Supplemental Figures

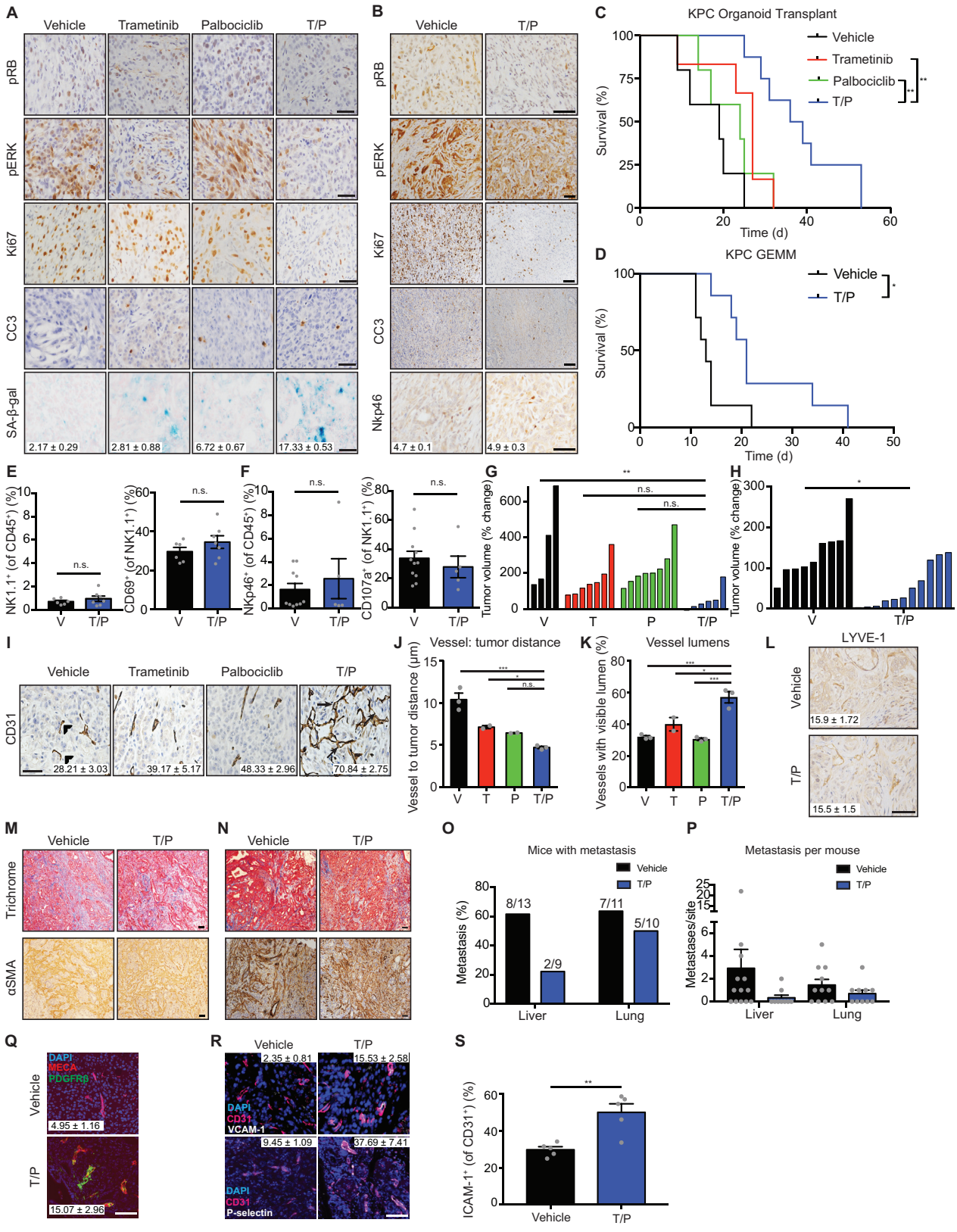

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Figure S1. Trametinib and Palbociclib Treatment Does Not Induce NK Cell-Mediated Tumor Responses but Rather Leads to Vascular Remodeling in PDAC Models, Related to Figure 1

(A) Immunohistochemical (IHC) staining and quantification of SA-β-gal positive area in KPC^{mut} organoid transplant tumors treated with vehicle (V), trametinib (T) (1 mg/kg), palbociclib (P) (100 mg/kg), or both (T/P) for 2 weeks (n = 3; T versus T/P and P versus T/P, $p < 0.0001$).

(B) IHC staining and quantification of $NKp46^{+}$ NK cells in KPC^{flox} GEMM tumors treated as in (A) (n = 2; $p = 0.592$).

(C and D) Kaplan-Meier survival curves of KPC^{mut} organoid transplant (C) and KPC^{flox} GEMM (D) mice treated as in (A) ($n \geq 5$). Values for V and T/P-treated cohorts in (D) are the same displayed in Figure 3F.

(E and F) Flow cytometry analysis of NK cell numbers and activation in KPC^{mut} transplant (E) or KPC^{flox} GEMM (F) tumors following treatment as in (A) ($n \geq 5$).

(G and H) Waterfall plots of the response of KPC^{mut} transplant (G) or KPC^{flox} GEMM (H) tumors following treatment as in (A) ($n \geq 4$). Values for V and T/P-treated cohorts in (H) are the same displayed in Figure 3E.

