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(71) **Demandeurs/Applicants:**
SHANGHAI HENGRUI PHARMACEUTICAL CO., LTD., CN;
JIANGSU HENGRUI MEDICINE CO., LTD., CN

(72) **Inventeurs/Inventors:**
QU, XIANGDONG, CN;
YE, XIN, CN;
YUAN, JIJUN, CN;
...

(54) **Titre : PROTEINE DIMERIQUE HETEROGENE IL-15 ET SES UTILISATIONS**
(54) **Title: IL-15 HETERODIMERIC PROTEIN AND USES THEREOF**

(57) **Abrégé/Abstract:**
Disclosed are an IL-15 heterogeneous dimer protein and uses thereof. The IL-15 heterogeneous dimer protein comprises protein (I) and protein (II). The protein (I) is formed by means of reconstruction between IL-15 or a modification thereof and a first Fc modification; the protein (II) a second Fc modification, or is formed by means of reconstruction between IL-15Ra or a modification thereof and the second Fc modification; and the first Fc modification and the second Fc modification are connected in a manner of Knob-into-Hole. The IL-15 heterogeneous dimer protein has obvious tumor suppression activity, and has better bioactivity and an extended in-vivo half-life period compared with a single IL-15 molecule.

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(72) **Inventeurs(suite)/Inventors(continued):** ZHANG, LEI, CN; CHEN, KAN, CN; ZHANG, LIANSHAN, CN; CAO, GUOQING, CN; XU, ZHIBIN, CN; TAO, WEIKANG, CN

(74) **Agent:** SIM & MCBURNEY

**Abstract**

The present invention relates to an IL-15 heterodimeric protein and uses thereof and further relates to an IL-15/IL-15Rα heterodimeric protein. The IL-15 heterodimeric protein comprises protein (I) and protein (II), wherein said protein (I) is reassembled and formed by IL-15 or its variant with a first Fc variant; wherein said protein (II) is the second Fc variant, or reassembled and formed by IL-15Rα or its variant with a second Fc variant. The first Fc variant and the second Fc variant are linked via "Knob-into-Hole" mode. The IL-15 heterodimeric protein of the present invention has significant anti-tumor activity, better biological activity and prolonged in vivo half-life compared to IL-15 molecule alone.

# IL-15 HETERODIMERIC PROTEIN AND USES THEREOF

## FIELD OF THE INVENTION

The present invention relates to an IL-15 heterodimeric protein and uses thereof, and further relates to an IL-15/IL-15Rα heterodimeric protein complex, and its use as a therapeutic agent particular as a therapeutic agent for cancer and autoimmune disease.

## BACKGROUND

Interleukin 15 (IL-15), discovered by Grabstein et al. in 1994, is a cytokine about 12-14kD, which plays a role in normal immune response in organism, such as promoting the proliferation of T cells, B cells and natural killer (NK) cells.

IL-15 is a member of small four α-helix bundle family of cytokines. IL-15 needs to bind with its receptor to exert the biological activity. IL-15 receptor consists of three receptor subunits: IL-15 receptor α (IL-15Rα), IL-2 receptor β (IL-2Rβ, also known as IL-15Rβ or CD122) and γc (also known as CD132). IL-15Rα contains a Sushi domain which is capable of binding with IL-15, and is essential for biological functions of IL-15 after binding.

Recently it is found that the complex formed by IL-15 and its receptor IL-15Rα can significantly enhance the biological activity of IL-15. Studies indicated that the complex formed by IL-15 and soluble IL-15Rα receptor is significantly superior to IL-15 alone in stimulating the proliferation of memory CD8+ T lymphocytes and NT/NKT cells. IL-15/IL-15Rα complex is more than 10 times stronger than IL-15 alone in stimulating proliferation of memory CD8+ T cell and in maintaining their survival, the mechanism may be related to cis presentation.

Since IL-15 has been shown good expectation in the field of Tumor immunotherapy, NIH first began the investigation of IL-15 treatment in tumor area, and tried to push it into clinical Phase I. However, IL-15 has disadvantages of small molecular weight, short in vivo half-life, hardly-controlled repeated dosage, and is likely to cause systemic immune side effect. There is an urgent need to find an approach which can increase the in vivo half-life, and promote or enhance the biological activity of IL-15 in vivo. There are many domestic and foreign companies or research institutions engaging in researches related to IL-15 immunotherapy, for example, patent CN100334112C (Shanghai Haixin Biotechnology Co., Ltd.) relates to IL-15-hIgG4Fc homodimeric protein in anti- microbial infection treatment; patent CN1942481A (Switzerland F.Hoffmann-LaRoche AG) relates to IL-15-Fc fusion expression system and its use; and patent CN101360827B (French Institute of Health and Medical Research) relates to

IL-15Ra (sushi + domain) -IL-15 fusion protein and its application in cancer treatment. Heterodimer molecules of the present application show better stability, prolonged in vivo half-life and improved biological activity by increasing intramolecular interactions. On the basis of the molecular design in the present application, targeting immune cytokines can be generated by fusing and inserting functional polypeptide via methods well known in the field, the present disclosure also relates to application of targeting immune cytokines in cancer and autoimmune disease therapy.

## SUMMARY OF THE INVENTION

The present invention provides a protein molecule having prolonged in vivo half-life, increased in vitro activity and significant anti-tumor activity designed and prepared via genetic engineering methods.

The present invention provides an IL-15 heterodimeric protein comprising:

protein (I) and protein (Π);

wherein the protein (I) is reassembled and formed by IL-15 or its variant with a first Fc variant;

protein (Π) is a second Fc variant, or protein (Π) is reassembled and formed by IL-15Rα or its variant with a second Fc variant;

protein (I) and protein (Π) form a stable heterodimeric protein by the interaction between the first Fc variant and the second Fc variant.

As described above, the term "reassembled and formed" means obtained by expressing recombinants of different proteins produced by genetic engineering methods.

In a preferred embodiment of the present invention, the first Fc variant and the second Fc variant are linked to the C-terminus of IL-15 and IL-15Rα.

In one embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein the sequence of IL-15 is SEQ ID NO: 1.

In a preferred embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein the protein (Π) is the second Fc variant.

In one embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein the protein (Π) is reassembled and formed by IL-15Rα or its variant with a second Fc variant.

In one embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein the IL-15Rα variant is an extracellular domain portion of IL-15Rα or functional fragment thereof, the functional fragment is preferably a truncated form of IL-15Rα extracellular

domain portion with 65 to 120 amino acids, more preferably a truncated form with 65 to 102 amino acids.

In one embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein the sequence of IL-15Rα variant is selected from the group consisting of SEQ ID NOs: 2-7.

In one embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein the sequence of IL-15Rα variant is selected from the group consisting of SEQ ID NOs: 3-7.

In one embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein the first Fc variant and the second Fc variant are respectively selected from Knob modified Fc and Hole modified Fc; or the first Fc variant and the second Fc variant are respectively selected from Hole modified Fc and Knob modified Fc. "Knob / Hole" mode helps the first Fc variant and the second Fc variant to form a heterodimeric protein after modification. When the first Fc variant is a Knob modified Fc, the second Fc variant is a Hole modified Fc; or when the second Fc variant is a Knob modified Fc, the first Fc variant is a Hole modified Fc.

In one embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein the sequence of the first Fc variant is selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 and SEQ ID NO: 29; and the sequence of the second Fc variant is selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 and SEQ ID NO: 29. The protein (I) and the protein (Π) form a heterodimer in "Knob/Hole" mode via the Fc variants set forth in SEQ ID NO: 26 and SEQ ID NO: 27, or in SEQ ID NO: 28 and SEQ ID NO: 29. For example, when the sequence of the first Fc variant is SEQ ID NO: 26, the sequence of the second Fc variant is SEQ ID NO: 27; or when the sequence of the second Fc variant is SEQ ID NO: 26, the sequence of the first Fc variant is SEQ ID NO: 27.

In one embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein the sequence of the protein (I) is selected from the group consisting of SEQ ID NO: 14-17, preferably SEQ ID NO: 14.

In one embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein the sequence of the protein (Π) is selected from the group consisting of SEQ ID NOs: 18-25 and 34-37, preferably SEQ ID NOs: 23 and 34-37, more preferably SEQ ID NOs: 34-37.

In one embodiment of the present invention, provided is an IL-15 heterodimeric protein, wherein he sequence of the protein (I) is selected from the group consisting of SEQ ID

NOs: 30-31; the sequence of the protein (Π) is selected from the group consisting of SEQ ID NOs: 32-33.

The IL-15 heterodimeric protein of the present invention is selected from the following dimeric proteins 3-17, wherein the dimeric proteins 3-17 are reassembled and formed by the corresponding protein (I) and protein (Π) as blow:

| No | Protein (I) | Protein (Π) |
|---|---|---|
| 3 | IL-15-Fc-Knob (SEQ ID No.14) | Fc-Hole (SEQ ID No.27) |
| 4 | IL-15-Fc-Hole (SEQ ID No.15) | Fc-Knob (SEQ ID No.26) |
| 5 | Fc-Knob -IL-15 (SEQ ID No.16) | Fc-Hole (SEQ ID No.27) |
| 6 | Fc-Hole -IL-15 (SEQ ID No.17) | Fc-Knob (SEQ ID No.26) |
| 7 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα ECD-Fc-Hole (SEQ ID No.19) |
| 8 | IL-15-Fc-Hole (SEQ ID No.15) | IL-15Rα ECD-Fc-Knob (SEQ ID No.18) |
| 9 | Fc-Knob -IL-15 (SEQ ID No.16) | Fc-Hole -IL-15Rα ECD (SEQ ID No.21) |
| 10 | Fc-Hole -IL-15 (SEQ ID No.17) | Fc-Knob -IL-15Rα ECD (SEQ ID No.20) |
| 11 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(77 )-Fc-Hole (SEQ ID No.23) |
| 12 | Fc-Knob(M)-IL-15 (SEQ ID No.30) | Fc-Hole(M)-IL-15Rα-sushi(65) (SEQ ID No.32) |
| 13 | IL-15-Fc-Knob(M) (SEQ ID No.31) | IL-15Rα-sushi (65) -Fc-Hole(M) (SEQ ID No.33) |
| 14 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(73 )-Fc-Hole (SEQ ID No.34) |
| 15 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(65)-Fc-Hole (SEQ ID No.35) |
| 16 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(86)-Fc-Hole (SEQ ID No.36) |
| 17 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(102)-Fc-Hole (SEQ ID No.37) |

The present invention also relates to a nucleic acid encoding the IL-15 heterodimeric protein mentioned above.

The present invention also relates to a DNA vector comprising the nucleic acid mentioned above.

The present invention also relates to a host cell transformed with the DNA vector mentioned in the present invention.

The present invention also relates to a method for preparing the IL-15 heterodimeric protein as described above, the method comprises: culturing the host cell of the present invention under the condition sufficient for the expression of the IL-15 heterodimeric protein as described above; expressing and purifying the IL-15 heterodimeric protein.

The present invention also relates to a pharmaceutical composition comprising the IL-15 heterodimeric protein of the present invention, and a pharmaceutically acceptable excipient, diluent or carrier

The present invention also relates to a targeting protein molecule comprising the IL-15 heterodimeric protein structure according to the present invention.

The present invention also relates to a method for stimulating or inhibiting the immune response in a mammal, comprising: administering to the mammal a therapeutically effective amount of the IL-15 heterodimeric protein according to the present invention, or the pharmaceutical composition according to the present invention, or the targeting protein molecule according to the present invention.

The present invention also relates to use of the IL-15 heterodimeric protein according to the present invention, or the pharmaceutical composition according to the present invention, or the targeting protein molecule according to the present invention, in the preparation of a medicament for treatment of IL-15-mediated diseases or disorders; wherein the disease is selected from the group consisting of infectious disease, cancer, blood disease and autoimmune disease. The cancer is selected from the group consisting of melanoma, colorectal cancer, skin cancer, lymphoma, renal cell carcinoma, solid tumor, liver cancer, lung cancer, stomach cancer, and breast cancer; the infectious disease is selected from the group consisting of variola virus infection, HIV infection, bacterial infection, fungal infection, and HBV infection; the blood disease is selected from the group consisting of anemia, acute myeloid leukemia, myelodysplastic syndrome, and T-cell large granular lymphocytic leukemia; the autoimmune disease is selected from the group consisting of multiple sclerosis disease, psoriasis, rheumatoid arthritis, inflammatory diseases, gastritis and mucosal inflammation. Wherein the drugs are the IL-15 heterodimeric protein according to the present invention, or the pharmaceutical composition according to the present invention administered in combination with small molecule inhibitor(s) or antibody(ies); the small molecule inhibitor(s) is/are preferably selected from the alkylating agent(s); the antibody(ies) is/are preferably selected from monoclonal antibody(ies), more preferably anti-CD20, anti-PD1, anti-PDL1, or anti-Her2 antibody(ies). Further, the drug according to the present invention is administered in combination with the therapeutically effective dose of drugs selected from the group consisting of temozolomide,

doxorubicin, paclitaxel, cisplatin, carboplatin, dacarbazine, topotecan, irinotecan, gemcitabine and bevacizumab.

The present invention also relates to use of the IL-15 heterodimeric protein according to the present invention, or the pharmaceutical composition according to the present invention, or the targeting protein molecule according to the present invention, for cell immunotherapy, especially for tumor immunotherapy of DC, CIK, DC-CIK, ECIK, NK, CAS-T, BiAb-T, TCR-T, and CAR-T.

Tumor immunotherapy is a hotspot of cancer therapy, which is considered as the fourth cancer treatment modality following surgery, chemotherapy and radiotherapy. The objective of tumor immunology therapy is to initiate or mobilize the body's immune system, enhance the anti-tumor immunity in the tumor microenvironment, and consequently control and kill tumor cells. It may be the most effective and safest approach for cancer treatment.

The mechanism underlying tumor immune escape is based on inhibitory effect of tumor per se on immune system to maintain or promote the tumor growth. Tumor immunotherapy is to uttermost enhance the patient's own immune system response to tumor. It not only needs to activate the existing immune system response in vivo, but also needs to maintain the duration and intensity of immune system response: this is the key of tumor immunotherapy.

Cytokines Immunotherapy is developed along with production of highly pure or recombinant cytokines. The principle is that after injection into the body, certain cytokines can regulate and enhance one or more functions of immune cells, and play a stronger anti-tumor immunity.

The present invention also relates to a method for treating or preventing a disease, cells express disease-associated antigen in said disease, the method comprises: administering to a patient the IL-15 heterodimeric protein according to the present invention, or the pharmaceutical composition according to the invention, or the targeting protein molecule of described in the invention; forming a specific binding complex between cells expressing disease-associated antigen and immune cells expressing IL-15Rα, sufficient for activating said immune cells; and killing said cells expressing disease-associated antigen via the immune cells. The cells expressing disease-associated antigen are preferably tumor cells or virus-infected cells. The immune cells are preferably T-cells, LAK cells or NK cells. The disease is selected from the group consisting of infectious disease, cancer, blood disease and autoimmune disease. The cancer is selected from the group consisting of melanoma, colorectal cancer, skin cancer, lymphoma, renal cell carcinoma, and solid tumor; the infectious disease is selected from the

group consisting of variola virus infection, HIV infection, bacterial infection, and fungal infection; the blood disease is selected from the group consisting of anemia, acute myeloid leukemia, myelodysplastic syndrome, and T-cell large granular lymphocytic leukemia; the autoimmune disease is selected from the group consisting of multiple sclerosis, psoriasis, rheumatoid arthritis, inflammation disease, gastritis, and mucosal inflammation.

The present invention also relates to a method for treating or preventing a disease, the method comprises a step of administering to a patient with the IL-15 heterodimeric protein of the invention, or the pharmaceutical composition according to the invention, or the targeting protein described in the present invention, and co-administrating with other drugs, such as small molecule inhibitor(s) or antibody(ies); the small molecule inhibitor(s) is/are selected from the alkylating agent(s); the antibody(ies) is/are selected from monoclonal antibody(ies), more preferably anti-CD20, anti-PD1, anti-PDL1, or anti-Her2 antibody(ies).

For better understanding of the present disclosure, certain technical and scientific terms are specifically defined below. Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the field to which this disclosure belongs.

**Terms**

As used herein, the single-letter code and the three-letter code for amino acids are as described in J. Biol. Chem, 243, (1968) p3558.

As used herein, "Heterodimeric protein" refers to a protein formed with the combination of two different monomeric proteins. In the present invention, two different monomeric proteins respectively contain Fc fragment or Fc variant fragment, and form a heterodimeric protein via the interaction of Fc fragments or Fc variant fragments.

In the present invention, "interaction" between the first Fc variant and the second Fc variant refers to the interaction between Fc variants. "Fc variant" refers to a Fc having one or more amino acid substitutions, insertions or deletions in appropriate site(s), causing changes in Fc structure or function. "Interaction between Fc variants" refers to space-filling effects, electrostatic Steering, hydrogen bonding, hydrophobic interactions and the like formed by mutated Fc variants. Interaction between Fc variants contributes to form a stable heterodimeric protein. A preferred mutation is designed to "Knob-into-Hole" mutant form.

In the present invention, heterodimeric protein is composed of "monomeric protein" (ie protein (I), protein (Π)) which can be a fusion protein or non-fusion protein.

As used herein, "fusion protein" refers to a protein product obtained by linking the coding regions of two or more genes using genetically recombinant method, chemical method or

other suitable methods; and expressing the recombinant gene under control of an identical regulatory sequence. In some embodiments of the present invention, the protein (I) is a fusion protein obtained by expressing the recombinant gene of IL-15 or a variant thereof with Fc variant; protein (II) can be a fusion protein obtained by expressing the recombinant gene of IL-15Rα with Fc variant. In the fusion proteins of the invention, the coding regions of two or more genes can be fused by sequence(s) encoding peptide linker(s) in one or several location(s). Peptide linker can also be used to construct the fusion protein of the invention.

As used herein, "IL-15" or "IL-15 peptide" can be any IL-15 (interleukin-15) or a mutant thereof, such as human or non-human mammalian IL-15 or non-mammal IL-15. Exemplary non-human mammals comprise such as pigs, rabbits, monkeys, chimpanzees, mice, and the like; non-mammals comprise such as chickens and the like. Preferably human interleukin-15 mature molecule is found in Database UniProtKB, Accession Number P40933, 49-162aa. The term "IL-15 variant" refers to a variant molecule with increased or decreased affinity to its receptor, or increased or decreased activity in stimulating T cells or NK cell, due to one or more amino acid substitutions, additions or deletions.

The "IL-15Rα" according to the present invention can be any species of IL-15Rα or a functional fragment thereof, such as human IL-15Rα or non-human mammal IL-15Rα or non-mammal IL-15Rα. Exemplary non-human mammals comprise such as pigs, rabbits, monkeys, chimpanzees, mice and the like; non-mammals comprise such as chickens. Preferably human IL-15Rα, more preferably an extracellular domain fragment of human interleukin-15 receptor α, called IL-15Rα ECD (SEQ ID NO: 2), see Database UniProtKB, Accession Number Q13261, 31-205aa. The term "IL-15Rα variant" refers to a functional mutant with one or more amino acid deletions, insertions or replacement mutations, which is capable of binding with its ligand molecule such as IL15. Preferably human IL15Rα molecule, more preferably a truncated form of human IL-15Rα extracellular domain fragment, which is a molecule having the activity of human interleukin-15 receptor α, and obtained by one or more amino acid deletions started from the C-terminal fragment of the extracellular domain, preferably a deletion mutant form retaining 65 to 120 amino acids, more preferably a deletion mutant truncated form retaining 65 to 102 amino acids, such as IL-15Rα-sushi (77) (SEQ ID NO: 3), IL-15Rα-sushi (65) (SEQ ID NO: 4).

