Co-stimulatory Signaling Determines Tumor Antigen Sensitivity and Persistence of CAR T Cells Targeting PSCA^{+} Metastatic Prostate Cancer

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Supplemental Figures:

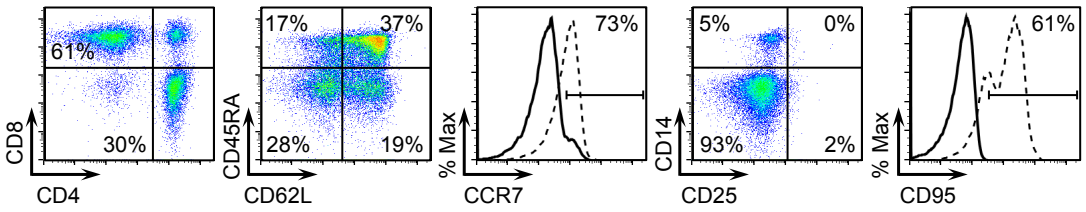

Figure S1: Cell-surface phenotype of PBMC. (a) Starting populations of PBMC were analyzed by flow cytometry for expression of CD4, CD8, CD45RA, CD62L, CCR7, CD14, CD25, and CD95. Representative FACS plots are shown.

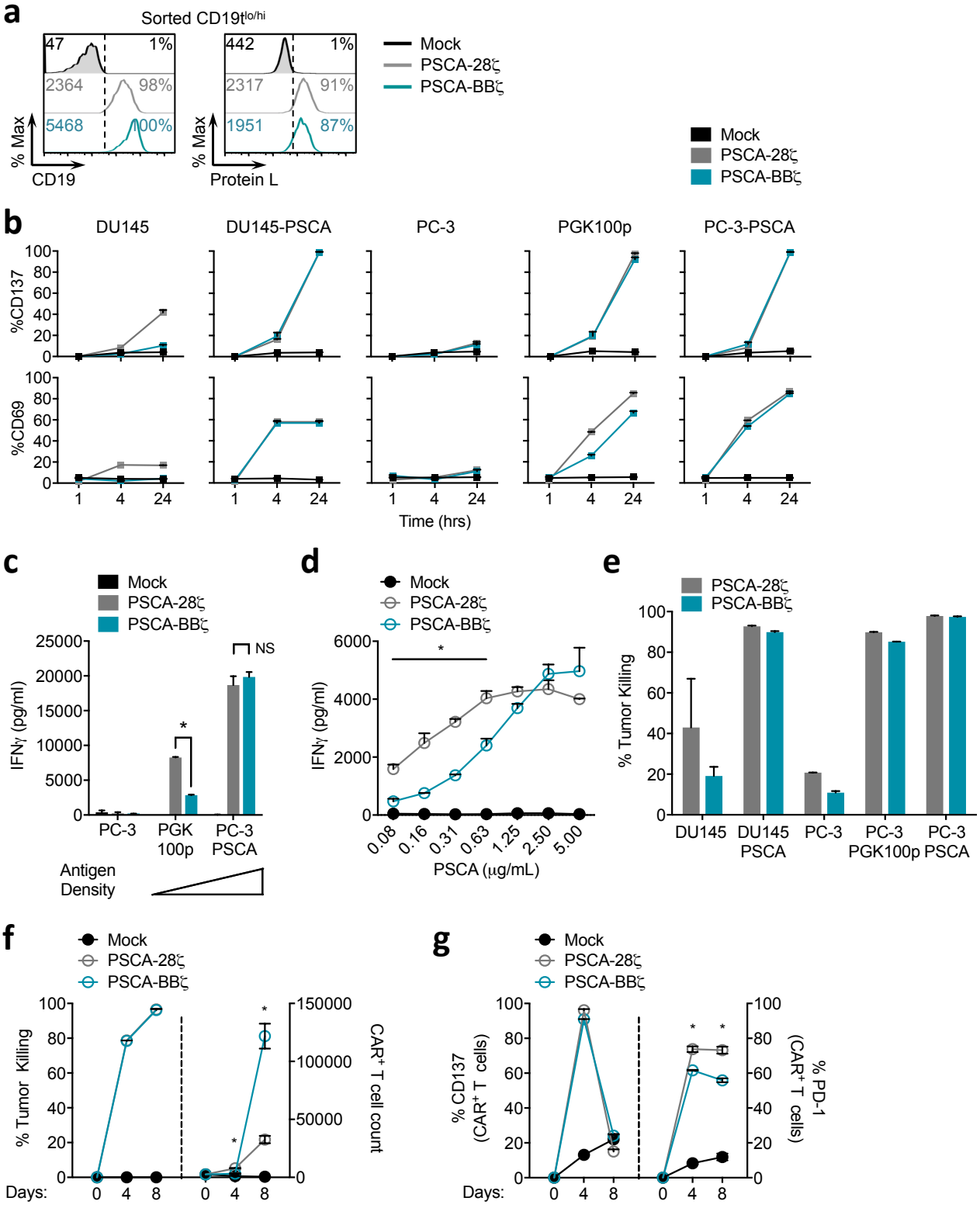

Figure S2: FACS sorted PSCA-28ζ and PSCA-BBζ CAR T cell function *in vitro*. **(a)** Mock (untransduced), PSCA-28ζ, or PSCA-BBζ CAR T cells were evaluated by flow cytometry for CD19t expression to detect lentiviral transduction of CARs (left) or Protein L to detect the scFv (right) following FACS-based sorting of selected CD19t-expressing populations. **(b)** Quantification of CD137 (top) and