(I–K) IHC staining of KPC^{mut} organoid transplant tumors treated as in (A). Quantification of blood vessels per field (T versus T/P and P versus T/P, $p < 0.01$) (I), vessel to tumor distance (J), and vessels with visible lumens (K) is shown (n = 3). Arrowhead, collapsed vessel; Arrow, visible lumen.

(L) IHC staining of KPC^{flox} GEMM tumors following treatment as in (A) (n = 3; $p = 0.88$).

(M and N) IHC staining of KPC^{flox} GEMM (M) or KPC^{mut} organoid transplant (N) tumors following treatment as in (A).

(O and P) PDAC-bearing KPC^{flox} GEMM mice were treated with vehicle or trametinib (1 mg/kg) and palbociclib (100 mg/kg) until survival endpoint, and the lungs and liver were subsequently analyzed for the presence (O) and number (P) of macrometastases ($n \geq 9$).

(Q and R) Immunofluorescence (IF) staining and quantification of PDGFRβ (Q) and P-selectin and VCAM-1 (R) colocalization with blood vessels in KPC^{mut} organoid transplant tumors treated as in (A) (n = 2-3; $p = 0.0322$, 0.0644, and 0.0396, respectively).

(S) Flow cytometry analysis of ICAM-1 expression on endothelial cells in KPC^{mut} organoid transplant tumors following treatment as in (A) (n = 5).

One-way ANOVA (A, G, and I–K). Log-rank test (C and D). Error bars, mean ± SEM. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. n.s., not significant. Scale bars, 50 μm.

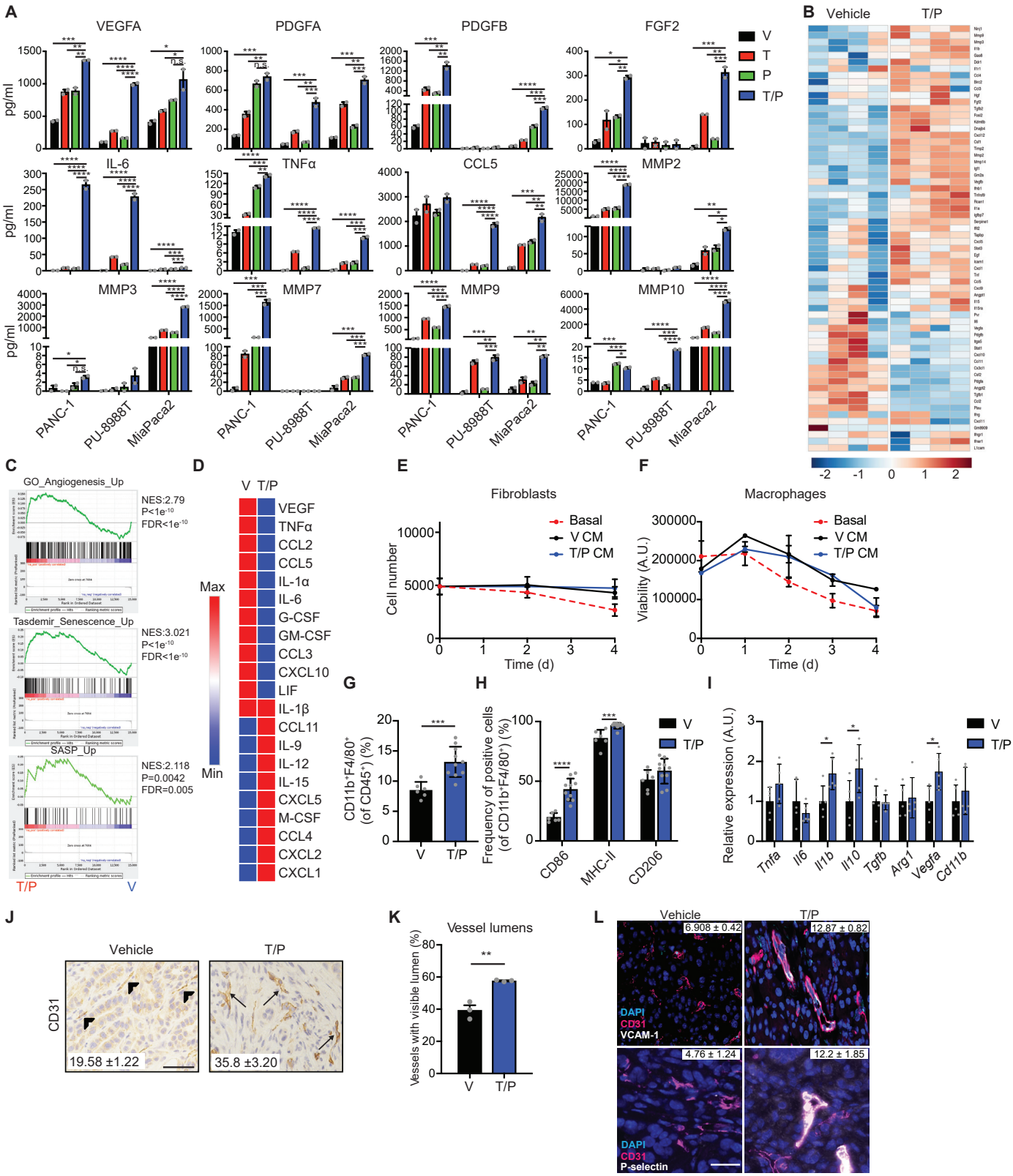

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Figure S2. Therapy-Induced Senescence Triggers a Pro-angiogenic SASP Exclusively in PDAC Tumor Cells, Related to Figure 2

(A) Cytokine array results from human KRAS mutant PDAC lines treated for 8 days with trametinib (25 nM) and/or palbociclib (500 nM) (n = 2).

