

Regulation of Hepatic Circadian Metabolism by the E3 ubiquitin ligase HRD1-controlled CREBH/PPAR α Transcriptional Program

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Online supplemental material

S-Table 1. Mouse gene primer information for qPCR used in this study

Gene name	Forward	Reverse
Hrd1	GCTTCCCCACCAATTCCTGA	GCATCAGGCTCTCCATCCTC
Sel1L	CTCCAAGACCCAGGAGGAAGA	TTCTTCAGCTTCAATGGCAGTC
CrebH	TCGTGCCAGTGCGAGTGT	AGCCACGCGGGATGC
LC3b	GCTCTTTGTTGGTGTGTA	TCTTCTGTTGCTGTTGTC
Atg7	CAGAAGAAGTTGAACGAGTA	CAGAGTCACCATTGTAGTAAT
Acot4	CTTTGCCACATTGGCTCTGG	ATGTCGAAGCATGTACCGCA
Bdh1	AGATGCGGCTAGTGGCAAAG	CAGTTCCTTGACCCCAGCAT
Cpt1a	AGAATCTCATTGGCCACCAG	CAG GGTCTCACTCTCCTTGC
Hmgcs1	GGAGAGTTTCCAGCGCTTTG	CTTGGCAACCTCAGCAACTT
Apoc2	CTC TGC TGG GCA CGG TGC A	GCC GCC GAG CTT TTG CTG TAC
Lcat	GCT CTG TGG CCA GTG GCA GG	AGG AGT GCG GTA GGC ACC CA
Fads2	GCT CTC AGA TCA CCG AGG AC	AGT GCC GAA GTA CGA GAG GA
Fgf21	GTG TCA AAG CCT CTA GGT TTC TT	GGT ACA CAT TGT AAC CGT CCT C
Ppara α	GAAGGGCACACGCGTGCGAGTTTTCAG	CTGTGATGACAACGTCTTGTTCCCGAACT
Pck1	CTCAGCTGCATAACGGTCTG	CTTCAGCTTGCGGATGACAC
G6pc	CTGTACCTGTGAGACCGGA	AGATGACGTTCAAACACCGGAA
β -actin	GATCTGGCACCACCTTCT	GGGGTGTGTAAGGTCTCAA

S-Table 2. Primer information for ChIP assay used in this study

Chromatin Immunoprecipitation assay		
Gene name	Forward	Reverse
Ms Hrd1	CCTGTTGTGATCCCCAACCAT	CCAAAGGGCTTACGACTCACA
Ms Sel1L	TGATGCAAACGTACAGCAGC	GCCACTCAAAGGGATTG

S-Figure 1

CREBH- or PPAR α - binding sites in the promoter regions of human and mouse *HRD1* and *Sell* genes. Highlighted red bases indicate transcription start sites. The primer locations and sequences for ChIP analyses were also indicated. CRE, CREBH-binding element; PPRE, PPAR α -binding element.

A. Human *HRD1* Promoter

(HPRM51308 NM_032431;name=SYVN1;Entrez_ID=84447)

GACCTGGCGCCAATCCTAGGACAGGTTTTGGTAAGCCTTTGAATTTCAATTGCCCCACGT
TTCGGGGGAGGGGGTAGCACCCCTAGCTCATAAACCTTAGTGATTGATGATTAAATGAG
ATGACGGAGGAAAACGCAAGGCACAAAGTGGATGCATTAGCTCCATTTTGTTAATCAGCA
GGCTTAGTTGGCTGCGACCCAGACACGAACTAAAATACAGTGCAGCCCAGGACCAGTGGG
GGTCTTGCTTATGGCTCAGAGCTGAACAACACATGGGCAGCAAAATCAGACACTGAGATG
CGGGCAGGCCTGCGACGC**TGAAGTCA**ATTCCCTTTGAACAAACAGAACACTTCCGTCCCAA
GATTAGCAGGAATTAATCTCCCAGTCTCGGGTACACCTGGTTGTCCCTCCCTGTCTGGC
GCGGCAAACGTTCCCGGAGGCCAGCCAGGGATCACTCGCCCAAGGACTGAGCTTTCCTTA
CTCTCAGCCAACTGGAGCGGGACCAGGGCCTAGGCAACGCAGCTGTCCGCCCTAACAAAC
CACTCACCTGCTTTCCTTTCTATAGGCCAGCAAAGGTACATTCTTTTTTCTTATTGGGC
CGCGTAACTTATCGCAACCAATCAGTGGCAGCCACGGGACCAACTCACTCCACACAAC
TTGTGGGGGTGATCATGGAGAAGACAAATTTTTGTTTTCCGCATCCAGTTCTCTCAGAGA
GCACCGTATTTGTCAAACCTGTTGTGACTCTCCCTAAATGTTTAAGAAAACATTTCAATCC
CCTCAGGCTTGTATAGTCTGTCCCTGGCCTACTCCCCGCTCCAGGTGGTACAGCCCGCAA
GCGGCTCCCCTTCCAGCTGCTCGCGGGGCCGAGTCCCCCAGTCCGAGGAGGCCACTCAG
CGCAGGAGCCATAACCATCTGTGACTAATAAATAATAGGGGGACCTCCGACTCCCCCTGT
TGCTTATTACCTTCCGACCACCTCTCGGACCTCTTGCCAGCCCTTCCCCGTAGACATC
ACCCAGATACGGTGGTACACCATTGCTATGGGCCACGTAGGGCGCAGTGCGAGCCAG
GGCAGGACGCACTTGGTACGACCCACGCCGCGCCCCGCGCCGCGGAAGTGAGGTGTC**TG**
ACCCCCGAAGTTCCGGTTCGCA**G**GGGGTGGGGAGTGTTGTTAACC GGAGGGGCAGCCGCA
GTCGCGCGGATTGAGCGGGCTCGCGGCGCTGGGTTCTGGTGAGTGGGGCGAAGTCTGGC

 PPRE
 CRE

B. Human *SEL1L* Promoter

(HPRM39386 M_001244984;name=SEL1L;Entrez_ID=6400)

