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Supplemental information

Nrf2 activation reprograms macrophage intermediary metabolism and suppresses the type I interferon response

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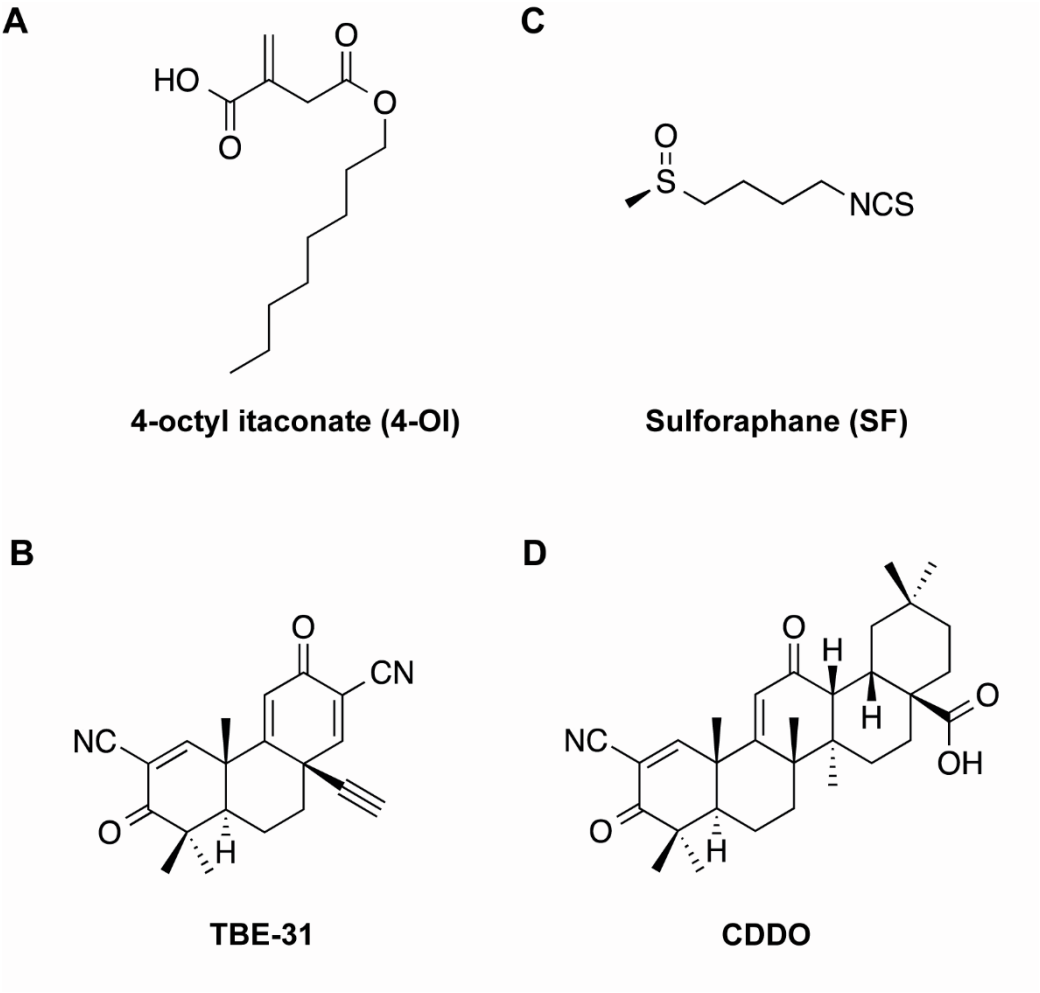

Figure S1 – Chemical structures of pharmacologic Nrf2 activators used in this study, related to STAR Methods

(A) 4-octyl itaconate (4-OI)

(B) Sulforaphane (SF)

(C) (±)-(4b*S*,8a*R*,10a*S*)-10a-ethynyl-4b,8,8-trimethyl-3,7-dioxo-3,4b,7,8,8a,9,10,10a-octahydrophenanthrene-2,6-dicarbonitrile (TBE-31)

(D) (+)-(4a*S*,6a*R*,6b*S*,8a*R*,12a*R*,14a*R*,14b*S*)-11-isocyano-2,2,6a,6b,9,9,12a-heptamethyl-10,14-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,12a,14,14a,14b-octadecahydropicene-4a-carboxylic acid (CDDO)