(B) Heatmap of RNA-seq analysis of SASP gene expression in tumors cells sorted from *KPC^{mut}* cell transplant mice treated for 2 weeks with vehicle or trametinib (1 mg/kg) and palbociclib (P) (100 mg/kg) (n = 4).

(C) Gene Set Enrichment Analysis (GSEA) of RNA-seq data from (B). NES, normalized enrichment score.

(D) Heatmap of cytokine array results from primary murine pancreatic fibroblasts following treatment as in (A). Data presented as mean of three biological replicates.

(E and F) Cell growth analysis of fibroblasts (E) or macrophages (F) harvested from C57BL/6 mice and cultured in basal or conditioned media from *KPC^{mut}* tumor cells treated for 8 days as in (A) (n = 3).

(G and H) Flow cytometry analysis of F4/80^{+} macrophage numbers and polarization in *KPC^{mut}* cell transplant tumors following 2-week treatment with vehicle or trametinib (1 mg/kg) and palbociclib (100 mg/kg) (n = 5).

(I) qRT-PCR analysis of M1 (*Tnfa, Il6, Il1b*), M2 (*Il10, Tgfb, Arg1*), and *Vegfa* gene expression in macrophages sorted from *KPC^{mut}* cell transplant tumors treated as in (G) (n = 5). Cd11b was used as a macrophage loading control. A.U., arbitrary units.

(J and K) IHC staining and quantification of total blood vessels (p = 0.009) (J) and those with visible lumens (K) in tumors harvested from *KPC^{mut}* organoids transplanted into NOD-*scid* IL2Rγ^{null} (NSG) mice treated as in (G) (n = 3).

(L) IF staining and quantification of P-selectin (p = 0.039) or VCAM-1 (p = 0.0126) colocalization with blood vessels in tumors from (J) (n = 4).

One-way ANOVA (A). Error bars, mean ± SEM. ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05. n.s., not significant. Scale bars, 50 μm.

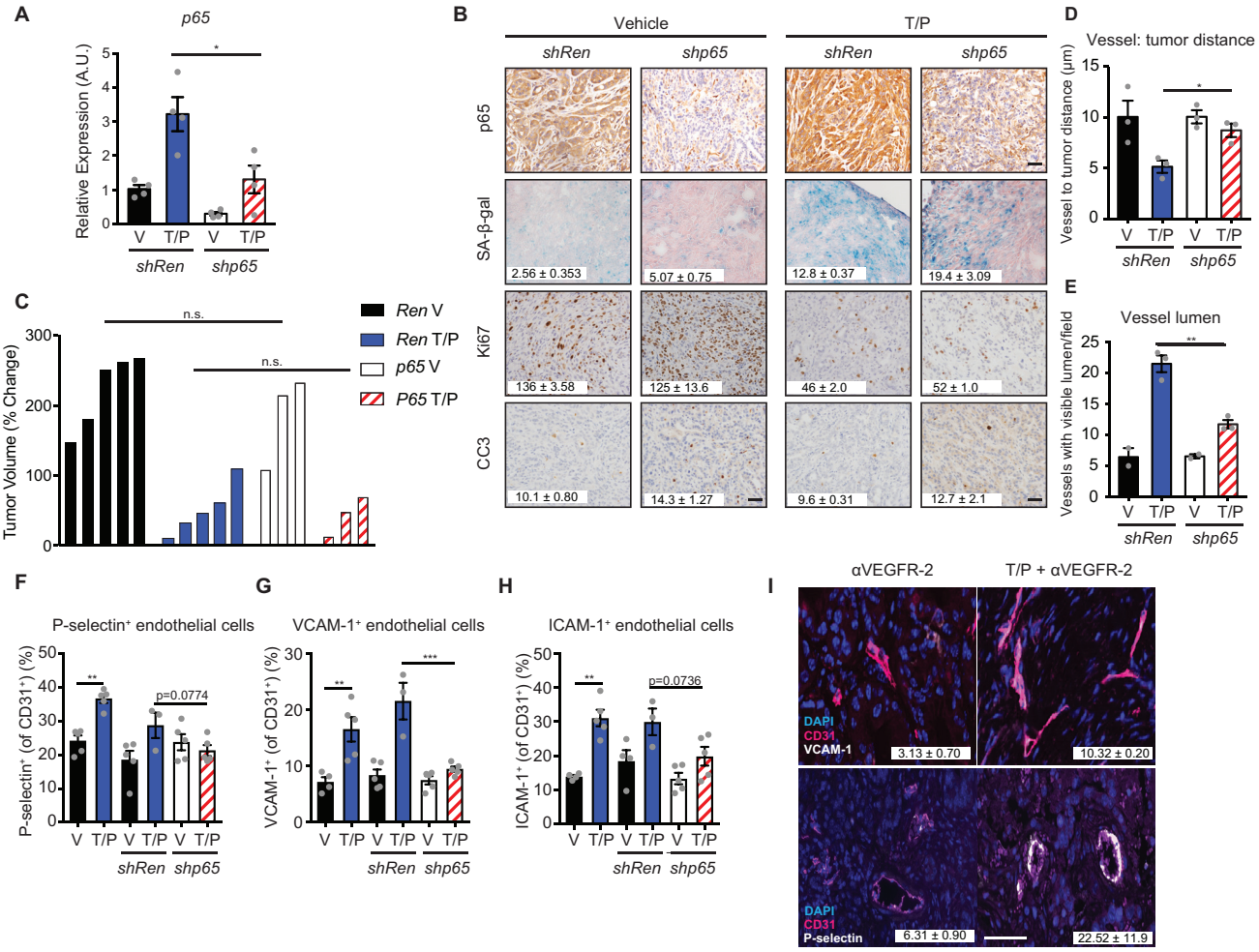

Figure S3. Multiple SASP Factors Contribute to Divergent Vascular Remodeling Phenotypes following T/P Treatment, Related to Figure 2

