

Supporting Information

p53 regulates skeletal muscle mitophagy and mitochondrial quality control following denervation-induced muscle disuse

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Table S1. List of antibodies

Antibody	Manufacturer	Catalog No.	Molecular Weight (kDa)
A-Tublin	Millipore-Sigma	CP06-100UG	60
ATF 5	Abcam	ab60126	31
ATF4 (CREB2)	Santa Cruz Biotechnology	SC-200	50
ATG 7	Sigma-Aldrich	A2856-25uL	75
Bax	Santa Cruz Biotechnology	sc23959	23
Beclin 1	Cell Signaling	3738S	60
Cathepsin D	Santa Cruz Biotechnology	SC-6486	25-37
CHOP	Santa Cruz Biotechnology	SC-7351	27
ClpP	Abcam	ab124822	37
COX I	Abcam	ab14705	39
CPN10	Enzo Life Sciences	ADI-SPA-110	10
H2B	Cell Signaling	2934S	15
LC3 (A/B)	Cell Signaling	4108S	14-16
LonP	Cell Signaling	28020S	100
mtHSP70	Enzo Life Sciences	ADI-SPS-825-F	75
p53	<i>made in house</i>		50
p62 (SQSTM1)	Abcam	ab56416	62
Parkin	Cell Signaling	4211S	52
PGC-1a	Millipore-Sigma	AB3242	100
PINK 1	Santa Cruz Biotechnology	sc-33796	50
TFE3	Sigma-Aldrich	HPA023881-100UL	72
TFEB	Bethyl Laboratories	A303-673A	50
TFEB	My Bio Source	MBS120432	50
YY1(H-10)	Santa Cruz Biotechnology	SC-7341	60

Table S2. List of primer oligonucleotide sequences used in real-time quantitative PCR analysis for *mus musculus*

Gene	Organism	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>PGC-1a</i> (<i>Ppargc1a</i>)	<i>Mus musculus</i>	TTC CAC CAA GAG CAA GTA T	CGC TGT CCC ATG AGG TAT T
<i>Atf4</i>	<i>Mus musculus</i>	GCC GGT TTA AGT TGT GTG CT	CTG GAT TCG AGG AAT GTG CT
<i>Atf5</i>	<i>Mus musculus</i>	TGG AGC GGG AGA TCC AGT A	GAC GCT GGA GAC AGA CGT ACA
<i>Tfeb</i>	<i>Mus musculus</i>	AGC TCC AAC CCG AGA AAG AGT TTG	CGT TCA GGT GGC TGC TAG AC
<i>Lc3</i> (<i>Maplc3a</i>)	<i>Mus musculus</i>	GCT TGC AGC TCA ATG CTA AC	CCT GCG AGG CAT AAA CCA TGT A
<i>p62</i> (<i>Sqstm1</i>)	<i>Mus musculus</i>	TGT GGT GGG AAC TCG CTA TAA	CAG CGG CTA TGA GAG AAG CTA T
<i>Parkin</i> (<i>Park2</i>)	<i>Mus musculus</i>	GTC TGC AAT TTG GTT TGG AGT A	GCA TCA TGG GAT TGT CTC TTA AA
<i>B2m</i>	<i>Mus musculus</i>	GGT CTT TCT GGT GCT TGT CT	TAT GTT CGG CTT CCC ATT CT
<i>Gapdh</i>	<i>Mus musculus</i>	AAC ACT GAG CAT CTC CCT CA	GTG GGT GCA GCG AAC TTT AT

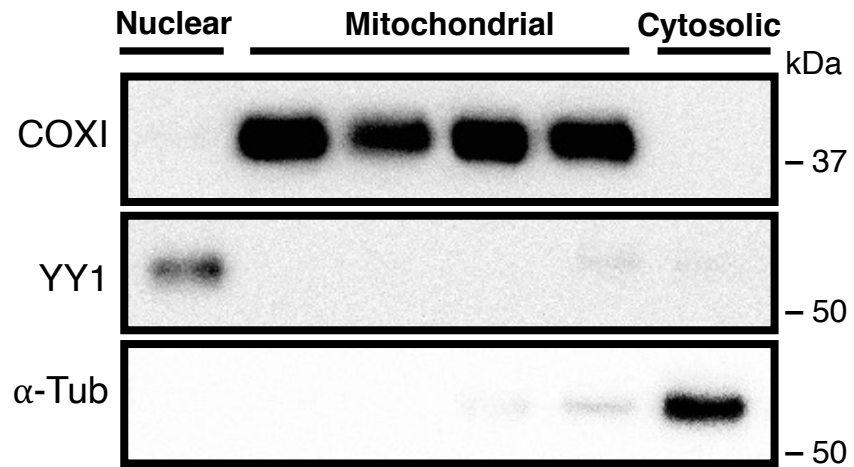


Figure S1. Nuclear, mitochondrial and cytosolic fraction purity in 7 day control and denervated gastrocnemius muscle.

