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(54) Title: ANTI-HUMAN HVEM (TNFRSF14) ANTIBODIES AND USES THEREOF

(57) Abstract: The invention discloses an antibody that binds an extracellular part of human HVEM on human HVEM-expressing cells, that prevents binding of BTLA to HVEM when the antibody is bound to said extracellular part of HVEM, wherein said antibody displaces BTLA bound to said extracellular part of HVEM. The invention also discloses the use of such an antibody in combating certain diseases.

Title: Anti-human HVEM (TNFRSF14) antibodies and uses thereof

FIELD OF THE INVENTION

The invention relates to the field of antibodies and the use of such antibodies. The invention in particular provides antibodies that bind HVEM. The invention also provides kits and compositions comprising an anti HVEM antibody and methods of treatment using an antibody as described herein.

BACKGROUND OF THE INVENTION

The activation of T cells requires the concomitant activation of at least two signals: the engagement of T cell receptor and an additional signal delivered by co-stimulatory molecules. Some co-stimulatory molecules belong to B7/CD28 and TNF/TNFR families, and play crucial roles in the modulation of immune responses and improvement of antitumor immunity. Tumors can escape immune surveillance by generating an immunosuppressive microenvironment, where antitumor T cell responses are attenuated by the lack of co-stimulatory molecules on and/or by overexpression of co-inhibitory molecules such as PD-L1/L2 on the surface of cancer cells. Targeting co-stimulatory and co-inhibitory pathways represent an attractive therapeutic strategy to enhance the antitumor immunity in several human cancers. Clinical trials targeting the co-inhibitory Ig molecules CTLA-4 and PD-1, have already given promising results in patients with melanoma, renal cell and prostate carcinoma, and non-Hodgkin's lymphoma, and resulted in drug approvals like Yervoy® and Opdivo®.

There is interest in evaluating the potential role of other co-stimulatory and co-inhibitory receptor/ligand interactions. One such molecule is Herpes virus entry mediator (HVEM/CD270/ TNFRSF14) and its ligands. The interactions between HVEM and its ligands are more complex than for instance PD-1/PD-L1, as there is evidence of bi-directional signalling. HVEM is a molecular switch between stimulatory and inhibitory signalling upon interaction with its ligands, which comprise BTLA (B- and T-lymphocyte attenuator), CD160, LIGHT (lymphotoxin-like, exhibits inducible expression, and competes with herpes simplex virus glycoprotein D for HVEM, a receptor expressed by T lymphocytes) and TNFß/LTα (tumor necrosis factor ß/lymphotoxin α). HVEM and its ligands have a role in the physiopathology of immune regulation. In the present invention it was shown that dysregulation of this network contributes to various diseases.

HVEM was initially discovered as the co-receptor for the glycoprotein D of the herpes simplex virus 1, allowing the entry of the virus in the cell. HVEM is found

to be widely expressed in tissues, with highest expression levels in lung, kidney, and liver. HVEM is also found to be expressed on T cells, B cells, NK cells and myeloid cells. Notably, HVEM expression is upregulated in several cancers. HVEM is known to interact with BTLA, CD160, LIGHT and TNFß. Most of the ligands, as well as HVEM itself can be expressed on either side of an immune synapse: CD160 is found to be expressed in NK cells, NKT cells and T cells, BTLA is highly expressed in activated T cells and rested B cells, and less in naïve T cells, NK cells, dendritic cells (DCs) and macrophages. LIGHT is found to be expressed by immature DCs, granulocytes, monocytes and activated T cells and TNFß is expressed in B cells and T cells and NK cells.

Without being bound by theory, it is believed that engagement of BTLA and CD160 on T lymphocytes by HVEM provides co-inhibitory signals to T lymphocytes through BTLA and CD160, ligation of HVEM on T lymphocytes by LIGHT and TNFβ delivers co-stimulatory signals through HVEM. These interactions are bidirectional: HVEM induces inhibitory signals in T cells after interaction with BTLA and CD160 on T cells, while both BTLA and CD160 act as activating ligands for HVEM resulting in NFκB activation. Furthermore, LIGHT delivers costimulatory signals to a T cell when interacting with HVEM expressed on the T cell, and HVEM has also been implicated to transmit costimulatory signals to a T cell when interacting with LIGHT expressed by the T cell. However, LIGHT does not contain an obvious signalling motif and its mechanism for signalling is incompletely defined.

When LIGHT and/or TNFß, BTLA and/or CD160 simultaneously interact with HVEM, the net effect is an inhibitory signal for T-cell activation. Many tumors (e.g., melanoma, esophageal squamous cell carcinoma, hepatocellular carcinoma, and colorectal cancer) overexpress HVEM. Hence, therapeutic blocking of the inhibitory interaction between HVEM on cancer cells with BTLA/CD160 on T cells, while leaving LIGHT-mediated signalling in HVEM expressing T cells intact, could enhance antitumor T cell responses. Antibodies have been described that can interfere with binding of BTLA to HVEM (WO2014184360A1). In one embodiment the present invention provides an antibody that can block binding of BTLA to HVEM and displace BTLA bound to HVEM. These and other antibodies are the subject of the present invention disclosure.

SUMMARY OF THE INVENTION

In one aspect the disclosure provides an antibody that binds an extracellular part of Herpesvirus entry mediator (HVEM) on HVEM expressing cells, and prevents binding of B- and T-lymphocyte attenuator (BTLA) to HVEM when said antibody is bound to said extracellular part of HVEM. In one aspect the antibody can displace a BTLA bound to said extracellular part of HVEM.

In one aspect the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 26-28 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 29-31 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions.

In one aspect, the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain region with the amino acid sequence of SEQ ID NO: 24 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 25 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions.

In one aspect the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 42-44 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 45-47 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions.

In one aspect, the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain region with the amino acid sequence of SEQ ID NO: 40 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 41 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions.

In one aspect the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 18-20 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 21-23 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions.

In one aspect, the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain region with the amino acid sequence of SEQ ID NO: 16 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 17 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions.

In one aspect the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with

the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 34-36 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 37-39 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions.

In one aspect, the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain region with the amino acid sequence of SEQ ID NO: 32 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 33 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions.

In one aspect the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 50-52 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 53-55 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions.

In one aspect, the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain region with the amino acid sequence of SEQ ID NO: 48 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 49 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions.

In one aspect the disclosure provides a nucleic acid molecule or nucleic acid molecules encoding an antibody as disclosed herein or an antigen binding fragment thereof as disclosed herein. Further provided is a nucleic acid encoding a variable region as disclosed herein.

In one aspect the disclosure provides a vector comprising a nucleic acid molecule as described herein. In one aspect the disclosure provides a cell comprising an antibody, a nucleic acid molecule or molecules and/or a vector as disclosed herein. Preferably, the host cell is a mammalian, insect, plant, bacterial or yeast cell. More preferably, the cell is a human cell. Preferably, the host cell is a hybridoma cell, a Chinese hamster ovary (CHO) cell, an NSO cell, or a PER-C6™ cell.

In one aspect the disclosure provides a method of producing the antibody as disclosed herein. The method includes harvesting of the antibody. Preferably, the antibodies are produced using a cell and harvested from said cell. Preferably said cell is a hybridoma cell, a Chinese hamster ovary (CHO) cell, an NSO cell, or a PER-C6™ cell. Preferably, the antibodies are produced synthetically.

In one aspect the disclosure provides a pharmaceutical composition comprising an antibody or antigen binding fragment thereof, nucleic acid and/or cell as disclosed. Preferably, the composition or antibody or antigen binding fragment thereof as disclosed herein are for use in the manufacture of a medicament. Preferably, the medicament is for the treatment and/or prophylaxis of cancer and immune-related disorders.

In one aspect the disclosure provides a method for the treatment of cancer and immune-related disorders in a subject comprising administering to the subject in need thereof a therapeutically effective amount of an antibody or antigen binding fragment thereof, a nucleic acid molecule or a vector as disclosed herein.

In one aspect the disclosure provides an antibody or antigen binding fragment thereof for use in the treatment of cancer and immune-related disorders.

In one aspect the disclosure provides a method for modulating HVEM signalling activity, comprising contacting HVEM expressing cells with an antibody or antigen binding fragment thereof, a nucleic acid molecule or a vector as disclosed herein.

In one aspect the disclosure provides a method for increasing an immune response in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a composition comprising an antibody or antigen binding fragment thereof, a nucleic acid molecule or a vector as disclosed herein.

In one aspect the disclosure provides a method for reducing tumor growth in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a composition comprising an antibody or antigen binding fragment thereof, a nucleic acid molecule or a vector as disclosed herein.

DETAILED DESCRIPTION OF THE DISCLOSED EMBODIMENTS

The disclosure describes antibodies that binds an extracellular part of human HVEM on HVEM-expressing cells and soluble HVEM. An antibody as described herein is useful to prevent binding of BTLA and CD160 to HVEM when the antibody is bound to said extracellular part of human HVEM. Several antibodies have been generated that binds to HVEM. Antibodies that specifically bind HVEM are known in the art. For example, the antibody eBio HVEM-122 (eBiosciences) is commercially available and referred to in example 2. Antibodies have been described that can interfere with binding of BTLA to HVEM (WO2014184360A1). The present invention provides antibodies that block binding of BTLA to HVEM and displace BTLA once bound to HVEM.

In one aspect the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells; prevents binding of BTLA to HVEM when said antibody is bound to said extracellular part of HVEM; and displaces a BTLA once bound to said extracellular part of HVEM.

The term "antibody" refers to an immunoglobulin molecule that is typically composed of two identical pairs of polypeptide chains, each pair having one "heavy" (H) chain and one "light" (L) chain. Human light chains are classified as kappa (κ) and lambda (λ). Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant regions of IgD, IgG, and IgA are comprised of three domains, CH1, CH2 and CH3, and the heavy chain constant regions of IgM and IgE are comprised of four domains, CH1, CH2, CH3, and CH4. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from the amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the light and heavy chain together form the antibody binding site and defines the specificity for the epitope. Various methods are known in the art to assign amino acids to a region or domain in an antibody. Well known methods include the Kabat method and the Chothia method (Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991); Chothia et al. Conformations of immunoglobulin hypervariable regions in Nature 1989; 342(6252):877-83). The assignment of the amino acids to each region or domain of this disclosure is in accordance with the definitions of Kabat.

The term "antibody" encompasses murine, humanized, deimmunized human and chimeric antibodies, and an antibody that is a multimeric form of antibodies, such as dimers, trimers, or higher-order multimers of monomeric antibodies. Antibody also encompasses monospecific, bispecific or multispecific antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity. It also encompasses an antibody that is linked or attached to a non-antibody moiety. Further, the term "antibody" is not limited by any particular method of producing the antibody. For example, it includes monoclonal antibodies, recombinant antibodies and polyclonal antibodies. The invention provides an antibody as described herein. Furthermore, the

invention provides a part, derivative and/or analogue of an antibody as disclosed herein. The part, derivative and/or analogue retains the antigen binding property of the antibody in kind, not necessarily in amount. Non-limiting examples of a part and/or derivative include a part of an antibody is an antigen binding part and typically contains one or more variable domains of the antibody. Non-limiting examples are the various Fab fragments. A part can also be a so-called single domain antibody fragment. A single-domain antibody fragment (sdAb, called Nanobody by Ablynx, the developer) is an antibody fragment with a single monomeric variable antibody domain. Like a whole antibody, it is able to bind selectively to a specific antigen. With a molecular weight of only 12–15 kDa, single-domain antibody fragments are much smaller than common antibodies (150–160 kDa) which are composed of two heavy protein chains and two light chains, and even smaller than Fab fragments (~50 kDa, one light chain and half a heavy chain) and single-chain variable fragments (~25 kDa, two variable regions, one from a light and one from a heavy chain). Single-domain antibodies by themselves are not much smaller than normal antibodies (being typically 90-100kDa). Single-domain antibody fragments are mostly engineered from heavy-chain antibodies found in camelids; these are called VHH fragments (Nanobodies®). Some fishes also have heavy-chain only antibodies (IgNAR, 'immunoglobulin new antigen receptor'), from which single-domain antibody fragments called VNAR fragments can be obtained. An alternative approach is to split the dimeric variable domains from common immunoglobulin G (IgG) from humans or mice into monomers. Although most research into single-domain antibodies is currently based on heavy chain variable domains, nanobodies derived from light chains have also been shown to bind specifically to target epitopes. A non-limiting example of an antibody part contains a variable domain of a heavy chain and/or a light chain of an antibody or an equivalent thereof. Non-limiting examples of such parts are VHH, Human Domain Antibodies (dAbs) and Unibodies. Preferred antibody parts or derivatives have at least a variable domain of a heavy chain and a light chain of an antibody as described herein. Non-limiting examples of a derivative or a part is are a F(ab)-fragment and a single chain Fv fragment. A functional part of a bispecific antibody comprises the antigen binding parts of the bispecific antibody, or a derivative and/or analogue of the binding parts.

A "single-chain antibody" (scFv) has a single polypeptide chain comprising a VL domain linked to a VH domain wherein VL domain and VH domain are paired to form a monovalent molecule. Single chain antibody can be prepared according to method known in the art (see, for example, Bird et al., (1988) Science 242:423-426 and Huston et al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). A "diabody" has two chains, each chain comprising a heavy chain variable region connected to a light chain variable region on the same polypeptide chain connected by a short peptide linker, wherein the two regions on the same chain do not pair with each other but with complementary domains on the other chain to form a bispecific molecule. Methods of preparing diabodies are known in the art (See, e.g., Holliger

P. et al., (1993) Proc.Natl. Acad. Sci. USA 90:6444-6448, and Poljak R. J. et al., (1994) Structure 2:1121-1123). Domain antibodies (dAbs) are small functional binding units of antibodies, corresponding to the variable regions of either the heavy or light chains of antibodies. Domain antibodies are well expressed in bacterial, yeast, and mammalian cell systems. Further details of domain antibodies and methods of production thereof are known in the art (see, for example, U.S. Patent Nos. 6,291,158; 6,582,915; 6,593,081; WO04/003019 and WO03/002609). Nanobodies are derived from the heavy chains of an antibody. A nanobody typically comprises a single variable domain and two constant domains (CH2 and CH3) and retains antigen-binding capacity of the original antibody. Nanobodies can be prepared by methods known in the art (see e.g., U.S. Patent No. 6,765,087, U.S. Patent No. 6,838,254, WO 06/079372). Unibodies have one light chain and one heavy chain of an IgG4 antibody. Unibodies may be made by the removal of the hinge region of IgG4 antibodies. Further details of unibodies and methods of preparing them may be found in WO2007/059782. The list of analogues to antibodies is increasing every year. With the sequence of the variable domains and the presently extensive knowledge of the 3D structure of many different antibodies the skilled person can convert an antibody of the invention to one or the other antibody analogue, part or derivative.

In addition to the binding molecule, the molecules of the invention may further comprise a moiety for increasing the in vivo half-life of the molecule, such as but not limited to polyethylene glycol (PEG), human serum albumin, glycosylation groups, fatty acids and dextran. Such further moieties may be conjugated or otherwise combined with the binding moiety using methods well known in the art. Also provided are chimeric antigen receptors (CAR) comprising a variable domain of an antibody as described herein. CAR are engineered receptors that combine a new specificity (typically an antigen binding part of an antibody or a derivative thereof) with an immune cell to target cells. The receptors are called chimeric because they are fused of parts from different sources (T lymphocytes genetically modified to express one or more chimeric antigen receptors (CARs; see, e.g., Eshhar, U.S. Patent No. 7,741,465; Eshhar, U.S. Patent Application Publication No. 2012/0093842). In some embodiments, the antibodies as disclosed herein can be coupled to an active compound for example a toxin. Furthermore, the antibodies or antigen binding fragments as disclosed may be coupled to a label, e.g., a fluorescent protein, chemical label, organic dye, coloured particle or enzyme. The antibodies as disclosed herein can be coupled to a drug to form an antibody-drug conjugate (ADC). The invention provides antibody analogues, antibody parts and antibody derivatives, also when these molecules are coupled to other molecules or incorporated.

In some embodiments an antibody as disclosed herein is a chimeric antibody. The term "chimeric antibody" refers to an antibody that comprises amino acid

sequences derived from two different species such as human and mouse, typically a combination of mouse variable (from heavy and light chains) regions and human constant (heavy and light chains) regions. A non-limiting example of generating such a chimeric antibody is described in the working examples (e.g., example 5). In this chimeric antibody the mouse IgG1/kappa constant region is exchanged for a human IgG/kappa constant domain. In some embodiments an antibody as disclosed herein is a humanized antibody. The term "humanized antibody" refers to an antibody that contains some or all of the CDRs from a non-human animal antibody while the framework and constant regions of the antibody contain amino acid residues derived from human antibody sequences. Humanized antibodies are typically produced by grafting CDRs from a mouse antibody into human framework sequences followed by back substitution of certain human framework residues for the corresponding mouse residues from the source antibody. The term “deimmunized antibody” also refers to an antibody of non-human origin in which, typically in one or more variable regions, one or more epitopes have been removed, that have a high propensity of constituting a human T-cell and/or B-cell epitope, for purposes of reducing immunogenicity. The amino acid sequence of the epitope can be removed in full or in part. However, typically the amino acid sequence is altered by substituting one or more of the amino acids constituting the epitope for one or more other amino acids, thereby changing the amino acid sequence into a sequence that does not constitute a human T-cell and/or B-cell epitope. The amino acids are substituted by amino acids that are present at the corresponding position(s) in a corresponding human variable heavy or variable light chain as the case may be. In some embodiments, an antibody as disclosed herein is a human antibody. The term "human antibody" refers to an antibody consisting of amino acid sequences of human immunoglobulin sequences only. A human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell or in a hybridoma derived from a mouse cell. Human antibodies may be prepared in a variety of ways known in the art. Chimeric, humanized, deimmunized and human antibodies are within the scope of the invention.

An antibody that binds human HVEM binds to HVEM under conditions that are normally used for antibody binding. When the antibody and human HVEM are contacted with each other under conditions suitable for antibody binding, the antibody will bind to human HVEM. The antibody binds to membrane bound human HVEM expressed on the HEK293F cells, while the antibody does not bind significantly to HEK293F cells that do not express human HVEM on their cell membrane. Binding of the antibody to a human HVEM expressing cell can be detected by methods known to the person skilled in the art. For example, by using a secondary antibody carrying a fluorescent label and measure labelled cells using flow cytometry (FACS).

HVEM, also known as tumor necrosis factor receptor superfamily member 14 (TNFRSF14) and CD270, is a human cell surface receptor of the TNF-receptor (tumor necrosis factor) superfamily. In humans, the protein is encoded by the *TNFRSF14* gene. HVEM can engage at least four distinct ligands, the TNFSF members LIGHT (TNFSF14) and TNFß/LTα (tumor necrosis factor β/lymphotoxin α) and immunoglobulin superfamily members B- and T- lymphocyte attenuator (BTLA) and CD160. For a reference sequence of human HVEM, we refer to SEQ ID NO.: 1 (Swiss-Prot no. Q92956.3; aa1-283). The reference is solely made to identify a HVEM gene/protein. It is not intended to limit the HVEM as described herein to the particular sequence of the database entry. Natural variants of HVEM that can bind BTLA, CD160, LIGHT and TNFß and can be bound by an antibody as described herein are within the scope of the invention. A recombinant human HVEM is also within the scope of the invention if it can bind BTLA, CD160, LIGHT and TNFß, and can bind an antibody as described herein.
HVEM is widely expressed in tissues, with highest expression levels in lung, kidney, and liver, and is found to be expressed on T cells, B cells, NK cells and myeloid cells. Expression of HVEM is found to be upregulated in several cancers. The term "HVEM expressing cells" refers to a cell that expresses HVEM. Exemplary cells are T cells, B cells, NK cells and myeloid cells. Binding of BTLA and/or CD160 to HVEM can have inhibitory effects, binding of LIGHT and/or TNFß to HVEM can have stimulatory effects.
The term "extra-cellular" literally means outside the cells. The term "extra-cellular part" refers to a part of a molecule that is on the outside of the cell membrane. This part of the molecule can be available for interactions with other molecules outside the cell. HVEM has an extracellular part defined by a cysteine-rich signature. The extracellular domain of HVEM contains four cysteine rich domains, namely CRD1-CRD4, and a linker. Without being bound by theory it is believed that interactions between BTLA and CD160 with HVEM occur via the CRD1 of HVEM, whereas interactions between LIGHT and TNFß with HVEM occur via the CRD2 and CRD3 of HVEM.

In one embodiment, an antibody of the invention binds the CRD1 of HVEM.

HVEM is present on the cell surface of most hematopoietic cell lineages, among which T cells, B cells, NK cells and myeloid cells. Expression of HVEM is found to be downregulated in T cells and B cells upon activation, and is upregulated in some cancers. Non limiting examples are: melanoma, esophageal squamous cell carcinoma, hepatocellular carcinoma, and colorectal cancer. The term "human HVEM expressing cell" refers to a cell that expresses human HVEM. Exemplary cells are T cells, B cells, NK cells and myeloid cells.

The term "to prevent binding" refers to the ability of the antibody or antigen-binding fragment thereof to prevent binding of a ligand to a protein, when said

protein is bound by said antibody. Where reference is made to "prevent binding", this can also be interpreted as "blocking". If binding of a ligand is prevented with more than (>)70% compared to binding of the ligand in absence of the antibody, binding of said ligand is said to be prevented. If binding of a ligand is prevented with more than or equals (≥) 30% but less than or equals (≤) 70% compared to binding of the ligand in absence of the antibody, binding said ligand is said to be partially prevented. Binding of a ligand is said not to be affected when binding of the ligand is prevented with less than (<) 30% compared to binding of the ligand in absence of the antibody. If binding of a ligand is increased with more than (>) 30%, compared to binding of the ligand in absence of the antibody, binding of said ligand is said to be enhanced.

An antibody or antigen-binding fragment as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Exemplary antibodies are: 45H6, 11H7, 36H12, 48H6, and 49G4.

In a further embodiment, an anti-HVEM antibody or antigen-binding fragment thereof as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and does not prevent or partially prevents binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Exemplary antibodies are: 45H6, 11H7, 36H12, 48H6, and 49G4.

In a further embodiment, an anti-HVEM antibody or antigen-binding fragment thereof as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and partially prevents binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Exemplary antibodies are: 45H6, 36H12, 48H6, and 49G4.

In a further embodiment, an anti-HVEM antibody or antigen-binding fragment thereof as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and does not prevent binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. An exemplary antibody is: 11H7.

In a further embodiment, an anti-HVEM antibody as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and partially prevents binding of CD160 to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Exemplary antibodies are: 45H6, 11H7, 36H12, 48H6, and 49G4.

In a further embodiment, an anti-HVEM antibody or antigen-binding fragment thereof as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells, partially prevents binding of CD160 and LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Exemplary antibodies are: 45H6, 36H12, 48H6, and 49G4.

In a further embodiment, an anti-HVEM antibody or antigen-binding fragment thereof as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells, partially prevents binding of CD160 to an extracellular part of HVEM on HVEM expressing cells, and does not prevent binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. An exemplary antibody is: 11H7.

Typically, an anti-HVEM antibody or antigen binding fragment thereof as described above binds the CRD1 domain of the HVEM protein.

Binding of BTLA, CD160 and LIGHT to HVEM is preferably measured with a method described in the examples. An exemplary method is described, for instance, in example 6b, of which the results are depicted in figure 6A-D. Preferably HEK293F cells, transfected with a full-length HVEM are used. Preferably, said cells stably express full length HVEM on the plasma membrane. A test antibody is examined using HVEM-expressing HEK293F cells. Cells are incubated with the anti-HVEM antibody of interest. After washing, cells are incubated with a biotin-labelled or his-tagged human BTLA, CD160 or LIGHT. After washing, the label or tag is detected with fluorescently labelled streptavidin or anti-his antibody. Binding of BTLA, CD160 or LIGHT to HVEM expressed on cells can be measured by detecting the fluorescence using a flow cytometer (FACS). The capacity to prevent binding is then determined by comparing the percentage of BTLA, CD160 or LIGHT bound to HVEM in presence of an anti-HVEM antibody to the percentage bound in presence of a control antibody that does not bind HVEM. Less binding of BTLA, CD160 or LIGHT to HVEM indicates a stronger blocking capacity of the antibody.

The term “to displace” refers to the capacity of a first entity to remove a second entity from its position, whereby the second entity is replaced by the first entity. If more than (>)70% of a ligand bound to the extracellular part of HVEM is displaced compared to presence of the ligand in absence of the antibody, said ligand is said to be displaced. If more than or equals (≥) 30% but less than or equals (≤) 70% of a ligand bound to the extracellular part of HVEM is displaced compared to presence of the ligand in absence of the antibody, said ligand is said to be partially displaced. A ligand is said not to be displaced if less than (<) 30% of the ligand

bound to the extracellular part of HVEM is displaced compared to presence of the ligand in absence of the antibody. If binding of a ligand is increased with more than (>) 30% compared to presence of the ligand in absence of the antibody, binding of said ligand is to be enhanced.

An antibody or antigen-binding fragment as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells. Said antibody preferably displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells. Exemplary antibodies are: 45H6, 11H7, 36H12, 48H6, and 49G4

In a further embodiment, an anti-HVEM antibody or antigen-binding fragment thereof as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and does not prevent or partially prevents binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells and does not displace LIGHT bound to the extracellular part of HVEM on HVEM expressing cells. Exemplary antibodies are: 45H6, 11H7, 36H12, 48H6, and 49G4.

In a further embodiment, an anti-HVEM antibody or antigen-binding fragment thereof as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and partially prevents binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells and does not displace LIGHT bound to the extracellular part of HVEM on HVEM expressing cells. Exemplary antibodies are: 45H6, 36H12, 48H6, and 49G4

In a further embodiment, an anti-HVEM antibody or antigen-binding fragment thereof as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and does not prevent binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells, and does not displace LIGHT bound to the extracellular part of HVEM on HVEM expressing cells. An exemplary antibody is: 11H7.

In a further embodiment, an anti-HVEM antibody as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and partially prevents binding of CD160 to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM

on HVEM expressing cells and partially displaces CD160 bound to the extracellular part of HVEM on HVEM expressing cells. Exemplary antibodies are: 45H6, 11H7, 36H12, 48H6, and 49G4

In a further embodiment, an anti-HVEM antibody or antigen-binding fragment thereof as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and partially prevents binding of CD160 and LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells, partially displaces CD160 bound to the extracellular part of HVEM on HVEM expressing cells, and does not displace LIGHT bound to the extracellular part of HVEM on HVEM expressing cells. Exemplary antibodies are: 45H6, 36H12, 48H6, and 49G4

In a further embodiment, an anti-HVEM antibody or antigen-binding fragment thereof as disclosed herein prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells, partially prevents binding of CD160 to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM, and does not prevent binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells, partially displaces CD160 bound to the extracellular part of HVEM on HVEM expressing cells, and does not displace LIGHT bound to the extracellular part of HVEM on HVEM expressing cells. An exemplary antibody is: 11H7.

Typically, an anti-HVEM antibody or antigen binding fragment thereof as described above binds the CRD1 domain of the HVEM protein.

In order to analyze if anti-HVEM antibodies as disclosed herein have the capacity to displace ligands bound to the extracellular part of HVEM, the skilled person can use a number of known suitable assays. One of the suitable methods is disclosed in the example section. The assay is described in detail in e.g. example 6c. Preferably HEK293F cells, transfected with full-length HVEM are used. Preferably, said cells are stably expressing full length HVEM on the plasma membrane. The cells are incubated with a soluble ligand (e.g., biotin-labelled or his-tagged BTLA, CD160 or LIGHT). Subsequently, the cells are incubated with an antibody of the invention, binding the extracellular part of HVEM. After washing, ligand bound to the cells is detected using fluorescently labelled streptavidin or anti-his antibody After washing, the fluorescent signal of the antibody bound to the ligand can be detected using a flow cytometer (FACS). The amount of ligand bound to the extracellular part of HVEM indicates the capacity of the anti-HVEM antibody to replace the

ligand bound to the extracellular part of HVEM of the antibody. A lower fluorescent signal of the ligand indicates a stronger capacity to replace of the anti-HVEM antibody. A preferred method is described in the examples of which the results are, for example, depicted in figure 7A-D. Percentages of displacement are typically given as a percentage compared to binding of the ligand to HVEM in presence of a non-specific antibody, under otherwise identical conditions. Displacement can be measured using metabolically active cells (for instance incubated overnight at 37 ^{0}C) or using metabolically inactive cells (for instance incubated at 4 ^{0}C in the presence of sodium azide).

Without being bound by theory, it is believed that activation of T cells is inhibited upon interaction of BTLA and/or CD160 with HVEM, even in presence of LIGHT and/or TNFß. An antibody as disclosed herein is useful to target cells expressing HVEM. Binding of an antibody as disclosed herein displaces BTLA and preferably at least partially displaces CD160 bound to the extracellular part of HVEM, but does not displace LIGHT bound to the extracellular part of HVEM. As a result, due to the ability to prevent binding of BTLA and CD160 and the capacity to displace BTLA and preferably partially displace CD160, the inhibitory effect of BTLA and CD160 on T-cell activation is suppressed. A cell that has bound an antibody as disclosed herein is available to respond to other stimuli, such as binding of LIGHT or TNFß to HVEM.

The full length cynomolgus (*Macaca fascicularis*) monkey HVEM protein (Met1-Ser280; NCBI Reference sequence: XP_005545061.1, see SEQ ID NO. 5) has a similar amino acid sequence as human HVEM and exhibits 82% on homology to the human HVEM protein (Met1-His283; Swiss-Prot no. Q92956.3, see SEQ ID NO. 1). The predicted amino acid sequence of the extracellular domain of cynomolgus monkey HVEM (i.e., Leu39-Val203; NCBI Reference Sequence: XP_005545061.1) shows 87% homology with the amino acid sequence of the extracellular domain of human HVEM protein (i.e., Leu 39-Val202; Swiss-Prot no. Q92956.3). Substitutions of amino acids of human HVEM with the corresponding amino acids of cynomolgus HVEM can be used to test the specificity and cross-specificity of the antibodies. In one embodiment, an antibody of the invention binds human HVEM and is cross-specific for cynomolgus (*Macaca fascicularis*) monkey HVEM. Exemplary antibodies are: 45H6, 11H7, 48H6 and 49G4. Without being bound by theory, it is believed that such antibodies are particularly suitable for binding to human HVEM carrying mutations. Furthermore, such antibodies are suitable for toxicity testing.

One aspect of the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 26-28 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable

region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 29-31 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions. Preferably, the antibody that binds an extracellular part of HVEM on HVEM expressing cells comprises a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 26-28 and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 29-31.

In a further aspect, the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain variable region with the amino acid sequence of SEQ ID NO: 24 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 25 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions. In a preferred embodiment, the 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions are located in the framework regions of the light and/or heavy chain variable region. Preferably, the antibody that binds an extracellular part of HVEM on HVEM expressing cell comprises a heavy chain variable region with the amino acid sequence of SEQ ID NO: 24 and a light chain variable region with the amino acid sequence of SEQ ID NO: 25. An exemplary antibody with these characteristics is 45H6.

One aspect of the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 42-44 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 45-47 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions. Preferably, the antibody that binds an extracellular part of HVEM on HVEM expressing cells comprises a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 42-44 and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 45-47.

In a further aspect, the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain variable region with the amino acid sequence of SEQ ID NO: 40 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 41 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions. In a preferred embodiment, the 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions are located in the framework regions of the light and/or heavy chain variable region. Preferably, the antibody that binds an extracellular part of HVEM on HVEM expressing cell comprises a heavy chain variable region with the amino acid sequence of SEQ ID NO: 40 and a light chain

variable region with the amino acid sequence of SEQ ID NO: 41. An exemplary antibody with these characteristics is 11H7.

One aspect of the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 18-20 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 21-23 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions. Preferably, the antibody that binds an extracellular part of HVEM on HVEM expressing cells comprises a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 18-20 and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 21-23.

In a further aspect, the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain variable region with the amino acid sequence of SEQ ID NO: 16 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 17 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions. In a preferred embodiment, the 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions are located in the framework regions of the light and/or heavy chain variable region. Preferably, the antibody that binds an extracellular part of HVEM on HVEM expressing cell comprises a heavy chain variable region with the amino acid sequence of SEQ ID NO: 16 and a light chain variable region with the amino acid sequence of SEQ ID NO: 17. An exemplary antibody with these characteristics is 36H12.

One aspect of the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 34-36 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 37-39 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions. Preferably, the antibody that binds an extracellular part of HVEM on HVEM expressing cells comprises a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 34-36 and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 37-39.

In a further aspect, the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain variable region with the amino acid sequence of SEQ ID NO: 32 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 33 with 0, 1, 2, 3, 4, 5,

6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions. In a preferred embodiment, the 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions are located in the framework regions of the light and/or heavy chain variable region. Preferably, the antibody that binds an extracellular part of HVEM on HVEM expressing cell comprises a heavy chain variable region with the amino acid sequence of SEQ ID NO: 32 and a light chain variable region with the amino acid sequence of SEQ ID NO: 33. An exemplary antibody with these characteristics is 48H6.

One aspect of the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 50-52 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 53-55 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions. Preferably, the antibody that binds an extracellular part of HVEM on HVEM expressing cells comprises a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 50-52 and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 53-55.

In a further aspect, the disclosure provides an antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain variable region with the amino acid sequence of SEQ ID NO: 48 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 49 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions. In a preferred embodiment, the 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions are located in the framework regions of the light and/or heavy chain variable region. Preferably, the antibody that binds an extracellular part of HVEM on HVEM expressing cell comprises a heavy chain variable region with the amino acid sequence of SEQ ID NO: 48 and a light chain variable region with the amino acid sequence of SEQ ID NO: 49. An exemplary antibody with these characteristics is 49G4.

