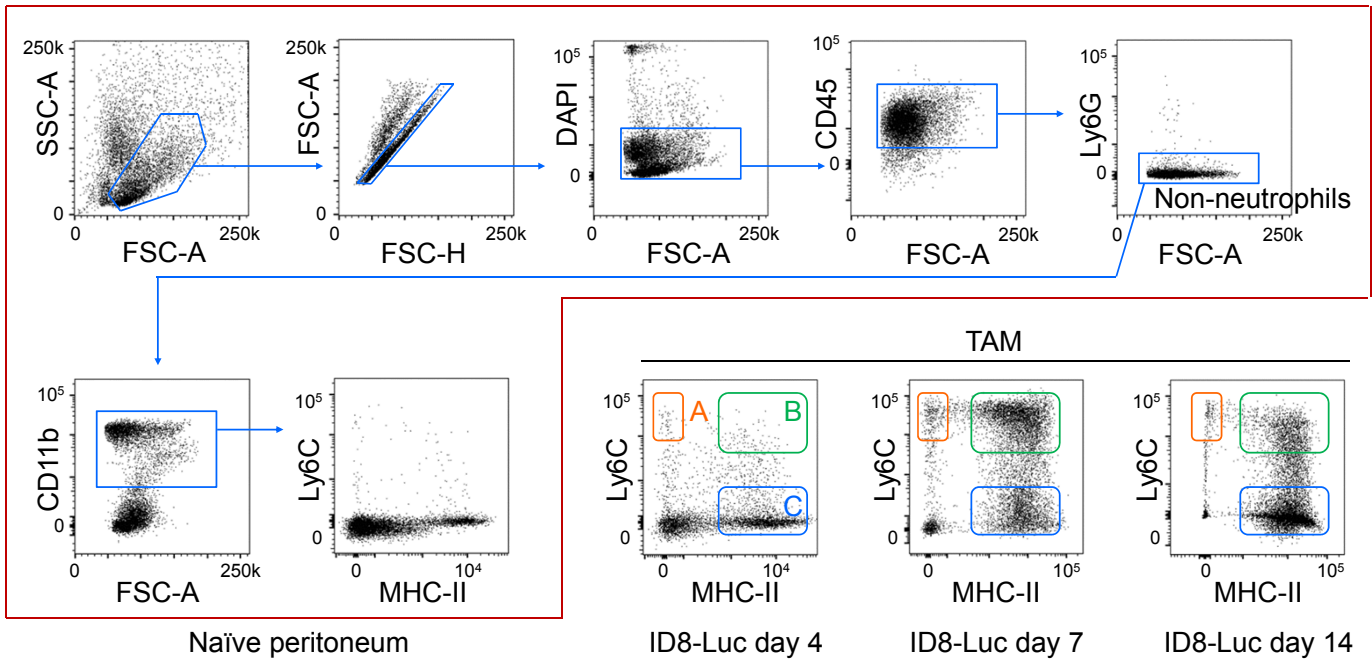
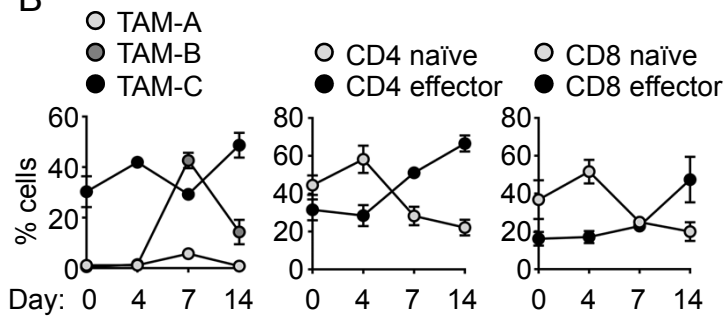


