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(54) Title: CHEMOKINE-CYTOKINE FUSION PROTEIN AND APPLICATION THEREOF

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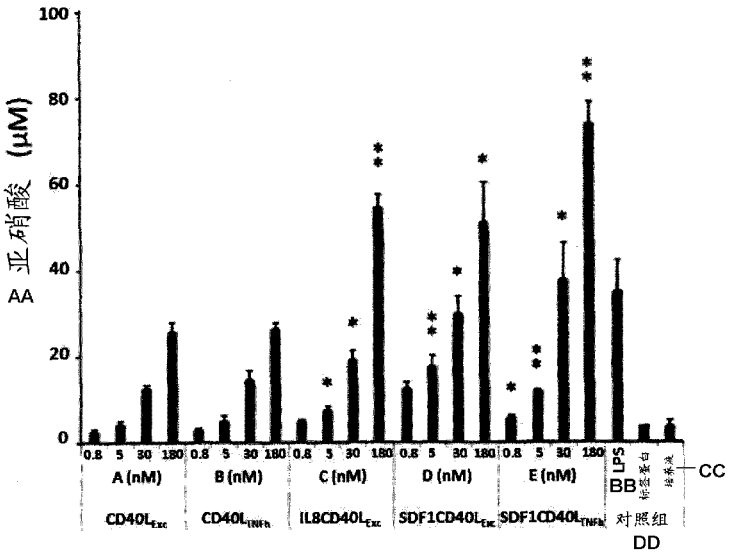

图 6 / Fig. 6

AA Nitrous acid
BB Tag protein
CC Culture medium
DD Control

(57) Abstract: The present invention provides a fusion protein comprising the chemokine polypeptide which is a chemokine or receptor-binding domain thereof, and the cytokine polypeptide linked to said chemokine polypeptide, the cytokine polypeptide is an interleukin, a TNF-superfamily cytokine or receptor-binding domain thereof, the chemokine polypeptide and the cytokine polypeptide possess same target cells, and the fusion protein has enhanced chemokine activity comparing with said chemokine polypeptide and enhanced cytokine activity comparing with said cytokine polypeptide.

(57) 摘要: 本发明提供一种融合蛋白，其包含趋化素多肽，其为一趋化素或其受体结合域；和连接至该趋化素多肽的细胞素多肽，其为一介白素、一 TNF-超家族细胞素或其受体结合域；其中该趋化素多肽和该细胞素多肽具有共同的标的细胞，且该融合蛋白具有相较于该趋化素多肽的增进的趋化素活性，以及相较于该细胞素多肽的增进的细胞素活性。

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TITLE OF THE INVENTION

CHEMOKINE-CYTOKINE FUSION PROTEIN AND ITS USE

FIELD OF THE INVENTION

[0001] The present invention relates to fusion protein, comprising a chemokine and a cytokine connected thereto, wherein the chemokine and the cytokine have a mutual target cell, and the fusion protein has an improved cytokine activity and an improved chemokine activity.

BACKGROUND OF THE INVENTION

[0002] Cytokines are a group of proteins that cells release upon excitation (only very few cytokines are expressed on cell membranes). Cytokines produced by cells can affect target cells nearby or through blood circulation at very low concentration. They have broad functions on promoting growth, differentiation and activation of target cells. Many cytokines can target immune cells and play a role in immune response. Based on structural and functional differences, cytokines may be broadly divided into chemokines, interleukins, growth factors, transforming growth factors, colony stimulating factors, tumor necrosis factors, and interferons, etc.

[0003] Chemokines are a group of cytokines being able to attract leukocytes, which are generally positively charged, secretory proteins having small molecule weights. Their main function is to attract immune cells to a region having tissue injuries or pathogen infection, allowing leukocytes to subsequently perform phagocytosis or elicit inflammation against pathogens at this specific site. Leukocytes attracted by chemokines may include neutrophils, monocytes/ macrophages, natural killer cells, dendritic cells and other leukocytes, which are of innate immunity; and T lymphocytes (T cells) or B lymphocytes (B cells) of adaptive immunity. Accordingly, chemokines play a very important role in the immune system of living organisms. Most chemokines have four highly conserved cysteine (C) forming disulfide bonds to stable their structure. Based on different numbers of amino acids between the first two Cs and the procession of the first C or not, they may be classified into four subfamilies of CXC (or α), CC (or β), C (or γ) and CX_3C. Stromal cell-derived factor-1 (SDF-1) is classified into the CXC subfamily of chemokines, and is also known as CXC ligand 12 (CXCL12). Having been observed in many species including humans, mice and cats of mammals and *Xenopus* of amphibians, and zebra fishes, it has little variation between different species and is highly conserved (Shirozu *et al.*, *Genomics* 28, 495-500). mRNAs transcribed from SDF-1 gene in mice and humans are subject to different splicings and thus two isoforms of SDF-1 may be observed: SDF-1α

and SDF-1β. The distribution of SDF-1 is very wide, and can be detected, including in lymphoid tissue, kidney, lung, liver, brain and muscle (Shirozu *et al.*, *Genomics* 28, 495-500). SDF-1 receptor CXCR4 not only constantly presents in organs, but can also be seen in hematopoietic stem cells, endothelial cells, dendritic cells, B cells and T cells. Therefore, these cells are attracted by SDF-1 to migrate to the site with high concentration of the chemokines (Bleul *et al.*, *Nature*, 382: 829-833; Oberlin et al., *Nature* 382: 833-835; Read *et al.*, *Developmental and comparative immunology*, 29, 143-152). Interleukin-8 (IL-8) is also classified into the CXC subfamily of chemokines (also known as CXCL8). After initial discovery in humans, it was successively observed in economic animals of pigs, cows and chickens. IL-8 at low concentration is able to attract several immune cells, including monocytes, macrophages, lymphocytes, neutrophils, etc.

[0004] CD40 ligand (CD40L) is a member of tumor necrosis factor (TNF) superfamily, which is a cytokine having functions on tumor necrosis and promoting differentiation, proliferation and apoptosis of white blood cells. CD40L is synthesized as a transmembrane protein. Take human CD40L as an example, the protein has a total of 261 amino acids, with first 22 amino-terminal amino acids being intracellular region, followed by 24 amino acids being transmembrane region, and 215 carboxy-terminal amino acids being extracellular (Exc) region, wherein the Exc region has at its carboxy terminus a TNF homology (TNFh) region conserved for all TNF superfamily proteins. CD40L presents mainly in the form of a transmembrane protein on the surface of activated $CD4^+$ T cells, and also presents on $CD8^+$ T cells, basophils, eosinophils, mast cells, natural killer cells, platelets, and even on the surface of CD40-expressing cells.

[0005] CD40, receptor of CD40L, is distributed on the surfaces of antigen presenting cells (APCs) of B cells, dendritic cells, macrophages, etc. Physiologically, these antigen presenting cells can be activated by CD40L expressed by T helper cells, promoting the expression of major histocompatibility complex class II (MHC-II) molecules and B7 molecules to assist in antigen presentation. CD40L activates signal transduction pathways by binding to CD40 on target cells. In addition to the aforementioned promotion of antigen presentation, effecting on B cells, CD40L can promote B cell proliferation, isotype switching of immunoglobulins, antibody secretion, memory B cell differentiation, or prevention of apoptosis; effecting on macrophages, CD40L can enhance their activation, production of interleukin-12 (IL-12) to activate T helper 1 (Thl), or secretion of chemokines, or the production of nitric oxide (NO) to promote microorganism defense ability of macrophages; effecting on dendritic cells, it can make them mature and activated, wherein the mature dendritic cells not only express a large amount of MHC-II molecules to

promote antigen presentation, but also secrete chemokines of TNF-α and IL-8, macrophage inflammatory protein la (MIP-1a), etc.

[0006] There are many researches that apply CD40L on vaccine adjuvant or treatment, for example, as adjuvants for duck hepatitis B virus (DHBV) vaccines (Gares *et al., Clin Vaccine Immunol* 13, 958-965), human immunodeficiency virus (HIV) DNA vaccines (Stone *et al.*, *J Virol* 80, 1762-1772), or in the treatment of human autoimmune diseases (Howard & Miller, *Autoimmunity* 37, 411-418), etc.

[0007] IL-2 is classified into the hematopoietin family, the family including a number of cell growth-related hormones or other cytokines, etc. Functions of IL-2 include: regulating the maturation and differentiation of T cells, stimulating proliferation and antibody secretion of B cells, promoting cytotoxicity of natural killer cells, and activating monocytes and macrophages, etc. IL-2 can also stimulate T cells and B cells to continue expressing MHC, and also stimulate natural killer cells to produce several different cytokines, including TNF-α, IFN-γ and granulocyte/macrophage colony stimulating factor (GM-CSF), etc. Studies have shown that IL-2 has anti-tumor and vaccine-enhancing effects.

[0008] However, there remains a need in the art for cytokines and chemokines with an improved activity.

BRIEF SUMMARY OF THE INVENTION

[0009] It was unexpectedly found in the present invention that a fusion protein comprising a chemokine and a cytokine connected to the chemokine has an improved cytokine activity and an improved chemokine activity.

[0010] Accordingly, the present invention provides a fusion protein, comprising a chemokine polypeptide, which a chemokine or a receptor binding domain thereof; and a cytokine polypeptide connected to the chemokine polypeptide, which is an interleukin, a TNF- superfamily cytokine, or a receptor binding domain thereof; wherein the chemokine polypeptide and the cytokine polypeptide have a common target cells, and the fusion protein has an improved chemokine activity as compared to the chemokine polypeptide, and an improved cytokine activity as compared to the cytokine polypeptide.

[0011] According to the present invention, the chemokine is a CXC chemokine, CC chemokine, C chemokine, and chemokine CX_3C, preferably CXC chemokine. According to one embodiment of the present invention, the chemokine may be a stromal cell derived factor (SDF-1) or IL-8.

[0012] According to the present invention, the cytokine polypeptide is an interleukin, a TNF- superfamily cytokine, or a receptor binding domain thereof. In one embodiment of

the present invention, the cytokine polypeptide is IL-2, CD40 ligand, or a receptor binding domain thereof.

[0013] In another aspect, the present invention provides an isolated nucleic acid molecule, which encodes a fusion protein of the present invention.

[0014] In yet another aspect, the present invention provides an expression vector, comprising a nucleic acid molecule of the invention.

[0015] The present invention also provides a host cell, comprising an expression vector of the invention or a nucleic acid molecule of the invention.

[0016] Details of various embodiments of the present invention are described below. Other features of the invention will be apparent from the detailed description of various embodiments and the claims.

[0017] Without further elaboration, it is believed that a person of ordinary skill in the art to which the present invention belongs can utilize the invention to its broadest extent based on the description above. It is to be understood that the following detailed description are exemplary and are not restrictive of the other disclosure in any way.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawing. In the drawings:

[0019] Figure 1 is a schematic diagram for chicken CD40L and its derivative proteins, $CD40L_{Exc}$ and$CD40L_{TNFh}$.

[0020] Figure 2 shows the results of SDS-PAGE and western blot analysis of the expressed chicken recombinant proteins. Lane1: IL8$CD40L_{Exc}$, expected size being 52 kDa; Lane 2: IL8IL2, expected size being 44 kDa; Lane 3: SDFl$CD40L_{Exc}$, expected size being 38 kDa; Lane 4: SDF1CD40LTNFh, expected size being 44 kDa; and Lane 5: SDF1IL2, expected size being 26 kDa.

[0021] Figure 3 shows the results of SDS-PAGE and western blot analysis for purified single proteins. Lane 1: tagged protein, expected size being 21 kDa; Lane 2: IL-8, expected size being 13 kDa; Lane 3: SDF-1, expected size being 11 kDa; Lane 4: IL-2, expected size being 32 kDa; Lane 5: $CD40L_{Exc}$, expected size being 42 kDa; and Lane 6: $CD40L_{TNFh}$, expected size being 33 kDa.

[0022] Figure 4 shows the chemotaxis of PBMCs by IL-8 derivative proteins. Cells attracted by the chemokine outside the agar would travel from the center of the agar to the surrounding. Cells are seen cloudy at low magnification. More cells at the surrounding

indicates greater degree of cell chemotaxis. At 2 μM, the chemotaxis extent of IL8CD40L$_{Exc}$ or IL8IL2 was significantly higher than single IL-8 protein.

[0023] Figure 5 shows the chemotaxis of PBMCs by SDF-1 derivative proteins. At 2 μM, the chemotaxis extent of SDF1CD40L$_{Exc}$, SDF1CD40L$_{TNFh}$, or SDF1IL2 was significantly higher than single SDF-1 protein.

[0024] Figure 6 shows the activities of CD40L derivative proteins on activating macrophages to produce NO. A: CD40L$_{Exc}$; B: CD40L$_{TNFh}$; C: IL8CD40L$_{Exc;}$ D : SDFlCD40L$_{Exc}$; E : SDFlCD40L$_{TNFh}$; and the control group: LPS (4 μg/ml) as a positive control group, tagged protein (250 nM) and the culture medium as negative control groups. * represent significantly higher activity as compared to single proteins ($^{*}p < 0.05$, $^{**}p < 0.01$).

