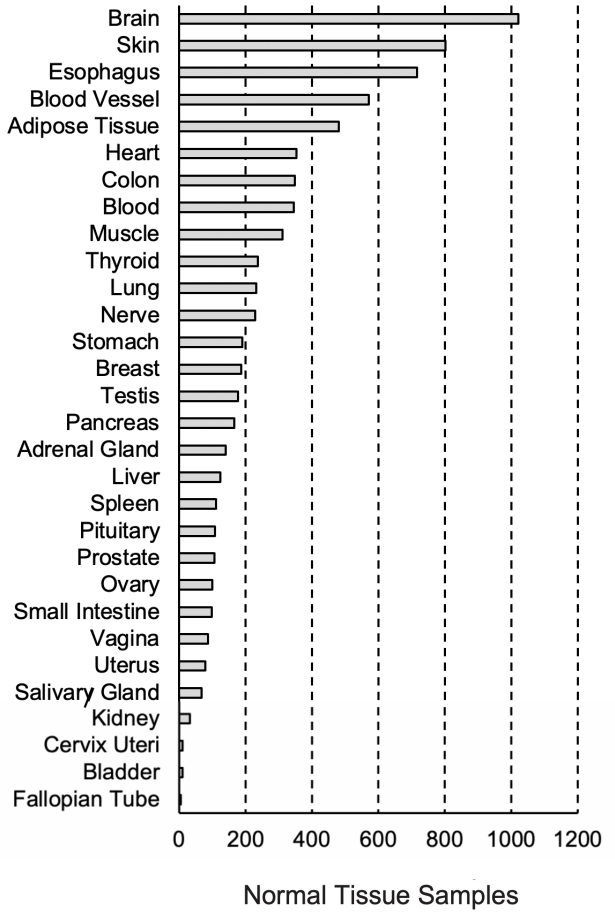
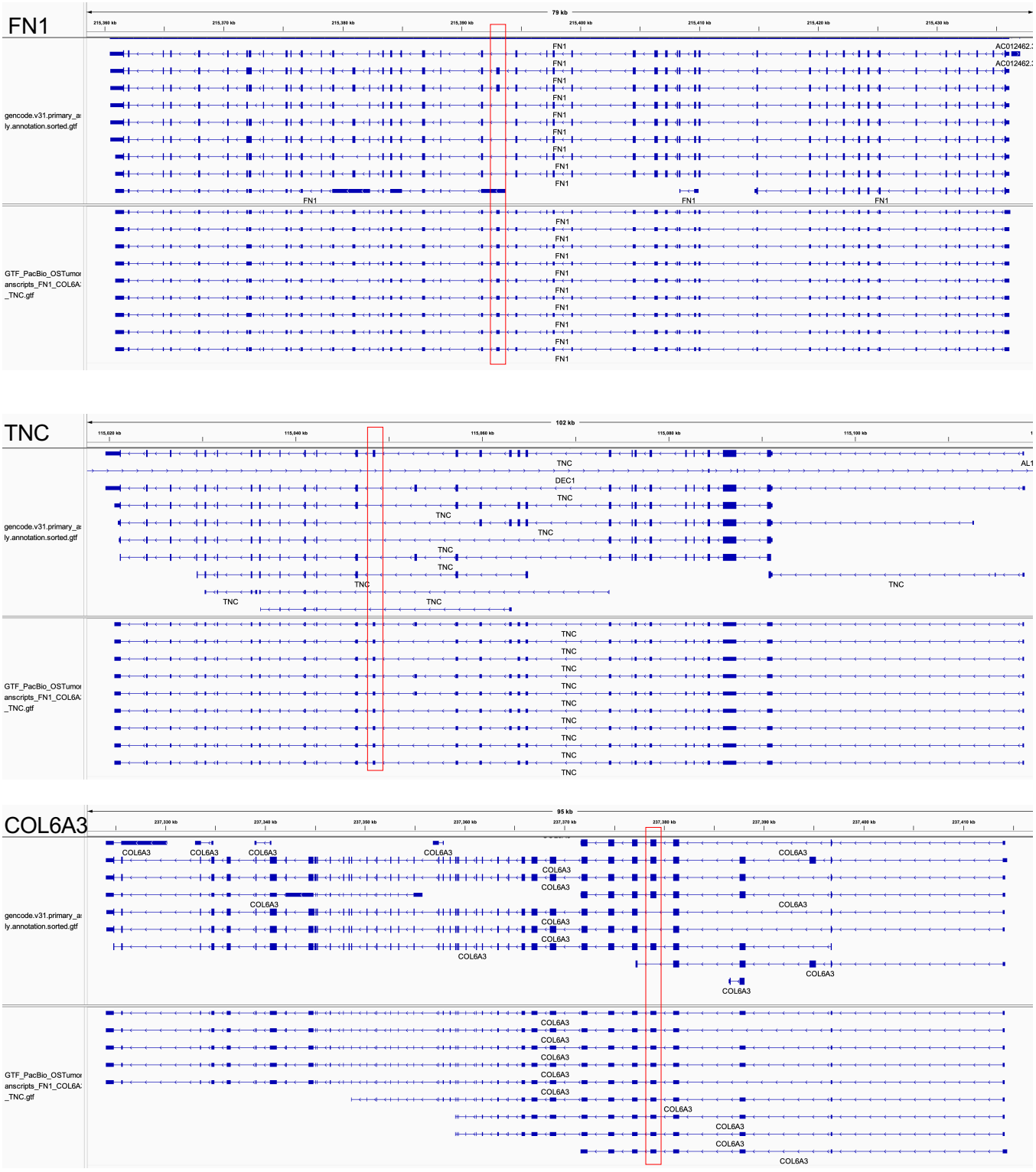
Supplementary Figure 1



