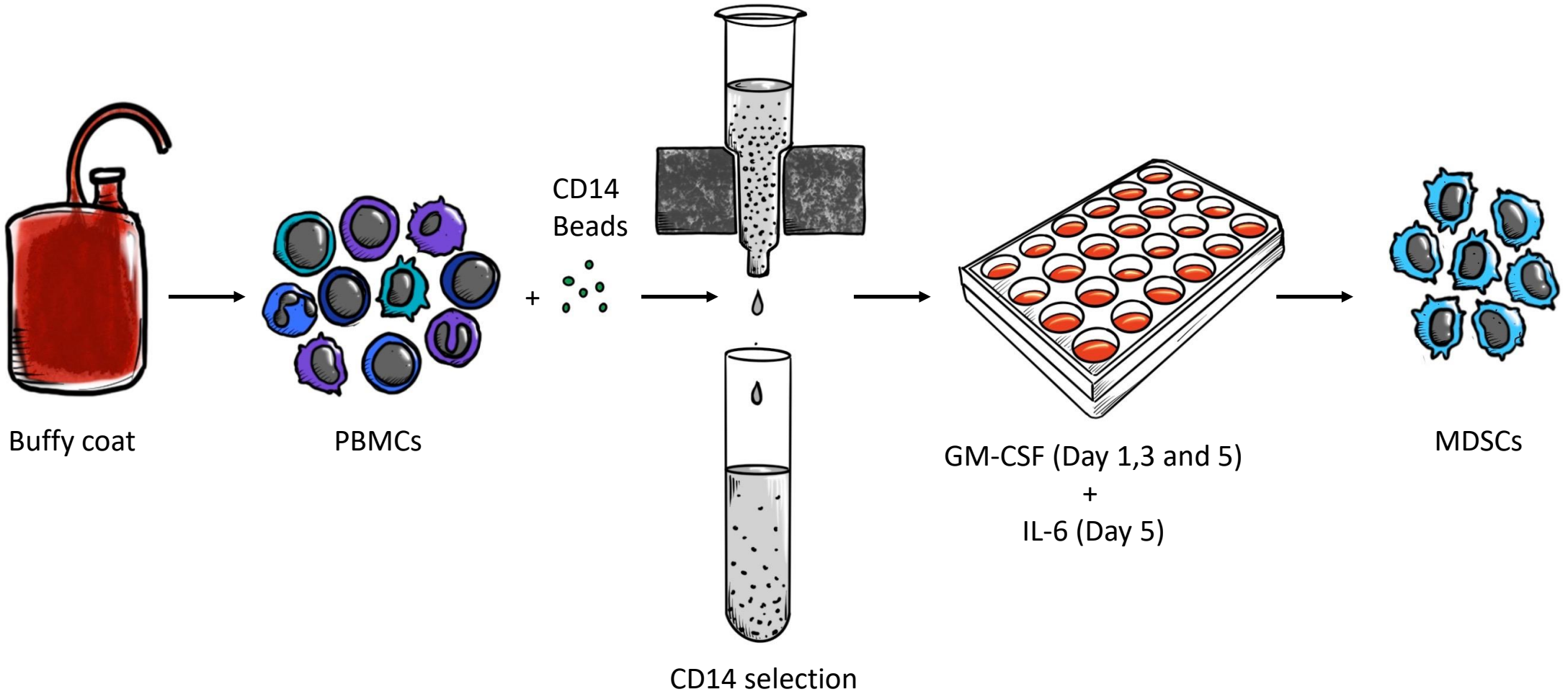
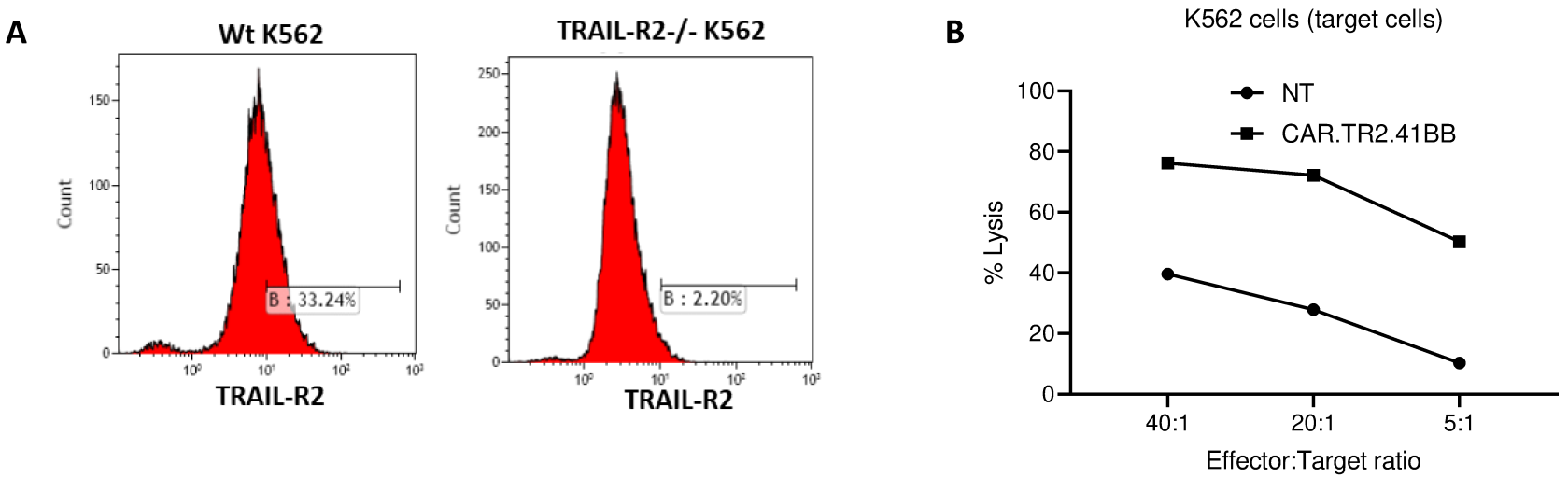
Supplemental Figure 1.



