(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 March 2017 (23.03.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/046200 A1

(51) **International Patent Classification**:
*C07K 14/54* (2006.01) *C12N 15/62* (2006.01)
*C07K 14/715* (2006.01)

(21) **International Application Number**:
PCT/EP2016/071759

(22) **International Filing Date**:
15 September 2016 (15.09.2016)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
15306418.3 16 September 2015 (16.09.2015) EP

(71) **Applicants**: **INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE)** [FR/FR]; 101, rue de Tolbiac, 75013 Paris (FR). **UNIVERSITÉ DE NANTES** [FR/FR]; 1, quai de Tourville, 44000 Nantes (FR). **UNIVERSITÉ D'ANGERS** [FR/FR]; 40 rue de Rennes, 75018 Paris (FR). **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS)** [FR/FR]; 3, Rue Michel Ange, 75016 Paris (FR). **CENTRE HOSPITALIER UNIVERSITAIRE DE NANTES** [FR/FR]; 5 allée de l'Ile Gloriette, 44000 Nantes (FR).

(72) **Inventors**: **MORTIER, Erwan**; U892 - Institut De Recherche En Sante IRS-UN 8, Quai Moncousu BP 70721, 44007 Nantes (FR). **MEGHNEM, Dihia**; U892 - Institut De Recherche En Sante IRS-UN 8, Quai Moncousu BP70721, 44007 Nantes (FR). **MORISSEAU, Sébastien**; U892 - Institut De Recherche En Sante IRS-UN 8, Quai Moncousu - BP 70721, 44007 Nantes (FR). **JACQUES, Yannick**; U892 - Institut De Recherche En Sante IRS-UN 8, Quai Moncousu - BP 70721, 44007 Nantes (FR).

(74) **Agent**: **COLLIN, Matthieu**; 7 rue Watt, 75013 Paris (FR).

(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, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, 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, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Published**:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2017/046200 A1

(54) **Title**: SPECIFIC INTERLEUKIN-15 (IL-15) ANTAGONIST POLYPEPTIDE AND USES THEREOF FOR THE TREATMENT OF INFLAMMATORY AND AUTO-IMMUNE DISEASES

(57) **Abstract**: The present invention relates to specific interleukin-15 (IL-15) antagonist polypeptides and uses thereof for the treatment of inflammatory and auto-immune diseases. In particular, the present invention relates to a specific interleukin-15 (IL-15) antagonist polypeptide comprising i) a IL15-Ralpha sushi-containing polypeptide comprising an amino acid sequence having at least 80% of identity with the amino acid sequence of SEQ ID NO:1 ii) a linker and iii) an IL- polypeptide comprising the amino acid sequence having at least at least 80% of identity with the amino acid sequence of SEQ ID NO:4 provided that the glutamine (Q) residue at position 108 is mutated.

# SPECIFIC INTERLEUKIN-15 (IL-15) ANTAGONIST POLYPEPTIDE AND USES THEREOF FOR THE TREATMENT OF INFLAMMATORY AND AUTO-IMMUNE DISEASES

## FIELD OF THE INVENTION:

The present invention relates to specific interleukin-15 (IL-15) antagonist polypeptides and uses thereof for the treatment of inflammatory and auto-immune diseases.

## BACKGROUND OF THE INVENTION:

IL-2 and IL-15 belong to a family of γc cytokines (IL-2, 4, 7, 9, 15, and 21) which are key regulators of lymphocyte homeostasis and function. They have the potential to promote lymphocyte proliferation and survival and thus overall enhance dominantly adaptive immune responses. Both cytokines share two receptor subunits, CD122 and CD132, β, and common γc chains, respectively. IL-2 exerts its pleiotropic activities through binding to either a dimeric receptor composed of CD122 and CD132 or a trimeric receptor composed of CD25, CD122 and CD132. For instance a complex of CD25, CD122 and CD132 binds IL-2 with high affinity (Ka 1011 M−1) and is present on activated T cells and T regulatory cells (Treg; CD3+CD4+CD25+Foxp3+). CD25 is constitutively expressed at high levels in Treg cells, enabling them to utilize very low background levels of IL-2. In contrast to IL-2, IL-15 is membrane-associated cytokine acting through cell-cell contact. The dominant mechanism of IL-15 signaling in vivo is trans-presentation of IL-15 by IL-15Rα (CD215) to a heterodimeric CD122/CD132 receptor. Unlike CD25, IL-15Rα binds IL-15 with a very high affinity (Ka 1011 M−1) that is not further increased in the presence of the complete IL-15 receptor, i.e., IL-15 has 100-fold higher affinity to IL-15Rα than IL-2 to CD25. IL-15Rα is expressed on T cells, NK cells, NKT cells, B cells, DCs, monocytes and macrophages, and in thymic and BM stromal cell lines. IL-2 and IL-15 shares many functions in both the adaptive and innate immune system, because both cytokines signal through the same CD122/CD132 receptor heterodimer. Both cytokines play pivotal roles in innate and adaptative immunity. Whereas initial in vitro experiments have shown a large functional overlap in the effects of the two cytokines (induction of the proliferation and cytotoxicity of activated lymphocytes and NK cells, co-stimulation of B cell proliferation and immunoglobulin synthesis, and chemoattraction of T cells), additional experiments have indicated that they can exert complementary or even contrasting actions in vivo. Whereas IL-2 or IL-2Rα knock-out in mice was associated with autoimmune phenotypes with increased populations of activated T and B cells, IL-15 and IL-15Rα knock-out resulted

in specific defects in NK, NK-T, intraepithelial lymphocytes, and memory CD8 T cells. Furthermore, IL-2 promotes peripheral tolerance by inducing activation-induced cell death. IL-2 is indeed critical to the development and function of Treg cells, whereas IL-15 inhibits IL-2-mediated activation-induced cell death, and, unlike IL-2, IL-15 is a survival factor for CD8 memory T cells. In line with these observations, it has been suggested that the major role of IL-2 is to limit continuous expansion of activated T cells, whereas IL-15 is critical for initiation of T cell division and survival of memory T cells. High levels of IL-15 expression have been associated to the pathogenesis of autoimmune and inflammatory diseases, like in Crohn's disease, psoriasis, leukemias, rheumatoid arthritis (RA) and graft rejection. Accordingly modulators of IL-15 represent a great interest in the therapeutic field. Fusion proteins (RLI and ILR), in which IL-15 and IL-15R alpha-sushi are attached by a flexible linker were described as potent adjuvants for the expansion of lymphocyte subsets (Mortier E. et al. J Biol Chem. 2006 Jan 20;281(3):1612-9 and WO2007046006). IL-15 antagonists could be a potential therapeutic to treat inflammatory diseases and several IL-15 antagonists have been described in the prior art. For example Ferrari-Lacraz S. et al. described an IL-15 antagonist consisting of an IL-15 mutant fused to a Fc domain of an immunoglobulin and demonstrated that said antagonist could be useful for the treatment of rheumatoid arthritis (Ferrari-Lacraz S. et al. J Immunol. 2004 Nov 1;173(9):5818-26.). However IL-15 antagonists described in the prior art also potentially antagonize the IL-2 signalling pathway and could thus be associated with adverse side effects (e.g. on tolerance) when they are injected in vivo.

**SUMMARY OF THE INVENTION:**

The present invention relates to specific interleukin-15 (IL-15) antagonist polypeptides and uses thereof for the treatment of inflammatory and auto-immune diseases. In particular, the present invention is defined by the claims.

**DETAILED DESCRIPTION OF THE INVENTION:**

The inventors surprisingly show that the RLI fusion protein described in Mortier E. et al. J Biol Chem. 2006 Jan 20;281(3):1612-9 and WO2007046006 wherein at least one glutamine (Q) residue at position 101 or 108 is mutated acts as an IL-15 antagonist but does not antagonize the interleukin-2 signalling pathway. This polypeptide is the first specific IL-15 antagonist targeting the common IL-2Rb/g receptor and thus offers huge advantages over the IL-15 antagonists described in the prior art since it can inhibit the proliferation of NK cells mediated by IL-15 without affecting the homeostasis of the T regulatory cells (Treg cells) that depends from the IL-2 signalling pathway.

Accordingly, the present invention relates to a specific interleukin-15 (IL-15) antagonist polypeptide comprising i) a IL15-Ralpha sushi-containing polypeptide comprising an amino acid sequence having at least 80% of identity with the amino acid sequence of SEQ ID NO:1 ii) a linker and iii) an IL-15 polypeptide comprising the amino acid sequence having at least at least 80% of identity with the amino acid sequence of SEQ ID NO:4 provided that the glutamine (Q) residue at position 108 is mutated.

As used herein, the expression "specific interleukin-15 (IL-15) antagonist polypeptide" refers to a polypeptide that is capable of inhibiting the IL-15 signalling pathway without antagonizing the IL-2 signalling pathway.

According to the invention a first amino acid sequence having at least 80% of identity with a second amino acid sequence means that the first sequence has 80; 81; 82; 83; 84; 85; 86; 87; 88; 89; 90; 91; 92; 93; 94; 95; 96; 97; 98; 99 or 100% of identity with the second amino acid sequence.

Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar are the two sequences. Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, Adv. Appl. Math., 2:482, 1981; Needleman and Wunsch, J. Mol. Biol., 48:443, 1970; Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A., 85:2444, 1988; Higgins and Sharp, Gene, 73:237-244, 1988; Higgins and Sharp, CABIOS, 5:151-153, 1989; Corpet et al. Nuc. Acids Res., 16:10881-10890, 1988; Huang et al., Comp. Appls Biosci., 8:155-165, 1992; and Pearson et al., Meth. Mol. Biol., 24:307-31, 1994). Altschul et al., Nat. Genet., 6:119-129, 1994, presents a detailed consideration of sequence alignment methods and homology calculations. By way of example, the alignment tools ALIGN (Myers and Miller, CABIOS 4:11-17, 1989) or LFASTA (Pearson and Lipman, 1988) may be used to perform sequence comparisons (Internet Program® 1996, W. R. Pearson and the University of Virginia, fasta20u63 version 2.0u63, release date December 1996). ALIGN compares entire sequences against one another, while LFASTA compares regions of local similarity. These alignment tools and their respective tutorials are available on the Internet at the NCSA Website, for instance. Alternatively, for comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function can be employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). The BLAST sequence

comparison system is available, for instance, from the NCBI web site; see also Altschul et al., J. Mol. Biol., 215:403-410, 1990; Gish. & States, Nature Genet., 3:266-272, 1993; Madden et al. Meth. Enzymol., 266:131-141, 1996; Altschul et al., Nucleic Acids Res., 25:3389-3402, 1997; and Zhang & Madden, Genome Res., 7:649-656, 1997.

According to the invention, the IL15-Ralpha sushi-containing polypeptide, the linker and the IL-15 polypeptide are fused in frame wherein the C-terminal end of the IL15-Ralpha sushi-containing polypeptide is fused to the N-terminal end of the linker and the C-terminal end of the linker is fused to the N-terminal end of the IL-15 polypeptide.

As used herein, the term "linker" has its general meaning in the art and refers to an amino acid sequence of a length sufficient to ensure that the proteins form proper secondary and tertiary structures.

In some embodiments, the linker is a peptidic linker which comprises at least one, but less than 30 amino acids e.g., a peptidic linker of 2-30 amino acids, preferably of 10-30 amino acids, more preferably of 15-30 amino acids, still more preferably of 19-27 amino acids, most preferably of 20-26 amino acids. In some embodiments, the linker has 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18; 19; 20; 21; 22; 23; 24; 25; 26; 27; 28; 29; 30 amino acid residues. Typically, linkers are those which allow the compound to adopt a proper conformation (i.e., a conformation allowing a proper signal transducing activity through the IL-15Rbeta/gamma signalling pathway). The most suitable linker sequences (1) will adopt a flexible extended conformation, (2) will not exhibit a propensity for developing ordered secondary structure which could interact with the functional domains of fusion proteins, and (3) will have minimal hydrophobic or charged character which could promote interaction with the functional protein domains. Typical surface amino acids in flexible protein regions include Gly, Asn and Ser (i.e., G, N or S). Virtually any permutation of amino acid sequences containing Gly, Asn and Ser would be expected to satisfy the above criteria for a linker sequence. Other near neutral amino acids, such as Thr, Ala, Leu, Gln (i.e., T, A, L, Q) may also be used in the linker sequence. The length of the linker sequence may vary without significantly affecting the biological activity of the fusion protein. If used for therapeutical purposes, the linker is preferably non-immunogenic. Exemplary linker sequences are described in U.S. Pat. Nos. 5,073,627 and 5,108,910.

In some embodiments, the linker of the present invention consists of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:3.

As used herein, the term mutation has its general meaning in the art and refers to a substitution, deletion or insertion. The term "substitution" means that a specific amino acid

residue at a specific position is removed and another amino acid residue is inserted into the same position. The term "deletion" means that a specific amino acid residue is removed. The term "insertion" means that one or more amino acid residues are inserted before or after a specific amino acid residue, more specifically, that one or more, preferably one or several, amino acid residues are bound to an a.-carboxyl group or an a,-amino group of the specific amino acid residue.

In some embodiments, the glutamine (Q) residue at position 108 is substituted by an amino acid residue selected from the group consisting of aspartic acid (D), glutamic acid (E), lysine (K), serine (S), threonine (T), asparagine (N), cysteine (C), alanine (A), glycine (G), valine (V), leucine (L), proline (P), methionine (M) and tryptophan (W). In some embodiments, the glutamine (Q) residue at position 108 is substituted by an aspartic acid (D) residue.

In some embodiments, the glutamine (Q) residue at position 101 in the IL-15 polypeptide is also mutated. In some embodiments, the glutamine (Q) residue at position 101 is substituted by an amino acid residue selected from the group consisting of aspartic acid (D) residue, lysine (K), and tryptophan (W). In some embodiments, the glutamine (Q) residue at position 101 is substituted by an aspartic acid (D) residue.

In some embodiments, both glutamine (Q) residues at position 101 and 108 are substituted. In some embodiments, both glutamine (Q) residues at position 101 and 108 are substituted by an aspartic acid (D) residue.

In some embodiments, the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention comprises an amino acid sequence having at least 80% of identity with the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6. In some embodiments, the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention consists of SEQ ID NO:5 or SEQ ID NO:6.

In some embodiments, the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention is fused to an immunoglobulin domain so as to form an immunoadhesin. As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention with the effector functions of immunoglobulin constant domains. In some embodiments, the immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM. In some embodiments, the immunoglobulin sequence is an immunoglobulin constant domain (Fc region). Immunoadhesins can possess many of the valuable chemical and biological properties of human antibodies. Since

immunoadhesins can be constructed from a human protein sequence with a desired specificity linked to an appropriate human immunoglobulin hinge and constant domain (Fc) sequence, the binding specificity of interest can be achieved using entirely human components. Such immunoadhesins are minimally immunogenic to the patient, and are safe for chronic or repeated use. The artisan skilled in the art can easily select the most appropriate Fc domain (Chan AC, Carter PJ. Therapeutic antibodies for autoimmunity and inflammation. Nat Rev Immunol. 2010 May;10(5):301-16. doi: 10.1038/nri2761. Review.). In some embodiments, the Fc region includes or not a mutation that inhibits complement fixation and/or Fc receptor binding (Zheng et al, Transplantation. 2006 Jan 15;81(1):109-16). In some embodiments, the Fc region is a native sequence Fc region. In some embodiments, the Fc region is a variant Fc region. In some embodiments, the Fc region is a functional Fc region. As used herein, the term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain, including native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. In some embodiments, the adhesion portion and the immunoglobulin sequence portion of the immunoadhesin are linked by a minimal linker.

According to the invention, the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention is produced by conventional automated peptide synthesis methods or by recombinant expression. General principles for designing and making proteins are well known to those of skill in the art. The specific interleukin-15 (IL-15) antagonist polypeptide of the present invention may be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols as described in Stewart and Young; Tam et al., 1983; Merrifield, 1986 and Barany and Merrifield, Gross and Meienhofer, 1979. The specific interleukin-15 (IL-15) antagonist polypeptide of the present invention may also be synthesized by solid-phase technology employing an exemplary peptide synthesizer such as a Model 433A from Applied Biosystems Inc. The purity of any given protein; generated through automated peptide synthesis or through recombinant methods may be determined using reverse phase HPLC analysis. Chemical authenticity of each peptide may be established by any method well known to those of skill in the art. As an alternative to automated peptide synthesis, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a protein of choice is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression as described herein below.

Recombinant methods are especially preferred for producing longer polypeptides. A variety of expression vector/host systems may be utilized to contain and express the peptide or protein coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors (Giga-Hama et al., 1999); insect cell systems infected with virus expression vectors (e.g., baculovirus, see Ghosh et al., 2002); plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g., Ti or pBR322 plasmid; see e.g., Babe et al., 2000); or animal cell systems. Those of skill in the art are aware of various techniques for optimizing mammalian expression of proteins, see e.g., Kaufman, 2000; Colosimo et al., 2000. Mammalian cells that are useful in recombinant protein productions include but are not limited to VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, COS cells (such as COS-7), W138, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562 and 293 cells. Exemplary protocols for the recombinant expression of the peptide substrates or fusion polypeptides in bacteria, yeast and other invertebrates are known to those of skill in the art and a briefly described herein below. Mammalian host systems for the expression of recombinant proteins also are well known to those of skill in the art. Host cell strains may be chosen for a particular ability to process the expressed protein or produce certain post-translation modifications that will be useful in providing protein activity. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, and the like have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

In some embodiments, it is contemplated that the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention is modified in order to improve their therapeutic efficacy. Such modification of therapeutic compounds may be used to decrease toxicity, increase circulatory time, or modify biodistribution. For example, the toxicity of potentially important therapeutic compounds can be decreased significantly by combination with a variety of drug carrier vehicles that modify biodistribution. A strategy for improving drug viability is the utilization of water-soluble polymers. Various water-soluble polymers have been shown to modify biodistribution, improve the mode of cellular uptake, change the permeability through physiological barriers; and modify the rate of clearance from the body. To achieve either a

targeting or sustained-release effect, water-soluble polymers have been synthesized that contain drug moieties as terminal groups, as part of the backbone, or as pendent groups on the polymer chain. For example, Pegylation is a well established and validated approach for the modification of a range of polypeptides. The benefits include among others : (a) markedly improved circulating half-lives *in vivo* due to either evasion of renal clearance as a result of the polymer increasing the apparent size of the molecule to above the glomerular filtration limit, and/or through evasion of cellular clearance mechanisms; (b) reduced antigenicity and immunogenicity of the molecule to which PEG is attached; (c) improved pharmacokinetics; (d) enhanced proteolytic resistance of the conjugated protein; and (e) improved thermal and mechanical stability of the PEGylated polypeptide.

Another object of the invention relates to an isolated, synthetic or recombinant nucleic acid encoding for a specific interleukin-15 (IL-15) antagonist polypeptide of the present invention.

As used herein, the term "nucleic acid " has its general meaning in the art and refers to a DNA or RNA molecule. However, the term captures sequences that include any of the known base analogues of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fiuorouracil, 5-bromouracil, 5- carboxymethylaminomethyl-2-thiouracil, 5-carboxymethyl-aminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1 -methyladenine, 1 -methylpseudouracil, 1-methylguanine, 1- methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5- methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5- methoxyamino-methyl-2-thiouracil, beta-D-mannosylqueosine, 5'- methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil- 5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, -uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

In some embodiments, the nucleic acid of the present invention is a DNA or RNA molecule, which may be included in any suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or a viral vector. The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of the introduced sequence.

So, another object of the invention relates to a vector comprising a nucleic acid of the invention.

Such vectors may comprise regulatory elements, such as a promoter, enhancer, terminator and the like, to cause or direct expression of said polypeptide upon administration to a subject. The vectors may further comprise one or several origins of replication and/or selectable markers. The promoter region may be homologous or heterologous with respect to the coding sequence, and provide for ubiquitous, constitutive, regulated and/or tissue specific expression, in any appropriate host cell, including for in vivo use. Examples of promoters include bacterial promoters (T7, pTAC, Trp promoter, etc.), viral promoters (LTR, TK, CMV-IE, etc.), mammalian gene promoters (albumin, PGK, etc), and the like. Examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like. Examples of viral vector include adenoviral, retroviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, GPenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in WO 95/14785, WO 96/22378, US 5,882,877, US 6,013,516, US 4,861,719, US 5,278,056 and WO 94/19478.

Another object of the present invention relates to a host cell which has been transfected, infected or transformed by a nucleic acid molecule and/or a vector according to the invention. The term "transformation" means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA has been "transformed".

The nucleic acid molecule of the invention may be used to produce a specific interleukin-15 (IL-15) antagonist polypeptide of the present invention in a suitable expression system. The term "expression system" means a host cell and compatible vector under suitable conditions, e.g. for the expression of a protein coded for by foreign DNA carried by the vector and introduced to the host cell. Common expression systems include *E. coli* host cells and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors. Other examples of host cells include, without limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, etc.). Specific examples include *E.coli*, *Kluyveromyces* or *Saccharomyces* yeasts, mammalian

cell lines (e.g., Vero cells, CHO cells, 3T3 cells, COS cells, etc.) as well as primary or established mammalian cell cultures (e.g., produced from lymphoblasts, fibroblasts, embryonic cells, epithelial cells, nervous cells, adipocytes, etc.). The construction of expression vectors in accordance with the invention, and the transformation of the host cells can be carried out using conventional molecular biology techniques. The specific interleukin-15 (IL-15) antagonist polypeptide of the present invention, can, for example, be obtained by culturing genetically transformed cells in accordance with the invention and recovering the polypeptide expressed by said cell, from the culture. They may then, if necessary, be purified by conventional procedures, known in themselves to those skilled in the art, for example by fractional precipitation, in particular ammonium sulfate precipitation, electrophoresis, gel filtration, affinity chromatography, etc. In particular, conventional methods for preparing and purifying recombinant proteins may be used for producing the polypeptides in accordance with the invention. Thus the present invention also relates to a method for producing a recombinant host cell expressing a specific interleukin-15 (IL-15) antagonist polypeptide of the present invention, said method comprising the steps consisting of: (i) introducing *in vitro* or *ex vivo* a recombinant nucleic acid or a vector as described above into a competent host cell, (ii) culturing *in vitro* or *ex vivo* the recombinant host cell obtained and (iii), optionally, selecting the cells which express and/or secrete the polypeptide of the invention. Such recombinant host cells can be used for the production of polypeptides and fusions proteins of the present invention. The invention further relates to a method of producing a specific interleukin-15 (IL-15) antagonist polypeptide of the present invention, which method comprises the steps consisting of: (i) culturing a transformed host cell according to the invention under conditions suitable to allow expression of said polypeptide or fusion protein; and (ii) recovering the expressed polypeptide or fusion protein.

