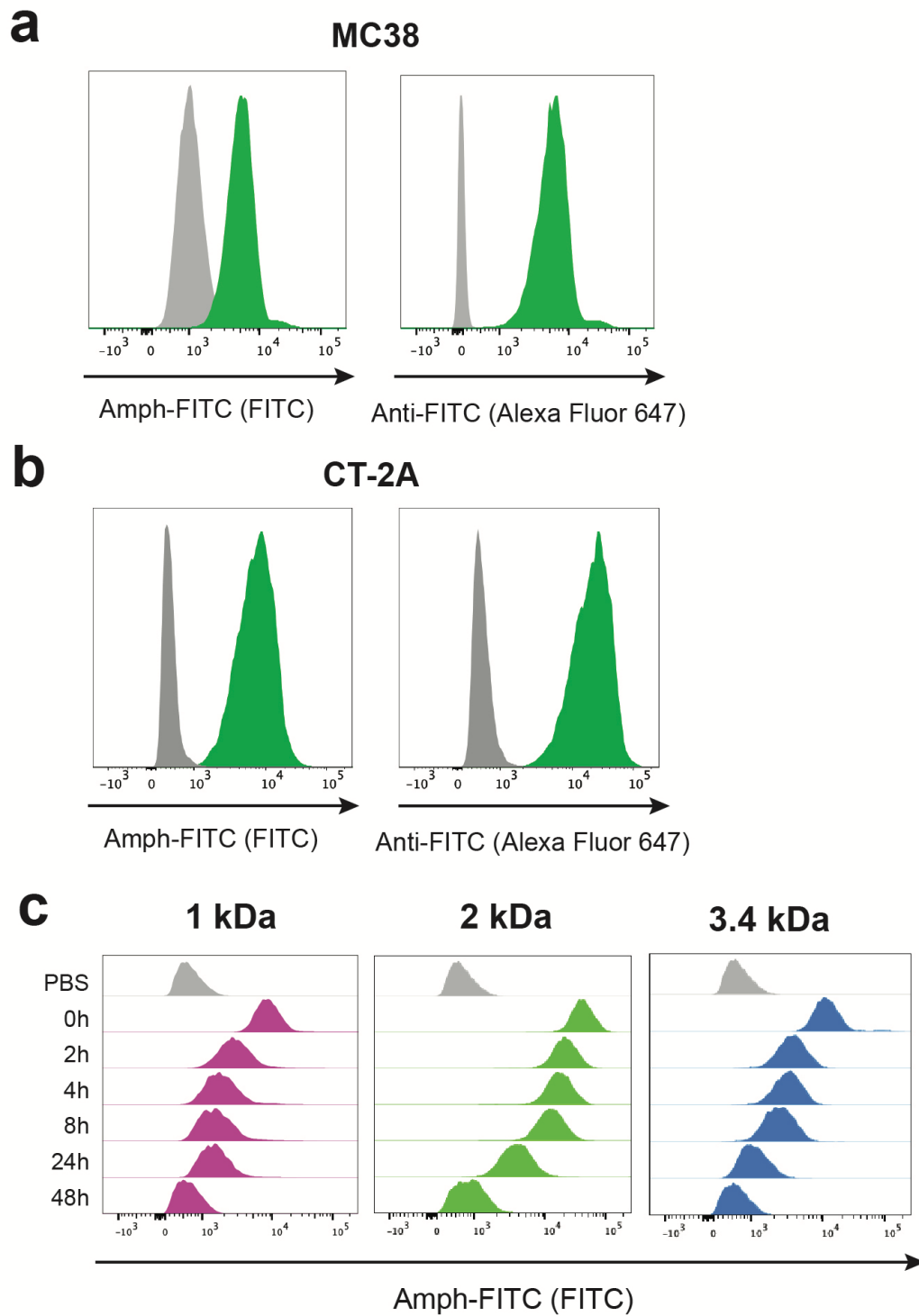


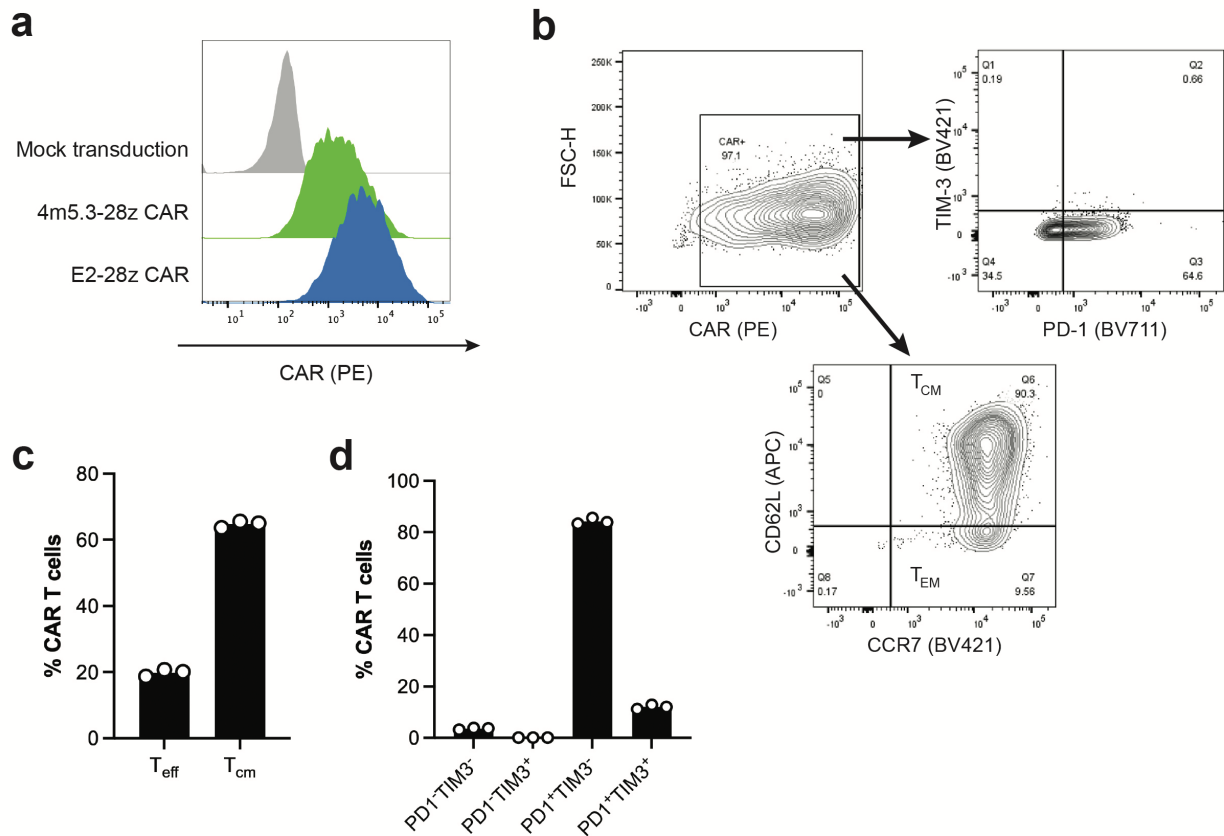


Universal redirection of CAR T cells against solid tumours via membrane-inserted ligands for the CAR