CTCAAATTCAACTAGCTGTGGCATTCTTCTCAGTGCTCTTATCACTATGCCCAATTTAAA
TTTTTTAAATAAAACAGGAAAAGATGCTAAACACATTACAAGTTCTTAAAATTATTCCTT
AATATTTATTTTGGGTCAATTTAAAAGGCTTGTGAATCCATAGCCTTGAGTTAATATTT
AGCCAGGTGTTAGTTAAATTTCAACTTCAACAGTCACCCGCACTTGTAATCTTGCCCT
ACGATGGACTACGTTTCCCAAGAGCACCCCGAGTTAATCACATGAAGCGCATTCTGGG
AATTGTAGTCTCGGATGAGCCTCTTCTCCAGTCTTGGACTCACGCGCTGATTCCGGTT
GAACCCAGGGGCTACCAATTAGCTGGGGCCATGTCCGTGAGTCGTGGTCCAACCGAGAAA
GCTGGTGTGGGAGCTTCGTACAATGATTGGCCAATGAA**TGACCCC**CGGGGCGGAGCAATG
GGGGTGGGCGGGGAGCCATGGTGATTGGACCAGAAGCCCGCGACGGCGGGCGGGGATTG

GCTGCGCGCTGGGTGAGGGAAGCCTGGGAAGGGGCGGAGGAAGGAGACTAGAGCAGGAAG
AGCAGCGGCGAGGCGGCGGTGGTGGCTGAGTCC

PPRE

C. Mouse *Hrd1* promoter (MPRM46266 NM_028769;name=Syvn1;Entrez_ID=74126)




ACCTGGGGGAGAGGAGGGATTGCCACAAAATGTAGGAGATTTTCAAGAATGGGGGAGGAT
GAGTGTGTAGGGTTAAAGGTAGCCAGTAGAAGTTCATAGCTAGCCTTATGGAGGAAGGAA
AGGGGAGCCATCTCGGGATGTTAACTGTTAAAGACAACAGGTGGTGGTGAAGATGGCTGA
GACCAAGAGCACAGGGCTGAGGGGCAGACAGGCACTGACACTGCTACCCTTAATACAGT
TCCTCCTGTTGTGATCCCCAACCATAATTACTTCGTTGCTACTTCATAACTGTAATTTTG
CTAGTTATGAATTGTAAGTAAACGTCTGATATGCAGGATATCTCATTTG**TGACCCCT**GTG
TAACGGTTTGATTCCCAAAGGGCTTACGACTCACAGGTTGAGAGCCAGCCACTGCCTTAA
AGTCGTCTAGAATCAGTTTTCTTTCTTTTTTGACAGACAAGATGTTTAATTCCGTTGTAC
TGAAGGAAAGCCATTTTATGTATTTTCTTAAGTGCTCTATCAGTAATGACAATTCTGAA
AGCCCCTGTGTTATATTTTAACAACACAGTCACCTCCGGTCTGTATTCACTGTCCGTGT
TGTGACTCCACAGTATAAATTCCTCCAGTTGATCTTCATGAATTCTTATATTTGATCCC
CCCCCCCCTTAGGCCTCTGAATTCCGAGTGAGTCCGAGTTAAAAATGGGAGGAGCACCCCT
CTAGCTGATAAACCTGGGTAATGAGGTGTCCGCTTTCAGTTTCCATTCTGTACGCGACTA
T**ACTGCTTGT**GTGAGCCCTAACAGACAGAATCAGCTCAGAACAAAGGGTCTGGCTATCTC
CCAGGGATGAACACGCACGCCGACTGAGCTTTTTGGGGTGTGAAAAGTCAACGCCTTCGC
ACAGAACTCTCCACCCCAACCTAGAAATAACTGGCGTTCTGTTTTATGTCAGTCCGGACA
CGCAAGCACTGCTCCTTTTTCGGGCCCCGTAAGCATCCCCCAGGCGGGATAGGGATCCC
CGGCCTATGGACTGCGCTTCTCAGCTGGCATCCAGCTGCCTTGGCACCCAGTCCGGGGC
CACTCTGCCTACAGACCCTAGCAACCACTCACCTGCTTTTCTTTCCCTATAGGCCAGAAA
TTTTTCCTTTCTTTTCTCATTGGTCCGCGTAACCTTTATCGCAACCAATCGGCGGTAGCCA
CGGGAACAAACTCACTCCTACACAACC**T**GCGTTGGGGGAGGTAACCTGGGAAGACCTAT
ATCTGTTTTCTGCACCGCTATTTTTTTCCGAGAAGCACTTAACCTTCTTACCGTGTCTAG
CTATCCCTGGAATGAGGCGCTTACACATTTTATTTCTTTTCATGCCTGACATAAAGTCTGG
CCCTTGCTCGCTCCTGCCCC

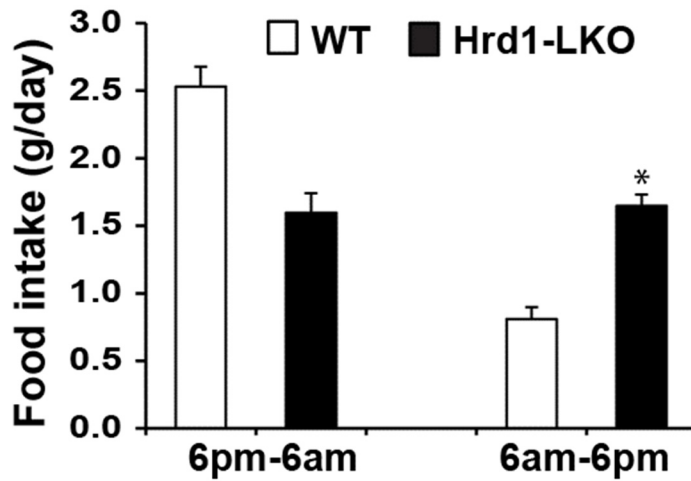
PPRE
Primer for PPRE
CRE
Primer for CRE

D. Mouse *Sel1L* promoter (MPRM31536 NM_001039089;name=Sel1l;Entrez_ID=20338)

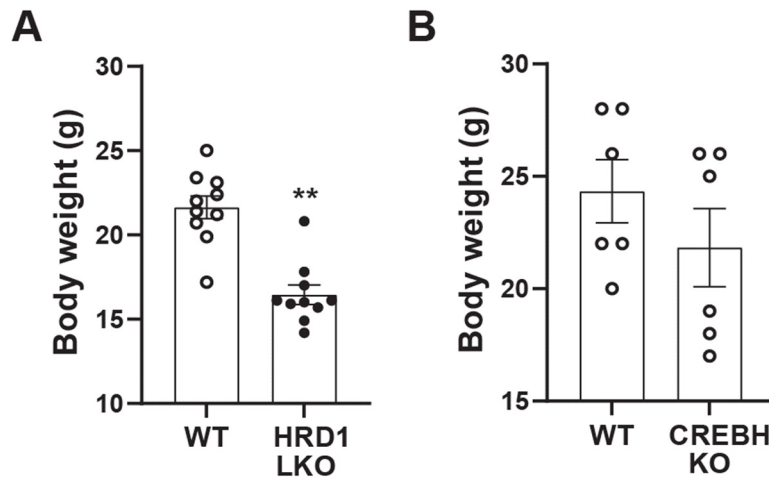
CTCCATCCTATACACCTTTATGCGCATGGTGCACGCACACACACACTGAGGGGGTGT
GGGGGCATGGGGGAAACTGTTCCCATGCCCTCTGATGCAAACGTACAGCAGCTAATTT
TTTAACATCTTCTTAAAAATAATGACTGCCAAAAAGATAGAAAACAAGTGTTCAGAGAA

