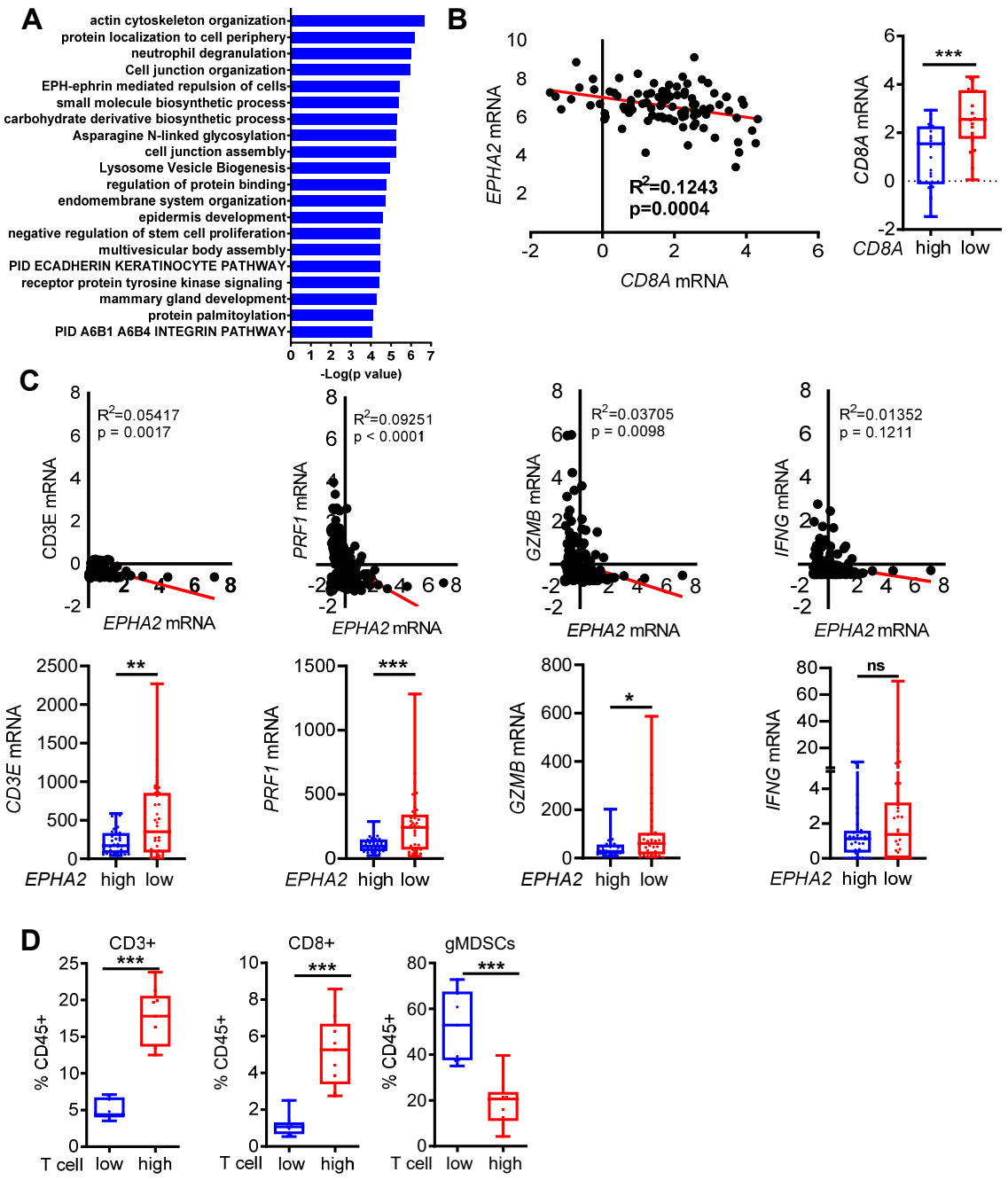

Figure S1. Expression of *EPHA2* correlates with the abundance of CD8+ T cells in PDA (Related to Figure 1). (**A**) Top pathways from Metascape analysis of genes negatively correlated with *CD8A* in human TCGA PDA dataset. (**B**) Correlation of transcript abundance for *CD8A* and *EPHA2* (left) and abundance of *CD8A* mRNA in the top and bottom 20% of *EPHA2* expression (right) in human PDA samples (QCMG_Nature_2016 dataset, cbioportal). (**C**) Correlation of transcript abundance (top row) and abundance of *CD3E, PRF1, GZMB* and *IFNG* transcripts (bottom row) in the top and bottom 20% of *EPHA2* expression, in human TCGA PDA dataset. (**D**) Flow cytometric analysis of immune cell populations in subcutaneously implanted T cell low and T cell high tumors derived from indicated clones (n=10/group). In (B-D), data presented as boxplots with horizontal lines and error bars indicating mean and range, respectively. Statistical analysis by Students' unpaired t-test (B, C, D) with significance indicated (*, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001; *ns*, not significant in this and all subsequent figures, unless otherwise indicated).