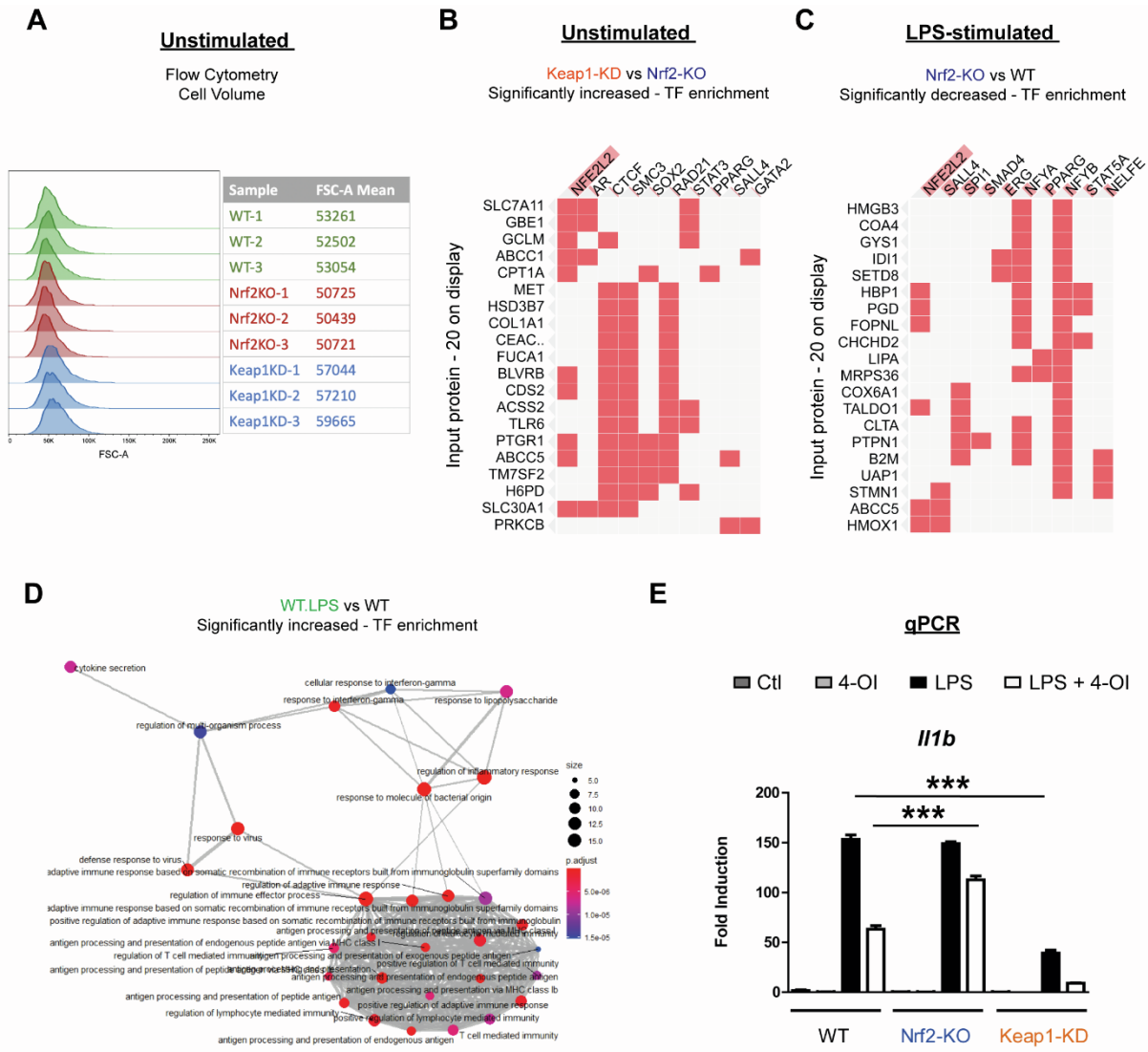

Figure S2 – LPS remodels macrophage proteome, related to Figure 1

(A) Flow cytometry analysis of cell size in unstimulated state

(B) TF enrichment of decreased targets in unstimulated Keap1-KD compared to Nrf2-KO

(C) TF enrichment of decreased targets in LPS stimulated Nrf2-KO compared to WT. ORA by Enricher. Top transcription factors ranked according to combined enrichment score (p value and Z score) using ENCODE and ChEA databases.