The specific interleukin-15 (IL-15) antagonist polypeptide of the present invention is particularly suitable for therapeutic purposes including the treatment of autoimmune diseases and inflammatory diseases.

Accordingly a further object of the present invention relates to the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention for use as a drug.

A further object of the present invention relates to a method of suppressing the immune response in a patient by administering a dose of a specific interleukin-15 (IL-15) antagonist polypeptide of the present invention, and thereby modulates IL-15 dependent immune responses.

In particular, the method of the present invention is suitable to treat a patient suffering from an autoimmune disease. Examples of autoimmune disease include, without limitation, acute disseminated encephalomyelitis (ADEM), acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, agammaglobulinemia, alopecia areata, amyloidosis, ankylosing spondylitis, anti-GBM/Anti-TBM nephritis, antiphospholipid syndrome (APS), autoimmune angioedema, autoimmune aplastic anemia, autoimmune dysautonomia, autoimmune hepatitis, autoimmune hyperlipidemia, autoimmune immunodeficiency, autoimmune inner ear disease (AIED), autoimmune myocarditis, autoimmune pancreatitis, autoimmune retinopathy, autoimmune thrombocytopenic purpura (ATP), autoimmune thyroid disease, autoimmune urticaria, axonal and neuronal neuropathies, Behcet's disease, bullous pemphigoid, autoimmune cardiomyopathy, Castleman disease, celiac disease, Chagas disease, chronic fatigue syndrome, chronic inflammatory demyelinating polyneuropathy (CIDP), chronic recurrent multifocal ostomyelitis (CRMO), Churg-Strauss syndrome, cicatricial pemphigoid/benign mucosal pemphigoid, Crohn's disease, Cogans syndrome, cold agglutinin disease, congenital heart block, coxsackie myocarditis, CREST disease, essential mixed cryoglobulinemia, demyelinating neuropathies, dermatitis herpetiformis, dermatomyositis, Devic's disease (neuromyelitis optica), discoid lupus, Dressler's syndrome, endometriosis, eosinophilic fasciitis, erythema nodosum, experimental allergic encephalomyelitis, Evans syndrome, fibromyalgia, fibrosing alveolitis, giant cell arteritis (temporal arteritis), glomerulonephritis, Goodpasture's syndrome, granulomatosis with polyangiitis (GPA), Graves' disease, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, hemolytic anemia, Henoch-Schonlein purpura, herpes gestationis, hypogammaglobulinemia, hypergammaglobulinemia, idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, IgG4-related sclerosing disease, immunoregulatory lipoproteins, inclusion body myositis, inflammatory bowel disease, insulin-dependent diabetes (type 1), interstitial cystitis, juvenile arthritis, Kawasaki syndrome, Lambert-Eaton syndrome, leukocytoclastic vasculitis, lichen planus, lichen sclerosus, ligneous conjunctivitis, linear IgA disease (LAD), lupus (SLE), Lyme disease, Meniere's disease, microscopic polyangiitis, mixed connective tissue disease (MCTD), monoclonal gammopathy of undetermined significance (MGUS), Mooren's ulcer, Mucha-Habermann disease, multiple sclerosis, myasthenia gravis, myositis, narcolepsy, neuromyelitis optica (Devic's), autoimmune neutropenia, ocular cicatricial pemphigoid, optic neuritis, palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus), paraneoplastic cerebellar degeneration, paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonnage-Turner syndrome, pars planitis

(peripheral uveitis), pemphigus, peripheral neuropathy, perivenous encephalomyelitis, pernicious anemia, POEMS syndrome, polyarteritis nodosa, type I, II, & III autoimmune polyglandular syndromes, polymyalgia rheumatica, polymyositis, postmyocardial infarction syndrome, postpericardiotomy syndrome, progesterone dermatitis, primary biliary cirrhosis, primary sclerosing cholangitis, psoriasis, psoriatic arthritis, idiopathic pulmonary fibrosis, pyoderma gangrenosum, pure red cell aplasia, Raynaud's phenomenon, reflex sympathetic dystrophy, Reiter's syndrome, relapsing polychondritis, restless legs syndrome, retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis, sarcoidosis, Schmidt syndrome, scleritis, scleroderma, Sjogren's syndrome, sperm & testicular autoimmunity, stiff person syndrome, subacute bacterial endocarditis (SBE), Susac's syndrome, sympathetic ophthalmia, Takayasu's arteritis, temporal arteritis/Giant cell arteritis, thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome, transverse myelitis, ulcerative colitis, undifferentiated connective tissue disease (UCTD), uveitis, vasculitis, vesiculobullous dermatosis, vitiligo, Waldenstrom's macroglobulinemia (WM), and Wegener's granulomatosis [Granulomatosis with Polyangiitis (GPA)].

More particularly, the method of the present invention is particularly suitable to treat a patient who is suffering from an autoimmune disease, including but not limited to the following: (1) a rheumatic disease such as rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, scleroderma, mixed connective tissue disease, dermatomyositis, polymyositis, Reiter's syndrome or Behcet's disease (2) type II diabetes (3) an autoimmune disease of the thyroid, such as Hashimoto's thyroiditis or Graves' Disease ( 4) an autoimmune disease of the central nervous system, such as multiple sclerosis, myasthenia gravis, or encephalomyelitis (5) a variety of phemphigus, such as phemphigus vulgaris, phemphigus vegetans, phemphigus foliaceus, Senear-Usher syndrome, or Brazilian phemphigus, ( 6) psoriasis, and (7) inflammatory bowel disease (e.g., ulcerative colitis or Crohn's Disease).

The administration of the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention may also be useful in the treatment of acquired immune deficiency syndrome (AIDS).

Another use for the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention includes the treatment of late phase HTLV (human T-cell lymphotrophic virus) I-induced adult T-cell leukemia-lymphoma, See Burton et al., Proc. Natl. Acad. Sci., 91:4935 (1994).

In some embodiments, the method of the present invention is used to treat a patient who has received a transplant of biological materials, such as an organ, tissue, or cell transplant. For

example, the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention may be particularly suitable, in promoting graft survival (allograft or xenograft). Typically the subject may have been transplanted with a graft selected from the group consisting of heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, bone, bone marrow, muscle, or bladder. The method of the present invention is also particularly suitable for preventing or suppressing an immune response associated with rejection of a donor tissue, cell, graft, or organ transplant by a recipient subject. Graft-related diseases or disorders include graft versus host disease (GVHD), such as associated with bone marrow transplantation, and immune disorders resulting from or associated with rejection of organ, tissue, or cell graft transplantation (e.g., tissue or cell allografts or xenografts), including e.g., grafts of skin, muscle, neurons, islets, organs, parenchymal cells of the liver, etc. Thus the method of the invention is useful for preventing Host-Versus-Graft-Disease (HVGD) and Graft-Versus-Host-Disease (GVHD). The specific interleukin-15 (IL-15) antagonist polypeptide of the present invention may be administered to the subject before, during and/or after transplantation (e.g., at least one day before transplantation, at least one day after transplantation, and/or during the transplantation procedure itself). In some embodiments, the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention may be administered to the subject on a periodic basis before and/or after transplantation.

Typically, the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention is typically administered in a therapeutically effective amount. By a "therapeutically effective amount" is meant a sufficient amount of the fusion protein or the antagonist of the invention to treat and/or to prevent the disease at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disease being treated and the severity of the disease; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied

over a wide range from 0.01 to 1,000 mg per adult per day. Preferably, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

A further object of the invention relates to pharmaceutical compositions comprising a specific interleukin-15 (IL-15) antagonist polypeptide of the present invention optionnaly with a pharmaceutically acceptable carrier or excipient. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms. Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions comprising

compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The specific interleukin-15 (IL-15) antagonist polypeptide of the present invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Sterile injectable solutions are prepared by incorporating the active polypeptides in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous

solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. The specific interleukin-15 (IL-15) antagonist polypeptide of the present invention may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. Multiple doses can also be administered. In addition to the compounds of the invention formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; liposomal formulations; time release capsules; and any other form currently used.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

**FIGURES:**

**Figure 1. Effect of mutated RLI or mutated IL-15 on cell proliferation.** Kit-225 (A), TF-1β (B) and NK-92 (C) cells were cultured for 72 h with increasing concentrations of IL-15 (■), RLI (●), RLI 101-108 (▲), RLI 65-69-101-108 (◇), RLI 65 (▽). Kit-225 (D) and NK-92 (E) cells were cultured for 72 h with increasing concentrations of IL-15 (■), IL-15 101 (○) or IL-15 108 (△). Cell proliferation was quantified by AlamarBlue® assay.

**Figure 2. Effect of mutated RLI on RLI induced cell proliferation.** Kit-225 (A), (A), TF-1β (B) and NK-92 (C) cells were cultured for 72 h with a fixed concentration of RLI (40 pM for Kit-225 and TF-1ß; 10 pM for NK-92) and increasing concentrations of RLI 101-108 (■), RLI 65-69-101-108 (○) or RLI 65 (●). Cell proliferation was quantified by AlamarBlue® assay.

**Figure 3. Effect of mutated RLI on IL-15 induced cell proliferation.** Kit-225 (A), (A), TF-1β (B) and NK-92 (C) cells were cultured for 72 h with a fixed concentration of IL-15

(80 pM for Kit-225, 40 pM for TF-1ß and NK-92) and increasing concentrations of RLI 101-108 (■), RLI 65-69-101-108 (○) or RLI 65 (●). Cell proliferation was quantified by AlamarBlue® assay.

**Figure 4. Effect of mutated IL-15 on IL-15 or IL-2 induced cell proliferation.** Kit-225 (left panels) and NK-92 (right panels) cells were cultured for 72 h with a fixed concentration of IL-15 (A) or IL-2 (B) (80 pM for Kit-225 and 40 pM for NK-92) and increasing concentrations of RLI 101-108 (■), IL-15 101 (●) or IL-15 108 (○). Cell proliferation was quantified by AlamarBlue® assay.

**Figure 5. Effect of mutated RLI on IL-2 induced cell proliferation in the presence of IL-2Ralpha blocking antibody.** Kit-225 (A) or NK-92 (B) cells were cultured for 72 hr with a fixed concentration of IL-2 (500 or 80 pM respectively), 33B3.1 blocking antibody (5nM) and increasing concentrations of RLI 101-108 (■) or RLI 65-69-101-108 (○). Cell proliferation was quantified by AlamarBlue® assay.

**Figure 6. Effect of mutated RLI before a brief stimulation of NK-92 cells by IL-15 or IL-2.** NK-92 cells were incubated with 200 nM of mutated RLI for 30 min before a 30 min stimulation with IL-15 (A) or IL-2 (B) (40 pM). Cells were then cultivated for 24 h and cell proliferation was quantified by AlamarBlue® assay.

**Figure 7. Effect of mutated RLI after a brief stimulation of NK-92 cells by IL-15 or IL-2.** NK-92 cells were incubated with 1 nM of IL-15 (A) or IL-2 (B) for 30 min. After 2 washes, cells were cultivated for 24 h with fixed concentration of mutated RLI (200 nM). Cell proliferation was quantified by AlamarBlue® assay.

**Figure 8. Effect of mutated RLI on the propagation of metabolic activity of NK-92 cells.** NK-92 cells were stimulated for 30 min with IL-15 (A) or IL-2 (B) (1 nM). After 2 washes, stimulated cells were cultivated with cytokine starved NK-92 cells in the presence of mutated RLI (50 nM) for 24 h. Cell proliferation was quantified by AlamarBlue® assay.

**Figure 9. In vivo effect of mutated RLI on NK and CD4 T-reg homeostasis.** PBS or mutated RLI were injected every day for 3 days (2 ug per injetion). Mice were sacrified one day later. Spleen and bone marrow were collected, and cells were isolated. NK cells were identified as NK1.1+ CD3- and T-reg cells were CD3+ CD4+ CD25+ FoxP3+.

**Figure 10. Molecular modelling based predicted effects of different mutations at position Q101 or Q108 on the antagonistic properties of IL-15.**

**Figure 11.** SPR sensorgrams of the binding (association and dissociation phases) of increasing concentrations of indicated molecules (3.1, 6.2, 12.5, 25, 50 and 100 nM) to immobilized CD122 (*A* and *C*) or Fc-IL-15Rα (*B* and *D*) chains. (*E*) SPR sensorgrams of

sequential binding of increasing concentrations of wt.RLI or $RLI_{Q108D}$ to immobilized CD122 chain, followed by the binding of CD132 chain.

**Figure 12** (*A*) Flow cytometric analysis of CD122 expression on the surface of $RLI_{Q108D}$ or $RLI_{N65K}$ pre-treated NK-92 cells revealed by blocking TU-27 or non-blocking CF-1 anti-CD122 mAbs. (*B*) Competition studies of indicated molecules with radioiodinated wt.RLI (1 nM) binding to NK-92 cells.

**Figure 13.** (*A*) Proliferation assay of Kit225 or (*B*) NK-92 cells cultured with increasing concentrations of indicated molecules.

**Figure 14.** (*A*) Flow cytometric analyses of wt.RLI-induced Stat5 and Stat3 phosphorylations in the absence or in the presence of $RLI_{Q108D}$ or $RLI_{N65K}$. Isotype control is shown. (*B*) Proliferation assay of Kit225, NK-92 and TF-1β cells cultured with a fixed concentration of wt.RLI (40 pM) and increasing concentrations of $RLI_{Q108D}$ or $RLI_{N65K}$.

**Figure 15.** (*A*) Analysis of p-Stat5 expression within Kit225 responding cells following 1 h co-culture with IL-15 (25 pM) loaded or un-loaded stably transfected IL-15R〈 HeLa presenting cells in the presence or in the absence of 50 nM of $RLI_{Q108D}$ or $IL\text{-}15_{Q108D}$. Numbers indicate the percentage of cells in the indicated gate. (*B*) Percentage of p-Stat5 positive Kit225 responding cells in response to increasing concentrations of IL-15 loaded on IL-15R〈 HeLa presenting cells in the presence of $RLI_{Q108D}$ (100 nM).

**Figure 16.** (*A*) Proliferation assay of TF-1β cells cultured with a fixed concentration of IL-2 (1 nM) and increasing concentrations of $RLI_{Q108D}$. (*B*) CD8 T cells were stimulated with 5 nM of indicated cytokines in the presence or in the absence of $RLI_{Q108D}$ (50 nM). (*C*) The proliferation of human PBMCs was evaluated by flow cytometry. Isolated PBMCs were labeled by VPD-450 and cultured with increasing concentrations of IL-15 or (*D*) IL-2 in the presence or in the absence of 100 nM of $RLI_{Q108D}$. CD8 T cells were identified as CD3+ CD8+ cells.

**Figure 17.** (*A*) Analysis of p-Stat5 within splenic NKp46+ CD3- NK and (B) CD8 T cells from mice treated as indicated. (n = 5 per conditions).

**Figure 18.** IL-2Rα impairs $RLI_{Q108D}$ inhibition of IL-2 signaling. (*A*) Inhibition assay of IL-15- and IL-2-induced Stat5 phosphorylation within Kit225 cells treated with $RLI_{Q108D}$ (white bars) for 1h or not (black bars), revealed by alpha screen technology. (*B*) Proliferation assay of Kit225 cells cultured with a fixed concentration of IL-2 (80 pM) and increasing concentrations of $RLI_{Q108D}$ or $RLI_{N65K}$. (*C*) Proliferation assay of Kit225 cells cultured with a fixed concentration of IL-2 (80 pM) and 33B3.1 anti-CD25 mAb (66 nM) and increasing concentrations of $RLI_{Q108D}$ or $RLI_{N65K}$. (*D*) Proliferation assay of Kit225 cells cultured with a

fixed concentration of IL-15 (40 pM) and increasing concentrations of $RLI_{Q108D}$ and/or IL-$15_{Q108D}$. (*E*) The proliferation of NK cells from human PBMCs was evaluated by flow cytometry. Isolated VPD-450-labeled PBMCs were cultured with a fixed concentration of IL-15 or (*F*) IL-2 in the presence or in the absence of 33B3.1 anti-CD25 mAb and increasing concentrations of $RLI_{Q108D}$. NK cells were identified as CD3- NKp46+ cells. (*G*) IL-2-induced proliferation of CD4 T cells was evaluated by VPD-450 dilution over CD25 expression in the presence or in the absence of $RLI_{Q108D}$. (*H*) IL-2-induced proliferation of CD25- or (*I*) CD25+ CD4+ CD3+ T cells was evaluated by VPD-450 dilution over CD25 expression in the presence or in the absence of 100 nM of $RLI_{Q108D}$.

**Figure 19.** (*A*) Mice were treated or not with $RLI_{Q108D}$ prior from administrating poly(I:C). NK cells in the blood from mice were analyzed for IFNγ and CD69 expression by flow cytometry. (*B*) Analysis of CD69 expression on CD8 and (*C*) CD4 T cells in the blood from mice treated as indicated.

**Figure 20.** (*A*) Percentage of FoxP3+ CD25+ CD4 T cells among total CD4 T cells from human PBMCs was analyzed by flow cytometry. (*B*) Ratio of proliferative CD4 regulatory T-cells over CD8 T cells among human PBMCs stimulated with indicated concentrations of IL-2 in the presence or in the absence of $RLI_{Q108D}$. (*C*) Splenic CD44+ CD8+ T cells or (*D*) CD3- NKp46+ NK cells from mice treated as indicated were analyzed by flow cytometry. (*E*) Numbers of splenic NK cells at the indicated stages of maturation from mice treated as indicated. (*F*) Splenic FoxP3+ CD25+ CD4 T cells were analyzed by flow cytometry and (*G*) graph showing the ratio of CD4 regulatory T-cells over NK cells among lymphoid gate.

**Figure 21.** (*A*) Twenty weeks old mice were treated with $RLI_{Q108D}$ and splenic FoxP3+ CD25+ CD4 T cells were analyzed. (*B*) Skin from Balb/c mice was grafted to DBA/2 recipient mice. Mice (n = 10, per conditions) were treated daily with 10 µg of $RLI_{Q108D}$ and graft survival was monitored.

**EXAMPLE 1:**

**Material & Methods**

***Cell culture***

The non adherent Kit-225 human T lymphoma cell line, obtained from Dr. D. Cantrell (University of Dundee, Scotland, U.K.), was cultured in RPMI-1640 medium (Gibco-Invitrogen) containing 6% heat-inactivated fetal calf serum, 2 mM glutamine, and 5 ng/mL human rIL-2. The nonadherent TF-1ß human erythroleukemia cell line, kindly provided by Dr. B. Azzarone (Institut Gustave-Roussy, Villejuif, France) was culture in RPMI 1640 medium containing 10% heat inactivated fetal calf serum, 2mM glutamine, 1 ng/mL GM-CSF ans 250

µg/mL geneticin. The NK-92 cell line, kindly provided by Henri Vié (Inserm U892, Nantes, France), was cultured in RPMI-1640 medium (Gibco-Invitrogen) containing 10% heat-inactivated human serum, 2 mM glutamine, and 5 ng/mL human rIL-2.

***Proliferation assays***

The proliferative responses of Kit-225, TF-1ß and NK-92 cells to cytokine was quantified by AlamarBlue® assay (AbD serotec, Kidlington, UK). Briefly, cells were starved in the culture medium without cytokine during 24 hrs for Kit-225 or 5 hrs for TF-1ß and NK-92. Cells were plated at $1.10^4$ cells in 100 µl and cultured for 48 h in medium supplemented with increasing concentrations of tested protein, or in medium supplemented with a fixed concentration of human rIL-15, human rIL-2 or RLI and increasing concentrations of tested antagonist. Cells were pulsed for 6 h with 10 µL per well of AlamarBlue. Cell proliferation was assessed by measuring fluorescence at 550/590 nm using Enspire plate reader (Perkin elmer, Hopkinton MA, USA).

***Short cytokine stimuli***

The proliferative responses of Kit-225, TF-1ß and NK-92 cells to a short cytokine stimulus was quantified by AlamarBlue® assay. Briefly, cells were starved in the culture medium without cytokine during 24 h. They were incubated at $2.10^5$ cells per mL with 40 pM or 1 nM of human rIL-15 or rIL-2 for 30 min, washed twice and cultured for 24 h in medium supplemented with fixed concentrations of tested protein. Cell metabolic activity was assayed by AlamarBlue as described previously. In a second set of experiments, the 30 min cytokine stimuli were realized on cells in contact with tested protein since 30 min.

***Propagation of viability signal***

The propagation of cell metabolic activity was detected by AlamarBlue® assay. Briefly, cells were starved in the culture medium without cytokine during 24hrs. Cells were incubated $2.10^5$ cells per mL with 1nM of human rIL-15 or rIL-2 for 30 minutes, washes twice and $2.10^4$ of these activated cells were cultured 24hrs with $2.10^4$ of starved in the presence of antagonist. Cell viability was assessed by AlamarBlue, as previously described.

