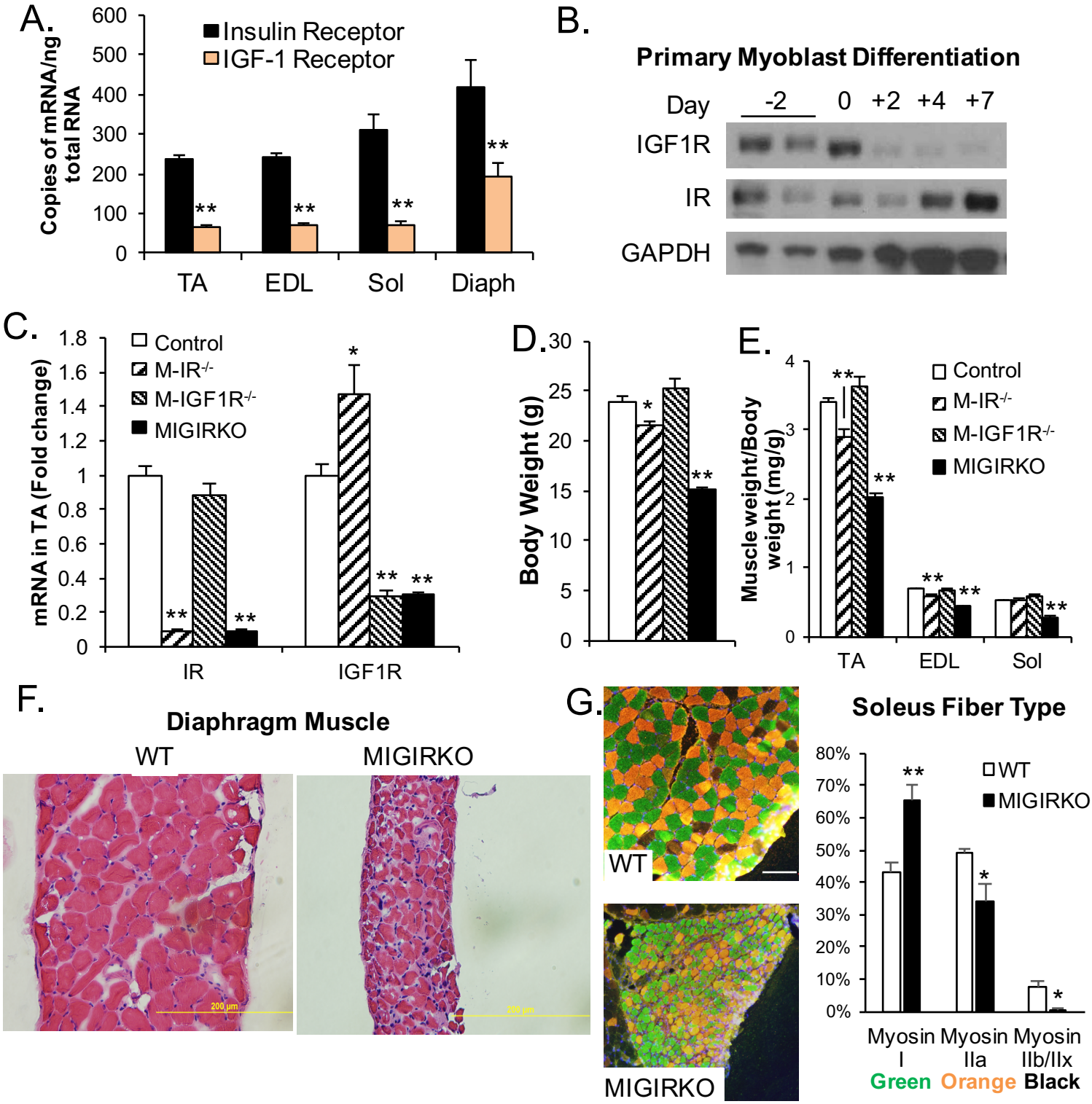


Figure S1. IR is the predominant receptor in differentiated muscle and Deletion of IR and IGF1R in muscle dramatically decreases muscle size and Function. (A) Copy number of insulin receptor and IGF-1 receptor mRNA determined by qPCR in muscle from 8-week-old male mice (n=7) was performed and quantitated using recombinant plasmid standard as in Figure 1A (**-$p<0.01$ vs. insulin receptor, TA data is the same as presented in Figure 1A). (B) IR and IGF1R western blot in primary mouse myoblasts during differentiation. (C) QPCR of quadriceps from fasted control, M-IR$^{-/-}$, M-IGF1R$^{-/-}$, and MIGIRKO mice (n=16 pooled controls and 4-8 knockouts). (D) Body weight and (E) muscle weights normalized to body weight from 7-8 week old control M-IR$^{-/-}$, M-IGF1R$^{-/-}$, and MIGIRKO. (F) H&E staining of diaphragm muscle from control and MIGIRKO mice (bar = 200 μm). (G) Fiber type density in soleus (bar = 100 μm). (*-$p<0.05$,**-$p<0.01$ vs. control; Student's t-test for 2 groups, ANOVA for 4)

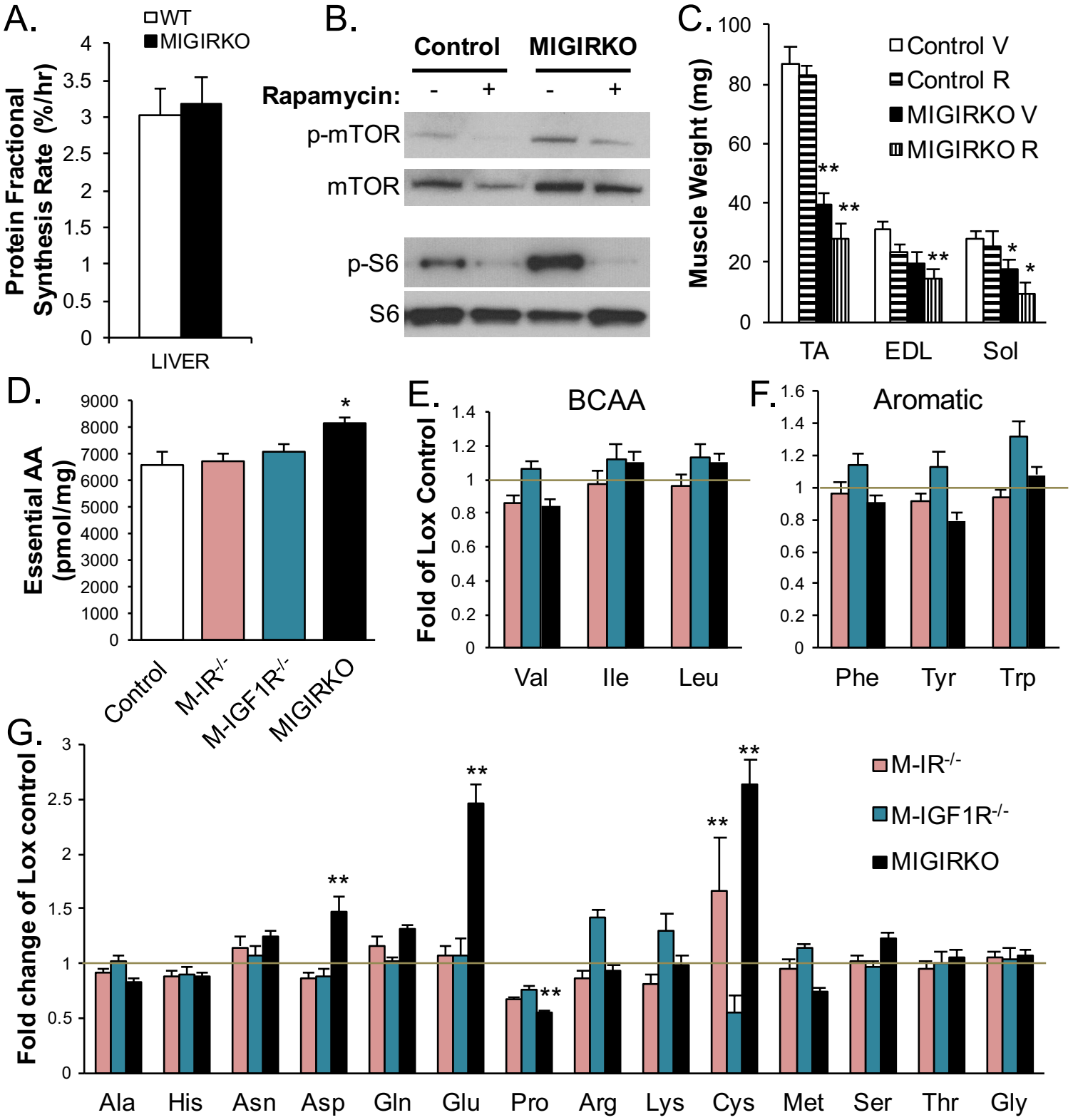


Figure S2. Protein synthesis, mTOR signaling and Muscle Amino Acid levels are increased in MIGIRKO mice. (A) Protein fractional synthesis rates were measured using ^{13}C-labeled phenylalinine in Liver (n=11). (B) Western blots for phosphorylation of mTOR and S6 in quadriceps from MIGIRKO and control mice treated with 1.2 mg/kg rapamycin for 12-14 days. (C) Muscle weight from MIGIRKO and control mice treated with rapamycin for 12-14 days (n=4-5 per group). (D) Essential amino acid (AA) levels measured by metabolomic analysis in hind limb muscle from M-IR$^{-/-}$, M-IGF1R$^{-/-}$, and MIGIRKO mice (n=18 pooled controls and 5-12 KO per group). Fold change of (E) Branch chain AA (BCAA), (F) Aromatic AA, and (G) other essential AA in knockout mice relative to control. (*-p<0.05,**-p<0.01 vs. control; ANOVA)

Supplemental Figure S3

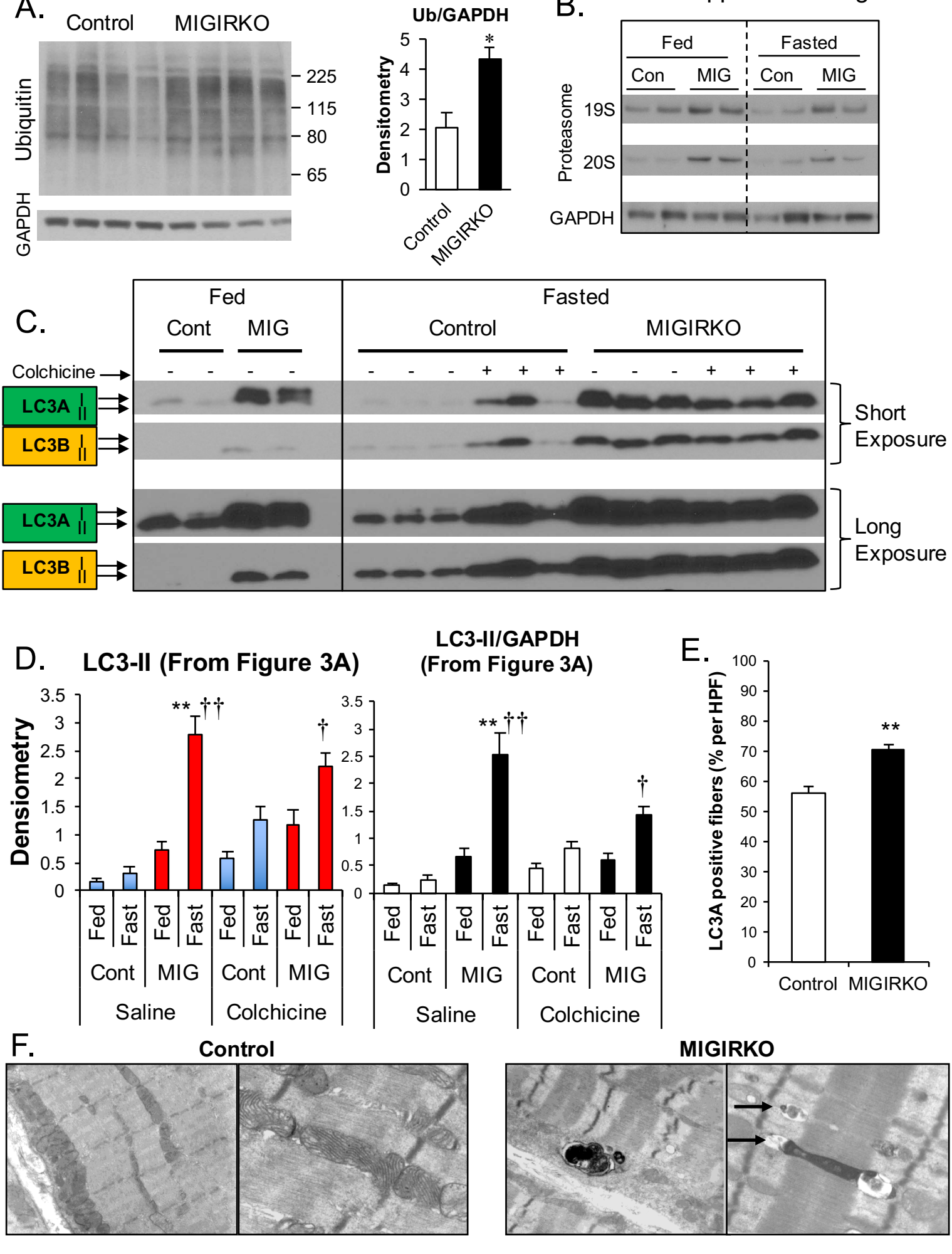


Figure S3. Ubiquitin-Proteasome subunits, LC3 isoforms,and autophagosomes are increased in MIGIRKO muscle. (A) Western blot and densitometry of total poly-ubiquitin (Ub) proteins in gastrocnemius from MIGIRKO and control mice treated with MG132 for 3 days. GAPDH normalizer blot is the same as depicted in Figure 2G as it was on the same gel. (B) Western blot of proteasomal subunits of the 19S regulatory complex (Rpt5/S6a) and the 20S core complex in quadriceps from MIGIRKO and control mice either fed or fasted overnight. (C) Western blots for LC3 using isoform-specific antibodies (LC3A – Cell Signaling #4599; LC3B – Cell Signaling #2775) in quadriceps muscle from fed or overnight-fasted MIGIRKO and control mice treated with either saline or 0.4 mg/kg colchicine for 2 days. (D) Densitometric analysis of LC3-II and LC3-II normalized to GAPDH from Figure 3 panel A using LC3A/B antibody (Cell Signaling #12741) (n=3-6 per group). (E) (F) Representative electron micrographs from soleus muscle of fed MIGIRKO and control mice showing double-membraned autophagosomes. (*p<0.05,**-p<0.01 control vs. MIGIRKO; †-p<0.05, ††-p<0.01 fed vs. fasted, t-test for 2 groups, ANOVA for more)

