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(54) Title: PEPTIDE EXCHANGE PROTEIN

(57) Abstract: This invention relates to peptide-exchange proteins comprising the luminal domain of TAP-binding protein-related (TAPBPR), which functions as a MHC class I peptide-exchange catalyst when presented to mammalian cells either as a soluble extracellular protein or as a membrane bound cell surface protein. This may be useful in modulating immune responses, including for example loading immunogenic peptide onto tumours or other disease cells to induce their recognition by T cells. Peptide-exchange proteins and methods for their use are provided.

Peptide Exchange Protein

Field

The present invention relates to peptide exchange catalysts and their use in modulating the peptide repertoire displayed by MHC class I molecules on the surface of mammalian cells.

Background

Although cancer immunotherapy has finally come of age, new therapies are in desperate need for patients with tumours resistant to current treatments [1]. With the use of immune checkpoint inhibitors such as anti-PD1 and anti-CTLA4, there is now potential to harness the function of T lymphocytes to recognise and destroy tumours [1,2]. However, such therapies are currently only beneficial for some patients particularly those with tumours of high mutational load [3-6]. Tumours can escape either natural or immunotherapy-induced immune control by a number of mechanisms including when the process of immunoediting selects for tumours with low immunogenicity [7]. Therefore, the ability to increase the immunogenicity of tumours may provide therapeutic benefit to a wider cohort of patients, including those with a lower mutational load.

As cytotoxic T lymphocytes recognise immunogenic peptides presented on MHC class I molecules, the ability to directly manipulate the antigens displayed on these molecules would be a fundamental step forward in our ability to boost both antitumour and antiviral immune responses. Over the past few years, we have been exploring the function TAPBPR, an IFN-γ-inducible MHC class I dedicated chaperone in the antigen processing and presentation pathway[7]. TAPBPR functions as a peptide editor on MHC class I molecules [8,9] and influences the final peptide repertoire expressed on the surface of cells[8]. Within the ER/cis-Golgi TAPBPR bridges UDP-glucose:glycoprotein glucosyltransferase 1 (UGT1), an resident enzyme which monitors glycoprotein folding, onto MHC class I to provide a quality control checkpoint[10]. Although TAPBPR resides intracellularly when expressed at natural levels, we have previously observed that over-expression of TAPBPR results in some of the TAPBPR protein being mislocalised to the cell surface[11].

Summary

The present inventors have unexpectedly discovered that the luminal domain of TAPBPR retains its ability to function as a MHC class I peptide-exchange catalyst when presented to mammalian cells either as a soluble extracellular protein or as a membrane bound cell surface protein. Soluble or cell surface peptide exchange catalysts may be useful in a range of therapeutic applications in the modulation of immune responses, including for example loading immunogenic peptide onto tumours or other disease cells to induce their recognition by T cells.

A first aspect of the invention provides a peptide-exchange protein comprising a fragment of TAP-binding protein-related (TAPBPR), said fragment consisting of the TAPBPR luminal domain.

In a particular embodiment, the invention provides an isolated peptide-exchange protein consisting of:

a fragment of TAP-binding protein-related (TAPBPR), said fragment consisting of a TAPBPR luminal domain and

a targeting domain that specifically binds to the surface of target cells,

wherein the targeting domain is an antibody molecule comprising an antibody antigen-binding domain, and consists of an amino acid sequence having at least 5070% sequence identity over its full length to the full length sequence of SEQ ID NO: 2 or SEQ ID NO: 22.

A peptide-exchange protein of the first aspect may be soluble or surface-bound. A surface-bound peptide-exchange protein of the first aspect may further comprise a heterologous transmembrane domain.

In a further embodiment, the peptide-exchange protein comprises:

(i) a fragment of TAP-binding protein-related (TAPBPR), said fragment comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22, and a TAPBPR transmembrane domain and a heterologous cell surface targeting sequence; or

(ii) a fragment of TAP-binding protein-related (TAPBPR) comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22 and a heterologous transmembrane domain, wherein the heterologous transmembrane domain localises the peptide-exchange protein to the plasma membrane; or.

(iii) a fragment of TAP-binding protein-related (TAPBPR) comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22, a heterologous transmembrane domain, and a heterologous cell surface targeting sequence; or

(iv) a fragment of TAP-binding protein-related (TAPBPR), said fragment consisting of a TAPBPR transmembrane domain and a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22,

wherein the TAPBPR transmembrane domain localises the peptide-exchange protein to the plasma membrane.

A second aspect of the invention provides a peptide-exchange protein comprising a TAPBPR fragment consisting of the TAPBPR luminal domain and TAPBPR transmembrane domain.

A surface-bound peptide-exchange protein of the first aspect or a peptide-exchange protein of the second aspect may further comprise a heterologous cell surface targeting sequence.

A third aspect of the invention provides a peptide-exchange protein comprising a TAPBPR fragment and a targeting domain, wherein the TAPBPR fragment comprises the TAPBPR luminal domain.

A peptide-exchange protein of third aspect may be soluble. The TAPBPR fragment may consist of the luminal domain.

A fourth aspect of the invention provides a nucleic acid encoding a peptide-exchange protein of the first, second, or third aspects.

A fifth aspect of the invention provides a vector comprising a nucleic acid of the fourth aspect.

A sixth aspect of the invention provides a mammalian cell comprising a peptide-exchange protein of the second aspect at its surface.

A seventh aspect provides an *in vitro, ex vivo,* or *in vivo* method of increasing the immunogenicity of mammalian cells comprising;

providing a population of mammalian cells having surface MHC class I molecules, and

contacting the population of mammalian cells with an immunogenic peptide and a peptide exchange protein of the first, second or third aspect,

such that the peptide exchange protein loads the immunogenic peptide onto MHC class I molecules on the surface of the cells in the population,

thereby increasing the immunogenicity of the mammalian cells.

The mammalian cells may be disease cells, such as cancer cells or cells infected with a pathogen.

An eighth aspect provides a method of increasing the immunogenicity of target cells in an individual comprising;

administering a peptide exchange protein of the third aspect to the individual, wherein the targeting domain of the peptide exchange protein binds to target cells in the individual, and

administering an immunogenic peptide to the individual, such that the peptide exchange protein loads the immunogenic peptide onto MHC class I molecules on the surface of the target cells,

thereby increasing the immunogenicity of said target cells.

In one embodiment, the method comprises:

providing a population of mammalian cells having surface MHC class I molecules, and contacting the population of mammalian cells with an immunogenic peptide and either

(a) a peptide exchange protein comprising a fragment of TAP-binding protein-related (TAPBPR), said fragment consisting of a TAPBPR luminal domain having an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22; or

(b) a mammalian cell comprising a peptide exchange protein at its surface, the peptide exchange protein comprising;

(i) a fragment of TAP-binding protein-related (TAPBPR), said fragment comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22 and a TAPBPR transmembrane domain; or

(ii) a fragment of TAP-binding protein-related (TAPBPR), said fragment comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22 and a heterologous transmembrane domain;

such that the peptide exchange protein loads the immunogenic peptide onto the surface MHC class I molecules of the cells in the population,

thereby increasing the immunogenicity of the mammalian cells.

The target cells may be disease cells, such as cancer cells or cells infected with a pathogen. A ninth aspect provides a method of stimulating or promoting an immune response in an individual comprising;

administering a peptide exchange protein of the third aspect to the individual, wherein the targeting domain of the peptide exchange protein binds to antigen presenting cells in the individual, and

administering an immunogenic peptide to the individual, such that the peptide exchange protein loads the immunogenic peptide onto surface MHC class I molecules of the antigen presenting cells and the antigen presenting cells stimulate or promote an immune response in the individual.

In one embodiment, the method comprises:

providing a population of antigen presenting cells previously obtained from the individual, and contacting the antigen presenting cells in vitro with an immunogenic peptide and either

(a) a peptide exchange protein comprising a fragment of TAP-binding protein-related (TAPBPR), said fragment consisting of a TAPBPR luminal domain having an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22; or

(b) a mammalian cell comprising a peptide exchange protein at its surface, the peptide exchange protein comprising;

(i) a fragment of TAP-binding protein-related (TAPBPR), said fragment comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22 and a TAPBPR transmembrane domain; or

(ii) a fragment of TAP-binding protein-related (TAPBPR), said fragment comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22 and a heterologous transmembrane domain;

such that the peptide exchange protein loads the immunogenic peptide onto surface MHC class I molecules of the antigen presenting cells.

A tenth aspect provides a method of producing antigen presenting cells for activating T cells comprising;

providing a population of antigen presenting cells previously obtained from an individual, and

contacting the antigen presenting cells with an immunogenic peptide and a peptide exchange protein of the first, second, or third aspect, such that the peptide exchange protein loads the immunogenic peptide onto MHC class I molecules on the surface of the antigen presenting cells.

A method of the tenth aspect may be an *in vitro* or *ex vivo* method.

In some embodiments, the loaded antigen presenting cells may be administered to an individual to stimulate a T cell immune response in the individual against the immunogenic peptide.

In other embodiments, the antigen presenting cells may be contacted with a population of T cells to activate the T cells against the immunogenic peptide. The activated T cells may be administered to an individual to stimulate a T cell immune response in the individual against the immunogenic peptide.

An eleventh aspect provides a method of reducing an immune response in an individual comprising;

administering a peptide exchange protein of the third aspect to the individual, wherein the targeting domain of the peptide exchange protein binds to target cells in the individual, and

administering a non-immunogenic peptide to the individual, such that the peptide exchange protein loads MHC class I molecules on the surface of target cells with the non-immunogenic peptide,

such that the immunogenicity of the target cells is reduced in the individual.

Suitable target cells include antigen presenting cells or cells associated with tissues or organs that elicit an immune response, such as an autoimmune response, in the individual. This may be useful for example in the treatment of autoimmune disease, immune-mediated inflammatory disease, or organ rejection in the individual.

A twelfth aspect of the invention provides an *in vitro* method of producing a MHC class I molecule displaying a target peptide comprising;

contacting an MHC class I molecule with a peptide exchange protein of the first or second aspect and an target peptide, such that the peptide exchange protein loads the target peptide onto the MHC class I molecule,

thereby producing an MHC class I molecule displaying the target peptide.

The MHC class I molecule may display an initial peptide that is replaced by the target peptide following contact with the peptide exchange protein.

In some embodiments, the MHC class I molecule displaying the target peptide may be contacted with a population of T cells to identify and/or isolate T cells that specifically bind to it.

A thirteenth aspect provides use of a peptide exchange protein as described herein in the manufacture of a medicament for increasing the immunogenicity of target cells in an individual according to claim 24 or stimulating an immune response in an individual according to claim 26.

Other aspects and embodiments of the invention are described in more detail below.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

Brief Description of the Figures

Figure 1 shows that peptide-receptive MHC class I is present on cells expressing surface TAPBPR. (a) Over-expression of TAPBPR results in its expression at the cell surface. IFN-γ treated HeLaM cells and HeLaM-TAPBPR^{KO} -/+ transduction with TAPBPR^{WT} were stained using the TAPBPR-specific mAb PeTe-4. (b-e) Cells over-expressing TAPBPR^{WT} show increased binding to exogenous peptide compared to control cells. IFN-γ treated cells were incubated with a HLA-A*68:02 specific fluorescent peptide ETVSK*QSNV or its nonbinding variant EGVSK*QSNG (in which the anchor residues are mutated) then analysed using flow cytometry. (b,c) Histograms of the typical peptide binding observed when cells were incubated with 10 nM (b) ETVSK*QSNV or (c) EGVSK*QSNG for 15 min at 37^{o}C. (d) Dose response curves and (e) time course showing the increased binding of exogenous peptide to cells over-expressing TAPBPR^{WT} compared to HeLaM, TAPBPR deficient (TAPBPR^{KO}), or HLA-A,-B,-C deficient (HLA-ABC^{KO}) variants when cells were treated with (d) increasing concentration of ETVSK*QSNV for 15 min or (e) 10 nM ETVSK*QSNV from 0-180 min at 37^{o}C. In (e) the binding observed with 10 nM of EGVSK*QSNG is included as a control. Line graphs show mean fluorescent intensity (MFI) -/+ s.e.m from three independent experiments.

Figure 2 shows that surface expressed TAPBPR enhances exogenous peptide association onto MHC class I molecules. (a,b) TAPBPR targeted to the plasma membrane (PM), but not the endoplasmic reticulum (ER), is detectable on the surface of cells and associates with MHC class I there. (a) Cell surface detection of TAPBPR using PeTe-4 on IFN-γ treated HeLaM-TAPBPR^{KO} cells -/+ transduction with TAPBPR^{WT}, TAPBPR^{PM}, TAPBPR^{ER} or TAPBPR^{TN5}. Note: As Transduction of TAPBPR^{PM} into HeLaM^{KO} cells resulted in extremely high surface expression of TAPBPR cells with a low transduction level were subsequently used to produce a cell line with similar TAPBPR surface expression as TAPBPR^{WT} expressing cells. (b) Immunoprecipitation of the cell surface pool of TAPBPR, by staining intact cells with PeTe-4 before lysis and addition of Protein-A sepharose, and the remaining intracellular TAPBPR pool, followed by Western blotting for TAPBPR, MHC class I (using HC10) and UGT1 on immunoprecipitates and lysates as indicated. (c, d) Cells expressing TAPBPR on their surface show a substantial enhancement in exogenous peptide association on MHC class I compared to cell with intracellular TAPBPR. IFN-γ treated cells were incubated with 10 nM ETVSK*QSNV, YVVPFVAK*V or EGVSK*QSNG for 15 min at 37^{o}C and analysed using flow cytometry. (e,f,g) Cells expressing tapasin target to the PM show a slight enhancement in exogenous peptide association compared to cells with

intracellular tapasin. IFN-γ treated HeLaMTAPBPR^{KO} -/+ transduction with tapasin^{WT} or tapasin^{PM} were either (e) stained with Pasta1 or (f,g) incubated with 10 nM ETVSK*QSNV, YVVPFVAK*V or EGVSK*QSNG for 15 min at 37°C, followed by flow cytometric analysis. (c,f) Histograms of the typical fluorescent peptide binding observed. (d,g) Bar charts show MFI -/+ s.e.m of fluorescent peptide binding from three independent experiments.*P ≤0.05, **P≤0.01, n/s not significant, using unpaired two-tailed t-test.

Figure 3 shows that surface TAPBPR functions as a MHC class I peptide exchange catalyst. Dissociation of the fluorescent peptide (a,b) YVVPKVAK*V (YVV*) or (c, d) ETVSK*QSNV (ETV*) from IFN-γ treated HeLa^{KO}TAPBPR^{WT} cells in the absence or presence of unlabelled competitor peptides YVVPFVAKV (YVV), ETVSEQSNV (ETV) or ETVSEQSNG (ETVΔ2/9). Cells were incubated with 10 nM fluorescent peptide for 15 min at 37°C, washed, then subsequently incubated with increasing concentrations of unlabelled competitor peptide for 15 min at 37°C. (a,c) Histograms show the typical dissociation of fluorescent peptide observed following incubation with 100 nM competitor peptide. (b,d) Line graphs show the percentage of fluorescent peptide remaining -/+ s.e.m following treatment with increasing concentrations of unlabelled peptide from (b) four and (d) three independent experiments.

Figure 4 shows that exogenous soluble TAPBPR enhances exogenous peptide association onto surface MHC class I. (a,b) Exogenous soluble TAPBPR binds to surface MHC class I molecules and (c-f) enhances the binding of exogenous peptide in an affinity based manner. IFN-γ treated (a,c) HeLaM and (b,e) HeLaM HLA-ABC^{KO} cells -/+ HLA-A*68:02 reconstitution were incubated in the absence or presence of 100 nM soluble TAPBPR^{WT} (sol WT) or TAPBPR^{TN5} (sol TN5) for 15 min at 37°C, followed by (a,b) detection of surface bound TAPBPR using PeTe-4 or (c-f) incubated with and without 10 nM ETVSK*QSNV (ETV*), YVVPFVAK*V (YVV*) or EGVSK*QSNG (ETVΔ2/9) for 15 min at 37°C and analysed using flow cytometry. (c,e) Histograms of the typical fluorescent peptide binding observed (d, f) Bar graphs show the MFI of fluorescent peptide binding -/+ s.e.m from three independent experiments. (g) Dose response curves of IFN-γ treated HeLaM and HeLaM-HLA-ABC^{KO} cells treated -/+ 100 nM TAPBPR with increasing concentrations of ETVSK*QSNV for 15 min at 37°C. Line graphs show MFI -/+ s.e.m from three independent experiments. (h & i) IFN-γ treated HeLaM-HLA-ABC^{KO} cells reconstituted with HLA-A*02:01 were incubated in the absence or presence of 1 μM soluble TAPBPRWT for 15 min at 37°C followed by incubation with and without 10 nM of the HLA-A2 binding peptide NLVPK*VATV (NLV*), CLGGK*LTMV (CLG*), YLLEK*LWRL (YLL*), and YVVPFVAK*V (YVV*), or as non-HLA-A2 binding controls ETVSK*QSNV (ETV*) (specific for HLA-A*A68:02) and SRYWK*IRTR (SRY*)(specific for HLA-B*27) for 15 min at 37°C. (h) Histograms of the typical fluorescent peptide binding observed using flow cytometry. (i) Bar graphs showing the MFI of fluorescent peptide binding to HeLaM HLA-ABC^{KO}+A2 -/+ s.e.m from two independent experiments with duplicates. ***P ≤ 0.001, ****P≤0.0001, n/s not significant, using unpaired two-tailed t-test.

Figure 5 shows antigenic peptides loaded onto MHC class I via TAPBPR are available to the T cell receptor. IFN-γ treated HeLaM HLA-ABC^{KO} cells reconstituted with HLA-A*02:01 were incubated in the absence or presence of 1 μM soluble TAPBPR^{WT} (sol WT) or TAPBPR^{TN5} (sol TN5) for 15 min at 37°C

followed by 15 min treatment with or without 10 nM unlabelled HLA-A2 binding peptide (a,b) YLLEMLWRL, (c,d) CLGGLLTMV or (e) NLVPMVATV. After washing, cells were either stained with the TCR-like mAb (a,b) L1 which recognises YLLEMLWRL/HLA-A2 complexes, (c,d) L2 which recognises CLGGLLTMV/HLA-A2 complexes or (e) irradiated followed by incubation with a HLA-A2 restricted NLVPMVATV specific CD8+ T cell line. Bar graphs (b, d) show the MFI of TCR-like mAb binding -/+ s.e.m from three independent experiments or (e) T cell activity measured by detecting IFN-γ secretion in fluorospot assays from triplicate wells representative of two independent experiments. ***P ≤ 0.001, ****P≤0.0001 using unpaired two-tailed t-test.

Figure 6 shows TAPBPR can load antigenic peptide onto tumour cells and induce their recognition by T cells. MCF-7 cells were treated -/+ 1 μM soluble $TAPBPR^{WT}$ or $TAPBPR^{TN5}$ for 15 min at 37°C followed by 60 min incubation -/+ 10 nM (6a) IMDQK*PFSV, ELAGK*GILTV, LLGRK*SFEV, or RLLQK*TELV, (6b) NLVPK*VATV or YLLEK*LWRL or (6c & d) YLLEMLWRL (YLL) followed by staining with the TCR-like mAb L1 specific for YLLEMLWRL/HLA-A2 complexes. (6d) The MFI of L1 binding to MCF-7 cells -/+ SD from three independent experiments. (6e) Bar graphs show T cell activity measured by IFN-γ secretion in fluorospot assays of a HLA-A2 restricted NLVPMVATV specific CD8+ T cell line when incubated with MCF-7 target cells as treated in Fig 6b with the exception that non-fluorescent NLVPMVATV peptide at 100 pM was used. Results are from triplicate wells representative of two independent experiments. Error bars -/+ SD. Note: In Figs 6a, 6b, & 6e IFNγ treated cells were used. Equivalent experiments of 6b-e were performed using HeLaM-$HLA\text{-}ABC^{KO}$ expressing HLA-A*02:01 and can be found in Fig. 16. *P ≤0.05, ***P ≤ 0.001, ****P≤0.0001 using unpaired two-tailed t-test.

Figure 7 shows constructs used for proof-of-concept of the chimeric peptide exchange approach. (Top) pDisplay-eGFP vector transduced into target cells to expressed GFP on the plasma membrane. (Bottom) sTAPBPR-linker-GFPNB. This construct makes soluble TAPBPR linked to a GFP nanobody. Three different variants have been made with varying linker regions to provide a flexible region between the antibody target and MHC class I. This insert is in the piggyBac vector and protein is produced in 293T cells. Yield = ~ 5mg/L (from adherent cell line) Note: TAPBPR in a non-glycosylated protein.

Figure 8 shows TAPBR binding and peptide exchange mediated by TAPBPR-fused to a nanobody specific for GFP on HeLa cells. (A) shows the surface expression of GFP on HeLaM and HeLaM transfected with the pDisplay-eGFP construct (pD-GFP) using an anti-GFP antibody. (B) Histograms and (C) Bar graphs compare the binding of recombinant soluble TAPBPR (TAPBPR) with recombinant TAPBPR-fused to a nanobody specific for GFP ($TAPBPR\text{-}GFP_{NB}$) to HeLaM cells (left) and HeLaM-pD-GFP cells (right). Histograms show the level of TAPBPR when the two cell lines were incubated with 100nM protein while bar graphs summarise results using a range of concentrations of protein (0.001-100 nM). (D & E) shows the binding of a fluorescent variant of an exogenous neoantigen peptide ETVSK*QSNV (ETV*) to HLA-A68 (MHC class I) expressed on HeLa when cells treated as in B & C where incubated with 10 nM peptide for 15 min after incubation with the indicated recombinant TAPBPR protein. Each bar represents mean and standard deviation of three independent experiments.

Figure 9 shows peptide exchange mediated by TAPBPR-fused to a nanobody specific for GFP on MCF-7 cells. (a) shows the expression of GFP on the surface of MCF-7 cells -/+ transfection with the pDisplay-eGFP construct. (b) shows the binding of TAPBPR to the MCF-7 cells -/+ surface GFP when incubated with 10nM recombinant protein. (c) shows the binding of a fluorescent exogenous viral peptide NLVPK*VATV (derivative of NLVPMVATV from CMV) to HLA-A2 (MHC class I) expressed on MCF-7 cells.

Figure 10 shows chimeric proteins comprising soluble TAPBPR linked to a Her2-specific-scFv. Three different variants were made with varying linker regions to provide a flexible region between the antibody target and MHC class I. The insert was in the piggyBac vector and protein was produced in 293T cells. Similar Yield as the GFP NB were obtained = ~ 5mg/L (from adherent cell line).

Figure 11 shows that soluble TAPBPR linked an anti-Her2 scFv targets TAPBPR binding to a tumour cell line in a Her2 dependent manner and functions to load immunogenic peptides onto the cell line. (A) shows the surface expression of Her on HeLaM and HeLaM over-expressing Her2 (+Her2) using an anti-Her2 antibody. Note, there is endogenous Her2 on HeLa. (B) Histograms and (C) Bar graphs compare the binding of recombinant soluble TAPBPR (TAPBPR) with recombinant TAPBPR-fused to a scFv specific for Her2 (TAPBPR-Her2-scFv) to HeLaM cells (left) and HeLaM+Her2 (right). Histograms show the level of TAPBPR when the two cell lines were incubated with 100nM protein while bar graphs summarise results using a range of concentrations of protein (0.001-100 nM). (D & E) shows the binding of a fluorescent variant of an exogenous neoantigen peptide ETVSK*QSNV (ETV*) to HLA-A68 (MHC class I) expressed on HeLa when cells treated as in B & C were incubated with 10 nM peptide for 15 min after incubation with the indicated recombinant TAPBPR protein. Each bar represents mean and standard deviation of three independent experiments.

Figure 12 shows that soluble TAPBPR linked to an anti-Her2 scFv targets TAPBPR binding to a tumour cell line in a Her2 dependent manner. (A) shows the surface expression of Her2 on HeLaM and HeLaM in which Her2 has been knocked out (HeLaM-$^{Her2\text{-}KO}$). (B) Histograms and (C) Bar graphs compare the binding of 100 nM recombinant soluble TAPBPR (TAPBPR) with 100 nM recombinant TAPBPR-fused to a scFv specific for Her2 (TAPBPR-Her2-scFv) to HeLaM cells (left) and HeLaM^{Her2KO} cells (right). (D & E) shows the binding of a fluorescent variant of an exogenous neoantigen peptide ETVSK*QSNV (ETV*) to HLA-A68 (MHC class I) expressed on HeLaM when cells treated as in B & C where incubated with 10 nM peptide for 15 min after incubation with the indicated recombinant TAPBPR protein. Each bar represents mean and standard deviation of three independent experiments.

Figure 13 shows that TAPBPR-Her2scFv can load immunogenic viral peptides which are recognised by viral specific T cell receptors. (A-D) shows the binding of a fluorescent variant of the viral peptides (A&B) NLVPK*VATV (NLV*) derived from pp65 protein from CMV or (C&D) YLLEK*LWRL (YLL*) derived from EBV onto HeLaM cells lacking expression of HLA-ABC (HeLaM-ABC^{KO}) -/+ HLA-A2 transduction, following incubation with 10 nM peptide for 15 min after incubation with the indicated recombinant TAPBPR protein. For the histograms in A & C 100 nM of the indicated recombinant TAPBPR protein was

used. (E) shows staining with the T cell receptor-like mAb LMP-1 on cells treated with TAPBPR as above incubated with non-fluorescent YLLEMLWRL peptide from EBV. The TCR is specific for YLLEMLWRL peptide presented on HLA-A2 molecules. Each bar represents mean and standard deviation of three independent experiments

Figure 14 shows TAPBPR-Her2-scFv fusion proteins work on human breast cell lines expressing Her2 including cells with low MHC class I. (A) Flow cytometric analysis of MHC class I (HLA-A2) and Her2 expression on the human breast cancer cell lines MCF-7 & SKBR3. Bar graphs show (B) the binding YLL* peptide and (C) the binding of TCR-like mAb reagent LMP-1 which recognises the EBV specific peptide YLL in the context of HLA-A2, when SKBR3 and MCF-7 cells were incubated with no peptide, peptide alone, or peptide in the presence of 100 nM soluble TAPBPR or 100nM TAPBPR-Her2 antibody fusion protein. These results clearly demonstrate that the TAPBPR-her 2 antibody fusion protein works extremely efficiently on both these breast cancer cell lines, despite expressing either low levels of MHC class I (SKBR3) or low levels of Her2 (MCF-7) and that the fusion protein is superior to the soluble TAPBPR protein alone.

Figure 15 shows that exogenous mouse TAPBPR can load immunogenic peptide onto human MHC class I. Top panel shows the binding of a fluorescent exogenous neoantigen peptide ETVSK*QSNV (ETV*) to HLA-A68 (MHC class I) expressed on HeLa cells in the absence and presence of mouse TAPBPR. Bottom panel shows the same experiment performed with human TAPBPR for comparison.

Figure 16 show expression levels and peptide editing functionality of TAPBPR with alterations to its cytoplasmic tail. (A) Shows the surface detection of TAPBPR and (B) show the binding of fluorescent peptide ETVSK*QSNV (ETV*) when cells are transduced with $TAPBPR^{WT}$, $TAPBPR^{tailless}$ which has the TMD of TAPBPR but lacks the cytoplasmic tail and $TAPBPR^{CD8tail}$ in which its cytoplasmic tail has been replaced with CD8. Staining on non-transduced cells are included as a control.

Figure 17 shows that soluble TAPBPR dissociates from cells upon high affinity peptide binding. IFN-γ treated HeLaM cell and HeLa-HLA-ABC^{KO} reconstituted with HLA-A*68:02 were incubated -/+ 100 nM soluble $TAPBPR^{WT}$ for 15 min at 37°C, followed by incubation with -/+ 10 nM EGVSK*QSNG (a non-HLA binding peptide), ETVSK*QSNV (a HLA binding peptide) or YVVPFVAK*V (a HLA binding peptide with high affinity) for 15 min at 37°C. Subsequently, the amount of TAPBPR remaining on the cell surface was detected by staining with the TAPBPR specific mAb PeTe4.

Figure 18 shows peptide loading and TCR recognition of HLA-A2 molecules expressed on HeLaM cells. HeLaM-HLA-ABC^{KO} cells reconstituted with HLA-A*02:01 were incubated -/+ 1 μM soluble $TAPBPR^{WT}$ or $TAPBPR^{TN5}$ for 15 min at 37°C followed by 60 min treatment -/+ 10 nM (16a) NLVPK*VATV or YLLEK*LWRL or (16b) YLLEMLWRL (YLL) followed by staining with the TCR-like mAb L1 specific for YLLEMLWRL/HLA-A2 complexes. (16c) The MFI of L1 binding to HeLaM-HLA-ABC^{KO} cells -/+ SD from three independent experiments. (16d) Bar graphs show T cell activity measured by IFN-γ secretion in fluorospot assays of a HLA-A2 restricted NLVPMVATV specific CD8+ T cell line when incubated with

HeLaM-HLA-ABC^{KO} target cells as treated in a with the exception that non-fluorescent NLVPMVATV peptide at 100 pM was used. Results are from triplicate wells representative of two independent experiments. Error bars -/+ SD. Note: In 18a & 18d IFNγ treated cells were used. ***P ≤ 0.001, ****P≤0.0001 using unpaired two-tailed t-test.

Figure 19 shows that soluble TAPBPR enhances T cell killing of tumour cells EL4 cells were incubated -/+ 1μM soluble TAPBPR^{WT} or TAPBPR^{TN5} for 15 min at 37°C, followed by (19a) detection of surface bound TAPBPR using PeTe-4, (19b) incubation -/+ 1 nM SIINFEK*L for 30 min at 37°C or (19c) incubation -/+ 1 nM non-labelled SIINFEKL peptide for 30 min, followed by staining with the 25-D1.16 mAb (recognises SIINFEKL/H-2K^{b} complexes). Histograms are representative of three independent experiments. (19d) Bar graphs show the MFI of 25-D1.16 -/+ SD from three independent experiments. (19e) OT1 killing of EL4 cells treated -/+ 1μM soluble TAPBPR^{WT} or TAPBPR^{TN5}, followed by incubation with 1 nM SIINFEKL peptide. Error bars -/+ s.e.m from triplicate wells. Data is representative of three independent experiments. Note: surface expressed H-2K^{b} are relatively more peptide receptive compared to human MHC I molecules. At 10 nM SIINFEKL, some exogenous peptide binding was observed in the absence of soluble TAPBPR^{WT}. As OT1 T cells are highly efficient cytotoxic cells, killing 80-100% of targets after 1-4 hours, we decreased the concentration of SIINFEKL used in these experiments to 1 nM in order to differentiate between TAPBPR-mediated and background peptide binding, otherwise we would not observe an additive effect of soluble TAPBPR on target cell killing.

Figure 20 shows that soluble TAPBPR linked to a PD-L1 specific nanobody targets TAPBPR binding to tumours in a PD-L1 dependent manner and functions to load immunogenic peptides onto a tumour cell line. (A) shows the surface expression of PD-L1 on HeLaM and HeLaM over-expressing PD-L1 (+PDL1). (B) Histograms and (C) Bar graphs compare the binding of recombinant soluble TAPBPR (TAPBPR) with recombinant TAPBPR-fused to a nanobody specific for PD-L1 (TAPBPR-PD-L1$_{NB}$) to HeLaM cells (left) and HeLaM+PDL1 (right). Histograms show the level of TAPBPR when the two cell lines were incubated with 100nM protein while bar graphs summarise results using a range of concentrations of protein (0.001-100 nM). (D & E) shows the binding of a fluorescent variant of an exogenous neoantigen peptide ETVSK*QSNV (ETV*) to HLA-A68 (MHC class I) expressed on HeLa when cells treated as in B & C where incubated with 10 nM peptide for 15 min after incubation with the indicated recombinant TAPBPR protein. Each bar represents mean and standard deviation of three independent experiments.

Figure 21 shows that TAPBPR fusion proteins with various lengths of linkers between the TAPBPR and antibody fragment all work efficiently. Three different linker sequences (see figure 7 for sequence) were inserted between the TAPBPR and antibody fragment for both the (A&C) TAPBPR-GFP$_{NB}$ fusion and (B&D) the TAPBPR-Her2-scFv fusion. (A & B) show the ability of TAPBPR-fusion to bind to cells expressing the antibody fragment ligand is similar regardless whether the short, long or extra-long linker is used. (C&D) shown that the ability of the TAPBPR-fusion to mediate peptide loading onto HLA molecules expressed on HeLaM cells is similar regardless of whether the short, long or extra-long linker is used. Note in this application, the long linker was selected for TAPBPR-antibody fusions unless indicated otherwise.

Figure 22 shows that TAPBPR can mediate peptide exchange on a wide range of HLA molecules, particularly HLA-A molecules. HeLa-HLA-ABC^{KO} cells expressing individual HLA I allomorphs were incubated with 1 μM TAPBPR for 15 min at 37°C, after which fluorescently-labelled peptide, specific for each corresponding HLA I allomorph, was added for an allele-dependent time period (15 min for A*68:02 and A*23:01 and 60 min for the others) and at an allele-dependent concentration (10 nM for A*68:02, A*02:01, A*23:01, A*32:01 and 100 nM for the others). Histograms shows the level of bound fluorescent peptide to each HLA I-expressing cell line when cells were incubated with peptide alone (black line) or with peptide and TAPBPR (blue line). A sample not treated with peptide was included as a negative control (solid grey line). TAPBPR was found to promote peptide exchange on all these HLA molecules.

Detailed Description

This invention relates to a recombinant peptide-exchange protein that comprises a fragment of TAP-binding protein-related (TAPBPR). The luminal domain of TAPBPR is shown herein to function as a peptide editor and a peptide-exchange protein comprising this domain acts as an extracellular or cell surface MHC class I peptide-exchange catalyst that is capable of loading exogenous peptide onto MHC class I molecules on the surface of a cell.

The peptide-exchange protein may comprise a fragment of TAP-binding protein-related (TAPBPR). A fragment is a truncated TAPBPR protein that lacks one or more amino acids of the full-length protein but retains peptide exchange activity. For example, a fragment may lack a contiguous sequence of 10 or more, 20 or more, 50 or more of 100 or more amino acids, relative to the full-length TABPR protein. In some embodiments, a TAPBPR fragment may lack the ectodomain and/or transmembrane domain of the full-length TAPBPR protein. A suitable TAPBPR fragment may comprise or consist of the luminal domain of the full-length TAPBPR protein.

TAPBPR may be mammalian TAPBPR, for example mouse or human TAPBPR, preferably human TAPBPR.

Human TAPBPR (Gene ID: 55080) is an intracellular peptide exchange catalyst that localises predominately to the endoplasmic reticulum (ER). Human TAPBPR may have the reference amino acid sequence of NCBI database entry NP_060479.3, XP_005253757.1 or SEQ ID NO: 18 and may be encoded by the reference nucleotide sequence of NM_018009.4 or SEQ ID NO: 17. 31 alleles of TAPBPR have been identified within the human population which result in changes to this protein. 6 are major isoforms which the individual variants being found at a frequency of 15-30% within different populations. Mouse TAPBPR (Gene ID: 213233) may have the reference amino acid sequence of NCBI database entry NP_663366.2 or SEQ ID NO: 20 and may be encoded by the reference nucleotide sequence of NM_145391.2 or SEQ ID NO: 19.

The TAPBPR fragment may comprise the luminal domain of TAPBPR. The luminal domain of TAPBPR is located at residues 22-405 of the full length human TAPBPR (including leader: for example SEQ ID NO:

18) and comprises an N-terminal unique domain, an IgV domain and an IgC domain. In some embodiments, the luminal domain may comprise residue I261 (TN5 patch), residues E205, R207, Q209, Q272 (TN6 patch), H210, K211 & R213 (TN7 patch), and residues 335-339, which are all involved in binding to MHC class I (Hermann et al (2013) J Immunology 191: 5743-5750). The luminal domain may further comprise residues 22-35, which form a loop which interacts with the peptide-binding groove of MHC class I. A suitable TAPBPR luminal domain may comprise the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 22 or a variant of one of these sequences. A TAPBPR luminal domain may be encoded by a nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 21 or a variant of one of these sequences.

A peptide-exchange protein as described herein may be soluble and not bound to a membrane either at the surface or within a mammalian cell. In particular, the peptide-exchange protein may lack transmembrane domains, membrane anchors or other features that might covalently attach it to an intracellular membrane or the cell membrane during or after expression.

In a soluble peptide-exchange protein as described herein, the TAPBPR fragment may consist of the luminal domain of TAPBPR. A soluble peptide-exchange protein may lack sequence from TAPBPR outside the luminal domain i.e. the TAPBPR fragment may be the only TAPBPR sequence in the peptide-exchange protein. For example, the peptide-exchange protein may lack the TAPBPR transmembrane domain, ectodomain or other non-luminal domains.

In other embodiments, a peptide-exchange protein as described herein may be bound to the plasma membrane at the surface of a mammalian cell. For example, the peptide-exchange protein may comprise a transmembrane domain (TMD) that attaches the protein to the plasma membrane. The TMD may be a TAPBPR TMD or a heterologous TMD. In some embodiments, the TMD may be sufficient to localise the peptide-exchange protein to the plasma membrane after expression. In other embodiments, the surface bound peptide-exchange protein may further comprise a cell surface targeting sequence that localises the peptide-exchange protein to the plasma membrane after expression.

In a surface bound peptide-exchange protein comprising a heterologous transmembrane domain, the TAPBPR fragment may comprise the luminal domain of TAPBPR. In other surface bound peptide-exchange proteins, the TAPBPR fragment may comprise both the luminal domain and TMD of TAPBPR. The TMD of TAPBPR is located at residues 407-426 of the full length human TAPBPR sequence (including leader) and may comprise the amino acid sequence of SEQ ID NO: 32 or a variant thereof. A suitable TAPBPR fragment may lack the cytoplasmic tail located at residues 427-468 of the full length human TAPBPR of the full length human TAPBPR sequence (as shown in SEQ ID NO: 18).

The TAPBPR fragment of the peptide-exchange protein displays peptide-exchange activity and is capable of loading cell-surface MHC class I molecules with an exogenous peptide.

A cell displaying MHC class I molecules may be exposed to (i) a soluble extracellular peptide-exchange protein as described herein (ii) a cell having a surface bound peptide-exchange protein as described

herein or (iii) a chimeric peptide-exchange protein as described herein that binds to the surface of the cells displaying the MHC class I molecules.

The loading of cell-surface MHC class I molecules as described herein may increase the number of MHC class I molecules on the surface of a cell which present the exogenous peptide relative to cells not treated with the peptide-exchange protein. For example, the number of MHC class I molecules on the surface of a cell which present the exogenous peptide may be increased by 30 fold or more, 40 fold or more, 50 fold or more, 60 fold or more, 70 fold or more, 80 fold or more, 90 fold or more, 100 fold or more, 150 fold or more or 200 fold or more exogenous peptide in the presence relative to the absence of TAPBPR. Cells may present none or substantially none of the exogenous peptide in the absence of treatment with the peptide-exchange protein.

In some embodiments, the endogenous peptides presented by the cell displaying MHC class I molecules may not have the same amino acid sequence as the exogenous peptide. In other embodiments, the cell may present low levels of endogenous peptide with the same amino acid sequence as the exogenous peptide. The peptide-exchange protein may increase the amount of peptide having the amino acid sequence that is presented by loading MHC class I molecules on the cell surface with exogenous peptide.

Sufficient exogenous peptide may be loaded onto cell-surface MHC class I molecules to stimulate a T cell response to the peptide in an individual.

