

SUPPLEMENTAL MATERIAL

Daley et al., <https://doi.org/10.1084/jem.20161707>

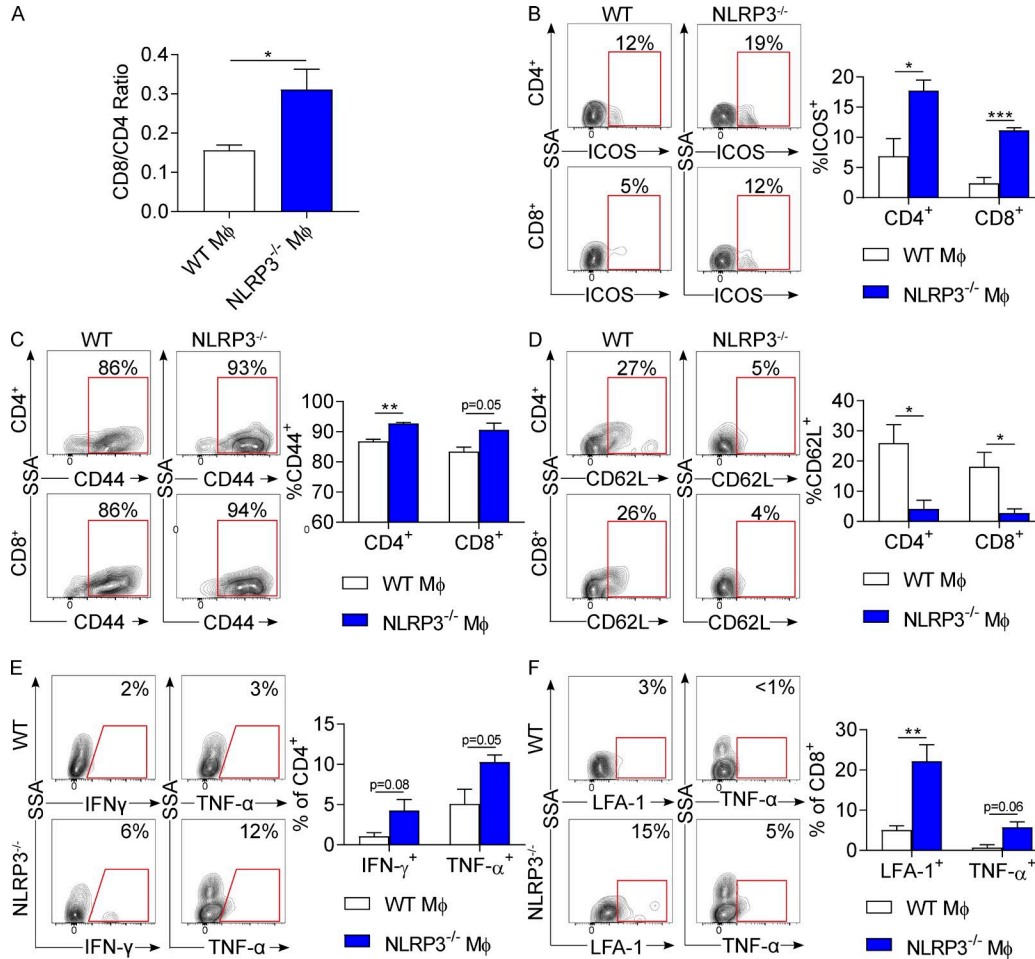


Figure S1. **Adoptive transfer of tumor-entrained NLRP3<sup>-/-</sup> macrophages enhances intratumoral T cell activation.** (A–F) NLRP3<sup>-/-</sup> mice were subcutaneously implanted with KPC-derived tumor cells admixed with PDA-entrained WT or NLRP3<sup>-/-</sup> macrophages. At 4 wk, tumors were harvested and analyzed by flow cytometry. (A) The CD8<sup>+</sup>/CD4<sup>+</sup> T cell ratio was calculated. (B–D) CD4<sup>+</sup> and CD8<sup>+</sup> T cells were analyzed for expression of inducible T cell co-stimulator (B), CD44 (C), and CD62L (D). (E) CD4<sup>+</sup> T cells were analyzed for expression of IFN- $\gamma$  and TNF. (F) CD8<sup>+</sup> T cells were analyzed for expression of LFA and TNF. This experiment was performed twice.  $n = 5$  mice/group. Unpaired Student's  $t$  test was used for statistical analyses. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . Data are presented as mean  $\pm$  standard error. ICOS, inducible T cell co-stimulator; M $\Phi$ , macrophage; SSA, side scatter.

