[0010] Additionally or alternatively, it is preferred that at least one of the amino acid residue(s) of the α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189, preferably from the group consisting of sequence

positions 70, 71, 72, and 187, is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

[0011] The present invention further provides a mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2), wherein at least one of the amino acid residue(s) of said β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312 is/are mutated.

[0012] Additionally or alternatively, it is preferred that said mutein comprises at least 90% sequence identity to the β -subunit of human Interleukin 12 (SEQ ID NO: 2).

[0013] Additionally or alternatively, it is preferred that at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 38, 39, 67, 103, 106, 115, 122, 123, 221, 232, and 312 is/are mutated, preferably from the group consisting of sequence positions 67, 115, 122, 123, 221, 232, and 312.

[0014] Additionally or alternatively, it is preferred that at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 103, 232, and 312, preferably at sequence position(s) 232 and/or 312, is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G).

[0015] Additionally or alternatively, it is preferred that the amino acid residue(s) of the β -subunit at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[0016] Additionally or alternatively, it is preferred that at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221, preferably from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221, more preferably from the group consisting of sequence positions 67, 115, and 221, is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[0017] The present invention further provides a mutein of human Interleukin 12, comprising an α -subunit (p35) and a β -subunit (p40), wherein the α -subunit is a mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1) according to the present invention and/or wherein the β -subunit is a mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2) according to the present invention.

[0018] The present invention further provides a nucleic acid molecule comprising i) a nucleotide sequence encoding the mutein of human Interleukin 12 according to the present invention or ii) a nucleotide sequence encoding a) the mutein of the α -subunit of human Interleukin 12 according to the present invention, and/or b) the mutein of the β -subunit of human Interleukin 12 according to the present invention, preferably wherein the nucleic acid molecule is operably linked to a regulatory sequence to allow expression of the nucleic acid molecule, wherein the regulatory sequence preferably comprises a promoter sequence.

[0019] The present invention further provides a pharmaceutical composition comprising a mutein according to the present invention, preferably further comprising a pharmaceutically acceptable carrier.

[0020] The present invention further provides a mutein according to the present invention for use as a medicament.

[0021] The present invention further provides a mutein according to the present invention for use in the treatment of a disease, wherein the disease is preferably a disease selected from the group consisting of an infectious disease, an autoimmune disease, cancer, a transplantation-related disease such as Graft-versus-Host-disease, a chronic inflammatory disease such as chronic inflammatory bowel disease, an acute inflammatory disease, sepsis, septic shock, diabetes or asthma.

[0022] These aspects of the invention will be more fully understood in view of the following drawings, detailed description and non-limiting examples.

BRIEF DESCRIPTION OF THE FIGURES

[0023] The accompanying drawings are included to further an understanding of the embodiments that are incorporated in and constitute a part of this description. The Figures illustrate embodiments and together with the description serve to explain principles of embodiments. Other embodiments and many of the intended advantages of embodiments will be readily appreciated, as they become better understood by reference to the detailed description. The elements of the drawings are not necessarily to scale relative to each other.

[0024] **Figure 1** shows modelling of the IL-12 : receptor complex. **Figure 1A** shows in (i) IL-12 superposed on the IL-23 : receptor complex. The IL-23 receptor structure is depicted in light grey, the IL-12 components (IL-12 α and IL-12 β) in dark grey. In (ii) of **Figure 1A**, both IL-12 receptor molecules (IL-12R β 1 and IL-12R β 2) were aligned. **Figure 1B** shows the root mean squared deviation (RMSD) curves for three subparts of the system, in reference to the starting frame.

[0025] **Figure 2** shows the analysis of important residues within IL-12 β for receptor engagement. **Figure 2A** shows the energetic contribution of every amino acid to the binding of IL-12 β to IL-12R β 1 computed with MM/GBSA. Amino acids marked with light dots were expected to enthalpically stabilize the IL-12 β : IL-12R β 1-complex. Black dots indicate amino acids that were tested due to entropic contributions to IL-12 β : IL-12R β 1 complex formation. Grey areas indicate the three domains of IL-12 β , white gaps the linkers between the domains. **Figure 2B** shows ten mutation candidates in IL-12 β that are marked as light dots in **Figure 2A**. Values indicate energetic contributions to complex formation and are given in units of kcal/mol. **Figure 2C** shows RMSF calculations for IL-12 β in receptor-bound, and unbound form. Black dots indicate selected candidates, which are also shown in **Figure 2A**.

[0026] **Figure 3** shows in **Figure 3A** the energetic contribution of every amino acid to the binding of IL-12 α to IL-12R β 2 computed with MM/GBSA, and in **Figure 3B** the top six mutation candidates in IL-12 α (most stabilizing contribution to binding). The values in **Figure 3B** indicate the energetic contribution to complex formation and are given in units of kcal/mol.

[0027] **Figure 4** shows *in silico* engineered IL-12 mutants forming disulfide-bonded IL-12 heterodimers with similar structural properties compared to the wild-type protein. **Figure 4A** shows the SDS-PAGE gels that confirmed purity and covalent linkage of IL-12 subunits for the different IL-12 mutants having one amino acid exchange in the IL-12 β subunit. Proteins were transiently expressed in ExpiCHO cells, with IL-12 α being C-terminally His-tagged. Supernatant was purified by chromatography (affinity and size exclusion column). Under reducing conditions ("red"; β -Me) both subunits, IL-12 α and IL-12 β , could be detected individually, whereas under non-reducing conditions ("non-red"; NEM) the covalently linked IL-12 protein was visible on the gel. MW, molecular weight. **Figure 4B** shows that far-UV spectra measured by circular dichroism (CD) spectroscopy confirmed a wildtype-like folding status of purified IL-12 proteins.

[0028] **Figure 5** shows that IL-12-muteins activated natural killer (NK) cells in a concentration-dependent manner, showing reduced activity for IL-12 β^{E221A} (SEQ ID NO: 7) and IL-12 β^{L103G} (SEQ ID NO: 4). **Figure 5A** shows the stimulation of NK-92 cells with purified IL-12-muteins (10 ng/ μ l) and measurement of STAT4 phosphorylation via immunoblotting. Stimulation with PBS served as negative control, STAT4 signals served as loading control. All mutants were run on the same blot. Lines between blots indicate a change of the sample order for better visualization and mutant comparison. **Figure 5B** shows that the signals of immunoblots shown in **Figure 5A** were quantified and normalized to IL-12 wild-type signals ($n=3$, $\pm$ SEM). Statistically significant changes in signal ratios are marked, with IL-12 β^{L103G} (SEQ ID NO: 4) showing reduced NK-92 cell signaling (One-way ANOVA, ** $p<0.005$). **Figure 5C** shows the same as in **Figure 5A**, but NK-92 cells were stimulated with 1 ng/ μ l IL-12. **Figure 5D** shows the same as in **Figure 5B**, with signal quantification of immunoblots shown in **Figure 5C**. IL-12 β^{E221A} (SEQ ID NO: 7) and IL-12 β^{L103G} (SEQ ID NO: 4) showed significantly less NK-92 cell activation. MW, molecular weight. **Figure 5E** shows concentration-dependent IL-12 receptor chain dimerization, measured by a NanoBRET™ assay. It shows significantly reduced IL-12 receptor dimerization by IL-12 β L103G and IL-12 β E221A compared to IL-12 wildtype at a stimulation with 1 nM cytokine (Two-way ANOVA, ** $p < 0.005$, **** $p < 0.00005$, $n = 3 \pm$ SD). Signals were normalized to PBS as negative control.

[0029] Figure 6 shows cell type specific IFN γ secretion of primary human CD8⁺ T and NK cells induced by attenuated IL-12-muteins. **Figure 6A** shows IL-12-mutein caused IFN γ production by CD8⁺ T cells. CD8⁺ T cells were isolated by MACS and stimulated with the indicated IL-12-muteins for 48 h. IFN γ secretion levels in the absence of IL-12 is shown as a horizontal dotted line. Supernatant was analyzed for IFN γ by ELISA. **Figure 6B** shows that NK cells showed reduced IFN γ secretion upon activation with IL-12-muteins. NK-cells were isolated by MACS and stimulated with the indicated IL-12-muteins for 48 h. The supernatant was analyzed for IFN γ by ELISA. A horizontal dotted line indicates IFN γ levels in the absence of IL-12. Mean and standard deviation of three biological replicates are shown for two independent experiments. Where indicated as “ns”, no statistically significant difference in comparison to wild-type IL-12 at 100 nM was observed by a two-way ANOVA test. **Figure 6C** shows exemplary EC50 graphs of cell responses after IL-12 stimulation of concentrations ranging from 0.01 pM to 10 nM, normalized to no IL-12 control. Arrows indicate differences of EC50 values of NK cell responses (squares) compared to CD8⁺ T cell responses (triangles) for the respective IL-12 variants. Factors were calculated by EC50 (NK cells) divided by EC50 (CD8⁺ T cells). **Figure 6D** shows resulting EC50 values for all tested donors calculated using a non-linear regression (log(agonist) vs. normalized response variable slope). The mean and 95 % confidence interval (CI) of three replicates of one experiment are displayed.

DETAILED DESCRIPTION OF THE INVENTION

[0030] The following language and descriptions of certain preferred embodiments of the present invention are provided in order to further an understanding of the principles of the present invention. However, it will be understood that no limitations of the present invention are intended, and that further alterations, modifications, and applications of the principles of the present invention are also included.

[0031] In general, the present invention relates to attenuated cytokine variants. More specifically, by combining computational modeling with insights obtained from experimental interleukin : receptor structures and dynamics, detailed molecular insights into the binding of human interleukins and human interleukin receptors were obtained. Based thereon, the inventors designed attenuated muteins of human

interleukin 12 (hIL-12). It has been surprisingly found that the muteins according to the present invention show less activation of NK cells while maintaining CD8⁺ T cell activation compared to “native” hIL-12. Thus, the muteins according to the present invention have the advantage of providing a solution for making hIL-12 amenable to (medical) uses.

[0032] In particular, the inventors generated a high-resolution computational model of the hIL-12 : receptor complex that provided for the first time the opportunity of obtaining detailed insights into structure and dynamics of the human IL-12 : receptor complex. These insights were successfully leveraged for engineering hIL-12 muteins for reduced receptor binding affinities compared to wild-type hIL-12 based on molecular dynamics simulations. Moreover, said hIL-12 muteins were experimentally validated showing that said muteins activate natural killer (NK) cells less compared to the hIL-12 wildtype while maintaining CD8⁺ T cell activation ability. This immunological signature is considered important for hIL-12-muteins suitable for cancer treatment, where natural killer cells contribute to severe side effects. More specifically, this holds promise as NK cells, which are considered critical for systemic toxicity of hIL-12 by secretion of IFN γ , have constant hIL-12 receptor levels, whereas the desired targets of hIL-12, T cells, increase surface expression of the hIL-12 receptor upon activation. The engineered attenuated hIL-12 muteins thus are expected to provide a therapeutic hIL-12 window by preferentially activating T cells. Hence, as the hIL-12 muteins according to the present invention are expected to elicit fewer side-effects while maintaining relevant biological activity compared to wild-type hIL-12, said innovative hIL-12 muteins are advantageous, e.g., for medical purposes.

[0033] In the context of the present invention, the term “mutein” refers to a variant of a native molecule, wherein said native molecule can be nucleic acid and/or amino acid molecule. For example, in case the mutein is a mutein of a native molecule being a nucleic acid molecule, said mutein has at least one nucleic acid residue mutated compared to the native molecule. Thus, the mutein has at least one nucleic acid residue replaced by a nucleic acid residue other than the respective nucleic acid residue of the native molecule’s nucleic acid sequence at the respective position. As another example, in case the mutein is a mutein of a native molecule being an amino

acid molecule, said mutein has at least one amino acid residue mutated compared to the native molecule. Thus, the mutein has at least one amino acid residue replaced by an amino acid residue other than the respective amino acid residue of the native molecule's amino acid sequence at the respective position.

[0034] Herein, a mutation refers to a deletion of a residue, an addition of one or more residues, and/or a replacement of a residue, wherein said replacement may be a replacement of one residue by another residue or a replacement of one residue by more than one residue(s). Preferably, a mutation refers to a replacement of one residue by a single residue any other than the replaced residue, preferably by alanine (A) or glycine (G).

[0035] As regards mutated amino acid residue(s) in a mutein according to the present invention, it is preferred that a mutated amino acid residue is an alanine (A) or glycine (G) that replaces the respective native amino acid residue. In particular, it is preferred in case of a mutein of hIL-12 α that a native amino acid residue of hIL-12 α is replaced by A. Additionally or alternatively, it is preferred in case of a mutein of hIL-12 β that a native amino acid residue of hIL-12 β is replaced by A or G.

[0036] A mutation can affect intracellular localization, functionality and/or activity of a molecule. Herein, it is preferred that the term "mutein" refers to a mutein of a native molecule, wherein said mutein exhibits a different cellular, biochemical and/or immunological effect compared to the native molecule. For example, in case the native molecule is a molecule that can initiate a signal cascade in a cell upon binding to a receptor, a mutein of said native molecule preferably exhibits a lower or higher potential of initiating the signal cascade upon binding to the receptor compared to the native molecule. Different mechanisms can be envisioned that can result in a change of the effect of a mutein compared to its native molecule. Preferably, muteins according to the present invention are secretion-competent muteins and thus, the muteins are able to perform a complete passage through the secretory pathway of a cell and through the cytoplasmic membrane.

[0037] Herein, "hIL-12 α " refers to the native α -subunit of human Interleukin 12 with the sequence set forth in SEQ ID NO: 1, and "hIL-12 β " refers to the native β -subunit of human Interleukin 12 with the sequence set forth in SEQ ID NO: 2.

[0038] Herein, the sequence set forth in SEQ ID NO: 1 refers to the “native” α -subunit of human Interleukin 12, and the sequence set forth in SEQ ID NO: 2 refers to the “native” β -subunit of human Interleukin 12. The term may be understood as “wild-type” and thus, the polypeptide sequences of hIL-12 α and hIL-12 β as set forth in SEQ ID NO: 1 and 2, respectively, may also be understood as wild-type hIL-12 α and wild-type hIL-12 β sequences. Sequences set forth in SEQ ID NO: 1 and 2 are also deposited in UniProtKB, with the sequence of hIL-12 α , SEQ ID NO: 1, being also deposited under UniProtKB accession number P29459, and the sequence of hIL-12 β , SEQ ID NO: 2, under UniProtKB accession number P29460.

[0039] Preferably, a mutein according to the present invention has an attenuated activity compared to the activity of the native molecule. This is advantageous as hIL-12 muteins with attenuated and thus, reduced activity compared to native hIL-12 can pave the way for making hIL-12 amenable for (medical) uses.

[0040] Muteins of the α -subunit of human Interleukin 12

[0041] In a first aspect, the present invention relates to a mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1), wherein at least one of the amino acid residue(s) of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 is/are mutated.

[0042] Accordingly, the mutein of hIL-12 α has the amino acid sequence of hIL-12 α as set forth in SEQ ID NO: 1, except that at least one of the amino acid residues selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 is mutated. In the context of the present invention, it may be that the numbering of sequence positions of said mutein of the α -subunit of human Interleukin 12 corresponds to the numbering of the sequence positions of the α -subunit of human Interleukin 12 according to SEQ ID NO: 1. In the context of the present invention, when referring to the mature protein of human IL-12 α -subunit according to SEQ ID NO: 1, wherein the signal sequence is cleaved, the numbering of sequence positions changes by 22 residues, i.e. position 70 then becomes position 48, respectively.

[0043] For example, the mutein of hIL-12 α can have the amino acid sequence of the native hIL-12 α as set forth in SEQ ID NO: 1, except that the amino acid residue(s) at

position(s) 70, 71, 72, 185, 187 and/or 189 is/are mutated. In a further example, the mutein of hIL-12 α can have the amino acid sequence of the native hIL-12 α as set forth in SEQ ID NO: 1, except that the amino acid residue(s) at position(s) 70, 71, 72, 185 and/or 187 is/are mutated. In a further example, the mutein of hIL-12 α can have the amino acid sequence of the native hIL-12 α as set forth in SEQ ID NO: 1, except that the amino acid residue(s) at position(s) 70, 71, 72 and/or 187 is/are mutated. In a further example, the mutein of hIL-12 α can have the amino acid sequence of the native hIL-12 α as set forth in SEQ ID NO: 1, except that the amino acid residue(s) at position(s) 71, 72 and/or 187 is/are mutated. In a further example, the mutein of hIL-12 α can have the amino acid sequence of the native hIL-12 α as set forth in SEQ ID NO: 1, except that the amino acid residue(s) at position(s) 72 and/or 187 is/are mutated.

[0044] As another example, the mutein of hIL-12 α can have the amino acid sequence of the native hIL-12 α as set forth in SEQ ID NO: 1, except that the amino acid residues at positions 70 and 71, 70 and 72, 71 and 72, 70 and 187, 71 and 187, or 72 and 187 are mutated.

[0045] As another example, the mutein of hIL-12 α can have the amino acid sequence of the native hIL-12 α as set forth in SEQ ID NO: 1, except that the amino acid residues at positions 70, 71 and 72; 70, 71 and 187; 70, 72 and 187; or 71, 72 and 187 are mutated.

[0046] As another example, the mutein of hIL-12 α can have the amino acid sequence of the native hIL-12 α as set forth in SEQ ID NO: 1, except that the amino acid residues at positions 70, 71, 72 and 187 are mutated.

[0047] Preferably, the mutein of hIL-12 α comprises at least 90% sequence identity to the α -subunit of human Interleukin 12 (SEQ ID NO: 1). Thus, the mutein of hIL-12 α has at least one of the amino acid residues of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 mutated and preferably comprises at least 90% sequence identity to hIL-12 α (SEQ ID NO: 1).

[0048] Accordingly, the mutein of hIL-12 α may have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at

least 98%, at least 99%, at least 99.5% or at least 99.9% sequence identity to the α -subunit of human Interleukin 12 (SEQ ID NO: 1).

[0049] Herein, the term "sequence identity" refers to a property of sequences that measures their similarity or relationship. The term "sequence identity" as used in the present invention means the percentage of pair-wise identical residues - following (homology) alignment of a nucleic acid and/or amino acid sequence with a given reference sequence - with respect to the number of residues in the longer of the two compared sequences. Sequence identity is measured by dividing the number of identical residues by the total number of residues and multiplying the product by 100.

[0050] The percentage of sequence homology or sequence identity can, for example, be determined herein using the program BLASTP, version blastp 2.2.5 (November 16, 2002; cf. Altschul, S. F. et al. (1997) Nucl. Acids Res.25, 3389-3402). For example, the percentage of homology may be based on the alignment of the entire polypeptide sequences (matrix: BLOSUM 62; gap costs: 11.1; cutoff value set to 10^{-3}) including the respective sequences. It may be calculated as the percentage of numbers of "positives" (homologous amino acid residues) indicated as result in the BLASTP program output divided by the total number of amino acid residues selected by the program for the alignment.

[0051] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein at least one of the amino acid residue(s) of the α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, and 187 is/are mutated.

[0052] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein at least one of the amino acid residue(s) of the α -subunit selected from the group consisting of sequence positions 70, 71, 72, and 187 is/are mutated.

[0053] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein i) at least one of the amino acid residue(s) of the α -subunit selected from the group consisting of sequence positions 70 to 72 is/are mutated, and/or wherein ii) the amino acid residue of the α -subunit at sequence position 187 is mutated.

[0054] According to some embodiments of the present invention, the mutein of hIL-12 α may be a mutein, wherein i) at least one of the amino acid residue(s) of the α -subunit selected from the group consisting of sequence positions 70 to 72 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, and/or wherein ii) the amino acid residue of the α -subunit at sequence position 187 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1.

[0055] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 70 is mutated.

[0056] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 70 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 α is a mutein, wherein the amino acid residue of the α -subunit at sequence position 70 is replaced by alanine. The amino acid sequence of the mutein hIL-12 α ^{D70A} is set forth herein as SEQ ID NO: 18.

[0057] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 71 is mutated.

[0058] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 71 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 α is a mutein, wherein the amino acid residue of the α -subunit at sequence position 71 is replaced by alanine. The amino acid sequence of the mutein hIL-12 α ^{H71A} is set forth herein as SEQ ID NO: 19.

[0059] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 72 is mutated.

[0060] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 72 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 α is a mutein, wherein the amino acid residue of the α -subunit at sequence position 72 is replaced by alanine. The amino acid sequence of the mutein hIL-12 α ^{E72A} is set forth herein as SEQ ID NO: 20.

[0061] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 185 is mutated.

[0062] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 185 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 α is a mutein, wherein the amino acid residue of the α -subunit at sequence position 185 is replaced by alanine. The amino acid sequence of the mutein hIL-12 α ^{E185A} is set forth herein as SEQ ID NO: 21.

[0063] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 187 is mutated.

[0064] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 187 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 α is a mutein, wherein the amino acid residue of the α -subunit at sequence position 187 is replaced by alanine. The amino acid sequence of the mutein hIL-12 α ^{D187A} is set forth herein as SEQ ID NO: 22.

[0065] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 189 is mutated.

[0066] According to one embodiment of the present invention, the mutein of hIL-12 α may be a mutein, wherein the amino acid residue of the α -subunit at sequence position 189 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 α is a mutein, wherein the amino acid residue of the α -subunit at sequence position 189 is replaced by alanine. The amino acid sequence of the mutein hIL-12 α ^{Y189A} is set forth herein as SEQ ID NO: 23.

[0067] Muteins of the β -subunit of human Interleukin 12

[0068] In a second aspect, the present invention relates to a mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2), wherein at least one of the amino acid residue(s) of said β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312 is/are mutated.

[0069] Accordingly, the mutein of hIL-12 β has the amino acid sequence of hIL-12 β as set forth in SEQ ID NO: 2, except that at least one of the amino acid residue(s) selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312 is/are mutated. In the context of the present invention, it may be that the numbering of sequence positions of said mutein of the β -subunit of human Interleukin 12 corresponds to the numbering of the sequence positions of the β -subunit of human Interleukin 12 according to SEQ ID NO: 2. In the context of the present invention, when referring to the mature protein of human IL-12 β -subunit according to SEQ ID NO: 2, wherein the signal sequence is cleaved, the numbering of sequence positions changes by 22 residues, i.e. position 37 then becomes position 22, respectively.

