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(54) **KV1.3 Binding immunoglobulins.**

(57) The present invention relates to immunoglobulins that specifically bind Kv1.3 and more in particular to polypeptides, nucleic acids encoding such polypeptides; to methods for preparing such polypeptides; to compositions and in particular to pharmaceutical compositions that comprise such polypeptides, for prophylactic, therapeutic or diagnostic purposes. In particular, the immunoglobulins of the present invention inhibit the activity of Kv1.3.

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Title: KV1.3 BINDING IMMUNOGLOBULINS

FIELD OF THE INVENTION

The present invention relates to immunoglobulins that bind Kv1.3 and more in particular to polypeptides, that comprise or essentially consist of one or more such immunoglobulins (also referred to herein as *"immunoglobulin(s) of the invention"*, and *"polypeptides of the invention"*, respectively).

The invention also relates to nucleic acids encoding such polypeptides (also referred to herein as "*nucleic acid(s) of the invention*"; to methods for preparing such polypeptides; to host cells expressing or capable of expressing such polypeptides; to compositions, and in particular to pharmaceutical compositions, that comprise such polypeptides, nucleic acids and/or host cells; and to uses of polypeptides, nucleic acids, host cells and/or compositions, in particular for prophylactic and/or therapeutic purposes, such as the prophylactic and/or therapeutic purposes mentioned herein.

Other aspects, embodiments, advantages and applications of the invention will become clear from the further description herein.

BACKGROUND ART

Ion channels are complex pore-forming membrane proteins that control the selective flux of ions across the membrane, thereby allowing the rapid movement of ions during electrical signaling processes. They are present in the membranes of all living cells and are key to establish a resting membrane potential, shape action potentials and other electrical signals by gating the flow of ions across the cell membrane.

Many channels are gated (i.e. opening and closing of the ion channel pore) such that ionic flow is only triggered in response to a specific stimulus e.g. ligand association, mechanical stress, pH or membrane voltage. As a consequence ion channels have been classified accordingly. A large group of ion channels (e.g., K, Na, Ca, HCN and TRP channels) that respond to potential differences across the cellular membrane for channel gating, share several structural similarities. These channels are thought to have evolved from a common ancestor and have been classified together as the "voltage-gated-like (VGL) ion channel chanome" (Yu et al., Pharmacol Rev 57(4):

387-95, 2005). Other ion channels, such as Cl channels, aquaporins and connexins have evolved quite separately and exhibit completely different structural properties to the VGL channels.

The Kv1.3 channel is a potassium selective voltage gated ion channel with an overall topology representative for these voltage gated like ion channels. Structurally, Kv1.3 exists in a homotetrameric configuration where each monomer consists of six transmembrane segments (S1 to S6) with the S5-S6 region forming the pore. The six transmembrane domains are interconnected by three extracellular loops, termed EL1-EL3, which are accessible from the external side. Both the N-terminal and the C-terminal end of the channel are located on the intracellular side of a cell and can associate with auxiliary subunits (Figure 1). The first extracellular loop EL1 connects the first and second transmembrane segment (S1-S2). The top of the voltage sensor formed by the connecting loop between the third and fourth transmembrane segment (S3-S4), constitutes the second extracellular accessible region EL2. As the voltage sensor moves substantially during the gating process, the conformation of this potential epitope is likely to be altered by the gating process of the channel. The last extracellular region EL3 is the pore region, delineated by the fifth and sixth transmembrane segments (S5-S6) of the four constituting subunits with the pore helix lining the top of the channel pore. The amino acid position of each transmembrane protein S1 to S6 is provided by the UniProtKB/Swiss-Prot database. The amino acid sequence of extracellular loop EL1 starts after the transmembrane region S1 and ends at S2. The amino acid sequence of extracellular loop EL2 starts after the transmembrane region S3 and ends at S4. The amino acid sequences of extracellular loop EL3 starts after the transmembrane region S5 and ends at S6. The mechanism by which potassium ions are transported through this ion channel and the molecular mechanisms for its selectivity towards potassium ions are described in Yellen et al., Q Rev Biophys 31(3):239-95, 1998; Morais-Cabral et al., Nature 414(6859):37, 2001; Kurata and Fedida, Prog Biophys Mol Biol 92(2):185, 2006 and Hoshi and Armstrong, J Gen Physiol 141(2):151, 2013.

Physiologically, this channel was originally identified in human islet cells and T lymphocytes, where, along with the calcium-activated potassium channel, it supports the activation of specific subsets of T cells. In particular, up-regulation and activation of Kv1.3 in activated effector memory T cell populations (TEM) allow sustained calcium influx into the cell through calciumrelease–activated channels, leading to the initiation of signaling cascades and gene regulation.

Blockade of the channel causes membrane depolarization, which attenuates intracellular Ca2+ levels required for lymphocyte activation upon T-cell stimulation, and inhibits immune responses in vivo. To date, the relevance of Kv1.3 to disease has been demonstrated by several animal and human studies, and its potential as a target to treat disease associated T cells in autoimmune settings such as MS, type 1 diabetes or rheumatoid arthritis has been widely investigated.

Modulators of Kv1.3 ion channel function typically include small molecules and peptide toxins derived from plants and venoms. Several natural toxin fragments have been identified and characterized to bind to the third extracellular (EL3) region of Kv1.3 i.e. the pore region (Zhu et al., Mol Cell Proteomics 10(2): M110.002832, 2011; Bergeron and Bingham, Toxins 4(11): 1082-1119, 2012). This region of Kv1.3 is considered of particular interest, since perturbation through EL3-binding is likely to affect conductance. Binding of the toxin peptides physically occludes the pore thereby eliminating the flow of K^+ ions. However, as the pore region is relatively conserved among family members of Kv1.3, these natural toxins tend to lack high specificity towards one family member. Given the ubiquitous distribution of Kv1.3 ion channels in living organisms, this lack of specificity for Kv1.3 is a major impediment for the use of natural toxins as a therapeutic agent. Researchers in the pharmaceutical industry and academia have invested considerable engineering efforts to improve this specificity drawback with variable success (Chi et al., Toxicon 59(4): 529-46, 2012; Berkut et al., J Biol Chem 289: 14331-14340, 2014; Takacs et al., PNAS 106(52): 22211-22216, 2009). Furthermore, such detailed engineering would be required for each individual toxin peptide, which is a time consuming and costly process.

Next to natural toxin peptides, also antibodies have been generated against ion channels in an attempt to block the channel in its function. Whilst antibodies are clearly desirable, due to their exquisite specificity, it has not been straightforward to generate functionally blocking antibodies. The lack of FDA approved antibody derived therapeutics against ion channels is exemplary in this regard.

So far, at least eleven ion channels have been targeted by the generation of antibodies binding to the third extracellular loop (Naylor and Beech, Methods Mol Biol 998:245-56, 2013) . Unfortunately, often these tool antibodies were polyclonal and attempts to isolate the functional monoclonal antibody within the pool has resulted in loss of all activity. Furthermore, limited efficacy in prohibiting ionic flow has been noted. As the pore region forms a cavity towards the

selectivity filter, it might be difficult for a full sized mAb to dock into this region such that it would directly block the ionic flow. One described antibody seemed to block the function of the channel but functioned more through the modulation of channel protein turn over (Yang et al., PlosOne 7(4): e36379, 2012).

There clearly remains a widely recognized need for improved potent and selective Kv1.3 inhibitors for use as therapeutic immunosuppressive agents. Moreover, there clearly remains a need for improved potent and selective Kv1.3 inhibitors which, in addition, do not compromise the protective immune response.

SUMMARY OF THE INVENTION

The present invention provides immunoglobulins with improved prophylactic, therapeutic and/or pharmacological properties, in addition to other advantageous properties (such as, for example, improved ease of preparation, good stability, and/or reduced costs of goods), compared to the prior art amino acid sequences and antibodies.

Based on extensive screening, characterization and combinatory strategies, the present inventors surprisingly observed that immunoglobulins recognizing particular epitopes on the first extracellular loop EL1 of Kv1.3 exhibited different modulating activities, highly improved interspecies cross-reactivity and exquisite selectivity properties. More specifically, the present inventors surprisingly observed that immunoglobulins that bind to the first extracellular loop (EL1) of Kv1.3 could modulate and/or partially or fully block the activity of this ion channel, as was e.g. demonstrated by electrophysiology (IonFluxTM, Molecular Devices), by their blocking of ^{125}I-margatoxin binding to Kv1.3 and by their capacity to inhibit T-cell activation and/or proliferation.

As described above, the pore channel of Kv1.3 is made up by the extracellular region EL3 of Kv1.3. Therefore, the finding of immunoglobulins that bind EL1 and still modulate, inhibit and/or block Kv1.3 activity was unexpected. The immunoglobulins of the present invention indeed cannot physically block the Kv1.3 pore channel, but would rather exert an indirect effect on the activity of the Kv1.3 pore, also referred to herein as allosteric modulation (as further defined herein).

Accordingly, the present invention relates to immunoglobulins that are directed against/and or that can specifically bind (as defined herein) to the EL1 extracellular loop of potassium channel 3 (Kv1.3), wherein the binding of said immunoglobulin to said EL1 extracellular

loop modulates the activity of Kv1.3 (in particular human Kv1.3). More particularly, the present invention provides immunoglobulins wherein the immunoglobulin modulates the activity of Kv1.3 by partially or fully blocking of Kv1.3 activity.

As described above, Kv1.3 channel is a potassium selective voltage gated ion channel. The partially or fully blocking of Kv1.3 activity will result in a reduction or even totally inhibiting the efflux of potassium ions from cells that have Kv1.3 channels. Accordingly, the present invention also relates to immunoglobulins that specifically bind to the EL1 extracellular loop Kv1.3, wherein the binding of said immunoglobulin to said EL1 extracellular loop modulates the activity of Kv1.3 by reducing or even totally inhibiting the efflux of potassium ions from T-cells.

In a particular aspect the efflux of potassium ions from T-cells was inhibited with an IC50 value of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower, as determined in Patch Clamp assay.

Furthermore, contrary to what is observed with natural toxins (which tend to lack high specificity towards one family member) the immunoglobulins of the present invention were shown highly specific for Kv1.3, with a more than 1000 fold selectivity over other related Kv ion family members. Accordingly, the present invention also relates to immunoglobulins that specifically bind to the EL1 extracellular loop Kv1.3, wherein the immunoglobulin has a more than 10 fold, more than 100 fold, preferably more than 1000 fold, and even up to 10000 fold or more selectivity over other related Kv ion channel family members for modulating and/or inhibiting the activity of Kv1.3.

In one aspect, the immunoglobulins of the invention have the same number of amino acids within their sequence compared to any one of SEQ ID NOs: 1-64 and have an amino acid sequence between position 8 and position 106 (according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 1-64.

In another aspect, the immunoglobulins of the invention have the same number of amino acids within their sequence compared to any one of SEQ ID NOs: 65-123 and have an amino acid sequence between position 8 and position 106 (according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 65-123.

In a preferred aspect, the immunoglobulins of the invention have the structure FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, in which CDR1, CDR2 and CDR3 are as defined herein, and FR1, FR2, FR3 and FR4 are framework sequences. Accordingly, the present invention relates to

immunoglobulins that (essentially) consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397.

Preferred immunoglobulins of the invention (essentially consist) of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 1 the G has been changed into L, or R;
- at position 2 the L has been changed into F, P, or I;
- at position 3 the L has been changed into P, or F;
- at position 4 the F has been changed into S, L, or I;
- at position 5 the S has been changed into I, or R;
- at position 6 the R has been changed into C, A, P, V, or L;
- at position 7 the N has been changed into H, P, I, M, Y, T or D:
- at position 8 the S has been changed into T, R, or I;

- at position 9 the A has been changed into V or T; and/or
- at position 10 the G has been changed into S, R, or V;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 1 the R has been changed into G, or C;
- at position 2 the I has been changed into V, T, S or L;
- at position 3 the R has been changed into G, or L;
- at position 4 the M has been changed into S, R, or T;
- at position 5 the G has been changed into V, S, or T;
- at position 7 the S has been changed into G, C, D, or E; and/or
- at position 8 the I has been changed into T, M, or R;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 1 the W has been changed into G;
- at position 3 the E has been changed into T, K, G, A, or I;
- at position 4 the G has been changed into E, or D;
- at position 5 the F has been changed into A, L, V, Y, T, or S;
- at position 6 the Y has been changed into F, or D:
- at position 7 the E has been changed into G, or K;
- at position 8 the Y has been changed into S or H; and/or
- at position 9 the W has been changed into S, G or C.

In particular, the immunoglobulins of the invention (essentially) consist of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-185; or

b) amino acid sequences that have 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 6 the R has been changed into A, or V; and/or
- at position 9 the A has been changed into V;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-271; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 2 the I has been changed into L;
- at position 4 the M has been changed into S or T;
- at position 5 the G has been changed into S or T; and/or
- at position 8 the I has been changed into T;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-398; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 3 the E has been changed into T or I;
- at position 4 the G has been changed into E;
- at position 5 the F has been changed into A; and/or
- at position 8 the Y has been changed into H.

In another aspect, the present invention relates to an immunoglobulin that (essentially) consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422.

Preferred immunoglobulins (essentially) consist of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214, wherein

- at position 1 the G has been changed into R, A, V, S, or K;
- at position 3 the T has been changed into N;
- at position 4 the F has been changed into L;
- at position 6 the N has been changed into S;
- at position 7 the F has been changed into Y:
- at position 8 the G has been changed into A; and/or
- at position 9 the M has been changed into V;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303, wherein

- at position 1 the A has been changed into T;
- at position 2 the I has been changed into V;
- at position 5 the T has been changed into S, or A;

- at position 6 the G has been changed into N, or A;
- at position 7 the G has been changed into S, or R;
- at position 8 the H has been changed into R, or Y;
- at position 9 the T has been changed into I, or K; and/or
- at position 10 the Y has been changed into F;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422, wherein

- at position 4 the F has been changed into Y, or S;
- at position 5 the G has been changed into D;
- at position 6 the D has been changed into G;
- at position 7 the G has been changed into D;
- at position 8 the T has been changed into A:
- at position 9 the Y has been changed into S;
- at position 10 the Y has been changed into F;
- at position 12 the Q has been changed into E;
- at position 14 the A has been changed into N, T, I, or R; and/or
- at position 17 the D has been changed into N, or G.

In a preferred aspect, the immunoglobulin of the invention is chosen from the group of polypeptides, wherein:

- CDR1 is SEQ ID NO: 182, CDR2 is SEQ ID NO: 269, and CDR3 is SEQ ID NO: 397;
- CDR1 is SEQ ID NO: 182, CDR2 is SEQ ID NO: 269, and CDR3 is SEQ ID NO: 394;
- CDR1 is SEQ ID NO: 185, CDR2 is SEQ ID NO: 271, and CDR3 is SEQ ID NO: 398;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 395;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 396;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 270, and CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 183, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 184, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 393;

- CDR1 is SEQ ID NO: 185, CDR2 is SEQ ID NO: 271, and CDR3 is SEQ ID NO: 398;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 290, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 292, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 293, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 294, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 216, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 419;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 297, and CDR3 is SEQ ID NO: 420;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298, and CDR3 is SEQ ID NO: 421;
- CDR1 is SEQ ID NO: 217, CDR2 is SEQ ID NO: 299, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 298, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 423;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 301, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 424;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 302, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 425;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 426;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 219, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 427;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 304, and CDR3 is SEQ ID NO: 428;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 421;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 429;
- CDR1 is SEQ ID NO: 221, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 222, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 430;

- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 306, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 223, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298 and CDR3 is SEQ ID NO: 431;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 432;
- CDR1 is SEQ ID NO: 224, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 307, and CDR3 is SEQ ID NO: 433;
- CDR1 is SEQ ID NO: 225, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 226, CDR2 is SEQ ID NO: 308, and CDR3 is SEQ ID NO: 434;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 301, and CDR3 is SEQ ID NO: 426;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 217, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298, and CDR3 is SEQ ID NO: 435; and
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 309, and CDR3 is SEQ ID NO: 418.

The immunoglobulins of the invention may (essentially) consist of an immunoglobulin single variable domain selected from a light chain variable domain sequence (*e.g.,* a V_L-sequence) and from a heavy chain variable domain sequence (*e.g.,* a V_H-sequence). The immunoglobulins of the invention may (essentially) consist of an immunoglobulin single variable domain selected from a heavy chain variable domain sequence that is derived from a conventional four-chain antibody and from a heavy chain variable domain sequence that is derived from heavy chain antibody. The immunoglobulins of the invention may (essentially) consists of an immunoglobulin single variable domain selected from a domain antibody (or an amino acid that is suitable for use as a domain antibody), a single domain antibody (or an amino acid that is suitable for use as a single domain antibody), a "dAb" (or an amino acid that is suitable for use as a dAb), a Nanobody , a VHH sequence, a camelized VH sequence, or a VHH sequence that has been obtained by affinity maturation. In a preferred aspect, the immunoglobulin of the invention (essentially) consists of a partially or fully humanized Nanobody, such as a partially or fully humanized VHH.

Preferred immunoglobulins of the invention are selected from any of SEQ ID NOs: 1-123 or immunoglobulins that have a sequence identity of more than 80% with any of SEQ ID NOs: 1-123.

The invention also relates to immunoglobulins directed against Kv1.3 that cross-block the binding to Kv1.3 of at least one of the immunoglobulin single variable domains with SEQ ID NOs: 1-123 and/or that are cross-blocked from binding to Kv1.3 by at least one of the immunoglobulin single variable domains with SEQ ID NOs: 1-123.

The immunoglobulins provided by the invention are preferably in essentially isolated form (as defined herein), or form part of a polypeptide (also referred to as "polypeptide of the invention"), which may comprise, or (essentially) consist of one or more immunoglobulins of the invention and which may optionally further comprise one or more further immunoglobulins (all optionally linked via one or more suitable linkers).

More particularly, the present invention provides multivalent polypeptide comprising, or (essentially) consisting of at least two immunoglobulins of the invention, wherein said at least two immunoglobulins can be the same or different and wherein said at least two immunoglobulins are directly linked to each other or linked to each other via a linker. Suitable linkers are selected from the group of linkers with SEQ ID NOs: 479-494.

In a preferred aspect, the invention relates to a multivalent polypeptide as defined above, which is selected from any of SEQ ID NOs: 451-473 or polypeptides that have a sequence identity of more than 80% with any of SEQ ID NOs: 451-473 (see Table A-3).

In another aspect, the invention relates to a compound or construct (also referred to herein as a "*compound of the invention*" or "*construct of the invention*", respectively) that comprises or (essentially) consists of one or more immunoglobulins or polypeptides of the invention (or suitable fragments thereof), and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more peptidic linkers. As will become clear to the skilled person from the further disclosure herein, such further groups, residues, moieties or binding units may or may not provide further functionality to the immunoglobulins of the invention (and/or to the compound, construct or polypeptide in which it is present) and may or may not modify the properties of the immunoglobulin of the invention.

In one specific aspect of the invention, a compound of the invention or a construct of the invention may have an increased half-life, compared to the corresponding immunoglobulin or polypeptide of the invention. Some preferred, but non-limiting examples of such compounds constructs will become clear to the skilled person based on the further disclosure herein, and for example comprise immunoglobulins or polypeptides of the invention that have been chemically

modified to increase the half-life thereof (for example, by means of pegylation); immunoglobulins or polypeptides of the invention that comprise at least one additional binding site for binding to a serum protein (such as serum albumin); or immunoglobulins or polypeptides of the invention that comprise at least one immunoglobulin or polypeptide of the invention that is linked to at least one moiety that increases the half-life of the immunoglobulin or polypeptide of the invention.

Examples of immunoglobulins or polypeptides of the invention that comprise such half-life extending moieties will become clear to the skilled person based on the further disclosure herein; and for example include, without limitation, polypeptides in which the one or immunoglobulins or polypeptides of the invention are suitable linked to one or more serum proteins or fragments thereof (such as (human) serum albumin or suitable fragments thereof) or to one or more binding units that can bind to serum proteins (such as, for example, domain antibodies, amino acids that are suitable for use as a domain antibody, single domain antibodies, amino acids that are suitable for use as a single domain antibody, "dAb"'s, amino acids that are suitable for use as a dAb, Nanobodies, VHH sequences, humanized VHH sequences, or camelized VH sequences that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG); reference is made to the further description and references mentioned herein); polypeptides in which an immunoglobulin or polypeptide of the invention is linked to an Fc portion (such as a human Fc) or a suitable part or fragment thereof; or polypeptides in which the one or more immunoglobulins or polypeptides of the invention are suitable linked to one or more small proteins or peptides that can bind to serum proteins (such as, without limitation, the proteins and peptides described in WO 91/01743, WO 01/45746, WO 02/076489).

Generally, the compounds or constructs of the invention with increased half-life preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding immunoglobulin or polypeptide of the invention per se.

In a preferred, but non-limiting aspect, such compounds or constructs of the invention have a serum half-life that is increased with more than 1 hour, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding immunoglobulin or polypeptide of the invention per se.

In another preferred, but non-limiting aspect, such compounds or constructs of the invention exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, compounds or polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

In a preferred aspect, the invention relates to a compound or construct as defined above, which is selected from any of SEQ ID NOs: 461-473 or compounds or constructs that have a sequence identity of more than 80% with any of SEQ ID NOs: 461-473 (see Table A-3).

The invention also relates to nucleic acids or nucleotide sequences that encode an immunoglobulin, a polypeptide, a compound and/or construct of the invention. Such a nucleic acid will also be referred to herein as "*nucleic acid(s) of the invention*" and may for example be in the form of a genetic construct, as further described herein. Accordingly, the present invention also relates to a nucleic acid or nucleotide sequence that is in the form of a genetic construct.

Nucleic acids encoding an immunoglobulin, a polypeptide, a compound and/or construct of the invention can be linked to obtain a nucleic acid encoding a multivalent polypeptide of the invention. Accordingly, the present invention also relates to the use of a nucleic acid or nucleotide sequence that encodes an immunoglobulin, a polypeptide, a compound and/or construct of the invention for the preparation of a genetic construct that encodes a multivalent polypeptide of the invention.

The invention further relates to a host or host cell that expresses (or that under suitable circumstances is capable of expressing) an immunoglobulin, a polypeptide, a compound and/or construct of the invention; and/or that contains a nucleic acid of the invention. Some preferred but non-limiting examples of such hosts or host cells will become clear from the further description herein.

The invention further relates to a composition containing or comprising at least one immunoglobulin, polypeptide, compound and/or construct of the invention and/or at least one nucleic acid of the invention, and optionally one or more further components of such compositions known per se, i.e. depending on the intended use of the composition. Such a

composition may for example be a pharmaceutical composition (as described herein) or a veterinary composition. Some preferred but non-limiting examples of such compositions will become clear from the further description herein.

The invention further relates to methods for preparing the immunoglobulins, polypeptides, compounds and/or constructs, nucleic acids, host cells, and composition described herein. The method for producing an immunoglobulin, polypeptide, compound and/or construct, nucleic acid, host cell, and composition of the invention may comprise the following steps:

a) expressing, in a suitable host cell or host organism or in another suitable expression system, a nucleic acid or nucleotide sequence of the invention, or a genetic construct of the invention; optionally followed by:

b) isolating and/or purifying the immunoglobulin, polypeptide, compound and/or construct of the invention thus obtained.

The invention further relates to applications and uses of the immunoglobulins, polypeptides, compound and/or constructs, nucleic acids, host cells, and compositions described herein, as well as to methods for the prevention and/or treatment of Kv1.3 associated diseases, disorders or conditions. Some preferred but non-limiting applications and uses will become clear from the further description herein.

As such, immunoglobulins, polypeptides, compounds and/or constructs and compositions of the present invention can be used for the prevention and/or treatment of Kv1.3 associated diseases, disorders or conditions. Patient groups susceptible to Kv1.3 associated diseases, disorders or conditions will be clear to the skilled person and for example include (without being limiting) multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity.

Accordingly, the present invention also relates to a method for the prevention and/or treatment of a Kv1.3 associated disease, disorder or condition in at least one of multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis,

systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one immunoglobulin, polypeptide, compound and/or construct of the invention or composition of the invention.

The invention also relates to the use of an immunoglobulin, polypeptide, compound and/or construct of the invention or composition of the invention in the preparation of a pharmaceutical composition for the prevention and/or treatment of Kv1.3 associated diseases, disorders or conditions in least one of multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity; and/or for use in one or more of the methods described herein.

The invention also relates to an immunoglobulin, polypeptide, compound and/or construct of the invention or a composition of the invention for prevention and/or treatment of Kv1.3 associated diseases, disorders or conditions in least one of multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity.

Other aspects, advantages, applications and uses of the polypeptides and compositions will become clear from the further disclosure herein. Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. Nothing herein is to be

construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

FIGURE LEGENDS

Figure 1: Planar membrane topology of a single potassium channel subunit of the Kv1.3 protein. Structurally, Kv1.3 channels exist as tetramers of four identical subunits. Each subunit is composed of six transmembrane domains S1 –S6 with the S5 – S6 region forming the K^+pore. These six transmembrane domains are interconnected by three extracellular loops, termed EL1-EL3. Both the N- and C-termini are on the cytoplasmic side of the cell membrane.

Figure 2A-2B: Schematic representation of the voltage protocol applied on the automated patch clamp IonFlux™ system, in either population or single cell mode. Kv1.3 potassium currents were elicited by a depolarization pulse to +40 mV from V_H of -80 mV every 30 s (A). The data points used in analysis represent normalized mean sustained current amplitudes ($I_{sustained}$) obtained from the area between the designated cursors (B).

Figure 3A-3F: Titration of monovalent anti-Kv1.3 Nanobodies (A: A019400003; B: A0194009G09) on human Kv1.3 expressed on CHO cells (closed triangles) and on parental CHO cells (closed circles). Cross species binding of a dilution series of anti Kv1.3 Nanobodies (C and E: A019400003; D and F: A0194009G09) to cyno Kv1.3 (C & D; parental CHO cells are depicted as closed circles; cyno Kv1.3 CHO cells are shown as closed squares) and rat Kv1.3 (E and F; parental CHO cells are depicted as closed squares; cyno Kv1.3 CHO cells are shown as closed circles). The MCF value (mean channel fluorescence) is plotted against the concentration of the Nanobody.

Figure 4A-4C: Effect of monovalent anti-Kv1.3 Nanobodies (A: A019400003; B: an irrelevant Nanobody (squares) and A0194009G09 (closed circles)) or the peptide toxin ShK (C) on binding of radiolabeled margatoxin to cyno Kv1.3 that is expressed on CHO cells. The counts per minute (cpm) value is plotted against the concentration of the Nanobody. Both anti-Kv1.3 Nanobodies completely block binding of 150 pM I125 margatoxin to cyno Kv1.3. The background (BG) is the control condition where no I125 margatoxin was added.

Figure 5A-5B: Dose-dependent effect of monovalent Nanobody A0194009G09 on human Kv1.3 channels stably expressed in HEK293H. Kv1.3 currents were recorded by automated population patch clamp using a depolarizing voltage protocol with a time interval of 30 s (as shown in Figure 2A) using the IonFlux™ system. A0194009G09 was sequentially applied to the same cell population by continuous perfusion of each concentration for 120 s. Representative Kv1.3 current

traces (A) show a clear dose-dependent inhibitory effect and the correlated concentration-response curves for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ are presented in (B).

Figure 6A-6B: Dose-dependent effect of monovalent Nanobody A01940020A06 on human Kv1.3 channels stably expressed in HEK293H. Kv1.3 currents were recorded by automated population patch clamp using a depolarizing voltage protocol with a time interval of 30 s (as shown in Figure 2A) using the IonFlux™ system. A01940020A06 was sequentially applied to the same cell population by continuous perfusion of each concentration for 120 s. Representative Kv1.3 current traces (A) show a clear dose-dependent inhibitory effect and the correlated concentration-response curves for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ are presented in (B).

Figures 7A-7B: Current recovery during washout of the selected monovalent Nanobodies. HEK293H cells stably expressing Kv1.3 were perfused with a single high dose of Nanobody (300 nM) for 120 s to achieve steady sate inhibition and subsequently superfused for at least 5 min with extracellular buffer. Kv1.3 currents were recorded by automated population patch clamp using a depolarizing voltage protocol with a time interval of 30 s, using the IonFlux™ system. The Kv1.3 currents recorded after washing the cell were superimposed on control Kv1.3. An almost full recovery from inhibition could be observed (A). For stability reasons and comparison, Kv1.3 currents were also recorded by automated single cell patch clamp. As expected, the inhibitory effect could be reversed for monovalent Nanobody A0194009G09 (B).

Figures 8A-8B: Schematic representation of the voltage protocol applied to each well before and after the application of test compound on the automated patch clamp IonWorks system. Kv1.3 potassium currents were elicited by a repeated gating voltage command protocol. K^+ currents were evoked by a train of 100 ms depolarizing steps to +50 mV from a holding potential of -80 mV, applied 15 times (P1 to P15) at 3 Hz (A). The data points used in analysis represent normalized mean sustained current amplitudes ($I_{sustained}$) obtained from the area between the designated cursors in P1 (B).

Figure 9A-9C: Dose-dependent effect of A0194009G09 on human Kv1.3 channels stably expressed in HEK293H at exemplified concentrations of 412 pM (A) and 100 nM (B). Kv1.3 currents were recorded by automated population patch clamp using the repeated gating voltage command protocol on the IonWorks system, as described in Example 5. Recordings were done in control

conditions (prior to compound addition). A0194009G09 was then incubated for 6 to 7 min prior to the second measurement using the identical pulse train. The correlated concentration-response curve for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ is presented in (C).

Figure 10A-10C: Kv1.3 current traces of A019400003 at low concentration (A) and high (B) concentration. The correlated concentration-response curve for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ is presented in (C).

Figure 11A-11D: Inhibition by monovalent anti-Kv1.3 Nanobodies and ShK [A: A019400003 (open circles) and A0194009G09 (closed circles); B: A0194020A06 (open circles); C: A0194009G09 (closed circles) and ShK (open squares); D: A0194020A06 (closed circles)] of IFNγ production (A and B) and CD25 expression (C and D) by $CCR7^-CD45RA^-$ T-cells after stimulation with OKT3. For the IFNγ readout, the IFNγ concentration (pg/ml) is plotted against the concentration of the Nanobody. For the measurement of CD25 expression, the MCF value (mean channel fluorescence) is plotted against the concentration of the Nanobody. The control condition where cells are not stimulated is indicated by 'no stim'.

Figure 12A-12D: Binding of a dilution series of multivalent Nanobodies to human, cyno and rat Kv1.3 expressed on CHO cells. A: A019400004; B: A019400013; C: A019400014; D: A019400015; closed circles: binding to parental CHO cells; closed squares: binding to cyno Kv1.3 CHO cells; closed up righted triangle: binding to rat Kv1.3 and closed down righted triangle: binding to human Kv1.3 CHO cells.

The MCF value (mean channel fluorescence) is plotted against the concentration of the Nanobody.

Figure 13A-13E: Effect of multivalent anti-Kv1.3 Nanobodies on binding of radiolabeled margatoxin to CHO expressed cyno Kv1.3. A: A019400013 (closed triangles) and unlabeled margatoxin (closed squares); B: A019400004 (closed squares); C: A019400012 (closed squares) and A019400014 (closed triangles); D: A019400015 (closed circles) and unlabeled margatoxin (closed squares); E: A019400032 (closed squares). The counts per minute (cpm) value is plotted against the concentration of the Nanobody.

Figure 14A-14C: Dose-dependent effect of bivalent A019400009 on human Kv1.3 channels stably expressed in HEK293H. Kv1.3 currents were recorded by automated population patch clamp using a depolarizing voltage protocol with a time interval of 30 s on the IonFlux™ system, as described in Figure 2A. A019400009 was sequentially applied to the same cell

population by continuous perfusion of each concentration for 120 s Kv1.3 current traces of A019400009 are depicted in (A). The correlated concentration-response curve for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ is presented in (B). The current recovery during washout of A019400009 is shown in (C)

Figure 15A-15C: Dose-dependent effect of biparatopic A019400012 on human Kv1.3 channels stably expressed in HEK293H. Kv1.3 currents were recorded by automated population patch clamp using a depolarizing voltage protocol with a time interval of 30 s on the IonFlux™ system, as described in Figure 2A. A019400012 was sequentially applied to the same cell population by continuous perfusion of each concentration for 120 s. Kv1.3 current traces of A019400012 are depicted in (A). The correlated concentration-response curve for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ is presented in (B). The current recovery during washout of A019400010 (= A019400012) is shown in (C).

Figure 16A-16C: Dose-dependent effect of biparatopic A019400014 on human Kv1.3 channels stably expressed in HEK293H. Kv1.3 currents were recorded by automated population patch clamp using a depolarizing voltage protocol with a time interval of 30 s on the IonFlux™ system, as described in Figure 2A. A019400014 was sequentially applied to the same cell population by continuous perfusion of each concentration for 120 s. Kv1.3 current traces of A019400014 are depicted in (A). The correlated concentration-response curve for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ is presented in (B). The current recovery during washout of A019400014 is shown in (C).

Figure 17A-17C to Figure 22A-22C: Dose-dependent effect of multivalent Nanobodies on human Kv1.3 channels stably expressed in HEK293H. Kv1.3 currents were recorded by automated population patch clamp using the repeated gating voltage command protocol on the IonWorks system, as described in Figure 8. Recordings were done in control conditions (prior to compound addition). The Nanobodies were then incubated for 6 to 7 min prior to the second measurement using the identical pulse train. Representative Kv1.3 current traces of the multivalent Nanobodies A019400004, A019400009, A019400012, A019400014, A019400015 and A019400032 are depicted in Figures 17A-17B to 22A-22B, respectively. The correlated concentration-response curves for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ are presented in Figures 17C to 22C, respectively.

Figure 23A to 23F: Inhibition by multivalent anti-Kv1.3 Nanobodies and Shk (A and D: A019400013 (open circles) and ShK (closed squares); B and E: A019400012 (open circles) and A019400014 (closed squares); C and F: A019400015 (closed squares)) of IFNγ production (A, B and C) and CD25 expression (D, E and F) by $CCR7^{-}CD45RA^{-}$ T-cells after stimulation with anti-CD3. For the IFNγ readout, the IFNγ concentration (pg/ml) is plotted against the concentration of the Nanobody. For the measurement of CD25 expression, the MCF value (mean channel fluorescence) is plotted against the concentration of the Nanobody. The control condition where cells are not stimulated is indicated by 'no stim'.

Figure 24A-24B: Histograms evaluating the binding of agitoxin-TAMRA to WT Kv1.3 (B) and a Kv1.3 EL1 mutant (A) expressed on HEK293H cells. The counts obtained in a FACS binding experiment are plotted against the fluorescence intensity of agitoxin-TAMRA. The histograms show that both constructs are bound by agitoxin indicating their functionality.

Figure 25: Dilutions series of monovalent Nanobodies (A019400003 (closed circles); A0194009G09 (closed triangles), irrelevant Nanobody (open circles)) and the ShK compound (closed checks) competing with a saturating concentration of FAM-labeled ShK for binding to human Kv1.3 expressed on HEK293H cells. The MCF value (mean channel fluorescence) is plotted against the concentration of the Nanobody.

Figure 26A to 26D: Binding to cyno Kv1.3 expressed on CHO cells by half-life extended bivalent constructs with the albumin binding Nanobody (Alb11) at different positions. Both A019400028 (A and C; Alb11 in the middle) and A019400024 (B and D; Alb11 at the C-terminus) bind similarly to cyno Kv1.3 expressed on CHO cells in absence of human serum albumin (HSA) (A and B); in presence of HSA (C and D), the potency is slightly decreased. The MCF value (mean channel fluorescence) is plotted against the concentration of the Nanobody.

Figure 27A-27C: Dose-dependent effect of trivalent A019400029 on human Kv1.3 channels stably expressed in HEK293H. Kv1.3 currents were recorded by automated population patch clamp using a depolarizing voltage protocol with a time interval of 30 s on the IonFlux™ system, as described in Figure 2A. A019400029 was sequentially applied to the same cell population by continuous perfusion of each concentration for 120 s. Kv1.3 current traces of A019400029 are depicted in (A). The correlated concentration-response curve for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ is presented in (B). The current recovery during washout of A019400029 is shown in (C).

Figure 28: Competition of the half-life extended bivalent A019400029 Nanobody construct with I125 margatoxin in absence (closed squares) and presence (closed circles) of HAS. Cyno Kv1.3 was expressed on CHO cells. The counts per minute (cpm) value is plotted against the concentration of the Nanobody. The background (BG) is the control condition where no I125 margatoxin was added and was evaluated in presence (down righted closed triangles) and absence of HSA (up righted closed triangles).

Figure 29A-29B: IFNγ production (A) and CD25 expression (B) by $CCR7^-CD45RA^-$ T-cells after stimulation with OKT3 in presence of a dilution series of the A019400024 Nanobody construct in absence (closed circles) and presence (closed squares) of HSA. For the IFNγ readout, the IFNγ concentration (pg/ml) is plotted against the concentration of the Nanobody. For the measurement of CD25 expression, the MCF value (mean channel fluorescence) is plotted against the concentration of the Nanobody. The control condition where cells are not stimulated is indicated by 'no stim'.

Figure 30A-30B: Inhibition of IFNγ production in anti-CD3 stimulated human PBMCs by a dilution series of the A019400029 Nanobody construct (closed circles) and ShK toxin (closed squares) (A). Same Nanobody and toxin do not inhibit IFNγ production in human PBMCs co-stimulated with anti-CD3 and anti-CD28 (B). The IFNγ concentration (pg/ml) is plotted against the concentration of the Nanobody or toxin. The control condition where cells are not stimulated is indicated by 'no stim'.

Figure 31A-31D: Effect of A019400029 on the voltage-dependence of activation on human Kv1.3 channels stably expressed in CHL cells. Kv currents were recorded by conventional planar patch clamp. Superimposed current traces were elicited by a 500 ms depolarizing pulse to +50 mV in 10 mV steps from a holding potential of -80 mV at 30 s intervals in the absence (B) and presence (C) of 10 nM A019400029. A schematic of the voltage protocols is given in Figure 31A. The data points used in analysis represent peak current amplitudes (I_{peak}) as indicated in B (arrow). The conductances were calculated as described in Example 10, normalized with their respective maximum conductance (Gmax) and plotted against the applied depolarization voltages (D). The Boltzmann equation was used to fit the curve (as described in Example 10).

Figure 32A-32B: Effect of A019400029 on association and washout of Kv1.3 currents on human Kv1.3 channels stably expressed in CHL cells. Kv currents were recorded by conventional planar patch clamp using a repeated gating voltage command protocol. Currents were provoked

by a test pulse from -80 mV to +40 mV every 15 s. The test pulse duration was either 20 ms (A) or 200 ms (B). Recordings were done in control conditions (prior to compound addition) and during a 3 to 5 min incubation of 10 nM A019400029, followed by compound washout. Peak and sustained current amplitudes were then plotted against the different time points.

Figure 33A-33B: Effect of A019400029 on association and washout of Kv1.3 currents on human Kv1.3 channels stably expressed in CHL cells. Kv currents were recorded by conventional planar patch clamp using a repeated gating voltage command protocol. Currents were provoked by a 200 ms test pulse from -80 mV to +40 mV every 15 s. Recordings were done in control conditions (prior to compound addition) and during a 3 to 5 min incubation of 10 nM A019400029, followed by compound washout. During this period of Nanobody incubation, the cells were held at a holding potential of either -80 mV (A) or -40 mV (B). Peak and sustained current amplitudes were then plotted against the different time points.

Figure 34A-34C: Effect of A019400029 on recovery from inactivation of Kv1.3 currents on human Kv1.3 channels stably expressed in CHL cells. Kv currents were recorded by conventional planar patch clamp. The recovery of inactivation from two inter-pulse potentials (-80 mV and -50 mV; C) was measured using a standard variable interval gapped pulse protocol (as shown in A). An initial 1 s pulse from -80 mV to +40 mV (P1) was followed by a second pulse from -80 mV to +40 mV for 150 ms (P2) after an interval of between 0.5 to 30 s. Representative traces in the absence and presence of 10 nM A019400029 are given in B. The percentage of recovery was calculated (see below) and plotted against pulse interval to show the recovery of inactivation (C).

$$\%\ \text{recovery} = \frac{\left(\text{P2}_{\text{peak}} - \text{P1}_{\text{sustained}}\right)}{\left(\text{P1}_{\text{peak}} - \text{P1}_{\text{sustained}}\right)} * 100$$

Figure 35A-35D: Schematic representation of the voltage protocols applied to each well before and after the application of test compound on the automated patch clamp IonWorks system. Kv1.5 and Kv1.6 potassium currents were elicited by a repeated gating voltage command protocol. K^+ currents were evoked by a train of 100 ms depolarizing steps to +50 mV from a holding potential of -80 mV, applied 15 times (P1 to P15) at 3 Hz (A). The data points used in analysis represent normalized mean sustained current amplitudes ($I_{sustained}$) obtained from the area between the designated cursors in P1 (B). The hERG currents were elicited by a pulse train of five pulses to +40 mV from V_H of -70 mV for 1 sec, then to -30 mV for 1 s, and back to the V_H of -70 mV,

at 03 Hz (C). The data points used in analysis represent normalized mean peak current amplitudes (I_{peak}) obtained from the area between the designated cursors in the tail step from pulse P5 (D).

Figure 36A-36D: Comparative pharmacology of the selected Nanobodies on human Kv1.3, Kv1.5, Kv1.6 and hERG K^+ channels. The Kv1.3 and Kv1.5 currents were recorded by automated population patch clamp (PPC) and the Kv1.6 and hERG K^+ current were recorded in single patch clamp mode (HT), using the repeated gating voltage command protocols on the IonWorks system, as described in Figure 35. Recordings were done in control conditions (prior to compound addition). The Nanobodies were then incubated for 6 to 7 min prior to the second measurement using identical pulse protocols. The obtained concentration-response relationships are shown in A, B, C and D for Kv1.5, Kv1.6, hERG and Kv1.3 respectively. All selected Nanobodies display profound (*i.e.* greater than 1.000 fold) selectivity for Kv1.3 over the other K^+ channels tested. The maximal inhibition at the highest concentration tested (*i.e.* 1 µM) was < 50 % in all other channels.

Figure 37: Study design for testing the efficacy of anti-Kv1.3 Nanobodies in a rat model of 2, 4-dinitrofluorobenzene (DNFB)-induced delayed-type hypersensitivity (DTH).

Figure 38: Ear swelling responses of the different treatment groups (n = 10 rats/group) in a rat model of 2, 4-dinitrofluorobenzene (DNFB)-induced delayed-type hypersensitivity. Animals received two subcutaneous injections of either vehicle, the reference compound ShK (10 µg/kg), the half-life extended anti-Kv1.3 Nanobody A019400029 (105 µg/kg) or the non-half-life extended Nanobody A019400032 (69.3 µg/kg) at 12 hours and 1 hour preceding the challenge, or one s.c. injection of A019400029 (105 µg/kg) at 1 hour before the challenge. Dexamethasone (Dex) was administered topically at 1 hour and 6 hours post-challenge (0.75 mg/ear) as positive control group. Data are represented as mean ± standard deviation. # $p < 0.05$ vs. vehicle group.

Figure 39: Ear swelling responses of the different treatment groups (n = 10 rats/group) in a rat model of 2, 4-dinitrofluorobenzene (DNFB)-induced delayed-type hypersensitivity. Animals received two subcutaneous injections of either vehicle, the reference compound ShK (100 µg/kg), or the half-life extended anti-Kv1.3 Nanobody A019400029 (1.05 mg/kg or 5.25 mg/kg) at 12 hours and 1 hour preceding the challenge. Dexamethasone (Dex) was administered topically at 1 hour and 6 hours post-challenge (0.75 mg/ear) as positive control group. Data are represented as mean ± standard deviation. a: $p < 0.05$ vs. vehicle group; b: non-inferior compared to ShK group; c: superior compared to ShK group.

DETAILED DESCRIPTION

Definitions

Unless indicated or defined otherwise, all terms used have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard handbooks, such as Sambrook et al. (Molecular Cloning: A Laboratory Manual (2nd.Ed.) Vols. 1-3, Cold Spring Harbor Laboratory Press, 1989), F. Ausubel et al. (Current protocols in molecular biology, Green Publishing and Wiley Interscience, New York, 1987), Lewin (Genes II, John Wiley & Sons, New York, N.Y., 1985), Old et al. (Principles of Gene Manipulation: An Introduction to Genetic Engineering (2nd edition) University of California Press, Berkeley, CA, 1981); Roitt et al. (Immunology (6th. Ed.) Mosby/Elsevier, Edinburgh, 2001), Roitt et al. (Roitt's Essential Immunology (10^{th} Ed.) Blackwell Publishing, UK, 2001), and Janeway et al. (Immunobiology (6th Ed.) Garland Science Publishing/Churchill Livingstone, New York, 2005), as well as to the general background art cited herein.

Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known per se, as will be clear to the skilled person. Reference is for example again made to the standard handbooks and the general background art mentioned herein and to the further references cited therein; as well as to for example the following reviews Presta (Adv. Drug Deliv. Rev. 58 (5-6): 640-56, 2006), Levin and Weiss (Mol. Biosyst. 2(1): 49-57, 2006), Irving et al. (J. Immunol. Methods 248(1-2): 31-45, 2001), Schmitz et al. (Placenta 21 Suppl. A: S106-12, 2000), Gonzales et al. (Tumour Biol. 26(1): 31-43, 2005), which describe techniques for protein engineering, such as affinity maturation and other techniques for improving the specificity and other desired properties of proteins such as immunoglobulins.

The term "sequence" as used herein (for example in terms like "immunoglobulin sequence", "antibody sequence", "variable domain sequence", "V_{HH} sequence" or "protein sequence"), should generally be understood to include both the relevant amino acid sequence as well as nucleic acids or nucleotide sequences encoding the same, unless the context requires a more limited interpretation.

Amino acid residues will be indicated according to the standard three-letter or one-letter amino acid code. Reference is made to Table A-2 on page 48 of WO 08/020079.

A nucleic acid or amino acid is considered to be "(in) (essentially) isolated (form)" - for example, compared to the reaction medium or cultivation medium from which it has been obtained - when it has been separated from at least one other component with which it is usually associated in said source or medium, such as another nucleic acid, another protein/polypeptide, another biological component or macromolecule or at least one contaminant, impurity or minor component. In particular, a nucleic acid or amino acid is considered "(essentially) isolated" when it has been purified at least 2-fold, in particular at least 10-fold, more in particular at least 100-fold, and up to 1000-fold or more. A nucleic acid or amino acid that is "in (essentially) isolated form" is preferably essentially homogeneous, as determined using a suitable technique, such as a suitable chromatographical technique, such as polyacrylamide-gel electrophoresis.

When a nucleotide sequence or amino acid sequence is said to "comprise" another nucleotide sequence or amino acid sequence, respectively, or to "essentially consist of" another nucleotide sequence or amino acid sequence, this may mean that the latter nucleotide sequence or amino acid sequence has been incorporated into the first mentioned nucleotide sequence or amino acid sequence, respectively, but more usually this generally means that the first mentioned nucleotide sequence or amino acid sequence comprises within its sequence a stretch of nucleotides or amino acid residues, respectively, that has the same nucleotide sequence or amino acid sequence, respectively, as the latter sequence, irrespective of how the first mentioned sequence has actually been generated or obtained (which may for example be by any suitable method described herein). By means of a non-limiting example, when a polypeptide of the invention is said to comprise an immunoglobulin single variable domain, this may mean that said immunoglobulin single variable domain sequence has been incorporated into the sequence of the polypeptide of the invention, but more usually this generally means that the polypeptide of the invention contains within its sequence the sequence of the immunoglobulin single variable domains irrespective of how said polypeptide of the invention has been generated or obtained. Also, when a nucleic acid or nucleotide sequence is said to comprise another nucleotide sequence, the first mentioned nucleic acid or nucleotide sequence is preferably such that, when it is expressed into an expression product (e.g. a polypeptide), the amino acid sequence encoded by the latter nucleotide sequence forms part of said expression product (in other words, that the latter nucleotide sequence is in the same reading frame as the first mentioned, larger nucleic acid or nucleotide sequence).

By "essentially consist of" is meant that the immunoglobulin single variable domain used in the method of the invention either is exactly the same as the polypeptide of the invention or corresponds to the polypeptide of the invention which has a limited number of amino acid residues, such as 1-20 amino acid residues, for example 1-10 amino acid residues and preferably 1-6 amino acid residues, such as 1, 2, 3, 4, 5 or 6 amino acid residues, added at the amino terminal end, at the carboxy terminal end, or at both the amino terminal end and the carboxy terminal end of the immunoglobulin single variable domain.

For the purposes of comparing two or more nucleotide sequences, the percentage of "sequence identity" between a first nucleotide sequence and a second nucleotide sequence may be calculated by dividing [the number of nucleotides in the first nucleotide sequence that are identical to the nucleotides at the corresponding positions in the second nucleotide sequence] by [the total number of nucleotides in the first nucleotide sequence] and multiplying by [100%], in which each deletion, insertion, substitution or addition of a nucleotide in the second nucleotide sequence - compared to the first nucleotide sequence - is considered as a difference at a single nucleotide (position). Alternatively, the degree of sequence identity between two or more nucleotide sequences may be calculated using a known computer algorithm for sequence alignment such as NCBI Blast v2.0, using standard settings. Some other techniques, computer algorithms and settings for determining the degree of sequence identity are for example described in WO 04/037999, EP 0967284, EP 1085089, WO 00/55318, WO 00/78972, WO 98/49185 and GB 2357768. Usually, for the purpose of determining the percentage of "sequence identity" between two nucleotide sequences in accordance with the calculation method outlined hereinabove, the nucleotide sequence with the greatest number of nucleotides will be taken as the "first" nucleotide sequence, and the other nucleotide sequence will be taken as the "second" nucleotide sequence.

For the purposes of comparing two or more amino acid sequences, the percentage of "sequence identity" between a first amino acid sequence and a second amino acid sequence (also referred to herein as "amino acid identity") may be calculated by dividing [the number of amino acid residues in the first amino acid sequence that are identical to the amino acid residues at the corresponding positions in the second amino acid sequence] by [the total number of amino acid residues in the first amino acid sequence] and multiplying by [100%], in which each deletion, insertion, substitution or addition of an amino acid residue in the second amino acid sequence -

compared to the first amino acid sequence - is considered as a difference at a single amino acid residue (position), *i.e.,* as an "amino acid difference" as defined herein. Alternatively, the degree of sequence identity between two amino acid sequences may be calculated using a known computer algorithm, such as those mentioned above for determining the degree of sequence identity for nucleotide sequences, again using standard settings. Usually, for the purpose of determining the percentage of "sequence identity" between two amino acid sequences in accordance with the calculation method outlined hereinabove, the amino acid sequence with the greatest number of amino acid residues will be taken as the "first" amino acid sequence, and the other amino acid sequence will be taken as the "second" amino acid sequence.

Also, in determining the degree of sequence identity between two amino acid sequences, the skilled person may take into account so-called "conservative" amino acid substitutions, which can generally be described as amino acid substitutions in which an amino acid residue is replaced with another amino acid residue of similar chemical structure and which has little or essentially no influence on the function, activity or other biological properties of the polypeptide. Such conservative amino acid substitutions are well known in the art, for example from WO 04/037999, GB 335768, WO 98/49185, WO 00/46383 and WO 01/09300; and (preferred) types and/or combinations of such substitutions may be selected on the basis of the pertinent teachings from WO 04/037999 as well as WO 98/49185 and from the further references cited therein.

Such conservative substitutions preferably are substitutions in which one amino acid within the following groups (a) – (e) is substituted by another amino acid residue within the same group: (a) small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro and Gly; (b) polar, negatively charged residues and their (uncharged) amides: Asp, Asn, Glu and Gln; (c) polar, positively charged residues: His, Arg and Lys; (d) large aliphatic, nonpolar residues: Met, Leu, Ile, Val and Cys; and (e) aromatic residues: Phe, Tyr and Trp. Particularly preferred conservative substitutions are as follows: Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into His; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

Any amino acid substitutions applied to the polypeptides described herein may also be based on the analysis of the frequencies of amino acid variations between homologous proteins of different species developed by Schulz et al. ("Principles of Protein Structure", Springer-Verlag, 1978), on the analyses of structure forming potentials developed by Chou and Fasman (Biochemistry 13: 211, 1974; Adv. Enzymol., 47: 45-149, 1978), and on the analysis of hydrophobicity patterns in proteins developed by Eisenberg et al. (Proc. Natl. Acad Sci. USA 81: 140-144, 1984), Kyte and Doolittle (J. Molec. Biol. 157: 105-132, 1981), and Goldman et al. (Ann. Rev. Biophys. Chem. 15: 321-353, 1986), all incorporated herein in their entirety by reference. Information on the primary, secondary and tertiary structure of Nanobodies is given in the description herein and in the general background art cited above. Also, for this purpose, the crystal structure of a V_{HH} domain from a llama is for example given by Desmyter et al. (Nature Structural Biology, 3: 803, 1996), Spinelli et al. (Natural Structural Biology, 3: 752-757, 1996) and Decanniere et al. (Structure, 7 (4): 361, 1999). Further information about some of the amino acid residues that in conventional V_H domains form the V_H/V_L interface and potential camelizing substitutions on these positions can be found in the prior art cited above.

Amino acid sequences and nucleic acid sequences are said to be "exactly the same" if they have 100% sequence identity (as defined herein) over their entire length.

When comparing two amino acid sequences, the term "amino acid difference" refers to an insertion, deletion or substitution of a single amino acid residue on a position of the first sequence, compared to the second sequence; it being understood that two amino acid sequences can contain one, two or more such amino acid differences. More particularly, in the amino acid sequences and/or polypeptides of the present invention, the term "amino acid difference" refers to an insertion, deletion or substitution of a single amino acid residue on a position of the CDR sequence specified in c), f) or i), compared to the CDR sequence of respectively a), d) or g); it being understood that the CDR sequence of c), f) and i) can contain one, two or maximal three such amino acid differences compared to the CDR sequence of respectively a), d) or g).

The "amino acid difference" can be any one, two or maximal three substitutions, deletions or insertions, or any combination thereof, that either improve the properties of the polypeptide of the invention or that at least do not detract too much from the desired properties or from the balance or combination of desired properties of the polypeptide of the invention. In this respect, the resulting polypeptide of the invention should at least bind Kv1.3 with the same,

about the same, or a higher affinity compared to the polypeptide comprising the one or more CDR sequences without the one, two or maximal three substitutions, deletions or insertions, said affinity as measured by surface plasmon resonance (SPR).

In this respect, the amino acid sequence according to c), f) and/or i) may be an amino acid sequence that is derived from an amino acid sequence according to a), d) and/or g) respectively by means of affinity maturation using one or more techniques of affinity maturation known *per se*.

For example, and depending on the host organism used to express the polypeptide of the invention, such deletions and/or substitutions may be designed in such a way that one or more sites for post-translational modification (such as one or more glycosylation sites) are removed, as will be within the ability of the person skilled in the art.

A "Nanobody family", "VHH family" or "family" as used in the present specification refers to a group of Nanobodies and/or VHH sequences that have identical lengths (i.e. they have the same number of amino acids within their sequence) and of which the amino acid sequence between position 8 and position 106 (according to Kabat numbering) has an amino acid sequence identity of 89% or more.

The terms "epitope" and "antigenic determinant", which can be used interchangeably, refer to the part of a macromolecule, such as a polypeptide or protein that is recognized by antigen-binding molecules, such as immunoglobulins, conventional antibodies, immunoglobulin single variable domains and/or polypeptides of the invention, and more particularly by the antigen-binding site of said molecules. Epitopes define the minimum binding site for an immunoglobulin, and thus represent the target of specificity of an immunoglobulin.

The part of an antigen-binding molecule (such as an immunoglobulin, a conventional antibody, an immunoglobulin single variable domain and/or a polypeptide of the invention) that recognizes the epitope is called a "paratope".

A polypeptide (such as an immunoglobulin, an antibody, an immunoglobulin single variable domain, a polypeptide of the invention, or generally an antigen binding molecule or a fragment thereof) that can "bind to" or "specifically bind to", that "has affinity for" and/or that "has specificity for" a certain epitope, antigen or protein (or for at least one part, fragment or epitope thereof) is said to be "against" or "directed against" said epitope, antigen or protein or is a

"binding" molecule with respect to such epitope, antigen or protein, or is said to be "anti"-epitope, "anti"-antigen or "anti"-protein (*e.g.,* "anti"-Kv1.3).

The term "specificity" has the meaning given to it in paragraph n) on pages 53-56 of WO 08/020079; and as mentioned therein refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding molecule or antigen-binding protein (such as an immunoglobulin single variable domain and/or a polypeptide of the invention) can bind. The specificity of an antigen-binding protein can be determined based on affinity and/or avidity, as described on pages 53-56 of WO 08/020079 (incorporated herein by reference), which also describes some preferred techniques for measuring binding between an antigen-binding molecule (such as an immunoglobulin single variable domain and/or polypeptide of the invention) and the pertinent antigen. Typically, antigen-binding proteins (such as the immunoglobulin single variable domains and/or polypeptides of the invention) will bind to their antigen with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles). Any K_D value greater than 10^4 mol/liter (or any K_A value lower than 10^4 M^{-1}) liters/mol is generally considered to indicate non-specific binding. Preferably, a monovalent polypeptide of the invention will bind to the desired antigen with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as *e.g.,* between 10 and 5 nM or less. Specific binding of an antigen-binding protein to an antigen or antigenic determinant can be determined in any suitable manner known *per se*, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known per se in the art; as well as the other techniques mentioned herein. As will be clear to the skilled person, and as described on pages 53-56 of WO 08/020079, the dissociation constant may be the actual or apparent dissociation constant. Methods for determining the dissociation constant will be clear to the skilled person, and for example include the techniques mentioned on pages 53-56 of WO 08/020079.

An immunoglobulin single variable domain and/or polypeptide is said to be "specific for" a first target or antigen compared to a second target or antigen when it binds to the first antigen with an affinity (as described above, and suitably expressed as a K_D value, K_A value, K_{off} rate

and/or K_{on} rate) that is at least 10 times, such as at least 100 times, and preferably at least 1000 times, and up to 10000 times or more better than the affinity with which the immunoglobulin single variable domain and/or polypeptide binds to the second target or antigen. For example, the immunoglobulin single variable domain and/or polypeptide may bind to the first target or antigen with a K_D value that is at least 10 times less, such as at least 100 times less, and preferably at least 1000 times less, such as 10000 times less or even less than that, than the K_D with which said immunoglobulin single variable domain and/or polypeptide binds to the second target or antigen. Preferably, when an immunoglobulin single variable domain and/or polypeptide is "specific for" a first target or antigen compared to a second target or antigen, it is directed against (as defined herein) said first target or antigen, but not directed against said second target or antigen.

The terms "(cross)-block", "(cross)-blocked", "(cross)-blocking", "competitive binding", "(cross)-compete", "(cross)-competing" and "(cross)-competition" are used interchangeably herein to mean the ability of an immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent to interfere with the binding of other immunoglobulins, antibodies, immunoglobulin single variable domains, polypeptides or binding agents to a given target. The extent to which an immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent is able to interfere with the binding of another to the target, and therefore whether it can be said to cross-block according to the invention, can be determined using competition binding assays. Particularly suitable quantitative cross-blocking assays are described in the Examples and include e.g. a fluorescence-activated cell sorting (FACS) binding assay with Kv1.3 expressed on cells. The extent of (cross)-blocking can be measured by the (reduced) channel fluorescence.

The following generally describes a suitable FACS assay for determining whether an immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent cross-blocks or is capable of cross-blocking according to the invention. It will be appreciated that the assay can be used with any of the immunoglobulins, antibodies, immunoglobulin single variable domains, polypeptides or other binding agents described herein. The FACS instrument (e.g. FACS Canto; Becton Dickinson) is operated in line with the manufacturer's recommendations.

To evaluate the "(cross)-blocking" or "(cross)-competition" between two binding agents (such as e.g. two immunoglobulin single variable domains and/or Nanobodies) for binding Kv1.3, a FACS competition experiment can be performed using cells (such as e.g. CHO cells or

HEK293H cells) overexpressing human Kv1.3 and the parental cells as background cell line. Different detection reagents can be used including e.g. monoclonal ANT-FLAG® M2 antibody (Sigma-Aldrich, cat# F1804), monoclonal anti-C-myc antibody (Sigma-Aldrich, cat# WH0004609M2), monoclonal ANTI-HIS TAG antibody (Sigma-Aldrich, cat# SAB1305538), each labeled differently. A wide range of fluorophores can be used as labels in flow cytometry (such as e.g PE (R-Phycoerythrin), 7-aminoactinomycin D (7-AAD), Acridine Orange, various forms of Alexa Fluor, Allophycocyanin (APC), AmCyan, Aminocoumarin, APC Cy5, APC Cy7, APC-H7, APC/Alexa Fluor 750, AsRed2, Azami-Green, Azurite, B ODIPY FL C5-ceramide, BCECF-AM, Bis-oxonol DiBAC2(3), BODIPY-FL, Calcein, Calcein AM, Caroxy-H2DCFDA, Cascade Blue, Cascade Yellow, Cell Tracker Green, Cerulean, CFSE, Chromomycin A3, CM-H2DCFDA, Cy2, Cy3, Cy3.5, Cy3B, Cy5, Cy5.5, Cy7, CyPet, DAF-FM DAF-FM diacetate, DAPI, DCFH (2'7'Dichorodihydrofluorescein), DHR, Dihydrocalcein AM, Dihydrorhoadamine, Dihydrothidium, DiLC1(5), DiOC6(3), DiOC7(3), dKeima-Red, DRAQ5, Dronpa-Green, various forms of DsRed dTomato, various forms of DyLight, E.coli BioParticles AF488, E2-Crimson, E2-Orange, EBFP2, ECFP, various forms of eFluor, EGFP, EGFP*, Emerald, eqFP650, eqFP670, ER-Tracker Blue-White DPX, Ethidium Bromide, Express2, EYFP, Fc OxyBurst Green, Fc OxyBurst Green 123, FITC, Fluo-3, Fluo-4, Fluorescein, Fura-2, Fura-Red, GFPuv, H2DCFDA, HcRed1, Hoechst Blue (33258), Hoechst Red (33342), Hydroxycoumarin, HyPer, Indo-1, Indo-1 Blue (Low Ca2+), Indo-1 Violet (High Ca2+), iRFP, J-Red, JC-1, JC-9, Katushka (TurboFP635), Katushka2 Kusabira-Orange, LDS 751, Lissamine Rhodamine B, various forms of Live/Dead, Lucifer yellow, Lucifer Yellow CH, Lyso Tracker Blue, Lyso Tracker Green, Lyso Tracker Red, mAmertrine, Marina Blue, mBanana, mCFP, mCherry, mCitrine, Methoxycoumarin, mHoneyDew, Midoriishi-Cyan, Mithramycin, Mito Tracker Deep Red, Mito Tracker Green, Mito Tracker Orange, Mito Tracker Red, MitoFluor Green, mKate (TagFP635), mKate2, mKeima, mKeima-Red, mKO, mKOk, mNeptune, Monochlorobimane, mOrange, mOrange2, mRaspberry, mPlum, mRFP1, mStrawberry, mTangerine, mTarquoise, mTFP1, mTFP1 (Teal), NBD, OxyBurst Green H2DCFDA, OxyBurst Green H2HFF BSA, Pacific Blue, PE (R-Phycoerythrin), PE Cy5, PE Cy5.5, PE Cy7, PE Texas Red, PE-Cy5 conjugates, PE-Cy7 conjugates, PerCP (Peridinin chlorphyll protein), PerCP Cy5.5, PhiYFP, PhiYFP-m, Propidium Iodide (PI), various forms of Qdot, Red 613, RFP Tomato, Rhod-2, S65A, S65C, S65L, S65T, Singlet Oxygen Sensor Green, Sirius, SNARF, Superfolder GFP, SYTOX Blue, SYTOX Green, SYTOX Orange, T-Sapphire, TagBFP, TagCFP, TagGFP, TagRFP, TagRFP657, TagYFP, tdTomato, Texas Red, Thiazole Orange, TMRE, TMRM, Topaz, TOTO-1, TO-PRO-1, TRITC, TRITC TruRed,

TurboFP602, TurboFP635, TurboGFP, TurboRFP, TurboYFP, Venus, Vybrant CycleDye Violet, Wild Type GFP, X-Rhodamin, Y66F, Y66H, Y66W, YOYO-1, YPet, ZsGreen1, ZsYellow1, Zymosan A BioParticles AF488 (see more at: http://www.thefcn.org/flow-fluorochromes). Fluorophores, or simply "fluors", are typically attached to the antibody (e.g. the immunoglobulin single variable domains and/or Nanobodies) that recognizes Kv1.3 or to the antibody that is used as detection reagent. Various conjugated antibodies are available, such as (without being limiting) for example antibodies conjugated to Alexa Fluor®, DyLight®, Rhodamine, PE, FITC, and Cy3. Each fluorophore has a characteristic peak excitation and emission wavelength. The combination of labels which can be used will depend on the wavelength of the lamp(s) or laser(s) used to excite the fluorophore and on the detectors available.

To evaluate the competition between two test binding agents (termed A and B) for binding to Kv1.3, a dilution series of cold (without any label) binding agent A is added to (e.g. 200 000) cells together with the labeled binding agent B*. The concentration of binding agent B* in the test mix should be high enough to readily saturate the binding sites on Kv1.3 expressed on the cells. The concentration of binding agent B that saturates the binding sites for that binding agent on Kv1.3 expressed on the cells can be determined with a titration series of binding agent B* on the Kv1.3 cells and determination of the EC_{50} value for binding. In order to work at saturating concentration, binding agent B* can be used at 100x the EC_{50} concentration.

After incubation of the cells with the mixture of binding agent A and binding agent B* and cells wash, read out can be performed on a FACS. First a gate is set on the intact cells as determined from the scatter profile and the total amount of channel fluorescence is recorded.

A separate solution of binding agent B* is also prepared. Binding agent B* in this solutions should be in the same buffer and at the same concentration as in the test mix (with binding agent A and B*). This separate solution is also added to the cells. After incubation and cells wash, read out can be performed on a FACS. First a gate is set on the intact cells as determined from the scatter profile and the total amount of channel fluorescence is recorded.

A reduction of fluorescence for the cells incubated with the mixture of binding agent A and B* compared to the fluorescence for the cells incubated with the separate solution of binding agent B* indicates that binding agent A (cross)-blocks binding by binding agent B* for binding to Kv1.3 expressed on the cells.

A cross-blocking immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent according to the invention is one which will bind to the Kv1.3 in the above FACS cross-blocking assay such that during the assay and in the presence of a second immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent the recorded fluorescence is between 80% and 0.1% (e.g. 80% to 4%) of the maximum fluorescence (measured for the separate labelled immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent), specifically between 75% and 0.1% (e.g. 75% to 4%) of the maximum fluorescence, and more specifically between 70% and 0.1% (e.g. 70% to 4%) of maximum fluorescence (as just defined above).

The competition between two test binding agents (termed A* and B*) for binding to Kv1.3 can also be evaluated by adding both binding agents, each labeled with a different fluorophore, to the Kv1.3 expressing cells. After incubation and cells wash, read out can be performed on a FACS. A gate is set for each fluorophore and the total amount of channel fluorescence is recorded. Reduction and/or absence of fluorescence of one of the fluorophore indicate (cross)-blocking by the binding agents for binding to Kv1.3 expressed on the cells.

Other methods for determining whether an immunoglobulin, antibody, immunoglobulin single variable domain, polypeptide or other binding agent directed against a target (cross)-blocks, is capable of (cross)-blocking, competitively binds or is (cross)-competitive as defined herein are described e.g. in Xiao-Chi Jia et al. (Journal of Immunological Methods 288: 91–98, 2004), Miller et al. (Journal of Immunological Methods 365: 118–125, 2011) and/or the methods described herein (see e.g. Example 7).

An amino acid sequence is said to be "cross-reactive" for two different antigens or antigenic determinants (such as *e.g.,* serum albumin from two different species of mammal, such as *e.g.,* human serum albumin and cyno serum albumin, such as *e.g.,* Kv1.3 from different species of mammal, such as e.g., human Kv1.3, cyno Kv1.3 and rat Kv1.3) if it is specific for (as defined herein) these different antigens or antigenic determinants.

In the context of the present invention, "modulating" or "to modulate" generally means reducing or inhibiting the activity of Kv1.3, as measured using a suitable in vitro, cellular or in vivo assay (such as those mentioned herein). In particular, "modulating" or "to modulate" may mean either reducing or inhibiting the activity of, or alternatively increasing the activity of Kv1.3, as measured using a suitable in vitro, cellular or in vivo assay (such as those mentioned herein), by

at least 1%, preferably at least 5%, such as at least 10% or at least 25%, for example by at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to activity of Kv1.3 in the same assay under the same conditions but without the presence of the immunoglobulin or polypeptide of the invention.

"Modulating" may also mean effecting a change with respect to one or more biological or physiological mechanisms, effects, responses, functions, pathways or activities in which Kv1.3 (or in which its substrate(s), ligand(s) or pathway(s) are involved, such as its signalling pathway or metabolic pathway and their associated biological or physiological effects) is involved. Again, as will be clear to the skilled person, such an action may be determined in any suitable manner and/or using any suitable (in vitro and usually cellular or in vivo assay) assay known per se, such as the assays described herein or in the prior art cited herein. In particular, an action may be such that an intended biological or physiological activity is increased or decreased, respectively, by at least 1%, preferably at least 5%, such as at least 10% or at least 25%, for example by at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to the biological or physiological activity in the same assay under the same conditions but without the presence of the immunoglobulin or polypeptide of the invention.

Modulating may for example involve reducing and/or inhibiting the efflux of potassium ions from T-cells. Modulation may involve the reduction and/or inhibition of T-cell activation and/or proliferation. Modulation may involve the reduction, inhibition and/or suppression of (unwanted) immune responses.

The term "allosteric modulation", "allosteric modulating", "allosteric modulator" as used in the present invention refers to the indirect modulation of the activity of Kv1.3. Allosteric modulators do not physically block the Kv1.3 channel, but rather bind at a site in Kv1.3 that is not directly involved in the activity of Kv1.3. Usually an allosteric modulator induces a conformational change within the protein structure of Kv1.3, which may ultimately also impose a structural stress on the pore channel. This may in its turn result in the blocking of the pore, the ion channel to adopt a non-functional state (resting or inactivated state) and/or maintain the ion channel in a non-functional state.

The term "potency" of a polypeptide of the invention, as used herein, is a function of the amount of polypeptide of the invention required for its specific effect to occur. It is measured simply as the inverse of the IC_{50} for that polypeptide. It refers to the capacity of said polypeptide of

the invention to modulate and/or partially or fully inhibit the function of Kv1.3. More particularly, it may refer to the capacity of said polypeptide to reduce or even totally inhibit the efflux of potassium ions from T-cells. As such, it may refer to the capacity of said polypeptide to inhibit proliferation of T-cells and/or suppress activation of T-cells resulting in the inhibition of certain immune responses in vivo.

The potency may be measured by any suitable assay known in the art or described herein. Without being limiting, various ion channel screening technologies are described e.g. by Dabrowski et al. (CNS & Neurological Disorders Drug Targets 7: 122, 2008), Lü and An (Comb. Chem. High Throughput Screen. 11:185-94, 2008), and Zheng et al. (Assay Drug Dev. Technol. 2: 543-52, 2004). Potency assays include (without being limiting) ion flux assays (Hanson et al. Br. J. Pharmacol. 126: 1707-16, 1999; Wang et al. Assay Drug Dev. Technol. 2: 525-34, 2004; Weaver et al. J. Biomol. Screen. 9: 671-7, 2004), radioligand binding studies (Felix et al. Biochemistry 38: 4922-30, 1999; Knaus et al. Biochemistry 34: 13627-13634, 1995; Helms et al. Biochemistry. 36: 3737-44, 1997), fluorescent dye assays, electrophysiology, such as voltage clamp (Huxley, Trends Neurosci. 25: 553-8, 2002), and in particular, the patch-clamp (Hamill et al. Pflügers Archiv European Journal of Physiology 391: 85–100, 1981) or high throughput versions thereof (Southan and Clark, Methods Mol. Biol. 565: 187-208, 2009), including PatchXpress (Molecular Devices; Ghetti et al. Methods Mol. Biol. 403: 59-69, 2007), Qpatch and Qpatch HTX (Sophion; Mathes et al. Comb. Chem. High Throughput Screen. 12: 78-95, 2009; Korsgaard et al. Comb. Chem. High Throughput Screen. 12: 51-63, 2009), PatchLiner (Nanion; Farre et al. Comb. Chem. High Throughput Screen 12: 24-37, 2009), IonWorks® HT, IonWorks® Quattro and IonFlux™ Systems (Molecular Devices; Jow et al. J Biomol. Screen. 12: 1059-67, 2007; Dale et al. Mol. Biosyst. 3: 714-22, 2007), T-cell activation assays (Nguyten et al. Molecular Pharmacology 50: 1672-1679, 1996; Hanson et al. Br. J. Pharmacol. 126: 1707-1716, 1999) and/or in vivo assays, such as Diabetes-prone Biobreeding Worchester rats (Beeton et al. Proc Natl Acad Sci U S A. 103: 17414-9, 2006), a rat model for allergic contact dermatitis (Azam et al. J. Invest. Dermatol. 127: 1419-29, 2007), and the animal model for T cell-mediated skin graft rejection (Ren et al. PLoS One 3:e4009, 2008).

In contrast, the "efficacy" of the polypeptide of the invention measures the maximum strength of the effect itself, at saturating polypeptide concentrations. Efficacy indicates the maximum response achievable from the polypeptide of the invention. It refers to the ability of a polypeptide to produce the desired (therapeutic) effect.

The "half-life" of a polypeptide of the invention can generally be defined as described in paragraph o) on page 57 of WO 08/020079 and as mentioned therein refers to the time taken for the serum concentration of the polypeptide to be reduced by 50%, *in vivo*, for example due to degradation of the polypeptide and/or clearance or sequestration of the polypeptide by natural mechanisms. The in vivo half-life of a polypeptide of the invention can be determined in any manner known per se, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally be as described in paragraph o) on page 57 of WO 08/020079. As also mentioned in paragraph o) on page 57 of WO 08/020079, the half-life can be expressed using parameters such as the t1/2-alpha, t1/2-beta and the area under the curve (AUC). Reference is for example made to the standard handbooks, such as Kenneth et al (Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists, John Wiley & Sons Inc, 1986) and M Gibaldi and D Perron ("Pharmacokinetics", Marcel Dekker, 2nd Rev. Edition, 1982). The terms "increase in half-life" or "increased half-life" are also as defined in paragraph o) on page 57 of WO 08/020079 and in particular refer to an increase in the t1/2-beta, either with or without an increase in the t1/2-alpha and/or the AUC or both.

Unless indicated otherwise, the terms "immunoglobulin" and "immunoglobulin sequence" - whether used herein to refer to a heavy chain antibody or to a conventional 4-chain antibody - is used as a general term to include both the full-size antibody, the individual chains thereof, as well as all parts, domains or fragments thereof (including but not limited to antigen-binding domains or fragments such as V_{HH} domains or V_H/V_L domains, respectively).

The term "domain" (of a polypeptide or protein) as used herein refers to a folded protein structure which has the ability to retain its tertiary structure independently of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins, and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain.

The term "immunoglobulin domain" as used herein refers to a globular region of an antibody chain (such as *e.g.,* a chain of a conventional 4-chain antibody or of a heavy chain antibody), or to a polypeptide that essentially consists of such a globular region. Immunoglobulin domains are characterized in that they retain the immunoglobulin fold characteristic of antibody molecules, which consists of a two-layer sandwich of about seven antiparallel beta-strands arranged in two beta-sheets, optionally stabilized by a conserved disulphide bond.

The term "immunoglobulin variable domain" as used herein means an immunoglobulin domain essentially consisting of four "framework regions" which are referred to in the art and herein below as "framework region 1" or "FR1"; as "framework region 2" or "FR2"; as "framework region 3" or "FR3"; and as "framework region 4" or "FR4", respectively; which framework regions are interrupted by three "complementarity determining regions" or "CDRs", which are referred to in the art and herein below as "complementarity determining region 1" or "CDR1"; as "complementarity determining region 2" or "CDR2"; and as "complementarity determining region 3" or "CDR3", respectively. Thus, the general structure or sequence of an immunoglobulin variable domain can be indicated as follows: FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4. It is the immunoglobulin variable domain(s) that confer specificity to an antibody for the antigen by carrying the antigen-binding site.

The term "immunoglobulin single variable domain", interchangeably used with "single variable domain", defines molecules wherein the antigen binding site is present on, and formed by, a single immunoglobulin domain. This sets immunoglobulin single variable domains apart from "conventional" immunoglobulins or their fragments, wherein two immunoglobulin domains, in particular two variable domains, interact to form an antigen binding site. Typically, in conventional immunoglobulins, a heavy chain variable domain (VH) and a light chain variable domain (VL) interact to form an antigen binding site. In this case, the complementarity determining regions (CDRs) of both VH and VL will contribute to the antigen binding site, i.e. a total of 6 CDRs will be involved in antigen binding site formation.

In view of the above definition, the antigen-binding domain of a conventional 4-chain antibody (such as an IgG, IgM, IgA, IgD or IgE molecule; known in the art) or of a Fab fragment, a F(ab')2 fragment, an Fv fragment such as a disulphide linked Fv or a scFv fragment, or a diabody (all known in the art) derived from such conventional 4-chain antibody, would normally not be regarded as an immunoglobulin single variable domain, as, in these cases, binding to the respective epitope of an antigen would normally not occur by one (single) immunoglobulin domain but by a pair of (associating) immunoglobulin domains such as light and heavy chain variable domains, *i.e.*, by a VH-VL pair of immunoglobulin domains, which jointly bind to an epitope of the respective antigen.

In contrast, immunoglobulin single variable domains are capable of specifically binding to an epitope of the antigen without pairing with an additional immunoglobulin variable domain.

The binding site of an immunoglobulin single variable domain is formed by a single VH/VHH or VL domain. Hence, the antigen binding site of an immunoglobulin single variable domain is formed by no more than three CDRs.

As such, the single variable domain may be a light chain variable domain sequence (*e.g.,* a VL-sequence) or a suitable fragment thereof; or a heavy chain variable domain sequence (*e.g.,* a VH-sequence or VHH sequence) or a suitable fragment thereof; as long as it is capable of forming a single antigen binding unit (*i.e.,* a functional antigen binding unit that essentially consists of the single variable domain, such that the single antigen binding domain does not need to interact with another variable domain to form a functional antigen binding unit).

In one embodiment of the invention, the immunoglobulin single variable domains are heavy chain variable domain sequences (*e.g.,* a VH-sequence); more specifically, the immunoglobulin single variable domains can be heavy chain variable domain sequences that are derived from a conventional four-chain antibody or heavy chain variable domain sequences that are derived from a heavy chain antibody.

For example, the immunoglobulin single variable domain may be a (single) domain antibody (or an amino acid that is suitable for use as a (single) domain antibody), a "dAb" or dAb (or an amino acid that is suitable for use as a dAb) or a Nanobody (as defined herein, and including but not limited to a VHH); other single variable domains, or any suitable fragment of any one thereof.

In particular, the immunoglobulin single variable domain may be a Nanobody® (as defined herein) or a suitable fragment thereof. [Note: Nanobody®, Nanobodies® and Nanoclone® are registered trademarks of Ablynx N.V.] For a general description of Nanobodies, reference is made to the further description below, as well as to the prior art cited herein, such as e.g. described in WO 08/020079 (page 16).

"VHH domains", also known as VHHs, V_HH domains, VHH antibody fragments, and VHH antibodies, have originally been described as the antigen binding immunoglobulin (variable) domain of "heavy chain antibodies" (*i.e.,* of "antibodies devoid of light chains"; Hamers-Casterman et al. Nature 363: 446-448, 1993). The term "VHH domain" has been chosen in order to distinguish these variable domains from the heavy chain variable domains that are present in conventional 4-chain antibodies (which are referred to herein as "V_H domains" or "VH domains") and from the light chain variable domains that are present in conventional 4-chain antibodies (which are

referred to herein as "V_L domains" or "VL domains"). For a further description of VHH's and Nanobodies, reference is made to the review article by Muyldermans (Reviews in Molecular Biotechnology 74: 277-302, 2001), as well as to the following patent applications, which are mentioned as general background art: WO 94/04678, WO 95/04079 and WO 96/34103 of the Vrije Universiteit Brussel; WO 94/25591, WO 99/37681, WO 00/40968, WO 00/43507, WO 00/65057, WO 01/40310, WO 01/44301, EP 1134231 and WO 02/48193 of Unilever; WO 97/49805, WO 01/21817, WO 03/035694, WO 03/054016 and WO 03/055527 of the Vlaams Instituut voor Biotechnologie (VIB); WO 03/050531 of Algonomics N.V. and Ablynx N.V.; WO 01/90190 by the National Research Council of Canada; WO 03/025020 (= EP 1433793) by the Institute of Antibodies; as well as WO 04/041867, WO 04/041862, WO 04/041865, WO 04/041863, WO 04/062551, WO 05/044858, WO 06/40153, WO 06/079372, WO 06/122786, WO 06/122787 and WO 06/122825, by Ablynx N.V. and the further published patent applications by Ablynx N.V. Reference is also made to the further prior art mentioned in these applications, and in particular to the list of references mentioned on pages 41-43 of the International application WO 06/040153, which list and references are incorporated herein by reference. As described in these references, Nanobodies (in particular VHH sequences and partially humanized Nanobodies) can in particular be characterized by the presence of one or more "Hallmark residues" in one or more of the framework sequences. A further description of the Nanobodies, including humanization and/or camelization of Nanobodies, as well as other modifications, parts or fragments, derivatives or "Nanobody fusions", multivalent constructs (including some non-limiting examples of linker sequences) and different modifications to increase the half-life of the Nanobodies and their preparations can be found e.g. in WO 08/101985 and WO 08/142164. For a further general description of Nanobodies, reference is made to the prior art cited herein, such as *e.g.,* described in WO 08/020079 (page 16).

"Domain antibodies", also known as "Dab"s, "Domain Antibodies", and "dAbs" (the terms "Domain Antibodies" and "dAbs" being used as trademarks by the GlaxoSmithKline group of companies) have been described in *e.g.,* EP 0368684, Ward et al. (Nature 341: 544-546, 1989), Holt et al. (Tends in Biotechnology 21: 484-490, 2003) and WO 03/002609 as well as for example WO 04/068820, WO 06/030220, WO 06/003388 and other published patent applications of Domantis Ltd. Domain antibodies essentially correspond to the VH or VL domains of non-camelid mammalians, in particular human 4-chain antibodies. In order to bind an epitope as a single

antigen binding domain, *i.e.,* without being paired with a VL or VH domain, respectively, specific selection for such antigen binding properties is required, e.g. by using libraries of human single VH or VL domain sequences. Domain antibodies have, like VHHs, a molecular weight of approximately 13 to approximately 16 kDa and, if derived from fully human sequences, do not require humanization for e.g. therapeutical use in humans.

It should also be noted that, although less preferred in the context of the present invention because they are not of mammalian origin, single variable domains can be derived from certain species of shark (for example, the so-called "IgNAR domains", see for example WO 05/18629).

Thus, in the meaning of the present invention, the term "immunoglobulin single variable domain" or "single variable domain" comprises polypeptides which are derived from a non-human source, preferably a camelid, preferably a camelid heavy chain antibody. They may be humanized, as previously described. Moreover, the term comprises polypeptides derived from non-camelid sources, e.g. mouse or human, which have been "camelized", as *e.g.,* described in Davies and Riechmann (FEBS 339: 285-290, 1994; Biotechnol. 13: 475-479, 1995; Prot. Eng. 9: 531-537, 1996) and Riechmann and Muyldermans (J. Immunol. Methods 231: 25-38, 1999).

The amino acid residues of a VHH domain are numbered according to the general numbering for V_H domains given by Kabat et al. ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, MD, Publication No. 91), as applied to VHH domains from Camelids, as shown *e.g.,* in Figure 2 of Riechmann and Muyldermans (J. Immunol. Methods 231: 25-38, 1999). Alternative methods for numbering the amino acid residues of V_H domains, which methods can also be applied in an analogous manner to VHH domains, are known in the art. However, in the present description, claims and figures, the numbering according to Kabat applied to VHH domains as described above will be followed, unless indicated otherwise.

It should be noted that - as is well known in the art for V_H domains and for VHH domains - the total number of amino acid residues in each of the CDRs may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering). This means that, generally, the numbering according to Kabat may or may not correspond to the actual numbering of the amino acid residues in the actual sequence.

The total number of amino acid residues in a VH domain and a VHH domain will usually be in the range of from 110 to 120, often between 112 and 115. It should however be noted that smaller and longer sequences may also be suitable for the purposes described herein.