The term "immunoglobulin Fc region" refers to an immunoglobulin chain constant region, especially C-terminus or a part of immunoglobulin heavy chain constant region, having no antigen-binding activity, is an site for the interaction of antibody molecule with effector molecules and cells. For example, an immunoglobulin Fc region may comprise two or more

the heavy chain CH1, CH2, CH3, CH4 domains in combination with an immunoglobulin hinge region. Fc may be derived from different species, preferably human immunoglobulins. According to the amino acid sequence of the heavy chain constant region, immunoglobulins can be divided into different categories, mainly comprising five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM. Some of them can be further divided into subclasses (isotypes), e.g. IgG-1, IgG-2, IgG-3, IgG-4; IgA-1 and IgA-2.

"Fc region" preferably comprises at least one immunoglobulin hinge region, and CH2 and CH3 regions of IgG. More preferably comprises a CH2 domain, a CH3 domain and an immunoglobulin hinge region of IgG1, the initial amino acid position of hinge region may be varied.

Mutation Design in Fc variant has been widely applied in the art for preparing bispecific antibody or heterologous dimeric Fc fusion protein. Representative examples comprise "Knob-into-Hole" form proposed by Cater et al. (Protein Engineering vol.9 no.7 pp.617-621, 1996); Fc-containing heterodimeric form generated by Amgen technical personnel by using Electrostatic Steering (US 20100286374 A1); heterodimeric form (SEEDbodies) via IgG/IgA strand exchange proposed by Jonathan H. Davis et al. (Protein Engineering, Design & Selection pp.1-8,2010); bispecific molecules formed by DuoBody platform technology of Genmab Company(Science, 2007.317 (5844)); heterodimeric proteins formed by Xencor company's technical staff via comprehensive analysis of structural calculation and Fc amino acid mutations, and different action modes (mAbs 3: 6,546-557; November/December 2011); heterodimeric protein forms obtained by Suzhou Corning Jerry company using Fc engineering methods on basis of charge network (CN201110459100.7); and other genetic engineering methods to form the heterologous dimeric functional protein on the basis of Fc amino acid change or function alteration. Knob/Hole structure on the Fc variant of the present invention refers to two Fc fragments respectively mutated, and then bound in the form of "Knob-into-Hole". Preferably using "knob-into-hole" model proposed by Cater et al to perform site mutations in the Fc region, so that the resulting first Fc variant and second Fc variant are able to be bound together in the form of "knob-into-hole" to form a heterodimer. It is within the scope of those skilled in the art to select a specific immunoglobulin Fc region from particular immunoglobulin classes and subclasses. Preferably select Fc region of human IgG1, IgG2, IgG3, and IgG4 antibody, more preferably Fc region of human IgG1 antibody. Randomly select one from the first Fc variant and the second Fc variant for knob mutation, the other for hole mutation. In an embodiment, the first Fc variant is mutated as knob mutation, such as the sequence shown in SEQ ID NO: 26; the second Fc variant is mutated as hole mutation, such as

the sequence shown in SEQ ID NO: 27.

The term "linker peptide (Linker)" in the present invention is a peptide used to connect IL-15 or IL-15Rα with Fc variant, in order to ensure the correct protein folding and stability. "Linker peptide" of the present invention is preferably (GGGGS) n, where n may be 0, 1, 2, 3, 4, 5 or more, preferably n is 2-4. If the sequence of the linker peptide is too short, the advanced structure folding of two proteins may be affected, and thus interfered with each other; if the sequence of the linker peptide is too long, the problem of immunogenicity is concerned, since the linker peptide sequence itself is a new antigen.

As used herein, "heterodimeric protein" is preferably a product of co-expressed genes, for example, co-expressed in prokaryotic cells such as E. coli; or co-expressed in eukaryotic cells, such as 293 and CHO.

As used herein, "coexpression" refers to multiple genes are expressed together in a cell to simultaneously generate their products. These genes can be simultaneously existing and separately or commonly controlled and expressed. In the present invention, two genes are preferably co-expressed in a eukaryotic cell. Product obtained by co-expression of genes is conducive to form a complex efficiently and easily; In the present invention, is conducive to form a heterodimeric protein.

As used herein, "Immunoglobulin" refers to a four-peptide chain structure connected together by disulfide bond between two identical heavy chains and two identical light chains. Different immunoglobulin heavy chain constant regions exhibit different amino acid compositions and rank orders, hence present different kinds of antigenicity. Accordingly, immunoglobulins can be divided into five categories, or called immunoglobulin isotypes, namely IgM, IgD, IgG, IgA and IgE. According to its amino acid composition of hinge region and the number and location of heavy chain disulfide bonds, the same type of Ig can be divided into different sub-categories, for example, IgG can be divided into IgG1, IgG2, IgG3, and IgG4. Light chain can be divided into κ or λ chain according to different constant region.

Targeting protein molecule refers to a class of proteins, which contains a fragment or a region capable of interacting with other proteins and introducing targeting peptides such as antibody fragments, ScFv or binding peptide of some classes of cell surface molecules and on the like on the basis of immune cytokines such as IL5, IL2, etc., or unique molecular design containing such cytokines such as molecule design in this application. For example, interaction between antibody and antigen, or interaction between ligand and receptor, makes molecules preferentially enriched in a particular tissue, organ or body part after entering into the body through targeting effect to exert their biological functions. A new molecule is formed by fusing

the free terminus of molecule according to the present application with some class of polypeptide; the method used herein can reasonably be derived and designed to generate a series of molecules in the art.

"Administration" or "treatment," as it applies to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic agent, diagnostic agent, or composition with the animal, human, subject, cell, tissue, organ, or biological fluid. "Administration" or "treatment" can refer, e.g., to therapeutic, pharmacokinetic, diagnostic, research, and experimental methods. Treatment of a cell encompasses contacting a reagent with the cell, as well as contacting a reagent with a fluid, where the fluid is in contact with the cell. "Administration" or "treatment" also means in vitro and ex vivo treatments, e.g., of a cell, with a reagent, diagnostic, binding compound, or with another cell. "Treatment," as it applies to a human, animal, or research subject, refers to therapeutic treatment, prophylactic or preventative measures, to research and diagnostic applications. "Treatment", as it applies to a human, animal, or research subject, or cell, tissue, or organ, encompasses contacting an IL15 agonist or IL15 antagonist with a human or animal, subject, a cell, tissue, physiological compartment, or physiological fluid. "Treatment of a cell" also encompasses situations where the IL15 agonist or IL15 antagonist contacts with IL15 receptor, e.g., in the fluid phase or colloidal phase, but also situations where the agonist or antagonist does not contact with the cell or the receptor.

"Treat" means to internally or externally administer a therapeutic agent, such as a composition containing IL-5 heterodimeric protein of the present invention to a patient suffering from one or more diseases or conditions. Said therapeutic agent is known to have therapeutic effect on these diseases or conditions. Typically, the therapeutic agent is administered in an amount effective to alleviate one or more diseases or conditions in the patient or population to be treated, either by inducing the regression of these diseases or conditions or by inhibiting the progression of such diseases or conditions to any clinically measurable degree. The amount of a therapeutic agent that is effective to alleviate any particular disease or condition (also referred to as the "therapeutically effective amount") may vary according to several factors such as the disease status, age, and weight of the patient, and the ability of the drug to elicit a desired response in the patient.

"Immune disease" or "immune disorder" includes e.g., pathological inflammation, inflammatory disorder, and autoimmune disease or disorder. "Immune disease" also refers to infection, persistent infection, and proliferative disorder such as cancer, tumor, and angiogenesis. "Cancerous disease" includes, e.g., cancer, cancer cells, tumor, angiogenesis, and

precancerous lesion, e.g., dysplasia.

As used herein, "polymerase chain reaction" or "PCR" refers to an amplification procedure or technique described in, e.g., U.S. Pat. No. 4,683,195. Generally, sequence information from the ends of the interest region or beyond the interest region needs to be available, such that oligonucleotide primers can be designed. These primers will be identical or similar in sequence to the strand opposite to the template to be amplified.

"Optional" or "optionally" means that the event or situation that follows may but does not necessarily occur, and the description includes the instances in which the event or circumstance does or does not occur. For example, "optionally contains 1-3 antibody heavy chain variable regions" means the antibody heavy chain variable region can be, but not necessarily to be, present. If any, may be 1, 2 or 3.

"Pharmaceutical composition" refers to a mixture containing one or more compounds according to the present invention or a physiologically/pharmaceutically acceptable salt or prodrug thereof with other chemical components, as well as additional components such as physiologically/pharmaceutically acceptable carriers and excipients. The pharmaceutical composition aims at promoting the administration to an organism, facilitating the absorption of the active indgredient and thereby exerting a biological effect.

Transformation of the host cell with the recombinant DNA may be carried out by conventional techniques well known to those skilled in the art. The obtained transformants are cultured by using conventional methods to express the polypeptide encoded by the gene of the invention. Culture medium may be selected from various conventional culture mediums based on the host cells used. The host cells grow under proper conditions.

## Brief description of the drawings

Figure 1. Relationship between dosing time and molar concentration in serum samples. ewe

Figure 2. In lung metastasis model of Test Example 3, the number of metastatic lumps in lung in dosing mice. * in the figure represents: p <0.05, vsPBS; ** represents: p <0.01, vsPBS; ## represents: p <0.01, vs IL-15.

Figure 3. In lung metastasis model of Test Example 3, the relative lung weight (lung weight/body weight) in dosing mice, ** in the figure represents: p <0.01, vs PBS; # represents: p <0.05, vs IL- Fifteen.

Figure 4. Curative effect of different administrated proteins on HCT-116 xenograft nude mice.

Figure 5. Curative effect of different administrated proteins on HCT-116 xenografts nude mice. Tumor weight on day 27. * in the figure represents: $p < 0.05$, vs blank.

Figure 6. Curative effect of different administrated proteins on HCT-116+PBMC xenograft SCID mice.

Figure 7. Curative effect of different administrated proteins on HCT-116+PBMC xenograft SCID mice, tumor weight on day 28. * in the figure represents: $p < 0.05$, vs PBMC.

Figure 8. Quantitative comparison of mice lung metastatic lumps after administration with dimeric protein 17, positive control (IL-15) and negative control (PBS), in the figure *: $P < 0.05$, **: $p < 0.01$, vs PBS.

Figure 9. Comparison of relative lung weight (lung weight/body weight) in mice after administration with dimeric protein 17, positive control (IL-15) and negative control (PBS).

Figure 10. Comparison of body weight in mice after administration with dimeric protein 17, positive control (IL-15) and negative control (PBS).

## DETAILED DESCRIPTION OF THE INVENTION

Hereinafter, the present invention is further described with reference to examples; however, the scope of the present invention is not limited thereto.

In the examples of the present invention, where specific conditions are not described, the experiments are generally conducted under conventional conditions, or under conditions proposed by the material or product manufacturers. See Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory; Current Protocols in Molecular Biology, Ausubel et al, Greene Publishing Associates, Wiley Interscience, NY. Where the source of the reagents is not specifically given, the reagents are commercially available conventional reagents.

### Example 1. Obtainment of heterodimeric IL-15 protein

Heterodimeric protein provided herein is formed by binding protein (I) and protein (II), the protein (I) is a fusion protein reassembled and formed by IL-15 or a variant thereof with the first Fc variant; the protein (II) may be the second Fc variant, or may be a fusion protein reassembled and formed by IL-15Rα ECD or a variant thereof with the second Fc variant; the binding is preferably binding the first Fc variant with the second Fc variant in a Knob/Hole form.

IL-15 used in the embodiments of the invention refers to the mature molecule of human interleukin-15 (SEQ ID NO: 1) or a variant thereof. IL-15Rα ECD used in the embodiments of the invention refers to the extracellular domain fragment of human

interleukin-15 receptor α (SEQ ID NO: 2); the variant thereof is preferably a truncated form, such as IL-15Rα-sushi (77) (SEQ ID NO: 3), IL-15Rα-sushi (65) (SEQ ID NO: 4). Fc fragment used in the embodiments of the invention may be Fc fragment of human antibody IgG1, IgG2, IgG3, IgG4, preferably Fc fragment of human IgG1. In the present invention, the first Fc variant or the second Fc variant preferably underwent Knob form mutation (SEQ ID NO: 26), or Hole form mutation (SEQ ID NO: 27). In the heterodimeric protein of the present invention, the protein (I) and protein (Π) form a dimer via Knob/Hole structure between the first Fc variant and the second Fc variant, preferably form a heterodimer via "Knob-into-Hole" action mode between the first Fc variant and the second Fc variant. Dimer containing only a single IL-15 effector molecule can also be fused to the first Fc variant by IL-15, and a heterodimeric protein, such as heterodimeric protein 3 of the invention is formed via Knob-into-Hole mode between the first Fc variant and the corresponding second Fc variant.

In the present invention, IL-15 or a variant thereof is fused to the first Fc fragment or the first Fc variant via a linker peptide to form a fusion protein, and in the present invention, IL-15Rα ECD or a variant thereof is fused to the second Fc fragment or the second Fc variant via a linker peptide to form a fusion protein, wherein the connection order of each protein component is not limited; the linker peptide may be a conventional flexible linker peptide in the art, preferably (GGGGS)n, wherein n is selected from 1-10, preferably selected from 1-5, most preferably 2.

**The corresponding protein sequences are as follows:**

IL-15 (protein sequence 1): (amino acid sequence of human interleukin 15, also known as sequence of control IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIH
DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

SEQ ID NO: 1.

IL-15Rα ECD (protein sequence 2): (amino acid sequence of extracellular domain of human interleukin 15 receptor alpha，also known as the indicated IL-15Ra segment in molecule H formed by fusing with Fc)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGS
QLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT

SEQ ID NO: 2.

IL-15Rα-sushi (77) (protein sequence 3): (a domain maintaining more than 90% of the binding activity in the extracellular domain of human interleukin-15 receptor alpha, known as the sushi domain plus a short linker peptide, belongs to a truncated form of IL-15Rα)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP SLKCIRDPALVHQRPAPP SEQ ID NO: 3.

IL-15Rα-sushi (65) (protein sequence 4): (a domain maintaining more than 90% of the binding activity in the extracellular domain of human interleukin-15 receptor alpha, known as the sushi domain, belongs to a truncated form of IL-15Rα)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP SLKCIR SEQ ID NO: 4.

IL15Ra-sushi (73) (protein sequence 5):

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP SLKCIRDPALVHQR SEQ ID NO: 5

IL15Ra-sushi (86) (protein sequence 6):

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP SLKCIRDPALVHQRPAPPSTVTTAGVT SEQ ID NO: 6

IL15Ra-sushi (102) (protein sequence 7):

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP SLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS SEQ ID NO: 7

IL-15-Fc (protein sequence 8): (a fusion protein formed by linking human interleukin-15 molecule with human IgG1-Fc sequence via a linker peptide, expressed as a bivalent homodimers, wherein the IL-15 molecule is at the N-terminus of the protein)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIH DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGG GGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

K SEQ ID NO: 8.

Fc-IL-15 (protein sequence 9): (a fusion protein formed by linking human interleukin-15 molecule with human IgG1-Fc sequence via a linker peptide, expressed as a bivalent homodimers, wherein the IL-15 molecule is at the C-terminus of the protein)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG
GGGSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVI
SLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN
TS SEQ ID NO: 9.

IL-15Rα ECD-Fc (protein sequence 10): (a fusion protein formed by linking extracellular domain of human interleukin 15 receptor alpha with human IgG1-Fc sequence via a linker peptide, expressed as a bivalent homodimers, wherein the IL-15Rα-ECD molecule is at the N-terminus of the protein)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGS
QLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTGGGGS
GGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK SEQ ID NO: 10.

Fc-IL-15Rα ECD (protein sequence 11): (a fusion protein formed by linking extracellular domain of human interleukin 15 receptor alpha with human IgG1-Fc sequence via a linker peptide, expressed as a bivalent homodimers, wherein the IL-15Rα-ECD molecule is at the C-terminus of the protein)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG

GGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKA
TNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTA
ATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQG
HSDTT SEQ ID NO: 11.

IL-15Rα-sushi (77) -Fc (protein sequence 12): (a fusion protein formed by linking sushi (77) fragment consisting of sushi domain of human interleukin-15 receptor alpha extracellular domainand and a linker peptide, with human IgG1-Fc sequence via a linker peptide, wherein the sushi (77) fragment is at the N-terminus)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRDPALVHQRPAPPSGGSGGGGSGGGSGGGGSLQEPKSSDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO: 12.

Fc- IL-15Rα-sushi (77) (protein sequence 13): (a fusion protein formed by linking sushi+ fragment consisting of sushi domain of human interleukin-15 receptor alpha extracellular domainand and a linker peptide, with human IgG1-Fc sequence via a linker peptide, wherein the sushi+ fragment is at the C-terminus)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKS
GGSGGGGSGGGSGGGGSLQITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTS
SLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPP SEQ ID NO: 13.

IL-15-Fc -Knob (protein sequence 14): (Fc portion of the above sequence 8 is mutated in a Knob form, and paired with another fusion molecule in a Hole form)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIH
DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGG
GGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPE

NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K SEQ ID NO:14.

IL-15-Fc-Hole (protein sequence 15): (Fc portion of the above sequence 8 is mutated in a Hole form, and paired with another fusion molecule in a Knob form)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIH
DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGG
GGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K SEQ ID NO: 15.

Fc-Knob -IL-15 (protein sequence 16): (Fc portion of the above sequence 9 is mutated in a Knob form, and paired with another fusion molecule in a Hole form)
EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG
GGGSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVI
SLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN
TS SEQ ID NO: 16.

Fc-Hole -IL-15 (protein sequence 17): (Fc portion of the above sequence 9 is mutated in a Hole form, and paired with another fusion molecule in a Knob form)
EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG
GGGSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVI
SLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN
TS SEQ ID NO: 17.

IL-15Rα ECD-Fc-Knob (protein sequence 18): (Fc portion of the above sequence 10 is

mutated in a Knob form, and paired with another fusion molecule in a Hole form)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGS
QLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTGGGGS
GGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK SEQ ID NO:18.

IL-15Rα ECD-Fc-Hole (protein sequence 19): (Fc portion of the above sequence 10 is mutated in a Hole form, and paired with another fusion molecule in a Knob form)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGS
QLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTGGGGS
GGGGSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP
GK SEQ ID NO: 19.

Fc-Knob-IL-15Rα ECD (protein sequence 20): (Fc portion of the above sequence 11 is mutated in a Knob form, and paired with another fusion molecule in a Hole form)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG
GGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKA
TNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTA
ATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQG
HSDTT SEQ ID NO:20.

Fc-Hole -IL-15Rα ECD (protein sequence 21): (Fc portion of the above sequence 11 is mutated in a Hole form, and paired with another fusion molecule in a Knob form)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG
GGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKA
TNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTA
ATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQG
HSDTT SEQ ID NO:21.