[0025] Figure 7 shows the results of test on IL-2 fusion proteins' promotion of lymphocyte proliferation. Stimulation index (SI) = OD of test groups / OD of those cultured with RPMI 1640 only. At a concentration of 0.625 - 160 nM, the SI value of SDF1IL2 fusion protein was significantly higher than the IL-2 alone group ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

[0026] Figure 8 shows that IL-2 fusion protein as an adjuvant of Newcastle disease (ND) vaccine can significantly promote cell immune response. Chickens of the group administered with IL-2 fusion protein adjuvant and ND vaccine exhibited a significantly higher proliferation of memory lymphocytes upon Newcastle disease virus (NDV) antigen re-stimulation, as compared to the ND vaccine group.

[0027] Figure 9 shows that the fusion proteins as adjuvants of infectious bronchitis (IB) vaccine can significantly promote cell immune response. Antigen re-stimulation tests were performed for infectious bronchitis virus (IBV). Chickens of the group administered with IL-2 fusion protein adjuvant and ND vaccine exhibited a significantly ($p < 0.01$) higher proliferation of memory lymphocytes, as compared to the IB vaccine group. Chickens of the group administered with CD40L$_{Exc}$ fusion protein adjuvant and ND vaccine exhibited a significantly ($p < 0.05$) higher proliferation of memory lymphocytes, as compared to the IB vaccine group.

DETAILED DESCRIPTION OF THE INVENTION

[0028] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person skilled in the art to which this invention belongs.

[0029] As used herein, the singular forms "a", "an", and "the" refer to one or more referents unless the context clearly dictates otherwise.

[0030] The term "chemokine polypeptide" as used herein refers to a polypeptide, which is a chemokine or a receptor binding domain thereof, wherein the chemokine includes but is not limited to CXC chemokines, CC chemokines, C chemokines and CX_3C chemokines.

[0031] The term "cytokine polypeptide" as used herein refers to a polypeptide, which is a cytokine or a receptor binding domain thereof, wherein the cytokine includes but is not limited to interleukins and cytokines of TNF-superfamily.

[0032] The term "chemokine activity" as used herein refers to the activities which chemokines possess or are able to exert *in vivo*, including but not limited to, chemotaxis of a variety of immune cells (including monocytes, macrophages, T cells , B cells, natural killer cells, dendritic cells and neutrophils, etc.).

[0033] The term "cytokine activity" as used herein refers to the activities which cytokines possess or are able to exert *in vivo*, including but not limited to the promotion of proliferation, immunoglobulin class switching and antibody secretion of B cells; differentiation of memory B cells, or prevention of their apoptosis; promoting macrophages' secretion of interleukin-12 to activate type I helper T cells or secrete chemokines; promoting macrophages to produce nitric oxide to enhance the defense capability against microorganisms; promoting the maturation and activation of dendritic cells; regulation of the maturation and differentiation of T cells; promoting the cytotoxicity and the production of a variety of different cytokines of natural killer cells; activation of monocytes and macrophages; and stimulation of T cells and B cells to continuously express MHC, etc.

[0034] The present invention provides a fusion protein, comprising a chemokine polypeptide, which is a chemokine or a receptor binding domain thereof, and a cytokine polypeptide connected to the chemokine polypeptide, which is a interleukin, a TNF-superfamily cytokine or a receptor binding domain thereof; wherein the chemokine polypeptide and the cytokine polypeptide have a common target cell, and the fusion protein has an improved chemokine activity as compared to the chemokine polypeptide, and an improved cytokine activity as compared to the cytokine polypeptide.

[0035] In preferred embodiments of the present invention, the chemokine polypeptide and the cytokine polypeptide are connected by a peptide linker. To join two proteins together and retain their original configurations and functions, an appropriate peptide linker may be added between the two proteins to reduce the interference with each other when the proteins fold. And such peptide linker may be a flexible peptide linker $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$ (usually n is less than 6) with a certain extent of flexibility and

hydrophilicity, or a hydrophilic helical peptide linker $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_n$ (usually n is less than 6).

[0036] In one embodiment of the invention, the chemokine is a CXC chemokine. In a certain embodiment, the chemokine is stromal cell-derived factor is (SDF-1). In another certain embodiment, the chemokine is IL-8.

[0037] In certain embodiments of the present invention, the chemokine polypeptide has an amino acid sequence selected from the following: SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, and a homolog thereof and an analog thereof.

[0038] In certain embodiments of the present invention, the cytokine is IL-2, CD40 ligand (CD40L) or a receptor binding domain thereof.

[0039] In certain embodiments of the present invention, the cytokine polypeptide has an amino acid sequence selected from the following: SEQ ID NO: 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and a homolog thereof and an analog thereof.

[0040] In certain embodiments of the present invention, the fusion protein of the present invention has an amino acid sequence selected from the following: SEQ ID NO: 40, 42, 44, 46 and 48.

[0041] In another aspect, the present invention provides an isolated nucleic acid molecule, which encodes a fusion protein of the present invention.

[0042] In certain embodiments of the present invention, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a chemokine polypeptide, selected from the following: SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, and a homolog thereof and an analog thereof.

[0043] In certain embodiments of the invention, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a chemokine polypeptide, selected from the following: SEQ ID NO: 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, and a homolog thereof and an analog thereof.

[0044] In certain embodiments of the invention, the isolated nucleic acid molecule has a sequence of one selected from the following: SEQ ID NO: 39, 41, 43, 45 and 47.

[0045] In yet another aspect, the present invention provides an expression vector, comprising a nucleic acid molecule of the invention.

[0046] The present invention also provides a host cell comprising an expression vector of the invention or a nucleic acid molecule of the invention.

[0047] The following examples are merely illustrative and not restrictive to the present invention.

[0048] **Example 1: Construction of prokaryotic expression vectors**

[0049] Previously constructed expression vectors for chicken SDF-1, IL-8, CD40L derivative proteins and IL-2 (Pei-Shan Wu, National Chung Hsing University, Institute of Veterinary Microbiology, 2008 Master's thesis, Studies on chicken CD40L and chemokines; Tsai *et al.*, *Taiwan Vet J* 31: 38-45) were used as a template for recombinant polymerase chain reactions, wherein the chicken CD40L (chCD40L) derivative protein includes an extracellular domain of CD40L ($CD40L_{Exc}$) or TNF homology domain of CD40L ($CD40L_{Exc}$) (see Figure 1). Genes sequences of conjugate proteins or fusion proteins $SDF1CD40L_{Exc}$, $SDF1CD40L_{TNFh}$, SDF1IL2, $IL8CD40L_{Exc}$, $IL8CD40L_{TNFh}$, IL8IL2, etc. were expanded therefrom.

[0050] The methods are briefly described as follows. Two specific primer pairs were designed based on the sequences of each of the genes. Forward primer of the first pair of primers has a gene sequence of an *Eco*R I restriction enzyme site and a front N-terminus of the fusion protein, and the reverse primer has a gene sequence of a helical peptide linker and a front C-terminus of the fusion protein. This pair of primers can specifically amplify DNA fragments encoding the front section of the fusion protein and the peptide linker. Forward primer of the second pair of primers has a gene sequence of a helical peptide linker and a rear N-terminus of the fusion protein, and the reverse primer has a gene sequence of a *Xho* I restriction enzyme site and a rear C-terminus of the fusion protein. This pair of primers can specifically amplify DNA fragments encoding the peptide linker and the rear section of the fusion protein. With the PCR products of this two primer pairs as templates, an additional PCR was performed using the forward primer of the first pair of primers and the reverse primer of the second pair of primers, and accordingly the two fragments were connected due to partial overlapping sequences of the helical peptide linker. The products obtained are fusion gene sequence comprising helical peptide linker gene therein.

[0051] After treating the above products with *Eco*R I and *Xho* I, a ligation with pET vectors (Novagen, Darmstad, Germany) treated by *Eco*R I and *Xho* I using T4 DNA ligase (Invitrogen) at 16 °C for 16 hour was performed. The constructed prokaryotic expression vector were respectively named as $pETSDFlCD40L_{Exc}$, $pETSDF1CD40L_{TNFh}$, pETSDF1IL2, $pETIL8CD40L_{Exc}$ and pETIL8IL2.

[0052] **Example 2: Expression of the recombinant proteins**

[0053] The constructed prokaryotic expression vector was transformed into *E. coli* expression strain BL21 (DE3), 0.5 mM IPTG was used to induce the expression of the recombinant protein, and collected bacteria cells by centrifugation with the removal culture medium. Subsequently, all of bacteria cells were resuspended in binding buffer, a high

pressure cell lysis instrument (French Pressure Cell Press, Thermo IEC, Needham, Height, MA, USA) was used to lysis the bacteria cells, and soluble proteins located in the supernatant after high speed centrifugation were isolated using nickel ion affinity column.

[0054] Insoluble protein located at the bottom pellet after centrifugation were treated with 8 M to unfold the proteins and they were dissolved in an aqueous solution, which was then subjected to a centrifugation of 12,000 rpm 30 minutes and dialysis of the supernatant was performed to slowly displace the urea, so that the proteins may refold to their original configurations. Lastly, the proteins were dissolved in phosphate buffer containing 10% glycerol (H 7.3), filtered through 0.22 μm membrane, concentration was determined by BCA protein assay kit (Pierce, Rockford, IL, USA), and stored at -20 °C. The isolated proteins were identified to be correct fusion proteins using MALDI-TOF mass spectrometer. SDS-PAGE and western blot (primary antibody being anti-His antibody, secondary antibody being AP- labeled goat anti-mouse IgG antibody, chromogenic reagent NBT/BCIP) analysis shows that the expressed chicken recombinant fusion proteins IL8CD40L$_{Exc}$, IL8IL2, SDF1CD40L$_{Exc}$, SDF1CD40L$_{TNFh}$ and SDF1IL2 etc. have the molecular weights as expected, respectively being 52 kDa, 44 kDa, 38 kDa, 44 kDa and 26 kDa (see Figure 2). In addition, single proteins were also prepared and isolated as controls by the methods as described above (Pei-Shan Wu, National Chung Hsing University, Institute of Veterinary Microbiology, 2008 Master's thesis, Studies on chicken CD40L and chemokines; Tsai *et al.*, *Taiwan Vet J* 31: 38-45).

[0055] **Example 3: Chemotactic activity assay**

[0056] Chemotactic activities of chemokines (SDF-1, or IL-8) and CD40L derivative proteins or IL-2-fused chemokines were accessed. Peripheral blood mononuclear cells were isolated using Histopaque 1077 (Sigma, Saint Louis, Mo, USA), washed twice with PBS, suspended with 10% FBS in RPMI 1640 (Gibco, Grand Island, NY, USA), and then added into 0.6% liquid agar, mixed well, resulting in a final concentration of 0.3% of the agar. Subsequently, 2 μl/well of the mixture were dripped in the center of the wells on a 48-well plate, and placed in a refrigerator for five minutes to solidify the agar, thus fixing the cells within the agar. Each well was then added with 250 μl medium containing a respective concentration protein to be tested, cultured overnight before observation.

[0057] Based on the minimum effective concentration (MEC) for each protein to exert chemotactic activity, with smaller MEC value indicating better chemotactic activity, IL-8 fused either with CD40L derivative protein or IL-2 (IL8CD40L$_{Exc}$ or IL8IL2) exhibited a smaller MEC value and a better chemotactic activity. The fusion proteins have a better chemotactic activity than IL-8 (see Table 1). SDF-1 fused either with CD40L derivative

protein or IL-2 (SDF1CD40L$_{Exc}$ or SDF1IL2) exhibited a smaller MEC value and a better chemotactic activity. The fusion proteins have a better chemotactic activity than SDF-1 (see Table 1). The chemotactic effects are better in higher concentrations of proteins. At the same concentration, the chemotactic extent of IL8CD40L$_{Exc}$ (with the best chemotactic activity) or IL8IL2 (with the second best chemotactic activity) were clearly higher than single IL8 protein (see Figure 4). And at the same concentration, the chemotactic extent of SDF1CD40L$_{Exc}$, SDF1CD40L$_{TNFh}$ or SDF1IL2 were clearly higher than simple mixture or single SDF-1 protein (see Figure 5).

[0058] Table 1: Minimum effective concentrations (MECs) of chemotactic activity

Group	Protein	MEC
A	IL-8	125 nM
B	IL8CD40L$_{Exc}$	62.5 nM
C	IL8IL2	62.5 nM
D	SDF-1	125 nM
E	SDF1CD40L$_{Exc}$	62.5 nM
F	SDF1CD40L$_{TNFh}$	62.5 nM
G	SDF1IL2	62.5 nM

[0059] Example 4: Analysis of activation of macrophages to produce nitric oxide (NO) by CD40L derivative proteins

[0060] Based on CD40L's property of being able to activate macrophages to produce NO, CD40L activities of a chemokine fused with a CD40L derivative protein were assessed. Peripheral blood mononuclear cells were isolated, washed twice with PBS, suspended in RPMI 1640 containing 10% FBS supplemented with 125 ng/ml chicken IL-2 and 4 μg/ml LPS at 2 x 10^6 cells/ml. One ml of cells were added to each well of a 24-well plates. One ml of fresh medium (also supplemented with 125 ng/ml chicken IL-2 and 4 μg/ml LPS) were added after 2 days. After 5 day simulation completed, monocytes differentiated into macrophages. After PBS washing 3 times to remove suspension cells, different concentrations of CD40L derivative proteins or fusion proteins were added, with the culture medium, tagged protein expressed by vectors and culture medium supplemented with 4 μg/ml LPS as negative and positive control groups. After 48 hour culture, 50 μl culture medium were taken and examined for nitrite (from NO) concentration using a commercially available kit (Griess Reagent System; Promega, Madison, WI, USA).