**Supplemental Figure 1. Expression of NFAT5 in the mouse models used in this study, as well as pro- and anti-inflammatory genes in wild-type and NFAT5-deficient macrophages.** (A) Suppression of NFAT5 mRNA expression in peritoneal CD11b macrophages of LysM-Cre *Nfat5*<sup>fl/fl</sup> mice. (B) Suppressed NFAT5 mRNA expression in different hematopoietic lineages (peritoneal macrophages, spleen T and B cells), but not in non-hematopoietic tissues (muscle and skin) in Vav-Cre *Nfat5*<sup>fl/fl</sup> mice. (C) Specific loss of NFAT5 mRNA in BMDM, T and B cells from Mx-Cre *Nfat5*<sup>fl/fl</sup> (KO) mice upon treatment with polyIC. (D) Western blots show representative experiments comparing NFAT5 protein expression in wild-type (W) and Vav-Cre *Nfat5*<sup>fl/fl</sup> BMDM or Mx-Cre *Nfat5*<sup>fl/fl</sup> BMDM (K). (E) Expression of the indicated genes in wild-type (Vav-Cre *Nfat5*<sup>+/+</sup>) and NFAT5-deficient (Vav-Cre *Nfat5*<sup>fl/fl</sup>) macrophages, either without pretreatment or pretreated 24h with IFN $\gamma$  or IL-4, and then stimulated with LPS as indicated. Values for each gene are represented relative to the respective stimulatory conditions that induced maximal expression in wild-type macrophages (100%), and show the mean  $\pm$  SEM of 3 independently performed experiments. Statistical significance was determined with an unpaired *t* test (A, C) or a two-way ANOVA test (E) (\**p* < 0.05; \*\**p* < 0.01).

