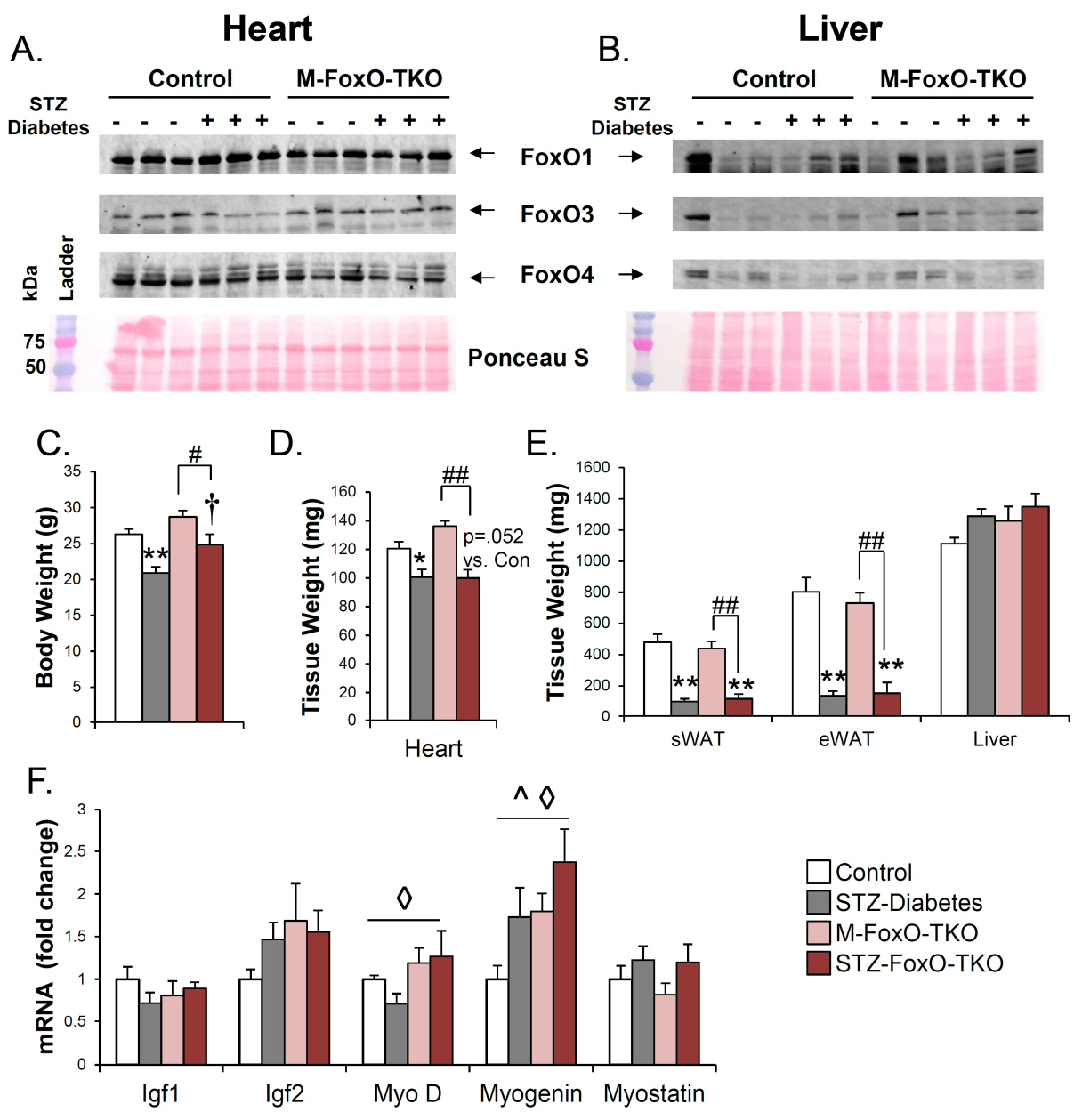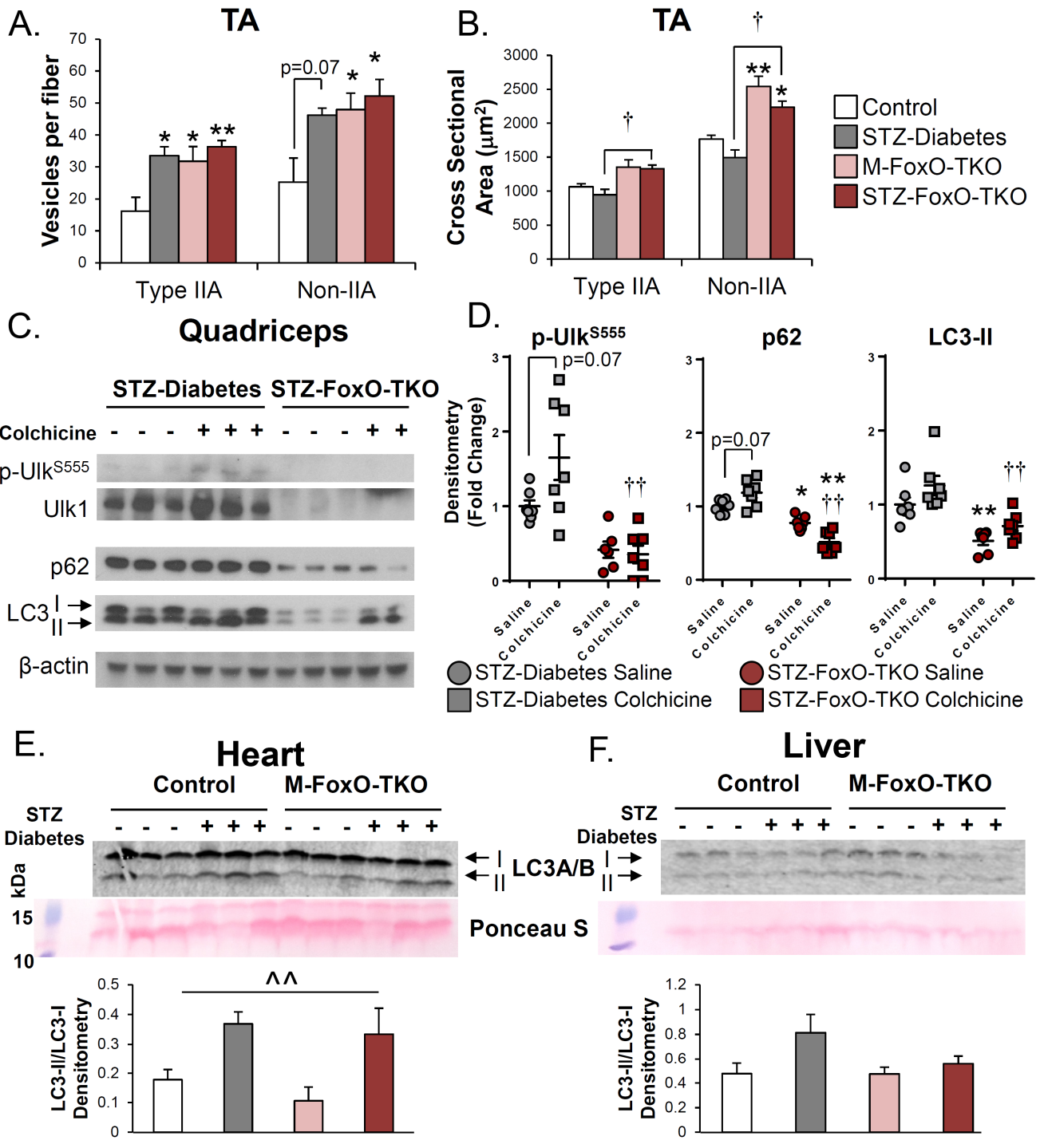SUPPLEMENTARY DATA

Supplementary Figure S1. Muscle-specific deletion of FoxOs mitigates weight loss, but does not prevent loss of cardiac/adipose tissue mass or alter myogenic factors in muscle in response to insulin-deficient diabetes. Western blot analysis for FoxO isoform expression in heart (A) and liver (B) from control and M-FoxO-TKO mice rendered diabetic with STZ. Body weight at sacrifice (C) in control, STZ-Diabetes, M-FoxO-TKO and STZ-FoxO-TKO mice (n=10-12). Heart weight (D), adipose and liver weights (E) after 12-15 days of diabetes after STZ treatment (n=10-12). mRNA levels of myogenic factors in quadriceps muscle (F) (n=4-6). (*-p<0.05, **-p<0.01 vs. control, †-p<0.05, STZ-Diabetes vs. STZ-FoxO-TKO, #-p<0.05, ##-p<0.01 as indicated, ◊-p<0.05 genotype main effect, ^-p<0.05 STZ main effect, Two-way ANOVA). sWAT – subcutaneous white adipose tissue (WAT), eWAT – epididymal WAT.
