

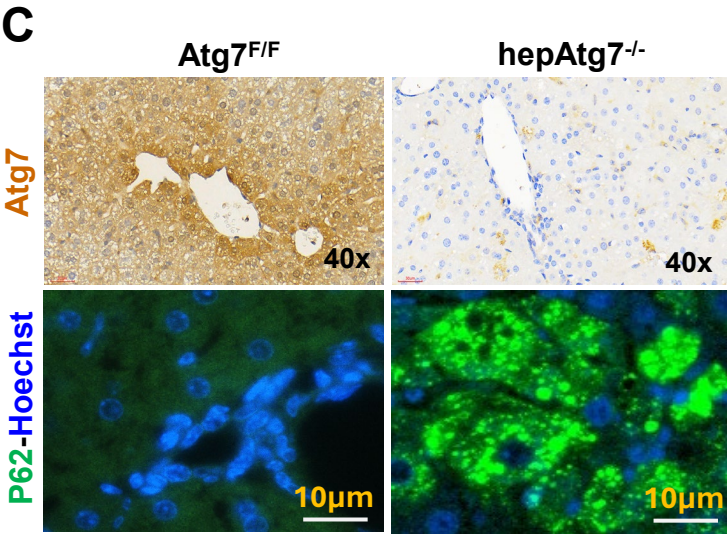
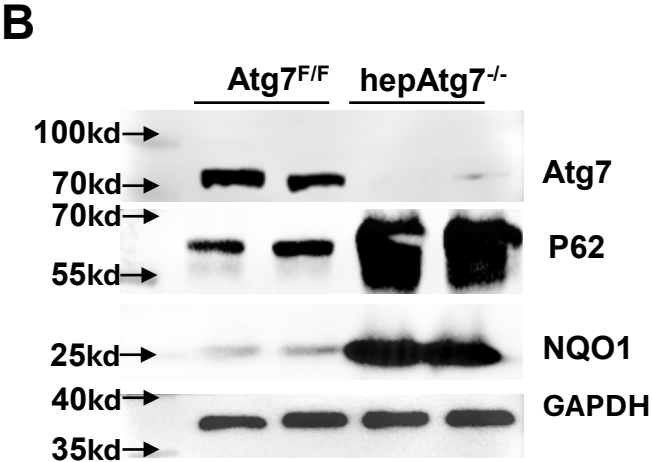
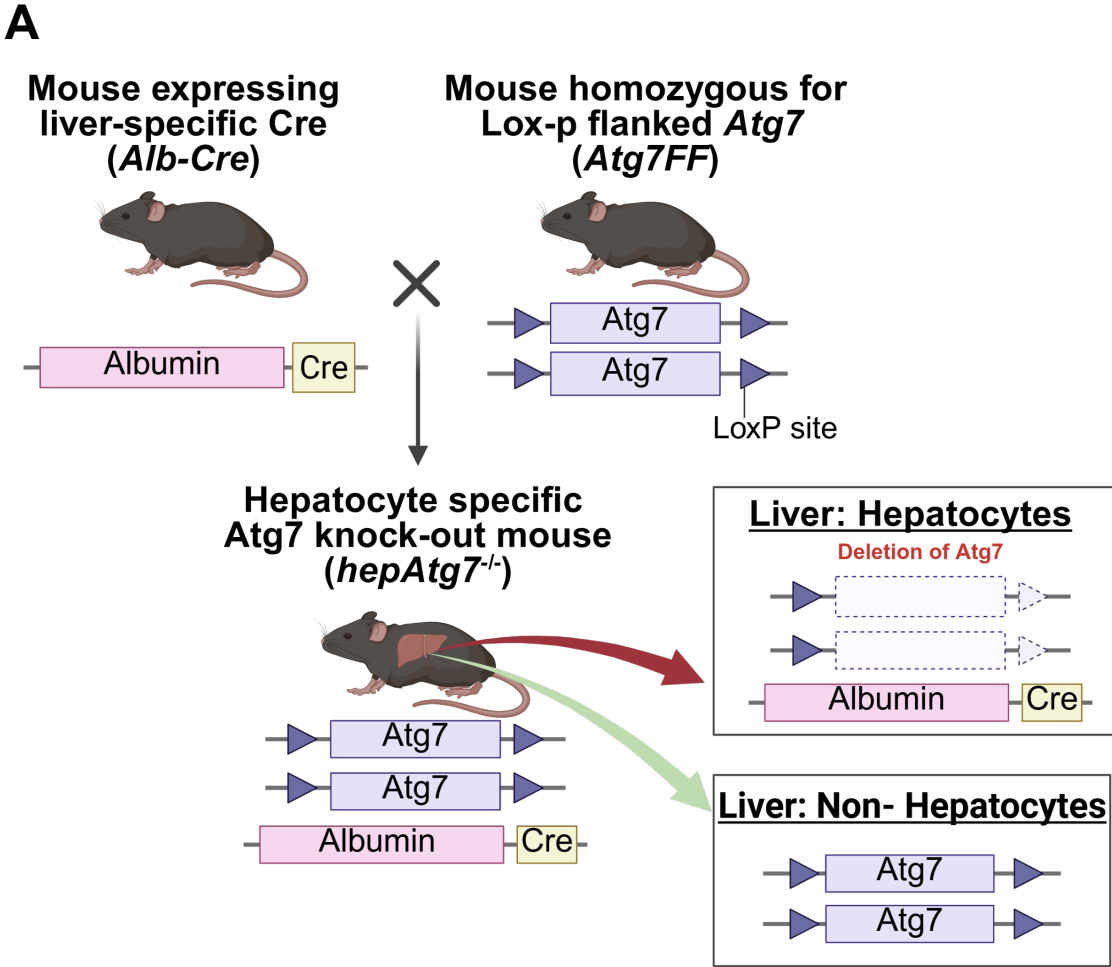
Autophagy Regulates the Hepatic Acetylome Through Modulating Acetyl-CoA Metabolite

Kamal Baral, Leah Spade, Shadie Shrestha, Kapil Thapa, Ramsey Rohner, Yuanyuan Li, Ghaidaa Majari,
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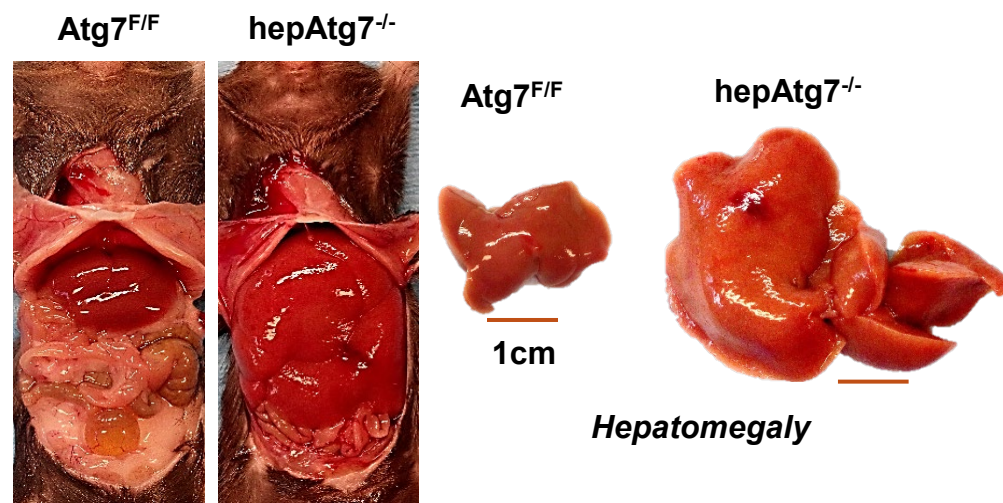
Supplementary Figures