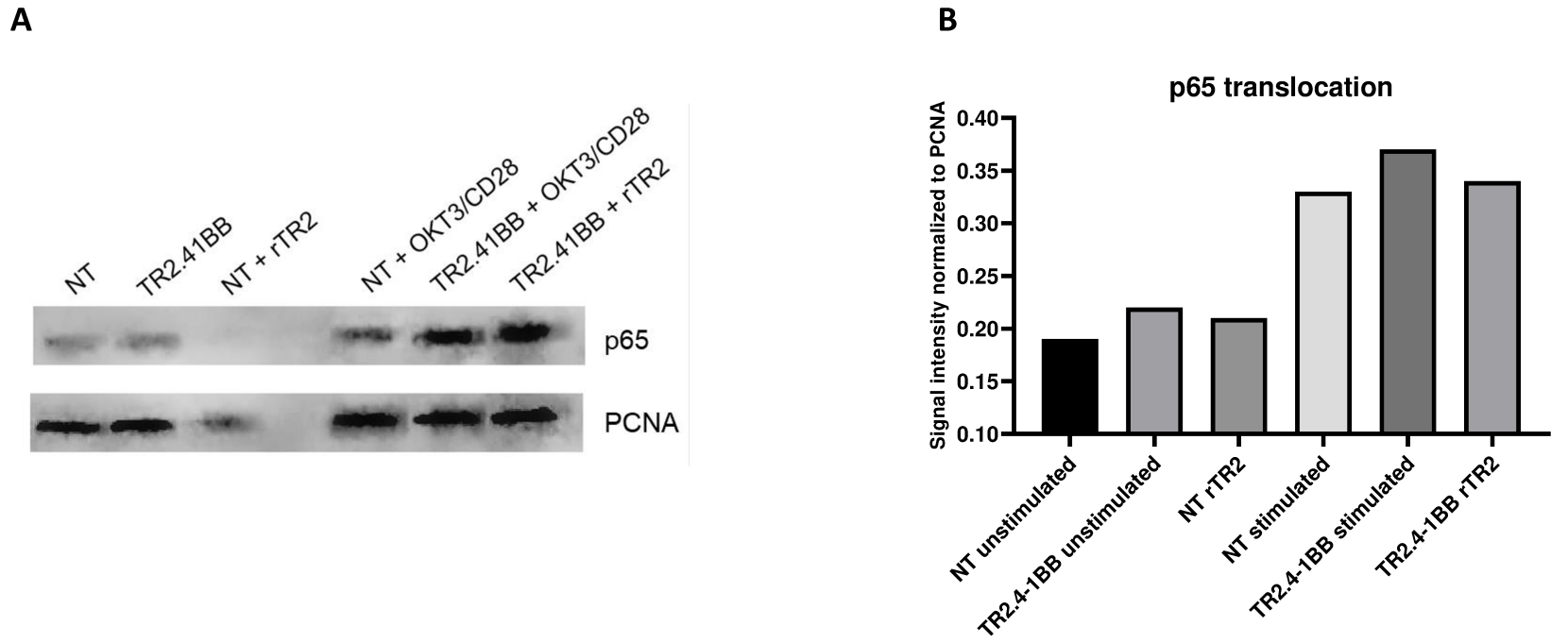
Suppl Fig 1. Generation of human MDSCs in vitro. PBMCs were obtained from healthy volunteers, CD14+ cells were isolated and then cultured for 7 days in the presence of GM-CSF and IL-6. GM-CSF was added on days 1, 3 and 5, whereas IL-6 was added only on day 5.