IL-15Rα-sushi (77) -Fc-Knob (protein sequence 22): (Fc portion of the above sequence 12 is mutated in a Knob form, and paired with another fusion molecule in a Hole form)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRDPALVHQRPAPPSGGSGGGGSGGGSGGGGSLQEPKSSDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO: 22.

IL-15Rα-sushi (77) -Fc-Hole (protein sequence 23): (Fc portion of the above sequence 12 is mutated in a Hole form, and paired with another fusion molecule in a Knob form)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRDPALVHQRPAPPSGGSGGGGSGGGSGGGGSLQEPKSSDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO: 23.

Fc-Knob - IL-15Rα-sushi (77) (protein sequence 24): (Fc portion of the above sequence 13 is mutated in a Knob form, and paired with another fusion molecule in a Hole form)
EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKS
GGSGGGGSGGGSGGGGSLQITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTS

SLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPP SEQ ID NO: 24.

Fc-Hole - IL-15Rα-sushi (77) (protein sequence 25): (Fc portion of the above sequence 13 is mutated in a Hole form, and paired with another fusion molecule in a Knob form)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKS
GGSGGGGSGGGSGGGGSLQITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTS
SLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPP SEQ ID NO: 25.

Fc-Knob (protein sequence 26): (Knob mutant form of human IgG1-Fc segment, which can be paired with IL-15-Fc-Hole/Fc-IL-15-Hole)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 26.

Fc-Hole (protein sequence 27): (Hole mutant form of human IgG1-Fc segment, which can be paired with IL-15-Fc-Knob/Fc-IL-15-Knob)

EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 27.

Fc-Knob (M) (protein sequence 28): Another form of Fc mutation,which can be paired with Fc-Hole (M) to form a heterodimer.

EPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 28

Fc-Hole (M) (protein sequence 29): Another form of Fc mutation,which can be paired with Fc-Knob (M) to form a heterodimer.

EPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 29

Fc-Knob (M) -IL-15 (protein sequence 30) (different mutation sites relative to Knob, another mutation manner of heterodimer)

EPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGG
GGSGGGGSGGGGSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAM
KCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQS
FVHIVQMFINTS SEQ ID NO: 30

IL-15-Fc- Knob (M) (protein sequence 31) (different mutation site relative to Knob, another mutation manner of heterodimer)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIH
DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGG
GGSEPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K SEQ ID NO: 31

Fc-Hole (M) -IL-15Rα-sushi (65) (protein sequence 32) (different mutation site relative to Hole, another mutation manner of heterodimer)

EPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK

TTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGG
GSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSL
TECVLNKATNVAHWTTPSLKCIR SEQ ID NO: 32

IL-15Rα-sushi (65) -Fc-Hole (M) (protein sequence 33) (different mutation site relative to Hole, another mutation manner of heterodimer)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRGGGGSGGGGSGGGGSGGGGSEPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLS
CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK SEQ ID NO: 33

IL-15Rα-sushi(73 )-Fc-Hole (protein sequence 34): (sushi (73) refers to a truncated form of IL15Rα containing the sushi domain with 73 amino acids in length)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRDPALVHQRSGGSGGGGSGGGGSGGGGSLQEPKSSDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK SEQ ID NO: 34

IL-15Rα-sushi(65)-Fc-Hole (protein sequence 35): (sushi (65) refers to a sushi domain with 65 amino acids in length)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRSGGSGGGGSGGGGSGGGGSLQEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK SEQ ID NO: 35

IL-15Rα-sushi(86)-Fc-Hole (protein sequence 36): (sushi (86) refers to a truncated form of IL15Rα containing the sushi domain with 86 amino acids in length
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP

SLKCIRDPALVHQRPAPPSTVTTAGVTSGGSGGGGSGGGSGGGGSLQEPKSSDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 36

IL-15Rα-sushi(102)-Fc-Hole (protein sequence 37): (sushi (102) refers to a truncated form of IL15Rα containing the sushi domain with 102 amino acids in length

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP
SLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSGGSGGGGSGGGSGGGG
SLQEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 37

**Example 2. Construction of related vectors**

*Materials:*

Eukaryotic expression vector pcDNA3.1 (+) (Life technologies, Cat. No. V790-20);

IL-15 (DNA sequence 1), IL-15Rα ECD (DNA sequence 2) and IgG1Fc (DNA sequence 3) DNA fragments were synthesized by gene synthesis company (GENEWIZ, Inc., Suzhou);

Primers DNA fragments were synthesized by gene synthesis company (GENEWIZ, Inc., Suzhou).

*Procedure*:

Fragments were spliced by the conventional PCR method.

1. Fragment ligation

IL-15-Fc fragment: By using overlapping PCR, an IL-15-Fc fragment (DNA sequence 4, SEQ ID NO: 42) was formed by joining three DNA fragments in order of IL-15, linker peptide, and Fc.

IL-15Rα ECD-Fc fragment: By using overlapping PCR, an IL-15Rα ECD-Fc fragment (DNA sequence 5, SEQ ID NO: 43) was formed by joining three DNA fragments in order of IL-15Rα ECD, linker peptide, and Fc.

Fc-IL-15 fragment: By using overlapping PCR, a Fc-IL-15 fragment (DNA Sequence 6, SEQ ID NO: 44) was formed by joining three DNA fragments in order of Fc, linker peptide, and IL-15.

Fc-IL-15Rα ECD fragment: By using overlapping PCR, a Fc-IL-15Rα ECD fragment (DNA Sequence 7, SEQ ID NO: 45) was formed by joining three DNA fragments in order of Fc, linker peptide, and IL-15Rα ECD.

IL-15-Fc-Knob fragment: By using overlapping PCR, an IL-15-Fc-Knob fragment (DNA Sequence 8, SEQ ID NO: 46) was formed by joining three DNA fragments in order of IL-15, linker peptide, and Fc-Knob.

IL-15-Fc-Hole fragment: By using overlapping PCR, an IL-15-Fc-Hole fragment (DNA Sequence 9, SEQ ID NO: 47) was formed by joining three DNA fragments in order of IL-15, linker peptide, and Fc-Hole.

Fc-Knob-IL-15 fragment: By using overlapping PCR, a Fc-Knob-IL-15 fragment (DNA sequence 10, SEQ ID NO: 48) was formed by joining three DNA fragments in order of Fc-Knob, linker peptide, and IL-15.

Fc-Hole-IL-15 fragment: By using overlapping PCR, a Fc-Hole-IL-15 fragment (DNA sequence 11, SEQ ID NO: 49) was formed by joining three DNA fragments in order of Fc-Hole, linker peptide, and IL-15.

IL-15Rα ECD-Fc-Knob fragment: By using overlapping PCR, an IL-15Rα ECD-Fc-Knob fragment (DNA Sequence 12, SEQ ID NO: 50) was formed by joining three DNA fragments in order of IL-15Rα ECD, linker peptide, and Fc-Knob.

IL-15Rα ECD-Fc-Hole fragment: By using overlapping PCR, an IL-15Rα ECD-Fc-Hole fragment (DNA Sequence 13, SEQ ID NO: 51) was formed by joining three DNA fragments in order of IL-15Rα ECD, linker peptide, and Fc-Hole.

Fc-Knob-IL-15Rα ECD fragment: By using overlapping PCR, a Fc-Knob-IL-15Rα ECD fragment (DNA Sequence 14, SEQ ID NO: 52) was formed by joining three DNA fragments in order of Fc-Knob, linker peptide, and IL-15Rα ECD.

Fc-Hole-IL-15Rα ECD fragment: By using overlapping PCR, a Fc-Hole-IL-15Rα ECD fragment (DNA Sequence 15, SEQ ID NO: 53) was formed by joining three DNA fragments in order of Fc-Hole, linker peptide, and IL-15Rα ECD.

Fc-Knob fragment, DNA sequence 16, SEQ ID NO: 54.

Fc-Hole fragment, DNA sequence 17, SEQ ID NO: 55.

Fc-Knob (M) -IL-15, DNA sequence 18, nucleotide sequence encoding the precursor of protein sequence 30, SEQ ID NO: 56.

IL-15-Fc-Knob (M), DNA sequence 19, nucleotide sequence encoding the precursor of protein sequence 31, SEQ ID NO: 57.

Fc-Hole (M) -IL-15Rα-sushi(65), DNA sequence 20, nucleotide sequence encoding the precursor of protein sequence 32, SEQ ID NO: 58.

IL15Rα-sushi (65) -Fc-Hole (M), DNA sequence 21, nucleotide sequence encoding the precursor of protein sequence 33, SEQ ID NO: 59.

IL-15Rα-sushi(73)-Fc-Hole, DNA sequence 22, nucleotide sequence encoding the precursor of protein sequence 34, SEQ ID NO: 60.

IL-15Rα-sushi(65)-Fc-Hole, DNA sequence 23, nucleotide sequence encoding the precursor of protein sequence 35, SEQ ID NO: 61.

IL-15Rα-sushi(86)-Fc-Hole, DNA sequences of 24, nucleotide sequence encoding the precursor of protein sequence 36, SEQ ID NO: 62.

IL-15Rα-sushi(102)-Fc-Hole, DNA sequence 25, nucleotide sequence encoding the precursor of protein sequence 37, SEQ ID NO: 63.

2. Inserting restriction sites and signal peptide sequence:

Restriction endonuclease KpnI site, Kozak sequence, and the signal peptide sequence were inserted at the 5'-terminus of the gene fragment by PCR method. The sequence between KpnI site and the gene fragment is shown in SEQ ID NO: 38:

ggtaccttgtgcccgggcgccac*atggagtttgggctgagctggctttttcttgtcgcgattcttaagggtgtccagtgc*

(The underline represents a KpnI restriction site, the italic represents signal peptide sequence); Termination codon TGA and NotI restriction enzyme site were inserted to the 3'-terminus of the three fragments respectively.

3. Construction of expression vectors

The gene fragments aforementioned were inserted into the vector pcDNA3.1 (+) respectively using restriction enzyme KpnI and NotI sites, to construct expression vectors such as pcDNA3.1-IL-15-Fc, pcDNA3.1-IL-15Rα ECD-Fc, pcDNA3.1-Fc, pcDNA3.1-Fc-IL-15, pcDNA3.1-Fc-IL-15Rα ECD and the like, to obtain the corresponding expression plasmids.

4. Site-directed mutagenesis in gene

Site-directed mutagenesis process was carried out by using KOD kit (TOYOBO Cat.KOD-201) 25μL system: 2.5μL 10×KOD buffer, 2.5μL 2mM dNTPs, 1μL primer 1 (10μM), 1μL primer 2 (10μM), 0.5μL KOD plus, 1μL 25mM MgSO4, 16μL ddH2O. Synthesis procedure: 94° C for 2 min, 94° C for 30 sec, 55° C for 30 sec, 68° C for 11 minutes, after 25 amplification cycles, PCR amplification process was terminated by another 11 minutes at 68° C. PCR product was digested for 5 hours with direct addition of 1μL of DpnI

(NEB Cat. R0176L), transformed into DH5a competent cells, clones were picked for sequencing to obtain desired plasmids. Protein 3 involved in the example of the present invention was obtained by expressing the expression vector containing DNA sequences 8 (SEQ ID NO: 46) and DNA sequence 17 (SEQ ID NO: 55); Protein 7 was obtained by expressing the expression vector containing DNA sequences 8 (SEQ ID NO: 46) and DNA sequence 13 (SEQ ID NO: 51). Proteins in other examples were coexpressed by the expression vector containing the DNA sequences.

*Constructing Nucleotide sequence for expression plasmid*

The following were DNA sequences used for vector construction, single line represents DNA sequences for signal peptide, dotted line represents DNA sequences for peptide linker, and the double line represents DNA sequences for Fc undergoing Knob/Hole mutation(s).

DNA Sequence 1: (IL-15, nucleotide sequence of human interleukin-15)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCAACTGGGTGAATGTAATTAGTGATTTGAAAAAAATTGAAGATCTTATT
CAATCTATGCATATTGATGCTACTTTATATACGGAAAGTGATGTTCACCCGAGTTGC
AAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTTATTTCACTTGAGTCC
GGCGATGCAAGTATTCATGATACAGTAGAAAATCTGATCATCTTAGCAAACAACAG
TTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGCAAAGAATGTGAGGAACTGG
AGGAAAAAAATATTAAAGAATTTTTGCAGAGTTTTGTACATATTGTCCAAATGTTCA
TCAACACTTCT SEQ ID NO: 39.

DNA Sequence 2: (IL-15Rα ECD, nucleotide sequence of the extracellular domain of human interleukin-15 receptor alpha)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCATCACCTGCCCTCCACCTATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCCGCGAGCGCTACATTTGTAACTCTGGTTTCAAGCGT
AAAGCCGGCACCTCCAGCCTGACCGAGTGCGTGTTGAACAAGGCCACCAATGTCGC
CCACTGGACAACCCCAAGTCTCAAATGCATTCGCGACCCTGCCCTGGTTCACCAACG
CCCAGCGCCACCATCCACAGTAACCACTGCAGGCGTGACCCCACAGCCAGAGAGCC
TCTCCCCTTCTGGCAAAGAGCCAGCAGCTTCATCTCCAAGCTCAAACAACACAGCG
GCCACAACAGCAGCTATTGTCCCGGGCTCCCAGCTGATGCCTTCAAAATCACCTTCC
ACAGGCACCACAGAGATCAGCAGTCATGAGTCCTCCCACGGCACCCCATCTCAGAC
AACAGCCAAGAACTGGGAACTCACAGCATCCGCCTCCCACCAGCCGCCAGGTGTGT
ATCCACAGGGCCACAGCGACACCACT SEQ ID NO: 40.

DNA Sequence 3: (Fc, nucleotide sequence of human IgG Fc)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCT
CCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACC
CTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAA
GATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAA
GACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGA
CTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAAC
AAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACG
GGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAG
TGTCACTTACATGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGG
AGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCA
GATGGTTCCTTTTTCTTGTACAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAA
GGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAA
AAATCTCTCAGCCTTTCTCCCGGCAAG SEQ ID NO: 41.

DNA Sequence 4: (IL-15-Fc, nucleotide sequence encoding the precursor of protein sequence 5)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCaactgggtgaatgtaattagtgatttgaaaaaaattgaagatcttattcaatctatgcatattgatgctactttatatacgga
aagtgatgttcacccgagttgcaaagtaacagcaatgaagtgctttcttggagttacaagttatttcacttgagtccggcgatgcaagtattc
atgatacagtagaaaatctgatcatcttagcaaacaacagtttgtcttctaatgggaatgtaacagaatctggatgcaaagaatgtgaggaact
ggaggaaaaaaaatattaaagaatttttgcagagttttgtacatattgtccaaatgttcatcaacacttctGGCGGAGGAGGCTCT
GGGGGCGGAGGAAGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTG
CCCAGCTCCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAA
GGATACCCTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTC
TCACGAAGATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACA
ATGCTAAGACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCA
GTGCTGACTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGT
GTCTAACAAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTC
AACCACGGGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAG
AATCAAGTGTCACTTACATGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTG
GAGTGGGAGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGC
TCGATTCAGATGGTTCCTTTTTCTTGTACAGCAAACTCACCGTTGACAAGAGTCGGT

GGCAGCAAGGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCAT
TATACCCAAAAATCTCTCAGCCTTTCTCCCGGCAAG SEQ ID NO: 42.

DNA Sequence 5: (IL-15Rα ECD-Fc, nucleotide sequence coding the precursor of protein sequence 7)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCatcACCtgccctCCACCTatgtccgtggaacacgcagacatctgggtcaagagctacagcttgtactccCG
CgagcgCtacatttgtaactctggtttcaagcgtaaagccggcACCtccagcctgACCgagtgcgtgttgaacAAGGCCAC
CaatgtcgcccactggacaaccccAagtctcaaatgcattCGCgaccctgccctggttcaccaaCGCccagcgccaccAtccaca
gtaACCACTgcaggCgtgaccccacagccagagagcctctccccttctggCaaagagccAgcagcttcatctccAagctcaaac
aacacagcggccacaacagcagctattgtcccgggctcccagctgatgccttcaaaatcaccttccacaggCaccacagagatCagcag
tcatgagtcctcccacggcaccccAtctcagacaacagccaagaactgggaactcacagcatccgcctcccaccagccgccaggtgtgt
atccacagggccacagcgacaccactGGCGGAGGAGGCTCTGGGGGCGGAGGAAGCGAACCTAA
GTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCTCCTGAGCTCTTGGGCGG
ACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACCCTTATGATCAGCAGAAC
ACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAAGATCCTGAAGTGAAATT
CAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACTAAGCCCCGTGAAG
AGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGACTGTTCTCCATCAGGACT
GGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAACAAGGCACTGCCCGCACCC
ATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACGGGAGCCACAGGTTTACAC
ATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAGTGTCACTTACATGTCTTG
TGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGGAGAGCAACGGACAACCA
GAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCAGATGGTTCCTTTTTCTTG
TACAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAAGGAAATGTGTTCAGCTG
TTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAAAAATCTCTCAGCCTTTC
TCCCGGCAAG SEQ ID NO: 43.

DNA Sequence 6: (Fc-IL-15, nucleotide sequence encoding the precursor of protein sequence 6)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCT
CCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACC
CTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAA
GATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAA
GACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGA

CTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAAC
AAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACG
GGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAG
TGTCACTTACATGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGG
AGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCA
GATGGTTCCTTTTTCTTGTACAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAA
GGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAA
AAATCTCTCAGCCTTTCTCCCGGCAAGGGCGGAGGAGGCTCTGGGGGCGGAGGAAG
Caactgggtgaatgta**ATT**agtgatttgaaaaaaattgaagatcttattcaatctatgcatattgatgctactttatatacggaaagtgatgttc
acCCGagttgcaaagtaacagcaatgaagtgctttctcttggagttacaagttatttcacttgagtccggcgatgcaagtattcatgatacag
tagaaaatctgatcatc**TTA**gcaaacaacagtttgtcttctaatgggaatgtaacagaatctggatgcaaagaatgtgaggaactggagga
aaaaaatattaaagaatttttgcagagttttgtacatattgtccaaatgttcatcaacacttct SEQ ID NO: 44.

DNA Sequence 7: (Fc-IL-15Rα ECD, nucleotide sequence encoding the precursor of protein sequence 8)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCT
CCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACC
CTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAA
GATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAA
GACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGA
CTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAAC
AAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACG
GGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAG
TGTCACTTACATGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGG
AGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCA
GATGGTTCCTTTTTCTTGTACAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAA
GGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAA
AAATCTCTCAGCCTTTCTCCCGGCAAGGGCGGAGGAGGCTCTGGGGGCGGAGGAAG
CatcACCtgccctCCACCTatgtccgtggaacacgcagacatctgggtcaagagctacagcttgtactccCGCgagcgCtaca
tttgtaactctggtttcaagcgtaaagccggcACCtccagcctgACCgagtgcgtgttgaacAAGGCCACCaatgtcgcccac
tggacaaccccAagtctcaaatgcattCGCgaccctgccctggttcaccaaCGCccagcgccaccAtccacagtaACCACTg
caggCgtgaccccacagccagagagcctctcccttctggCaaagagccAgcagcttcatctccAagctcaaacaacacagcggcca
caacagcagctattgtcccgggctcccagctgatgccttcaaaatcaccttccacaggCaccacagagatCagcagtcatgagtcctccc

acggcaccccAtctcagacaacagccaagaactgggaactcacagcatccgcctcccaccagccgccaggtgtgtatccacagggcca
cagcgacaccact SEQ ID NO: 45.