(A) qRT-PCR analysis of *p65* gene expression in *KPC*^{mut} cells harboring *Renilla* (*Ren*) or *p65* shRNAs and treated for 8 days with vehicle or trametinib (25nM) and palbociclib (500nM). A.U., arbitrary units.

(B) IHC staining of *KPC*^{mut} organoid transplant tumors harboring *Ren* or *p65* shRNAs and treated for 2 weeks with vehicle or trametinib (1 mg/kg) and palbociclib (100 mg/kg) (n = 2-3).

(C) Waterfall plot of the response of *KPC*^{mut} organoid transplant tumors harboring *Ren* or *p65* shRNAs following treatment as in (B) ($n \geq 3$).

(D and E) Quantification of vessel to tumor distance (D) and vessels with visible lumens per field (E) in *KPC*^{mut} organoid transplant tumors harboring *Ren* or *p65* shRNAs and treated as in (B) (see Figure 2E) (n = 2-3).

(F–H) Flow cytometry analysis of surface marker expression on endothelial cells in indicated *KPC*^{mut} cell transplant tumors treated as in (B) (n = 3-5).

(I) IF staining and quantification of P-selectin (p = 0.17) or VCAM-1 (p = 0.01) colocalization with blood vessels in *KPC*^{mut} organoid transplant tumors treated for 2 weeks with vehicle, trametinib (1 mg/kg), palbociclib (100 mg/kg), and/or a VEGFR-2 blocking antibody (DC101; 800 μg) (n = 4).

One-way ANOVA (C–H). Error bars, mean ± SEM. ***p < 0.001, **p < 0.01, *p < 0.05. n.s., not significant. Scale bars, 50 μm.

