

## SUPPLEMENTAL MATERIALS

for

### **SLC45A2: A MELANOMA ANTIGEN WITH HIGH TUMOR SELECTIVITY AND REDUCED POTENTIAL FOR AUTOIMMUNE TOXICITY**

#### SUPPLEMENTAL FIGURE LEGENDS

**Figure S1. Ectopic expression of HLA allotypes in melanoma cell line Mel888.** Parental Mel888 cells were transduced to over-express either HLA-A\*0201 or HLA-A\*2402, stained with A\*0201- or A\*2402-specific antibodies, and analyzed by flow cytometry.

**Figure S2. SLC45A2 expression is restricted to melanoma tumor cells. (A)** SLC45A2 expression in non-melanoma tumor cell lines including colon cancer, breast cancer, prostate cancer, lung cancer and hematologic malignancies, as determined by RT-PCR analysis. **(B)** Summary of RT-PCR results showing SLC45A2, MART-1, PMEL, and tyrosinase expression in non-melanoma cell lines.

**Figure S3. SLC45A2 gene expression in Cancer Cell Line Encyclopedia (CCLE) cell lines.** Gene expression analysis in a diverse array of CCLE tumor cell lines shows SLC45A2 expression is melanoma tumor-specific.

**Figure S4. Quantitative comparison of SLC45A2 gene expression in various normal tissues and cancers.** RNAseq analysis derived from the GTex Portal and TCGA databases showing SLC45A2 transcript expression in normal tissues and various cancers, respectively. TPM, transcripts per million.

**Figure S5. Generation of HLA-A\*0201-restricted SLC45A2 antigen-specific CTLs from multiple normal donor PBMC. (A)** See Figure 3 legend for description of SLC45A2 antigen-specific T-cell generation. **(B)** Phenotype of SLC45A2-specific CTLs 14 days post-REP, as determined by CD45RA, CCR7, CD62L and CD28 staining and flow cytometry.

**Figure S6. SLC45A2-specific CTLs demonstrate lytic activity against uveal and mucosal melanoma cell lines.** (A) Expression analysis of MDAs SLC45A2, MART-1, PMEL and tyrosinase in 5 different uveal melanoma cell lines, as determined by RT-PCR. (B) Cytotoxic activity of SLC45A2-specific CTLs against HLA-A\*0201-positive or negative uveal melanoma cell lines. Cytotoxic activity of MART1- or PMEL-specific CTLs also was tested against these uveal cell lines. (C) Cytotoxic activity of SLC45A2-specific CTLs against HLA-A\*2402-positive or negative uveal melanoma cell lines. Significant killing is observed for OMM1 (HLA-A2+, SLC45A2+) uveal melanoma line, but not for HLA-A2-negative melanoma lines (92.1, 202, UPMD2). UPMD1 is an HLA-A2+ line with markedly reduced expression of SLC45A2, which is reflected in significantly lower lytic sensitivity. (D) Expression analysis of MDAs in 2 mucosal melanoma cell lines, as determined by RT-PCR. (E) Cytotoxic activity of SLC45A2-, MART1-, or PMEL-specific CTLs against HLA-A\*0201-positive or negative mucosal melanoma cell lines. All results show one representative experiment of at least 2 performed.

**Figure S7. IFN-gamma release by MDA-specific CTLs in response to titrated HLA-A\*0201 restricted peptides.** T2 cells pulsed with titrated concentrations of different MDA peptides were co-cultured with SLC45A2-, MART1-, or PMEL-specific CTLs. Forty-eight hours later, IFN- $\gamma$  concentration was measured by ELISA.

**Figure S8. Primary melanocytes express HLA-A\*0201 but are only killed by SLC45A2 T cells after peptide pulsing.** (A) Comparison of surface HLA-A\*0201 expression in Mel526, A375, and primary melanocytes 3C and 4C following staining with mAb BB7.2 and flow cytometric analysis. (B) Primary melanocyte lines 3C and 4C were pulsed with 1 $\mu$ g/ml SLYSYFQKV peptide and used as targets for HLA-A\*0201-restricted SLC45A2-specific CTLs in standard <sup>51</sup>Cr release assay.

**Figure S9. SLC45A2-specific CTLs from different donors preferentially kill melanoma tumor cells over primary melanocytes.** Results of standard <sup>51</sup>Cr release assay showing cytolytic activity of HLA-A\*0201-restricted SLC45A2-specific CTLs derived from 3 different PBMC donors against A\*0201-expressing Mel 526 (SLC45A2+), A375 (SLC45A2-), and two melanocyte cell lines (3C and 4C).