In one aspect, an anti-HVEM antibody or antigen-binding fragment referred to herein by sequence prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells. Exemplary antibodies are: 45H6, 11H7, 36H12, 48H6, and 49G4

In a further aspect, an anti-HVEM antibody or antigen-binding fragment referred to herein by sequence prevents binding of BTLA to an extracellular part of HVEM

on HVEM expressing cells and partially prevents binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells and does not displace LIGHT bound to the extracellular part of HVEM on HVEM expressing cells. Exemplary antibodies are: 45H6, 36H12, 48H6, and 49G4

In a further aspect, an anti-HVEM antibody or antigen-binding fragment referred to herein by sequence prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and does not prevent binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells, and does not displace LIGHT bound to the extracellular part of HVEM on HVEM expressing cells. An exemplary antibody is: 11H7.

In a further aspect, an anti-HVEM antibody or antigen-binding fragment referred to herein by sequence prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and partially prevents binding of CD160 to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells and partially displaces CD160 bound to the extracellular part of HVEM on HVEM expressing cells. Exemplary antibodies are: 45H6, 11H7, 36H12, 48H6, and 49G4

In a further aspect, an anti-HVEM antibody or antigen-binding fragment referred to herein by sequence prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells and partially prevents binding of CD160 and LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM. Preferably said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells, partially displaces CD160 bound to the extracellular part of HVEM on HVEM expressing cells, and does not displace LIGHT bound to the extracellular part of HVEM on HVEM expressing cells. Exemplary antibodies are: 45H6, 36H12, 48H6, and 49G4

In a further aspect, an anti-HVEM antibody or antigen-binding fragment referred to herein by sequence prevents binding of BTLA to an extracellular part of HVEM on HVEM expressing cells, partially prevents binding of CD160 to an extracellular part of HVEM on HVEM expressing cells, and does not prevent binding of LIGHT to an extracellular part of HVEM on HVEM expressing cells when said antibody is bound to said extracellular part of HVEM, wherein said antibody displaces BTLA bound to the extracellular part of HVEM on HVEM expressing cells, partially displaces CD160 bound to the extracellular part of HVEM on HVEM expressing

cells, and does not displace LIGHT bound to the extracellular part of HVEM on HVEM expressing cells. An exemplary antibody is: 11H7

Typically, an anti-HVEM antibody or antigen binding fragment thereof referred to herein by sequence binds the CRD1 domain of the HVEM protein.

An anti-HVEM antibody or antigen binding fragment thereof of the disclosure preferably comprises a heavy chain variable region and a light chain variable region as described herein. Such an antibody has good characteristics. It is of course possible to generate variants of such an original antibody by modifying one or more amino acids therein. Many of such variants will behave more or less similar when compared to said original. Such variants are also included in the scope of the disclosure.

Variants can have amino acid substitutions, insertions, deletions, or additions with respect to the sequence of the original antibody. An amino acid substitution is the replacement of an amino acid with another amino acid. Preferably, the amino acid is preplaced by an amino acid having similar chemical properties, which is often called conservative substitution. Amino acid deletions result in the deletion of one or multiple amino acids form the sequence. Amino acid insertions result in one or more additional amino acids in the sequence. Amino acid addition results in one or more amino acids at the start or end of the amino acid sequence.

A non-limiting example of such a modification is an antibody comprising a pyroglutamate instead of a glutamate. Other non-limiting examples of such modifications are an insertion, deletion, inversion and/or substitution of one or more amino acids when compared to said original antibody. Preferably amino acid substitutions, insertions, deletions, or additions are outside the CDR's of the variable domain. Preferably amino acid substitutions, insertions, deletions, or additions are within the framework regions of the variable region and/or in the constant region of the antibody. HVEM binding of variants can be tested as described herein.

In some embodiments, the constant region of an antibody of the invention is the constant region of an IgG, IgA, IgD, IgE or IgM antibody, such as IgG1, IgG2, IgG3 or IgG4 antibody. The constant regions may comprise modifications such as amino acid substitutions to confer specific properties to the constant regions. For instance, mutation of the IgG4 hinge region to render the antibody more stable towards the exchange of half-molecules. Other modifications affect half-life of the antibody, add or remove a glycosylation site, improve production, improve the homogeneity of the antibody product produced in large scale fermenters etc.

An antibody of the invention is preferably a murine IgG1, a human IgG1 mutated in the constant region to reduce or prevent complement activation or Fc receptor interactions, or a human IgG4, or a human IgG4 mutated to prevent the exchange of half-molecules with other IgG4 molecules.

Some variation in the CDRs (CDR1-CDR2-CDR3) of an antibody as disclosed herein is allowed. Typically, between about 0-2 amino acid substitutions, insertions, deletions, or additions are allowed in one CDR. Often more amino acid changes than 2 are allowed.

An antibody of the invention can have a heavy chain CDR1, CDR2, or CDR3 with 0-5, preferably 0-2, more preferably 2, 1, or 0 amino acid substitutions, insertions, deletions, or additions with respect to naturally occurring heavy chain CDR1, CDR2, or CDR3.

Such an antibody can have a light chain CDR1, CDR2, or CDR3 with 0-5, preferably 0-2, more preferably 0-1, more preferably 0 amino acid substitutions, insertions, deletions, or additions with respect to naturally occurring light chain CDR1, CDR2, or CDR3.

Some variation in the variable regions of an antibody as disclosed herein is allowed. Typically, between about 0-10 amino acid substitutions are allowed in a variable chain. Often more amino acid changes than 10 are allowed.

An antibody of the invention can have a heavy chain variable region with 0-15, preferably 0-10, more preferably 0-5, more preferably 5, 4, 3, 2, 1, or 0 amino acid substitutions, insertions, deletions, or additions with respect to a naturally occurring variable heavy chain.

Such an antibody can have a light chain variable region with 0-15, preferably 0-10, more preferably 0-5, more preferably 5, 4, 3, 2, 1, or 0 amino acid substitutions, insertions, deletions, or additions with respect to a naturally occurring light chain variable region.

Some variations in the constant region of an antibody as disclosed herein is allowed. Typically, between about 0-10 amino acid substitutions are allowed in the constant region. Often more amino acid changes than 10 are allowed. An antibody of the invention can have a heavy chain constant region (CH1-CH2-CH3) with 0-15, preferably 0-10, more preferably 0-5, more preferably 5, 4, 3, 2, 1, or 0 amino acid substitutions with respect to a naturally occurring heavy chain constant region (CH1-CH2-CH3). Such an antibody can have a light chain constant region with 0-5, preferably 5, 4, 3, 2, 1, or 0 amino acid substitutions with respect to a naturally occurring light chain constant region.

Some variation in IgG4 occurs in nature and/or is allowed without changing the immunological properties of the resulting antibody. An antibody with an IgG4 constant region or a mutated IgG1 constant region has at least most of the pharmacological properties of an antibody but does not bind complement, and will thus not induce depletion of the cells its binds to *in vivo*. Preferably said constant region is a constant region of a human antibody (chimeric).

Preferably, said constant region is a region that is deficient in complement activation, preferably a human IgG_4 constant region or a mutated human IgG_1 constant region.

HVEM binding by an antibody and antigen binding fragments thereof disclosed herein can be confirmed in a number of suitable assays known to the skilled person. Such assays include, e.g., affinity assays, e.g., western blots, radioimmunoassay, FACS, and ELISA (enzyme-linked immunosorbent assay). The examples (e.g., Example 2 c and 6a) describe in detail some of the many assays which can be used to measure HVEM binding, as well as a method to determine the relative binding affinity of an antibody for human HVEM.

In a further aspect, the disclosure provides a nucleic acid molecule or nucleic acid molecules encoding an antibody as disclosed herein or an antigen binding fragment thereof as disclosed herein. Further provided is a nucleic acid molecule encoding a variable region as disclosed herein. A nucleic acid as used in the disclosure is typically but not exclusively a ribonucleic acid (RNA) or a deoxyribonucleic acid (DNA). Based on the genetic code, a skilled person can determine the nucleic acid sequence which encode an antibody variant as disclosed herein. Based on the degeneracy of the genetic code, sixty-four codons may be used to encode twenty amino acids and translational terminal signal. As is known to a skilled person, codon usage bias in different organisms can affect gene expression level. Various computational tools are available to the skilled person in order to optimize codon usage depending on which organisms the desired nucleic acid will be expressed.

In a further aspect, the disclosure provides a vector comprising a nucleic acid sequence molecule as described herein. The term “vector” as used herein refers to a nucleic acid molecule, such as a plasmid, bacteriophage or animal virus, capable of introducing a heterologous nucleic acid sequence into a host cell. A vector according to the invention allows the expression or production of an antibody of the invention encoded by the heterologous nucleic acid sequence in a host cell. A vector used in accordance with the invention is for instance derived from an animal virus, examples of which include, but not limited to, vaccinia virus (including attenuated derivatives such as the Modified Vaccinia virus Ankara, MVA), Newcastle Disease virus (NDV), adenovirus or retrovirus. A vector according to the invention preferably comprises an expression cassette comprising a promoter that is suitable for initiation of transcription of an antibody according to the invention in the selected host cells. Examples of suitable promoters for expression of polypeptides according to the invention in eukaryotic host cells include, but are not limited to, beta-actin promoter, immunoglobin promoter, 5S RNA promoter, or virus derived promoters such as cytomegalovirus (CMV), Rous sarcoma virus (RSV) and Simian virus 40 (SV40) promoters for mammalian hosts.

When a nucleic acid molecule or nucleic acid molecules as disclosed herein is/are expressed in a cell, the cell may produce an antibody according to the disclosure. Therefore, in one embodiment, a cell is provided comprising an antibody, a nucleic acid molecule or molecules and/or a vector according to the

disclosure. The host cells may be a mammalian, insect, plant, bacterial or yeast cell. Said cell is preferably an animal cell, preferably a mammalian cell, most preferably a human cell. Examples of mammalian cell lines suitable as host cells include a hybridoma cell, a Chinese hamster ovary (CHO) cell, an NSO cell, or a PER-C6™ cell. For the purpose of the disclosure a suitable cell is any cell capable of comprising and preferably of producing said antibodies and/or said nucleic acids. The disclosure further encloses cell cultures that comprise said cells.

The term "host cell" refers to a cell into which an expression vector has been introduced. The term encompasses not only the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in successive generations due to either environmental influences or mutation, such progeny may not be identical to the parent cell but are still included within the scope of the term "host cell."

An antibody as disclosed herein can be produced by any method known to a skilled person. In a preferred embodiment, the antibodies are produced using a cell, preferably wherein the cell is a hybridoma cell, a CHO cell, an NS0 cell or a PER-C6™ cell. In a particular preferred embodiment said cell is a CHO cell, preferably said cell is cultured in serum free medium. This includes harvesting said antibody form said culture. The antibody is preferably purified form the medium, preferably said antibody is affinity purified. Alternatively, said antibodies can be generated synthetically.

Various institutions and companies have developed cell lines for the large-scale production of antibodies, for instance for clinical use. These cells are also used for other purposes such as the production of proteins. Cell lines developed for industrial scale production of proteins and antibodies are herein further referred to as industrial cell lines. Thus, a preferred embodiment of the disclosure provides the use of a cell line developed for the large-scale production of said antibodies.

An antibody according to the invention exhibits a number of activities that can be advantageously used in therapeutic and non-therapeutic uses. In particular, antibodies according to the invention are useful for the treatment of an individual. Preferably, the antibodies according to the invention are useful for the treatment of immune related diseases or prevention against immune related diseases. In a preferred embodiment the antibodies according to the invention are useful for the treatment of cancer. In some embodiments, an antibody according to the invention is preferably used in therapy, preferably human therapy. In some embodiments, an antibody as disclosed herein may be used for research purposes. For example, in in vitro experiments, cell culture, organotypic culture and in vivo models.

Also described are methods for treatment of cancer. Examples of cancers are e.g.: melanoma, esophageal squamous cell carcinoma, hepatocellular carcinoma, and colorectal cancer.

The invention provides a method for the treatment of a subject suffering from cancer comprising administering to said subject a therapeutically effective amount of an antibody as disclosed herein. Further provided is a method for the preparation of a medicament for the treatment of a subject suffering from cancer. The disclosure describes methods for preventing inhibition of T-cell activation by preventing binding of BTLA and HVEM, and CD160 and HVEM.

The invention provides a method for the treatment of a subject suffering from inflammatory diseases comprising administering to said subject a therapeutically effective amount of an antibody as disclosed herein. Further provided is a method for the preparation of a medicament for the treatment of a subject suffering from inflammatory diseases. The disclosure describes methods for preventing inhibition of T-cell activation by preventing binding of BTLA and HVEM, and CD160 and HVEM.

The disclosure further comprises a pharmaceutical composition comprising an antibody or antigen binding fragment thereof as disclosed herein, or a nucleic acid encoding same, or a cell comprising an antibody or antigen binding fragment thereof as disclosed herein, or a nucleic acid encoding same. Provided are pharmaceutical compositions comprising a polypeptide according to the invention or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier, diluent and/or excipient. Such compositions are especially suited for use as a medicament. The compositions may be in any suitable forms, such as liquid, semi-solid and solid dosage forms. The dosage and scheduling for the formulation, which is selected can be determined by standard procedures, well known by a skilled person. Such procedures involve extrapolating and estimating dosing schedule form animal models, and then determining the optimal dosage in a human clinical dose ranging study. The dosage in pharmaceutical compositions will vary depending upon a number of factors, such as the desired release and pharmacodynamic characteristics.

One embodiment of the disclosure provides a pharmaceutical composition as described herein for use as a prophylactic or in the treatment of cancer and/or immune–related disorders.

Further provided are methods to modulate HVEM signalling activity. The term “to modulate” refers to the activity of adjusting an output signal of a system. The output signal or output can be adjusted in such a way that an inhibitory output signal becomes stimulatory and vice versa. The invention provides a method for the modulation of HVEM signalling, comprising administering to said subject a therapeutically effective amount of an antibody as disclosed herein. Without being bound by theory, it is believed that HVEM delivers coinhibitory signals to a T cell expressing BTLA or CD160. LIGHT and TNFß, on the other hand deliver costimulatory signals to a T cell when interacting with HVEM expressed on the T cell. When LIGHT and/or TNFß, BTLA and/or CD160 simultaneously interact with

HVEM, the net result is an inhibitory signal for T-cell activation. This interaction is bidirectional: HVEM induces inhibitory signals in T cells after interaction with BTLA and CD160 on T cells, while both BTLA and CD160 act as activating ligands for HVEM resulting in NFκB activation. Furthermore, LIGHT delivers costimulatory signals to a T cell when interacting with HVEM expressed on the T cell, and HVEM has also been implicated to transmit costimulatory signals to a T cell when interacting with LIGHT expressed by the T cell. However, LIGHT does not contain an obvious signalling motif and its mechanism for signalling is incompletely defined.

Costimulatory and co-inhibitory signalling relayed by HVEM and BTLA in a T cell can be measured in levels of NFκB or NFAT, as well as the release of IL-2, TNFα and IFNγ. Methods to measure levels of NFAT are known in the art. An exemplary method is described, for instance, in example 3c, of which the results are depicted in figure 4A-B. Methods to measure levels of NFκB are known in the art. An exemplary method is described, for instance in example 6f, of which the results are depicted in figure 8A-C. Methods to measure levels of IL-2, TNFα and IFNγ are known in the art. An exemplary method is described, for instance, in example 6k, of which the results are depicted in figure 11A-C.

In one aspect the disclosure provides a method for modulating HVEM signalling activity, comprising contacting HVEM expressing cells with an antibody or antigen binding fragment thereof, a nucleic acid molecule or a vector as disclosed herein. In one aspect the disclosure provides a method for increasing an immune response in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a composition comprising an antibody or antigen binding fragment thereof, a nucleic acid molecule or a vector as disclosed herein.

In one aspect the disclosure provides a method for reducing tumor growth in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a composition comprising an antibody or antigen binding fragment thereof, a nucleic acid molecule or a vector as disclosed herein.

As used herein, an “subject” is a human or an animal. Subjects include, but are not limited to, mammals such as humans, pigs, ferrets, seals, rabbits, cats, dogs, cows and horses, and birds such as chickens, ducks, geese and turkeys. In a preferred embodiment of the invention, a subject is a mammal. In a particularly preferred embodiment, the subject is a human.

The term "antigen-binding fragment" of an antibody refers to one or more portions of a full-length antibody that retain the ability to bind to the same antigen (i.e., human HVEM) that the antibody binds to. The term "antigen-binding fragment" also encompasses a portion of an antibody that is part of a larger

molecule formed by non-covalent or covalent association or of the antibody portion with one or more additional molecular entities. Examples of additional molecular entities include amino acids, peptides, or proteins, such as the streptavidin core region, which may be used to make a tetrameric scFv molecule (Kipriyanov et al. Hum Antibodies Hybridomas 1995; 6(3): 93-101). An exemplary antigen-binding fragment is a VH and/or a VL of an antibody. Antigen-binding fragments include Fab, F(ab'), $F(ab')_2$, complementarity determining region (CDR) fragments, single-chain antibodies (scFv), bivalent single-chain antibodies, and other antigen recognizing immunoglobulin fragments. In some instances, the term “antibody” as used herein can be understood to also include an antigen binding fragment thereof.

The term "human antibody" refers to an antibody consisting of amino acid sequences of human immunoglobulin sequences only. A human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell or in a hybridoma derived from a mouse cell. Human antibodies may be prepared in a variety of ways known in the art.

The term "epitope" refers to the part of an antigen that is capable of specific binding to an antibody, or T-cell receptor or otherwise interacting with a molecule. "Epitope" is also referred to in the art as the "antigenic determinant". An epitope generally consists of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains. An epitope may be "linear" or "non-linear/conformational". Once a desired epitope is determined (e.g., by epitope mapping), antibodies to that epitope can be generated. The generation and characterization of antibodies may also provide information about desirable epitopes. From this information, it is then possible to screen antibodies for those which bind to the same epitope e.g. by conducting cross-competition studies to find antibodies that competitively bind with one another, i.e., the antibodies compete for binding to the antigen.

As used herein, "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, the verb “to consist” may be replaced by “to consist essentially of” meaning that a compound or adjunct compound as defined herein may comprise additional component(s) than the ones specifically identified, said additional component(s) not altering the unique characteristic of the invention.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

The word “approximately” or “about” when used in association with a numerical value (approximately 10, about 10) preferably means that the value may be the given value of 10 more or less 1% of the value.

As used herein, the terms "treatment," "treat," and "treating" refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (e.g., in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example to prevent or delay their recurrence.

For the purpose of clarity and a concise description features are described herein as part of the same or separate embodiments. However, it will be appreciated that the scope of the invention may include embodiments having combinations of all or some of the features described.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

The invention is further explained in the following examples. These examples do not limit the scope of the invention, but merely serve to clarify the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Flow-cytometric binding characteristics of mouse anti-human HVEM antibodies to membrane-bound full-length human HVEM, to membrane-bound human HVEM deleted for CRD1, or to membrane-bound full-length cynomolgus HVEM on HEK293F cells. Dashed line represents background (i.e., no binding of mouse anti-human HVEM antibodies).

Figure 2. Effect of mouse anti-human HVEM antibodies on binding of (A) soluble human BTLA and of (B) soluble human LIGHT to membrane-bound full-length human HVEM on HEK293F cells. Dashed line represents negative controls (i.e., ligand/receptor binding without addition of mouse anti-human antibody or with addition of a mouse IgG1 negative isotype control = 100% binding of ligands to HVEM receptor).

Figure 3. (A) Effect of mouse anti-human HVEM antibodies on NFκB signalling in membrane-bound human HVEM expressing cells. Soluble human LIGHT ligand was included as reference. (B) Effect of mouse anti-human HVEM antibodies on

soluble human LIGHT (≈EC80)-induced NFκB signalling in membrane-bound human HVEM expressing cells. Mean ± SD (n = 2) are shown.

Figure 4. (A) Assay principle of the NFAT-response element-luciferase (RE-luc) human BTLA/HVEM Blockade Bioassay: a combination of (1) membrane human HVEM and proprietary membrane human T cell receptor (TCR) activator expressing CHO-K1 Activator cells (i.e., artificial antigen-presenting cells (aAPC)) and of (2) membrane human BTLA and membrane human TCR complex expressing NFAT-RE-luc Jurkat Effector T cells is used to examine the ability of mouse anti-human HVEM antibodies to block the BTLA/HVEM-mediated inhibition of TCR-induced NFAT signalling. (B) Effect of mouse anti-human HVEM antibodies on membrane-bound human BTLA/human HVEM-mediated inhibition of TCR-induced NFAT signalling in membrane-bound human BTLA/human TCR expressing Jurkat Effector T cells. Mean ± SD (n = 2) are shown.

Figure 5. Flow-cytometric binding characteristics of purified BTLA/HVEM blocking mouse versus chimeric mouse/human anti-human HVEM antibodies to membrane-bound (full-length) human HVEM on HEK293F cells. Mean ± SD (n = 2) are shown.

Figure 6. Effect of purified BTLA/HVEM blocking mouse versus chimeric mouse/human anti-human HVEM antibodies on binding of (A) soluble human BTLA, of (B) soluble human CD160, of (C) soluble human LIGHT, and of (D) soluble human TNFβ to membrane-bound full-length human HVEM on HEK293F cells. Dashed line represents negative controls (i.e., ligand/receptor binding with addition of a mouse IgG1 or a human IgG4 negative isotype control = 100% binding of ligands to HVEM receptor). Mean ± SD (n = 2-3) are shown from one (D), two (A and B), or three (C) independent performed experiments.

Figure 7. Effect of purified BTLA/HVEM chimeric mouse/human anti-human HVEM antibodies on displacement of pre-bound (A) soluble human BTLA, of (B) soluble human CD160, of (C) soluble human LIGHT, and of (D) soluble human TNFβ from membrane-bound full-length human HVEM on HEK293F cells. Dashed line represents negative controls (i.e., ligand/receptor binding with addition of a human IgG4 negative isotype control = 100% binding of ligands to HVEM receptor). Mean ± SD (n = 2) are shown.

Figure 8. (A) Effect of purified BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on NFκB signalling in membrane-bound human HVEM expressing cells. Soluble human LIGHT ligand was included as reference. (B) Effect of non-cross-linked versus cross-linked purified BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on NFκB signalling in membrane-bound human HVEM expressing cells. Soluble human LIGHT ligand was included as reference. (C) Effect of purified BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on soluble human LIGHT (≈EC80)-induced NFκB signalling in membrane-bound human HVEM expressing cells.

Mean ± SD (n = 2) are shown from one (B) or two (A and C) independent performed experiments.

Figure 9. (A) Effect of soluble human TNFβ ligand on NFκB signalling in membrane-bound human HVEM expressing cells. (B) Effect of purified BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on soluble human TNFβ (≈EC80)-induced NFκB signalling in membrane-bound human HVEM expressing cells. Mean ± SD (n = 3) are shown.

Figure 10. Effect of purified BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on membrane-bound human BTLA/human HVEM-mediated inhibition of TCR-induced NFAT signalling in membrane-bound human BTLA/human TCR expressing Jurkat Effector T cells. Mean ± SD (n = 2) are shown from two independent performed experiments.

Figure 11. Effect of purified BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on membrane-bound human BTLA/human HVEM-mediated inhibition of TCR-induced (figures upper row) and of TCR/CD28-induced (figures lower row) IL-2 (A), TNFα (B) or IFNγ (C) release from membrane-bound human BTLA/human TCR expressing primary naïve human T cells enriched from 6 healthy donors (donor A, C, D, G, H and K). Dashed line represents basal cytokine release (i.e., exposure to human IgG4/κ negative isotype control). Mean ± SD (n=5) are shown.

EXAMPLES

Example 1. Generation of mouse anti-human HVEM monoclonal antibodies

(a). Generation of Sf9 insect cells and HEK293F cells expressing surface human HVEM

cDNA encoding for human HVEM protein (Swiss-Prot no. Q92956.3; see SEQ ID NO. 1) was optimized for mammalian expression and synthesized by GENEART, Regensburg, Germany (see SEQ ID NO. 2). This cDNA was subcloned in baculovirus transfer plasmid pVL1393 (BD transfection kit cat no. 560129; BD Biosciences). Subsequently, Sf9 insect cells (Spodoptera frugiperda) were transfected with transfer plasmid pVL1393 containing cDNA encoding human HVEM using the baculoCOMPLETE all-in-one kit (Oxford Expression Technologies), and then incubated at 27°C for 4-5 days. After this transfection step, supernatant was collected and stored at 4°C, and used to infect more Sf9 insect cells for virus amplification. For this purpose, Sf9 insect cells were transfected with amplified recombinant baculovirus, and then incubated at 27°C for 3-5 days. These Sf9 insect cells were harvested, washed with sterile PBS, and aliquoted at 20.0 x 10^6 cells/mL in PBS and stored at -80°C to obtain cell lysates. Prior to storage,

human HVEM surface expression on transfected Sf9 insect cells was confirmed using 1:20 diluted phycoerythrin (PE)-conjugated mouse anti-human HVEM antibody (clone eBioHVEM-122; eBioscience) and flow cytometry.

cDNA encoding for human HVEM protein (Swiss-Prot no. Q92956.3; see SEQ ID NO. 1) was optimized for mammalian expression and synthesized by GENEART, Regensburg, Germany (see SEQ ID NO. 2). This cDNA was subcloned in a pcDNA3.1-derived expression plasmid. This full-length human HVEM plasmid was transfected in FreeStyle™ 293F cells (Life Technologies) using the FreeStyle™ 293 Expression System (Life Technologies). Stable human full-length HVEM-transfected HEK293F cells (clone no. 128) were selected using 125 µg/mL G418 (Gibco). These HEK293F cells were harvested, washed with sterile PBS, and aliquoted at 19.0 x 10^6 cells/mL in PBS and stored at -80°C to obtain cell lysates. Prior to storage, human HVEM surface expression on transfected HEK293F cells was confirmed using 1:20 diluted phycoerythrin (PE)-conjugated mouse anti-human HVEM antibody (clone eBioHVEM-122; eBioscience) and flow cytometry.

(b). Immunization and generation of mouse anti-human HVEM monoclonal antibodies

Two immunization protocols were applied:

During the first immunization protocol, BALB/c mice (females, 6-8 weeks of age; Charles River Laboratories) were subcutaneously injected with ≈ 500 µL soluble recombinant C-terminal polyhistidine-tagged human extracellular HVEM domain (NCBI Ref SEQ NP_003811.2; Sino Biological Inc) in water-in-oil emulsified Complete Freund's Adjuvant (CFA; Sigma) or in oil-in-water emulsified Sigma Adjuvant System® (SAS; Sigma) on Day 0; each mouse was injected with 25 µg recombinant human HVEM in 250 µL PBS mixed with 250 µL CFA or SAS. On Day 21, antibody responses were boosted by subcutaneous injections with recombinant human HVEM in Incomplete Freund's Adjuvant (IFA; Sigma) or SAS; each mouse was injected with 25 µg recombinant human HVEM in 250 µL PBS mixed with 250 µL IFA or SAS. On Day 42 and on Day 87, mice were boosted again by subcutaneous injections with recombinant human HVEM and human HVEM-transfected Sf9 insect cell lysate in IFA or SAS; each mouse was injected with 25 µg recombinant human HVEM and human HVEM-transfected Sf9 insect cell lysate (prepared from 1.8 x 10^6 viable and membrane-bound HVEM expressing cells) in 250 µL PBS mixed with 250 µL IFA or SAS. Finally, mice were intraperitoneally injected with recombinant human HVEM and human HVEM-transfected Sf9 insect cell lysate without adjuvant on Day 98 and on Day 99; each mouse was injected with 25 µg recombinant human HVEM and human HVEM-transfected Sf9 insect cell lysate (prepared from 1.8 x 10^6 viable and membrane-bound HVEM expressing cells) in 250 µL PBS. On Day 102, splenocytes from immunized mice were fused with SP2/0-Ag14 myeloma cells (DSMZ) using standard hybridoma technology

originally described by Köhler and Milstein (Nature 1975, 256: 495) as described below.

During the second immunization protocol, BALB/c mice (females, 6-8 weeks of age; Charles River Laboratories) were subcutaneously injected with ≈ 500 µL soluble recombinant C-terminal polyhistidine-tagged human extracellular HVEM domain (NCBI Ref SEQ NP_003811.2; Sino Biological Inc) and human HVEM-transfected Sf9 insect cell lysate or human HVEM-transfected HEK293F cell lysate in CFA or SAS or without adjuvant on Day 0; each mouse was injected with 10-20 µg recombinant human HVEM and human HVEM-transfected Sf9 insect cell or HEK293F cell lysate (both prepared from 5.0 x 10^6 viable and membrane-bound HVEM expressing cells) in 250 µL PBS mixed with or without 250 µL CFA or SAS. On Day 21 and on Day 42, antibody responses were boosted by subcutaneous injections with recombinant human HVEM and human HVEM-transfected Sf9 insect cell lysate or human HVEM-transfected HEK293F cell lysate in IFA or SAS or without adjuvant; each mouse was injected with 10-20 µg recombinant human HVEM and human HVEM-transfected Sf9 insect cell or HEK293F cell lysate (both prepared from 5.0 x 10^6 viable and membrane-bound HVEM expressing cells) in 250 µL PBS mixed with or without 250 µL IFA or SAS. Finally, mice were intraperitoneally injected with recombinant human HVEM and human HVEM-transfected Sf9 insect cell lysate or human HVEM-transfected HEK293F cell lysate without adjuvant on Day 59 and on Day 64; each mouse was injected with 10-20 µg recombinant human HVEM and human HVEM-transfected Sf9 insect cell or HEK293F cell lysate (both prepared from 5.0 x 10^6 viable and membrane-bound HVEM expressing cells) in 250 µL PBS. On day 67, splenocytes from immunized mice were fused with SP2/0-Ag14 myeloma cells (DSMZ) using standard hybridoma technology originally described by Köhler and Milstein (Nature 1975, 256: 495). Briefly, immunized mice were sacrificed. Splenocytes were teased from spleens, and washed in serum-free opti-MEM® I with GlutaMax medium (SF medium; Invitrogen). Logarithmically growing SP2/0-Ag14 myeloma cells were washed in SF medium, and added to the splenocytes yielding a 5:1 ratio of splenocytes-to-myeloma cells. The cells were then pelleted, and the supernatant was removed. One ml of a **37%** (v/v) solution of polyethylene glycol 4000 (Merck) was then added dropwise over a 60-sec period, after which the cells were incubated for another 60-sec at 37°C. Eight ml SF medium, followed by 5 ml opti-MEM® I with GlutaMax/10% (v/v) fetal calf serum (FCS; Bodinco), was then slowly added with gentle agitation. After 30 minutes at room temperature (RT), the cells were pelleted, washed in opti-MEM® I with GlutaMax/10% FCS to remove residual polyethylene glycol, and finally plated at a concentration of 0.1 x 10^6 cells/200 µL per well in aminopterin selection medium, i.e., opti-MEM® I with GlutaMax/10% FCS that was supplemented with 50x Hybri-Max™ aminopterin (a *de novo* DNA synthesis inhibitor; Sigma). From Day 7, aminopterin selection medium was

replenished every 2-3 days, and on Day 13-14, aminopterin selection medium was replaced by opti-MEM I with GlutaMax/10% FCS.

(c). Screening for the presence mouse anti-human HVEM monoclonal antibodies

From Day 13 after fusion, supernatants from growing hybridomas were screened for the presence of mouse anti-human HVEM antibodies (i.e., 'high affinity' IgGs, as opposed to 'low affinity' IgMs) using an ELISA with soluble recombinant C-terminal polyhistidine-tagged human HVEM (rhuHVEM; Sino Biological) as target protein. To this end, rhuHVEM was coated at 1 µg/mL in PBS (50 ng/50 µL/well) using half-area flat-bottomed 96-wells EIA plates (Corning) during 16-24 hours at 4-8°C. After extensive washing with PBS/0.05%, w/v, Tween 20, plates were blocked with PBS/0.05% Tween 20/1%, w/v, bovine serum albumin (BSA; Roche) for 1 hour at RT. Subsequently, plates were incubated with 50 µL undiluted hybridoma supernatant/well for 1 hour at RT. After extensive washing in PBS/0.05% Tween 20, binding of antibodies was determined with 1:5000 diluted horseradish peroxidase-conjugated goat anti-mouse IgG Fcγ-specific antibodies (Jackson ImmunoResearch) for 1 hour at RT, followed by a ready-to-use solution of TMB substrate (Invitrogen) for colorimetric detection. After adding 1 M H_2SO_4, binding of antibodies was measured at wavelength of 450 nm (reference wavelength of 655 nm) using a microplate reader (model iMark; BioRad).

From Day 13 after fusion, supernatants from growing hybridomas were also screened and confirmed for mouse anti-human HVEM antibodies (i.e., 'high affinity' IgGs, as opposed to 'low affinity' IgMs) production using a cell-based ELISA with membrane-bound human HVEM as target protein. To this end, stable human full-length HVEM-transfected HEK293F cells (clone no. 128; see above Example 1a) were coated at 2 x 10^6 viable cells/mL in PBS (0.1 x 10^6 viable cells/50 µL/well) using half-area flat-bottomed 96-wells EIA plates (Corning) during 16-24 hours at 4-8°C. Non-transfected (i.e., negative for membrane-bound human HVEM expression) wild type (WT) HEK293F coated cells were run in parallel as a negative control. After extensive washing with PBS/0.05%, w/v, Tween 20, plates were blocked with PBS/0.05% Tween 20/1%, w/v, BSA (Roche) for 1 hour at RT. Subsequently, plates were incubated with 50 µL undiluted hybridoma supernatant/well for 1 hour at RT. After extensive washing in PBS/0.05% Tween 20, binding of antibodies was determined with 1:5000 diluted horseradish peroxidase-conjugated goat anti-mouse IgG Fcγ-specific antibodies (Jackson ImmunoResearch) for 1 hour at RT, followed by a ready-to-use solution of TMB substrate (Invitrogen) for colorimetric detection. After adding 1 M H_2SO_4, binding of antibodies was measured at wavelength of 450 nm (reference wavelength of 655 nm) using a microplate reader (model iMark; BioRad).