[0061] IL8CD40L$_{Exe}$ fusion protein exhibited a significantly better activity as compared to the group added CD40L$_{Exe}$ alone (5-30 nM, $p < 0.05$; 180 nM, $p < 0.01$). The effects of the fusion protein SDFlCD40L$_{Exc}$ were significantly better than CD40L$_{Exc}$ single protein (5 nM, $p < 0.01$; 30-180 nM, $p < 0.05$). For the combination of SDF-1 and CD40L$_{TNFh}$, similar results were obtained that SDF1CD40L$_{TNFh}$ fusion protein had a significantly better effects than a single CD40LTNFh protein (5 nM and 180 nM, $p < 0.01$; 0.8 nM and 30 nM, $p < 0.05$) (see Figure 6).

[0062] Example 5: Activity of IL2 fusion protein on promoting lymphocyte proliferation

[0063] Based on the activity of IL2 on promoting lymphocyte proliferation, IL-2 activities of a chemokine fused with IL-2 were accessed. In view of that the activity of intracellular acid phosphatase are proportional to cell number, chromogenic substrate p-nitrophenyl phosphate (pNpp) was used. Peripheral blood mononuclear cells were isolated, and then cultured in RPMI 1640 containing 10% FBS supplemented with different concentrations of proteins, 10 μg/ml ConA (positive control group), or 10 nM tagged protein (negative control group) on 96-well plate at 2×10^5/well. After culture for 3 days, the culture was subjected to 3000 rpm centrifugation for 10 minutes, removed the culture medium, 100 μl chromogenic reagent (0.1 M sodium acetate, H 5.5, 0.1% Triton X- 100, and 10 mM pNpp) were added to each well, and incubated at 37 °C for two hours. 10 μl 1 N NaOH were then added to terminate the reaction. Absorbance at a wavelength of 405 nm was read and used to calculate the stimulation index (SI), where SI = OD of experimental group / OD of RPMI 1640 culture only. The results show that at 0.625-160 nM SDF1IL2 fusion proteins exhibited significantly higher activity on proliferation promotion than IL-2 (0.625 nM, $p < 0.05$; 1.25 – 80 nM, $p < 0.001$; 160 nM, $p < 0.01$). These results show that IL-2 activity was significantly improved after fused with a chemokine (see Figure 7).

[0064] Example 6: Fusion protein as adjuvant of Newcastle disease (ND) vaccine adjuvant to promote vaccine-induced immune responses

[0065] IL-2 fusion protein was used as an adjuvant of avian Newcastle disease (ND) vaccine and administered to chickens. After administration of the vaccine, the blood of the chickens was drawn for the culture of lymphocytes, and inactivated Newcastle disease virus (NDV) were added as antigen to perform antigen re-stimulation assay. 10 μg/ml ConA were added to the culture medium as the positive control group. The proliferation of memory lymphocytes that can recognize NDV antigen of each group of chickens was compared. The methods for determining proliferation state are the same as described in

Example 5. Proliferation rate = (OD of test groups / OD of RPMI 1640 culture only) × 100%. Compared with the group vaccinated with ND vaccine only, the groups vaccinated with IL-2 fusion proteins as ND vaccine adjuvant (SDF1IL2 + ND vaccine) had a significantly enhanced proliferation of antigen-specific memory lymphocytes upon antigen re-stimulation (see Figure 8).

[0066] Example 7: Fusion protein as adjuvant of avian infectious bronchitis (IB) vaccine to promote vaccine-induced immune responses

[0067] IL-2 fusion proteins or $CD40L_{Exc}$ fusion proteins were used as an adjuvant of avian infectious bronchitis (IB) vaccine. After administration of the vaccine, the blood of the chickens was drawn for the culture of lymphocytes, and inactivated infectious bronchitis virus (IBV) were added as antigen to perform antigen re-stimulation assay. The proliferation of memory lymphocytes that can recognize IBV antigen of each group of chickens was compared. The methods for determining proliferation state are the same as described in Example 5. Proliferation rate = (OD of test groups / OD of RPMI 1640 culture only) × 100%. Compared with the group vaccinated with IB vaccine only, the groups vaccinated with IL-2 fusion proteins as IB vaccine adjuvant (SDF1IL2 + IB vaccine) ($p < 0.01$) or vaccinated with $CD40L_{Exc}$ fusion proteins as IB vaccine adjuvant (SDF1$CD40L_{Exc}$ + IB vaccine) ($p < 0.05$) had a significantly enhanced proliferation of antigen-specific memory lymphocytes upon antigen re-stimulation (see Figure 9).

[0068] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the appended claims.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A fusion protein, comprising:
a chemokine polypeptide, which is a chemokine or a receptor binding domain thereof; and
a cytokine polypeptide connected to the chemokine polypeptide by a hydrophilic helical peptide linker, the cytokine polypeptide being IL-2, CD40 ligand or a receptor binding domain thereof;
wherein the chemokine polypeptide and the cytokine polypeptide have a common target cell, and the fusion protein has an improved chemokine activity as compared to the chemokine polypeptide, and an improved cytokine activity as compared to the cytokine polypeptide.

2. The fusion protein according to claim 1, wherein the chemokine is selected from the group consisting of a CXC chemokine, a CC chemokine, a C chemokine and a CX_3C chemokine.

3. The fusion protein according to claim 2, wherein the chemokine is a CXC chemokine.

4. The fusion protein according to claim 3, wherein the chemokine is stromal cell-derived factor or IL-8.

5. The fusion protein according to claim 1, wherein the chemokine polypeptide has an amino acid sequence selected from the following: SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, a homolog of any one thereof and an analog of any one thereof.

6. The fusion protein according to claim 1, wherein the cytokine polypeptide has an amino acid sequence selected from the following: SEQ ID NO: 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, a homolog of any one thereof and an analog of any one thereof.

7. The fusion protein according to claim 1, which has an amino acid sequence selected from the following: SEQ ID NO: 40, 42, 44, 46 and 48.

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8. An isolated nucleic acid molecule encoding a fusion protein according to any one of claims 1 to 4.

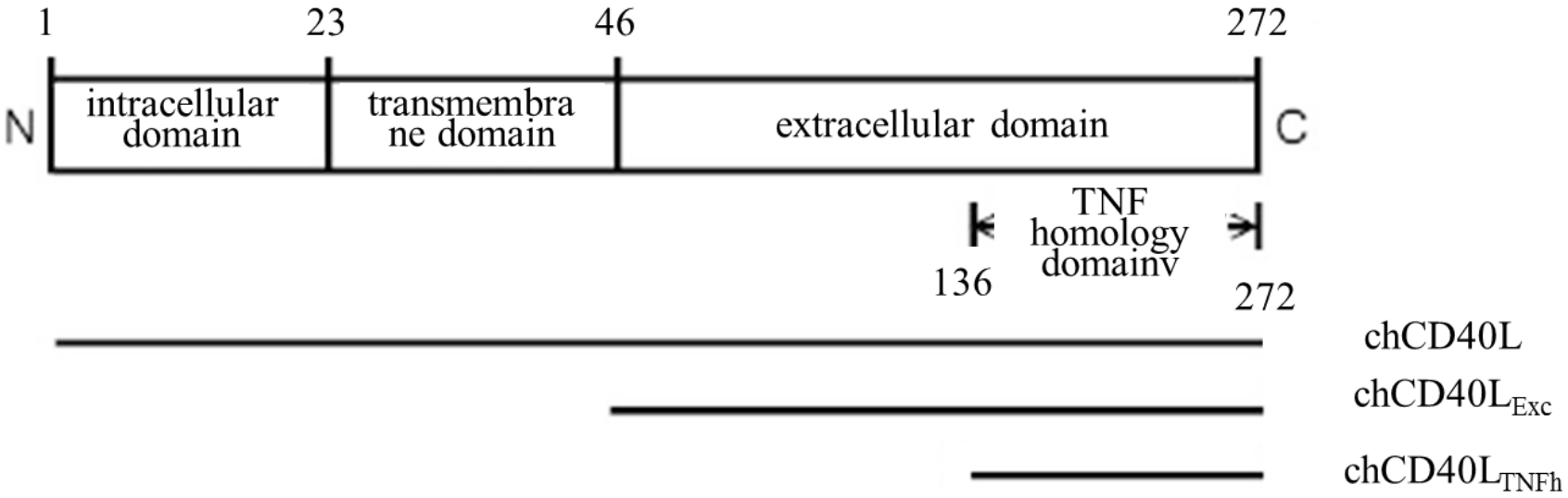
9. The isolated nucleic acid molecule according to claim 8, which comprises a nucleotide sequence encoding a chemokine polypeptide, selected from the following: SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, a homolog of any one thereof and an analog of any one thereof.

10. The isolated nucleic acid molecule according to claim 8, which comprises a nucleotide sequence encoding a chemokine polypeptide, selected from the following: SEQ ID NO: 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, a homolog of any one thereof and an analog of any one thereof.

11. The isolated nucleic acid molecule according to claim 8, which has a sequence selected from one of the following: SEQ ID NO: 39, 41, 43, 45 and 47.

12. An expression vector comprising the nucleic acid molecule according to any one of claims 8 to 11.

13. A host cell comprising a nucleic acid molecule according to any one of claims 8 to 11, optionally comprising the expression vector of claim 12.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transmembra ne domain
extracellular domain
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272
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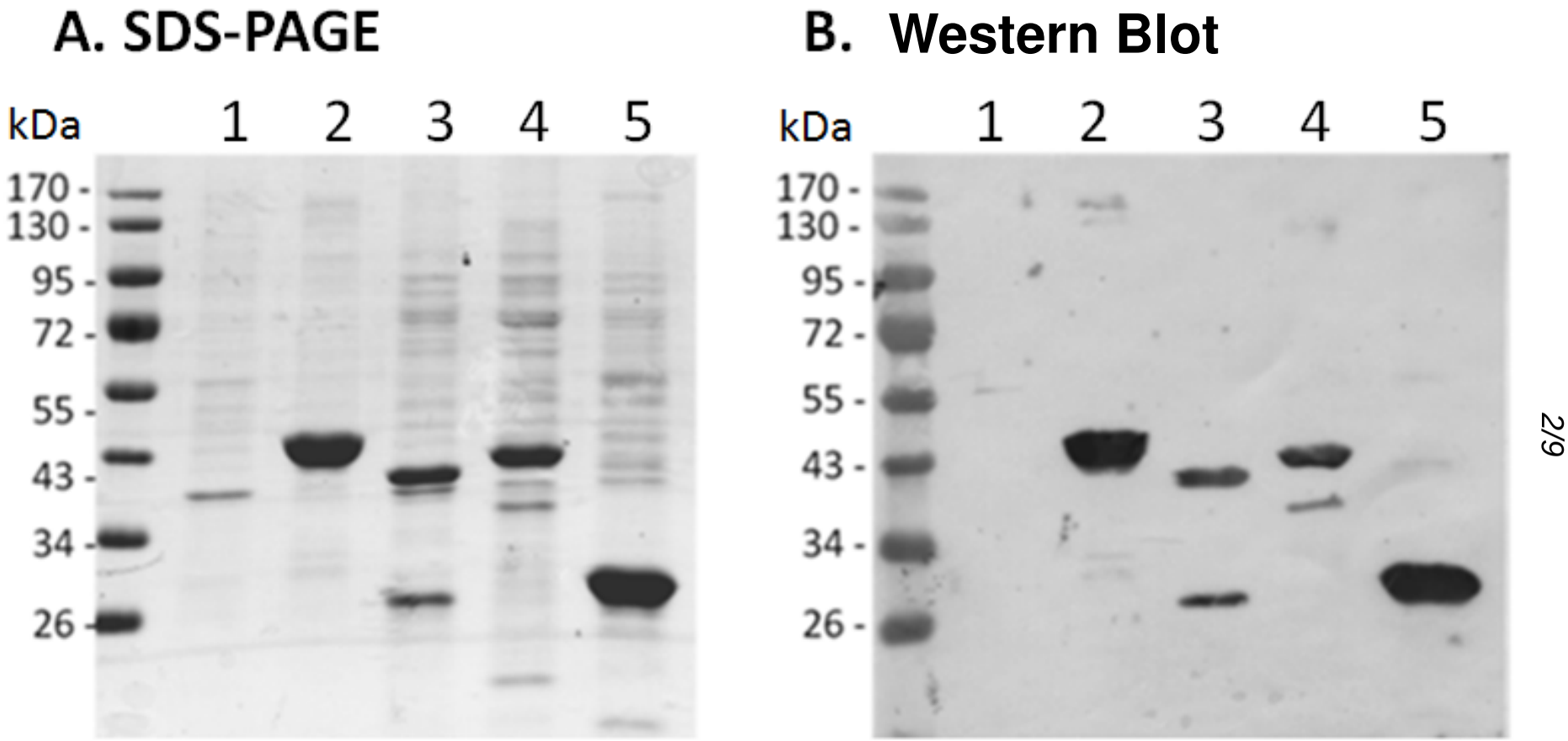
chCD40L$_{Exc}$
chCD40L$_{TNFh}$

