SUPPLEMENTAL MATERIAL

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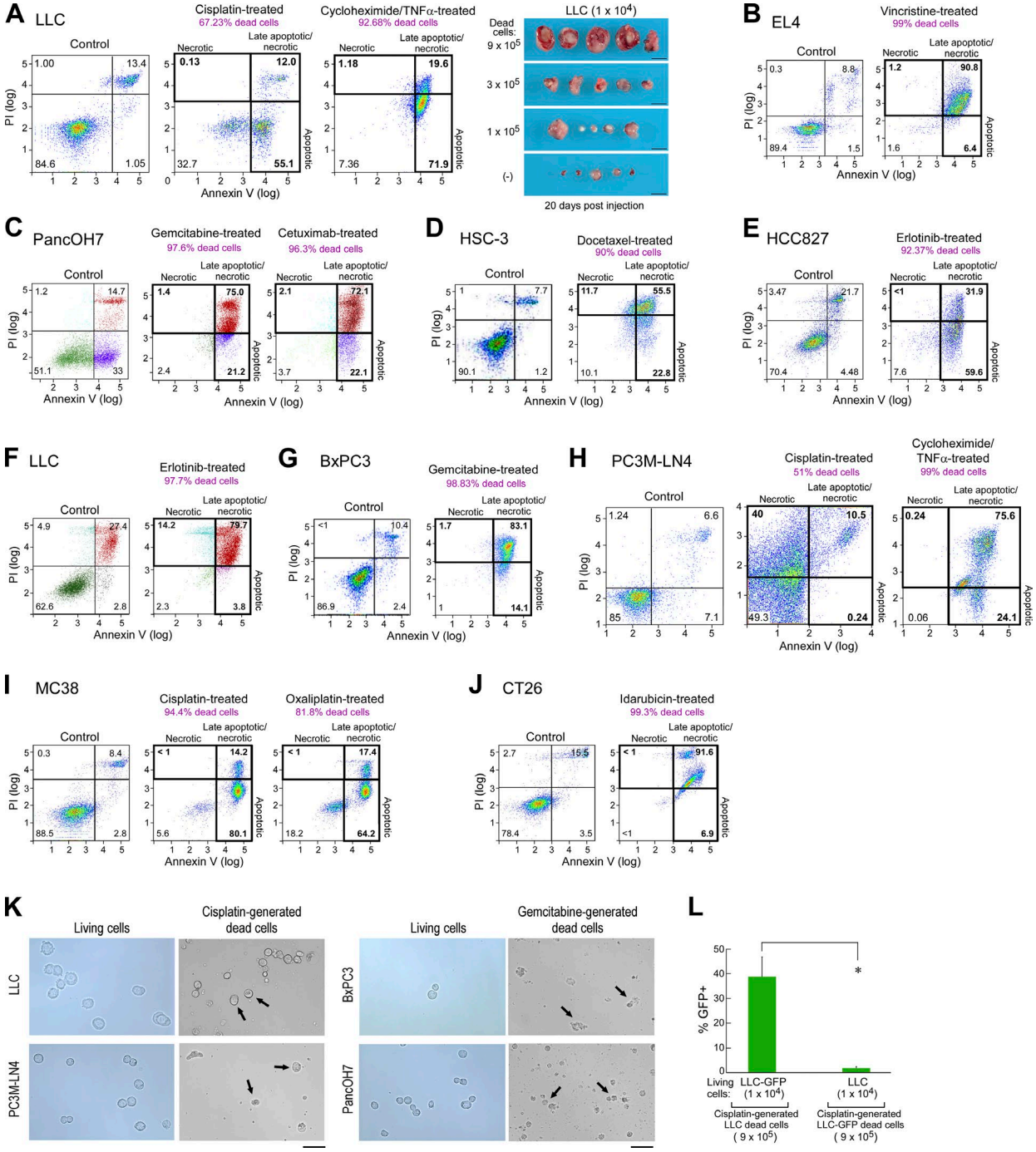

Figure S1. **Characterization of therapy-generated tumor cell debris by annexin V/PI flow cytometry analysis. (A)** Flow cytometry analysis of apoptotic (annexin V^+PI^-; bottom right quadrant), necrotic (annexin V^-PI^+; top left quadrant), and late apoptotic/necrotic (annexin V^+PI^+; top right quadrant) cell debris from in vitro cultures of cisplatin- or cycloheximide plus TNFα–treated LLC; images on the right show representative debris-stimulated LLC tumors at 20 d after injection. Bars, 1 cm. **(B–J)** Same as in A, but in vincristine-treated EL4 (B); gemcitabine- or cetuximab-treated PancOH7 (C), docetaxel-treated HSC-3 (D), erlotinib-treated HCC827 (E), LLC (F), gemcitabine-treated BxPC3 (G), cisplatin- or cycloheximide plus TNFα–treated PC3M-LN4 (H), cisplatin- or oxaliplatin-treated MC38 (I), and idarubicin-treated