Cell Metabolism

Supplemental Information

Serine metabolism supports macrophage IL-1β production

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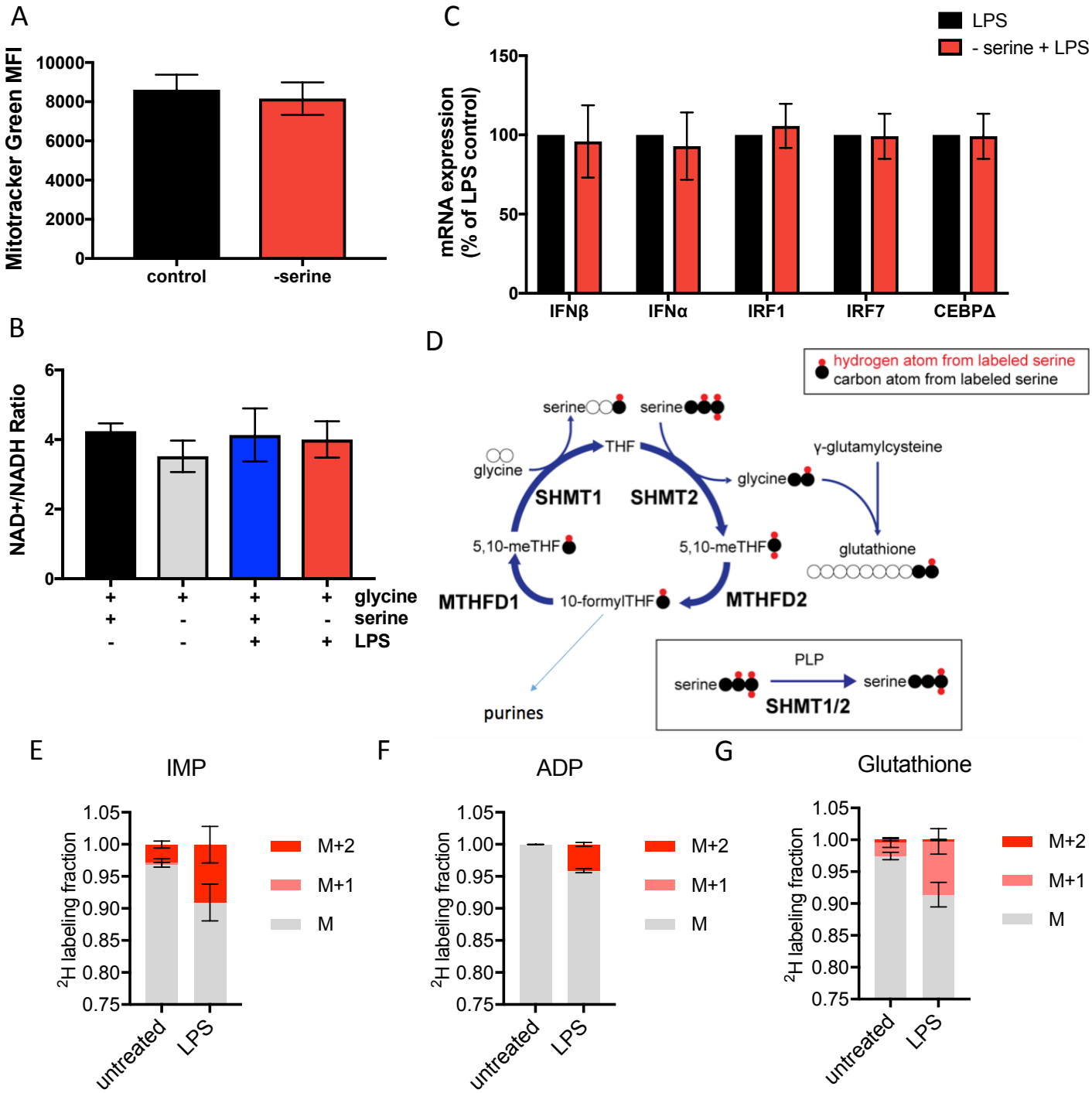

Figure S1, related to Figure 1. Serine deprivation does not affect mitochondrial mass or interferon signaling. 2,3,3-D3 serine carbon labeling shows that upon LPS stimulation, serine is incorporated into GSH.

(A) Mean fluorescence intensity of Mitotracker Green in peritoneal macrophages in control media compared to serine deprived media (n=3).

