

SUPPLEMENTARY INFORMATION

HNF1A is a novel BRD4 target and critical for BET-inhibitor response in pancreatic ductal adenocarcinoma

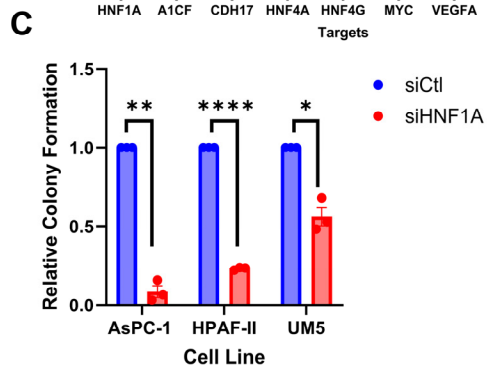
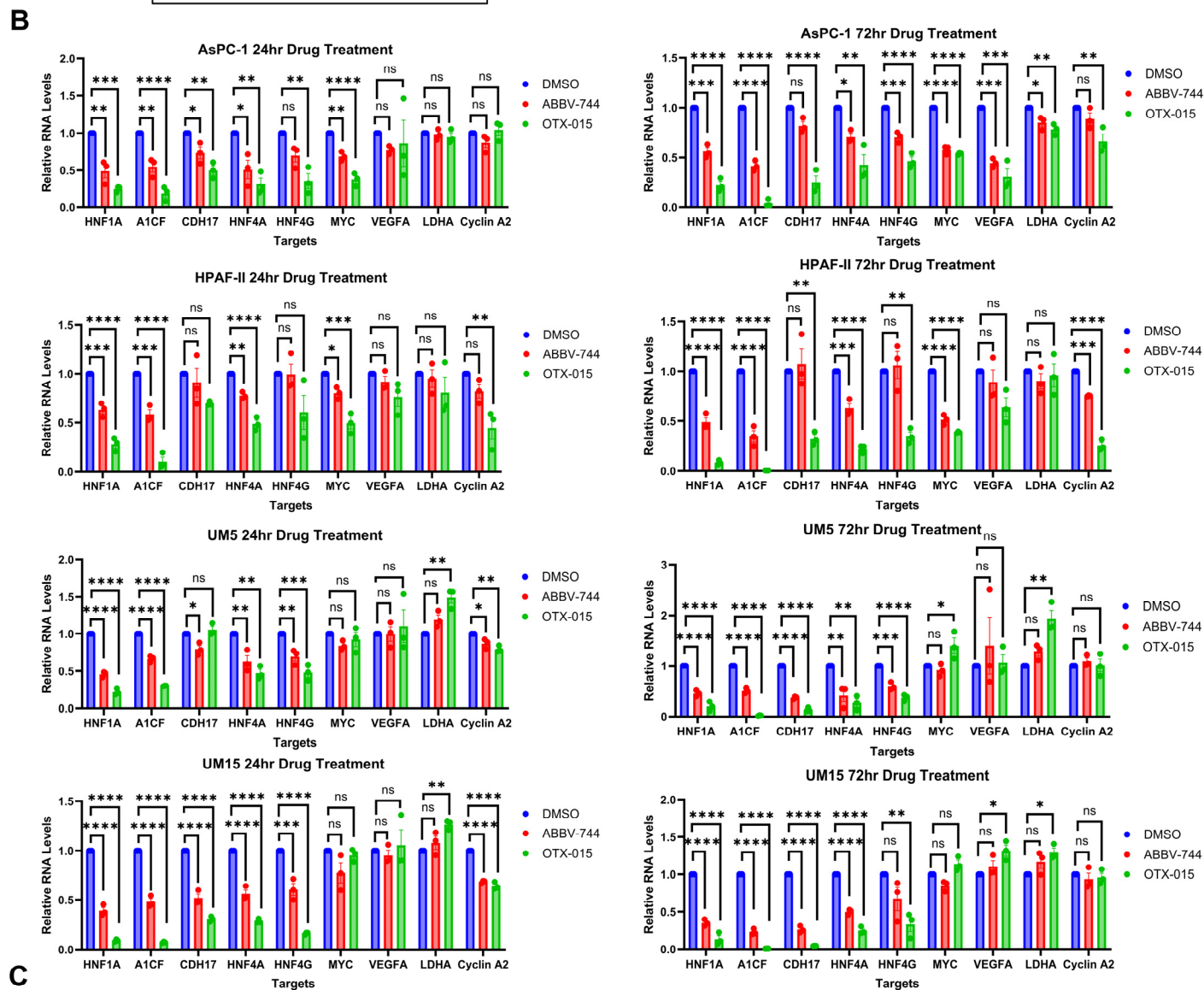
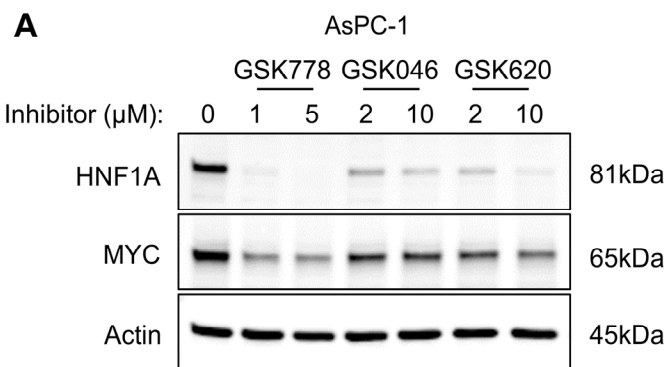
Kennedy S. Humphrey^{1*}, Katherine J. Crawford^{1*}, Bharani Muppavarapu¹, Melanie M. Mayberry¹, William Morris¹, Evan Torres¹, Mark D. Long², Jianxin Wang¹, Erik S. Knudsen¹, Agnieszka K. Witkiewicz¹, Ethan V. Abel^{1#}

Institutional Affiliations:

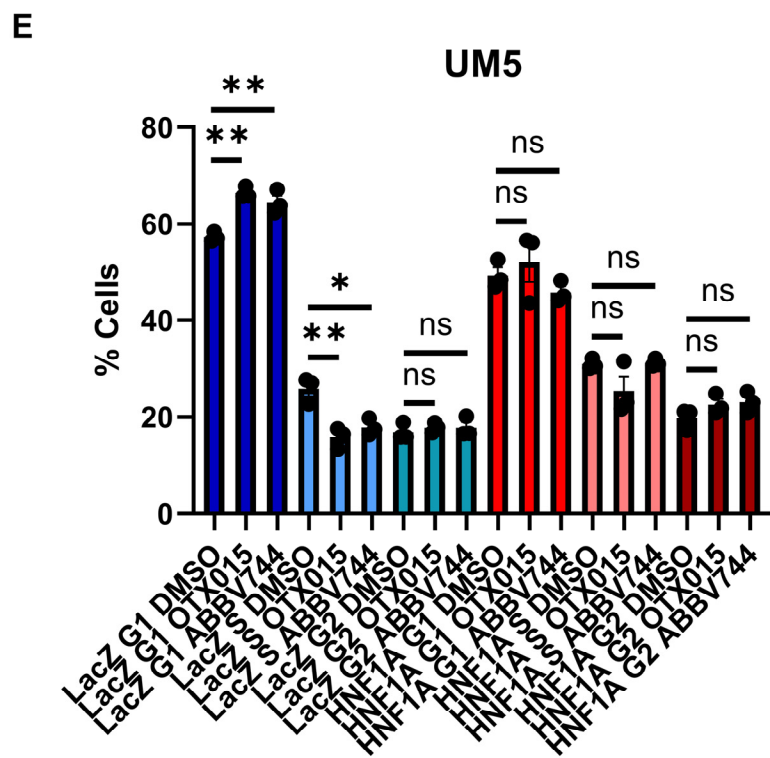
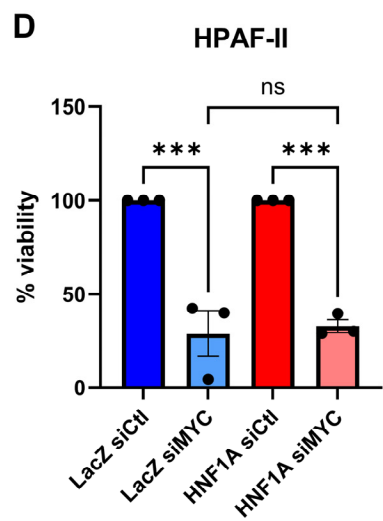
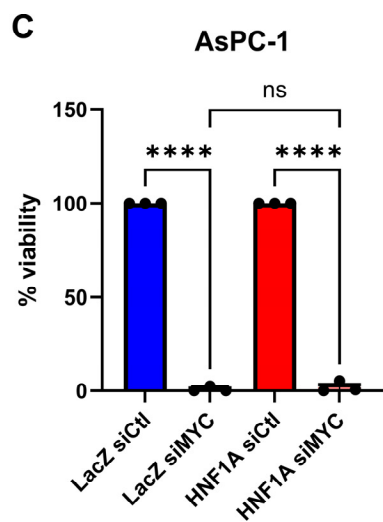
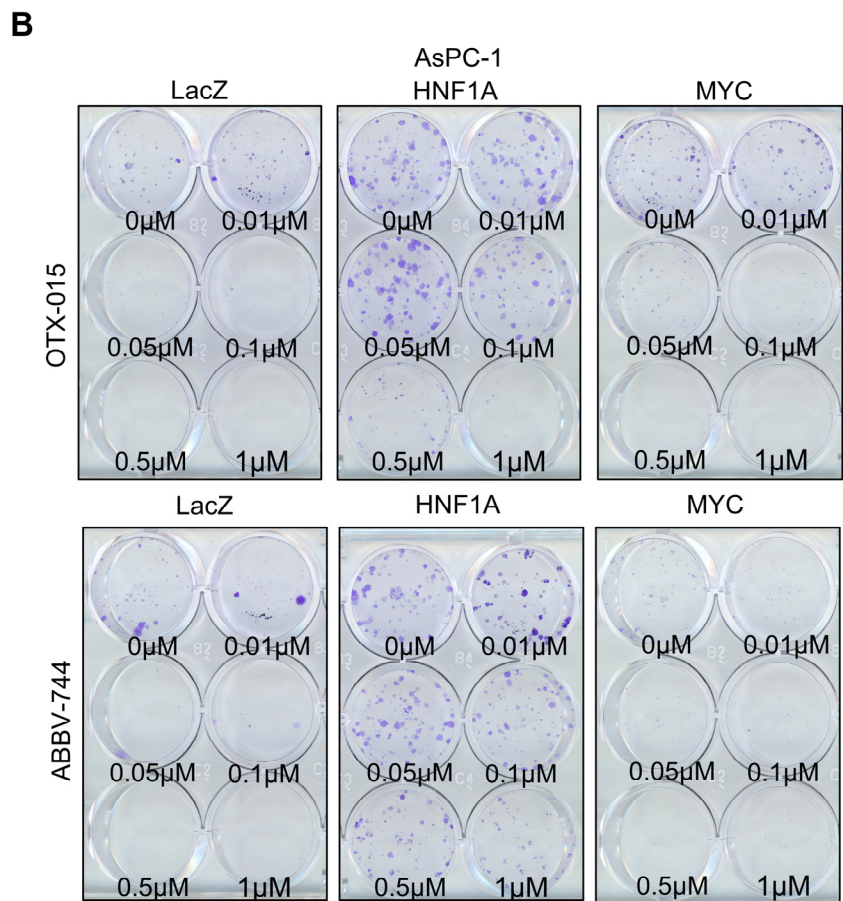
¹Department of Molecular and Cellular Biology, Roswell Park Comprehensive Cancer Center, Buffalo, New York. ²Department of Biostatistics and Bioinformatics, Roswell Park Comprehensive Cancer Center, Buffalo, New York, United States of America.