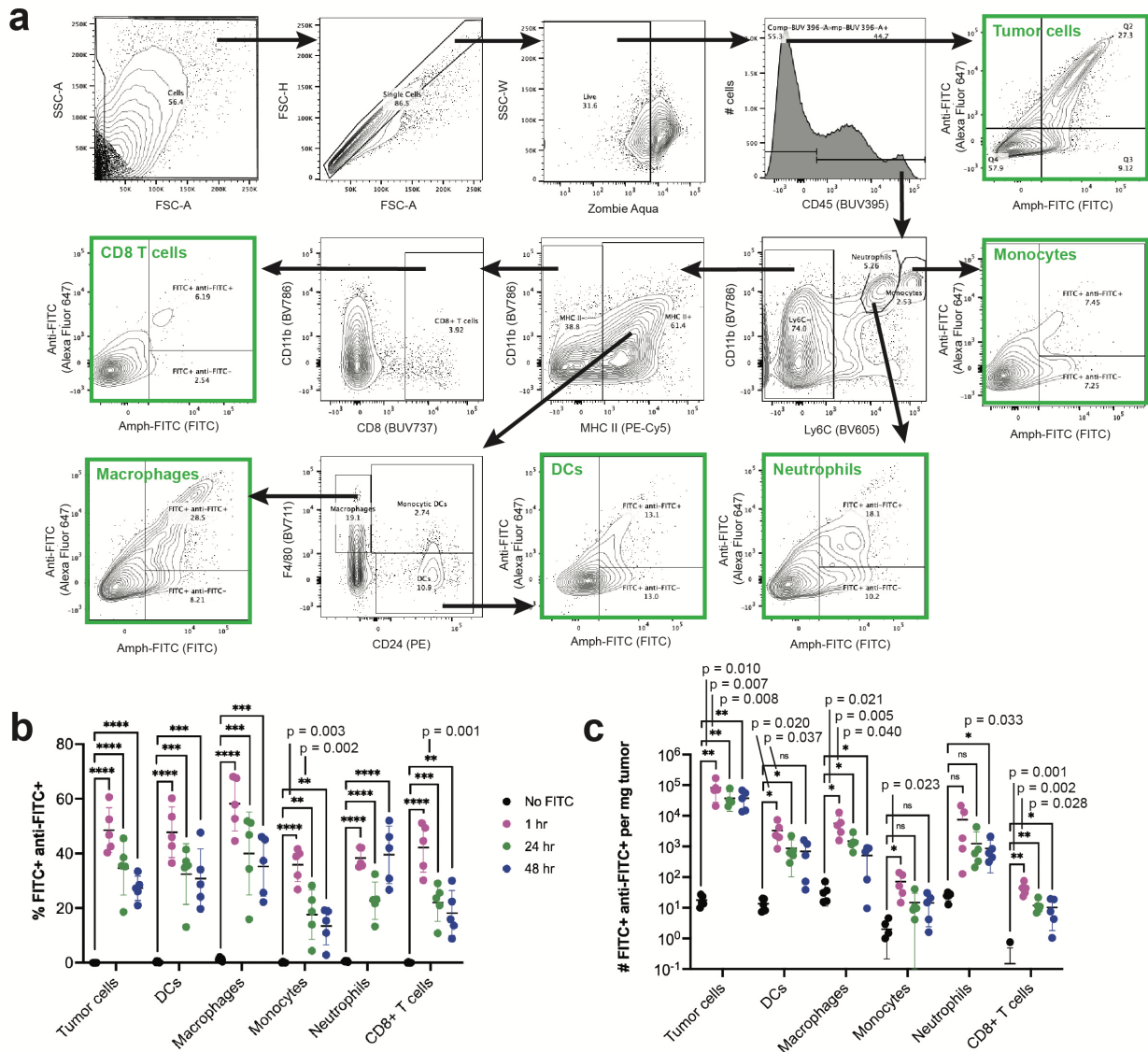
In the format provided by the authors and unedited



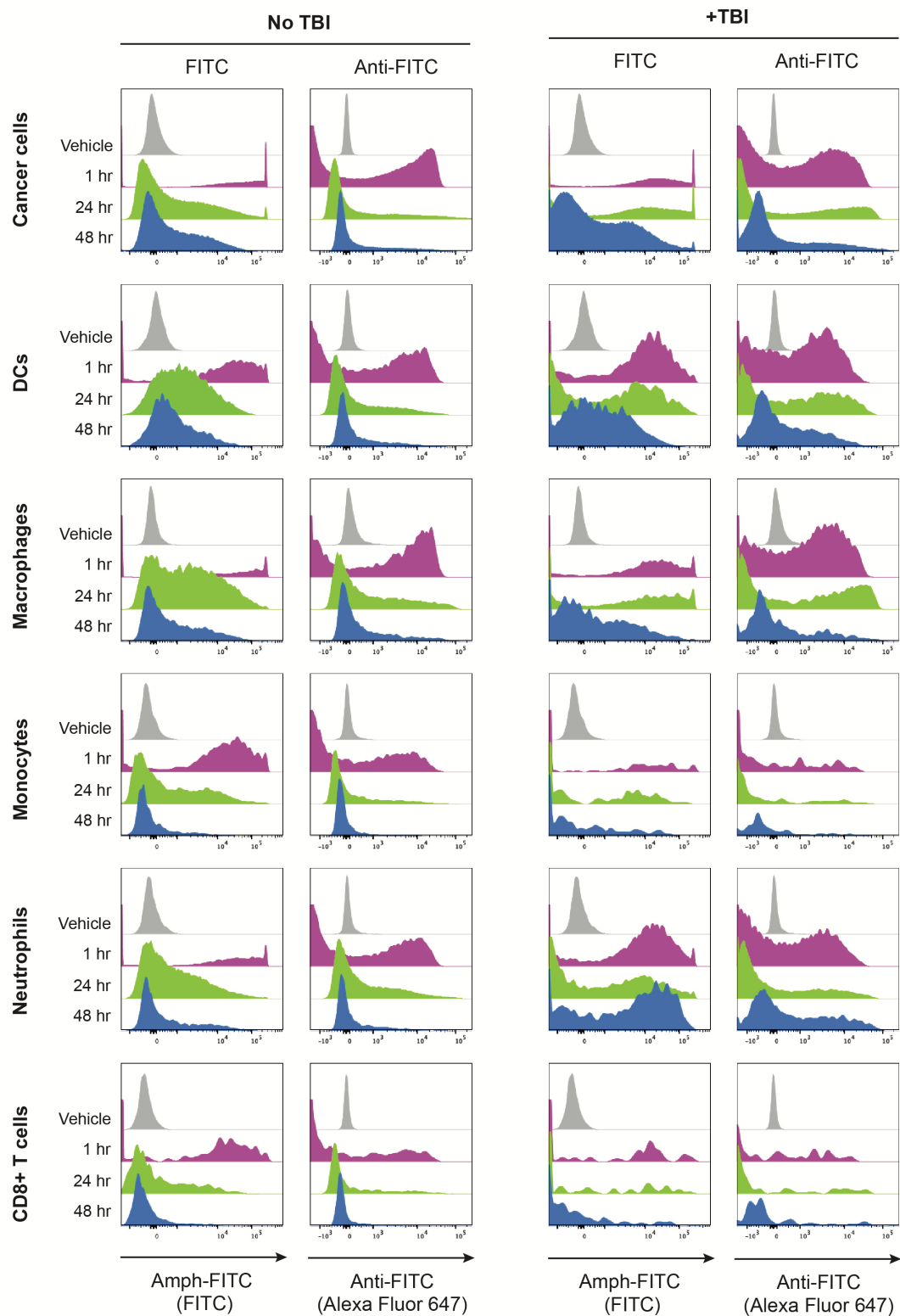
Supplementary Fig. 1 | Characterization of kinetics of amph-FITC tagging. **a,b** MC38 murine colon carcinoma cells (**a**) and CT-2A murine glioma cells (**b**) were incubated with amph-FITC at 100 nM for 30 min at 37°C in PBS, stained with an anti-FITC antibody, and analyzed by flow cytometry. Shown are representative histograms of total FITC (left) and anti-FITC signals (right). **c** Kinetic analysis of amph-FITC MFI of amph-FITC⁺ B16F10 cultured in RPMI following tagging.