CT26 (J). **(K)** Representative images of dead cell bodies (black arrows) in cultures of cisplatin (LLC or PC3M-LN4)- and gemcitabine (BxPC3 or PancOH7)- generated debris. Bars, 100 µm. **(L)** Flow cytometry analysis (percent GFP^+) of debris-stimulated LLC-GFP tumors (left) versus debris-stimulated LLC tumors (right). $n = 5$ mice/group. *, $P < 0.05$. Error bars represent SEM.

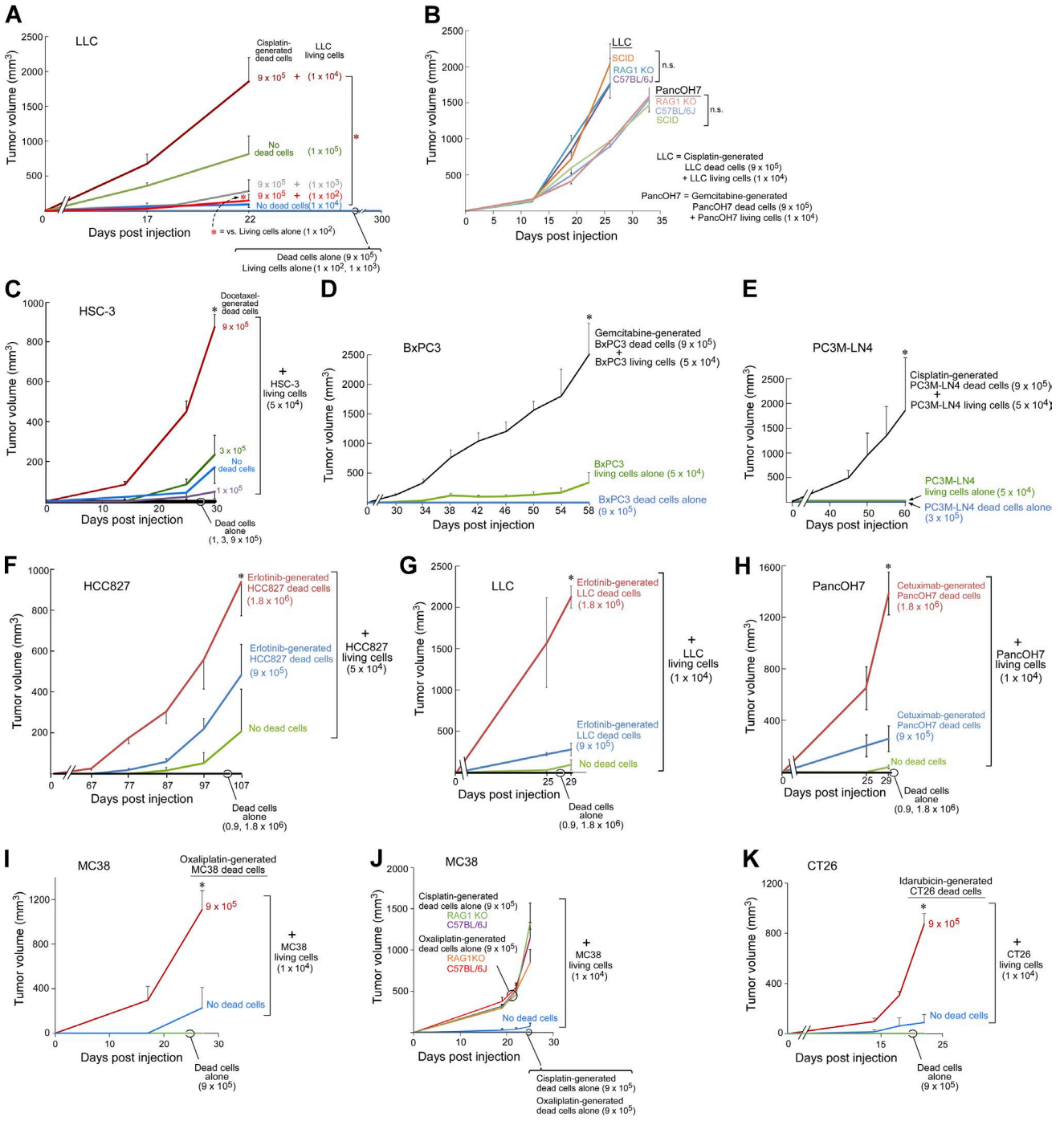

Figure S2. **Chemotherapy- and targeted therapy–generated tumor cell debris stimulates primary tumor growth. (A)** Cisplatin-generated LLC debris coinjected with a subthreshold inoculum of LLC living cells. *n* = 5–15 mice/group. Two-way repeated-measures mixed-effects ANOVAs for tumor growth rates and two-tailed Student's *t* test for final tumor measurements were used throughout unless specified. *, $P < 0.05$ versus living cells alone ("No dead cells;" blue line; red asterisk). **(B)** Chemotherapy-generated dead cells coinjected with a subthreshold inoculum of 10^4 LLC or PancOH7 living cells into C57BL/6J, RAG1 KO, or SCID mice. *n* = 10–15 mice/group. **(C–E)** Debris-stimulated HSC-3 (C), BxPC3 (D), or PC3M-LN4 (E) tumor growth from chemotherapy-generated debris coinjected with a subthreshold inoculum of 5×10^4 living cells. *n* = 5–15 mice/group. *, $P < 0.05$ versus "no dead cells" (blue) or living cells alone (green). **(F–H)** Debris-stimulated HCC827 (F), LLC (G), or PancOH7 (H) from targeted therapy–generated dead cells coinjected with a subthreshold inoculum of living cells (5×10^4 or 10^4 cells). *n* = 5–8 mice/group. *, $P < 0.05$ versus no dead cells (green). **(I–K)** Debris-stimulated MC38 (I and J) or CT26 (K) from oxaliplatin-, cisplatin-, or idarubicin-generated dead cells coinjected with a subthreshold inoculum of living tumor cells (10^4 cells). *n* = 5 mice/group. *, $P < 0.05$ versus no dead cells (blue). Error bars represent SEM.