*Authors contributed equally to this work

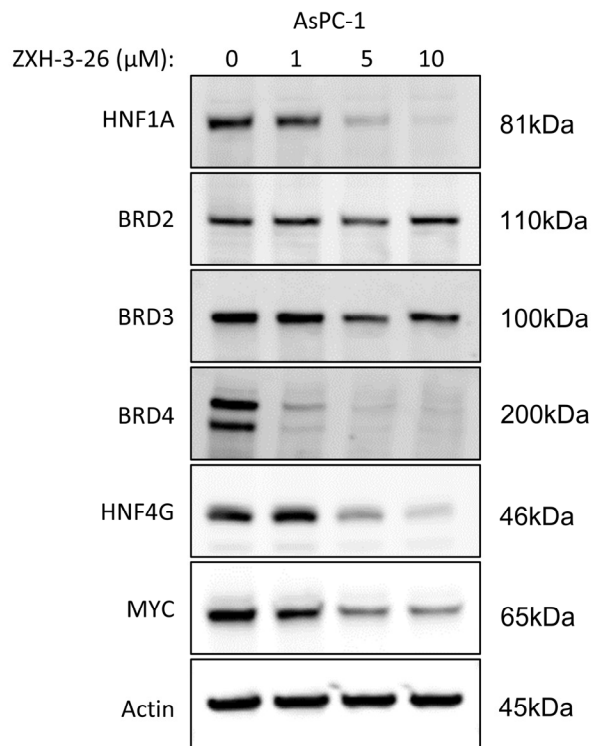
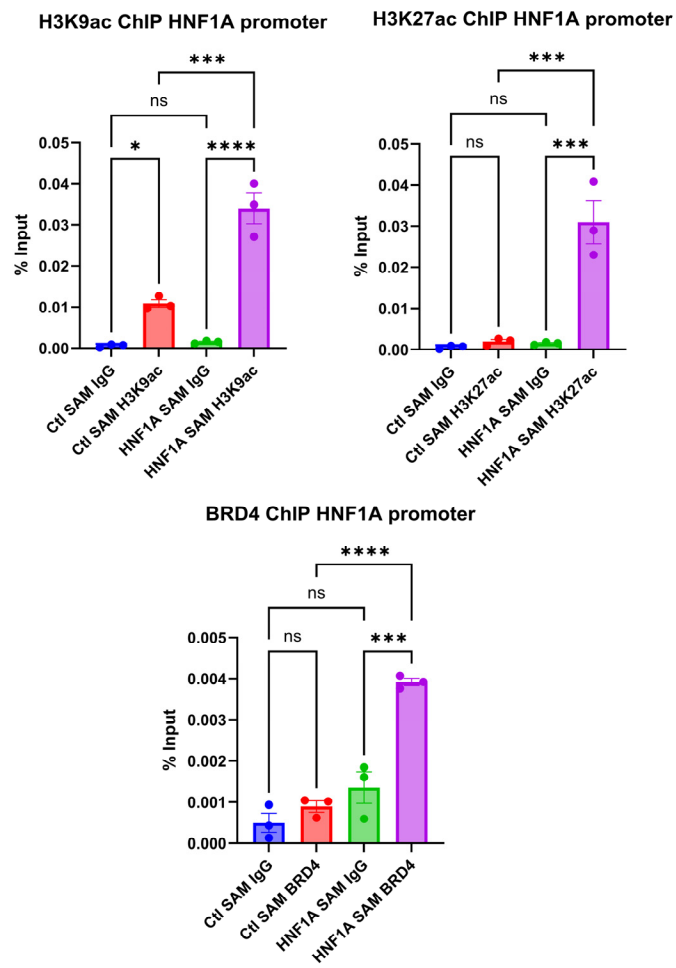
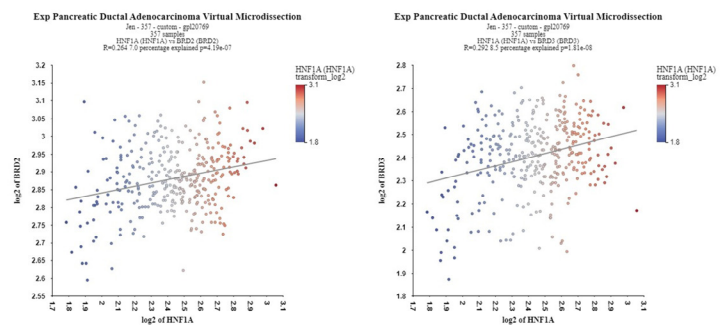
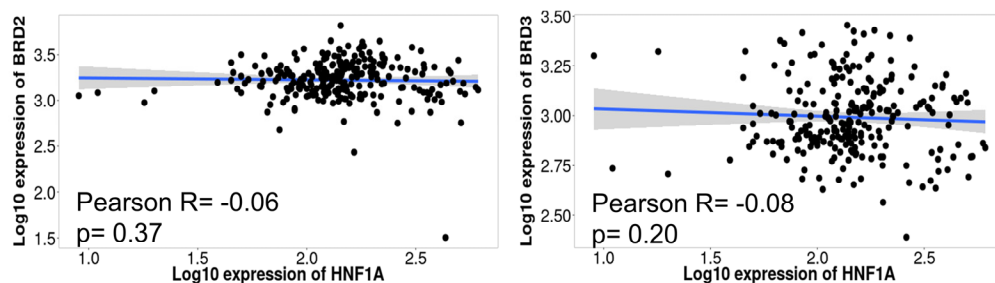
#Corresponding Author: Ethan V. Abel, Roswell Park Comprehensive Cancer Center, Elm and Carlton Streets, Buffalo, NY 14263. E-mail: Ethan.Abel@RoswellPark.org



Supplemental Figure 1. Inhibition of HNF1A expression by BD-selective BET-inhibitors and effects of HNF1A loss on colony formation. **A**, Western blotting of HNF1A, MYC, and Actin for AsPC-1 cells were treated with the indicated concentrations of GSK778 (BD1-selective), GSK046 (BD2-selective), or GSK620 (BD2-selective) for 72 hours. **B**, quantitative RT-PCR analysis of HNF1A, HNF1A-target genes (*A1CF*, *CDH17*, *HNF4A*, *HNF4G*), *MYC*, and MYC-target genes (*VEGFA*, *LDHA*, cyclin A2 (*CCNA2*)) mRNA levels following 24 hours of treatment of AsPC-1, HPAF-II, UM5, and UM15 cells with either DMSO, 0.1 μ M ABBV-744, or 0.5 μ M OTX-015. Bar graphs represent the mean $\pm$ SEM, n=3. Statistical difference was determined by Student t-test with Welch's correction; ns = non-significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **C**, Colony formation of AsPC-1, HPAF-II, and UM5 following knockdown of HNF1A. Bar graphs represent the mean $\pm$ SEM, n=3. Statistical difference was determined by one-way ANOVA with Dunnett's multiple comparisons test (DMSO as control); *p<0.05, **p<0.01, ****p<0.0001.