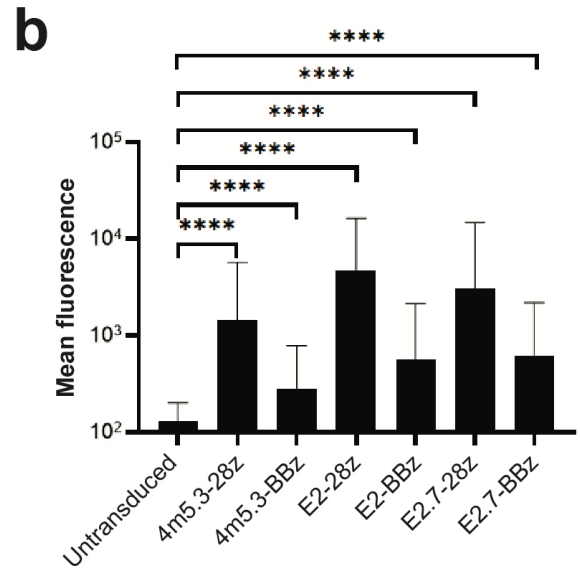
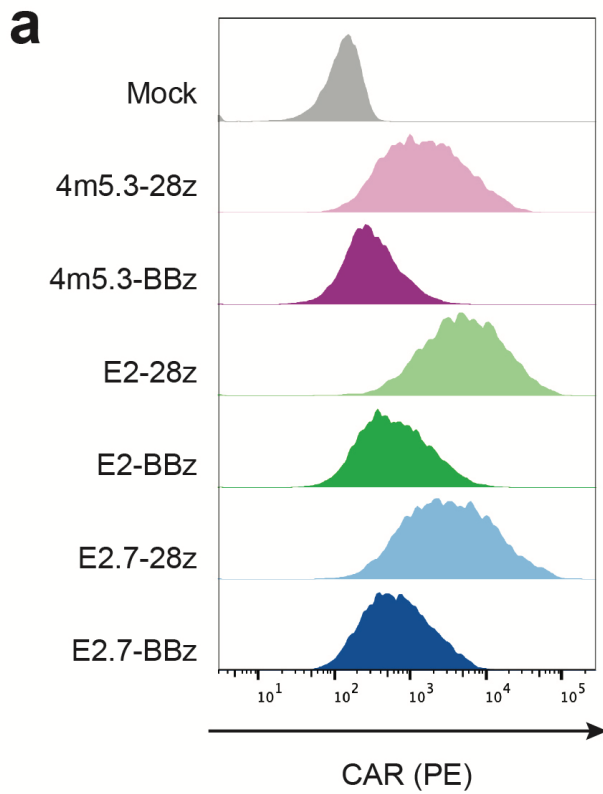
Supplementary Fig. 2 | Murine T cells are effectively transduced to express FITC-specific CARs. a CAR expression in murine CD8⁺ 4m5.3-28z and E2-28z FITC CAR T cells, detected by staining of Myc tag. **b** Gating for exhaustion and memory phenotypes of FITC CAR T cells two days post-transduction. **c** CAR T cell phenotypes prior to adoptive transfer. ($n = 3$ technical replicates) **d** Mean PD-1/TIM-3 expression by CAR T cells prior to adoptive transfer. ($n = 3$ technical replicates)



Supplementary Fig. 3 | Biodistribution of intratumorally injected amph-FITC. a Gating strategy for tumor immunophenotyping. **b,c** Percent FITC⁺ anti-FITC⁺ double-positive cells (**b**) and density of FITC⁺ anti-FITC⁺ cells (**c**) in B16F10 tumors in C57BL/6 mice lymphodepleted with 5 Gy TBI at 1, 24, and 48 hr following amph-FITC injection ($n = 5$ animals/group). p values were determined by unpaired Student's t test. Error bars represent standard deviation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supplementary Fig. 4 | Sample histograms of labeled cell types. Representative histograms of FITC and anti-FITC signal of cancer cells and immune cell subsets in lymphoreplete (left) and lymphodepleted (TBI, right) mice at 1, 24, and 48 hr following amph-FITC injection.



Supplementary Fig. 5 | Expression of CAR variants in murine CD8⁺ T cells. a, b Histograms (a) and quantification (mean \pm SD) (b) of CAR expression 24 hr post-transduction of primary murine CD8⁺ T cells for FITC scFvs of varying affinity and with CD28 vs. 4-1BB costimulatory domains. *P* values were determined by unpaired Student's *t* test. Error bars represent standard deviation. *****p* < 0.0001.