DNA Sequence 8: (IL-15-Fc-Knob, nucleotide sequence encoding the precursor of protein sequence 11)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCaactgggtgaatgtaATTagtgatttgaaaaaaattgaagatcttattcaatctatgcatattgatgctactttatatacg
gaaagtgatgttcacCCGagttgcaaagtaacagcaatgaagtgctttcttggagttacaagttatttcacttgagtccggcgatgcaagt
attcatgatacagtagaaaatctgatcatcTTAgcaaacaacagtttgtcttctaatgggaatgtaacagaatctggatgcaaagaatgtga
ggaactggaggaaaaaaatattaaagaatttttgcagagttttgtacatattgtccaaatgttcatcaacacttctGGCGGAGGAGG
CTCTGGGGGCGGAGGAAGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCC
CCTGCCCAGCTCCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGC
CCAAGGATACCCTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGAC
GTTTCTCACGAAGATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGT
GCACAATGCTAAGACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCG
TTTCAGTGCTGACTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGC
AAGGTGTCTAACAAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAA
GGGTCAACCACGGGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGA
CAAAGAATCAAGTGTCACTTTACTGTCTTGTGAAGGGCTTCTACCCCTCAGACATCG
CCGTGGAGTGGGAGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCC
TGTGCTCGATTCAGATGGTTCCTTTTTCTTGTACAGCAAACTCACCGTTGACAAGAG
TCGGTGGCAGCAAGGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACA
ACCATTATACCCAAAAATCTCTCAGCCTTTCTCCCGGCAAG SEQ ID NO: 46.

DNA Sequence 9: (IL-15-Fc-Hole, nucleotide sequence encoding the precursor of protein sequence 12)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCaactgggtgaatgtaATTagtgatttgaaaaaaattgaagatcttattcaatctatgcatattgatgctactttatatacg
gaaagtgatgttcacCCGagttgcaaagtaacagcaatgaagtgctttcttggagttacaagttatttcacttgagtccggcgatgcaagt
attcatgatacagtagaaaatctgatcatcTTAgcaaacaacagtttgtcttctaatgggaatgtaacagaatctggatgcaaagaatgtga
ggaactggaggaaaaaaatattaaagaatttttgcagagttttgtacatattgtccaaatgttcatcaacacttctGGCGGAGGAGG
CTCTGGGGGCGGAGGAAGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCC
CCTGCCCAGCTCCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGC
CCAAGGATACCCTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGAC

GTTTCTCACGAAGATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGT
GCACAATGCTAAGACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCG
TTTCAGTGCTGACTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGC
AAGGTGTCTAACAAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAA
GGGTCAACCACGGGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGA
CAAAGAATCAAGTGTCACTTACATGTCTTGTGAAGGGCTTCTACCCCTCAGACATCG
CCGTGGAGTGGGAGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCC
TGTGCTCGATTCAGATGGTTCCTTTTTCTTGACCAGCAAACTCACCGTTGACAAGAG
TCGGTGGCAGCAAGGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACA
ACCATTATACCCAAAAATCTCTCAGCCTTTCTCCCGGCAAG SEQ ID NO: 47.

DNA Sequence 10: (Fc-Knob-IL-15, nucleotide sequence encoding the precursor of protein sequence 13)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCC
GGCTCTCGGTGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCA
GCTCCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGAT
ACCCTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCAC
GAAGATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGC
TAAGACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGC
TGACTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCT
AACAAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACC
ACGGGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATC
AAGTGTCACTTTACTGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGT
GGGAGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGA
TTCAGATGGTTCCTTTTTCTTGTACAGCAAACTCACCGTTGACAAGAGTCGGTGGCA
GCAAGGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATA
CCCAAAAATCTCTCAGCCTTTCTCCCGGCAAGaactgggtgaatgtaATTagtgatttgaaaaaaattgaa
gatcttattcaatctatgcatattgatgctactttatatacggaaagtgatgttcacCCGagttgcaaagtaacagcaatgaagtgctttctctt
ggagttacaagttatttcacttgagtccggcgatgcaagtattcatgatacagtagaaaatctgatcatcTTAgcaaacaacagtttgtcttct
aatgggaatgtaacagaatctggatgcaaagaatgtgaggaactggaggaaaaaaatattaaagaatttttgcagagttttgtacatattgtcc
aaatgttcatcaacacttct SEQ ID NO: 48.

DNA Sequence 11: (Fc-Hole -IL-15, nucleotide sequence encoding the precursor of protein sequence 14)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCC
GGCTCTCGGTGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCA
GCTCCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGAT
ACCCTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCAC
GAAGATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGC
TAAGACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGC
TGACTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCT
AACAAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACC
ACGGGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATC

AAGTGTCACTTACATGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGT
GGGAGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGA
TTCAGATGGTTCCTTTTTCTTGACCAGCAAACTCACCGTTGACAAGAGTCGGTGGCA
GCAAGGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATA
CCCAAAAATCTCTCAGCCTTTCTCCCGGCAAGaactgggtgaatgtaATTagtgatttgaaaaaaattgaa
gatcttattcaatctatgcatattgatgctactttatatacggaaagtgatgttcacCCGagttgcaaagtaacagcaatgaagtgctttctctt
ggagttacaagttatttcacttgagtccggcgatgcaagtattcatgatacagtagaaaatctgatcatcTTAgcaaacaacagtttgtcttct
aatgggaatgtaacagaatctggatgcaaagaatgtgaggaactggaggaaaaaaatattaaagaatttttgcagagttttgtacatattgtcc
aaatgttcatcaacacttct SEQ ID NO: 49.

DNA Sequence 12: (IL-15Rα ECD-Fc-Knob, nucleotide sequence encoding the precursor of protein sequence 15)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCatcACCtgccctCCACCTatgtccgtggaacacgcagacatctgggtcaagagctacagcttgtactccCG
CgagcgCtacatttgtaactctggtttcaagcgtaaagccggcACCtccagcctgACCgagtgcgtgttgaacAAGGCCAC
CaatgtcgcccactggacaaccccAagtctcaaatgcattCGCgaccctgccctggttcaccaaCGCccagcgccaccAtccaca
gtaACCACTgcaggCgtgaccccacagccagagagcctctccccttctggCaaagagccAgcagcttcatctccAagctcaaac
aacacagcggccacaacagcagctattgtcccgggctcccagctgatgccttcaaaatcaccttccacaggCaccacagagatCagcag
tcatgagtcctcccacggcaccccAtctcagacaacagccaagaactgggaactcacagcatccgcctcccaccagccgccaggtgtgt
atccacagggccacagcgacaccactGGCGGAGGAGGCTCTGGGGGCGGAGGAAGCGAACCTAA
GTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCTCCTGAGCTCTTGGGCGG
ACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACCCTTATGATCAGCAGAAC
ACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAAGATCCTGAAGTGAAATT
CAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACTAAGCCCCGTGAAG
AGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGACTGTTCTCCATCAGGACT
GGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAACAAGGCACTGCCCGCACCC
ATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACGGGAGCCACAGGTTTACAC
ATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAGTGTCACTTTACTGTCTTGT
GAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGGAGAGCAACGGACAACCAG
AAAACAACTACAAGACCACACCTCCTGTGCTCGATTCAGATGGTTCCTTTTTCTTGT
ACAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAAGGAAATGTGTTCAGCTGT
TCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAAAAATCTCTCAGCCTTTCT
CCCGGCAAG SEQ ID NO: 50.

DNA Sequence 13: (IL-15Rα ECD-Fc-Hole, nucleotide sequence encoding the precursor of protein sequence 16)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC

TCTCGGTGCatcACCtgccctCCACCTatgtccgtggaacacgcagacatctgggtcaagagctacagcttgtactccCG
CgagcgCtacatttgtaactctggtttcaagcgtaaagccggcACCtccagcctgACCgagtgcgtgttgaacAAGGCCAC
CaatgtcgcccactggacaaccccAagtctcaaatgcattCGCgaccctgccctggttcaccaaCGCccagcgccaccAtccaca
gtaACCACTgcaggCgtgaccccacagccagagagcctctccccttctggCaaagagccAgcagcttcatctccAagctcaaac
aacacagcggccacaacagcagctattgtcccgggctcccagctgatgccttcaaaatcaccttccacaggCaccacagagatCagcag
tcatgagtcctcccacggcaccccAtctcagacaacagccaagaactgggaactcacagcatccgcctcccaccagccgccaggtgtgt
atccacagggccacagcgacaccactGGCGGAGGAGGCTCTGGGGGCGGAGGAAGCGAACCTAA
GTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCTCCTGAGCTCTTGGGCGG
ACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACCCTTATGATCAGCAGAAC
ACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAAGATCCTGAAGTGAAATT
CAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACTAAGCCCCGTGAAG
AGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGACTGTTCTCCATCAGGACT
GGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAACAAGGCACTGCCCGCACCC
ATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACGGGAGCCACAGGTTTACAC
ATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAGTGTCACTTACATGTCTTG
TGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGGAGAGCAACGGACAACCA
GAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCAGATGGTTCCTTTTTCTTG
ACCAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAAGGAAATGTGTTCAGCTG
TTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAAAAATCTCTCAGCCTTTC
TCCCGGCAAG SEQ ID NO: 51.

DNA Sequence 14: (Fc-Knob-IL-15Rα ECD, nucleotide sequence encoding the precursor of protein sequence 17)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCT
CCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACC
CTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAA
GATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAA
GACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGA
CTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAAC
AAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACG
GGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAG
TGTCACTTTACTGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGG
AGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCA

GATGGTTCCTTTTTCTTGTACAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAA
GGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAA
AAATCTCTCAGCCTTTCTCCCGGCAAGatcACCtgccctCCACCTatgtccgtggaacacgcagacatct
gggtcaagagctacagcttgtactccCGCgagcgCtacatttgtaactctggtttcaagcgtaaagccggcACCtccagcctgACC
gagtgcgtgttgaacAAGGCCACCaatgtcgcccactggacaaccccAagtctcaaatgcattCGCgaccctgccctggttca
ccaaCGCccagcgccaccAtccacagtaACCACTgcaggCgtgaccccacagccagagagcctctccccttctggCaaagag
ccAgcagcttcatctccAagctcaaacaacacagcggccacaacagcagctattgtcccgggctcccagctgatgccttcaaaatcacct
tccacaggCaccacagagatCagcagtcatgagtcctcccacggcaccccAtctcagacaacagccaagaactgggaactcacagca
tccgcctcccaccagccgccaggtgtgtatccacagggccacagcgacaccact SEQ ID NO: 52.

DNA Sequence 15: (Fc-Hole -IL-15Rα ECD, nucleotide sequence encoding the precursor of protein sequence 18)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCT
CCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACC
CTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAA
GATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAA
GACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGA
CTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAAC
AAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACG
GGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAG
TGTCACTTACATGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGG
AGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCA
GATGGTTCCTTTTTCTTGACCAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAA
GGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAA
AAATCTCTCAGCCTTTCTCCCGGCAAGatcACCtgccctCCACCTatgtccgtggaacacgcagacatct
gggtcaagagctacagcttgtactccCGCgagcgCtacatttgtaactctggtttcaagcgtaaagccggcACCtccagcctgACC
gagtgcgtgttgaacAAGGCCACCaatgtcgcccactggacaaccccAagtctcaaatgcattCGCgaccctgccctggttca
ccaaCGCccagcgccaccAtccacagtaACCACTgcaggCgtgaccccacagccagagagcctctccccttctggCaaagag
ccAgcagcttcatctccAagctcaaacaacacagcggccacaacagcagctattgtcccgggctcccagctgatgccttcaaaatcacct
tccacaggCaccacagagatCagcagtcatgagtcctcccacggcaccccAtctcagacaacagccaagaactgggaactcacagca
tccgcctcccaccagccgccaggtgtgtatccacagggccacagcgacaccact SEQ ID NO: 53.

DNA Sequence 16: (Fc-Knob, nucleotide sequence encoding the precursor of protein sequence

23)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCT
CCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACC
CTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAA
GATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAA
GACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGA
CTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAAC
AAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACG
GGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAG
TGTCACTTTACTGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGG
AGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCA
GATGGTTCCTTTTTCTTGTACAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAA
GGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAA
AAATCTCTCAGCCTTTCTCCCGGCAAG SEQ ID NO: 54.

DNA Sequence 17: (Fc-Hole, nucleotide sequence encoding the precursor of protein sequence

24)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCT
CCTGAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACC
CTTATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAA
GATCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAA
GACTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGA
CTGTTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAAC
AAGGCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACG
GGAGCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAG
TGTCACTTACATGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGG
AGAGCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCA
GATGGTTCCTTTTTCTTGACCAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAA
GGAAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAA
AAATCTCTCAGCCTTTCTCCCGGCAAG SEQ ID NO: 55.

DNA Sequence 18: (Fc-Knob (M) -IL-15, nucleotide sequence encoding the precursor of protein

sequence 30)

ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTCGCGATTCTTAAGGGTGTCCAGTGC
GAGCCCAAATCTAGTGACAAAACTCACACGTCCCCACCGTCCCCAGCACCTGAACT
CCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT
CTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTG
AGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG
CCGCGGGAAGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCT
GCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCC
CTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACC
ACAGGTGTACACCCTGCCCCCATGCCGGGATGAGCTGACCAAGAACCAGGTCAGCC
TGTGGTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
AATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGG
CTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGA
ACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
AGCCTCTCCCTGTCTCCGGGTAAAGGCGGAGGAGGCTCTGGCGGTGGTGGCAGTGG
TGGCGGAGGGTCAGGAGGTGGTGGAAGCAACTGGGTGAATGTAATTAGTGATTTGA
AAAAAATTGAAGATCTTATTCAATCTATGCATATTGATGCTACTTTATATACGGAAA
GTGATGTTCACCCGAGTTGCAAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTAC
AAGTTATTTCACTTGAGTCCGGCGATGCAAGTATTCATGATACAGTAGAAAATCTGA
TCATCTTAGCAAACAACAGTTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGCA
AAGAATGTGAGGAACTGGAGGAAAAAAAATATTAAAGAATTTTTGCAGAGTTTTGTA
CATATTGTCCAAATGTTCATCAACACTTCT SEQ ID NO: 56

DNA Sequence 19: (IL-15-Fc-Knob (M), nucleotide sequence encoding the precursor of protein sequence 31)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCAACTGGGTGAATGTAATTAGTGATTTGAAAAAAATTGAAGATCTTATT
CAATCTATGCATATTGATGCTACTTTATATACGGAAAGTGATGTTCACCCGAGTTGC
AAAGTAACAGCAATGAAGTGCTTTCTCTTGGAGTTACAAGTTATTTCACTTGAGTCC
GGCGATGCAAGTATTCATGATACAGTAGAAAATCTGATCATCTTAGCAAACAACAG
TTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGCAAAGAATGTGAGGAACTGG
AGGAAAAAAAATATTAAAGAATTTTTGCAGAGTTTTGTACATATTGTCCAAATGTTCA
TCAACACTTCTGGCGGAGGAGGCTCTGGGGGCGGAGGATCCGAGCCCAAATCTAGT
GACAAAACTCACACGAGCCCACCGAGCCCAGCACCTGAACTCCTGGGGGGACCGTC

AGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGA
GGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACT
GGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAAGAGCA
GTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGC
TGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATC
GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCC
TGCCCCCATGCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGTGGTGCCTGGTC
AAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGA
GAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTA
CAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT
CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTC
CGGGTAAA SEQ ID NO: 57

DNA Sequence 20: (Fc-Hole (M) -IL-15Rα-sushi(65), nucleotide sequence encoding the precursor of protein sequence 32)

ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTCGCGATTCTTAAGGGTGTCCAGTGC
GAGCCCAAATCTAGTGACAAAACTCACACGTCCCCACCGTCCCCAGCACCTGAACT
CCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT
CTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTG
AGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG
CCGCGGGAAGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCT
GCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCC
CTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACC
ACAGGTGTGCACCCTGCCCCCAtccCGGGATGAGCTGACCAAGAACCAGGTCAGCCT
GAGCTGCGCCGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCA
ATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGC
TCCTTCTTCCTCGTGAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAA
CGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGA
GCCTCTCCCTGTCTCCGGGTAAAGGCGGAGGAGGCTCTGGCGGTGGTGGCAGTGGT
GGCGGAGGGTCAGGAGGTGGTGGAAGCATCACCTGCCCTCCACCTATGTCCGTGGA
ACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTACTCCCGCGAGCGCTACATTT
GTAACTCTGGTTTCAAGCGTAAAGCCGGCACCTCCAGCCTGACCGAGTGCGTGTTG
AACAAGGCCACCAATGTCGCCCACTGGACAACCCCAAGTCTCAAATGCATTCGC
SEQ ID NO: 58

DNA Sequence 21: (IL15Rα-sushi (65) -Fc-Hole (M), nucleotide sequence encoding the precursor of protein sequence 33)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCATCACCTGCCCTCCACCTATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCCGCGAGCGCTACATTTGTAACTCTGGTTTCAAGCGT
AAAGCCGGCACCTCCAGCCTGACCGAGTGCGTGTTGAACAAGGCCACCAATGTCGC
CCACTGGACAACCCCAAGTCTCAAATGCATTCGCGGAGGGGGTGGCAGCGGCGGGG
GAGGTTCAGGCGGAGGTGGGTCTGGAGGCGGTGGATCCGAGCCCAAATCTAGTGAC
AAAACTCACACGTCCCCACCGTCCCCAGCACCTGAACTCCTGGGGGGACCGTCAGT
CTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGT
CACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT
ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAAGAGCAGTA
CAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGA
ATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAG
AAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTGCACCCTGCC
CCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGAGCTGCGCCGTCAAAG
GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC
AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCGTGAGC
AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGT
GATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
GTAAA SEQ ID NO: 59

DNA Sequence 22: (IL-15Rα-sushi(73 )-Fc-Hole, nucleotide sequence encoding the precursor of protein sequence 34)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCATCACCTGCCCTCCACCTATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCCGCGAGCGCTACATTTGTAACTCTGGTTTCAAGCGT
AAAGCCGGCACCTCCAGCCTGACCGAGTGCGTGTTGAACAAGGCCACCAATGTCGC
CCACTGGACAACCCCAAGTCTCAAATGCATTCGCGACCCTGCCCTGGTTCACCAACG
CTCCGGCGGATCAGGAGGTGGTGGCAGCGGGGGGTGGTTCCGGTGGAGGGGGCTCCT
TGCAGGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCTCCTG
AGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACCCTTA
TGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAAGAT

CCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGAC
TAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGACTGT
TCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAACAAGG
CACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACGGGAG
CCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAGTGTC
ACTTACATGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGGAGA
GCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCAGAT
GGTTCCTTTTTCTTGACCAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAAGG
AAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAAAA
ATCTCTCAGCCTTTCTCCCGGCAAG SEQ ID NO: 60