Kamal Baral et al 2026

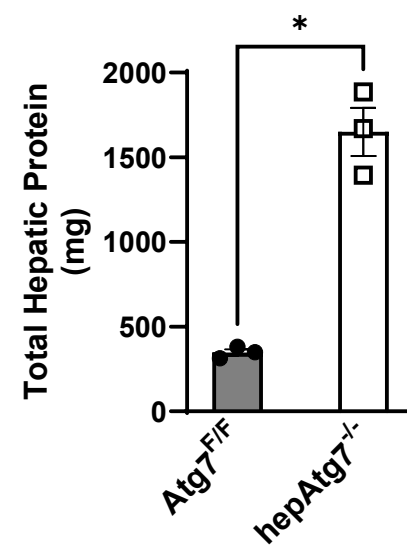
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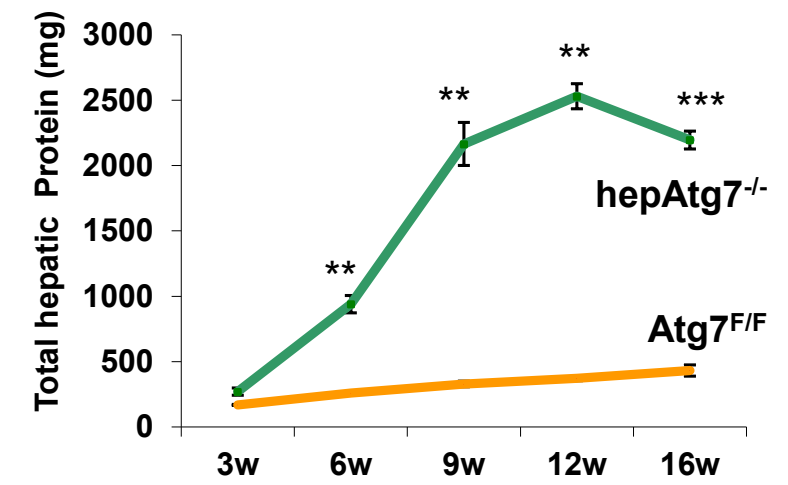
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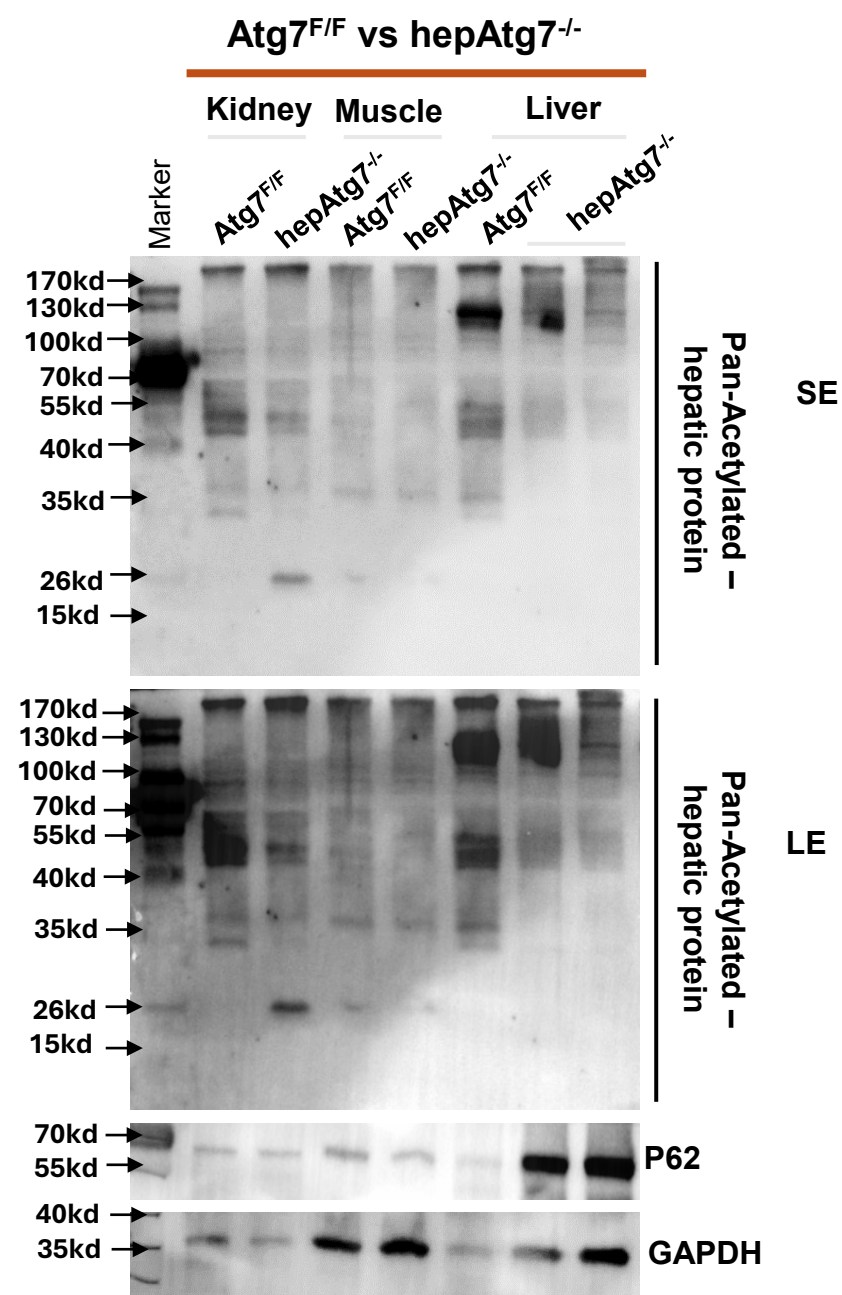
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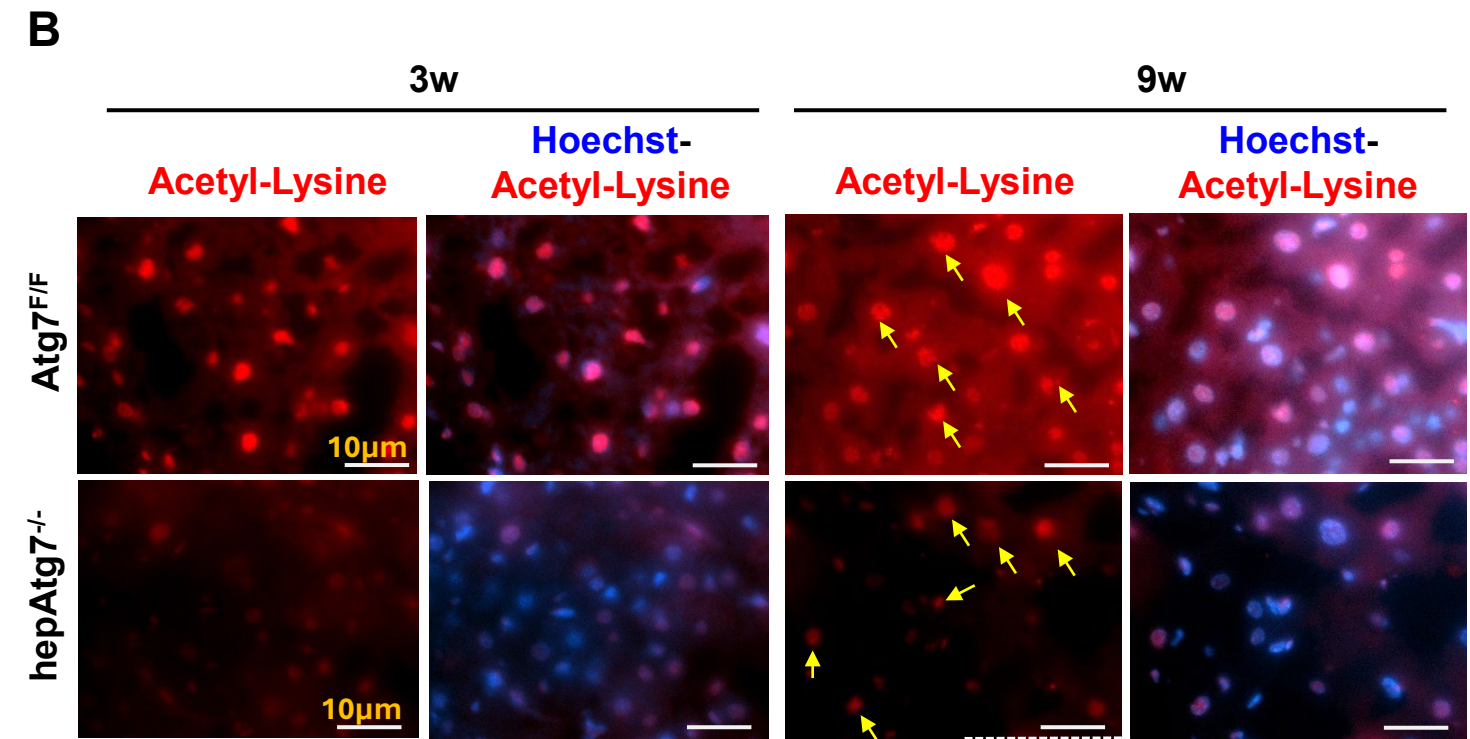
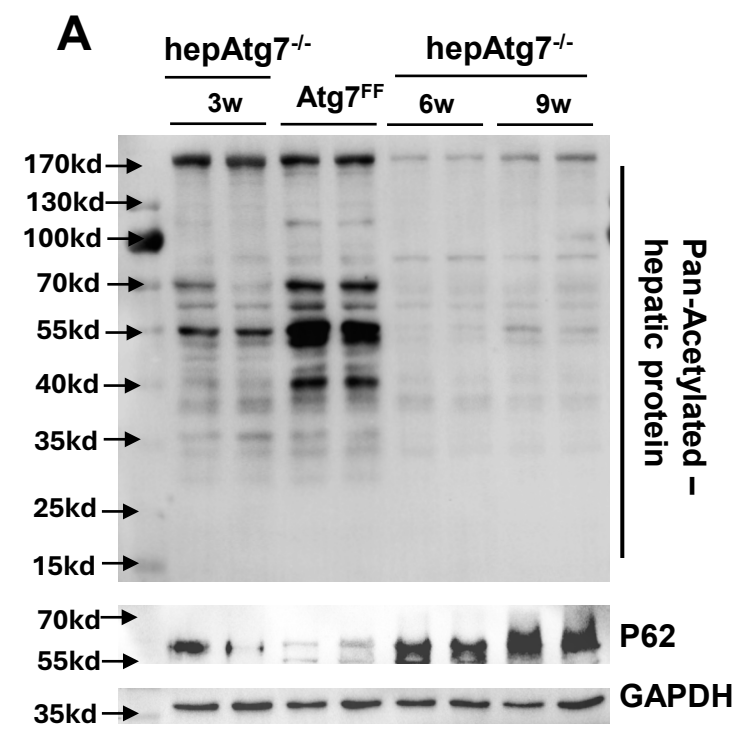
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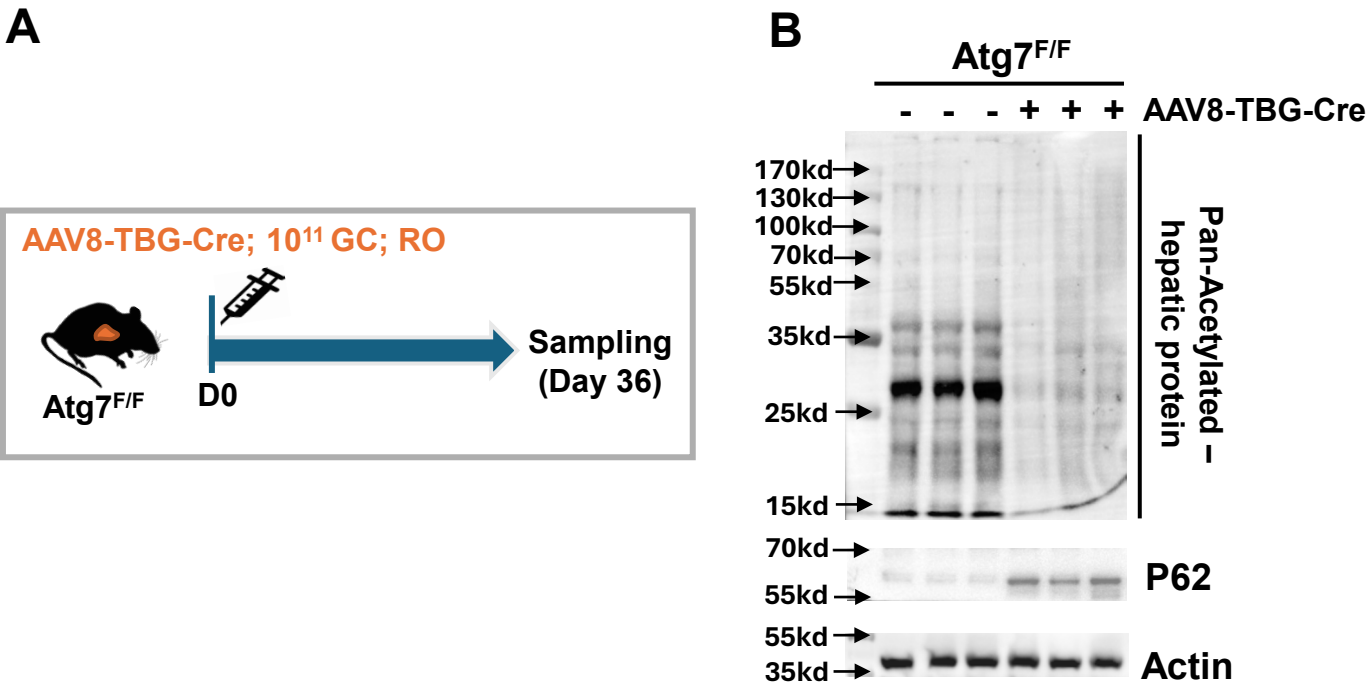
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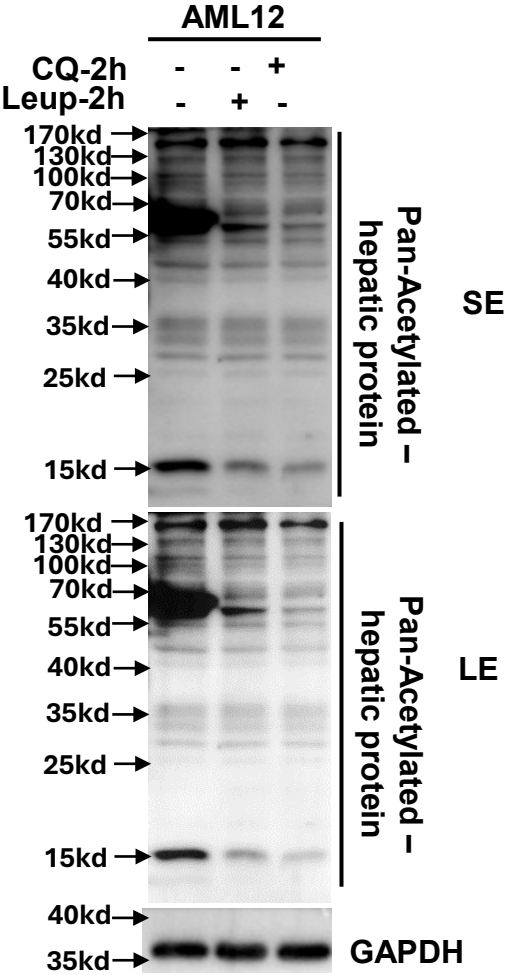
Supplementary Figure 3.



Supplementary Figure 4.



Supplementary Figure 5.



Supplementary Figure 6.

Gene Symbol	Atg5 ^{FF}	hepAtg5 ^{-/-}	SEM	T-test
<i>Sirt1</i>	1	0.79	0.04	0.03
<i>Sirt2</i>	1	0.91	0.08	0.44
<i>Sirt3</i>	1	0.99	0.08	0.95
<i>Sirt4</i>	1	0.85	0.03	0.15
<i>Sirt5</i>	1	1.63	0.10	0.01
<i>Sirt6</i>	1	0.65	0.03	0.01
<i>Sirt7</i>	1	1.05	0.07	0.8