GTCAAGACACTGAGACAGGAAAGACTTTCCCCCTAACCCCTGCTGAGTTCTGTATGCA
GTGCTGGAAAATAAATCTTAGAAGGATTCAATTTGATTAAGAAGACTGAAAAGCATCAAG
ATGCTGCCCTGCCACTCAAAGGGATTGTGCGTGCATCGGTACTIONACTACATAACTATTGC
AATCCCTGAAGTGCCTTGGCTCTCACCTCTGTGGCCACACAAACCATCTAATCCCTTTTC
TGGTCAAGGAAAAAGAGAGCATTGCAATATTTCACTAGAGAAGAATGAGGACCAGTGTTT
GAAGGGTTTGGGAGAGCTGTTAGAACATTTATTGAGTGGCTAATATATATGGAAGCCTTA
GTGAAAACAATCACAGGAGCTGAACTAATCTCCACTGGTAGGAATTTCTTCCCTTTTGT
TTATTAGGGAAATGACAAGCGGCATTGTCTTGTACTGCTGGCTCTGTAAACTTCACGTAT
TGCTTCACCTAAGCTTTTTTTTTTTAAGACTTAAGTATTTTTTTTTTAATAAATATTTAAA
ATTTTCAGTTCAGGTATAGCTTATTTCTCAGCGTTCATATCATGTCCAGTTCAAGTTCTC
AAAATAATTAAGAAATACTTAGCAACCTTGCAAGTTCTTAACCGTTTGATATCTGTT
CCTTGATAATTATTCTGGTTCAAACGCAAGAGTCCAGTGAATCATTCTTGAAACTCCT
TATGCCCTCAGCCACCTGCGGCTTCTAACCTGACTTTATGCTAAACTGCATCTCCAAGA
GCATCGGCCGAGAAAATCTAGTGCGGTGCATGGTGGGAGTTGTAGTCCTGGATGAGGGT
TCCCCCGCCTTCGAGCCGCTGATTCAGGCGCTACCAATTAGCTGGGGCCATGTCGGTGAG
GCCGTGGTCCAACCGGAAAGCTGGTATGACAACGTACACAATGATTGGCTAAAAGATG
ACATAGGGGGCGTGACTATGTGTGGGGTGGGGCGATCACGGCCATTGGCCAAGGAGGACG
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GGAACCTGAGCAGGAAGAGCGGCTGCGAGGCGGCGGCACAGAGGCAG

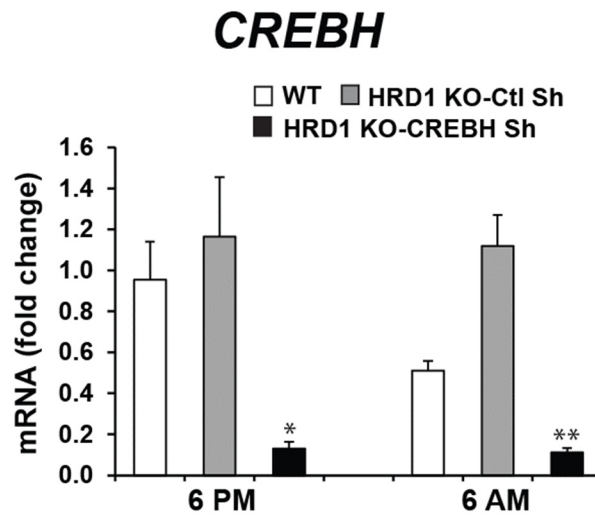
 PPRE 1
 Primer for PPRE 1
 PPRE 2



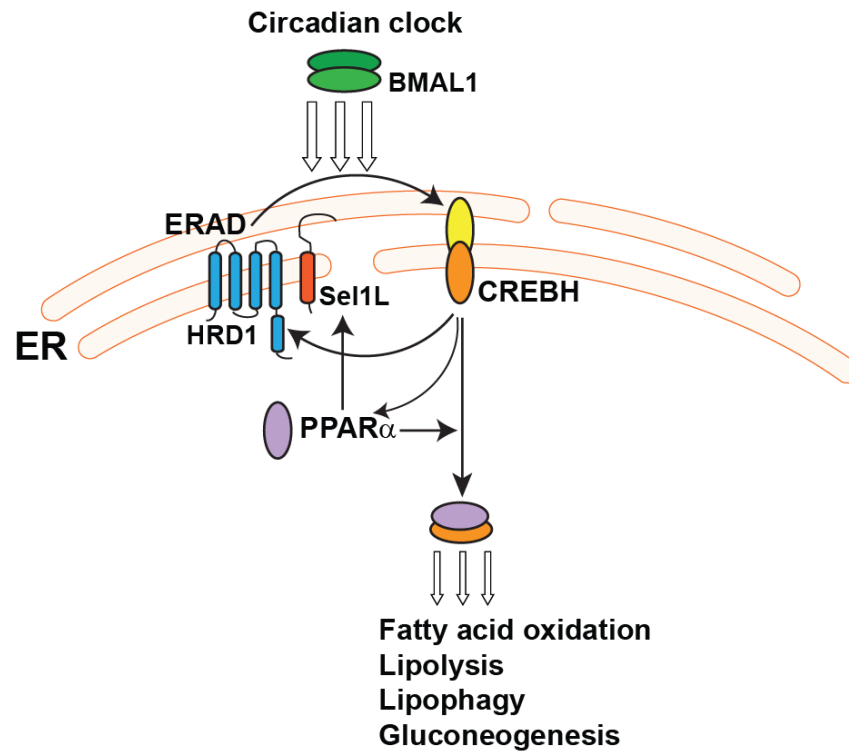
S-Figure 2. Profiles of food intakes of HRD1 LKO and WT control mice under the circadian clock. Circadian rhythmic profiles of food consumption of HRD1 LKO vs WT control mice under the circadian clock, determined through the comprehensive metabolic cage PhenoMaster System. The graphs show quantification of food intakes of HRD1 LKO and WT control mice during the nighttime (6 PM – 6 AM) and daytime (6 AM – 6 PM) periods. Each bar donates mean \pm SEM (n=6 mice). * $p < 0.05$.



