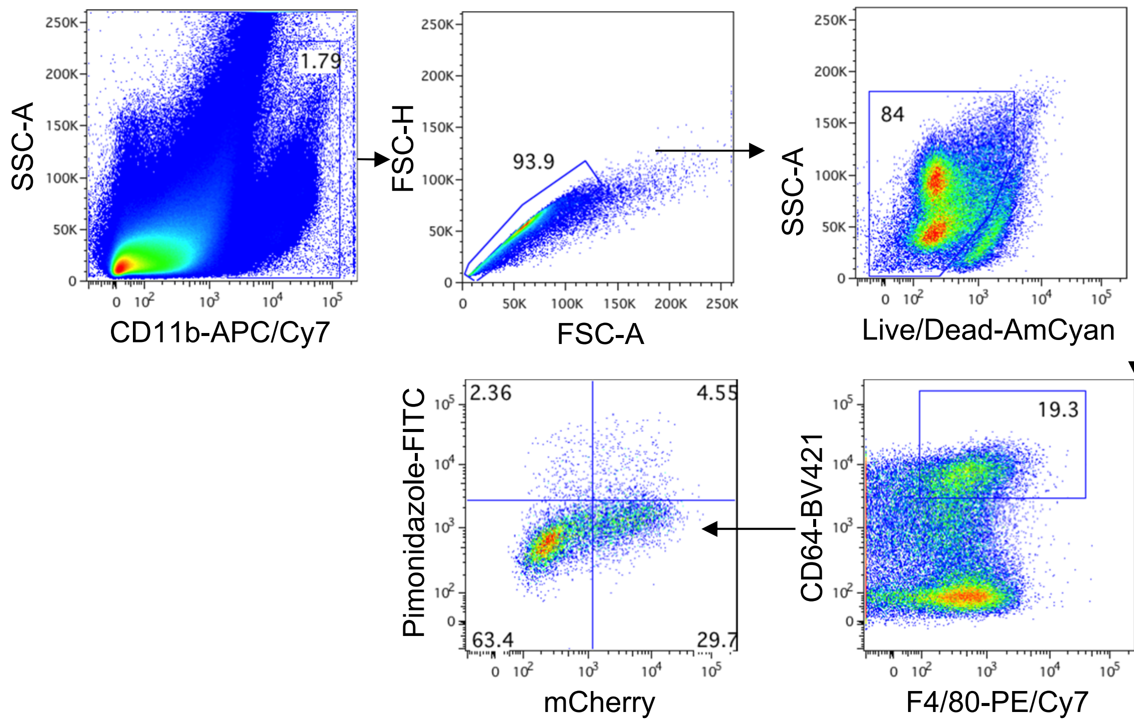
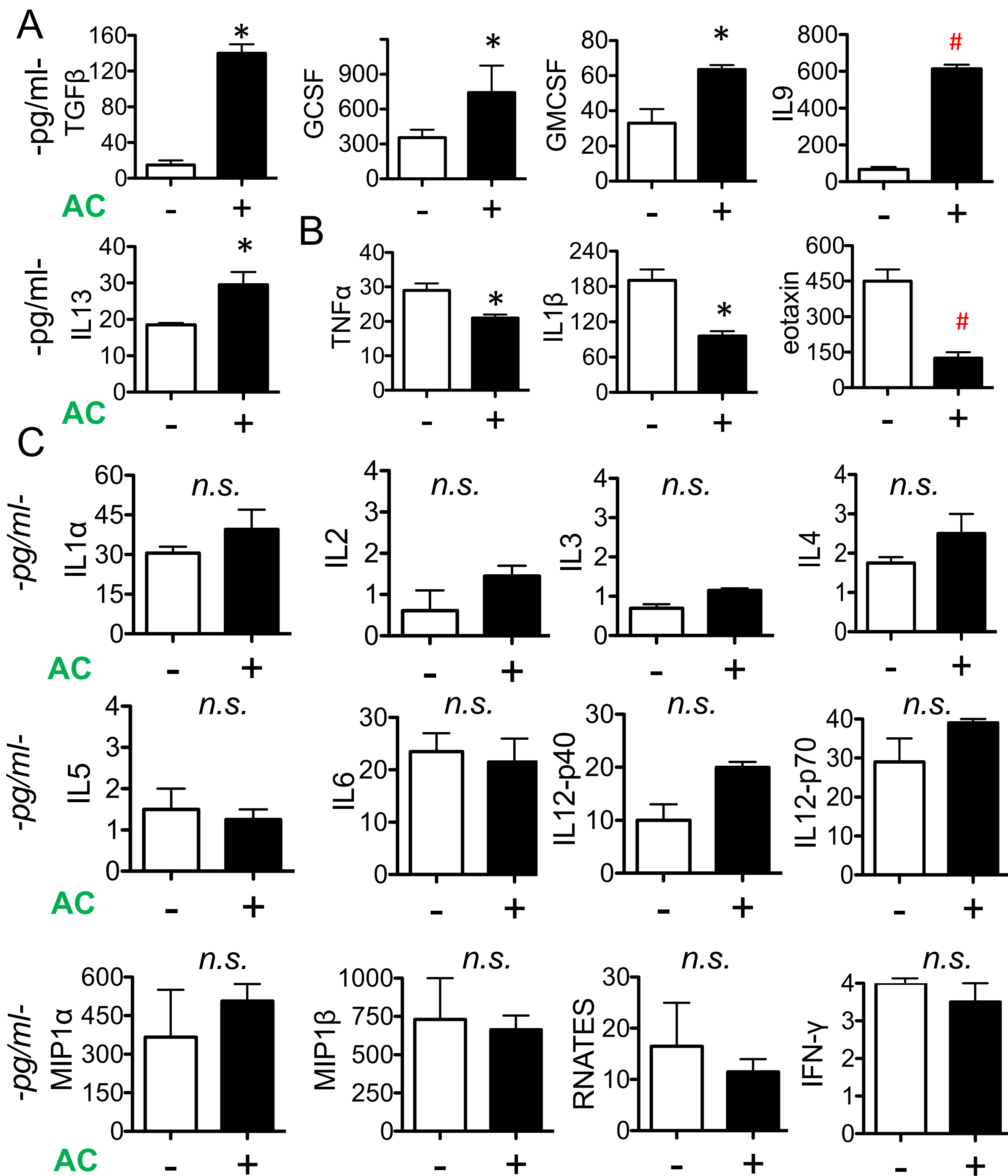
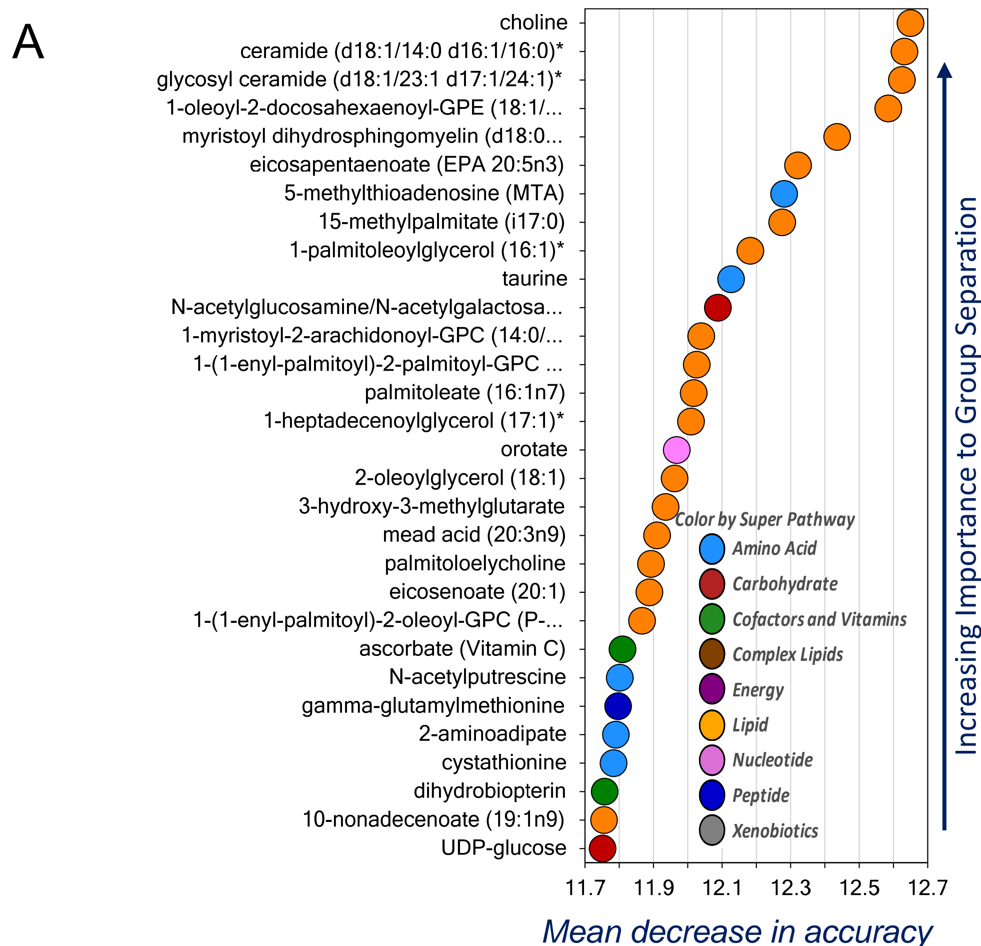
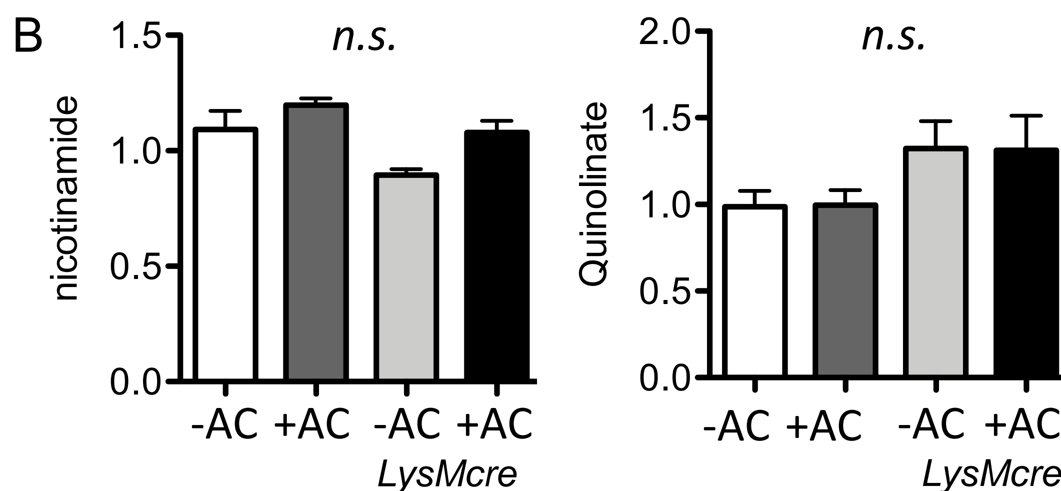


Supplemental Figure 1. Gating strategy for sorting pimonidazole positive and mCherry positive cardiac macrophages. Flow cytometry gating strategy for macrophages (CD45+Ly6G-Ly6C-F4/80+CD64+) after coronary ligation of MHCmcherry mice and collagenase treatment of myocardial extracts.