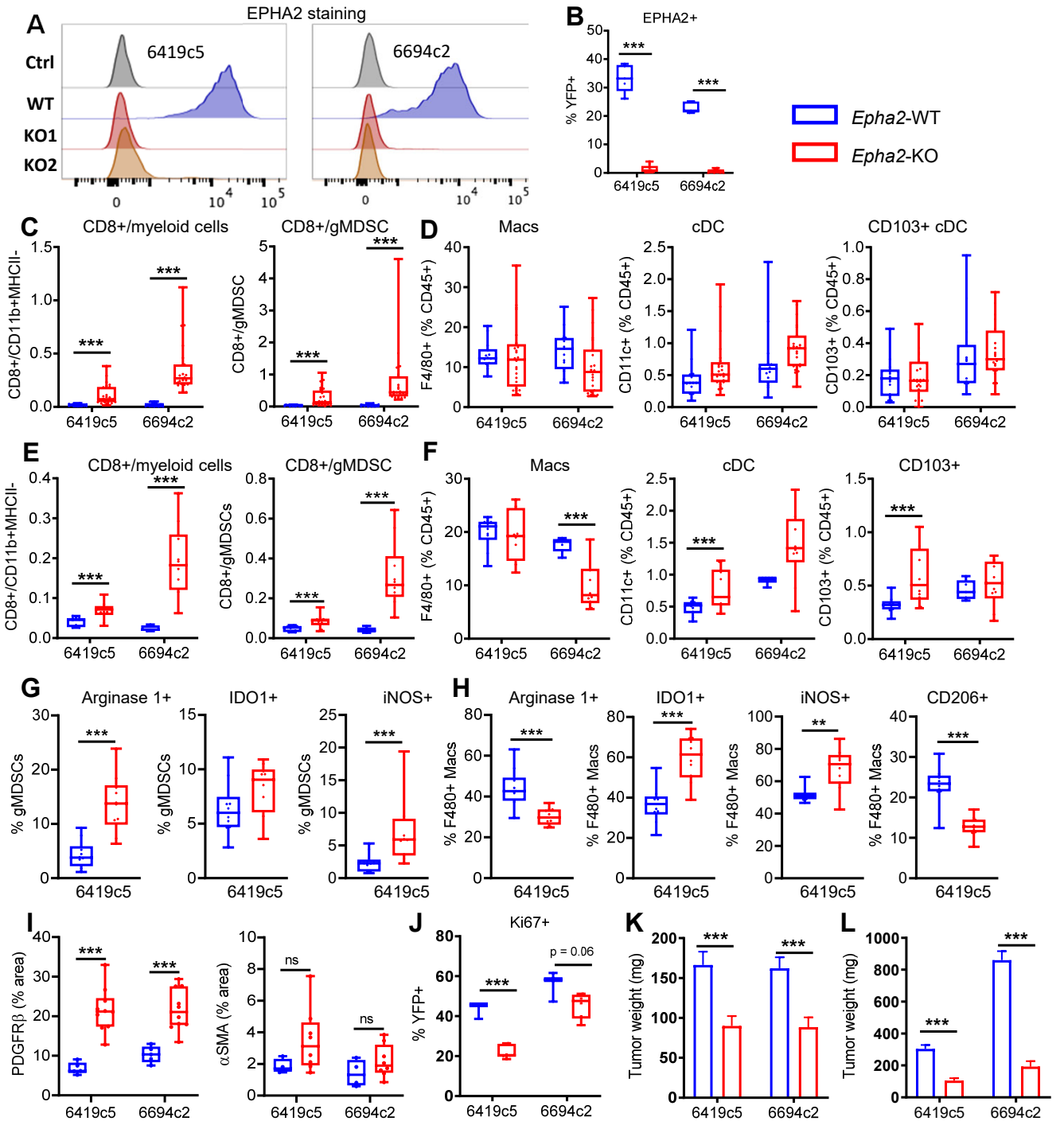

Figure S2. Tumor cell-intrinsic *Epha2* regulates T cell infiltration (Related to Figure 2). (**A-B**) Representative histogram (**A**) and quantification (**B**) of EPHA2 protein expression in subcutaneous *Epha2*-WT and *Epha2*-KO tumors from indicated clones (n=5/group). (**C-F**) Flow cytometric analysis of immune cell populations in subcutaneous (**C-D**, n=12-25/group) and orthotopic (**E-F,** n=5-10/group) tumors from *Epha2*-WT and *Epha2*-KO tumors from indicated clones. (**G-H**) Flow cytometric analysis of gMDSCs and Macs in subcutaneous *Epha2*-WT and *Epha2*-KO tumors from indicated clone (n=10/group). (**I**) Quantification of αSMA and PDGFRβ stainings in subcutaneous *Epha2*-WT and *Epha2*-KO tumors from indicated clones (n=4-10/group). (**J**) Proportions of Ki67+ tumor cells among cultured *Epha2*-WT and *Epha2*-KO tumor cells, by flow cytometry (n=3-6/group). (**K-L**) Weights of implanted subcutaneous (**K**, n=12-25/group) and orthotopic (**L**, n=5-10/group) *Epha2*-WT and *Epha2*-KO tumors from indicated clones, 21 days post implantation. Statistical difference between groups calculated using Student's unpaired t-test (B-L).