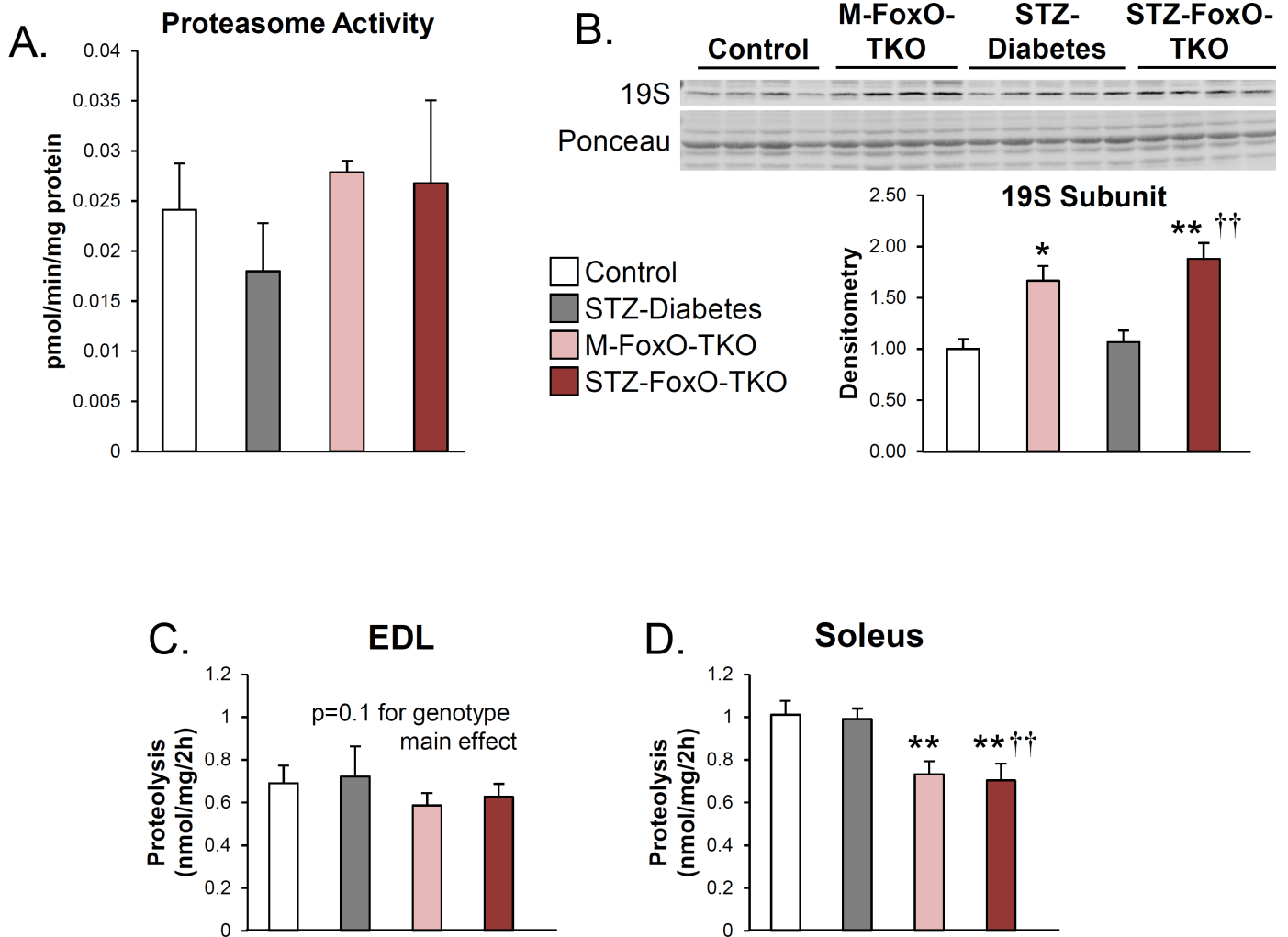
SUPPLEMENTARY DATA

Supplementary Figure S2. FoxO Deletion prevents upregulation of autophagy markers in skeletal muscle. Quantification of total LC3A vesicles per fiber (A) and fiber cross-sectional area (B) from TA images in Figure 3A (n=5-6). Western blot analysis (C) and densitometry (D) of autophagy markers in Quad from STZ-Diabetes and STZ-FoxO-TKO mice treated with Saline or Colchicine for 2 days prior to sacrifice (n=7 per group). Western blot analysis and densitometric ratio of LC3-II/LC3-I in heart (E) and liver (F) from control, STZ-Diabetes, M-FoxO-TKO and STZ-FoxO-TKO mice. (*-p<0.05, **-p<0.01 vs. control, †-p<0.05, ††-p<0.01, STZ-Diabetes vs. STZ-FoxO-TKO, ^^-p<0.01 STZ main effect, Two-way ANOVA)

Supplementary Figure S3. Muscle Specific FoxO Deletion reduces proteolysis in Soleus muscle, but proteasome activity is unchanged and a 19S proteasome subunit is increased in Quad. Proteasome activity (A) and western of a 19S proteasomal subunit (B) in Quadriceps muscle. Proteolysis measured by tyrosine release in ex vivo EDL (C) and Soleus (D) muscle (n=4-5). (*-$p<0.05$, **-$p<0.01$ vs. control, †-$p<0.05$, ††-$p<0.01$, STZ-Diabetes vs. STZ-FoxO-TKO, Two-way ANOVA)