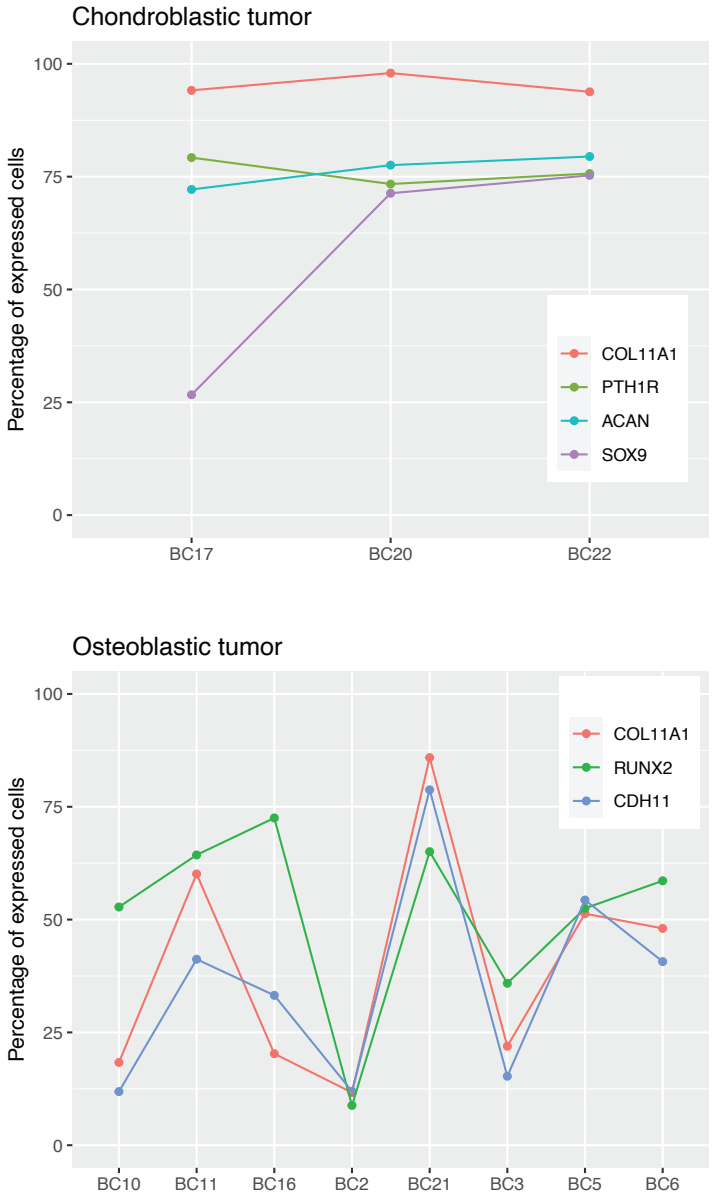
Supplementary Figure 1: Normal tissue types and their distribution in 7,460 RNA-seq data downloaded from GTEx portal version 7.

