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(71) Applicant: **THE CHILDREN'S HOSPITAL OF PHILADELPHIA** [US/US]; 3401 Civic Center Boulevard, Philadelphia, PA 19104 (US).

(72) Inventors: **SGOURAKIS, Nikolaos**; 3401 Civic Center Boulevard, Philadelphia, PA 19104 (US). **HWANG, Daniel**; 3401 Civic Center Boulevard, Philadelphia, PA 19104 (US).

(74) Agent: **CRUZ, Richard L.**; Ice Miller, LLP, 1735 Market Street, Suite 3900, Philadelphia, Pennsylvania 19103 (US).

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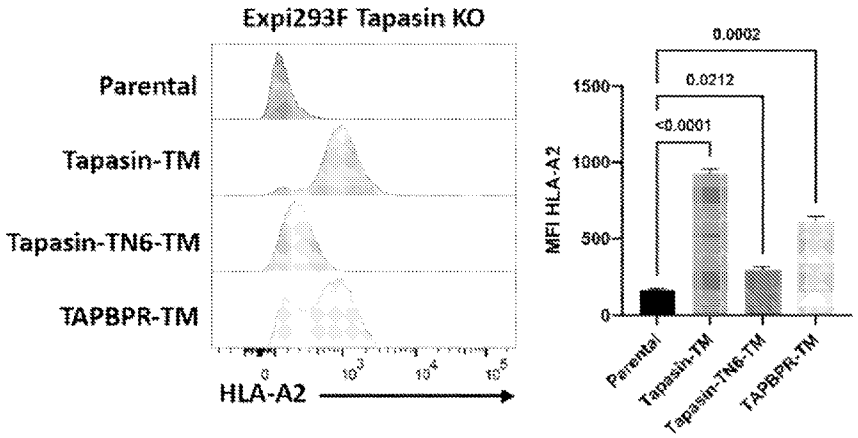


FIG. 1A

(57) Abstract: The present invention relates to engineered variants of chaperone proteins to mediate mature peptide human leukocyte antigen (pHLA) complex production and design and method for making and using the same.

PLATFORM FOR INCREASING HLA CLASS I ANTIGEN PRESENTATION UTILIZING ENGINEERED CHAPERONE FUSION PROTEINS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 63/597,186, filed November 8, 2023. The disclosure of which is herein incorporated by reference in its entirety.

INCORPORATION BY REFERENCE

[0002] All documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0003] This application contains a sequence listing, which is submitted electronically. The contents of the electronic sequence listing (074313.8WO1 Sequence Listing.xml; Size: 21,795; and Date of Creation: November 4, 2024) is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0004] The present invention relates to engineered variants of chaperone proteins to mediate mature peptide human leukocyte antigen (pHLA) complex production.

BACKGROUND OF THE INVENTION

[0005] While T cell-based immunotherapies have brought about a groundbreaking shift in the clinic, their efficacy still fundamentally depends on the immune system's capacity to recognize specific MHC class I molecules presenting peptide neoantigens on the surface of malignant cells. Therefore, mutations to the MHC-I antigen processing and presentation (APP) pathway have emerged as a common mechanism for immune evasion in multiple indications.

[0006] Neuroblastoma is an immunologically "cold tumor" characterized by low mutational burden, leading to a paucity of neoantigens that is further compounded by aberrant MHC-I antigen presentation. Such diseases are highly refractory to T cell-based immunotherapy, including checkpoint blockade, often lacking suitable surface-expressed antigens that can be targeted via chimeric antigen receptors (CARs). While the development of

peptide-centric CARs (which recognize pHLA complexes) can provide access to intracellular targets, they do not address the low antigen density problem, thereby limiting therapeutic efficacy.

[0007] Thus, there is an unmet need for approaches to increase HLA expression levels toward both T- and CAR-T based immunotherapies.

[0008] Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

[0009] The invention relates to methods of increasing surface human leukocyte antigen (HLA) expression which may comprise fusing a nucleic acid encoding a chaperone to a nucleic acid encoding a protein of interest, forming a fused nucleic acid encoding a chaperone fusion protein (CFP), administering the nucleic acid encoding the CFP to a cell, allowing the nucleic acid to express the CFP in the cell, wherein the CFP increases HLA expression in the cell.

[0010] In one embodiment, the chaperone is a HLA class I chaperone. In another embodiment, the chaperone is tapasin or transporter associated with antigen processing (TAP)-binding protein related (TAPBPR). In another embodiment, the tapasin or the TAPBR is fused to a transmembrane (TM) domain. In another embodiment, the TM domain is a human leukocyte antigen (HLA) TM domain. In another embodiment, the HLA is HLA-G.

[0011] In an advantageous embodiment, the nucleic acid encoding the TM domain of HLA-G is SEQ ID NO: 3. In another advantageous embodiment, the nucleic acid encoding the CFP is SEQ ID NO: 4 or SEQ ID NO: 5.

[0012] In one embodiment, the protein of interest is an antigen, advantageously a tumor antigen.

[0013] The invention also relates to an immune adjuvant produced by a T cell in a T cell based immunotherapy comprising a T cell transduced with any of the herein disclosed CFPs. In one embodiment, the T cell based immunotherapy is chimeric antigen receptor (CAR)-T, T-cell receptor (TCR)-T or tumor infiltrating lymphocyte (TIL) therapy.

[0014] The invention also relates to a vaccine adjuvant comprising any of the herein disclosed CFPs. In one embodiment, the vaccine is an anti-viral vaccine or a cancer vaccine.

[0015] The invention also relates to a multivalent molecule comprising any of the herein disclosed CFPs conjugated to a protein or molecule that mediates targeting of the CFP to a target cell. In one embodiment, the protein that mediates targeting of the CFP to a target cell is

a single chain fragment variable (scFV) antibody. In another embodiment, the target cell is a tumor cell or a viral infected cell.

[0016] The invention also encompasses a pharmaceutical composition which may comprise any of the herein disclosed CFPs, any of the herein disclosed immune adjuvants, any of the herein disclosed vaccine adjuvants, or any of the herein disclosed multivalent molecules and a pharmaceutically acceptable carrier.

[0017] The invention also encompasses a method of treating or preventing a disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of any of the herein disclosed CFPs, any of the herein disclosed immune adjuvants, any of the herein disclosed vaccine adjuvants, any of the herein disclosed multivalent molecules or a pharmaceutical composition which may comprise any of the herein disclosed CFPs, any of the herein disclosed immune adjuvants, any of the herein disclosed vaccine adjuvants, or any of the herein disclosed multivalent molecules and a pharmaceutically acceptable carrier.

[0018] The invention also encompasses the use of any of the herein disclosed CFPs, any of the herein disclosed immune adjuvants, any of the herein disclosed vaccine adjuvants, any of the herein disclosed multivalent molecules or a pharmaceutical composition which may comprise any of the herein disclosed CFPs, any of the herein disclosed immune adjuvants, any of the herein disclosed vaccine adjuvants, or any of the herein disclosed multivalent molecules and a pharmaceutically acceptable carrier to increase surface human leukocyte antigen (HLA) expression.

[0019] The invention also encompasses methods for enhancing peptide presentation and identification in tumor cells. The methods comprise (a) contacting tumor cells with a nucleic acid encoding a chaperone fused to a nucleic acid encoding a protein of interest to form a fused nucleic acid encoding a chaperone fusion protein (CFP); (b) expressing the CFP in the tumor cells; (c) upregulating human leukocyte antigen (HLA) expression; (d) capturing and identifying peptides that are bound on the upregulated HLAs. In certain embodiments, the HLA is HLA-I.

[0020] In one embodiment, the chaperone is a HLA class I chaperone. In another embodiment, the chaperone is tapasin or transporter associated with antigen processing (TAP)-binding protein related (TAPBPR). In another embodiment, the tapasin or the TAPBR is fused to a transmembrane (TM) domain. In another embodiment, the TM domain is a human leukocyte antigen (HLA) TM domain. In another embodiment, the HLA is HLA-G.

[0021] In an advantageous embodiment, the nucleic acid encoding the TM domain of HLA-G is SEQ ID NO: 3. In another advantageous embodiment, the nucleic acid encoding the CFP is SEQ ID NO: 4 or SEQ ID NO: 5.

[0022] In one embodiment, the protein of interest is an antigen, advantageously a tumor antigen.

[0023] Accordingly, it is an object of the invention not to encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product. It may be advantageous in the practice of the invention to be in compliance with Art. 53(c) EPC and Rule 28(b) and (c) EPC. All rights to explicitly disclaim any embodiments that are the subject of any granted patent(s) of applicant in the lineage of this application or in any other lineage or in any prior filed application of any third party is explicitly reserved. Nothing herein is to be construed as a promise.

[0024] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0025] These and other embodiments are disclosed or are obvious from and encompassed by the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0027] The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings.

[0028] **FIGS. 1A-1D. CFPs enhance increase HLA-A2 expression in tapasin knockout and neuroblastoma cell lines.** Cell lines were transduced with lentiviral CFP constructs and then assayed for HLA-A2 expression by flow cytometry. **FIG. 1A.** Expression of HLA-A2 in Expi293F Tapasin KO cells and **FIG. 1B.** Expression of FLAG-TAG in Expi293F Tapasin KO cells. **FIG. 1C (top).** EBc1 neuroblastoma cell line. EBc1 cells were transduced with 10 mL of lentivirus per 250k cells. **FIG. 1C (bottom).** Expression of FLAG-TAG in EBc1-GFP Tapasin KO cells. Quantification of median fluorescence intensity (MFI) is shown. Statistical significance was determined by one-way ANOVA followed by multiple comparisons testing versus parental cell lines. **FIG. 1D.** Titration of Tapasin-TM and Tapasin-TN6 lentivirus in EBc1 cells. Quantification of HLA-A2 MFI is shown.

[0029] **FIG. 2. Expression of Tapasin and TAPBPR in neuroblastoma cell lines.** RNA expression of Tapasin and TAPBPR in neuroblastoma cell lines with EBc1 is boxed in red.

[0030] **FIG. 3A-3H. T cell derived CFPs increase efficiency of T cell killing. FIG. 3A** Simultaneous transduction and purification of 1G4 and/or CFP expressing cells. Cells were transduced with 1G4 and CFP lentiviruses. 1G4 expressing cells are labeled with PE-conjugated HLA-A2/NYESO$_{157\text{-}165}$ tetramers and purified with anti-PE microbeads. Similarly, CFP expressing 1G4 T cells are purified using FLAG-TAG antibody (right). Column flow through shows negative population. **FIG. 3B.** To measure killing of EBc-1 tumor cells, they were first labeled with CellTrace Violet as an identifying marker before plating. The following day they were first pulsed with 10ug/mL NYESO$_{157\text{-}165}$, extensively washed, then co-cultured overnight with $CD8^+$ T cells transduced with 1G4 +/- CFPs. Quantification of number of surviving EBc-1 cells is shown. **FIG. 3C.** Expression of 4-1BB on CD8 T cells after co-culture. **FIG. 3D.** Cell surface FLAG expression on EBc1 cells after co-culture with CFP transduced CD8 T cells. **FIG. 3E.** MFI of CD69 on CD8 T cells after co-culture. **FIG. 3F.** Frequency of 4-1BB expression on CD8 T cells. **FIG. 3G.** Frequency of EBc1 cells expressing FLAG on the cell surface after co-culture. **FIG. 3H.** Transduction of NY-ESO-1 peptide and CD58 in SUDHL4 cells which are NY-ESO-1^-/$CD58^-$. Significance for FIGS. 3E-G was determined by one-way ANOVA. Significance for FIG. 3B was determined by two-way ANOVA followed by multiple comparisons testing.

[0031] FIGS. 4A-4F. T cell derived CFPs increase efficiency of T cell killing. FIG. 4A. NYESO-1 specific killing of SUDHL4 cells transduced to express NYESO-1. 1G4 T cells were mixed at different effector to target ratios and incubated overnight with SUDHL4 NYESO-1 cells and SUDHL4 cell transduced with empty vector. NYESO-1 specific killing is calculated by subtracting the number of surviving cells in SUDHL4 NYESO-1 cells from the number of surviving cells in SUDHL4 Empty Vector cells and divided by surviving cells in SUDHL4 Empty Vector. **Fig. 4B.** Number of surviving SUDHL4 NYESO-1 cells with 1G4 T cells and CFPs Tapasin-TM or TAPBPR-TM. **Fig. 4C.** Number of surviving SUDHL4 Empty Vector cells with 1G4 T cells and CFPs Tapasin-TM or TAPBPR-TM. **FIG. 4D.** Frequency of surviving tumor cells expressing FLAG TAG. **Fig. 4E.** MFI of 4-1BB in CD8 T cells: SUDHL4 NYESO-1 on top and SUDHL4 Empty Vector on bottom. **Fig. 4F.** MFI of CD69 in CD8 T cells: SUDHL4 NYESO-1 on top and SUDHL4 Empty Vector on bottom. Significance was determined by two-way ANOVA followed by multiple comparisons testing. Average of n=4 technical replicates is shown.

[0032] FIGs. 5A-5C. CFPs enhance the immunogenicity of neuroblastoma cells. FIG. 5A. Schematic of T cell killing assay using $NYESO_{157-165}$ pulsed EBc-1 cells and 1G4 TCR-T cells. **FIG. 5B.** Number of surviving cells after overnight culture for different effector to target ratios is shown (right). **FIG. 5C.** T cell killing assay using EBc-1 cells transduced with full length NYESO-1 or with a control lentivirus. NYESO-1 specific lysis with a 1:2 E:T ratio is shown. Killing is measured using a flow cytometry based assay. **FIG. 5D.** Kinetics of NYESO-1 specific killing performed as in C but measured with xCELLigence-based impedance measurements.

[0033] FIGs. 6A-6C. Tapasin interactions stabilize MHC-I complexes on the cell surface. Parental EbC1 cells and those transduced with tapasin variants were treated with **(FIG. 6A)** ribosomal inhibitor cycloheximide, **(FIG. 6B)** transport inhibitor brefeldin A, and **(FIG. 6C)** lysosomal inhibitor bafilomycin A1. MFI of surface HLA-A2 is shown.

[0034] FIGs. 7A-7C. CFP expressing T cells kill B cell lymphoma cells more efficiently. SUDHL4 B cell lymphoma cells transduced with NYESO-1 or empty vector were first labeled with CellTrace Violet as an identifying marker before plating. Cells were then co-cultured overnight with $CD8^+$ T cells transduced with 1G4 +/- CFPs. **FIG. 7A.** Quantification of NYESO-1 specific killing is shown. **FIG. 7B.** Expression of 4-1BB on CD8 T cells after co-culture. **FIG. 7C.** Cell surface FLAG expression on SUDHL4 NYESO-1 cells after co-culture with CFP transduced CD8 T cells.

[0035] FIG. 8. CFPs enhance immunoprecipitation of folded HLA-I molecules for immunopeptidomics applications. Parental EBc1 cells and those transduced with tapasin WT, Tapasin-TM CFP, and negative control tapasin-TN6-TM were lysed and samples collected prior to immunoprecipitation as well as following immunoprecipitation with anti-HLA-A2 antibody BB7.2. SDS-PAGE and native gel electrophoresis was performed prior to western blot probing for B2M or vinculin as loading control.

[0036] TABLE 1. Features and Sequences of Tapasin-TM and TAPBPR-TM. The features of Tapasin-TM (SEQ ID NO: 4) and TAPBPR-TM (SEQ ID NO: 5) include the signal peptide of HLA (underlined; SEQ ID NO: 1), FLAG Tag (bolded; SEQ ID NO: 2), and short peptide linker GGS (italicized) at the N'-terminus of both proteins, and the transmembrane (TM) domain of HLA-G (bold and italicized; SEQ ID NO: 3) and a stop codon (indicated by *) at the C'-terminus of both proteins.

Feature:	**Sequence or Symbol:**
Signal peptide of HLA (SEQ ID NO: 1)	MAVMAPRTLVLLLSGALALTQTWAGS
FLAG tag (SEQ ID NO: 2)	DYKDDDDK
Linker	GGS
TM domain of HLA-G (SEQ ID NO: 3)	SGAGSAIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD
Stop Codon	*
Protein:	**Sequence:**
Human Tapasin-TM (SEQ ID NO: 4)	MAVMAPRTLVLLLSGALALTQTWAGS**DYKDDDDK***GGS*GPAVIECWFVEDASG KGLAKRPGALLLRQGPGEPPPRPDLDPELYLSVHDPAGALQAAFRRYPRGAPA PHCEMSRFVPLPASAKWASGLTPAQNCPRALDGAWLMVSISSPVLSLSSLLRP QPEPQQEPVLITMATVVLTVLTHTPAPRVRLGQDALLDLSFAYMPPTSEAASSL APGPPPFGLEWRRQHLGKGHLLLAATPGLNGQMPAAQEGAVAFAAWDDDEP WGPWTGNGTFWLPTVQPFQEGTYLATIHLPYLQGQVTLELAVYKPPKVSLMPA TLARAAPGEAPPELLCLVSHFYPSGGLEVEWELRGGPGGRSQKAEGQRWLSA LRHHSDGSVSLSGHLQPPPVTTEQHGARYACRIHHPSLPASGRSAEVTLEVAG LSGPSLEDS***SGAGSAIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD****
Human TAPBPR-TM (SEQ ID NO: 5)	MAVMAPRTLVLLLSGALALTQTWAGS**DYKDDDDK***GGS*KPHPAEGQWRAVDV VLDCFLVKDGAHRGALASSEDRARASLVLKQVPVLDDGSLEDFTDFQGGTLAQ DDPPIIFEASVDLVQIPQAEALLHADCSGKEVTCEISRYFLQMTETTVKTAAWFM ANVQVSGGGPSISLVMKTPRVAKNEVLWHPTLNLPLSPQGTVRTAVEFQVMTQ TQSLSFLLGSSASLDCGFSMAPGLDLISVEWRLQHKGRGQLVYSWTAGQGQA VRKGATLEPAQLGMARDASLTLPGLTIQDEGTYICQITTSLYRAQQIIQLNIQASP KVRLSLANEALLPTLICDIAGYYPLDVVVTWTREELGGSPAQVSGASFSSLRQS VAGTYSISSSLTAEPGSAGATYTCQVTHISLEEPLGASTQVVPPERR***SGAGSAI PIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD****

[0037] TABLE 2. Sequences of TAPBPR-TM variants and orthologs. The sequences of the luminal portion of TAPBPR orthologs: Human (*Homo sapiens*; reference Q9BX59), Chicken (*Gallus gallus*; reference NP_001382952.1); and Mouse (*Mus musculus*; reference XP_030111162.1); and chicken tapasin (chTapasin; UniProt reference A4F5A9) are listed

(SEQ ID NOs: 6-10, respectively). The combination of the TAPBPR orthologs and chTapasin with the TM domain of HLA-G (bold and italicized; SEQ ID NO: 3) are also listed (SEQ ID NOs. 11-14, respectively), as well as the combination of the TAPBPR orthologs and chTapasin with all features from Table 1: the signal peptide of HLA (underlined; SEQ ID NO: 1), FLAG Tag (bolded; SEQ ID NO: 2), and short peptide linker GGS (italicized) at the N'-terminus of both proteins, and the transmembrane (TM) domain of HLA-G (bold and italicized; SEQ ID NO: 3) (SEQ ID NOs. 5, 15, 16, and 17, respectively).