***SLC45A2: A melanoma tumor antigen  
with high tumor selectivity and  
reduced potential for autoimmune toxicity***

***SUPPLEMENTAL FIGURES AND TABLES***

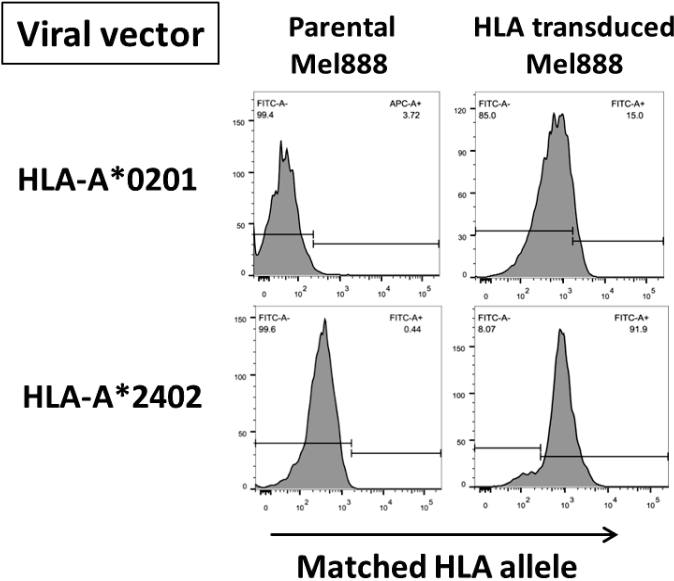
**Supplemental Table S1.** Human MDA gene primer pair sequences used for RT-PCR.

<b>Gene</b>	<b>Sense</b>	<b>Anti-sense</b>	<b>size</b>
SLC45A2	CTGGCCGCCACATCTATAAAT	GTAGCAGAACTCTCTTCCGAAC	125 bp
MART-1	ACAGTGATCCTGGGAGTCTTAC	TTGAAGAGACACTTTGCTGTCC	168 bp
gp100	AGGTGCCTTTCTCCGTGAG	GCTTCAGCCAGATAGCCACT	128bp
Tyrosinase	GCAAAGCATACCATCAGCTCA	GCAGTGCATCCATTGACACAT	145bp
GAPDH	AAT CCC ATC ACC ATC TTC CA	TGG ACT CCA CGA CGT ACT CA	94bp

**Supplemental Table S2.** SLC45A2-derived peptides detected by mass spectrometric analysis of melanoma cell lines.

SLC45A2 peptide	Peptide position	Number of melanomas in which peptide was detected	HLA restriction element	Worldwide HLA prevalence	Predicted HLA binding affinity (nM)
SLYSYFQKV	382 - 390	6	A*0201	28%	7
RLLGTEFQV	209 - 217	8			11
SYIGLKGLYF	393 - 402	3	A*2402	34%	51
VWFLSPILGF	73 - 82	2			76
ALIANPRRK	129 - 137	2	A*0301	8%	108
SGQAGRHIY	5 - 13	2	A*3002	3%	41

**Supplemental Figure S1:** Ectopic expression of HLA allotypes in melanoma cell line Mel888.

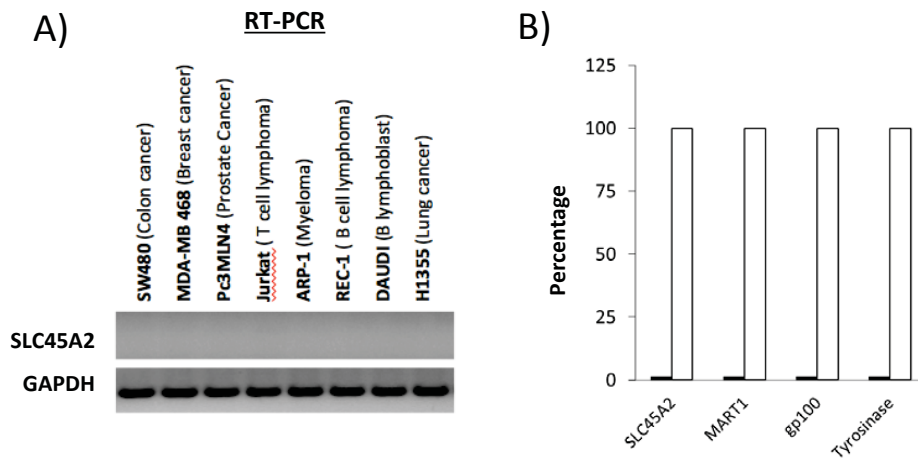


**Supplemental Table S3.** Confirmation of natural SLC45A2 peptide processing and presentation by HLA transduction.

SLC45A2 peptide	HLA-transduced SLC45A2 <sup>+</sup> melanoma cell line (Peptide ion score)			Predicted HLA-peptide binding affinity (nM)		
	Mel888 PARENTAL	Mel888 A*0201	Mel888 A*2402	A*0101	A*0201	A*02401
SLYSYFQKV	-	20	-	19814	7	12016
RLLGTEFQV	-	27	-	21492	11	14058
SYIGLKGLYF	34	23	61	12377	24841	51
VWFLSPILGF	-	-	17	11843	18995	76