***In vivo effect of mutated RLI on NK and T-reg populations.***

PBS or mutated RLI were injected every day for 3 days (2 ug per injetion). Mice were sacrified one day later. Spleen and bone marrow were collected, and cells were isolated. NK cells were identified as NK1.1+ CD3- and T-reg cells were CD3+ CD4+ CD25+ FoxP3+, by flow cytometry.

**Results**

In order to inhibit IL-15 acting through trans-presentation, we have generated a fusion molecule that binds to CD122 with a higher infinity that IL-15 alone (RLI), and mutated at the CD132 interface ($RLI_{Q108D}$). In consequence, the mutated RLI binds efficiently CD122 chain but is unable to transduce a signal within responding cells. We observed that Kit-225, TF-1b and NK-92 cells proliferate in response to both wild-type IL-15 and RLI, whereas no proliferation signal was detected in response to the mutated RLI (Figure 1). Then, we tested whether the mutated RLI was able to inhibit wild-type RLI-dependent proliferation that directly targets CD122/CD132 dimeric receptor. We found that the mutated RLI efficiently inhibits RLI-dependent cell proliferation, reaching a IC50 at 2 nM (Figure 2). Surprisingly, we observed that the mutated RLI efficiently abrogated IL-15-dependent proliferation (Figure 3), whereas no inhibition was observed on IL-2-dependent proliferation (Figure 4). We found that the mutated RLI was able to inhibit IL-2 dependent signal only when CD25 was blocked (33B3.1 blocking Ab) (Figure 5). To confirm the potency of the mutated RLI to inhibit IL-15 signal, we assessed a "pulse stimulation" assay by incubating cells with cytokines for 30 min, then after washes to remove unbound cytokine, cells were resuspended in cytokine-free media for 24h. We observed that the mutated RLI efficiently decreased cell responses to IL-15 and not to IL-2 (Figure 7 and 8). Lastly, we investigated whether the mutated RLI could affect NK and T-reg population on mice. For that purpose, we injected the mutated RLI or PBS every day for 3 days (2ug per injection). One day later, mice were sacrified and NK and T-reg were isolated from spleen and bone marrow. We observed that NK population decreased after injection of the mutated RLI in the spleen. Whereas T-regs population remained unchanged. No difference was observed in the bone marrow (Figure9).

**EXAMPLE 2:**

**Materials and Methods :**

**Cell lines**

The non-adherent Kit225 human T lymphoma cell line was cultured in RPMI-1640 medium (Gibco-Invitrogen) containing 6% heat-inactivated fetal calf serum, 2 mM glutamine, and 5 ng/mL human rIL-2. The NK-92 cell line, kindly provided by Henri Vié (CRCNA, Nantes, France), was cultured in RPMI-1640 medium (Gibco-Invitrogen) containing 10% heat-inactivated human serum, 2 mM glutamine, and 5 ng/mL human rIL-2. The non-adherent TF-1ß human erythroleukemia cell line was cultured in RPMI 1640 medium containing 10% heat inactivated fetal calf serum, 2 mM glutamine, 1 ng/mL GM-CSF and 250 µg/mL geneticin.

Healthy donor blood samples were collected from the French blood bank (Etablissement Français du Sang (EFS)). Peripheral Blood Mononuclear Cells (PBMC) were isolated on a Ficoll gradient. All cell lines were maintained at 37 °C, in a humidified, 5% CO2 atmosphere.

**Surface Plasmonic Resonance studies**

The SPR experiments were performed with a BIAcore 3000 biosensor (BIAcore, Uppsala, Sweden). Recombinant proteins (hCD122, hCD132, Fc-IL-15Rα) were covalently linked to CM5 sensor chips, and the binding of increasing concentrations of ligands (IL-15, IL-$15_{Q108D}$, RLI, $RLI_{Q108D}$, $RLI_{N65K}$) was monitored. Analysis of sensorgrams was performed using BIAlogue kinetics evaluation software.

**Alpha Screen SureFire Assay**

Kit225 cells were starved for 12 h in a serum-free medium without IL-2 and plated in 96-well plates at a density of $2.10^5$ cells/well. Kit225 cells were incubated with $RLI_{Q108D}$ for 30 minutes then stimulated with 40 pM of IL-15 or IL-2 for 1 h. Stat5 phosphorylation was measured using the p-Stat5 Alphascreen Surefire kit (PerkinElmer Life Sciences). After stimulation, cells were lysed. Then, 4 µl of lysates were transferred to a 384-well white Optiplate (PerkinElmer Life Sciences), and Stat5 phosphorylation was measured according to the manufacturer's instructions. Stat5 phosphorylation values obtained from untreated condition were subtracted from values obtained in presence of ligands for specific Stat5 phosphorylation values.

**Proliferation assay**

The proliferative responses of Kit225 cells to cytokine were quantified by alamarBlue assay (AbD serotec, Kidlington, UK). Briefly, cells were starved in a cytokine-free culture medium overnight for Kit225 cells. Cells were plated at $10^4$ cells in 100 µl and cultured for 72 hrs in medium supplemented with a fixed concentration of human rIL-15, human rIL-2 or RLI and increasing concentrations of $RLI_{Q108D}$ antagonist. Cells were pulsed for 6 hrs with 10 µL per well of AlamarBlue. Cell proliferation was assessed by measuring fluorescence at 550/590 nm using Enspire plate reader (Perkin Elmer, Hopkinton MA, USA).

**Flow cytometry**

Cell surface expression of h/mCD3, h/mCD8, h/mCD4, h/mNKp46, mNK1.1, mCD27, mCD11b, mCD44, h/mCD132, mCD122, h/mCD25, hIL-15Rα was performed using commercial antibodies. Before staining, Fc receptors were blocked for 15 min at 4°C with PBS 1% human sera. Cells were incubated for 30 min at 4°C with antibodies for cell surface staining. Analyses of Stat5 and Stat3 phosphorylations were performed following the protocol of the

manufacturer using Lyse/Fix Buffer and Perm Buffer III (BD Biosciences). FoxP3 staining was performed following the protocol outlined in the Foxp3 kit (eBioscience). Samples were acquired on an 8-colors FACS Canto II cytometer (BD bioscience). Analyses were performed using FlowJo software 9.9 (Beckman Coulter).

To study the inhibition of IL-15 *trans*-presentation by $RLI_{Q108D}$, IL-15Rα stably transfected HeLa cell lines were incubated with IL-15 for 1 hour at 37°C, to allow IL-15 to bind to IL-15Rα and were washed to remove soluble IL-15. Starved Kit225 were incubated with $RLI_{Q108D}$ for 1h and then cocultured with presenting HeLa cells during 1 hour. The cells were fixed and permeabilized for intracellular p-Stat5 expression analysis.

For Proliferation assay of human PBMC, cells were isolated on a Ficoll gradient. Then, cells were washed in PBS and $10.10^6$ PBMC were incubated for 10 min at 37°C with a 1 ⌈M final concentration of BD Horizon™ Dye 450 (VPD-450, BD Biosciences). Reaction was stopped by 10-fold dilution with PBS and cells were washed in complete medium R10 (RPMI 1640 medium supplemented with 1 mM sodium/pyruvate, 1 mM non essential amino acids, 100 IU/mL penicillin/streptomycin, 2 mM L-Glutamine and 10% FBS). PBMC were seeded in a 96-well round bottom plate at $1.10^6$ cells/mL in complete medium R10 and incubated at 37°C at indicated concentrations of cytokines and variants.

***In vivo* models**

C57BL/6, Balb/c, and DBA/2 mice were purchased from the Janvier Laboratory. All mice were usually used between the age of 6 and 12 weeks. All animal experiments were approved by the Regional Ethics Committee in Animal experimentation (Pays de la Loire, France) and performed according to European Union Guidelines (authorization number no. B44-278).

For *in vivo* Stat5 phosphorylation assay, 10 µg of $RLI_{Q108D}$ or PBS were administrated i.p. to C57BL/6 mice, 30 min prior from another 30 min i.p. stimulation with 2 µg of IL-2. Mice were sacrificed and subsequently isolated splenocytes were fixed. Cell suspensions from spleen were prepared by mechanical dilacerations followed by staining for phenotypic analysis. Membrane NKp46 and CD8 staining were performed before cell permeabilization and intracellular p-Stat5 staining.

For poly(I:C) activation, mice were pretreated or not overnight with 10 µg of $RLI_{Q108D}$. Then, mice were activated with 25 µg/g poly(I:C) together or not with 10 µg of $RLI_{Q108D}$ and studied for NK and CD8 T cell activation in the blood. Surface CD69 expression and intracellular IFNγ after 6 h were analyzed by flow cytometry.

Transplants experiments were performed using sex-matched animals. Mice were anesthetized with a mixture of 75 mg/kg of ketamine and 15 mg/ kg of xylazine. The skin transplantation was performed on the right lateral flank of DBA/2 (H-$2^d$) recipient mice with a full-thickness tail skin of Balb/c (H-$2^d$) donor mice. The skin graft was secured with an adhesive bandage for the initial 5 days. Mice were divided into two groups: untreated and $RLI_{Q108D}$-treated groups. Mice were treated with daily intraperitoneal dose (10 µg/mouse) of $RLI_{Q108D}$. Graft survival was then followed by daily visual inspection. All treatments were started on day before the day of skin graft. Rejection was defined as a loss of > 80% of the transplanted skin tissue.

**Results:**

**$RLI_{Q108D}$: an IL-15.IL-15Rα fusion molecule with increased affinity for CD122 and impaired recruitment of CD132.**

In order to inhibit IL-2 and IL-15 dependent effector functions, we developed an original approach targeting the CD122/CD132 dimer, by combining an increased affinity for CD122 and an impaired recruitment of CD132. On one hand, we found that the low affinity of IL-15 for soluble CD122 (Kd = 25 nM) (Figure 11A) was increased by 14-fold when IL-15 was covalently linked to IL-15Rα (RLI fusion molecule). On the other hand, the mutation of Q108 residue to D on IL-15 has been shown to abrogate its binding to the CD132 chain. This mutation does not affect IL-15/IL-15Rα complex formation, as an $IL\text{-}15_{Q108D}$ mutein bound immobilized Fc-IL-15Rα with a similar affinity as wild-type IL-15 (Figure 11B). We have thus generated $RLI_{Q108D}$, a fusion molecule between $IL\text{-}15_{Q108D}$ mutein covalently linked to the soluble IL-15Rα sushi domain. As a control, $RLI_{N65K}$ was produced in which CD122 recruitment was abrogated by introducing the N65K mutation in the IL-15 moiety. Surface Plasmonic Resonance analyses showed, as expected, that $RLI_{Q108D}$ retained a similar affinity for CD122 chain as wild-type RLI (Figure 11A and 11C), whereas no binding to immobilized Fc-IL-15Rα was observed (Figure 11D). By contrast, $RLI_{N65K}$ was unable to bind CD122 (Figure 11C). Furthermore, soluble CD132 binding to RLI/CD122 complex was completely abrogated when introducing in RLI the Q108D substitution ($RLI_{Q108D}$) (Figure 11E). In the context of cell membrane-anchored receptors (using Kit-225 and NK-92 cells expressing CD122, CD132, IL-15Rα and IL-2Rα (CD25)), the binding of TU-27 (an anti-CD122 mAb that blocks the binding of IL-2 and IL-15 to CD122) was significantly inhibited by $RLI_{Q108D}$, whereas $RLI_{N65K}$ had no effect. As a control, the binding of the non-blocking CF-1 anti-CD122 mAb was unaffected by either $RLI_{Q108D}$ or $RLI_{N65K}$ (Figure 12A). Accordingly, $RLI_{Q108D}$ competed out radio-iodinated

wt.RLI binding to CD122/CD132 dimeric receptor on NK-92 cells, with IC50 (20 nM) that was however 10-fold higher than that of wt.RLI (2 nM), due to the absence of recruitment of CD132 (Figure 12B). Thus, $RLI_{Q108D}$ is an original IL-15 derived molecule that combines an increased affinity for CD122 and an impaired recruitment of CD132.

**$RLI_{Q108D}$ inhibits both IL-15 and IL-2 signaling through their common CD122/CD132 dimeric receptor.**

Regarding the biological activity of the molecules, as expected, wt.RLI, by acting through the CD122/CD132 dimer, induced Kit225 and NK-92 proliferations (EC50 = 50 pM), whereas $RLI_{Q108D}$, $RLI_{N65K}$ and $IL\text{-}15_{Q108D}$ were inactive (Figure 13A and 13B), confirming the necessity of the recruitment of both CD122 and CD132 chains. As expected from the binding studies, $RLI_{Q108D}$ efficiently inhibited both wt.RLI-induced Stat3 and Stat5 phosphorylations (Figure 14A), as well as Kit225, NK-92 and TF-1β cell proliferations (Figure 14B), with an IC50 of 5 nM. No inhibitory activity of $RLI_{N65K}$ was observed (Figure 14A and 14B), reinforcing the interest of targeting CD132 recruitment, instead of CD122 recruitment.

Moreover, IL-15 *trans*-presentation by IL-15Rα transfected Hela cells to CD122/CD132 on Kit225 cells was significantly abrogated by $RLI_{Q108D}$ revealing its potency to inhibit IL-15 signaling even during cell-cell interaction (Figure 15A and 15B). By contrast, and most importantly, $IL\text{-}15_{Q108D}$ was unable to exert such inhibitory effect (Figure 15A), highlighting the importance of the increased affinity of $RLI_{Q108D}$ over $IL\text{-}15_{Q108D}$ for targeting the CD122/CD132 dimer and exerting an inhibitory action.

To test $RLI_{Q108D}$ efficiency on IL-2 signaling through CD122/CD132 dimeric receptor, we used TF-1β cells that express CD122 and CD132 but not IL-2Rα. $RLI_{Q108D}$ efficiently inhibited IL-2-dependend TF-1β cell proliferation with an IC50 of 3 nM (Figure 16A). Then, we extended our analysis to human primary cells. VPD-450 labeled PBMCs were stimulated with IL-15 or IL-2 in the presence of $RLI_{Q108D}$. We found that CD8 T cell proliferations in response to IL-15 and IL-2 were both inhibited by $RLI_{Q108D}$ (Figures 16B-D).

The inhibitory efficiency of $RLI_{Q108D}$ was further confirmed *in vivo*. When $RLI_{Q108D}$ was administrated into mice prior to injection of wt.RLI, the level of Stat5 phosphorylation within splenic NK cells and CD8 T cell was found to be reduced as compared to that of NK cells from untreated mice (Figure 17A an 17B). Thus, IL-2 and IL-15 signaling through the CD122/CD132 dimeric receptor was efficiently inhibited by $RLI_{Q108D}$ *in vitro* and *in vivo*.

**IL-2Ra impairs $RLI_{Q108D}$ inhibition of IL-2 signaling through its IL-2Ra/CD122/CD132 trimeric receptor.**

To address the question whether IL-2Rα and IL-15Rα chains could impact the blocking effect of $RLI_{Q108D}$, we again took advantage of Kit225, NK-92 and TF-1β cells that respond to exogenous IL-2 and IL-15 through their specific trimeric receptor (IL-2Rα/CD122/CD132 for IL-2 and IL-15Rα/CD122/CD132 for IL-15; *cis*-presentation). Surprisingly, IL-15-induced, but not IL-2-induced, Stat5 phosphorylation in Kit225 cells was inhibited by $RLI_{Q108D}$ (Figure 18A). Accordingly, $RLI_{Q108D}$ left IL-2-induced cell proliferation unaffected (Figure 18B). Interestingly, an inhibitory activity of $RLI_{Q108D}$ on IL-2 -induced cell proliferation was revealed when adding an antibody (33B3.1) that blocks IL-2 binding to CD25, suggesting that the presence of IL-2Rα precluded $RLI_{Q108D}$ inhibitory action (Figure 18C). In both assays, $RLI_{N65K}$ was inactive (Figure 18B and 18C).

$RLI_{Q108D}$ inhibited IL-15-induced cell proliferation, with an IC50 of 30 nM, 6-fold higher than that of RLI-induced proliferation. Moreover, we found that blocking IL-15Rα by $IL\text{-}15_{Q108D}$ enhanced $RLI_{Q108D}$ inhibitory activity (Figure 18D). This indicates that $RLI_{Q108D}$ inhibitory activity was slightly impaired in the context of the trimeric receptor, and therefore that the presence of IL-15Rα could affect its inhibitory action.

We extended our analysis to human primary cells. IL-15-induced NK cell proliferation was fully abrogated in the presence of $RLI_{Q108D}$ with an IC50 of 5 nM (Figure 18E). By contrast, IL-2-induced NK cell proliferation was only partially inhibited by $RLI_{Q108D}$, with a high degree of variability among experiments. However, when the blocking anti-CD25 33B3.1 mAb was added to the culture, IL-2-induced NK cell proliferation by $RLI_{Q108D}$ was fully efficient, with a similar IC50 as for inhibition of IL-15-induced proliferation (Figure 18F). These results confirm the impairment of $RLI_{Q108D}$ action by IL-2Rα. Accordingly to what observed previously, $RLI_{Q108D}$ inhibited the IL-2-induced proliferation of CD4 CD25- T cells, but not that of CD25 expressing conventional CD4 T cells (Figures 18G-I). Taken together, these results indicate that $RLI_{Q108D}$ was less efficient in the context of the trimeric receptor, and therefore that the presence of IL-15Rα can also affect its inhibitory action, although not as dramatically as IL-2Rα.

**Inhibition of CD122/CD132 dimeric receptor by $RLI_{Q108D}$ limits cell activation and promotes tolerance.**

IL-15 and IL-15Rα are induced during inflammation. To further describe the potency of $RLI_{Q108D}$ to inhibit inflammation related cell activation, poly(I:C) was administrated to mice. IFNγ expression by NK cells from poly(I:C) stimulated mice was significantly impaired by $RLI_{Q108D}$, without affecting CD69 expression at the cell surface (Figure 19A). However, CD69

expression was decreased on CD8 and CD4 T cells when $RLI_{Q108D}$ was injected together with poly(I:C) (Figure 19B and 19C). Thus, TLR stimulation *in vivo* requires CD122/CD132 dimeric receptor for supporting NK and T cells activation and is inhibited by $RLI_{Q108D}$.

IL-15R〈 is broadly expressed independently of CD122 and CD132, whereas IL-2Rα expression is restricted mainly to CD4 regulatory T cells (T-reg) and activated T cells. Accordingly to our observations, enrichment by IL-2 of CD4 T cells in FoxP3+ positive cells from human PBMCs was not affected by $RLI_{Q108D}$ (Figure 20A). As a net result, $RLI_{Q108D}$ treatment shifted the T-reg over CD8 T cell ratio in favor of T-regs (Figure 20B), highlighting its potential in the promotion of tolerance.

In order to evaluate $RLI_{Q108D}$ efficiency *in vivo*, $RLI_{Q108D}$ was administrated in mice together with IL-2. As expected, IL-2 injection led to an increase of CD44+ CD8 T and NK cells that was completely abrogated by $RLI_{Q108D}$ (Figure 20C and 20D). Moreover, by studying NK cell maturation, we found that the CD11b+ CD27- mature NK cell population was dramatically decreased (Figure 20E). Interestingly, the CD4 T-reg population was not inhibited but even promoted by $RLI_{Q108D}$ when injected together with IL-2 (Figure 20F), leading to a balance in favor to T-reg cells over NK cells in wild-type mice (Figure 20G), confirming the results obtained with human PBMCs. Interestingly, high amount of T-reg cells were found within aged mice, and injection of $RLI_{Q108D}$ by itself significantly enhanced splenic T-reg population (Figure 21A).

Regulatory T cells could prevent allogeneic graft rejection by inhibiting T cell activation. Thus, we wondered whether $RLI_{Q108D}$, that favors by it-self tolerance over cell activation, could present a potential therapeutical benefit using a transplantation model in which skin from Balb/c mice was grafted to DBA/2 recipient. We found that all untreated mice rejected the allogeneic skin graft by day 14. However, a daily treatment with $RLI_{Q108D}$ post-graft resulted in an increase of graft survival (Figure 21B), supporting the idea that $RLI_{Q108D}$ could favor tolerance.

**SEQUENCES:**

SEQ ID NO:1

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNV AHWTTPSLKCIRDPALVHQRPAPP

SEQ ID NO:2

SGGSGGGGSGGGSGGGGSLQ

SEQ ID NO:3

SGGSGGGGSGGGSGGGGSGG

SEQ ID NO:4

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLES
GDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

SEQ ID NO:5

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNV
AHWTTPSLKCIRDPALVHQRPAPPSGGSGGGGSGGGSGGGGSGGNWVNVISDLKKIE
DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANN
SLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIV**D**MFINTS

SEQ ID NO:6

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNV
AHWTTPSLKCIRDPALVHQRPAPPSGGSGGGGSGGGSGGGGSGGNWVNVISDLKKIE
DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANN
SLSSNGNVTESGCKECEELEEKNIKEFL**D**SFVHIV**D**MFINTS

**REFERENCES:**

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

## CLAIMS:

1. A specific interleukin-15 (IL-15) antagonist polypeptide comprising i) a IL15-Ralpha sushi-containing polypeptide comprising an amino acid sequence having at least 80% of identity with the amino acid sequence of SEQ ID NO:1 ii) a linker and iii) an IL-15 polypeptide comprising the amino acid sequence having at least at least 80% of identity with the amino acid sequence of SEQ ID NO:4 provided that the glutamine (Q) residue at position 108 is mutated.

2. The specific IL-15 antagonist polypeptide of claim 1 wherein the linker is a peptidic linker of 2-30 amino acids, preferably of 10-30 amino acids, more preferably of 15-30 amino acids, still more preferably of 19-27 amino acids, most preferably of 20-26 amino acids.