S-Figure 3. Body weights of HRD1 LKO, CREBH KO and their WT control mice. (A) Body weights of HRD1 LKO and WT control mice. Each bar donates mean \pm SEM (n=10 mice). ** p < 0.01. (B) Body weights of CREBH KO and WT control mice. Each bar donates mean \pm SEM (n=6 mice).



S-Figure 4. Expression levels of the *Crebh* mRNA in the livers of WT or HRD1 LKO mice infected with recombinant adenovirus expressing CREBH shRNA or control shRNA at the representative nighttime (6 PM) or daytime (6 AM) period. Levels of mRNA were determined by qPCR. Fold changes of mRNA levels were calculated by comparing to the level in one of the WT mice at 6 PM. Data are mean \pm SEM (n = 3 mice/genotype/time point). *P < 0.05 and **P < 0.01 for HRD1 KO-CREBH shRNA vs. HRD1 KO-ctl shRNA.



S-Figure 5. Illustration of circadian-controlled HRD1/Se1L-CREBH/PPAR α regulatory axis in maintaining energy homeostasis.

Uncropped immunoblots for Figure 1A

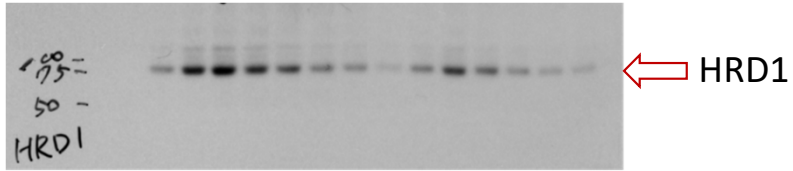


Figure 1A: Western blot for HRD1. The arrow shows the cropped ROI.

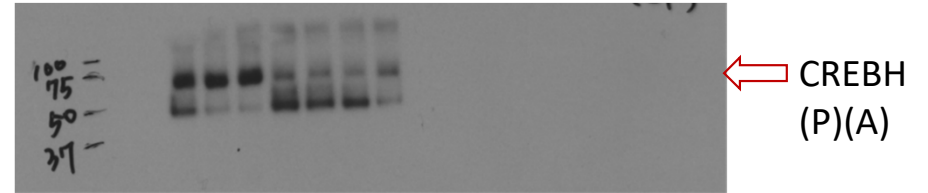


Figure 1A: Western blot for CREBH. The arrow shows the cropped ROI.

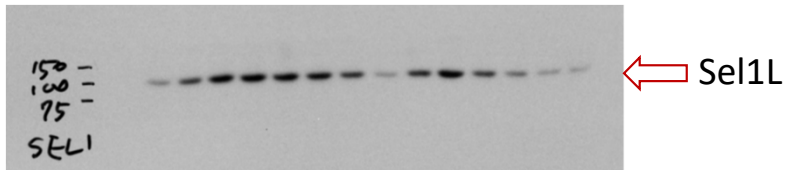


Figure 1A: Western blot for Sel1L. The arrow shows the cropped ROI.

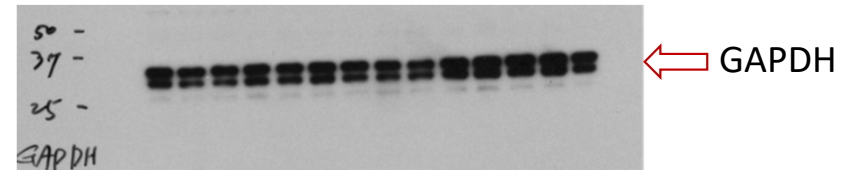


Figure 1A: Western blot for GAPDH. The arrow shows the cropped ROI.

Uncropped images for **Figure 2B, 2C, 2D**

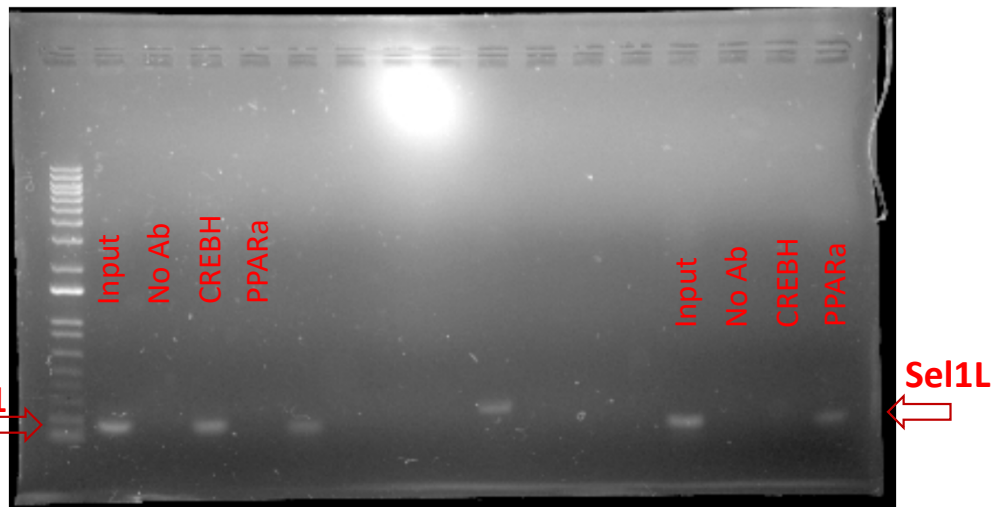


Figure 2B: ChIP assay image for CREBH- and PPAR α -binding activities in *HRD1* or *Sel1L* gene promoters.