**Supplemental Figure S2:** SLC45A2 expression is restricted to melanoma tumor cells

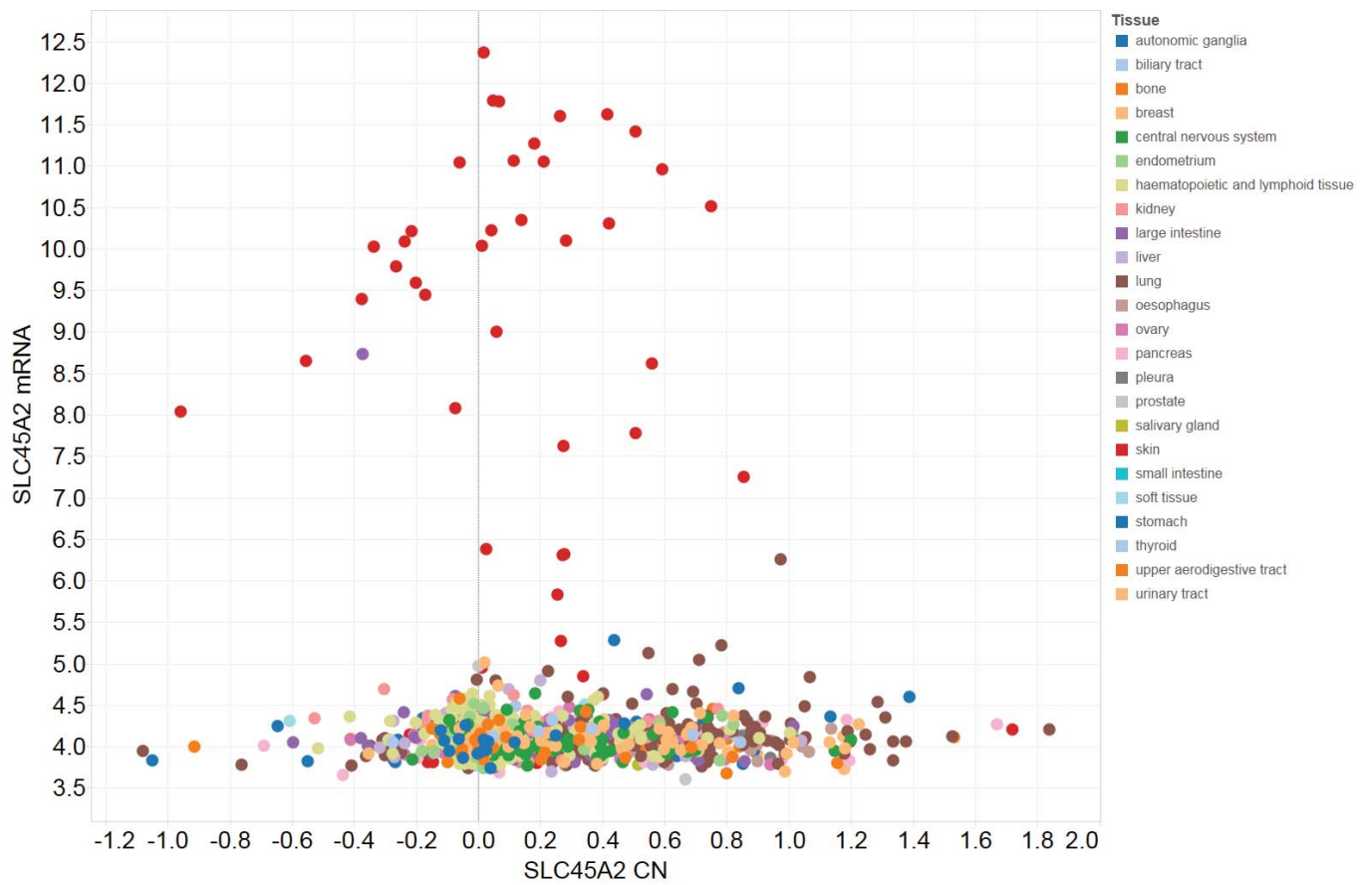
**Non-melanoma cell line summary**





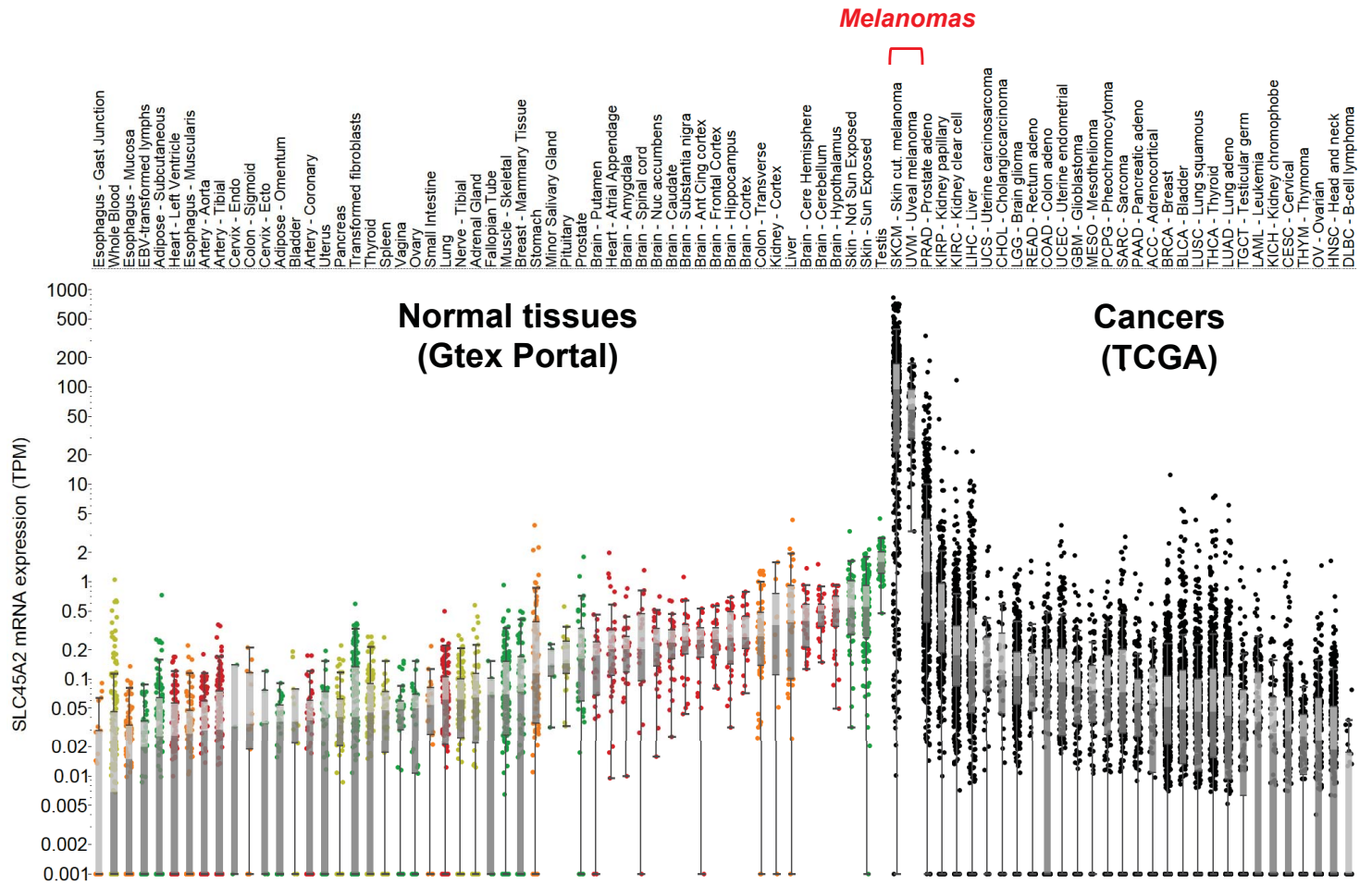
**Supplemental Figure S3:** SLC45A2 expression