

# Supplementary Information to

## A ketogenic diet can mitigate SARS-CoV-2 induced systemic reprogramming and inflammation

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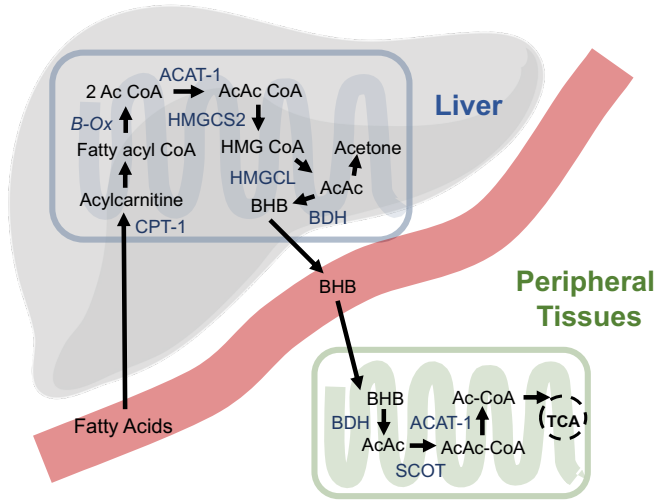
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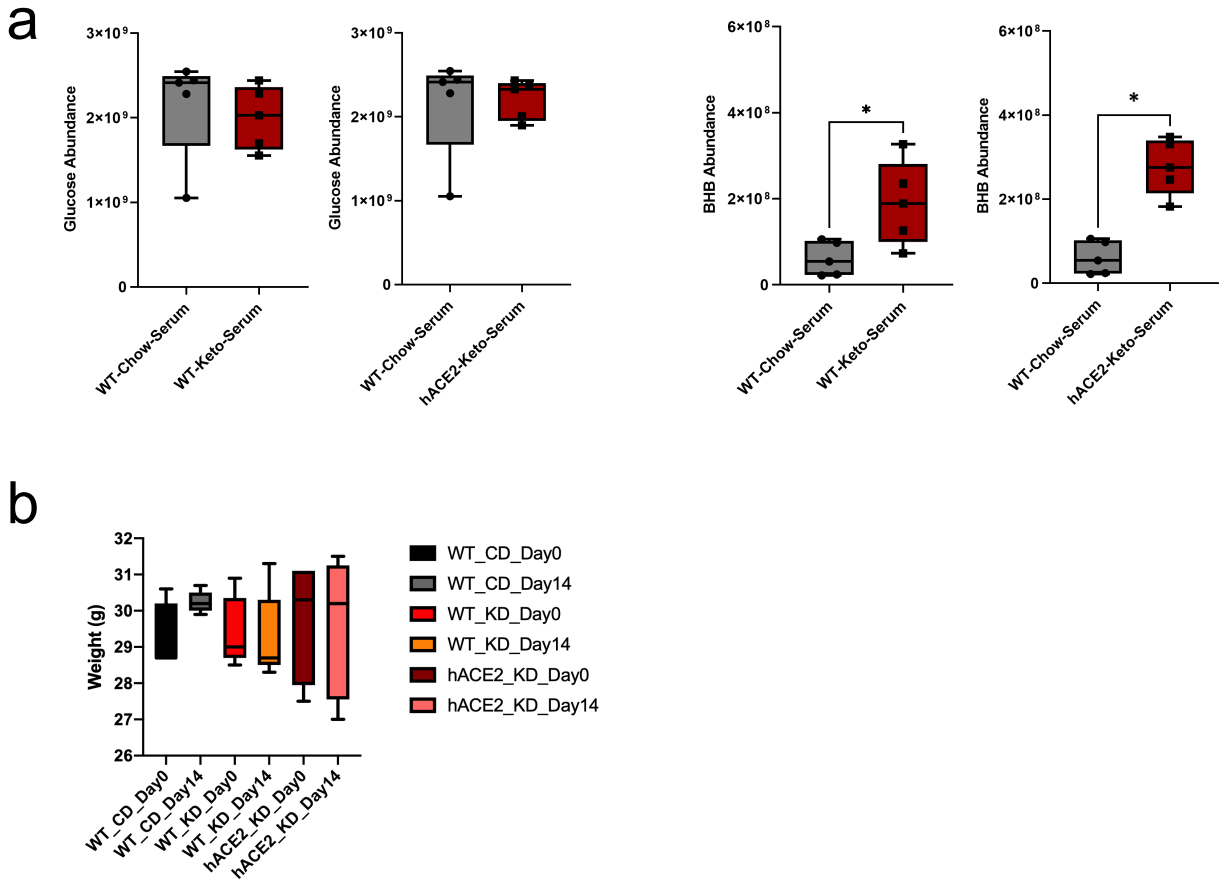
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Ketogenesis