Protein:	**Sequence:**
Luminal Human TABPR (SEQ ID NO: 6)	KPHPAEGQWRAVDVVLDCFLVKDGAHRGALASSEDRARASLVLKQVPVLDD GSLEDFTDFQGGTLAQDDPPIIFEASVDLVQIPQAEALLHADCSGKEVTCEISR YFLQMTETTVKTAAWFMANVQVSGGGPSISLVMKTPRVAKNEVLWHPTLNL PLSPQGTVRTAVEFQVMTQTQSLSFLLGSSASLDCGFSMAPGLDLISVEWRL QHKGRGQLVYSWTAGQGQAVRKGATLEPAQLGMARDASLTLPGLTIQDEGT YICQITTSLYRAQQIIQLNIQASPKVRLSLANEALLPTLICDIAGYYPLDVVVTWT REELGGSPAQVSGASFSSLRQSVAGTYSISSSLTAEPGSAGATYTCQVTHISL EEPLGASTQVVPPERR
Luminal Chicken TABPR (SEQ ID NO: 7)	VEGLTPVPELRRVDVVLGCSYVWEGGLSRAFGGSEHPATLVLRGLSVTDDG TLGDVTDYEIPQADHSSSPPIIFEASEQLVSIPYAEALLHVDASGEEVSCELSP YSFQQEGNGLCSASWFLATIRLSSGISIVLLLRGPSCSSQKEGHDVTLHPKLRI PMSKEGTLLTTVEFQSSSNNTSLRTRLGSSITLDCHFALAPSFLLSSLEWRRQ HRGSGRSLFRYRVGNAGLTAQPKVHVDVEQLLGNGDASLTLQEATVNDEGT YICLVSTAQHQVQHNIQLLVSEPPRVRVFPTEASLKRDETITLTCNIAGYYPLDI SVSWTQKTPEDEVEISPSNTRFSSHRQSQDGTYSINSYLSVNLATAQAPATY TCHVSHVALEAPISISTHLKAPEHTELE
Luminal Mouse TABPR (SEQ ID NO: 8)	DPPTAPTTAERQRQPTDIILDCFLVTEDRHRGAFASSGDRERALLVLKQVPVL DDGSLEGITDFQGSTETKQDSPVIFEASVDLVQIPQAEALLHADCSGKAVTCEI SKYFLQARQEATFEKAHWFISNMQVSRGGPSVSMVMKTLRDAEVGAVRHPT LNLPLSAQGTVKTQVEFQVTSETQTLNHLLGSSVSLHCSFSMAPGLDLTGVE WRLQHKGSGQLVYSWKTGQGQAKRKGATLEPEELLRAGNASLTLPNLTLKD EGNYICQISTSLYQAQQIMPLNILAPPKIQLHLANKDPLPSLVCSIAGYYPLDVG VTWIREELGGIPAQVSGASFSSLRQSTMGTYSISSTVMADPGPTGATYTCQV AHVSLEEPLTTSMRVLPNPEQR
Chicken tapasin (full sequence) (SEQ ID NO: 9)	MAAGLRLLLAGLCWSQFRVEDAASPPPPPAPVRCALLEGVGRGGGLPGGG NARPALLRFGGDAETPPEPGPEPEVTFNVSDPWGTLTPLGVPPRTPPSCELN PTNPQTGSDPWSRPLHPDARSPPTAGGQWWVAAVGTPQYGVTALLQGGM GTEGTITAAVALAVLTHTPTLRARVGSPIHLHCAFAAPPSSFVLEWRHQNRGA GRVLLAYDSSTARAPRATPGAELLLGTRDGDGVTAVTLRLARPSPGDEGTYI CSVFLPHGHTQTVLQLHVFEPPKVTLSPKNLVVAPGMSAELRCHVSGFYPLD VTVTWQRRAGGSGTSRSPRDTVMDSWTSGHRQAADGTYSRTAAARLIPAR PQHHGDVYSCVVTHTALAKPMRVSVRLLLAGTEGPHLEDITGLFLVAFVLCGL IRWLYPKAARPKEETKKSQ
Chick tapasin without native transmembrane (SEQ ID NO: 10)	MAAGLRLLLAGLCWSQFRVEDAASPPPPPAPVRCALLEGVGRGGGLPGGG NARPALLRFGGDAETPPEPGPEPEVTFNVSDPWGTLTPLGVPPRTPPSCELN PTNPQTGSDPWSRPLHPDARSPPTAGGQWWVAAVGTPQYGVTALLQGGM GTEGTITAAVALAVLTHTPTLRARVGSPIHLHCAFAAPPSSFVLEWRHQNRGA GRVLLAYDSSTARAPRATPGAELLLGTRDGDGVTAVTLRLARPSPGDEGTYI CSVFLPHGHTQTVLQLHVFEPPKVTLSPKNLVVAPGMSAELRCHVSGFYPLD VTVTWQRRAGGSGTSRSPRDTVMDSWTSGHRQAADGTYSRTAAARLIPAR PQHHGDVYSCVVTHTALAKPMRVSVRLLL
Protein + HLA-G TM only:	**Sequence:**
Luminal Human TAPBPR + HLA-G TM (SEQ ID NO: 11)	KPHPAEGQWRAVDVVLDCFLVKDGAHRGALASSEDRARASLVLKQVPVLDD GSLEDFTDFQGGTLAQDDPPIIFEASVDLVQIPQAEALLHADCSGKEVTCEISR YFLQMTETTVKTAAWFMANVQVSGGGPSISLVMKTPRVAKNEVLWHPTLNL

	PLSPQGTVRTAVEFQVMTQTQSLSFLLGSSASLDCGFSMAPGLDLISVEWRL QHKGRGQLVYSWTAGQGQAVRKGATLEPAQLGMARDASLTLPGLTIQDEGT YICQITTSLYRAQQIIQLNIQASPKVRLSLANEALLPTLICDIAGYYPLDVVVTWT REELGGSPAQVSGASFSSLRQSVAGTYSISSSLTAEPGSAGATYTCQVTHISL EEPLGASTQVVPPERR***SGAGSAIPIMGIVAGLVVLAAVVTGAAVAAVLWRKK SSD***
Luminal Chicken TABPR + HLA-G TM (SEQ ID NO: 12)	VEGLTPVPELRRVDVVLGCSYVWEGGLSRAFGGSEHPATLVLRGLSVTDDG TLGDVTDYEIPQADHSSSPPIIFEASEQLVSIPYAEALLHVDASGEEVSCELSP YSFQQEGNGLCSASWFLATIRLSSGISIVLLLRGPSCSSQKEGHDVTLHPKLRI PMSKEGTLLTTVEFQSSSNNTSLRTRLGSSITLDCHFALAPSFLLSSLEWRRQ HRGSGRSLFRYRVGNAGLTAQPKVHVDVEQLLGNGDASLTLQEATVNDEGT YICLVSTAQHQVQHNIQLLVSEPPRVRVFPTEASLKRDETITLTCNIAGYYPLDI SVSWTQKTPEDEVEISPSNTRFSSHRQSQDGTYSINSYLSVNLATAQAPATY TCHVSHVALEAPISISTHLKAPEHTELE***SGAGSAIPIMGIVAGLVVLAAVVTGA AVAAVLWRKKSSD***
Luminal Mouse TABPR + HLA-G TM (SEQ ID NO: 13)	DPPTAPTTAERQRQPTDIILDCFLVTEDRHRGAFASSGDRERALLVLKQVPVL DDGSLEGITDFQGSTETKQDSPVIFEASVDLVQIPQAEALLHADCSGKAVTCEI SKYFLQARQEATFEKAHWFISNMQVSRGGPSVSMVMKTLRDAEVGAVRHPT LNLPLSAQGTVKTQVEFQVTSETQTLNHLLGSSVSLHCSFSMAPGLDLTGVE WRLQHKGSGQLVYSWKTGQGQAKRKGATLEPEELLRAGNASLTLPNLTLKD EGNYICQISTSLYQAQQIMPLNILAPPKIQLHLANKDPLPSLVCSIAGYYPLDVG VTWIREELGGIPAQVSGASFSSLRQSTMGTYSISSTVMADPGPTGATYTCQV AHVSLEEPLTTSMRVLPNPEQR***SGAGSAIPIMGIVAGLVVLAAVVTGAAVAA VLWRKKSSD***
Chick tapasin + HLA-G TM (SEQ ID NO: 14)	MAAGLRLLLAGLCWSQFRVEDAASPPPPPAPVRCALLEGVGRGGGLPGGG NARPALLRFGGDAETPPEPGPEPEVTFNVSDPWGTLTPLGVPPRTPPSCELN PTNPQTGSDPWSRPLHPDARSPPTAGGQWWVAAVGTPQYGVTALLQGGM GTEGTITAAVALAVLTHTPTLRARVGSPIHLHCAFAAPPSSFVLEWRHQNRGA GRVLLAYDSSTARAPRATPGAELLLGTRDGDGVTAVTLRLARPSPGDEGTYI CSVFLPHGHTQTVLQLHVFEPPKVTLSPKNLVVAPGMSAELRCHVSGFYPLD VTVTWQRRAGGSGTSRSPRDTVMDSWTSGHRQAADGTYSRTAAARLIPAR PQHHGDVYSCVVTHTALAKPMRVSVRLLL***SGAGSAIPIMGIVAGLVVLAAVVT GAAVAAVLWRKKSSD***
Protein + all features:	**Sequence:**
Human TAPBPR-TM (SEQ ID NO: 5)	MAVMAPRTLVLLLSGALALTQTWAGS**DYKDDDDK***GGS*KPHPAEGQWRAVD VVLDCFLVKDGAHRGALASSEDRARASLVLKQVPVLDDGSLEDFTDFQGGTL AQDDPPIIFEASVDLVQIPQAEALLHADCSGKEVTCEISRYFLQMTETTVKTAA WFMANVQVSGGGPSISLVMKTPRVAKNEVLWHPTLNLPLSPQGTVRTAVEF QVMTQTQSLSFLLGSSASLDCGFSMAPGLDLISVEWRLQHKGRGQLVYSWT AGQGQAVRKGATLEPAQLGMARDASLTLPGLTIQDEGTYICQITTSLYRAQQII QLNIQASPKVRLSLANEALLPTLICDIAGYYPLDVVVTWTREELGGSPAQVSG ASFSSLRQSVAGTYSISSSLTAEPGSAGATYTCQVTHISLEEPLGASTQVVPP ERR***SGAGSAIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD***
Chicken TABPR-TM (SEQ ID NO: 15)	MAVMAPRTLVLLLSGALALTQTWAGS**DYKDDDDK***GGS*VEGLTPVPELRRVD VVLGCSYVWEGGLSRAFGGSEHPATLVLRGLSVTDDGTLGDVTDYEIPQAD HSSSPPIIFEASEQLVSIPYAEALLHVDASGEEVSCELSPYSFQQEGNGLCSA SWFLATIRLSSGISIVLLLRGPSCSSQKEGHDVTLHPKLRIPMSKEGTLLTTVEF QSSSNNTSLRTRLGSSITLDCHFALAPSFLLSSLEWRRQHRGSGRSLFRYRV GNAGLTAQPKVHVDVEQLLGNGDASLTLQEATVNDEGTYICLVSTAQHQVQH NIQLLVSEPPRVRVFPTEASLKRDETITLTCNIAGYYPLDISVSWTQKTPEDEV EISPSNTRFSSHRQSQDGTYSINSYLSVNLATAQAPATYTCHVSHVALEAPISI STHLKAPEHTELE***SGAGSAIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD***
Mouse TABPR-TM (SEQ ID NO: 16)	MAVMAPRTLVLLLSGALALTQTWAGS**DYKDDDDK***GGS*DPPTAPTTAERQRQ PTDIILDCFLVTEDRHRGAFASSGDRERALLVLKQVPVLDDGSLEGITDFQGS TETKQDSPVIFEASVDLVQIPQAEALLHADCSGKAVTCEISKYFLQARQEATFE KAHWFISNMQVSRGGPSVSMVMKTLRDAEVGAVRHPTLNLPLSAQGTVKTQ VEFQVTSETQTLNHLLGSSVSLHCSFSMAPGLDLTGVEWRLQHKGSGQLVY SWKTGQGQAKRKGATLEPEELLRAGNASLTLPNLTLKDEGNYICQISTSLYQA QQIMPLNILAPPKIQLHLANKDPLPSLVCSIAGYYPLDVGVTWIREELGGIPAQV

	SGASFSSLRQSTMGTYSISSTVMADPGPTGATYTCQVAHVSLEEPLTTSMRV LPNPEQR***SGAGSAIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSSD***
Chick tapasin-TM (SEQ ID NO: 17)	MAVMAPRTLVLLLSGALALTQTWAGS**DYKDDDDK***GGS*MAAGLRLLLAGLCW SQFRVEDAASPPPPPAPVRCALLEGVGRGGGLPGGGNARPALLRFGGDAET PPEPGPEPEVTFNVSDPWGTLTPLGVPPRTPPSCELNPTNPQTGSDPWSRP LHPDARSPPTAGGQWWVAAVGTPQYGVTALLQGGMGTEGTITAAVALAVLT HTPTLRARVGSPIHLHCAFAAPPSSFVLEWRHQNRGAGRVLLAYDSSTARAP RATPGAELLLGTRDGDGVTAVTLRLARPSPGDEGTYICSVFLPHGHTQTVLQ LHVFEPPKVTLSPKNLVVAPGMSAELRCHVSGFYPLDVTVTWQRRAGGSGT SRSPRDTVMDSWTSGHRQAADGTYSRTAAARLIPARPQHHGDVYSCVVTHT ALAKPMRVSVRLLL***SGAGSAIPIMGIVAGLVVLAAVVTGAAVAAVLWRKKSS D***

DETAILED DESCRIPTION OF THE INVENTION

[0038] Tapasin, a member of the PLC, and a related protein TAPBPR are HLA class I chaperones that are frequently mutated or dysregulated in cancers [Shionoya, Y., et al., Loss of tapasin in human lung and colon cancer cells and escape from tumor-associated antigen-specific CTL recognition. Oncoimmunology, 2017. 6(2): p. e1274476. PMC5353923 PMID: 28344889]. These chaperones have three major functions in mediating production of mature pHLA: 1) directly stabilizing nascent HLA molecules, which are intrinsically unstable when empty; 2) enabling exchange of low affinity peptides for high affinity ones (peptide editing), and 3) bridge the gap with the TAP transporter which translocates peptides into the ER for loading on HLA-I [Paulsson, K.M., et al., Association of tapasin and COPI provides a mechanism for the retrograde transport of major histocompatibility complex (MHC) class I molecules from the Golgi complex to the endoplasmic reticulum. J Biol Chem, 2002. 277(21): p. 18266-71. PMID: 11884415, Grandea, A.G., 3rd and L. Van Kaer, Tapasin: an ER chaperone that controls MHC class I assembly with peptide. Trends Immunol, 2001. 22(4): p. 194-9. PMID: 11274924]. Cells lacking tapasin have reduced HLA class I expression on the cell surface and a less focused peptide repertoire, with diminished loading of high-affinity peptides [Reeves, E. and E. James, Antigen processing and immune regulation in the response to tumours. Immunology, 2017. 150(1): p. 16-24. PMC5341504 PMID: 27658710]. TAPBPR is an auxiliary chaperone that is not restricted to the PLC but instead actively participates in quality control through the reglycosylation of sub optimally loaded MHC molecules by recruiting them to UGGT1 [Neerincx, A., et al., TAPBPR bridges UDP-glucose:glycoprotein glucosyltransferase 1 onto MHC class I to provide quality control in the antigen presentation pathway. Elife, 2017. 6. PMC5441866 PMID: 28425917]. These critical functions of HLA-I chaperones in regulating antigen presentation, and by extension immunosurveillance, are frequently disrupted across cancer types to evade immune detection [Shionoya, Y., et al., Loss

of tapasin in human lung and colon cancer cells and escape from tumor-associated antigen-specific CTL recognition. Oncoimmunology, 2017. 6(2): p. e1274476. PMC5353923 PMID: 28344889]. It therefore follows that the immunogenicity of cold tumors may be restored by increasing the density of tumor antigens presented via HLA class I at the cell surface. In theory this may be accomplished either by increasing the quantity of pHLA to reach the cell surface and/or focusing the peptide repertoire such that tumor antigens are presented at higher frequency relative to other less immunogenic peptides. Indeed, increasing the quantity of pHLA at the cell surface, or exchange of peptides for tumor antigens enhances the ability for CTLs to recognize and kill tumor cells [Chen, X., et al., A membrane-associated MHC-I inhibitory axis for cancer immune evasion. Cell, 2023. 186(18): p. 3903-3920 e21. PMID: 37557169].

[0039] Here Applicants propose a novel approach to increase surface HLA class I expression by expanding the function of chaperones which mediate mature pHLA complex production, effectively enhancing the endogenous APP pathway. Applicants have engineered a library of the chaperones tapasin and TAPBPR, including HLA allotype-agnostic variants, which travel along the secretory pathway together with HLA molecules, enabling them to exert their chaperoning function in non-native subcellular compartments, including at the cell surface. Applicants show that these Chaperone Fusion Proteins (CFPs) can increase HLA-A2 expression in multiple cell lines, including in EBc-1 neuroblastoma cells. Furthermore, Applicants show that CFPs can be produced and delivered by activated T cells, resulting in enhanced T cell recognition, and pHLA-restricted killing of target cells. This platform may be used in any context in which antigen presentation is dysregulated or would need enhancement. These applications may include the following:

[0040] As an immune adjuvant produced by T cells in T cell-based immunotherapy, including but not limited to CAR-T, TCR-T or tumor infiltrating lymphocyte (TIL) therapies. In this application, T cells are transduced and stably produce CFPs. These cells then transfer CFPs via extracellular vesicles, resulting in surface expression of the CFP (FIG. 2). This then enhances the immunological recognition of the target by the T cell, as evidenced by enhanced killing (FIG. 2)

[0041] As a vaccine adjuvant: Optimal induction of a vaccine-mediated immune response requires presentation of antigens to T cells. This both applies to anti-viral and cancer vaccines. Thus, CFPs may be included as a component of vaccines that can adjuvant a cell-mediated response.