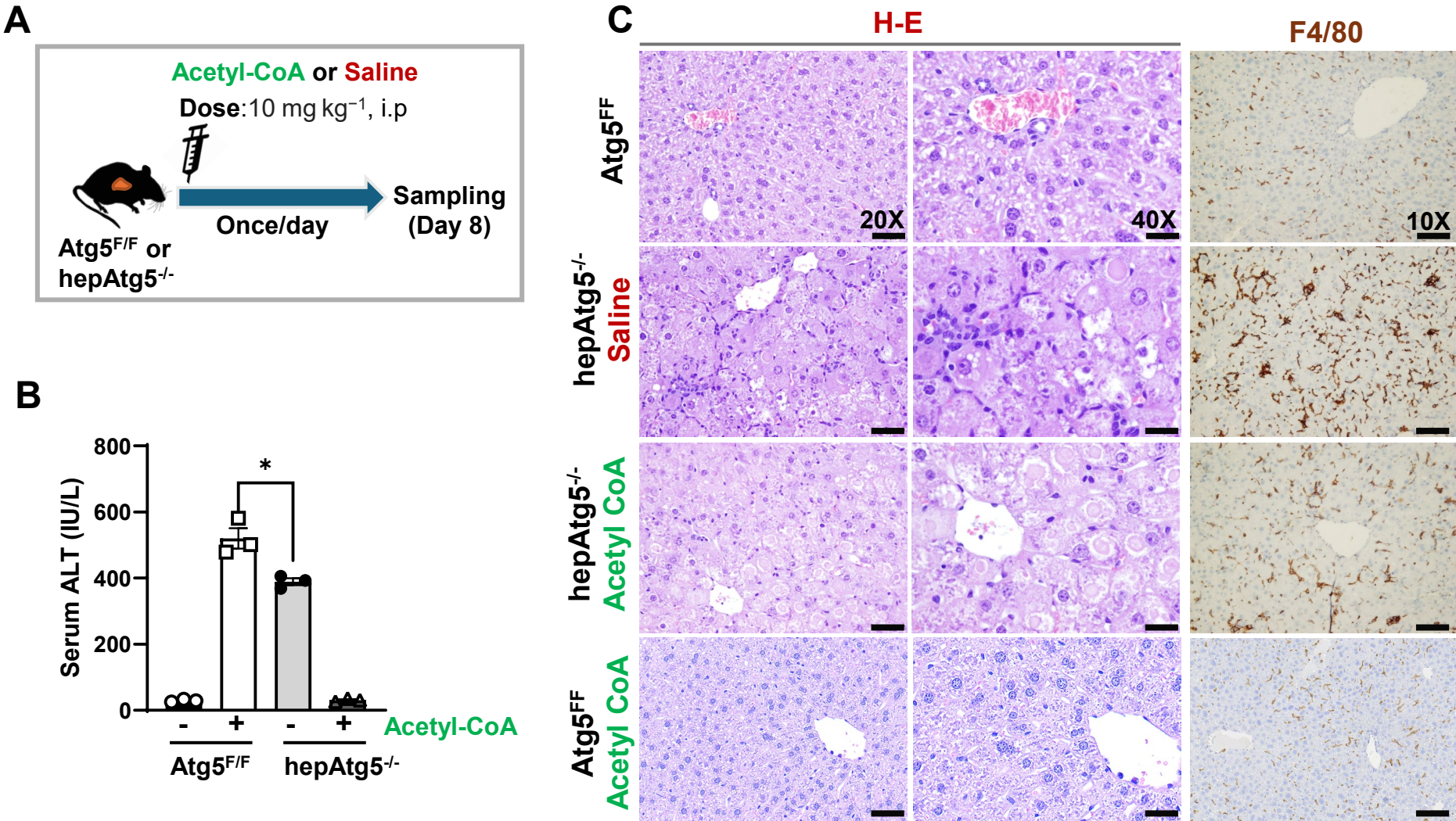
Fold change

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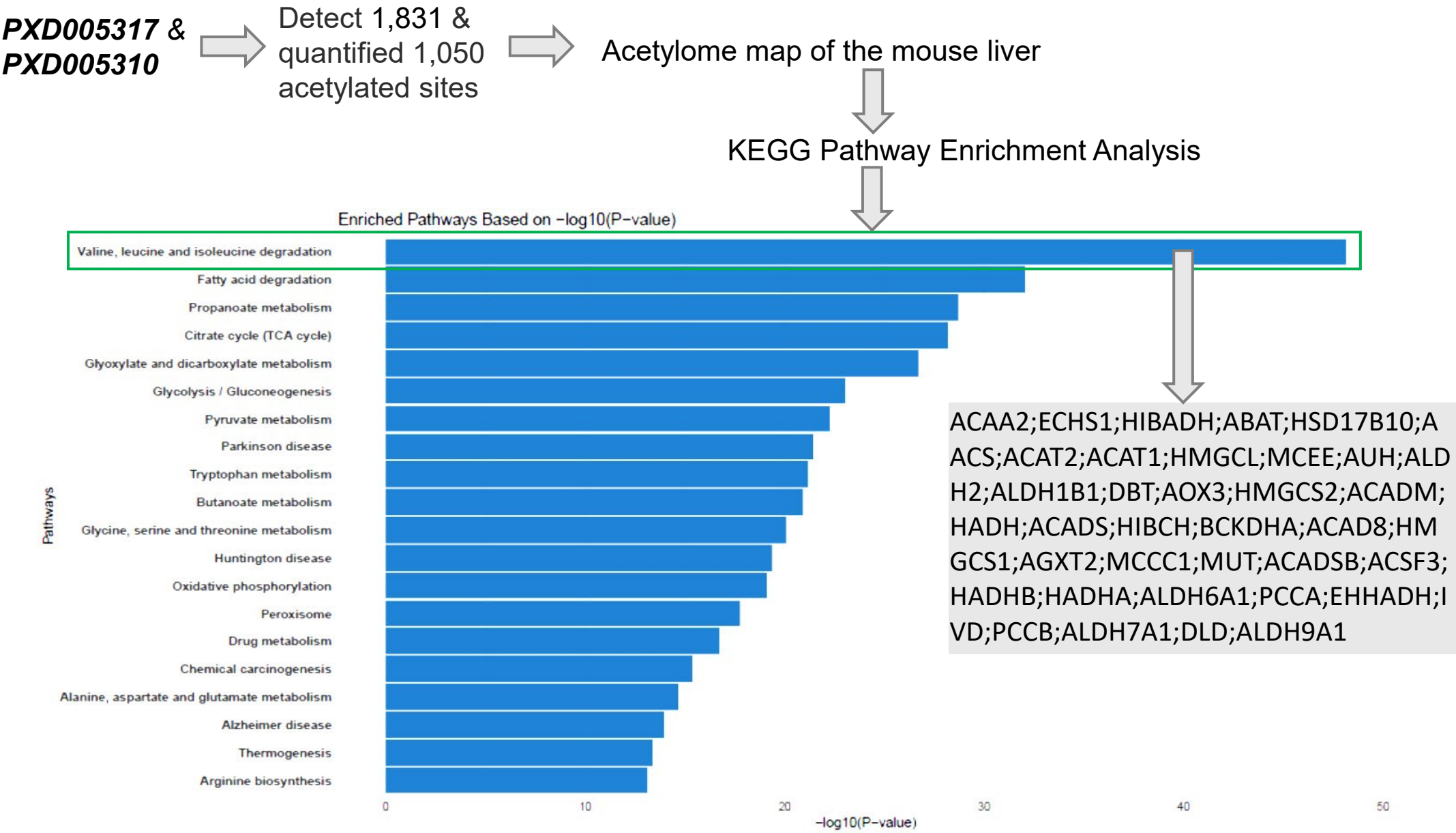
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Supplementary Figure 7.



Supplementary Figure 8.



Supplementary Figure Legends

Supplementary Figure 1. Hepatic protein accumulation in autophagy-deficient livers.

(A) Schematic illustrating the generation of liver-specific autophagy-deficient mice (hepAtg7^{-/-}). (B) Immunoblot analysis of liver lysates from control (Atg7^{F/F}) and hepAtg7^{-/-} mice showing expression levels of autophagy-related proteins (Atg7, P62/SQSTM1, NQO1) and GAPDH as a loading control. (C) Immunohistochemistry (IHC) for Atg7 (40×) and immunofluorescence (IF) for P62/SQSTM1 (green) with Hoechst nuclear staining (blue) in liver sections. hepAtg7^{-/-} livers lack Atg7 and exhibit P62/SQSTM1 accumulation (scale bar: 10 μm). Images are representative of three biological replicates per group. (D) Representative gross morphology of livers harvested from 9-week-old Atg7^{F/F} and hepAtg7^{-/-} mice (n = 3 per group). (E) Quantification of total hepatic protein content in 9-week-old Atg7^{F/F} and hepAtg7^{-/-} mice (n = 3 per group). Total protein was measured using the BCA assay and normalized to whole liver weight (LW). (F) Time-course analysis of total hepatic protein levels in Atg7^{F/F} and hepAtg7^{-/-} mice at 3, 6, 9, 12, and 16 weeks of age. Data represents mean ± SEM (n ≥ 3 biological replicates per group at each time point). Statistical significance: ns, not significant; *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Figure 2. Tissue-specific analysis of protein acetylation in autophagy-deficient mice. Immunoblot analysis of kidney, skeletal muscle, and liver tissues from 9-week-old Atg7^{F/F} (control) and hepAtg7^{-/-} mice. Tissue lysates were probed for acetyl-lysine (to assess global protein acetylation), P62/SQSTM1 (autophagy marker),

and GAPDH (loading control) to evaluate tissue-specific changes in protein acetylation associated with autophagy deficiency. SE, short exposure; LE, long exposure.

Supplementary Figure 3. Age-dependent analysis of hepatic protein acetylation in autophagy-deficient mice. (A) Immunoblot analysis of liver lysates from 3-, 6-, and 9-week-old Atg7^{F/F} and hepAtg7^{-/-} mice. Samples were probed for acetyl-lysine (acetylation), P62/SQSTM1 (autophagy marker), and GAPDH (loading control) to assess dynamic changes in the hepatic protein acetylation over time. (B) Immunohistochemical staining of liver sections from the indicated genotypes using anti-acetyl-lysine antibody. Arrowheads indicate nuclear localization of acetylated proteins.

Supplementary Figure 4. Hepatic protein acetylation following AAV8-TBG-Cre-mediated Atg7 deletion. (A) Schematic showing AAV8-TBG-Cre administration in Atg7^{F/F} mice to induce liver-specific Atg7 deletion. AAV8-TBG-Cre was delivered via retroorbital (RO) injection at a dose of 10¹¹ genomic copies (GC). (B) Immunoblot analysis of liver lysates from Atg7^{F/F} mice with or without AAV8-TBG-Cre infection. Blots were probed for acetyl-lysine (to assess global protein acetylation), P62/SQSTM1 (autophagy marker), and Actin (loading control) to determine the impact of Cre-mediated autophagy deficiency on hepatic protein acetylation.

Supplementary Figure 5. Analysis of cellular protein acetylation in an in vitro hepatic cell model. Cell lysates from AML12 hepatocyte cells treated with or without chloroquine (CQ) or leupeptin (Leup) for 2 hours were analyzed by immunoblotting for acetyl-lysine (acetylation) and GAPDH (loading control). SE-Short exposure; LE-Long exposure.

Supplementary Figure 6. Downregulation of sirtuin gene expression in Atg5-deficient livers. Heatmap showing the relative mRNA expression levels of various sirtuins in liver tissues from Atg5^{F/F} and hepAtg5^{-/-} mice.

Supplementary Figure 7: Acetyl-CoA Supplementation Attenuates Liver Injury and Inflammation in Atg5-Deficient Mice. (A) Schematic representation of the acetyl-CoA administration protocol in Atg5^{F/F} and hepAtg5^{-/-} mice. (B) Serum alanine aminotransferase (ALT) levels were measured in 9-week-old Atg5^{F/F} and hepAtg5^{-/-} mice, with or without acetyl-CoA treatment. (C) Liver sections were analyzed by hematoxylin and eosin (H&E) staining and immunostaining for F4/80 to assess tissue morphology and macrophage infiltration (original magnification, 10X, 20X, 40X). Data are presented as mean ± SEM (n = 3). Statistical significance: *P ≥ 0.05.

Supplementary Figure 8: Experimental workflow for hepatic acetylome profiling and pathway enrichment analysis. Experimental strategy used to characterize the hepatic acetylome. Quantitative acetylome profiling was performed using datasets PXD005317 and PXD005310, yielding 1,831 detected acetylated peptides and 1,050 confidently quantified acetylation sites. The resulting mouse liver acetylome map was subsequently analyzed using KEGG pathway enrichment, revealing multiple significantly enriched metabolic pathways. Among these, valine, leucine, and isoleucine degradation—the branched-chain amino acid (BCAA) degradation pathway—was the most strongly enriched, as visualized in the bar graph ranked by $-\log_{10}(\text{p-value})$. The accompanying annotation box highlights representative acetylated enzymes contributing to this pathway, including ACAA2, ECHS1, HIBADH, ABAT, and HSD17B10. Together, these findings

illustrate how hepatic protein acetylation extensively targets central metabolic pathways, and they suggest that these BCAA-associated proteins may be among those potentially downregulated in autophagy-deficient liver.

Supplementary Table 1: List of Antibodies

Antibody/Species	Vendor/Catalog #/Dilution
Atg5/Rabbit	Proteintech/10181-2-AP/1:3000
Atg7/Rabbit	Proteintech/10088-2-AP/1:5000
Nqo1/Rabbit	Cell signaling/ D6H3A/1:1000
P62/rabbit	Proteintech/84826-1-RR/1:5000
Gapdh/Mouse	Proteintech/60004-1-Ig/1:10000
Acetyl Lysine/Rabbit	Cell signaling/ 9441S/1:1000
Acetyl Lysine/Mouse	Novus biologicals/NB110-96876/1:1000
Ubiquitin/Rabbit	Proteintech/10201-2-AP/1:5000
SUMO2/3/Rabbit	Proteintech/11251-1-AP/1:2000
Methyl lysine/Rabbit	Cell Signaling/14679S/1:1000
Phospho-tyrosine/Rabbit	Biotechne/MAB1676/1:1000
B-Actin/	Cell Signaling/3700S/1:2000
Acly/Rabbit	Proteintech/15421-1-AP/1:2000
Acss/Rabbit	Proteintech/16087-1-AP/1:2000
Pdha1/Rabbit	Proteintech/18068-1-AP/1:2000
Mlycd/Rabbit	Proteintech/15265-1-AP/1:2000
HRP-labeled anti-Mouse 2nd Ab/Goat	Jackson ImmunoResearch Laboratories Inc/115-035-062/1:5000
HRP-labeled anti-Rabbit 2nd Ab/Goat	Jackson ImmunoResearch Laboratories Inc/111-035-045/1:5000