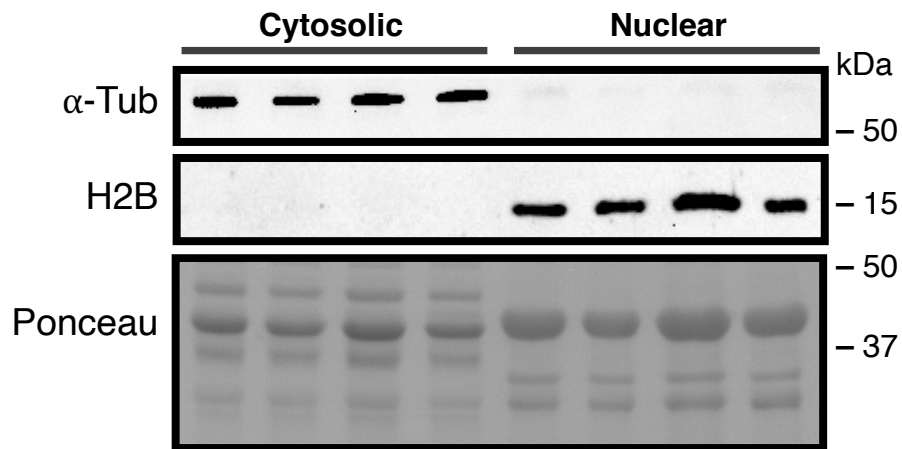


Figure S2. Nuclear and cytosolic fraction purity in 1 day control and denervated gastrocnemius muscle.

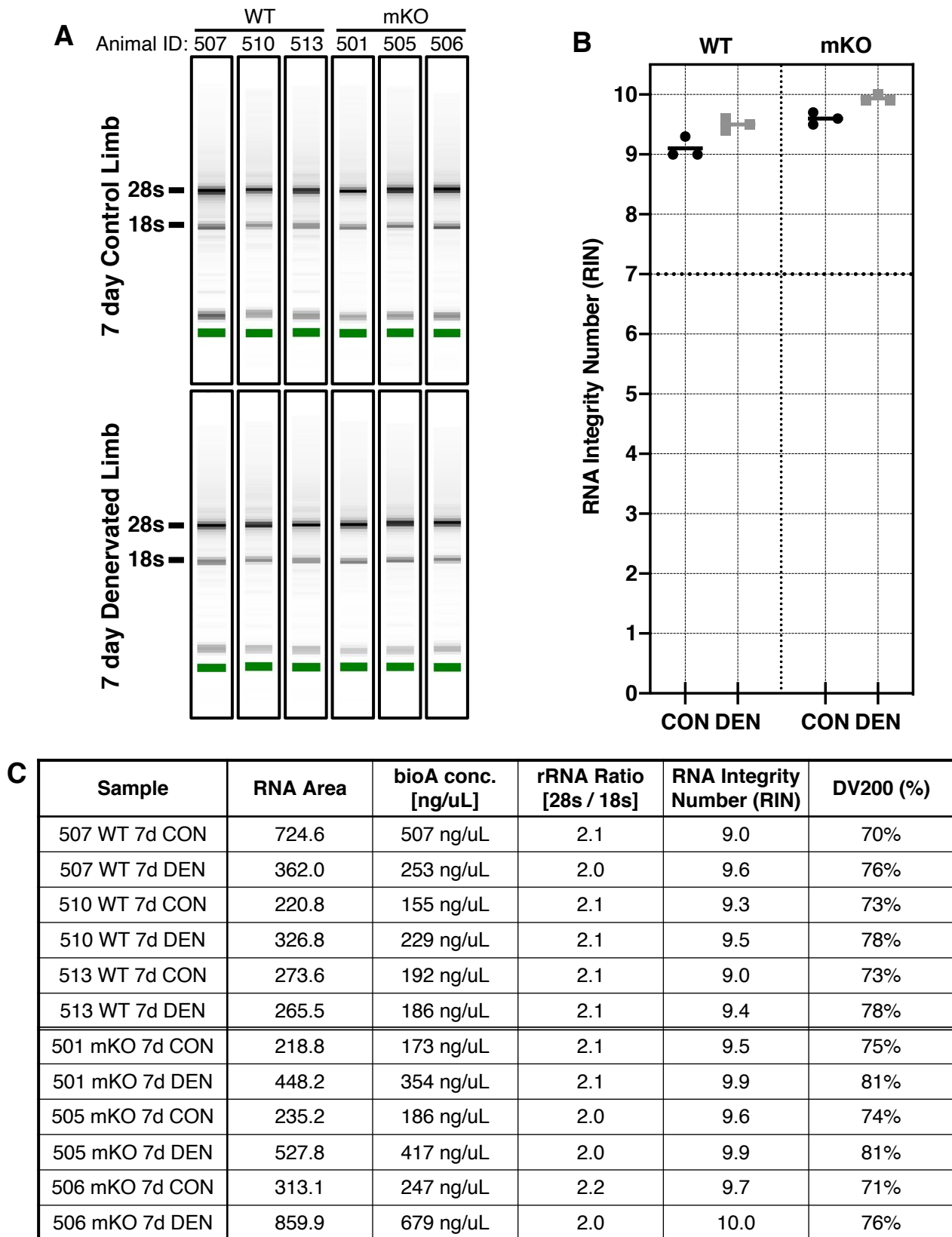


Figure S3. Bioanalyzer quality control report for mRNA and cDNA samples prepared for RNA sequencing. **(A)** Representative RIN tracings from all samples used in RNAseq analysis. **(B)** RNA integrity was similar for across all samples, with thresholds set at rRNA ratio ≥ 2.0 ; RIN ≥ 7 ; and DV200% $\geq 70\%$ **(C)**.