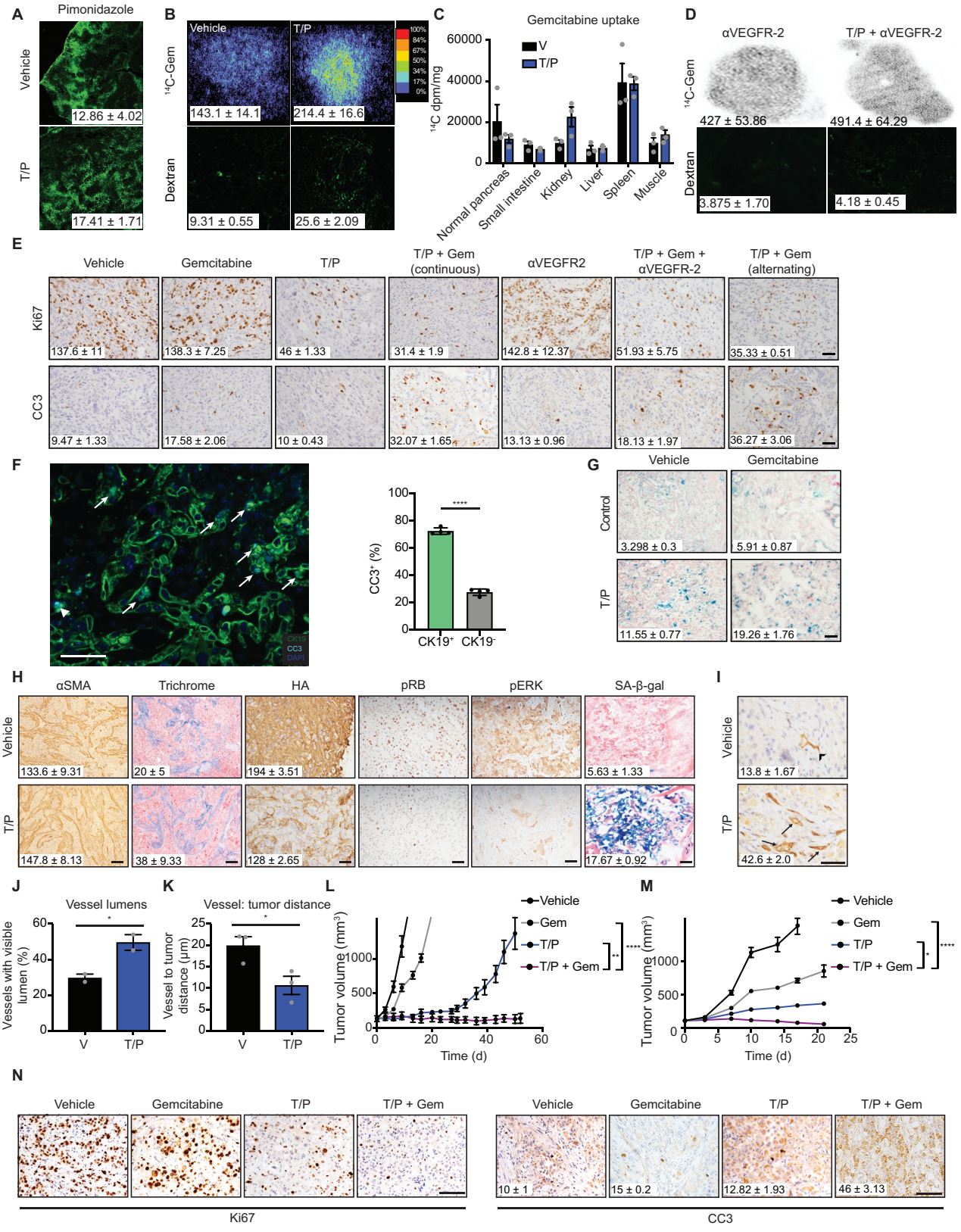

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Figure S4. Trametinib and Palbociclib Treatment Enhances Gemcitabine Cytotoxicity in Both Mouse and Human PDAC via Vascular Remodeling, Related to Figure 3

(A) IF staining and quantification of activated pimonidazole in *KPC^{mut}* organoid transplant tumors treated with vehicle or trametinib (1 mg/kg) and palbociclib (100 mg/kg) for 2 weeks (n = 2; p = 0.41).

(B) *KPC^{flox}* GEMM mice were pretreated with vehicle or trametinib (1 mg/kg) and palbociclib (100 mg/kg) for 2 weeks, and subsequently injected with ^{14}C-labeled gemcitabine (Gem) and dextran prior to tissue harvest. Heatmap representation of autoradiograph showing distribution and quantification of ^{14}C-Gem uptake into tumors (n = 3; p = 0.03). Below: dextran staining and quantification (n = 3-4; p = 0.0003).

(C) Quantification of ^{14}C-Gem levels in indicated tissues harvested from *KPC^{mut}* organoid transplant mice following treatment as in (B) (n = 3).

(D) *KPC^{mut}* organoid transplant mice were pretreated with vehicle, trametinib (1 mg/kg), palbociclib (100 mg/kg), and/or a VEGFR-2 blocking antibody (DC101; 800 μg) for 2 weeks, and subsequently injected with ^{14}C-labeled gemcitabine and dextran prior to tissue harvest. Autoradiograph showing distribution and quantification of ^{14}C-Gem uptake into tumors (n = 2-3; p = 0.485). Below: dextran staining and quantification (n = 2-3; p = 0.844).

(E) IHC staining of *KPC^{mut}* organoid transplant tumors treated for 2 weeks with vehicle, trametinib (1 mg/kg), palbociclib (100 mg/kg), gemcitabine (100 mg/kg), and/or a VEGFR-2 blocking antibody (DC101; 800 μg) either continuously (C) or on an alternating (A) schedule. Quantification of Ki67$^+$ (Gem versus T/P/G (C), p = 0.0244; Gem versus T/P/G (A), p = 0.0029; T/P/G (C) versus T/P/G/DC101, p = 0.0727) and cleaved caspase-3 (CC3$^+$) cells (Gem versus T/P/G (C), p = 0.0054; Gem versus T/P/G (A), p = 0.0072; T/P/G (C) versus T/P/G/DC101, p = 0.0056) per field is shown (n = 3).

(F) IF staining of a *KPC^{flox}* GEMM tumors treated for 2 weeks with trametinib (1 mg/kg), palbociclib (100 mg/kg), and gemcitabine (100 mg/kg). Right: quantification of the percentage of CC3$^+$ cells in the epithelial (CK19$^+$) versus stromal (CK19$^-$) compartments (n = 4).

(G) Staining and quantification of SA-β-gal positive area in *KPC^{mut}* organoid transplant tumors treated for 2 weeks with vehicle, trametinib (1 mg/kg), palbociclib (100 mg/kg), and/or gemcitabine (100 mg/kg) (n = 3; T/P versus T/P/G, p = 0.0183).

(H) IHC staining of PR-07 patient-derived xenograft (PDX) PDAC tumors treated as in (A). Quantification of αSMA (p = 0.3140), collagen (trichrome) (p = 0.2312), and hyaluronic acid (HA) (p = 0.0001) staining intensity, as well as SA-β-gal positive area (p = 0.0017), is shown (n = 3).

(I–K) IHC staining and quantification of blood vessels per field (p = 0.0004) (I), vessel to tumor distance (J), and vessels with visible lumens (K) in PR-07 PDX PDAC tumors treated as in (A) (n = 2-3). Arrowhead, collapsed vessel; Arrow, visible lumen.

(L and M) Tumor volumes of mice bearing PR-07 (L) or PR-05 (M) PDAC PDX tumors treated with vehicle, trametinib (1 mg/kg), palbociclib (100 mg/kg), and/or gemcitabine (100 mg/kg) for indicated times (n = 7).

(N) IHC staining and quantification of CC3$^+$ cells in PR-07 PDAC PDX tumors treated as in (L) (n = 3; T/P versus T/P/G, p = 0.0046).

One-way ANOVA (E and G). Two-way ANOVA (L and M). Error bars, mean ± SEM. ****p < 0.0001, **p < 0.01, *p < 0.05. Scale bars, 50 μm.