Supplemental Figure 2.



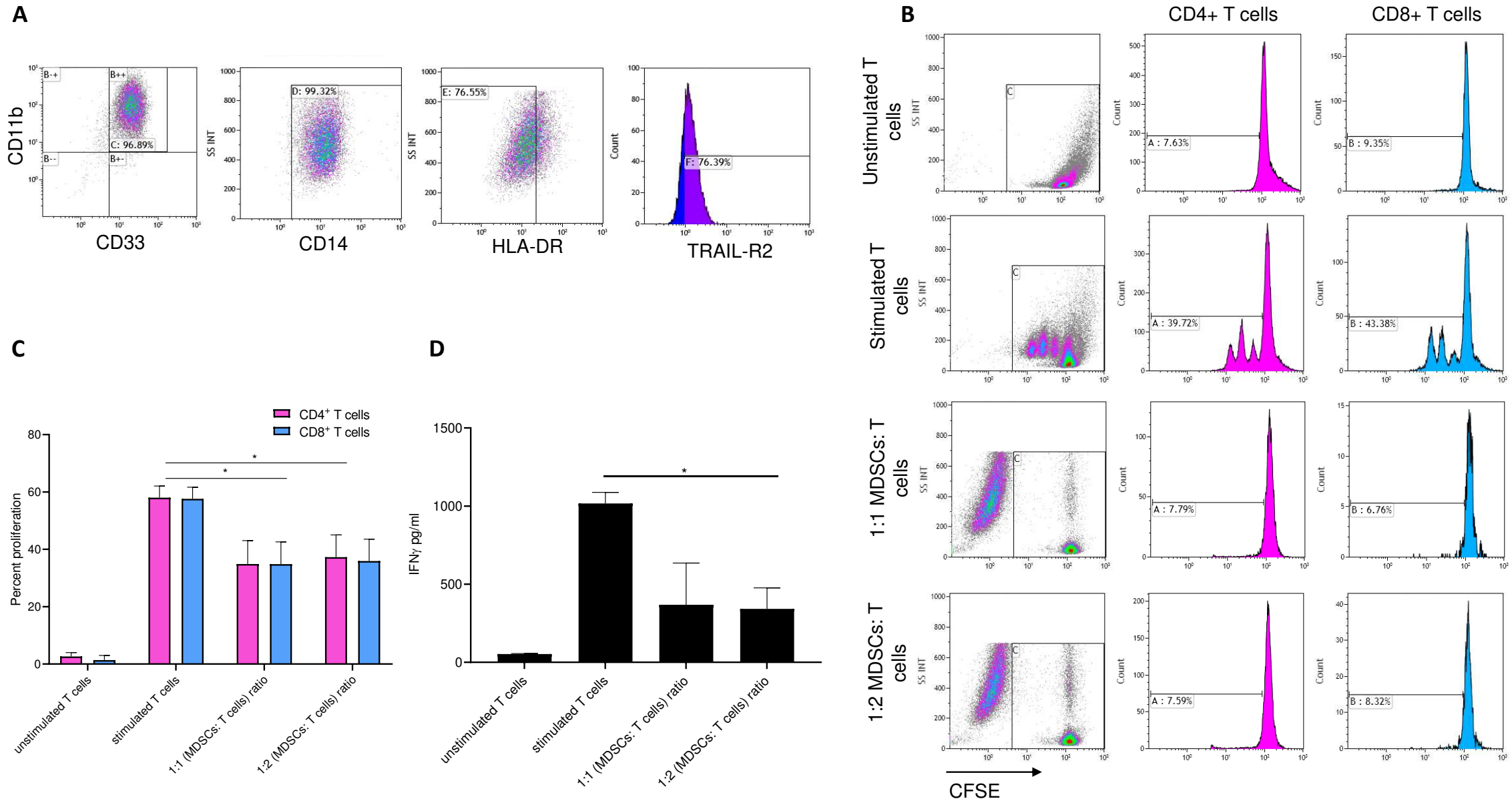
Suppl Fig 2. The novel TR2.41BB co-stimulatory receptor specifically targets TR2. (a) CRISPR knockout of TR2 in K562 cell line. (b) in vitro cytolytic function of control (NT) and CAR.TR2.41BB T cells assessed in a 6 hr ^{51}Cr-release assay at effector: targets of 5:1 to 40:1 using K562 cells transduced with TR2 ectodomain as targets.