Determination of CDR regions may also be done according to different methods. In the CDR determination according to Kabat, FR1 of a VHH comprises the amino acid residues at positions 1-30, CDR1 of a VHH comprises the amino acid residues at positions 31-35, FR2 of a VHH comprises the amino acids at positions 36-49, CDR2 of a VHH comprises the amino acid residues at positions 50-65, FR3 of a VHH comprises the amino acid residues at positions 66-94, CDR3 of a VHH comprises the amino acid residues at positions 95-102, and FR4 of a VHH comprises the amino acid residues at positions 103-113.

In the present application, however, CDR sequences were determined according to Kontermann and Dübel (Eds., Antibody Engineering, vol 2, Springer Verlag Heidelberg Berlin, Martin, Chapter 3, pp. 33-51, 2010). According to this method, FR1 comprises the amino acid residues at positions 1-25, CDR1 comprises the amino acid residues at positions 26-35, FR2 comprises the amino acids at positions 36-49, CDR2 comprises the amino acid residues at positions 50-58, FR3 comprises the amino acid residues at positions 59-94, CDR3 comprises the amino acid residues at positions 95-102, and FR4 comprises the amino acid residues at positions 103-113.

Immunoglobulin single variable domains such as Domain antibodies and Nanobodies (including VHH domains) can be subjected to humanization. In particular, humanized immunoglobulin single variable domains, such as Nanobodies (including VHH domains) may be immunoglobulin single variable domains that are as generally defined for in the previous paragraphs, but in which at least one amino acid residue is present (and in particular, in at least one of the framework residues) that is and/or that corresponds to a humanizing substitution (as defined herein). Potentially useful humanizing substitutions can be ascertained by comparing the sequence of the framework regions of a naturally occurring V_{HH} sequence with the corresponding framework sequence of one or more closely related human V_H sequences, after which one or more of the potentially useful humanizing substitutions (or combinations thereof) thus determined can be introduced into said V_{HH} sequence (in any manner known per se, as further described herein) and the resulting humanized V_{HH} sequences can be tested for affinity for the target, for stability, for ease and level of expression, and/or for other desired properties. In this way, by means of a limited degree of trial and error, other suitable humanizing substitutions (or suitable combinations

thereof) can be determined by the skilled person based on the disclosure herein. Also, based on the foregoing, (the framework regions of) an immunoglobulin single variable domain, such as a Nanobody (including VHH domains) may be partially humanized or fully humanized.

Immunoglobulin single variable domains such as Domain antibodies and Nanobodies (including VHH domains and humanized VHH domains), can also be subjected to affinity maturation by introducing one or more alterations in the amino acid sequence of one or more CDRs, which alterations result in an improved affinity of the resulting immunoglobulin single variable domain for its respective antigen, as compared to the respective parent molecule. Affinity-matured immunoglobulin single variable domain molecules of the invention may be prepared by methods known in the art, for example, as described by Marks et al. (Biotechnology 10:779-783, 1992), Barbas, et al. (Proc. Nat. Acad. Sci, USA 91: 3809-3813, 1994), Shier et al. (Gene 169: 147-155, 1995), Yelton et al. (Immunol. 155: 1994-2004, 1995), Jackson et al. (J. Immunol. 154: 3310-9, 1995), Hawkins et al. (J. Mol. Biol. 226: 889 896, 1992), Johnson and Hawkins (Affinity maturation of antibodies using phage display, Oxford University Press, 1996).

The process of designing/selecting and/or preparing a polypeptide, starting from an immunoglobulin single variable domain such as a Domain antibody or a Nanobody, is also referred to herein as "formatting" said immunoglobulin single variable domain; and an immunoglobulin single variable domain that is made part of a polypeptide is said to be "formatted" or to be "in the format of" said polypeptide. Examples of ways in which an immunoglobulin single variable domain can be formatted and examples of such formats will be clear to the skilled person based on the disclosure herein; and such formatted immunoglobulin single variable domain form a further aspect of the invention.

For example, and without limitation, one or more immunoglobulin single variable domains may be used as a "binding unit", "binding domain" or "building block" (these terms are used interchangeable) for the preparation of a polypeptide, which may optionally contain one or more further immunoglobulin single variable domains that can serve as a binding unit (*i.e.,* against the same or another epitope on Kv1.3 and/or against one or more other antigens, proteins or targets than Kv1.3).

Monovalent polypeptides comprise or essentially consist of only one binding unit (such as *e.g.,* immunoglobulin single variable domains). Polypeptides that comprise two or more binding units (such as *e.g.,* immunoglobulin single variable domains) will also be referred to herein as

"multivalent" polypeptides, and the binding units/immunoglobulin single variable domains present in such polypeptides will also be referred to herein as being in a "multivalent format". For example a "bivalent" polypeptide may comprise two immunoglobulin single variable domains, optionally linked via a linker sequence, whereas a "trivalent" polypeptide may comprise three immunoglobulin single variable domains, optionally linked via two linker sequences; whereas a "tetravalent" polypeptide may comprise four immunoglobulin single variable domains, optionally linked via three linker sequences, etc..

In a multivalent polypeptide, the two or more immunoglobulin single variable domains may be the same or different, and may be directed against the same antigen or antigenic determinant (for example against the same part(s) or epitope(s) or against different parts or epitopes) or may alternatively be directed against different antigens or antigenic determinants; or any suitable combination thereof. Polypeptides that contain at least two binding units (such as *e.g.,* immunoglobulin single variable domains) in which at least one binding unit is directed against a first antigen (*i.e.,* Kv1.3) and at least one binding unit is directed against a second antigen (*i.e.,* different from Kv1.3) will also be referred to as "multispecific" polypeptides, and the binding units (such as *e.g.,* immunoglobulin single variable domains) present in such polypeptides will also be referred to herein as being in a "multispecific format". Thus, for example, a "bispecific" polypeptide of the invention is a polypeptide that comprises at least one immunoglobulin single variable domain directed against a first antigen (*i.e.,* Kv1.3) and at least one further immunoglobulin single variable domain directed against a second antigen (*i.e.,* different from Kv1.3), whereas a "trispecific" polypeptide of the invention is a polypeptide that comprises at least one immunoglobulin single variable domain directed against a first antigen (*i.e.,* Kv1.3), at least one further immunoglobulin single variable domain directed against a second antigen (*i.e.,* different from Kv1.3) and at least one further immunoglobulin single variable domain directed against a third antigen (*i.e.,* different from both Kv1.3 and the second antigen); etc.

"Multiparatopic polypeptides", such as *e.g.,*" biparatopic polypeptides" or "triparatopic polypeptides", comprise or essentially consist of two or more binding units that each have a different paratope (as will be further described herein; see chapter on multivalent polypeptides of the invention).

<u>Immunoglobulins that bind extracellular loop EL1 of voltage gated Kv1.3 potassium channel</u>

The present invention provides immunoglobulins (also referred to herein as "immunoglobulins of the invention") and/or polypeptides (also referred to herein as "polypeptides of the invention") that have specificity for and/or that bind Kv1.3, preferably human Kv1.3. Kv1.3 also known as KCNA3; MK3; HGK5; HLK3; PCN3; HPCN3; HUKIII, is a protein that, in humans, is encoded by the KCNA3 gene (Accession No. P22001, human KCNA3), which is located at chromosome 1p13.3. Thus, the immunoglobulins and/or polypeptides of the invention preferably bind to human Kv1.3 (SEQ ID NO: 474).

In one aspect of the invention, the immunoglobulins and/or polypeptides of the present invention bind to the the first extracellular loop EL1 of Kv1.3. The amino acid sequence of extracellular loop EL1 starts after the transmembrane region S1 and ends at S2. More specifically, the extracellular loop EL1 of Kv1.3 spans position 254 to position 294 of SEQ ID NO: 474.

The present inventors surprisingly observed that the immunoglobulins and/or polypeptides of the invention that bind this part of Kv1.3 exhibited different modulating activities on Kv1.3, such as partial or full blocking of Kv1.3, inhibition of T-cell activation and/or proliferation and/or suppression of (unwanted) immune reaction in vivo. In addition, these immunoglobulins showed highly improved interspecies cross-reactivity and exquisite selectivity properties.

Accordingly, the present invention relates to an immunoglobulin and/or polypeptide that specifically binds to the EL1 extracellular loop of potassium channel 3 (Kv1.3), and wherein the binding of said immunoglobin to said EL1 extracellular loop modulates and/or inhibits the activity of Kv1.3. As the pore channel of Kv1.3 is made up by the extracellular region EL3 of Kv1.3, the finding of immunoglobulins that bind EL1 and still modulate, inhibit and/or block Kv1.3 activity (i.e. without physical interaction with and/or blocking of EL3) was unexpected.

Preferred immunoglobulins and/or polypeptides of the invention include immunoglobulins (such as heavy chain antibodies, conventional 4-chain antibodies (such as IgG, IgM, IgA, IgD or IgE molecules), Fab fragments, F(ab')2 fragments, Fv fragments such as disulphide linked Fv or scFv fragments, or diabodies derived from such conventional 4-chain antibody, the individual chains thereof, as well as all parts, domains or fragments thereof (including but not limited to antigen-binding domains or fragments such as immunoglobulin single variable domains), monovalent polypeptides of the invention, or other binding agents).

Binding of the immunoglobulins and/or polypeptides of the invention to Kv1.3 can be measured in binding assays that preserve that conformation of the Kv1.3 target. Typical assays

include (without being limiting) assays in which Kv1.3 is exposed on a cell surface (such as e.g. CHO cells (e.g. CHO-K1), HEK cells, HeLa cells, Chinese Hamster Lung (CHL) cells, Caki cells etc.). A preferred assay for measuring binding of the immunoglobulins and/or polypeptides of the invention to Kv1.3 is a FACS assay, such as e.g. the FACS assay as described in the examples, wherein binding of the immunoglobulins and/or polypeptides of the invention to Kv1.3 expressed on CHO-K1 cells and/or HEK293H cells is determined. Some preferred EC50 values for binding of the immunoglobulins and/or polypeptides of the invention to Kv1.3 will become clear from the further description and examples herein.

In such FACS binding assay, the immunoglobulins and/or polypeptides of the present invention may have EC50 values in binding human Kv1.3 of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such FACS binding assay, the immunoglobulins and/or polypeptides of the present invention may have EC50 values in binding human Kv1.3 between 10^{-10} M and 10^{-8} M, such as between 10^{-9} M and 10^{-8} M or between 10^{-10} M and 10^{-9} M.

In such FACS binding assay, the immunoglobulins and/or polypeptides of the present invention may have EC50 values in binding cyno Kv1.3 of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such FACS binding assay, the polypeptides of the present invention may have EC50 values in binding cyno Kv1.3 between 10^{-10} M and 10^{-7} M, such as between 10^{-10} M and 10^{-8} M, between 10^{-10} M and 10^{-9} M.

In such FACS binding assay, the immunoglobulins and/or polypeptides of the present invention may have EC50 values in binding rat Kv1.3 of 10^{-6} M or lower, preferably of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such FACS binding assay, the polypeptides of the present invention may have EC50 values in binding rat Kv1.3 between 10^{-10} M and 10^{-6} M, such as between 10^{-10} M and 10^{-7} M, between 10^{-10} M and 10^{-8} M, between 10^{-10} M and 10^{-9} M.

The immunoglobulins and/or polypeptides of the invention bind EL1 of Kv1.3 and modulate and/or (partially or fully) inhibit the function of Kv1.3. More particularly, the immunoglobulins and/or polypeptides of the present invention may depolarize the T cell membrane and/or reduce or even totally inhibit the efflux of potassium ions from T-cells. As such, the immunoglobulins and/or polypeptides of the invention may partially or fully inhibit

proliferation of T-cells and/or suppress activation of T-cells resulting of the inhibition of certain immune responses in vivo.

More particularly, the immunoglobulins and/or polypeptides of the invention may indirectly modulate the function of Kv1.3, i.e. as an allosteric modulator (as defined herein). For example, the immunoglobulins and/or polypeptides of the invention may induce a conformational change within the structure of the Kv1.3 pore channel.

Accordingly, in one aspect, the present invention relates to an immunoglobulin and/or polypeptide that specifically binds to the EL1 extracellular loop of Kv1.3, and wherein the binding of said immunoglobulin and/or polypeptide to said EL1 extracellular loop modulates and/or (partially or fully) inhibits the activity of Kv1.3 by allosteric modulation of the activity of Kv1.3. More particularly, the immunoglobulins and/or polypeptides of the present invention may allosterically depolarize the T cell membrane and/or reduce or even totally inhibit the efflux of potassium ions from T-cells. As such, the immunoglobulins and/or polypeptides of the invention may inhibit proliferation of T-cells and/or suppress activation of T-cells resulting of the inhibition of certain immune responses in vivo.

Modulation and/or inhibition of the efflux of potassium ions can be determined by a variety of ion channel screening technologies including (without being limiting) ion flux assays, radioligand binding studies, fluorescent dye assays, and electrophysiology, such as voltage clamp, and in particular, the patch-clamp. An overview of different ion channel technologies is provided by e.g. Dabrowski et al. (CNS & Neurological Disorders Drug Targets 7: 122, 2008), Lü and An (Comb. Chem. High Throughput Screen. 11:185-94, 2008), and Zheng et al. (Assay Drug Dev. Technol. 2: 543-52, 2004).

Voltage clamp (Huxley, Trends Neurosci. 25: 553-8, 2002) is used to measure the ion currents through the membrane of excitable cells. The patch-clamp variant of this technique (Hamill et al. Pflügers Archiv European Journal of Physiology 391: 85–100, 1981) allows the study of single or multiple ion channels in cells.

Higher throughput electrophysiological platforms have been developed ranging from medium throughput systems to higher throughput platforms (see e.g. Southan and Clark, Methods Mol. Biol. 565: 187-208, 2009), including PatchXpress (Molecular Devices; Ghetti et al. Methods Mol. Biol. 403: 59-69, 2007), Qpatch and Qpatch HTX (Sophion; Mathes et al. Comb. Chem. High Throughput Screen. 12: 78-95, 2009; Korsgaard et al. Comb. Chem. High Throughput Screen. 12:

51-63, 2009), PatchLiner (Nanion; Farre et al. Comb. Chem. High Throughput Screen 12: 24-37, 2009), IonWorks® HT, IonWorks® Quattro and IonFlux™ Systems (Molecular Devices; Jow et al. J Biomol. Screen. 12: 1059-67, 2007; Dale et al. Mol. Biosyst. 3: 714-22, 2007). Some preferred IC50 values for the polypeptides of the invention in these assays will become clear from the further description and examples herein.

On the IonFlux™ (Molecular Devices) using Kv1.3-expressing HEK293H cells, for example, the immunoglobulins and/or polypeptides of the invention have IC50 values of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in this automated Patch Clamp assay, the polypeptides of the present invention may have IC50 values between 10^{-10} M and 10^{-7} M, between 10^{-10} M and 10^{-8} M, between 10^{-10} M and 10^{-9} M, such as e.g. between 10^{-9} M and 10^{-7} M, between 10^{-9} M and 10^{-8} M, between 10^{-8} M and 10^{-7} M or between 10^{-10} M and 10^{-9} M.

Accordingly, the present invention relates to an immunoglobulin and/or polypeptide that specifically binds to the EL1 extracellular loop of Kv1.3, and that modulates and/or inhibits the activity of Kv1.3 by (allosteric) modulation of the efflux of potassium ions, with a potency of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower, as measured on IonFlux™ (Molecular Devices).

On the IonWorks® Quattro (Molecular Devices) using Kv1.3-expressing Chinese Hamster Lung (CHL) cells, for example, the immunoglobulins and/or polypeptides of the invention have IC50 values of 10^{-} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, on this high-throughput planar perforated patch clamp, the polypeptides of the present invention may have IC50 values between 10^{-10} M and 10^{-7} M, between 10^{-10} M and 10^{-8} M, between 10^{-10} M and 10^{-9} M, such as e.g. between 10^{-8} M and 10^{-7} M, between 10^{-9} M and 10^{-7} M or between 10^{-10} M and 10^{-9} M.

Accordingly, the present invention relates to an immunoglobulin and/or polypeptide that specifically binds to the EL1 extracellular loop of Kv1.3, and wherein the binding of said immunoglobulin and/or polypeptide to said EL1 extracellular loop inhibits the activity of Kv1.3 by (allosteric) modulation of the efflux of potassium ions, with a potency of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower, as measured on IonWorks® Quattro (Molecular Devices).

Modulation and/or inhibition of Kv1.3 by the polypeptides of the invention can also be assessed in radioligand binding studies. Binding studies with tritiated correolide (e.g. C20-29-[3H]dihydrocorreolide (diTC)) to a single class of sites in membranes prepared from CHO/Kv1.3 cells has been described by Felix et al. (Biochemistry 38: 4922-30, 1999). Knaus et al. (Biochemistry 34: 13627-13634, 1995) describes, for example, the binding of monoiodotyrosinyl margatoxin (125I-margatoxin) to heterotetrameric Kv channels in rat brain synaptic plasma membranes. Binding studies of 125I-margatoxin to plasma membranes prepared from either Jurkat cells, a human leukemic T cell line, or CHO cells stably transfected with the Shaker-type voltage-gated K+ channel, K(V)1.3 have been described by Helms et al. (Biochemistry. 36: 3737-44, 1997). Some preferred IC50 values for blocking 125I-margatoxin binding to Kv1.3 by the polypeptides of the invention will become clear from the further description and examples herein.

The immunoglobulins and/or polypeptides of the present invention may block binding of 125I-margatoxin to cynomolgus Kv1.3 overexpressing CHO cells with IC50 values of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such 125I-margatoxin blocking assay, the immunoglobulins and/or polypeptides of the present invention may have IC50 values between 10^{-10} M and 10^{-8} M, between 10^{-10} M and 10^{-9} M, such as e.g. between 10^{-9} M and 10^{-8} M or between 10^{-10} M and 10^{-9} M.

Accordingly, the present invention relates to an immunoglobulin and/or polypeptide that specifically binds to the EL1 extracellular loop of Kv1.3, and wherein the binding of said immunoglobulin and/or polypeptide to said EL1 extracellular loop block binding of 125I-margatoxin to cynomolgus Kv1.3 overexpressing CHO cells with a potency of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower.

Other flux assays for measuring modulation and/or inhibition of Kv1.3 by the polypeptides of the invention include (without being limiting) the high-throughput efflux assay with radiolabelled^{86} Rubidium described by Hanson et al. (Br. J. Pharmacol. 126: 1707-16, 1999), the nonradioactive rubidium (Rb(+)) efflux assay described by Wang et al. (Assay Drug Dev. Technol. 2: 525-34, 2004) and a fluorescence-based thallium flux assay (Weaver et al. J. Biomol. Screen. 9: 671-7, 2004).

Inhibition of T-cell activation and/or proliferation by the polypeptides of the present invention can be measured in T-cell activation assays. Without being limiting, T-cell activation assays have been described by Nguyten et al. (Molecular Pharmacology 50: 1672-1679, 1996) and

Hanson et al. (Br. J. Pharmacol. 126: 1707–1716, 1999). Some preferred IC50 values for inhibition of T-cell activation and/or proliferation by the monovalent polypeptides of the invention will become clear from the further description and examples herein.

In a T-cell activation assay with CCR7$^-$ CD45RA$^-$ T cells stimulated with anti-CD3 antibody OKT3 (as described in the Examples 4.4 and 5.5), the immunoglobulins and/or polypeptides of the invention have IC50 values for inhibiting IFNgamma production of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, 10^{-10} M or lower, or even of 10^{-11} M or lower. For example, in this T-cell activation assay, the immunoglobulins and/or polypeptides of the present invention inhibit IFNgamma production with IC50 values between 10^{-11} M and 10^{-7} M, between 10^{-11} M and 10^{-8} M, between 10^{-11} M and 10^{-9} M, such as e.g. between 10^{-8} M and 10^{-7} M, between 10^{-11} M and 10^{-9} M, between 10^{-10} M and 10^{-9} M, or between 10^{-11} M and 10^{-10} M.

Accordingly, the present invention relates to an immunoglobulin and/or polypeptide that specifically binds to the EL1 extracellular loop of Kv1.3, and wherein the binding of said immunoglobulin and/or polypeptide to said EL1 extracellular loop inhibits IFNgamma production in T cells with a potency of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower, or even of 10^{-11} M or lower, as measured in a T-cell activation assay with CCR7$^-$CD45RA$^-$ T cells stimulated with anti-CD3 antibody OKT3 (as described in the Examples 4.4 and 5.5).

In this T-cell activation assay with CCR7$^-$CD45RA$^-$ T cells stimulated with anti-CD3 antibody OKT3 (as described in the Examples 4.4 and 5.5), the immunoglobulins and/or polypeptides of the invention have IC50 values for inhibiting CD25 upregulation of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, 10^{-10} M or lower, or even of 10^{-11} M or lower. For example, in this T-cell activation assay, the immunoglobulins and/or polypeptides of the present invention inhibit CD25 upregulation with IC50 values between 10^{-11} M and 10^{-7} M, between 10^{-11} M and 10^{-8} M, between 10^{-11} M and 10^{-9} M, such as e.g. between 10^{-8} M and 10^{-7} M, between 10^{-11} M and 10^{-9} M, between 10^{-10} M and 10^{-9} M or between 10^{-11} M and 10^{-10} M.

Accordingly, the present invention relates to an immunoglobulin and/or polypeptide that specifically binds to the EL1 extracellular loop of Kv1.3, and wherein the binding of said immunoglobulin and/or polypeptide to said EL1 extracellular loop inhibits CD25 upregulation in T cells with a potency of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or

lower, or even of 10^{-10} M or lower, or even of 10^{-11} M or lower, as measured in a T-cell activation assay with $CCR7^{-}CD45RA^{-}$ T cells stimulated with anti-CD3 antibody OKT3 (as described in the Examples 4.4 and 5.5).

In a cell activation assay with peripheral blood mononucleated cells (PBMCs) stimulated with anti-CD3 antibody OKT3 and anti-CD28 (as described in the Example 9), the polypeptides of the invention do not block IFNgamma production.

Accordingly, the present invention relates to an immunoglobulin and/or polypeptide that specifically binds to the EL1 extracellular loop of Kv1.3, and wherein the binding of said immunoglobulin and/or polypeptide to said EL1 extracellular does not block INFgamma production in a cell activation assay with peripheral blood mononucleated cells (PBMCs) stimulated with anti-CD3 antibody OKT3 and anti-CD28 antibody (as described in the Example 9).

Immunosuppressive effects of the polypeptides of the invention can further be evaluated in in vivo models, such as e.g. in rats, pigs and/or primates. Diabetes-prone Biobreeding Worchester rats have been used as a model for autoimmune diabetes (Beeton et al. Proc Natl Acad Sci U S A. 103: 17414-9, 2006). A rat model for allergic contact dermatitis, an animal model for psoriasis, has been described by Azam et al. (J. Invest. Dermatol. 127: 1419-29, 2007). Immunodeficient mice reconstituted with human T cells have been used as animal model for T cell-mediated skin graft rejection (Ren et al. PLoS One 3:e4009, 2008). For example, in the rat model for allergic contact dermatitis as described in Example 12 and 13, the polypeptides of the invention (significantly) reduce the increase in ear thickness with at least about 0.085-0.102 mm and at least about 0.147-0.164 mm versus vehicle, respectively.

Accordingly, the present invention relates to an immunoglobulin and/or polypeptide that specifically binds to the EL1 extracellular loop of Kv1.3, and wherein the binding of said immunoglobulin and/or polypeptide to said EL1 extracellular reduces the increase in ear thickness with at least about 0.085-0.102 mm and at least about 0.147-0.164 mm versus vehicle in a rat model for allergic contact dermatitis as described in Example 12 and Example 13, respectively.

The immunoglobulins and/or polypeptides that specifically bind to the EL1 extracellular loop of Kv1.3 show more than 1000 fold, and even up to 10000 fold selectivity for modulating and/or inhibiting the activity of Kv1.3 over other related Kv ion channel family members. The selective inhibition by the immunoglobulin and/or polypeptide of the present invention can be determined e.g. by comparing the concentration of immunoglobulin and/or polypeptide needed

for inhibiting the respective channel with the concentration of immunoglobulin and/or polypeptide needed for inhibiting Kv1.3. Ion channel family members include hERG, KCa3.1 (SK4), Kv4.3/KChIP2.2, Kv1.2, Kv1.4, Cav1.3/b3/a2d1, Kir2.1, KCa2.2, KCa2.3, Kv7.2/Kv7.3, Kv1.1, Kv1.5, Kv3.4, Nav1.1, Nav1.2 and Nav1.6.

More in particular the immunoglobulins and/or polypeptides show a more than 1000 fold, and even up to 10000 fold selectivity over Kv1.5, Kv1.6, and hERG.

Monovalent polypeptides of the invention

The present invention provides stretches of amino acid residues (SEQ ID NOs: 181-210, SEQ ID NOs: 268-289, SEQ ID NOs: 393-415 and SEQ ID NOs: 211-227, SEQ ID NOs: 290-309, SEQ ID NOs: 416-435; Table A-2) that are particularly suited for binding to the EL1 extracellular loop of Kv1.3. In particular, the invention provides stretches of amino acid residues which bind to the EL1 extracellular loop of human Kv1.3 and wherein the binding of said stretches to said EL1 extracellular loop inhibits the activity of Kv1.3 (as described above). These stretches of amino acid residues may be present in, and/or may be incorporated into, a polypeptide of the invention, in particular in such a way that they form (part of) the antigen binding site of the polypeptide of the invention. These stretches of amino acid residues have been generated as CDR sequences of heavy chain antibodies or V_{HH} sequences that were raised against Kv1.3. These stretches of amino acid residues are also referred to herein as "*CDR sequence(s) of the invention*" (*i.e.,* as "CDR1 sequence(s) of the invention", "CDR2 sequence(s) of the invention" and "CDR3 sequence(s) of the invention", respectively).

It should however be noted that the invention in its broadest sense is not limited to a specific structural role or function that these stretches of amino acid residues may have in a polypeptide of the invention, as long as these stretches of amino acid residues allow the polypeptide of the invention to bind to Kv1.3 with a certain affinity and potency (as defined herein). Thus, generally, the invention in its broadest sense provides monovalent polypeptides (also referred to herein as "*monovalent polypeptide(s) of the invention*") that are capable of binding to Kv1.3 with a certain specified affinity, avidity, efficacy and/or potency and that comprises one or more CDR sequences as described herein and, in particular a suitable combination of two or more such CDR sequences, that are suitably linked to each other via one or more further amino acid sequences, such that the entire polypeptide forms a binding domain

and/or binding unit that is capable of binding to Kv1.3. It should however also be noted that the presence of only one such CDR sequence in a monovalent polypeptide of the invention may by itself already be sufficient to provide the monovalent polypeptide of the invention the capacity of binding to Kv1.3; reference is for example again made to the so-called "Expedite fragments" described in WO 03/050531.

In a specific, but non-limiting aspect, the monovalent polypeptide of the invention, may comprise at least one stretch of amino acid residues that is chosen from the group consisting of:

i) CDR1 sequences:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182;

and/or

ii) CDR2 sequences:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269;

and/or

iii) CDR3 sequences:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397.

In a further aspect, the monovalent polypeptide of the invention, may comprise at least one stretch of amino acid residues that is chosen from the group consisting of:

i) CDR1 sequences:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 1 the G has been changed into L, or R;
- at position 2 the L has been changed into F, P, or I;
- at position 3 the L has been changed into P, or F;
- at position 4 the F has been changed into S, L, or I;

- at position 5 the S has been changed into I, or R;
- at position 6 the R has been changed into C, A, P, V, or L;
- at position 7 the N has been changed into H, P, I, M, Y, T or D:
- at position 8 the S has been changed into T, R, or I;
- at position 9 the A has been changed into V or T; and/or
- at position 10 the G has been changed into S, R, or V;

and/or

ii) CDR2 sequences:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 1 the R has been changed into G, or C;
- at position 2 the I has been changed into V, T, S or L;
- at position 3 the R has been changed into G, or L;
- at position 4 the M has been changed into S, R, or T;
- at position 5 the G has been changed into V, S, or T;
- at position 7 the S has been changed into G, C, D, or E; and/or
- at position 8 the I has been changed into T, M, or R;

and/or

iii) CDR3 sequences:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 1 the W has been changed into G;
- at position 3 the E has been changed into T, K, G, A, or I;
- at position 4 the G has been changed into E, or D;
- at position 5 the F has been changed into A, L, V, Y, T, or S;
- at position 6 the Y has been changed into F, or D:
- at position 7 the E has been changed into G, or K;
- at position 8 the Y has been changed into S or H; and/or
- at position 9 the W has been changed into S, G or C.

In a further aspect, the monovalent polypeptide of the invention, may comprise at least one stretch of amino acid residues that is chosen from the group consisting of:

i) CDR1 sequences:

a) SEQ ID NOs: 181-185; or

b) amino acid sequences that have 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 6 the R has been changed into A, or V; and/or
- at position 9 the A has been changed into V;

and/or

ii) CDR2 sequences:

c) SEQ ID NOs: 268-271; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 2 the I has been changed into L;
- at position 4 the M has been changed into S or T;
- at position 5 the G has been changed into S or T; and/or
- at position 8 the I has been changed into T;

and/or

iii) CDR3 sequences:

e) SEQ ID NOs: 393-398; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 3 the E has been changed into T or I;
- at position 4 the G has been changed into E;
- at position 5 the F has been changed into A; and/or
- at position 8 the Y has been changed into H.

In a further aspect, the monovalent polypeptide of the invention, may comprise at least one stretch of amino acid residues that is chosen from the group consisting of:

i) CDR1 sequences:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214;

and/or

ii) CDR2 sequences:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303;

and/or

iii) CDR3 sequences:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422.

In a further aspect, the monovalent polypeptide of the invention, may comprise at least one stretch of amino acid residues that is chosen from the group consisting of:

i) CDR1 sequences:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214, wherein

- at position 1 the G has been changed into R, A, V, S, or K;
- at position 3 the T has been changed into N;
- at position 4 the F has been changed into L;
- at position 6 the N has been changed into S;
- at position 7 the F has been changed into Y:
- at position 8 the G has been changed into A; and/or
- at position 9 the M has been changed into V;

and/or

ii) CDR2 sequences:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303, wherein

- at position 1 the A has been changed into T;

- at position 2 the I has been changed into V;
- at position 5 the T has been changed into S, or A;
- at position 6 the G has been changed into N, or A;
- at position 7 the G has been changed into S, or R;
- at position 8 the H has been changed into R, or Y;
- at position 9 the T has been changed into I, or K; and/or
- at position 10 the Y has been changed into F;

and/or

iii) CDR3 sequences:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422, wherein

- at position 4 the F has been changed into Y, or S;
- at position 5 the G has been changed into D;
- at position 6 the D has been changed into G;
- at position 7 the G has been changed into D;
- at position 8 the T has been changed into A:
- at position 9 the Y has been changed into S;
- at position 10 the Y has been changed into F;
- at position 12 the Q has been changed into E;
- at position 14 the A has been changed into N, T, I, or R; and/or
- at position 17 the D has been changed into N, or G.

In particular, a monovalent polypeptide of the invention may be a monovalent polypeptide that comprises one antigen binding site, wherein said antigen binding site comprises at least one stretch of amino acid residues that is chosen from the group consisting of the CDR1 sequences, CDR2 sequences and CDR3 sequences as described above (or any suitable combination thereof). In a preferred aspect, however, the monovalent polypeptide of the invention comprises more than one, such as two or more stretches of amino acid residues chosen from the group consisting of the CDR1 sequences of the invention, the CDR2 sequences of the invention and/or the CDR3 sequences of the invention. Preferably, the monovalent polypeptide of the invention comprises three stretches of amino acid residues chosen from the group consisting of the CDR1

sequences of the invention, the CDR2 sequences of the invention and the CDR3 sequences of the invention, respectively. The combinations of CDR's that are mentioned herein as being preferred for the monovalent polypeptides of the invention are listed in Table A-2.

It should be further noted that the invention is not limited as to the origin of the monovalent polypeptide of the invention (or of the nucleic acid of the invention used to express it), nor as to the way that the monovalent polypeptide or nucleic acid of the invention is (or has been) generated or obtained. Thus, the monovalent polypeptides of the invention may be naturally occurring monovalent polypeptides (from any suitable species) or synthetic or semi-synthetic monovalent polypeptides.

Furthermore, it will also be clear to the skilled person that it is possible to "graft" one or more of the CDR's mentioned above onto other "scaffolds", including but not limited to human scaffolds or non-immunoglobulin scaffolds. Suitable scaffolds and techniques for such CDR grafting will be clear to the skilled person and are well known in the art, see for example US 7,180,370, WO 01/27160, EP 0605522, EP 0460167, US 7,054,297, Nicaise et al. (Protein Science 13: 1882-1891, 2004), Ewert et al. (Methods 34: 184-199, 2004), Kettleborough et al. (Protein Eng. 4: 773-783, 1991), O'Brien and Jones (Methods Mol. Biol. 207: 81-100, 2003), Skerra (J. Mol. Recognit. 13: 167-187, 2000) and Saerens et al. (J. Mol. Biol. 352: 597-607, 2005) and the further references cited therein. For example, techniques known per se for grafting mouse or rat CDR's onto human frameworks and scaffolds can be used in an analogous manner to provide chimeric proteins comprising one or more of the CDR sequences defined herein for the monovalent polypeptides of the invention and one or more human framework regions or sequences. Suitable scaffolds for presenting amino acid sequences will be clear to the skilled person, and for example comprise, without limitation, to binding scaffolds based on or derived from immunoglobulins (i.e. other than the immunoglobulin sequences already described herein), protein scaffolds derived from protein A domains (such as Affibodies™), tendamistat, fibronectin, lipocalin, CTLA-4, T-cell receptors, designed ankyrin repeats, avimers and PDZ domains (Binz et al. Nat. Biotech., 23: 1257, 2005), and binding moieties based on DNA or RNA including but not limited to DNA or RNA aptamers (Ulrich et al. Comb. Chem. High Throughput Screen 9: 619-32, 2006).

In said monovalent polypeptides of the invention, the CDR's may be linked to further amino acid sequences and/or may be linked to each other via amino acid sequences, in which said

amino acid sequences are preferably framework sequences or are amino acid sequences that act as framework sequences, or together form a scaffold for presenting the CDR's.

According to a preferred, but non-limiting embodiment, the monovalent polypeptides of the invention comprise at least three CDR sequences linked to at least two framework sequences, in which preferably at least one of the three CDR sequences is a CDR3 sequence, with the other two CDR sequences being CDR1 or CDR2 sequences, and preferably being one CDR1 sequence and one CDR2 sequence. According to one specifically preferred, but non-limiting embodiment, the monovalent polypeptides of the invention have the structure FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, in which CDR1, CDR2 and CDR3 are as defined herein for the monovalent polypeptides of the invention, and FR1, FR2, FR3 and FR4 are framework sequences. In such a monovalent polypeptide of the invention, the framework sequences may be any suitable framework sequences, and examples of suitable framework sequences will be clear to the skilled person, for example on the basis the standard handbooks and the further disclosure and prior art mentioned herein.

Accordingly, a monovalent polypeptide of the present invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397.

In particular, according to this preferred but non-limiting aspect, a monovalent polypeptide of the present invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182;

and

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269;

and

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397.

In a further aspect, a monovalent polypeptide of the present invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 1 the G has been changed into L, or R;
- at position 2 the L has been changed into F, P, or I;
- at position 3 the L has been changed into P, or F;
- at position 4 the F has been changed into S, L, or I;
- at position 5 the S has been changed into I, or R;
- at position 6 the R has been changed into C, A, P, V, or L;
- at position 7 the N has been changed into H, P, I, M, Y, T or D:

- at position 8 the S has been changed into T, R, or I;
- at position 9 the A has been changed into V or T; and/or
- at position 10 the G has been changed into S, R, or V;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 1 the R has been changed into G, or C;
- at position 2 the I has been changed into V, T, S or L;
- at position 3 the R has been changed into G, or L;
- at position 4 the M has been changed into S, R, or T;
- at position 5 the G has been changed into V, S, or T;
- at position 7 the S has been changed into G, C, D, or E; and/or
- at position 8 the I has been changed into T, M, or R;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 1 the W has been changed into G;
- at position 3 the E has been changed into T, K, G, A, or I;
- at position 4 the G has been changed into E, or D;
- at position 5 the F has been changed into A, L, V, Y, T, or S;
- at position 6 the Y has been changed into F, or D:
- at position 7 the E has been changed into G, or K;
- at position 8 the Y has been changed into S or H; and/or
- at position 9 the W has been changed into S, G or C.

In particular, according to this preferred but non-limiting aspect, a monovalent polypeptide of the present invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 1 the G has been changed into L, or R;
- at position 2 the L has been changed into F, P, or I;
- at position 3 the L has been changed into P, or F;
- at position 4 the F has been changed into S, L, or I;
- at position 5 the S has been changed into I, or R;
- at position 6 the R has been changed into C, A, P, V, or L;
- at position 7 the N has been changed into H, P, I, M, Y, T or D:
- at position 8 the S has been changed into T, R, or I;
- at position 9 the A has been changed into V or T; and/or
- at position 10 the G has been changed into S, R, or V;

and

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 1 the R has been changed into G, or C;
- at position 2 the I has been changed into V, T, S or L;
- at position 3 the R has been changed into G, or L;
- at position 4 the M has been changed into S, R, or T;
- at position 5 the G has been changed into V, S, or T;
- at position 7 the S has been changed into G, C, D, or E; and/or
- at position 8 the I has been changed into T, M, or R;

and

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 1 the W has been changed into G;
- at position 3 the E has been changed into T, K, G, A, or I;
- at position 4 the G has been changed into E, or D;
- at position 5 the F has been changed into A, L, V, Y, T, or S;
- at position 6 the Y has been changed into F, or D:
- at position 7 the E has been changed into G, or K;
- at position 8 the Y has been changed into S or H; and/or
- at position 9 the W has been changed into S, G or C.

In a further aspect, a monovalent polypeptide of the present invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-185; or

b) amino acid sequences that have 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 6 the R has been changed into A, or V; and/or
- at position 9 the A has been changed into V;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-271; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 2 the I has been changed into L;
- at position 4 the M has been changed into S or T;
- at position 5 the G has been changed into S or T; and/or
- at position 8 the I has been changed into T;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-398; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 3 the E has been changed into T or I;
- at position 4 the G has been changed into E;
- at position 5 the F has been changed into A; and/or
- at position 8 the Y has been changed into H.

In particular, according to this preferred but non-limiting aspect, a monovalent polypeptide of the present invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-185; or

b) amino acid sequences that have 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 6 the R has been changed into A, or V; and/or
- at position 9 the A has been changed into V;

and

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-271; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 2 the I has been changed into L;
- at position 4 the M has been changed into S or T;
- at position 5 the G has been changed into S or T; and/or
- at position 8 the I has been changed into T;

and

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-398; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 3 the E has been changed into T or I;
- at position 4 the G has been changed into E;
- at position 5 the F has been changed into A; and/or
- at position 8 the Y has been changed into H.

In a further aspect, a monovalent polypeptide of the present invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422.

In particular, according to this preferred but non-limiting aspect, a monovalent polypeptide of the present invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214;

and

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303;

and

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422.

In a further aspect, a monovalent polypeptide of the present invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214, wherein

- at position 1 the G has been changed into R, A, V, S, or K;
- at position 3 the T has been changed into N;
- at position 4 the F has been changed into L;
- at position 6 the N has been changed into S;
- at position 7 the F has been changed into Y:
- at position 8 the G has been changed into A; and/or
- at position 9 the M has been changed into V;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303, wherein

- at position 1 the A has been changed into T;
- at position 2 the I has been changed into V;
- at position 5 the T has been changed into S, or A;
- at position 6 the G has been changed into N, or A;
- at position 7 the G has been changed into S, or R;
- at position 8 the H has been changed into R, or Y;
- at position 9 the T has been changed into I, or K; and/or
- at position 10 the Y has been changed into F;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422, wherein

- at position 4 the F has been changed into Y, or S;
- at position 5 the G has been changed into D;
- at position 6 the D has been changed into G;
- at position 7 the G has been changed into D;
- at position 8 the T has been changed into A:
- at position 9 the Y has been changed into S;
- at position 10 the Y has been changed into F;
- at position 12 the Q has been changed into E;
- at position 14 the A has been changed into N, T, I, or R; and/or
- at position 17 the D has been changed into N, or G.

In particular, according to this preferred but non-limiting aspect, a monovalent polypeptide of the present invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214, wherein

- at position 1 the G has been changed into R, A, V, S, or K;
- at position 3 the T has been changed into N;
- at position 4 the F has been changed into L;
- at position 6 the N has been changed into S;
- at position 7 the F has been changed into Y:
- at position 8 the G has been changed into A; and/or
- at position 9 the M has been changed into V;

and

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303, wherein

- at position 1 the A has been changed into T;
- at position 2 the I has been changed into V;
- at position 5 the T has been changed into S, or A;
- at position 6 the G has been changed into N, or A;
- at position 7 the G has been changed into S, or R;
- at position 8 the H has been changed into R, or Y;
- at position 9 the T has been changed into I, or K; and/or
- at position 10 the Y has been changed into F;

and

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422, wherein

- at position 4 the F has been changed into Y, or S;
- at position 5 the G has been changed into D;
- at position 6 the D has been changed into G;
- at position 7 the G has been changed into D;
- at position 8 the T has been changed into A:
- at position 9 the Y has been changed into S;
- at position 10 the Y has been changed into F;
- at position 12 the Q has been changed into E;
- at position 14 the A has been changed into N, T, I, or R; and/or
- at position 17 the D has been changed into N, or G.

In one specific aspect, the monovalent polypeptide of the invention is chosen from the group of amino acid sequences, wherein:

- CDR1 is SEQ ID NO: 182, CDR2 is SEQ ID NO: 269, and CDR3 is SEQ ID NO: 397;
- CDR1 is SEQ ID NO: 182, CDR2 is SEQ ID NO: 269, and CDR3 is SEQ ID NO: 394;
- CDR1 is SEQ ID NO: 185, CDR2 is SEQ ID NO: 271, and CDR3 is SEQ ID NO: 398;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 393;

- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 395;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 396;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 270, and CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 183, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 184, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 393; and
- CDR1 is SEQ ID NO: 185, CDR2 is SEQ ID NO: 271, and CDR3 is SEQ ID NO: 398.

In a further aspect, the monovalent polypeptide of the invention is chosen from the group of amino acid sequences, wherein:

- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 290, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 292, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 293, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 294, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 216, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 419;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 297, and CDR3 is SEQ ID NO: 420;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298, and CDR3 is SEQ ID NO: 421;
- CDR1 is SEQ ID NO: 217, CDR2 is SEQ ID NO: 299, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 298, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 423;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 301, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 424;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 302, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 425;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 426;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 417;

- CDR1 is SEQ ID NO: 219, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 427;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 304, and CDR3 is SEQ ID NO: 428;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 421;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 429;
- CDR1 is SEQ ID NO: 221, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 222, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 430;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 306, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 223, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298 and CDR3 is SEQ ID NO: 431;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 432;
- CDR1 is SEQ ID NO: 224, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 307, and CDR3 is SEQ ID NO: 433;
- CDR1 is SEQ ID NO: 225, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 226, CDR2 is SEQ ID NO: 308, and CDR3 is SEQ ID NO: 434;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 301, and CDR3 is SEQ ID NO: 426;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 217, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298, and CDR3 is SEQ ID NO: 435; and
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 309, and CDR3 is SEQ ID NO: 418.

Representative polypeptides of the present invention having the CDRs described above are shown in Table A-2.

In one aspect, the monovalent polypeptide has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 1-64. In another aspect, the monovalent polypeptide has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) that has 89% or more sequence identity compared to any one of SEQ ID NOs: 1-64. Preferably, the monovalent polypeptide has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 1-64 and the monovalent polypeptide has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) that has 89% or more sequence identity compared to any one of SEQ ID NOs: 1-64. In another preferred aspect,

the monovalent polypeptide belongs to family 12, such as e.g. a monovalent polypeptide selected from any one of SEQ ID NOs: 1-64.

In one aspect, the monovalent polypeptide has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 65-123. In another aspect, the monovalent polypeptide has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) that has 89% or more sequence identity compared to any one of SEQ ID NOs: 65-123. Preferably, the monovalent polypeptide has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 65-123 and the monovalent polypeptide has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) that has 89% or more sequence identity compared to any one of SEQ ID NOs: 65-123. In another preferred aspect, the monovalent polypeptide belongs to family 1, such as e.g. a monovalent polypeptide selected from any one of SEQ ID NOs: 65-123.Monovalent polypeptides comprising one or more of the above specified stretches of amino acid residues may modulate and/or partially or fully inhibit the function of Kv1.3. More particularly, these polypeptides of the present invention may depolarize the T cell membrane and/or reduce or even totally inhibit the efflux of potassium ions from T-cells. As such, these polypeptides of the invention may inhibit proliferation of T-cells and/or suppress activation of T-cells resulting of the inhibition of certain immune responses in vivo.

In one particular aspect, the polypeptides of the invention indirectly modulate the function of Kv1.3, i.e. as an allosteric modulator (as defined herein). More specifically, the polypeptides of the invention may induce a conformational change within the structure of the Kv1.3 pore.

Binding of the polypeptides of the invention to Kv1.3 can be measured in binding assays that preserve the conformation of the Kv1.3 target. Typical assays include (without being limiting) assays in which Kv1.3 is exposed on a cell surface (such as e.g. CHO cells, HEK cells, HeLa cells, Chinese Hamster Lung (CHL) cells, Caki cells, etc.). A preferred assay for measuring binding of the polypeptides of the invention to Kv1.3 is a FACS assay, such as e.g. the FACS assay as described in the examples, wherein binding of the polypeptides of the invention to Kv1.3 expressed on CHO-K1 cells and/or HEK293H cells is determined. Some preferred EC50 values for binding of the polypeptides of the invention to Kv1.3 will become clear from the further description and examples herein.

In such FACS binding assay, the monovalent polypeptides of the present invention may have EC50 values in binding human Kv1.3 of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such FACS binding assay, the monovalent polypeptides of the present invention may have EC50 values in binding human Kv1.3 between 10^{-9} M and 10^{-8} M.

In such FACS binding assay, the monovalent polypeptides of the present invention may have EC50 values in binding cyno Kv1.3 of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such FACS binding assay, the monovalent polypeptides of the present invention may have EC50 values in binding cyno Kv1.3 between 10^{-10} M and 10^{-7} M, such as between 10^{-9} M and 10^{-8} M.

In such FACS binding assay, the monovalent polypeptides of the present invention may have EC50 values in binding rat Kv1.3 of 10^{-6} M or lower, preferably of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such FACS binding assay, the monovalent polypeptides of the present invention may have EC50 values in binding rat Kv1.3 between 10^{-10} M and 10^{-6} M, such as between 10^{-7} M and 10^{-6} M.

Modulation and/or inhibition of the efflux of potassium ions can be determined by a variety of ion channel screening technologies including (without being limiting) ion flux assays, radioligand binding studies, fluorescent dye assays, and electrophysiology, such as voltage clamp, and in particular, the patch-clamp. An overview of different ion channel technologies is provided by e.g. Dabrowski et al. (CNS & Neurological Disorders Drug Targets 7: 122, 2008), Lü and An (Comb. Chem. High Throughput Screen. 11:185-94, 2008), and Zheng et al. (Assay Drug Dev. Technol. 2: 543-52, 2004).

Voltage clamp (Huxley, Trends Neurosci. 25: 553-8, 2002) is used to measure the ion currents through the membrane of excitable cells. The patch-clamp variant of this technique (Hamill et al. Pflügers Archiv European Journal of Physiology 391: 85–100, 1981) allows the study of single or multiple ion channels in cells.

Higher throughput electrophysiological platforms have been developed ranging from medium throughput systems to higher throughput platforms (see e.g. Southan and Clark, Methods Mol. Biol. 565: 187-208, 2009), including PatchXpress (Molecular Devices; Ghetti et al. Methods Mol. Biol. 403: 59-69, 2007), Qpatch and Qpatch HTX (Sophion; Mathes et al. Comb. Chem. High Throughput Screen. 12: 78-95, 2009; Korsgaard et al. Comb. Chem. High Throughput Screen. 12:

51-63, 2009), PatchLiner (Nanion; Farre et al. Comb. Chem. High Throughput Screen 12: 24-37, 2009), IonWorks® HT, IonWorks® Quattro and IonFlux™ Systems (Molecular Devices; Jow et al. J Biomol. Screen. 12: 1059-67, 2007; Dale et al. Mol. Biosyst. 3: 714-22, 2007). Some preferred IC50 values for the polypeptides of the invention in these assays will become clear from the further description and examples herein.

On the IonFlux™ (Molecular Devices) using Kv1.3-expressing HEK293H cells, for example, the monovalent polypeptides of the invention have IC50 values of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in this automated Patch Clamp assay, the monovalent polypeptides of the present invention may have IC50 values between 10^{-10} M and 10^{-7} M, between 10^{-10} M and 10^{-8} M, between 10^{-10} M and 10^{-9} M, such as e.g. between 10^{-9} M and 10^{-7} M.

On the IonWorks® Quattro (Molecular Devices) using Kv1.3-expressing Chinese Hamster Lung (CHL) cells, for example, the monovalent polypeptides of the invention have IC50 values of 10^{-} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, on this high-throughput planar perforated patch clamp, the monovalent polypeptides of the present invention may have IC50 values between 10^{-10} M and 10^{-7} M, between 10^{-10} M and 10^{-8} M, between 10^{-10} M and 10^{-9} M, such as e.g. between 10^{-8} M and 10^{-7} M. More in particular, the monovalent polypeptides that belong to family 12 may have an IC50 value on the IonWorks® Quattro (Molecular Devices) between 10^{-8} M and 10^{-7} M.

Modulation and/or inhibition of Kv1.3 by the polypeptides of the invention can also be assessed in radioligand binding studies. Binding studies with tritiated correolide (e.g. C20-29-[3H]dihydrocorreolide (diTC)) to a single class of sites in membranes prepared from CHO/Kv1.3 cells has been described by Felix et al. (Biochemistry 38: 4922-30, 1999). Knaus et al. (Biochemistry 34: 13627-13634, 1995) describes, for example, the binding of monoiodotyrosinyl margatoxin (125I-margatoxin) to heterotetrameric Kv channels in rat brain synaptic plasma membranes. Binding studies of 125I-margatoxin to plasma membranes prepared from either Jurkat cells, a human leukemic T cell line, or CHO cells stably transfected with the Shaker-type voltage-gated K+ channel, K(V)1.3 have been described by Helms et al. (Biochemistry. 36: 3737-44, 1997). Some preferred IC50 values for blocking 125I-margatoxin binding to Kv1.3 by the polypeptides of the invention will become clear from the further description and examples herein.

The monovalent polypeptides of the present invention may block binding of 125I-margatoxin to cynomolgus Kv1.3 overexpressing CHO cells with IC50 values of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such 125I-margatoxin blocking assay, the monovalent polypeptides of the present invention may have IC50 values between 10^{-10} M and 10^{-8} M, such as e.g. between 10^{-9} M and 10^{-8} M.

Other flux assays for measuring modulation and/or inhibition of Kv1.3 by the polypeptides of the invention include (without being limiting) the high-throughput efflux assay with radiolabelled^{86} Rubidium described by Hanson et al. (Br. J. Pharmacol. 126: 1707-16, 1999), the nonradioactive rubidium (Rb(+)) efflux assay described by Wang et al. (Assay Drug Dev. Technol. 2: 525-34, 2004) and a fluorescence-based thallium flux assay (Weaver et al. J. Biomol. Screen. 9: 671-7, 2004).

Inhibition of T-cell activation and/or proliferation by the polypeptides of the present invention can be measured in T-cell activation assays. Without being limiting, T-cell activation assays have been described by Nguyten et al. (Molecular Pharmacology 50: 1672-1679, 1996) and Hanson et al. (Br. J. Pharmacol. 126: 1707–1716, 1999). Some preferred IC50 values for inhibition of T-cell activation and/or proliferation by the monovalent polypeptides of the invention will become clear from the further description and examples herein.

In a T-cell activation assay with $CCR7^-$ $CD45RA^-$ T cells stimulated with anti-CD3 antibody OKT3 (as described in the Examples 4.4 and 5.5), the monovalent polypeptides of the invention have IC50 values for inhibiting IFNgamma production of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, 10^{-10} M or lower, or even of 10^{-11} M or lower. For example, in this T-cell activation assay, the monovalent polypeptides of the present invention inhibit IFNgamma production with IC50 values between 10^{-11} M and 10^{-7} M, between 10^{-11} M and 10^{-8} M, between 10^{-11} M and 10^{-9} M, such as e.g. between 10^{-8} M and 10^{-7} M. More in particular, the monovalent polypeptides that belong to family 12 may inhibit IFNgamma production with IC50 values between 10^{-8} M and 10^{-7} M.

In this T-cell activation assay with $CCR7^-CD45RA^-$ T cells stimulated with anti-CD3 antibody OKT3 (as described in the Examples 4.4 and 5.5), the monovalent polypeptides of the invention have IC50 values for inhibiting CD25 upregulation of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, 10^{-10} M or lower, or even of 10^{-11} M or lower. For example, in this T-cell activation assay, the monovalent polypeptides of the present invention

inhibit CD25 upregulation with IC50 values between 10^{-11} M and 10^{-7} M, between 10^{-11} M and 10^{-8} M, between 10^{-11} M and 10^{-9} M, such as e.g. between 10^{-8} M and 10^{-7} M. More in particular, the monovalent polypeptides that belong to family 12 may inhibit CD25 upregulation with IC50 values between 10^{-8} M and 10^{-7} M.

The invention also relates to a monovalent polypeptide which has at least 80% amino acid identity (or sequence identity as defined herein), preferably at least 85% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even (essentially) 100% amino acid identity with at least one of the amino acid sequences of SEQ ID NOs: 1-123.

In one specific, but non-limiting aspect, the monovalent polypeptide of the invention may be a monovalent polypeptide that comprises an immunoglobulin fold or a monovalent polypeptide that, under suitable conditions (such as physiological conditions) is capable of forming an immunoglobulin fold (*i.e.,* by folding). Reference is *inter alia* made to the review by Halaby et al. (J. Protein Eng. 12: 563-71, 1999). Preferably, when properly folded so as to form an immunoglobulin fold, the stretches of amino acid residues may be capable of properly forming the antigen binding site for binding Kv1.3. Accordingly, in a preferred aspect the monovalent polypeptide of the invention is an immunoglobulin, such as e.g. an immunoglobulin single variable domain.

Accordingly, the framework sequences are preferably (a suitable combination of) immunoglobulin framework sequences or framework sequences that have been derived from immunoglobulin framework sequences (for example, by sequence optimization such as humanization or camelization). For example, the framework sequences may be framework sequences derived from an immunoglobulin single variable domain such as a light chain variable domain (*e.g.,* a V_L-sequence) and/or from a heavy chain variable domain (*e.g.,* a V_H-sequence). In one particularly preferred aspect, the framework sequences are either framework sequences that have been derived from a V_{HH}-sequence (in which said framework sequences may optionally have been partially or fully humanized) or are conventional V_H sequences that have been camelized (as defined herein).

The framework sequences may preferably be such that the monovalent polypeptide of the invention is an immunoglobulin single variable domain such as a Domain antibody (or an amino acid sequence that is suitable for use as a domain antibody); is a single domain antibody (or

an amino acid that is suitable for use as a single domain antibody); is a "dAb" (or an amino acid that is suitable for use as a dAb); is a Nanobody® (including but not limited to V_{HH}), is a humanized V_{HH} sequence, is a camelized V_H sequence; or is a V_{HH} sequence that has been obtained by affinity maturation. Again, suitable framework sequences will be clear to the skilled person, for example on the basis the standard handbooks and the further disclosure and prior art mentioned herein.

In particular, the framework sequences present in the monovalent polypeptides of the invention may contain one or more of Hallmark residues (as defined in WO 08/020079 (Tables A-3 to A-8)), such that the monovalent polypeptide of the invention is a Nanobody. Some preferred, but non-limiting examples of (suitable combinations of) such framework sequences will become clear from the further disclosure herein (see *e.g.,* Table A-2). Generally, Nanobodies (in particular V_{HH} sequences and partially humanized Nanobodies) can in particular be characterized by the presence of one or more "*Hallmark residues*" in one or more of the framework sequences (as *e.g.,* further described in WO 08/020079, page 61, line 24 to page 98, line 3).

More in particular, a Nanobody can be an immunoglobulin and/or polypeptide with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and which:

i) have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NOs: 1-123 (see Table A-1), in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded. In this respect, reference is also made to Table A-2, which lists the framework 1 sequences (SEQ ID NOs: 124-180), framework 2 sequences (SEQ ID NOs: 227-267), framework 3 sequences (SEQ ID NOs: 310-392) and framework 4 sequences (SEQ ID NOs: 436-450) of the immunoglobulin single variable domains of SEQ ID NOs: 1-123 (see Table A-1); or

ii) combinations of framework sequences as depicted in Table A-2;

and in which:

iii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-3 to Table A-8 of WO 08/020079.

In a preferred aspect, the present invention provides an immunoglobulin or monovalent polypeptide that is selected from any of SEQ ID NOs: 1-123.

The present invention also relates to monovalent polypeptides and/or immunoglobulin single variable domains directed against K1.3, that cross-blocks the binding to Kv1.3 of at least one of the immunoglobulins with SEQ ID NOs: 1-123 and/or that are cross-blocked from binding to Kv1.3 by at least one of the immunoglobulins with SEQ ID NOs: 1-123.

The invention further relates to monovalent polypeptides and/or immunoglobulin single variable domains directed against Kv1.3 that bind the same epitope as is bound by the monovalent polypeptides of the present invention, more particularly by the monovalent polypeptides with SEQ ID NOs: 1-123.

In a particular aspect, the invention relates to monovalent polypeptides and/or immunoglobulin single variable domains directed against Kv1.3 that bind the same epitope as is bound by the monovalent polypeptides of the present invention that belong to family 12, more particularly by the monovalent polypeptides with SEQ ID NO: 1-64.

In another particular aspect, the invention relates to monovalent polypeptides and/or immunoglobulin single variable domains directed against Kv1.3 that bind the same epitope as is bound by the monovalent polypeptides of the present invention that belong to family 1, more particularly by the monovalent polypeptides with SEQ ID NO: 65-123.

Again, such monovalent polypeptides may be an immunoglobulin, such as an immunoglobulin single variable domain, derived in any suitable manner and from any suitable source, and may for example be naturally occurring V_{HH} sequences (*i.e.,* from a suitable species of Camelid) or synthetic or semi-synthetic amino acid sequences, including but not limited to "humanized" (as defined herein) Nanobodies or VHH sequences, "camelized" (as defined herein) immunoglobulin sequences (and in particular camelized heavy chain variable domain sequences), as well as Nanobodies that have been obtained by techniques such as affinity maturation (for example, starting from synthetic, random or naturally occurring immunoglobulin sequences), CDR grafting, veneering, combining fragments derived from different immunoglobulin sequences, PCR assembly using overlapping primers, and similar techniques for engineering immunoglobulin

sequences well known to the skilled person; or any suitable combination of any of the foregoing as further described herein. Also, when an immunoglobulin comprises a V_{HH} sequence, said immunoglobulin may be suitably humanized, as further described herein, so as to provide one or more further (partially or fully) humanized immunoglobulins of the invention. Similarly, when an immunoglobulin comprises a synthetic or semi-synthetic sequence (such as a partially humanized sequence), said immunoglobulin may optionally be further suitably humanized, again as described herein, again so as to provide one or more further (partially or fully) humanized immunoglobulins of the invention.

These monovalent polypeptides of the invention, and in particular the immunoglobulins comprising the CDR sequences of the invention are particularly suited for use as building block or binding unit for the preparation of multivalent polypeptides.

Accordingly, the monovalent polypeptides of the invention that bind Kv1.3 can be in essentially isolated form (as defined herein), or they may form part of a protein or polypeptide, which may comprise or essentially consist of one or more monovalent polypeptides that bind Kv1.3 and which may optionally further comprise one or more further amino acid sequences (all optionally linked via one or more suitable linkers). The present invention also relates to a protein or polypeptide that comprises or essentially consists of one or more monovalent polypeptides of the invention (or suitable fragments thereof).

The one or more monovalent polypeptides of the invention are thus used as a binding unit or building block in such a protein or polypeptide, so as to provide a monovalent, multivalent or multiparatopic polypeptide of the invention, respectively, all as described herein. The present invention thus also relates to a polypeptide which is a monovalent construct comprising or essentially consisting of one monovalent polypeptide of the invention. The present invention thus also relates to a polypeptide which is a multivalent polypeptide, such as *e.g.*, a bivalent or trivalent polypeptide comprising or essentially consisting of two or more monovalent polypeptides of the invention (for multivalent and multispecific polypeptides containing one or more VHH domains and their preparation, reference is also made to Conrath et al., J. Biol. Chem. 276: 7346-7350, 2001, as well as to for example WO 96/34103, WO 99/23221 and WO 2010/115998).

Multivalent polypeptides of the invention

The invention further relates to a multivalent polypeptide (also referred to herein as a "*multivalent polypeptide(s) of the invention*") that comprises or (essentially) consists of at least one immunoglobulin single variable domain (or suitable fragments thereof) directed against Kv1.3, preferably human Kv1.3, and one additional immunoglobulin single variable domain.

In a preferred aspect, the multivalent polypeptide of the invention comprises or essentially consists of two or more immunoglobulin single variable domains directed against Kv1.3. The two or more immunoglobulin single variable domains may optionally be linked via one or more peptidic linkers.

In the multivalent polypeptide of the invention, the two or more immunoglobulin single variable domains or Nanobodies may be the same or different, and may be directed against the same antigen or antigenic determinant (for example against the same part(s) or epitope(s) or against different parts or epitopes) or may alternatively be directed against different antigens or antigenic determinants; or any suitable combination thereof. For example, a bivalent polypeptide of the invention may comprise (a) two identical immunoglobulin single variable domains or Nanobodies; (b) a first immunoglobulin single variable domain or Nanobody directed against a first antigenic determinant of a protein or antigen and a second immunoglobulin single variable domain or Nanobody directed against the same antigenic determinant of said protein or antigen which is different from the first immunoglobulin single variable domain or Nanobody; (c) a first immunoglobulin single variable domain or Nanobody directed against a first antigenic determinant of a protein or antigen and a second immunoglobulin single variable domain or Nanobody directed against another antigenic determinant of said protein or antigen; or (d) a first immunoglobulin single variable domain or Nanobody directed against a first protein or antigen and a second immunoglobulin single variable domain or Nanobody directed against a second protein or antigen (i.e. different from said first antigen). Similarly, a trivalent polypeptide of the invention may, for example and without being limited thereto. comprise (a) three identical immunoglobulin single variable domains or Nanobodies; (b) two identical immunoglobulin single variable domains or Nanobodies against a first antigenic determinant of an antigen and a third immunoglobulin single variable domain or Nanobody directed against a different antigenic determinant of the same antigen; (c) two identical immunoglobulin single variable domains or Nanobodies against a first antigenic determinant of an antigen and a third immunoglobulin single variable domain or Nanobody directed against a second antigen different from said first antigen; (d) a first

immunoglobulin single variable domain or Nanobody directed against a first antigenic determinant of a first antigen, a second immunoglobulin single variable domain or Nanobody directed against a second antigenic determinant of said first antigen and a third immunoglobulin single variable domain or Nanobody directed against a second antigen different from said first antigen; or (e) a first immunoglobulin single variable domain or Nanobody directed against a first antigen, a second immunoglobulin single variable domain or Nanobody directed against a second antigen different from said first antigen, and a third immunoglobulin single variable domain or Nanobody directed against a third antigen different from said first and second antigen.

Polypeptides of the invention that contain at least two immunoglobulin single variable domains and/or Nanobodies, in which at least one immunoglobulin single variable domain or Nanobody is directed against a first antigen (i.e. against Kv1.3) and at least one immunoglobulin single variable domain or Nanobody is directed against a second antigen (i.e. different from Kv1.3), will also be referred to as "multispecific" polypeptides of the invention, and the immunoglobulin single variable domains or Nanobodies present in such polypeptides will also be referred to herein as being in a "multispecific format". Thus, for example, a "bispecific" polypeptide of the invention is a polypeptide that comprises at least one immunoglobulin single variable domain or Nanobody directed against a first antigen (i.e. Kv1.3) and at least one further immunoglobulin single variable domain or Nanobody directed against a second antigen (i.e. different from Kv1.3), whereas a "trispecific" polypeptide of the invention is a polypeptide that comprises at least one immunoglobulin single variable domain or Nanobody directed against a first antigen (i.e. Kv1.3), at least one further immunoglobulin single variable domain or Nanobody directed against a second antigen (i.e. different from Kv1.3) and at least one further immunoglobulin single variable domain or Nanobody directed against a third antigen (i.e. different from both Kv1.3, and the second antigen); etc.

Accordingly, in its simplest form, a bispecific polypeptide of the invention is a bivalent polypeptide of the invention (as defined herein), comprising a first immunoglobulin single variable domain or Nanobody directed against Kv1.3, and a second immunoglobulin single variable domain or Nanobody directed against a second antigen, in which said first and second immunoglobulin single variable domain or Nanobody may optionally be linked via a linker sequence (as defined herein); whereas a trispecific polypeptide of the invention in its simplest form is a trivalent polypeptide of the invention (as defined herein), comprising a first immunoglobulin single variable

domain or Nanobody directed against Kv1.3, a second immunoglobulin single variable domain or Nanobody directed against a second antigen and a third immunoglobulin single variable domain or Nanobody directed against a third antigen, in which said first, second and third immunoglobulin single variable domain or Nanobody may optionally be linked via one or more, and in particular two, linker sequences.

In a preferred aspect, the polypeptide of the invention is a trivalent, bispecific polypeptide. A trivalent, bispecific polypeptide of the invention in its simplest form may be a trivalent polypeptide of the invention (as defined herein), comprising two identical immunoglobulin single variable domains or Nanobodies against Kv1.3 and a third immunoglobulin single variable domain or Nanobody directed against another antigen (e.g. serum albumin), in which said first, second and third immunoglobulin single variable domain or Nanobody may optionally be linked via one or more, and in particular two, linker sequences. Particularly preferred trivalent, bispecific polypeptides in accordance with the invention are those shown in the Examples described herein and in Table A-3.

In another aspect, the polypeptide of the invention is a bispecific polypeptide. A bispecific polypeptide of the invention in its simplest form may be a bivalent polypeptide of the invention (as defined herein), comprising a immunoglobulin single variable domain or Nanobody against Kv1.3 and a second immunoglobulin single variable domain or Nanobody directed against another antigen, in which said first and second immunoglobulin single variable domain or Nanobody may optionally be linked via a linker sequence.

In a further aspect, the polypeptide of the invention is a multiparatopic polypeptide (also referred to herein as "*multiparatopic polypeptide(s) of the invention*"), such as *e.g.,* (a) "*biparatopic polypeptide(s) of the invention*" or "*triparatopic polypeptide(s) of the invention*". The term "*multiparatopic*" (antigen-) binding molecule or "*multiparatopic*" polypeptide as used herein shall mean a polypeptide comprising at least two (i.e. two or more) immunoglobulin single variable domains, wherein a "first" immunoglobulin single variable domain is directed against Kv1.3 and a "second" immunoglobulin single variable domain is directed against Kv1.3, and wherein these "first" and "second" immunoglobulin single variable domains have a different paratope. Accordingly, the multiparatopic polypeptide comprises or consists of two or more immunoglobulin single variable domains that are directed against Kv1.3, wherein at least one "first" immunoglobulin single variable domain is directed against a first epitope on Kv1.3 and at

least one “second” immunoglobulin single variable domain is directed against a second epitope on Kv1.3 different from the first epitope on Kv1.3.

In a preferred aspect, the polypeptide of the invention is a biparatopic polypeptide. The term "*biparatopic*" (antigen-)binding molecule or "*biparatopic*" polypeptide as used herein shall mean a polypeptide comprising a “first” immunoglobulin single variable domain directed against Kv1.3 and a “second” immunoglobulin single variable domain directed against Kv1.3, wherein these “first” and “second” immunoglobulin single variable domains have a different paratope. Accordingly, the biparatopic polypeptide comprises or consists of two or more immunoglobulin single variable domains that are directed against Kv1.3, wherein a “first” immunoglobulin single variable domain is directed against a first epitope on Kv1.3 and a “second” immunoglobulin single variable domain is directed against a second epitope on Kv1.3 different from the first epitope on Kv1.3.

In another further aspect, the polypeptide of the invention is a triparatopic polypeptide. The term "*triparatopic*" (antigen-)binding molecule or "*triparatopic*" polypeptide as used herein shall mean a polypeptide comprising a “first” immunoglobulin single variable domain directed against Kv1.3, a “second” immunoglobulin single variable domain directed against Kv1.3 and a “third” immunoglobulin single variable domain directed against Kv1.3, wherein these “first”, “second” and “third” immunoglobulin single variable domains have a different paratope. Accordingly, the triparatopic polypeptide comprises or consists of three or more immunoglobulin single variable domains that are directed against Kv1.3, wherein a “first” immunoglobulin single variable domain is directed against a first epitope on Kv1.3, a “second” immunoglobulin single variable domain is directed against a second epitope on Kv1.3 different from the first epitope on Kv1.3, and a “third” immunoglobulin single variable domain is directed against a third epitope on Kv1.3 different from the first and second epitope on Kv1.3.

The two or more immunoglobulin single variable domains present in the multivalent polypeptide of the invention may consist of a light chain variable domain sequence (e.*g.,* a V_L-sequence) or of a heavy chain variable domain sequence (*e.g.,* a V_H-sequence); they may consist of a heavy chain variable domain sequence that is derived from a conventional four-chain antibody or of a heavy chain variable domain sequence that is derived from heavy chain antibody. In a preferred aspect, they consist of a Domain antibody (or an amino acid that is suitable for use as a domain antibody), of a single domain antibody (or an amino acid that is suitable for use as a single

domain antibody), of a "dAb" (or an amino acid that is suitable for use as a dAb), of a Nanobody® (including but not limited to V_{HH}), of a humanized V_{HH} sequence, of a camelized V_H sequence; or of a V_{HH} sequence that has been obtained by affinity maturation. The two or more immunoglobulin single variable domains may consist of a partially or fully humanized Nanobody or a partially or fully humanized VHH. In a preferred aspect of the invention, the immunoglobulin single variable domains encompassed in the multivalent polypeptide of the invention are one or more monovalent polypeptides of the invention, as defined herein.

In a preferred aspect of the invention, the first immunoglobulin single variable domain and the second immunoglobulin single variable domain present in the multiparatopic (preferably biparatopic or triparatopic) polypeptide of the invention do not (cross)-compete with each other for binding to Kv1.3 and, as such, belong to different families. Accordingly, the present invention relates to a multiparatopic (preferably biparatopic) polypeptide comprising two or more immunoglobulin single variable domains wherein each immunoglobulin single variable domain belongs to a different family. In one aspect, the first immunoglobulin single variable domain of this preferred multiparatopic (preferably biparatopic) polypeptide of the invention does not cross-block the binding to Kv1.3 of the second immunoglobulin single variable domain of this preferred multiparatopic (preferably biparatopic) polypeptide of the invention and/or the first immunoglobulin single variable is not cross-blocked from binding to Kv1.3 by the second immunoglobulin single variable domain. In another aspect, the first immunoglobulin single variable domain of this preferred multiparatopic (preferably biparatopic) polypeptide of the invention cross-blocks the binding to Kv1.3 of the second immunoglobulin single variable domain of this preferred multiparatopic (preferably biparatopic) polypeptide of the invention and/or the first immunoglobulin single variable is cross-blocked from binding to Kv1.3 by the second immunoglobulin single variable domain.

Preferred combination of immunoglobulin single variable domains present in the multiparatopic (such as biparatopic or triparatopic) polypeptide of the invention may encompass any of the following:

- the first immunoglobulin single variable domain cross-blocks the binding to Kv1.3 of at least one of the immunoglobulin single variable domains with SEQ ID NOs: 1-64 [family 12] and/or is cross-blocked from binding to Kv1.3 by at least one of the immunoglobulin single variable domains with SEQ ID NOs: 1-64 [family 12]; and the second immunoglobulin single variable

domain cross-blocks the binding to Kv1.3 of at least one of the immunoglobulin single variable domains with SEQ ID NOs: 65-123 [family 1] and/or is cross-blocked from binding to Kv1.3 by at least one of immunoglobulin single variable domains with SEQ ID NOs: 65-123 [family 1];

- the first immunoglobulin single variable domain cross-blocks the binding to Kv1.3 of at least one of the immunoglobulin single variable domains with SEQ ID NOs: 65-123 [family 1] and/or is cross-blocked from binding to Kv1.3 by at least one of the immunoglobulin single variable domains with SEQ ID NOs: 65-123 [family 1]; and the second immunoglobulin single variable domain cross-blocks the binding to Kv1.3 of at least one of the immunoglobulin single variable domains with SEQ ID NOs: 1-64 [family 12] and/or is cross-blocked from binding to Kv1.3 by at least one of the immunoglobulin single variable domains with SEQ ID NOs: 1-64 [family 12];
- the first immunoglobulin single variable domain cross-blocks the binding to Kv1.3 of at least one of the immunoglobulin single variable domains with SEQ ID NOs: 1-64 [family 12] and/or is cross-blocked from binding to Kv1.3 by at least one of the immunoglobulin single variable domains with SEQ ID NOs: 1-64 [family 12]; and the second immunoglobulin single variable domain cross-blocks the binding to Kv1.3 of at least one of the immunoglobulin single variable domains with SEQ ID NOs: 1-64 [family 12] and/or is cross-blocked from binding to Kv1.3 by at least one of the immunoglobulin single variable domains with SEQ ID NOs: 1-64 [family 12];
- the first immunoglobulin single variable domain cross-blocks the binding to Kv1.3 of at least one of the immunoglobulin single variable domains with SEQ ID NOs: 65-123 [family 1] and/or is cross-blocked from binding to Kv1.3 by at least one of the immunoglobulin single variable domains with SEQ ID NOs: 65-123 [family 1]; and the second immunoglobulin single variable domain cross-blocks the binding to Kv1.3 of at least one of the immunoglobulin single variable domains with SEQ ID NOs: 65-123 [family 1] and/or is cross-blocked from binding to Kv1.3 by at least one of the immunoglobulin single variable domains with SEQ ID NOs: 65-123 [family 1];
- the first immunoglobulin single variable domain binds the same epitope as is bound by SEQ ID NOs: 1-64 [family 12] and the second immunoglobulin single variable domain binds the same epitope as is bound by SEQ ID NOs: 65-123 [family 1];
- the first immunoglobulin single variable domain binds the same epitope as is bound by SEQ ID NOs: 65-123 [family 1] and the second immunoglobulin single variable domain binds the same epitope as is bound by SEQ ID NOs: 1-64 [family 12];

- the first immunoglobulin single variable domain binds the same epitope as is bound by SEQ ID NOs: 1-64 [family 12] and the second immunoglobulin single variable domain binds the same epitope as is bound by SEQ ID NOs: 1-64 [family 12]; or
- the first immunoglobulin single variable domain binds the same epitope as is bound by SEQ ID NOs: 65-123 [family 1] and the second immunoglobulin single variable domain binds the same epitope as is bound by SEQ ID NOs: 65-123 [family 1].

In a further aspect, the invention relates to a multiparatopic (preferably biparatopic) polypeptide comprising two or more immunoglobulin single variable domains directed against Kv1.3 that bind the same epitope as is bound by any one of SEQ ID NOs: 1-123.

Different families (1 and 12) exhibiting different functional profiles have been identified amongst the monovalent polypeptides of the invention (see Tables A-4 and A-5). Accordingly, the present invention relates to a multiparatopic polypeptide comprising two or more immunoglobulin single variable domains wherein each immunoglobulin single variable domain belongs to a different family as defined herein.

Preferred immunoglobulin single variable domains for use in these multiparatopic (preferably biparatopic) polypeptides of the invention are the monovalent polypeptides of the invention (see Table A-1), belonging to families 1 and 12. Particularly preferred biparatopic polypeptides in accordance with the invention are those shown in the Examples described herein and in Table A-3.

Accordingly, preferred combination of immunoglobulin single variable domains that belong to families 1 and 12 present in the multiparatopic (such as biparatopic or triparatopic) polypeptide of the invention may encompass any of the following:

- the first immunoglobulin single variable domain belongs to family 12; and the second immunoglobulin single variable belongs to family 1;
- the first immunoglobulin single variable domain belongs to family 1; and the second immunoglobulin single variable belongs to family 12;
- the first immunoglobulin single variable domain belongs to family 1; and the second immunoglobulin single variable belongs to family 1;
- the first immunoglobulin single variable domain belongs to family 12; and the second immunoglobulin single variable belongs to family 12;

- the first immunoglobulin single variable domain has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 1-64 and has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 1-64; and the second immunoglobulin single variable domain has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 65-123 and has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) wih 89% or more sequence identity compared to any one of SEQ ID NOs: 65-123;
- the first immunoglobulin single variable domain has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 65-123 and has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 65-123; and the second immunoglobulin single variable domain has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 1-64 and has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 1-64;
- the first immunoglobulin single variable domain has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 65-123 and has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 65-123; and the second immunoglobulin single variable domain has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 65-123 and has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 65-123; or
- the first immunoglobulin single variable domain has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 1-64 and has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 1-64; and the second immunoglobulin single variable domain has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 65-123 and has an amino acid sequence between position 8 and position 106

(according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 1-64.

The multivalent polypeptides of the invention may modulate and/or partially or fully inhibit the function of Kv1.3. More particularly, the multivalent polypeptides of the present invention may depolarize the T cell membrane and/or reduce or even totally inhibit the efflux of potassium ions from T-cells. As such, the multivalent polypeptides of the invention may inhibit proliferation of T-cells and/or suppress activation of T-cells resulting of the inhibition of certain immune responses in vivo.

In one particular aspect, the multivalent polypeptides of the invention indirectly modulate the function of Kv1.3, i.e. as an allosteric modulator (as defined herein). More specifically, the multivalent polypeptides of the invention may induce a conformational change within the structure of the Kv1.3 pore.

Binding of the multivalent polypeptides of the invention to Kv1.3 can be measured in binding assays that preserve that conformation of the Kv1.3 target. Typical assays include (without being limiting) assays in which Kv1.3 is exposed on a cell surface (such as e.g. CHO cells, HEK cells, HeLa cells, Chinese Hamster Lung (CHL) cells, etc.). A preferred assay for measuring binding of the multivalent polypeptides of the invention to Kv1.3 is a FACS assay, such as e.g. the FACS assay as described in the examples, wherein binding of the multivalent polypeptides of the invention to Kv1.3 expressed on CHO-K1 cells and/or HEK293H cells is determined. Some preferred EC50 values for binding of the multivalent polypeptides of the invention to Kv1.3 will become clear from the further description and examples herein.

In such FACS binding assay, the multivalent polypeptides of the present invention may have EC50 values in binding human Kv1.3 of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such FACS binding assay, the immunoglobulins and/or polypeptides of the present invention may have EC50 values in binding human Kv1.3 between 10^{-10} M and 10^{-8} M, such as between 10^{-9} M and 10^{-8} M or between 10^{-10} M and 10^{-9} M. More particularly, multivalent polypeptides of the present invention that comprise 2 or more monovalent polypeptides belonging to family 12 may have EC50 values in binding human Kv1.3 between 10^{-10} M and 10^{-8} M, such as between 10^{-10} M and 10^{-9} M. Biparatopic polypeptides of the present invention that comprise 2 monovalent polypeptides belonging to different families (e.g. one monovalent polypeptide belonging to family 1 and one monovalent polypeptide belonging to

family 12) 12 may have EC50 values in binding human Kv1.3 between 10^{-10} M and 10^{-8} M, such as between 10^{-10} M and 10^{-9} M. Multivalent polypeptides of the present invention that comprise 2 or more monovalent polypeptides belonging to family 1 may have EC50 values in binding human Kv1.3 between 10^{-10} M and 10^{-8} M, such as between 10^{-9} M and 10^{-8} M.

In such FACS binding assay, the multivalent polypeptides of the present invention may have EC50 values in binding cyno Kv1.3 of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such FACS binding assay, the multivalent polypeptides of the present invention may have EC50 values in binding cyno Kv1.3 between 10^{-10} M and 10^{-7} M, such as between 10^{-10} M and 10^{-9} M.

In such FACS binding assay, the multivalent polypeptides of the present invention may have EC50 values in binding rat Kv1.3 of 10^{-6} M or lower, preferably of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such FACS binding assay, the multivalent polypeptides of the present invention may have EC50 values in binding rat Kv1.3 between 10^{-10} M and 10^{-8} M, such as between 10^{-10} M and 10^{-9} M, or between 10^{-9} M and 10^{-8} M. More particularly, multivalent polypeptides of the present invention that comprise 2 or more monovalent polypeptides belonging to family 12 may have EC50 values in binding rat Kv1.3 between 10^{-10} M and 10^{-8} M, such as between 10^{-10} M and 10^{-9} M. Biparatopic polypeptides of the present invention that comprise 2 monovalent polypeptides belonging to different families (e.g. one monovalent polypeptide belonging to family 1 and one monovalent polypeptide belonging to family 12) 12 may have EC50 values in binding rat Kv1.3 between 10^{-10} M and 10^{-8} M, such as between 10^{-10} M and 10^{-9} M. Multivalent polypeptides of the present invention that comprise 2 or more monovalent polypeptides belonging to family 1 may have EC50 values in binding ray Kv1.3 between 10^{-10} M and 10^{-8} M, such as between 10^{-9} M and 10^{-8} M.

Modulation and/or inhibition of the efflux of potassium ions can be determined by a variety of ion channel screening technologies including (without being limiting) ion flux assays, radioligand binding studies, fluorescent dye assays, and electrophysiology, such as voltage clamp, and in particular, the patch-clamp. An overview of different ion channel technologies is provided by e.g. Dabrowski et al. (CNS & Neurological Disorders Drug Targets 7: 122, 2008), Lü and An (Comb. Chem. High Throughput Screen. 11:185-94, 2008), and Zheng et al. (Assay Drug Dev. Technol. 2: 543-52, 2004).

Voltage clamp (Huxley, Trends Neurosci. 25: 553-8, 2002) is used to measure the ion currents through the membrane of excitable cells. The patch-clamp variant of this technique (Hamill et al. Pflügers Archiv European Journal of Physiology 391: 85–100, 1981) allows the study of single or multiple ion channels in cells.

Higher throughput electrophysiological platforms have been developed ranging from medium throughput systems to higher throughput platforms (see e.g. Southan and Clark, Methods Mol. Biol. 565: 187-208, 2009), including PatchXpress (Molecular Devices; Ghetti et al. Methods Mol. Biol. 403: 59-69, 2007), Qpatch and Qpatch HTX (Sophion; Mathes et al. Comb. Chem. High Throughput Screen. 12: 78-95, 2009; Korsgaard et al. Comb. Chem. High Throughput Screen. 12: 51-63, 2009), PatchLiner (Nanion; Farre et al. Comb. Chem. High Throughput Screen 12: 24-37, 2009), IonWorks® HT, IonWorks® Quattro and IonFlux™ Systems (Molecular Devices; Jow et al. J Biomol. Screen. 12: 1059-67, 2007; Dale et al. Mol. Biosyst. 3: 714-22, 2007). Some preferred IC50 values for the polypeptides of the invention in these assays will become clear from the further description and examples herein.

On the IonFlux™ (Molecular Devices) using Kv1.3-expressing HEK293H cells, for example, the multivalent polypeptides of the invention have IC50 values of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in this automated Patch Clamp assay, the multivalent polypeptides of the present invention may have IC50 values between 10^{-10} M and 10^{-7} M, between 10^{-10} M and 10^{-8} M, between 10^{-10} M and 10^{-9} M, such as e.g. between 10^{-9} M and 10^{-8} M, between 10^{-8} M and 10^{-7} M or between 10^{-10} M and 10^{-9} M. More particularly, multivalent polypeptides of the present invention that comprise 2 or more monovalent polypeptides belonging to family 12 may have IC50 values on the IonFlux™ (Molecular Devices) between 10^{-10} M and 10^{-7} M, such as between 10^{-10} M and 10^{-9} M. Biparatopic polypeptides of the present invention that comprise 2 monovalent polypeptides belonging to different families (e.g. one monovalent polypeptide belonging to family 1 and one monovalent polypeptide belonging to family 12) may have IC50 values on the IonFlux™ (Molecular Devices) between 10^{-10} M and 10^{-7} M, such as between 10^{-9} M and 10^{-7} M.

On the IonWorks® Quattro (Molecular Devices) using Kv1.3-expressing Chinese Hamster Lung (CHL) cells, for example, the immunoglobulins and/or polypeptides of the invention have IC50 values of 10^{-} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, on this high-throughput planar perforated patch

clamp, the polypeptides of the present invention may have IC50 values between 10^{-10} M and 10^{-7} M, between 10^{-10} M and 10^{-8} M, between 10^{-10} M and 10^{-9} M, such as e.g. between 10^{-9} M and 10^{-8} M, between 10^{-8} M and 10^{-7} M or between 10^{-10} M and 10^{-9} M. More particularly, multivalent polypeptides of the present invention that comprise 2 or more monovalent polypeptides belonging to family 12 may have IC50 values on the IonWorks® Quattro (Molecular Devices) between 10^{-10} M and 10^{-7} M, such as between 10^{-10} M and 10^{-9} M. Biparatopic polypeptides of the present invention that comprise 2 monovalent polypeptides belonging to different families (e.g. one monovalent polypeptide belonging to family 1 and one monovalent polypeptide belonging to family 12) may have IC50 values on IonWorks® Quattro (Molecular Devices) between 10^{-10} M and 10^{-7} M, such as between 10^{-9} M and 10^{-7} M.