[0070] For example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have

the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 67, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 67, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 67, 103, 115, 122, 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 67, 103, 122, 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 103, 122, 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 122, 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 123, 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 216, 221, 232, and/or 312 is/are mutated. In a further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 221, 232, and/or 312 is/are mutated. In a

further example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue(s) at positions 232, and/or 312 is/are mutated.

[0071] As another example, the mutein of hIL-12 β can have the amino acid sequence of the native hIL-12 β as set forth in SEQ ID NO: 2, except that the amino acid residue at positions 67 and 115, 67 and 122, 67 and 123, 67 and 221, 67 and 232, 67 and 312, 115 and 122, 115 and 123, 115 and 221, 115 and 232, 115 and 312, 122 and 123, 122 and 221, 122 and 232, 122 and 312, 123 and 221, 123 and 232, 123 and 312, 221 and 232, 221 and 312, or 232 and 312 are mutated.

[0072] Preferably, the mutein of hIL-12 β comprises at least 90% sequence identity to the β -subunit of human Interleukin 12 (SEQ ID NO: 2). Thus, the mutein of hIL-12 β has at least one of the amino acid residue(s) of said β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312 mutated and preferably comprises at least 90% sequence identity to hIL-12 β (SEQ ID NO: 2).

[0073] Accordingly, the mutein of hIL-12 β may have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5% or at least 99.9% sequence identity to the β -subunit of human Interleukin 12 (SEQ ID NO: 2).

[0074] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 38, 39, 67, 103, 106, 115, 122, 123, 221, 232, and 312 is/are mutated.

[0075] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 67, 115, 122, 123, 221, 232, and 312 is/are mutated.

[0076] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 103, 232 and 312 is/are

mutated, preferably at sequence positions 232 and/or 312. This is advantageous as it has been shown that a mutation of any of these sequence positions can entropically contribute to receptor binding and thus, IL-12 β : IL-12R β 1 complex formation. Without being bound by theory it is believed that these three amino acid residues represent rigid positions in an unbound hIL-12 β subunit juxtaposed to flexible domains of strongly fluctuating sites. Replacing any of the amino acid residues at positions 103, 232 and 312, preferably at sequence positions 232 and/or 312, by any other amino acid residue than the one at the respective position in SEQ ID NO: 2, preferably by glycine (G), can alter the dynamics of hIL-12 β and thus, can impede receptor binding. Thus, said muteins are also advantageous as they allow engineering attenuated IL-12-muteins.

[0077] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residues of the β -subunit selected at sequence positions 122 and/or 123 is/are mutated. This is advantageous, as it has been shown that a mutation of any of these sequence positions can entropically contribute to receptor binding and thus, IL-12 β : IL-12R β 1 complex formation. Without being bound by theory, it is believed that an amino acid residue replacement at any of these two sequence positions by any other amino acid residue than the amino acid residue at the respective position in SEQ ID NO: 2, preferably by alanine (A), can entropically destabilize receptor binding. Thus, said muteins are also advantageous as they allow engineering attenuated IL-12-muteins.

[0078] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216 and 221 is/are mutated. This is advantageous, as it has been shown that a mutation of any of these sequence positions can enthalpically contribute to receptor binding and thus, IL-12 β : IL-12R β 1 complex formation. Replacing any of the amino acid residues at positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221 by any other amino acid residue than the one at the respective position in SEQ ID NO: 2, preferably by alanine (A), can alter the dynamics of hIL-12 β and thus, can impede receptor binding. Thus, said muteins are also advantageous as they allow engineering attenuated IL-12-muteins. As regards the mutated and/or replaced

amino acid residues at sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221 in the mutein, it is preferred that at least one of the amino acid residue(s) of the β -subunit selected from

- the group consisting of sequence positions 38, 39, 67, 106, 115, and 221,
- the group consisting of sequence positions 39, 67, 106, 115 and 221,
- the group consisting of sequence positions 39, 67, 115 and 221, or
- the group consisting of sequence positions 67, 115 and 221,

is/are mutated, preferably replaced by any other amino acid residue than the one at the respective position in SEQ ID NO: 2, preferably replaced by alanine (A).

[0079] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein

- i) at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 103, 232, and 312 is/are mutated, and/or wherein
- ii) the amino acid residue(s) of the β -subunit at sequence position(s) 122 and/or 123 is/are mutated, and/or wherein
- iii) at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221 is/are mutated.

[0080] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein

- i) at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 103, 232, and 312 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G), and/or wherein
- ii) the amino acid residue(s) of the β -subunit at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or wherein
- iii) at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106,

115, 216, and 221 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[0081] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein

- i) at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 103, 232, and 312 is/are mutated, and/or wherein
- ii) the amino acid residue(s) of the β -subunit at sequence position(s) 122 and/or 123 is/are mutated, and/or wherein
- iii) at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221 is/are mutated.

[0082] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein

- i) at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 103, 232, and 312 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (G), and/or wherein
- ii) the amino acid residue(s) of the β -subunit at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or wherein
- iii) at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[0083] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein

- i) the amino acid residue(s) of the β -subunit at sequence position(s) 232 and/or 312 is/are mutated, and/or wherein

- ii) the amino acid residue(s) of the β -subunit at sequence position(s) 122 and/or 123 is/are mutated, and/or wherein
- iii) at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 67, 115, and 221 is/are mutated.

[0084] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein

- i) the amino acid residue(s) of the β -subunit at sequence position(s) 232 and/or 312 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G), and/or wherein
- ii) the amino acid residue(s) of the β -subunit at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or wherein
- iii) at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 67, 115, and 221 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[0085] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 103 is mutated.

[0086] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 103 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 103 is replaced by glycine. The amino acid sequence of the mutein hIL-12 β ^{L103G} is set forth herein as SEQ ID NO: 4.

[0087] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 232 is mutated.

[0088] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 232 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 232 is replaced by glycine. The amino acid sequence of the mutein hIL-12 β ^{I232G} is depicted herein as SEQ ID NO: 8.

[0089] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 312 is mutated.

[0090] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 312 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 312 is replaced by glycine. The amino acid sequence of the mutein hIL-12 β ^{D312G} is depicted herein as SEQ ID NO: 9.

[0091] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 122 is mutated.

[0092] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 122 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 122 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{E122A} is depicted herein as SEQ ID NO: 10.

[0093] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 123 is mutated.

[0094] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 123 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 123 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{P123A} is depicted herein as SEQ ID NO: 11.

[0095] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 82 is mutated.

[0096] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 82 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 82 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{F82A} is depicted herein as SEQ ID NO: 12.

[0097] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 37 is mutated.

[0098] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 37 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 37 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{W37A} is depicted herein as SEQ ID NO: 13.

[00099] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 39 is mutated.

[00100] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 39 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 39 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{P39A} is depicted herein as SEQ ID NO: 14.

[00101] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 106 is mutated.

[00102] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 106 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 106 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{K106A} is depicted herein as SEQ ID NO: 15.

[00103] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 81 is mutated.

[00104] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 81 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 81 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{E81A} is depicted herein as SEQ ID NO: 16.

[00105] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 115 is mutated.

[00106] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 115 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 115 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{D115A} is depicted herein as SEQ ID NO: 5.

[00107] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 67 is mutated.

[00108] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 67 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 67 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{E67A} is depicted herein as SEQ ID NO: 3.

[00109] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 216 is mutated.

[00110] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 216 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 216 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{H216A} is depicted herein as SEQ ID NO: 6.

[00111] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 38 is mutated.

[00112] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 38 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 38 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{Y38A} is depicted herein as SEQ ID NO: 17.

[00113] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 221 is mutated.

[00114] According to one embodiment of the present invention, the mutein of hIL-12 β may be a mutein, wherein the amino acid residue of the β -subunit at sequence position 221 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A). In one embodiment of the present invention, the mutein of hIL-12 β is a mutein, wherein the amino acid residue of the β -subunit at sequence position 221 is replaced by alanine. The amino acid sequence of the mutein hIL-12 β ^{E221A} is depicted herein as SEQ ID NO: 7.

[00115] Muteins of human Interleukin 12

[00116] In a third aspect, the present invention relates to a mutein of human Interleukin 12, comprising an α -subunit (p35) and a β -subunit (p40), wherein the α -subunit is a mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1) according to the present invention and/or wherein the β -subunit is a mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2) according to the present invention. As regards the nature of the hIL-12 α mutein and/or hIL-12 β mutein according to the present invention, the same applies as it has been described herein in connection with the muteins of the α -subunit of human Interleukin 12 (SEQ ID NO: 1) according to the present invention and/or the muteins of the β -subunit of human Interleukin 12 (SEQ ID NO: 2) according to the present invention, respectively. Moreover, also the

other features of such a hIL-12 α mutein and/or hIL-12 β mutein can be as described herein.

[00117] For example, the mutein of hIL-12 according to the present invention may comprise an α -subunit (p35), wherein said α -subunit may be a mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1) according to the present invention. For example, the mutein of hIL-12 according to the present invention may comprise an α -subunit (p35), wherein said α -subunit may be a mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1), wherein at least one of the amino acid residue(s) of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 is mutated, preferably selected from the group consisting of sequence positions 70, 71, 72, and 187.

[00118] According to one embodiment of the present invention, the mutein of hIL-12 according to the present invention comprises an α -subunit (p35), wherein said α -subunit may be a mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1) comprising at least 90% sequence identity to the α -subunit of human Interleukin 12 (SEQ ID NO: 1).

[00119] According to one embodiment of the present invention, the hIL-12 α mutein comprised in the hIL-12 mutein may have at least one of the amino acid residue(s) selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189, preferably selected from the group consisting of sequence positions 70, 71, 72, and 187, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

[00120] According to one embodiment of the present invention, the hIL-12 α mutein comprised in the hIL-12 mutein may have an amino acid residue at sequence positions 70, 71, 72, 185, 187, and/or 189, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A). According to one further embodiment of the present invention, the hIL-12 α mutein comprised in the hIL-12 mutein may have an amino acid residue at sequence positions 70, 71, 72, 185, and/or 187, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A). According to one further embodiment of the

present invention, the hIL-12 α mutein comprised in the hIL-12 mutein may have an amino acid residue at sequence positions 70, 71, 72, and/or 187, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A). According to one further embodiment of the present invention, the hIL-12 α mutein comprised in the hIL-12 mutein may have an amino acid residue at sequence positions 71, 72, and/or 187, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A). According to one further embodiment of the present invention, the hIL-12 α mutein comprised in the hIL-12 mutein may have an amino acid residue at sequence positions 72 and/or 187, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

[00121] For example, the mutein of hIL-12 according to the present invention comprises a β -subunit (p40), wherein said β -subunit may be a mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2) according to the present invention. For example, the mutein of hIL-12 according to the present invention may comprise a β -subunit (p40), wherein said β -subunit may be a mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2), wherein at least one of the amino acid residue(s) of the β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312 is/are mutated, preferably wherein the at least one of the amino acid residue(s) of the β -subunit is selected from the group consisting of sequence positions 39, 67, 103, 106, 115, 122, 123, 221, 232, and 312, more preferably wherein the at least one amino acid residue(s) of the β -subunit is selected from the group consisting of sequence positions 39, 67, 103, 115, 122, 123, 221, 232, and 312 and/or is selected from the group consisting of sequence positions 67, 115, 122, 123, 221, 232, and 312. According to one embodiment of the present invention, the mutein of hIL-12 according to the present invention comprises a β -subunit (p40), wherein said β -subunit may be a mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2) comprising at least 90% sequence identity to the β -subunit of human Interleukin 12 (SEQ ID NO: 2).

[00122] According to one embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have at least one of the amino acid residue(s) selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312, preferably the at least one of the amino acid residue(s) is/are selected from the group consisting of sequence positions 39, 67, 103, 106, 115, 122, 123, 221, 232, and 312, more preferably the at least one of the amino acid residue(s) is/are selected from the group consisting of sequence positions 39, 67, 103, 115, 122, 123, 221, 232, and 312 and/or is/are selected from the group consisting of sequence positions 67, 115, 122, 123, 221, 232, and 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G).

[00123] According to one embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence

position(s) 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 82, 103, 106, 115, 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 103, 106, 115, 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 103, 115, 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 103, 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 122, 123, 216, 221, 232, and/or 312, which is/are replaced by an amino

acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 123, 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 216, 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 221, 232, and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G). According to one further embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have (an) amino acid residue(s) at sequence position(s) 232 and/or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) or glycine (G).

[00124] According to one embodiment of the present invention, the hIL-12 β mutein comprised in the hIL-12 mutein may have an amino acid residue at sequence position 103, 232 or 312, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G) and/or an amino acid residue at sequence position(s) 37, 38, 39, 67, 81, 82, 106, 115, 122, 123, 216, and/or 221, which is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00125] Molecules and cells carrying information of the muteins

[00126] The muteins according to the present invention may be encoded by a nucleic acid molecule. As used herein, the term "nucleic acid molecule" refers to a, natural or artificial, single and/or double stranded nucleic acid molecule. Said molecule is preferably a deoxyribonucleic acid (DNA) molecule and thus, preferably

is built up of adenosine, guanosine, cytidine, and/or thymidine residues, herein also referred to as nucleotides. A nucleic acid molecule is advantageous to store genetic information that can be transcribed into ribonucleic acid (RNA) polyribonucleotides that carry the genetic information for protein synthesis, which can be translated into proteins. Thus, nucleic acid molecules are also advantageous to enable expression of proteins like muteins according to the present invention, for example, when being introduced into a suitable host cell.

[00127] Accordingly, the present invention also provides a nucleic acid molecule comprising a nucleotide sequence encoding a mutein of the α -subunit of human Interleukin 12 according to the present invention and/or a mutein of the β -subunit of human Interleukin 12 according to the present invention. In one embodiment of the present invention, the nucleic acid molecule comprises a nucleotide sequence encoding a mutein of human Interleukin 12 according to the present invention. As regards the nature of such a mutein of the α -subunit of human Interleukin 12, of the β -subunit of human Interleukin 12, and/or of the human Interleukin 12, the same applies as it has been described herein in connection with the hIL-12 α , hIL-12 β and hIL-12 muteins according to the present invention, respectively. Moreover, also the other features of such muteins can be as described herein.

[00128] Preferably, the nucleic acid molecule of the present invention is operably linked to a regulatory sequence to allow expression of the nucleic acid molecule. The regulatory sequence may comprise a promoter sequence. Herein, the term "promoter sequence" refers to a DNA sequence, which initiates and directs the transcription of a gene into an RNA transcript in cells.

[00129] The nucleic acid molecule(s) according to the present invention may be comprised in a vector. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid molecule, preferably a nucleic acid molecule according to the present invention, to which it has been linked. One type of vector may be a plasmid, which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector may be a viral vector, wherein additional DNA segments can be ligated into the viral genome.

[00130] Accordingly, the present invention also provides a vector comprising the nucleic acid molecule according to the present invention. As regards the nature of said nucleic acid molecule, the same applies as it has been described herein in connection with the nucleic acid molecule according to the present invention. Moreover, also the other features of such a nucleic acid molecule can be as described herein.

[00131] The present invention also provides a host cell comprising the nucleic acid molecule according to the present invention and/or the vector according to the present invention. Herein, a host cell can be any prokaryotic (e.g., *E. coli*) or eukaryotic cell (e.g., insect cells, yeast or mammalian cells). Preferably, said host is a suitable host cell, more preferably a host cell suitable for expression of a nucleic acid molecule and/or of a vector according to the present invention. As regards the nature of the nucleic acid molecule and/or the vector, the same applies as it has been described herein in connection with the nucleic acid molecule according to the present invention and the vector according to the present invention, respectively. Moreover, also the other features of such a nucleic acid molecule and/or of such a vector can be as described herein.

[00132] Immune modulators and pharmaceutical compositions comprising muteins

[00133] The present invention also provides an immune modulator comprising a mutein according to the present invention. Herein, the term “immune modulator” is intended to be understood as referring to any protein, substance or composition that is able to carry out immunomodulation, which is the adjustment of the immune-response to a desired level, as e.g. in immunopotentialization, immunosuppression, or induction of immunologic tolerance. Thus, the immune modulator according to the present invention comprises a hIL-12 α , hIL-12 β and/or hIL-12 mutein according to the present invention. As regards the nature of said mutein of the α -subunit of human Interleukin 12, of the β -subunit of human Interleukin 12, and/or of the human Interleukin 12, the same applies as it has been described herein in connection with the hIL-12 α , hIL-12 β and hIL-12 muteins according to the present invention, respectively. Moreover, also the other features of such muteins can be as described herein.

[00134] The present invention also provides a pharmaceutical composition comprising a mutein according to the present invention and/or an immune modulator according to the present invention. Thus, the pharmaceutical composition according to the present invention comprises a hIL-12 α , hIL-12 β and/or hIL-12 mutein according to the present invention and/or an immune modulator according to the present invention. As regards the nature of said immune modulator and/or the mutein of the α -subunit of human Interleukin 12, of the β -subunit of human Interleukin 12, and/or of the human Interleukin 12, the same applies as it has been described herein in connection with the immune modulator and the hIL-12 α , hIL-12 β and hIL-12 muteins according to the present invention, respectively. Moreover, also the other features of such muteins and/or immune modulators can be as described herein.

[00135] Preferably, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier. Herein, the term “pharmaceutically acceptable carrier” refers to chemical compounds, materials, ingredients, and/or compositions, which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a mammal, preferably of a human, without (excessive) toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Thus, a pharmaceutically acceptable carrier relates to an inactive substance formulated alongside the pharmaceutically active substance, herein a mutein and/or an immune modulator according to the present invention, for facilitating its handling in view of dosage, adsorption, solubility or pharmacokinetic considerations. Examples of suitable pharmaceutical acceptable carriers are well known in the art and include phosphate buffered saline solutions, buffer, water, emulsions, such as oil/water emulsions, various wetting agents, and sterile solutions.

[00136] Preferably, the pharmaceutical composition comprises a mutein according to the present invention and/or an immune modulator according to the present invention in an effective amount, i.e. an amount sufficient to induce a detectable beneficial and/or therapeutic response in a mammal, preferably a human, to which the pharmaceutical composition is to be administered.

[00137] The present invention also provides the use of a mutein according to the present invention for the manufacture of a medicament for treating a disease in a

mammal, preferably a human, wherein the disease is preferably a disease selected from the group consisting of an infectious disease, an autoimmune disease, cancer, a transplantation-related disease such as Graft-versus-Host-disease, a chronic inflammatory disease such as chronic inflammatory bowel disease, an acute inflammatory disease, sepsis, septic shock, diabetes or asthma. Thus, for the manufacture of said medicament, a hIL-12 α , hIL-12 β and/or hIL-12 mutein according to the present invention is to be used. As regards the nature of the muteins of the α -subunit of human Interleukin 12, of the β -subunit of human Interleukin 12, and/or of the human Interleukin 12, the same applies as it has been described herein in connection with the hIL-12 α , hIL-12 β and hIL-12 muteins according to the present invention, respectively. Moreover, also the other features of such muteins can be as described herein.

[00138] hIL-12 muteins as medicaments

[00139] The present invention also relates to a mutein according to the present invention for use as a medicament. Thus, a hIL-12 α , hIL-12 β and/or hIL-12 mutein according to the present invention are disclosed herein for use as a medicament. As regards the nature of said mutein of the α -subunit of human Interleukin 12, of the β -subunit of human Interleukin 12, and/or of the human Interleukin 12, the same applies as it has been described herein in connection with the hIL-12 α , hIL-12 β and hIL-12 muteins according to the present invention, respectively. Moreover, also the other features of such muteins can be as described herein.

[00140] Preferably, the medicament is a medicament capable of preventing and/or treating a disease, preferably a Interleukin 12-mediated disease and/or a disease selected from the group consisting of an infectious disease, an autoimmune disease, cancer, a transplantation-related disease such as Graft-versus-Host-disease, a chronic inflammatory disease such as chronic inflammatory bowel disease, an acute inflammatory disease, sepsis, septic shock, diabetes or asthma.

[00141] The present invention also relates to a mutein according to the present invention for use in the treatment of a disease, wherein the disease is preferably a disease selected from the group consisting of an infectious disease, an autoimmune disease, cancer, a transplantation-related disease such as Graft-versus-Host-

disease, a chronic inflammatory disease such as chronic inflammatory bowel disease, an acute inflammatory disease, sepsis, septic shock, diabetes or asthma. Thus, a hIL-12 α , hIL-12 β and/or hIL-12 mutein according to the present invention are disclosed herein for use in the treatment of a disease. As regards the nature of said mutein of the α -subunit of human Interleukin 12, of the β -subunit of human Interleukin 12, and/or of the human Interleukin 12, the same applies as it has been described herein in connection with the hIL-12 α , hIL-12 β and hIL-12 muteins according to the present invention, respectively. Moreover, also the other features of such muteins can be as described herein.

[00142] The present invention also relates to a method of treating an Interleukin 12-mediated disease in a mammal, preferably a human, wherein the disease is preferably a disease selected from the group consisting of an infectious disease, an autoimmune disease, cancer, a transplantation-related disease such as Graft-versus-Host-disease, a chronic inflammatory disease, such as chronic inflammatory bowel disease, an acute inflammatory disease, sepsis, septic shock, diabetes or asthma, comprising the step of administering a composition comprising a mutein according to the present invention and/or a pharmaceutical composition according to the present invention and/or an immune modulator according to the present invention to said mammal in need thereof. Thus, a hIL-12 α , hIL-12 β and/or hIL-12 mutein according to the present invention, optionally comprised in a pharmaceutical composition and/or an immune modulator according to the present invention, are disclosed herein for use in the treatment of a disease. As regards the nature of said mutein of the α -subunit of human Interleukin 12, of the β -subunit of human Interleukin 12, and/or of the human Interleukin 12, pharmaceutical composition and/or immune modulator, the same applies as it has been described herein in connection with the hIL-12 α , hIL-12 β and hIL-12 muteins according to the present invention, pharmaceutical composition according to the present invention and/or immune modulator according to the present invention, respectively. Moreover, also the other features of such muteins, pharmaceutical compositions and/or immune modulators can be as described herein.

[00143] As regards possible routes of administration, it is envisioned herein that a composition comprising a mutein, a pharmaceutical composition and/or an immune

modulator according to the present invention may be administered via a wide range of classes of forms of administration known to the skilled person, such as (needle) injection, by means of an inhalator, or in form of a cream, foam, gel, lotion and/or ointment. Dose and duration of action have to be deliberately adjusted in each case.