In some embodiments, the peptide-exchange protein may consist of the TAPBPR fragment. This may be useful for example in altering the immunogenicity of mammalian cells *in vitro* or *ex vivo*. In a soluble peptide-exchange protein, the TAPBPR fragment may comprise the TAPBPR luminal domain. For example, the TAPBPR fragment may lack the TAPBPR TMD and the TAPBPR cytoplasmic tail and may for example consist of the luminal domain. In a surface bound peptide-exchange protein, the TAPBPR fragment may comprise the TAPBPR luminal domain and TMD. The TAPBPR fragment may lack the TAPBPR cytoplasmic tail and may for example consist of the TAPBPR luminal domain and TMD.

In other embodiments, the peptide-exchange protein may further comprise one or more domains in addition to the TAPBPR fragment. The one or more additional domains may be heterologous domains (i.e. amino acid sequences not derived from TAPBPR). For example, a surface-bound peptide-exchange protein may comprise a heterologous TMD and/or cell surface targeting sequence. In some embodiments, the peptide-exchange protein may be a fusion protein comprising the TAPBPR fragment and one or more heterologous domains.

The absence of the TAPBPR cytoplasmic tail may be sufficient to localise a peptide-exchange protein comprising a TAPBPR or heterologous TMD to the cell membrane. Suitable heterologous TMDs may include the platelet derived growth factor receptor (PDGFR) TMD, the influenza hemagglutinin TMD and the influenza neuraminidase TMD. In other embodiments, the peptide-exchange protein may further comprise a heterologous cell surface targeting sequence. A cell surface targeting sequence is an amino

acid sequence that directs a protein expressed in a cell to the plasma membrane. Suitable cell surface targeting sequences may include the cytoplasmic domains of CD8, MHC class I molecules, Transferrin receptor, CD147, VSVG, NCAM, CD44 or E-cadherin. Examples of suitable peptide exchange proteins may include the TAPBPR-CD8 construct of SEQ ID NO: 34 or a variant thereof.

The term "heterologous" refers to a polypeptide or nucleic acid that is foreign to a particular biological system, such as a host cell, and is not naturally occurring in that system. A heterologous polypeptide or nucleic acid may be introduced to a biological system by artificial means, for example using recombinant techniques. For example, heterologous nucleic acid encoding a polypeptide may be inserted into a suitable expression construct which is in turn used to transform a host cell to produce the polypeptide. A heterologous polypeptide or nucleic acid may be synthetic or artificial or may exist in a different biological system, such as a different species or cell type. A recombinant polypeptide may be expressed from heterologous nucleic acid that has been introduced into a cell by artificial means, for example using recombinant techniques. A recombinant polypeptide may be identical to a polypeptide that is naturally present in the cell or may be different from the polypeptides that are naturally present in that cell.

The term "endogenous“ refers to a peptide, polypeptide or nucleic acid or other factor that is generated by natural processes in a biological system, such as a host cell. The term "exogenous“ refers to a peptide, polypeptide or nucleic acid that is not generated by natural processes in a biological system and is produced and/or introduced to the system by artificial means, for example by administration or recombinant expression. An exogenous factor may be synthesised using conventional techniques, such as solid-phase synthesis. An exogenous factor may be identical to a factor that is naturally present in a biological system (i.e. an endogenous factor) or may be different from the factors that are naturally present in that biological system.

Preferably, the peptide-exchange protein further comprises a targeting domain. The peptide-exchange protein described herein may be a chimeric protein or fusion protein comprising a targeting domain and a TAPBPR fragment comprising or consisting of the TAPBPR luminal domain. Chimeric peptide-exchange proteins as described herein are preferably soluble and may be useful for example in altering the immunogenicity of mammalian cells in vivo, as well as for in vitro and ex vivo applications.

In some embodiments, the TAPBPR fragment may be at the N terminal of the peptide exchange protein and the targeting domain may be at the C terminal of the peptide exchange protein. In other embodiments, the TAPBPR fragment may be at the C terminal of the peptide exchange protein and the targeting domain may be at the N terminal of the peptide exchange protein.

The targeting domain may be directly connected to the TAPBPR fragment or may be connected via a linker.

Suitable linkers are well-known in the art and include chemical and peptidyl linkers. For example, a peptidyl linker may comprise a sequence of amino acid residues, for example, 5 to 30 or 5 to 22 amino acid residues, preferably 10 to 20 amino acid residues, more preferably about 12 amino acid residues.

Any linker sequence may be employed. Preferably, the linker sequence is a heterologous sequence. Suitable linker amino acid sequences are well known in the art and may include the amino acid sequences GGGGS, $(GGGGS)_3$ or GSTVAAPSTVAAPSTVAAPSGS, HVGGGGSGGGGSGGGGSTS or variants thereof.

The targeting domain allows the chimeric peptide exchange protein to selectively target a specific population of target cells in an individual. The targeting domain of the chimeric peptide exchange protein binds specifically to the target cells. Preferably, the targeting domain of the chimeric peptide exchange protein binds selectively to target cells relative to non-target cells i.e. it shows increased binding to target cells relative to non-target cells. Binding of the targeting domain to the target cells allows the TAPBPR fragment of the chimeric protein to act selectively at the surface of the target cells relative to non-target cells (i.e. cells to which the targeting domain does not bind), for example to load MHC class I molecules on the surface of the target cell with exogenous peptide.

Target cells may comprise MHC class I molecules on the cell surface. MHC class I molecules are heterodimers comprising an α chain and β2-microglobulin. MHC class I molecules are expressed on all nucleated human cells. An individual inherits a set of HLA-A, -B and –C genes from each parent. These genes are co-dominantly expressed and nucleated cells in mammals express up to 6 different classical MHC class I molecules. MHC class I molecules are highly polymorphic within the α chain and there is huge variation within the population. MHC class I molecules may include HLA-A molecules, HLA-B molecules, such as HLA-B51, HLA-B15, HLA-B38, and HLA-B57 and HLA-C molecules, such as HLA-Cw1. Preferred MHC class I molecules include HLA-A.

In some embodiments, the target cells may be disease cells, such as cancer cells, cells infected with a pathogen, or other cells that cause disease. Increasing the immunogenicity of disease cells in an individual using a chimeric peptide exchange protein may generate or increase the strength of immune responses against the disease cells in the individual. This may lead to a reduction or eradication of disease cells in the individual and may exert a therapeutic effect.

In other embodiments, the target cells may be antigen presenting cells. Loading the surface MHC I molecules of antigen presenting cells with an exogenous immunogenic peptide useful in increasing or eliciting immune responses, for example T cell immune responses, against disease cells in vivo, thereby exerting a therapeutic effect.

In other embodiments, the target cells may be host cells that elicit an immune reaction, such as an autoimmune or auto inflammatory response. Loading the surface MHC I molecules of the host cells with an exogenous non-immunogenic peptide may be useful in reducing or preventing autoimmune or immune mediated inflammatory responses against the cells in vivo, thereby exerting a therapeutic effect.

The targeting domain may specifically bind to a marker, such a receptor or antigen that is present on the surface of a target cell of the individual. The binding affinity of the targeting domain for its target cell marker may be higher than the binding affinity of TAPBPR for MHC class I molecules.

Suitable targeting domains include any molecule that are capable of specific binding to a cell marker. For example, the targeting domain may be a ligand for a receptor on the surface of the target cell or an antibody molecule that specifically binds to an antigen on the surface of the target cell.

In some preferred embodiments, a chimeric peptide-exchange protein comprising a targeting domain may show no binding or substantially no binding to MHC class I molecules on the surface of a cell if the target cell marker that is bound by the targeting domain is not present on the surface of the cell.

An antibody molecule is a polypeptide or protein comprising an antibody antigen-binding site. The term encompasses any immunoglobulin whether natural or partly or wholly synthetically produced. Antibody molecules may have been isolated or obtained by purification from natural sources, or else obtained by genetic recombination, or by chemical synthesis, and that they may contain unnatural amino acids.

Suitable antibody molecules may include whole antibodies and fragments thereof. Fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) single-domain antibodies (sdAb) (also called nanobodies (Nb)) (Ward *et al.* (1989) Nature 341, 544-546; McCafferty *et al.*, (1990) Nature, 348, 552-554; Holt *et al.* (2003) Trends in Biotechnology 21, 484-490), which consist of either a monomeric VH domain or a monomeric VL domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird *et al.* (1988) Science, 242, 423-426; Huston *et al.* (1988) PNAS USA, 85, 5879-5883); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; Holliger *et al.* (1993a), Proc. Natl. Acad. Sci. USA 90 6444-6448).

Fv, scFv, diabody, sdAb and other antibody molecules may be stabilized by the incorporation of disulphide bridges, for example linking the VH and VL domains (Reiter *et al.* (1996), Nature Biotech, 14, 1239-1245). Minibodies comprising a scFv joined to a CH3 domain may also be made (Hu *et al.* (1996), Cancer Res., 56(13):3055-61). Other examples of binding fragments are Fab', which differs from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain, including one or more cysteines from the antibody hinge region, and Fab'-SH, which is a Fab' fragment in which the cysteine residue(s) of the constant domains bear a free thiol group.

In some preferred embodiments, the targeting domain may specifically bind to a target molecule, such as a tumour antigen, on a cancer cell. For example, the targeting domain may be an antibody molecule that binds to a tumour antigen.

The expression of one or more antigens (i.e. tumour antigens) may distinguish cancer cells from normal somatic cells in an individual. Normal somatic cells in an individual may not express the one or more antigens or may express them in a different manner, for example at lower levels, in different tissue and/or at a different developmental stage. Tumour antigens may therefore be used to target chimeric peptide exchange proteins specifically to cancer cells.

Tumour antigens expressed by cancer cells may include, for example, cancer-testis (CT) antigens encoded by cancer-germ line genes, such as MAGE-A1, MAGE-A2, MAGE- A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, GAGE-I, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, BAGE-I, RAGE- 1, LB33/MUM-1, PRAME, NAG, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE- C1/CT7, MAGE-C2, NY-ESO-I, LAGE-I, SSX-I, SSX-2(HOM-MEL-40), SSX-3, SSX-4, SSX-5, SCP-I and XAGE and immunogenic fragments thereof (Simpson et al. Nature Rev (2005) 5, 615-625, Gure et al., Clin Cancer Res (2005) 11, 8055-8062; Velazquez et al., Cancer Immun (2007) 7, 1 1 ; Andrade et al., Cancer Immun (2008) 8, 2; Tinguely et al., Cancer Science (2008); Napoletano et al., Am J of Obstet Gyn (2008) 198, 99 e91-97).

Other tumour antigens include, for example, overexpressed, upregulated or mutated proteins and differentiation antigens particularly melanocyte differentiation antigens such as p53, ras, CEA, MUC1, PMSA, PSA, tyrosinase, Melan-A, MART-1, gp100, gp75, alpha-actinin-4, Bcr-Abl fusion protein, Casp-8, beta-catenin, cdc27, cdk4, cdkn2a, coa-1, dek-can fusion protein, EF2, ETV6-AML1 fusion protein, LDLR-fucosyltransferaseAS fusion protein, HLA-A2, HLA-A11, hsp70-2, KIAAO205, Mart2, Mum-2, and 3, neo-PAP, myosin class I, OS-9, pml-RAR.alpha. fusion protein, PTPRK, K-ras, N-ras, Triosephosphate isomerase, GnTV, Herv-K-mel, NA-88, SP17, and TRP2-Int2, (MART-I), E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, ErbB2/her2, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens E6 and E7, TSP-180, MAGE-4, MAGE-5, MAGE-6, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, PDL1, CD20, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, alpha.-fetoprotein, 13HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, G250, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB\170K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein\cyclophilin C-associated protein), TAAL6, TAG72, TLP, TPS, tyrosinase related proteins such as TRP-1, TRP-2 and ABC transporters expressed on the surface of tumours that are the mediators of drug resistance, such as. P-gp, BCRP and MRP1,

Other tumour antigens include out-of-frame peptide-MHC complexes generated by the non-AUG translation initiation mechanisms employed by “stressed” cancer cells (Malarkannan et al. Immunity (1999) 10(6):681-90).

Other tumour antigens are well-known in the art (see for example WO00/20581; Cancer Vaccines and Immunotherapy (2000) Eds Stern, Beverley and Carroll, Cambridge University Press, Cambridge) The sequences of these tumour antigens are readily available from public databases but are also found in WO1992/020356 A1, WO1994/005304 A1, WO1994/023031 A1, WO1995/020974 A1, WO1995/023874 A1 and WO1996/026214 A1.

Suitable targeting domains, such as antibody molecules that specifically bind to tumour antigens, are well known in the art and may be generated using conventional techniques. For example, a suitable targeting domain that specifically binds to ErbB2 (Her2) may comprise the VH and VL domains of SEQ ID NOs: 24 and 25 (trastuzumab) or the set of CDRs therein or the scFv of SEQ ID NO:23; a suitable targeting domain that specifically binds to PD-L1 may comprise PD-1, the antibody antigen-binding domain of atezolizumab or durvalumab or a nanobody sequence of any of SEQ ID NOs 25 to 31; and a suitable targeting domain that specifically binds to CD20 may comprise the antibody antigen-binding domain of rituximab. Other suitable targeting domains, for example nanobody targeting domains, are publically available (see for example Zuo et al. iCAN: Institute Collection and Analysis of Nanobodies).

In other embodiments, the targeting domain may specifically bind to a marker, such as a receptor, on an antigen presenting cell, such as a dendritic cell. For example, the targeting domain may be an Fc region that binds to an Fc receptor on the antigen presenting cell. Suitable Fc regions are well known in the art.

The targeting domain may be an antibody molecule that binds to a surface marker on the antigen presenting cell or a ligand or binding protein of the surface marker. Antigen presenting cells may include dendritic cells of any sub-type. XCR1+ dendritic cells mediate the cross-presentation of antigen for the activation of effector CD8+ T cells. Surface markers on XCR1+ dendritic cells may include XCR1, DNGR1 (CLEC9A) and BDCA3 (also known as CD141). CD172α+ dendritic cells induce T helper 2 (TH2) or TH17 cells, and promote of humoral immune responses. Surface markers on CD172α+ dendritic cells include CD172α and BDCA1 (also known as CD1c). Plasmacytoid DCs produce of type I interferon (IFN) during viral infections. Surface markers on plasmacytoid DCs include BDCA2 and BDCA4. Monocyte-derived DCs promote local T cell responses and enhance inflammation and chemokine production. Surface markers on monocyte-derived DCs include FcεRI and FcγRI expression is upregulated on activation. Macrophages eliminate pathogens and promote tissue homeostasis. Surface markers on macrophages include CD68. Expression of FcγRI is also upregulated on activation. Other suitable markers for dendritic cells include CD19, CD20, CD38, CD14 and/or Langerin/CD207.

In other preferred embodiments, the targeting domain may specifically bind to an antigen on a pathogen-infected cell. For example, the targeting domain may bind to a pathogen protein or a host cell protein whose surface expression is up-regulated by pathogen infection. For an HIV infected cell, the targeting domain may specifically bind to a marker on the cell surface, such as gp120 or gp41. Suitable targeting domains include antibody molecules or CD4, which specifically binds to surface gp120. For a CMV infected cell, the targeting domain may specifically bind to a viral protein such as UL11, UL142, UL9, UL1, UL5, UL16, UL55 (gB), UL74 (gO), UL75 (gH), UL155 (gL), which are all found on the surface of

infected cells (Weekes et al (2014) Cell 157:1460-1472). Host proteins whose expression is upregulated or induced on the surface of infected cells include inhibitory NK receptor KLRG-1, which may be specifically bound using an E-cadherin (CDH1) targeting domain (Weekes et al (2014) Cell 157:1460-1472).

Examples of suitable chimeric peptide exchange proteins may include the TAPBPR-LONG-FcIgG1 construct of SEQ ID NO: 4, the sTAPBPR-sPD1 construct of SEQ ID NO: 6, the TAPBPR-Her2scFv construct of SEQ ID NO: 8, and the sTAPBPR-GFP sdAb construct of SEQ ID NO: 10; the sTAPBPR-LONG-PD-L1-NB1 construct of SEQ ID NO: 12; the sTAPBPR-LONG-PD-L1-NB2 construct of SEQ ID NO: 14; the sTAPBPR-LONG-PD-L1-NB4 construct of SEQ ID NO: 16; and variants of any of these reference sequences.

A protein described herein that is a variant of a reference sequence, such as a peptide exchange protein sequence described above, may have 1 or more amino acid residues altered relative to the reference sequence. For example, 50 or fewer amino acid residues may be altered relative to the reference sequence, preferably 45 or fewer, 40 or fewer, 30 or fewer, 20 or fewer, 15 or fewer, 10 or fewer, 5 or fewer or 3 or fewer, 2 or 1. For example, a variant described herein may comprise the sequence of a reference sequence with 50 or fewer, 45 or fewer, 40 or fewer, 30 or fewer, 20 or fewer, 15 or fewer, 10 or fewer, 5 or fewer, 3 or fewer, 2 or 1 amino acid residues mutated. For example, a chimeric protein described herein may comprise an amino acid sequence with 50 or fewer, 45 or fewer, 40 or fewer, 30 or fewer, 20 or fewer, 15 or fewer, 10 or fewer, 5 or fewer, 3 or fewer, 2 or 1 amino acid residue altered relative to any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 34.

An amino acid residue in the reference sequence may be altered or mutated by insertion, deletion or substitution, preferably substitution for a different amino acid residue. Such alterations may be caused by one or more of addition, insertion, deletion or substitution of one or more nucleotides in the encoding nucleic acid.

A protein as described herein that is a variant of a reference sequence, such as a peptide exchange protein sequence described above, may share at least 50% sequence identity with the reference amino acid sequence, at least 55%, at least 60%, at least 65%, at least 70%, at least about 80%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity. For example, a variant of a protein described herein may comprise an amino acid sequence that has at least 50% sequence identity with the reference amino acid sequence, at least 55%, at least 60%, at least 65%, at least 70%, at least about 80%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity with the reference amino acid sequence, for example one or more of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22.

Sequence identity is commonly defined with reference to the algorithm GAP (Wisconsin GCG package, Accelerys Inc, San Diego USA). GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4. Use of GAP may

be preferred but other algorithms may be used, e.g. BLAST (which uses the method of Altschul *et al.* (1990) *J. Mol. Biol.* 215: 405-410), FASTA (which uses the method of Pearson and Lipman (1988) *PNAS USA* 85: 2444-2448), or the Smith-Waterman algorithm (Smith and Waterman (1981) *J. Mol Biol. 147*: 195-197), or the TBLASTN program, of Altschul et al. (1990) supra, generally employing default parameters. In particular, the psi-Blast algorithm may be used (Nucl. Acids Res. (1997) 25 3389-3402). Sequence identity and similarity may also be determined using Genomequest™ software (Gene-IT, Worcester MA USA).

Sequence comparisons are preferably made over the full-length of the relevant sequence described herein.

A peptide exchange protein described herein may further comprise one or more heterologous amino acid sequences additional to the TAPBPR fragment and optional targeting domain and/or linker. For example, the peptide exchange protein may further comprise one or more additional domains which improve stability, pharmacokinetics, targeting, affinity, purification and/or production properties.

In some embodiments, the peptide exchange protein described herein may further comprise a protease recognition site located between the targeting domain and the TAPBPR fragment. This may be useful for example, in clearing TAPBPR from the target cell, if required. Suitable proteases may include trypsin, chymotrypsin, factor Xa, tobacco etch virus (TEV) protease, thrombin and papain. Other suitable site specific proteases are well-known in the art and any site-specific endoprotease may be used.

In some embodiments, the peptide exchange protein may further comprise a reactive moiety to permit the use of “click chemistry” for conjugation with the targeting domain or other domain. Click-chemistry may for example involve the Cu(I)-catalysed coupling between two components, one containing an azido group and the other a terminal acetylene group, to form a triazole ring. Since azido and alkyne groups are inert to the conditions of other coupling procedures and other functional groups found in proteins are inert to click chemistry conditions, click-chemistry allows the controlled attachment of almost any linker or chemical group to the peptide exchange protein under mild conditions and in particular allows the chemical conjugation of a targeting domain to a TAPBPR fragment. For example, cysteine residues of the peptide exchange protein may be reacted with a bifunctional reagent containing a thiol-specific reactive group at one end (e.g. iodoacetamide, maleimide or phenylthiosulfonate) and an azide or acetylene at the other end. Label groups may be attached to the terminal azide or acetylene using click-chemistry. For example, a second linker with either an acetylene or azide group on one end of a linker and a chelate (for metal isotopes) or leaving group (for halogen labelling) on the other end (Baskin, J. (2007) *PNAS* 104(43)16793-97) may be employed.

Peptide exchange proteins as described herein may be provided using synthetic or recombinant techniques which are standard in the art.

In some embodiments, the peptide exchange protein described herein may be produced with an affinity tag, which may, for example, be useful for purification. An affinity tag is a heterologous peptide sequence which forms one member of a specific binding pair. Polypeptides containing the tag may be purified by the binding of the other member of the specific binding pair to the polypeptide, for example in an affinity column. For example, the tag sequence may form an epitope which is bound by an antibody molecule. Suitable affinity tags include for example, glutathione-S-transferase, (GST), maltose binding domain (MBD), MRGS$(H)_6$, DYKDDDDK (FLAG™), T7-, S- (KETAAAKFERQHMDS), poly-Arg (R_{5-6}), poly-His (H_{2-10}), poly-Cys (C_4) poly-Phe(F_{11}) poly-Asp(D_{5-16}), SUMO tag (Invitrogen Champion pET SUMO expression system), Strept-tag II (WSHPQFEK), c-myc (EQKLISEEDL), Influenza-HA tag (Murray, P. J. et al (1995) *Anal Biochem* 229, 170-9), Glu-Glu-Phe tag (Stammers, D. K. et al (1991) *FEBS Lett* 283, 298-302), Tag.100 (Qiagen; 12 aa tag derived from mammalian MAP kinase 2), Cruz tag 09™ (MKAEFRRQESDR, Santa Cruz Biotechnology Inc.) and Cruz tag 22™ (MRDALDRLDRLA, Santa Cruz Biotechnology Inc.). Known tag sequences are reviewed in Terpe (2003) Appl. Microbiol. Biotechnol. 60 523-533. In preferred embodiments, a poly-His tag such as $(H)_6$, His-SUMO tag (Invitrogen Champion pET SUMO expression system), or MRGS$(H)_6$ may be used.

The affinity tag sequence may be separated from the peptide exchange protein described herein after purification, for example, using a site-specific protease.

In some embodiments, the peptide exchange protein described herein may be coupled to a leader peptide to direct secretion of the peptide exchange protein from cell into the culture medium as a precursor protein.
A range of suitable leader peptides are known in the art. The leader peptide may be heterologous to the TAPBPR fragment described herein i.e. it may be a non-TAPBPR leader sequence. For example, an α-factor secretion signal or BiP leader sequence may be employed. The leader peptide is located at the N terminus of the precursor protein. After expression of the precursor, the leader peptide is then removed by post-translational processing after expression of the precursor to generate the mature peptide exchange protein.

Peptide exchange proteins as described herein may be isolated, in the sense of being free from contaminants, such as other polypeptides and/or cellular components.

Peptide exchange proteins load MHC class I molecules on the surface of the cells with exogenous peptide. An exogenous peptide is a peptide that is not generated naturally by the cells with the MHC class I molecules. For example, it may have been administered to the individual. Exogenous peptide may have the same amino acid sequence as an endogenous peptide that is generated naturally by the cells or a different amino acid sequence.

In some embodiments, the immunogenicity of the exogenous peptide may be different to the immunogenicity of endogenous peptides displayed in the MHC class I molecules (i.e. it may be higher or lower). For example, an exogenous peptide as described herein may be immunogenic or non-

immunogenic, depending on the application. In other embodiments, the immunogenicity of the exogenous peptide may be the same as the immunogenicity of one or more endogenous peptides displayed in the MHC class I molecules. For example, the exogenous peptide may have the same amino acid sequence as one or more endogenous peptides. Loading of MHC class I molecules with the exogenous peptide as described here may increase the total amount of peptide with the amino acid sequence that is displayed on the cells and may thereby increase or reduce the immunogenicity of the cells.

Peptides that are displayed by MHC class I molecules are well-known in the art (see for example the on-line Immune Epitope Database and Analysis Resource (IEDB); Vita et al Nucl Acid Res 2014 Oct 9 piii:gku938) and further peptides may be identified using immunopeptidomic techniques. Direct binding of peptides to MHC class I molecules may be confirmed by testing the binding of labelled peptides in cellular assays or using MHC beads. Binding of non-labelled peptide to MHC class I molecules may be determined by staining treated cells with TCR-tetramers specific for the peptide.

An immunogenic peptide is an exogenous peptide that is capable of generating an immune response in an individual when loaded onto an MHC class I molecule. For example, the immunogenic peptide/MHC class I complex may be recognised by T cells. The presence of MHC class I molecules loaded with immunogenic peptide on the surface of target cells may induce or increase immune responses against the target cells.

Suitable immunogenic peptides are known in the art and may for example be candidates in vaccines for cancer or infection. In some embodiments, immunogenic peptides for loading onto MHC class I may be antigens naturally expressed on a patient's own tumour; neoantigens or other peptides derived from tumours; or peptides derived from pathogens, such as viruses.

In some embodiments, the immunogenic peptide may comprise an antigen or an epitope that is characteristic of a disease cell. For example, the immunogenic peptide may comprise an antigen or an epitope that is characteristic of a cancer cell or a pathogen-infected cell.

Epitopes that are characteristic of cancer cells are well known in the art and include epitopes from tumour antigens. Suitable antigens and epitopes are described elsewhere herein. Preferred tumour antigens from which immunogenic peptides may be derived include neoantigens, tumour-specific, differentiation and overexpressed proteins, such as ErbB2/Her2 (e.g. RLLQETELV), gp100 (e.g. IMDQVPFSV and YLEPGPVTA), NY-Eso-1 (e.g. SLLMWITQC), p53 (e.g. LLGRNSFEV), MART1 (e.g. ELAGIGILTV), MAGE-10 (e.g. GLYDGMEHL), human AFP (e.g. FMNKFIYEI), Mesothelin (e.g. SLLFLLFSL), MAGE-A4 (e.g. GVYDGREHTV), MART-1 (e.g EAAGIGILTV, ELAGIGILTV) and 5T4 (e.g. FLTGNQLAV, RLARLALVL).

Other tumour antigens and epitopes are well known in the art (see for example the Cancer Research Institute NY on-line peptide database; Tumor T cell antigen database, Olsen et al (2017) Cancer

Immunol Immunother. doi: 10.1007/s00262-017-1978-y; Immune Epitope and Analysis Resource, Vita et al Nucleic Acids Res. 2014 Oct 9. pii: gku938).

Epitopes that are characteristic of pathogen-infected cells are well known in the art and include epitopes from viral proteins. Suitable epitopes are described elsewhere herein and may include influenza epitopes (e.g. GILGFVFTL, AIMDKNIIL), HIV epitopes (e.g. ILKEPVHGV, SLYNTVATL, KLTPLCVTL), hepatitis B epitopes (e.g. FLPSDFFPSV, WLSLLVPFV), Human cytomegalovirus (CMV) epitopes (e.g. NLVPMVATV, VLEETSVML), Epstein Barr virus (EBV) epitopes (e.g. YLLEMLWRL, CLGGLLTMV), Varicella-zoster virus epitopes (e.g. ILIEGIFFV), Measles epitopes (e.g. ILPGQDLQYV), ZIKA (e.g. FLVEDHGFGV, KSYFVRAAK), and Ebola virus epitopes. Other viral epitopes are well known in the art (see for example Immune Epitope and Analysis Resource, Vita et al Nucleic Acids Res. 2014 Oct 9. pii: gku938).

In some embodiments, MHC class I molecules on cancer cells may be loaded with immunogenic peptides comprising one or more viral epitopes. This may be useful in eliciting anti-viral immune responses against the cancer cells.

In other embodiments, the immunogenic peptide may comprise an antigen or an epitope that is not characteristic of a disease cell but is still capable of eliciting an immune response against cells displaying it at the cell surface. For example, the immunogenic peptide may comprise a synthetic epitope. Suitable synthetic epitopes are well known in the art. A synthetic epitope may be generated for example by replacing an amino acid exposed to the TCR in a peptide displayed on MHC class I molecules with an artificial amino acid, such as 3-cyclohexylalanine (CHA).

A non-immunogenic peptide is an exogenous peptide that does not generate an immune response in an individual when loaded onto an MHC class I molecule. The presence of MHC class I molecules loaded with non-immunogenic peptide on the surface of target cells may prevent or reduce immune responses against the target cells.

Suitable exogenous peptides may be 8-15mers, for example 8-11mers, preferably 9mers. The exogenous peptide may be compatible with some or all of the MHC class I molecules present on the surface of the target cells. For example, the sequence of the exogenous peptide may include suitable anchor residues required for association with some or all of the MHC class I molecules on the surface of the target cells.

The MHC class I molecules on the surface of the target cells may include HLA-A molecules, such as HLA-A68 and HLA-A2. MHC class I molecules may be identified using conventional techniques, such as tissue typing or flow cytometry.

Suitable sequences for display by the MHC class I molecules on the surface of the target cells may be determined using standard techniques. For example, when HLA-A2 molecules are present on the surface

of the target cells, the exogenous peptide may have the sequence xLxxxxxxV/L, where X is independently any amino acid; when HLA-A*03:01 molecules are present on the surface of the target cells, the exogenous peptide may have the sequence xL/I/VxxxxxxK/R, where X is independently any amino acid; when HLA-A*68:02 molecules are present on the surface of the target cells, the exogenous peptide may have the sequence xT/VxxxxxxL/V, where X is independently any amino acid; when HLA-B*27:05 molecules are present on the surface of the target cells, the exogenous peptide may have the sequence xRxxxxxxx, where X is independently any amino acid; when HLA-B*51:01 molecules are present on the surface of the target cells, the exogenous peptide may have the sequence xPxxxxxI/V, where X is independently any amino acid; and when HLA-B*15:03 molecules are present on the surface of the target cells, the exogenous peptide may have the sequence xQ/KxxxxxXF/YL, where X is independently any amino acid.

Other aspects of the invention provide a nucleic acid encoding a peptide exchange protein described herein as described above and a vector comprising such a nucleic acid.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Preferably, the vector contains appropriate regulatory sequences to drive the expression of the nucleic acid in mammalian cells. A vector may also comprise sequences, such as origins of replication, promoter regions and selectable markers, which allow for its selection, expression and replication in bacterial hosts such as *E. coli*. Vectors may be plasmids, viral e.g. phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 3rd edition, Russell et al., 2001, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, are described in detail in Current Protocols in Molecular Biology, Ausubel et al. eds. John Wiley & Sons, 1992.

A nucleic acid or vector as described herein may be introduced into a host cell. Another aspect of the invention provides a recombinant cell comprising a nucleic acid or vector that expresses a peptide exchange protein as described above. A range of host cells suitable for the production of recombinant peptide exchange protein are known in the art. Suitable host cells may include prokaryotic cells, in particular bacteria such as *Escherichia coli* and *Lactococcus lactis* and eukaryotic cells, including mammalian cells such as CHO and CHO-derived cell lines (Lec cells), HeLa, COS, HEK293 and HEK-EBNA cells, amphibian cells such as Xenopus oocytes, insect cells such as *Trichoplusia ni*, Sf9 and Sf21 and yeast cells, such as *Pichia pastoris*.

Techniques for the introduction of nucleic acid into cells are well established in the art and any suitable technique may be employed, in accordance with the particular circumstances. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. adenovirus, AAV, lentivirus or vaccinia. For bacterial cells, suitable techniques may include calcium chloride

transformation, electroporation and transfection using bacteriophage. Marker genes such as antibiotic resistance or sensitivity genes may be used in identifying clones containing nucleic acid of interest, as is well-known in the art.

The introduction may be followed by expression of the nucleic acid to produce the encoded peptide exchange protein. In some embodiments, host cells (which may include cells actually transformed although more likely the cells will be descendants of the transformed cells) may be cultured *in vitro* under conditions for expression of the nucleic acid, so that the encoded serpin polypeptide is produced. When an inducible promoter is used, expression may require the activation of the inducible promoter.

The expressed polypeptide comprising or consisting of the peptide exchange protein may be isolated and/or purified, after production. This may be achieved using any convenient method known in the art. Techniques for the purification of recombinant polypeptides are well known in the art and include, for example HPLC, FPLC or affinity chromatography. In some embodiments, purification may be performed using an affinity tag on the polypeptide as described above.

Another aspect of the invention provides a method of producing a peptide exchange protein described herein comprising expressing a heterologous nucleic acid encoding the peptide exchange protein in a host cell and optionally isolating and/or purifying the peptide exchange protein thus produced. After production, the peptide exchange protein may be investigated further, for example the pharmacological properties and/or activity may be determined. Methods and means of protein analysis are well-known in the art.

A peptide exchange protein described herein as described herein may be useful in therapy. For example, the peptide exchange protein may be administered to an individual to modulate the immunogenicity of target cells or the peptide exchange protein may be used to modulate the immunogenicity of cells in vitro or ex vivo, which are then administered to an individual. A peptide exchange protein for administration to an individual is preferably a chimeric peptide exchange protein comprising a targeting domain. This allows the immunogenicity of target cells in the individual to be modulated.

Whilst the peptide exchange protein may be administered alone, it will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the chimeric peptide exchange protein. Thus pharmaceutical compositions may comprise, in addition to the peptide exchange protein itself, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. The term “pharmaceutically acceptable” as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g., human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be “acceptable” in the sense of being compatible with the other ingredients of the formulation. The precise nature of the carrier or other material

will depend on the route of administration, which may be by bolus, infusion, injection or any other suitable route, as discussed below.

The peptide exchange protein may be administered in combination with an exogenous peptide, preferably an immunogenic peptide. In some embodiments, the peptide exchange protein and the exogenous peptide may be formulated in the same pharmaceutical composition. In other embodiments, the peptide exchange protein and the exogenous peptide may be formulated in separate pharmaceutical compositions.

In some embodiments, the peptide exchange protein and/or exogenous peptide may be provided in a lyophilised form for reconstitution prior to administration. For example, a lyophilised peptide exchange protein and/or exogenous peptide may be re-constituted in sterile water and mixed with saline prior to administration to an individual.

For parenteral, for example sub-cutaneous, intra-tumoural, intra-muscular or intra-venous administration, e.g. by injection, the pharmaceutical composition comprising the peptide exchange protein and/or exogenous peptide described herein, nucleic acid or cell may be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles, such as Sodium Chloride Injection, Ringer's Injection, and Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be employed as required including buffers such as phosphate, citrate and other organic acids; antioxidants, such as ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3'-pentanol; and m-cresol); low molecular weight polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone; amino acids, such as glycine, glutamine, asparagines, histidine, arginine, or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose or dextrins; chelating agents, such as EDTA; sugars, such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions, such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants, such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Suitable carriers, excipients, etc. can be found in standard pharmaceutical texts, for example, Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, Easton, Pa., 1990.

Pharmaceutical compositions and formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the chimeric protein described herein with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

A pharmaceutical composition comprising a peptide exchange protein and/or exogenous peptide as described herein may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Peptide exchange proteins described herein may be useful in modulating the immunogenicity of mammalian cells in vivo, in vitro or ex vivo. For example a method may comprise;

providing a population of mammalian cells having surface MHC class I molecules,

contacting the population of mammalian cells with an exogenous peptide and a peptide exchange protein comprising a TAPBPR fragment consisting of the luminal domain of TAPBPR,

such that the peptide exchange protein loads the exogenous peptide onto the surface MHC class I molecules of the cells in the population,

thereby modulating the immunogenicity of the mammalian cells.

Surface MHC class I molecules that have been loaded with immunogenic exogenous peptides are accessible to T cell receptors. The loading of the surface MHC class I molecules with immunogenic peptides may induce or increase T cell recognition of the cells of the mammalian cells and may increase the immunogenicity of the mammalian cells.

In some embodiments, the mammalian cells may be antigen presenting cells (APCs), such as dendritic cells. The loading of the surface MHC class I molecules with immunogenic exogenous peptides may increase the ability of the APCs to induce immune responses, for example immune responses against the antigenic epitopes contained in the immunogenic peptide. APCs loaded with immunogenic peptide as described above may be used to stimulate T cells in vitro or ex vivo or administered to an individual to stimulate T cells in vivo.

A method of producing antigen presenting cells for generating or increasing an immune response in an individual may comprise;

providing a population of antigen presenting cells previously obtained from the individual, and

contacting the antigen presenting cells with a peptide exchange protein and an immunogenic peptide, such that the peptide exchange protein loads the immunogenic peptide onto surface MHC class I molecules of the antigen presenting cells,

the antigen presenting cells being capable of stimulating T cells to generate an immune response

The method may an in vitro or an ex vivo method.

The immunogenic peptide may comprise one or more antigenic epitopes. The antigen presenting cells may activate T cells against the antigenic epitopes of the immunogenic peptide. For example, the antigenic epitopes of the immunogenic peptide may be present on disease cells in the individual. The antigen presenting cells may activate T cells capable of generating an immune response against the disease cells in the individual.

In some embodiments, following production, the antigen presenting cells may be administered to an individual to activate T cells and generate or increase a T cell immune response in the individual.

In other embodiments, following production, the antigen presenting cells may be contacted with a population of T cells to activate the T cells against the one or more antigenic epitopes of the immunogenic peptide in vivo or ex vivo. The activated T cells may be administered to an individual to generate a T cell immune response in the individual. The individual may be the individual from which the population of T cells was obtained (autologous) or a different individual (allogeneic).

Suitable antigen presenting cells include any cell that expresses the MHC class I molecules against which the immune response is to be directed. In some embodiments, dendritic cells may be preferred.

Peptide exchange proteins described herein may be useful in modulating the immunogenicity of mammalian cells in vivo. This may be useful in immunotherapeutic applications, for example in which the generation or enhancement of an immune response might have a therapeutic effect. Suitable applications might include the treatment of conditions associated with the presence of populations of disease cells in an individual. These conditions might include cancer and infection with an intracellular pathogen. Other suitable applications might include the treatment of autoimmune or auto inflammatory conditions in which the reduction in immunogenicity of a cell or tissue might have a therapeutic effect. The targeting domain of a peptide exchange protein as described above may preferentially or selectively direct the protein to target cells within the individual relative to non-target cells.

A peptide exchange protein described herein may be used in a method of treatment of the human or animal body, including therapeutic and prophylactic or preventative treatment (e.g. treatment before the onset of a condition in an individual to reduce the risk of the condition occurring in the individual; delay its onset; or reduce its severity after onset). Prophylactic or preventative treatment may include vaccination. The method of treatment may comprise administering a peptide exchange protein described herein and an immunogenic peptide to an individual in need thereof.

A method of increasing the immunogenicity of target cells in an individual may comprise;

administering a peptide exchange protein as described above to the individual, wherein peptide exchange protein comprises a targeting domain which binds to target cells in the individual, and

administering an immunogenic peptide to the individual, such that the peptide exchange protein loads the immunogenic peptide onto surface MHC class I molecules of the target cells,

thereby increasing the immunogenicity of the target cells.

An individual suitable for treatment as described above may be a mammal, such as a rodent (e.g. a guinea pig, a hamster, a rat, a mouse), murine (e.g. a mouse), canine (e.g. a dog), feline (e.g. a cat), equine (e.g. a horse), a primate, simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon), an ape (e.g. gorilla, chimpanzee, orang-utan, gibbon), or a human.