(D) Enrichment map of GO: biological processes of significantly increased proteins with LPS stimulation in WT

(E) qPCR (n = 3 biological replicates) validation of 4-OI (125 μM) inhibition of IL-1β **(A, E)** (n = 3 biological replicates). Data are mean ± SEM. p value determined by one-way ANOVA, corrected for multiple comparisons by Tukey statistical test. p <0.05*; p <0.01**; p < 0.001***. **(D)** ORA by clusterProfiler, FDR correction by Bonferroni test **(B-C)** (combined score – p value and z score).

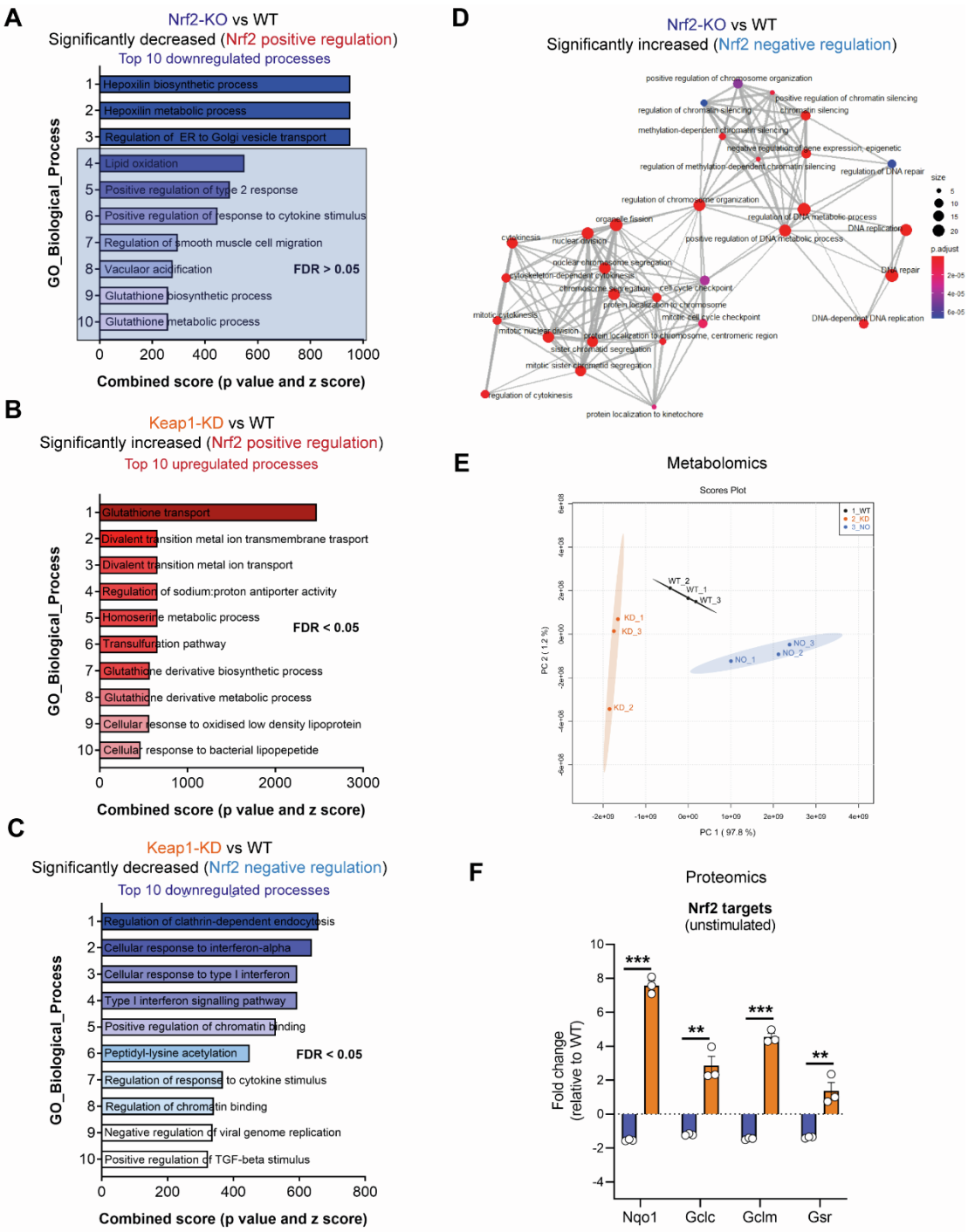

Figure S3 – Enrichment analysis of unstimulated macrophages, related to Figure 2

(A) Enrichment of GO: biological processes of Nrf2 positively regulated targets (Nrf2-KO vs WT)

(B) Enrichment of GO: biological processes of Nrf2 positively regulated targets (Keap1-KD vs WT)

(C) Enrichment of Reactome pathways of Nrf2 negatively regulated targets (Keap1-KD vs WT) **(A-C)** ORA by Enrichr and FDR correction by Bonferroni test. Top processes ranked according to combined enrichment score (p value and Z score).

(D) Enrichment map of GO: biological processes of Nrf2 negatively regulated targets (Nrf2-KO vs WT). ORA by clusterProfiler, FDR correction by Bonferroni test.

(E) PCA plot of metabolomics. Determined by Metaboanalyst 5.0.

(F) Fold change of prototypical Nrf2 targets (Nrf2-KO vs WT or Keap1-KD vs WT). Data are mean ± SEM. p value determined by multiple t tests - one per row, corrected for multiple comparisons by Holm-Sidak test. p <0.05*; p <0.01**; p < 0.001***.