[00144] Production of hIL-12 muteins

[00145] The present invention also relates to a method for producing a mutein according to the present invention as described herein, comprising the steps of:

- (a) introducing into a nucleic acid molecule encoding a polypeptide, said polypeptide being
 - (i) the human Interleukin 12 α -subunit polypeptide (SEQ ID NO: 1), or
 - (ii) a polypeptide comprising at least 90% sequence identity to the human Interleukin 12 α -subunit polypeptide (SEQ ID NO: 1), or
 - (iii) the human Interleukin 12 β -subunit polypeptide (SEQ ID NO: 2), or
 - (iv) a polypeptide comprising at least 90% sequence identity to the human Interleukin 12 β -subunit polypeptide (SEQ ID NO: 2), or
 - (v) the human Interleukin 12 polypeptide comprising an α -subunit (p35), preferably of (i) or (ii), and a β -subunit (p40), preferably of (iii) or (iv), a nucleotide sequence mutating at least one amino acid residue(s) of said polypeptide in case of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 and/or in case of said 12 β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312, and
- (b) introducing the obtained nucleic acid molecule for expression into a host cell or into a cell extract or into a cell lysate.

[00146] As regards the method according to the present invention, it is preferred that, in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of said α -subunit of hIL-12 selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189.

[00147] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a

nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of said α -subunit of hIL-12 selected from the group consisting of sequence positions 70, 71, 72, 185, and 187.

[00148] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating at least one of the amino acid residues of said α -subunit of hIL-12 selected from the group consisting of sequence positions 70, 71, 72, and 187.

[00149] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating i) at least one of the amino acid residue(s) of the α -subunit of hIL-12 selected from the group consisting of sequence positions 70 to 72, and/or ii) the amino acid residue of the α -subunit of hIL-12 at sequence position 187.

[00150] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing i) at least one of the amino acid residue(s) of the α -subunit of hIL-12 selected from the group consisting of sequence positions 70 to 72 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A), and/or ii) the amino acid residue of the α -subunit of hIL-12 at sequence position 187 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

[00151] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of hIL-12 at sequence position 70.

[00152] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit

of hIL-12 at sequence position 70 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

[00153] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of hIL-12 at sequence position 71.

[00154] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of hIL-12 at sequence position 71 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

[00155] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of hIL-12 at sequence position 72.

[00156] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of hIL-12 at sequence position 72 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

[00157] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of hIL-12 at sequence position 185.

[00158] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of hIL-12 at sequence position 185 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

[00159] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of hIL-12 at sequence position 187.

[00160] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of hIL-12 at sequence position 187 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

[00161] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of hIL-12 at sequence position 189.

[00162] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of hIL-12 at sequence position 189 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

[00163] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v) a nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312.

[00164] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 38, 39, 67, 103, 106, 115, 122, 123, 221, 232, and 312.

[00165] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a

nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 67, 115, 122, 123, 221, 232, and 312.

[00166] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating

- i) at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 103, 232, and 312, and/or
- ii) the amino acid residue(s) of the β -subunit of hIL-12 at sequence position(s) 122 and/or 123, and/or
- iii) at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221.

[00167] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing

- i) at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 103, 232, and 312 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G),
- ii) the amino acid residue(s) of the β -subunit of hIL-12 at sequence position(s) 122 and/or 123 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or
- iii) at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00168] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating

- i) at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 103, 232, and 312,
- ii) the amino acid residue(s) of the β -subunit of hIL-12 at sequence position(s) 122 and/or 123, and/or
- iii) at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221.

[00169] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing

- i) at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 103, 232, and 312 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G),
- ii) the amino acid residue(s) of the β -subunit of hIL-12 at sequence position(s) 122 and/or 123 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or
- iii) at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00170] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating

- i) the amino acid residue(s) of the β -subunit of hIL-12 at sequence position(s) 232 and/or 312,

- ii) the amino acid residue(s) of the β -subunit of hIL-12 at sequence position(s) 122 and/or 123, and/or
- iii) at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 67, 115, and 221.

[00171] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing

- i) the amino acid residue(s) of the β -subunit of hIL-12 at sequence position(s) 232 and/or 312 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G),
- ii) the amino acid residue(s) of the β -subunit of hIL-12 at sequence position(s) 122 and/or 123 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or
- iii) at least one of the amino acid residue(s) of the β -subunit of hIL-12 selected from the group consisting of sequence positions 67, 115, and 221 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00172] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 103.

[00173] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 103 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G).

[00174] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a

nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 232.

[00175] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 232 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G).

[00176] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 312.

[00177] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 312 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G).

[00178] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 122.

[00179] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 122 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00180] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 123.

[00181] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 123 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00182] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 82.

[00183] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 82 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00184] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 37.

[00185] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 37 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00186] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 39.

[00187] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit

of hIL-12 at sequence position 39 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00188] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 106.

[00189] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 106 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00190] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 81.

[00191] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 81 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00192] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 115.

[00193] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 115 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00194] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 67.

[00195] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 67 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00196] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 216.

[00197] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 216 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00198] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 38.

[00199] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 38 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00200] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a

nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of hIL-12 at sequence position 221.

[00201] According to one embodiment of the present invention, it is preferred that in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of hIL-12 at sequence position 221 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

[00202] Sequences, as used herein, are depicted in **Table 1** below.

SEQ ID NO:	Subunit or respective mutein	Sequence
1	hIL-12 α	MCPARSLLLVATLVLLDHLSLARNLPVATPDPGMF PCLHHSQNLLRAVSNNMLQKARQTLEFYPTSEEID HEDITKDKTSTVEACLPLELTKNESCLNSRETSFIT NGSCLASRKTSFMMALCLSSIEDLKMYQVEFKT MNAKLLMDPKRQIFLDQNMMLAVIDELMQALNFNSE TVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRV MSYLNAS
2	hIL-12 β	MCHQQLVISWFSVLFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGR FTCWMLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLAERVRGDNKEYEYSVEQCQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
3	hIL-12 β^{E67A}	MCHQQLVISWFSVLFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSAVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGR FTCWMLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLAERVRGDNKEYEYSVEQCQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR

SEQ ID NO:	Subunit or respective mutein	Sequence
		YYSSSWSEWASVPCS
4	hIL-12 β ^{L103G}	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLGL HKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGR FTCWLLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLAERVRGDNKEYEYSVECQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
5	hIL-12 β ^{D115A}	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTAILKDQKEPKNKTFRLCEAKNYSGR FTCWLLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLAERVRGDNKEYEYSVECQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
6	hIL-12 β ^{H216A}	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGR FTCWLLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLAERVRGDNKEYEYSVECQEDSACPAAEESLPI EVMVDAVAKLKYENYTSSFFIRDIIKPDPPKNLQLK

SEQ ID NO:	Subunit or respective mutein	Sequence
		PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
7	hIL-12 β ^{E221A}	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLAERVRGDNKEYEYSVECQEDSACPAAEESLPI EVMVDAVHKLKYANYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
8	hIL-12 β ^{I232G}	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLAERVRGDNKEYEYSVECQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDGIKPDPPKNLQL KPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQV QGKSKREKKDRVFTDKTSATVICRKNASISVRAQD RYYSSSWSEWASVPCS
9	hIL-12 β ^{D312G}	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA

SEQ ID NO:	Subunit or respective mutein	Sequence
		TLSAERVVRGDNKEYEYSVEQCQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQGR YYSSSWSEWASVPCS
10	hIL-12 β ^{E122A}	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKAPKNKTFLRCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLSAERVVRGDNKEYEYSVEQCQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
11	hIL-12 β ^{P123A}	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEAKNKTFLRCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLSAERVVRGDNKEYEYSVEQCQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
12	hIL-12 β ^{F82A}	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEAGDAGQYTCHKGGEVLSHSLLLL

SEQ ID NO:	Subunit or respective mutein	Sequence
		HKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA Tlsaervrgdnkeyeysvecqedsacpaaeeslpi EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
13	hIL-12 β ^{W37A}	MCHQQLVISWFSVLFLASPLVAIWELKKDVYVVEL DAYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA Tlsaervrgdnkeyeysvecqedsacpaaeeslpi EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
14	hIL-12 β ^{P39A}	MCHQQLVISWFSVLFLASPLVAIWELKKDVYVVEL DWYADAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA Tlsaervrgdnkeyeysvecqedsacpaaeeslpi EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS

SEQ ID NO:	Subunit or respective mutein	Sequence
15	hIL-12 β ^{K106A}	MCHQQLVISWFSVLFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HAKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLAERVRGDNKEYEYSVEQCQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
16	hIL-12 β ^{E81A}	MCHQQLVISWFSVLFLASPLVAIWELKKDVYVVEL DWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKAFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLAERVRGDNKEYEYSVEQCQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
17	hIL-12 β ^{Y38A}	MCHQQLVISWFSVLFLASPLVAIWELKKDVYVVEL DWAPDAPGEMVVLTCDTPEEDGITWTLDQSSEVL GSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL HKKEDGIWSTDILKDQKEPKNKTFRLCEAKNYSGR FTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAA TLAERVRGDNKEYEYSVEQCQEDSACPAAEESLPI EVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLK PLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ

SEQ ID NO:	Subunit or respective mutein	Sequence
		GKSKREKKDRVFTDKTSATVICRKNASISVRAQDR YYSSSWSEWASVPCS
18	hIL-12 α ^{D70A}	MCPARSLLLVATLVLLDHLSLARNLPVATPDPGMF PCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIA HEDITKDKTSTVEACLPLELTKNESCLNSRETSFIT NGSCLASRKTSFMMALCLSSIEDLKMYQVEFKT MNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSE TVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRV MSYLNAS
19	hIL-12 α ^{H71A}	MCPARSLLLVATLVLLDHLSLARNLPVATPDPGMF PCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEID AEDITKDKTSTVEACLPLELTKNESCLNSRETSFIT NGSCLASRKTSFMMALCLSSIEDLKMYQVEFKT MNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSE TVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRV MSYLNAS
20	hIL-12 α ^{E72A}	MCPARSLLLVATLVLLDHLSLARNLPVATPDPGMF PCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEID HADITKDKTSTVEACLPLELTKNESCLNSRETSFIT NGSCLASRKTSFMMALCLSSIEDLKMYQVEFKT MNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSE TVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRV MSYLNAS
21	hIL-12 α ^{E185A}	MCPARSLLLVATLVLLDHLSLARNLPVATPDPGMF PCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEID HEDITKDKTSTVEACLPLELTKNESCLNSRETSFIT NGSCLASRKTSFMMALCLSSIEDLKMYQVEFKT

SEQ ID NO:	Subunit or respective mutein	Sequence
		MNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSE TVPQKSSLEAPDFYKTKIKLCILLHAFRIRAVTIDRV MSYLNAS
22	hIL-12 α ^{D187A}	MCPARSLLL VATLVLLDHLSLARNLPVATPDPGMF PCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEID HEDITKDKTSTVEACLPLELTKNESCLNSRETSFIT NGSCLASRKTSFMMALCLSSIEDLKMYQVEFKT MNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSE TVPQKSSLEEPAFYKTKIKLCILLHAFRIRAVTIDRV MSYLNAS
23	hIL-12 α ^{Y189A}	MCPARSLLL VATLVLLDHLSLARNLPVATPDPGMF PCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEID HEDITKDKTSTVEACLPLELTKNESCLNSRETSFIT NGSCLASRKTSFMMALCLSSIEDLKMYQVEFKT MNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSE TVPQKSSLEEPDFAKTKIKLCILLHAFRIRAVTIDRV MSYLNAS

Examples

[00203] Materials and Methods

[00204] Structural modelling of the human IL-12 (hIL-12) receptor

[00205] The structure of the human IL-12 (hIL-12) receptor was modelled based on the crystal structures for human IL-12 (PDB: 1f45, Yoon *et al.* 2000) and the human IL-23 receptor (PDB: 6wdq, Glassman *et al.* 2021). This procedure was motivated by the similar molecular setup of IL-12 and IL-23 receptor complexes (Y. Bloch *et al.* 2017; Glassman *et al.* 2021). IL-12 and IL-23 both bind to the IL-12R β 1 receptor chain, but IL-12 further assembles with IL-12R β 2, while IL-23 binds to IL-23R to each form a fully functional receptor complex. Missing residues in the hIL-12 structure were incorporated using the MODELLER software (Webb and Sali 2016).

[00206] To build a structural model for the IL-12 : receptor complex, the resulting hIL-12 structure was superposed on hIL-23 in the IL-23 receptor complex. Superpositioning was carried out with PyMOL (The PyMOL Molecular Graphics System Version 2.0 Schrödinger LLC). For IL-12R β 1, the first two, and for IL-12R β 2, the first three extracellular domains were considered for the final model. Note that only one domain is resolved for IL-12R β 1 in the hIL-23 receptor complex. Thus alpha-fold (Jumper *et al.* 2021) was used to build a model for the first two domains of IL-12R β 1. Then the alpha-fold model for IL-12R β 1 was superposed onto the first domain of IL-12R β 1 in the IL-23 receptor complex. The IL-12R β 2 model-structure was superposed onto IL-23R. In combination, the superposed structures yield the final model for the hIL-12 : receptor complex for further analyses.

[00207] Molecular dynamics simulations

[00208] The resulting hIL-12 : receptor complex pdb-structure was processed with the pdb2pqr server (Dolinsky *et al.* 2004) to capture protonation states, and afterwards the simulation system was generated with the xleap-module of the Amber18 package (Case *et al.* 2014). This included definition of disulfide bonds and solvation of the complex with the TIP3P water model (Jorgensen *et al.* 1983) into an octahedral simulation system with a minimum distance of 1 nm between protein and box boundary. Sodium counterions were added to electrostatically minimize the

system and a final salt concentration of 250 mM NaCl was adjusted. The protein was described with the ff14SB force field (Maier *et al.* 2015). Hydrogen-mass repartitioning was activated with the parmed module. The generated simulation system was energy-minimized in two steps. First, the system was minimized in 3,000 cycles (1,500 cycles steepest descent and 1,500 conjugate gradient method) employing harmonic position restraints with a force constant of 10.0 kcal/mol/Å². Second, the system was minimized in 20,000 cycles (10,000 cycles steepest descent and 10,000 conjugate gradient method) in absence of restraining forces.

[00209] Subsequently, the system was equilibrated in 8 consecutive molecular dynamics (MD) simulations. In the first three simulations, the system was equilibrated from 100 K to 300 K (Berendsen-thermostat) in steps of 100 K, employing positional restraints with a force constant of 25.0 kcal/mol/Å² on the protein atoms. The restraints were gradually removed in the subsequent five simulations and a Berendsen-barostat with a reference pressure of 1 bar was switched on. The whole equilibration phase covers a simulation-time of 2.1 ns. The output structure of the equilibration phase was used as starting structure for the production run, which covers a simulation time of 100 ns. In the production run, the atoms were free of external restraints and data was written out every 5,000 steps.

[00210] Expression and purification of IL-12-muteins

[00211] IL-12-muteins were produced using the ExpiCHO Expression System (Gibco) according to the manufacturer's protocol. Subunit sequences for IL-12α and IL-12β were optimized for *C. cricetus* (GeneArt, ThermoFisher), and expressed from pcDNA 3.4 TOPO vectors (Thermo Fisher). IL-12α was C-terminally His-tagged, connected with a linker containing a TEV cleavage site, and amino acid exchanges in IL-12β were generated by site-directed mutagenesis PCR. Transient transfections in ExpiCHO cells were conducted with a subunit ratio of 1:1 for 5 days at 32 °C. After expression, the medium was supplemented with SigmaFAST protease inhibitor (Sigma-Aldrich), centrifuged (5,000 g, 30 min, 4 °C) and applied to a HisTrap HP column (Cytiva) in phosphate-buffered saline (PBS) buffer, pH 7.4. Elution was performed with an imidazole concentration gradient from 0 mM to 500 mM in PBS. His-tag cleavage was performed by TEV protease (containing a His-tag itself) overnight at 4 °C and followed by a subsequent HisTrap HP column in PBS buffer. A

final purification step was performed by size exclusion chromatography using a HiLoad 26/600 Superdex 200 pg column (GE Healthcare) in PBS buffer, pH 7.4.

[00212] Purity and redox status were assessed by 12% SDS-PAGE, supplementing proteins with 0.2 volumes 5x Laemmli buffer with 10% β -mercaptoethanol (β -Me, reducing) or 100 mM *N*-ethylmaleimide (NEM, non-reducing) instead, and Coomassie staining of the gel.

[00213] Spectroscopic analyses of IL-12-muteins

[00214] Far-UV circular dichroism (CD) spectra were measured with Jasco J-1500 spectropolarimeter at 25 °C in a 0.2 mm quartz cuvette with a protein concentration of 0.4 mg/ml in PBS, pH 7.4. Spectra were recorded 10 times, averaged and buffer-corrected.

[00215] Functional analyses of IL-12-muteins

[00216] Functionality of IL-12 heterodimers was assessed in different immune cells and with a receptor dimerization assay:

[00217] First, STAT4 phosphorylation in the human NK-92 cell line (ATCC[®] CRL-2407[™]) was monitored. Cells were cultured in Alpha-MEM without ribonucleosides (Gibco) with 1.5 g/l NaHCO₃, 12.5% fetal bovine serum (FBS, Gibco), 12.5% horse serum (Gibco), 0.2 mM myo-inositol, 0.1 mM β -Me, 0.02 mM folic acid, and 100 U/ml IL-2 (Peprotech) in a humidified incubator at 37 °C and 5% CO₂. Before cell stimulation, cells were starved overnight in medium without FBS, horse serum, and IL-2. After seeding cells in a tissue-culture treated 48-well plate with a cell number of 0.5×10^6 /well, stimulation was performed with 10 ng/ μ l or 1 ng/ μ l IL-12 protein for 30 minutes. Stimulation with PBS, pH 7.4 served as negative control. Cells were transferred into Eppendorf reaction tubes, centrifuged (300 g, 5 min, 4 °C) and lysed with RIPA buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% NP40, 0.5% DOC, 0.1% SDS) supplemented with protease (Roche) and phosphatase (Serva) inhibitor for 20 min, 4 °C. After centrifugation (20,000 g, 5 min, 4 °C), lysates were supplemented with 0.2 volumes 5x Laemmli buffer with β -Me and heated to 95 °C for 5 min. For immunoblots, samples were run on 12% SDS-PAGE gels, transferred to PVDF membranes, blocked with 5% (w/v) BSA in TBS with 0.1% Tween-20 and blotted

with α STAT4 (Cell Signaling, #2653S, 1:1,000 in 5% BSA) or α PSTAT4 (Cell Signaling #5267S, 1:1,000 in 5% BSA). Species-specific HRP-conjugated secondary antibodies (Santa Cruz Biotechnology, 1:10,000 in 5% BSA) were used to detect the proteins. Blots were detected using Amersham ECL prime (Cytiva) and a Fusion-FX7.Edge V0.70 imager (Vilber).

[00218] Second, the inventors used the NanoBRET™ assay for analysis of receptor chain dimerization upon cytokine binding. COS-7 cells were seeded in uncoated tissue culture 6-well plates (VWR) and transfected using Metafectene PRO (Biontex) according to manufacturer's protocol. In total, 2 μ g receptor chain DNA (pHTC HaloTag® (HT) CMV-neo IL-12R β 2; pNLF1-C [CMV/Hygro] NanoLuc® luciferase (NL) IL-12R β 1; Promega) were transfected per well with a ratio of 100:1 HT:NL. After 20 h, transfected cells were detached by Accutase® (Sigma-Aldrich) and resuspended in assay medium (DMEM w/o Phenolred, 4 % (v/v) FBS) to 2.2×10^5 cells/ml. After division into two pools, 1 μ l HaloTag® NanoLuc® 618 Ligand (Promega) or 1 μ l DMSO per ml cells was added. 2×10^4 cells/well were seeded into a white bottom 96-well plate and incubated for 20 h. The IL-12 heterodimer variants or PBS were used to stimulate the cells for 30 min at final concentrations of 10–0.01 nM. After addition of the Nano-Glo® substrate, a CLARIOstar® platereader (BMG Labtech) was used for measurement of the bioluminescence resonance energy transfer (BRET) signal. The normalized NanoBRET™ ratios were calculated by dividing blank-corrected acceptor emission (610 nm) by blank-corrected donor luminescence (450–480 nm) and multiplication by 1000. Normalized NanoBRET™ signals were determined averaging the ratios and subtracting the DMSO mean ratio from the experimental mean ratio. Samples are measured in technical triplicates.

[00219] Third, primary human CD8+ T cells and primary human NK cells were used for functional analyses as follows: Human peripheral blood mononuclear cells (PBMCs) were isolated from an in-house blood donor after informed consent by SepMate™-50 tubes (StemCell Technologies) with Pancoll human (Pan-biotech) density gradient centrifugation. Fresh blood was diluted with Roswell Park Memorial Institute Medium 1640 (RPMI 1640, Gibco) containing 1% penicillin/streptomycin (Gibco). The isolated cells were stored frozen at -80 °C in freezing media containing 90% fetal calve serum (FCS, Gibco) with 10% DMSO (Sigma). Frozen cells were

thawed and rested overnight in RPMI 1640 (Gibco) containing 10% FCS (Gibco), 1% sodium pyruvate (100 mM, Gibco), 1% L-glutamin (200 mM, Gibco), 1x MEM non-essential amino acids (100x, Gibco), 1% penicillin/streptomycin (10000 IU/ml, Gibco), 0.01 M HEPES pH 7.4 (1 M, Gibco), 0.015 mg/ml Gentamicin (40 mg/ml, ratiopharm) and in the presence of 20 IU/ml IL-2 (Clingen). CD8⁺ T cells and NK cells were then isolated from PBMCs by magnetic-activated cell sorting (MACS) separation using CD8⁺ T cell isolation kit (Miltenyi) and NK cell isolation kit with LS magnetic columns (Miltenyi Biotec).

[00220] For the IFN γ induction assay, CD8⁺ T cells were seeded at 80,000 cells/well in a 96-well flat bottom plate coated with 2 μ g/ml anti-human CD3 antibody (eBioscience) and 0.5 μ g/ml anti-human CD28 antibody (eBioscience). Cells were stimulated with 100 IU/mL IL-2 with or without addition of the respective IL-12-mutein. NK cells were seeded at 40,000 cells/well in a 96-well flat bottom plate, stimulated with 50 ng/mL IL-18 (R&D System) with or without addition of the respective IL-12-mutein. In each case, the supernatant was analyzed after 48 h using the IFN γ human uncoated ELISA kit (ThermoFisher) and measured with an INFINITE F PLEX plate reader (TECAN).