3. The specific IL-15 antagonist polypeptide of claim 1 wherein the linker consists of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:3.

4. The specific IL-15 antagonist polypeptide of claim 1 wherein the glutamine (Q) residue at position 108 in the IL-15 polypeptide is substituted by an amino acid residue selected from the group consisting of aspartic acid (D), glutamic acid (E), lysine (K), serine (S), threonine (T), asparagine (N), cysteine (C), alanine (A), glycine (G), valine (V), leucine (L), proline (P), methionine (M) and tryptophan (W).

5. The specific IL-15 antagonist polypeptide of claim 4 wherein the glutamine (Q) residue at position 108 is substituted by an aspartic acid (D) residue.

6. The specific IL-15 antagonist polypeptide of claim 1 wherein the glutamine (Q) residue at position 101 in the IL-15 polypeptide is also mutated.

7. The specific IL-15 antagonist polypeptide of claim 6 wherein the glutamine (Q) residue at position 101 is substituted by an amino acid residue selected from the group consisting of aspartic acid (D) residue, lysine (K), and tryptophan (W).

8. The specific IL-15 antagonist polypeptide of claim 6 wherein the glutamine (Q) residue at position 101 is substituted by an aspartic acid (D) residue.

9. The specific IL-15 antagonist polypeptide of claim 1 wherein both glutamine (Q) residues at position 101 and 108 in the IL-15 polypeptide are substituted by an aspartic acid (D) residue.

10. The specific IL-15 antagonist polypeptide of claim 1 comprises an amino acid sequence having at least 80% of identity with the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:6.

11. The specific IL-15 antagonist polypeptide of claim 1 comprises the specific interleukin-15 (IL-15) antagonist polypeptide of the present invention consists of SEQ ID NO:5 or SEQ ID NO:6.

12. The specific IL-15 antagonist polypeptide of claim 1 which is fused to an immunoglobulin domain

13. The specific IL-15 antagonist polypeptide of claim 1 wherein the immunoglobulin domain is an immunoglobulin constant domain (Fc region).

14. A nucleic acid encoding for the specific interleukin-15 (IL-15) antagonist polypeptide of claim 1.

15. A vector comprising the nucleic acid of claim 14.

16. A host cell a host cell which has been transfected, infected or transformed by the nucleic acid of claim 14 and/or the claim 15.

17. The specific interleukin-15 (IL-15) antagonist polypeptide of claim 1 for use as a drug.

18. A method of suppressing the immune response in a patient by administering a dose of the specific interleukin-15 (IL-15) antagonist polypeptide of claim 1, and thereby modulates IL-15 dependent immune responses.

19. The method of claim 18 wherein the patient suffers from an autoimmune disease.

20. The method of claim 19 wherein the patient suffers from an autoimmune disease selected from the group consisting of acute disseminated encephalomyelitis (ADEM), acute necrotizing hemorrhagic leukoencephalitis, Addison's disease, agammaglobulinemia, alopecia areata, amyloidosis, ankylosing spondylitis, anti-

GBM/Anti-TBM nephritis, antiphospholipid syndrome (APS), autoimmune angioedema, autoimmune aplastic anemia, autoimmune dysautonomia, autoimmune hepatitis, autoimmune hyperlipidemia, autoimmune immunodeficiency, autoimmune inner ear disease (AIED), autoimmune myocarditis, autoimmune pancreatitis, autoimmune retinopathy, autoimmune thrombocytopenic purpura (ATP), autoimmune thyroid disease, autoimmune urticaria, axonal and neuronal neuropathies, Behcet's disease, bullous pemphigoid, autoimmune cardiomyopathy, Castleman disease, celiac disease, Chagas disease, chronic fatigue syndrome, chronic inflammatory demyelinating polyneuropathy (CIDP), chronic recurrent multifocal ostomyelitis (CRMO), Churg-Strauss syndrome, cicatricial pemphigoid/benign mucosal pemphigoid, Crohn's disease, Cogans syndrome, cold agglutinin disease, congenital heart block, coxsackie myocarditis, CREST disease, essential mixed cryoglobulinemia, demyelinating neuropathies, dermatitis herpetiformis, dermatomyositis, Devic's disease (neuromyelitis optica), discoid lupus, Dressler's syndrome, endometriosis, eosinophilic fasciitis, erythema nodosum, experimental allergic encephalomyelitis, Evans syndrome, fibromyalgia, fibrosing alveolitis, giant cell arteritis (temporal arteritis), glomerulonephritis, Goodpasture's syndrome, granulomatosis with polyangiitis (GPA), Graves' disease, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, hemolytic anemia, Henoch-Schonlein purpura, herpes gestationis, hypogammaglobulinemia, hypergammaglobulinemia, idiopathic thrombocytopenic purpura (ITP), IgA nephropathy, IgG4-related sclerosing disease, immunoregulatory lipoproteins, inclusion body myositis, inflammatory bowel disease, insulin-dependent diabetes (type 1), interstitial cystitis, juvenile arthritis, Kawasaki syndrome, Lambert-Eaton syndrome, leukocytoclastic vasculitis, lichen planus, lichen sclerosus, ligneous conjunctivitis, linear IgA disease (LAD), lupus (SLE), Lyme disease, Meniere's disease, microscopic polyangiitis, mixed connective tissue disease (MCTD), monoclonal gammopathy of undetermined significance (MGUS), Mooren's ulcer, Mucha-Habermann disease, multiple sclerosis, myasthenia gravis, myositis, narcolepsy, neuromyelitis optica (Devic's), autoimmune neutropenia, ocular cicatricial pemphigoid, optic neuritis, palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus), paraneoplastic cerebellar degeneration, paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonnage-Turner syndrome, pars planitis (peripheral uveitis), pemphigus, peripheral neuropathy, perivenous encephalomyelitis, pernicious anemia, POEMS syndrome,

polyarteritis nodosa, type I, II, & III autoimmune polyglandular syndromes, polymyalgia rheumatica, polymyositis, postmyocardial infarction syndrome, postpericardiotomy syndrome, progesterone dermatitis, primary biliary cirrhosis, primary sclerosing cholangitis, psoriasis, psoriatic arthritis, idiopathic pulmonary fibrosis, pyoderma gangrenosum, pure red cell aplasia, Raynaud's phenomenon, reflex sympathetic dystrophy, Reiter's syndrome, relapsing polychondritis, restless legs syndrome, retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis, sarcoidosis, Schmidt syndrome, scleritis, scleroderma, Sjogren's syndrome, sperm & testicular autoimmunity, stiff person syndrome, subacute bacterial endocarditis (SBE), Susac's syndrome, sympathetic ophthalmia, Takayasu's arteritis, temporal arteritis/Giant cell arteritis, thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome, transverse myelitis, ulcerative colitis, undifferentiated connective tissue disease (UCTD), uveitis, vasculitis, vesiculobullous dermatosis, vitiligo, Waldenstrom's macroglobulinemia (WM), and Wegener's granulomatosis [Granulomatosis with Polyangiitis (GPA)].

21. The method of claim 19 for promoting graft survival and for treating patients with graft versus host disease

22. A pharmaceutical composition comprising specific IL-15 antagonist polypeptide of claim 1.

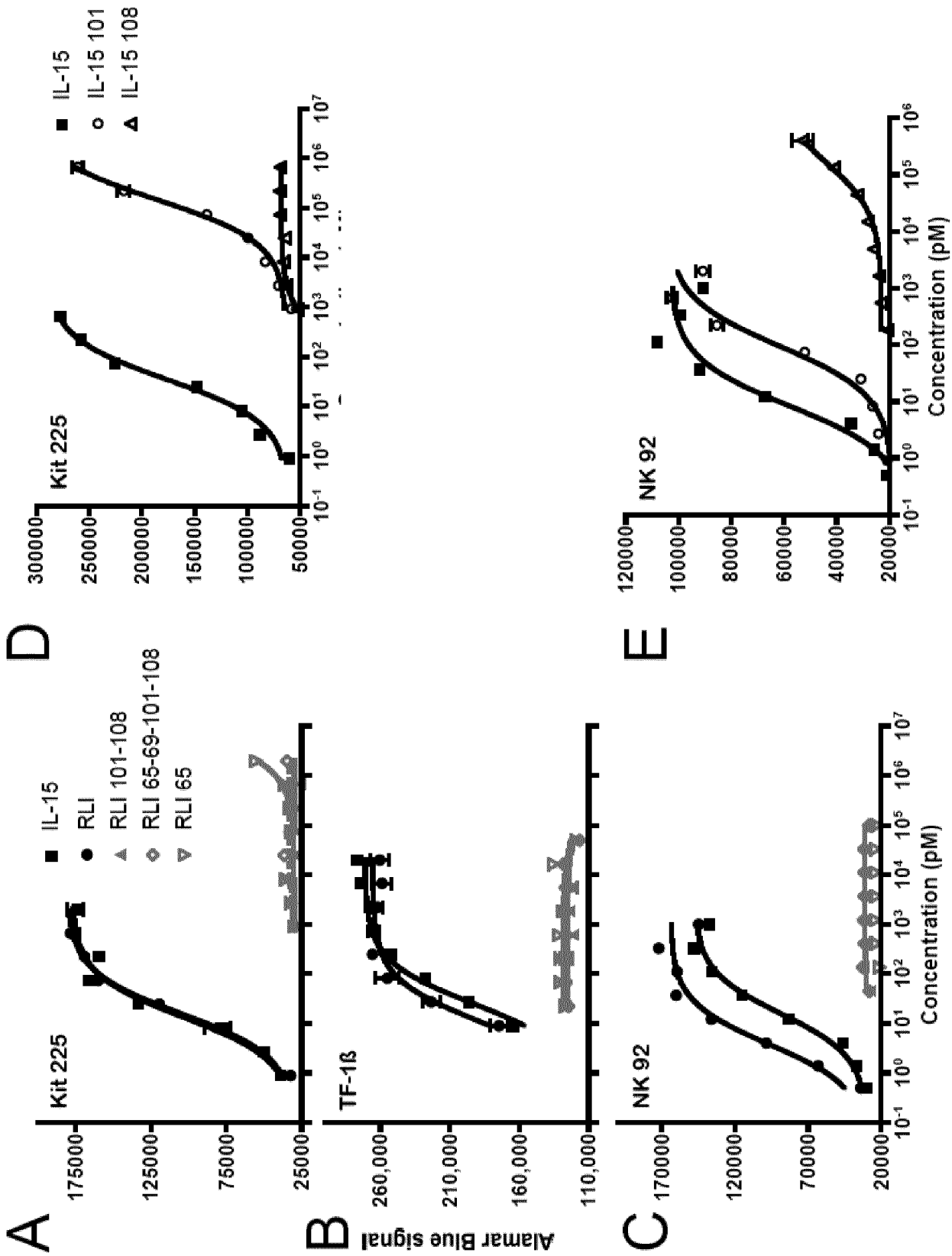

A
Kit 225
IL-15
RLI
RLI 101-108
RLI 65-69-101-108
RLI 65
175000
125000
75000
25000
B
TF-1ß
Alamar Blue signal
260,000
210,000
160,000
110,000
C
NK 92
170000
120000
70000
20000
Concentration (pM)
D
Kit 225
IL-15
IL-15 101
IL-15 108
300000
250000
200000
150000
100000
50000
E
NK 92
120000
100000
80000
60000
40000
20000
Concentration (pM)

Figure 1

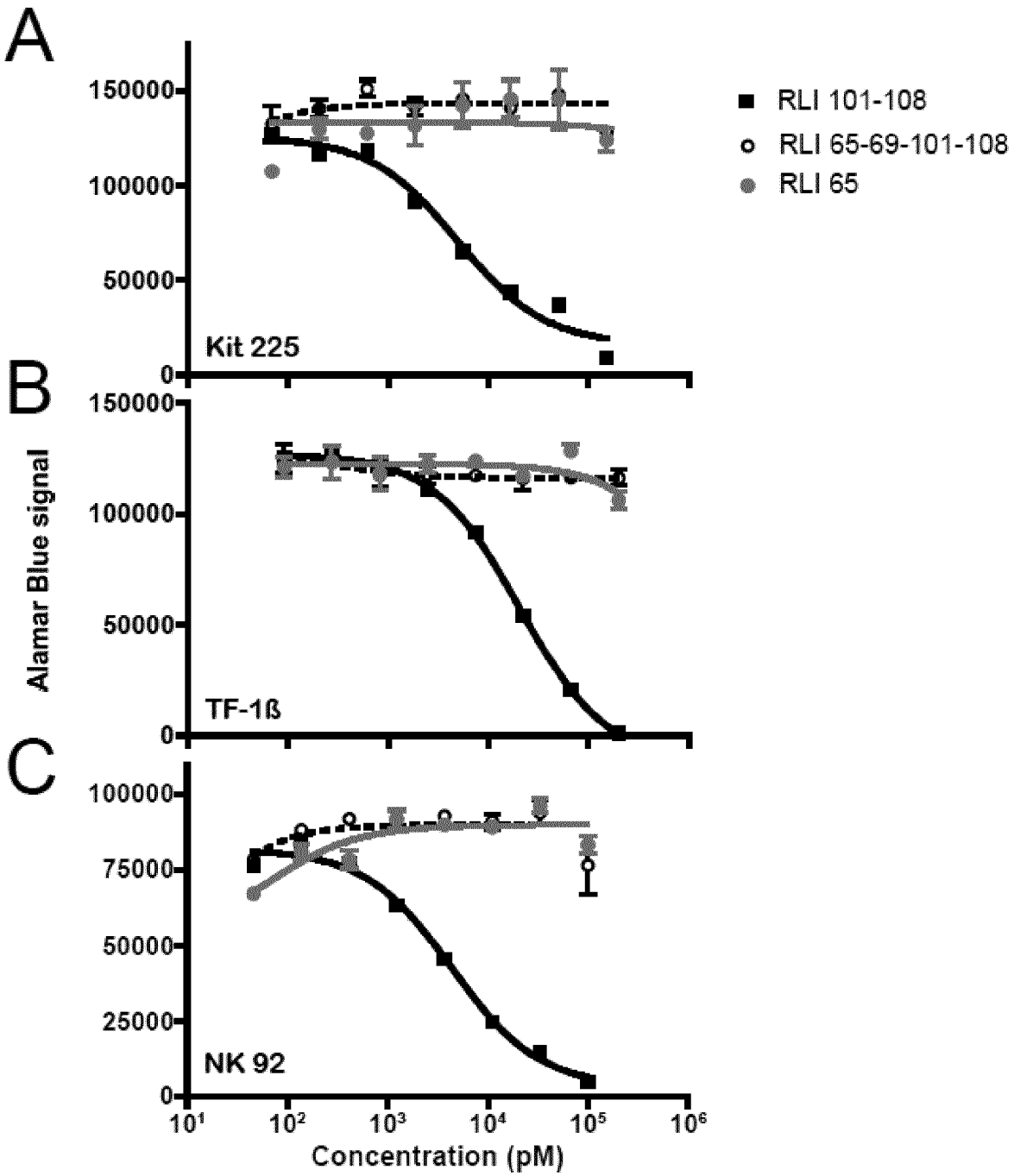

A
B
C
Alamar Blue signal
150000
100000
50000
0
Kit 225
TF-1ß
100000
75000
50000
25000
NK 92
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$
Concentration (pM)
RLI 101-108
RLI 65-69-101-108
RLI 65

Figure 2

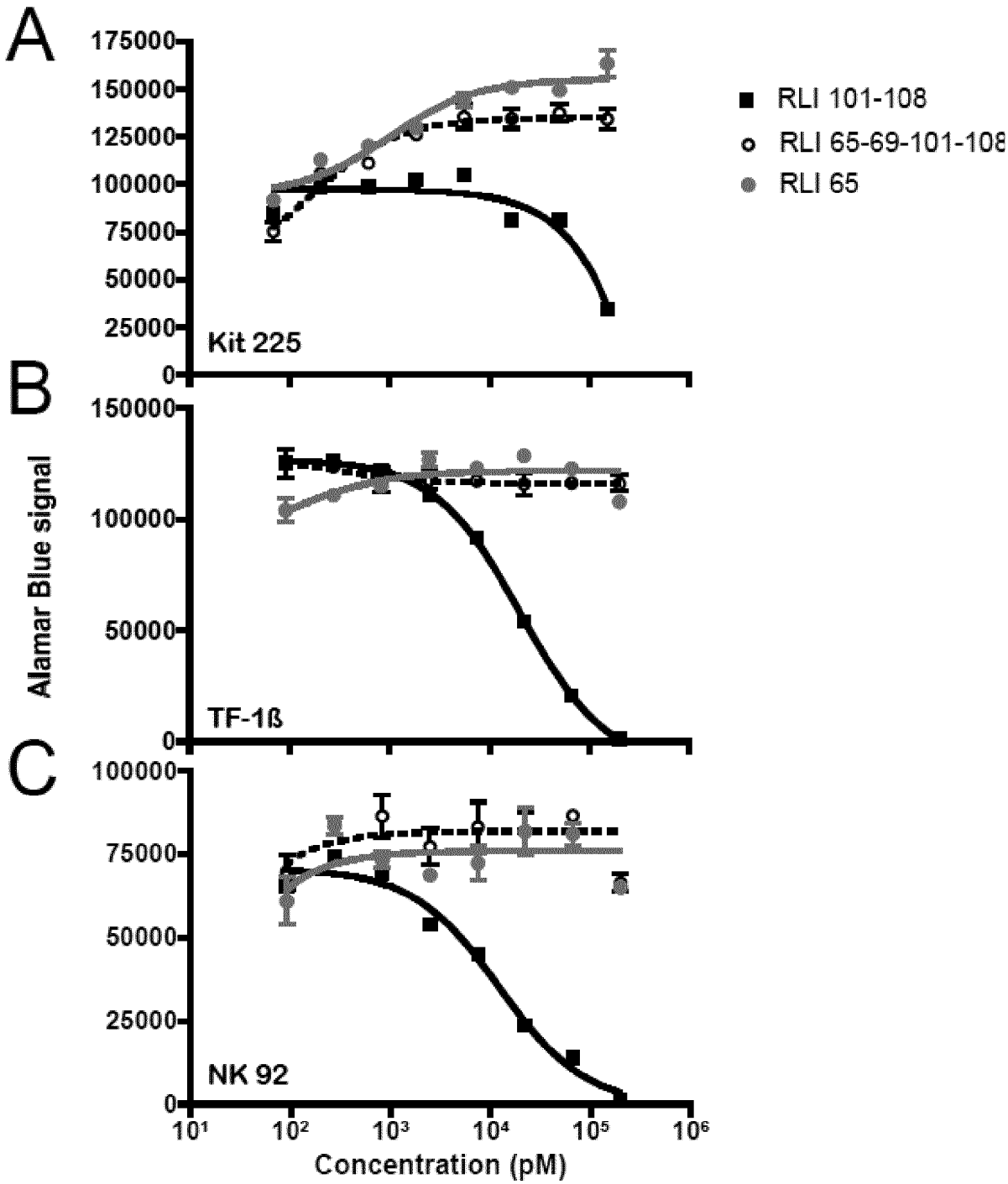

A
175000
150000
125000
100000
75000
50000
25000
0
Kit 225
B
150000
100000
50000
0
TF-1ß
C
100000
75000
50000
25000
0
NK 92
Alamar Blue signal
$10^1$ $10^2$ $10^3$ $10^4$ $10^5$ $10^6$
Concentration (pM)
RLI 101-108
RLI 65-69-101-108
RLI 65

Figure 3

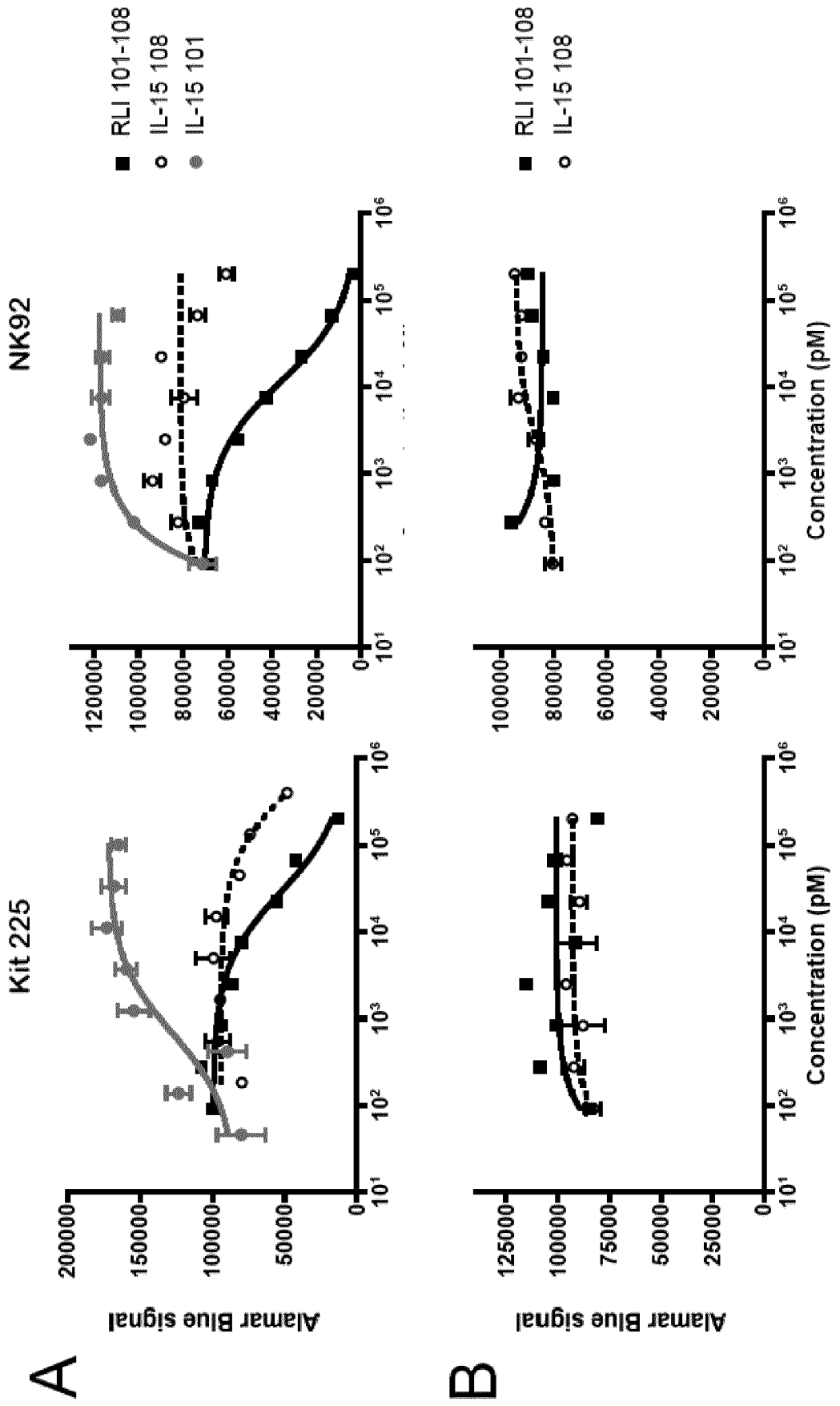

A
B
NK92
Kit 225
RLI 101-108
IL-15 108
IL-15 101
Alamar Blue signal
Concentration (pM)
0
20000
40000
60000
80000
100000
120000
50000
150000
200000
25000
75000
125000
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
$10^6$