Supplementary Figure 2



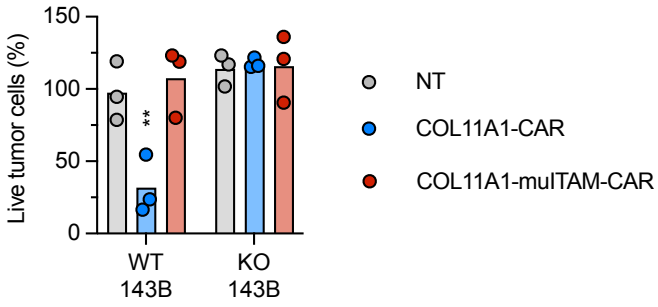
Supplementary Figure 2: Iso-seq full-length transcripts for three AS targets. FN1, COL6A3 and TNC are expressed at high levels in 83%, 34%, and 29% OS samples. For each gene. **Top panel:** annotated genecode transcripts. **Bottom panel:** 3-top transcript sequence that match the AS targets for each sample. The AS targets are highlighted with red boxes.

Supplementary Figure 3



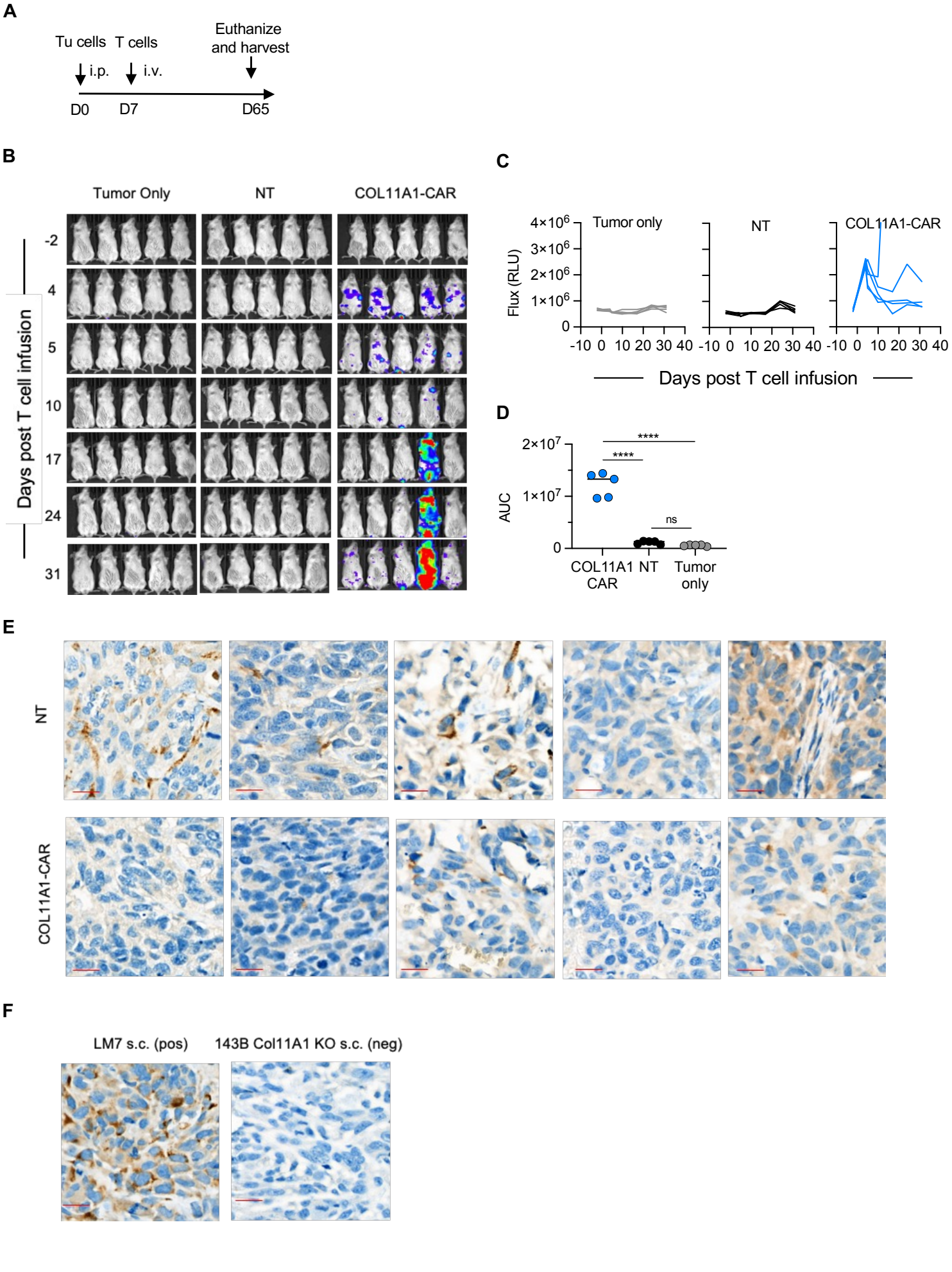
Supplementary Figure 3. **Expression prevalence of *COL11A1* in osteosarcoma tumor cells using single-cell RNA-seq data from Zhou *et al* (PMID 33303760).** The tumor cells were identified based on marker genes expressed in chondroblast cells and osteoblast cells for 3 chondroblastic OS tumors, and 8 osteoblastic OS tumors, respectively, as documented by Zhou et al. The marker genes for chondroblastic OS are PTH1R, ACAN, SOX9 while those for osteoblastic OS are RUNX2 and CDH11. *COL11A1* expression at the single cell level is comparable to marker genes in all samples except for BC16.