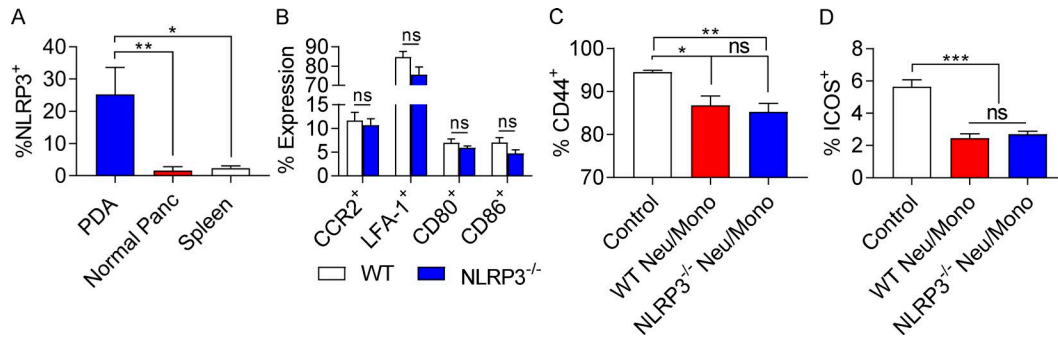


Figure S2. **Phenotype and T cell inhibitory effects of PDA-infiltrating neutrophils and inflammatory monocytes in the context of NLRP3 deletion.** (A) Gr1<sup>+</sup>CD11b<sup>+</sup> neutrophils and inflammatory monocytes from WT control pancreata (Panc) or pancreata and spleen of littermate WT mice harboring orthotopic KPC tumor were tested for expression of NLRP3 on day 21. (B) Gr1<sup>+</sup>CD11b<sup>+</sup> neutrophils (Neu) and inflammatory monocytes (Mono) from orthotopic KPC tumor in WT and NLRP3<sup>-/-</sup> hosts were tested for diverse surface marker expression. (C and D) Antigen-restricted CD4<sup>+</sup> OT-II T cells were stimulated with OVA<sub>323-339</sub> alone or in co-culture with Gr1<sup>+</sup>CD11b<sup>+</sup> cells harvested from orthotopic PDA tumors in WT or NLRP3<sup>-/-</sup> hosts. T cells activation at 96 h was determined by expression of CD44 (C) and inducible T cell co-stimulator (ICOS; D). Experiments were repeated twice and performed in quadruplicate. Unpaired Student's *t* test was used for statistical analyses. \*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001. Data are presented as mean ± standard error.

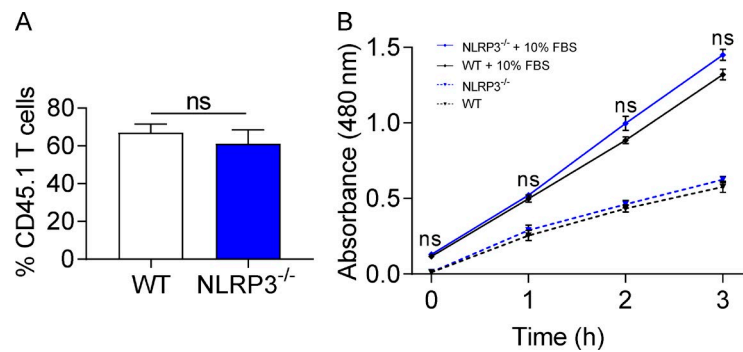


Figure S3. **T cells do not exhibit increased migration in PDA in NLRP3<sup>-/-</sup> hosts.** (A) Day-18 PDA-bearing WT and NLRP3<sup>-/-</sup> mice were administered 10<sup>7</sup> CD45.1<sup>+</sup> T cells via retroorbital injection. The fraction of PDA-infiltrating CD45.1<sup>+</sup> T cells was determined at 36 h. (B) T cells from tumor-bearing WT and NLRP3<sup>-/-</sup> hosts were harvested and serum-starved overnight before plating over a 3- $\mu$ m cellular insert in the presence or absence of 10% FBS. T cell migration was measured at various time points. Each experiment was performed twice using five replicates. Unpaired Student's *t* test was used for statistical analyses (*P* = NS). Data are presented as mean ± standard error.