In some preferred embodiments, the individual is a human. In other preferred embodiments, non-human mammals, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (*e.g.* murine, primate, porcine, canine, or rabbit animals) may be employed.

Suitable target cells may include disease cells i.e. cells that are associated with a disease condition in the individual, such as cells infected with virus or other intracellular pathogen, or cancer or tumour cells.

In some preferred embodiments, the target cells are cancer cells. A method of treatment of cancer in an individual may comprise;

administering a peptide exchange protein described above to the individual, wherein peptide exchange protein comprises a targeting domain which binds to cancer cells in the individual, and

administering an immunogenic peptide to the individual, such that the peptide exchange protein loads the immunogenic peptide onto surface MHC class I molecules of the cancer cells of the individual,

thereby eliciting or increasing an immune response in the individual against the cancer cells.

Cancer may be characterised by the abnormal proliferation of malignant cancer cells and may include leukaemias, such as AML, CML, ALL and CLL, lymphomas, such as Hodgkin lymphoma, non-Hodgkin lymphoma and multiple myeloma, and solid cancers such as sarcomas, skin cancer, melanoma, bladder cancer, brain cancer, breast cancer, uterus cancer, ovary cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer, liver cancer, head and neck cancer, oesophageal cancer, pancreas cancer, renal cancer, adrenal cancer, stomach cancer, testicular cancer, cancer of the gall bladder and biliary tracts, thyroid cancer, thymus cancer, cancer of bone, and cerebral cancer, as well as cancer of unknown primary (CUP).

In some embodiments, cancer cells within an individual may be immunologically distinct from normal somatic cells in the individual (i.e. the cancerous tumour may be immunogenic). For example, the cancer cells may be capable of eliciting a systemic immune response in the individual against one or more antigens expressed by the cancer cells. The tumour antigens that elicit the immune response may be specific to cancer cells or may be shared by one or more normal cells in the individual. In other embodiments, cancer cells within an individual may not be immunologically distinct from normal somatic cells in the individual until MHC class I molecules on the surface of the cancer cells are loaded with exogenous immunogenic peptide using a peptide exchange protein as described herein.

In some embodiments, the individual may have minimal residual disease (MRD) after an initial cancer treatment.

An individual with cancer may display at least one identifiable sign, symptom, or laboratory finding that is sufficient to make a diagnosis of cancer in accordance with clinical standards known in the art. Examples of such clinical standards can be found in textbooks of medicine such as Harrison's Principles of Internal Medicine, 15th Ed., Fauci AS et al., eds., McGraw-Hill, New York, 2001. In some instances, a diagnosis

of a cancer in an individual may include identification of a particular cell type (e.g. a cancer cell) in a sample of a body fluid or tissue obtained from the individual.

In particular, treatment may include inhibiting cancer growth, including complete cancer remission, and/or inhibiting cancer metastasis. Cancer growth generally refers to any one of a number of indices that indicate change within the cancer to a more developed form. Thus, indices for measuring an inhibition of cancer growth include a decrease in cancer cell survival, a decrease in tumour volume or morphology (for example, as determined using computed tomographic (CT), sonography, or other imaging method), a delayed tumour growth, a destruction of tumour vasculature, improved performance in delayed hypersensitivity skin test, an increase in the activity of T cells, and a decrease in levels of tumour-specific antigens. Administration of T cells modified as described herein may improve the capacity of the individual to resist cancer growth, in particular growth of a cancer already present the subject and/or decrease the propensity for cancer growth in the individual.

In other preferred embodiments, the target cells are pathogen-infected cells.

A method of treatment of pathogen infection in an individual may comprise;

administering a peptide exchange protein described above to the individual, wherein peptide exchange protein comprises a targeting domain which binds to pathogen-infected cells in the individual, and

administering an immunogenic peptide to the individual, such that the peptide exchange protein loads the immunogenic peptide onto surface MHC class I molecules of the pathogen-infected cells of the individual,

thereby eliciting or increasing an immune response in the individual against the pathogen-infected cells.

Pathogen infection may include viral infection, for example HIV, EBV, CMV or hepatitis infection.

In other preferred embodiments, the target cells are antigen presenting cells, such as dendritic cells. Antigen presenting cells present antigenic epitopes to T cells to activate a T cell response against the antigen. A method of treatment of a condition associated with disease cells in an individual may comprise;

administering a peptide exchange protein described herein to the individual, wherein the peptide exchange protein comprises a targeting domain that binds to antigen presenting cells in the individual, and

administering an immunogenic peptide to the individual, such that the peptide exchange protein loads the immunogenic peptide onto surface MHC class I molecules of the antigen presenting cells,

such that said antigen presenting cells generate or increase an immune response in the individual against the disease cells.

Disease cells may include cancer cells or pathogen-infected cells. For example, this may be useful in treating pathogen infections in which a peptide vaccine is currently used to induce CD8+ T cells responses, such as infections of HIV, EBV, CMV, hepatitis viruses, influenza, polio, human papilloma virus, measles, mumps, rubella, chicken pox, ebola, or zika; or cancer, for example by boosting the number of T cells capable of recognising a particular antigen.

In other embodiments, methods described herein may be useful in reducing immunogenicity. A method of reducing an immune response in an individual may comprise;

administering a chimeric peptide exchange protein to the individual, wherein the targeting domain of the chimeric peptide exchange protein binds to target cells in the individual,

administering a non-immunogenic peptide to the individual, such that the peptide exchange protein replaces immunogenic peptides in surface MHC class I molecules with non-immunogenic peptides and the immunogenicity of the target cells is reduced in the individual.

The chimeric peptide exchange protein may for example, reduce the immunogenicity of the donor organ and/or antigen presentation cells removing recognition of self/donor-peptides (e.g. alloantigens/minor histocompatibility antigens) which are the target of the immune recognition.

In some preferred embodiments, the individual may have an autoimmune disease or immune-mediated inflammatory disease. A method of treatment of autoimmune or immune-mediated inflammatory disease in an individual may comprise;

administering a peptide exchange protein described above to the individual,

wherein peptide exchange protein comprises a targeting domain which binds to cells in the individual having surface MHC class I molecules displaying an immunogenic peptide, and

administering an non-immunogenic peptide to the individual, such that the peptide exchange protein replaces the immunogenic peptide in the surface MHC class I molecules with the non-immunogenic peptide,

thereby preventing or reducing an immune response in the individual against the cells.

In other preferred embodiments, methods described herein may be useful in organ or tissue transplantation. A method of treating diseases associated with MHC class I molecules in an individual may comprise;

administering a chimeric peptide exchange protein described herein to the individual, wherein the targeting domain of the chimeric peptide exchange protein binds to target cells in the individual which have disease associated MHC class I molecules on their surface,

administering an exogenous peptide to the individual, such that the peptide exchange protein loads the surface MHC class I molecules with the exogenous peptide,

such that the MHC class I molecules are stabilised by the exogenous peptide.

In other embodiments, methods described herein may be useful in treating diseases associated with MHC class I molecules. A method of treating diseases associated with MHC class I molecules in an individual may comprise;

administering a chimeric peptide exchange protein described herein to the individual, wherein the targeting domain of the chimeric peptide exchange protein binds to target cells in the individual which have disease associated MHC class I molecules on their surface, and

administering an exogenous peptide to the individual, such that the peptide exchange protein loads the surface MHC class I molecules with the exogenous peptide,

such that the MHC class I molecules are stabilised by the exogenous peptide.

MHC class I associated diseases may include the spondyloarthropathies (associated with HLA-B27), Behcet's disease (associated with HLA-B51), Birdshot Chorioretinopathy (associated with HLA-A29) psoriasis and psoriatic arthritis (associated with HLA-Cw6).

Administration is normally in a "therapeutically effective amount" or "prophylactically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the composition, the method of administration, the scheduling of administration and other factors known to medical practitioners.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the circumstances of the individual to be treated. For example, a composition may be administered in combination with vaccination, immune checkpoint inhibition, other immunotherapies and potentially chemotherapy and radiotherapy.

Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors and may depend on the severity of the symptoms and/or progression of a disease being treated. Appropriate doses of therapeutic polypeptides are well known in the art (Ledermann J.A. et al. (1991) Int. J. Cancer 47: 659-664; Bagshawe K.D. et al. (1991) Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-922). Specific dosages may be indicated herein or in the Physician's Desk Reference (2003) as appropriate for the type of medicament being administered may be used. A therapeutically effective amount or suitable dose of a chimeric protein described herein may be determined by comparing its *in vitro* activity and *in vivo* activity in an animal model. Methods for extrapolation of effective dosages in mice and other test animals to humans are known. The precise dose will depend upon a number of factors, including whether the chimeric protein described herein is for prevention or for treatment, the size and location of the area to be treated, the precise nature of the chimeric protein described herein and the nature of any detectable label or other molecule attached to the chimeric protein described herein.

A typical dose of a peptide exchange protein will be in the range of 0.1 mg/kg to 100mg/kg. For example, a dose in the range 100 µg to 1 g may be used for systemic applications. An initial higher loading dose, followed by one or more lower doses, may be administered. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician.

In some embodiments, pre-vaccination and/or re-vaccination with tumour or viral antigens may be required before administration of the TAPBPR/peptide combination intended to be delivered to the tumour/infection site. The vaccination strategy may employ TAPBPR to load peptides or may involve standard vaccination regimens.

The treatment schedule for an individual may be dependent on the pharmocokinetic and pharmacodynamic properties of the peptide exchange protein described herein composition, the route of administration and the nature of the condition being treated.

Treatment may be periodic, and the period between administrations may be about 12 hours or more, 24 hours or more, 36 hours or more, 48 hours or more, 96 hours or more, or one week or more. Suitable formulations and routes of administration are described above.

Treatment may be any treatment and therapy, whether of a human or an animal (e.g. in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition or delay of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, cure or remission (whether partial or total) of the condition, preventing, delaying, abating or arresting one or more symptoms and/or signs of the condition or prolonging survival of a subject or patient beyond that expected in the absence of treatment.

Treatment may also be prophylactic (i.e. prophylaxis). For example, an individual susceptible to or at risk of the occurrence or re-occurrence of disease may be treated as described herein. Such treatment may prevent or delay the occurrence or re-occurrence of the disease in the individual.

Other aspects of the invention relate to kits for use in increasing immunogenicity or stimulating immune responses as described herein. A kit may comprise a peptide exchange protein and an immunogenic peptide as described above.

A kit may further comprise an additional therapeutic agent, such as a vaccine or immune checkpoint inhibitor.

Other aspects of the invention relate to methods and reagents for identifying, characterising or isolating T cells *in vitro* or *ex vivo* using MHC class I molecules that display a target peptide.

A method of producing a MHC class I molecule displaying a target peptide may comprise;

contacting an MHC class I molecule with a peptide exchange protein described above and an target peptide, such that the peptide exchange protein loads the target peptide onto the MHC class I molecule,

thereby producing an MHC class I molecule displaying the target peptide.

Preferably, the MHC class I molecule is contacted with a soluble peptide exchange protein. Soluble peptide exchange proteins are described in detail above.

The target peptide may be a peptide which is capable of specific binding to a T cell when displayed by a MHC class I molecule. For example, the target peptide may comprise a viral, bacterial, cancer or autoimmune antigenic epitope. MHC class I molecules displaying the target peptide may be useful in identifying, quantifying, characterising or isolating T cells within a population of T cells that specifically bind to the MHC class I molecule/target peptide complex.

The MHC class I molecule may display an initial peptide that is replaced by the target peptide following contact with the peptide exchange protein. The sequence of the initial peptide is independent of the target peptide being employed, and any convenient peptide that can be displayed by MHC class I molecules may be employed.

In some embodiments, the MHC class I molecule may be immobilised on a solid support, such as a bead. The MHC class I molecule may be a member of a population of MHC class I molecules immobilised on the solid support. For example, the peptide exchange protein may be contacted with a population of MHC class I molecules immobilised on the solid support may load target peptide onto the MHC class I molecules in the immobilised population.

MHC class I molecules may be immobilised on the solid support by any convenient technique. For example, the MHC class I molecules may be biotinylated and may be bound to the support through a biotin/streptavidin interaction.

In other embodiments, the MHC class I molecule may be in solution, for example as a sub-unit of a multimer. The peptide exchange protein may be contacted in solution with a multimer that comprises multiple MHC class I molecules. Preferred multimers include tetramers of biotinylated MHC class I molecules linked by streptavidin. The streptavidin may be labelled, for example with a fluorophore such as phycoerythrin. Tetramers of biotinylated MHC class I molecules are well known in the art (Altman et al Science 1996, 274: 94-96).

In some embodiments, the MHC class I molecules displaying the target peptide may be contacted with a population of T cells, for example a population of T cells previously obtained from an individual. The binding of the MHC class I molecules to T cells in the population may be determined. Binding may be determined by any convenient technique, such as flow cytometry.

The frequency or number of T cells within the population that bind to the MHC class I molecules displaying the target molecule may be determined. This may be useful in research or for diagnostic or prognostic applications.

T cells that bind to the MHC class I molecules displaying the target molecule may be isolated and/or expanded *in vitro*, for example for use in therapeutic applications.

Other aspects and embodiments of the invention provide the aspects and embodiments described above with the term "comprising" replaced by the term "consisting of" and the aspects and embodiments described above with the term "comprising" replaced by the term "consisting essentially of".

It is to be understood that the application discloses all combinations of any of the above aspects and embodiments described above with each other, unless the context demands otherwise. Similarly, the application discloses all combinations of the preferred and/or optional features either singly or together with any of the other aspects, unless the context demands otherwise.

Modifications of the above embodiments, further embodiments and modifications thereof will be apparent to the skilled person on reading this disclosure, and as such, these are within the scope of the present invention.

All documents and sequence database entries mentioned in this specification are incorporated herein by reference in their entirety for all purposes.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

Experimental

1. Materials and Methods

1.1 Constructs

The production of full-length TAPBPR^{WT} and TAPBPR^{TN5} was performed using the lentiviral vector pHRSIN-C56W-UbEM, which produces TAPBPR under the control of the spleen focus-forming virus (SFFV) promoter and the GFP derivative emerald under the control of an ubiquitin promoter, as previously described [11,15]. The cloning of the chimeric constructs TAPBPR^{PM} and tapasin^{PM} was performed in the same lentiviral vector, by a twostep PCR procedure, where the ectodomain and transmembrane domain of either TAPBPR or tapasin were amplified and then fused to the cytoplasmic tail of CD8. TAPBPR^{ER} was created using a similar procedure, in which the ectodomain of TAPBPR was fused to the transmembrane and cytoplasmic domains of tapasin. To produce secreted versions of TAPBPR^{WT} or TAPBPR^{TN5} , the luminal domains of both were cloned into a piggyback transposon-based mammalian cell expression system as decribed in Li et al [22].

1.2 Cell culture

HeLaM cells, a variant HeLa cell line that is more responsive to IFN [23], their modified variants, HEK-293T cells and MCF7 cells were maintained in Dulbecco's Modified Eagle's medium (DMEM; Sigma-Aldrich, UK) supplemented with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific), 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, Thermo Fisher Scientific) at 37°C with 5% CO2. To induce expression of endogenously expressed TAPBPR and up-regulate other components of the MHC class I antigen processing and presentation pathway, HeLaM and MCF7 cells were treated with 200 U/ml IFN-γ (Peprotech, UK) for 48–72 h where indicated.

1.3 Antibodies

The following TAPBPR-specific antibodies were used: PeTe4, a mouse monoclonal antibody (mAb) specific for the native conformation of TAPBPR, raised against amino acids 22–406 of human TAPBPR 11 that does not cross-react with tapasin 15, and ab57411, a mouse mAb raised against amino acids 23–122 of TAPBPR that is reactive to denatured TAPBPR (Abcam, UK). The following MHC class I-specific antibodies were used: W6/32, a pan- MHC class I mAb that recognises a conformation-specific epitope on the MHC class I α2 domain, independently of the presence of β2m and peptide 24; HC10, a MHC class I-specific mAb that recognises HLA-A, -B, and -C molecules containing a PxxWDR motif at amino acids 57–62 in the α1 domain 25,26; biotinylated anti-HLA-A68-reactive mAb, specific for HLA-A2 and -A68 heavy chain/β2m heterodimers (One Lambda, Thermo Fisher Scientific, Canoga Park, CA); BB7.2, an antibody specific for HLA-A2 heavy chain/β2m heterodimer. Other antibodies used include: Pasta-1, the tapasin-specific mAb (Dick et al., 2002); rabbit anti-calnexin (Enzo Life Sciences, UK); rabbit mAb to UGT1 (ab124879, Abcam); IgG2a isotype control as a negative control (Sigma-Aldrich).

1.4 Lentiviral transduction and transfections

Lentivirus was produced by transfecting HEK-293T cells with lentiviral vectors along with the packaging vector pCMVΔR8.91 and the envelope vector pMD.G using Fugene (Promega, UK). Viral supernatant was collected at 48 h and used to transduce a previously described TAPBPR-knockout HeLaM cell line (HeLaM-TAPBPR^{KO}) (Neerincx et al., 2017). TAPBPR^{WT}, TAPBPR^{TN5}, TAPBPRP^{PM}, TAPBPR^{ER}, tapasin^{WT} and tapasin^{PM} were reconstituted in the HeLaM-TAPBPR^{KO} cell line.

1.5 MHC class I-binding peptides

The following MHC-class I specific peptides were used: HLA-A*68:02-binding peptide ETVSEQSNV, its derivative EGVSEQSNG, obtained by replacing its anchor residues (amino acids on positions 2 and 9) with glycine, as well as their fluorescently-labelled versions ETVSK^{TAMRA}QSNV and respectively EGVSK^{TAMRA}QSNG, obtained by replacing the glutamate on position 5 with a lysine, labelled with 5-carboxytetramethylrhodaime [TAMRA] (from Peptide Synthetics, UK); HLA-A*02:01 binding peptides NLVPMVATV, YLLEMLWRL, CLGGLLTMV and YVVPFVAKV, together with their fluorescently-labelled variants NLVPK^{TAMRA}VATV, FMVFK^{TAMRA}QTHI, CLGGK^{TAMRA}LTMV, YLLEK^{TAMRA}LWRL and respectively YVVPFVAK^{TAMRA}V (from Peptide Synthetics, UK); HLA-B*27:05 specific peptide SRYWAIRTR and its fluorescently-labelled variant SRYWK^{TAMRA}IRTR (from Peptide Synthetics, UK).

1.6 Flow cytometry

Following trypsinisation, cells were washed in 1% bovine serum albumin (BSA), dissolved in 1x PBS at 4°C and then stained for 30 min at 4°C in 1% BSA containing one of the following antibodies: W6/32, pete4, pasta-1, anti-HLA-A68-reactive mAb, BB7.2 or with an isotype control antibody. After washing the cells to remove excess unbound antibody, the primary antibodies bound to the cells were detected by incubation at 4°C for 25 min with either goat anti-mouse Alexa-Fluor 647 IgG (Invitrogen Molecular Probes, Thermo Fisher Scientific) or with Alexa-Fluor 647-conjugated streptavidin (Invitrogen Molecular Probes, Thermo Fisher
Scientific) for the biotinylated anti-HLA-A68 mAb. After subsequent three rounds of washing, the fluorescence levels were detected using a BD FACScan analyser with Cytek modifications and analysed using FlowJo (FlowJo, LLC, Ashland, OR).

1.7 Immunoprecipitation, gel electrophoresis and western blotting

Cells were harvested then washed in phosphate-buffered saline (PBS). For surface TAPBPR immunoprecipitation experiments, cells were incubated with 2 µg Pete4 antibody in 1% BSA in 1x PBS for 1 h with rotation at 4°C. Excess antibody was removed by washing the cells 5 times in 1x PBS at 4°C. Cells were then lysed and the intracellular TAPBPR immunoprecipitation was further performed similarly to the TAPBPR immunoprecipitation from the whole cell lysates.

For TAPBPR immunoprecipitation experiments from whole cell lysates, cells were lysed in 1% triton X-100 (VWR, Radnor, PN), Tris-buffered saline (TBS) (20 mM Tris-HCl, 150 mM NaCl, 2.5 mM CaCl2) supplemented with 10 mM NEM, 1 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich), and protease inhibitor cocktail (Roche, UK) for 30 min at 4°C. Nuclei and cell debris were pelleted by centrifugation at 13,000 × g for 15 min and supernatants were collected. Immunoprecipitation was performed with Pete4 antibody coupled to protein A sepharose (GE Healthcare) for 2 h at 4°C with rotation. Following immunoprecipitation, beads were washed thoroughly in 0.1% detergent-TBS to remove unbound protein. For separation by gel electrophoresis, the samples were heated at 94°C for 10 min in sample buffer (125 mM Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 0.04% bromophenol blue), supplemented with 100 mM β-mercaptoethanol. In order to analyse the samples by western blotting, proteins were transferred onto an Immobilon transfer membrane (Merck Millipore). Membranes were blocked using 5% (w/v) dried milk and 0.1% (v/v) Tween 20 in PBS for 30 min and subsequently incubated with the indicated primary antibody for 1–16 h. After washing, membranes were incubated with species-specific HRP-conjugated secondary antibodies, washed and detected by enhanced chemiluminescence using Western Lightning (Perkin Elmer, UK) and Super RX film (Fujifilm, UK). Films were scanned on a CanoScan8800F using MX Navigator Software (Canon, UK).

1.8 Expression and purification of TAPBPR protein

Secreted forms of either $TAPBPR^{WT}$ or $TAPBPR^{TN5}$ were expressed in 293T cells, using the PiggyBac expression system as described in 22. For that, the C-terminally His-tagged ectodomain of either protein was cloned into a modified version of the PB-T-PAF vector, lacking the protein A using NheI and NotI (Thermo Fisher Scientific). In brief, $2x10^5$ 293T cells were transfected with 100ng PB-RN plasmid, 100ng

PBase plasmid and 800ng PB-T-TAPBPR or PB-T-TAPASIN. 48h after transfection, cells were selected for stable integration using Dulbecco's Modified Eagle's medium (DMEM; Sigma-Aldrich, UK) supplemented with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific), 1xP/S, 3µg/ml Puromycin (Invivogen) and 700µg G418 (Gibco, Thermo Scientific) for 7 days. For protein production, $6x10^7$ cells were induced with 2µg/ml Doxycycline for 5 to 7 days in 200ml DMEM supplemented with 5% FCS and 100U/ml penicillin. After 7 days, the media was collected and TAPBPR was purified using NiSepharose™excel beads (GE Lifesciences). Proteins were eluted with 250mM imidazole in PBS (Sigma) and subsequently dialysed against PBS (Sigma) for 48h. For purity assessment, elution fractions were analysed by SDSPAGE, followed by Coomassie staining.

1.9 Peptide binding

Target cell lines were seeded at 25,000-30,000 cells/well in 12-well plates and stimulated with IFN-γ. Following the stimulation period, the cells were washed 3 times with 1x PBS and incubated with 300 µL pre-warmed opti-MEM (Thermo Fisher Scientific, UK). In case the peptide binding was done in the presence of recombinant TAPBPR, the cells were then treated with or without recombinant TAPBPR (100 nM for HLA-A*68:02 or 1 µM for HLA-A* 02:01). After 15 min, the desired TAMRA-labelled peptide was added to the cells and incubated at 37°C (15 min for HLA-A*68:02 or 60 min for HLA-A*02:01). In case the peptide binding was facilitated by over-expressed TAPBPR, the labelled peptide was directly added to the cells, without using recombinant TAPBPR. Following the peptide treatment, the cells were washed three times in 1x PBS and harvested. The level of bound peptide/cell was determined by flow cytometry, using the YelFL1 channel (Cytek).

1.10 Peptide exchange

Cells were seeded at 25,000 cells/well and stimulated with IFN-γ for 48 hours, then washed and treated with 10 nM TAMRA-labelled peptide of interest diluted in opti-MEM for 15 min at 37°C, as described above. Following the binding step, the peptide-containing media was removed, the cells were washed and then treated with media alone or with different concentrations of non-labelled peptide for another 15 min at 37°C. The cells were then washed and harvested and the level of bound peptide per cell was determined by flow cytometry, using the YelFL1 channel (Cytek).

1.11 TCR-like mAb staining

TCR-like mAb specific for peptides derived from then Epstein-Barr Virus (EBV) latent gene products Latent Membrane Protein 2A ($LMP2A_{426–434}$: CLGGLLTMV) and Latent Membrane Protein 1 ($LMP1_{125–133}$: YLLEMLWRL) in association with HLA-A*02:01 (Sim et al., 2013) were used to stain target cells, following treatment with 10 nM of the corresponding peptide, in the presence of recombinant TAPBPR. After washing, the level of bound TCR-like mAb bound was detected using the goat anti-mouse Alexa-Fluor 647 IgG and subsequently measured by flow cytometry.

1.12 FluoroSpot T cell assay

Target cells (MCF-7 cells or HeLaM cells deficient of HLA heavy chain A, B and C and reconstituted with HLA-A*02:01 heavy chain) were seeded at 80,000 cells/well of a 6-well plate and stimulated with 200

units of IFN-γ (Peprotech, UK) for 72 hours. Cells were then washed 3 times with 1x PBS (Sigma-Aldrich, UK) and incubated with 600 µL pre-warmed opti-MEM, containing either recombinant WT TAPBPR, TN5 TAPBPR mutant, or without TAPBPR. After 15 min, 100 pM NLVPMVATV peptide was added to the desired samples and incubated for another 60 min. Following the peptide treatment, cells were washed 3 times in 1x PBS and harvested. Each sample was then washed again twice in 1x PBS and resuspended in X-VIVO 15 medium at 1 mil cells/mL. Target cells were first irradiated and then added to a 96-well plate, pre-blocked overnight, at 50,000 cells/well together with 8,000 T cells/well. The plate was incubated overnight at 37°C and developed the following day.

2. Results

2.1 Peptide-receptive MHC class I is present on cells expressing surface TAPBPR

To explore whether surface TAPBPR was potentially capable of functioning as a peptide editor on surface MHC class I molecules, we first asked whether the fluorescent peptide ETVSK*QSNV, a variant of the neoantigen ETVSEQSNV that binds to HLA-A*68:02 with high affinity, exhibited increased binding to cells expressing surface TAPBPR compared to cells lacking surface TAPBPR. While IFN-γ treated HeLaM-TAPBPR^{KO} (HeLaM^{KO}) and HeLaM cells do not express any TAPBPR at their cell surface, over-expression of TAPBPR^{WT} in HeLaM-TAPBPR^{KO} resulted in a significant amount of surface expressed TAPBPR (Figure 1a). When cells expressing surface TAPBPR were incubated with 10 nM exogenous peptide for 15 min at 37°C, we observed a significant enhancement in fluorescence indicative of peptide binding to these cells (Figure 1b). In contrast, we did not observe any binding of ETVSK*QSNV to the cell lines lacking surface TAPBPR under identical conditions (Figure 1b). To ensure cellular fluorescence observed was a direct consequence of peptide binding to MHC class I, we also incubated cells with a variant fluorescent peptide (EGVSK*QSNG) in which the anchor residues permitting binding to HLA-A*68:02 were mutated. This did not bind to any of the cell lines (Figure 1c).The enhancement in peptide binding to IFN-γ treated HeLa^{KO}TAPBPR^{WT} compared to HeLaM or HeLaM^{KO} cells was observed over a wide range of peptide concentrations (Figure 1d). At higher peptide concentrations, exogenous peptide binding to cells lacking surface TAPBPR was observed (Figure 1d). However, this TAPBPR-independent peptide binding to cells was ~120 times less efficient as ~1.2 µM peptide was required to obtain a similar fluorescence as observed with 10 nM peptide in a TAPBPR-promoted manner required (Figure 1d). Peptide binding to cells was dependent on MHC class I given HLA-A,B,C deficient HeLaM cells were unable to bind exogenous peptide until concentrations of 10µM were used. When we explored the kinetics of TAPBPR-promoted peptide binding over time, we observed a striking increase in the ability of cells to load exogenous peptide onto surface MHC class I compared to TAPBPR-independent conditions upon incubation with 10 nM peptide (Figure 1e). For examples, after 60 min we observed a 21-fold increase in the level of fluorescent peptide bound to HeLaKO-TAPBPR^{WT} compared to the HeLaM and HeLaM^{KO} controls (Figure 1e). These findings are supportive of a role of surface TAPBPR in loading antigenic peptides onto surface MHC class I molecules. Furthermore, we also observed high levels of exogenous peptide loading on HeLaM^{KO}TAPBPR^{WT} cells at 4°C, which inhibits membrane trafficking, further suggesting peptide loading was occurring directly at the cell surface, rather than in endocytic vesicles.

2.2 Surface expressed TAPBPR enhances exogenous peptide association onto MHC class I molecules

To provide definitive proof that the surface pool of TAPBPR, rather than over-expression of the protein, was responsible for loading exogenous peptide onto MHC class I, we produced two chimeric TAPBPR constructs to target TAPBPR to different subcellular sites. Plasma membrane (PM) targeting of the lumen portion of TAPBPR was achieved by replacing cytoplasmic tail of TAPBPR with that of CD8 ($TAPBPR^{PM}$)[12] while TAPBPR was retained in the endoplasmic reticulum (ER) by replacing its transmembrane domain and cytoplasmic tail with those of tapasin ($TAPBPR^{ER}$) [13,14]. In contrast to $TAPBPR^{PM}$, which was expressed at very high levels on the cell surface, $TAPBPR^{ER}$ was not detectable on the plasma membrane (Figure 2a). Immunoprecipitation of the surface pool of TAPBPR indicated that MHC class I was associated with surface expressed TAPBPR on $TAPBPR^{WT}$ and $TAPBPR^{PM}$ transduced cells but not from those transduced with $TAPBPR^{TN5}$, a mutated TAPBPR variant which does not bind to MHC class I[15] (Figure 2b). As the amount of surface TAPBPR isolated (Figure 2b) closely correlated with surface TAPBPR expression observed using flow cytometry (Figure 2a) with barely detectable quantities isolated from cells expressing $TAPBPR^{ER}$, and

UGT1 was not detectable in the surface pull-downs (Figure 2b) we appeared to have isolated only the surface pool of TAPBPR, and not the intracellular pool. Isolation of the intracellular TAPBPR pool, from cells post-surface TAPBPR preclear, confirmed all TAPBPR variants were expressed and that $TAPBPR^{WT}$, $TAPBPR^{PM}$ and $TAPBPR^{ER}$ molecules exhibited strong associations with MHC class I (Figure 2b). In contrast to $TAPBPR^{PM}$, a significant association of UGT1 with both $TAPBPR^{WT}$ and $TAPBPR^{ER}$ was observed, supportive of the predicted subcellular localisation of the chimeric proteins (Figure 2b). When the ability of the cell lines to bind to two exogenous HLA-A*68:02 specific fluorescent peptides, ETVSK*QSNV and YVVPFVAK*V, was tested, only cells expressing surface TAPBPR exhibited significant peptide association in the presence of 10 nM exogenous peptide for 15 min at 37°C (Figure 2c & d). No fluorescent peptide binding was observed on cells expressing $TAPBPR^{ER}$ (Figure 2c & d). These results provide indication that surface TAPBPR, rather than its over-expression, is responsible for the loading of exogenous peptide onto MHC class I.

2.3 Surface expressed tapasin also enhances exogenous peptide association onto MHC class I molecules, but to a lesser extent than TAPBPR

As tapasin is also an MHC class I peptide editor, we asked whether this molecule could similarly load exogenous peptide onto MHC class I when expressed at the cell surface. In contrast to the overexpression of $TAPBPR^{WT}$, the overexpression of $tapasin^{WT}$ does not result in this protein being expressed at the cell surface (Figure 2e), most likely due to the ER retention motif found in its cytoplasmic tail [13,14]. Therefore, we replaced the cytoplasmic tail of tapasin with that of CD8 ($tapasin^{PM}$) which resulted in its expression at the cell surface (Figure 2e). When the ability of tapasin overexpressing cells to bind to ETVSK*QSNV and

YVVPFVAK*V was tested, a slight but significant increase in exogenous fluorescent peptide binding to HLA-A*68:02 was observed with cells expressing tapasin at the cell surface (Figure 2f & g). These results provide indication that surface tapasin is also capable of enhancing exogenous peptide association onto

MHC class I. However, exogenous peptide binding observed with tapasin^{PM} was ~10 times less than that observed with TAPBPR^{PM}.

2.4 Surface TAPBPR functions as peptide exchange catalyst on surface MHC class I molecules

There are two conceivable mechanisms by which surface expressed TAPBPR could promote the loading of exogenous peptide onto MHC class I; it may drag peptide-receptive MHC class I molecules with it through the secretory pathway to the cell surface and/or it may retain its ability to function as a peptide exchange catalyst in this atypical location. To explore this further, we developed an assay to determine whether surface expressed TAPBPR was capable of promoting peptide exchange on MHC class I molecules. First, cells were incubated with 10 nM fluorescently-labelled peptide for 15 min at 37°C to allow surface MHC class I molecules to bind to labelled peptides. The, after extensive washing to remove any unbound fluorescent peptide, the ability of the cells to exchange the labelled peptide was assessed by incubating the cell with various unlabelled competitor peptides for 15 min at 37°C. Using this method we observed dissociation of both YVVPKVAK*V (Figure 3a & b) and ETVSK*QSNV (Figure 3c & d) in the presence of high affinity unlabelled competitor peptide either (ETVSEQSNV or YVVPFVAKV). No dissociation of the fluorescent peptide from HLA-A*68:02 was observed on HeLa^{KO}TAPBPR^{WT} cells in the presence of
EGVAK*QSNG, which cannot bind to HLA-A*68:02 (Figure 3). Our results provide indication surface TAPBPR can promote peptide exchange on surface MHC class I molecules in a peptide affinity (YVVPFVAKV> ETVSEQSNV>EGVSEQSNQ) and peptide-concentration dependent manner (Figure 3b & d). These findings demonstrate TAPBPR still retains its ability to function as a peptide exchange catalyst when expressed on the cell surface and that it is capable of peptide exchange on surface MHC class I molecules.

2.5 Exogenous soluble TAPBPR binds to surface MHC class I molecules

As plasma-membrane bound TAPBPR functions as a peptide exchange catalyst on HLA-A*68:02, we were curious whether exogenous soluble TAPBPR added to cells was also capable of the same catalytic function, or whether it needed its membrane anchor for proper orientation. First, we tested whether exogenous TAPBPR, which consists of its N-terminally IgV and IgC domains, but lacking the transmembrane and cytoplasmic tail, could bind to surface MHC class I molecules. When HeLaM cells were incubated with 100 nM of exogenous TAPBPR^{WT} for 15 min at 37°C, TAPBPR was clearly detectable on the cell surface using the TAPBPR-specific mAb PeTe-4 (Figure 4a). The binding of TAPBPR to cells appeared to be entirely dependent on it association with MHC class I since: 1) Exogenous TAPBPR^{TN5} (a mutant that cannot bind to MHC class I[15]) did not bind to cells (Figure 4a), 2) Exogenous TAPBPR^{WT} could no longer bind HeLaM lacking classical MHC class I expression (HeLaM-HLA-ABC^{KO}) (Figure 4b) and 3) The binding of exogenous TAPBPR^{WT} to HeLaMHLA-ABC^{KO} cells was restored when HLA-A*A68:02 expression was reconstituted (Figure 4b). These results demonstrate that exogenous soluble TAPBPR can bind to HLA-A*68:02 expressed on the cell surface.

2.6 Exogenous soluble TAPBPR enhances peptide association onto surface MHC class I

We next asked whether the exogenous TAPBPR bound to surface MHC class I molecules was capable of peptide exchange similarly to the membrane anchored version. Following incubation of cells for 15 min at 37°C in the presence or absence of 100 nM exogenous TAPBPR, cells were treated with or without 10 nM fluorescent peptide for an additional 15 mins. In contrast to the extremely low levels of exogenous peptide binding observed on HeLaM cells in the absence of $TAPBPR^{WT}$ or when treated with $TAPBPR^{TN5}$, a significant enhancement in the binding of exogenous fluorescent peptides ETVSK*QSNV and YVVPKVAK*V to HeLaM was observed when cells were treated with exogenous $TAPBPR^{WT}$ (Figure 4c &d). No association of EGVAK*QSNG was observed in any of the conditions tested (Figure 4c & d). The binding of exogenous peptide to cells via TAPBPR was shown to be mediate via HLA-A*68:02 since no binding of ETVSK*QSNV (Figure 4e & f) or YVVPKVAK*V was observed to cell lacking MHC class I expression and peptide association was restored when HLA-A*68:02 expression was reconstituted (Figure 4e & f). Soluble $TAPBPR^{WT}$ enhanced exogenous peptide binding to cells over a wide range of peptide concentrations (Figure 4g). These results clearly demonstrate that exogenous TAPBPR can load peptides on HLA-A*68:02 and that the luminal domain of TAPBPR is sufficient for the position of TAPBPR onto MHC class I molecules.

To ensure the results observed were not an anomaly of HLA-A*68:02, we extended our analysis to test the ability of TAPBPR to load a range of exogenous peptides onto another human MHC class I molecule, HLA-A*02:01. Exogenous $TAPBPR^{WT}$ significantly promoted the binding of fluorescent variants of NLVPMVATV (an immunogenic peptide derived from the CMV protein $pp65^{16}$), YVVPFVAKV (derived from human CCR4-NOT transcription complex subunit 1^{8}) and YLLEMLWRL (an immunogenic peptide derived from the EBV protein Latent membrane protein 1 (LMP1)[17](Figure 4h & i). The TAPBPR promoted loading of these exogenous peptides was dependent on HLA-A2 as no fluorescent peptide binding was observed on HLA-A2 negative cells. Exogenous $TAPBPR^{WT}$ also appeared to slightly promote the binding of a fluorescent variant of CLGGLLTMV (an immunogenic peptide derived from the EBV protein Latent membrane protein 1) although not to significant levels (Figure 4h & i) but did not promote the binding of peptides specific for other MHC class I molecules onto HLA-A2 (Figure 4i). Exogenous $TAPBPR^{TN5}$, which cannot bind to MHC class I, did not promote the binding of any peptides to HLA-A2 (Figure 4i). Together, our data strongly suggests exogenous TAPBPR can promote the loading exogenous peptide onto surface MHC class I in an affinity-based manner.

TAPBPR was found to promote peptide exchange on a wide range of different HLA molecules, particularly HLA-A molecules (Figure 22), including A*68:02, A*23:01, A*02:01, A*32:01, A*03:01, A*68:01, and A*11:01.

2.7 Antigenic peptides loaded onto MHC class I via TAPBPR are available to the T cell receptor

We subsequently determined whether the peptides loaded via TAPBPR were available for T cell receptor (TCR) detection. Encouragingly, soluble TAPBPR was found to dissociate from cells upon high affinity peptide binding onto surface MHC I molecules (Fig. 17), raising the possibility that TAPBPR-loaded peptide:MHC complexes might be fully accessible for T cell receptors (TCR) detection..