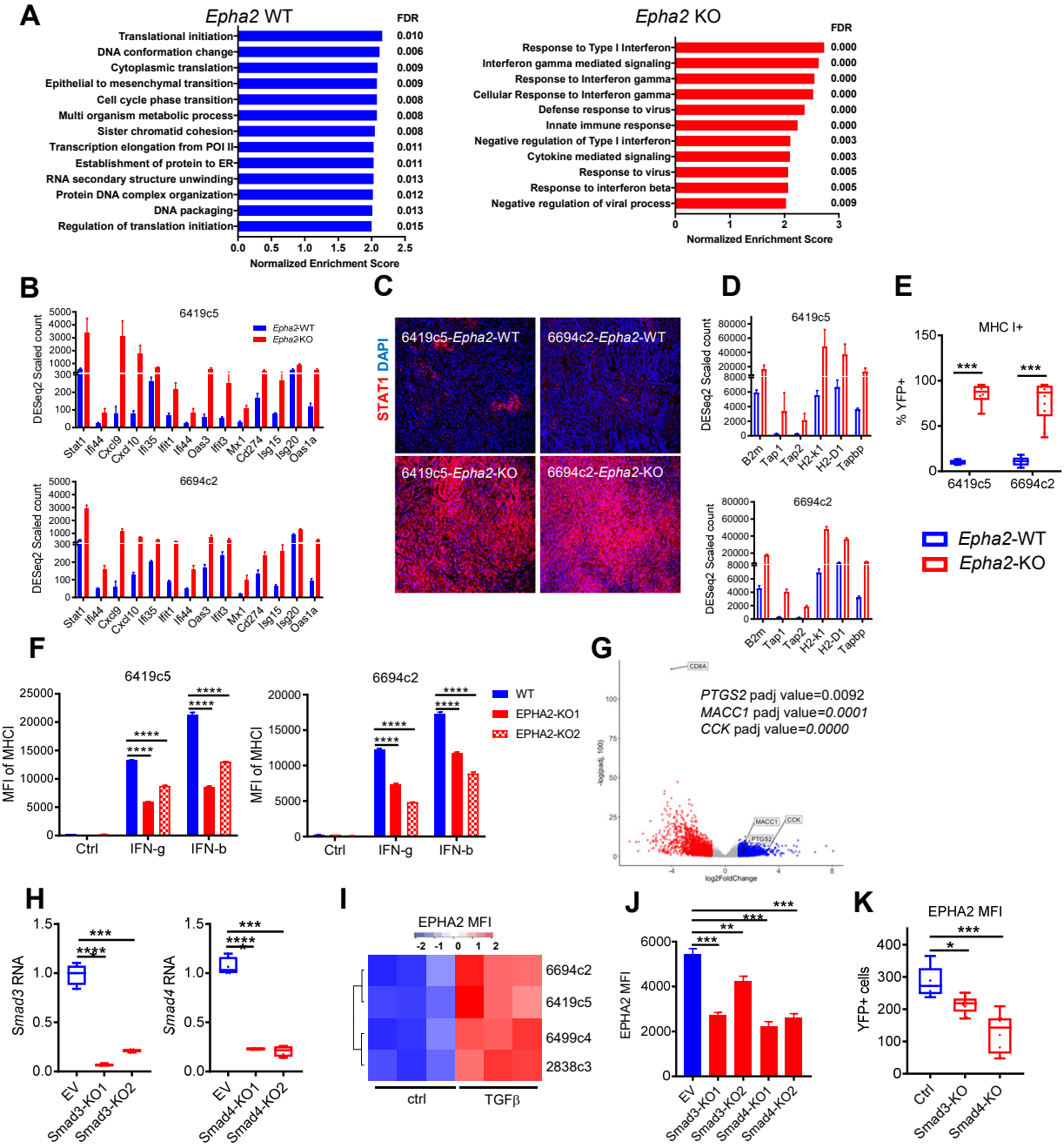

Figure S3. TGFβ-SMAD3, 4-PTGS2 axis as potential tumor cell-intrinsic regulator of TME (Related to Figure 4). (**A**) Gene set enrichment analysis of differentially expressed genes in *Epha2-WT* or *Epha2*-KO tumor cells using the GO biological processes gene sets (n=3-8/group). (**B**) Interferon responsive genes in tumor cells from implanted tumors (n=3-8/group). (**C**) Representative IF images of STAT1 staining (red) in tumors (n=3 /group). (**D**) Antigen presentation genes on implanted tumor cells (n=3-8/group). (**E**) Proportions of MHC I+ YFP+ tumor cells in implanted tumors (n=5/group). (**F**) MHC I protein expression on YFP+ tumor cells *in vitro* after 24 hours with either no treatment (ctrl) or IFNγ, or IFNβ treatments (n=3/group). (**G**) Differentially expressed genes identified in human T cell high (red) and T cell low (blue) PDA tumors (top and bottom 20% of *CD8A* expression). Genes with padj<0.05 and log2foldChange>2 shown. (**H**) Relative expression of *Smad3 and Smad4* in control (EV) and KO tumor cell clones generated from 6419c5. Data from n=4 independent experiments. (**I**) EPHA2 protein expression in PDA tumor cell clones treated with either PBS or TGFβ for 72 hours. Data from n=3 independent experiments. Color key represents the normalized Z score. (**J**) EPHA2 protein expression on *Smad3* and *Smad4* KO cell lines *in vitro* (n=3/group). (**K**) EPHA2 protein expression in YFP+ cells from control and *Smad3* and *Smad4* KO tumors (n=5-9/group). In (E, H, K) data presented as boxplots with horizontal lines and error bars indicating mean and range, respectively. In (B, D, F and J), data presented as mean with error bars indicating SEM. Statistical difference between two groups calculated by Students' unpaired t-test (E), between multiple groups determined by one-way ANOVA with Tukey's HSD post-test (F, H, J, K).