Figure 1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55
43
34
26

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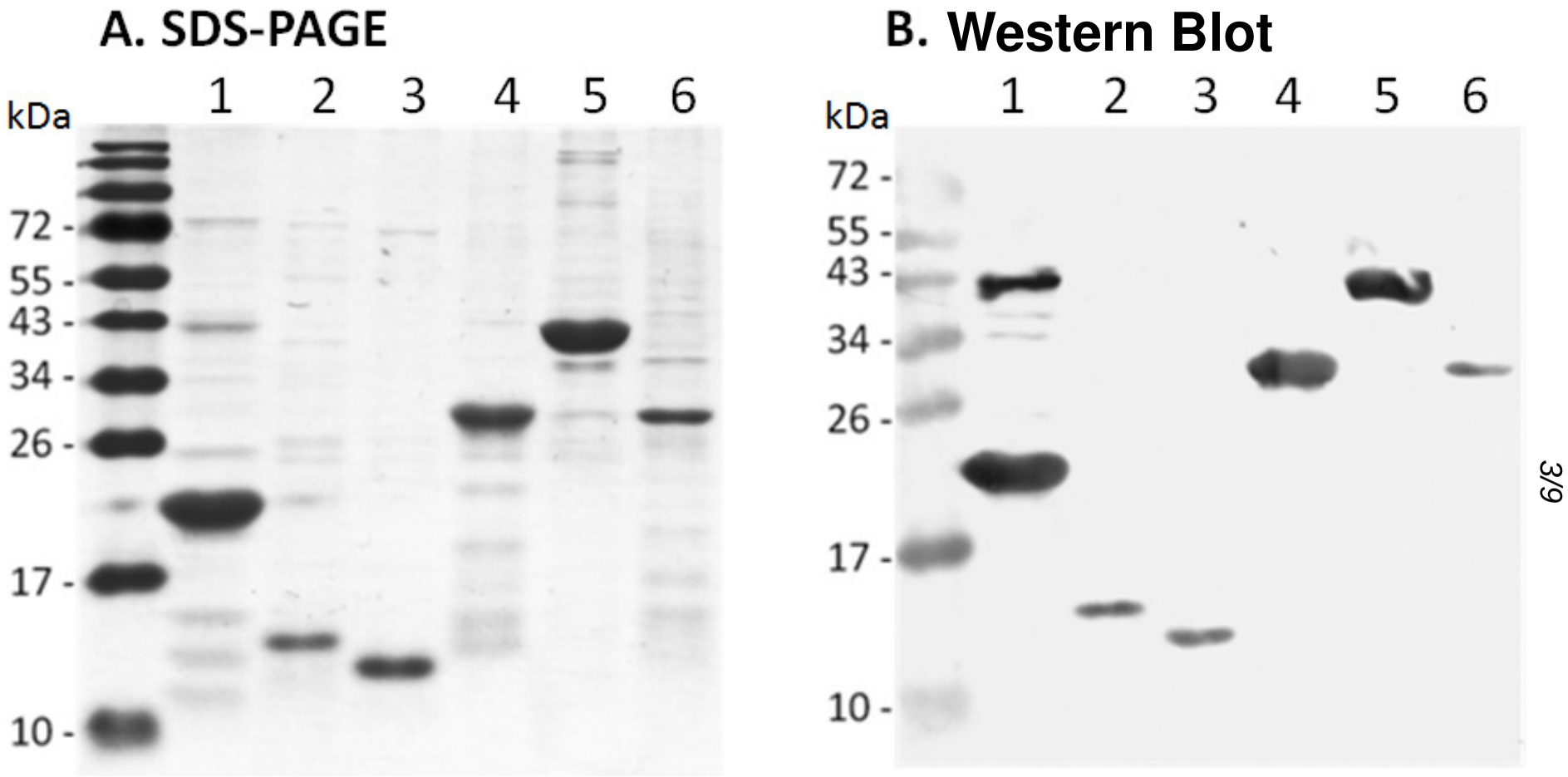
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55 -
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34 -
26 -
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43 -
34 -
26 -
17 -
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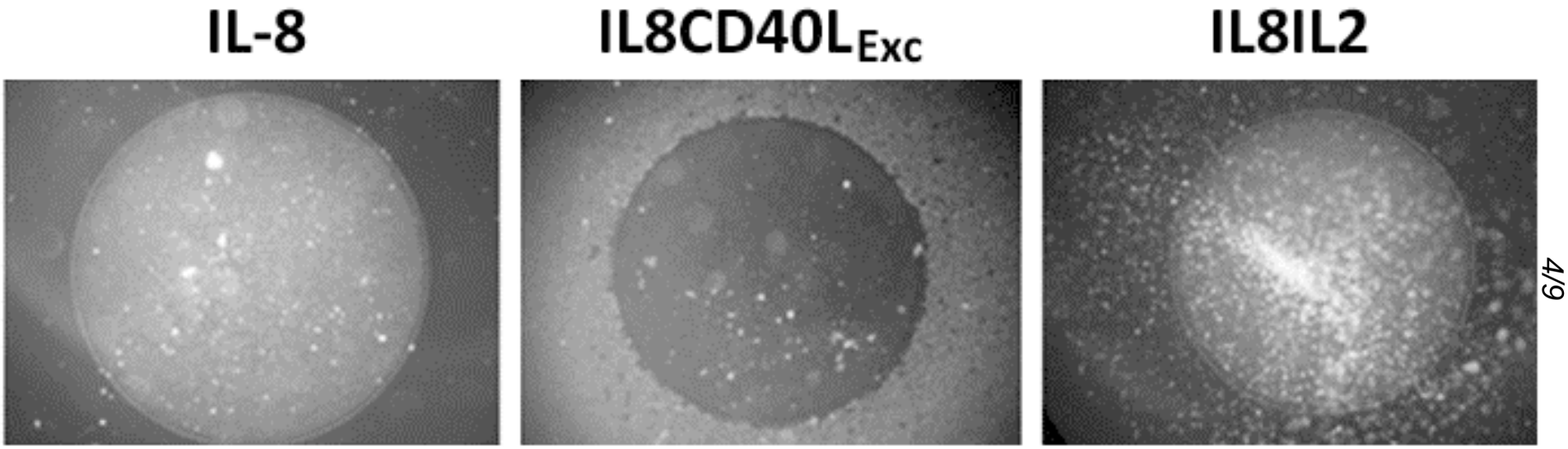

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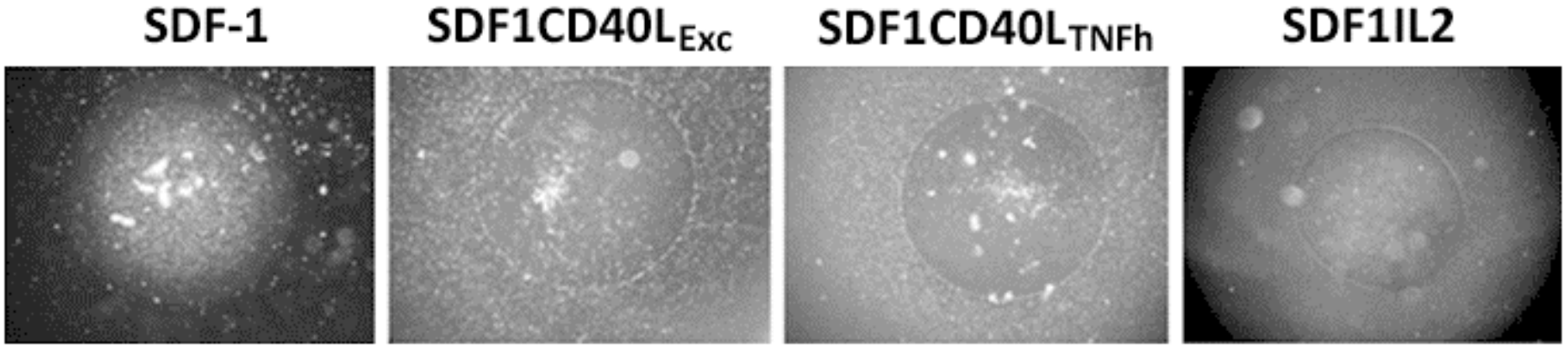

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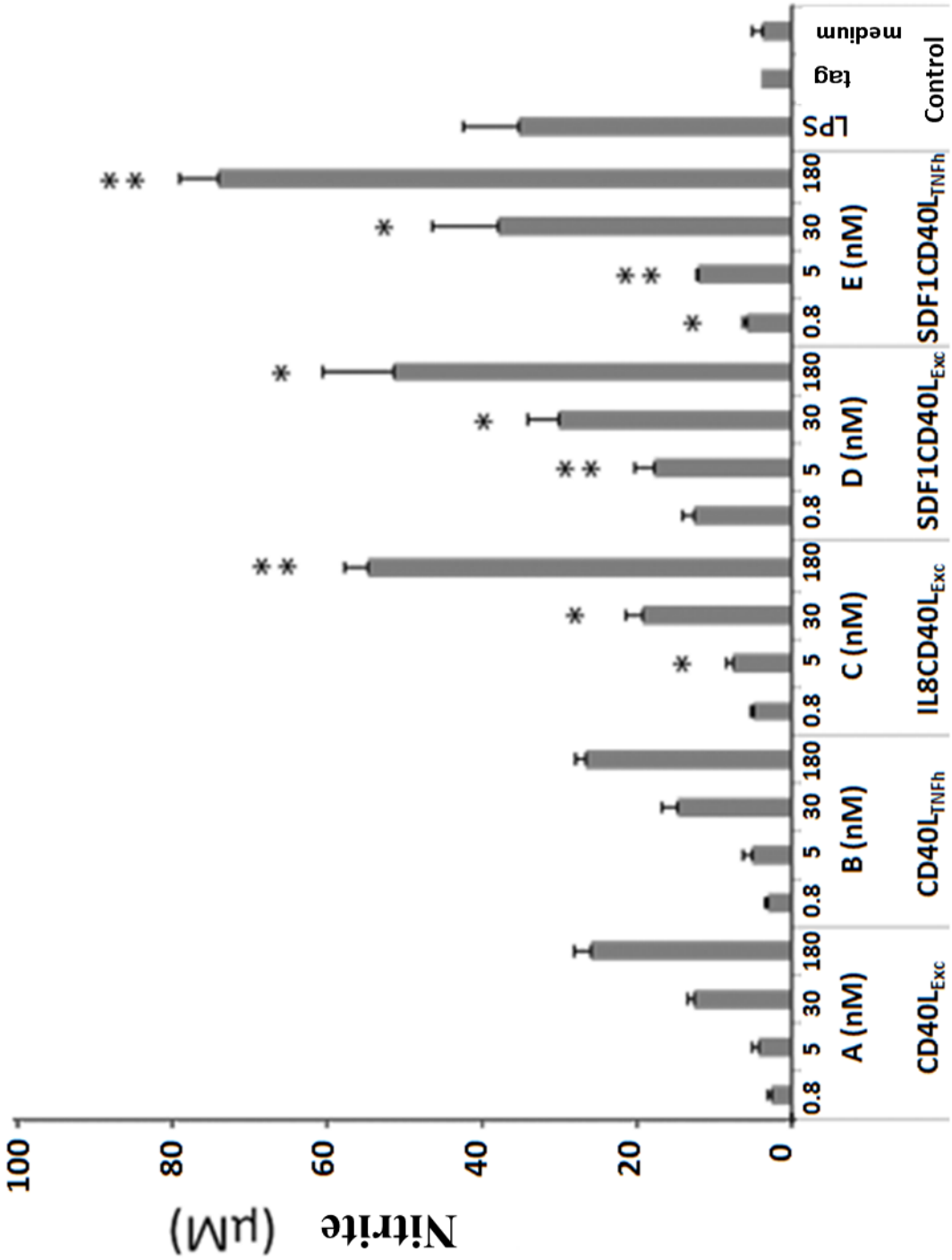
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40
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0
CD40L$_{Exc}$
A (nM)
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5
30
180
CD40L$_{TNFh}$
B (nM)
0.8
5
30
180
IL8CD40L$_{Exc}$
C (nM)
0.8
5
30
180
SDF1CD40L$_{Exc}$
D (nM)
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30
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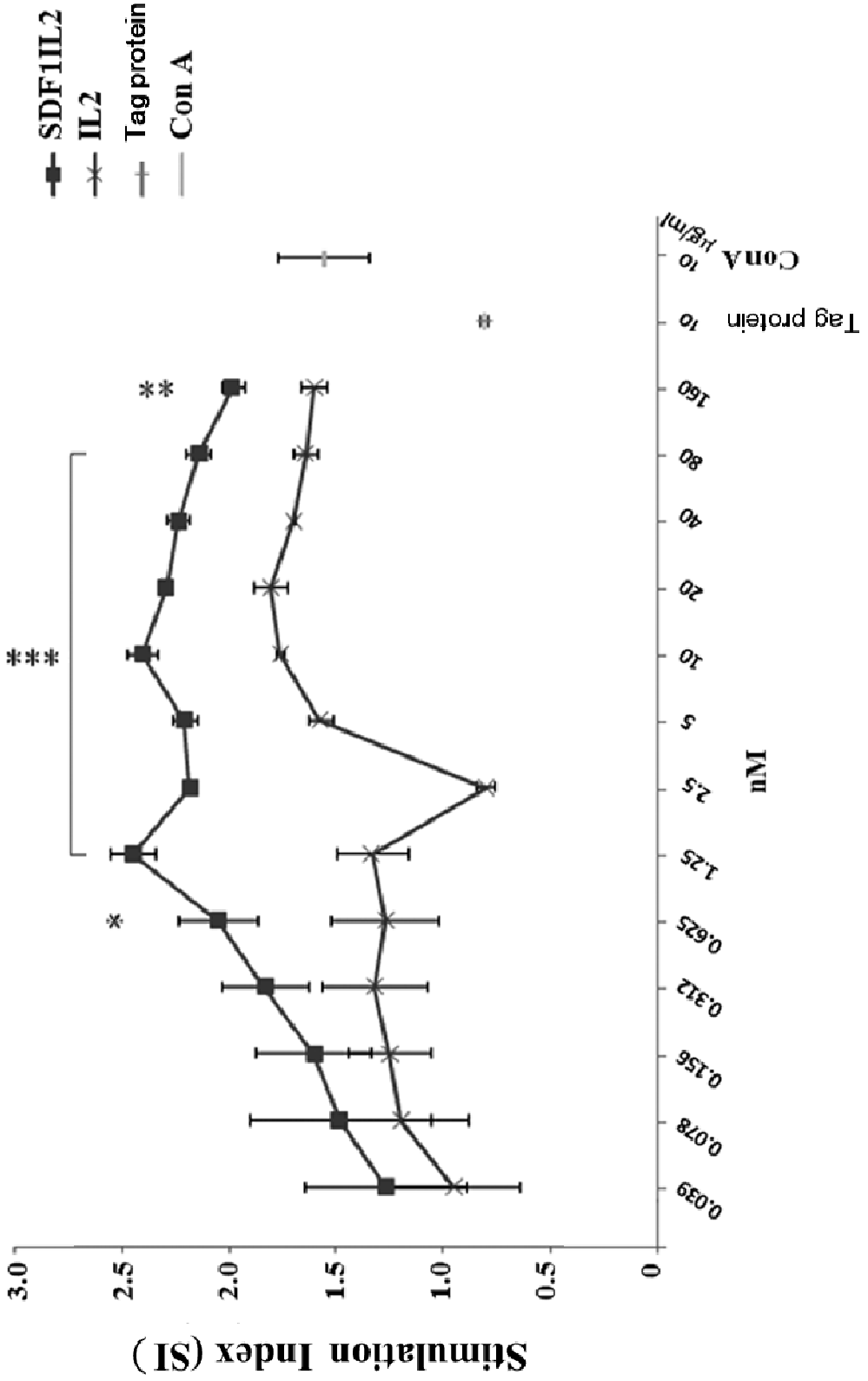
LPS
tag
medium
Control