To explore this, we first asked if two anti-EBV TCR-like mAbs L1 and L2, specific for $LMP1_{125-133}$ and $LMP2_{426-434}$ derived peptides presented on HLA-A* 02:01 respectively[17], could recognise YLLEMLWRL and CLGGLLTMV loaded onto surface HLA-A2 by exogenous TAPBPR. A significant increase in TCR detection of their respective peptide observed on HeLa-HLA-ABC^{KO}A2+ cells in the presence of exogenous $TAPBPR^{WT}$, compared to cells treated with peptide alone or incubated with peptide following treatment with exogenous $TAPBPR^{TN5}$ (Figure 5a-d). The ability of the TCR-like mAb to recognise peptide loaded onto HLA-A2 via TAPBPR strongly reflects the level of peptide binding (Figure 4h&i), with the largest increase in recognition observed with L1-TCR recognition of YLLEMLWRL (Figure 5a&b). Next, we tested the ability of TAPBPR loaded peptide to stimulate T cells by using FluoroSpot assays to measure IFN-γ production from a HLA-A2 restricted CD8+ T cell line specific for the immunogenic peptide NLVPMVATV derived from the cytomegalovirus (CMV) protein pp65[16] (). We observed a significant increase in the stimulation of the T cells incubated with NLVPMVATV after treatment with exogenous $TAPBPR^{WT}$, compared to cells incubated with peptide alone or to cells incubated with peptide following treatment with exogenous $TAPBPR^{TN5}$ (Figure 5e). These results not only demonstrate that peptide loaded onto MHC class I via TAPBPR is present on the cell surface, but that it is also accessible for recognition by CD8+ T cells.

2.8 TAPBPR can load antigenic peptide onto tumour cells and induce their recognition by T cells

As the ability to load immunogenic peptide onto tumour cells would prove very useful for cancer immunotherapy, we tested the ability of TAPBPR to load tumour or viral peptides onto the breast cancer cell line MCF-7.

We found that soluble $TAPBPR^{WT}$ significantly enhanced the loading of fluorescent derivatives of the tumour antigens IMDQVPFSV (derived from gp100)[28], ELAGIGILTV (from Melan-A/MART-1)[29], *LLGRNSFEV* (derived from p53)[30] and RLLQETELV (from HER-2/neu)[31] (Fig. 6a) onto HLA-A*02:01 naturally expressed on MCF-7, a breast cancer cell line. Exogenous soluble $TAPBPR^{WT}$ also enhanced the association of both YLLEK*LWRL (from EBV LMP1) and NLVPK*VATV (from CMV) onto HLA-A2 molecules expressed on the MCF-7 cells compared to those treated with peptide alone or incubated with peptide following treatment with exogenous $TAPBPR^{TN5}$ (Figure 6b).

We found that YLLEMLWRL loaded onto MCF-7 cells by TAPBPR was strongly detected by the anti-EBV TCR-like mAb L1, specific for $LMP1_{125-133}$ presented on HLA-A*02:01 (17) (Fig. 6c & 6d). Furthermore, NLVPMVATV loaded onto MCF-7 cells by soluble TAPBPR significantly increased the stimulation, measured by IFNγ secretion, of human CD8+ T cells specific for $pp65_{495–503}$ presented on HLA-A2 (16) when incubated with unlabelled peptide, compared to MCF-7 cells incubated with peptide alone or peptide with exogenous $TAPBPR^{TN5}$ (Fig. 6e). We have further verified these findings using HeLaM-HLA-ABC^{KO}-A2+ (Fig. 18). These results demonstrate that soluble TAPBPR can efficiently load antigenic peptides onto tumour cell lines for recognition by CD8+ T cells and could be used to enhance T cell responses to tumours.

2.9 Chimeric TAPBPR

TAPBPR-linker-GFP nanobody fusion products (with a range of linkers) were generated (Figure 7). These products were used to demonstrate that TAPBPR-Ab fusion products retain full functionality. TAPBPR fusion proteins with various lengths of linkers between the TAPBPR and the antibody fragment were shown to work efficiently (Figure 21).

2.10 Peptide Loading on Cervical Cancer cells

We found TAPBPR fused to the GFP-specific nanobody bound to HeLaM cells expressing GFP on their cell surface but was unable to bind or load peptides onto HeLaM cells without surface GFP i.e. bystander cells (Figure 8). At 100 nM recombinant protein, while soluble TAPBPR alone bound at low levels to HeLaM cells due to its interaction with HLA-A*68:02, the TAPBPR-GFP_{NB} fusion protein exhibited extremely high levels of binding to HeLaM cells expression surface GFP (Fig 8B & 8C). However, at 100 nM the TAPBPR-GFP_{NB} fusion protein exhibited no binding to HeLaM cells lacking GFP (Fig 8B and 8C). At 10 nM recombinant protein, while soluble TAPBPR alone did not exhibit any binding to either of the HeLaM cell lines (Fig 8C), we found that the TAPBPR-GFP_{NB} fusion protein exhibited high levels of binding, specifically to cells expressing GFP (Fig 8C). This demonstrates the majority of this TAPBPR fusion protein binding comes from the antibody tag.

While the TAPBPR-GFP nanobody fusion was unable to load exogenous peptide efficiently onto HeLaM cells in the absence of surface GFP, it was extremely efficient at loading peptides onto surface GFP positive cells (Figure 8D &8E). Even at 1 nM the TAPBPR-GFP_{NB} fusion was capable of loading exogenous peptides onto antibody-target expressing cells (Fig 8E). Soluble TAPBPR exhibited no peptide loading at using similar concentrations of protein (Fig 8E). Data shown is for TAPBPR fusion product with the long linker, but similar results were found for all three different linkers (short, long and extra-long). Together, these results suggest that the MHC I binding site on TAPBPR is masked by the antibody fragment in the non-surface bound state. However, upon the antibody fragment binding to its target, the MHC class I binding site on TAPBPR is exposed, allowing the TAPBPR to function as a peptide exchange catalyst on cell surface expressed MHC class I molecules. This provides essential proof-of-concept data that TAPBPR functionality can be directed to desired cell types expressing a specific marker

2.11 Peptide Loading on Breast Cancer cells

The effect of TAPBPR fused to the GFP nanobody on the breast cancer cell line MCF-7 was determined. MCF-7 expresses HLA-A2, a very common MHC class I molecule. The cells were made to express GFP on their cell surface (Figure 9a) and the ability of the TAPBPR-GFP nanobody fusion to bind to the cells was tested (Figure 9b). Again, this revealed the TAPBPR-GFP-nanobody fusion would bind to cells expressing GFP on their cell surface, but did not bind to GFP-negative cells (Fig 9b), demonstrating the ability to target TAPBPR to a specific cell population which expresses a particular cell surface marker. TAPBPR-GFP-nanobody fusion was subsequently tested for its ability to load an immunogenic viral peptide (NLVPMVATV peptide derived from CMV) to MCF-7 cells (Figure 9c). This demonstrated that the TAPBPR fusion product was capable of loading viral peptides on this breast cancer cell line in a targeted manner (dependent on the specificity of the attached antibody).

2.12 Chimeric proteins with tumour specificity

While the data above with the TAPBPR-GFP-nanobody provide proof-of-concept data regarding the ability to selectively target TAPBPR, we next designed a TAPBPR-antibody conjugated which would permit TAPBPR to be targeted to a marker found naturally on tumour cell lines. We therefore produced TAPBPR linked to a scFv with specificity for the tumour cell marker ErbB2 (Her2) (Figure 10).

Soluble TAPBPR linked to the anti-Her2 scFv was shown to bind to tumour cell lines in a Her2 dependent manner and functioned to load immunogenic peptides onto the cell lines (Figure 11 & 12). Very strong binding of TAPBPR-Her2-scFv was observed to cells over-expressing Her2 (Figure 11B & 11C). Furthermore, we observed increased binding of the TAPBPR-Her2-scFv to HeLaM cells which naturally express Her2 (see Figure 11A for endogenous Her2 level on HeLaM) compared to soluble TAPBPR alone (Figure 11B and 11C). Data shown is for TAPBPR fusion product with the long linker, but similar results were found this the short and extra-long linker variants.

To confirm the binding of the TAPBPR-Her2-scFv to HeLaM cells was dominantly via the scFv binding to Her2, as opposed to TAPBPR binding to MHC I, we produced a HeLaM cell line in which Her2 was knocked out (Figure 12A). Upon treatment with the recombinant TAPBPR proteins, we observed no binding at all of the TAPBPR-Her2-scFv protein to the Her2-deficient HeLaM cell (Fig 12B and 12C). In contrast, soluble TAPBPR alone was still capable of binding to the HeLaM cells lacking Her2 (Fig 12B & 12C). Together, these results demonstrate that the TAPBPR-Her2-scFv fusion binds to cells in a Her2-dependent manner and that this fusion protein is incapable of binding to cells that lack surface Her2 expression i.e bystander cells which are MHC class I positive. Therefore, the TAPBPR-Her2-scFv shows specificity for Her2, which may be useful therapeutically to target TAPBPR to Her2 positive tumour cell lines, while leaving healthy cells alone.

Next, we tested whether the TAPBPR-Her2-scFv was capable of promoting the loading of exogenous peptides onto cells in a Her2-dependent manner. Our results show that the TAPBPR-Her2-scFv fusion protein is very efficient at loading exogenous peptide onto the surface of HeLaM cells in a Her2-depedent manner (Fig 11D, 11E, 12D &12E). We found it efficiently loaded a fluorescent variant of the neo-antigen ETVSEQSNV (ETV*) onto surface expressed HLA-A*68:02 molecules in a Her2 dependent manner (Fig 11D, 11E, 12D &12E). Furthermore, we tested if the TAPBPR-Her2-scFv was capable of loading viral peptides onto another MHC class I molecule, HLA-A2. We found that the TAPBPR-Her2-scFV could load fluorescent variants of both the CMV-derived peptide NLVPMVATV (NLV) (Fig 13A & 13B) and the EBV derived peptide YLLEMLWRL (YLL) (Fig 13C &13D) onto surface expressed HLA-A2 molecules. This peptide loading was dependent expression of HLA-A2 as no peptide loading was observed on cells lacking HLA-A, -B and –C expression (Fig 13). Furthermore, the TAPBPR-Her2-scFv was more efficient at loading these peptides than soluble TAPBPR alone (Fig 13A-D).

In contrast to soluble TAPBPR, which dissociates from the cells following loading peptide onto MHC class I, it was likely that TAPBPR-antibody fusion proteins would remain bound to the surface of cells via the antibody:target interaction. Therefore, we next tested whether the T cell receptor would have access to

peptide:MHC I complexes on cells treated with TAPBPR-Her2-scFv fusion protein. This revealed that the TCR-like mAb LMP-1 could recognise YLLEMLWRL /HLA-A2 complexes on cells treated with the TAPBPR-Her2-scFv (Figure 13E). In fact, the TCR-like mAb LMP-1 bound better to cells treated with the TAPBPR-Her2-scFv fusion + peptide, that to those treated with soluble TAPBPR + peptide (Fig 13E). This likely reflect the fact that the TAPBPR-Her2-scFv is more efficient than soluble TAPBPR at loading YLL peptide onto these cells (Figure 13D).

Having shown that the TAPBPR-Her2-scFv exhibits exclusive specificity to cell expressing Her2, we explored whether the TAPBPR-Her2-scFv may be useful therapeutically to target Her2 positive tumour cell lines (Fig 14). We tested the ability of the TAPBPR-Her2-scFv to load immunogenic peptides onto two breast cancer cell lines; MCF-7 cells (which express low levels of Her2 but high MHC class I) and SKBR3 (which express high levels of Her2 but low levels of MHC class I) (Figure 14). Our results demonstrate that the TAPBPR-Her2-scFv efficiently loads antigenic peptides onto both of these breast cancer cell lines, that it is more efficient that soluble TAPBPR, and that the level of peptide loading observed is proportional to the expression of Her2 on the cell lines (Fig 14B).

Given that many tumours down-regulate MHC I expression, it is extremely promising to observe efficient peptide loading using the TAPBPR-Her2-scFV protein on the SKBR3 cell line which is known to have low MHC I expression. This implies that low level MHC class I expression may not be a barrier to being able to use TAPBPR-antibody fusion proteins therapeutically. Finally, we tested the ability of the TCR to detect peptide:MHC I complexes loaded by TAPBPR. This revealed the TCR-like specific mAb LMP-1 bound extremely well to cells treated with the TAPBPR-Her2-scFV and peptide (Fig 14C). In fact, significantly more YLL/HLA-A2 complexes were accessible for TCR binding when cells were treated with the TAPBPR-Her2-scFv + peptide, than those treated with soluble TAPBPR+ peptide or peptide alone (Fig 14C). Together this data demonstrates that the TAPBPR-Her2-scFV fusion protein is extremely efficient at loading exogenous, immunogenic peptides onto the surface of Her-2 positive tumour cell lines and that the resultant peptide:MHC I complexes are accessible to TCR binding. This data support the concept that a TAPBPR-Her2-scFv protein may have the translational potential to selectively increase the immunogenicity of Her2 positive tumours and induce the recognition of such tumours by viral specific T cells, even on tumours with low MHC class I expression.

We have also produced recombinant TAPBPR linked to a PD-L1 specific nanobody (Figure 20). This was also shown to target TAPBPR binding to tumours in a PD-L1 dependent manner (Fig 20B & 20C), and to load immunogenic peptides onto a tumour cell line (Figure 20D & 20E).

2.13 Steric hindrance using the TAPBPR-antibody fusion fragments

We have observed that the TAPBPR-antibody fusion proteins appear to be unable to bind to MHC class I, and consequently unable to mediate peptide exchange, in the absence of the ligand for the antibody. Our findings suggest that the MHC class I binding site on TAPBPR is masked by the antibody fragment (either a nanobody or scFV) in the non-plasma membrane bound state, but is subsequently exposed upon the antibody binding its ligand on the cell surface. Data supporting steric hindrance by the antibody fusion

fragment in the non-bound state can found for the TAPBPR-GFP-nanobody fusion protein in Figure 8 (on HeLa cells i.e not expressing surface GFP) and for the TAPBPR-Her2-scFv fusion protein in Figure 12 (on the HeLa cell in which Her2 has been knocked out). In both Figure 8 and 12 we observed that while soluble TAPBPR alone bound well to MHC class I expressed on HeLa/HeLa-Her2KO cells, the TAPBPR-antibody fusions were unable to bind to HLA molecules expressed on these cells (Fig 8B & C on HeLa, Fig 12B & C on HeLa-Her2KO cells). However, when the ligand for the antibody was present on the cells, the TAPBPR fusion proteins were able to bind extremely well to the cells (Fig 8B & C on HeLa+GFP, Fig 12B & C on HeLa). Similarly, when we tested the ability of the TAPBPR-antibody fusion proteins to mediate exogenous peptide loading, we observed in both Figure 8 and 12 that while soluble TAPBPR alone could efficiently mediate exogenous peptide loading on MHC class I expressed on HeLa/HeLa-Her2KO cells, the TAPBPR-antibody fusions were unable to load peptides on these cells (Fig 8B & C on HeLa, Fig 12 B & C on HeLa-Her2KO cells). Again, when the ligand for the antibody was present on the cells, the TAPBPR fusion proteins were now capable of efficiently mediating exogenous peptide loading (Fig 8B & C on HeLa+GFP, Fig 12 B & C on HeLa). This interesting observation is promising when considering the therapeutic application of TAPBPR-fusion proteins, as it suggests that such products would be highly selective for chosen target and would have limited effects on healthy cells only expressing MHC class I.

2.14 Viral peptides loaded onto tumour cells

TAPBPR-Her2-svFc was shown to load viral peptides onto HeLa cells expressing HLA-A2 (Figure 13a-d). In addition, the T cell receptor (TCR) was shown to recognise the loaded cells (Figure 13e). This shows that the TAPBPR-Her2-svFc is functional and the resultant peptide/MHC class I complexes are accessible for the T cell to bind.

2.15 Exogenous mouse TAPBPR can also be utilised to load immunogenic peptide onto human MHC class I molecules.

The luminal domains of mouse TAPBPR were shown to load fluorescent human neoantigen peptide ETVSK*QSNV (ETV*) to HLA-A68 (MHC class I) expressed on HeLa cells (Figure 15). These results demonstrate that exogenous mouse TAPBPR can also be utilised in the same way as human TAPBPR to load immunogenic peptide onto human MHC class I molecule. Although less efficient than human TAPBPR, mouse TAPBPR is still capable of loading enough immunogenic peptide that would trigger T cell responses.

2.16 Removal of cytoplasmic tail targets TAPBPR to the cell surface

Cells transduced with $TAPBPR^{WT}$, $TAPBPR^{tailless}$ which has the TMD of TAPBPR but lacks the cytoplasmic tail and $TAPBPR^{CD8tail}$ in which its cytoplasmic tail has been replaced with CD8, were shown to express TAPBPR on the cell surface (Figure 16A) and bind fluorescent peptide ETVSK*QSNV (ETV*) (Figure 16B) relative to cells not transduced with TAPBPR. The results in Figure 16 demonstrate that TAPBPR lacking any cytoplasmic tail is efficiently expressed at the cell surface (Note: Cells expressing $TAPBPR^{CD8tail}$ have a lower level of transduction but the addition of the CD8 cytoplasmic tail onto TAPBPR allows for more efficient surface expression at the lower transduction efficiency). When

TAPBPR expressing cells are gated on equivalent population (see gate on A), all TAPBPR variants are capable of a similar degree of peptide editing (B).

2.17 Soluble TAPBPR induces tumour cell killing by CD8+ T lymphocytes

Although the results above provide indication that soluble TAPBPR could be utilised to decorate target cells with immunogenic peptides and enhance T cell responses against tumours, we next determined whether this could result in enhanced killing of tumour cells. We assessed killing of murine EL4 tumour cells by OT1 T cells in the presence of human TAPBPR and very low concentrations of SIINFEKL peptide. Soluble human $TAPBPR^{WT}$ bound to EL4 cells (Fig. 19a) and significantly enhanced the loading of SIINFEKL onto H-$2K^b$ expressed on EL4 (Fig. 19b, 19c & 19d). When we tested the ability of OT1 cytotoxic T cells, which recognise SIINFEKL in the context of H-$2K^b$, to lyse peptide-pulsed EL4 target cells, we observed a significant enhancement in killing in the presence of soluble human $TAPBPR^{WT}$, but not in the presence of $TAPBPR^{TN5}$ (Fig. 19e). These results demonstrate that TAPBPR can be utilised to enhance the killing of tumours by peptide-specific CD8+ T lymphocytes.

Although TAPBPR usually functions as a peptide editor intracellular within cells, we reveal that when given access to the surface pool of MHC class I molecules, either through targeting membrane-bound TAPBPR to plasma membrane or by adding exogenous soluble TAPBPR to cells, TAPBPR retains its function as a peptide exchange catalyst. While, tapasin targeted to the plasma membrane also appeared to assist in the loading of exogenous peptide, the level of peptide binding achieved were significantly lower than TAPBPR. Therefore, this may be due to tapasin dragging a small pool of peptide-receptive molecules with it though the secretory pathway, rather than retaining its peptide-editing activity on the cell surface. A few known factors may help explain why extracellular TAPBPR is the superior peptide editor on surface MHC class I. First, unlike tapasin [18,19], TAPBPR is able to bind to MHC class I in the absence of any other co-factors or leucine zippering [8,9], a finding verified in the recent crystal structures of the TAPBPR:MHC class I complex [20,21]. Second, TAPBPR has higher affinity for MHC class I than tapasin [9]. Third, TAPBPR can also interact with MHC class I in a glycan-independent manner, thus broadening the species of MHC class I it can bind, including those on the cell surface, compared to tapasin.

By initially exploring the artefactual expression of surface expressed TAPBPR, we have developed a novel cellular based peptide-exchange assay, in addition to those already established [18,19], which may help us better define both the mechanism of peptide editing and the peptide selection criteria exerted by TAPBPR. However, the most exciting implications of our discoveries relates to the potential translational opportunities of utilising TAPBPR to load immunogenic peptides onto cells to target their recognition by the immune system.

Although exogenous peptide alone can bind to MHC class I in a passive manner, the presence of extracellular TAPBPR permits peptide loading in an active mechanism, speeding up the process so that it is almost instantaneously and permitting it to occur at very low peptide concentration. Therefore, extracellular TAPBPR has the potential to enable us to override natural peptide selection processes that occur within cells via the MHC class I antigen processing and pathway. This could be beneficial in a

number of clinical situation which is it desirable to induce T cells responses. First, targeting TAPBPR to the surface of professional antigen presenting cells, such as dendritic cells, may prove useful in combination with peptide-based vaccination strategies, to boast the pool of pathogen or tumour-specific lymphocytes in the circulation. Second, targeting TAPBPR to tumours could be utilised to boast the level of tumour antigens including neoantigens displayed directly on the tumour thus improve the recognition of tumours by the pre-existing tumour specific lymphocytes. Third, TAPBPR could be used to load immunogenic peptides derived from pathogens such as viruses onto the surface of tumours permitting pathogen-specific T cells to mediate an antitumour immune response. Fourth, the loading immunogenic peptides onto cells harbouring latent/persistent pathogens via TAPBPR may prove beneficial in infection control.

TAPBPR may also remove peptides which are the targets in CD8+ T cell immune-mediate inflammatory diseases. The ability to switch the exogenous immunogenic peptide displayed on the surface of a cell in the face of immunoediting or immune evasion may be a major advance for future of immunotherapy.

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Sequences

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SEQ ID NO: 1 –nucleotide sequence encoding TAPBPR luminal domain (mature sequence without 21 aa leader sequence)

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SEQ ID NO: 2 –TAPBPR luminal domain (mature sequence without 21 aa leader sequence)

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SEQ ID NO: 6 sTAPBPR-sPD1 (mature TAPBPR domain underlined)

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SEQ ID NO: 8 TAPBPR-Her2scFv (mature TAPBPR domain underlined)

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aaa

SEQ ID NO: 9 nucleotide sequence encoding TAPBPR-GFP-NB

MGTQEGWCLLLCLALSGAAETKPHPAEGQWRAVDVVLDCFLVKDGAHRGALASSEDRARASLVLKQVPVLDDGSLED
FTDFQGGTLAQDDPPIIFEASVDLVQIPQAEALLHADCSGKEVTCEISRYFLQMTETTVKTAAWFMANVQVSGGGPS
ISLVMKTPRVAKNEVLWHPTLNLPLSPQGTVRTAVEFQVMTQTQSLSFLLGSSASLDCGFSMAPGLDLISVEWRLQH
KGRGQLVYSWTAGQGQAVRKGATLEPAQLGMARDASLTLPGLTIQDEGTYICQITTSLYRAQQIIQLNIQASPKVRL
SLANEALLPTLICDIAGYYPLDVVVTWTREELGGSPAQVSGASFSSLRQSVAGTYSISSSLTAEPGSAGATYTCQVT
HISLEEPLGASTQVVPPERRHVGGGGSGGGGSGGGGSTSQVQLVESGGALVQPGGSLRLSCAASGFPVNRYSMRWYR
QAPGKEREWVAGMSSAGDRSSYEDSVKGRFTISRDDARNTVYLQMNSLKPEDTAVYYCNVNVGFEYWGQGTQVTVSS
K*

SEQ ID NO: 10 TAPBPR-GFP-NB (mature TAPBPR domain underlined)

atgggcacacaggagggctggtgcctgctgctctgcctggctctatctggagcagcagaaaccaagccccacccagc
agaggggcagtggcgggcagtggacgtggtcctagactgtttcctggtgaaggacggtgcgcaccgtggagctctcg
ccagcagtgaggacagggcaagggcctcccttgtgctgaagcaggtgccagtgctggacgatggctccctggaggac
ttcaccgatttccaaggggcacactggcccaagatgacccacctattatctttgaggcctcagtggacctggtcca
gattccccaggccgaggccttgctccatgctgactgcagtgggaaggaggtgacctgtgagatctcccgctactttc
tccagatgacagagaccactgttaagacagcagcttggttcatggccaacgtgcaggtctctggaggggacctagc
atctccttggtgatgaagactcccagggtcgccaagaatgaggtgctctggcacccaacgctgaacttgccactgag
cccccaggggactgtgcgaactgcagtggagttccaggtgatgacacagacccaatccctgagcttcctgctggggt
cctcagcctccttggactgtggcttctccatggcaccgggcttggacctcatcagtgtggagtggcgactgcagcac
aagggcaggggtcagttggtgtacagctggaccgcagggcaggggcaggctgtgcggaagggcgctaccctggagcc
tgcacaactgggcatggccagggatgcctccctcaccctgcccggcctcactatacaggacgaggggacctacattt
gccagatcaccacctctctgtaccgagctcagcagatcatccagctcaacatccaagcttcccctaaagtacgactg

agcttggcaaacgaagctctgctgcccaccctcatctgcgacattgctggctattaccctctggatgtggtggtgac
gtggacccgagaggagctgggtgggtccccagcccaagtctctggtgcctccttctccagcctcaggcaaagcgtgg
caggcacctacagcatctcctcctctctcaccgcagaacctggctctgcaggtgccacttacacctgccaggtcaca
cacatctctctggaggagccccttggggccagcacccaggttgtcccaccagagcggagacacgtgggtggcggtgg
ctccggtggcggtggctccggtggcggtggctccactagtgaggtgcagctggtggagagcggcggcggcctggtgc
agcccggcggcagcctgagactgagctgcgccgccagcggcttcaccctggactactacgccatcggctggttcaga
caggcccccggcaaggagagagagtgggccagcagcatcagcagcagcgacggcagcacctactacgccgacagcgt
gaagggcagattcaccatcagcagagacaacgccaagaacaccgtgttcctgcagatgaacagcctgaagcccgagg
acaccgccgtgtacagctgcgccgccagccaggcccccatcaccatcgccaccatgatgaagcccttctacgactac
tggggccagggcacccaggtgaccgtgagcagcggcggaggaggatct

SEQ ID NO: 11 nucleotide sequence encoding sTAPBPR-LONG-PD-L1-NB1

MGTQEGWCLLLCLALSGAAETKPHPAEGQWRAVDVVLDCFLVKDGAHRGALASSEDRARASLVLKQVPVLDDGSLED
FTDFQGGTLAQDDPPIIFEASVDLVQIPQAEALLHADCSGKEVTCEISRYFLQMTETTVKTAAWFMANVQVSGGGPS
ISLVMKTPRVAKNEVLWHPTLNLPLSPQGTVRTAVEFQVMTQTQSLSFLLGSSASLDCGFSMAPGLDLISVEWRLQH
KGRGQLVYSWTAGQGQAVRKGATLEPAQLGMARDASLTLPGLTIQDEGTYICQITTSLYRAQQIIQLNIQASPKVRL
SLANEALLPTLICDIAGYYPLDVVVTWTREELGGSPAQVSGASFSSLRQSVAGTYSISSSLTAEPGSAGATYTCQVT
HISLEEPLGASTQVVPPERRHVGGGGSGGGGSGGGGSTSEVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFR
QAPGKEREWASSISSSDGSTYYADSVKGRFTISRDNAKNTVFLQMNSLKPEDTAVYSCAASQAPITIATMMKPFYDY
WGQGTQVTVSSGGGGS*

SEQ ID NO: 12 sTAPBPR-LONG-PD-L1-NB1 (mature TAPBPR domain underlined)

atgggcacacaggagggctggtgcctgctgctctgcctggctctatctggagcagcagaaaccaagccccacccagc
agaggggcagtggcgggcagtggacgtggtcctagactgtttcctggtgaaggacggtgcgcaccgtggagctctcg
ccagcagtgaggacagggcaagggcctcccttgtgctgaagcaggtgccagtgctggacgatggctccctggaggac
ttcaccgatttccaaggggcacactggcccaagatgacccacctattatctttgaggcctcagtggacctggtcca
gattccccaggccgaggccttgctccatgctgactgcagtgggaaggaggtgacctgtgagatctcccgctactttc
tccagatgacagagaccactgttaagacagcagcttggttcatggccaacgtgcaggtctctggaggggacctagc
atctccttggtgatgaagactcccagggtcgccaagaatgaggtgctctggcacccaacgctgaacttgccactgag
cccccaggggactgtgcgaactgcagtggagttccaggtgatgacacagacccaatccctgagcttcctgctggggt
cctcagcctccttggactgtggcttctccatggcaccgggcttggacctcatcagtgtggagtggcgactgcagcac
aagggcaggggtcagttggtgtacagctggaccgcagggcaggggcaggctgtgcggaagggcgctaccctggagcc
tgcacaactgggcatggccagggatgcctccctcaccctgcccggcctcactatacaggacgaggggacctacattt
gccagatcaccacctctctgtaccgagctcagcagatcatccagctcaacatccaagcttcccctaaagtacgactg
agcttggcaaacgaagctctgctgcccaccctcatctgcgacattgctggctattaccctctggatgtggtggtgac
gtggacccgagaggagctgggtgggtccccagcccaagtctctggtgcctccttctccagcctcaggcaaagcgtgg
caggcacctacagcatctcctcctctctcaccgcagaacctggctctgcaggtgccacttacacctgccaggtcaca
cacatctctctggaggagccccttggggccagcacccaggttgtcccaccagagcggagacacgtgggtggcggtgg
ctccggtggcggtggctccggtggcggtggctccactagtgaggtgcagctggtggagagcggcggcggcctggtgc
agcccggcggcagcctgagactgagctgcgccgccagcggcttcaccctggactactacgccaagtgctggttcaga
caggcccccggcaaggagagagagtgggtgagctgcatcagcagcagcgacggcagcacctactacgccgacagcgt
gaagggcagattcaccatcagcagagacaacgccaagaacaccgtgtacctgcagatgaacagcctgaagcccgagg
acaccgccgtgtacttctgcgccgccagacacggcggccccctgaccgtggagtacttcttcgactactggggccag
ggcacccaggtgaccgtgagcagcggcggaggaggatct

SEQ ID NO: 13 nucleotide sequence encoding sTAPBPR-LONG-PD-L1-NB2

MGTQEGWCLLLCLALSGAAETKPHPAEGQWRAVDVVLDCFLVKDGAHRGALASSEDRARASLVLKQVPVLDDGSLED
FTDFQGGTLAQDDPPIIFEASVDLVQIPQAEALLHADCSGKEVTCEISRYFLQMTETTVKTAAWFMANVQVSGGGPS
ISLVMKTPRVAKNEVLWHPTLNLPLSPQGTVRTAVEFQVMTQTQSLSFLLGSSASLDCGFSMAPGLDLISVEWRLQH
KGRGQLVYSWTAGQGQAVRKGATLEPAQLGMARDASLTLPGLTIQDEGTYICQITTSLYRAQQIIQLNIQASPKVRL
SLANEALLPTLICDIAGYYPLDVVVTWTREELGGSPAQVSGASFSSLRQSVAGTYSISSSLTAEPGSAGATYTCQVT
HISLEEPLGASTQVVPPERRHVGGGGSGGGGSGGGGSTSEVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAKCWFR
QAPGKEREWVSCISSSDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYFCAARHGGPLTVEYFFDYWGQ
GTQVTVSSGGGGS*

SEQ ID NO: 14 sTAPBPR-LONG-PD-L1-NB2 (mature TAPBPR domain underlined)

atgggcacacaggagggctggtgcctgctgctctgcctggctctatctggagcagcagaaaccaagccccacccagc
agaggggcagtggcgggcagtggacgtggtcctagactgtttcctggtgaaggacggtgcgcaccgtggagctctcg
ccagcagtgaggacagggcaagggcctcccttgtgctgaagcaggtgccagtgctggacgatggctccctggaggac
ttcaccgatttccaaggggcacactggcccaagatgacccacctattatctttgaggcctcagtggacctggtcca
gattccccaggccgaggccttgctccatgctgactgcagtgggaaggaggtgacctgtgagatctcccgctactttc
tccagatgacagagaccactgttaagacagcagcttggttcatggccaacgtgcaggtctctggaggggacctagc

atctccttggtgatgaagactcccagggtcgccaagaatgaggtgctctggcacccaacgctgaacttgccactgag
cccccaggggactgtgcgaactgcagtggagttccaggtgatgacacagacccaatccctgagcttcctgctggggt
cctcagcctccttggactgtggcttctccatggcaccgggcttggacctcatcagtgtggagtggcgactgcagcac
aagggcaggggtcagttggtgtacagctggaccgcagggcaggggcaggctgtgcggaagggcgctaccctggagcc
tgcacaactgggcatggccagggatgcctccctcaccctgcccggcctcactatacaggacgaggggacctacattt
gccagatcaccacctctctgtaccgagctcagcagatcatccagctcaacatccaagcttcccctaaagtacgactg
agcttggcaaacgaagctctgctgcccaccctcatctgcgacattgctggctattaccctctggatgtggtggtgac
gtggacccgagaggagctgggtgggtccccagcccaagtctctggtgcctccttctccagcctcaggcaaagcgtgg
caggcacctacagcatctcctcctctctcaccgcagaacctggctctgcaggtgccacttacacctgccaggtcaca
cacatctctctggaggagccccttggggccagcacccaggttgtcccaccagagcggagacacgtgggtggcggtgg
ctccggtggcggtggctccggtggcggtggctccactagtgaggtgcagctggtggagagcggcggcggcctggtgc
aggccggcggcagcctgagactgagctgcgccgccagcggcagcaccttcagccagtacgacgtgggctggtacaga
caggcccccggcaagcagagagagctggtggccttcagcagcagcggcggcagaaccatctaccccgacagcgtgaa
gggcagattcaccttcagcagagacaacaccaagaacaccgtgtacctgcagatgaccagcctgaagcccgaggaca
ccgccgtgtactactgcaagatcgactggtacctgaacagctactggggccagggcacccaggtgaccgtgagcagc
ggcggaggaggatct

SEQ ID NO: 15 nucleotide sequence encoding sTAPBPR-LONG-PD-L1-NB4

MGTQEGWCLLLCLALSGAAETKPHPAEGQWRAVDVVLDCFLVKDGAHRGALASSEDRARASLVLKQVPVLDDGSLED
FTDFQGGTLAQDDPPIIFEASVDLVQIPQAEALLHADCSGKEVTCEISRYFLQMTETTVKTAAWFMANVQVSGGGPS
ISLVMKTPRVAKNEVLWHPTLNLPLSPQGTVRTAVEFQVMTQTQSLSFLLGSSASLDCGFSMAPGLDLISVEWRLQH
KGRGQLVYSWTAGQGQAVRKGATLEPAQLGMARDASLTLPGLTIQDEGTYICQITTSLYRAQQIIQLNIQASPKVRL
SLANEALLPTLICDIAGYYPLDVVVTWTREELGGSPAQVSGASFSSLRQSVAGTYSISSSLTAEPGSAGATYTCQVT
HISLEEPLGASTQVVPPERRHVGGGGSGGGGSGGGGSTSEVQLVESGGGLVQAGGSLRLSCAASGSTFSQYDVGWYR
QAPGKQRELVAFSSSGGRTIYPDSVKGRFTFSRDNTKNTVYLQMTSLKPEDTAVYYCKIDWYLNSYWGQGTQVTVSS
GGGGS*

SEQ ID NO: 16 sTAPBPR-LONG-PD-L1-NB4 (mature TAPBPR domain underlined)

atgggcacacaggagggctggtgcctgctgctctgcctggctctatctggagcagcagaaaccaagccccacccagc
agaggggcagtggcgggcagtggacgtggtcctagactgtttcctggtgaaggacggtgcgcaccgtggagctctcg
ccagcagtgaggacagggcaagggcctcccttgtgctgaagcaggtgccagtgctggacgatggctccctggaggac
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tccagatgacagagaccactgttaagacagcagcttggttcatggccaacgtgcaggtctctggaggggacctagc
atctccttggtgatgaagactcccagggtcgccaagaatgaggtgctctggcacccaacgctgaacttgccactgag
cccccaggggactgtgcgaactgcagtggagttccaggtgatgacacagacccaatccctgagcttcctgctggggt
cctcagcctccttggactgtggcttctccatggcaccgggcttggacctcatcagtgtggagtggcgactgcagcac
aagggcaggggtcagttggtgtacagctggaccgcagggcaggggcaggctgtgcggaagggcgctaccctggagcc
tgcacaactgggcatggccagggatgcctccctcaccctgcccggcctcactatacaggacgaggggacctacattt
gccagatcaccacctctctgtaccgagctcagcagatcatccagctcaacatccaagcttcccctaaagtacgactg
agcttggcaaacgaagctctgctgcccaccctcatctgcgacattgctggctattaccctctggatgtggtggtgac
gtggacccgagaggagctgggtgggtccccagcccaagtctctggtgcctccttctccagcctcaggcaaagcgtgg
caggcacctacagcatctcctcctctctcaccgcagaacctggctctgcaggtgccacttacacctgccaggtcaca
cacatctctctggaggagccccttggggccagcacccaggttgtcccaccagagcggagaacagccttgggagtcat
ctttgccagcagtctcttccttcttgcactgatgttcctggggcttcagagacggcaagcacctacaggacttgggc
tgcttcaggctgaacgctgggagaccacttcctgtgctgacacacagagctcccatctccatgaagaccgcacagcg
cgtgtaagccagcccagctga

SEQ ID NO: 17 nucleotide sequence encoding hTAPBPR full length (with TMD and cytoplasmic tail) (V41_G151_V169 polymorphisms as used in our studies)

MGTQEGWCLLLCLALSGAAETKPHPAEGQWRAVDVVLDCFLVKDGAHRGALASSEDRARASLVLKQVPVLDDGSLED
FTDFQGGTLAQDDPPIIFEASVDLVQIPQAEALLHADCSGKEVTCEISRYFLQMTETTVKTAAWFMANVQVSGGGPS
ISLVMKTPRVAKNEVLWHPTLNLPLSPQGTVRTAVEFQVMTQTQSLSFLLGSSASLDCGFSMAPGLDLISVEWRLQH
KGRGQLVYSWTAGQGQAVRKGATLEPAQLGMARDASLTLPGLTIQDEGTYICQITTSLYRAQQIIQLNIQASPKVRL
SLANEALLPTLICDIAGYYPLDVVVTWTREELGGSPAQVSGASFSSLRQSVAGTYSISSSLTAEPGSAGATYTCQVT
HISLEEPLGASTQVVPPERR*TALGVIFASSLFLLALMFLGL*QRRQAPTGLGLLQAERWETTSCADTQSSHLHEDRTA
RVSQPS**

SEQ ID NO: 18 hTAPBPR full length (with TMD and cytoplasmic tail) (V41_G151_V169 polymorphisms as used herein) (mature TAPBPR domain full underline; TMD italics; cytoplasmic tail dotted underline)

atgggcttggagcccagctggtatctgctgctctgtttggctgtctctggggcagcagggactgaccctcccacagc
gcccaccacagcagaaagacagcggcagcccacggacatcatcttagactgcttcttggtgacagaagacaggcacc
gcggggcttttgccagcagtggggacagggagagggccttgcttgtgctgaagcaggtaccagtgctggatgatggc
tccctggaaggcatcacagatttccaggggagcactgagaccaaacaggattcacctgttatctttgaggcctcagt
ggacttggtacagattccccaggcagaggcgttgctccatgctgactgcagcgggaaggcagtgacctgcgagatct
ccaagtatttcctccaggccagacaagaggccacttttgagaaagcacattggttcatcagcaacatgcaggtttct
agaggtggccccagtgtctccatggtgatgaagactctaagagatgctgaagttggagctgtccggcaccctacact
gaacctacctctgagtgcccagggcacagtgaagactcaagtggagttccaggtgacatcagagacccaaaccctga
accacctgctggggtcctctgtctccctgcactgcagtttctccatggcaccagacctggacctcactggcgtggag
tggcggctgcagcataaaggcagcggccagctggtgtacagctggaagacagggcaggggcaggccaagcgcaaggg
cgctacactggagcctgaggagctactcagggctggaaacgcctctctcaccttacccaacctcactctaaaggatg
aggggacctacatctgccagatctccacctctctgtatcaagctcaacagatcatgccacttaacatcctggctccc
cccaaagtacaactgcacttggcaaacaaggaccctctgccttccctcgtctgcagcattgccggctactatcctct
ggatgtgggagtgacgtggattcgagaggagctgggtggaattccagcccaagtctctggtgcctccttctccagcc
tcaggcagagcacgatgggaacctacagcatttcttccacggtgatggctgacccaggccccacaggtgccacttat
acctgccaagtcgcccacgtctccctggaggagccccttacaaccagcatgagggttttgccaaatccagagcagag
aggaaccttgggagtcatctttgccagcatcatcttcctttctgcgctgttgttgtttctgggacttcacagacagc
aagcttcttcgtcaaggtccaccaggcctatgaggcattctgggtga

