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(54) **Title:** MHO IB-MEDIATED MYELIN-SPECIFIC IMMUNOSUPPRESSION AS A NOVEL TREATMENT FOR MULTIPLE SCLEROSIS AND MOG ANTIBODY DISEASE

(57) **Abstract:** The present invention relates to therapeutical uses of non-classical human major histocompatibility complex (MHC) molecules (also named MHC class lb molecules) in combination with myelin-associated peptide antigens for the treatment of multiple sclerosis (MS), MOG antibody disease and MOG antibody positive neuromyelitis optica. The invention more specifically relates to recombinant polypeptides comprising peptide antigens and one or more domains of a non-classical MHC class lb molecule. The invention also relates to methods of producing such recombinant polypeptides, pharmaceutical compositions comprising the same, as well as their uses for treating multiple sclerosis (MS), MOG antibody disease and MOG antibody positive neuromyelitis optica.

MHC Ib-mediated myelin-specific immunosuppression as a novel treatment for multiple sclerosis and MOG antibody disease

FIELD OF THE INVENTION

The present invention relates to therapeutical uses of non-classical human major histocompatibility complex (MHC) molecules (also named MHC class Ib molecules) in combination with peptide antigens for the treatment of multiple sclerosis (MS), MOG antibody disease and MOG antibody positive neuromyelitis optica. The invention more specifically relates to recombinant polypeptides comprising peptide antigens and one or more domains of a non-classical MHC class Ib molecule. The invention also relates to methods of producing such recombinant polypeptides, pharmaceutical compositions comprising the same, as well as their uses for treating multiple sclerosis (MS), MOG antibody disease and MOG antibody positive neuromyelitis optica.

BACKGROUND

Multiple sclerosis (MS) and MOG encephalomyelitis (also known as myelin-oligodendrocyte glycoprotein antibody disease, MOG antibody disease, MOGAD) are autoimmune diseases in which T cells of the immune system attack myelin sheaths in the central nervous system, resulting in gradually progressive neurodegeneration. The relapsing flare-up of disease activity characteristic of MS can be effectively suppressed by immunomodulatory therapies. For the rare MOGAD, which has long been considered a subtype of MS, there are no approved therapeutics, so therapy initially consists of watching and waiting for the disease to progress. In both diseases, slowly progressive neurodegeneration occurs. Some therapeutics effective in MS, such as the antibodies natalizumab or ocrelizumab or the orally bioavailable S1P inhibitor fingolimod, even carry the risk that a persistent and normally harmless intracerebral infection with JC virus can no longer be controlled, resulting in an often fatal progressive multifocal leukoencephalopathy. In this respect, the problem of either insufficient immunomodulation or immunomodulation with too severe side effects remains unsolved in both diseases, especially with regard to slow progression. Common biologics do not cross the blood-brain barrier and thus cannot have an anti-inflammatory effect in situ. In contrast, regulatory cells have been described to cross it well (Schneider-Hohendorf et al., Eur J Immunol. 2010 Dec;40(12):3581-90).

Two strategies have been evaluated so far, at least in early clinical studies. Similar to hyposensitization strategies in allergy, large amounts of antigens have been administered by different routes to induce antigen-specific tolerance. However, in autoimmune diseases, these strategies have caused severe side effects and have not been clinically successful. Attempts to induce tolerance by adoptive transfer of antigen-specific regulatory T cells or antigen-loaded tolerogenic dendritic cells appear more promising. However, these strategies are extremely complex and expensive, requiring GMP-compliant production and quality control processes for each individual patient. Therefore, even if small clinical trials have been successful, it is highly questionable whether adoptive transfer therapies will be available for many patients in the foreseeable future.

Immunosuppressive MHC class Ib molecules such as HLA-G are critical for tolerance induction during pregnancy. They exert immunosuppressive effects on various immune cells via immunosuppressive receptors such as ILT2, ILT4 and Kir2DL4. WO 2018/215340 relates to combinations of MHC class Ib molecules and peptides for targeted therapeutic immunomodulation.

Taken together, there remains a need for improved drugs for the treatment of multiple sclerosis (MS) and MOG antibody disease. Similarly, there also remains a need for improved drugs for the treatment of MOG antibody positive neuromyelitis optica (NMO).

DESCRIPTION OF THE INVENTION

The inventors have found that human MHC class Ib molecules such as HLA-G possess the ability to induce antigen-specific tolerance towards presented peptide antigens. Thus, albeit being of similar structure and sequence as classical human MHC class Ia molecules which induce antigen peptide-specific immune responses, MHC class Ib molecules can advantageously be used according to the invention to suppress immune responses in an antigen-specific manner. Additionally, the inventors have found that for the suppression of immune responses according to the invention, molecules other than naturally occurring MHC class Ib molecules, and in particular polypeptides which only comprise at least one domain of an MHC class Ib molecule, preferably at least an [alpha]3 domain of an MHC class Ib molecule, can be used: The [alpha]1 and [alpha]2 domains of variable class I a molecules can be combined with the [alpha]3 domain of a human MHC class Ib molecule in order to suppress immune responses towards peptides presented by these antigens.

Antigen-loaded HLA-G molecules can be unstable. Thus, the inventors designed soluble recombinant polypeptides comprising a peptide antigen, an MHC class Ib molecule such as HLA-G and β2-microglobulin (b2m), and connected these three components covalently (e.g., via covalent linkers). Alternatively, the antigen-binding α1 and α2 domains of an MHC class Ib molecule such as HLA-G were exchanged by the respective domains of other MHC molecules to enhance the flexibility and versatility of these recombinant polypeptides (see, for instance, Figure 2). These alternative recombinant polypeptides can be designed with antigen-binding domains of other human HLA molecules. It was previously found that constructs comprising the α1 and α2 domains of murine H2-K^b can present the ovalbumin-derived peptide SIINFEKL to OT-1 T cells. (OT-1 T cells express a transgenic T cell receptor that specifically recognizes this antigen) (WO 2018/215340).

Surprisingly, the inventors have found that by using the recombinant polypeptides of the invention, immune responses against human myelin-oligodendrocyte glycoprotein (MOG), human myelin basic protein (MBP), human myelin-associated glycoprotein (MAG), or human myelin proteolipid protein (PLP1) can be

suppressed. Thus, according to the invention, multiple sclerosis (MS), myelin-oligodendrocyte glycoprotein antibody disease (MOG antibody disease) and MOG antibody positive neuromyelitis optica can be treated by the recombinant polypeptides of the invention.

Experimental data of the inventors show that a suitable peptide antigen and the presence of an [alpha]3 domain of an MHC class Ib molecule (e.g. HLA-G) are required to achieve the desired effect. Therefore, this approach goes beyond previously described strategies that either use antigenic peptides in the absence of costimulation (resulting in anergic rather than tolerogenic T cells), or MHC class Ib molecules in an antigen-unspecific setting.

Furthermore, according to the invention, the recombinant polypeptides of the invention are expected to be highly advantageous in the immunoterapeutic treatment of multiple sclerosis (MS), MOG antibody disease and MOG antibody positive neuromyelitis optica, because are expected to show an improved safety profile as compared to conventional drugs aganst these diseases, which may cause severe side effects such as progressive multifocal leukoencephalopathy.

Moreover the inventors have surprisingly found that the recombinant polypeptides of the invention do not only modulate T-cell responses but also prevent the formation of MOG-specific autoantibodies in model experiments. It is expected that this finding will translate into a clinical improvement in patients having multiple sclerosis (MS), MOG antibody disease and MOG antibody positive neuromyelitis optica, because MOG-specific autoantibodies are involved in the pathology of these diseases.

Accordingly, the invention relates to the following preferred embodiments:

1. A recombinant polypeptide capable of presenting a peptide antigen, the recombinant polypeptide comprising, in an N- to C-terminal order,
   i) a peptide antigen presented by said recombinant polypeptide, wherein the peptide antigen is a peptide of human myelin-oligodendrocyte glycoprotein (MOG), human myelin basic protein (MBP), human myelin-associated glycoprotein (MAG), or human myelin proteolipid protein (PLP1);
   ii) optionally a linker sequence;
   iii) optionally a sequence of a human polypeptide domain comprising a sequence of a human β2 microglobulin, or an amino acid sequence at least 90% identical to the amino acid sequence of human β2 microglobulin represented by SEQ ID NO: 5;
   iv) optionally a linker sequence;
   v) optionally an [alpha] 1 domain of an MHC molecule;
   vi) optionally an [alpha] 2 domain of an MHC molecule;

vii) an [alpha] 3 domain of an MHC class Ib molecule or a derivative of an [alpha] 3 domain of an MHC class Ib molecule, said derivative being capable of binding to ILT2 or ILT4;
viii) optionally a protease cleavage site;
ix) optionally a spacer sequence; and
x) optionally an affinity tag.

2. The recombinant polypeptide according to item 1, wherein said peptide antigen according to i) is 7 to 11 amino acids in length, preferably 8-10 amino acids in length.
3. The recombinant polypeptide according to item 1 or 2, wherein said peptide antigen according to i) consists of an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33.
4. The recombinant polypeptide according to item 1 or 2, wherein said peptide antigen according to i) consists of an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30.
5. The recombinant polypeptide according to any one of the preceding items, wherein said peptide antigen according to i) is a peptide of human myelin-oligodendrocyte glycoprotein (MOG).
6. The recombinant polypeptide according to any one of items 1-2 and 4-5, wherein said peptide antigen consists of the amino acid sequence of SEQ ID NO: 2.
7. The recombinant polypeptide according to any one of the preceding items, wherein said [alpha]1 domain according to (v) and said [alpha]2 domain according to (vi) are from a human MHC class Ia molecule or from a human MHC class Ib molecule.
8. The recombinant polypeptide according to item 7, wherein said [alpha]1 domain according to (v) and said [alpha]2 domain according to (vi) are from a human MHC class Ia molecule.
9. The recombinant polypeptide according to item 8, wherein said [alpha]1 domain according to (v) and said [alpha]2 domain according to (vi) are from a human HLA-A2 molecule.
10. The recombinant polypeptide according to item 7, wherein said [alpha]1 domain according to (v) and said [alpha]2 domain according to (vi) are from a human MHC class Ib molecule.
11. The recombinant polypeptide according to any one of the preceding items, wherein the [alpha] 3 domain of the MHC class Ib molecule according to (vii) is an [alpha] 3 domain of human HLA-E, human HLA-F or human HLA-G.
12. The recombinant polypeptide according to any one of the preceding items, wherein the [alpha] 3 domain of the MHC class Ib molecule according to (vii) is an [alpha] 3 domain of human HLA-G.
13. The recombinant polypeptide according to any one of the preceding items, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 80% amino acid sequence identity, preferably at least 90% amino acid sequence identity, with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.

14. The recombinant polypeptide according to item 13, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 92% amino acid sequence identity with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
15. The recombinant polypeptide according to item 13, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 94% amino acid sequence identity with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
16. The recombinant polypeptide according to item 13, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 96% amino acid sequence identity with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
17. The recombinant polypeptide according to item 13, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 98% amino acid sequence identity with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
18. The recombinant polypeptide according to item 13, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 99% amino acid sequence identity with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
19. The recombinant polypeptide according to item 13, wherein the [alpha]3 domain according to (vii) is identical to the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
20. The recombinant polypeptide according to any one of the preceding items, wherein the linker sequence according to (ii) and/or the linker sequence according to (iv) comprises the amino acid sequence (GGGGS)n, wherein n is an integer equal to or higher than 1.
21. The recombinant polypeptide according to item 20, wherein the linker sequence according to (ii) comprises the amino acid sequence (GGGGS)n, and wherein n is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 and is preferably selected from the group consisting of 2, 3, 4 and 5.
22. The recombinant polypeptide according to item 20 or 21, wherein the linker sequence according to (iv) comprises the amino acid sequence (GGGGS)n, and wherein n is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 and is preferably selected from the group consisting of 2, 3, 4 and 5.
23. The recombinant polypeptide according to any one of the preceding items, wherein said sequence of a human polypeptide domain according to (iii) is at least 95% identical to the amino acid sequence of SEQ ID NO: 5, preferably at least 98% identical to the amino acid sequence of SEQ ID NO: 5 and more preferably identical to the amino acid sequence of SEQ ID NO: 5.
24. The recombinant polypeptide according to any one of the preceding items, wherein said polypeptide is dimeric or multimeric.
25. The recombinant polypeptide according to any one of the preceding items, wherein the polypeptide comprises or consists of all of the components i) to vii)

26. The recombinant polypeptide according to any one of the preceding items, wherein the polypeptide does not comprise components viii) to x).
27. The recombinant polypeptide according to any one of items 1 to 25, wherein the polypeptide comprises or consists of all of the components i) to x).
28. The recombinant polypeptide according to any one of the preceding items, further comprising an N-terminal secretion signal peptide sequence.
29. The recombinant polypeptide according to any one of items 1-27, wherein the recombinant polypeptide consists of an amino acid sequence consisting of the following ((a) and (b)) in an N- to C-terminal order:

(a) a peptide antigen selected from the group consisting of the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33; and

(b) the amino acid sequence of SEQ ID NO: 16.

30. The recombinant polypeptide according to any one of the preceding items, wherein the recombinant polypeptide is soluble.
31. A nucleic acid encoding one or more polypeptides according to any one of the preceding items.
32. The nucleic acid according to item 31, wherein the nucleic acid is a vector.
33. A pharmaceutical composition comprising at least one nucleic acid according to items 31 or 32.
34. A pharmaceutical composition or kit comprising at least one recombinant polypeptide according to any one of items 1-30.
35. The pharmaceutical composition or kit according to item 34, wherein the pharmaceutical composition or kit comprises at least two different recombinant polypeptides according to any one of items 1-30, and wherein each of the different polypeptides comprises a different peptide antigen as defined in any one of items 3 to 6.
36. A pharmaceutical composition or kit according to any one of items 33-35, for use in the treatment of multiple sclerosis (MS), MOG antibody disease or MOG antibody positive neuromyelitis optica in a human patient.
37. The pharmaceutical composition or kit for use according to item 36, wherein the treatment is a treatment of multiple sclerosis (MS).
38. The pharmaceutical composition or kit for use according to item 36, wherein the treatment is a treatment of MOG antibody disease.
39. The pharmaceutical composition or kit for use according to item 36, wherein the treatment is a treatment of MOG antibody positive neuromyelitis optica.
40. The pharmaceutical composition or kit for use according to any one of items 36-39, wherein the treatment is treatment by immunotherapy.

41. The pharmaceutical composition or kit for use according to any one of items 36-40, wherein the treatment is by inducing immunological tolerance against human myelin-oligodendrocyte glycoprotein.
42. The pharmaceutical composition or kit for use according to any one of items 36-41, wherein the treatment is for reducing plasma or cerebrospinal fluid levels of autoantibodies against human myelin-oligodendrocyte glycoprotein.
43. The pharmaceutical composition or kit for use according to any one of items 36-42, wherein the human patient is a patient who had plasma or cerebrospinal fluid autoantibodies against myelin-oligodendrocyte glycoprotein prior to the start of the treatment.
44. The pharmaceutical composition or kit for use according to any one of items 36-43, wherein the treatment is by inducing myelin-specific regulatory T cells.
45. A recombinant host cell comprising a nucleic acid or a vector according to item 31 or 32 and expressing the recombinant polypeptide according to any one of items 1-30.
46. A method for obtaining pharmaceutical composition comprising a polypeptide according to any one of items 1-30, the method comprising the steps of (a) culturing the recombinant host cell of item 45 under conditions allowing expression of the recombinant polypeptide from the nucleic acid molecule, (b) recovering the recombinant polypeptide, (c) purifying the recombinant polypeptide, and (d) formulating the recombinant polypeptide into a pharmaceutical composition.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Depiction of a peptide-loaded soluble MHC Ib molecule suitable to achieve therapeutic antigen-specific immunomodulation.

The presented peptide antigenis depicted in dotted spheres, the HLA-G alpha1-3 domains are sketched in light-grey, and the beta2microglobulin domain is shown in dark grey. An optional linker connecting the antigenic peptide with the beta2microglobulin molecule is displayed in grey stick style, and an optional disulfide trap is depicted in black spheres. This figure was generated using Pymol and is adapted from structures published in Clements et al., Proc Natl Acad Sci U S A. 2005 Mar 1;102(9):3360-5 and Hansen et al., Trends Immunol. 2010 Oct;31(10):363-9.

Figure 2: Example for a vector-based construct encoding a single chain MHC Ib molecule suitable for therapeutic peptide-specific immunomodulation.

HLA-G1 and HLA-G5 each consist of 3 [alpha] domains (here in black), a non-covalently associated beta 2-microglobulin subunit (here in dark grey) and the antigenic peptide presented on HLA-G (short black arrow). HLA-G1 further contains a transmembrane domain and a short intracellular chain (not shown here). As shown here, the [alpha]-3 domain is capable of binding to the receptors ILT2 (see Shiroishi et al., Proc Natl Acad Sci U S A. 2003 July 22;100(15):8856-8861) and ILT4 (see Shiroishi et al., Proc Natl Acad Sci U S A. 2006 Oct 31;103(44):16412-7) on immune cells. Physiologically, these sequences form a non-covalently linked MHC

class 1 complex. To simplify purification of the complex MHC Ib molecule, one or more protein tags (such as SpotTag, myc tag and/or His(6x) tag) may be introduced. They may be introduced in such a way as to enable their later optional removal via cleavage using an optional Factor Xa or Furin cleavage site. Furthermore, the antigenic peptide, beta 2-microglobulin and MHC Ib [alpha]chain can be linked in order to increase the stability. The vector map was generated using Snapgene Viewer Software.

Figure 3: Surrogates of recombinant polypeptides of the invention induce IL10 secreting Treg in mice.
In this experiment, 100µg of surrogate molecules consisting of a viral (Gp34) or Ovalbumin (Ova) model peptide antigen, murine H2-K^b alpha1 and 2 domains, and human HLA-G alpha 3 domain and beta-2-microglobulin were injected i.p. into 12 week old C57BL/6 mice. After 14 days, mice were sacrificed and splenocytes isolated via Ficoll gradient were rechallenged with 5µg/ml of either Gp34 or Ova peptide in an 48h standard murine IL-10 ELISpot assay (Mabtech mouse IL-10 HRP ELISpot Kit) (A).
A significant increase in regulatory T cells that secreted IL-10 only in response to a rechallenge with the peptide towards which tolerance was induced via surrogate molecule injection was detected (B).

Figure 4: Surrogates of recombinant polypeptides of the invention prevent CD8+ T-cell driven EAE in mice.
In this MS mouse model, the model antigen ovalbumin (OVA) is expressed in oligodendrocytes under the control of the myelin basic protein (MBP) promoter (ODC-OVA). This leads to the presentation of the OVA257-264 peptide on H-2Kb MHC molecules on oligodendrocytes. OT-I mice express a T cell receptor (OT-I) on their CD8+ T cells, which recognizes exactly this peptide-MHC combination. When CD8+ T cells from these mice are transferred into 10 day old ODC-OVA mice, these develop an experimental autoimmune encephalomyelitis (EAE) which resembles in many aspects the pathogenesis and symptomatology of MS (Na et al., *Brain*, Volume 131, Issue 9, September 2008, Pages 2353–2365). In this experiment, 500µg of surrogate molecules consisting of a viral (Gp34) or Ovalbumin (Ova) model peptide antigen, murine H2-K^b alpha1 and 2 domains, and human HLA-G alpha 3 domain and beta-2-microglobulin or just PBS were injected the same day. EAE was scored according to Bittner et al., J Vis Exp . 2014 Apr 15;(86):51275.
Only Ovalbumin-tolerance inducing surrogate molecules almost completely prevented EAE symptoms.
(A) experimental design; (B) results.

Figure 5: Surrogates of recombinant polypeptides of the invention can result in effective bystander immunosuppression.
In this eperiment, it was tested wether the the Mog44 peptide surrogate molecule could induce protective T cells that can inhibit cytotoxic T cells targeting another peptide presented by the same cells. 250µg per mouse of this molecule described in Figure 4 were injected into with OT-I cells into ODC-OVA mice as described in Figure 4.

With this lower dose of surrogate molecules, EAE symptoms could not be completely prevented, but significantly reduced both with molecules inducing tolerance towards the directly targeted CD8 epitope (Ova_KbG) or towards another epitope (Mog44) expressed on the same cells (Mog44_DbG).
(A) EAE score; (B): body weight.

Figure 6: Some surrogates of recombinant polypeptides of the invention selectively prevent $CD4^+$ T cell driven EAE in mice.
(A) experimental design In this model, a strong, myelin-specific autoimmune response is triggered by administration of MOG 35-55 peptide in combination with Complete Freund's adjuvant, which activates CD4+ Th17 cells, and pertussis toxin, which makes the blood-brain barrier more permeable (Protocol: Bittner et al., J Vis Exp . 2014 Apr 15;(86):51275). Here, CD4+ cells as well as antibodies play a crucial role in the development of EAE (Tigno-Aranjuez et al., J Immunol November 1, 2009, 183 (9) 5654-5661). In addition, 100µg/mouse of surrogate molecules consisting of a viral (Gp34) or two Mog peptide antigens (Mog37 or Mog44), murine $H2\text{-}D^b$ alpha1 and 2 domains, and human HLA-G alpha 3 domain and beta-2-microglobulin or just PBS were injected the first day.
The Mog44 peptide containing surrogate molecule significantly reduced EAE symptoms (B) and weight loss (C).

Figure 7: Mog44 surrogates of recombinant polypeptides of the invention prevented inflammation and CD8 T cell infiltration in the spinal cord. (A) Toluidine; (B) CD8-DAB
10 µm fresh frozen sections were stained with comercial Toluidine 1x staining reagent for 1h at room temperature. A strong infiltration of immune cells was detected in EAE, but prevented by Mog44_Db_G.
10 µm fresh frozen sections were briefly dried at room temperature, fixed with acetone, blocked with 5% BSA 10% normal goat serum in PBS, stained with 1:100 anti-CD8 antibody, secondary antibody coupled to HRP and DAB solution (detailed methods: Karikari et al., Brain Behav Immun. 2022 Jan 12;101:194-210). Mog35-55 induced EAE lead to a strong infiltration of $CD8^+$ cells into the spinal cord which was prevented by MOG44_Db_G surrogate molecule.
(A) Toluidine; (B) CD8-DAB

Figure 8: Detection of anti-MOG35-55 antibodies in Mog-EAE mice treated with surrogates of recombinant polypeptides of the invention ("AIM Bio")
Murine serum was collected from heart puncture after mice were sacrificed and diluted 1:50 in PBS. 10µg/ml Mog35-55 in PBS were used for coating over night, wells were then blocked using 1%BSA for 2h, before diluted sera were added for 1h. Anti-Mog35-55 antibodies were detected using the indicated secondary HRP coupled antibodies (dilution 1:5000 in PBS). Mog35-55 induced EAE correlated with high levels of Mog35-55 specific IgG autoantibodies, which were not detectable in animals treated with 100µg MOG44_Db_G surrogate molecule.

Figure 9: List of the human MS & MOGAD recombinant polypeptide candidates.