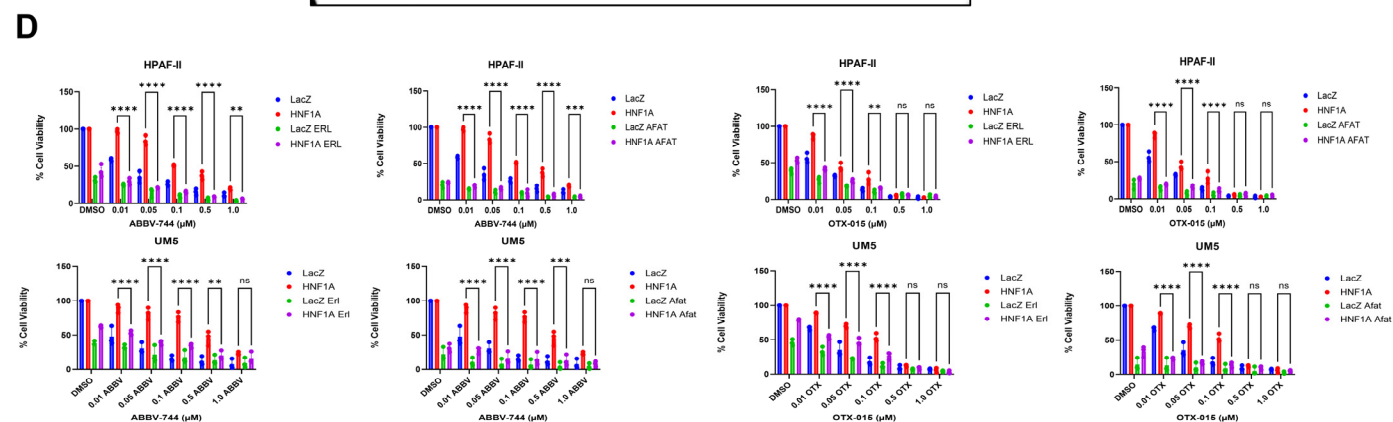
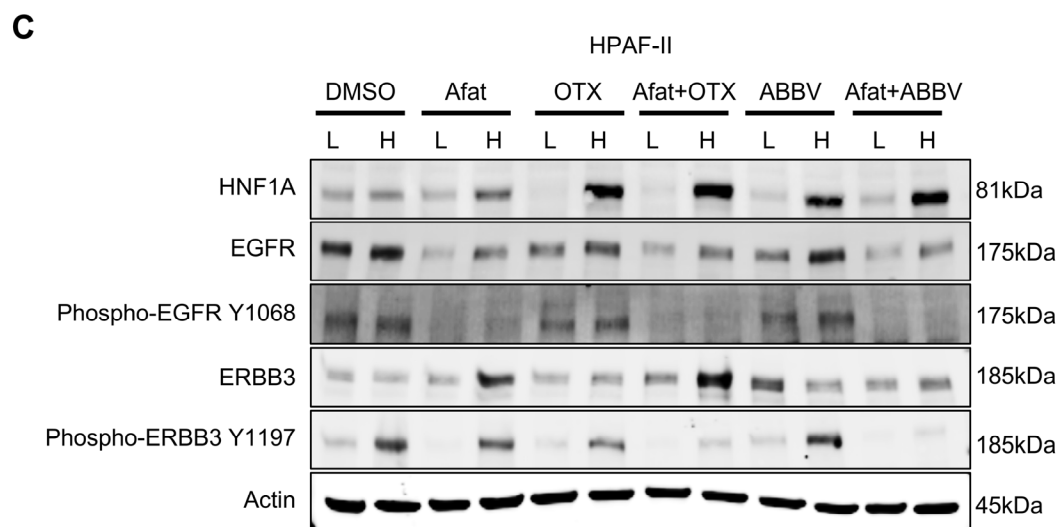
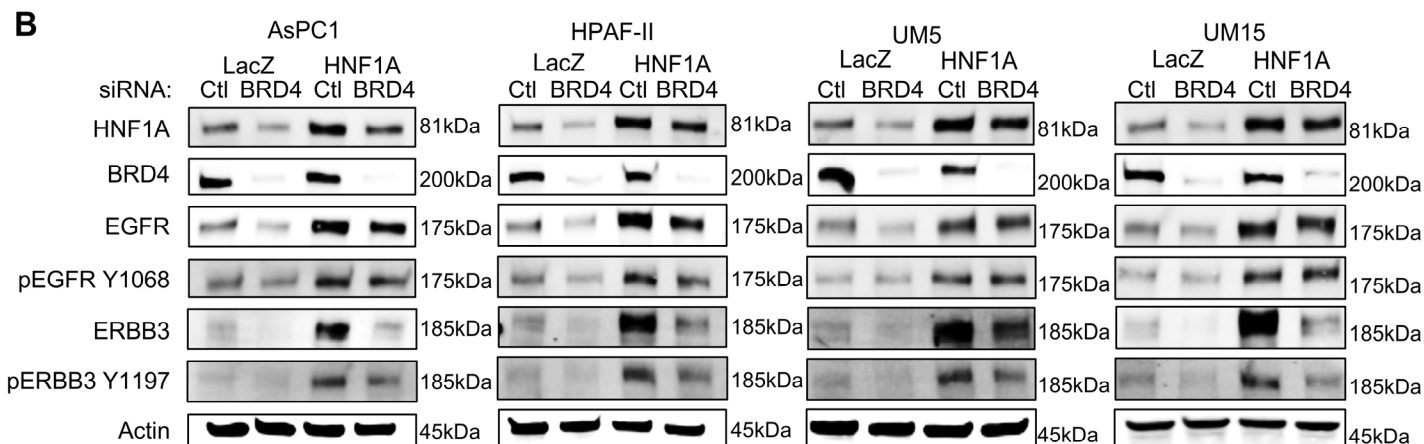
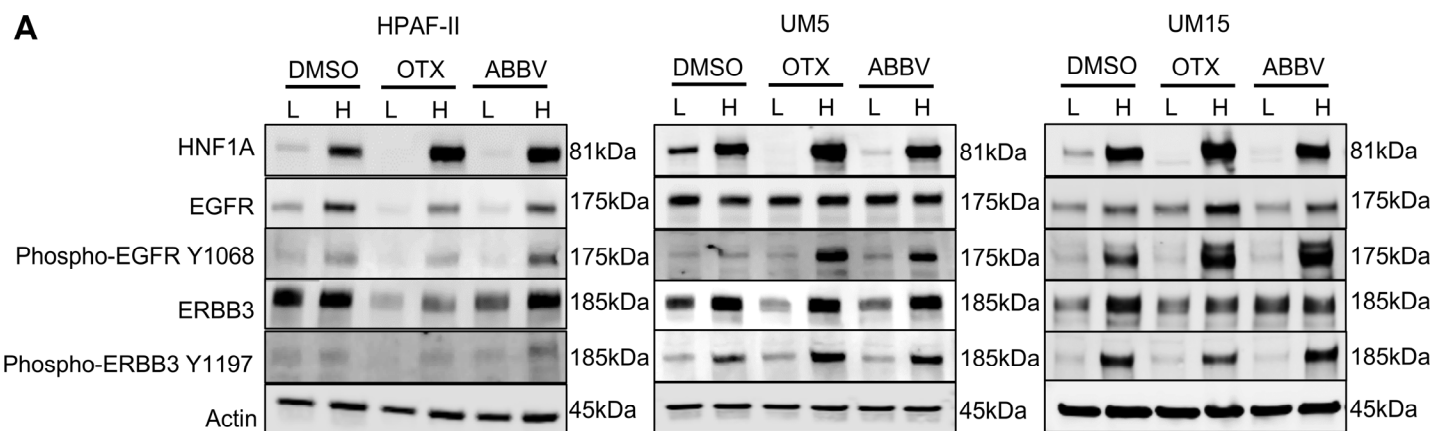