Modulation and/or inhibition of Kv1.3 by the multivalent polypeptides of the invention can also be assessed in radioligand binding studies. Binding studies with tritiated correolide (e.g. C20-29-[3H]dihydrocorreolide (diTC)) to a single class of sites in membranes prepared from CHO/Kv1.3 cells has been described by Felix et al. (Biochemistry 38: 4922-30, 1999). Knaus et al. (Biochemistry 34: 13627-13634, 1995) describes, for example, the binding of monoiodotyrosinyl margatoxin (125I-margatoxin) to heterotetrameric Kv channels in rat brain synaptic plasma membranes. Binding studies of 125I-margatoxin to plasma membranes prepared from either Jurkat cells, a human leukemic T cell line, or CHO cells stably transfected with the Shaker-type voltage-gated K+ channel, K(V)1.3 have been described by Helms et al. (Biochemistry. 36: 3737-44, 1997). Some preferred IC50 values for blocking 125I-margatoxin binding to Kv1.3 by the multivalent polypeptides of the invention will become clear from the further description and examples herein.

The multivalent polypeptides of the present invention may block binding of 125I-margatoxin to cynomolgus Kv1.3 overexpressing CHO cells with IC50 values of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower. For example, in such 125I-margatoxin blocking assay, the multivalent polypeptides of the present invention may have IC50 values between 10^{-10} M and 10^{-8} M, such as e.g. between 10^{-10} M and 10^{-9} M.

Other flux assays for measuring modulation and/or inhibition of Kv1.3 by the multivalent polypeptides of the invention include (without being limiting) the high-throughput efflux assay with radiolabelled^{86} Rubidium described by Hanson et al. (Br. J. Pharmacol. 126: 1707-16, 1999), the nonradioactive rubidium (Rb(+)) efflux assay described by Wang et al. (Assay Drug

Dev. Technol. 2: 525-34, 2004) and a fluorescence-based thallium flux assay (Weaver et al. J. Biomol. Screen. 9: 671-7, 2004).

Inhibition of T-cell activation and/or proliferation by the multivalent polypeptides of the present invention can be measured in T-cell activation assays. Without being limiting, T-cell activation assays have been described by Nguyten et al. (Molecular Pharmacology 50: 1672-1679, 1996) and Hanson et al. (Br. J. Pharmacol. 126: 1707–1716, 1999). Some preferred IC50 values for inhibition of T-cell activation and/or proliferation by the multivalent polypeptides of the invention will become clear from the further description and examples herein.

In a T-cell activation assay with $CCR7^-$ $CD45RA^-$ T cells stimulated with anti-CD3 antibody OKT3 (as described in the Examples 4.4 and 5.5), the polypeptides of the invention have IC50 values for inhibiting IFNgamma production of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, 10^{-10} M or lower, or even of 10^{-11} M or lower. For example, in this T-cell activation assay, the polypeptides of the present invention inhibit IFNgamma production with IC50 values between 10^{-11} M and 10^{-7} M, between 10^{-11} M and 10^{-8} M, between 10^{-11} M and 10^{-9} M, such as e.g. between 10^{-11} M and 10^{-9} M, between 10^{-10} M and 10^{-9} M, or between 10^{-11} M and 10^{-10} M. More particularly, multivalent polypeptides of the present invention that comprise 2 or more monovalent polypeptides belonging to family 12 may inhibit IFNgamma production with IC50 values between 10^{-10} M and 10^{-9} M, such as between 10^{-11} M and 10^{-10} M. Biparatopic polypeptides of the present invention that comprise 2 monovalent polypeptides belonging to different families (e.g. one monovalent polypeptide belonging to family 1 and one monovalent polypeptide belonging to family 12) may inhibit IFNgamma production with IC50 values between 10^{-17} M and 10^{-9} M, such as between 10^{-10} M and 10^{-9} M.

In this T-cell activation assay with $CCR7^-CD45RA^-$ T cells stimulated with anti-CD3 antibody OKT3 (as described in the Examples 4.4 and 5.5), the multivalent polypeptides of the invention have IC50 values for inhibiting CD25 upregulation of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, 10^{-10} M or lower, or even of 10^{-11} M or lower. For example, in this T-cell activation assay, the multivalent polypeptides of the present invention inhibit CD25 upregulation with IC50 values between 10^{-11} M and 10^{-7} M, between 10^{-11} M and 10^{-8} M, between 10^{-11} M and 10^{-9} M, such as e.g. between 10^{-11} M and 10^{-9} M, between 10^{-10} M and 10^{-9} M or between 10^{-11} M and 10^{-10} M.

In a cell activation assay with peripheral blood mononucleated cells (PBMCs) stimulated with anti-CD3 antibody OKT3 and anti-CD28 (as described in Example 9), the multivalent polypeptides of the invention do not block IFNgamma production.

Immunosuppressive effects of the multivalent polypeptides of the invention can further be evaluated in in vivo models, such as e.g. in rats, pigs and/or primates. Diabetes-prone Biobreeding Worchester rats have been used as a model for autoimmune diabetes (Beeton et al. Proc Natl Acad Sci U S A. 103: 17414-9, 2006). A rat model for allergic contact dermatitis, an animal model for psoriasis, has been described by Azam et al. (J. Invest. Dermatol. 127: 1419-29, 2007). Immunodeficient mice reconstituted with human T cells have been used as animal model for T cell-mediated skin graft rejection (Ren et al. PLoS One 3:e4009, 2008). For example, in the rat model for allergic contact dermatitis as described in Example 12 and 13, the polypeptides of the invention (significantly) reduce the increase in ear thickness with at least about 0.085-0.102 mm and at least about 0.147-0.164 mm versus vehicle, respectively.

Furthermore, the multivalent polypeptides of the invention demonstrated a dramatically improved interspecies cross-reactivity and potency. In addition, a more than 1000 fold, and even up to 10000 fold selectivity (as defined herein) over the closest related ion channels (such as e.g. hERG, KCa3.1 (SK4), Kv4.3/KChIP2.2, Kv1.2, Kv1.4, Cav1.3/b3/a2d1, Kir2.1, KCa2.2, KCa2.3, Kv7.2/Kv7.3, Kv1.1, Kv1.5, Kv3.4, Nav1.1, Nav1.2 and Nav1.6) was reported. More specifically, the multivalent polypeptides show more than 1000 fold, and even up to 10000 fold selectivity for modulating and/or inhibiting the activity of Kv1.3 over other related Kv ion channel family members. The selective inhibition by the multivalent polypeptides of the present invention can be determined e.g. by comparing the concentration of polypeptide needed for inhibiting the respective channel with the concentration of polypeptide needed for inhibiting Kv1.3. More in particular the multivalent polypeptides show a more than 1000 fold, and even up to 10000 fold selectivity over Kv1.5, Kv1.6, and hERG.

Compounds, constructs and/or polypeptides of the invention

The monovalent polypeptide of the invention and the multivalent polypeptide of the invention, may or may not further comprise one or more other groups, residues, moieties or binding units (these monovalent polypeptides as well as multivalent polypeptides (with or without additional groups, residues, moieties or binding units) are all referred to as (*"compound(s) of the*

invention, construct(s) of the invention and/or polypeptide(s) of the invention"). If present, such further groups, residues, moieties or binding units may or may not provide further functionality to the immunoglobulin single variable domain (and/or to the polypeptide in which it is present) and may or may not modify the properties of the immunoglobulin single variable domain.

For example, such further groups, residues, moieties or binding units may be one or more additional amino acid sequences, such that the polypeptide is a (fusion) protein or (fusion) polypeptide. In a preferred but non-limiting aspect, said one or more other groups, residues, moieties or binding units are immunoglobulins. Even more preferably, said one or more other groups, residues, moieties or binding units are immunoglobulin single variable domains chosen from the group consisting of Domain antibodies, amino acids that are suitable for use as a domain antibody, single domain antibodies, amino acids that are suitable for use as a single domain antibody, "dAb"'s, amino acids that are suitable for use as a dAb, Nanobodies (such as e.g. VHH, humanized VHH or camelized VH sequences).

As described above, additional binding units, such as immunoglobulin single variable domains having different antigen specificity can be linked to form multispecific polypeptides. By combining immunoglobulin single variable domains of two or more specificities, bispecific, trispecific etc. constructs can be formed. For example, a polypeptide according to the invention may comprise one, two or more immunoglobulin single variable domains directed against Kv1.3 and one immunoglobulin single variable domain against another target. Such constructs and modifications thereof, which the skilled person can readily envisage, are all encompassed by the term *"compound of the invention, construct of the invention and/or polypeptide of the invention"* as used herein.

In the compounds, constructs and/or polypeptides described above, the one, two or more immunoglobulin single variable domains and the one or more groups, residues, moieties or binding units may be linked directly to each other and/or via one or more suitable linkers or spacers. For example, when the one or more groups, residues, moieties or binding units are amino acid sequences, the linkers may also be amino acid sequences, so that the resulting polypeptide is a fusion (protein) or fusion (polypeptide).

The one or more further groups, residues, moieties or binding units may be any suitable and/or desired amino acid sequences. The further amino acid sequences may or may not change, alter or otherwise influence the (biological) properties of the polypeptide of the invention,

and may or may not add further functionality to the polypeptide of the invention. Preferably, the further amino acid sequence is such that it confers one or more desired properties or functionalities to the polypeptide of the invention.

Example of such amino acid sequences will be clear to the skilled person, and may generally comprise all amino acid sequences that are used in peptide fusions based on conventional antibodies and fragments thereof (including but not limited to ScFv's and single domain antibodies). Reference is for example made to the review by Holliger and Hudson (Nature Biotechnology 23: 1126-1136, 2005).

For example, such an amino acid sequence may be an amino acid sequence that increases the half-life, the solubility, or the absorption, reduces the immunogenicity or the toxicity, eliminates or attenuates undesirable side effects, and/or confers other advantageous properties to and/or reduces the undesired properties of the compound, construct and/or polypeptide of the invention, compared to polypeptide of the invention per se. Some non-limiting examples of such amino acid sequences are serum proteins, such as human serum albumin (see for example WO 00/27435) or haptenic molecules (for example haptens that are recognized by circulating antibodies, see for example WO 98/22141).

In one specific aspect of the invention, a polypeptide is prepared that has an increased half-life, compared to the corresponding polypeptide of the invention. Examples of polypeptides of the invention that comprise such half-life extending moieties for example include, without limitation, polypeptides in which the immunoglobulin single variable domains are suitable linked to one or more serum proteins or fragments thereof (such as (human) serum albumin or suitable fragments thereof) or to one or more binding units that can bind to serum proteins (such as, for example, Domain antibodies, amino acids that are suitable for use as a domain antibody, single domain antibodies, amino acids that are suitable for use as a single domain antibody, "dAb"'s, amino acids that are suitable for use as a dAb, Nanobodies, VHH sequences, humanized VHH sequences or camelized VH sequences) that can bind to serum proteins (such as serum albumin (such as human serum albumin)), serum immunoglobulins (such as IgG), transferrin or one of the other serum proteins listed in WO 04/003019; polypeptides in which the immunoglobulin single variable domain is linked to an Fc portion (such as a human Fc) or a suitable part or fragment thereof; or polypeptides in which the one or more immunoglobulin single variable domains are suitable linked to one or more small proteins or peptides that can bind to serum proteins (such as,

without limitation, the proteins and peptides described in WO 91/01743, WO 01/45746 or WO 02/076489). Reference is also made to the dAb's described in WO 03/002609 and WO 04/003019 and to Harmsen et al. (Vaccine 23: 4926-42, 2005); to EP 0368684, as well as to WO 08/028977, WO 08/043821, WO 08/043822 by Ablynx N.V. and WO 08/068280.

According to a specific, but non-limiting aspect of the invention, the polypeptides of the invention may contain, besides the one or more immunoglobulin single variable domains and/or monovalent polypeptides of the invention against Kv1.3, at least one immunoglobulin single variable domain against human serum albumin. These immunoglobulin single variable domains against human serum albumin may be as generally described in the applications by Ablynx N.V. cited above (see for example WO 04/062551). Some particularly preferred Nanobodies that provide for increased half-life and that can be used in the polypeptides of the invention include the Nanobodies ALB-1 to ALB-10 disclosed in WO 06/122787 (see Tables II and III) of which ALB-8 (SEQ ID NO: 62 in WO 06/122787) is particularly preferred, as well as the Nanobodies disclosed in WO 2012/175400 (SEQ ID NOs: 1-11 of WO 2012/175400).

The polypeptide of the invention may, for example, be a trivalent, bispecific polypeptide, comprising two immunoglobulin single variable domains, preferably monovalent polypeptides of the invention against Kv1.3 and a third immunoglobulin single variable domain directed against (human) serum albumin, in which said first, second and third immunoglobulin single variable domain may optionally be linked via one or more, and in particular two, linker sequences.

According to one specific aspect, one or more polypeptides of the invention may be linked (optionally via a suitable linker or hinge region) to one or more constant domains (for example, 2 or 3 constant domains that can be used as part of/to form an Fc portion), to an Fc portion and/or to one or more antibody parts, fragments or domains that confer one or more effector functions to the polypeptide of the invention and/or may confer the ability to bind to one or more Fc receptors. For example, for this purpose, and without being limited thereto, the one or more further amino acid sequences may comprise one or more C_H2 and/or C_H3 domains of an antibody, such as from a heavy chain antibody (as described herein) and more preferably from a conventional human 4-chain antibody; and/or may form (part of) and Fc region, for example from IgG (e.g. from IgG1, IgG2, IgG3 or IgG4), from IgE or from another human Ig such as IgA, IgD or IgM. For example, WO 94/04678 describes heavy chain antibodies comprising a Camelid V_{HH}

domain or a humanized derivative thereof (i.e. a Nanobody), in which the Camelidae C_H2 and/or C_H3 domain have been replaced by human C_H2 and C_H3 domains, so as to provide an immunoglobulin that consists of 2 heavy chains each comprising a Nanobody and human C_H2 and C_H3 domains (but no C_H1 domain), which immunoglobulin has the effector function provided by the C_H2 and C_H3 domains and which immunoglobulin can function without the presence of any light chains. Other amino acid sequences that can be suitably linked to the polypeptides of the invention so as to provide an effector function will be clear to the skilled person, and may be chosen on the basis of the desired effector function(s). Reference is for example made to WO 04/058820, WO 99/42077, WO 02/056910 and WO 05/017148, as well as the review by Holliger and Hudson, supra; and to WO 09/068628. Coupling of a polypeptide of the invention to an Fc portion may also lead to an increased half-life, compared to the corresponding polypeptide of the invention. For some applications, the use of an Fc portion and/or of constant domains (*i.e.,* C_H2 and/or C_H3 domains) that confer increased half-life without any biologically significant effector function may also be suitable or even preferred. Other suitable constructs comprising one or more polypeptides of the invention and one or more constant domains with increased half-life *in vivo* will be clear to the skilled person, and may for example comprise polypeptides linked to a C_H3 domain, optionally via a linker sequence. Generally, any fusion protein or derivatives with increased half-life will preferably have a molecular weight of more than 50 kD, the cut-off value for renal absorption.

In another specific, but non-limiting, aspect, the polypeptides of the invention may be linked (optionally via a suitable linker or hinge region) to naturally occurring, synthetic or semi-synthetic constant domains (or analogs, variants, mutants, parts or fragments thereof) that have a reduced (or essentially no) tendency to self-associate into dimers (i.e. compared to constant domains that naturally occur in conventional 4-chain antibodies). Such monomeric (i.e. not self-associating) Fc chain variants, or fragments thereof, will be clear to the skilled person. For example, Helm et al. (J. Biol. Chem. 271: 7494, 1996), describe monomeric Fc chain variants that can be used in the polypeptide chains of the invention.

Also, such monomeric Fc chain variants are preferably such that they are still capable of binding to the complement or the relevant Fc receptor(s) (depending on the Fc portion from which they are derived), and/or such that they still have some or all of the effector functions of the Fc portion from which they are derived (or at a reduced level still suitable for the intended use).

Alternatively, in such a polypeptide chain of the invention, the monomeric Fc chain may be used to confer increased half-life upon the polypeptide chain, in which case the monomeric Fc chain may also have no or essentially no effector functions.

Generally, the polypeptides of the invention with increased half-life preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding immunoglobulin single variable domain or polypeptide of the invention *per se*.

Generally, the polypeptides of the invention with increased half-life preferably have a half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the half-life of the corresponding immunoglobulin single variable domain or polypeptide of the invention *per se*.

In another preferred, but non-limiting aspect, such polypeptides of the invention exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

The further amino acid residues may or may not change, alter or otherwise influence other (biological) properties of the polypeptide of the invention and may or may not add further functionality to the polypeptide of the invention. For example, such amino acid residues:

a) can comprise an N-terminal Met residue, for example as result of expression in a heterologous host cell or host organism.

b) may form a signal sequence or leader sequence that directs secretion of the polypeptide from a host cell upon synthesis (for example to provide a pre-, pro- or prepro- form of the polypeptide of the invention, depending on the host cell used to express the polypeptide of the invention). Suitable secretory leader peptides will be clear to the skilled person, and may be as further described herein. Usually, such a leader sequence will be linked to the N-

terminus of the polypeptide, although the invention in its broadest sense is not limited thereto;

c) may form a "tag", for example an amino acid sequence or residue that allows or facilitates the purification of the polypeptide, for example using affinity techniques directed against said sequence or residue. Thereafter, said sequence or residue may be removed (e.g. by chemical or enzymatical cleavage) to provide the polypeptide (for this purpose, the tag may optionally be linked to the amino acid sequence or polypeptide sequence via a cleavable linker sequence or contain a cleavable motif). Some preferred, but non-limiting examples of such residues are multiple histidine residues, glutathione residues and a myc-tag such as AAAEQKLISEEDLNGAA (SEQ ID NO: 206);

d) may be one or more amino acid residues that have been functionalized and/or that can serve as a site for attachment of functional groups. Suitable amino acid residues and functional groups will be clear to the skilled person and include, but are not limited to, the amino acid residues and functional groups mentioned herein for the derivatives of the polypeptides of the invention.

The multivalent polypeptides of the invention can generally be prepared by a method which comprises at least the step of suitably linking the immunoglobulin single variable domain and/or monovalent polypeptide of the invention to one or more further immunoglobulin single variable domains and/or monovalent polypeptides of the invention, optionally via the one or more suitable linkers, so as to provide the multivalent polypeptide of the invention. Polypeptides of the invention can also be prepared by a method which generally comprises at least the steps of providing a nucleic acid that encodes a polypeptide of the invention, expressing said nucleic acid in a suitable manner, and recovering the expressed polypeptide of the invention. Such methods can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the methods and techniques further described herein.

A method for preparing multivalent polypeptides of the invention may comprise at least the steps of linking two or more immunoglobulin single variable domains and/or monovalent polypeptides of the invention and for example one or more linkers together in a suitable manner. The immunoglobulin single variable domains and/or monovalent polypeptides of the invention (and linkers) can be coupled by any method known in the art and as further described herein. Preferred techniques include the linking of the nucleic acid sequences that encode the

immunoglobulin single variable domains and/or monovalent polypeptides of the invention (and linkers) to prepare a genetic construct that expresses the multivalent polypeptide. Techniques for linking amino acids or nucleic acids will be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook et al. and Ausubel et al., mentioned above, as well as the Examples below.

Accordingly, the present invention also relates to the use of an immunoglobulin single variable domain and/or monovalent polypeptide of the invention in preparing a multivalent polypeptide of the invention. The method for the preparation of a multivalent polypeptide will comprise the linking of an immunoglobulin single variable domain and/or monovalent polypeptide of the invention to at least one further immunoglobulin single variable domain and/or monovalent polypeptide of the invention, optionally via one or more linkers. The immunoglobulin single variable domain and/or monovalent polypeptide of the invention is then used as a binding domain or binding unit in providing and/or preparing the multivalent polypeptide comprising two (*e.g.,* in a bivalent polypeptide), three (*e.g.,* in a trivalent polypeptide), four (e.g., in a tetravalent) or more (*e.g.,* in a multivalent polypeptide) binding units. In this respect, the immunoglobulin singe variable domain and/or the monovalent polypeptide of the invention may be used as a binding domain or binding unit in providing and/or preparing a multivalent, such as bivalent, trivalent or tetravalent polypeptide of the invention comprising two, three, four or more binding units.

Accordingly, the present invention also relates to the use of an immunoglobulin single variable domain and/or particularly, a monovalent polypeptide of the invention (as described herein) in preparing a multivalent polypeptide. The method for the preparation of the multivalent polypeptide will comprise the linking of the immunoglobulin single variable domain and/or monovalent polypeptide of the invention to at least one further immunoglobulin single variable domain and/or monovalent polypeptide of the invention, optionally via one or more linkers.

Suitable spacers or linkers for use in multivalent polypeptides of the invention will be clear to the skilled person, and may generally be any linker or spacer used in the art to link amino acid sequences. Preferably, said linker or spacer is suitable for use in constructing polypeptides that are intended for pharmaceutical use.

Some particularly preferred spacers include the spacers and linkers that are used in the art to link antibody fragments or antibody domains. These include the linkers mentioned in the general background art cited above, as well as for example linkers that are used in the art to

construct diabodies or ScFv fragments (in this respect, however, it should be noted that, whereas in diabodies and in ScFv fragments, the linker sequence used should have a length, a degree of flexibility and other properties that allow the pertinent V_H and V_L domains to come together to form the complete antigen-binding site, there is no particular limitation on the length or the flexibility of the linker used in the polypeptide of the invention, since each immunoglobulin single variable domain by itself forms a complete antigen-binding site).

For example, a linker may be a suitable amino acid sequence, and in particular amino acid sequences of between 1 and 50, preferably between 1 and 30, such as between 1 and 10 amino acid residues. Some preferred examples of such amino acid sequences include gly-ser linkers, for example of the type $(gly_xser_y)_z$, such as (for example $(gly_4ser)_3$ or $(gly_3ser_2)_3$, as described in WO 99/42077, hinge-like regions such as the hinge regions of naturally occurring heavy chain antibodies or similar sequences (such as described in WO 94/04678).

Some other particularly preferred linkers are mentioned in Table A-8, of which GS35 (SEQ ID NO: 489) is particularly preferred.

Other suitable linkers generally comprise organic compounds or polymers, in particular those suitable for use in proteins for pharmaceutical use. For instance, poly(ethyleneglycol) moieties have been used to link antibody domains, see for example WO 04/081026.

It is encompassed within the scope of the invention that the length, the degree of flexibility and/or other properties of the linker(s) used (although not critical, as it usually is for linkers used in ScFv fragments) may have some influence on the properties of the final polypeptide of the invention, including but not limited to the affinity, specificity or avidity for Kv1.3, or for one or more of the other antigens. Based on the disclosure herein, the skilled person will be able to determine the optimal linker(s) for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

It is also within the scope of the invention that the linker(s) used confer one or more other favourable properties or functionality to the polypeptides of the invention, and/or provide one or more sites for the formation of derivatives and/or for the attachment of functional groups (*e.g.*, as described herein for the derivatives of the polypeptides of the invention). For example, linkers containing one or more charged amino acid residues can provide improved hydrophilic properties, whereas linkers that form or contain small epitopes or tags can be used for the purposes of detection, identification and/or purification. Again, based on the disclosure herein,

the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

Finally, when two or more linkers are used in the polypeptides of the invention, these linkers may be the same or different. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

Usually, for ease of expression and production, a polypeptide of the invention will be a linear polypeptide. However, the invention in its broadest sense is not limited thereto. For example, when a polypeptide of the invention comprises three of more amino acid sequences or Nanobodies, it is possible to link them by use of a linker with three or more "arms", which each "arm" being linked to an amino acid sequence or Nanobody, so as to provide a "star-shaped" construct. It is also possible, although usually less preferred, to use circular constructs.

Also encompassed in the present invention are compounds, constructs and/or polypeptides that comprise an immunoglobulin or polypeptide of the invention and further comprising tags or other functional moieties, *e.g.,* toxins, labels, radiochemicals, etc..

Alternatively, the additional groups, residues, moieties or binding units may for example be chemical groups, residues, moieties, which may or may not by themselves be biologically and/or pharmacologically active. For example, and without limitation, such groups may be linked to the one or more immunoglobulin single variable domains or monovalent polypeptides of the invention so as to provide a "derivative" of the polypeptide of the invention.

Accordingly, the invention in its broadest sense also comprises compounds, constructs and/or polypeptides that are derivatives of the polypeptides of the invention. Such derivatives can generally be obtained by modification, and in particular by chemical and/or biological (*e.g.,* enzymatical) modification, of the polypeptides of the invention and/or of one or more of the amino acid residues that form polypeptide of the invention.

Examples of such modifications, as well as examples of amino acid residues within the polypeptide sequences that can be modified in such a manner (i.e. either on the protein backbone but preferably on a side chain), methods and techniques that can be used to introduce such modifications and the potential uses and advantages of such modifications will be clear to the skilled person (see also Zangi et al., Nat Biotechnol 31(10):898-907, 2013).

For example, such a modification may involve the introduction (*e.g.,* by covalent linking or in any other suitable manner) of one or more functional groups, residues or moieties into or onto the polypeptide of the invention, and in particular of one or more functional groups, residues or moieties that confer one or more desired properties or functionalities to the polypeptide of the invention. Example of such functional groups will be clear to the skilled person.

For example, such modification may comprise the introduction (*e.g.,* by covalent binding or in any other suitable manner) of one or more functional groups that that increase the half-life, the solubility and/or the absorption of the polypeptide of the invention, that reduce the immunogenicity and/or the toxicity of the polypeptide of the invention, that eliminate or attenuate any undesirable side effects of the polypeptide of the invention, and/or that confer other advantageous properties to and/or reduce the undesired properties of the polypeptide of the invention; or any combination of two or more of the foregoing. Examples of such functional groups and of techniques for introducing them will be clear to the skilled person, and can generally comprise all functional groups and techniques mentioned in the general background art cited hereinabove as well as the functional groups and techniques known per se for the modification of pharmaceutical proteins, and in particular for the modification of antibodies or antibody fragments (including ScFv's and single domain antibodies), for which reference is for example made to Remington (Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, PA, 1980). Such functional groups may for example be linked directly (for example covalently) to a polypeptide of the invention, or optionally via a suitable linker or spacer, as will again be clear to the skilled person.

One specific example is a derivative polypeptide of the invention wherein the polypeptide of the invention has been chemically modified to increase the half-life thereof (for example, by means of pegylation). This is one of the most widely used techniques for increasing the half-life and/or reducing the immunogenicity of pharmaceutical proteins and comprises attachment of a suitable pharmacologically acceptable polymer, such as poly(ethyleneglycol) (PEG) or derivatives thereof (such as methoxypoly(ethyleneglycol) or mPEG). Generally, any suitable form of pegylation can be used, such as the pegylation used in the art for antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv's); reference is made to for example Chapman (Nat. Biotechnol. 54: 531-545, 2002), Veronese and Harris (Adv. Drug Deliv. Rev. 54: 453-456, 2003), Harris and Chess (Nat. Rev. Drug. Discov. 2: 214-221, 2003) and WO

04/060965. Various reagents for pegylation of proteins are also commercially available, for example from Nektar Therapeutics, USA.

Preferably, site-directed pegylation is used, in particular via a cysteine-residue (see for example Yang et al. (Protein Engineering 16: 761-770, 2003). For example, for this purpose, PEG may be attached to a cysteine residue that naturally occurs in a polypeptide of the invention, a polypeptide of the invention may be modified so as to suitably introduce one or more cysteine residues for attachment of PEG, or an amino acid sequence comprising one or more cysteine residues for attachment of PEG may be fused to the N- and/or C-terminus of a polypeptide of the invention, all using techniques of protein engineering known per se to the skilled person.

Preferably, for the polypeptides of the invention, a PEG is used with a molecular weight of more than 5000, such as more than 10,000 and less than 200,000, such as less than 100,000; for example in the range of 20,000-80,000.

Another, usually less preferred modification comprises N-linked or O-linked glycosylation, usually as part of co-translational and/or post-translational modification, depending on the host cell used for expressing the polypeptide of the invention.

Yet another modification may comprise the introduction of one or more detectable labels or other signal-generating groups or moieties, depending on the intended use of the labelled polypeptide of the invention. Suitable labels and techniques for attaching, using and detecting them will be clear to the skilled person, and for example include, but are not limited to, fluorescent labels (such as fluorescein, isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine and fluorescent metals such as ^{152}Eu or others metals from the lanthanide series), phosphorescent labels, chemiluminescent labels or bioluminescent labels (such as luminal, isoluminol, theromatic acridinium ester, imidazole, acridinium salts, oxalate ester, dioxetane or GFP and its analogs), radio-isotopes (such as ^{3}H, ^{125}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{36}Cl, ^{57}Co, ^{58}Co, ^{59}Fe, and ^{75}Se), metals, metals chelates or metallic cations (for example metallic cations such as ^{99m}Tc, ^{123}I, ^{111}In, ^{131}I, ^{97}Ru, ^{67}Cu, ^{67}Ga, and ^{68}Ga or other metals or metallic cations that are particularly suited for use in *in vivo*, *in vitro* or *in situ* diagnosis and imaging, such as (^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe)), as well as chromophores and enzymes (such as malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, biotinavidin peroxidase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β-

galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholine esterase). Other suitable labels will be clear to the skilled person, and for example include moieties that can be detected using NMR or ESR spectroscopy.

Such labelled polypeptides of the invention may for example be used for *in vitro*, *in vivo* or *in situ* assays (including immunoassays known per se such as ELISA, RIA, EIA and other "sandwich assays", etc.) as well as *in vivo* diagnostic and imaging purposes, depending on the choice of the specific label.

As will be clear to the skilled person, another modification may involve the introduction of a chelating group, for example to chelate one of the metals or metallic cations referred to above. Suitable chelating groups for example include, without limitation, diethyl-enetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

Yet another modification may comprise the introduction of a functional group that is one part of a specific binding pair, such as the biotin-(strept)avidin binding pair. Such a functional group may be used to link the polypeptide of the invention to another protein, polypeptide or chemical compound that is bound to the other half of the binding pair, i.e. through formation of the binding pair. For example, a polypeptide of the invention may be conjugated to biotin, and linked to another protein, polypeptide, compound or carrier conjugated to avidin or streptavidin. For example, such a conjugated polypeptide of the invention may be used as a reporter, for example in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin. Such binding pairs may for example also be used to bind the polypeptide of the invention to a carrier, including carriers suitable for pharmaceutical purposes. One non-limiting example are the liposomal formulations described by Cao and Suresh (Journal of Drug Targeting 8: 257, 2000). Such binding pairs may also be used to link a therapeutically active agent to the polypeptide of the invention.

Other potential chemical and enzymatical modifications will be clear to the skilled person. Such modifications may also be introduced for research purposes (e.g. to study function-activity relationships). Reference is for example made to Lundblad and Bradshaw (Biotechnol. Appl. Biochem. 26: 143-151, 1997).

Preferably, the compounds, constructs, polypeptides and/or derivatives are such that they bind to Kv1.3, with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50}

value, as further described herein) that is as defined herein (i.e. as defined for the polypeptides of the invention). Such derivatives will usually also have a Kv1.3 blocking efficacy and/or potency as defined herein.

Such compounds, constructs and/or polypeptides of the invention and derivatives thereof may also be in essentially isolated form (as defined herein).

The invention further relates to methods for preparing the compounds, constructs, polypeptides, nucleic acids, host cells, and compositions described herein.

The polypeptides and nucleic acids of the invention can be prepared in a manner known per se, as will be clear to the skilled person from the further description herein. For example, the polypeptides of the invention can be prepared in any manner known per se for the preparation of antibodies and in particular for the preparation of antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments). Some preferred, but non-limiting methods for preparing the polypeptides and nucleic acids include the methods and techniques described herein.

The method for producing a polypeptide of the invention may comprise the following steps:

- the expression, in a suitable host cell or host organism (also referred to herein as a "*host of the invention*") or in another suitable expression system of a nucleic acid that encodes said polypeptide of the invention (also referred to herein as a "*nucleic acid of the invention*"),

optionally followed by:

- isolating and/or purifying the polypeptide of the invention thus obtained.

In particular, such a method may comprise the steps of:

- cultivating and/or maintaining a host of the invention under conditions that are such that said host of the invention expresses and/or produces at least one polypeptide of the invention;

optionally followed by:

- isolating and/or purifying the polypeptide of the invention thus obtained.

Accordingly, the present invention also relates to a nucleic acid or nucleotide sequence that encodes a polypeptide of the invention (also referred to as "*nucleic acid of the invention*"). A nucleic acid of the invention can be in the form of single or double stranded DNA or RNA, and is preferably in the form of double stranded DNA. For example, the nucleotide sequences of the

invention may be genomic DNA, cDNA or synthetic DNA (such as DNA with a codon usage that has been specifically adapted for expression in the intended host cell or host organism).

According to one embodiment of the invention, the nucleic acid of the invention is in essentially isolated from, as defined herein. The nucleic acid of the invention may also be in the form of, be present in and/or be part of a vector, such as for example a plasmid, cosmid or YAC, which again may be in essentially isolated form.

The nucleic acids of the invention can be prepared or obtained in a manner known per se, based on the information on the polypeptides of the invention given herein, and/or can be isolated from a suitable natural source. Also, as will be clear to the skilled person, to prepare a nucleic acid of the invention, also several nucleotide sequences, such as at least two nucleic acids encoding an immunoglobulin single variable domain or a monovalent polypeptide of the invention and for example nucleic acids encoding one or more linkers can be linked together in a suitable manner.

Techniques for generating the nucleic acids of the invention will be clear to the skilled person and may for instance include, but are not limited to, automated DNA synthesis; site-directed mutagenesis; combining two or more naturally occurring and/or synthetic sequences (or two or more parts thereof), introduction of mutations that lead to the expression of a truncated expression product; introduction of one or more restriction sites (e.g. to create cassettes and/or regions that may easily be digested and/or ligated using suitable restriction enzymes), and/or the introduction of mutations by means of a PCR reaction using one or more "mismatched" primers. These and other techniques will be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook et al. and Ausubel et al., mentioned above, as well as to the Examples below.

The nucleic acid of the invention may also be in the form of, be present in and/or be part of a genetic construct, as will be clear to the person skilled in the art. Such genetic constructs generally comprise at least one nucleic acid of the invention that is optionally linked to one or more elements of genetic constructs known per se, such as for example one or more suitable regulatory elements (such as a suitable promoter(s), enhancer(s), terminator(s), etc.) and the further elements of genetic constructs referred to herein. Such genetic constructs comprising at least one nucleic acid of the invention will also be referred to herein as "*genetic constructs of the invention*".

The genetic constructs of the invention may be DNA or RNA (, and are preferably double-stranded DNA. The genetic constructs of the invention may also be in a form suitable for transformation of the intended host cell or host organism, in a form suitable for integration into the genomic DNA of the intended host cell or in a form suitable for independent replication, maintenance and/or inheritance in the intended host organism. For instance, the genetic constructs of the invention may be in the form of a vector, such as for example a plasmid, cosmid, YAC, a viral vector or transposon. In particular, the vector may be an expression vector, i.e. a vector that can provide for expression in vitro and/or in vivo (e.g. in a suitable host cell, host organism and/or expression system).

In a preferred but non-limiting embodiment, a genetic construct of the invention comprises

a) at least one nucleic acid of the invention; operably connected to

b) one or more regulatory elements, such as a promoter and optionally a suitable terminator; and optionally also

c) one or more further elements of genetic constructs known per se;

in which the terms "regulatory element", "promoter", "terminator" and "operably connected" have their usual meaning in the art (as further described herein); and in which said "further elements" present in the genetic constructs may for example be 3'- or 5'-UTR sequences, leader sequences, selection markers, expression markers/reporter genes, and/or elements that may facilitate or increase (the efficiency of) transformation or integration. These and other suitable elements for such genetic constructs will be clear to the skilled person, and may for instance depend upon the type of construct used; the intended host cell or host organism; the manner in which the nucleotide sequences of the invention of interest are to be expressed (e.g. via constitutive, transient or inducible expression); and/or the transformation technique to be used. For example, regulatory sequences, promoters and terminators known per se for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments) may be used in an essentially analogous manner.

Preferably, in the genetic constructs of the invention, said at least one nucleic acid of the invention and said regulatory elements, and optionally said one or more further elements, are "operably linked" to each other, by which is generally meant that they are in a functional relationship with each other. For instance, a promoter is considered "operably linked" to a coding

sequence if said promoter is able to initiate or otherwise control/regulate the transcription and/or the expression of a coding sequence (in which said coding sequence should be understood as being "under the control of" said promoter). Generally, when two nucleotide sequences are operably linked, they will be in the same orientation and usually also in the same reading frame. They will usually also be essentially contiguous, although this may also not be required.

Preferably, the regulatory and further elements of the genetic constructs of the invention are such that they are capable of providing their intended biological function in the intended host cell or host organism.

For instance, a promoter, enhancer or terminator should be "operable" in the intended host cell or host organism, by which is meant that (for example) said promoter should be capable of initiating or otherwise controlling/regulating the transcription and/or the expression of a nucleotide sequence - *e.g.,* a coding sequence - to which it is operably linked (as defined herein).

Some particularly preferred promoters include, but are not limited to, promoters known per se for the expression in the host cells mentioned herein; and in particular promoters for the expression in the bacterial cells, such as those mentioned herein and/or those used in the Examples.

A selection marker should be such that it allows - *i.e.,* under appropriate selection conditions - host cells and/or host organisms that have been (successfully) transformed with the nucleotide sequence of the invention to be distinguished from host cells/organisms that have not been (successfully) transformed. Some preferred, but non-limiting examples of such markers are genes that provide resistance against antibiotics (such as kanamycin or ampicillin), genes that provide for temperature resistance, or genes that allow the host cell or host organism to be maintained in the absence of certain factors, compounds and/or (food) components in the medium that are essential for survival of the non-transformed cells or organisms.

A leader sequence should be such that - in the intended host cell or host organism - it allows for the desired post-translational modifications and/or such that it directs the transcribed mRNA to a desired part or organelle of a cell. A leader sequence may also allow for secretion of the expression product from said cell. As such, the leader sequence may be any pro-, pre-, or prepro-sequence operable in the host cell or host organism. Leader sequences may not be required for expression in a bacterial cell. For example, leader sequences known per se for the

expression and production of antibodies and antibody fragments (including but not limited to single domain antibodies and ScFv fragments) may be used in an essentially analogous manner.

An expression marker or reporter gene should be such that - in the host cell or host organism - it allows for detection of the expression of (a gene or nucleotide sequence present on) the genetic construct. An expression marker may optionally also allow for the localisation of the expressed product, *e.g.,* in a specific part or organelle of a cell and/or in (a) specific cell(s), tissue(s), organ(s) or part(s) of a multicellular organism. Such reporter genes may also be expressed as a protein fusion with the amino acid sequence or polypeptide of the invention. Some preferred, but non-limiting examples include fluorescent proteins such as GFP.

Some preferred, but non-limiting examples of suitable promoters, terminator and further elements include those that can be used for the expression in the host cells mentioned herein; and in particular those that are suitable for expression in bacterial cells, such as those mentioned herein and/or those used in the Examples below. For some (further) non-limiting examples of the promoters, selection markers, leader sequences, expression markers and further elements that may be present/used in the genetic constructs of the invention - such as terminators, transcriptional and/or translational enhancers and/or integration factors - reference is made to the general handbooks such as Sambrook et al. and Ausubel et al. mentioned above, as well as to the examples that are given in WO 95/07463, WO 96/23810, WO 95/07463, WO 95/21191, WO 97/11094, WO 97/42320, WO 98/06737, WO 98/21355, US 7,207,410, US 5,693,492 and EP 1085089. Other examples will be clear to the skilled person. Reference is also made to the general background art cited above and the further references cited herein.

The genetic constructs of the invention may generally be provided by suitably linking the nucleotide sequence(s) of the invention to the one or more further elements described above, for example using the techniques described in the general handbooks such as Sambrook et al. and Ausubel et al., mentioned above.

Often, the genetic constructs of the invention will be obtained by inserting a nucleotide sequence of the invention in a suitable (expression) vector known per se. Some preferred, but non-limiting examples of suitable expression vectors are those used in the Examples below, as well as those mentioned herein.

The nucleic acids of the invention and/or the genetic constructs of the invention may be used to transform a host cell or host organism, *i.e.,* for expression and/or production of the

polypeptide of the invention. Suitable hosts or host cells will be clear to the skilled person, and may for example be any suitable fungal, prokaryotic or eukaryotic cell or cell line or any suitable fungal, prokaryotic or (non-human) eukaryotic organism, for example:

- a bacterial strain, including but not limited to gram-negative strains such as strains of *Escherichia coli*; of *Proteus*, for example of *Proteus mirabilis*; of *Pseudomonas*, for example of *Pseudomonas fluorescens*; and gram-positive strains such as strains of *Bacillus*, for example of *Bacillus subtilis* or of *Bacillus brevis*; of *Streptomyces*, for example of *Streptomyces lividans*; of *Staphylococcus*, for example of *Staphylococcus carnosus*; and of *Lactococcus*, for example of *Lactococcus lactis*;
- a fungal cell, including but not limited to cells from species of *Trichoderma*, for example from *Trichoderma reesei*; of *Neurospora*, for example from *Neurospora crassa*; of *Sordaria*, for example from *Sordaria macrospora*; of *Aspergillus*, for example from *Aspergillus niger* or from *Aspergillus sojae*; or from other filamentous fungi;
- a yeast cell, including but not limited to cells from species of *Saccharomyces*, for example of *Saccharomyces cerevisiae*; of *Schizosaccharomyces*, for example of *Schizosaccharomyces pombe*; of *Pichia*, for example of *Pichia pastoris* or of *Pichia methanolica*; of *Hansenula*, for example of *Hansenula polymorpha*; of *Kluyveromyces*, for example of *Kluyveromyces lactis*; of *Arxula*, for example of *Arxula adeninivorans*; of *Yarrowia*, for example of *Yarrowia lipolytica*;
- an amphibian cell or cell line, such as *Xenopus oocytes*;
- an insect-derived cell or cell line, such as cells/cell lines derived from lepidoptera, including but not limited to *Spodoptera* SF9 and Sf21 cells or cells/cell lines derived from *Drosophila*, such as Schneider and Kc cells;
- a plant or plant cell, for example in tobacco plants; and/or
- a mammalian cell or cell line, for example a cell or cell line derived from a human, a cell or a cell line from mammals including but not limited to CHO-cells (for example CHO-K1 cells), BHK-cells and human cells or cell lines such as HeLa, COS, Caki and HEK293H cells;

as well as all other host cells or (non-human) hosts known per se for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments), which will be clear to the skilled person. Reference is also made to the general background art cited hereinabove, as well as to for example WO 94/29457; WO 96/34103; WO 99/42077; Frenken et al. (Res Immunol. 149: 589-99, 1998); Riechmann and

Muyldermans (1999), supra; van der Linden (J. Biotechnol. 80: 261-70, 2000); Joosten et al. (Microb. Cell Fact. 2: 1, 2003); Joosten et al. (Appl. Microbiol. Biotechnol. 66: 384-92, 2005); and the further references cited herein.

The polypeptides of the invention may also be expressed as so-called "intrabodies", as for example described in WO 94/02610, WO 95/22618 and US 7,004,940; WO 03/014960; in Cattaneo and Biocca ("Intracellular Antibodies: Development and Applications" Landes and Springer-Verlag, 1997); and in Kontermann (Methods 34: 163-170, 2004).

The polypeptides of the invention can for example also be produced in the milk of transgenic mammals, for example in the milk of rabbits, cows, goats or sheep (see for example US 6,741,957, US 6,304,489 and US 6,849,992 for general techniques for introducing transgenes into mammals), in plants or parts of plants including but not limited to their leaves, flowers, fruits, seed, roots or tubers (for example in tobacco, maize, soybean or alfalfa) or in for example pupae of the silkworm *Bombix mori*.

Furthermore, the polypeptides of the invention can also be expressed and/or produced in cell-free expression systems, and suitable examples of such systems will be clear to the skilled person. Some preferred, but non-limiting examples include expression in the wheat germ system; in rabbit reticulocyte lysates; or in the *E. coli* Zubay system.

Preferably, in the invention, an (*in vivo* or *in vitro*) expression system, such as a bacterial expression system, is used that provides the polypeptides of the invention in a form that is suitable for pharmaceutical use, and such expression systems will again be clear to the skilled person. As also will be clear to the skilled person, polypeptides of the invention suitable for pharmaceutical use can be prepared using techniques for peptide synthesis.

For production on industrial scale, preferred heterologous hosts for the (industrial) production of immunoglobulin single variable domains or immunoglobulin single variable domain-containing polypeptide therapeutics include strains of *E. coli*, *Pichia pastoris*, *S. cerevisiae* that are suitable for large scale expression/production/fermentation, and in particular for large scale pharmaceutical expression/production/fermentation. Suitable examples of such strains will be clear to the skilled person. Such strains and production/expression systems are also made available by companies such as Biovitrum (Uppsala, Sweden).

Alternatively, mammalian cell lines, in particular Chinese hamster ovary (CHO) cells, can be used for large scale expression/production/fermentation, and in particular for large scale

pharmaceutical expression/production/fermentation. Again, such expression/production systems are also made available by some of the companies mentioned above.

The choice of the specific expression system would depend in part on the requirement for certain post-translational modifications, more specifically glycosylation. The production of an immunoglobulin single variable domain-containing recombinant protein for which glycosylation is desired or required would necessitate the use of mammalian expression hosts that have the ability to glycosylate the expressed protein. In this respect, it will be clear to the skilled person that the glycosylation pattern obtained (*i.e.,* the kind, number and position of residues attached) will depend on the cell or cell line that is used for the expression. Preferably, either a human cell or cell line is used (*i.e.,* leading to a protein that essentially has a human glycosylation pattern) or another mammalian cell line is used that can provide a glycosylation pattern that is essentially and/or functionally the same as human glycosylation or at least mimics human glycosylation. Generally, prokaryotic hosts such as *E. coli* do not have the ability to glycosylate proteins, and the use of lower eukaryotes such as yeast usually leads to a glycosylation pattern that differs from human glycosylation. Nevertheless, it should be understood that all the foregoing host cells and expression systems can be used in the invention, depending on the desired polypeptide to be obtained.

Thus, according to one non-limiting embodiment of the invention, the polypeptide of the invention is glycosylated. According to another non-limiting embodiment of the invention, the polypeptide of the invention is non-glycosylated.

According to one preferred, but non-limiting embodiment of the invention, the polypeptide of the invention is produced in a bacterial cell, in particular a bacterial cell suitable for large scale pharmaceutical production, such as cells of the strains mentioned above.

According to another preferred, but non-limiting embodiment of the invention, the polypeptide of the invention is produced in a yeast cell, in particular a yeast cell suitable for large scale pharmaceutical production, such as cells of the species mentioned above.

According to yet another preferred, but non-limiting embodiment of the invention, the polypeptide of the invention is produced in a mammalian cell, in particular in a human cell or in a cell of a human cell line, and more in particular in a human cell or in a cell of a human cell line that is suitable for large scale pharmaceutical production, such as the cell lines mentioned hereinabove.

When expression in a host cell is used to produce the polypeptides of the invention, the polypeptides of the invention can be produced either intracellullarly (*e.g.,* in the cytosol, in the periplasma or in inclusion bodies) and then isolated from the host cells and optionally further purified; or can be produced extracellularly (*e.g.,* in the medium in which the host cells are cultured) and then isolated from the culture medium and optionally further purified. When eukaryotic host cells are used, extracellular production is usually preferred since this considerably facilitates the further isolation and downstream processing of the polypeptides obtained. Bacterial cells such as the strains of *E. coli* mentioned above normally do not secrete proteins extracellularly, except for a few classes of proteins such as toxins and hemolysin, and secretory production in E. coli refers to the translocation of proteins across the inner membrane to the periplasmic space. Periplasmic production provides several advantages over cytosolic production. For example, the N-terminal amino acid sequence of the secreted product can be identical to the natural gene product after cleavage of the secretion signal sequence by a specific signal peptidase. Also, there appears to be much less protease activity in the periplasm than in the cytoplasm. In addition, protein purification is simpler due to fewer contaminating proteins in the periplasm. Another advantage is that correct disulfide bonds may form because the periplasm provides a more oxidative environment than the cytoplasm. Proteins overexpressed in E. coli are often found in insoluble aggregates, so-called inclusion bodies. These inclusion bodies may be located in the cytosol or in the periplasm; the recovery of biologically active proteins from these inclusion bodies requires a denaturation/refolding process. Many recombinant proteins, including therapeutic proteins, are recovered from inclusion bodies. Alternatively, as will be clear to the skilled person, recombinant strains of bacteria that have been genetically modified so as to secrete a desired protein, and in particular a polypeptide of the invention, can be used.

Thus, according to one non-limiting embodiment of the invention, the polypeptide of the invention is a polypeptide that has been produced intracellularly and that has been isolated from the host cell, and in particular from a bacterial cell or from an inclusion body in a bacterial cell. According to another non-limiting embodiment of the invention, the polypeptide of the invention is a polypeptide that has been produced extracellularly, and that has been isolated from the medium in which the host cell is cultivated.

Some preferred, but non-limiting promoters for use with these host cells include:

- for expression in E. coli: lac promoter (and derivatives thereof such as the lacUV5 promoter); arabinose promoter; left- (PL) and rightward (PR) promoter of phage lambda; promoter of the trp operon; hybrid lac/trp promoters (tac and trc); T7-promoter (more specifically that of T7-phage gene 10) and other T-phage promoters; promoter of the Tn10 tetracycline resistance gene; engineered variants of the above promoters that include one or more copies of an extraneous regulatory operator sequence;
- for expression in S. cerevisiae: constitutive: ADH1 (alcohol dehydrogenase 1), ENO (enolase), CYC1 (cytochrome c iso-1), GAPDH (glyceraldehydes-3-phosphate dehydrogenase), PGK1 (phosphoglycerate kinase), PYK1 (pyruvate kinase); regulated: GAL1,10,7 (galactose metabolic enzymes), ADH2 (alcohol dehydrogenase 2), PHO5 (acid phosphatase), CUP1 (copper metallothionein); heterologous: CaMV (cauliflower mosaic virus 35S promoter);
- for expression in Pichia pastoris: the AOX1 promoter (alcohol oxidase I);
- for expression in mammalian cells: human cytomegalovirus (hCMV) immediate early enhancer/promoter; human cytomegalovirus (hCMV) immediate early promoter variant that contains two tetracycline operator sequences such that the promoter can be regulated by the Tet repressor; Herpes Simplex Virus thymidine kinase (TK) promoter; Rous Sarcoma Virus long terminal repeat (RSV LTR) enhancer/promoter; elongation factor 1α (hEF-1α) promoter from human, chimpanzee, mouse or rat; the SV40 early promoter; HIV-1 long terminal repeat promoter; β-actin promoter;

Some preferred, but non-limiting vectors for use with these host cells include:

- vectors for expression in mammalian cells: pMAMneo (Clontech), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593), pBPV-1 (8-2) (ATCC 37110), pdBPV-MMTneo (342-12) (ATCC 37224), pRSVgpt (ATCC37199), pRSVneo (ATCC37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460) and 1ZD35 (ATCC 37565), as well as viral-based expression systems, such as those based on adenovirus;
- vectors for expression in bacterial cells: pET vectors (Novagen) and pQE vectors (Qiagen);
- vectors for expression in yeast or other fungal cells: pYES2 (Invitrogen) and Pichia expression vectors (Invitrogen);
- vectors for expression in insect cells: pBlueBacII (Invitrogen) and other baculovirus vectors

- vectors for expression in plants or plant cells: for example vectors based on cauliflower mosaic virus or tobacco mosaic virus, suitable strains of Agrobacterium, or Ti-plasmid based vectors.

Some preferred, but non-limiting secretory sequences for use with these host cells include:

- for use in bacterial cells such as E. coli: PelB, Bla, OmpA, OmpC, OmpF, OmpT, StII, PhoA, PhoE, MalE, Lpp, LamB, and the like; TAT signal peptide, hemolysin C-terminal secretion signal;
- for use in yeast: α-mating factor prepro-sequence, phosphatase (pho1), invertase (Suc), etc.;
- for use in mammalian cells: indigenous signal in case the target protein is of eukaryotic origin; murine Ig κ-chain V-J2-C signal peptide; etc.

Suitable techniques for transforming a host or host cell of the invention will be clear to the skilled person and may depend on the intended host cell/host organism and the genetic construct to be used. Reference is again made to the handbooks and patent applications mentioned above.

After transformation, a step for detecting and selecting those host cells or host organisms that have been successfully transformed with the nucleotide sequence/genetic construct of the invention may be performed. This may for instance be a selection step based on a selectable marker present in the genetic construct of the invention or a step involving the detection of the polypeptide of the invention, *e.g.,* using specific antibodies.

The transformed host cell (which may be in the form or a stable cell line) or host organisms (which may be in the form of a stable mutant line or strain) form further aspects of the present invention.

Preferably, these host cells or host organisms are such that they express, or are (at least) capable of expressing (*e.g.,* under suitable conditions), a polypeptide of the invention (and in case of a host organism: in at least one cell, part, tissue or organ thereof). The invention also includes further generations, progeny and/or offspring of the host cell or host organism of the invention, that may for instance be obtained by cell division or by sexual or asexual reproduction.

To produce/obtain expression of the polypeptides of the invention, the transformed host cell or transformed host organism may generally be kept, maintained and/or cultured under conditions such that the (desired) polypeptide of the invention is expressed/produced. Suitable

conditions will be clear to the skilled person and will usually depend upon the host cell/host organism used, as well as on the regulatory elements that control the expression of the (relevant) nucleotide sequence of the invention. Again, reference is made to the handbooks and patent applications mentioned above in the paragraphs on the genetic constructs of the invention.

Generally, suitable conditions may include the use of a suitable medium, the presence of a suitable source of food and/or suitable nutrients, the use of a suitable temperature, and optionally the presence of a suitable inducing factor or compound (*e.g.,* when the nucleotide sequences of the invention are under the control of an inducible promoter); all of which may be selected by the skilled person. Again, under such conditions, the polypeptides of the invention may be expressed in a constitutive manner, in a transient manner, or only when suitably induced.

It will also be clear to the skilled person that the polypeptide of the invention may (first) be generated in an immature form (as mentioned above), which may then be subjected to post-translational modification, depending on the host cell/host organism used. Also, the polypeptide of the invention may be glycosylated, again depending on the host cell/host organism used.

The polypeptide of the invention may then be isolated from the host cell/host organism and/or from the medium in which said host cell or host organism was cultivated, using protein isolation and/or purification techniques known per se, such as (preparative) chromatography and/or electrophoresis techniques, differential precipitation techniques, affinity techniques (*e.g.,* using a specific, cleavable amino acid sequence fused with the polypeptide of the invention) and/or preparative immunological techniques (i.e. using antibodies against the polypeptide to be isolated).

Compositions of the invention

Generally, for pharmaceutical use, the compounds, constructs and/or polypeptides of the invention may be formulated as a pharmaceutical preparation or composition comprising at least one compound, construct and/or polypeptide of the invention and at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally one or more further pharmaceutically active polypeptides and/or compounds. By means of non-limiting examples, such a formulation may be in a form suitable for oral administration, for parenteral administration (such as by intravenous, intramuscular or subcutaneous injection or intravenous

infusion), for topical administration, for administration by inhalation, by a skin patch, by an implant, by a suppository, etc, wherein the parenteral administration is preferred. Such suitable administration forms - which may be solid, semi-solid or liquid, depending on the manner of administration - as well as methods and carriers for use in the preparation thereof, will be clear to the skilled person, and are further described herein. Such a pharmaceutical preparation or composition will generally be referred to herein as a "pharmaceutical composition". A pharmaceutical preparation or composition for use in a non-human organism will generally be referred to herein as a "veterinary composition".

Thus, in a further aspect, the invention relates to a pharmaceutical composition that contains at least one amino acid of the invention, at least one immunoglobulin of the invention, at least one polypeptide of the invention, at least one compound of the invention, at least one construct of the invention or at least one nucleic acid of the invention and at least one suitable carrier, diluent or excipient (i.e., suitable for pharmaceutical use), and optionally one or more further active substances. In a particular aspect, the invention relates to a pharmaceutical composition that contains at least one of SEQ ID NOs: 1-123 and451-473 and at least one suitable carrier, diluent or excipient (i.e., suitable for pharmaceutical use), and optionally one or more further active substances.

Generally, the immunoglobulins, polypeptides, compounds and/or constructs of the invention can be formulated and administered in any suitable manner known per se. Reference is for example made to the general background art cited above (and in particular to WO 04/041862, WO 04/041863, WO 04/041865, WO 04/041867 and WO 08/020079) as well as to the standard handbooks, such as Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Company, USA (1990), Remington, the Science and Practice of Pharmacy, 21st Edition, Lippincott Williams and Wilkins (2005); or the Handbook of Therapeutic Antibodies (S. Dubel, Ed.), Wiley, Weinheim, 2007 (see for example pages 252-255).

The immunoglobulins, polypeptides, compounds and/or constructs of the invention may be formulated and administered in any manner known per se for conventional antibodies and antibody fragments (including ScFv's and diabodies) and other pharmaceutically active proteins. Such formulations and methods for preparing the same will be clear to the skilled person, and for example include preparations suitable for parenteral administration (e.g. intravenous,

intraperitoneal, subcutaneous, intramuscular, intraluminal, intra-arterial or intrathecal administration) or for topical (*i.e.*, transdermal or intradermal) administration.

Preparations for parenteral administration may for example be sterile solutions, suspensions, dispersions or emulsions that are suitable for infusion or injection. Suitable carriers or diluents for such preparations for example include, without limitation, those mentioned on page 143 of WO 08/020079. Usually, aqueous solutions or suspensions will be preferred.

The immunoglobulins, polypeptides, compounds and/or constructs of the invention can also be administered using methods of delivery known from gene therapy, see, *e.g.*, U.S. Patent No. 5,399,346, which is incorporated by reference for its gene therapy delivery methods. Using a gene therapy method of delivery, primary cells transfected with the gene encoding an immunoglobulin, polypeptide, compound and/or construct of the invention can additionally be transfected with tissue specific promoters to target specific organs, tissue, grafts, tumors, or cells and can additionally be transfected with signal and stabilization sequences for subcellularly localized expression.

Thus, the immunoglobulins, polypeptides, compounds and/or constructs of the invention may be systemically administered, *e.g.*, orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the immunoglobulins, polypeptides, compounds and/or constructs of the invention may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of the immunoglobulin, polypeptide, compound and/or construct of the invention. Their percentage in the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of the immunoglobulin, polypeptide, compound and/or construct of the invention in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain binders, excipients, disintegrating agents, lubricants and sweetening or flavoring agents, for example those mentioned on pages 143-144 of WO 08/020079. When the unit dosage form is a capsule, it may contain, in

addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the immunoglobulins, polypeptides, compounds and/or constructs of the invention, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the immunoglobulins, polypeptides, compounds and/or constructs of the invention may be incorporated into sustained-release preparations and devices.

Preparations and formulations for oral administration may also be provided with an enteric coating that will allow the constructs of the invention to resist the gastric environment and pass into the intestines. More generally, preparations and formulations for oral administration may be suitably formulated for delivery into any desired part of the gastrointestinal tract. In addition, suitable suppositories may be used for delivery into the gastrointestinal tract.

The immunoglobulins, polypeptides, compounds and/or constructs of the invention may also be administered intravenously or intraperitoneally by infusion or injection. Particular examples are as further described on pages 144 and 145 of WO 08/020079 or in PCT/EP2010/062975 (entire document).

For topical administration, the immunoglobulins, polypeptides, compounds and/or constructs of the invention may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologic acceptable carrier, which may be a solid or a liquid. Particular examples are as further described on page 145 of WO 08/020079.

Useful dosages of the immunoglobulins, polypeptides, compounds and/or constructs of the invention can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see US 4,938,949.

Generally, the concentration of the immunoglobulins, polypeptides, compounds and/or constructs of the invention in a liquid composition, such as a lotion, will be from about 0.1-25 wt-

%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the immunoglobulins, polypeptides, compounds and/or constructs of the invention required for use in treatment will vary not only with the particular immunoglobulin, polypeptide, compound and/or construct selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician. Also the dosage of the immunoglobulins, polypeptides, compounds and/or constructs of the invention varies depending on the target cell, tumor, tissue, graft, or organ.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced administrations.

An administration regimen could include long-term, daily treatment. By "long-term" is meant at least two weeks and preferably, several weeks, months, or years of duration. Necessary modifications in this dosage range may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. See Remington's Pharmaceutical Sciences (Martin, E.W., ed. 4), Mack Publishing Co., Easton, PA. The dosage can also be adjusted by the individual physician in the event of any complication.

Uses of the immunoglobulins, polypeptides, compounds and/or constructs of the invention

The invention further relates to applications and uses of the immunoglobulins, polypeptides, compounds and/or constructs, nucleic acids, host cells and compositions described herein, as well as to methods for the prevention and/or treatment of Kv1.3 associated diseases, disorders or conditions. Some preferred but non-limiting applications and uses will become clear from the further description herein.

The immunoglobulin, polypeptide, compound and/or construct of the invention can generally be used to modulate the activity of Kv1.3; such as partially or fully inhibit or partially or fully block the activity of Kv1.3. In particular, the immunoglobulin, polypeptide, compound and/or construct of the invention can modulate the activity of Kv1.3 such that it decreases the activity by at least 1%, preferably at least 5%, such as at least 10%, or at least 25%, preferably, at least 50%,

at least 60%, at least 70%, at least 80%, or at least 90% or more, such as 100% compared to the activity of Kv1.3 in the absence of the immunoglobulin, polypeptide, compound and/or construct of the invention as determined by a suitable assay, such as those described herein.

In one aspect, the immunoglobulin, polypeptide, compound and/or construct of the invention can reduce the flow of ions through Kv1.3 by at least 1%, preferably at least 5%, such as at least 10%, or at least 25%, preferably, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% or more, such as 100% compared to the flow of ions through the Kv1.3 pore channel in the absence of the immunoglobulin, polypeptide, compound and/or construct of the invention, as determined by a suitable assay, such as those described herein.

In another aspect, the invention relates to a method for the prevention and/or treatment of at least one Kv1.3 associated disease, disorder or condition, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an immunoglobulin of the invention, of a polypeptide of the invention, of a compound of the invention, of a construct of the invention and/or of a pharmaceutical composition comprising the same.

In the context of the present invention, the term "prevention and/or treatment" not only comprises preventing and/or treating the disease, but also generally comprises preventing the onset of the disease, slowing or reversing the progress of disease, preventing or slowing the onset of one or more symptoms associated with the disease, reducing and/or alleviating one or more symptoms associated with the disease, reducing the severity and/or the duration of the disease and/or of any symptoms associated therewith and/or preventing a further increase in the severity of the disease and/or of any symptoms associated therewith, preventing, reducing or reversing any physiological damage caused by the disease, and generally any pharmacological action that is beneficial to the patient being treated.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases, disorders and conditions mentioned herein.

The invention relates to a method for the prevention and/or treatment of at least one disease, disorder or condition that is associated with Kv1.3, with its biological or pharmacological activity, and/or with the biological pathways or signaling in which Kv1.3 is involved, said method

comprising administering, to a subject in need thereof, a pharmaceutically active amount of an immunoglobulin of the invention, of a polypeptide of the invention, of a compound of the invention, of a construct of the invention and/or of a pharmaceutical composition comprising the same. In particular, the invention relates to a method for the prevention and/or treatment of at least one disease, disorder or condition that can be prevented and/or treated by modulating Kv1.3, its biological or pharmacological activity, and/or the biological pathways or signaling in which Kv1.3 is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an immunoglobulin of the invention, of a polypeptide of the invention, of a compound of the invention, of a construct of the invention, and/or of a pharmaceutical composition comprising the same.

In particular, said pharmaceutically effective amount may be an amount that is sufficient to modulate Kv1.3, its biological or pharmacological activity, and/or the biological pathways or signaling in which Kv1.3 is involved; and/or an amount that provides a level of the immunoglobulin of the invention, of the polypeptide of the invention, of the compound of the invention, and/or of the construct of the invention in the circulation that is sufficient to modulate Kv1.3, its biological or pharmacological activity, and/or the biological pathways or signaling in which Kv1.3 is involved.

The invention also relates to a method for the prevention and/or treatment of at least one disease, disorder and/or condition that can be prevented and/or treated by administering of an immunoglobulin of the invention, of a polypeptide of the invention, of a compound of the invention and/or of a construct of the invention to a patient, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an immunoglobulin of the invention, of a polypeptide of the invention, of a compound of the invention, of a construct of the invention and/or of a pharmaceutical composition comprising the same.

More in particular, the invention relates to a method for the prevention and/or treatment of at least one disease, disorder and/or condition chosen from the group consisting of the diseases, disorders and conditions listed herein, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an immunoglobulin of the invention, of a polypeptide of the invention, of a compound of the invention, of a construct of the invention and/or of a pharmaceutical composition comprising the same.

In particular, the present invention relates to a method for the prevention and/or treatment of multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity.

In another particular aspect, the present invention relates to a method for the prevention and/or treatment of multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity, said method comprising administering a pharmaceutically active amount of at least one of SEQ ID NOs: 1-123 and 451-473, and/or of a pharmaceutical composition comprising the same.

In a further aspect, the invention relates to a method for immunotherapy, and in particular for passive immunotherapy, which method comprises administering, to a subject suffering from or at risk of the diseases and disorders mentioned herein, a pharmaceutically active amount of an immunoglobulin of the invention, of a polypeptide of the invention, of a compound of the invention, of a construct of the invention and/or of a pharmaceutical composition comprising the same.

In the above methods, the immunoglobulins, polypeptides, compounds and/or constructs of the invention and/or the compositions comprising the same can be administered in any suitable manner, depending on the specific pharmaceutical formulation or composition to be used. Thus, the immunoglobulins, polypeptides, compounds and/or constructs of the invention and/or the compositions comprising the same can for example be administered orally, intraperitoneally (e.g. intravenously, subcutaneously, intramuscularly, or via any other route of administration that circumvents the gastrointestinal tract), intranasally, transdermally, topically, by means of a suppository, by inhalation, again depending on the specific pharmaceutical formulation or composition to be used. The clinician will be able to select a suitable route of

administration and a suitable pharmaceutical formulation or composition to be used in such administration, depending on the disease, disorder or condition to be prevented or treated and other factors well known to the clinician.

The immunoglobulins, polypeptides, compounds and/or constructs of the invention and/or the compositions comprising the same are administered according to a regime of treatment that is suitable for preventing and/or treating the disease, disorder or condition to be prevented or treated. The clinician will generally be able to determine a suitable treatment regimen, depending on factors such as the disease, disorder or condition to be prevented or treated, the severity of the disease to be treated and/or the severity of the symptoms thereof, the specific immunoglobulins, polypeptides, compounds and/or constructs of the invention to be used, the specific route of administration and pharmaceutical formulation or composition to be used, the age, gender, weight, diet, general condition of the patient, and similar factors well known to the clinician.

Generally, the treatment regimen will comprise the administration of one or more immunoglobulins, polypeptides, compounds and/or constructs of the invention, or of one or more compositions comprising the same, in one or more pharmaceutically effective amounts or doses. The specific amount(s) or doses to be administered can be determined by the clinician, again based on the factors cited above.

Generally, depending on the specific disease, disorder or condition to be treated, the potency of the specific immunoglobulin, polypeptide, compound and/or construct of the invention to be used, the specific route of administration and the specific pharmaceutical formulation or composition used, the clinician will be able to determine a suitable daily dose.

Usually, in the above method, an immunoglobulin, polypeptide, compound and/or construct of the invention will be used. It is however within the scope of the invention to use two or more immunoglobulins, polypeptides, compounds and/or constructs of the invention in combination.

The immunoglobulins, polypeptides, compounds and/or constructs of the invention may be used in combination with one or more further pharmaceutically active compounds or principles, i.e., as a combined treatment regimen, which may or may not lead to a synergistic effect.

Again, the clinician will be able to select such further compounds or principles, as well as a suitable combined treatment regimen, based on the factors cited above and his expert judgment.

In particular, the immunoglobulins, polypeptides, compounds and/or constructs of the invention may be used in combination with other pharmaceutically active compounds or principles that are or can be used for the prevention and/or treatment of the diseases, disorders and conditions cited herein, as a result of which a synergistic effect may or may not be obtained. Examples of such compounds and principles, as well as routes, methods and pharmaceutical formulations or compositions for administering them will be clear to the clinician.

When two or more substances or principles are to be used as part of a combined treatment regimen, they can be administered via the same route of administration or via different routes of administration, at essentially the same time or at different times (e.g. essentially simultaneously, consecutively, or according to an alternating regime). When the substances or principles are to be administered simultaneously via the same route of administration, they may be administered as different pharmaceutical formulations or compositions or part of a combined pharmaceutical formulation or composition, as will be clear to the skilled person.

Also, when two or more active substances or principles are to be used as part of a combined treatment regimen, each of the substances or principles may be administered in the same amount and according to the same regimen as used when the compound or principle is used on its own, and such combined use may or may not lead to a synergistic effect. However, when the combined use of the two or more active substances or principles leads to a synergistic effect, it may also be possible to reduce the amount of one, more or all of the substances or principles to be administered, while still achieving the desired therapeutic action. This may for example be useful for avoiding, limiting or reducing any unwanted side-effects that are associated with the use of one or more of the substances or principles when they are used in their usual amounts, while still obtaining the desired pharmaceutical or therapeutic effect.

The effectiveness of the treatment regimen used according to the invention may be determined and/or followed in any manner known per se for the disease, disorder or condition involved, as will be clear to the clinician. The clinician will also be able, where appropriate and on a case-by-case basis, to change or modify a particular treatment regimen, so as to achieve the desired therapeutic effect, to avoid, limit or reduce unwanted side-effects, and/or to achieve an

appropriate balance between achieving the desired therapeutic effect on the one hand and avoiding, limiting or reducing undesired side effects on the other hand.

Generally, the treatment regimen will be followed until the desired therapeutic effect is achieved and/or for as long as the desired therapeutic effect is to be maintained. Again, this can be determined by the clinician.

In another aspect, the invention relates to the use of an immunoglobulin, polypeptide, compound and/or construct of the invention in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one disease, disorder and condition associated with Kv1.3; and/or for use in one or more of the methods of treatment mentioned herein.

The invention also relates to the use of an immunoglobulin, polypeptide, compound and/or construct of the invention, in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one of the diseases, disorders and conditions associated with Kv1.3 and/or with the signaling pathways and/or the biological functions and responses in which Kv1.3 are involved; and/or for use in one or more of the methods described herein.

The invention also relates to the use of an immunoglobulin, polypeptide, compound and/or construct of the invention in the preparation of a pharmaceutical composition for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by modulating Kv1.3, its biological or pharmacological activity, and/or the biological pathways or signaling in which Kv1.3 is involved.

The invention also relates to the use of an immunoglobulin, polypeptide, compound and/or construct of the invention in the preparation of a pharmaceutical composition for the prevention and/or treatment of at least one disease, disorder or condition that can be prevented and/or treated by administering an immunoglobulin, polypeptide, compound and/or construct of the invention to a patient.

More in particular, the invention relates to the use of an immunoglobulin, polypeptide, compound and/or construct of the invention in the preparation of a pharmaceutical composition for the prevention and/or treatment of Kv1.3 related disorders, and in particular for the prevention and treatment of multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome,

Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity.

The invention further relates to an immunoglobulin, polypeptide, compound and/or construct of the invention or a pharmaceutical composition comprising the same for use in the prevention and/or treatment of at least one Kv1.3 related disease, disorder and/or condition.

The invention further relates to an immunoglobulin, polypeptide, compound and/or construct of the invention or a pharmaceutical composition comprising the same for use in the prevention and/or treatment of at least one disease, disorder and/or condition associated with Kv1.3, with its biological or pharmacological activity, and/or with the biological pathways or signaling in which Kv1.3 involved.

The invention further relates to an immunoglobulin, polypeptide, compound and/or construct of the invention or a pharmaceutical composition comprising the same for use in the prevention and/or treatment of at least one disease, disorder and/or condition that can be prevented and/or treated by modulating Kv1.3, its biological or pharmacological activity, and/or the biological pathways or signaling in which Kv1.3 is involved.

The invention also relates to an immunoglobulin, polypeptide, compound and/or construct of the invention or a pharmaceutical composition comprising the same for use in the prevention and/or treatment of at least one disease, disorder and/or condition that can be prevented and/or treated by administering of an immunoglobulin, polypeptide, compound and/or construct of the invention to a patient.

The invention further relates to immunoglobulin, polypeptide, compound and/or construct of the invention or a pharmaceutical composition comprising the same for use in the prevention and/or treatment of multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. In veterinary applications, the subject to be treated includes any animal raised for commercial purposes or kept as a pet. As will be clear to

the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases, disorders and conditions mentioned herein.

Again, in such a pharmaceutical composition, the one or more immunoglobulins, polypeptides, compounds and/or constructs of the invention, or nucleotide encoding the same, and/or a pharmaceutical composition comprising the same, may also be suitably combined with one or more other active principles, such as those mentioned herein.

The invention also relates to a composition (such as, without limitation, a pharmaceutical composition or preparation as further described herein) for use, either in vitro (e.g. in an in vitro or cellular assay) or in vivo (e.g. in an a single cell or multi-cellular organism, and in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a disease, disorder or condition of the invention).

The immunoglobulins, polypeptides, compounds and/or constructs of the present invention ameliorate the effects of inflammation in a relevant delayed-type hypersensitivity (DTH) rat model. Based on their mode of action, the immunoglobulins, polypeptides, compounds and/or constructs of the present invention may be useful in the treatment of other Kv1.3 associated diseases, including but not limited to multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity.

It is to be understood that reference to treatment includes both treatment of established symptoms and prophylactic treatment, unless explicitly stated otherwise.

The invention will now be further described by means of the following non-limiting preferred aspects, examples and figures.

The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced hereinabove.

ITEMS

1. An immunoglobulin that specifically binds to the EL1 extracellular loop of potassium channel 3 (Kv1.3), wherein the binding of said immunoglobulin to said EL1 extracellular loop modulates the activity of Kv1.3.

2. The immunoglobulin according to item 1, wherein the immunoglobulin modulates the activity of Kv1.3 by partially or fully blocking of Kv1.3 activity.

3. The immunoglobulin according to any one of items 1 to 2, wherein the immunoglobulin modulates the activity of Kv1.3 by reducing or even totally inhibiting the efflux of potassium ions from T-cells.

4. The immunoglobulin according to item 3, wherein the immunoglobulin reduces or inhibits the efflux of potassium ions from T-cells with an IC50 value of 10^{-7} M or lower, preferably of 10^{-8} M or lower, more preferably of 10^{-9} M or lower, or even of 10^{-10} M or lower, as determined in Patch Clamp assay.

5. The immunoglobulin according to any one of items 1 to 4, wherein the immunoglobulin modulates and/or inhibits the activity of Kv1.3 allosterically.

6. The immunoglobulin according to any one of items 1 to 5, wherein the immunoglobulin has a more than 10 fold, more than 100 fold, preferably more than 1000 fold selectivity over other related Kv ion channel family members for modulating and/or inhibiting the activity of Kv1.3.

7. The immunoglobulin according to any one of items 1 to 6, wherein the immunoglobulin has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 1-64 and wherein the immunoglobulin has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 1-64.

8. The immunoglobulin according to any one of items 1 to 6, wherein the immunoglobulin has the same number of amino acids within its sequence compared to any one of SEQ ID NOs: 65-

123 and wherein the immunoglobulin has an amino acid sequence between position 8 and position 106 (according to Kabat numbering) with 89% or more sequence identity compared to any one of SEQ ID NOs: 65-123.

9. The immunoglobulin according to any one of items 1 to 6, that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397.

10. The immunoglobulin according to item 9, that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182;

and

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269;

and

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397.

11. The immunoglobulin according to any one of items 9 to 10, in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 1 the G has been changed into L, or R;
- at position 2 the L has been changed into F, P, or I;
- at position 3 the L has been changed into P, or F;
- at position 4 the F has been changed into S, L, or I;
- at position 5 the S has been changed into I, or R;
- at position 6 the R has been changed into C, A, P, V, or L;
- at position 7 the N has been changed into H, P, I, M, Y, T or D:
- at position 8 the S has been changed into T, R, or I;
- at position 9 the A has been changed into V or T; and/or
- at position 10 the G has been changed into S, R, or V;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 1 the R has been changed into G, or C;
- at position 2 the I has been changed into V, T, S or L;

- at position 3 the R has been changed into G, or L;
- at position 4 the M has been changed into S, R, or T;
- at position 5 the G has been changed into V, S, or T;
- at position 7 the S has been changed into G, C, D, or E; and/or
- at position 8 the I has been changed into T, M, or R;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 1 the W has been changed into G;
- at position 3 the E has been changed into T, K, G, A, or I;
- at position 4 the G has been changed into E, or D;
- at position 5 the F has been changed into A, L, V, Y, T, or S;
- at position 6 the Y has been changed into F, or D:
- at position 7 the E has been changed into G, or K;
- at position 8 the Y has been changed into S or H; and/or
- at position 9 the W has been changed into S, G or C.

12. The immunoglobulin according to item 11, in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-210; or

b) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 1 the G has been changed into L, or R;
- at position 2 the L has been changed into F, P, or I;
- at position 3 the L has been changed into P, or F;
- at position 4 the F has been changed into S, L, or I;
- at position 5 the S has been changed into I, or R;
- at position 6 the R has been changed into C, A, P, V, or L;
- at position 7 the N has been changed into H, P, I, M, Y, T or D:

- at position 8 the S has been changed into T, R, or I;
- at position 9 the A has been changed into V or T; and/or
- at position 10 the G has been changed into S, R, or V;

and

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-289; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 1 the R has been changed into G, or C;
- at position 2 the I has been changed into V, T, S or L;
- at position 3 the R has been changed into G, or L;
- at position 4 the M has been changed into S, R, or T;
- at position 5 the G has been changed into V, S, or T;
- at position 7 the S has been changed into G, C, D, or E; and/or
- at position 8 the I has been changed into T, M, or R;

and

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-415; or

f) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 1 the W has been changed into G;
- at position 3 the E has been changed into T, K, G, A, or I;
- at position 4 the G has been changed into E, or D;
- at position 5 the F has been changed into A, L, V, Y, T, or S;
- at position 6 the Y has been changed into F, or D:
- at position 7 the E has been changed into G, or K;
- at position 8 the Y has been changed into S or H; and/or
- at position 9 the W has been changed into S, G or C.

13. The immunoglobulin according to any one of items 9 to 12, in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-185; or

b) amino acid sequences that have 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 6 the R has been changed into A, or V; and/or
- at position 9 the A has been changed into V;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-271; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 2 the I has been changed into L;
- at position 4 the M has been changed into S or T;
- at position 5 the G has been changed into S or T; and/or
- at position 8 the I has been changed into T;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-398; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 3 the E has been changed into T or I;
- at position 4 the G has been changed into E;
- at position 5 the F has been changed into A; and/or
- at position 8 the Y has been changed into H.

14. The immunoglobulin according to item 13, in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 181-185; or

b) amino acid sequences that have 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 182, wherein

- at position 6 the R has been changed into A, or V; and/or
- at position 9 the A has been changed into V;

and

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 268-271; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 269, wherein

- at position 2 the I has been changed into L;
- at position 4 the M has been changed into S or T;
- at position 5 the G has been changed into S or T; and/or
- at position 8 the I has been changed into T;

and

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 393-398; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 397, wherein

- at position 3 the E has been changed into T or I;
- at position 4 the G has been changed into E;
- at position 5 the F has been changed into A; and/or
- at position 8 the Y has been changed into H.

15. The immunoglobulin according to any one of items 1 to 6 or items 9 to 14, wherein said immunoglobulin is chosen from the group of polypeptides, wherein:

- CDR1 is SEQ ID NO: 182, CDR2 is SEQ ID NO: 269, and CDR3 is SEQ ID NO: 397;
- CDR1 is SEQ ID NO: 182, CDR2 is SEQ ID NO: 269, and CDR3 is SEQ ID NO: 394;
- CDR1 is SEQ ID NO: 185, CDR2 is SEQ ID NO: 271, and CDR3 is SEQ ID NO: 398;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 395;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 396;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 270, and CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 183, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 184, CDR2 is SEQ ID NO: 268, and CDR3 is SEQ ID NO: 393; and
- CDR1 is SEQ ID NO: 185, CDR2 is SEQ ID NO: 271, and CDR3 is SEQ ID NO: 398.

16. The immunoglobulin according to any one of items 1 to 3, that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422.

17. The immunoglobulin according to item 16, that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214;

and

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303;

and

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422.

18. The immunoglobulin according to any one of items 16 or 17, in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214, wherein

- at position 1 the G has been changed into R, A, V, S, or K;
- at position 3 the T has been changed into N;
- at position 4 the F has been changed into L;
- at position 6 the N has been changed into S;
- at position 7 the F has been changed into Y:
- at position 8 the G has been changed into A; and/or
- at position 9 the M has been changed into V;

and/or

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303, wherein

- at position 1 the A has been changed into T;
- at position 2 the I has been changed into V;
- at position 5 the T has been changed into S, or A;
- at position 6 the G has been changed into N, or A;
- at position 7 the G has been changed into S, or R;
- at position 8 the H has been changed into R, or Y;
- at position 9 the T has been changed into I, or K; and/or
- at position 10 the Y has been changed into F;

and/or

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422, wherein

- at position 4 the F has been changed into Y, or S;
- at position 5 the G has been changed into D;
- at position 6 the D has been changed into G;
- at position 7 the G has been changed into D;
- at position 8 the T has been changed into A:
- at position 9 the Y has been changed into S;
- at position 10 the Y has been changed into F;
- at position 12 the Q has been changed into E;
- at position 14 the A has been changed into N, T, I, or R; and/or
- at position 17 the D has been changed into N, or G.

19. The immunoglobulin according to item 18, in which:

i) CDR1 is chosen from the group consisting of:

a) SEQ ID NOs: 211-227; or

b) amino acid sequences that have 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 214, wherein

- at position 1 the G has been changed into R, A, V, S, or K;
- at position 3 the T has been changed into N;
- at position 4 the F has been changed into L;
- at position 6 the N has been changed into S;
- at position 7 the F has been changed into Y:
- at position 8 the G has been changed into A; and/or
- at position 9 the M has been changed into V;

and

ii) CDR2 is chosen from the group consisting of:

c) SEQ ID NOs: 290-309; or

d) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 303, wherein

- at position 1 the A has been changed into T;
- at position 2 the I has been changed into V;
- at position 5 the T has been changed into S, or A;
- at position 6 the G has been changed into N, or A;
- at position 7 the G has been changed into S, or R;
- at position 8 the H has been changed into R, or Y;
- at position 9 the T has been changed into I, or K; and/or
- at position 10 the Y has been changed into F;

and

iii) CDR3 is chosen from the group consisting of:

e) SEQ ID NOs: 416-435; or

f) amino acid sequences that have 4, 3, 2, or 1 amino acid(s) difference with the amino acid sequence of SEQ ID NO: 422, wherein

- at position 4 the F has been changed into Y, or S;
- at position 5 the G has been changed into D;
- at position 6 the D has been changed into G;
- at position 7 the G has been changed into D;
- at position 8 the T has been changed into A:
- at position 9 the Y has been changed into S;
- at position 10 the Y has been changed into F;
- at position 12 the Q has been changed into E;
- at position 14 the A has been changed into N, T, I, or R; and/or
- at position 17 the D has been changed into N, or G.

20. The immunoglobulin according to any one of items 1 to 6 or items 16 to 19, wherein said immunoglobulin is chosen from the group of polypeptides, wherein:

- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 290, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 417;

- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 292, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 293, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 294, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 216, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 419;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 297, and CDR3 is SEQ ID NO: 420;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298, and CDR3 is SEQ ID NO: 421;
- CDR1 is SEQ ID NO: 217, CDR2 is SEQ ID NO: 299, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 298, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 423;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 301, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 424;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 302, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 425;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 426;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 219, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 427;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 304, and CDR3 is SEQ ID NO: 428;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 421;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 429;
- CDR1 is SEQ ID NO: 221, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 222, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 430;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 306, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 223, CDR2 is SEQ ID NO: 303, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298 and CDR3 is SEQ ID NO: 431;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 296, and CDR3 is SEQ ID NO: 432;

- CDR1 is SEQ ID NO: 224, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 307, and CDR3 is SEQ ID NO: 433;
- CDR1 is SEQ ID NO: 225, CDR2 is SEQ ID NO: 300, and CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 226, CDR2 is SEQ ID NO: 308, and CDR3 is SEQ ID NO: 434;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 295, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 301, and CDR3 is SEQ ID NO: 426;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 217, CDR2 is SEQ ID NO: 305, and CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298, and CDR3 is SEQ ID NO: 435; and
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 309, and CDR3 is SEQ ID NO: 418.