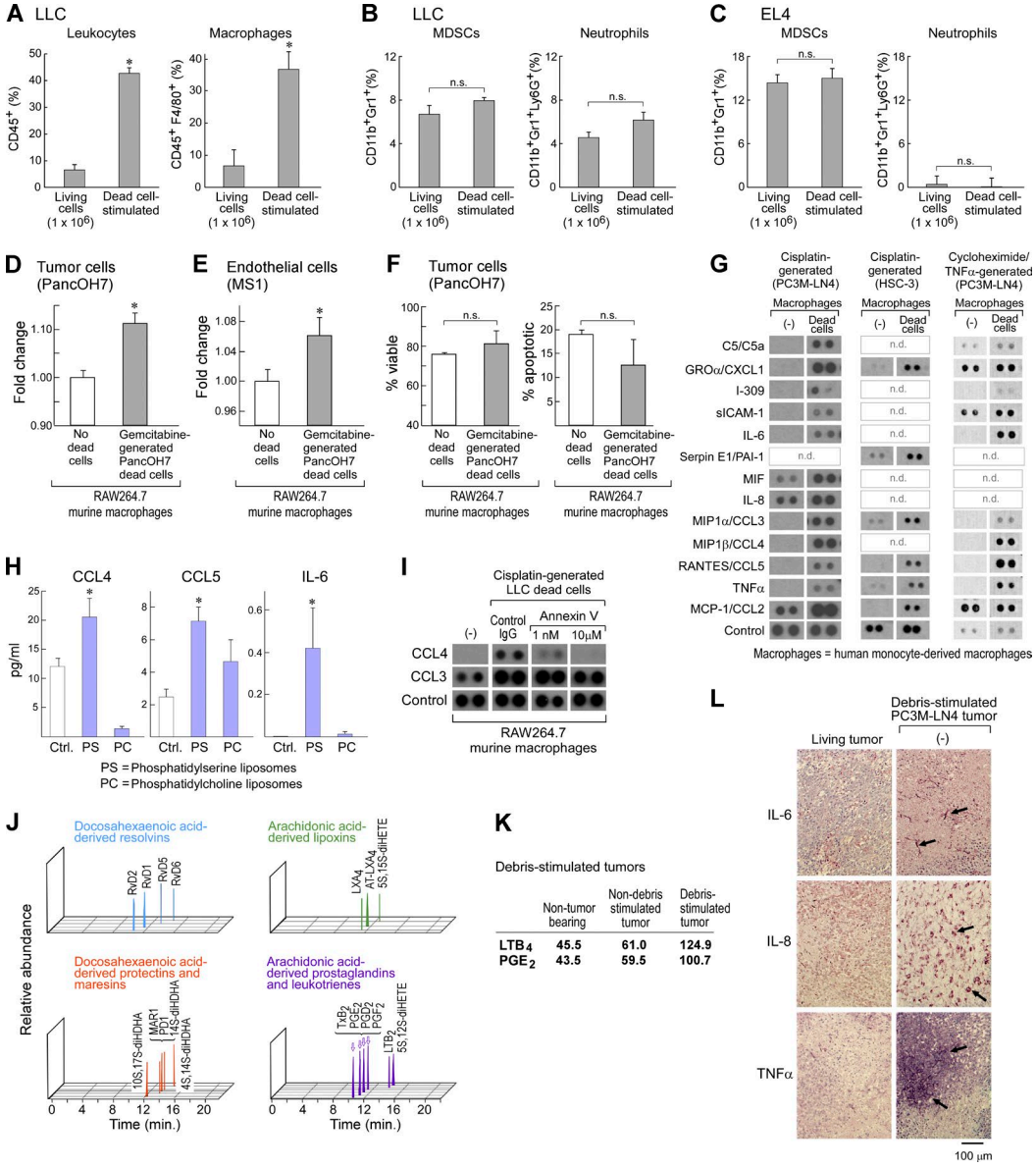

Figure S3. **Apoptotic debris stimulates primary tumor growth via macrophage secretion of proinflammatory cytokines and lipid autacoid mediators. (A)** Flow cytometry analysis of nondebris (10^6 living cells/mouse; left) or debris-stimulated (9×10^5 cisplatin-generated LLC dead cells coinjected with 10^4 LLC living cells/mouse; right) LLC tumors for leukocytes ($CD45^+$) and macrophages ($CD45^+F4/80^+$). *, $P < 0.05$ versus control (10^6 living cells). On the left is total leukocyte (% $CD45^+$) population (including % $CD45^+F4/80^+$ cells), and on the right is macrophage population (% $CD45^+F4/80^+$ cells only). $n = 8$ mice/group. **(B and C)** Flow cytometry analysis of nondebris (10^6 living cells/mouse; left) or debris-stimulated (9×10^5 cisplatin-generated LLC or vincristine-generated EL4 dead cells coinjected with 10^4 LLC or EL4 living cells/mouse, respectively; right) tumors for myeloid-derived suppressor cells (MDSCs; $CD11b^+Gr1^+$) and neutrophils ($CD11b^+Gr1^+Ly6G^+$). $n = 5$–10 mice/group. **(D–F)** PancOH7 tumor cell proliferation ($n = 12$/group), viability ($n = 3$/group), apoptosis ($n = 3$/group), and MS1 endothelial cell proliferation ($n = 12$/group) from conditioned media of RAW264.7 macrophages cocultured for 24 h with tumor cell debris. *, $P < 0.05$ versus no dead cells (macrophages) alone. Data are representative of two biological repeats. **(G)** Cytokine/chemokine profile of conditioned media from human monocyte-derived macrophages exposed to cisplatin-generated (PC3M-LN4 or HSC-3) or cycloheximide plus TNFα–generated (PC3M-LN4) dead cells compared with macrophages