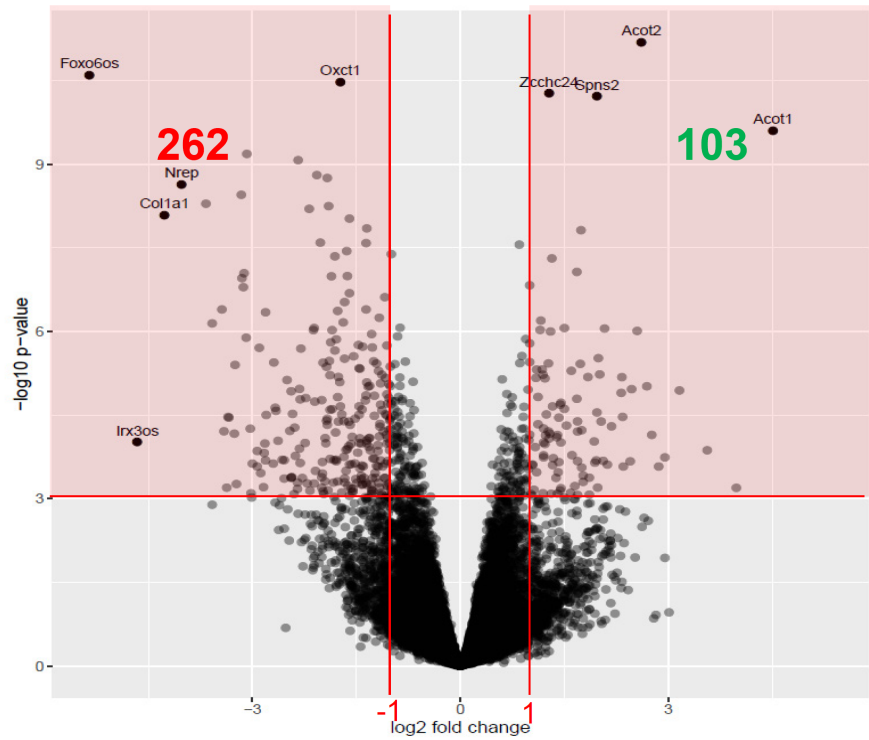
Supplementary Figure S4. Despite regulation of 4EBP1, FoxO Deletion does not restore the suppressed protein synthesis in STZ-Diabetes, and upregulation of autophagy markers by STZ-Diabetes is muscle-type specific. Western blot (A) and densitometry (B) of phospho- and total 4EPB1 protein in Quad. Protein synthesis by SUnSET method (C) and densitometry (D) in Quad (n=2-6 per group, Fed and Fasted mice treated +/- puromycin were used as positive and negative controls; ND=not diabetic and therefore excluded). qRT-PCR of autophagy/lysosomal genes and E3-ubiquitin ligases in EDL (E) and Soleus (F) from control, STZ-Diabetes, M-FoxO-TKO, and STZ-FoxO-TKO mice. (n=5-6 per group. *-p<0.05, **-p<0.01 vs. control, †-p<0.05, ††-p<0.01, STZ-Diabetes vs. STZ-FoxO-TKO, ^-p<0.05, ^^-p<0.01 STZ main effect, Two-way ANOVA)
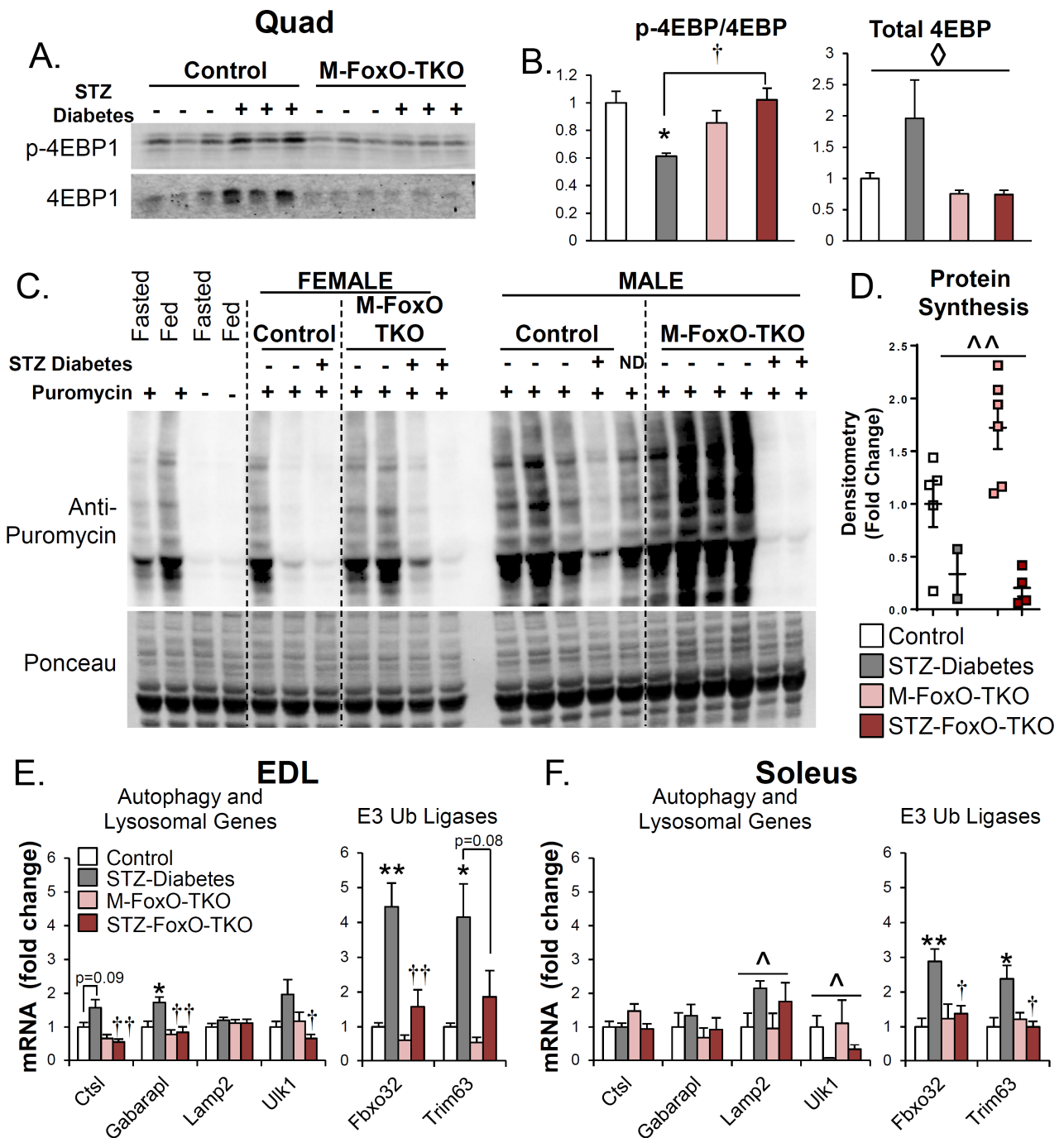
SUPPLEMENTARY DATA