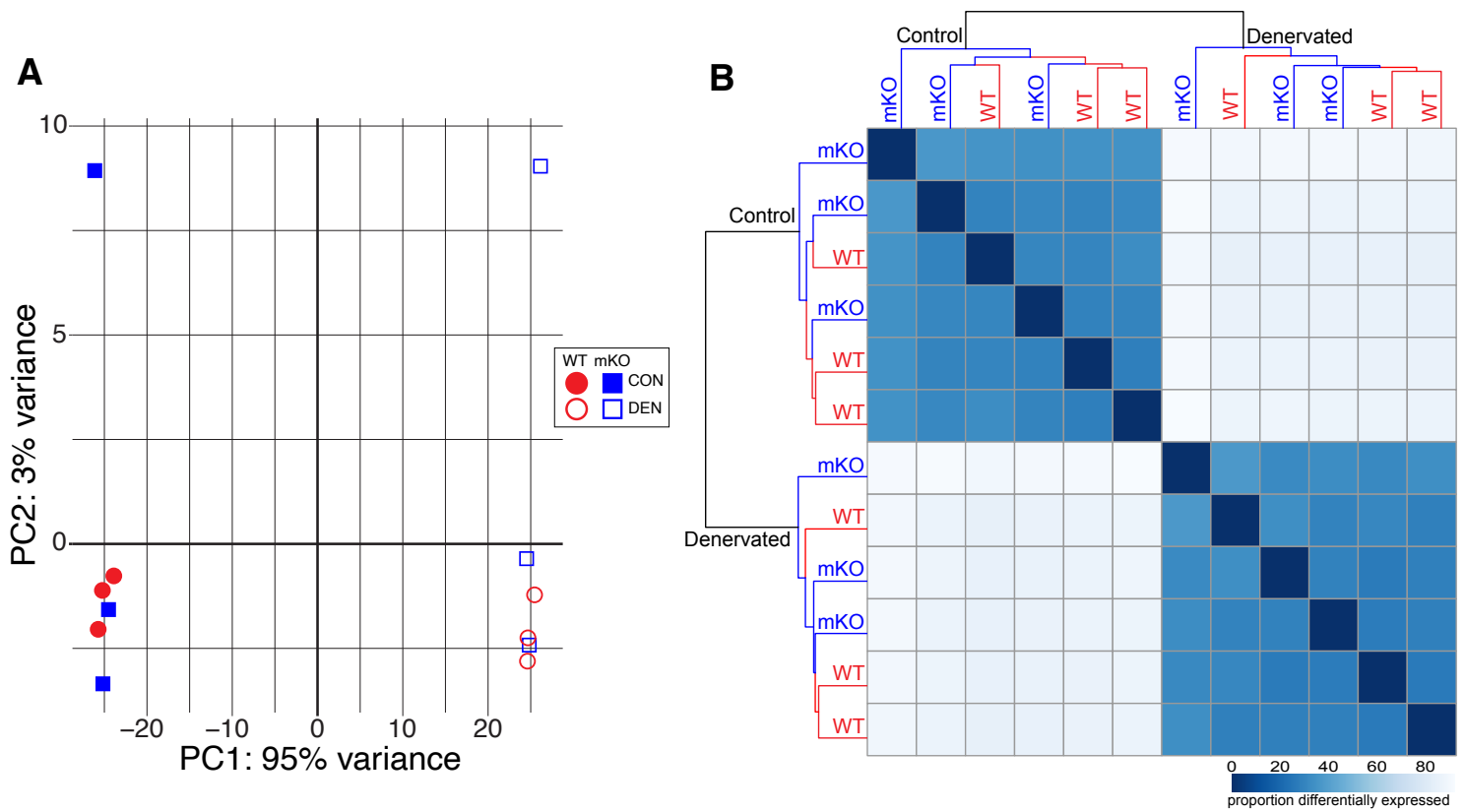


Figure S4. Principal-Component Analysis. **(A)** Principal-component 1 appears to segregate data based on denervation, whereas the principal-component 2 separates data based on genotype, albeit to a much lesser degree of variance between samples. **(B)** The sample clustering heatmap for visualization of sample similarity via the number of differentially expressed transcripts in pairwise comparisons.

