Fig. S5

A

		Clone 9	Clone 4	Clone 7
Positive Cell Pellet		Staining	Staining	Staining
Epithelium	Esophageal and salivary gland (cytoplasm)	1-2+ (occas)	No staining	No staining
	Testis (***membrane*** & cytoplasm)	No staining	***1-3+ (rare to occas)***	No staining
	Pancreas, skin, tonsil, anterior pituitary (cytoplasm)	No staining	1-3+ (rare to occas)	No staining
Myoepithelium	Mammary gland (cytoplasm)	1-3+ (freq)	No staining	No staining
Mononuclear cells	Esophagus, pancreas, pituitary (interstitium), and tonsil (***membrane*** & cytoplasm)	***1-3+ (rare to occas)***	No staining	No staining
Reticular cells and fibers	Lymph node and spleen (***membrane*** & cytoplasm)	***2-4+ (freq)***	No staining	No staining
Vascular and/or intrinsic smooth myocytes	> 30 human tissues (cytoplasm)	1-4+ (occas to freq)	No staining	No staining
Adipocytes	Pancreas (cytoplasm)	No staining	1-3+ (rare)	No staining

B

		Clone 4	Clone 7
Positive Cell Pellet		Staining	Staining
Negative Cell Pellet		No Staining	No Staining
Epithelium	**Pancreas, acini and duct (membrane & cytoplasm)**	**1-3+ (occas), all 3 donors**	No staining
	Testis, Spermatogenic cells (membrane & cytoplasm)	1-3+ (rare to freq), all 3 donors	No staining
Amnion	Placenta (membrane & cytoplasmic)	1-3+ (freq), 1 of 3 donors	No staining

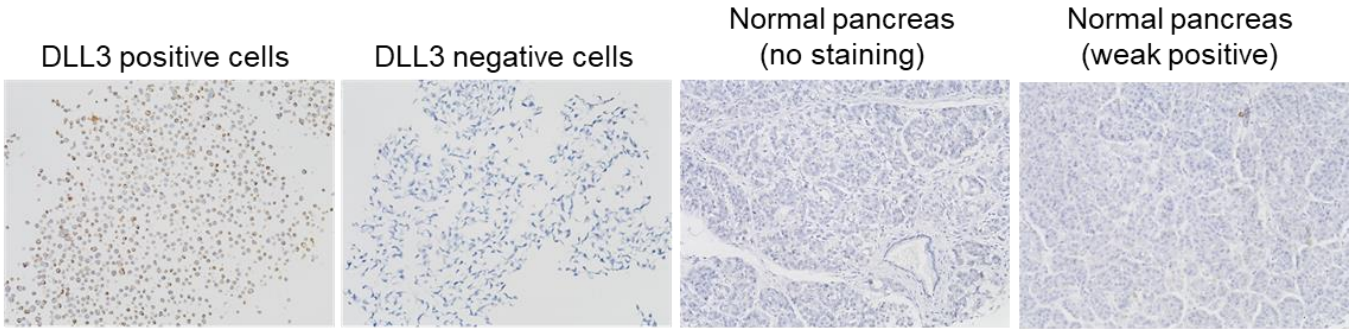
C

		Clone 4(Lot 1)	Clone 4(Lot 2)
Positive Cell Pellet		Staining	Staining
Negative Cell Pellet		No Staining	No Staining
Pancreas	**Membrane staining**	**No staining (all 8 donors)**	**No staining (all 8 donors)**
	Extracellular material in duct and acinar lumina	1-3+, rare (1 of 8 donors)	1-3+, rare (1 of 8 donors)
	Cytoplasm of duct epithelial cells	No staining	1+, rare (1 of 8 donors)

For panels A-C, 1+ = weak, 2+ = moderate, 3+ = strong, 4+ = intense, occas = occasional, freq = frequent. Frequency modifiers were included to provide the approximate percentage staining of expected numbers of that cell type or tissue element at that location. The frequency of cells with staining was identified as follows: very rare (<1% of cells of a particular cell type); rare (1-5% of cells of a particular cell type); rare to occasional (>5-25% of cells of a particular cell type); occasional (>25-50% of cells of a particular type); occasional to frequent (>50-75% of cells of a particular cell type); frequent (>75-100% of cells of a particular cell type).