Figure 6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Con A
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0.078
0.156
0.312
0.625
1.25
2.5
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20
40
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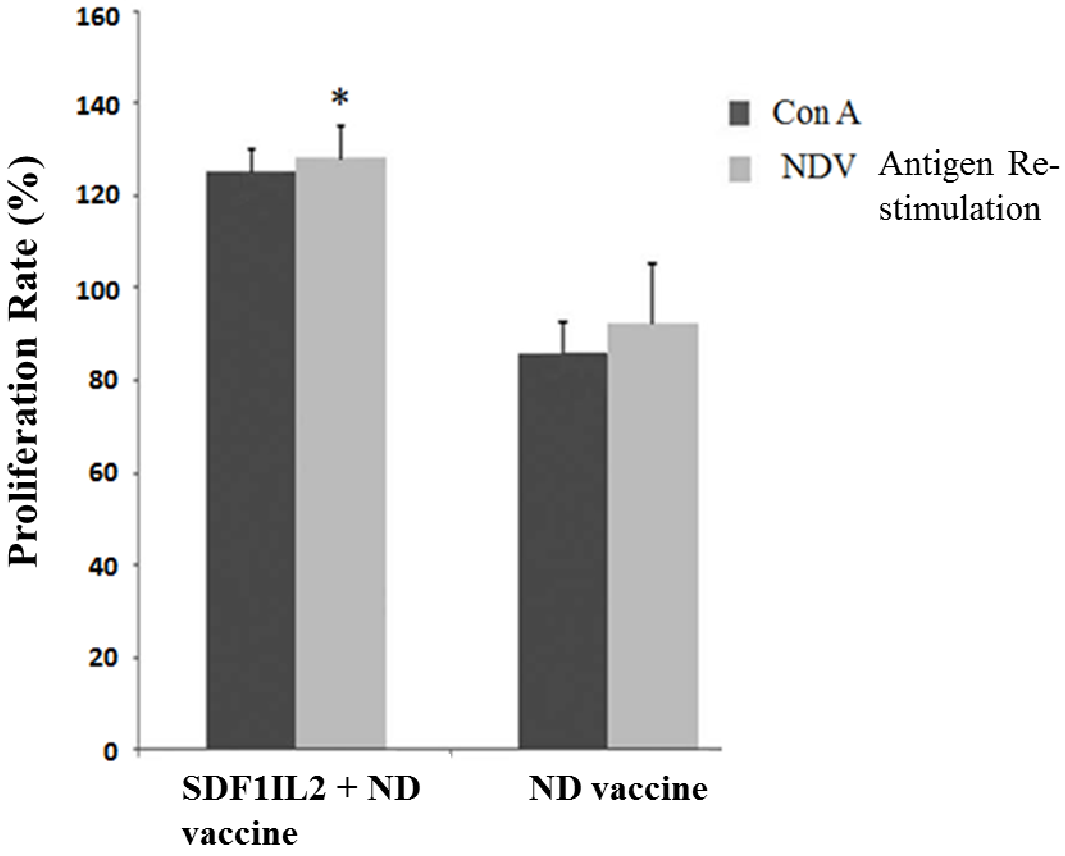
nM
Tag protein 10
ConA 10 μg/ml
*
**
***
#

Figure 7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140
120
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Proliferation Rate (%)
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Con A
NDV Antigen Re-stimulation
SDF1IL2 + ND vaccine
ND vaccine

Figure 8

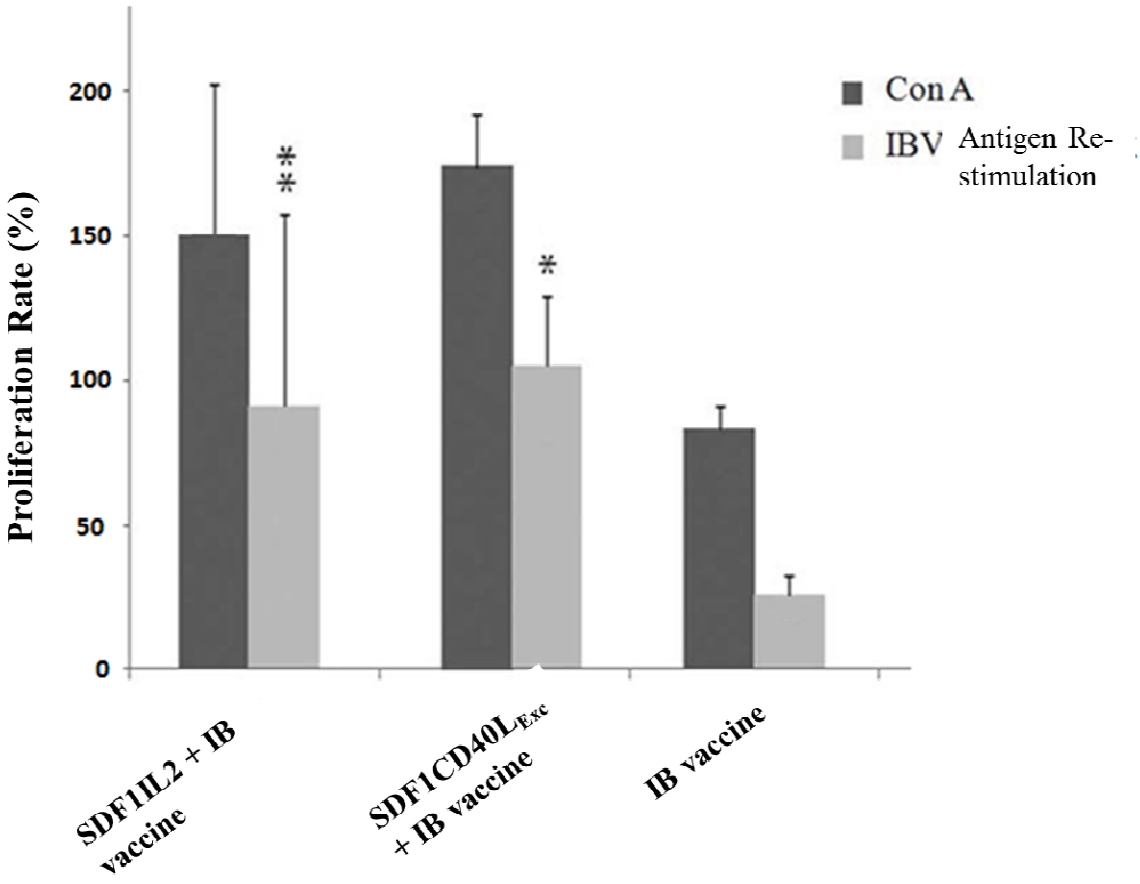
Proliferation Rate (%)
200
150
100
50
0
**
*
Con A
IBV Antigen Re-stimulation
SDF1IL2 + IB vaccine
SDF1CD40L$_{Exc}$ + IB vaccine
IB vaccine

Figure 9

Sequence Listing

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Met Asn Glu Ala Tyr Ser Pro Ala Ala Pro Arg Pro Met Gly Ser Thr
1               5                   10                  15

Ser Pro Ser Thr Met Lys Met Phe Met Cys Phe Leu Ser Val Phe Met
            20                  25                  30

Val Val Gln Thr Ile Gly Thr Val Leu Phe Cys Leu Tyr Leu His Met
        35                  40                  45
```

```
Lys Met Asp Lys Met Glu Glu Val Leu Ser Leu Asn Glu Asp Tyr Ile
    50                  55                  60

Phe Leu Arg Lys Val Gln Lys Cys Gln Thr Gly Glu Asp Gln Lys Ser
65                  70                  75                  80

Thr Leu Leu Asp Cys Glu Lys Val Leu Lys Gly Phe Gln Asp Leu Gln
                85                  90                  95

Cys Lys Asp Arg Thr Ala Ser Glu Glu Leu Pro Lys Phe Glu Met His
            100                 105                 110

Arg Gly His Glu His Pro His Leu Lys Ser Arg Asn Glu Thr Ser Val
        115                 120                 125

Ala Glu Glu Lys Arg Gln Pro Ile Ala Thr His Leu Ala Gly Val Lys
    130                 135                 140

Ser Asn Thr Thr Val Arg Val Leu Lys Trp Met Thr Thr Ser Tyr Ala
145                 150                 155                 160

Pro Thr Ser Ser Leu Ile Ser Tyr His Glu Gly Lys Leu Lys Val Glu
                165                 170                 175

Lys Ala Gly Leu Tyr Tyr Ile Tyr Ser Gln Val Ser Phe Cys Thr Lys
            180                 185                 190

Ala Ala Ala Ser Ala Pro Phe Thr Leu Tyr Ile Tyr Leu Tyr Leu Pro
        195                 200                 205

Met Glu Glu Asp Arg Leu Leu Met Lys Gly Leu Asp Thr His Ser Thr
    210                 215                 220

Ser Thr Ala Leu Cys Glu Leu Gln Ser Ile Arg Glu Gly Gly Val Phe
225                 230                 235                 240

Glu Leu Arg Gln Gly Asp Met Val Phe Val Asn Val Thr Asp Ser Thr
                245                 250                 255

Ala Val Asn Val Asn Pro Gly Asn Thr Tyr Phe Gly Met Phe Lys Leu
            260                 265                 270
```

<210> 21
<211> 786
<212> DNA

<213> Sus scrofa

<400> 21
atgatcgaaa cgtacagcca accttcgccc cgctctgtgg ccgctggacc acccgtcagt 60

atgaaaatct ttatgtattt acttactgtt tttcttatca cccagatgat tgggtcagca 120

ctttttgcag cgtaccttca cagaagattg gacaagatag aagatgaaag gaatcttcat 180

gaagattttg tgttcataaa aacgatacag agatgcaagc aaggagaggg gtccttatcc 240

ttattgaact gtgaggaaat cagaagccag tttgaagacc tggtcaaggg tataatgcaa 300

agcaaagaag tgaagaagaa agaaaaaagc tttgaaatgc acaaaggcga tcaggatcct 360

caaattgcgg cacatgtcat aagcgaggcc agtagtaaaa cagcatctgt cctgcagtgg 420

gcccccaaag ggtactacac cctcagcacc aacttggtga ccctggaaaa cgggagacag 480

ctggccgtca aaagacaagg aatctattac atctacgccc aagtcacctt ctgctccaac 540

cgggacgccg cgggtcaagc tcccttcata gccagcctct gcctgaggtc cccaagcggg 600

tcggagagaa tcttactccg cgcggccaac acccacagtt cctccaagcc ctgcgggcag 660

caatccattc acttgggcgg agtcttcgag ttgcaacccg gcgcttcggt gttcgtcaac 720

gtgactgatc caagccaagt gagccacggg accggcttca cgtcttttgg cctcctcaaa 780

ctctga 786

<210> 22
<211> 261
<212> PRT
<213> Sus scrofa

<400> 22

Met Ile Glu Thr Tyr Ser Gln Pro Ser Pro Arg Ser Val Ala Ala Gly
1 5 10 15

Pro Pro Val Ser Met Lys Ile Phe Met Tyr Leu Leu Thr Val Phe Leu
20 25 30

Ile Thr Gln Met Ile Gly Ser Ala Leu Phe Ala Ala Tyr Leu His Arg
35 40 45

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
50 55 60

Phe Ile Lys Thr Ile Gln Arg Cys Lys Gln Gly Glu Gly Ser Leu Ser
65 70 75 80

Leu Leu Asn Cys Glu Glu Ile Arg Ser Gln Phe Glu Asp Leu Val Lys
85 90 95

