# Supplementary Data for:

## PGC-1α contributes to denervation-induced mitophagy in skeletal muscle

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Fig.S1

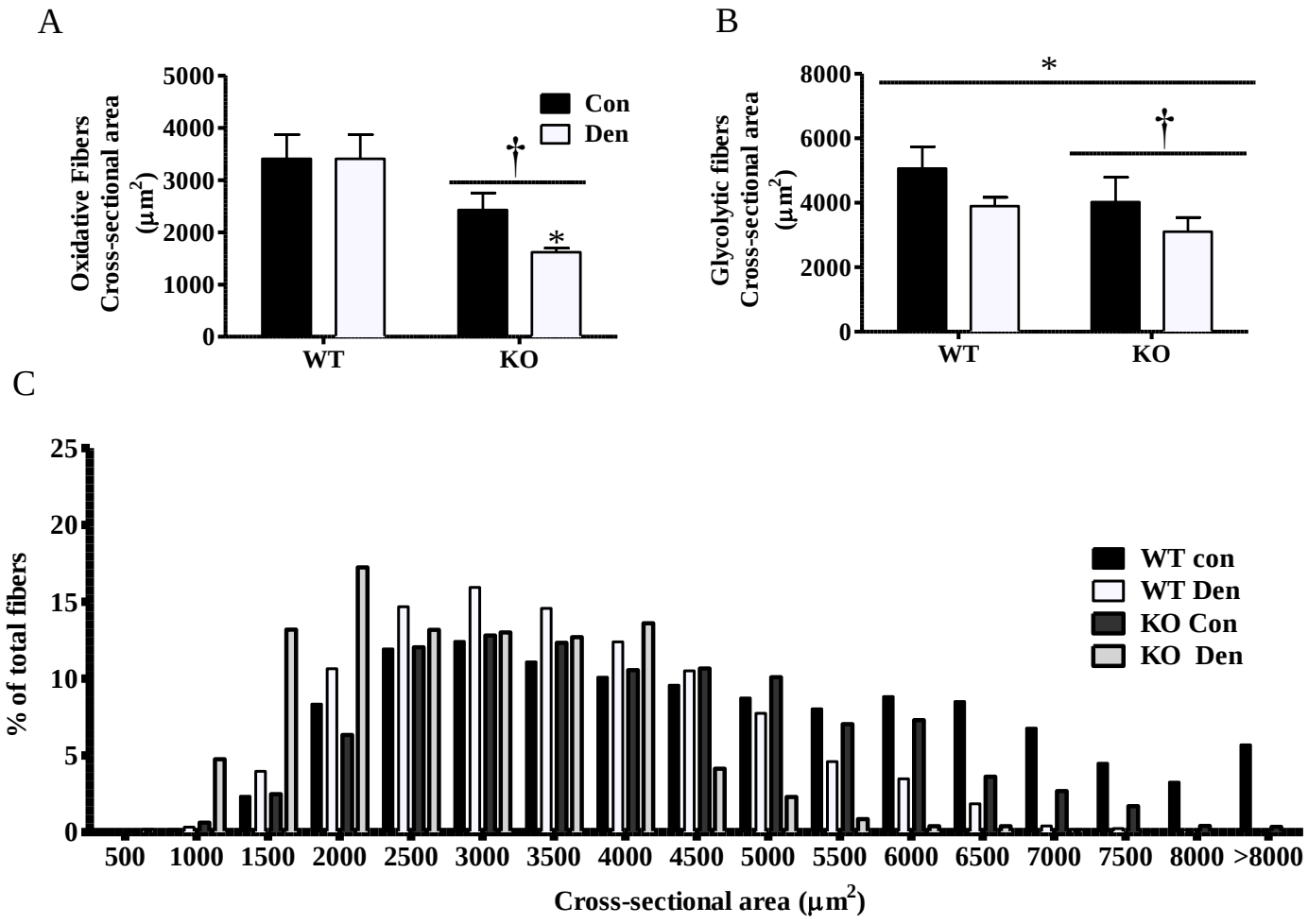


**Fig.S1 PGC-1α KO animals have lower muscle cross-sectional area .** A-C. TA muscle fiber cross sectional area of WT KO Con and Den was measured from serial cross sections stained with SDH. A. Average size of oxidative fibers. B. Average size of glycolytic fibers. C. Fiber size distribution . *P<0.05, significant effect of denervation .† P<0.05, significant effect of genotype (n=4 for all groups).