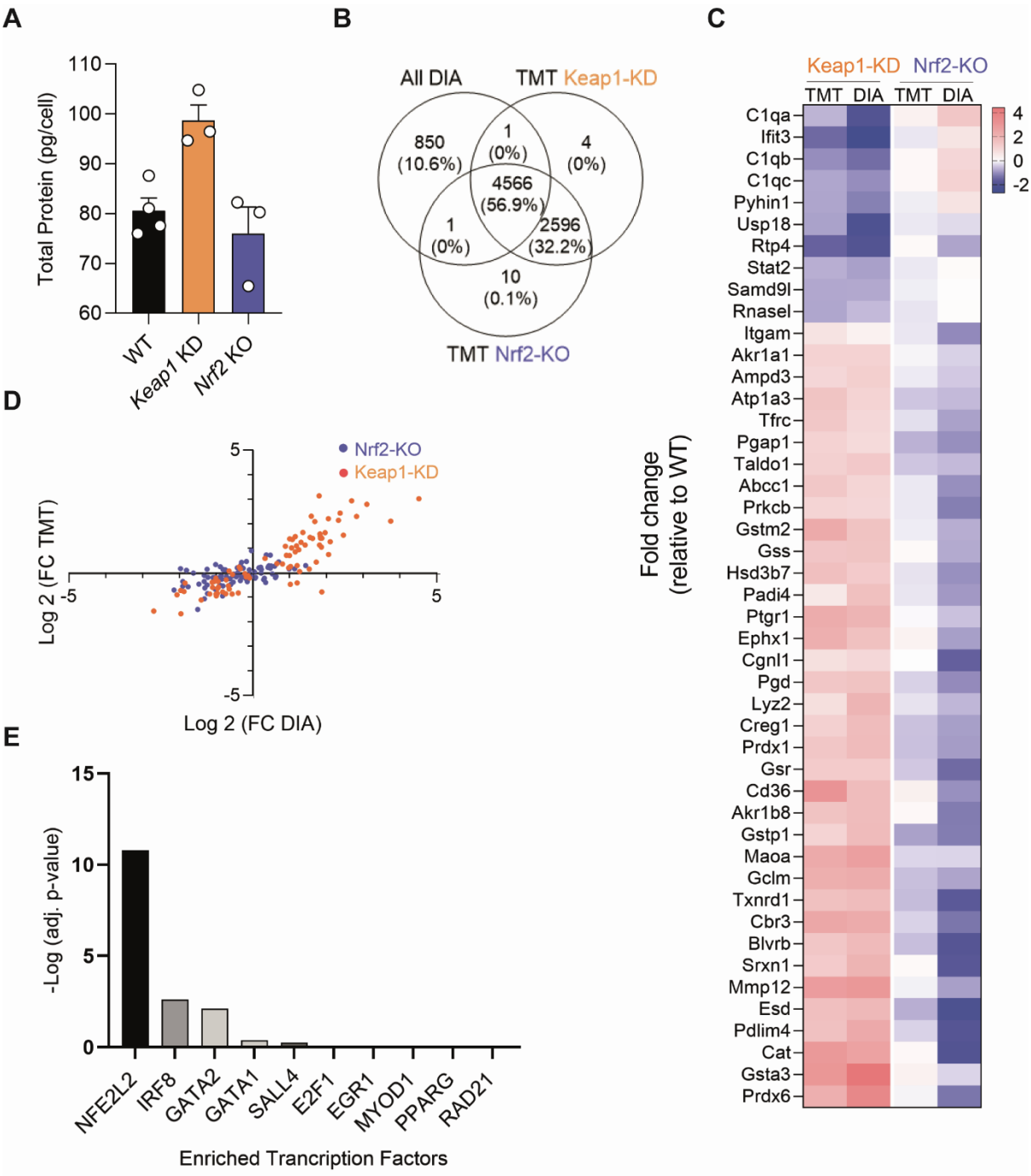

Figure S4 – Data-independent acquisition (DIA) proteomics, related to Figure 2

(A) Total protein content of WT, Nrf2-KO and Keap1-KD macrophages from DIA proteomics. Data are mean ± SEM.

(B) Venn diagram comparing protein hits from TMT and DIA datasets. DIA dataset includes proteins identified in both Nrf2-KO and Keap1-KD.

(C) Heatmap comparing significantly differentially regulated targets identified in TMT and DIA datasets. Data are fold change of Keap1-KD or Nrf2-KO relative to WT.

(D) Correlation plot comparing Log_2FC values of significantly differentially regulated targets identified in TMT and DIA datasets.

(E) TF enrichment of differentially regulated targets in Keap1-KD compared to WT. ORA by Enrichr. Top 10 targets ranked according to adjusted p value.