21. The immunoglobulin according to any one of items 1 to 20, wherein said immunoglobulin essentially consists of a domain antibody, an immunoglobulin that is suitable for use as a domain antibody, a single domain antibody, an immunoglobulin that is suitable for use as a single domain antibody, a dAb, an immunoglobulin that is suitable for use as a dAb, a Nanobody, a VHH sequence, a humanized VHH sequence, a camelized VH sequence, or a VHH sequence that has been obtained by affinity maturation.

22. The immunoglobulin according to any one of items 1 to 21, wherein said immunoglobulin is chosen from the group consisting of immunoglobulins with SEQ ID NOs: 1-123 or immunoglobulins that have a sequence identity of more than 80% with SEQ ID NOs: 1-123.

23. The immunoglobulin according to any one of items 1 to 22, wherein said immunoglobulin is chosen from the group consisting of SEQ ID NOs: 1-123.

24. The immunoglobulin according to any one of items 1 to 23, wherein said Kv1.3 is human Kv1.3.

25. The immunoglobulin according to any one of items 1 to 24, wherein said immunoglobulin cross-blocks the binding to Kv1.3 of at least one of the polypeptides with SEQ ID NOs: 1-123 and/or is cross-blocked from binding to Kv1.3 by at least one of the polypeptides with SEQ ID NOs: 1-123.

26. A polypeptide that comprises or essentially consists of one or more immunoglobulins according to any one of items 1 to 25.

27. The polypeptide according to item 26, that comprises or essentially consists of at least two immunoglobulins according to any one of items 1 to 25.

28. The polypeptide according to item 27, wherein said at least two immunoglobulins can be the same or different.

29. The polypeptide according to any one of items 27 to 28, wherein said at least two immunoglobulins are directly linked to each other or linked to each other via a linker.

30. The polypeptide according to item 29, in which the linker is selected from the group of linkers with SEQ ID NOs: 479 to 494.

31. The polypeptide according to any one of items 26 to 30, wherein said polypeptide is chosen from the group of polypeptides with SEQ ID NOs: 451-473 or polypeptides that have a sequence identity of more than 80% with SEQ ID NOs: 451-473.

32. The polypeptide according to any one of items 26 to 31, wherein said polypeptide is chosen from the group consisting of SEQ ID NOs: 451-473.

33. A compound or construct that comprises or essentially consists of an immunoglobulin according to any one of items 1 to 25 or a polypeptide according to any one of items 26 to 32, and which further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more peptidic linkers.

34. The compound or construct according to item 33, which has an increased half-life compared to the corresponding immunoglobulin according to any one of items 1 to 25 or polypeptide according to any one of items 26 to 32, per se.

35. The compound or construct according to item 34, in which said one or more other groups, residues, moieties or binding units provide the polypeptide with increased half-life, compared to the corresponding immunoglobulin according to any one of items 1 to 25 or polypeptide according to any one of items 26 to 32.

36. The compound or construct according to item 35, in which said one or more other groups, residues, moieties or binding units that provide the immunoglobulin or polypeptide with increased half-life is chosen from the group consisting of a polyethylene glycol molecule, serum proteins or fragments thereof, binding units that can bind to serum proteins, an Fc portion, and small proteins or peptides that can bind to serum proteins.

37. The compound or construct according to any one of items 35 or 36, in which said one or more other groups, residues, moieties or binding units that provide the immunoglobulin or polypeptide with increased half-life is chosen from the group consisting of human serum albumin or fragments thereof.

38. The compound or construct according to any one of items 35 to 36, in which said one or more other binding units that provide the polypeptide with increased half-life are chosen from the group consisting of binding units that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

39. The compound or construct according to item 38, in which said one or more other binding units that provide the polypeptide with increased half-life are chosen from the group consisting of domain antibodies, amino acids that are suitable for use as a domain antibody, single domain antibodies, amino acids that are suitable for use as a single domain antibody, "dAb"'s, amino acids that are suitable for use as a dAb, Nanobodies, VHH sequences, humanized VHH sequences, or camelized VH sequences that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

40. The compound or construct according to item 39, wherein said compound or construct is chosen from the group consisting of compounds or constructs with SEQ ID NOs: 461-473 or

compounds or constructs that have a sequence identity of more than 80% with SEQ ID NOs: 461-473.

41. The compound or construct according to any one of items 39 to 40, wherein said compound or construct is chosen from the group consisting of SEQ ID NOs: 461-473.

42. The compound or construct according to any one of items 38 to 41, that has a serum half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding immunoglobulin according to any one of items 1 to 25 or polypeptide according to any of itemss 26 to 32, per se.

43. The compound or construct according to any one of items 38 to 42, that has a serum half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding immunoglobulin according to any one of items 1 to 25 or polypeptide according to any one of items 26 to 32, per se.

44. The compound or construct according to any one of items 38 to 43, that has a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more; for example, of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

45. A nucleic acid encoding an immunoglobulin according to any one of items 1 to 25, a polypeptide according to any one of items 26 to 32, or a compound or construct according to any one of items 33 to 44.

46. An expression vector comprising a nucleic acid according to item 45.

47. A host or host cell comprising a nucleic acid according to item 45, or an expression vector according to item 46.

48. A composition comprising at least one immunoglobulin according to any one of items 1 to 25, a polypeptide according to any one of items 26 to 32, a compound or construct according to any one of items 33 to 44, or a nucleic acid according to item 45.

49. The composition according to item 48, which is a pharmaceutical composition.

50. The composition according to item 49, which further comprises at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally comprises one or more further pharmaceutically active polypeptides and/or compounds.

51. The composition according to any one of items 48 to 50, the immunoglobulin according to any one of items 1 to 25, the polypeptide according to any one of items 26 to 32, the compound or construct according to any one of items 33 to 44, for use as a medicament.

52. The composition according to any one of items 48 to 50, the immunoglobulin according to any one of items 1 to 25, the polypeptide according to any one of items 26 to 32, or the compound or construct according to any one of items 33 to 44, for use in the treatment or prevention of a Kv1.3 associated disease, disorder or condition.

53. The composition, the immunoglobulin, the polypeptide, or the compound or construct according to item 52, wherein the disease, disorder or condition is selected from multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity.

54. A method for producing an immunoglobulin according to any one of items 1 to 25 or a polypeptide according to any one of items 26-32, said method at least comprising the steps of:

a) expressing, in a suitable host cell or host organism or in another suitable expression system, a nucleic acid sequence according to item 45; optionally followed by:

b) isolating and/or purifying the immunoglobulin according to any one of items 1 to 25, or the polypeptide according to any one of items 26 to 32.

55. A method for the prevention and or treatment of a Kv1.3 associated disease, disorder or condition, wherein said method comprises administering, to a subject in need thereof, a pharmaceutically active amount of at least one immunoglobulin according to any one of items 1 to 25, a polypeptide according to any one of items 26 to 32, a compound or construct according to any one of items 33 to 44, or a composition according to any one of items 48 to 53.

56. The method according to item 55, wherein the disease, disorder or condition is selected from multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity.

57. Use of an immunoglobulin according to any one of items 1 to 25, a polypeptide according to any one of items 26 to 32, a compound or construct according to any one of items 33 to 44, or a composition according to any one of items 48 to 53, in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one Kv1.3 associated disease, disorder or condition; and/or for use in one or more of the methods according to items 55 to 56.

58. Use of an immunoglobulin according to item 57, wherein the disease, disorder or condition is selected from multiple sclerosis, rheumatoid arthritis, type-1 diabetes mellitus, type-2 diabetes mellitus, psoriasis, inflammatory bowel disease, contact-mediated dermatitis, psoriatic arthritis, asthma, allergy, restenosis, systemic sclerosis, fibrosis, scleroderma, glomerulonephritis, chronic obstructive pulmonary disease (COPD), Sjogren's syndrome, Alzheimer's disease, inflammatory bone resorption, systemic lupus erythematosus, ulcerative colitis, obesity, graft-versus host disease, transplant rejection, and delayed type hypersensitivity.

EXAMPLES

Example 1: Immunization of llamas with Kv1.3, cloning of the heavy chain-only antibody fragment repertoires and preparation of phage

1.1 Immunizations

After approval of the Ethical Committee (University Antwerp, Belgium), 3 llamas (*llama glama*) were immunized with a pVAX1-human Kv1.3 plasmid vector (Invitrogen, Carlsbad, CA, USA) (2x150µg/dose) according to standard protocols by 5/8 bilateral, intradermal *in vivo* electroporation at biweekly intervals. After the 6^{th} (5^{th} for 1 llama) injection, the llamas received 1 subcutaneous injection of HEK293H (DSMZ, ACC 635) or Caki cells (Nguyen et al., Adv Immunol 79: 261-296, 2001) overexpressing human Kv1.3 (2E07 cells/dose). Cells were re-suspended in D-PBS and kept on ice prior to injection. 1 animal received also human Kv1.3 expressing VLP's (Molecular Integral, INT-793A) after the cell boost.

1.2 Cloning of heavy chain-only antibody fragment repertoires and preparation of phage

Following the final injection of each subset, immune tissues as the source of B-cells that produce the heavy-chain antibodies were collected from the immunized llamas. Blood samples, collected few days after the last injection of each subset, were collected per animal. From the blood samples, peripheral blood lymphocytes (PBLs) were prepared using Ficoll-Hypaque according to the manufacturer's instructions (Amersham Biosciences, Piscataway, NJ, USA). From the PBLs and the lymph node biopsy (LN), total RNA was extracted, which was used as starting material for RT-PCR to amplify the VHH encoding DNA segments. For each immunized llama,

libraries were constructed by pooling the total RNA isolated from samples originating from a certain subset of the immunization schedule i.e. after one type of immunization antigen.

In short, the PCR-amplified VHH repertoire was cloned via specific restriction sites into a vector designed to facilitate phage display of the VHH library. The vector was derived from pUC119 which contains a resistance gene for ampicillin or carbenicillin and the lac promoter followed by the coding sequence of the pIII protein signal peptide in frame with a downstream Nanobody cloning site (pAX212). In frame with the VHH coding sequence, the vector encodes a C-terminal 3xFLAG and His6 tag and a coliphage pIII protein. Phage were prepared according to standard protocols (see for example WO 04/041865, WO 04/041863, WO 04/062551, WO 05/044858 and other prior art and applications filed by Ablynx N.V. cited herein) and stored after filter sterilization at 4°C or at -80°C in 20 % glycerol for further use.

Example 2: Selection of Kv1.3 specific VHHs via phage display

VHH repertoires obtained from all llamas and cloned as phage library were used in different selection strategies, applying a multiplicity of selection conditions. Variables included i) the presentation form of the Kv1.3 (on different cell backgrounds or on liposomes/VLPs), ii) the antigen presentation method (in solution when using cells or coated onto plates when using VLPs), iii) the antigen concentration, iv) the orthologue used (human or cynomolgus), and v) the number of selection rounds. All solid coated phase selections were done in Maxisorp 96-well plates (Nunc, Wiesbaden, Germany).

Selections were performed as follows: Kv1.3 antigen preparations for solid and solution phase selection formats were presented as described above at multiple concentrations. After 2h incubation with the phage libraries followed by extensive washing, bound phages were eluted with trypsin (1 mg/mL) for 15 minutes. When trypsin was used for phage elution, the protease activity was immediately neutralized by applying 0.8 mM protease inhibitor ABSF. As control, selections without antigen were performed in parallel.

Phage outputs were used to infect *E. coli* which were then in turn used to prepare phage for the next selection round (phage rescue). The phage outputs were also used to infect *E. coli* which were then plated on agar plates (LB+carb+glucose$^{2\%}$) for analysis of individual VHH clones. In order to screen a selection output for specific binders, single colonies were picked from the agar plates and grown in 1 mL 96-deep-well plates. LacZ-controlled VHH expression was

induced by adding IPTG (1mM final) in the absence of glucose. Periplasmic extracts (in a volume of ~ 80 uL) were prepared according to standard protocols (see for example WO 03/035694, WO 04/041865, WO 04/041863, WO 04/062551 and other prior art and applications filed by Ablynx N.V. cited herein).

Example 3: Screening of periplasmic extracts

3.1 Screening for Kv1.3 binding Nanobodies in a flow cytometry assay

Periplasmic extracts were screened for cell expressed Kv1.3 binding in a FACS assay using in house made Kv1.3-expressing CHO-K1 and/or HEK293H cells. 2×10^5 cells were incubated in 1:5 diluted periplasmic extracts for 30 min at 4°C, and then thoroughly washed. Next, cells were incubated with 1 µg/ml monoclonal ANT-FLAG® M2 antibody (Sigma-Aldrich, cat# F1804) for 30 min at 4°C, washed again, and incubated for 30 min at 4°C with goat anti-mouse PE labelled antibody (1:1000). Samples were washed, resuspended in FACS Buffer (D-PBS from Gibco, with 10% FBS from Sigma and 0.05% sodium azide from Merck) supplemented with 5 nM TOPRO3 (Molecular Probes cat# T3605). Cell suspensions were then analyzed on a FACS Array. Gating was set on live, intact cells using forward/side scatter and TOPRO3 channel fluorescence parameters. Live cell PE channel mean channel fluorescence values higher than those obtained for control experiments including an irrelevant specificity binding Nanobody indicated that a clone bound the cell line. In addition absence of binding to the parental cell line was checked.

3.2 Screening for inhibitory Kv1.3 Nanobodies in electrophysiology

Periplasmic extracts were electrophysiologically screened for inhibitory effects on the voltage-gated potassium channel Kv1.3 on the IonFlux™ automated Patch Clamp using Kv1.3-expressing HEK293H cells. The complete procedure for evaluating the modulatory effect of periplasmic extracts on human Kv1.3 via electrophysiological recordings is given below.

IonFlux™ 16

The IonFlux™ (Molecular Devices) is an automated patch clamp system with integrated Well Plate Microfluidic™ Technology, temperature control and continuous perfusion and voltage clamp. The IonFlux™ 16 has sixteen parallel amplifiers and uses 96-well IonFlux plates conform to the Society for Biomolecular Sciences. This system allows both population and single cell patch clamp.

Solutions and Nanobodies handling

Extracellular solution contained (in mM): 132 NaCl, 5.4 KCl, 1.8 CaCl2, 0.8 MgCl2, 10 HEPES, 5 glucose (pH 7.2 with NaOH, and 285 – 290 mOsmolar). Intracellular solution contained (in mM): 40 KF, 100 KCl, 2 MgCl2, 10 HEPES, 5 EGTA (pH 7.45 with CsOH, and 300 – 315 mOsmolar). These solutions were freshly made, stocked for no longer than a month at 4 °C and filtered prior to use. Periplasmic extract were 1:5 diluted in extracellular solution and transferred to a V-bottom deep well square well plate (Westburg, #AB0932).

Cell preparation

HEK293H cells stably expressing the human Kv1.3 channel were generated in house. Cells were cultured in T-175 cell culture flasks (Greinerbio-one, #660160) using standard culture medium DMEM Glutamax™ (GIBCO, #31966) containing 10 % FBS (Sigma-Aldrich, #F7524), 1% penicillin + streptomycin (GIBCO, #15140-122), 1mg/ml G418 (GIBCO, #10131-027). Cells were seeded at a density of 25.000 cells/cm² or 12.000 cells/cm² for 2 or 3 days respectively before being used on the IonFlux™ 16 (Fluxion, Molecular Devices). Optimal Cell confluence prior to harvesting never exceeded 80 %. The cells were washed twice with d-PBS without Ca2+ and Mg2+ (Invitrogen, #14190) and detached with 3ml Trypsin/EDTA 0.25% (Invitrogen, Cat 25200-056) for 5 to 10 min at 37°C. DMEM Glutamax™ containing 10 % FBS was added to inactivate the enzymatic reaction triggered by the trypsin. Subsequently, the cell pellet was re-suspended in 20 ml d-PBS + 10 % FBS and centrifuged at 200 x g during 10 min at RT in 50 ml conical CELLSTAR® tube (Greinerbio-one, #227-261). Cells were counted (Casy TT, Roche), suspended at 1 million cells/ml, transferred to a new 50 ml conical CELLSTAR® tube and gently shaken at RT for approximately 20 min. One million cells were centrifuged for 2 min at 200 x g. The pellet was gently resuspended in 5 ml extracellular buffer and centrifuged a second time for 2 min at 200 x g. Finally, the pellet was resuspended in 2000 µl extracellular buffer and immediately tested on the IonFlux™.

IonFlux™ 16 procedure and the human Kv1.3 assay

250 µl of sterile cell culture grade water was dispensed into every well of the IonFlux 96-well plate except the outlet wells, using an 8 channel multi-pipette. Before rinsing the plate, excess water present on the rim of the plate was wiped off. The designated plate was inserted into the IonFlux system and subsequently rinsed 4 times according to a standard Water Rinse protocol. After rinsing, the plate was emptied. The inlet wells were then manually filled with extracellular buffer, trap wells with intracellular buffer and the diluted Nanobodies or selective peptides were

distributed into the compounds wells (250 µl/well). Subsequently, the plate was primed before the actual experiment according to the plate specific protocols. For population plates (Molecular Devices, #910-0098): 1) traps and compounds at 5 psi for t= 0 – 160 s and 2 psi for t= 160 – 175 s, 2) traps but not compounds at 2 psi for t= 175 – 180 s, and 3) main channel at 1 psi for t= 0 – 160 s and 0.3 psi for t= 162 – 180 s. For single cell plates (Molecular Devices, # 910-0100): 1) traps but not compounds at 11 psi for t= 0 – 350 s and 1.5 psi for t= 625 - 630 2) traps and compounds at 5 psi for t= 350 – 600 s and 1.5 psi for t= 600 – 625 s, and 3) main channel at 0.5 psi for t= 0 – 350 s and 1 psi for t= 350 – 600 s, and 0.3 psi for t= 600 – 627 s. After priming, the out- and inlet wells were emptied and 250 µl of the prepared cell suspension (i.e. approximately 1 million cells) was distributed into the inlet wells of the designated plate. After introduction of the cells, the plate was reprimed: 1) traps and compounds at 5 psi for t= 0 – 20 s and 2 psi for t= 25 – 50 s, 2) traps not with compounds at 2 psi for t= 50 – 55 s, and 3) main channel at 1 for t= 0 – 30 s and 0.4 psi for t= 30 – 55 s. Then, cells were introduced to the main channel and trapped at lateral trapping sites with the trapping protocol: 1) trapping vacuum of 8 mmHg for t= 0 to 76 s, 2) main channel pressure of 0.2 psi for t= 0 – 2 s, followed by 13 repeated square pulses of 0 – 0.2 psi with baseline duration of 4.5 s and pulse duration of 0.8 s, followed by 0.2 psi for 2 s. Whole cell access was achieved by rupturing the patch of the membrane over the hole using the following break protocol: 1) breaking vacuum of 8 mmHg for t= 0 – 15 s, followed by a pulse square pulse of 8 – 16 mmHg with a pulse duration of 10 s, and followed by 5 mmHg for 10 s, and 2) main channel pressure at 0.15 psi for t= 0 – 35 s. After whole cell configuration, the vacuum pressure was held at 5 mmHg and the main channel pressure at 0.1 psi until the end of the experiment. Cells were first allowed to dialyze for 240 s, before compounds were tested. A time course protocol was applied to assess the effect of the compounds on potassium currents elicited by a depolarizing pulse protocol. In order to be able to perform an off-line linear leak subtraction, cells were clamped at -80 mV for 10 ms then hyperpolarized to -100 mV for 50 ms, and repolarized to -80 mV for 30 ms. Subsequently, potassium currents were provoked by a depolarizing step from -80 mV to +40 mV for 250 ms at 30 s interval (as shown in Figure 2A). After the stabilizing period, extracellular buffer was continuously perfused during 120 s as a negative control, followed by sequential perfusion of periplasmic extracts, different concentrations of Nanobodies or selective peptides. The interval between several compound additions was 120 s. The inhibitory responses were recorded at room temperature (21°C – 24°C) with a minimum of n= 2 at each compound.

IonFlux data inclusion criteria and data analysis

Data points were accepted if:

A) Automated Population Patch

1) Individual membrane resistance quality and stability was > 50 MΩ during data acquisition
2) Current amplitude quality and stability was > 5 nA at +40 mV after negative control
3) Run-up/run-down < 10 % during data acquisition
4) Standard IC_{50} value within anticipated range

B) Automated Single cell patch

1) Individual membrane resistance quality and stability was > 500 MΩ during data acquisition
2) Current amplitude quality and stability was > 200 pA at +40 mV after negative control
3) Run-up/run-down < 10% during data acquisition
4) Standard IC_{50} value within anticipated range

Currents were measured using IonFlux software (Fluxion Biosciences), monitored continuously during the exposure to the compounds, and outliers were excluded to filter out recordings that were lost. Measured currents were normalized by the mean sustained current corrected amplitude prior to compound addition (Figure 2B). Current inhibition was estimated by the residual response after 120 s of each compound application. IonFlux software (Fluxion Biosciences), Microsoft Excel (Microsoft) and Prism 6 (GraphPad Software) were used for current analysis.

3.3 Screening for Nanobodies blocking 125I-margatoxin binding to Kv1.3 expressing cells

Periplasmic extracts were screened in a radioligand 125I-margatoxin competition assay to assess the blocking capacity of the expressed Nanobodies. Cynomolgous Kv1.3 was presented on CHO cells overexpressing Kv1.3.

In order to detect binding of margatoxin to cell expressed Kv1.3, a radiolabeled toxin was used (125I-margatoxin; MgTX; Perkin Elmer, NEX083). To setup the assay, first a titration series of the radiolabeled 125I-margatoxin was performed on the CHO-cyKv1.3 and parental CHO K1 cells. To have maximum sensitivity of the screening, the EC_{30} concentration (150 pM) was chosen for competition during screening and later on, also characterization.

In brief, 35 µl of periplasmic extract was added to 150 pM labeled margatoxin (50µl) and 40000 CHO-cyKv1.3 cells that were seeded the day before in a poly-D-lysine coated 96-well

plate (BD Biocoat, Cat354620) in a total of 200 µl. After two hours incubation at RT, cells were washed two times before read out was performed with 100 µl/well MicroScint-20 (Perkin Elmer) on a TopCount device (Perkin Elmer).

As reference coumpounds, a dilution series of ShK-1aJ (Smartox, #08SHK001) and unlabeled margatoxin (Alamone labs, Cat RTM-325) were included. As controls, conditions were taken along where there was no Nanobody present in the peri extract or a known irrelevant Nanbody and samples were included where excess cold margatoxin was included. For each sample the percentage block was determined using the control samples to determine the assay window.

3.4 Conclusion

Nanobodies which scored positive in the flow cytometric screening, the ephys assay or 125I-Margatoxin competition assay were sequenced. Corresponding amino acid sequences are shown in Table A-1. Clones were clustered into sequence families based on their overall sequence. 2 distinct families (Family 1 and 12) belonging to 2 different B-cell lineages of Kv1.3 binders were identified. Corresponding alignments are provided in Table A-4 and Table A-5, respectively.

Example 4: Characterization of purified Nanobodies

Binding/inhibitory anti-Kv1.3 Nanobodies selected from the screening described in Example 3 supra were further purified and characterized. Selected Nanobodies were expressed in E. coli TG1 as triple Flag, His6-tagged proteins. Expression was induced by addition of 1 mM IPTG and allowed to continue for 4 hours at 37°C. After spinning the cell cultures, periplasmic extracts were prepared by freeze-thawing the pellets. These extracts were used as starting material and Nanobodies were purified via IMAC and size exclusion chromatography (SEC) resulting in 95% purity as assessed via SDS-PAGE.

4.1 Binding of anti-Kv1.3 Nanobodies to human, cyno and rat Kv1.3 expressed on CHO cells

Binding of 2 exemplified monovalent Nanobodies to human, cyno and rat Kv1.3 expressed on CHO cells was evaluated on FACS as outlined in Example 3. Dilution series of anti-Kv1.3 Nanobodies starting from 1 µM down to 10 pM were applied to the cells. As a control, the parental CHO cell line was included (see Figure 3 A-F). Both Nanobodies clearly bind to human,

cyno and rat Kv1.3 although with slightly lower potency to the latter. The EC_{50} values obtained from the dose response curve are depicted in Table B-1.

Table B-1: EC_{50} (M) of anti-Kv1.3 monovalent Nanobodies in binding on cyno, rat, and human Kv1.3 expressed on CHO cells as determined in FACS.

VHH ID	CHO cyKv1.3	CHO ratKv1.3	CHO huKv1.3
A019400003	2.5E-09	3.8E-07	6.2E-09
A0194009G09	1.1E-08	2.4E-07	7.9E-09

4.2 Inhibition by monovalent anti-Kv1.3 Nanobodies of 125I margatoxin binding to cyno Kv1.3 expressed on CHO cells

The blocking capacity towards radiolabeled margatoxin by the Nanobodies was evaluated in a human 125I-MgTX competition assay as outlined in Example 3.3, with the difference that here a dilution series of the purified Nanobodies/toxins was applied (Figure 4 A-C). The IC_{50} values for the Nanobodies/toxin (Shk, Smartox, #08SHK001) in blocking the interaction of MgTX to human Kv1.3 are depicted in Table B-2.

Table B-2: IC_{50} (M) of anti-Kv1.3 monovalent Nanobodies and ShK or MgTX compound for Inhibition of radiolabeled 125I-MgTX binding to cyKv1.3 expressed on CHO cells by binding.

VHH ID/compound	IC_{50} (in M)
A019400003	1.3E-09
A0194009G09	9.8E-09
ShK	2.4E-10
MgTX	2.4E-10

4.3 Electrophysiological characterization of monovalent inhibitory Kv1.3 Nanobodies on human Kv1.3 expressing HEK293H

IonFlux™

Selected Nanobodies were electrophysiologically characterized on human Kv1.3 on the IonFlux™ automated Patch Clamp using Kv1.3-expressing HEK293H cells. The procedure for evaluating the modulatory effect of the purified Nanobodies on human Kv1.3 via

electrophysiological recordings is given in Example 3.2 supra. A time course protocol was applied to assess the Nanobody potencies (IC_{50}) on potassium currents elicited by a depolarizing pulse protocol (Figure 2A). After the stabilizing period, extracellular buffer was continuously perfused during 120 s as a negative control, followed by sequential perfusion of different concentrations of Nanobodies or the selective hKv1.3 channel blocker *Stichodactyla helianthus* (ShK-1aj Smartox, #08SHK001). The interval between several additions of compound concentrations was 120 s. The half maximal inhibitory concentrations (IC_{50}) were calculated at room temperature from seven-point (unless stated otherwise) concentration-response curves with a minimum of n= 2 at each concentration.

In a "wash-off" experiment, a single high dose (300 nM) was applied during 120 s, followed by a continuous perfusion of extracellular buffer for at least 5 min, in order to assess the rate of current recovery during washout. In these experiments both population and single cell automated patch clamp were used to record current amplitudes.

Measured currents were normalized by the mean sustained current corrected amplitude prior to Nanobody addition (as shown in Figure 2B). Current inhibition was estimated by the residual response after 120 s of each Nanobody concentration application. The IC_{50} and hill slope for compound concentration were then fit to the following formula:

Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*Hill Slope))

IonFlux software (Fluxion Biosciences), Microsoft Excel (Microsoft) and Prism 6 (GraphPad Software) were used to analyze and present IC_{50} values and currents.

The results show a dose-dependent inhibition of the selected Nanobodies with almost complete current recovery. Data are given below in Table B-3, and the typical experiments are shown in Figures 5 to 7.

Table B-3: Characterization of monovalent human Kv1.3 channel inhibitory Nanobodies on Ionflux™

Nanobody ID	**Average IC_{50}* (M)**
A0194009G09	8.2E-09
A01940016B04	8.4E-08

A01940020A06	1.34E-08
Reference compound	**Average IC_{50} (M)**
ShK-1aJ (Smartox, #08SHK001)	7.6E-11

(*)IC_{50} values generated on the IonFlux™ system (compound concentration at which Kv1.3 Ion channel current is 50% of the current in absence of the compound)

IonWorks

Selected Nanobodies were electrophysiologically characterized on human Kv1.3 on the IonWorks automated perforated-Patch Clamp using Kv1.3-expressing Chinese Hamster Lung (CHL) cells. The procedure for evaluating the modulatory effect of the purified Nanobodies on human Kv1.3 via electrophysiological recordings is given below.

IonWorks Quattro

IonWorks Quattro (Molecular Devices) is a second-generation screening instrument that provides membrane voltage control and provides a direct electrophysiological assay for screening and characterization of compounds. It is an automated, high-throughput planar perforated patch clamp that uses a 384-well PatchPlate™ substrate.

Solutions and Nanobody handling

Extracellular solution contained (in mM): 138 NaCl, 2.7 KCl, 0.9 CaCl2, 0.5 MgCl2, 8 Na_2HPO_4, and 1.5 KH_2PO_4 (pH 7.3 with NaOH, and 285 – 290 mOsmolar). The intracellular solution contained (in mM): 100 K-gluconate, 40 KCl, 3.2 EGTA, 5 HEPES and 3.2 MgCl2 (pH 7.3 with KOH, and 300 – 315 mOsmolar). These solutions were freshly made, stocked for no longer than a month at 4°C and filtered prior to use. The selected Nanobodies were directly diluted in extracellular solution to obtain a 3 µM sample solution. A 96 well master plate was prepared by transferring 300 µL of 3 µM sample solution and an up plate dilution was carried out (1:3). For the Kv1.3 assay, 50 µl of each sample was transferred to columns of a 384-well plate (Costar polypropylene, # 3657). Quinidine standard curves were included, along with vehicle (low) and quinidine (high: 300 µM final assay concentration) controls.

Cell preparation

Chinese Hamster Lung (CHL; Essen Bioscience) cell lines stably expressing the full length human Kv1.3 channel were cultured in T-175 cell culture flasks (Greinerbio-one, #660160) using standard culture medium DMEM (Invitrogen, #41965) containing 10% FBS (HyClone, # SH3007103), 1% non-essential amino acids (Invitrogen, #11140), 1% sodium-pyruvate (Invitrogen, #C11360), 1% penicillin + streptomycin (Invitrogen, #C10378), 200 µg/ml G418 (Invitrogen, #10131), 20 mM HEPES (Invitrogen, #15630-114), and 29 mM KCl (Sigma, #P5405). Cells were seeded at a density of 25.000 cells/cm² or 12.000 cells/cm² for 2 or 3 days respectively before being used on the IonWorks (Essen Bioscience). Optimal Cell confluence prior to harvesting was 50-80 %. The cells were washed with 20 ml PBS without Ca^{2+} and Mg^{2+} (GibCo, # 14190-094) and detached with 2 ml Trypsin/EDTA 0.25% (GibCo, #25200-056) for 6 min at 37°C. The cells were diluted with 10 ml of external buffer (GibCo, #14040). The suspension was transferred to a 15 ml centrifuge tube and centrifuged for 2 minutes at 200 x g. The supernatant was removed and the pellet re-suspended in 4.5 ml of extracellular buffer. Following approximately 3 titrations with a 5 ml Corning Costar® stripette (Sigma-Aldrich, #CLS4487), a further 70 titrations were conducted with a 200 µl pipette. The cell suspension (at a density of 3-5M cells per ml) was added to the cell boat within the IonWorks and the experiment instigated.

IonWorks procedure and assay

The basic principles of IonWorks automated patch clamp electrophysiology are described by Schroeder et al. (J Biomol Screen 8(1):50-64, 2003). The experiments summarized therein used the population patch clamp (PPC) configuration described by Finkel et al. (J Biomol Screen 11(5):488-96, 2006). Either single cell single cell mode (HT) or population (PPC) mode was used in the assays, depending on the K_v ion channel. In PPC mode, an ensemble average of the current from up to 64 cells per well was recorded.

Electrical access was achieved using 100 µg/ml amphotericin (Sigma, #A4888) in the internal solution to obtain the perforated-patch clamp configuration. Cells were initially held for a period of 30s at -80mV. A pulse train of fifteen depolarizing steps (P1 to P15) from -80 mV to +50 mV for 100 ms at 3 Hz pulse intervals was performed in control conditions (prior to compound addition). The Nanobodies were then incubated for 6 to 7 min prior to the second measurement using the identical pulse train (as shown in Figure 8A).

IonWorks data inclusion criteria and data analysis

Data points were accepted if the following well and plate Quality Control criteria were met:

1) Individual seal resistances > 20 MΩ on pre- and post-compound reads
2) Individual peak Kv1.x current amplitude >500 pA
3) Plate Z' value > 0.4 (where determined)
4) Plate average seal resistance > 30 MΩ
5) Plate average mean current amplitude > 0.5 nA
6) Standard IC_{50} value within anticipated range

Currents were measured using IonWorks software v.2.0.4.4. (Fluxion Biosciences). Kv1.3 currents were measured as sustained currents in the gating step pulse P1 (Figure 8B). The effects of the compounds were quantified by dividing the current in the presence of the compound by the pre-compound current. The selective hKv1.3 channel blocker ShK-1aJ was used as reference standard in the hKv1.3 assay, while for the Kv1.5, Kv1.6 and hERG assay quinidine was used. This percentage of inhibition value was then normalized by using the following equation:

$$\text{Norm \% I} = \frac{100 - \left[\left(\frac{\text{post}}{\text{pre}}\right) \text{x } 100\right]}{\text{Average } \left[\left(\frac{\text{DMSOpost}}{\text{DMSOpre}}\right) \text{x } 100\right]}$$

Subsequently, the Kv1.3 data were further normalized to the maximal block control to remove the impact of the small (~10%) residual outward currents unblocked by quinidine. IonWorks software (Molecular devices), Microsoft Excel (Microsoft) and Prism 6 (GraphPad Software) were used to analyze and present IC_{50} values and currents.

Representative Kv1.3 current traces of Nanobody A0194009G09 reveal a potent concentration-dependent inhibition with nearly full block at highest tested dose (Figure 9A and 9B). The correlated concentration-response curve for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ is presented in Figure 9C.

Representative Kv1.3 current traces of Nanobody A019400003 demonstrate a biphasic modulatory effect on Kv1.3 ion channels with at low concentrations (*e.g.* 130 pM) an attenuated cumulative pulse to pulse interaction (Figure 10A), and at higher concentrations (*e.g.* 100 nM) an inhibitory effect (Figure 10B). The correlated concentration-response curve for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ is presented in Figure 10C. Corresponding IC_{50} values are given in Table B-4.

Table B-4: Characterization of monovalent human Kv1.3 inhibitory Nanobodies on IonWorks

Nanobody ID	Average IC_{50}* (M)
A0194009G09	3.7E-08
A0194016B04	3.4E-08
A019400003	> 1 μM
Reference compound	**Average IC_{50} (M)**
ShK-1aJ (Smartox, #08SHK001)	4E-10

(*)IC_{50} values generated on the IonWorks (compound concentration at which Kv1.3 Ion channel current is 50% of the current in absence of the compound)

4.4 Inhibition by monovalent anti-Kv1.3 Nanobodies of IFNγ production and CD25 expression of $CCR7^-CD45RA^-$ T cells after stimulation with anti-CD3

The purified anti-Kv1.3 Nanobodies were characterized in a T Cell-activation assay. Human T Cells were first collected from Buffy Coat blood (from healthy volunteers, Bloodbank Gent) using RosetteSep (StemCell Technologies, #15061) followed by an enrichment on Ficoll-Paque™ PLUS (GE Healthcare #17-1440-03). CCR7-CD45RA- T cells were isolated through negative selection using biotinylated antibodies against CD45RA (BD Bioscience #624008) and CCR7 (BD Bioscence #624009) in addition to the Dynabeads Biotin Binder (Invitrogen #110.47). The purity of the population was afterwards checked with anti-CD3 (eBioscience # 12-0037-73); anti-CD8 (BD Bioscience #345775); anti-CD4 (BD Bioscience #345771); anti-CD45RO (BD Bioscience #555493); anti-CD45RA (BD Bioscience # 550855); anti-rat IgG (Jackson ImmunoResearch Laboratories #112-116-143); anti-CD19 (BD Bioscience #555413) and anti-Human CCR7 (R&D Systems #MAB197) labeled antibodies in flow cytometric assay.

Isolated $CCR7^-CD45RA^-$ T Cells were then stimulated on anti-CD3 (eBioscience 16-0037-85; 540 ng/ml) coated 96-well plates at a concentration of 200 000 cells/well in absence or presence of dilution series of anti-Kv1.3 antibodies and ShK positive control (Smartox, #08SHK001). After 72h, IFN-gamma production was measured with anti-human IFNγ antibody capture in ELISA (BD Bioscence #551221) combined with biotinylated anti-human IFNγ (BD Bioscience, #554550) and streptavidin-HRP (Dakocytomation #P0397) as detection (Figure 11A-11B).

In addition, also CD25 expression was measured in flow cytometry, using an anti CD25 antibody (BD Pharmingen, cat 557138) (Figure 11C-11D). The A019400003 Nanobody did not block

the stimulation of T-cells, whereas A0194009G09 and A0194020A06 clearly inhibited the responses, however with lower potency compared to ShK.

The average inhibitory IC_{50} values of the anti-Kv1.3 monovalent Nanobodies are depicted in Table B-5.

Table B-5: Inhibition by monovalent anti-Kv1.3 Nanobodies of IFNγ secretion and CD25 expression by $CCR7^{-}CD45RA^{-}$ T cells after stimulation with plate bound anti-CD3

VHH ID/compound	IC_{50} (in M) IFNγ read out	IC_{50} (in M) CD25 read out
irrelevant Nb	no effect	no effect
MgTX	9,4E-11	no fit
ShK	7,6E-11	2,7E-10
A0194009G09	4,7E-08	6,2E-08
A019400003	no effect	no effect
A01940020A06	3,0E-08	3,6E-08

Example 5: Generation and screening of multivalent Kv1.3 blocking Nanobodies

5.1 Construction of bivalent and trivalent, mono- and bispecific formats

In order to increase potency and/or efficacy, bi- and trivalent molecules were constructed by genetic engineering. Two or three Nanobodies were genetically linked together with a 35GS linker in between the building blocks and subsequently expressed in Pichia according to standard conditions.

Different multivalent constructs were made as listed in Table A-6.

5.2 Binding of multivalent anti-Kv1.3 Nanobodies to human, cyno and rat Kv1.3 expressed on CHO cells

Binding of the bi- and trivalent constructs to human, cyno and rat Kv1.3 was performed as outlined in Example 4.1 and is presented in Figure 12A-12D. The data indicate an improved binding of the formatted variants on all targets compared to their monovalent counterparts (see Figure 3). The EC_{50} values obtained from the dose response curve are depicted in Table B-6.

Table B-6: EC_{50} (M) of anti-Kv1.3 multivalent Nanobodies for binding on cyno, rat, and human Kv1.3 expressed on CHO cells as determined in FACS

VHH ID	CHO cyKv1.3	CHO ratKv1.3	CHO huKv1.3
A019400004	4.4E-10	1.1E-09	2.2E-09
A019400013	2.4E-10	4.9E-10	3.2E-10
A019400014	4.1E-10	7.4E-10	1.1E-09
A019400015	3.1E-10	4.4E-10	6.6E-10

5.3 Inhibition by multivalent anti-Kv1.3 Nanobodies of 125I Margatoxin binding to cyno Kv1.3 expressed on CHO cell

The inhibition of margatoxin binding to cyno Kv1.3 was investigated for the different formats as described in Example 3.3 and Example 4.2 (Figure 13A-13E). Anti-Kv1.3 Nanobodies completely block binding of 150 pM I125 margatoxin to cyno Kv1.3. The background (BG) is the control condition where no I125 margatoxin was added. A clearly improved potency was observed compared to its monovalent counterparts (Figure 4). An overview of the obtained IC_{50} values is shown in Table B-7.

Table B-7: Inhibition of radiolabeled 125I-MgTX binding to cyno Kv1.3 expressed on CHO cells by anti-Kv1.3 multivalent Nanobodies

VHH ID/compound	IC_{50} (in M)
A019400013	6.6E-10
A019400004	6.4E-10
A019400012	5.0E-10
A019400014	6.1E-10
A019400015	2.0E-10
A019400032	5.6E-10

5.4 Electrophysiological characterization of multivalent inhibitory Kv1.3 Nanobodies on human Kv1.3 expressing HEK293H

IonFlux™

Selected Nanobodies were electrophysiologically characterized on human Kv1.3 on the IonFlux™ automated Patch Clamp using Kv1.3-expressing HEK293H cells. The complete procedure

for evaluating the modulatory effect of the purified Nanobodies on human Kv1.3 via electrophysiological recordings is given in Examples 3 and 4. A time course protocol was applied to assess the Nanobody potencies (IC_{50}) on potassium currents elicited by a depolarizing pulse protocol (Figure 2A). In a "wash-off" experiment, a single high dose of Nanobody (300 nM) was applied during 120 s, followed by a continuous perfusion of extracellular buffer for at least 5 min, in order to assess the rate of current recovery during washout. In these experiments both population and single cell automated patch clamp were used to record current amplitudes.

The selected Nanobodies A019400009, A019400012 and A019400014 produced a concentration-dependent inhibition with partial to full block at highest dose, and no current recovery could be observed after at least 5 min washout with extracellular buffer (Figures 14-16). Corresponding IC_{50} values are given in Table B-8.

Table B-8: Characterization of multivalent human Kv1.3 channel inhibitors

Nanobody ID	**Nanobody format**	**Average IC_{50} *(M)**
A019400009	Bivalent	7.3E-10
A019400012	Biparatopic	4.2E-09
A019400014	Biparatopic	1.4E-08
Reference compound		**Average IC_{50} (M)**
ShK-1aJ (Smartox, #08SHK001)		7.6E-11

(*)IC_{50} values generated on the IonFlux™ system (compound concentration at which Kv1.3 Ion channel current is 50% of the current in absence of the compound)

IonWorks

Selected Nanobodies were electrophysiologically characterized on human Kv1.3 on the IonWorks automated perforated Patch Clamp using Kv1.3-expressing Chinese Hamster Lung (CHL) cells. The procedure for evaluating the modulatory effect of the purified Nanobodies on human Kv1.3 via electrophysiological recordings is given in Example 4. Repeated gating voltage-command protocols were employed to determine Nanobody potencies (IC_{50}). Kv1.3 currents were measured as sustained currents in the first gating step pulse P1 (see Figure 8).

The half maximal inhibitory concentrations (IC_{50}) were calculated at room temperature from eight-point concentration-response curves with an n= 4 at each concentration. The effects of

the compounds were quantified by dividing the current in the presence of the compound by the pre-compound current. The selective hKv1.3 channel blocker ShK-1aJ (Smartox, #08SHK001) was used as reference standard in the hKv1.3 assay. This percentage of inhibition value was then normalized as described in Example 4.3. The Kv1.3 data was then further normalized to the maximal block control to remove the impact of the small (~10%) residual outward currents unblocked by ShK-1aJ. IonWorks software v.2.0.4.4. (Molecular devices), Microsoft Excel (Microsoft) and Prism 6 (GraphPad Software) were used to analyze and present IC_{50} values and currents.

Results are presented in Figures 17 to 22. Kv1.3 current traces of Nanobody A019400004 demonstrate a biphasic modulatory effect on Kv1.3 ion channels with, at low concentrations (*e.g.* 130 pM), an attenuated cumulative pulse to pulse interaction (Figure 17A), and at higher concentrations (*e.g.* 100 nM), an inhibitory effect (Figure 17B). The correlated concentration-response curve for the inhibition of human Kv1.3 channels, measured as normalized mean $I_{sustained}$ is presented in Figure 17C.

Representative Kv1.3 current traces of the multivalent Nanobodies A019400009, A019400012, A019400014, A019400015 and A019400032 reveal a concentration-dependent inhibition with partial to full block at highest tested dose (Figures 18A-18B to 22A-22B, respectively) and the correlated concentration-response curves for the inhibition of human $K_v1.3$ channels, measured as normalized mean $I_{sustained}$ are presented in Figures 18C to 22C, respectively. The IC_{50} values are given in Table B-9.

Table B-9: Characterization of the human Kv1.3 channel inhibitors

Nanobody ID	**Nanobody format**	**Average IC_{50}* (M)**
A019400004	Bivalent	> 1 µM
A019400009	Bivalent	8.1E-10
A019400012	Biparatopic	4.2E-09
A019400014	Biparatopic	1.4E-08
A019400015	Trivalent	8E-10
A019400032	bivalent	1.2 E-09
Reference compound		**Average IC_{50} (M)**
ShK-1aJ (Smartox, #08SHK001)		4E-10

(*)IC_{50} values generated on the IonWorks (compound concentration at which Kv1.3 Ion channel current is 50% of the current in absence of the compound)

5.5 Inhibition by multivalent anti-Kv1.3 Nanobodies of IFNγ production and CD25 expression by CCR7$^-$CD45RA$^-$ T cells after stimulation with anti-CD3

The inhibition of CCR7$^-$CD45RA$^-$ T cell activation after stimulation with anti-CD3 was evaluated for the bi- and trivalent constructs. An identical assay setup was used as described above (see Example 4.4). Obtained results are summarized in Table B-10 and Figure 23A-23F. The formatted bivalent (A019400013) and trivalent (A019400015) Nanobodies inhibited IFNγ secretion and CD25 expression with a similar potency compared to ShK. The biparatopic Nanobodies (A019400012 and A019400014) were slightly less potent.

Table B-10: Inhibition by multivalent anti-Kv1.3 Nanobodies of IFNγ secretion and CD25 expression by CCR7$^-$CD45RA$^-$ T cells after stimulation with plate bound anti-CD3.

VHH ID/compound	IC_{50} (in M) IFNγ read out	IC_{50} (in M) CD25 read out
A019400004	no effect	no effect
A019400013	8,3E-11	1,2E-10
A019400015	3,2E-11	5,9E-11
A019400012	3,1E-10	3,1E-10
A019400014	3,2E-10	1,1E-10
A019400032	6,9E-11	1,2E-10

Example 6: Mapping of the binding epitope of the anti-Kv1.3 Nanobodies

In order to determine the binding epitope of the Nanobodies belonging to different B-cell lineages; binding of anti-Kv1.3 Nanobodies to mutant Kv1.3 constructs expressed on HEK293H cells was checked in flow cytometry. In these mutants the first extracellular loop (EL1) was replaced by an irrelevant amino acid stretch. Expression of these constructs was evaluated with fluorescently labeled agitoxin (rAgitoxin-2-Cys-TAMRA (Alomone Labs #RTA-420-T)) in flow cytometry (see Figure 24). The experiment was performed as outlined in Example 4.1 with the difference that cells were used with expression of Kv1.3 EL1 mutants instead of WT human Kv1.3.

None of the evaluated samples showed detectable binding to the Kv1.3 EL1 mutants (data not shown).

Example 7: Allosteric binding

To evaluate the competition between the toxin ShK and the Nanobodies for binding to Kv1.3, a FACS competition experiment was performed using HEK293H cells overexpressing human Kv1.3 and the parental HEK293H cells as background cell line. As detection reagent, FAM- labeled ShK was used (6-FAM-AEEAc-Stichodactyla helianthus Neurotoxin (ShK) (Bachem, H-6088, 1046522, PRT00000366/01/01)). To setup the assay, first a titration series of the labeled Shk-FAM was performed on the HEK293H Kv1.3 cells in order to determine the EC_{50} value for binding. In order to determine allosteric competition, so working at saturating concentration, the labeled ShK-FAM was used at 100x the EC_{50} concentration in the competition experiment (70 nM).

In brief, a dilution series of ShK or Nanobody were added together with the labeled toxin to 200 000 cells in a 96-well plate. After 90 minutes incubation at 4°C, cells were washed three times before read out was performed on a FACS Canto (Becton Dickinson). First a gate was set on the intact cells as determined from the scatter profile. Next, dead cells were gated out by their fluorescence profile from the TOPRO stain (5 nM, Molecular probes, T3605). Results are provided in Figure 25. The monovalent A019400003 and A0194009G09 only partially blocked binding of FAM labeled ShK to Kv1.3 whereas the unlabeled ShK completely blocked binding indicating that the monovalent Nanobodies allosteric compete with the ShK toxin. The percentage of inhibition for Nanobodies blocking the interaction of ShK to human Kv1.3 is depicted in Table B-11.

Table B-11: FACS competition assay: competition between high concentration of ShK-Fam (70nM) and anti-Kv1.3 Nanobodies for binding to HEK 293H hu Kv1.3 cells

	% inhibition
A019400003	87,13
A0194009G09	40,79
ShK (non labeled)	102,27

Example 8:Exploration of half-life extension

Alb11, a Nanobody binding to human serum albumin was linked to the multivalent Kv1.3 Nanobodies to increase the *in vivo* half-life of the formatted molecules (WO 06/122787). Different formats were made including different positioning of the different composing Nanobodies. An overview of the explored formats is shown in Table A-3.

As the binding of human serum albumin (HSA) to the Alb11 Nanobody might have an impact on the potency of the (multivalent) Nanobody, the half-life extended Nanobodies were characterized in several assays in the presence of HSA (see Examples 8.3, 8.4 and 8.5 infra).

8.1 Evaluation of positioning of the Alb11 Nanobody in FACS

Analogous as described in Example 4.1, binding of half-life extended anti-Kv1.3 Nanobodies to cyno and rat Kv1.3 expressed on CHO cells was explored in a flow cytometric assay (Figure 26A-26B). The EC_{50} values obtained in this assay are listed in Table B-12.

8.2 Evaluation of positioning of the Alb11 Nanobody using automated Patch Clamp electrophysiology

The half-life (HLE) extended Nanobody was electrophysiologically characterized on the human Kv1.3 on the IonFlux™ automated Patch Clamp using Kv1.3-expressing HEK293H cells. The complete procedure for evaluating the modulatory effect of the purified Nanobodies on human Kv1.3 via electrophysiological recordings is given in Examples 4 and 5. A time course protocol was applied to assess the Nanobody potency (IC_{50}) on potassium currents elicited by a depolarizing pulse protocol (as shown in Figure 2A). In a "wash-off" experiment, a single high dose was applied during 120 s, followed by a continuous perfusion of extracellular buffer for at least 5 min; this in order to assess the rate of current recovery during washout. In this experiment the single cell automated patch clamp was used to record current amplitudes.

The selected Nanobody produced a concentration-dependent inhibition with full block at highest dose (Figure 27A-27B), and no current recovery could be observed after at least 5 min washout with extracellular buffer (Figure 27C). The IC_{50} value for the trivalent Nanobody A019400029 was 3.8E-09 M.

8.3 Impact of human serum albumin on the potency in binding FACS

The half-life extended Nanobodies were evaluated for binding to cyno and rat Kv1.3 expressed on CHO cells in a flow cytometric assay as outlined in Example 4.1. In addition, HSA (50µM; Sigma, Cat A8763) was added to all reagents and buffers that were used during the assay to allow binding of HSA to Alb11 (Figure 26C-26D). The EC_{50} values are shown in Table B-12.

Table B-12: EC_{50} (M) values of half-life extended Nanobodies for binding Kv1.3 (cyno and rat) in FACS assay in absence and presence of HSA

Construct ID	EC_{50} on cyno Kv1.3	EC_{50} on rat Kv1.3
A019400013	3,3E-10	7,04E-10
A019400013 + HSA	4,5E-10	8,5E-10
A019400023	9,4E-10	1,2E-09
A019400023 + HSA	2,1E-09	3,7E-09
A019400024	8,1E-10	1,3E-09
A019400024 + HSA	1,6E-09	3,2E-09
A019400027	4,6E-10	8,3E-10
A019400027 + HSA	8,1E-10	2,0E-09
A019400025	7,8E-10	1,1E-09
A019400025 + HSA	7,6E-09	2,3E-08
A019400026	4,4E-10	7,2E-10
A019400026 + HSA	2,1E-09	7,3E-09
A019400028	7,2E-10	1,2E-09
A019400028 + HSA	5,8E-09	2,0E-08

8.4 Impact of human serum albumin on the potency in 125I margatoxin competition

The half-life extended Nanobodies were also evaluated for competition with binding of 125I margatoxin to cyno Kv1.3 expressed on CHO cells in presence of HSA, as previously described in Examples 4.2 and 5.3. First, the influence of 25 µM HSA (Sigma, Cat A8763) was evaluated on the binding of radiolabeleld I125 margatoxin, to confirm that HSA did not affect the dose response curve of I125 MgTX (data not shown). Next, the competition was performed in the absence and presence of 25 µM HSA for comparison (Sigma, Cat A8763). The data presented in Figure 28 show that HSA does not influence the potency of the construct. The IC_{50} values are shown in Table B-13.

Table B-13: IC_{50} values of half-life extended Nanobodies in 125I MgTX competition assay in presence and absence of HSA

Construct ID	IC_{50} on cyno Kv1.3
A019400029	9.0E-10
A019400029 + HSA	1.4E-09

8.5 Impact of human serum albumin on the potency of the half-life extended Nanobodies in T cell assay

The half-life extended Nanobodies were also tested in the T cell assay as outlined in Example 5.5. The Nanobodies were tested both in absence and presence of 10 µM HSA (Sigma, Cat A8763) (see Figure 29). The obtained IC_{50} values in both IFNγ and CD25 read out are shown in Table B-14.

Table B-14: IC_{50} values of half-life extended Nanobodies for IFNγ production and CD25 expression in T cell activation assay in the presence and absence of HAS

VHH ID/compound	IC_{50} (in M) IFNγ read out	IC_{50} (in M) CD25 read out
A019400013	8,3E-11	1,2E-10
A019400013 + HSA	3,1E-11	3,7E-11
A019400032	6,9E-11	1,2E-10
A019400032 + HSA	8,6E-11	3,3E-10
A019400024	1,9E-10	1,4E-10
A019400024 + HSA	6,6E-10	5,5E-10
A019400028	1,0E-10	1,4E-10
A019400028 + HSA	2,7E-09	5,1E-09
A019400027	3,8E-10	6,4E-10
A019400027 + HSA	9,8E-10	3,4E-09
A019400026	3,6E-10	1,1E-10
A019400026 + HSA	1,8E-09	3,2E-10
A019400029	1.9E-10	1.2E-10
A019400029 + HSA	6,2E-10	1.2E-10

8.6 Human and rat HSA binding in Surface Plasmon Resonance (SPR)

Binding of the half-life extended format to human and rat serum albumin (SA) was evaluated in SPR (surface plasmon resonance) on a BIAcore T100 instrument. For comparison, also the monovalent Alb11 Nanobody was tested for binding to human and rat SA.

Briefly, human and rat HSA (Sigma; #8763 and #A6272) were immobilized directly on a CM5 chip at respectively 320 and 2978 RU. The Nanobodies were then injected at different concentrations (between 1.6nM and 1000nM) for 120s and allowed to dissociate for 900s. Evalution of the binding curves was done using BIAcore T100 Evaluation software V2.0.3. Kinetic analysis was performed by fitting a 1:1 interaction model (Langmuir binding) (R_{max} = global; R_I = constant, offset = 0). Obtained K_D values can be found in Table B-15.

Table B-15: Binding of HLE Nanobody to Human and Rat serum albumin

	human SA			**rat SA**			**K_D on rat / K_D on human**
	k_a (1/Ms)	k_d (1/s)	K_D (M)	k_a (1/Ms)	k_d (1/s)	K_D (M)	
A019400029	4.0E+05	6.3E-03	1.6E-08	1.1E+05	4.9E-01	4.5E-06	281
ALB00011	6.0E+05	1.6E-03	2.8E-09	2.8E+05	2.3E-01	8.3E-07	296

Example 9:Effect of anti-Kv1.3 Nanobodies on IFNγ production by human PBMC's after anti-CD28 and anti-CD3 stimulation

The anti-Kv1.3 inhibitory Nanobodies were evaluated for their effect on cytokine secretion by plate-bound anti-CD3/CD28 stimulated PBMCs. ShK was included as reference compound (Figure 30). This co-stimulation of T-cells with anti-CD3 and anti-CD28 reflects strong immune stimulation as encountered during an acute infection. Single stimulation with anti-CD3 imitates rather the moderate immune stimulation resembling the situation during e.g. an autoimmune disease.

Briefly, PBMCs were first collected from Buffy Coat blood (from healthy volunteers, Bloodbank Gent) using RosetteSep (StemCell Technologies, #15061) followed by an enrichment on Ficoll-Paque™ PLUS (GE Healthcare #17-1440-03). The purity of the population was afterwards checked with anti-CD3 (eBioscience # 12-0037-73); anti-CD8 (BD Bioscience #345775); anti-CD4 (BD Bioscience #345771); anti-CD45RO (BD Bioscience #555493); anti-CD45RA (BD Bioscience #

550855) and anti-CD19 (BD Bioscience #555413) fluorescently labeled antibodies in a flow cytometric assay. The isolated PBMCs were then stimulated on anti-CD3 (eBioscience 16-0037-85; 540 ng/ml) coated 96-well plates at a concentration of 200 000 cells/well in absence or presence of anti-CD28 (1 µg/ml, Sanguin, M1650) and a dilution series of anti-Kv1.3 Nanobodies or ShK reference compound. After 72h, IFN-gamma production was measured with anti-human IFNγ antibody capture in ELISA (BD Bioscence #551221) combined with biotinylated anti-human IFNγ (BD Bioscience, #554550) and streptavidin-HRP (Dakocytomation #P0397) as detection.

As shown in Figure 30, the anti-Kv1.3 Nanobodies did not block IFNγ production of human PBMC's after anti-CD28 and anti-CD3 stimulation but show inhibition of the single anti-CD3 stimulation of these primary cells.

Example 10: Effect of inhibitory Nanobodies on the electrophysiological properties (Mode-Of-Action) of the Kv1.3 ion channel measured by conventional planar patch clamp electrophysiology

The effects of HLE Nanobody A019400029 on the electrophysiological properties of the Kv1.3 K^+ channel were evaluated. Current recordings were performed by conventional planar patch clamp electrophysiology using overexpressing Kv1.3 CHL cells. This procedure, together with the detailed voltage command protocols are given below.

Solutions and Nanobodies handling

Extracellular solution contained (in mM): 140 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose (pH 7.4 with NaOH, and 310 – 330 mOsmolar). Intracellular solution contained (in mM): 140 KCl, 1 MgCl2, 20 HEPES, 1 EGTA (pH 7.3 with KOH, and 295 – 310 mOsmolar). These solutions were filtered and stored for no longer than 6 weeks at 4°C. On each day of recording, an aliquot of the selected Nanobody was diluted with extracellular solution containing 0.1% BSA (Sigma, #A4503) to give a final concentration of 10 nM.

Cell preparation

Chinese Hamster Lung (CHL; Essen Bioscience) cell lines stably expressing the full length human Kv1.3 channel were cultured in T-175 cell culture flasks (Greinerbio-one, #660160) using standard culture medium DMEM (Invitrogen, #41965) containing 10% FBS (HyClone, #

SH3007103), 1% non-essential amino acids (Invitrogen, #11140), 1% sodium-pyruvate (Invitrogen, #C11360), 1% penicillin + streptomycin (Invitrogen, #C10378), 200 µg/ml G418 (Invitrogen, #10131), 20 mM HEPES (Invitrogen, #15630-114), and 29 mM KCl (Sigma, #P5405). Optimal Cell confluence prior to harvesting was 50-80 %. The cells were washed with 20 ml PBS without Ca2+ and Mg2+ (GibCo, # 14190-094) and detached with 2 ml Trypsin/EDTA 0.25% (GibCo, #25200-056) for 6 min at 37°C. The cells were diluted with 10 ml of standard cell culture medium containing 10% FBS, 1% non-essential amino acids, 1% sodium-pyruvate, 1% penicillin + streptomycin, 200 µg/ml G418, 20 mM HEPES, and 29 mM KCl. The suspension was transferred to a 15 ml centrifuge tube and centrifuged for 2 minutes at 200 x g. The supernatant was removed and the pellet was re-suspended in the same medium as described above. Cells were seeded at a density of 25.000 cells/cm² or 12.000 cells/cm² on poly-D-lysine coated glass coverslips 1 or 2 days prior to recordings.

Conventional planar Patch Clamp Electrophysiology

KV1.3 expressing CHL cells grown on poly-D-lysine coated glass coverslips were placed in the recording chamber perfused with extracellular solution and visualized on a Nikon Eclipse inverted microscope. Currents were recorded using standard whole-cell voltage-clamp techniques (Hamill et al., Pflugers Arch 391:85-100, 1981), at room temperature using an Axopatch 200B amplifier, converted to a digital signal using a digidata 1440A analogue-to-digital converter (Molecular Devices) and low pass Bessel filtered at 5 kHz and digitized at 10 kHz. Recording electrodes were pulled from borosilicate glass pipettes on a Sutter P-97 horizontal pipette puller yielding resistances of 2-6 MΩ when filled with intracellular solution. After formation of a tight seal (> 1GΩ) by manual suction in the voltage clamp mode, the command voltage set to -80 mV and pipette capacitance was compensated. The cell membrane was ruptured and compensation circuitry employed to minimize capacitance transients and 80-85% of series resistance errors (mean whole cell capacitance of 15 ± 3 pF and series resistance of 6.0 ± 2.3 MΩ; n=36). Leak currents were subtracted using the P/4 protocol supplied with the pClamp10 software. Membrane potentials were not corrected for junction potentials (4.1 mV as determined by Clampex 10 software). Descriptions of the voltage protocols to elucidate the Nanobody mechanism of action are provided in the figure legends. Samples were applied using a micro-injection needle coupled to a pressurized solenoid controller (ALA Scientific Instruments, ALA-

VM8/BPS-8 valve control system) positioned close to the recording cell (~200 µm). Correct positioning was confirmed by observing small movement of the cells upon switching.

Data analysis

The activation-conductance plot was fitted using a Boltzmann function: g_K/g_K max=1/[1+exp($V_{½}$-V/k)], where g_K is the conductance which is normalized relative to the maximum conductance (gK max), V½ is the membrane potential at which half the channels are activated and k is the slope of the curve. To allow the construction of inactivation curves the current was normalized (I) to that produced by a depolarization from -80 mV to +40 mV (I_{max}) and plotted against the conditioning pulse potential. The inactivation curves were fitted according to the Boltzmann function: I/Imax=1/[1+exp(V-$V_{½}$)/k], where V is the conditioning pulse potential, $V_{½}$ is the membrane potential at which half the channels are inactivated and k is the slope. Current amplitudes were determined as either peak outward current during the depolarizing pulse or the sustained current taken as the mean amplitude during the final 5 ms of the voltage step. The effects of the Nanobody or vehicle control were quantified by first dividing the KV1.3 current amplitude in the presence of treatment at the end of the incubation period by the amplitude of the KV1.3 current at the end of the pre-addition control period, multiplied by 100 yielding a % control current value. The % inhibition was determined by subtracting the % control current from 100. All data analyses were performed using Axon pClamp10, Microsoft Excel v7.0 and GraphPad Prism v5.0. The recovery from inactivation and/or inhibition studies used normalized currents employing the equation: % recovery = ($P2_{peak}$ – $P1s_{ustained}$)/($P1_{peak}$ – $P1_{sustained}$) x100, where $P2_{peak}$ is the maximal current from the test pulse, $P1_{sustained}$ is the current amplitude at the end of the conditioning pulse and $P1_{peak}$ is the maximal current from the conditioning pulse (see Figure 34).

Voltage protocols and results

Effects on voltage-dependence of activation were evaluated by determining the current voltage-relationship prior Nanobody application and after 5 minutes incubation of A019400029. Kv currents were provoked by a 500 ms depolarizing pulse to +50 mV in 10 mV steps from a holding potential of -80 mV at 30 s intervals in the absence (Figure 31B) and presence of 10 nM A019400029 (Figure 31C). A schematic of the voltage protocols is given in Figure 31A. The data points used in analysis represent peak current amplitudes as indicated in Figure 31B (arrow). In order to determine voltage dependence of block, an I-V plot and calculation of fractional block at

each test potential was performed (data not shown). A Boltzmann analysis (G/V) was done to measure effects on activation gating.

In order to evaluate the effect of A019400029 on the association and washout of Kv1.3 currents on human Kv1.3 channels stably expressed in CHL cells, Kv currents were provoked by a test pulse from -80 mV to +40 mV every 15 s. The test pulse duration was either 20 ms or 200 ms to determine whether kinetics of block were dependent on period of activation/inactivation (Figure 32A-B). Recordings were done in control conditions (prior to compound addition) and during a 3 to 5 min incubation of 10 nM A019400029, followed by compound washout. Peak and sustained current amplitudes were then plotted against the different time points. In addition, to investigate the effect of A019400029 on voltage-dependence of inactivation, the cells were held at either -80 mV or -50mV during the 3 to 5 minutes incubation with A019400029. Currents were provoked by a 200 ms test pulse from -80 mV to +40 mV every 15 s (Figure 33A-B). Peak and Sustained current amplitudes were then plotted against the different time points.

Application of 10 nM A019400029 markedly increased current decay following channel activation, but did not alter the voltage dependency of activation (Figure 31C). The inhibitory Nanobody A019400029 produced cumulative block of Kv1.3 currents when channels were repeatedly gated. Within each pulse, inhibition of both the early peak and sustained current was observed, however the effect on the sustained current was more rapid and pronounced. The rate of onset of the Nanobody blocking effect was slower when shorter pulses were employed. No current recovery could be observed after washout with extracellular buffer. Inhibition did not require channel inactivation (Figure 32) and using different pulse durations and holding potentials during Nanobody incubation showed that the inhibition induced by A019400029 appeared to be dependent on channel gating (Figure 33).

The recovery of inactivation from two inter-pulse potentials (-80 mV and -50 mV; Figure 34C) was measured using a standard variable interval gapped pulse protocol (as shown in Figure 34A). An initial 1 s pulse from -80 mV to +40 mV (P1) was followed by a second pulse from -80 mV to +40 mV for 150 ms (P2) after an interval of between 0.5 to 30 s. Representative traces in the absence and presence of 10 nM A019400029 are given in Figure 34B. The percentage of recovery was calculated (as described above) and plotted against pulse interval to show the recovery of inactivation (Figure 34C). In the presence of A019400029 both recovery from inactivation and inhibition could be observed when an inter-pulse potential of -80 mV has been employed, whereas

on application of an inter-pulse potential of -50 mV, an attenuation of recovery could be detected. Thus it appears that the relief of inhibition by 10 nM of A019400029 is voltage-dependent (Figure 34).

Example 11: Comparative pharmacology of inhibitory Kv1.3 Nanobodies on Kv1.3, Kv1.5, Kv1.6 and Kv11.1 K^+ (hERG) channels measured by automated patch clamp electrophysiology

Electrophysiological recordings were made from Chinese Hamster Lung (CHL) cell lines expressing the full length Kv1.3, Kv1.5 and hERG K^+ channels or Chinese Hamster Ovary (CHO) cells transiently transfected with Kv1.6 cDNA (ChanTest EZcells™ TT, #CT7220). Either single cell (HT) for hERG and Kv1.6 or population (PPC) patch clamp for Kv1.3 and Kv1.5 were made in the perforated patch clamp configuration using the IonWorks Quattro instrument. The more detailed procedure is explained in Example 4, together with cell culture conditions, cell preparations, the composition of the intracellular and extracellular solution used in these experiments. However, frozen Human Kv1.6-CHO EZcells™ TT were thawed very rapidly in a 37°C water bath and transferred to a 50 ml conical tube. Ten ml growth media Ham's/F12 (GibCo, #31765-027) containing 10% FBS (HyClone, # SH3007103), and 1% penicillin + streptomycin (Invitrogen, #C10378) and cells were centrifuged at 250 x g for 5 min. Pellet was resuspended in fresh 20 ml fresh medium and titrated to disperse cell clumps. The cell suspension (at a density of 3-5M cells per ml) was added to the cell boat within the IonWorks and the experiment was then initiated.

Additional note: for the hERG recordings the intracellular solution contained (mM): 140 KCl, 1 MgCl2, 1 EGTA, 20 HEPES (pH 7.3 with CsOH, and 300 – 315 mOsmolar). Kv1.3, Kv1.5 and Kv1.6 currents were elicited by a pulse train of fifteen depolarizing steps from -80 mV to +50 mV for 100 ms at 3 Hz pulse intervals in control conditions (prior to compound addition). The hERG currents were elicited by a pulse train of five pulses to +40 mV from V_H of -70 mV for 1 sec, then to -30 mV for 1 s, and then to -70 mV, every 3 s. The schematics of the voltage protocols are given in Figure 35. The Nanobodies were then incubated for 6 to 7 min prior to the second measurement using the identical pulse train. The selected Nanobodies were tested at 8 concentrations in up to 4 wells per concentration with repeated gating voltage-command.

Data points were accepted if the following well and plate Quality Control criteria were met.

A) Kv1.3 and Kv1.5

7) Individual seal resistances > 20 MΩ on pre- and post-compound reads

8) Individual peak Kv1.x current amplitude >500 pA

9) Plate Z' value > 0.4 (where determined)

10) Plate average seal resistance > 30 MΩ

11) Plate average mean current amplitude > 0.5 nA

12) Standard IC_{50} value within anticipated range

B) hERG and Kv1.6

1) Individual seal resistance > 50 MΩ on pre- and post-compound reads

2) Individual peak hERG tail current amplitude > 150 pA or peak Kv1.6 outward current amplitude > 400 pA

3) Plate average seal resistance > 100 MΩ

4) Plate average mean current amplitude > 0.3 nA

Currents were first measured under control conditions and after an incubation period of 6 to 7 min with the Nanobody using an identical protocol. Kv1.3, Kv1.5 and Kv1.6 currents were measured as peak and sustained currents in the first gating step pulse P1 and pulse 15. The hERG currents were measured at the peak in the tail step from pulse P5 (as shown in Figure 35). The effects of the compounds were quantified by dividing the current in the presence of the compound by the pre-compound current. This percentage of inhibition value was then normalized as described in Example 4. The Kv1.3 and Kv1.5 data was then further normalized to the maximal block control to remove the impact of the small (~10%) residual outward currents unblocked by quinidine. For Kv1.6 the small (mean current 0.24 nA) non-specific outward current was subtracted from all currents prior analysis.

Based on the comparison of the Nanobody concentration needed for inhibiting the channel, all selected Nanobodies displayed profound selectivity (*i.e.* greater than 1.000 fold) for Kv1.3 with no evidence for off target effects against Kv1.5, Kv1.6 and hERG K^+ channels. The maximal block at highest concentration tested (*i.e.* 1 μM) was less than 50 % in all other channels (Figure 36).

Example 12: Evaluation of the Kv1.3 Nanobodies in a delayed-type hypersensitivity (DTH) rat model

A delayed-type hypersensitivity (DTH) reaction is an expression of T cell-mediated immunity in response to cutaneous sensitization and challenge with reactive haptens like 2, 4-dinitrofluorobenzene (DNFB), largely mediated by skin-homing effector memory T cells (Azam P et al., J Invest Dermatol 127(6):1419-29, 2007; Matheu MP et al., Immunity 29(4):602-14, 2008). The voltage-gated potassium channel Kv1.3 is expressed in T cells, and is important in maintaining T cell activation (predominantly effector memory T cells).

For the purpose of *in vivo* proof-of-concept, the efficacy of anti-Kv1.3 Nanobodies on DNFB-induced delayed type hypersensitivity in Wistar rats was evaluated. The DTH response in the rats was elicited as follows (see Figure 37): on day 0 (start of the in-life) and day 1, 100 µL of 1 % (wt/vol) DNFB prepared in 4:1 acetone/olive oil was applied to the shaved dorsum for sensitization. On day 5, animals were challenged on both sides of the right ear pinnae of the animals with 50 µL of 0.5% (wt/vol) DNFB prepared in 4:1 acetone: olive oil. Animals (n = 10 rats/group) received one or two subcutaneous (s.c.) injection(s) of either vehicle, the reference compound ShK or an anti-Kv1.3 Nanobody (A019400029) at 12 hours and/or 1 hour preceding the challenge. As positive control, animals were treated with dexamethasone (topical, 0.75 mg at 1 hour and 6 hours post-challenge). On day 5 before DNFB challenge, the baseline right ear pinna thickness was measured, and the net ear swelling response was determined 24 hours after challenge with a spring-loaded micrometer.

The results of the experiments are shown in Figure 38. The vehicle-treated control animals showed a mean increase in right ear thickness of 0.280±0.037 mm. Rats from the positive control group, treated with dexamethasone 1h and 6h post-challenge (topical), showed a clear reduction in the ear swelling response (mean increase in ear thickness of 0.027±0.017 mm). Rats treated with two s.c. injections of 10 µg/kg of the reference compound ShK showed a statistical significant reduction of the ear swelling response versus vehicle (mean increase in ear thickness of 0.213±0.019 mm). Also the three Nanobody-treated groups showed a comparable and significant reduction in ear swelling versus the vehicle-treated animals: (i) animals treated with two injections (12h and 1h pre-challenge) of an equimolar dose of 105 µg/kg of the half-life extended anti-Kv1.3 Nanobody A019400029 showed a mean increase in ear thickness of 0.178±0.013 mm; (ii) animals treated with only one administration of A019400029 (105 µg/kg, 1h before challenge) showed a

similar ear swelling response (mean increase in ear thickness of 0.184±0.033 mm); (iii) animals treated with two injections (12h and 1h pre-challenge) of an equimolar dose of 69.3 µg/kg of the non-half-life extended anti-Kv1.3 Nanobody A019400032 showed a mean increase in ear thickness of 0.195±0.038 mm. There were no statistical significant differences between any of the three Nanobody-treated groups, or between the ShK-treated group and any of the Nanobody-treated groups.

In conclusion, treatment with anti-Kv1.3 Nanobodies resulted in a significant reduction of the DTH response in rats versus the vehicle group, at equimolar doses compared to the reference compound ShK. These results highlight the immunosuppressant potential of the anti-Kv1.3 Nanobodies in auto-immune diseases.

Example 13: *In vivo* proof-of-concept and benchmarking study in a delayed-type hypersensitivity (DTH) rat model

The *in vivo* efficacy of an anti-Kv1.3 Nanobody (A019100029) on DNFB-induced delayed type hypersensitivity in Wistar rats was evaluated and compared with the anti-Kv1.3 peptide toxin ShK (Stichodactyla toxin). The study was designed to prove non-inferiority of the Nanobody versus ShK with 80% power, based on a non-inferiority margin which was derived from previously obtained results of the Nanobody and ShK in the same DTH model (see Example 12). The DTH response in the rats was elicited as follows (see Figure 37): on day 0 (start of the in-life) and day 1, 100 µL of 1 % (wt/vol) DNFB prepared in 4:1 acetone/olive oil was applied to the shaved dorsum for sensitization. On day 5, animals were challenged on both sides of the right ear pinnae of the animals with 50 µL of 0.5% (wt/vol) DNFB prepared in 4:1 acetone: olive oil. Animals (n = 10 rats/group) received two subcutaneous (s.c.) injections of either vehicle, the benchmark compound ShK or the anti-Kv1.3 Nanobody at 12 hours and/or 1 hour preceding the challenge. As positive control, animals were treated with dexamethasone (topical, 0.75 mg at 1 hour and 6 hours post-challenge). On day 5 before DNFB challenge, the baseline right ear pinna thickness was measured, and the net ear swelling response was determined 24 hours after challenge with a spring-loaded micrometer.

The results of the experiments are shown in Figure 39. The vehicle-treated control animals showed a mean increase in right ear thickness of 0.266±0.027 mm. Rats from the positive control group, treated with dexamethasone 1h and 6h post-challenge (topical), showed a

pronounced reduction in the ear swelling response (mean increase in ear thickness of 0.018±0.016 mm). Two s.c. injections of 100 µg/kg of the reference compound ShK resulted in a statistical significant reduction of the ear swelling response versus vehicle (mean increase in ear thickness of 0.136±0.024 mm). Rats treated with an equimolar dose of the Nanobody (1.05 mg/kg) showed a comparable and significant reduction in ear swelling (mean increase in ear thickness of 0.120±0.022 mm). This response was statistically non-inferior compared to the benchmark ShK group. On the other hand, rats treated with a 5-fold higher dose of the Nanobody (5.25 mg/kg) showed statistical superiority on the 5% significance level in reducing the ear swelling response compared to the benchmark ShK group at 100 µg/kg (mean increase in ear thickness of 0.102±0.014 mm).

In conclusion, these results demonstrate that the anti-Kv1.3 Nanobody is superior compared to ShK for the treatment of the DTH response in rats, and highlight its immunosuppressant potential for the treatment of auto-immune diseases.