Supplementary Figure 4



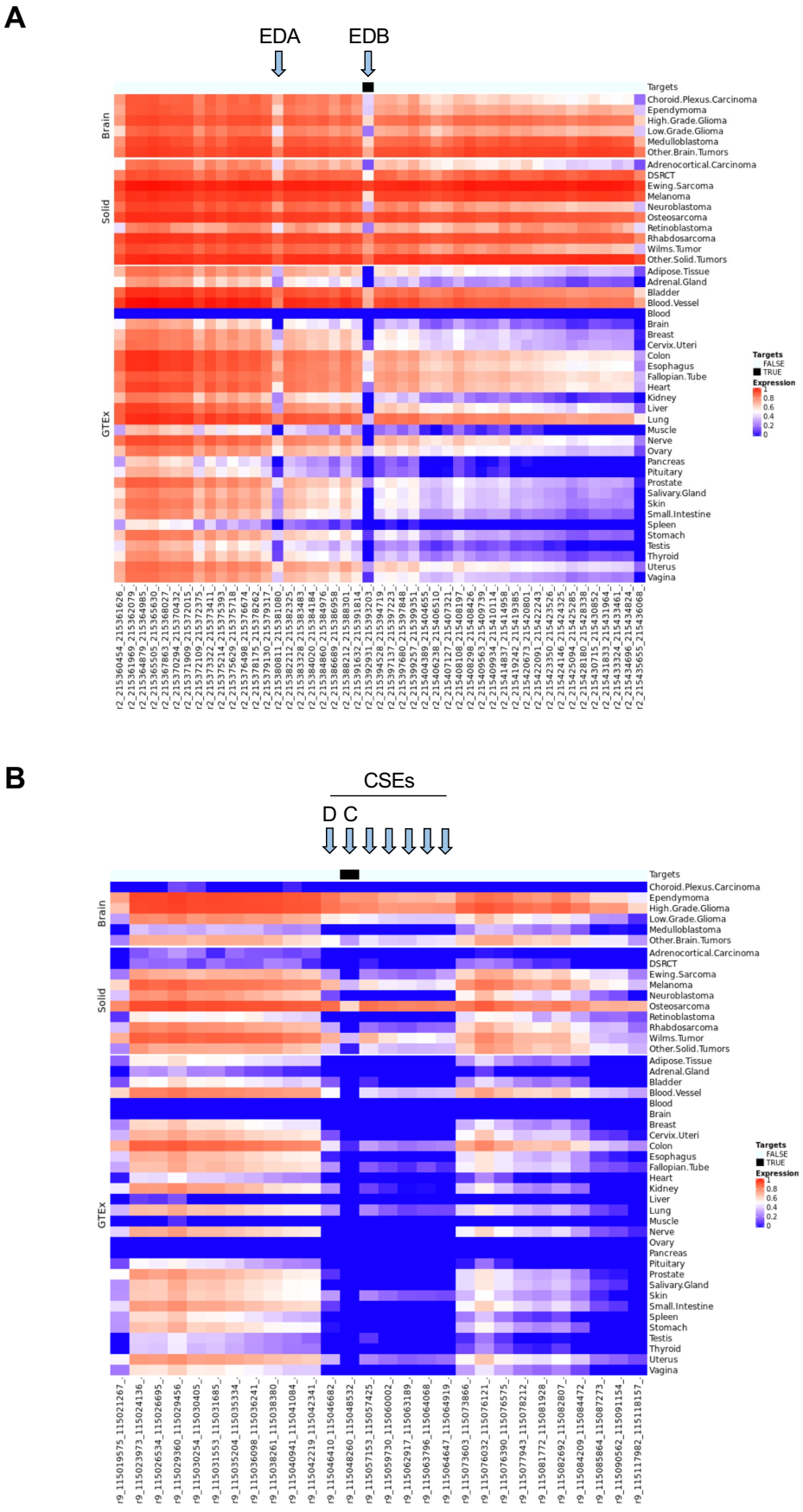
Supplementary Figure 4: Recognition of target cells by COL11A1-CAR T cells depends on the expression of COL11A1 and functional COL11A1-CARs. NT, COL11A1-CAR T-cells, and CoL11A1-mulTAM-CAR T cells were cocultured with indicated tumor cells at a 4:1 E:T ratio. After 72 hours live tumor cells were determined by MTS assay (n=3-4 donors, two-way ANOVA, **p<0.01,****p<0.0001).