Supplementary Figure S5. A majority of the changes in transcript levels in response to STZ-Diabetes are normalized by deletion of FoxOs in muscle. Volcano plots of transcripts comparing STZ-Diabetes to Controls (A) and STZ-FoxO-TKO to M-FoxO-TKO (B) from quadriceps and gastrocnemius muscles determined by RNA-Seq (n=4-6 per group). Red shaded boxes indicate transcripts that are increased or decreased by more than 2 fold with a p-value less than 0.001. Red numbers indicate the total number of downregulated transcripts while green numbers indicate upregulated transcripts.

Supplementary Figure S6. A subset of transcripts in the ubiquitin-proteasome pathway is increased by diabetes in a FoxO-dependent manner. Heat map of all 125 genes from the "Ubiquitin mediated proteolysis" KEGG pathway with cluster analysis based on Z-score.

Heat map of 125 Genes from KEGG Pathway "Ubiquitin Mediated Proteolysis"
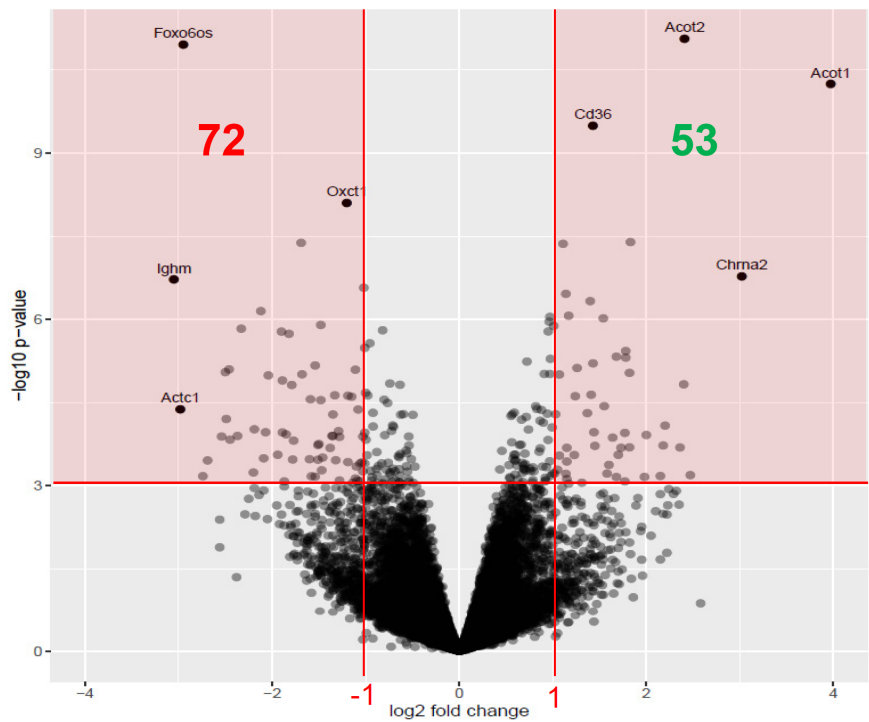
Supplementary Figure S7. A subset of transcripts in the Biosynthesis of Unsaturated Fatty Acids, Fatty Acid Metabolism and PPAR Signaling pathways is increased by diabetes independent of FoxOs. Heat map of all genes from the "Biosynthesis of Unsaturated Fatty Acids", "Fatty Acid Metabolism", and "PPAR Signaling" KEGG pathways from Figure 5E with cluster analysis based on Z-score.