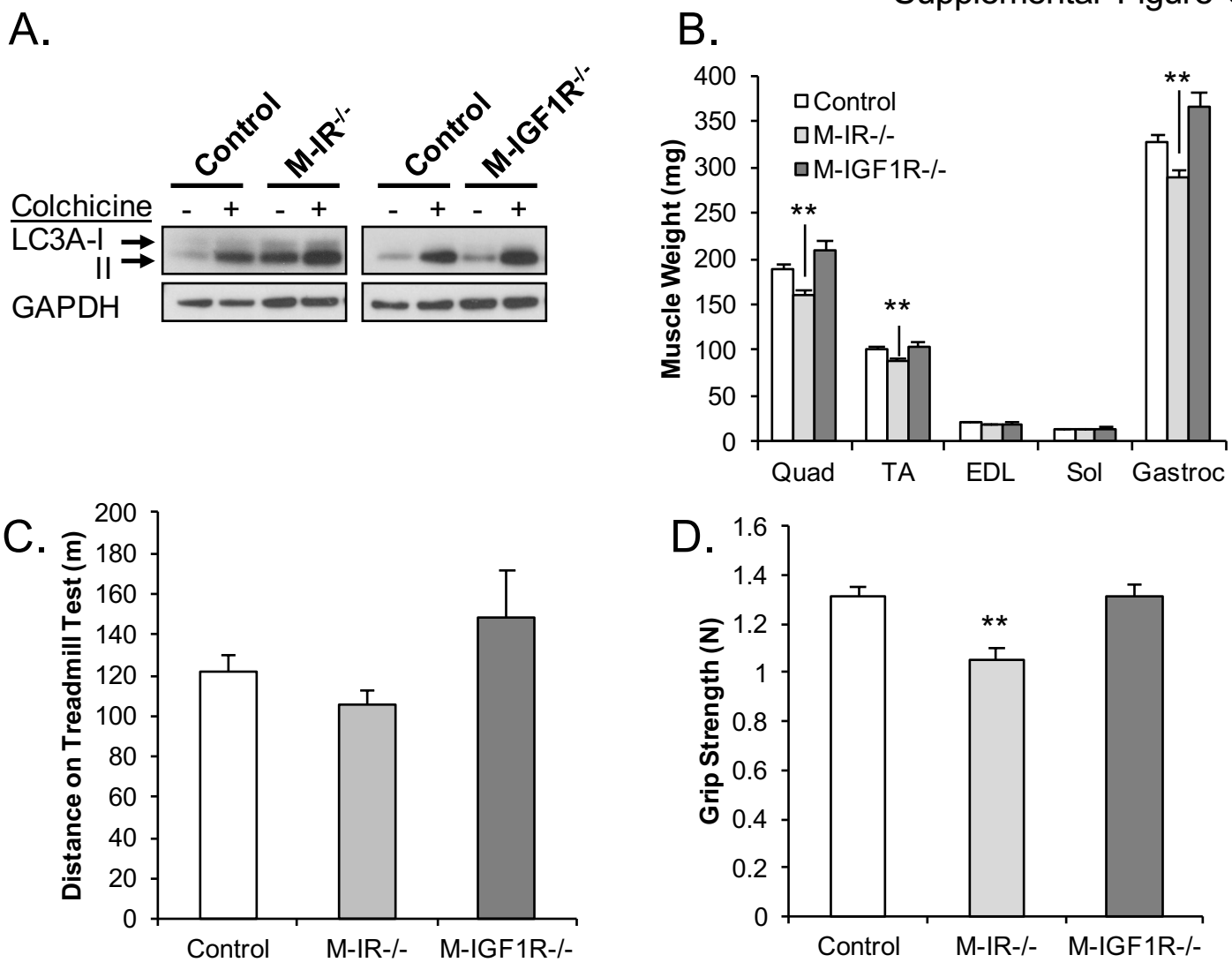


Figure S4. M-IR$^{-/-}$ mice display muscle atrophy, muscle dysfunction and impaired autophagy as they age. (A) Western blot of LC3A in quadriceps from 52-week old M-IR$^{-/-}$, M-IGF1R$^{-/-}$, and control mice treated for 2 days with colchicine or saline. (B) Muscle weights from 52-week-old M-IR$^{-/-}$, M-IGF1R$^{-/-}$, and control mice. (C) Distance run on acute treadmill test and (D) grip strength from 52-week old M-IR$^{-/-}$, M-IGF1R$^{-/-}$, and control mice. (**-p<0.01 vs. control, student's t-test)

Supplemental Figure S5

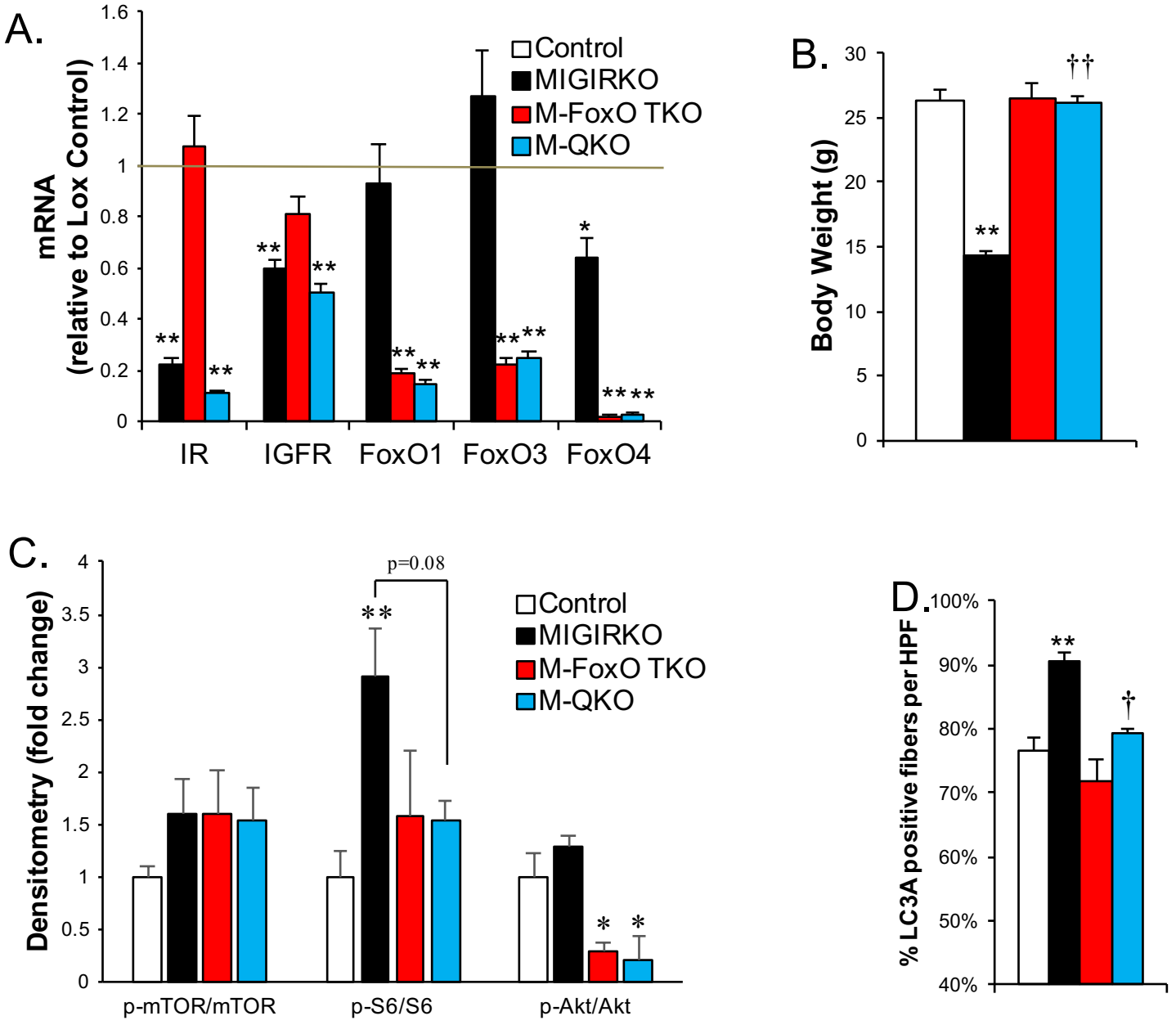


Figure S5. Deletion of FoxO1/3/4 in MIGIRKO mice normalizes body weight, attenuates mTOR signaling and decreases Akt activation. (A) mRNA levels of IR, IGF1R and FoxO isoforms in MIGIRKO, M-FoxO TKO, and M-QKO TA muscle relative to littermate lox controls. (B) Body weights of control and knockout mice (n=5-7 per group). (C) Densitometry of western blots from Figure 6B (n=5-6 per group). (D) Percent of LC3A-positive myofibers per high power field from Figure 6 panel C. (*-p<0.05,**-p<0.01 vs. Control, †-p<0.05, ††-p<0.01 MIGIRKO vs. M-QKO, ANOVA).

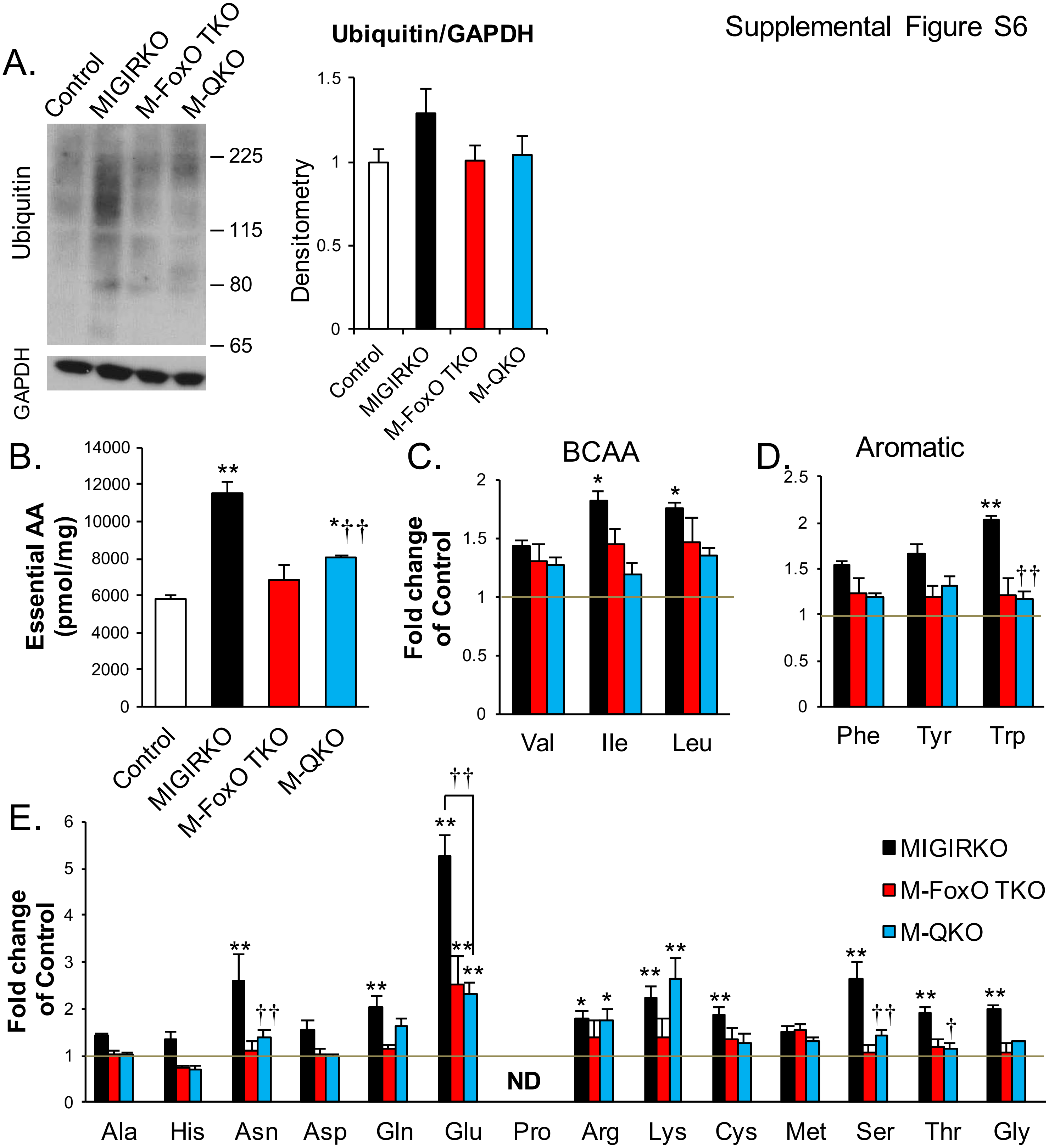


Figure S6. Deletion of FoxO1/3/4 in MIGIRKO mice attenuates ubiquitination and Amino Acid differences. (A) Western Blot and densitometry of total poly-ubiquitin proteins in Control, MIGIRKO, M-FoxO TKO, and M-QKO quadriceps (n=5-6 per group). (B) Essential amino acid (AA) levels in hind limb muscle from MIGIRKO, M-FoxO TKO, and M-QKO (n=6 per group). Fold change of (C) Branch chain AA (BCAA), (D) Aromatic AA, and (E) other essential AA in knockout mice relative to control. (*-p<0.05,**-p<0.01 vs. Control,††-p<0.01 MIGIRKO vs. M-QKO, ANOVA). ND – not determined.