Fig.S2

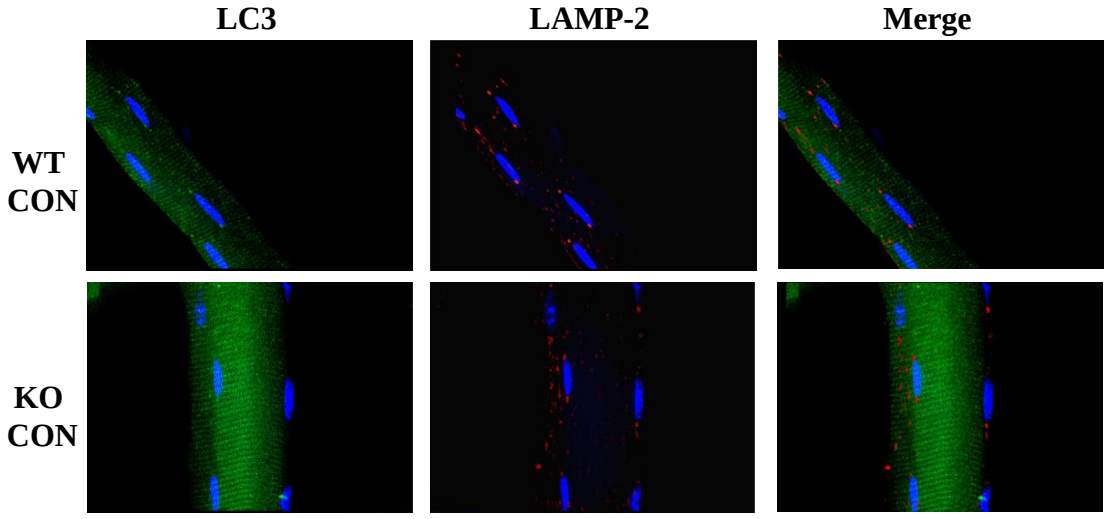


**Fig.S2** Confocal images of fixed single fibers immuno-stained for LC3 (green) and lysosomal Lamp-2 (red). Colocalization is shown in yellow (Merge) which represents autophagosomes within lysosomes. Nuclei are in blue (Dapi).

Fig.S3

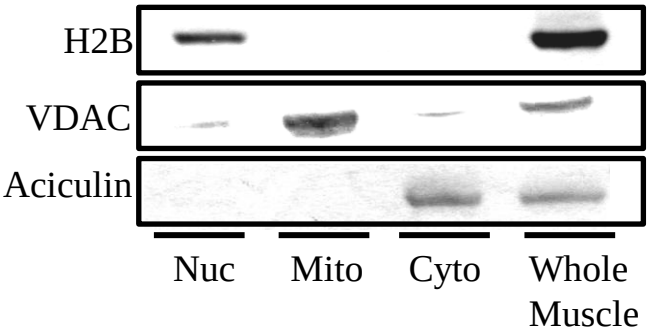


**Fig S3.** Fraction purity. Representative blots demonstrating the quality of cellular fractionation in nuclear (Nuc), mitochondrial (Mito), cytosolic (Cyto) cellular subfractions as well as whole muscle lysates, for positive control. H2B is a nuclear factor, VDAC is a mitochondrial protein and Aciculin is a cytosolic protein. Equal amounts of protein were loaded for each.