Figure 4

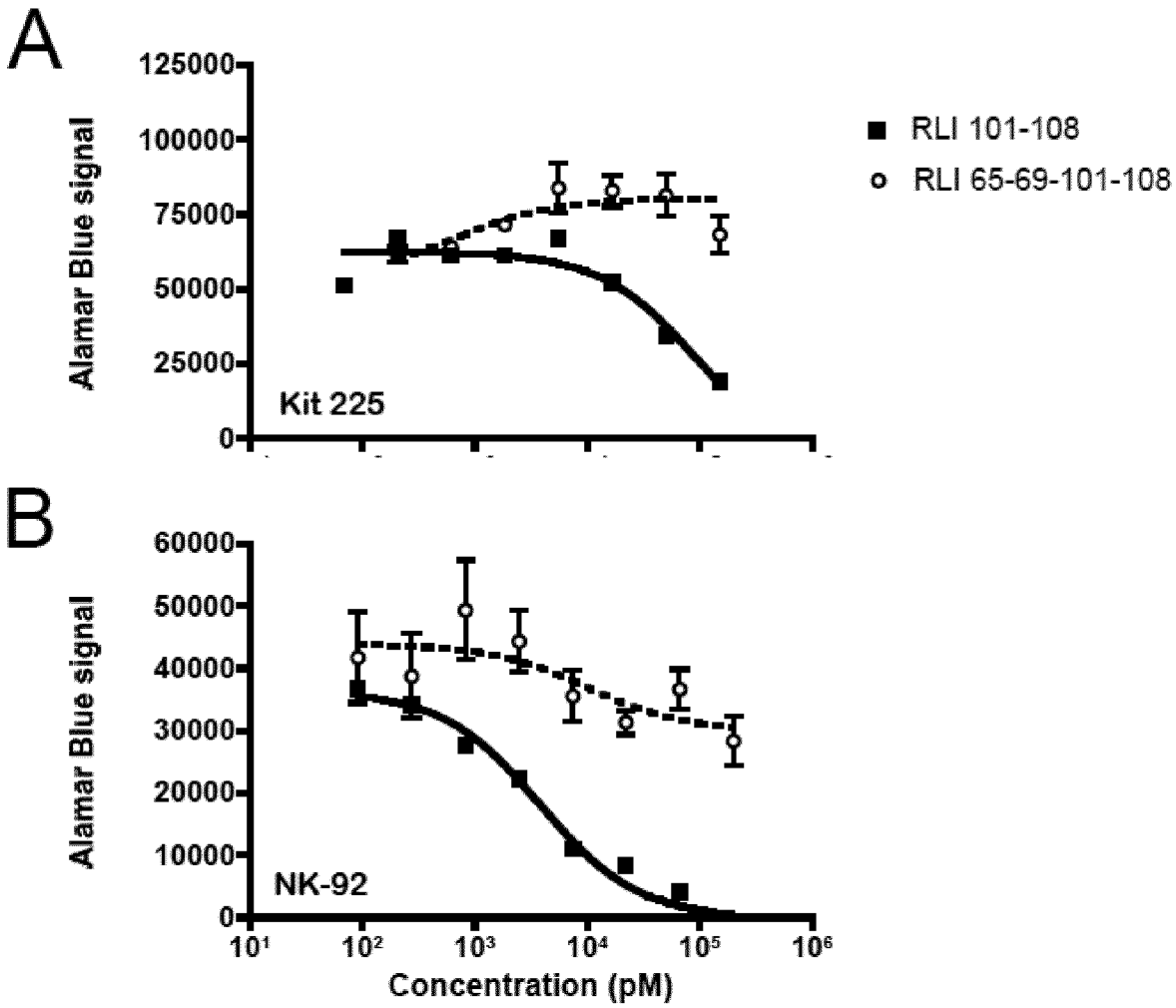

A
125000
100000
75000
50000
25000
0
Alamar Blue signal
Kit 225
RLI 101-108
RLI 65-69-101-108
B
60000
50000
40000
30000
20000
10000
0
Alamar Blue signal
NK-92
10¹
10²
10³
10⁴
10⁵
10⁶
Concentration (pM)

**Figure 5**

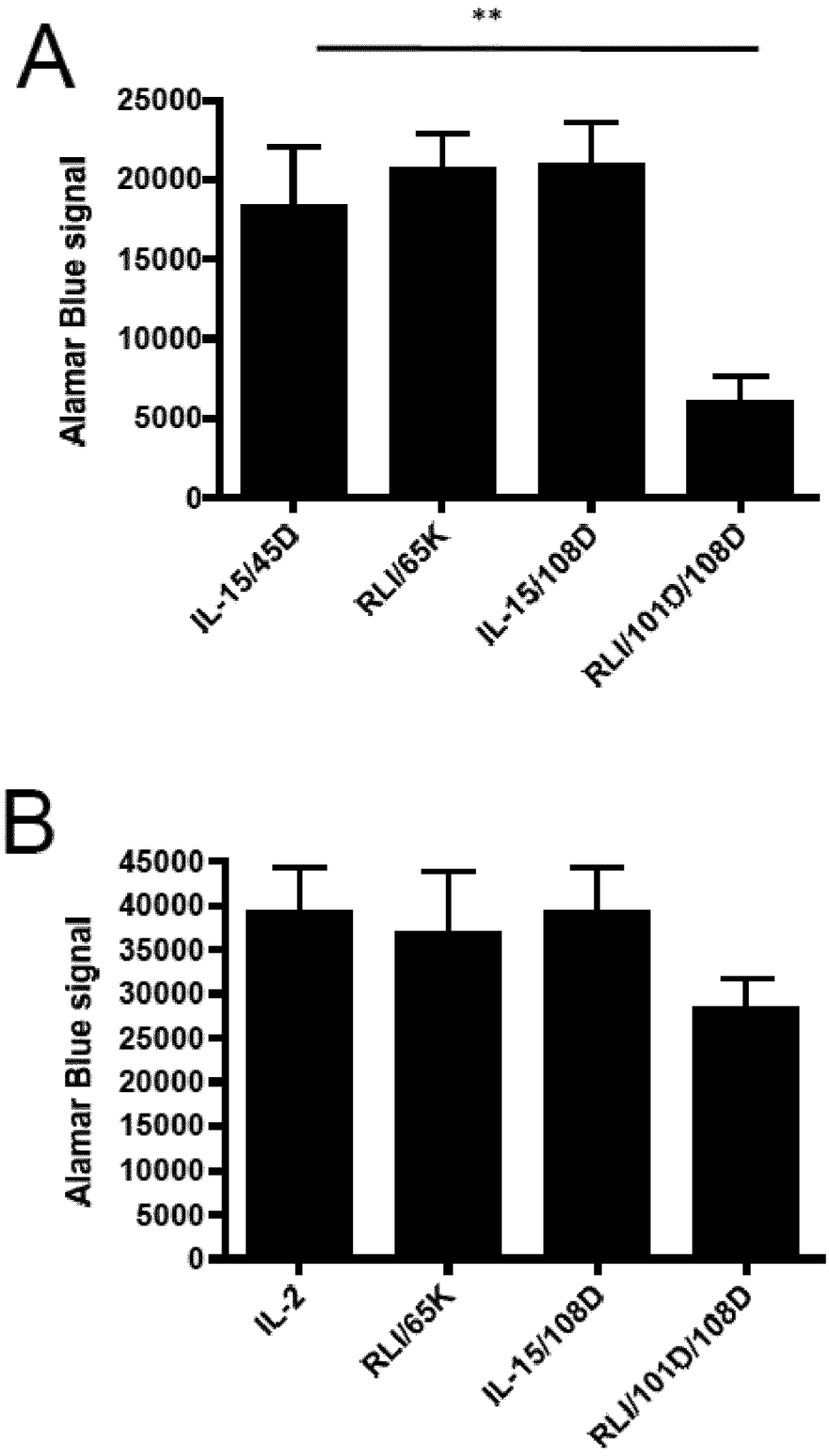

A
**
Alamar Blue signal
25000
20000
15000
10000
5000
0
IL-15/45D
RLI/65K
IL-15/108D
RLI/101D/108D
B
Alamar Blue signal
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
IL-2
RLI/65K
IL-15/108D
RLI/101D/108D

Figure 6

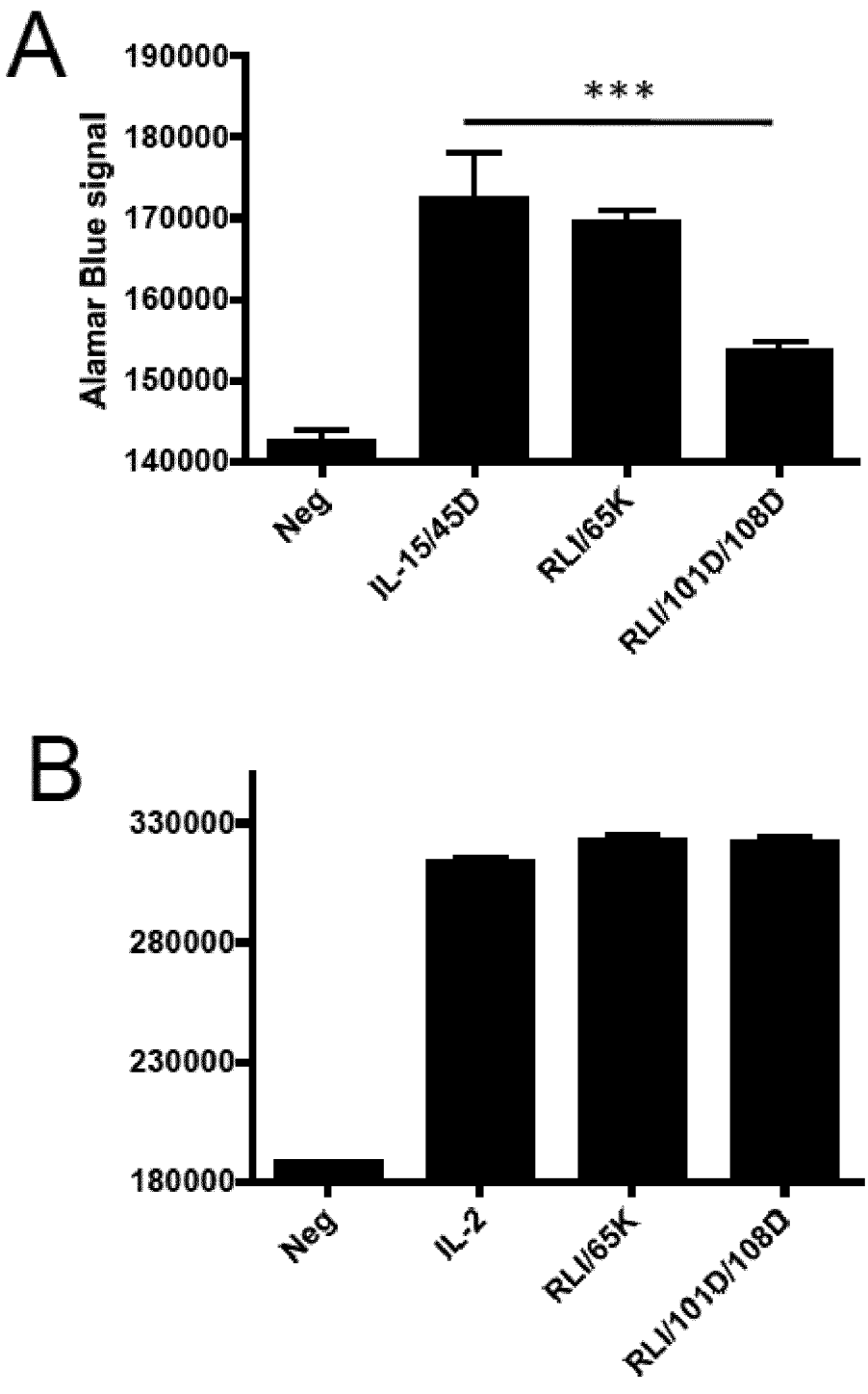

A
Alamar Blue signal
190000
180000
170000
160000
150000
140000
***
Neg
IL-15/45D
RLI/65K
RLI/101D/108D
B
330000
280000
230000
180000
Neg
IL-2
RLI/65K
RLI/101D/108D

Figure 7

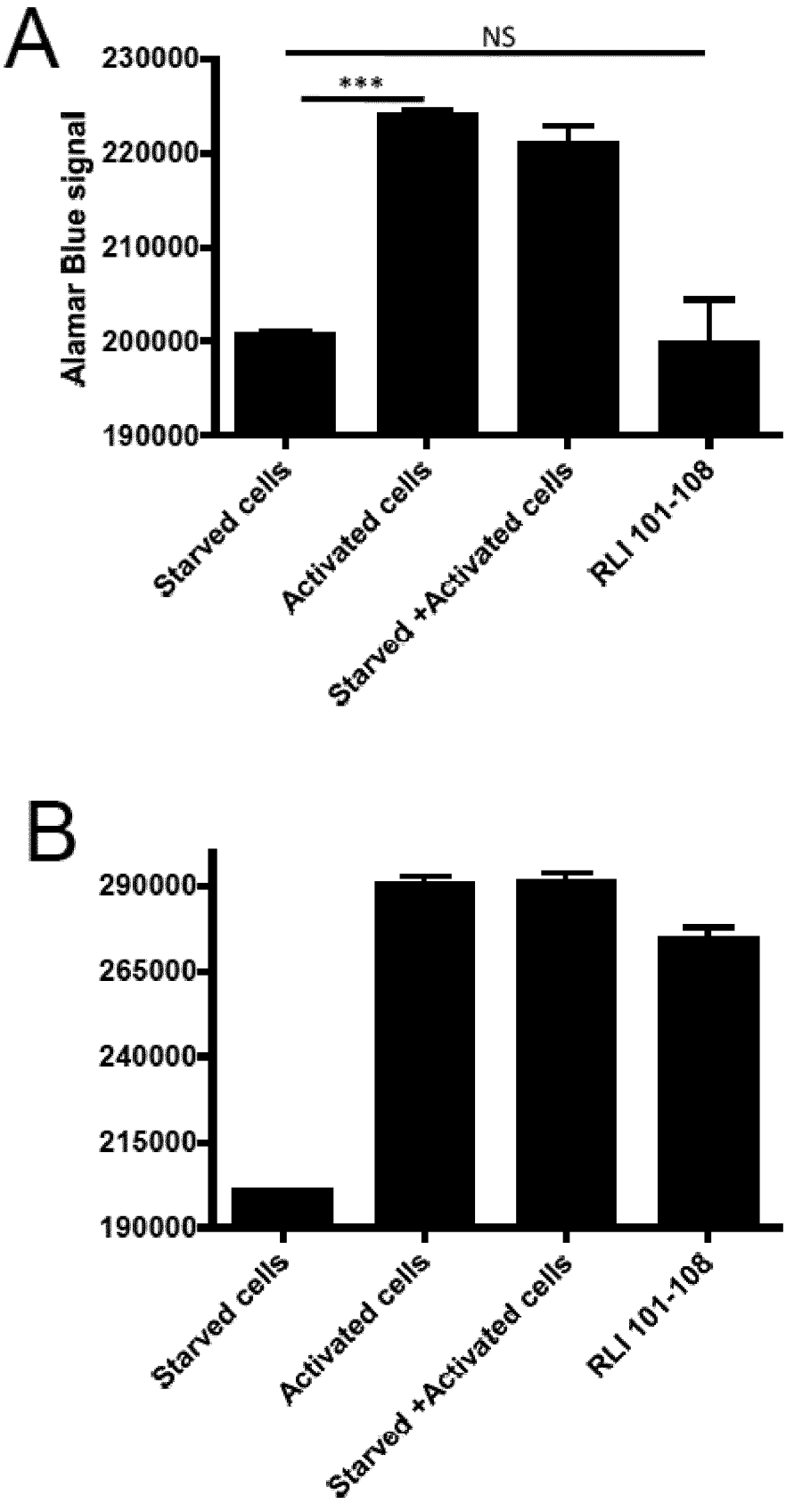

A
NS
***
230000
220000
210000
200000
190000
Alamar Blue signal
Starved cells
Activated cells
Starved +Activated cells
RLI 101-108
B
290000
265000
240000
215000
190000
Starved cells
Activated cells
Starved +Activated cells
RLI 101-108

Figure 8

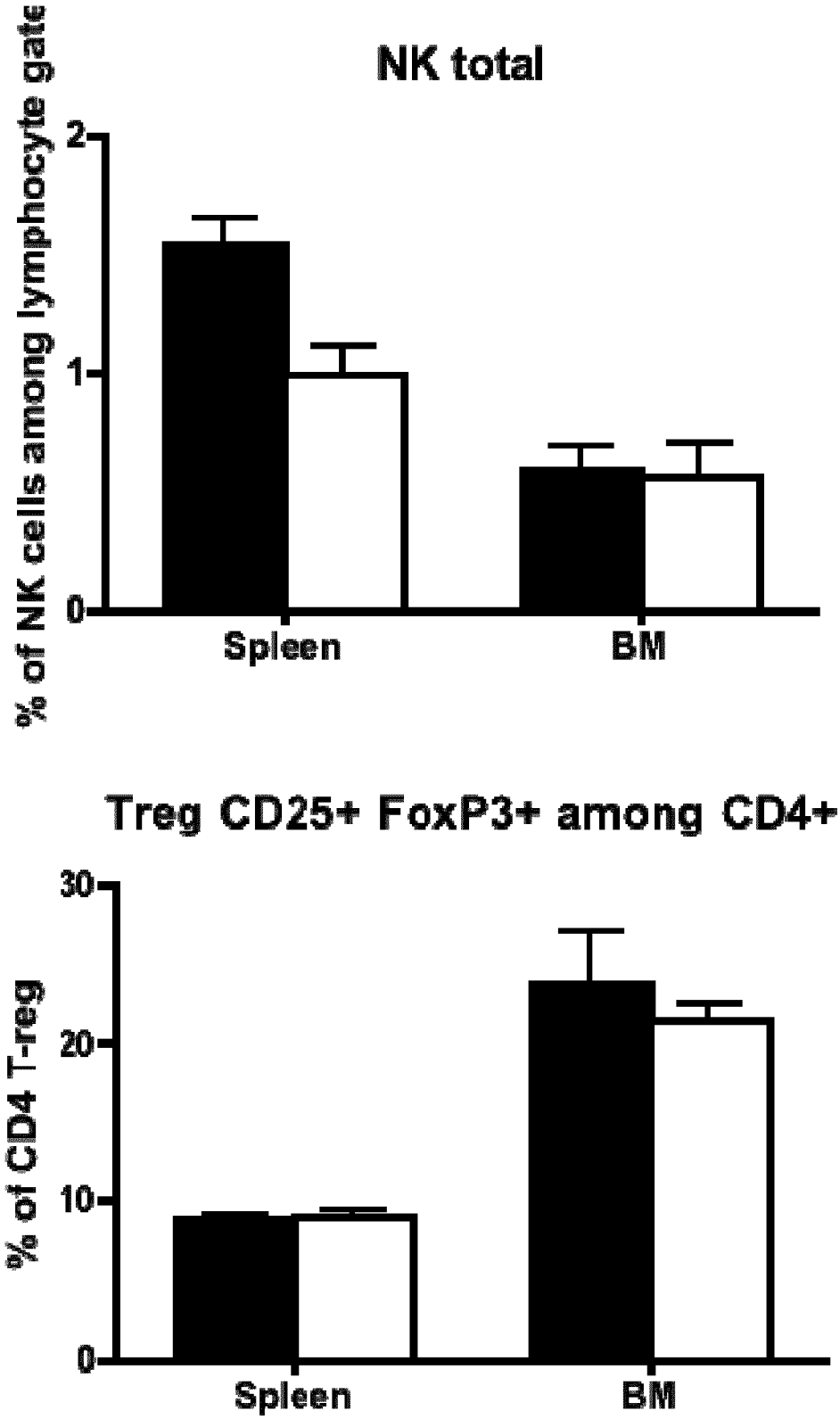

NK total
% of NK cells among lymphocyte gate
2
1
0
Spleen
BM
Treg CD25+ FoxP3+ among CD4+
% of CD4 T-reg
30
20
10
0
Spleen
BM