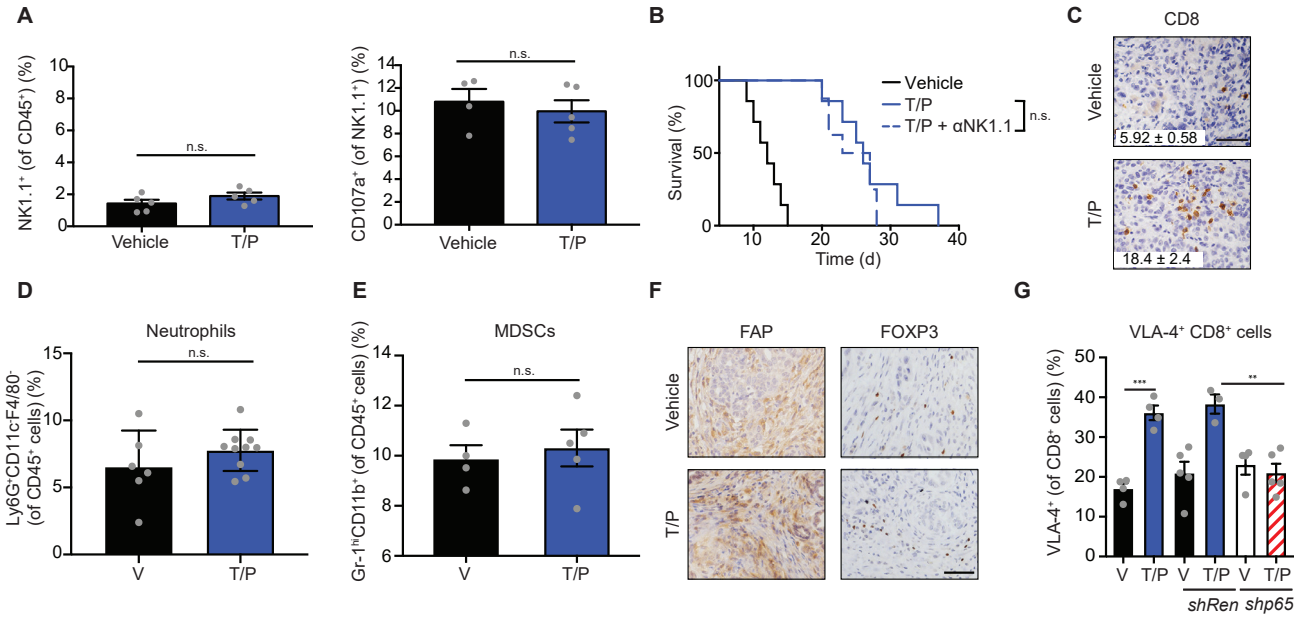

Figure S5. Therapy-Induced Senescence Directly Promotes CD8$^+$ T Cell Accumulation in PDAC, Related to Figure 4

(A) Flow cytometry analysis of NK cell numbers (left) and activity (right) in *KPC^{mut}* cell transplant tumors following 2-week treatment with vehicle or trametinib (1 mg/kg) and palbociclib (100 mg/kg) (n = 4-5).

(B) Kaplan-Meier survival curve of *KPC^{mut}* cell transplant mice treated with vehicle or trametinib (1 mg/kg) and palbociclib (100 mg/kg) in the presence or absence of a NK1.1 depleting antibody (PK136; 250 ug) (n $\geq$ 7).

(C) IHC staining and quantification of CD8$^+$ T cells per field in *KPC^{mut}* organoid transplant tumors treated as in (A) (n = 2-3; p = 0.037).

(D and E) Flow cytometry analysis of neutrophils (D) and MDSCs (E) in *KPC^{mut}* cell transplant tumors treated as in (A) (n = 4-5).

(F) IHC staining of *KPC^{mut}* organoid transplant tumors treated as in (A). Representative images of 3 biological replicates.

(G) Flow cytometry analysis of VLA-4 expression on CD8$^+$ T cells in indicated *KPC^{mut}* cell transplant tumors treated as in (A) (n = 3-5).

One-way ANOVA (G). Log-rank test (B). Error bars, mean ± SEM. ***p < 0.001, **p < 0.01. n.s., not significant. Scale bars, 50 μm.