Supplementary Table 2: List of Primers

Gene	Forward Primer	Reverse Primer
<i>Hdac1</i>	5'-TCCAACATGACCAACCAGAA-3'	5'-TTGTGAGGGTGCTCCTCATC-3'
<i>Hdac2</i>	5'-TGCTGTCAATTTTCCCATGA-3'	5'-GCGCTAGGCTGGTACATCTC-3'
<i>Hdac3</i>	5'-AATGTGCCCTTACGAGATGG-3'	5'-GTAGCCACCACCTCCCAGTA-3'
<i>Hdac4</i>	5'-CAGACAGCAAGCCCTCCTAC-3'	5'-AGACCTGTGGTGAACCTTGG-3'
<i>Hdac5</i>	5'-AGTGAGAGCAGCCAGGAAGA-3'	5'-GTACACCTGGAGGGGCTGTA-3'
<i>Hdac6</i>	5'-CTGGCTAAGGGAGTCAGTGC-3'	5'-TAGACAGGCTTCTTCCACTT-3'
<i>Hdac7</i>	5'-TGAAGAATGGCTTTGCTGTG-3'	5'-CACTGGGGTCTGGTAGAAA-3'
<i>Sirt1</i>	5'-TCTCTGTGGGATTCCTGAC-3'	5'-GATCCTTTGGATTCTGCAA-3'
<i>Sirt2</i>	5'-TAGACACGCTGGAACGAGTG-3'	5'-CCAGCCCATCGTGTATTCTT-3'
<i>Sirt3</i>	5'-AGGTGGAGGAAGCAGTGAGA-3'	5'-GCTTGGGGTTGTGAAAGAAA-3'
<i>Sirt4</i>	5'-CGAGCAAAGCTCCCAATAG-3'	5'-GTTTCATTCCAGCCTTTTGA-3'
<i>Sirt5</i>	5'-CATCACCCAGAACATTGACG-3'	5'-CAGGATCCAGGTTTCTCCA-3'
<i>Sirt6</i>	5'-GGCTACGTGGATGAGGTGAT-3'	5'-GGCTCAGCCTTGAGTGCTAC-3'
<i>Sirt7</i>	5'-CAGAAAGGAAGGCTGTGAG-3'	5'-GAAGGGCAGTACGCTCAGTC-3'
<i>Acc1</i>	5'-GCGGCTACAGGGACTATACTG-3'	5'-CGGAAGTAAGAGCTACTAGCGG-3'
<i>Acc2</i>	5'-GGGCTCCCTGGATGACAAC-3'	5'-GCTCTTCCGGGAGGAGTTCT-3'
<i>Srebp1c</i>	5'- GGAGCCATGGATTGCACATT-3'	5'- GCTTCCAGAGAGGAGGCCAG-3'
<i>Srebp2</i>	5'-AGCCAAGGAGAGCCTGTACTG-3'	5'-GAGAGCGCACAGCTGCATCG-3'
<i>Chrebp</i>	5'-CCTTCGCCAAGCTCAGCACTT-3'	5'-TGGCTTGCTCAGGCACAA-3'
<i>Acat1</i>	5'-ATGCCAGCACACTGAACGA-3'	5'-AATCAATGGGGTCTACGGCAG-3'
<i>Hmgcs2</i>	5'-TGGTTCAAGACAGGGACACAGAAC-3'	5'-AGAGGAATACCAGGGCCCAACAT-3'
<i>Hmgcl</i>	5'-GCACCTTGCCAAAGCAGGTGAAG-3'	5'-CGGAAAGCATGTGCATCAGCCT-3'
<i>Bdh1</i>	5'-AGGCTGTGACTCTGGATTGGG-3'	5'-CTGGATGGTTCTCAGTCGGTCA-3'
<i>Mct1</i>	5'-CATTGGTGTTATTGGAGGTC-3'	5'-GAAAGCCTGATTAAAGTGAG-3'
<i>Acly</i>	5'-AAGAAGGAGGGGAAGCTGAT-3'	5'-TCGCATGTCTGGGTTGTTA-3'
<i>Acss1</i>	5'-GAGCATGAGCAGTGAAGACA-3'	5'-CATGGCGGCATACAGTAGATAG-3'
<i>Acss2</i>	5'-GGAGACCACCAAGATCACATAC-3'	5'-CCCTTCTGAATGCCCTGTTTA-3'
<i>Mlycd</i>	5'-TTTCAAGCCTGTGCGCTATC-3'	5'-GGCACTCTGAGTCTGTGAATAG-3'
<i>Pdha1</i>	5'-AGAGAGGATGGGCTCAAGTA-3'	5'-CAAGTGACAGAAACCACGAATG-3'
<i>Nqo1</i>	5'-AGCGTTCGGTATTACGATCC-3'	5'-AGTACAATCAGGGCTCTTCTCG-3'
<i>Atg7</i>	5'-TCCGTTGAAGTCTCTGCTT-3'	5'-CCAAGTGAAGTTCACCATCCT-3'
<i>Acat2</i>	5'-CTGGAGGCATGGAGAATATGAG-3'	5'-CTGTGAGACCATCAGAGATAG-3'
<i>Hmgcs1</i>	5'-CTGTTTGAGGAGTCTGGGAATAC-3'	5'-CCTGCAACTACCAGAGCATATC-3'
<i>Hmgcr</i>	5'-CGTAACCCAAAGGGTCAAGAT-3'	5'-CTGTGCTGTTCTGAGGAGAA-3'
<i>Nqo1_PS1</i>	5'-CCCTCAGGCACAACTGTAA-3'	5'-GCTTCATCAGGAAAGGCTATCT-3'
<i>Nqo1_PS2</i>	5'-GAAGCAGCCTGTGGAACCTA-3'	5'-CGCACTTCCCAAACAAAGAAA-3'
<i>Nqo1_PS3</i>	5'-CAAGAATGGGTGTTACAAGTTCAG-3'	5'-AGTCTAACAGGTACCCGAGAA-3'
<i>Acss1_PS1</i>	5'-AGGTGTAGGAACTGGCAAAG-3'	5'-GCAAAGAACCACAGAGAGA-3'
<i>Acss1_PS2</i>	5'-CCCTCCAAGGAAACTACCTATC-3'	5'-GGCCTGAGTCCAGTAACAATA-3'
<i>Acss1_PS3</i>	5'-GGTTGCTCATCAGCCCTTTA-3'	5'-GATTGGGAACCGCTCTAAGT-3'
<i>Acss1_PS4</i>	5'-GGGCCGTGGAGACTCTTA-3'	5'-GCACTCACCAGACACATTCA-3'
<i>Mlycd_PS1</i>	5'-TCTCTGGCTGACCTAGACTTAC-3'	5'-CTCTTGGCTTGCATCCTTACA-3'
<i>Mlycd_PS2</i>	5'-GAGGCGGTGACTCTCAGAA-3'	5'-CAGCTGCTAGCGGGAGA-3'
<i>Mlycd_PS3</i>	5'-CGGCGAGAAACCGGAAG-3'	5'-GTCTTCTGCGCAGCTC-3'
<i>Pdha1_PS1</i>	5'-TCTCTCTCTCTCTCTCTCTCT-3'	5'-CCAAGTGCAGGCAGGAATTA-3'
<i>Pdha1_PS2</i>	5'-CCCAAGTGACCTCTAGAAATG-3'	5'-GCAGAATCCTGTATCTCCTGTG-3'
<i>Pdha1_PS3</i>	5'-GTCGCGACTCCGTCACT-3'	5'-GGATCAGGCGACCTCAC-3'

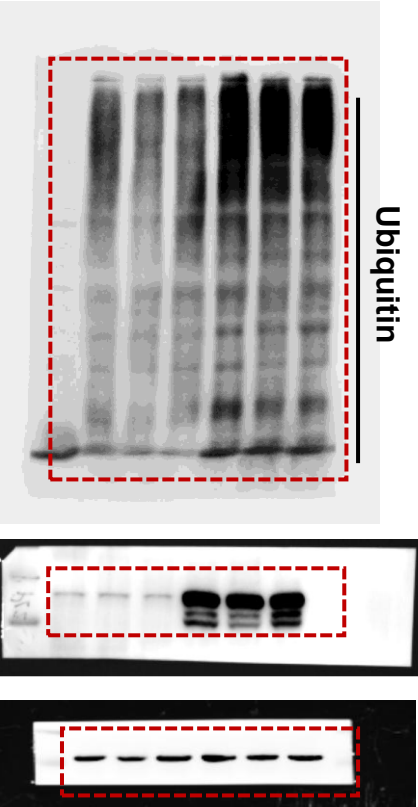
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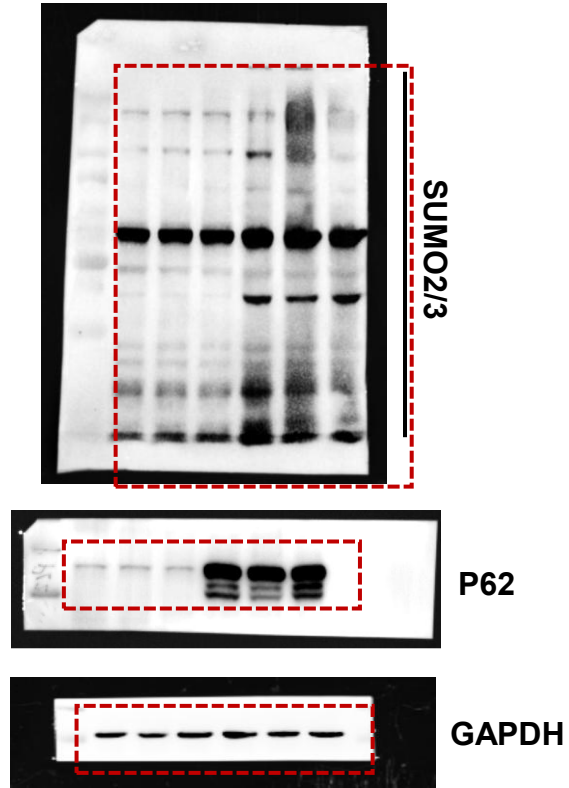
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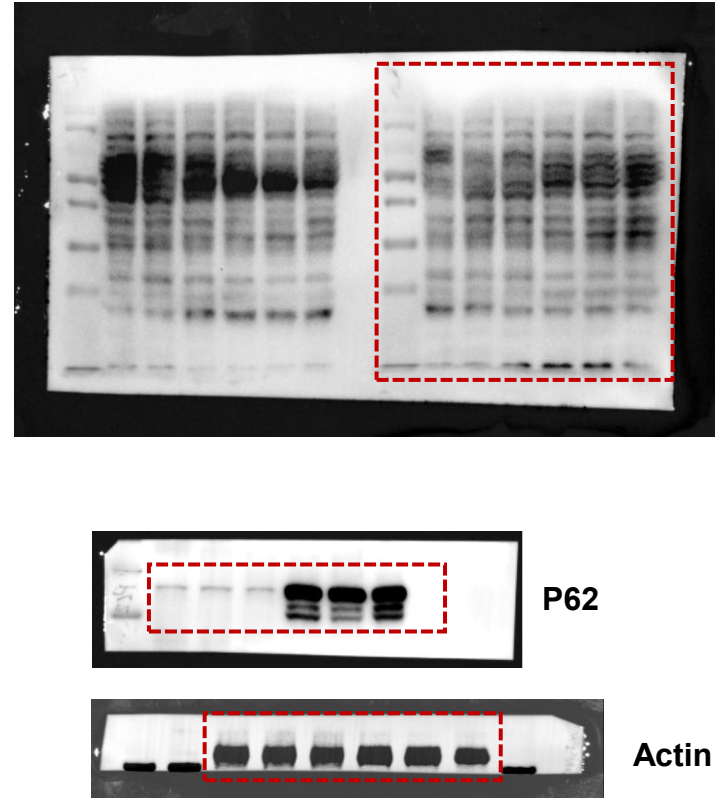
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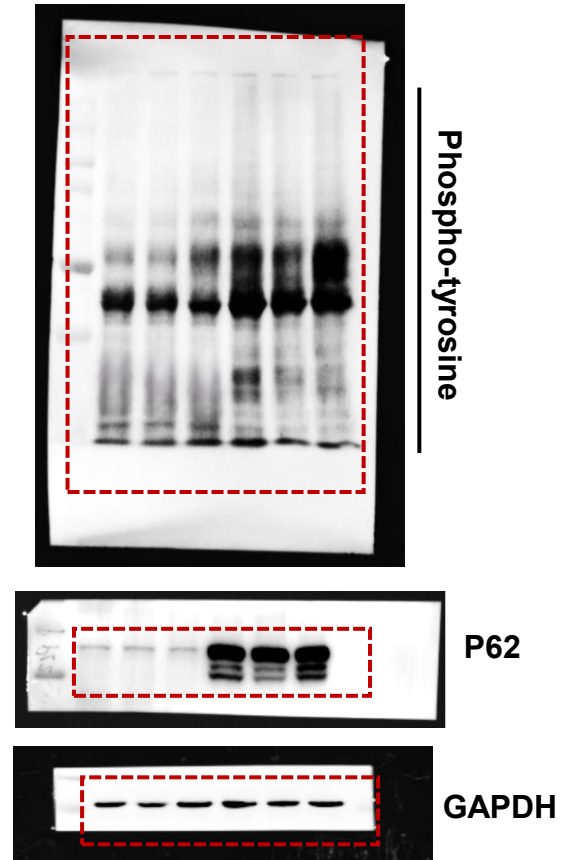
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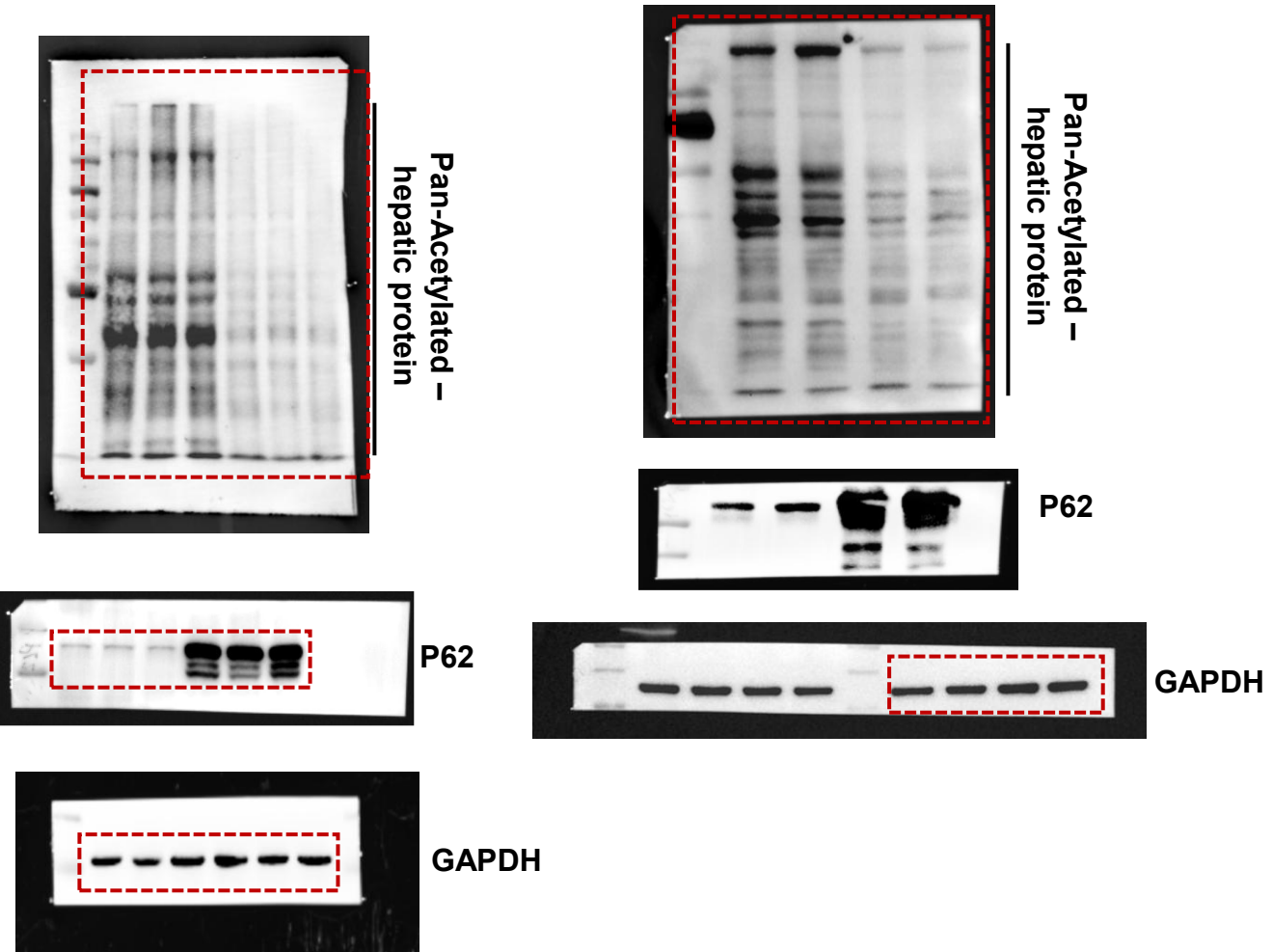
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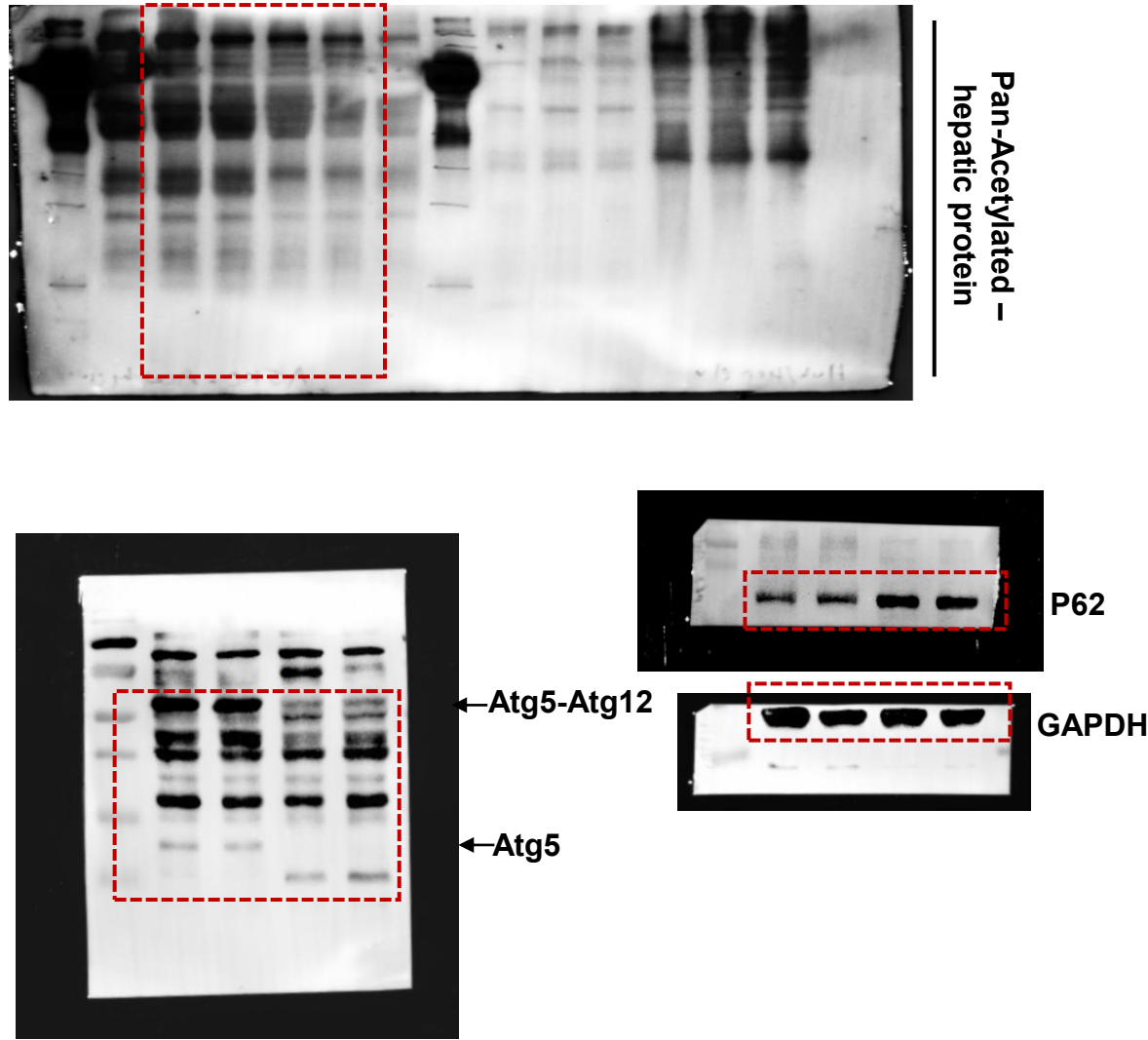
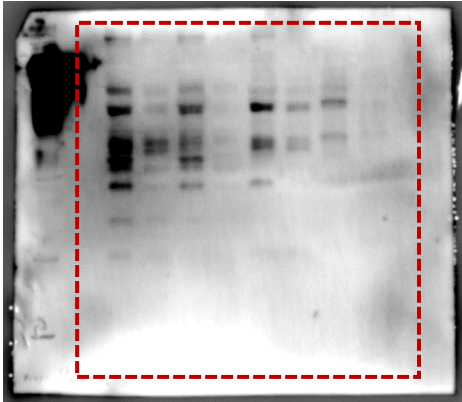
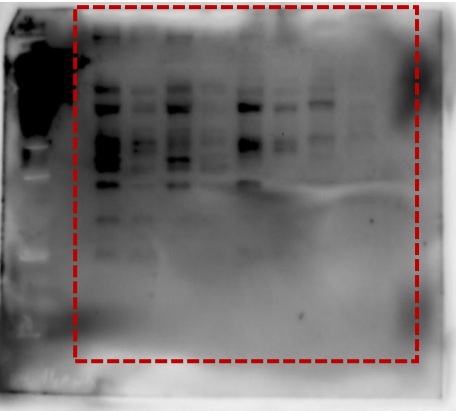


