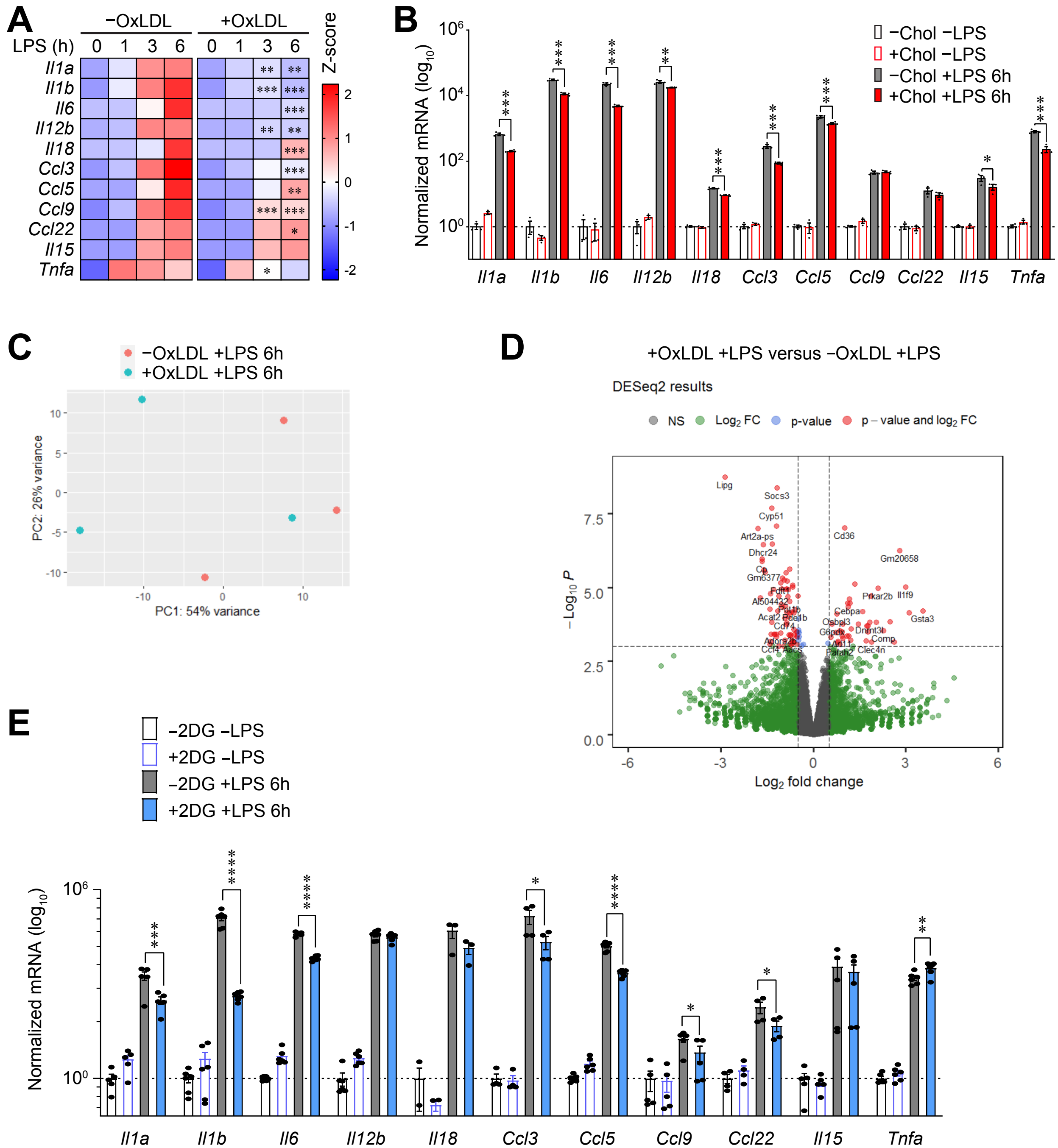
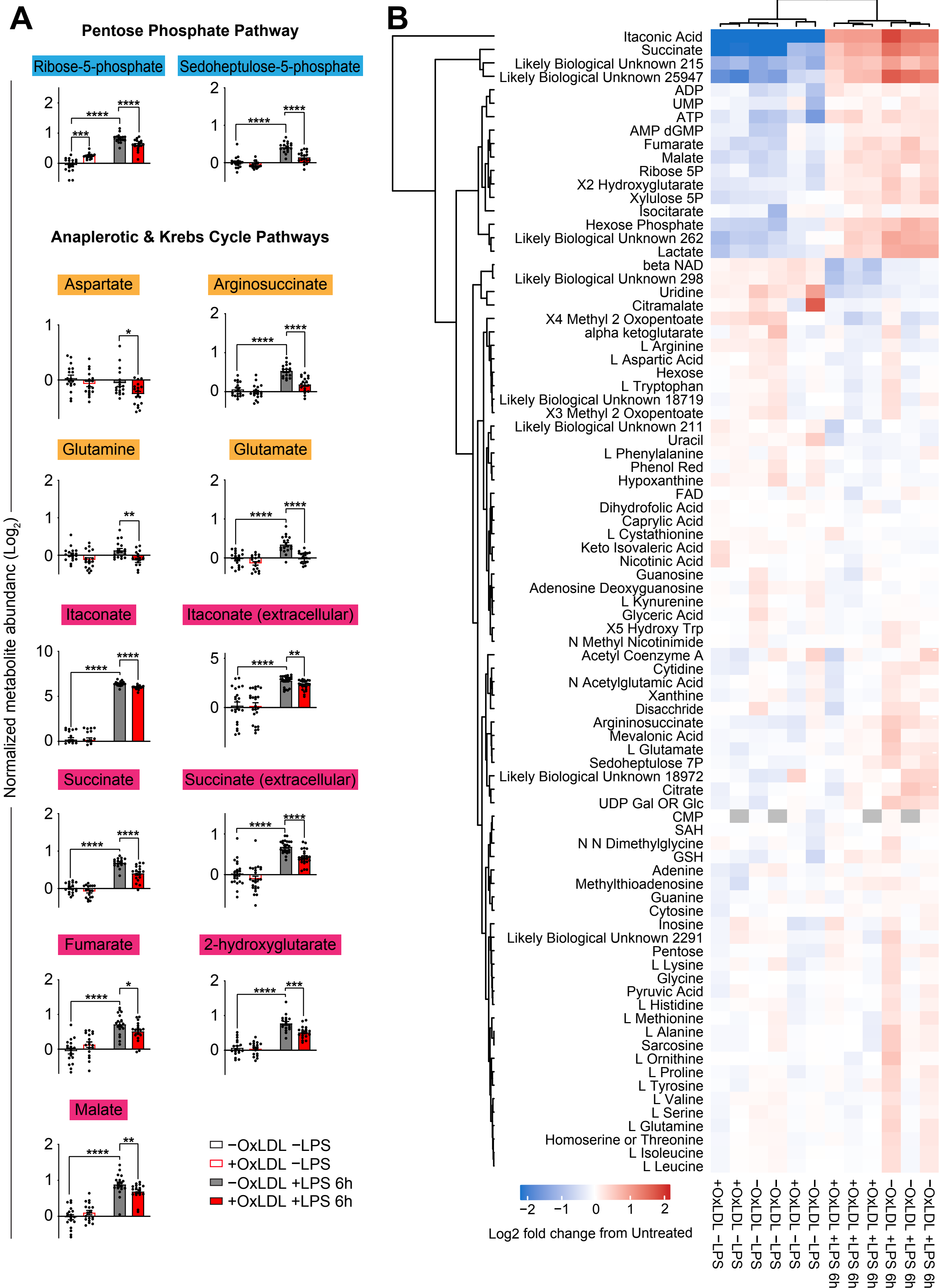
Supplemental Figure 1



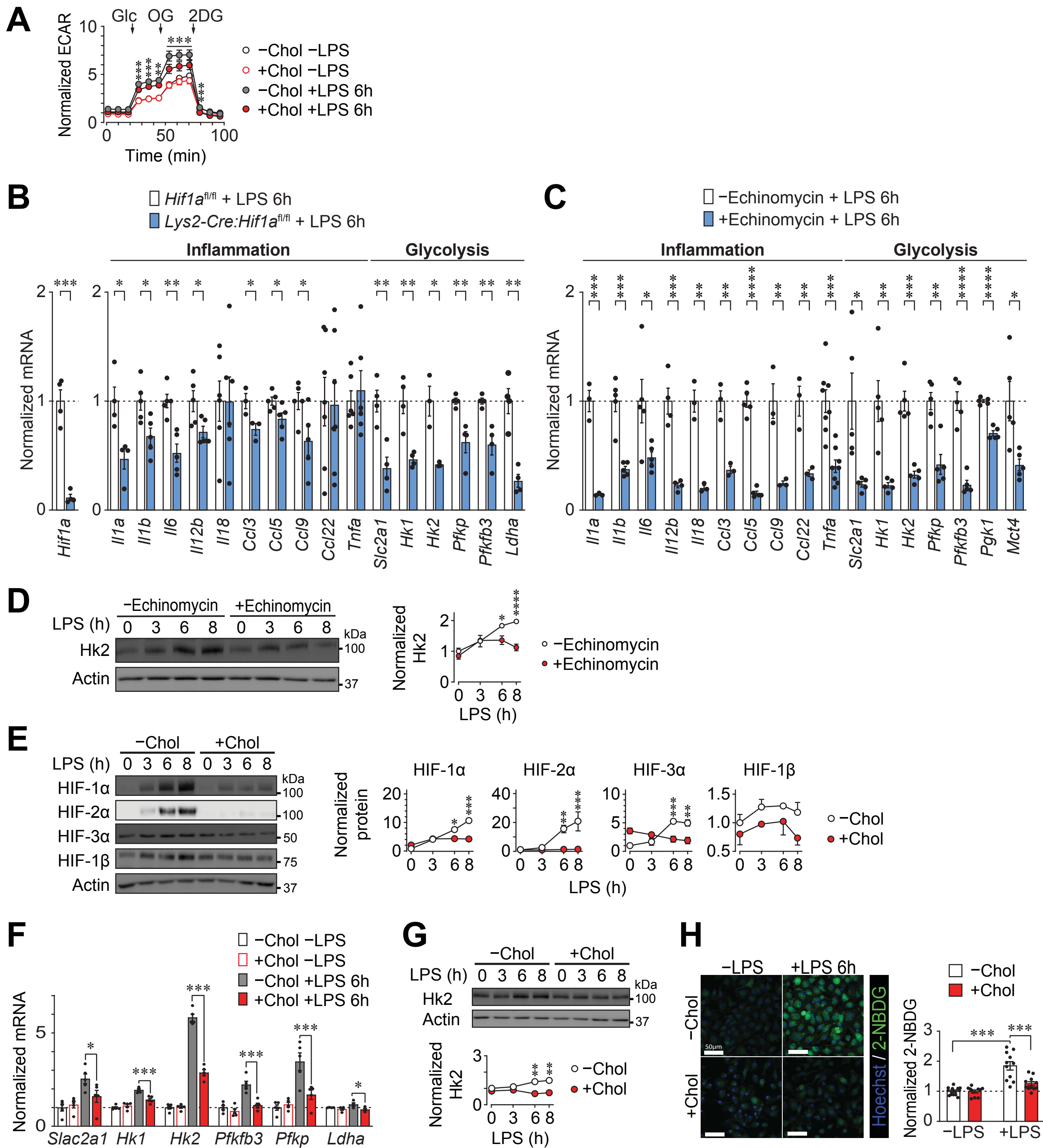
(A and B) Effect of lipid accumulation in PMφs and LPS stimulation on inflammatory gene mRNA expression. mRNA was analyzed by qPCR. (A) ±OxLDL accumulation (heat map z scores, n=3-4), (B) ±cholesterol (Chol) accumulation. In the -Chol group, PMφs were cultured for 24 h in media containing ethanol without cholesterol. For each gene, values were normalized to values without cholesterol and LPS (assigned a value of 100, dashed line, n=3). **(C and D)** Transcriptomic analysis of LPS-stimulated PMφs with and without oxLDL accumulation. Both groups were assessed 6 h after stimulation with LPS. (C) Principal component analysis of sample variance (n = 3) and (D) volcano plot illustrating enrichment of genes in the two groups. Genes with adjusted p values of <0.1 and a log2FC of <-0.5 or >0.5 are highlighted. **(E)** Effect of 