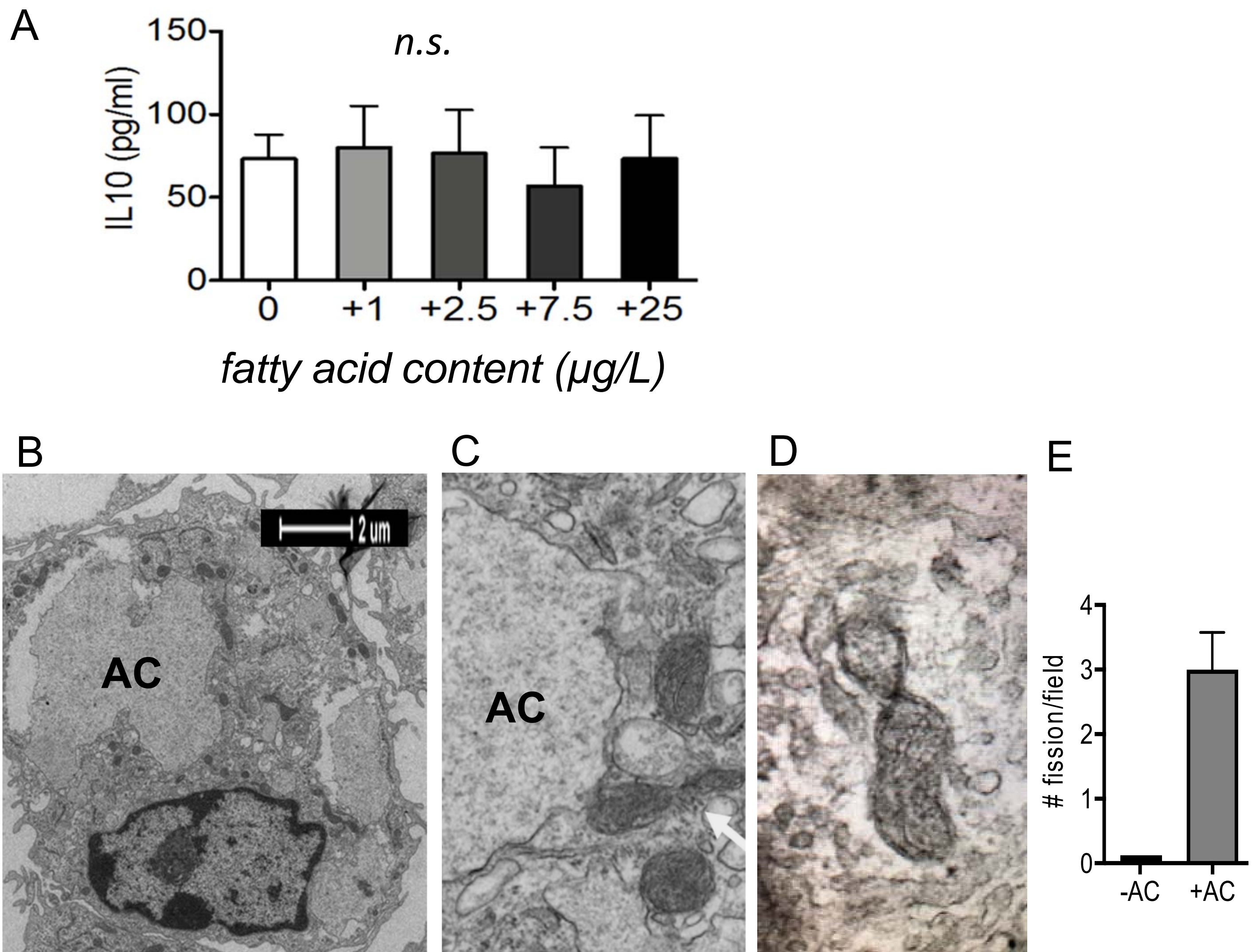


Supplemental Figure 2. Apoptotic cells induce a polarized anti-inflammatory cytokine phenotype in activated macrophages. Elicited primary macrophages were co-cultivated with early (Annexin V positive, Propidium Iodide negative) apoptotic cells (ACs). Non engulfed cells were removed from adherent phagocytes and cell culture media analyzed for secreted cytokines. (**A**) Induced efferocytic cytokines are as follows: TGFβ, fibroblast growth factor β; GCSF, granulocyte colony stimulating factor; GMCSF, granulocyte-macrophage colony-stimulating factor; IL9, interleukin 9; IL13, interleukin 13. (**B**) Suppressed cytokine response is as follows: TNFα, tumor necrosis factor α, IL1β, interleukin 1β. Asteriscks indicate $p<0.05$. # indicates newly identified efferocytic-induced molecules. **(C)** *Non-significant cytokine responses after efferocytosis.* (ACs). IL interleukin; MIP1α, macrophage inflammatory protein 1α, MIP1β, macrophage inflammatory protein 1β; RANTES, chemokine ligand 5 (regulated on activation, normal T cell expressed and secreted); IFNγ, interferon γ. *n.s.* indicates not statistically significant.