SEQ ID NO: 19 nucleotide sequence encoding mouse TAPBPR full length

MGLEPSWYLLLCLAVSGAAGTDPPTAPTTAERQRQPTDIILDCFLVTEDRHRGAFASSGDRERALLVLKQVPVLDDG
SLEGITDFQGSTETKQDSPVIFEASVDLVQIPQAEALLHADCSGKAVTCEISKYFLQARQEATFEKAHWFISNMQVS
RGGPSVSMVMKTLRDAEVGAVRHPTLNLPLSAQGTVKTQVEFQVTSETQTLNHLLGSSVSLHCSFSMAPDLDLTGVE
WRLQHKGSGQLVYSWKTGQGQAKRKGATLEPEELLRAGNASLTLPNLTLKDEGTYICQISTSLYQAQQIMPLNILAP
PKVQLHLANKDPLPSLVCSIAGYYPLDVGVTWIREELGGIPAQVSGASFSSLRQSTMGTYSISSTVMADPGPTGATY
TCQVAHVSLEEPLTTSMRVLPNPEQRGTLGVIFASIIFLSALLLFLGLHRQQASSSRSTRPMRHSG*

SEQ ID NO: 20 mouse TAPBPR full length

gaccctcccacagcgcccaccacagcagaaagacagcggcagcccacggacatcatcttagactgcttcttggtgac
agaagacaggcaccgcggggcttttgccagcagtggggacagggagagggccttgcttgtgctgaagcaggtaccag
tgctggatgatggctccctggaaggcatcacagatttccaggggagcactgagaccaaacaggattcacctgttatc
tttgaggcctcagtggacttggtacagattccccaggcagaggcgttgctccatgctgactgcagcgggaaggcagt
gacctgcgagatctccaagtatttcctccaggccagacaagaggccacttttgagaaagcacattggttcatcagca
acatgcaggtttctagaggtggccccagtgtctccatggtgatgaagactctaagagatgctgaagttggagctgtc
cggcaccctacactgaacctacctctgagtgcccagggcacagtgaagactcaagtggagttccaggtgacatcaga
gacccaaaccctgaaccacctgctggggtcctctgtctccctgcactgcagtttctccatggcaccagacctggacc
tcactggcgtggagtggcggctgcagcataaaggcagcggccagctggtgtacagctggaagacagggcaggggcag
gccaagcgcaagggcgctacactggagcctgaggagctactcagggctggaaacgcctctctcaccttacccaacct
cactctaaaggatgaggggacctacatctgccagatctccacctctctgtatcaagctcaacagatcatgccactta
acatcctggctccccccaaagtacaactgcacttggcaaacaaggaccctctgccttccctcgtctgcagcattgcc
ggctactatcctctggatgtgggagtgacgtggattcgagaggagctgggtggaattccagcccaagtctctggtgc
ctccttctccagcctcaggcagagcacgatgggaacctacagcatttcttccacggtgatggctgacccaggcccca
caggtgccacttatacctgccaagtcgcccacgtctccctggaggagccccttacaaccagcatgagggttttgcca
aatccagagcagagaggaacc

SEQ ID NO: 21 nucleotide sequence encoding luminal domains of mouse TAPBPR

DPPTAPTTAERQRQPTDIILDCFLVTEDRHRGAFASSGDRERALLVLKQVPVLDDGSLEGITDFQGSTETKQDSPVI
FEASVDLVQIPQAEALLHADCSGKAVTCEISKYFLQARQEATFEKAHWFISNMQVSRGGPSVSMVMKTLRDAEVGAV
RHPTLNLPLSAQGTVKTQVEFQVTSETQTLNHLLGSSVSLHCSFSMAPDLDLTGVEWRLQHKGSGQLVYSWKTGQGQ
AKRKGATLEPEELLRAGNASLTLPNLTLKDEGTYICQISTSLYQAQQIMPLNILAPPKVQLHLANKDPLPSLVCSIA
GYYPLDVGVTWIREELGGIPAQVSGASFSSLRQSTMGTYSISSTVMADPGPTGATYTCQVAHVSLEEPLTTSMRVLP
NPEQRGT*

SEQ ID NO: 22 luminal domains of mouse TAPBPR

ADLGSRAMAQVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIAWVRQMPGKGLEYMGLIYPGDSDTKYSPSFQGQV
TISVDKSVSTAYLQWSSLKPSDSAVYFCARHDVGYCSSSNCAKWPEYFQHWGQGTLVTVSSGGGGSGGGGSGGGGSQ
SVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDHTNRPAGVPDRFSGSKSGTSASLAISG
FRSEDEADYYCASWDYTLSGWVFGGGTKLTVLGAAA

SEQ ID NO: 23 Anti-HER2 svFc

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISS
LQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL
QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
SEQ ID NO: 24 Anti-HER2 (trastuzumab) Light chain

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKN
TAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPPKSCDKTHTCP
PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 25 Anti-HER2 (trastuzumab) Heavy chain

EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKEREWASSISSSDGSTYYADSVKGRFTISRDNAKN
TVFLQMNSLKPEDTAVYSCAASQAPITIATMMKPFYDYWGQGTQVTVSS
SEQ ID NO: 26 Anti-PDL1 nanobody

EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAKCWFRQAPGKEREWVSCISSSDGSTYYADSVKGRFTISRDNAKN
TVYLQMNSLKPEDTAVYFCAARHGGPLTVEYFFDYWGQGTQVTVSS
SEQ ID NO: 27 Anti-PDL1 nanobody

EVQLVESGGGLVQPGGSLRLSCAASGFTFDYYAIGWFRQAPGKAREGVSCISGGDNSTYYADSVKGRFTISRDNAKN
TVYLQMNSLKPEDTAVYYCATGGWKYCSGYDPEYIYWGQGTQVTVSS
SEQ ID NO: 28 Anti-PDL1 nanobody

EVQLVESGGGLVQAGGSLRLSCAASGSTFSQYDVGWYRQAPGKQRELVAFSSSGGRTIYPDSVKGRFTFSRDNTKNT
VYLQMTSLKPEDTAVYYCKIDWYLNSYWGQGTQVTVSS
SEQ ID NO: 29 Anti-PDL1 nanobody

EVQLVESGGGLVQAGGSLRLSCAASGVDASNSAMGWYRQAPGKQREWVARITGGGLIAYTDSVKGRFTISRDNAKST
VYLQMWSLEPEDTAVYYCNTINSRDGWGQGTQVTVSS
SEQ ID NO: 30 Anti-PDL1 nanobody

EVQLVESGGGLVQAGGSLTISCAASGITFSDSIVSWYRRARGKQREWVAGISNGGTTKYAESVLGRFTISRDNAKNM
VYLQMWGLNPEDTAVYLCKVRQYWGQGTQVTVSS
SEQ ID NO: 31 Anti-PDL1 nanobody

TALGVIFASSLFLLALMFLGL
SEQ ID NO: 32 Human TAPBPR TMD

atgggcacacaggagggctggtgcctgctgctctgcctggctctatctggagcagcagaaaccaagccccacccagc
agaggggcagtggcgggcagtggacgtggtcctagactgtttcctggtgaaggacggtgcgcaccgtggagctctcg
ccagcagtgaggacagggcaagggcctcccttgtgctgaagcaggtgccagtgctggacgatggctccctggaggac
ttcaccgatttccaaggggcacactggcccaagatgacccacctattatctttgaggcctcagtggacctggtcca
gattccccaggccgaggccttgctccatgctgactgcagtgggaaggaggtgacctgtgagatctcccgctactttc
tccagatgacagagaccactgttaagacagcagcttggttcatggccaacgtgcaggtctctggaggggacctagc
atctccttggtgatgaagactcccagggtcgccaagaatgaggtgctctggcacccaacgctgaacttgccactgag
cccccaggggactgtgcgaactgcagtggagttccaggtgatgacacagacccaatccctgagcttcctgctggggt
cctcagcctccttggactgtggcttctccatggcaccgggcttggacctcatcagtgtggagtggcgactgcagcac
aagggcaggggtcagttggtgtacagctggaccgcagggcaggggcaggctgtgcggaagggcgctaccctggagcc
tgcacaactgggcatggccagggatgcctccctcaccctgcccggcctcactatacaggacgaggggacctacattt
gccagatcaccacctctctgtaccgagctcagcagatcatccagctcaacatccaagcttcccctaaagtacgactg
agcttggcaaacgaagctctgctgcccaccctcatctgcgacattgctggctattaccctctggatgtggtggtgac
gtggacccgagaggagctgggtgggtccccagcccaagtctctggtgcctccttctccagcctcaggcaaagcgtgg
caggcacctacagcatctcctcctctctcaccgcagaacctggctctgcaggtgccacttacacctgccaggtcaca
cacatctctctggaggagccccttggggccagcacccaggttgtcccaccagagcggagaacagccttgggagtcat
ctttgccagcagtctcttccttcttgcactgatgttcctggggcttcagagacgaagacgtgtttgcaaatgtcccc
ggcctgtggtcaaatcgggagacaagcccagcctttcggcgagatacgtctaa
SEQ ID NO: 33 nucleotide sequence encoding hTAPBPR-CD8 cytoplasmic tail

MGTQEGWCLLLCLALSGAAETKPHPAEGQWRAVDVVLDCFLVKDGAHRGALASSEDRARASLVLKQVPVLDDGSLED
FTDFQGGTLAQDDPPIIFEASVDLVQIPQAEALLHADCSGKEVTCEISRYFLQMTETTVKTAAWFMANVQVSGGGPS
ISLVMKTPRVAKNEVLWHPTLNLPLSPQGTVRTAVEFQVMTQTQSLSFLLGSSASLDCGFSMAPGLDLISVEWRLQH
KGRGQLVYSWTAGQGQAVRKGATLEPAQLGMARDASLTLPGLTIQDEGTYICQITTSLYRAQQIIQLNIQASPKVRL
SLANEALLPTLICDIAGYYPLDVVVTWTREELGGSPAQVSGASFSSLRQSVAGTYSISSSLTAEPGSAGATYTCQVT
HISLEEPLGASTQVVPPERRTALGVIFASSLFLLALMFLGLQRRRRVCKCPRPVVKSGDKPSLSARYV*

SEQ ID NO: 34 TAPBPR-CD8 cytoplasmic tail

The claims defining the invention are as follows:

1. An isolated peptide-exchange protein consisting of;

a fragment of TAP-binding protein-related (TAPBPR), said fragment consisting of a TAPBPR luminal domain and

a targeting domain that specifically binds to the surface of target cells,

wherein the targeting domain is an antibody molecule comprising an antibody antigen-binding domain, and the TAPBPR luminal domain consists of an amino acid sequence having at least 70% sequence identity over its full length to the full length sequence of SEQ ID NO: 2 or SEQ ID NO: 22.

2. An isolated peptide-exchange protein according to claim 1 wherein the targeting domain is linked to the TAPBPR fragment via a linker.

3. An isolated peptide-exchange protein according to claim 1 or claim 2 wherein the target cells are cancer cells.

4. An isolated peptide-exchange protein according to claim 3 wherein the targeting domain specifically binds to a target molecule on the surface of the cancer cells.

5. An isolated peptide-exchange protein according to claim 4 wherein the target molecule is ErbB2, PDL1 or CD20.

6. An isolated peptide-exchange protein according to claim 1 or claim 2 wherein the target cells are pathogen infected cells.

7. An isolated peptide-exchange protein according to claim 6 wherein the pathogen infected cells are cells infected with HIV, CMV, EBV, HPV, Influenza or hepatitis.

8. An isolated peptide-exchange protein according to claim 6 or claim 7 wherein the targeting domain specifically binds to a pathogen antigen or a molecule upregulated by pathogen infection on the surface of the infected cells.

9. An isolated peptide-exchange protein according to claim 8 wherein the pathogen antigen is an HIV antigen selected from gp120 and gp41; a CMV antigen selected from UL11, UL142, UL9, UL1, UL5, UL16, UL55, UL74, UL75 and UL155 (gL) or an influenza antigen selected from hemagglutinin and neuramidase.

10. An isolated peptide-exchange protein according to any one of claims 1 to 9 wherein the target cells are antigen presenting cells, optionally dendritic cells.

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11. An isolated peptide-exchange protein according to any one of claims 1 to 10 wherein the targeting domain is an scFv or nanobody.

12. An isolated peptide-exchange protein according to any one of claims 1 to 11 comprising an amino acid sequence having at least 70% sequence identity over its full length to the full length sequence of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16.

13. A peptide-exchange protein comprising;

(i) a fragment of TAP-binding protein-related (TAPBPR), said fragment comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22, a TAPBPR transmembrane domain and a heterologous cell surface targeting sequence; or

(ii) a fragment of TAP-binding protein-related (TAPBPR) comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22 and a heterologous transmembrane domain, wherein the heterologous transmembrane domain localises the peptide-exchange protein to the plasma membrane; or

(iii) a fragment of TAP-binding protein-related (TAPBPR) comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22, a heterologous transmembrane domain, and a heterologous cell surface targeting sequence; or

(iv) a fragment of TAP-binding protein-related (TAPBPR), said fragment consisting of a TAPBPR transmembrane domain and a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22,

wherein the TAPBPR transmembrane domain localises the peptide-exchange protein to the plasma membrane.

14. An isolated peptide-exchange protein according to claim 13 wherein the heterologous cell surface targeting sequence comprises the cytoplasmic domain of CD8.

15. An isolated peptide-exchange protein according to claim 14 comprising an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 34,

16. A nucleic acid encoding an isolated peptide-exchange protein according to any one of claims 1 to 15.

17. A vector comprising a nucleic acid according to claim 16.

18. A mammalian cell comprising a nucleic acid according to claim 16 or a vector according to claim 17.

19. A mammalian cell comprising a peptide-exchange protein according to any one of claims 13 to 15 at its surface.

20. A method of increasing the immunogenicity of mammalian cells comprising;

providing a population of mammalian cells having surface MHC class I molecules, and

contacting the population of mammalian cells with an immunogenic peptide and either

(a) a peptide exchange protein comprising a fragment of TAP-binding protein-related (TAPBPR), said fragment consisting of a TAPBPR luminal domain having an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22; or

(b) a mammalian cell comprising a peptide exchange protein at its surface, the peptide exchange protein comprising;

(i) a fragment of TAP-binding protein-related (TAPBPR), said fragment comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22 and a TAPBPR transmembrane domain; or

(ii) a fragment of TAP-binding protein-related (TAPBPR), said fragment comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22 and a heterologous transmembrane domain;

such that the peptide exchange protein loads the immunogenic peptide onto the surface MHC class I molecules of the cells in the population, thereby increasing the immunogenicity of the mammalian cells.

21. A method of producing antigen presenting cells to stimulate an immune response in an individual comprising;

providing a population of antigen presenting cells previously obtained from the individual, and

contacting the antigen presenting cells *in vitro* with an immunogenic peptide and either

(a) a peptide exchange protein comprising a fragment of TAP-binding protein-related (TAPBPR), said fragment consisting of a TAPBPR luminal domain having an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22; or

(b) a mammalian cell comprising a peptide exchange protein at its surface, the peptide exchange protein comprising;

(i) a fragment of TAP-binding protein-related (TAPBPR), said fragment comprising a TAPBPR luminal domain consisting of an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22 and a TAPBPR transmembrane domain; or

(ii) a fragment of TAP-binding protein-related (TAPBPR), said fragment comprising a TAPBPR luminal domain consisting of an amino acid sequence having at

least 70% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 22 and a heterologous transmembrane domain;

such that the peptide exchange protein loads the immunogenic peptide onto surface MHC class I molecules of the antigen presenting cells.

22. A method according to claim 21 wherein the antigen presenting cells are dendritic cells, optionally wherein the method further comprises activating a population of T cells with the antigen presenting cells.

23. A method according to any one of claims 20 to 22 further comprising administering the mammalian cells, antigen presenting cells or activated T cells to an individual.

24. A method of increasing the immunogenicity of target cells in an individual comprising;

administering a peptide exchange protein according to any one of claims 1 to 12 to the individual, wherein the peptide exchange protein comprises a targeting domain that binds to target cells in the individual, and

administering an immunogenic peptide to the individual, such that the peptide exchange protein loads the immunogenic peptide onto surface MHC class I molecules of the target cells,

thereby increasing the immunogenicity of said target cells

25. A method according to claim 24 wherein the target cells are disease cells, optionally cancer cells or pathogen infected cells.

26. A method of stimulating an immune response in an individual comprising;

administering a peptide exchange protein according to any one of claims 1 to 12 to the individual, wherein the targeting domain of the peptide exchange protein binds to antigen presenting cells in the individual, and

administering an immunogenic peptide to the individual, such that the peptide exchange protein loads the immunogenic peptide onto surface MHC class I molecules of the antigen presenting cells,

such that said antigen presenting cells stimulate an immune response in the individual.

27. A method according to claim 26 wherein the antigen presenting cells are dendritic cells.

28. A method according to any one of claims 20-27 wherein the immunogenic peptide is a vaccine.

29. Use of a peptide exchange protein according to any one of claims 1 to 12 in the manufacture of a medicament for increasing the immunogenicity of target cells in an individual according to claim 24 or stimulating an immune response in an individual according to claim 26.

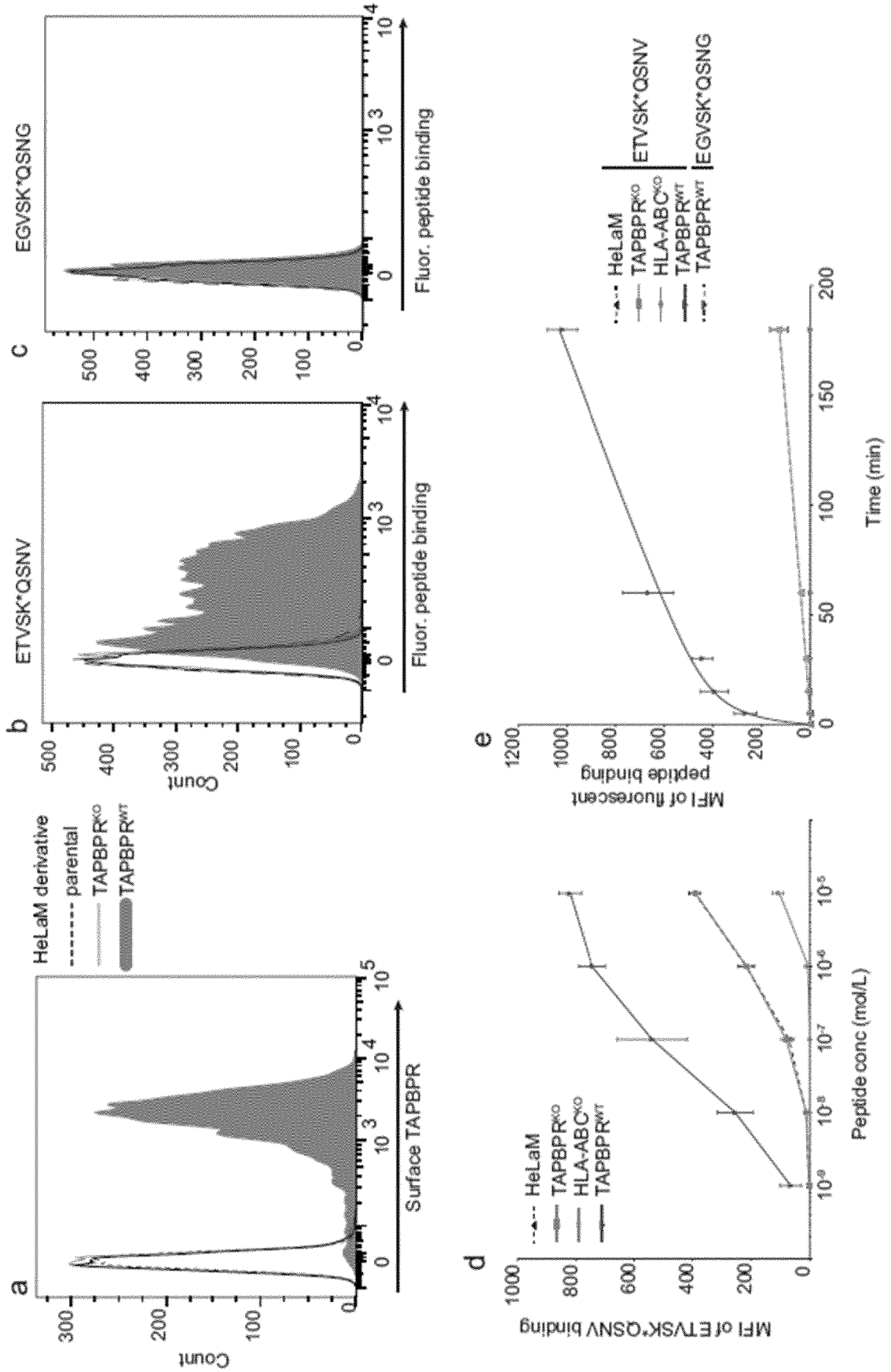

a
b
c
d
e
HeLaM derivative
parental
TAPBPR^KO
TAPBPR^WT
Count
Surface TAPBPR
ETVSK*QSNV
EGVSK*QSNG
Fluor. peptide binding
HeLaM
HLA-ABC^KO
MFI of ETVSK*QSNV binding
Peptide conc (mol/L)
MFI of fluorescent peptide binding
Time (min)

Figure 1

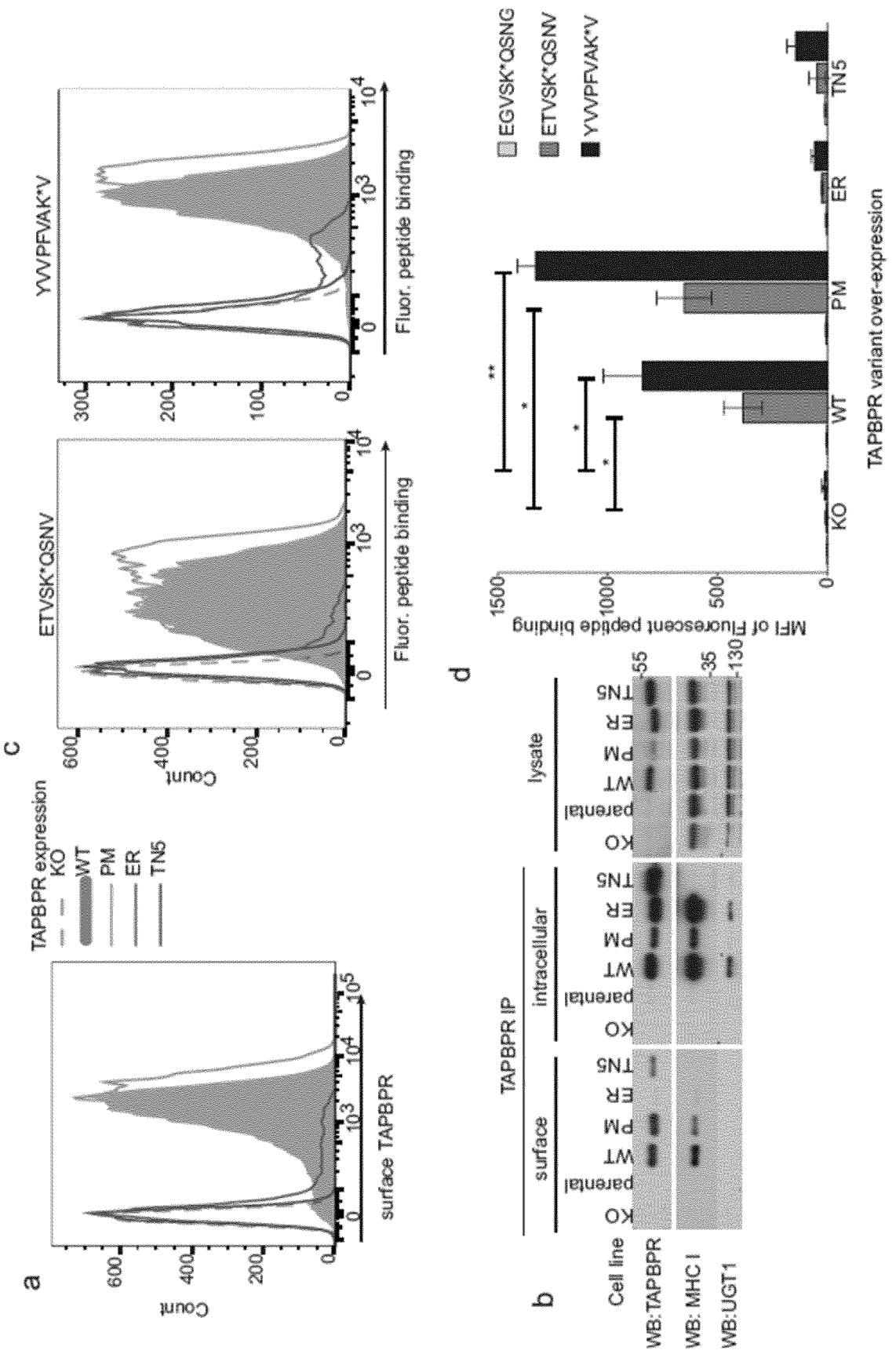

a
Count
surface TAPBPR
TAPBPR expression
KO
WT
PM
ER
TN5
c
ETVSK*QSNV
YVVPFVAK*V
Count
Fluor. peptide binding
d
EGVSK*QSNG
ETVSK*QSNV
YVVPFVAK*V
MFI of Fluorescent peptide binding
TAPBPR variant over-expression
KO
WT
PM
ER
TN5
b
TAPBPR IP
surface
intracellular
lysate
Cell line
parental
KO
WT
PM
ER
TN5
WB:TAPBPR
WB: MHC I
WB:UGT1
55
35
130

Figure 2

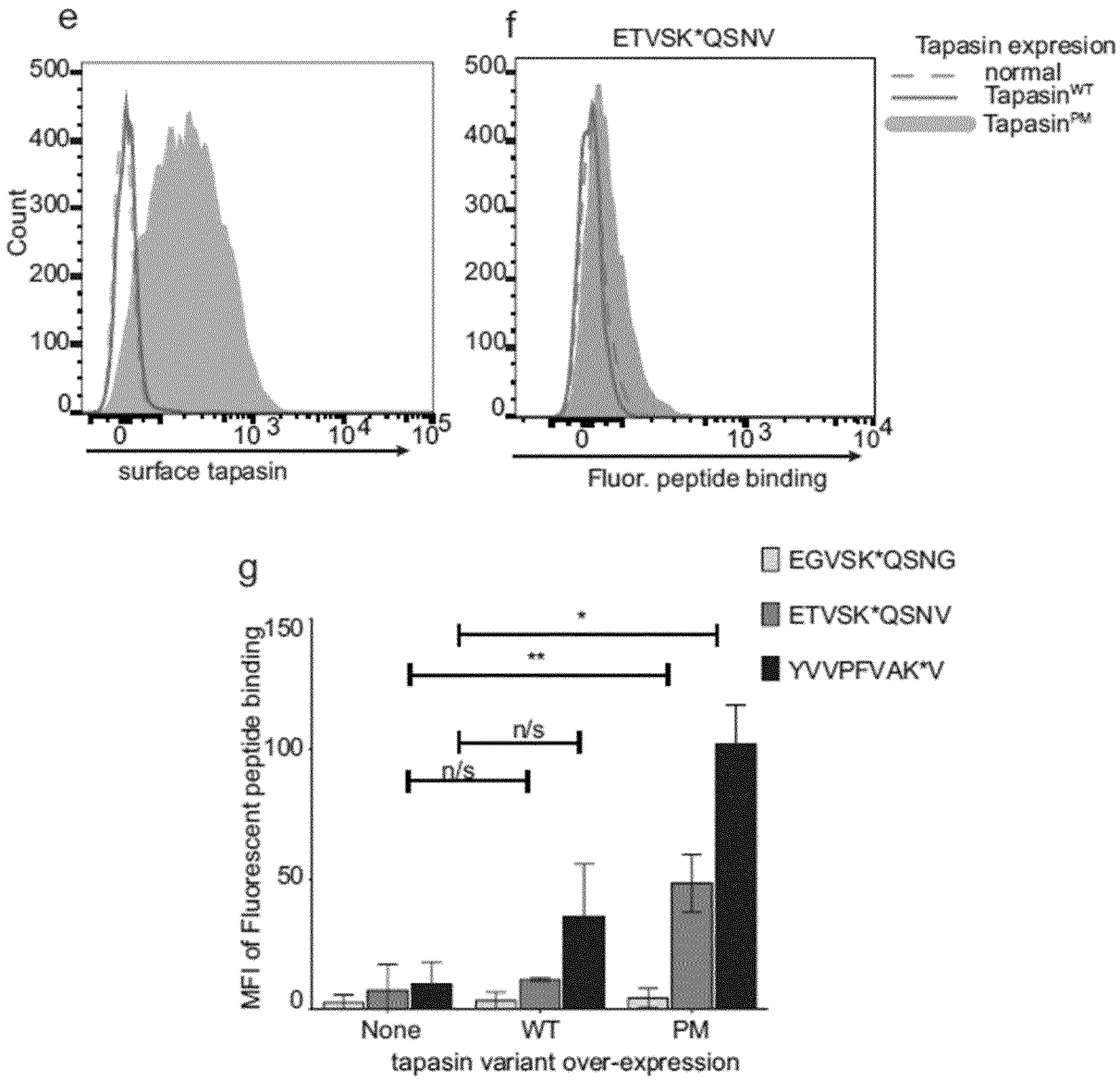


Figure 2 continued

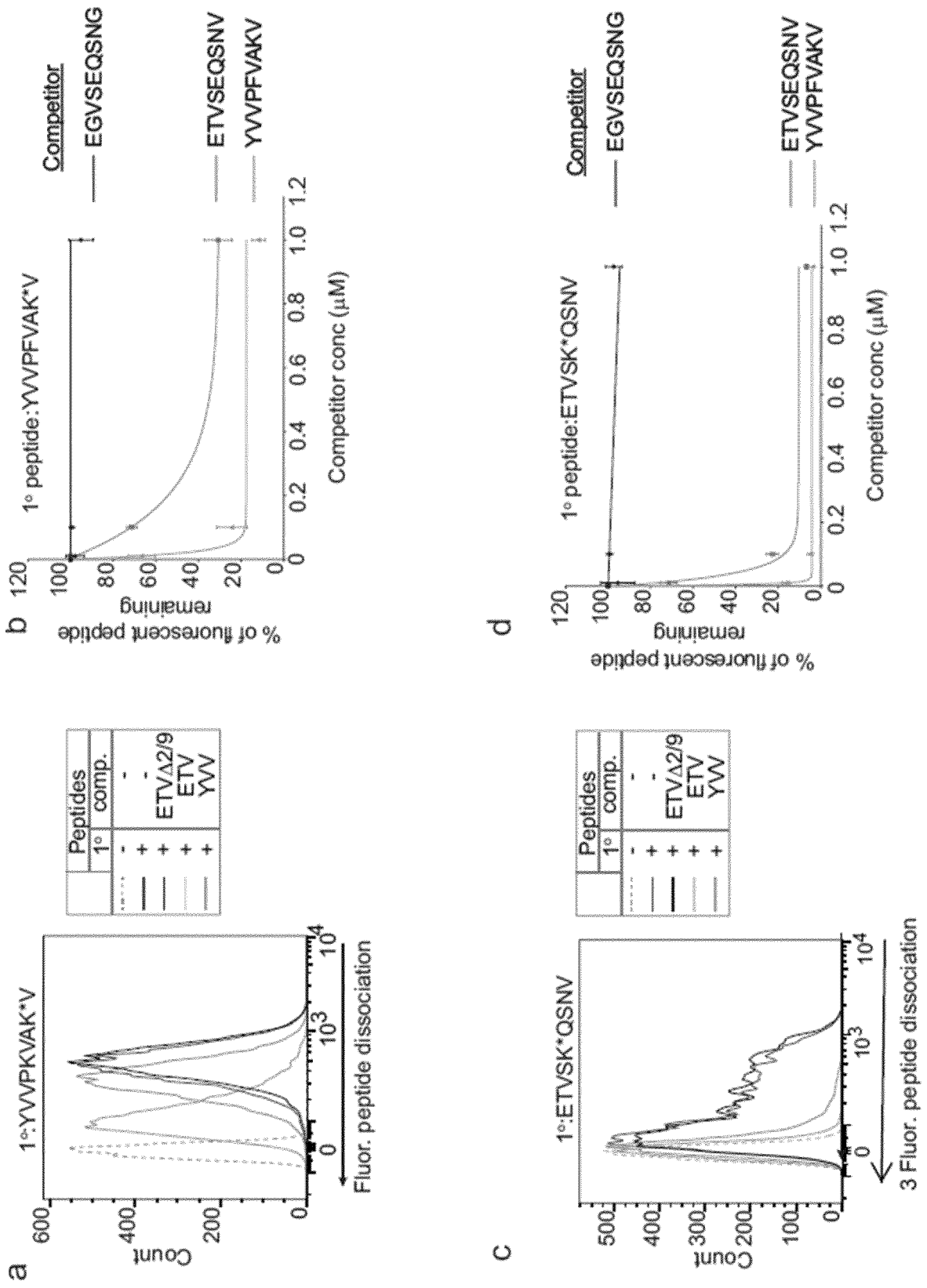

a
1°:YVVPKVAK*V
Count
Fluor. peptide dissociation
Peptides
1°
comp.
ETV/Δ2/9
ETV
YVV
b
1° peptide:YVVPFVAK*V
% of fluorescent peptide remaining
Competitor conc (μM)
Competitor
EGVSEQSNG
ETVSEQSNV
YVVPFVAKV
c
1°:ETVSK*QSNV
3 Fluor. peptide dissociation
d
1° peptide:ETVSK*QSNV

Figure 3

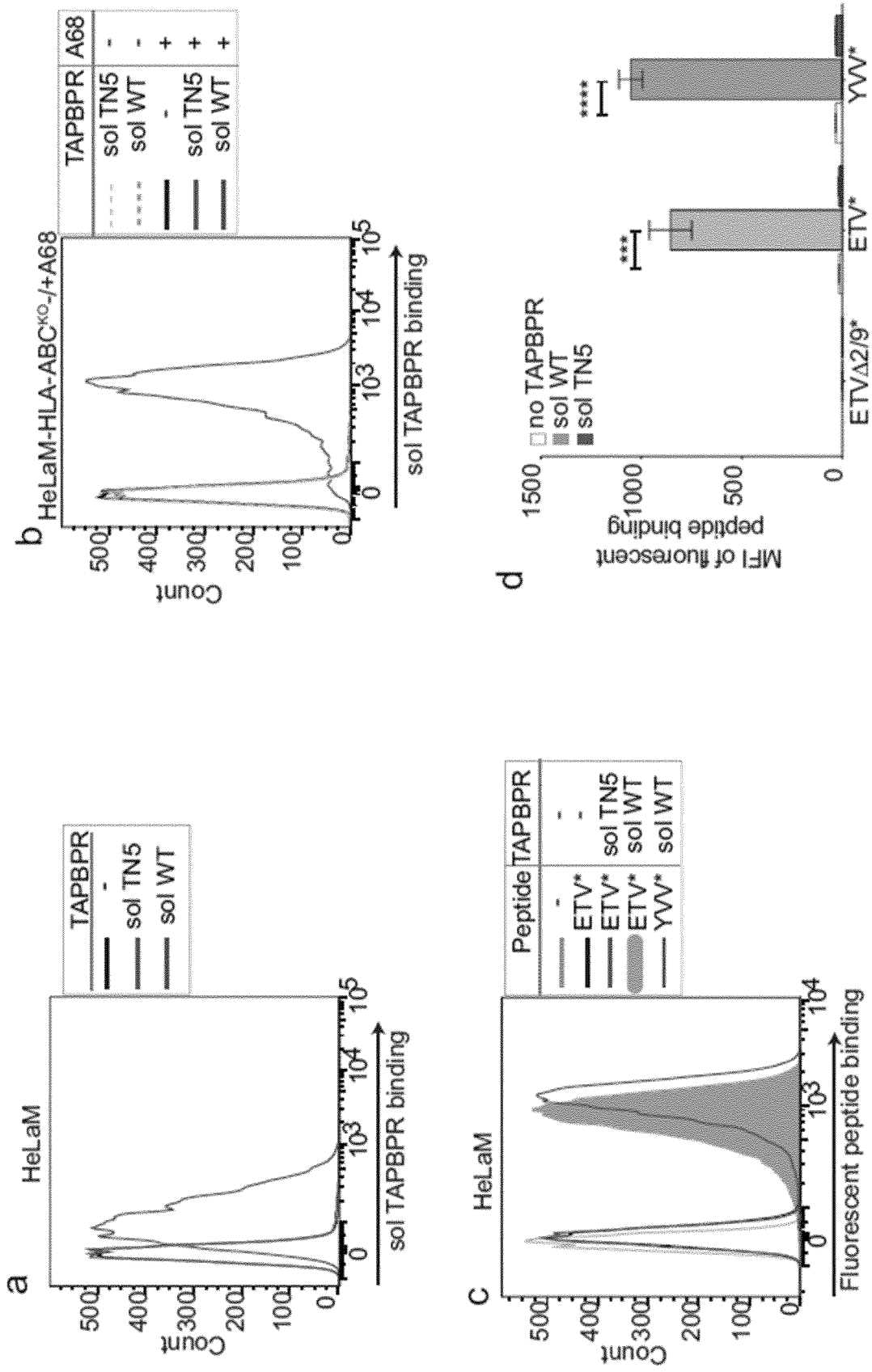

a
HeLaM
TAPBPR
-
sol TN5
sol WT
Count
sol TAPBPR binding
b
HeLaM-HLA-ABC^KO-/+A68
TAPBPR
A68
sol TN5
sol WT
Count
sol TAPBPR binding
c
HeLaM
Peptide
TAPBPR
ETV*
ETV*
ETV*
YVV*
sol TN5
sol WT
sol WT
Count
Fluorescent peptide binding
d
no TAPBPR
sol WT
sol TN5
MFI of fluorescent peptide binding
***
****
ETVΔ2/9*
ETV*
YVV*