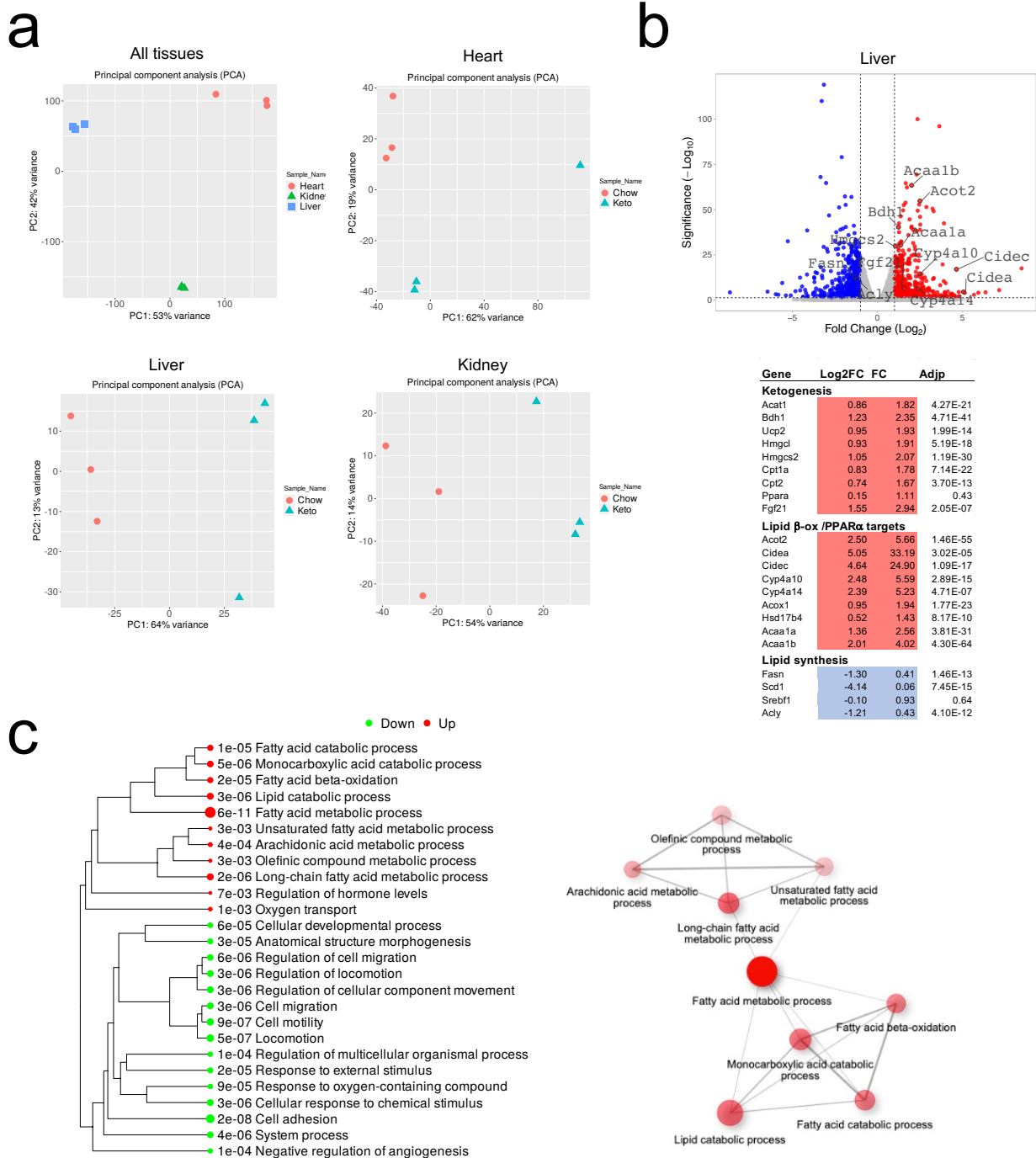


Ketolysis

Supplementary Figure 1. Schematization of ketogenic metabolism.