alone. Cytokines with no difference in control versus dead cell exposure are labeled n.d. **(H)** ELISA quantification of CCL4, CCL5, and IL-6 in mouse macrophage (RAW264.7)-conditioned media after incubation with PS or PC liposomes (100 μM). $n = 4$/group ($n = 3$ biological repeats). *, $P < 0.05$ versus macrophages alone. **(I)** Cytokine/chemokine profile of mouse macrophage (RAW264.7)-conditioned media after incubation with annexin V or control IgG–treated cisplatin-generated LLC debris compared with macrophages alone. **(J and K)** LC-MS-MS profiling of plasma from nontumor-bearing mice, mice with nondebris-stimulated tumors (10^6 LLC living cells/mouse), and mice with debris-stimulated tumors (9×10^5 cisplatin-generated LLC dead cells coinjected with 10^4 living cells/mouse); numbers represent pg/2 ml plasma. $n = 5$ mice/group. **(L)** Immunohistochemical analysis of inflammatory cytokines IL-6, IL-8, and TNFα in debris-stimulated PC3M-LN4 (3×10^5 cisplatin-generated dead cells coinjected with 5×10^4 living cells) tumors compared with nondebris tumors generated only from living tumor cells (5×10^4 living cells). Error bars represent SEM.

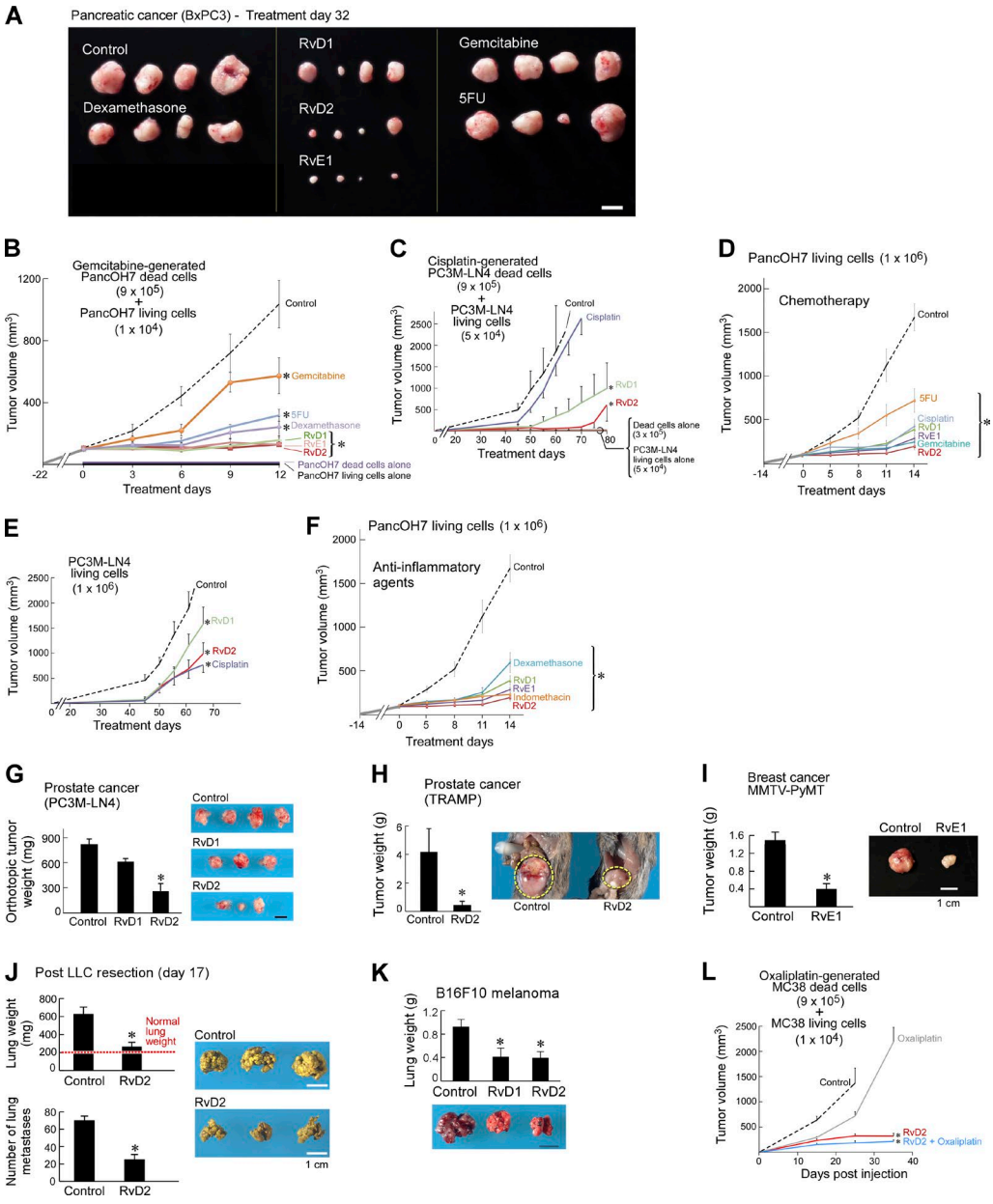

Figure S4. **Resolvins inhibit primary tumor growth in debris-stimulated and nondebris tumors including orthotopic and genetically engineered models. (A)** Debris-stimulated BxPC3 tumors (9×10^5 gemcitabine-generated dead cells + 5×10^4 living cells) after 32 d of RvD1, RvD2, RvE1, dexamethasone, gemcitabine, 5-FU, or control treatment. Bar, 1 cm. **(B, D, and F)** Debris (B) or nondebris-stimulated PancOH7 tumor growth (D and F) with systemic administration