CD69 (bottom) expression on Mock, PSCA-28ζ, and PSCA-BBζ CAR T cells following a 1, 4, or 24 hour co-culture with the indicated tumor targets at a 1:2 effector:tumor (E:T) ratio. **(c)** IFNγ production quantified by ELISA in supernatants from PSCA-CAR T cells cultured overnight with with PC-3, PGK100p, or PC-3-PSCA tumor cells. **(d)** IFNγ production quantified by ELISA in supernatants from PSCA-CAR T cells cultured overnight on plate-bound recombinant human PSCA at varying protein concentrations. **(e)** Quantification of tumor killing by PSCA-28ζ or PSCA-BBζ CAR T cells at a 1:2 E:T ratio following a 3-day co-culture with indicated tumor cells compared to Mock. **(f**) Quantification of tumor killing and CAR+ T cell count after 4 or 8 days of co-culture with PC-3-PSCA at a 1:20 E:T ratio. (**g**) Quantification of CD137 and PD-1 expression on PSCA-CAR T cells from (f).

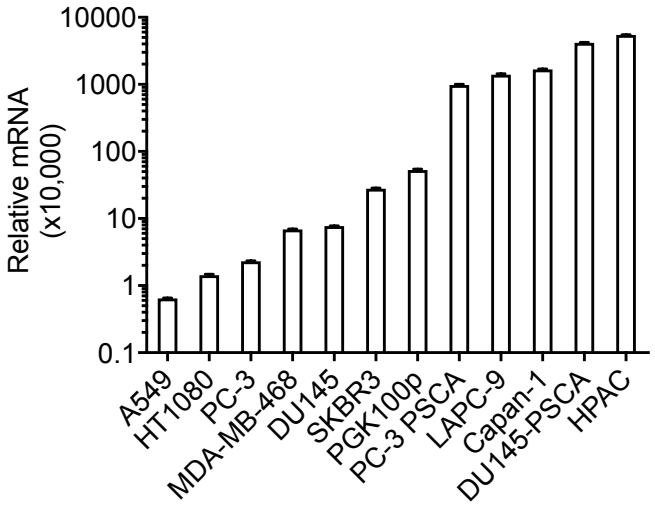

Figure S3: mRNA expression analysis of PSCA in tumor cell lines. (a) qPCR performed on various prostate and non-prostate cancer cell lines to quantify PSCA expression. PSCA mRNA was normalized to GAPDH mRNA.