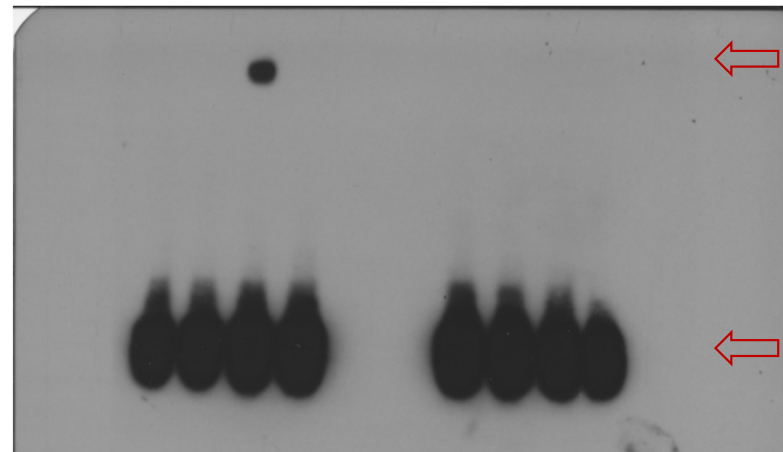


Figure 2C: EMSA assay image. The arrow shows the cropped ROI.

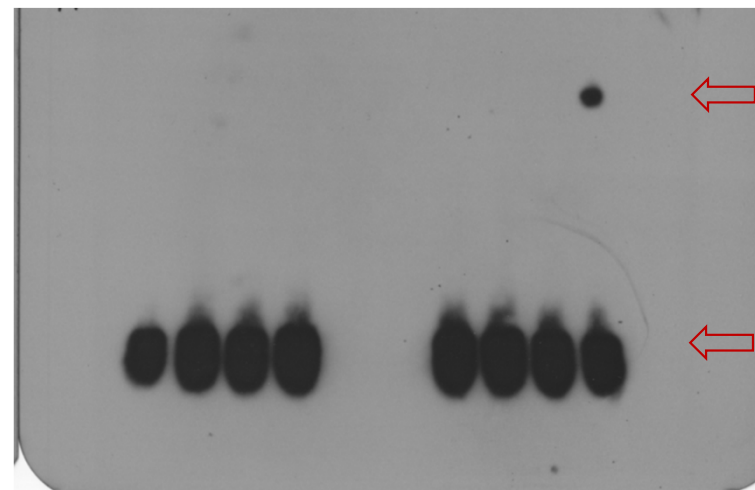


Figure 2D: EMSA assay image. The arrow shows the cropped ROI.

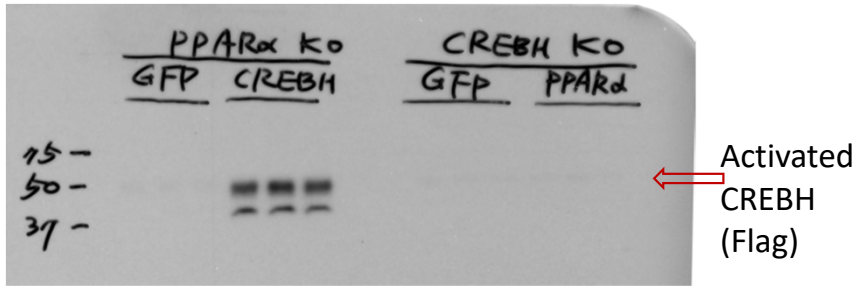


Figure 2G: Western blot image for activated CREBH. The arrow shows the cropped ROI.

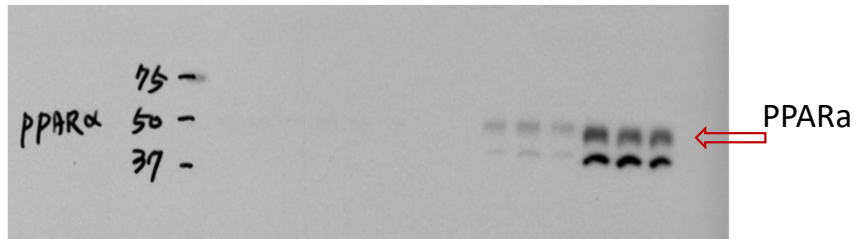


Figure 2G: Western blot image for PPARα. The arrow shows the cropped ROI.

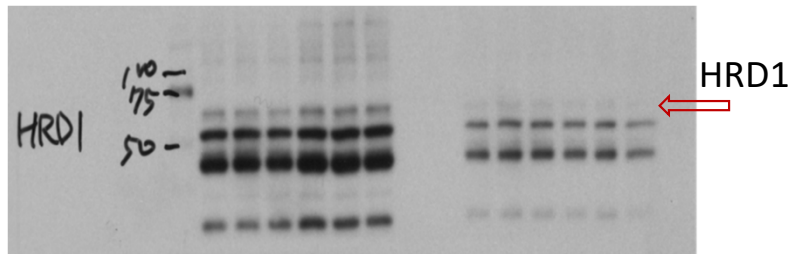


Figure 2G: Western blot image for HRD1. The arrow shows the cropped ROI.

Uncropped immunoblots for Figure 2G

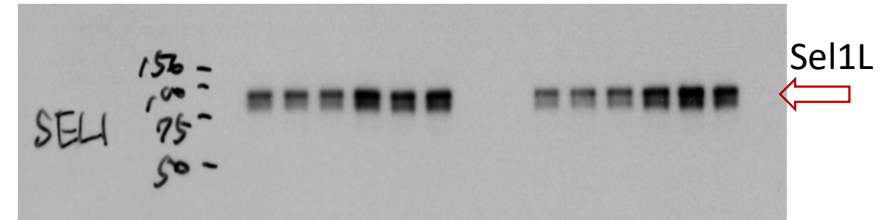


Figure 2G: Western blot image for Sel1L. The arrow shows the cropped ROI.

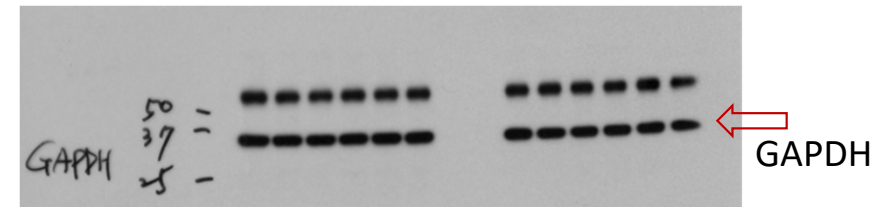


Figure 2G: Western blot image for GAPDH. The arrow shows the cropped ROI.