The myelin (MAG, MBP, MOG, PLP) peptide and MHC class I presenting molecules are as follows:

Recombinant polypeptide	Peptide antigen sequence	SEQ ID NO:
MAG160_A2G_His	MVPDNCPEL	25
MAG160_G_His	MVPDNCPEL	25
MAG237_G_Spt	KYPPVIVEM	34
MOG104_A2G_Spt	AIGEGKVTL	26
MOG104_A2G_Spt	AIGEGKVTL	26
MBP29_G_Spt	FLPRHRDTG	35
MBP75_G_Spt	RSQPGLCNM	27
MBP169_G_Spt	KGVDAQGTL	36
MBP244_A2G_Spt	SLSRFSWGA	28
MOG38_G_Spt	RHPIRALVG	31
MOG42_G_Spt	RALVGDEVEL	32
MOG44_G_Spt	FSRVVHLYRNG	37
MOG70_G_Spt	RPPFSRVVHL	38
MOG102_G_Spt	KDAIGEGKVTL	29
MOG104_A2G_Spt	AIGEGKVTL	26
MOG157_A2G_Spt	VLLAVLPVL	2
MOG157_G_Spt	VLLAVLPVL	2
MOG163_G_Spt	VSPGVLVLL	39
MOG185_G_His	KLRAEIENL	40
MOG203_A2G_His	RVPCWKITL	41
MOG203_G_His	RVPCWKITL	41
PLP3_A2G_His	LLLECCARCL	33
PLP3_G_His	LLLECCARCL	33
PLP80_A2G_His	FLYGALLLA	42
PLP105_G_Spt	KTTICGKGL	43
PLP127_G_His	RGQHQAHSL	44
PLP151_G_spt	KFVGITYAL	45

It is further shown which combinations of myelin peptides and antigen-presenting MHC class I alpha 1 and 2 domains (HLAG= HLA-G, A2G = HLA-A2 presenting domains + HLA-G alpha 3 domain) lead to good results with regards to expression/production, ELISpot based prioritization in healthy blood donors (described in

Figure 10) or AlphaFold2 prediction. His indicates a 6-Histidine Tag, Spt indicates a Spot-Tag.

Figure 10: Upregulation of CD8 Treg in healthy blood donors by a recombinant polypeotide of the invention containing the VLLAVLPVL antigen ("Mog157_A2G")

In vitro Treg induction mediated by AIM Biologicals was carried out as follows:

PBMCs from healthy donors were purified via density centrifugation was performed on white blood cells from a leukocyte reduction chamber using Ficoll. Cells were centrifuged for 20 min at 1200 x g without brake followed by collection of the interphase ring that was washed with 1x PBS (5 min, 300 x g). PBMC were frozen till further use.

PBMCs were thawed 1 day prior to PBMC pulsing (d-1) and kept over night in 5 ml X-VIVO 15 medium containing 5% human AB serum in a well of a 6 well plate at 37°C.

On the next day (d0), cells were counted and resuspended in X-VIVO 15 complete medium (5% hAB serum & cytokine cocktail: 20 ng/ml hIL-2, 20 ng/ml hGM-CSF, 10 ng/ml hIL-4 & 10 ng/ml hTGF-b1) at a cell density of $3x10^6$ cells/ml. For experiments, $3x10^6$ cells were seeded in the respective wells of a 12-well plate with a final volume of 1000 µl X-VIVO complete medium with cytokine cocktail and 5 µg/ml of an AIM Bio molecule or the respective controls.

On day 3, 1 ml complete medium (with cytokines) was added, on day 6, a second pulse with 5 µg/ml AIM Bio molecule was performed (after removing medium). On days 7, 10 & 12, 1 ml complete medium (with cytokines) was added.

On day 13, ELISPOT plate PVDF membrane was activated with 50 µl/well EtOH (35% v/v) for 1 min followed by 5x washing with 200 µl distilled sterile water. Plate was coated with 100 µl/well anti-hIL10 (clone 9D-7, 1:500 dilution in PBS, sterile filtered) at 4°C over night. On the next day, unbound coating antibody was removed, 5 washing steps were performed with 200 µl PBS and 200 µl blocking buffer (X-VIVO 15 5% hAB serum) was added and the plate incubated for 30 min - 2 h at room temperature.

Day 14, 200,000 cells were seeded per well on the ELISPOT plates in duplicates, including negative controls (cells plus PBS) and a positive control (e.g. LPS).

Secondary antibody was prepared: 1 µg/ml alL-10-biotinylated antibody in 0.5% BSA/1x PBS (1:1000 dilution) and horseradish peroxidase-conjugated streptavidin (1:750 in 0.5% BSA/PBS), tetramethylbenzidine solution was filtered using a 0.45 µm filter and stored at 4°C till use.

Cell supernatant was removed and 5x washed using 100 µl PBS. Last excess buffer was removed using paper towels.

25 µl diluted HRP-streptavidin (1:750) was added per well and incubated for 1 h at room temperature in the dark followed by 5 washing steps using sterile 1xPBS.

100 µl of filtered TMB substrate was added per well for 15-25 min till blue sports developed. Reaction was stopped by washing the wells thoroughly with tapped water.

Plastic underdrains of the plates was removed and the bottom and sides of the plates were washed with tap water and dried.

MOG157_A2G_Spt induced at least 30% more IL-10 secreting T reg in 75% of of all healthy blood donors.

Figure 11: Control experiment for Figure 8 and showing that total IgG is not reduced by MOG47_Db_G surrogate molecule treatment. Easy-Titer™ Human IgG (gamma chain) Assay Kit (Thermo Fisher) was used to quantify total IgG according to the manjufacturers instructions. This experiments in conjunction with Figure 8 indicates that selective antibody responses can be suppressed using single-chain MHC Ib molecules.

Figure 12: Stability of purified single-chain MHC Ib molecules. After purification of the single chain MHC Ib molecules, their stability was analysed after 1 and 3 freeze-thawing cycles, storage for 5 days at room temperature and heating up to a temperature of 50°C for 30 min. For this, A) a Coomassie gel staining of a 12% polyacrylamide gel using 2 µg AIM Bio and B) an aHLA-G Western blot using the 2A12aHLA-G antibody (1:1000) blot using 1 µg protein was performed under non-reducing conditions. Both monomers and dimers are detectable.

Figure 13: Single-chain MHC Ib molecules are thermally stable. For the Thermal Shift Assay (TSA), 3 µg of the respective single chain MHC Ib molecule or Motavizumab as control molecule were diluted with PBS and 5x SYPRO Orange dye (stock 5000x, final concentration: 5x) to a volume of 25 µl. A melting curve program was set up on a StepOnePlus Instrument using the StepOnePlus Software 2.3. The start temperature was 25°C for one minute followed by a temperature increase of 1°C per minute to a final temperature of 95°C for 2 min, thereby measuring the autofluorescence as arbitrary unit. Data were exported and graphs were drawn in Prism V7.04. For determination of the melting temperature (Tm), the Boltzman sigmoidal function was used.

Figure 14: Single-chain MHC Ib molecules induce Treg in a dose-dependent manner. OT-I mice were injected i.p. with indicated amounts of single-chain H2_K^b alpha1+2 and HLA-G alpha3 domain constructs with human beta-2-microglobulin and the indicated peptide or carrier (PBS). Ova is the cognate peptide for the OT-I TCR in these mice, Gp34 is an irrelevant, virus derived control peptide. After 14 days, mice were sacrificed and splenocytes tested for IL10 secreting cells in a recall mouse IL-10 ELISpot (200,000 cells per well, MabTech mouse IL-10 ELISpot kit, 5 µg/ml of the indicated peptide or only PBS were added, 48h). A clear induction of IL-10 secreting cells reactive to Ova peptide was observed when 50 and 500 µg mouse adapted Ova_KbG were injected.

Figure 15: Single-chain MHC Ib molecules inhibit T cell lysis in a dose-dependent manner. 10 mio OT-1/ml for 3 days in the presence of single-chain MHC Ib molecules 2 h, 37°C, shaking at 125 rpm, OT-1:Panc02 ratio 50:1.

Methods:

Splenocyte isolation and treatment

OT1/BL6 Mice were sacrificed and splenocytes were collected and washed once in RPMI 5% FCS. Red blood cells were removed with 2ml 1x sterile RBC lysis buffer for 3 min. Cells were cultured in high density culture (10mio cells/ml) for 72h in RPMI 10% FCS medium with GMCSF 20 ng/mL, IL-2 20ng/ml and IL-4 10 ng/ml and increasing doses of Ova_KbG. Cells are then scraped from the plates, CD8+ cells are then purified via magnetic beads.

Luciferase assay (48h)

Use sterile 96-well white plate. Load Panc02 fluc+ target cells with 20µg/ml Ova peptide (SIINFEKL) for 60 min at 37°C with 500 rpm shaking. Mix effector CD8+ T cells 50:1 with PancO2 target cells (5000 target cells), add luciferin. Measure luminescence at 0h, 24h, 48h.

Figure 16: Serum cytokines from EAE-ODC Ova mice. Serum cytokines from EAE-ODC Ova mice were measured with Th1/Th2 10plex Flowcytomix Kit (eBioscience) according to the manufacturer's instruction. The kit was used for the simultaneous detection of mouse granulocyte-macrophage colony-stimulating factor (GMCSF), interleukin 1 alpha (IL-1a), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), t interleukin-17 (IL-17), and tumor necrosis factor (TNF) in a single sample. This array kit provides a mixture of ten capture beads with distinct fluorescent intensities that have been coated with capture antibodies specific for each cytokine. Beads coated with ten specific capture antibodies were mixed. Subsequently, 25 µL of the mixed captured beads, 25 µL of the unknown serum sample or standard dilutions, and 25 µL of phycoerythrin (PE) detection reagent were added consecutively to each well in 96-V bottom well plates and incubated for 2 h at room temperature in the dark. The samples were washed with 1 mL of wash buffer for 5 min and centrifuged. The bead pellet was resuspended in 200 µL buffer after discarding the supernatant. Samples were measured on the Attune™ NxT Flow Cytometer and analyzed Attune Cytometric Software (Thermo Fisher Scientific).

Figure 17: Immunofluorescence analyses of a spinal cord of a MOG-induced EAE model
A, B: Caspase 3 infiltration in MOG-induced EAE spinal cord. A: Images; B: Quantitation
C, D: Lesions in MOG-induced EAE white matter in spinal cord. C: Images; D: Quantitation
E, F : CD3 infiltration in MOG-induced EAE spinal cord. E: Images; F: Quantitation

Figure 18: Increase in IL10 spots after treatment with MOG157_A2G

DETAILED DESCRIPTION OF THE INVENTION

Definitions and General Techniques

Unless otherwise defined below, the terms used in the present invention shall be understood in accordance with their common meaning known to the person skilled in the art. All publications, patents and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes. Publications referred to herein may be cited either by specifying the full literature reference in the text.

All proteins in accordance with the invention, including the recombinant polypeptides of the invention, can be obtained by methods known in the art. Such methods include methods for the production of recombinant polypeptides. The recombinant polypeptides of the invention can be expressed in recombinant host cells according to the invention. Recombinant host cells of the invention are preferably mammalian cells such as CHO and HEK cells.
It will be understood that the recombinant polypeptides of the invention are meant to optionally include a secretion signal peptide sequence. Similarly, the recombinant polypeptides of the invention are meant to also optionally include affinity tags, e.g. in order to facilitate purification, and optional protease cleavage sites between the tag and the polypeptide, e.g. in order to facilitate removal of the tags by protease cleavage.
It is also understood that any reference to amino acid sequences referred to herein is meant to encompass not only the unmodified amino acid sequence but also typical posttranslational modifications of these amino acid sequences (e.g., glycosylation or deamidation of amino acids, the clipping of particular amino acids or other posttranslational modifications) occurring in cellular expression systems known in the art, including mammalian cells such as CHO and HEK cells.

Likewise, it will be understood that the recombinant polypeptides of the invention are meant to optionally include the respective pro-peptides.

It will also be understood that the recombinant polypeptides of the invention can be in form of their soluble or their membrane-bound form. Whether a recombinant polypeptide is “soluble” under these conditions can be determined by methods known in the art, e.g., by measuring the turbidity of the recombinant polypeptide under the above-indicated reference conditions. As used herein, soluble means that at least 95% of the recombinant polypeptide is determined to be soluble under these reference conditions.
Single chain MHC molecules can be stored, for instance, in PBS at -80°C (with or without 0.1% human albumin as carrier, depending on the protein concentration) or in 50% glycerol at -20°C.
According to the invention, MHC molecules are preferably human MHC molecules.

The recombinant polypeptides of the invention are preferably isolated recombinant polypeptides.

It will be understood how a recombinant polypeptide capable of binding and presenting an peptide antigen according to the invention can be prepared. For example, peptide antigen-binding domains such as [alpha]1 and [alpha]2 domains are well-known, and modifications of these domains can be made. The capability of a peptide antigen to bind to the polypeptides and MHC molecules according to the invention can be determined by techniques known in the art, including but not limited to explorative methods such as MHC peptide elution followed by Mass spectrometry and bio-informatic prediction in silico, and confirmative methods such as MHC peptide multimere binding methods and stimulation assays.

In accordance with the invention, the recombinant polypeptides, pharmaceutical compositions and kits of the invention are preferably suitable for use in a human patient.
In accordance with the invention, the recombinant polypeptides, pharmaceutical compositions and kits of the invention are preferably suitable for use in the treatment of multiple sclerosis (MS), MOG antibody disease or MOG antibody positive neuromyelitis optica in a human patient.
In accordance with the invention, the recombinant polypeptides, pharmaceutical compositions and kits of the invention are preferably suitable for inducing immunological tolerance against human myelin-oligodendrocyte glycoprotein (MOG), human myelin basic protein (MBP), human myelin-associated glycoprotein (MAG), or human myelin proteolipid protein (PLP1), e.g., in a human patient.
It is understood that in accordance with the invention, the recombinant polypeptides, pharmaceutical compositions and kits of the invention are stable.

It will be understood that in connection with the peptide antigens used in accordance with the invention, any lenghts of these peptide antigens referred to herein (e.g. "7 to 11 amino acids in length") are meant to refer to the length of the peptide antigens themselves. Thus, the lenghts of peptide antigens referred to herein do not include the length conferred by additional amino acids which are not part of the peptide antigens such as additional amino acids from possible linker sequences etc.

In accordance with the present invention, each occurrence of the term "comprising" may optionally be substituted with the term "consisting of".

Methods and Techniques
Generally, unless otherwise defined herein, the methods used in the present invention (e.g. cloning methods or methods relating to antibodies) are performed in accordance with procedures known in the art, e.g. the procedures described in Sambrook et al. ("Molecular Cloning: A Laboratory Manual.", 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York 1989), Ausubel et al. ("Current Protocols in Molecular Biology." Greene Publishing Associates and Wiley Interscience; New York 1992), and Harlow and Lane ("Antibodies: A Laboratory Manual" Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York 1988), all of which are incorporated herein by reference.

Protein-protein binding, such as binding of antibodies to their respective target proteins, can be assessed by methods known in the art. Protein-protein binding is preferably assessed by surface plasmon resonance spectroscopy measurements.

For instance, binding of MHC class Ib molecules or recombinant polypeptides according to the invention to their receptors, including ILT2 and ILT4, is preferably assessed by surface plasmon resonance spectroscopy

measurements. More preferably, binding of MHC class Ib molecules or recombinant polypeptides according to the invention to their receptors is assessed by surface plasmon resonance measurements at 25°C. Appropriate conditions for such surface plasmon resonance measurements have been described by Shiroishi et al., Proc Natl Acad Sci U S A. 2003 July 22;100(15):8856-8861.

Sequence Alignments of sequences according to the invention are performed by using the BLAST algorithm (see Altschul et al.(1990) "Basic local alignment search tool." Journal of Molecular Biology 215. p. 403-410.; Altschul et al.: (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.). Appropriate parameters for sequence alignments of short peptides by the BLAST algorithm, which are suitable for peptide antigens in accordance with the invention, are known in the art. Most software tools using the BLAST algorithm automatically adjust the parameters for sequence alignments for a short input sequence. In one embodiment, the following parameters are used: Max target sequences 10; Word size 3; BLOSUM 62 matrix; gap costs: existence 11, extension 1; conditional compositional score matrix adjustment. Thus, when used in connection with sequences, terms such as "identity" or "identical" preferably refer to the identity value obtained by using the BLAST algorithm.

Preparation of pharmaceutical compositions of the Invention

Pharmaceutical compositions of the present invention are prepared in accordance with known standards for the preparation of pharmaceutical compositions.

For instance, the pharmaceutical compositions are prepared in a way that they can be stored and administered appropriately. The pharmaceutical compositions of the invention may therefore comprise pharmaceutically acceptable components such as carriers, excipients and/or stabilizers.

Such pharmaceutically acceptable components are not toxic in the amounts used when administering the pharmaceutical composition to a human patient. The pharmaceutical acceptable components added to the pharmaceutical compositions may depend on the chemical nature of the active ingredients present in the composition, the particular intended use of the pharmaceutical compositions and the route of administration. In general, the pharmaceutically acceptable components used in connection with the present invention are used in accordance with knowledge available in the art, e.g. from Remington's Pharmaceutical Sciences, Ed. AR Gennaro, 20th edition, 2000, Williams & Wilkins, PA, USA. Pharmaceutical compositions comprising the nucleic acids of the invention (e.g., RNAs) may also be formulated in accordance with knowledge available in the art, e.g. using liposomal formulations targeting dendritic cells.

Peptide Antigens in Accordance with the Invention

The peptide antigens which can be used in accordance with the invention, including the peptide antigens as defined above, are not particularly limited other than by their ability to be presented on MHC molecules. It is understood that a "peptide antigen presented by said recombinant polypeptide" as referred to in relation to the

invention is a peptide antigen that is presented by said recombinant polypeptide to human T cells, if such T cells are present, in a way that it binds to a T cell receptor on the human T-cells.

Peptides which are able to be presented on MHC molecules can be generated as known in the art (see, for instance, Rammensee, Bachmann, Emmerich, Bachor, Stevanović. SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics. 1999 Nov;50(3-4):213-9; Pearson et al. MHC class I-associated peptides derive from selective regions of the human genome. J Clin Invest. 2016 Dec 1;126(12):4690-4701; and Rock, Reits, Neefjes. Present Yourself! By MHC Class I and MHC Class II Molecules. Trends Immunol. 2016 Nov;37(11):724-737).

Peptide antigens are generally known in the art. Generally, the peptide antigens in accordance with the invention are capable of binding to MHC class I proteins. It will be understood by a person skilled in the art that for each MHC class Ib molecule or polypeptide capable of presenting peptides in accordance with the invention, peptide antigens which are capable of binding to said MHC class Ib molecule or recombinant polypeptide will preferably be used. These peptide antigens can be selected based on methods known in the art.

Binding of peptide antigens to MHC class Ib molecules or to polypeptides capable of peptide antigen binding in accordance with the invention can be assessed by methods known in the art, e.g. the methods of:

Rammensee, Bachmann, Emmerich, Bachor, Stevanović. SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics. 1999 Nov;50(3-4):213-9;

Pearson et al. MHC class I-associated peptides derive from selective regions of the human genome. J Clin Invest. 2016 Dec 1;126(12):4690-4701; and

Rock, Reits, Neefjes. Present Yourself! By MHC Class I and MHC Class II Molecules. Trends Immunol. 2016 Nov;37(11):724-737.

Such methods include experimental methods and methods for the prediction of peptide antigen binding.

Anchor residues which serve to anchor the peptide antigen on the MHC class I molecule and to ensure binding of the peptide antigen to the MHC class I molecule are known in the art.

In a preferred embodiment in accordance with all embodiments of the invention, the peptide antigen used in accordance with the invention contain any of the anchor or preferred amino acid residues in the positions as predicted for MHC class I molecules.

Such predictions can preferably be made in as described in any one of the following publications:

- Rammensee et al, SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics (1999) 50: 213-219
- Nielsen et al, Protein Sci (2003) 12:1007-1017
- Neefjes et al. Nat Rev Immunol. 2011 Nov 11;11(12):823-36

- Diehl et al. Curr Biol. 1996 Mar 1;6(3):305-14,
- Lee et al. Immunity. 1995 Nov;3(5):591-600.
- Desai & Kulkarni-Kale, T-cell epitope prediction methods: an overview. Methods Mol Biol. 2014;1184:333-64.
- Jumper *et al.* Highly accurate protein structure prediction with AlphaFold. Nature 2021;596:583–589

In the invention, the peptide antigen is from human myelin-oligodendrocyte glycoprotein (MOG), human myelin basic protein (MBP), human myelin-associated glycoprotein (MAG), or human myelin proteolipid protein (PLP1).

It is understood that the non-anchor amino acid residues of the peptide antigen of the invention may or may not contain conservative substitutions, preferably not more than two conservative substitutions, more preferably one conservative subsitution with respect to the corresponding amino acid sequence of a peptide antigen from human myelin-oligodendrocyte glycoprotein (MOG), human myelin basic protein (MBP), human myelin-associated glycoprotein (MAG) or human myelin proteolipid protein (PLP1).

Peptide antigens of the invention preferably consist of naturally occurring amino acids. However, non-naturally occurring amino acids such as modified amino acids can also be used. For instance, in one embodiment, a peptide antigen of the invention encompasses the peptidomimetic of the indicated peptide antigen amino acid sequence of human myelin-oligodendrocyte glycoprotein (MOG), human myelin basic protein (MBP), human myelin-associated glycoprotein (MAG), or human myelin proteolipid protein (PLP1).

Methods for the synthesis of peptide antigens, including peptide antigens in accordance with the invention, are well known in the art.

Therapeutic applications of the invention

The recombinant polypeptides of the invention can be used for the treatment of multiple sclerosis, MOG antibody disease and MOG antibody positive neuromyelitis optica.

The treatment can be a treatment by inducing myelin-specific regulatory T cells. As such regulatory T cells (e.g., CD8-positive regulatory T cells) are activated in myelinated structures, they confer target cell protection against cytotoxic T cells recognizing the same or another myelin antigen. Regulatury T-cells (e.g., CD8-positive regulatory T cells) are known in the art and can be detected, for instance, by their secretion of IL-10.

While CD8-positive regulatory T cells are not as well known as CD4CD25 regulatory T cells, they have even been described to be more potent. See, for instance:

Junfeng Liu , Dacan Chen, Golay D. Nie and Zhenhua Dai CD8+CD122+ T-Cells: A Newly Emerging Regulator with Central Memory Cell Phenotypes. Front. Immunol. doi: 10.3389/fimmu.2015.00494; and

Niederlova, V., Tsyklauri, O., Chadimova, T. and Stepanek, O. (2021), $CD8^+$ Tregs revisited: A heterogeneous population with different phenotypes and properties. Eur. J. Immunol., 51: 512-530. https://doi.org/10.1002/eji.202048614

While these are characterized by expression of CD122 and CD8 in mice, their human counterparts have been described to be CD8 and CXCR3 positive. See, for instance:

Shi Z, Okuno Y, Rifa'i M, Endharti AT, Akane K, Isobe K, et al. Human CD8+CXCR3+ T cells have the same function as murine CD8+CD122+ Treg. *Eur J Immunol* (2009) **39**:2106–2119. doi:10.1002/eji.200939314).

The treatment can be a treatment for reducing plasma or cerebrospinal fluid levels of autoantibodies against human myelin-oligodendrocyte glycoprotein. The human patient can be a patient who had plasma or cerebrospinal fluid autoantibodies against myelin-oligodendrocyte glycoprotein prior to the start of the treatment.

In accordance with the invention, the autoantibodies can be detected by various methods known in the art. A preferred approach are cell-based assays (CBAs) where the suspected target antigen of the autoantibodies (e.g., myelin-oligodendrocyte glycoprotein) is overexpressed in HEK293 or CHO cells which are then incubated with serum or cerebrospinal fluid, typically for 1 h at room temperature. Mock-transfected sister cells serve as controls. Autoantibodies that bind to the cells are detected with different fluorescently labeled anti-human specific secondary antibodies that recognize total human IgG (heavy and light chain), IgG-Fc (constant chain) or IgG1. Binding is quantified by either flow cytometry (CBA-FACS) or visual scoring by microscopic evaluation of the immunofluorescence (CBA-IF), which is often titrated. In the majority of established cell-based assays for detection of MOG-antibodies, the 218 amino acid a1 isoform of MOG is used, although an alternative in which a truncated version of MOG that contains only the extracellular immunoglobulin and the transmembrane domain has also been tested. Other approaches like enzyme-linked immunosorbent assays (ELISAs) or Western Blots are also possible, but often less sensitive, as conformation-sensitive antibodies may not be detected by these methods. Suitable approaches have been described in

Waters, P., Pettingill, P. & Lang, B. Detection methods for neural autoantibodies. Handb. Clin. Neurol.133, 147–163 (2016).

A detailed overview on Myelin oligodendrocyte glycoprotein (MOG) antibodies can be found in Reindl M, Waters P. Myelin oligodendrocyte glycoprotein antibodies in neurological disease. Nat Rev Neurol. 2019 Feb;15(2):89-102. doi: 10.1038/s41582-018-0112-x. PMID: 30559466.