(B) NAD+/NADH ratio in peritoneal macrophages with or without serine after stimulation with LPS for 4 hours (n=4).

(C) Ifnα, Ifnβ, Ifnγ, Irf1, Irf7 and C/EBPΔ mRNA expression in peritoneal macrophages deprived of serine and treated with 100 ng/mL LPS for 2 hours normalized to LPS treated macrophages. (n=5)

For A-B, E-G, Peritoneal macrophages were treated for 4 hours with 100ng/mL LPS. For A-C, data are shown as mean ± SEM. p values were calculated using a paired one-way ANOVA (A,B) or a two tailed Student's t test (C) compared to control. *p<0.05

(D) Schematic of 2,3,3-D3 Serine labeling.

(E) 2,3,3-D3 serine labeling of IMP in peritoneal macrophages at 4 hours post-LPS.

(F) 2,3,3-D3 serine labeling of ADP in peritoneal macrophages at 4 hours post-LPS.

(G) 2,3,3-D3 serine labeling of glutathione in peritoneal macrophages at 4 hours post LPS. For E-G, data are shown as mean ± SD.

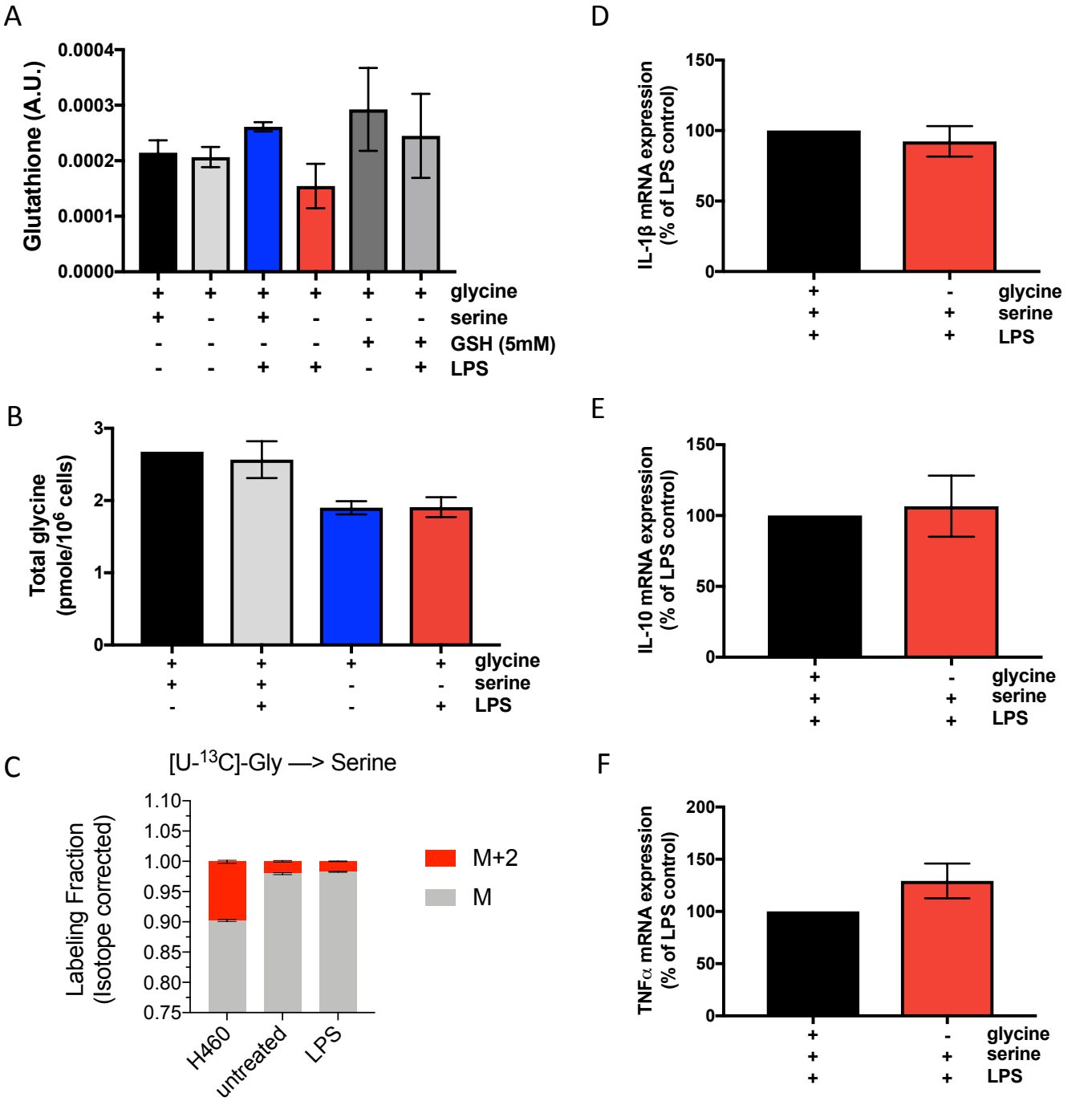

Figure S2, related to Figure 2. GSH ethyl ester supplementation restored GSH levels in peritoneal macrophages. Glycine deprivation did not alter cytokine expression in BMDMs.

(A) GSH levels in peritoneal macrophages treated with LPS for 4 hours in serine depleted media with or without 5mM GSH ethyl ester supplementation (n=5).

(B) Concentration of glycine in peritoneal macrophages (n=3) at 4 hours post LPS with or without serine.

(C) U-[^{13}C]-Glycine labeling of serine in H460 lung cancer cells and peritoneal macrophages treated with LPS for 4 hours (n=3).

(D) IL-1β mRNA expression in BMDMs stimulated with LPS in media with or without extracellular glycine (n=5)

(E) IL-10 mRNA expression in BMDMs stimulated with LPS in media with or without extracellular glycine (n=4)

(F) TNFα mRNA expression in BMDMs stimulated with LPS in media with or without extracellular glycine (n=5)

For B and C, data are shown as mean ± SD.

For D-F, BMDMs were treated for 4 hours with 100ng/mL LPS in the presence or absence of glycine. For A, D-F, data are shown as mean ± SEM. p values were calculated using a paired one-way ANOVA compared to control (A-B) or a two tailed Student's t test (D-F). *p<0.05