From Day 13 after fusion, supernatants from growing hybridomas were also screened and confirmed for mouse anti-human HVEM antibodies (i.e., 'high affinity' IgGs, as opposed to 'low affinity' IgMs) production using FACS with membrane-bound human HVEM as target protein. To this end, stable human full-length HVEM-transfected HEK293F cells (clone no. 128; see above Example 1a) were put at 10 x 10^6 cells/mL in ice-chilled phosphate-buffered saline containing 0.1% BSA (Sigma)/0.05% NaN_3 (PBS/BSA/NaN_3) supplemented with 50 µg/mL human IgGs (blocking of possible Fcγ receptors; Sigma) for 10 minutes at 4°C. Then, 10 µL/tube (i.e., 0.1 x 10^6 cells) of these cells were incubated with 100 µL undiluted hybridoma supernatant/tube for 30 minutes at 4°C. Non-transfected (i.e., negative for membrane-bound human HVEM expression) WT HEK293F cells were run in parallel as a negative control to determine antibody specificity. After extensive washing in PBS/BSA/NaN_3, cells were subsequently incubated with 1:200 diluted PE-conjugated goat anti-mouse IgG Fcγ-specific antibodies (Jackson ImmunoResearch) for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were fixed in 2% formaldehyde in PBS/BSA/NaN_3 for 30 minutes at 4°C. Binding of antibodies was measured using a flow cytometer (model FACSCalibur; BD Biosciences).

Triple HVEM positive (i.e., rhuHVEM+ in ELISA, membrane HVEM+ HEK293F cells in ELISA, and membrane HVEM+ HEK293F cells in FACS) hybridomas were expanded and cryopreserved. Supernatants from these triple HVEM positive hybridomas showed no reactivity with non-transfected (i.e., negative for membrane-bound human HVEM expression) WT HEK293F cells. Using this approach yielded 18 anti-human HVEM-specific antibody-producing hybridomas. Mouse antibodies were purified from these anti-human HVEM-specific antibody-producing hybridoma supernatants using protein G columns (GE Healthcare). Heavy and light chains were typed for isotype class using the IsoStrip™ Mouse Monoclonal Antibody Isotyping kit (Roche). Subsequently, supernatants and/or purified antibodies from these anti-human HVEM-specific antibody-producing hybridomas were tested for their effect on human HVEM-ligand (i.e., human BTLA and human LIGHT) binding to membrane-bound human HVEM, for their binding to membrane-bound human HVEM deleted for cysteine-rich domain 1 (CRD1), and for their cross-reactivity to membrane-bound cynomolgus monkey HVEM, as described in Example 2. In addition, a selection of these purified antibodies from these anti-human HVEM-specific antibody-producing hybridomas were tested for their effect on NFκB signalling in membrane human HVEM expressing cells, on soluble human LIGHT-induced NFκB signalling in membrane human HVEM expressing cells, and on membrane human BTLA/human HVEM-mediated inhibition of TCR-induced NFAT signalling in membrane human BTLA/human TCR expressing cells, as described in Example 3.

Example 2. Flow cytometric characterization of mouse anti-human HVEM monoclonal antibodies

(a). Binding of mouse anti-human HVEM antibodies to membrane-bound full-length human HVEM and to membrane-bound human HVEM deleted for CRD1

In order to analyse the fine specificity of mouse anti-human HVEM antibodies, the location of epitope(s) recognized by the generated mouse anti-human HVEM antibodies was determined by domain mapping. The ability of mouse anti-human HVEM antibodies to bind to the CRD1 truncated human HVEM, expressed on the surface of (HEK-derived) 293F cells, was determined by FACS analysis.

Based on literature (Swiss-Prot no. Q92956.3; Montgomery et al. Cell 1996; 87:427-436; Hsu et al. J Biochem Chem 1997; 272: 13471-13474; Naismith et al. Trends Biochem Sci 1998; 23: 74-79; Carfi et al. Molecul Cell 2001; 8: 169-179; Bodmer et al. Trends Biochem Sci 2002; 27: 19-26; Compaan et al. J Biochem Chem 2005; 280: 39553-39561), cysteine-rich domains (CRD) in the extracellular region of human HVEM were identified, and are coded CRD1, CRD2, CRD3, and (truncated) CRD4. CRDs contain topologically distinct types of modules, called an A-module and a B-module. A-modules are C-shaped structures, and B-modules are S-shaped structures. A typical CRD is usually composed of A1-B2-modules or A2-B1-modules (or, less frequently, a different pair of modules, like A1-B1) with 6 conserved cysteine residues, wherein the numeral denotes the number of disulphide bridges within each module. Human HVEM-CRD1 comprises A1-B2-modules (42-75, see SEQ ID NO. 1), human HVEM-CRD2 comprises A1-B2-modules (78-119, see SEQ ID NO. 1), human HVEM-CRD3 comprises an A2-module and a non-canonical (reminiscent of a B1-module) B0-module (121-162, see SEQ ID NO. 1), and human truncated HVEM-CRD4 comprises only an A1-module (165-179, see SEQ ID NO. 1). Two different human HVEM constructs were generated and expressed: (1) full-length human HVEM construct, which starts with N-terminal CRD1 (i.e., CRD1 A1-B2-modules cover amino acids 42-75, see SEQ ID NO. 1), and therefore denoted as 'full-length', and comprised amino acids 1-283 (see SEQ ID NO. 1), and (2) 'CRD1 truncated' construct, which starts with N-terminal CRD2 (i.e., CRD2 A1-B2-modules cover amino acids 22-63, see SEQ ID NO. 3), and comprised amino acids 20-227 linked to a mouse Ig signal peptide amino acids 1-19 (see SEQ ID NO. 3). cDNA encoding for CRD1 truncated human HVEM protein (Swiss-Prot no. Q92956.3) was optimized for mammalian expression and synthesized by GENEART, Regensburg, Germany (see SEQ ID NO. 4). This cDNA was subcloned in a pcDNA3.1-derived expression plasmid.

Generation of human 'full-length' HVEM-transfected HEK293F cells (clone no. 128) is described in Example 1a. Using the FreeStyleTM 293 Expression System (Invitrogen), FreeStyleTM 293F cells (Invitrogen) were transiently transfected with the 'CRD1 truncated' variants of human HVEM. After 72 hours,

surface human HVEM expression on transfected cells was analysed by FACS analysis. To this end, stable human full-length HVEM-transfected HEK293F cells and transient human 'CRD1 truncated' HVEM-transfected HEK293F cells were put at 10 x 10^6 cells/mL in ice-chilled phosphate-buffered saline containing 0.1% BSA (Sigma)/0.05% NaN_3 (PBS/BSA/NaN_3) supplemented with 50 µg/mL human IgGs (blocking of possible Fcγ receptors; Sigma) for 10 minutes at 4°C. Then, 10 µL/tube (i.e., 0.1 x 10^6 cells) of these cells were incubated with 100 µL undiluted hybridoma supernatant/tube for 30 minutes at 4°C. Phycoerythrin (PE)-conjugated mouse anti-human HVEM antibody (clone eBioHVEM-122; eBioscience) at 1:20 was run as a positive control antibody. After extensive washing in PBS/BSA/NaN_3, cells were subsequently incubated with 1:200 diluted PE-conjugated goat anti-mouse IgG Fcγ-specific antibodies (Jackson ImmunoResearch) for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were fixed in 2% formaldehyde in PBS/BSA/NaN_3 for 30 minutes at 4°C. Binding of antibodies was measured using a flow cytometer (model FACSCalibur; BD Biosciences).

As shown in figure 1, all 18-examined mouse anti-human HVEM antibodies recognized full-length human HVEM on transfected HEK293F cells, whereas most (15/18) of these mouse anti-human HVEM antibodies showed no binding on 'CRD1 truncated' human HVEM on transfected HEK293F cells. In contrast, mouse anti-human HVEM antibodies no. 38G10, 39B9 and 47E10 recognized 'CRD1 truncated' human HVEM on transfected HEK293F cells.

These results demonstrated that mouse anti human HVEM antibodies no. 38G10, 39B9 and 47E10 seemed to recognize linear and/or non-linear/conformational epitopes in CRD2, CRD3, CRD4, and/or the 'linker' fragment (aa sequence 124-146, see SEQ ID NO. 3) of the extracellular domain of human HVEM, whereas all other generated mouse anti-human HVEM antibodies (15/18) seemed to recognize linear and/or non-linear/conformational epitopes in CRD1 of the extracellular domain of human HVEM.

(b). Binding of mouse anti-human HVEM antibodies to membrane-bound cynomolgus monkey HVEM

In order to analyse the multispecies cross-reactivity of mouse anti-human HVEM antibodies, the ability of mouse anti-human HVEM antibodies to bind to the cynomolgus monkey HVEM, expressed on the surface of (HEK-derived) 293F cells, was determined by FACS analysis.

cDNA encoding for cynomolgus monkey HVEM protein (see SEQ ID NO. 5; NCBI Reference Sequence XP_005545061.1) was optimized for mammalian expression and synthesized by GENEART, Regensburg, Germany (see SEQ ID NO. 6). This cDNA was subcloned in a pcDNA3.1-derived expression plasmid.

Generation of human 'full-length' HVEM-transfected HEK293F cells (clone no. 128) is described in Example 1a. Using the FreeStyle™ 293 Expression System (Invitrogen), FreeStyle™ 293F cells (Invitrogen) were transiently transfected with cynomolgus monkey (full-length) HVEM. After 72 hours, surface cynomolgus monkey HVEM expression on transfected cells was analysed by FACS analysis. To this end, stable human full-length HVEM-transfected HEK293F cells and transient cynomolgus monkey full-length HVEM-transfected HEK293F cells were put at 10 x 10^6 cells/mL in ice-chilled phosphate-buffered saline containing 0.1% BSA (Sigma)/0.05% NaN_3 (PBS/BSA/NaN_3) supplemented with 50 µg/mL human IgGs (blocking of possible Fcγ receptors; Sigma) for 10 minutes at 4°C. Then, 10 µL/tube (i.e., 0.1 x 10^6 cells) of these cells were incubated with 100 µL undiluted hybridoma supernatant/tube for 30 minutes at 4°C. Phycoerythrin (PE)-conjugated mouse anti-human HVEM antibody (clone eBioHVEM-122; eBioscience) at 1:20 was run as a positive control antibody. After extensive washing in PBS/BSA/NaN_3, cells were subsequently incubated with 1:200 diluted PE-conjugated goat anti-mouse IgG Fcγ-specific antibodies (Jackson ImmunoResearch) for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were fixed in 2% formaldehyde in PBS/BSA/NaN_3 for 30 minutes at 4°C. Binding of antibodies was measured using a flow cytometer (model FACSCalibur; BD Biosciences).

As shown in figure 1, all 18-examined mouse anti-human HVEM antibodies recognized full-length human HVEM on transfected HEK293F cells and most (14/18) of these mouse anti-human HVEM antibodies showed cross-reactivity - to a variable degree - against cynomolgus monkey full-length HVEM on transfected HEK293F cells. In contrast, mouse anti-human HVEM antibodies no. 36H12, 37D11, 41F11 and 49A11 did not recognize cynomolgus monkey full-length HVEM on transfected HEK293F cells.

These results demonstrated that most (14/18) of generated mouse anti-human HVEM antibodies seemed to recognize linear and/or non-linear/conformational epitopes in CRD1, CRD2, CRD3, CRD4, and/or the 'linker' fragment (aa sequence 180-203, see SEQ ID NO. 5) of the extracellular domain of cynomolgus monkey full-length HVEM.

Predicted amino acid sequence of full-length cynomolgus monkey HVEM protein (Met1 – Ser280; NCBI Reference Sequence: XP_005545061.1) shows 82% homology with amino acid sequence of human HVEM protein (Met1 – His283; Swiss-Prot no. Q92956.3), and predicted amino acid sequence of extracellular region of cynomolgus monkey HVEM (i.e., Leu39 – Val203; NCBI Reference Sequence: XP_005545061.1) shows 87% homology with amino acid sequence of extracellular region human HVEM protein (i.e., Leu39 – Val202; Swiss-Prot no. Q92956.3). These results demonstrated that most (14/18) of the generated mouse

anti-human HVEM monoclonal antibodies cross-reacted with homologous cynomolgus monkey HVEM on transfected HEK293F cells.

(c). Effect of mouse anti-human HVEM antibodies on binding of human BTLA and human LIGHT to membrane-bound human HVEM

Extracellular HVEM has two spatially ligand-binding regions (Cai et al. Immunol Rev 2009; 229: 244-258; Steinberg et al. Immunol Rev 2011; 244: 169-187; Pasero et al. Curr Opin Pharmacol 2012; 12: 478-485), one region for canonical ligands, which belong to the TNF superfamily (i.e., LIGHT and TNFβ), and another region for non-canonical ligands, which belong to the Ig superfamily (i.e., BTLA and CD160). Mutational analysis and molecular modelling revealed that BTLA and CD160 interact with CRD1, whereas LIGHT and TNFβ binding reside in CRD2 and CRD3 on the opposite face of HVEM.

In order to analyse the effect of mouse anti-human HVEM antibodies on binding of human BTLA and human LIGHT to membrane-bound human HVEM, the ability of mouse anti-human HVEM antibodies to sterically hinder the interaction of human BTLA and of human LIGHT on human full-length HVEM, expressed on the surface of (HEK-derived) 293F cells, was determined by FACS analysis.

Generation of human 'full-length' HVEM-transfected HEK293F cells (clone no. 128) is described in Example 1a. Binding of soluble human BTLA of soluble human CD160, and of soluble human LIGHT on surface human HVEM-transfected cells was analysed by FACS analysis. To this end, stable human full-length HVEM-transfected HEK293F cells were put at 10 x 10^6 cells/mL in ice-chilled phosphate-buffered saline containing 0.1% BSA (Sigma)/0.05% NaN_3 (PBS/BSA/NaN_3) supplemented with 50 µg/mL human IgGs (blocking possible Fcγ receptors; Sigma) for 10 minutes at 4°C. Then, 10 µL/tube (i.e., 0.1 x 10^6 cells) of these cells were incubated with or without 100 µL protein G purified mouse anti-HVEM antibody at 10 µg/mL/tube or a negative control mouse IgG1 (BD Biosciences) at 10 µg/mL/tube for 30 minutes at 4°C. After this (i.e., without washing), cells were subsequently incubated with 1 µg/mL soluble human BTLA-human Fcγ fusion protein (Sino Biological Inc) or with 0.1 µg/mL soluble his-tagged human LIGHT (R&D Systems) in PBS/BSA/NaN_3 for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were incubated with biotinylated mouse anti-human IgG Fcγ-specific antibody (detection BTLA; Southern Biotech) at 10 µg/mL or with biotinylated mouse anti-his antibody (detection LIGHT; R&D Systems) at 5 µg/mL for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were incubated with 1:200 diluted PE-conjugated streptavidin (Jackson ImmunoResearch) for 30 minutes at 4°C. After extensive washing in

PBS/BSA/NaN_3, cells were fixed in 2% formaldehyde in PBS/BSA/NaN_3 for 30 minutes at 4°C. Binding of ligands BTLA and LIGHT on membrane human HVEM was measured using a flow cytometer (model FACSCalibur; BD Biosciences).

As shown in figure 2 and Table 1, four types of mouse anti-human HVEM antibodies were found: (type 1 antibodies; 6/18) non-blocking BTLA/HVEM and LIGHT/HVEM interactions, (type 2 antibodies; 3/18) blocking BTLA/HVEM interaction and non-blocking LIGHT/HVEM interaction, (type 3 antibodies; 4/18) non-blocking BTLA/HVEM interaction and blocking LIGHT/HVEM interaction, and (type 4 antibody; 5/18) blocking BTLA/HVEM and LIGHT/HVEM interactions.

Table 1. Summary of mouse anti-human HVEM antibody blocking effects on soluble human BTLA (sBTLA) ligand, soluble human CD160 (sCD160) and soluble human LIGHT (sLIGHT) ligand binding to human HVEM membrane receptor (mHVEM).

	Blocking ligand-mHVEM interaction			
Anti-HVEM Ab	**sBTLA**	**sCD160**	**sLIGHT**	**binding to CRD1 truncated HVEM**
8H5	+++	++	+	-
29C2	-	-*	++	-
36H12	+++	+++	+++	-
37D11	-	-	-	-
38B12	-	-	+	-
38G10	-	-	-	-/+
39B9	-	-	-	+
41D7	-	-	-	-
43E10	-	-	-	-
45C6	-*	-	+	-
45H6	+++	+++	+++	-
47E10	-	-	-	-/+

48H6	+++	+++	++	-
11H7	+++	+++	-	-
41F11	++	++	-/+	-
49A11	++	++	-	-
49G4	+++	+++	+++	-
52D3	-*	-*	++	-

- = no blocking (-* = enhanced binding of ligand to human HVEM) of ligand-receptor interaction, + = weak blocking of ligand-receptor interaction, ++ = intermediate blocking of ligand-receptor interaction, +++ = strong blocking of ligand-receptor interaction; last column binding to CRD1 truncated human HVEM. All antibodies bind to full length human HVEM.

These results demonstrated that a panel of human ligand-blocking and ligand-non-blocking mouse anti-human HVEM antibodies with multiple characteristics (i.e., type 1-4 mouse anti-human HVEM antibodies (see above)) was generated. Of note, antibodies that bind CRD1 like (36H12, 45H6) may or may not (52D3) block binding of sBTLA or sCD160. CRD1 antibody 52D3 indeed appears to enhance binding of the ligand(s). In addition, it clearly shows the different functional activity of the CRD1 targeting antibodies.

Example 3. Biological characterization of mouse anti-human HVEM monoclonal antibodies

(a). Effect of mouse anti-human HVEM antibodies on NFκB signalling in membrane human HVEM expressing cells

HVEM signalling can induce the activation of NFκB in multiple HVEM expressing cells from the immune system, like T and B lymphocytes, and dendritic cells, which turns on several genes important to their cell function. Ligation of HVEM on T lymphocytes by LIGHT provides positive co-stimulatory signals, which leads to survival, proliferation, and differentiation of and IFNγ secretion from T lymphocytes (Del Rio et al. Journal Leukocyte Biology 2010; 87: 223-235). Ligation of HVEM on B lymphocytes by LIGHT co-stimulates CD40L-mediated proliferation and antibody secretion, thereby enhancing humoral responses (Del Rio et al. Journal Leukocyte Biology 2010; 87: 223-235). Ligation of HVEM on immature dendritic cells by LIGHT co-stimulates CD40L-mediated maturation, cytokine secretion (IL-12, IL-6, and TNF-α), and priming of specific anti-tumour CTLs and their production of IFN-γ (Del Rio et al. Journal Leukocyte Biology 2010; 87: 223-235).

In order to analyse the effect of mouse anti-human HVEM antibodies on membrane-bound human HVEM-mediated NFκB signalling, the NFκB-response element-luciferase (RE-luc) human HVEM Bioassay Reporter Cells (HEK293; Promega) were used to examine the ability of mouse anti-human HVEM antibodies to activate HVEM-mediated NFκB signalling.

Briefly, human HVEM expressing NFκB-RE-luc cells were plated at 35,000 cells/well in flat-bottomed TC-treated white-solid 96-wells plates (Corning), and were incubated overnight at 37°C/5% CO_2. Next day, these cells were washed, and subsequently incubated with or without 0.0015-10 µg/mL (3-fold dilution steps) mouse anti-human HVEM antibodies. Titrated (i.e., 0, 0.0015-10 µg/mL (3-fold dilution steps)) soluble his-tagged human LIGHT (R&D Systems) was run in parallel for reference purposes. After 6 hours incubation at 37°C/5% CO_2, luciferase production in human HVEM expressing NFκB- NFκB-RE-luc cells was measured using the Bio-Glo™ Luciferase Assay System (Promega) in a luminometer.

As shown in figure 3A and Table 2, several examined (7/12) mouse anti-human HVEM antibodies induced dose-dependent NFκB activation in human HVEM expressing NFκB-RE-luc cells (i.e., compared to soluble LIGHT-mediated NFκB induction) to a variable degree (rank order; no. 48H6 > 36H12 > 29C2 > 8H5 = 45H6 = 49G4 > 52D3), which demonstrated their agonistic activity. Control soluble human LIGHT also showed dose-dependent NFκB activation in these human HVEM expressing NFκB-RE-luc cells. In contrast, mouse anti-human HVEM antibodies no. 11H7, 41F11, 43E10, 47E10, and 49A11 showed no agonistic activity in human HVEM expressing NFκB-RE-luc cells (i.e., compared to soluble LIGHT-mediated NFκB induction). Interestingly, there seemed to be a relationship (see Table 2) between the ability of these examined mouse anti-human HVEM antibodies to sterically block the soluble human LIGHT/human HVEM interaction (see Example 2c) and their agonistic activity (i.e., NFκB induction) on membrane human HVEM expressing cells.

Table 2. Relationship of mouse anti-human HVEM antibody blocking effects on soluble human LIGHT (sLIGHT) ligand binding to human HVEM membrane receptor (mHVEM; see Example 2c) and their agonistic activity (i.e., compared to sLIGHT-mediated NFκB induction) on membrane human HVEM expressing cells.

Anti-HVEM Ab	**NFκB induction**	**Blocking interaction sLIGHT/mHVEM**
8H5	+	+

29C2	+/++	++
36H12	++/+++	+++
43E10	-	-
45H6	+	+++
47E10	-	-
48H6	+++	++
11H7	-	-
41F11	-	-/+
49A11	-	-
49G4	+	+++
52D3	-/+	++

- = no blocking of LIGHT/HVEM interaction or agonistic activity, + = weak blocking of LIGHT/HVEM interaction or agonistic activity, ++ = intermediate blocking of LIGHT/HVEM interaction or agonistic activity, +++ = strong blocking of LIGHT/HVEM interaction or agonistic activity.

These results demonstrated that mouse anti-human HVEM antibodies, which blocked the human LIGHT/human HVEM interaction (see Example 2c) were able to mimic soluble human LIGHT/human HVEM-mediated NKκB signalling. Noteworthy, soluble human LIGHT has been shown to be much less potent than membrane-bound LIGHT expressing cells for activating human HVEM expressed on cells (Cheung et al. PNAS 2009; 106: 6244-6249).

(b). Effect of mouse anti-human HVEM antibodies on soluble human LIGHT-induced NFκB signalling in membrane human HVEM expressing cells

In order to analyse the effect of mouse anti-human HVEM antibodies on soluble human LIGHT-induced NFκB signalling in membrane human HVEM expressing cells, the NFκB-RE-luc human HVEM Bioassay Reporter Cells (HEK293; Promega) were used to examine the ability of mouse anti-human HVEM antibodies to interfere (e.g., blocking, additive or synergistic effect) with soluble LIGHT/membrane HVEM-mediated NFκB signalling.

Briefly, human HVEM expressing NFκB-RE-luc cells were plated at 35,000 cells/well in flat-bottomed TC-treated white-solid 96-wells plates (Corning), and were incubated overnight at 37˚C/5% CO_2. Next day, these cells were washed, and subsequently incubated with or without 0.0015-10 µg/mL (3-fold dilution steps) mouse anti-human HVEM antibodies in combination with soluble his-tagged

human LIGHT (R&D Systems) at 0.3 µg/mL (≈ EC80; see Example 2a and Figure 3A). After 6 hours incubation at 37°C/5% CO_2, luciferase production in human HVEM expressing NFκB- NFκB-RE-luc cells was measured using the Bio-Glo™ Luciferase Assay System (Promega) in a luminometer.

As shown in figure 3B and Table 3, a very weak agonist but intermediate LIGHT/HVEM interaction blocker (see Table 2) mouse anti-human HVEM antibody no. 52D3 was able to weakly but dose-dependently inhibit soluble human LIGHT-mediated NFκB activation in human HVEM expressing NFκB-RE-luc cells. Surprisingly, non-agonist and LIGHT/HVEM interaction non-blocker (see Table 2) mouse anti-human HVEM antibody no. 49A11 was also able to weakly but dose-dependently inhibit soluble human LIGHT-mediated NFκB activation in human HVEM expressing NFκB-RE-luc cells. In addition, non-agonists and LIGHT/HVEM interaction non-blockers (see Table 2) mouse anti-human HVEM antibodies no. 11H7, 41F11, 43E10, and 47E10 showed no effect on soluble human LIGHT-mediated NFκB activation in human HVEM expressing NFκB-RE-luc cells, whereas weak/intermediate/strong agonists and weak/intermediate/strong LIGHT/HVEM interaction blockers (see Table 2) mouse anti-human HVEM antibodies no. 8H5, 29C2, 36H12, 45H6, 48H6 and 49G4 showed possible additive but no synergistic effects on soluble human LIGHT-mediated NFκB activation in human HVEM expressing NFκB-RE-luc cells.

Table 3. Summary of mouse anti-human HVEM antibody effects on soluble human LIGHT (sLIGHT) ligand binding to human HVEM membrane receptor (mHVEM; see Example 2c) and their effect on soluble human LIGHT-induced agonistic activity (i.e., NFκB induction) on membrane human HVEM expressing cells.

Anti-HVEM Ab	**Blocking sLIGHT NFκB induction**	**Blocking interaction sLIGHT/mHVEM**
8H5	-*	+
29C2	-*	++
36H12	-*	+++
43E10	-	-
45H6	-*	+++
47E10	-	-
48H6	-*	++
11H7	-	-
41F11	-	-/+
49A11	+	-

49G4	-*	+++
52D3	+	++

- = no blocking of LIGHT/HVEM interaction or of LIGHT-induced agonistic activity, + = weak blocking of LIGHT/HVEM interaction or of LIGHT-induced agonistic activity, ++ = intermediate blocking of LIGHT/HVEM interaction or of LIGHT-induced agonistic activity, +++ = strong blocking of LIGHT/HVEM interaction or of LIGHT-induced agonistic activity. * = Agonistic effect of either LIGHT and/or mouse anti-human HVEM antibody.

These results demonstrated that mouse anti-human HVEM antibodies, which block the human LIGHT/human HVEM interaction (except for LIGHT/HVEM interaction non-blocker mouse anti-human HVEM antibody no. 49A11; see also Example 2c) were either able to block or to mimic soluble human LIGHT/human HVEM-mediated NKκB signalling.

(c). Effect of mouse anti-human HVEM antibodies on membrane human BTLA/membrane human HVEM-mediated inhibition of TCR-induced NFAT signalling in membrane human BTLA/membrane human TCR expressing T cells

Like described above, ligation of HVEM on T lymphocytes by LIGHT delivers positive co-stimulatory signals through HVEM, whereas engagement of BTLA on T lymphocytes by HVEM provides negative co-inhibitory signals to T lymphocytes via BTLA (Del Rio et al. Journal Leukocyte Biology 2010; 87: 223-235). This BTLA/HVEM pathway down-regulates TCR-mediated signalling in both CD4 and CD8 T lymphocytes, and results in decreased T lymphocyte proliferation and cytokine production. Engagement of BTLA on B lymphocytes by HVEM reduces activation of signalling molecules downstream of the BCR and attenuates B cell proliferation (Vendel et al. Journal Immunology 2009; 182:1509-1517). Unlike PD-1 and CTLA4, BTLA is not expressed on regulator T (Treg) lymphocytes (Del Rio et al. Journal Leukocyte Biology 2010; 87: 223-235).

In order to analyse the effect of mouse anti-human HVEM antibodies on membrane human BTLA/membrane human HVEM-mediated inhibition of membrane human TCR-induced NFAT signalling, the NFAT-response element-luciferase (RE-luc) human BTLA/HVEM Blockade Bioassay (containing a combination of (1) membrane human HVEM and a proprietary membrane human TCR activator expressing CHO-K1 Activator cells (artificial antigen-presenting cells), and of (2) membrane human BTLA and membrane human TCR expressing NFAT-RE-luc Jurkat Effector T cells; Promega) was used to examine the ability of mouse anti-human HVEM antibodies to block the BTLA/HVEM-mediated inhibition of TCR-induced NFAT signalling.

In this BTLA/HVEM Blockade Bioassay, membrane human BTLA and membrane human TCR expressing NFAT-RE-luc Jurkat Effector T cells are used as effector cells, and membrane human HVEM and a proprietary membrane human TCR activator expressing CHO-K1 Activator cells are used as artificial antigen-presenting cells. When these two cells are co-cultivated, TCR complexes on effector cells are activated by TCR activator expressing artificial antigen-presenting cells, resulting in expression of the NFAT luciferase reporter. However, BTLA and HVEM ligation prevents TCR activation and suppresses the NFAT-responsive luciferase activity. This inhibition can be specifically reversed by exposure to blocking anti-HVEM antibodies. Neutralizing anti-HVEM antibodies block BTLA/HVEM interaction and promote T cell activation (i.e., "releasing the brake"), resulting in reactivation of the NFAT responsive luciferase reporter (see Figure 4A).

Briefly, human HVEM and proprietary human TCR activator expressing CHO-K1 Activator cells were plated at 40,000 cells/well in flat-bottomed TC-treated white-solid 96-wells plates (Corning), and were incubated overnight at 37°C/5% CO_2. Next day, these cells were washed, and subsequently incubated with or without 0.0015-10 µg/mL (3-fold dilution steps) mouse anti-human HVEM antibodies. Then, human BTLA and human TCR expressing NFAT-RE-luc Jurkat Effector T cells were added at 50,000 cells/well. After 6 hours incubation at 37°C/5% CO_2, luciferase production in human BTLA and human TCR expressing NFAT-RE-luc Jurkat Effector T cells was measured using the Bio-Glo™ Luciferase Assay System (Promega) in a luminometer.

As shown in figure 4B and Table 4, several examined (8/12) mouse anti-human HVEM antibodies dose-dependently blocked the BTLA/HVEM-mediated inhibition of TCR-induced NFAT signalling in human BTLA and human TCR expressing NFAT-RE-luc Jurkat Effector T cells to a variable degree (rank order; no. 45H6 > 49G4 > 36H12 > 11H7 > 8H5 = 41F11 = 48H6 = 49A11). In contrast, mouse anti-human HVEM antibodies no. 29C2, 43E10, 47E10, and 52D3 showed no effect on the BTLA/HVEM-mediated inhibition of TCR-induced NFAT signalling in human BTLA and human TCR expressing NFAT-RE-luc Jurkat Effector T cells. Interestingly, there seemed to be a relationship (see Table 4) between the ability of these examined mouse anti-human HVEM antibodies to sterically block the soluble human BTLA/human HVEM interaction (see Example 2c) and their blocking capacity (i.e., abrogation of BTLA/HVEM-mediated inhibition of TCR-induced NFAT signalling) in the NFAT-RE-luc human BTLA/HVEM Blockade Bioassay.

Table 4. Relationship of mouse anti-human HVEM antibody blocking effects on soluble human BTLA (sBTLA) ligand binding to human HVEM membrane receptor (mHVEM; see Example 2c) and their blocking effect on membrane human BTLA/membrane human HVEM-induced (mBTLA/mHVEM) inhibition of TCR-

induced NFAT signalling in membrane human BTLA/TCR expressing Effector T cells.

Anti-HVEM Ab	Blocking mBTLA/mHVEM TCR-NFAT inhibition	Blocking interaction sBTLA/mHVEM
8H5	+	+++
29C2	-	-
36H12	++	+++
43E10	-	-
45H6	+++	+++
47E10	-	-
48H6	+	+++
11H7	+/++	+++
41F11	+	++
49A11	+	++
49G4	++/+++	+++
52D3	-	-*

- = no blocking of BTLA/HVEM interaction or of BTLA/HVEM-induced TCR-NFAT inhibition, + = weak blocking of BTLA/HVEM interaction or of BTLA/HVEM-induced TCR-NFAT inhibition, ++ = intermediate blocking of BTLA/HVEM interaction or of BTLA/HVEM-induced TCR-NFAT inhibition, +++ = strong blocking of BTLA/HVEM interaction or of BTLA/HVEM-induced TCR-NFAT inhibition. * = Enhanced binding of BTLA to human HVEM.

These results demonstrated that mouse anti-human HVEM antibodies, which blocked the human BTLA/human HVEM interaction (see Example 2c) were able to block the human BTLA/human HVEM-mediated inhibition of TCR-induced NFAT signalling

Example 4. Molecular genetic characterization of BTLA/HVEM blocking mouse anti-human HVEM monoclonal antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4

Hybridoma cells producing BTLA/HVEM blocking mouse anti-human HVEM antibody no. 36H12, 45H6, 48H6, 11H7, and 49G4 were washed with PBS, and aliquoted in microvials containing 5 x 10^6 cells, and stored as pellets at -80°C. These cell pellets were used to isolate RNA by using RNeasy Mini Isolation Kit (QIAGEN). RNA concentration was determined (A260 nm), and RNA was stored at -80°C. By reverse transcriptase, cDNA was synthesized from 1 µg of RNA using the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas), and stored at -80°C. Based on the isotype mouse IgG1/kappa, primers as shown in Table 5 were

designed to amplify the variable (V) regions of BTLA/HVEM blocking mouse anti-human HVEM antibody no. 36H12, 45H6, 48H6, 11H7, and 49G4.

Table 5. PCR primers used to amplify cDNA of BTLA/HVEM blocking mouse anti-human HVEM antibody no. 36H12, 45H6, 48H6, 11H7, and 49G4.

