Supplementary Figures and Legends

Supplemental Fig. S1: Representative patterns of tumor cell HLA class I positivity and T cell infiltration

Representative photomicrographs from four separate HNSCC tumors of merged immunofluorescence images assessing the expression of HLA class I (yellow) or cytokeratin (red) positive tumor cells and localization of CD3 (green) T cells. Also shown is a representative photomicrograph of a negative control tumor (no primary antibody stain).
Supplemental Fig. S2: Validation of double positive engineered HNSCC cell lines

Flow cytometry was used to assess HLA-A*02 and HPV 16 E7 (via NGFR tag) expression for engineered (A) JHU-029, (B) UM-SCC-1, (C) HPV+ UM-SCC-47 and (D) A*02+ UM-SCC-109
HNSCC cell lines. Representative flow cytometry dot plots shown in the left panels. Right panels, qPCR was used to assess expression of HPV 16 E7 in HPV- cell lines engineered to express E7.
Supplemental Fig. S3: Other tumor cells lacking APPM that escape T cell killing expressed increased PD-L1
A, different proportions of A*02+E7+ tumor cells with tumor cells lacking A*02 or E7, or genetically lacking β2M were assessed for their susceptibility to killing by E7 TCR engineered T cells via real-time impedance analysis. Proportion of the tumor cell population lacking A*02, E7 or β2M is shown in each legend. Time 0 on the x-axis is the time at which E7 TCR engineered T cells were added to the co-culture. T cells were added at a fixed 2:1 E:T ratio. Representative impedance plots from one of at least three independent experiments with similar results are shown. B, following 24 hours of co-culture with T cells or media alone, mixtures of tumor cells composed of 50% of cells expressing and 50% of cells lacking A*02, E7 or β2M were harvested and assessed by flow cytometry for expression of A*02, E7 (NGFR) and PD-L1. Non-adherent cells were washed away before tumor cell harvest. Representative dot plots of viable (sytox blue negative) tumor cells are shown. Pie graphs depicting the frequency of HLA or antigen negative tumor cells (orange) relative to positive tumor cells (blue) are inset in each dot plot. C, Representative histograms and quantification of median fluorescent intensity (MFI) of PD-L1 expression on tumor cells with and without exposure to T cells are shown in the right panels. Experiments were performed in technical triplicate and reproduced in at least two independent experiments. ***, p < 0.001, t-test.
Supplemental Fig. S4: β2M knockout cell lines lose expression of cell surface HLA-A*02

CRISPR/Cas9 gene editing was used to delete β2M in (A) JHU-029 and (B) UM-SCC-109 cells. Representative dot plots of baseline (JHU-029) and IFNγ-inducible (UM-SCC-109) HLA-A*02 expression are shown on the left. To verify a lack of effect of the IFNγ response pathway, PD-L1 expression was assessed concurrently. Representative baseline and IFNγ-inducible PD-L1 expression histograms and MFI quantification are shown on the right. (C) A*02+ indicates a
homogenous UM-SCC-47 HLA A*02+ population and 0.5A*02− indicates a population of 50% HLA A*02− cells and 50% HLA A*02+ cells. (D) E7+ indicates a homogenous UM-SCC-109 HPV 16 E7+ population and 0.5E7− indicates a population of 50% HPV 16 E7− cells and 50% HPV 16 E7+ cells. Supernatants were collected from each condition following 24 hours of co-culture with TCR engineered T cells (2:1 E:T ratio). IFNγ concentration was measured by ELISA. ***, p < 0.001, ANOVA.
Supplemental Fig. S5: IFNγ pre-treatment primes T cell escape variant tumor cells for killing by PD-L1 t-haNKs

T cell escape variant JHU-029 (A), UM-SCC-1 (B), UM-SCC-47 (C) or UM-SCC-109 (D) cells were stimulated with IFNγ 20ng/mL for 24 hours, then assessed for PD-L1 expression by flow cytometry (Left panels). The ability of haNKs (lacking the PD-L1 CAR) or PD-L1 t-haNKs were assessed for their ability to kill T cell escape variant T cells with and without IFNγ pretreatment at a fixed 2:1 E:T ratio. Representative impedance plots and quantification of % loss of cell index 12 hours after the addition of the effectors are shown. Time 0 on the impedance plots represents the time at which effectors were added. **, p < 0.01; ***, p < 0.001, ANOVA.
Supplemental Fig. S6: PD-L1 t-haNKs prevented clonal outgrowth of more escape variant tumor cells selected by TCR engineered T cells.

Mixtures of (A) UM-SCC-1 or UM-SCC-47 tumor cells expressing (25%) or lacking (75%) A*02, (B) JHU-029 or UM-SCC-109 cells expressing or lacking E7, or (C) JHU-029 cells expressing or lacking β2M were assessed for their susceptibility to killing by TCR engineered T cells with or without PD-L1 t-haNKs via real-time impedance analysis. Time 0 on the x-axis is the time at which TCR engineered T cells were added to the co-culture. PD-L1 t-haNKs were added to the co-culture 12 hours after the addition of TCR engineered T cells (red lines). All effector T and NK cells were added at a 2:1 E:T ratio. Representative impedance plots from one
of at least three independent experiments with similar results are shown. Mixtures of JHU-029 (D), UM-SCC-1 (E), UM-SCC-47 (F) or UM-SCC-109 (G) tumor cell expressing (25%) or lacking (75%) HLA A*02, HPV 16 E7, or β2M were assessed for their susceptibility to killing by TCR engineered T cells with or without PD-L1 t-haNKs via real-time impedance analysis. PD-L1 t-haNKs were added to the co-culture 12 hours after the addition of E7 TCR engineered T cells. Data shown is normalized to the timepoint at which NK cells were added. All effector cells were added at a 2:1 E:T ratio. Representative impedance plots from one of at least two independent experiments with similar results are shown on the right. Quantification of normalized cell index 8 hours after the addition of PD-L1 t-haNKs is shown on the left. ***, p < 0.001, ANOVA.
Supplemental Fig. S7: Loss of IFN signaling conveys resistance to killing by PD-L1 t-haNKs but not TCR engineered T cells.

JHU-029 or UM-SCC-109 cells expressing A*02 and E7 but genetically deleted of IFNGR1 (A, B) or STAT1 (C, D) were assessed for their susceptibility to killing by TCR engineered T cells. CRISPR/Cas9 gene editing was used to delete IFNGR1 or STAT1 in JHU-029 and UM-SCC-109 cells. MFI graphs of baseline and IFNγ-inducible HLA class I and PD-L1 expression are shown for each engineered model. E, JHU-029 cells lacking E7 and genetically deleted of STAT1 were assessed for their susceptibility to killing by TCR engineered T cells. Time 0 on the x-axis is the time at which E7 TCR engineered T cells were added to the co-culture. PD-L1 t-haNKs were added to the co-culture 12 hours after the addition of E7 TCR engineered T cells.
(red line). All effector cells were added at a 2:1 E:T ratio. Representative impedance plots from one of at least three independent experiments with similar results are shown. *, p < 0.05; **, p < 0.01; ***, p < 0.001, ANOVA.