[0042] As used herein, the term "multivalent" refers to an engineered molecule that incorporates two or more biologically active segments. The protein fragments forming the multivalent molecule optionally may be linked through a linker or a spacer which attaches the constituent parts and permits each to function independently. The linker or a spacer allow separate domains to fold independent of each other. In one embodiment, the linker is a protein linker which may be alanine, glycine, proline or serine-based linkers or a combination thereof and may be as short as 8 amino acids to as long as 50 amino acids in length.

[0043] As a component of a multivalent molecule for enhancing antigen presentation in target cells. In this application, CFPs are conjugated to another protein that mediates targeting of the CFP to the target cell (e.g. tumor or viral infected cells). For example, single chain fragment variable (scFv) antibodies can be conjugated to CFPs. The scFv mediates targeting of the CFP to the target cell (e.g. tumor or viral infected cells), allowing for target cell specific upregulation of HLA.

[0044] The present invention is described with reference to particular embodiments having various features. It will be apparent to those skilled in the art that various modifications and variations can be made in the practice of the present invention without departing from the scope or spirit of the invention. One skilled in the art will recognize that these features may be used singularly or in any combination based on the requirements and specifications of a given application or design. One skilled in the art will recognize that the systems and devices of embodiments of the invention can be used with any of the methods of the invention and that any methods of the invention can be performed using any of the systems and devices of the invention. Embodiments comprising various features may also consist of or consist essentially of those various features. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention. The description of the invention provided is merely exemplary in nature and, thus, variations that do not depart from the essence of the invention are intended to be within the scope of the invention.

[0045] Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

[0046] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as would be commonly understood or used by one of ordinary skill in the art encompassed by this technology and methodologies.

[0047] The invention provides a nucleic acid molecule comprising a nucleic acid sequence encoding any of the peptides or proteins of the invention. The nucleic acid may be cDNA. Such a nucleic acid molecule can be synthesized in accordance with methods known in the art. Due to the degeneracy of the genetic code, one of ordinary skill in the art will appreciate that nucleic acid molecules of different nucleotide sequence can encode the same amino acid sequence.

[0048] The invention provides a vector comprising a nucleic acid sequence according to the third aspect of the invention. The vector may include, in addition to a nucleic acid sequence encoding only a peptide of the invention, one or more additional nucleic acid sequences encoding one or more additional peptides. Such additional peptides may, once expressed, be fused to the N-terminus or the C-terminus of the peptide of the invention. In one embodiment, the vector includes a nucleic acid sequence encoding a peptide or protein tag such as, for example, a biotinylation site, a FLAG-tag, a MYC-tag, an HA-tag, a GST-tag, a Strep-tag or a poly-histidine tag.

[0049] Suitable vectors are known in the art as is vector construction, including the selection of promoters and other regulatory elements, such as enhancer elements. The vector utilized in the context of the present invention desirably comprises sequences appropriate for introduction into cells. For instance, the vector may be an expression vector, a vector in which the coding sequence of the polypeptide is under the control of its own cis-acting regulatory elements, a vector designed to facilitate gene integration or gene replacement in host cells, and the like.

[0050] A used herein, a "vector" is a tool that allows or facilitates the transfer of an entity from one environment to another. It is a replicon, such as a plasmid, phage, or cosmid, into which another DNA segment may be inserted so as to bring about the replication of the inserted segment. Generally, a vector is capable of replication when associated with the proper control elements. In general, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. Vectors include, but are not limited to, nucleic acid molecules that are single-chain, single-stranded, double-stranded, or partially double-stranded; nucleic acid molecules that comprise one or more free ends, no free ends (e.g. circular); nucleic acid molecules that comprise DNA, RNA, or both; and other varieties of polynucleotides known in the art. One type of vector is a "plasmid," which refers to a circular

double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Another type of vector is a viral vector, wherein virally-derived DNA or RNA sequences are present in the vector for packaging into a virus (e.g. retroviruses, replication defective retroviruses, adenoviruses, replication defective adenoviruses, and adeno-associated viruses (AAVs)). Viral vectors also include polynucleotides carried by a virus for transfection into a host cell. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g. bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors." Common expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In an advantageous embodiment, the vector is a lentivector.

[0051] Recombinant expression vectors can comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory elements, which may be selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory element(s) in a manner that allows for expression of the nucleotide sequence (e.g. in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). With regards to recombination and cloning methods, mention is made of U.S. patent application Ser. No. 10/815,730, published Sep. 2, 2004 as US 2004-0171156 A1, the contents of which are herein incorporated by reference in their entirety.

[0052] The term "cancer" according to the invention comprises leukemias, seminomas, melanomas, teratomas, lymphomas, neuroblastomas, glioblastomas, gliomas, rectal cancer, endometrial cancer, kidney cancer, adrenal cancer, thyroid cancer, blood cancer, skin cancer, cancer of the brain, cervical cancer, intestinal cancer, liver cancer, colon cancer, stomach cancer, intestine cancer, head and neck cancer, gastrointestinal cancer, lymph node cancer, esophagus cancer, colorectal cancer, pancreas cancer, ear, nose and throat (ENT) cancer, breast cancer, prostate cancer, cancer of the uterus, ovarian cancer and lung cancer and the metastases thereof. Examples thereof are lung carcinomas, mamma carcinomas, prostate carcinomas,

colon carcinomas, renal cell carcinomas, cervical carcinomas, or metastases of the cancer types or tumors described above. The term cancer according to the invention also comprises cancer metastases and relapse of cancer.

[0053] The term "autoimmunity" according to the invention relates a system of immune responses of an organism against its own healthy cells, tissues and other normal body constituents. Any disease resulting from this type of immune response is termed an "autoimmune disease." Prominent examples include, but are not limited to, celiac disease, post-infectious IBS, diabetes mellitus type 1, Henoch–Schönlein purpura (HSP) sarcoidosis, systemic lupus erythematosus (SLE), Sjögren syndrome, eosinophilic granulomatosis with polyangiitis, Hashimoto's thyroiditis, Graves' disease, idiopathic thrombocytopenic purpura, Addison's disease, rheumatoid arthritis (RA), ankylosing spondylitis, polymyositis (PM), dermatomyositis (DM), Alopecia Areata and multiple sclerosis (MS).

[0054] The term "infectious disease" according to the invention relates to a transmissible disease or communicable disease, or an illness resulting from an invasion of tissues by pathogens, their multiplication, and the reaction of host tissues to the infectious agent and the toxins they produce. Infectious diseases are caused by infectious agents (pathogens) such as, but not limited to: bacteria (e.g. Mycobacterium tuberculosis, Staphylococcus aureus, Escherichia coli, Clostridium botulinum, and Salmonella spp.), viruses and related agents such as viroids. (e.g. HIV, Rhinovirus, Lyssaviruses such as Rabies virus, Ebolavirus and Severe acute respiratory syndrome coronavirus 2), fungi, further subclassified into: Ascomycota, including yeasts such as Candida (the most common fungal infection); filamentous fungi such as Aspergillus; Pneumocystis species; and dermatophytes, a group of organisms causing infection of skin and other superficial structures in humans, basidiomycota, including the human-pathogenic genus Cryptococcus, parasites, which are usually divided into: unicellular organisms (e.g. malaria, Toxoplasma, Babesia), macroparasites (worms or helminths) including nematodes such as parasitic roundworms and pinworms, tapeworms (cestodes), and flukes (trematodes, such as schistosomes), arthropods such as ticks, mites, fleas, and lice, can also cause human disease, which conceptually are similar to infections, but invasion of a human or animal body by these macroparasites is usually termed infestation, and prions (although they do not secrete toxins).

[0055] The term "peptide disease target" according to the invention relates to compositions such as, but not limited to, allotypes, peptides, epitopes, cell populations, ligands, receptors, or

binders that interact, or affect the expression of, a target of interest, which may be specific to a desired disease.

[0056] The invention relates to methods of increasing surface human leukocyte antigen (HLA) expression which may comprise fusing a nucleic acid encoding a chaperone to a nucleic acid encoding a protein of interest, forming a fused nucleic acid encoding a chaperone fusion protein (CFP), administering the nucleic acid encoding the CFP to a cell, allowing the nucleic acid to express the CFP in the cell, wherein the CFP increases HLA expression in the cell.

[0057] In one embodiment, the chaperone is a HLA class I chaperone. In another embodiment, the chaperone is tapasin or transporter associated with antigen processing (TAP)-binding protein related (TAPBPR). In another embodiment, the tapasin or the TAPBR is fused to a transmembrane (TM) domain. In another embodiment, the TM domain is a human leukocyte antigen (HLA) TM domain. In another embodiment, the HLA is HLA-G.

[0058] In an advantageous embodiment, the nucleic acid encoding the TM domain of HLA-G is SEQ ID NO: 3. In another advantage embodiment, the nucleic acid encoding the CFP is SEQ ID NO: 4 or SEQ ID NO: 5.

[0059] In one embodiment, the protein of interest is an antigen, advantageously a tumor antigen. As used herein, an antigen may encompass a protein or a fragment, derivative or variant thereof as long as an immune response is capable of being elicited by the antigen or the fragment, derivative or variant thereof.

[0060] A tumor antigen may be a tumor-specific antigen or a tumor-associated antigen. Tumor antigens include, but are not limited to, alphafetoprotein (AFP), carcinoembryonic antigen (CEA), CA-125, MUC-1, epithelial tumor antigen (ETA), tyrosinase, melanoma-associated antigen (MAGE), and abnormal products of ras, p53.

[0061] The invention also relates to an immune adjuvant produced by a T cell in a T cell-based immunotherapy comprising a T cell transduced with any of the herein disclosed CFPs. In one embodiment, the T cell-based immunotherapy is a chimeric antigen receptor (CAR)-T (see, e.g., international patent publication WO2019210153A1), T-cell receptor (TCR)-T (see, e.g., international patent publication WO2020038492A1) or tumor infiltrating lymphocyte (TIL) therapy (see, e.g., international patent publication WO2018182817A1).

[0062] The invention also relates to a vaccine adjuvant comprising any of the herein disclosed CFPs. In one embodiment, the vaccine is an anti-viral vaccine or a cancer vaccine.

[0063] The invention also relates to a multivalent molecule comprising any of the herein disclosed CFPs conjugated to a protein or molecule that mediates targeting of the CFP to a

target cell. In one embodiment, the protein that mediates targeting of the CFP to a target cell is a single chain fragment variable (scFV) antibody. In another embodiment, the target cell is a tumor cell or a viral infected cell.

[0064] The therapeutically active agents, vaccines and compositions described herein may be administered via any conventional route, including by injection or infusion. The administration may be carried out, for example, orally, intravenously, intraperitoneally, intramuscularly, subcutaneously or transdermally. In one embodiment, administration is carried out intranodally such as by injection into a lymph node. Other forms of administration envision the in vitro transfection of antigen presenting cells such as dendritic cells with nucleic acids described herein followed by administration of the antigen presenting cells.

[0065] The agents described herein are administered in effective amounts. An "effective amount" refers to the amount which achieves a desired reaction or a desired effect alone or together with further doses. In the case of treatment of a particular disease or of a particular condition, the desired reaction preferably relates to inhibition of the course of the disease. This comprises slowing down the progress of the disease and, in particular, interrupting or reversing the progress of the disease. The desired reaction in a treatment of a disease or of a condition may also be delay of the onset or a prevention of the onset of said disease or said condition.

[0066] An effective amount of an agent described herein will depend on the condition to be treated, the severeness of the disease, the individual parameters of the patient, including age, physiological condition, size and weight, the duration of treatment, the type of an accompanying therapy (if present), the specific route of administration and similar factors. Accordingly, the doses administered of the agents described herein may depend on various of such parameters. In the case that a reaction in a patient is insufficient with an initial dose, higher doses (or effectively higher doses achieved by a different, more localized route of administration) may be used.

[0067] The pharmaceutical compositions of the invention are preferably sterile and contain an effective amount of the therapeutically active substance to generate the desired reaction or the desired effect.

[0068] The pharmaceutical compositions of the invention are generally administered in pharmaceutically compatible amounts and in pharmaceutically compatible preparation. The term "pharmaceutically compatible" refers to a nontoxic material which does not interact with the action of the active component of the pharmaceutical composition. Preparations of this kind may usually contain salts, buffer substances, preservatives, carriers, supplementing immunity-

enhancing substances such as adjuvants, e.g. CpG oligonucleotides, cytokines, chemokines, saponin, GM-CSF and/or RNA and, where appropriate, other therapeutically active compounds. When used in medicine, the salts should be pharmaceutically compatible. However, salts which are not pharmaceutically compatible may be used for preparing pharmaceutically compatible salts and are included in the invention. Pharmacologically and pharmaceutically compatible salts of this kind comprise in a non-limiting way those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic acids, and the like. Pharmaceutically compatible salts may also be prepared as alkali metal salts or alkaline earth metal salts, such as sodium salts, potassium salts or calcium salts.

[0069] A pharmaceutical composition of the invention may comprise a pharmaceutically compatible carrier. The term "carrier" refers to an organic or inorganic component, of a natural or synthetic nature, in which the active component is combined in order to facilitate application. According to the invention, the term "pharmaceutically compatible carrier" includes one or more compatible solid or liquid fillers, diluents or encapsulating substances, which are suitable for administration to a patient. The components of the pharmaceutical composition of the invention are usually such that no interaction occurs which substantially impairs the desired pharmaceutical efficacy.

[0070] The pharmaceutical compositions of the invention may contain suitable buffer substances such as acetic acid in a salt, citric acid in a salt, boric acid in a salt and phosphoric acid in a salt.

[0071] The pharmaceutical compositions may, where appropriate, also contain suitable preservatives such as benzalkonium chloride, chlorobutanol, paraben and thimerosal.

[0072] The pharmaceutical compositions are usually provided in a uniform dosage form and may be prepared in a manner known per se. Pharmaceutical compositions of the invention may be in the form of capsules, tablets, lozenges, solutions, suspensions, syrups, elixirs or in the form of an emulsion, for example.

[0073] Compositions suitable for parenteral administration usually comprise a sterile aqueous or nonaqueous preparation of the active compound, which is preferably isotonic to the blood of the recipient. Examples of compatible carriers and solvents are Ringer solution and isotonic sodium chloride solution. In addition, usually sterile, fixed oils are used as solution or suspension medium.

[0074] The invention also encompasses a use of any one of the herein disclosed complexes, nucleic acids, vectors or pharmaceutical compositions to screen a panel of candidate epitopic peptides to identify relevant peptide disease targets. In one embodiment, the disease is cancer, autoimmunity or an infectious disease.

[0075] The invention also encompasses methods for enhancing peptide presentation and identification in tumor cells. The methods comprise (a) contacting tumor cells with a nucleic acid encoding a chaperone fused to a nucleic acid encoding a protein of interest to form a fused nucleic acid encoding a chaperone fusion protein (CFP); (b) expressing the CFP in the tumor cells; (c) upregulating human leukocyte antigen (HLA) expression; (d) capturing and identifying peptides that are bound on the upregulated HLAs. In certain embodiments, the HLA is HLA-I. Capturing and identifying peptides that are bound on the upregulated HLA peptides can, for example, be done using MHC I immunopeptidome isolation and analysis methods, such as those described in Purcell et al., Nat. Protocol. 14(6):1687-1707 (2019); Kuznetsov et al., Molecules 25(22):5409 (2020); Illing et al., Current Opinion Immunol. 77:102216 (2022); and Kovalchik et al., bioRxiv (2020); and those demonstrated in the Examples below. Briefly, MHC-I molecules can be immunoprecipitated using a pan allelic antibody, such as W6/32), and peptides will be eluted under mild acidic conditions and analyzed using liquid chromatography coupled with mass spectrometry (LC-MS). Software such as MSFragger (Kong et al., Nat. Methods 14(5):513-520 (2017)) can be used to search the MS raw data for peptides using reference protein databases, further validated using MhcVizPipe (Kovalchik et al., Mol. Cell Proteomics 21(1):100178 (2022)).

[0076] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined in the appended claims.

[0077] The present invention will be further illustrated in the following Examples which are given for illustration purposes only and are not intended to limit the invention in any way.

EMBODIMENTS

[0078] Embodiment 1 is a method of increasing surface human leukocyte antigen (HLA) expression comprising fusing a nucleic acid encoding a chaperone to a nucleic acid encoding a protein of interest, forming a fused nucleic acid encoding a chaperone fusion protein (CFP), administering the nucleic acid encoding the CFP to a cell, allowing the nucleic acid to express the CFP in the cell, wherein the CFP increases HLA expression in the cell.

[0079] Embodiment 2 is the method of embodiment 1, wherein the chaperone is a HLA class I chaperone.

[0080] Embodiment 3 is the method of embodiment 2, wherein the chaperone is tapasin or transporter associated with antigen processing (TAP)-binding protein related (TAPBPR).

[0081] Embodiment 4 is the method of embodiment 3, wherein the tapasin or the TAPBR is fused to a transmembrane (TM) domain.

[0082] Embodiment 5 is the method of embodiment 4, wherein the TM domain is a human leukocyte antigen (HLA) TM domain.

[0083] Embodiment 6 is the method of embodiment 5, wherein the HLA is HLA-G.

[0084] Embodiment 7 is the method of embodiment 6, wherein the nucleic acid encoding the TM domain of HLA-G is SEQ ID NO: 3.

[0085] Embodiment 8 is the method of embodiment 7, wherein the nucleic acid encoding the CFP is SEQ ID NO: 4 or SEQ ID NO: 5.

[0086] Embodiment 9 is the method of any one of embodiments 1-8, wherein the protein of interest is an antigen.

[0087] Embodiment 10 is the method of embodiment 9, wherein the antigen is a tumor antigen.

[0088] Embodiment 11 is an immune adjuvant produced by a T cell in a T cell based immunotherapy comprising a T cell transduced with the CFP of any one of embodiments 1-10.

[0089] Embodiment 12 is the immune adjuvant of embodiment 11, wherein the T cell based immunotherapy is chimeric antigen receptor (CAR)-T, T-cell receptor (TCR)-T or tumor infiltrating lymphocyte (TIL) therapy.

[0090] Embodiment 13 is a vaccine adjuvant comprising the CFP of any one of embodiments 1-10.

[0091] Embodiment 14 is the vaccine adjuvant of embodiment 13, wherein the vaccine is an anti-viral vaccine or a cancer vaccine.

[0092] Embodiment 15 is a multivalent molecule comprising the CFP of any one of embodiments 1-10 conjugated to a protein or molecule that mediates targeting of the CFP to a target cell.

[0093] Embodiment 16 is the multivalent molecule of embodiment 15, wherein the protein that mediates targeting of the CFP to a target cell is a single chain fragment variable (scFV) antibody.

