Supplemental Figures

Figure S1. CD68 colocalizes with LAMP-1 positive vesicles in peritoneal macrophages.

Zymosan (100 μg/ml, 1h) treated PECs were permeablized and then stained for CD68 (green; left panel) and LAMP-1 (red, middle panel). Nuclei were counter stained with DAPI (blue; right panel) and visualized on the 60x objective from a Nikon Eclipse TE300 microscope. A merged image (left panel) reveals that CD68 and LAMP-1 staining vesicles co-localize.

Figure S2. Leukocyte distribution is normal in CD68 knockout mice.

Leukocytes were harvested from the peritoneum (**A)** spleen (**B, C**) of 8 week old wt and ko mice and evaluated by FACS (see Fig. 1). No significant differences were observed in the distribution specific cell populations (percentages indicated for each gate) either in these or older mice (data not shown; see also Table 1a & 1b). There were no significant differences in peritoneal resident macrophages (CD11b+, Gr-1-; **A**), peritoneal PMNs (CD11b+, Gr-1+; **A**), splenic macrophages (CD11b+, Gr-1-; **B**), splenic monocytes (CD11b+; **B**) or splenic DCs (CD11c+, MHC-II^{hi}; **C**), including the lymphoid (i.e., CD11c+, CD11b-, CD8α+) and myeloid (CD11c+, CD11b+, CD8α-) DC subpopulations (20). Percentages for each corresponding population are indicated with each gate.

Figure S3. The expression *cd68* proximal *eifa1* and *mpdu.1* are not affected by CD68 knockout.

eifa1 and *mpdu.1* gene expression was evaluated in wt, het and CD68 ko PECs and BMMs by RT-PCR after treatment Ox-LDL (10 μg/ml, 4h), as detailed in Figure 3. GAPDH expression serves as a control. No RT represents a control sample were reverse transcriptase was not added.

Figure S4. Ox-LDL uptake in wt and CD68[-/-] PECs.

(**A**) Wt and ko PECs, loaded with native LDL or Ox-LDL, were stained with oil red O.

(**B**) Wt and ko PECs were loaded with increasing doses DiI labeled Ox-LDL and evaluated by FACS (same data as in Fig. 3A, but plotted as maximal fluorescence for each population). PECs were either not treated (shaded gray area) or treated with 0.1 μg/ml (dotted grey line), 1.0 μg/ml (solid grey line), or 10 μg/ml DiI-Ox-LDL (solid black line) for 4 hours at 37°C.

Figure S5. CD68 BMMs polarize normally to M1 and M2 subsets.

(A) Wt and ko BMMs were polarized to M1 (LPS, 100 ng/ml + IFN-γ, 50 U/ml; 24h) and M2 (IL-4 10 ng/ml; 48h) phenotypes and evaluated for changes in the surface expression of CD206 (mannose receptor; eBioscience), a M2 marker.

(**B**) Wt and ko PECs were evaluated for IL-4 (10 ng/ml; 24h) dependent expression of arginase 1 (Arg1), an M2 gene, by Q-PCR, as previously reported.

(**C**) Wt and ko BMMs were evaluated for IL-4 (10 ng/ml; 24h) and LPS (1 μg/ml, 24h) dependent expression of IL-10 by ELISA (eBioscience).

Figure S1 (Song, et al)