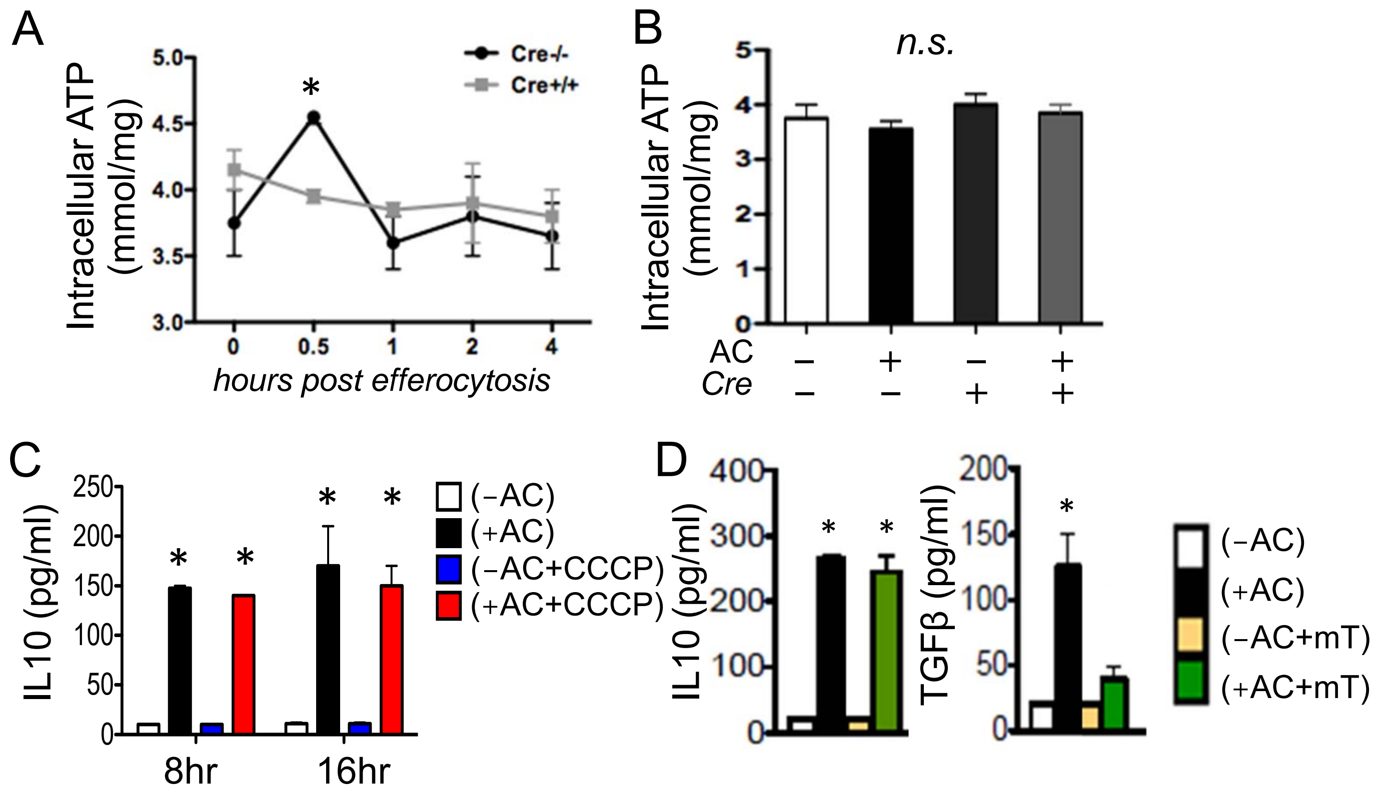




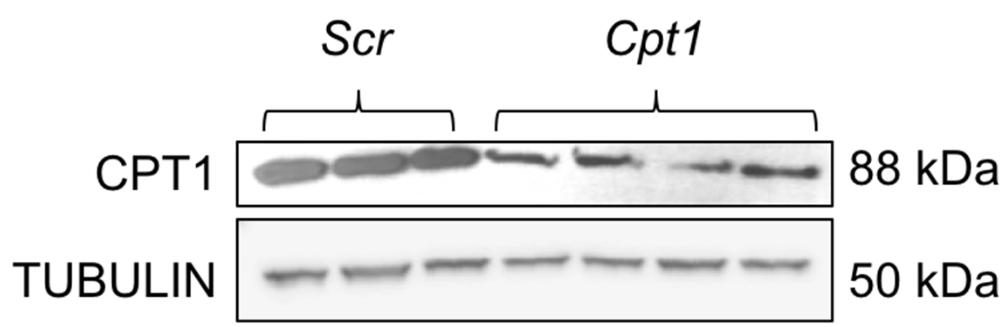
Supplemental Figure 3. Biochemical importance plot of efferocytosis and NAD metabolites during efferocytosis and in the absence of *Risp*. (A) Biochemical importance plot after non-biased Random Forest Analysis (RFA) for biomarker identification of most significant variables between macrophages at steady state, versus during efferocytosis. Y axis is increasing importance to group separation. Variables with larger mean decrease in accuracy are more important for data classification. Accuracy of prediction was determined after identifying the biomarker that changes per specific macrophage group. (B) Metabolites involved in nicotinate metabolism pathways are plotted as relative values minus and plus apoptotic cells (ACs) and in *Risp fl/fl and Risp fl/fl LysMcre* macrophages.