Uncropped immunoblots for **Figure 3B**

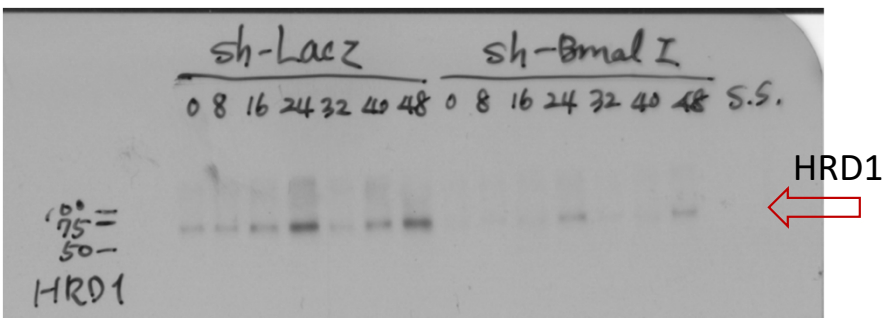


Figure 3B: Western blot for HRD1. The arrow shows the cropped ROI.

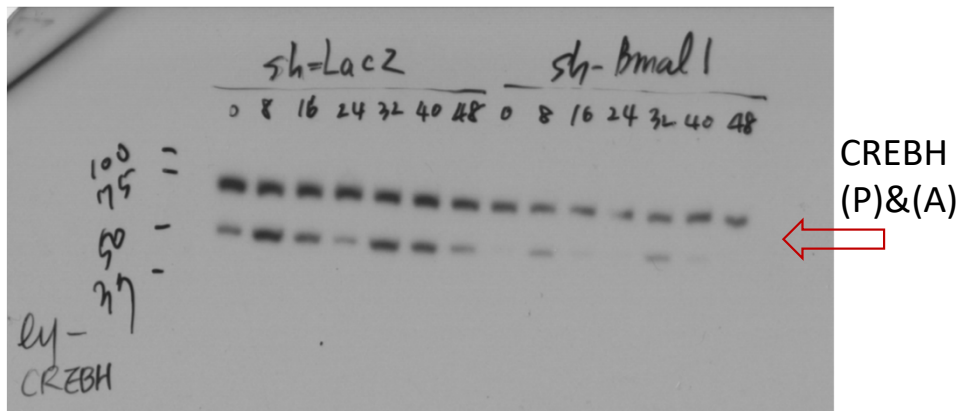


Figure 3B: Western blot for CREBH. The arrow shows the cropped ROI.

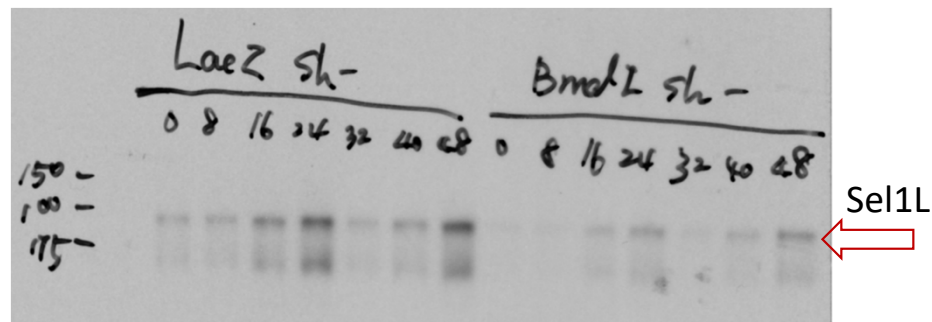


Figure 3B: Western blot for Sel1L. The arrow shows the cropped ROI.

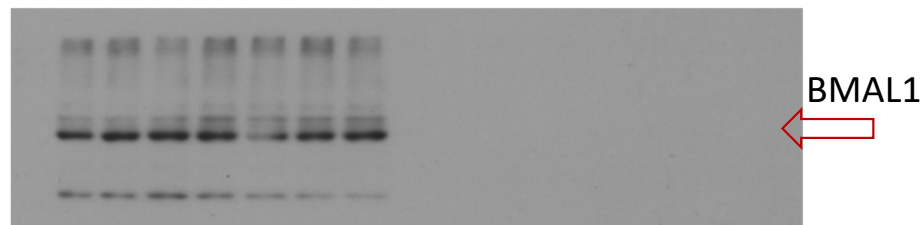


Figure 3B: Western blot for BMAL1. The arrow shows the cropped ROI.

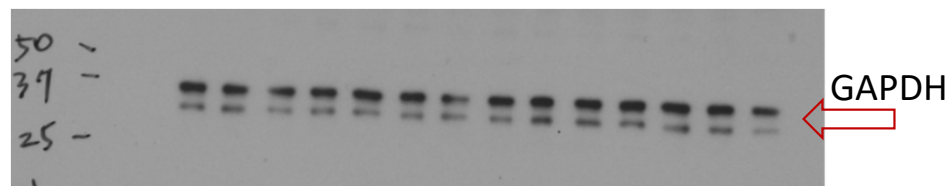


Figure 3B: Western blot for GAPDH. The arrow shows the cropped ROI.

Uncropped immunoblots for **Figure 3C**

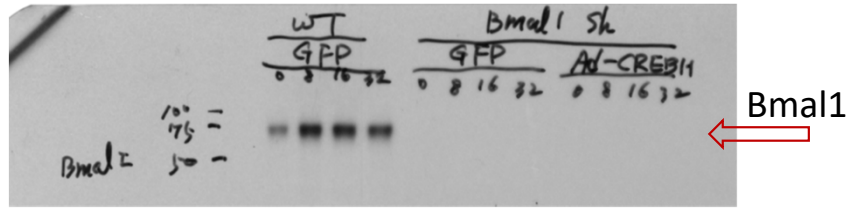


Figure 3C: Western blot image for Bmal1. The arrow shows the cropped ROI.

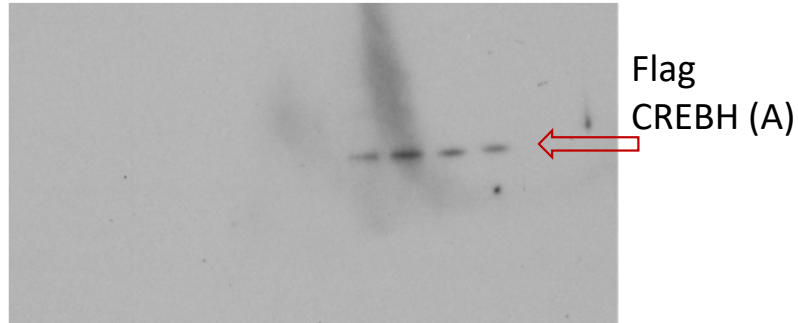


Figure 3C: Western blot image for Flag-CREBH(A). The arrow shows the cropped ROI.