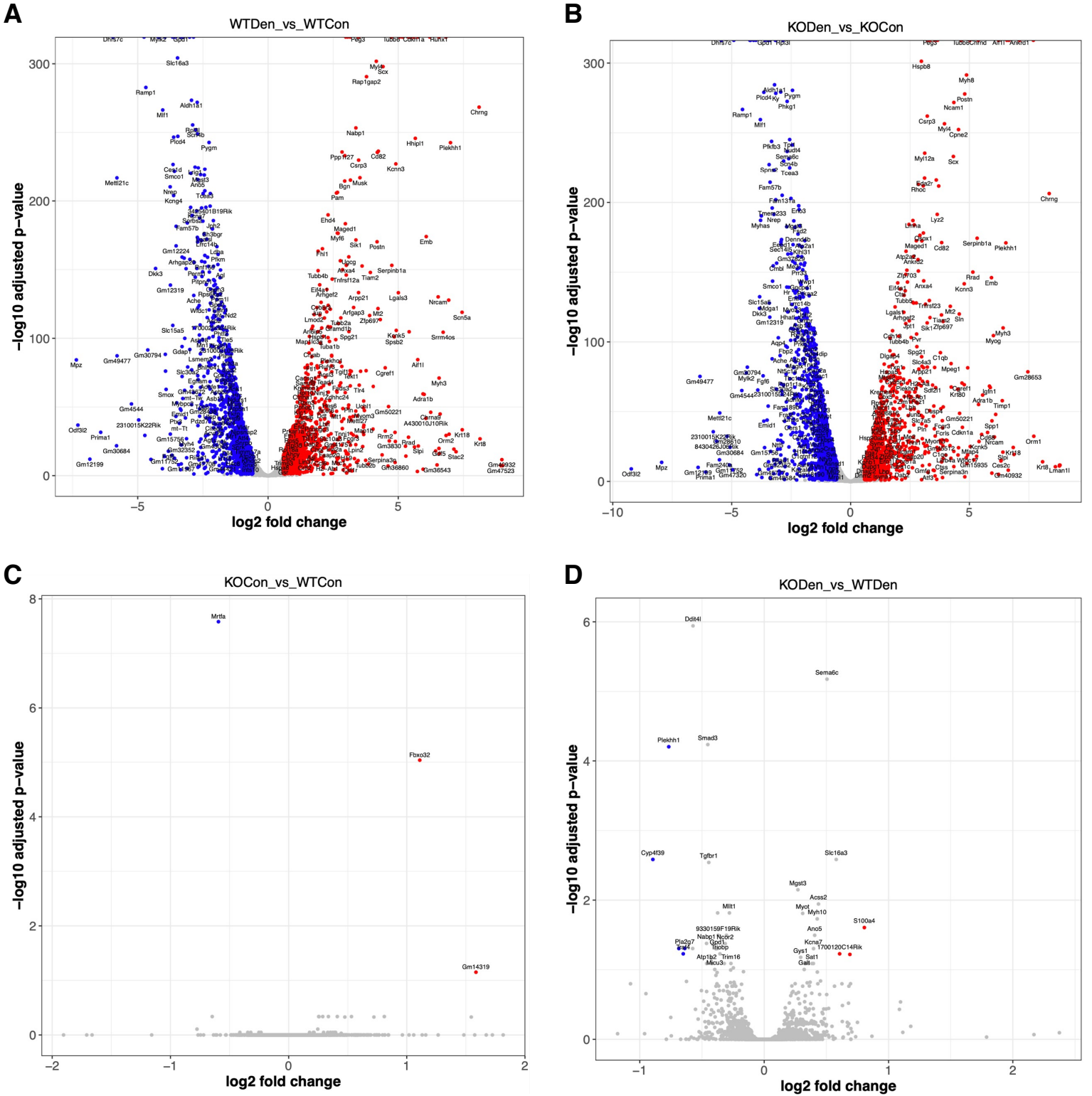


Figure S5. Volcano plots for the four comparisons: **(A)** WT CON vs DEN; **(B)** mKO CON vs DEN; **(C)** WT CON vs mKO CON; and **(D)** WT DEN vs mKO DEN. Plots were generated using R v.3.6.1, using the differential gene expression results obtained from DESeq2. The genes with less than 3 FPKM in both comparison groups from each result set were filtered out before plotting. Genes which had $abs(\text{Log}_2\text{FC}) > 0.58$ ($=1.5x$ FC) and padj (FDR) < 0.1 were colored red or blue depending on the direction of the fold change.

Table S3. RNAseq results for select genes involved in mitochondrial biogenesis, autophagy/mitophagy, lysosomal biogenesis, the UPR^{mt} and atrogenes/apoptotic factors along with corresponding heat map depicting log₂FC (Scale is -2 to +2, blue to red). Only genes with p-value and FDR < 0.05 were considered differentially expressed