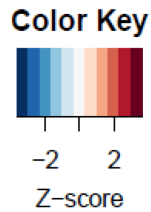
Heat map of All Genes from KEGG Pathways "Biosynthesis of Unsaturated Fatty Acids", "Fatty Acid Metabolism", and "PPAR Signaling Pathway"
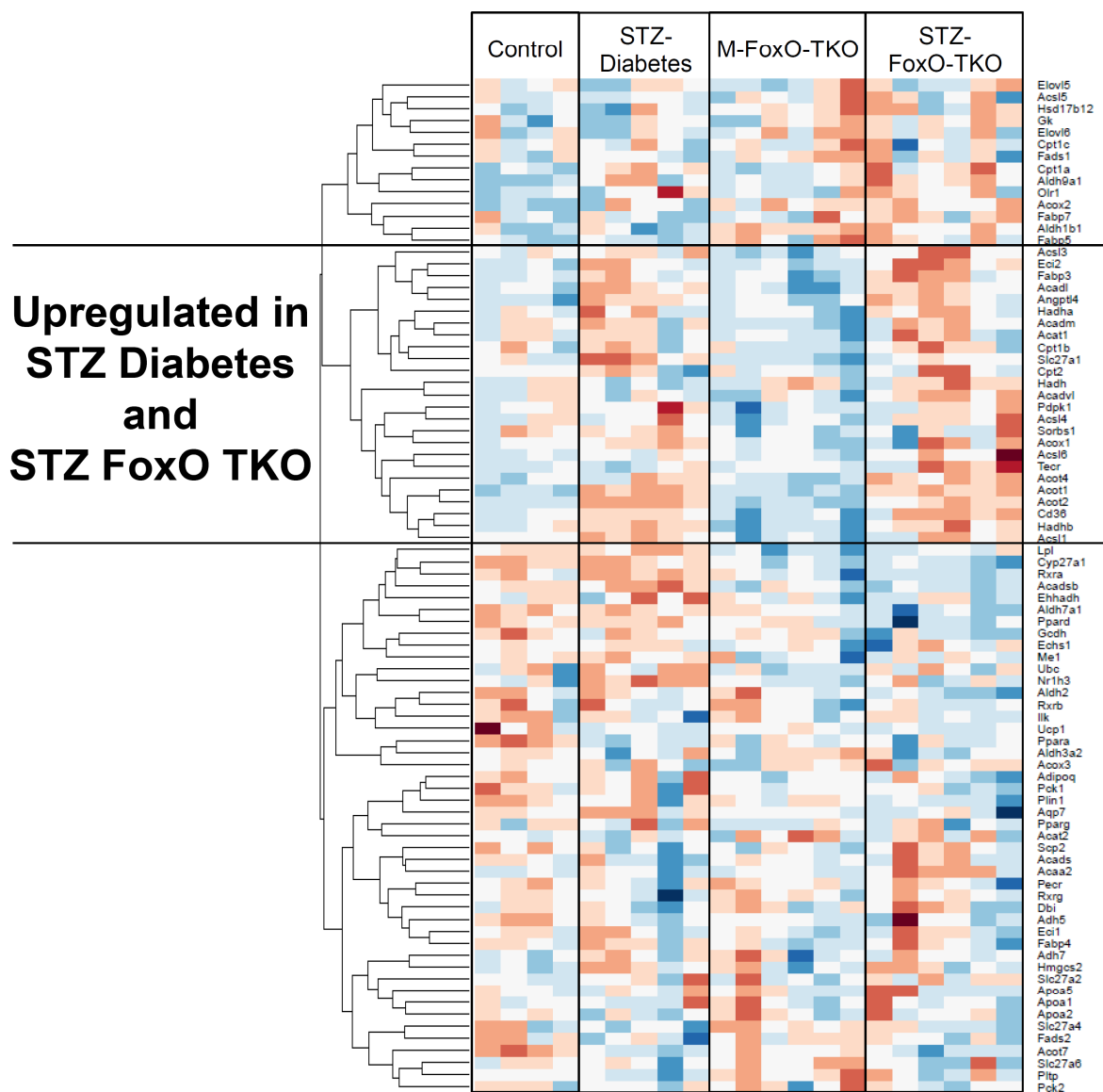
Supplementary Table S1. Primers for QPCR of Mouse genes.