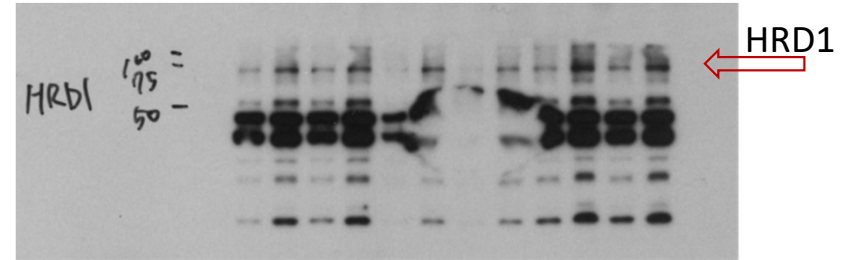


Figure 3C: Western blot image for HRD1. The arrow shows the cropped ROI.

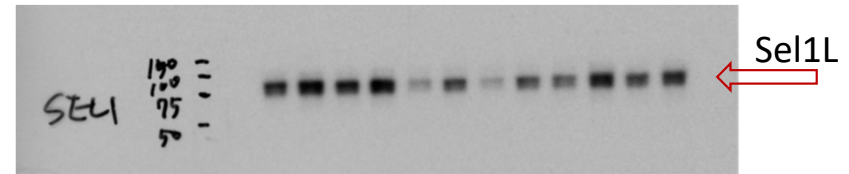


Figure 3C: Western blot image for Sel1L. The arrow shows the cropped ROI.

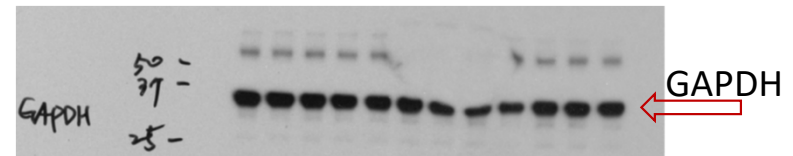


Figure 3C: Western blot image for GAPDH. The arrow shows the cropped ROI.

Uncropped immunoblots for Figure 7B

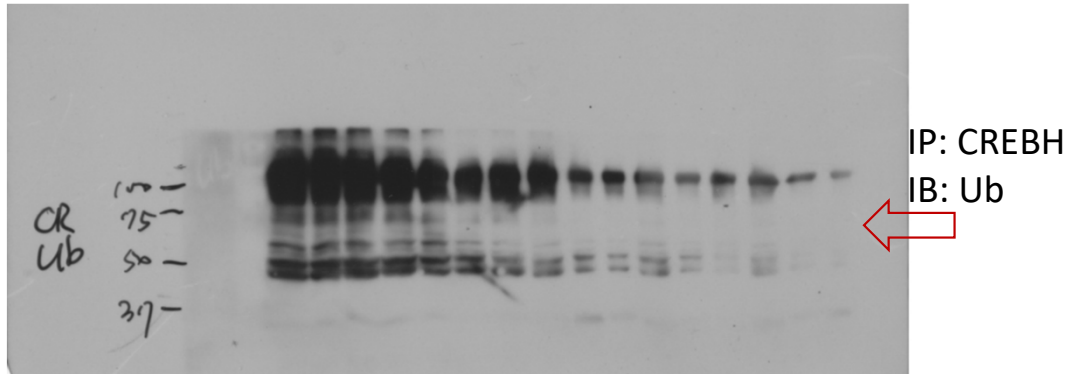


Figure 7B: IP-Western blot: CREBH IP and Ub IB. The arrow shows the cropped ROI.

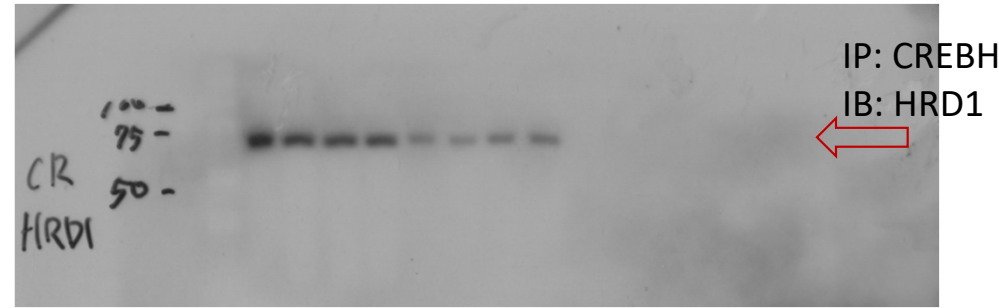


Figure 7B: IP-Western blot: CREBH IP and HRD1 IB. The arrow shows the cropped ROI.

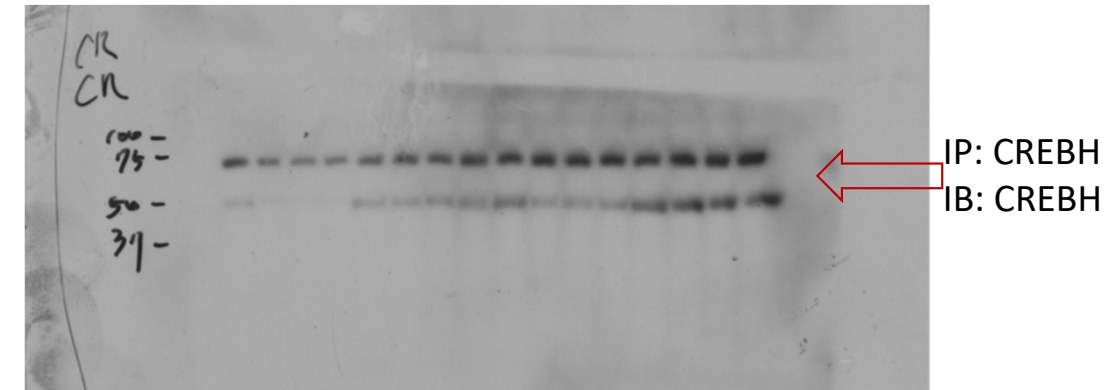


Figure 7B: IP-Western blot: CREBH IP and CREBH IB. The arrow shows the cropped ROI.