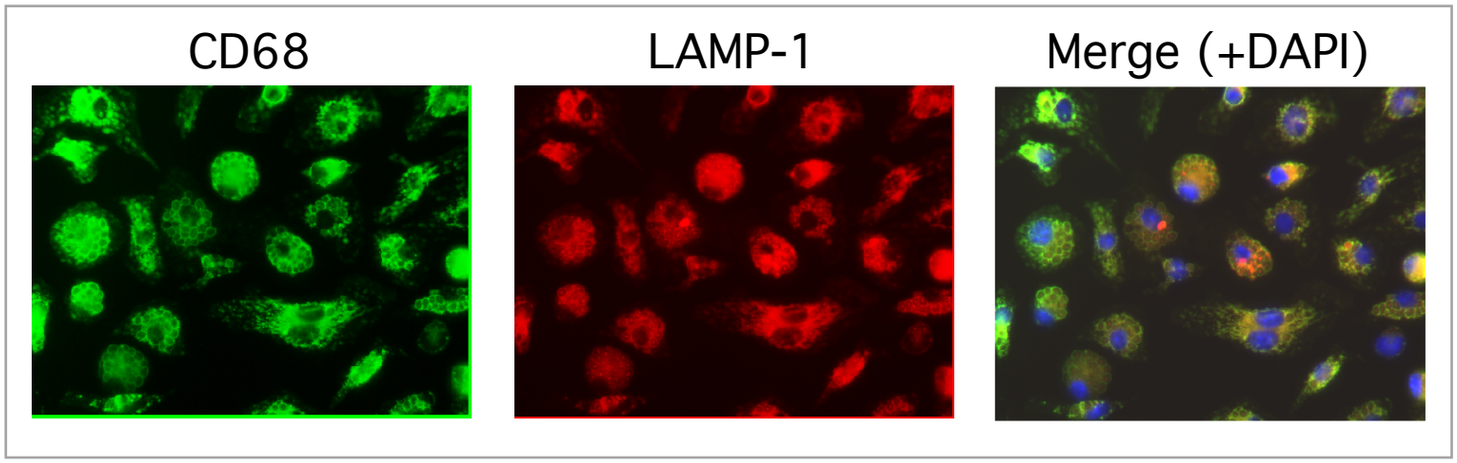

Figure S2 (Song, et al)

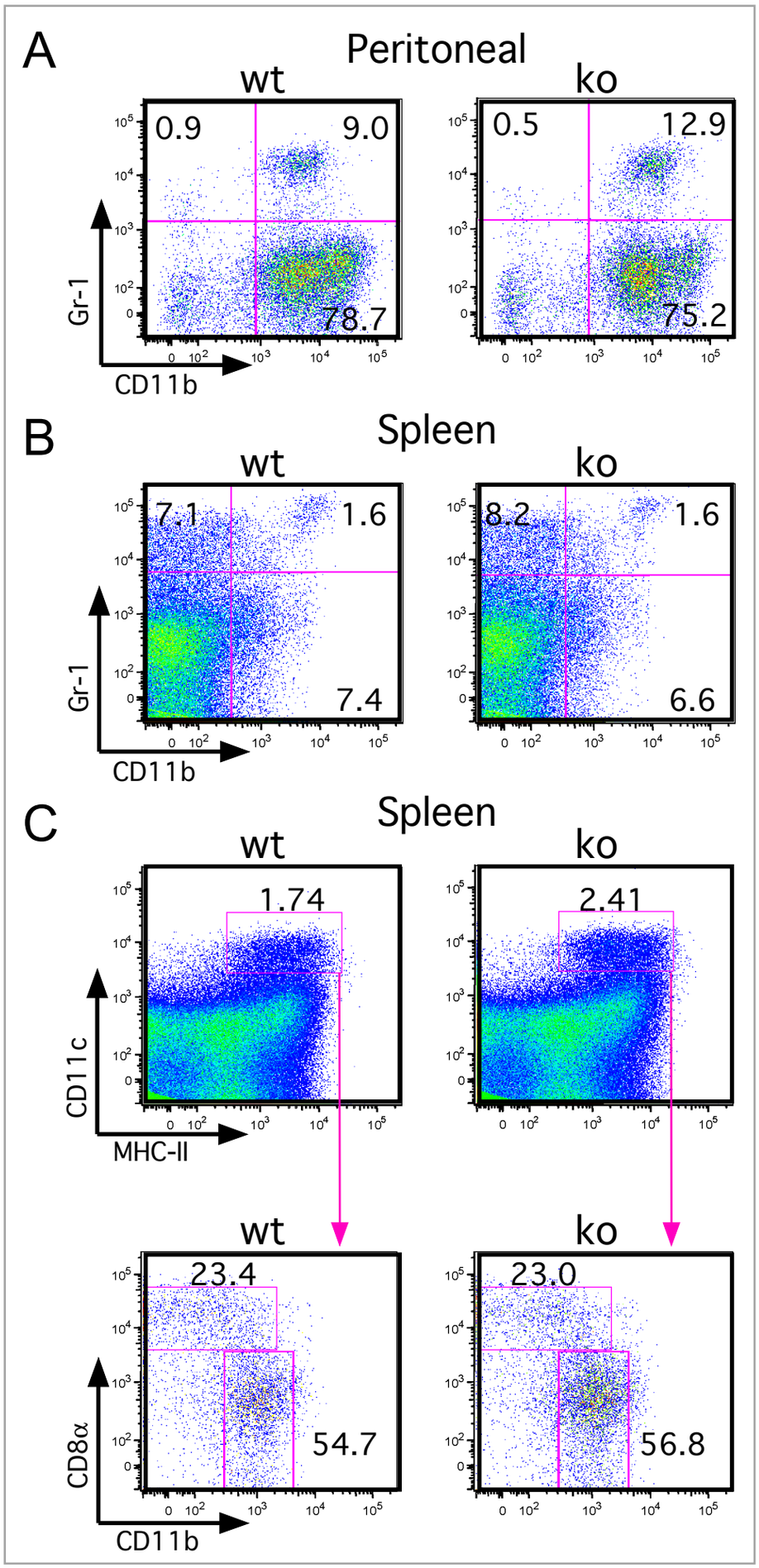

Figure S3 (Song, et al)