DNA Sequence 23: (IL-15Rα-sushi(65)-Fc-Hole, nucleotide sequence encoding the precursor of protein sequence 35)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCATCACCTGCCCTCCACCTATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCCGCGAGCGCTACATTTGTAACTCTGGTTTCAAGCGT
AAAGCCGGCACCTCCAGCCTGACCGAGTGCGTGTTGAACAAGGCCACCAATGTCGC
CCACTGGACAACCCCAAGTCTCAAATGCATTCGCTCCGGCGGATCAGGAGGTGGTG
GCAGCGGGGGTGGTTCCGGTGGAGGGGGCTCCTTGCAGGAACCTAAGTCCTCTGAT
AAGACCCACACATGTCCCCCCTGCCCAGCTCCTGAGCTCTTGGGCGGACCTTCCGTG
TTTCTGTTCCCCCCAAAGCCCAAGGATACCCTTATGATCAGCAGAACACCCGAAGTT
ACTTGCGTGGTCGTGGACGTTTCTCACGAAGATCCTGAAGTGAAATTCAACTGGTAC
GTGGATGGCGTGGAGGTGCACAATGCTAAGACTAAGCCCCGTGAAGAGCAGTACA
ACTCTACCTACCGGGTCGTTTCAGTGCTGACTGTTCTCCATCAGGACTGGCTCAACG
GGAAGGAGTATAAGTGCAAGGTGTCTAACAAGGCACTGCCCGCACCCATCGAGAA
GACCATTTCTAAGGCCAAGGGTCAACCACGGGAGCCACAGGTTTACACATTGCCTC
CCAGTCGGGAGGAGATGACAAAGAATCAAGTGTCACTTACATGTCTTGTGAAGGGC
TTCTACCCCTCAGACATCGCCGTGGAGTGGGAGAGCAACGGACAACCAGAAAACAA
CTACAAGACCACACCTCCTGTGCTCGATTCAGATGGTTCCTTTTTCTTGACCAGCAA
ACTCACCGTTGACAAGAGTCGGTGGCAGCAAGGAAATGTGTTCAGCTGTTCTGTGA
TGCACGAGGCCCTGCACAACCATTATACCCAAAAATCTCTCAGCCTTTCTCCCGGCA
AG SEQ ID NO: 61

DNA Sequence 24: (IL-15Rα-sushi(86)-Fc-Hole, nucleotide sequence encoding the precursor of

protein sequence 36)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCATCACCTGCCCTCCACCTATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCCGCGAGCGCTACATTTGTAACTCTGGTTTCAAGCGT
AAAGCCGGCACCTCCAGCCTGACCGAGTGCGTGTTGAACAAGGCCACCAATGTCGC
CCACTGGACAACCCCAAGTCTCAAATGCATTCGCGACCCTGCCCTGGTTCACCAACG
CCCAGCGCCACCATCCACAGTAACCACTGCAGGCGTGACCTCCGGCGGATCAGGAG
GTGGTGGCAGCGGGGGTGGTTCCGGTGGAGGGGGCTCCTTGCAGGAACCTAAGTCC
TCTGATAAGACCCACACATGTCCCCCCTGCCCAGCTCCTGAGCTCTTGGGCGGACCT
TCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACCCTTATGATCAGCAGAACACCC
GAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAAGATCCTGAAGTGAAATTCAAC
TGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACTAAGCCCCGTGAAGAGCA
GTACAACTCTACCTACCGGGTCGTTTCAGTGCTGACTGTTCTCCATCAGGACTGGCT
CAACGGGAAGGAGTATAAGTGCAAGGTGTCTAACAAGGCACTGCCCGCACCCATCG
AGAAGACCATTTCTAAGGCCAAGGGTCAACCACGGGAGCCACAGGTTTACACATTG
CCTCCCAGTCGGGAGGAGATGACAAAGAATCAAGTGTCACTTACATGTCTTGTGAA
GGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGGAGAGCAACGGACAACCAGAAA
ACAACTACAAGACCACACCTCCTGTGCTCGATTCAGATGGTTCCTTTTTCTTGACCA
GCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAAGGAAATGTGTTCAGCTGTTCT
GTGATGCACGAGGCCCTGCACAACCATTATACCCAAAAATCTCTCAGCCTTTCTCCC
GGCAAG SEQ ID NO: 62

DNA Sequence 25: (IL-15Rα-sushi(102)-Fc-Hole, nucleotide sequence encoding the precursor of protein sequence 37)

ATGGACATGCGGGTGCCAGCCCAGCTGCTGGGCCTGTTGCTGCTGTGGTTCCCCGGC
TCTCGGTGCATCACCTGCCCTCCACCTATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCCGCGAGCGCTACATTTGTAACTCTGGTTTCAAGCGT
AAAGCCGGCACCTCCAGCCTGACCGAGTGCGTGTTGAACAAGGCCACCAATGTCGC
CCACTGGACAACCCCAAGTCTCAAATGCATTCGCGACCCTGCCCTGGTTCACCAACG
CCCAGCGCCACCATCCACAGTAACCACTGCAGGCGTGACCCCACAGCCAGAGAGCC
TCTCCCCTTCTGGCAAAGAGCCAGCAGCTTCAGGCGGAGGAGGCTCTGGGGGCGGA
GGAAGCGAACCTAAGTCCTCTGATAAGACCCACACATGTCCCCCCTGCCCAGCTCCT
GAGCTCTTGGGCGGACCTTCCGTGTTTCTGTTCCCCCCAAAGCCCAAGGATACCCTT
ATGATCAGCAGAACACCCGAAGTTACTTGCGTGGTCGTGGACGTTTCTCACGAAGA

TCCTGAAGTGAAATTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGA
CTAAGCCCCGTGAAGAGCAGTACAACTCTACCTACCGGGTCGTTTCAGTGCTGACTG
TTCTCCATCAGGACTGGCTCAACGGGAAGGAGTATAAGTGCAAGGTGTCTAACAAG
GCACTGCCCGCACCCATCGAGAAGACCATTTCTAAGGCCAAGGGTCAACCACGGGA
GCCACAGGTTTACACATTGCCTCCCAGTCGGGAGGAGATGACAAAGAATCAAGTGT
CACTTACATGTCTTGTGAAGGGCTTCTACCCCTCAGACATCGCCGTGGAGTGGGAGA
GCAACGGACAACCAGAAAACAACTACAAGACCACACCTCCTGTGCTCGATTCAGAT
GGTTCCTTTTTCTTGACCAGCAAACTCACCGTTGACAAGAGTCGGTGGCAGCAAGG
AAATGTGTTCAGCTGTTCTGTGATGCACGAGGCCCTGCACAACCATTATACCCAAAA
ATCTCTCAGCCTTTCTCCCGGCAAG SEQ ID NO: 63

**Example 3. Protein Expression**

IL-15 protein was transiently transfected and expressed using FreeStyle 293 cells (GIBCO, Cat#R79007). FreeStyle 293 cells were suspension cultured in Freestyle 293 expression medium (GIBCO, Cat#12338018), followed by addition of Ultra Low IgG Fetal Bovine Serum (ultra low immunoglobulins FBS, GIBCO, Cat # 16250078) with a final concentration of 1%. The corresponding expression plasmids described in Example 1 and transfection reagent PEI (Polysciences, Cat#239662) were prepared, plasmids amount was 100ug/100ml cells, ratio of plasmid to PEI was 1:2 by mass. Cell density on the day of transfection was $1\times10^6$/ml. 1L of FreeStyle 293 cells were prepared to be transfected. 50ml of Opti-MEM (GIBCO, Cat # 11058021) medium was mixed with the plasmid, kept still for 5min, and filtered; another 50ml of Opti-MEM medium was mixed with PEI, kept still for 5min and filtered. The plasmid was mixed with PEI and kept still for 15min. The mixture of plasmid and PEI was slowly added to the cells and cultured in shaking incubator at 130rpm at 37° C, 8% $CO_2$ . 5 days later, the supernatant was collected by centrifugation for protein purification.

**Example 4 Protein purification**

(1) IL-15 heterodimeric protein for affinity chromatography:

Supernatant was collected from cell culture after high speed centrifugation and subjected to affinity chromatography using Protein A column from GE. The equilibration buffer used in chromatography was 1×PBS (pH7.4), after cell supernatant was loaded and bound, washing with PBS until UV returned to baseline, and then eluting the target protein with elution buffer 0.1M glycine (pH3.0) . The pH was adjusted to neutral with Tris, and the target protein was stored;

(2) IL-15 heterodimeric protein for ion exchange chromatography:

The pH of the product obtained during the affinity chromatography was adjusted to be 1-2 pH units lower or higher than pI, appropriately diluted to control the conductivity of sample less 5ms/cm. Utilizing suitable buffer corresponding to pH, such as phosphate buffer, acetate buffer, and others, the product was NaCl-gradient eluted under corresponding pH condition utilizing conventional ion-exchange column chromatography methods in the art such as cation exchange or anion exchange, the tube containing the target protein was selected according to SDS-PAGE and stored.

(3) IL-15 heterodimeric protein for size exclusion chromatography:

The product obtained during the ion exchange chromatography was concentrated by ultrafiltration and loaded for size exclusion chromatography, by using such as GE Superdex200 gel to remove possible polymer and other components, in order to obtain the desired product with high purity. Purity of the obtained protein can be detected by SDS-PAGE and SEC-HPLC. Protein concentration was determined by UV spectrophotometry.

The obtained protein sequences were described in Example 1, each specific heterodimeric protein was composed of one or two protein sequence(s) selected from the above sequences, preferably heterodimeric protein formed with Knob/Hole pairing, which was co-expressed in cells, and obtained by purification; alternatively, a bivalent protein may be composed of Fc chain region without mutations. In a preferred embodiment, for example, molecule 3 was formed by pairing fusion protein IL-15-Fc-Knob with Fc-Hole (obtained by purification after co-expression), molecule 7 in the examples was formed by pairing fusion proteinsIL-15-Fc-Knob with IL-15RαECD-Fc-Hole (obtained by purification after co-expression). Non-limiting examples of the dimeric protein according to the present invention are shown in Table 1 below:

Table 1. Design for each dimeric protein

| No | Protein (I) | Protein (П) |
|---|---|---|
| 1 | IL-15-Fc (SEQ ID No.8) | IL-15-Fc (SEQ ID No.8) |
| 2 | Fc-IL-15 (SEQ ID No.9) | Fc-IL-15 (SEQ ID No.9) |
| 3 | IL-15-Fc-Knob (SEQ ID No.14) | Fc-Hole (SEQ ID No.27) |
| 4 | IL-15-Fc-Hole (SEQ ID No.15) | Fc-Knob (SEQ ID No.26) |
| 5 | Fc-Knob -IL-15 (SEQ ID No.16) | Fc-Hole (SEQ ID No.27) |
| 6 | Fc-Hole -IL-15 (SEQ ID No.17) | Fc-Knob (SEQ ID No.26) |
| 7 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα ECD-Fc-Hole (SEQ ID No.19) |

| | | |
|---|---|---|
| 8 | IL-15-Fc-Hole (SEQ ID No.15) | IL-15Rα ECD-Fc-Knob (SEQ ID No.18) |
| 9 | Fc-Knob -IL-15 (SEQ ID No.16) | Fc-Hole -IL-15Rα ECD (SEQ ID No.21) |
| 10 | Fc-Hole -IL-15 (SEQ ID No.17) | Fc-Knob -IL-15Rα ECD (SEQ ID No.20) |
| 11 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(77 )-Fc-Hole (SEQ ID No.23) |
| 12 | Fc-Knob (M) -IL-15 (SEQ ID No.30) | Fc-Hole(M)-IL-15Rα-sushi(65)(SEQ ID No.32) |
| 13 | IL-15-Fc-Knob (M) (SEQ ID No.31) | IL-15Rα-sushi (65) -Fc-Hole (M) (SEQ ID No.33) |
| 14 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(73 )-Fc-Hole (SEQ ID No.34) |
| 15 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(65)-Fc-Hole (SEQ ID No.35) |
| 16 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(86)-Fc-Hole (SEQ ID No.36) |
| 17 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(102)-Fc-Hole (SEQ ID No.37) |

Table 1 specifically shows 17 dimeric proteins involved in the present invention, named as dimeric proteins 1 to 17, respectively, each was reassembled and formed by the corresponding protein (I) and protein (II) shown in the table.

**Test Examples**

**Test Example 1. Proliferation Assay *in vitro***

**Test the proliferation of fresh human peripheral blood mononuclear cells (PBMC) affected by IL-15, dimeric proteins 1 to 17 of the present invention.**

Fresh PBMCs were cultured in RPMI1640 medium containing 10% FBS, centrifuged and resuspended to cell density of $5\times10^5$ cells/ml, 90ul was added into each well of 96-well plate, samples were diluted at certain multiple to different concentrations with PBS, 10ul was added into each well of 96-well plate, and cultured in the incubator at 37 ° C, 5% $CO_2$ for 48 hours. Thereafter, 50 μl was taken for detection of cell proliferation with CellTiter-Glo® Luminescent Cell Viability Assay kit. The results are shown in Table 2:

Table 2. Results of the test of *in vitro* proliferation

| Sample | PBMC relative activity |
|---|---|
| IL-15 (Control) | 100 |

| | |
|---|---|
| 1 | 79 |
| 2 | 46 |
| 3 | 367 |
| 5 | 138 |
| 7 | 1100 |
| 8 | 183 |
| 9 | 26 |
| 10 | 11 |
| 11 | 1392 |
| 12 | 210 |
| 13 | 206 |
| 14 | 1243 |
| 15 | 1338 |
| 16 | 1024 |
| 17 | 2677 |

Experimental results in the test example: human PBMC proliferation assay showed that the effects of heterodimeric protein 3,7,11 and 14-17 of the present invention on promoting proliferation are obviously superior to that of control IL-15, as well as are obviously superior to that of the homodimers 1 and 2. In addition, the fusion protein with Fc variant located at the C-terminus has a better effect than the one with Fc variant located at the N-terminus. The heterodimeric proteins 12 and 13 of other variant molecules also have better effect on promoting proliferation than control IL-15.

**Test Example 2. In vivo Detecting PK of IL-15 heterologous dimer**

*Purpose*

Evaluate the pharmacokinetics of IL-15 heterodimeric proteins 3 and 7 in mice.

*Material and methods*

1. Test compounds, that is samples

IL-15, IL-15 heterodimeric proteins 3 and 7.

2. Animals

C57BL/6 mice, SPF, 15-16g, ♂, available from Shanghai Super B&K Laboratory Animal Corp. Ltd.

3. Animal test procedure

27 of C57BL/6 mice were divided into 3 groups, 9 in each group, and 3 per cage. Blood samples of 3 mice were collected at each time point, blood was sampled from circulation. 2μg of IL-15,10μg of dimeric protein 3 and 20μg of dimeric protein 7 (IL-15, dimeric protein 3 and dimeric protein 7 have the same molar of 0.15nmol) were intraperitoneally injected. Blood was sampled at 30min, 1, 2, 4, 8, 24, 48, 72, and 120h after administration, 50-100μl of orbital blood sample was taken at each time point. Serum was used for human IL-15 ELISA.

3. Results and discussion

After administration of equimolar IL-15, dimeric protein 3 and dimeric protein 7, IL-15 reached a maximal concentration within 30 minutes, and then was rapidly metabolized over time and completely metabolized 24 hours after administration. The dimeric protein 3 reached a maximal concentration 2 hours after administration, and then is slowly metabolized over time and completely metabolized 120 hours after administration. The dimeric protein 7 reached a maximal amount 2 hours after administration, and then is slowly metabolized over time. 120 hours later, high concentration of protein was still detected.

PK detection (Figure 1) showed that after administration, the maximal molar concentrations of both dimeric protein 3 and dimeric protein 7 in the serum were less than that of IL-15, but their retention times in the serum were superior to that of IL-15, exhibiting a significant long-term effect.

Detecting *in vivo* the efficacy of IL-15

This example is to test the efficacy of IL-15, dimeric protein 3 and dimeric protein 7 in three models of lung metastasis model, nude mice tumor-bearing model and severe combined immunodeficient NOD-SCID mice model. The results are shown in the following test examples.

**Test Example 3. Lung metastasis model**

*Purpose*

Establish mouse lung metastasis model using B16F10 cells to assess the impact on

tumor metastasis and growth after administration of IL-15 drug.

*Material and protocol*

1. Test protein

IL-15, dimeric protein 3 and dimeric protein 7.

2. Test animals

C57BL/6 mice, SPF, 10-16g, ♂, available from Shanghai Super B&K Laboratory Animal Corp. Ltd.

3. Animal test procedure

Dosage regimen: 32 of C57BL/6 mice were divided into 4 groups, 8 in each group. $1.5\times10^5$ of B16F10 cells were intravenously injected via tail-vein. PBS, 2μg of IL-15,11μg of dimeric protein 3 and 14μg of dimeric protein 7 (IL-15, dimeric protein 3 and dimeric protein 7 have the same molar of 0.16nmol) were intraperitoneally injected on day 1, 2, and 10.

Mice were sacrificed on day 21. Lungs were removed, weighed, observed the black lung lumps, photographed, fixed in neutral formalin, and counted for the number of black lumps.

3. Results

In the dosage regimen, lungs of mice in PBS group showed growth of a large number of metastatic melanoma (175 ± 23); lungs of IL-15 group showed a large number of melanoma lumps (157 ± 20), about 90% of that in PBS group; lungs of dimeric protein 3 group exhibited a few metastatic melanoma lumps (26 ± 6), about 15% of that in PBS group; lungs of dimeric protein 7 group showed more visible lung metastatic melanoma lumps ( 83 ± 28), about 49% of that in PBS group. The number of lung lumps in PBS group was significantly higher than that in dimeric protein 3 group and dimeric protein 7 group, but showed no significantly different from that in IL-15 group. The number of lung lumps in IL-15 group was significantly higher than that in dimeric protein 3 group. The number of lung lumps in dimeric protein 7 group was significantly higher than that in dimeric protein 3 group, as shown in Fig 2. The relative lung weight in PBS group was significantly higher than that in dimeric protein 3 group and dimeric protein 7 group, but showed no significantly different from that in IL-15 group. The relative lung weight in IL-15 group was significantly higher than that in dimeric protein 3 group and dimeric protein 7 group, as shown in FIG 3.

In summary, in B16F10 mouse model, the efficacies of three kinds of proteins were as follows: dimeric protein 3> dimeric protein 7> IL-15.

**Test Example 4. Nude mouse tumor-bearing model**

*Purpose*

Establish nude mouse tumor-bearing model using HCT-116 (human colon carcinoma) cells to assess the impact on tumor growth after administration of IL-15 drugs.

*Material and protocol*

1. Test protein

IL-15, dimeric protein 3 and dimeric protein 7.

2. Test animal

BALB/cA-nude mice, SPF, 16-20 g, ♀, available from Shanghai Super B&K Laboratory Animal Corp. Ltd.

3. Animal test procedure

(1) Nude mice were to adapt to the laboratory environment for 10 days.

(2) Tumor cells transplantation

Nude mice were inoculated subcutaneously in the right rib with HCT-116 cells ($5\times10^6$/mouse). Tumor grew for 20 days. When the volume of tumor grew to 100±15 $mm^3$, animals were randomly grouped (d0), n= 6.