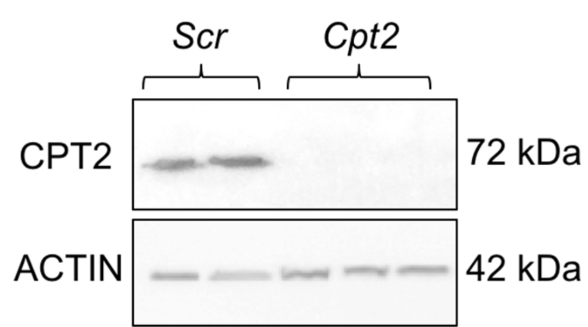


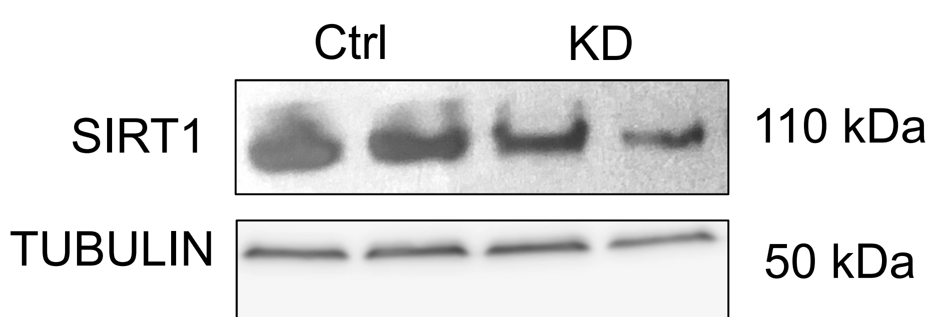
Supplemental Figure 4. IL10 production in T cells treated with fatty acids and TEM of efferocytosis. (**A**) Jurkat T cells were cultured in media with increasing fatty acid content as indicated (microgram/L fatty acid per liter of media) for 24 hrs. Culture media was then collected and IL10 was measured by ELISA. (B) Transmission electron micrographs of elicited primary peritoneal-derived macrophages during efferocytosis with apoptotic cells (AC). (**B**) Image depicts macrophage engulfing apoptotic cell. (**C**) Focus is on mitochondrion (white arrow) of the phagocyte, proximal to the engulfed AC. (**D**) Evidence for mitochondrial fission in an efferocyte. (**E**) Quantification of mitochondria displaying morphology consistent with fission per field as displayed in Panel C.