TABLES

Table A-1: Amino acid sequences of monovalent anti-Kv1.3 Nanobodies ("ID" refers to the SEQ ID NO as used herein)

Name	ID	Amino acid sequence
A0194009B01	1	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRDFVA RIRSGGSTNYADSVKGRFTISRDKATNTVYLQMNSLKPEDTAVYYCSSWRT GAYEYWGQGTLVTVSS
A0194009B06	2	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRDFVA RIRSGGSTNYADSVKGRFTISRDKATNTVYLQMNSLKPEDTAVYYCSSWRT GAYEYWGQGTQVTVSS
A0194009G09	3	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVA RIRMGGSINYADTVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSWR TGFYEYWGQGTQVTVSS
A0194016A05	4	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRDFVA RIRSGGSTNYADSVKGRFTISRDKATNTVHLQMNSLKPEDTAVYYCGSWR TGAYEYWGQGTLVTVSS
A0194016B04	5	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRDFVA RIRSGGSTNYADSVKGRFTISRDKATNTLYLQMNNLKPEDTAVYYCSSWRT EAYEYWGRGTLVTVSS
A0194016B06	6	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRDFVA RIRSGGSTNYADSVKGRFTISRDKATNTVYLQMNSLKPEDTAVYYCSSWRT GAYEHWGQGTLVTVSS
A0194016C03	7	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRDFVA RIRSGGSTNYADSVKGRFTISRDKATNTLYLQVNNLKPEDTAVYYCSSWRT EAYEYWGRGTLVTVSS

Name	ID	Amino acid sequence
A0194016C10	8	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRDFVA RIRSSGSTNYADSVKGRFTISRDKATNTVYLQMNSLKPEDTAVYYCSSWRT GAYEYWGQGAQVTVSS
A0194016F09	9	EVQLVESGGGLVQAGGSLGLSCSASGLLFSVNSAGWYRQAPGKQRDFVA RIRSGGSTNYADSVKGRFTISRDKATNTVYLQMNSLKPEDTAVYYCSSWRT GAYEYWGQGTQVTVSS
A0194016F11	10	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRDLVA RIRSGGSTNYADSVKGRFTISRDKATNTLYLQMNNLKPEDTAVYYCSSWRT EAYEYWGRGTQVTVSS
A0194016G07	11	EVQLVESGGGLVQAGGSLELSCSASGLLFSRNSVGWYRQAPGKKRDFVAR IRSGGSTNYADSVKGRFIISRDNAKNTLYLQMNALKPEDTGVYYCSSWRTG AYEYWGQGTLVTVSS
A0194016G08	12	EVQLVESGGGLVQAGGSLRLSCSASGLLFSANSAGWYRQAPGKQRDFVA RIRSGGSTNYADSVKGRFTVSRDNAKNTMYLQMNGLKPEDTAVYYCSSW RTGAYEYWGQGTQVTVSS
A0194020A06	13	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVAR IRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGW REGFYEYWGQGTLVTVSS
A0194020A07	14	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRRAPGKQREFVAR IRMGGSINYADSVKGRFTTSRDNAKNTIYLQMNSLKPEDTAVYYCSSWRE GFYEYWGQGTLVTVSS
A0194020B10	15	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSVGWYRRAPGKQREFVAR LRTTGSTNYAQSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCSAWRI EAYEYWGQGTQVTVSS

Name	ID	Amino acid sequence
A0194020C03	16	EVQLVESGGGLVQPGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVAR IRMGGSINYADSVKGRFTTSRDNAKNTIYLQMNSLKPEDTAVYYCSSWRE GFYEYWGQGTLVTVSS
A0194020C04	17	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVA RIRMGGSINYGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAKYFCSSW REGFYEYWGQGTLVTVSS
A0194020F09	18	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVA RIRMGGSINYADSVKGRFTTSRDNAKNTIYLQMNSLKPEDTAVYYCSSWR EGFYEYWGQGTQVTVSS
7257f0227ef9f636dc76 30192ad6e1c2	19	EVQLVESAGGLVQAGGSPGLSSSDSGLLFSRHSAGWYRPAPGKRREFVARI RMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGW REGFYEYWGQGTQVTVSS
5e260b33fe6df6b5c84 88d5a032f4852	20	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRRELVAR IRMGGSINYADSVKGRFTVSRDNAKNMMYLLMNDLNREYTAVYFCSGW REGFFEYWGQGTQVTVSS
afc519230558a133019 113e9509a672d	21	EVQLVESGGGLVQAGGALGLPCSASGLLFIRNSASWYRQAPGKRREFVARI RMGGSINYADSVKGRFTVSRDNAKNMMYLQMIDLKPEDTTVYCCSGWR EGFYEYWGQGTQVTVSS
728cbff15841abbeb52 d482b0016f638	22	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVA RIRMGGSINYADSVKGRFTTSRDNAKNTIYLQMNSLKPEDTAVYYCSSWR EGFYEYWGQVTQVTVSS
f678139d1aa501f05ed 990e993ff2875	23	EVQLVESGGGLAQAGGSLGLSCSASGLLFSRNSAGRYRQAPGKRPEFFARV RMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGW REGFYEYSGQGTQVTVSS

Name	ID	Amino acid sequence
46dd642501e86a0a2d d21551e7faf09d	24	EVQLVESGGGFVQAGGSLGHSCSASGLLSSCNTAGWYRQAPGKRREFVA RIRMVGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSG WREGFYGYWGQGTQVTVSS
7e991df80879caf6e5e2 47f65a8a8511	25	EVQLVESGGGLVQAGGSLGASRSASGLPLSRNSAGWYRQGPGKRREFVA RIRMGGSINYADSVKGRFTVSGDNAKNMMYLQMNDLKPEDTAGYFCSG WREGFYEYWGQGTQVTVSS
9e441891a0fa341c415 27504c05ed15b	26	EVQLVESGGGLVQAGGSLGLSCSAPGLLFSRNSAGWCRQAPGKRREFVAR TRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNGLKPEDTAVCLCSG WREGFYEYWRQGTQVTVSS
efd395f1c79fe9a34e97 926be4a9338a	27	EVQLVESGGCLVQAGGSLGLSCSASLLFFSRNSARWYRQAPVKRREFVGRI RMGGSINYADSGKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGW REGFYEYWGQGTQVTVSS
343640744296c3de37b 6202ce181a132	28	EVQLVESGGGLVQAGGSLGLACSASGLLISRNSAGWYRHAPGKQRAFVAR VRMGGSINYGDAVKGRFTASRDIAKNTMYLQMNDLKPEDTAIYFCSSWR EGFYEYWGQGTQVTVSS
4f9e2c0a8018ba1c7e1 6fe3b803d0f49	29	EVQLVESGGGLVQAGGALGLSCSASGLLFSRNSAGWYRQAPGKKRDFVA RIRMGGSINYGVSVKGRFTVSRDIAKNTMYPQMNDLKPEDAAKYFCSSW RKGFYEYWGQGTQVTVSS
213bd8ef8a6baa96fc7d 30be8aafff99	30	EVQLVESGGGSAQPGESLRLSCSASGLLFSRNSAGWYRQAPGKQREFVAR IRMGGSINYGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAKYFCSSWRE GFYEYWGQGTQVTVSS
c12bd10881dd3a7abf9 89d49cdf44468	31	EVQLVESGGGLVQAGGALGFTCSDSRLLFSRNSAGWYRQAAGKQREFVA RIRMGGSINYGDSVKGRFTVSRDIAKNTMYLRMNDLKPEDTAIYFCSSWR EGFYEYWGQGTQVTVSS

Name	ID	Amino acid sequence
36af9242c324d9ba4c518859deec3094	32	EVQLVESGGALVQAGGSLGLPCSAPGLLFSRNSAGWYRQAPGKQREFVAGVRMGGSINYGGSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAVYFCSSWRGGFYEYWGQGTQVTVSS
f9bb0eb8f0f497fa30f6d5bc51778ee1	33	EVQLVESGGGFDQAGGSLGLPCSAPGLLFSRNRVGWYRQAPGKQRDFVARIRMGGSTNYADSVKGRFTISRDNAKNMVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTQVTVSS
74c0c7da4aae9a3f64407f504af93fa6	34	EVQLVESGGDLVQAGGALGLSCPASGLLFSRNSAGWYRQAPGKQRELVACIRMGGSINYGDSVKGRFTVSRDIAKNTMYLQVDDLKPEDTAIYFCSSWREGFYEYWGQGTQVTVSS
cf31a849d3da18aeedc44dd4d393fef0	35	EVQLVESGGGLVKAGGSLGLSCSASGLLFSRNSVGWYRQAPGKQREFVARIRMGGSINYGGSVKGRFTVSRDIAKNTMYLQMNDLKPEETAWYFCSSWREGFYEYWSQRTQVTVSS
b077a6b050fff43ea3758b7a56dafb9b	36	EVQLVESAGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYGDSVKGRFTVSRDIAKDTRYMQINDLKPEDTAKYFCRSWREGLYEYWGQGTQVTVSS
d800d638e066182304b00f51542faf38	37	EVQLVESGGGLLQAGGSLGLSCSASGLLLSRNRARWYRQAPGKQREFVARIRMGGSMNDGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAIYFCRSWREGFYEYWGQGTQVTVSS
45e8d1be704b2eef9ed8156abc03c6d8	38	EVQLVESGGGLVQAGGALGLSCSASGLLFSRNSAGWHRQAPGKQREFVARIRMGGSINYGDSVKGRFTVSRDIAKNTMYLQMNELKPEDTAKYLCRSWREGFYESWGRGTQVTVSS
32a3666f797cfcd58659bc01153ccb38	39	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRPAPGKQREFVARIRMGGSINYGDSVKGRFTVARDIAKNTMYLQMNDLKPEETAIYFCSSWREGVYKYGGKGTQVTVSS

Name	ID	Amino acid sequence
1584d2e29498ea04b8cefac5c34d263d	40	EVQLVESGGGLVQAGGRRGLSCSASGFLFRRPSAGWYRQDPGKQREFVARIRMGGSINYGDSVKGRFTVSRDIAKNTMYLQMNDVKPEDTAKYFCSSWREGFYEYWGQGTQVTVSS
2b7789fd646b01f675945e09acc89530	41	EVQLVESGGALVQAGGSLGLSCSASGLLFIRNSAGCYRQAPGKHREFVARIRMGGSINYGDSVKGRFTVSRDIAKNTVYLQMNDLKPEYTAIYFCSSWREGYYEYWGQGTQVTVSS
97276e7c5dea225b4fbb58426d23b2e5	42	EVQLVESGGGLVEAGGSLGLSCSASGLLFSRISAGWYREAPGQQRECVARIRMGGSINYGDSVKGRFTVSRDIAKNTMYLQMNDLKPEHTAKYLCSSWREGFYEYWGQGTQVTVSS
a0615e93638f77818f6936045f94977f	43	EVQLVESGEGLVQAGGSLGLSCSASGLLFSPNSAGWYRQAPGKQRKFVARIRRGGGINYGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAIYFCSSWREGFDEYWAQGTQVTVSS
5f18d5599843f072a1eed218d2a74048	44	EVQLVESGGGLVQAGGPLGLSCSASGLLFSRNSAGWYRQAPGKQLAFVGRIGMGGCINYGDSVKGRFTVSRDIAKNTMYLQMNGLKPEDTAKYFCSSWREGFYEYWGQGTQVTVSS
0f4d6d2f274ddd6f2beb50c53e54d4f4	45	EVQLVESGGGLVQAAGSLGLPCSASGLLFSRMSARWYRQAPGEQREFVARIRMGGSINYGDSVKGRFTVTRDIAKNTMYLQMNDLKPEDTAKYFCSSWREGFYEYWCQGTQVTVSS
545db63aa3771946a230075631c4d56d	46	EVQLVESGEGLVPAGGSLGPSCSASGLLFSRYSAGWYRQAPGKQREFVARSRMGGSINYGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAEYFCSSWREGFYEYWGIGTQVTVSS
aff17a29c9e12331adb924c5c79b1643	47	EVQLVESGGALVQAGGPPGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINDGDSVKGRFTVSRDIDKNTMYLQMNDLKPENTAKYFCSSWREGFYKYWGQGTQVTVSS

Name	ID	Amino acid sequence
ef8dd380aee92f426ec4a6b86dcba935	48	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRQAPGQKREFVARIRTGGSTNYADSVKGRFTVSRGNAKNTVYLQMNNLKPEDTAVYYCSSWRTGAYEYWGQGTQVTVSS
6812576932a2c1e0f08ec106e0d0a04e	49	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNIAGWYRPAPGKQREFVARIRMGGSINYGDSVKGRFNGSRDIAKNTMYLQMDDLKPEDTAICFCSSGREGFYEYWGQGTQVTVSS
01ebe14f074a754cc51561530766ead4	50	EVQLVESAGGLVQAGGPLGLSCSSSGLLFSLNSAGCSRQPPGKQREFVARIRMGGSINYGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAKYFCSSWREGFYEYWGQGTQVTVSS
eb93eef0aa2380097f9dc5733bc6dd43	51	EVQLVESGGALMQAGGSLGPPCPASGPLFRRTRAGWYRQAPGKQREFVARIRMGGSINYGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAKYFCSSWREGFYEYWGQGTQVTVSS
28c4a7cd04f53076c4f5acb03236ef61	52	EVQLVESGGCLVQAGGSLGLSCSAPGLLFSPNSAGWYRQAPGKQREFVARILMGGSINYGDSVKGRFTVSRDIAKNTMYLQMKDLKPEDTAKYFCSRWREGFYEYCGQGTQVTVSS
8c06f7a5597c4192b17846077a8fce8a	53	EVQLVESGGGLVQAGGPLGLSCSASGIPFSRNSAGWYRQAPGKQREFVARIRMGGSRNYGDSVKGRFTVSGDIAKNTMYLQMNDLKPEDTAKDFGSSWREGFYEYWGQGTQVTVSS
63394ed3c69537d64eb90d1f6971fc43	54	EVQLVESGGGLVQTGGSLTLSCSASGLLFSVNSAGWYRQAPGKQRDFVARIRRGGDTNYAESVRGRFTVSRDKAKNTMYLQMNSLKPEDTAVYYCASWRTGSYEYWGQGTQVTVSS
fc796afb415f19e50d23537a96a99ae0	55	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRIFVARLRRGGDTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSAWRAGTYEYWGQVTQVTVSS

Name	ID	Amino acid sequence
af47752f179d4b7a61f3a0536bbf4cfc	56	EVQLVESGGGSVHPGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRIFVARLRRGGDTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSAWRAGTYEYWGQGTQVTVSS
4738fe2c7cdba35563859b889c0914be	57	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSVGWYRRAPGKQREFVARLRTTGSTNYAESVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCSAWRIEAYEYWGQGTQVTVSS
7ca2189f04d2906f692762b2d3820dd2	58	EVQLVESGGGLEQAGGSLRLSCSASGLLFSVNSAGWYRQAPGKQRDFVARIRSGGSTNYADSVKGRSTVSRDNAKNTLYLQLYSLKPEDTAVYYCSSWRTGAYEYWGQWTQVTVSS
3f009bd0371fd5a057e1dd514a697a0d	59	EVQLVESGGGLVKAGGSLRLSCSASGLLFRVNSVGWYRQAPGKQRDFVARIRRGGSTNYADTVKGRVTISRDNAKNTVYLQMNSLSPADTGVYYCSSWREGAYEYWGQGTQVTVSS
8d7c16aaec812b89b63ecfe25917a02d	60	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSAGWYRRAPGKQREFVARLRRGGETNYGDSVKGRFTISRDKATNTLYLQMNSLRTEDTAVYYCSSWRTGSYEYWGQGTQVTVSS
0049b650b8e87b0381fc26f80b9c4525	61	EVQLVESGGGLVQAGGSLRLSCSASGLLFRVNSAGWYRQAQGKQREFVARIRSGGSTNYADSVKGRFIISRDNAQNTLYLQMNNLSPEDTAAYYCSSWRIDAYEYWGQGTQVTVSS
a0f1f7e657eebacae2796f435e4e4fda	62	EVQLVESAGGLVPAGGSLRLPCSAPGLLFSVDSAGWYRQAPGKQRDFVARIRSGGSTNDADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGAYEYWGQGTQVTVSS
d9f77b8d61469fbeacad1fffa6142a31	63	EVQLVESGGGFVQAGGSLRLSCSASGLLFSVNSTVSYRQAPGKQRDCVARIRSGGSTNYADSVKGRFTVSRDNAKNTVYLQMNSLKHEDTAVYYCSSWRTGAYEYWGQGTQVTVSS

Name	ID	Amino acid sequence
2f69e44b1b5912bda2c7dee779e5c265	64	EVQLVESGEGLVKAGESLRLSCSASGLLFSVDSTGWYRQAPGKQREFFARIRSGGSINYGDSVKGRFTISRDKATNTLYLQMSNLNPEDTAVYYCSSWRIGSYEYWGQGTQVTVSS
A0194003A02	65	EVQLVESGGGLVQAGDSLSLSCTASRGTFRNFGMGWFRQAQGKEREFVAAISRSGGHKYYSDSVKGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAARFRFDDGTSYYQRAFYDFWGQGTLVTVSS
A0194003A04	66	EVQLVESGGGLVQAGDSLSLSCTASRGTFRNFGMGWSRQAQGKEREFVAAISRSGGHKYYSDSVKGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAARFRFDDGTSYYQRAFYDFWGQGTLVTVSS
A0194003A08	67	EVQLVESGGGLVQAGDSLSLSCTASAGTFRNFGMGWFRQARGEEREFVATISRSARHTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARFRSDDGTYYYQRAFYDFWGQGTQVTVSS
A0194003A09	68	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNYGMGWFRQAQGKEREFVAAISRSAGRTYYSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAARFRFDDGTYYYQRAFYDFWGQGTLVTVSS
A0194003A12	69	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNYGMGWFRQAQGKEREFVAAISRSASRTYYSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAARFRFDDGTYYYQRAFYDFWGQGTQVTVSS
A0194003B01	70	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFGMGWFRQAQGKEREFVAAISRSGGHIYYSDSVKGRFTIARANAKNMVSLQMNSLKPEDTAVYYCAARFRFDDGTYYYQRAFYDFWGQGTQVTVSS
A0194003B06	71	EVQLVESGGGLVQAGGSLSLSCTVSGGNFRNFGMGWFRQAHGKEREFVAAISRSGGRTYYADSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYSCAARFRSDDGTYYYQRAFYDFWGQGTQVTVSS

Name	ID	Amino acid sequence
A0194003B09	72	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNYGMGWFRQAQGKEREFV AAISRSAGRTYYSDSVKGRFTISKNNAKNVMSLQMNSLKPEDTAVYYCAA RFRFDDGTYYYQRAFYDFWGQGTLVTVSS
A0194003B11	73	EVQLVESGGGLVRAGGSLSLSCTSSSGTFRNFAMGWFRQAQGKEREFVA TISRSGGHTYYSDSVKGRFTISRDNAKNLVSLQMNSLKPEDTAVYYCAARF RFDDDTYYYQRAFYDFWGQGTLVTVSS
A0194003C08	74	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNYGMGWFRQAQGKEREFV AAISRSGGRTYYSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAAR FRFDDGTYYYQRAFYDFWGQGTQVTVSS
A0194003F08	75	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFGMGWFRQDQGQEREFV AAISRSGGRTYFSDSVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAARF RFDDGTYYYQRAFYDFWGQGTQVTVSS
A0194004F06	76	EVQLVESGGGLVQAGGSLSLSCTASRGTFRNFGMGWFRQAQGKEREFVA AISRSAGHTYYSDSVKGRFTISRDNAKNMVSLQMDSLKPEDTAVYYCAARF RFDDGTYYYQRTFYDFWGQGTQVTVSS
A0194007A01	77	EVQLVESGGGLAQAGGSLSLSCTASGGNFRNFGMGWFRQVQGTEREFV AAISRTGGRTYFSDSVKGRFTISRDNAKNMVSLQMNSLKPEDSAVYYCAA RFRFGDGTYYYQRNFYDFWGQGTQVTVSS
A0194007B04	78	EVQLVESGGGLVQAGDSLSLSCTASRGTFRNFGMGWFRQAQGKEREFVA AISRSGGHKYYSDSVQGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAAR FRFDDGTSYYQRAFYDFWGQGTQVTVSS
A0194007B09	79	EVQLVESGGGLVQTGGSLSLSCTASGGTFRNYGMGWFRQAQGKEREFVA AISRSAGRTYYSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAARF RFDDGTYYYQRAFYDFWGQGTQVTVSS

Name	ID	Amino acid sequence
A0194007B12	80	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNYAMGWFRQAQGKEREFVA TISRSGGYTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RFGDGTYYYQRAFYDFWGQGTQVTVSS
A0194007C01	81	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFGMGWFRQAQGKEREFVA AISRSGGRTYYSDSVKGRFTISKDNAKNIMSLQMNSLRPEDTAVYYCAARF RFDDGTYYYQRAFYDFWGQGTQVTVSS
A0194007D01	82	EVQLVESGGGSVQAGGSLSLSCTASRGTFRNFGMGWFRQAQGKEREFVA AISRTGGRTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAIYYCAARF RFDDGTYYYQRAFYDFWGQGTLVTVSS
A0194007D10	83	EVQLVESGGGLVQAGDSLSLSCTASAGTFRNFGMGWFRQARGEEREFVA TISRSARHTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RSDGGTYYYQRAFYDFWGQGTQVTVSS
A0194007D11	84	EVQLVESGGGLVQAGGSLSLSCTASAGTFRNFGMGWFRQAQGKEREFVA AISRSGGHTYYSDSVKGRFTISKDNAKNMVSLKMNSLKPEDTADYYCAARF RFDDGTYYYQRAFYDFWGQGTQVTVSS
A0194007E04	85	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFGMGWFRQVQGKEREFVA AISRSGGHIFYSDSVKGRFTISKDNAKNILFLQMNSLKPEDTAVYYCAARFRF DDGTYYYQRAFYDFWGQGTQVTVSS
A0194007E08	86	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNYGMGWFRQAQGKEREFV AAISRSAGRTYYSDSAKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAAR FRFDDGTYYYQRAFYDFWGQGTLVTVSS
A0194007E12	87	EVQLVESGGGLVQAGGSLSLSCTASGGNFRNFGMGWFRQAQGKEREFV AAISRSGGHTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA RFRYGDGTYYYQRAFYDFWGQGTLVTVSS

Name	ID	Amino acid sequence
A0194007F01	88	EVQLVESGGGLVQAGDSLSLSCTASRGTFRNFGMGWFRQAQGKEREFVA AISRSGGRKYYSDSVKGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAARF RFDDGTSYYQRAFYDFWGQGTLVTVSS
A0194007G09	89	EVQLVESGGGLVEAGGSLSLSCTASAGTFRNFGMGWFRQARGEEREFVA TISRSARHTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RSDDGTYYYQRAFYDFWGQGTQVTVSS
A0194008A01	90	EVQLVESGGGLVQAGDSLSLSCTASAGTLRNFGMGWFRQARGEEREFVA TISRSARHTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RSDDGTYYYQRRFYDFWGQGTQVTVSS
A0194008A02	91	EVQLVESGGGLVQAGDSLSLSCTASAGTLRNFGMGWFRQARGEEREFVA TISRSARHTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RSGDGTYYYQRAFYDFWGQGTQVTVSS
A0194008A09	92	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNYGMGWFRQAQGKEREFV AAISRTGGHTYYSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAAR FRFGDGTYYYQRAFYDFWGQGTLVTVSS
A0194008A11	93	EVQLVESGGGLVQAGDSLSLSCTASAGTLRNFGMGWFRQARGEEREFVA TISRSARHTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RSDDGTYYYQRAFYDFWGQGTQVTVSS
A0194008B01	94	EVQLVESGGGLVQAGGSLSLSCTSSKGTFRNFAMGWFRQAQGKEREFVA TISRSGGHTYYSDSVKGRFAISRDNAKNLVSLQMNSLNAEDTAVYYCAARF RSDDDTYYYQRAFYDFWGQGTLVTVSS
A0194008C01	95	EVQLVESGGGLVQAGGSLSLPCTASGGTFRNYGMGWFRQAQGKEREFV AAISRSAGRTYYSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAAR FRFDDGTYYYQRAFYDFWGQGTLVTVSS

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A0194008C07	96	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNYGMGWFRQAQGKERDFV AAISRTNGHIYYSDSVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAARF RFGDGTYYYQRTFYDFWGQGTLVTVSS
A0194008C08	97	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFAMGWFRQAQGKEREFVA TISRSGGHIYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RFDDGTSYYQRAFYDFWGQGTLVTVSS
A0194008D08	98	EVQLVESGGDLVQPGGSLRLSCTASGGTFRNYGMGWFRQAQGKEREFV AAISRTGGHTYYSDSVKGRFTISKDNAKNIVSLQMNSLKPEDTAVYYCAAR FRFGDGTYYYQRNFYDFWGQGTQVTVSS
A0194008F05	99	EVQLVESGGGLVQAGGPLSLSCTASGGTFRNYGMGWFRQAQGKEREFV AAISRSAGRTYYSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAAR FRFDDGTYYYQRAFYDFWGQGTLVTVSS
A0194008G10	100	EVQLVESGGGSVQAGGSLRLVCAFSGGTFRNFAMGWFRQAQGKEREFV ATISRSGGHTYYSDSVKGRFTISKDNAKNMVYLQMYSLKPEDTAVYYCAGR FRFGDGAYYYQRTFYDFWGQGTLVTVSS
KV13BIIPMP026D09	101	EVQLVESGGGLVQAGGSLSLSCTASRGTFRNFAMGWFRQAQGKEREFVA TISRSGGHIYYSDSVKGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAARF RFDDGTSYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP026F08	102	EVQLVESGGGLVQAGGSLSLSCTASRGTFRNYAMGWFRQAQGKEREFVA TISRSGGHIYYSDSVKGRFAISKDNAKNMMSLRMNSLKPEDTAVYYCAARF RFDDGTYFYQRAFYDFWGQGTQVTVSS
KV13BIIPMP027A11	103	KVQLVESRGGLVQAGGSLRLSCTASGGTFRNYGMGWFRQAQGKEREFV AAVSRTGGRTYYSDSVKGRFTISRDNAKNMVSLQMNKLKPEDTAVYYCAA RFRFDDGTYYYQRAFYDFWGQGTQVTVSS

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KV13BIIPMP027B10	104	EVQLVESGGGLVQAGGSLSLSCTASGGTFRSYGMGWFRQAQGKEREFVA AISRTGGHTYYSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAARF RFGDGTYYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP027B12	105	EVQLVESGGGLVQAGGSLSLSCTASGGNFRNFGMGWFRQAQGTEREFV AAISRTGGRTYFSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA RFRFDDGTYYYQRNFYDFWGQGAQVTVSS
KV13BIIPMP027D09	106	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFAMGWFREAQGKEREFVA TISRSGGHTYYSDSVKGRFTISRDNAKNMVALQMNSLKPEDTAVYYCAAR FRFDDGTYYYQRAFYNLWGQGTQVTVSS
KV13BIIPMP027D10	107	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNYGMGWFRQAQGKEREFV AAISRTGGHTYYSDSVKGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAA RFRFGDGTYYYQRNFYDFWGQGTQVTVSS
KV13BIIPMP027E08	108	EVQLVESGGGLVQAGGSLSLSCTASVGNFRNFGMGWFRQAQGKEREFV AAISRSGGHTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA RFRFDDGTYYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP027E11	109	EVQLVESGGGLVQAGGSLSLSCTVSGGNFRNFGMGWFRQAHGKEREFV AAISRSGGRTYYADSVKGRFTVSRDNAKNMVSLQMNSLKPEDTAVYSCAA RFRSDDGTYYYQRAFYDFWGQGTQVTVSS
A019400003	110	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFGMGWFRQAQGKEREFVA AISRTGGHTYYQDSVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAARF RFGDGTYYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP027H08	111	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFAMGWFRQAQGKEREFVA TISRSGGHIFYSESVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RSDDGTYYYQRIFYDFWGQGTQVTVSS

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KV13BIIPMP027H09	112	EVQLVESGGGLVQPGGSLSLSCTASAGTFRNFGMGWFRQARGEEREFVA TISRSARHTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RSDDGTYYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP027H10	113	EVQLVESGGGSVQAGGSLSLSCTASRGTFRNYGMGWFRQAQGKEREFVA AISRSGGHTYYSDSVKGRFTISKDNAKNIMSLQMNSLKPEDTAVYYCAARF RFDDGTYYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP049B09	114	EVQLVESGGGLVQAGGSLSLSCTVSGGNFRNFGVGWFRQAHGKEREFVA AISRAGGRTYYADSVKGRFAISRDNAKNMVSLQMNSLKPEDTAVYTCAAR FRSGDGTSYYERAFYDFWGQGTQVTVSS
KV13BIIPMP049E10	115	KVQLVESGGGLVQPGGSLSLSCTASAGTFRNFGMGWFRQAQGTEREFVA AISRSGGRTYFSDSVKGRFTISKDNAKNMLSLQMNSLKPEDTAVYYCAARF RSDDGTYYYQRAFYDFWGQGAQVTVSS
KV13BIIPMP050A07	116	EVQLVESGGGLVQAGGSLSLSCTVSGGTFRNYGMGWFRQFQGKEREFVA AISRSGGHIFYSESVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RSGDGTYYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP050A09	117	EVQLVESGGGLVQAGGSLSLSCTASAGTFRNFGMGWFRQARGEAREFVA TISRSGGHIYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RSDDGTYYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP050A10	118	EVQLVESGGGLVRAGGSLSLSCTSSSGTFRNFAMGWFRQAQGKEREFVA TISRSGGHTYYSDSVKGRFTISRDNAKNLVSLQMSSLKPEDTAVYYCAARFR FDDDTYYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP050B11	119	EVQLVESGGGSVQAGNSLSLSCTASGGTFRNYAMGWFRQAQGKEREFV ATISRSGGHIYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAAYYCAAR FRFGDGTYYYQRAFYDFWGQGTQVTVSS

Name	ID	Amino acid sequence
KV13BIIPMP050C09	120	EVQLVESGGGLVQAGDSLSLSCTASRGTFRNFGMGWFRQAQGKEREFVA AISRSGGHKYYSDSVKGRSTISKDNAKNMVSLQMNSLKPEDTAVYYCAAR FRFDDGTSYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP050D09	121	EVQLVESGGGLAQAGGSLSLSCTASGGNFRNFGMGWFRQVQGTEREFV AAISRTGGRTYFSDSVKGRFTISRDNAKNMVSLQMNSLKPEDSAVYYCAA RFRFGDGTYYYQRNFYGFWGQGAQVTVSS
KV13BIIPMP050E12	122	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNYGMGWFRQAQGKEREFV AAISRSAGHIYYLNSVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAARF RFDDGTYYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP050F11	123	EVQLVESGGGLVQAGNSLSLSCTASAGTLRNFGMGWFRQARGEEREFVA TISRSARHTYYSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAARF RSDDGTYYYQRAFYDFWGQGTQVTVSS

Table A-2: Sequences for CDRs and frameworks, plus preferred combinations as provided in formula I, namely FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 (the following terms: "ID" refers to the given SEQ ID NO)

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
1	A0194009B01	124	EVQLVESGGGLVQAGGSLRLSCSAS	181	GLLFSVNSAG	227	WYRQAPGKQRDFVA	268	RIRSGGSTN	310	YADSVKGRFTISRDKATNTVYLQMNSLKPEDTAVYYCSS	393	WRTGAYEY	436	WGQGTLVTVSS
2	A0194009B06	124	EVQLVESGGGLVQAGGSLRLSCSAS	181	GLLFSVNSAG	227	WYRQAPGKQRDFVA	268	RIRSGGSTN	310	YADSVKGRFTISRDKATNTVYLQMNSLKPEDTAVYYCSS	393	WRTGAYEY	437	WGQGTQVTVSS
3	A0194009G09	125	EVQLVESGGGLVQAGGSLGLSCSAS	182	GLLFSRNSAG	228	WYRQAPGKQREFVA	269	RIRMGGSIN	311	YADTVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSS	394	WRTGFYEY	437	WGQGTQVTVSS
4	A0194016A05	124	EVQLVESGGGLVQAGGSLRLSCSAS	181	GLLFSVNSAG	227	WYRQAPGKQRDFVA	268	RIRSGGSTN	312	YADSVKGRFTISRDKATNTVHLQMNSLKPEDTAVYYCGS	393	WRTGAYEY	436	WGQGTLVTVSS
5	A0194016B04	124	EVQLVESGGGLVQAGGSLRLSCSAS	181	GLLFSVNSAG	227	WYRQAPGKQRDFVA	268	RIRSGGSTN	313	YADSVKGRFTISRDKATNTLYLQMNNLKPEDTAVYYCSS	395	WRTEAYEY	438	WGRGTLVTVSS
6	A0194016B06	124	EVQLVESGGGLV	181	GLLFSVN	227	WYRQAPGK	268	RIRSGG	310	YADSVKGRFTISRDKATN	396	WRTGA	436	WGQG

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
			QAGGSLRLSCSAS		SAG		QRDFVA		STN		TVYLQMNSLKPEDTAVYYCSS		YEH		TLVTVSS
7	A0194016C03	124	EVQLVESGGGLVQAGGSLRLSCSAS	181	GLLFSVNSAG	227	WYRQAPGKQRDFVA	268	RIRSGGSTN	314	YADSVKGRFTISRDKATNTLYLQVNNLKPEDTAVYYCSS	395	WRTEAYEY	438	WGRGTLVTVSS
8	A0194016C10	124	EVQLVESGGGLVQAGGSLRLSCSAS	181	GLLFSVNSAG	227	WYRQAPGKQRDFVA	270	RIRSSGSTN	310	YADSVKGRFTISRDKATNTVYLQMNSLKPEDTAVYYCSS	393	WRTGAYEY	439	WGQGAQVTVSS
9	A0194016F09	125	EVQLVESGGGLVQAGGSLGLSCSAS	181	GLLFSVNSAG	227	WYRQAPGKQRDFVA	268	RIRSGGSTN	310	YADSVKGRFTISRDKATNTVYLQMNSLKPEDTAVYYCSS	393	WRTGAYEY	437	WGQGTQVTVSS
10	A0194016F11	124	EVQLVESGGGLVQAGGSLRLSCSAS	181	GLLFSVNSAG	229	WYRQAPGKQRDLVA	268	RIRSGGSTN	313	YADSVKGRFTISRDKATNTLYLQMNNLKPEDTAVYYCSS	395	WRTEAYEY	440	WGRGTQVTVSS
11	A0194016G07	126	EVQLVESGGGLVQAGGSLELSCSAS	183	GLLFSRNSVG	230	WYRQAPGKKRDFVA	268	RIRSGGSTN	315	YADSVKGRFIISRDNAKNTLYLQMNALKPEDTGVYYCSS	393	WRTGAYEY	436	WGQGTLVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
12	A0194016G08	124	EVQLVESGGGLVQAGGSLRLSCSAS	184	GLLFSANSAG	227	WYRQAPGKQRDFVA	268	RIRSGGSTN	316	YADSVKGRFTVSRDNAKNTMYLQMNGLKPEDTAVYYCSS	393	WRTGAYEY	437	WGQGTQVTVSS
13	A0194020A06	125	EVQLVESGGGLVQAGGSLGLSCSAS	182	GLLFSRNSAG	231	WYRQAPGKRREFVA	269	RIRMGGSIN	317	YADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSG	397	WREGFYEY	436	WGQGTLVTVSS
14	A0194020A07	125	EVQLVESGGGLVQAGGSLGLSCSAS	182	GLLFSRNSAG	232	WYRRAPGKQREFVA	269	RIRMGGSIN	318	YADSVKGRFTTSRDNAKNTIYLQMNSLKPEDTAVYYCSS	397	WREGFYEY	436	WGQGTLVTVSS
15	A0194020B10	124	EVQLVESGGGLVQAGGSLRLSCSAS	185	GLLFSVNSVG	232	WYRRAPGKQREFVA	271	RLRTTGSTN	319	YAQSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCSA	398	WRIEAYEY	437	WGQGTQVTVSS
16	A0194020C03	127	EVQLVESGGGLVQPGGSLGLSCSAS	182	GLLFSRNSAG	228	WYRQAPGKQREFVA	269	RIRMGGSIN	318	YADSVKGRFTTSRDNAKNTIYLQMNSLKPEDTAVYYCSS	397	WREGFYEY	436	WGQGTLVTVSS
17	A0194020C04	125	EVQLVESGGGLVQAGGSLGLSCSAS	182	GLLFSRNSAG	228	WYRQAPGKQREFVA	269	RIRMGGSIN	320	YGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAKYFCSS	397	WREGFYEY	436	WGQGTLVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
18	A0194020F09	125	EVQLVESGGGLV QAGGSLGLSCSAS	182	GLLFSRN SAG	228	WYRQAPGK QREFVA	269	RIRMG GSIN	318	YADSVKGRFTTSRDNAK NTIYLQMNSLKPEDTAVY YCSS	397	WREGF YEY	437	WGQG TQVTV SS
19	7257f0227ef9 f636dc76301 92ad6e1c2	128	EVQLVESAGGLV QAGGSPGLSSSD S	186	GLLFSRH SAG	233	WYRPAPGK RREFVA	269	RIRMG GSIN	317	YADSVKGRFTVSRDNAK NMMYLQMNDLKPEDT AVYFCSG	397	WREGF YEY	437	WGQG TQVTV SS
20	5e260b33fe6 df6b5c8488d 5a032f4852	125	EVQLVESGGGLV QAGGSLGLSCSAS	182	GLLFSRN SAG	234	WYRQAPGK RRELVA	269	RIRMG GSIN	321	YADSVKGRFTVSRDNAK NMMYLLMNDLNREYTA VYFCSG	399	WREGF FEY	437	WGQG TQVTV SS
21	afc519230558 a133019113e 9509a672d	129	EVQLVESGGGLV QAGGALGLPCSA S	187	GLLFIRN SAS	231	WYRQAPGK RREFVA	269	RIRMG GSIN	322	YADSVKGRFTVSRDNAK NMMYLQMIDLKPEDTT VYCCSG	397	WREGF YEY	437	WGQG TQVTV SS
22	728cbff15841 abbeb52d482 b0016f638	125	EVQLVESGGGLV QAGGSLGLSCSAS	182	GLLFSRN SAG	228	WYRQAPGK QREFVA	269	RIRMG GSIN	318	YADSVKGRFTTSRDNAK NTIYLQMNSLKPEDTAVY YCSS	397	WREGF YEY	441	WGQV TQVTV SS
23	f678139d1aa 501f05ed990 e993ff2875	130	EVQLVESGGGLA QAGGSLGLSCSAS	188	GLLFSRN SAG	235	RYRQAPGKR PEFFA	272	RVRMG GSIN	317	YADSVKGRFTVSRDNAK NMMYLQMNDLKPEDT AVYFCSG	397	WREGF YEY	442	SGQGT QVTVS S

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
24	46dd642501e86a0a2dd21551e7faf09d	131	EVQLVESGGGFVQAGGSLGHSCSAS	189	GLLSSCNTAG	231	WYRQAPGKRREFVA	273	RIRMVGSIN	317	YADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSG	400	WREGFYGY	437	WGQGTQVTVSS
25	7e991df80879caf6e5e247f65a8a8511	132	EVQLVESGGGLVQAGGSLGASRSAS	190	GLPLSRNSAG	236	WYRQGPGKRREFVA	269	RIRMGGSIN	323	YADSVKGRFTVSGDNAKNMMYLQMNDLKPEDTAGYFCSG	397	WREGFYEY	437	WGQGTQVTVSS
26	9e441891a0fa341c41527504c05ed15b	133	EVQLVESGGGLVQAGGSLGLSCSAP	182	GLLFSRNSAG	237	WCRQAPGKRREFVA	274	RTRMGGSIN	324	YADSVKGRFTVSRDNAKNMMYLQMNGLKPEDTAVCLCSG	397	WREGFYEY	443	WRQGTQVTVSS
27	efd395f1c79fe9a34e97926be4a9338a	134	EVQLVESGGCLVQAGGSLGLSCSASS	191	LLFFSRNSAR	238	WYRQAPVKRREFVG	269	RIRMGGSIN	325	YADSGKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSG	397	WREGFYEY	437	WGQGTQVTVSS
28	343640744296c3de37b6202ce181a132	135	EVQLVESGGGLVQAGGSLGLACSAS	192	GLLISRNSAG	239	WYRHAPGKQRAFVA	272	RVRMGGSIN	326	YGDAVKGRFTASRDIAKNTMYLQMNDLKPEDTAIYFCSS	397	WREGFYEY	437	WGQGTQVTVSS
29	4f9e2c0a8018ba1c7e16fe3b803d0f49	136	EVQLVESGGGLVQAGGALGLSCSAS	182	GLLFSRNSAG	230	WYRQAPGKKRDFVA	269	RIRMGGSIN	327	YGVSVKGRFTVSRDIAKNTMYPQMNDLKPEDAAKYFCSS	401	WRKGFYEY	437	WGQGTQVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
30	213bd8ef8a6baa96fc7d30be8aafff99	137	EVQLVESGGGSAQPGESLRLSCSAS	182	GLLFSRNSAG	228	WYRQAPGKQREFVA	269	RIRMGGSIN	320	YGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAKYFCSS	397	WREGFYEY	437	WGQGTQVTVSS
31	c12bd10881dd3a7abf989d49cdf44468	138	EVQLVESGGGLVQAGGALGFTCSDS	193	RLLFSRNSAG	240	WYRQAAGKQREFVA	269	RIRMGGSIN	328	YGDSVKGRFTVSRDIAKNTMYLRMNDLKPEDTAIYFCSS	397	WREGFYEY	437	WGQGTQVTVSS
32	36af9242c324d9ba4c518859deec3094	139	EVQLVESGGALVQAGGSLGLPCSAP	182	GLLFSRNSAG	228	WYRQAPGKQREFVA	275	GVRMGGSIN	329	YGGSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAVYFCSS	402	WRGGFYEY	437	WGQGTQVTVSS
33	f9bb0eb8f0f497fa30f6d5bc51778ee1	140	EVQLVESGGGFDQAGGSLGLPCSAP	194	GLLFSRNRVG	227	WYRQAPGKQRDFVA	276	RIRMGGSTN	330	YADSVKGRFTISRDNAKNMVYLQMNSLKPEDTAVYYCSS	394	WRTGFYEY	437	WGQGTQVTVSS
34	74c0c7da4aae9a3f64407f504af93fa6	141	EVQLVESGGDLVQAGGALGLSCPAS	182	GLLFSRNSAG	241	WYRQAPGKQRELVA	277	CIRMGGSIN	331	YGDSVKGRFTVSRDIAKNTMYLQVDDLKPEDTAIYFCSS	397	WREGFYEY	437	WGQGTQVTVSS
35	cf31a849d3da18aeedc44dd4d393fef0	142	EVQLVESGGGLVKAGGSLGLSCSAS	183	GLLFSRNSVG	228	WYRQAPGKQREFVA	269	RIRMGGSIN	332	YGGSVKGRFTVSRDIAKNTMYLQMNDLKPEETAWYFCSS	397	WREGFYEY	444	WSQRTQVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
36	b077a6b050fff43ea3758b7a56dafb9b	143	EVQLVESAGGLVQAGGSLGLSCSAS	182	GLLFSRNSAG	228	WYRQAPGKQREFVA	269	RIRMGGSIN	333	YGDSVKGRFTVSRDIAKDTRYMQINDLKPEDTAKYFCRS	403	WREGLYEY	437	WGQGTQVTVSS
37	d800d638e066182304b00f51542faf38	144	EVQLVESGGGLLQAGGSLGLSCSAS	195	GLLLSRNRAR	228	WYRQAPGKQREFVA	278	RIRMGGSMN	334	DGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAIYFCRS	397	WREGFYEY	437	WGQGTQVTVSS
38	45e8d1be704b2eef9ed8156abc03c6d8	136	EVQLVESGGGLVQAGGALGLSCSAS	182	GLLFSRNSAG	242	WHRQAPGKQREFVA	269	RIRMGGSIN	335	YGDSVKGRFTVSRDIAKNTMYLQMNELKPEDTAKYLCRS	404	WREGFYES	440	WGRGTQVTVSS
39	32a3666f797cfcd58659bc01153ccb38	125	EVQLVESGGGLVQAGGSLGLSCSAS	182	GLLFSRNSAG	243	WYRPAPGKQREFVA	269	RIRMGGSIN	336	YGDSVKGRFTVARDIAKNTMYLQMNDLKPEETAIYFCSS	405	WREGVYKY	445	GGKGTQVTVSS
40	1584d2e29498ea04b8cefac5c34d263d	145	EVQLVESGGGLVQAGGRRGLSCSAS	196	GFLFRRPSAG	244	WYRQDPGKQREFVA	269	RIRMGGSIN	337	YGDSVKGRFTVSRDIAKNTMYLQMNDVKPEDTAKYFCSS	397	WREGFYEY	437	WGQGTQVTVSS
41	2b7789fd646b01f675945e09acc89530	146	EVQLVESGGALVQAGGSLGLSCSAS	197	GLLFIRNSAG	245	CYRQAPGKHREFVA	269	RIRMGGSIN	338	YGDSVKGRFTVSRDIAKNTVYLQMNDLKPEYTAIYFCSS	406	WREGYYEY	437	WGQGTQVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
42	97276e7c5dea225b4fbb58426d23b2e5	147	EVQLVESGGGLVEAGGSLGLSCSAS	198	GLLFSRISAG	246	WYREAPGQQRECVA	269	RIRMGGSIN	339	YGDSVKGRFTVSRDIAKNTMYLQMNDLKPEHTAKYLCSS	397	WREGFYEY	437	WGQGTQVTVSS
43	a0615e93638f77818f6936045f94977f	148	EVQLVESGEGLVQAGGSLGLSCSAS	199	GLLFSPNSAG	247	WYRQAPGKQRKFVA	279	RIRRGGGIN	340	YGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAIYFCSS	407	WREGFDEY	446	WAQGTQVTVSS
44	5f18d5599843f072a1eed218d2a74048	149	EVQLVESGGGLVQAGGPLGLSCSAS	182	GLLFSRNSAG	248	WYRQAPGKQLAFVG	280	RIGMGGCIN	341	YGDSVKGRFTVSRDIAKNTMYLQMNGLKPEDTAKYFCSS	397	WREGFYEY	437	WGQGTQVTVSS
45	0f4d6d2f274ddd6f2beb50c53e54d4f4	150	EVQLVESGGGLVQAAGSLGLPCSAS	200	GLLFSRMSAR	249	WYRQAPGEQREFVA	269	RIRMGGSIN	342	YGDSVKGRFTVTRDIAKNTMYLQMNDLKPEDTAKYFCSS	397	WREGFYEY	447	WCQGTQVTVSS
46	545db63aa3771946a230075631c4d56d	151	EVQLVESGEGLVPAGGSLGPSCSAS	201	GLLFSRYSAG	228	WYRQAPGKQREFVA	281	RSRMGGSIN	343	YGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAEYFCSS	397	WREGFYEY	448	WGIGTQVTVSS
47	aff17a29c9e12331adb924c5c79b1643	152	EVQLVESGGALVQAGGPPGLSCSAS	182	GLLFSRNSAG	228	WYRQAPGKQREFVA	269	RIRMGGSIN	344	DGDSVKGRFTVSRDIDKNTMYLQMNDLKPENTAKYFCSS	408	WREGFYKY	437	WGQGTQVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
48	ef8dd380aee92f426ec4a6b86dcba935	124	EVQLVESGGGLVQAGGSLRLSCSAS	181	GLLFSVNSAG	250	WYRQAPGQKREFVA	282	RIRTGGSTN	345	YADSVKGRFTVSRGNAKNTVYLQMNNLKPEDTAVYYCSS	393	WRTGAYEY	437	WGQGTQVTVSS
49	6812576932a2c1e0f08ec106e0d0a04e	125	EVQLVESGGGLVQAGGSLGLSCSAS	202	GLLFSRNIAG	243	WYRPAPGKQREFVA	269	RIRMGGSIN	346	YGDSVKGRFNGSRDIAKNTMYLQMDDLKPEDTAICFCSS	409	GREGFYEY	437	WGQGTQVTVSS
50	01ebe14f074a754cc51561530766ead4	153	EVQLVESAGGLVQAGGPLGLSCSSS	203	GLLFSLNSAG	251	CSRQPPGKQREFVA	269	RIRMGGSIN	320	YGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAKYFCSS	397	WREGFYEY	437	WGQGTQVTVSS
51	eb93eef0aa2380097f9dc5733bc6dd43	154	EVQLVESGGALMQAGGSLGPPCPAS	204	GPLFRRTRAG	228	WYRQAPGKQREFVA	269	RIRMGGSIN	320	YGDSVKGRFTVSRDIAKNTMYLQMNDLKPEDTAKYFCSS	397	WREGFYEY	437	WGQGTQVTVSS
52	28c4a7cd04f53076c4f5acb03236ef61	155	EVQLVESGGCLVQAGGSLGLSCSAP	199	GLLFSPNSAG	228	WYRQAPGKQREFVA	283	RILMGGSIN	347	YGDSVKGRFTVSRDIAKNTMYLQMKDLKPEDTAKYFCSR	397	WREGFYEY	449	CGQGTQVTVSS
53	8c06f7a5597c4192b17846077a8fce8a	149	EVQLVESGGGLVQAGGPLGLSCSAS	205	GIPFSRNSAG	228	WYRQAPGKQREFVA	284	RIRMGGSRN	348	YGDSVKGRFTVSGDIAKNTMYLQMNDLKPEDTAKDFGSS	410	WREGFYEY	437	WGQGTQVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
54	63394ed3c69537d64eb90d1f6971fc43	156	EVQLVESGGGLVQTGGSLTLSCSAS	181	GLLFSVNSAG	227	WYRQAPGKQRDFVA	285	RIRRGGDTN	349	YAESVRGRFTVSRDKAKNTMYLQMNSLKPEDTAVYYCAS	411	WRTGSYEY	437	WGQGTQVTVSS
55	fc796afb415f19e50d23537a96a99ae0	124	EVQLVESGGGLVQAGGSLRLSCSAS	181	GLLFSVNSAG	252	WYRQAPGKQRIFVA	286	RLRRGGDTN	350	YADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSA	412	WRAGTYEY	441	WGQVTQVTVSS
56	af47752f179d4b7a61f3a0536bbf4cfc	157	EVQLVESGGGSVHPGGSLRLSCSAS	181	GLLFSVNSAG	252	WYRQAPGKQRIFVA	286	RLRRGGDTN	350	YADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSA	412	WRAGTYEY	437	WGQGTQVTVSS
57	4738fe2c7cdba35563859b889c0914be	124	EVQLVESGGGLVQAGGSLRLSCSAS	185	GLLFSVNSVG	232	WYRRAPGKQREFVA	271	RLRTTGSTN	351	YAESVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCSA	398	WRIEAYEY	437	WGQGTQVTVSS
58	7ca2189f04d2906f692762b2d3820dd2	158	EVQLVESGGGLEQAGGSLRLSCSAS	181	GLLFSVNSAG	227	WYRQAPGKQRDFVA	268	RIRSGGSTN	352	YADSVKGRSTVSRDNAKNTLYLQLYSLKPEDTAVYYCSS	393	WRTGAYEY	450	WGQWTQVTVSS
59	3f009bd0371fd5a057e1dd514a697a0d	159	EVQLVESGGGLVKAGGSLRLSCSAS	206	GLLFRVNSVG	227	WYRQAPGKQRDFVA	287	RIRRGGSTN	353	YADTVKGRVTISRDNAKNTVYLQMNSLSPADTGVYYCSS	413	WREGAYEY	437	WGQGTQVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
60	8d7c16aaec812b89b63ecfe25917a02d	124	EVQLVESGGGLVQAGGSLRLSCSAS	181	GLLFSVNSAG	232	WYRRAPGKQREFVA	288	RLRRGGETN	354	YGDSVKGRFTISRDKATNTLYLQMNSLRTEDTAVYYCSS	411	WRTGSYEY	437	WGQGTQVTVSS
61	0049b650b8e87b0381fc26f80b9c4525	124	EVQLVESGGGLVQAGGSLRLSCSAS	207	GLLFRVNSAG	253	WYRQAQGKQREFVA	268	RIRSGGSTN	355	YADSVKGRFIISRDNAQNTLYLQMNNLSPEDTAAYYCSS	414	WRIDAYEY	437	WGQGTQVTVSS
62	a0f1f7e657eebacae2796f435e4e4fda	160	EVQLVESAGGLVPAGGSLRLPCSAP	208	GLLFSVDSAG	227	WYRQAPGKQRDFVA	268	RIRSGGSTN	356	DADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCSS	393	WRTGAYEY	437	WGQGTQVTVSS
63	d9f77b8d61469fbeacad1fffa6142a31	161	EVQLVESGGGFVQAGGSLRLSCSAS	209	GLLFSVNSTV	254	SYRQAPGKQRDCVA	268	RIRSGGSTN	357	YADSVKGRFTVSRDNAKNTVYLQMNSLKHEDTAVYYCSS	393	WRTGAYEY	437	WGQGTQVTVSS
64	2f69e44b1b5912bda2c7dee779e5c265	162	EVQLVESGEGLVKAGESLRLSCSAS	210	GLLFSVDSTG	255	WYRQAPGKQREFFA	289	RIRSGGSIN	358	YGDSVKGRFTISRDKATNTLYLQMSNLNPEDTAVYYCSS	415	WRIGSYEY	437	WGQGTQVTVSS
65	A0194003A02	163	EVQLVESGGGLVQAGDSLSLSCTAS	211	RGTFRNFGMG	256	WFRQAQGKEREFVA	290	AISRSGGHKY	359	YSDSVKGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAA	416	RFRFDDGTSYYQRAFY	436	WGQGTLVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
													DF		
66	A0194003A04	163	EVQLVESGGGLVQAGDSLSLSCTAS	211	RGTFRNFGMG	257	WSRQAQGKEREFVA	290	AISRSGGHKY	359	YSDSVKGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAA	416	RFRFDDGTSYYQRAFYDF	436	WGQGTLVTVSS
67	A0194003A08	163	EVQLVESGGGLVQAGDSLSLSCTAS	212	AGTFRNFGMG	258	WFRQARGEEREFVA	291	TISRSARHTY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	417	RFRSDDGTYYYQRAFYDF	437	WGQGTQVTVSS
68	A0194003A09	164	EVQLVESGGGLVQAGGSLSLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	292	AISRSAGRTY	361	YSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	436	WGQGTLVTVSS
69	A0194003A12	164	EVQLVESGGGLVQAGGSLSLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	293	AISRSASRTY	361	YSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	437	WGQGTQVTVSS
70	A0194003B01	164	EVQLVESGGGLV	214	GGTFRN	256	WFRQAQGK	294	AISRSG	362	YSDSVKGRFTIARANAKN	418	RFRFD	437	WGQG

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
			QAGGSLSLSCTAS		FGMG		EREFVA		GHIY		MVSLQMNSLKPEDTAVYYCAA		DGTYYYQRAFYDF		TQVTVSS
71	A0194003B06	165	EVQLVESGGGLVQAGGSLSLSCTVS	215	GGNFRNFGMG	259	WFRQAHGKEREFVA	295	AISRSGGRTY	363	YADSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYSCAA	417	RFRSDDGTYYYQRAFYDF	437	WGQGTQVTVSS
72	A0194003B09	164	EVQLVESGGGLVQAGGSLSLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	292	AISRSAGRTY	364	YSDSVKGRFTISKNNAKNVMSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	436	WGQGTLVTVSS
73	A0194003B11	166	EVQLVESGGGLVRAGGSLSLSCTSS	216	SGTFRNFAMG	256	WFRQAQGKEREFVA	296	TISRSGGHTY	365	YSDSVKGRFTISRDNAKNLVSLQMNSLKPEDTAVYYCAA	419	RFRFDDDTYYYQRAFYDF	436	WGQGTLVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
74	A0194003C08	164	EVQLVESGGGLVQAGGSLSLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	295	AISRSGGRTY	361	YSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	437	WGQGTQVTVSS
75	A0194003F08	164	EVQLVESGGGLVQAGGSLSLSCTAS	214	GGTFRNFGMG	260	WFRQDQGQEREFVA	295	AISRSGGRTY	366	FSDSVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	437	WGQGTQVTVSS
76	A0194004F06	164	EVQLVESGGGLVQAGGSLSLSCTAS	211	RGTFRNFGMG	256	WFRQAQGKEREFVA	297	AISRSAGHTY	367	YSDSVKGRFTISRDNAKNMVSLQMDSLKPEDTAVYYCAA	420	RFRFDDGTYYYQRTFYDF	437	WGQGTQVTVSS
77	A0194007A01	167	EVQLVESGGGLAQAGGSLSLSCTAS	215	GGNFRNFGMG	261	WFRQVQGTEREFVA	298	AISRTGGRTY	368	FSDSVKGRFTISRDNAKNMVSLQMNSLKPEDSAVYYCAA	421	RFRFGDGTYYYQRNFYDF	437	WGQGTQVTVSS
78	A0194007B04	163	EVQLVESGGGLVQAGDSLSLSCTAS	211	RGTFRNFGMG	256	WFRQAQGKEREFVA	290	AISRSGGHKY	369	YSDSVQGRFTISKDNAKNMVSLQMNSLKPEDTAVY	416	RFRFDDGTSYYQRAFY	437	WGQGTQVTV

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
											YCAA		DF		SS
79	A0194007B09	168	EVQLVESGGGLV QTGGSLSLSCTAS	213	GGTFRN YGMG	256	WFRQAQGK EREFVA	292	AISRSA GRTY	361	YSDSVKGRFTISKNNAKN IMSLQMNSLKPEDTAVY YCAA	418	RFRFD DGTYY YQRAF YDF	437	WGQG TQVTV SS
80	A0194007B12	164	EVQLVESGGGLV QAGGSLSLSCTAS	217	GGTFRN YAMG	256	WFRQAQGK EREFVA	299	TISRSG GYTY	360	YSDSVKGRFTISRDNAKN MVSLQMNSLKPEDTAVY YCAA	422	RFRFG DGTYY YQRAF YDF	437	WGQG TQVTV SS
81	A0194007C01	164	EVQLVESGGGLV QAGGSLSLSCTAS	214	GGTFRN FGMG	256	WFRQAQGK EREFVA	295	AISRSG GRTY	370	YSDSVKGRFTISKDNAKN IMSLQMNSLRPEDTAVY YCAA	418	RFRFD DGTYY YQRAF YDF	437	WGQG TQVTV SS
82	A0194007D01	169	EVQLVESGGGSV QAGGSLSLSCTAS	211	RGTFRNF GMG	256	WFRQAQGK EREFVA	298	AISRTG GRTY	371	YSDSVKGRFTISRDNAKN MVSLQMNSLKPEDTAIY YCAA	418	RFRFD DGTYY YQRAF YDF	436	WGQG TLVTVS S
83	A0194007D10	163	EVQLVESGGGLV	212	AGTFRNF	258	WFRQARGE	291	TISRSA	360	YSDSVKGRFTISRDNAKN	423	RFRSD	437	WGQG

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
			QAGDSLSLSCTAS		GMG		EREFVA		RHTY		MVSLQMNSLKPEDTAVYYCAA		GGTYYYQRAFYDF		TQVTVSS
84	A0194007D11	164	EVQLVESGGGLVQAGGSLSLSCTAS	212	AGTFRNFGMG	256	WFRQAQGKEREFVA	300	AISRSGGHTY	372	YSDSVKGRFTISKDNAKNMVSLKMNSLKPEDTADYYCAA	418	RFRFDDGTYYYQRAFYDF	437	WGQGTQVTVSS
85	A0194007E04	164	EVQLVESGGGLVQAGGSLSLSCTAS	214	GGTFRNFGMG	262	WFRQVQGKEREFVA	301	AISRSGGHIF	373	YSDSVKGRFTISKDNAKNILFLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	437	WGQGTQVTVSS
86	A0194007E08	164	EVQLVESGGGLVQAGGSLSLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	292	AISRSAGRTY	374	YSDSAKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	436	WGQGTLVTVSS
87	A0194007E12	164	EVQLVESGGGLVQAGGSLSLSCTAS	215	GGNFRNFGMG	256	WFRQAQGKEREFVA	300	AISRSGGHTY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	424	RFRYGDGTYYYQRAF	436	WGQGTLVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
													YDF		
88	A0194007F01	163	EVQLVESGGGLVQAGDSLSLSCTAS	211	RGTFRNFGMG	256	WFRQAQGKEREFVA	302	AISRSGGRKY	359	YSDSVKGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAA	416	RFRFDDGTSYYQRAFYDF	436	WGQGTLVTVSS
89	A0194007G09	170	EVQLVESGGGLVEAGGSLSLSCTAS	212	AGTFRNFGMG	258	WFRQARGEEREFVA	291	TISRSARHTY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	417	RFRSDDGTYYYQRAFYDF	437	WGQGTQVTVSS
90	A0194008A01	163	EVQLVESGGGLVQAGDSLSLSCTAS	218	AGTLRNFGMG	258	WFRQARGEEREFVA	291	TISRSARHTY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	425	RFRSDDGTYYYQRRFYDF	437	WGQGTQVTVSS
91	A0194008A02	163	EVQLVESGGGLVQAGDSLSLSCTAS	218	AGTLRNFGMG	258	WFRQARGEEREFVA	291	TISRSARHTY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	426	RFRSGDGTYYYQRAFYDF	437	WGQGTQVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
92	A0194008A09	164	EVQLVESGGGLVQAGGSLSLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	303	AISRTGGHTY	361	YSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAA	422	RFRFGDGTYYYQRAFYDF	436	WGQGTLVTVSS
93	A0194008A11	163	EVQLVESGGGLVQAGDSLSLSCTAS	218	AGTLRNFGMG	258	WFRQARGEEREFVA	291	TISRSARHTY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	417	RFRSDDGTYYYQRAFYDF	437	WGQGTQVTVSS
94	A0194008B01	171	EVQLVESGGGLVQAGGSLSLSCTSS	219	KGTFRNFAMG	256	WFRQAQGKEREFVA	296	TISRSGGHTY	375	YSDSVKGRFAISRDNAKNLVSLQMNSLNAEDTAVYYCAA	427	RFRSDDDTYYYQRAFYDF	436	WGQGTLVTVSS
95	A0194008C01	172	EVQLVESGGGLVQAGGSLSLPCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	292	AISRSAGRTY	361	YSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	436	WGQGTLVTVSS
96	A0194008C07	164	EVQLVESGGGLVQAGGSLSLSCTAS	213	GGTFRNYGMG	263	WFRQAQGKERDFVA	304	AISRTNGHIY	376	YSDSVKGRFTISKDNAKNILSLQMNSLKPEDTAVYY	428	RFRFGDGTYYYQRTFY	436	WGQGTLVTVS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
											CAA		DF		S
97	A0194008C08	164	EVQLVESGGGLVQAGGSLSLSCTAS	220	GGTFRNFAMG	256	WFRQAQGKEREFVA	305	TISRSGGHIY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	416	RFRFDDGTSYYQRAFYDF	436	WGQGTLVTVSS
98	A0194008D08	173	EVQLVESGGDLVQPGGSLRLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	303	AISRTGGHTY	377	YSDSVKGRFTISKDNAKNIVSLQMNSLKPEDTAVYYCAA	421	RFRFGDGTYYYQRNFYDF	437	WGQGTQVTVSS
99	A0194008F05	174	EVQLVESGGGLVQAGGPLSLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	292	AISRSAGRTY	361	YSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	436	WGQGTLVTVSS
100	A0194008G10	175	EVQLVESGGGSVQAGGSLRLVCAFS	220	GGTFRNFAMG	256	WFRQAQGKEREFVA	296	TISRSGGHTY	378	YSDSVKGRFTISKDNAKNMVYLQMYSLKPEDTAVYYCAG	429	RFRFGDGAYYYQRTFYDF	436	WGQGTLVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
101	KV13BIIPMP026D09	164	EVQLVESGGGLVQAGGSLSLSCTAS	221	RGTFRNFAMG	256	WFRQAQGKEREFVA	305	TISRSGGHIY	359	YSDSVKGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAA	416	RFRFDDGTSYYQRAFYDF	437	WGQGTQVTVSS
102	KV13BIIPMP026F08	164	EVQLVESGGGLVQAGGSLSLSCTAS	222	RGTFRNYAMG	256	WFRQAQGKEREFVA	305	TISRSGGHIY	379	YSDSVKGRFAISKDNAKNMMSLRMNSLKPEDTAVYYCAA	430	RFRFDDGTYFYQRAFYDF	437	WGQGTQVTVSS
103	KV13BIIPMP027A11	176	KVQLVESRGGLVQAGGSLRLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	306	AVSRTGGRTY	380	YSDSVKGRFTISRDNAKNMVSLQMNKLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	437	WGQGTQVTVSS
104	KV13BIIPMP027B10	164	EVQLVESGGGLVQAGGSLSLSCTAS	223	GGTFRSYGMG	256	WFRQAQGKEREFVA	303	AISRTGGHTY	361	YSDSVKGRFTISKNNAKNIMSLQMNSLKPEDTAVYYCAA	422	RFRFGDGTYYYQRAFYDF	437	WGQGTQVTVSS
105	KV13BIIPMP027B12	164	EVQLVESGGGLVQAGGSLSLSCTAS	215	GGNFRNFGMG	264	WFRQAQGTEREFVA	298	AISRTGGRTY	381	FSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVY	431	RFRFDDGTYYYQRNF	439	WGQGAQVTV

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
											YCAA		YDF		SS
106	KV13BIIPMP027D09	164	EVQLVESGGGLVQAGGSLSLSCTAS	220	GGTFRNFAMG	265	WFREAQGKEREFVA	296	TISRSGGHTY	382	YSDSVKGRFTISRDNAKNMVALQMNSLKPEDTAVYYCAA	432	RFRFDDGTYYYQRAFYNL	437	WGQGTQVTVSS
107	KV13BIIPMP027D10	164	EVQLVESGGGLVQAGGSLSLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	303	AISRTGGHTY	359	YSDSVKGRFTISKDNAKNMVSLQMNSLKPEDTAVYYCAA	421	RFRFGDGTYYYQRNFYDF	437	WGQGTQVTVSS
108	KV13BIIPMP027E08	164	EVQLVESGGGLVQAGGSLSLSCTAS	224	VGNFRNFGMG	256	WFRQAQGKEREFVA	300	AISRSGGHTY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	437	WGQGTQVTVSS
109	KV13BIIPMP027E11	165	EVQLVESGGGLVQAGGSLSLSCTVS	215	GGNFRNFGMG	259	WFRQAHGKEREFVA	295	AISRSGGRTY	383	YADSVKGRFTVSRDNAKNMVSLQMNSLKPEDTAVYSCAA	417	RFRSDDGTYYYQRAFYDF	437	WGQGTQVTVSS
110	A019400003	164	EVQLVESGGGLV	214	GGTFRN	256	WFRQAQGK	303	AISRTG	384	YQDSVKGRFTISKDNAK	422	RFRFG	437	WGQG

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
			QAGGSLSLSCTAS		FGMG		EREFVA		GHTY		NILSLQMNSLKPEDTAVYYCAA		DGTYYYQRAFYDF		TQVTVSS
111	KV13BIIPMP027H08	164	EVQLVESGGGLVQAGGSLSLSCTAS	220	GGTFRNFAMG	256	WFRQAQGKEREFVA	307	TISRSGGHIF	385	YSESVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	433	RFRSDDGTYYYQRIFYDF	437	WGQGTQVTVSS
112	KV13BIIPMP027H09	177	EVQLVESGGGLVQPGGSLSLSCTAS	212	AGTFRNFGMG	258	WFRQARGEEREFVA	291	TISRSARHTY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	417	RFRSDDGTYYYQRAFYDF	437	WGQGTQVTVSS
113	KV13BIIPMP027H10	169	EVQLVESGGGSVQAGGSLSLSCTAS	225	RGTFRNYGMG	256	WFRQAQGKEREFVA	300	AISRSGGHTY	386	YSDSVKGRFTISKDNAKNIMSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	437	WGQGTQVTVSS
114	KV13BIIPMP049B09	165	EVQLVESGGGLVQAGGSLSLSCTVS	226	GGNFRNFGVG	259	WFRQAHGKEREFVA	308	AISRAGGRTY	387	YADSVKGRFAISRDNAKNMVSLQMNSLKPEDTAVYTCAA	434	RFRSGDGTSYYERAFYD	437	WGQGTQVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
													F		
115	KV13BIIPMP049E10	178	KVQLVESGGGLVQPGGSLSLSCTAS	212	AGTFRNFGMG	264	WFRQAQGTEREFVA	295	AISRSGGRTY	388	FSDSVKGRFTISKDNAKNMLSLQMNSLKPEDTAVYYCAA	417	RFRSDDGTYYYQRAFYDF	439	WGQGAQVTVSS
116	KV13BIIPMP050A07	165	EVQLVESGGGLVQAGGSLSLSCTVS	213	GGTFRNYGMG	266	WFRQFQGKEREFVA	301	AISRSGGHIF	385	YSESVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	426	RFRSGDGTYYYQRAFYDF	437	WGQGTQVTVSS
117	KV13BIIPMP050A09	164	EVQLVESGGGLVQAGGSLSLSCTAS	212	AGTFRNFGMG	267	WFRQARGEAREFVA	305	TISRSGGHIY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVYYCAA	417	RFRSDDGTYYYQRAFYDF	437	WGQGTQVTVSS
118	KV13BIIPMP050A10	166	EVQLVESGGGLVRAGGSLSLSCTSS	216	SGTFRNFAMG	256	WFRQAQGKEREFVA	296	TISRSGGHTY	389	YSDSVKGRFTISRDNAKNLVSLQMSSLKPEDTAVYYCAA	419	RFRFDDDTYYYQRAFYDF	437	WGQGTQVTVSS

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
119	KV13BIIPMP050B11	179	EVQLVESGGGSVQAGNSLSLSCTAS	217	GGTFRNYAMG	256	WFRQAQGKEREFVA	305	TISRSGGHIY	390	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAAYYCAA	422	RFRFGDGTYYYQRAFYDF	437	WGQGTQVTVSS
120	KV13BIIPMP050C09	163	EVQLVESGGGLVQAGDSLSLSCTAS	211	RGTFRNFGMG	256	WFRQAQGKEREFVA	290	AISRSGGHKY	391	YSDSVKGRSTISKDNAKNMVSLQMNSLKPEDTAVYYCAA	416	RFRFDDGTSYYQRAFYDF	437	WGQGTQVTVSS
121	KV13BIIPMP050D09	167	EVQLVESGGGLAQAGGSLSLSCTAS	215	GGNFRNFGMG	261	WFRQVQGTEREFVA	298	AISRTGGRTY	368	FSDSVKGRFTISRDNAKNMVSLQMNSLKPEDSAVYYCAA	435	RFRFGDGTYYYQRNFYGF	439	WGQGAQVTVSS
122	KV13BIIPMP050E12	164	EVQLVESGGGLVQAGGSLSLSCTAS	213	GGTFRNYGMG	256	WFRQAQGKEREFVA	309	AISRSAGHIY	392	YLNSVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAA	418	RFRFDDGTYYYQRAFYDF	437	WGQGTQVTVSS
123	KV13BIIPMP050F11	180	EVQLVESGGGLVQAGNSLSLSCTAS	218	AGTLRNFGMG	258	WFRQARGEEREFVA	291	TISRSARHTY	360	YSDSVKGRFTISRDNAKNMVSLQMNSLKPEDTAVY	417	RFRSDDGTYYYQRAF	437	WGQGTQVTV

ID	Nanobody	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
											YCAA		YDF		SS

Table A-3: Amino acid sequences of selected multivalent anti-Kv1.3 Nanobodies

Name	ID	Amino acid sequence
A019400004 (A019400003-40GS-A019400003)	451	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFGMGWFRQAQGKEREFVAAISRTGGHTYYQD SVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAARFRFGDGTYYYQRAFYDFWGQGTQVTVS SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLS LSCTASGGTFRNFGMGWFRQAQGKEREFVAAISRTGGHTYYQDSVKGRFTISKDNAKNILSLQ MNSLKPEDTAVYYCAARFRFGDGTYYYQRAFYDFWGQGTQVTVSS
A019400009 (A0194009G09-35GS-A0194009G09)	452	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAG WYRQAPGKQREFVARIRMGGSINYADTVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSW RTGFYEYWGQGTLVTVSS
A019400010 (A0194009G09-35GS- A019400003)	453	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLSLSCTASGGTFRNFGMG WFRQAQGKEREFVAAISRTGGHTYYQDSVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAAR FRFGDGTYYYQRAFYDFWGQGTLVTVSS

Name	ID	Amino acid sequence
A019400011 (A019400003-35GS-A0194009G09)	454	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFGMGWFRQAQGKEREFVAAISRTGGHTYYQD SVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAARFRFGDGTYYYQRAFYDFWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSA SGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTVKGRFTISRDNAKNTVYLQMNSLKP EDTAVYYCSSWRTGFYEYWGQGTLVTVSS
A019400012 (A0194009G09-35GS- A019400003)	455	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLSLSCTASGGTFRNFGMG WFRQAQGKEREFVAAISRTGGHTYYQDSVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAAR FRFGDGTYYYQRAFYDFWGQGTLVTVSS
A019400013 (A0194009G09-35GS-A0194009G09)	456	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAG WYRQAPGKQREFVARIRMGGSINYADTVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSW RTGFYEYWGQGTLVTVSS

Name	ID	Amino acid sequence
A019400014 (A019400003-35GS-A0194009G09)	457	EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFGMGWFRQAQGKEREFVAAISRTGGHTYYQD SVKGRFTISKDNAKNILSLQMNSLKPEDTAVYYCAARFRFGDGTYYYQRAFYDFWGQGTLVTVS SGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSA SGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTVKGRFTISRDNAKNTVYLQMNSLKP EDTAVYYCSSWRTGFYEYWGQGTLVTVSS
A019400015 (A0194009G09-35GS-A0194009G09-35GS- A0194009G09)	458	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAG WYRQAPGKQREFVARIRMGGSINYADTVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSW RTGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTVKGRFTISR DNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSS

Name	ID	Amino acid sequence
A019400016 (A0194009G09-35GS-A0194009G09-35GS-A0194009G09-35GS-A0194009G09)	459	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAG WYRQAPGKQREFVARIRMGGSINYADTVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSW RTGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTVKGRFTISR DNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPG KQREFVARIRMGGSINYADTVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEY WGQGTLVTVSS
A019400032 (A0194020A06-35GS-A0194020A06)	460	DVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSV KGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSA GWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFC SGWREGFYEYWGQGTLVTVSS

Name	ID	Amino acid sequence
A019400023 (A0194020B10-35GS-A0194020B10-35GS-ALB11)	461	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSVGWYRRAPGKQREFVARLRTTGSTNYAQSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCSAWRIEAYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSVGWYRRAPGKQREFVARLRTTGSTNYAQSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCSAWRIEAYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
A019400024 (A0194020A06-35GS-A0194020A06-35GS-ALB11)	462	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
A019400025 (A0194020B10-9GS-ALB11-9GS-A0194020B10)	463	EVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSVGWYRRAPGKQREFVARLRTTGSTNYAQSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCSAWRIEAYEYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLRLSCSASGLLFSVNSVGWYRRAPGKQREFVARLRTTGSTNYAQSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCSAWRIEAYEYWGQGTLVTVSS

Name	ID	Amino acid sequence
A019400026 (A0194009G09-9GS-ALB11-9GS-A0194009G09)	464	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSSGGGGSGGGSE VQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSV KGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQLV ESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTVKGRFTI SRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSS
A019400027 (A0194009G09-35GS-A0194009G09-35GS-ALB11)	465	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKQREFVARIRMGGSINYADTV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSWRTGFYEYWGQGTLVTVSSGGGGSGGGG SGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAG WYRQAPGKQREFVARIRMGGSINYADTVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCSSW RTGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTIS RDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
A019400028 (A0194020A06-9GS-ALB11-9GS-A0194020A06)	466	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVK GRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGG SEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADS VKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGSEVQL VESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFT VSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSS

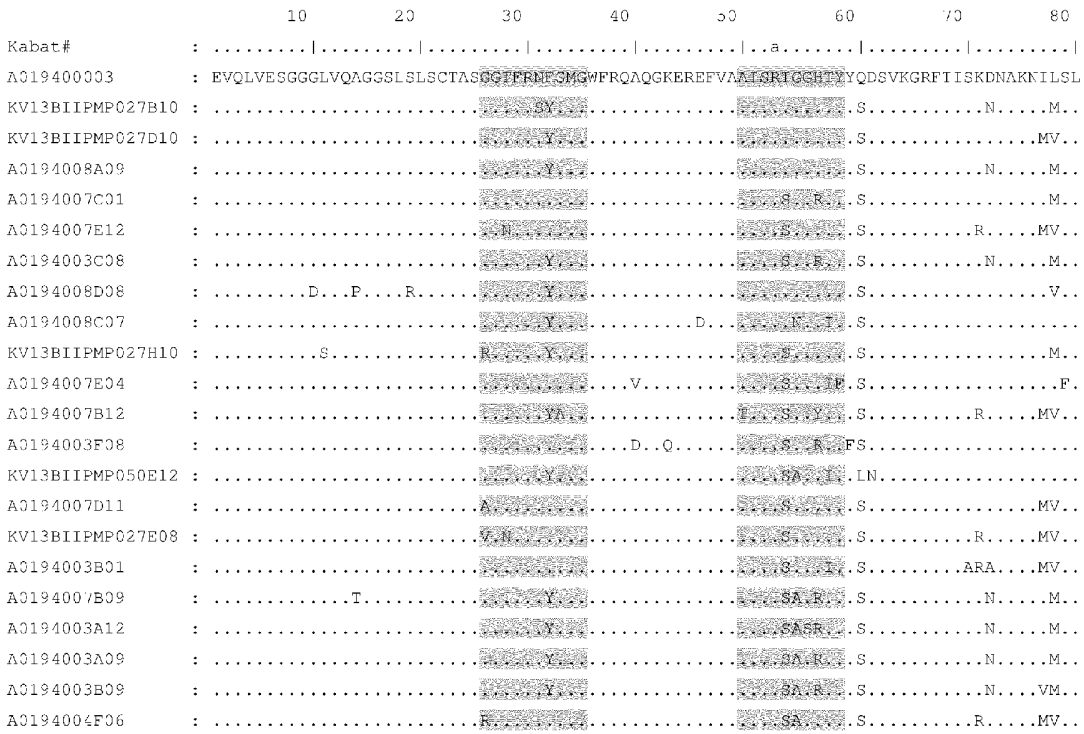
Name	ID	Amino acid sequence
A019400029 (A0194020A06-35GS-A0194020A06-35GS-ALB11)	467	DVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
A019400034 (A0194020A06-9GS-A0194020A06-35GS-ALB11)	468	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS

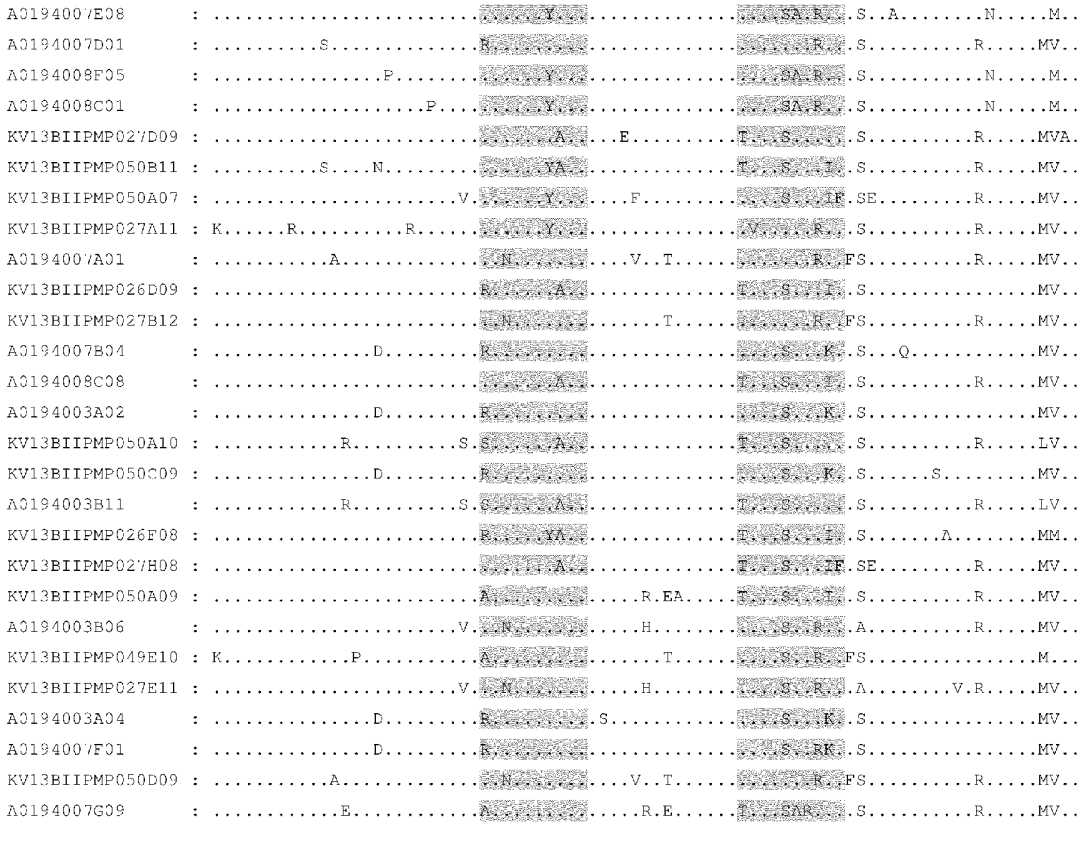
Name	ID	Amino acid sequence
A019400035 (A0194020A06-15GS-A0194020A06-35GS-ALB11)	469	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
A019400036 (A0194020A06-20GS-A0194020A06-35GS-ALB11)	470	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS

Name	ID	Amino acid sequence
A019400037 (A0194020A06-25GS-A0194020A06-35GS-ALB11)	471	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
A019400038 (A0194020A06-40GS-A0194020A06-35GS-ALB11)	472	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS

Name	ID	Amino acid sequence
A019400039 (A0194020A06-35GS-ALB11-35GS-A0194020A06)	473	EVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQAGGSLGLSCSASGLLFSRNSAGWYRQAPGKRREFVARIRMGGSINYADSVKGRFTVSRDNAKNMMYLQMNDLKPEDTAVYFCSGWREGFYEYWGQGTLVTVSS

Table A-4: Sequence alignment of Kv1.3 Family 1 binders




A0194007E08 : .....Y.....SA.R..S..A.....N.....M..
A0194007D01 : .....S.....R.....R..S.....R.....MV..
A0194008F05 : .....P.....Y.....SA.R..S.....N.....M..
A0194008C01 : .....P.....Y.....SA.R..S.....N.....M..
KV13BIIPMP027D09 : .....A...E.....T..S....S.....R.....MVA.
KV13BIIPMP050B11 : .....S....N.....YA.....T..S..I.S.....R.....MV..
KV13BIIPMP050A07 : .....V.....Y....F.....S..IE.SE.....R.....MV..
KV13BIIPMP027A11 : K.....R.....R.....Y.....V...R..S.....R.....MV..
A0194007A01 : .....A.....N.....V..T.....R.FS.....R.....MV..
KV13BIIPMP026D09 : .....A.....T..S..I.S.....MV..
KV13BIIPMP027B12 : .....N.....T.....R.FS.....R.....MV..
A0194007B04 : .....D.....K.....S..K.S...Q.....MV..
A0194008C08 : .....A.....T..S..I.S.....R.....MV..
A0194003A02 : .....D.....K.....S..K.S.....MV..
KV13BIIPMP050A10 : .....R.....S.....A.....T..S....S.....R.....LV..
KV13BIIPMP050C09 : .....D.....K.....S..K.S.....S.....MV..
A0194003B11 : .....R.....S.....A.....T..S....S.....R.....LV..
KV13BIIPMP026F08 : .....R.....YA.....T..S..I.S.....A.....MM..
KV13BIIPMP027H08 : .....A.....T..S..IE.SE.....R.....MV..
KV13BIIPMP050A09 : .....A.....R.EA.....T..S..I.S.....R.....MV..
A0194003B06 : .....V..N.....H.....S..R..A.....R.....MV..
KV13BIIPMP049E10 : K.....P.....A.....T.....S..R..FS.....M...
KV13BIIPMP027E11 : .....V..N.....H.....S..R..A.....V.R.....MV..
A0194003A04 : .....D.....R.....S.....S..K.S.....MV..
A0194007F01 : .....D.....R.....S..RK.S.....MV..
KV13BIIPMP050D09 : .....A.....N.....V..T.....R.FS.....R.....MV..
A0194007G09 : .....E.....A.....R.E.....T..SMK..S.....R.....MV..

(continued)

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                          10        20        30        40        50        60        70        80
Kabat#           : .........|.........|.........|.........|.........|..a.......|.........|.........|
A0194000D3       : EVQLVESGGGLVQAGGSLSLSCTASGGTFRNFSMGWFRQAQGKEREFVAAISRTGGHTYYQDSVKGRFTISKDNAKNILSL
KV13BIIPMP027H09 : .............P...........A.L.....A.....R.E......T..SAR....S..........R.....MV..
A0194008A02      : ...............D.........A.L.....A.....R.E......T..SAR....S..........R.....MV..
A0194003A08      : ...............D.........A.L.....A.....R.E......T..SAR....S..........R.....MV..
KV13BIIPMP050F11 : ...............N.........A.L.....A.....R.E......T..SAR....S..........R.....MV..
A0194008A11      : ...............D.........A.L.....A.....R.E......T..SAR....S..........R.....MV..
A0194007D10      : ...............D.........A.L.....A.....R.E......T..SAR....S..........R.....MV..
KV13BIIPMP049B09 : ........................V..N....V.....H.........T..A.R..A.......A..R.....MV..
A0194008A01      : ...............D.........A.L.....A.....R.E......T..SAR....S..........R.....MV..
A0194008B01      : ........................S.K.....A.............T..S.....S.......A..R.....LV..
A0194008G10      : ..........S.......R.V.AF.....A.....A.............T..S.....S..............MVY.
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Table A-4: Sequence alignment of Kv1.3 Family 1 binders

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                                90        100               110
Kabat#           : ..abc.......|.........|abcdefghij.........|...
A0194000D3       : QMNSLKPEDTAVYYCAARFRFGDGTYYYQRAFYDFWGQGTQVTVSS
KV13BIIPMP027B10 : ................A.....A.....A.....A.....A.....
KV13BIIPMP027D10 : ................A.....A.....N..A.....A.....
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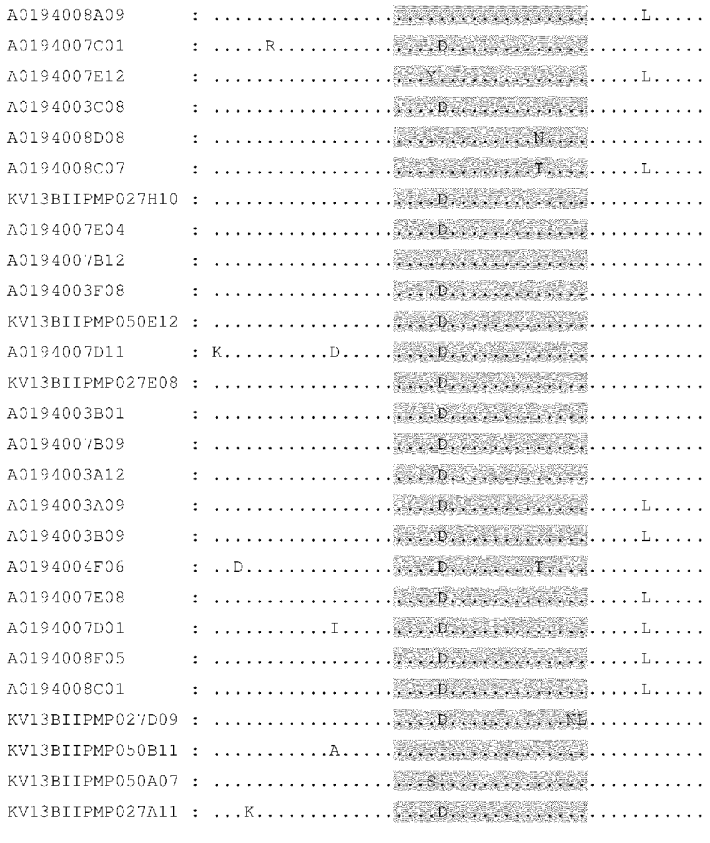
A0194008A09 : .....L.....
A0194007C01 : .....R.....D.....
A0194007E12 : .....Y.....L.....
A0194003C08 : .....D.....
A0194008D08 : .....N.....
A0194008C07 : .....T.....L.....
KV13BIIPMP027H10 : .....D.....
A0194007E04 : .....D.....
A0194007B12 : .....
A0194003F08 : .....D.....
KV13BIIPMP050E12 : .....D.....
A0194007D11 : K.....D.....D.....
KV13BIIPMP027E08 : .....D.....
A0194003B01 : .....D.....
A0194007B09 : .....D.....
A0194003A12 : .....D.....
A0194003A09 : .....D.....L.....
A0194003B09 : .....D.....L.....
A0194004F06 : ..D.....D.....T.....
A0194007E08 : .....D.....L.....
A0194007D01 : .....I.....D.....L.....
A0194008F05 : .....D.....L.....
A0194008C01 : .....D.....L.....
KV13BIIPMP027D09 : .....D.....NE.....
KV13BIIPMP050B11 : .....A.....
KV13BIIPMP050A07 : .....S.....
KV13BIIPMP027A11 : ...K.....D.....