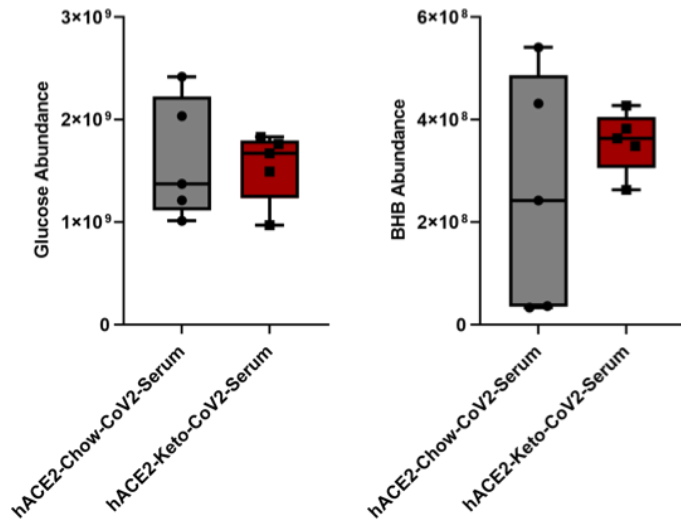


**Supplementary Figure 2. a)** glucose and  $\beta$ -hydroxybutyrate (BHB) abundance in CD, KD WT and hACE2+ KD mice in serum (\* for significance  $p < 0.05$ ,  $n=5/\text{group}$ ); **b)** Body weight changes during KD induction in WT and hACE2+ mice, and CD control (followed over 2 weeks of diet).

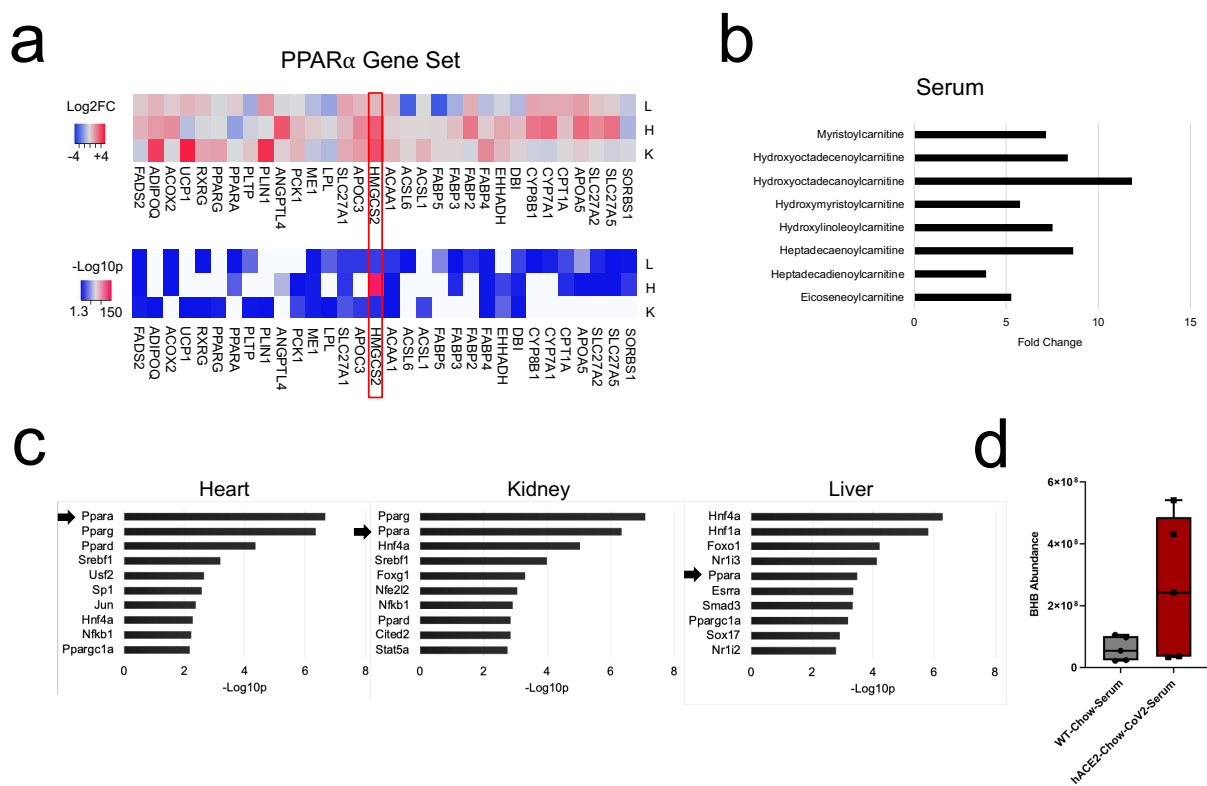


**Supplementary Figure 3. a)** principal component analysis of RNA-seq data for heart, liver and kidney in hACE2+ mice under CD or KD (both uninfected); **b)** volcano plot of differentially regulated genes in hACE2+ KD vs hACE2+ CD mice (both uninfected) and table showing log2FC

and adjusted p values for up- and down-regulated genes in the ketogenesis, lipid  $\beta$ -oxidation/PPAR- $\alpha$  targets and lipid synthesis pathways; **c)** GO gene set network enrichment analysis of differentially regulated genes in RNA-sequencing data (liver) in hACE2+ KD Mock vs hACE2+ CD Mock mice (red, upregulated pathways, green, downregulated pathways).