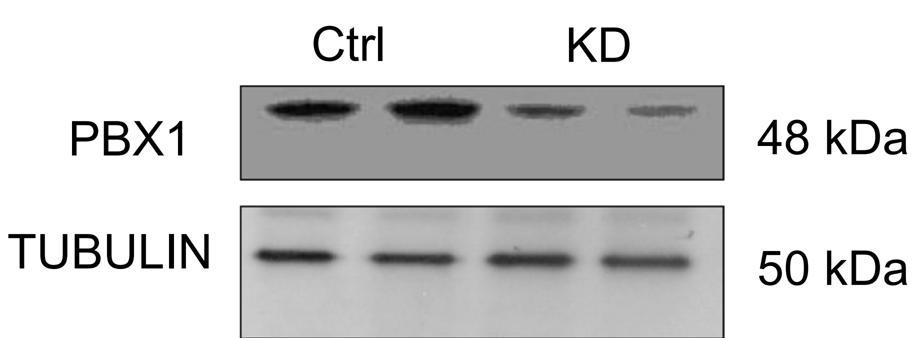


Supplemental Figure 5. ATP kinetics and effects of targeting mitochondrial membrane potential and reactive oxygen species during efferocytosis in *Risp-deficient* macrophages. (**A**) Kinetics of intracellular ATP levels after efferocytosis in *Rispfl/fl* macrophages, plus or minus *LysMcre*. (**B**) Quantification of intracellur ATP in *Rispfl/fl* and *Rispfl/fl LysMcre* macrophages, plus or minus apoptotic cells (AC) at one hour post efferocytosis (**C**) Efferocytosis and IL10 measurement by ELISA +/- carbonyl cyanide m-chlorophenyl hydrazine/CCCP to interfere with mitochondrial membrane potential. (**D**) IL10 and TGFβ was measured by ELISA after treatment of efferocytes with mitochondrial-targeted antioxidant mitoTEMPO/mT. *n.s.* = not statistically significant. * $p < 0.05$.