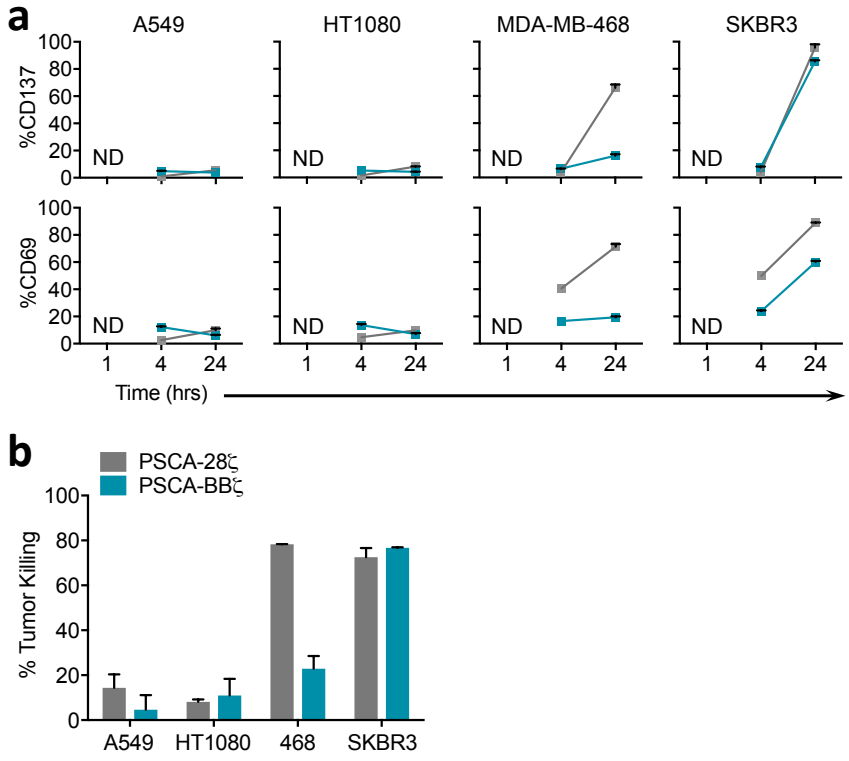

Figure S4: PSCA-28ζ and PSCA-BBζ CAR T cell function against non-prostate cancer cells *in vitro*. (a) Quantification of CD137 (top) and CD69 (bottom) expression on PSCA-28ζ and PSCA-BBζ CAR T cells following a 4 or 24 hour co-culture with the indicated tumor targets at a 1:2 effector:tumor (E:T) ratio. **(b)** Quantification of tumor killing by PSCA-28ζ or PSCA-BBζ CAR T cells at a 1:2 E:T ratio following a 3-day co-culture with indicated tumor cells compared to Mock.