[0094] Embodiment 17 is the multivalent molecule of embodiment 15 or 16, wherein the target cell is a tumor cell or a viral infected cell.

[0095] Embodiment 18 is a pharmaceutical composition comprising the CFP of any one of embodiments 1-10, the immune adjuvant of embodiment 11 or embodiment 12, the vaccine adjuvant of embodiment 13 or embodiment 14, or the multivalent molecule of any one of embodiments 15-17 and a pharmaceutically acceptable carrier.

[0096] Embodiment 19 is a method of treating or preventing a disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the CFP of any one of embodiments 1-10, the immune adjuvant of embodiment 11 or embodiment 12, the vaccine adjuvant of embodiment 13 or embodiment 14, the multivalent molecule of any one of embodiments 15-17 or the pharmaceutical composition of embodiment 18.

[0097] Embodiment 20 is a use of the CFP of any one of embodiments 1-10, the immune adjuvant of embodiment 11 or embodiment 12, the vaccine adjuvant of embodiment 13 or embodiment 14, the multivalent molecule of any one of embodiments 15-17 or the pharmaceutical composition of embodiment 18 to increase surface human leukocyte antigen (HLA) expression.

[0098] Embodiment 21 is a method for enhancing peptide presentation and identification in tumor cells, the method comprising:

(a) contacting tumor cells with a nucleic acid encoding a chaperone fused to a nucleic acid encoding a protein of interest to form a fused nucleic acid encoding a chaperone fusion protein (CFP);

(b) expressing the CFP in the tumor cells;

(c) upregulating human leukocyte antigen (HLA) expression;

(d) capturing and identifying peptides bound on the upregulated HLAs.

[0099] Embodiment 21a is the method of embodiment 21, wherein the HLA is HLA-I.

[00100] Embodiment 22 is the method of embodiment 21, wherein the chaperone is a HLA class I chaperone.

[00101] Embodiment 23 is the method of embodiment 22, wherein the chaperone is tapasin or transporter associated with antigen processing (TAP)-binding protein related (TAPBR).

[00102] Embodiment 24 is the method of embodiment 23, wherein the tapasin or the TAPBR is fused to a transmembrane (TM) domain.

[00103] Embodiment 25 is the method of embodiment 24, wherein the TM domain is a human leukocyte antigen (HLA) TM domain.

[00104] Embodiment 26 is the method of embodiment 25, wherein the HLA is HLA-G.

[00105] Embodiment 27 is the method of embodiment 26, wherein the nucleic acid encoding the TM domain of HLA-G comprises SEQ ID NO:3.

[00106] Embodiment 28 is the method of embodiment 27, wherein the nucleic acid encoding the CFP comprises SEQ ID NO:4 or SEQ ID NO:5.

[00107] Embodiment 29 is the method of any one of embodiments 21-28, wherein the protein of interest is an antigen.

Examples

Example 1: Materials and Methods

[00108] Lentiviral Production. Lentivirus was produced by co-transfection of Lenti-X 293T cells (Takara Bio) with pSFFV transfer vector containing gene of interest along with psPAX2 packaging vector and pMD2.G envelope vector. Transfections were performed using lipofectamine 3000 according to manufacturer instructions. Virus containing supernatant was then collected each day for up to 3 days and concentrated using Lenti-X concentrator (Takara Bio) according to manufacturer instructions.

[00109] T cell Activation and Transduction and Purification. CD8+ T cells were obtained from the Human Immunology Core of the University of Pennsylvania. T cells were then stimulated with anti-CD3/anti-CD28 Dynabeads at a 1:1 ratio in complete RPMI medium supplemented with 10 ng/mL IL-2 for 24 hours prior to transduction. T cells were then replated on tissue culture treated plates coated with 50 ug/mL retronectin (Takara Bio) along with lentivirus. After an additional 48 hours of culture, dynabeads were removed and cells were expanded by adding fresh media supplemented with IL-2. Cells were expanded for several days before purification. 1G4 purification was performed by labeling transduced cells with NYESO with PE-conjugated, HLA-A01*01 tetramer refolded with $NYESO_{157-165}$ followed by magnetically activated cell sorting (MACS) using anti-PE microbeads. CFP expressing cells were then purified by a second round of MACS using anti-FLAG-PE antibody conjugate. Tumor cells were also transduced using the same method as above.

[00110] T cell Killing Assays. 1G4 T cells were co-cultured at different E:T ratios with tumor cells overnight. Killing was assayed by flow cytometry. Cell number was determined using CountBright counting beads (Thermo Fisher).

Example 2: CFPs Tapasin-TM and TAPBPR-TM increase cell surface pHLAs

[00111] In theory, increased cell surface pHLA may be achieved by leveraging the chaperoning functions of tapasin and TAPBPR. To explore this possibility, Tapasin and TAPBPR variants were engineered that are targeted to the plasma membrane (called **Tapasin-TM & TAPBPR-TM**), allowing them to associate with HLAs from their point of synthesis in the ER to the cell surface [7]. This has been accomplished by replacing the endogenous transmembrane (TM) domain, which normally retains tapasin and TAPBPR within the ER/Golgi compartments, with the HLA-G TM domain, allowing for trafficking along the secretory pathway (Table 1). Applicants term these molecules chaperone fusions proteins (**CFPs**).

[00112] When expressed in tapasin deficient Expi293 cells, CFPs dramatically increased median surface expression of HLA-A2, by ~6-fold (Fig. 1A) with concurrent increase in FLAG surface expression (Fig. 1B). Applicants subsequently tested this system in a neuroblastoma tumor cell line. The baseline expression of Tapasin and TABPR in neuroblastoma tumor cell lines is shown in Fig. 2. Expression of CFPs in the EBc1 neuroblastoma cell line showed similar results, with ~3-fold upregulation of HLA-A2 expression in cells expressing Tapasin-TM (Fig. 1C) and concurrent increase in FLAG surface expression (Fig. 1D).

[00113] Further titration of transduction conditions showed that Tapasin-TM expression had dose-dependent effects on HLA-A2 expression. The optimal dose of Tapasin-TM resulted in a ~5-fold increase in HLA-A2 expression (Fig. 1E). Higher doses resulted in diminished HLA-A2, but still higher than expression in parental EBc1 cells. Lastly, HLA-A2 upregulation could be abrogated by interfering with CFP interaction with HLA, for example, as exhibited with the Tapasin-TN6-TM variant which only mildly upregulated HLA-A2.

Example 3: CFP delivery to T cells

[00114] To demonstrate that CFPs can, at least in principle, be delivered to tumor cells via T cells, huCD8 T cells were co-transduced with 1G4 TCR and either tapasin-TM or TAPBPR-TM CFPs. These T cells were then purified to near 100% purity by PE-conjugated HLA-A2/NY-ESO-$1_{157\text{-}165}$ tetramer staining followed by magnetically activated cell sorting (MACS) with anti-PE microbeads (Fig 3A). CFP expressing cells were then purified by a second round of MACS using the same principle (Fig. 3A, right). Given that this is the most likely application of CFPs, Applicants then sought to directly test whether these cells had enhanced capacity to kill target tumor cells. Indeed, CFP expressing 1G4 T cells have superior ability to recognize and kill EBc1 cells pulsed with NYESO$1_{157\text{-}165}$ (Figs. 3B, 3C). Importantly, EBc1 cells were washed thoroughly to remove all excess peptide prior to incubation with 1G4 T cells. Improved

killing correlated with increased expression activation markers CD69 and 4-1BB (Figs. 3E, 3F). Furthermore, FLAG-TAG could be detected on the surface of EBc1 cells (Figs. 3D, 3G). The likely mode of this transfer is via EVs, as other markers CD8 T cell membrane markers were detected on the surface of EBc1 cells, including CD8 (data not shown). Peptide/MHC and CD58 are both required for efficient tumor killing, as shown by transduction of NY-ESO-1 peptide and CD58 in SUDHL4 cells which are NY-ESO-1$^-$/CD58$^-$ (Fig. 3H).

Example 4: CFPs enhance T-cell mediated tumor killing

[00115] Applicants then tested whether CFPs could enhance killing of cancer cells which have intrinsic resistance to T cell-mediated death. SUDHL4 cells are a diffuse B cell lymphoma cell line which are a liquid cancer. SUDHL4 cells that were either transduced with NYESO-1 or empty vector were utilized. In contrast to 1G4 T cells which had only 20% NYESO-1 specific killing, 1G4 T cells transduced with TAPBPR-TM had enhanced ability to kill target SUDHL4 cells, with ~40% NYESO-1 specific killing (Fig. 4A). Number of surviving NYESO-1 SUDHL4 cells and Empty vector SUDHL4 cells with 1G4 T cells and CFPs Tapasin-TM or TAPBPR-TM shown in Figs. 4B and 4C. CFPs were detected on the surface of SUDHL4 cells, in a E:T ratio dependent manner (Fig. 4D). Improved killing correlated with increased expression of 4-1BB (Fig. 4E, top), suggesting improved T cell activation. Increased expression of CD69 was also exhibited (Fig. 4F, top). MFI of 4-1BB and CD69 in the SUDHL4 empty vector controls are shown in the bottom panels of Figs. 4E and 4F, respectively.

[00116] *Example 5: CFPs enhance the immunogenicity of neuroblastoma cells.*

[00117] EBc-1 cells were pulsed with NYESO-$1_{157\text{-}165}$ and co-cultured with 1G4 TCR-T cells, and it was found that expression of CFPs enhanced T cell-mediated killing **(Fig. 5A,B)**. Similarly, cells presenting NYESO-1 antigen via the endogenous pathway also showed increased sensitivity to 1G4-TCR-T cell killing **(Fig. 5C)**. To further confirm these results, the kinetics of killing in overnight cultures of cells presenting NYESO-1 antigen via the endogenous pathway was also measured through xCELLigence-based impedance measurements. Similarly, enhanced killing of EBc1 cells expressing the Tapasin-TM CFP was observed. These data suggest that CFPs can increase the immunogenicity of neuroblastoma, a *bona fide* cold tumor model.

[00118] *Example 6: Tapasin interactions stabilize MHC-I complexes on the cell surface.*

[00119] To determine the mechanism by which CFPs upregulate HLA-I molecules, the synthesis of new MHC-I molecules was inhibited using the ribosomal inhibitor cycloheximide, which revealed that MHC-I molecules in cells transduced with Tapasin-TM had longer half-

life than either parental, or even WT tapasin transduced cells **(Fig. 6A)**. Similarly, inhibiting anterograde trafficking of MHC-I from the Golgi with brefeldin A revealed enhanced stability of MHC-I molecules in Tapasin-TM transduced cells **(Fig. 6B)**. Lastly, inhibiting the degradation of MHC-I molecules using the lysosome inhibitor bafilomycin A1 showed that Tapasin-TM transduced cells had diminished sensitivity to bafilomycin A1 treatment, suggesting that fewer MHC-I molecules are targeted to the lysosome in those cells **(Fig. 6C)**.

[00120] *Example 7: CFP expressing T cells kill B cell lymphoma cells more efficiently.*

[00121] To extend the findings to B cell lymphomas, SUDHL4 cells were transduced with either NYESO-1 or the empty vector and co-cultured with 1G4 T cells that express CFPs. CFP expressing 1G4 T cells had superior ability to recognize and kill SUDHL4 cells expressing NYESO-1 **(Fig. 7A)**. Enhanced killing correlated with increased expression of T cell activation marker 4-1BB, suggesting superior target recognition **(Fig. 7B)**. Lastly, CFPs could be detected on the surface of surviving target cells, showing they have been transferred to the target cells **(Fig. 7C)**.

[00122] *Example 8: CFPs enhance immunoprecipitation of folded HLA-I molecules for immunopeptidomics.*

[00123] Methods—Immunoprecipitation of HLA-A2 molecules

[00124] Cell pellets of the neuroblastoma cell line EBc1 were lysed and HLA-A2 molecules bound to BB7.2 antibody conjugated CNBr-activated Sepharose substrate. pMHC complexes were then eluted with trifluoroacetic acid prior to SDS-PAGE and native gel electrophoresis. Subsequently, lysates were analyzed by western blot for the presence of beta-2-microglobulin.

[00125] Results

[00126] Increased surface expression of HLA-I and HLA-A2 in NB cells implied that total production of folded HLA-I complexes was increased. This suggested that CFPs could be used as an enhancer of immunoprecipitation (IP) for HLA-I, which may be useful for immunopeptidomic applications. To test this, it was sought to determine if CFPs enhanced immunoprecipitation of HLA-A2 molecules from cells with low HLA-I density. Parental EBc1 cells and those transduced with tapasin WT, Tapasin-TM CFP, and negative control tapasin-TN6-TM were lysed and folded HLA-A2 molecules were precipitated using Sepharose substrate conjugated to BB7.2 antibody. Following elution, the presence of HLA-I proteins was analyzed by western blot for beta-2-microglobulin (B2M) from SDS-PAGE gels and native gels. A significant increase in B2M in cells transduced with CFPs was observed over all other conditions, including WT tapasin both from pre-IP lysates and post-IP lysates (top) and in

native gels (bottom). Loading control blots for vinculin showed no difference in the amount of protein loaded (right) indicating a specific increase in HLA-I in cells transduced with CFPs.

References for Examples 1-4:

[00127] 1. Shionoya, Y., et al., *Loss of tapasin in human lung and colon cancer cells and escape from tumor-associated antigen-specific CTL recognition.* Oncoimmunology, 2017. **6**(2): p. e1274476. PMC5353923 PMID: 28344889

[00128] 2. Paulsson, K.M., et al., *Association of tapasin and COPI provides a mechanism for the retrograde transport of major histocompatibility complex (MHC) class I molecules from the Golgi complex to the endoplasmic reticulum.* J Biol Chem, 2002. **277**(21): p. 18266-71. PMID: 11884415

[00129] 3. Grandea, A.G., 3rd and L. Van Kaer, *Tapasin: an ER chaperone that controls MHC class I assembly with peptide.* Trends Immunol, 2001. **22**(4): p. 194-9. PMID: 11274924

[00130] 4. Reeves, E. and E. James, *Antigen processing and immune regulation in the response to tumours.* Immunology, 2017. **150**(1): p. 16-24. PMC5341504 PMID: 27658710

[00131] 5. Neerincx, A., et al., *TAPBPR bridges UDP-glucose:glycoprotein glucosyltransferase 1 onto MHC class I to provide quality control in the antigen presentation pathway.* Elife, 2017. **6.** PMC5441866 PMID: 28425917

[00132] 6. Chen, X., et al., *A membrane-associated MHC-I inhibitory axis for cancer immune evasion.* Cell, 2023. *186*(18): p. 3903-3920 e21. PMID: 37557169

[00133] 7. McShan, A.C., et al., *Molecular determinants of chaperone interactions on MHC-I for folding and antigen repertoire selection.* Proc Natl Acad Sci USA, 2019. **116**(51): p. 25602-25613. PMC6926029 PMID: 31796585

* * *

[00134] Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

WHAT IS CLAIMED IS:

1. A method of increasing surface human leukocyte antigen (HLA) expression comprising fusing a nucleic acid encoding a chaperone to a nucleic acid encoding a protein of interest, forming a fused nucleic acid encoding a chaperone fusion protein (CFP), administering the nucleic acid encoding the CFP to a cell, allowing the nucleic acid to express the CFP in the cell, wherein the CFP increases HLA expression in the cell.

2. The method of claim 1, wherein the chaperone is a HLA class I chaperone.

3. The method of claim 2, wherein the chaperone is tapasin or transporter associated with antigen processing (TAP)-binding protein related (TAPBPR).

4. The method of claim 3, wherein the tapasin or the TAPBR is fused to a transmembrane (TM) domain.

5. The method of claim 4, wherein the TM domain is a human leukocyte antigen (HLA) TM domain.

6. The method of claim 5, wherein the HLA is HLA-G.

7. The method of claim 6, wherein the nucleic acid encoding the TM domain of HLA-G is SEQ ID NO: 3.

8. The method of claim 7, wherein the nucleic acid encoding the CFP is SEQ ID NO: 4 or SEQ ID NO: 5.

9. The method of any one of claims 1-8, wherein the protein of interest is an antigen.

10. The method of claim 9, wherein the antigen is a tumor antigen.

11. An immune adjuvant produced by a T cell in a T cell based immunotherapy comprising a T cell transduced with the CFP of any one of claims 1-10.

12. The immune adjuvant of claim 11, wherein the T cell based immunotherapy is chimeric antigen receptor (CAR)-T, T-cell receptor (TCR)-T or tumor infiltrating lymphocyte (TIL) therapy.

13. A vaccine adjuvant comprising the CFP of any one of claims 1-10.

14. The vaccine adjuvant of claim 13, wherein the vaccine is an anti-viral vaccine or a cancer vaccine.

15. A multivalent molecule comprising the CFP of any one of claims 1-10 conjugated to a protein or molecule that mediates targeting of the CFP to a target cell.

16. The multivalent molecule of claim 15, wherein the protein that mediates targeting of the CFP to a target cell is a single chain fragment variable (scFV) antibody.

17. The multivalent molecule of claim 15 or 16, wherein the target cell is a tumor cell or a viral infected cell.

18. A pharmaceutical composition comprising the CFP of any one of claims 1-10, the immune adjuvant of claim 11 or claim 12, the vaccine adjuvant of claim 13 or claim 14, or the multivalent molecule of any one of claims 15-17 and a pharmaceutically acceptable carrier.

19. A method of treating or preventing a disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the CFP of any one of claims 1-10, the immune adjuvant of claim 11 or claim 12, the vaccine adjuvant of claim 13 or claim 14, the multivalent molecule of any one of claims 15-17 or the pharmaceutical composition of claim 18.

20. Use of the CFP of any one of claims 1-10, the immune adjuvant of claim 11 or claim 12, the vaccine adjuvant of claim 13 or claim 14, the multivalent molecule of any one of claims 15-17 or the pharmaceutical composition of claim 18 to increase surface human leukocyte antigen (HLA) expression.

21. A method for enhancing peptide presentation and identification in tumor cells, the method comprising:

(a) contacting tumor cells with a nucleic acid encoding a chaperone fused to a nucleic acid encoding a protein of interest to form a fused nucleic acid encoding a chaperone fusion protein (CFP);

(b) expressing the CFP in the tumor cells;

(c) upregulating human leukocyte antigen (HLA) expression;

(d) capturing and identifying peptides bound on the upregulated HLAs.

22. The method of claim 21, wherein the HLA is HLA-I.

23. The method of claim 21, wherein the chaperone is a HLA class I chaperone.

24. The method of claim 23, wherein the chaperone is tapasin or transporter associated with antigen processing (TAP)-binding protein related (TAPBR).