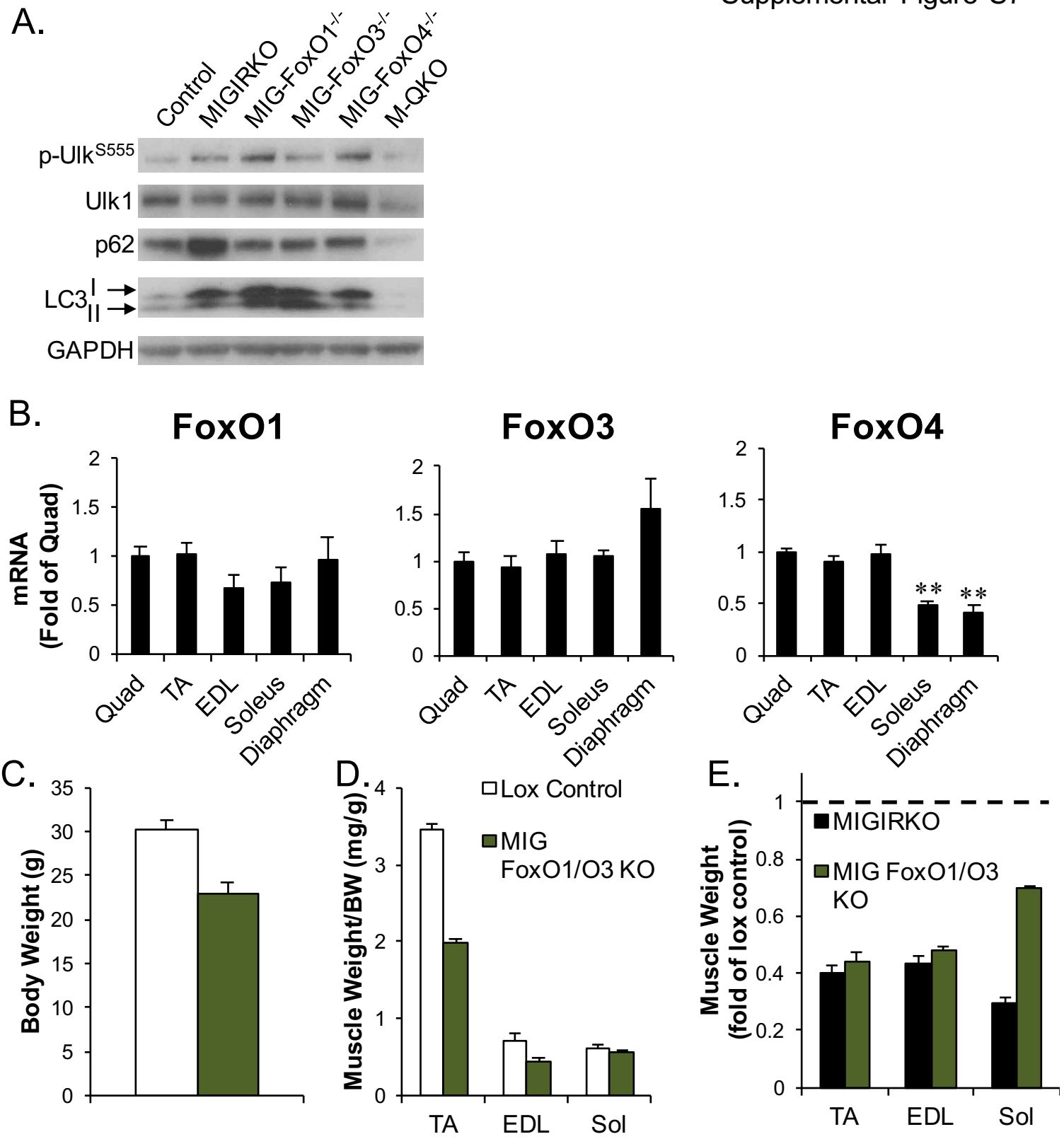


Figure S7. Deletion of individual FoxOs does not reverse autophagy in MIGIRKO, while deletion of both FoxO1 and FoxO3 partially rescues oxidative muscle atrophy in MIGIRKO. (A) Western Blot of autophagy markers in TA muscle from mice in Figure 7F-7G. GAPDH normalizer blot is the same as depicted in Figure 7F as it was on the same gel. (B) mRNA levels of FoxO isoforms in 5 different muscle groups from control mice (n=4-8). (C) Body weights and (D) muscle weight normalized to body weight of control and MIG-FoxO1/O3 KO mice at 8 weeks of age (n=2). (E) Percent decrease in muscle mass in MIGIRKO and MIG-FoxO1/O3 KO relative to littermate controls (n=2). (**-$p<0.01$ vs. Quad, Student's t-test)

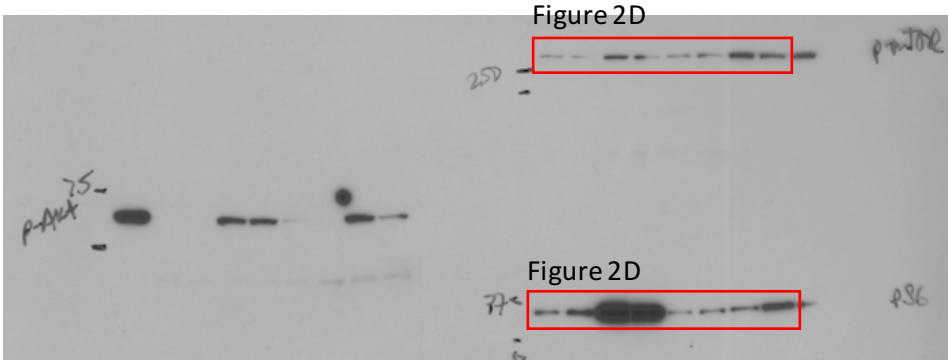
Supplemental Table S1. Primers for QPCR of mouse genes.

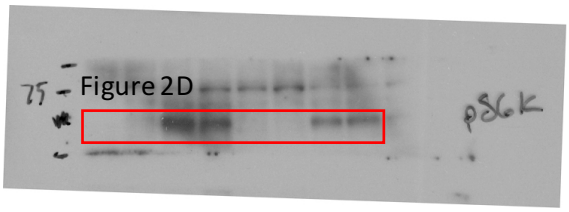
Common name	Gene name	5' primer	3' primer
mRNA Primers			
IR	*Insr*	AAATGCAGGAACTCTCGGAAGCCT	ACCTTCGAGGATTTGGCAGACCTT
IGF1R	*Igf1r*	ATCGCGATTTCTGCGCCAACA	TTCTTCTCTTCATCGCCGCAGACT
IGF-1	*Igf1*	GACCGAGGGGCTTTTACTTC	GGGGCACAGTACATCTCCA
Myf5	*Myf5*	AATGCCATCCGCTACATTGAGAGC	TGTCAAAGCTGCTGTTCTTTCGGG
MyoD	*Myod1*	AGCACTACAGTGGCGACTCAGAT	TCCACTATGCTGGACAGGCAGT
Myogenin	*Myog*	TTGCTCAGCTCCCTCAACCAGGA	AGATTGTGGGCGTCTGTAGGGTCA
Myostatin	*Mstn*	TGGCTCAAACAGCCTGAATCCAAC	TGGGTGTGTCTGTCACCTTGACTT
Follistatin	*Fst*	AGTGACTTACTCCAGCGCCT	TTACTGTCAGGACACAGCTCATCG
Actin (SkM)	*Acta1*	TTCAACGTGCCTGCCATGTATGTG	ATGATGGCGTGTGGCAGGGCATA
Desmin	*Des*	ACCAGGACCTGCTCAATGTGAAGA	TCGGAAGTTGAGAGCAGAGAAGGT
MCK	*Ckm*	GCAGCAGCTCATTGATGACCACTT	ACCTCCTTCATATTGCCTCCCTTC
Myosin I	*Myh7*	ACCAGGCCCTTTGACCTCAAGAAA	TCTTGTCGAACTTGGGTGGGTTCT
Myosin IIa	*Myh2*	TCACATCCAACAAGAAGCCAGAGC	CCCTGGCTGACAAATGGGTAATCA
Myosin IIb	*Myh4*	AGTCCCAGGTCAACAAGCTG	TTTCTCCTGTCACCTCTCAACA
Myosin IIx	*Myh1*	AGTCCCAGGTCAACAAGCTG	CACATTTTGCTCATCTCTTTG
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
p62	*Sqstm1*	TCTGGGGTAGTGGGTGTCAG	AGAATGTGGGGGAGAGTGTG
LC3A	*Map1lc3a*	TCTGGTCCCAGACCATGTTA	GGTTGACCAGCAGGAAGAAG
LC3B	*Map1lc3b*	CACTGCTCTGTCTTGTGTAGGTTG	TCGTTGTGCCTTTATTAGTGCATC
Lamp2a	*Lamp2*	ACAACCTGACTCCTGTCGTTCAGA	AGTTGGAGTTGGAGTGGGTGTTGA
Gabarapl 1	*Gabarapl 1*	GTCATCGTGGAGAAGGCTCCTAAA	GGAGGGATGGTGTTGTTGACAAAG
Psma1	*Psma1*	CGTTCTCAATCAGCTCGTACTT	CTGCAGGGAGTGTTTCTCTT
Psmb4	*Psmb4*	TGTGGACATGCTTGGTGTAG	CTGGCTGCTTCTCTAGAACTT
Psmc4	*Psmc4*	CTTGGAAGCTGTGGATCAGAA	TCCCGGTCGATGGTACTC
Psmd11	*Psmd11*	CTAGATATGGAAGCAGCCACAG	CCAATGCTTGGCGTAAGAAAG
Atrogin-1	*Fbxo32*	CTGTGCTGGTGGGCAACATTAACA	CGTCACTCAGCCTCTGCATGAT
MuRF-1	*Trim63*	ATGAAGTGATCATGGACCGGCA	TTGCACAAGGAGCAAGTAGGCA
MUSA1	*Fbxo30*	TCG TGG AAT GGT AAT CTT GC	CCT CCC GTT TCT CTA TCA CG
SMART	*Fbxo21*	TCA ATA ACC TCA AGG CGT TC	GTT TTG CAC ACA AGC TCC A
UBC	*Ubc*	CGTCGAGCCCAGTGTTACCACC	ACCTCCCCCATCACACCCAAGA
FoxO1	*Foxo1*	TGCTGTGAAGGGACAGATTG	GAGTGGATGGTGAAGAGCGT
FoxO3	*Foxo3*	ACAAACGGCTCACTTTGTCCCAGA	TCTTGCCCGTGCCTTCATTCT
FoxO4	*Foxo4*	GGTGCCCTACTTCAAGGACA	AGCTTGCTGCTGCTATCCAT
TBP	*Tbp*	ACCCTTCACCAATGACTCCTATG	TGACTGCAGCAAATCGCTTGG
GAPDH	*Gapdh*	TGTCGTGGAGTCTACTGGTGTCTT	TCTCGTGGTTCACACCCATCACAA

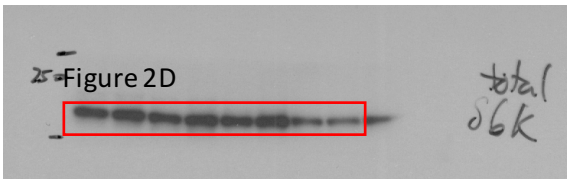
Supplemental Table S2. Antibodies used.

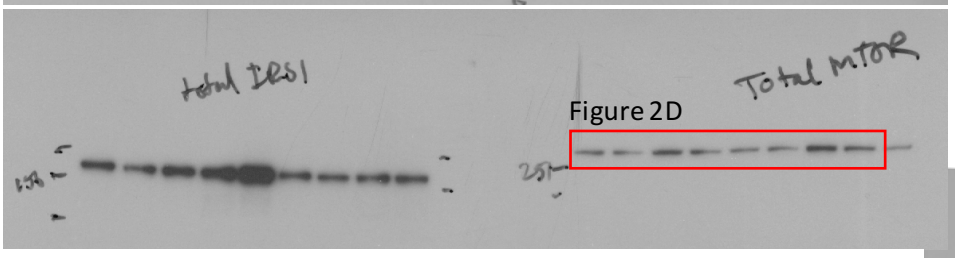
Protein	Vendor	Catalog number	Dilution
Immunofluorescence Antibodies			
LC3A	Cell Signaling	4599	1:200
Laminin	Sigma	L9393	1:200
Myosin IIa	DSHB University of Iowa	SC-71	1:200
Myosin heavy chain, slow	DSHB University of Iowa	BA-F8	1:200
Alexa-Fluor-594 Goat anti mouse	Life Technologies	#A-11032	1:500
Alexa-Fluor-488 Goat anti rabbit	Life Technologies	#A-11008	1:500
Western Blot Antibodies			
p-mTOR S2448	Cell Signaling	5536	1:1000
mTOR	Cell Signaling	2983	1:1000
p-S6K T389	Cell Signaling	9205	1:1000
S6K	Cell Signaling	9202	1:1000
p-S6	Cell Signaling	2211	1:1000
S6	Cell Signaling	2317	1:1000
FoxO1	Cell Signaling	2880	1:1000
FoxO3	Cell Signaling	12829	1:1000
FoxO4	Abcam	128908	1:1000
GAPDH	Cell Signaling	5174	1:1000
IGF1R	Cell Signaling	3027	1:1000
IR	Santa Cruz	SC-711	1:1000
19S proteasome subunit	Enzo Life Sciences	BML-PW8870	1:1000
20S proteasome subunit	Enzo Life Sciences	BML-PW8155-0025	1:1000
LC3A/B (Used for all blots except Supplemental Fig S3C)	Cell Signaling	12741	1:1000
LC3A	Cell Signaling	4599	1:1000
LC3B	Cell Signaling	2775	1:1000
p62/SQSTM1	Cell Signaling	5114	1:500
p-ULK S555	Cell Signaling	5869	1:1000
Ulk1	Cell Signaling	8054	1:1000
PARP	Cell Signaling	9542	1:500
p-Akt S473	Cell Signaling	9271	1:1000
Akt	Cell Signaling	4685	1:1000