Fig. S5 continued

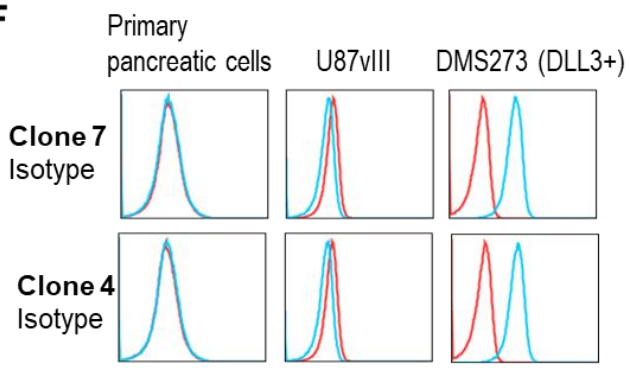
D



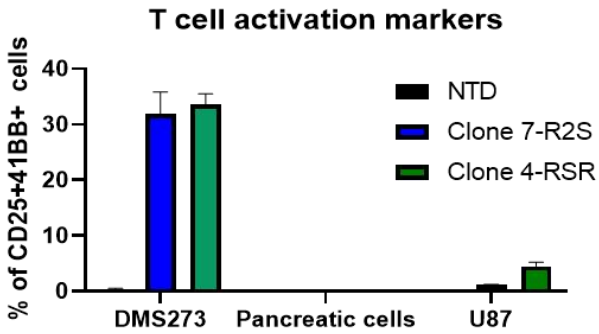
E

Normal pancreatic tissue (N=20)	**N=16**	**No staining**
	N=2	Equivocal positive staining (cytoplasmic), rare
	N=2	Weak positive staining (cytoplasmic), rare

F



G



H

	Age matched control (n=3)	Clone 4-RSR (n=5)
Infiltration	No infiltration	Minimal (n=1) Mild (n=1)
Decreased secretory material (acinar cell)	No findings	Minimal (n=1) Mild (n=4)

Fig. S5. Clone 4 showed pancreatic membrane staining in one of three TCR studies but other derisking studies suggest the staining may be artifactual. (**A**) Clone 9 showed off-target binding to multiple tissues in initial TCR study and was eliminated as a candidate. The scFv binding domains of clone 4, 7 and 9 were fused to a human IgG2 Fc fragment and assayed using immunohistochemistry (IHC) against a panel of 36 human tissues to evaluate binding from 1 donor. Results are shown for staining at 10 mg/mL. DLL3-positive and negative cell pellets were utilized as controls. Staining was scored by a board-certified pathologist on both intensity and frequency. Scoring is shown for control cell pellets and for tissues in which positive staining with either CARs 9 or 4 was observed. (**B**) Membrane staining of clone 4 was observed in epithelial cells in the pancreas (acini, ducts), amnion in the placenta and spermatogenic cells in the testis. The scFv binding domains of clone 4 and 7 were tested using methods described in (A) against a panel of 36 human tissues to evaluate binding from 3 donors. Results are shown for staining at 10 μg/mL. Scoring is shown for control cell pellets and for tissues in which membrane staining with clone 4 was observed. (**C**) Follow-up study to further evaluate pancreatic membrane staining was performed using the same protocol with 8 additional human donors and two lots of clone 4 soluble binding domain. No pancreatic membrane staining was observed in any donors. (**D**) Additional IHC work was performed in-house and no pancreatic membrane staining was observed. Representative images of clone 4 staining on DLL3-positive cells, DLL3-negative cells and 2 normal pancreas samples were shown. (**E**) In-house IHC staining of 20 normal pancreas samples (US Biomax) was reviewed by a pathologist. Staining pattern and intensity was summarized in the table. (**F**) Clone 4 and clone 7 showed binding to DLL3 positive DMS273 cells but not to human primary pancreatic cells. Data from one representative donor is shown. (**G**) Clone 4 and clone 7 CAR T cells are not activated after co-cultured with primary pancreatic cells for 2 days at an E:T of 2:1. Error bars represent standard deviation, n=4 technical replicates. Experiments were performed 3 times with DLL3 CAR Ts generated from different donors. Data from one representative donor is shown. (**H**) DLL3 CAR T cells-related changes in pancreas are limited to minimal/mild zymogen decrease and lymphocyte infiltrates. Animals bearing SHP-77 subcutaneous tumors received a single dose of NTD or DLL3 CAR T cells ($5x10^6$ CAR+ per animal). After tumor clearance, pancreas tissues were collected, H&E stained and analyzed by a pathologist.