Figure 4

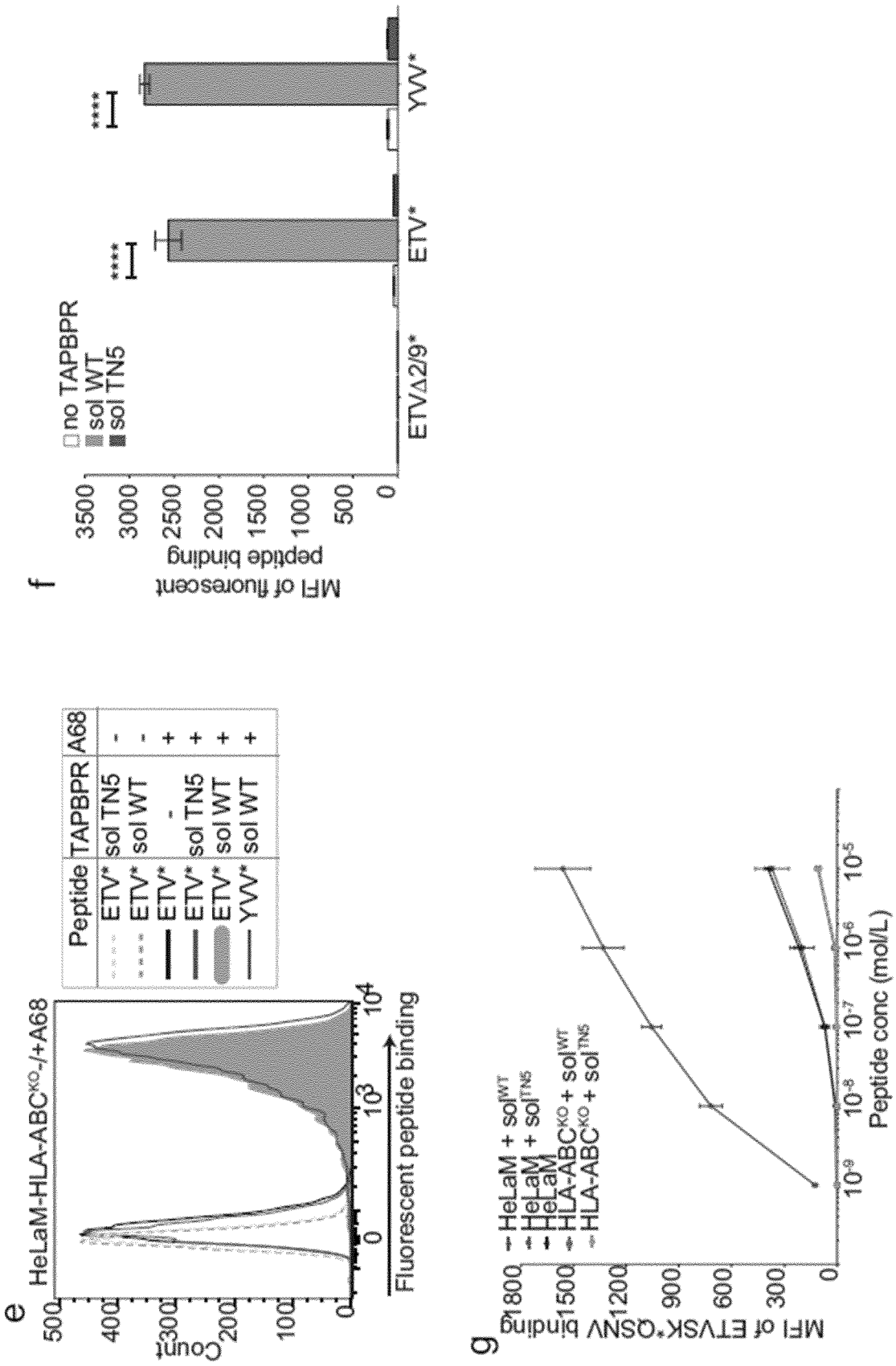
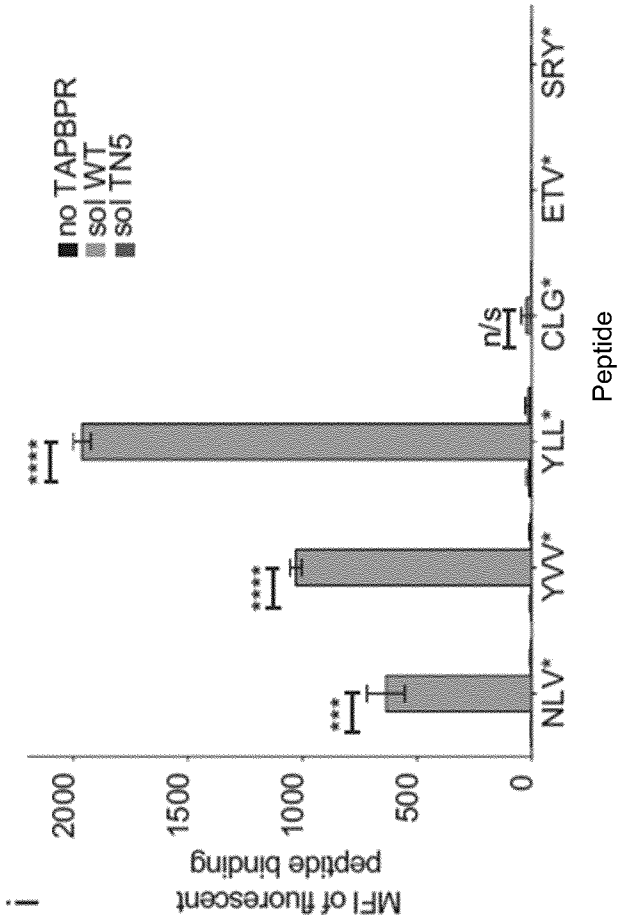


Figure 4 continued



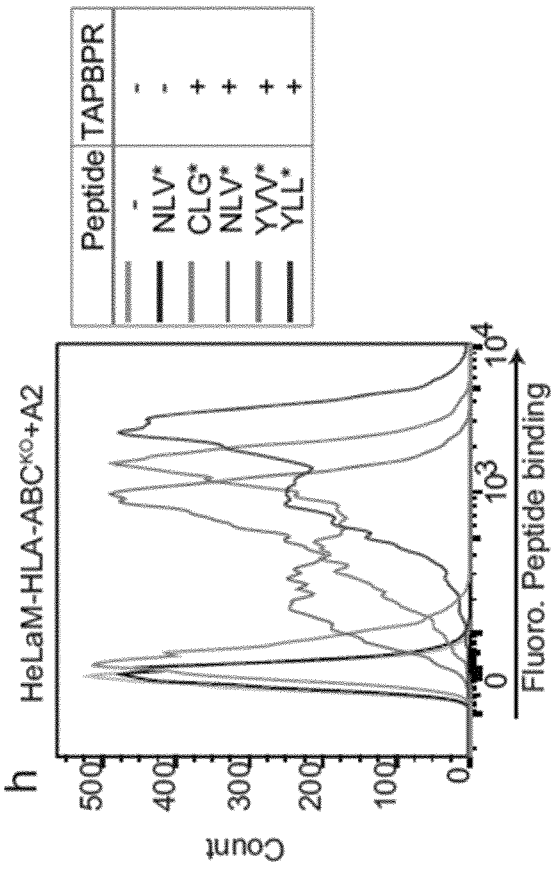


Figure 4 continued

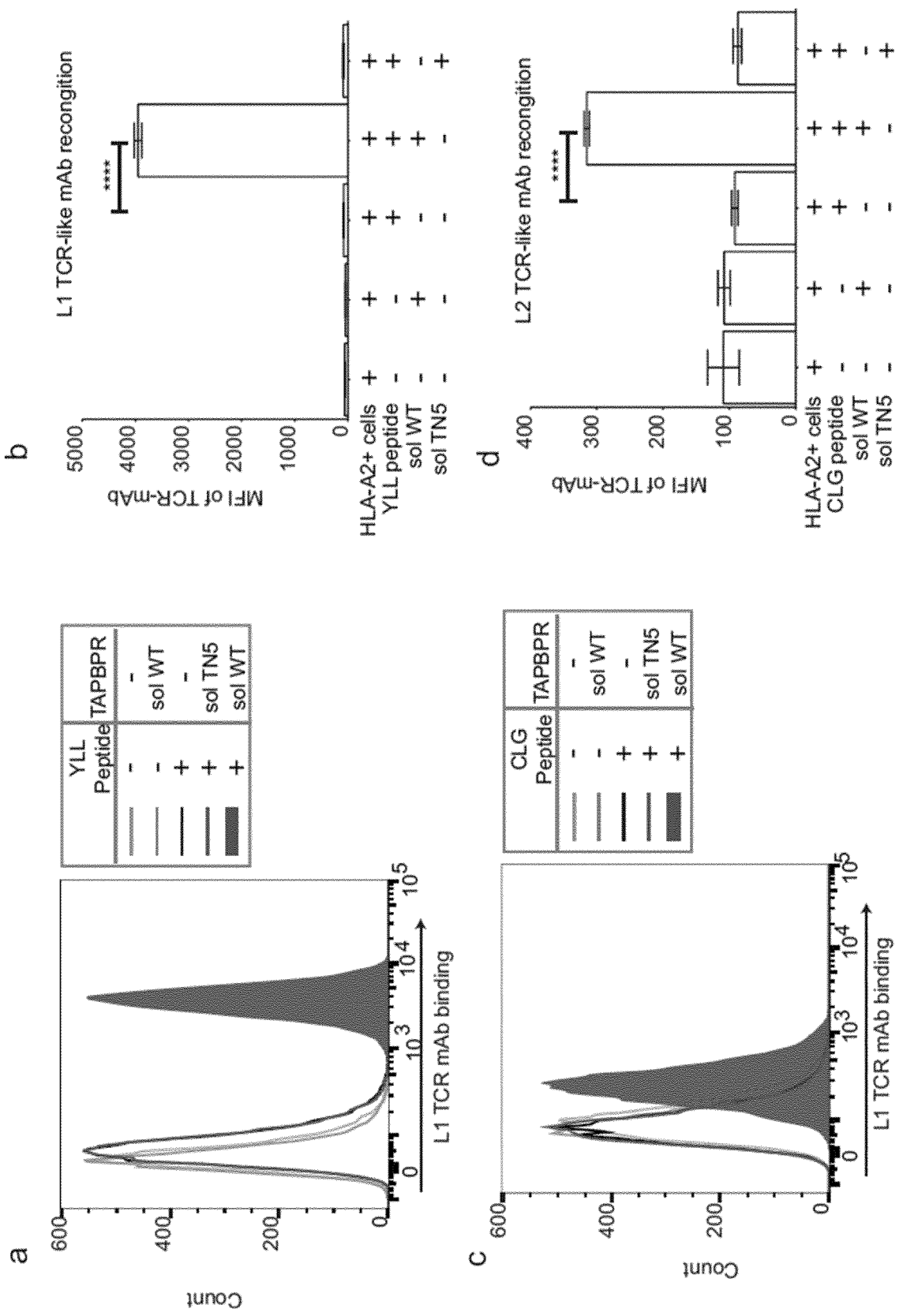

a
Count
L1 TCR mAb binding
YLL Peptide
TAPBPR
- -
- sol WT
+ -
+ sol TN5
+ sol WT
b
L1 TCR-like mAb recongition
MFI of TCR-mAb
****
HLA-A2+ cells + + + +
YLL peptide - - + +
sol WT - + - +
sol TN5 - - - +
c
Count
L1 TCR mAb binding
CLG Peptide
TAPBPR
- -
- sol WT
+ -
+ sol TN5
+ sol WT
d
L2 TCR-like mAb recongition
MFI of TCR-mAb
****
HLA-A2+ cells + + + +
CLG peptide - - + +
sol WT - + - +
sol TN5 - - - +

Figure 5

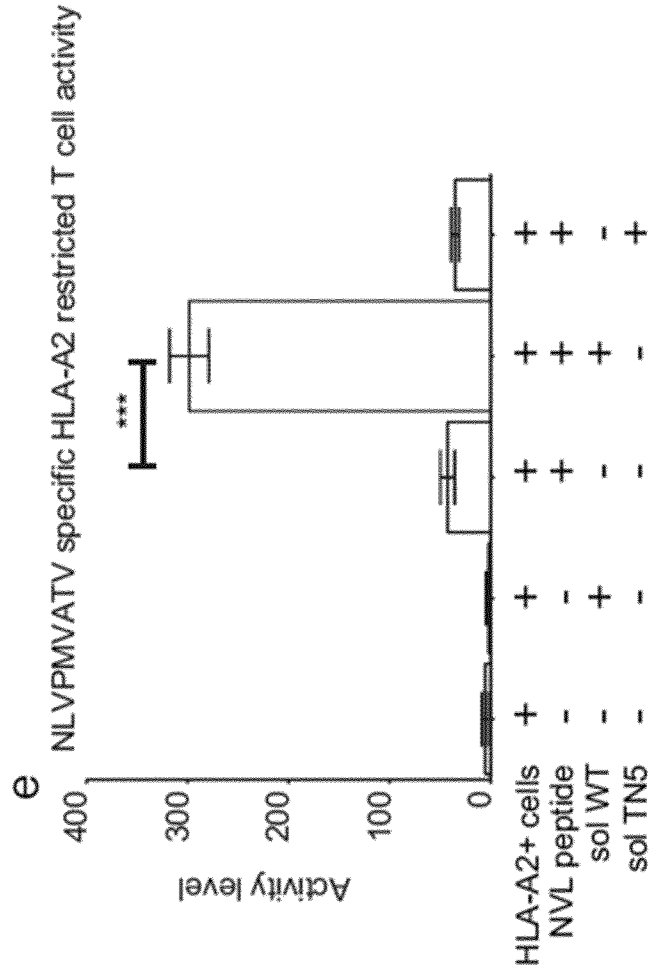


Figure 5 continued

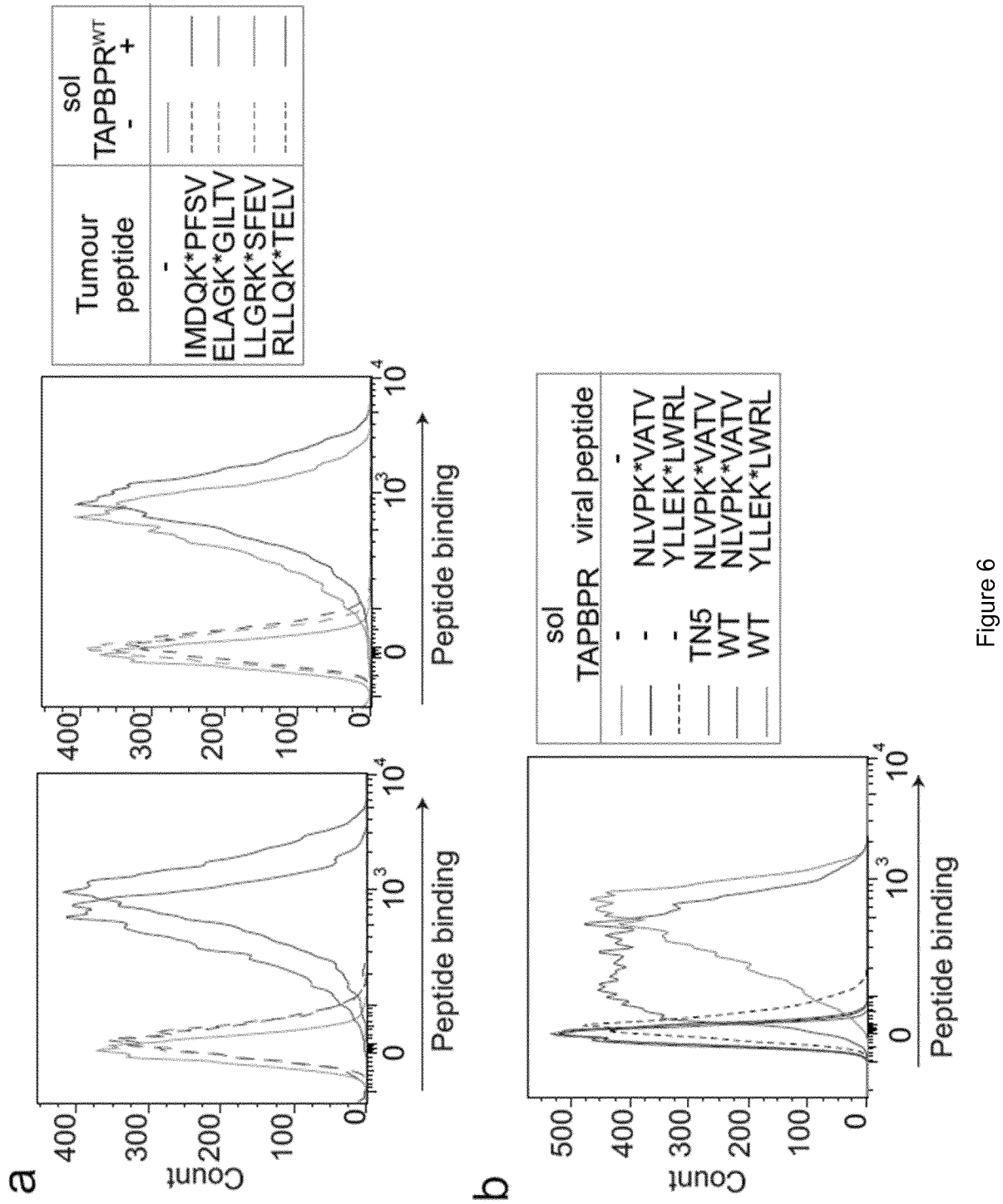

a
b
Count
Peptide binding
Tumour peptide
sol TAPBPR^WT
-
+
IMDQK*PFSV
ELAGK*GILTV
LLGRK*SFEV
RLLQK*TELV
sol TAPBPR
viral peptide
NLVPK*VATV
YLLEK*LWRL
TN5
WT

Figure 6

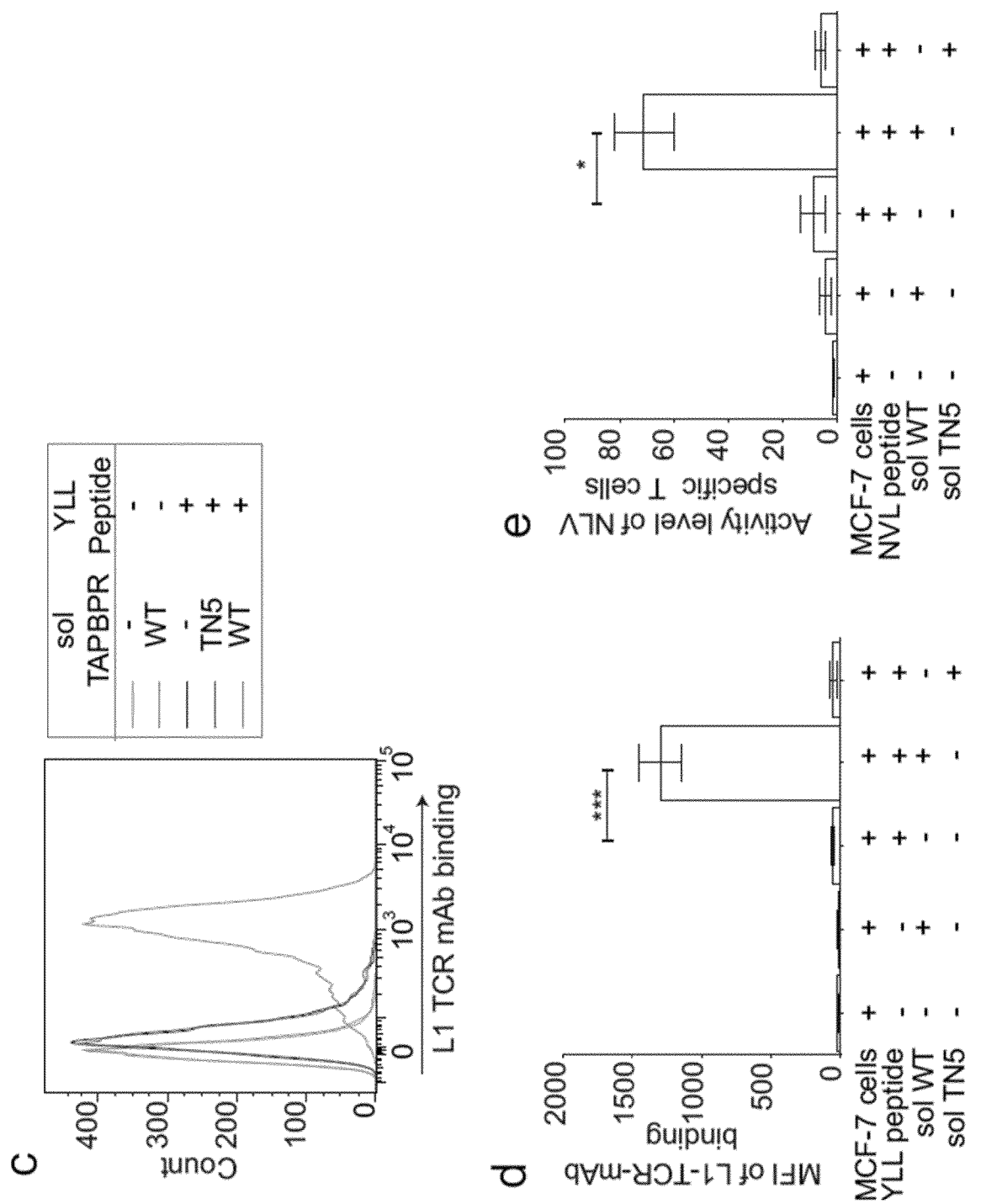


Figure 6 continued

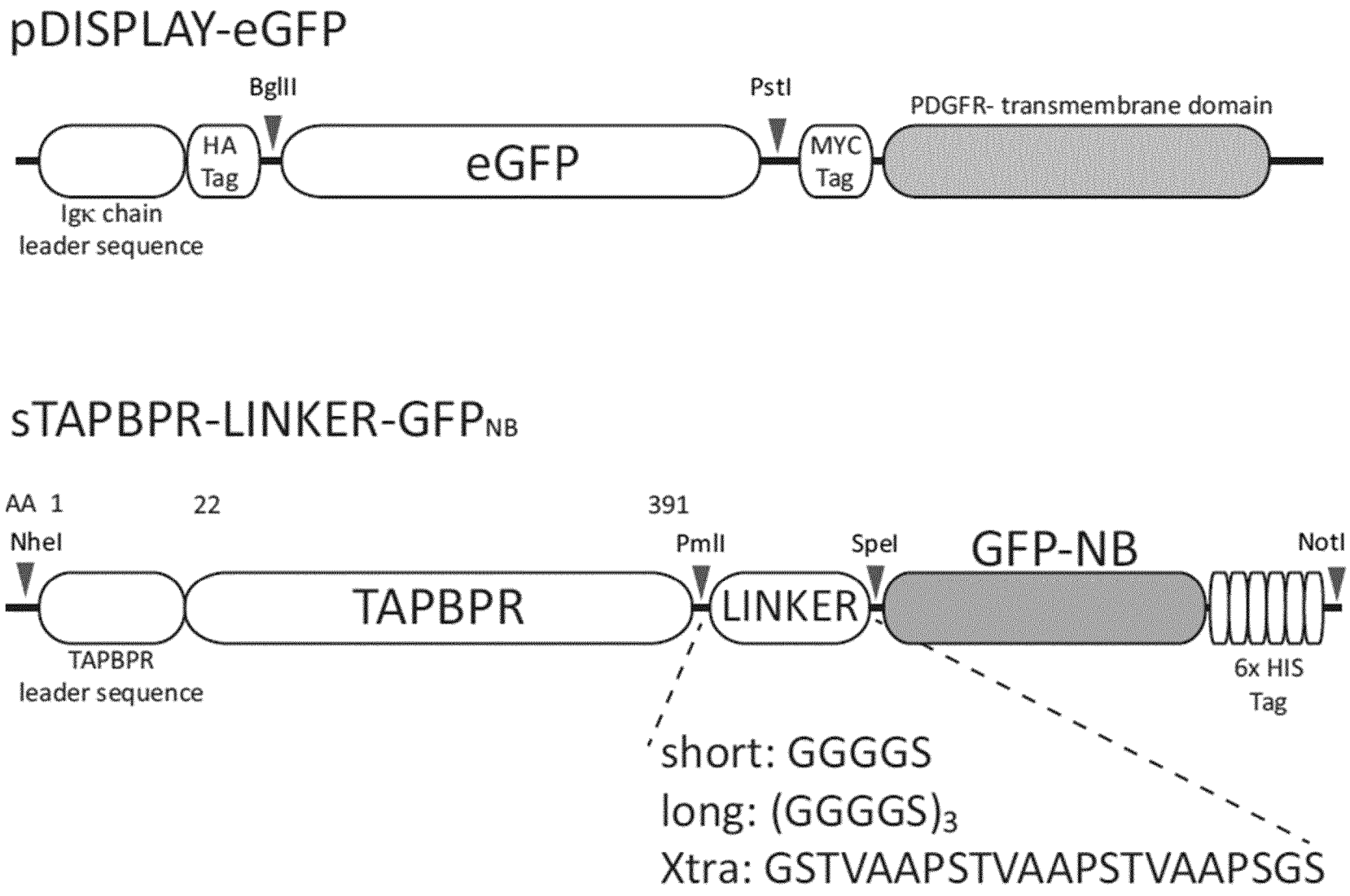


Figure 7

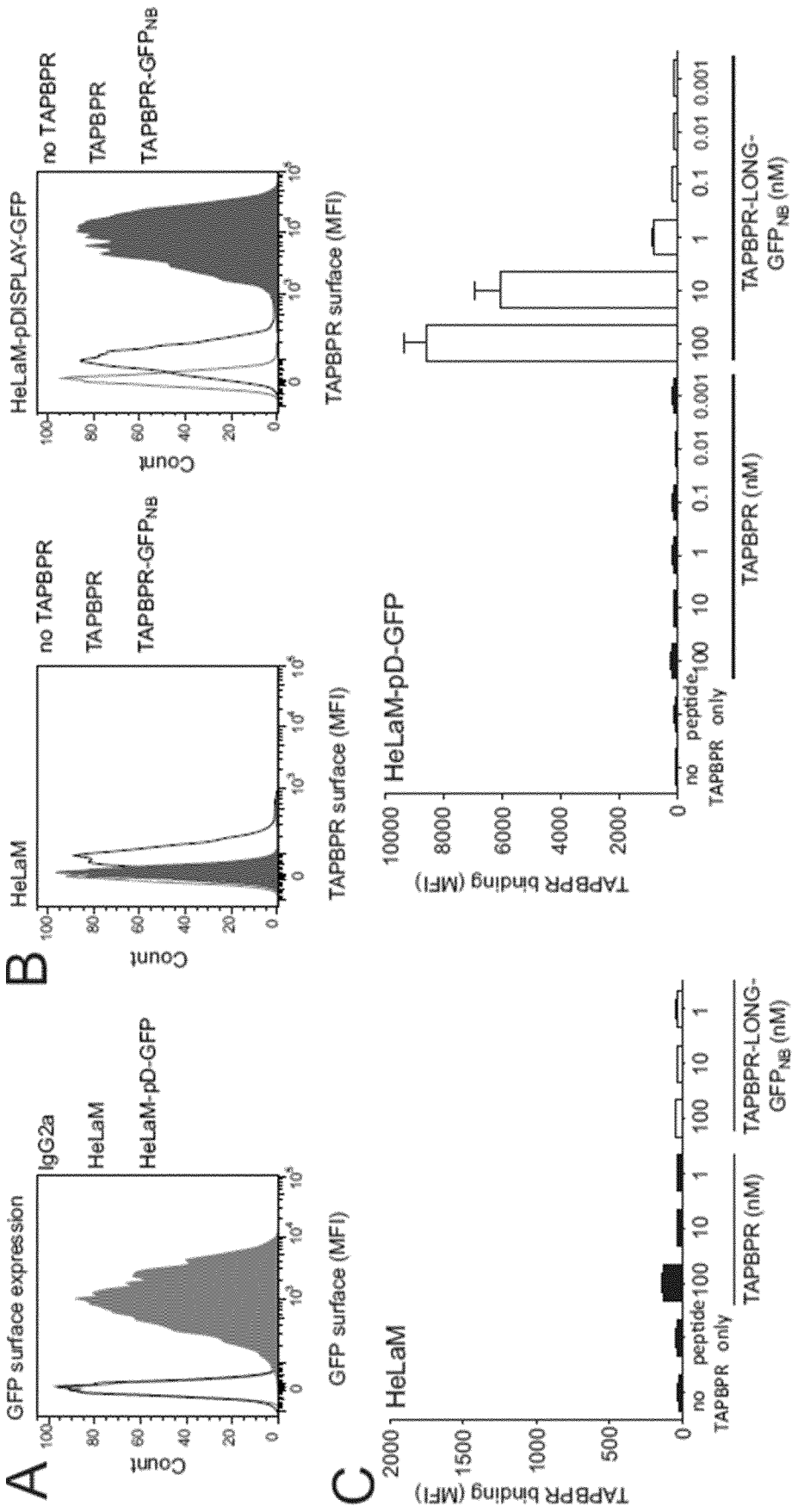

A
GFP surface expression
IgG2a
HeLaM
HeLaM-pD-GFP
Count
B
HeLaM
no TAPBPR
TAPBPR
TAPBPR-GFPNB
TAPBPR surface (MFI)
HeLaM-pDISPLAY-GFP
C
HeLaM
GFP surface (MFI)
TAPBPR binding (MFI)
no peptide
TAPBPR only
TAPBPR (nM)
TAPBPR-LONG-GFPNB (nM)
HeLaM-pD-GFP

Figure 8

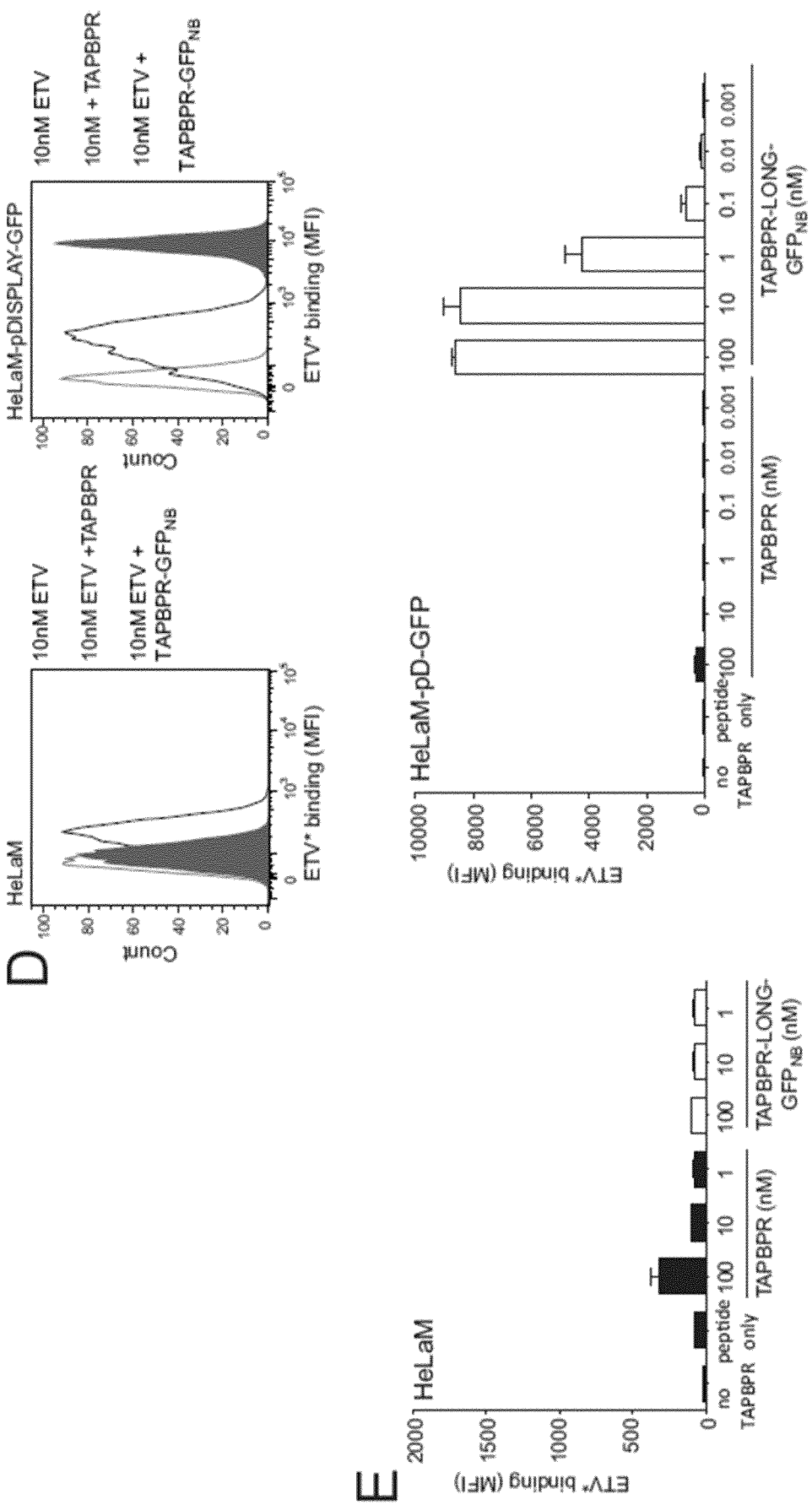


Figure 8 continued

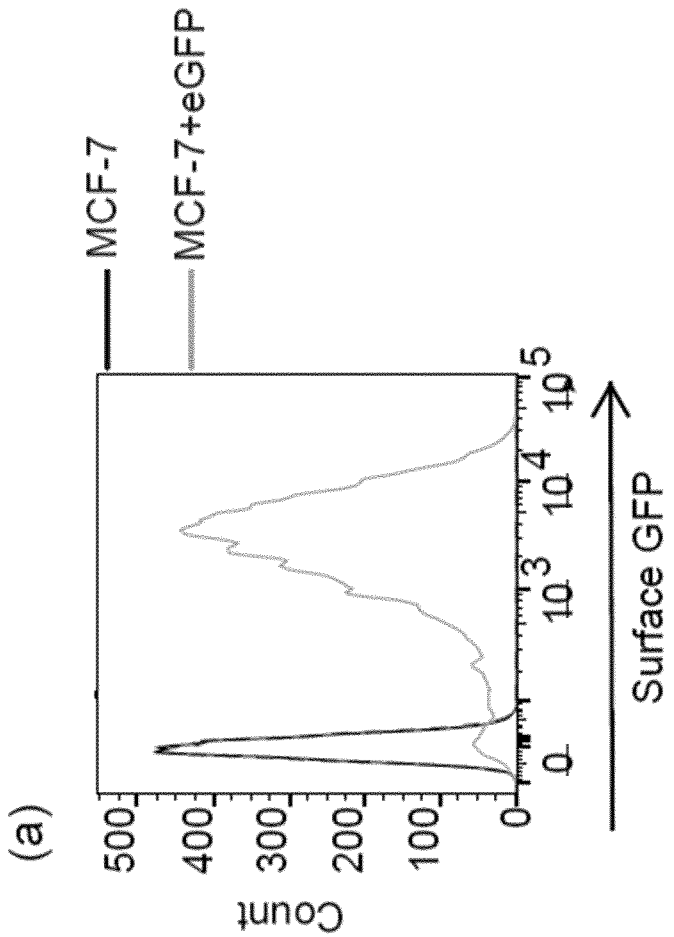
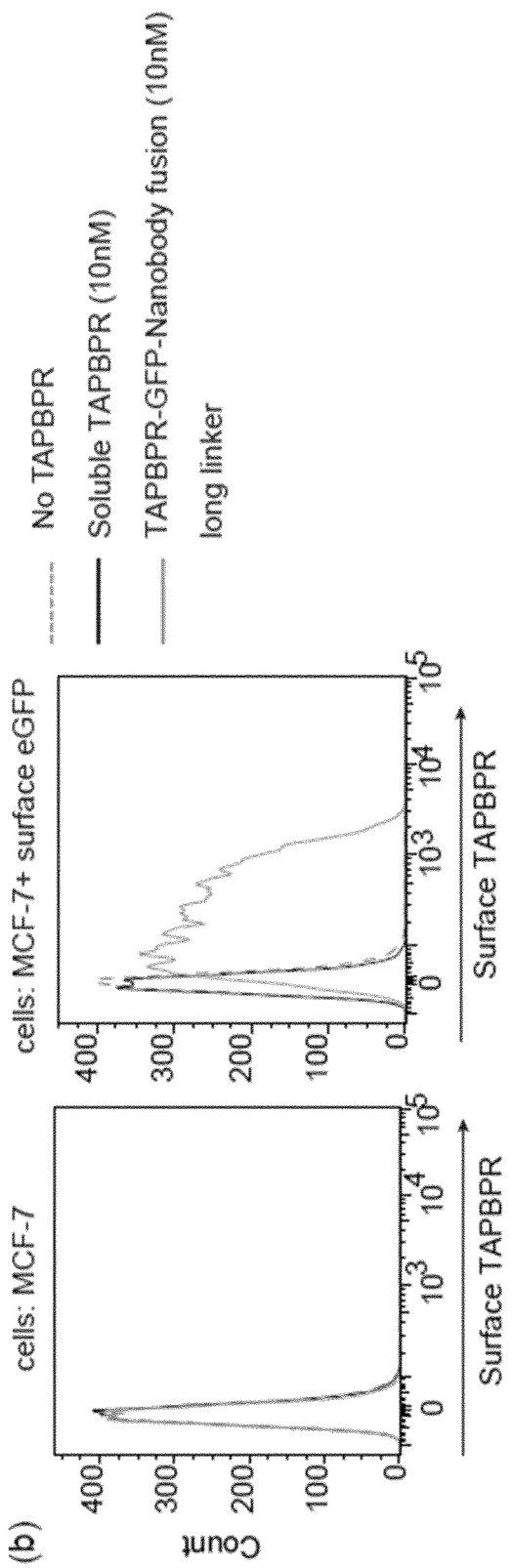

(a)
MCF-7
MCF-7+eGFP
Count
500
400
300
200
100
0
0
10^3
10^4
10^5
Surface GFP

Figure 9



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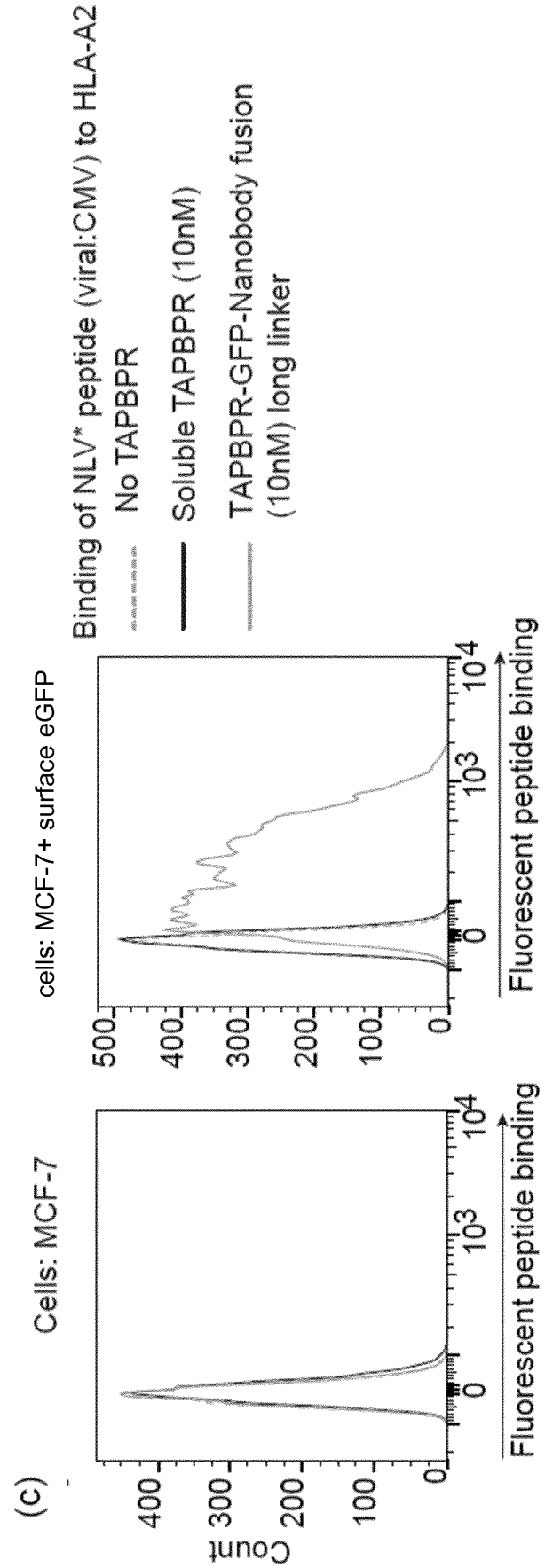