Sequences

Preferred amino acid sequences referred to in the present application can be independently selected from the following sequences. The sequences are represented in an N-terminal to C-terminal order; and they are represented in the one-letter amino acid code.

Exemplary sequences which are part of of the recombinant polypeptides of the invention:

Optional leader Peptide (absent from the recombinant polypeptide due to processing during cellular expression): e.g. MSRSVALAVLALLSLSGLEA (SEQ ID NO: 1)

Peptide antigen: any MHC class I peptide corresponding to MHC class I [alpha] 1&2 domains, e.g. VLLAVLPVL (SEQ ID NO: 2) (most preferred)

First linker: For instance GGGGSGGGGSGGGGS (SEQ ID NO: 3) or GCGASGGGGSGGGGS (SEQ ID NO: 4)

beta 2 Microglobulin, for instance:
IQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTE KDEYACRVNHVTLSQPKIVKWDRDM (SEQ ID NO: 5, human beta 2 Microglobulin)

Second Linker, for instance:
GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 6)

[Alpha] 1 & 2 domain derived either from human HLA-G or from any other MHC class I [alpha]1&2 domain suitable to present the selected antigenic peptide, Y84 may be C in DT variant

e.g. [Alpha] 1 & 2 domain derived from human HLA-G: E.g.,
GSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDSDSACPRMEPRAPWVEQEGPEYWEEETRNTKAH AQTDRMNLQTLRGCYNQSEASSHTLQWMIGCDLGSDGRLLRGYEQYAYDGKDYLALNEDLRSWTAADTAA QISKRKCEAANVAEQRRAYLEGTCVEWLHRYLENGKEMLQRA (SEQ ID NO: 7)

Or: Human HLA-A2 [alpha]1 & 2 domain: E.g.,
GSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDGETRKVKAH SQTHRVDLGTLRGCYNQSEAGSHTVQRMYGCDVGSDWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAA QTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYLENGKETLQRT (SEQ ID NO: 8)

Human HLA-G [alpha]3 domain (or any MHC Ib [alpha]3 domain, such as HLA-F, which also interacts with ILT2 and ILT4 receptors), for instance:
DPPKTHVTHHPVFDYEATLRCWALGFYPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGE EQRYTCHVQHEGLPEPLMLRWSKEGDGGIMSVRESRSLSEDL (SEQ ID NO: 9; sequence of HLA-G [alpha]3).

Note that the following underlined amino acids of this sequence are relevant for ILT2 or ILT4 receptor interaction:
DPPKTHVTHH**PVFDYE**ATLRCWALGFYPAEIILTWQRDGEDQTQD**V**ELVETRPAGDGTFQKWAAV**V**VPSGE EQRYTCHVQHEGLPEPLMLRWSKEGDGGIMSVRESRSLSEDL

Alternatively, a shorter form of a human HLA-G [alpha]3 domain may be used which lacks the optional C-terminal amino acid sequence from intron 4 (SKEGDGGIMSVRESRSLSEDL; SEQ ID NO: 20), i.e.: DPPKTHVTHHPVFDYEATLRCWALGFYPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGE EQRYTCHVQHEGLPEPLMLRW (SEQ ID NO: 21),
Factor Xa restriction site: IEGRTGTKLGP (SEQ ID NO: 10)
SpotTag: PDRVRAVSHWSSC (SEQ ID NO: 11)
Myc tag: EQKLISEEDL (SEQ ID NO: 12)
His tag: HHHHHH* (SEQ ID NO: 13)
Spacer sequence: e.g. NSAVD (SEQ ID NO: 14) or GS

Further (alternative) exemplary peptide antigens which can be part of the recombinant polypeptides of the invention are as follows:

Table 1: Further preferred peptide antigens

Peptide antigen is a peptide of:	Peptide sequence	SEQ ID NO:	remarks
MAG	MVPDNCPEL	25	Preferably used in recombinant polypeptides containing human HLA-A2 [alpha]1 & 2 domain
MOG	AIGEGKVTL	26	Preferably used in recombinant polypeptides contructs containing human HLA-A2 [alpha]1 & 2 domain
MBP	RSQPGLCNM	27	Preferably used in recombinant polypeptides contructs containing [Alpha] 1 & 2 domain derived from human HLA-G
MBP	SLSRFSWGA	28	Preferably used in recombinant polypeptides contructs containing human HLA-A2 [alpha]1 & 2 domain
MOG	KDAIGEGKVTL	29	Preferably used in recombinant polypeptides contructs containing [Alpha] 1 & 2 domain derived from human HLA-G
MOG	AIGEGKVTL	30	Preferably used in recombinant polypeptides contructs containing human HLA-A2 [alpha]1 & 2 domain
MOG	RHPIRALVG		Preferably used in recombinant

		31	polypeptides contructs containing [Alpha] 1 & 2 domain derived from human HLA-G
MOG	RALVGDEVEL	32	Preferably used in recombinant polypeptides contructs containing [Alpha] 1 & 2 domain derived from human HLA-G
PLP	LLLECCARCL	33	Preferably used in recombinant polypeptides contructs containing [Alpha] 1 & 2 domain derived from human HLA-G

Example for a recombinant polypeptide of the invention (with the optional leader peptide):
MSRSVALAVLALLSLSGLEAVLLAVLPVLGCGASGGGGSGGGGSIQRTPKIQVYSRHPAENGKSNFLNCYVS
GFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDMGG
GGSGGGGSGGGGSGGGGSGSHSMRYFSAAVSRPGRGEPRFIAMGYVDDTQFVRFDSDSACPRMEPRAP
WVEQEGPEYWEEETRNTKAHAQTDRMNLQTLRGCYNQSEASSHTLQWMIGCDLGSDGRLLRGYEQYAYD
GKDYLALNEDLRSWTAADTAAQISKRKCEAANVAEQRRAYLEGTCVEWLHRYLENGKEMLQRADPPKTHVT
HHPVFDYEATLRCWALGFYPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHV
QHEGLPEPLMLRWSKEGDGGIMSVRESRSLSEDLGSPDRVRAVSHWSSC* (SEQ ID NO: 15; note that the asterisk denotes the stop codon)

Note that the sequence of the peptide antigen (here: VLLAVLPVL) of the above full length recombinant polypeptide can be substituted by any peptide antigen sequence in accordance with the invention, i.e. by any peptide antigen presented by said recombinant polypeptide, wherein the peptide antigen is a peptide of human myelin-oligodendrocyte glycoprotein (MOG), human myelin basic protein (MBP), human myelin-associated glycoprotein (MAG), or human myelin proteolipid protein (PLP1). That is, recombinant polypeptides of the invention may consist of a sequence consisting of a peptide antigen which is a peptide of human myelin-oligodendrocyte glycoprotein (MOG), human myelin basic protein (MBP), human myelin-associated glycoprotein (MAG), or human myelin proteolipid protein (PLP1)(e.g., any one of the peptide antigens of SEQ ID NO: 2, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33), followed by the sequence of

GCGASGGGGSGGGGSIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFS
KDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDMGGGGSGGGGSGGGGSGGGGSGSHSMRY
FSAAVSRPGRGEPRFIAMGYVDDTQFVRFDSDSACPRMEPRAPWVEQEGPEYWEEETRNTKAHAQTDRMN
LQTLRGCYNQSEASSHTLQWMIGCDLGSDGRLLRGYEQYAYDGKDYLALNEDLRSWTAADTAAQISKRKCE
AANVAEQRRAYLEGTCVEWLHRYLENGKEMLQRADPPKTHVTHHPVFDYEATLRCWALGFYPAEIILTWQRD

GEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPLMLRWSKEGDGGIMSVRESRS
LSEDLGSPDRVRAVSHWSSC* (SEQ ID NO: 16; note that the asterisk denotes the stop codon)
These recombinant polypeptides of the invention may also contain the optional leader peptide as exemplified above.

The receptors ILT2 (also known as LILRB1) and ILT4 (also known as LILRB2) are known in the art. Preferred sequences of these receptors in accordance with the invention are as follows:

ILT2:
MTPILTVLICLGLSLGPRTHVQAGHLPKPTLWAEPGSVITQGSPVTLRCQGGQETQEYRL
YREKKTALWITRIPQELVKKGQFPIPSITWEHAGRYRCYYGSDTAGRSESSDPLELVVTG
AYIKPTLSAQPSPVVNSGGNVILQCDSQVAFDGFSLCKEGEDEHPQCLNSQPHARGSSRA
IFSVGPVSPSRRWWYRCYAYDSNSPYEWSLPSDLLELLVLGVSKKPSLSVQPGPIVAPEE
TLTLQCGSDAGYNRFVLYKDGERDFLQLAGAQPQAGLSQANFTLGPVSRSYGGQYRCYGA
HNLSSEWSAPSDPLDILIAGQFYDRVSLSVQPGPTVASGENVTLLCQSQGWMQTFLLTKE
GAADDPWRLRSTYQSQKYQAEFPMGPVTSAHAGTYRCYGSQSSKPYLLTHPSDPLELVVS
GPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGVVIGILVAVILLLLLLLLLF
LILRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQ
PEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMD
TEAAASEAPQDVTYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 17)

ILT4:
MTPIVTVLICLGLSLGPRTHVQTGTIPKPTLWAEPDSVITQGSPVTLSCQGSLEAQEYRL
YREKKSASWITRIRPELVKNGQFHIPSITWEHTGRYGCQYYSRARWSELSDPLVLVMTGA
YPKPTLSAQPSPVVTSGGRVTLQCESQVAFGGFILCKEGEEEHPQCLNSQPHARGSSRAI
FSVGPVSPNRRWSHRCYGYDLNSPYVWSSPSDLLELLVPGVSKKPSLSVQPGPVVAPGES
LTLQCVSDVGYDRFVLYKEGERDLRQLPGRQPQAGLSQANFTLGPVSRSYGGQYRCYGAH
NLSSECSAPSDPLDILITGQIRGTPFISVQPGPTVASGENVTLLCQSWRQFHTFLLTKAG
AADAPLRLRSIHEYPKYQAEFPMSPVTSAHAGTYRCYGSLNSDPYLLSHPSEPLELVVSG
PSMGSSPPPTGPISTPAGPEDQPLTPTGSDPQSGLGRHLGVVIGILVAVVLLLLLLLLLF
LILRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKDTQ
PEDGVEMDTRAAASEAPQDVTYAQLHSLTLRRKATEPPPSQEREPPAEPSIYATLAIH (SEQ ID NO: 18)

The sequences of human myelin-oligodendrocyte glycoprotein (MOG), human myelin basic protein (MBP), human myelin-associated glycoprotein (MAG), or human myelin proteolipid protein (PLP1) are known in the art. Preferred amino acid sequences of human myelin-oligodendrocyte glycoprotein (MOG), human myelin

basic protein (MBP), human myelin-associated glycoprotein (MAG), or human myelin proteolipid protein (PLP1) are as follows:

human myelin-oligodendrocyte glycoprotein (MOG):
>NP_996532.2 myelin-oligodendrocyte glycoprotein isoform alpha1 precursor [Homo sapiens]
MASLSRPSLPSCLCSFLLLLLLQVSSSYAGQFRVIGPRHPIRALVGDEVELPCRISPGKNATGMEVGWYR
PPFSRVVHLYRNGKDQDGDQAPEYRGRTELLKDAIGEGKVTLRIRNVRFSDEGGFTCFFRDHSYQEEAAM
ELKVEDPFYWVSPGVLVLLAVLPVLLLQITVGLIFLCLQYRLRGKLRAEIENLHRTFDPHFLRVPCWKIT
LFVIVPVLGPLVALIICYNWLHRRLAGQFLEELRNPF
(SEQ ID NO: 19)

human myelin basic protein (MBP):
>NP_001020252.1 myelin basic protein isoform 1 [Homo sapiens]
MASQKRPSQRHGSKYLATASTMDHARHGFLPRHRDTGILDSIGRFFGGDRGAPKRGSGKVPWLKPGRSPL
PSHARSQPGLCNMYKDSHHPARTAHYGSLPQKSHGRTQDENPVVHFFKNIVTPRTPPPSQGKGRGLSLSR
FSWGAEGQRPGFGYGGRASDYKSAHKGFKGVDAQGTLSKIFKLGGRDSRSGSPMARR
(SEQ ID NO: 22)

human myelin-associated glycoprotein (MAG):
>NP_002352.1 myelin-associated glycoprotein isoform a precursor [Homo sapiens]
MIFLTALPLFWIMISASRGGHWGAWMPSSISAFEGTCVSIPCRFDFPDELRPAVVHGVWYFNSPYPKNYP
PVVFKSRTQVVHESFQGRSRLLGDLGLRNCTLLLSNVSPELGGKYYFRGDLGGYNQYTFSEHSVLDIVNT
PNIVVPPEVVAGTEVEVSCMVPDNCPELRPELSWLGHEGLGEPAVLGRLREDEGTWVQVSLLHFVPTREA
NGHRLGCQASFPNTTLQFEGYASMDVKYPPVIVEMNSSVEAIEGSHVSLLCGADSNPPPLLTWMRDGTVL
REAVAESLLLELEEVTPAEDGVYACLAENAYGQDNRTVGLSVMYAPWKPTVNGTMVAVEGETVSILCSTQ
SNPDPILTIFKEKQILSTVIYESELQLELPAVSPEDDGEYWCVAENQYGQRATAFNLSVEFAPVLLLESH
CAAARDTVQCLCVVKSNPEPSVAFELPSRNVTVNESEREFVYSERSGLVLTSILTLRGQAQAPPRVICTA
RNLYGAKSLELPFQGAHRLMWAKIGPVGAVVAFAILIAIVCYITQTRRKKNVTESPSFSAGDNPPVLFSS
DFRISGAPEKYESERRLGSERRLLGLRGEPPELDLSYSHSDLGKRPTKDSYTLTEELAEYAEIRVK
(SEQ ID NO: 23)

human myelin proteolipid protein (PLP1):
>NP_000524.3 myelin proteolipid protein isoform 1 [Homo sapiens]
MGLLECCARCLVGAPFASLVATGLCFFGVALFCGCGHEALTGTEKLIETYFSKNYQDYEYLINVIHAFQY
VIYGTASFFFLYGALLLAEGFYTTGAVRQIFGDYKTTICGKGLSATVTGGQKGRGSRGQHQAHSLERVCH

CLGKWLGHPDKFVGITYALTVVWLLVFACSAVPVYIYFNTWTTCQSIAFPSKTSASIGSLCADARMYGVL
PWNAFPGKVCGSNLLSICKTAEFQMTFHLFIAAFVGAAATLVSLLTFMIAATYNFAVLKLMGRGTKF
(SEQ ID NO: 24)

The present invention is further illustrated by the following non-limiting examples:

EXAMPLES

Example 1:

Methods for producing recombinant polypeptides of the invention

- Expi-293F cells (Thermo Fisher), grown in Expi-293™ expression medium (Thermo Fisher): transfection of 1 µg DNA into 2.5x10^6 cells/ml using the Expifectamine™ 293 Transfection kit (Thermo Fisher) using Opti-MEM (Thermo Fisher) for complexation of DNA with Expifectamine, after 18-20 h, addition of enhancer according to the protocol, harvesting of the supernatant after 4-6 days (37°C, 8% CO_2, humidified incubator), 19 mm² orbital shaker 125 rpm
- Spot-tag protein purification: equilibration of Spot-Cap resin: transfer of desired slurry amount into an appropriate tube, sediment beads by centrifugation (4°C, 4 min, 2500 g), remove & discard supernatant, add 10 bed volumes PBS (cold) to beads, invert to mix, sediment beads by centrifugation (4°C, 4 min, 2500 g), remove & discard supernatant, repeat 2 times
- Add required volume beads to supernatant, incubate ON, 4°C on a rotator, wash beads by repeated centrifugation (4°C, 4 min, 2500 g), and removal of supernatant
- Prepare a 500 µM Spot-peptide solution in PBS, remove the supernatant, incubate with 1/3rd of the spot-peptide solution for 5-10 min

Sediment beads by centrifugation. Use Amicon Ultra-4 centrifugal filters (15 kDa cutoff) for Protein concentration and spot-peptide removal with 15 kDa Amicon cutoff columns

Rinse the Amicon Ultra-4 centrifugal filters (15 kDa cutoff) with PBS followed by 0.1 N NaOH (centrifugation at 4000 g, 4°C) to remove trace amounts of glycerine.

ELISPOT:

1) Cell culture

A) PBMC isolation (under a laminar flow hood)

To isolate peripheral blood mononuclear cells (PBMC), a density centrifugation was performed with white blood cells from a leukocyte reduction chamber and density gradient medium (e.g. Ficoll, or ROTI Sep 1077). Cells were centrifuged for 20 min at 1200 x g without brake followed by collection of the interphase ring that was washed with 1x PBS (5 min, 300 x g). PBMC were frozen till further use.

B) PBMC pulsing (under a laminar flow hood)

PBMCs were thawed 1 day prior to PBMC pulsing (d-1) and kept over night in 5 ml X-VIVO 15 medium containing 5% human AB serum in a well of a 6 well plate at 37°C.

On the next day (d0) cells were counted and resuspended in X-VIVO 15 complete medium (5% hAB serum & cytokine cocktail: 20 ng/ml hIL-2, 20 ng/ml hGM-CSF, 10 ng/ml hIL-4 & 10 ng/ml hTGF-b1) at a cell density of $3x10^6$ cells/ml.

For experiments, $3x10^6$ cells were seeded in the respective wells of a 12-well plate with a final volume of 1000 µl X-VIVO complete medium with cytokine cocktail and

5 µg/ml of an AIM Bio molecule or the respective controls.

On day 3, 1 ml complete medium (with cytokines) was added, on day 6, a second pulse with 5 µg/ml of a recombinant polypeptide of the invention or a surrogate thereof (collectively referred to as "AIM Bio" molecule) was performed (after removing medium). On days 7, 10 & 12, 1 ml complete medium (with cytokines) was added.

Materials:

X-VIVO 15 medium + 5% human AB serum

X-VIVO 15 complete medium: X-VIVO 15 medium + 2% human AB serum supplemented with cytokine cocktail: 10 ng/ml TGF-b1, 10 ng/ml IL-4, 20 ng/ml IL-2, 20 ng/ml GM-CSF

6 and 12 well plate

2) ELISPOT

On day 13, ELISPOT plates were coated using anti-hIL10 (clone 9D-7, 1:500 dilution in PBS, sterile filtered) and aIL10 (10G8-biotin) and on day 14, 200,000 cells were seeded per well on the ELISPOT plates in duplicates, including negative controls (cells plus PBS) and a positive control (e.g. LPS).

The PFDF membrane was activated with 50 µl/well EtOH (35% v/v) for 1 min followed by 5x washing with 200 µl distilled sterile water. Plate was coated with 100 µl/well antibody solution at 4°C over night. On the next day, unbound coating antibody was removed, 5 washing steps were performed with 200 µl PBS and 200 µl blocking buffer (X-VIVO 15 5% hAB serum) was added and the plate incubated for 30 min - 2 h at room temperature.

The respective antigenic peptide (e.g.MOG157) in DMSO or DMSO as a control were prepared, and a final amount of 5 µg peptide/ml was added to the final volume of 100 µl/well. 150,000 cells were seeded per well in X-VIVO 15 medium with 5% human AB serum. Blocking buffer (X VIVO 15 medum + 5% hAB serum) was carefully removed, and medium with PBS as negative control and stimulants (5 µg/ml total volume in each well) were added to the other wells and incubated at 37°C over night.

Outside the laminar flow hood

Secondary antibody was prepared: 1 µg/ml aIL-10-biotinylated antibody in 0.5% BSA/1x PBS (1:1000 dilution) and horseradish peroxidase-conjugated streptavidin (1:750 in 0.5% BSA/PBS), tetramethylbenzidine solution was filtered using a 0.45 µm filter and stored at 4°C till use.

Cell supernatant was removed and 5x washed using 100 µl PBS. Last excess buffer was removed using paper towels.

25 µl diluted HRP-streptavidin (1:750) was added per well and incubated for 1 h at room temperature in the dark followed by 5 washing steps using sterile 1xPBS.
100 µl of filtered TMB substrate was added per well for 15-25 min till blue sports developed. Reaction was stopped by washing the wells thoroughly with tapped water.
Plastic underdrains of the plates was removed and the bottom and sides of the plates were washed with tap water and dried.
Plates were read out using an ImmunoSpot S6 Ultra-V Analyzer (Cellular Technology Limited), analysed in Excel and graphs/statistics were done in Graphad Prism.
Required: Capture antibodies: anti-hIL10 (Clone: 9D-7, Mabtech #3430-3-250; 1:500 dilution), anti-hIL10-biotinylated (Mabtech, #3430-6-250), 1x PBS (sterile), 35% EtOH (v/v), Blocking buffer: X-vivo 5% hAB serum (sterile) [blocking is done in the same medium as cell culture], Dilution buffer: 0.5% BAS in PBS, Washing buffer: 1x PBS, Medium: for T cells, X-VIVO 15 medium (Lonza), Filter syringe: Millex GV, ELISPOT PVDF plate (#MSIP4510, Millipore), TMB substrate

Example 2: Surrogates of recombinant polypeptides of the invention induce IL10 secreting Treg in mice
Wild type black 6 mice were injected with 100µg recombinant polypeptides (also referred to as "AIMBio") having the following sequences,
Ova_KbG
SIINFEKLGCGASGGGGSGGGGSIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVE
HSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDMGGGGSGGGGSGGGGSGGGGS
GPHSLRYFVTAVSRPGLGEPRYMEVGYVDDTEFVRFDSDAENPRYEPRARWMEQEGPEYWERETQKAKG
NEQSFRVDLRTLLGCYNQSKGGSHTIQVISGCEVGSDGRLLRGYQQYAYDGCDYIALNEDLKTWTAADMAAL
ITKHKWEQAGEAERLRAYLEGTCVEWLRRYLKNGNATLLRTDPPKTHVTHHPVFDYEATLRCWALGFYPAEII
LTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPLMLRWSKEGDGGIM
SVRESRSLSEDLGSPDRVRAVSHWSSC (SEQ ID NO: 46)
and
Gp34_KbG
AVYNFATMGCGASGGGGSGGGGSIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKV
EHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDMGGGGSGGGGSGGGGSGGGGS
GPHSLRYFVTAVSRPGLGEPRYMEVGYVDDTEFVRFDSDAENPRYEPRARWMEQEGPEYWERETQKAKG
NEQSFRVDLRTLLGCYNQSKGGSHTIQVISGCEVGSDGRLLRGYQQYAYDGCDYIALNEDLKTWTAADMAAL
ITKHKWEQAGEAERLRAYLEGTCVEWLRRYLKNGNATLLRTDPPKTHVTHHPVFDYEATLRCWALGFYPAEII
LTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPLMLRWSKEGDGGIM
SVRESRSLSEDL GSPDRVRAVSHWSSC (SEQ ID NO: 47)
for inducing tolerance towards an OVA peptide or an viral Gp34 peptide, respectively. Gp34 is a well-characterized T cell epitope derived from Lymphocytic Choriomeningitis virus (LCMV) Glycoprotein. While this epitope was traditionally named Gp33, the epitope presented on H2-K^b was later found to comprise just amino

acids 34-41. (An epitope beginning at amino acid 33 is, in contrast, presented on H2-K^d.) Therefore, we call the H2-K^b epitope Gp34, which is in line with the most recent recommendations. Still, there is an ambiguous use of the Gp33 and Gp34 nomenclature in the literature. The first eight amino acids of SEQ ID NO: 47 show the respective peptide sequence. After 2 weeks, mice were sacrificed, and splenocytes re-challenged either with the matching or a mismatching peptide. IL-10 secreting cells were quantified by ELIspot. The results are shown in Figure 3.