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A0194007A01      : .........S.....................D.....................
KV13BIIPMP026D09 : ...................D..S.....N.....................
KV13BIIPMP027B12 : ...................D.....D.....N.....A.....
A0194007B04      : ...................D..S.....N.....................
A0194008C08      : ...................D..S.....N.....L.....
A0194003A02      : ...................D..S.....N.....L.....
KV13BIIPMP050A10 : ..S................D.D.....N.....................
KV13BIIPMP050C09 : ...................D..S.....N.....................
A0194003B11      : ...................D.D.....N.....L.....
KV13BIIPMP026F08 : R..................D..S.....N.....................
KV13BIIPMP027H08 : ...................SD.....N.....I.....
KV13BIIPMP050A09 : ...................SD.....N.....................
A0194003B06      : .............S.....SD.....N.....................
KV13BIIPMP049E10 : ...................SD.....N.....A.....
KV13BIIPMP027E11 : .............S.....SD.....N.....................
A0194003A04      : ...................D..S.....N.....L.....
A0194007F01      : ...................D..S.....N.....L.....
KV13BIIPMP050D09 : .........S.....................N..G.....A.....
A0194007G09      : ...................SD.....N.....................
```

(continued)

```
                                 90        100                 110
Kabat#           : ..abc.......|.........|abcdefghij.........|...
A0194000D3       : QMNSLKPEDTAVYYCAARFRFGDGIYYYGMAFYDFWGQGTQVTVSS
KV13BIIPMP027H09 : ...................SD.....N.....................
A0194008A02      : ...................S.....N.....................
```

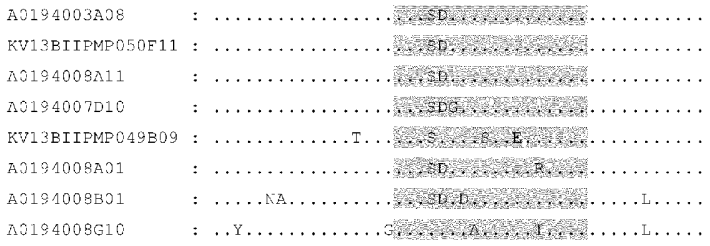
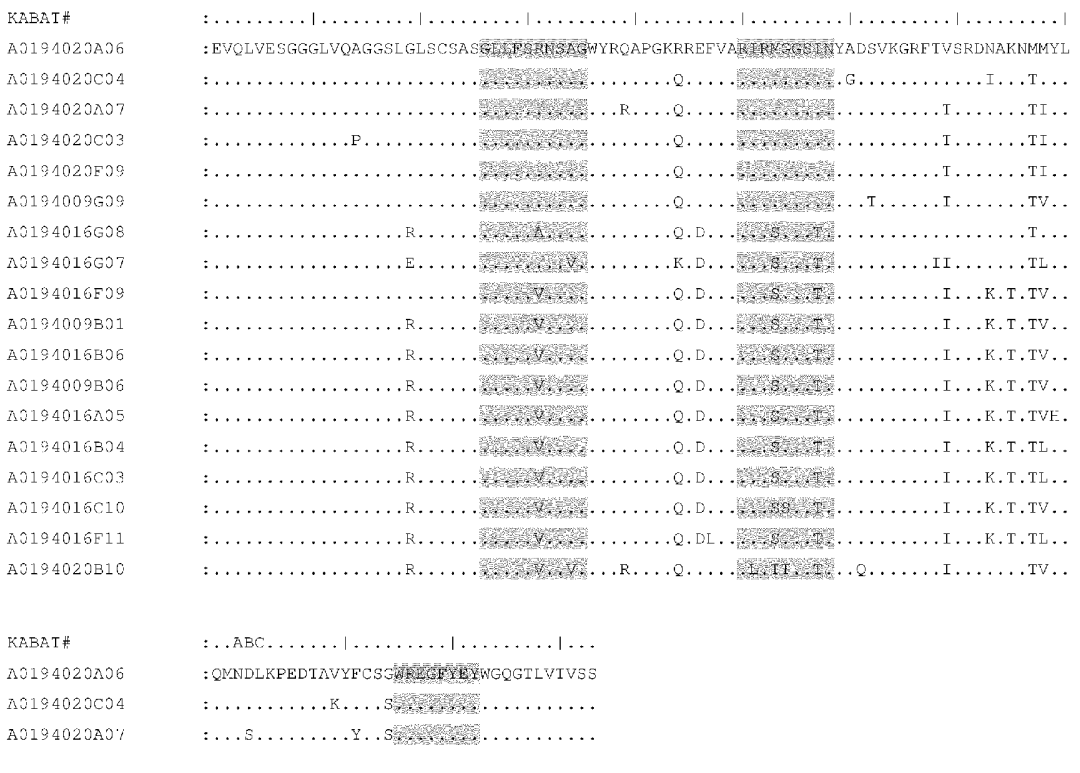

A0194003A08 : ..... SD. .....
KV13BIIPMP050F11 : ..... SD. .....
A0194008A11 : ..... SD. .....
A0194007D10 : ..... SDG. .....
KV13BIIPMP049B09 : .....T... S. . E. .....
A0194008A01 : ..... D. . R. .....
A0194008B01 : .....NA..... D.D. .....L.....
A0194008G10 : ..Y.....G. . A. . I. .....L.....

Table A-5: Sequence alignment of Kv1.3 Family 12 binders



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A0194020C03       :...S.........Y..S..TEA.....Q.....
A0194020F09       :...S.........Y..S..TEA.....Q.....
A0194009G09       :...S.........Y..S..TEA.....Q.....
A0194016G08       :...G.........Y..S..TEA.....Q.....
A0194016G07       :...A......G..Y..S..TEA.....Q.....
A0194016F09       :...S.........Y..S..TEA.....Q.....
A0194009B01       :...S.........Y..S..TEA.....Q.....
A0194016B06       :...S.........Y..S..TEA.....Q.....
A0194009B06       :...S.........Y..S..TEA.....Q.....
A0194016A05       :...S.........Y.GS..TEA.....Q.....
A0194016B04       :...N.........Y..S..TEA.....R.....
A0194016C03       :.V.N.........Y..S..TEA.....R.....
A0194016C10       :...S.........Y..S..TEA.....AQ.....
A0194016F11       :...N.........Y..S..TEA.....R..Q.....
A0194020B10       :...N.........Y..A..TEA.....Q.....
```

Table A-6: Representative multivalent formats

Construct ID	VHH identity	Family	Linker	VHH identity	Family	Linker	VHH identity	Family
A019400004	A019400003	1	40 GS	A019400003	1			
A019400012 A019400010	A0194009G09	12	35 GS	A019400003	1			
A019400013 A019400009	A0194009G09	12	35 GS	A0194009G09	12			
A019400014 A019400011	A019400003	1	35 GS	A0194009G09	12			
A019400015	A0194009G09	12	35 GS	A0194009G09	12	35 GS	A0194009G09	12
A019400032	A0194020A06	12	35 GS	A0194020A06	12			
A019400023	A0194020B10	12	35GS	A0194020B10	12	35GS	Alb11	
A019400024	A0194020A06	12	35GS	A0194020A06	12	35GS	Alb11	
A019400025	A0194020B10	12	9GS	Alb11		9GS	A0194020B10	12
A019400026	A0194009G09	12	9GS	Alb11		9GS	A0194009G09	12
A019400027	A0194009G09	12	35GS	A0194009G09	12	35GS	Alb11	
A019400028	A0194020A06	12	9GS	Alb11		9GS	A0194020A06	12
A019400029	A0194020A06	12	35GS	A0194020A06	12	35GS	Alb11	

Table A-7: Kv1.3 sequences from various species ("ID" refers to the SEQ ID NO as used herein)

Prot ID	Species	ID	Sequence
P22001	Homo sapiens	474	MDERLSLLRSPPPPSARHRAHPPQRPASSGGAHTLVNHGYAEPAAGRELPPDMTVVPGDHLLEPEVADGGGA PPQGGCGGGGCDRYEPLPPSLPAAGEQDCCGERVVINISGLRFETQLKTLCQFPETLLGDPKRRMRYFDPLRNE YFFDRNRPSFDAILYYYQSGGRIRRPVNVPIDIFSEEIRFYQLGEEAMEKFREDEGFLREEERPLPRRDFQRQVWL LFEYPESSGPARGIAIVSVLVILISIVIFCLETLPEFRDEKDYPASTSQDSFEAAGNSTSGSRAGASSFSDPFFVVETLC IIWFSFELLVRFFACPSKATFSRNIMNLIDIVAIIPYFITLGTELAERQGNGQQAMSLAILRVIRLVRVFRIFKLSRHSK GLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEADDPTSGFSSIPDAFWWAVVTMTTVGYGDMHPVTIGG KIVGSLCAIAGVLTIALPVPVIVSNFNYFYHRETEGEEQSQYMHVGSCQHLSSSAEELRKARSNSTLSKSEYMVIEE GGMNHSAFPQTPFKTGNSTATCTTNNNPNSCVNIKKIFTDV
P78352	Rattus norvegicus	475	MTVVPGDHLLEPEAAGGGGGDPPQGGCVSGGGCDRYEPLPPALPAAGEQDCCGERVVINISGLRFETQLKTLC QFPETLLGDPKRRMRYFDPLRNEYFFDRNRPSFDAILYYYQSGGRIRRPVNVPIDIFSEEIRFYQLGEEAMEKFRE DEGFLREEERPLPRRDFQRQVWLLFEYPESSGPARGIAIVSVLVILISIVIFCLETLPEFRDEKDYPASPSQDVFEAA NNSTSGASSGASSFSDPFFVVETLCIIWFSFELLVRFFACPSKATFSRNIMNLIDIVAIIPYFITLGTELAERQGNGQ QAMSLAILRVIRLVRVFRIFKLSRHSKGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEADDPSSGFNSIPDA FWWAVVTMTTVGYGDMHPVTIGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYHRETEGEEQAQYMHVGSCQ HLSSSAEELRKARSNSTLSKSEYMVIEEGGMNHSAFPQTPFKTGNSTATCTTNNNPNSCVNIKKIFTDV
P16390	Mus musculus	476	MTVVPGDHLLEPEAAGGGGGDPPQGGCGSGGGGGGCDRYEPLPPALPAAGEQDCCGERVVINISGLRFETQL KTLCQFPETLLGDPKRRMRYFDPLRNEYFFDRNRPSFDAILYYYQSGGRIRRPVNVPIDIFSEEIRFYQLGEEAMEK FREDEGFLREEERPLPRRDFQRQVWLLFEYPESSGPARGIAIVSVLVILISIVIFCLETLPEFRDEKDYPASPSQDVFE AANNSTSGAPSGASSFSDPFFVVETLCIIWFSFELLVRFFACPSKATFSRNIMNLIDIVAIIPYFITLGTELAERQGNG

			QQAMSLAILRVIRLVRVFRIFKLSRHSKGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEADDPSSGFNSIPD AFWWAVVTMTTVGYGDMHPVTIGGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYHRETEGEEQAQYMHVGSC QHLSSSAEELRKARSNSTLSKSEYMVIEEGGMNHSAFPQTPFKTGNSTATCTTNNNPNSCVNIKKIFTDV
XP_005542459	Macaca fascicularis	477	MDEHLSLLRSPPPPSARHRAHPAQRPASSGGAHTLVNPGYAEPAAGPELPPDMTVVPGDHLLEPEVADGGGA PPQGGCGGGGCDRYEPLPPSLPAAGEQDCCGERVVINISGLRFETQLKTLCQFPETLLGDPKRRMRYFDPLRNE YFFDRNRPSFDAILYYYQSGGRIRRPVNVPIDIFSEEIRFYQLGEEAMEKFREDEGFLREEERPLPRRDFQRQVWL LFEYPESSGPARGIAIVSVLVILISIVIFCLETLPEFRDEKDYPASPSQDSFDAAGNSTSGAAAGASSFSDPFFVVETL CIIWFSFELLVRFFACPSKATFSRNIMNLIDIVAIIPYFITLGTELAERQGNGQQAMSLAILRVIRLVRVFRIFKLSRHS KGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEADDPTSGFSSIPDAFWWAVVTMTTVGYGDMHPVTIG GKIVGSLCAIAGVLTIALPVPVIVSNFNYFYHRETEGEEQAQYMHVGSCQHLSSSAEELRKARSNSTLSKSEYMVI EEGGMNHSAFPQTPFKTGNSTATCTTNNNPNSCVNIKKIFTDV
AFH32312	Macaca mulatta	477	MDEHLSLLRSPPPPSARHRAHPAQRPASSGGAHTLVNPGYAEPAAGPELPPDMTVVPGDHLLEPEVADGGGA PPQGGCGGGGCDRYEPLPPSLPAAGEQDCCGERVVINISGLRFETQLKTLCQFPETLLGDPKRRMRYFDPLRNE YFFDRNRPSFDAILYYYQSGGRIRRPVNVPIDIFSEEIRFYQLGEEAMEKFREDEGFLREEERPLPRRDFQRQVWL LFEYPESSGPARGIAIVSVLVILISIVIFCLETLPEFRDEKDYPASPSQDSFDAAGNSTSGAAAGASSFSDPFFVVETL CIIWFSFELLVRFFACPSKATFSRNIMNLIDIVAIIPYFITLGTELAERQGNGQQAMSLAILRVIRLVRVFRIFKLSRHS KGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEADDPTSGFSSIPDAFWWAVVTMTTVGYGDMHPVTIG GKIVGSLCAIAGVLTIALPVPVIVSNFNYFYHRETEGEEQAQYMHVGSCQHLSSSAEELRKARSNSTLSKSEYMVI EEGGMNHSAFPQTPFKTGNSTATCTTNNNPNSCVNIKKIFTDV

Table A-8: Various amino acid sequences ("ID" refers to the SEQ ID NO as used herein)

Name	ID	Amino acid sequence
Alb-11	478	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAP GKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMN SLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
5GS linker	479	GGGGS
7GS linker	480	sggsggs
8GS linker	481	ggggcgggs
9GS linker	482	GGGGSGGGS
10GS linker	483	GGGGSGGGGS
15GS linker	484	GGGGSGGGGSGGGGS
18GS linker	485	GGGGSGGGGSGGGGGGGS
20GS linker	486	GGGGSGGGGSGGGGSGGGGS
25GS linker	487	GGGGSGGGGSGGGGSGGGGSGGGGS
30GS linker	488	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
35GS linker	489	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
40GS linker	490	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGG GS
G1 hinge	491	EPKSCDKTHTCPPCP
9GS-G1 hinge	492	GGGGSGGGSEPKSCDKTHTCPPCP
Llama upper long hinge region	493	epktpkpqpaaa
G3 hinge	494	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCP RCPEPKSCDTPPPCPRCP

Equivalents

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as an illustration of certain aspects and embodiments of the invention. Other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages and objects of the invention are not necessarily encompassed by each embodiment of the invention.

CONCLUSIES

1. Een immunoglobuline dat specifiek bindt aan de EL1 extracellulaire loop van kalium kanaal 3 (Kv1.3), waarbij de binding van het genoemde immunoglobuline aan de genoemde EL1 extracellulaire loop de activiteit van Kv1.3 moduleert.

2. Het immunoglobuline volgens conclusie 1, waarbij het immunoglubuline de activiteit van Kv1.3 moduleert door gedeeltelijke of volledige blokkering van de activiteit van Kv1.3.

3. Het immunoglobuline volgens een van de conclusies 1-2, waarbij het immunoglobuline de activiteit van Kv1.3 moduleert door de efflux van kalium-ionen uit T-cellen te reduceren of zelfs volledig te inhiberen.

4. Het immunoglobuline volgens conclusie 3, waarbij het immunoglobuline de efflux van kalium-ionen uit T-cellen reduceert of inhibeert met een IC50 waarde van 10^{-7} M of lager, 10^{-8} M of lager, liefst 10^{-9} M of lager, of zelfs 10^{-10} M of lager, zoals bepaald in Patch Clamp assay.

5. Het immunoglobuline volgens een van de conclusies 1-4, waarbij het immunoglobuline de activiteit van Kv1.3 allosterisch moduleert en/of inhibeert.

6. Het immunoglobuline volgens een van de conclusies 1-5, waarbij het immunoglobuline meer dan 10 maal, meer dan 100 maal, liefst meer dan 1000 maal selectief is over ander gerelateerde Kv ionkanalen families voor het moduleren en /of inhiberen van de activiteit van Kv1.3.

7. Het immunoglobuline volgens een van de conclusies 1-6, waarbij het immunoglobuline hetzelfde aantal aminozuurresiduen telt in vergelijking met een van SEQ ID NOs: 1-64 en waarbij het immunoglobuline een aminozuursequentie tussen positie 8 en positie 106 (volgens Kabat nummering) heeft van 89% of meer sequentie identiteit in vergelijking met een van SEQ ID NOs: 1-64.

8. Het immunoglobuline volgens een van de conclusies 1-6, waarbij het immunoglobuline hetzelfde aantal aminozuurresiduen telt in vergelijking met een van SEQ ID NOs: 65-123 en waarbij het immunoglobuline een aminozuursequentie tussen positie 8 en positie 106 (volgens Kabat nummering) heeft van 89% of meer sequentie identiteit in vergelijking met een van SEQ ID NOs: 65-123.

9. Het immunoglobuline volgens een van de conclusies 1-6, dat bestaat uit 4 raamwerkregio's, respectievelijk FR1 tot FR4, en 3 complementariteit bepalende regio's, respectievelijk CDR1 tot CDR3, waarbij:

i) CDR1 is gekozen uit de groep bestaande uit:

a) SEQ ID NOs: 181-210; of

b) aminozuursequenties die 4, 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 182;

en/of

ii) CDR2 is gekozen uit de groep bestaande uit:

c) SEQ ID NOs: 268-289; of

d) aminozuursequenties die 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 269;

en/of

iii) CDR3 is gekozen uit de groep bestaande uit:

e) SEQ ID NOs: 393-415; of

f) aminozuursequenties die 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 397.

10. Het immunoglobuline volgens conclusie 9, waarbij:

i) CDR1 is gekozen uit de groep bestaande uit:

a) SEQ ID NOs: 181-210; of

b) aminozuursequenties die 4, 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 182, waarbij

- op positie 1 de G is veranderd in L, of R;
- op positie 2 de L is veranderd in F, P, of I;

- op positie 3 de L is veranderd in P, of F;
- op positie 4 de F is veranderd in S, L, of I;
- op positie 5 de S is veranderd in I, of R;
- op positie 6 de R is veranderd in C, A, P, V, of L;
- op positie 7 de N is veranderd in H, P, I, M, Y, T of D:
- op positie 8 de S is veranderd in T, R, of I;
- op positie 9 de A is veranderd in V of T; en/of
- op positie 10 de G is veranderd in S, R, of V;

en/of

ii) CDR2 is gekozen uit de groep bestaande uit:

c) SEQ ID NOs: 268-289; of

d) aminozuursequenties die 4, 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 269, waarbij

- op positie 1 de R is veranderd in G, of C;
- op positie 2 de I is veranderd in V, T, S of L;
- op positie 3 de R is veranderd in G, of L;
- op positie 4 de M is veranderd in S, R, of T;
- op positie 5 de G is veranderd in V, S, of T;
- op positie 7 de S is veranderd in G, C, D, of E; en/of
- op positie 8 de I is veranderd in T, M, of R;

en/of

iii) CDR3 is gekozen uit de groep bestaande uit:

e) SEQ ID NOs: 393-415; of

f) aminozuursequenties die 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 397, waarbij

- op positie 1 de W is veranderd in G;
- op positie 3 de E is veranderd in T, K, G, A, of I;
- op positie 4 de G is veranderd in E, of D;
- op positie 5 de F is veranderd in A, L, V, Y, T, of S;
- op positie 6 de Y is veranderd in F, of D:
- op positie 7 de E is veranderd in G, of K;

- op positie 8 de Y is veranderd in S of H; en/of
- op positie 9 de W is veranderd in S, G of C.

11. Het immunoglobuline volgens een van de conclusies 1-6 of een van de conclusies 9-11, waarbij het genoemde immunoglobuline is gekozen uit de groep van eiwitten, waarbij:

- CDR1 is SEQ ID NO: 182, CDR2 is SEQ ID NO: 269, en CDR3 is SEQ ID NO: 397;
- CDR1 is SEQ ID NO: 182, CDR2 is SEQ ID NO: 269, en CDR3 is SEQ ID NO: 394;
- CDR1 is SEQ ID NO: 185, CDR2 is SEQ ID NO: 271, en CDR3 is SEQ ID NO: 398;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, en CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, en CDR3 is SEQ ID NO: 395;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 268, en CDR3 is SEQ ID NO: 396;
- CDR1 is SEQ ID NO: 181, CDR2 is SEQ ID NO: 270, en CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 183, CDR2 is SEQ ID NO: 268, en CDR3 is SEQ ID NO: 393;
- CDR1 is SEQ ID NO: 184, CDR2 is SEQ ID NO: 268, en CDR3 is SEQ ID NO: 393; en
- CDR1 is SEQ ID NO: 185, CDR2 is SEQ ID NO: 271, en CDR3 is SEQ ID NO: 398.

12. Het immunoglobuline volgens een van de conclusies 1-3, dat bestaat uit 4 raamwerkregio's, respectievelijk FR1 tot FR4, en 3 complementariteit bepalende regio's, respectievelijk CDR1 tot CDR3, waarbij:

i) CDR1 is gekozen uit de groep bestaande uit:

a) SEQ ID NOs: 211-227; of

b) aminozuursequenties die 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 214;

en/of

ii) CDR2 is gekozen uit de groep bestaande uit:

c) SEQ ID NOs: 290-309; of

d) aminozuursequenties die 4, 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 303;

en/of

iii) CDR3 is gekozen uit de groep bestaande uit:

e) SEQ ID NOs: 416-435; of

f) aminozuursequenties die 4, 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 422.

13. Het immunoglobuline volgens conclusie 12, waarbij:

i) CDR1 is gekozen uit de groep bestaande uit:

a) SEQ ID NOs: 211-227; of

b) aminozuursequenties die 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 214, waarbij

- op positie 1 de G is veranderd in R, A, V, S, of K;
- op positie 3 de T is veranderd in N;
- op positie 4 de F is veranderd in L;
- op positie 6 de N is veranderd in S;
- op positie 7 de F is veranderd in Y:
- op positie 8 de G is veranderd in A; en/of
- op positie 9 de M is veranderd in V;

en/of

ii) CDR2 is gekozen uit de groep bestaande uit:

c) SEQ ID NOs: 290-309; of

d) aminozuursequenties die 4, 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 303, waarbij

- op positie 1 de A is veranderd in T;
- op positie 2 de I is veranderd in V;
- op positie 5 de T is veranderd in S, of A;
- op positie 6 de G is veranderd in N, of A;
- op positie 7 de G is veranderd in S, of R;
- op positie 8 de H is veranderd in R, of Y;
- op positie 9 de T is veranderd in I, of K; en/of
- op positie 10 de Y is veranderd in F;

en/of

iii) CDR3 is gekozen uit de groep bestaande uit:

e) SEQ ID NOs: 416-435; of

f) aminozuursequenties die 4, 3, 2, of 1 aminozuur residues verschillen met de aminozuursequentie van SEQ ID NO: 422, waarbij

- op positie 4 de F is veranderd in Y, of S;
- op positie 5 de G is veranderd in D;
- op positie 6 de D is veranderd in G;
- op positie 7 de G is veranderd in D;
- op positie 8 de T is veranderd in A:
- op positie 9 de Y is veranderd in S;
- op positie 10 de Y is veranderd in F;
- op positie 12 de Q is veranderd in E;
- op positie 14 de A is veranderd in N, T, I, of R; en/of
- op positie 17 de D is veranderd in N, of G.

14. Het immunoglobuline volgens een van de conclusies 1-6 of volgens een van de conclusies 12-13, waarbij het genoemde immunoglobuline is gekozen uit de groep van eiwitten, waarbij:

- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 303, en CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 290, en CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 291, en CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 292, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 293, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 294, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 295, en CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 216, CDR2 is SEQ ID NO: 296, en CDR3 is SEQ ID NO: 419;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 295, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 295, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 297, en CDR3 is SEQ ID NO: 420;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298, en CDR3 is SEQ ID NO: 421;
- CDR1 is SEQ ID NO: 217, CDR2 is SEQ ID NO: 299, en CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 298, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 291, en CDR3 is SEQ ID NO: 423;

- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 300, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 214, CDR2 is SEQ ID NO: 301, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 300, en CDR3 is SEQ ID NO: 424;
- CDR1 is SEQ ID NO: 211, CDR2 is SEQ ID NO: 302, en CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, en CDR3 is SEQ ID NO: 425;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, en CDR3 is SEQ ID NO: 426;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 303, en CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 218, CDR2 is SEQ ID NO: 291, en CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 219, CDR2 is SEQ ID NO: 296, en CDR3 is SEQ ID NO: 427;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 304, en CDR3 is SEQ ID NO: 428;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 305, en CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 303, en CDR3 is SEQ ID NO: 421;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 296, en CDR3 is SEQ ID NO: 429;
- CDR1 is SEQ ID NO: 221, CDR2 is SEQ ID NO: 305, en CDR3 is SEQ ID NO: 416;
- CDR1 is SEQ ID NO: 222, CDR2 is SEQ ID NO: 305, en CDR3 is SEQ ID NO: 430;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 306, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 223, CDR2 is SEQ ID NO: 303, en CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298 en CDR3 is SEQ ID NO: 431;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 296, en CDR3 is SEQ ID NO: 432;
- CDR1 is SEQ ID NO: 224, CDR2 is SEQ ID NO: 300, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 220, CDR2 is SEQ ID NO: 307, en CDR3 is SEQ ID NO: 433;
- CDR1 is SEQ ID NO: 225, CDR2 is SEQ ID NO: 300, en CDR3 is SEQ ID NO: 418;
- CDR1 is SEQ ID NO: 226, CDR2 is SEQ ID NO: 308, en CDR3 is SEQ ID NO: 434;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 295, en CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 301, en CDR3 is SEQ ID NO: 426;
- CDR1 is SEQ ID NO: 212, CDR2 is SEQ ID NO: 305, en CDR3 is SEQ ID NO: 417;
- CDR1 is SEQ ID NO: 217, CDR2 is SEQ ID NO: 305, en CDR3 is SEQ ID NO: 422;
- CDR1 is SEQ ID NO: 215, CDR2 is SEQ ID NO: 298, en CDR3 is SEQ ID NO: 435; en
- CDR1 is SEQ ID NO: 213, CDR2 is SEQ ID NO: 309, en CDR3 is SEQ ID NO: 418.

15. Het immunoglobuline volgens een van de conclusies 1-14, waarbij het genoemde immunoglobuline bestaat uit een domein antilichaam, een enkel-domein antilichaam, een dAb, een Nanobody, een VHH sequentie, een gehumanizeerde VHH sequentie, een gekamelizeerde VH sequentie, of een VHH sequentie dat werd verkregen door affiniteitsmaturatie.

16. Het immunoglobuline volgens een van de conclusies 1-15, waarbij het genoemde immunoglobuline is gekozen uit de groep bestaande uit SEQ ID NOs: 1-123.

17. Het immunoglobuline volgens een van de conclusies 1-16, waarbij het genoemde Kv1.3 is humaan Kv1.3.

18. Een eiwit dat één of meer immunoglobulines omvat volgens een van de conclusies 1-17.

19. Het eiwit volgens conclusie 18, dat tenminste twee immunoglobulines omvat volgens een van de conclusies 1-17.

20. Het eiwit volgens conclusie 19, waarbij de genoemde twee immunoglobulines hetzelfde of verschillend kunnen zijn.

21. Het eiwit volgens een van de conclusies 18-20, waarbij het eiwit is gekozen uit de groep bestaande uit SEQ ID NOs: 451-473.

22. Een fusie-eiwit of construct dat een immunoglobuline omvat volgens een van de conclusies 1-17, of een eiwit volgens een van de conclusies 18-21, en dat verder één of meer groepen, residues of delen bevat, optioneel verbonden via één of meer linkers.

23. Het fusie-eiwit of construct volgens conclusie 22, dat een verhoogd half-leven heeft in vergelijking met het overeenkomstige immunoglobuline volgens een van de conclusies 1-17 of eiwit volgens een van de conclusies 18-21, per se.

24. Het fusie-eiwit of construct volgens conclusie 23, waarin de genoemde één of meer andere groepen, residues, of delen het eiwit voorzien met een verhoogd half-leven, in vergelijking met het overeenkomstige immunoglobuline volgens een van de conclusies 1-17 of eiwit volgens een van de conclusies 18-21.

25. Het fusie-eiwit of construct volgens een van de conclusies 22-24, waarin het fusie-eiwit of construct is gekozen uit de groep bestaande uit SEQ ID NOs: 461-473.

26. Het immunoglobuline volgens een van de conclusies 1-17, het eiwit volgens een van de conclusies 18-21, of het fusie-eiwit of construct volgens een van de conclusies 22 - 25, voor gebruik in de behandeling of preventie van een Kv1.3 gerelateerde ziekte.

27. Het immunoglobuline, het eiwit, of het fusie-eiwit of construct volgens conclusie 26, waarin de ziekte is gekozen uit multiple sclerose, gewrichtsreuma, diabetes type-1 mellitus, diabetes type-2 mellitus, psoriasis, de ziekte van Crohn (IBD), contactdermatitis, artritis psoriatica, astma, allergie, restenose, systemische sclerodermie, fibrose, sclerodermie, glomerulonefritis, chronische obstructieve longziekte (COPD), het syndroom van Sjögren, Alzheimer, botresorptie, systemische lupus erythematosus, colitis ulcerosa, obesitas, graft-versus-host ziekte, transplantatie afstoting, en vertraagd-type overgevoeligheid.

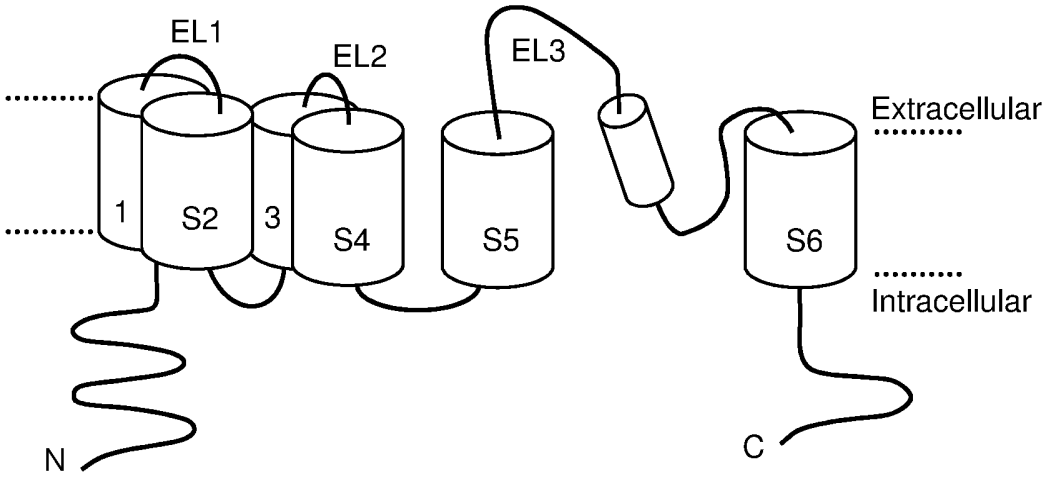


FIG. 1


+40 mV
-80 mV
-100 mV
100 ms

FIG. 2A

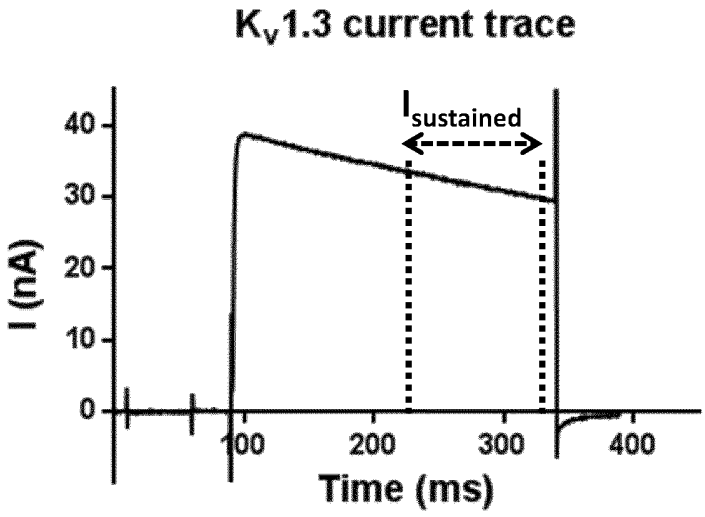

K_V1.3 current trace
$I_{sustained}$
I (nA)
40
30
20
10
0
100
200
300
400
Time (ms)

FIG. 2B

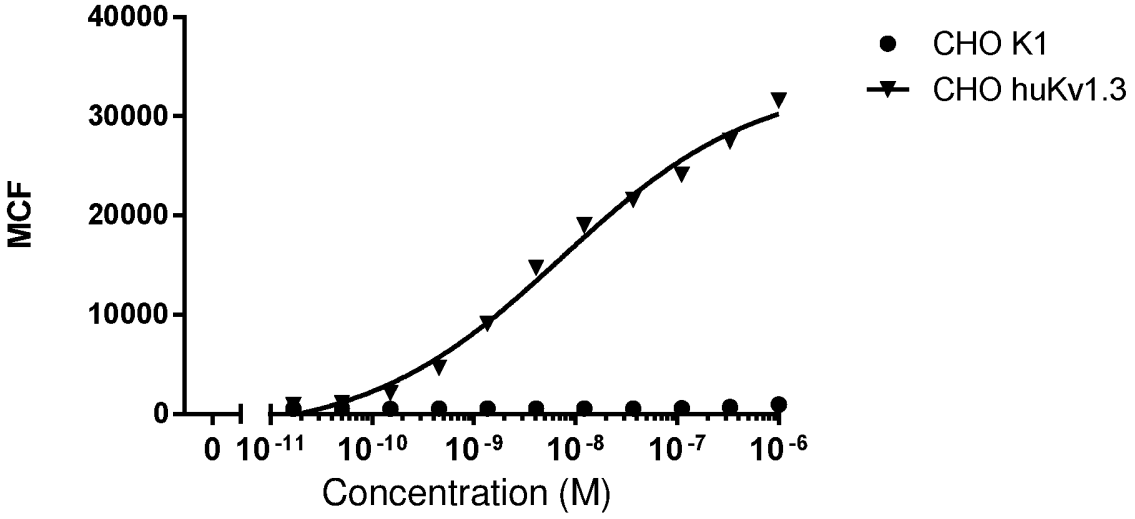

40000
30000
20000
10000
0
MCF
0 10-11 10-10 10-9 10-8 10-7 10-6
Concentration (M)
CHO K1
CHO huKv1.3

FIG. 3A

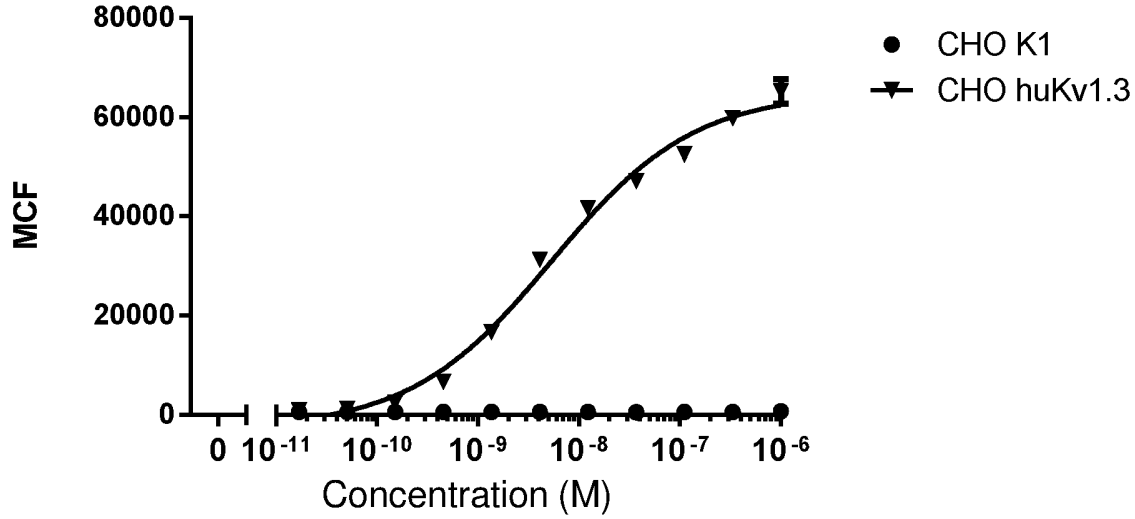

80000
60000
40000
20000
0
MCF
0 10-11 10-10 10-9 10-8 10-7 10-6
Concentration (M)
CHO K1
CHO huKv1.3

FIG. 3B

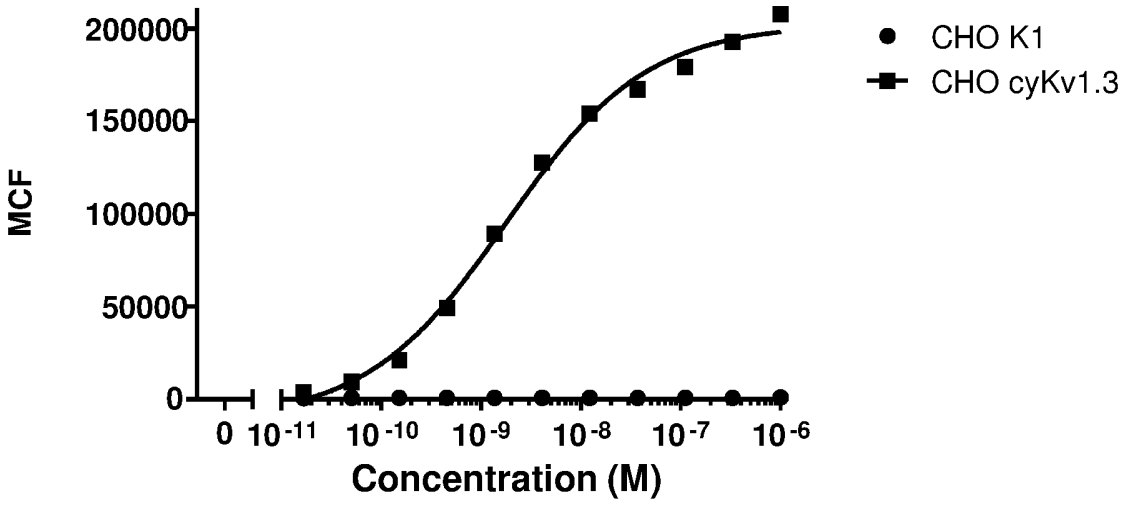

200000
150000
100000
50000
0
MCF
0 10-11 10-10 10-9 10-8 10-7 10-6
Concentration (M)
CHO K1
CHO cyKv1.3

FIG. 3C

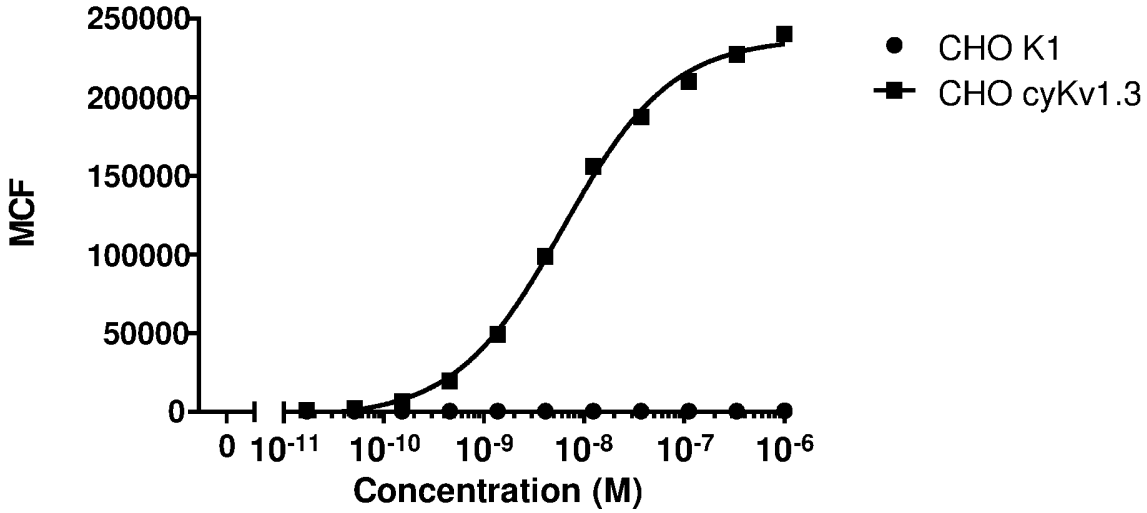

250000
200000
150000
100000
50000
0
MCF
0 10-11 10-10 10-9 10-8 10-7 10-6
Concentration (M)
CHO K1
CHO cyKv1.3

FIG. 3D

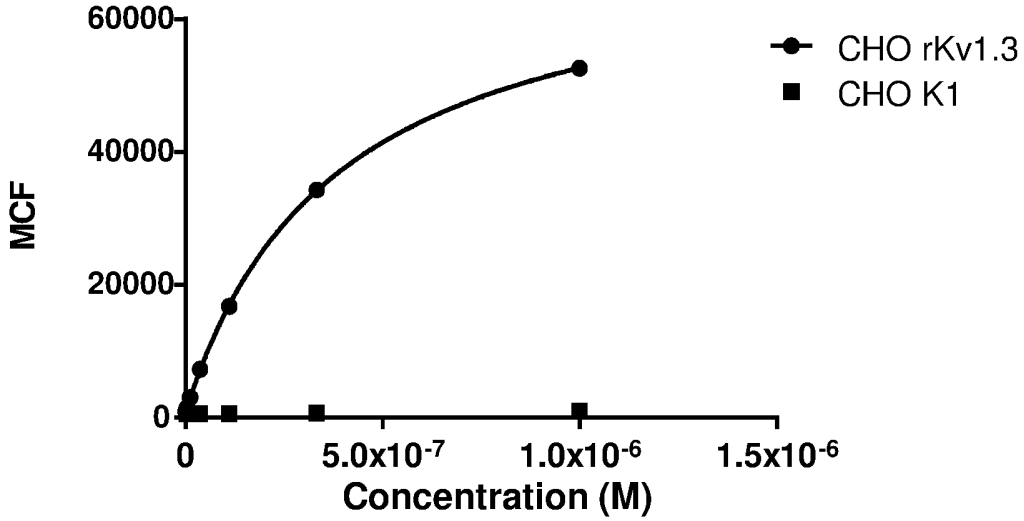

60000
40000
20000
0
MCF
0
5.0x10-7
1.0x10-6
1.5x10-6
Concentration (M)
CHO rKv1.3
CHO K1

FIG. 3E

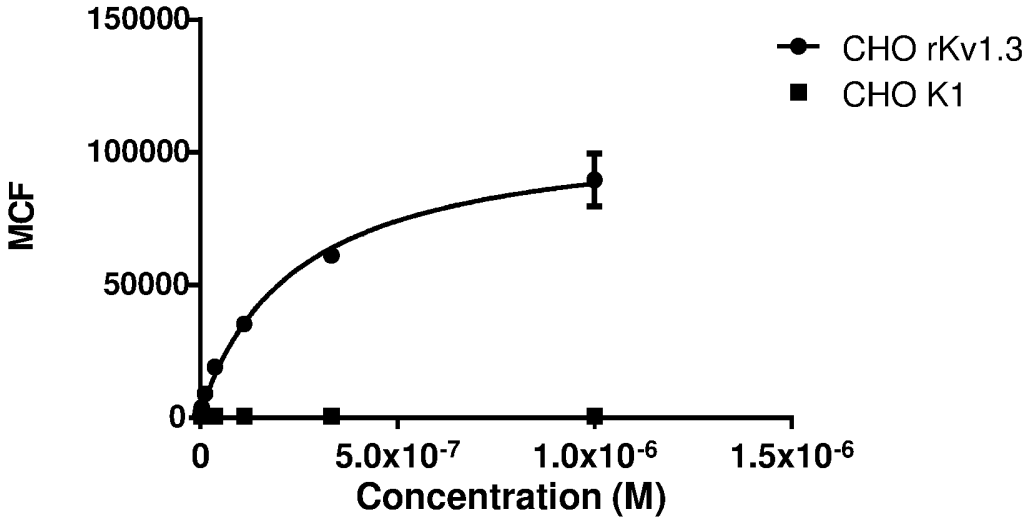

150000
100000
50000
0
MCF
0
5.0x10-7
1.0x10-6
1.5x10-6
Concentration (M)
CHO rKv1.3
CHO K1

FIG. 3F

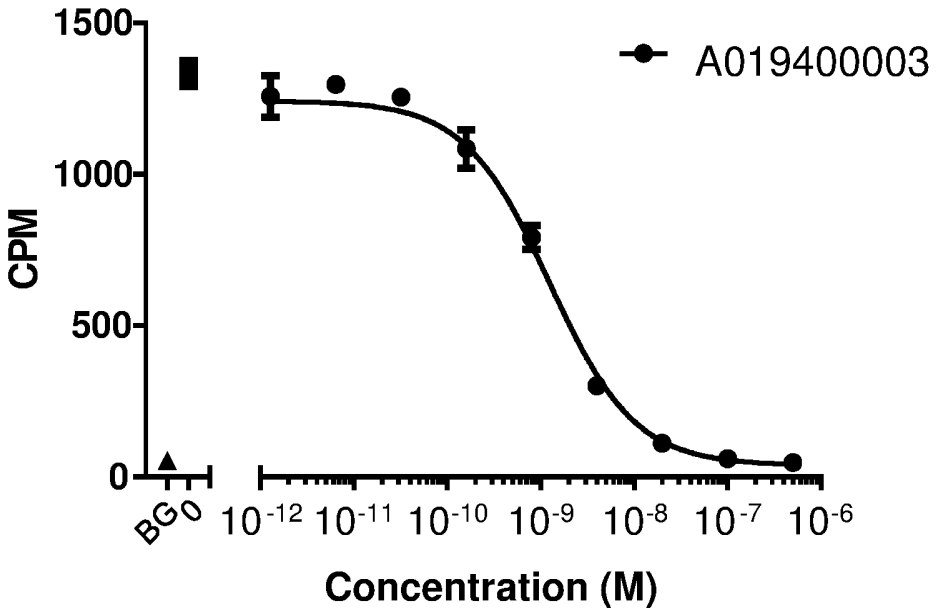

1500
1000
500
0
CPM
A019400003
BG
0
10-12
10-11
10-10
10-9
10-8
10-7
10-6
Concentration (M)

FIG. 4A

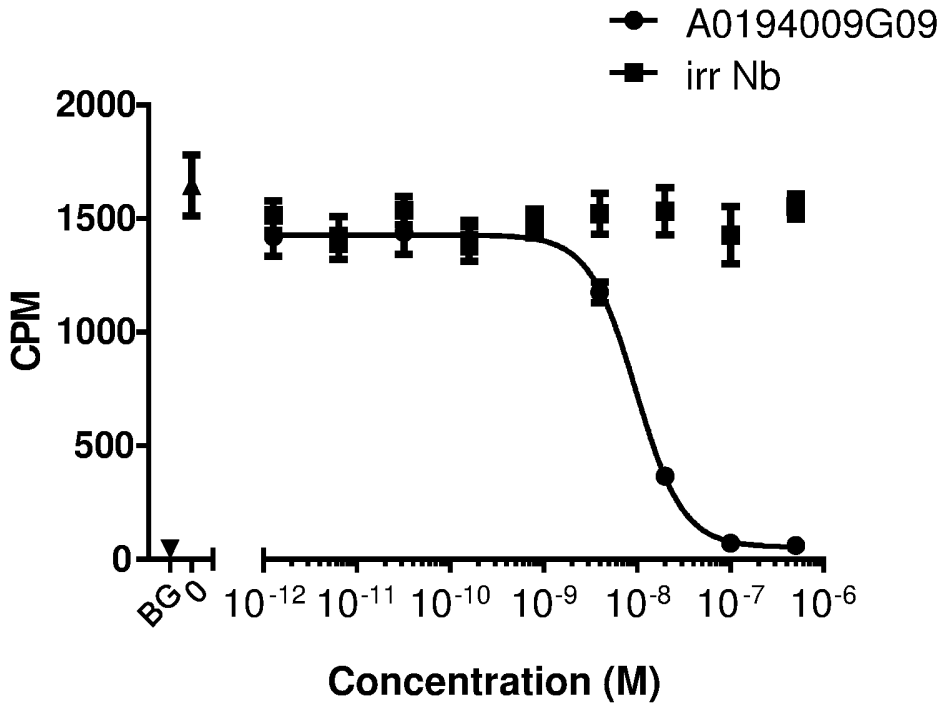

A0194009G09
irr Nb
2000
1500
1000
500
0
CPM
BG
0
10-12
10-11
10-10
10-9
10-8
10-7
10-6
Concentration (M)

FIG. 4B

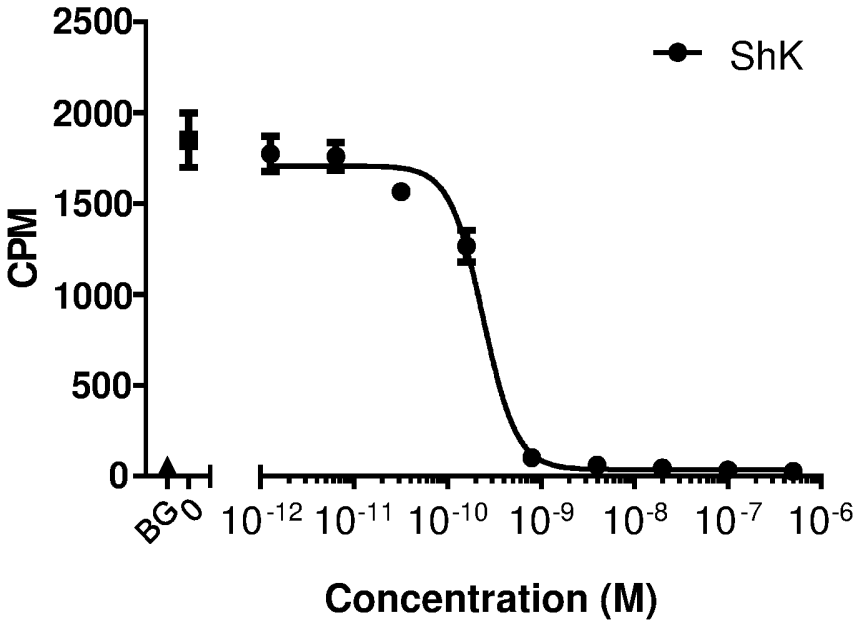

2500
2000
1500
1000
500
0
CPM
ShK
BG
0
10-12
10-11
10-10
10-9
10-8
10-7
10-6
Concentration (M)

FIG. 4C

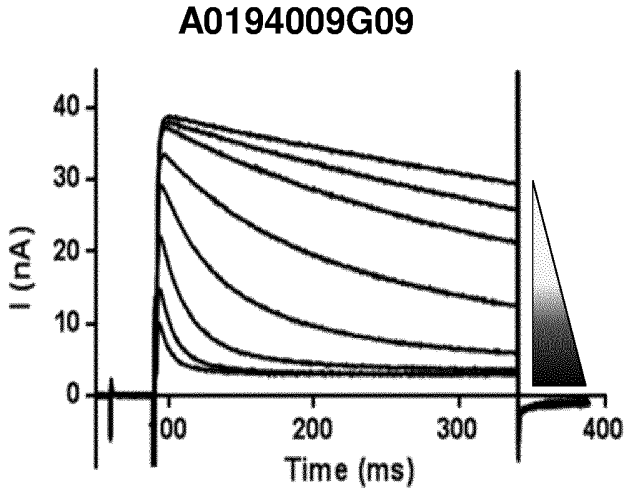

A0194009G09
40
30
20
10
0
I (nA)
100
200
300
400
Time (ms)

FIG. 5A

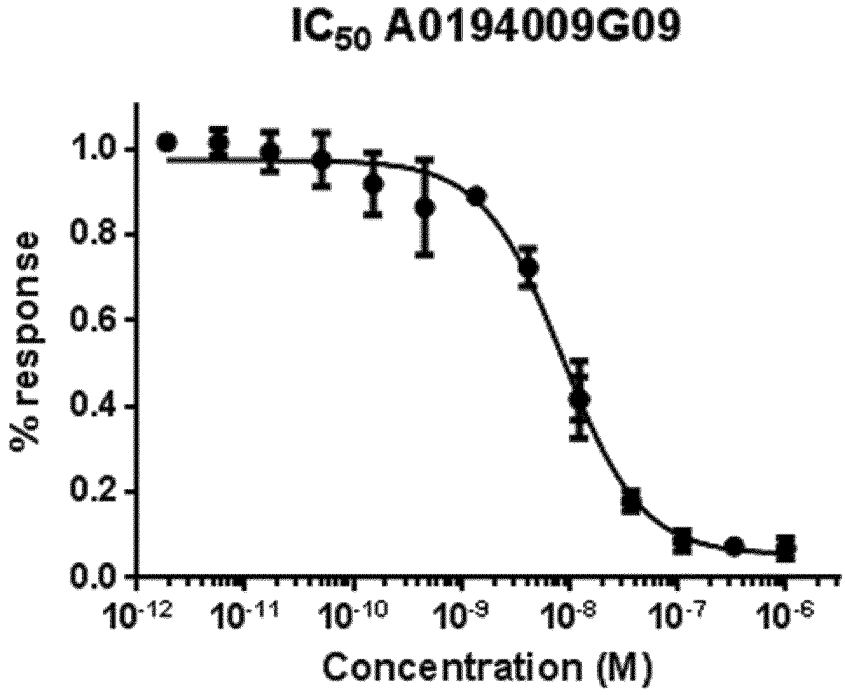

IC50 A0194009G09
1.0
0.8
0.6
0.4
0.2
0.0
% response
10-12
10-11
10-10
10-9
10-8
10-7
10-6
Concentration (M)

FIG. 5B

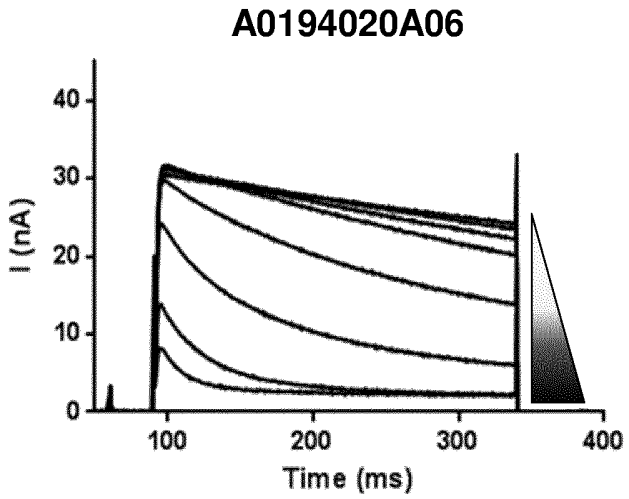

A0194020A06
I (nA)
40
30
20
10
0
100
200
300
400
Time (ms)

FIG. 6A

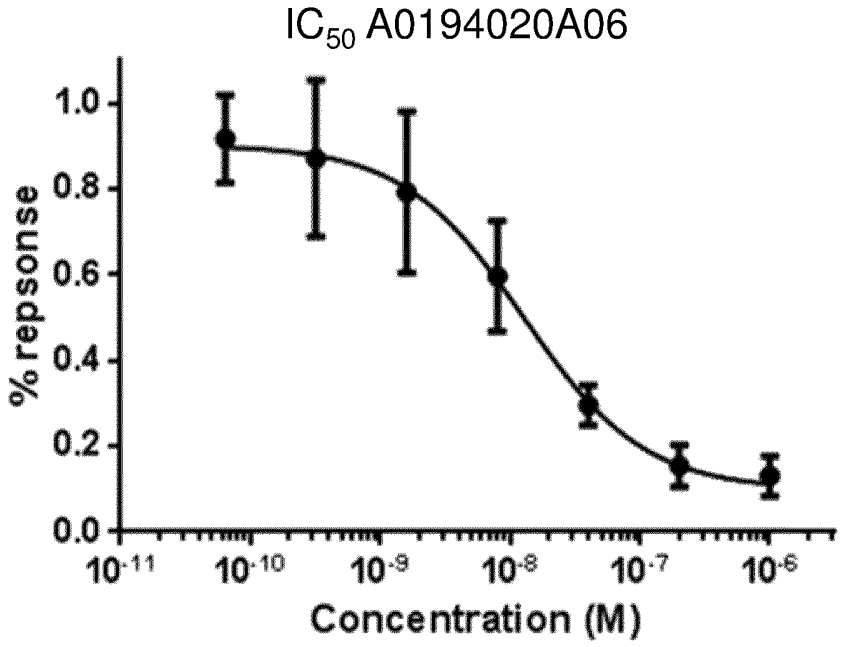

IC50 A0194020A06
% repsonse
1.0
0.8
0.6
0.4
0.2
0.0
10-11
10-10
10-9
10-8
10-7
10-6
Concentration (M)

FIG. 6B

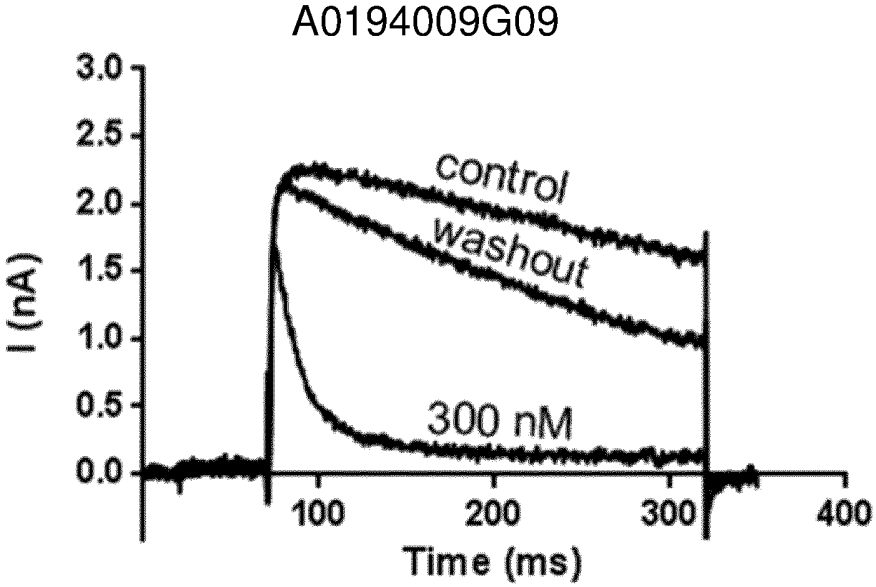

A0194009G09
3.0
2.5
2.0
1.5
1.0
0.5
0.0
I (nA)
control
washout
300 nM
100
200
300
400
Time (ms)

FIG. 7A

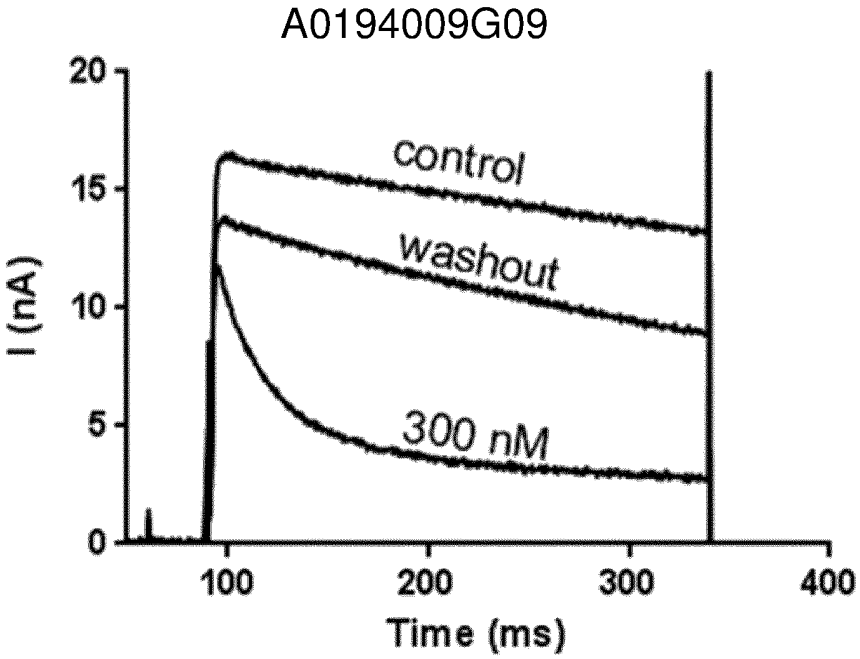

A0194009G09
20
15
10
5
0
I (nA)
control
washout
300 nM
100
200
300
400
Time (ms)

FIG. 7B

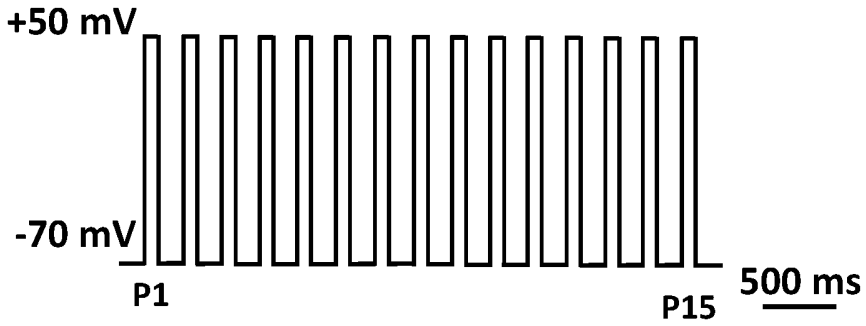

+50 mV
-70 mV
P1
P15
500 ms

FIG. 8A

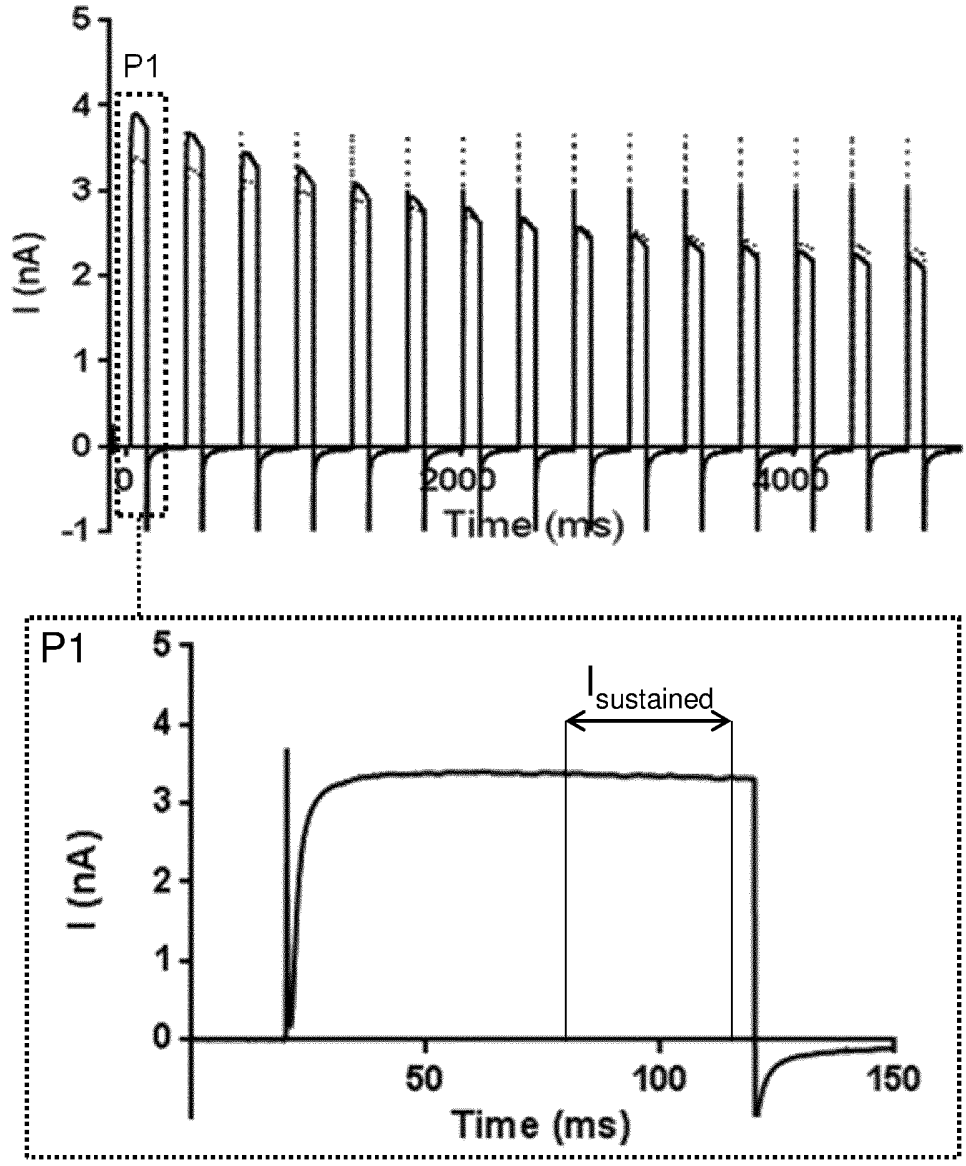

P1
I (nA)
Time (ms)
0
2000
4000
P1
I sustained
I (nA)
Time (ms)
50
100
150

FIG. 8B

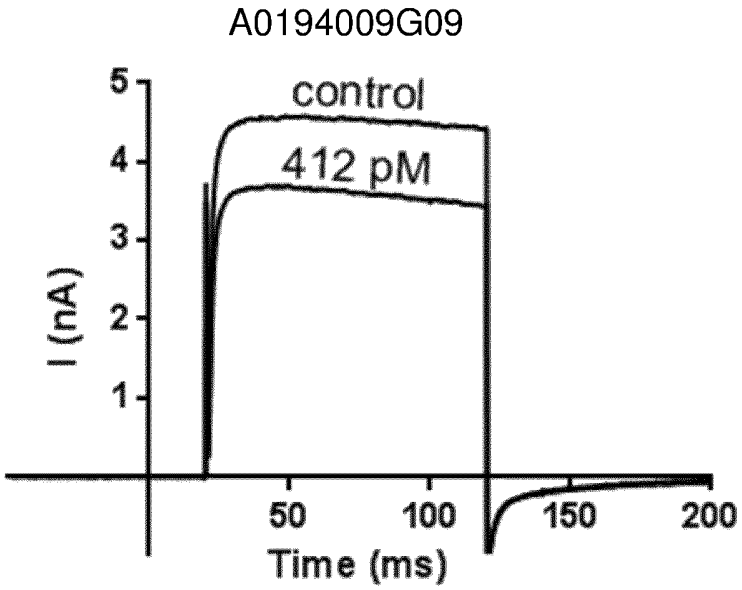

A0194009G09
control
412 pM
I (nA)
5
4
3
2
1
50
100
150
200
Time (ms)

FIG. 9A

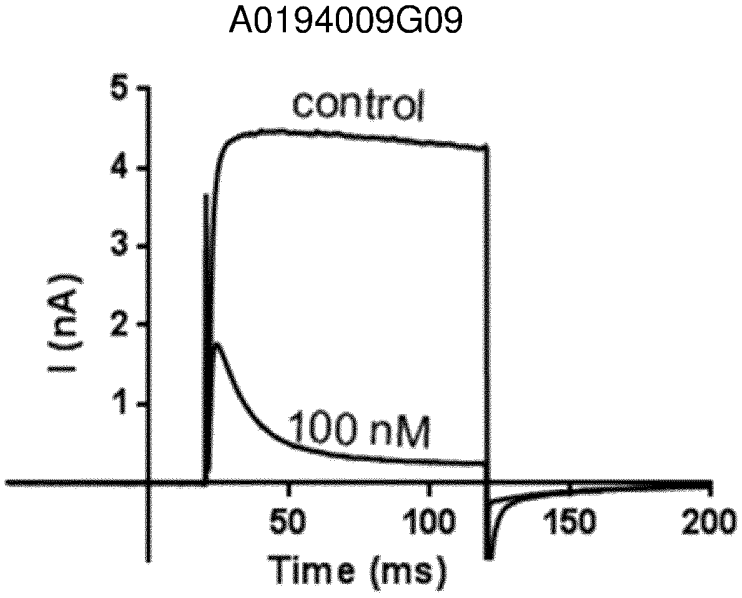

A0194009G09
control
100 nM
I (nA)
5
4
3
2
1
50
100
150
200
Time (ms)

FIG. 9B

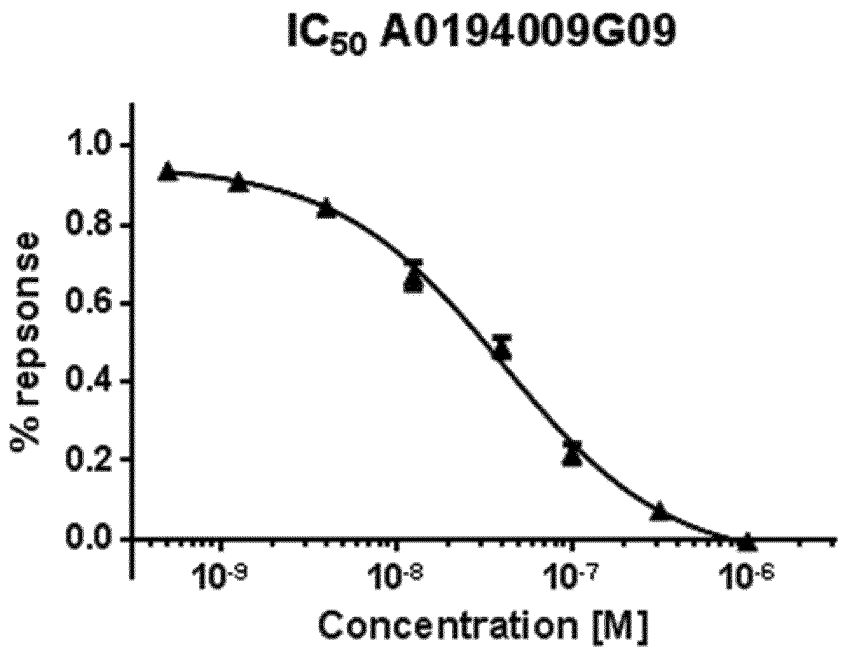

IC50 A0194009G09
% repsonse
1.0
0.8
0.6
0.4
0.2
0.0
10-9
10-8
10-7
10-6
Concentration [M]

FIG. 9C

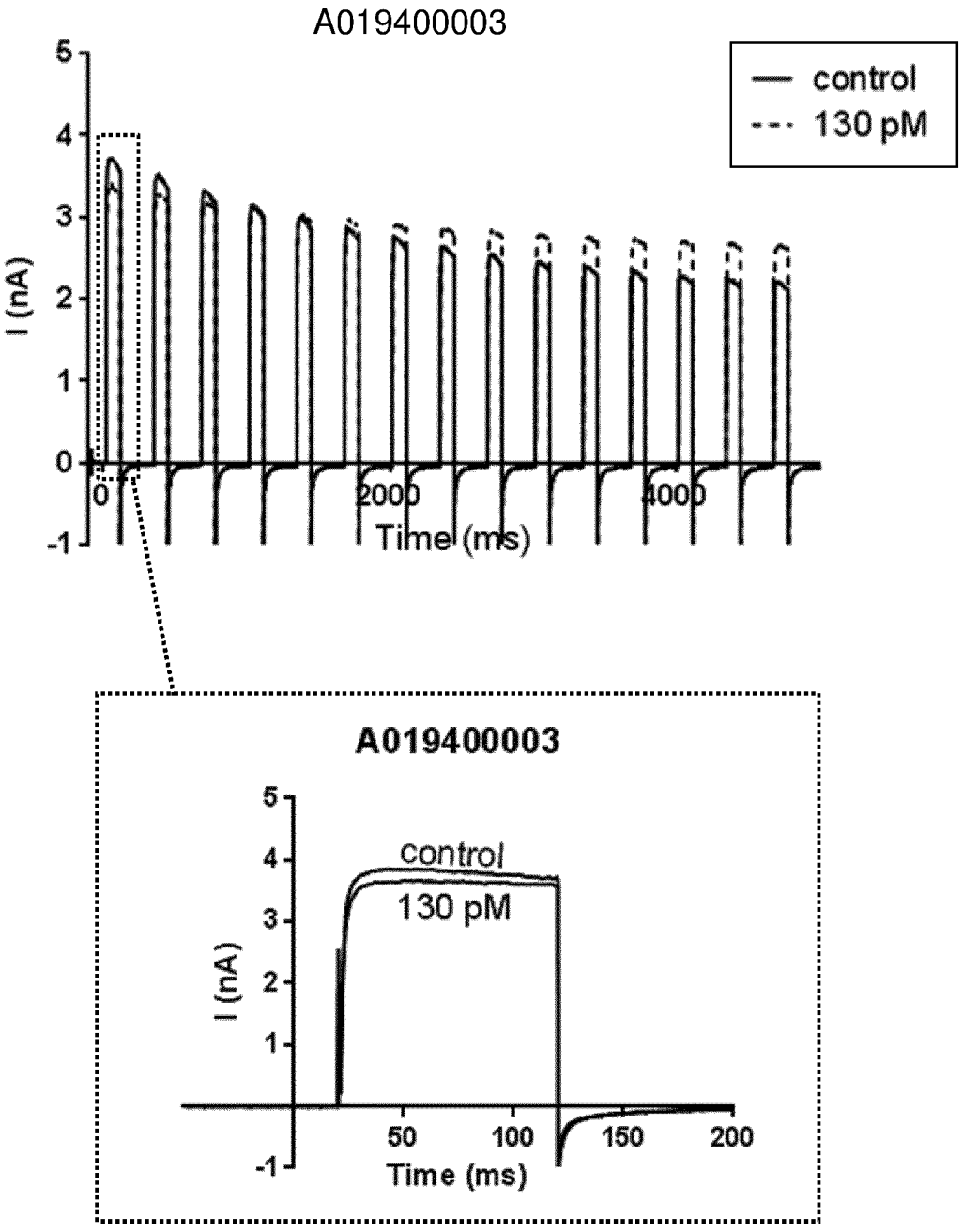

A019400003
control
130 pM
I (nA)
Time (ms)
A019400003
control
130 pM
I (nA)
Time (ms)

FIG. 10A

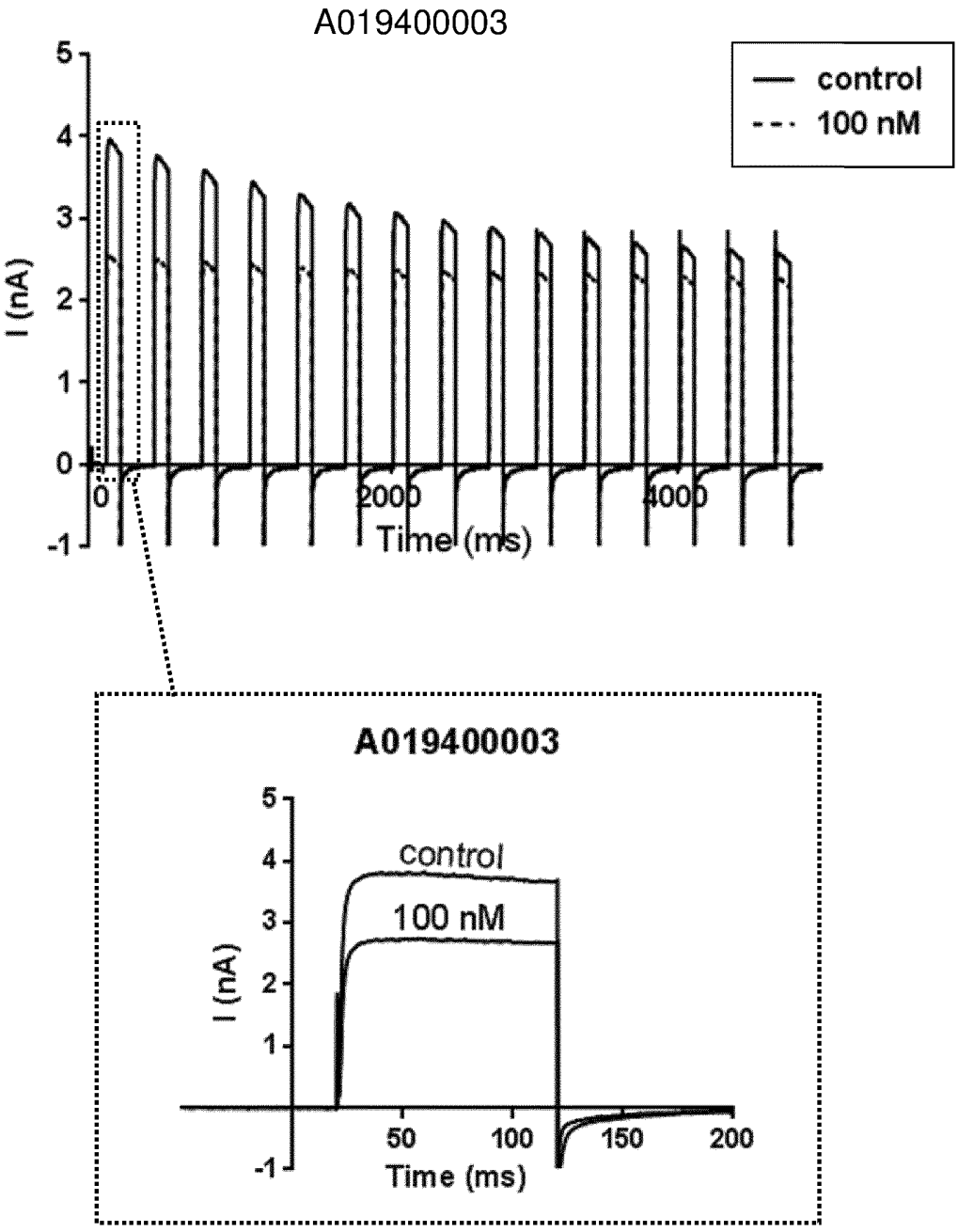

A019400003
control
100 nM
I (nA)
Time (ms)
0
2000
4000
A019400003
control
100 nM
I (nA)
Time (ms)
50
100
150
200

FIG. 10B

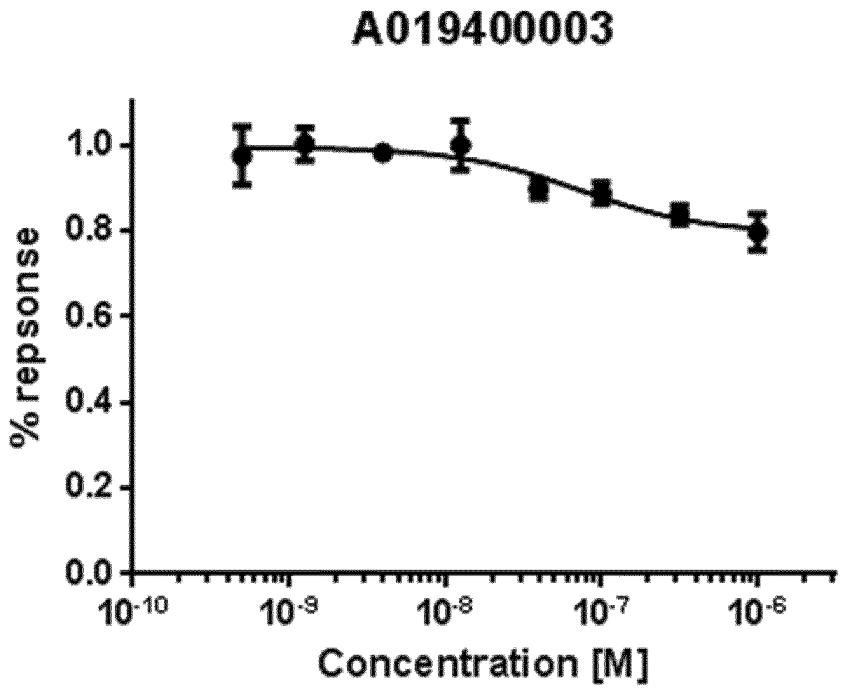

A019400003
% repsonse
1.0
0.8
0.6
0.4
0.2
0.0
10-10
10-9
10-8
10-7
10-6
Concentration [M]