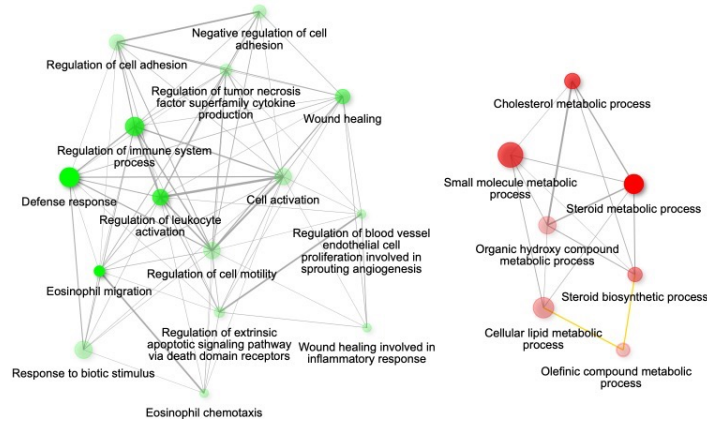


**Supplementary Figure 4.** Glucose and  $\beta$ -hydroxybutyrate (BHB) levels in mice serum in CD or KD SARS-CoV2 infected mice ( $n=5$ /group, significance threshold  $p < 0.05$ ).



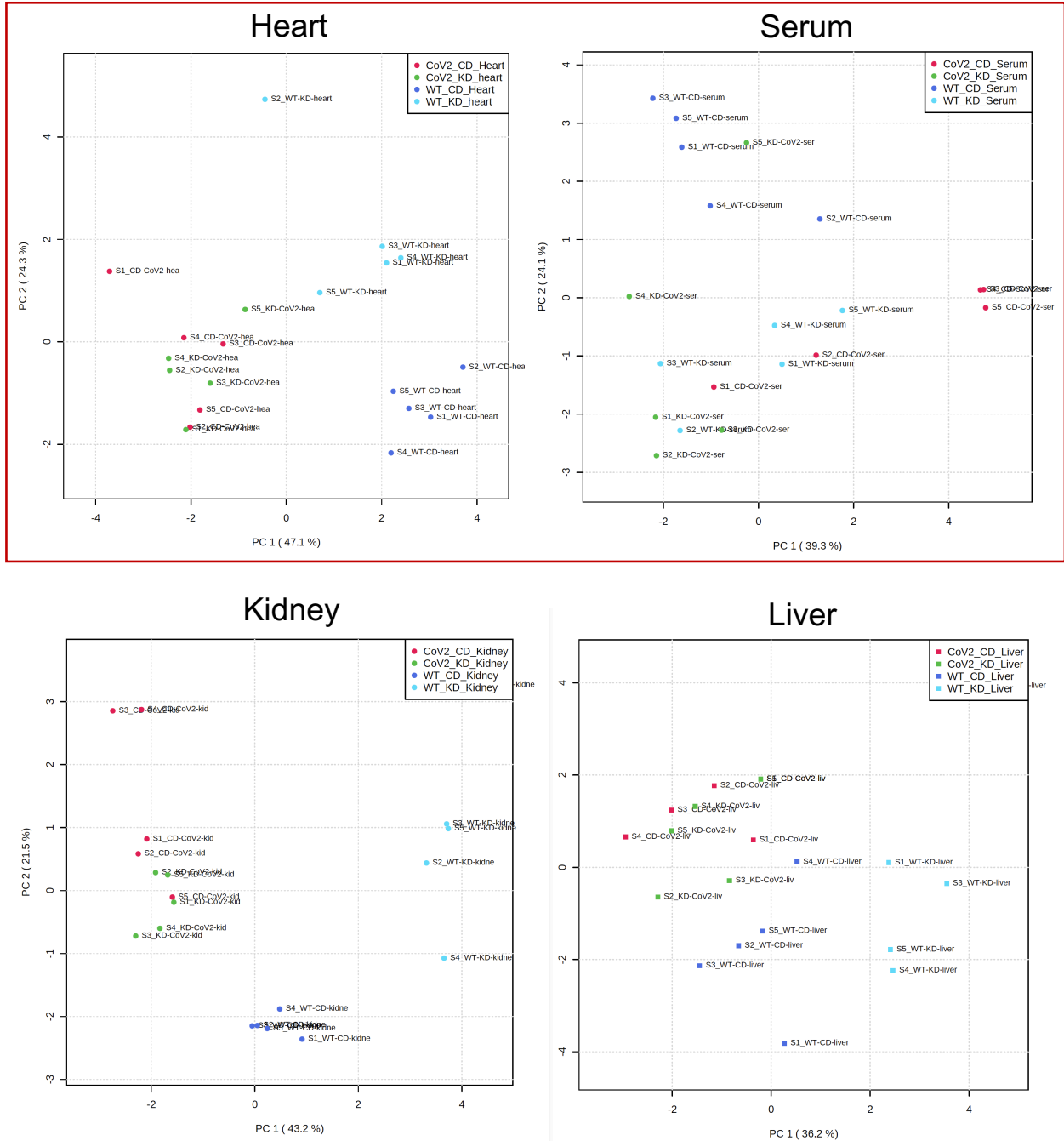
**Supplementary Figure 5. a)** PPAR $\alpha$  gene set enrichment analysis for liver, heart and kidney tissues at 7 days from infection showing significant up regulation of hydroxymethylglutaryl-CoA synthase 2 (HMGCS2) across all tissues in CD mice ( $n=3/\text{group}$ ,  $p < 0.05$  and fold change  $> |1|$ ); **b)** fold change of acylcarnitine abundances in mice liver after 7 days from infection in CD (significance  $p < 0.05$ , fold change  $> |2|$ ) ( $n=5/\text{group}$ ); **c)** transcriptional regulatory network analysis of RNA-sequencing data (first 500 DEGs ranked according to adjusted significance  $p$  value) from mice liver, heart, and kidney, after 7 days from infection with SARS-CoV2, constructed using the TRRUST v2 dataset. Bars represent  $-\log_{10}$  of transcriptional regulatory network significance, arrows highlight the PPAR $\alpha$  transcriptional regulatory network for each tissue ( $n=3/\text{group}$ ); **d)** serum levels of 3 $\beta$ -hydroxybutyrate (BHB) after 7 days from infection in CD mice ( $n=5/\text{group}$ ).

