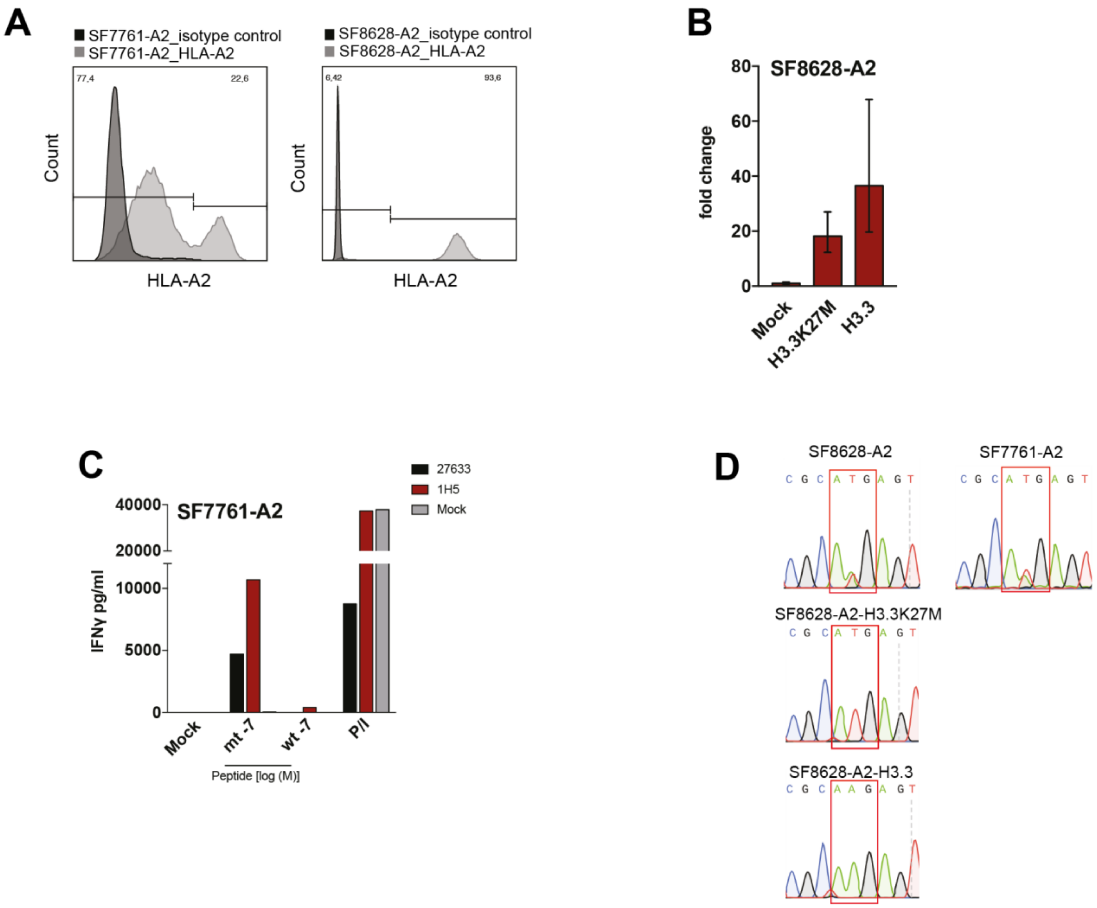
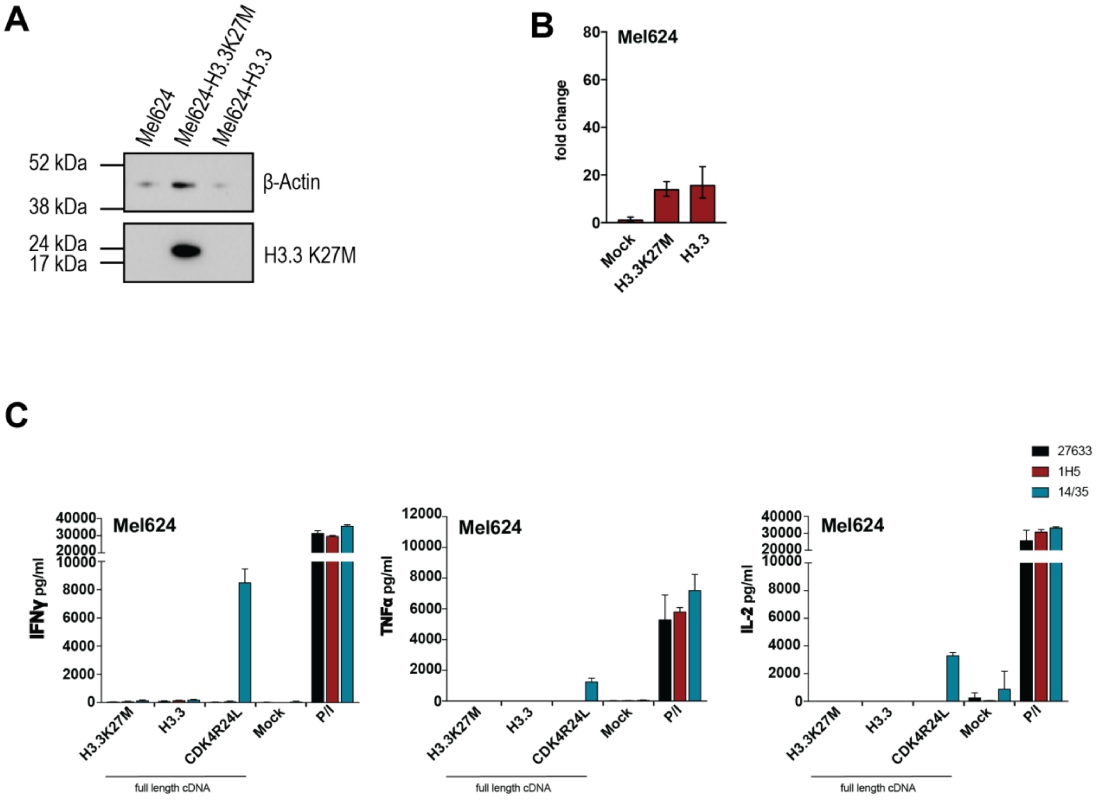
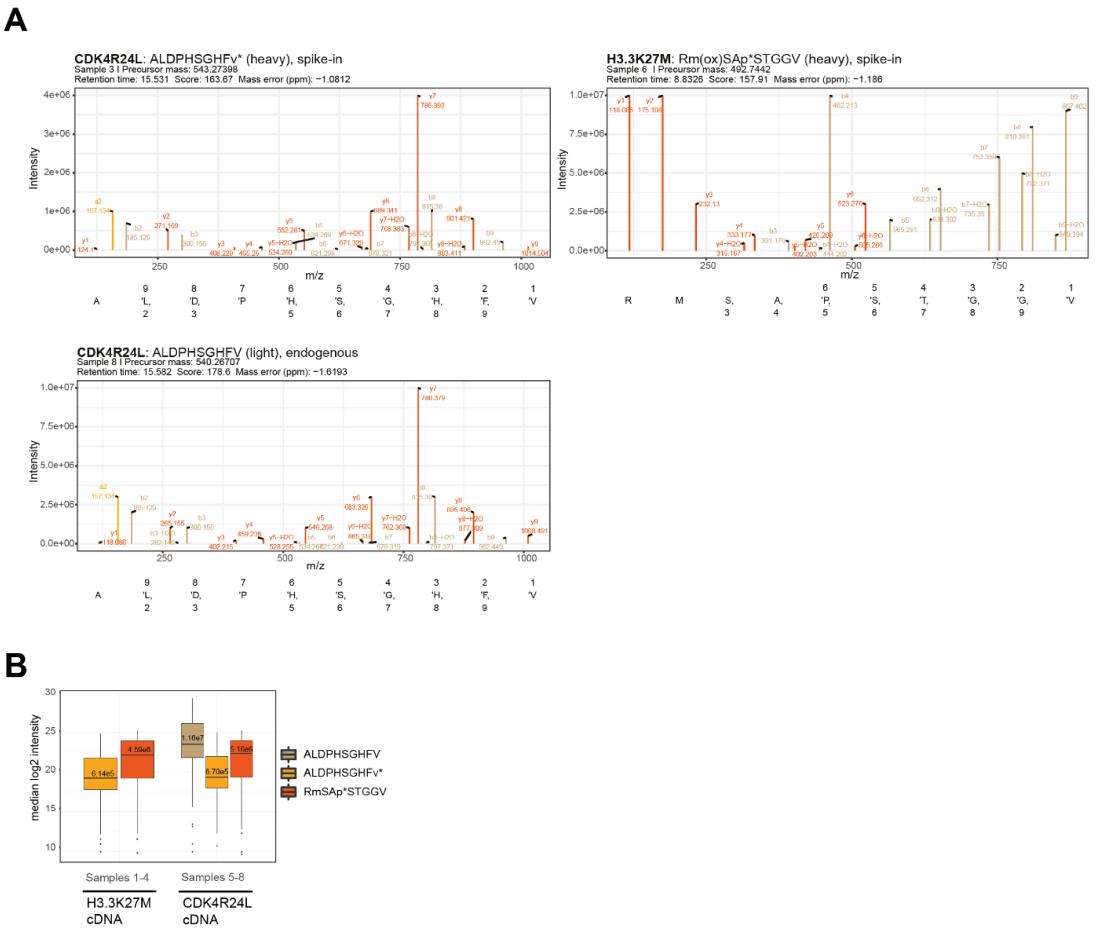
Supplementary Figures



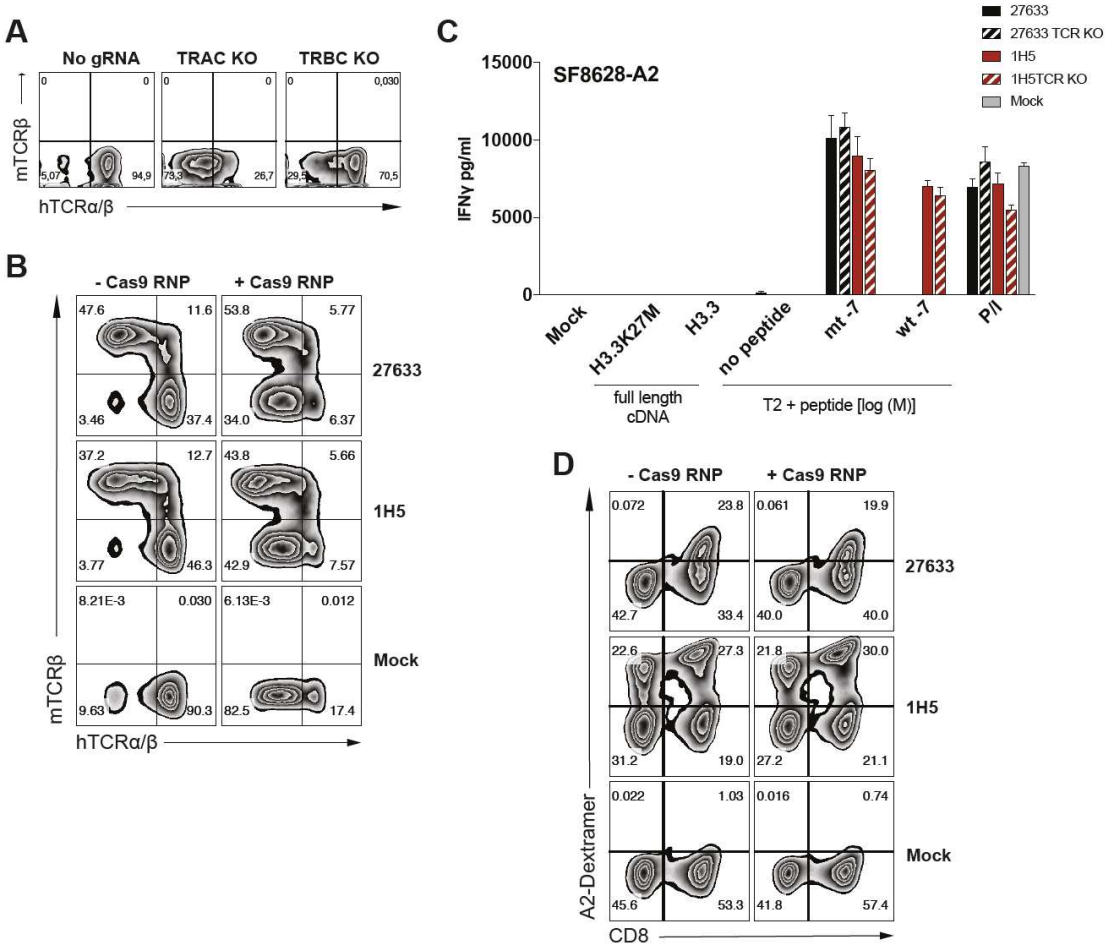
Supplementary Figure 1 **Mutant and wild type H3.3 overexpression profiles and failure of H3.3K27M TCR-transduced T cells to recognize DIPG cell line SF7761 naturally expressing mutant H3.3 histone.** **(A)** The glioma cells lines SF7761 and SF8628 were retrovirally transduced to express HLA-A*02:01. Expression levels were determined by flow cytometry using a HLA-A*02 specific antibody. **(B)** Relative quantitation of all H3.3 transcripts in SF8628_A2 cells. Bars represent fold change of H3.3 