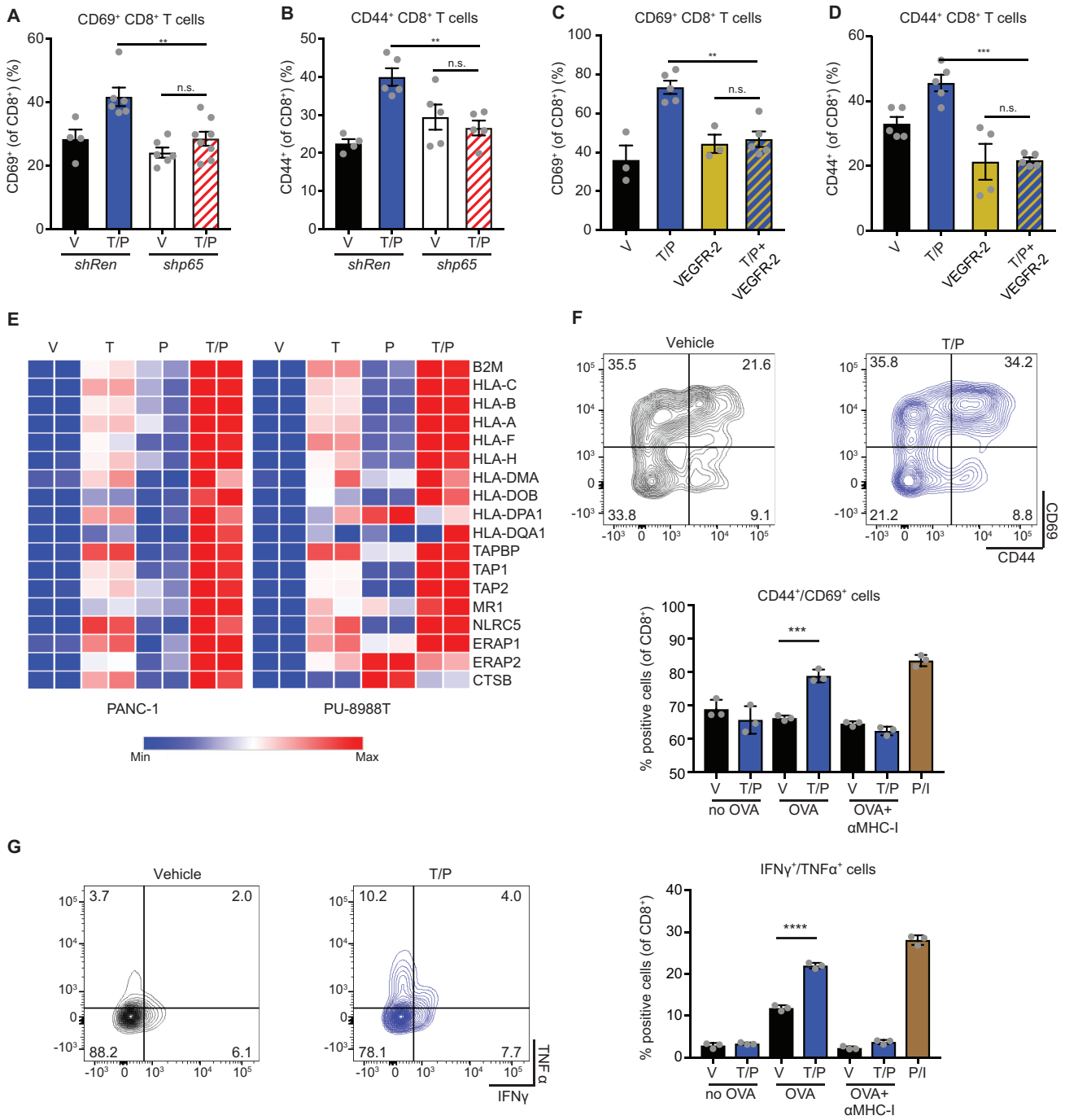

Figure S6. Therapy-Induced Senescence Stimulates CD8+ T Cell Activation, Related to Figure 5

(A and B) Flow cytometry analysis of CD8+ T cells in *KPC^{mut}* PDAC cell transplant tumors harboring control *Ren* or *p65*-targeting shRNAs and treated with vehicle or trametinib (1 mg/kg) and palbociclib (100 mg/kg) for 2 weeks (n = 4-5 mice).

(C and D) Flow cytometry analysis of CD8+ T cells in *KPC^{mut}* cell transplant tumors treated with vehicle, trametinib (1 mg/kg), palbociclib (100 mg/kg), and/or a VEGFR-2 blocking antibody (DC101; 800 μg) for 2 weeks (n = 3-5).

(E) Heatmap of expression of genes related to antigen processing and presentation in human KRAS mutant PDAC lines following 8 day treatment with trametinib (25 nM) and/or palbociclb (500 nM) as assessed from analysis of RNA-seq data generated in Ruscetti et al. (2018). Two biological replicates per cell line are shown.

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(F and G) Reprentative histograms and quantification of T cell activation (F) and effector function (G) markers following flow cytometry analysis of OTI $CD8^+$ T cells co-cultured with control or chicken ovalbumin (OVA)-expressing KPC^{mut} tumor cells pretreated with vehicle or trametinib (25 nM) and palbociclb (500 nM) for 8 days. PMA/ionomycin (P/I) stimulation served as a positive control for T cell activation, and MHC-I activity was functionally blocked with an H-$2k^b$ blocking antibody (Y-3; 40 μg/ml) (n = 3).

One-way ANOVA (A–D, F, and G). Error bars, mean ± SEM. ****p < 0.0001, ***p < 0.001, **p < 0.01. n.s., not significant.