[00221] Quantification and statistical analyses

[00222] Immunoblots were quantified using the Bio-1D software (Vilber) and statistical analyses were performed with Prism (GraphPad software). Statistical significance was analyzed with statistical tests as stated in the description of the respective experiment.

[00223] Results**[00224] Table 2:** Tabular overview of mutated sequence positions.

Subunit	Position	Amino acid in SEQ ID sequence ("native")	Mutated amino acid	Preferred mutated amino acid leading to an amino acid sequence according to SEQ ID NOs in brackets
IL-12α (SEQ ID NO: 1)	70	D	Any amino acid other native one	A (SEQ ID NO: 18)
	71	H		A (SEQ ID NO: 19)
	72	E		A (SEQ ID NO: 20)
	185	E		A (SEQ ID NO: 21)
	187	D		A (SEQ ID NO: 22)
	189	Y		A (SEQ ID NO: 23)
IL-12β (SEQ ID NO: 2)	37	W	Any amino acid other native one	A (SEQ ID NO: 13)
	38	Y		A (SEQ ID NO: 17)
	39	P		A (SEQ ID NO: 14)
	67	E		A (SEQ ID NO: 3)
	81	E		A (SEQ ID NO: 16)
	82	F		A (SEQ ID NO: 12)
	103	L		G (SEQ ID NO: 4)
	106	K		A (SEQ ID NO: 15)
	115	D		A (SEQ ID NO: 5)
	122	E		A (SEQ ID NO: 10)
	123	P		A (SEQ ID NO: 11)
	216	H		A (SEQ ID NO: 6)
	221	E		A (SEQ ID NO: 7)
	232	I		G (SEQ ID NO: 8)
	312	D		G (SEQ ID NO: 9)

[00225] A high-resolution model of the human IL-12 : receptor complex

[00226] High-resolution structures of cytokine : receptor complexes allow to rationally engineer binding interfaces for desired properties. Thus far, the best available cryo-EM structure of the human IL-12 : receptor complex has a resolution of ~10 Å (Glassman *et al.* 2021), limiting design efforts that require atomistic insights. In contrast, for key parts of the IL-23 : receptor complex, high resolution

crystallographic structures have been available (Y. Bloch *et al.* 2017; Glassman *et al.* 2021). Since IL-12 and IL-23 are structurally related and share the IL-12R β 1 receptor chain, this was used in an approach to obtain a high-resolution computational model of the human IL-12 : receptor complex (see **Fig. 1A**), where the IL-23 : receptor complex served as a basis (for details see Materials and Methods).

[00227] To assess stability of the models, root mean squared deviations (RMSD) were first computed for the IL-12 molecule alone, the IL-12 : IL12R β 1-complex and the entire IL-12 : IL12R β 1/IL12R β 2-complex in reference to the starting structures as a function of time (see **Fig. 1B**). In particular, for IL-12 alone and in complex with IL-12R β 1, rapid convergence on the timescale of the simulations was achieved. Thus, the last 10 ns of the simulation were considered to represent reliable data to compute observables for the system, and the molecular dynamics (MD) data supported the validity of our structural model.

[00228] Computational design of IL-12-variants that reduce receptor affinity

[00229] Structural and biochemical data suggest that binding of the IL-12 β -subunit to IL-12R β 1 is the key for inducing IL-12 receptor heterodimerization and downstream signaling (Georgy *et al.* 2021; Glassman *et al.* 2021). Furthermore, NK cells have lower levels of cell-surface IL-12R β 1 than activated T cells (Glassman *et al.* 2021). Engineering IL-12 β for reduced IL-12R β 1 affinity thus provides a route towards more T cell-selective IL-12-muteins. Thus, the goal of the computational analyses was to identify amino acids in the IL-12 β -subunit that stabilize the IL-12 : IL-12R β 1-complex as a basis for mutation candidates to weaken receptor binding affinity.

[00230] For this purpose, Molecular Mechanics Generalized Born Surface Area (MM/GBSA) calculations were performed for the IL-12 β : IL-12R β 1-complex based on the last 10 ns of the performed molecular dynamics (MD) simulation applying the method of Genheden and Ryde from 2015. This method facilitated the computation of ligand binding affinities. Interactions between the binding partners were evaluated according to the force field parameters. The solvent was described as a continuum with two terms contributing to the binding free energy: nonpolar (to solvate the molecules in absence of electrostatics) and polar free energies (turning on

electrostatics). The Generalized Born (GB) model was selected by setting $igb = 5$, and an implicit salt concentration of 250 mM was adjusted (Onufriev *et al.* 2004). These calculations allowed extraction of the energetic contribution of each amino acid to the complex formation (see **Fig. 2A**). Negative values indicated a stabilizing contribution of the specific amino acid to complex formation and hence, indicated potential mutation candidates. The top ten candidates within IL-12 β obeying these criteria are listed in **Fig. 2B**. In total, six candidates were identified and predicted to attenuate IL-12 α : IL-12R β 2 binding through MM/GBSA calculations (see **Fig. 3A** and **3B**).

[00231] The performed *in silico* screen also suggested further amino acids with a stabilizing role for binding of IL-12 β to IL-12R β 1 (see **Fig. 2A** and **B**). Besides energetic contributions, it was of interest to investigate entropic effects that may influence IL-12 : receptor binding. Ligand : receptor binding generally reduces flexibility and hence entropy of the binding partners. However, individual residues may also become more flexible upon complex formation due to allosteric mechanisms. In addition to this, specific rigid sequences, in particular when juxtaposed to flexible domains, may also be important for complex formation, as they can regulate the global dynamics in a way to optimize compatibility with binding to the receptor. Both scenarios were considered by analyzing the flexibility of all amino acids of the IL-12 β subunit in its unbound as well as in its receptor-bound state. Flexibilities were quantified by computing root mean squared fluctuations (RMSF) in the uncomplexed and receptor-bound form (see **Fig. 2C**).

[00232] For most amino acids, fluctuations were larger in the unbound form compared to the bound form as expected, but a few residues also showed increased fluctuations in complex with the receptor. As two amino acids with larger fluctuations in complex with the receptor compared to the unbound state, E122 and P123 were identified with both amino acids lying between the first and second domain of IL-12 β (see **Fig. 2A**). Hypothetically, substituting E122 by an alanine (A) may decrease the local flexibility and may also diminish the differences in flexibility between receptor bound and unbound form. In this scenario, E122A would entropically destabilize receptor binding. Similarly, mutating P123 by an alanine may lead to structures with

a localized higher helicity possibly attenuating receptor binding. Based on these considerations, E122A and P123A were identified as additional candidates.

[00233] For the second hypothesis, that states that rigid sites juxtaposed to flexible domains may stabilize complex formation, three rigid positions in unbound IL-12 β (low RMSF) juxtaposed to strongly fluctuating sites (high RMSF) were identified. Mutating these amino acids to glycines (G) may increase their flexibility, which may also impact fluctuations of the neighboring domains. Thus, such mutations may alter the dynamics of IL-12 β , and hence also impede receptor binding. The following amino acids within IL-12 β were identified to qualify as candidates to attenuate human IL-12 receptor binding: L103, I232, and D312 (see **Fig. 2C**). It is to be noted that in the selection of such entropic candidates, it was also included as a further condition that their enthalpic contribution to receptor binding was low (see **Fig. 2A**).

[00234] Structure-guided engineering provided IL-12-muteins with reduced NK cell activation while maintaining T cell activation

[00235] The model of the IL-12 : receptor complex provided several candidates for mutation to reduce receptor binding of IL-12. To test these predictions experimentally, wild-type IL-12 and nine of its variants were expressed in CHO cells and purified by metal-affinity and size exclusion chromatography. Each mutein could be purified and formed disulfide-bridged covalent heterodimers as expected for IL-12 (Yoon *et al.* 2000) (see **Fig. 4A**, wherein the amino acid sequence of “IL-12^{wt}” corresponds to the SEQ ID NO: 2, wherein the amino acid sequence of “IL-12^{E67A}” corresponds to the SEQ ID NO: 3, wherein the amino acid sequence of “IL-12^{D115A}” corresponds to the SEQ ID NO: 5, wherein the amino acid sequence of “IL-12^{H216A}” corresponds to the SEQ ID NO: 6, wherein the amino acid sequence of “IL-12^{E221A}” corresponds to the SEQ ID NO: 7, wherein the amino acid sequence of “IL-12^{L103G}” corresponds to the SEQ ID NO: 4, wherein the amino acid sequence of “IL-12^{E122A}” corresponds to the SEQ ID NO: 10, wherein the amino acid sequence of “IL-12^{P123A}” corresponds to the SEQ ID NO: 11, wherein the amino acid sequence of “IL-12^{I232G}” corresponds to the SEQ ID NO: 8, and wherein the amino acid sequence of “IL-12^{D312G}” corresponds to the SEQ ID NO: 9). The muteins gave rise to far-UV circular dichroism (CD) spectra highly similar to the wild-type protein (see **Fig. 4B**) indicating that none of the muteins compromised structure formation.

[00236] Using the selected panel of IL-12-muteins, their capability was tested to induce STAT4 phosphorylation in human NK-92 cells. At cytokine concentrations of 10 ng/μl, most muteins showed wild-type-like signaling capabilities in NK cells (see **Fig. 5A** and **5B**). One exception in the investigated muteins was the L103G-mutein (SEQ ID NO: 4), that, even by this single point mutation, reduced signaling in NK cells by approximately 75% (see **Fig. 5A, B**). However, when a lower cytokine concentration of 1 ng/μl were used, two muteins showed slightly higher signaling, whereas two others of the designed muteins showed reduced STAT4 phosphorylation in NK-92 cells. The latter included an enthalpic mutein (E221A, SEQ ID NO: 7) as well as again the entropic mutein L103G (SEQ ID NO: 4) (see **Fig. 5C** and **D**). To verify that changes in receptor binding cause the differences in STAT4 phosphorylation, the inventors used a NanoBRET™ assay to measure IL-12 receptor chain dimerization. In this assay, both variants showed significantly less receptor binding, thus less receptor dimerization, compared to IL-12 wildtype at a stimulation concentration of 1 nM (**Fig. 5E**).

[00237] Based on these findings, L103G (SEQ ID NO: 4) and E221A (SEQ ID NO: 7) were further analyzed for cell type-specific activation of primary human lymphocytes isolated from peripheral blood to identify muteins with the following feature: a decreased production of IFNγ by the NK cells, indicating preferred T cell activation with attenuated NK cell activation that indicates a reduced systemic toxicity *in vivo* compared to wild-type IL-12. To test for said feature, IL-12-mutein concentrations ranging from 0.001 nM to 100 nM were used to activate CD8⁺ T cells and NK cells for 48 h. Depending on the cell type, different additives were used to induce IFNγ release. For CD8⁺ T cells, human anti-CD3, anti-CD28 antibodies, and IL-2 were added to enable a stimulatory signal mediated by the T cell receptor, while NK cells were incubated with additional IL-18 (Malek 2008; Oka *et al.* 2020; Romee *et al.* 2012). The concentration of produced IFNγ in the supernatant was determined by ELISA.

[00238] Both L103G (SEQ ID NO: 4) and E221A (SEQ ID NO: 7) induced IFNγ secretion from CD8⁺ T cells in a concentration dependent manner with significantly reduced secretion activity compared to the wild-type (see **Fig. 6A**). NK cells were significantly less activated compared to the wild-type especially for lower

concentrations of the muteins, with L103G (SEQ ID NO: 4) showing a stronger differentiation than E221A (SEQ ID NO: 7) (see **Fig. 6B**). Additionally, the IL-12 muteins showed substantial differences between the half-maximum effective concentration (EC50) and therefore in the potency on the different cell types (**Fig. 6C**). In general, L103G showed a stronger discrimination between NK cells and CD8+ T cells than E221A, although the magnitude of this effect was donor-dependent (**Fig. 6D**). Together, these data indicate that the tested IL-12 muteins are able to induce cell type-specific IFN γ production as intended.

[00239] These findings indicated that the tested IL-12-muteins were indeed able to induce a cell-type-specific IFN γ production.

[00240] **Conclusion**

[00241] IL-12 is a key cytokine for immunity against intracellular pathogens and a highly promising candidate to reactive immune reactions in the immunosuppressive tumor microenvironment. As such, insights into the structure and dynamics of its receptor binding are of great interest. Using recently published high-resolution structures of IL-23 bound to its receptor (Y. Bloch *et al.* 2017; Glassman *et al.* 2021), a high-resolution structural model of the human IL-12 : receptor complex is provided by the inventors. Using this model combined with computer simulations, residues were identified that may reduce IL-12 : receptor binding. The chosen approach included flexibility analyses and thus entropic contributions to receptor binding, which have not been used thus far to engineer attenuated IL-12-muteins. These muteins may be of interest to provide IL-12 molecules with less systemic toxicity.

The invention is further characterized by the following items:

1. A mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1), wherein at least one of the amino acid residue(s) of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 is/are mutated.
2. The mutein of item 1, wherein said mutein comprises at least 90% sequence identity to the α -subunit of human Interleukin 12 (SEQ ID NO: 1).
3. The mutein of item 1 or item 2, wherein at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70, 71, 72, 185, and 187 is/are mutated.
4. The mutein of any one of the preceding items, wherein at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70, 71, 72, and 187 is/are mutated.
5. The mutein of any one of the preceding items, wherein i) at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70 to 72 is/are mutated, and/or wherein ii) the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187 is mutated.
6. The mutein of any one of the preceding items, wherein i) at least one of the amino acid residues of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70 to 72 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, and/or wherein ii) the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1.
7. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 70 is mutated.
8. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 70 is replaced by

an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A) (SEQ ID NO: 18).

9. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 71 is mutated.
10. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 71 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A) (SEQ ID NO: 19).
11. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 72 is mutated.
12. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 72 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A) (SEQ ID NO: 20).
13. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 185 is mutated.
14. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 185 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A) (SEQ ID NO: 21).
15. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187 is mutated.
16. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A) (SEQ ID NO: 22).
17. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 189 is mutated.

18. The mutein of any one of the preceding items, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 189 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A) (SEQ ID NO: 23).
19. A mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2), wherein at least one of the amino acid residue(s) of said β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312 is/are mutated.
20. The mutein of item 19, wherein said mutein comprises at least 90% sequence identity to the β -subunit of human Interleukin 12 (SEQ ID NO: 2).
21. The mutein of item 19 or item 20, wherein at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 38, 39, 67, 103, 106, 115, 122, 123, 221, 232, and 312 is/are mutated.
22. The mutein of any one of items 19 to 21, wherein at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 67, 115, 122, 123, 221, 232, and 312 is/are mutated.
23. The mutein of any one of items 19 to 22, wherein i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312 is/are mutated, and/or wherein ii) the amino acid residue(s) of the β -subunit at sequence position(s) 122 and/or 123 is/are mutated, and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221 is/are mutated.
24. The mutein of any one of items 19 to 23, wherein i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312 is/are replaced by an amino acid other than the amino acid at the respective sequence position in

SEQ ID NO: 2, preferably by glycine (G), and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or wherein iii) at least one of the amino acid residues of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

25. The mutein of any one of items 19 to 24, wherein i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312 is/are mutated, and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are mutated, and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221 is/are mutated.
26. The mutein of any one of items 19 to 25, wherein i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (G), and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
27. The mutein of any one of items 19 to 26, wherein i) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 232 and/or 312

is/are mutated, and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are mutated, and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 67, 115, and 221 is/are mutated.

28. The mutein of any one of items 19 to 27, wherein i) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 232 and/or 312 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G), and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 67, 115, and 221 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
29. The mutein of any one of items 19 to 28, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 103 is mutated.
30. The mutein of any one of items 19 to 29, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 103 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G) (SEQ ID NO: 4).
31. The mutein of any one of items 19 to 30, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 232 is mutated.
32. The mutein of any one of items 19 to 31, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 232 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G) (SEQ ID NO: 8).

33. The mutein of any one of items 19 to 32, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 312 is mutated.
34. The mutein of any one of items 19 to 33, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 312 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G) (SEQ ID NO: 9).
35. The mutein of any one of items 19 to 34, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 122 is mutated.
36. The mutein of any one of items 19 to 35, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 122 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 10).
37. The mutein of any one of items 19 to 36, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 123 is mutated.
38. The mutein of any one of items 19 to 37, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 123 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 11).
39. The mutein of any one of items 19 to 38, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 82 is mutated.
40. The mutein of any one of items 19 to 39, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 82 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 12).
41. The mutein of any one of items 19 to 40, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 37 is mutated.
42. The mutein of any one of items 19 to 41, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 37 is replaced by an

amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 13).

43. The mutein of any one of items 19 to 42, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 39 is mutated.
44. The mutein of any one of items 19 to 43, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 39 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 14).
45. The mutein of any one of items 19 to 44, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 106 is mutated.
46. The mutein of any one of items 19 to 45, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 106 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 15).
47. The mutein of any one of items 1 to 46, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 81 is mutated.
48. The mutein of any one of items 19 to 47, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 81 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 16).
49. The mutein of any one of items 19 to 48, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 115 is mutated.
50. The mutein of any one of items 19 to 49, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 115 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 5).
51. The mutein of any one of items 19 to 50, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 67 is mutated.

52. The mutein of any one of items 19 to 51, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 67 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 3).
53. The mutein of any one of items 19 to 52, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 216 is mutated.
54. The mutein of any one of items 19 to 53, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 216 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 6).
55. The mutein of any one of items 19 to 54, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 38 is mutated.
56. The mutein of any one of items 19 to 55, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 38 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 17).
57. The mutein of any one of items 19 to 56, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 221 is mutated.
58. The mutein of any one of items 19 to 57, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 221 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 7).
59. A mutein of human Interleukin 12, comprising an α -subunit (p35) and a β -subunit (p40), wherein the α -subunit is a mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1) of any one of items 1 to 18 and/or wherein the β -subunit is a mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2) of any one of items 19 to 58.
60. The mutein of item 59, wherein the α -subunit is a mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1) of any one of items 1 to 18.

61. The mutein of item 59 or item 60, wherein at least one of the amino acid residue(s) of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 is/are mutated.
62. The mutein of any one of items 59 to 61, wherein said α -subunit comprises at least 90% sequence identity to the α -subunit of human Interleukin 12 (SEQ ID NO: 1).
63. The mutein of any one of items 59 to 62, wherein at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70, 71, 72, 185, and 187 is/are mutated.
64. The mutein of any one of items 59 to 63, wherein at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70, 71, 72, and 187 is/are mutated.
65. The mutein of any one of items 59 to 64, wherein i) at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70 to 72 is/are mutated, and/or wherein ii) the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187 is mutated.
66. The mutein of any one of items 59 to 65, wherein i) at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70 to 72 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or wherein ii) the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
67. The mutein of any one of items 59 to 66, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 70 is mutated.
68. The mutein of any one of items 59 to 67, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 70 is replaced by an

amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 18).

69. The mutein of any one of items 59 to 68, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 71 is mutated.
70. The mutein of any one of items 59 to 69, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 71 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 19).
71. The mutein of any one of items 59 to 70, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 72 is mutated.
72. The mutein of any one of items 59 to 71, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 72 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 20).
73. The mutein of any one of items 59 to 72, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 185 is mutated.
74. The mutein of any one of items 59 to 73, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 185 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 21).
75. The mutein of any one of items 59 to 74, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187 is mutated.
76. The mutein of any one of items 59 to 75, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 22).
77. The mutein of any one of items 59 to 76, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 189 is mutated.

78. The mutein of any one of items 59 to 77, wherein the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 189 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 23).
79. The mutein of any one of items 59 to 78, wherein the β -subunit is a mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2) of any one of items 19 to 58.
80. The mutein of any one of items 59 to 79, wherein at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312 is/are mutated.
81. The mutein of any one of items 59 to 80, wherein said β -subunit comprises at least 90% sequence identity to the β -subunit of human Interleukin 12 (SEQ ID NO: 2).
82. The mutein of any one of items 59 to 81, wherein at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 38, 39, 67, 103, 106, 115, 122, 123, 221, 232, and 312 is/are mutated.
83. The mutein of any one of items 59 to 82, wherein at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 67, 115, 122, 123, 221, 232, and 312 is/are mutated.
84. The mutein of any one of items 59 to 83, wherein i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312 is/are mutated, and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are mutated, and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221 is/are mutated.

85. The mutein of any one of items 59 to 84, wherein i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G), and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
86. The mutein of any one of items 59 to 85, wherein i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312 is/are mutated, and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are mutated, and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221 is/are mutated.
87. The mutein of any one of items 59 to 86, wherein i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G), and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221 is/are replaced by an amino acid other than the

amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

88. The mutein of any one of items 59 to 87, wherein i) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 232 and/or 312 is/are mutated, and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are mutated, and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 67, 115, and 221 is/are mutated.
89. The mutein of any one of items 59 to 88, wherein i) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 232 and/or 312 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G), and/or wherein ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or wherein iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 67, 115, and 221 is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
90. The mutein of any one of items 59 to 89, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 103 is mutated.
91. The mutein of any one of items 59 to 90, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 103 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G) (SEQ ID NO: 4).
92. The mutein of any one of items 59 to 91, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 232 is mutated.

93. The mutein of any one of items 59 to 92, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 232 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G) (SEQ ID NO: 8).
94. The mutein of any one of items 59 to 93, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 312 is mutated.
95. The mutein of any one of items 59 to 94, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 312 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G) (SEQ ID NO: 9).
96. The mutein of any one of items 59 to 95, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 122 is mutated.
97. The mutein of any one of items 59 to 96, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 122 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 10).
98. The mutein of any one of items 59 to 97, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 123 is mutated.
99. The mutein of any one of items 59 to 98, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 123 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 11).
100. The mutein of any one of items 59 to 99, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 82 is mutated.
101. The mutein of any one of items 59 to 100, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 82 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 12).