Supplemental Figure 3.



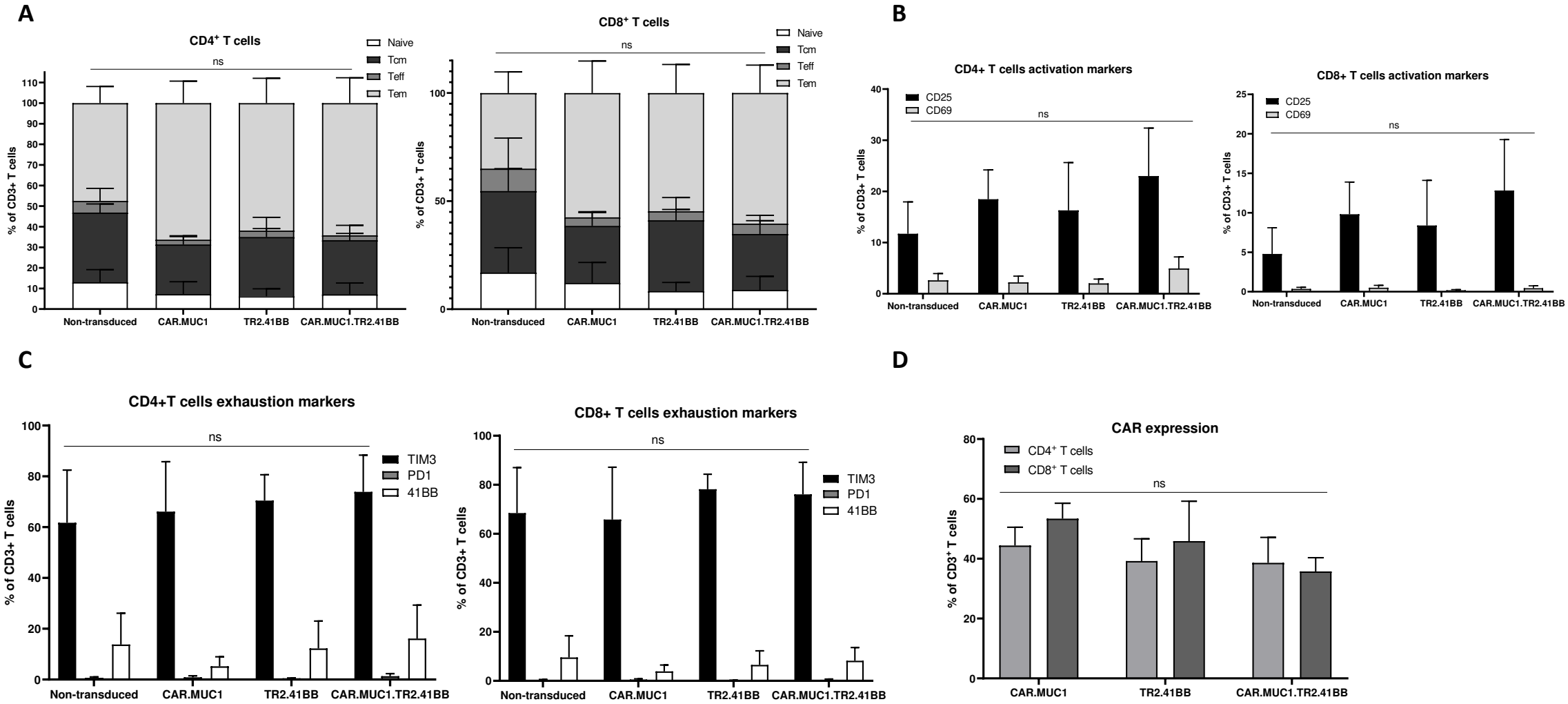
Suppl Fig 3. TR2.41BB co-stimulatory receptor induces translocation of NFkB upon TR2 engagement. (a) Cells were harvested at 120 minutes, the nuclear fraction was extracted, and a western blot was performed to measure the translocation of NFκB into the nucleus (b) Quantification of p65 in the nuclear extract. The membrane was imaged on Licor, and p65 translocation was normalized to the internal control, PCNA (n=1).