FIG. 10C

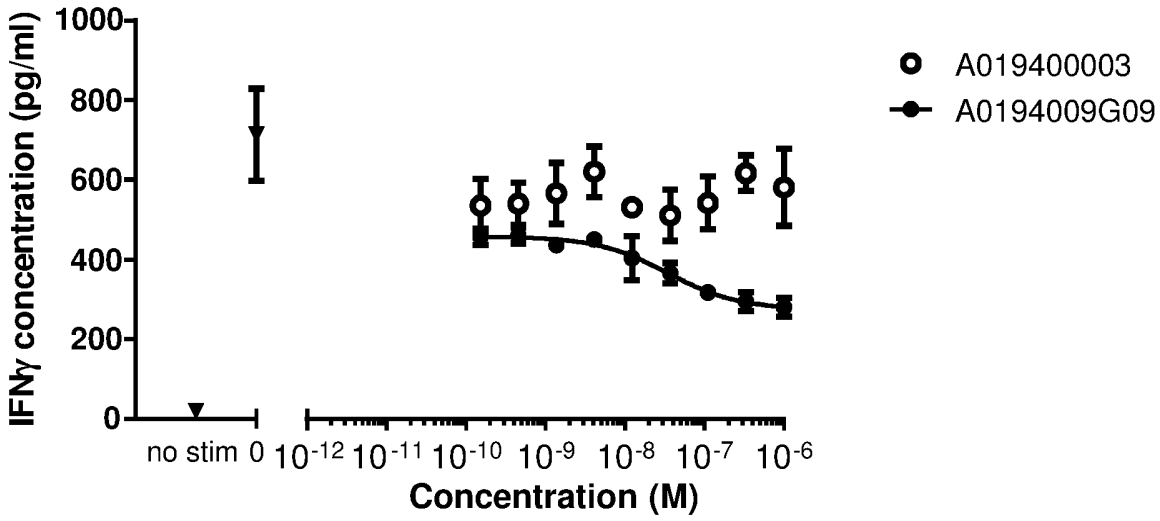


FIG. 11A

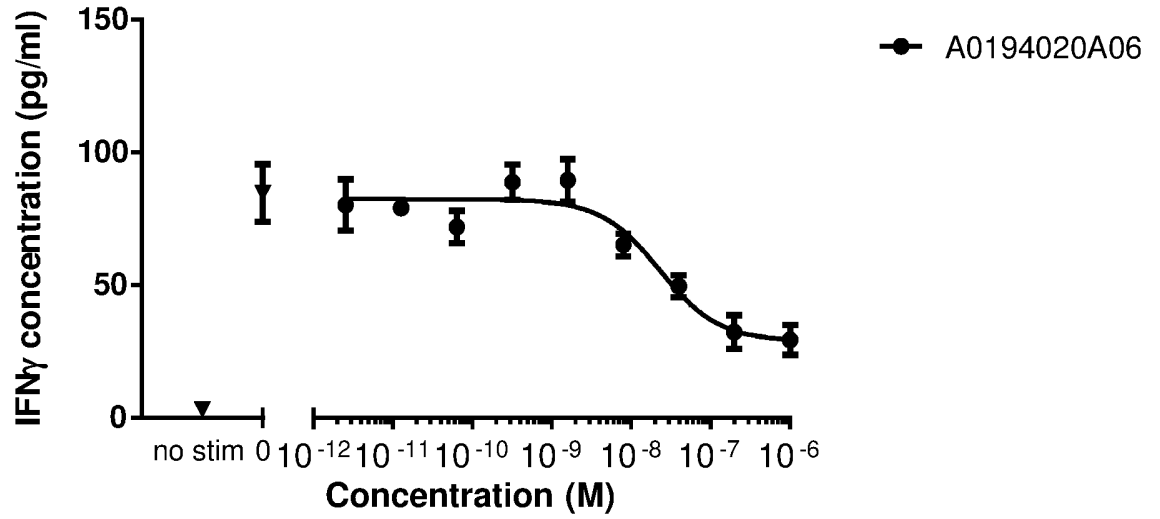


FIG. 11B

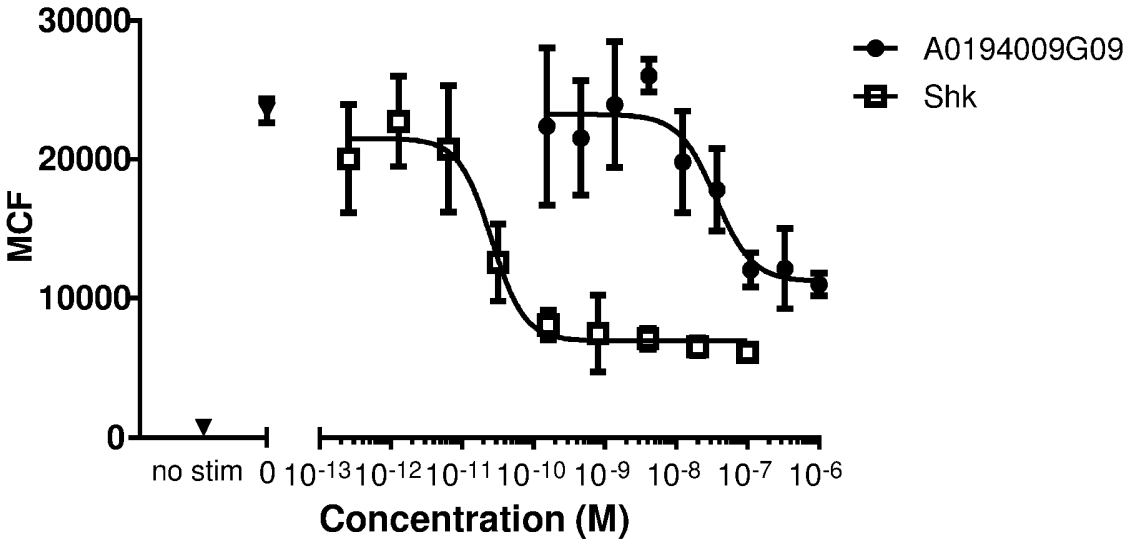


FIG. 11C

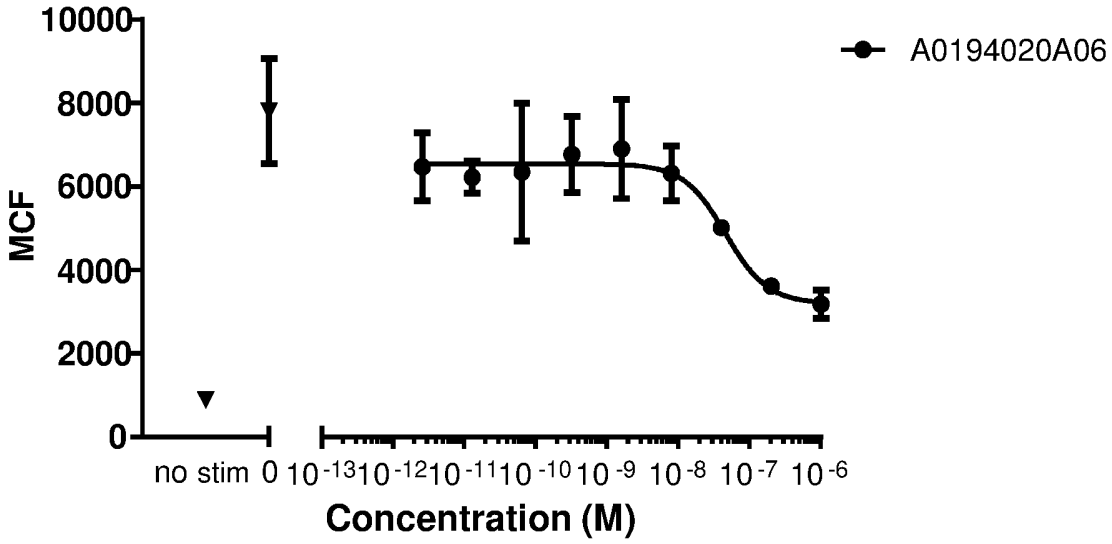


FIG. 11D

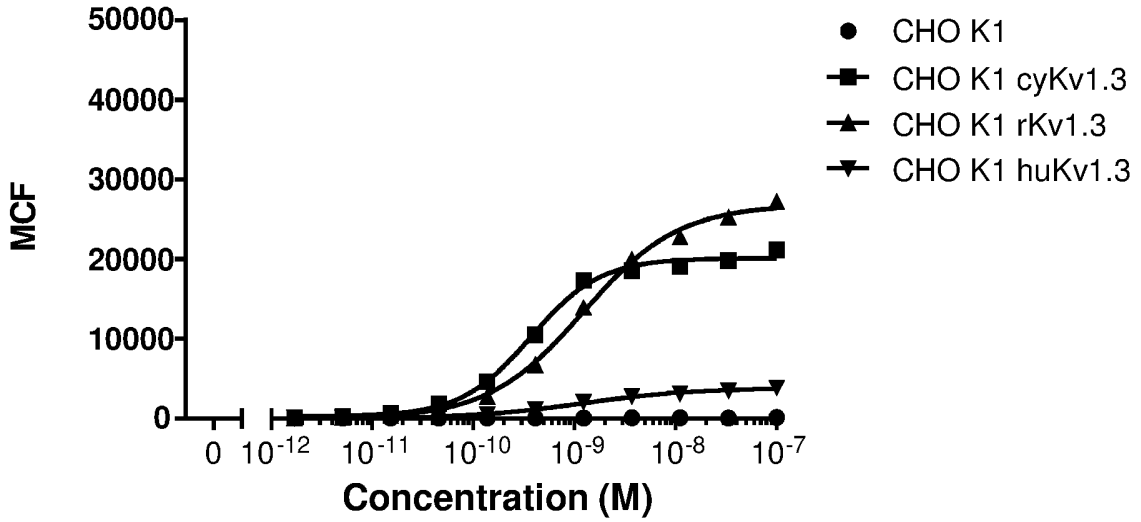


FIG. 12A

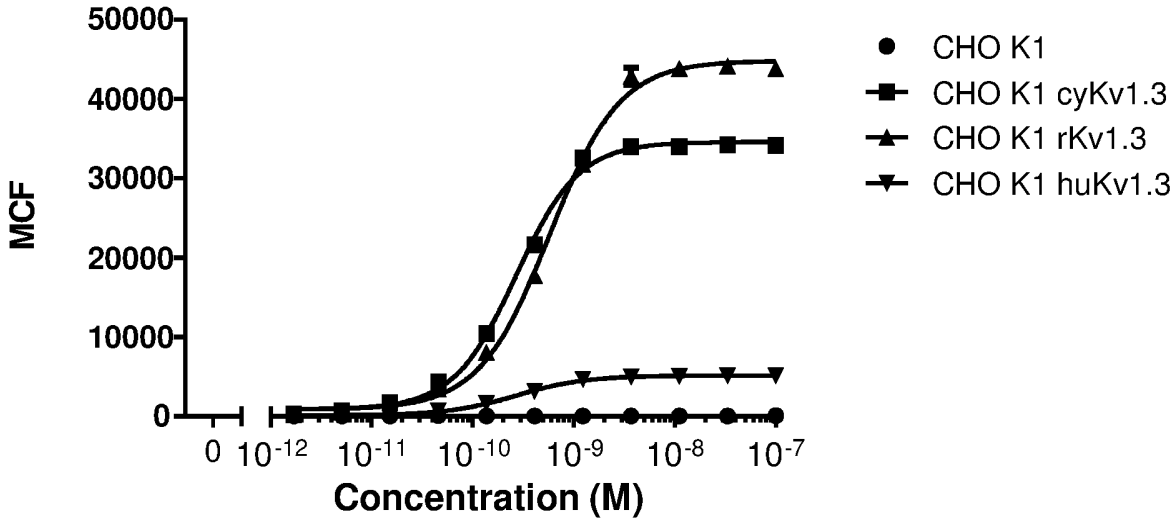


FIG. 12B

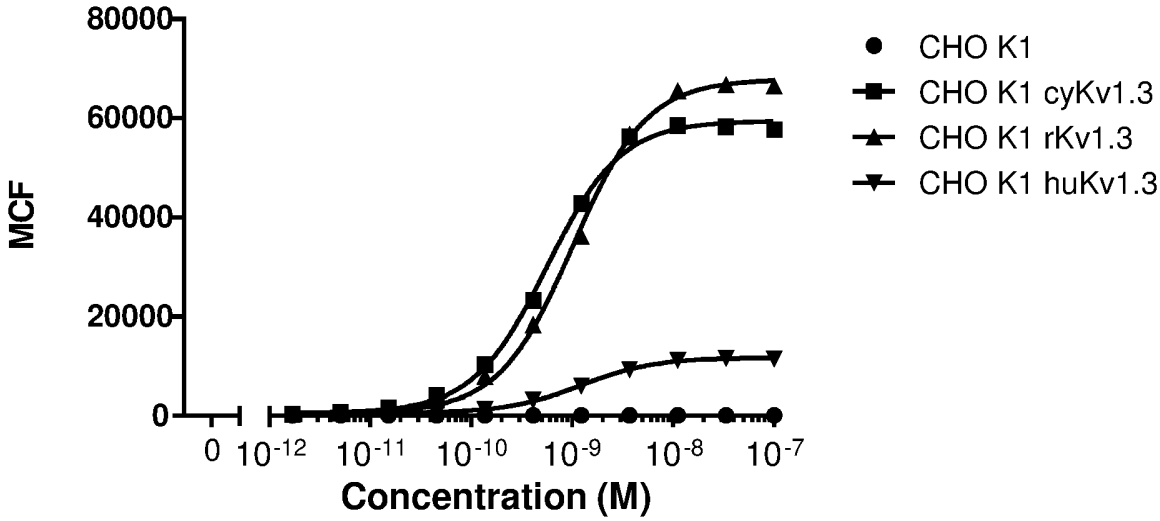


FIG. 12C

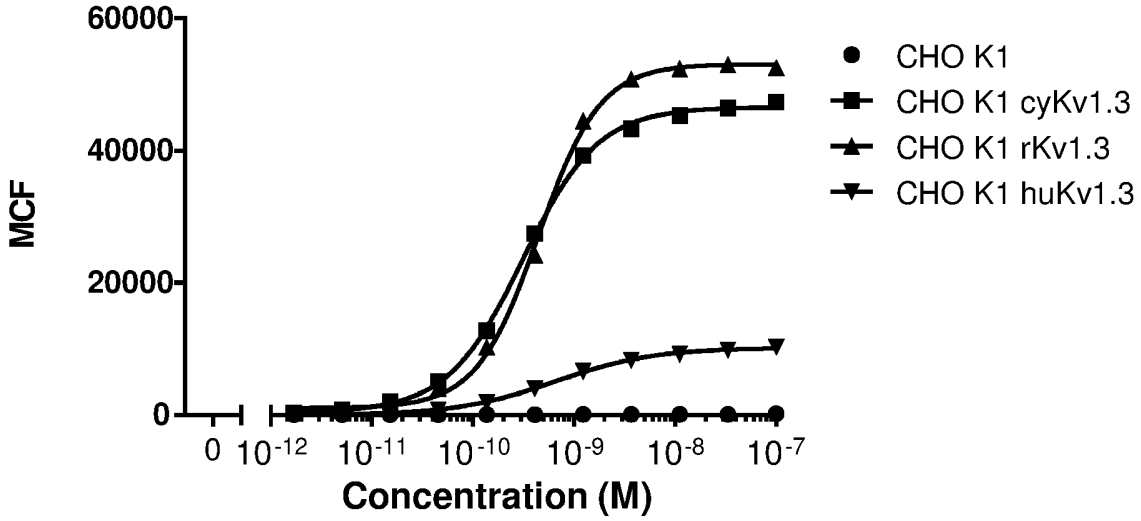


FIG. 12D

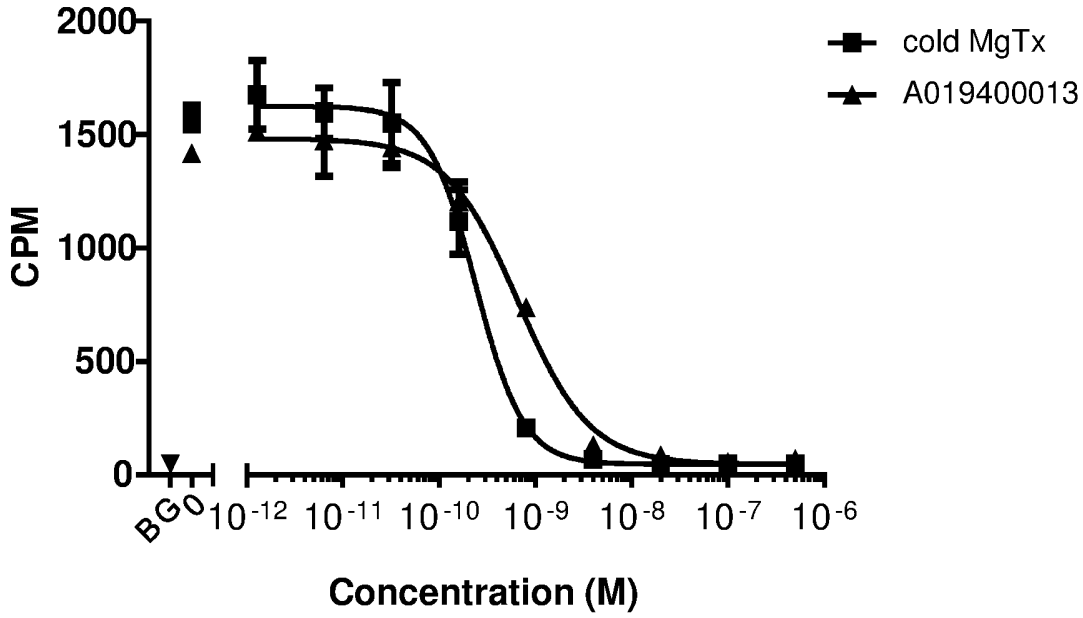


FIG. 13A

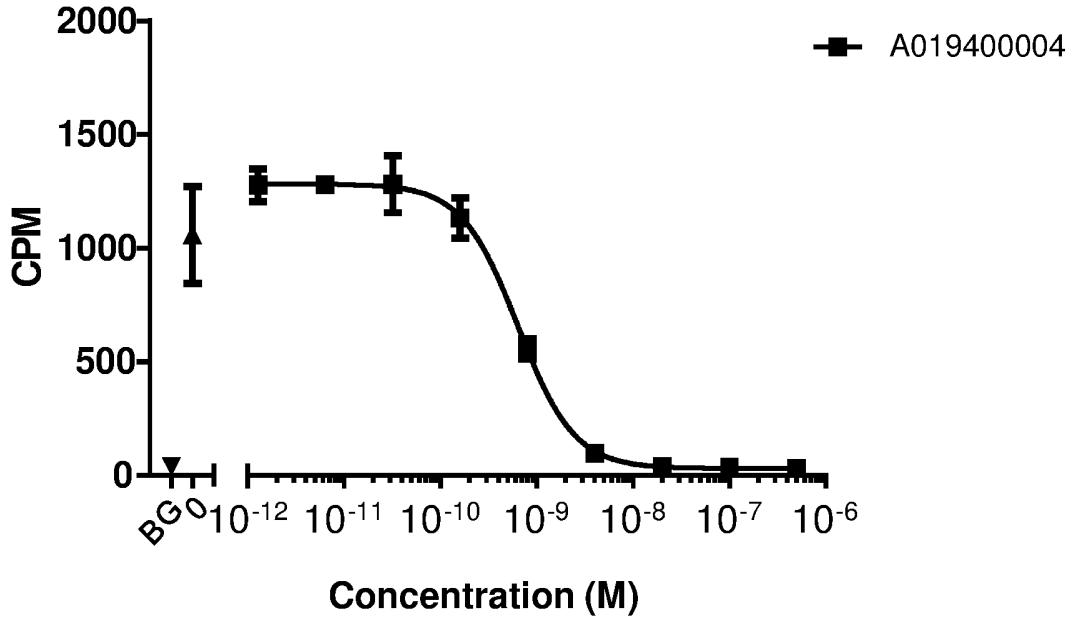


FIG. 13B

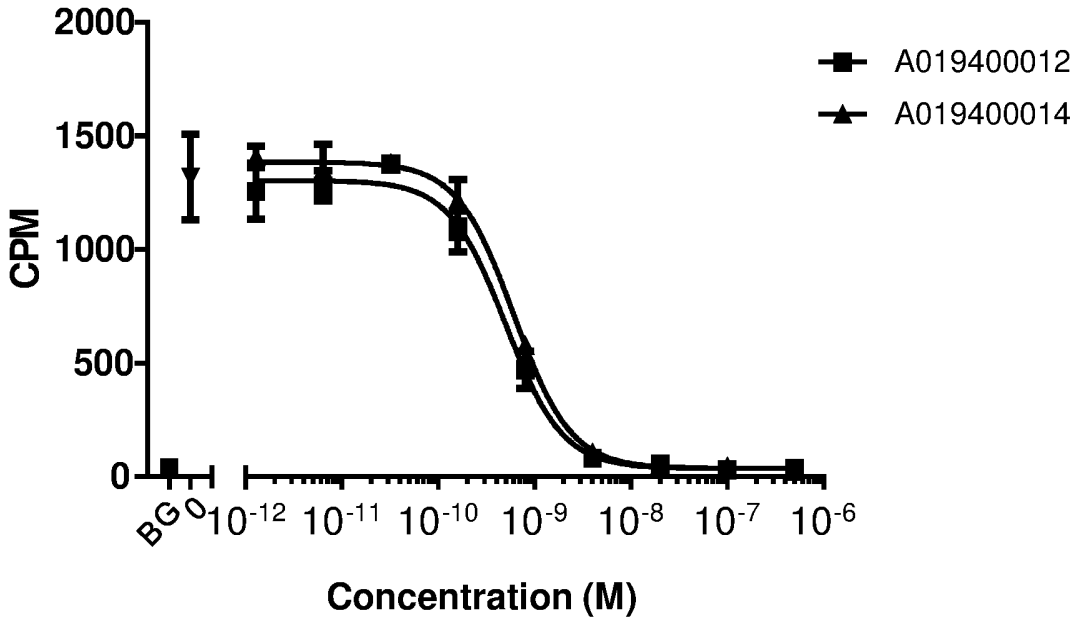


FIG. 13C

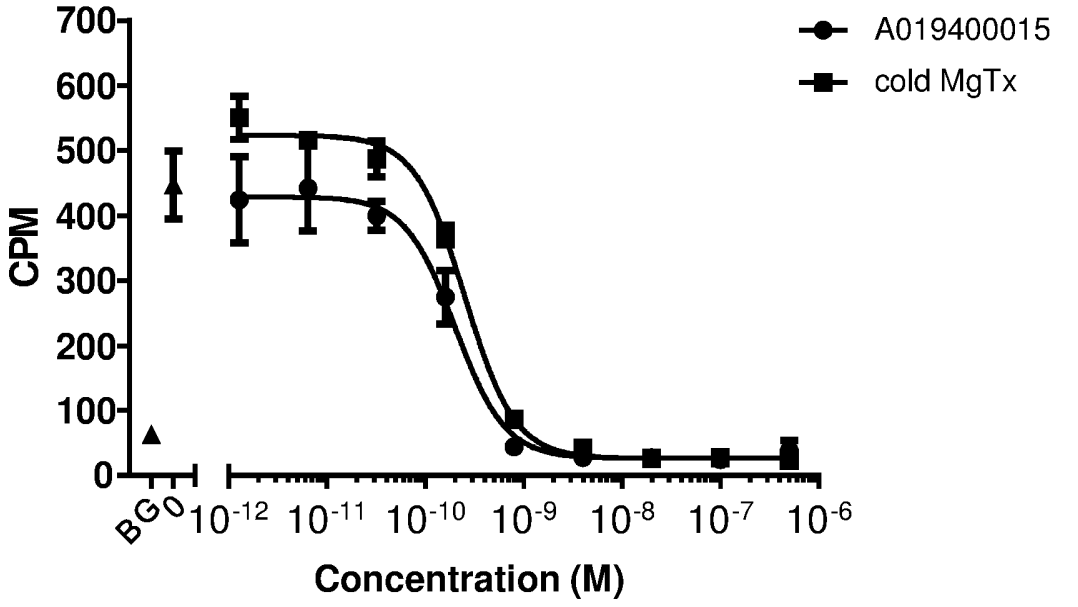


FIG. 13D

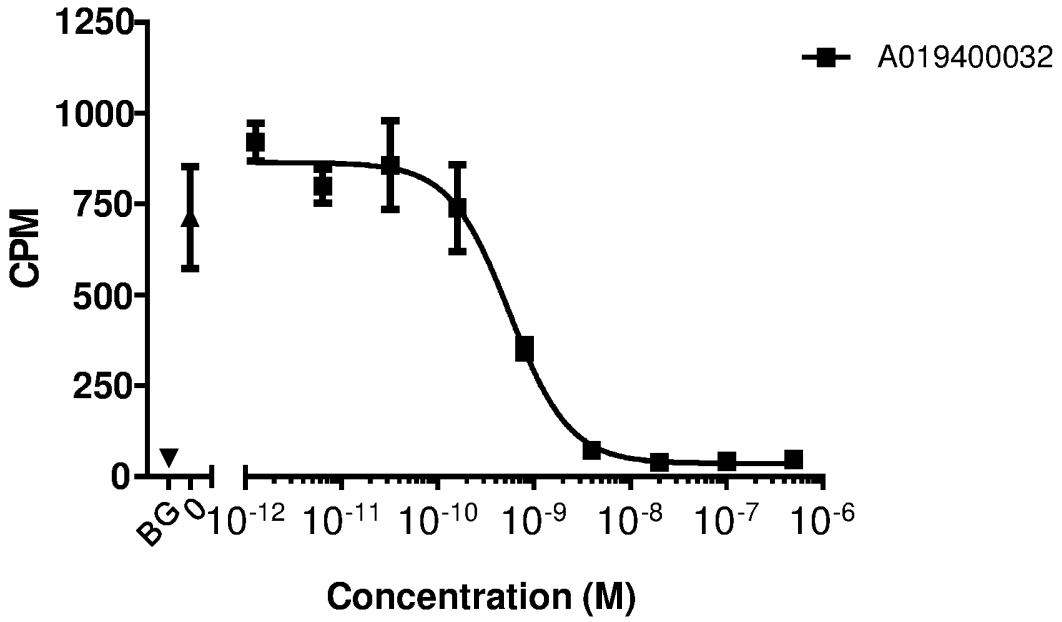

1250
1000
750
500
250
0
CPM
A019400032
BG
0
10-12
10-11
10-10
10-9
10-8
10-7
10-6
Concentration (M)

FIG. 13E

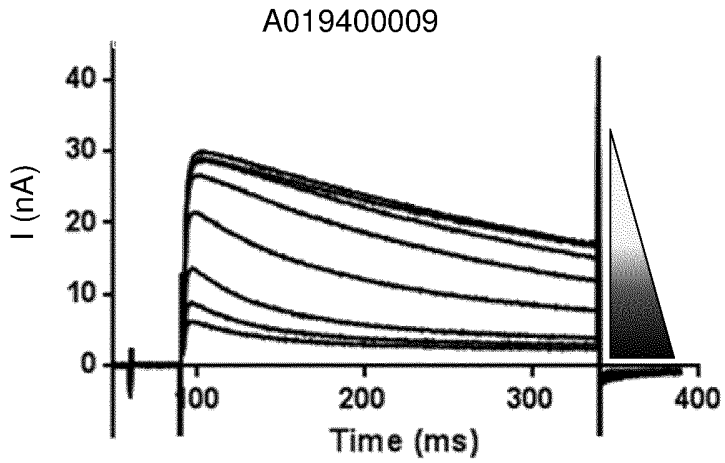


FIG. 14A

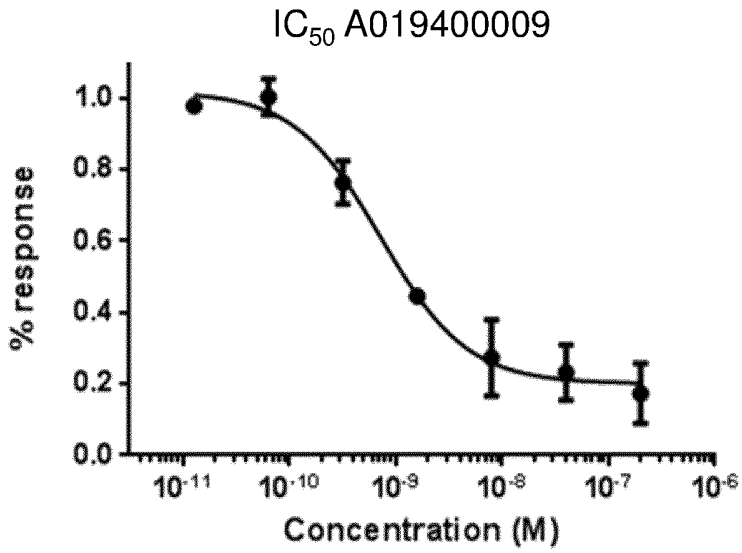


FIG. 14B

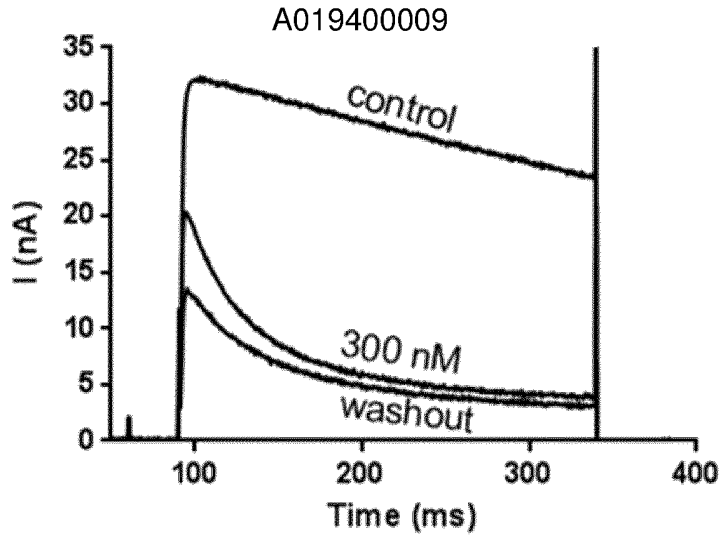


FIG. 14C

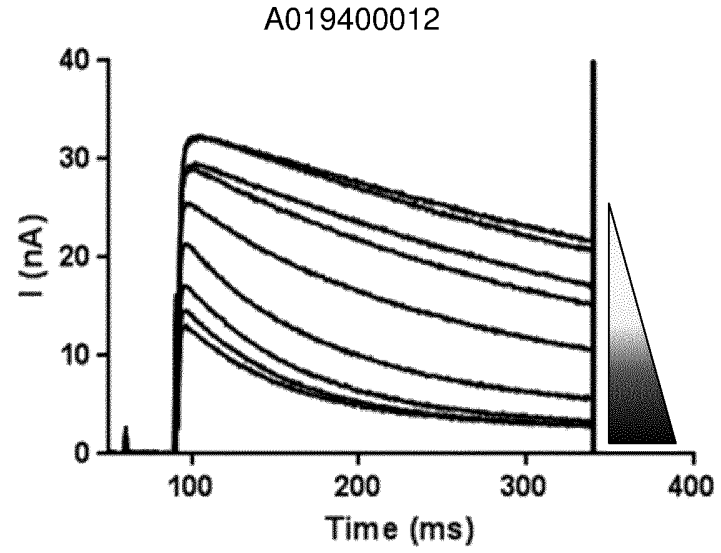


FIG. 15A

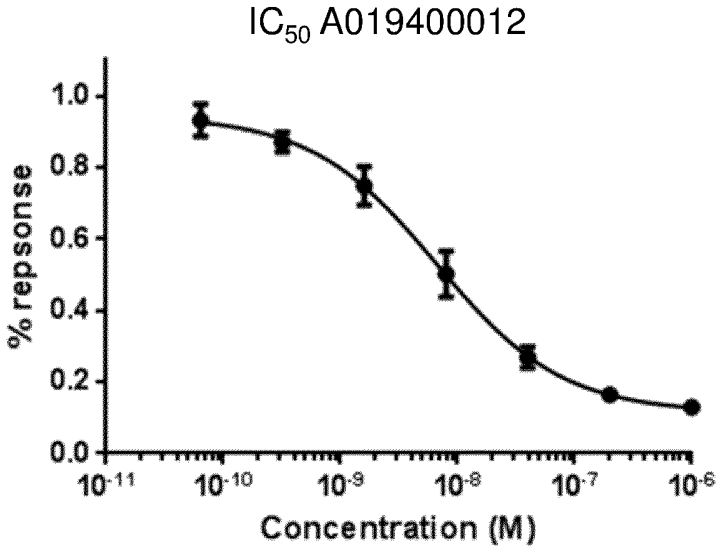


FIG. 15B

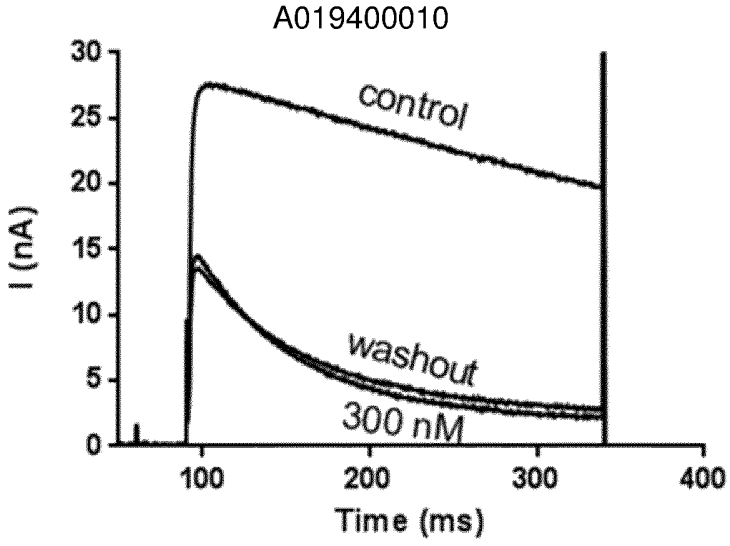


FIG. 15C

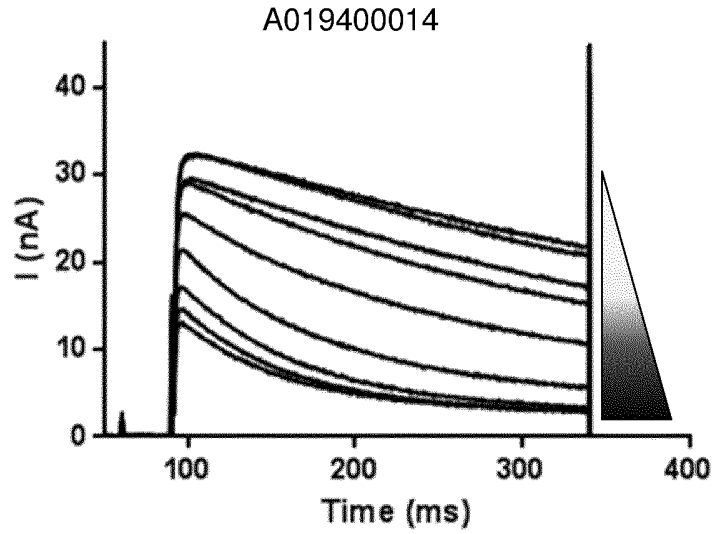


FIG. 16A

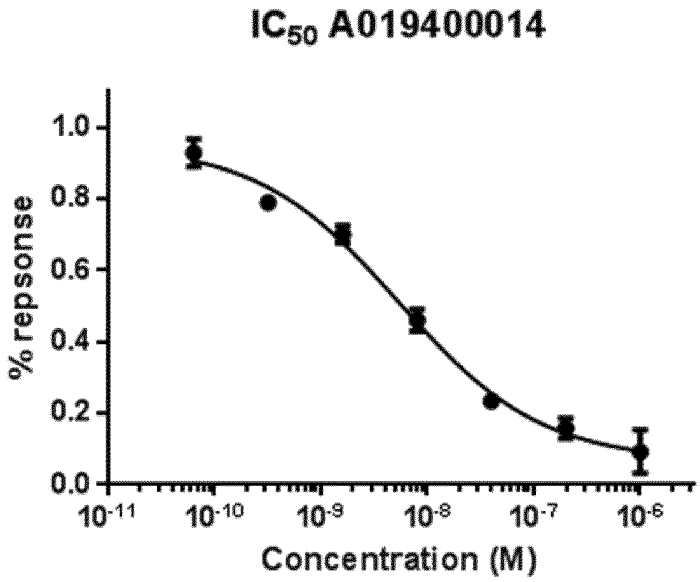


FIG. 16B

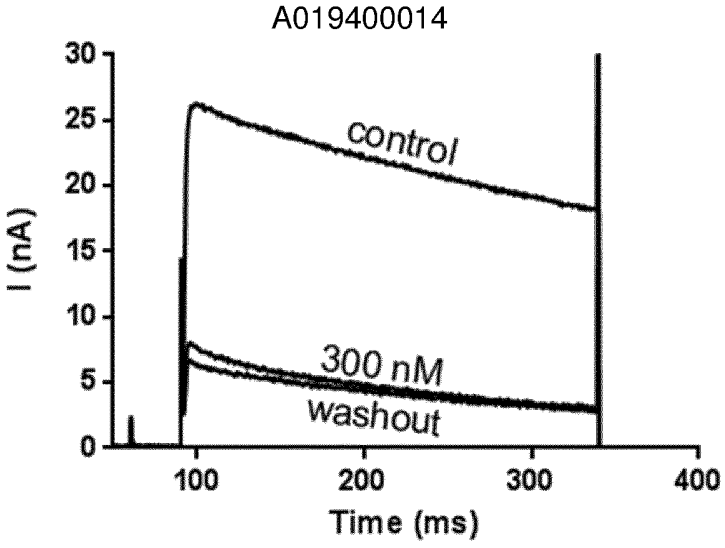


FIG. 16C

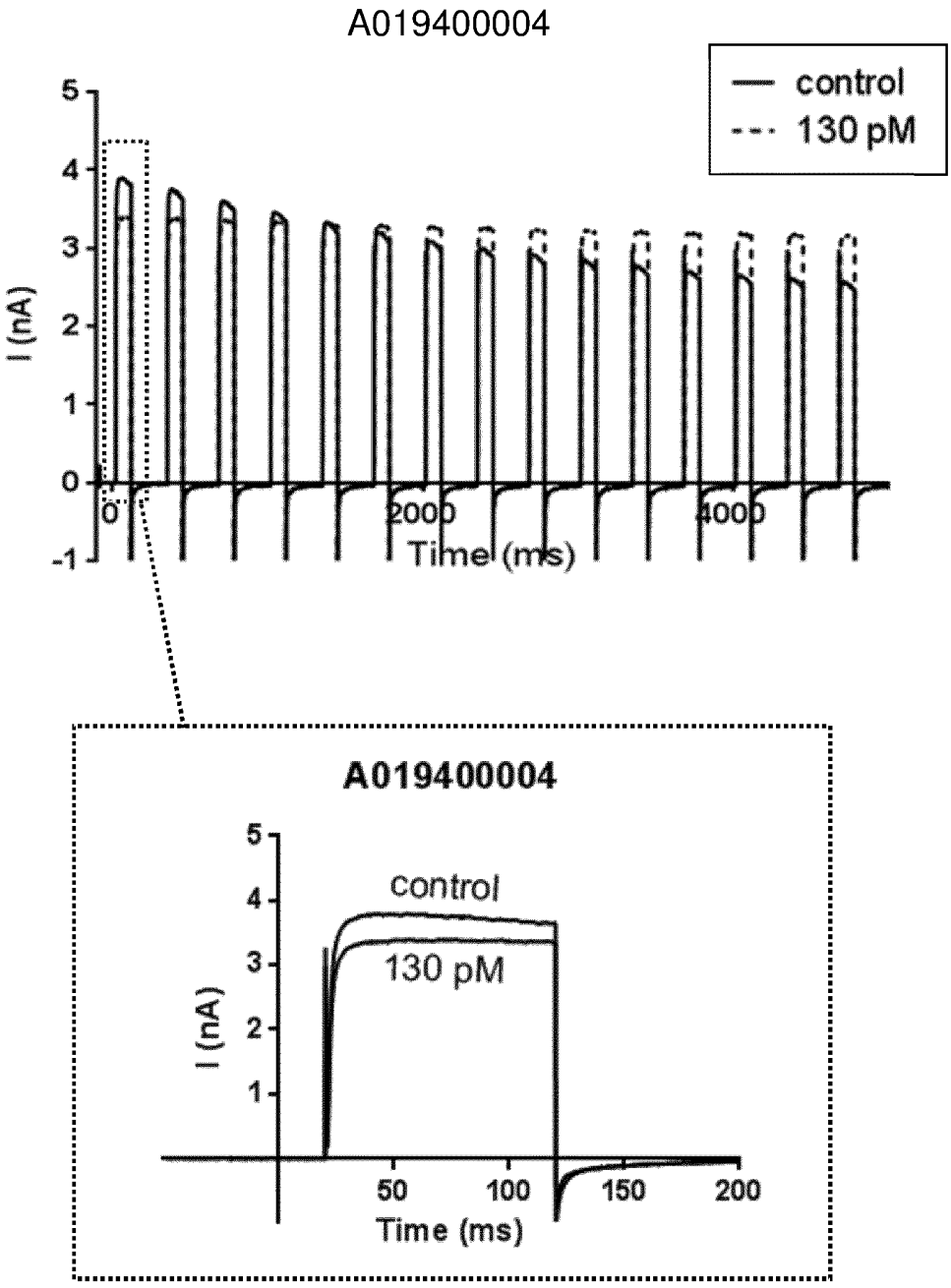

A019400004
control
130 pM
I (nA)
Time (ms)
A019400004
control
130 pM
I (nA)
Time (ms)

FIG. 17A

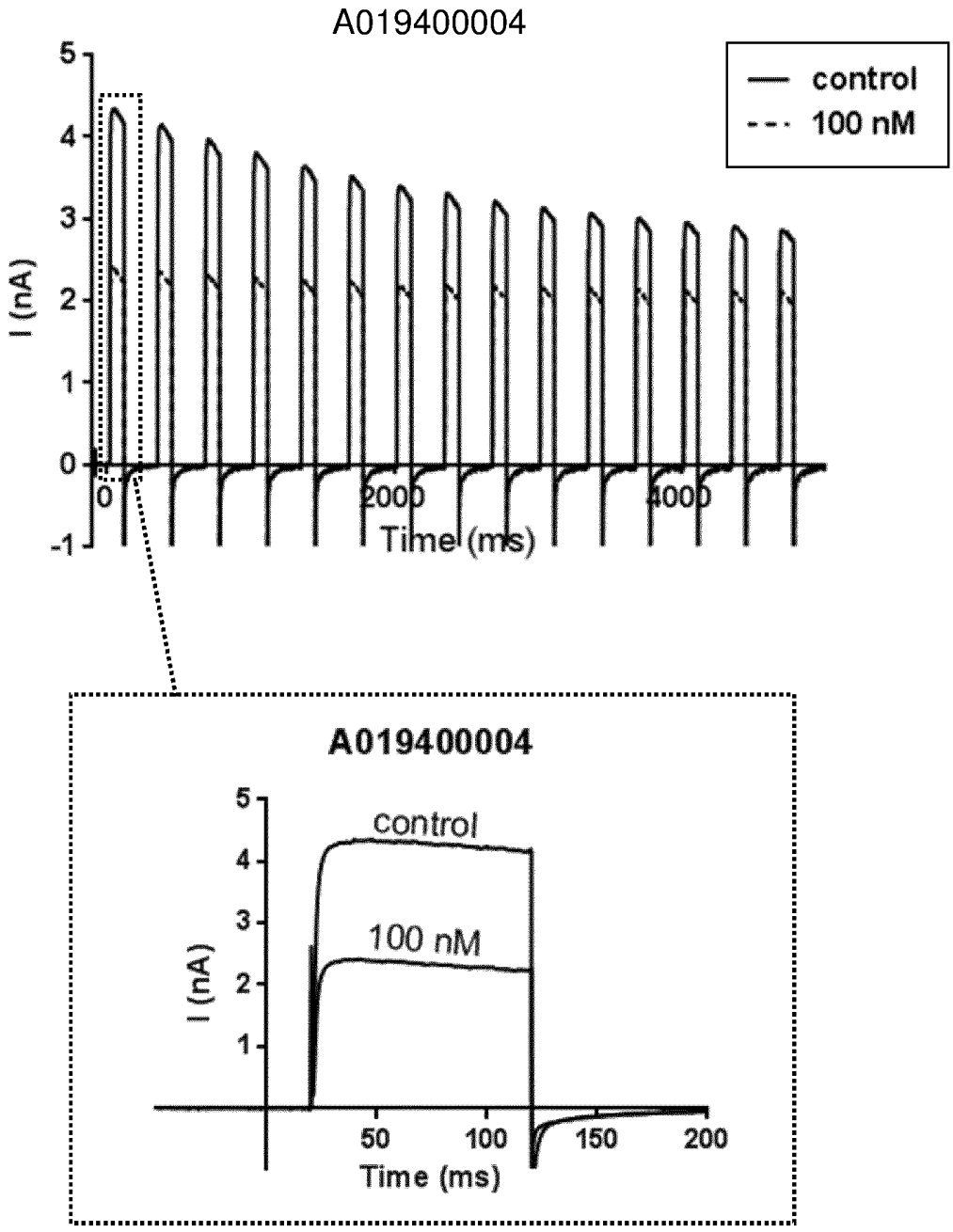

A019400004
control
100 nM
I (nA)
Time (ms)
A019400004
control
100 nM
I (nA)
Time (ms)

FIG. 17B

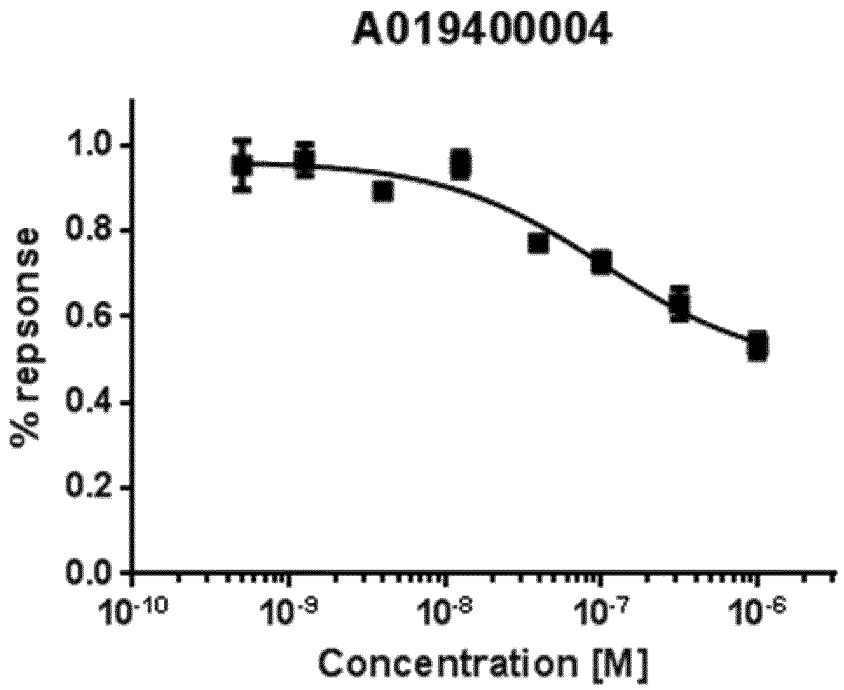

A019400004
% repsonse
1.0
0.8
0.6
0.4
0.2
0.0
10-10
10-9
10-8
10-7
10-6
Concentration [M]

FIG. 17C

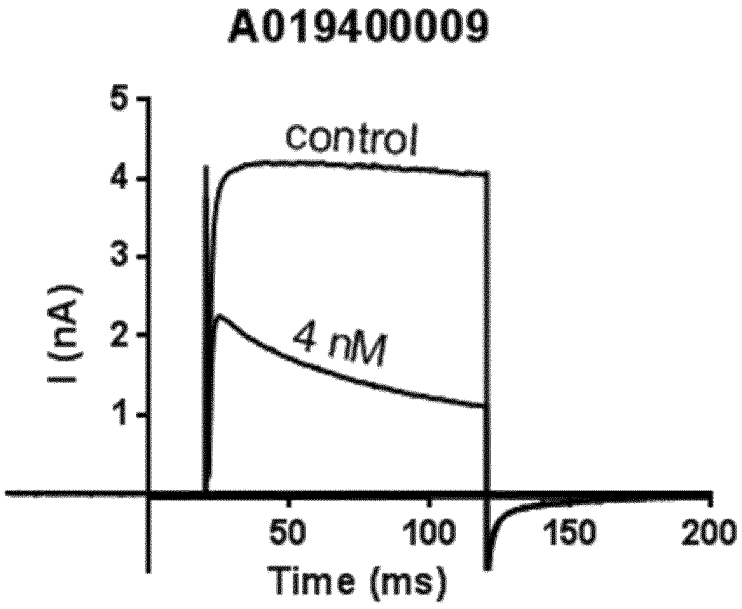


FIG. 18A

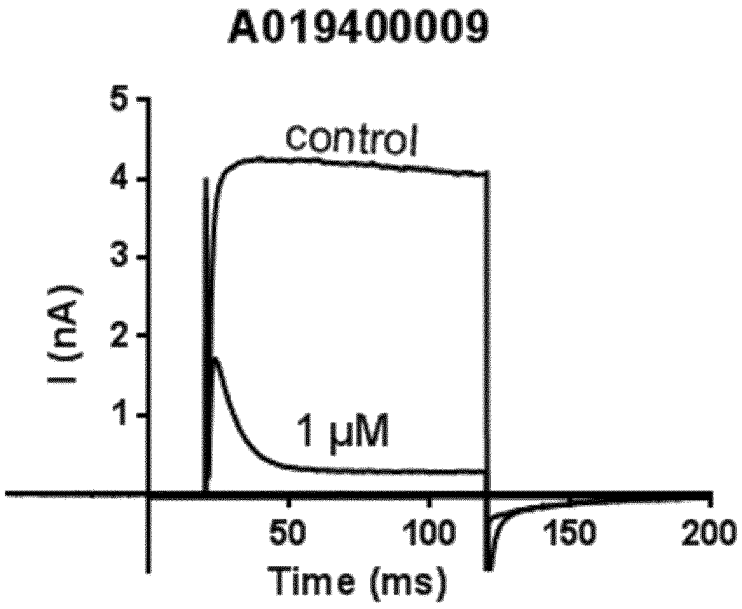


FIG. 18B

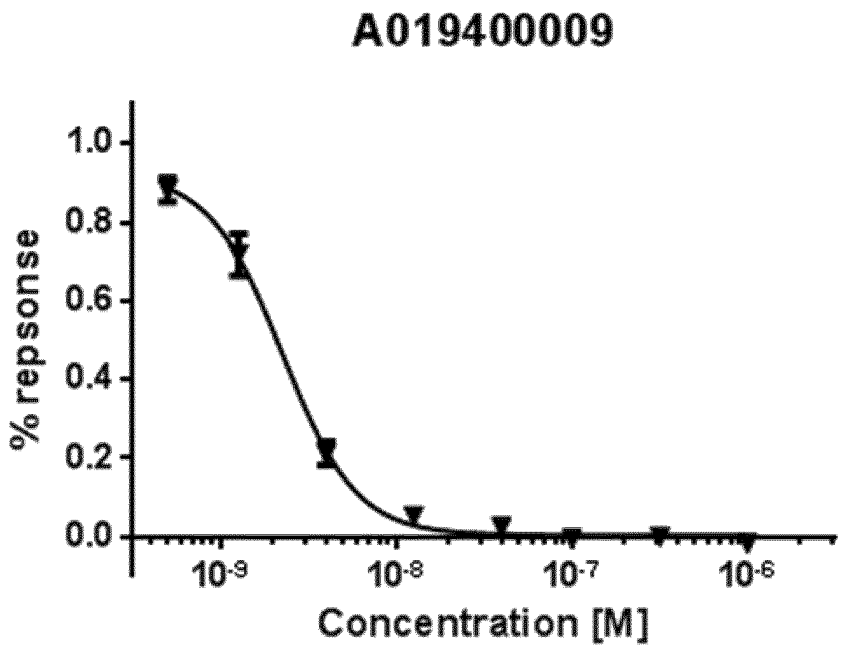

A019400009
1.0
0.8
0.6
0.4
0.2
0.0
% repsonse
10-9
10-8
10-7
10-6
Concentration [M]

FIG. 18C

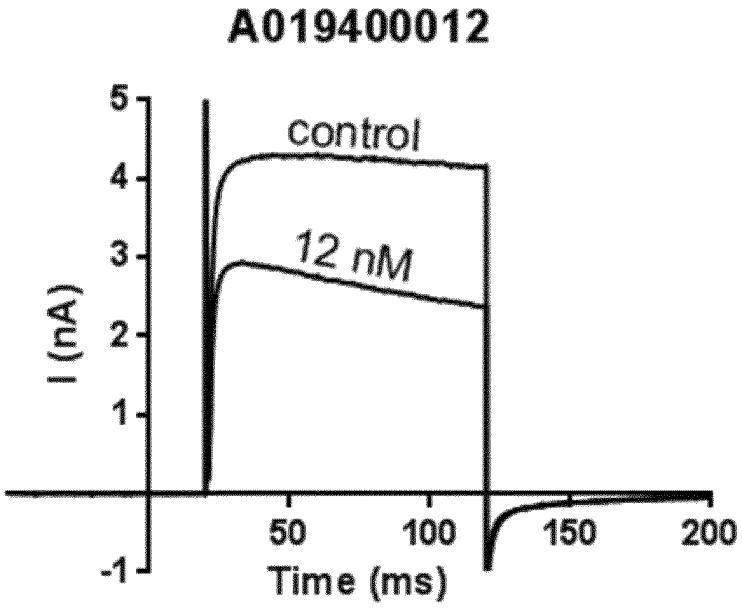


FIG. 19A

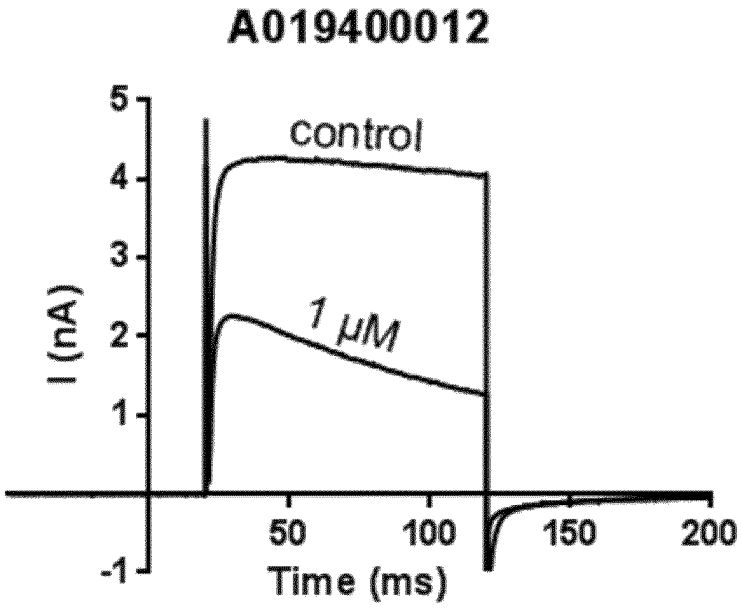


FIG. 19B

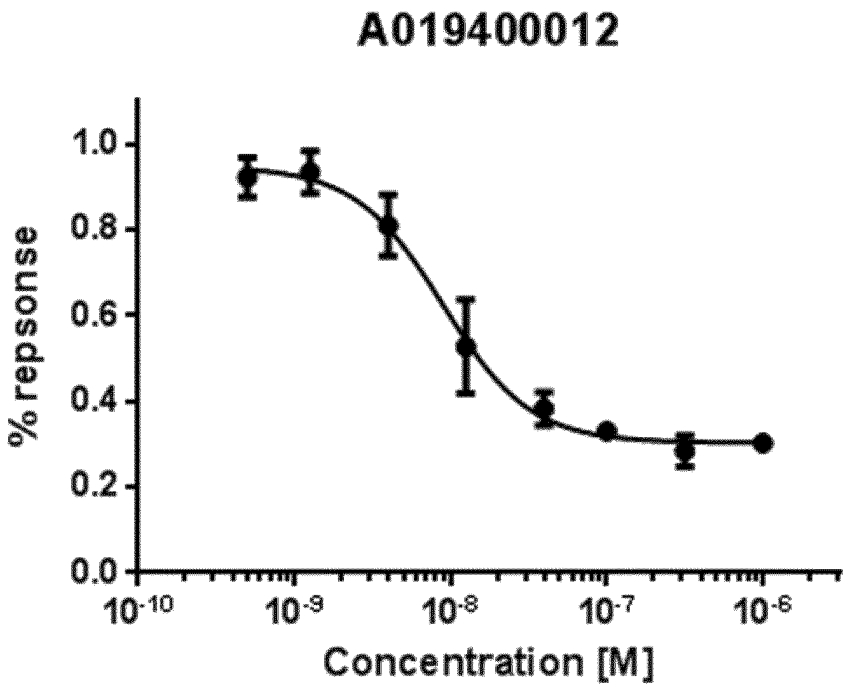

A019400012
% repsonse
1.0
0.8
0.6
0.4
0.2
0.0
10-10
10-9
10-8
10-7
10-6
Concentration [M]

FIG. 19C

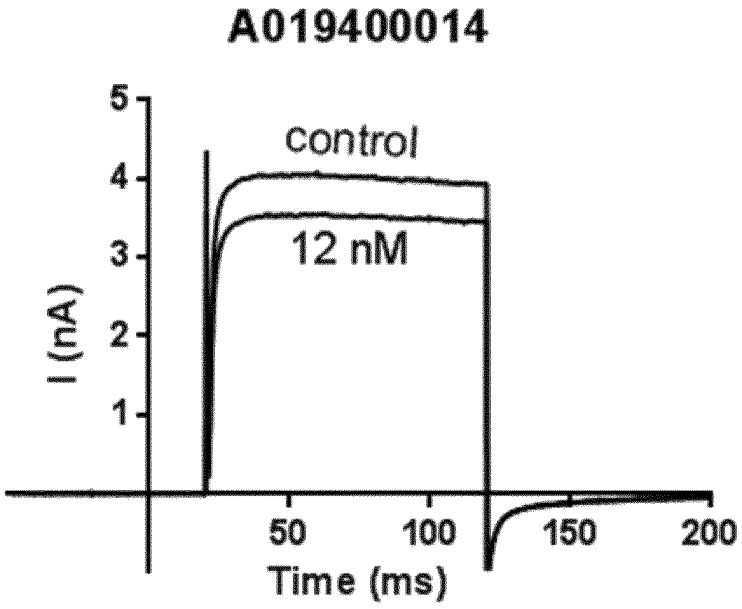


FIG. 20A

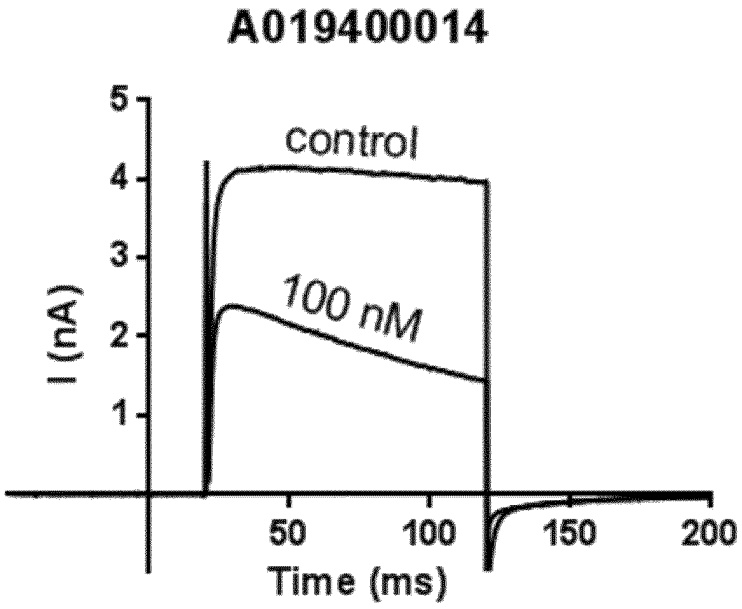


FIG. 20B

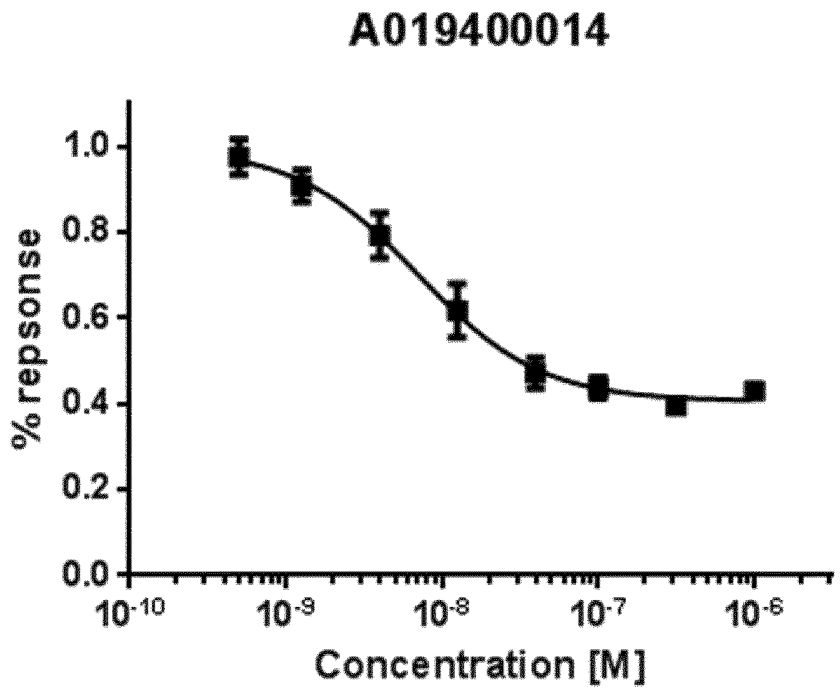

A019400014
% repsonse
1.0
0.8
0.6
0.4
0.2
0.0
10-10
10-9
10-8
10-7
10-6
Concentration [M]

FIG. 20C

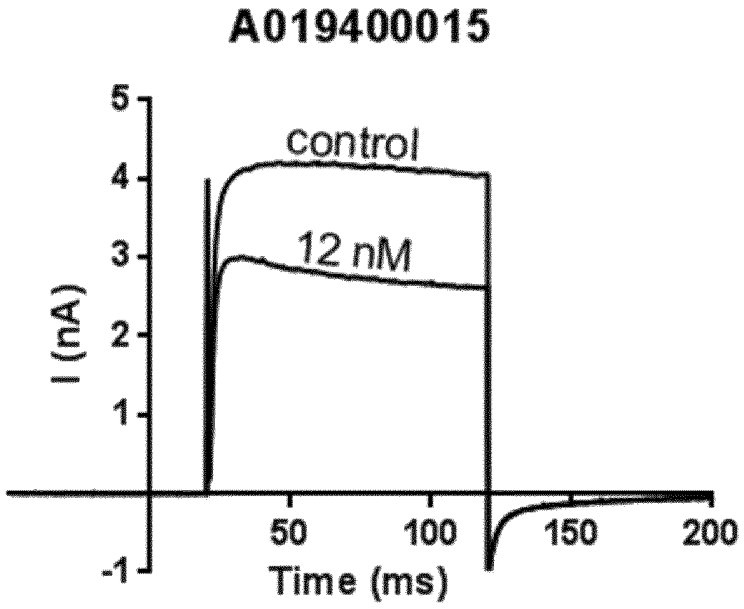


FIG. 21A

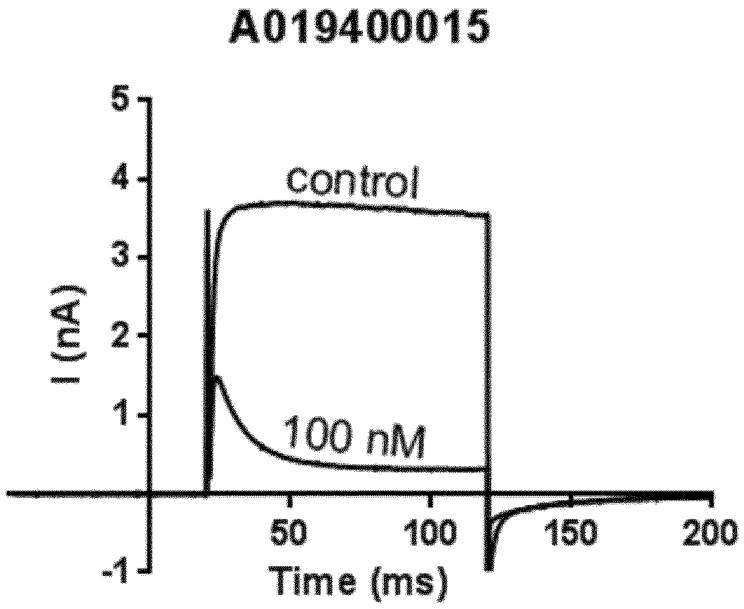


FIG. 21B

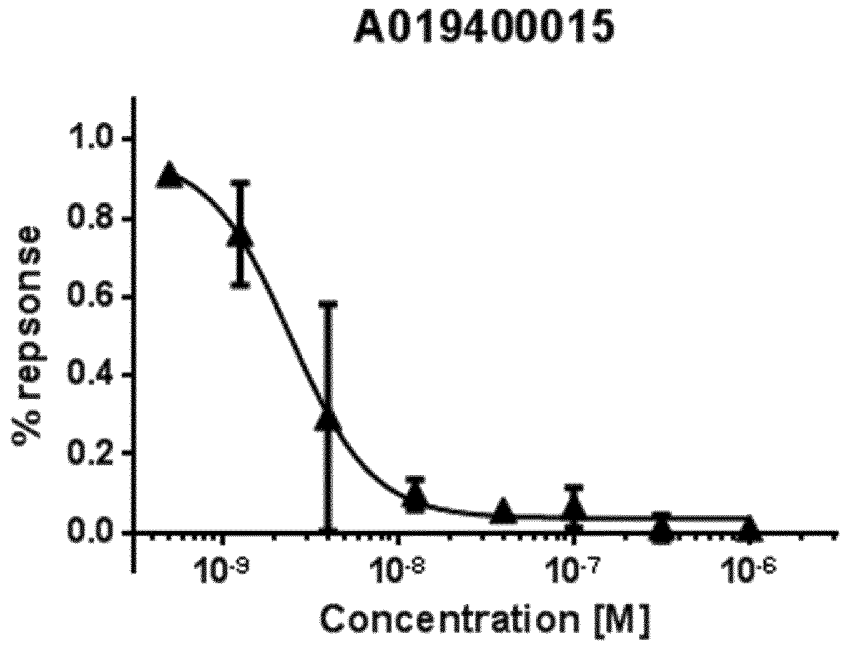

A019400015
1.0
0.8
0.6
0.4
0.2
0.0
% repsonse
10-9
10-8
10-7
10-6
Concentration [M]

FIG. 21C

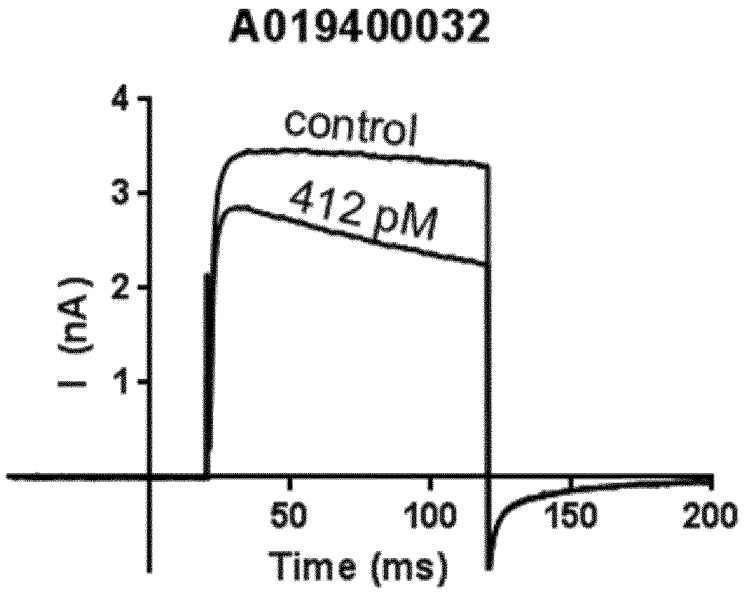


FIG. 22A

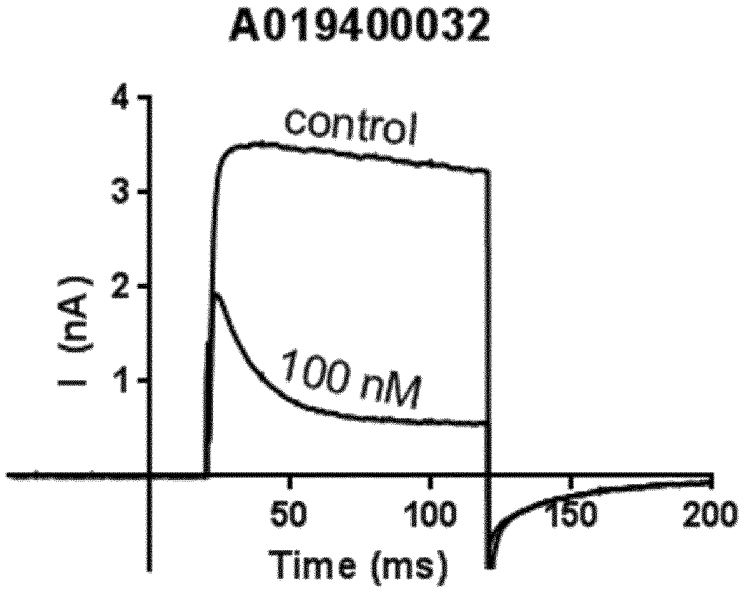


FIG. 22B

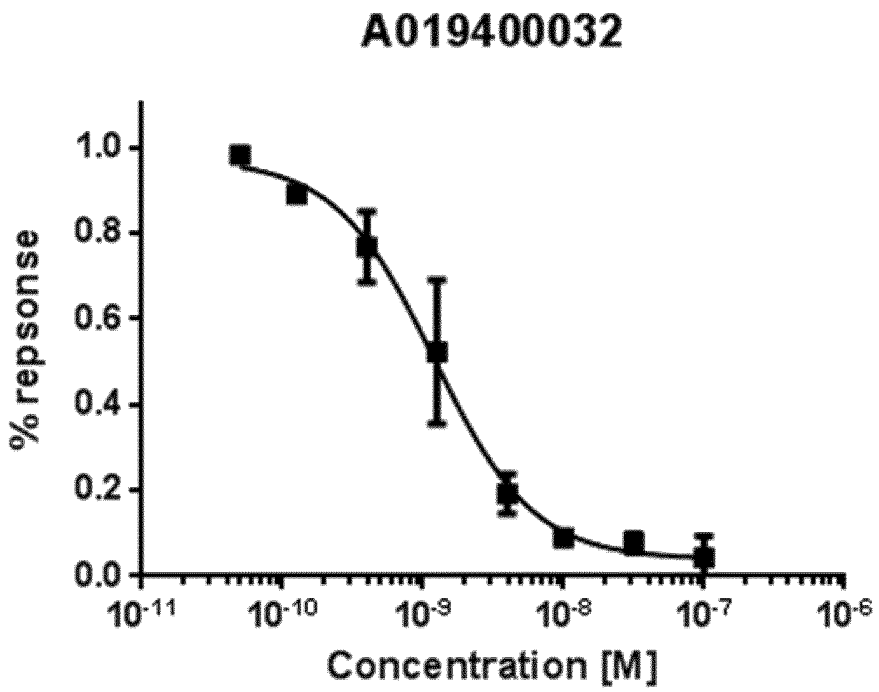

A019400032
% repsonse
1.0
0.8
0.6
0.4
0.2
0.0
10-11
10-10
10-9
10-8
10-7
10-6
Concentration [M]

FIG. 22C

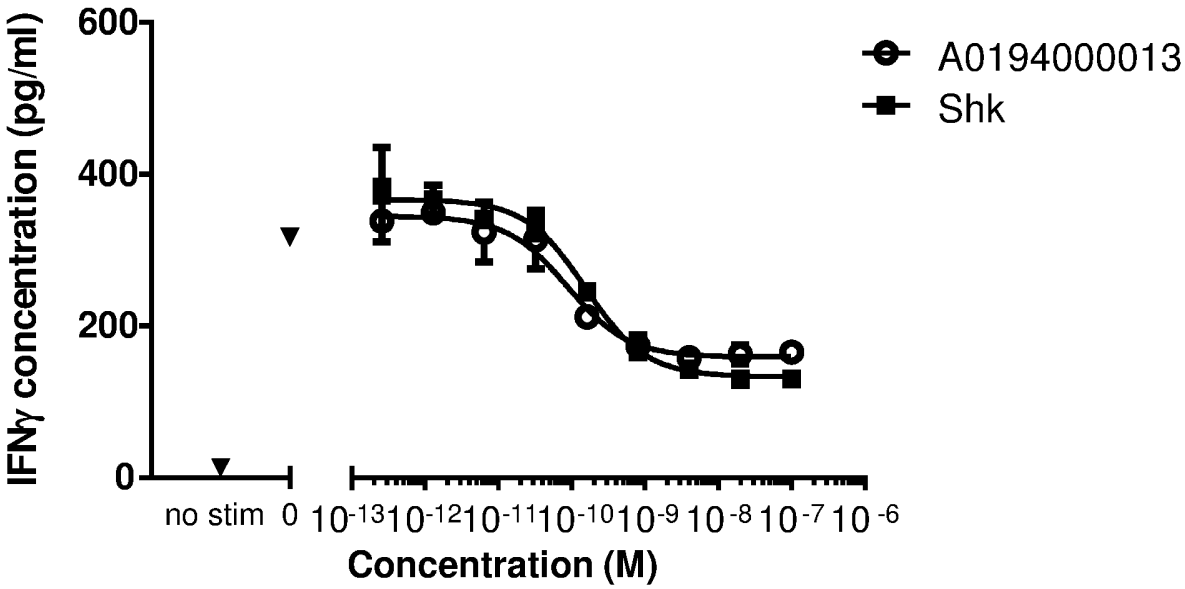


FIG. 23A

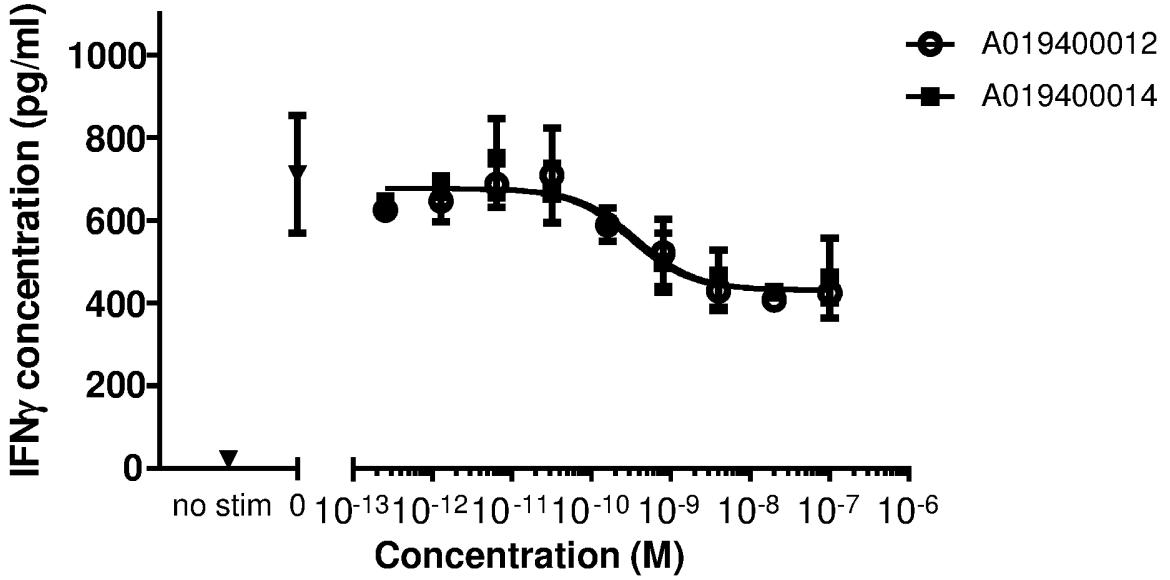


FIG. 23B

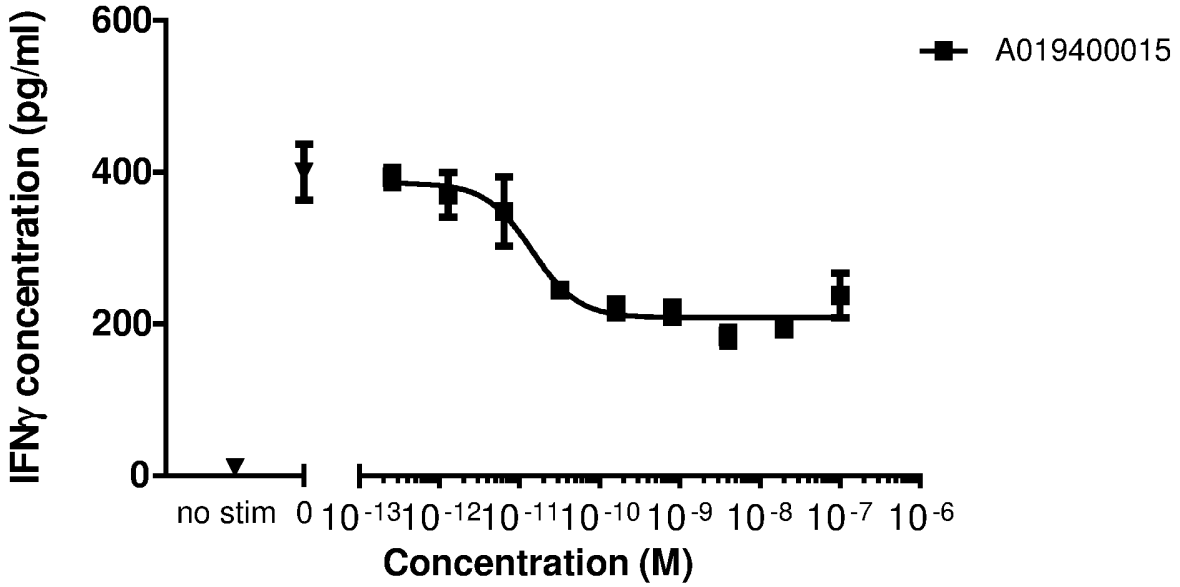


FIG. 23C

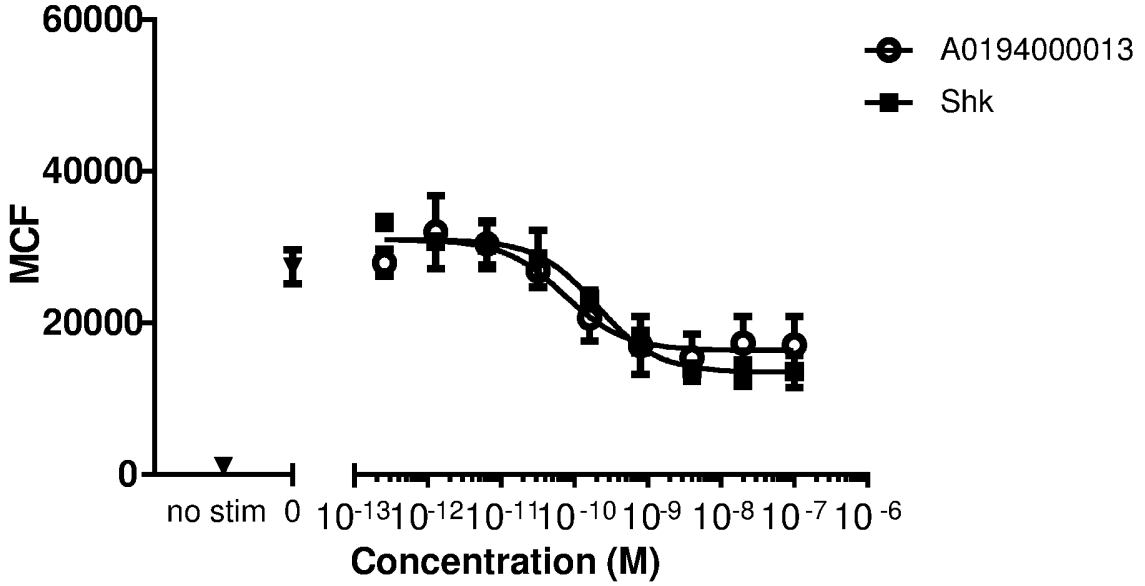


FIG. 23D

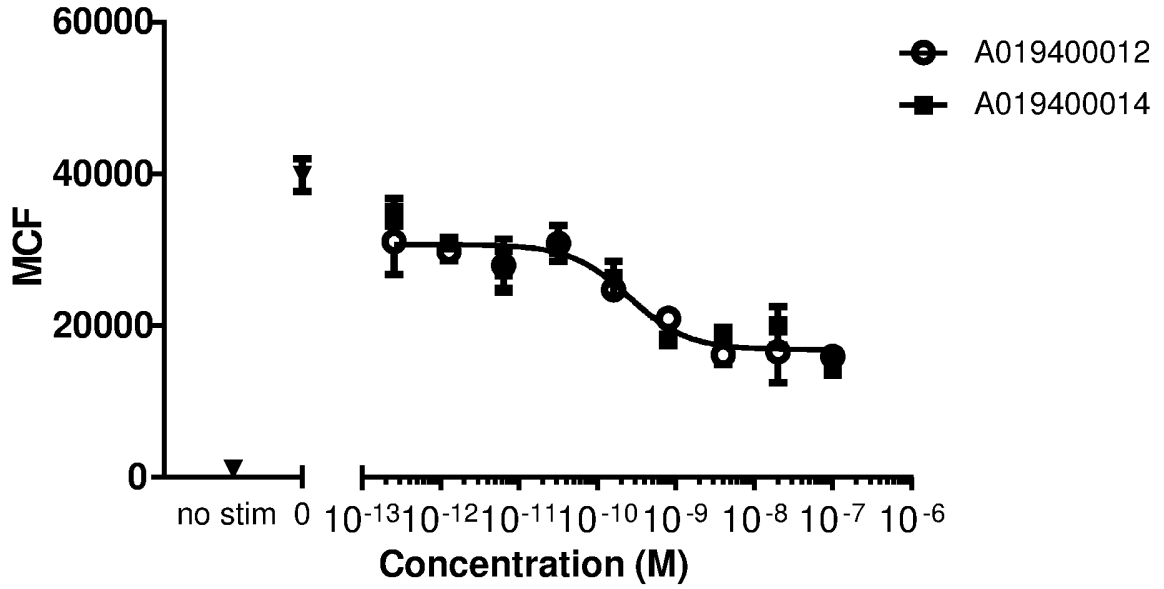


FIG. 23E

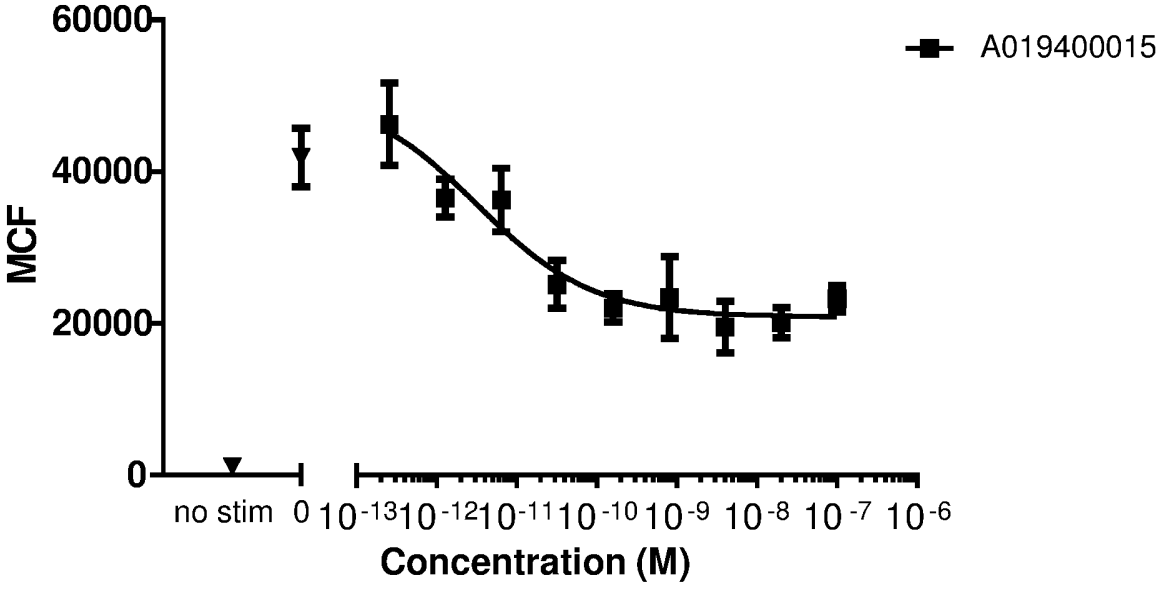


FIG. 23F

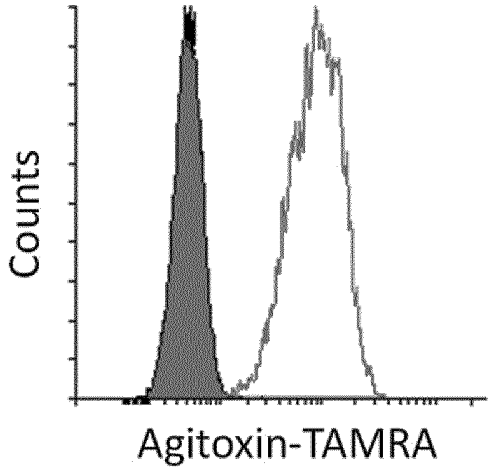


FIG. 24A

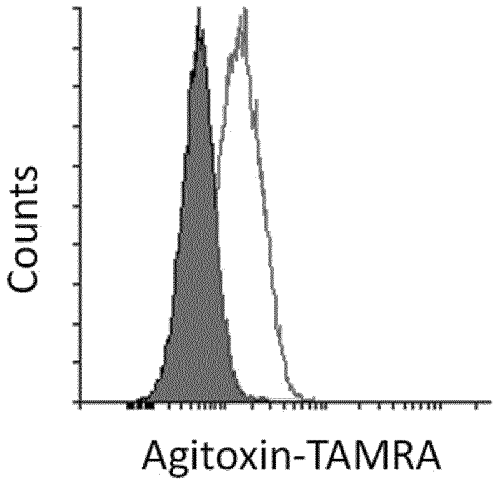


FIG. 24B

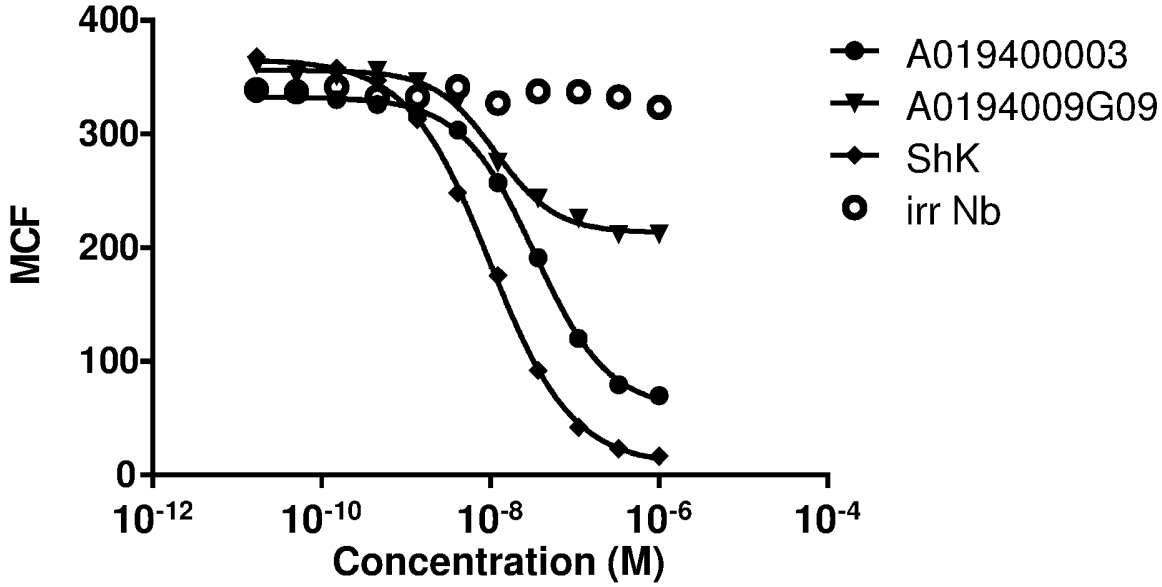

400
300
200
100
0
MCF
10-12
10-10
10-8
10-6
10-4
Concentration (M)
A019400003
A0194009G09
ShK
irr Nb