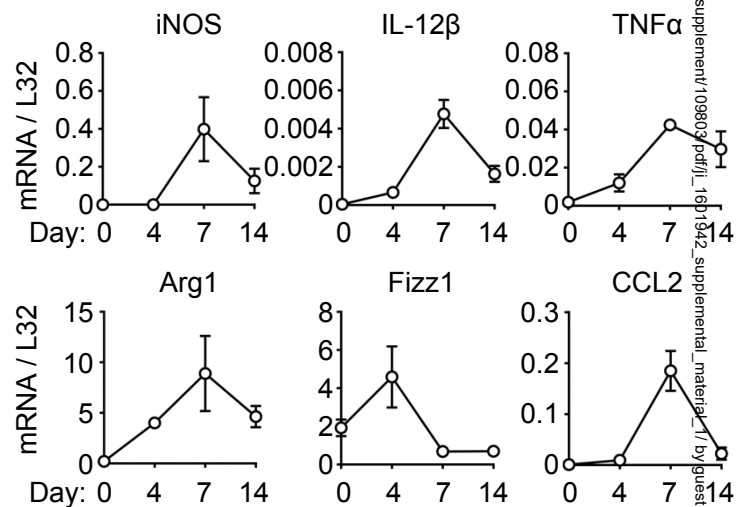
A


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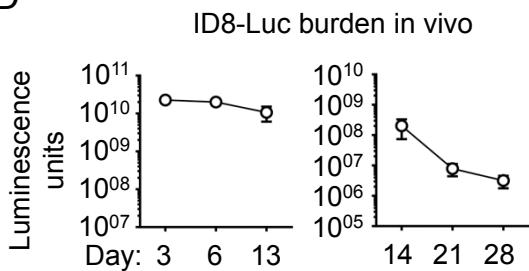
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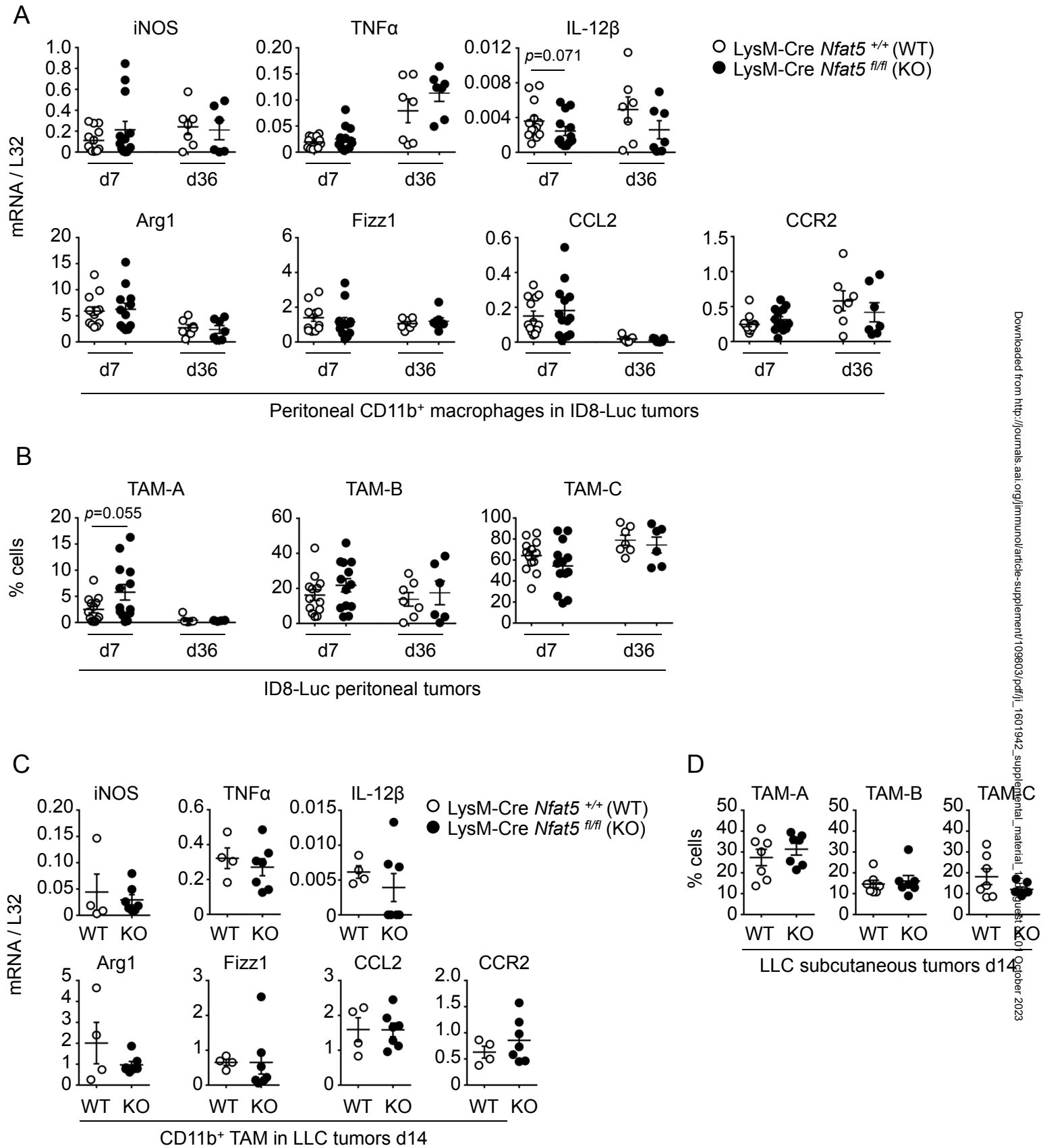
C



D


**Supplemental Figure 2. Responsiveness of wild-type peritoneal macrophages to ID8-Luc ovarian cancer cells.**

(A) Flow cytometry gating strategy for the analysis of peritoneal macrophage subsets, as well as peritoneal macrophage subset composition in naïve peritoneum and different days after ID8-Luc inoculation. (B) Proportions of tumor associated macrophage (TAM) subsets, and naïve and effector CD4 and CD8 T cells in naïve peritoneum and different days after ID8-Luc inoculation. Note that values for day 0 correspond to resident peritoneal macrophage subsets in the naïve peritoneum, analyzed with the same markers and gating strategy used to define TAM subsets in ID8-Luc-bearing mice. (C) mRNA expression of the indicated genes in CD11b<sup>+</sup> macrophages in naïve peritoneum and different days after ID8-Luc inoculation. (D) ID8-Luc tumor burden was assessed by intravital luminescence. Results in B through D show the mean  $\pm$  SEM of 3 to 4 analyzed mice.



**Supplemental Figure 3. Analysis of wild-type and NFAT5-deficient tumor-associated macrophages in ID8-Luc and LLC tumor models.** (A) Comparison of mRNA levels of the indicated genes in peritoneal CD11b<sup>+</sup> macrophages isolated at days 7 and 36 after ID8-Luc inoculation. (B) proportion of tumor-associated macrophage (TAM) subsets in peritoneal exudates of the same mice. Results in (A) and (B) correspond to experiments shown in Figure 6A. Statistical significance was determined with an unpaired Mann Whitney test (A) and an unpaired *t* test (B). (C and D) Gene expression (C) and proportion of TAM subsets (D) in CD11b<sup>+</sup> macrophages isolated from LLC tumors from the same mice (4 WT, 7 KO in (C), and 7 mice of each genotype in (D)) shown in Figure 6B.