in Cancer Cell Line Encyclopedia (CCLE) cell lines.

### SLC45A2 transcript expression (microarray)



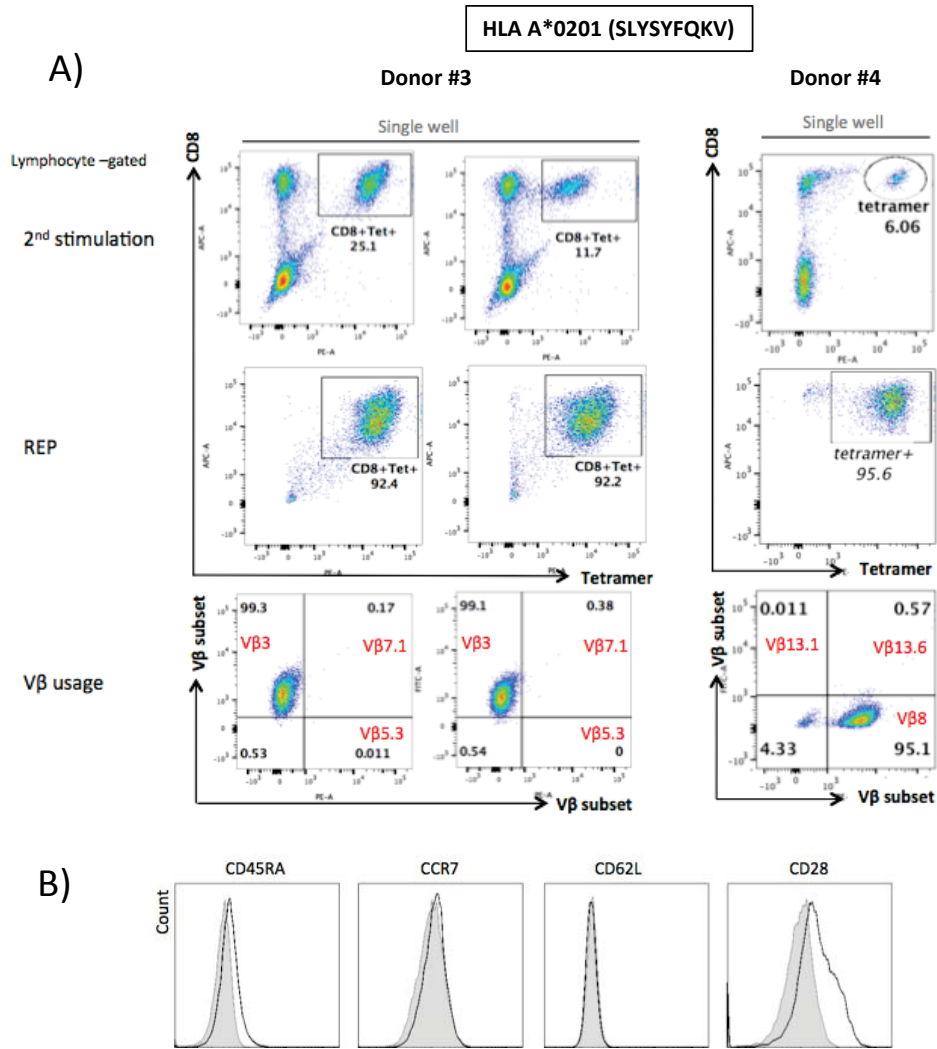
Supplemental Figure S4: Quantitative comparison of SLC45A2 gene expression in various normal tissues and cancers