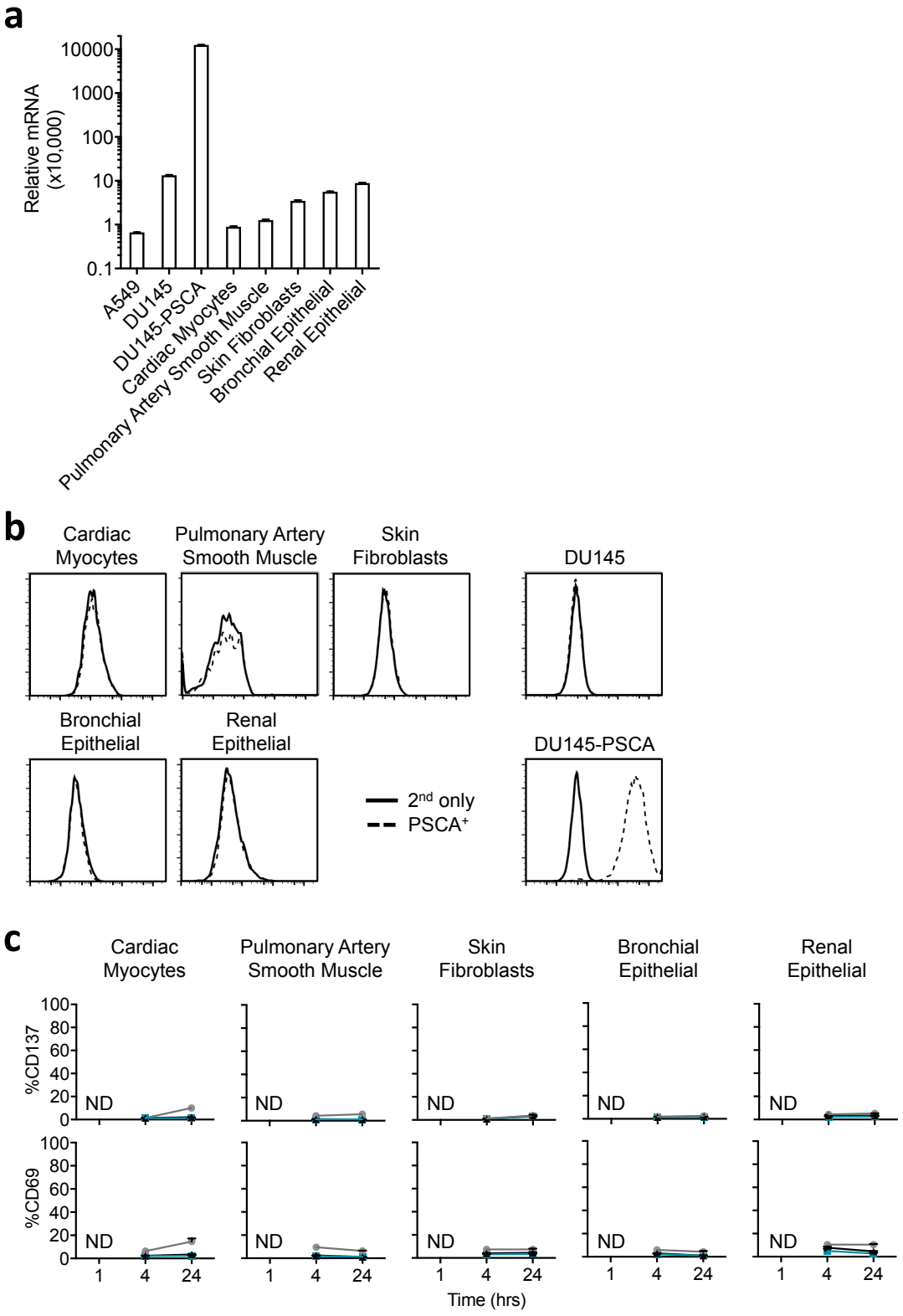

Figure S5: PSCA-28ζ and PSCA-BBζ CAR T cell function against normal human cell lines *in vitro*. (a) qPCR performed on various normal human cell lines to quantify PSCA expression. PSCA mRNA was normalized to GAPDH mRNA. **(b)** Flow cytometric analysis of PSCA expression in normal

human cell lines. DU145 and DU145-PSCA serve as controls. **(c)** Quantification of CD137 (top) and CD69 (bottom) expression on PSCA-28ζ and PSCA-BBζ CAR T cells following a 4 or 24 hour co-culture with the indicated tumor targets at a 1:2 effector:tumor (E:T) ratio.