Example 3: Surrogates of recombinant polypeptides of the invention prevent CD8+ T-cell driven EAE in mice
As described in (Na et al, Brain. 2008 Sep;131(Pt 9):2353-65.), the adoptive transfer of $CD8^+$ OT-I T cells that recognize an ovalbumin epitope in the context of H2-K^b into mice which express ovalbumin in oligodendrocytes leads to experimental autoimmune encephalomyelitis which recapitulates many MS and MOGAD symptoms. In this animal model, a single injection of 500µg of recombinant polypeptides surrogate molecules (also referred to as "AIMBio") that induce tolerance towards the targeted ovalbumin epitope almost completely prevented EAE symptoms, while a surrogate molecule presenting a control peptide hat no significant protective effects (Figure 4). The sequences of the recombinant polypeptide surrogate molecules were as shown in Example 2.
In the same model, the inventors were further able to show that using an antigen peptide also presented of the target tissue or cell can result in effective bystander immunosuppression. Here, 250µg of recombinant polypeptide surrogate molecules (also referred to as "AIMBio") were injected per mouse (Figure 5). The sequences of the recombinant polypeptide surrogate molecules were as follows:
Mog44_DbG
FSRVVHLYRNGGCGASGGGGSGGGGSIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERI
EKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDMGGGGSGGGGSGGGGSGG
GGSGPHSMRYFETAVSRPGLEEPRYISVGYVDNKEFVRFDSDAENPRYEPRAPWMEQEGPEYWERETQKA
KGQEQWFRVSLRNLLGCYNQSAGGSHTLQQMSGCDLGSDWRLLRGYLQFAYEGRDYIALNEDLKTWTAAD
MAAQITRRKWEQSGAAEHYKAYLEGECVEWLHRYLKNGNATLLRTDPPKTHVTHHPVFDYEATLRCWALGF
YPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPLMLRWSKEGD
GGIMSVRESRSLSEDLGSPDRVRAVSHWSSC (SEQ ID NO: 48)

Mog37_DbG
VGWYRSPFSRGCGASGGGGSGGGGSIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERI
EKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDMGGGGSGGGGSGGGGSGG
GGSGPHSMRYFETAVSRPGLEEPRYISVGYVDNKEFVRFDSDAENPRYEPRAPWMEQEGPEYWERETQKA
KGQEQWFRVSLRNLLGCYNQSAGGSHTLQQMSGCDLGSDWRLLRGYLQFAYEGRDYIALNEDLKTWTAAD
MAAQITRRKWEQSGAAEHYKAYLEGECVEWLHRYLKNGNATLLRTDPPKTHVTHHPVFDYEATLRCWALGF
YPAEIILTWQRDGEDQTQDVELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPLMLRWSKEGD
GGIMSVRESRSLSEDLGSPDRVRAVSHWSSC (SEQ ID NO: 49)

Example 4: Some surrogates of recombinant polypeptides of the invention selectively prevent $CD4^+$ T cell driven EAE in mice

At the day zero, 33µg or 100µg recombinant polypeptide of the invention surrogate molecule ("AIM Bio") was injected i.p., 100 µl MOG35-55 peptide/CFA (Complete Freund's Adjuvance; final concentration Mycobacterium Tuberculosis H37RA and peptide each 1 mg/ml) emulsion were injected each left and right s.c. into the flank and 250ng pertussis toxin (in 200µl PBS) intraperitoneally. A second pertussis toxin injection was given 3 days later. In this animal model, a single injection AIM Bio surrogate molecules that induce tolerance towards the a Mog epitope (Mog44_DbG) significantly reduced EAE symptoms, while a surrogate molecule presenting a control peptide (Gp34) or a non-functional Mog peptide (Mog37) hat no significant protective effects (Figure 6). In this model Mog44 AIM Bio also prevented inflammation and CD8 T cell infiltration in the spinal cord (Figure 7). The sequences of the recombinant polypeptide surrogate molecules were shown in Example 3.

In this model Mog44 AIM Bio also completely prevented the formation of MOG-specific autoantibodies in the serum as tested by ELISA (Figure 8 and Figure 11). This is a strong indicator that the recombinant polypeptides of the invention are effective therapeutics in MS and MOGAD, which are often characterized by antibody responses against MOG. Thus, the patient population is defined by a common autoimmune-related antigen.

Mog-reactive antibodies in sera of AIM Bio (33 or 100µg) treated mice were detected via standard ELISA protocol, with 3 washes in between each step. Briefly, ELISA plates were coated with 10µg/ml Mog35-55 peptide, blocked with PBS 1% BSA, before mouse sera diluted 1:25 in PBS 1% BSA were added for 1h. Anti-mouse IgG-HRP or anti-mouse heavy and light chain – HRP antibodies diluted 1:5000 were used for detection.

Example 5: Human recombinant polypeptide candidates of the invention for MS and MOGAD

The recombinant polypeptides of the invention are newly developed protein complexes derived from the pregnancy-associated immunosuppressive MHC molecule HLA-G. It is likely that HLA-G enables an embryo to influence the maternal immune system to tolerate embryonic antigens but further antagonize antigens from pathogens. The recombinant polypeptides of the invention containing variable peptides were able to selectively eliminate peptide-specific cytotoxic effector T cells as well as induce peptide-specific regulatory T cells in the test tube.

Figure 9 shows a list of the human MS & MOGAD recombinant polypeptide candidates.

The inventors' findings show that single-chain proteins containing myelin peptide antigens and a HLA-G alpha 3 domain can induce tolerogenic T cells in healthy donors. Thus, CD8 Treg were upregulated by at least 30%

in 75% of all healthy blood donors by a recombinant polypeotide of the invention containing the VLLAVLPVL antigen (also referred to as "Mog157_A2G"; see Figure 10).

Example 6: Further proof-of-principle of stability and effects of the recombinant polypeptides of the invention. Additionally, the inventors set out to obtain and test recombinant polypeptidies having the general structure of the recombinant polypeptides of the invention but containing various different peptide antigens, in order to obtain further proof-of-principle that recombinant polypeptidies of the invention and surrogates thereof are stable and efficacious. As shown in Figures 12 and 13, respectively, the tested recombinant polypeptidies are stable during freeze-thawing and storage and are thermally stable. Further, they induce Treg in a dose-dependent manner (Figure 14) and inhibit T cell lysis in a dose-dependent manner (Figure 15). Effects of the recombinant polypeptidies on the serum cyctokine profile in EAE-ODC Ova mice are shown in Figure 16. There is an induction of IL-10 and possibly IL-4, both known to be immunosuppressive cytokines downregulating immune responses in inflammatory settings. This requires an HLA-G alpha3 domain plus a cognate peptide.. IL-2 seems to be induced in response to presenting the cells with a cognate peptide that is irrespective of the alpha3 domain. IL-2 is needed for T cell activation and survival.

Example 7: MOG-tolerance inducing recombinant polypeptides of the invention prevent apoptosis (cleaved caspase 3), $CD3^+$ immune cell infiltration and myelin lesions in the spimal cord. The mouse model is described in Figure 6.

Immunofluorescence analyses of a spinal cord of a MOG-induced EAE model.

Materials:

Primary Antibodies

α-CD3i clone CD3-12 (1:100); Rat monoclonal (rat anti-human, cross-react to mouse, Bio-rad); α-MBP (1:300, Rabbit monoclonal, MBL); α-cleaved caspase3 (1:400, Rabbit monoclonal, Cell Signalling)

DAPI (1:500, Sigma # D8417)

Secondary Antibodies

α- rabbit-Cy3 (red, 1:300, Dianova #111-165-144); α- rat-AF488 (green, 1:300, Invitrogen) or α- rabbit-Cy3 (red, 1:300, Dianova#112-165-167)

Protocol

Warm frozen sections at RT for 5 min, mark the staining areas with Pap-Pen, fix in acetone, 10 min at -20 °C

Wash: 3 times with 1X PBS

Block 1h: 5% BSA+0.2% Triton-X100+5% NGS in 1X PBS

Primary Antibody: 1% BSA+1%NGS+0.2% Triton-X100; 4°C, Overnight

Wash: 3 times with 1X PBS

Secondary Antibody : 1% BSA+1%NGS+0.2% Triton-X100; RT, 1h

Wash: 3 times with 1X PBS

DAPI: 1:300 in 1X PBS, 10 min, RT
Wash: 2 times with 1X PBS
Mount with Aquapolymount

Detailed Results:
Figure 17 A, B: Caspase 3 activation (apoptosis) can be prevented in MOG-induced EAE spinal cord. A: Images; B: Quantitation
Figure 17 C, D: Lesions in MOG-induced EAE white matter in spinal cord can be prevented. C: Images; D: Quantitation
Figure 17 E, F : CD3 infiltration into the spinal cord in MOG-induced EAE can be prevented. E: Images; F: Quantitation

Example 8: Increase in IL10 spots after treatment with MOG157_A2G
Figure 18 shows an increase in IL10 spots after treatment with MOG157_A2G, which indicates that tolerogenic Treg have been induced. This is an alternative depiction of Figure 10.

INDUSTRIAL APPLICABILITY
The pharmaceutical compositions, polypeptides, nucleic acids, cells, and products for use in the invention are industrially applicable. For example, they can be used in the manufacture of, or as, pharmaceutical products.

CLAIMS

1. A recombinant polypeptide capable of presenting a peptide antigen, the recombinant polypeptide comprising, in an N- to C-terminal order,
   i) a peptide antigen presented by said recombinant polypeptide, wherein the peptide antigen is a peptide of human myelin-oligodendrocyte glycoprotein (MOG), human myelin basic protein (MBP), human myelin-associated glycoprotein (MAG), or human myelin proteolipid protein (PLP1);
   ii) optionally a linker sequence;
   iii) optionally a sequence of a human polypeptide domain comprising a sequence of a human β2 microglobulin, or an amino acid sequence at least 90% identical to the amino acid sequence of human β2 microglobulin represented by SEQ ID NO: 5;
   iv) optionally a linker sequence;
   v) optionally an [alpha] 1 domain of an MHC molecule;
   vi) optionally an [alpha] 2 domain of an MHC molecule;
   vii) an [alpha] 3 domain of an MHC class Ib molecule or a derivative of an [alpha] 3 domain of an MHC class Ib molecule, said derivative being capable of binding to ILT2 or ILT4;
   viii) optionally a protease cleavage site;
   ix) optionally a spacer sequence; and
   x) optionally an affinity tag.
2. The recombinant polypeptide according to claim 1, wherein said peptide antigen according to i) is 7 to 11 amino acids in length, preferably 8-10 amino acids in length.
3. The recombinant polypeptide according to claim 1 or 2, wherein said peptide antigen according to i) consists of an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33.
4. The recombinant polypeptide according to claim 1 or 2, wherein said peptide antigen according to i) consists of an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30.
5. The recombinant polypeptide according to any one of the preceding claims, wherein said peptide antigen according to i) is a peptide of human myelin-oligodendrocyte glycoprotein (MOG).
6. The recombinant polypeptide according to any one of claims 1-2 and 4-5, wherein said peptide antigen consists of the amino acid sequence of SEQ ID NO: 2.
7. The recombinant polypeptide according to any one of the preceding claims, wherein said [alpha]1 domain according to (v) and said [alpha]2 domain according to (vi) are from a human MHC class Ia molecule or from a human MHC class Ib molecule.

8. The recombinant polypeptide according to claim 7, wherein said [alpha]1 domain according to (v) and said [alpha]2 domain according to (vi) are from a human MHC class Ia molecule.
9. The recombinant polypeptide according to claim 8, wherein said [alpha]1 domain according to (v) and said [alpha]2 domain according to (vi) are from a human HLA-A2 molecule.
10. The recombinant polypeptide according to claim 7, wherein said [alpha]1 domain according to (v) and said [alpha]2 domain according to (vi) are from a human MHC class Ib molecule.
11. The recombinant polypeptide according to any one of the preceding claims, wherein the [alpha] 3 domain of the MHC class Ib molecule according to (vii) is an [alpha] 3 domain of human HLA-E, human HLA-F or human HLA-G.
12. The recombinant polypeptide according to any one of the preceding claims, wherein the [alpha] 3 domain of the MHC class Ib molecule according to (vii) is an [alpha] 3 domain of human HLA-G.
13. The recombinant polypeptide according to any one of the preceding claims, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 80% amino acid sequence identity, preferably at least 90% amino acid sequence identity, with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
14. The recombinant polypeptide according to claim 13, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 92% amino acid sequence identity with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
15. The recombinant polypeptide according to claim 13, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 94% amino acid sequence identity with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
16. The recombinant polypeptide according to claim 13, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 96% amino acid sequence identity with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
17. The recombinant polypeptide according to claim 13, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 98% amino acid sequence identity with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
18. The recombinant polypeptide according to claim 13, wherein the [alpha]3 domain or derivative according to (vii) is identical to or has at least 99% amino acid sequence identity with the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
19. The recombinant polypeptide according to claim 13, wherein the [alpha]3 domain according to (vii) is identical to the [alpha]3 domain having the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 21.
20. The recombinant polypeptide according to any one of the preceding claims, wherein the linker sequence according to (ii) and/or the linker sequence according to (iv) comprises the amino acid sequence (GGGGS)n, wherein n is an integer equal to or higher than 1.

21. The recombinant polypeptide according to claim 20, wherein the linker sequence according to (ii) comprises the amino acid sequence (GGGGS)n, and wherein n is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 and is preferably selected from the group consisting of 2, 3, 4 and 5.
22. The recombinant polypeptide according to claim 20 or 21, wherein the linker sequence according to (iv) comprises the amino acid sequence (GGGGS)n, and wherein n is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 and is preferably selected from the group consisting of 2, 3, 4 and 5.
23. The recombinant polypeptide according to any one of the preceding claims, wherein said sequence of a human polypeptide domain according to (iii) is at least 95% identical to the amino acid sequence of SEQ ID NO: 5, preferably at least 98% identical to the amino acid sequence of SEQ ID NO: 5 and more preferably identical to the amino acid sequence of SEQ ID NO: 5.
24. The recombinant polypeptide according to any one of the preceding claims, wherein said polypeptide is dimeric or multimeric.
25. The recombinant polypeptide according to any one of the preceding claims, wherein the polypeptide comprises or consists of all of the components i) to vii)
26. The recombinant polypeptide according to any one of the preceding claims, wherein the polypeptide does not comprise components viii) to x).
27. The recombinant polypeptide according to any one of claims 1 to 25, wherein the polypeptide comprises or consists of all of the components i) to x).
28. The recombinant polypeptide according to any one of the preceding claims, further comprising an N-terminal secretion signal peptide sequence.
29. The recombinant polypeptide according to any one of claims 1-27, wherein the recombinant polypeptide consists of an amino acid sequence consisting of the following ((a) and (b)) in an N- to C-terminal order:

(a) a peptide antigen selected from the group consisting of the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33; and

(b) the amino acid sequence of SEQ ID NO: 16.

30. The recombinant polypeptide according to any one of the preceding claims, wherein the recombinant polypeptide is soluble.
31. A nucleic acid encoding one or more polypeptides according to any one of the preceding claims.
32. The nucleic acid according to claim 31, wherein the nucleic acid is a vector.
33. A pharmaceutical composition comprising at least one nucleic acid according to claims 31 or 32.
34. A pharmaceutical composition or kit comprising at least one recombinant polypeptide according to any one of claims 1-30.

35. The pharmaceutical composition or kit according to claim 34, wherein the pharmaceutical composition or kit comprises at least two different recombinant polypeptides according to any one of claims 1-30, and wherein each of the different polypeptides comprises a different peptide antigen as defined in any one of claims 3 to 6.
36. A pharmaceutical composition or kit according to any one of claims 33-35, for use in the treatment of multiple sclerosis (MS), MOG antibody disease or MOG antibody positive neuromyelitis optica in a human patient.
37. The pharmaceutical composition or kit for use according to claim 36, wherein the treatment is a treatment of multiple sclerosis (MS).
38. The pharmaceutical composition or kit for use according to claim 36, wherein the treatment is a treatment of MOG antibody disease.
39. The pharmaceutical composition or kit for use according to claim 36, wherein the treatment is a treatment of MOG antibody positive neuromyelitis optica.
40. The pharmaceutical composition or kit for use according to any one of claims 36-39, wherein the treatment is treatment by immunotherapy.
41. The pharmaceutical composition or kit for use according to any one of claims 36-40, wherein the treatment is by inducing immunological tolerance against human myelin-oligodendrocyte glycoprotein.
42. The pharmaceutical composition or kit for use according to any one of claims 36-41, wherein the treatment is for reducing plasma or cerebrospinal fluid levels of autoantibodies against human myelin-oligodendrocyte glycoprotein.
43. The pharmaceutical composition or kit for use according to any one of claims 36-42, wherein the human patient is a patient who had plasma or cerebrospinal fluid autoantibodies against myelin-oligodendrocyte glycoprotein prior to the start of the treatment.
44. The pharmaceutical composition or kit for use according to any one of claims 36-43, wherein the treatment is by inducing myelin-specific regulatory T cells.
45. A recombinant host cell comprising a nucleic acid or a vector according to claim 31 or 32 and expressing the recombinant polypeptide according to any one of claims 1-30.
46. A method for obtaining pharmaceutical composition comprising a polypeptide according to any one of claims 1-30, the method comprising the steps of (a) culturing the recombinant host cell of claim 45 under conditions allowing expression of the recombinant polypeptide from the nucleic acid molecule, (b) recovering the recombinant polypeptide, (c) purifying the recombinant polypeptide, and (d) formulating the recombinant polypeptide into a pharmaceutical composition.

Figure 1

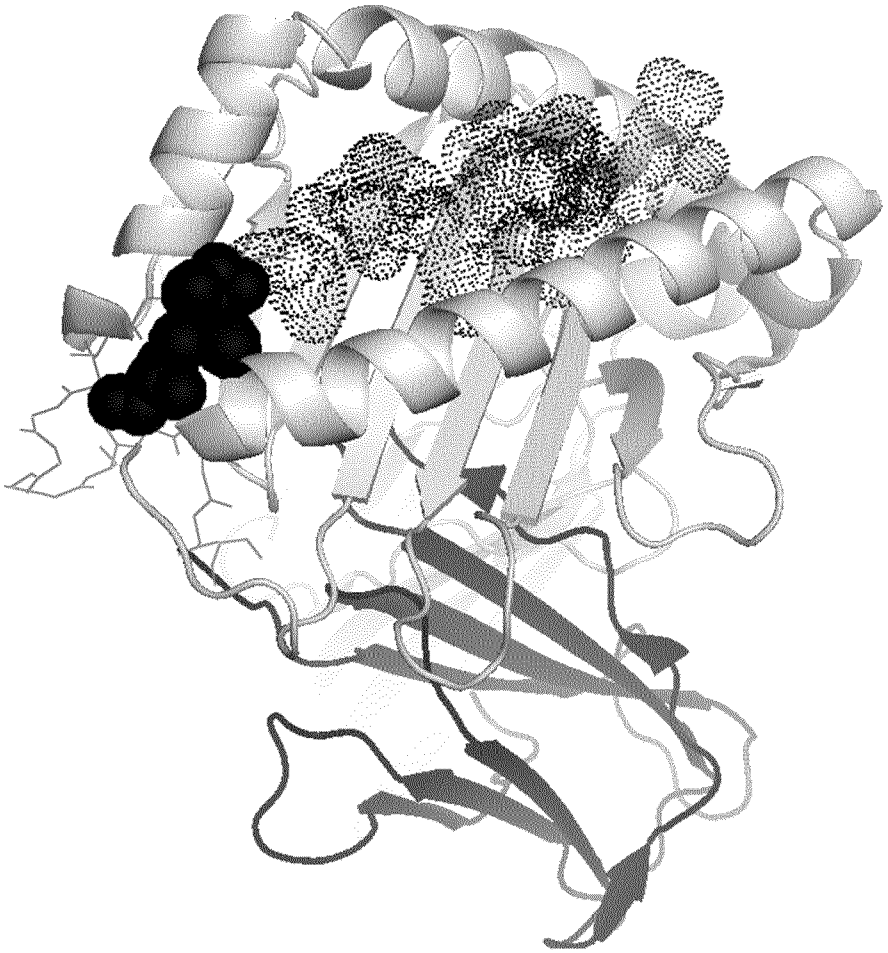


Figure 2

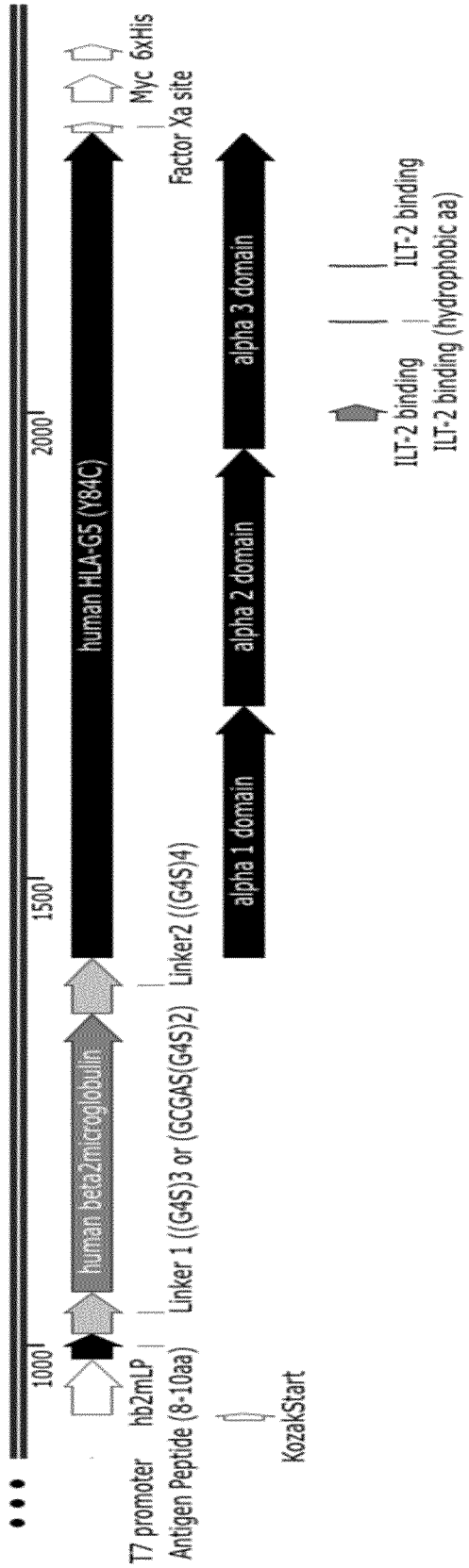

1000
1500
2000
T7 promoter
Antigen Peptide (8-10aa)
hb2mLP
KozakStart
human beta2microglobulin
Linker 1 ((G4S)3 or (GCGAS(G4S)2)
human HLA-G5 (Y84C)
Linker2 ((G4S)4)
alpha 1 domain
alpha 2 domain
alpha 3 domain
Myc 6xHis
Factor Xa site
ILT-2 binding
ILT-2 binding (hydrophobic aa)
ILT-2 binding