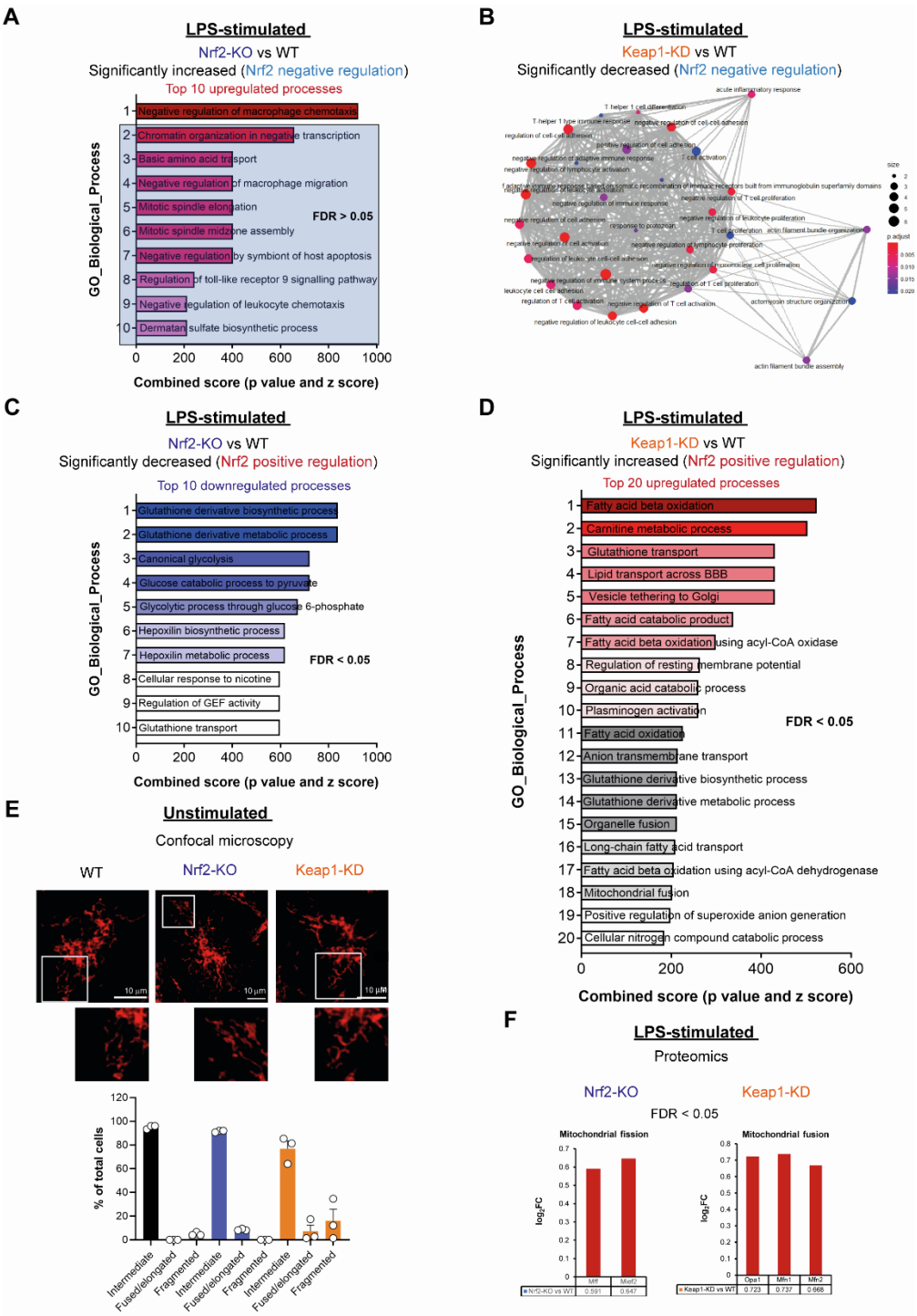

Figure S5 – Enrichment analysis of unstimulated macrophages, related to Figure 3

(A) Enrichment map of GO: biological processes of Nrf2 negatively regulated targets (Nrf2-KO vs WT)

(B) Enrichment map of GO: biological processes of Nrf2 negatively regulated targets (Keap1-KD vs WT) **(A-B)** ORA by clusterProfiler, FDR correction by Bonferroni test.

(C) Enrichment of GO: biological processes of Nrf2 positively regulated targets (Nrf2-KO vs WT)

(D) Enrichment of GO: biological processes of Nrf2 positively regulated targets (Keap1-KD vs WT)

(C-D) ORA by Enrichr and FDR correction by Bonferroni test. Top processes ranked according to combined enrichment score (p value and Z score).

(E) Confocal microscopy of mitochondrial morphology using Tom20 (images are representative, bar plot n = 3 biological replicates). Scale bar 10 μm. Data are mean ± SEM. p value determined by one-way ANOVA, corrected for multiple comparisons by Tukey statistical test.