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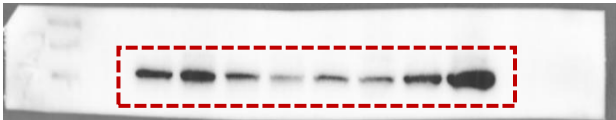
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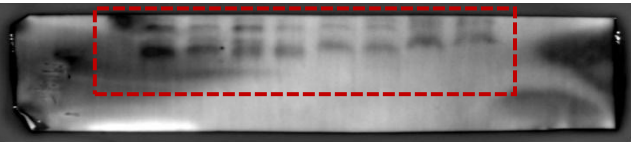
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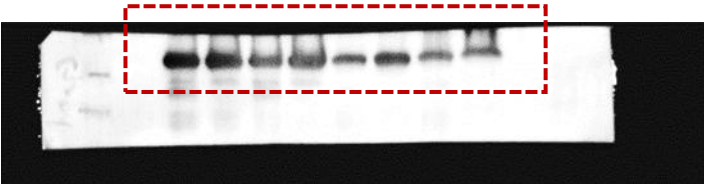
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Calreticulin



Histone



GAPDH

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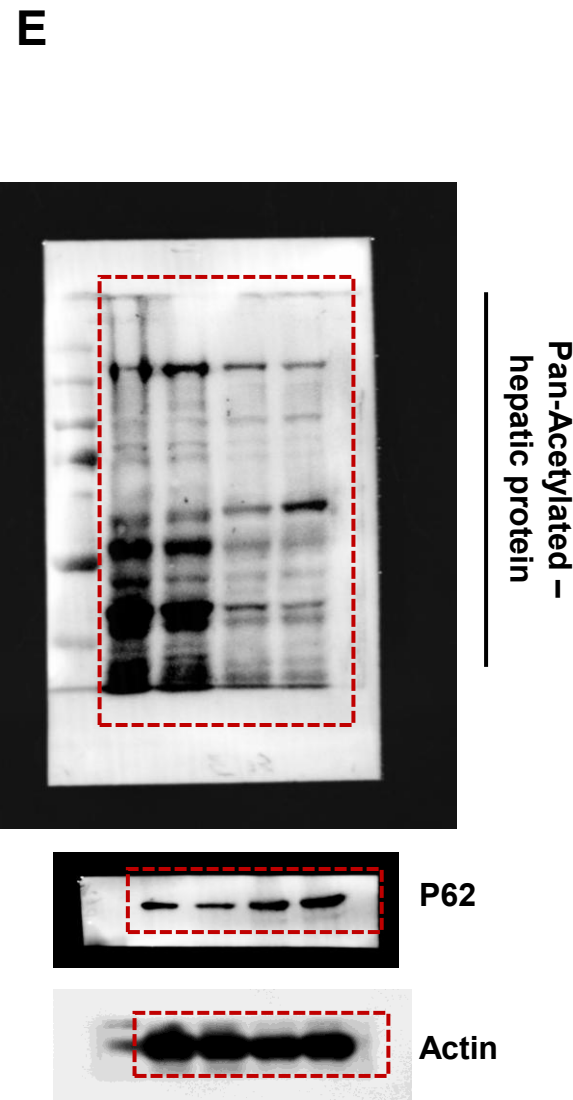
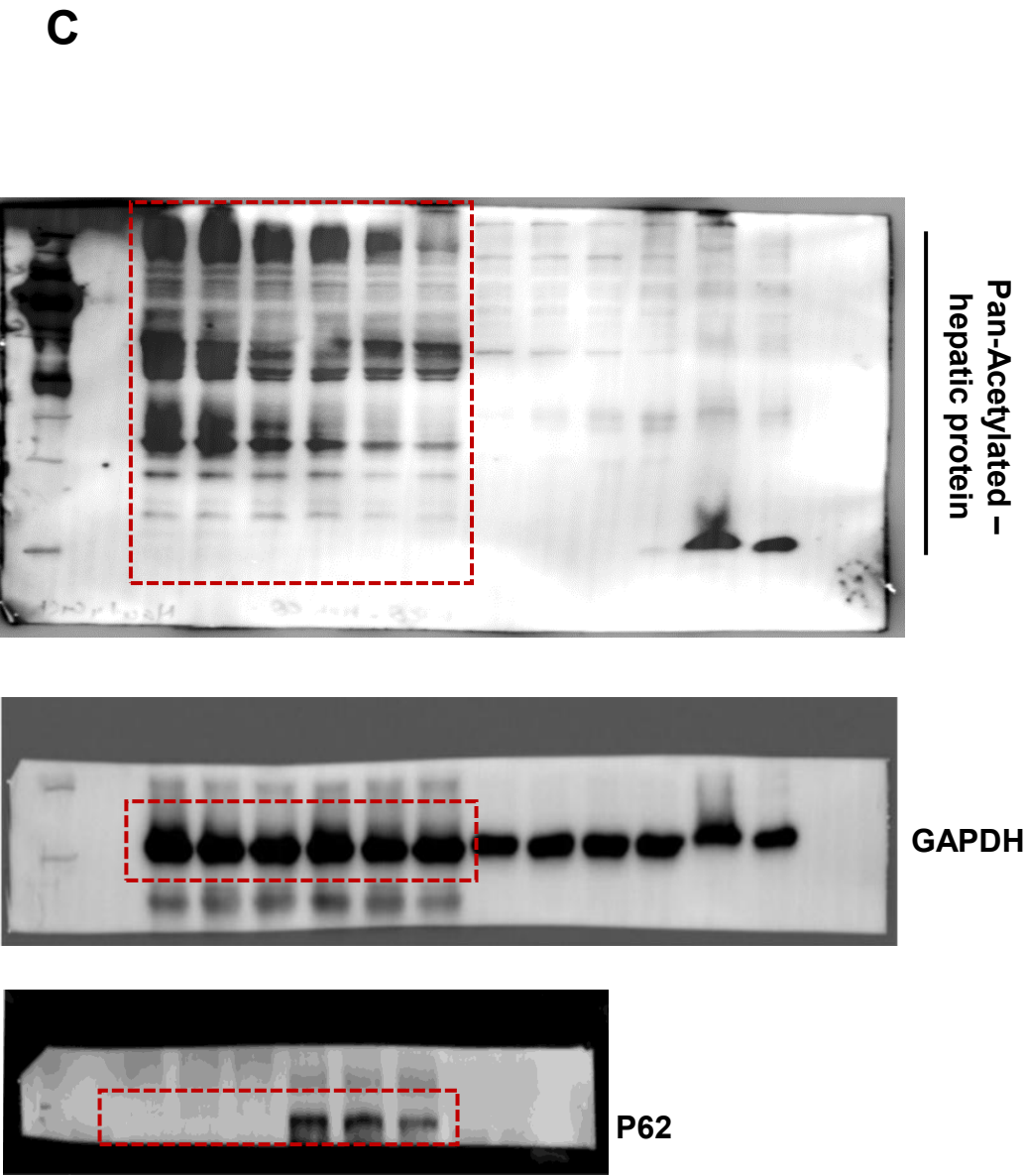


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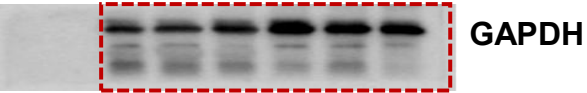
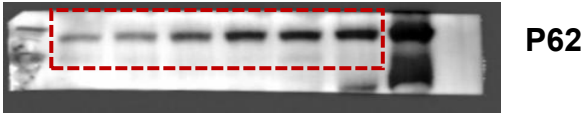
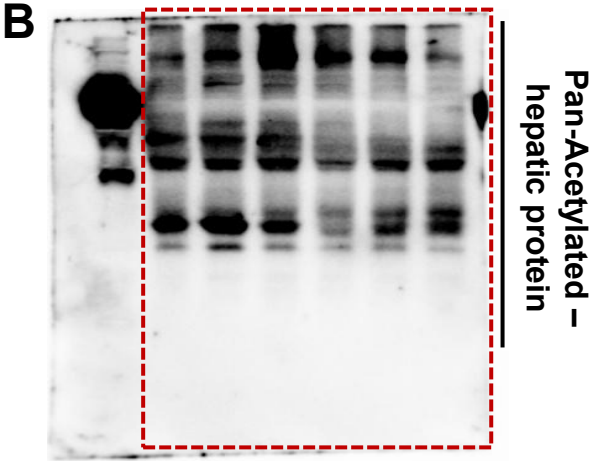
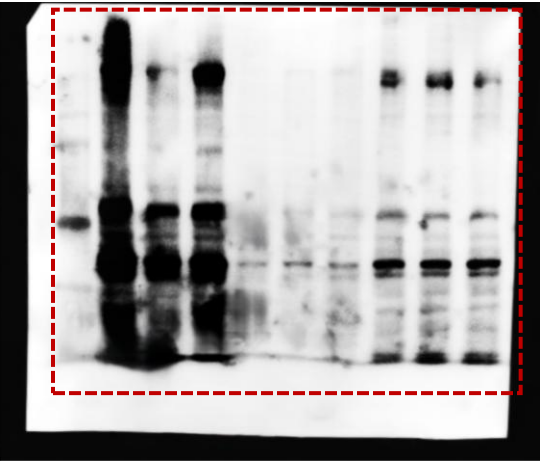
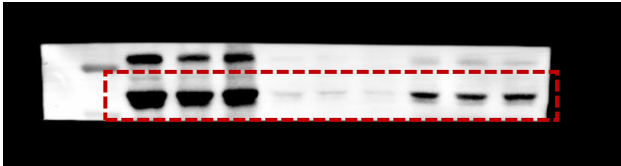


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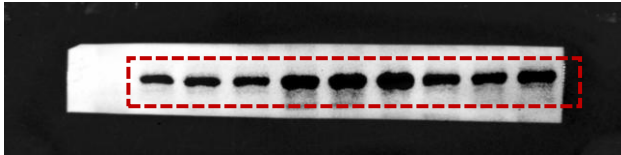
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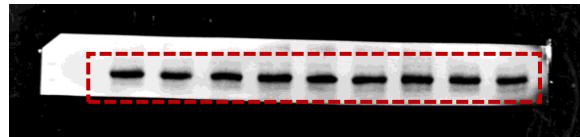
Pan-Acetylated -
hepatic protein



Atg7



P62



Actin

Figure 6.