102. The mutein of any one of items 59 to 101, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 37 is mutated.
103. The mutein of any one of items 59 to 102, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 37 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 13).
104. The mutein of any one of items 59 to 103, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 39 is mutated.
105. The mutein of any one of items 59 to 104, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 39 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 14).
106. The mutein of any one of items 59 to 105, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 106 is mutated.
107. The mutein of any one of items 59 to 106, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 106 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 15).
108. The mutein of any one of items 59 to 107, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 81 is mutated.
109. The mutein of any one of items 59 to 108, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 81 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 16).
110. The mutein of any one of items 59 to 109, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 115 is mutated.
111. The mutein of any one of items 59 to 110, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 115 is replaced by

an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 5).

112. The mutein of any one of items 59 to 111, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 67 is mutated.
113. The mutein of any one of items 59 to 112, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 67 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 3).
114. The mutein of any one of items 59 to 113, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 216 is mutated.
115. The mutein of any one of items 59 to 114, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 216 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 6).
116. The mutein of any one of items 59 to 115, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 38 is mutated.
117. The mutein of any one of items 59 to 116, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 38 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 17).
118. The mutein of any one of items 59 to 117, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 221 is mutated.
119. The mutein of any one of items 59 to 118, wherein the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 221 is replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A) (SEQ ID NO: 7).
120. A nucleic acid molecule comprising i) a nucleotide sequence encoding the mutein of human Interleukin 12 of any one of items 59 to 119 or ii) a nucleotide

sequence encoding a) the mutein of the α -subunit of human Interleukin 12 of any one of items 1 to 18 and/or b) the mutein of the β -subunit of human Interleukin 12 of any one of items 19 to 58.

121. The nucleic acid molecule of item 120, wherein the nucleotide sequence encodes a mutein of the α -subunit of human Interleukin 12 comprising at least 90% sequence identity to the α -subunit of human Interleukin 12 (SEQ ID NO: 1).
122. The nucleic acid molecule of item 120 or 121, comprising a nucleotide sequence encoding a mutein of the α -subunit of human Interleukin 12 of any one of SEQ ID NOs: 18 to 24.
123. The nucleic acid molecule of any one of items 120 to 122, wherein the nucleotide sequence encodes a mutein of the β -subunit of human Interleukin 12 comprising at least 90% sequence identity to the β -subunit of human Interleukin 12 (SEQ ID NO: 2).
124. The nucleic acid molecule of any one of items 120 to 123, comprising a nucleotide sequence encoding a mutein of the β -subunit of human Interleukin 12 of any one of SEQ ID NOs: 3 to 17.
125. The nucleic acid molecule of any one of items 120 to 124, wherein the nucleic acid molecule is operably linked to a regulatory sequence to allow expression of the nucleic acid molecule.
126. The nucleic acid molecule of item 125, wherein the regulatory sequence comprises a promoter sequence.
127. A vector comprising the nucleic acid molecule of any one of items 120 to 126.
128. A host cell comprising the nucleic acid molecule of any one of items 120 to 126 and/or the vector of item 127.
129. An immune modulator comprising a mutein of any one of items 1 to 119.

130. A pharmaceutical composition comprising a mutein of any one of items 1 to 119 and/or an immune modulator of item 129, preferably further comprising a pharmaceutically acceptable carrier.
131. Use of a mutein of any one of items 1 to 119 for the manufacture of a medicament for treating a disease in a mammal, preferably a human, wherein the disease is preferably a disease selected from the group consisting of an infectious disease, an autoimmune disease, cancer, a transplantation-related disease such as Graft-versus-Host-disease, a chronic inflammatory disease such as chronic inflammatory bowel disease, an acute inflammatory disease, sepsis, septic shock, diabetes or asthma.
132. The mutein of any one of items 1 to 119 for use as a medicament.
133. The mutein of any one of items 1 to 119 for use in the treatment of a disease, wherein the disease is preferably a disease selected from the group consisting of an infectious disease, an autoimmune disease, cancer, a transplantation-related disease such as Graft-versus-Host-disease, a chronic inflammatory disease such as chronic inflammatory bowel disease, an acute inflammatory disease, sepsis, septic shock, diabetes or asthma.
134. A method of treating an Interleukin 12-mediated disease in a mammal, preferably a human, wherein the disease is preferably a disease selected from the group consisting of an infectious disease, an autoimmune disease, cancer, a transplantation-related disease such as Graft-versus-Host-disease, a chronic inflammatory disease, such as chronic inflammatory bowel disease, an acute inflammatory disease, sepsis, septic shock, diabetes or asthma, comprising the step of administering a composition comprising a mutein of any one of items 1 to 119 and/or the pharmaceutical composition of item 130 and/or the immune modulator of item 129 to said mammal in need thereof.
135. Method for producing a mutein according to any one of items 1 to 119, comprising the steps of:
- (a) introducing into a nucleic acid molecule encoding a polypeptide, said polypeptide being
 - (i) the human Interleukin 12 α -subunit polypeptide (SEQ ID NO: 1), or

- (ii) a polypeptide comprising at least 90% sequence identity to the human Interleukin 12 α -subunit polypeptide (SEQ ID NO: 1), or
 - (iii) the human Interleukin 12 β -subunit polypeptide (SEQ ID NO: 2), or
 - (iv) a polypeptide comprising at least 90% sequence identity to the human Interleukin 12 β -subunit polypeptide (SEQ ID NO: 2), or
 - (v) the human Interleukin 12 polypeptide comprising an α -subunit (p35), preferably of (i) or (ii), and a β -subunit (p40), preferably of (iii) or (iv), a nucleotide sequence mutating at least one amino acid residue(s) of said polypeptide in case of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189 and/or in case of said 12 β -subunit selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312, and
- (b) introducing the obtained nucleic acid molecule for expression into a host cell or into a cell extract or into a cell lysate.

136. The method of item 135, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, 187, and 189.

137. The method of item 135 or item 136, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, 185, and 187.

138. The method of any one of items 135 to 137, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of said α -subunit selected from the group consisting of sequence positions 70, 71, 72, and 187.

139. The method of any one of items 135 to 138, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide

sequence is introduced mutating i) at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70 to 72, and/or ii) the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187.

140. The method of any one of items 135 to 139, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing i) at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70 to 72 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A), and/or ii) the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).
141. The method of any one of items 135 to 140, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 70.
142. The method of any one of items 135 to 141, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 70 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).
143. The method of any one of items 135 to 142, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 71.
144. The method of any one of items 135 to 143, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 71 by an amino acid other than the

amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

145. The method of any one of items 135 to 144, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 72.
146. The method of any one of items 135 to 145, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 72 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).
147. The method of any one of items 135 to 146, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 185.
148. The method of any one of items 135 to 147, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 185 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).
149. The method of any one of items 135 to 148, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187.
150. The method of any one of items 135 to 149, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 187 by an amino acid other than the

amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).

151. The method of any one of items 135 to 150, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 189.
152. The method of any one of items 135 to 151, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (i) or (ii) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the α -subunit of human Interleukin 12 at sequence position 189 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).
153. The method of any one of items 135 to 152, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232, and 312.
154. The method of any one of items 135 to 153, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 38, 39, 67, 103, 106, 115, 122, 123, 221, 232, and 312.
155. The method of any one of items 135 to 154, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 67, 115, 122, 123, 221, 232, and 312.

156. The method of any one of items 135 to 155, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating
- i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312, and/or
 - ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123, and/or
 - iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221.
157. The method of any one of items 135 to 156, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing
- i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G),
 - ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or
 - iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 106, 115, 216, and 221 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
158. The method of any one of items 135 to 157, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating

- i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312,
 - ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123, and/or
 - iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221.
159. The method of any one of items 135 to 158, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing
- i) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232, and 312 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G),
 - ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or
 - iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 38, 39, 67, 106, 115, and 221 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
160. The method of any one of items 135 to 159, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating
- i) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 232 and/or 312,
 - ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123, and/or

- iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 67, 115, and 221.
161. The method of any one of items 135 to 160, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing
- i) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 232 and/or 312 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G),
 - ii) the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A), and/or
 - iii) at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 67, 115, and 221 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
162. The method of any one of items 135 to 161, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 103.
163. The method of any one of items 135 to 162, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 103 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G).
164. The method of any one of items 135 to 163, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide

sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 232.

165. The method of any one of items 135 to 164, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 232 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G).
166. The method of any one of items 135 to 165, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 312.
167. The method of any one of items 135 to 166, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 312 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by glycine (G).
168. The method of any one of items 135 to 167, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 122.
169. The method of any one of items 135 to 168, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 122 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
170. The method of any one of items 135 to 169, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide

sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 123.

171. The method of any one of items 135 to 170, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 123 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
172. The method of any one of items 135 to 171, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 82.
173. The method of any one of items 135 to 172, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 82 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
174. The method of any one of items 135 to 173, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 37.
175. The method of any one of items 135 to 174, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 37 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
176. The method of any one of items 135 to 175, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide

sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 39.

177. The method of any one of items 135 to 176, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 39 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
178. The method of any one of items 135 to 177, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 106.
179. The method of any one of items 135 to 178, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 106 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
180. The method of any one of items 135 to 179, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 81.
181. The method of any one of items 135 to 180, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 81 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
182. The method of any one of items 135 to 181, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide

sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 115.

183. The method of any one of items 135 to 182, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 115 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
184. The method of any one of items 135 to 183, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 67.
185. The method of any one of items 135 to 184, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 67 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
186. The method of any one of items 135 to 185, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 216.
187. The method of any one of items 135 to 186, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 216 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
188. The method of any one of items 135 to 187, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide

sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 38.

189. The method of any one of items 135 to 188, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 38 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).
190. The method of any one of items 135 to 189, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced mutating the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 221.
191. The method of any one of items 135 to 190, wherein in step (a) into the nucleic acid molecule encoding the polypeptide of (iii) or (iv) or (v), a nucleotide sequence is introduced replacing the amino acid residue of the β -subunit of human Interleukin 12 at sequence position 221 by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 2, preferably by alanine (A).

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Claims

1. A mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1), wherein at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70, 71, 72, 185, 187 and 189 is/are mutated.
2. The mutein of claim 1, wherein said mutein comprises at least 90% sequence identity to the α -subunit of human Interleukin 12 (SEQ ID NO: 1).
3. The mutein of claim 1 or 2, wherein at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70, 71, 72, 185, and 187 is/are mutated, preferably wherein at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70, 71, 72 and 187 is/are mutated.
4. The mutein of any one of the preceding claims, wherein at least one of the amino acid residue(s) of the α -subunit of human Interleukin 12 selected from the group consisting of sequence positions 70, 71, 72, 185, 187 and 189, preferably selected from the group consisting of sequence positions 70, 71, 72 and 187, is/are replaced by an amino acid other than the amino acid at the respective sequence position in SEQ ID NO: 1, preferably by alanine (A).
5. A mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2), wherein at least one of the amino acid residue(s) of said β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 37, 38, 39, 67, 81, 82, 103, 106, 115, 122, 123, 216, 221, 232 and 312 is/are mutated.
6. The mutein of claim 5, wherein said mutein comprises at least 90% sequence identity to the β -subunit of human Interleukin 12 (SEQ ID NO: 2).
7. The mutein of claim 5 or claim 6, wherein at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 38, 39, 67, 103, 106, 115, 122, 123, 221, 232,

and 312, preferably selected from the group consisting of sequence positions 67, 115, 122, 123, 221, 232 and 312, is/are mutated.

8. The mutein of any one of claims 5 to 7, wherein at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence positions 103, 232 and 312, preferably at sequence position(s) 232 and/or 312, is/are replaced by an amino acid other than the amino acid at the respective sequence position(s) in SEQ ID NO: 2, preferably is/are replaced by glycine (G).
9. The mutein of any one of claims 5 to 8, wherein the amino acid residue(s) of the β -subunit of human Interleukin 12 at sequence position(s) 122 and/or 123 is/are replaced by an amino acid other than the amino acid at the respective sequence position(s) in SEQ ID NO: 2, preferably is/are replaced by alanine (A).
10. The mutein of any one of claims 5 to 9, wherein at least one of the amino acid residue(s) of the β -subunit of human Interleukin 12 selected from the group consisting of sequence position(s) 37, 38, 39, 67, 81, 82, 106, 115, 216 and 221, preferably selected from the group consisting of sequence position(s) 38, 39, 67, 106, 115 and 221, more preferably selected from the group consisting of sequence positions 67, 115 and 221, is/are replaced by an amino acid other than the amino acid at the respective sequence position(s) in SEQ ID NO: 2, preferably is/are replaced by alanine (A).
11. A mutein of human Interleukin 12, comprising an α -subunit (p35) and a β -subunit (p40), wherein the α -subunit is a mutein of the α -subunit of human Interleukin 12 (SEQ ID NO: 1) of any one of claims 1 to 4, and/or wherein the β -subunit is a mutein of the β -subunit of human Interleukin 12 (SEQ ID NO: 2) of any one of claims 5 to 10.
12. A nucleic acid molecule comprising i) a nucleotide sequence encoding the mutein of human Interleukin 12 of claim 11 or ii) a nucleotide sequence encoding a) the mutein of the α -subunit of human Interleukin 12 of any one of claims 1 to 4, and/or b) the mutein of the β -subunit of human Interleukin 12 of any one of claims 5 to 10, preferably wherein the nucleic acid molecule is

operably linked to a regulatory sequence to allow expression of the nucleic acid molecule, wherein the regulatory sequence preferably comprises a promoter sequence.

13. A pharmaceutical composition comprising a mutein of any one of claims 1 to 11, preferably further comprising a pharmaceutically acceptable carrier.
14. The mutein of any one of claims 1 to 11, for use as a medicament.
15. The mutein of any one of claims 1 to 11, for use in the treatment of a disease, wherein the disease is preferably a disease selected from the group consisting of an infectious disease, an autoimmune disease, cancer, a transplantation-related disease, such as Graft-versus-Host-disease, a chronic inflammatory disease, such as chronic inflammatory bowel disease, an acute inflammatory disease, sepsis, septic shock, diabetes or asthma.

Figures

Figure 1

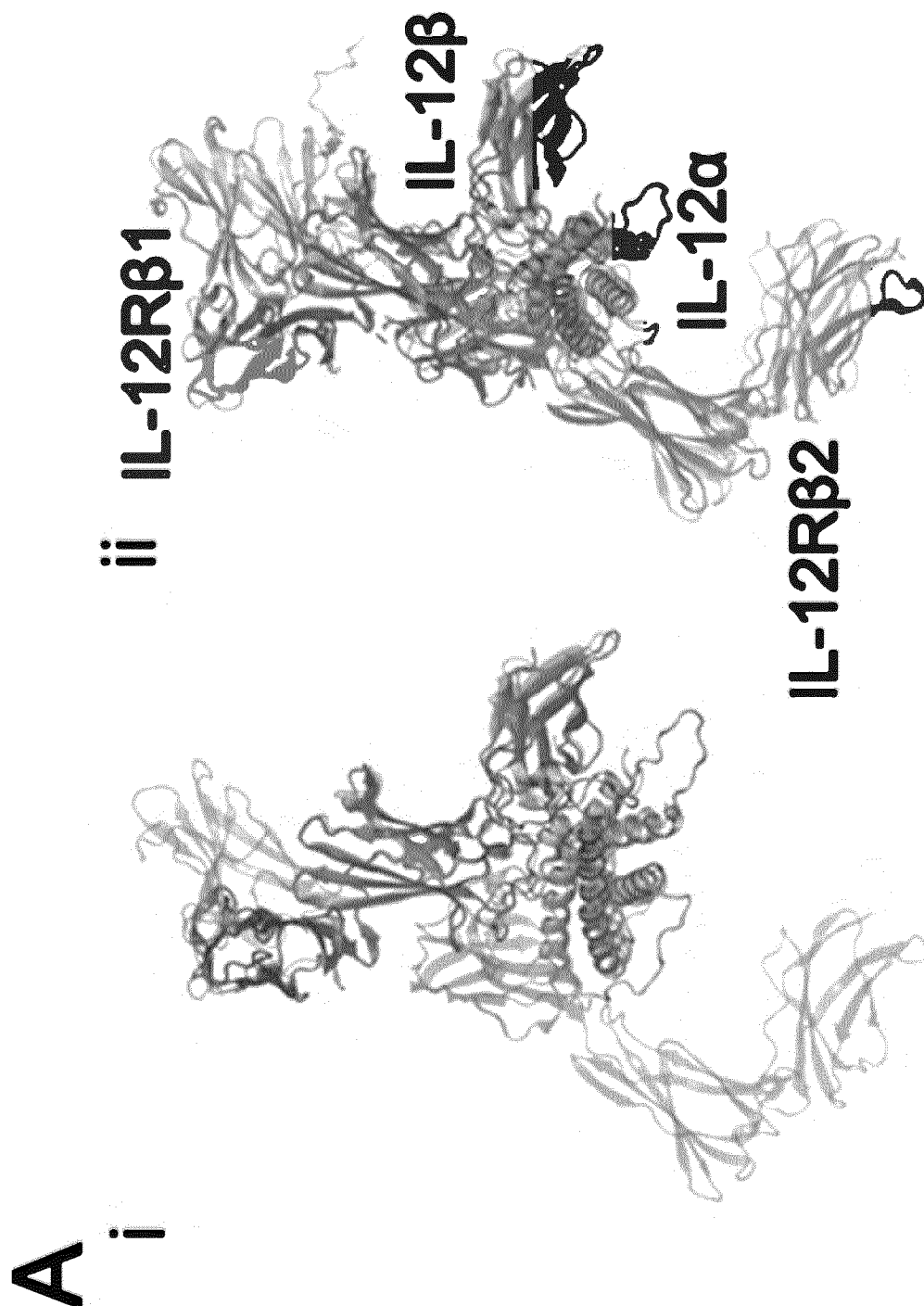


Figure 1 (cont.)