```
Gly Ile Met Gln Ser Lys Glu Val Lys Lys Lys Glu Lys Ser Phe Glu
            100                 105                 110

Met His Lys Gly Asp Gln Asp Pro Gln Ile Ala Ala His Val Ile Ser
        115                 120                 125

Glu Ala Ser Ser Lys Thr Ala Ser Val Leu Gln Trp Ala Pro Lys Gly
    130                 135                 140

Tyr Tyr Thr Leu Ser Thr Asn Leu Val Thr Leu Glu Asn Gly Arg Gln
145                 150                 155                 160

Leu Ala Val Lys Arg Gln Gly Ile Tyr Tyr Ile Tyr Ala Gln Val Thr
                165                 170                 175

Phe Cys Ser Asn Arg Asp Ala Ala Gly Gln Ala Pro Phe Ile Ala Ser
            180                 185                 190

Leu Cys Leu Arg Ser Pro Ser Gly Ser Glu Arg Ile Leu Leu Arg Ala
        195                 200                 205

Ala Asn Thr His Ser Ser Ser Lys Pro Cys Gly Gln Gln Ser Ile His
    210                 215                 220

Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
225                 230                 235                 240

Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe
                245                 250                 255

Gly Leu Leu Lys Leu
            260
```

<210> 23
<211> 786
<212> DNA
<213> Bos taurus

<400> 23
atgatcgaaa catacagtca accttctccc cgctccgtgg ccactggacc acctgtcagt 60

atgaaaattt ttatgtattt acttacagtt tttcttatca cccagatgat tgggtcagcg 120

ctttttgctg tgtatcttca cagacgattg gacaagatag aagacgaaag gaatcttcat 180

gaagattttg tgttcatgaa aacgatacag agatgcaata aaggagaggg gtccttatcc 240

ttactgaact gtgaggaaat tagaagccgg tttgaagact tggtcaagga tataatgcaa 300

aacaaagaag taaagaagaa agaaaaaaac tttgaaatgc acaagggtga tcaggagcct 360

cagatagcgg cacatgtcat cagtgaggcc agtagtaaaa caacctctgt tctccagtgg 420

gcccccaaag gatactacac cctaagcaac aacctggtaa ccctcgaaaa cgggaaacag 480

ctggccgtga aaagacaagg attctattac atctacaccc aagtcacctt ctgttccaat 540

cgggaaactt tgagtcaagc tccatttata gccagcctct gcctgaagtc cccaagtgga 600

tcagagagaa tcttactgag agctgcaaac acccacagtt cttccaaacc atgcgggcag 660

caatccattc acttaggagg agtctttgaa ttgcaatcgg gtgcttcggt gtttgtcaat 720

gtgactgatc caagtcaagt gagccacggg acgggcttca catcatttgg cttactcaaa 780

ctctga 786

<210> 24
<211> 261
<212> PRT
<213> Bos taurus

<400> 24

Met Ile Glu Thr Tyr Ser Gln Pro Ser Pro Arg Ser Val Ala Thr Gly
1 5 10 15

Pro Pro Val Ser Met Lys Ile Phe Met Tyr Leu Leu Thr Val Phe Leu
20 25 30

Ile Thr Gln Met Ile Gly Ser Ala Leu Phe Ala Val Tyr Leu His Arg
35 40 45

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
50 55 60

Phe Met Lys Thr Ile Gln Arg Cys Asn Lys Gly Glu Gly Ser Leu Ser
65 70 75 80

Leu Leu Asn Cys Glu Glu Ile Arg Ser Arg Phe Glu Asp Leu Val Lys
85 90 95

Asp Ile Met Gln Asn Lys Glu Val Lys Lys Lys Glu Lys Asn Phe Glu
100 105 110

Met His Lys Gly Asp Gln Glu Pro Gln Ile Ala Ala His Val Ile Ser
115 120 125

Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Pro Lys Gly
130 135 140

```
Tyr Tyr Thr Leu Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
145                 150                 155                 160

Leu Ala Val Lys Arg Gln Gly Phe Tyr Tyr Ile Tyr Thr Gln Val Thr
                165                 170                 175

Phe Cys Ser Asn Arg Glu Thr Leu Ser Gln Ala Pro Phe Ile Ala Ser
            180                 185                 190

Leu Cys Leu Lys Ser Pro Ser Gly Ser Glu Arg Ile Leu Leu Arg Ala
        195                 200                 205

Ala Asn Thr His Ser Ser Ser Lys Pro Cys Gly Gln Gln Ser Ile His
    210                 215                 220

Leu Gly Gly Val Phe Glu Leu Gln Ser Gly Ala Ser Val Phe Val Asn
225                 230                 235                 240

Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe
                245                 250                 255

Gly Leu Leu Lys Leu
            260
```

<210> 25
<211> 786
<212> DNA
<213> Homo sapiens

<400> 25
atgatcgaaa catacaacca aacttctccc cgatctgcgg ccactggact gcccatcagc 60

atgaaaattt ttatgtattt acttactgtt tttcttatca cccagatgat tgggtcagca 120

ctttttgctg tgtatcttca tagaaggttg gacaagatag aagatgaaag gaatcttcat 180

gaagattttg tattcatgaa aacgatacag agatgcaaca caggagaaag atccttatcc 240

ttactgaact gtgaggagat taaaagccag tttgaaggct ttgtgaagga tataatgtta 300

aacaaagagg agacgaagaa agaaaacagc tttgaaatgc aaaaaggtga tcagaatcct 360

caaattgcgg cacatgtcat aagtgaggcc agcagtaaaa caacatctgt gttacagtgg 420

gctgaaaaag gatactacac catgagcaac aacttggtaa ccctggaaaa tgggaaacag 480

ctgaccgtta aaagacaagg actctattat atctatgccc aagtcacctt ctgttccaat 540

cgggaagctt cgagtcaagc tccatttata gccagcctct gcctaaagtc ccccggtaga 600

ttcgagagaa tcttactcag agctgcaaat acccacagtt ccgccaaacc ttgcgggcaa 660

caatccattc acttgggagg agtatttgaa ttgcaaccag gtgcttcggt gtttgtcaat 720

gtgactgatc caagccaagt gagccatggc actggcttca cgtcctttgg cttactcaaa 780

ctctga 786

<210> 26
<211> 261
<212> PRT
<213> Homo sapiens

<400> 26

Met Ile Glu Thr Tyr Asn Gln Thr Ser Pro Arg Ser Ala Ala Thr Gly
1 5 10 15

Leu Pro Ile Ser Met Lys Ile Phe Met Tyr Leu Leu Thr Val Phe Leu
20 25 30

Ile Thr Gln Met Ile Gly Ser Ala Leu Phe Ala Val Tyr Leu His Arg
35 40 45

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
50 55 60

Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
65 70 75 80

Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys
85 90 95

Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu
100 105 110

Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser
115 120 125

Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
130 135 140

Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
145 150 155 160

Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
165 170 175

Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser
180 185 190

```
Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala
        195                 200                 205

Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His
    210                 215                 220

Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
225                 230                 235                 240

Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe
                245                 250                 255

Gly Leu Leu Lys Leu
            260
```

<210> 27
<211> 783
<212> DNA
<213> Mus musculus

<400> 27

```
atgatagaaa catacagcca accttccccc agatccgtgg caactggact tccagcgagc       60

atgaagattt ttatgtattt acttactgtt ttccttatca cccaaatgat tggatctgtg      120

ctttttgctg tgtatcttca tagaagattg gataaggtcg aagaggaagt aaaccttcat      180

gaagattttg tattcataaa aaagctaaag agatgcaaca aaggagaagg atctttatcc      240

ttgctgaact gtgaggagat gagaaggcaa tttgaagacc ttgtcaagga tataacgtta      300

aacaaagaag agaaaaaaga aaacagcttt gaaatgcaaa gaggtgatga ggatcctcaa      360

attgcagcac acgttgtaag cgaagccaac agtaatgcag catccgttct acagtgggcc      420

aagaaaggat attataccat gaaaagcaac ttggtaatgc ttgaaaatgg gaaacagctg      480

acggttaaaa gagaaggact ctattatgtc tacactcaag tcaccttctg ctctaatcgg      540

gagccttcga gtcaacgccc attcatcgtc ggcctctggc tgaagcccag cagtggatct      600

gagagaatct tactcaaggc ggcaaatacc cacagttcct cccagctttg cgagcagcag      660

tctgttcact tgggcggagt gtttgaatta caagctggtg cttctgtgtt tgtcaacgtg      720

actgaagcaa gccaagtgat ccacagagtt ggcttctcat cttttggctt actcaaactc      780

tga                                                                    783
```

<210> 28
<211> 260
<212> PRT
<213> Mus musculus

<400> 28

```
Met Ile Glu Thr Tyr Ser Gln Pro Ser Pro Arg Ser Val Ala Thr Gly
1               5                  10                  15

Leu Pro Ala Ser Met Lys Ile Phe Met Tyr Leu Leu Thr Val Phe Leu
            20                  25                  30

Ile Thr Gln Met Ile Gly Ser Val Leu Phe Ala Val Tyr Leu His Arg
        35                  40                  45

Arg Leu Asp Lys Val Glu Glu Glu Val Asn Leu His Glu Asp Phe Val
    50                  55                  60

Phe Ile Lys Lys Leu Lys Arg Cys Asn Lys Gly Glu Gly Ser Leu Ser
65                  70                  75                  80

Leu Leu Asn Cys Glu Glu Met Arg Arg Gln Phe Glu Asp Leu Val Lys
                85                  90                  95

Asp Ile Thr Leu Asn Lys Glu Glu Lys Lys Glu Asn Ser Phe Glu Met
            100                 105                 110

Gln Arg Gly Asp Glu Asp Pro Gln Ile Ala Ala His Val Val Ser Glu
        115                 120                 125

Ala Asn Ser Asn Ala Ala Ser Val Leu Gln Trp Ala Lys Lys Gly Tyr
    130                 135                 140

Tyr Thr Met Lys Ser Asn Leu Val Met Leu Glu Asn Gly Lys Gln Leu
145                 150                 155                 160

Thr Val Lys Arg Glu Gly Leu Tyr Tyr Val Tyr Thr Gln Val Thr Phe
                165                 170                 175

Cys Ser Asn Arg Glu Pro Ser Ser Gln Arg Pro Phe Ile Val Gly Leu
            180                 185                 190

Trp Leu Lys Pro Ser Ser Gly Ser Glu Arg Ile Leu Leu Lys Ala Ala
        195                 200                 205

Asn Thr His Ser Ser Ser Gln Leu Cys Glu Gln Gln Ser Val His Leu
    210                 215                 220

Gly Gly Val Phe Glu Leu Gln Ala Gly Ala Ser Val Phe Val Asn Val
225                 230                 235                 240
```

```
Thr Glu Ala Ser Gln Val Ile His Arg Val Gly Phe Ser Ser Phe Gly
                245                 250                 255


Leu Leu Lys Leu
            260
```

<210> 29
<211> 429
<212> DNA
<213> Gallus gallus

<400> 29

```
atgatgtgca aagtactgat ctttggctgt atttcggtag caatgctaat gactacagct       60

tatggagcat ctctatcatc agcaaaaagg aaacctcttc aaacattaat aaaggattta      120

gaaatattgg aaaatatcaa gaacattcat ctcgagctct acacaccaac tgagacccag      180

gagtgcaccc agcaaactct gcagtgttac ctgggagaag tggttactct gaagaaagaa      240

actgaagatg acactgaaat taaagaagaa tttgtaactg ctattcaaaa tatcgataag      300

aacctcaaga gtcttacggg tctaaatcac accggaagtg aatgcaaggt ctgtgaagct      360

aacaacaaga aaaaatttcc tgattttctc catgaactga ccaactttgt gagatatctg      420

caaaaataa                                                              429
```

<210> 30
<211> 142
<212> PRT
<213> Gallus gallus

<400> 30

```
Met Met Cys Lys Val Leu Ile Phe Gly Cys Ile Ser Val Ala Met Leu
1               5                   10                  15


Met Thr Thr Ala Tyr Gly Ala Ser Leu Ser Ser Ala Lys Arg Lys Pro
            20                  25                  30


Leu Gln Thr Leu Ile Lys Asp Leu Glu Ile Leu Glu Asn Ile Lys Asn
        35                  40                  45


Ile His Leu Glu Leu Tyr Thr Pro Thr Glu Thr Gln Glu Cys Thr Gln
    50                  55                  60


Gln Thr Leu Gln Cys Tyr Leu Gly Glu Val Val Thr Leu Lys Lys Glu
65                  70                  75                  80