Supplemental Figure 2. HNF1A overexpression protects against BETi. **A**, biological replicate dose curve colony formation experiments (n=3) for LacZ and HNF1A overexpressing HPAF-II cells. Cells were plated at 200 cells per well and treated with the indicated concentrations of OTX-015 or ABBV-744 for 2 weeks. Resultant colonies were quantitated by AlamarBlue viability assay. Statistical difference was determined by two-way ANOVA; ns = non-significant, *p<0.05, ****p<0.0001. **B**, representative LacZ, HNF1A, and MYC overexpressing AsPC-1 colonies after 2 weeks of BETi treatment. The radius of the wells is indicated below. **C, D**, colony viability of AsPC-1 (**C**) and HPAF-II (**D**) LacZ and HNF1A overexpressing cells transfected with MYC siRNA for 2 weeks. **E**, quantitation of propidium iodide staining of LacZ and HNF1A overexpressing UM5 cells treated with 0.5 μ M OTX-015 or 0.1 μ M ABBV-744 for 72 hours. Relative percentages of G0/G1, S, and G2/M phase cells are shown. Statistical difference was determined by one-way ANOVA with Tukey's multiple comparisons test; ns = non-significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

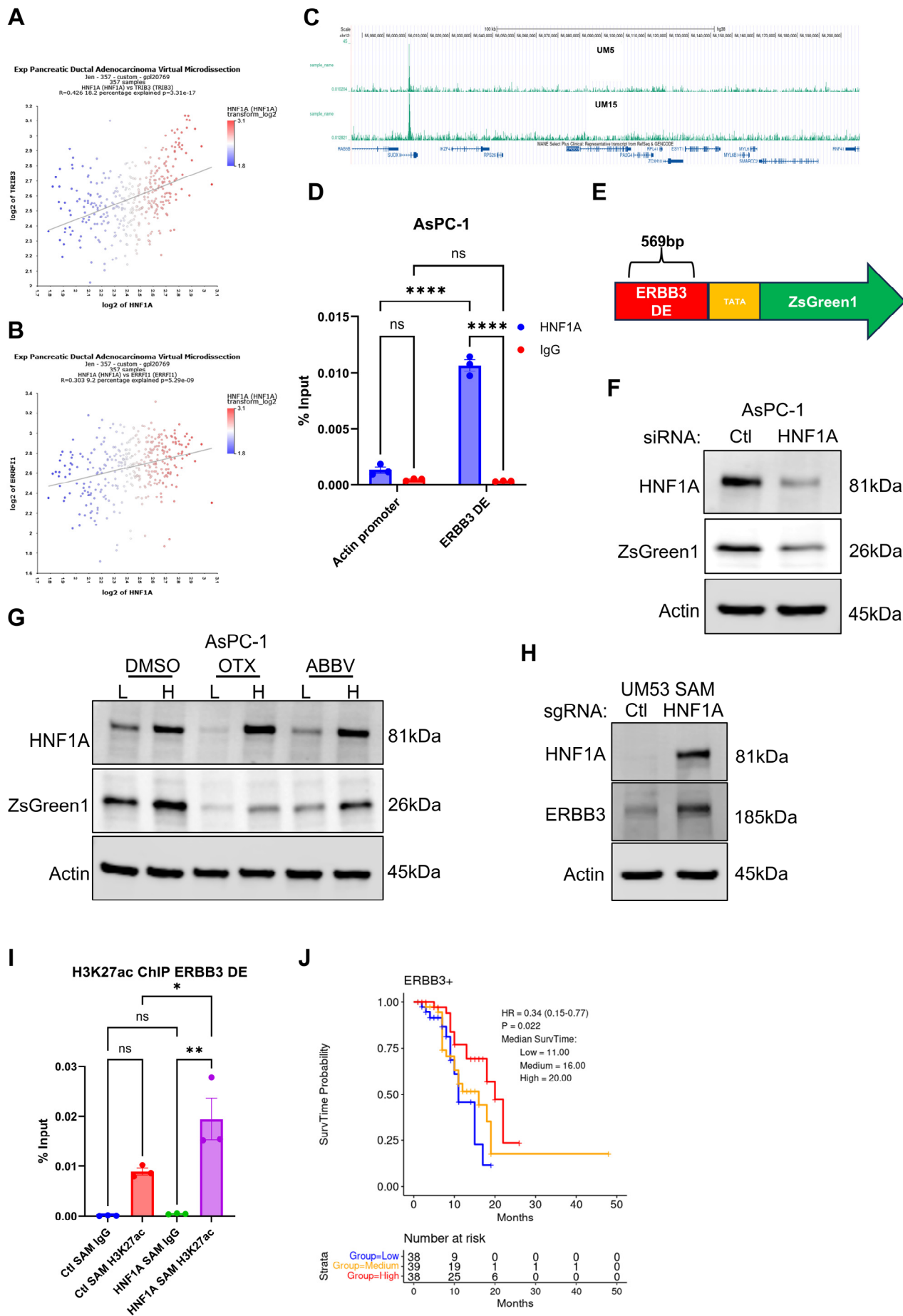
A**B****C****D**

Supplemental Figure 3. Association of HNF1A with BET-family proteins in PDAC. **A**, Western blotting of HNF1A, BRD2, BRD3, BRD4, HNF4G, MYC, and Actin in AsPC-1 cells treated with the indicated concentrations of BRD4-selective degrader ZXH-3-26 for 72 hours. **B**, ChIP-PCR was performed in UM53 SAM cells expressing