expression, normalized to H3.3 expression in the parental cell line. Expression in parental cell line was arbitrarily set to 1. Error bars represent minimum and maximum relative level of gene expression. **(C)** Levels of IFNγ secretion of 27633 and 1H5 TCR-transduced PBMCs, co-cultured with the human SF7761 glioma cell line, harboring a heterozygous copy of mutant H3.3 histone. The cells were retrovirally transduced to express HLA-A*02:01 (SF7761-A2). A total of 10^4 $CD8^+$ transduced T cells were co-cultured at an E:T ratio of 1:1. The experiment was performed twice and representative data are shown. **(D)** Presence of naturally expressed or overexpressed H3.3 wild type or H3.3K27M transcripts in SF8628-A2 (left panel) and SF7761-A2 (right panel) cells, was verified by RT-PCR and Sanger sequencing.



Supplementary Figure 2 **Lack of IFNγ, TNFα and IL-2 production by H3.3K27M TCR-transduced T cells indicates failure to recognize Mel624 cells overexpressing the mutant H3.3K27M histone.** **(A)** H3.3K27M protein was detected by western blot. Top panel shows β-actin (45 kDa), lower panel shows H3.3K27M (17 kDa) in parental or transduced HLA-A*02:01^{+} Mel624 cells. **(B)** Relative quantitation of all H3.3 transcripts in Mel624 cells. Bars represent fold change of H3.3 expression, normalized to H3.3 expression in the parental cell line. Expression in parental cell line was arbitrarily set to 1. Error bars represent minimum and maximum relative level of gene expression. **(C)** Levels of IFNγ, TNFα and IL-2 secretion of 27633 and 1H5 TCR-transduced PBMCs, co-cultured with the human Mel624 melanoma cell line retrovirally transduced to express either H3.3K27M full length cDNA, H3.3 wild type full length cDNA or CDK4R24L full length cDNA. A total of 10^4 CD8^{+} transduced cells were co-cultured at a 1:1 E:T ratio. A CDK4R24L-specific TCR (14/35) served as positive control for functional antigen processing and presenting machinery in Mel624 cells. P/I represents maximum IFNγ secretion from PBMCs stimulated with PMA/Ionomycin. Graphs represent means of triplicate cultures ±SD.