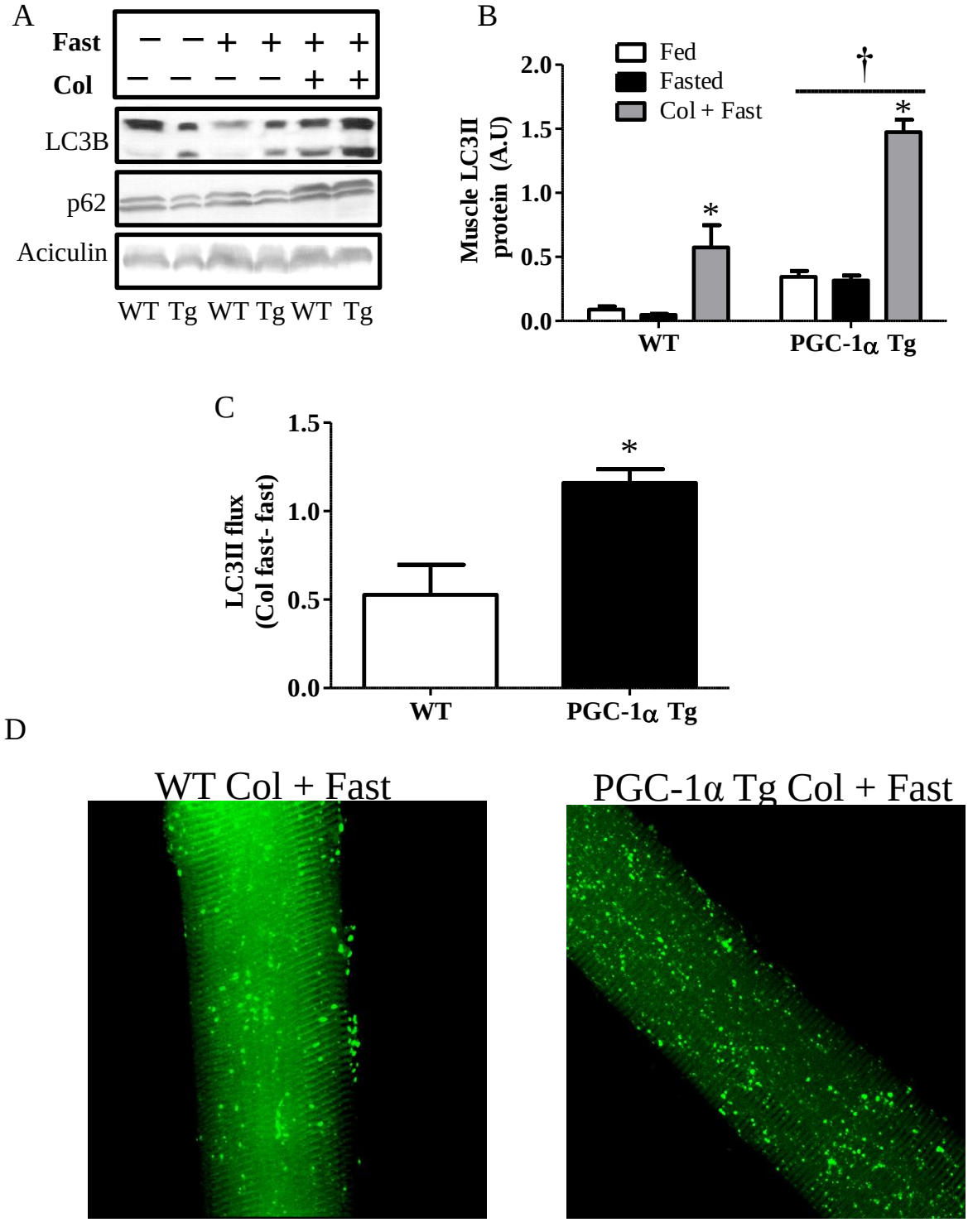


**Fig. S4: Autophagy protein expression and flux.** A) Representative blots of autophagic proteins and B-C) quantification of protein expression and autophagy flux in WT and PGC-1αTg animals that were fed (Control) or fasted (24h), with or without colchicine (Col) treatment; B) LC3II; C) autophagy flux. GAPDH was used as loading control. D) Representative images of single fibers isolated from FDB muscle transfected with LC3-YFP (Green) showing increased fasting-induced autophagsome accumulation in PGC-1αTg treated with colchicine as compared to WT. *P<0.05 significant effect of treatment. †P<0.05 significant effect of genotype. Aciculin was used as a loading control (n=3 for all groups).