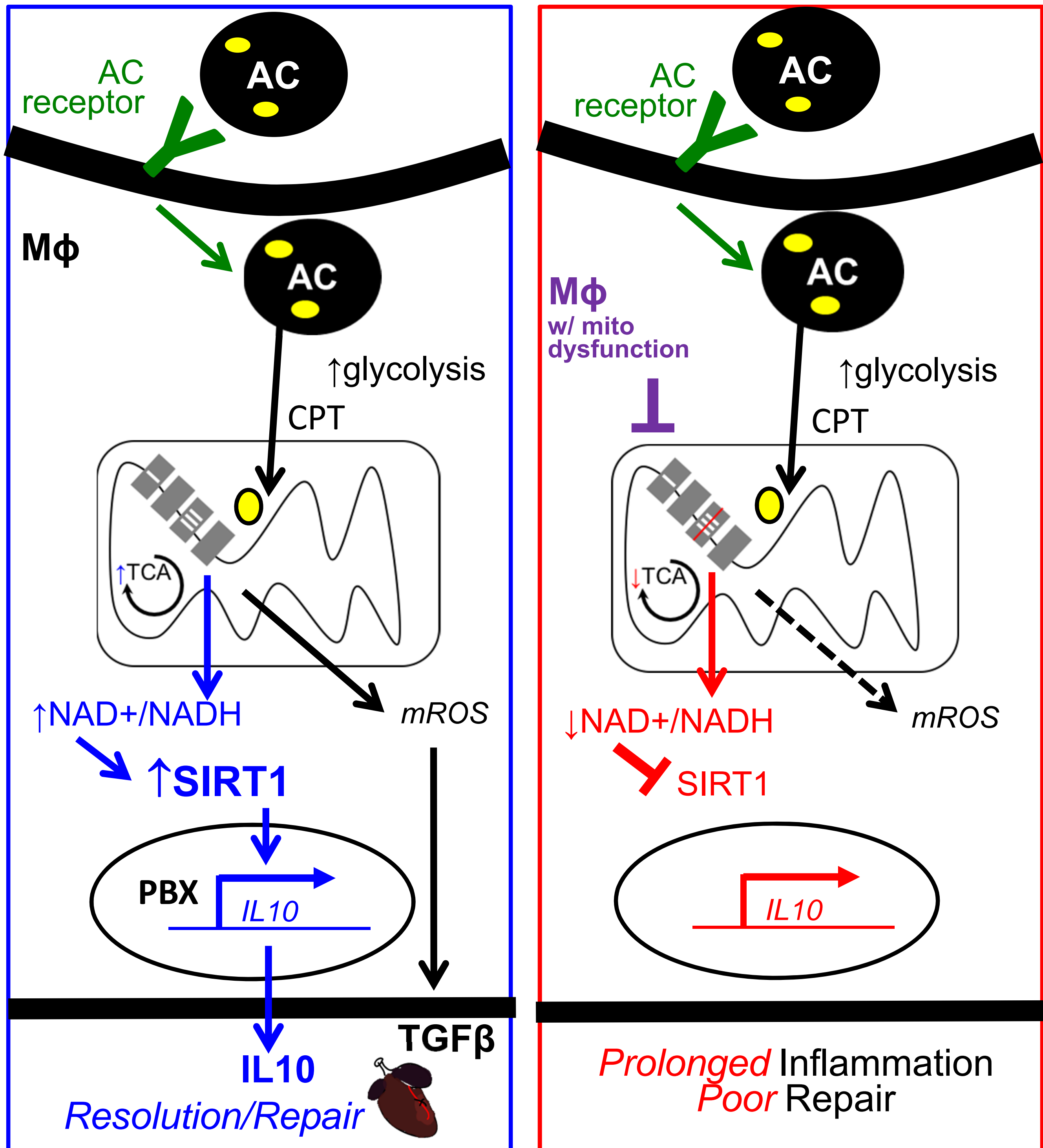








Supplemental Figure 6. Western blots after siRNA knockdown in peritoneal macrophages. Macrophages were treated with indicated siRNA or control siRNA for 72hr before protein lysate was harvested to check for knockdown efficiency.



Supplemental Figure 7. Working model of metabolic efferocytic reprogramming through the mitochondrial electron transport chain. Depicted in schematic is a working model from the experimental data. Shown are macrophages (Mϕs), during the process of apoptotic cell (AC) metabolism, and effects on wound healing. Electron transport I and III complexes are depicted, as well as the TCA cycle. NAD+ is nicotinamide adenine dinucleotide. SIRT1 is Sirtuin1. IL10 is interleukin 10. TGFβ is transforming growth factor beta. mROS is mitochondrial reactive oxygen species. See text for details.