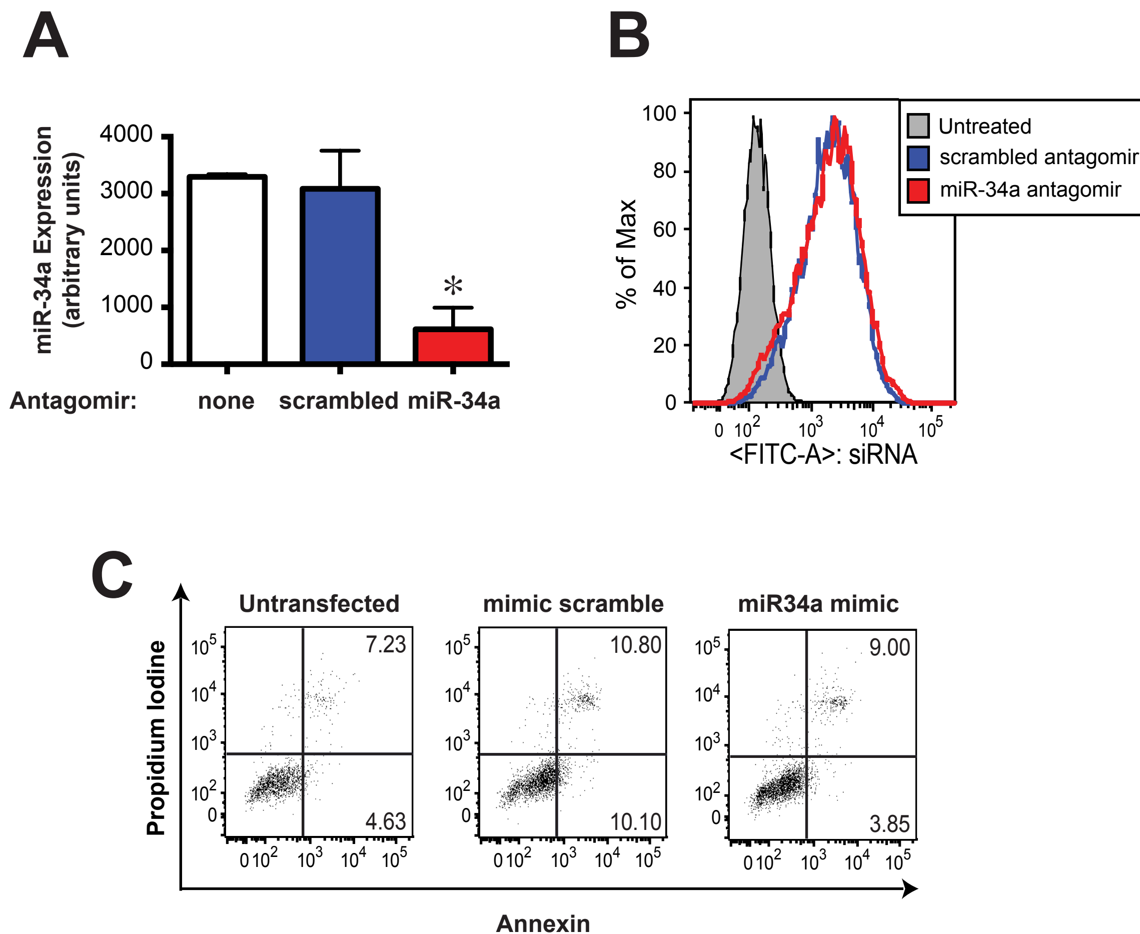
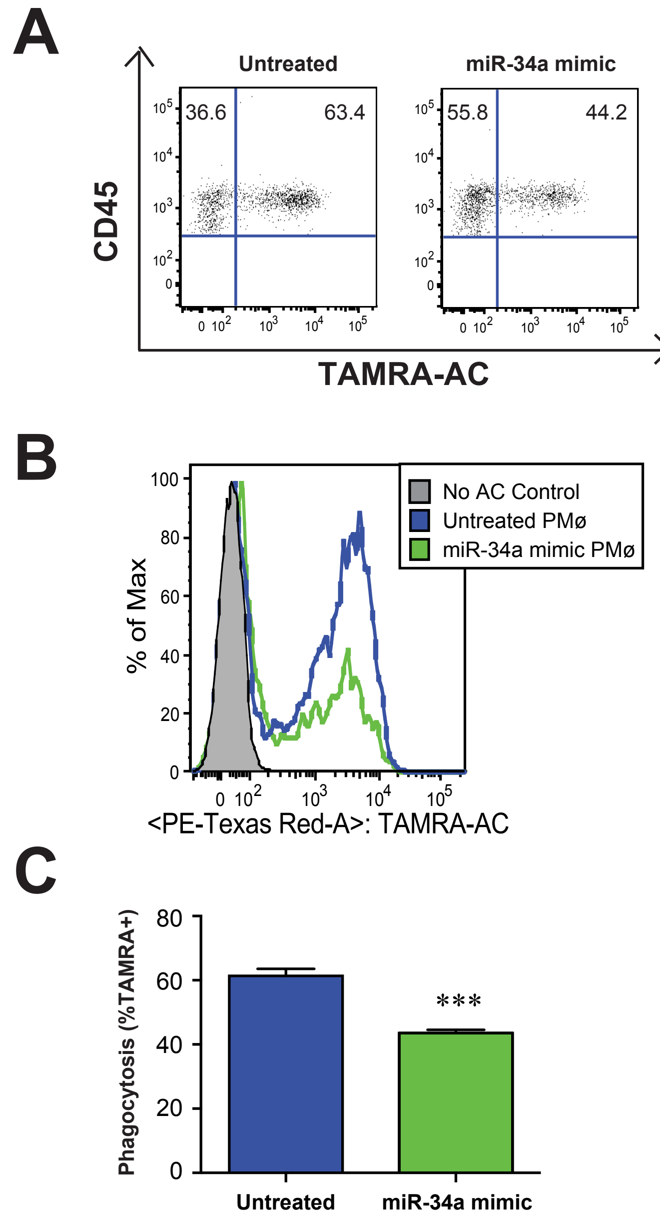