of resolvins (RvD1, RvD2, or RvE1), cisplatin, gemcitabine, 5-FU, dexamethasone, or indomethacin. Treatment was initiated once tumors reached 100–200 mm^3 throughout unless otherwise specified. $n = 5$ mice/group. Two-way repeated-measures mixed-effects ANOVAs for tumor growth rates assessed by the F test with two-tailed Student's t test for final tumor measurements were used throughout unless otherwise specified. **(C and E)** Debris-stimulated (C) or nondebris (E) PC3M-LN4 tumor growth with systemic resolvins (RvD1 or RvD2) or cisplatin. $n = 5$ mice/group. Treatment was initiated on the day of tumor cell injection. **(G)** Orthotopic (PC3M-LN4) tumor burden after treatment with RvD1 or RvD2. Treatment was initiated on the day of tumor cell injection. $n = 5$–7 mice/group. Images show representative tumors after 28 d of treatment. Bars, 1 cm. **(H)** Tumor burden in RvD2-treated TRAMP mice. Treatment was initiated when mice were 8 wk of age. Images show representative tumors after 56 d of treatment (dashed circles). $n = 5$–10 mice/group. **(I)** Tumor burden in RvE1-treated MMTV-PyMT mice. Treatment for 84 d initiated when mice were 8 wk of age. Images show representative tumors. **(J)** Lung weight (top) and number of metastases (bottom) of spontaneous LLC lung metastasis 17 d after primary tumor removal (LLC resection). Representative photographs on day 17 after LLC resection are shown. $n = 6$ mice/group. **(K)** RvD1 or RvD2 in melanoma metastasis (B16F10). Metastatic burden was measured as lung weight after RvD1 or RvD2 treatment for 21 d. The blue inset shows representative lung metastasis. Bar, 1 cm. $n = 5$ mice/group. **(L)** Oxaliplatin-stimulated MC38 tumor growth with systemic RvD2 and/or oxaliplatin. Treatment was initiated on the day of tumor cell injection. $n = 5$ mice/group. *, $P < 0.05$ versus control. Error bars represent SEM.