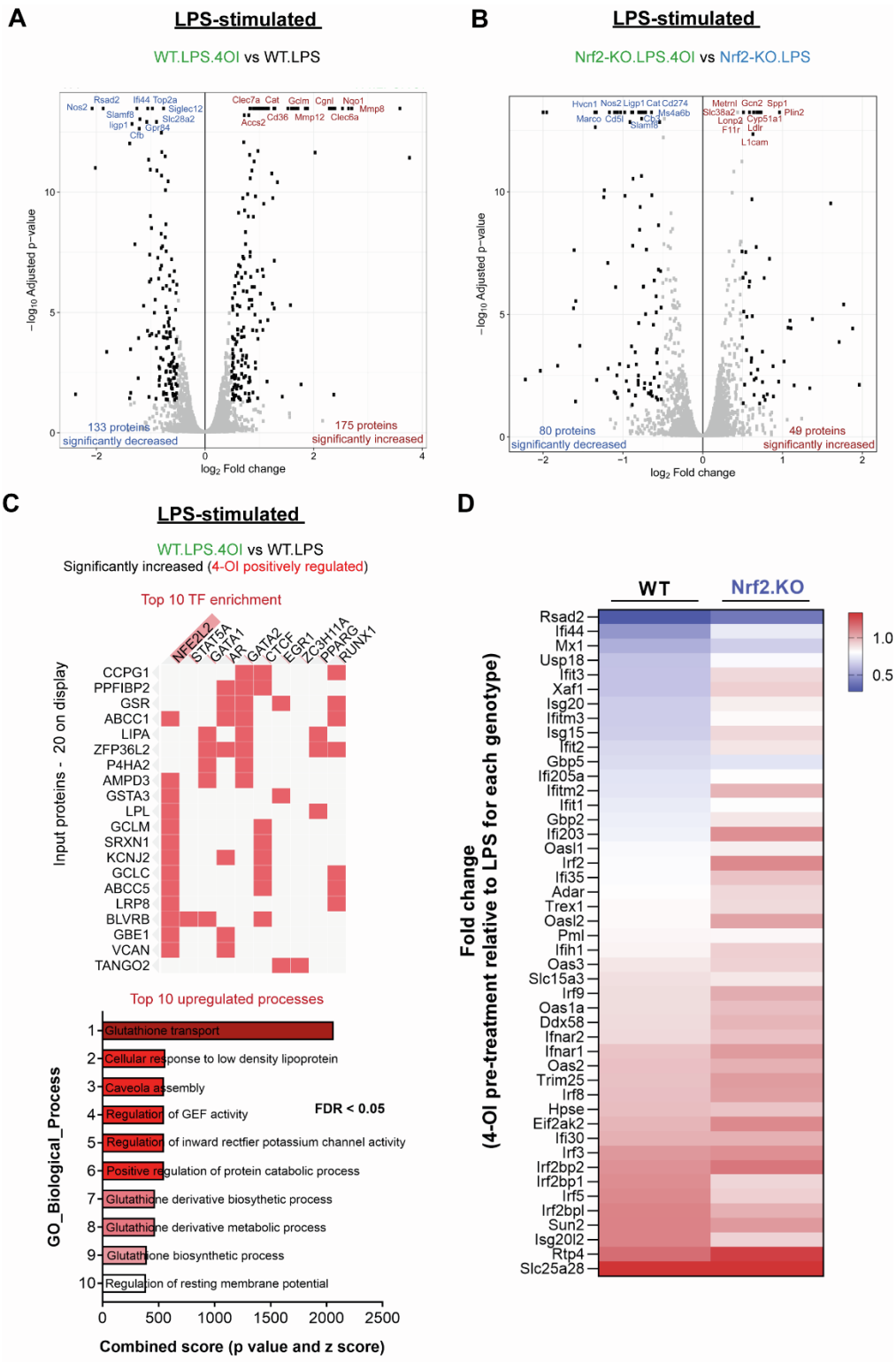

Figure S6 – 4-OI activates Nrf2 and suppresses type I IFN, related to Figure 4

(A) Volcano plot of LPS with 4-OI compared to LPS only (WT)

(B) Volcano plot of LPS with 4-OI compared to LPS only (Nrf2-KO)

(C) Enrichment of TF and GO: biological processes of 4-OI positively regulated targets compared to LPS only (WT). ORA by Enrichr, FDR correction by Bonferroni test. Top processes ranked according to combined enrichment score (p value and Z score).

(D) Heatmap of type I interferon stimulated proteins comparing 4-OI pre-treatment of LPS activated cells to LPS only for each genotype. Data are mean of fold change from 3 biological replicates per condition.