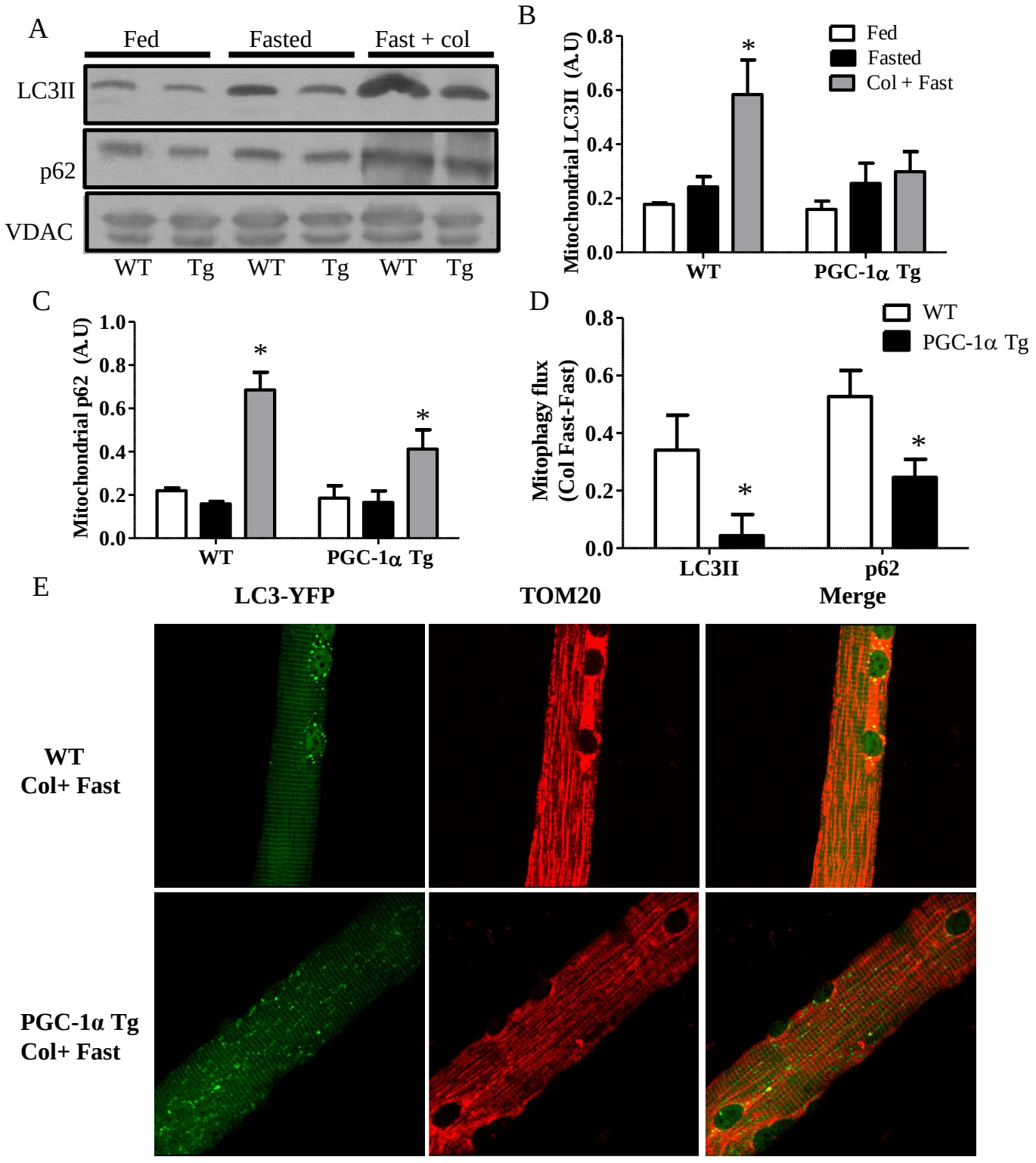


**Fig. S5: Nutrient deprivation-induced Mitophagy flux is reduced in Tg animals** A) Representative blots of autophagic proteins on isolated mitochondria in WT and PGC-1αTg animals that were fed or fasted, with or without colchicine (Col) treatment; B-C) quantification of protein expression and autophagy flux; B)LC3II; C)p62; D) mitophagy flux. VDAC was used as loading control. E) Single fibers isolated from the FDB muscle of animals that were fasted and treated with colchicine. Muscle was transfected with LC3-YFP (green) and immunostained for mitochondrial marker TOM20 (Red). Colocalization of LC3 with TOM20 (yellow) represents mitochondria within autophagosomes, which are more prevalent in WT animals when compared to Tg. *P<0.05 significant effect of treatment. †P<0.05 significant effect of genotype (n=3 for all groups).

**Table S1. Primer sequences based on gene transcripts available in GenBank.**

| Gene | Forward primer (5’ → 3’) | Reverse primer (5’ → 3’) |
|---|---|---|
| *Coxiv* | CTCCAACGAATGGAAGACAG | TGACAACCTTCTTAGGGAAC |
| *Mul1* | GCCTTGCTGATGTAGTTGTCTG | CAGGCCCAGCTTATGATAGAA |
| *Bnip3l* | GGAAAGCGGCACAGAGAA | GAATGACGCCAGTGCTGAT |
| *Park2* | GTCTGCAATTTGGTTTGGAGTA | GCATCATGGGATTGTCTCTTAAA |
| *Sqstm1* | TGTGGTGGGAACTCGCTATAA | CAGCGGCTATGAGAGAAGCTAT |