Gene Common Name / Description	Gene	DeSeq									EdgeR														
		WT			mKO			WT 7d Con vs 7d Den			mKO 7d Con vs 7d Den			WT			mKO			WT 7d Con vs 7d Den			mKO 7d Con vs 7d Den		
		logFC	logFC	logFC	p-value	FDR	logFC	p-value	FDR	logFC	p-value	FDR	logFC	p-value	FDR	logFC	p-value	FDR	logFC	p-value	FDR	logFC	p-value	FDR	
PGC-1a	Ppargc1a			-0.68	0.00	0.00	-0.44	0.00	0.00			-0.67	0.00	0.00	-0.42	0.13	0.18								
PGC-1B	Ppargc1b			-0.72	0.00	0.00	-0.38	0.01	0.02			-0.71	0.00	0.00	-0.36	0.00	0.00								
Tfam	Tfam			-0.29	0.00	0.01	-0.32	0.00	0.00			-0.28	0.00	0.00	-0.30	0.00	0.00								
Tim 22	Timm22			0.31	0.16	0.52	0.05	0.69	0.78			0.33	0.03	0.11	0.06	0.42	0.50								
Tim 23	Timm23			-0.47	0.00	0.00	-0.50	0.00	0.00			-0.46	0.00	0.00	-0.48	0.00	0.00								
TIMDC1	Timmdc1			0.04	0.82	1.77	-0.01	0.93	0.96			0.07	0.63	1.40	0.01	0.86	0.90								
Tim13	Timm13			-0.27	0.00	0.01	-0.29	0.00	0.01			-0.26	0.00	0.00	-0.28	0.00	0.00								
Tom 40	Tomm40			-0.03	0.89	1.86	0.08	0.45	0.58			0.00	0.98	1.99	0.10	0.18	0.25								
Tom 20	Tomm20			0.20	0.02	0.05	0.10	0.24	0.36			0.22	0.00	0.01	0.12	0.06	0.09								
TOM22	Tomm22			0.42	0.00	0.00	0.50	0.00	0.00			0.43	0.00	0.00	0.52	0.00	0.00								
CI - Ubiquinone oxidoreductase subunit A13	Ndufa13			-0.85	0.00	0.00	-0.91	0.00	0.00			-0.83	0.00	0.00	-0.89	0.00	0.00								
CI - Ubiquinone oxidoreductase subunit S1	Ndufs1			-1.18	0.00	0.00	-1.24	0.00	0.00			-1.16	0.00	0.00	-1.22	0.00	0.00								
CI - Ubiquinone oxidoreductase subunit V1	Ndufv1			-0.96	0.00	0.00	-1.08	0.00	0.00			-0.95	0.00	0.00	-1.06	0.00	0.00								
CI - NADH dehydrogenase 3, mitochondrial	mt-Nd3			-2.09	0.22	0.67	-1.19	0.12	0.21			-2.07	0.06	0.18	-1.15	0.02	0.04								
CI - NADH dehydrogenase 2, mitochondrial	mt-Nd2			-1.63	0.00	0.00	-1.72	0.00	0.00			-1.61	0.00	0.00	-1.70	0.00	0.00								
CI - succinate dehydrogenase complex, subunit A, flavoprotein	Sdha			-1.40	0.00	0.00	-1.45	0.00	0.00			-1.39	0.00	0.00	-1.43	0.00	0.00								
CI - succinate dehydrogenase complex, subunit B, flavoprotein	Sdhb			-0.97	0.00	0.00	-1.05	0.00	0.00			-0.95	0.00	0.00	-1.04	0.00	0.00								
CI - succinate dehydrogenase complex, subunit C, flavoprotein	Sdhc			-0.94	0.00	0.00	-0.99	0.00	0.00			-0.92	0.00	0.00	-0.97	0.00	0.00								
CI - succinate dehydrogenase complex assembly factor 1	Sdhaf1			0.00	1.00	2.00	0.00	0.99	1.00			0.02	0.90	1.87	0.02	0.83	0.87								
CI - succinate dehydrogenase complex assembly factor 2	Sdhaf2			-0.14	0.45	1.17	0.07	0.51	0.63			-0.12	0.35	0.87	0.08	0.22	0.29								
CI - ubiquinol cytochrome c reductase core protein 2	Uqcrc2			-0.77	0.00	0.00	-0.81	0.00	0.00			-0.76	0.00	0.00	-0.79	0.00	0.00								
CI - ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	Uqcrc1			-0.73	0.00	0.00	-0.84	0.00	0.00			-0.72	0.00	0.00	-0.82	0.00	0.00								
CI - ubiquinol-cytochrome c reductase, complex III subunit VII	Uqcrcq			-0.73	0.00	0.00	-0.89	0.00	0.00			-0.72	0.00	0.00	-0.87	0.00	0.00								
CI - ubiquinol-cytochrome c reductase core protein 1	Uqcrc1			-1.24	0.00	0.00	-1.27	0.00	0.00			-1.23	0.00	0.00	-1.25	0.00	0.00								
CI - cytochrome b, mitochondrial	mt-Cytb			-1.01	0.00	0.00	-1.17	0.00	0.00			-1.00	0.00	0.00	-1.15	0.00	0.00								
CIV - SCO2 cytochrome c oxidase assembly protein	Sco2			0.71	0.04	0.16	0.63	0.00	0.00			0.37	0.01	0.02	0.64	0.00	0.00								