Supplemental Figure 1. Efficacy of Mø transfection to manipulate miR-34a expression without impacting viability

A. Resident murine AMø were transfected with control or miR-34a-specific antagomirs using RNAiMAX lipofectamine for 24 h, then RNA was harvested and assayed for miR-34a expression relative to sno-142 using quantitative real-time RT-PCR. Data are mean ± SEM from n=3-7 mice from one or more experiments; *, statistically significant, $p<0.05$ by one-way ANOVA with Bonferroni post-hoc testing. B. Resident murine AMø were transfected with fluorescein-labeled control antagomirs or miR-34a-specific antagomirs using RNAiMAX lipofectamine for 24 h, then analyzed by flow cytometry, gating on all CD45+ cells. Stacked overlays of histograms showing FITC fluorescence, representative of two experiments with similar results. C. Resident murine PMø were transfected with control mimics or miR-34a-specific mimics using RNAiMAX lipofectamine for 24 h, then viability was assayed by annexin-FITC and propidium iodine staining and flow cytometry; numbers show the percentage of events in that quadrant. Representative of two experiments with similar results.



Supplemental Figure 2. Flow cytometric confirmation that miR-34a negatively regulates efferocytosis.

PMø were transfected with control or miR-34a-specific mimics using RNAiMAX lipofectamine for 48 h, then exposed to TAMRA-labeled AC for 1 h. PMø were removed from culture, surface-stained with CD45, then analyzed by flow cytometry.

A. Representative dot plot of AC engulfment showing CD45+ versus TAMRA+ staining on CD45+ gated cells; numbers show the percentage of events in that quadrant.

B. Representative histogram showing TAMRA+ staining on CD45+ gated cells.

C. AC uptake measured as percent of TAMRA+ cells. Data shown are mean ± SEM from n=2-4 mice assayed individually in each of two independent experiments. ***, statistically significant, $p<0.0001$ by unpaired Student t test.