FIG. 25

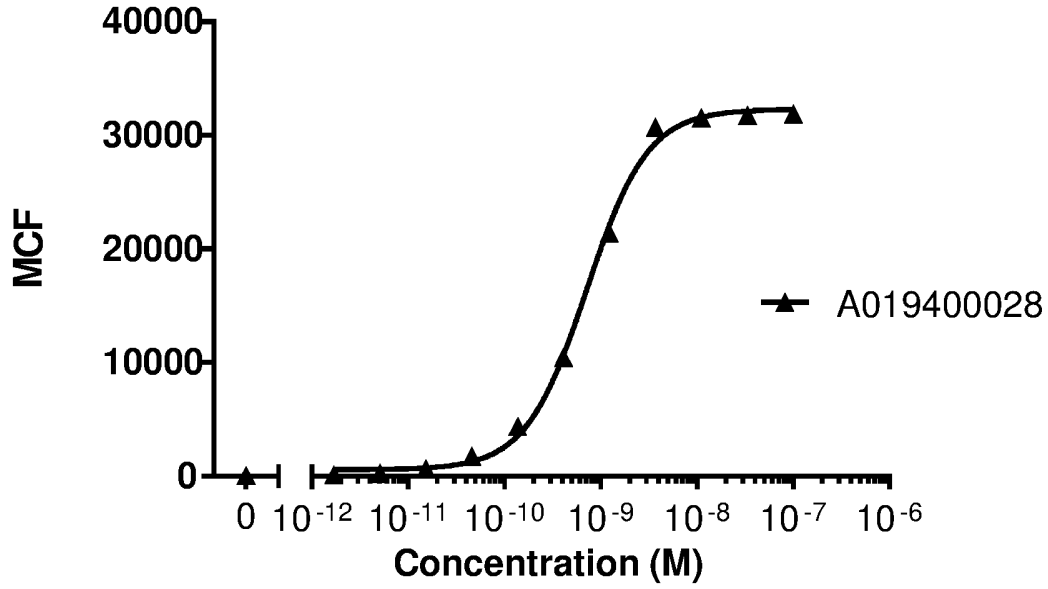


FIG. 26A

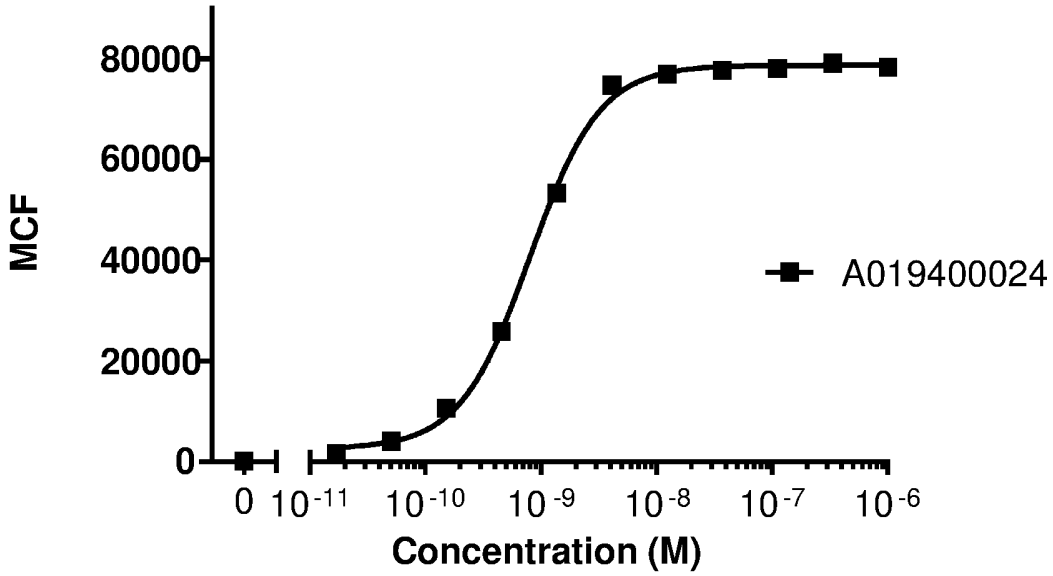


FIG. 26B

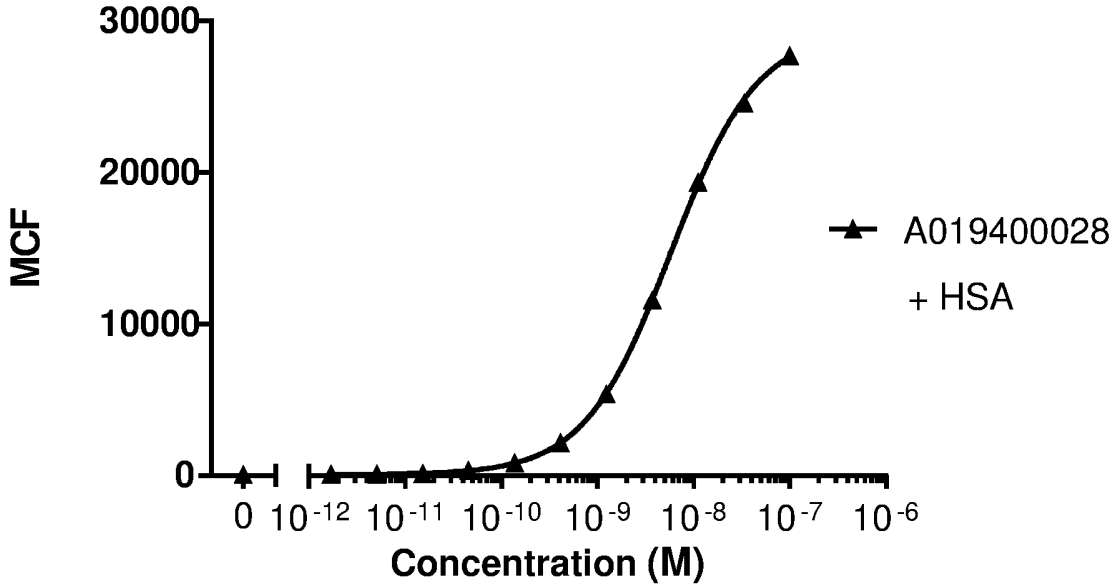


FIG. 26C

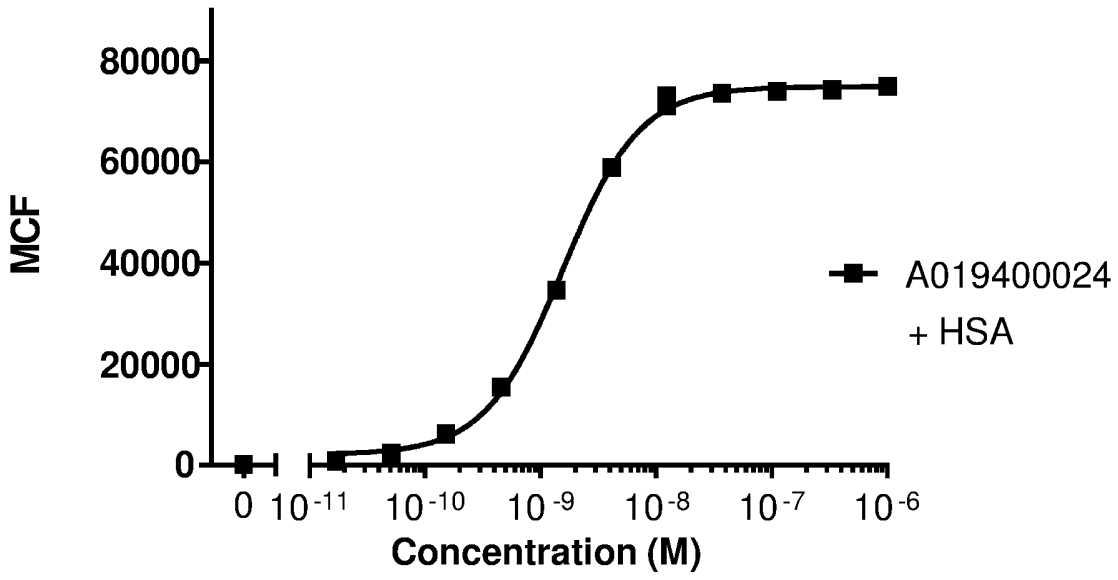


FIG. 26D

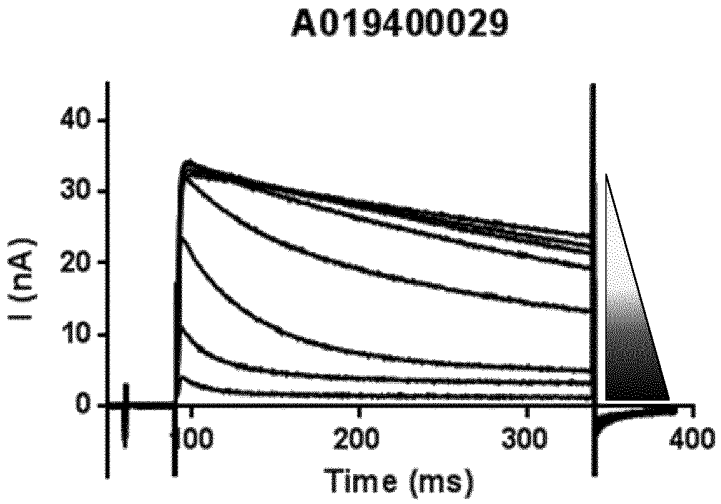


FIG. 27A

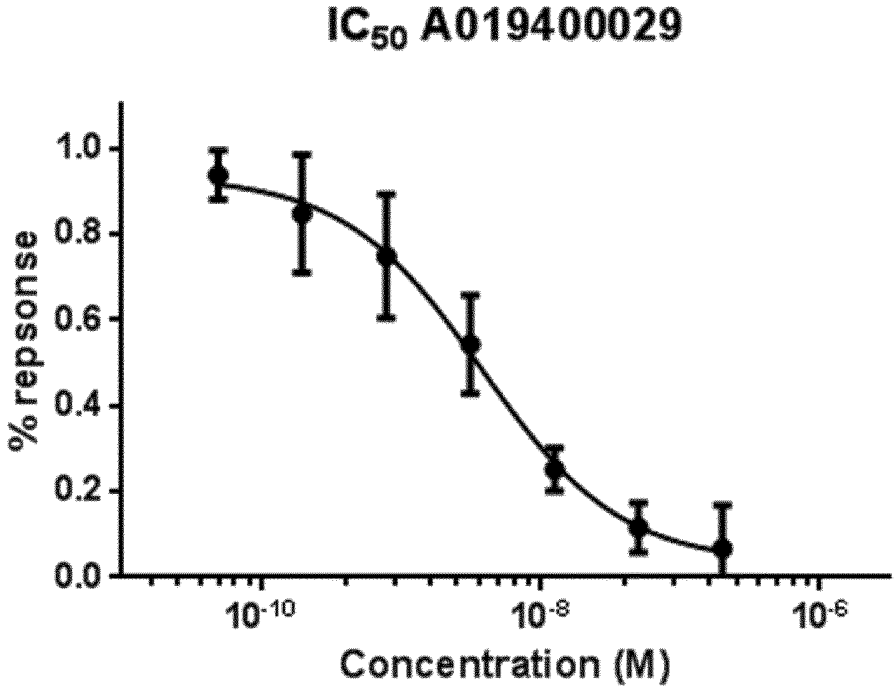


FIG. 27B

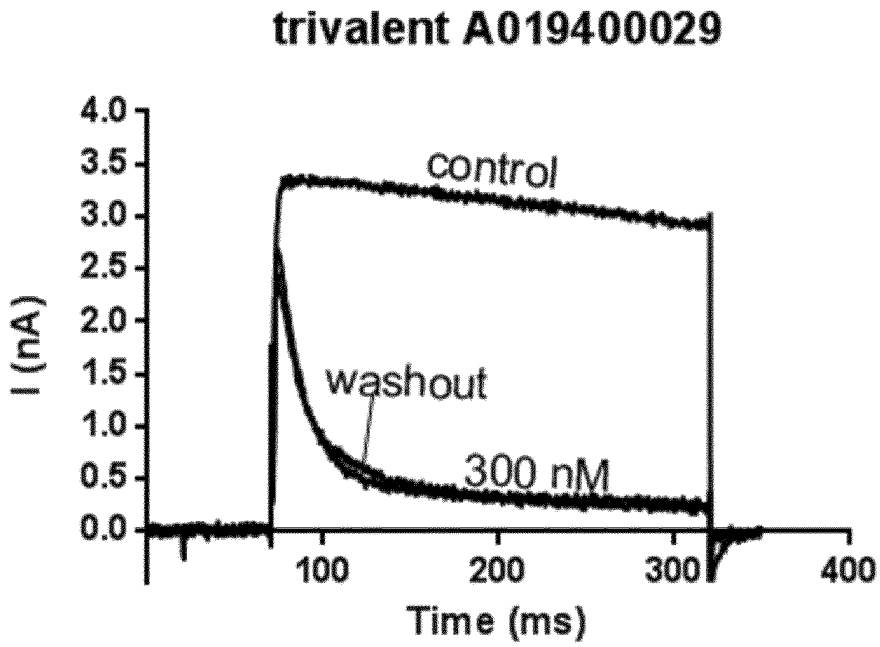


FIG. 27C

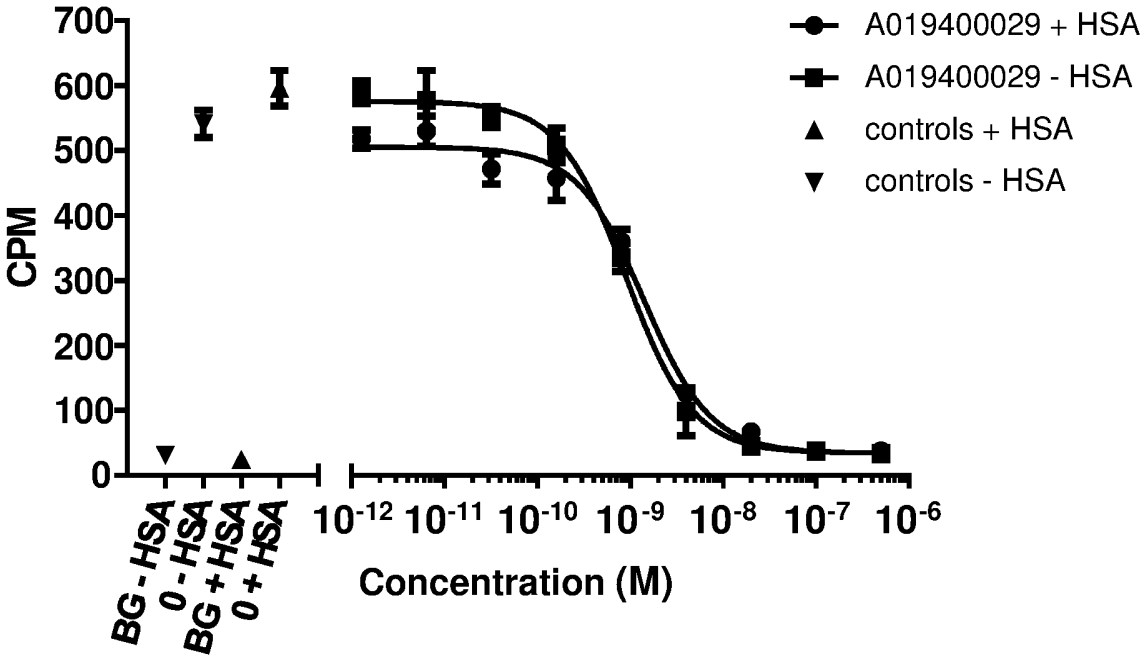


FIG. 28

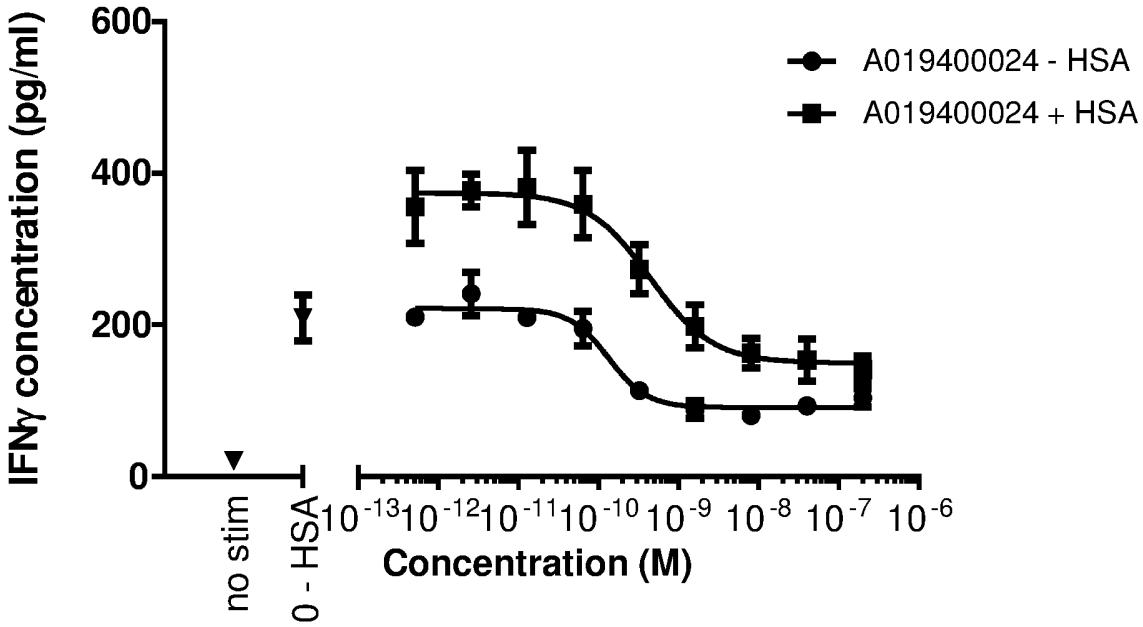


FIG. 29A

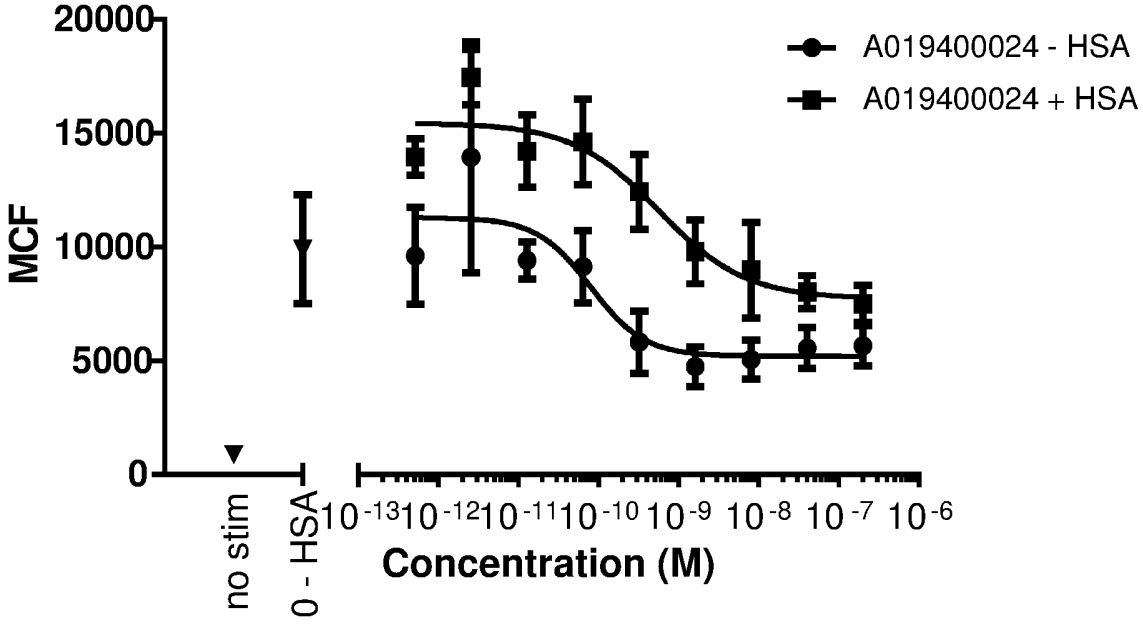


FIG. 29B

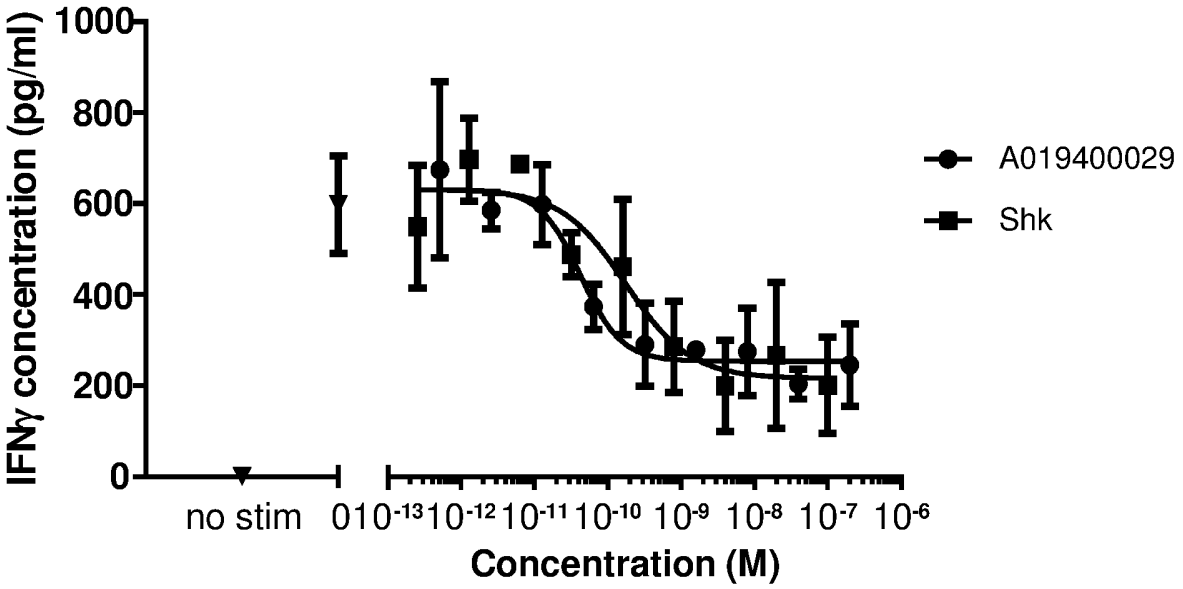


FIG. 30A

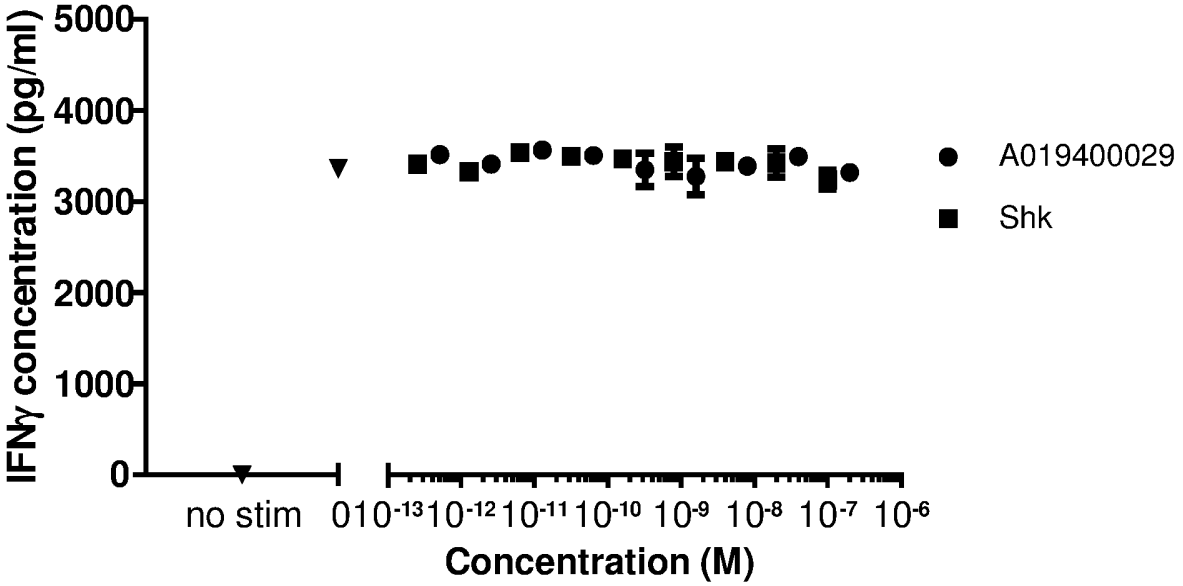


FIG. 30B

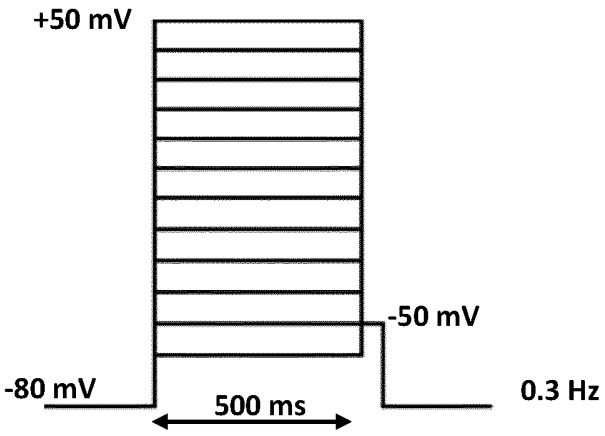


FIG. 31A

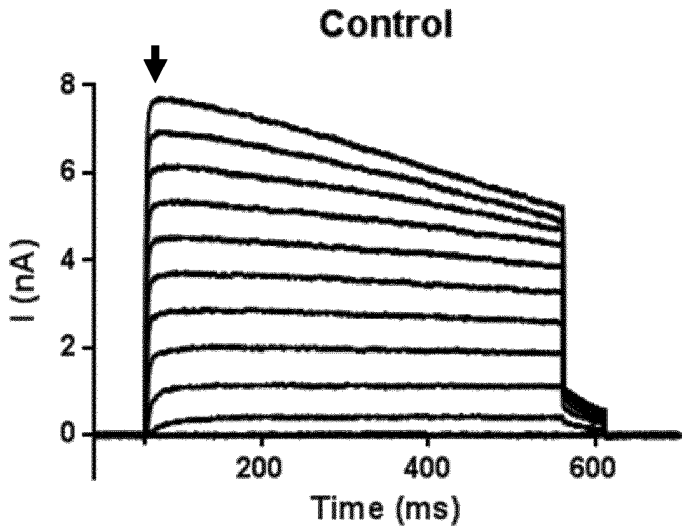


FIG. 31B

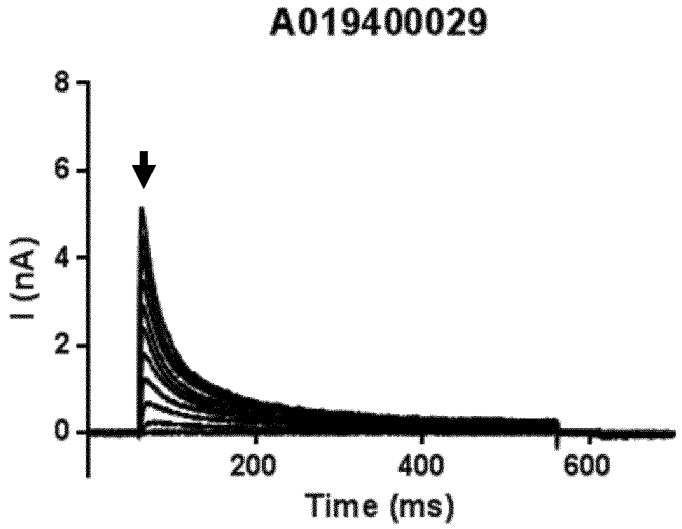


FIG. 31C

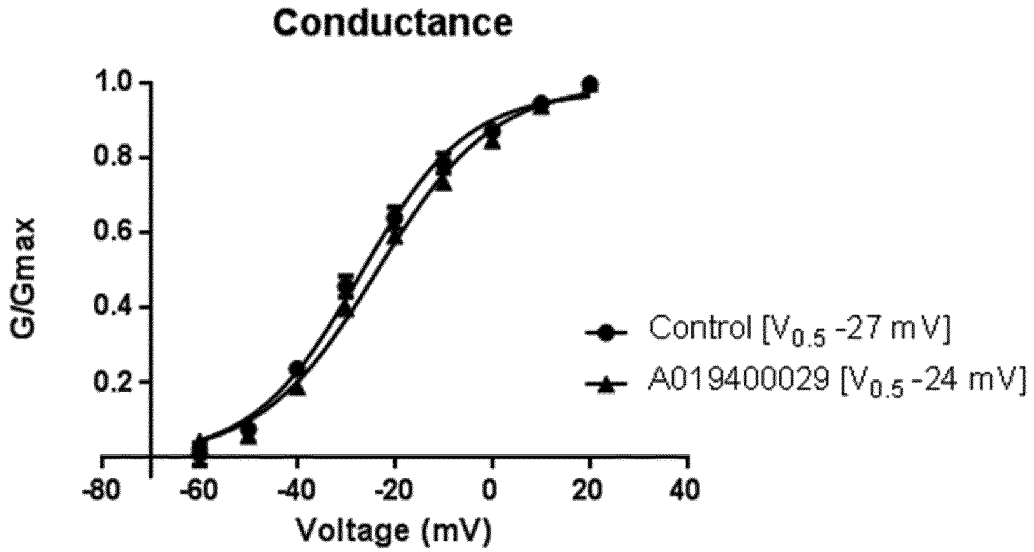


FIG. 31D

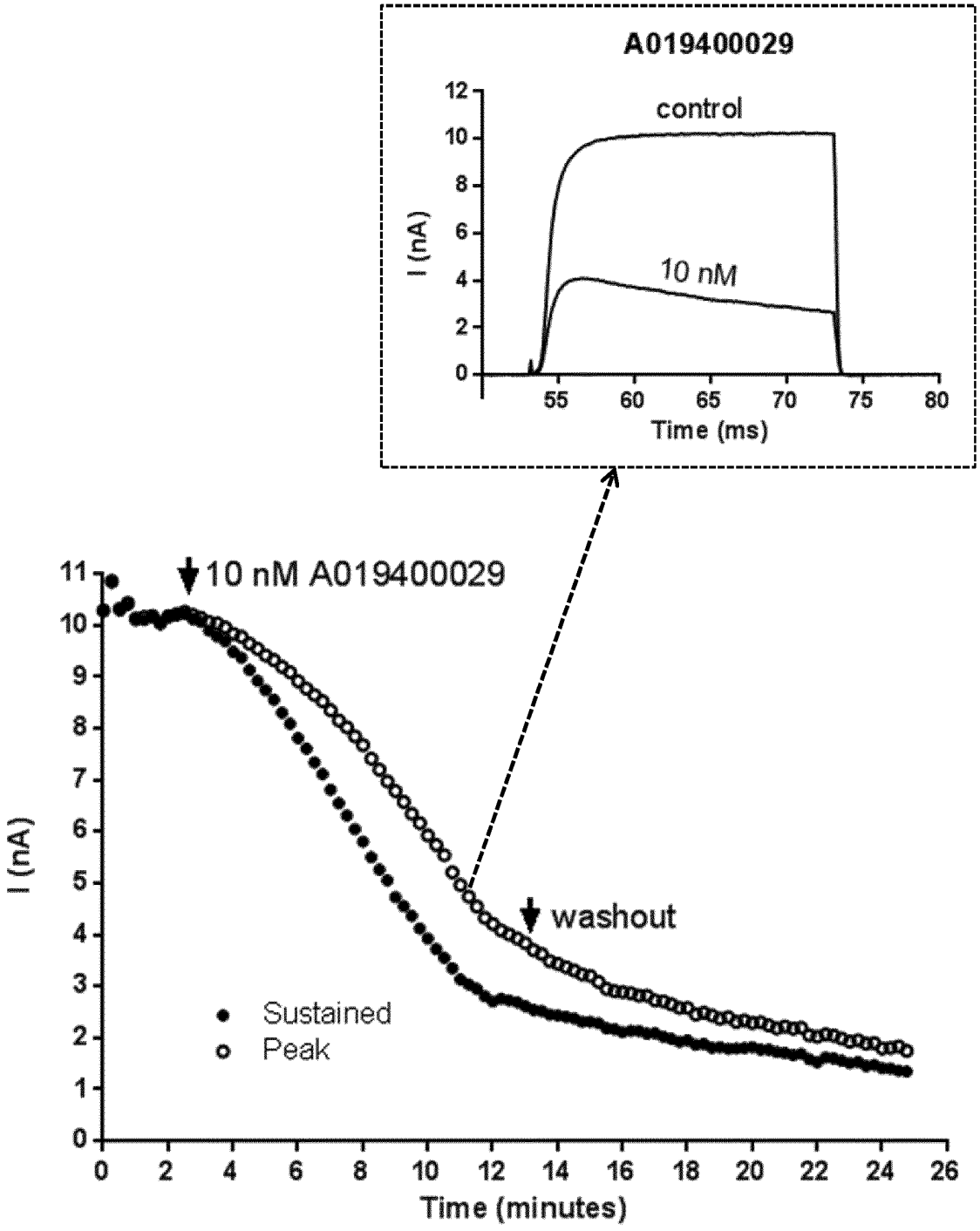

A019400029
control
10 nM
I (nA)
Time (ms)
10 nM A019400029
washout
Sustained
Peak
I (nA)
Time (minutes)

FIG. 32A

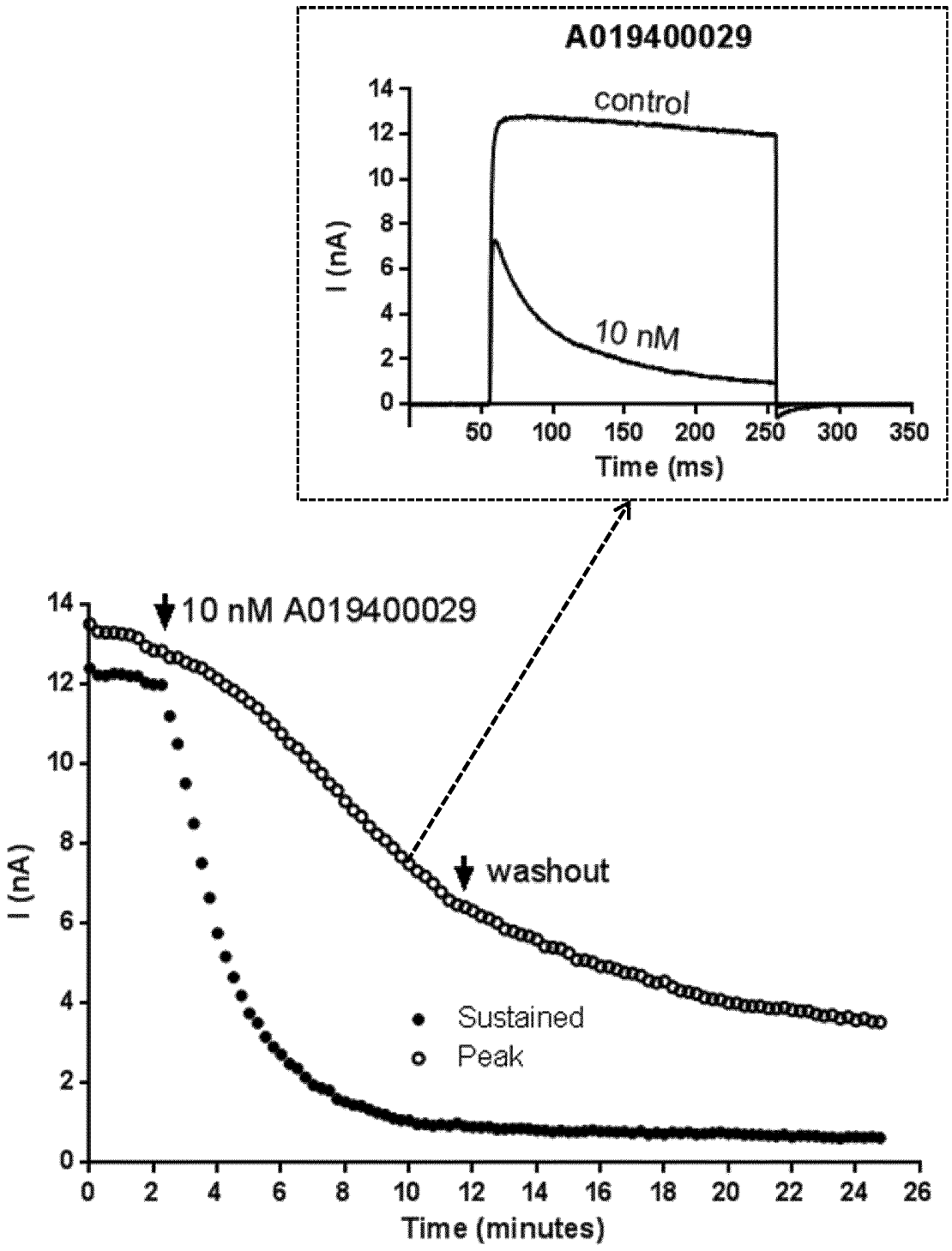

A019400029
control
10 nM
I (nA)
Time (ms)
10 nM A019400029
washout
Sustained
Peak
I (nA)
Time (minutes)

FIG. 32B

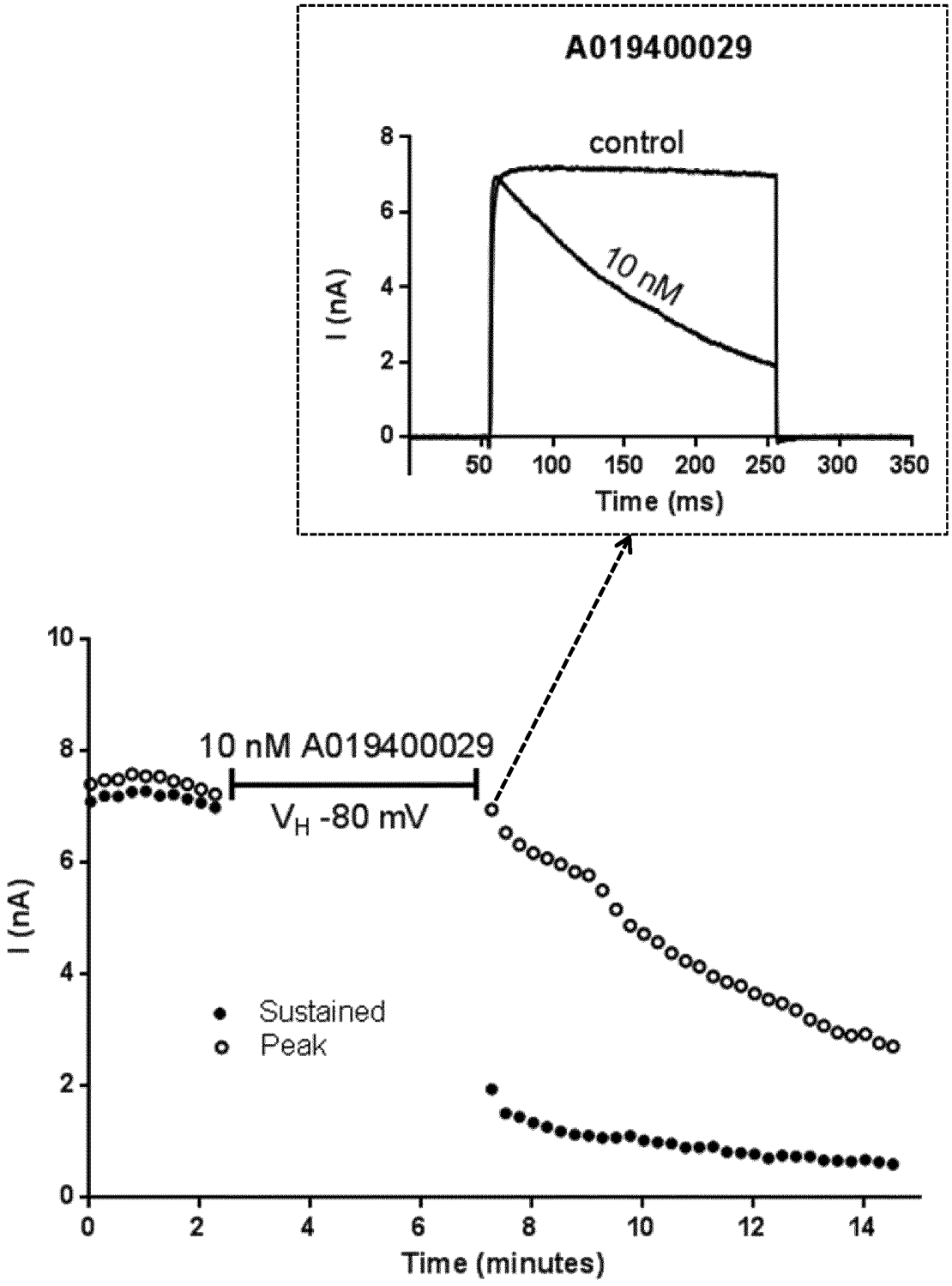

A019400029
control
10 nM
I (nA)
Time (ms)
10 nM A019400029
V_H -80 mV
Sustained
Peak
I (nA)
Time (minutes)

FIG. 33A

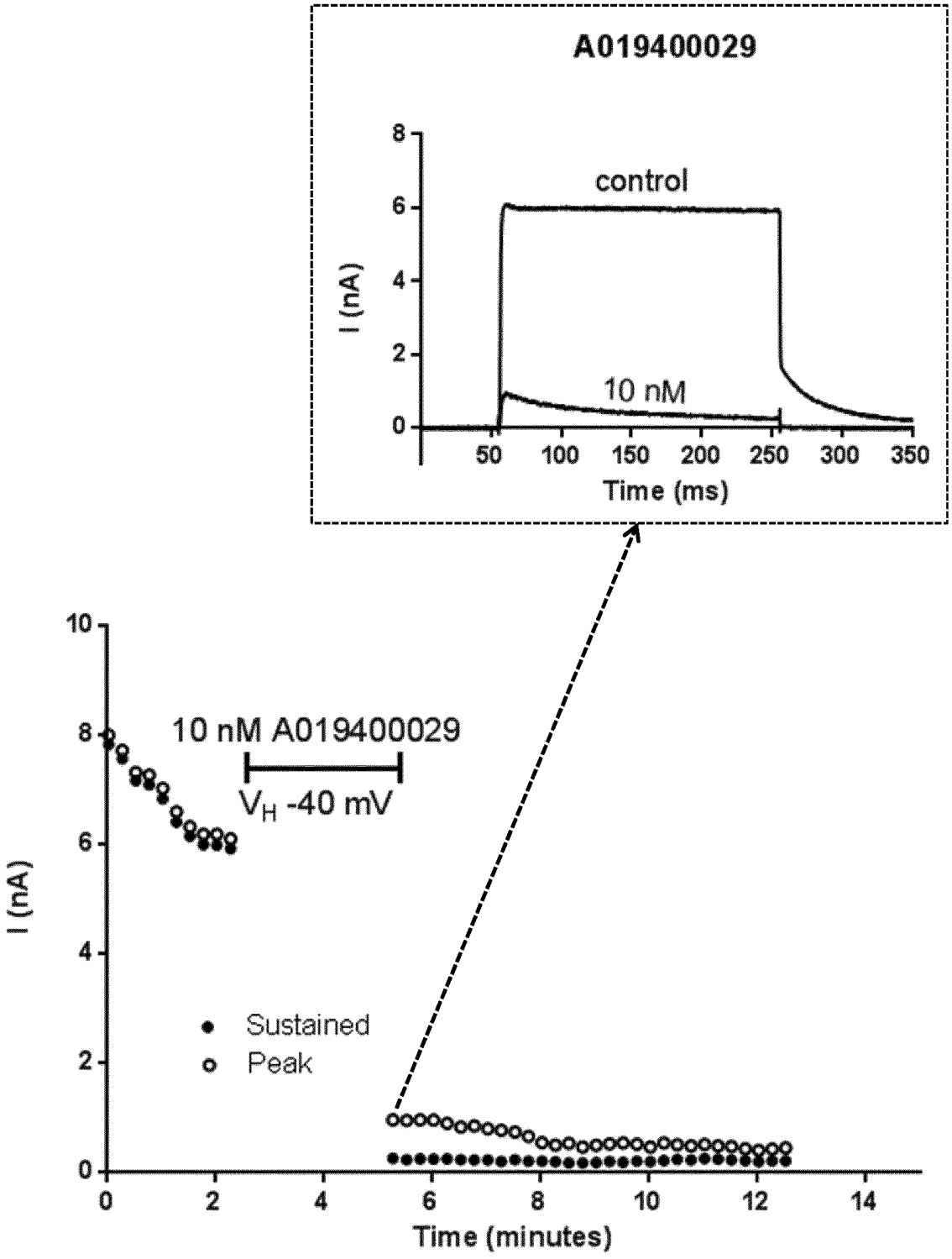

A019400029
control
10 nM
I (nA)
Time (ms)
10 nM A019400029
V_H -40 mV
Sustained
Peak
I (nA)
Time (minutes)

FIG. 33B

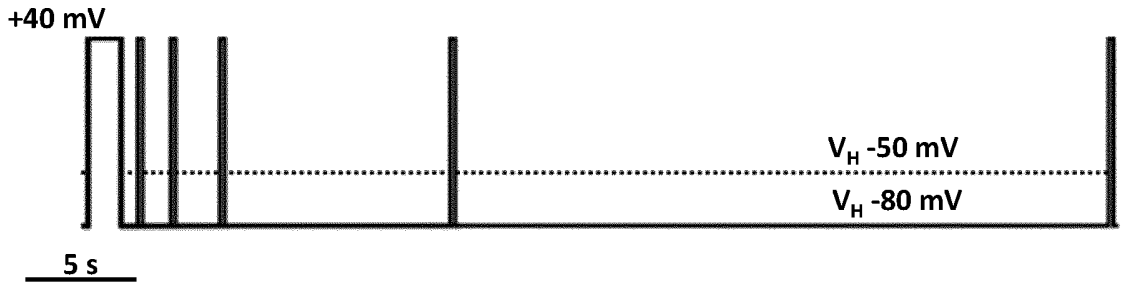


FIG. 34A

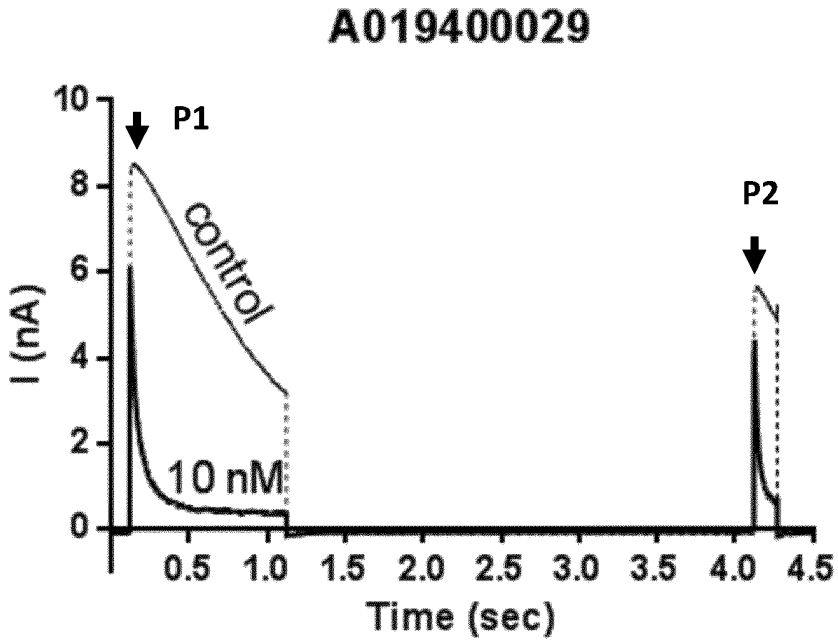


FIG. 34B

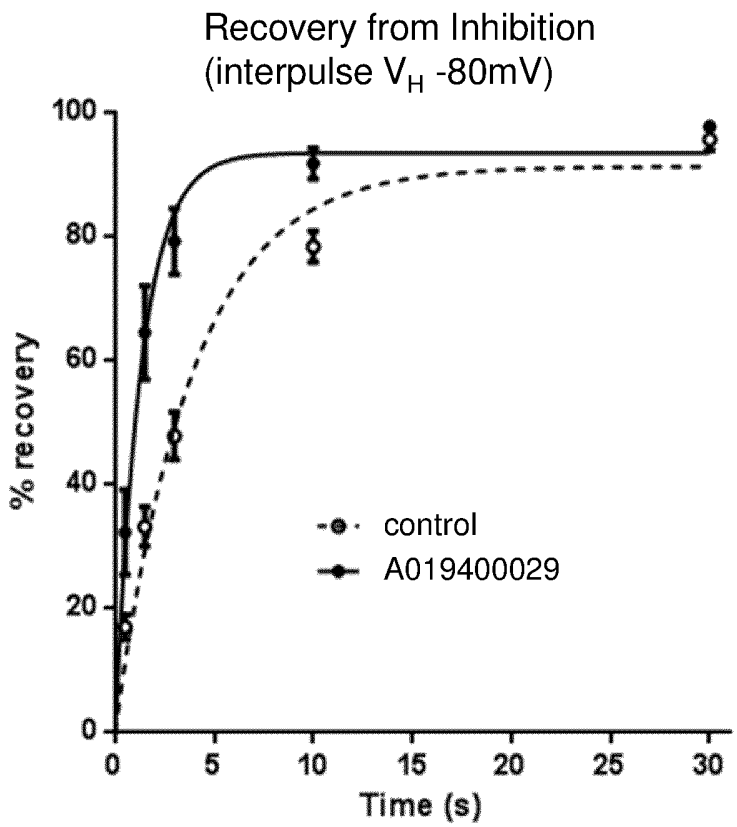
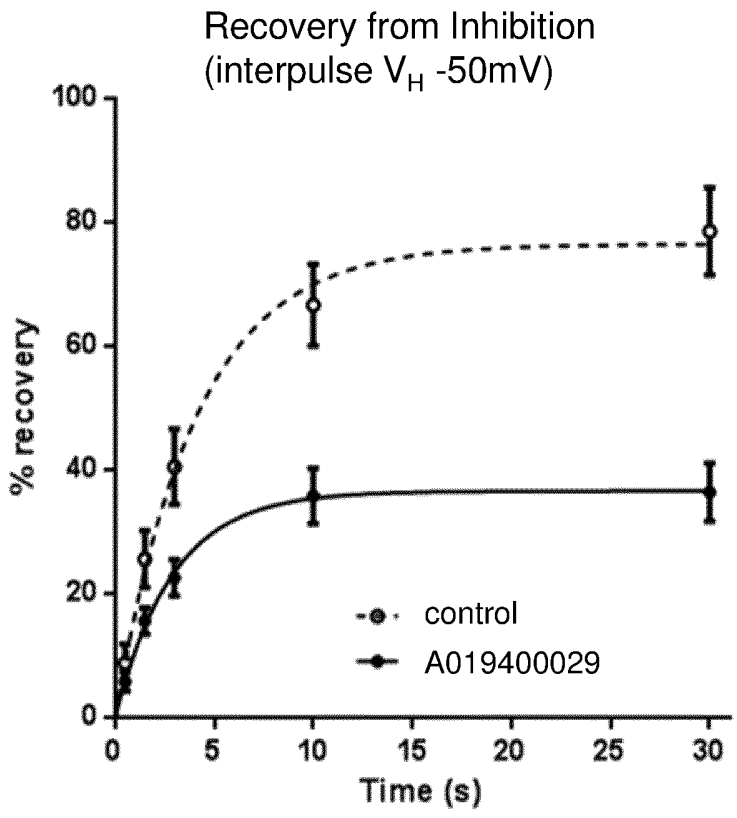

Recovery from Inhibition
(interpulse V_H -80mV)
% recovery
Time (s)
control
A019400029
0
20
40
60
80
100
0
5
10
15
20
25
30

FIG. 34C


Recovery from Inhibition
(interpulse V_H -50mV)
% recovery
Time (s)
control
A019400029
0
20
40
60
80
100
0
5
10
15
20
25
30

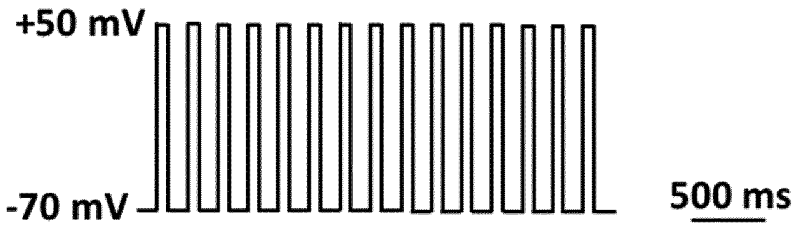


FIG. 35A

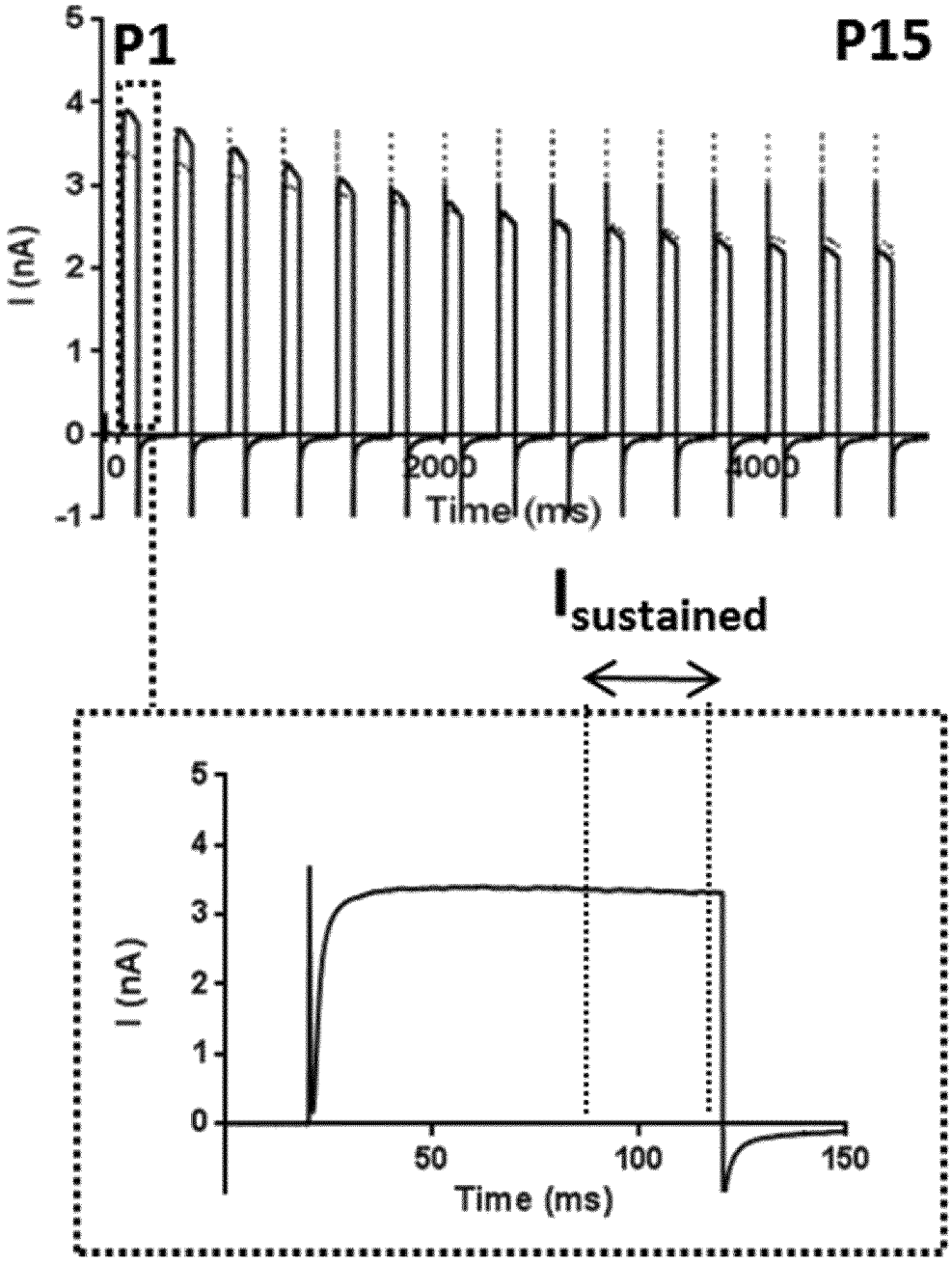


FIG. 35B

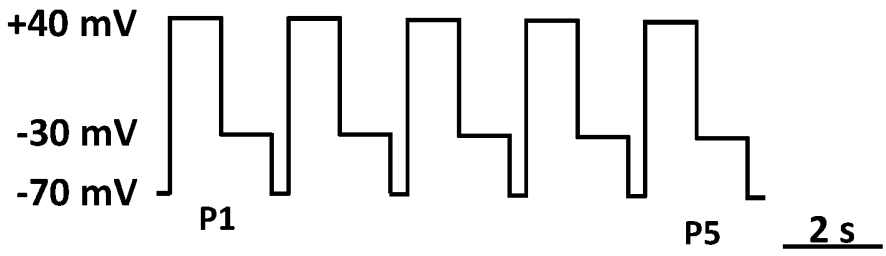


FIG. 35C

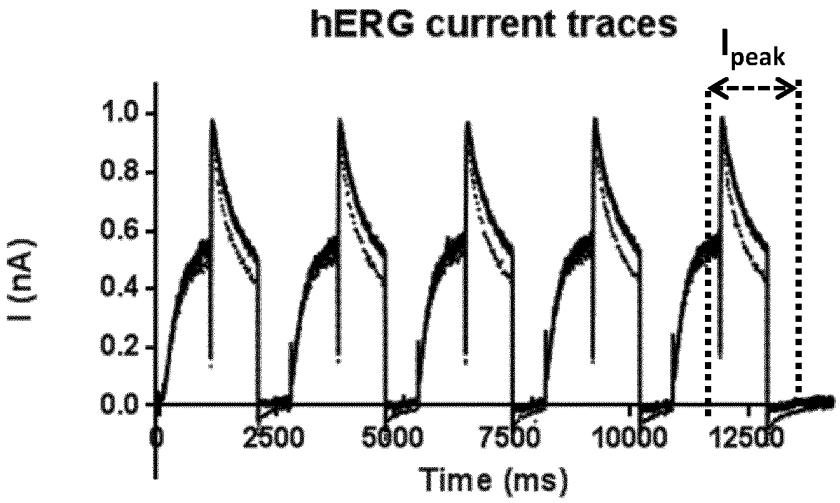


FIG. 35D

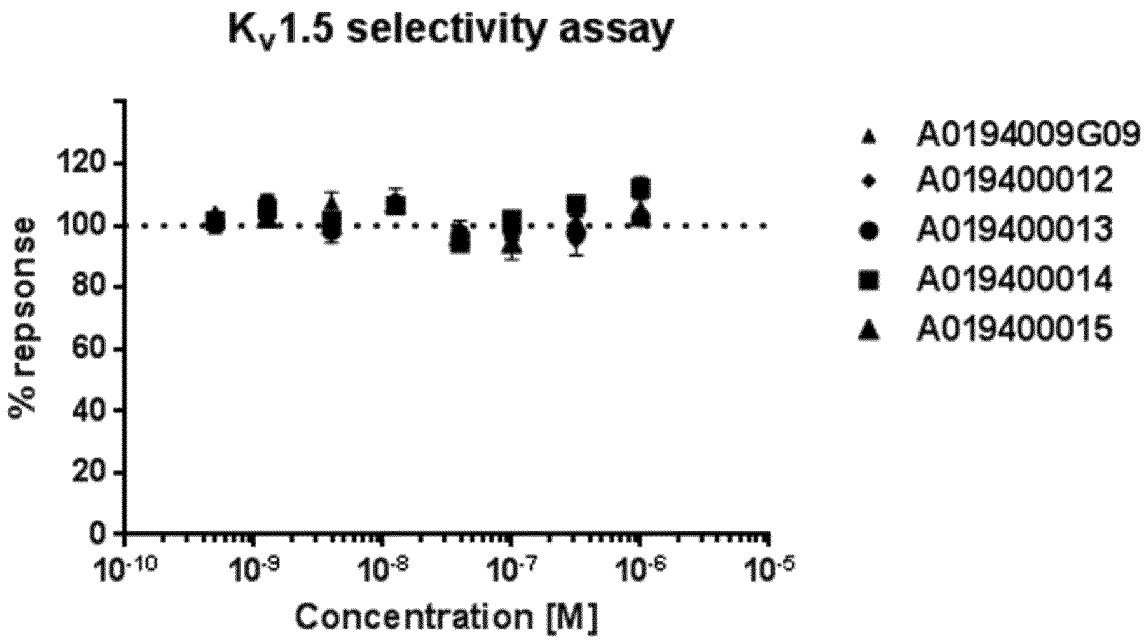


FIG. 36A

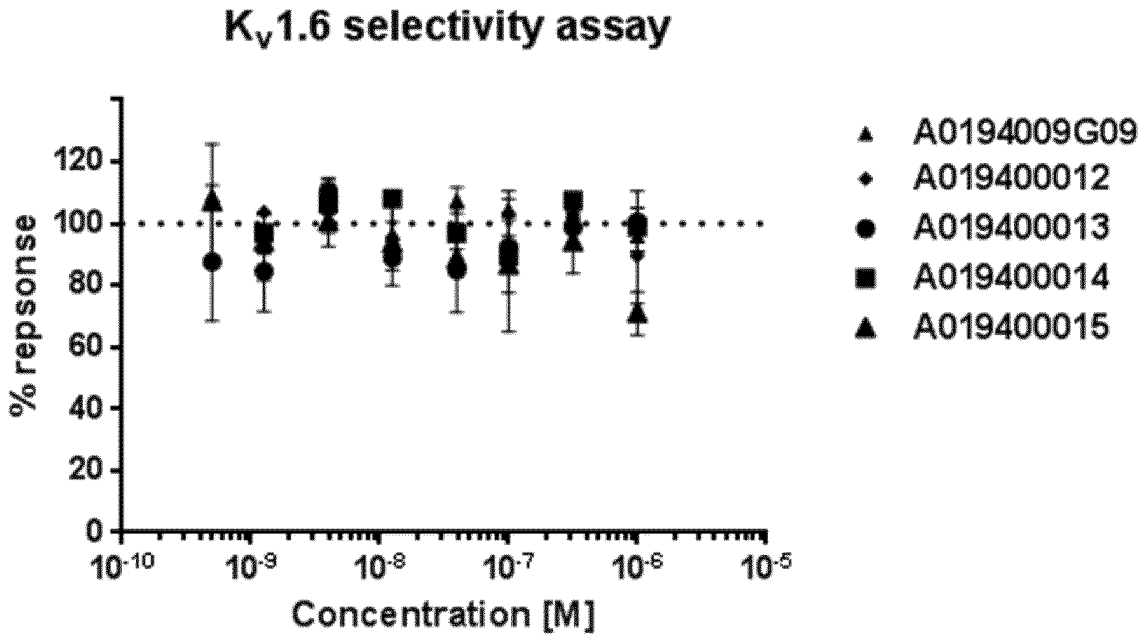


FIG. 36B

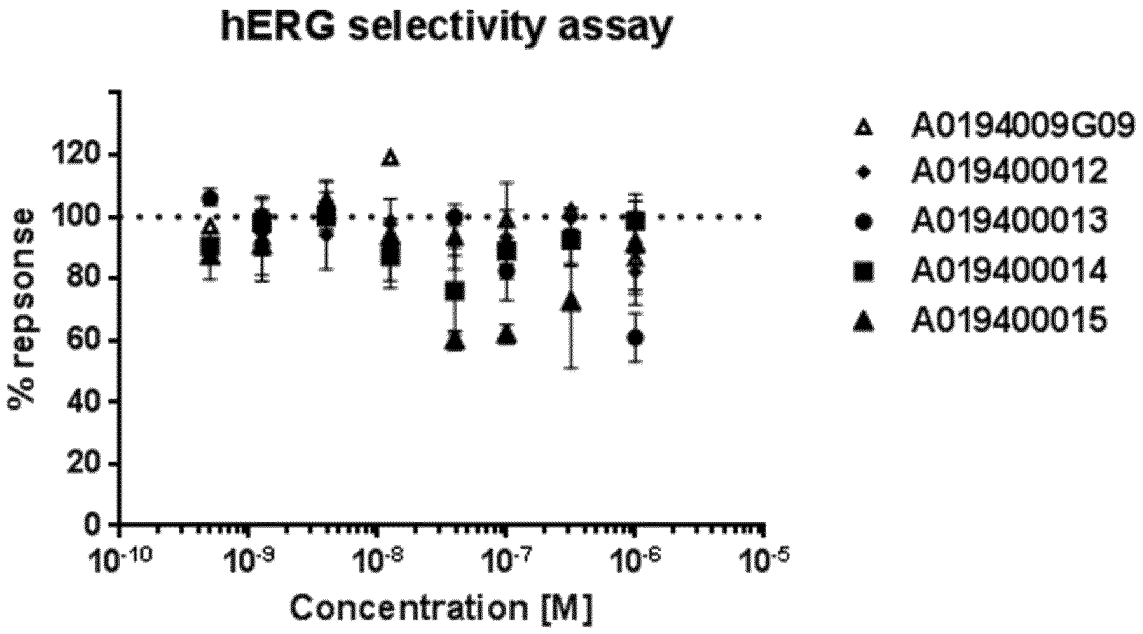


FIG. 36C

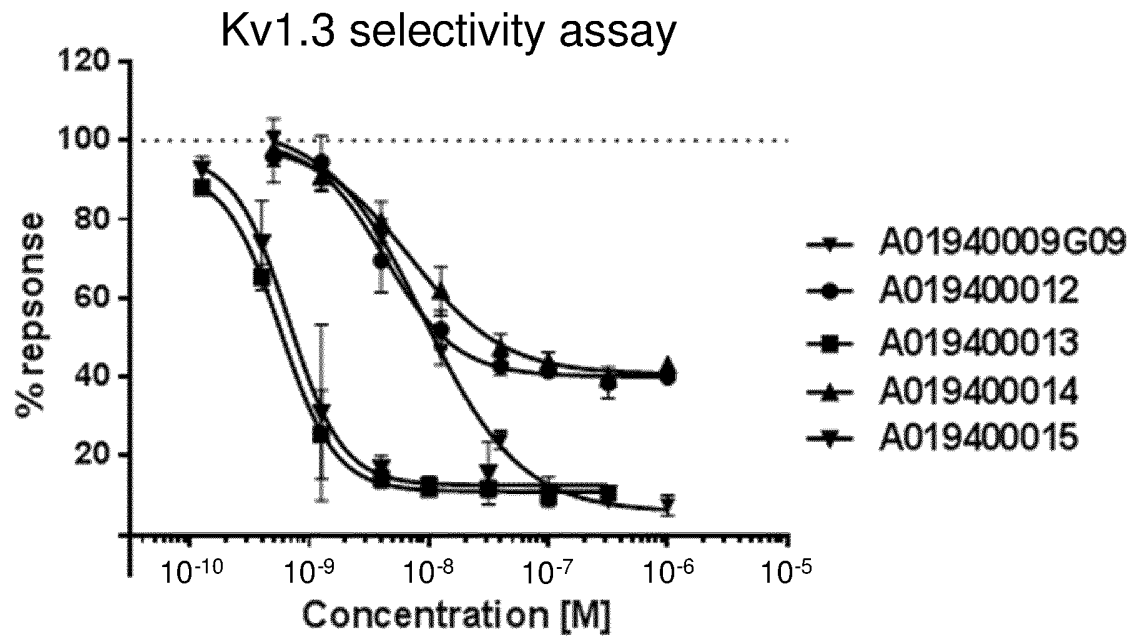


FIG. 36D

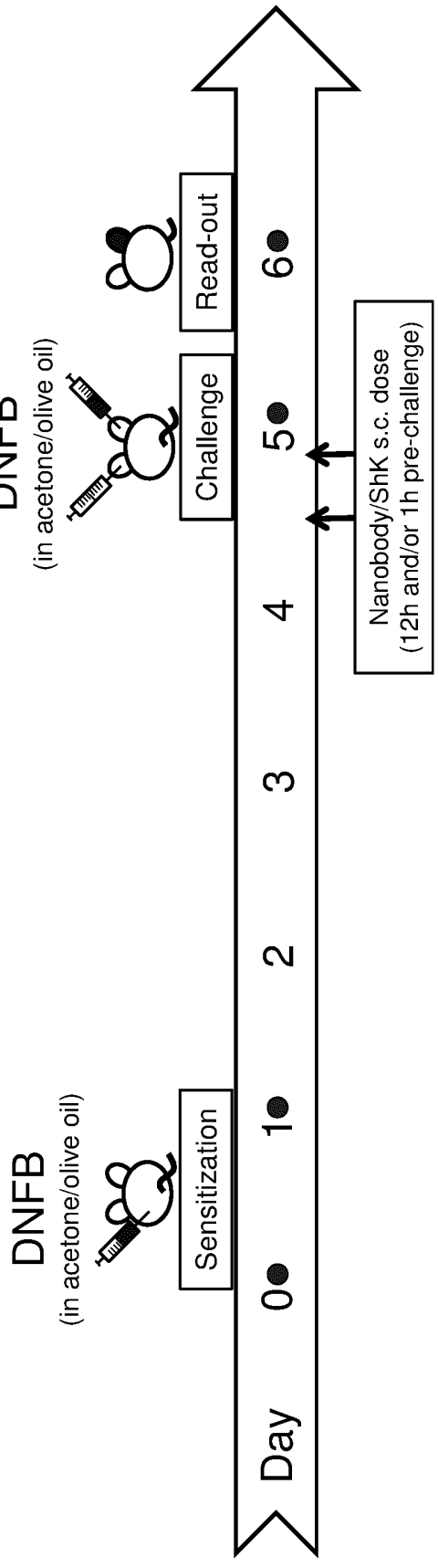

DNFB
(in acetone/olive oil)
Sensitization
DNFB
(in acetone/olive oil)
Challenge
Read-out
Nanobody/ShK s.c. dose
(12h and/or 1h pre-challenge)
Day
0
1
2
3
4
5
6

FIG. 37

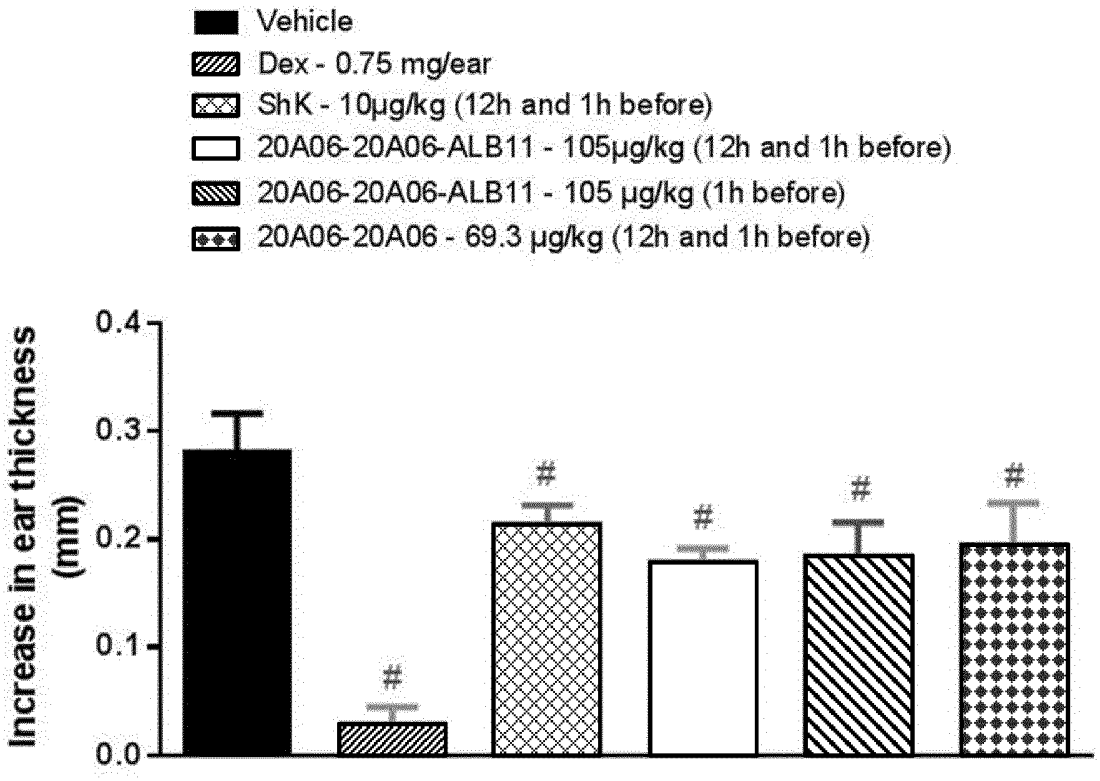

Vehicle
Dex - 0.75 mg/ear
ShK - 10µg/kg (12h and 1h before)
20A06-20A06-ALB11 - 105µg/kg (12h and 1h before)
20A06-20A06-ALB11 - 105 µg/kg (1h before)
20A06-20A06 - 69.3 µg/kg (12h and 1h before)
Increase in ear thickness (mm)
0.4
0.3
0.2
0.1
0.0
#
#
#
#
#

FIG. 38

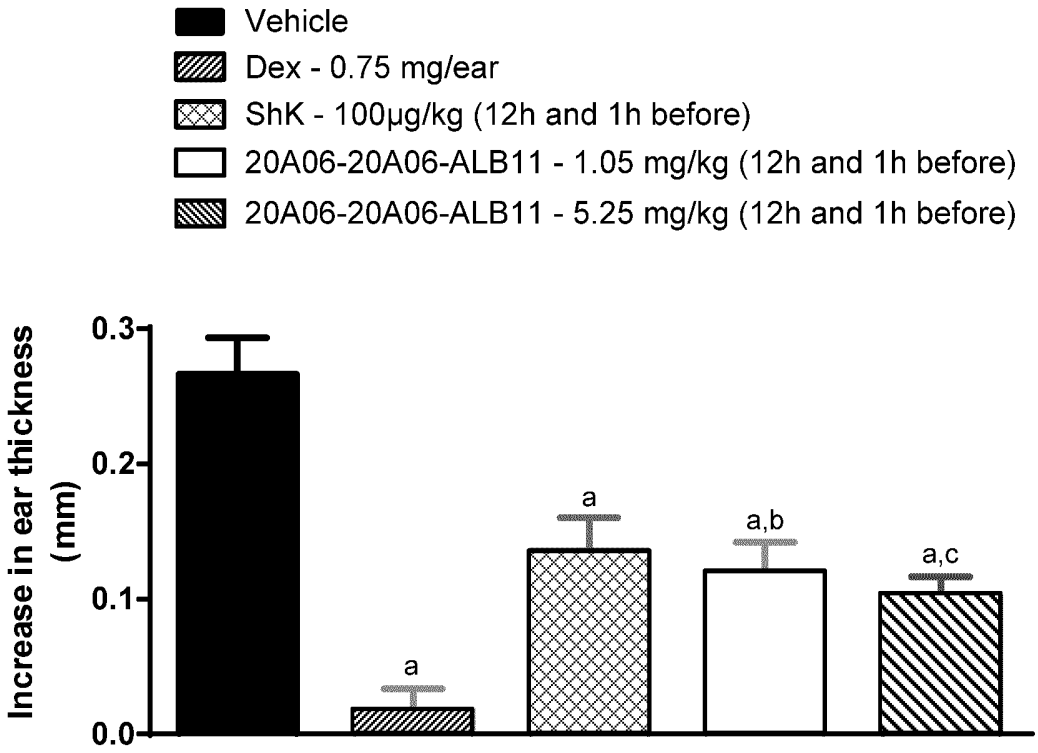

Vehicle
Dex - 0.75 mg/ear
ShK - 100µg/kg (12h and 1h before)
20A06-20A06-ALB11 - 1.05 mg/kg (12h and 1h before)
20A06-20A06-ALB11 - 5.25 mg/kg (12h and 1h before)
Increase in ear thickness (mm)
0.3
0.2
0.1
0.0
a
a
a,b
a,c

FIG. 39

Title: **KV1.3 BINDING IMMUNOGLOBULINS**

ABSTRACT

The present invention relates to immunoglobulins that specifically bind Kv1.3 and more in particular to polypeptides, nucleic acids encoding such polypeptides; to methods for preparing such polypeptides; to compositions and in particular to pharmaceutical compositions that comprise such polypeptides, for prophylactic, therapeutic or diagnostic purposes. In particular, the immunoglobulins of the present invention inhibit the activity of Kv1.3.

Agentschap NL
Ministerie van Economische Zaken

OCTROOIAANVRAAG NR.:
NO 139213
NL 2013661

ONDERZOEKSRAPPORT

BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK

RELEVANTE LITERATUUR

Categorie[1]	Literatuur met, voor zover nodig, aanduiding van speciaal van belang zijnde tekstgedeelten of figuren.	Van belang voor conclusie(s) nr:	Classificatie (IPC)
X	ALVARO DUQUE ET AL: "Expression of Kv1.3 potassium channels regulates density of cortical interneurons", DEVELOPMENTAL NEUROBIOLOGY, 1 juli 2013 (2013-07-01), bladzijden n/a-n/a, XP055188884, ISSN: 1932-8451, DOI: 10.1002/dneu.22105 * bladzijde 843, linker kolom, alinea 2 *	1-27	INV. C07K16/28
A	JING ZHU ET AL: "Allowed N-glycosylation sites on the Kv1.2 potassium channel S1-S2 linker: implications for linker secondary structure and the glycosylation effect on channel function", BIOCHEMICAL JOURNAL, deel 375, nr. 3, 1 november 2003 (2003-11-01), bladzijden 769-775, XP055188895, ISSN: 0264-6021, DOI: 10.1042/BJ20030517 * figuur 6A *	1-27	
A	XIAO-FANG YANG ET AL: "The Antibody Targeting the E314 Peptide of Human Kv1.3 Pore Region Serves as a Novel, Potent and Specific Channel Blocker", PLOS ONE, deel 7, nr. 4, 27 april 2012 (2012-04-27), bladzijde e36379, XP055188882, DOI: 10.1371/journal.pone.0036379 * figuren 3, 4 * * bladzijde 1, laatste alinea - bladzijde 2, alinea 1 *	1-27	Onderzochte gebieden van de techniek: C07K

-/--

Indien gewijzigde conclusies zijn ingediend, heeft dit rapport betrekking op de conclusies ingediend op:

Plaats van onderzoek:	Datum waarop het onderzoek werd voltooid:	Bevoegd ambtenaar:
's-Gravenhage	13 mei 2015	Brouns, Gaby

[1] CATEGORIE VAN DE VERMELDE LITERATUUR

X: de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur
Y: de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht
A: niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft
O: niet-schriftelijke stand van de techniek
P: tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur
T: na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding
E: eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven
D: in de octrooiaanvrage vermeld
L: om andere redenen vermelde literatuur
&: lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

1

EOB FORM 02.83 (P0414B)

RELEVANTE LITERATUUR

Categorie[1]	Literatuur met, voor zover nodig, aanduiding van speciaal van belang zijnde tekstgedeelten of figuren.	Van belang voor conclusie(s) nr:
A L	CN 102 180 950 A (WUHAN UNION HOSPITAL) 14 september 2011 (2011-09-14) * conclusies 1, 2 * -& Kun Liu ET AL: "Human-derived voltage-gated potassium channel 1.3 immunogenic peptide fragment and application thereof" In: 14 september 2011 (2011-09-14), XP055189342, bladzijden 1-14, -----	1-27
A	Y. YANG ET AL: "Specific Kv1.3 blockade modulates key cholesterol-metabolism-associated molecules in human macrophages exposed to ox-LDL", THE JOURNAL OF LIPID RESEARCH, deel 54, nr. 1, 24 oktober 2012 (2012-10-24), bladzijden 34-43, XP055189220, ISSN: 0022-2275, DOI: 10.1194/jlr.M023846 -----	1-27

1

[1] CATEGORIE VAN DE VERMELDE LITERATUUR

X: de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur
Y: de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht
A: niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft
O: niet-schriftelijke stand van de techniek
P: tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur
T: na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding
E: eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven
D: in de octrooiaanvrage vermeld
L: om andere redenen vermelde literatuur
&: lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

EOB FORM 02.83 (P0414C)

AANHANGSEL BEHORENDE BIJ HET RAPPORT BETREFFENDE HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK, UITGEVOERD IN DE OCTROOIAANVRAGE NR.

NO 139213
NL 2013661

Het aanhangsel bevat een opgave van elders gepubliceerde octrooiaanvragen of octrooien (zogenaamde leden van dezelfde octrooifamilie), die overeenkomen met octrooischriften genoemd in het rapport.
De opgave is samengesteld aan de hand van gegevens uit het computerbestand van het Europees Octrooibureau per
De juistheid en volledigheid van deze opgave wordt noch door het Europees Octrooibureau, noch door het Bureau voor de Industriële eigendom gegarandeerd; de gegevens worden verstrekt voor informatiedoeleinden.

13-05-2015

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
CN 102180950 A	14-09-2011	GEEN	

EPO FORM P0466

Algemene informatie over dit aanhangsel is gepubliceerd in de 'Official Journal' van het Europees Octrooibureau nr 12/82 blz 448 ev

SCHRIFTELIJKE OPINIE

DOSSIER NUMMER	INDIENINGSDATUM	VOORRANGSDATUM	AANVRAAGNUMMER
NO139213	21.10.2014		NL2013661

CLASSIFICATIE
INV. C07K16/28

AANVRAGER
Ablynx N.V.

Deze schriftelijke opinie bevat een toelichting op de volgende onderdelen:

- ☒ Onderdeel I Basis van de schriftelijke opinie
- ☐ Onderdeel II Voorrang
- ☐ Onderdeel III Vaststelling nieuwheid, inventiviteit en industriële toepasbaarheid niet mogelijk
- ☐ Onderdeel IV De aanvraag heeft betrekking op meer dan één uitvinding
- ☒ Onderdeel V Gemotiveerde verklaring ten aanzien van nieuwheid, inventiviteit en industriële toepasbaarheid
- ☐ Onderdeel VI Andere geciteerde documenten
- ☒ Onderdeel VII Overige gebreken
- ☒ Onderdeel VIII Overige opmerkingen

	DE BEVOEGDE AMBTENAAR

Aanvraag nr.:

SCHRIFTELIJKE OPINIE

NL2013661

Onderdeel I Basis van de Schriftelijke Opinie

1. Deze schriftelijke opinie is opgesteld op basis van de meest recente conclusies ingediend voor aanvang van het onderzoek.

2. Met betrekking tot **nucleotide en/of aminozuur sequenties** die genoemd worden in de aanvraag en relevant zijn voor de uitvinding zoals beschreven in de conclusies, is dit onderzoek gedaan op basis van:

   a. type materiaal:

   ☒ sequentie opsomming

   ☐ tabel met betrekking tot de sequentie lijst

   b. vorm van het materiaal:

   ☐ op papier

   ☒ in elektronische vorm

   c. moment van indiening/aanlevering:

   ☒ opgenomen in de aanvraag zoals ingediend

   ☐ samen met de aanvraag elektronisch ingediend

   ☐ later aangeleverd voor het onderzoek

3. ☐ In geval er meer dan één versie of kopie van een sequentie opsomming of tabel met betrekking op een sequentie is ingediend of aangeleverd, zijn de benodigde verklaringen ingediend dat de informatie in de latere of additionele kopieën identiek is aan de aanvraag zoals ingediend of niet meer informatie bevatten dan de aanvraag zoals oorspronkelijk werd ingediend.

4. Overige opmerkingen:

Aanvraag nr.:

SCHRIFTELIJKE OPINIE

NL2013661

Onderdeel V Gemotiveerde verklaring ten aanzien van nieuwheid, inventiviteit en industriële toepasbaarheid

1. Verklaring

Nieuwheid	Ja:	Conclusies 7-16, 19-27
	Nee:	Conclusies 1-6, 17, 18
Inventiviteit	Ja:	Conclusies
	Nee:	Conclusies 1-27
Industriële toepasbaarheid	Ja:	Conclusies 1-27
	Nee:	Conclusies

2. Citaties en toelichting:

Zie aparte bladzijde

Onderdeel VII Overige gebreken

De volgende gebreken in de vorm of inhoud van de aanvraag zijn opgemerkt:

Zie aparte bladzijde

Onderdeel VIII Overige opmerkingen

De volgende opmerkingen met betrekking tot de duidelijkheid van de conclusies, beschrijving, en figuren, of met betrekking tot de vraag of de conclusies nawerkbaar zijn, worden gemaakt:

Zie aparte bladzijde

The present application discloses VHH nanobodies obtained from immunisation of llama with human Voltage-Gated Potassium Channel Subunit Kv1.3. A number of nanobodies have been obtained and analysed for binding to human, rat and cynomolgus monkey Kv1.3, inhibition of binding of radiolabeled 125I-MgTX to Kv1.3, inhibition of ion channel activity, as well as interferon gamma and CD25 expression on T cells activated with anti-CD3 and an effect on delayed-type hypersensitivity (DTH).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 ALVARO DUQUE ET AL: "Expression of Kv1.3 potassium channels regulates density of cortical interneurons", DEVELOPMENTAL NEUROBIOLOGY, 1 juli 2013 (2013-07-01), bladzijden n/a-n/a, XP055188884

D2 JING ZHU ET AL: "Allowed N-glycosylation sites on the Kv1.2 potassium channel S1-S2 linker: implications for linker secondary structure and the glycosylation effect on channel function", BIOCHEMICAL JOURNAL, deel 375, nr. 3, 1 november 2003 (2003-11-01), bladzijden 769-775, XP055188895

D3 XIAO-FANG YANG ET AL: "The Antibody Targeting the E314 Peptide of Human Kv1.3 Pore Region Serves as a Novel, Potent and Specific Channel Blocker", PLOS ONE, deel 7, nr. 4, 27 april 2012 (2012-04-27), bladzijde e36379, XP055188882, DOI: 10.1371/journal.pone.0036379

The claimed subject-matter relates to immunoglobulins binding to the first extracellular loop (EL1) of Kv1.3, defined by functional features in claims 1-6 and structural features in claims 1-14 and 16. Only for a small number of structurally defined immunoglobulins it has been shown that they comprise the desired functional features.

In particular, inhibition of Kv1.3 activity is only shown for nanobodies A0194009G09 (SEQ ID NO:3), A0194020A06 (SEQ ID NO:13) in monomeric or multimeric form, A0194016B04 (SEQ ID NO:5) only in monomeric form and A0194020B10 (SEQ ID NO:15) only in multimeric form.
In contrast, for A019400003 (SEQ ID NO:110) it is shown that said nanobody binds

human, rat and cynomolgus Kv1.3 expressed on CHO cells (table B-1) and blocks the interaction with radiolabeled margatoxin (table B-2), but no effect on efflux of potassium ions from activated T cells is shown (table B-5).

For all other immunoglobulins falling under the scope of the claims no effect has been shown. It is well known that even a single amino acid substitution can have a significant effect on the binding specificity and function of an immunoglobulin, therefore it is not possible to generalise the amino acid sequences of immunoglobulins for which an effect has been shown.

NOVELTY

D1 discloses an immunoglobulin specifically binding to a peptide corresponding to amino acids 263-276 of human Kv1.3, which is located within the first extracellular loop (EL1) spanning aa 254-294 (**D2**, fig. 6A). Said immunoglobulins have the technical features of *claims 1-6, 17 and 18* the subject-matter of said claims thus lacks novelty. However, the subject-matter indicated above relating to defined immunoglobulins for which a technical effect has been shown, appears not to be disclosed in the prior art.

INVENTIVE STEP

D3 discloses a rabbit polyclonal antibody directed against aa 420-433 of human Kv1.3, which are located in the third extracellular loop of said protein (p.1, last §-p2, §1). It is shown that said immunoglobulins are capable of inhibiting Kv1.3 activity in HEK293 cells transfected with Kv1.3 (3), as well as in Jurkat T cells (fig. 4).
From this the subject-matter of the present invention differs in that immunoglobulins capable of inhibiting Kv1.3 activity recognise EL1. No effect for said difference has been demonstrated in a comparative example, therefore the problem to be solved may be seen as how to provide further immunoglobulins capable of inhibiting Kv1.3 activity.

The solution as claimed comprises an inventive step for those immunoglobulins defined above for which a technical effect has been demonstrated. Said immunoglobulins may not be obviously derived from the prior art.
Immunoglobulins for which it has not been shown that they inhibit Kv1.3 activity are merely obvious alternatives for anti-EL1 antibodies known from D1, devoid of inventive step.

Re Item VII

Certain defects in the application

Claim 27 relates to diseases which are to be treated or prevented using the immunoglobulins of the invention. However, the application does not demonstrate in any way that indeed said diseases may be thus treated or prevented, with the exception of DTH (exp.13).
It is considered to be undue burden on the skilled person to practise the invention for all diseases except for DTH. Said subject-matter is thus insufficiently disclosed and supported.

Re Item VIII

Certain observations on the application

The terms 'modulate' and 'inhibit' in claims 1 and 5 does not allow the skilled person to define immunoglobulins falling under the scope of the claims.

Similarly, the term 'partial blocking' in claim 2 and 'reduction of efflux of potassium ions' in claim 3 lack clarity.

The skilled person is not enabled to identify when the half-life is 'increased' (claims 23, 24).

'A disease related to Kv1.3' is no well defined medical condition (claim 26).

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