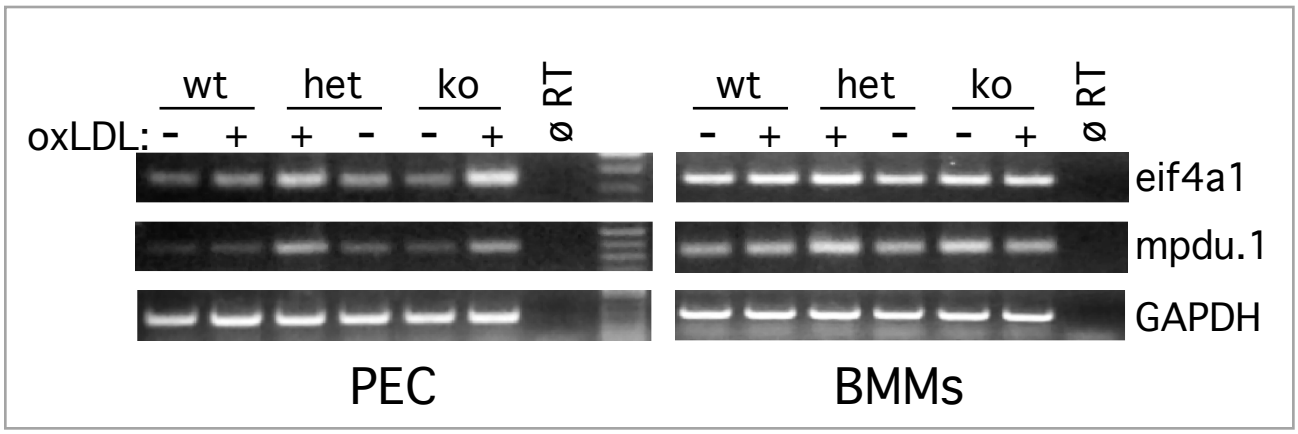

Figure S4 (Song, et al)

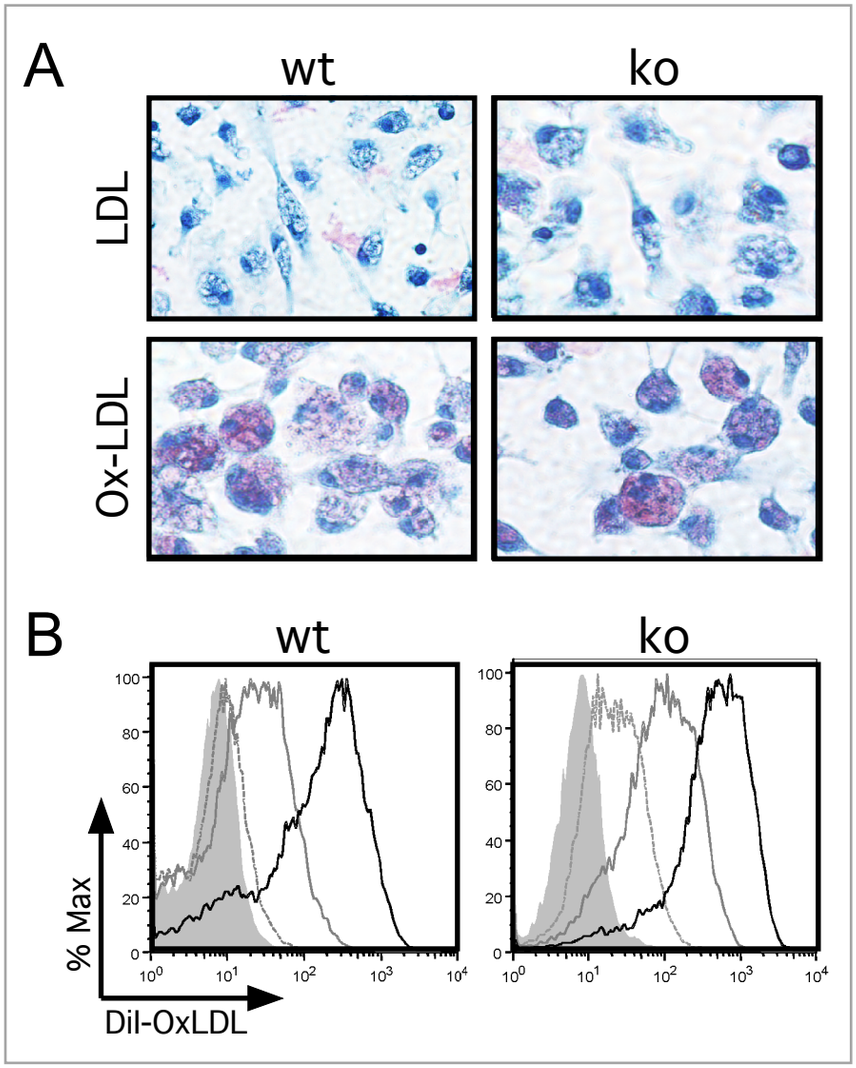

Figure S5 (Song, et al)