Supplemental Figure 4.



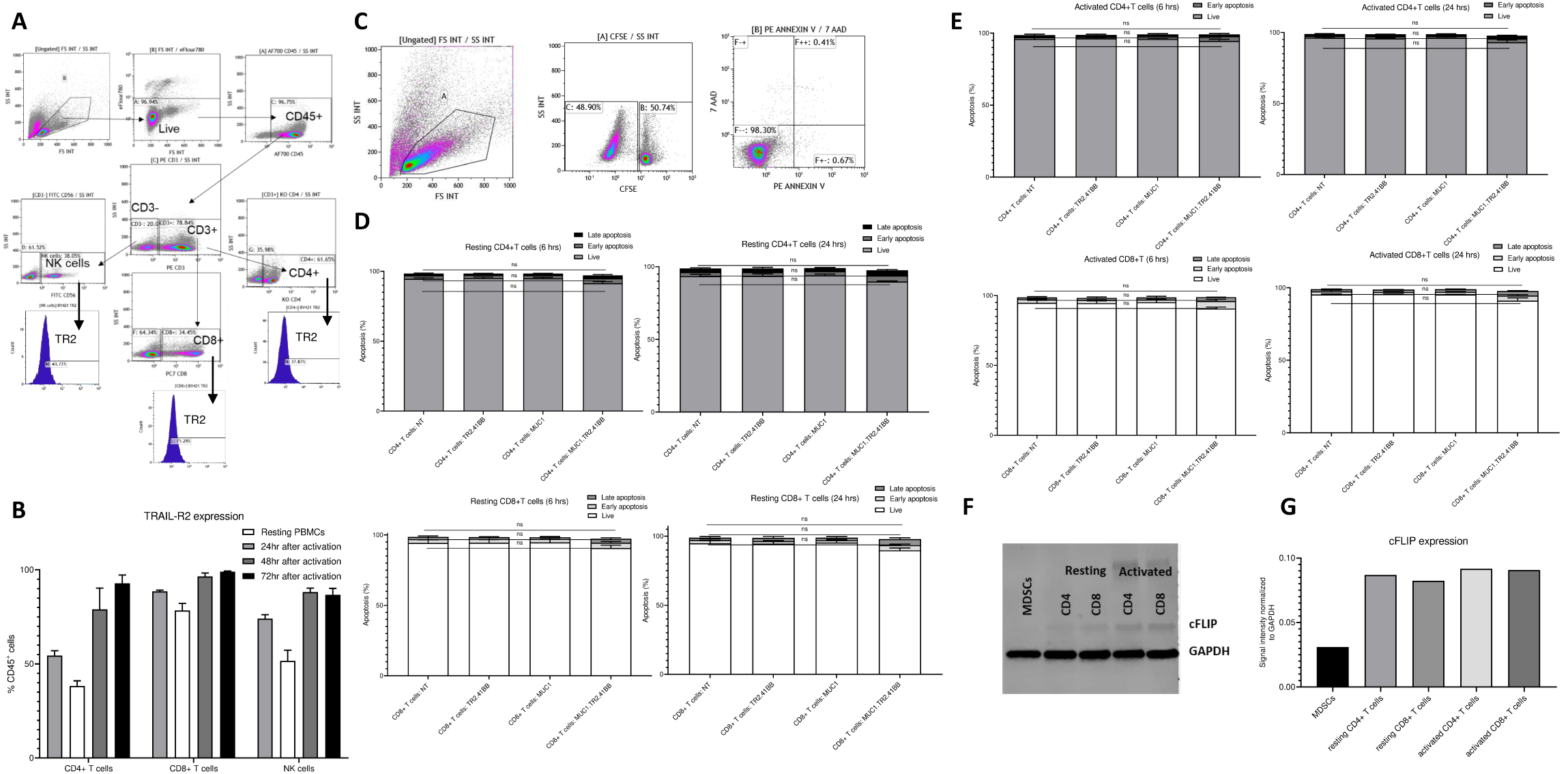
Suppl Fig 4. In vitro generated myeloid derived suppressor cells (MDSCs) of the monocytic lineage demonstrated suppressive potential. (a) MDSCs were harvested after 7 days of culture with GM-CSF and IL-6. Flow cytometry was performed to validate the presence of CD33+ CD11b+ CD14+ HLA-DR^{low} TR2+ cells. (b) MDSCs were co-cultured with autologous CFSE-labeled T cells at a 1:1 and 1:2 ratio in the presence of T cell stimuli. After 3 days T cell proliferation was measured as CFSE-dilution using flow cytometry (n=15). (c) Quantification of T cell proliferation in the co-culture suppression assay. (d) Co-culture supernatants were collected from the suppression assay cultures and IFNγ was detected by ELISA. Data represent mean ± SEM (n=2). Statistics: two-way ANOVA followed by Tukey's multiple comparisons (C, D); *p<0.05.