non-targeting (Ctl) or HNF1A promoter-targeting (HNF1A) sgRNAs using normal IgG, acetyl-histone H3 (K9), acetyl-histone H3 (K27), or BRD4 antibody. Enrichment of the HNF1A promoter (% input) is shown. Bar graphs represent the mean $\pm$ SEM, n=3. Statistical difference was determined by one-way ANOVA with Tukey's multiple comparisons test; ns = non-significant, *p<0.05, ***p<0.001, ****p<0.0001. **C**, correlations of HNF1A mRNA expression and BRD2 and BRD3 mRNA expressions from Moffitt *et al.*, 2015³². **D**, correlations of HNF1A mRNA expression and BRD2 and BRD3 mRNA expressions from PDAC tumors from TNMplot.com⁴⁰.

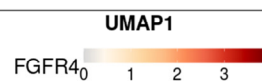
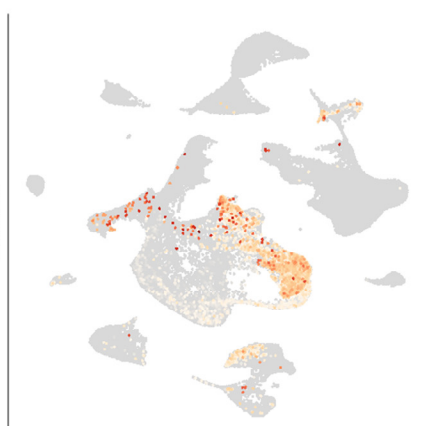
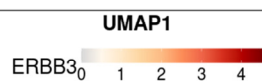
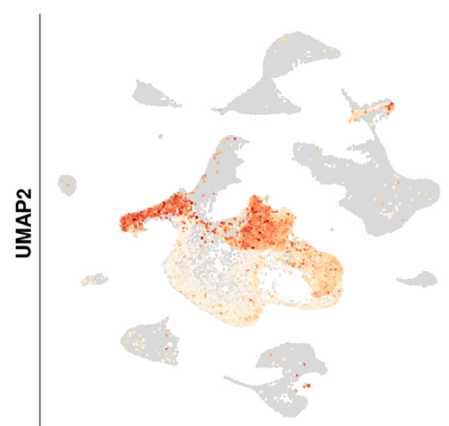
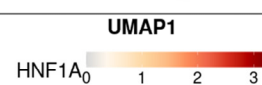
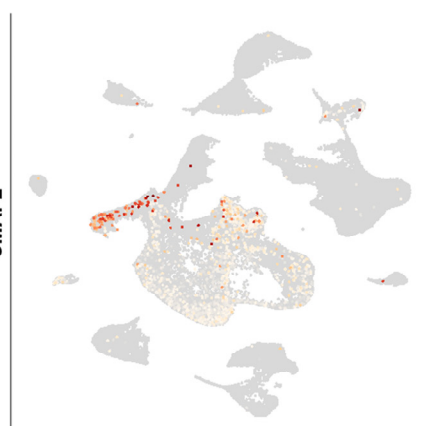
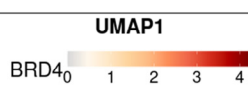
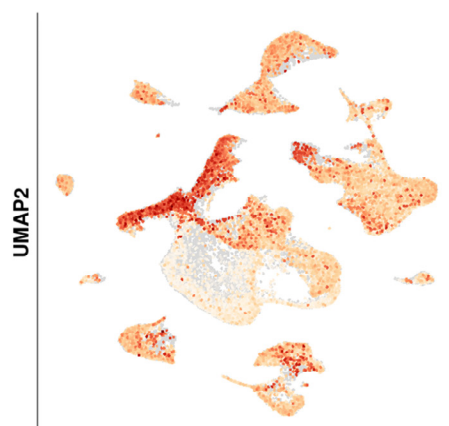
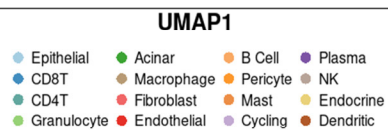
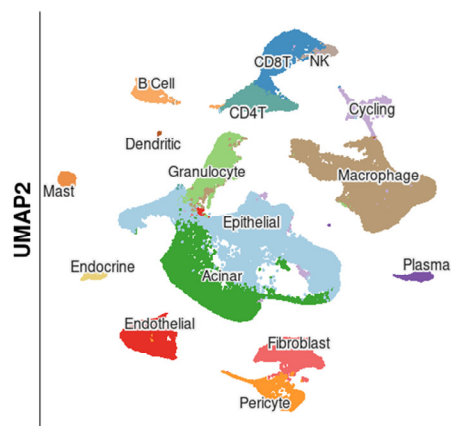
Supplemental Figure 4. Transcriptomic and pathway analysis of BETi-responsive/HNF1A-dependent genes. **A**, **B**, Venn diagrams showing overlap of transcripts downregulated by HNF1A rescue in OTX-015 and ABBV-744 treated cells with transcripts upregulated by knockdown of HNF1A in AsPC-1 cells (**A**) and previously reported HNF1A-repressed genes²³ (**B**). **C**, clustered GSEA heatmap for pathways differentially regulated by OTX-015 or ABBV-744 treatment and HNF1A rescue in AsPC-1 cells.