Common name	Gene name	5' primer	3' primer
Mouse QPCR Primers			
FoxO1	*Foxo1*	TGCTGTGAAGGGACAGATTG	GAGTGGATGGTGAAGAGCGT
FoxO3	*Foxo3*	ACAAACGGCTCACTTTGTCCCAGA	TCTTGCCCGTGCCTTCATTCT
FoxO4	*Foxo4*	GGTGCCCTACTTCAAGGACA	AGCTTGCTGCTGCTATCCAT
IGF-1	*Igf1*	GACCGAGGGGCTTTTACTTC	GGGGCACAGTACATCTCCA
IGF-2	*Igf2*	GGACCGCGGCTTCTACTT	ACGTCCCTCTCGGACTTG
MyoD	*Myod1*	AGCACTACAGTGGCGACTCAGAT	TCCACTATGCTGGACAGGCAGT
Myogenin	*Myog*	TTGCTCAGCTCCCTCAACCAGGA	AGATTGTGGGCGTCTGTAGGGTCA
Myostatin	*Mstn*	TGGCTCAAACAGCCTGAATCCAAC	TGGGTGTGTCTGTCACCTTGACTT
Psme4	*Psme4*	AATTTTCTCCCATAAAGTCGGA	CAGATCAAGAGCAACCTGGG
Ube4a	*Ube4a*	GTTTGCAGCAATCCAGAAAGAG	CAGGCTCTCTGACACACTATTATC
Ubeq2q	*Ubeq2q*	GAATCGCTCGTGGTTCTTGT	GCTGGAGATGAGGGGAAGA
Itch	*Itch*	CCA CCC ACC CCA CGA AGA CC	CTA GGG CCC GAG CCT CCA GA
Psmb3	*Psmb3*	GAGCAAATGAAGGGCTTGAA	GGCCAACCTCCTGTATGAGA
Psmd8	*Psmd8*	GGCTGACTCAGGAAGCTGTT	CCTGTGCAAAGACATTCCCT
Psmd11	*Psmd11*	CTAGATATGGAAGCAGCCACAG	CCAATGCTTGGCGTAAGAAAG
CD36	*Cd36*	CCTGCAAATGTCAGAGGAAA	GCGACATGATTAATGGCACA
FATP1	*Slc27a1*	AGCCGAACACGAATCAGAAC	TTCTGTGTGTACGTGGGTGG
Acsl1	*Acsl1*	ACCATCAGTGGTACCCGCTA	CGCTCACCACCTTCTGGTAT
Acot1	*Acot1*	CCCCTGTGACTATCCTGAGAA	CAAACACTCACTACCCAACTGT
Acot2	*Acot2*	ATGGTGGCCTCGTCTTTCG	GAGCGGCGGAGGTACAAAC
p27 Kip1	*Cdkn1b*	GGGGAACCGTCTGAAACATT	AGTGTCCAGGGATGAGGAAG
Gadd45a	*Gadd45a*	GGATCCTTCCATTGTGATGAA	TGCTACTGGAGAACGACGC
4E-BP	*Eif4ebp1*	CCT CCT TGT GCC TGT GTC TA	GCC TAA GGA AAG ATG GGT GT
Cathepsin L	*Ctsl*	TATCCCTCAGCAAGAGAAAGCCCT	TCCTTCATAGCCATAGCCCACCAA
LC3A	*Map1lc3a*	TCTGGTCCCAGACCATGTTA	GGTTGACCAGCAGGAAGAAG
LC3B	*Map1lc3b*	CACTGCTCTGTCTTGTGTAGGTTG	TCGTTGTGCCTTTATTAGTGCATC
Lamp2a	*Lamp2*	ACAACCTGACTCCTGTCGTTCAGA	AGTTGGAGTTGGAGTGGGTGTTGA
Gabarapl	*Gabarapl1*	GTCATCGTGGAGAAGGCTCCTAAA	GGAGGGATGGTGTTGTTGACAAAG
Bnip3	*Bnip3*	CCCAGACACCACAAGATACCAACA	GGTCGACTTGACCAATCCCATATCCA
Bnip3L	*Bnip3l*	CACCACAAGAAGATGGGCAGATCA	TGGACCAGTCTGATACCCAGT
SMART	*Fbxo21*	TCA ATA ACC TCA AGG CGT TC	GTT TTG CAC ACA AGC TCC A
MUSA1	*Fbxo30*	TCG TGG AAT GGT AAT CTT GC	CCT CCC GTT TCT CTA TCA CG
Atrogin-1	*Fbxo32*	CTGTGCTGGTGGGCAACATTAACA	CGTCACTCAGCCTCTGCATGAT
MuRF-1	*Trim63*	ATGAAGTGATCATGGACCGGCA	TTGCACAAGGAGCAAGTAGGCA
Ulk1	*Ulk1*	TGCAAATGGTACAATCAGCTGCCC	AGCAGGGCTTTGTGATATCTCGGT
TBP	*Tbp*	ACCCTTCACCAATGACTCCTATG	TGACTGCAGCAAATCGCTTGG
GAPDH	*Gapdh*	TGTCGTGGAGTCTACTGGTGTCTT	TCTCGTGGTTCACACCCATCACAA

Supplementary Table S2. Primers for QPCR of Human genes.

