Supplementary Figure Legends

Supplementary Figure 1. Phenotypic analysis of FAP$^+$ stromal cells in untreated TC1 flank tumors in C57BL/6 mice. Untreated TC1 tumors were harvested and digested with a mixture of collagenases to make single cell suspension. Cells were stained with anti-CD45, anti-CD90 and anti-FAP. Propidium iodide was used to exclude dead cells.

Supplementary Figure 2. Depletion of FAP$^+$ cells. FAP-CAR T cells were injected intravenously into AE17.ova tumor-bearing mice when tumors reached ~100 mm^3. At 3 days after T cell infusion, tumors were harvested and digested to determine the amount of depletion of **(A)** FAP$^+$ cells; **(B)** FAP^{hi} cells; and **(C)** FAP^{lo} cells by FAP-CAR T cells. * Denotes statistical significance between untreated, MigR1 and FAP-CAR-treated samples, p value < 0.05.

Supplementary Figure 3. Persistence and antitumor activities of FAP-CAR T cells employing either human 4-1BB (73.3-hBBz) or mouse CD28 (73.3-m28z) co-stimulatory domain in mice and their *in vitro* activity. Mouse FAP-CAR T cells were produced expressing different intracellular activation domains including the mouse CD3ζ chain (73.3-mz), the mouse CD3ζ plus mouse CD28 domains (73.3-m28z), the human CD3ζ chain (73.3-hz), and the human CD3ζ plus human 41BB domains (73.3-hBBz). (**A**) Persistence of FAP-CAR T cells (73.3-hBBz) over time. AE17.ova tumor-bearing mice were injected with 10 million FAP-CAR T cells through the tail vein when the tumors reached ~100 mm^3. Tumors were harvested 3, 7 and 10 days after adoptive transfer, to assess the frequencies of GFP$^+$ CD3$^+$ FAP-CAR T cells (n=5). * Denotes statistical significance in lower percent CAR TILs compared to the 3 day time point, p value < 0.05. **(B)** To determine target-specific cytolytic activity of two FAP-CAR T cells,

various Effector:Target ratio of MigR1 and FAP-CAR T cells were reacted with 3T3.FAP fibroblasts for 18 hours. (**C**) IFNγ production from FAP-CAR T cells were determined by reacting 3T3.mFAP at effector:target ratio of 10:1 for 18 hours. *Denotes statistical significance between the first generation (CD3ζ constructs) versus the corresponding second generation (4-1BB and CD28ζ) constructs, p value < 0.05. (**D**) FAP-CAR T cells with different co-stimulatory domain persisted similarly *in vivo*. The FAP-CAR-hBBz and FAP-CAR-m28z constructs were transduced into congenic Thy1.1 C57BL/6 mouse T cells to determine their trafficking and persistence in tumor-bearing mice. AE17.ova tumors were injected into Thy1.2 C57BL/6 mice. When tumors reached ~100-125 mm^3, a single dose (10 million) of $Thy1.1^+$ FAP-CAR T cells were injected through the tail vein into mice. Tumors were harvested 3 days after adoptive transfer, digested and dissociated into single cell suspensions. Cells were stained with fluorochrome-conjugated anti-Thy1.1, together with anti-CD3 antibody to determine percent FAP-CAR T cells in tumors. (**E**) *In vivo* antitumor activity of two FAP-CAR T cells. AE17.ova tumor-bearing mice were injected with 10 million FAP-CAR T cells through the tail vein when tumors reached ~150 mm^3. Tumor measurements followed. * Denotes statistical significance between untreated and FAP-CAR-treated samples, p value < 0.05.

Supplementary Figure 4. Deletion of DGKζ enhanced effector functions and *in vivo* persistence of FAP-CAR T cells. Splenic T cells were isolated from WT C57BL/6 mice, as well as DGKζ knockout mice, activated, transduced with FAP-CAR and then expanded. A week later, the FAP-CAR T cells with or without DGKζ deletion were reacted with 3T3 or 3T3.FAP fibroblasts for 18 hours to determine **(A)** cytotoxicity and **(B)** IFNγ production. * Denotes statistical significance between untreated and two FAP-CAR treated samples, p value < 0.05.

