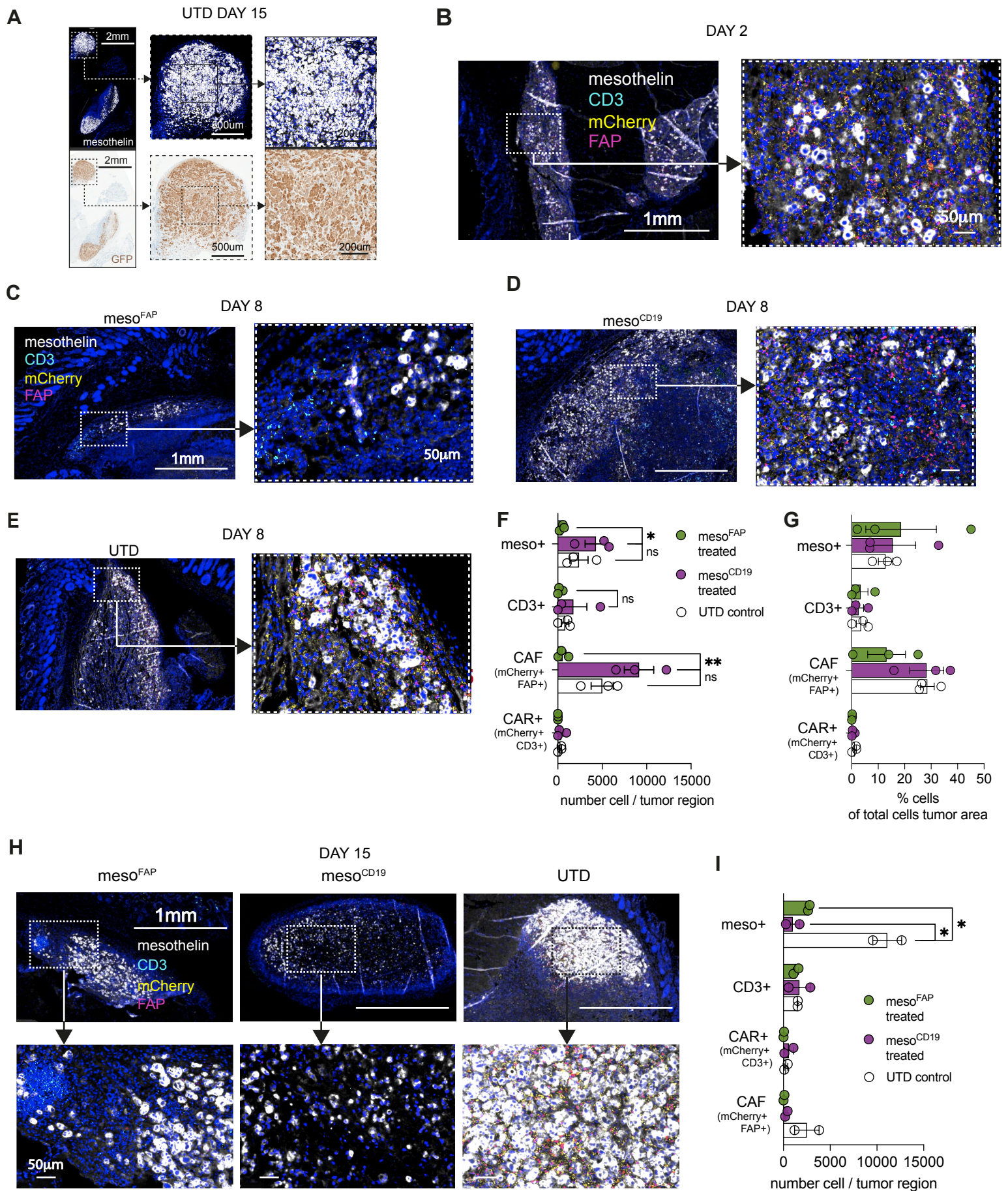


Supplementary Figure S5



Suppl. Fig. S5. Intratumoral multiplex RNA-ISH IF imaging and analysis of CAR-TEAM treated mice. **A.** Representative colocalization of GFP and mesothelin in AsPC-1 transduced with GFP by IF imaging for mesothelin (white) and IHC for GFP (brown) on day 15 (day 13 after UTD treatment). **B–E.** Multiplex ISH-IF imaging with mesothelin (white), CD3+ (cyan), FAP+ (red), mCherry+ (yellow), and DAPI+ (blue) on day 2 (**B**) and (**C–E**) on day 8 treated with (**C**) meso^{FAP}, (**D**) meso^{CD19}, or (**E**) UTD. **F.** Quantification of **C–E** as cell numbers per tumor region from 3 representative examples. **G.** Analysis of mesothelin+ (meso+) PDAC cells, CD3+ T-cells, mCherry+CD3+ CAR T-cells (CAR+) or mCherry+FAP+ CAFs as percentage of the tumor areas measured by multiplex ISH IF on day 8. **H.** Representative images on day 15. **I.** Analysis of mesothelin+, CD3+, CAR+ (mCherry+ and CD3+), or CAF+ (mCherry+ and FAP+), shown as the number of cells per tumor region on day 15. Data represent mean +/-SEM. Stars indicate significance as determined by unpaired two-tailed t-tests. *p < 0.05, **p < 0.01, ns = not significant (panels **F**, **G**, and **I**). N=4-5 animals/group, repeated with T-cells from two healthy donors.