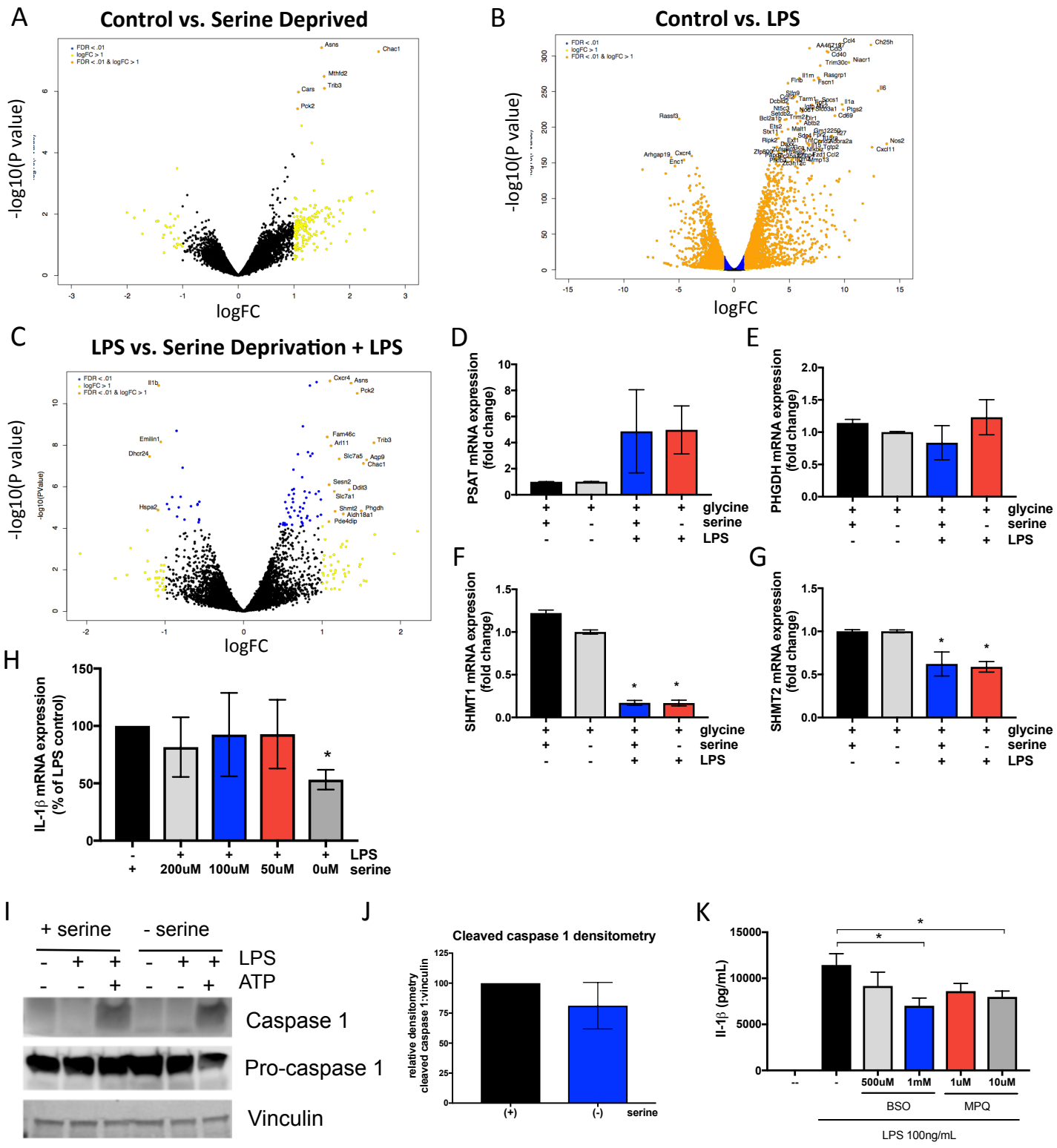

Figure S3, related to Figure 3. Serine deprivation diminishes IL-1β mRNA expression without altering the NLRP3 inflammasome.

(A) RNAseq Volcano plot of genes in Control vs. serine deprived BMDMs for 4 hours (n=4).

(B) RNAseq Volcano plot of genes in Control vs. LPS treated BMDMs for 4 hours (n=4).

(C) RNAseq Volcano plot of genes in LPS treated vs. LPS treated, serine deprived BMDMs for 4 hours (n=4).

(D) mRNA fold change (ΔΔCt normalized to untreated) of PSAT n=5 (E) PHGDH n=7 (F) SHMT1 n=9 (G) SHMT2 n=9 in BMDMs with or without serine stimulated with 100ng/mL LPS for 4 hours normalized to untreated macrophages.

(H) IL-1β mRNA expression in BMDMs stimulated with 100ng/mL LPS for 4 hours in various concentrations of extracellular serine

(I) Representative western blot of cleaved and pro-caspase 1 in BMDMs

(J) Densitometry of cleaved caspase 1 western blot (5 blots)

(K) Protein secretion of IL-1β, n=6 in BMDMs pretreated for 1 hour with BSO or mitoparaquat (MPQ)

For A-K BMDMs were cultured in media with 400μM glycine and with or without 400μM serine.

For I-K, in BMDMs were treated with 100ng/mL LPS for 6 hours and 5mM ATP for 30 minutes.

For D-H, J-K, Data are shown as mean ± SEM. p values were calculated using a paired one-way ANOVA compared to LPS stimulated cells, or a two tailed Student's t test (J). *p<0.05