### SLC45A2 mRNA transcript expression (RNAseq)



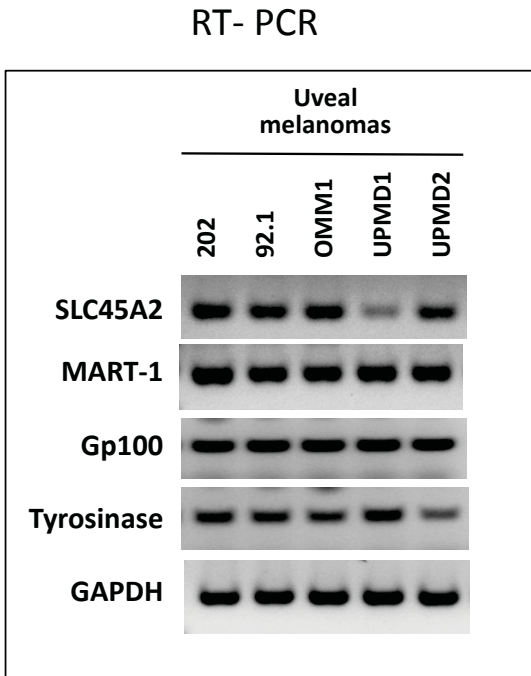
**Supplemental Figure S5:**

Generation of HLA-A\*0201-restricted SLC45A2 antigen-specific CTLs from multiple normal donor PBMC.