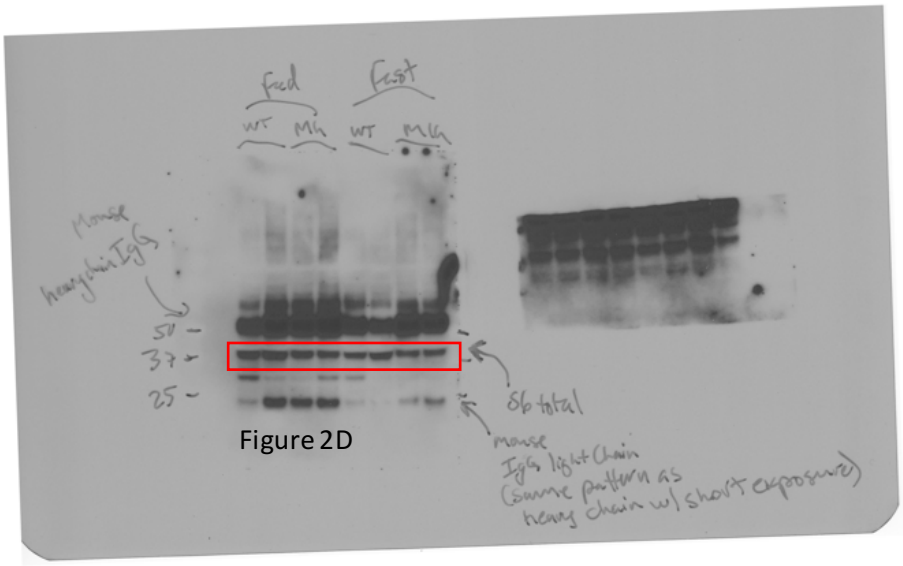
Full Uncut Gels

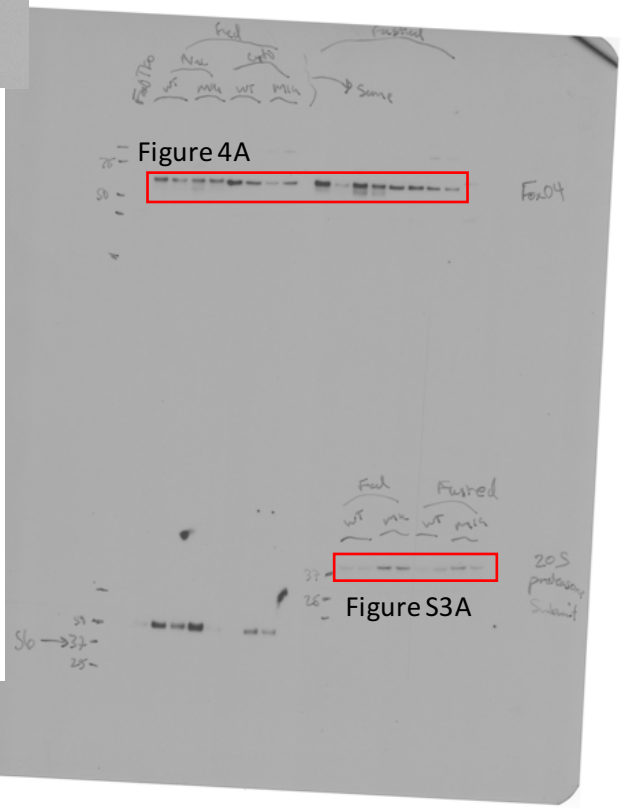


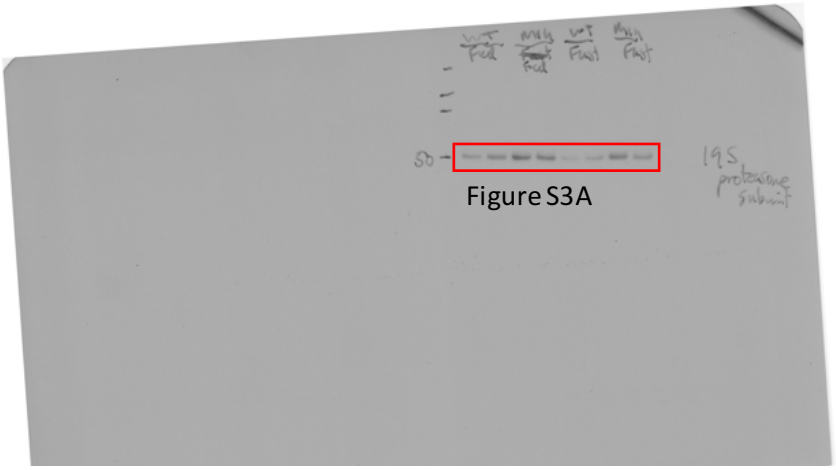


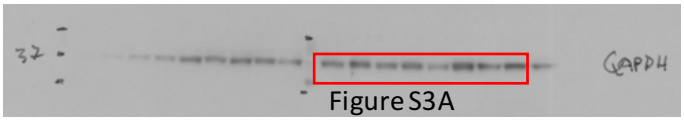


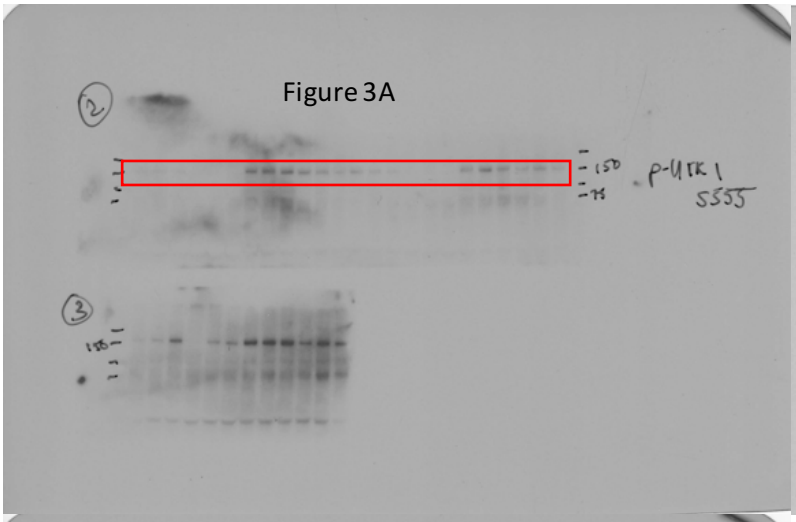








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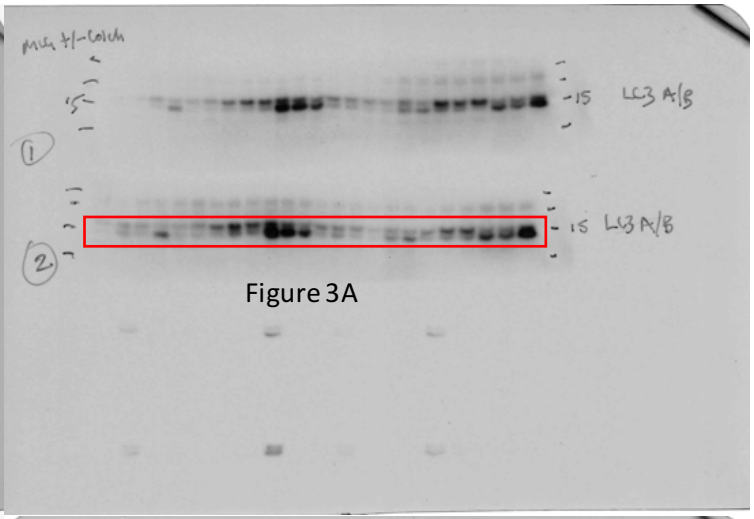
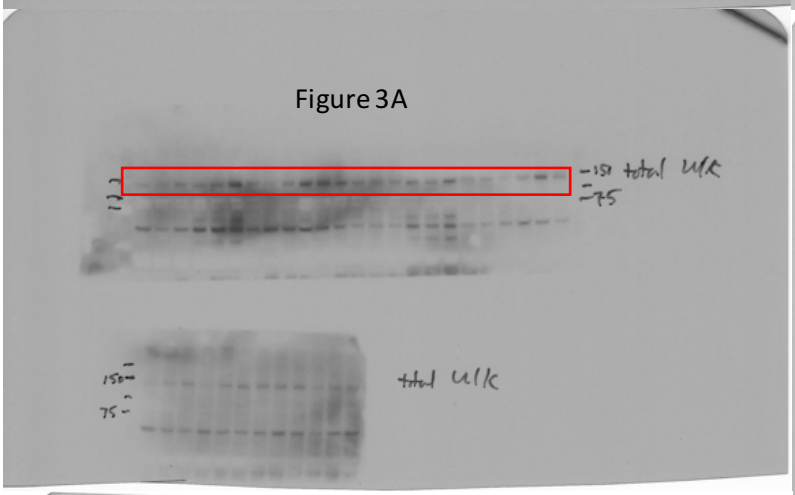

LC3 A/B
LC3 A/B
Figure 3A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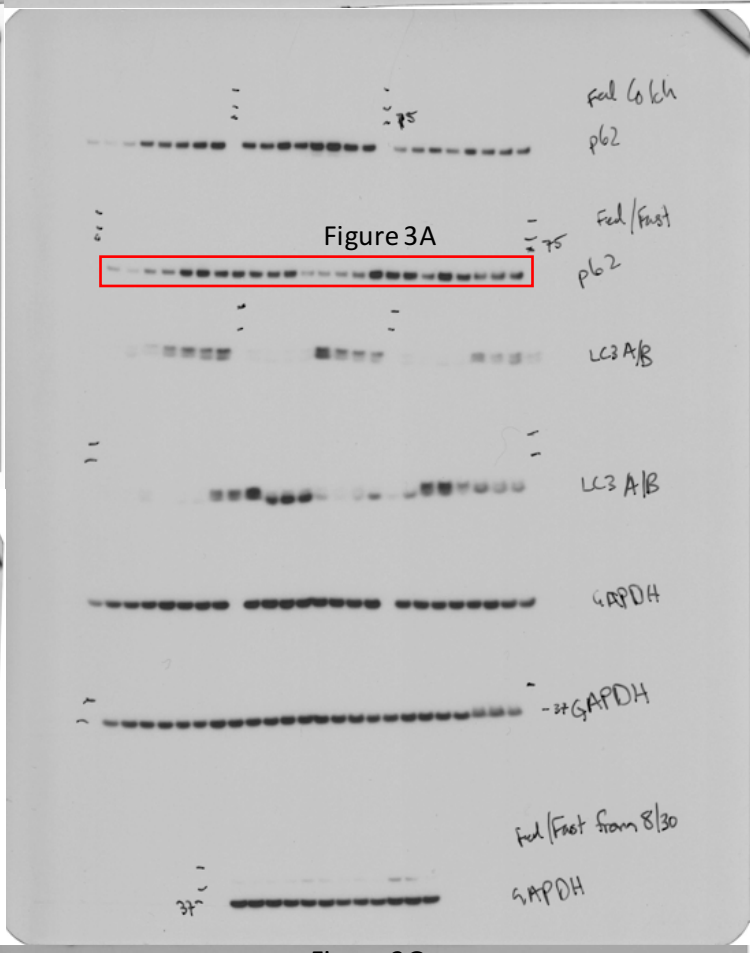
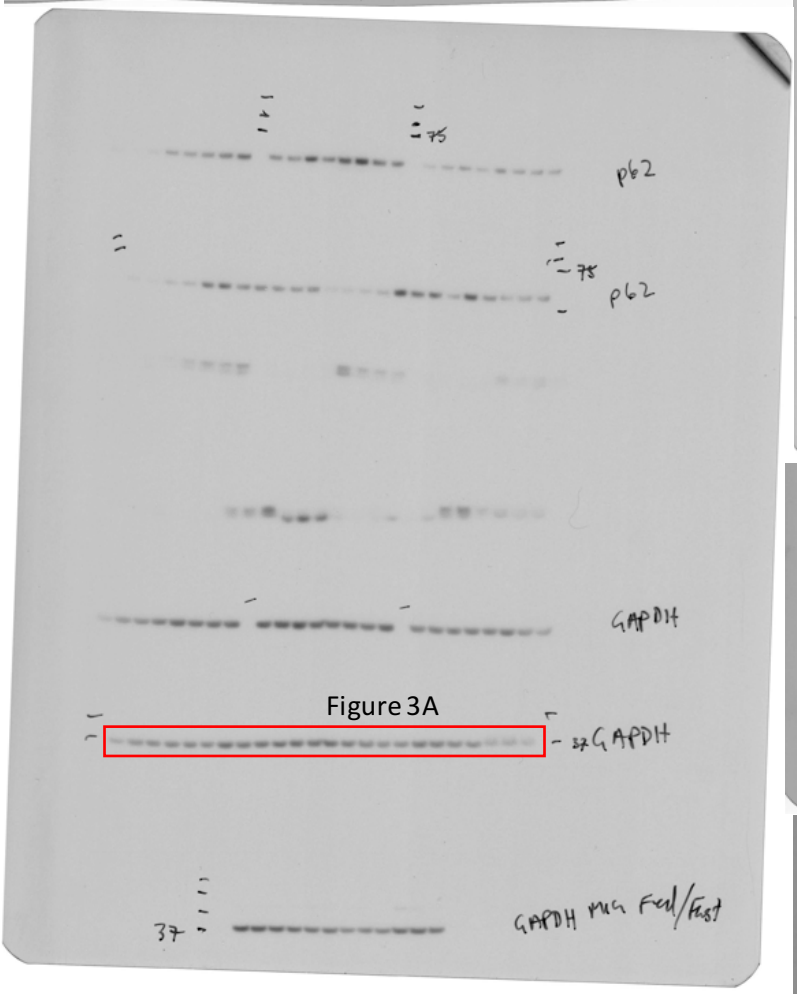
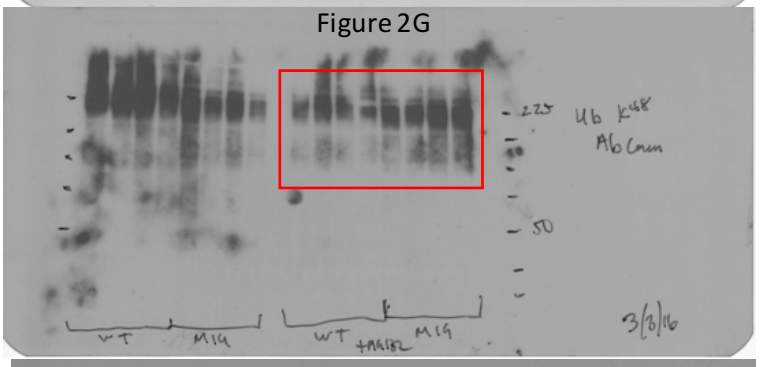

Fed Golch
p62
Fed/Fast
Figure 3A
p62
LC3 A/B
LC3 A/B
GAPDH
GAPDH
Fed/Fast from 8/30
GAPDH


p62
p62
GAPDH
Figure 3A
GAPDH
GAPDH MG Fed/Fast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Ub K48
3/8/16

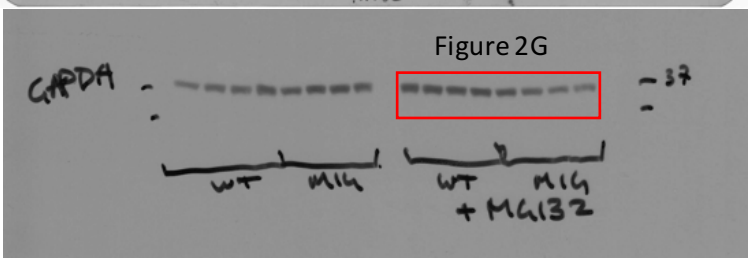
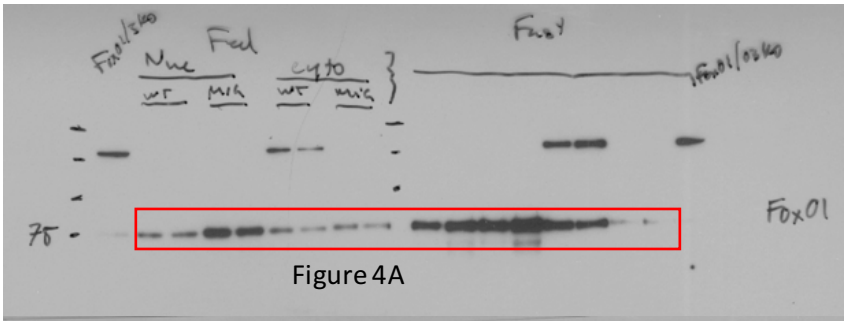
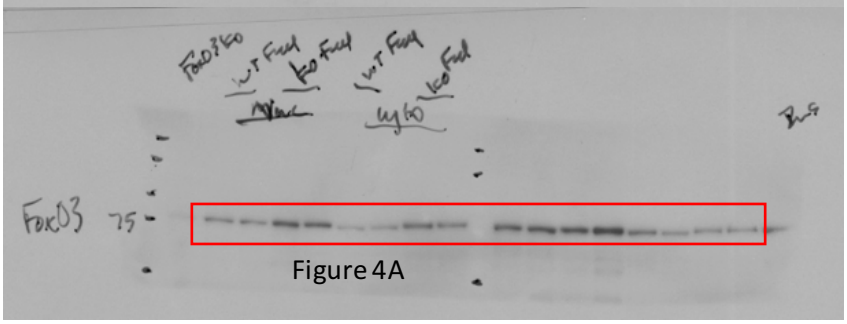
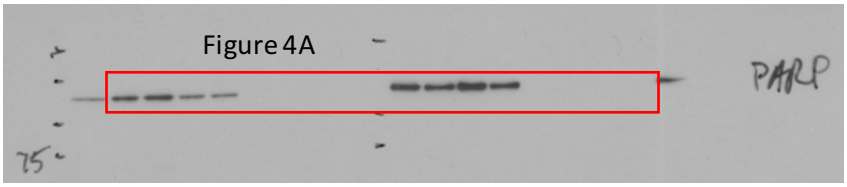