Supplemental Figure 3
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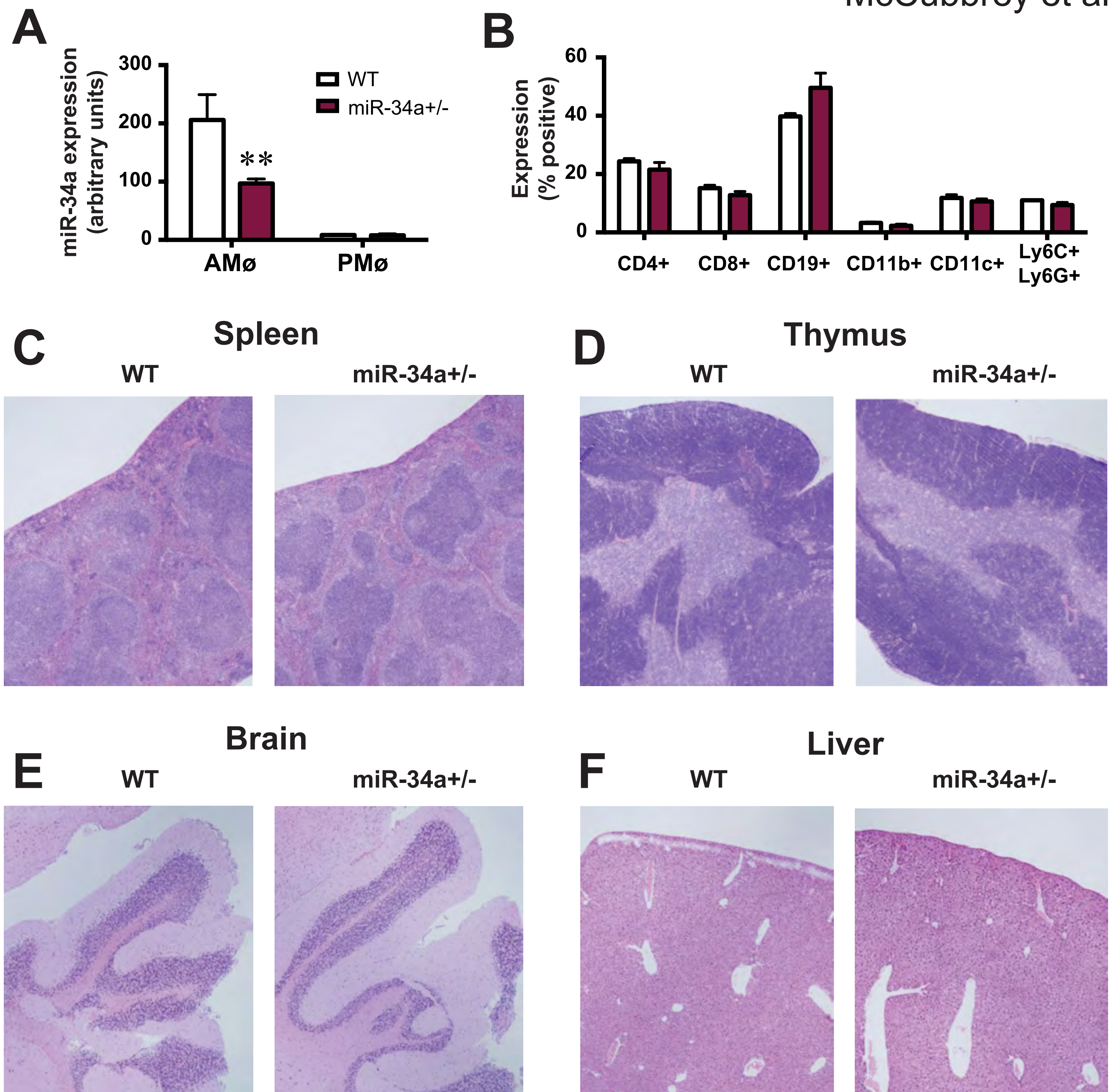

Supplemental Figure 3. Phenotype of MiR-34a+/- mice.
MiR-34aflox mice were crossed with LysM cre mice to develop miR-34a flox/- LysM cre mice (miR-34a+/-).
A. Resident AMø and PMø were harvested from miR-34a+/- mice (red) and wt C57BL/6 mice (WT) (white), total RNA was harvested and assessed for miR-34a expression, relative to sno-142, using quantitative real-time RT-PCR. Data are mean ± SEM of 6 mice of each genotype assayed individually in two independent experiments. **, statistically significant, $p<0.01$ by one-way ANOVA with Bonferroni post-hoc testing. B. Splenocytes were stained with mAbs and analyzed by flow cytometry, gating on CD45+ cells. Data are mean ± SE of 3 mice of each genotype. C-F. Various organs were harvested from wt mice and miR-34a+/- mice, processed for histology using paraffin-embedding and H&E staining. Representative examples of 3 mice of each genotype are shown at 40X final magnification; (C) spleen, (D) thymus, (E) brain, (F) liver.

