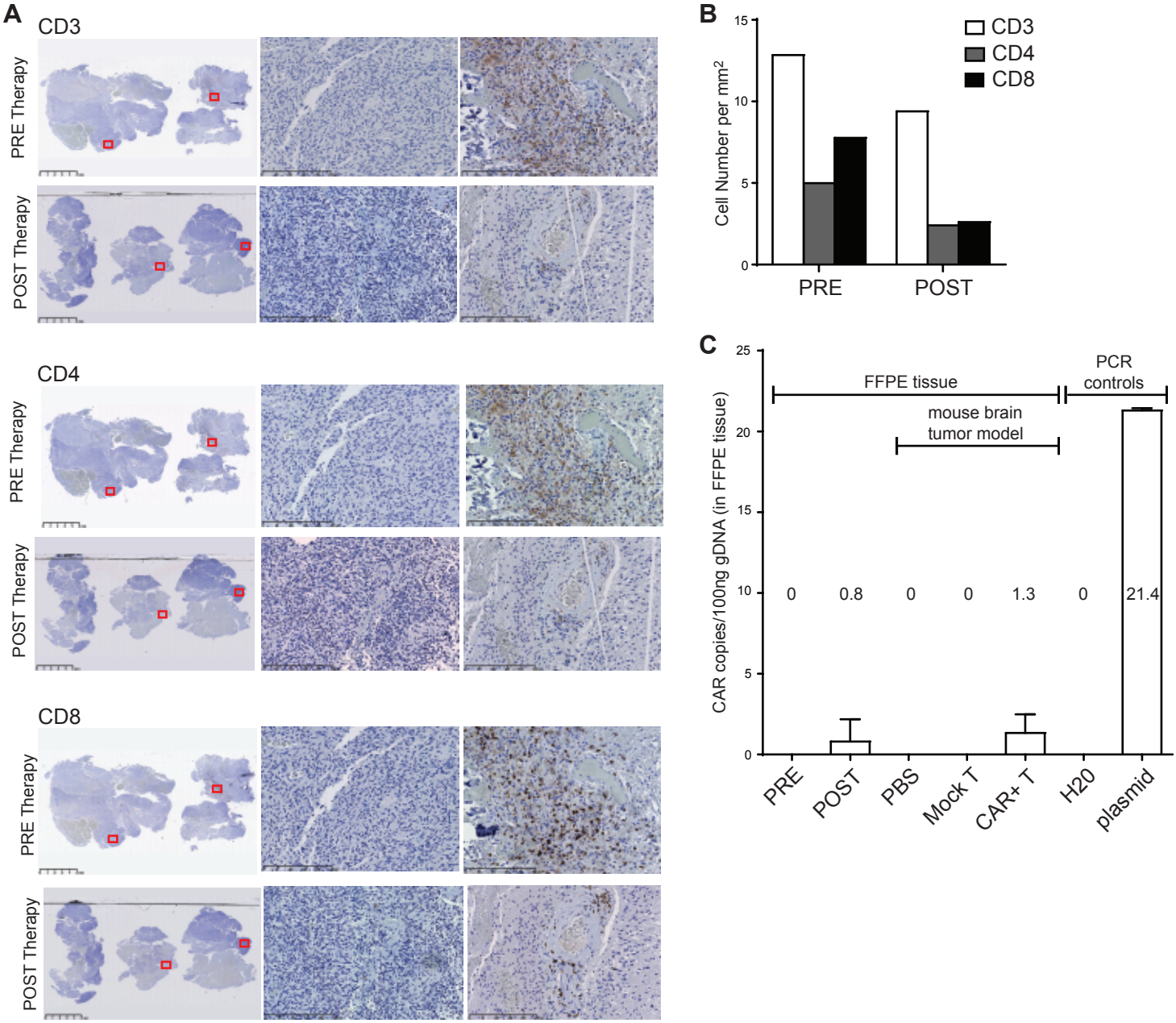

Fig. S5. T cell detection in tumor tissue from UPN031. A, Immunohistochemical staining of CD3, CD8 or CD4 on formalin-fixed, paraffin-embedded (FFPE) primary patient derived brain tumor tissue excised from UPN031 at day 8 (PRE) and day 184 (POST) according to the timeline depicted in Figure S2B. Red boxes indicate location of enlarged images to the right. **B**, Quantification of cell numbers that stained positive for either human CD3, CD4 or CD8 on whole slide sections as depicted in (A), leftmost panels. Total CD3 T cells, including both CD4 and CD8 T cell subsets, were not increased post-therapy. **C**, IL13-zetakine/HyTK transgene copy numbers per 100ng gDNA from the indicated FFPE tissue or 6.4 x 10^{-5} fg of the IL13-zetakine/HyTk plasmid DNA as evaluated by digital droplet PCR (ddPCR). H_2O and plasmid DNA were included as ddPCR controls; brain tissue from mice that had been treated with either PBS, mock-transduced T cells, or CAR-expressing T cells as previously described (Brown et al. Clin Cancer Res, 2012) were included as FFPE controls. Mean copy number values (+ S.D. error bars) are indicated for each bar/plot. By this analysis we estimate less than 1 therapeutic CAR T cell per 20,000 total cells in the tumor tissue, assuming 100 ng of gDNA is equivalent to approximately 16,700 human cells (assuming a diploid cell contains 6 pg gDNA). Together, these IHC and ddPCR data suggest that few therapeutic CAR T cells persisted in the recurrent tumor 14-weeks after the last T cell infusion.