Figure 3

A

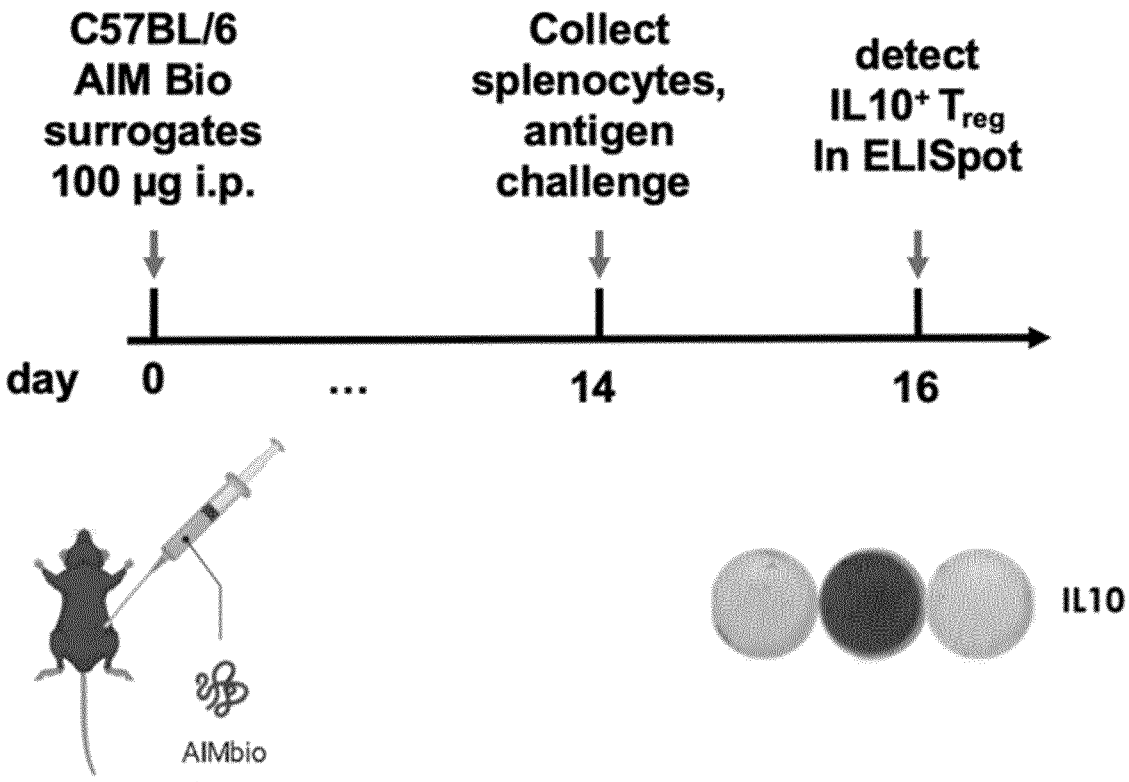

C57BL/6
AIM Bio
surrogates
100 µg i.p.
Collect
splenocytes,
antigen
challenge
detect
IL10+ Treg
In ELISpot
day
0
...
14
16
AIMbio
IL10

Figure 3, continued

B

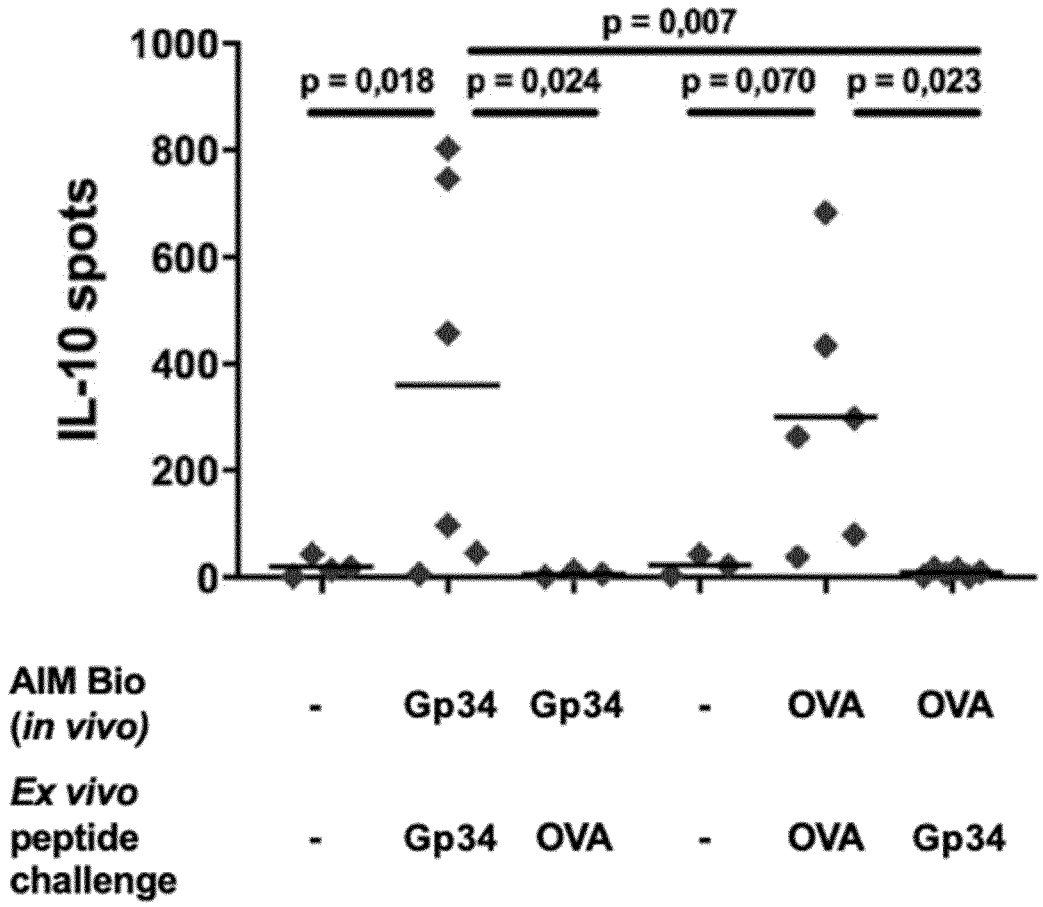


Figure 4

A

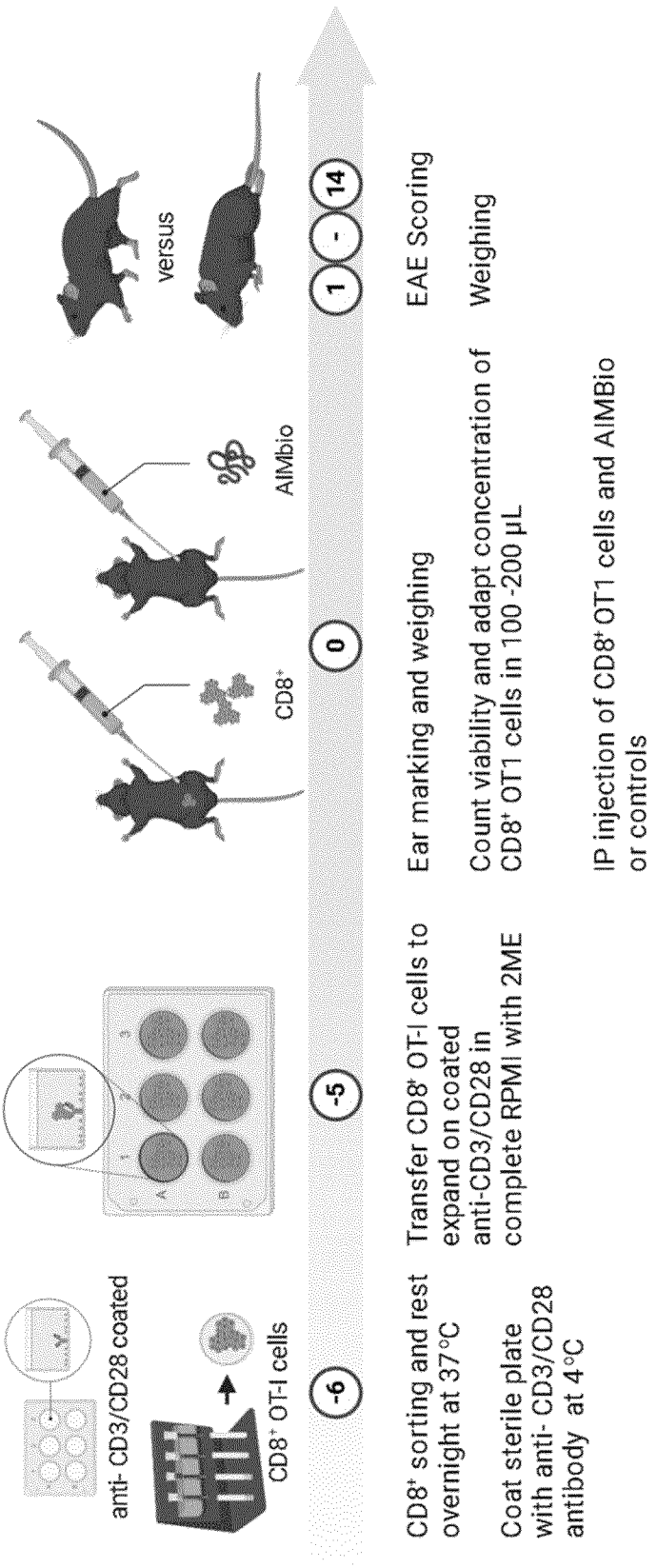

anti- CD3/CD28 coated
CD8+ OT-I cells
CD8+ sorting and rest overnight at 37°C
Coat sterile plate with anti- CD3/CD28 antibody at 4°C
-6
Transfer CD8+ OT-I cells to expand on coated anti-CD3/CD28 in complete RPMI with 2ME
-5
CD8+
0
AIMbio
Ear marking and weighing
Count viability and adapt concentration of CD8+ OT1 cells in 100 -200 µL
IP injection of CD8+ OT1 cells and AIMBio or controls
1 - 14
EAE Scoring
Weighing
versus

Figure 4, continued

B

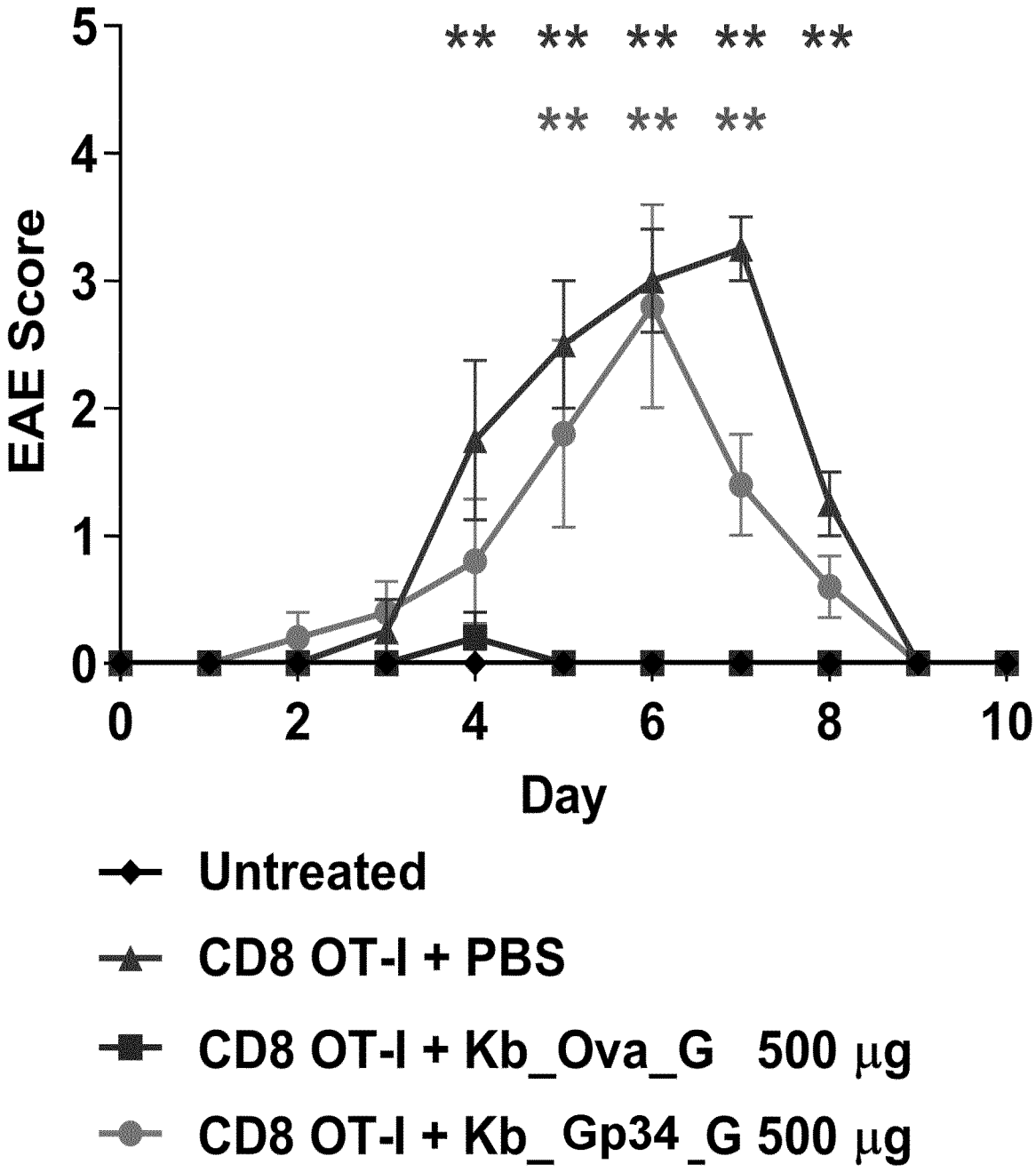


Figure 5

A

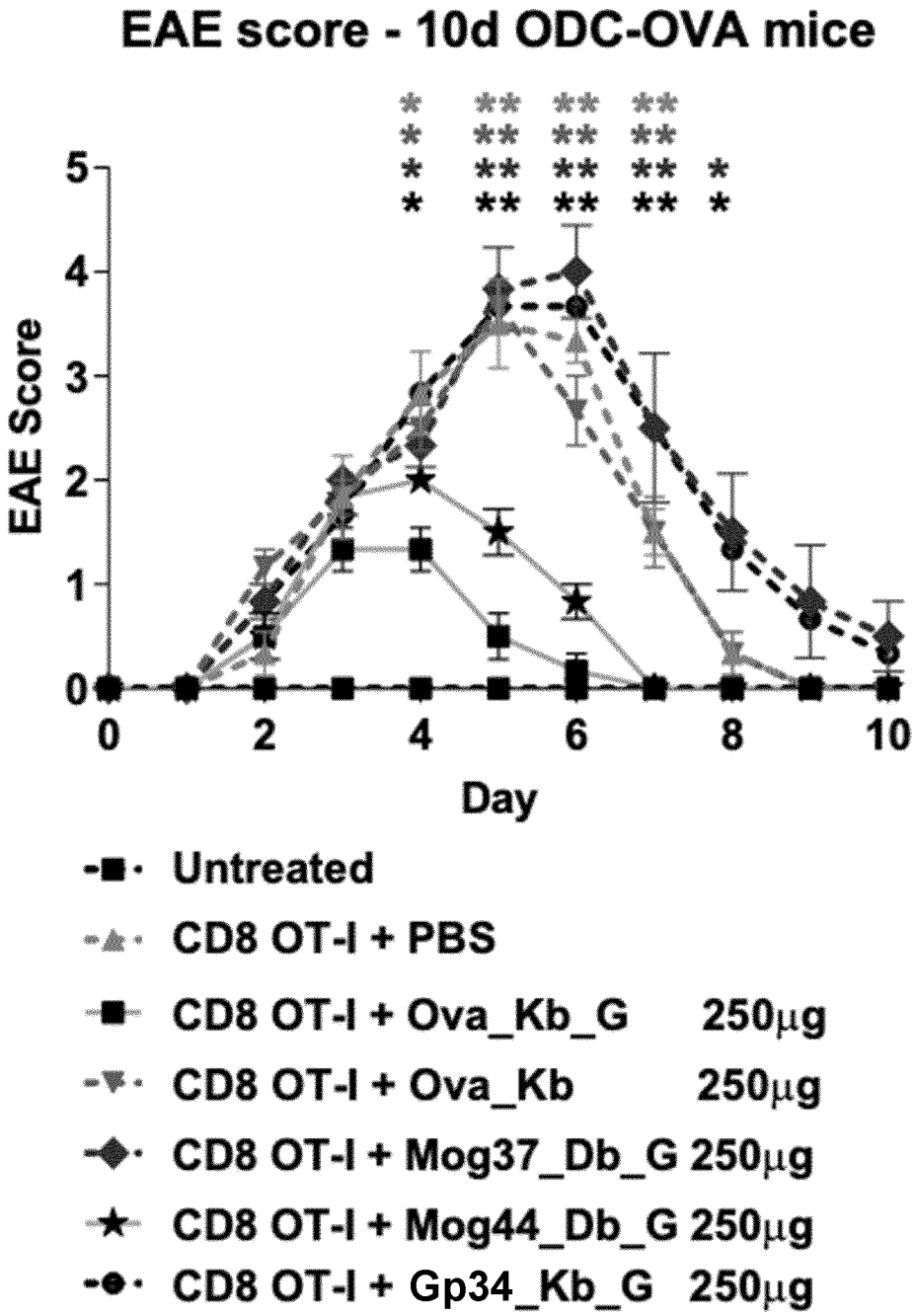

EAE score - 10d ODC-OVA mice
EAE Score
Day
0
2
4
6
8
10
0
1
2
3
4
5
Untreated
CD8 OT-I + PBS
CD8 OT-I + Ova_Kb_G 250µg
CD8 OT-I + Ova_Kb 250µg
CD8 OT-I + Mog37_Db_G 250µg
CD8 OT-I + Mog44_Db_G 250µg
CD8 OT-I + Gp34_Kb_G 250µg

Figure 5, continued

B

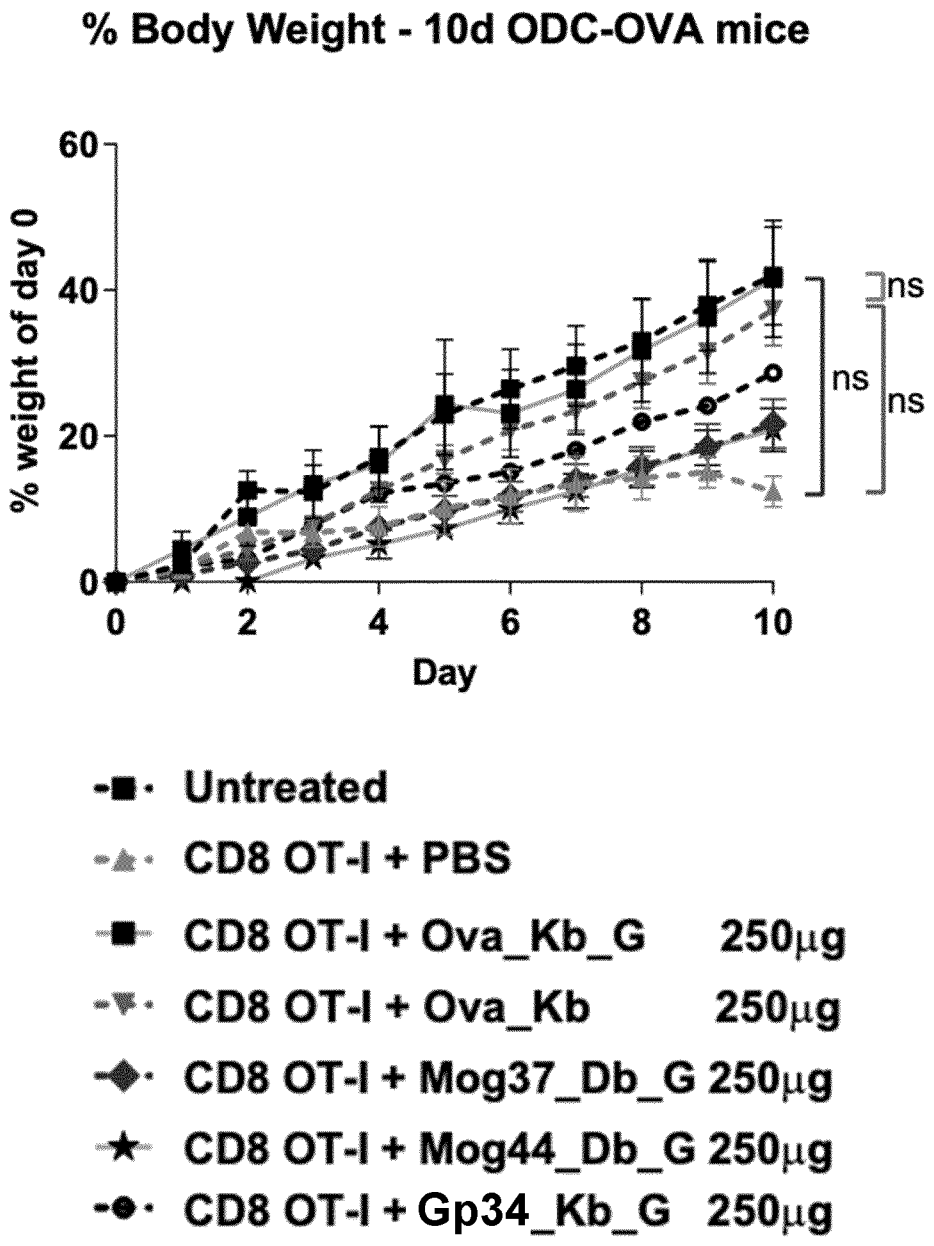


Figure 6

A

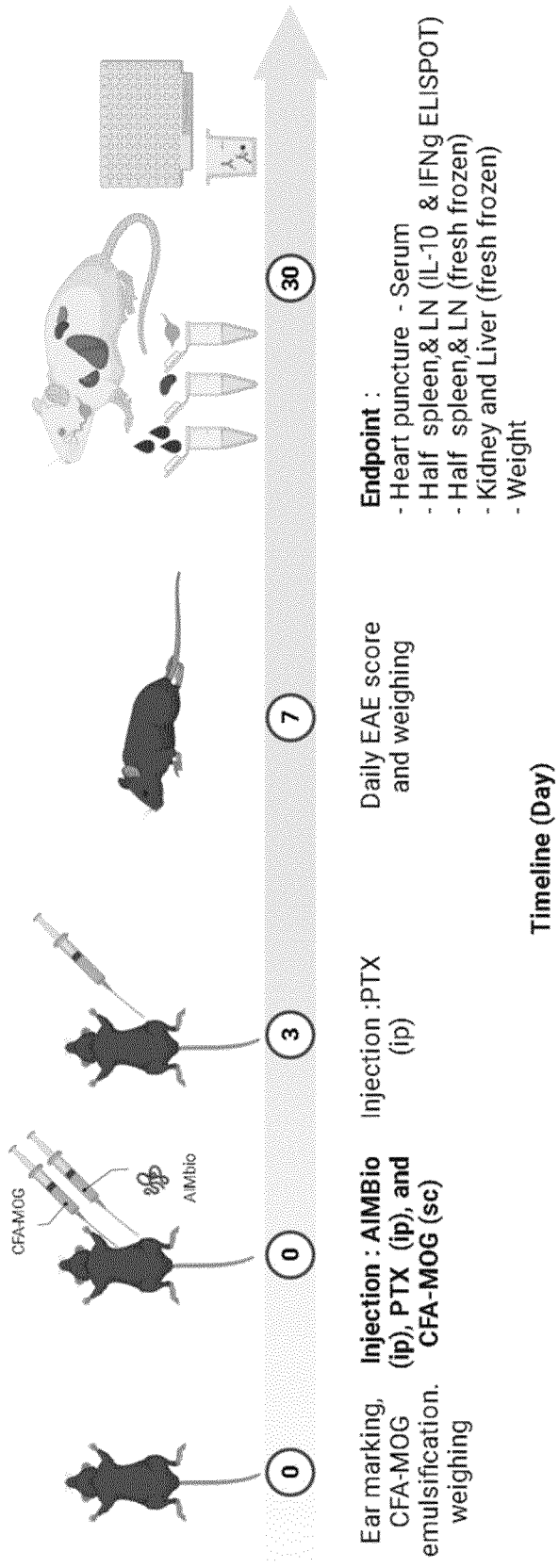

CFA-MOG
AIMbio
0
3
7
30
Ear marking, CFA-MOG emulsification. weighing
Injection : AIMBio (ip), PTX (ip), and CFA-MOG (sc)
Injection :PTX (ip)
Daily EAE score and weighing
Endpoint :
- Heart puncture - Serum
- Half spleen,& LN (IL-10 & IFNg ELISPOT)
- Half spleen,& LN (fresh frozen)
- Kidney and Liver (fresh frozen)
- Weight
Timeline (Day)