CIV - SCO1 cytochrome c oxidase assembly protein	Sco1			0.60	0.07	0.25	0.32	0.05	0.10			0.31	0.01	0.02	0.33	0.00	0.01								
CIV - cytochrome c oxidase I, mitochondrial	mt-Co1			-1.23	0.00	0.00	-1.33	0.00	0.00			-1.22	0.00	0.00	-1.31	0.00	0.00								
CIV - cytochrome oxidase subunit III	mt-Co3			-1.24	0.00	0.00	-1.36	0.00	0.00			-1.22	0.00	0.00	-1.35	0.00	0.00								
CIV - cytochrome c oxidase subunit 4I2	Cox4i2			-1.56	0.04	0.17	-0.90	0.02	0.04			-0.76	0.00	0.01	-0.87	0.00	0.00								
CV - ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1	Atp5a1			-1.12	0.00	0.00	-1.17	0.00	0.00			-1.11	0.00	0.00	-1.15	0.00	0.00								
CV - ATP synthase mitochondrial F1 complex assembly factor 1	Atpaf1			-0.72	0.00	0.00	-0.70	0.00	0.00			-0.71	0.00	0.00	-0.68	0.00	0.00								
CV - ATP synthase mitochondrial F1 complex assembly factor 2	Atpaf2			-0.49	0.00	0.00	-0.45	0.00	0.00			-0.47	0.00	0.00	-0.43	0.00	0.00								
CV - ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit	Atp5d			-0.43	0.00	0.00	-0.51	0.00	0.00			-0.42	0.00	0.00	-0.49	0.00	0.00								
CV - ATP synthase 8, mitochondrial	mt-Atp8			-1.85	0.18	0.56	-1.37	0.11	0.18			-1.77	0.05	0.15	-1.31	0.02	0.03								
Beclin1	Becn1			-0.14	0.41	1.09	-0.22	0.01	0.03			-0.12	0.33	0.83	-0.20	0.01	0.01								
ULK1	Ulk1			-0.41	0.00	0.00	-0.75	0.00	0.00			-0.40	0.00	0.00	-0.73	0.00	0.00								
Atg5	Atg5			0.37	0.16	0.52	0.05	0.72	0.81			0.20	0.03	0.05	0.07	0.45	0.53								
Atg7	Atg7			0.82	0.00	0.00	0.76	0.00	0.00			0.83	0.00	0.00	0.78	0.00	0.00								
Atg12	Atg12			0.38	0.00	0.00	0.22	0.04	0.07			0.40	0.00	0.00	0.24	0.00	0.01								
LC3 - microtubule-associated protein 1 light chain 3 alpha	Map1lc3a			1.59	0.00	0.00	1.25	0.00	0.00			1.60	0.00	0.00	1.26	0.00	0.00								
LC3 - microtubule-associated protein 1 light chain 3 beta	Map1lc3b			-0.25	0.11	0.39	-0.32	0.00	0.00			-0.23	0.07	0.23	-0.30	0.00	0.00								
p62	Sqstm1			0.82	0.00	0.00	0.51	0.00	0.00			0.83	0.00	0.00	0.53	0.00	0.00								
Parkin	Prkn			0.97	0.00	0.00	0.88	0.00	0.00			0.98	0.00	0.00	0.90	0.00	0.00								
PINK1	Pink1			-1.48	0.00	0.00	-1.59	0.00	0.00			-1.47	0.00	0.00	-1.57	0.00	0.00								
FoxO1	Foxo1			0.68	0.00	0.00	0.03	0.82	0.88			0.69	0.00	0.00	0.03	0.86	0.89								
FoxO3	Foxo3			0.37	0.09	0.31	0.03	0.78	0.86			0.19	0.01	0.02	0.05	0.54	0.61								
TFEB	Tfeb			-1.43	0.00	0.00	-1.59	0.00	0.00			-1.42	0.00	0.00	-1.57	0.00	0.00								
TFE3	Tfe3			0.37	0.22	0.66	0.05	0.76	0.84			0.40	0.05	0.17	0.07	0.48	0.56								
Mucolin1	Mcoln1			-0.12	0.61	1.45	-0.09	0.46	0.59			-0.10	0.53	1.22	-0.07	0.36	0.44								
Cathepsin D	Ctsd			1.16	0.00	0.00	1.04	0.00	0.00			1.17	0.00	0.00	1.06	0.00	0.00								
Cathepsin B	Ctsb			0.66	0.00	0.00	0.71	0.00	0.00			0.67	0.00	0.00	0.73	0.00	0.00								
Lamp1	Lamp1			-0.23	0.00	0.00	-0.38	0.00	0.00			-0.22	0.00	0.00	-0.36	0.00	0.00								
Lamp2	Lamp2			0.48	0.00	0.00	0.42	0.00	0.00			0.49	0.00	0.00	0.44	0.00	0.00								
ATPase, H+ transporting, lysosomal V0 subunit D2	Atp6v0d2			5.25	0.00	0.00	3.72	0.00	0.00			4.92	0.00	0.00	3.66	0.00	0.00								
ATPase, H+ transporting, lysosomal V1 subunit E1	Atp6v1e1			0.58	0.00	0.00	0.50	0.00	0.00			0.59	0.00	0.00	0.52	0.00	0.00								
ATPase, H+ transporting, lysosomal V1 subunit H	Atp6v1h			1.13	0.00	0.00	1.14	0.00	0.00			1.14	0.00	0.00	1.16	0.00	0.00								
HSC70	Hspa8			0.46	0.00	0.00	0.47	0.00	0.00	</															