2-deoxyglucose (2DG) pretreatment (1 h) on inflammatory gene expression in PMφs. Four groups were studied; with or without 2DG (+2DG or -2DG) and with or without 6 h LPS stimulation (+LPS 6 h or -LPS). mRNA was quantified by qPCR. For each gene, data were normalized to the -2DG -LPS group (assigned a value of 1, dashed line, n=3-6). The mean ± SEM is plotted in all graphs. Statistical significance was determined by two-way ANOVA with Bonferroni correction (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

Supplemental Figure 2



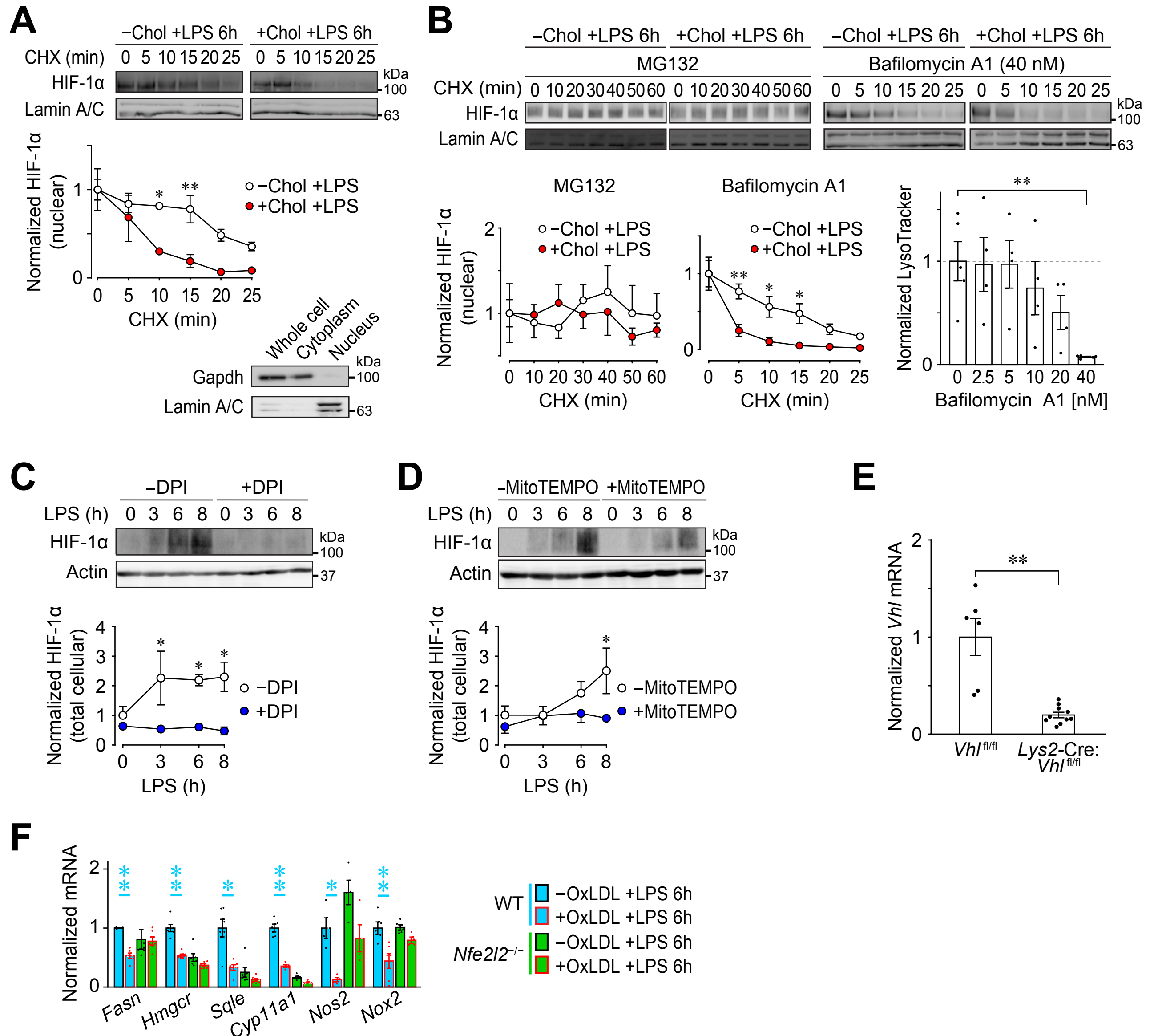
(A and B) Steady-state metabolomics from four groups of PMφs: ±oxLDL (24 h) and ±LPS stimulation (6 h). (A) Bar graphs showing metabolites with significant differences between groups. Values for each metabolite were normalized to the -oxLDL -LPS group (log2 scale, n=18). Metabolites are organized by metabolic pathways: pentose phosphate pathway, Krebs cycle and anaplerotic (intermediary) reactions (glycolysis pathway metabolites are shown in Figure 2). The mean ± SEM is plotted. Statistical significance was determined by two-way ANOVA with Bonferroni correction (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001). (B) Complete metabolomics data expressed as a heat-map with unbiased clustering analysis (n=18).

Supplemental Figure 3



(A) Seahorse analysis of PMφs. Glycolysis stress tests showing ECAR normalized to baseline (assigned a value of 1) ±Chol ±LPS (n=16). The addition of glucose (Glc), oligomycin (OG) and 2-deoxyglucose (2DG) is indicated. Asterisks indicate significant differences in LPS-stimulated cells ± cholesterol (Chol). **(B and C)** Effect of *Hif1a* deficiency (B) and HIF-1α inhibition by echinomycin (C) on inflammatory and glycolysis gene mRNA expression in LPS-stimulated (6 h) Mφs. (B) BMDMφs from *Lys2*-Cre:*Hif1a*$^{fl/fl}$ and *Hif1a*$^{fl/fl}$ Cre-negative littermates were stimulated with LPS for 6 h. (C) WT PMφs were treated with echinomycin or buffer containing DMSO (-echinomycin control) and stimulated with LPS. mRNA was quantified by qPCR. For each gene, values were normalized to the control group (*Hif1a*$^{fl/fl}$ or – echinomycin, assigned a value of 1, dotted line, n=3-7). **(D)** Effect echinomycin on Hk2 protein expression in LPS-stimulated (0 to 8 h) PMφs. A representative immunoblot and quantification showing a LPS time course. For each time point, Hk2 values were normalized to corresponding actin value and the 0 h LPS time point of the DMSO control (– echinomycin) group (assigned a value of 1, n=4 for each data point). **(E)** Effect of cholesterol accumulation and LPS stimulation on the expression of HIF family members in PMφs. Representative immunoblots and quantification of HIF-1α, HIF-2α, HIF-3α, HIF-1β in a LPS time course. For each time point, values were normalized to the corresponding actin value and the -cholesterol 0 h LPS time point (assigned a value of 1, n=3-4). **(F)** Effect of cholesterol accumulation and LPS stimulation on glycolysis gene mRNA expression. mRNA abundance in PMφs was analyzed by qPCR. For each gene, the data were normalized to values of PMφs without cholesterol and LPS stimulation (assigned a value of 1, dashed line, n=4-5). **(G)** Effect of cholesterol accumulation and LPS stimulation on Hk2 protein in PMφs. Representative immunoblots and quantification of Hk2 protein in PMφs ± cholesterol and LPS (n=3, normalization as in E). **(H)** Effect of cholesterol and LPS stimulation on glucose accumulation in PMφs. Representative images of 2-NBDG accumulation (green) and quantification (n=16). Values are normalized to the -cholesterol -LPS group (assigned a value of 1, dashed line). The mean ± SEM is plotted in all graphs. Statistical significance (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001) was determined by two-way ANOVA with Bonferroni correction (A, D-H) or by an unpaired two-tailed student's t-test (B, C).