Hearth KD	CoV-2 vs CD	CoV-2			
Direction	adj.Pval	nGenes	Pathways		Genes
Down regulated	0.00116021	11	Defense response		Timp1, Hmox1, Adam8, Lcn2, Lrp8, Clec4d, Ccl2, Thbs1, Chl3, Mmp12, Lgals3,
Down regulated	0.00116021	3	Eosinophil migration		Adam8, Ccl2, Lgals3
Down regulated	0.00217031	10	Regulation of immune system process		Hmox1, Fstl3, Adam8, Lrp8, Gpnmb, Clec4d, Ccl2, Thbs1, Mmp12, Lgals3
Down regulated	0.00217031	7	Regulation of leukocyte activation		Hmox1, Adam8, Gpnmb, Clec4d, Ccl2, Thbs1, Lgals3
Down regulated	0.00312756	6	Wound healing		Timp1, Hmox1, Tnc, Ccl2, Thbs1, Mmp12
Down regulated	0.00453005	3	Regulation of extrinsic apoptotic signaling pathway via death domain receptors		Hmox1, Thbs1, Lgals3
Down regulated	0.00453005	4	Regulation of tumor necrosis factor superfamily cytokine production		Adam8, Gpnmb, Ccl2, Thbs1
Down regulated	0.00453005	2	Regulation of blood vessel endothelial cell proliferation involved in sprouting angiogenesis		Hmox1, Thbs1
Down regulated	0.00453005	8	Regulation of cell motility		Timp1, Hmox1, Adam8, Gpnmb, Ccl2, Thbs1, Mmp12, Lgals3
Down regulated	0.00453005	5	Negative regulation of cell adhesion		Adam8, Tnc, Gpnmb, Thbs1, Mmp12
Down regulated	0.00453005	9	Response to biotic stimulus		Ankrd1, Adam8, Lcn2, Lrp8, Clec4d, M2, Ccl2, Mmp12, Lgals3
Down regulated	0.00453005	8	Cell activation		Timp1, Hmox1, Adam8, Gpnmb, Clec4d, Ccl2, Thbs1, Lgals3
Down regulated	0.00453005	7	Regulation of cell adhesion		Fstl3, Adam8, Tnc, Gpnmb, Ccl2, Thbs1, Mmp12
Down regulated	0.00453005	2	Wound healing involved in inflammatory response		Timp1, Hmox1
Down regulated	0.00453005	2	Eosinophil chemotaxis		Ccl2, Lgals3
Up regulated	1.80E-06	11	Steroid metabolic process		Cyp17a1, Ear1, Srd5a1, Soat2, Tm7sf2, Hmgcs2, Hnf1a, Scd1, Serpina12, Dhcr7, Saa1
Up regulated	9.11E-05	7	Cholesterol metabolic process		Soat2, Tm7sf2, Hmgcs2, Hnf1a, Serpina12, Dhcr7, Saa1
Up regulated	0.00011135	20	Small molecule metabolic process		Cyp17a1, Acaca, Srd5a1, Soat2, Acat2, Tm7sf2, Dhkd1, Hmgcs2, Hnf1a, Bco2, Amt, Scd1, Serpina12, Nage, Dhcr7, Cyp2c70, Gdf2, Cyp4f15, Saa1, Urad
Up regulated	0.00071679	6	Steroid biosynthetic process		Cyp17a1, Srd5a1, Tm7sf2, Hmgcs2, Hnf1a, Dhcr7
Up regulated	0.00189709	13	Cellular lipid metabolic process		Ear1, Acaca, Srd5a1, Soat2, Acat2, Hmgcs2, Hnf1a, Plk3c2g, Bco2, Scd1, Serpina12, Cyp2c70, Cyp4f15
Up regulated	0.00307265	9	Organic hydroxy compound metabolic process		Srd5a1, Soat2, Tm7sf2, Hmgcs2, Hnf1a, Serpina12, Dhcr7, Cyp4f15, Saa1
Up regulated	0.00421454	5	Olefinic compound metabolic process		Cyp17a1, Srd5a1, Bco2, Cyp2c70, Cyp4f15