Figure 9 continued

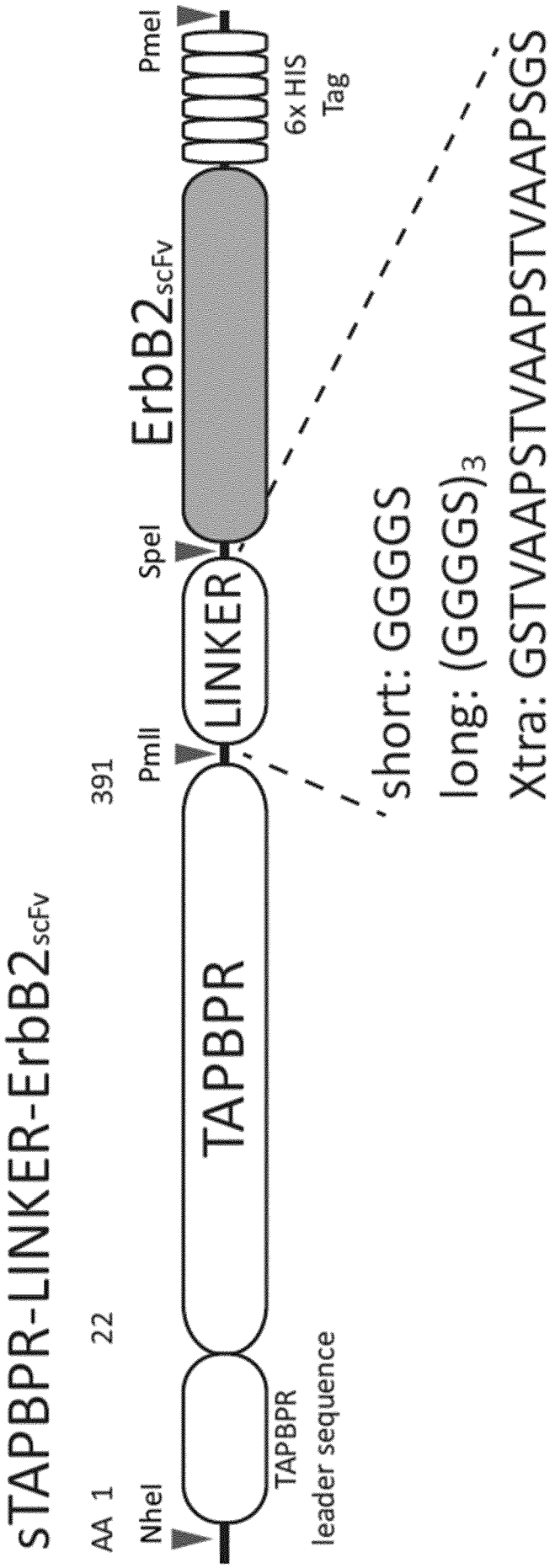

sTAPBPR-LINKER-ErbB2scFv
AA 1
22
391
NheI
TAPBPR
leader sequence
TAPBPR
PmlI
LINKER
SpeI
ErbB2scFv
PmeI
6x HIS
Tag
short: GGGGS
long: (GGGGS)3
Xtra: GSTVAAPSTVAAPSGS

Figure 10

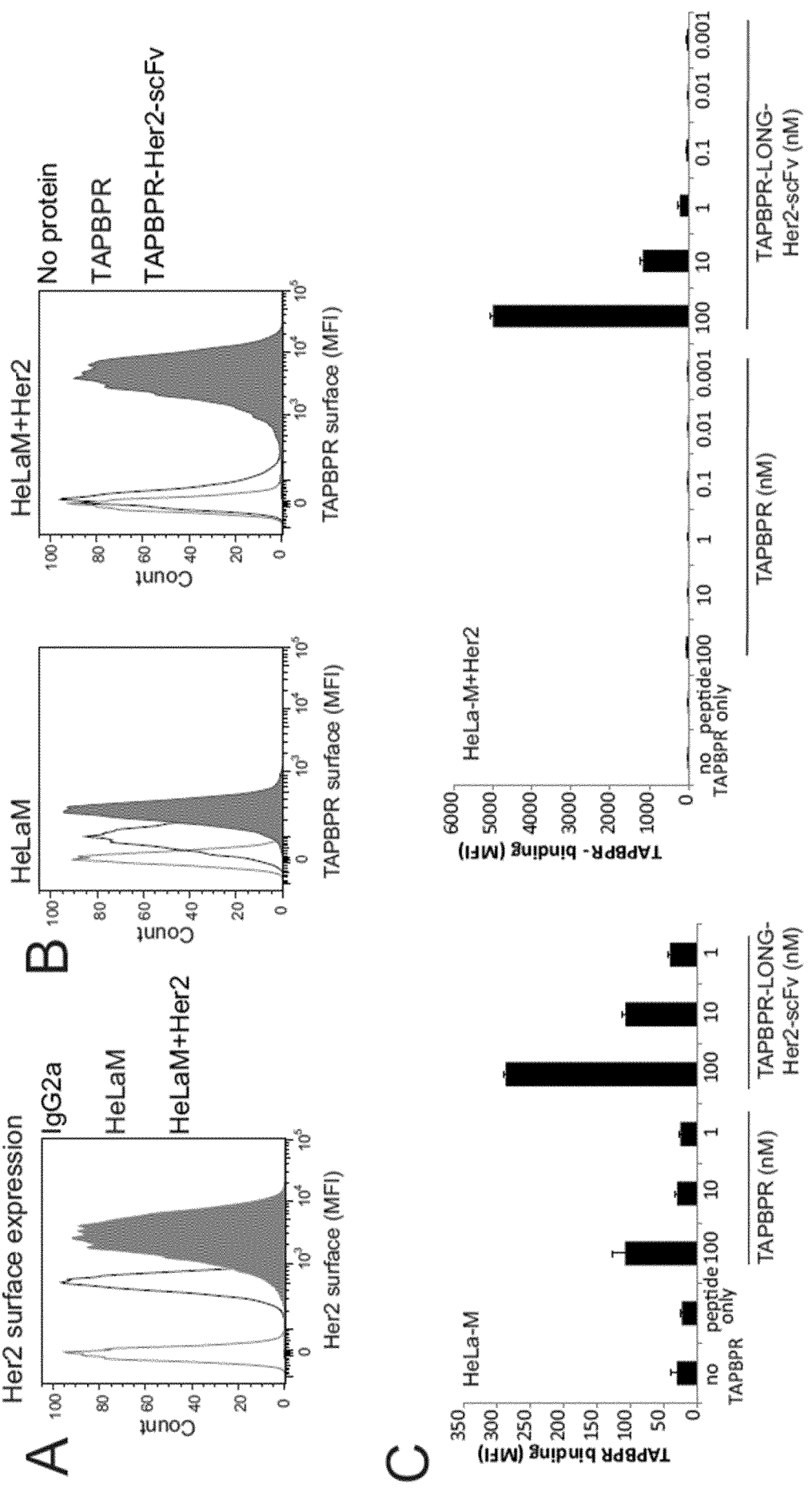

A
Her2 surface expression
IgG2a
HeLaM
HeLaM+Her2
Count
Her2 surface (MFI)
B
HeLaM
HeLaM+Her2
No protein
TAPBPR
TAPBPR-Her2-scFv
Count
TAPBPR surface (MFI)
C
HeLa-M
TAPBPR binding (MFI)
no peptide
TAPBPR only
TAPBPR (nM)
TAPBPR-LONG-Her2-scFv (nM)
HeLa-M+Her2
TAPBPR- binding (MFI)

Figure 11

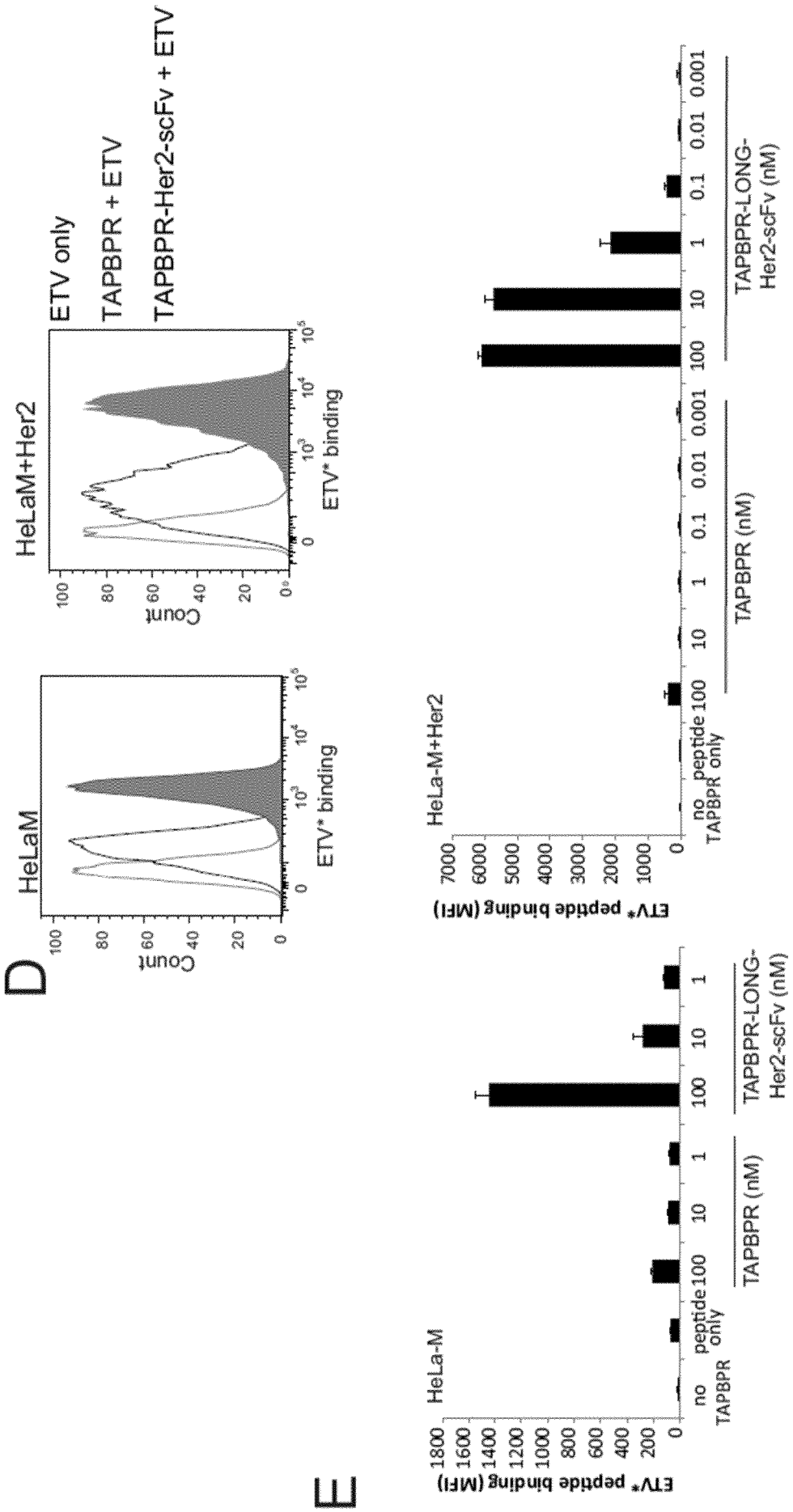


Figure 11 continued

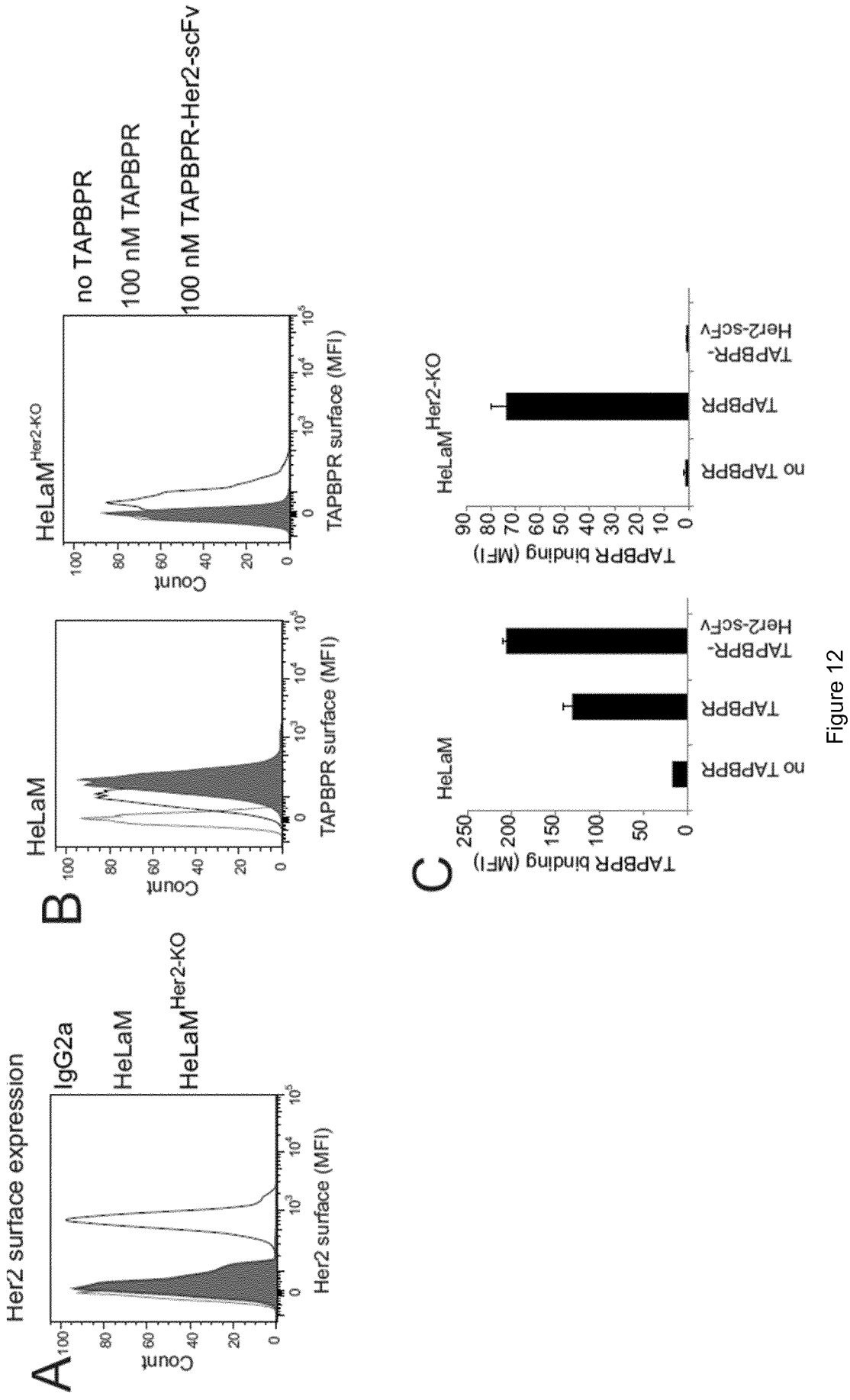

A
Her2 surface expression
IgG2a
HeLaM
HeLaM Her2-KO
Count
Her2 surface (MFI)
B
HeLaM
HeLaM Her2-KO
no TAPBPR
100 nM TAPBPR
100 nM TAPBPR-Her2-scFv
Count
TAPBPR surface (MFI)
C
HeLaM
HeLaM Her2-KO
TAPBPR binding (MFI)
no TAPBPR
TAPBPR
TAPBPR-Her2-scFv

Figure 12

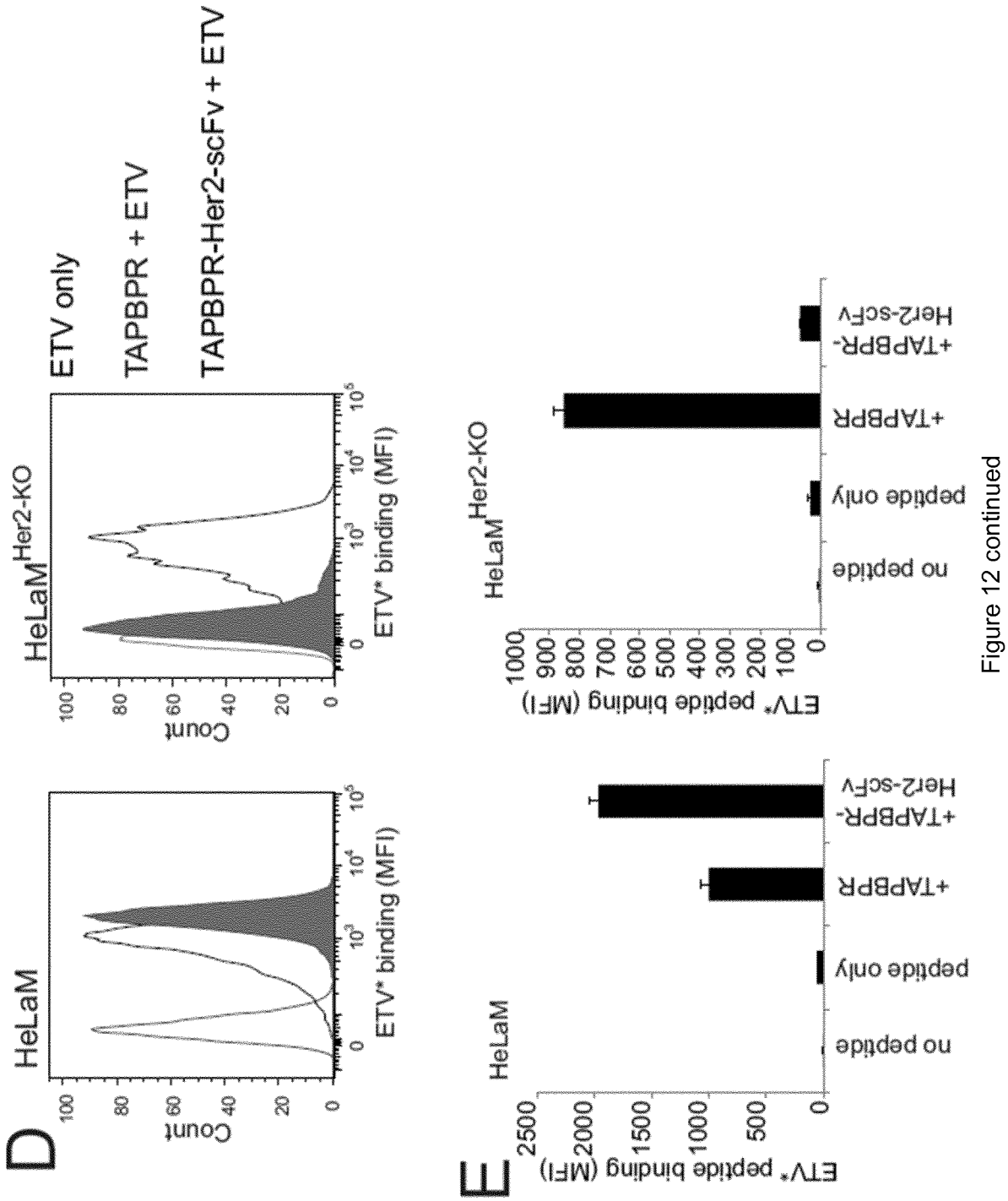


Figure 12 continued

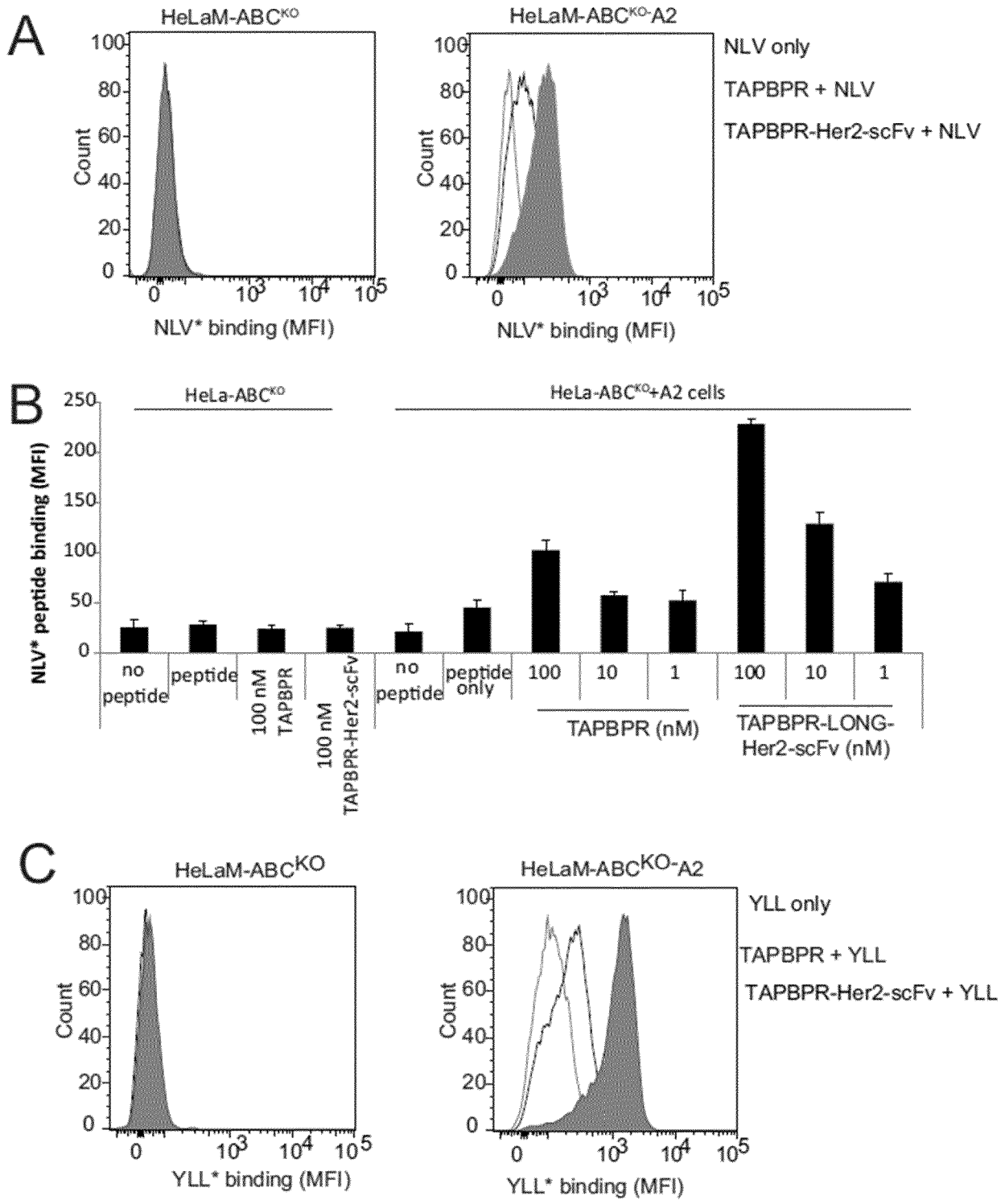

A
HeLaM-ABC^KO
HeLaM-ABC^KO-A2
Count
NLV* binding (MFI)
NLV only
TAPBPR + NLV
TAPBPR-Her2-scFv + NLV
B
HeLa-ABC^KO
HeLa-ABC^KO+A2 cells
NLV* peptide binding (MFI)
no peptide
peptide
100 nM TAPBPR
100 nM TAPBPR-Her2-scFv
peptide only
TAPBPR (nM)
TAPBPR-LONG-Her2-scFv (nM)
C
HeLaM-ABC^KO
HeLaM-ABC^KO-A2
YLL* binding (MFI)
YLL only
TAPBPR + YLL
TAPBPR-Her2-scFv + YLL

Figure 13

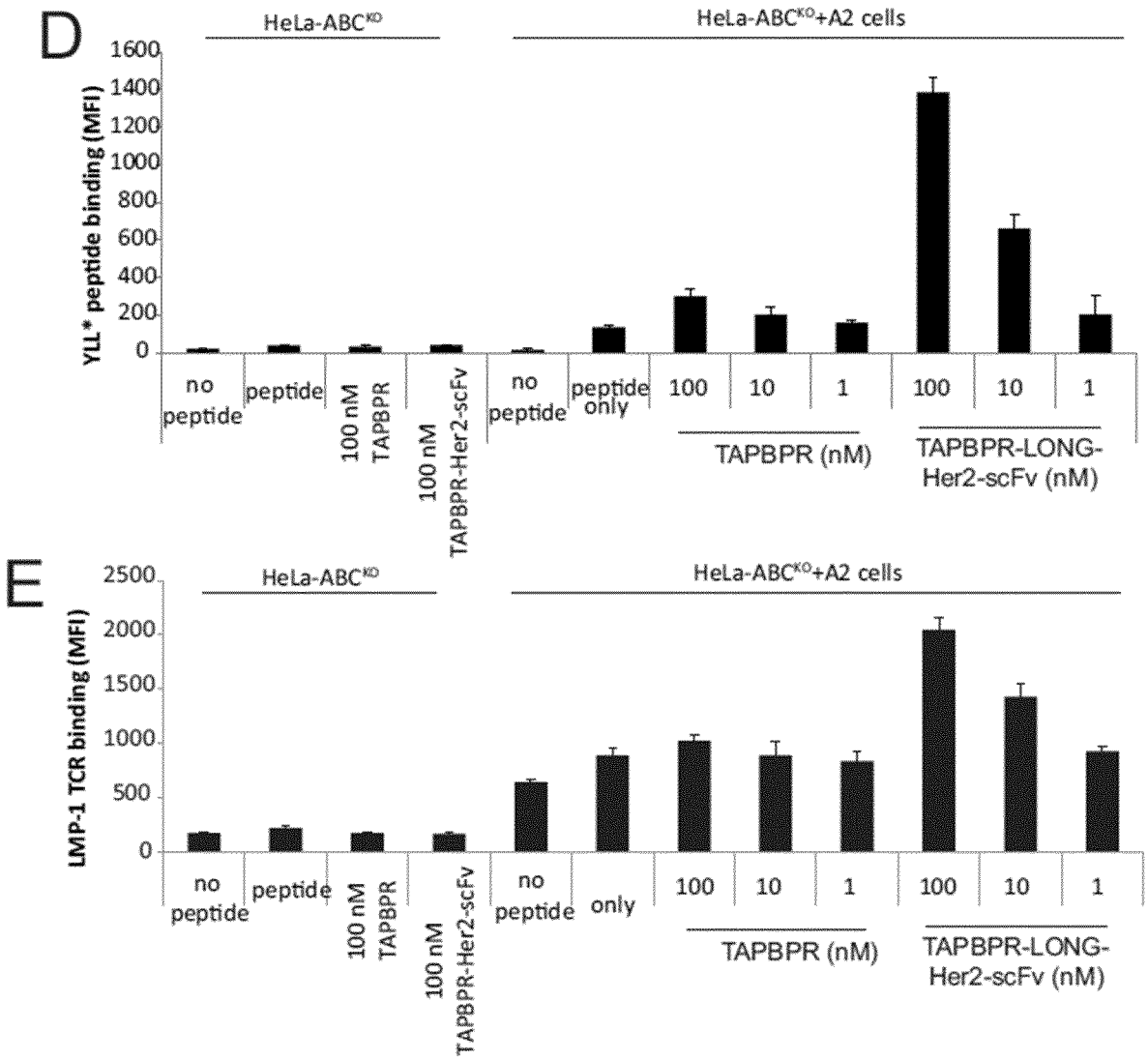


Figure 13 continued

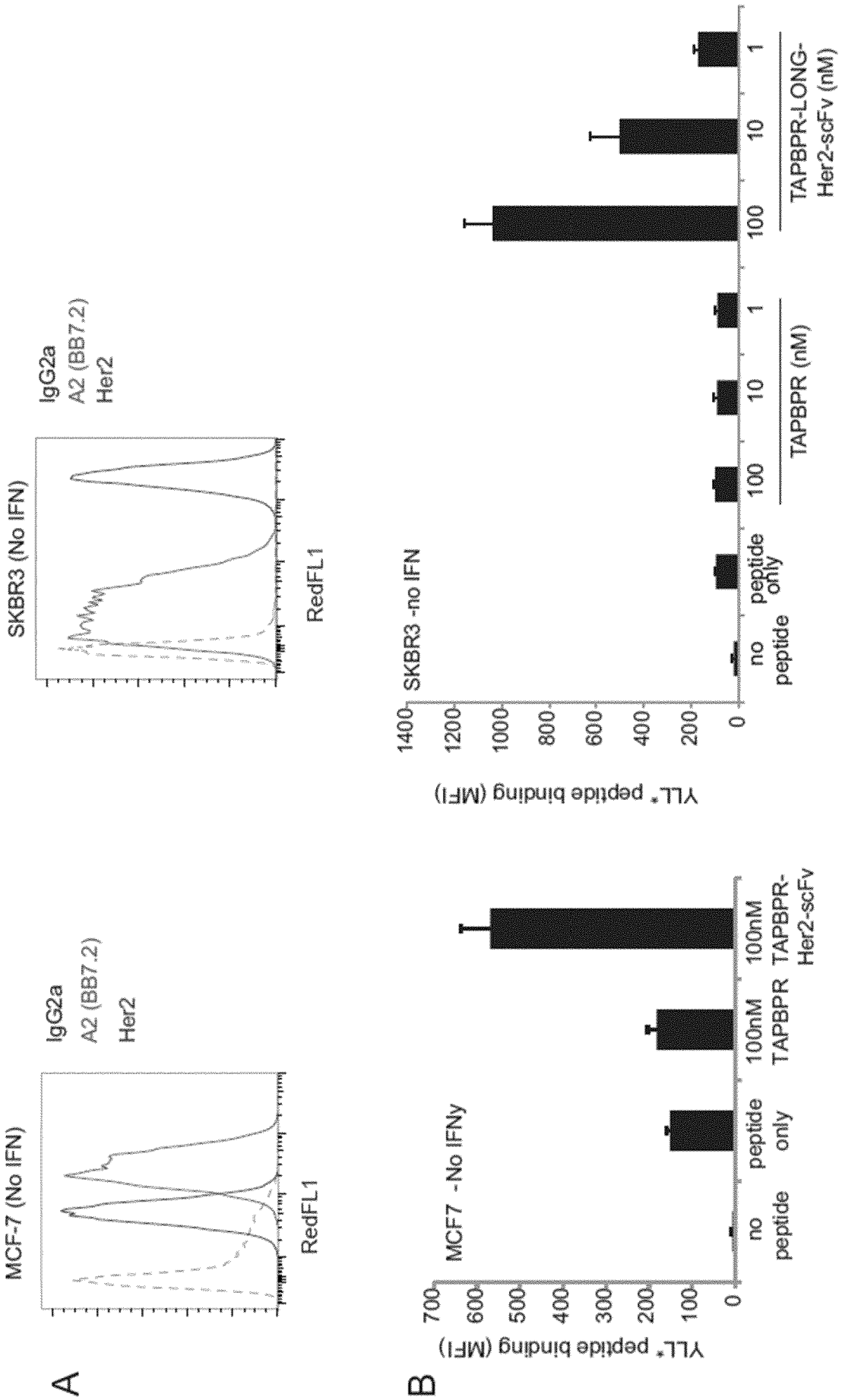

A
MCF-7 (No IFN)
IgG2a
A2 (BB7.2)
Her2
RedFL1
SKBR3 (No IFN)
IgG2a
A2 (BB7.2)
Her2
RedFL1
B
MCF7 -No IFNy
YLL* peptide binding (MFI)
0
100
200
300
400
500
600
700
no peptide
peptide only
100nM TAPBPR
100nM TAPBPR-Her2-scFv
SKBR3 -no IFN
YLL* peptide binding (MFI)
0
200
400
600
800
1000
1200
1400
no peptide
peptide only
100
10
1
TAPBPR (nM)
100
10
1
TAPBPR-LONG-Her2-scFv (nM)

Figure 14

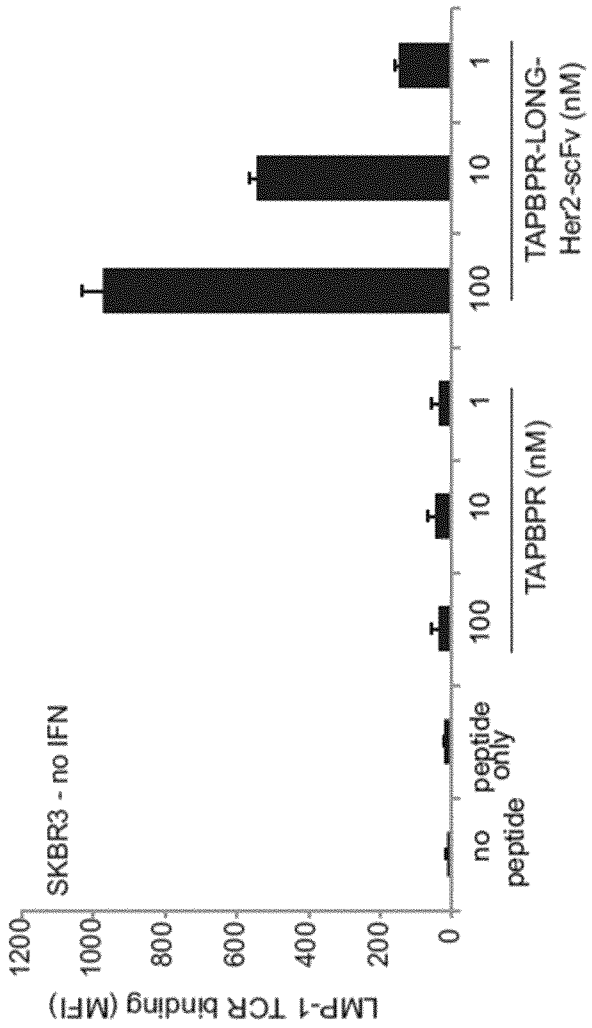
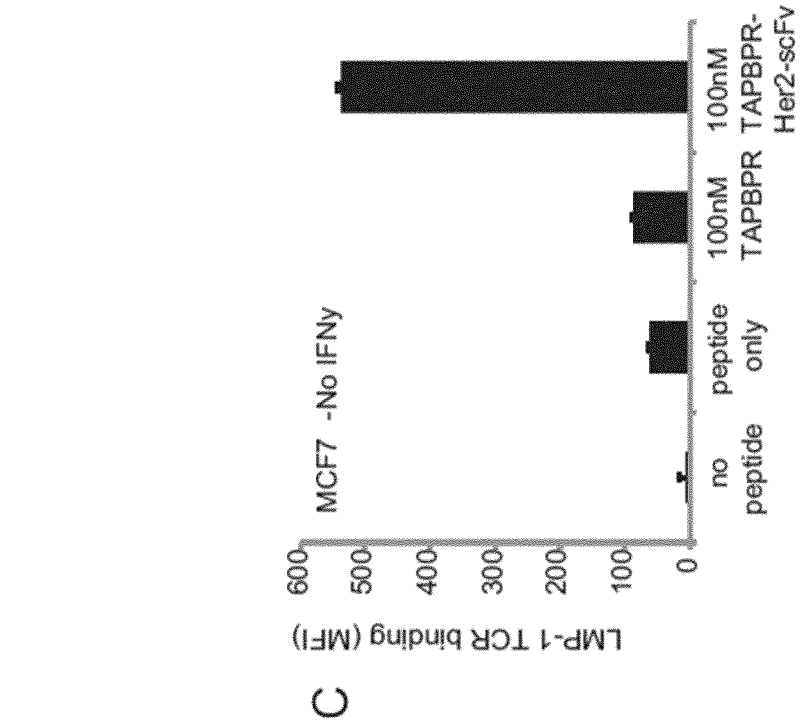


Figure 14 continued



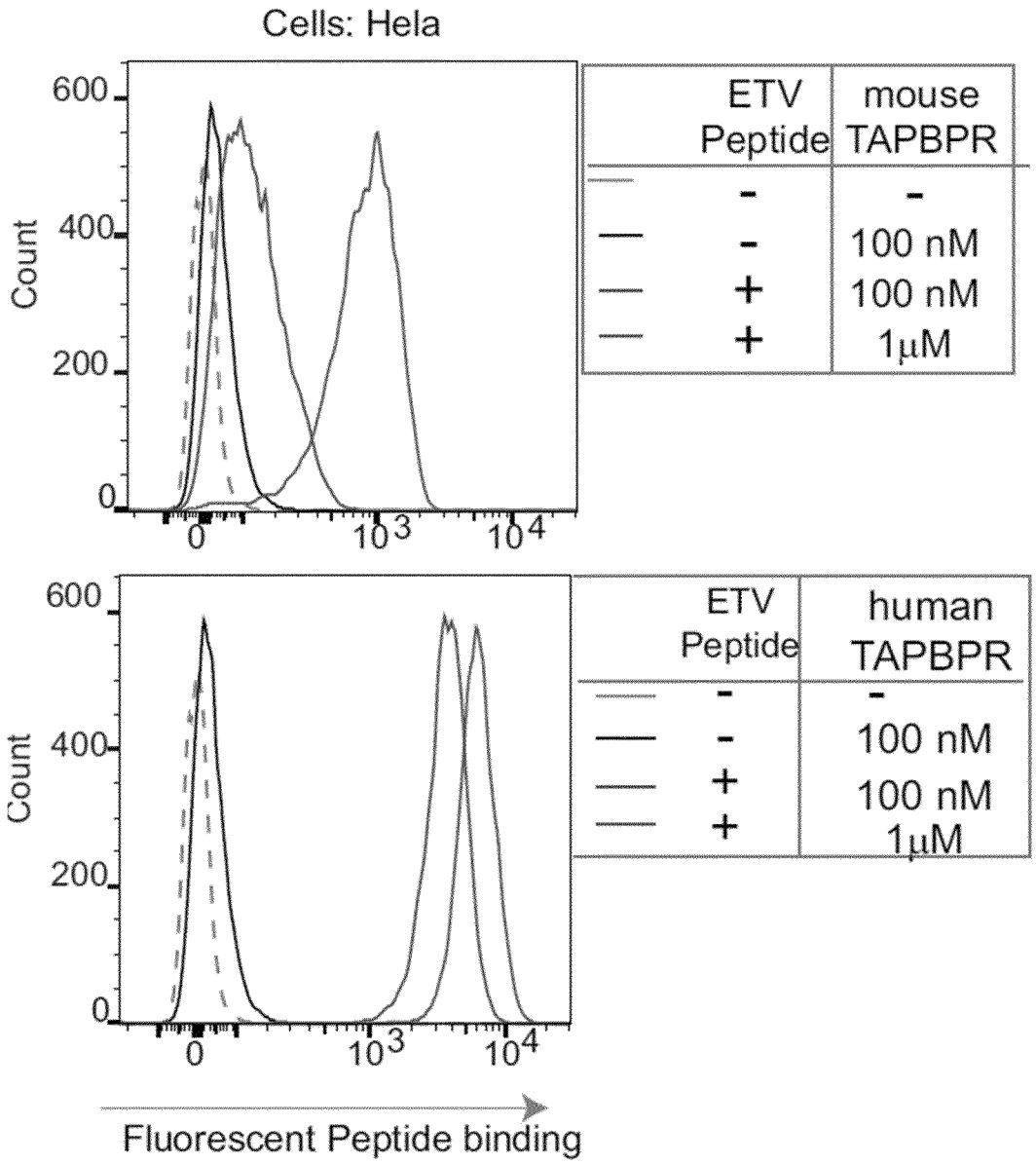

Cells: Hela
Count
600
400
200
0
0
10 3
10 4
ETV Peptide
mouse TAPBPR
-
-
100 nM
+
100 nM
+
1µM
Count
600
400
200
0
0
10 3
10 4
ETV Peptide
human TAPBPR
-
-
100 nM
+
100 nM
+
1µM
Fluorescent Peptide binding