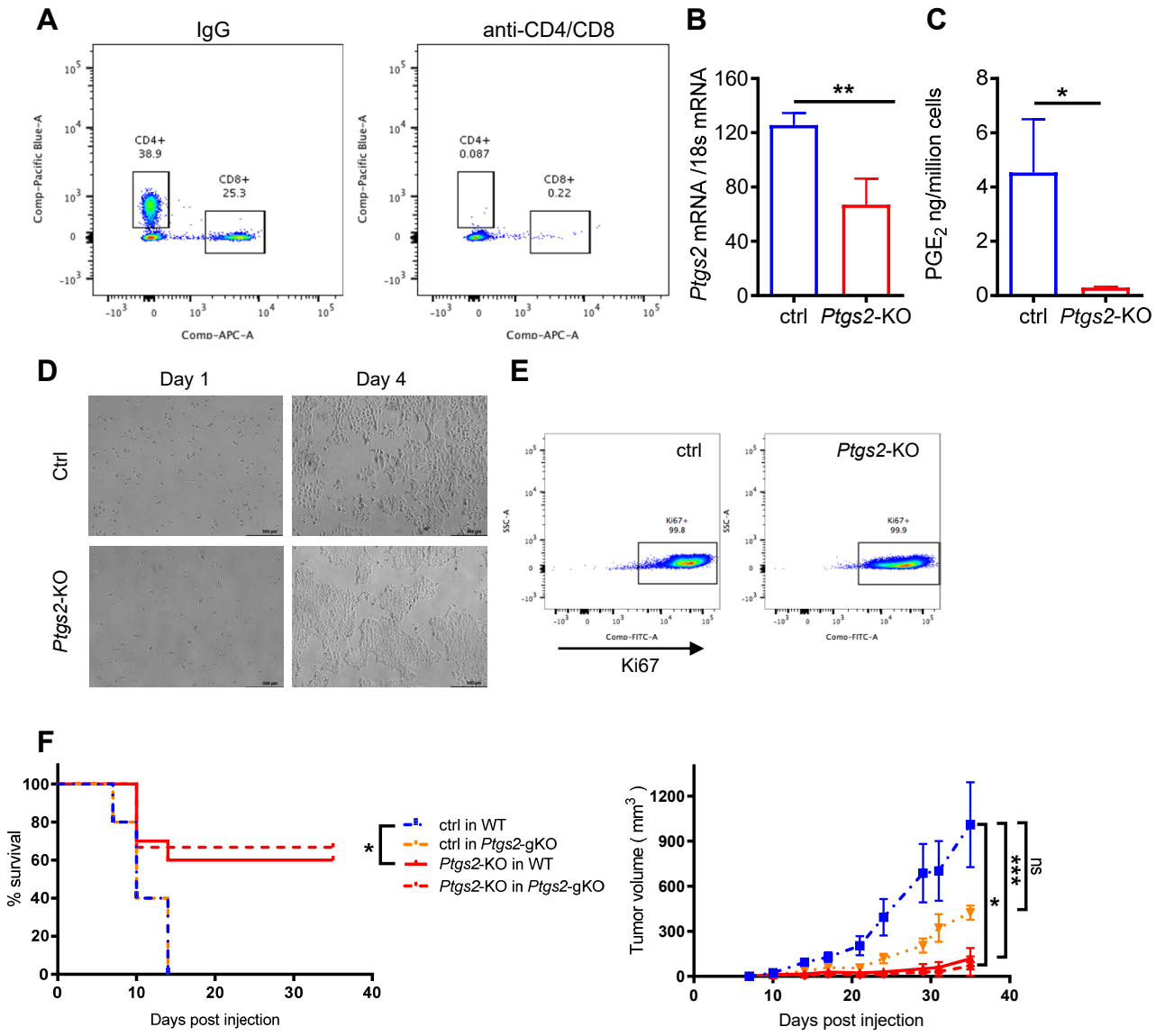

Figure S4. Effect of *Ptgs2* deletion on PDA cells in vitro and in vivo (Related to Figure 5). (**A**) T cell depletion check by flow cytometry on day 35 post tumor implantation. (**B-C**) Relative *Ptgs2* mRNA expression (B) and extracellular PGE2 levels (C) measured in control (ctrl, Cas9, no gRNA, transduced) and *Ptgs2*-KO (Cas9/ *Ptges2* gRNA transduced) KPC cell line conditioned media (n=3/group). (**D**) Control (ctrl) and *Ptgs2*-KO KPC cells plated 50000 cells/well on 6 well plates. Phase contrast 4X images taken on day 1 and day 4 in culture (representative image of 3/group). (**E**) Ki67+ control (ctrl) and *Ptgs2*-KO cells, shown as percent of live cells; flow cytometry performed on day 3 in culture. (**F**) Control (ctrl) and *Ptgs2*-KO KPC cell lines injected in WT and global *Ptgs2* knockout (*Ptgs2*-gKO) mice (n=5-10/group); tumor free survival (left) and tumor growth (right). In (B-C) data presented as mean with error bars indicating SEM. Statistical differences calculated using Students' unpaired t-test (B-C) and linear mixed-effects model with Tukey's HSD post-test using the lme4 and survival package in R (F).