Figure 6, continued

B

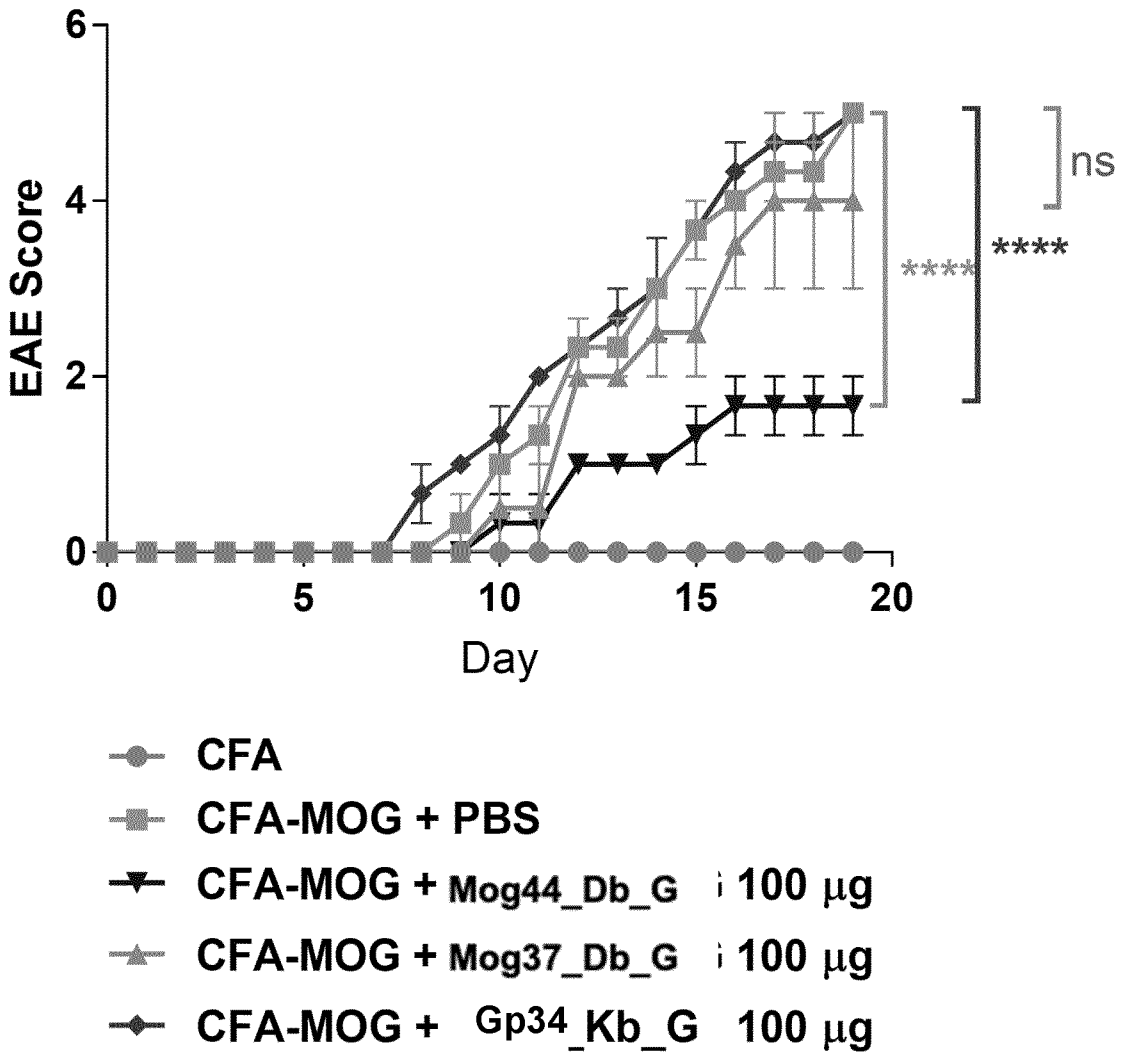


Figure 6, continued

C

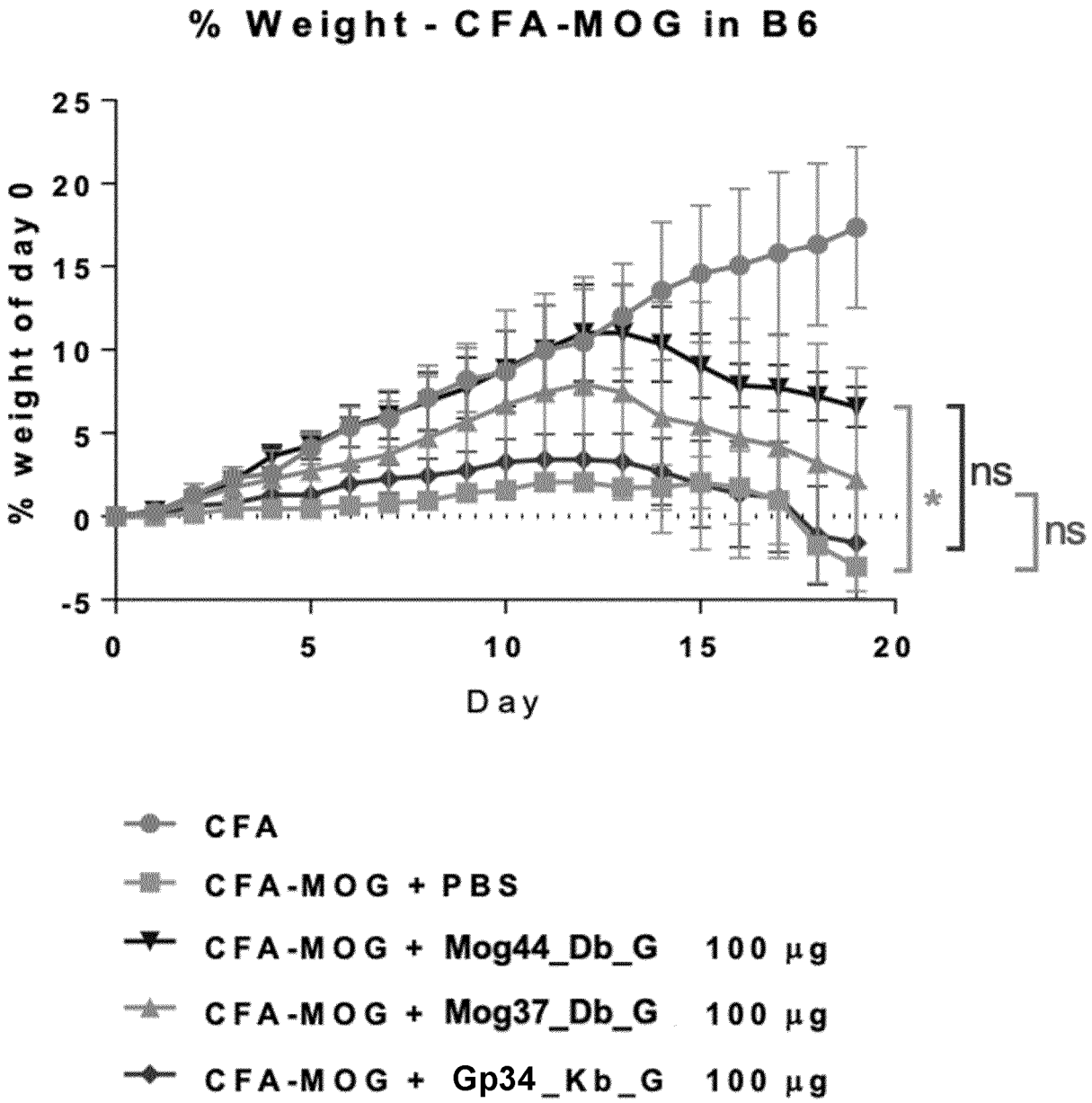
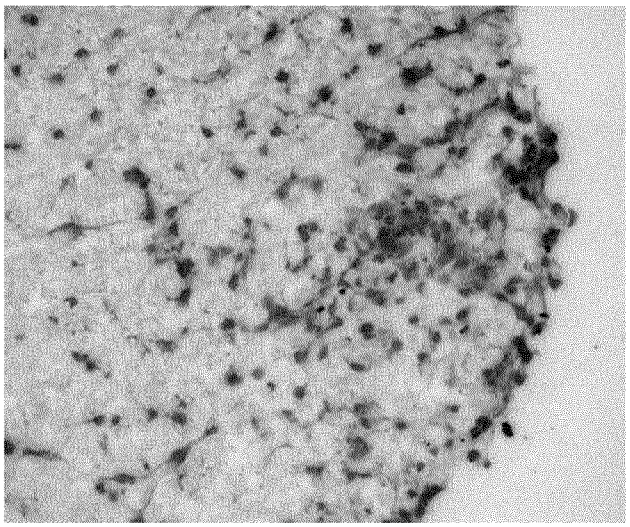


Figure 7

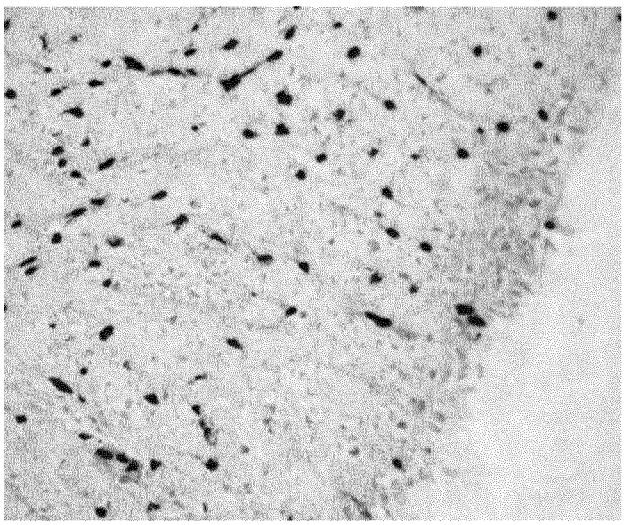
A

Spinal cord (Toluidine)

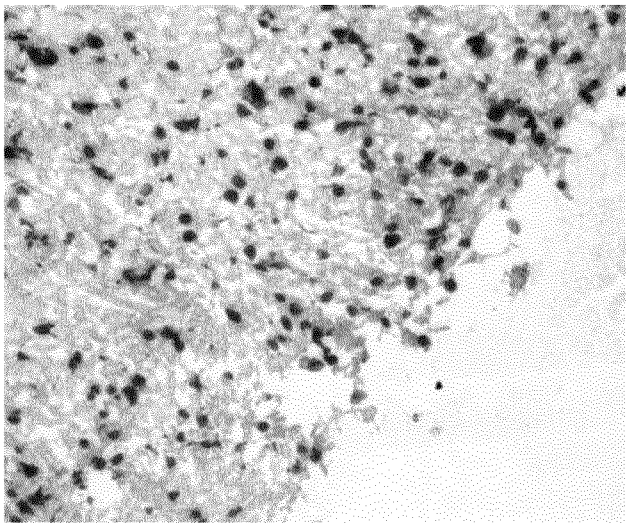
EAE + PBS



EAE + MOG44_Db_G



EAE + MOG37_Db_G



CFA control

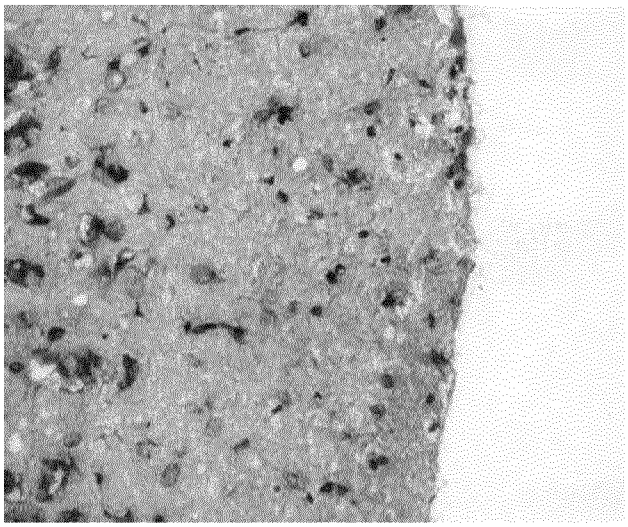
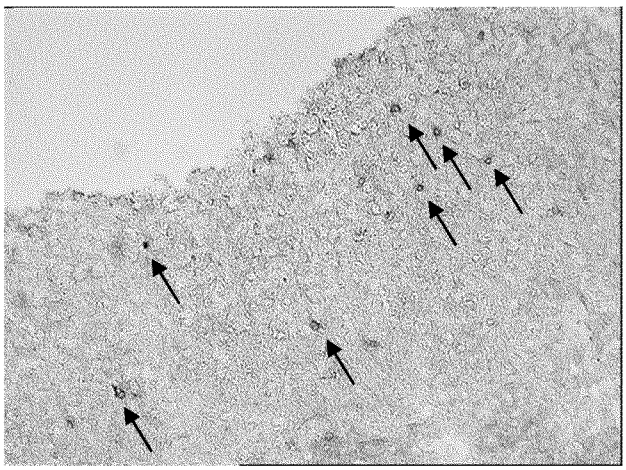


Figure 7, continued

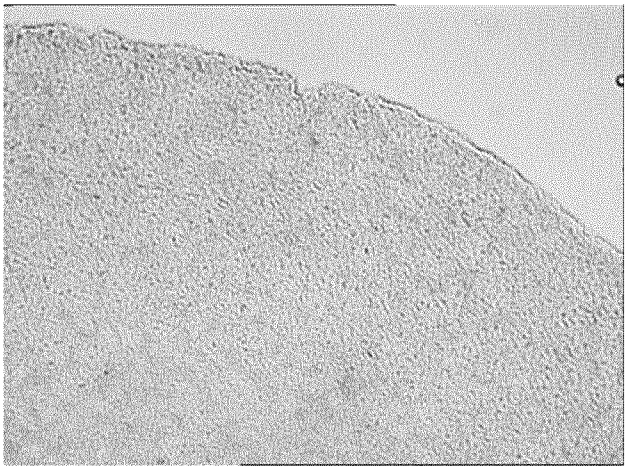
B

Spinal cord (CD8-DAB)

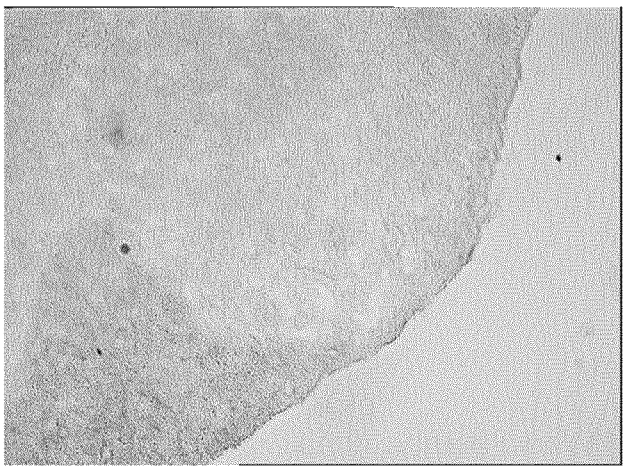
EAE + PBS



EAE + MOG44_Db_G



no EAE (CFA) control



↑ = CD8 T cell

Figure 8

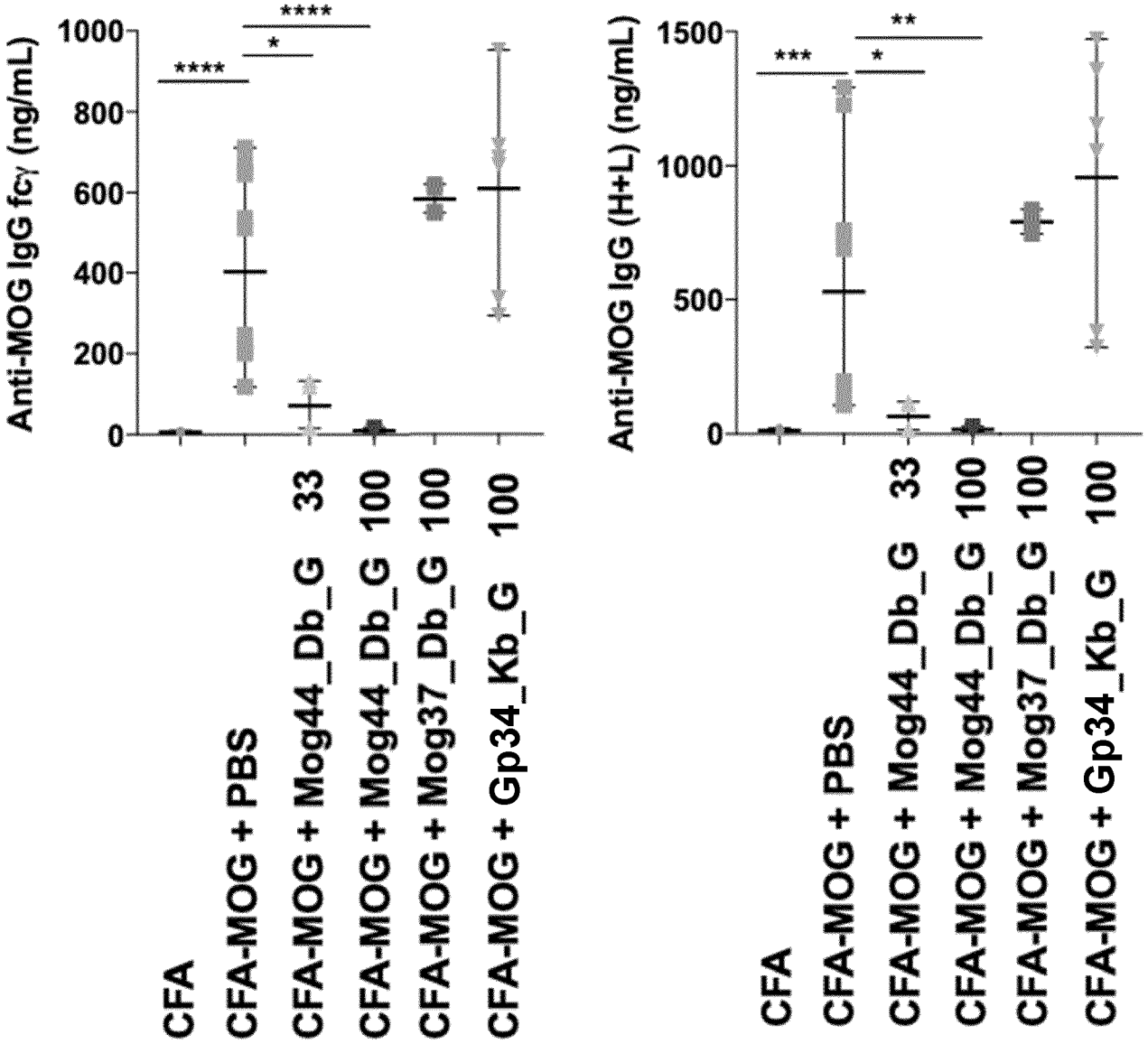

Anti-MOG IgG fcγ (ng/mL)
1000
800
600
400
200
0
****
****
*
CFA
CFA-MOG + PBS
CFA-MOG + Mog44_Db_G 33
CFA-MOG + Mog44_Db_G 100
CFA-MOG + Mog37_Db_G 100
CFA-MOG + Gp34_Kb_G 100
Anti-MOG IgG (H+L) (ng/mL)
1500
1000
500
0
***
**
*
CFA
CFA-MOG + PBS
CFA-MOG + Mog44_Db_G 33
CFA-MOG + Mog44_Db_G 100
CFA-MOG + Mog37_Db_G 100
CFA-MOG + Gp34_Kb_G 100

Figure 9

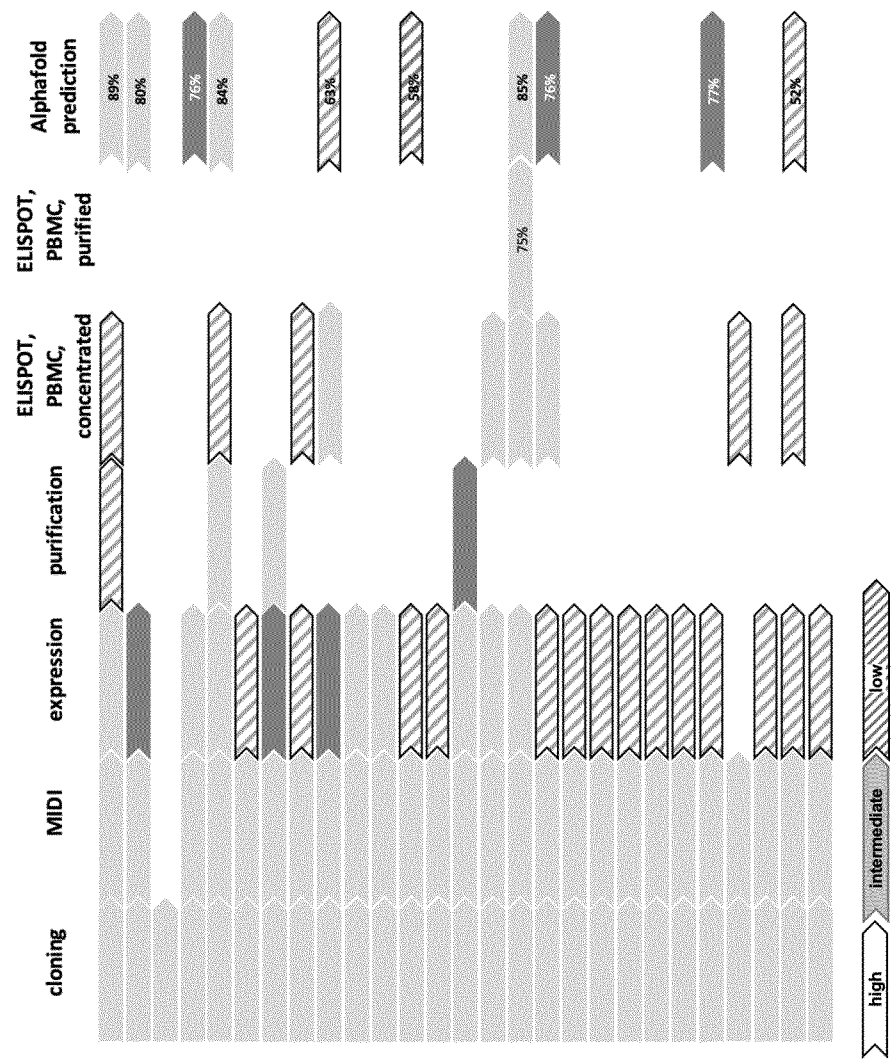

MS AIMbio
name AIMbio
peptide sequence
cloning
MIDI
expression
purification
ELISPOT, PBMC, concentrated
ELISPOT, PBMC, purified
Alphafold prediction
MS-01 MAG160_A2G_His MVPDNCPEL
MS-02 MAG160_G_His MVPDNCPEL
MS-03 MAG237_G_Spt KYPPVIVEM
MS-04 MAG281_G_Spt REAVAESLL
MS-05 MAG287_A2G_Spt SLLLELEEV
MS-06 MBP29_G_Spt FLPRHRDTG
MS-07 MBP75_G_Spt RSQPGLCNM
MS-08 MBP169_G_Spt KGVDAQGTL
MS-09 MBP244_A2G_Spt SLSRFSWGA
MS-10 MOG38_G_Spt RHPIRALVG
MS-11 MOG42_G_Spt RALVGDEVEL
MS-12 MOG44_G_Spt FSRVVHLYRNG
MS-13 MOG70_G_Spt RPPFSRVVHL
MS-14 MOG102_G_Spt KDAIGEGKVTL
MS-15 MOG104_A2G_Spt AIGEGKVTL
MS-16 MOG157_A2G_Spt VLLAVLPVL
MS-17 MOG157_G_Spt VLLAVLPVL
MS-18 MOG163_G_Spt VSPGVLVLL
MS-19 MOG185_G_His KLRAEIENL
MS-20 MOG203_A2G_His RVPCWKITL
MS-21 MOG203_G_His RVPCWKITL
MS-22 PLP3_A2G_His LLLECCARCL
MS-23 PLP3_G_His LLLECCARCL
MS-24 PLP80_A2G_His FLYGALLLA
MS-25 PLP105_G_Spt KTTICGKGL
MS-26 PLP127_G_His RGQHQAHSL
MS-27 PLP151_G_spt KFVGITYAL
89%
80%
76%
84%
63%
58%
75%
85%
76%
77%
52%
priority
high
intermediate
low