(3) Administration dosage and method

Each group was intraperitoneally injected once every two days (three times a week) with test drug IL-15 (2μg/mouse), or dimeric protein 3 (10μg/mouse), or dimeric protein 7 (20μg/mouse) (IL-15, dimeric protein 3 and dimeric protein 7 have the same molar of 0.15nmol).

(4) Determination of tumor volume and weight of nude mice

Mice were measured for tumor volume 2-3 times per week (Fig. 4), weighed, and recorded. On day 27, mice were sacrificed to collect the tumor.

(5) Statistics

Excel statistical software: means are calculated as avg; SD is calculated as STDEV; SEM is calculated as STDEV/SQRT; P value between different groups is calculated as TTEST.

Tumor volume (V) is calculated as: $V = 1/2 \times L_{length} \times L_{short}^2$

Relative volume (RTV) $= V_T/V_0$

Inhibition rate (%) = $(C_{RTV}-T_{RTV}) / C_{RTV}$ (%)

$V_0$ and $V_T$ represent the tumor volume at the beginning of the experiment and at the end of the experiment, respectively. $C_{RTV}$ and $T_{RTV}$ represent blank control group (Blank) and relative tumor volume at the end of the experiment, respectively.

3. Results

The effect of IL-15 protein in inhibiting growth of HCT-116 tumor was shown in Table 3 and Figure 4. Equimolar IL-15, dimeric protein 3 and dimeric protein 7 were

continuously administrated for 27days, once every two days. IL-15, dimeric protein 3 and dimeric protein 7 could inhibit the growth of transplanted HCT-16 tumor, the inhibition rates of which are 32%, 45%, and 20%, respectively. But no significant difference was seen compared to the control group, because of significant individual differences. No deaths occurred during the administration, no significant decrease in body weight was seen in each group during the administration, suggesting that the administration dosage at present does not have significant toxicity.

On day 27, tumors in each group were stripped and weighed, as shown in Figure 5, * in the figure presents: $p<0.05$ compared to Blank, which indicated that the tumor weight in dimeric protein 3 group was significantly decreased compared to that in Blank, the tumor weights in groups of IL-15 and dimeric protein 7 were comparable with that in Blank group.

In summary, in HCT-116 nude model, the inhibitory efficacies of 3 kinds of protein was: dimeric protein 3> IL-15> dimeric protein 7.

Table 3. Therapeutic effects of administrated proteins on HCT-116 xenografts nude mice

| Group | Mean tumor volume ($mm^3$) | | Mean tumor volume ($mm^3$) | | Relative tumor volume | | % tumor inhibition D28 | p |
|---|---|---|---|---|---|---|---|---|
| | D0 | SEM | D27 | SEM | D27 | SEM | | vs blank |
| BLANK | 89.82 | 8.84 | 1493.16 | 182.76 | 17.37 | 2.60 | | |
| IL-15 | 96.05 | 11.65 | 1098.37 | 177.68 | 11.73 | 1.47 | 32 | 0.09 |
| Dimeric protein 3 | 86.79 | 12.56 | 777.77 | 279.39 | 9.63 | 4.06 | 45 | 0.14 |
| Dimeric protein 7 | 96.30 | 16.22 | 1260.47 | 219.72 | 13.87 | 2.01 | 20 | 0.31 |

**Test Example 5. Severe combined immunodeficient NOD-SCID mice**

*Purpose*

HCT-116 cells were mixed with human peripheral blood mononuclear cells (PBMC) in vitro, and inoculated into severe combined immunodeficient NOD-SCID mice. The impact on tumor growth after the administration of IL-15 was evaluated. Nod-SCID mice theoretically lack T cells and NK cells, as a result, after administration of IL-15, human PBMC cells (including T

and NK cells) can be activated to kill cancer cells and inhibit tumor cell growth. Nod-SCID mouse model is much closer to the mimic of human immune system for killing tumor cells.

*Material and protocol*

1. Test protein

IL-15 protein purchased from Novoprotein Scientific Inc;

IL-15, dimeric protein 3 and dimeric protein 7.

2. Test animal

NOD SCID female mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Lot number: 11400700006527), 6-8 weeks old, about 20g, 5 in each drug group.

3. Animal test procedure

(1) SCID mice were to adapt to the laboratory environment for 10 days.

(2) PBMC Cells Isolation

6ml of lymphocyte separation medium (Lymphocyte Separation Medium, LSM) was aseptically transferred to a 15ml-centrifuge tube (before, LSM was gently mixed by inverting the bottle);

4ml of venous blood anti-coagulation treated with heparin was mixed with saline, 8ml of the diluted blood was carefully added into LSM in the centrifuge tube (at room temperature, slowly added to form a clear stratification between blood and the LSM, rather than mixed into the LSM);

After centrifugation at room temperature at 400g for 15-30 minutes, red blood cells and polymorphonuclear leukocytes were precipitated, meanwhile, a layer of mononuclear cells was formed above the LSM;

Plasma was aspirated from 4-6cm above the lymphocytes ;

Lymphocyte layer and half of LSM below it were aspirated and transferred to another centrifuge tube, followed by addition of equal volume of balanced salt buffer PBS, and centrifuged for 10 minutes at room temperature at 1000rpm;

Cells were washed with PBS buffer or RPMI-1640 medium, and resuspended with RPMI-1640 medium;

After 3-4 hours of incubation at 37 ° C, suspended cells were collected and counted.

(3) Tumor cell transplantation

HCT-116 cells were mixed uniformly with PBMC cell at a ratio of 4:1, and inoculated subcutaneously on the right rib of SCID mice (HCT-116 cells: $5\times10^6$/mouse, PBMC: $1.25\times10^6$/mouse). Tumor was grown for 28 days.

(4) Administration dosage and method

Drugs were intraperitoneally injected from the next day after inoculation of HCT-116+PBMC cells, once every two days, for consecutive 10 doses, dosage of IL-15 was 2μg/mouse, dosage of dimeric protein 3 was 10μg/mouse, dosage of dimeric protein 7 was 20μg/mouse (IL-15, dimeric protein 3 and dimeric protein 7 have the same molar of 0.15nmol).

(5) Measurement of tumor volume and body weight of SCID mice

From day 12, mice were measured for tumor volume, weighed, and recorded every 2 to 3 days. On day 28, mice were sacrificed to obtain the tumor.

(6) Statistics

Excel statistical software: means were calculated as avg; SD value was calculated as STDEV; SEM value was calculated as STDEV/SQRT; P value between different groups was calculated as TTEST.

Tumor volume (V) is calculated as: $V = 1/2 \times L_{length} \times L_{short}^2$

Inhibition rate (%) = $(V_T - V_0) / V_T$ (%)

$V_0$ and $V_T$ represent the tumor volume at the beginning of the experiment and at the end of the experiment, respectively.

3. Results

From the next day after the inoculation of cells, equimolar IL-15, dimeric protein 3 and the dimeric protein 7 were administered continuously for 20 days, once every two days. The effect of IL-15 protein on inhibiting HCT-116+PBMC tumor growth was shown in Table 4 and Figure 6: no tumor was found in each group in the first week. From day 12 (D12), tumor in each group was found, the tumor volumes in IL-15 group, dimeric protein 3 group, and dimeric protein 7 group were less than that in PBMC group. On day 28 (D28), IL-15 and dimeric protein 3 could significantly inhibit the growth of transplanted HCT-16 tumor cells, with inhibition rates of 42% and 41% respectively; the inhibition rate of dimeric protein-7 group was 27%, exhibiting no significant difference compared to PBMC group. No deaths occurred during the administration, no significant decrease in body weight was seen in each group during the administration, suggesting that the administration dosage at present does not have significant toxicity.

On day 28, tumors in each group were stripped and weighed, as shown in Figure 7, the tumor weight in dimeric protein 3 group was significantly decreased compared to that in PBMC group; the tumor weight in IL-15 group was decreased compared to that in PBMC group, without significant difference; and the tumor weight in dimeric protein 7 group was comparable to that in PBMC group.

In summary, in HCT-116+PBMC SCID mouse model, the inhibitory effect of 3 kinds of protein was: dimeric protein 3≥ IL-15> dimeric protein 7.

Table 4. Efficacy of administration proteins on HCT-116+PBMC SCID xenografts

| Group | Mean tumor volume ($mm^3$) | | Mean tumor volume ($mm^3$) | | % inhibition ratio D28 | p |
|---|---|---|---|---|---|---|
| | D12 | SEM | D28 | SEM | (vs PBMC) | (vs PBMC) |
| PBMC | 230.33 | 32.16 | 1746.91 | 173.76 | 0 | |
| PBMC+IL-15 (2 μg/mouse) | 115.09 | 35.82 | 1015.25 | 180.70 | 42 | 0.0193 * |
| PBMC+ Dimeric protein 3 (10 μg/mouse) | 107.27 | □□□□ 2 | 1030.68 | 131.82 | 41 | 0.0111 * |
| PBMC+ Dimeric protein 7 (20 μg/mouse) | 94.68 | 13.98 | 1271.80 | 172.97 | 27 | 0.0886 |

* in the figure presents: $p<0.05$,vs PBMC.

**Test Example 6 Proliferation assay of Mo7e cells in vitro**

1. Main materials

Mo7e (human megakaryocyte leukemia cell line) purchased from Peking Union Medical College;

IL-15 purchased from Novoprotein, Item No. C016, IL-15 analogs (dimeric protein 11-17) from internal preparation;

Cell Counting Kit-8 (CCK-8) purchased from WST, Cat No. EX660;

GM-CSF purchased from NOVOProtein, Cat No. CC79.

2. Procedure

1) Mo7e was cultured in the incubator with modified RPMI-1640 medium at 37° C (5% $CO_2$) (containing 2.05mM L-glutamine, 10% FBS and 15ng/ml GM-CSF);

2) Mo7e cells in good condition were centrifuged at room temperature, 150 × g for 5min. The supernatant was discarded;

3) The cell pellet was washed with GM-CSF-free medium twice and then counted;

4) Cell concentration was adjusted and plated in 96-well plate with a cell number of $2\times10^4$ /well and a volume of 90μl (GM-CSF-free), kept in the cell incubator for culture;

5) IL-15 and its analogs (dimeric proteins 11 to 17) were 4-multiple proportion diluted with PBS, 10μl/well was added to the cell culture system after 2 hours incubation of cells in 96-well plates. Each concentration was titrated in triplicate well as well as blank wells (titrated with only PBS);

6) Cell plates were cultured in the incubator for 3 days;

7) All test wells were added with 10ul of CCK-8, and incubated in the incubator for 3 hours;

8) Absorbance at 450nm (OD450) was detected.

| Sample | **EC50 (nM)** relative activity |
|---|---|
| **IL-15** (Control) | 100.00 |
| **11** | 951.57 |
| **12** | 186.96 |
| **13** | 400.38 |
| **14** | 2526.19 |
| **15** | 2496.47 |
| **16** | 2988.73 |
| **17** | 2060.19 |

Mo7e proliferation assay showed that test dimeric proteins 11-17 were significantly superior to the control IL15.

**Test Example 7 Mouse lung metastasis model**

1. Procedure

32 of C57BL/6 mice (SPF, available from Shanghai Super B&K Laboratory Animal

Corp. Ltd) were intravenously injected with $1.5\times10^5$ of B16F10 cells (Shanghai Life Sciences Research Institute of Cell Resource Center, lot No TCM36), and divided into 4 groups, 8 in each group. Each group were intraperitoneally injected with PBS, 2μg of IL-15, or 4.2 μg, 12.5μg of dimeric protein 17 on Day 1, weighed once every 2-3 days, and one mouse from each group was sacrificed on day 14 for observation of lung metastasis. All C57BL/6 mice were sacrificed on day 16. Lungs were collected, weighed, observed for black lumps in lungs, photographed, fixed in methanol, and counted the number of black lumps.

2. Results

Lungs of mice in PBS group showed a large number of metastatic melanoma lumps (73±43). Lungs in IL-15 administration group showed a large number of metastatic melanoma lumps (65±29), which was 90% of that in PBS group. Lungs in 4.2 μ g of dimeric protein 17 administration group exhibited several of metastatic melanoma lumps (32±24), which was 44% of that in PBS group; Lungs in 12.5 μg of dimeric protein 17 administration group exhibited fewer metastatic melanoma lumps (14±14), which was 19% of that in PBS group.

In the present B16F10 model, the efficacy of dimeric protein 17 was significantly superior to that of IL-15, as shown in Figure 8 which represents the number of lung metastatic lumps in each group, compared to PBS group, * represents $p<0.05$, ** represents $p<0.01$.

The relative lung weight in PBS group is higher than that in dimeric protein 17 administration group, as shown in Figure 9.

The body weight of mice in 12.5ug of dimeric protein 17 group was slightly decreased on day 5 after administration, and then gradually recovered, as shown in Figure 10.

In summary, dimeric protein 17 can inhibit lung metastatic B16F10 cells in mice and exhibit dose effect.

**CLAIMS**:

1. An IL-15 heterodimeric protein, comprising:
protein (I) and protein (Π);
wherein said protein (I) is reassembled and formed by IL-15 or its variant with a first Fc variant;
said protein (Π) is a second Fc variant, or is reassembled and formed by IL-15Rα or its variant with a second Fc variant;
protein (I) and protein (Π) form a stable heterodimeric protein by the interaction between the first Fc variant and the second Fc variant.

2. The IL-15 heterodimeric protein according to claim 1, wherein the sequence of IL-15 is SEQ ID NO: 1.

3. The IL-15 heterodimeric protein according to claim 1, wherein the protein (Π) is a second Fc variant.

4. The IL-15 heterodimeric protein according to claim 1, wherein the protein (Π) is reassembled and formed by IL-15Rα or its variant with a second Fc variant.

5. The IL-15 heterodimeric protein according to claim 1, wherein IL-15Rα variant is an extracellular domain portion of IL-15Rα or functional fragment thereof, said functional fragment is preferably a truncated form of IL-15Rα extracellular domain portion with 65 to 120 amino acids, more preferably a truncated form with 65 to 102 amino acids.

6. The IL-15 heterodimeric protein according to claim 1, wherein the sequence of IL-15Rα variant is selected from the group consisting of SEQ ID NOs: 2-7, more preferably selected from the group consisting of SEQ ID NOs: 3-7.

7. The IL-15 heterodimeric protein according to claim 1, wherein:
the first Fc variant is selected from Knob modified Fc and Hole modified Fc; or the second Fc variant is selected from Hole modified Fc and Knob modified Fc;
when the first Fc variant is a Knob modified Fc, the second Fc variant is a Hole modified Fc; or when the second Fc variant is a Knob modified Fc, the first Fc variant is a Hole modified Fc;
preferably, the first Fc variant and the second Fc variant are linked to the C-terminus of IL-15

and IL-15Rα.

8. The IL-15 heterodimeric protein according to claim 1, wherein the sequence of the first Fc variant is selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 and SEQ ID NO: 29; and the sequence of the second Fc variant is selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28 and SEQ ID NO: 29.

9. The IL-15 heterodimeric protein according to claim 1, wherein the sequence of the protein (I) is selected from the group consisting of SEQ ID NOs: 14-17, preferably SEQ ID NO: 14; the sequence of the protein (Π) is selected from the group consisting of SEQ ID NOs: 18-25 and 34-37, preferably selected from the group consisting of SEQ ID NOs: 23 and 34-37, more preferably selected from the group consisting of SEQ ID NOs: 34-37.

10. The IL-15 heterodimeric protein according to claim 1, wherein the sequence of the protein (I) is selected from the group consisting of SEQ ID NOs: 30-31; the sequence of the protein (Π) is selected from the group consisting of SEQ ID NOs: 32-33.

11. The IL-15 heterodimeric protein according to claim 1, which is selected from the group consisting of the following dimeric proteins 3-17, wherein the dimeric proteins 3-17 are reassembled and formed by the corresponding protein (I) and protein (Π) as below:

| No | Protein (I) | Protein (Π) |
|---|---|---|
| 3 | IL-15-Fc-Knob (SEQ ID No.14) | Fc-Hole (SEQ ID No.27) |
| 4 | IL-15-Fc-Hole (SEQ ID No.15) | Fc-Knob (SEQ ID No.26) |
| 5 | Fc-Knob -IL-15 (SEQ ID No.16) | Fc-Hole (SEQ ID No.27) |
| 6 | Fc-Hole -IL-15 (SEQ ID No.17) | Fc-Knob (SEQ ID No.26) |
| 7 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα ECD-Fc-Hole (SEQ ID No.19) |
| 8 | IL-15-Fc-Hole (SEQ ID No.15) | IL-15Rα ECD-Fc-Knob (SEQ ID No.18) |
| 9 | Fc-Knob -IL-15 (SEQ ID No.16) | Fc-Hole -IL-15Rα ECD (SEQ ID No.21) |
| 10 | Fc-Hole -IL-15 (SEQ ID No.17) | Fc-Knob -IL-15Rα ECD (SEQ ID No.20) |
| 11 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(77 )-Fc-Hole (SEQ ID No.23) |

| | | |
|---|---|---|
| 12 | Fc-Knob(M)-IL-15 (SEQ ID No.30) | Fc-Hole(M)-IL-15Rα-sushi(65) (SEQ ID No.32) |
| 13 | IL-15-Fc-Knob(M) (SEQ ID No.31) | IL-15Rα-sushi (65) -Fc-Hole(M) (SEQ ID No.33) |
| 14 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(73 )-Fc-Hole (SEQ ID No.34) |
| 15 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(65)-Fc-Hole (SEQ ID No.35) |
| 16 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(86)-Fc-Hole (SEQ ID No.36) |
| 17 | IL-15-Fc –Knob (SEQ ID No.14) | IL-15Rα-sushi(102)-Fc-Hole (SEQ ID No.37) . |

12. A nucleic acid encoding the IL-15 heterodimeric protein according to any one of claims 1-11.

13. A DNA vector comprising the nucleic acid according to claim 12.

14. A host cell, which is transformed with said DNA vector according to claim 13.

15. A method for preparing the IL-15 heterodimeric protein according to any one of claims 1-11, comprising:
culturing the host cell according to claim 14 under the condition sufficient for the expression of the IL-15 heterodimeric protein according to any one of claims 1-11; expressing and purifying the IL-15 heterodimeric protein.

16. A pharmaceutical composition, comprising the IL-15 heterodimeric protein according to any one of claims 1-11, and a pharmaceutically acceptable excipient, diluent or carrier.

17. A targeting protein molecule, comprising the IL-15 heterodimeric protein according to any one of claims 1-11.

18. A method for stimulating or inhibiting the immune response in a mammal, comprising:
administering to the mammal a therapeutically effective amount of the IL-15 heterodimeric protein according to any one of claims 1-11, or the pharmaceutical composition according to claim 16, or the targeting protein molecule according to claim 17.

19. The use of the IL-15 heterodimeric protein according to any one of claims 1-11, or the pharmaceutical composition according to claim 16, or the targeting protein molecule according to claim 17, in the preparation of a medicament for treatment of IL-15-mediated diseases or disorders.