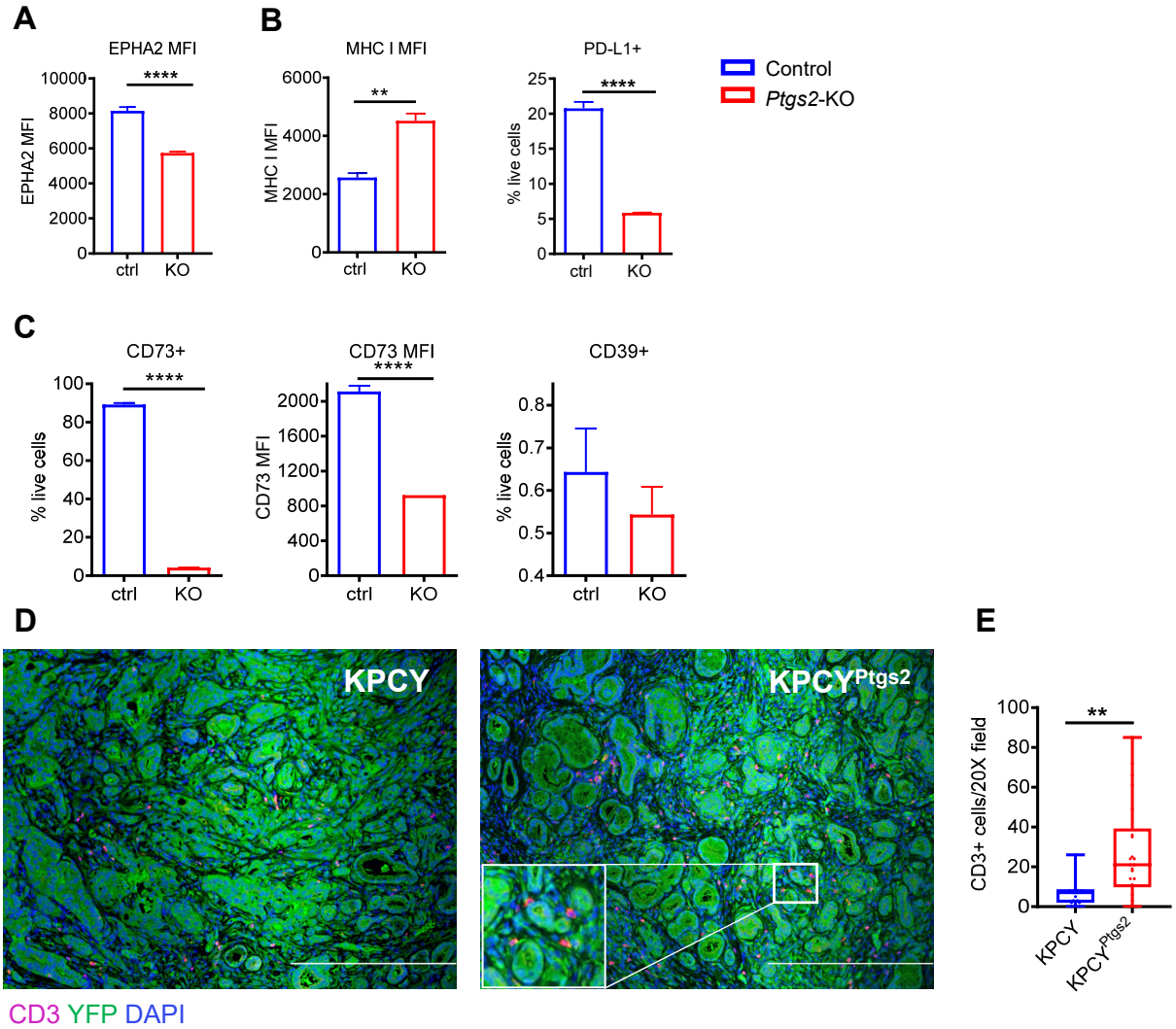

Figure S5. Effect of *Ptgs2* deletion on tumor cells and TME in vitro and in vivo (Related to Figure 6). (**A-C**) Flow cytometric analysis of control (ctrl) and *Ptgs2*-KO tumor cells after 48 hours in culture (representative experiment of 2, n=3/group). (**D-E**) Representative 20X images (**D**) and quantification (**E**) of CD3+ cells (red) in autochthonous tumors from KPCY and KPCY^{Ptgs2} mice (n=20-25 fields/group). YFP+ cells and nuclei stained in green and blue, respectively. Small square in (D) represents the cropped 20X area shown in the inset (big square). Data presented as mean with error bars indicating SEM (A-C) and boxplots with horizontal lines and error bars indicating mean and range, respectively (E). Statistical differences calculated using Students' unpaired t-test in GraphPad Prism.