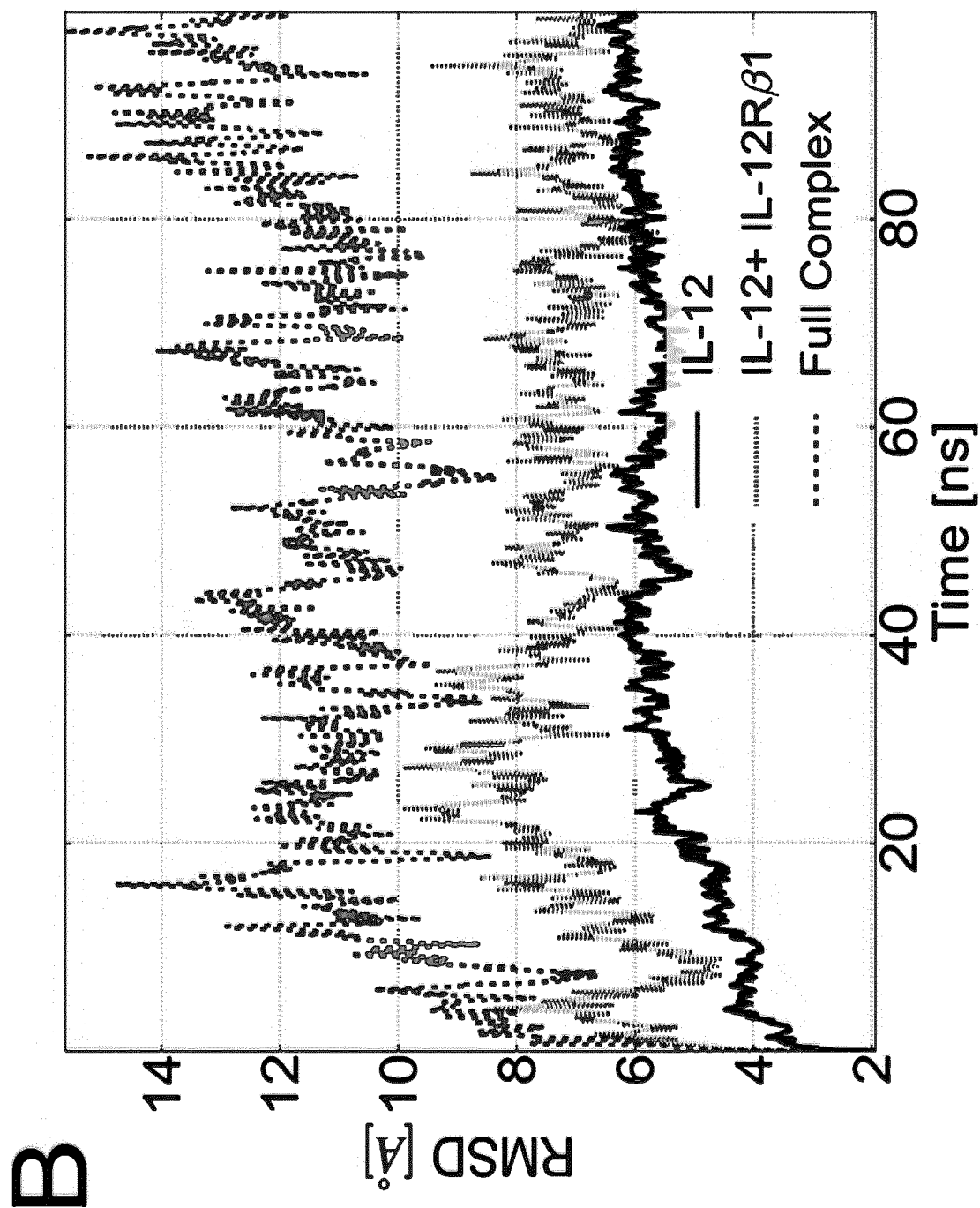


FIGURE 2

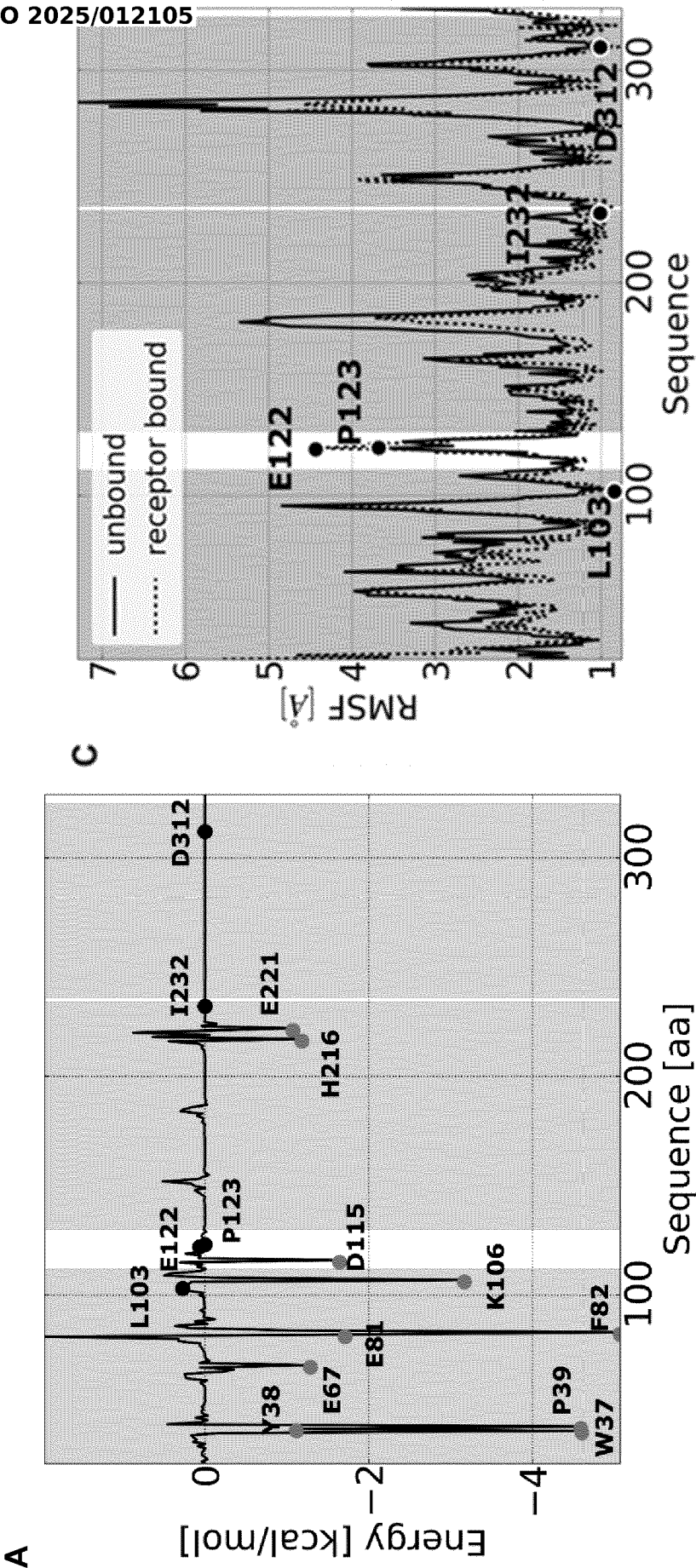
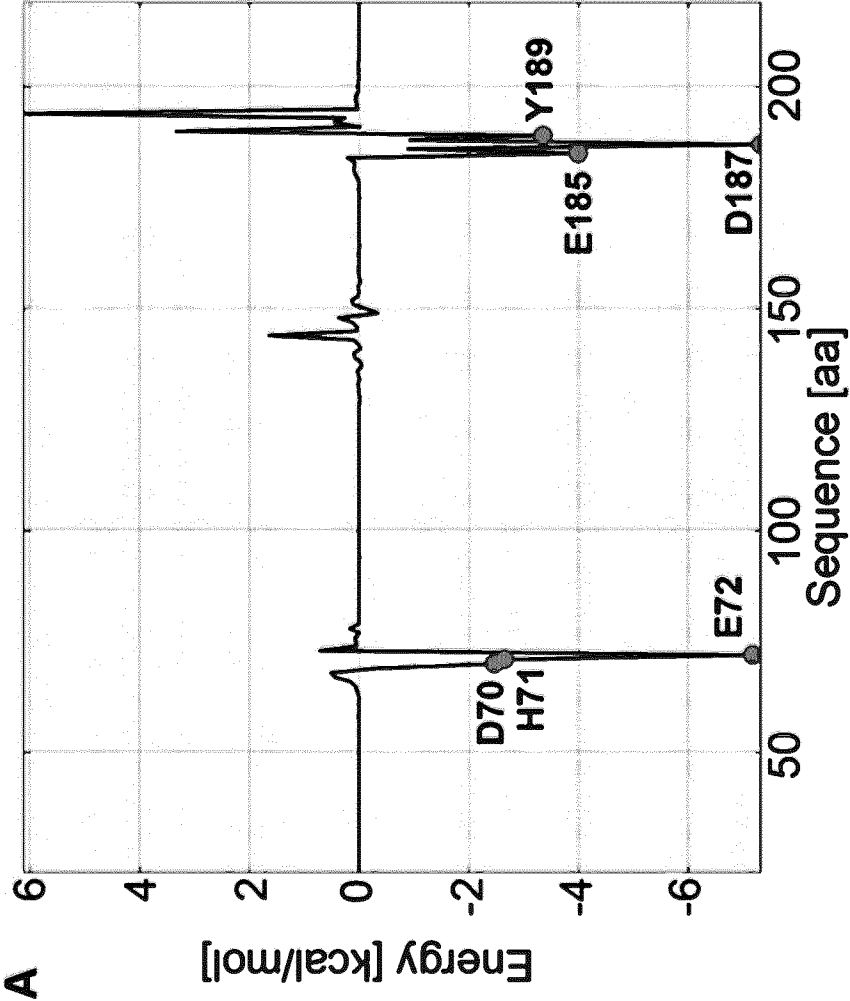


Figure 3



B

residue	D187	E72	E185	Y189 ¹	H71	D70
energy [kcal/mol]	-7.3	-7.2	-4.0	-3.3	-2.6	-2.5

Figure 4

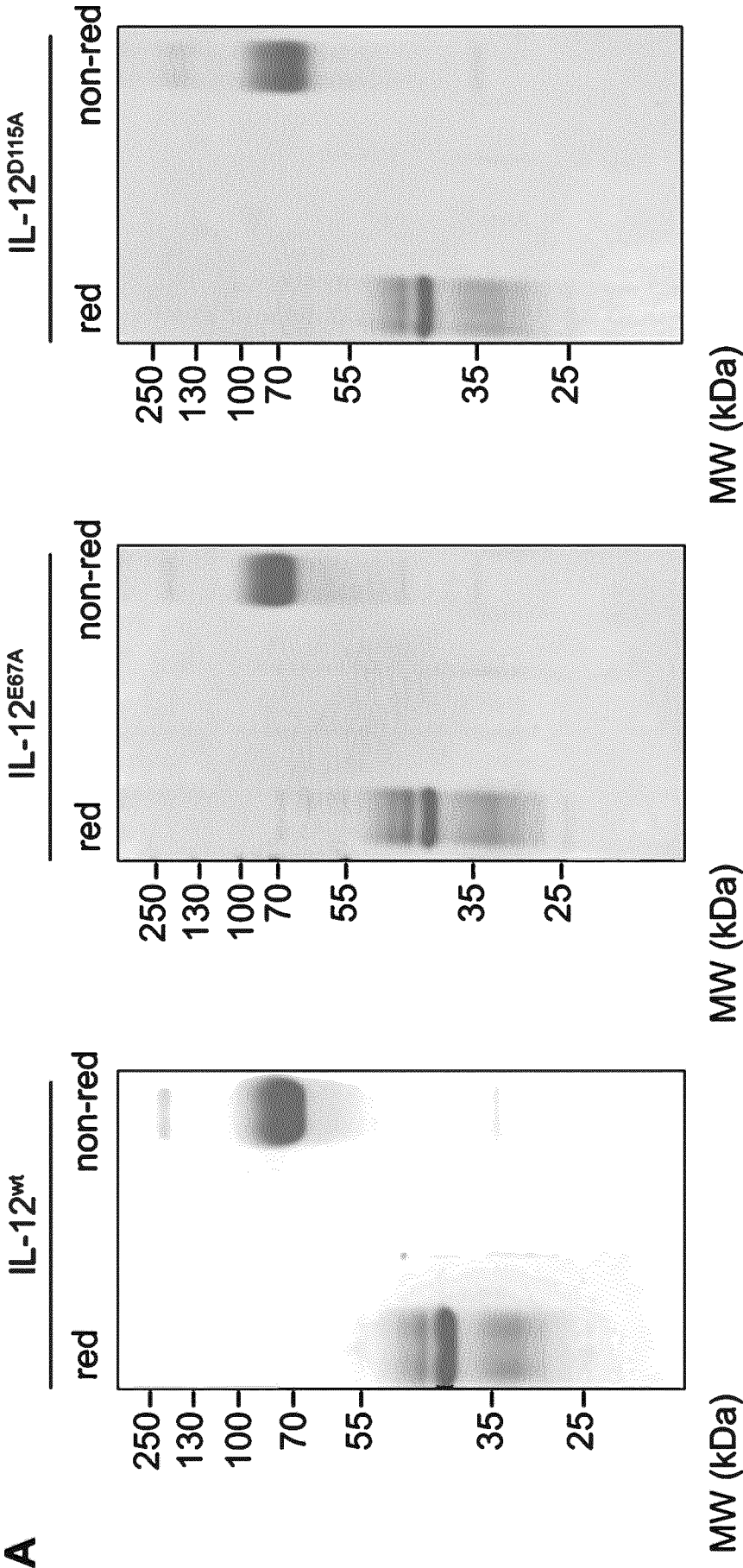


Figure 4A (cont.)

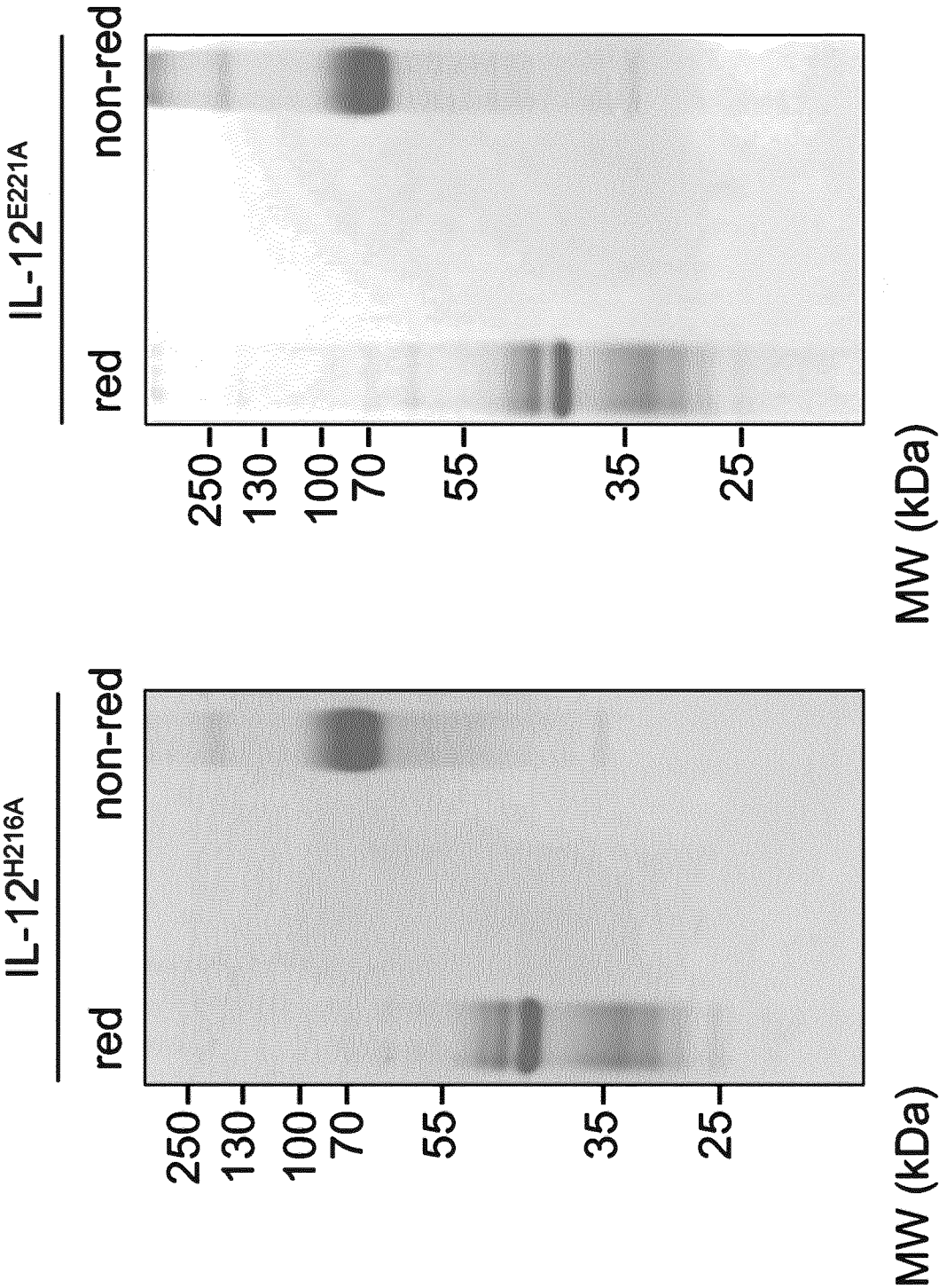


Figure 4A (cont.)

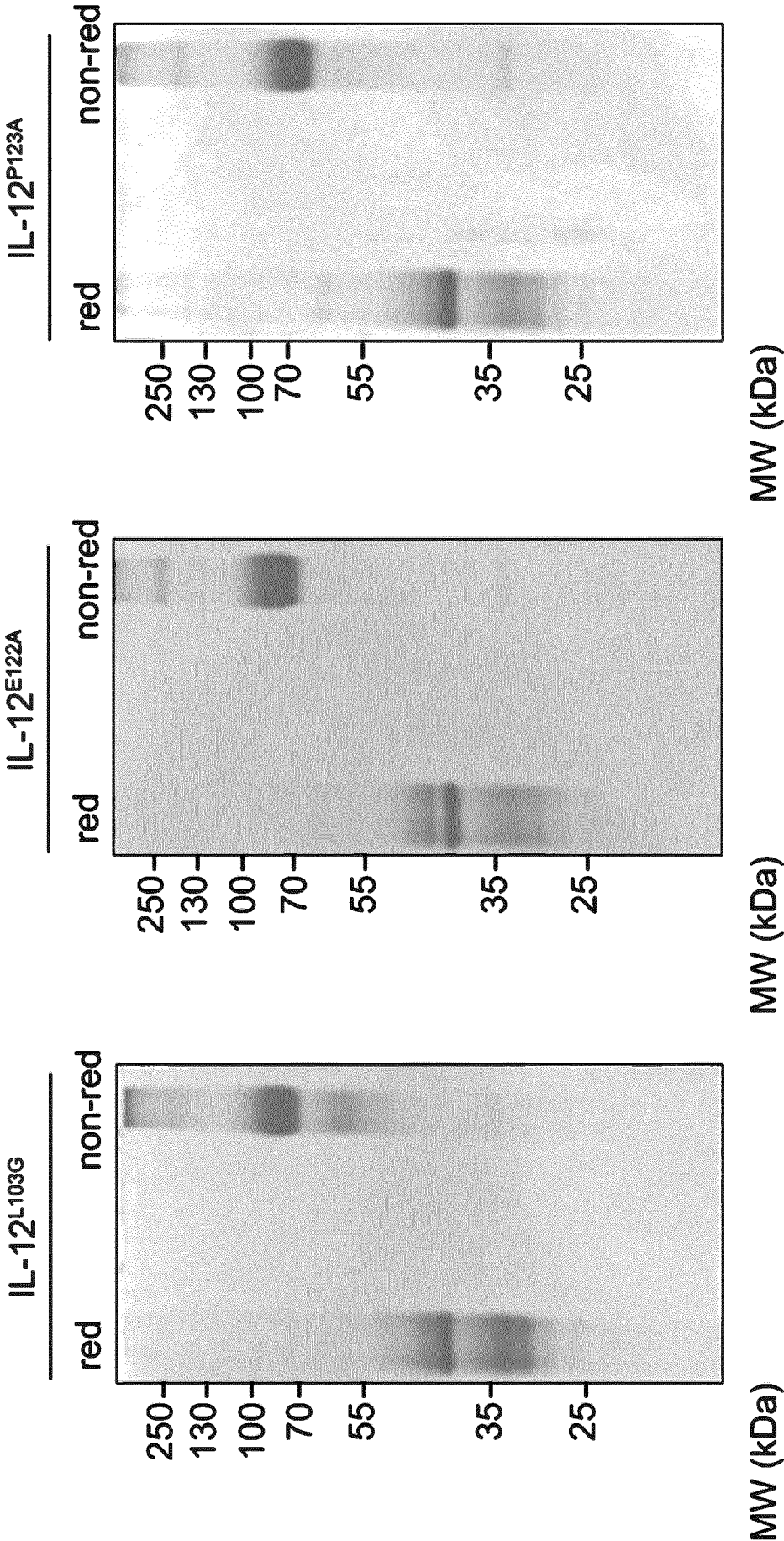


Figure 4A (cont.)

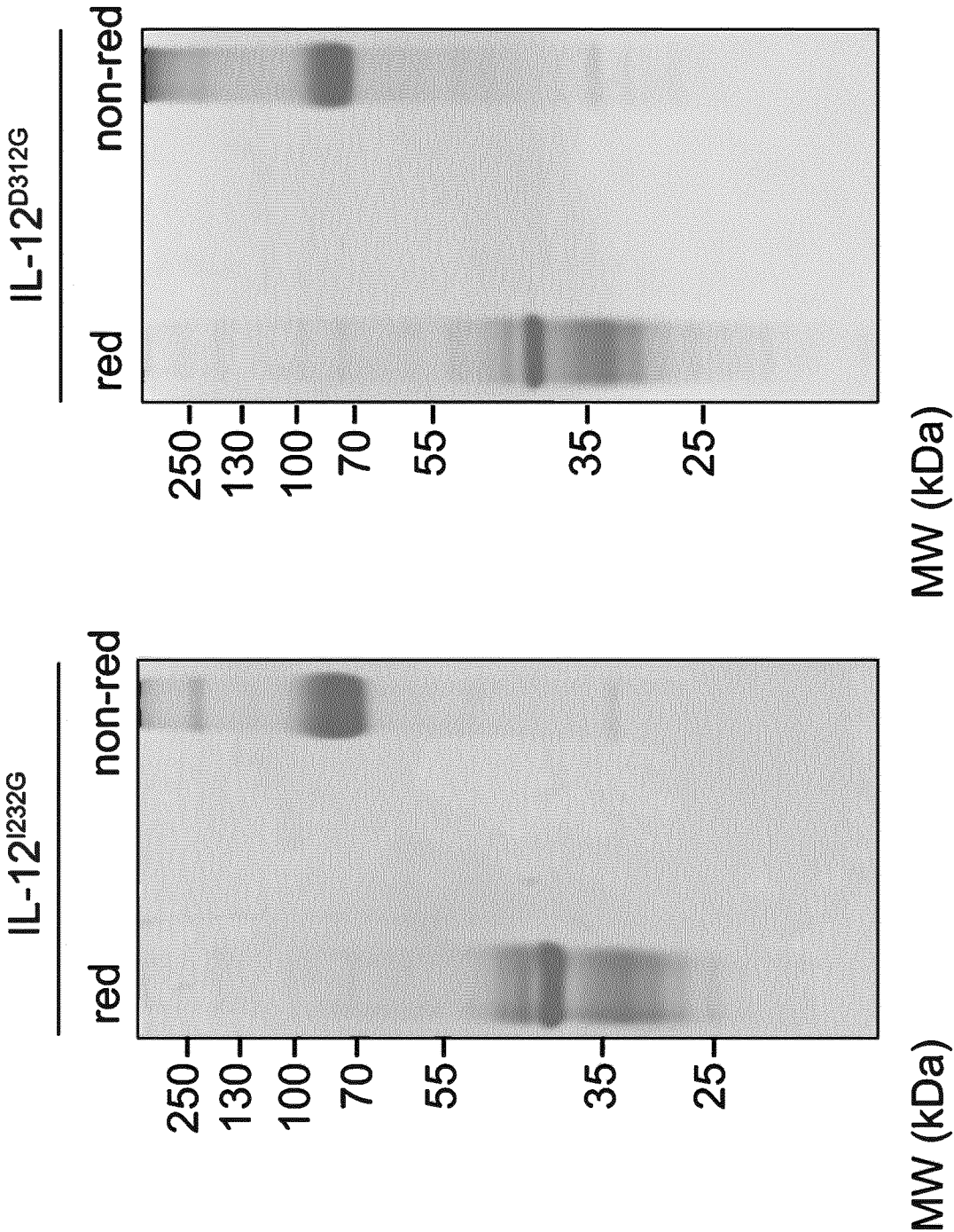


Figure 4 (cont.)

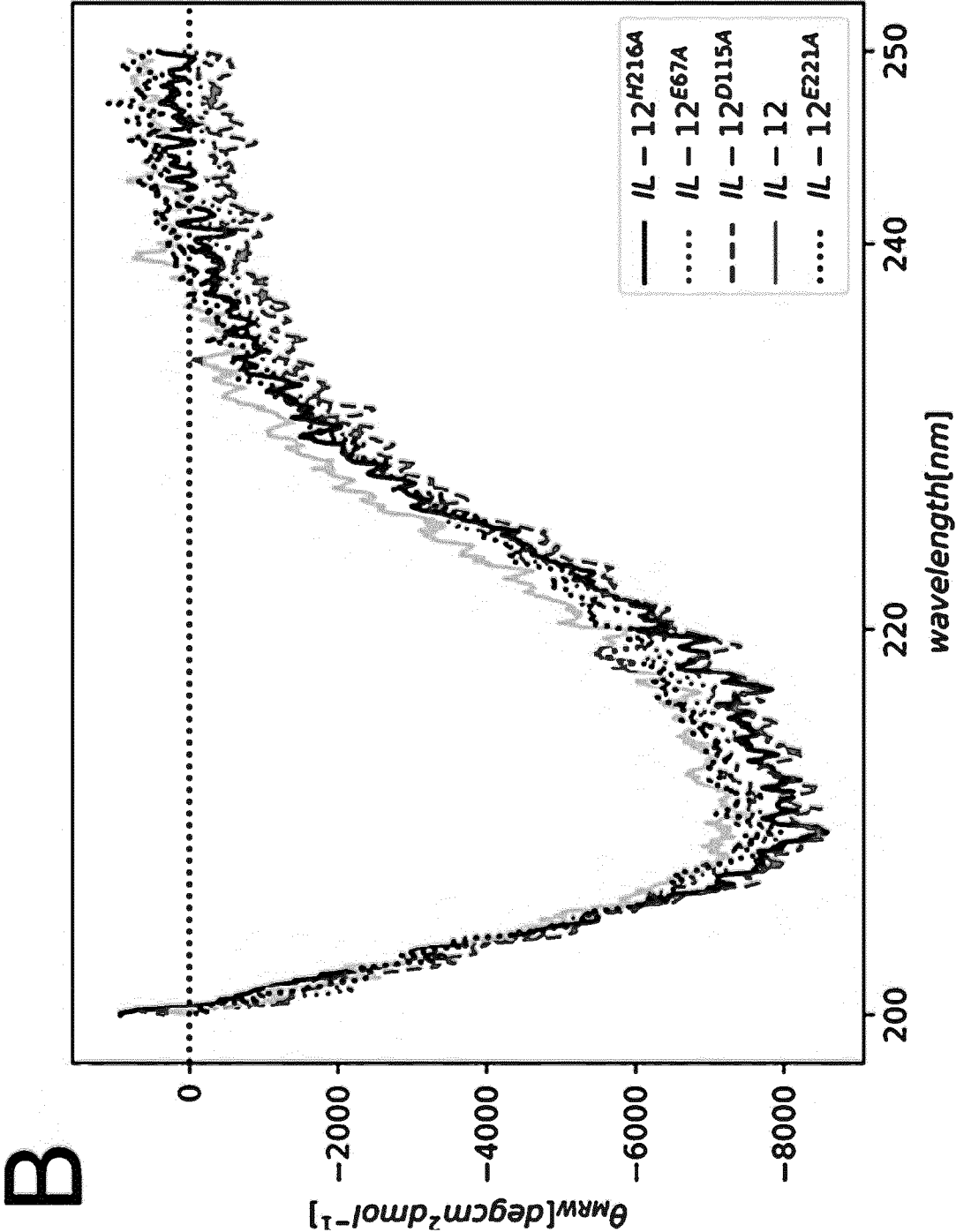


Figure 4B (cont.)