| *Maplc3b* | GCTTGCAGCTCAATGCTAAC | CCTGCGAGGCATAAACCATGTA |
| *Atg7* | TTTCTGTCACGGTTCGATAATG | TGAATCCTTCTCGCTCGTACT |
| *Lamp2* | GCTGAACAACAGCCAAATTA | CTGAGCCATTAGCCAAATACAT |
| *Catsd* | TTTGCCAATGCTGTCGTACT | AGCGAGTGTGACTATGTGTGAG |
| *Foxo3* | ATGGACGACCTGCTGGATAAC | GGAGCTCTTGGCGGTATATG |
| *Actb* | TGTGACGTTGACATCCGTAA | GCTAGGAGCCAGAGCAGTAA |
| *Gapdh* | AACACTGAGCATCTCCCTCA | GTGGGTGCAGCGAACTTTAT |

## Table S2. Antibodies list

| Antibody | Manufacturer | Product number |
|---|---|---|
| Goat anti-Rat Alexa Flour® 647 | Life Technologies | A-21247 |
| Goat anti-Rabbit Alexa Fluor® 488 | Life Technologies | A-11034 |
| Atg7 | Sigma-Aldrich | A2856 |
| Beclin1 | Cell Signaling Technology | 3738 |
| Cathepsin D | Santa Cruz Biotechnology | Sc6486 |
| COXIV | Abcam | Ab14744 |
| GAPDH | Abcam | Ab8245 |
| Histone-2B/H2B | Cell Signaling Technology | 2934 |
| Lamp-2 | Abcam | Ab13524 |
| LC3B/Maplc3b | Cell Signaling Technology | 2775 |
| NIX/Bnip3l | Abcam | Ab109414 |
| p62/Sqstm1 | Sigma-Aldrich | P0067 |
| Parkin | Cell Signaling Technology | 4211 |
| PGC-1α/Ppargc1a | Millipore Corporation | AB3242 |
| T-eIF2α | Cell Signaling Technoogy | 9722 |
| TFEB | MyBioSource Inc | MBS120432 |
| Ubiquitin (Ub) | Enzo Life Sciences | ADI-SPA-203 |
| VDAC/Porin | Abcam | 14734 |