Figure 15

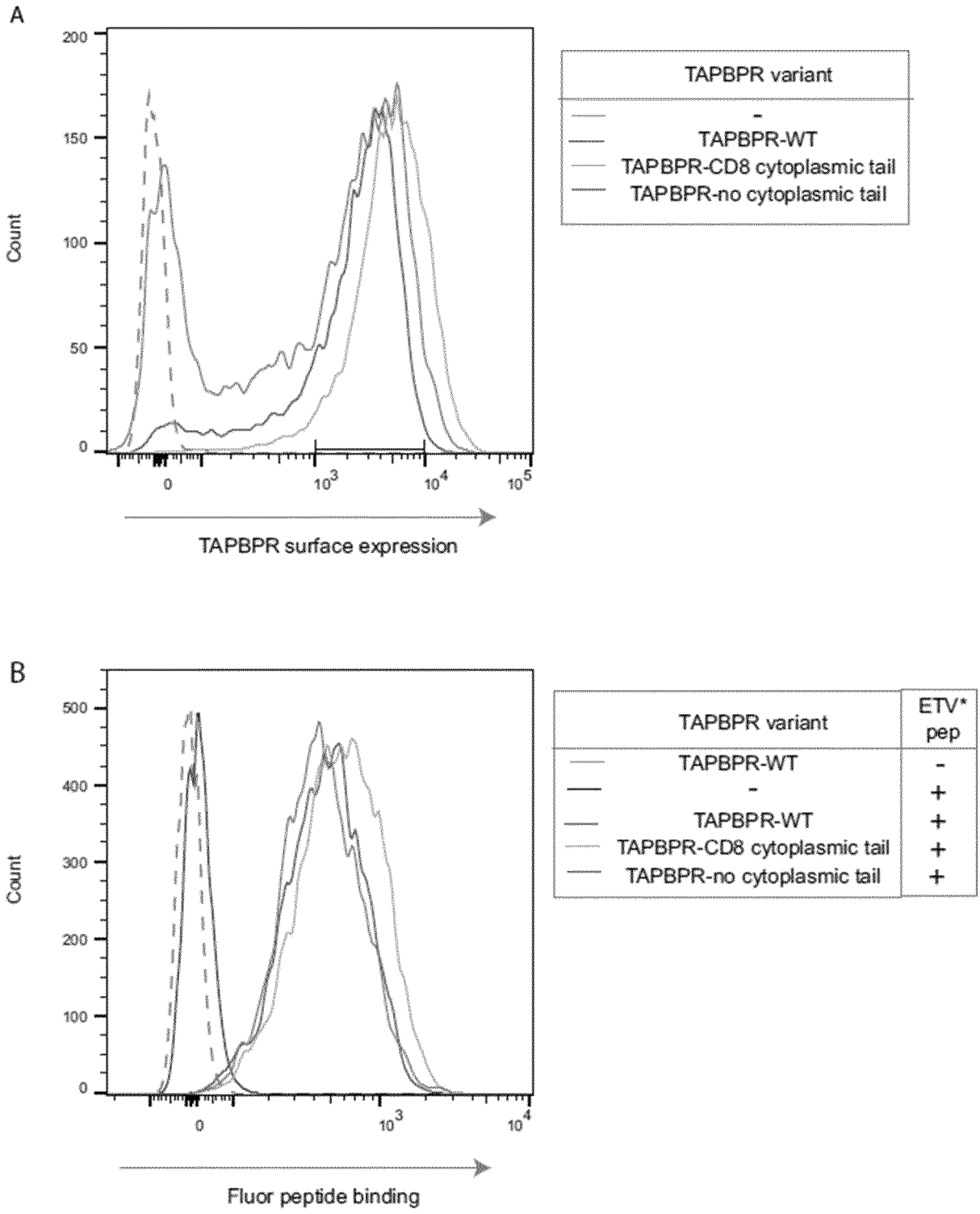

A
Count
200
150
100
50
0
0
10^3
10^4
10^5
TAPBPR surface expression
TAPBPR variant
-
TAPBPR-WT
TAPBPR-CD8 cytoplasmic tail
TAPBPR-no cytoplasmic tail
B
Count
500
400
300
200
100
0
0
10^3
10^4
Fluor peptide binding
TAPBPR variant
ETV* pep
TAPBPR-WT -
- +
TAPBPR-WT +
TAPBPR-CD8 cytoplasmic tail +
TAPBPR-no cytoplasmic tail +

Figure 16

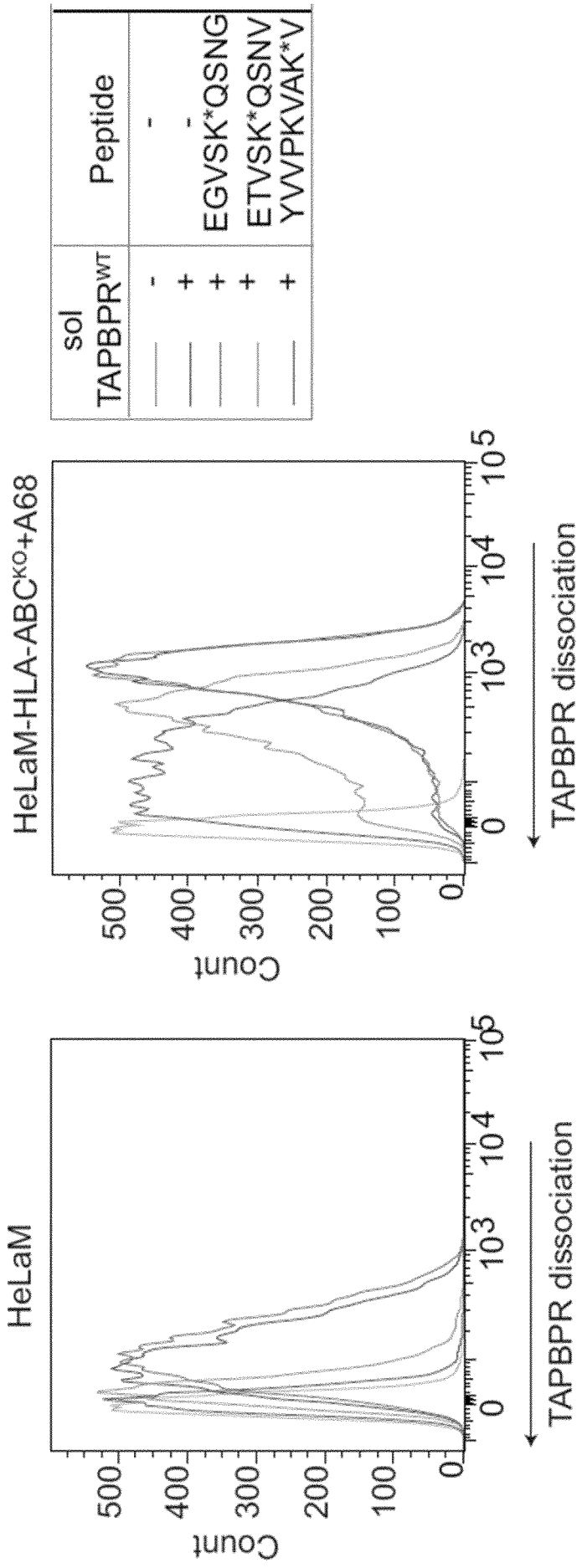

sol TAPBPR^WT
Peptide
-
-
+
-
+
EGVSK*QSNG
+
ETVSK*QSNV
+
YVVPKVAK*V
HeLaM-HLA-ABC^KO+A68
HeLaM
Count
0
100
200
300
400
500
0
10^3
10^4
10^5
TAPBPR dissociation

Figure 17

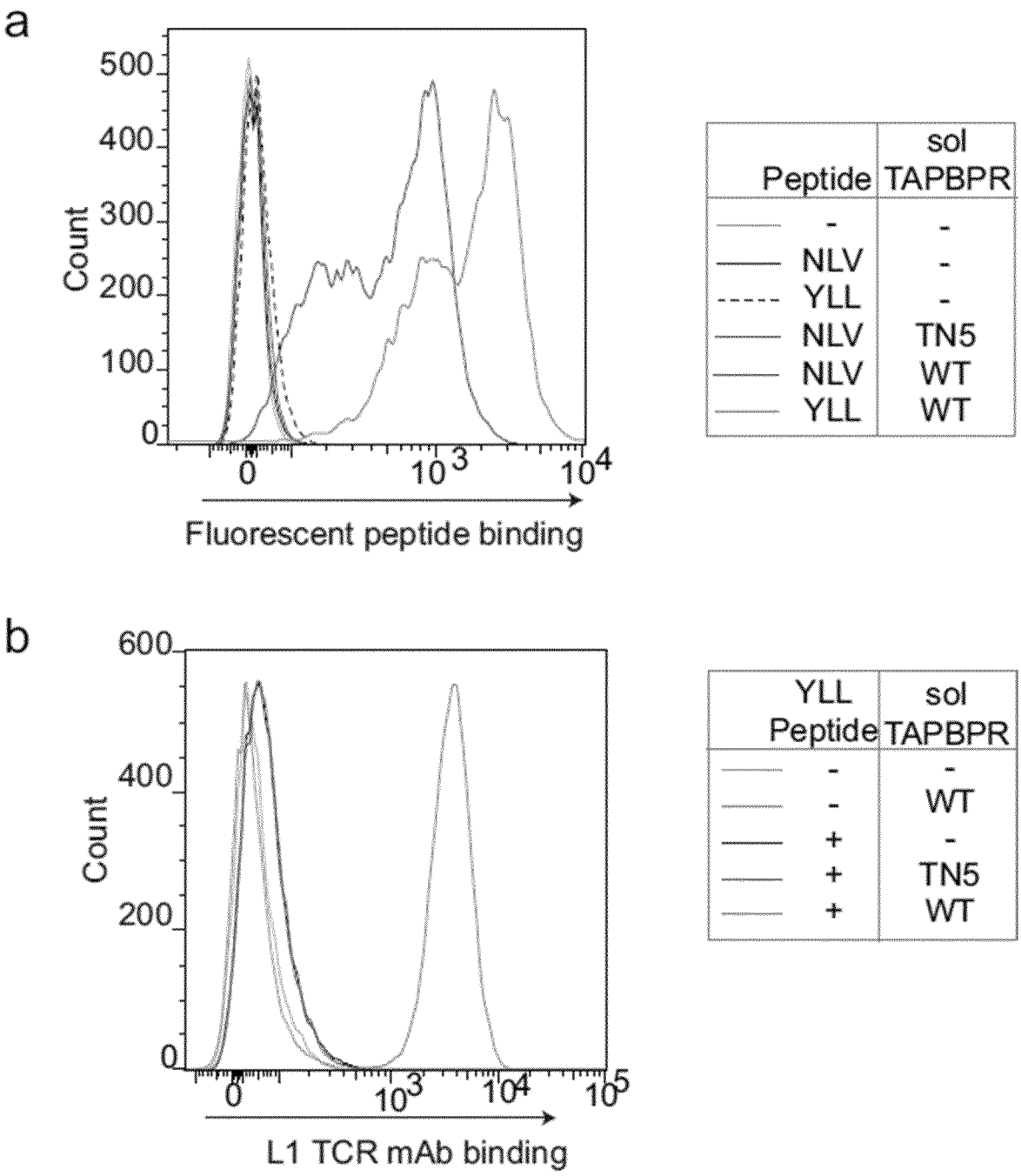

a
500
400
300
200
100
0
Count
0
10 3
10 4
Fluorescent peptide binding
Peptide
sol TAPBPR
-
-
NLV
-
YLL
-
NLV
TN5
NLV
WT
YLL
WT
b
600
400
200
0
Count
0
10 3
10 4
10 5
L1 TCR mAb binding
YLL Peptide
sol TAPBPR
-
-
-
WT
+
-
+
TN5
+
WT

Figure 18

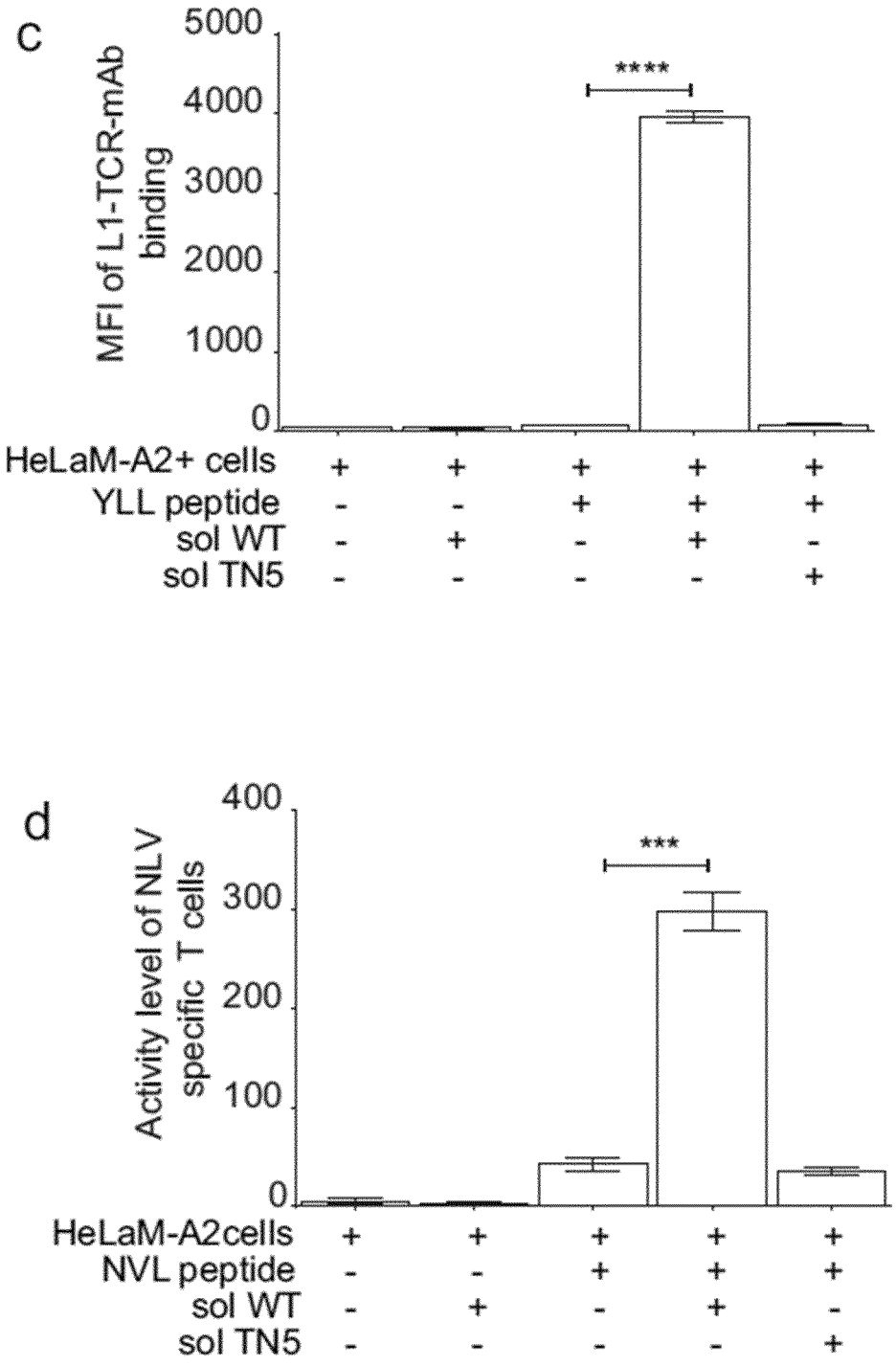


Figure 18 continued

5

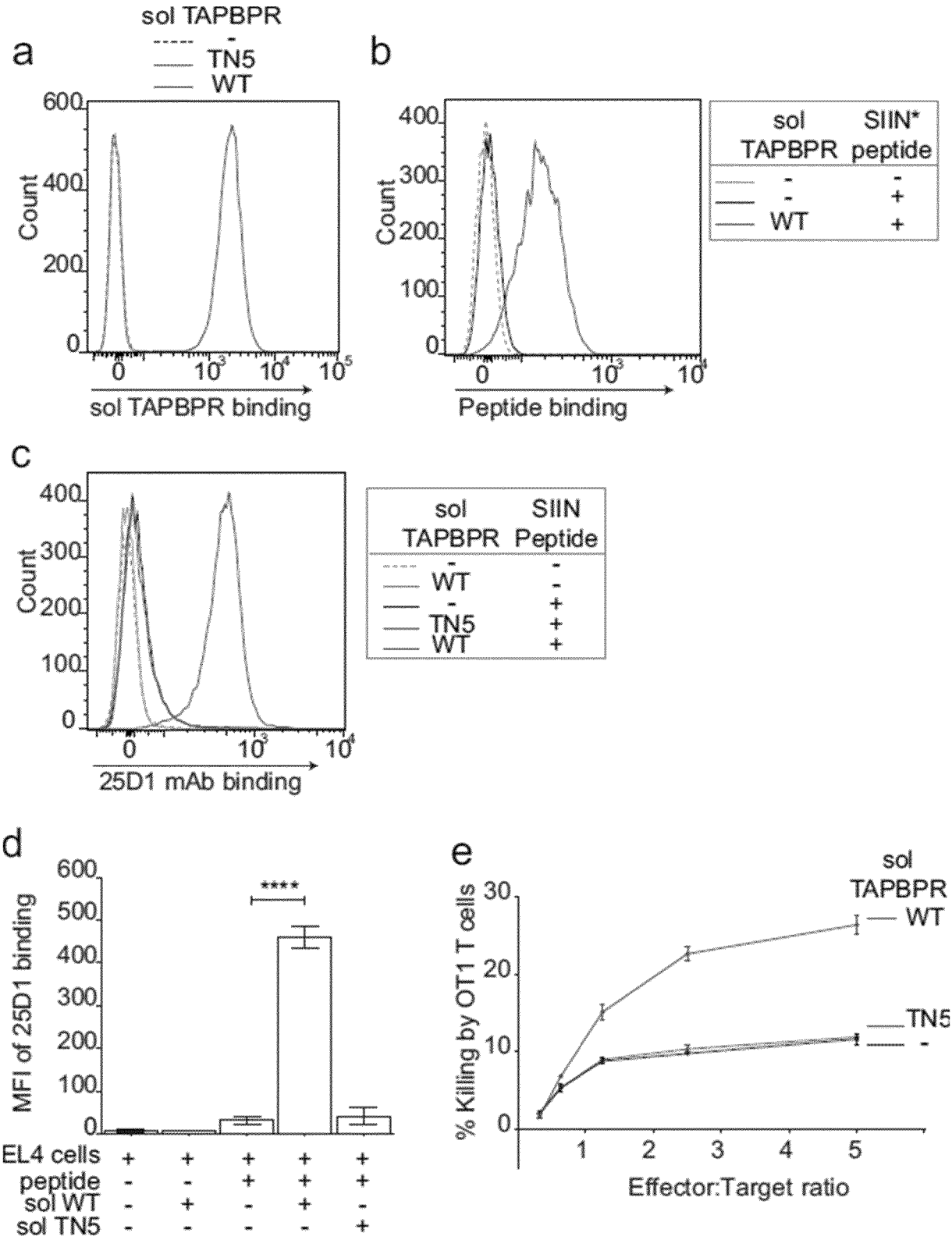

a
sol TAPBPR
-
TN5
WT
Count
sol TAPBPR binding
b
Count
Peptide binding
sol TAPBPR
SIIN* peptide
WT
c
Count
25D1 mAb binding
sol TAPBPR
SIIN Peptide
WT
TN5
d
MFI of 25D1 binding
****
EL4 cells
peptide
sol WT
sol TN5
e
% Killing by OT1 T cells
Effector:Target ratio
sol TAPBPR
WT
TN5

Figure 19

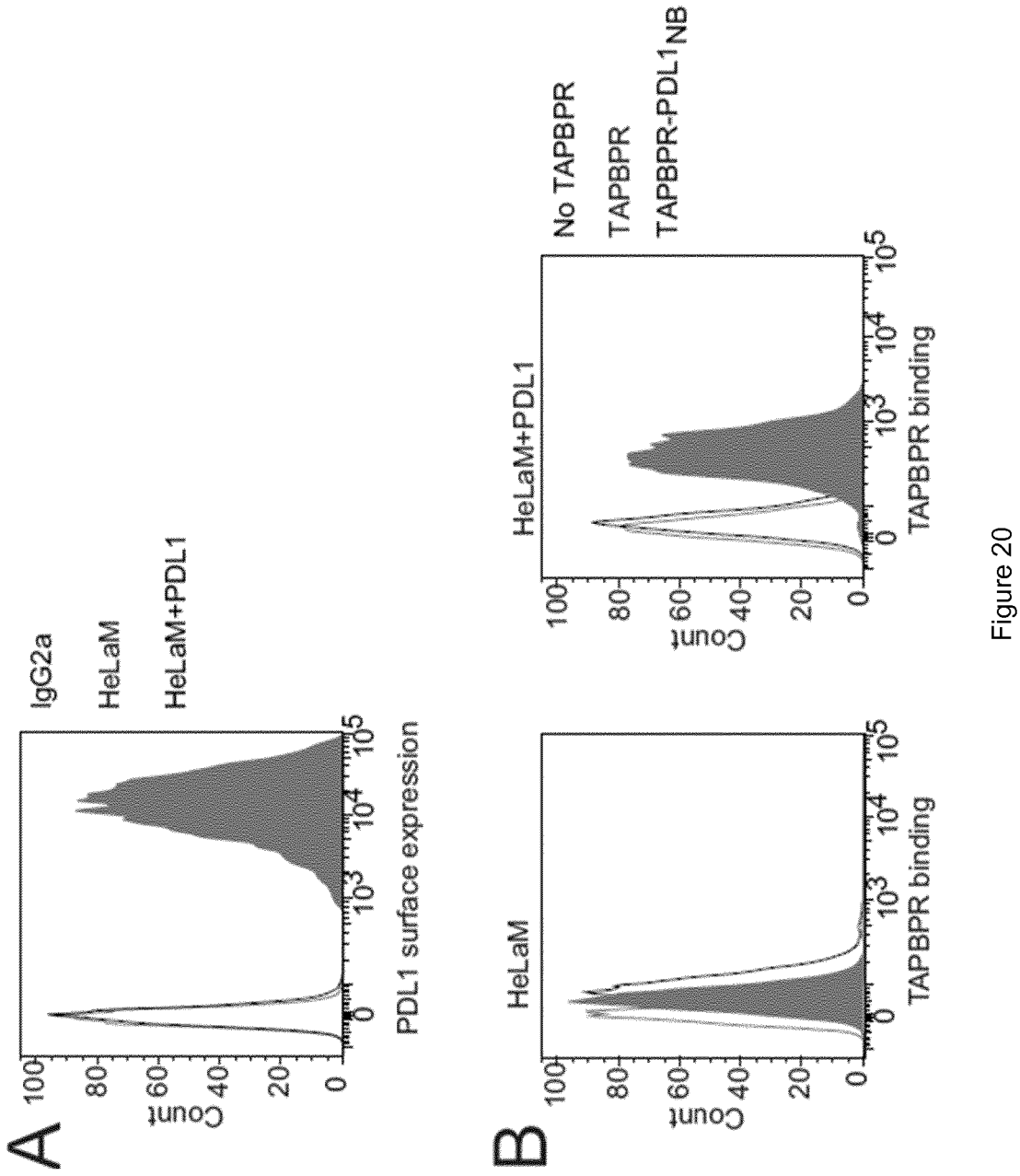

A
IgG2a
HeLaM
HeLaM+PDL1
Count
PDL1 surface expression
B
HeLaM
HeLaM+PDL1
No TAPBPR
TAPBPR
TAPBPR-PDL1NB
TAPBPR binding

Figure 20

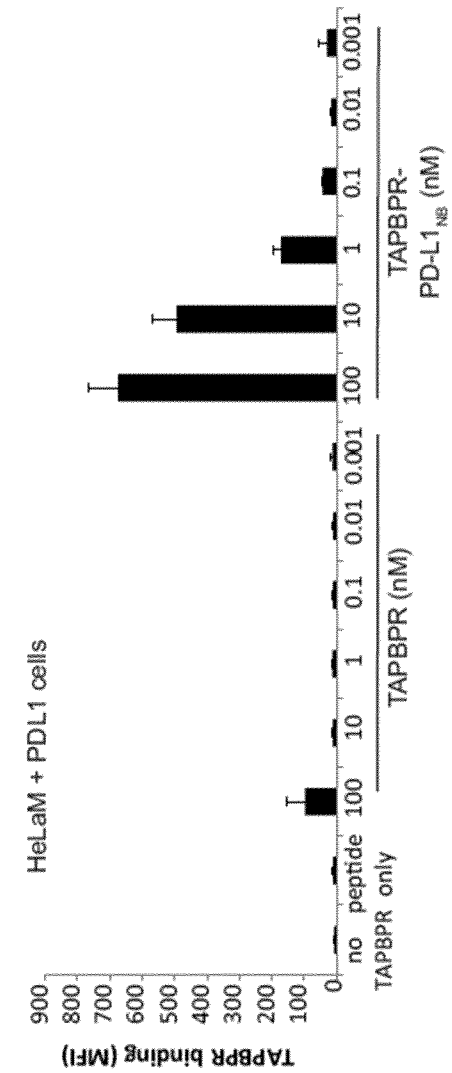
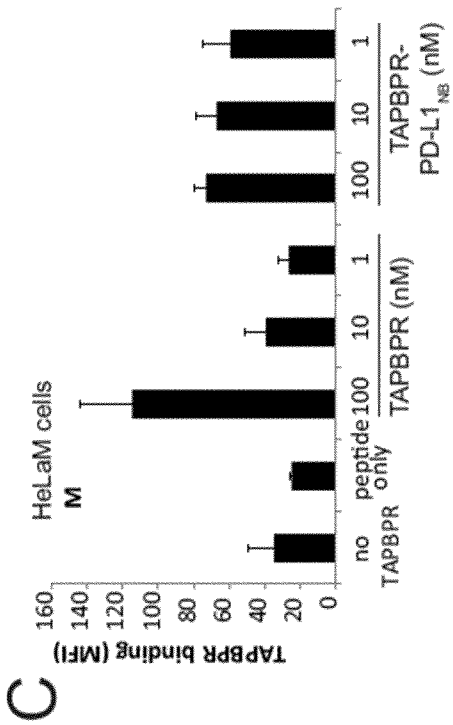


Figure 20 continued



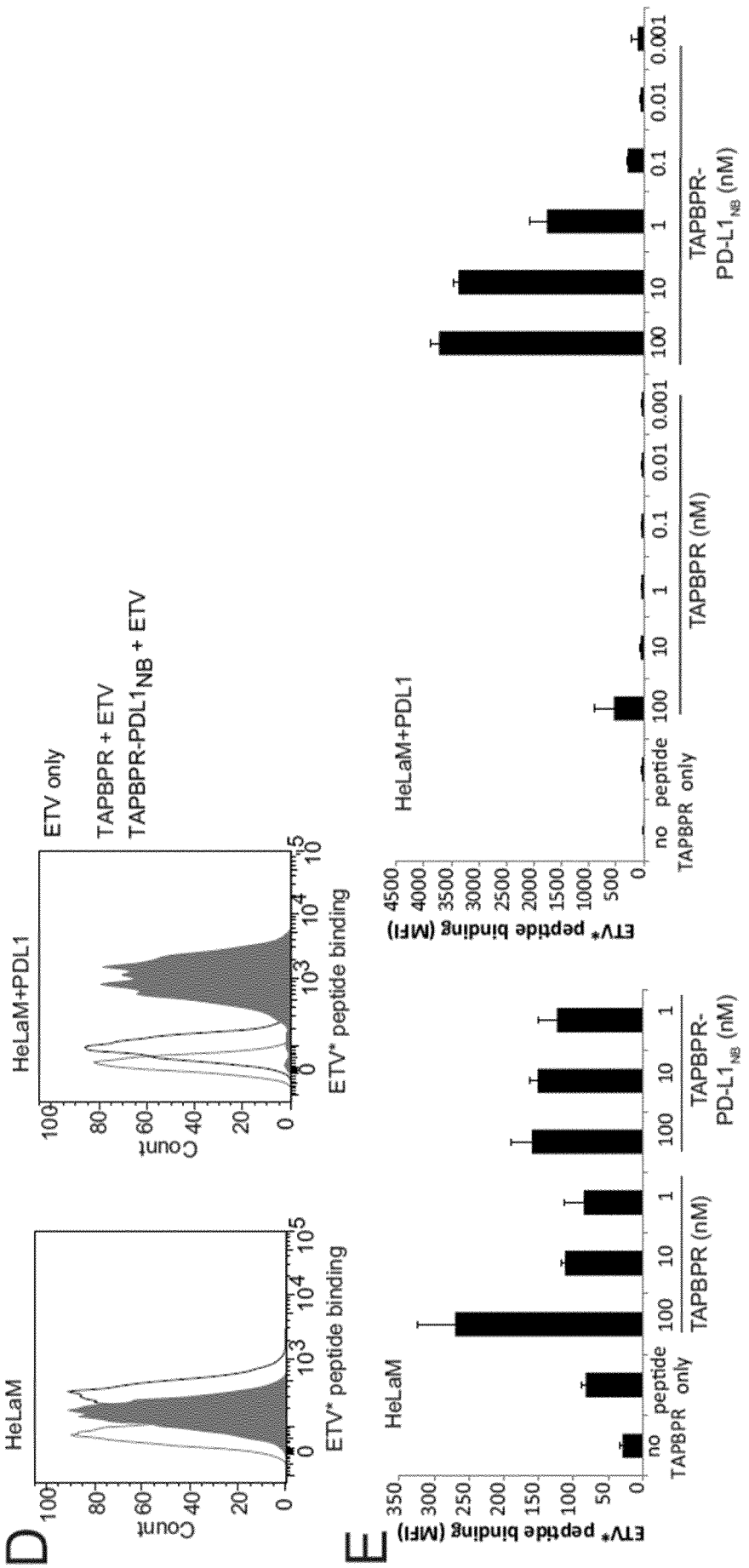


Figure 20 continued

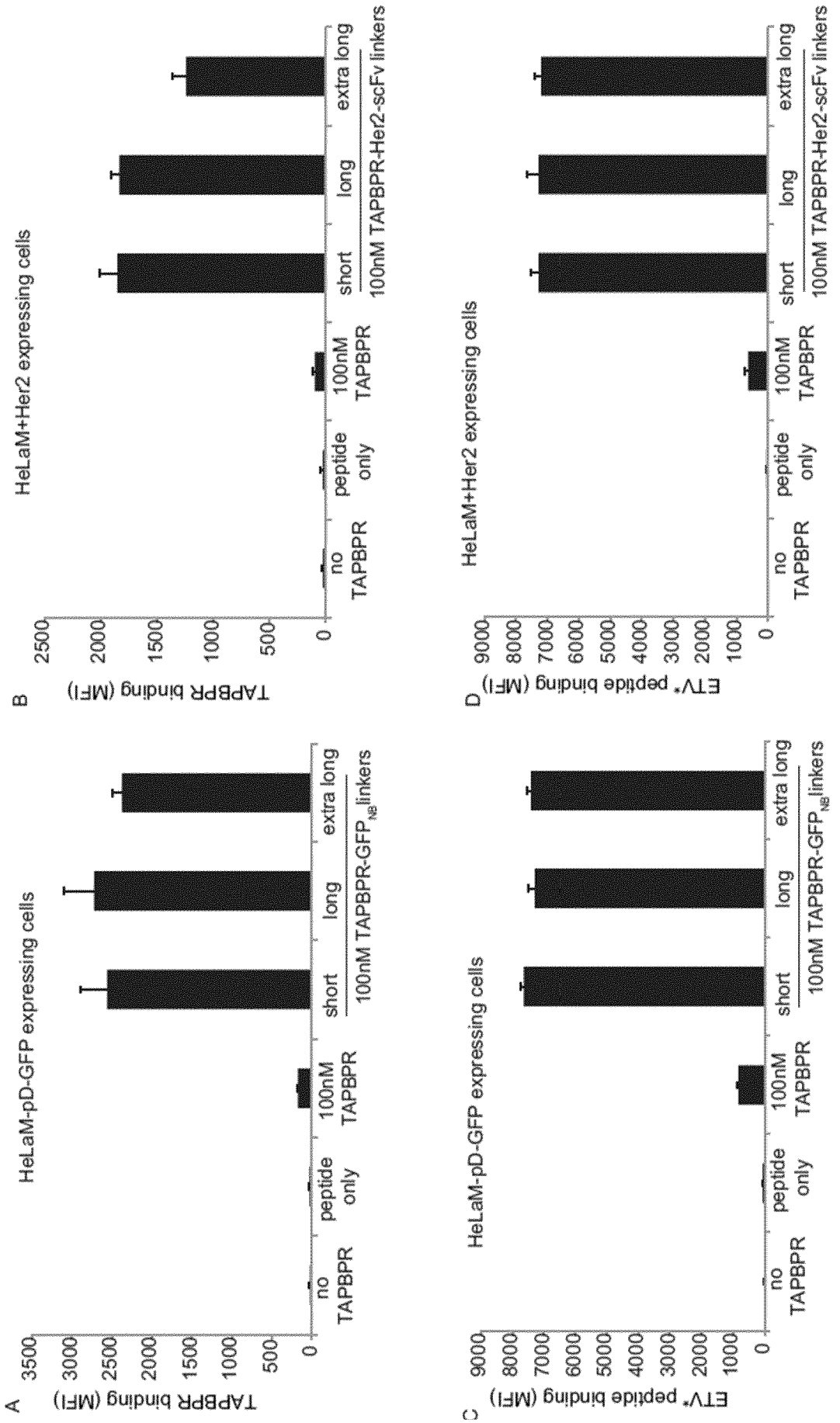

A
HeLaM-pD-GFP expressing cells
TAPBPR binding (MFI)
3500
3000
2500
2000
1500
1000
500
0
no TAPBPR
peptide only
100nM TAPBPR
short
long
extra long
100nM TAPBPR-GFP$_{NB}$ linkers
B
HeLaM+Her2 expressing cells
TAPBPR binding (MFI)
2500
2000
1500
1000
500
0
no TAPBPR
peptide only
100nM TAPBPR
short
long
extra long
100nM TAPBPR-Her2-scFv linkers
C
HeLaM-pD-GFP expressing cells
ETV* peptide binding (MFI)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
no TAPBPR
peptide only
100nM TAPBPR
short
long
extra long
100nM TAPBPR-GFP$_{NB}$ linkers
D
HeLaM+Her2 expressing cells
ETV* peptide binding (MFI)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
no TAPBPR
peptide only
100nM TAPBPR
short
long
extra long
100nM TAPBPR-Her2-scFv linkers

Figure 21

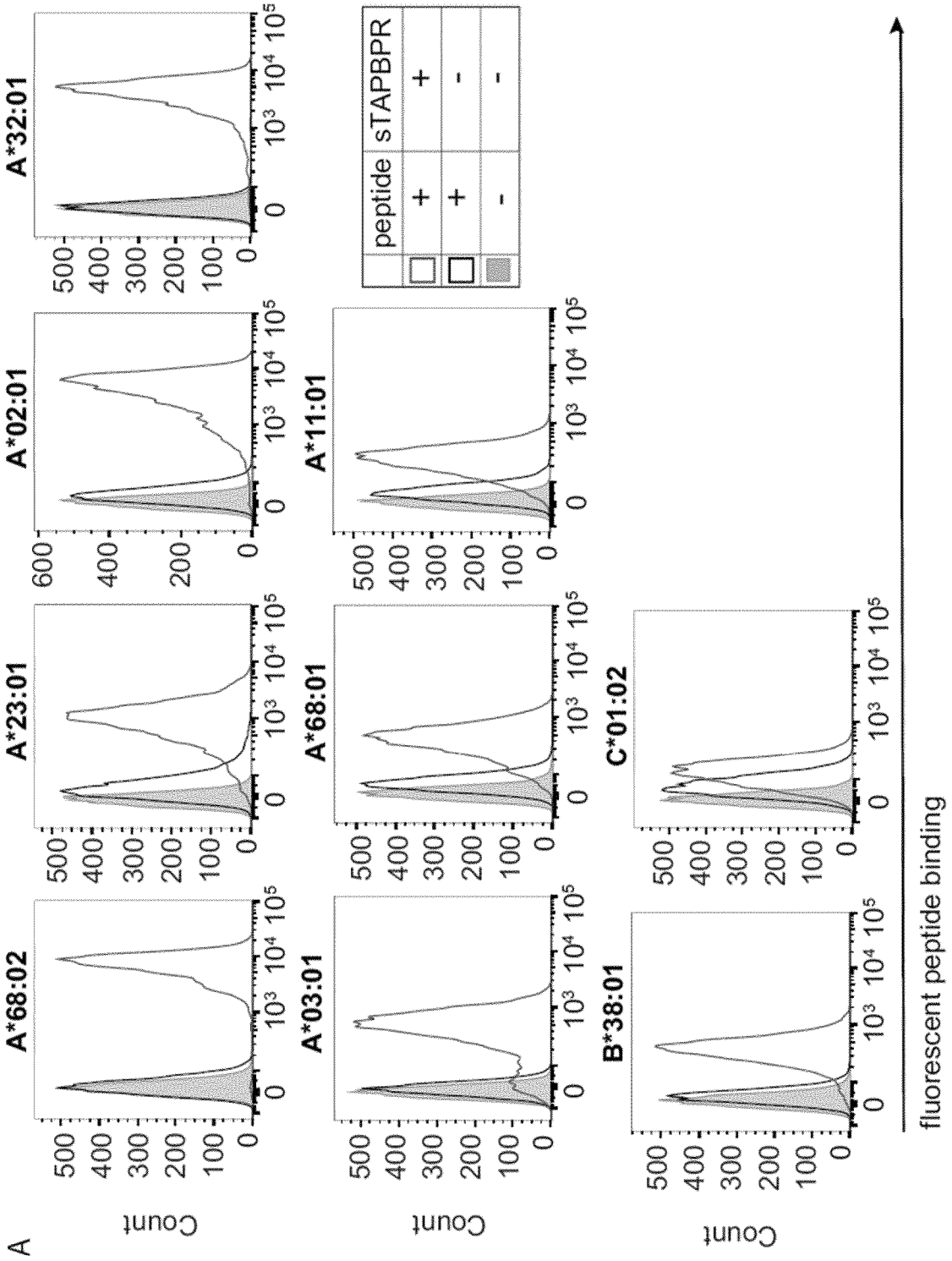

A
A*68:02
A*23:01
A*02:01
A*32:01
A*03:01
A*68:01
A*11:01
B*38:01
C*01:02
Count
peptide
sTAPBPR
fluorescent peptide binding

Figure 22