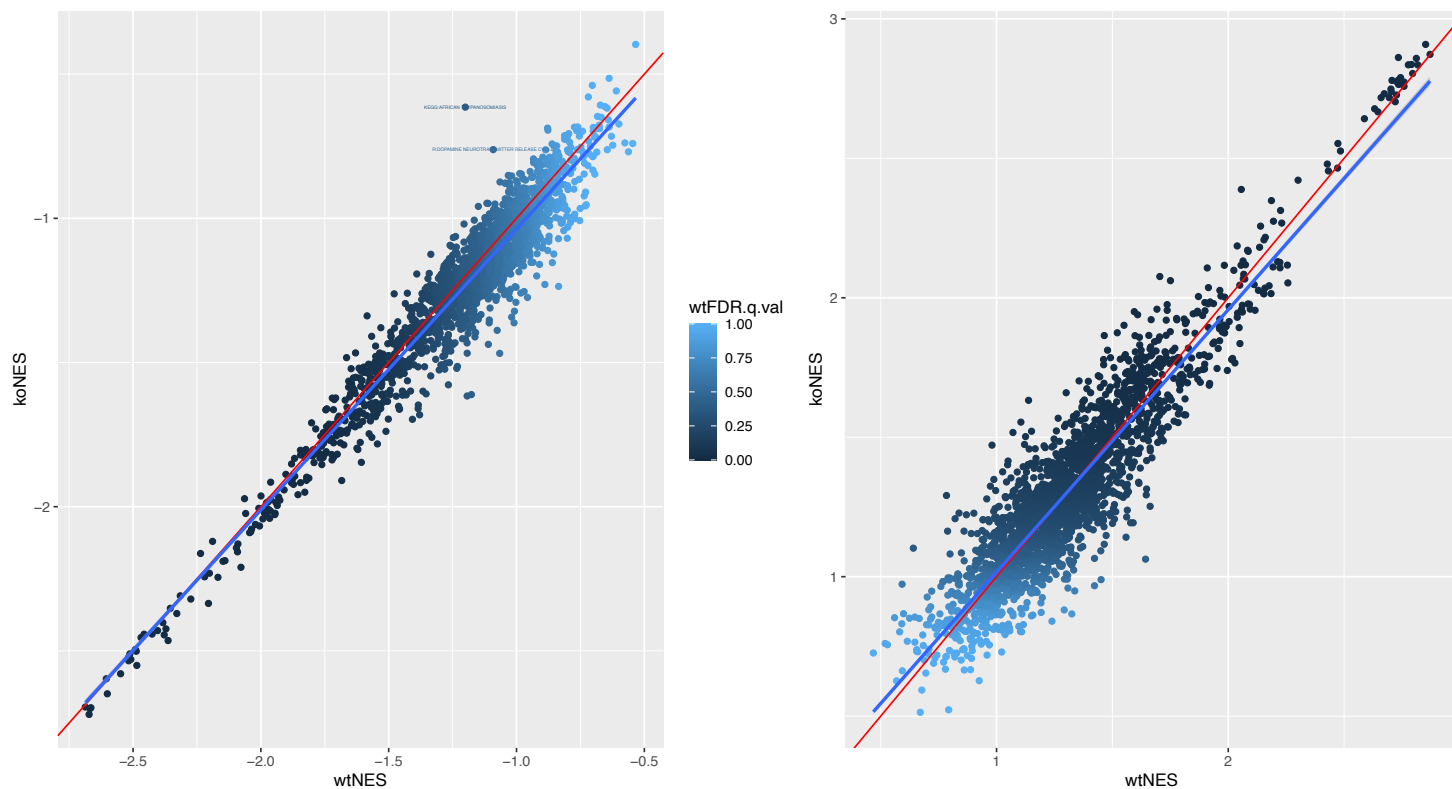


Figure S6. A plot of the GSEA normalized enrichment score for the two independent comparisons was generated, which did not reveal any gene-sets with large differences between the two comparisons. Correlation between normalized enrichment scores (NES) for the WT: denervated vs control, and KO: denervated vs control. Red line represents identity, the blue line is the best fit line from linear regression. Top panels: gene-sets with positive NES; Bottom panels: gene-sets with negative NES.

Table S4. To test whether genotype affected gene expression differentially in the two treatments, an interaction term was included in the statistical model for detection of differentially expressed genes. GSEA enrichment analysis revealed the top gene sets enriched in up- and down-regulate genes.

Supplemental Table 4A. The top-ranking gene sets enriched in up-regulated genes

GS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
GO: extracellular matrix structural constituent	125	0.56	2.58	0.000	0.000	0.000
GO: collagen trimer	70	0.58	2.41	0.000	0.000	0.000
GO: collagen-containing extracellular matrix	271	0.47	2.41	0.000	0.000	0.000
R: collagen biosynthesis and modifying enzymes	60	0.59	2.37	0.000	0.000	0.000
R: collagen chain trimerization	41	0.63	2.33	0.000	0.000	0.000
KEGG: staphylococcus aureus infection	41	0.63	2.32	0.000	0.000	0.001
R: collagen formation	79	0.54	2.31	0.000	0.000	0.001
GO: extracellular matrix	378	0.43	2.27	0.000	0.000	0.002
GO: myeloid leukocyte mediated immunity	79	0.51	2.16	0.000	0.002	0.015
R: assembly of collagen fibrils and other multimeric structures	51	0.55	2.15	0.000	0.002	0.017
R: extracellular matrix organization	247	0.43	2.15	0.000	0.002	0.018
KEGG: glycosaminoglycan degradation	16	0.73	2.14	0.000	0.002	0.021
R: homologous dna pairing and strand exchange	40	0.57	2.09	0.000	0.003	0.047
GO: granulocyte migration	106	0.46	2.07	0.000	0.004	0.058
GO: chromosome separation	83	0.48	2.06	0.000	0.004	0.065
GO: negative regulation of nuclear division	58	0.51	2.06	0.000	0.004	0.071
GO: phagocytosis	163	0.43	2.06	0.000	0.004	0.073
R: chemokine receptors bind chemokines	30	0.59	2.05	0.000	0.004	0.079
GO: antigen processing and presentation of peptide antigen	46	0.53	2.03	0.000	0.005	0.108
GO: metaphase/anaphase transition of cell cycle	56	0.51	2.02	0.000	0.006	0.121