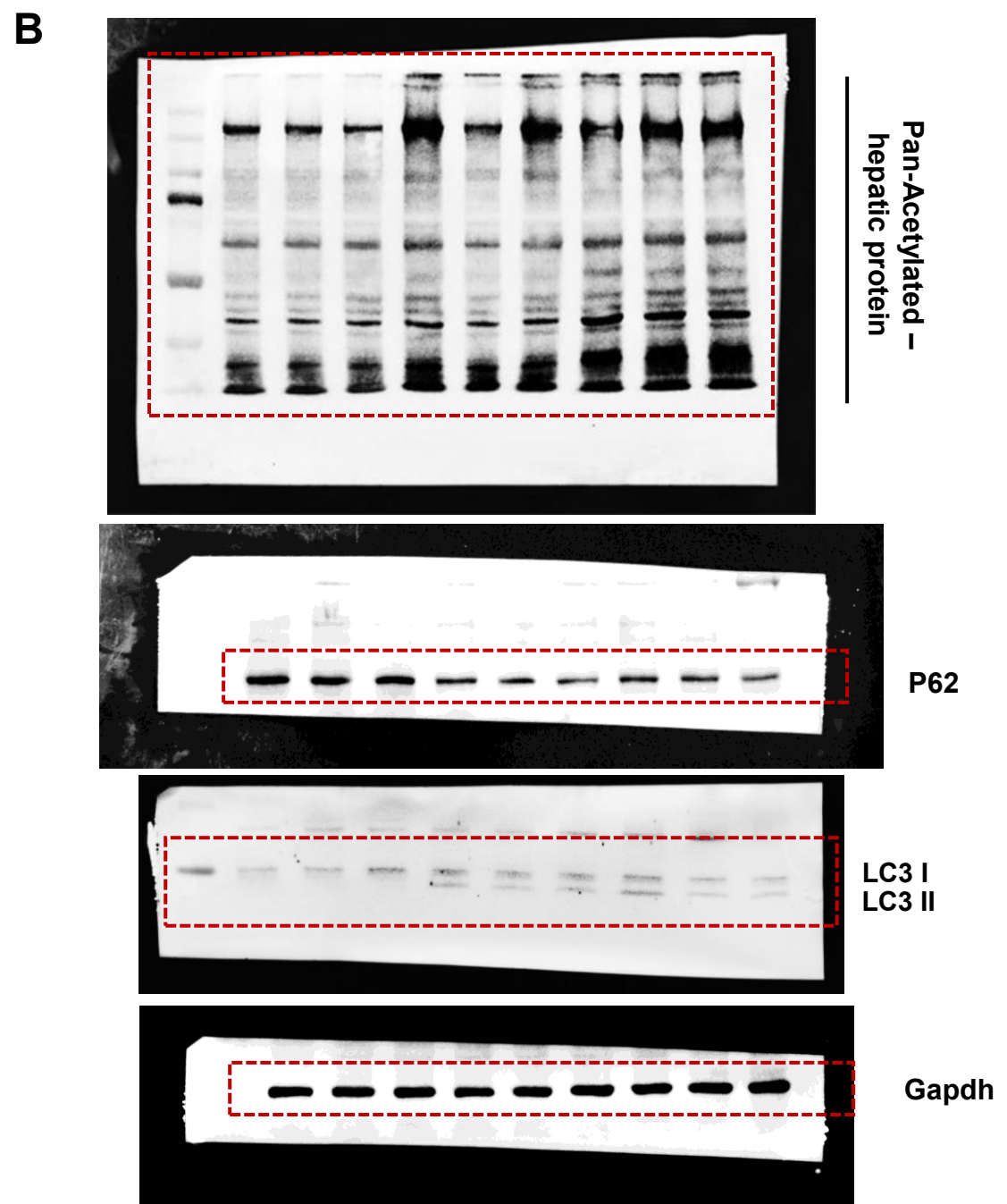
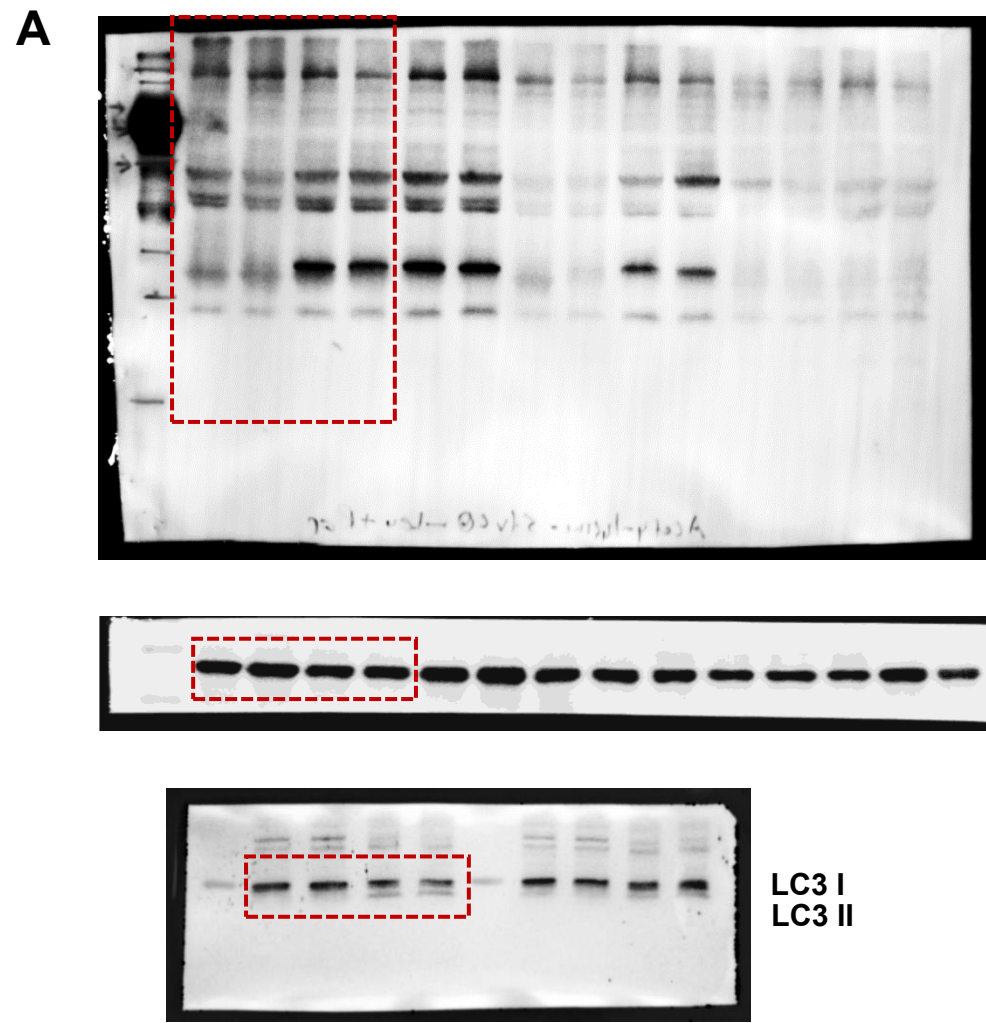
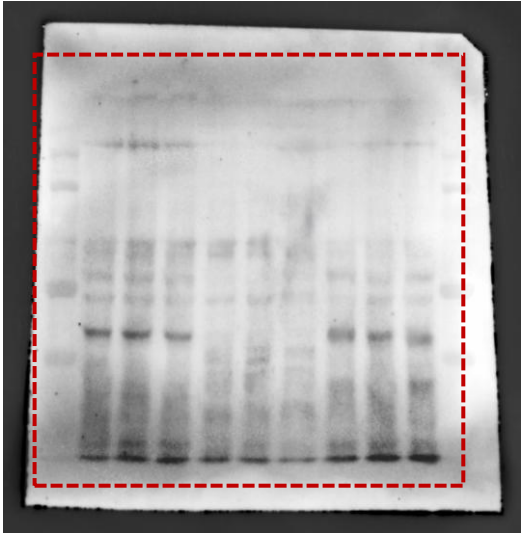
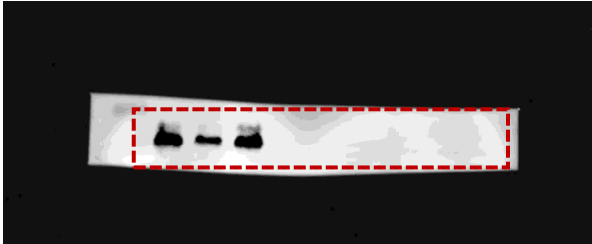


Figure 8.

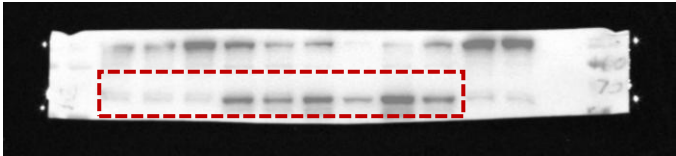
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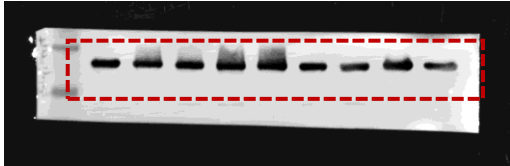
Pan-Acetylated –
hepatic protein



Atg7

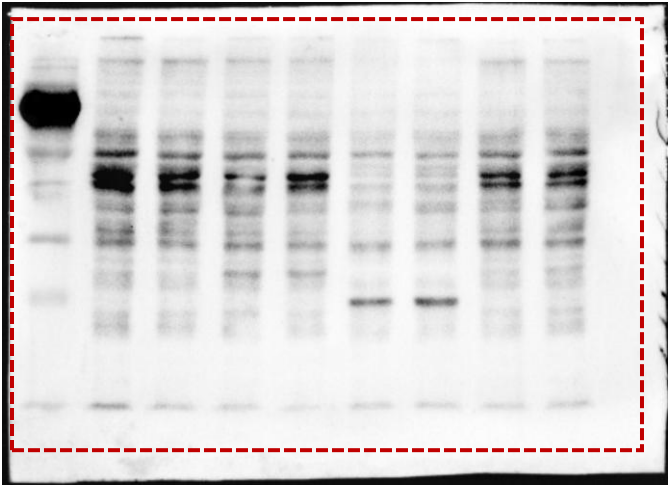


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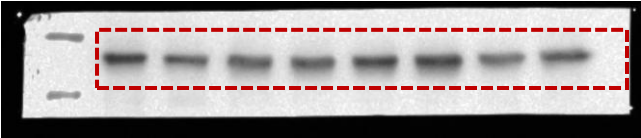