Supplemental Figure 4
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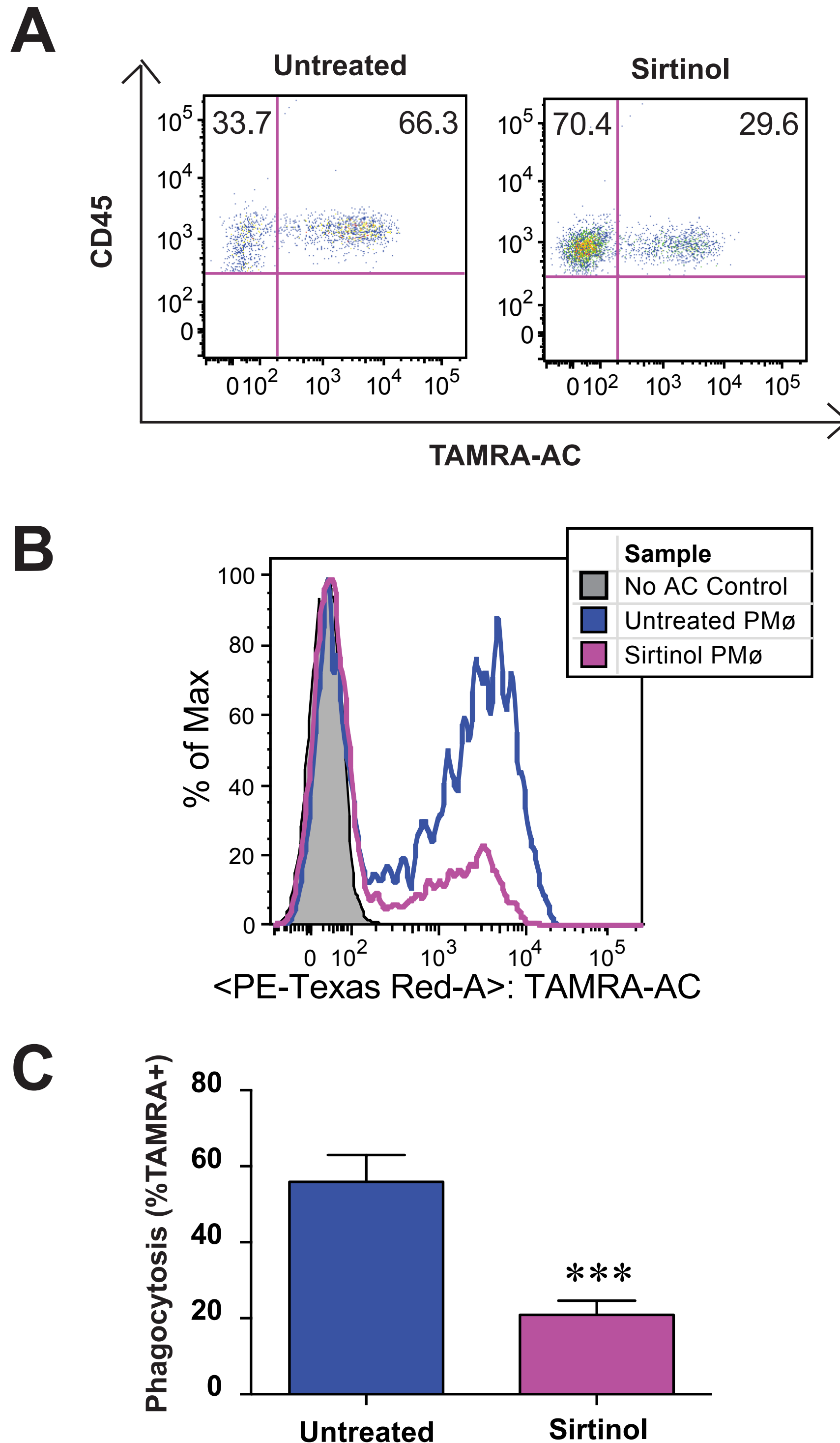

Supplemental Figure 4. Flow cytometric analysis confirms that sirtinol inhibits efferocytosis. PMø were treated with 10 μM Sirtinol for 24 h, then exposed to TAMRA-labeled apoptotic thymocytes for 1 h. PMø were removed from culture and surface stained with CD45, then assessed by flow cytometry. A. Representative dot plot of apoptotic cell engulfment showing CD45+ versus TAMRA+ staining on CD45+ gated cells. B. Representative histogram showing TAMRA+ staining on CD45+ gated cells. C. Quantified uptake measured as percentage of TAMRA+ cells. Data are mean ± SEM of n=5 mice, assayed individually in four independent experiments. **, statistically significant, $p<0.0001$ by paired Student t test.