25. The method of claim 24, wherein the tapasin or the TAPBR is fused to a transmembrane (TM) domain.

26. The method of claim 25, wherein the TM domain is a human leukocyte antigen (HLA) TM domain.

27. The method of claim 26, wherein the HLA is HLA-G.

28. The method of claim 27, wherein the nucleic acid encoding the TM domain of HLA-G comprises SEQ ID NO:3.

29. The method of claim 28, wherein the nucleic acid encoding the CFP comprises SEQ ID NO:4 or SEQ ID NO:5.

30. The method of any one of claims 21-29, wherein the protein of interest is an antigen.

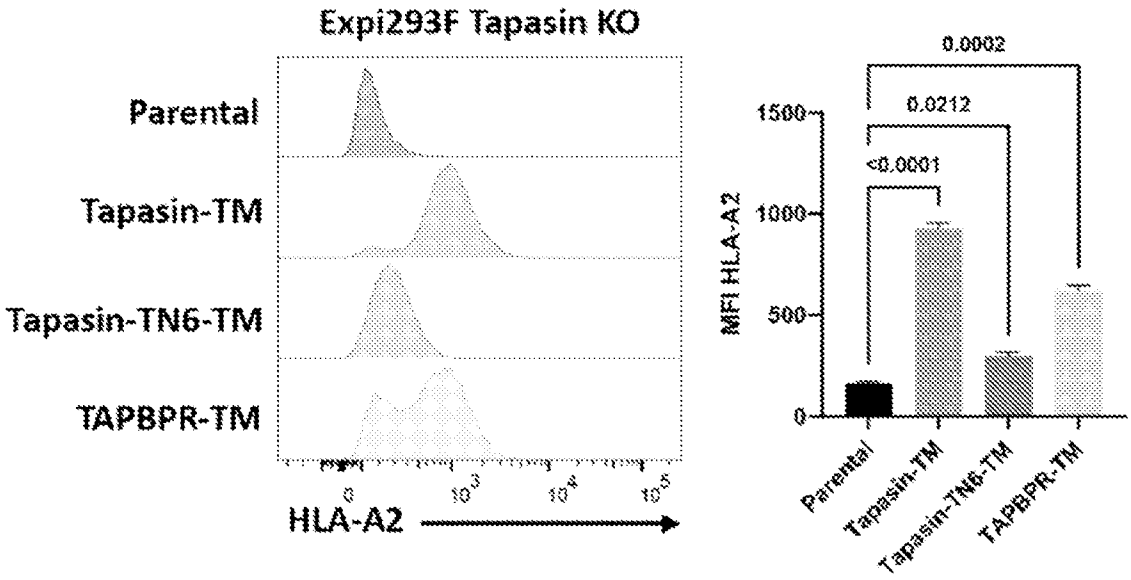
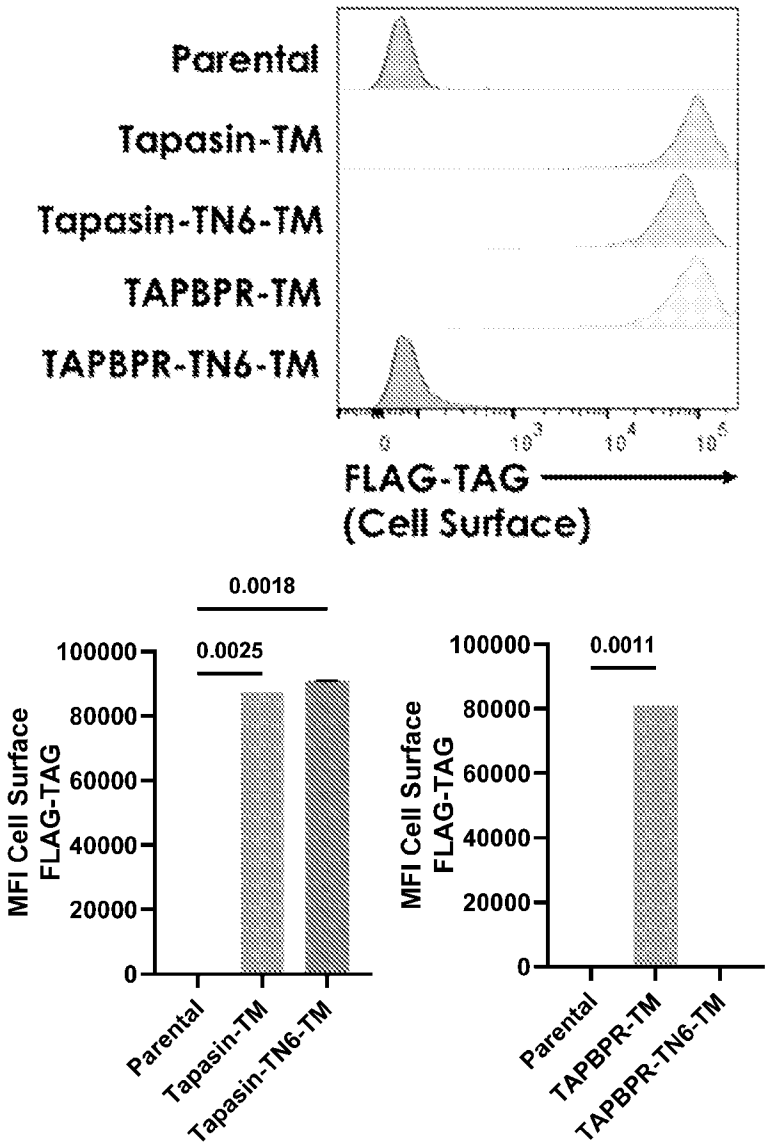

Expi293F Tapasin KO
Parental
Tapasin-TM
Tapasin-TN6-TM
TAPBPR-TM
0
10^3
10^4
10^5
HLA-A2
MFI HLA-A2
1500
1000
500
0
0.0002
0.0212
<0.0001
Parental
Tapasin-TM
Tapasin-TN6-TM
TAPBPR-TM

FIG. 1A


Parental
Tapasin-TM
Tapasin-TN6-TM
TAPBPR-TM
TAPBPR-TN6-TM
0
10^3
10^4
10^5
FLAG-TAG
(Cell Surface)
0.0018
0.0025
MFI Cell Surface FLAG-TAG
100000
80000
60000
40000
20000
0
Parental
Tapasin-TM
Tapasin-TN6-TM
0.0011
MFI Cell Surface FLAG-TAG
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80000
60000
40000
20000
0
Parental
TAPBPR-TM
TAPBPR-TN6-TM

FIG. 1B

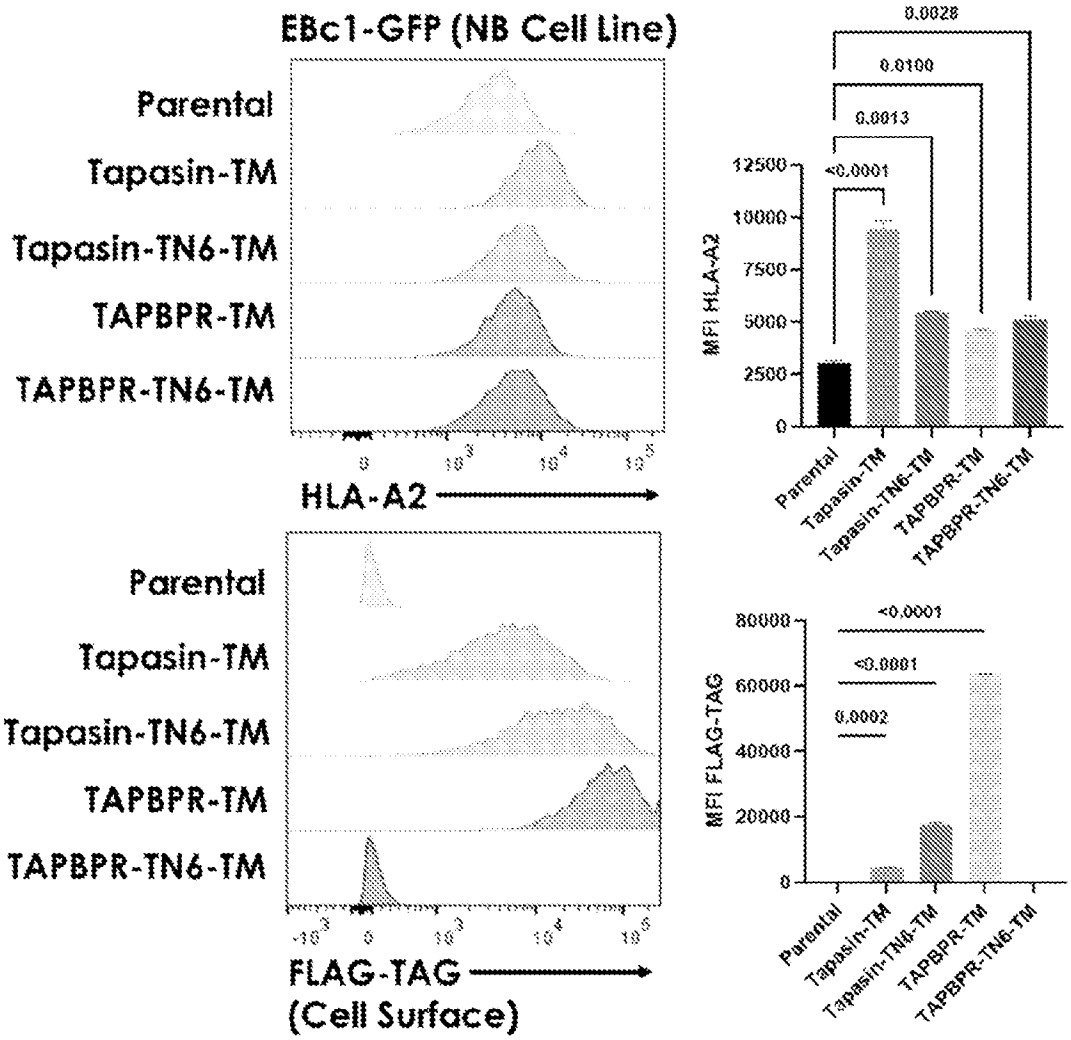

EBc1-GFP (NB Cell Line)
Parental
Tapasin-TM
Tapasin-TN6-TM
TAPBPR-TM
TAPBPR-TN6-TM
HLA-A2
Parental
Tapasin-TM
Tapasin-TN6-TM
TAPBPR-TM
TAPBPR-TN6-TM
FLAG-TAG
(Cell Surface)
0.0028
0.0100
0.0013
<0.0001
MFI HLA-A2
12500
10000
7500
5000
2500
0
Parental
Tapasin-TM
Tapasin-TN6-TM
TAPBPR-TM
TAPBPR-TN6-TM
<0.0001
<0.0001
0.0002
MFI FLAG-TAG
80000
60000
40000
20000
0
Parental
Tapasin-TM
Tapasin-TN6-TM
TAPBPR-TM
TAPBPR-TN6-TM

FIG. 1C

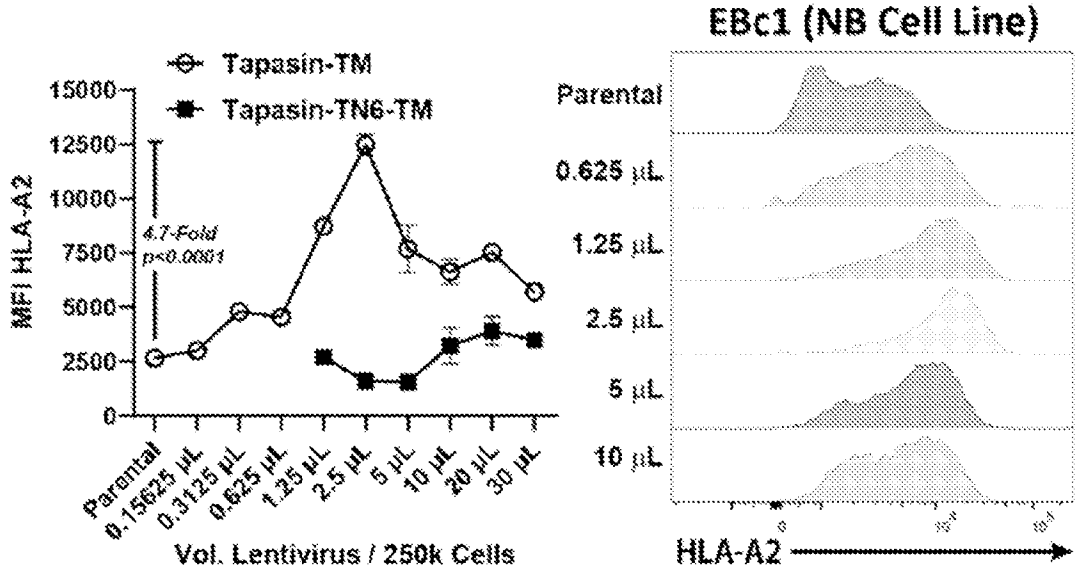

EBc1 (NB Cell Line)
Tapasin-TM
Tapasin-TN6-TM
MFI HLA-A2
15000
12500
10000
7500
5000
2500
0
4.7-Fold
p<0.0001
Parental
0.15625 µL
0.3125 µL
0.625 µL
1.25 µL
2.5 µL
5 µL
10 µL
20 µL
30 µL
Vol. Lentivirus / 250k Cells
Parental
0.625 µL
1.25 µL
2.5 µL
5 µL
10 µL
HLA-A2

FIG. 1D

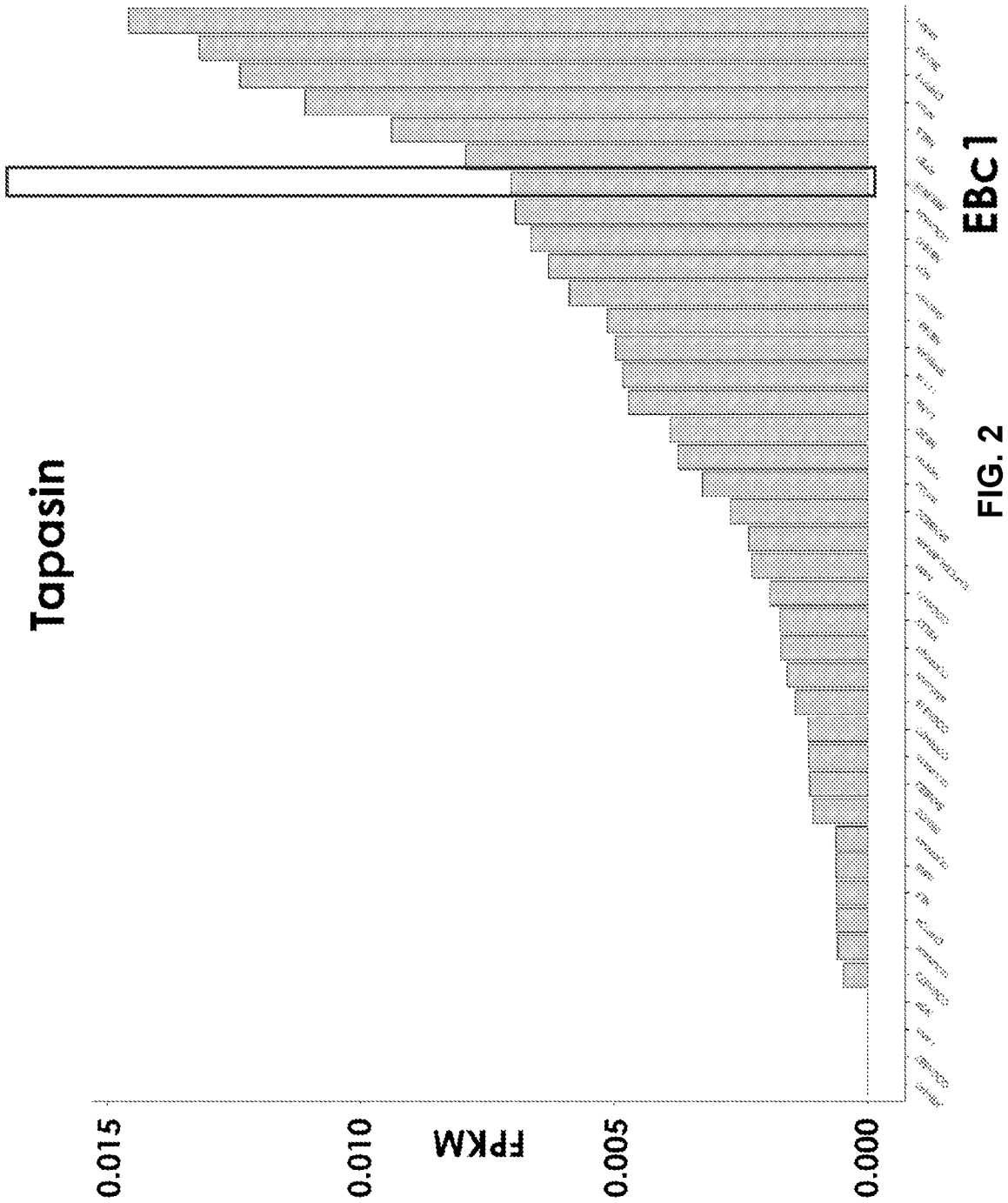


FIG. 2

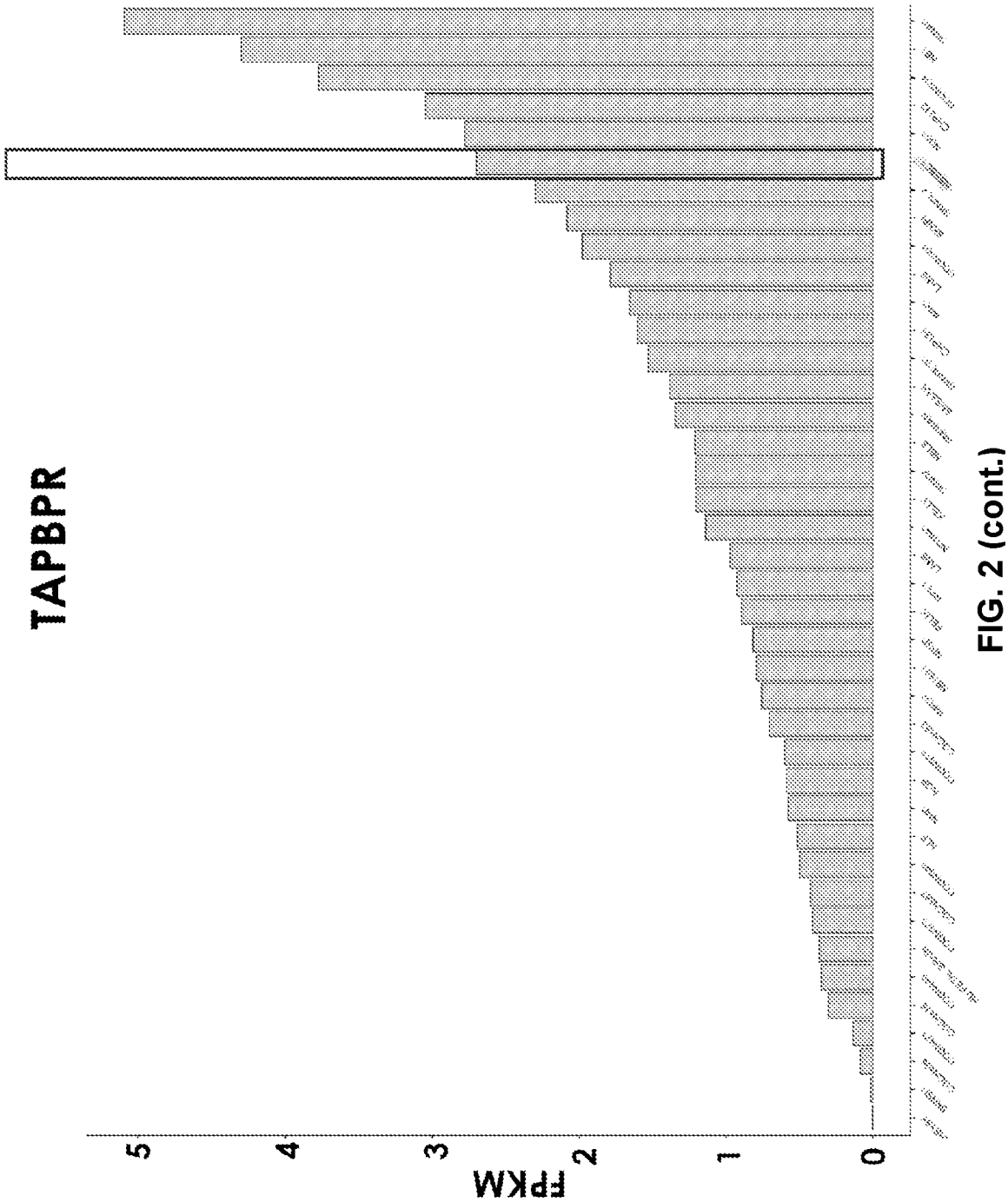


FIG. 2 (cont.)