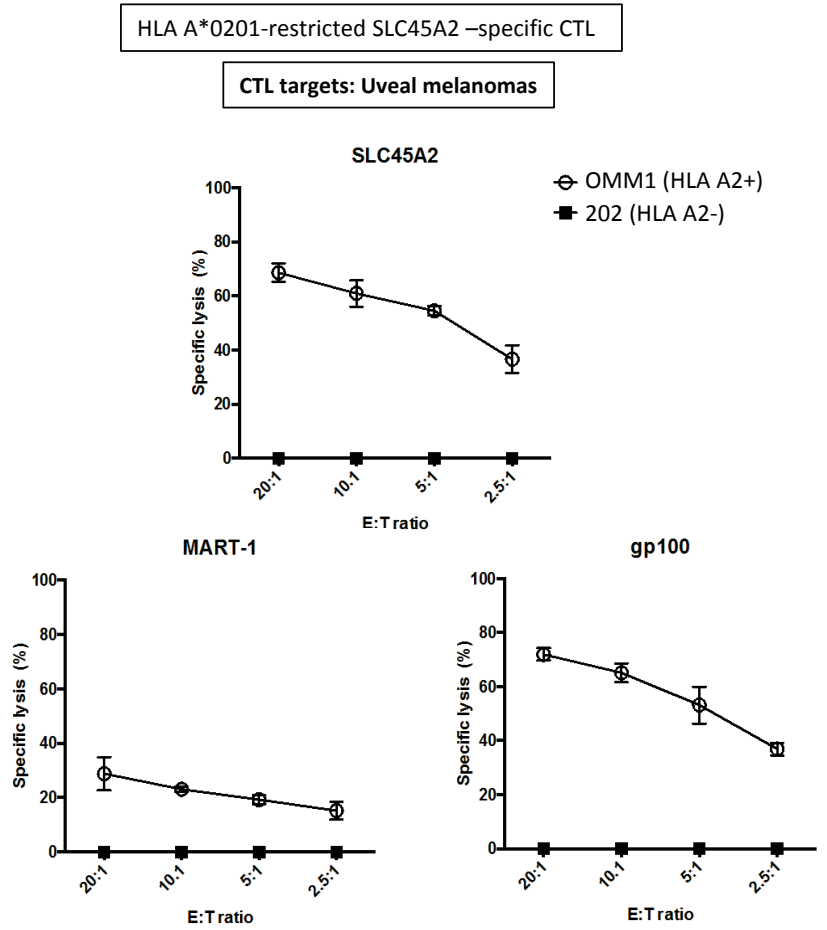


**Supplemental Figure S6: SLC45A2-specific CTL demonstrate lytic activity against uveal and mucosal melanoma cell lines.**

A)



B)



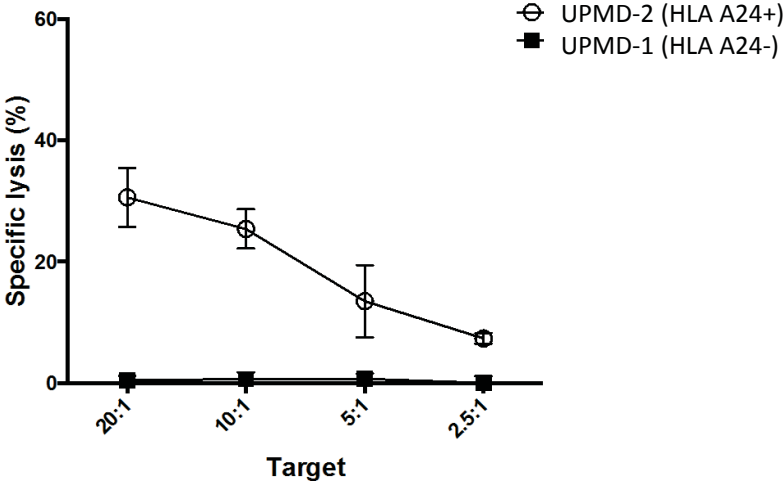
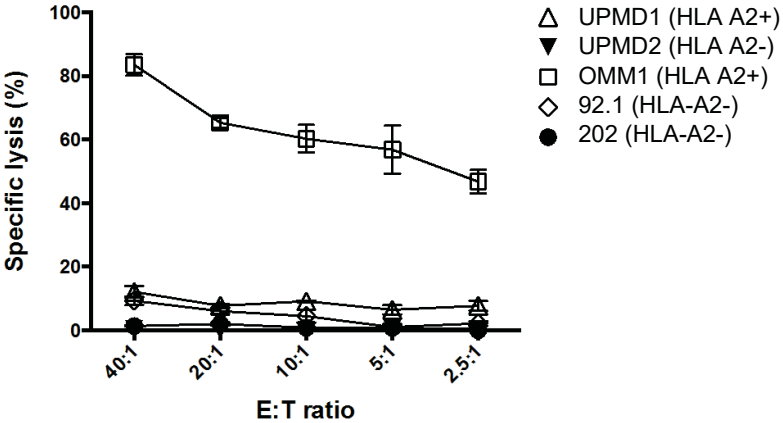
**Supplemental Figure S6: SLC45A2-specific CTL demonstrate lytic activity against uveal and mucosal melanoma cell lines.**

CTL targets: Uveal melanomas

C) HLA A\*0201-restricted SLC45A2 –specific CTL

D)

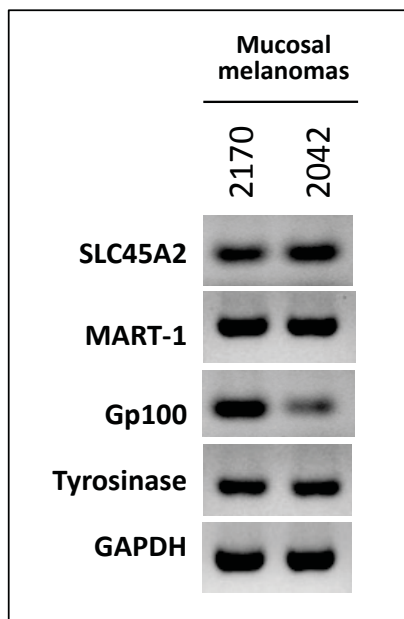
HLA A\*2402-restricted SLC45A2 –specific CTL



**Supplemental Figure S6: SLC45A2-specific CTL demonstrate lytic activity against uveal and mucosal melanoma cell lines.**

D)