No.*	Sequence	s/as	Gene
383	ATGAAGTTGCCTGTTAGGCTGTTGGTGCTG (SEQ ID NO. 7)	s	VL
387	ATGGATTTWCAGGTGCAGATTWTCAGCTTC (SEQ ID NO. 8)	s	VL
389	ATGGGCWTCAAAGATGGAGTCACA (SEQ ID NO. 9)	s	VL
394	ACTGGATGGTGGGAAGATGG (SEQ ID NO.10)	as	Ck
404	ATGAAATGCAGCTGGGGCATSTTCTTC (SEQ ID NO. 11)	s	VH
407	ATGRACTTTGGGYTCAGCTTGRTTT (SEQ ID NO. 12)	s	VH
408	ATGGGACTCCAGGCTCAATTTAGTTTTCCTT (SEQ ID NO. 13)	s	VH
409	ATGGCTTGTCYTTRGSGCTRCTCTTCTGC (SEQ ID NO. 14)	s	VH
416	CAGTGGATAGACAGATGGGGG (SEQ ID NO. 15)	as	CH

s = sense; as = antisense; VL = variable light chain region; VH = variable heavy chain region; Ck = constant mouse kappa (κ) light chain region; CH = constant mouse IgG1 heavy chain region; * Numbering according to Bioceros BV internal coding system; degenerated primers: K = G or T, S = G or C, R = A or G, M = A or C, W = A or T, Y = C or T, H = A or C or T, and N = any base.

Primers 383, 387, and 389 are sense primers designed to anneal with the signal peptide of the light chain of a mouse antibody; primer 394 is an antisense primer annealing with the constant region of mouse κ light chain. Primers 404, 407, 408, and 409 are sense primers annealing with the signal peptide of the heavy chain of a mouse antibody; primer 416 is antisense primer designed to anneal with the constant region of mouse IgG1 heavy chain. Various PCRs were done using primer combinations shown in Table 5. Generated PCR products were subcloned in pCR™-Blunt II-TOPO® vector. Subsequently, cloned inserts were sequenced.

From heavy chain and light chain sequence reactions, a total of 8 and 4 informative sequences, respectively, were obtained of amino acid sequences of mouse anti-human HVEM antibody 36H12. Based on this information, consensus amino acid sequences of VH and VL regions of mouse anti-human HVEM antibody 36H12 were determined, and are set forth in SEQ ID NO. 16 and 17, respectively. The amino acid sequences of the CDRs of VH and VL regions of mouse anti-human HVEM antibody 36H12 are set forth in SEQ ID NO. 18-20 and 21-23, respectively.

From both heavy chain and light chain sequence reactions, a total of 4 informative sequences were obtained of mouse anti-human HVEM antibody 45H6. Based on this information, consensus amino acid sequences of VH and VL regions of mouse anti-human HVEM antibody 45H6 were determined, and are set forth in SEQ ID NO. 24 and 25, respectively. The amino acid sequences of the CDRs of VH and VL regions of mouse anti-human HVEM antibody 45H6 are set forth in SEQ ID NO. 26-28 and 29-31, respectively.

From heavy chain and light chain sequence reactions, a total of 5 and 4 informative sequences, respectively, were obtained of mouse anti-human HVEM antibody 48H6. Based on this information, consensus amino acid sequences of VH and VL regions of mouse anti-human HVEM antibody 48H6 were determined, and are set forth in SEQ ID NO. 32 and 33, respectively. The amino acid sequences of the CDRs of VH and VL regions of mouse anti-human HVEM antibody 48H6 are set forth in SEQ ID NO. 34-36 and 37-39, respectively.

From heavy chain and light chain sequence reactions, a total of 9 and 3 informative sequences, respectively, were obtained of mouse anti-human HVEM antibody 11H7. Based on this information, consensus amino acid sequences of VH and VL regions of mouse anti-human HVEM antibody 11H7 were determined, and are set forth in SEQ ID NO. 40 and 41, respectively. The amino acid sequences of the CDRs of VH and VL regions of mouse anti-human HVEM antibody 11H7 are set forth in SEQ ID NO. 42-44 and 45-47, respectively.

From heavy chain and light chain sequence reactions, a total of 5 and 3 informative sequences, respectively, were obtained of mouse anti-human HVEM antibody 49G4. Based on this information, consensus amino acid sequences of VH and VL regions of mouse anti-human HVEM antibody 49G4 were determined, and are set forth in SEQ ID NO. 48 and 49, respectively. The amino acid sequences of the CDRs of VH and VL regions of mouse anti-human HVEM antibody 49G4 are set forth in SEQ ID NO. 50-52 and 53-55, respectively.

Example 5. Generation of BTLA/HVEM blocking chimeric mouse/human IgG4/kappa (i.e., exchanging mouse constant IgG1/kappa

regions for human constant IgG4/kappa regions) anti-human HVEM monoclonal antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4

Based on determined mouse V-regions (see Example 4 above) of BTLA/HVEM blocking mouse anti-human HVEM antibodies, a design was made to generate chimeric mouse/human anti-human HVEM antibody versions. To this end, for mammalian expression-optimized cDNA sequences, SEQ ID NO. 56 (coding for chimeric mouse/human heavy IgG4 chain 36H12), NO. 57 (coding for chimeric mouse/human heavy IgG4 chain 45H6), NO. 58 (coding for chimeric mouse/human heavy IgG4 chain 48H6), NO. 59 (coding for chimeric mouse/human heavy IgG4 chain 11H7), and NO. 60 (coding for chimeric mouse/human heavy IgG4 chain 49G4), and SEQ ID NO. 61 (coding for chimeric mouse/human light κ chain 36H12), NO. 62 (coding for chimeric mouse/human light κ chain 45H6), NO. 63 (coding for chimeric mouse/human light κ chain 48H6), NO. 64 (coding for chimeric mouse/human light κ chain 11H7), and NO. 65 (coding for chimeric mouse/human light κ chain 49G4), were ordered at GENEART (Regensburg, Germany), which encoded a human signal peptide followed by either the mouse VH chain linked to the human stabilized IgG4 constant region (i.e., S239P; according Angal et al in Mol. Immunol., Vol. 30, No. 1, pp. 105-108, 1993), or followed by the mouse VL chain linked to the human kappa constant region. Using suitable restriction enzymes, generated cDNAs were subcloned in pcDNA3.1-derived expression plasmids. Subsequently, chimeric antibodies were transiently expressed in 293-F cells (Invitrogen) using the FreeStyle™ 293 Expression System (Invitrogen). Expressed chimeric anti-human HVEM antibodies were purified from supernatants using conventional affinity chromatography protein A columns. After this, LPS levels were determined using the LAL chromogenic endpoint assay (Hycult Biotech), and all our purified chimeric mouse/human anti-human HVEM antibodies (i.e., 36H12, 45H6, 48H6, 11H7, and 49G4) contained < 0.0005 EU LPS/μg chimeric IgG.

For chimeric amino acid sequences, see SEQ ID NO. 66 (chimeric mouse/human heavy IgG4 chain 36H12), NO. 67 (chimeric mouse/human heavy IgG4 chain 45H6), NO. 68 (chimeric mouse/human heavy IgG4 chain 48H6), NO. 69 (chimeric mouse/human heavy IgG4 chain 11H7), and NO. 70 (chimeric mouse/human heavy IgG4 chain 49G4), and SEQ ID NO. 71 (chimeric mouse/human light κ chain 36H12), NO. 72 (chimeric mouse/human light κ chain 45H6), NO. 73 (chimeric mouse/human light κ chain 48H6), NO. 74 (chimeric mouse/human light κ chain 11H7), and NO. 75 (chimeric mouse/human light κ chain 49G4).

Example 6. Binding and biological characterization of BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4

(a). Relative binding affinity of BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies for membrane-bound human HVEM

In order to determine the relative binding affinity of purified BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 for human HVEM, FACS analysis was used.

To this end, stable human full-length HVEM-transfected HEK293F cells (clone no. 128; see Example 1(a) above) were put at 10 x 10^6 cells/mL in ice-chilled PBS containing 0.1% BSA (Sigma)/0.05% NaN_3 (PBS/BSA/NaN_3) supplemented with 50 µg/mL human IgGs (blocking possible Fcγ receptors; Sigma) for 10 minutes at 4°C. Then, 10 µL/tube (i.e., 0.1 x 10^6 cells) of these cells were incubated with or without 100 µL titrated (in PBS/BSA/NaN_3) purified BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibody/tube for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were subsequently incubated with 1:200 diluted PE-conjugated goat anti-human IgG Fcγ-specific antibodies (Jackson ImmunoResearch) for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN3, cells were fixed in 4% formaldehyde in PBS/BSA/NaN_3 for 30 minutes at 4°C. Binding (geo-mean fluorescence intensity) of chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 on membrane human HVEM was measured using a flow cytometer (FACSCalibur; BD Biosciences). For comparison, purified BTLA/HVEM blocking mouse anti-human HVEM antibody counterparts no. 36H12, 45H6, 48H6, 11H7, and 49G4 were run in parallel, and their binding was monitored as described in Example 2 (a).

As shown in Figure 5, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 dose-dependently bound to membrane human HVEM. Based on their binding profile, the following relative affinity ranking was found (from high to lower affinity): 45H6 = 49G4 > 36H12 = 48H6 > 11H7. For comparison, their BTLA/HVEM blocking mouse anti-human HVEM antibody counterparts no. 36H12, 45H6, 48H6, 11H7, and 49G4 also showed dose-dependent binding to membrane human HVEM and demonstrated a very similar relative affinity ranking, i.e., 45H6 = 49G4 > 36H12 = 48H6 > 11H7. More specifically, chimeric mouse/human anti-human HVEM antibody no. 45H6, 49G4, 36H12, 48H6, and 11H7 resulted in the following relative affinities (i.e., half-maximum binding EC50) of 306, 312, 433, 472, and 630 ng/mL, respectively, while corresponding mouse anti-human HVEM antibody no. 45H6, 49G4, 36H12, 48H6, and 11H7 resulted in relative affinities of 260, 266, 430, 356, and 532 ng/mL, respectively, which indicated that binding affinities of the

BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibody no. 36H12, 45H6, 48H6, 11H7, and 49G4 against membrane-bound HVEM seemed to remain unaltered during the chimerization process.

b). Effect of BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on binding of human BTLA, human CD160, human LIGHT, and human TNFβ to membrane-bound human HVEM

Extracellular HVEM has two spatially ligand-binding regions (Cai et al. Immunol Rev 2009; 229: 244-258; Steinberg et al. Immunol Rev 2011; 244: 169-187; Pasero et al. Curr Opin Pharmacol 2012; 12: 478-485), one region for canonical ligands, which belong to the TNF superfamily (i.e., LIGHT and TNFβ), and another region for non-canonical ligands, which belong to the Ig superfamily (i.e., BTLA and CD160). Mutational analysis and molecular modelling revealed that BTLA and CD160 interact with CRD1, whereas LIGHT and TNFβ binding reside in CRD2 and CRD3 on the opposite face of HVEM.

In order to analyse the effect of purified BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 on binding of human BTLA, human CD160, human LIGHT, and human TNFβ to membrane-bound human HVEM, the ability of chimeric mouse/human anti-human HVEM antibodies to sterically hinder the interaction of human BTLA, of human CD160, of human LIGHT, and of human TNFβ (or also called LTα) on human full-length HVEM, expressed on the surface of (HEK-derived) 293F cells, was determined by FACS analysis.

Generation of human 'full-length' HVEM-transfected HEK293F cells (clone no. 128) is described in Example 1a. Binding of soluble human BTLA, of soluble human CD160, of soluble human LIGHT, and of soluble human TNFβ on surface human HVEM-transfected cells was analysed by FACS analysis. To this end, stable human full-length HVEM-transfected HEK293F cells were put at 10 x 10^6 cells/mL in ice-chilled phosphate-buffered saline containing 0.1% BSA (Sigma)/0.05% NaN_3 (PBS/BSA/NaN_3) supplemented with 50 µg/mL human IgGs (blocking possible Fcγ receptors; Sigma) for 10 minutes at 4°C. Then, 10 µL/tube (i.e., 0.1 x 10^6 cells) of these cells were incubated with or without 100 µL purified chimeric mouse/human anti-HVEM antibody at 10 µg/mL/tube or a human IgG4/κ (Sigma) negative isotype control at 10 µg/mL/tube for 30 minutes at 4°C. After this (i.e., without washing), cells were subsequently incubated with 1 µg/mL soluble biotinylated human BTLA (Sino Biological Inc), with 10 µg/mL soluble his-tagged human CD160 (Sino Biological Inc), with 1 µg/mL soluble his-tagged human LIGHT (Sino Biological Inc), or with 0.1 µg/mL soluble biotinylated human TNFβ (Sino Biological Inc) in PBS/BSA/NaN_3 for 30 minutes at 4°C. After extensive

washing in PBS/BSA/NaN_3, cells were incubated with 1:200 diluted PE-conjugated streptavidin (detection BTLA and TNFβ; Jackson ImmunoResearch) or with biotinylated mouse anti-his antibody (detection CD160 and LIGHT; R&D Systems) at 10 µg/mL for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were incubated with 1:200 diluted PE-conjugated streptavidin (detection CD160 and LIGHT; Jackson ImmunoResearch) for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were fixed in 4% formaldehyde in PBS/BSA/NaN_3 for 30 minutes at 4°C. Binding of ligands BTLA, CD160, LIGHT and TNFβ on membrane human HVEM was measured using a flow cytometer (model FACSCalibur; BD Biosciences). For comparison, purified BTLA/HVEM blocking mouse anti-human HVEM antibody counterparts no. 36H12, 45H6, 48H6, 11H7, and 49G4 at 10 µg/mL/tube and a mouse IgG1 (BD Biosciences) negative isotype control at 10 µg/mL/tube were run in parallel.

As shown in figure 6A, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 prevented (i.e., > 95% blocking) human BTLA binding to membrane human HVEM, which was comparable to their BTLA/HVEM blocking mouse anti-human HVEM antibody counterparts no. 36H12, 45H6, 48H6, 11H7, and 49G4 (i.e., > 95% blocking).

As shown in figure 6B, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 partially prevented (i.e., ≈60-65% blocking) human CD160 binding to membrane human HVEM, which was comparable to their BTLA/HVEM blocking mouse anti-human HVEM antibody counterparts no. 36H12, 45H6, 48H6, 11H7, and 49G4 (i.e., ≈45-55% blocking).

As shown in figure 6C, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, and 49G4 partially prevented (i.e., ≈30-50% blocking) human LIGHT binding to membrane human HVEM, which was comparable to their BTLA/HVEM blocking mouse anti-human HVEM antibody counterparts no. 36H12, 45H6, 48H6, and 49G4 (i.e., ≈40-50% blocking). In contrast, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibody no. 11H7 did not prevent but surprisingly seemed to enhance or stabilize (i.e., ≈20% enhancement) human LIGHT binding to membrane human HVEM, which was comparable to their BTLA/HVEM blocking mouse anti-human HVEM antibody counterpart no. 11H7 (i.e., ≈10% enhancement).

As shown in figure 6D, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 (partially) prevented human TNFβ binding to membrane human HVEM to a variable degree (order; no. 36H12 = 45H6 = 48H6 (i.e., > 94% blocking) > 49G4 (i.e., > 80%

blocking) > 11H7 (i.e., > 55% blocking)), which was comparable to their BTLA/HVEM blocking mouse anti-human HVEM antibody counterparts no. 36H12, 45H6, 48H6, 11H7, and 49G4 (rank order; no. 36H12 = 45H6 = 48H6 (i.e., > 95% blocking) > 49G4 (i.e., > 85% blocking) > 11H7 (i.e., > 60% blocking)).

c). Effect of BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on displacement of pre-bound human BTLA, human CD160, human LIGHT, and human TNFβ from membrane-bound human HVEM

In order to analyse whether purified BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 were able to displace pre-bound human BTLA, human CD160, human LIGHT, and human TNFβ from membrane-bound human HVEM, the effect of chimeric mouse/human anti-human HVEM antibodies on human BTLA, on human CD160, on human LIGHT, and on human TNFβ (or also called LTα) displacement from human full-length HVEM, expressed on the surface of (HEK-derived) 293F cells, was determined by FACS analysis.

Generation of human 'full-length' HVEM-transfected HEK293F cells (clone no. 128) is described in Example 1a. Displacement of pre-bound soluble human BTLA, soluble human CD160, soluble human LIGHT, and soluble human TNFβ from surface human HVEM-transfected cells was analysed by FACS analysis. To this end, stable human full-length HVEM-transfected HEK293F cells were put at 10 x 10^6 cells/mL in ice-chilled phosphate-buffered saline containing 0.1% BSA (Sigma)/0.05% NaN_3 (PBS/BSA/NaN_3) supplemented with 50 µg/mL human IgGs (blocking possible Fcγ receptors; Sigma) for 10 minutes at 4°C. Then, 10 µL/tube (i.e., 0.1 x 10^6 cells) of these cells were incubated without or with 50 µL soluble biotinylated human BTLA-human Fcγ fusion protein (Sino Biological Inc) at 2 µg/mL/tube, with soluble his-tagged human CD160 (Sino Biological Inc) at 20 µg/mL/tube, with soluble his-tagged human LIGHT (Sino Biological Inc) at 2 µg/mL/tube, or with soluble biotinylated human TNFβ (Sino Biological Inc) at 0.2 µg/mL/tube in PBS/BSA/NaN_3 for 30 minutes at 4°C. After this (i.e., without washing), cells were subsequently incubated with 50 µL purified chimeric mouse/human anti-HVEM antibody at 20 µg/mL/tube or a human IgG4/κ (Sigma) negative isotype control at 20 µg/mL/tube for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were incubated with 1:200 diluted PE-conjugated streptavidin (detection BTLA and TNFβ; Jackson ImmunoResearch) or with biotinylated mouse anti-his antibody (detection CD160 and LIGHT; R&D Systems) at 10 µg/mL for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were incubated with 1:200 diluted PE-conjugated streptavidin (detection BTLA and TNFβ; Jackson ImmunoResearch) or with biotinylated mouse anti-his antibody (detection CD160 and LIGHT; R&D Systems) at 10 µg/mL for 30 minutes

at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were incubated with 1:200 diluted PE-conjugated streptavidin (detection CD160 and LIGHT; Jackson ImmunoResearch) for 30 minutes at 4°C. After extensive washing in PBS/BSA/NaN_3, cells were fixed in 4% formaldehyde in PBS/BSA/NaN_3 for 30 minutes at 4°C. Residual binding of ligands BTLA, CD160, LIGHT and TNFβ on membrane human HVEM was measured using a flow cytometer (model FACSCalibur; BD Biosciences). For comparison, purified BTLA/HVEM blocking mouse anti-human HVEM antibody counterparts no. 36H12, 45H6, 48H6, 11H7, and 49G4 at 10 µg/mL/tube and a mouse IgG1 (BD Biosciences) negative isotype control at 10 µg/mL/tube were run in parallel.

As shown in figure 7A, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 displaced (i.e., > 95% displacement) pre-bound human BTLA from membrane human HVEM.

As shown in figure 7B, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 partially displaced (i.e., ≈50-60% displacement) pre-bound human CD160 from membrane human HVEM.

As shown in figure 7C, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, and 49G4 did not displace (i.e., <20% displacement) pre-bound human LIGHT from membrane human HVEM. In contrast, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibody no. 11H7 did not displace pre-bound human LIGHT from membrane human HVEM but surprisingly seemed to enhance or stabilize pre-bound (i.e., ≈30% enhancement) human LIGHT to membrane human HVEM.

As shown in figure 7D, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, and 49G4 (partially) displaced pre-bound human TNFβ from membrane human HVEM to a variable degree (rank order; no. 36H12 = 45H6 = 48H6 (i.e., > 90% displacement) > 49G4 (i.e., > 55% displacement)). In contrast, BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibody no. 11H7 did not seem to displace pre-bound human TNFβ from membrane human HVEM (i.e., < 20% displacement).

(d). Effect of BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on NFκB signalling in membrane human HVEM expressing cells

In order to analyse the effect of chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 on membrane-bound human HVEM-mediated NFκB signalling, the NFκB-response element-luciferase

(RE-luc) human HVEM Bioassay Reporter Cells (HEK293; Promega) were used to examine the ability of mouse anti-human HVEM antibodies to activate HVEM-mediated NFκB signalling.

Briefly, human HVEM expressing NFκB-RE-luc cells were plated at 35,000 cells/well in flat-bottomed TC-treated white-solid 96-wells plates (Corning), and were incubated overnight at 37°C/5% CO_2. Next day, these cells were washed, and subsequently incubated with or without 0.0015-10 µg/mL (3-fold dilution steps) chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4. Titrated (i.e., 0, 0.0015-10 µg/mL (3-fold dilution steps)) soluble his-tagged human LIGHT (R&D Systems) was run in parallel for reference purposes. After 6 hours incubation at 37°C/5% CO_2, luciferase production in human HVEM expressing NFκB- NFκB-RE-luc cells was measured using the Bio-Glo™ Luciferase Assay System (Promega) in a luminometer.

As shown in figure 8A, only chimeric mouse/human mouse anti-human HVEM antibody no. 48H6 induced a weak dose-dependent NFκB activation in human HVEM expressing NFκB-RE-luc cells (i.e., compared to soluble LIGHT-mediated NFκB induction), whereas chimeric mouse/human mouse anti-human HVEM antibodies no. 36H12, 45H6, 11H7, and 49G4 showed no or very weak agonistic activity in human HVEM expressing NFκB-RE-luc cells (i.e., compared to soluble LIGHT-mediated NFκB induction). Control soluble human LIGHT also showed dose-dependent NFκB activation in these human HVEM expressing NFκB-RE-luc cells. Noteworthy, soluble human LIGHT has been shown to be much less potent than membrane-bound LIGHT expressing cells for activating human HVEM expressed on cells (Cheung et al. PNAS 2009; 106: 6244-6249).

Although the binding affinities of BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibody no. 36H12, 45H6, 48H6, 11H7, and 49G4 against membrane-bound HVEM seemed to remain unaltered during the chimerization process (see Example 6a), it was surprising that BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibody no. 36H12, 45H6, 48H6, and 49G4 with their human constant IgG4 Fc-tail were not or only weakly able to mimic soluble human LIGHT/human HVEM-mediated NKκB signalling as opposed to their mouse anti-human HVEM antibody IgG1 counterparts, which clearly showed NFκB signalling activity (see Example 3a).

(e). Effect of cross-linked BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on NFκB signalling in membrane human HVEM expressing cells

It is well known that cross-linking of antibodies against human CD40 and OX40/CD134 (both members of the TNF receptor superfamily, like human HVEM/CD270) can enhance their agonistic activity (i.e., mimicking CO40L and OX40L mediating effects, respectively) upon binding to membrane-bound CD40 and OX40 expressing cells (Xu et al. Cancer Cell 2018; 33: 664-675; Zhang et al. J Biol Chem 2016; 291: 27134-27146). Because of above-described surprising results with BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibody no. 36H12, 45H6, 48H6, and 49G4 (i.e., not or weakly able to mimic soluble human LIGHT/human HVEM-mediated NKκB signalling as opposed to their mouse anti-human HVEM antibody counterparts, which clearly showed NFκB signalling activity (see Example 6b and 3a, respectively)), the degree of antibody aggregation of (1) chimeric mouse/human and (2) fully mouse versions anti-human HVEM antibody no. 36H12, 45H6, 48H6, 11H7, and 49G4 was determined using size exclusion chromatography analysis, and demonstrated the following: (1) 2.3%, 0.7%, 1.2%, 1.6%, and 9.4% aggregation for chimeric mouse/human anti-human HVEM antibody no. 36H12, 45H6, 48H6, 11H7, and 49G4, respectively, and (2) 36.3%, 25.8%, 19.9%, 12.4%, and 14.8% aggregation for fully mouse anti-human HVEM antibody no. 36H12, 45H6, 48H6, 11H7, and 49G4, respectively. This relatively high degree of antibody aggregation in mouse anti-human HVEM antibody no. 36H12, 45H6, 48H6, and 49G4 preparations strongly suggested that the agonistic activity of mouse anti-human HVEM antibody no. 36H12, 45H6, 48H6, and 49G4 in NFκB-response element-luciferase (RE-luc) human HVEM Bioassay Reporter Cells was an artefactual effect caused by antibody aggregation (i.e, mimicking antibody cross-linking effect). To substantiate this hypothesis, non-cross-linked and cross-linked BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibody no. 36H12, 45H6, 48H6, 11H7, and 49G4 (with a relatively low degree of antibody aggregation) were examined using the NFκB-response element-luciferase (RE-luc) human HVEM Bioassay Reporter Cells.

Briefly, human HVEM expressing NFκB-RE-luc cells were plated at 35,000 cells/well in flat-bottomed TC-treated white-solid 96-wells plates (Corning), and were incubated overnight at 37˚C/5% CO_2. Next day, these cells were washed, and subsequently incubated with or without 0.016-10 µg/mL (5-fold dilution steps) chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4, which were pre-treated with or without 10 µg/mL cross-linking goat anti-human IgG Fcγ-specific antibodies (Jackson ImmunoResearch) for 15-30 minutes at RT. Titrated (i.e., 0, 0.016-10 µg/mL (5-fold dilution steps)) soluble his-tagged human LIGHT (Sino Biological Inc) was run in parallel for reference purposes. After 6 hours incubation at 37˚C/5% CO_2, luciferase production in human HVEM expressing NFκB- NFκB-RE-luc cells was measured using the Bio-Glo™ Luciferase Assay System (Promega) in a luminometer.

As shown in figure 8B, only non-cross-linked chimeric mouse/human mouse anti-human HVEM antibody no. 48H6 induced a weak dose-dependent NFκB activation in human HVEM expressing NFκB-RE-luc cells (i.e., compared to soluble LIGHT-mediated NFκB induction), whereas non-cross-linked chimeric mouse/human mouse anti-human HVEM antibodies no. 36H12, 45H6, 11H7, and 49G4 showed no or very weak agonistic activity in human HVEM expressing NFκB-RE-luc cells (i.e., compared to soluble LIGHT-mediated NFκB induction). In contrast, all examined cross-linked chimeric mouse/human mouse anti-human HVEM antibodies induced dose-dependent NFκB activation in human HVEM expressing NFκB-RE-luc cells (i.e., compared to soluble LIGHT-mediated NFκB induction). Control soluble human LIGHT also showed dose-dependent NFκB activation in these human HVEM expressing NFκB-RE-luc cells. Noteworthy, soluble human LIGHT has been shown to be much less potent than membrane-bound LIGHT expressing cells for activating human HVEM expressed on cells (Cheung et al. PNAS 2009; 106: 6244-6249).

These results demonstrated that non-cross-linked chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 (with a relatively low degree of antibody aggregation) were not or only weakly able to mimic soluble human LIGHT/human HVEM-mediated NKκB signalling, whereas, upon cross-linking, these chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 were able to mimic soluble human LIGHT/human HVEM-mediated NKκB signalling. Noteworthy, soluble human LIGHT has been shown to be much less potent than membrane-bound LIGHT expressing cells for activating human HVEM expressed on cells (Cheung et al. PNAS 2009; 106: 6244-6249).

(f). Effect of BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on soluble human LIGHT-induced NFκB signalling in membrane human HVEM expressing cells

In order to analyse the effect of chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 on soluble human LIGHT-induced NFκB signalling in membrane human HVEM expressing cells, the NFκB-RE-luc human HVEM Bioassay Reporter Cells (HEK293; Promega) were used to examine the ability of mouse anti-human HVEM antibodies to interfere (e.g., blocking, additive or synergistic effect) with soluble LIGHT/membrane HVEM-mediated NFκB signalling.

Briefly, human HVEM expressing NFκB-RE-luc cells were plated at 35,000 cells/well in flat-bottomed TC-treated white-solid 96-wells plates (Corning), and were incubated overnight at 37°C/5% CO_2. Next day, these cells were washed, and subsequently incubated with or without 0.0015-10 µg/mL (3-fold dilution steps)

chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 with soluble his-tagged human LIGHT (Sino Biological Inc) at 0.3 µg/mL (≈ EC80; see Example 2a and Figure 3A). After 6 hours incubation at 37°C/5% CO_2, luciferase production in human HVEM expressing NFκB- NFκB-RE-luc cells was measured using the Bio-Glo™ Luciferase Assay System (Promega) in a luminometer.

As shown in figure 8C, chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 showed no effect on soluble human LIGHT-mediated NFκB activation in human HVEM expressing NFκB-RE-luc cells.

These results demonstrated that chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 were not able to affect soluble human LIGHT/human HVEM-mediated NKκB signalling.

(g). Effect of BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on soluble human TNFβ-induced NFκB signalling in membrane human HVEM expressing cells

TNFβ/LTα has been reported to have a weak binding to HVEM, and its exact functional role in the HVEM pathway is still unclear (Cai et al. Immunol Rev 2009; 229: 244-258), although there is a general consensus that the TNFβ/HVEM pathway (as with the LIGHT/HVEM pathway) provides costimulatory signals, which results in enhanced immune responses (Cai et al. Immunol Rev 2009; 229: 244-258; Steinberg et al. Immunol Rev 2011; 244: 169-187; Pasero et al. Curr Opin Pharmacol 2012; 12: 478-485; Del Rio et al. Am J Transplant 2013; 13:541-551; Schaer et al. J Immunother Cancer 2014; 2: 7).

In order to analyse the effect of chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 on soluble human TNFβ-induced NFκB signalling in membrane human HVEM expressing cells, the NFκB-RE-luc human HVEM Bioassay Reporter Cells (HEK293; Promega) were used to examine the ability of mouse anti-human HVEM antibodies to interfere (e.g., blocking, additive or synergistic effect) with soluble TNFβ/membrane HVEM-mediated NFκB signalling.

Briefly, human HVEM expressing NFκB-RE-luc cells were plated at 32,000 cells/well in flat-bottomed TC-treated white-solid 96-wells plates (Corning), and were incubated overnight at 37°C/5% CO_2. Next day, these cells were washed, and subsequently incubated with or without 0.0015-10 µg/mL (3-fold dilution steps) chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6,

11H7, and 49G4 with soluble recombinant human TNFβ (Sino Biological) at 0.05 µg/mL (≈ EC80; see Figure 9A). Titrated (i.e., 0, 0.000026-2 µg/mL (5-fold dilution steps)) soluble recombinant human TNFβ was run in parallel for reference purposes. After 6 hours incubation at 37°C/5% CO_2, luciferase production in human HVEM expressing NFκB- NFκB-RE-luc cells was measured using the Bio-Glo™ Luciferase Assay System (Promega) in a luminometer.

As shown in figure 9B, chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 showed no effect on soluble human TNFβ -mediated NFκB activation in NFκB-RE-luc cells, which expressed relatively low levels of membrane-bound human HVEM (i.e., a signal to noise ratio of < 5 using PE-conjugated mouse anti-human HVEM antibody (clone eBioHVEM-122; eBioscience) at 1:20). This observation was surprising because chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 prevented soluble human TNFβ binding to and/or displaced pre-bound soluble human TNFβ (see Example 5 (b) and Example 5(c), respectively) from HEK293F cells clone no. 128, which expressed relatively high levels of membrane-bound human HVEM (i.e., overexpression; a signal to noise ratio of ≈ 1000 using PE-conjugated mouse anti-human HVEM antibody (clone eBioHVEM-122; eBioscience) at 1:20); see Figure 1) . However, human TNFβ has been reported to have a high affinity binding to human TNFR1/CD120a and human TNFR2/CD120b (Medvedev et al. J Biol Chem 1996; 16: 9778-9784). Interesting, HEK293 cells endogenously express low levels of human TNFR1/CD120a (Murphy et al. Cell Death Differ. 1998; 5 :497-505; McFarlane et al. FEBS Letters 2002; 515: 119-126; Razonable et al. Antimicrob Agents Chemother 2005; 49: 1617-1621). Most likely, human TNFβ preferentially binds to these 'high affinity' human TNFR1 (as opposed to 'low/weak affinity' human HVEM) on these low HVEM+/TNFR1+ co-expressing cells, thereby triggering preferentially soluble human TNFβ/human TNFR1-mediated (as opposed to soluble human TNFβ/human HVEM-mediated) NFκB activation. Under such condition, chimeric mouse/human anti-human HVEM antibodies will be ineffective.

These results demonstrated that chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 were not able to affect soluble human TNFβ-mediated NKκB signalling when membrane-bound human HVEM and membrane-bound human TNFR1 are co-expressed at relatively low levels on cells.

(h). Effect of BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on membrane human BTLA/membrane human HVEM-mediated inhibition of TCR-induced NFAT signalling in membrane human BTLA/membrane human TCR expressing T cells

In order to analyse the effect of chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 on membrane human BTLA/membrane human HVEM-mediated inhibition of membrane human TCR-induced NFAT signalling, the NFAT-response element-luciferase (RE-luc) human BTLA/HVEM Blockade Bioassay (see Example 3(c) above) was used to examine the ability of mouse anti-human HVEM antibodies to block the BTLA/HVEM-mediated inhibition of TCR-induced NFAT signalling.

Briefly, human HVEM and proprietary human TCR activator expressing CHO-K1 Activator cells were plated at 40,000 cells/well in flat-bottomed TC-treated white-solid 96-wells plates (Corning), and were incubated overnight at 37°C/5% CO_2. Next day, these cells were washed, and subsequently incubated with or without 0.0015-10 µg/mL (3-fold dilution steps) chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4. Then, human BTLA and human TCR expressing NFAT-RE-luc Jurkat Effector T cells were added at 50,000 cells/well. After 6 hours incubation at 37°C/5% CO_2, luciferase production in human BTLA and human TCR expressing NFAT-RE-luc Jurkat Effector T cells was measured using the Bio-Glo™ Luciferase Assay System (Promega) in a luminometer.

As shown in figure 10, all examined chimeric mouse/human anti-human HVEM antibodies dose-dependently blocked the BTLA/HVEM-mediated inhibition of TCR-induced NFAT signalling in human BTLA and human TCR expressing NFAT-RE-luc Jurkat Effector T cells to a variable degree (in this order; no. 45H6 > 49G4 > 11H7 > 36H12 >> 48H6).

These results demonstrated that chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 were able to block the human BTLA/human HVEM-mediated inhibition of TCR-induced NFAT signalling.