Supplemental Figure 4



(A) Effect of cholesterol (Chol) accumulation on HIF-1α stability in the nucleus of LPS-stimulated (6 h) PMφs. Representative immunoblots of nuclear extracts show a time course after cycloheximide (CHX) treatment. Values normalized to the corresponding Lamin A/C and the respective pre-CHX (0 min) time point are plotted (the 0 min time point was assigned a value of 1, n=3-4). The immunoblot below shows Gapdh and Lamin A/C abundance in whole cell, cytoplasmic and nuclear subcellular fractionated lysates. **(B)** Proteasomes, not lysosomes, are the primary organelles for HIF-1α degradation in cholesterol-loaded PMφs. PMφs were cultured in media with cholesterol or with ethanol (carrier) for 24 h, then stimulated with LPS (6 h). MG132 or Bafilomycin A1 (40 nM) were added 5 h after LPS stimulation to block protein degradation by the proteasome or lysosome, respectively. After 1 h (6 h post-LPS stimulation) cells were treated with CHX to block protein translation and nuclei were harvested at indicated intervals. Representative immunoblots and quantification are shown. Values normalized to the corresponding Lamin A/C and the respective pre-CHX (0 min) time point are plotted (the 0 min time point was assigned a value of 1, n=5). The graph on the far-right shows the normalized mean fluorescent intensity of lysotracker dye in LPS-stimulated PMφs treated with increasing concentrations of Bafilomycin A1 (timing was as described above). Based on this experiment, the 40 nM concentration was selected for all experiments. **(C, D)** HIF-1α protein levels are dependent on cytoplasmic and mitochondrial reactive oxygen species (ROS). Representative immunoblots and quantification of a LPS time course show the effect of blocking NADPH oxidases with diphenyleneiodonium (DPI) (C) or scavenging of mitochondria-derived ROS by mitoTEMPO (D) on LPS-induced HIF-1α protein levels in PMφs. DPI or mitoTEMPO were added 1 h prior to LPS treatment. Values are normalized to the corresponding actin and the pre-LPS (0 min) time point of the control (-inhibitor) group (the 0 min time point was assigned a value of 1, n=3 for DPI and n=4 for MitoTEMPO). **(E)** *Vhl* mRNA expression in *Vhl*$^{fl/fl}$ and *Lys2*-Cre:*Vhl*$^{fl/fl}$ BMDMφs. qPCR analysis was performed and the data are normalized to mean values of the *Vhl*$^{fl/fl}$ group (assigned a value of 1, n=6-10, unpaired Student's t-test). **(F)** OxLDL accumulation suppresses mRNA expression of NADPH-requiring apoenzymes in LPS-stimulated (6 h) WT, but not *Nfe2l2*$^{-/-}$, BMDMφs. For each gene, qPCR data were normalized to WT cells without oxLDL (assigned a value of 1). Statistical comparisons using the Mann-Whitney U test were within each genotype (p values are blue for WT and green for *Nfe2l2*$^{-/-}$, n=3-6). The mean ± SEM is plotted in all graphs. Unless indicated otherwise statistical significance was determined by a two-way ANOVA with Bonferroni correction (*P<0.05, **P<0.01).