FIG. 3C

FIG. 3B

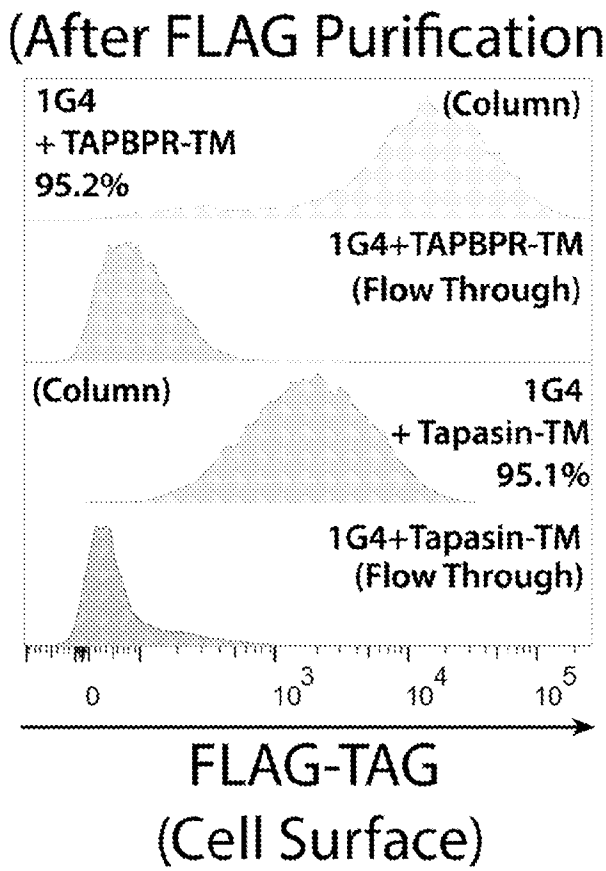
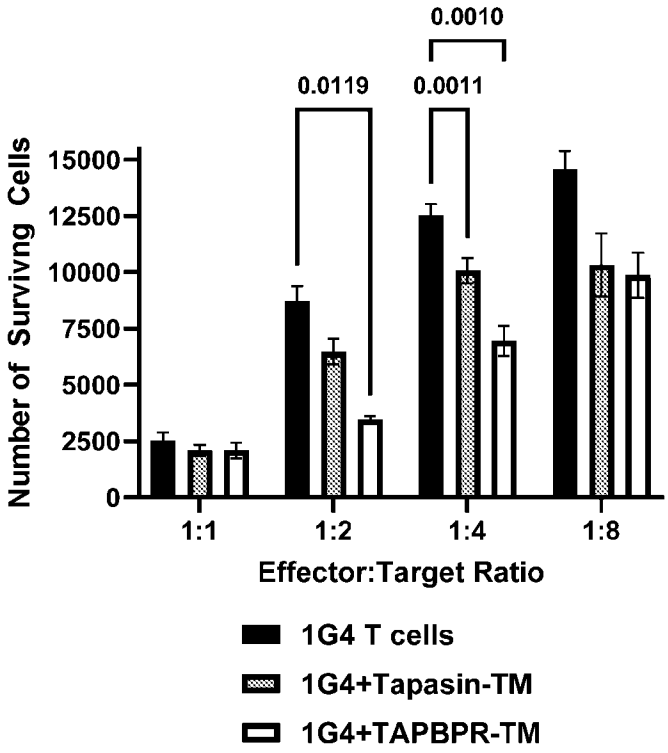

(After FLAG Purification)
1G4
+ TAPBPR-TM
95.2%
(Column)
1G4+TAPBPR-TM
(Flow Through)
(Column)
1G4
+ Tapasin-TM
95.1%
1G4+Tapasin-TM
(Flow Through)
0
10^3
10^4
10^5
FLAG-TAG
(Cell Surface)

FIG. 3A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0.0119
0.0011
Number of Survivng Cells
15000
12500
10000
7500
5000
2500
0
1:1
1:2
1:4
1:8
Effector:Target Ratio
1G4 T cells
1G4+Tapasin-TM
1G4+TAPBPR-TM

FIG. 3B

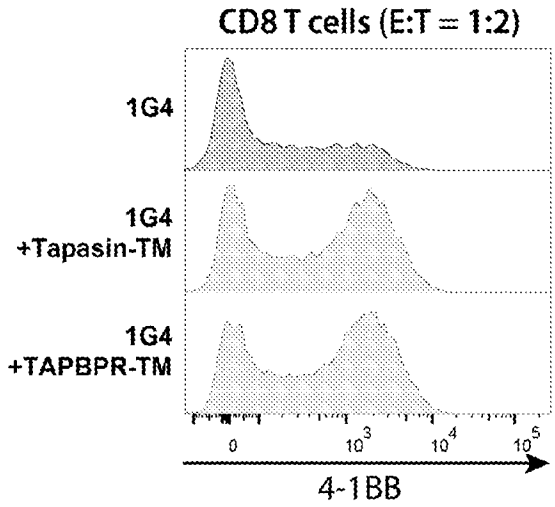
CD8 T cells (E:T = 1:2)
1G4
1G4
+Tapasin-TM
1G4
+TAPBPR-TM
0
10^3
10^4
10^5
4-1BB

FIG. 3C

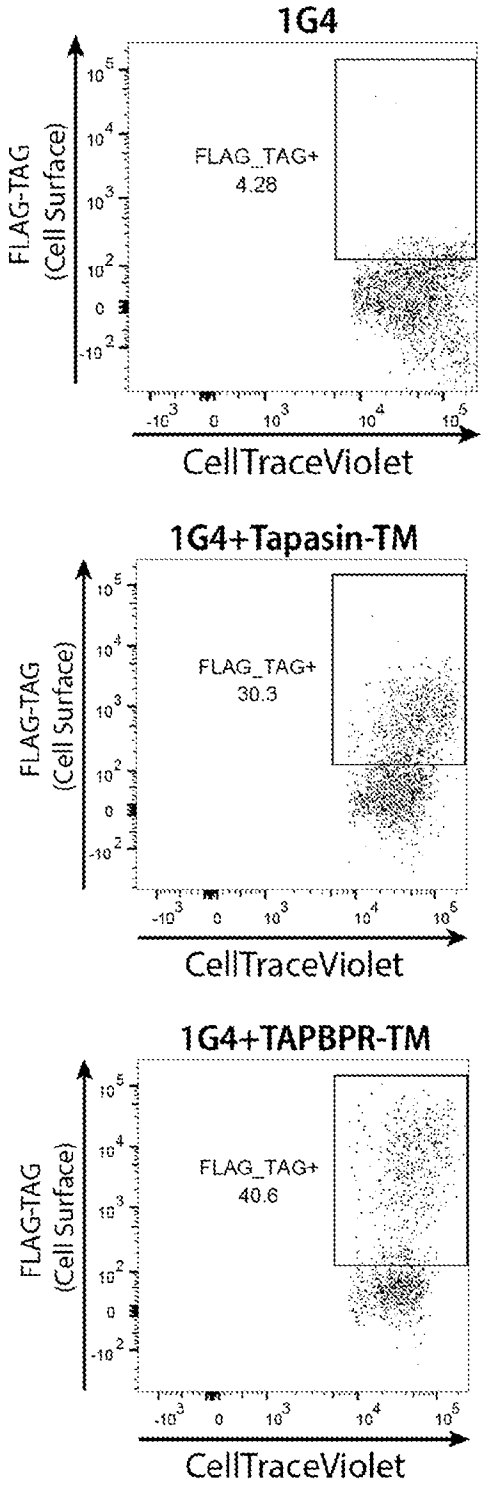
1G4
FLAG_TAG+
4.28
FLAG-TAG
(Cell Surface)
CellTraceViolet
1G4+Tapasin-TM
FLAG_TAG+
30.3
FLAG-TAG
(Cell Surface)
CellTraceViolet
1G4+TAPBPR-TM
FLAG_TAG+
40.6
FLAG-TAG
(Cell Surface)
CellTraceViolet

FIG. 3D

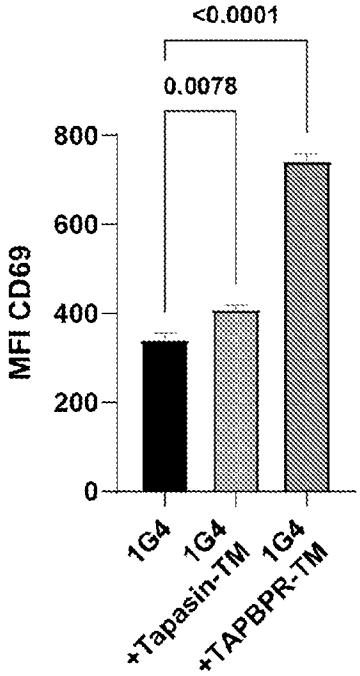
<0.0001
0.0078
800
600
400
200
0
MFI CD69
1G4
1G4 +Tapasin-TM
1G4 +TAPBPR-TM

FIG. 3E

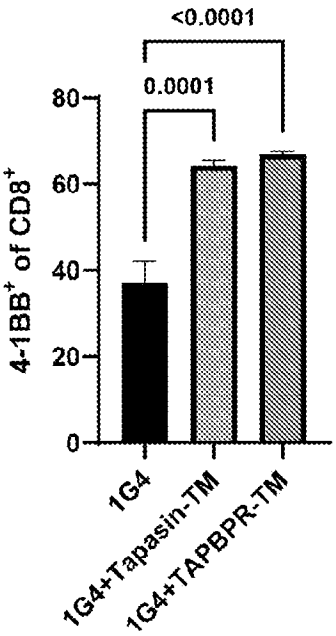
<0.0001
0.0001
80
60
40
20
0
4-1BB+ of CD8+
1G4
1G4+Tapasin-TM
1G4+TAPBPR-TM

FIG. 3F

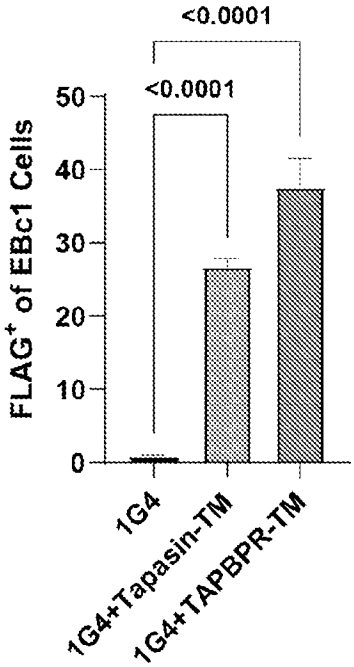
<0.0001
<0.0001
FLAG+ of EBc1 Cells
50
40
30
20
10
0
1G4
1G4+Tapasin-TM
1G4+TAPBPR-TM

FIG. 3G

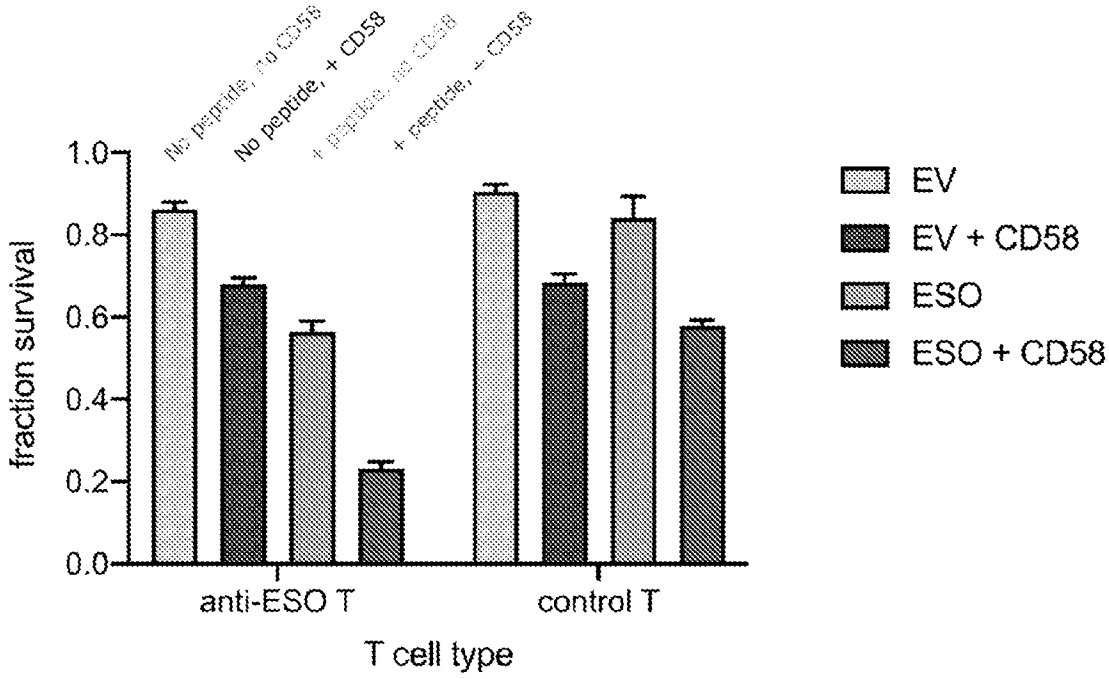
No peptide, + CD58
fraction survival
1.0
0.8
0.6
0.4
0.2
0.0
anti-ESO T
control T
T cell type
EV
EV + CD58
ESO
ESO + CD58

FIG. 3H

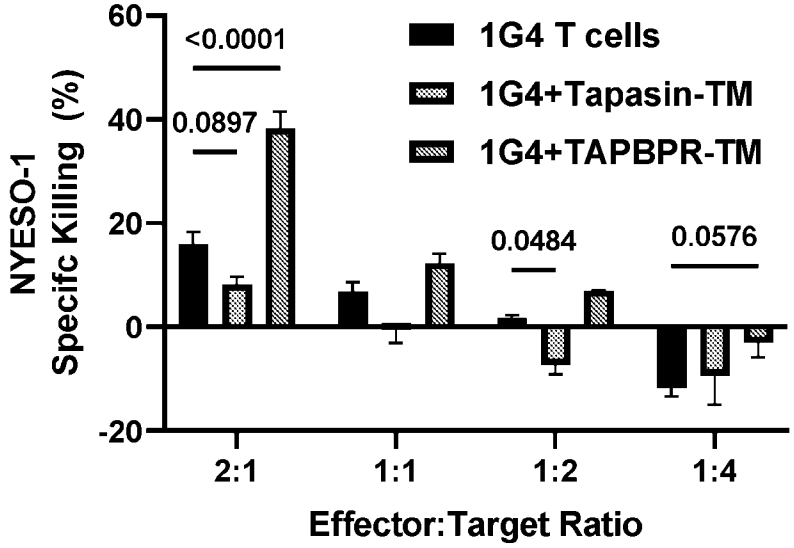
60
40
20
0
-20
NYESO-1
Specifc Killing (%)
<0.0001
0.0897
0.0484
0.0576
1G4 T cells
1G4+Tapasin-TM
1G4+TAPBPR-TM
2:1
1:1
1:2
1:4
Effector:Target Ratio

FIG. 4A

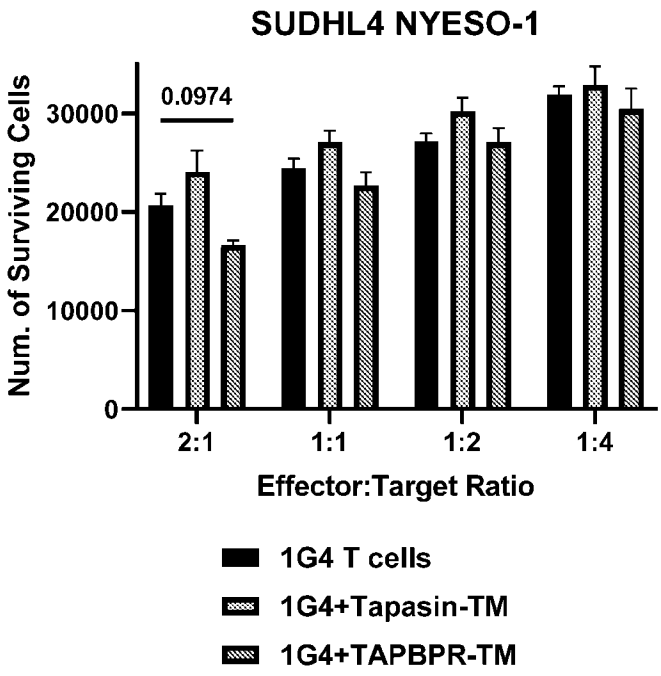
SUDHL4 NYESO-1
Num. of Surviving Cells
30000
20000
10000
0
0.0974
2:1
1:1
1:2
1:4
Effector:Target Ratio
1G4 T cells
1G4+Tapasin-TM
1G4+TAPBPR-TM