Thr Glu Asp Asp Thr Glu Ile Lys Glu Glu Phe Val Thr Ala Ile Gln
```

85 90 95

Asn Ile Asp Lys Asn Leu Lys Ser Leu Thr Gly Leu Asn His Thr Gly
100 105 110

Ser Glu Cys Lys Val Cys Glu Ala Asn Asn Lys Lys Lys Phe Pro Asp
115 120 125

Phe Leu His Glu Leu Thr Asn Phe Val Arg Tyr Leu Gln Lys
130 135 140

<210> 31
<211> 465
<212> DNA
<213> Sus scrofa

<400> 31
atgtataaga tgcagctctt gtgttgcatt gcactaaccc ttgcactcat ggcaaacggt 60

gcacctactt caagctctac aaagaacaca aagaaacaac tggagccatt gctgctggat 120

ttacagttgc ttttgaagga agttaagaat tacgagaatg ctgatctctc caggatgctc 180

acatttaaat tttacatgcc caagcaggct acagaattga aacaccttca gtgtttagta 240

gaagaactca aagctctgga gggagtgcta aatttaggtc aaagcaaaaa ctctgactca 300

gcaaatatca aggaatcaat gaacaatatc aacgtaacag ttttggaact aaagggatct 360

gaaacaagtt tcgaatgtga atatgatgat gagacagtaa ctgctgttga atttctgaac 420

aaatggatta ccttttgtca aagcatctac tcaacactga cttga 465

<210> 32
<211> 154
<212> PRT
<213> Sus scrofa

<400> 32

Met Tyr Lys Met Gln Leu Leu Cys Cys Ile Ala Leu Thr Leu Ala Leu
1 5 10 15

Met Ala Asn Gly Ala Pro Thr Ser Ser Ser Thr Lys Asn Thr Lys Lys
20 25 30

Gln Leu Glu Pro Leu Leu Leu Asp Leu Gln Leu Leu Leu Lys Glu Val
35 40 45

Lys Asn Tyr Glu Asn Ala Asp Leu Ser Arg Met Leu Thr Phe Lys Phe
50 55 60

```
Tyr Met Pro Lys Gln Ala Thr Glu Leu Lys His Leu Gln Cys Leu Val
65                  70                  75                  80

Glu Glu Leu Lys Ala Leu Glu Gly Val Leu Asn Leu Gly Gln Ser Lys
                85                  90                  95

Asn Ser Asp Ser Ala Asn Ile Lys Glu Ser Met Asn Asn Ile Asn Val
            100                 105                 110

Thr Val Leu Glu Leu Lys Gly Ser Glu Thr Ser Phe Glu Cys Glu Tyr
        115                 120                 125

Asp Asp Glu Thr Val Thr Ala Val Glu Phe Leu Asn Lys Trp Ile Thr
    130                 135                 140

Phe Cys Gln Ser Ile Tyr Ser Thr Leu Thr
145                 150
```

<210> 33
<211> 405
<212> DNA
<213> Bos taurus

<400> 33

```
gcacctactt caagctctac ggggaacaca atgaaagaag tgaagtcatt gctgctggat       60

ttacagttgc ttttggagaa agttaaaaat cctgagaacc tcaagctctc caggatgcat      120

acatttgact tttacgcgcc caaggttaac gctacagaat tgaaacatct taagtgttta      180

ctagaagaac tcaaacttct agaggaagtg ctaaatttag ctccaagcaa aaacttgaac      240

cccagagaga tcaaggattc aatggacaat atcaagagaa tcgttttgga actacaggga      300

tctgaaacaa gattcacatg tgaatatgat gatgcaacag taaacgctgt agaatttctg      360

aacaaatgga ttacctttg tcaaagcatc tactcaacaa tgact                       405
```

<210> 34
<211> 135
<212> PRT
<213> Bos taurus

<400> 34

```
Ala Pro Thr Ser Ser Ser Thr Gly Asn Thr Met Lys Glu Val Lys Ser
1               5                   10                  15

Leu Leu Leu Asp Leu Gln Leu Leu Leu Glu Lys Val Lys Asn Pro Glu
            20                  25                  30

Asn Leu Lys Leu Ser Arg Met His Thr Phe Asp Phe Tyr Ala Pro Lys
```

```
        35                  40                  45

Val Asn Ala Thr Glu Leu Lys His Leu Lys Cys Leu Leu Glu Glu Leu
    50                  55                  60

Lys Leu Leu Glu Glu Val Leu Asn Leu Ala Pro Ser Lys Asn Leu Asn
65                  70                  75                  80

Pro Arg Glu Ile Lys Asp Ser Met Asp Asn Ile Lys Arg Ile Val Leu
                85                  90                  95

Glu Leu Gln Gly Ser Glu Thr Arg Phe Thr Cys Glu Tyr Asp Asp Ala
            100                 105                 110

Thr Val Asn Ala Val Glu Phe Leu Asn Lys Trp Ile Thr Phe Cys Gln
        115                 120                 125

Ser Ile Tyr Ser Thr Met Thr
    130                 135
```

<210> 35
<211> 462
<212> DNA
<213> Homo sapiens

<400> 35

```
atgtacagga tgcaactcct gtcttgcatt gcactaagtc ttgcacttgt cacaaacagt       60

gcacctactt caagttctac aaagaaaaca cagctacaac tggagcattt actgctggat      120

ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc      180

acatttaagt tttacatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa      240

gaagaactca aacctctgga ggaagtgcta aatttagctc aaagcaaaaa ctttcactta      300

agacccaggg acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa      360

acaacattca tgtgtgaata tgctgatgag acagcaacca ttgtagaatt tctgaacaga      420

tggattacct tttgtcaaag catcatctca acactgactt ga                         462
```

<210> 36
<211> 153
<212> PRT
<213> Homo sapiens

<400> 36

```
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1               5                   10                  15
```

```
Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
            20                  25                  30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
        35                  40                  45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
    50                  55                  60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65                  70                  75                  80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
                85                  90                  95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            100                 105                 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
        115                 120                 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu Thr
145                 150
```

<210> 37
<211> 500
<212> DNA
<213> Mus musculus

<400> 37

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atgcagctcg catcctgtgt cacattgaca cttgtgctcc ttgtcaacag cgcacccact       60

tcaagctcca cttcaagctc tacagcggaa gcacagcagc agcagcagca gcagcagcag      120

cagcagcagc acctggagca gctgttgatg gacctacagg agctcctgag caggatggag      180

aattacagga acctgaaact ccccaggatg ctcaccttca aattttactt gcccaagcag      240

gccacagaat tgaaagatct tcagtgccta gaagatgaac ttggacctct gcggcatgtt      300

ctggatttga ctcaaagcaa aagctttcaa ttggaagatg ctgagaattt catcagcaat      360

atcagagtaa ctgttgtaaa actaaagggc tctgacaaca catttgagtg ccaattcgat      420

gatgagtcag caactgtggt ggactttctg aggagatgga tagccttctg tcaaagcatc      480

atctcaacaa gccctcaata                                                  500
```

<210> 38
<211> 169
<212> PRT
<213> Mus musculus

<400> 38

Met Tyr Ser Met Gln Leu Ala Ser Cys Val Thr Leu Thr Leu Val Leu
1 5 10 15

Leu Val Asn Ser Ala Pro Thr Ser Ser Ser Thr Ser Ser Ser Thr Ala
20 25 30

Glu Ala Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln His Leu
35 40 45

Glu Gln Leu Leu Met Asp Leu Gln Glu Leu Leu Ser Arg Met Glu Asn
50 55 60

Tyr Arg Asn Leu Lys Leu Pro Arg Met Leu Thr Phe Lys Phe Tyr Leu
65 70 75 80

Pro Lys Gln Ala Thr Glu Leu Lys Asp Leu Gln Cys Leu Glu Asp Glu
85 90 95

Leu Gly Pro Leu Arg His Val Leu Asp Leu Thr Gln Ser Lys Ser Phe
100 105 110

Gln Leu Glu Asp Ala Glu Asn Phe Ile Ser Asn Ile Arg Val Thr Val
115 120 125

Val Lys Leu Lys Gly Ser Asp Asn Thr Phe Glu Cys Gln Phe Asp Asp
130 135 140

Glu Ser Ala Thr Val Val Asp Phe Leu Arg Arg Trp Ile Ala Phe Cys
145 150 155 160

Gln Ser Ile Ile Ser Thr Ser Pro Gln
165

<210> 39
<211> 672
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence of fusion protein

<400> 39
gctctgtcgc aaggtaggac gctggtaaag atggggaatg agctgcggtg ccagtgcatt 60

agcactcatt ctaagttcat ccaccctaaa tccattcaag atgtgaagct gacgccaagc 120

ggcccccact gcaagaatgt tgaaatcata gctactctaa aggatggaag agaggtgtgc 180

ttgaacccca ctgctccctg ggtacagctg atcgtaaagg cacttatggc caaggctcag 240

ctcaattctg atgcaccact ggaagctgcg gcaaaagagg cagctgcgaa ggaagcggca 300

gcgaaagcat ctctatcatc agcgaaaagg aaacctcttc aaacattaat aaaggattta 360

gaaatattgg aaaatatcaa gaacaagatt catctcgagc tctacacacc aactgagacc 420

caggagtgca cccagcaaac tctgcagtgt tacctgggag aagtggttac tctgaagaaa 480

gaaactgaag atgacactga aattaaagaa gaatttgtaa ctgctattca aaatatcgaa 540

aagaacctca agagtcttac gggtctaaat cacaccggaa gtgaatgcaa gatctgtgaa 600

gctaacaaca agaaaaaatt tcctgatttt ctccatgaac tgaccaactt tgtgagatat 660

ctgcaaaaat aa 672

<210> 40
<211> 223
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence of fusion protein

<400> 40

Ala Leu Ser Gln Gly Arg Thr Leu Val Lys Met Gly Asn Glu Leu Arg
1 5 10 15

Cys Gln Cys Ile Ser Thr His Ser Lys Phe Ile His Pro Lys Ser Ile
20 25 30

Gln Asp Val Lys Leu Thr Pro Ser Gly Pro His Cys Lys Asn Val Glu
35 40 45

Ile Ile Ala Thr Leu Lys Asp Gly Arg Glu Val Cys Leu Asn Pro Thr
50 55 60

Ala Pro Trp Val Gln Leu Ile Val Lys Ala Leu Met Ala Lys Ala Gln
65 70 75 80

Leu Asn Ser Asp Ala Pro Leu Glu Ala Ala Ala Lys Glu Ala Ala Ala
85 90 95

Lys Glu Ala Ala Ala Lys Ala Ser Leu Ser Ser Ala Lys Arg Lys Pro
100 105 110

```
Leu Gln Thr Leu Ile Lys Asp Leu Glu Ile Leu Glu Asn Ile Lys Asn
        115                 120                 125

Lys Ile His Leu Glu Leu Tyr Thr Pro Thr Glu Thr Gln Glu Cys Thr
    130                 135                 140

Gln Gln Thr Leu Gln Cys Tyr Leu Gly Glu Val Val Thr Leu Lys Lys
145                 150                 155                 160

Glu Thr Glu Asp Asp Thr Glu Ile Lys Glu Glu Phe Val Thr Ala Ile
                165                 170                 175

Gln Asn Ile Glu Lys Asn Leu Lys Ser Leu Thr Gly Leu Asn His Thr
            180                 185                 190

Gly Ser Glu Cys Lys Ile Cys Glu Ala Asn Asn Lys Lys Lys Phe Pro
        195                 200                 205

Asp Phe Leu His Glu Leu Thr Asn Phe Val Arg Tyr Leu Gln Lys
    210                 215                 220
```

<210> 41
<211> 990
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence of fusion protein

<400> 41

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gctctgtcgc aaggtaggac gctggtaaag atggggaatg agctgcggtg ccagtgcatt       60

agcactcatt ctaagttcat ccaccctaaa tccattcaag atgtgaagct gacgccaagc      120

ggcccccact gcaagaatgt tgaaatcata gctactctaa aggatggaag agaggtgtgc      180

ttgaacccca ctgctccctg ggtacagctg atcgtaaagg cacttatggc caaggctcag      240

ctcaattctg atgcaccact ggaagctgcg gcaaaagagg cagctgcgaa ggaagcggca      300

gcgaaacaca tgaagatgga taagatggaa gaggtgttga gtttaaatga agattacatc      360

tttctgagaa aagtgcagaa atgtcagacg ggagaagatc agaagtcgac attattggac      420

tgtgaaaaag ttctaaaagg cttccaggac ctccaatgca gggataggac agccagtgag      480

gagttgccaa aatttgaaat gcacagaggt catgagcacc cccacttgaa gagtaggaat      540

gagacatctg tggcagagga gaagaggcag ccgatcgcaa cacacctggc aggggtgaag      600

agcaacacaa cagtgagagt gctgaagtgg atgacgacgg gctacgcccc aacgagcagc      660

ttgatatcct accatgaggg gaagctgaag gtggagaaag cagggctcta ctacatctac      720
```

tcacaagtca gcttctgcac caaggcggcg gcttcggcgc cattcaccct ctatatttat 780

ttgtacctcc ccatggaaga ggaccggctc ctgatgaagg gacttgacac gcacagcacc 840

tccacggctc tctgtgagct ccagtccatc cgggagggcg gtgtcttcga gctgcggcag 900

ggcgacatgg actttgtcaa tgtgacggac tcaacagcag tgaacgtcaa ccctggcaac 960

acctactttg gcatgttcaa gctgtagtaa 990

<210> 42
<211> 328
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence of fusion protein

<400> 42

Ala Leu Ser Gln Gly Arg Thr Leu Val Lys Met Gly Asn Glu Leu Arg
1 5 10 15

Cys Gln Cys Ile Ser Thr His Ser Lys Phe Ile His Pro Lys Ser Ile
20 25 30

Gln Asp Val Lys Leu Thr Pro Ser Gly Pro His Cys Lys Asn Val Glu
35 40 45

Ile Ile Ala Thr Leu Lys Asp Gly Arg Glu Val Cys Leu Asn Pro Thr
50 55 60

Ala Pro Trp Val Gln Leu Ile Val Lys Ala Leu Met Ala Lys Ala Gln
65 70 75 80

Leu Asn Ser Asp Ala Pro Leu Glu Ala Ala Ala Lys Glu Ala Ala Ala
85 90 95

Lys Glu Ala Ala Ala Lys His Met Lys Met Asp Lys Met Glu Glu Val
100 105 110

Leu Ser Leu Asn Glu Asp Tyr Ile Phe Leu Arg Lys Val Gln Lys Cys
115 120 125

Gln Thr Gly Glu Asp Gln Lys Ser Thr Leu Leu Asp Cys Glu Lys Val
130 135 140

Leu Lys Gly Phe Gln Asp Leu Gln Cys Arg Asp Arg Thr Ala Ser Glu
145 150 155 160