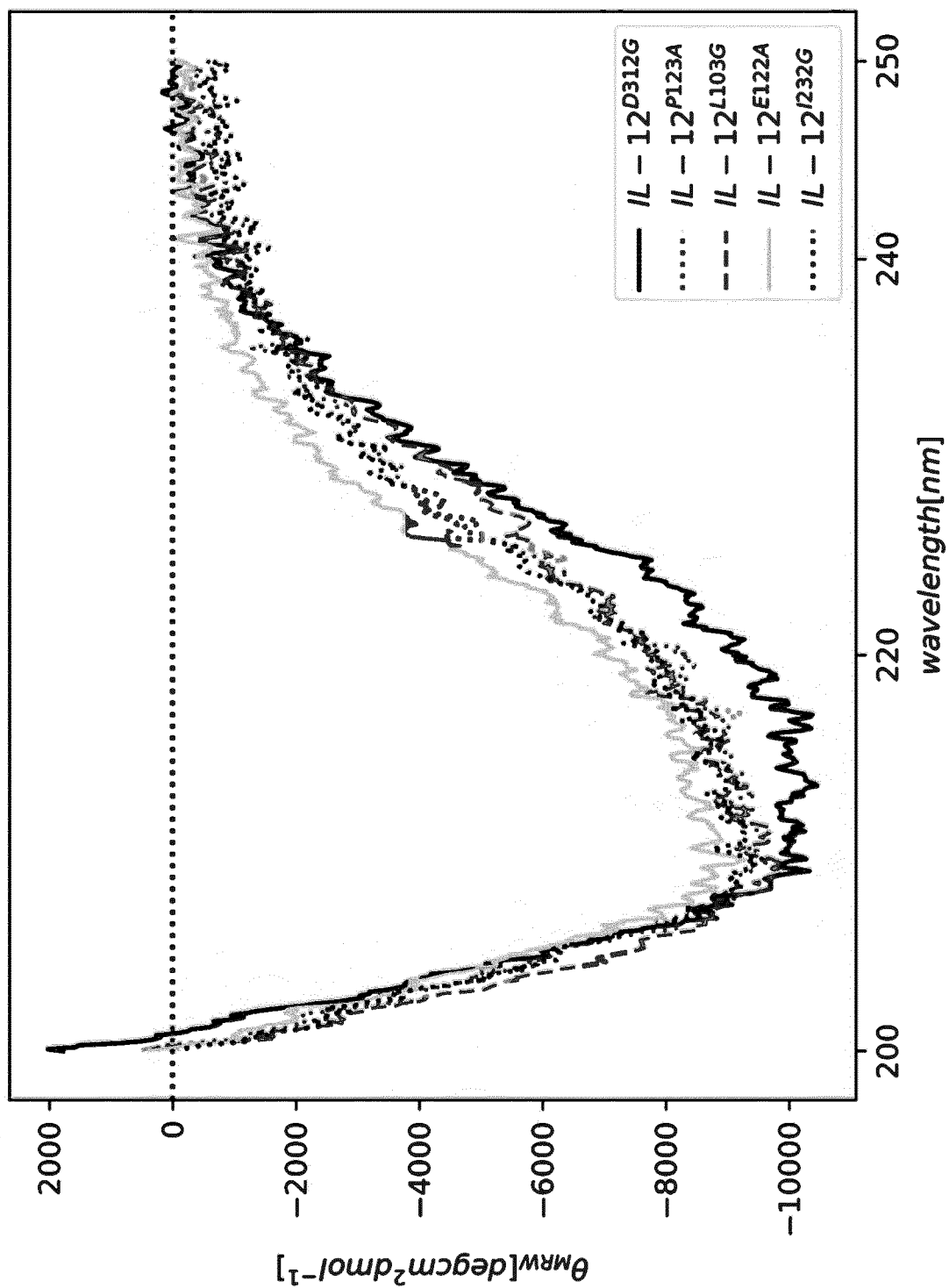


Figure 5

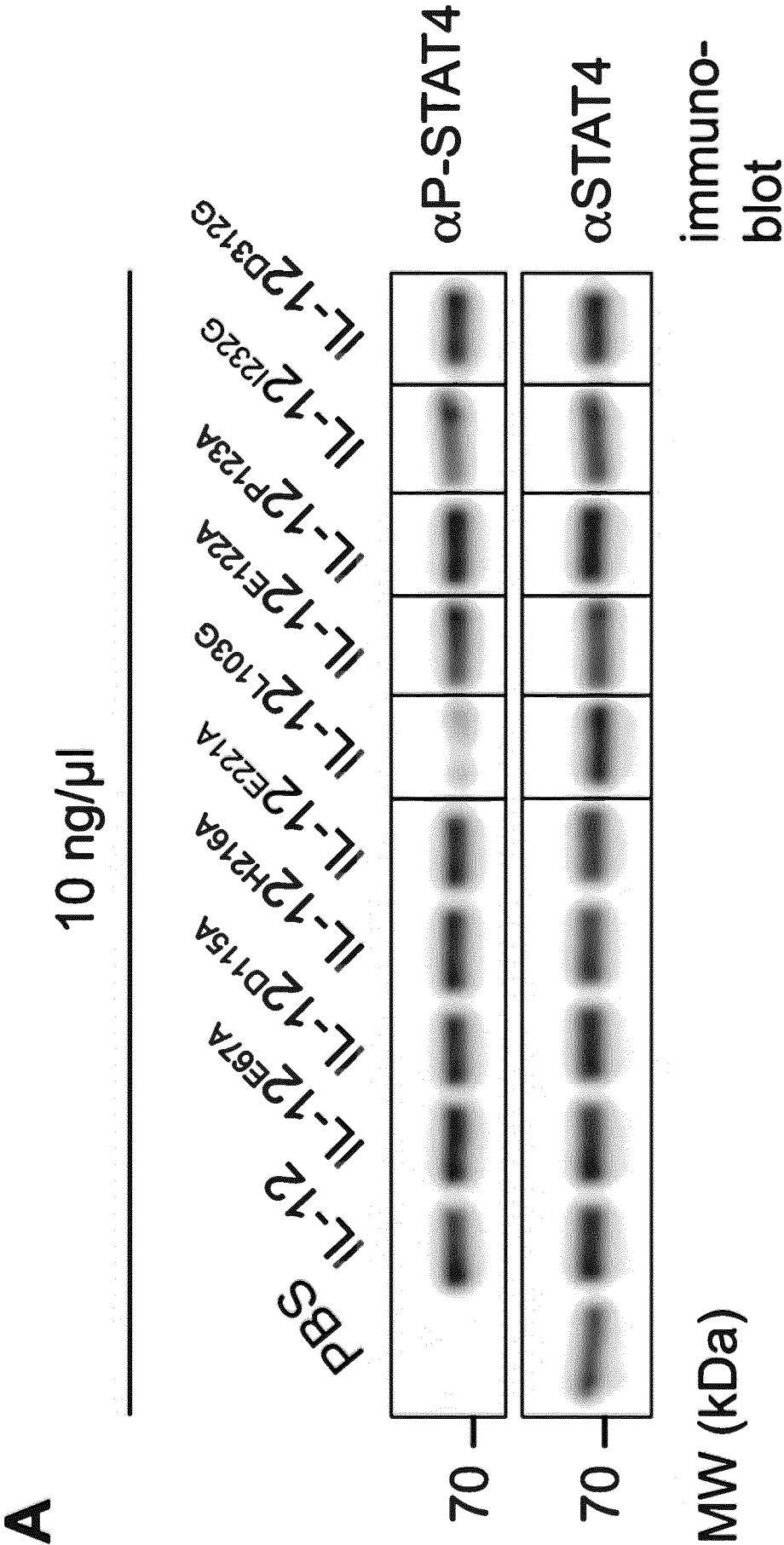


Figure 5 (cont.)

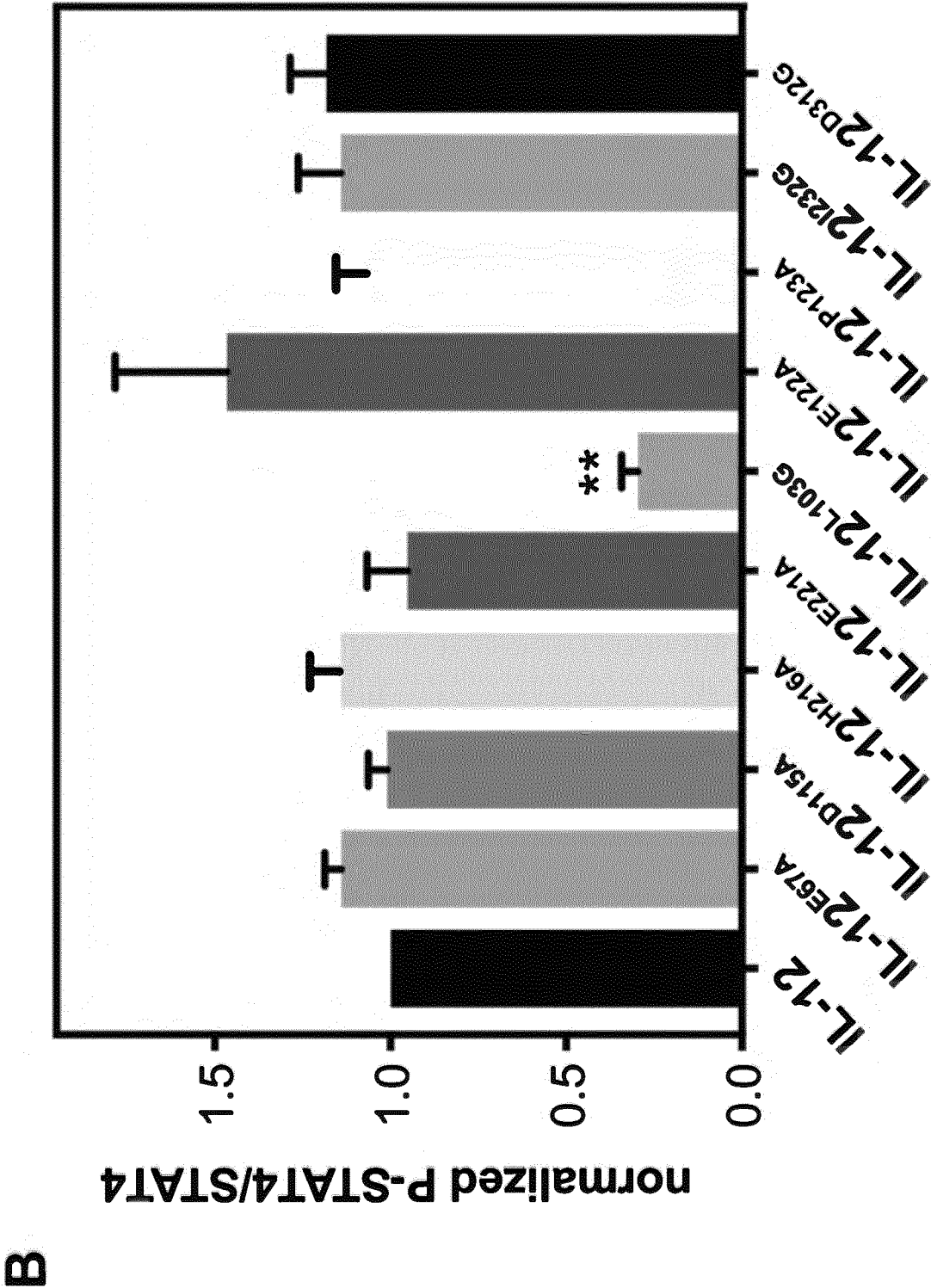


Figure 5 (cont.)

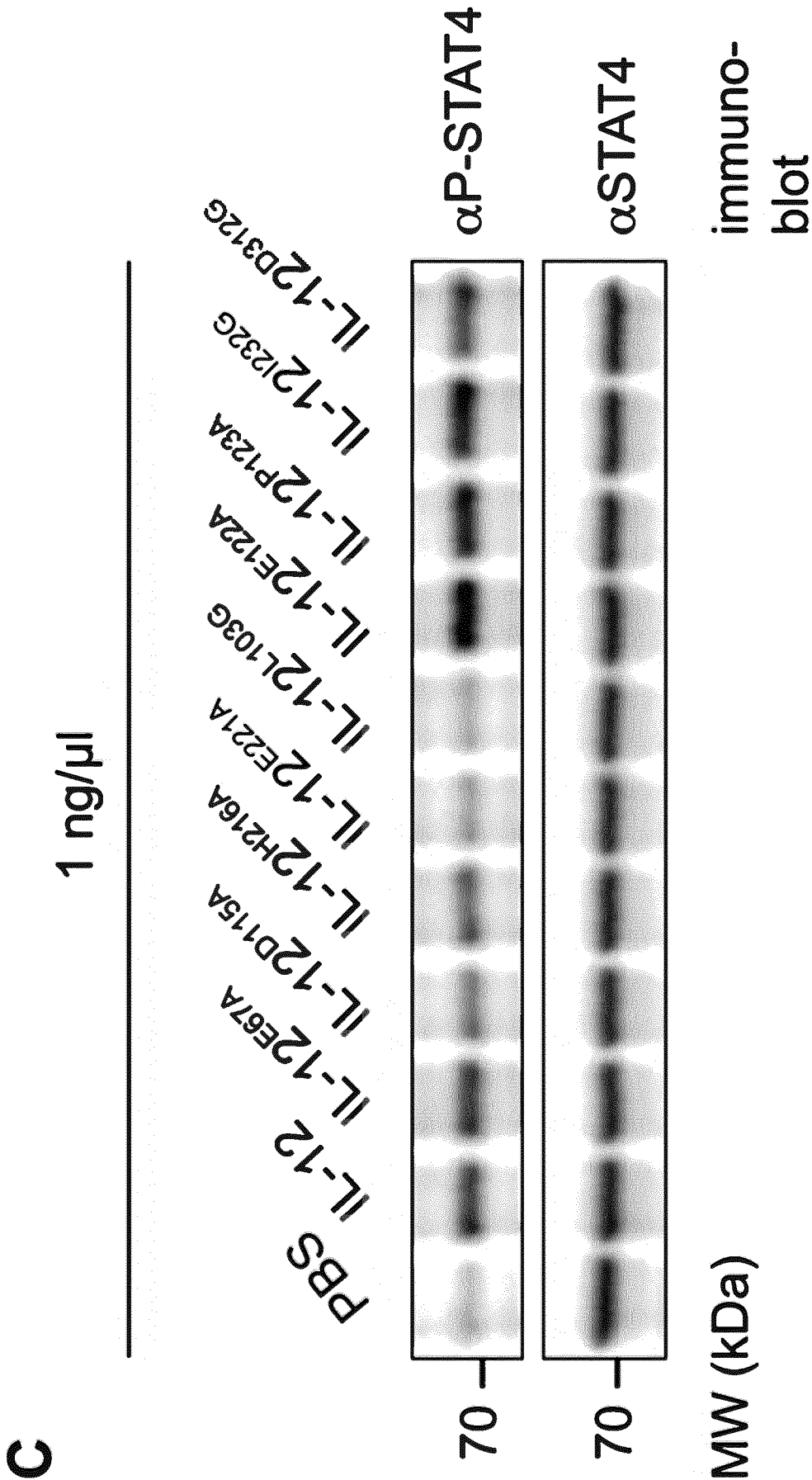


Figure 5 (cont.)

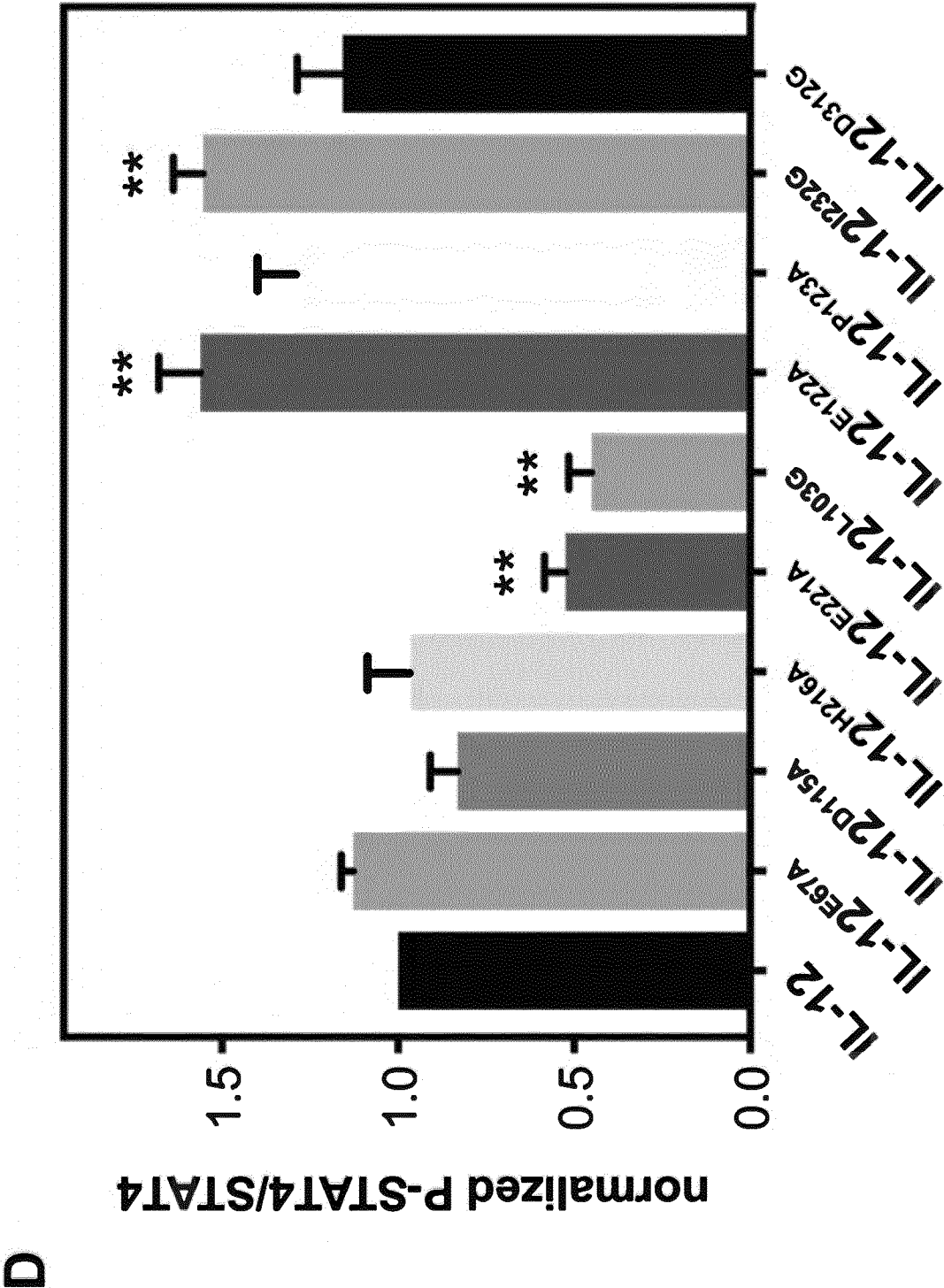


Figure 5 (cont.)