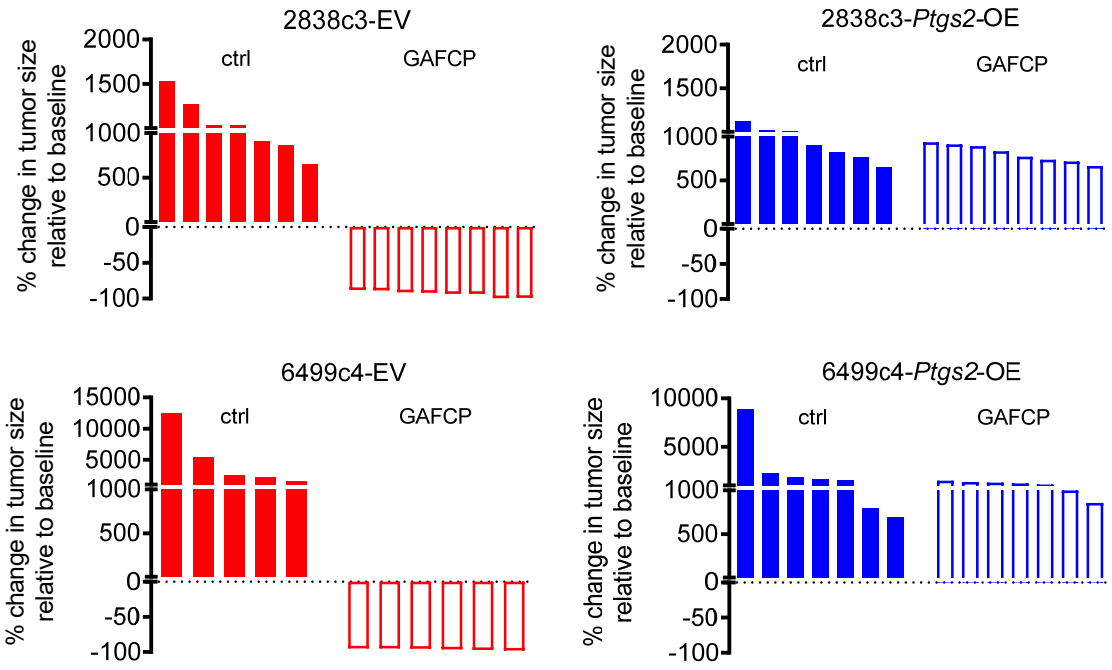

Figure S6. Tumor cell-intrinsic *Ptgs2* promotes resistance to immunotherapy in PDA (Related to Figure 7). Tumor size change relative to the baseline in parental (EV) and *Ptgs2*-OE T cell high tumors with or without the GAFCP treatment. Tumor cells implanted subcutaneously into C57BL/6 mice (n=5-8/group). GAFCP treatment started 9 days post implantation at 3-5 mm tumor diameter.

Supplemental Table 1. Experimental Models: Tumor Cells and Mouse Strains

Tumor Cells	Source
PENN 6419c5	Generated/B.Z. Stanger
PENN 6694c2	Generated/B.Z. Stanger
PENN 2838c3	Generated/B.Z. Stanger
PENN 6499c4	Generated/B.Z. Stanger
PENN 4662	Generated/R.H. Vonderheide
Mouse Strains	**Source**
$Kras^{LSL\text{-}G12D/+}$;$Trp53^{LSL\text{-}R172H/+}$;$Pdx1\text{-}Cre^{+/-}$; $Rosa26^{YFP/YFP}$	Generated
$Kras^{LSL\text{-}G12D/+}$;$Trp53^{LSL\text{-}R172H/+}$;$Pdx1\text{-}Cre^{+/-}$; $Rosa26^{YFP/YFP}$; $Ptgs2^{flox/flox}$	Generated/R.H. Vonderheide
Tamoxifen-inducible $Cre^{+/-}$ $Ptgs2^{flox/flox}$	Generated/G.A. FitzGerald

Supplemental Table 2. Antibodies for Flow Cytometry

Target and clone	Source	Identifier
CD335 (NKp46) FITC (29A1.4)	Biolegend	137606
CD279 (PD-1) FITC (29F.1A12)	Biolegend	135214
FOXP3 PE (MF23)	BD	560408
FOXP3 APC (FJK-16s)	eBioscience	17-5773-82
CD8a PE-CF594 (53-6.7)	BD	562283
CD8a APC-C7 (53-6.7)	BD Pharmingen	557654
CD103 PE/Dazzle 594 (2E7)	Biolegend	121430
CD103 PE-Cy7 (2E7)	Biolegend	121426
CD223 (Lag-3) PE/Dazzle 594 (C9B7W)	Biolegend	125224
CD3e PE/Cy5 (145-2C11)	Biolegend	100310
CD11c PE/Cy5 (N418)	Biolegend	117316
CD45 AF700 (30-F11)	Biolegend	103128
CD8a PE/Cy7 (53-6.7)	Biolegend	100722
I-A/I-E (MHCII) PE/Cy7 (M5/114.15.2)	Biolegend	107630
CD44 V450 (IM7)	BD	560451
Ly-6G V450 (1A8)	BD	560603
H-2Kb/H-2Db (MHCI) AF647 (28-8-6)	Biolegend	114612
Granzyme B AF647 (GB11)	Biolegend	515406
F4/80 APC/Cy7 (BM8)	Biolegend	123118
CD279 (PD-1) APC/Cy7 (29F.1A12)	Biolegend	135224
Ki67 AF700 (B56)	BD	561227
CD11b PerCP-Cy5.5 (M1/70)	BD	550993
CD11b APC (M1/70)	BD	553312
CD366 (Tim-3) PerCP-Cy5.5 (RMT3-23)	Biolegend	119718
Nur77 PerCP-eFluor710 (12.14)	eBioscience	46-5965-82
CD19 BV605 (6D5)	Biolegend	115540