20. The use according to claim 19, wherein:
the disease or the disorder is selected from the group consisting of infectious disease, cancer, blood disease, inflammatory disease and autoimmune disease;
the cancer is selected from the group consisting of melanoma, colorectal cancer, skin cancer, lymphoma, renal cell carcinoma, liver cancer, lung cancer, stomach cancer, and breast cancer;
the infectious disease is selected from the group consisting of variola virus infection, HIV infection, bacterial infection, fungal infection, and HBV infection;
the blood disease is selected from the group consisting of anemia, acute myeloid leukemia, myelodysplastic syndrome, and T-cell large granular lymphocytic leukemia;
the autoimmune disease is selected from the group consisting of multiple sclerosis, psoriasis, rheumatoid arthritis, gastritis, and mucositis.

21. The use according to claim 19, wherein the IL-15 heterodimeric protein according to any one of claims 1-11, or the pharmaceutical composition according to claim 16, or the targeting protein molecule according to claim 17 is administered in combination with small molecule inhibitor or antibody; the small molecule inhibitor is selected from the alkylating agent; the antibody is selected from monoclonal antibody, more preferably selected from anti-CD20, anti-PD1, anti-PDL1, and anti-Her2 antibody.

22. Use of the IL-15 protein complex according to any one of claims 1-11, or the pharmaceutical composition according to claim 16, or the targeting protein molecule according to claim 17, in the preparation of a medicament for cell immunotherapy, especially in the preparation of a medicament for tumor immunotherapy of DC, CIK, DC-CIK, ECIK, NK, CAS-T, BiAb-T, TCR-T, and CAR-T.