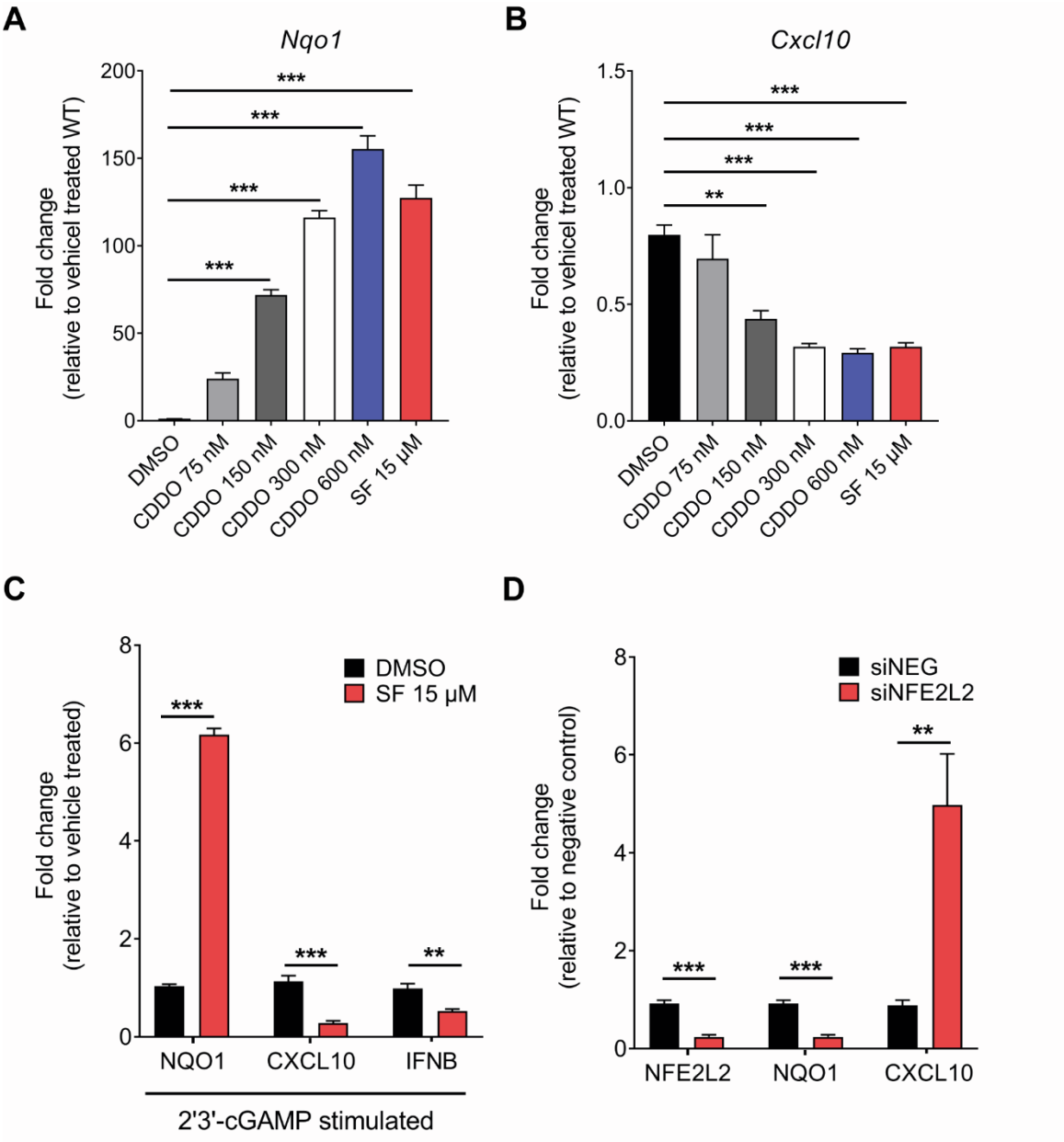

Figure S7 – The pharmacologic Nrf2 activators SF and CDDO downregulate type I IFN response, related to Figure 4

(A) mRNA levels for NQO1 in RAW264.7 cells that had been treated with sulforaphane (SF) or increasing concentrations of CDDO for 24 h (n = 3 biological replicates).

(B) mRNA levels for CXCL10 in RAW264.7 cells that had been treated with sulforaphane (SF) or increasing concentrations of CDDO for 24 h (n = 3 biological replicates). Data are mean ± SEM. p value determined by one-way ANOVA, corrected for multiple comparisons by Tukey statistical test. p <0.05*; p <0.01**; p < 0.001***.

(C) mRNA levels for NQO1, CXCL10 and IFNB in PMA-differentiated THP1 cells that had been treated with SF for 24 h, and then transfected with 2'3'-cGAMP for a further 6 h (n = 3 biological replicates).

(D) mRNA levels for NFE2L2, NQO1 and CXCL10 in PMA-differentiated THP1 cells that had been treated with si*NFE2L2* or siNeg control and harvested 48 h post-siRNA treatment. Note in each case the reciprocal relation between the expression of NQO1 and CXCL10.

Data are mean ± SEM. p value determined by multiple t tests - one per row, corrected for multiple comparisons by Holm-Sidak test. p <0.05*; p <0.01**; p < 0.001***.