(k). Effect of BTLA/HVEM blocking chimeric mouse/human anti-human HVEM antibodies on membrane human BTLA/membrane human HVEM-mediated inhibition of TCR-induced cytokine release from membrane human BTLA/membrane human TCR expressing primary naïve human T cells

Like described above, ligation of HVEM on T lymphocytes by LIGHT delivers positive co-stimulatory signals through HVEM, whereas engagement of BTLA on T lymphocytes by HVEM provides negative co-inhibitory signals to T lymphocytes via BTLA (Del Rio et al. Journal Leukocyte Biology 2010; 87: 223-235). This BTLA/HVEM pathway down-regulates TCR-mediated signalling in both CD4 and CD8 T lymphocytes, and results in decreased T lymphocyte proliferation and cytokine production.

In order to analyse the effect of chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 on membrane human BTLA/membrane human HVEM-mediated inhibition of membrane human TCR-induced cytokine release, a co-culture of (1) stable human full-length HVEM-transfected HEK293F cells (clone no. 128; see above Example 1a), which were transiently transfected with membrane-bound anti-human CD3 (OKT3) single-chain variable fragment (scFv) TCR activator as described previously (Chen et al. Front Immunol 2017; 8: 793; artificial antigen-presenting cells) with slight modifications, and of (2) membrane human BTLA and membrane human TCR complex expressing primary human naïve T cells (responder cells) was used to examine the ability of mouse anti-human HVEM antibodies to attenuate/reverse the BTLA/HVEM-mediated inhibition of TCR-induced cytokine release (for assay principle, see also Figure 4A, except human HVEM expressing CHO-K1 artificial antigen-presenting cells and human BTLA expressing Jurkat Effector T cells were exchanged for human HVEM expressing HEK293F artificial antigen-presenting cells and human BTLA expressing primary human naïve T cells, respectively).

Briefly, cDNA encoding membrane-bound anti-human CD3 (OKT3) scFv TCR activator protein (SEQ ID NO. 76) was optimized for mammalian expression and synthesized by GENEART, Regensburg, Germany (see SEQ ID NO. 77). This cDNA was subcloned in a pcDNA3.1-derived expression plasmid. This anti-human CD3 (OKT3) scFv TCR activator protein plasmid was transfected in stable human full-length HVEM-transfected HEK293F cells (clone no. 128; see above Example 1a) using the FreeStyle™ 293 Expression System (Life Technologies). After 2 days, these HEK293F cells were harvested and resuspended at 1.0 x 10^6 cells/mL in RPMI-1640 culture medium (Gibco) containing 10% fetal calf serum (Capricorn) and 50 µg/mL gentamycin (Gibco). Prior to co-culturing, anti-human CD3 (OKT3) scFv TCR activator protein surface expression on transiently transfected human HVEM expressing HEK293F cells (i.e., used as artificial antigen-presenting cells) was confirmed using 1:200 diluted PE-conjugated goat anti-human IgG Fcγ-specific antibodies (Jackson ImmunoResearch) and flow cytometry.

Human peripheral blood mononuclear cells (PBMC) from healthy donors (informed consent) were isolated by density centrifugation on Lymphoprep™ (1.077 g/mL; Nycomed). Subsequently, human T lymphocytes (i.e., CD4 and CD8) were enriched from this PBMC fraction using the Dynabeads™ Untouched™ Human T Cells Kit (Invitrogen), and resuspended at 1.0 x10^6 cells/mL in RPMI-1640 culture medium (Gibco) containing 10% fetal calf serum (Capricorn) and 50 µg/mL gentamycin (Gibco). Prior to co-culturing, human BTLA surface expression on enriched human naïve T lymphocytes (i.e., used as responder cells) was confirmed using 1:20 diluted PE-conjugated mouse anti-human BTLA-specific antibody (BD Biosciences) and flow cytometry.

Human HVEM expressing artificial antigen-presenting HEK293F cells at 1.0 x 10^6 cells/mL were pre-treated with 40 µg/mL chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 for 15-30 minutes at RT. In parallel, 40 µg/mL human IgG4/κ (Sigma) was run as a negative isotype control. After this (i.e., without washing), these chimeric mouse/human anti-human HVEM antibody pre-treated artificial antigen-presenting HEK293F cells and enriched human naïve T lymphocytes were co-cultured at an 1:1 ratio (i.e., 50,000 T cells/50,000 artificial antigen-presenting cells/200 µL/well) in flat-bottomed TC-treated transparent 96-wells plates (Corning) with and without 0.5 µg/mL co-stimulatory mouse anti-human CD28 antibody (clone CD28.2; BD Biosciences) in the presence of 20 µg/mL chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 48H6, 11H7, and 49G4 or 20 µg/mL human IgG4/κ (Sigma) negative isotype control at 37°C/5% CO_2 for 2 days. After a 2-day culture, supernatants were harvested and frozen at -80°C until use.

Release of human IL-2, human TNFα, and human IFNγ from primary human naïve T lymphocytes was determined in these supernatants using in-house developed conventional sandwich ELISAs, i.e., (I) for IL-2 ELISA, a combination of rat anti-human IL-2 monoclonal coating antibody (clone MQ1-17H12; eBioscience), titrated rhuIL-2 standards (PeproTech), and biotinylated rabbit anti-human IL-2 polyclonal detection antibodies (eBioscience) was used, (II) for TNFα ELISA, a combination of mouse anti-human TNFα monoclonal antibody coating (clone MAb11; Biolegend), titrated rhuTNFα standards (PeproTech), and biotinylated mouse anti-human TNFα monoclonal detection antibody (clone MAb11; Biolegend) was used, and (III) for IFNγ ELISA, a combination of mouse anti-human IFNγ monoclonal coating antibody (clone NIB42; eBioscience), titrated rhuIFNγ standards (PeproTech), and biotinylated mouse anti-human IFNγ monoclonal detection antibody (clone 4S.B3; eBioscience) was used.

Supernatants from artificial antigen-presenting HEK293F cells or enriched human naïve T lymphocytes, which are not co-cultured but are cultured separately for 2 days (i.e., 50,000 artificial antigen-presenting cells/200 µL/well or 50,000 T cells//200 µL/well), show no measurable human IL-2, human TNFα, or human IFNγ levels.

As shown in figure 11A, all examined chimeric mouse/human anti-human HVEM antibodies attenuated/reversed the BTLA/HVEM-mediated inhibition of TCR/CD28-induced IL-2 release from co-cultured human BTLA expressing primary human naïve T lymphocytes enriched from all 6 healthy donors to a variable degree (rank order; no. 45H6 > 11H7 > 36H12 > 49G4 >> 48H6). In addition, chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, 11H7, and 49G4 attenuated/reversed the BTLA/HVEM-mediated inhibition of TCR-induced IL-2 release (if any) from co-cultured human BTLA expressing primary human naïve T

lymphocytes enriched from 2/6 healthy donors (i.e., donor A and H) to a variable degree (rank order; no. 45H6 > 11H7 > 36H12 > 49G4).

As shown in figure 11B, chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, and 11H7 attenuated/reversed the BTLA/HVEM-mediated inhibition of TCR/CD28-induced TNFα release from co-cultured human BTLA expressing primary human naïve T lymphocytes enriched from all 6 healthy donors to a variable degree (rank order; no. 45H6 > 11H7 > 36H12). In addition, chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, and 11H7 attenuated/reversed the BTLA/HVEM-mediated inhibition of TCR-induced TNFα release (if any) from co-cultured human BTLA expressing primary human naïve T lymphocytes enriched from 4/6 healthy donors (i.e., donor A, D, H and K) to a variable degree (rank order; no. 45H6 > 11H7 = 36H12).

As shown in figure 11C, chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, and 11H7 attenuated/reversed the BTLA/HVEM-mediated inhibition of TCR/CD28-induced IFNγ release from co-cultured human BTLA expressing primary human naïve T lymphocytes enriched from all 6 healthy donors to a variable degree (rank order; no. 45H6 > 11H7 > 36H12). In addition, chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, and 11H7 attenuated/reversed the BTLA/HVEM-mediated inhibition of TCR-induced IFNγ release (if any) from co-cultured human BTLA expressing primary human naïve T lymphocytes enriched from 4/6 healthy donors (i.e., donor A, D, H and K) to a variable degree (rank order; no. 45H6 = 11H7 > 36H12).

These results demonstrated that chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, and 11H7 were able to attenuate/reverse the human BTLA/human HVEM-mediated inhibition of TCR/CD28-induced IL-2, TNFα and IFNγ release from human BTLA expressing primary naïve human T cells. In addition, these results demonstrated that chimeric mouse/human anti-human HVEM antibodies no. 36H12, 45H6, and 11H7 were able to attenuate/reverse the human BTLA/human HVEM-mediated inhibition of TCR-induced IL-2, TNFα and IFNγ release (if any) from human BTLA expressing primary naïve human T cells.

DESCRIPTION OF SEQUENCES

SEQ ID NO. 1

Amino acid sequence human HVEM (Swiss-Prot no. Q92956.3; aa 1-283)

```
  1  MEPPGDWGPP PWRSTPKTDV LRLVLYLTFL GAPCYAPALP SCKEDEYPVG SECCPKCSPG
 61  YRVKEACGEL TGTVCEPCPP GTYIAHLNGL SKCLQCQMCD PAMGLRASRN CSRTENAVCG
121  CSPGHFCIVQ DGDHCAACRA YATSSPGQRV QKGGTESQDT LCQNCPPGTF SPNGTLEECQ
```

```
181  HQTKCSWLVT KAGAGTSSSH WVWWFLSGSL VIVIVCSTVG LIICVKRRKP RGDVVKVIVS
241  VQRKRQEAEG EATVIEALQA PPDVTTVAVE ETIPSFTGRS PNH
```

Signal peptide (aa sequence 1-38), extracellular domain (aa sequence 39-202, comprising CRD1 (aa sequence 42-75), CRD2 (aa sequence 78-119), CRD3 (aa sequence 121-162), and truncated CRD4 (aa sequence 165-179), 'linker' (aa sequence 180-202)), transmembrane domain (aa sequence 203-223), and cytoplasmic domain (aa sequence 224-283)

SEQ ID NO. 2

cDNA sequence coding for human HVEM protein (optimized for mammalian expression)

```
  1  ATGGAGCCCC CTGGCGATTG GGGACCTCCA CCTTGGAGAA GCACCCCCAA GACCGACGTG
 61  CTGCGGCTGG TGCTGTACCT GACCTTTCTG GGCGCTCCCT GTTACGCCCC TGCCCTGCCT
121  AGCTGCAAAG AGGACGAGTA CCCTGTGGGC AGCGAGTGCT GCCCTAAGTG CAGCCCTGGC
181  TACAGAGTGA AAGAGGCCTG CGGCGAGCTG ACCGGCACCG TGTGTGAACC TTGTCCCCCT
241  GGCACCTATA TCGCCCACCT GAACGGCCTG AGCAAGTGCC TGCAGTGCCA GATGTGCGAC
301  CCCGCTATGG GCCTGAGAGC CAGCAGAAAC TGCAGCCGGA CCGAGAATGC CGTGTGCGGC
361  TGTTCTCCTG GCCACTTCTG CATCGTGCAG GACGGCGATC ACTGCGCCGC CTGTAGAGCC
421  TACGCCACAT CTAGCCCAGG CCAGAGAGTG CAGAAGGGCG GCACCGAGAG CCAGGATACC
481  CTGTGCCAGA ATTGCCCTCC CGGCACCTTC AGCCCCAACG GCACACTGGA AGAGTGCCAG
541  CACCAGACCA AGTGCAGCTG GCTCGTGACC AAAGCCGGCG CTGGCACAAG CAGCTCTCAC
601  TGGGTGTGGT GGTTTCTGAG CGGCAGCCTC GTGATCGTGA TTGTGTGCAG CACCGTGGGC
661  CTGATCATCT GCGTGAAGCG GAGAAAGCCC AGAGGCGACG TCGTGAAAGT GATCGTGTCC
721  GTGCAGCGGA AGCGGCAGGA AGCCGAAGGC GAGGCCACAG TGATTGAGGC CCTGCAGGCT
781  CCCCCTGACG TGACAACAGT GGCCGTGGAA GAGACAATCC CCAGCTTCAC CGGCAGATCC
841  CCCAACCAC
```

SEQ ID NO. 3

Amino acid sequence CRD1 truncated human HVEM

```
  1  MEWSGVFMFL LSVTAGVHSE PCPPGTYIAH LNGLSKCLQC QMCDPAMGLR ASRNCSRTEN
 61  AVCGCSPGHF CIVQDGDHCA ACRAYATSSP GQRVQKGGTE SQDTLCQNCP PGTFSPNGTL
121  EECQHQTKCS WLVTKAGAGT SSSHWVWWFL SGSLVIVIVC STVGLIICVK RRKPRGDVVK
181  VIVSVQRKRQ EAEGEATVIE ALQAPPDVTT VAVEETIPSF TGRSPNH
```

Mouse Ig signal peptide (aa sequence 1-19), extracellular domain (aa sequence 20-146, comprising CRD2 (aa sequence 22-63), CRD3 (aa sequence 65-106), and truncated CRD4 (aa sequence 109-123), 'linker' fragment (aa

sequence 124-146)), transmembrane domain (aa sequence 147-167), and cytoplasmic domain (aa sequence 168-227)

SEQ ID NO. 4
cDNA sequence coding for CRD1 truncated human HVEM protein (optimized for mammalian expression)

```
  1  ATGGAGTGGA GCGGCGTGTT CATGTTCCTG CTGAGCGTGA CAGCCGGCGT GCACAGCGAA
 61  CCTTGTCCCC CTGGCACCTA TATCGCCCAC CTGAACGGCC TGAGCAAGTG CCTGCAGTGC
121  CAGATGTGCG ACCCCGCTAT GGGCCTGAGA GCCAGCAGAA ACTGCAGCCG GACCGAGAAT
181  GCCGTGTGCG GCTGTTCTCC TGGCCACTTC TGCATCGTGC AGGACGGCGA TCACTGCGCC
241  GCCTGTAGAG CCTACGCCAC ATCTAGCCCT GGCCAGAGAG TGCAGAAGGG CGGCACCGAG
301  AGCCAGGATA CCCTGTGCCA GAATTGCCCT CCCGGCACCT TCAGCCCCAA CGGCACACTG
361  GAAGAGTGCC AGCACCAGAC CAAGTGCAGC TGGCTCGTGA CCAAAGCCGG CGCTGGCACA
421  AGCAGCTCTC ACTGGGTGTG GTGGTTTCTG AGCGGCAGCC TCGTGATCGT GATTGTGTGC
481  AGCACCGTGG GCCTGATCAT CTGCGTGAAG CGGAGAAAGC CCAGAGGCGA CGTCGTGAAA
541  GTGATCGTGT CCGTGCAGCG GAAGCGGCAG GAAGCCGAAG GCGAGGCCAC AGTGATTGAG
601  GCCCTGCAGG CTCCCCCTGA CGTGACAACA GTGGCCGTGG AAGAGACAAT CCCCAGCTTC
661  ACCGGCAGAT CCCCCAACCA C
```

SEQ ID NO. 5
Amino acid sequence cynomolgus monkey HVEM (NCBI Reference Sequence: XP_005545061.1; aa 1-280)

```
  1  MEPPGGWGSP PRRPAPKADI LTLVLYLTFL GSPCYAPALP SCKEDEYPVG SECCPKCGPG
 61  FHVRQACGEQ TGTVCEPCSP GTYIAHFNGL SKCLQCQMCD PAMGLRTSRN CSTTANALCG
121  CSPGHFCIIQ DGDHCAACRA YATSSPGQRV QKGGTESQDT LCQNCPPGTF SSNGTLEECQ
181  HGTKCSKWLV TEAGPGTSSF RWVWWLLSGT LIVIIVGLIL GLIYVKRRKS RGDVVKVIVS
241  VQRKRQEAEG EAIVTEALQA PPDITTVAVE ETEPAFTGRS
```

SEQ ID NO. 6
cDNA sequence coding for cynomolgus monkey HVEM protein (optimized for mammalian expression)

```
  1  ATGGAGCCTC CAGGCGGATG GGGATCTCCC CCAAGAAGGC CTGCCCCCAA GGCCGATATC
 61  CTGACCCTGG TGCTGTACCT GACCTTCCTG GGCAGCCCTT GTTACGCCCC TGCCCTGCCT
121  AGCTGCAAAG AGGACGAGTA CCCTGTGGGC AGCGAGTGCT GCCCTAAGTG CGGCCCTGGA
181  TTTCATGTGC GGCAGGCCTG TGGCGAGCAG ACCGGCACAG TGTGCGAGCC TTGTAGCCCC
241  GGCACCTATA TCGCCCACTT CAACGGCCTG AGCAAGTGCC TGCAGTGCCA GATGTGCGAC
301  CCCGCTATGG GCCTGCGGAC CAGCAGAAAT TGCAGCACCA CCGCCAATGC CCTGTGCGGC
```

```
361  TGTTCTCCTG GCCACTTCTG CATTATTCAG GACGGCGACC ACTGCGCCGC CTGCAGAGCC
421  TATGCCACAT CTAGCCCTGG CCAGCGGGTG CAGAAGGGCG GAACAGAGTC TCAGGACACC
481  CTGTGCCAGA ACTGCCCCCC TGGCACCTTC AGCAGCAACG GCACCCTGGA AGAGTGCCAG
541  CACGGCACCA AGTGCAGCAA GTGGCTCGTG ACAGAGGCCG GACCTGGCAC CAGCAGCTTC
601  AGATGGGTGT GGTGGCTGCT GAGCGGCACA CTGATCGTGA TCATCGTGGG CCTGATCCTG
661  GGACTGATCT ACGTGAAGCG GCGGAAGTCC AGAGGCGACG TCGTGAAAGT GATCGTGTCC
721  GTGCAGCGGA AGAGACAGGA AGCCGAGGGC GAGGCCATTG TGACCGAAGC CCTGCAGGCC
781  CCTCCCGACA TTACAACCGT GGCCGTGGAA GAAACCGAGC CCGCCTTTAC CGGCAGATCC
```

SEQ ID NO. 7
PCR primer

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTG

SEQ ID NO. 8
PCR primer

ATGGATTTWCAGGTGCAGATTWTCAGCTTC

SEQ ID NO. 9
PCR primer

ATGGGCWTCAAAGATGGAGTCACA

SEQ ID NO. 10
PCR primer

ACTGGATGGTGGGAAGATGG

SEQ ID NO. 11
PCR primer

ATGAAATGCAGCTGGGGCATSTTCTTC

SEQ ID NO. 12
PCR primer

ATGRACTTTGGGYTCAGCTTGRTTT

SEQ ID NO. 13
PCR primer

ATGGGACTCCAGGCTCAATTTAGTTTTCCTT

SEQ ID NO. 14
PCR primer

ATGGCTTGTCYTTRGSGCTRCTCTTCTGC

SEQ ID NO. 15
PCR primer

CAGTGGATAGACAGATGGGGG

SEQ ID NO. 16
Consensus amino acid sequence of heavy chain variable region of mouse anti-human HVEM antibody 36H12

```
  1  EVQLQQSGAG LVKPGASVKL SCTASGFNIK DTYMHWVRQR PEQGLEWIGR IDPATANTKY
 61  DPKFQGKATL TTDTSSNTAY LQLSSLTSED TAVYYCVTYG YDVSWFAYWG LGALVTVSA
```

SEQ ID NO. 17
Consensus amino acid sequence of light chain variable region of mouse anti-human HVEM antibody 36H12

```
  1  DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV HSNGITYLEW YLQKPGQSPK LLIYKVSNRF
 61  SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP LTFGAGTKLE LK
```

Complementarity determining regions (CDRs) of mouse anti-human HVEM antibody 36H12: SEQ ID NO. 18-23

SEQ ID NO. 18
Amino acid sequence heavy chain CDR1 of 36H12

DTYMH

SEQ ID NO. 19
Amino acid sequence heavy chain CDR2 of 36H12

RIDPATANTKYDPKFQG

SEQ ID NO. 20
Amino acid sequence heavy chain CDR3 of 36H12

YGYDVSWFAY

SEQ ID NO. 21
Amino acid sequence light chain CDR1 of 36H12

RSSQSIVHSNGITYLE

SEQ ID NO. 22
Amino acid sequence light chain CDR2 of 36H12

KVSNRFS

SEQ ID NO. 23
Amino acid sequence light chain CDR3 of 36H12

FQGSHVPLT

SEQ ID NO. 24
Consensus amino acid sequence of heavy chain variable region of mouse anti-human HVEM antibody 45H6

```
  1  DVQLVESGGG LVQPGGSRKL SCAASGFTFS SFGMHWVRQA PEKGLEWVAY ISSGNSNIYY
 61  VDTVKGRFTI SRDNPKNTLF LQMTSLRSED TAMYYCARKR AYGDYSGFSM DYWGQGTSVT
121  VSS
```

SEQ ID NO. 25
Consensus amino acid sequence of light chain variable region of mouse anti-human HVEM antibody 45H6

```
  1  DIVMTQSQKF MSTSVGDRVS VTCKASQNVD TNVAWYQQKP GQSPKALIYS ASYRYSGVPD
 61  RFTGSGSGTD FTLTISNVQS EDLAEYFCQQ YNKFPLTFGG GTKLEIK
```

Complementarity determining regions (CDRs) of mouse anti-human HVEM antibody 45H6:
SEQ ID NO. 26-31

SEQ ID NO. 26
Amino acid sequence heavy chain CDR1 of 45H6

SFGMH

SEQ ID NO. 27
Amino acid sequence heavy chain CDR2 of 45H6

YISSGNSNIYYVDTVKG

SEQ ID NO. 28
Amino acid sequence heavy chain CDR3 of 45H6

KRAYGDYSGFSMDY

SEQ ID NO. 29
Amino acid sequence light chain CDR1 of 45H6

KASQNVDTNVA

SEQ ID NO. 30
Amino acid sequence light chain CDR2 of 45H6

SASYRYS

SEQ ID NO. 31

Amino acid sequence light chain CDR3 of 45H6

QQYNKFPLT

SEQ ID NO. 32
Consensus amino acid sequence of heavy chain variable region of mouse anti-human HVEM antibody 48H6

```
  1  EVKLVESGGG LVKPGGSLKL SCAASGFTFS GYAMSWVRQT PEKRLEWVAS ISSGGSTYYP
 61  DSVKGRFTIP RDDARNILYL QMSSLRSEDT AIYYCARGGH GSSYVYWGQG TTLTVSS
```

SEQ ID NO. 33
Consensus amino acid sequence of light chain variable region of mouse anti-human HVEM antibody 48H6

```
  1  DIVMSQSPSS LAVSVGEKVT MSCKSSQSLL YSSNQKNYLA WYQQKPGQSP KLLIYWASTR
 61  ESGVPDRFTG SGSGTDFTLT ISSVKAEDLA VYYCHQYYSY PLTFGAGTKL ELN
```

Complementarity determining regions (CDRs) of mouse anti-human HVEM antibody 48H6:
SEQ ID NO. 34-39

SEQ ID NO. 34
Amino acid sequence heavy chain CDR1 of 48H6

GYAMS

SEQ ID NO. 35
Amino acid sequence heavy chain CDR2 of 48H6

SISSGGSTYYPDSVKG

SEQ ID NO. 36
Amino acid sequence heavy chain CDR3 of 48H6

GGHGSSYVY

SEQ ID NO. 37
Amino acid sequence light chain CDR1 of 48H6

KSSQSLLYSSNQKNYLA

SEQ ID NO. 38
Amino acid sequence light chain CDR2 of 48H6

WASTRES

SEQ ID NO. 39
Amino acid sequence light chain CDR3 of 48H6

HQYYSYPLT

SEQ ID NO. 40
Consensus amino acid sequence of heavy chain variable region of mouse anti-human HVEM antibody 11H7

```
  1  QVQLKQSGPG LVQPSQSLSI TCTVSGFSLT IYGVHWVRQS PGKGLEWLGV IWSGGSTDYN
 61  AAFISRLSIS KDNSKSQVFF KMNSLQANDT AIYYCARRDY GSRSFYYAMD YWGQGTSVTV
121  SS
```

SEQ ID NO: 41
Consensus amino acid sequence of light chain variable region of mouse anti-human HVEM antibody 11H7

```
  1  EIVLTQSPAL MAASPGEKVT ITCSVSSSIS SSNLHWYQQK SETSPKPWIY GTSNLASGVP
 61  VRFSGSGSGT SYSLTISSME AEDAATYYCQ QWSSYPLTFG GGTKLEIK
```

Complementarity determining regions (CDRs) of mouse anti-human HVEM antibody 11H7:
SEQ ID NO. 42-47

SEQ ID NO. 42
Amino acid sequence heavy chain CDR1 of 11H7

IYGVH

SEQ ID NO. 43
Amino acid sequence heavy chain CDR2 of 11H7

VIWSGGSTDYNAAFIS

SEQ ID NO. 44
Amino acid sequence heavy chain CDR3 of 11H7

RDYGSRSFYYAMDY

SEQ ID NO. 45
Amino acid sequence light chain CDR1 of 11H7

SVSSSISSSNLH

SEQ ID NO. 46
Amino acid sequence light chain CDR2 of 11H7

GTSNLAS

SEQ ID NO. 47
Amino acid sequence light chain CDR3 of 11H7

QQWSSYPLT

SEQ ID NO. 48
Consensus amino acid sequence of heavy chain variable region of mouse anti-human HVEM antibody 49G4

```
  1  EVQLQQSGAE LVKPGASVKL SCRASGFNIK DTYMHWVKQR PEQGLEWIGR IDPARGNTKY
 61  DPKFQGKATI TADTSSNTAY LQLSSLTSED TAVYYCASAM DYWGQGTSVT VSS
```

SEQ ID NO. 49
Consensus amino acid sequence of light chain variable region of mouse anti-human HVEM antibody 49G4

```
  1  DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV HSNGNTYLEW FLQKPGQSPK LLIYKVSNRF
 61  SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP LTFGAGTKLE LK
```

Complementarity determining regions (CDRs) of mouse anti-human HVEM antibody 49G4:
SEQ ID NO. 50-55

SEQ ID NO. 50
Amino acid sequence heavy chain CDR1 of 49G4

DTYMH

SEQ ID NO. 51
Amino acid sequence heavy chain CDR2 of 49G4

RIDPARGNTKYDPKFQG

SEQ ID NO. 52
Amino acid sequence heavy chain CDR3 of 49G4

AMDY

SEQ ID NO. 53
Amino acid sequence light chain CDR1 of 49G4

RSSQSIVHSNGNTYLE

SEQ ID NO. 54
Amino acid sequence light chain CDR2 of 49G4

KVSNRFS

SEQ ID NO. 55

Amino acid sequence light chain CDR3 of 49G4

FQGSHVPLT

SEQ ID NO. 56

cDNA sequence coding for chimeric mouse VH 36H12 and human constant heavy IgG4 chain

```
    1  ATGGAGCTGG GCCTGAGCTG GATTTTTCTG CTGGCCATCC TGAAGGGCGT
GCAGTGCGAA
   61  GTTCAGCTGC AGCAATCTGG CGCCGGACTG GTTAAGCCTG GCGCCTCTGT
GAAGCTGAGC
  121  TGTACCGCCA GCGGCTTCAA CATCAAGGAC ACCTACATGC ACTGGGTCCG
ACAGAGGCCT
  181  GAGCAGGGAC TCGAATGGAT CGGCAGAATC GATCCCGCCA CCGCCAACAC
CAAATACGAC
  241  CCCAAGTTCC AGGGCAAAGC CACACTGACC ACCGACACCA GCAGCAACAC
AGCCTACCTG
  301  CAGCTGTCTA GCCTGACCAG CGAAGATACC GCCGTGTACT ACTGCGTGAC
CTACGGCTAC
  361  GATGTGTCTT GGTTTGCCTA CTGGGGACTG GGCGCCCTGG TTACAGTTTC
TGCCGCCTCT
  421  ACAAAGGGCC CCAGCGTTTT CCCACTGGCT CCCTGTAGCA GAAGCACCAG
CGAATCTACA
  481  GCCGCTCTGG GCTGCCTGGT CAAGGACTAC TTTCCTGAGC CTGTGACCGT
GTCCTGGAAC
  541  TCTGGCGCTC TGACATCTGG CGTGCACACC TTTCCAGCCG TGCTGCAAAG
CAGCGGCCTG
  601  TACTCTCTGA GCAGCGTGGT CACAGTGCCT AGCTCTAGCC TGGGCACCAA
GACCTACACC
  661  TGTAATGTGG ACCACAAGCC TAGCAACACC AAGGTGGACA AGCGCGTGGA
ATCTAAGTAC
  721  GGCCCTCCTT GTCCTCCATG TCCTGCACCT GAGTTTCTCG GCGGACCCTC
CGTGTTCCTG
  781  TTTCCTCCAA AGCCTAAGGA CACCCTGATG ATCAGCAGAA CCCCTGAAGT
GACCTGCGTG
  841  GTGGTGGACG TTTCCCAAGA GGACCCTGAG GTGCAGTTCA ATTGGTACGT
GGACGGCGTG
  901  GAAGTGCACA ACGCCAAGAC CAAGCCTAGA GAGGAACAGT TCAACAGCAC
CTACAGAGTG
  961  GTGTCCGTGC TGACAGTGCT GCACCAGGAT TGGCTGAACG GCAAAGAGTA
CAAGTGCAAG
 1021  GTGTCCAACA AGGGCCTGCC TAGCAGCATC GAGAAAACCA TCAGCAAGGC
CAAGGGCCAG
 1081  CCAAGAGAAC CCCAGGTGTA CACACTGCCT CCAAGCCAAG AGGAAATGAC
CAAGAACCAG
```

1141 GTGTCCCTGA CCTGCCTCGT GAAGGGCTTC TACCCTTCCG ATATCGCCGT GGAATGGGAG
1201 AGCAATGGCC AGCCTGAGAA CAACTACAAG ACAACCCCTC CTGTGCTGGA CAGCGACGGC
1261 TCATTCTTCC TGTACAGCAG ACTGACCGTG GACAAGAGCA GATGGCAAGA GGGCAACGTG
1321 TTCTCCTGCA GCGTGATGCA CGAGGCCCTG CACAACCACT ACACCCAGAA GTCTCTGTCC
1381 CTGTCTCTGG GCAAG

SEQ ID NO. 57
cDNA sequence coding for chimeric mouse VH 45H6 and human constant heavy IgG4 chain

1 ATGGAGCTGG GCCTGAGCTG GATTTTTCTG CTGGCCATCC TGAAGGGCGT GCAGTGTGAT
61 GTGCAGCTGG TGGAATCTGG CGGAGGACTG GTTCAACCTG GCGGCAGCAG AAAGCTGTCT
121 TGTGCCGCCA GCGGCTTCAC CTTCAGCAGC TTTGGAATGC ACTGGGTCCG ACAGGCCCCT
181 GAGAAAGGCC TTGAGTGGGT CGCCTACATC AGCAGCGGCA ACAGCAACAT CTACTACGTG
241 GACACCGTGA AGGGCAGATT CACCATCTCC AGAGACAACC CCAAGAATAC CCTGTTCCTG
301 CAGATGACCA GCCTGCGGAG CGAGGATACC GCCATGTACT ACTGCGCCCG GAAAAGAGCC
361 TACGGCGACT ACAGCGGCTT TAGCATGGAT TACTGGGGCC AGGGCACCAG CGTGACAGTG
421 TCTAGCGCCT CTACAAAGGG CCCTAGCGTG TTCCCTCTGG CTCCTTGTAG CAGAAGCACC
481 AGCGAGTCTA CAGCCGCTCT GGGCTGTCTG GTCAAGGACT ACTTTCCCGA GCCTGTGACC
541 GTGTCCTGGA ATTCTGGCGC TCTGACAAGC GGCGTGCACA CCTTTCCAGC TGTGCTGCAA
601 AGCAGCGGCC TGTACTCTCT GAGCAGCGTG GTCACAGTGC CTAGCTCTAG CCTGGGCACC
661 AAGACCTACA CCTGTAATGT GGACCACAAG CCTAGCAACA CCAAGGTGGA CAAGCGCGTG
721 GAATCTAAGT ACGGCCCTCC TTGTCCTCCA TGTCCTGCTC CAGAGTTTCT CGGCGGACCC
781 TCCGTGTTCC TGTTTCCTCC AAAGCCTAAG GACACCCTGA TGATCAGCAG AACCCCTGAA
841 GTGACCTGCG TGGTGGTGGA CGTTTCCCAA GAGGACCCTG AGGTGCAGTT CAATTGGTAC
901 GTGGACGGCG TGGAAGTGCA CAACGCCAAG ACCAAGCCTA GAGAGGAACA GTTCAACAGC

961 ACCTACAGAG TGGTGTCCGT GCTGACCGTG CTGCACCAGG ATTGGCTGAA CGGCAAAGAG
1021 TACAAGTGCA AGGTGTCCAA CAAGGGCCTG CCTAGCAGCA TCGAGAAAAC CATCAGCAAG
1081 GCCAAGGGCC AGCCAAGAGA ACCCCAGGTG TACACACTGC CTCCAAGCCA AGAGGAAATG
1141 ACCAAGAACC AGGTGTCCCT GACCTGCCTG GTTAAGGGCT TCTACCCCTC CGATATCGCC
1201 GTGGAATGGG AGAGCAATGG CCAGCCTGAG AACAACTACA AGACCACACC ACCTGTGCTG
1261 GACAGCGACG GCTCATTCTT CCTGTACAGC AGACTGACCG TGGACAAGAG CAGATGGCAA
1321 GAGGGCAACG TGTTCAGCTG CAGCGTGATG CACGAGGCCC TGCACAACCA CTACACCCAG
1381 AAGTCTCTGA GCCTGTCTCT GGGCAAG

SEQ ID NO. 58

cDNA sequence coding for chimeric mouse VH 48H6 and human constant heavy IgG4 chain