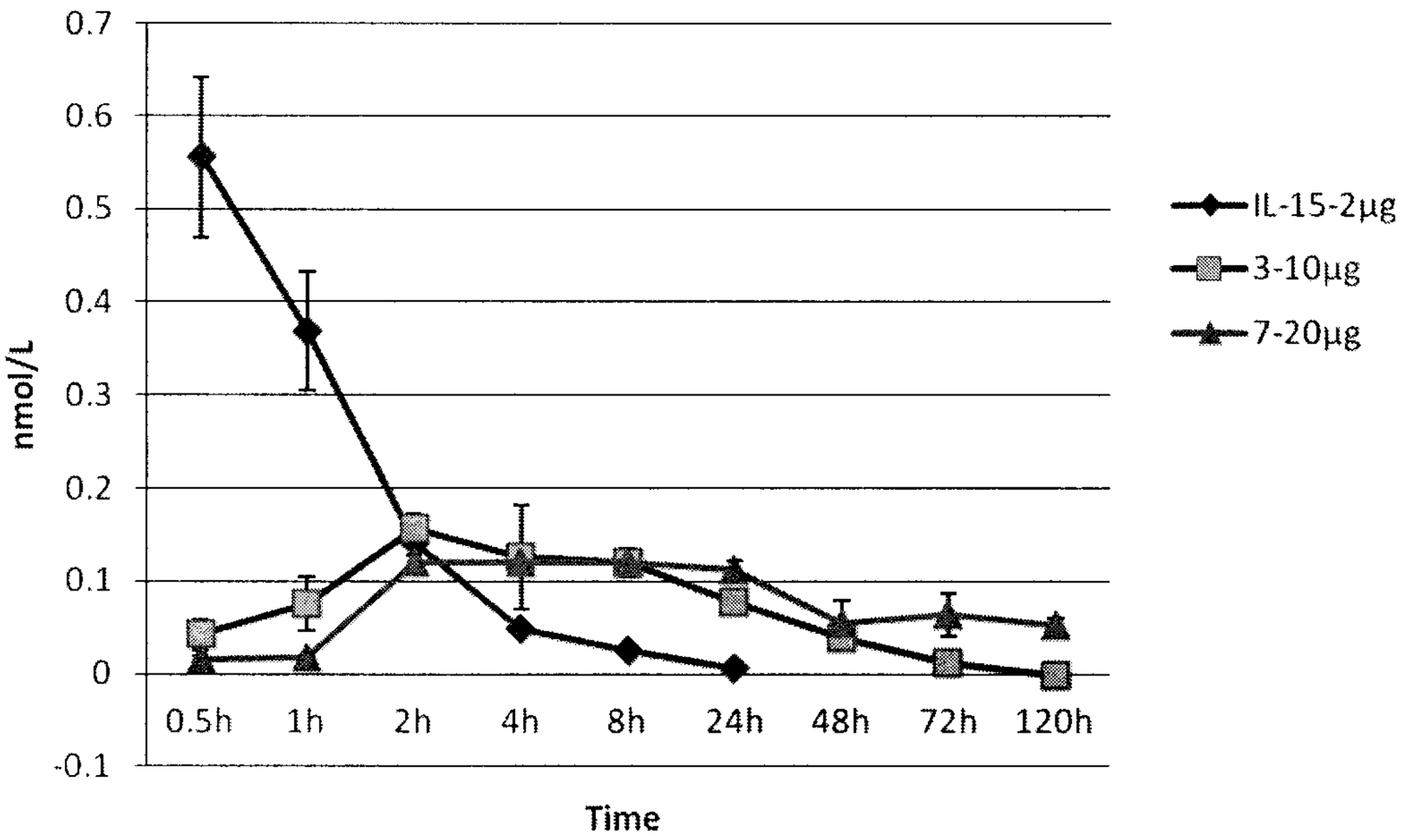



Figure **1**

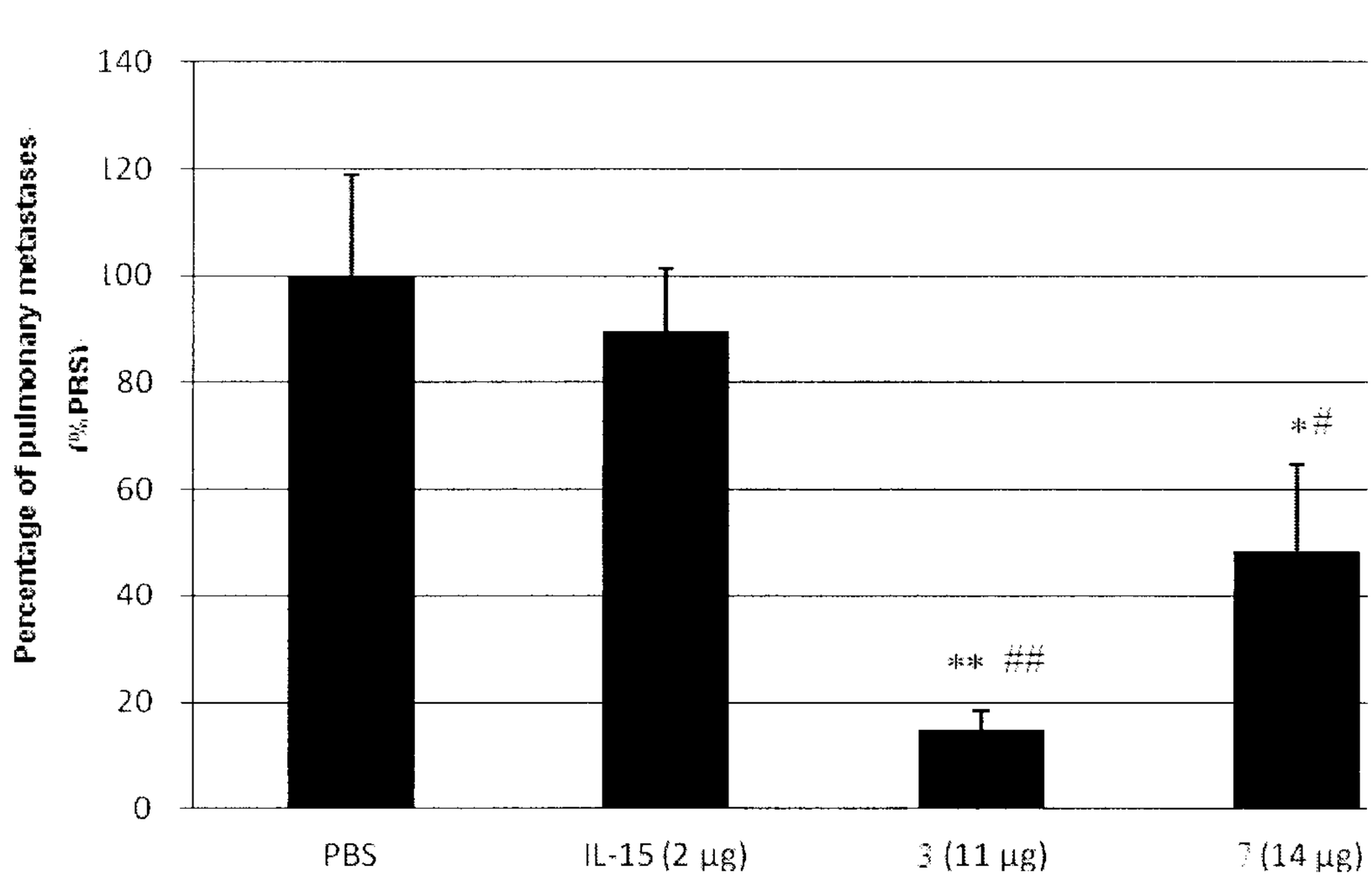



Figure **2**

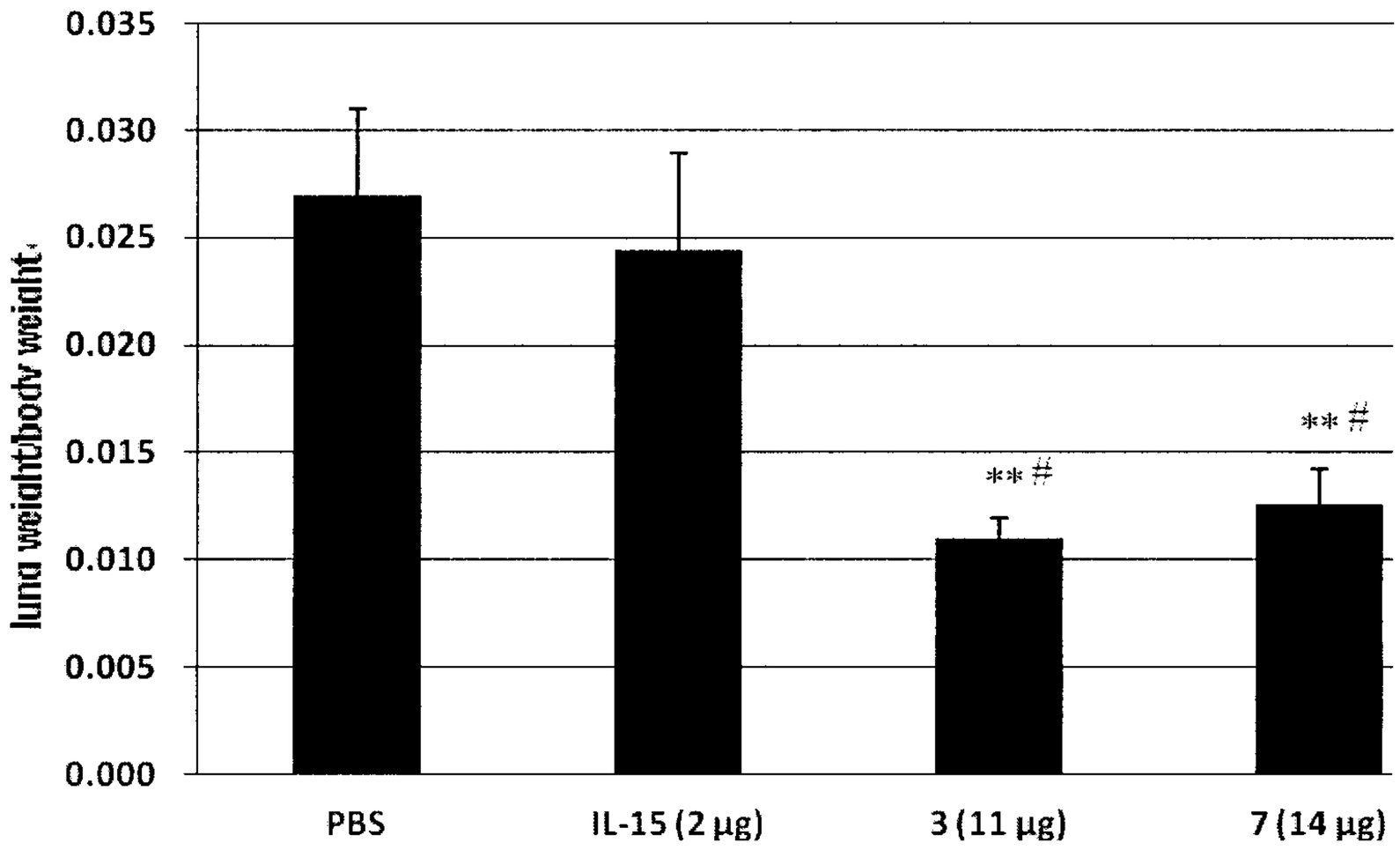



Figure **3**

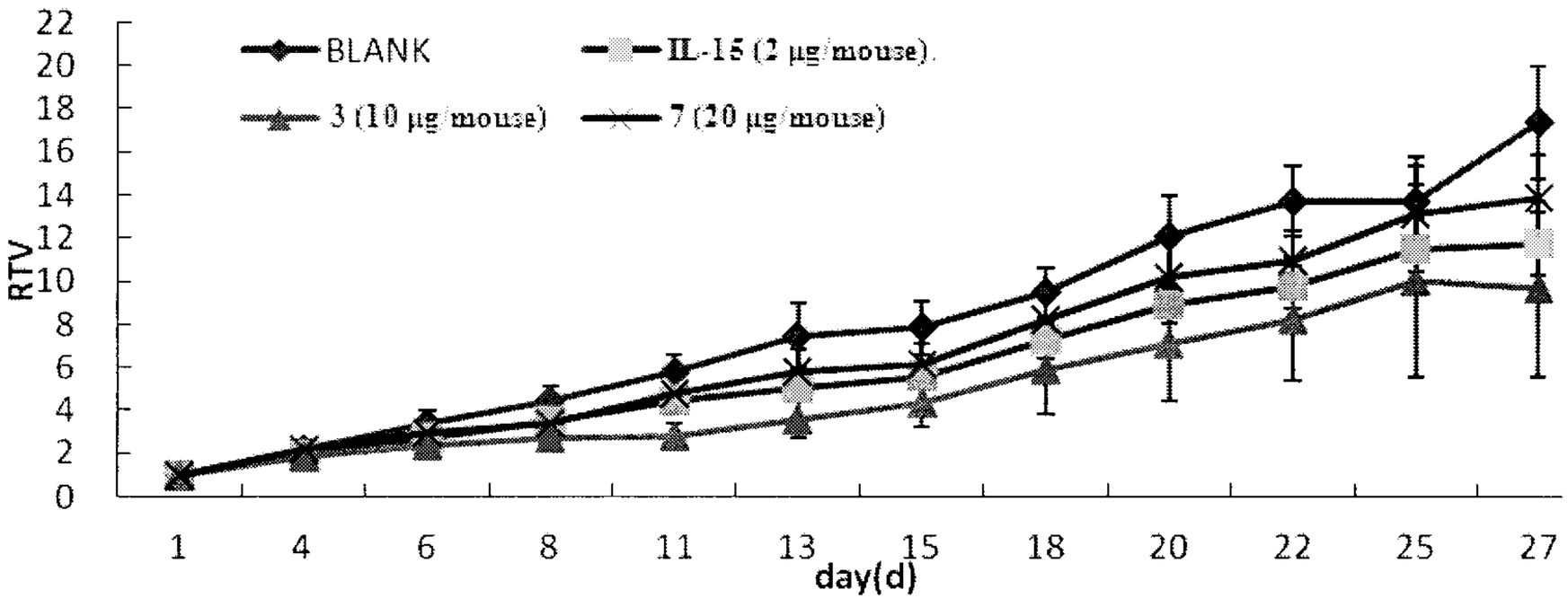



Figure **4**

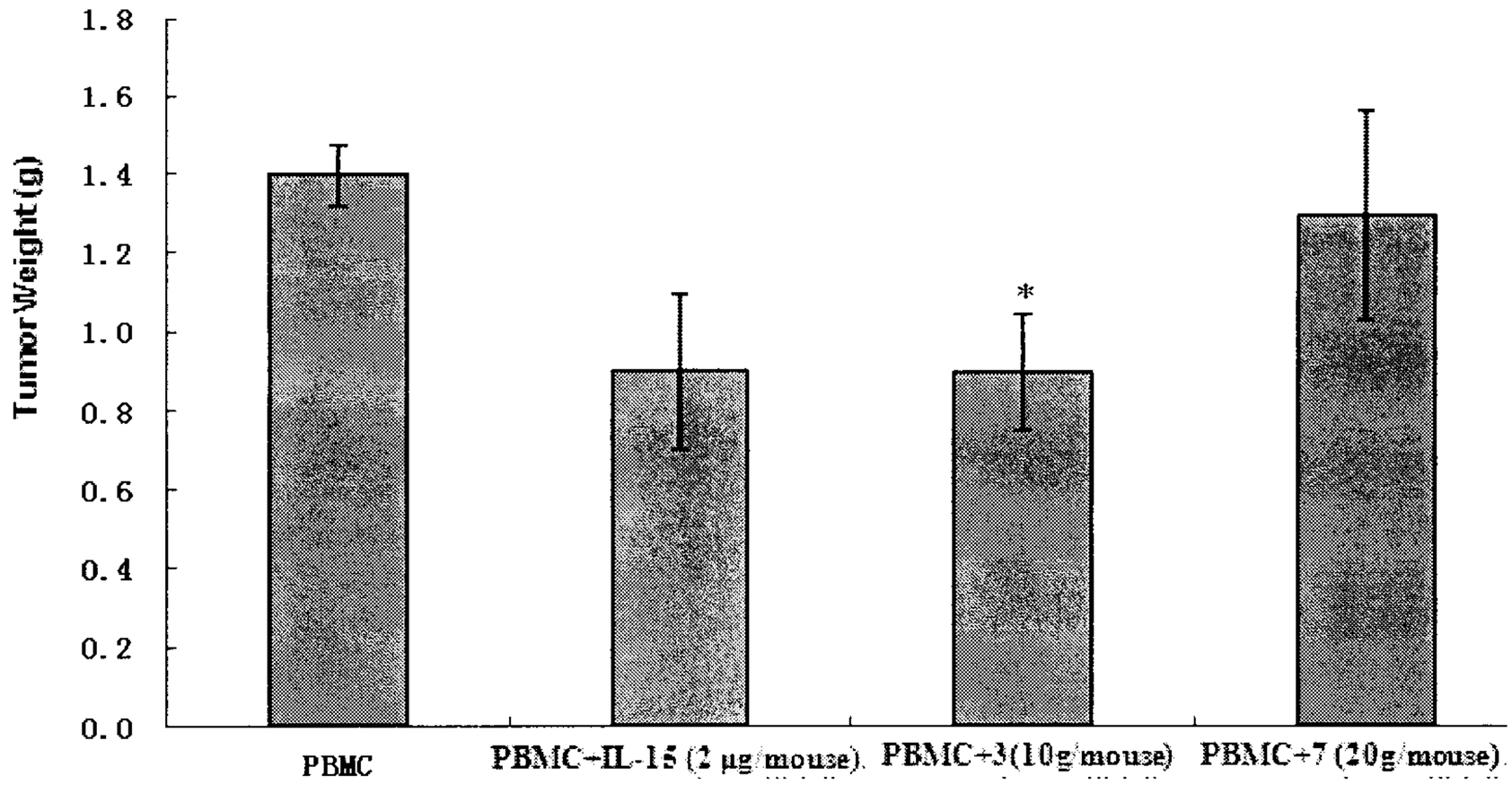



Figure **5**

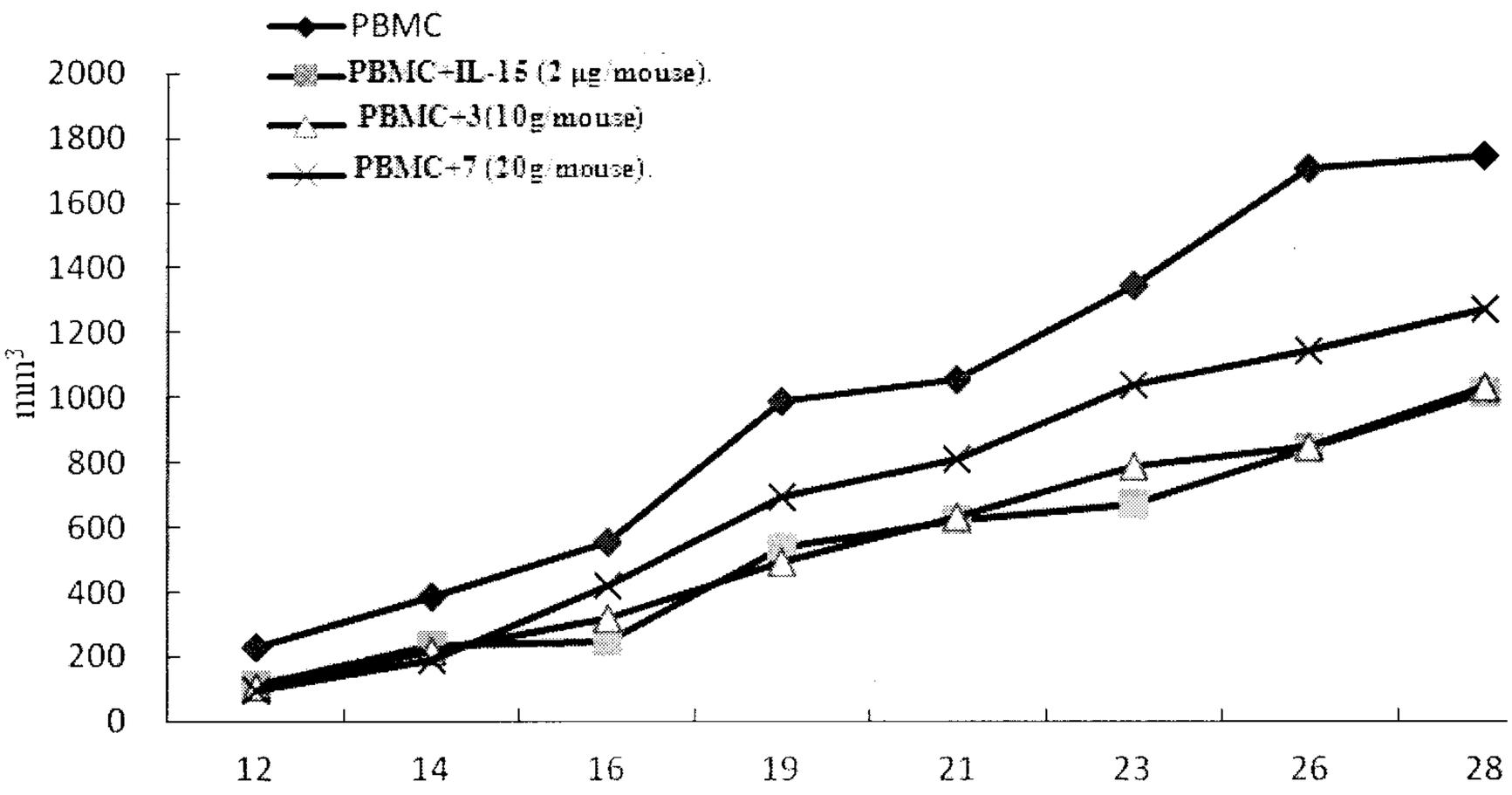



Figure **6**

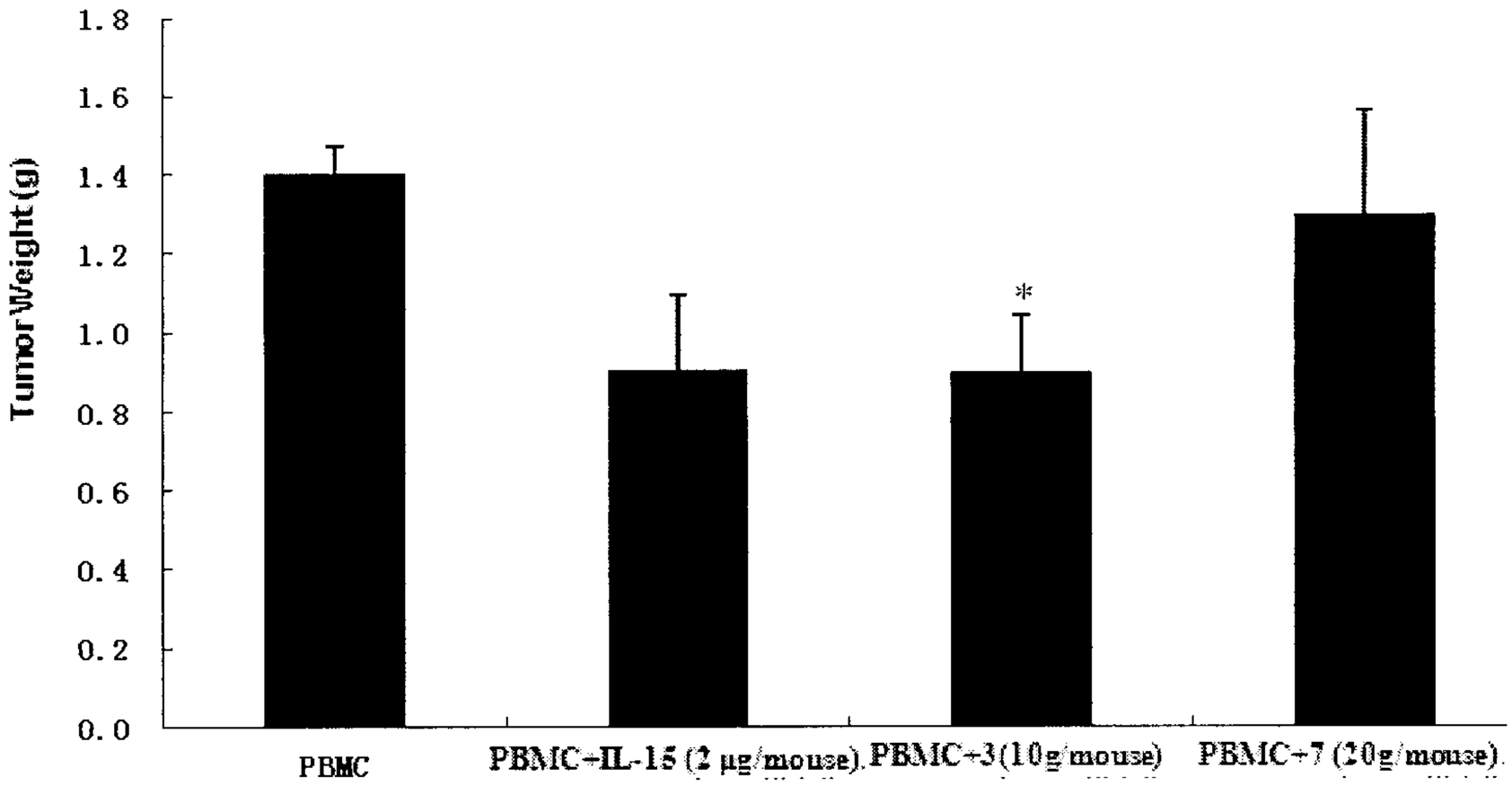



Figure 7

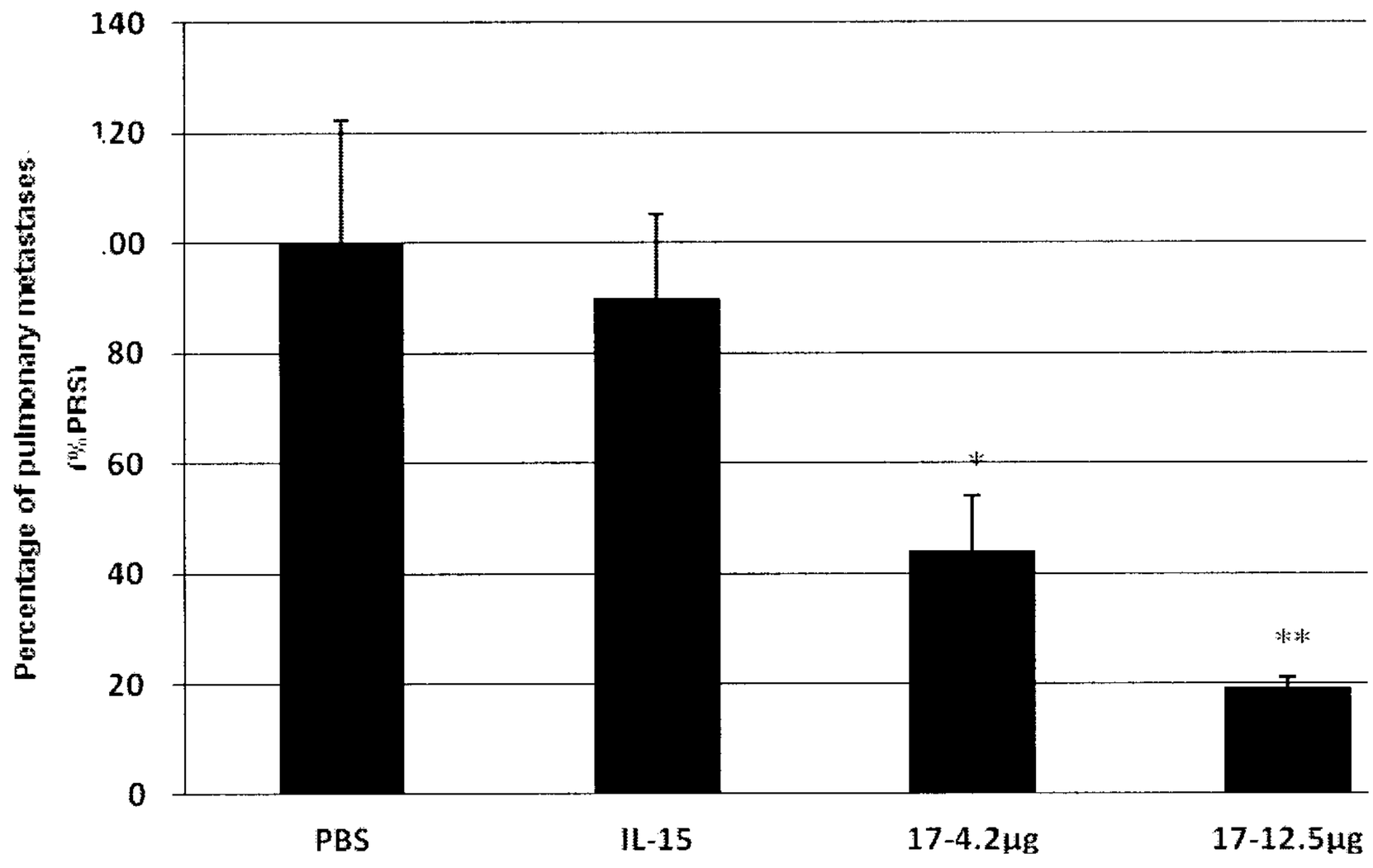



Figure 8

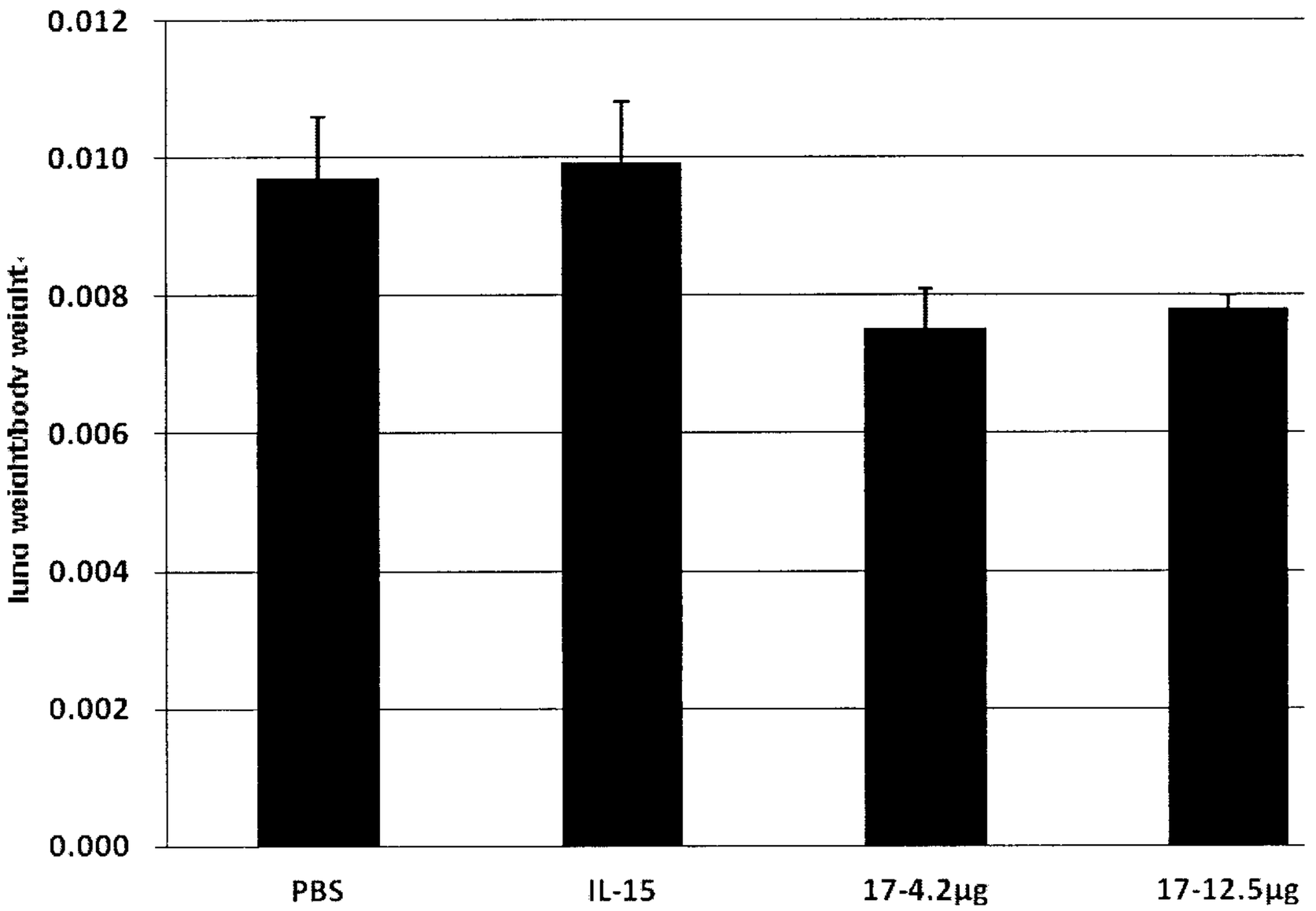



Figure **9**

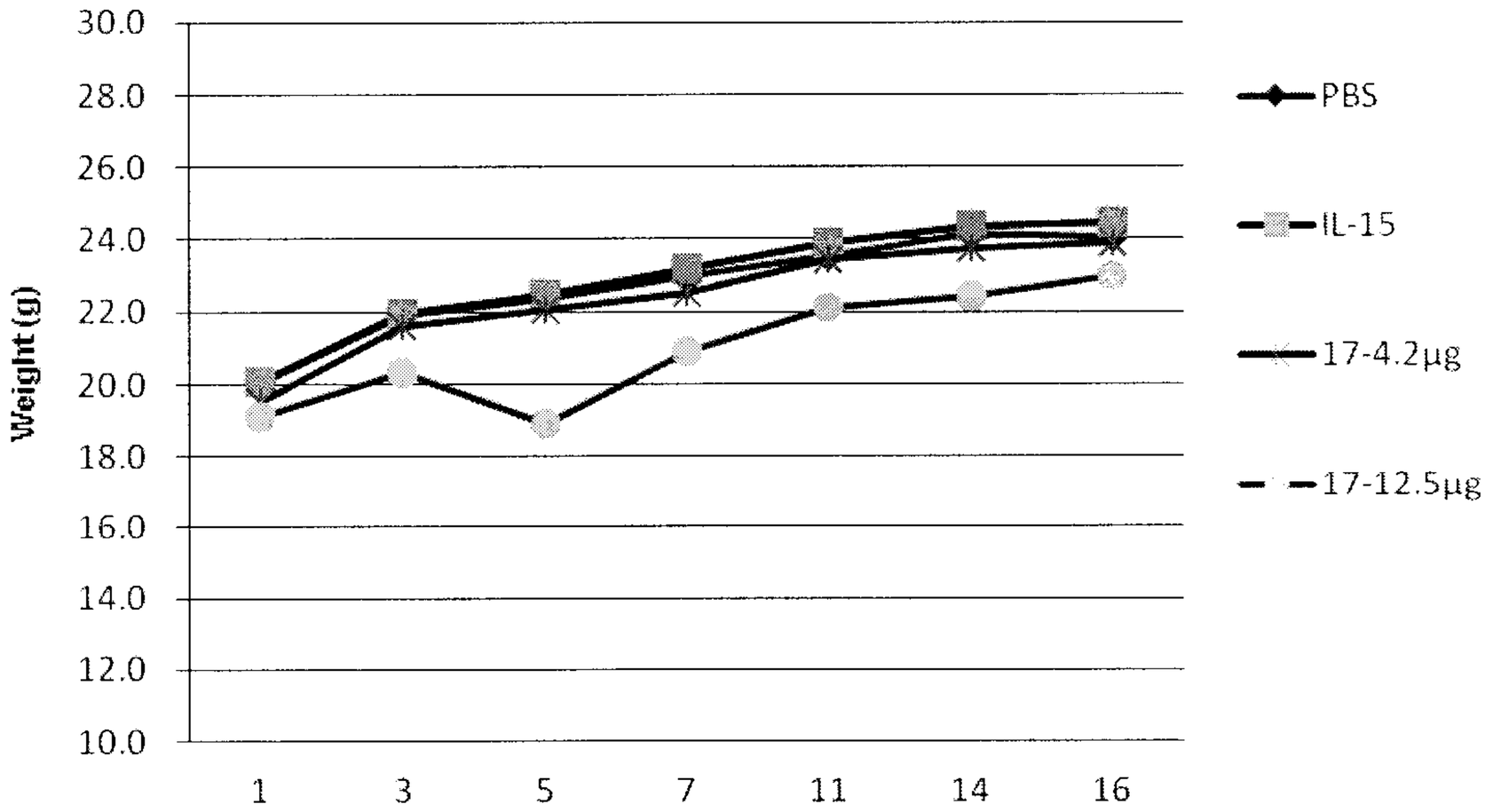



Figure **10**