Figure 2G
GAPDA
WT
MG
WT
MG
+MG132







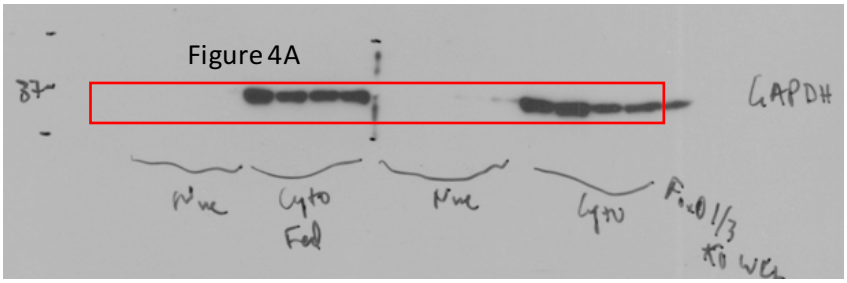
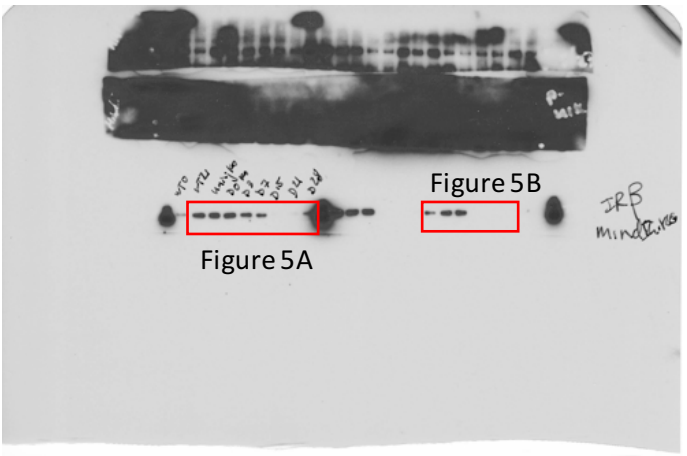
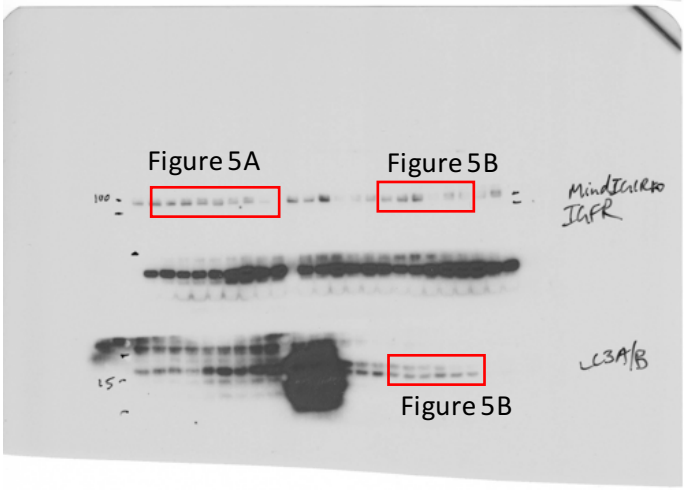
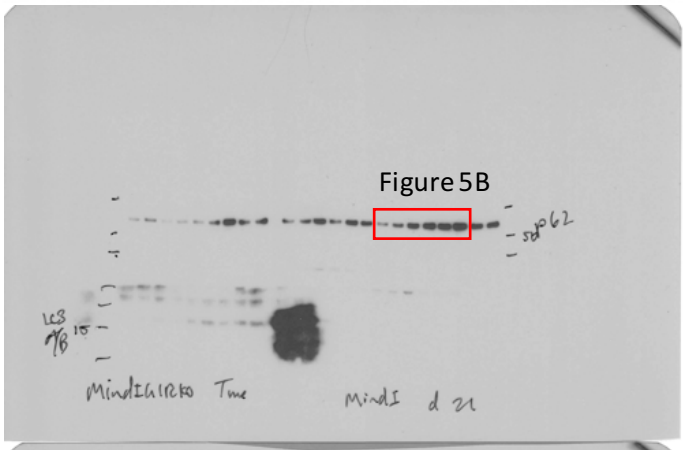
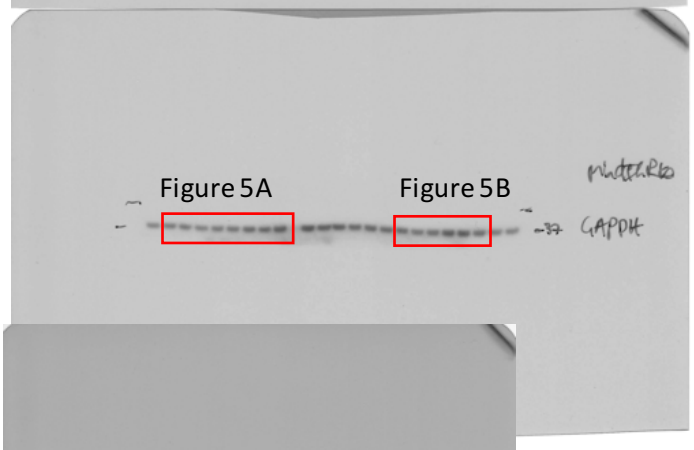