Figure 10

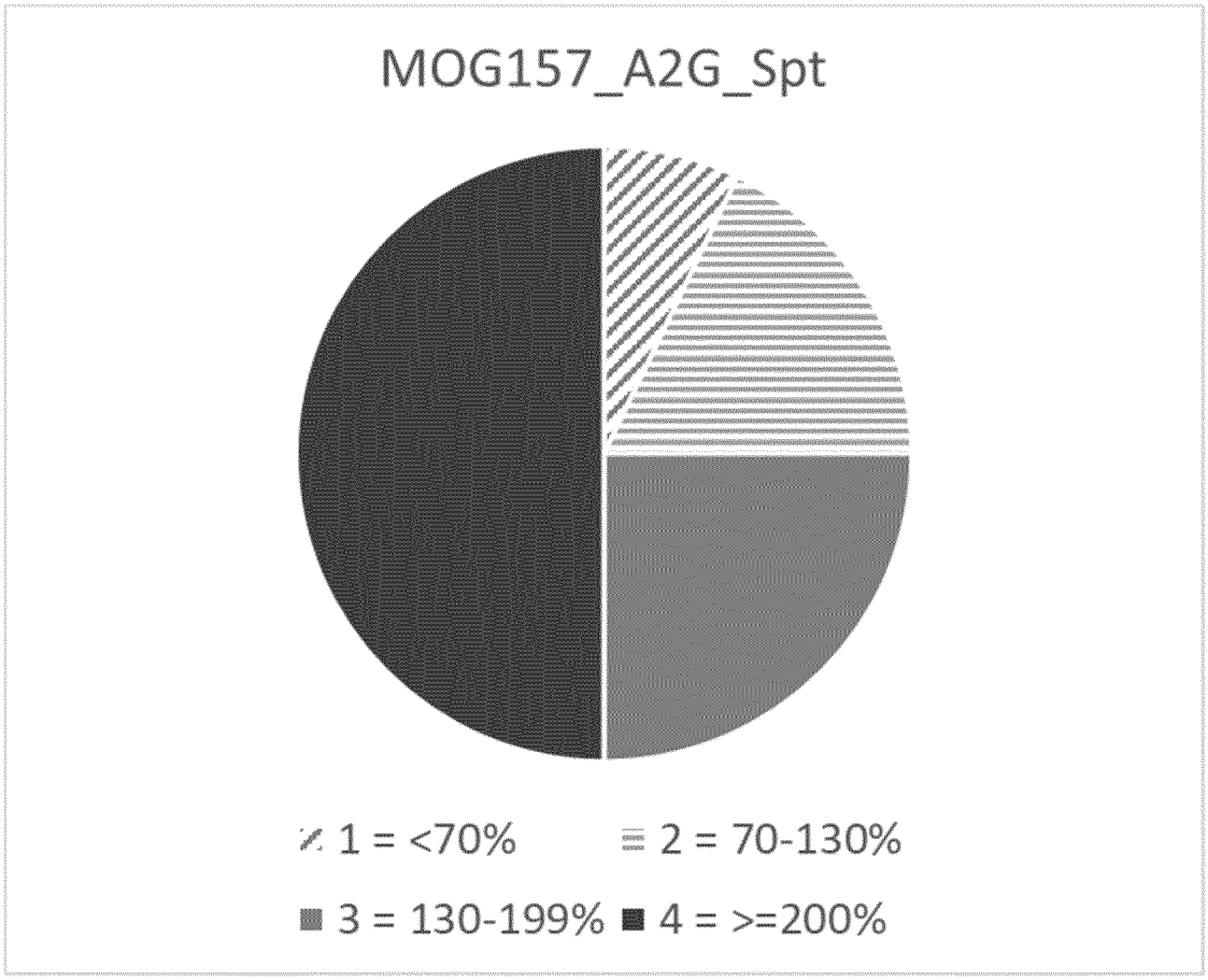


Figure 11

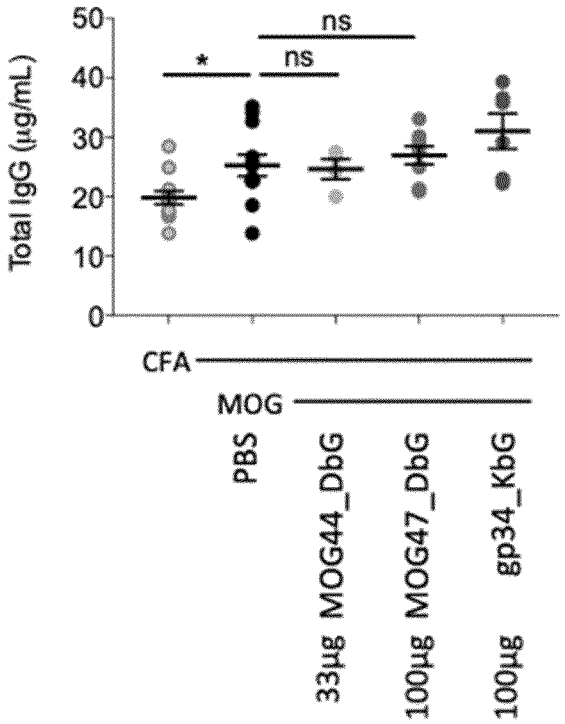

Total IgG (µg/mL)
50
40
30
20
10
0
*
ns
ns
CFA
MOG
PBS
33µg MOG44_DbG
100µg MOG47_DbG
100µg gp34_KbG