Supplemental Figure 5. Regulation of EGFR/ERBB3-signaling by HNF1A, BETi, and BRD4. **A**, Western blotting for phospho-EGFR Y1068, EGFR, phospho-ERBB3 Y1197, ERBB3, HNF1A, and Actin from LacZ (L) and HNF1A (H) overexpressing HPAF-II, UM5, and UM15 cells treated with DMSO, 0.5 μ M OTX-015, or 0.1 μ M ABBV-744 for 72 hours. **B**, Western blotting for phospho-EGFR Y1068, EGFR, phospho-ERBB3 Y1197, ERBB3, HNF1A, and Actin from LacZ and HNF1A overexpressing AsPC-1, HPAF-II, UM5, and UM15 cells knocked down for BRD4. **C**, Western blotting for phospho-EGFR Y1068, EGFR, phospho-ERBB3 Y1197, ERBB3, HNF1A, and Actin from LacZ (L) and HNF1A (H) overexpressing HPAF-II cells treated with DMSO, 1 μ M afatinib, 0.5 μ M OTX-015, 0.1 μ M ABBV-744, or combinations of afatinib and either OTX-015 or ABBV-744 for 72 hours. **D**, dose curves colony formation experiments for LacZ, and HNF1A overexpressing HPAF-II and UM5 cells treated with DMSO, 1 μ M erlotinib, 0.5 μ M OTX-015, 0.1 μ M ABBV-744, or combinations of erlotinib and either OTX-015 or ABBV-744 (left panels) or BETi in combination with 1 μ M afatinib (right panel) for two weeks. Bar graphs represent the mean $\pm$ SEM, n=3. Statistical difference was determined by one-way ANOVA with Tukey's multiple comparisons test; ns = non-significant, *p<0.05, ***p<0.001, ****p<0.0001.