Figure 9

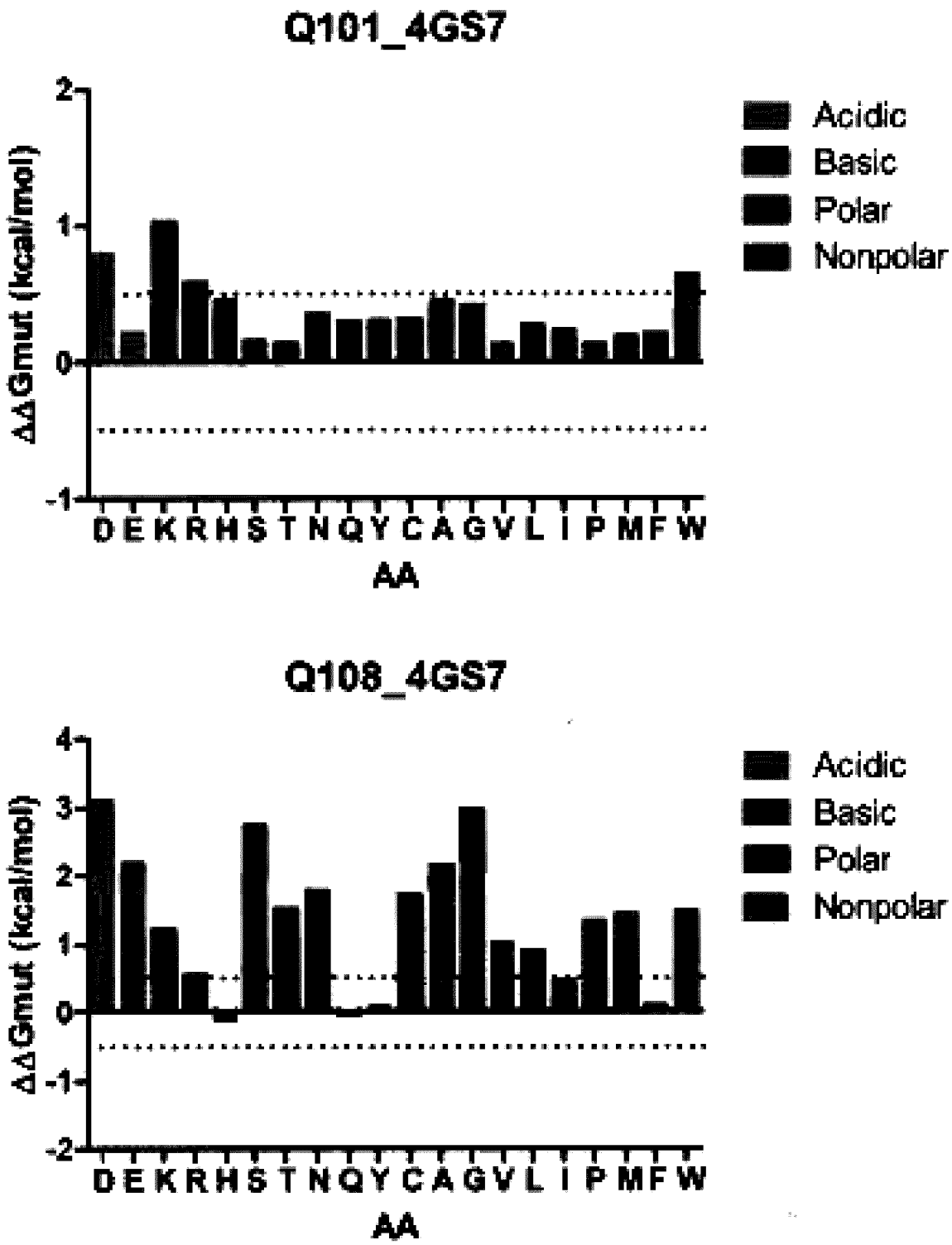
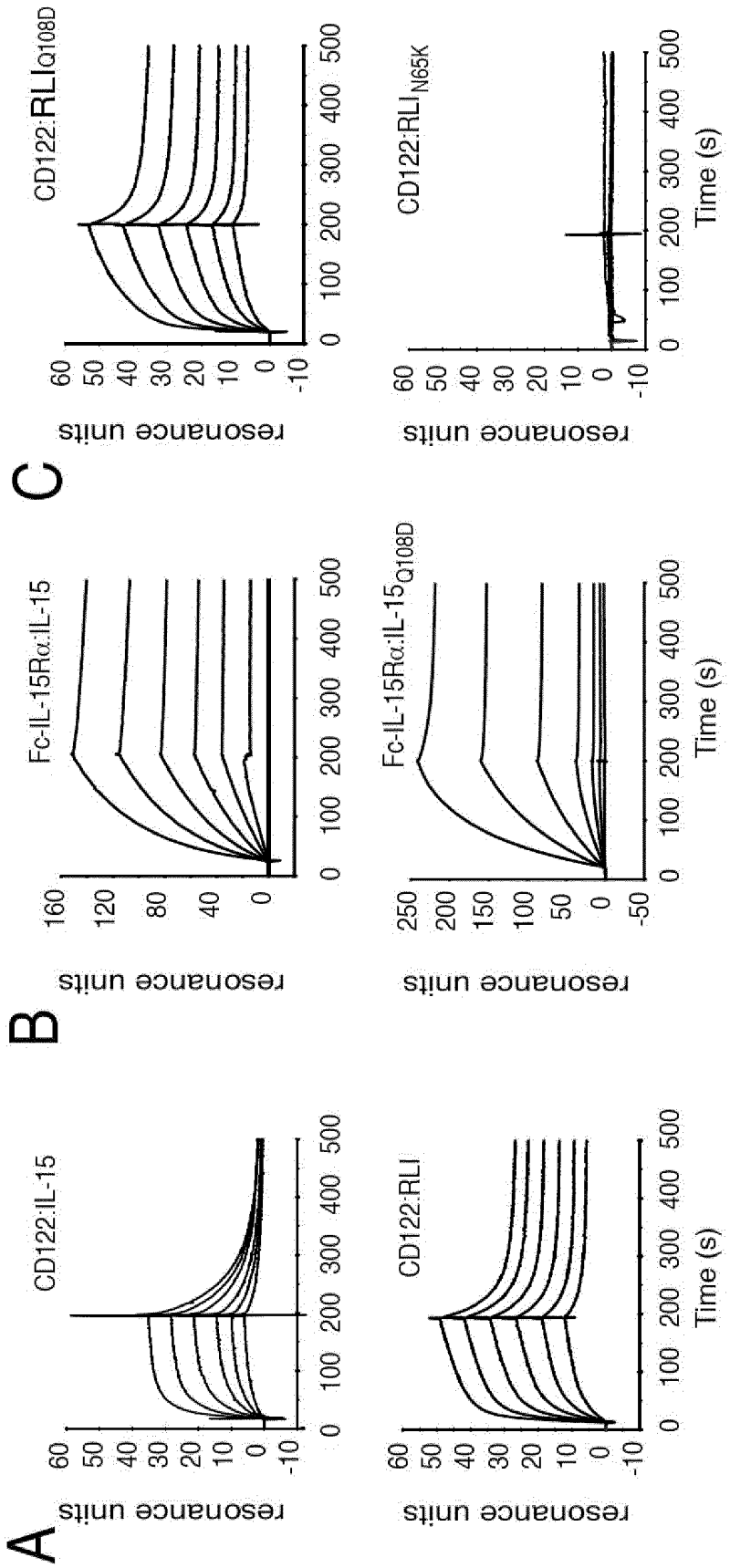
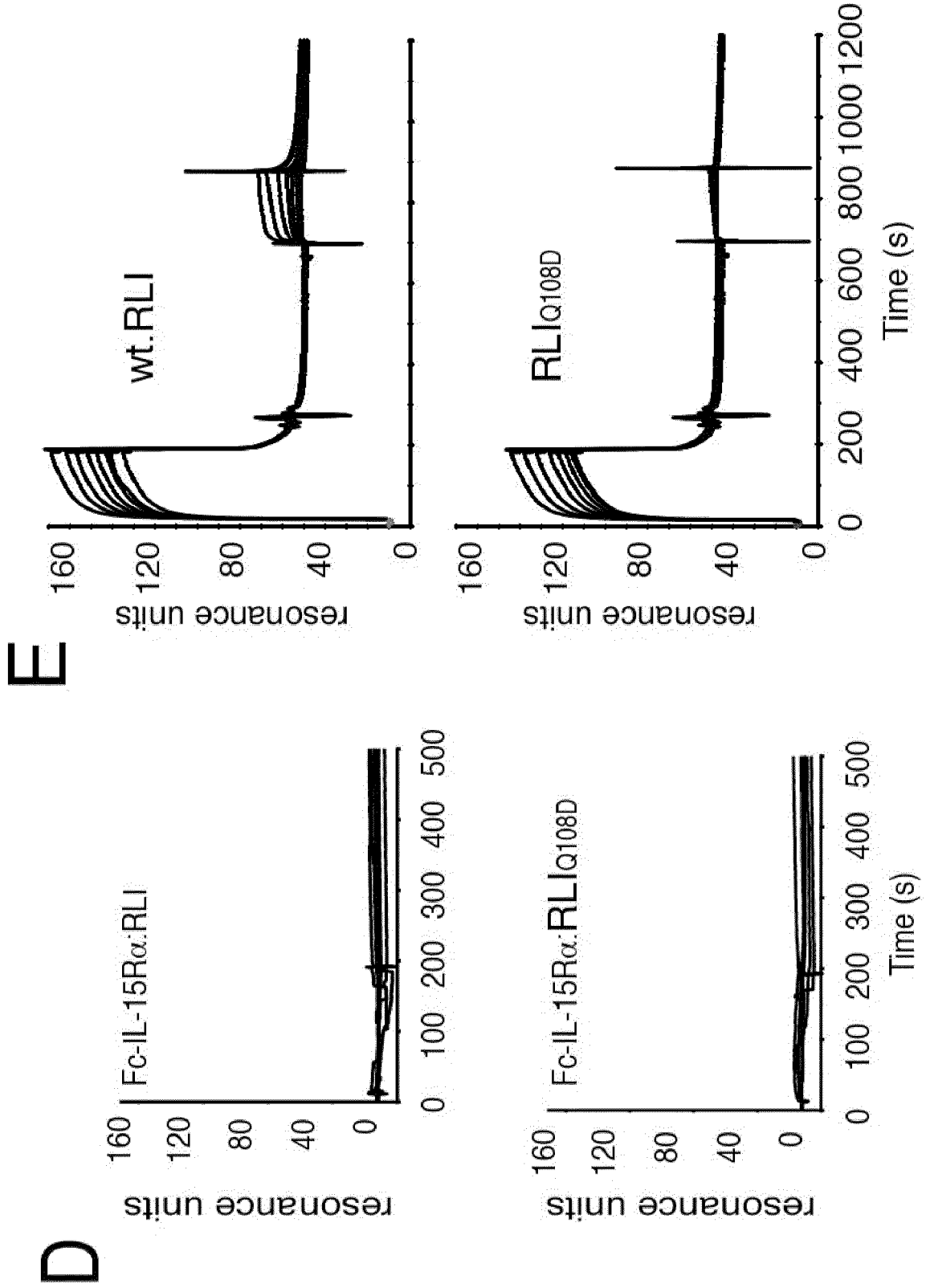

Q101_4GS7
ΔΔGmut (kcal/mol)
2
1
0
-1
D E K R H S T N Q Y C A G V L I P M F W
AA
Acidic
Basic
Polar
Nonpolar
Q108_4GS7
ΔΔGmut (kcal/mol)
4
3
2
1
0
-1
-2
D E K R H S T N Q Y C A G V L I P M F W
AA
Acidic
Basic
Polar
Nonpolar

Figure 10




Figures 11A-C




Figures 11D-E

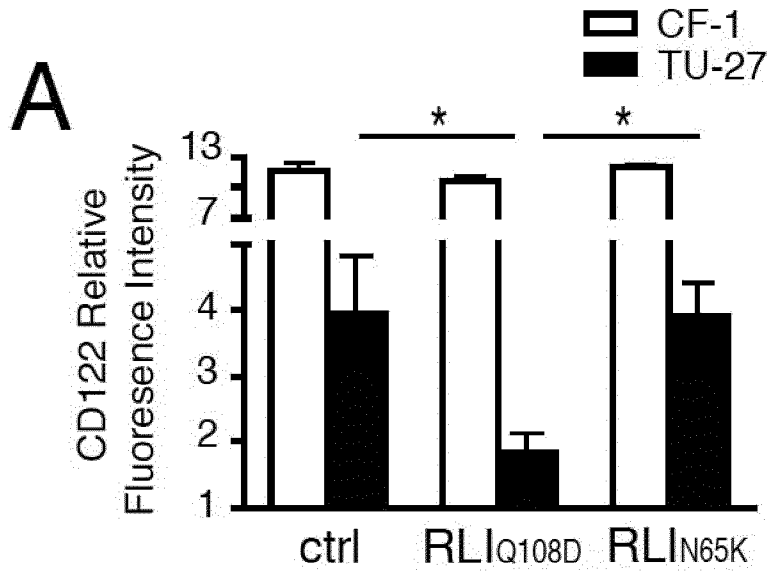



Figure 12A

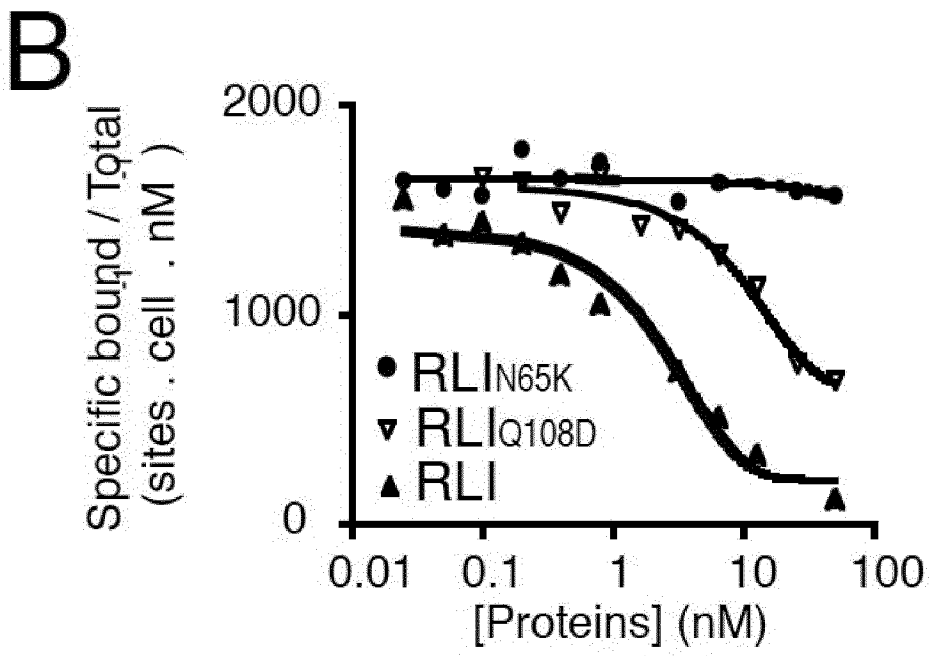



Figure 12B

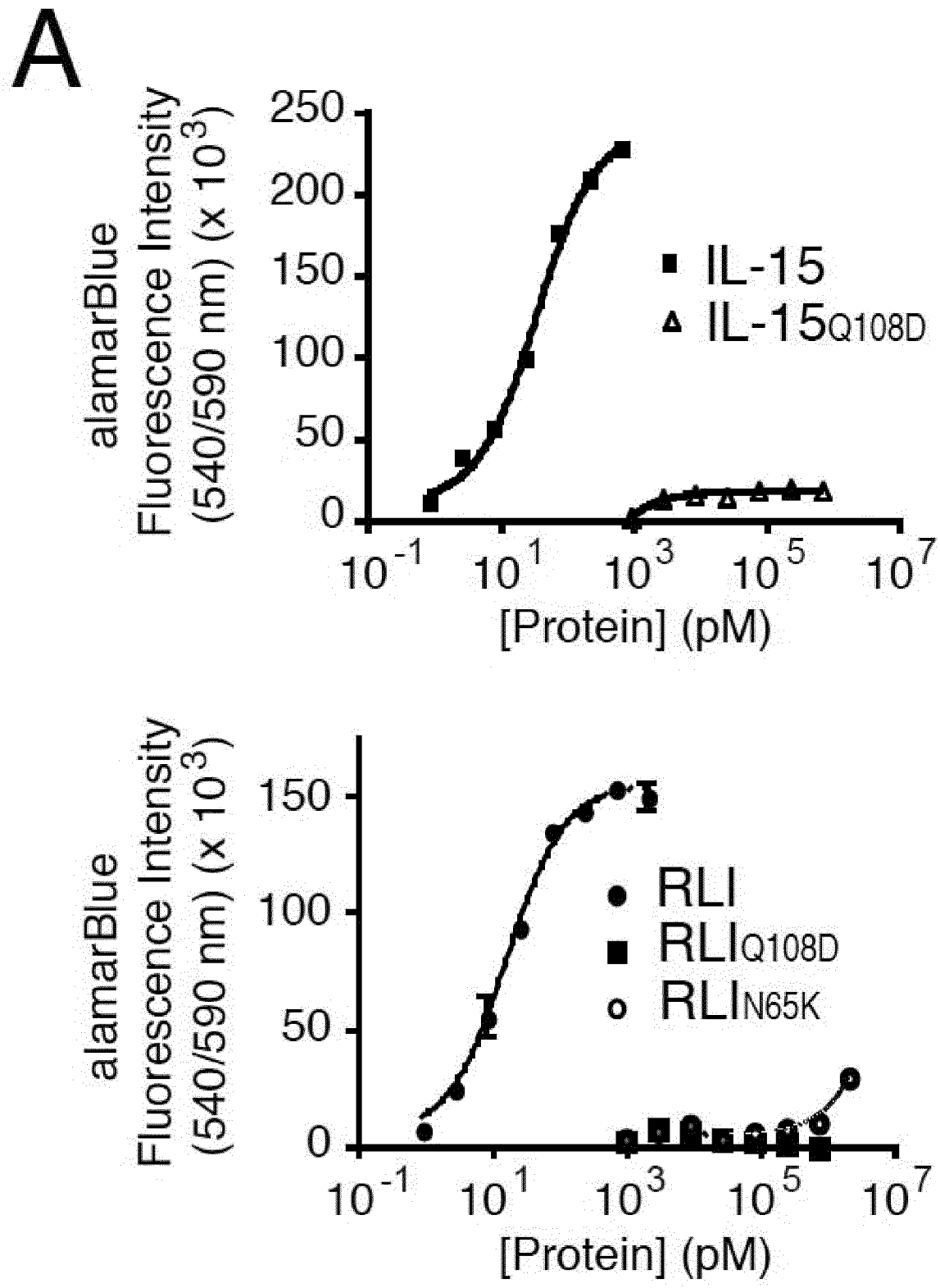

A
alamarBlue
Fluorescence Intensity
(540/590 nm) (x 10^3)
250
200
150
100
50
0
IL-15
IL-15Q108D
10^-1
10^1
10^3
10^5
10^7
[Protein] (pM)
alamarBlue
Fluorescence Intensity
(540/590 nm) (x 10^3)
150
100
50
0
RLI
RLIQ108D
RLIN65K
10^-1
10^1
10^3
10^5
10^7
[Protein] (pM)

Figure 13A

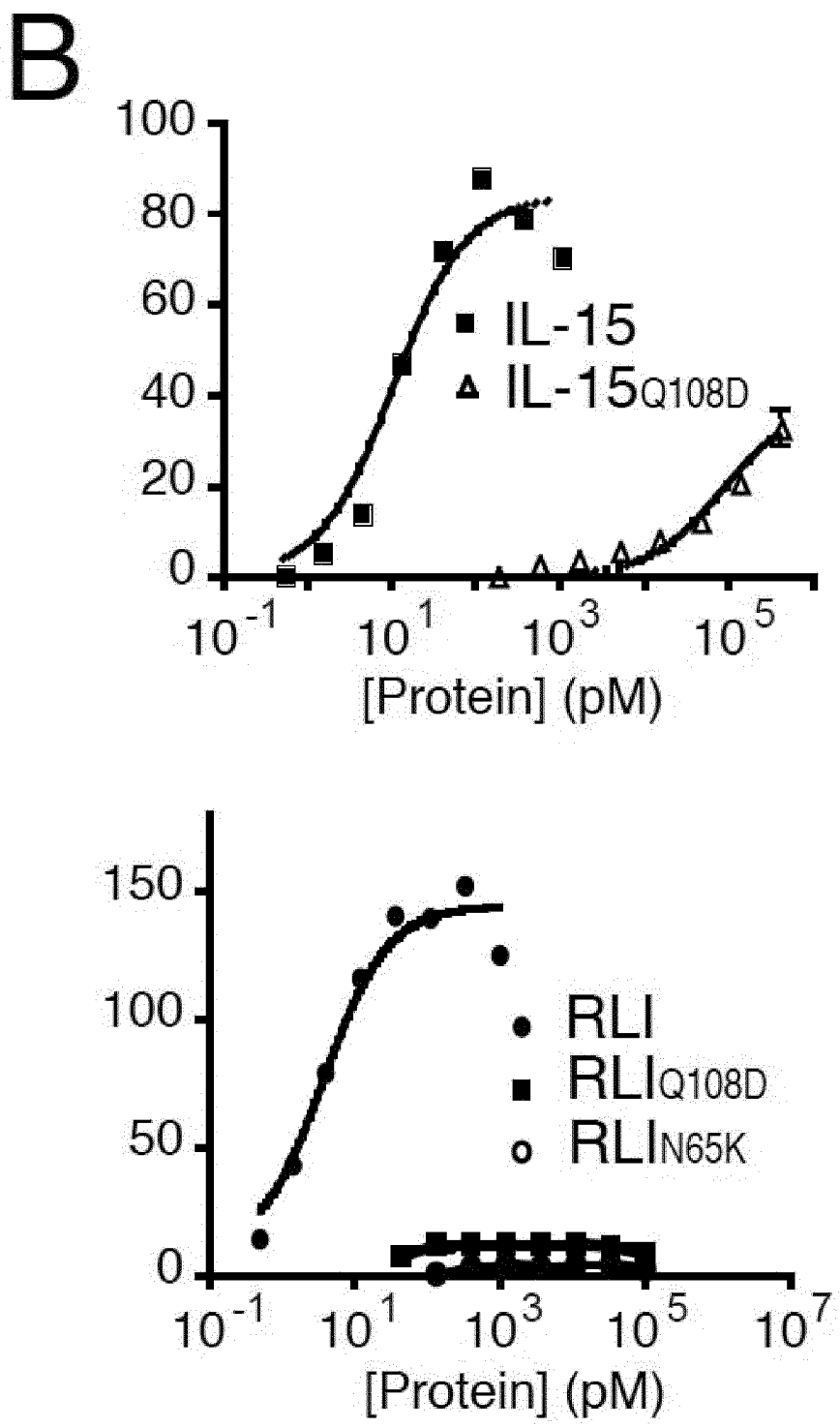

B
100
80
60
40
20
0
IL-15
IL-15Q108D
10-1
101
103
105
[Protein] (pM)
150
100
50
0
RLI
RLIQ108D
RLIN65K
10-1
101
103
105
107
[Protein] (pM)