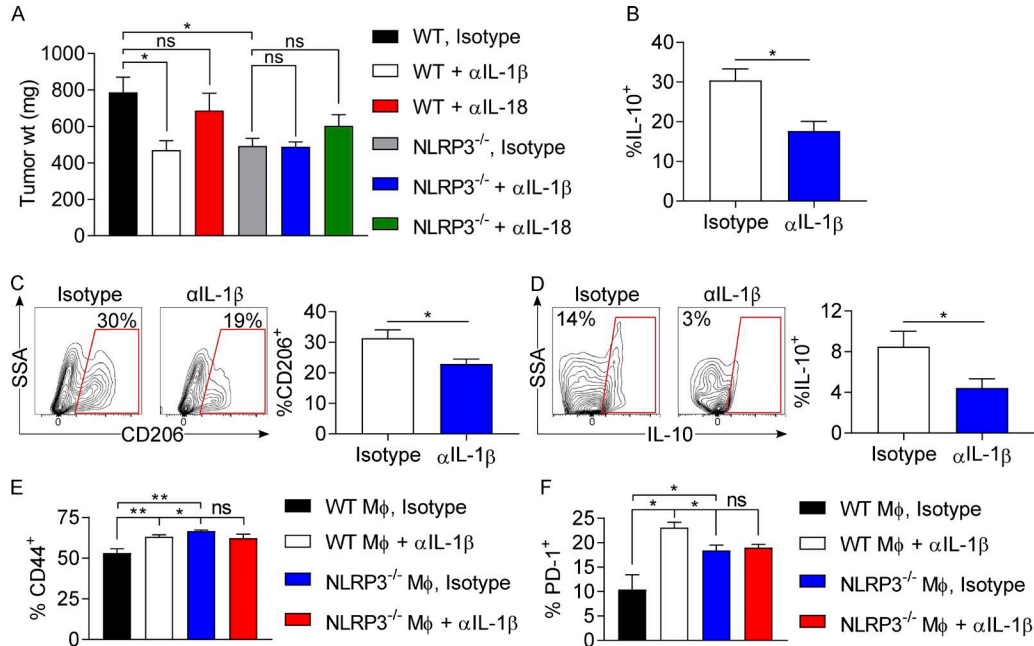


Figure S4. **IL-1 $\beta$  blockade protects against PDA and enhances the capacity of TAMs to activate T cells.** (A) WT and NLRP3<sup>-/-</sup> mice were orthotopically implanted with KPC-derived tumor cells and serially treated with neutralizing  $\alpha$ IL-1 $\beta$  or  $\alpha$ IL-18 mAbs or isotype control. Cohorts of mice were sacrificed on day 21, and pancreas tumors were weighed.  $n = 5$ /group. This experiment was repeated twice. (B) Splenic macrophages were cultured with a neutralizing IL-1 $\beta$  mAb or isotype control. IL-10 expression was measured at 24 h by flow cytometry. This experiment was performed using five replicates. (C and D) WT mice were orthotopically implanted with KPC-derived tumor cells and serially treated with neutralizing  $\alpha$ IL-1 $\beta$  mAb or isotype control. Tumors were harvested on day 21, and TAMs were analyzed for expression of CD206 (C) and IL-10 (D).  $n = 10$ /group. (E and F) TAMs were harvested from orthotopic KPC tumors in WT and NLRP3<sup>-/-</sup> hosts, pulsed with OVA<sub>323-339</sub> peptide, and plated with CD4<sup>+</sup> OT-II T cells. Activation of T cells at 96 h was determined by expression of CD44 (E) and PD-1 (F). In vitro and in vivo experiments were performed twice. Littermate controls were used. Unpaired Student's  $t$  test was used for statistical analyses. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ . Data are presented as mean  $\pm$  standard error. M $\Phi$ , macrophage; SSA, side scatter.