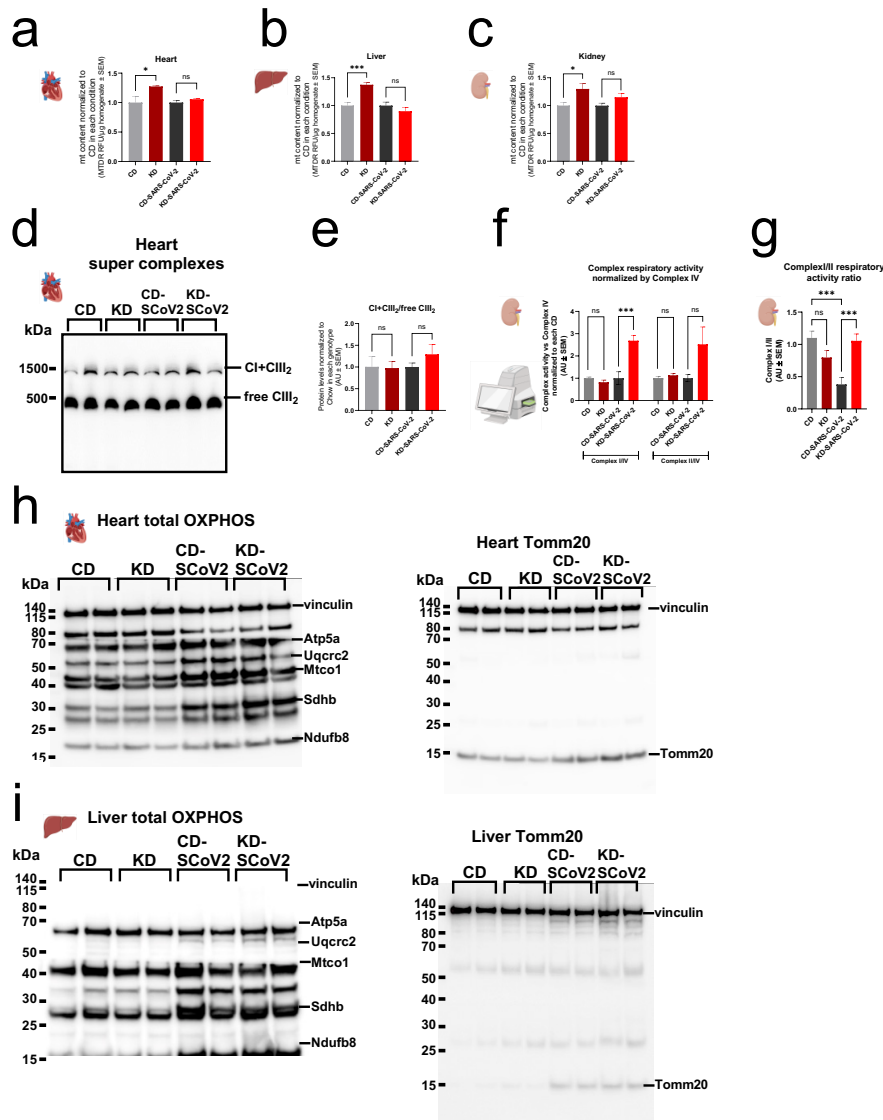


**Supplementary Figure 6.** GO gene set network enrichment analysis of differentially regulated genes in RNA-sequencing data in heart in SARS-CoV2 infected mice (KD vs CD). Enrichment analysis for DEGs was performed using the DESeq2 method with FDR cutoff 0.1 and minimum fold change 2, and gene sets from the GO Biological Process repository. Two nodes (pathways) are connected if they share >30% genes, red represents up regulated pathways, darker nodes are more significantly enriched gene sets, bigger nodes represent larger gene sets, ticker edges represent more overlapping genes.