```
Glu Leu Pro Lys Phe Glu Met His Arg Gly His Glu His Pro His Leu
                165                 170                 175

Lys Ser Arg Asn Glu Thr Ser Val Ala Glu Glu Lys Arg Gln Pro Ile
            180                 185                 190

Ala Thr His Leu Ala Gly Val Lys Ser Asn Thr Thr Val Arg Val Leu
        195                 200                 205

Lys Trp Met Thr Thr Ser Tyr Ala Pro Thr Ser Ser Leu Ile Ser Tyr
    210                 215                 220

His Glu Gly Lys Leu Lys Val Glu Lys Ala Gly Leu Tyr Tyr Ile Tyr
225                 230                 235                 240

Ser Gln Val Ser Phe Arg Thr Lys Ala Ala Ala Ser Ala Pro Phe Thr
                245                 250                 255

Leu Tyr Ile Tyr Leu Tyr Leu Pro Met Glu Glu Asp Arg Leu Leu Met
            260                 265                 270

Lys Gly Leu Asp Thr His Ser Ala Ser Thr Ala Leu Cys Glu Leu Gln
        275                 280                 285

Ser Ile Arg Glu Gly Gly Val Phe Glu Leu Arg Gln Gly Asp Met Val
    290                 295                 300

Phe Val Asn Val Thr Asp Ser Thr Ala Val Asn Val Asn Pro Gly Asn
305                 310                 315                 320

Thr Tyr Phe Gly Met Phe Lys Leu
                325
```

<210> 43
<211> 615
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence of fusion protein

<400> 43

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aagcctgtca gcctgactta ccgatgcccc tgtcgattct tcgagagcaa cgtggcgagg       60

gccaacatta agcacctcaa aatcctttcc actcccaact gctcgcttca gattgttgca      120

aggctcaaga gcaacagcaa gcaagtgtgc attgatccca agctaaagtg gatccaggaa      180

tatctggaga aagctttaaa caaggaagct gcggcaaaag aggcagctgc gaaggaagcg      240
```

gcagcgaaag catctctatc atcagcgaaa aggaaacctc ttcaaacatt aataaaggat 300

ttagaaatat tggaaaatat caagaacaag attcatctcg agctctacac accaactgag 360

acccaggagt gcacccagca aactctgcag tgttacctgg gagaagtggt tactctgaag 420

aaagaaactg aagatgacac tgaaattaaa gaagaatttg taactgctat tcaaaatatc 480

gaaaagaacc tcaagagtct tacgggtcta aatcacaccg gaagtgaatg caagatctgt 540

gaagctaaca acaagaaaaa atttcctgat tttctccatg aactgaccaa ctttgtgaga 600

tatctgcaaa aataa 615

<210> 44
<211> 204
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence of fusion protein

<400> 44

Lys Pro Val Ser Leu Thr Tyr Arg Cys Pro Cys Arg Phe Phe Glu Ser
1 5 10 15

Asn Val Ala Arg Ala Asn Ile Lys His Leu Lys Ile Leu Ser Thr Pro
20 25 30

Asn Cys Ser Leu Gln Ile Val Ala Arg Leu Lys Ser Asn Ser Lys Gln
35 40 45

Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln Glu Tyr Leu Glu Lys
50 55 60

Ala Leu Asn Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala
65 70 75 80

Ala Ala Lys Ala Ser Leu Ser Ser Ala Lys Arg Lys Pro Leu Gln Thr
85 90 95

Leu Ile Lys Asp Leu Glu Ile Leu Glu Asn Ile Lys Asn Lys Ile His
100 105 110

Leu Glu Leu Tyr Thr Pro Thr Glu Thr Gln Glu Cys Thr Gln Gln Thr
115 120 125

Leu Gln Cys Tyr Leu Gly Glu Val Val Thr Leu Lys Lys Glu Thr Glu
130 135 140

```
Asp Asp Thr Glu Ile Lys Glu Glu Phe Val Thr Ala Ile Gln Asn Ile
145                 150                 155                 160

Glu Lys Asn Leu Lys Ser Leu Thr Gly Leu Asn His Thr Gly Ser Glu
                165                 170                 175

Cys Lys Ile Cys Glu Ala Asn Asn Lys Lys Lys Phe Pro Asp Phe Leu
            180                 185                 190

His Glu Leu Thr Asn Phe Val Arg Tyr Leu Gln Lys
        195                 200
```

<210> 45
<211> 933
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence of fusion protein

<400> 45

```
aagcctgtca gcctgactta ccgatgcccc tgtcgattct tcgagagcaa cgtggcgagg       60

gccaacatta agcacctcaa aatcctttcc actcccaact gctcgcttca gattgttgca      120

aggctcaaga gcaacagcaa gcaagtgtgc attgatccca agctaaagtg gatccaggaa      180

tatctggaga aagctttaaa caaggaagct gcggcaaaag aggcagctgc gaaggaagcg      240

gcagcgaaac acatgaagat ggataagatg gaagaggtgt tgagtttaaa tgaagattac      300

atctttctga gaaaagtgca gaaatgtcag acgggagaag atcagaagtc gacattattg      360

gactgtgaaa aagttctaaa aggcttccag gacctccaat gcagggatag gacagccagt      420

gaggagttgc caaaatttga aatgcacaga ggtcatgagc acccccactt gaagagtagg      480

aatgagacat ctgtggcaga ggagaagagg cagccgatcg caacacacct ggcaggggtg      540

aagagcaaca caacagtgag agtgctgaag tggatgacga cgggctacgc cccaacgagc      600

agcttgatat cctaccatga ggggaagctg aaggtggaga aagcagggct ctactacatc      660

tactcacaag tcagcttctg caccaaggcg gcggcttcgg cgccattcac cctctatatt      720

tatttgtacc tccccatgga agaggaccgg ctcctgatga agggacttga cacgcacagc      780

acctccacgg ctctctgtga gctccagtcc atccgggagg gcggtgtctt cgagctgcgg      840

cagggcgaca tggactttgt caatgtgacg gactcaacag cagtgaacgt caaccctggc      900

aacacctact ttggcatgtt caagctgtag taa                                   933
```

<210> 46
<211> 309

<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence of fusion protein

<400> 46

```
Lys Pro Val Ser Leu Thr Tyr Arg Cys Pro Cys Arg Phe Phe Glu Ser
1               5                  10                  15

Asn Val Ala Arg Ala Asn Ile Lys His Leu Lys Ile Leu Ser Thr Pro
            20                  25                  30

Asn Cys Ser Leu Gln Ile Val Ala Arg Leu Lys Ser Asn Ser Lys Gln
        35                  40                  45

Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln Glu Tyr Leu Glu Lys
    50                  55                  60

Ala Leu Asn Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala
65                  70                  75                  80

Ala Ala Lys His Met Lys Met Asp Lys Met Glu Glu Val Leu Ser Leu
                85                  90                  95

Asn Glu Asp Tyr Ile Phe Leu Arg Lys Val Gln Lys Cys Gln Thr Gly
            100                 105                 110

Glu Asp Gln Lys Ser Thr Leu Leu Asp Cys Glu Lys Val Leu Lys Gly
        115                 120                 125

Phe Gln Asp Leu Gln Cys Arg Asp Arg Thr Ala Ser Glu Glu Leu Pro
    130                 135                 140

Lys Phe Glu Met His Arg Gly His Glu His Pro His Leu Lys Ser Arg
145                 150                 155                 160

Asn Glu Thr Ser Val Ala Glu Glu Lys Arg Gln Pro Ile Ala Thr His
                165                 170                 175

Leu Ala Gly Val Lys Ser Asn Thr Thr Val Arg Val Leu Lys Trp Met
            180                 185                 190

Thr Thr Ser Tyr Ala Pro Thr Ser Ser Leu Ile Ser Tyr His Glu Gly
        195                 200                 205

Lys Leu Lys Val Glu Lys Ala Gly Leu Tyr Tyr Ile Tyr Ser Gln Val
```

```
    210                 215                 220

Ser Phe Arg Thr Lys Ala Ala Ala Ser Ala Pro Phe Thr Leu Tyr Ile
225                 230                 235                 240

Tyr Leu Tyr Leu Pro Met Glu Glu Asp Arg Leu Leu Met Lys Gly Leu
                245                 250                 255

Asp Thr His Ser Ala Ser Thr Ala Leu Cys Glu Leu Gln Ser Ile Arg
            260                 265                 270

Glu Gly Gly Val Phe Glu Leu Arg Gln Gly Asp Met Val Phe Val Asn
        275                 280                 285

Val Thr Asp Ser Thr Ala Val Asn Val Asn Pro Gly Asn Thr Tyr Phe
    290                 295                 300

Gly Met Phe Lys Leu
305
```

<210> 47
<211> 666
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence of fusion protein

<400> 47

```
aagcctgtca gcctgactta ccgatgcccc tgtcgattct tcgagagcaa cgtggcgagg       60

gccaacatta agcacctcaa aatcctttcc actcccaact gctcgcttca gattgttgca      120

aggctcaaga gcaacagcaa gcaagtgtgc attgatccca agctaaagtg gatccaggaa      180

tatctggaga aagctttaaa caaggaagct gcggcaaaag aggcagctgc gaaggaagcg      240

gcagcgaaaa tcgcaacaca cctggcaggg gtgaagagca acacaacagt gagagtgctg      300

aagtggatga cgacgggcta cgccccaacg agcagcttga tatcctacca tgaggggaag      360

ctgaaggtgg agaaagcagg gctctactac atctactcac aagtcagctt ctgcaccaag      420

gcggcggctt cggcgccatt caccctctat atttatttgt acctccccat ggaagaggac      480

cggctcctga tgaagggact tgacacgcac agcacctcca cggctctctg tgagctccag      540

tccatccggg agggcggtgt cttcgagctg cggcagggcg acatggactt tgtcaatgtg      600

acggactcaa cagcagtgaa cgtcaaccct ggcaacacct actttggcat gttcaagctg      660

tagtaa                                                                 666
```

<210> 48
<211> 220
<212> PRT
<213> Artificial Sequence

<220>
<223> Amino acid sequence of fusion protein

<400> 48

```
Lys Pro Val Ser Leu Thr Tyr Arg Cys Pro Cys Arg Phe Phe Glu Ser
1               5                  10                  15

Asn Val Ala Arg Ala Asn Ile Lys His Leu Lys Ile Leu Ser Thr Pro
            20                  25                  30

Asn Cys Ser Leu Gln Ile Val Ala Arg Leu Lys Ser Asn Ser Lys Gln
        35                  40                  45

Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln Glu Tyr Leu Glu Lys
    50                  55                  60

Ala Leu Asn Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala
65                  70                  75                  80

Ala Ala Lys Ile Ala Thr His Leu Ala Gly Val Lys Ser Asn Thr Thr
                85                  90                  95

Val Arg Val Leu Lys Trp Met Thr Thr Ser Tyr Ala Pro Thr Ser Ser
            100                 105                 110

Leu Ile Ser Tyr His Glu Gly Lys Leu Lys Val Glu Lys Ala Gly Leu
        115                 120                 125

Tyr Tyr Ile Tyr Ser Gln Val Ser Phe Arg Thr Lys Ala Ala Ala Ser
    130                 135                 140

Ala Pro Phe Thr Leu Tyr Ile Tyr Leu Tyr Leu Pro Met Glu Glu Asp
145                 150                 155                 160

Arg Leu Leu Met Lys Gly Leu Asp Thr His Ser Ala Ser Thr Ala Leu
                165                 170                 175

Cys Glu Leu Gln Ser Ile Arg Glu Gly Gly Val Phe Glu Leu Arg Gln
            180                 185                 190

Gly Asp Met Val Phe Val Asn Val Thr Asp Ser Thr Ala Val Asn Val
        195                 200                 205
```

```
Asn Pro Gly Asn Thr Tyr Phe Gly Met Phe Lys Leu
    210                 215                 220
```