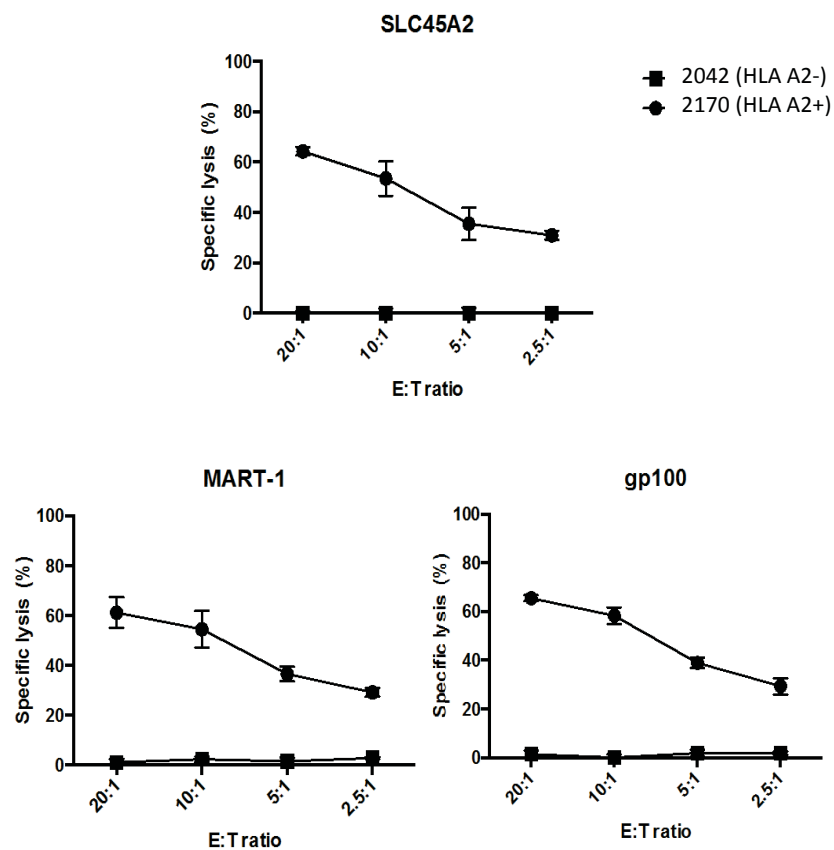
RT- PCR



E)

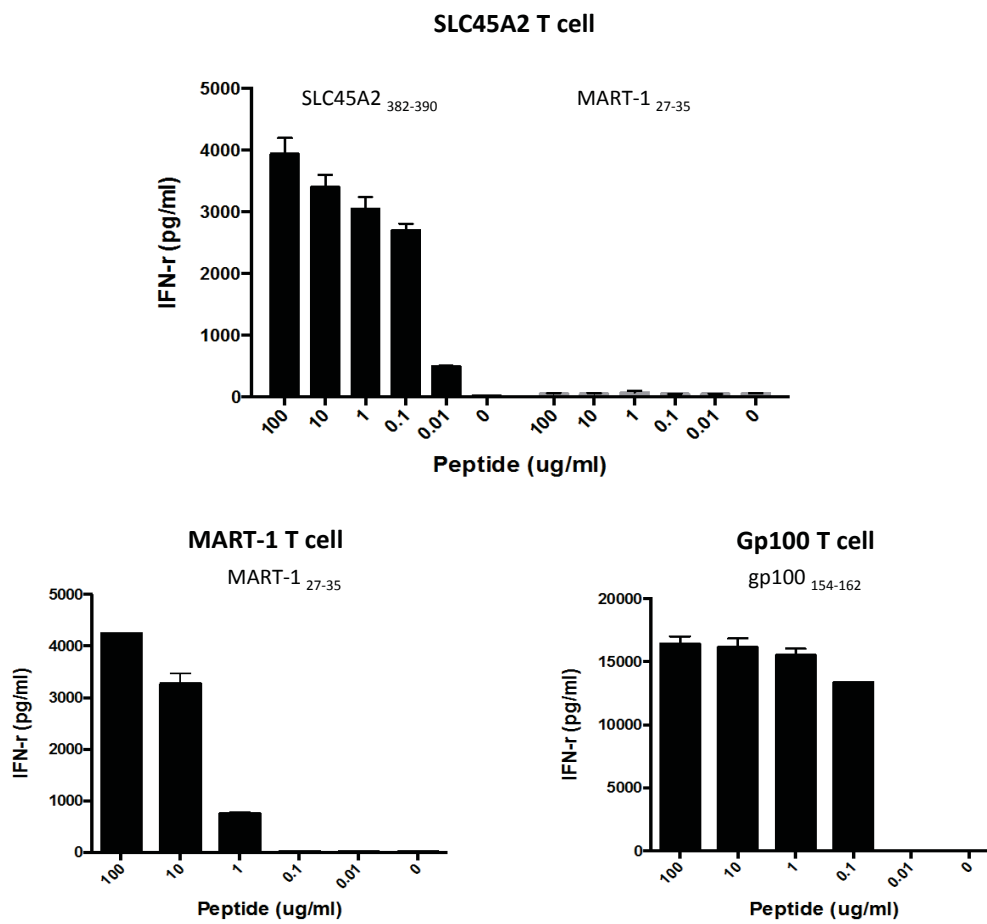
HLA A\*0201-restricted SLC45A2 –specific CTL

CTL targets: Mucosal melanomas



**Supplemental Figure S7:**

IFN-gamma release by MDA-specific CTL in response to titrated HLA-A\*0201 restricted MDA peptides.