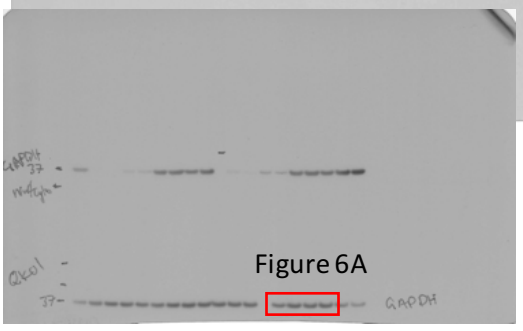
Figure 4A – See page 1 for FoxO4 blot

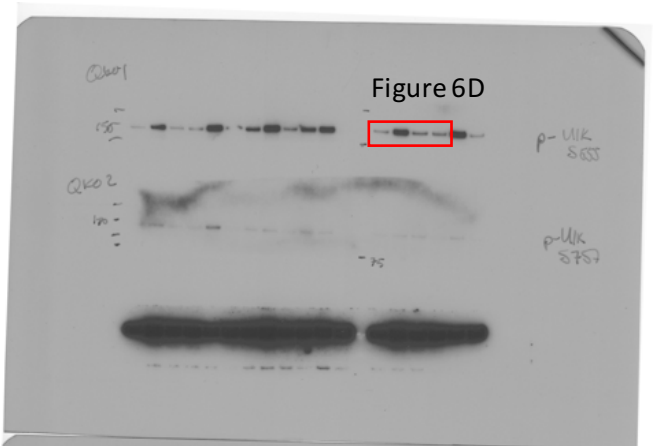


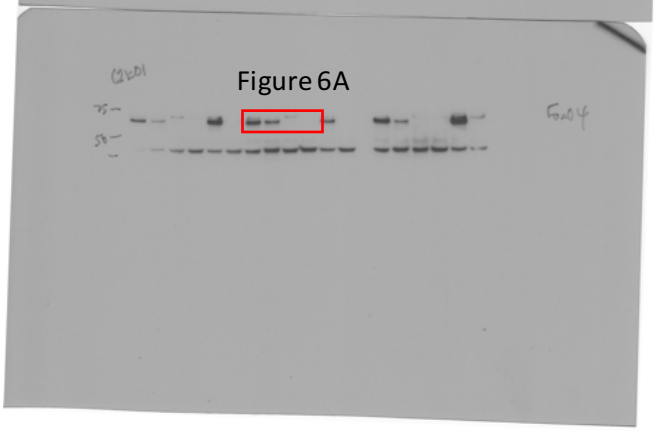












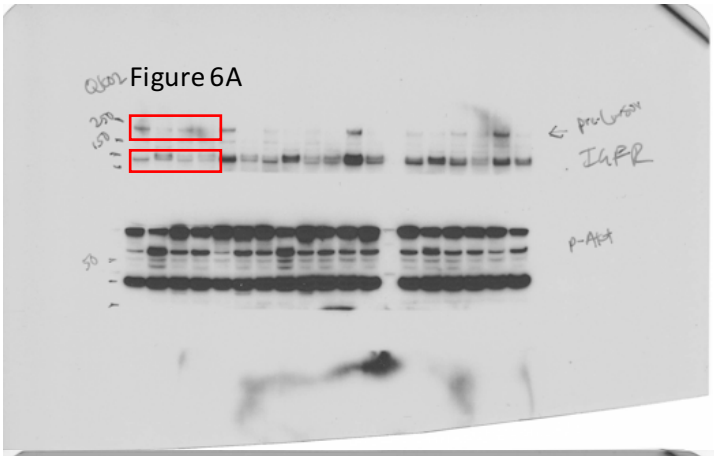


Figure 6A
IGFR
p-Akt

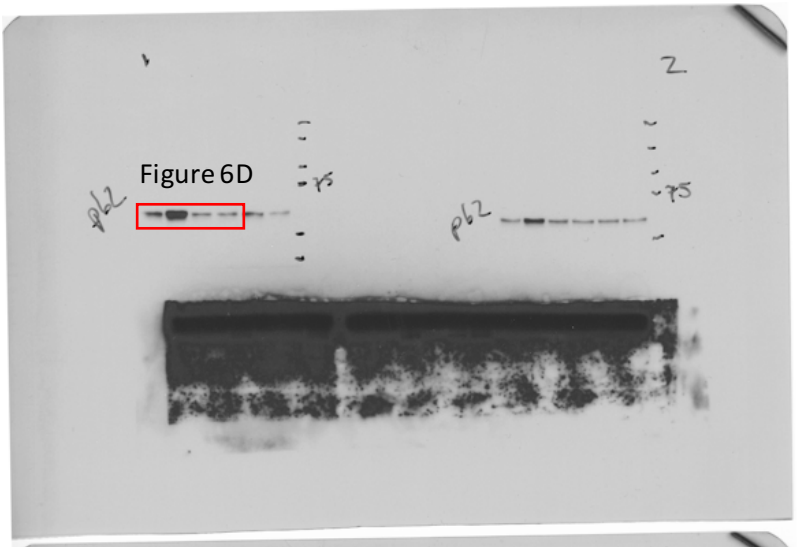


Figure 6D
p62
p62

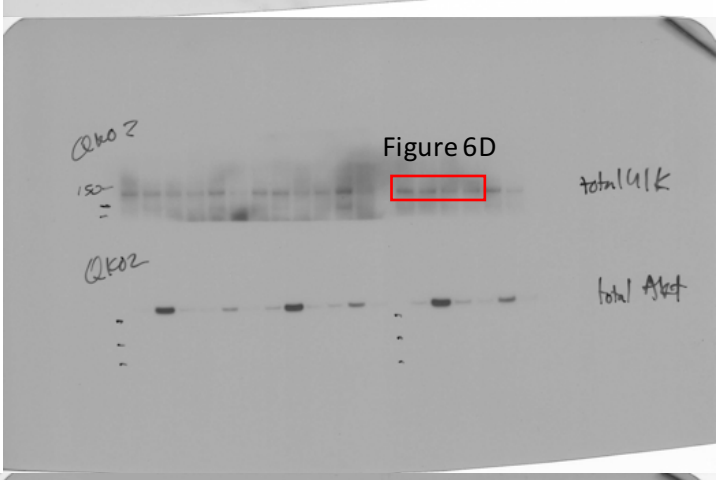


Figure 6D
total IGFR
total Akt

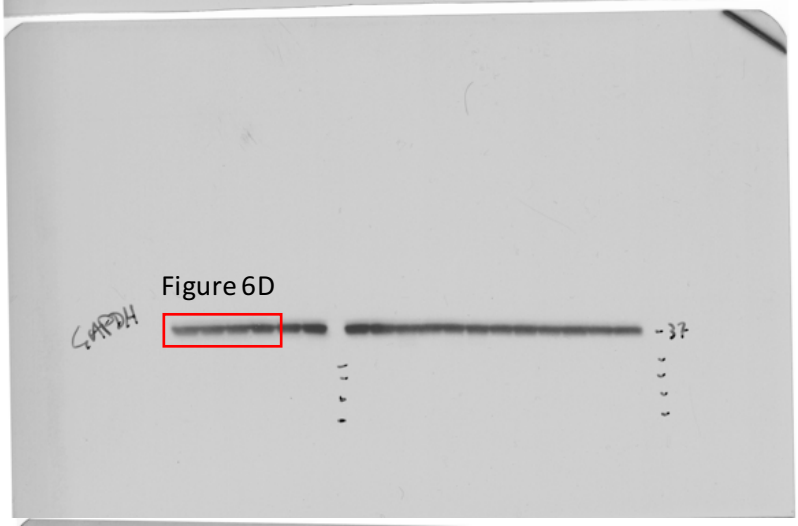
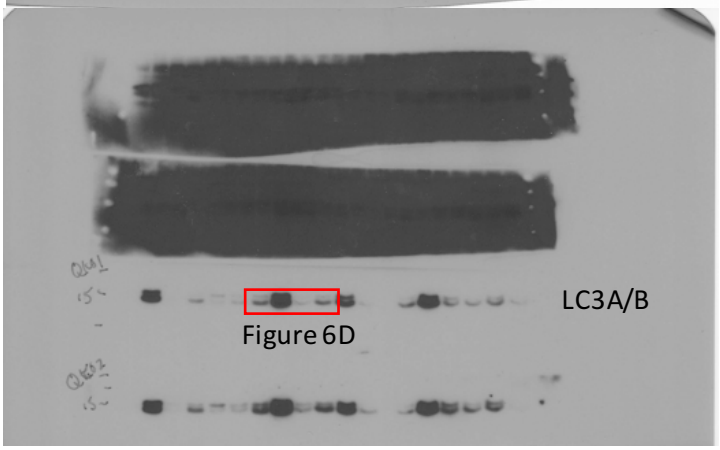
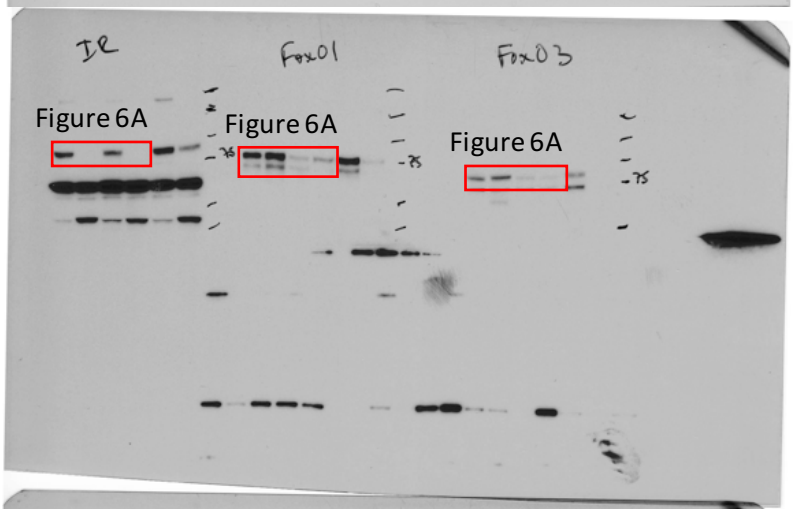
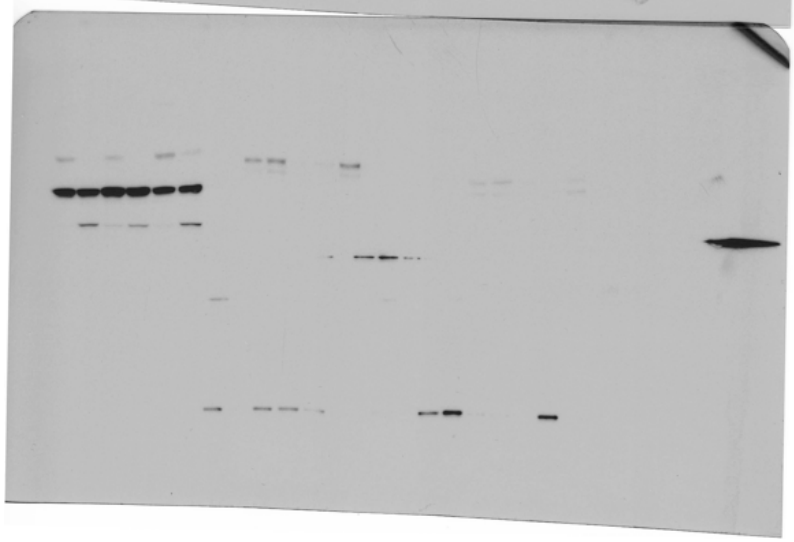


Figure 6D
GAPDH


LC3A/B
Figure 6D


IR
FoxO1
FoxO3
Figure 6A
Figure 6A
Figure 6A



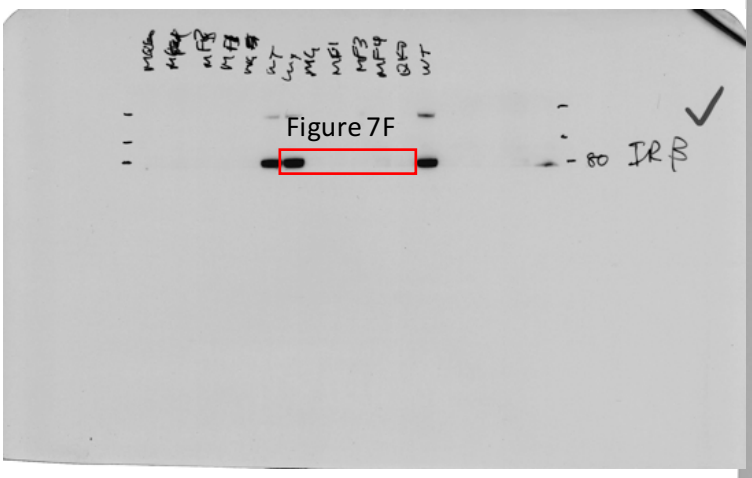
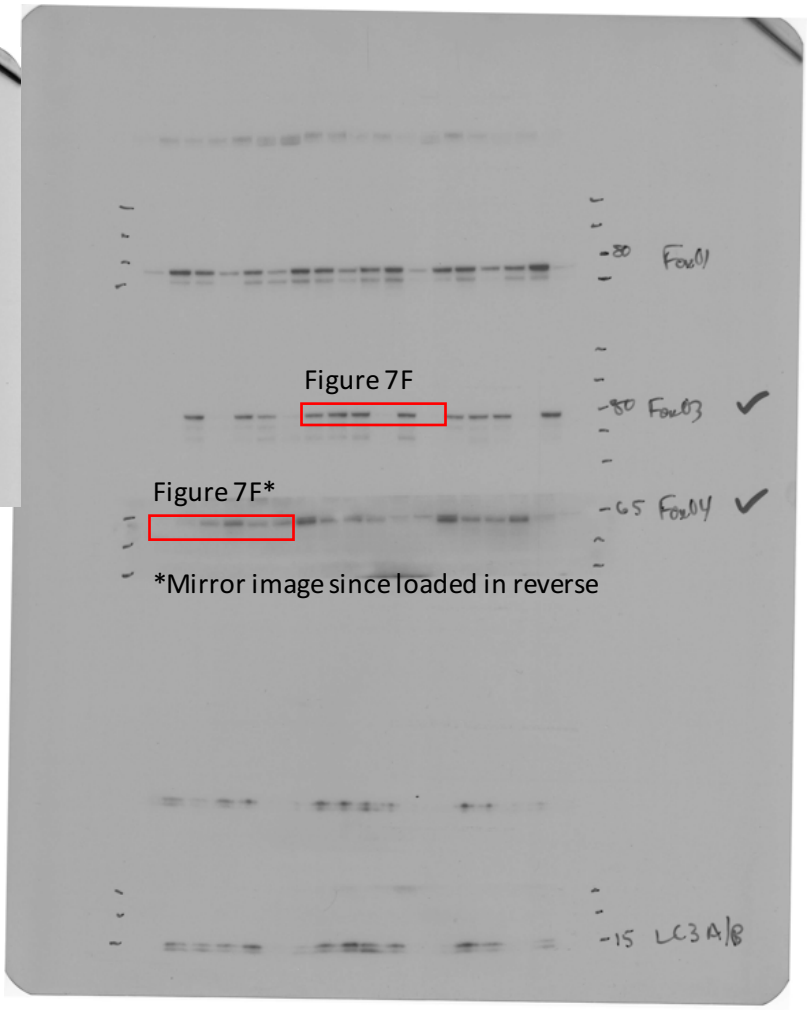