FIG. 4B

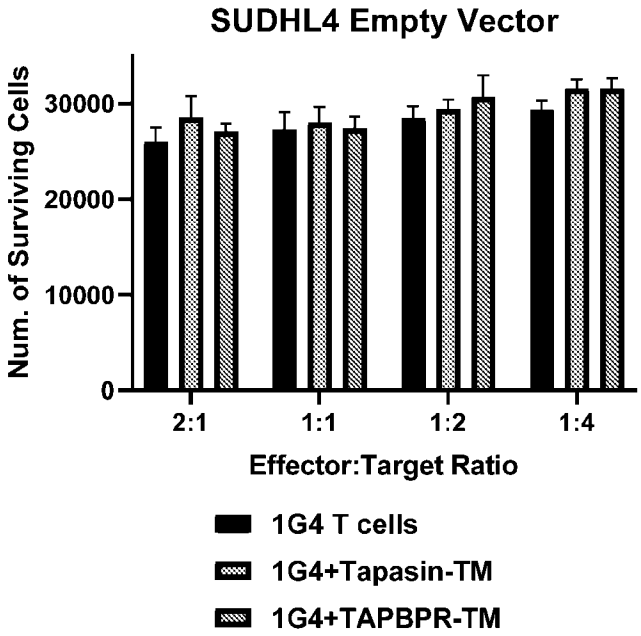
SUDHL4 Empty Vector
Num. of Surviving Cells
30000
20000
10000
0
2:1
1:1
1:2
1:4
Effector:Target Ratio
1G4 T cells
1G4+Tapasin-TM
1G4+TAPBPR-TM

FIG. 4C

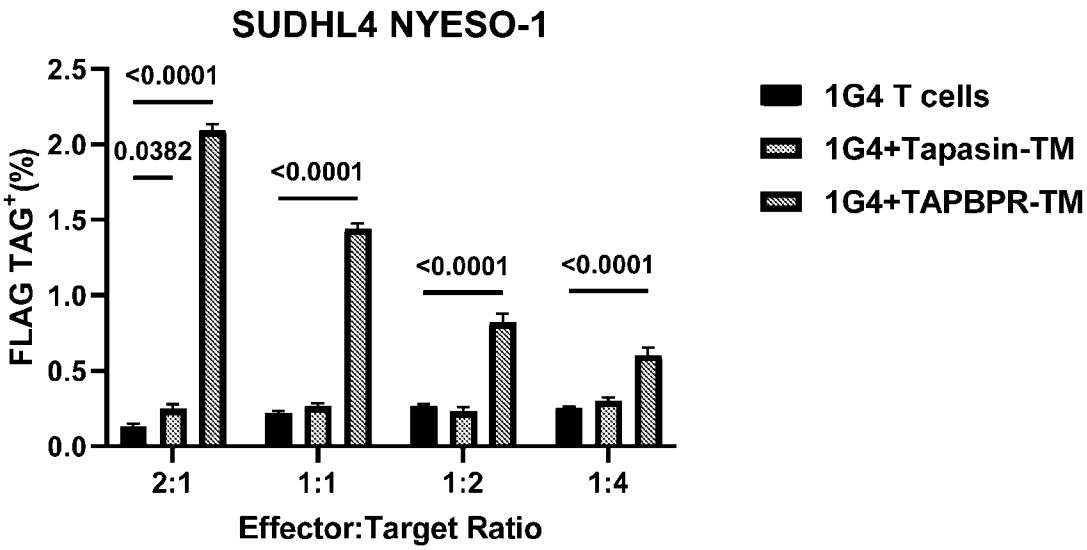
SUDHL4 NYESO-1
FLAG TAG+(%)
2.5
2.0
1.5
1.0
0.5
0.0
<0.0001
0.0382
<0.0001
<0.0001
<0.0001
2:1
1:1
1:2
1:4
Effector:Target Ratio
1G4 T cells
1G4+Tapasin-TM
1G4+TAPBPR-TM

FIG. 4D

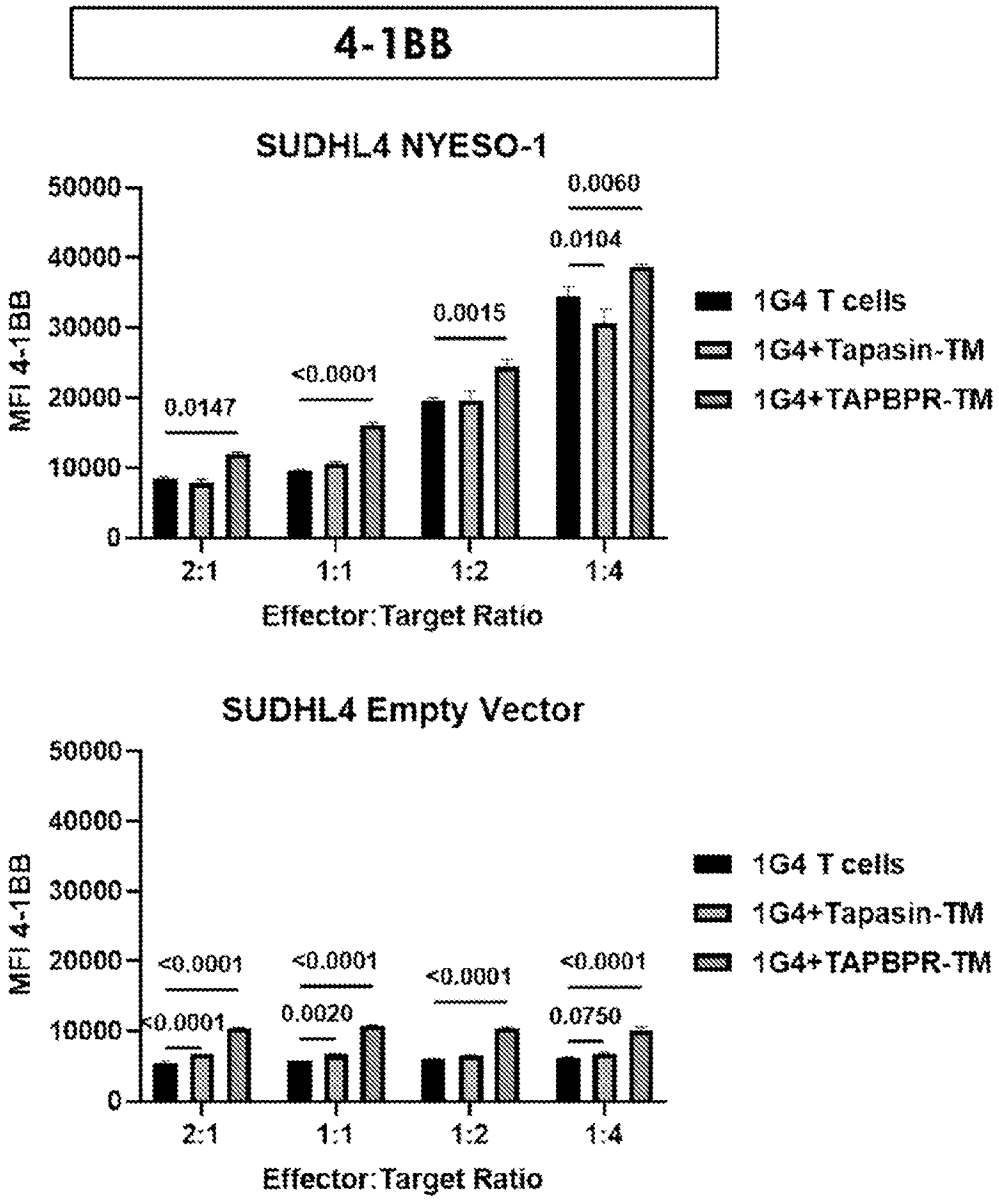
4-1BB
SUDHL4 NYESO-1
MFI 4-1BB
50000
40000
30000
20000
10000
0
0.0147
<0.0001
0.0015
0.0104
0.0060
2:1
1:1
1:2
1:4
Effector:Target Ratio
1G4 T cells
1G4+Tapasin-TM
1G4+TAPBPR-TM
SUDHL4 Empty Vector
MFI 4-1BB
50000
40000
30000
20000
10000
0
<0.0001
<0.0001
<0.0001
<0.0001
<0.0001
0.0020
0.0750
2:1
1:1
1:2
1:4
Effector:Target Ratio
1G4 T cells
1G4+Tapasin-TM
1G4+TAPBPR-TM

FIG. 4E

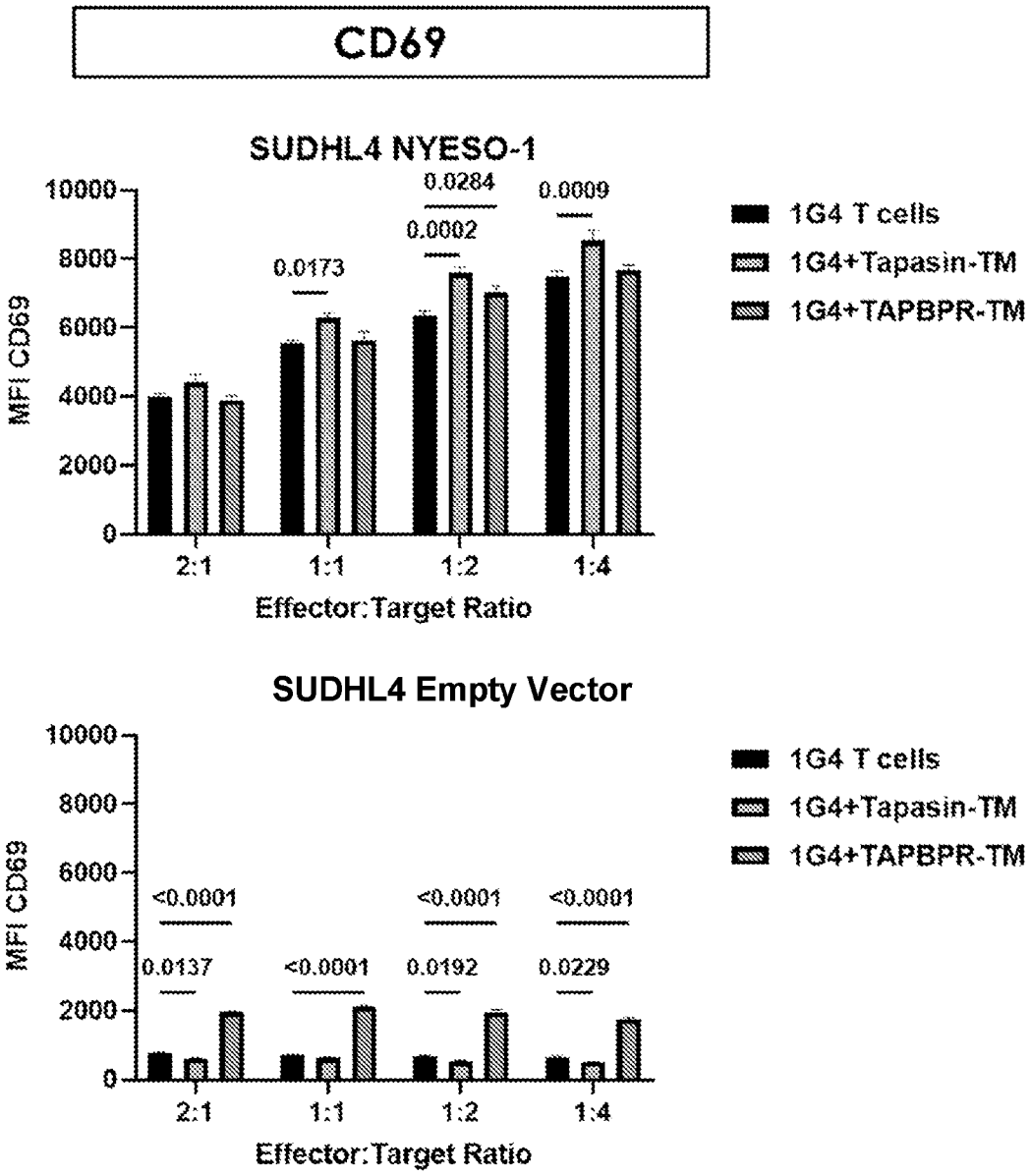
CD69
SUDHL4 NYESO-1
MFI CD69
10000
8000
6000
4000
2000
0
0.0173
0.0284
0.0002
0.0009
2:1
1:1
1:2
1:4
Effector:Target Ratio
1G4 T cells
1G4+Tapasin-TM
1G4+TAPBPR-TM
SUDHL4 Empty Vector
MFI CD69
10000
8000
6000
4000
2000
0
<0.0001
<0.0001
<0.0001
0.0137
<0.0001
0.0192
0.0229
2:1
1:1
1:2
1:4
Effector:Target Ratio
1G4 T cells
1G4+Tapasin-TM
1G4+TAPBPR-TM

FIG. 4F

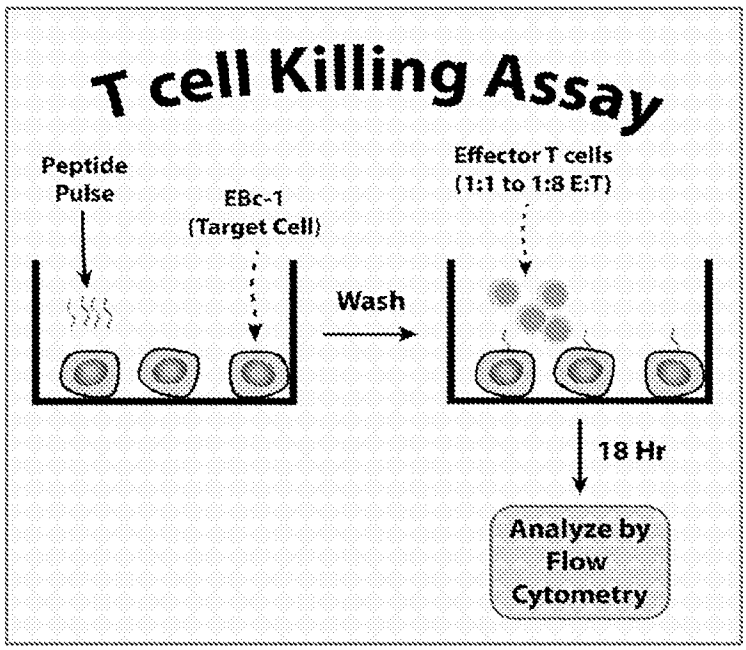
T cell Killing Assay
Peptide Pulse
EBc-1 (Target Cell)
Effector T cells (1:1 to 1:8 E:T)
Wash
18 Hr
Analyze by Flow Cytometry

FIG. 5A

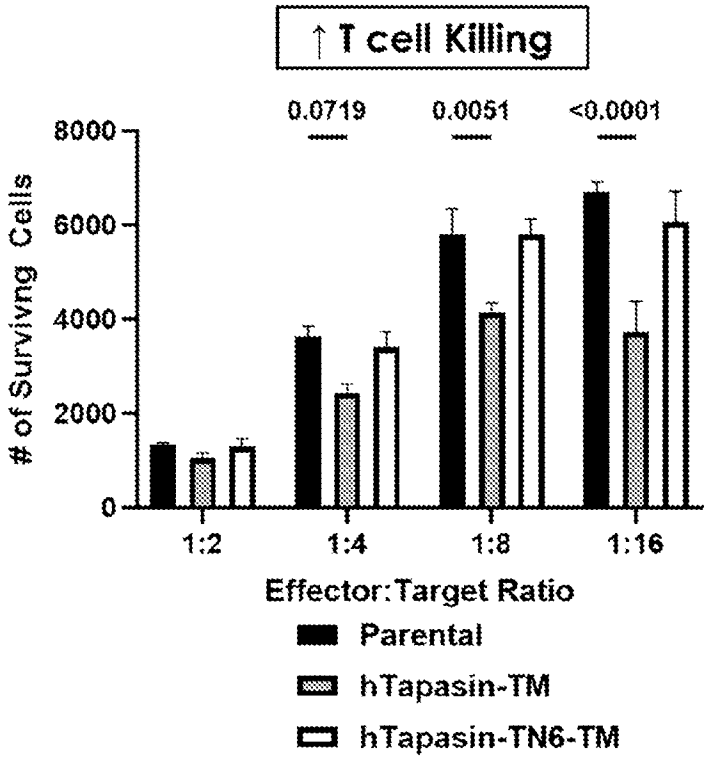
↑ T cell Killing
0.0719
0.0051
<0.0001
8000
6000
4000
2000
0
# of Survivng Cells
1:2
1:4
1:8
1:16
Effector:Target Ratio
Parental
hTapasin-TM
hTapasin-TN6-TM

FIG. 5B

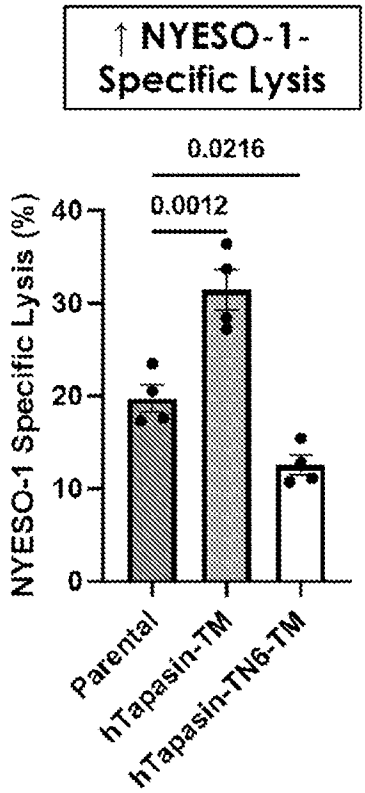
↑ NYESO-1-Specific Lysis
0.0216
0.0012
NYESO-1 Specific Lysis (%)
40
30
20
10
0
Parental
hTapasin-TM
hTapasin-TN6-TM