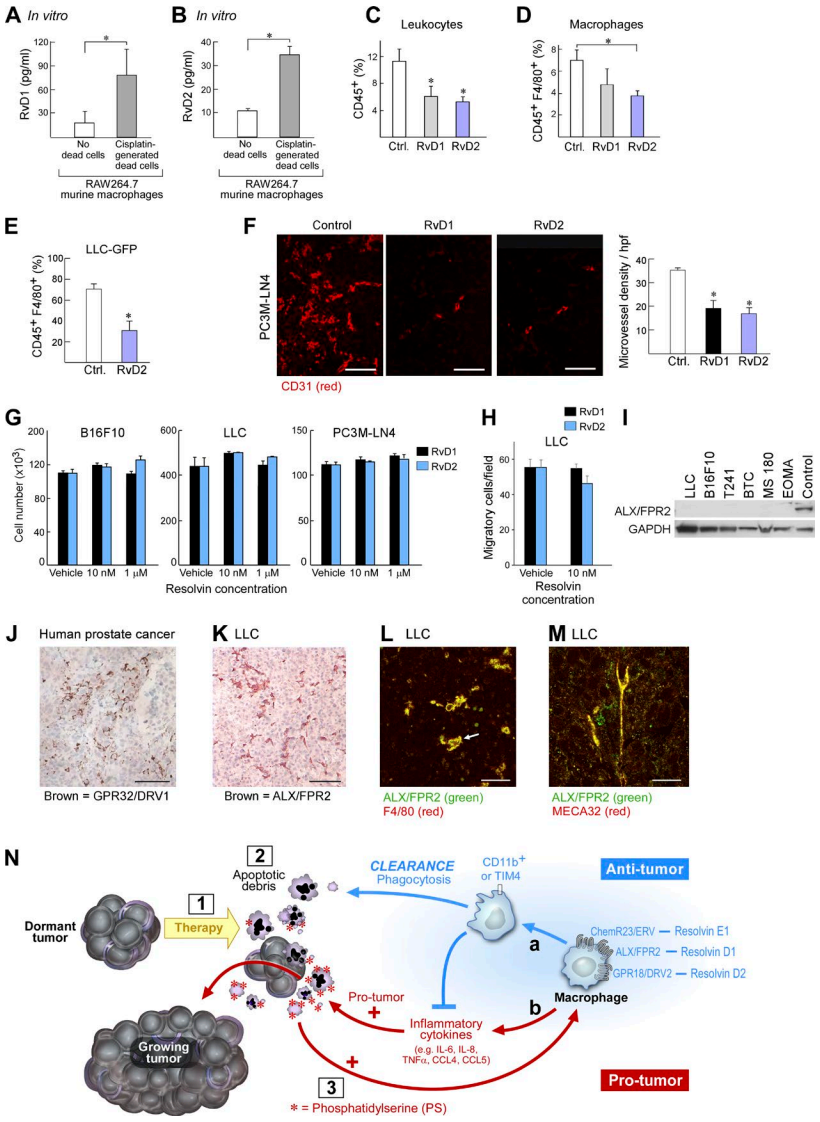

Figure S5. **Resolvins target the tumor stroma. (A and B)** RvD1 (A) and RvD2 (B) levels in mouse macrophage (RAW264.7)-conditioned media after coincubation with cisplatin-generated LLC debris. $n = 4$/group ($n = 2$ biological repeats). **(C and D)** Leukocyte (C) and macrophage (D) infiltration measured by flow cytometry in Matrigel plugs from mice treated systemically with RvD1 or RvD2 (15 ng/mouse/d); infiltrating leukocytes ($CD45^+$), or infiltrating macrophages ($CD45^+F4/80^+$). $n = 8$–10 Matrigel plugs/group. **(E)** Infiltrating macrophages ($CD45^+F4/80^+$) in RvD2-treated LLC-GFP tumors. $n = 3$ mice/group. **(F)** Immunofluorescent staining for $CD31^+$ vessels (red) in PC3M-LN4 tumors (10^6 living cells) from resolvin (RvD1 or RvD2; 15 ng/d)- or vehicle-treated mice (day 60 of treatment). Bars, 50 µm. Immunohistochemistry for $CD31^+$ blood vessels (confirmed via vessel structure morphology) in resolvin- or vehicle-treated nondebris PC3M-LN4 tumors (10^6 living cells; day 60 treatment). *, $P < 0.05$ versus control. **(G)** Proliferation of B16F10, LLC, or PC3M-LN4 tumors cells treated with RvD1 (black bars) or RvD2 (blue bars). $n = 4$–8/group, representative of three biological replicates. **(H)** Migration of LLC tumor cells treated with RvD1 (black bars) or RvD2 (blue bars). $n = 4$/group, representative of two biological replicates. **(I)** RvD1 receptor ALX/FPR2 expression