Supplemental Figure 5.



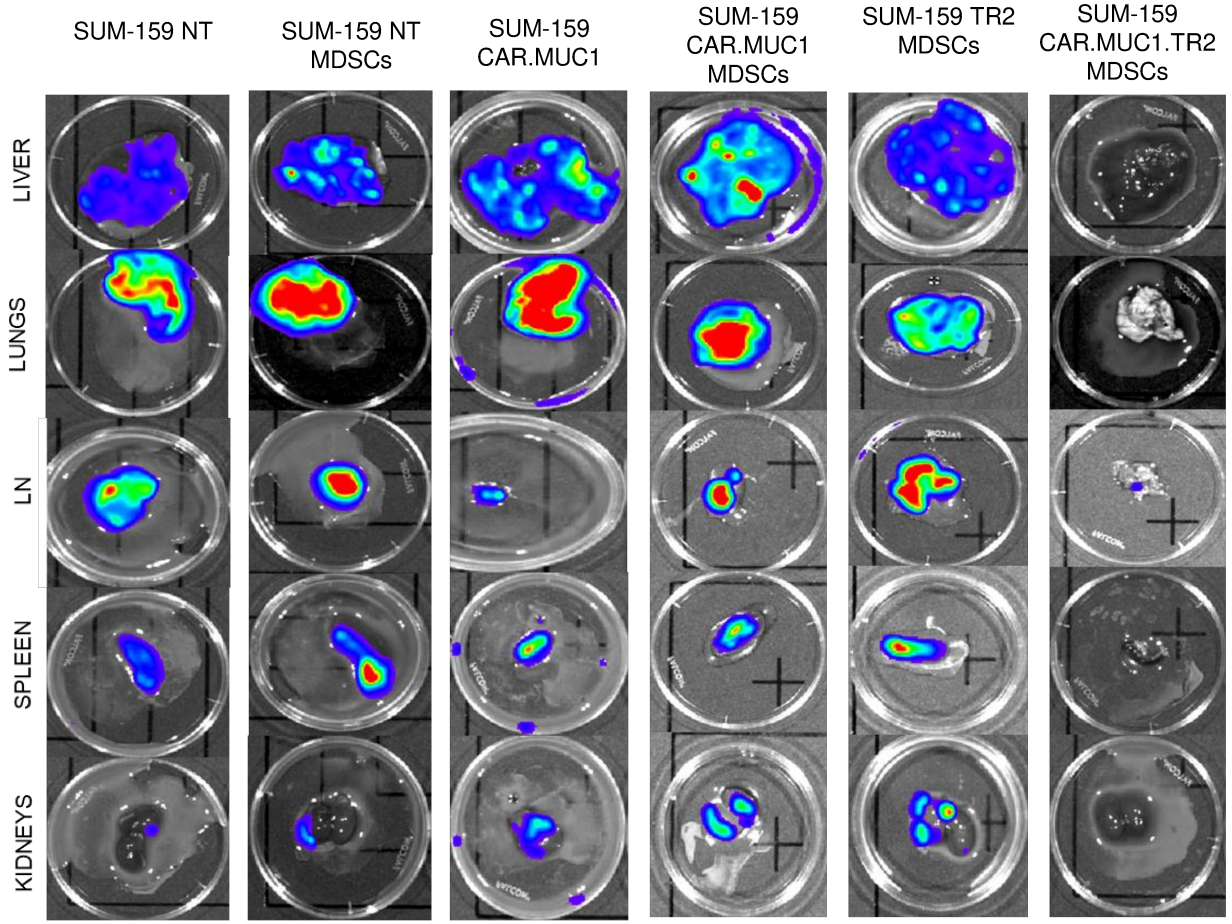
Suppl Fig 5. MUC1.TR2.41BB CAR T cells show similar differentiation phenotype and exhaustion/activation status compared to CAR.MUC1 or TR2.41BB cells alone. Activated T cells were either transduced with CAR.MUC1 or TR2.41BB constructs alone or sequentially with CAR.MUC1 and TR2.41BB, and flow cytometry was performed on day 14 after transduction to assess (a) differentiation phenotype, (b) exhaustion markers, (c) activation markers, and (d) CAR expression. Data represent mean ± SEM, (n=3). Statistics: two-way ANOVA followed by Tukey's multiple comparisons (A, B, C & D); ns= not significant.

Supplemental Figure 6.