1 ATGGAGCTGG GCCTGAGCTG GATTTTTCTG CTGGCCATCC TGAAGGGCGT GCAGTGCGAA
61 GTGAAGCTGG TGGAATCTGG CGGCGGACTG GTTAAGCCTG GCGGATCTCT GAAGCTGAGC
121 TGTGCCGCCA GCGGCTTTAC CTTTAGCGGC TACGCCATGA GCTGGGTCCG ACAGACACCC
181 GAGAAGAGAC TGGAATGGGT CGCCAGCATC AGCAGCGGCG GCAGCACATA TTACCCCGAC
241 TCTGTGAAGG GCCGCTTTAC AATCCCCAGA GATGACGCCC GGAACATCCT GTACCTGCAG
301 ATGAGCAGCC TGCGGAGCGA GGATACCGCC ATCTACTATT GTGCCAGAGG CGGCCACGGC
361 AGCAGCTATG TTTATTGGGG CCAGGGCACC ACACTGACCG TGTCTAGCGC CTCTACAAAG
421 GGCCCTAGCG TGTTCCCTCT GGCTCCTTGT AGCAGAAGCA CCAGCGAGTC TACAGCCGCT
481 CTGGGCTGTC TGGTCAAGGA CTACTTTCCC GAGCCTGTGA CAGTGTCCTG GAACTCTGGC
541 GCTCTGACAA GCGGCGTGCA CACATTTCCA GCCGTGCTGC AAAGCAGCGG CCTGTACTCT
601 CTGAGCAGCG TGGTCACAGT GCCTAGCTCT AGCCTGGGCA CCAAGACCTA CACCTGTAAT
661 GTGGACCACA AGCCTAGCAA CACCAAGGTG GACAAGCGCG TGGAATCTAA GTACGGCCCT
721 CCTTGTCCTC CATGTCCTGC ACCTGAGTTT CTCGGCGGAC CCTCCGTGTT CCTGTTTCCT

781 CCAAAGCCTA AGGACACCCT GATGATCAGC AGAACCCCTG AAGTGACCTG
CGTGGTGGTG
841 GACGTTTCCC AAGAGGACCC TGAGGTGCAG TTCAATTGGT ACGTGGACGG
CGTGGAAGTG
901 CACAACGCCA AGACCAAGCC TAGAGAGGAA CAGTTCAACA GCACCTACAG
AGTGGTGTCC
961 GTGCTGACAG TGCTGCACCA GGATTGGCTG AACGGCAAAG AGTACAAGTG
CAAGGTGTCC
1021 AACAAGGGCC TGCCTAGCAG CATCGAGAAA ACCATCAGCA AGGCCAAGGG
CCAGCCAAGA
1081 GAACCCCAGG TGTACACACT GCCTCCAAGC CAAGAGGAAA TGACCAAGAA
CCAGGTGTCC
1141 CTGACCTGCC TCGTGAAGGG CTTCTACCCT TCCGATATCG CCGTGGAATG
GGAGAGCAAT
1201 GGCCAGCCTG AGAACAACTA CAAGACAACC CCTCCTGTGC TGGACAGCGA
CGGCTCATTC
1261 TTCCTGTACA GCAGGCTGAC CGTGGACAAG AGCAGATGGC AAGAGGGCAA
CGTGTTCAGC
1321 TGCAGCGTGA TGCACGAGGC CCTGCACAAC CACTACACCC AGAAGTCTCT
GAGCCTGTCT
1381 CTGGGCAAG

SEQ ID NO. 59

cDNA sequence coding for chimeric mouse VH 11H7 and human constant heavy IgG4 chain

1 ATGGAGCTGG GCCTGAGCTG GATTTTTCTG CTGGCCATCC TGAAGGGCGT
GCAGTGTCAG
61 GTGCAGCTGA AGCAGTCTGG ACCTGGACTG GTGCAGCCTA GCCAGAGCCT
GAGCATCACC
121 TGTACCGTGT CCGGCTTCAG CCTGACCATC TATGGCGTGC ACTGGGTCCG
ACAGAGCCCT
181 GGCAAAGGAC TGGAATGGCT GGGAGTGATT TGGAGCGGCG GCAGCACCGA
TTACAACGCC
241 GCCTTTATCA GCAGACTGAG CATCTCCAAG GACAACAGCA AGAGCCAGGT
GTTCTTCAAG
301 ATGAACTCCC TGCAGGCCAA CGACACCGCC ATCTACTACT GCGCCAGAAG
AGACTACGGC
361 AGCCGGTCCT TCTACTACGC TATGGACTAT TGGGGCCAGG GCACCAGCGT
GACAGTGTCT
421 AGCGCCTCTA CAAAGGGCCC TAGCGTGTTC CCTCTGGCTC CTTGTAGCAG
AAGCACCAGC
481 GAGTCTACAG CCGCTCTGGG CTGTCTGGTC AAGGACTACT TTCCCGAGCC
AGTGACCGTG
541 TCCTGGAATT CTGGCGCTCT GACAAGCGGC GTGCACACCT TTCCAGCTGT
GCTGCAAAGC
601 AGCGGCCTGT ACTCTCTGAG CAGCGTGGTC ACAGTGCCTA GCTCTAGCCT
GGGCACCAAG

661 ACCTACACCT GTAATGTGGA CCACAAGCCT AGCAACACCA AGGTGGACAA
GCGCGTGGAA
721 TCTAAGTACG GCCCTCCTTG TCCTCCATGT CCTGCTCCAG AGTTTCTCGG
CGGACCCTCC
781 GTGTTCCTGT TTCCTCCAAA GCCTAAGGAC ACCCTGATGA TCAGCAGAAC
CCCTGAAGTG
841 ACCTGCGTGG TGGTGGACGT TTCCCAAGAG GACCCTGAGG TGCAGTTCAA
TTGGTACGTG
901 GACGGCGTGG AAGTGCACAA CGCCAAGACC AAGCCTAGAG AGGAACAGTT
CAACAGCACC
961 TACAGAGTGG TGTCCGTGCT GACCGTGCTG CATCAGGATT GGCTGAACGG
CAAAGAGTAC
1021 AAGTGCAAGG TGTCCAACAA GGGCCTGCCT AGCAGCATCG AGAAAACCAT
CAGCAAGGCC
1081 AAGGGCCAGC CAAGAGAACC CCAGGTGTAC ACACTGCCTC CAAGCCAAGA
GGAAATGACC
1141 AAGAATCAGG TGTCCCTGAC CTGCCTCGTG AAGGGCTTCT ACCCTTCCGA
TATCGCCGTG
1201 GAATGGGAGA GCAATGGCCA GCCTGAGAAC AACTACAAGA CAACCCCTCC
TGTGCTGGAC
1261 AGCGACGGCT CATTCTTCCT GTACTCCAGA CTGACCGTGG ACAAGAGCAG
ATGGCAAGAG
1321 GGCAACGTGT TCAGCTGCTC CGTGATGCAC GAGGCCCTGC ACAACCACTA
CACCCAGAAG
1381 TCCCTGAGCC TGTCTCTGGG CAAA

SEQ ID NO. 60

cDNA sequence coding for chimeric mouse VH 49G4 and human constant heavy IgG4 chain

1 ATGGAGCTGG GCCTGAGCTG GATTTTTCTG CTGGCCATCC TGAAGGGCGT
GCAGTGCGAA
61 GTTCAGCTGC AGCAGTCTGG CGCCGAGCTT GTGAAACCTG GCGCCTCTGT
GAAGCTGAGC
121 TGTAGAGCCA GCGGCTTCAA CATCAAGGAC ACCTACATGC ACTGGGTCAA
GCAGAGGCCT
181 GAGCAGGGCC TCGAATGGAT CGGCAGAATC GATCCCGCCA GAGGCAACAC
CAAATACGAC
241 CCCAAGTTCC AGGGCAAAGC CACCATCACC GCCGACACCT CTAGCAACAC
AGCCTACCTG
301 CAGCTGTCCA GCCTGACCTC TGAAGATACC GCCGTGTACT ACTGCGCCAG
CGCTATGGAT
361 TATTGGGGCC AGGGCACAAG CGTGACCGTG TCTAGCGCCT CTACAAAGGG
CCCTAGCGTG

```
 421  TTCCCACTGG CTCCCTGTAG CAGAAGCACC AGCGAATCTA CAGCCGCTCT
GGGCTGCCTG
 481  GTCAAGGACT ACTTTCCTGA GCCTGTGACA GTGTCCTGGA ACTCTGGCGC
TCTGACAAGC
 541  GGCGTGCACA CATTTCCAGC CGTGCTGCAA AGCAGCGGCC TGTACTCTCT
GAGCAGCGTG
 601  GTCACAGTGC CTAGCTCTAG CCTGGGCACC AAGACCTACA CCTGTAATGT
GGACCACAAG
 661  CCTTCCAACA CCAAGGTGGA CAAGCGCGTG GAATCTAAGT ACGGCCCTCC
TTGTCCTCCA
 721  TGTCCTGCAC CTGAGTTTCT CGGCGGACCC TCCGTGTTCC TGTTTCCTCC
AAAGCCTAAG
 781  GACACCCTGA TGATCAGCAG AACCCCTGAA GTGACCTGCG TGGTGGTGGA
CGTTTCCCAA
 841  GAGGACCCTG AGGTGCAGTT CAATTGGTAC GTGGACGGCG TGGAAGTGCA
CAACGCCAAG
 901  ACCAAGCCTA GAGAGGAACA GTTCAACAGC ACCTACAGAG TGGTGTCCGT
GCTGACAGTG
 961  CTGCACCAGG ATTGGCTGAA CGGCAAAGAG TACAAGTGCA AGGTGTCCAA
CAAGGGCCTG
1021  CCTAGCAGCA TCGAGAAAAC CATCAGCAAG GCCAAGGGCC AGCCAAGAGA
ACCCCAGGTG
1081  TACACACTGC CTCCAAGCCA AGAGGAAATG ACCAAGAACC AGGTGTCCCT
GACCTGCCTC
1141  GTGAAGGGCT TCTACCCTTC CGATATCGCC GTGGAATGGG AGAGCAATGG
CCAGCCTGAG
1201  AACAACTACA AGACAACCCC TCCTGTGCTG GACTCCGACG GCTCATTCTT
CCTGTACAGC
1261  AGACTGACCG TGGACAAGAG CAGATGGCAA GAGGGCAACG TGTTCTCCTG
CAGCGTGATG
1321  CACGAAGCCC TGCACAACCA CTACACCCAG AAGTCTCTGT CCCTGTCTCT GGGCAAG
```

SEQ ID NO. 61

cDNA sequence coding for chimeric mouse VL 36H12 and human constant light kappa chain

```
   1  ATGGACATGA GAGTTCCCGC TCAGCTGCTG GGACTGCTGC TGCTTTGGTT TCCTGGCGCT
  61  AGATGCGACG TGCTGATGAC CCAGACACCT CTGAGCCTGC CTGTGTCTCT GGGAGATCAG
 121  GCCAGCATCA GCTGCAGATC CAGCCAGAGC ATCGTGCACA GCAACGGCAT CACCTACCTG
 181  GAATGGTATC TGCAGAAGCC CGGACAGAGC CCCAAGCTGC TGATCTACAA GGTGTCCAAC
 241  CGGTTCAGCG GCGTGCCCGA TAGATTTTCT GGCAGCGGCT CTGGCACCGA CTTCACCCTG
 301  AAGATCTCCA GAGTGGAAGC CGAGGACCTG GGCGTGTACT ACTGCTTCCA AGGCTCTCAC
 361  GTGCCCCTGA CATTTGGAGC CGGCACCAAG CTGGAACTGA AGAGAACAGT GGCCGCTCCT
 421  AGCGTGTTCA TCTTCCCACC TTCCGACGAG CAGCTGAAAA GCGGCACAGC CTCTGTCGTG
 481  TGCCTGCTGA ACAACTTCTA CCCCAGAGAA GCCAAGGTGC AGTGGAAGGT GGACAACGCC
 541  CTGCAGAGCG GCAATAGCCA AGAGAGCGTG ACCGAGCAGG ACAGCAAGGA CTCTACCTAC
```

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601  AGCCTGAGCA GCACCCTGAC ACTGAGCAAG GCCGACTACG AGAAGCACAA AGTGTACGCC
661  TGCGAAGTGA CCCACCAGGG CCTTTCTAGC CCTGTGACCA AGAGCTTCAA CCGGGGCGAA
721  TGT
```

SEQ ID NO. 62
cDNA sequence coding for chimeric mouse VL 45H6 and human constant light kappa chain

```
  1  ATGGACATGA GAGTTCCCGC TCAGCTGCTG GGACTGCTGC TGCTTTGGTT TCCTGGCGCT
 61  AGATGCGACA TCGTGATGAC CCAGAGCCAG AAATTCATGA GCACCAGCGT GGGCGACAGA
121  GTGTCCGTGA CATGTAAAGC CAGCCAGAAC GTGGACACCA ACGTGGCCTG GTATCAGCAG
181  AAGCCTGGAC AGAGCCCCAA GGCTCTGATC TACAGCGCCA GCTACAGATA CAGCGGCGTG
241  CCCGATAGAT TCACAGGCAG CGGCTCTGGC ACCGACTTCA CCCTGACAAT CAGCAACGTG
301  CAGAGCGAGG ACCTGGCCGA GTATTTCTGC CAGCAGTACA ACAAGTTCCC TCTGACCTTC
361  GGCGGAGGCA CCAAGCTGGA AATCAAGAGA ACAGTGGCCG CTCCTAGCGT GTTCATCTTC
421  CCACCTTCCG ACGAGCAGCT GAAAAGCGGC ACAGCCTCTG TCGTGTGCCT GCTGAACAAC
481  TTCTACCCCA GAGAAGCCAA GGTGCAGTGG AAGGTGGACA ACGCCCTGCA GAGCGGCAAT
541  AGCCAAGAGA GCGTGACCGA GCAGGACAGC AAGGACTCTA CCTACAGCCT GAGCAGCACA
601  CTGACCCTGA GCAAGGCCGA CTACGAGAAG CACAAAGTGT ACGCCTGCGA AGTGACCCAC
661  CAGGGCCTTT CTAGCCCTGT GACCAAGAGC TTCAACCGGG GCGAATGT
```

SEQ ID NO. 63
cDNA sequence coding for chimeric mouse VL 48H6 and human constant light kappa chain

```
  1  ATGGACATGA GAGTTCCCGC TCAGCTGCTG GGACTGCTGC TGCTTTGGTT TCCTGGCGCT
 61  AGATGCGACA TCGTGATGTC TCAGAGCCCT AGCAGCCTGG CCGTGTCTGT GGGAGAGAAA
121  GTGACCATGA GCTGCAAGAG CAGCCAGAGC CTGCTGTACT CCAGCAACCA GAAGAACTAC
181  CTGGCCTGGT ATCAGCAGAA GCCCGGACAG TCTCCCAAGC TGCTGATCTA CTGGGCCAGC
241  ACCAGAGAAA GCGGCGTGCC CGATAGATTC ACAGGCAGCG GCAGCGGAAC CGACTTCACC
301  CTGACAATCA GCAGCGTGAA GGCCGAGGAC CTGGCTGTGT ACTACTGCCA CCAGTATTAC
361  AGCTACCCTC TGACCTTCGG AGCCGGCACC AAGCTGGAAC TGAACAGAAC AGTGGCCGCT
421  CCTAGCGTGT TCATCTTCCC ACCTTCCGAC GAGCAGCTGA AGTCTGGCAC AGCCTCTGTC
481  GTGTGCCTGC TGAACAACTT CTACCCCAGA GAAGCCAAGG TGCAGTGGAA GGTGGACAAC
541  GCCCTGCAGA GCGGCAATAG CCAAGAGAGC GTGACCGAGC AGGACAGCAA GGACTCTACC
601  TACAGCCTGA GCAGCACACT GACCCTGAGC AAGGCCGACT ACGAGAAGCA CAAAGTGTAC
661  GCCTGCGAAG TGACCCACCA GGGCCTTTCT AGCCCTGTGA CCAAGAGCTT CAACCGGGGC
721  GAATGT
```

SEQ ID NO. 64
cDNA sequence coding for chimeric mouse VL 11H7 and human constant light kappa chain

```
  1  ATGGACATGA GAGTTCCCGC TCAGCTGCTG GGACTGCTGC TGCTTTGGTT TCCTGGCGCT
 61  AGATGCGAGA TCGTGCTGAC ACAGAGCCCT GCTCTGATGG CTGCTTCCCC TGGCGAGAAA
121  GTGACCATCA CCTGTAGCGT GTCCAGCAGC ATCAGCAGCT CCAACCTGCA CTGGTATCAG
181  CAGAAGTCCG AGACAAGCCC CAAGCCTTGG ATCTACGGCA CCAGCAATCT GGCCAGCGGA
241  GTGCCTGTCA GATTTTCTGG CAGCGGCTCT GGCACCAGCT ACAGCCTGAC AATCAGCAGC
301  ATGGAAGCCG AGGATGCCGC CACCTACTAC TGCCAGCAGT GGTCTAGCTA CCCTCTGACC
361  TTTGGCGGAG GCACCAAGCT GGAAATCAAG CGGACAGTGG CCGCTCCTAG CGTGTTCATC
421  TTTCCACCTA GCGACGAGCA GCTGAAGTCT GGCACAGCCT CTGTCGTGTG CCTGCTGAAC
481  AACTTCTACC CCAGAGAAGC CAAGGTGCAG TGGAAGGTGG ACAACGCCCT GCAGAGCGGC
541  AATAGCCAAG AGAGCGTGAC CGAGCAGGAC AGCAAGGACT CCACCTATAG CCTGAGCAGC
601  ACCCTGACAC TGAGCAAGGC CGACTACGAG AAGCACAAAG TGTACGCCTG CGAAGTGACC
661  CACCAGGGCC TTTCTAGCCC TGTGACCAAG AGCTTCAACC GGGGCGAATG T
```

SEQ ID NO. 65

cDNA sequence coding for chimeric mouse VL and 49G4 human constant light kappa chain

```
  1  ATGGACATGA GAGTTCCCGC TCAGCTGCTG GGACTGCTGC TGCTTTGGTT TCCTGGCGCT
 61  AGATGCGACG TGCTGATGAC CCAGACACCT CTGAGCCTGC CTGTGTCTCT GGGAGATCAG
121  GCCAGCATCA GCTGCAGATC CAGCCAGAGC ATCGTGCACA GCAACGGCAA TACCTACCTG
181  GAATGGTTCC TGCAGAAGCC CGGACAGAGC CCCAAGCTGC TGATCTACAA GGTGTCCAAC
241  CGGTTCAGCG GCGTGCCCGA TAGATTTTCT GGCAGCGGCT CTGGCACCGA CTTCACCCTG
301  AAGATCTCCA GAGTGGAAGC CGAGGACCTG GGCGTGTACT ACTGCTTCCA AGGCTCTCAC
361  GTGCCCCTGA CATTTGGAGC CGGCACCAAG CTGGAACTGA AGAGAACAGT GGCCGCTCCT
421  AGCGTGTTCA TCTTCCCACC TTCCGACGAG CAGCTGAAAA GCGGCACAGC CTCTGTCGTG
481  TGCCTGCTGA ACAACTTCTA CCCCAGAGAA GCCAAGGTGC AGTGGAAGGT GGACAACGCC
541  CTGCAGAGCG GCAATAGCCA AGAGAGCGTG ACCGAGCAGG ACAGCAAGGA CTCTACCTAC
601  AGCCTGAGCA GCACCCTGAC ACTGAGCAAG GCCGACTACG AGAAGCACAA AGTGTACGCC
661  TGCGAAGTGA CCCACCAGGG CCTTTCTAGC CCTGTGACCA AGAGCTTCAA CCGGGGCGAA
721  TGT
```

SEQ ID NO. 66

Amino acid sequence of chimeric mouse VH 36H12 and human constant heavy IgG4 chain

```
  1  MELGLSWIFL LAILKGVQCE VQLQQSGAGL VKPGASVKLS CTASGFNIKD TYMHWVRQRP
 61  EQGLEWIGRI DPATANTKYD PKFQGKATLT TDTSSNTAYL QLSSLTSEDT AVYYCVTYGY
121  DVSWFAYWGL GALVTVSAAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN
181  SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY
241  GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV
301  EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ
361  PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
```

```
421  SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
```

SEQ ID NO. 67

Amino acid sequence of chimeric mouse VH 45H6 and human constant heavy IgG4 chain

```
  1  MELGLSWIFL LAILKGVQCD VQLVESGGGL VQPGGSRKLS CAASGFTFSS FGMHWVRQAP
 61  EKGLEWVAYI SSGNSNIYYV DTVKGRFTIS RDNPKNTLFL QMTSLRSEDT AMYYCARKRA
121  YGDYSGFSMD YWGQGTSVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT
181  VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV
241  ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY
301  VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK
361  AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
421  DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
```

SEQ ID NO. 68

Amino acid sequence of chimeric mouse VH 48H6 and human constant heavy IgG4 chain

```
  1  MELGLSWIFL LAILKGVQCE VKLVESGGGL VKPGGSLKLS CAASGFTFSG YAMSWVRQTP
 61  EKRLEWVASI SSGGSTYYPD SVKGRFTIPR DDARNILYLQ MSSLRSEDTA IYYCARGGHG
121  SSYVYWGQGT TLTVSSASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG
181  ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTKTYTCN VDHKPSNTKV DKRVESKYGP
241  PCPPCPAPEF LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSQEDPEVQ FNWYVDGVEV
301  HNAKTKPREE QFNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKGLPSSIEK TISKAKGQPR
361  EPQVYTLPPS QEEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
421  FLYSRLTVDK SRWQEGNVFS CSVMHEALHN HYTQKSLSLS LGK
```

SEQ ID NO. 69

Amino acid sequence of chimeric mouse VH 11H7 and human constant heavy IgG4 chain

```
  1  MELGLSWIFL LAILKGVQCQ VQLKQSGPGL VQPSQSLSIT CTVSGFSLTI YGVHWVRQSP
 61  GKGLEWLGVI WSGGSTDYNA AFISRLSISK DNSKSQVFFK MNSLQANDTA IYYCARRDYG
121  SRSFYYAMDY WGQGTSVTVS SASTKGPSVF PLAPCSRSTS ESTAALGCLV KDYFPEPVTV
181  SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTK TYTCNVDHKP SNTKVDKRVE
241  SKYGPPCPPC PAPEFLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV
301  DGVEVHNAKT KPREEQFNST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA
361  KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD
421  SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK
```

SEQ ID NO. 70

Amino acid sequence of chimeric mouse VH 49G4 and human constant heavy IgG4 chain

```
  1  MELGLSWIFL LAILKGVQCE VQLQQSGAEL VKPGASVKLS CRASGFNIKD TYMHWVKQRP
 61  EQGLEWIGRI DPARGNTKYD PKFQGKATIT ADTSSNTAYL QLSSLTSEDT AVYYCASAMD
121  YWGQGTSVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS
181  GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP
241  CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK
301  TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV
361  YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS
421  RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK
```

SEQ ID NO. 71

Amino acid sequence of chimeric mouse VL 36H12 and human constant light kappa chain

```
  1  MDMRVPAQLL GLLLLWFPGA RCDVLMTQTP LSLPVSLGDQ ASISCRSSQS IVHSNGITYL
 61  EWYLQKPGQS PKLLIYKVSN RFSGVPDRFS GSGSGTDFTL KISRVEAEDL GVYYCFQGSH
121  VPLTFGAGTK LELKRTVAAP SVFIFPPSDE QLKSGTASVV CLLNNFYPRE AKVQWKVDNA
181  LQSGNSQESV TEQDSKDSTY SLSSTLTLSK ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE
241  C
```

SEQ ID NO. 72

Amino acid sequence of chimeric mouse VL 45H6 and human constant light kappa chain

```
  1  MDMRVPAQLL GLLLLWFPGA RCDIVMTQSQ KFMSTSVGDR VSVTCKASQN VDTNVAWYQQ
 61  KPGQSPKALI YSASYRYSGV PDRFTGSGSG TDFTLTISNV QSEDLAEYFC QQYNKFPLTF
121  GGGTKLEIKR TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN
181  SQESVTEQDS KDSTYSLSST LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC
```

SEQ ID NO. 73

Amino acid sequence of chimeric mouse VL 48H6 and human constant light kappa chain

```
  1  MDMRVPAQLL GLLLLWFPGA RCDIVMSQSP SSLAVSVGEK VTMSCKSSQS LLYSSNQKNY
 61  LAWYQQKPGQ SPKLLIYWAS TRESGVPDRF TGSGSGTDFT LTISSVKAED LAVYYCHQYY
121  SYPLTFGAGT KLELNRTVAA PSVFIFPPSD EQLKSGTASV VCLLNNFYPR EAKVQWKVDN
181  ALQSGNSQES VTEQDSKDST YSLSSTLTLS KADYEKHKVY ACEVTHQGLS SPVTKSFNRG
241  EC
```

SEQ ID NO. 74

Amino acid sequence of chimeric mouse VL 11H7 and human constant light kappa chain

```
  1  MDMRVPAQLL GLLLLWFPGA RCEIVLTQSP ALMAASPGEK VTITCSVSSS ISSSNLHWYQ
 61  QKSETSPKPW IYGTSNLASG VPVRFSGSGS GTSYSLTISS MEAEDAATYY CQQWSSYPLT
121  FGGGTKLEIK RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
181  NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC
```

SEQ ID NO. 75

Amino acid sequence of chimeric mouse VL 49G4 and human constant light kappa chain

```
  1  MDMRVPAQLL GLLLLWFPGA RCDVLMTQTP LSLPVSLGDQ ASISCRSSQS IVHSNGNTYL
 61  EWFLQKPGQS PKLLIYKVSN RFSGVPDRFS GSGSGTDFTL KISRVEAEDL GVYYCFQGSH
121  VPLTFGAGTK LELKRTVAAP SVFIFPPSDE QLKSGTASVV CLLNNFYPRE AKVQWKVDNA
181  LQSGNSQESV TEQDSKDSTY SLSSTLTLSK ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE
241  C
```

SEQ ID NO. 76

Amino acid sequence of membrane-bound anti-human CD3 scFv (signal peptide followed by OKT3 scFv linked to CH2-CH3 domains of human IgG1 and human CD80 cytoplasmic tail)

```
  1  MKWVTFISLL FLFSSAYSQV QLQQSGAELA RPGASVKMSC KASGYTFTRY TMHWVKQRPG
 61  QGLEWIGYIN PSRGYTNYNQ KFKDKATLTT DKSSSTAYMQ LSSLTSEDSA VYYCARYYDD
121  HYCLDYWGQG TTLTVSSGGG GSGGGGSGGG GSQIVLTQSP AIMSASPGEK VTMTCSASSS
181  VSYMNWYQQK SGTSPKRWIY DTSKLASGVP AHFRGSGSGT SYSLTISGME AEDAATYYCQ
241  QWSSNPFTFG SGTKLEINRA AAPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF
301  NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT
361  ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
421  PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GWAITLISVN
481  GIFVICCLTY CFAPRCRERR RNERLRRESV RPV
```

SEQ ID NO. 77

cDNA sequence coding for membrane-bound anti-human CD3 scFv