Supplemental Table 4B. The top-ranking gene sets enriched in down-regulated genes

GS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
R: foxo-mediated transcription of cell cycle genes	16	-0.8	-2.32	0.000	0.000	0.001
R: autophagy	118	-0.5	-2.23	0.000	0.001	0.003
GO: autophagosome	63	-0.55	-2.23	0.000	0.001	0.004
GO: fatty acid catabolic process	82	-0.53	-2.21	0.000	0.001	0.004
R: constitutive signaling by akt1 e17k in cancer	26	-0.67	-2.2	0.000	0.001	0.007
R: macroautophagy	105	-0.5	-2.19	0.000	0.001	0.007
GO: polyubiquitin modification-dependent protein binding	48	-0.58	-2.18	0.000	0.001	0.009
GO: autophagosome assembly	88	-0.52	-2.18	0.000	0.001	0.010
GO: lipid oxidation	88	-0.51	-2.17	0.000	0.001	0.012
GO: ubiquitin binding	69	-0.53	-2.17	0.000	0.001	0.013
GO: macroautophagy	184	-0.45	-2.16	0.000	0.001	0.013
GO: autophagosome organization	92	-0.5	-2.16	0.000	0.001	0.013
KEGG: oxidative phosphorylation	123	-0.48	-2.16	0.000	0.001	0.013
KEGG: mitophagy - animal	65	-0.53	-2.13	0.000	0.001	0.020
GO: mitochondrion disassembly	62	-0.53	-2.13	0.000	0.001	0.020
GO: fatty acid oxidation	86	-0.5	-2.13	0.000	0.001	0.020
GO: regulation of tor signaling	89	-0.5	-2.13	0.000	0.001	0.021
GO: respiratory chain	87	-0.5	-2.13	0.000	0.001	0.022
GO: autophagy of mitochondrion	62	-0.53	-2.12	0.000	0.001	0.023

Table S5. Linear regression analysis comparing the fold-change (FC) in whole muscle (WM) LC3-II or p62 protein expression with the fold-change in autophagy flux measured via LC3-II or p62 accumulation. Comparisons were derived from previously published work cited within column from our laboratory, in which flux was measured using either colchicine injections or bafilomycin A treatment in order to block lysosomal degradation of the autophagosome.

Referenced Paper	Conditions	Model	Flux Measure	Comparison	LC3-II		p62	
					FC in Protein	FC in Flux	FC in Protein	FC in Flux
(13) Vainshtein, A. et al. <i>Skelet. Muscle.</i> , 2015	- 7d Denervation - PGC-1a KO vs WT	Mice	Colchicine injections	WT: Den / Con	2.8	0.4	1.5	0.5
				KO: Den / Con	2.2	0.1	0.7	>3
				KO: Con / WT Con	0.7	0.6	1.3	0.1
				KO: Den / WT Den	0.5	1.1	0.6	1.4
(46) Vainshtein, A. et al. <i>Am. J. Physiol. Cell Physiol.</i> , 2015	- PGC-1a KO vs WT - Acute Exercise (Ex) - Acute Ex + Recovery (Ex+R)	Mice	Colchicine injections	WT: Ex / Con	2.1	1.7	0.9	1.2
				WT: Ex+R/Con	1.5	1.9	1.7	1.4
				KO: Ex / Con	0.7	1.4	1.5	1.4
				KO: Ex+R / Con	1.7	0.5	1.8	1.0
				KO: Ex+R / Ex	2.4	0.4	1.2	0.7
				Con: KO / WT	1.1	0.9	0.9	1.0
(47) Parousis, A. et al. <i>Autophagy</i> , 2016	- Chronic Contractile Activity	C2C12	Bafilomycin A treatment	CCA / Con	-	-	1.2	0.6
(48) Carter, H.N. et al. <i>J. Physiol.</i> , 2018	- Aged vs Young - 7d Chronic Contractile Activity	Rats	Colchicine injections	Young: CCA / Con	1.26	0.8	-	-
				Con: Aged / Young	1.5	1.3	-	-
				Aged: CCA / Con	1.18	1.0	-	-
(49) Kim, Y et al. <i>Pflügers Arch.</i> , 2018	- 1, 2, 5, & 7-days of Chronic Contractile Activity	Rats	Colchicine injections	1d CCA / Con	0.7	2.2	0.6	1.6
				2d CCA / Con	0.5	2.2	1.8	0.7
				5d CCA / Con	0.4	2.4	1.5	0.8
				7d CCA / Con	0.4	2.3	1.3	1.4
(50) Triolo, M. et al. <i>J. Physiol.</i> , 2022	- 1, 3, & 7d Denervation	Mice	Colchicine injections	1d Den / Con	1.1	1.3	1.2	0.9
				3d Den / Con	1.3	1.9	3.4	0.7
				7d Den / Con	2.1	0.7	2.3	0.1
WM LC3-II vs LC3-II Flux					R2 = 0.408		p=0.002	
WM p62 vs p62 Flux					R2 = 0.221		p=0.049	