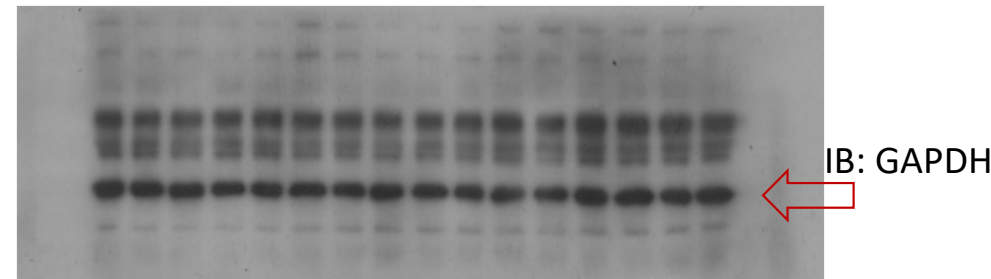


Figure 7B: Western blot (IB): GAPDH. The arrow shows the cropped ROI.

Uncropped immunoblots for **Figure 7C**

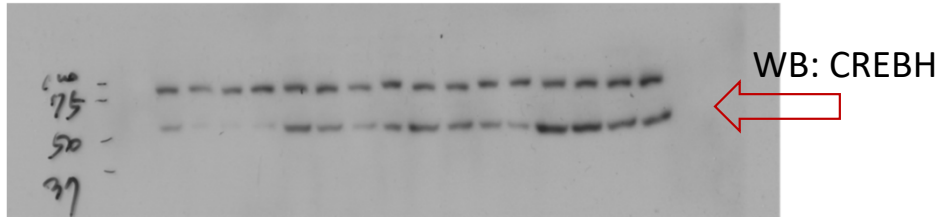


Figure 7C: Western blot for CREBH. The arrow shows the cropped ROI.

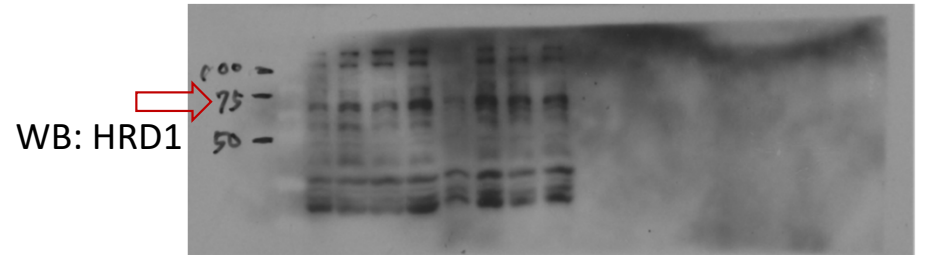


Figure 7C: Western blot for HRD1. The arrow shows the cropped ROI.

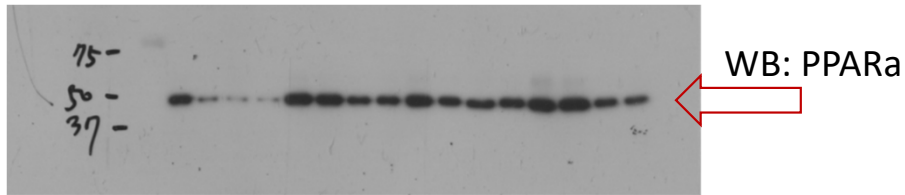


Figure 7C: Western blot for PPARa. The arrow shows the cropped ROI.

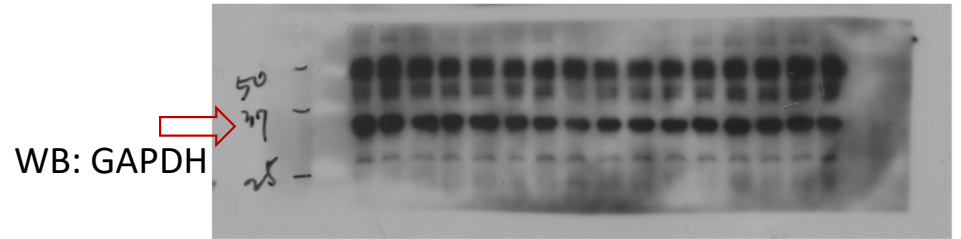


Figure 7C: Western blot for GAPDH. The arrow shows the cropped ROI.

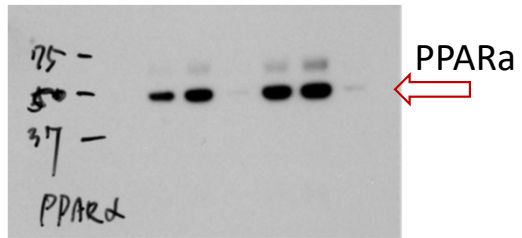


Figure 8A: Western blot image for PPARα. The arrow shows the cropped ROI.

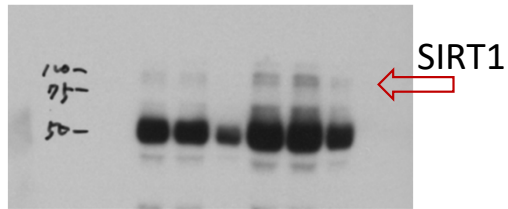


Figure 8A: Western blot image for SIRT1. The arrow shows the cropped ROI.

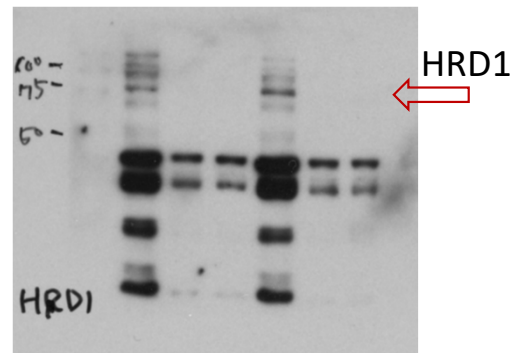


Figure 8A: Western blot image for HRD1. The arrow shows the cropped ROI.

Uncropped immunoblots for **Figure 8A**

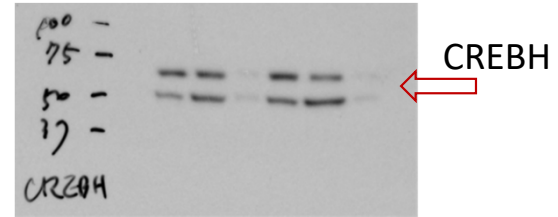


Figure 8A: Western blot image for CREBH. The arrow shows the cropped ROI.

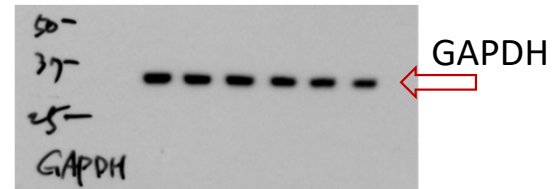


Figure 8A: Western blot image for GAPDH. The arrow shows the cropped ROI.