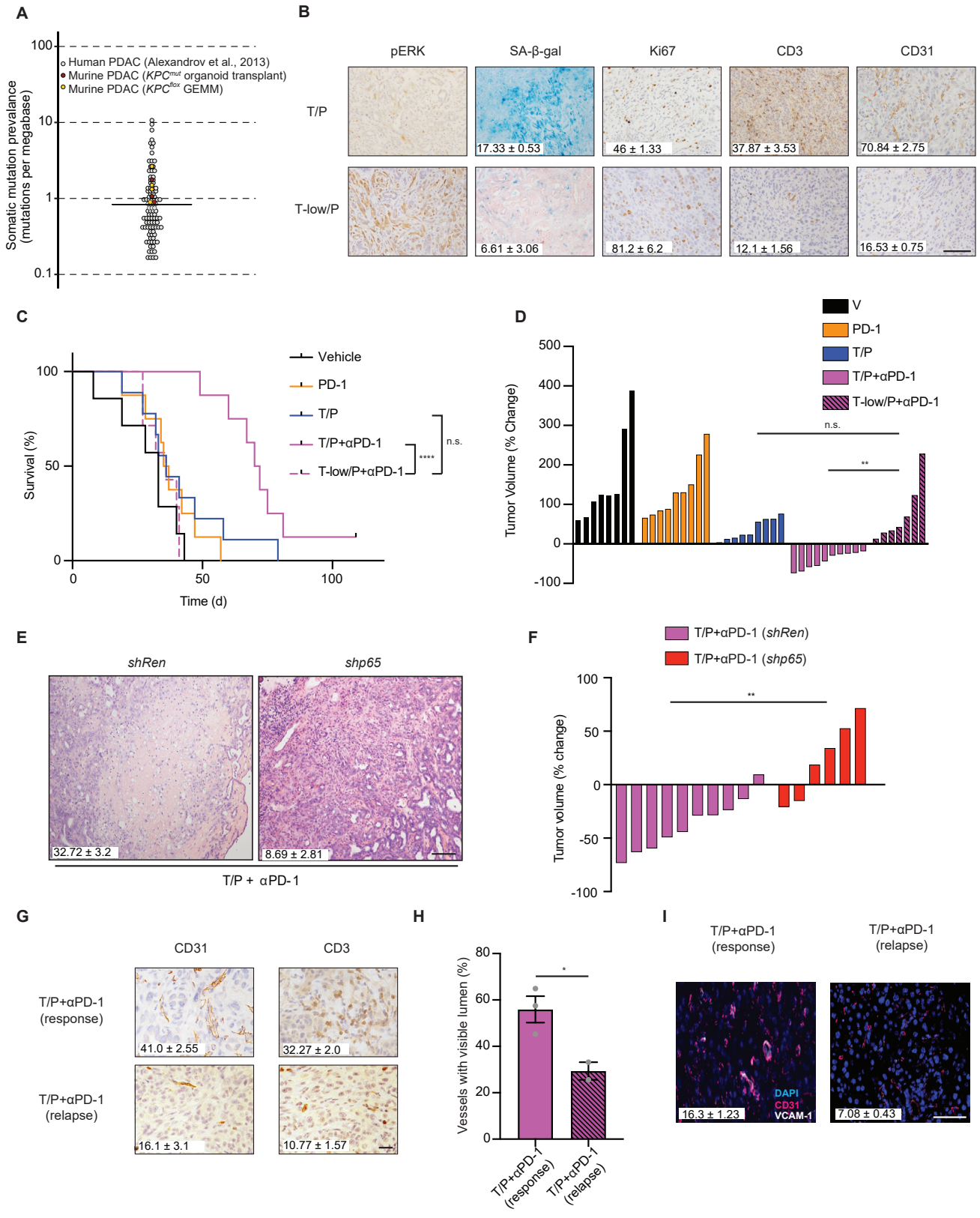

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Figure S7. Synergy between T/P Treatment and PD-1 Checkpoint Blockade Is Dependent on SASP-Mediated Vascular Remodeling, Related to Figure 7

(A) Quantification of number of somatic mutations (per megabase) in KPC^{flox} GEMM (yellow dots; n = 4) and KPC^{mut} organoid transplant tumors (red dots; n = 3) as compared to human PDAC tumors (gray dots) from Alexandrov et al. (2013).

(B) IHC staining and quantification of SA-β-gal$^+$ (p = 0.0028), Ki67$^+$ (p = 0.031), CD3$^+$ (p = 0.0026), and CD31$^+$ (p < 0.0001) cells per field in KPC^{mut} organoid transplant tumors treated with palbociclib (100 mg/kg) in combination with standard (1 mg/kg) and low dose (T-low) (0.3 mg/kg) trametinib (n = 2-3). Values for SA-β-gal, CD31, and Ki67 in the T/P-treated cohort are the same displayed in Figures S1A, S1I, and S4E.

(C) Kaplan-Meier survival curve of KPC^{mut} PDAC organoid transplant mice treated with vehicle, trametinib (1 or 0.3 (T-low) mg/kg), palbociclib (100 mg/kg), and/or a PD-1 blocking antibody (RMP1-14; 200 μg) ($n \geq 7$). Values for V, T/P, PD-1, and T/P/PD-1-treated cohorts are the same displayed in Figure 7F.

(D) Waterfall plot of the response of KPC^{mut} PDAC organoid transplant tumors after 2 weeks of treatment as in (C) ($n \geq 7$). Values for V, T/P, PD-1, and T/P/PD-1-treated cohorts are the same displayed in Figure 7D.

(E and F) KPC^{mut} organoid transplant tumors harboring *Ren* or *p65* shRNAs were treated for 2 weeks with trametinib (1 mg/kg), palbociclib (100 mg/kg), and a PD-1 blocking antibody (RMP1-14; 200 μg). (E) H&E staining and quantification of percent of tumor covered in necrosis (n = 2-3; p = 0.03). (F) Waterfall plot of the response of individual tumors to treatment ($n \geq 6$).

(G–I) IHC and IF staining of KPC^{mut} organoid transplant tumors following acute 2 week treatment (response) or > 80 days of treatment (relapse) with trametinib (1 mg/kg), palbociclib (100 mg/kg), and a PD-1 blocking antibody (RMP1-14; 200 μg). (G) Quantification of CD31$^+$ (p = 0.0085) and CD3$^+$ (p = 0.0048) cells per field (n = 2-3). (H) Quantification of blood vessels with visible lumens (n = 2-3). (I) Quantification of VCAM-1 (p = 0.0195) colocalization with blood vessels (n = 4). One-way ANOVA (D). Log-rank test (C). Error bars, mean ± SEM. ****p < 0.0001, **p < 0.01, *p < 0.05. n.s., not significant. Scale bars, 50 μm.