FIG. 5C

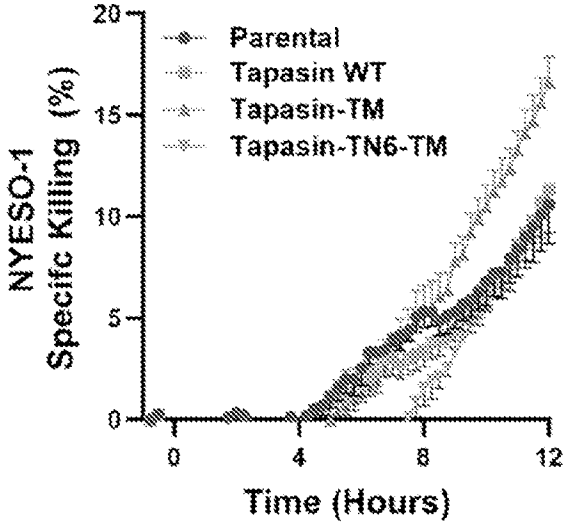
Parental
Tapasin WT
Tapasin-TM
Tapasin-TN6-TM
NYESO-1 Specific Killing (%)
20
15
10
5
0
0
4
8
12
Time (Hours)

FIG. 5D

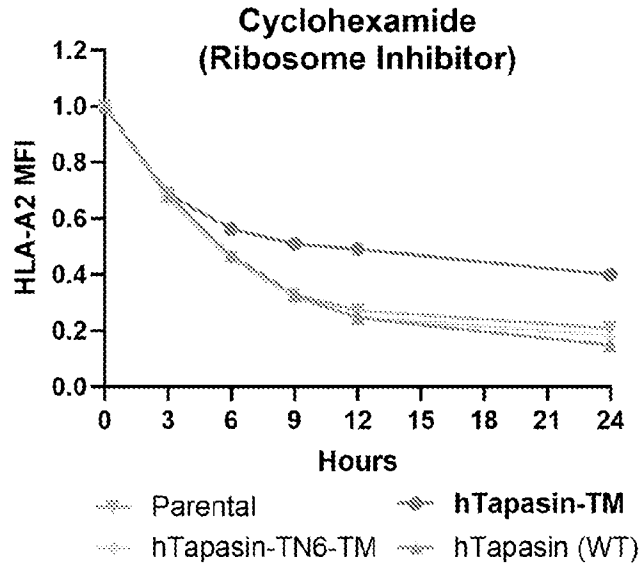
Cyclohexamide
(Ribosome Inhibitor)
HLA-A2 MFI
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
3
6
9
12
15
18
21
24
Hours
Parental
hTapasin-TM
hTapasin-TN6-TM
hTapasin (WT)

FIG. 6A

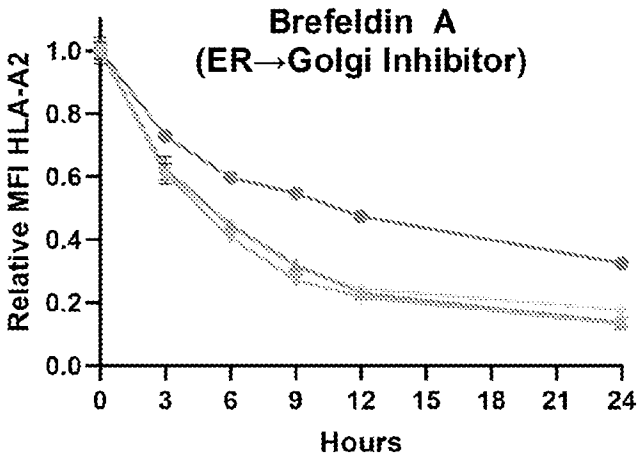
Brefeldin A
(ER→Golgi Inhibitor)
Relative MFI HLA-A2
1.0
0.8
0.6
0.4
0.2
0.0
0
3
6
9
12
15
18
21
24
Hours

FIG. 6B

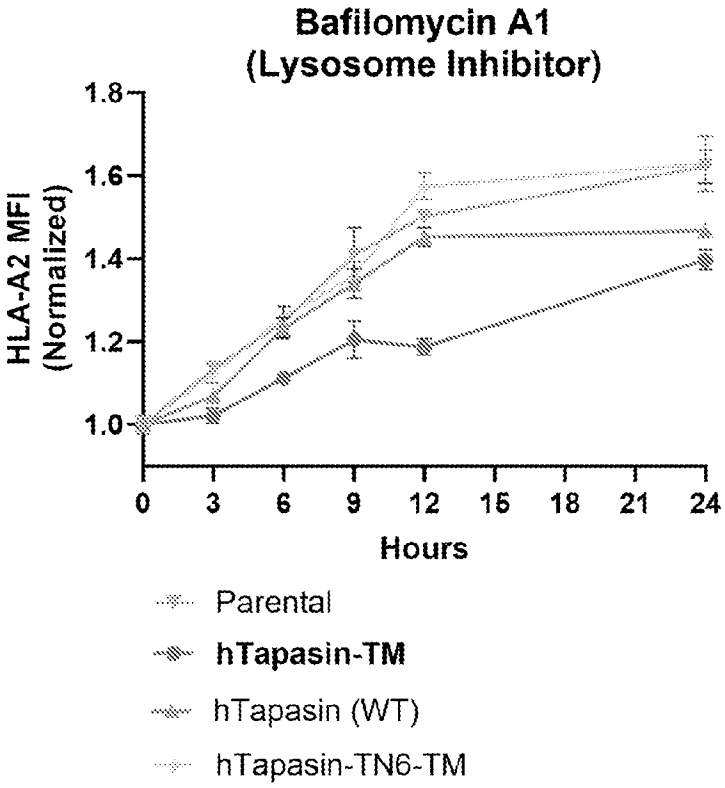
Bafilomycin A1
(Lysosome Inhibitor)
HLA-A2 MFI
(Normalized)
1.8
1.6
1.4
1.2
1.0
0
3
6
9
12
15
18
21
24
Hours
Parental
hTapasin-TM
hTapasin (WT)
hTapasin-TN6-TM

FIG. 6C

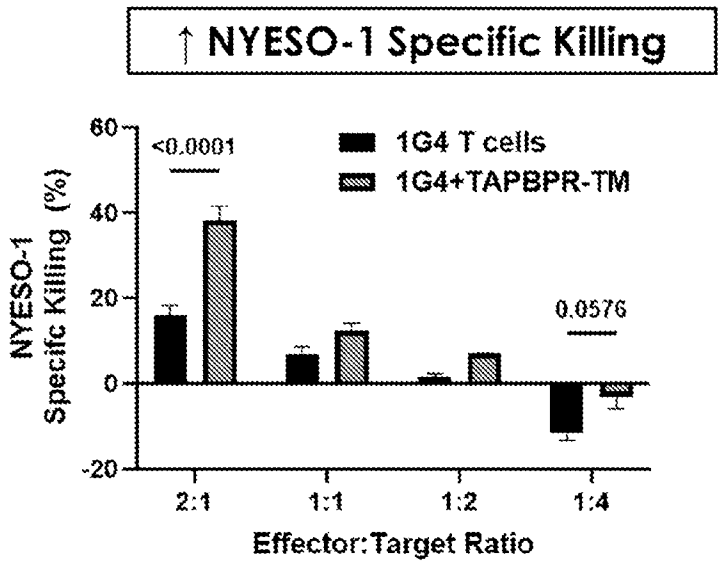
↑ NYESO-1 Specific Killing
NYESO-1
Specific Killing (%)
60
40
20
0
-20
<0.0001
0.0576
1G4 T cells
1G4+TAPBPR-TM
2:1
1:1
1:2
1:4
Effector:Target Ratio

FIG. 7A

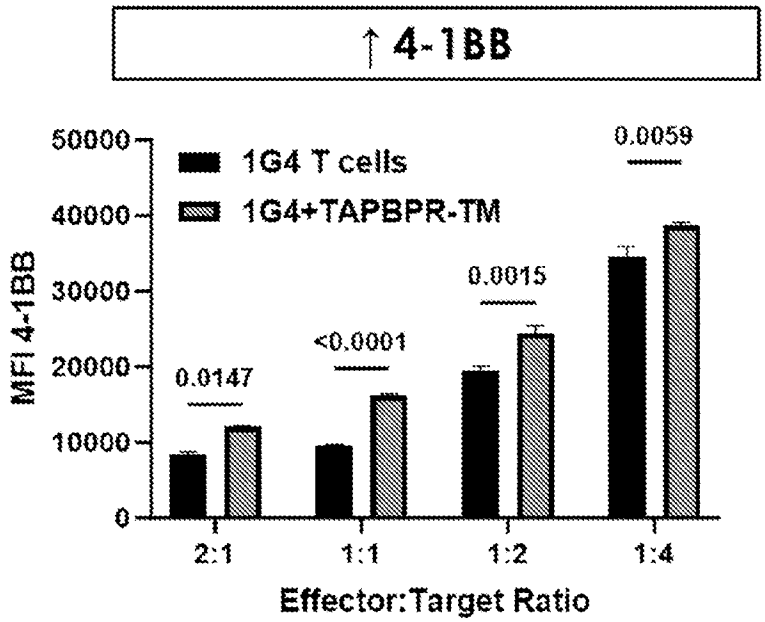
↑ 4-1BB
MFI 4-1BB
50000
40000
30000
20000
10000
0
1G4 T cells
1G4+TAPBPR-TM
0.0147
<0.0001
0.0015
0.0059
2:1
1:1
1:2
1:4
Effector:Target Ratio

FIG. 7B

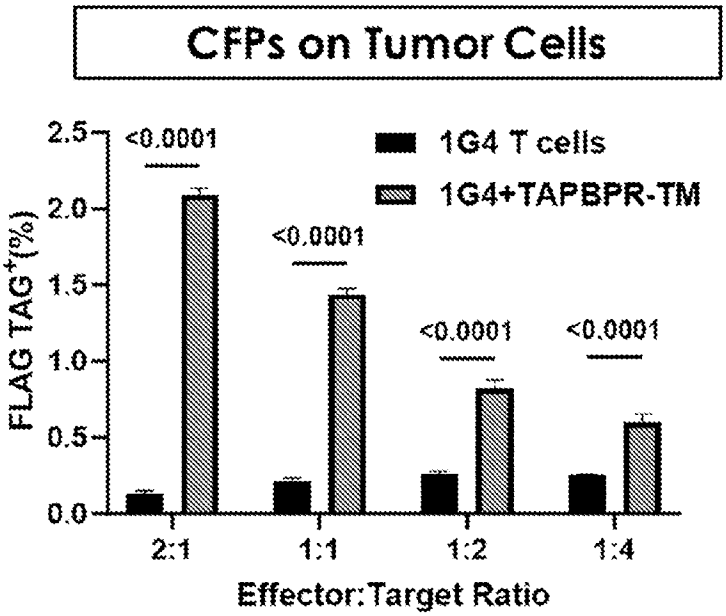
CFPs on Tumor Cells
FLAG TAG+(%)
2.5
2.0
1.5
1.0
0.5
0.0
1G4 T cells
1G4+TAPBPR-TM
<0.0001
<0.0001
<0.0001
<0.0001
2:1
1:1
1:2
1:4
Effector:Target Ratio

FIG. 7C

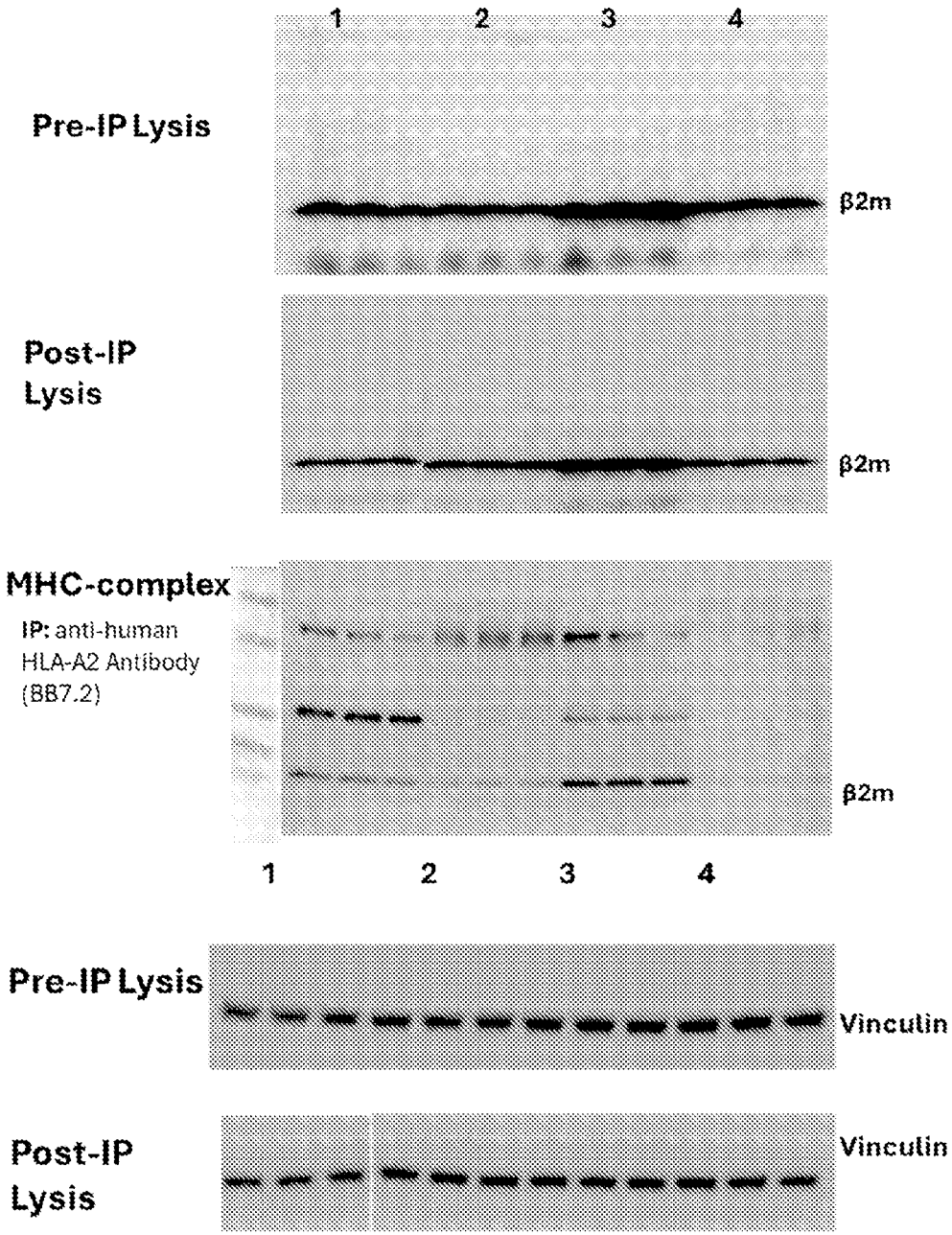
1
2
3
4
Pre-IP Lysis
β2m
Post-IP Lysis
β2m
MHC-complex
IP: anti-human HLA-A2 Antibody (BB7.2)
β2m
1
2
3
4
Pre-IP Lysis
Vinculin
Post-IP Lysis
Vinculin

FIG. 8

Group:

1. Ebc1 NYESO-1 GFP (Parental)
2. Ebc1 NYESO-1 GFP / hTapasin-CT (WT tapasin)
3. Ebc1 NYESO-1 GFP / hTapasin-TM (Engineered tapasin)
4. Ebc1 NYESO-1 GFP / hTapasin-TN6-TM (negative control)

Group	ID	Protein(μg/μL)	Total protein (mg)
1	p1	5.3	20.2
	p2	4.4	16.7
	p3	3.3	12.5
2	ct1	7.6	28.7
	ct2	4.1	15.8
	ct3	3.7	14
3	tm1	4.2	15.9
	tm2	3.6	13.7
	tm3	5.2	19.9
4	tn tm1	5.7	21.6
	tn tm2	4	15.3
	tn tm3	6.6	24.9

Total protein per IP: 12.5 mg

FIG. 8 (cont.)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/055092

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C07K 14/705*** (2024.01); ***C12N 15/62*** (2024.01); ***A61K 39/00*** (2024.01); ***C07K 14/47*** (2024.01); *A61P 35/00* (2024.01)

CPC: ***C07K 14/70539; A61K 39/0011; C07K 14/4748; C12N 15/62;*** *A61P 35/00; C07K 2319/03; C07K 2319/40; C07K 2319/70*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2023/163980 A1 (THE CHILDREN'S HOSPITAL OF PHILADELPHIA et al.) 31 August 2023 (31.08.2023) entire document	1-6, 9, 10
A	US 2020/0148743 A1 (COMMISSARIAT A L'ENERGIE ATOMIQUE ET AUX ENERGIES ALTERNATIVES et al.) 14 May 2020 (14.05.2020) entire document	1-10
A	US 2017/0252417 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 07 September 2017 (07.09.2017) entire document	1-10
A	US 2021/0054046 A1 (SANGAMO THERAPEUTICS INC. et al.) 25 February 2021 (25.02.2021) entire document	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 December 2024 (13.12.2024)	**11 February 2025 (11.02.2025)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450** **UNITED STATES OF AMERICA**	**TAINA MATOS**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/055092

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/055092

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☑ Claims Nos.: **11-20**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claims 1-10 are drawn to methods of increasing surface human leukocyte antigen (HLA) expression.

Group II: claims 21-30 are drawn to methods for enhancing peptide presentation and identification in tumor cells.

The inventions listed in Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, methods of increasing surface human leukocyte antigen (HLA) expression, are not present in Group II; and the special technical features of Group II, methods for enhancing peptide presentation and identification in tumor cells, are not present in Group I.

Additionally, even if Groups I and II were considered to share the technical features of fusing a nucleic acid encoding a chaperone to a nucleic acid encoding a protein of interest, forming a fused nucleic acid encoding a chaperone fusion protein (CFP), administering the nucleic acid encoding the CFP to a cell, allowing the nucleic acid to express the CFP in the cell, wherein the CFP increases HLA expression in the cell, these shared technical features do not represent a contribution over the prior art.

Specifically, WO 2023/163980 A1 to The Children's Hospital Of Philadelphia et al. teaches fusing a nucleic acid encoding a chaperone to a nucleic acid encoding a protein of interest, forming a fused nucleic acid encoding a chaperone fusion protein (CFP) (The invention encompasses a nucleic acid encoding any one of the herein disclosed isolated ligand exchange protein, Para. [0018]; the ligand exchange protein may be a fusion protein comprising the TAPBPR fragment and one or more heterologous domains, Para. [0094]), administering the nucleic acid encoding the CFP to a cell, allowing the nucleic acid to express the CFP in the cell (A recombinant polypeptide may be expressed from heterologous nucleic acid that has been introduced into a cell by artificial means, Para. [0097]), wherein the CFP increases HLA expression in the cell (a method of producing a MHC class I molecule displaying a target peptide comprising contacting an MHC class I molecule with any one of the herein disclosed ligand exchange proteins and a target peptide, such that the ligand exchange protein loads the target peptide onto the MHC class I molecule thereby producing an MHC class I

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

molecule displaying the target peptide, Para. [0030]; MHC class I molecules may include HLA-A molecules, Para. [00105]).

The inventions listed in Groups I and II therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-10**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.