Supplemental Table 1. Primers used for RT-qPCR.

Target gene		Primer sequence
<i>Arg1</i>	Forward	5'-CAC ACT GAC ATC AAC ACT CC-3'
<i>Arg1</i>	Reverse	5'-TCT CGC AAG CCA ATG TAC AC-3'
<i>Ccl2</i>	Forward	5'-CTC AGC CAG ATG CAG TTA ACG-3'
<i>Ccl2</i>	Reverse	5'-CAG ACC TCT CTC TTG AGC TTG G-3'
<i>Ccr2</i>	Forward	5'-ACC TCA GTT CAT CCA CGG CAT AC-3'
<i>Ccr2</i>	Reverse	5'-CAA GCT CCA ATT TGC TTC ACA-3'
<i>Cd163</i>	Forward	5'-CTG ATG GAG CAG ATC TGG AAC-3'
<i>Cd163</i>	Reverse	5'-CAG ATC CAC ATC CAA GCT GAC-3'
<i>Chi3l3</i>	Forward	5'-TCC ATG ATC CTA AGG ATG GC-3'
<i>Chi3l3</i>	Reverse	5'-ATG AGC TTC TCA GAA GCT GC-3'
<i>Gas6</i>	Forward	5'-GAG GAC ATC TTA CCA TGT GTGC-3'
<i>Gas6</i>	Reverse	5'-TGA AGC CTC TTG AAG CGT AG-3'
<i>Ifng</i>	Forward	5'-CTC AAG TGG CAT AGA TGT GG-3'
<i>Ifng</i>	Reverse	5'-CAG GTG TGA TTC AAT GAC GC-3'
<i>Il12b</i>	Forward	5'-AGA TGA AGG AGA CAG AGG AG-3'
<i>Il12b</i>	Reverse	5'-ACT TGC TGC ATG AGG AAT TG-3'
<i>Il1b</i>	Forward	5'-TGA AGA AGA GCC CAT CCT CTG-3'
<i>Il1b</i>	Reverse	5'-AGC TTT CAG CTC ATA TGG GTC-3'
<i>Il2</i>	Forward	5'-AGC TGT TGA TGG ACC TAC AG-3'
<i>Il2</i>	Reverse	5'-AAA TCC AGA ACA TGC CGC AG-3'
<i>Il4</i>	Forward	5'-CTC ACA GCA ACG AAG AAC ACC-3'
<i>Il4</i>	Reverse	5'-GCT TAT CGA TGA ATC CAG GC-3'
<i>Il6</i>	Forward	5'-GCC AGA GTC CTC CAG AGA GAT AC-3'
<i>Il6</i>	Reverse	5'-CCA CTC CTT CTG TGA CTC CAG C-3'
<i>L32</i>	Forward	5'-ACC AGT CAG ACC GAT ATG TG-3'
<i>L32</i>	Reverse	5'-ATT GTG GAC CAG GAA CTT GC-3'
<i>Mrc1</i>	Forward	5'-GGA CTC TGG ATT GGA CTC AAC AG-3'
<i>Mrc1</i>	Reverse	5'-GCT CTG ATG ATG GAC TTC CTG G-3'
<i>Nfat5</i>	Forward	5'-CAG CCA AAA GGG AAC TGG AG-3'
<i>Nfat5</i>	Reverse	5'-GAA AGC CTT GCT GTG TTC TG-3'
<i>Nos2</i>	Forward	5'-AGC TGG GCT GTA CAA ACC TT-3'
<i>Nos2</i>	Reverse	5'-CTC CCA TGT TGC ATT GGA AG-3'
<i>Retnla</i>	Forward	5'-CAG CTG ATG GTC CCA GTG AAT A-3'
<i>Retnla</i>	Reverse	5'-GGC CCA TCT GTT CAT AGT CTT GAC-3'
<i>Tnfa</i>	Forward	5'-TCG TAG CAA ACC ACC AAG TG-3'
<i>Tnfa</i>	Reverse	5'-GGA GTA GAC AAG GTA CAA CC-3'