in tumor cell lines in vitro; LLC, B16F10, T241 fibrosarcoma, BTC, MS-180 (sarcoma), and EOMA. Mouse spleen was used as control. **(J)** Human RvD1 receptor GPR32/DRV1 (DAB staining) expression in human clinical prostate cancer specimens. **(K)** Mouse RvD1 receptor ALX/FPR2 (DAB staining) expression in LLC tumor infiltrating stromal and inflammatory cells. Bars, 100 µm. **(L)** Immunofluorescent double staining for ALX/FPR2 (green) and macrophage marker F4/80 (red) in LLC tumors. Colocalization of fluorescence (yellow) indicates tumor macrophages expressing ALX/FPR2. **(M)** Immunofluorescent double staining for ALX/FPR2 (green) and the endothelial cell marker MECA-32 (red) in LLC tumors. Colocalization of fluorescence (yellow) indicates tumor endothelial cells in tumor blood vessels expressing ALX/FPR2. Bars, 10 µm. Error bars represent SEM. **(N)** Schematic of the role of resolvins in countering therapy-generated debris-stimulated dormant tumor growth. Current cancer therapy, such as chemotherapy or targeted therapy (1), generates apoptotic tumor cell debris that expresses PS (2). Macrophages in the tumor stroma then react to the PS "eat me" signal on the debris (3). If resolvins bind their receptor (ALX/FPR2, GPR18/DRV2, or ChemR23/ERV), macrophages will (a) stimulate phagocytosis ($CD11b^+$) or efferocytosis ($TIM4^+$) of debris and counterregulate proinflammatory cytokines. This pathway will ultimately clear the apoptotic signal that can stimulate tumor growth. However, if resolvins are not present, then macrophages will (b) release proinflammatory, protumorigenic cytokines, promoting tumor growth of the living tumor cell population.