Figure 12

stability of purified single-chain MHC Ib molecules

A

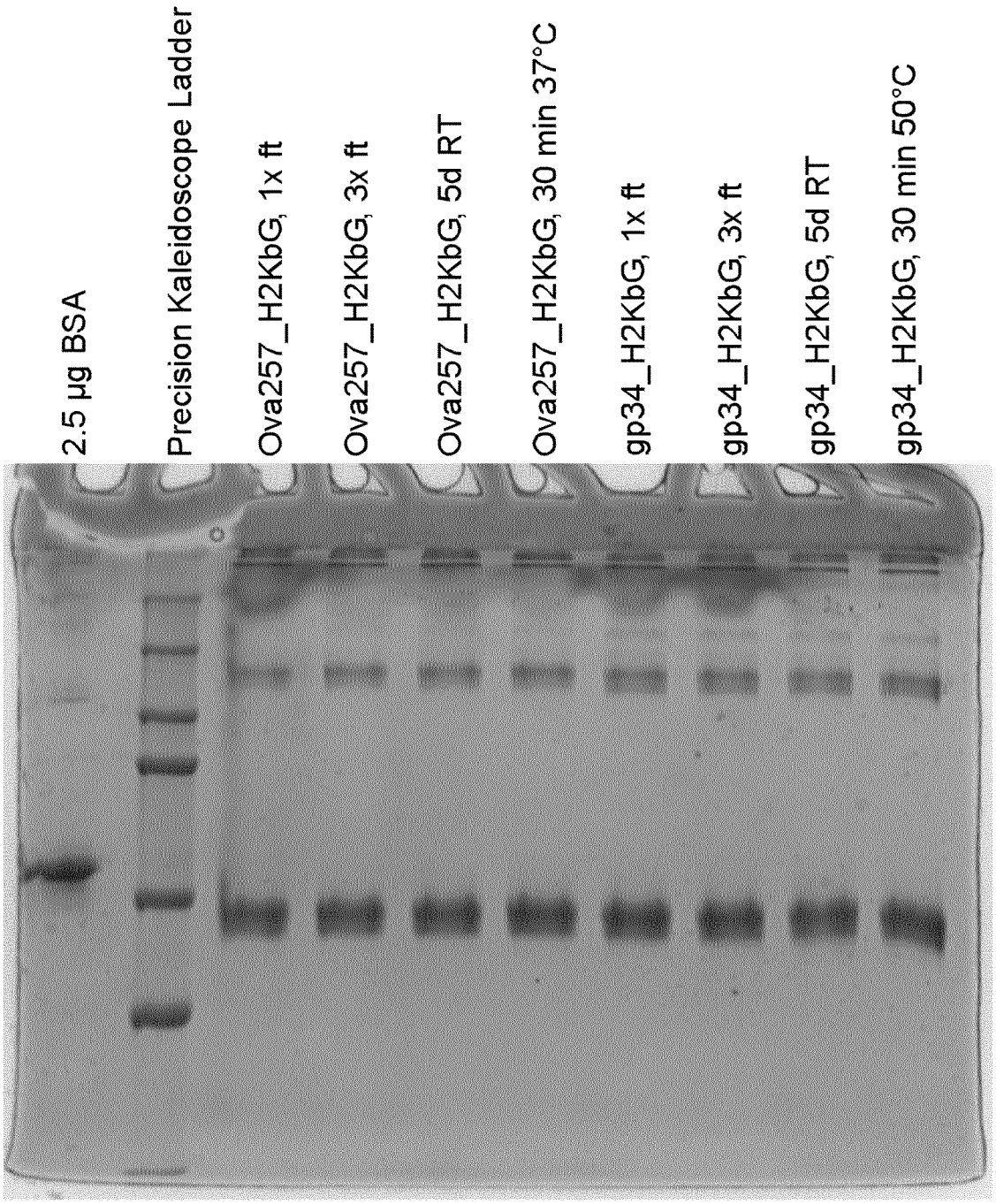


Figure 12, continued

B

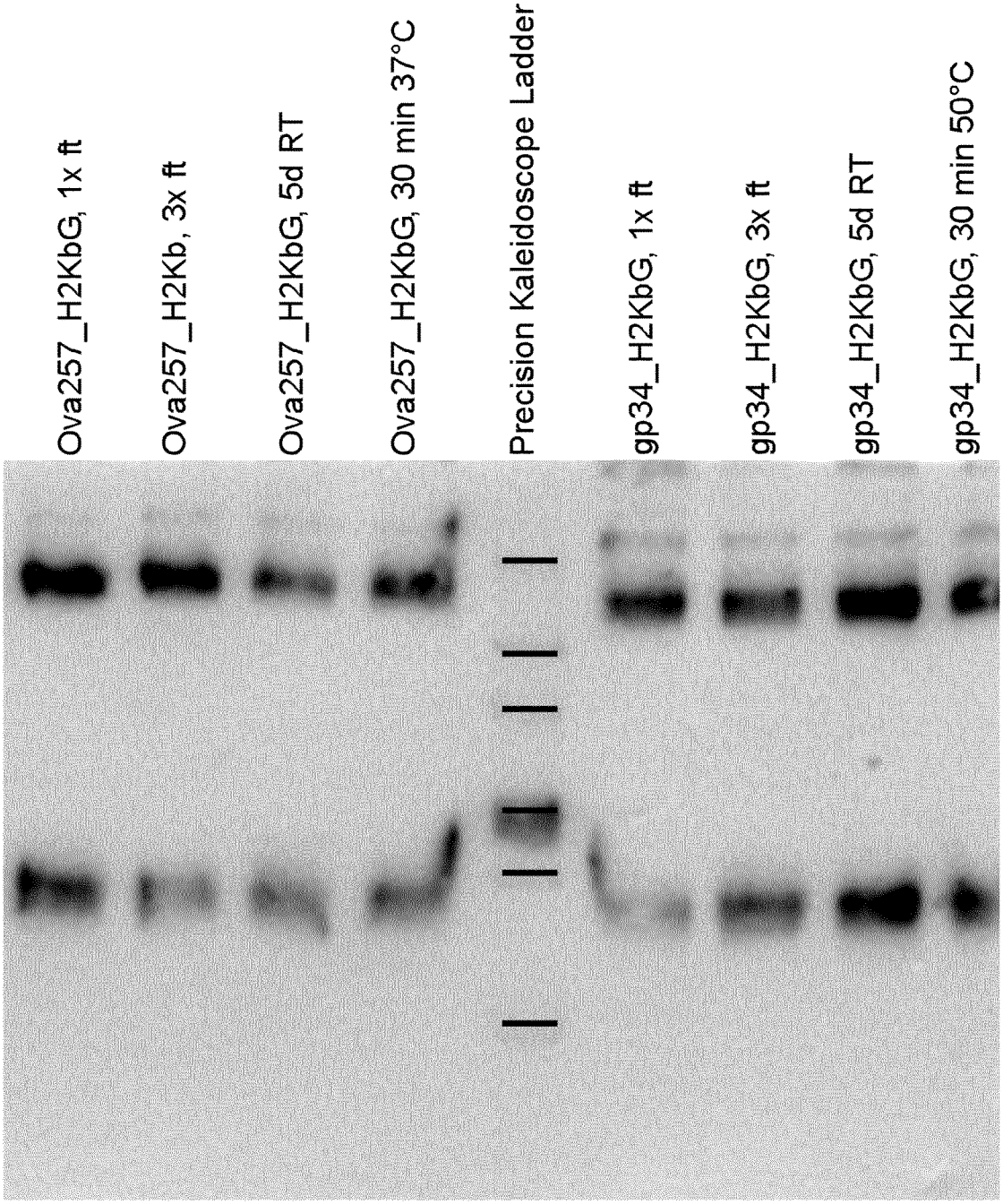


Figure 13

single-chain MHC Ib molecules are thermally stable

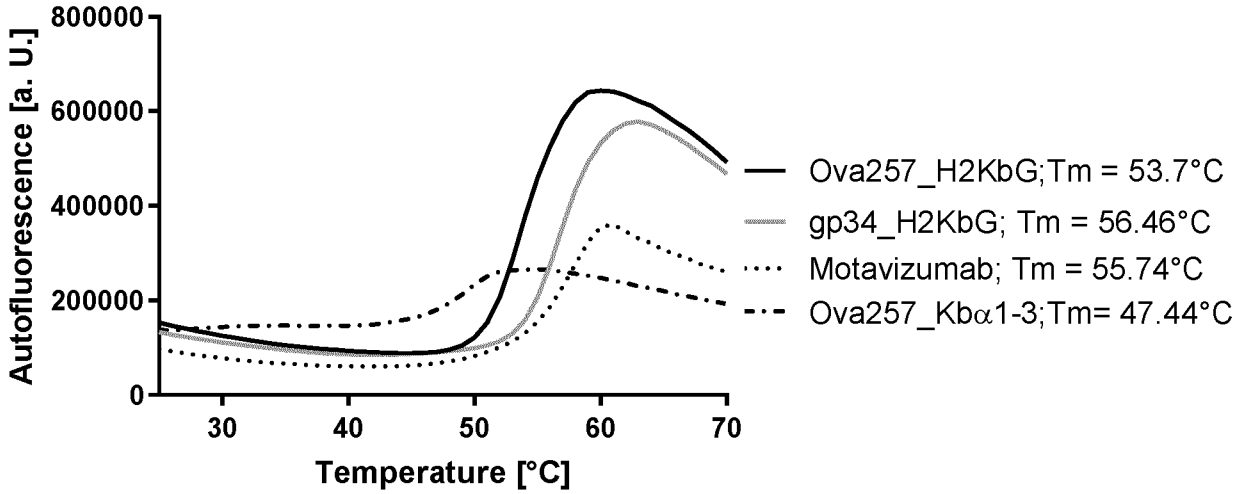


Figure 14

single-chain MHC Ib molecules induce Treg in a dose-dependent manner

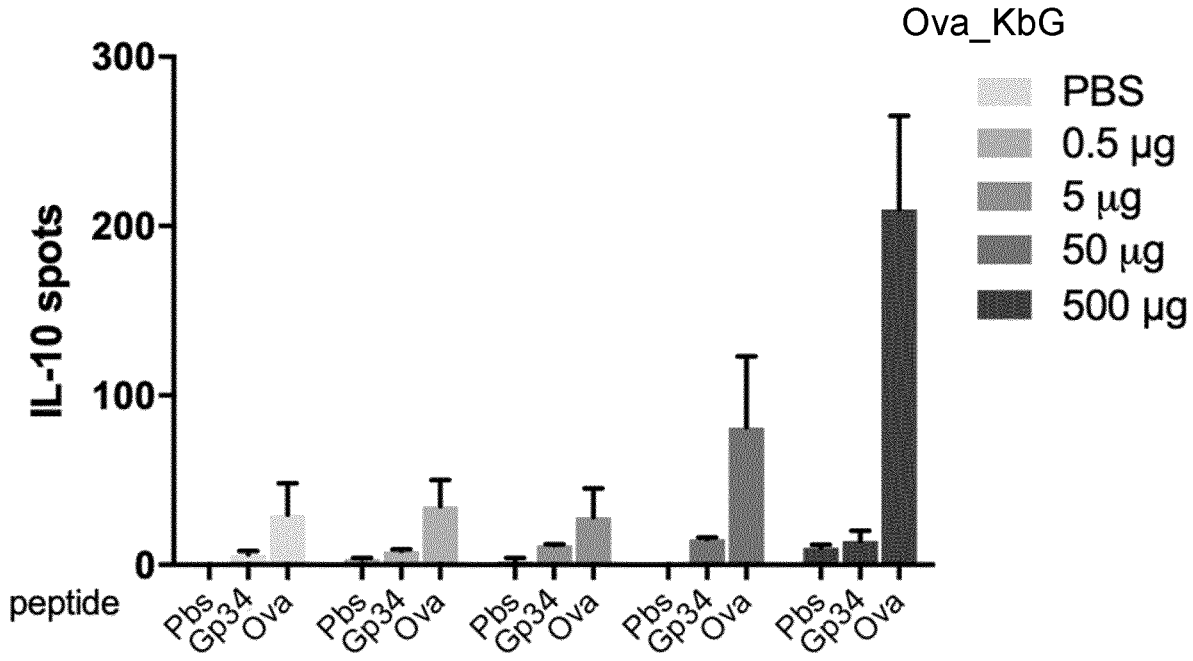


Figure 15

Single-chain MHC Ib molecules inhibit T cell lysis in a dose-dependent manner

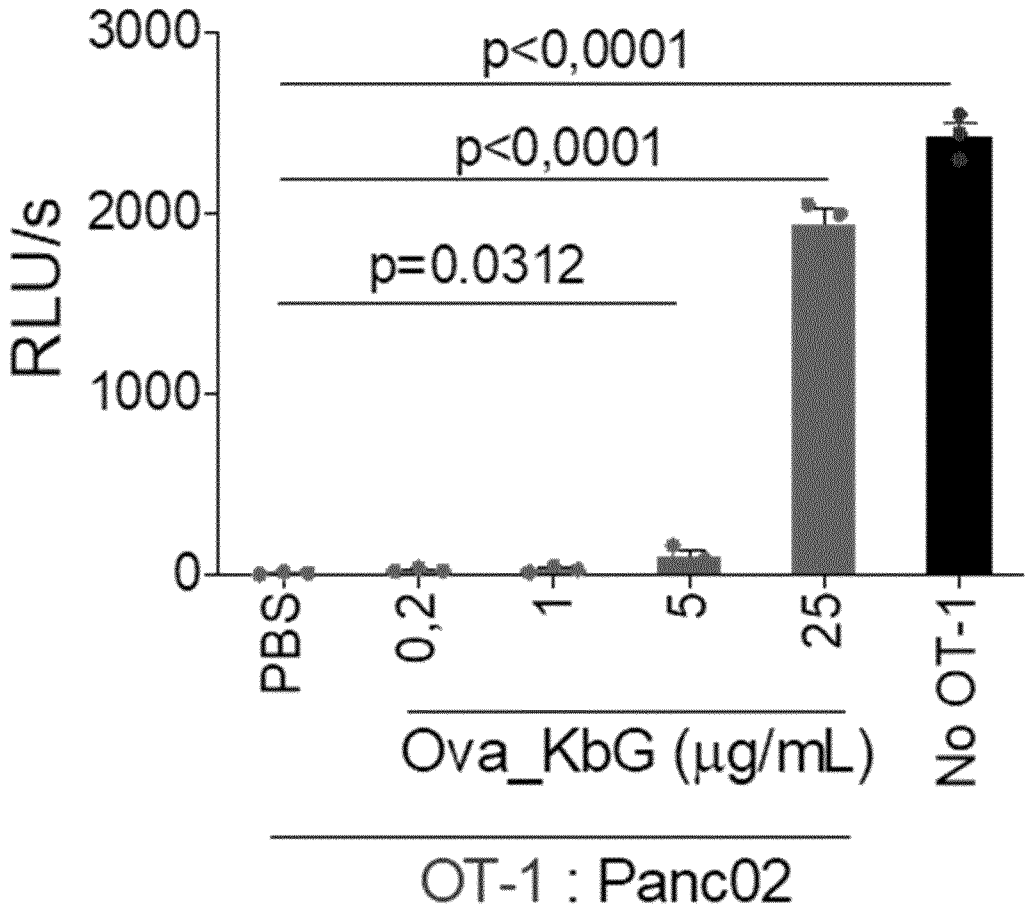


Figure 16

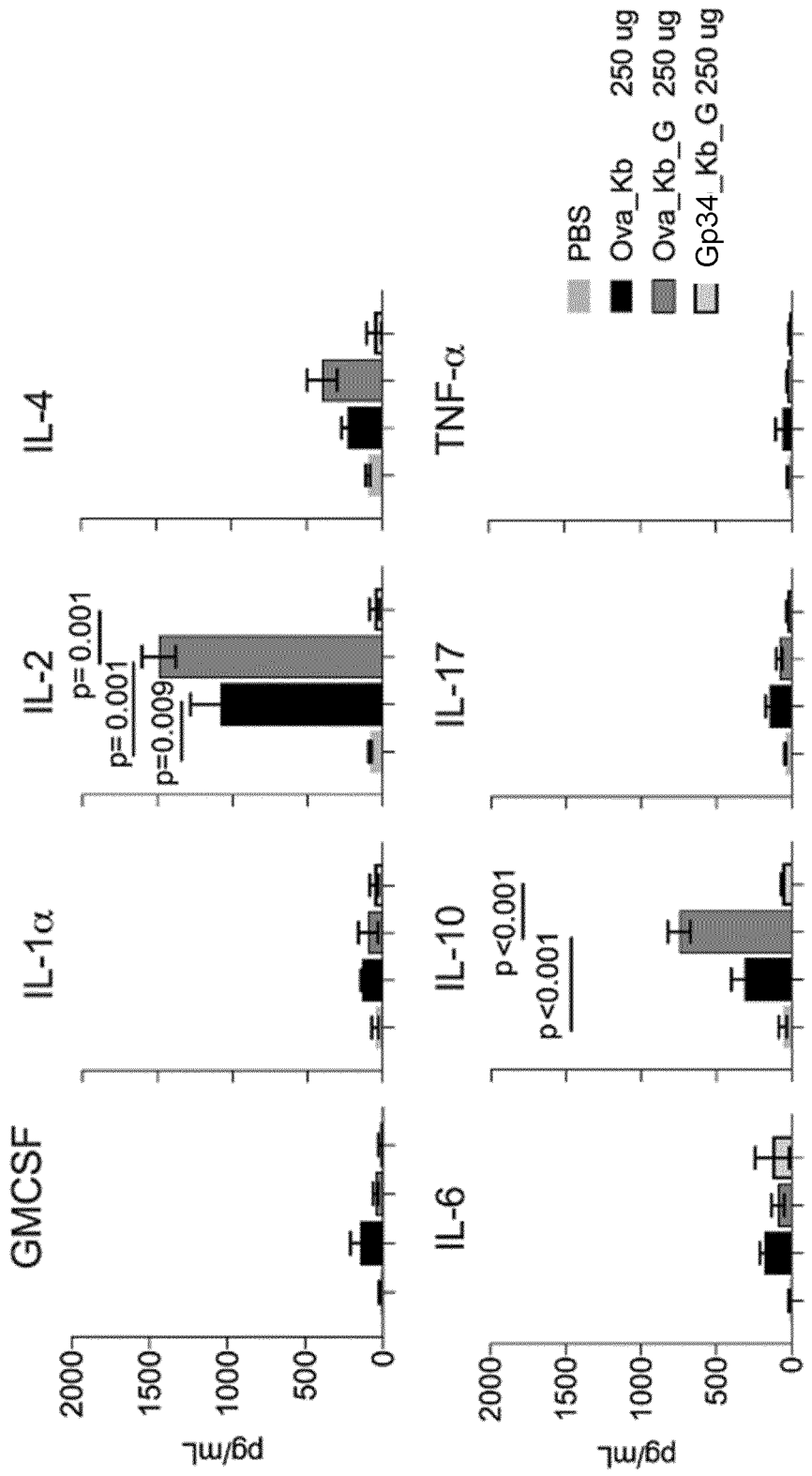
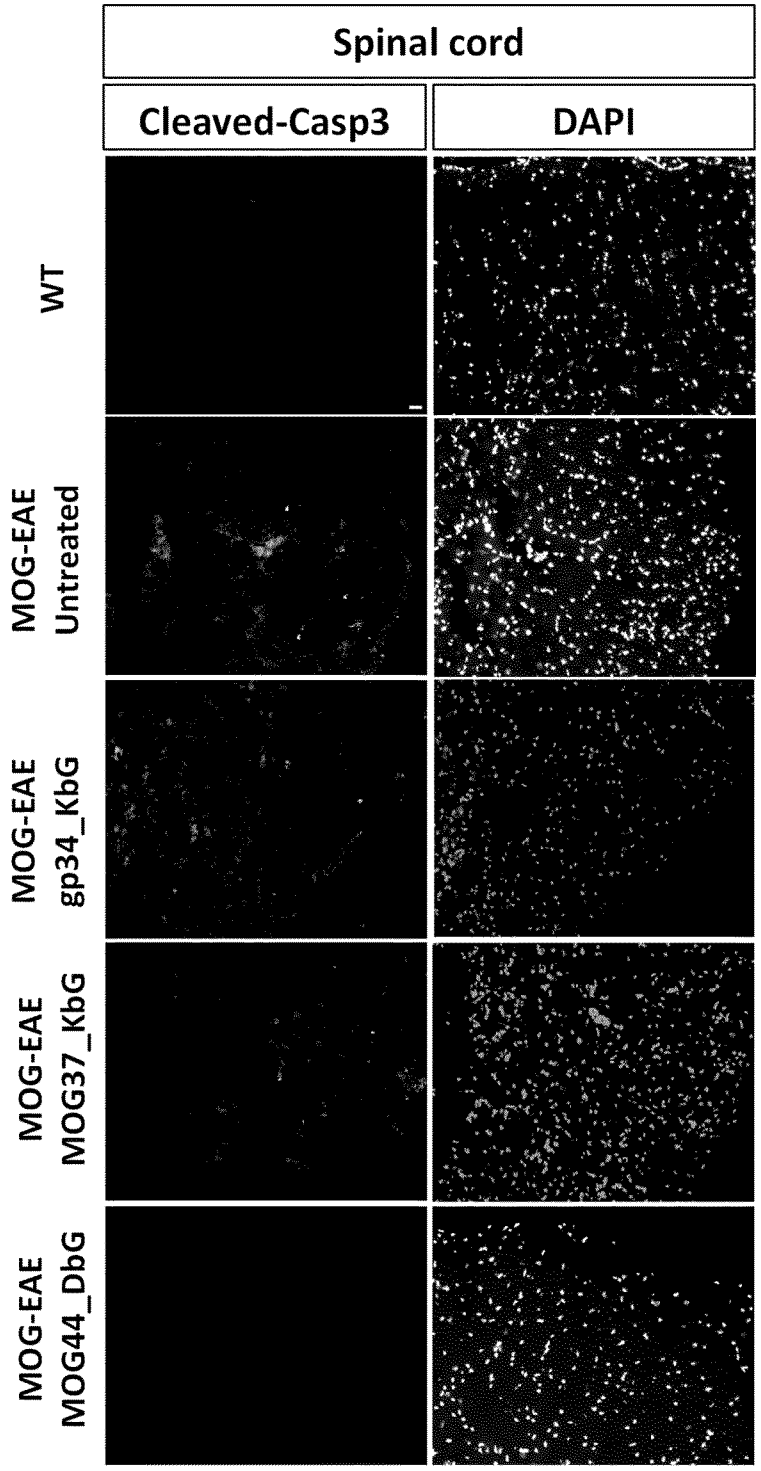

Gp34
PBS
Ova_Kb 250 ug
Ova_Kb_G 250 ug
Gp34_Kb_G 250 ug
GMCSF
IL-1α
IL-2
IL-4
IL-6
IL-10
IL-17
TNF-α
p= 0.001
p= 0.001
p=0.009
p<0.001
p<0.001
pg/mL
0
500
1000
1500
2000

Figure 17

A



Scale bar: 25 µm

Figure 17, continued

B

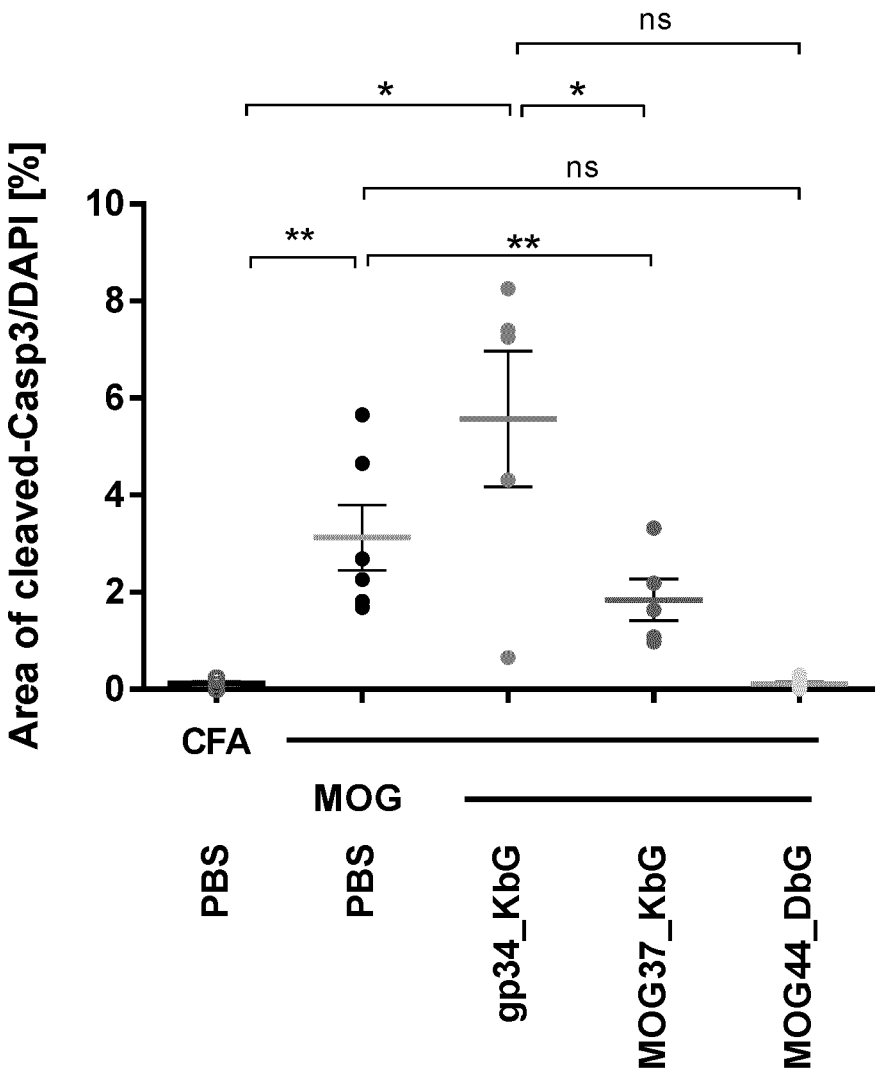


Figure 17, continued

C

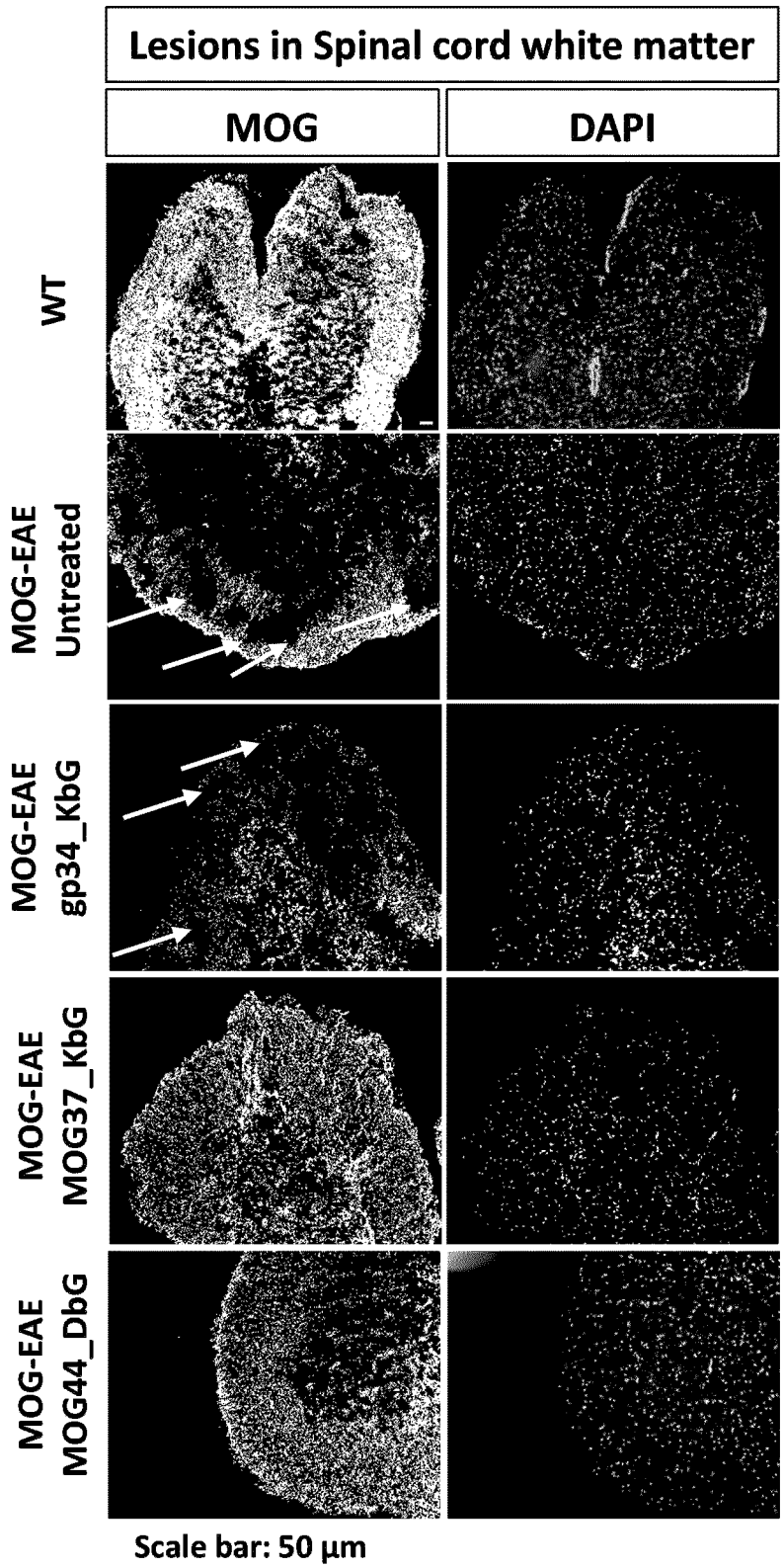
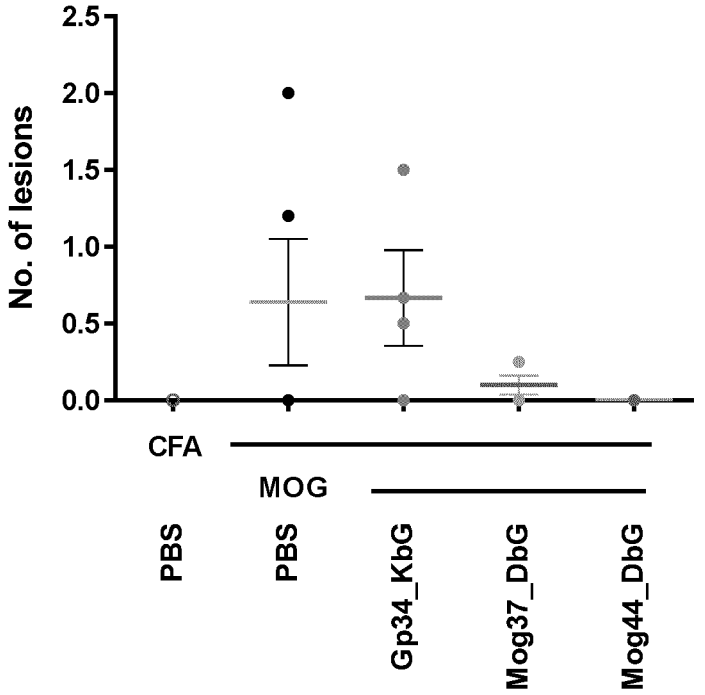


Figure 17, continued

D



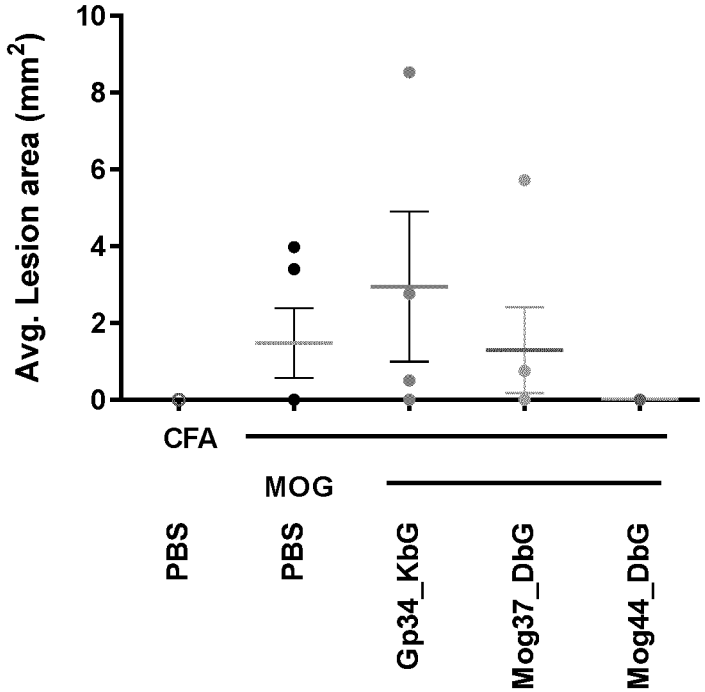


Figure 17, continued

E

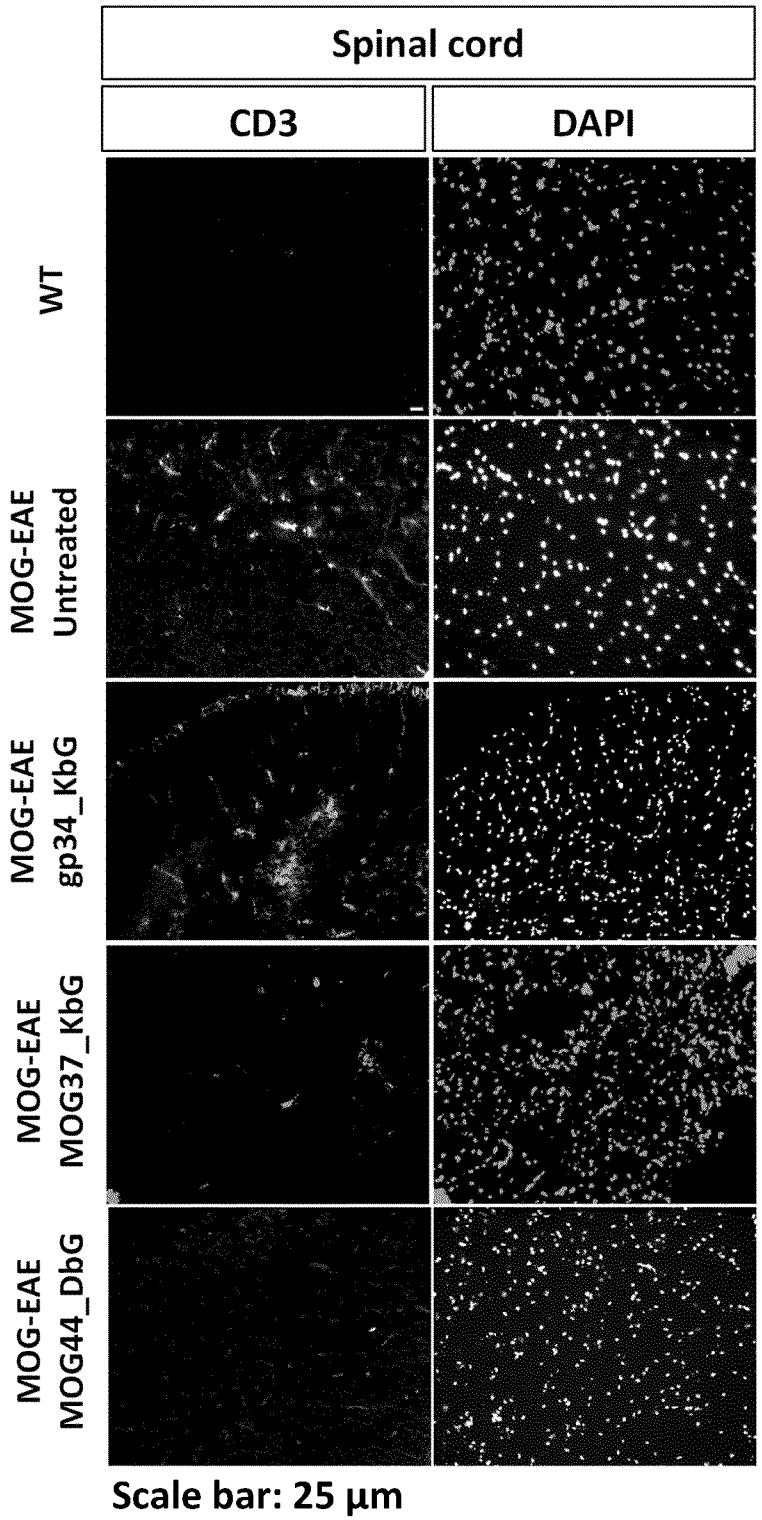


Figure 17, continued

F

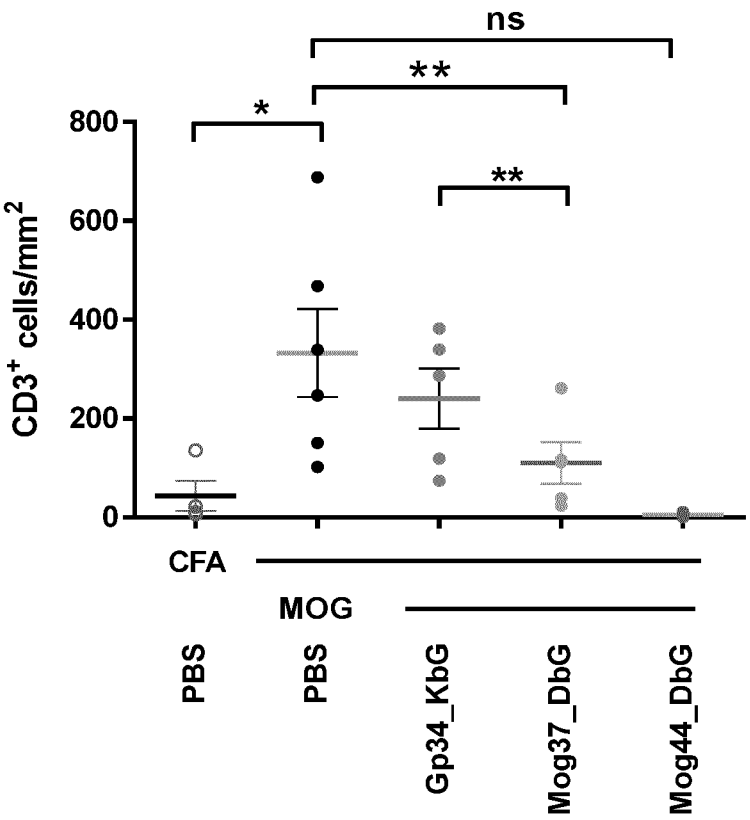
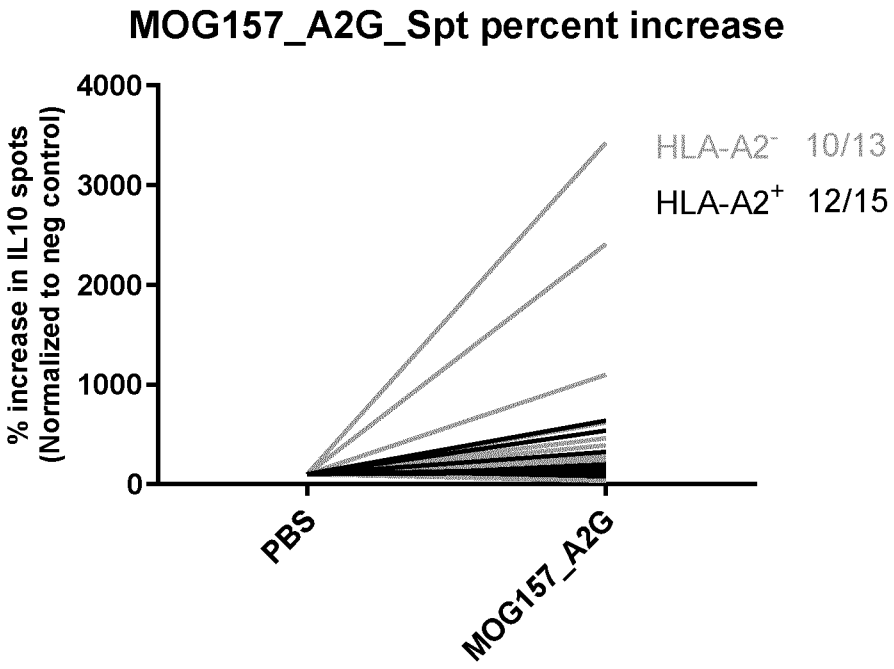


Figure 18



INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2023/057679

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed.

   b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      ☒ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/057679

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/435 C07K16/28
ADD.

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2018/215340 A1 (BRUTTEL VALENTIN [DE]; UNIV WUERZBURG J MAXIMILIANS [DE]) 29 November 2018 (2018-11-29) the whole document in particular, claims 22 and 44 -----	1-46
Y	US 2020/138928 A1 (CHEN SWEY-SHEN [US]) 7 May 2020 (2020-05-07) the whole document in particular, paragraphs [0033], [0048], [0056] to [0057], [0097], and [0110] -----	1-46

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

[x] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 2023	07/07/2023

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

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/057679

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WISCHHUSEN JORG ET AL: "alpha-synuclein peptides presented on chimeric MHC class Ib molecules prevent loss of substantia nigra neurons in an animal model for Parkinson's disease", JOURNAL OF IMMUNOLOGY, vol. 208, no. 1, 15 March 2022 (2022-03-15), XP055965217, Retrieved from the Internet: URL:https://www.jimmunol.org/content/208/1_Supplement/60.16> the whole document -----	1-46
A	TAJFIROUZ DEENA A. ET AL: "Clinical Characteristics and Treatment of MOG-IgG-Associated Optic Neuritis", CURRENT NEUROLOGY AND NEUROSCIENCE REPORTS, vol. 19, no. 12, 26 November 2019 (2019-11-26), XP93057252, New York ISSN: 1528-4042, DOI: 10.1007/s11910-019-1014-z Retrieved from the Internet: URL:http://link.springer.com/article/10.1007/s11910-019-1014-z/fulltext.html> the whole document -----	1-46

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2023/057679

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2020157175 A1	21-05-2020
		WO 2018215340 A1	29-11-2018
US 2020138928 A1	07-05-2020	NONE	