Figure 13B

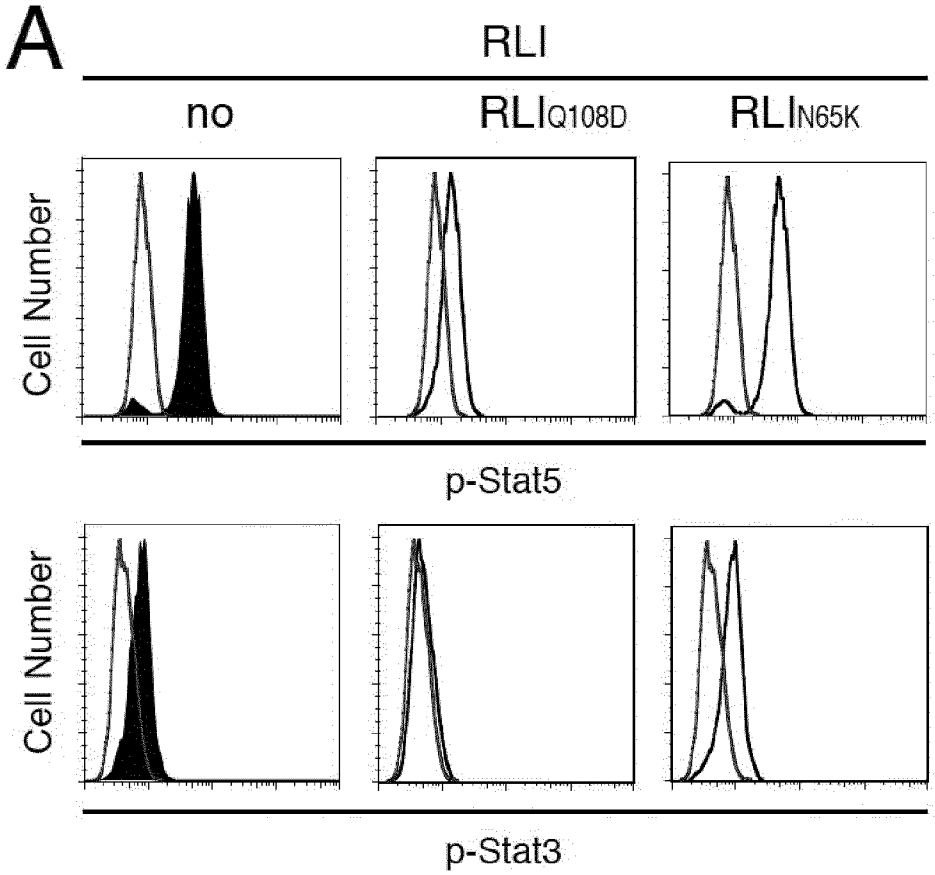

A
RLI
no
RLIQ108D
RLIN65K
Cell Number
p-Stat5
Cell Number
p-Stat3

**Figure 14A**

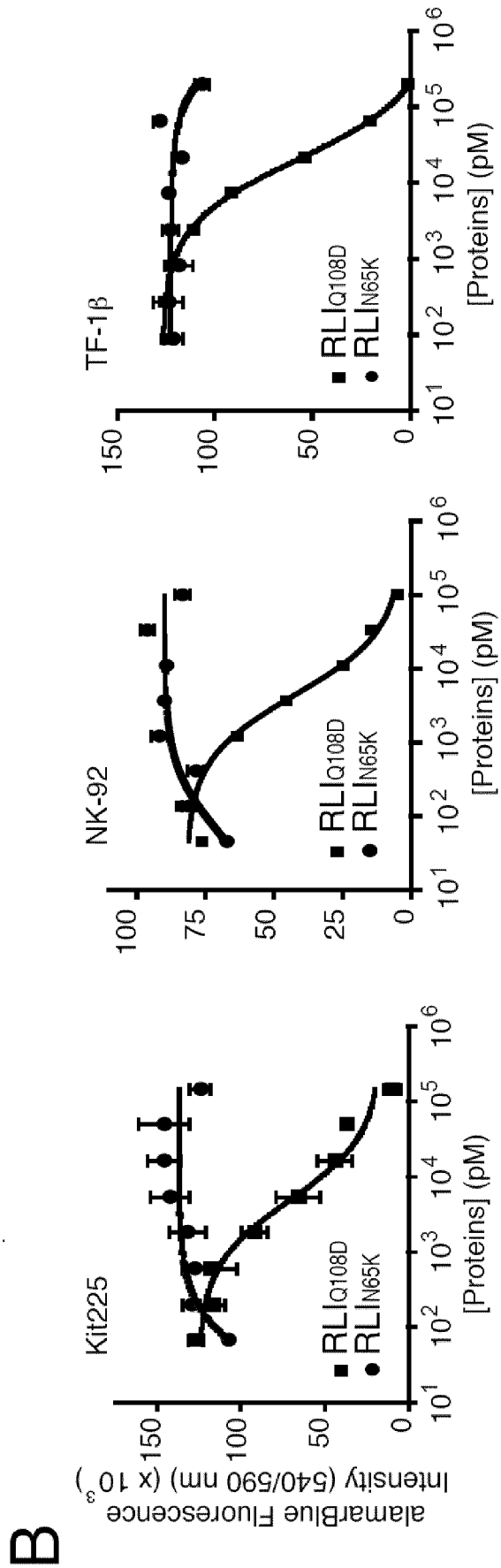

B
alamarBlue Fluorescence Intensity (540/590 nm) (x 10³)
Kit225
NK-92
TF-1β
RLIQ108D
RLIN65K
[Proteins] (pM)
0
25
50
75
100
150
10¹
10²
10³
10⁴
10⁵
10⁶

Figure 14B

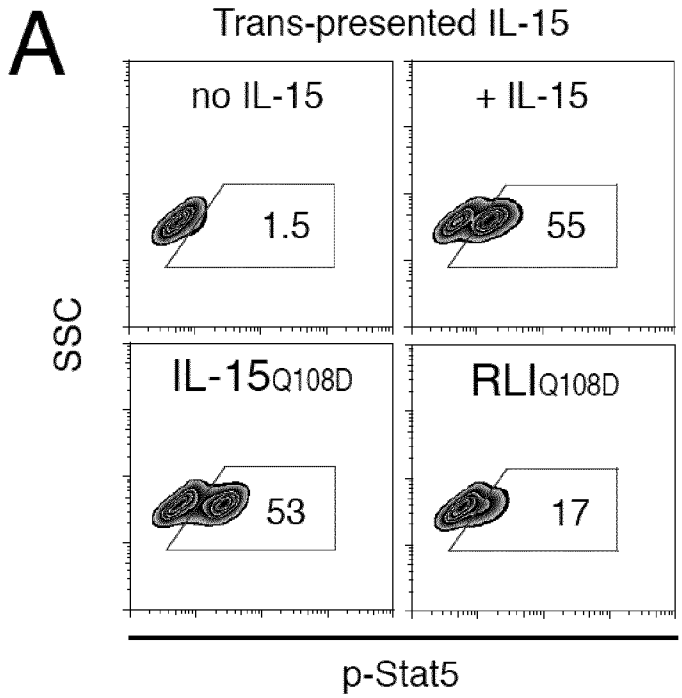



Figure 15A

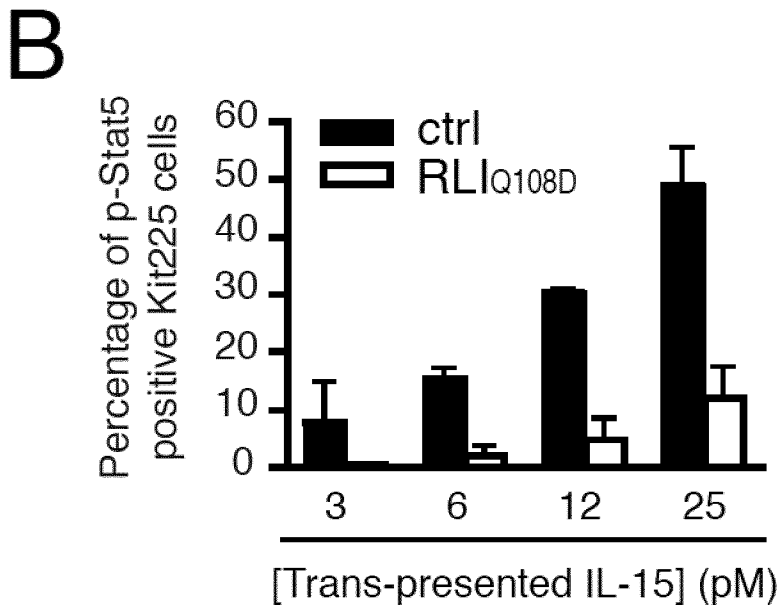



Figure 15B

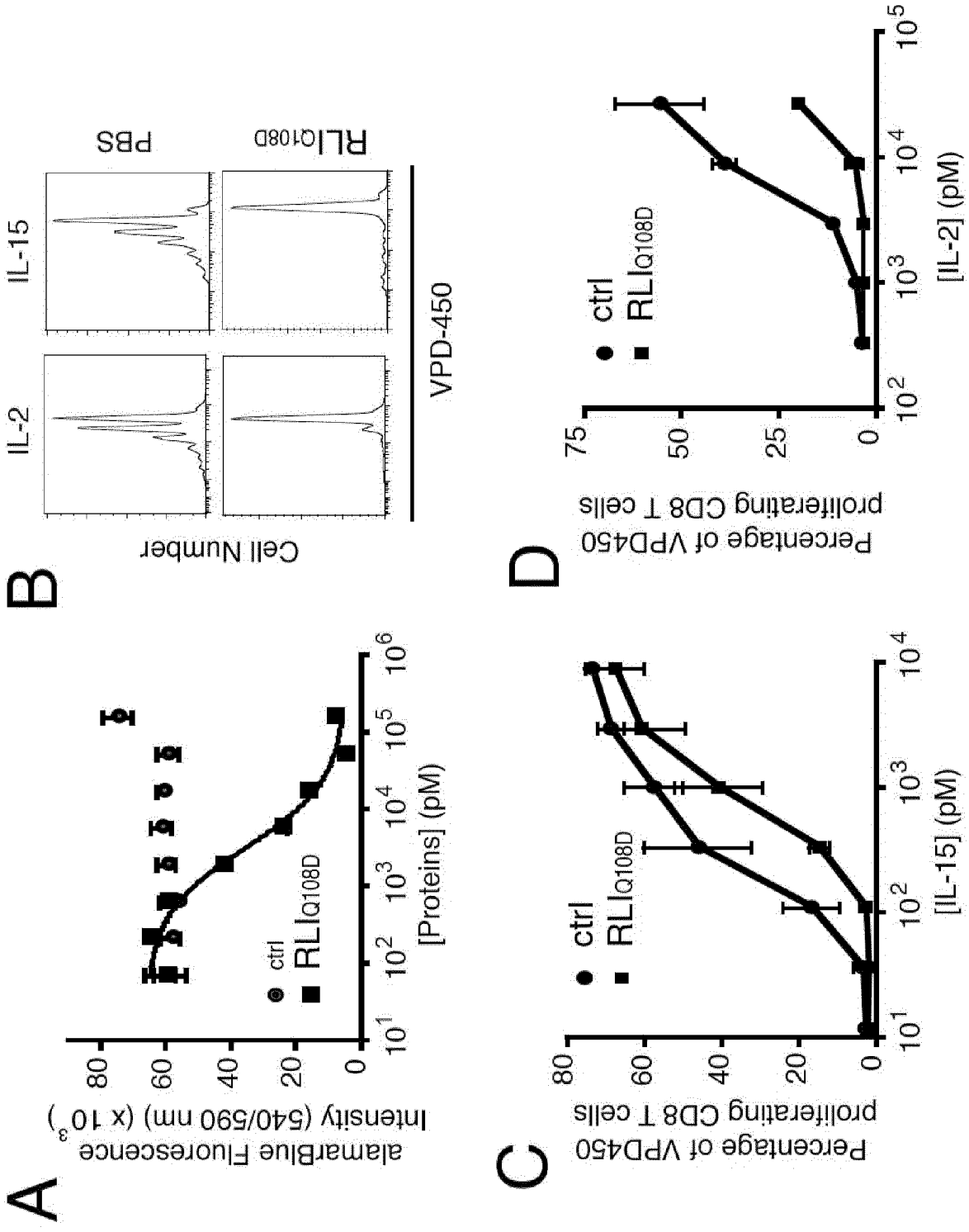

A
alamarBlue Fluorescence Intensity (540/590 nm) (x 10³)
ctrl
RLIQ108D
[Proteins] (pM)
B
IL-2
IL-15
Cell Number
VPD-450
RLIQ108D
PBS
C
Percentage of VPD450 proliferating CD8 T cells
ctrl
RLIQ108D
[IL-15] (pM)
D
Percentage of VPD450 proliferating CD8 T cells
ctrl
RLIQ108D
[IL-2] (pM)

Figure 16

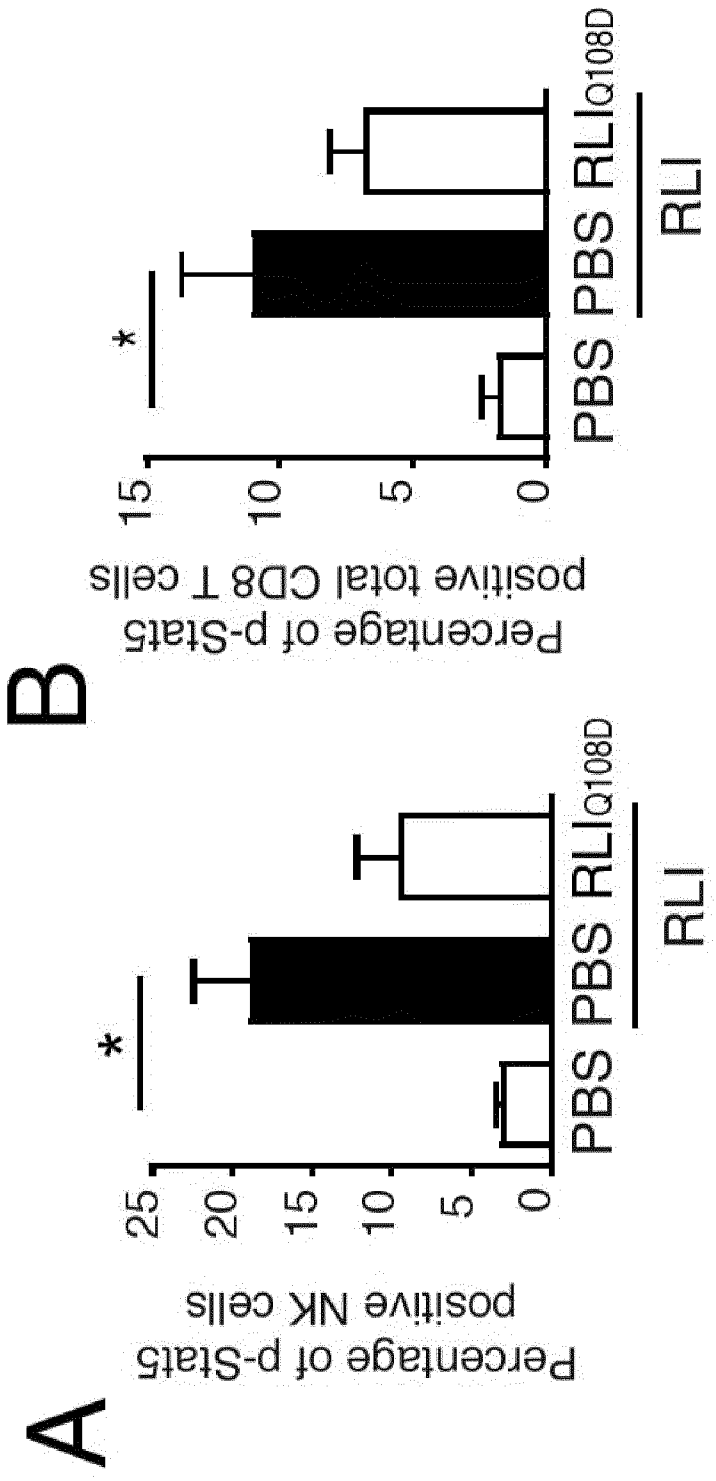
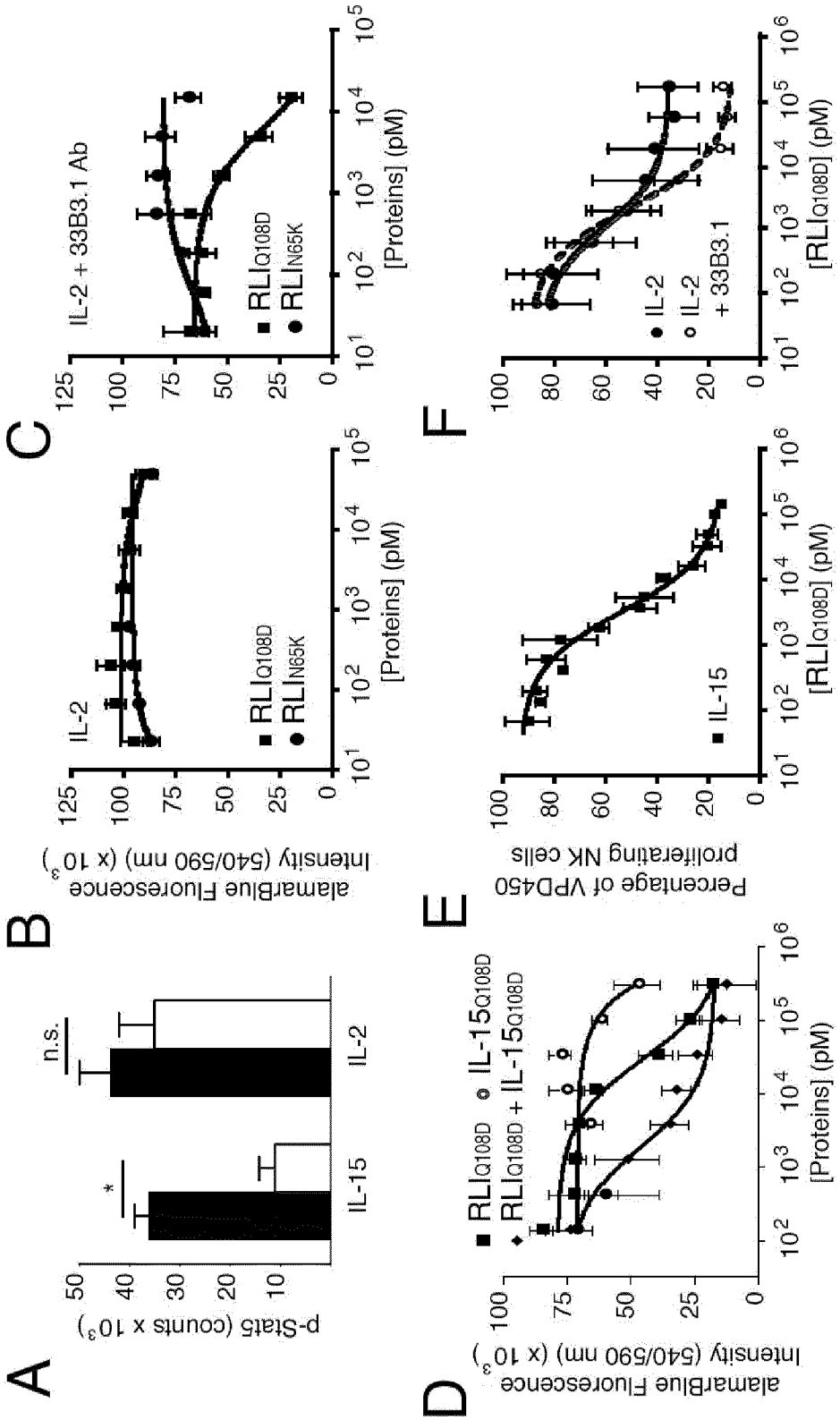
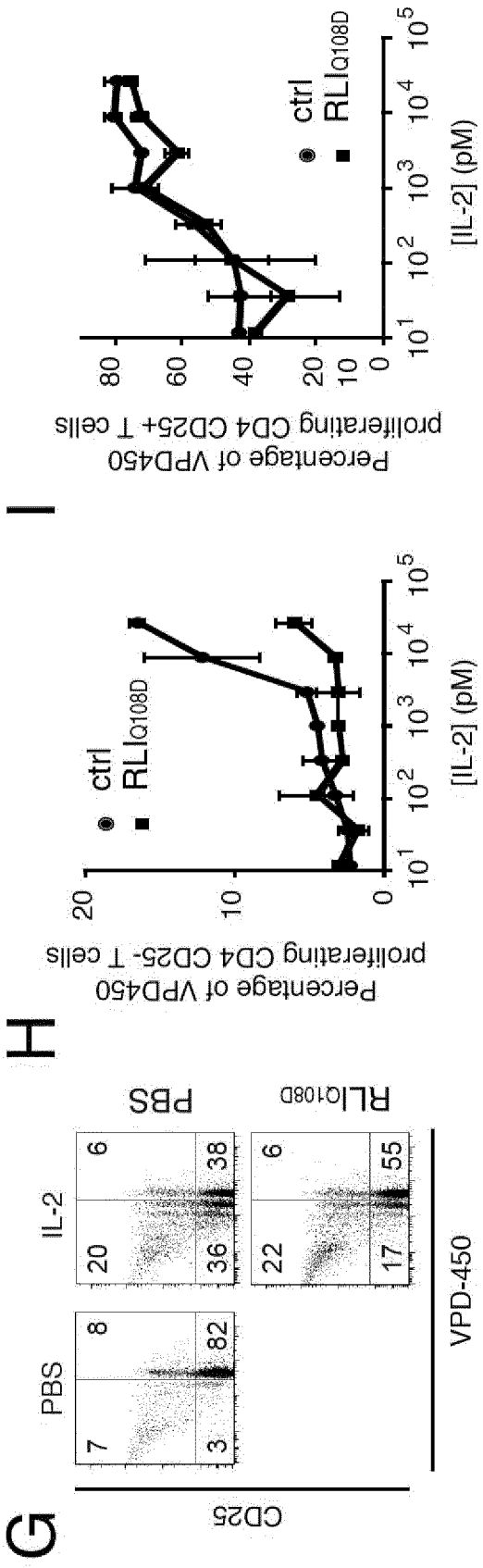