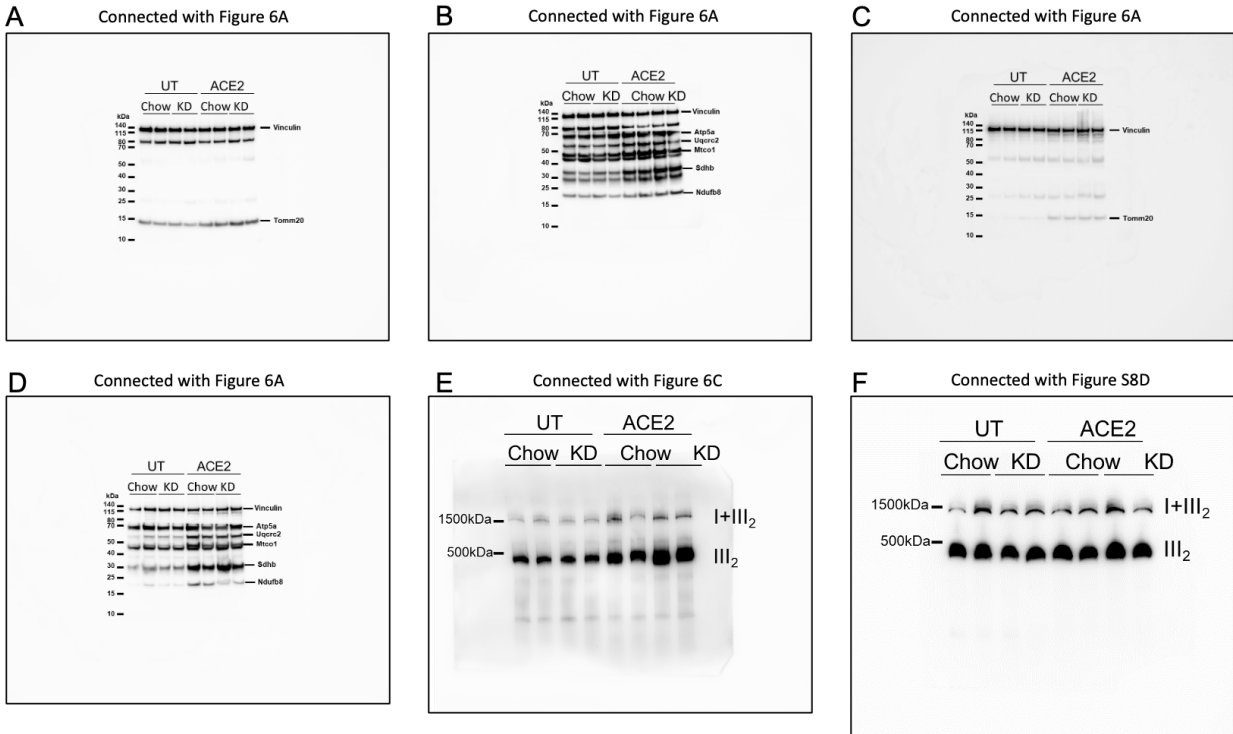




**Supplementary Figure 7.** Principal component analysis of metabolomics data showing significant changes in at least one tissue/condition ( $p < 0.05$  and  $FC > |1|$ ) for heart, serum, kidney, and liver in SARS-CoV-2 infected/uninfected (control) mice under KD or CD ( $n=5$ /group).



**Supplementary Figure 8.** Mitochondrial mass analysis in **a)** heart, **b)** liver, **c)** kidney. **d)** Immunoblot of blue native gel electrophoresis stained for Uqcrc2 in the heart. **e)** Quantification of assembled CI+CIII<sub>2</sub> versus unassembled CIII<sub>2</sub> respirasome ratios in the heart normalized to each CD condition. Graphs represent mean ±SEM of N=4 biological replicates. **f)** Respiratory activity of complexes I and II normalized by complex IV and each CD condition in the kidney measured by RIFS. Graphs represent mean ±SEM of N=5-10 biological replicates. **g)** Respiratory ratios of complex I/II in the kidney. Graphs represent mean ±SEM of N=5-10 biological replicates. **h, i)** Whole membrane images of total OXPHOS staining (complex I=Ndufb8, complex II=Sdhd, complex III=Uqcrc2, complex IV=Mtco1, complex V=Atp5a, Tomm20) and vinculin from heart and liver. Statistical significance for pairwise comparisons in A, B, C, E, and F was determined using unpaired 2-tailed Student's t-test or Mann-Whitney test where appropriate. Statistical significance of group comparisons in G was determined by One-way ANOVA with Holm-Šidák's multiple comparisons test. \* = p < 0.05, \*\* = p < 0.01, \*\*\* = p < 0.001.