```
   1  ATGAAATGGG TCACCTTTAT CTCCCTGCTG TTCCTGTTCT CCAGCGCCTA
CTCTCAGGTC
  61  CAGTTGCAGC AGTCTGGCGC CGAATTGGCT AGACCTGGCG CCTCCGTGAA
GATGTCCTGC
 121  AAGGCTTCCG GCTACACCTT CACCAGATAC ACCATGCACT GGGTCAAGCA
GAGGCCTGGA
 181  CAGGGCCTTG AGTGGATCGG CTACATCAAC CCTTCTCGGG GCTACACCAA
CTACAACCAG
```

241 AAGTTCAAGG ACAAGGCTAC CCTGACAACC GACAAGTCCT CCTCCACCGC
CTACATGCAG
301 CTGTCCAGCC TGACCTCTGA GGACTCCGCC GTGTACTACT GTGCCCGGTA
CTACGACGAC
361 CACTACTGCC TGGATTATTG GGGCCAGGGC ACCACACTGA CAGTGTCTAG
CGGAGGCGGA
421 GGATCTGGTG GTGGTGGATC TGGCGGCGGA GGTTCTCAGA TTGTGCTGAC
CCAGTCTCCT
481 GCCATCATGT CCGCTTCTCC CGGCGAGAAA GTGACAATGA CCTGCTCCGC
CTCTTCCTCC
541 GTGTCCTACA TGAACTGGTA TCAGCAGAAG TCCGGCACCT CTCCTAAGCG
GTGGATCTAC
601 GACACCTCCA AGCTGGCATC TGGCGTGCCC GCTCACTTTA GAGGCTCTGG
CTCTGGCACC
661 AGCTACTCCC TGACCATCTC TGGCATGGAA GCCGAGGATG CCGCCACCTA
CTATTGCCAG
721 CAGTGGTCTA GCAACCCCTT CACCTTCGGC TCCGGCACCA AGCTGGAAAT
CAACAGAGCC
781 GCCGCTCCTT CCGTGTTTCT GTTCCCTCCA AAGCCTAAGG ACACCCTGAT
GATCTCTCGG
841 ACCCCTGAAG TGACCTGCGT GGTGGTCGAT GTGTCTCACG AGGACCCAGA
AGTGAAGTTC
901 AATTGGTACG TGGACGGCGT GGAAGTGCAC AACGCCAAGA CCAAGCCTAG
AGAGGAACAG
961 TACAACTCCA CCTACAGAGT GGTGTCCGTG CTGACCGTGC TGCACCAGGA
TTGGCTGAAC
1021 GGCAAAGAGT ACAAGTGCAA GGTGTCCAAC AAGGCTCTGC CCGCTCCTAT
CGAAAAGACC
1081 ATCTCCAAGG CCAAGGGCCA GCCTAGGGAA CCCCAGGTTT ACACCCTGCC
TCCAAGCCGG
1141 GAAGAGATGA CCAAGAACCA GGTGTCCCTG ACCTGCCTGG TCAAGGGCTT
CTACCCTTCC
1201 GATATCGCCG TGGAATGGGA GAGCAATGGC CAGCCTGAGA ACAACTACAA
GACCACACCT
1261 CCTGTGCTGG ACTCCGACGG CTCATTCTTC CTGTACAGCA AGCTGACAGT
GGACAAGTCC
1321 AGATGGCAGC AGGGCAACGT GTTCTCCTGC TCCGTGATGC ACGAGGCCCT
GCACAATCAC
1381 TACACACAGA AGTCCCTGTC TCTGTCCCCT GGCTGGGCTA TCACCCTGAT
CTCTGTGAAC
1441 GGCATCTTCG TGATCTGCTG CCTGACCTAC TGCTTCGCCC CTAGATGCAG
AGAGCGGCGG
1501 AGAAACGAAC GGCTGCGGAG AGAATCTGTC CGGCCTGTG

Claims

1. An antibody that binds the extracellular cysteine-rich domain-1 (CRD1) of Herpesvirus entry mediator (HVEM) on HVEM expressing cells, and prevents binding of B- and T-lymphocyte attenuator (BTLA) to HVEM when the antibody is bound to said extracellular part of HVEM, and displaces BTLA bound to said extracellular part of HVEM.

2. The antibody of claim 1, wherein said antibody does not displace LIGHT bound to said extracellular part of HVEM when said antibody is bound to said extracellular part of HVEM.

3. The antibody of claim 1, wherein the antibody partially prevents binding of CD160 to HVEM when said antibody is bound to said extracellular part of HVEM and partially displaces CD160 bound to said extracellular part of HVEM.

4. The antibody of claims 1-3, that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 26-28 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 29-31 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions.

5. The antibody of claims 1-4 that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain region with the amino acid sequence of SEQ ID NO: 24 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 25 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions.

6. An antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 42-44 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 45-47 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions.

7. An antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain region with the amino acid sequence of SEQ ID NO: 40 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 41 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions.

8. An antibody that binds an extracellular part of HVEM on HVEM expressing cells comprising a heavy chain variable region with the CDR1, CDR2 and CDR3 sequence of SEQ ID NO: 18-20 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions, and a light chain variable region with the CDR1, CDR2, CDR3 sequence of SEQ ID NO: 21-23 with 0, 1 or 2 amino acid insertions, deletions, substitutions or additions.

9. An antibody that binds an extracellular part of HVEM on HVEM expressing cell comprising a heavy chain region with the amino acid sequence of SEQ ID NO: 16 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions and a light chain variable region with the amino acid sequence of SEQ ID NO: 17 with 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid insertions, deletions, substitutions or additions.

10. The antibody of claims 1-3, 6, 7, wherein said antibody does not prevent binding of LIGHT to said extracellular part of HVEM when said antibody is bound to said extracellular part of HVEM.

11. The antibody of claims 1-10, wherein said antibody is selected from a monoclonal antibody, a polyclonal antibody, a humanized antibody, and an antigen-binding fragment thereof.

12. One or more nucleic acid molecules comprising a nucleotide sequence encoding an antibody according to any one of claims 4-10 and 11.

13. A cell comprising one or more nucleic acids according to claim 12, wherein said one or more nucleic acids are capable of assembly into an antibody according to any one of claims 1-11.

14. An antibody according to any one of claims 1-11 for use in the treatment of a disease.

15. A pharmaceutical composition comprising an antibody or antigen-binding fragment thereof or a nucleic acid molecule according to any of claims 1-12 and a pharmaceutically acceptable carrier and/or diluent.

16. The pharmaceutical composition of claim 15 for use as prophylactic or in the treatment of cancer and/or immune-related disorders.