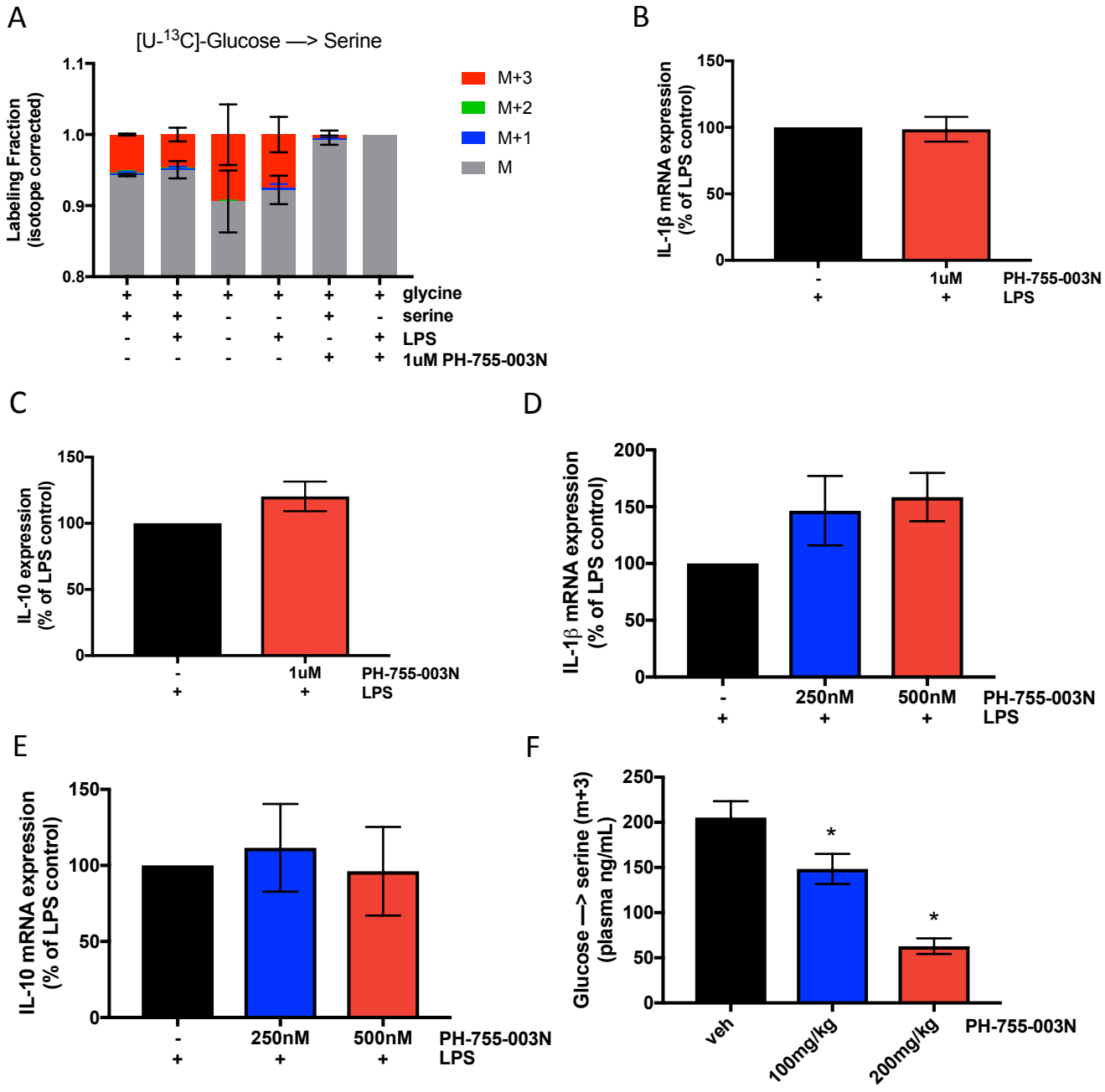

Figure S4, related to Figure 4. PHGDH inhibition in vitro does not replicate in vivo findings.

(A) [U^{13}C]-Glucose incorporation into serine after peritoneal macrophages were stimulated with LPS for 4 hours (n=3). PH-755-003N was given for 1 hr before LPS and for the duration of the LPS treatment.

(B) mRNA expression of IL-1β in peritoneal macrophages after 1 hr pretreatment with 1μM PH-755-003 and LPS stimulation for 4 hrs (n=7)

(C) mRNA expression of IL-10 in peritoneal macrophages after 1 hr pretreatment with 1μM PH-755-003 and LPS stimulation for 4 hrs (n=6)

(D) mRNA expression of IL-1β in BMDMs after 1 hr pretreatment with 1μM PH-755-003 and LPS stimulation for 4 hrs (n=4)

(E) mRNA expression of IL-10 in BMDMs after 1 hr pretreatment with 1μM PH-755-003 and LPS stimulation for 4 hrs (n=4)

(F) Incorporation of [U^{13}C]-Glucose (m+6) into serine (m+3) in the plasma of mice treated with a single oral dose with PH-755-003N at 100 and 200mg/kg.

For B-C, peritoneal macrophages were treated in mouse plasma like media (MPLM) which has physiologic concentrations of 95μM serine, 217μM glycine, and 4.4mM glucose. In D-E, BMDMs were treated in MEM media with 400uM serine and 400uM glycine and 17mM glucose.

For A, Data shown as as mean ± SD.

For B-F, data are shown as mean ± SEM. For B-C, p values were calculated using a two tailed Student's t test compared to LPS stimulated cells. *p<0.05. For D-E, p values were calculated using a paired one-way ANOVA compared to LPS stimulated cells. For F, p values were calculated using an unpaired one-way ANOVA compared to vehicle treated mice. *p<0.05.