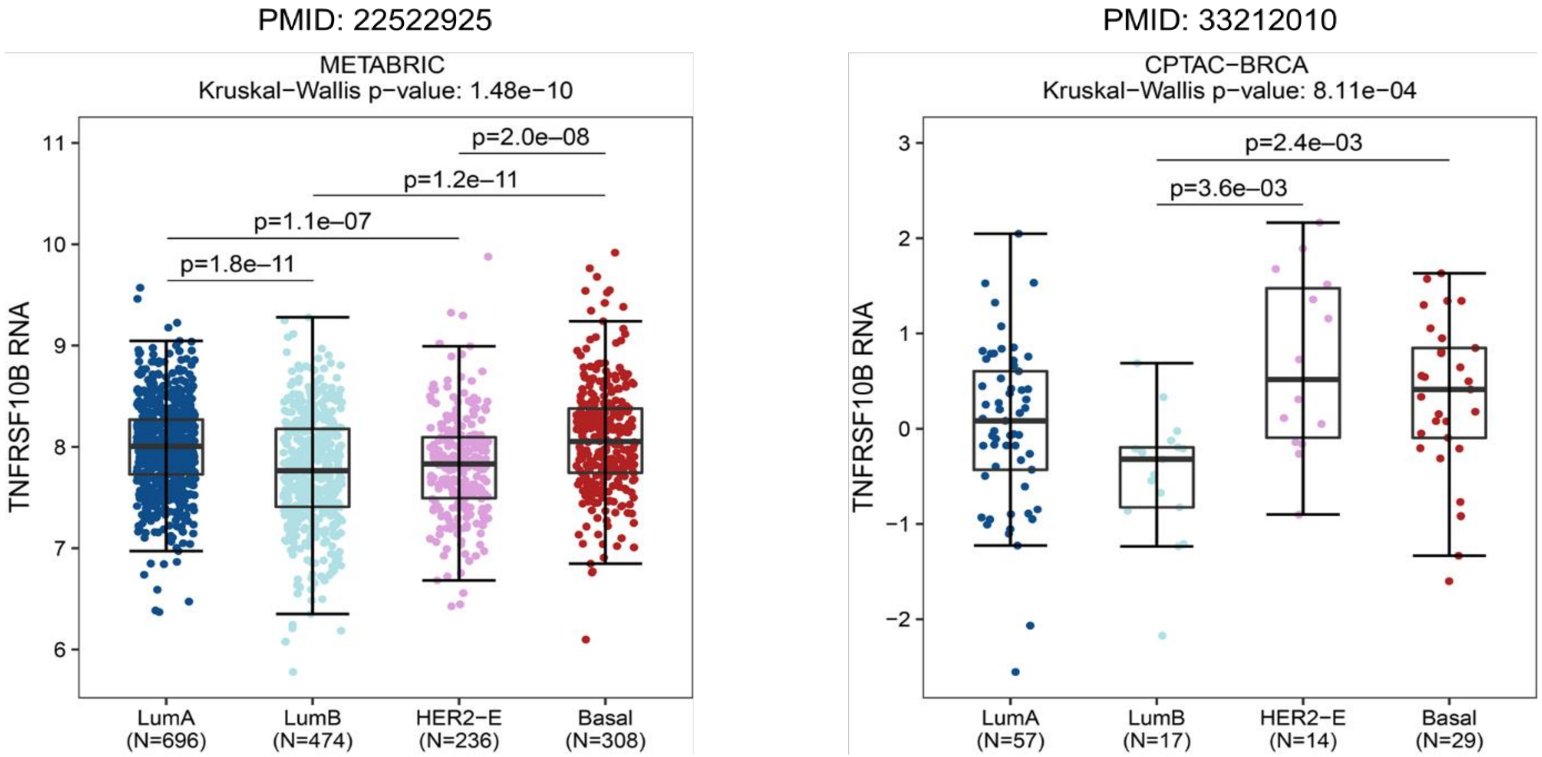
Suppl Fig 6. TR2.41BB does not induce toxicity in resting and activated CD4+ and CD8+ T cells owing to increased expression of cFLIP. Resting and activated PBMCs were harvested at 24, 48 and 72 hrs and flow cytometry was performed to assess TR2 expression on CD4+, CD8+T cells and NK cells. (a) A representative flow gating and (b) TR2 quantification, mean ± SEM, (n=3). Resting and activated T cells (72hr) were labeled with CFSE and co-cultured in the presence of T cells transduced with either CAR.MUC1 or TR2.41BB constructs or both. Cells were harvested after 6 and 24 hr and flow cytometry was performed using 7-AAD and annexin to assess the percentage of apoptosis. (c) Representative flow gating and percentage of apoptosis in (d) resting and (e) activated CD4+ and CD8+T cells, mean±SEM, (n=2-3). (f) Protein lysate from resting T cells, activated T cells, and MDSCs was collected, and western blot was performed. (g) Quantification of cFLIP protein expression. The membrane was imaged on Licor, and cFLIP expression was normalized to the internal control, GAPDH (n=1). Statistics: two-way ANOVA followed by Tukey's multiple comparisons (D, E); ns= not significant.

Supplemental Figure 7.



Suppl Fig 7. MUC1.TR2.41BB CAR T cells control primary tumor growth and avert metastases. NSG mice were transplanted with GFP.ffLuc labeled SUM-159 cells with or without MDSCs and treated with NT, CAR.MUC1, TR2.41BB, or CAR.MUC1.TR2.41BB T cells. Mice were sacrificed at the end of the study (day 49), and organs were harvested and imaged for metastatic spread. Representative images shown, n=4-5.

Supplemental Figure 8.



Suppl Fig 8. TRAIL-R2 RNA expression in human breast cancer datasets

Bulk transcriptomic data was analyzed from two publicly available human breast cancer datasets; METABRIC (microarray) and CPTAC-BRCA (RNA-seq). Kruskal-Wallis followed by pairwise Wilcoxon tests were performed between all group combinations with significant differences indicated by p-values adjusted for multiple comparisons. Boxplots depict interquartile range (IQR) with lower whiskers representing Q1 – 1.5*IQR and upper whiskers Q3 + 1.5*IQR.