A
Percentage of p-Stat5 positive NK cells
25
20
15
10
5
0
*
PBS
PBS
RLI
RLIQ108D
RLI
B
Percentage of p-Stat5 positive total CD8 T cells
15
10
5
0
*
PBS
PBS
RLI
RLIQ108D
RLI

Figure 17




**Figures 18A-F**




Figures 18G-I

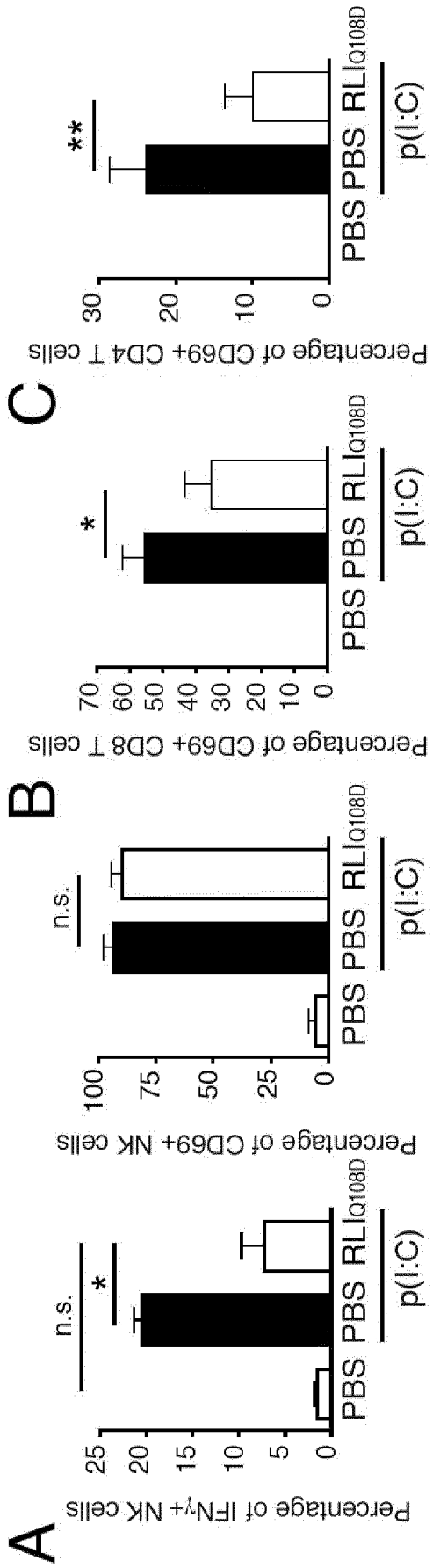
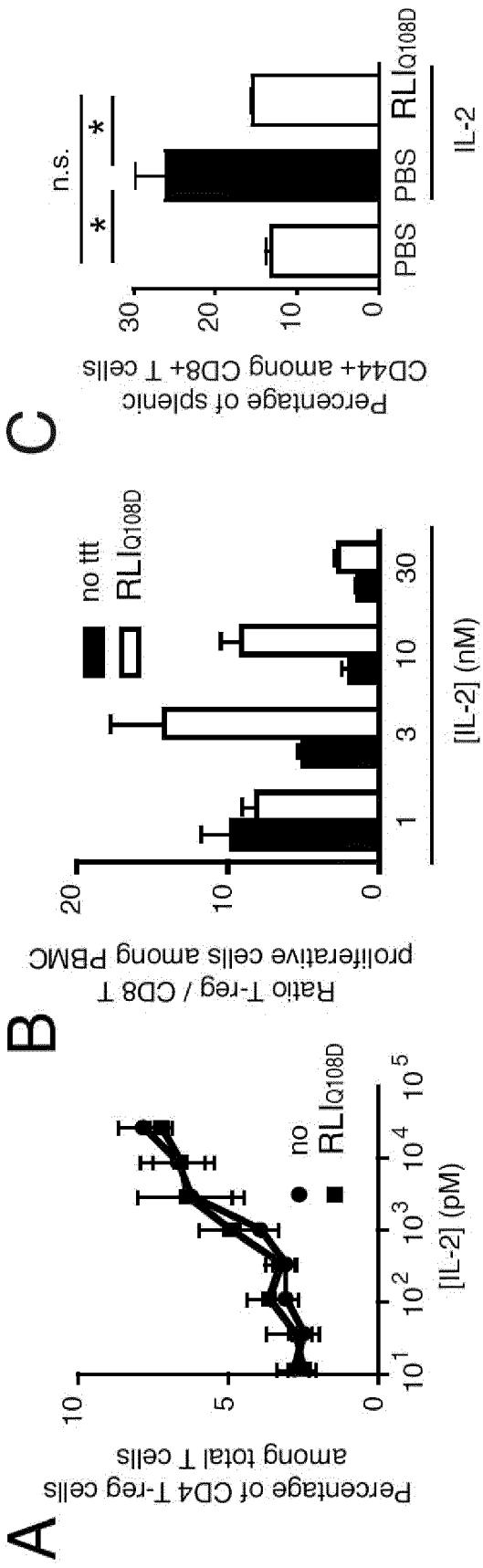
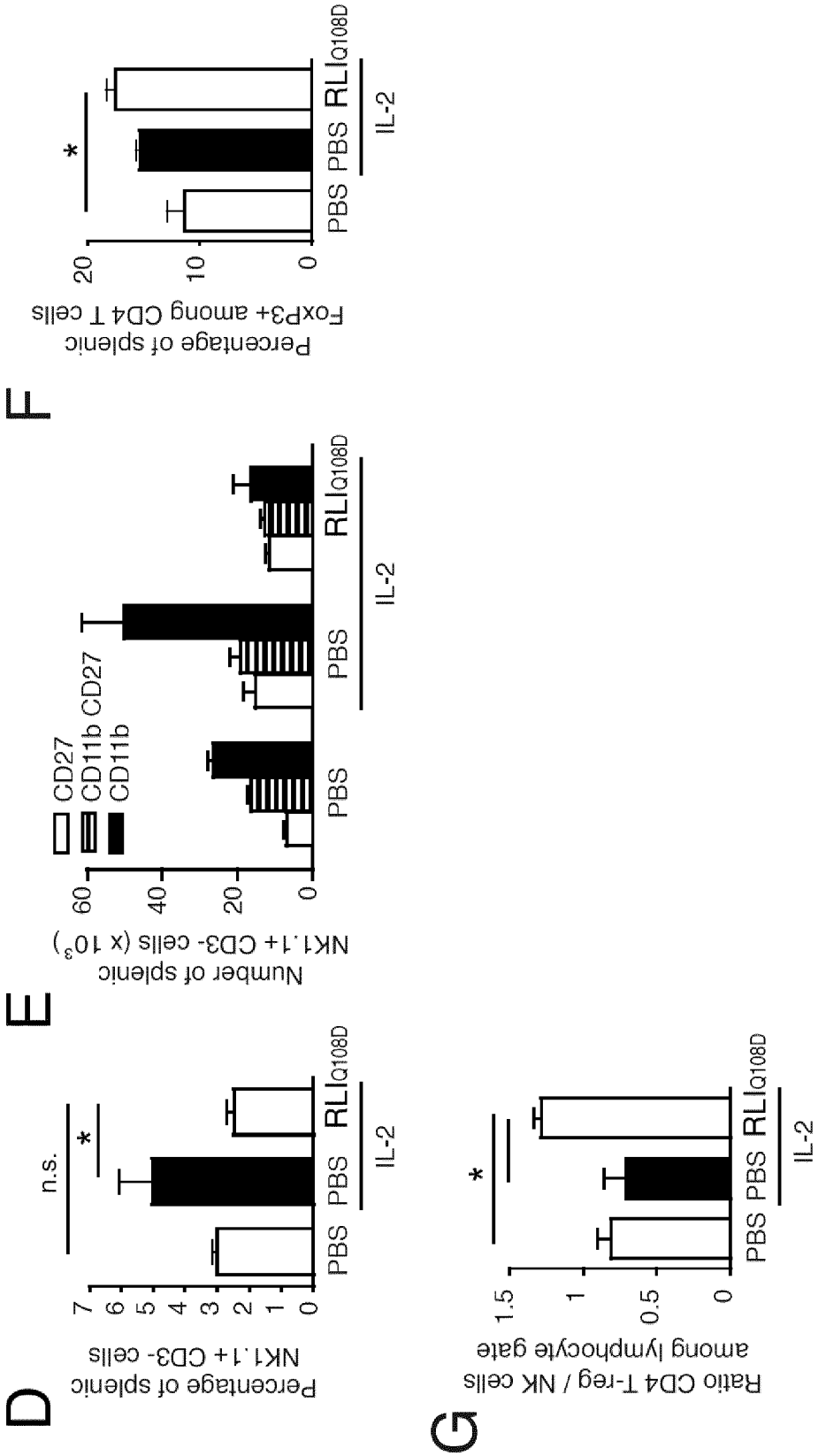

A
Percentage of IFNγ+ NK cells
25
20
15
10
5
0
n.s.
*
PBS PBS RLIQ108D
p(I:C)
B
Percentage of CD69+ NK cells
100
75
50
25
0
n.s.
PBS PBS RLIQ108D
p(I:C)
C
Percentage of CD69+ CD8 T cells
70
60
50
40
30
20
10
0
*
PBS PBS RLIQ108D
p(I:C)
Percentage of CD69+ CD4 T cells
30
20
10
0
**
PBS PBS RLIQ108D
p(I:C)

**Figure 19**




**Figures 20A-C**




**Figures 20D-G**

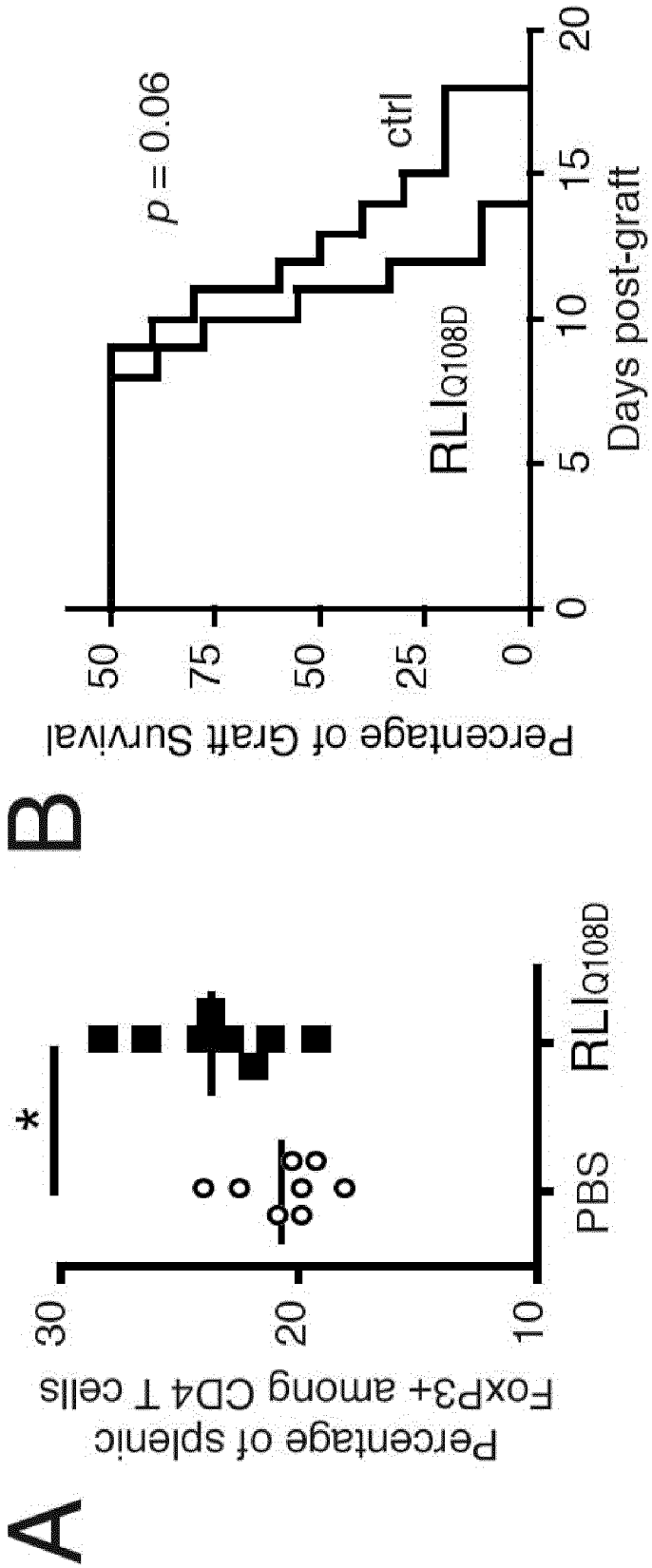

A
Percentage of splenic FoxP3+ among CD4 T cells
30
20
10
*
PBS
RLIQ108D
B
Percentage of Graft Survival
50
75
50
25
0
0
5
10
15
20
Days post-graft
p = 0.06
ctrl
RLIQ108D

Figure 21

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/071759

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/54 C07K14/715 C12N15/62
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 2008/143794 A1 (ALTOR BIOSCIENCE CORP [US]; WONG HING C [US]; RHODE PETER [US]; ZHU XI) 27 November 2008 (2008-11-27) claim 1; figure 2c; example 2 | 1-10, 12-22 |
| Y | EP 2 915 569 A1 (CYTUNE PHARMA [FR]) 9 September 2015 (2015-09-09) abstract; claims 1,11-14 | 1-10, 12-22 |
| Y | WO 2015/018529 A1 (CYTUNE PHARMA [FR]; INST GUSTAVE ROUSSY IGR [FR]) 12 February 2015 (2015-02-12) claims 1-11 | 1-10, 12-22 |
| Y | WO 2015/018528 A1 (CYTUNE PHARMA [FR]; UNIV PARIS DESCARTES [FR]; ASSIST PUBL HOPITAUX DE) 12 February 2015 (2015-02-12) claims 1-16 | 1-10, 12-22 |

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 27 October 2016 | 07/11/2016 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Gurdjian, Didier |

1

Form PCT/ISA/210 (second sheet) (April 2005)

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/071759

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 2006/020849 A2 (BETH ISRAEL HOSPITAL [US]; HOFFMANN LA ROCHE [CH]; ZHENG XIN XIAO [US]) 23 February 2006 (2006-02-23) abstract; claims 1-7,36-75; sequence 2 page 2 - page 3 | 1-10, 12-22 |
| Y | WO 2014/170032 A1 (CYTUNE PHARMA [FR]; INST GUSTAVE ROUSSY IGR [FR]) 23 October 2014 (2014-10-23) abstract page 94; claims 1-29; sequence 17 | 1-10, 12-22 |
| Y | WO 2007/046006 A2 (INST NAT SANTE RECH MED [FR]; JACQUES YANNICK [FR]; PLET ARIANE [FR];) 26 April 2007 (2007-04-26) cited in the application abstract; claims 1-47; figures 1-42 | 1-10, 12-22 |

1

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/071759

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 2008143794 A1 | 27-11-2008 | AU 2008253720 A1 | 27-11-2008 |
| | | CA 2690825 A1 | 27-11-2008 |
| | | CN 101743249 A | 16-06-2010 |
| | | CN 104109200 A | 22-10-2014 |
| | | DK 2160401 T3 | 20-10-2014 |
| | | DK 2388266 T3 | 26-05-2014 |
| | | EP 2160401 A1 | 10-03-2010 |
| | | EP 2388266 A2 | 23-11-2011 |
| | | EP 2769984 A1 | 27-08-2014 |
| | | ES 2470772 T3 | 24-06-2014 |
| | | ES 2518393 T3 | 05-11-2014 |
| | | JP 5501222 B2 | 21-05-2014 |
| | | JP 5841623 B2 | 13-01-2016 |
| | | JP 2010527919 A | 19-08-2010 |
| | | JP 2014113159 A | 26-06-2014 |
| | | JP 2016094419 A | 26-05-2016 |
| | | PT 2160401 E | 30-10-2014 |
| | | PT 2388266 E | 23-06-2014 |
| | | US 2009324538 A1 | 31-12-2009 |
| | | US 2010278774 A1 | 04-11-2010 |
| | | US 2012230946 A1 | 13-09-2012 |
| | | US 2014219955 A1 | 07-08-2014 |
| | | US 2015132257 A1 | 14-05-2015 |
| | | WO 2008143794 A1 | 27-11-2008 |
| EP 2915569 A1 | 09-09-2015 | AU 2015226504 A1 | 08-09-2016 |
| | | CA 2938649 A1 | 11-09-2015 |
| | | EP 2915569 A1 | 09-09-2015 |
| | | WO 2015131994 A1 | 11-09-2015 |
| WO 2015018529 A1 | 12-02-2015 | CA 2920539 A1 | 12-02-2015 |
| | | CN 105579062 A | 11-05-2016 |
| | | EP 3030262 A1 | 15-06-2016 |
| | | JP 2016527286 A | 08-09-2016 |
| | | KR 20160040632 A | 14-04-2016 |
| | | US 2016184399 A1 | 30-06-2016 |
| | | WO 2015018529 A1 | 12-02-2015 |
| WO 2015018528 A1 | 12-02-2015 | AU 2014304931 A1 | 25-02-2016 |
| | | CA 2919725 A1 | 12-02-2015 |
| | | CN 105612175 A | 25-05-2016 |
| | | EA 201690333 A1 | 30-06-2016 |
| | | EP 3030575 A1 | 15-06-2016 |
| | | KR 20160042922 A | 20-04-2016 |
| | | US 2016175459 A1 | 23-06-2016 |
| | | WO 2015018528 A1 | 12-02-2015 |
| WO 2006020849 A2 | 23-02-2006 | AR 050293 A1 | 11-10-2006 |
| | | AR 050693 A1 | 15-11-2006 |
| | | US 2006057102 A1 | 16-03-2006 |
| | | US 2006057680 A1 | 16-03-2006 |
| | | WO 2006017853 A2 | 16-02-2006 |
| | | WO 2006020849 A2 | 23-02-2006 |
| WO 2014170032 A1 | 23-10-2014 | CA 2909576 A1 | 23-10-2014 |
| | | CN 105324124 A | 10-02-2016 |
| | | EP 2986312 A1 | 24-02-2016 |
| | | JP 2016518361 A | 23-06-2016 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/071759

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| | | KR 20150145260 A | 29-12-2015 |
| | | US 2016068584 A1 | 10-03-2016 |
| | | WO 2014170032 A1 | 23-10-2014 |
| WO 2007046006 A2 | 26-04-2007 | AT 527365 T | 15-10-2011 |
| | | BR PI0619300 A2 | 27-09-2011 |
| | | CA 2625694 A1 | 26-04-2007 |
| | | CN 101360827 A | 04-02-2009 |
| | | DK 1934353 T3 | 09-01-2012 |
| | | EP 1777294 A1 | 25-04-2007 |
| | | EP 1934353 A2 | 25-06-2008 |
| | | ES 2374166 T3 | 14-02-2012 |
| | | JP 5394742 B2 | 22-01-2014 |
| | | JP 5886247 B2 | 16-03-2016 |
| | | JP 2009512433 A | 26-03-2009 |
| | | JP 2013226155 A | 07-11-2013 |
| | | PT 1934353 E | 10-01-2012 |
| | | US 2009238791 A1 | 24-09-2009 |
| | | WO 2007046006 A2 | 26-04-2007 |