#Denotes statistical significance between WT and DGKζ KO FAP-CAR-treated samples, p value < 0.05. To determine their persistence *in vivo,* AE17.ova tumor-bearing mice were injected with 10 million wild-type or DGKζ knockout FAP-CAR T cells when tumors reached ~100 mm3. Tumors were harvested 11 days post-injection. **(C)** Percent FAP-CAR T cells were determined using flow cytometry. * Denotes statistical significance between WT and DGKζ KO FAP-CAR-treated samples, p value < 0.05.

Supplementary Figure 5. Body weight of FAP-CAR T cells-treated mice remained constant or increased. Wild type FAP-CAR T cells were injected into mice bearing tumors derived from AE17.ova (**A**), TC1 (**B**), LKRM2 (**C**), 4T1 (**D**) and CT26 (**E**) cells and weights were monitored. No loss of body weight was observed in any group. (**F**): Mice bearing AE17.ova tumors were injected with either wild-type FAP-CAR T cells or "hyperactive" DGKζ knockout FAP-CAR T cells. Again, no weight loss was observed.

Supplementary Figure 6. Histology of bone marrows and pancreas following treatment with FAP-CAR T cells. Representative H&E sections of organs harvested one week after T cell infusion from tumor-bearing mice treated with wildtype or DGKζ knockout FAP-CAR T cells. Femur bones from mice treated with **(A)** control untreated; **(B)** MigR1 T cells; **(C)** FAP-CAR T cells. Pancreas from mice treated with **(D)** untreated control; **(E)** wild-type FAP-CAR T cells; and **(F)** DGKζ knockout FAP-CAR T cells. Pancreatic Islets of Langerhans are marked by an asterisk. Blood vessels are labeled with a "V". No changes were seen in the organs treated with WT FAP-CAR T cells **(E)**. In mice treated with DGKζ knockout FAP-CAR T cells **(F)**, focal areas of lymphocytes were noted in a peri-islet (white arrows) and peri-vascular (white

arrowheads) location.

Supplementary Figure 7. Activation-induced cell death of FAP-CAR T cells. (A) Comparison of *in vivo* persistence between MigR1 and FAP-CAR T cells. Tumors were harvested 3 and 8 days after injection of 10 million FAP-CAR T cells into AE17.ova tumor bearing mice. * Denotes statistical significance in lower percent TILs compared to the 3 day time point, p value < 0.05. #Denotes statistical significance in lower percent FAP-CAR TILs compared to MigR1 TILs at the same time point, p value <0.05. **(B)** FAP-CAR T cells died soon after antigen stimulation. MigR1 and FAP-CAR T cells with either human or mouse intracellular domains were exposed to either BSA- or FAP-coated beads. Amount of live cells were counted every day for 3 days, by trypan blue staining.

Supplementary Figure 8. Differential FAP expression on tumor and pancreatic FAP^+ stromal cells. Tumors and pancreas from different tumor-bearing mice were harvested and digested to form single cell suspensions. Cells were stained with biotin-conjugated anti-FAP antibody and streptavidin-conjugated fluorochrome, together with anti-CD90 and anti-CD45 antibodies. The mean fluorescence intensity of FAP expression on $CD90^+$CD45- cells in tumors (red) and pancreas (blue) were compared.

Supplementary Figure 9. mAb 73.3 and mAb FAP5 are specific for distinct epitopes of murine FAP expressed by fibroblasts. **(A)** The indicated FAP antibodies (73.3 produced as described herein, FAP5, generously provided by Boehringer Ingelheim (30), and sheep polyclonal anti-human FAP that cross reacts with murine FAP purchased from R&D (26) were

reacted with 3T3 and 3T3.FAP cells. **(B)** Pre-incubation with the indicated concentrations of 73.3 had no effect on the binding of FAP5 to 3T3.FAP cells indicating that they are binding to different epitopes.