Gapdh

E

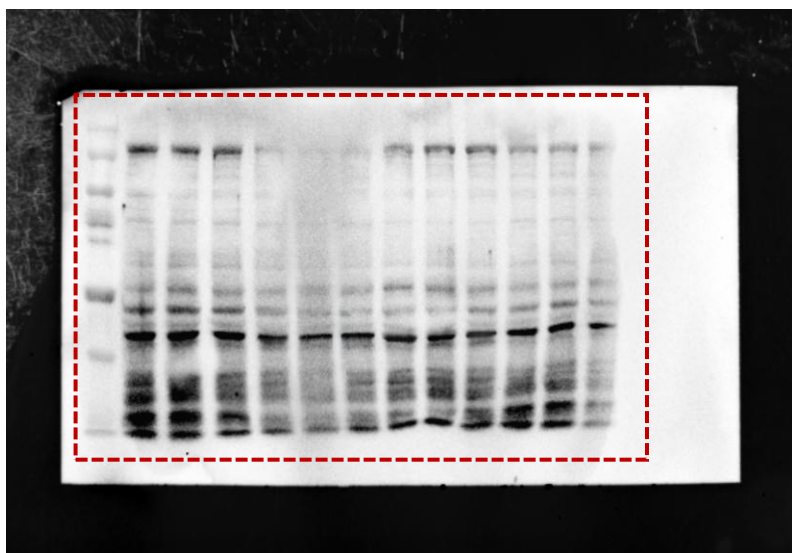


Pan-Acetylated –
hepatic protein



Gapdh

G



Pan-Acetylated -
hepatic protein



Atg7

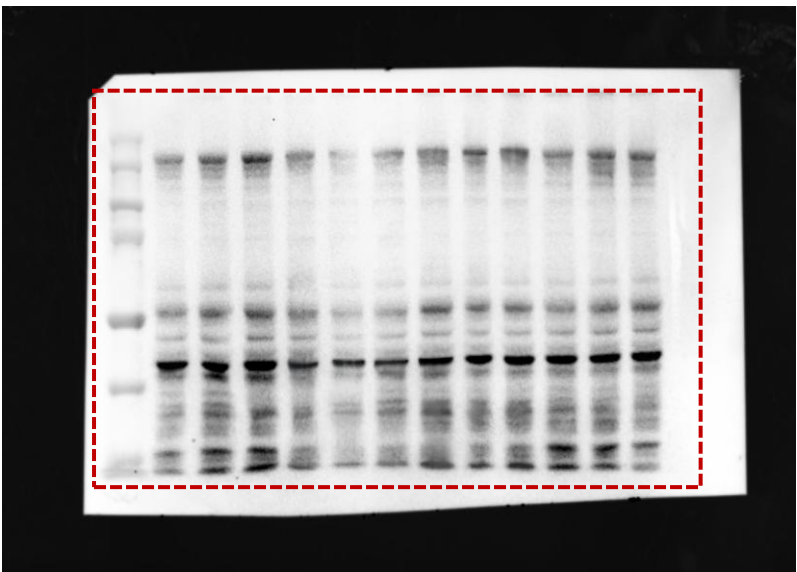


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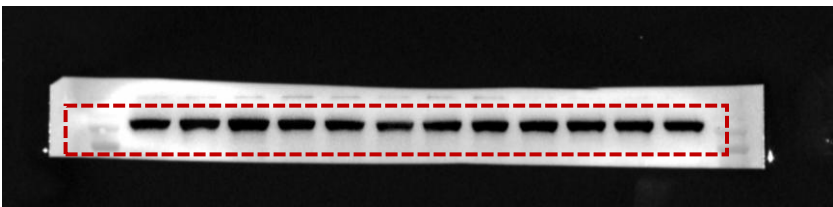


Actin

H



Pan-Acetylated –
hepatic protein



Atg7



P62



Gapdh

Figure 9.

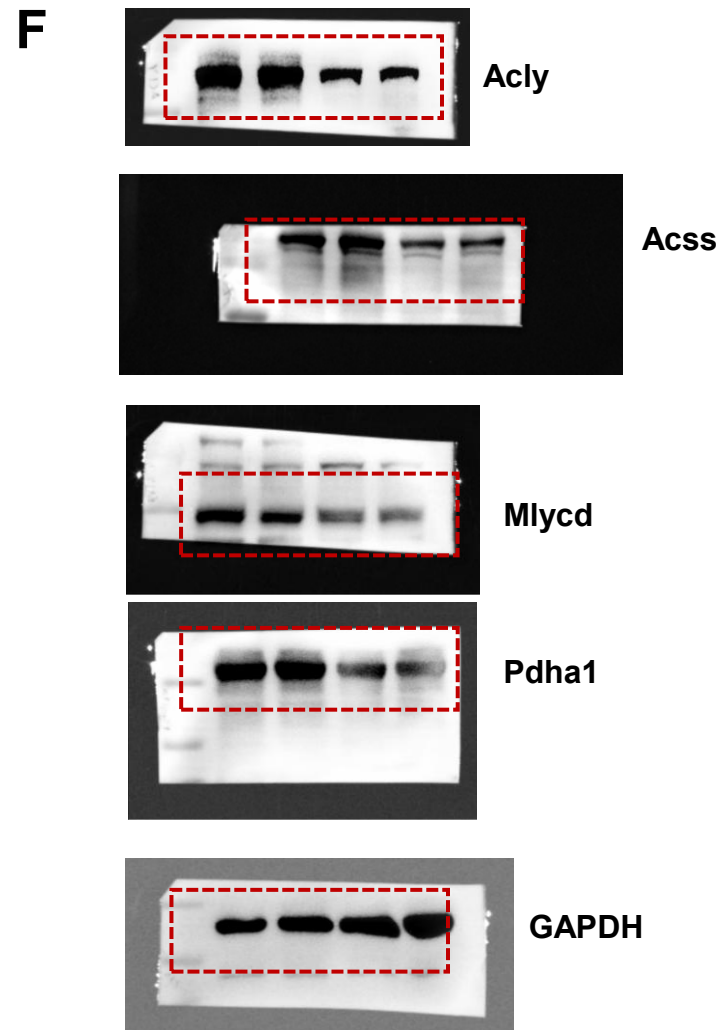


Figure 10.

B

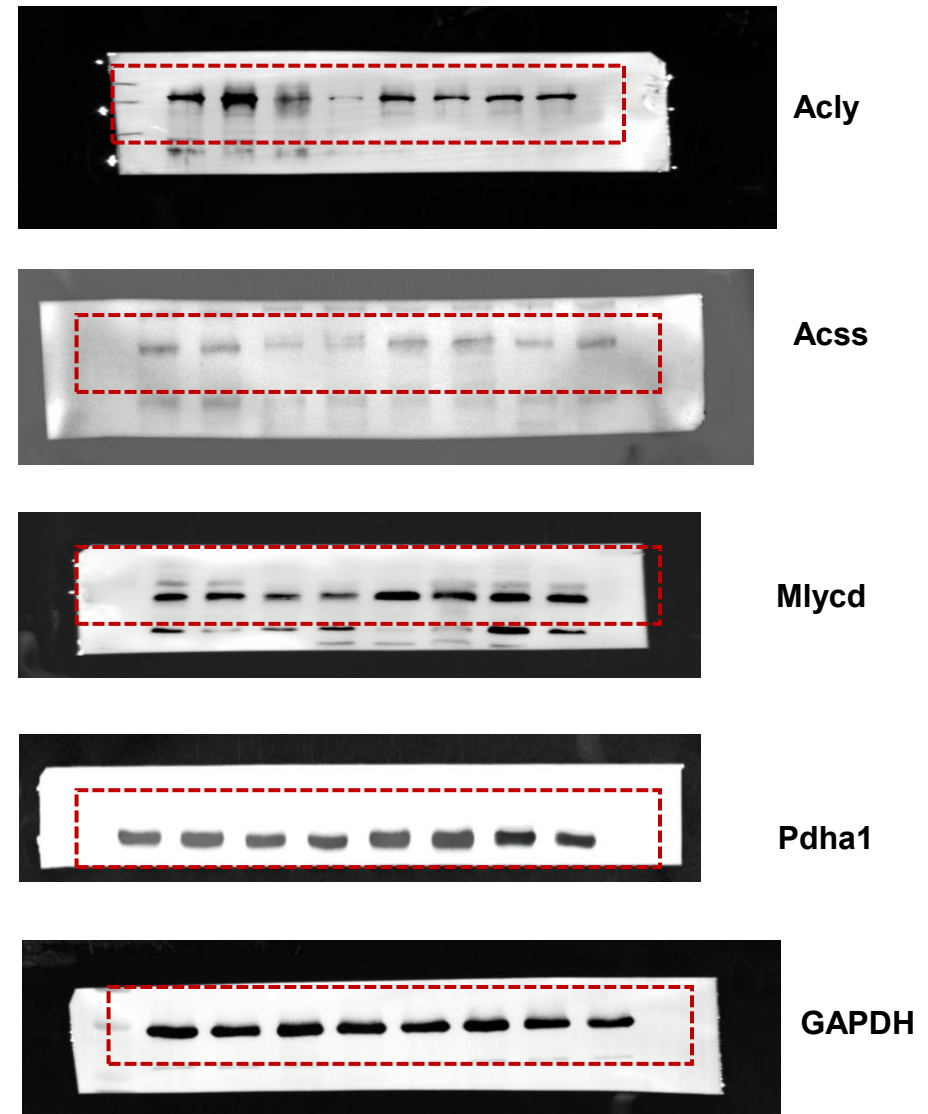
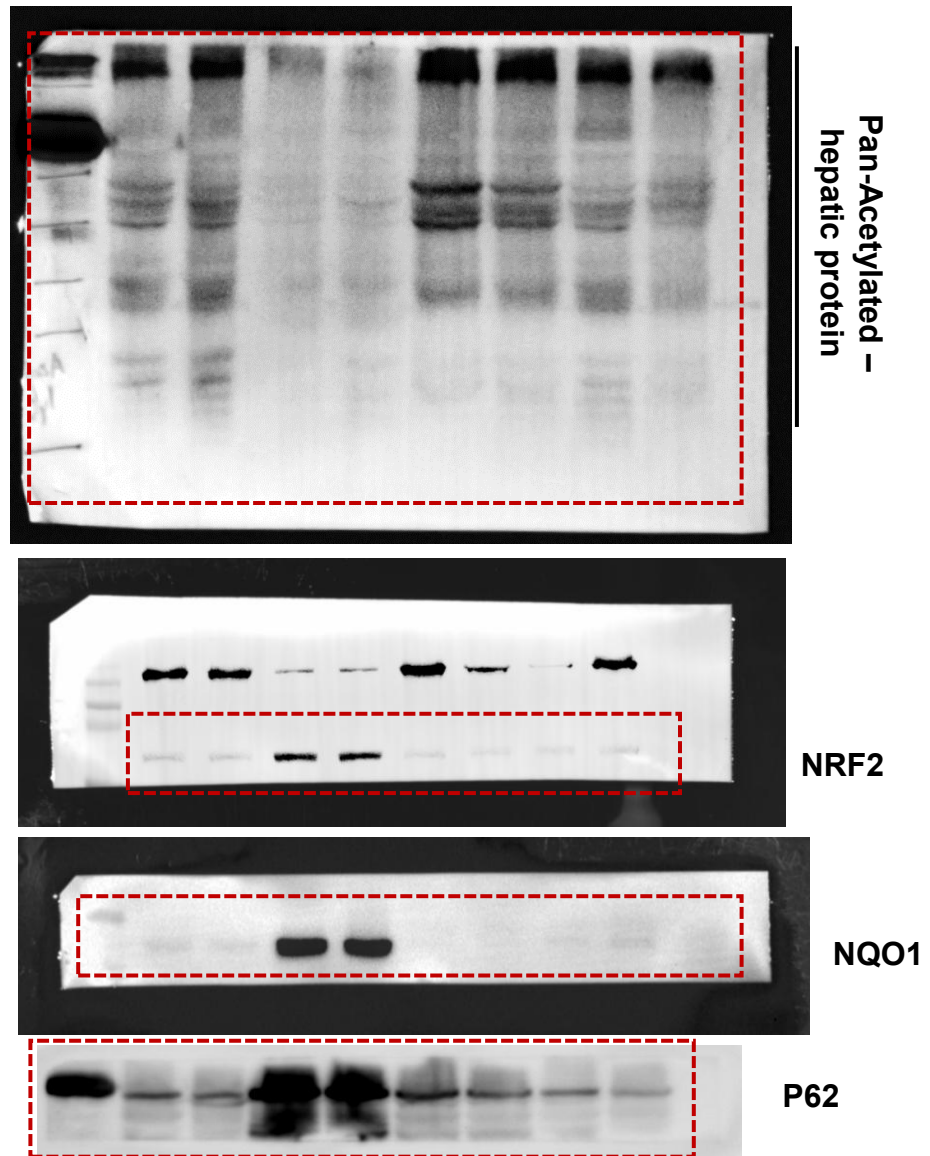


Figure 11.

A

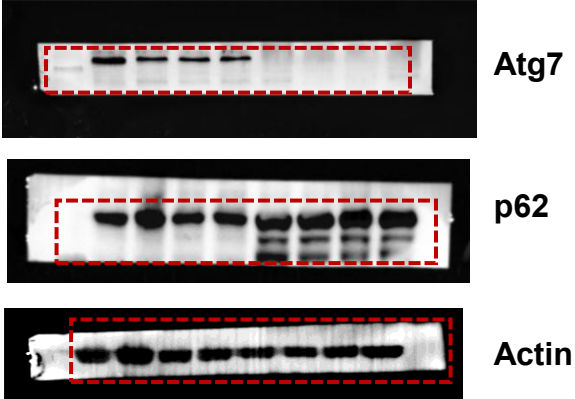
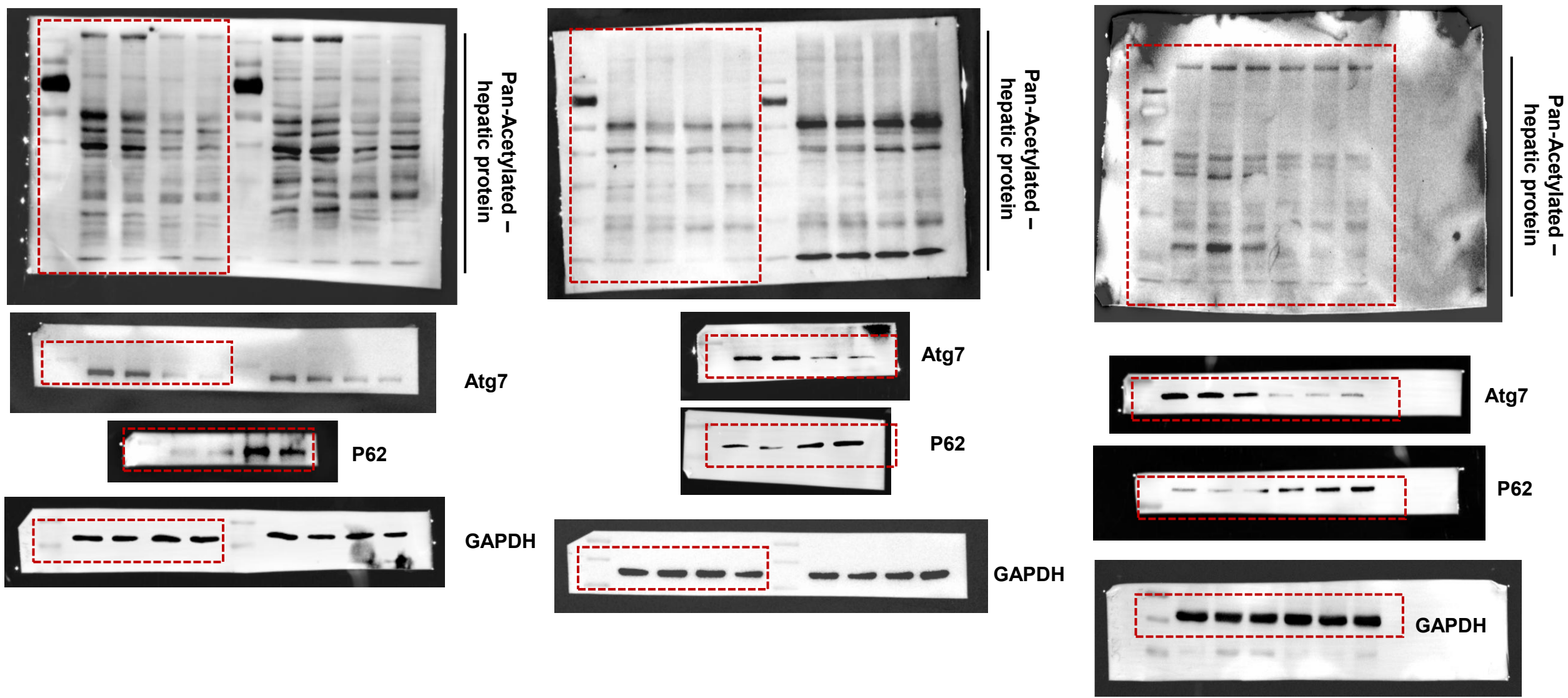
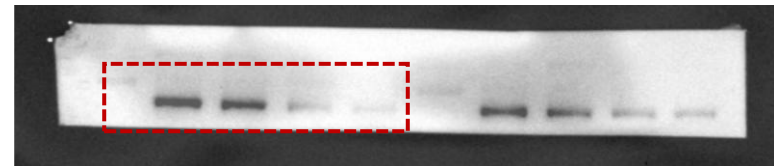