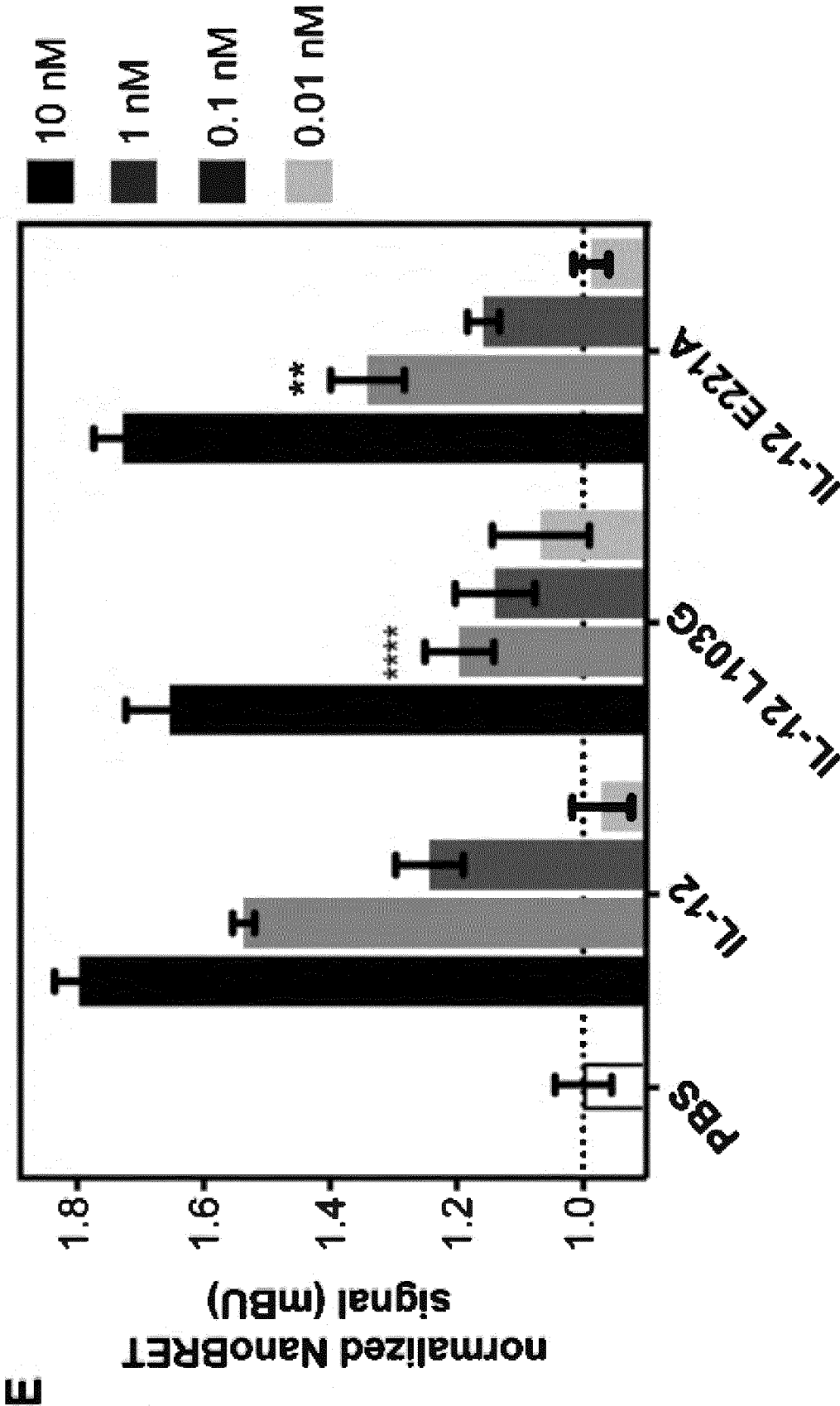


Figure 6

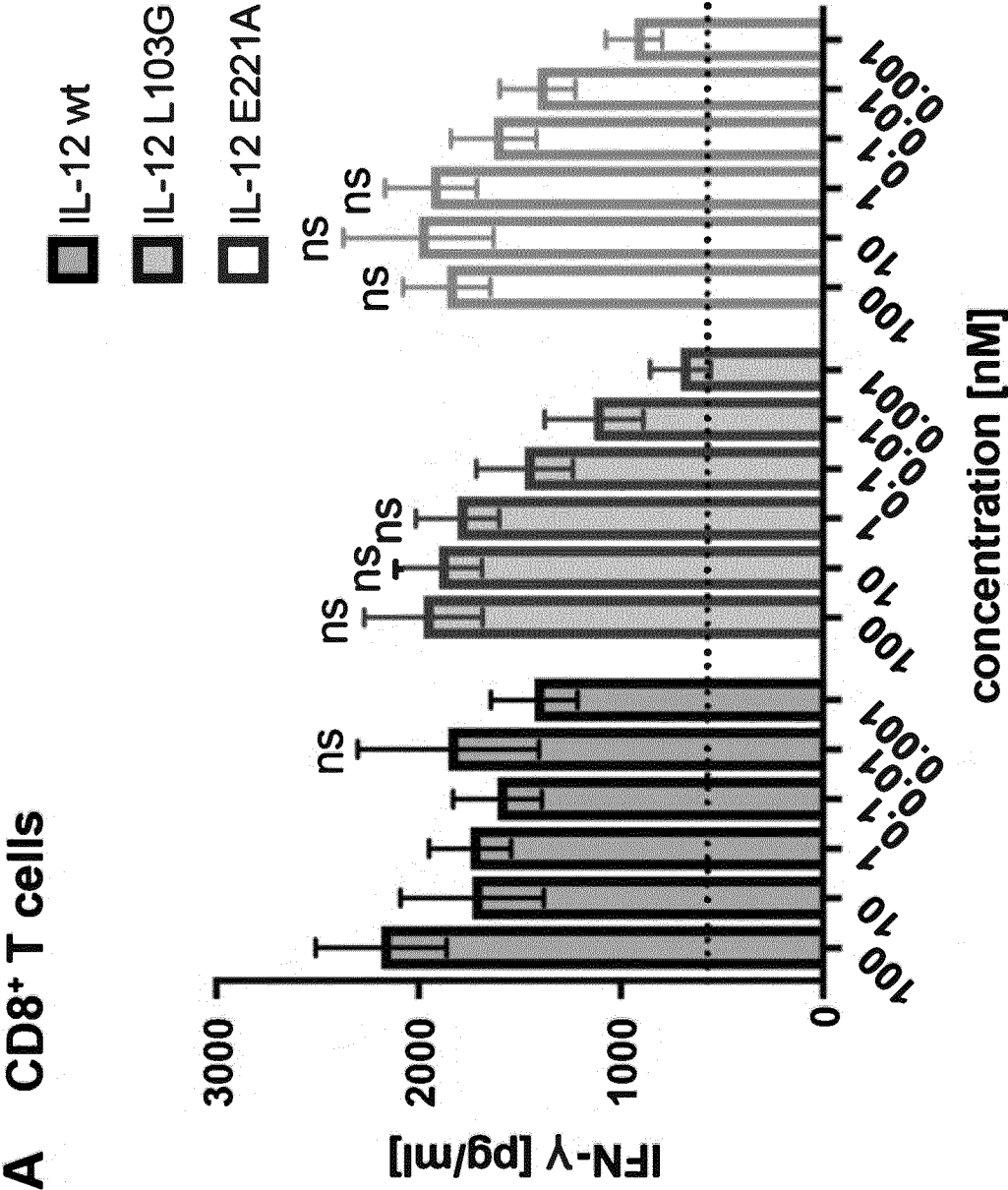


Figure 6 (cont.)

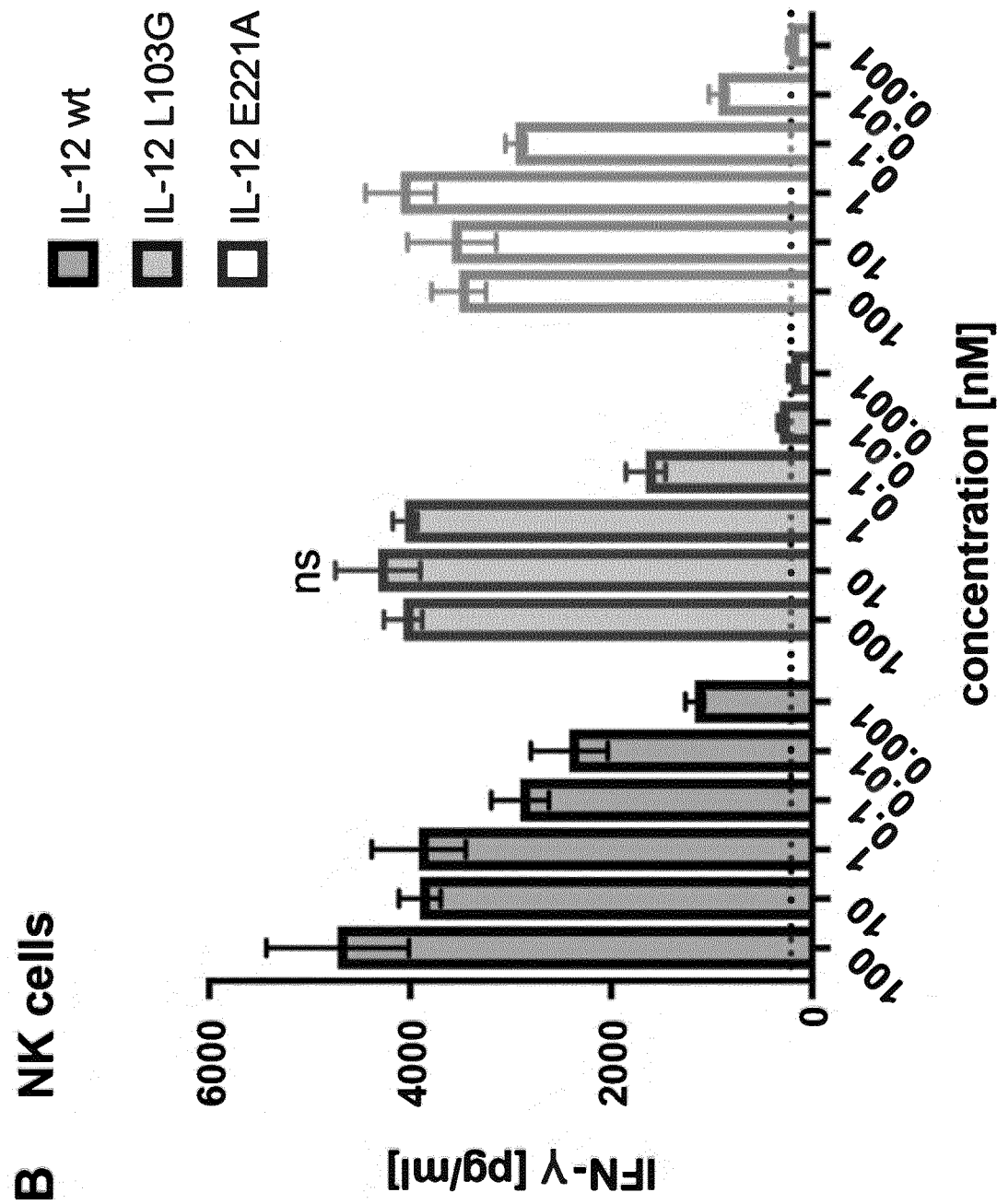


Figure 6 (cont.)

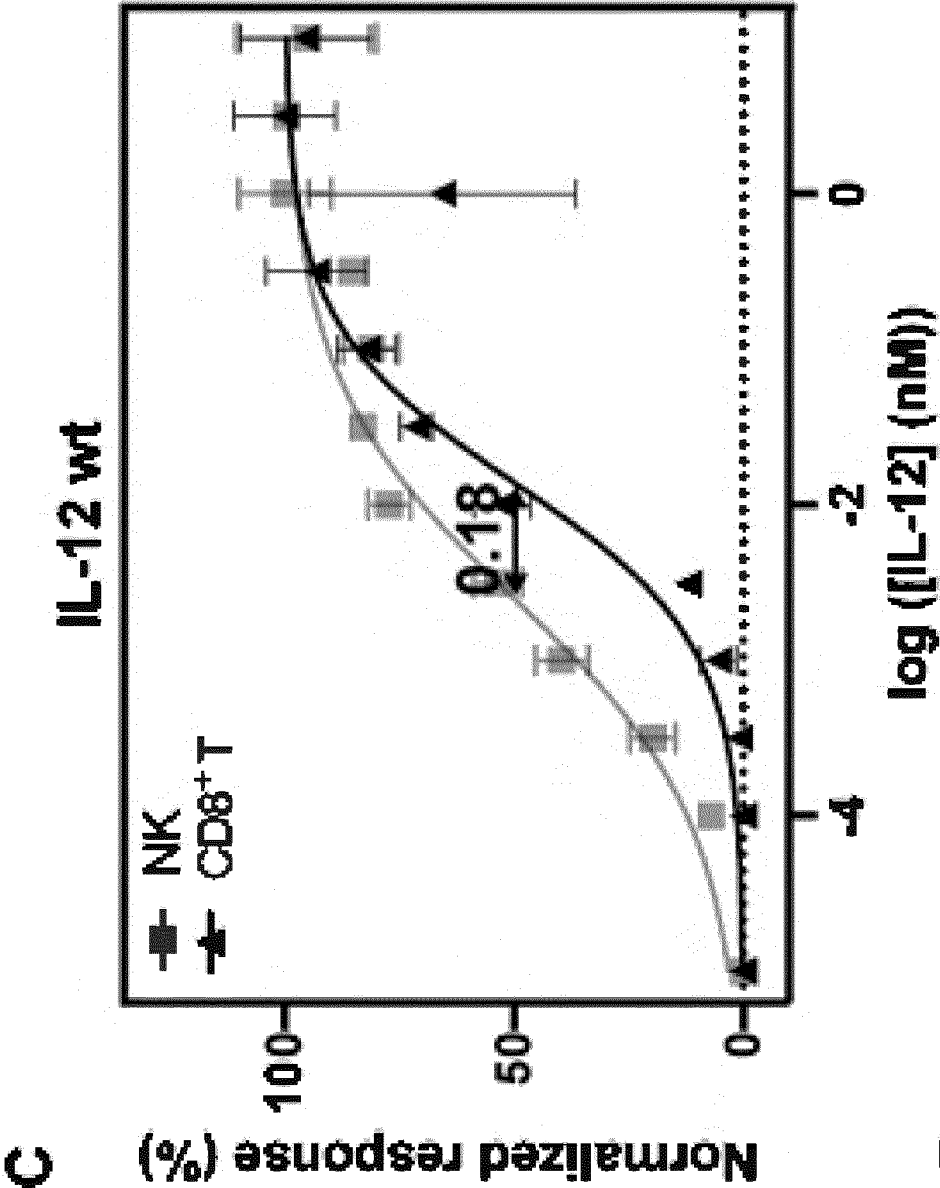


Figure 6C (cont.)

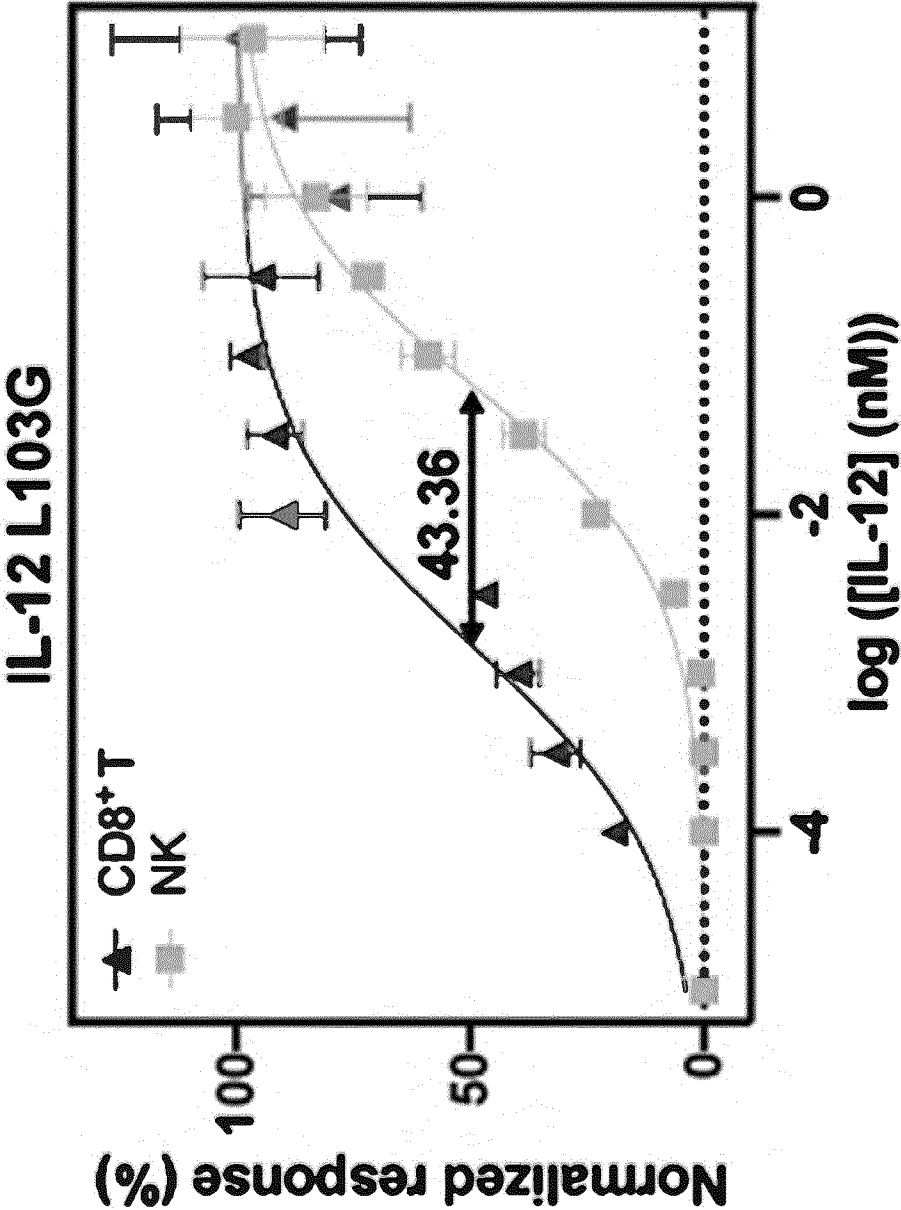


Figure 6C (cont.)

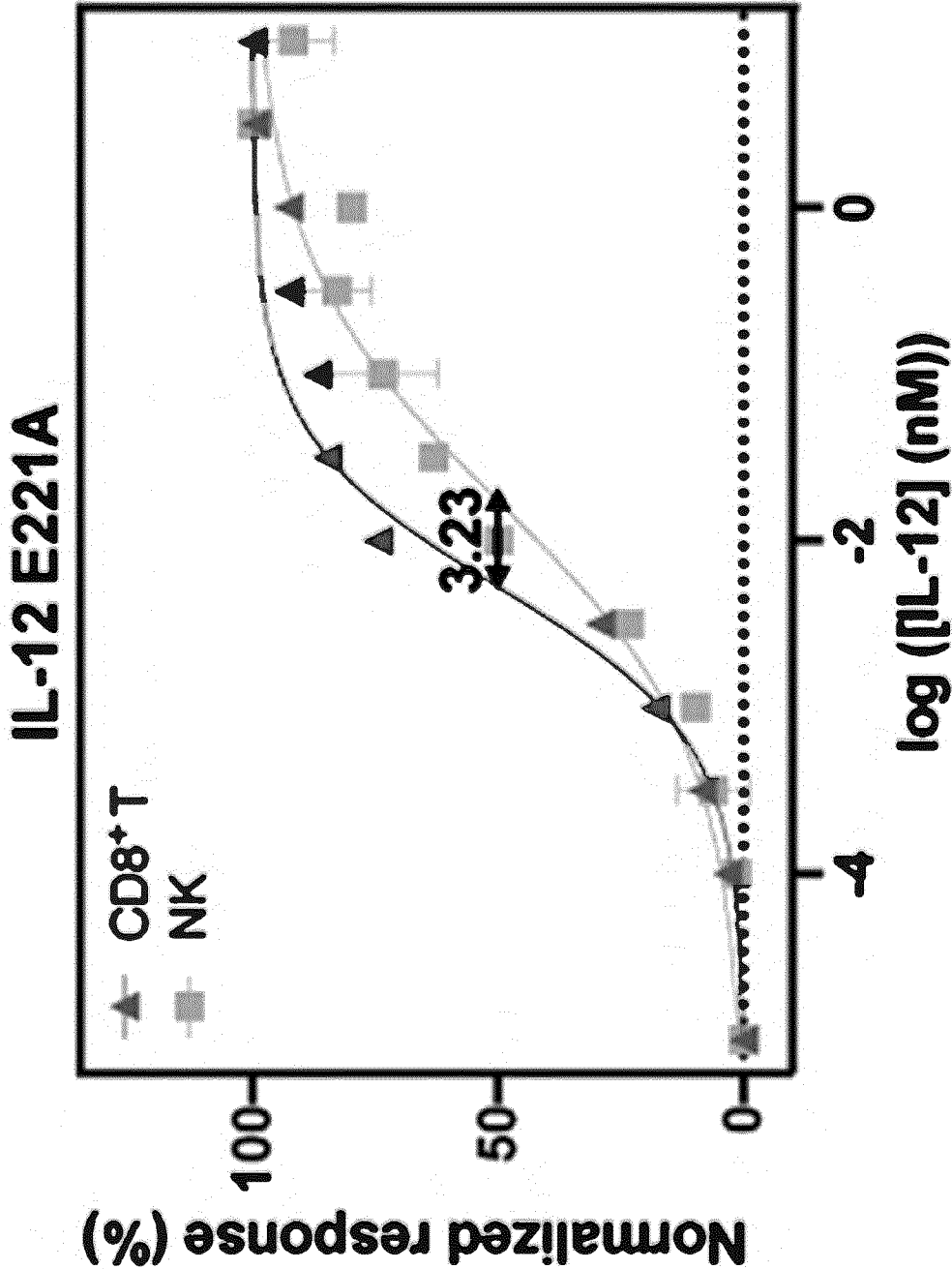


Figure 6 (cont.)

D

	NK cells					
	wt		L103G		E221A	
	EC50	95% CI	EC50	95% CI	EC50	95% CI
Donor 1	0.0025	0.0018 to 0.0034	0.0670	0.0530 to 0.0848	0.0172	0.0129 to 0.0230
Donor 2	0.0109	0.0075 to 0.0159	2.2230	1.8030 to 2.7520	0.4936	0.4148 to 0.5861
Donor 3	0.0005	0.0003 to 0.0007	0.0530	0.0401 to 0.0704	0.0103	0.0074 to 0.0145
Donor 4	0.0223	0.0126 to 0.0384	0.1540	0.1332 to 0.1796	0.0422	0.0303 to 0.0582

CD8+ T cells						factor (EC50 NK/EC50 CD8+ T)	
wt		L103G		E221A		wt	E221A
EC50	95% CI	EC50	95% CI	EC50	95% CI		
0.0139	0.0087 to 0.0232	0.0015	0.0009 to 0.0027	0.0053	0.0040 to 0.0071	0.1791	43.3571
0.0033	0.0019 to 0.0061	0.9512	0.6612 to 1.3530	0.1281	0.0698 to 0.2219	3.3091	2.3370
0.0063	0.0048 to 0.0084	0.0019	0.0007 to 0.0048	0.0027	0.0018 to 0.0039	0.0757	27.8571
n.d.	n.d.	0.0270	0.0141 to 0.0517	0.0065	0.0029 to 0.0136	n.d.	5.7037
							6.4892