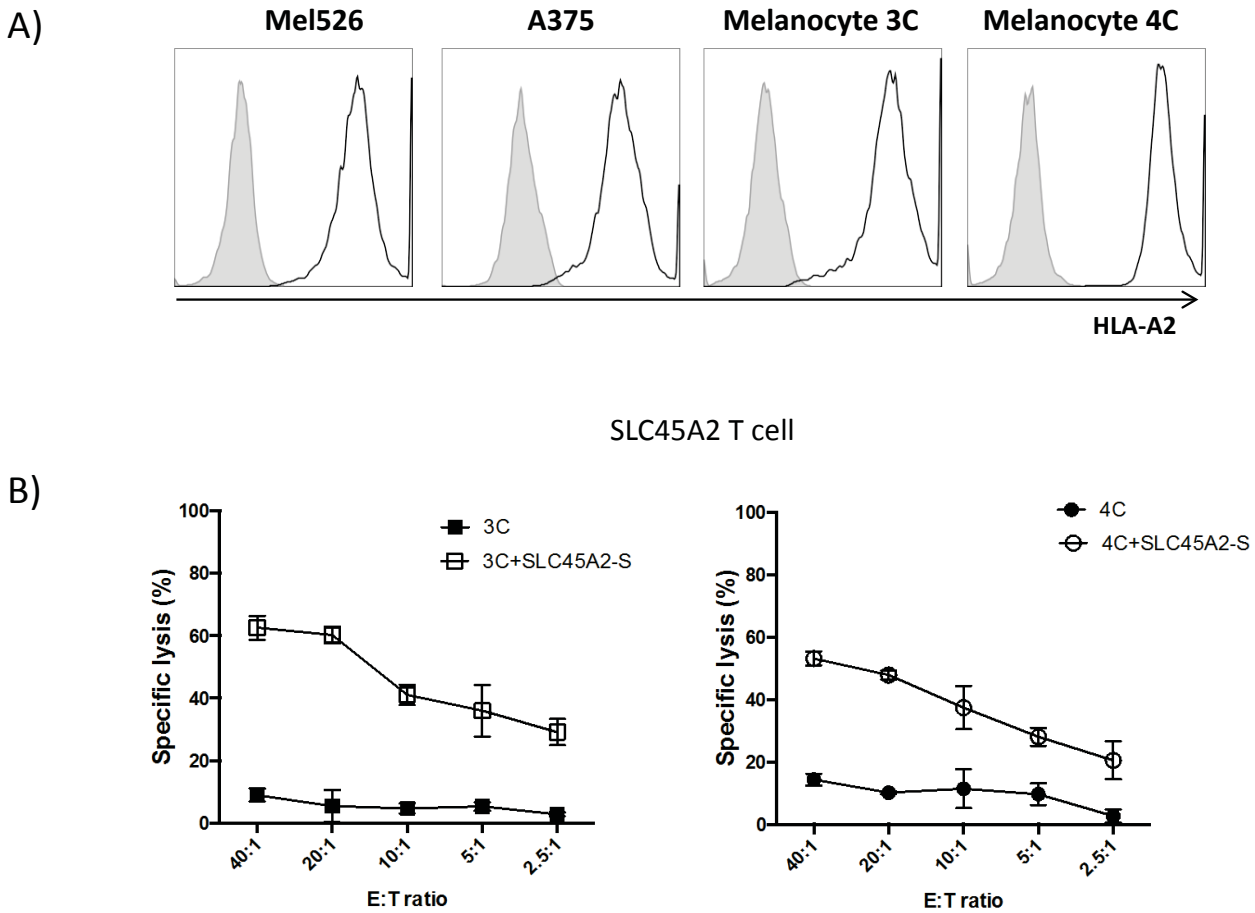
**Supplemental Table S4.** Sequences and predicted binding affinities of HLA-A2-restricted MDA peptides.

<b>MDA Protein</b>	<b>Peptide position</b>	<b>Peptide seq</b>	<b>Affinity (nM)</b>	<b>HLA-type</b>
<b>SLC45A2</b>	382-390	<b>SLYSYFQKV</b>	7	HLA-A*0201
<b>gp100</b>	154-162	<b>KTWGQYWQV</b>	9	HLA-A*0201
<b>MART-1</b>	27-35	<b>AAGIGILTV</b>	2498	HLA-A*0201



**Supplemental Figure S8:**

Primary melanocytes express HLA-A\*0201 but are only killed by SLC45A2 T cells after peptide pulsing.



**Supplemental Figure S9:**

SLC45A2-specific CTLs from different donors kill melanoma tumor cells preferentially over primary melanocytes.

HLA A\*0201 restricted SLC45A2 –specific CTL