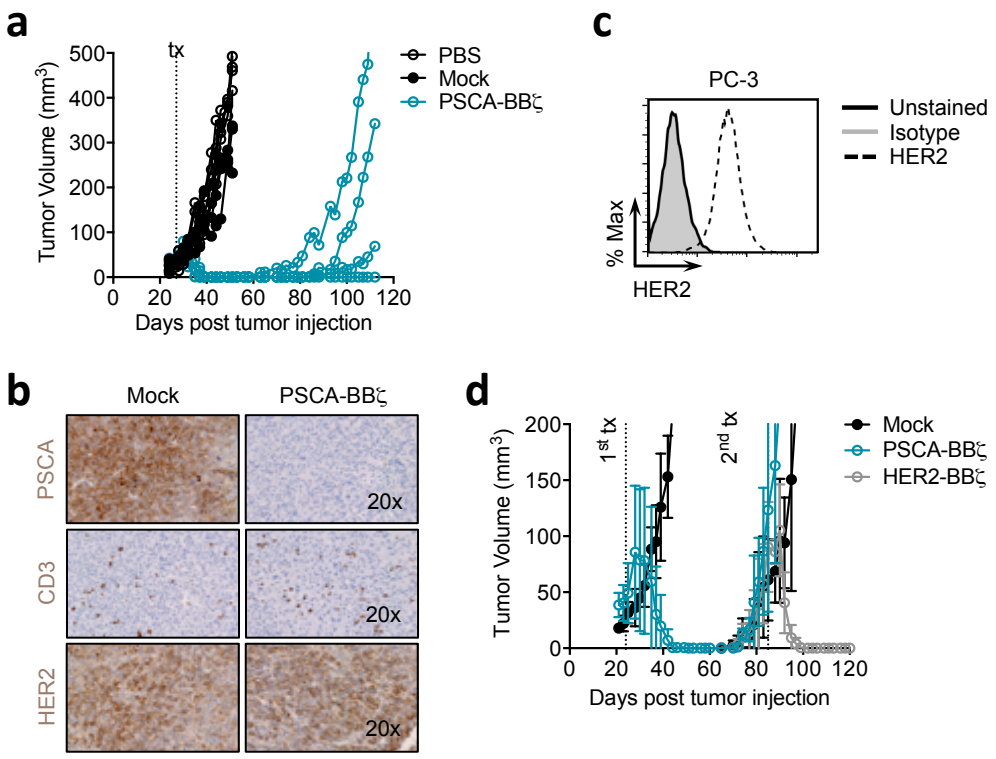

Figure S6: Treatment of PSCA-negative tumor recurrences with HER2-specific CAR T cells. (a) Kinetics of tumor recurrences in PSCA-BBζ treated PC-3-PSCA tumor bearing mice. Each line represents an individual mouse per group. N = 4 per group. Data are representative of at least two independent studies. **(b)** Immunohistochemistry of PC-3-PSCA tumors harvested from Mock-treated (at primary endpoint) or recurrent PSCA-BBζ-treated tumors stained with human PSCA, CD3 and HER2. **(c)** HER2 expression in PC-3-PSCA tumor cells, assessed by flow cytometry. **(d)** Tumor volume (mm^3) in mice bearing PC-3-PSCA tumors treated i.v. with 5 x 10^6 Mock or PSCA-BBζ CAR T cells (N = 6 per group) on day 24 ("1st tx"). On day 81, when CAR T cell-treated mice showed tumor recurrence (50 - 100 mm^3), mice were assigned to a second treatment ("2nd tx") receiving i.t. injections of either 5 x 10^6 Mock, PSCA-BBζ, or HER2-BBζ CAR T cells (N = 2 per group).

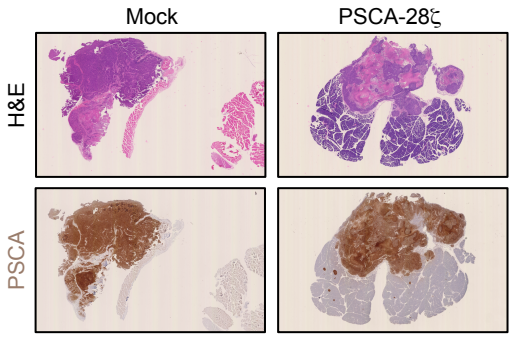

Figure S7: PSCA expression in tumors from Mock- and PSCA-28ζ-treated mice. Immunohistochemistry of LAPC-9 tumors harvested from Mock-treated (at primary endpoint, day 62) and recurrent PSCA-28ζ-treated tumors (day 77) stained with H&E (upper panel) or human PSCA (lower panel).