Supplementary Figure 3 **The mutated CDK4 peptide, but not mutated H3.3 peptide is detectable in U87MG glioblastoma cells overexpressing the respective cDNAs.** Samples 1-4 recombinantly express H3.3K27M, samples 5-8 express CDK4R24L. **(A)** Representative annotated MS2 spectra generated from the MaxQuant output for the mutated CDK4 and H3.3 heavy synthetic peptides (top panel) and the endogenous CDK4R24L counterpart eluted from HLA-A*02:01^{+}/CDK4R24L^{+} cells after W6/32 HLA pulldowns (bottom panel). Endogenous mutant H3.3 peptide could not be detected in any of the samples. Peaks represent b ions in ochre and y ions in red. **(B)** Boxplot of the MS2 intensities by group.



Supplementary Figure 4 **CRISPR/Cas9 knockout of endogenous human TCR does not lead to recognition of the H3.3K27M mutation. (A)** Expression of transduced TCR (mTCRβ, y-axis) and endogenous TCR (hTCRα/β, x-axis) after knockout of either endogenous TCR alpha or beta chain with CRISPR/Cas9 in mock-transduced T cells. S.p.HiFiCas9 V3 enzyme and gRNAs targeting human TRAC or TRBC loci were complexed and nucleofected into T cells. Endogenous (hTCRα/β) and therapeutic (mTCRβ) TCR expression was assessed by flow cytometry after 4 days in culture on 10 IU/ml IL-2, cells were gated on $CD3^+$. **(B)** 27633-, 1H5- and mock-transduced T cells were nucleofected with sgRNAs and Cas9 protein (right panel) or without Cas9 (left panel) targeting the endogenous human TRAC and TRBC locus together. Endogenous (hTCRα/β) and therapeutic (mTCRβ) TCR expression was assessed by flow cytometry after 4 days in culture on 10 IU/ml IL-2, cells were gated on $CD3^+$. Even though the proportion of $hTCR\alpha/\beta^+mTCR^+$ double positive cells in the therapeutic TCR-transduced PBMCs is quite low to begin with, probably due to stronger expression and preferential pairing of transduced TCR chains, the successful knockout is also reflected in the $hTCR\alpha/\beta^+mTCR^-$ populations. **(C)** Cells described in (B) were co-cultured with SF8628-A2, SF8628-A2-H3.3K27M, SF8628-A2-H3.3 wild type or with peptide-loaded T2 cells (10^{-7} M mutant or wt peptide, respectively). Graphs represent mean IFNγ secretion of triplicate cultures ±SD. **(D)** Staining of the TCRs with H3.3K27M dextramer (y-axis) and CD8 (x-axis) after knockout of human TCR alpha and beta chains with CRISPR/Cas9. FACS staining was performed after 4 days in culture on 10IU/ml IL-2, cells were gated on $CD3^+$**.**

Supplementary Table 1 **ERAP1 allotype identity in cell lines.**

	ERAP 1 polymorphisms								
Cell line	**E56K**	**R127P**	**G346D**	**M349V**	**K528R**	**D575N**	**R725Q**	**Q730E**	**Allotype**
Mel21a	E	R	**D**	M	**R**	D	R	**E**	*019
	E	R	**D**	M	**R**	D	R	**E**	*019
Mel624	E	R	G	M	K	D	R	**E**	*018
	E	R	G	M	K	D	R	**E**	*018
SF8628	E	R	G	M	K	D	R	Q	*002
	K	**P**	G	M	**R**	D	R	**E**	*014
U87MG	E	**P**	G	M	K	D	R	Q	*013
	E	**P**	G	M	**R**	D	R	**E**	*015

Bold type shows variants at the indicated amino acid position.