Figure 7F
80
IRβ


80
FoxO1
Figure 7F
80
FoxO3
Figure 7F*
65
FoxO4
*Mirror image since loaded in reverse
15
LC3A/B

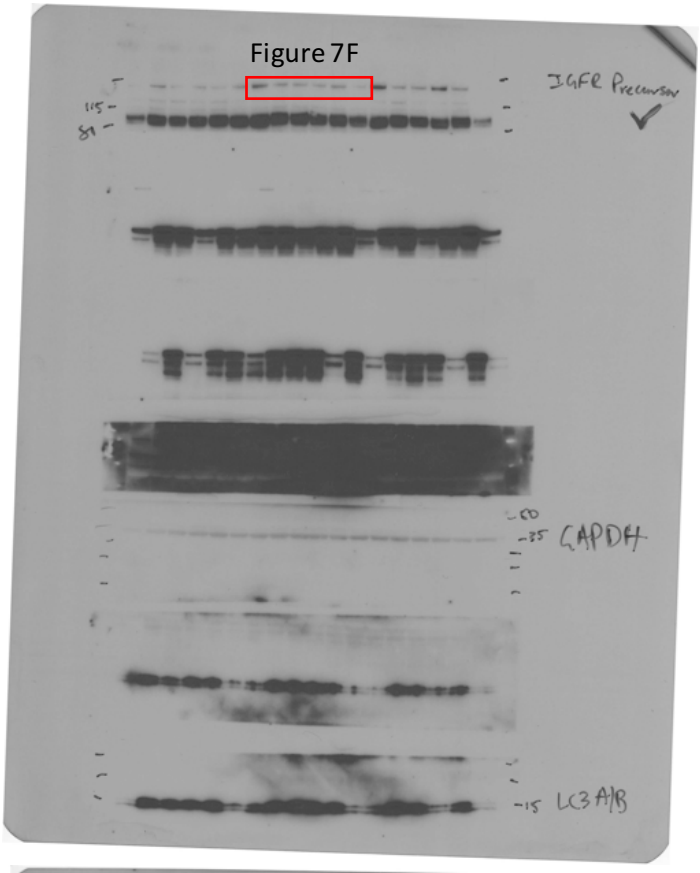


Figure 7F
IGFR Precursor
115
85
50
35
GAPDH
15
LC3A/B

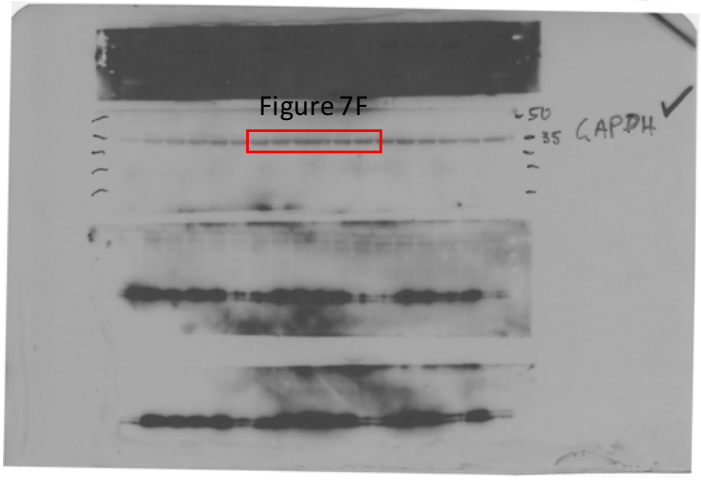


Figure 7F
50
35
GAPDH

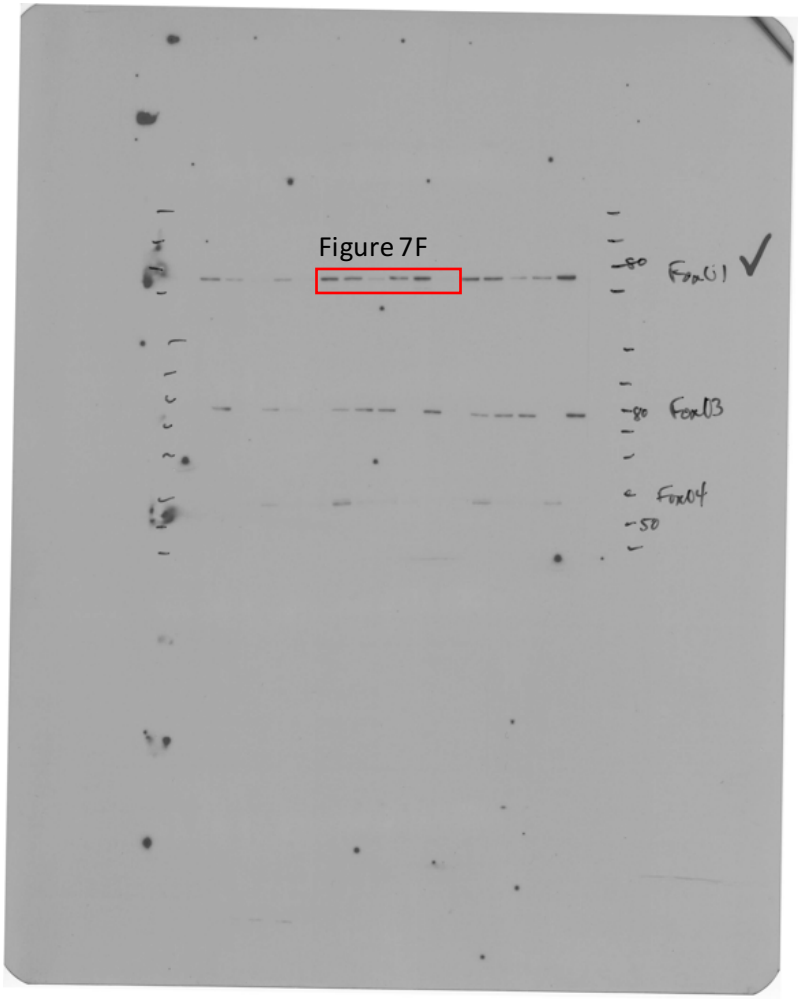


Figure 7F
80
FoxO1
80
FoxO3
FoxO4
50

Figure 7F

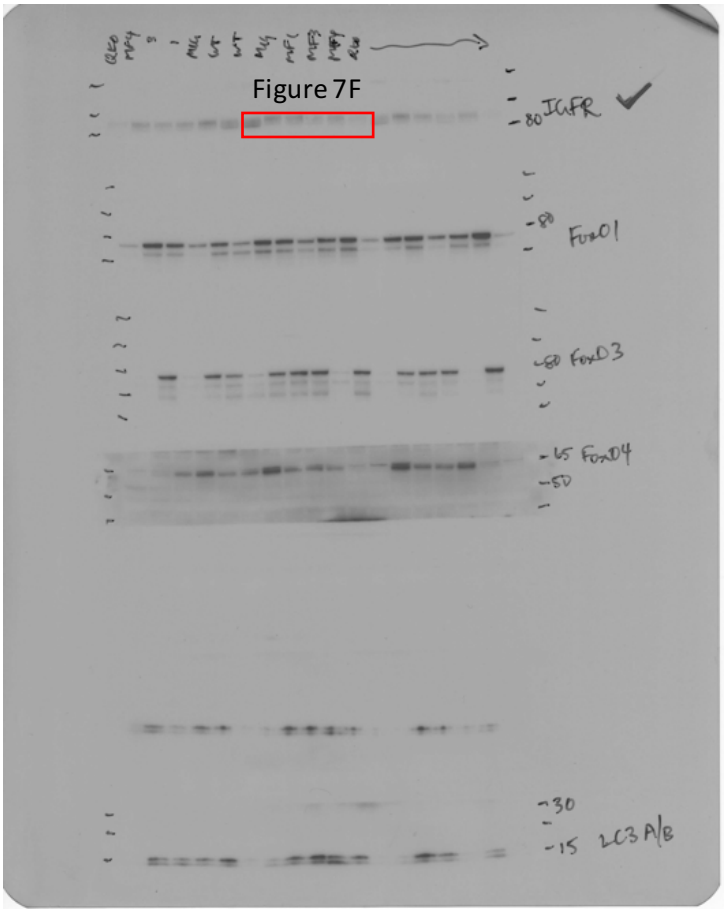


Figure 6B

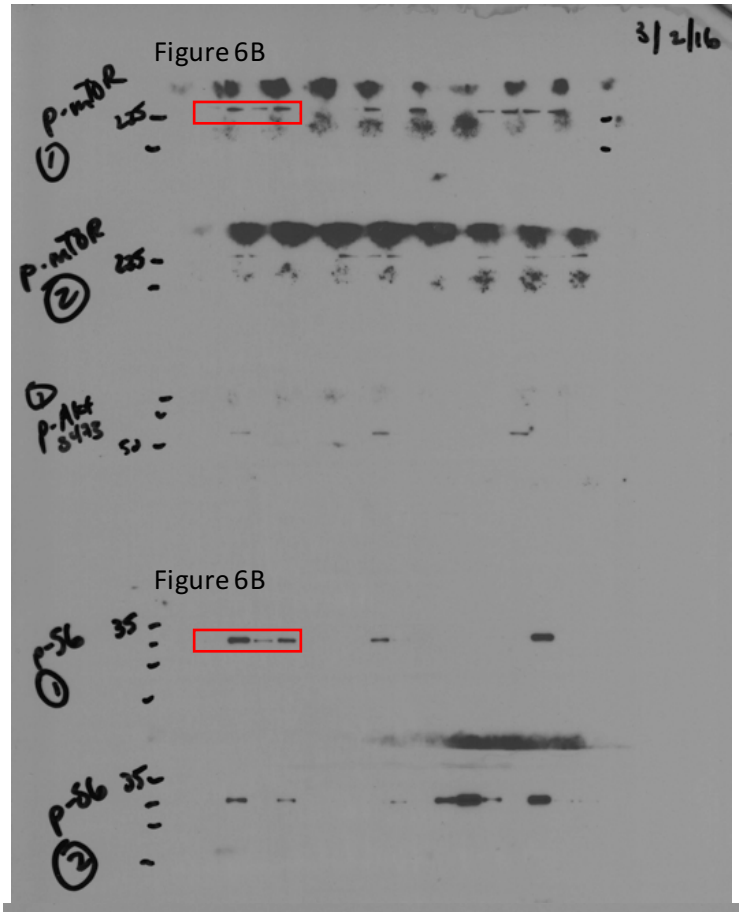


Figure 6B

Figure 6B

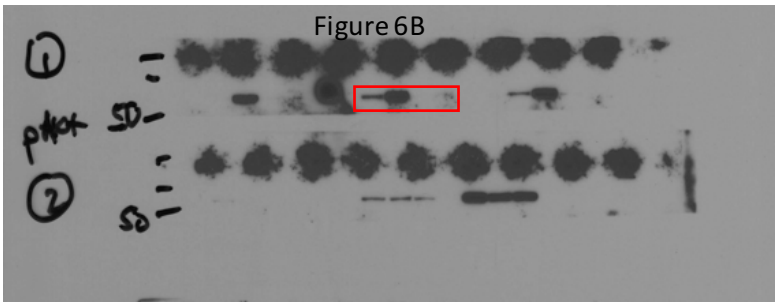


Figure 6B

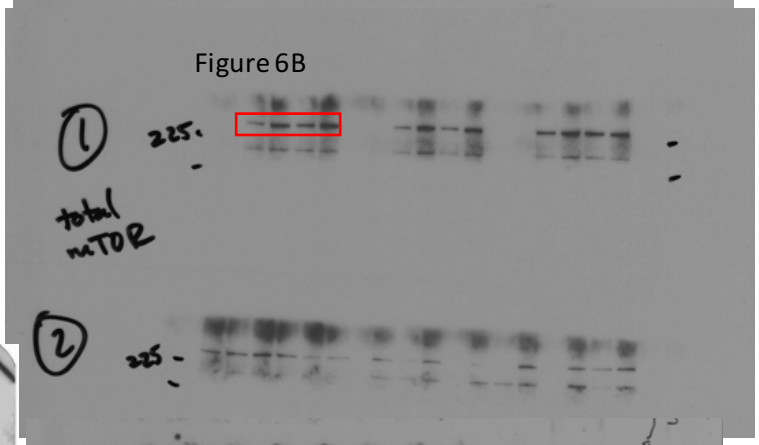


Figure 6B

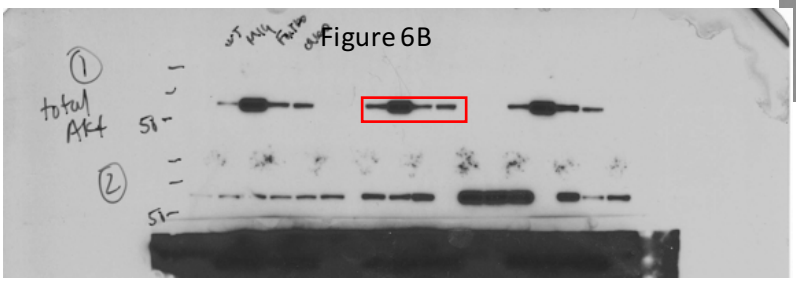
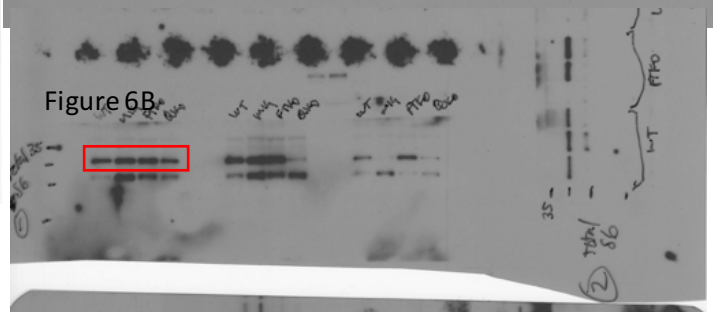
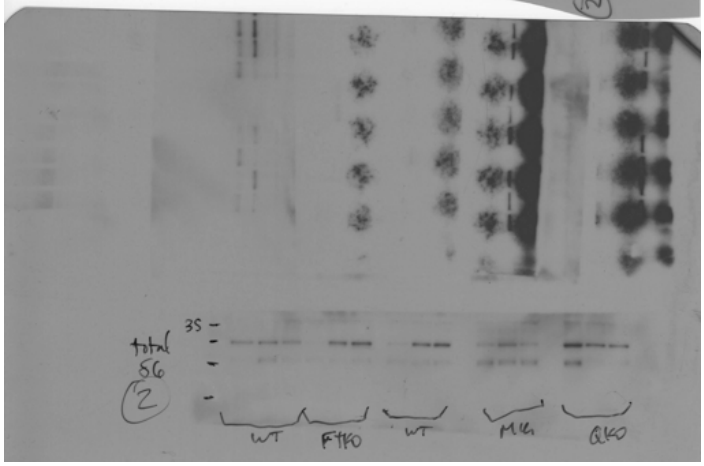
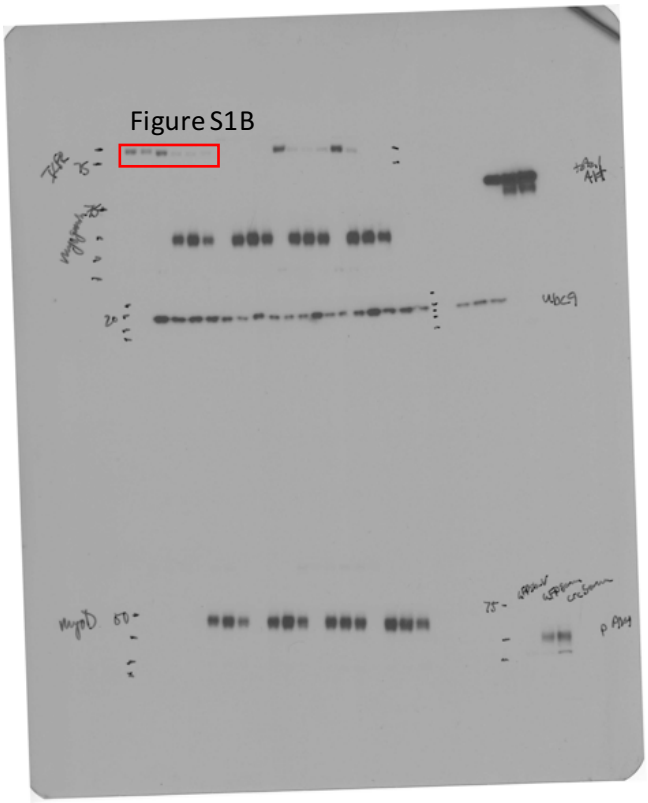


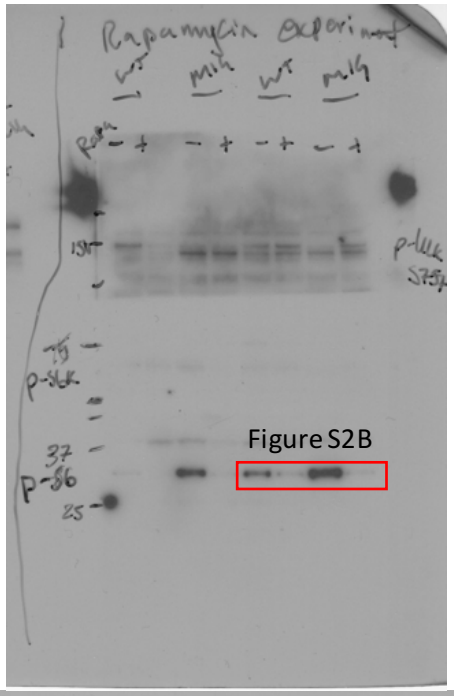
Figure 6B

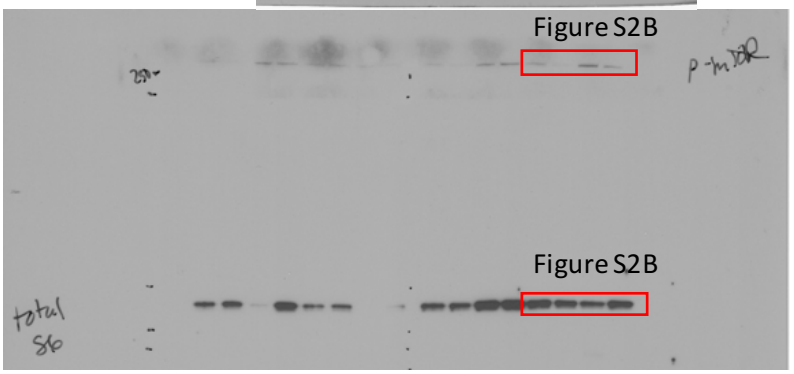


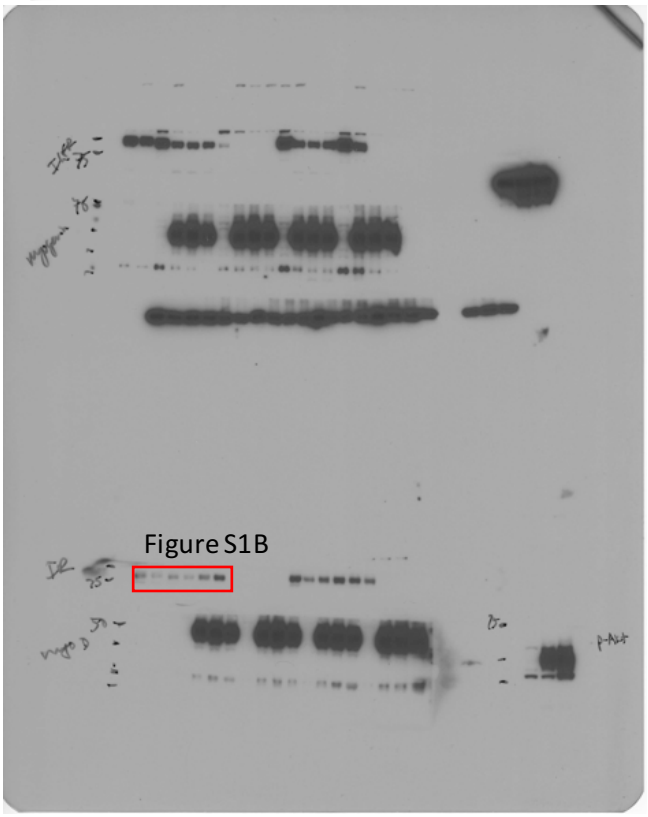


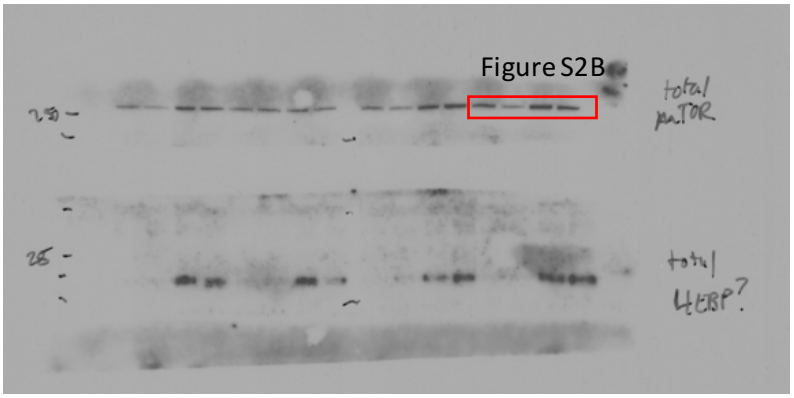
Full Uncut Gels from Supplemental Figures