**Supplementary Figure 9. a-f) uncropped seahorse immunoblots.**

(CoV+ vs Mock) CD						
Gene	Liver		Kidney		Heart	
	log2FoldCha	padj	log2FoldCha	padj	log2FoldCha	padj
App	-0.3423153	0.00483703	-0.238524	0.0054805	-0.0446387	0.7856388
Casp1	-0.1093449	0.7651363	0.818861	0.03854891	0.3002827	0.5885465
Hsp90ab1	0.3233307	0.03626698	-0.5408839	3.5627E-07	-0.195772	0.09447505
Mefv	-0.1652302	0.8009388	0.1839004	0.9303023	-1.627225	0.3714338
Nlrp3	0.5238939	0.2897234	0.3420884	0.6782744	0.1504231	0.8987734
P2rx7	-0.3889939	0.1690553	0.2183965	0.5485049	-0.1858189	0.5549637
Panx1	-0.1365616	0.890656	0.3118183	0.6757288	0.01509982	0.9867074
Pstpip1	0.5233991	0.6164818	-0.0121071	0.9899481	0.07730529	0.9294771
Pycard	-0.2876032	0.6650046	0.08115971	0.8126235	-0.8352595	0.0025423
Sugt1	-0.0480264	0.8199031	-0.14305	0.2834837	0.1887355	0.274736
Txn1	0.1468964	0.3849401	-0.011905	0.9402218	0.3965358	0.01726207
Txnip	-0.7109239	0.00205565	0.94375542	5.1151E-17	1.358478	0.00560716

(CoV+ vs Mock) KD						
Gene	Liver		Kidney		Heart	
	log2FoldCha	padj	log2FoldCha	padj	log2FoldCha	padj
App	-0.0900725	0.6216856	-0.2873438	0.00158866	-0.1307284	0.6251281
Casp1	0.95826911	0.00050974	1.3639762	0.00229168	0.2272381	0.7156191
Hsp90ab1	0.1536993	0.2454463	-0.4073162	9.59E-06	-0.0437195	0.851374
Mefv	0.6655186	0.5156681	-0.0239098	0.5	-0.1954177	0.5
Nlrp3	1.49572097	9.37E-05	0.3343609	0.7462106	0.6352095	0.6748814
P2rx7	-0.0481552	0.9063406	-0.1650734	0.6885929	-0.1651845	0.790371
Panx1	0.6544571	0.3913517	0.7336121	0.2883224	0.07396773	0.9536502
Pstpip1	0.7776461	0.3616439	-0.0733855	0.9357134	0.3401629	0.7749411
Pycard	0.3681263	0.5261097	0.60198056	0.00701464	-0.4982977	0.3441938
Sugt1	0.0060055	0.9828179	0.1892025	0.1758379	-0.0574606	0.8659103
Txn1	-0.2943571	0.03422323	0.01261635	0.9539017	-0.0863247	0.8135332
Txnip	-0.3579914	0.3803984	0.1414272	0.3366593	0.1910114	0.3029226

(CoV+ KD vs Mock CD)						
Gene	Liver		Kidney		Heart	
	log2FoldCha	padj	log2FoldCha	padj	log2FoldCha	padj
App	-0.0458794	0.9442773	0.00017191	0.9997431	0.00694549	0.9974641
Casp1	0.83098482	0.0273742	0.4409857	0.7344761	0.06868844	0.9783912
Hsp90ab1	-0.0203087	0.9859957	0.24088217	0.30376346	0.08495171	0.8707698
Mefv	-0.6621344	0.5	-0.5956393	0.5	-0.1259762	0.5
Nlrp3	0.3953297	0.7403654	0.06067101	0.5	0.15296	0.9728533
P2rx7	0.2412895	0.8244944	-0.1564205	0.9482943	-0.0603667	0.9783419
Panx1	0.6120604	0.5	0.3964755	0.5	0.01937028	0.9974641
Pstpip1	0.7151182	0.5	0.02577697	0.9972775	-0.3726863	0.8546449
Pycard	0.94850844	0.31653556	0.2420692	0.8535377	-0.3919385	0.7044471
Sugt1	0.09159173	0.9126346	0.1262393	0.848884	-0.0975446	0.9137739
Txn1	0.1465255	0.778751	-0.0745583	0.937866	-0.0020518	0.9997147
Txnip	-0.0101596	0.9941421	-0.0766517	0.946901	-0.2102992	0.34853195

**Supplementary Figure 10.** NLRP3 inflammasome transcriptional gene set analysis for infected mice under CD or KD. In red:  $p < 0.05$  ( $n=3$ ).