Supplementary Figure 5



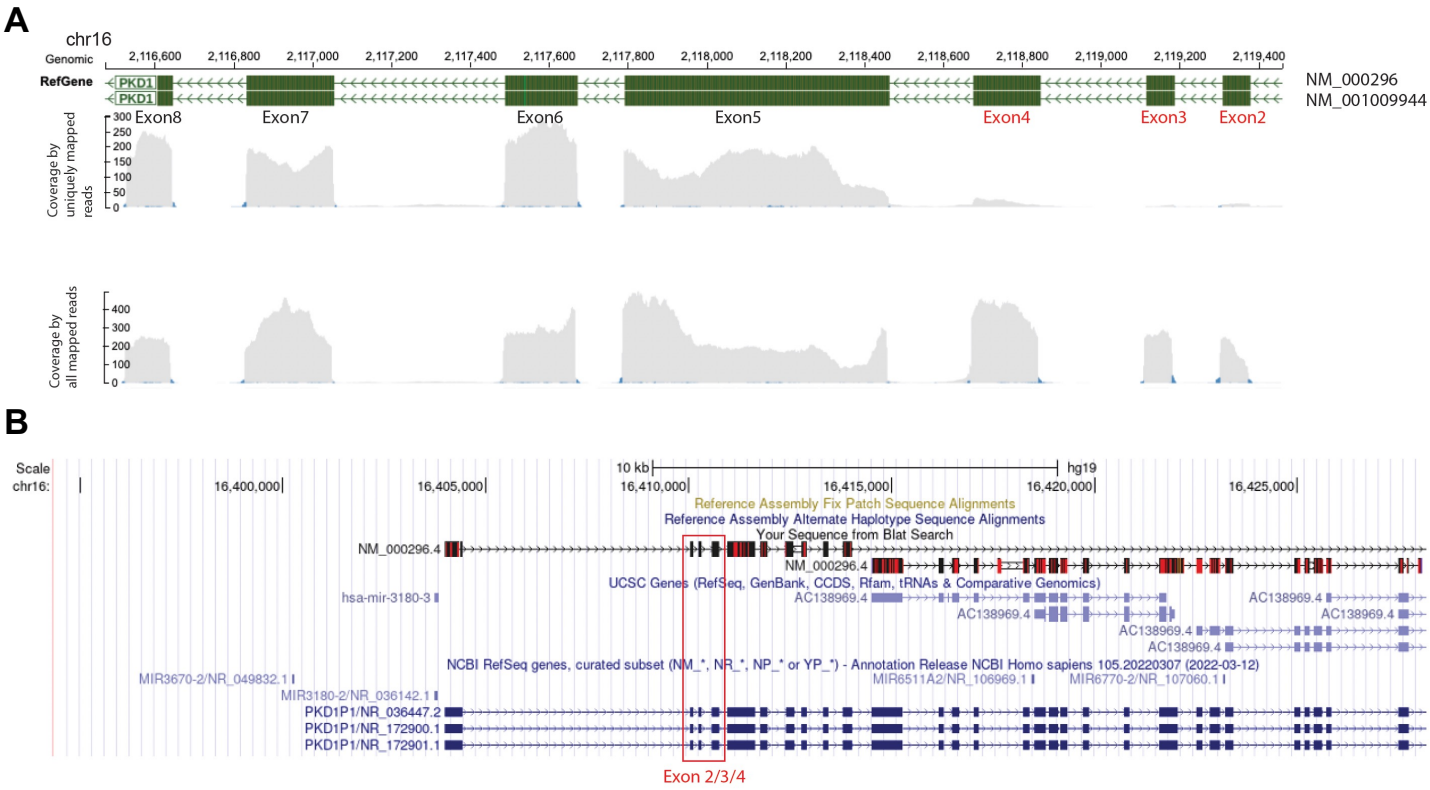
Supplementary Figure 5. Transient *in vivo* expansion of COL11A1-CAR T cells and recurrence of tumors with decreased expression of COL11A1. (**A**) Schematic of animal experiment. Day 0: i.p. injection of $1x10^6$ LM7 cells; D7: i.v. injection of $3x10^6$ NT or CAR T cells genetically modified to express eGFP.ffLuc (n=5 mice per group). (**B**) Representative bioluminescence images. (**C**) Quantitative bioluminescence data. (**D**) Area under the curve analysis for the first 10 days post T cell infusion, two-way ANOVA, ****p<0.0001, ns: not significant. (**E**) Representative IHC images for Col11A1 of tumors harvested after 65 days post T cell injection; 32x magnification, scale bar, 50 μm (red). (**E**) Representative IHC images for Col11A1 expression of positive control tumor (LM7; subcutaneous tumor: s.c.), and negative control tumor (143B-COL11A1-KO; s.c.) used for all IHC experiments throughout the manuscript. 32x magnification; scale bar, 50 μm (red)