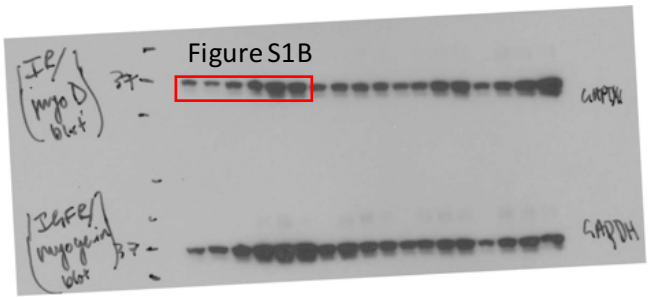


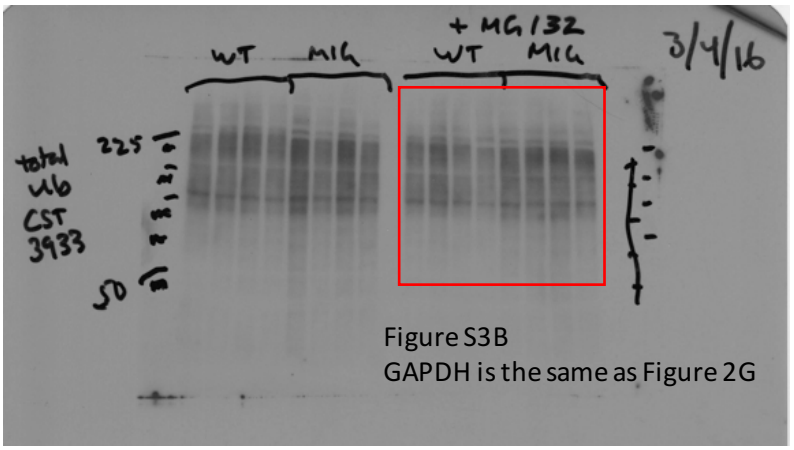












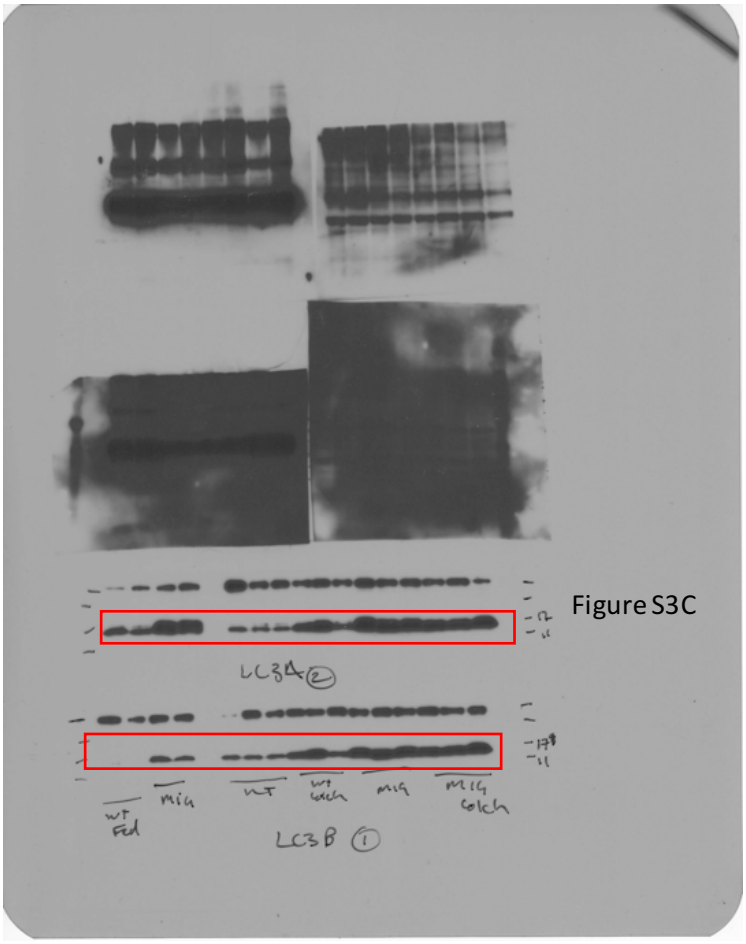
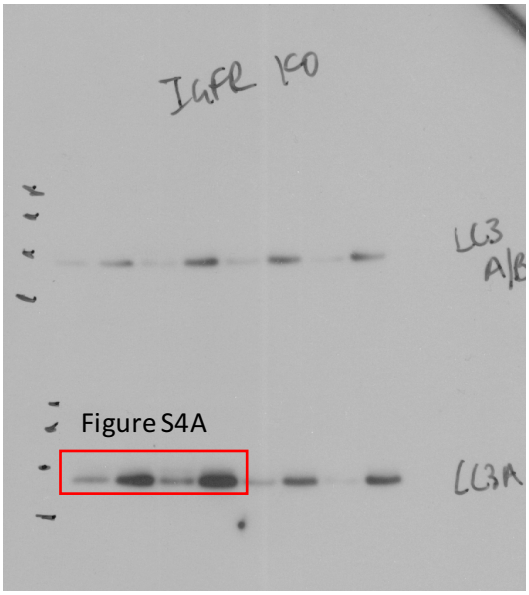


Figure S3C
LC3A
LC3B


IGFR KO
LC3 A/B
Figure S4A
LC3A

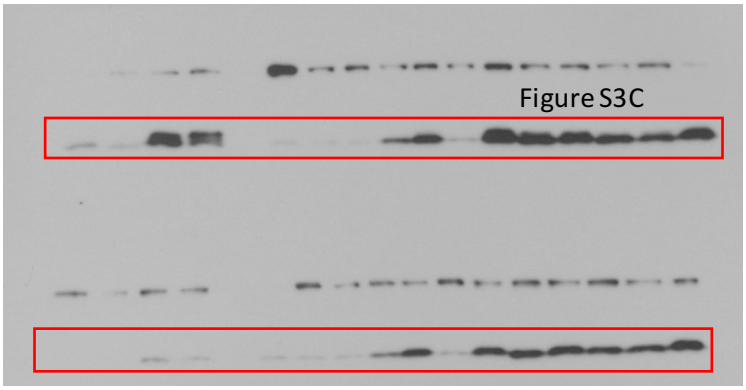
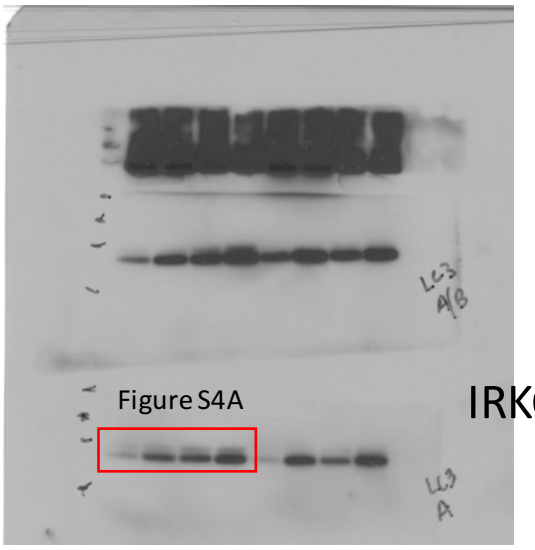
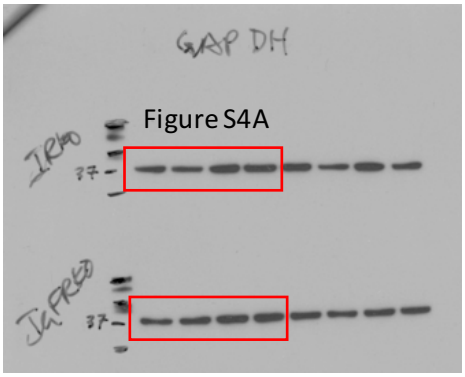
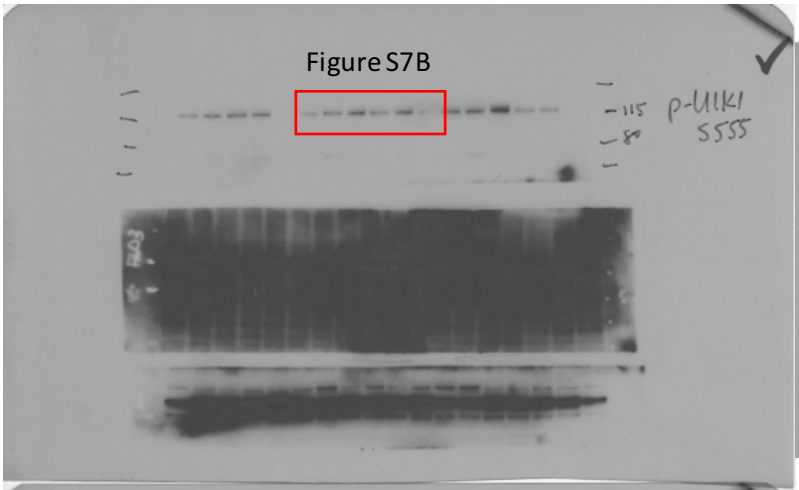


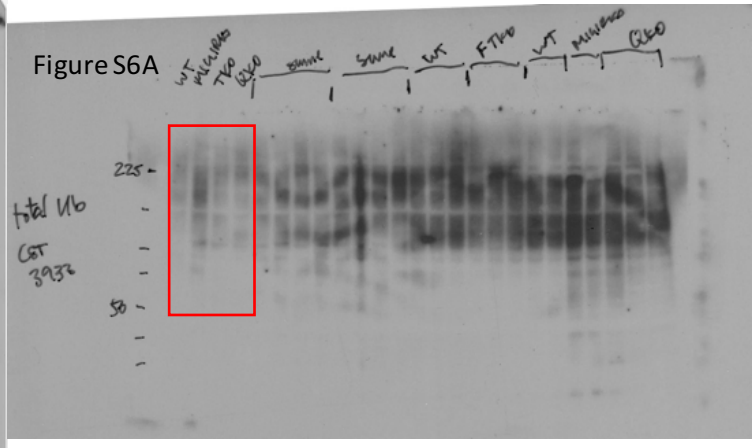
Figure S3C

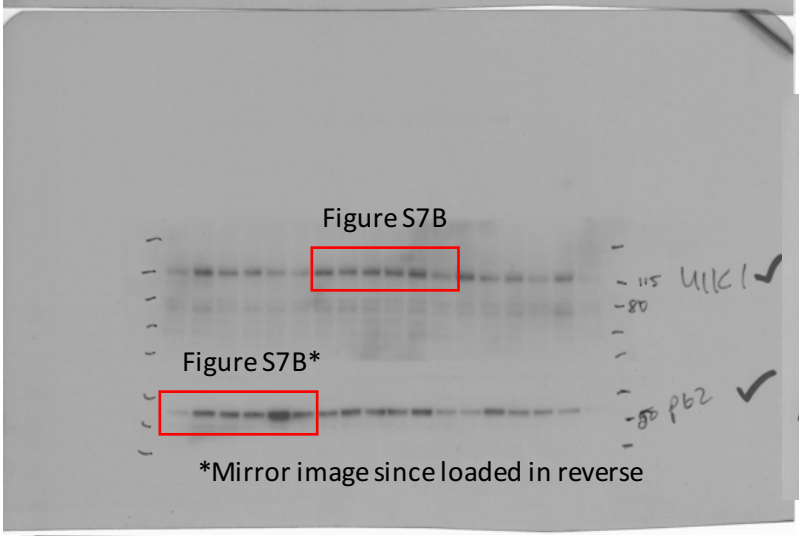

LC3 A/B
Figure S4A
LC3 A

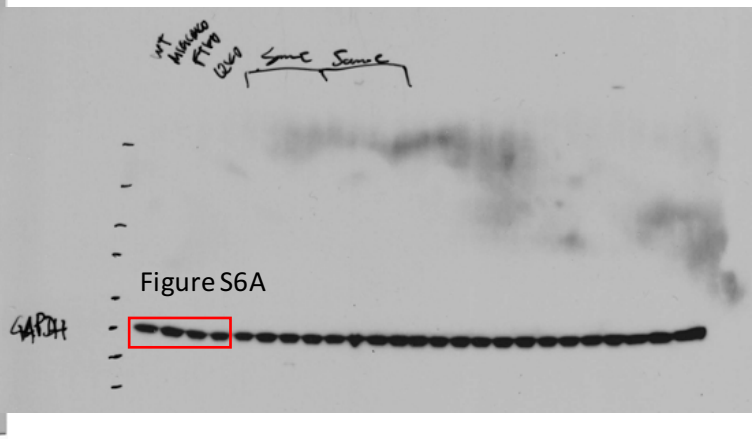
IRKO

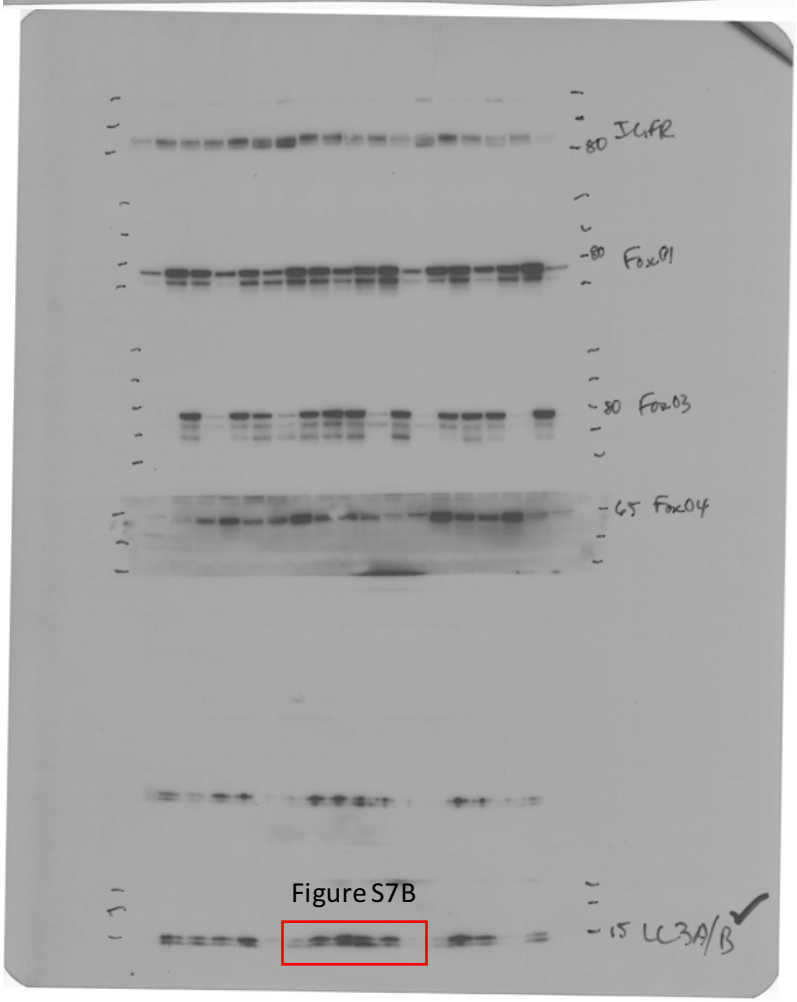

GAP DH
Figure S4A
IRKO
IGFRKO











GAPDH for Figure S6A is the same as Figure 7F above since they were run on the same gels.