Common name	Gene name	5' primer	3' primer
Human **QPCR Primers**			
p27 Kip1	*CDKN1B*	TTCATCAAGCAGTGATGTATCTGA	AAGAAGCCTGGCCTCAGAAG
Gadd45b	*GADD45B*	GGATGAGCGTGAAGTGGATT	GTGTACGAGTCGGCCAAGTT
4E-BP	*EIF4EBP1*	AGTTCCGACACTCCATCAGG	CGGGGACTACAGCACGAC
Cathepsin L	*CTSL*	AAAGGCAGCAAGGATGAGTG	GCGCGTGACTGGTTGAG
LC3B	*MAP1LC3B*	TTGTTTTATCCAGAACAGGAAGC	CGGAGAAGACCTTCAAGCAG
LC3C	*MAP1LC3C*	CCCAAGCGTCAGACCCTTC	GGGGAACTTTGCCCGGATT
Lamp2	*LAMP2*	CAATGCATAAGACCGCACAG	GGTGTTGCAGCTGTTGTTGT
Gabarapl	*GABARAPL1*	TGGCCAACAGTAAGGTCAGA	TCGGAAAAAGGAAGGAGAAA
SMART	*FBXO21*	TGCGTTTGGGATGTAGTGAG	ATAACGTGCTGGTGGAGGAC
MUSA1	*FBXO30*	TTCACACAATGGGAATGCTG	GTCCAGGGCAGCTAGGG
Atrogin-1	*FBXO32*	ATGCCACTCAGGGATGTGA	TTCTCAACTGCCATTCTGGA
MuRF-1	*TRIM63*	CTTCGTGCTCCTTGCACAT	ATCGTCACGGAGTGTACGG
Bnip3	*BNIP3*	CTGTGCGTCCAGCAGTATTT	GATGCAGGAGGAGAGCCTG
Bnip3L	*BNIP3L*	CGCACTTTTCTTCAAAGCCT	GATGTGGAAATGCACACCAG
TBP	*TBP*	GCCATAAGGCATCATTGGAC	AACAACAGCCTGCCACCTTA

SUPPLEMENTARY DATA

Supplementary Table S3. Antibodies used.

Protein	Vendor	Catalog number	Dilution
Immunofluorescence Antibodies			
LC3A	Cell Signaling	4599	1:200
Laminin	Sigma	L9393	1:200
Myosin IIa	DSHB University of Iowa	SC-71	1:200
Alexa-Fluor-594 Goat anti mouse	Life Technologies	#A-11032	1:500
Alexa-Fluor-488 Goat anti rabbit	Life Technologies	#A-11008	1:500
Western Blot Antibodies			
FoxO1	Cell Signaling	2880	1:1000
FoxO3	Cell Signaling	12829	1:1000
FoxO4	Abcam	128908	1:1000
β-Actin	Cell Signaling	4970	1:1000
GAPDH	Cell Signaling	5174	1:1000
LC3A/B	Cell Signaling	12741	1:1000
p62/SQSTM1	Cell Signaling	5114	1:500
p-ULK^{S555}	Cell Signaling	5869	1:1000
ULK1	Cell Signaling	8054	1:500
Ubiquitin	Cell Signaling	3933	1:1000
19S Proteasome Subunit	Enzo Life Sciences	BML-PW8870	1:1000
p-4EBP1	Cell Signaling	9451	1:1000
4EBP1	Cell Signaling	9452	1:1000
Anti-Puromycin Sera (non-concentrated)	DSHB University of Iowa	PMY-2A4-S	1:20
Secondary for Puromycin	Jackson ImmunoResearch	115-035-208	1:10,000
Rabbit secondary antibody	Thermo Fisher	SA5-35571	1:10,000
Mouse secondary antibody	Thermo Fisher	SA5-35521	1:10,000