Supplemental Table 3. Antibodies for Flow Cytometry

Target and clone	Source	Identifier
CD11c BV605 (N418)	Biolegend	117334
CD152 (CTLA-4) BV605 (UC10-4B9)	Biolegend	106323
Ly-6C BV570 (HK1.4)	Biolegend	128030
CD45 BV570 (30-F11)	Biolegend	103136
CD4 BV650 (RM4-5)	Biolegend	100546
CD8a BV785 (53-6.7)	Biolegend	100750
CD90.1 BV650 (OX-7)	Biolegend	202533
CD45.1 APC-Cy7 (A20)	Biolegend	110715
Arginase1 PE (A1exF5)	eBioscience	12-3697-82
IDO AF647 (2E2/IDO1)	Biolegend	654004
iNOS AF488 (CXNFT)	eBioscience	53-5920-82
IL-10 FITC (JES5-16E3)	Biolegend	505006
PD-L1 PE-Cy7 (10F.9G2)	Biolegend	124314
PD-1 PE-Cy7 (RMPI-30)	Biolegend	109110
IL-12 PECy7 (C15.6)	Biolegend	505210
G-CSF eFluor660 (9B4CSF)	ThermoFisher Scientific	50-7353-82
GM-CSF PE (MP1-22E9)	Biolegend	505406
NK1.1 APC Cy7 (PK136)	Biolegend	108724
XCR1 FITC (ZET)	Biolegend	148210
CD39 PE-Cy7 (Duha 59)	Biolegend	143806
CD73 BV605 (Ty/11.8)	Biolegend	127215
GR1 PE (RB6-8C5)	Biolegend	108408

Supplemental Table 4. Antibodies for Flow Cytometry

Target and clone	Source	Identifier
SIRPa APC-Cy7 (P84)	Biolegend	144014
CCR7 PE-Cy5 (4B12)	Biolegend	120114
CD31 APC-Cy7 (ER-MP12)	Abcore	AC16-0061-05
RORgT FITC (4G419)	Abcam	Ab104906
Caspase 3 activated FITC (A7R34)	BD	550480
CD86 BV785 (GL-1)	Biolegend	105043
GATA3 PE-Cy7 (L50-823)	BD	560405
T-bet Pacific Blue (4B10)	Biolegend	644808
B220 BV711 (RA3-6B2)	BD	563892
CD64 PE-CF594 (145-2C11)	BD	562286
Live/Dead Fixable Aqua Dead Cell stain kit	ThermoFisher Scientific	L34957

Supplemental Table 5. Gating Strategies

Gating Strategies	
Tumor cells	Live CD45- CD31-
Th1	Live CD45+ CD3+ CD4+ T-bet+
Th2	Live CD45+ CD3+ CD4+ GATA3+
Treg	Live CD45+ CD3+ CD4+ FOXP3+
Th17	Live CD45+ CD3+ CD4+ RORgt+
Macrophages	Live CD45+ F4/80+ CD11b+
cDC	Live CD45+ F4/80- lineage- MHC II+ CD11c+
cDC1	Live CD45+ F4/80- lineage- MHC II+ CD11c+ xCR1+
cDC2	Live CD45+ F4/80- lineage- MHC II+ CD11c+ SIRPa+
MDSC	Live CD45+ CD11b+ Gr1+
Neutrophils	Live CD45+ F4/80- Gr1+
Myeloid cells	Live CD45+ CD11b+ MHCII- or Live CD45+ CD11b+
gMDSC	Live CD45+ CD11b+ MHCII- Ly6G+ Ly6C+

Supplemental Table 6. Antibodies for Immunofluorescent and Immunohistochemistry Stainings

Target and antibody dilution	Source	Identifier
CD3 Rabbit 1:50	Abcam	ab5690
GFP Goat 1:500	Abcam	ab6673
COX-2 rabbit monoclonal antibody 1:100	ThermoFisher Scientific	RM-9121-R7
αSMA 1:100	Abcam	ab5694
PDGFRβ 1:100	Abcam	ab32570