Figure 12.

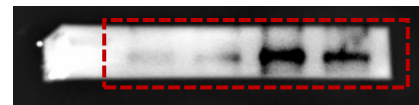
A



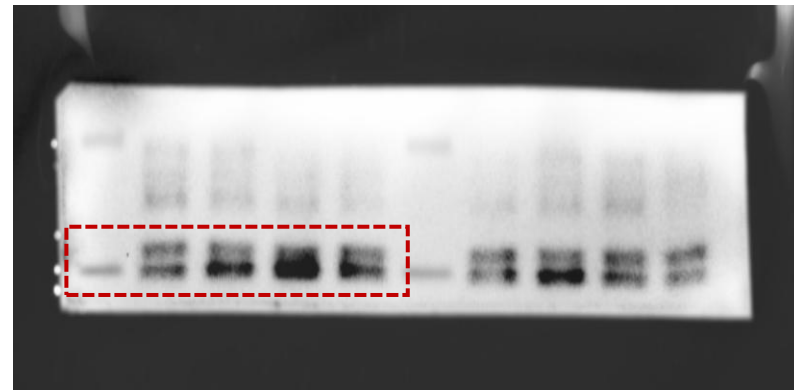
C



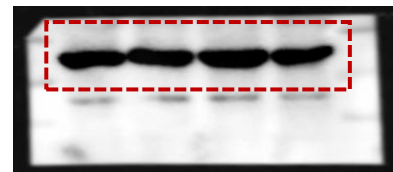
Atg7



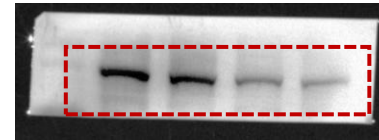
P62



**LC3B I
LC3B II**



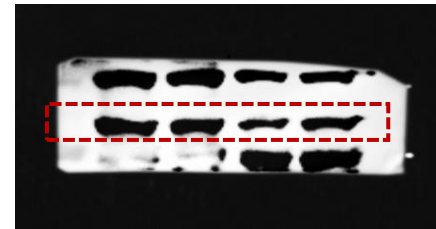
GAPDH



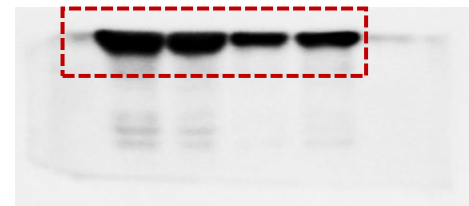
Acly



Acss



Mlycd



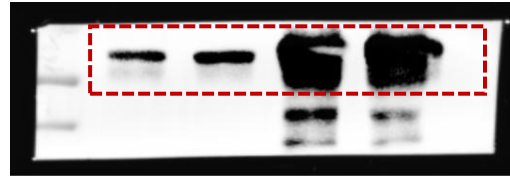
Pdha1

Supplementary Figure 1.

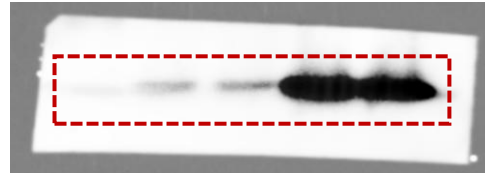
A



Atg7



P62

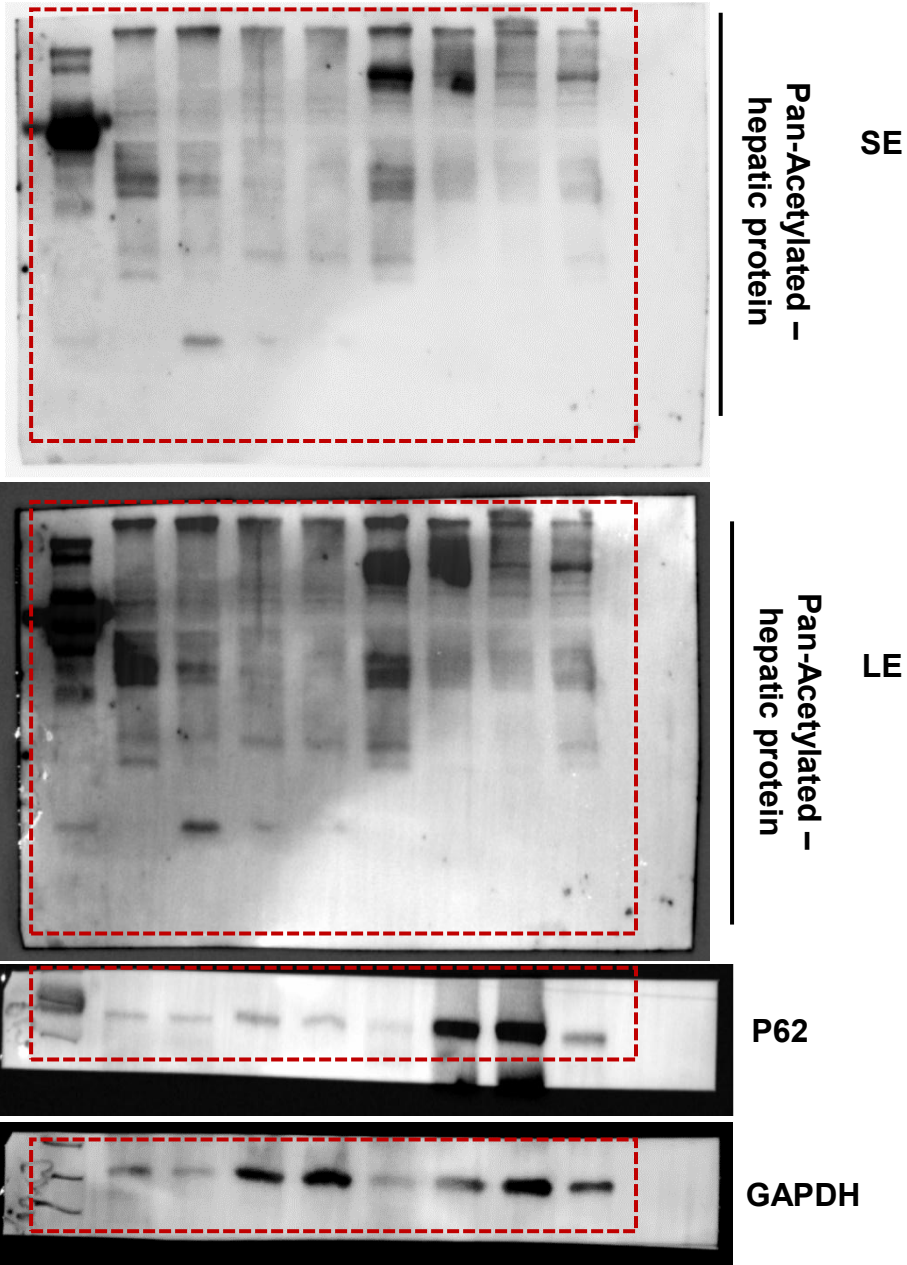


NQO1



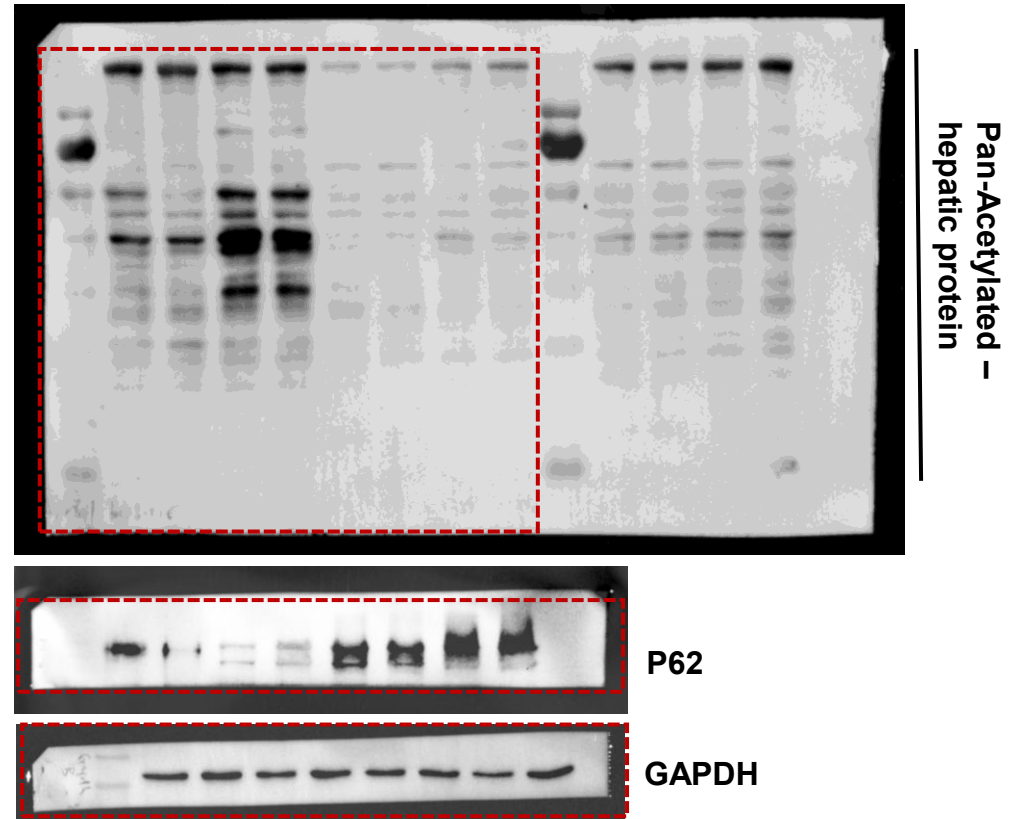
GAPDH

Supplementary Figure 2.



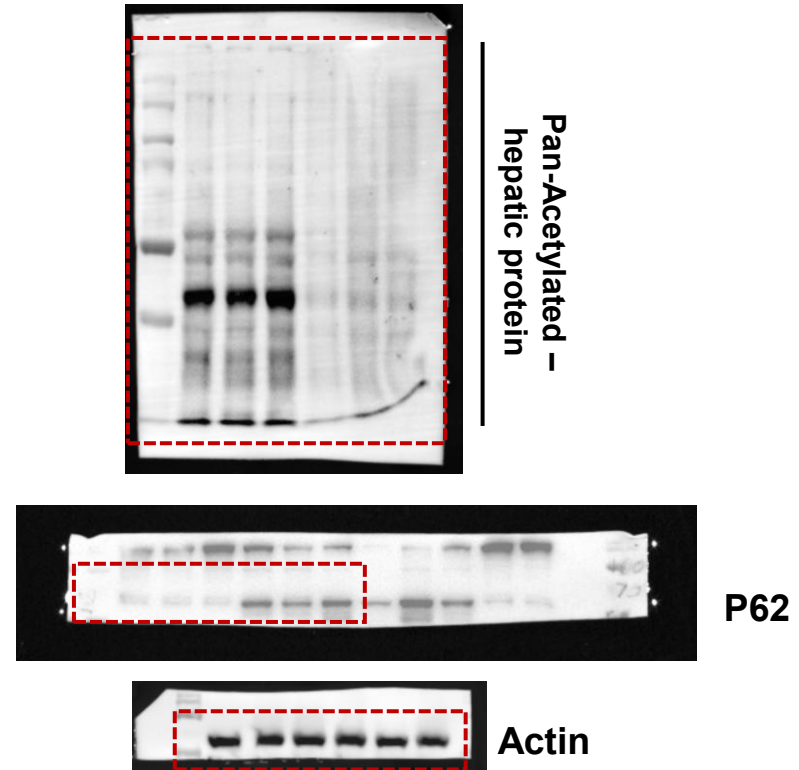
Supplementary Figure 3.

A



Supplementary Figure 4.

B



Supplementary Figure 5.