17. A method for modulating HVEM signalling activity, comprising contacting HVEM expressing cells with an antibody or antigen-binding fragment thereof or a nucleic acid molecule according to of any one of claims 1-12.

18. A method for the treatment of a human or an animal suffering from a medical indication, said method comprising administering to the human or animal a therapeutically effective amount of an antibody or antigen-binding fragment thereof or a nucleic acid molecule according to any one of claims 1-12.

19. A method for reducing tumor growth in a subject comprising administering to the subject in need a therapeutically effective amount of a composition comprising an antibody or antigen-binding fragment thereof or a nucleic acid molecule according to any one of claims 1-12.

Figure 1

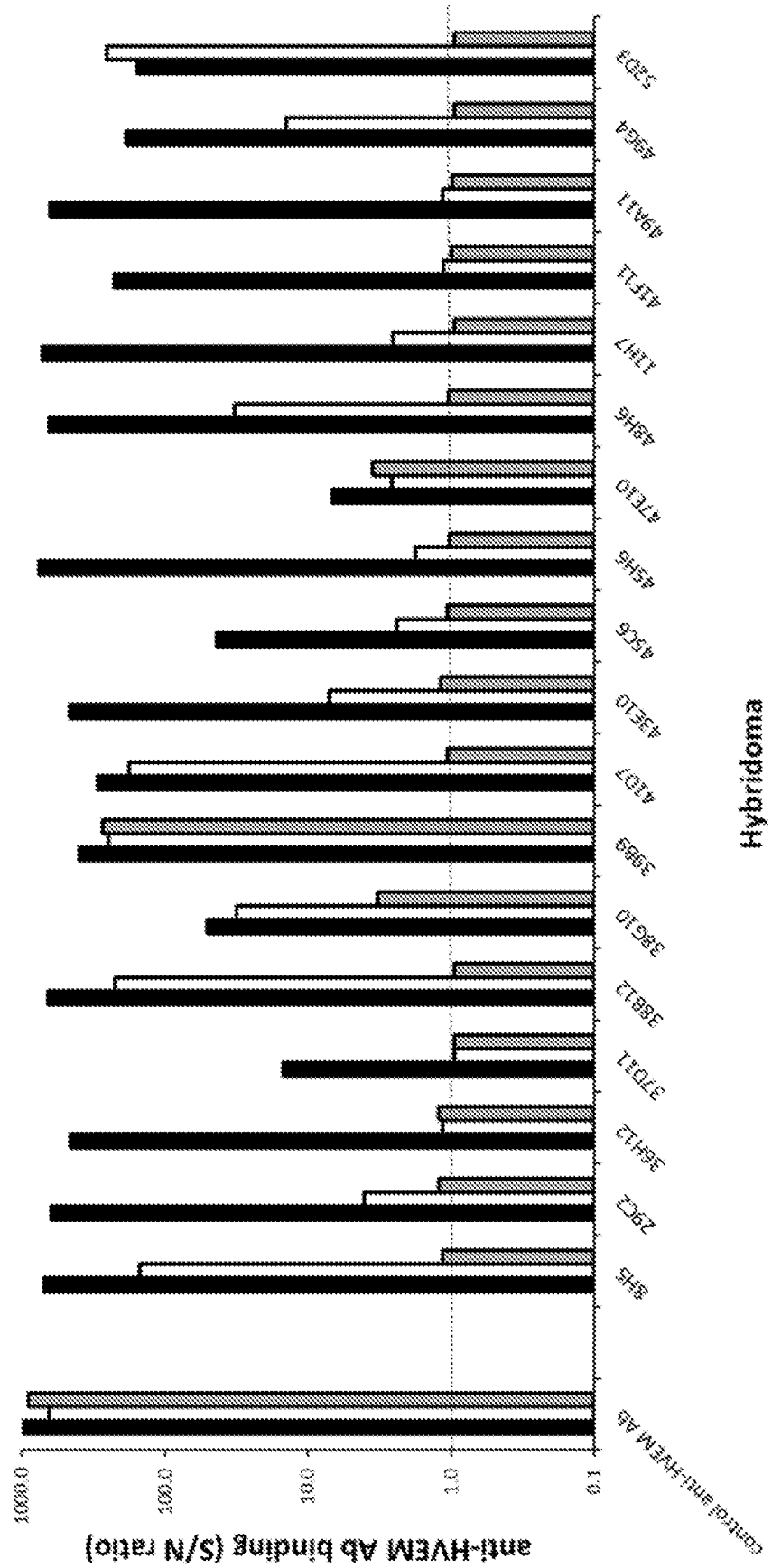

full length human HVEM
cynomolgus HVEM
CRD1 truncated human HVEM
anti-HVEM Ab binding (S/N ratio)
1000.0
100.0
10.0
1.0
0.1
Control anti-HVEM Ab
8H5
29C2
35H12
37D11
38B12
38G10
39B9
41D7
43E10
45C6
45H5
47E10
48H5
51H7
41F11
49A11
42E4
52D3
Hybridoma

Figure 2A

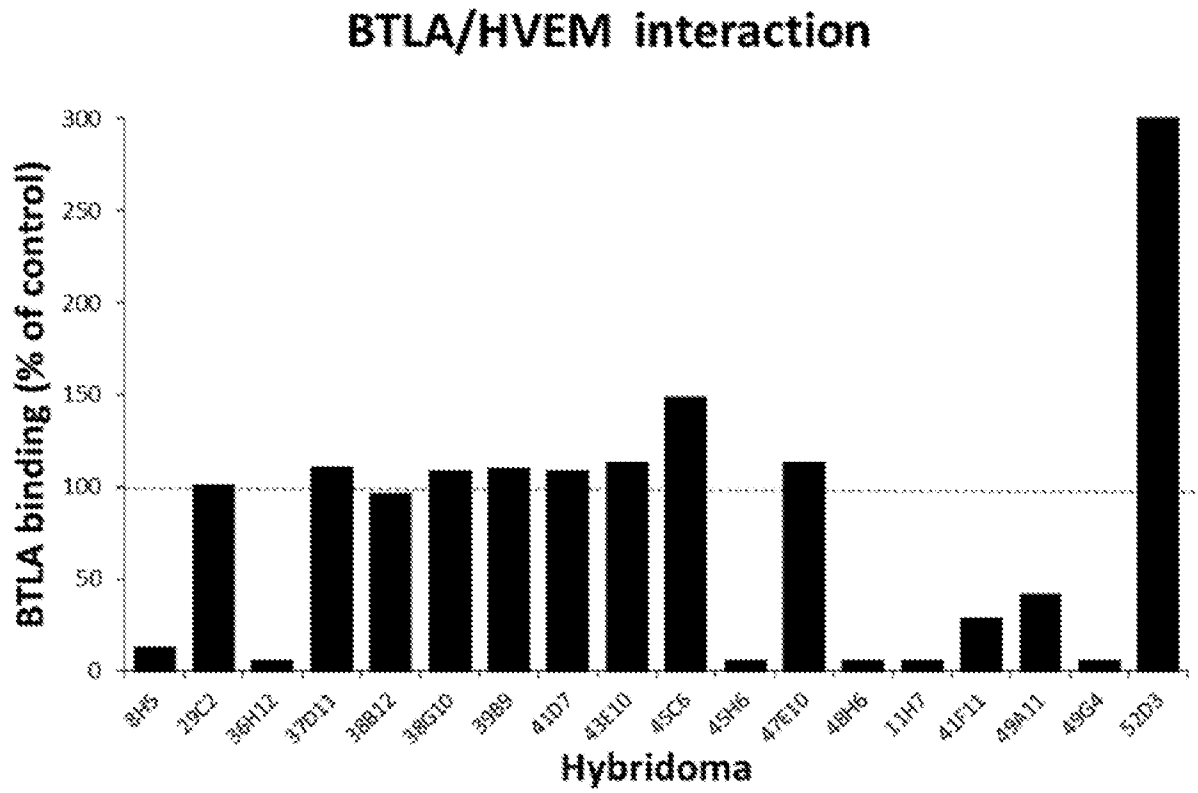

BTLA/HVEM interaction
BTLA binding (% of control)
300
250
200
150
100
50
0
3H5
21C2
36H12
37D13
38B12
38G10
3989
43D7
43E10
45C6
45H6
47E10
48H6
51H7
41F11
49A11
49G4
52D3
Hybridoma

Figure 2B

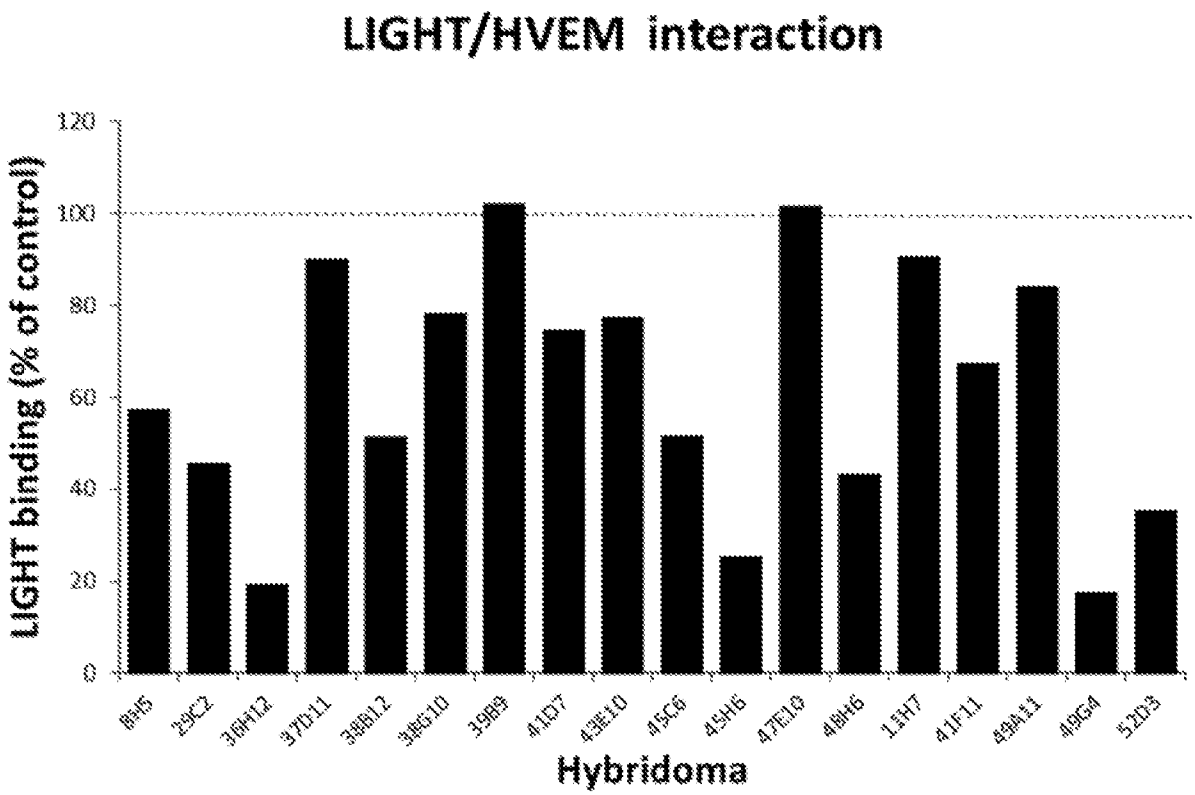

LIGHT/HVEM interaction
LIGHT binding (% of control)
120
100
80
60
40
20
0
8H5
29C2
36H12
37D11
38B12
38G10
39B9
41D7
43E10
45C6
45H6
47E10
48H6
1.5H7
41F11
49B11
49G4
52D3
Hybridoma

Figure 3A

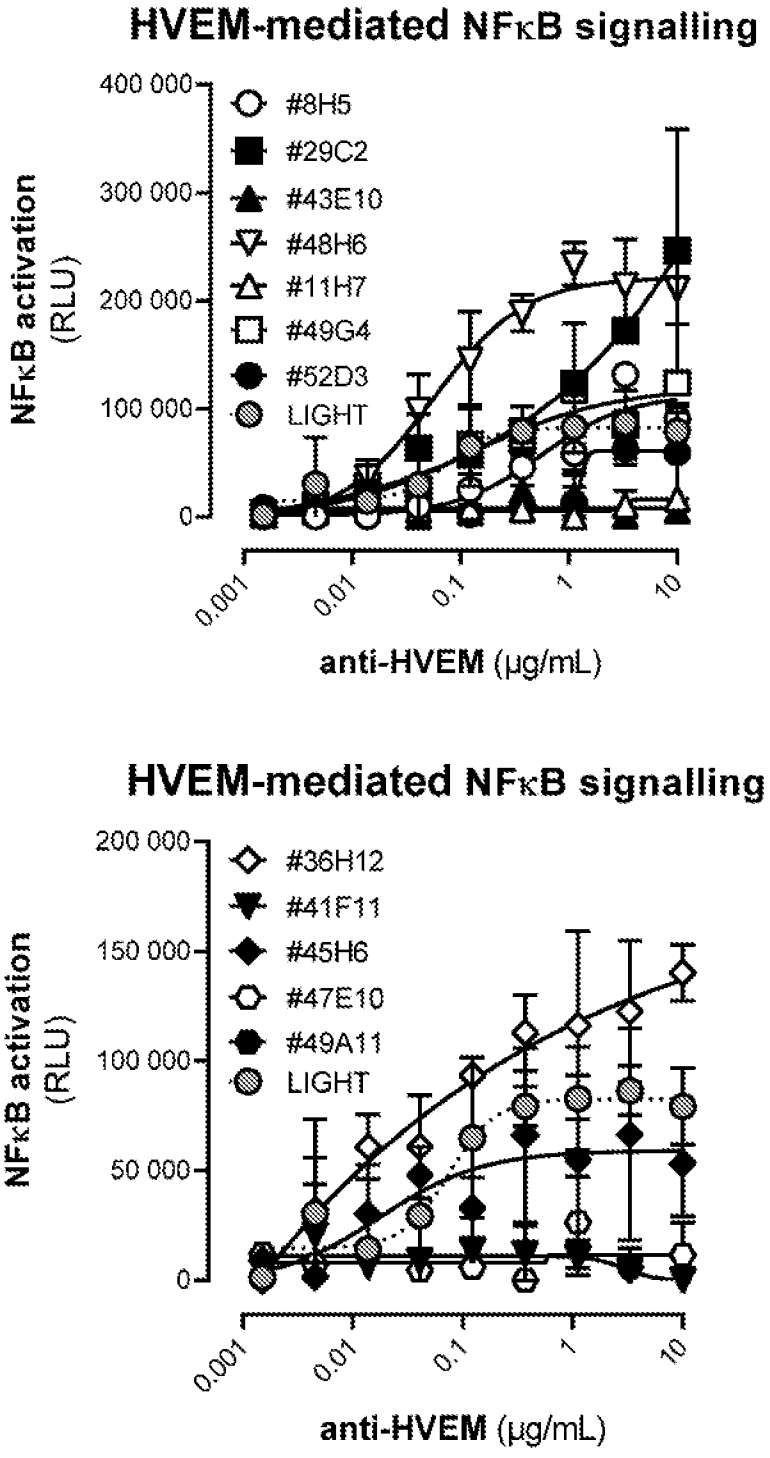

HVEM-mediated NFκB signalling
NFκB activation (RLU)
400 000
300 000
200 000
100 000
0
#8H5
#29C2
#43E10
#48H6
#11H7
#49G4
#52D3
LIGHT
0.001
0.01
0.1
1
10
anti-HVEM (µg/mL)
HVEM-mediated NFκB signalling
NFκB activation (RLU)
200 000
150 000
100 000
50 000
0
#36H12
#41F11
#45H6
#47E10
#49A11
LIGHT
0.001
0.01
0.1
1
10
anti-HVEM (µg/mL)

Figure 3B

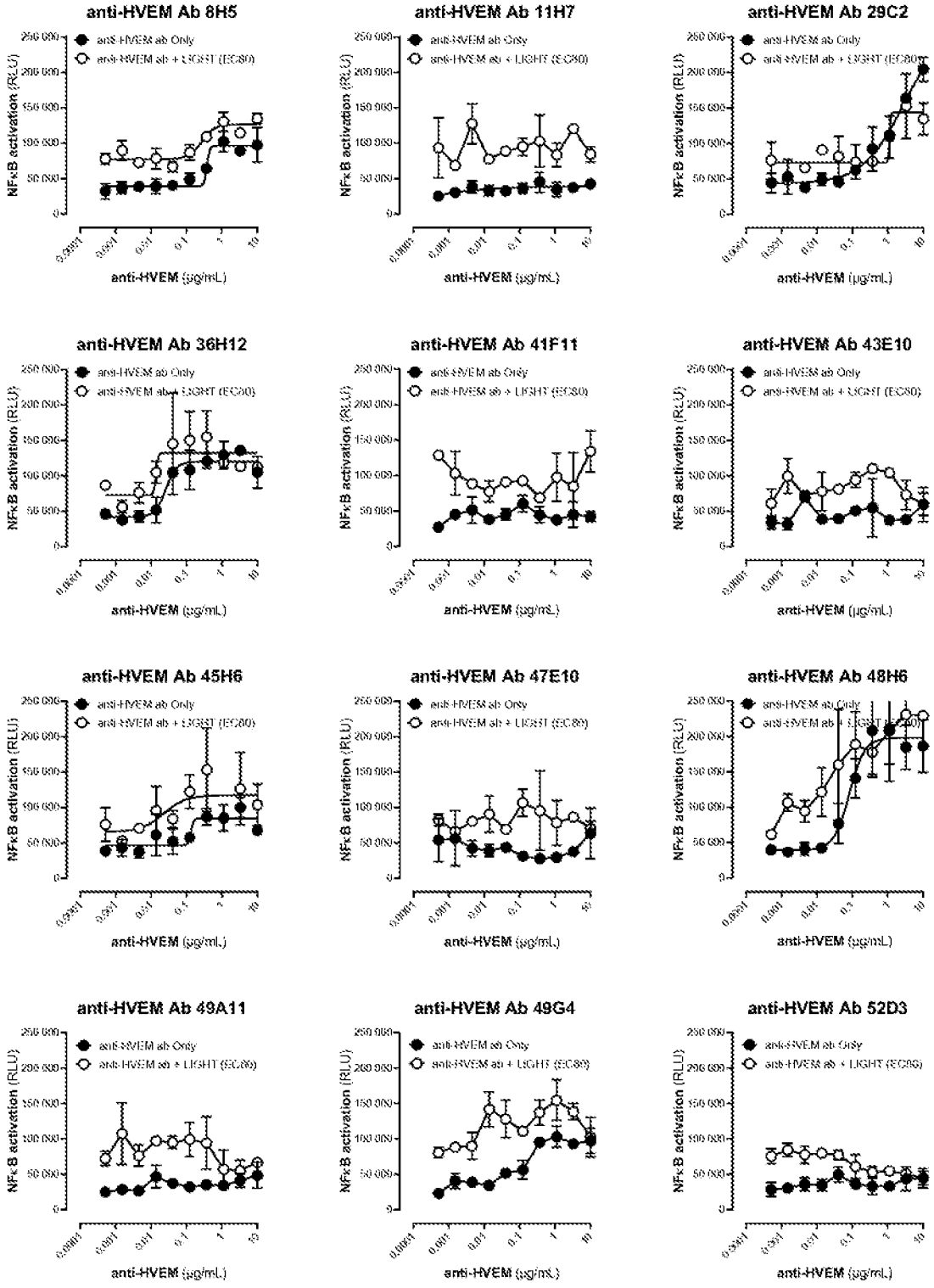

anti-HVEM Ab 8H5
anti-HVEM Ab 11H7
anti-HVEM Ab 29C2
anti-HVEM Ab 36H12
anti-HVEM Ab 41F11
anti-HVEM Ab 43E10
anti-HVEM Ab 45H6
anti-HVEM Ab 47E10
anti-HVEM Ab 48H6
anti-HVEM Ab 49A11
anti-HVEM Ab 49G4
anti-HVEM Ab 52D3
anti-HVEM ab Only
anti-HVEM ab + LIGHT (EC80)
NFκB activation (RLU)
anti-HVEM (µg/mL)

Figure 4A

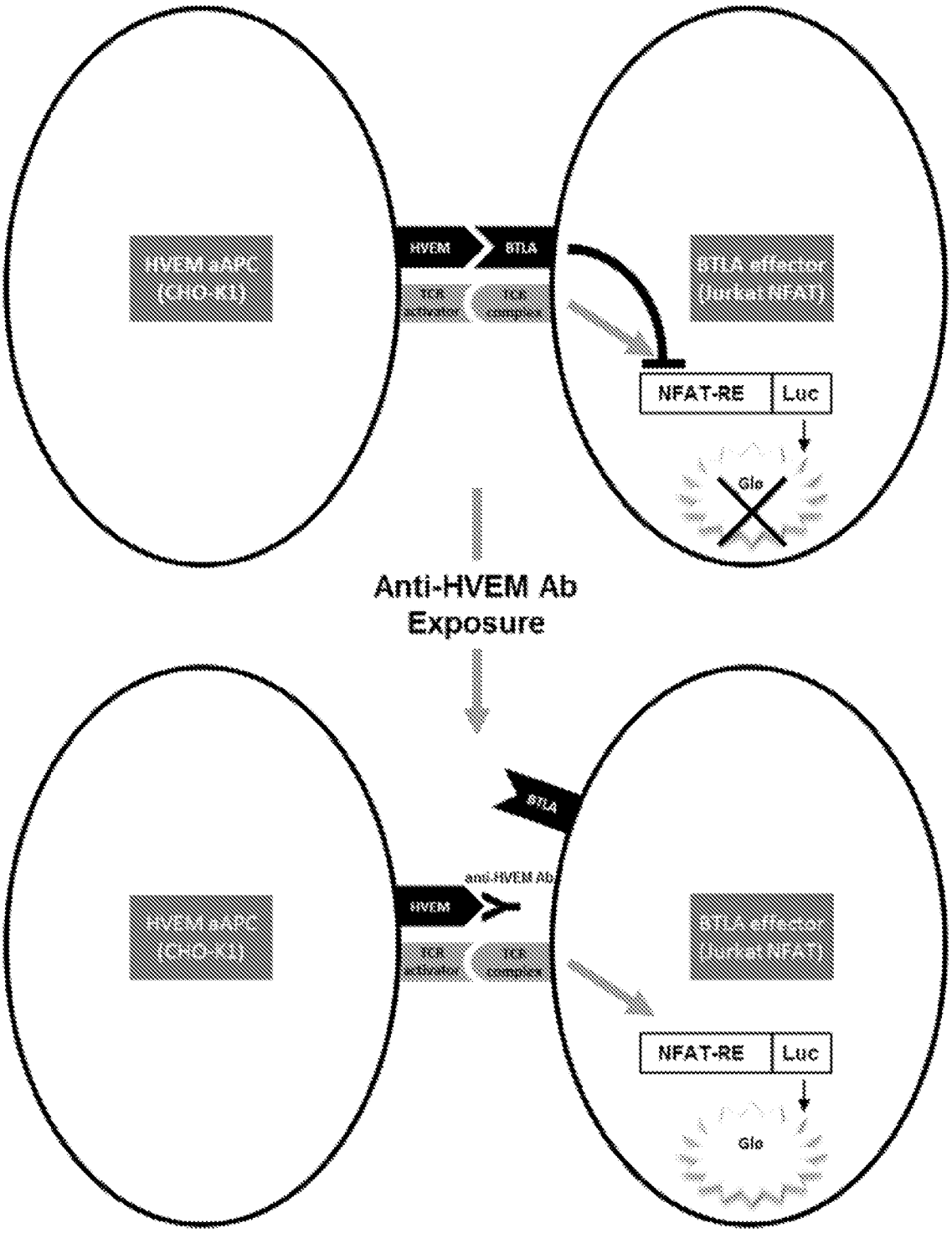

HVEM aAPC (CHO-K1)
HVEM
BTLA
TCR activator
TCR complex
BTLA effector (Jurkat NFAT)
NFAT-RE
Luc
Glo
Anti-HVEM Ab Exposure
BTLA
anti-HVEM Ab
HVEM aAPC (CHO-K1)
HVEM
TCR activator
TCR complex
BTLA effector (Jurkat NFAT)
NFAT-RE
Luc
Glo

Figure 4B

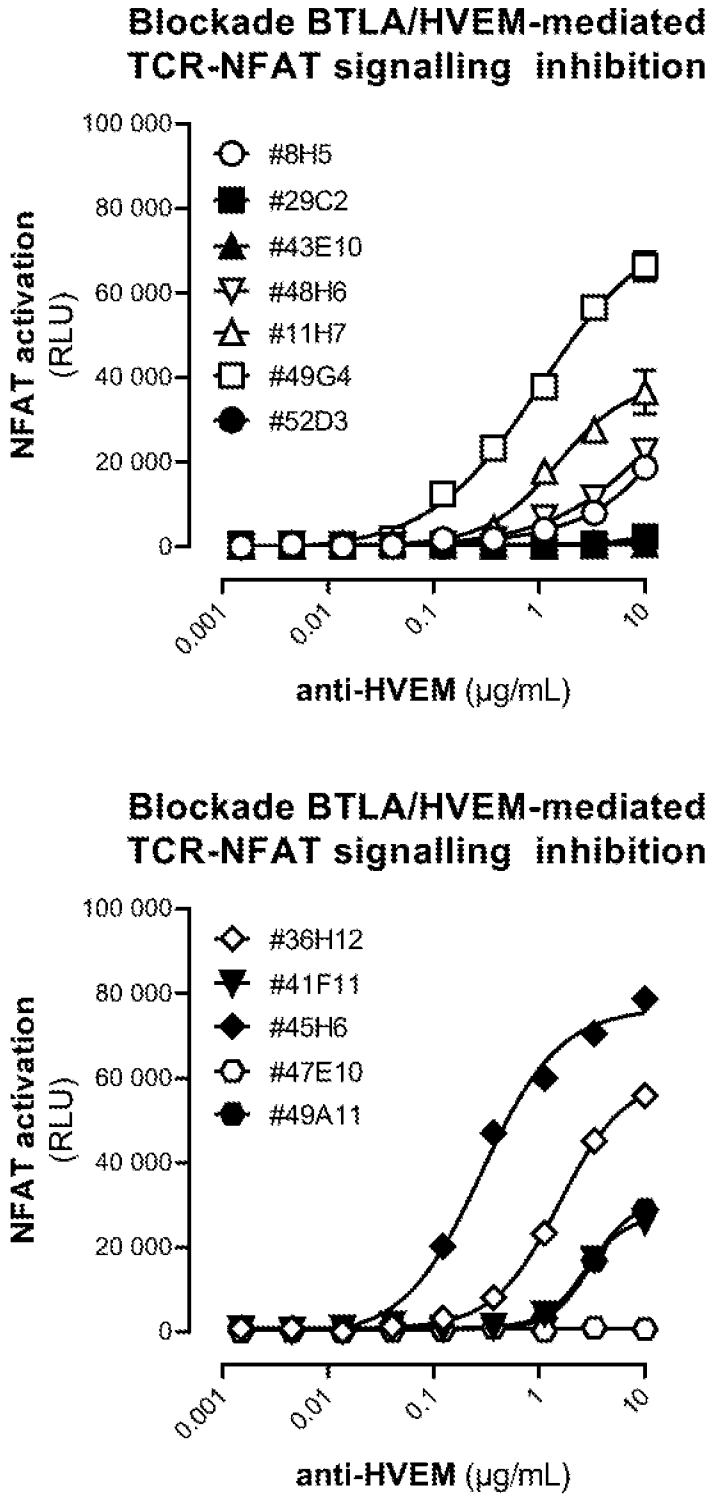

Blockade BTLA/HVEM-mediated
TCR-NFAT signalling inhibition
NFAT activation (RLU)
100 000
80 000
60 000
40 000
20 000
0
#8H5
#29C2
#43E10
#48H6
#11H7
#49G4
#52D3
0.001
0.01
0.1
1
10
anti-HVEM (µg/mL)
Blockade BTLA/HVEM-mediated
TCR-NFAT signalling inhibition
NFAT activation (RLU)
100 000
80 000
60 000
40 000
20 000
0
#36H12
#41F11
#45H6
#47E10
#49A11
0.001
0.01
0.1
1
10
anti-HVEM (µg/mL)

Figure 5

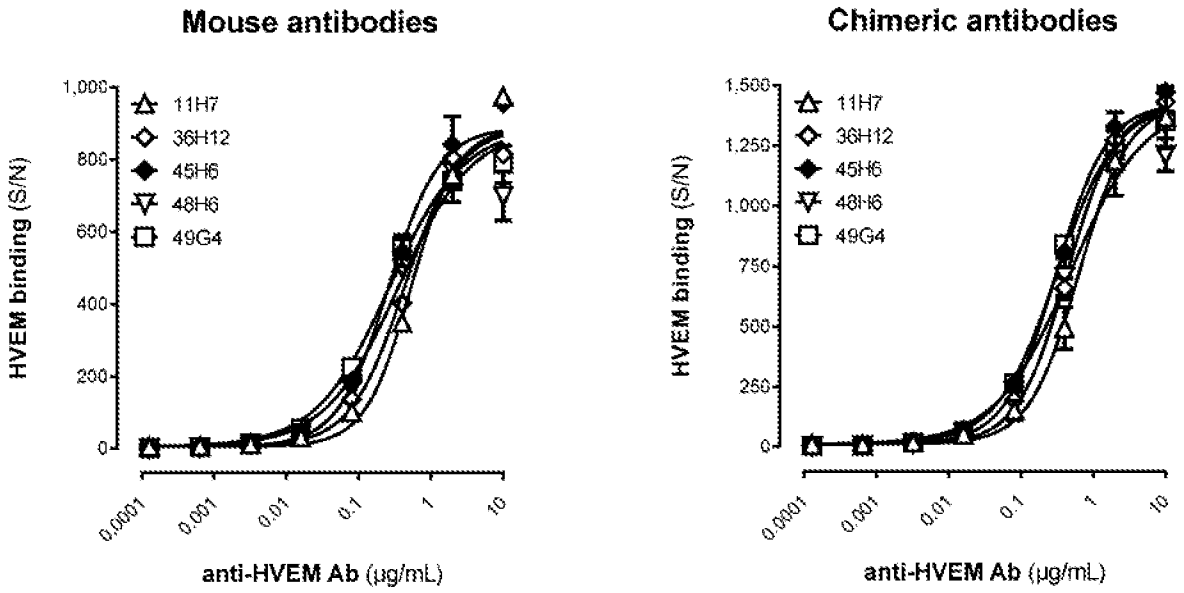
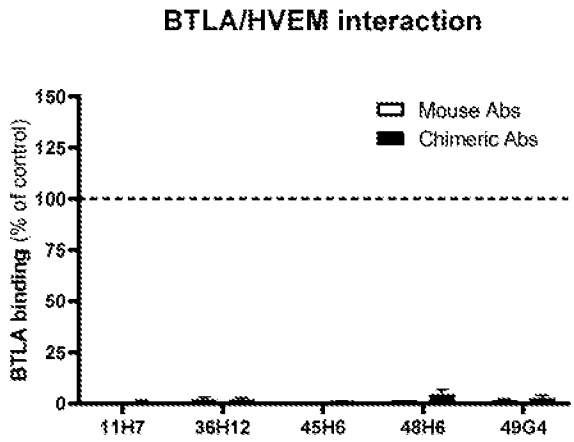
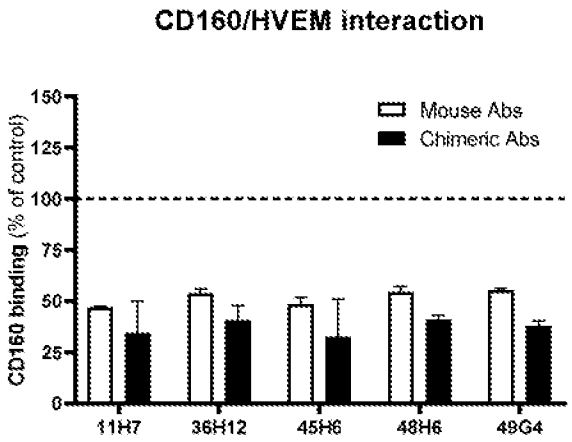

Mouse antibodies
HVEM binding (S/N)
1,000
800
600
400
200
0
11H7
36H12
45H6
48H6
49G4
0.0001
0.001
0.01
0.1
1
10
anti-HVEM Ab (µg/mL)
Chimeric antibodies
HVEM binding (S/N)
1,500
1,250
1,000
750
500
250
0
11H7
36H12
45H6
48H6
49G4
0.0001
0.001
0.01
0.1
1
10
anti-HVEM Ab (µg/mL)

Figure 6

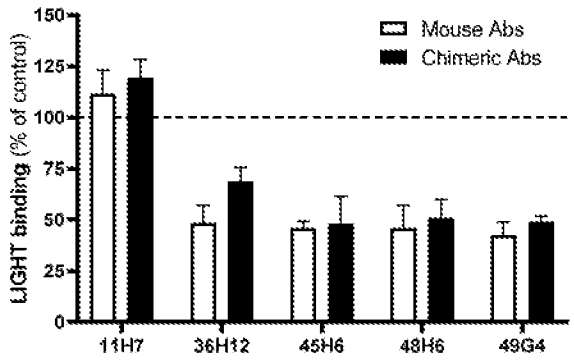
A



B



C



D

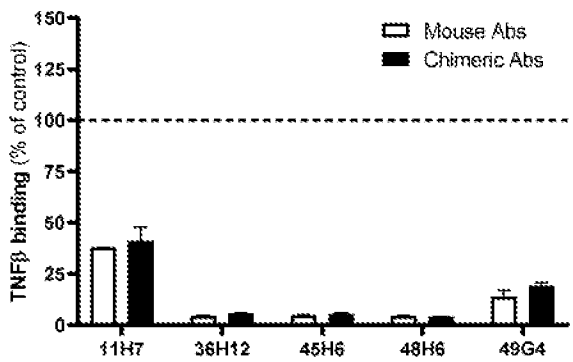
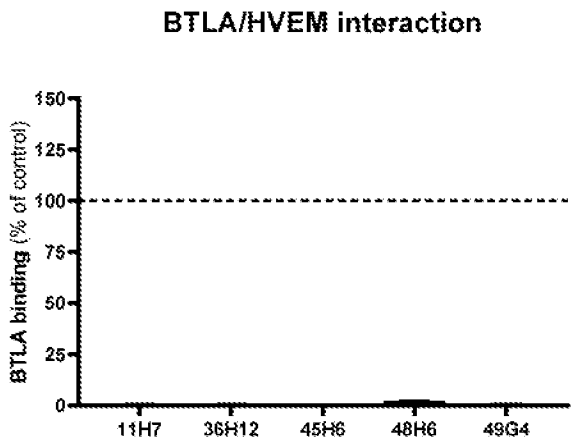
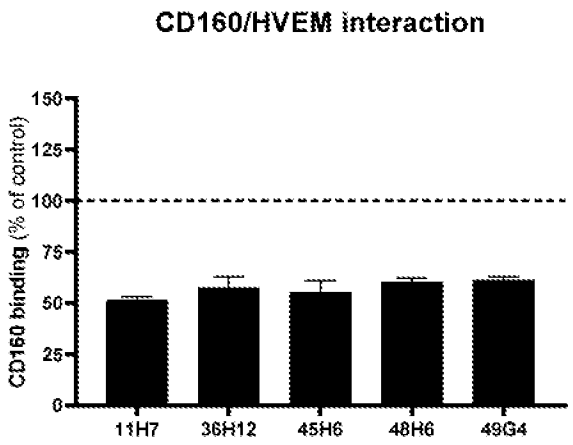


Figure 7

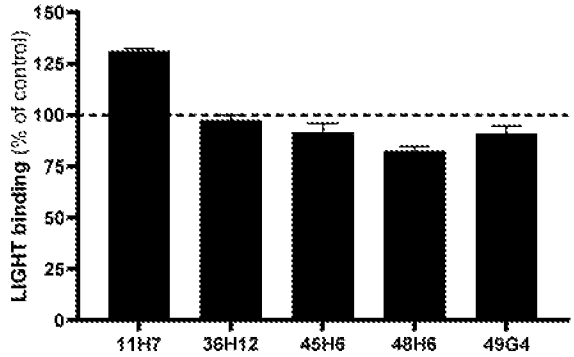
A



B



C



D

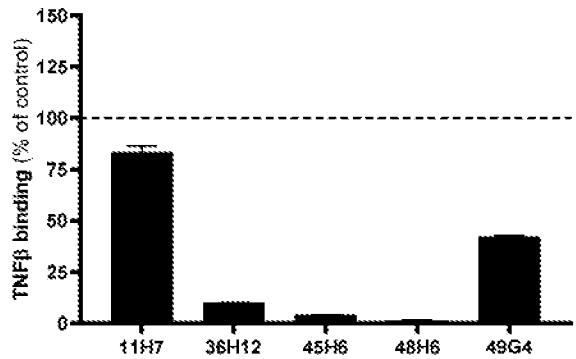
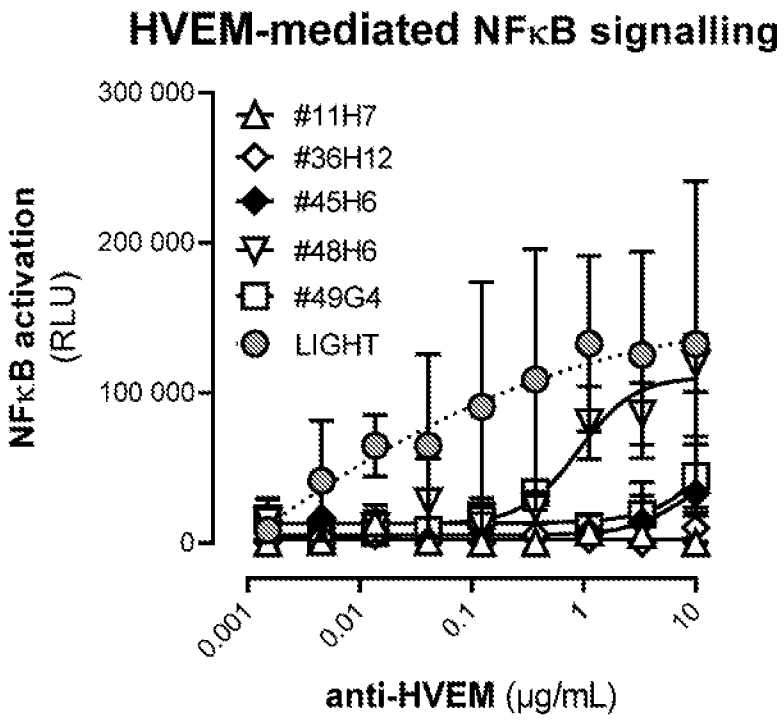
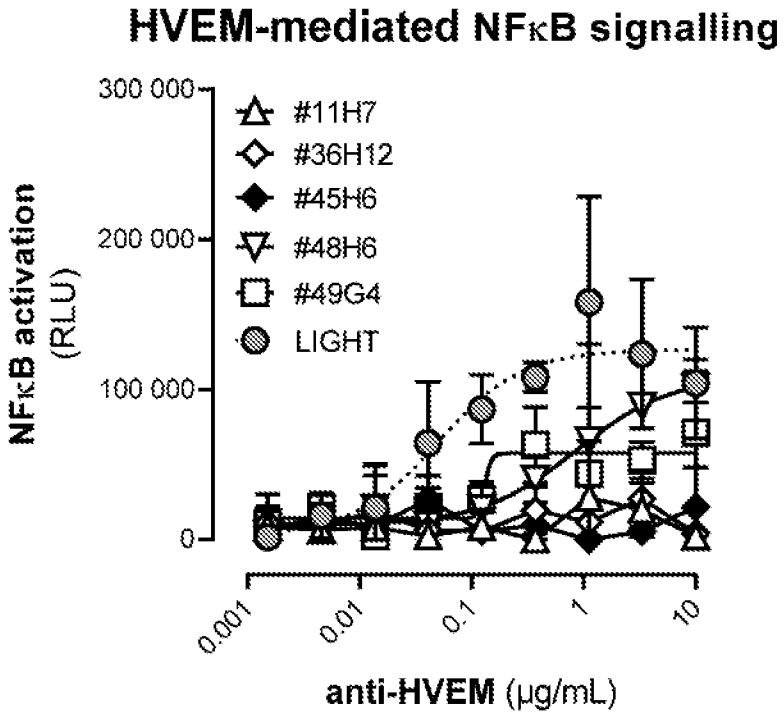


Figure 8A (Experiment 1 & 2)





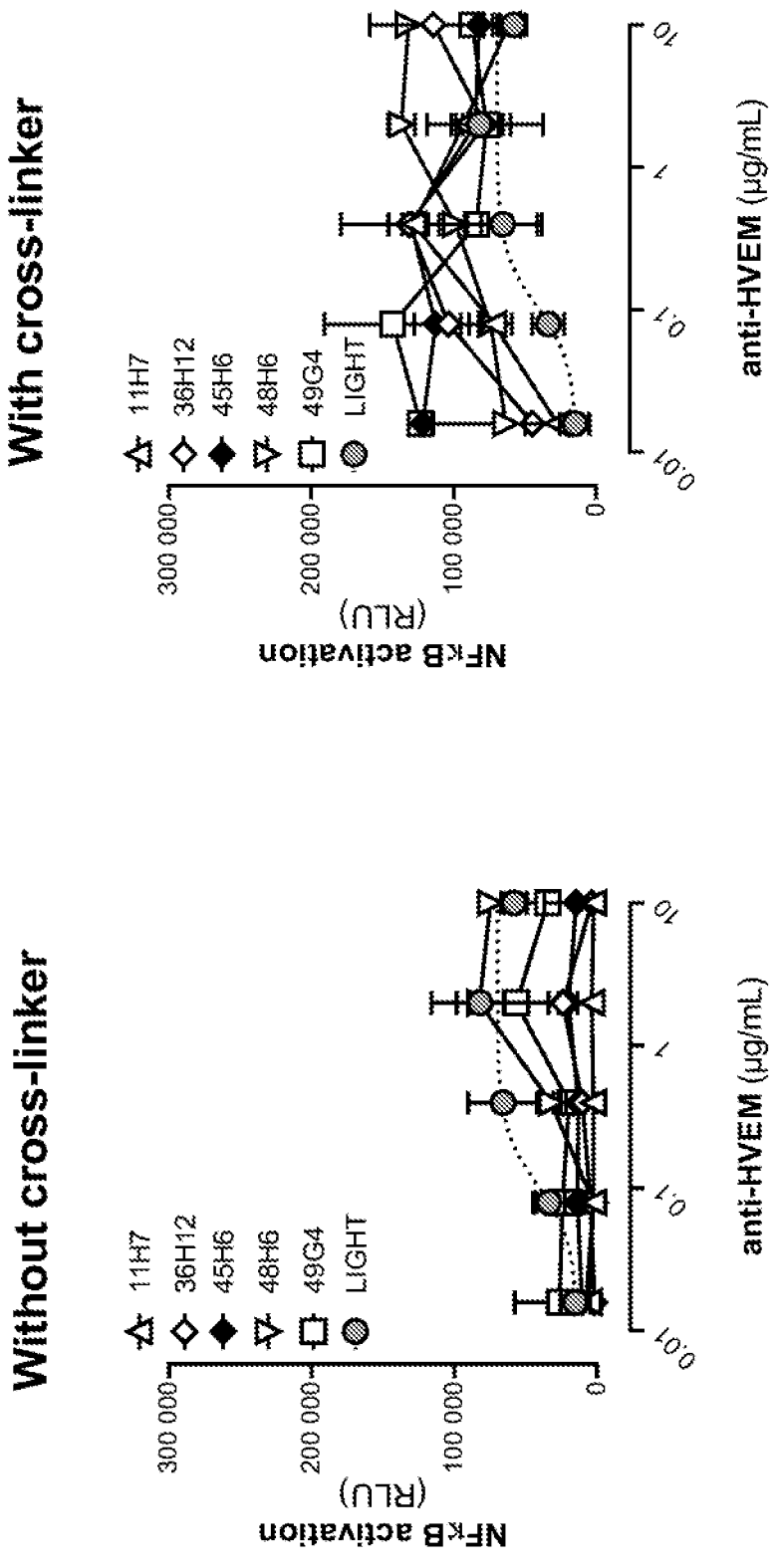

With cross-linker
11H7
36H12
45H6
48H6
49G4
LIGHT
NFκB activation (RLU)
300 000
200 000
100 000
0
10
1
0,1
0,01
anti-HVEM (µg/mL)
Without cross-linker
11H7
36H12
45H6
48H6
49G4
LIGHT
NFκB activation (RLU)
300 000
200 000
100 000
0
10
1
0,1
0,01
anti-HVEM (µg/mL)

Figure 8B

Figure 8C (Experiment 1)

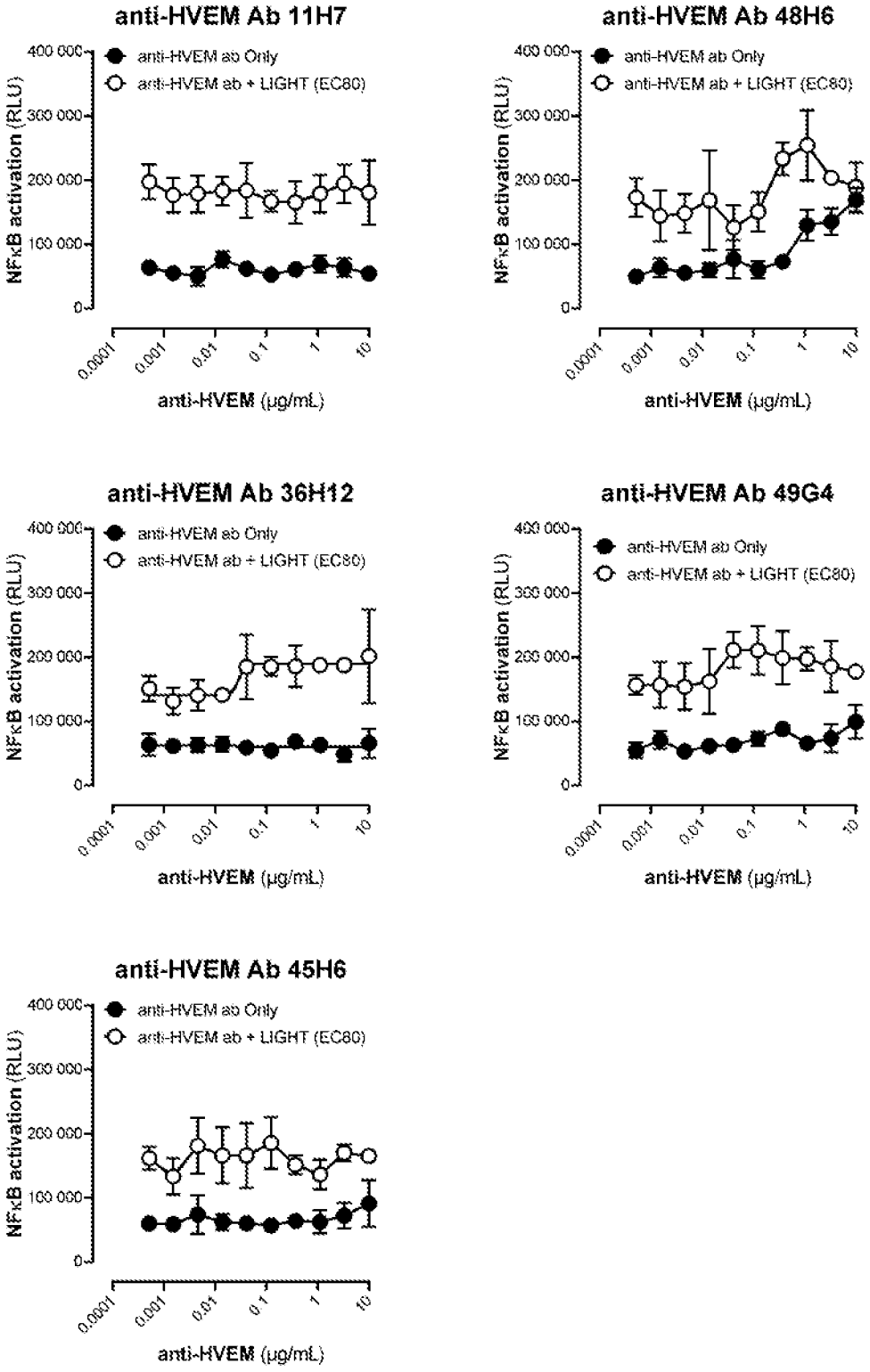


Figure 8C continued (Experiment 2)

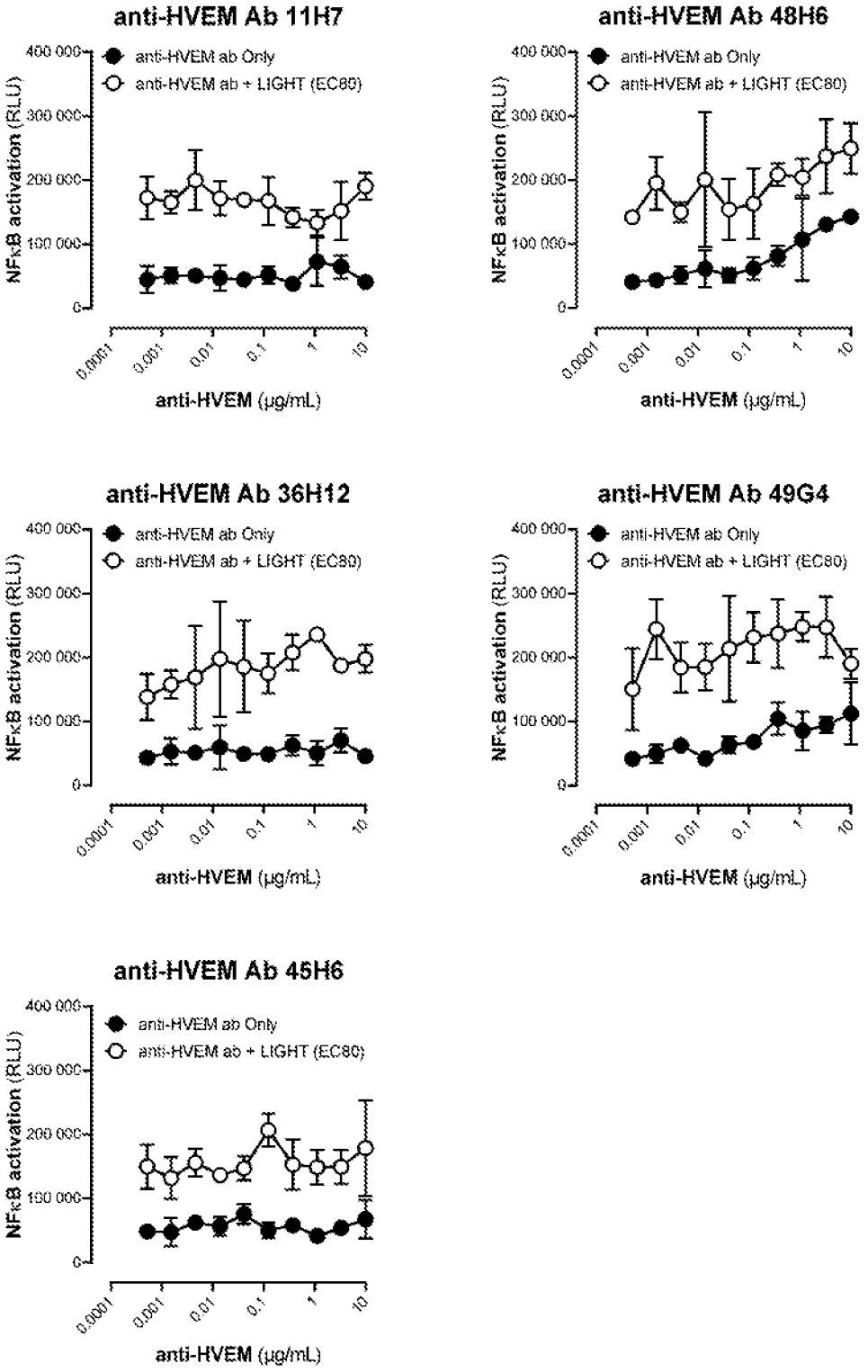
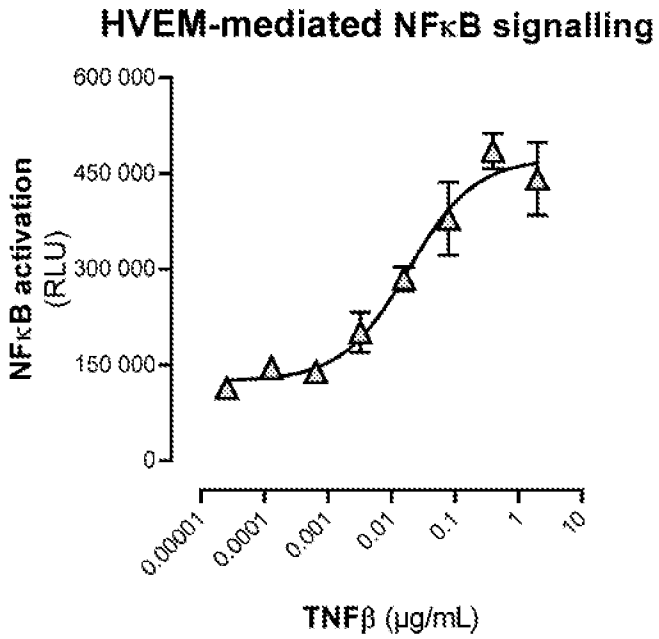


Figure 9

A


HVEM-mediated NFκB signalling
NFκB activation (RLU)
600 000
450 000
300 000
150 000
0
0.00001
0.0001
0.001
0.01
0.1
1
10
TNFβ (µg/mL)

B

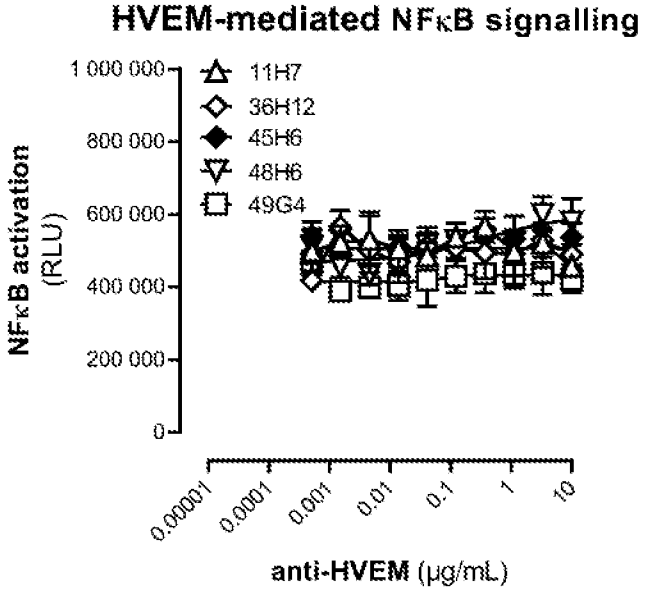
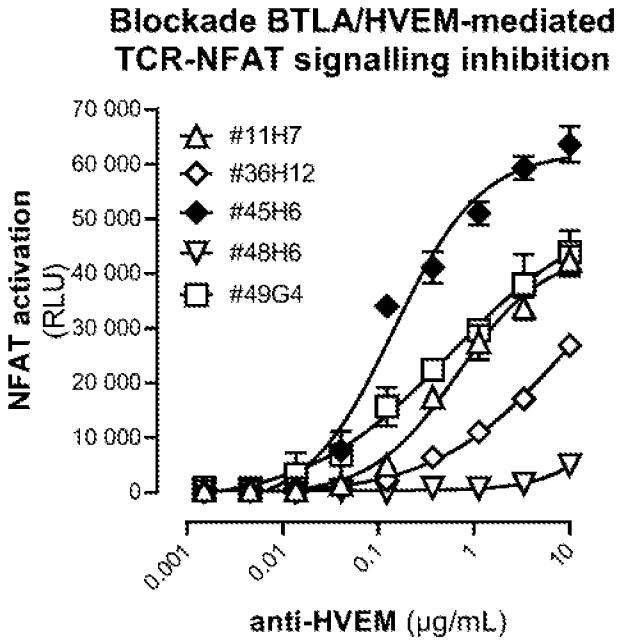

HVEM-mediated NFκB signalling
NFκB activation (RLU)
1 000 000
800 000
600 000
400 000
200 000
0
11H7
36H12
45H6
48H6
49G4
0.00001
0.0001
0.001
0.01
0.1
1
10
anti-HVEM (µg/mL)

Figure 10 (Experiment 1 & 2)



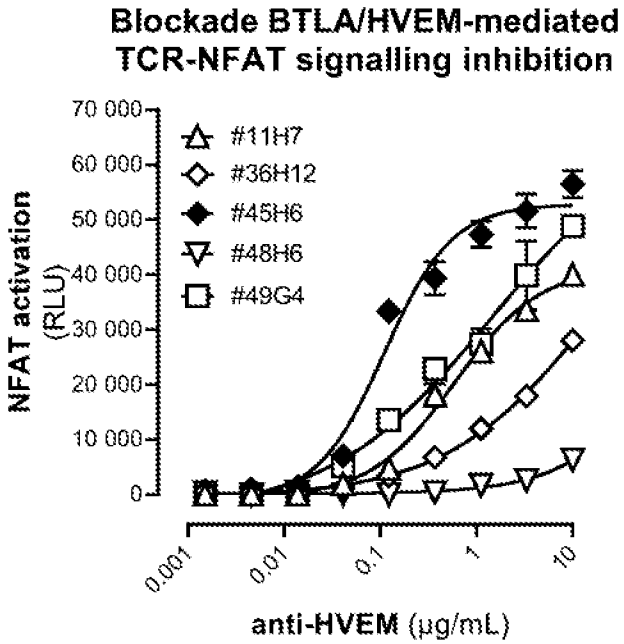


Figure 11A

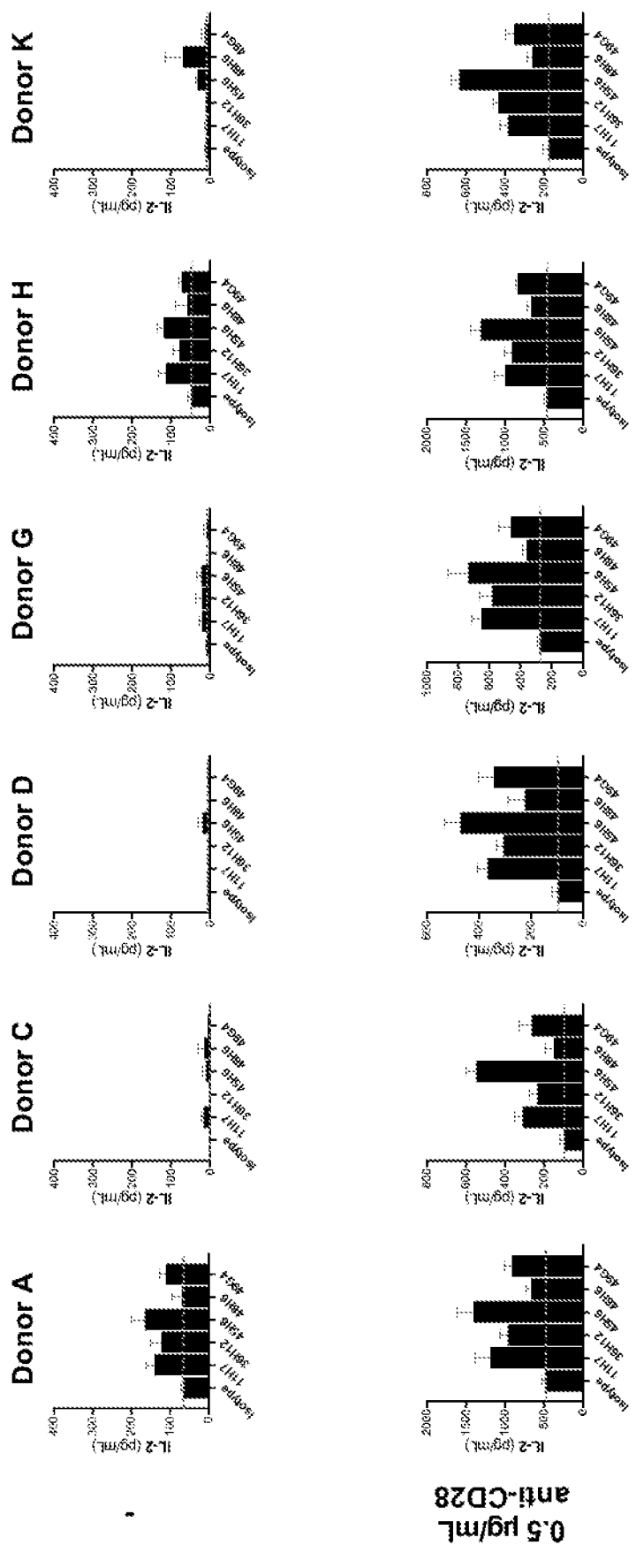

Donor A
Donor C
Donor D
Donor G
Donor H
Donor K
-
0.5 µg/mL anti-CD28
IL-2 (pg/mL)
Isotype
1H7
38H12
45H6
48H6
49G4

Figure 11B

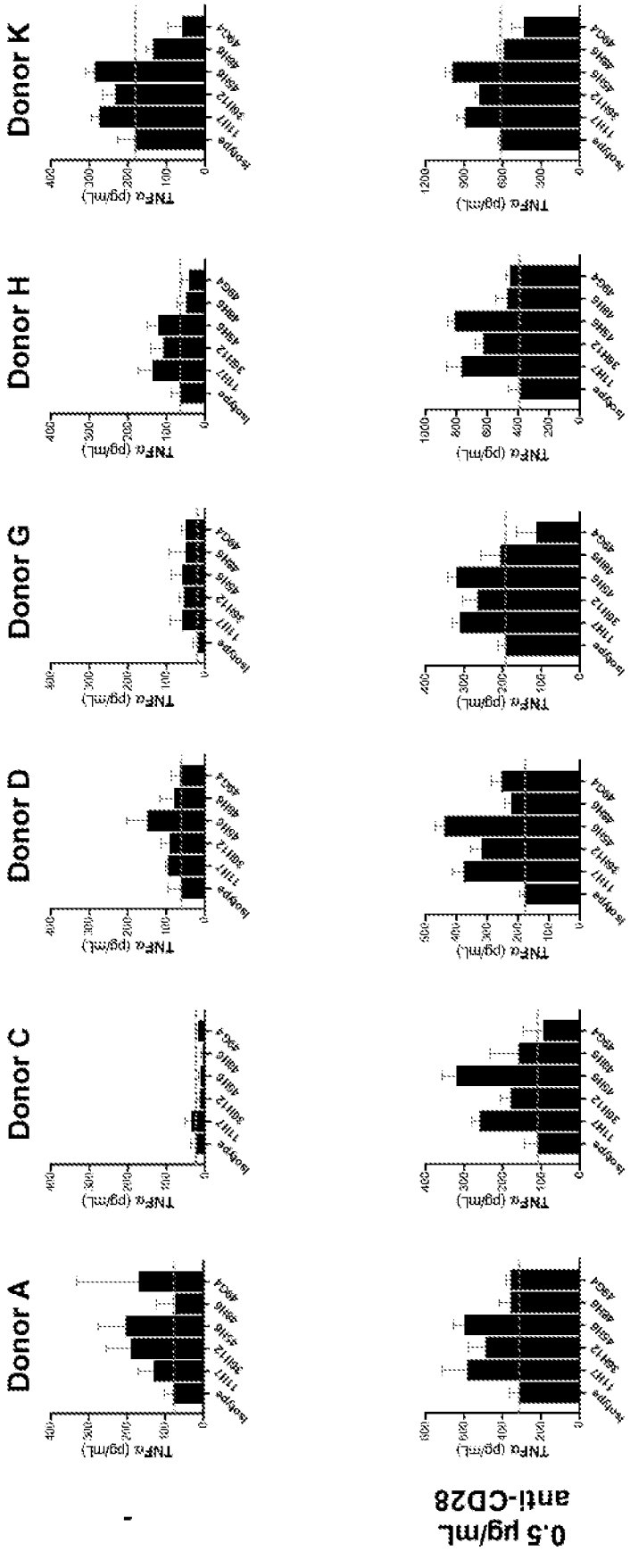
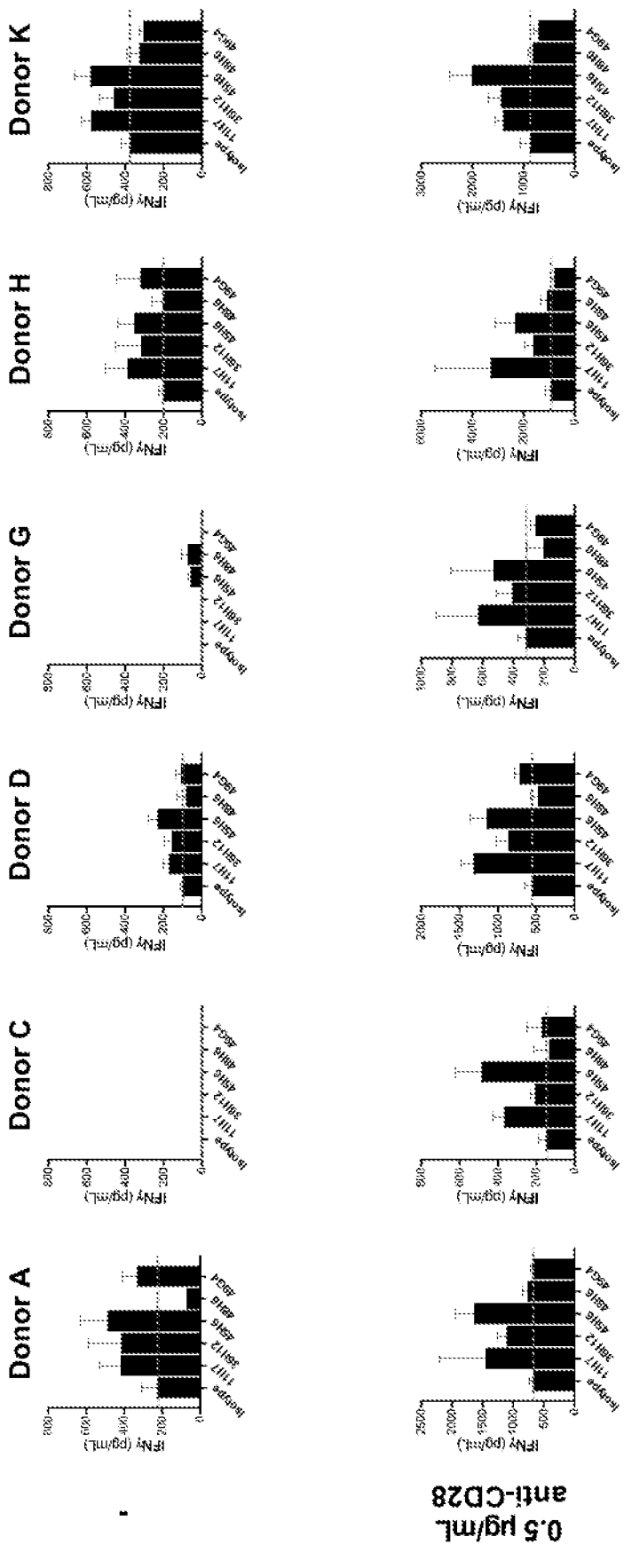

-
0.5 µg/mL anti-CD28
Donor A
Donor C
Donor D
Donor G
Donor H
Donor K
TNFα (pg/mL)
Isotype
11t7
3a1t12
4s1t6
4s1t6
4q1t6
4q1t6
4pG4

Figure 11C


Donor A
Donor C
Donor D
Donor G
Donor H
Donor K
-
0.5 µg/mL anti-CD28
IFNγ (pg/mL)
Isotype
1111T
3811T2
4516
4816
4816
4SC4