Supplementary Figure 6



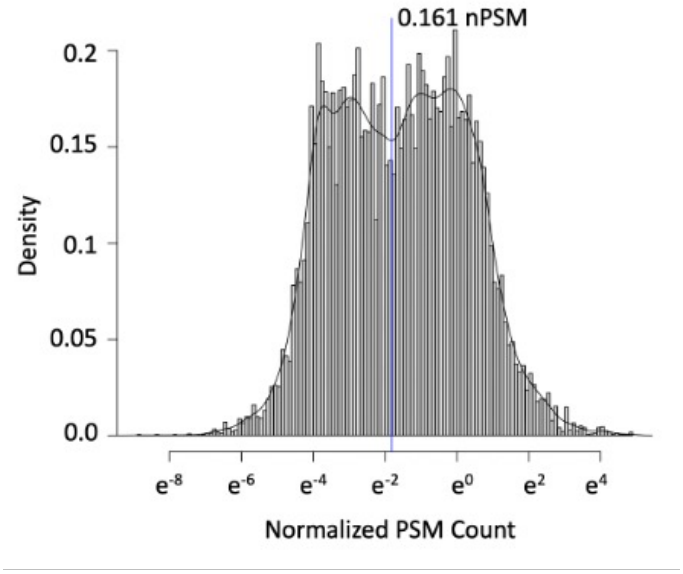
Supplementary Figure 7: CSE targets in FN1 and TNC. Heatmaps showing exon expression across normal and disease tissue types for (**A**) **FN1** and (**B**) TNC. (**A**) Exclusion of exon that encodes extra domain (EDB) due to expression in normal tissues. (**B**) Seven CSEs were identified in TNC, including the CSEs encoding the C and D domain of TNC, respectively.

Supplementary Figure 7



Supplementary Figure 7: PKD1 exon expression quantification affected by uniqueness of RNA-seq mapping. (**A**) Coverage of RNA-seq of a GTEx brain cerebellum sample (SRR1307770) using the uniquely mapped reads (MAPQ ≥10, used as a default by htseq, top panel) and all mapped reads (MAPQ ≥0, bottom panel). Exons 2-4 labeled in red text shown no coverage using uniquely mapped reads (top) in contrast to comparable coverage using all reads (bottom panel). (**B**) Blat search of PKD1 mRNA shown that exons 2-4 of PDK1 share 100% sequence identity to the three exons in PKD1P1, a known pseudo gene located 14Mb downstream. This explains the non-unique mapping of these exons in RNA-seq data.

Supplementary Figure 8



Supplementary Figure 8: Normalized PSM distribution in GTEx. A cutoff was determined based on the bimodal distribution was used to define highly expressed peptides at 0.161.