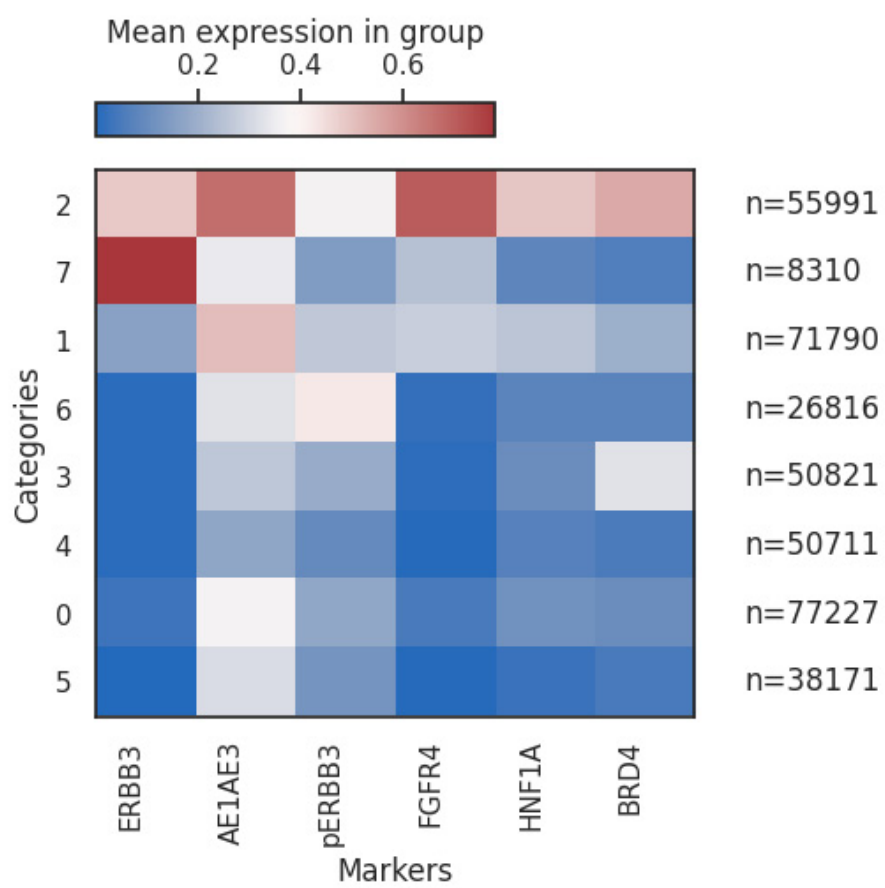


Supplemental Figure 6. Association of HNF1A with EGFR/ERBB3-signaling components. **A, B**, Correlation of *TRIB3* (**A**) and *ERRFI1* (**B**) mRNA expressions with *HNF1A* mRNA expression from Moffitt *et al.*, 2015³². **C**, UCSC Genome Browser HNF1A ChIP-seq tracks from UM5 and UM15 cells showing the ERBB3 locus and adjacent gene bodies. **D**, ChIP-PCR was performed on AsPC-1 cells using normal IgG or HNF1A antibody. Enrichment of the ERBB3 distal enhancer (DE) was compared to the ACTB (Actin) promoter as an active promoter control. Bar graphs represent the mean $\pm$ SEM, n=3. Statistical difference was determined by one-way ANOVA with Tukey's multiple comparisons test; ns = non-significant, ****p<0.0001. **E**, schematic representation of the ERBB3 DE reporter construct. A 569bp region of flanking the HNF1A ChIP-seq peak was cloned upstream of a minimal (TA) promoter-ZsGreen1 cassette. **F**, Western blot for HNF1A, ZsGreen1, and actin following HNF1A knockdown (72 hours) in AsPC-1 cells transduced with the ERBB3 DE reporter virus. **G**, Western blot for HNF1A, ZsGreen1, and actin following BETi treatment (72 hours) in LacZ- or HNF1A-overexpressing AsPC-1 cells transduced with the ERBB3 DE reporter virus. **H**, Western blot for HNF1A, ERBB3, and actin in UM53 SAM cells expressing non-targeting (Ctl) or HNF1A promoter sgRNAs. **I**, ChIP-PCR was performed on UM53 SAM Ctl or HNF1A sgRNA cells using normal IgG or acetyl-histone H3 (Lys27) antibody. Bar graphs represent the mean $\pm$ SEM, n=3. Statistical difference was determined by one-way ANOVA with Tukey's multiple comparisons test; ns = non-significant, *p<0.05, **p<0.01. **J**, Kaplan-Meier plot for PDAC patient survival correlated with ERBB3 staining intensity. Patients were stratified by low, medium, and high expression of ERBB3. N=115 patients.



Supplemental Figure 7. Single cell expressions of BRD4, HNF1A, ERBB3, FGFR4 in PDAC patients. UMAP representation of single cell RNA-sequencing from 30 PDAC patients⁴³. Clusters for PDAC (Epithelial) and other pancreas cell types are indicated, as are the relative expression levels of BRD4, HNF1A, ERBB3, and FGFR4. UMAPs were downloaded directly from

https://pascadimaglianolab.shinyapps.io/SC_Pancreas_Atlas/⁴³.



leiden



leiden

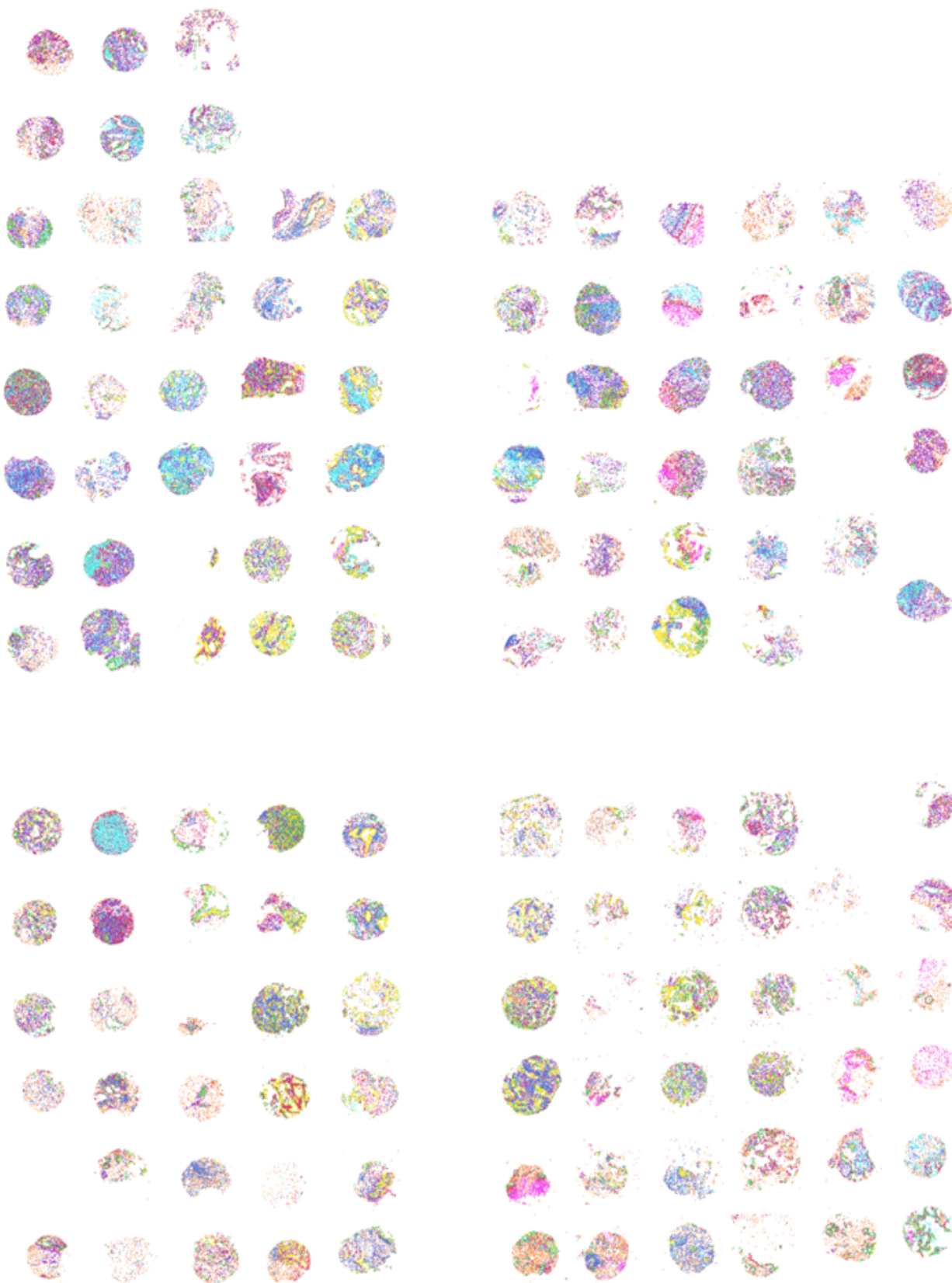


Figure 1

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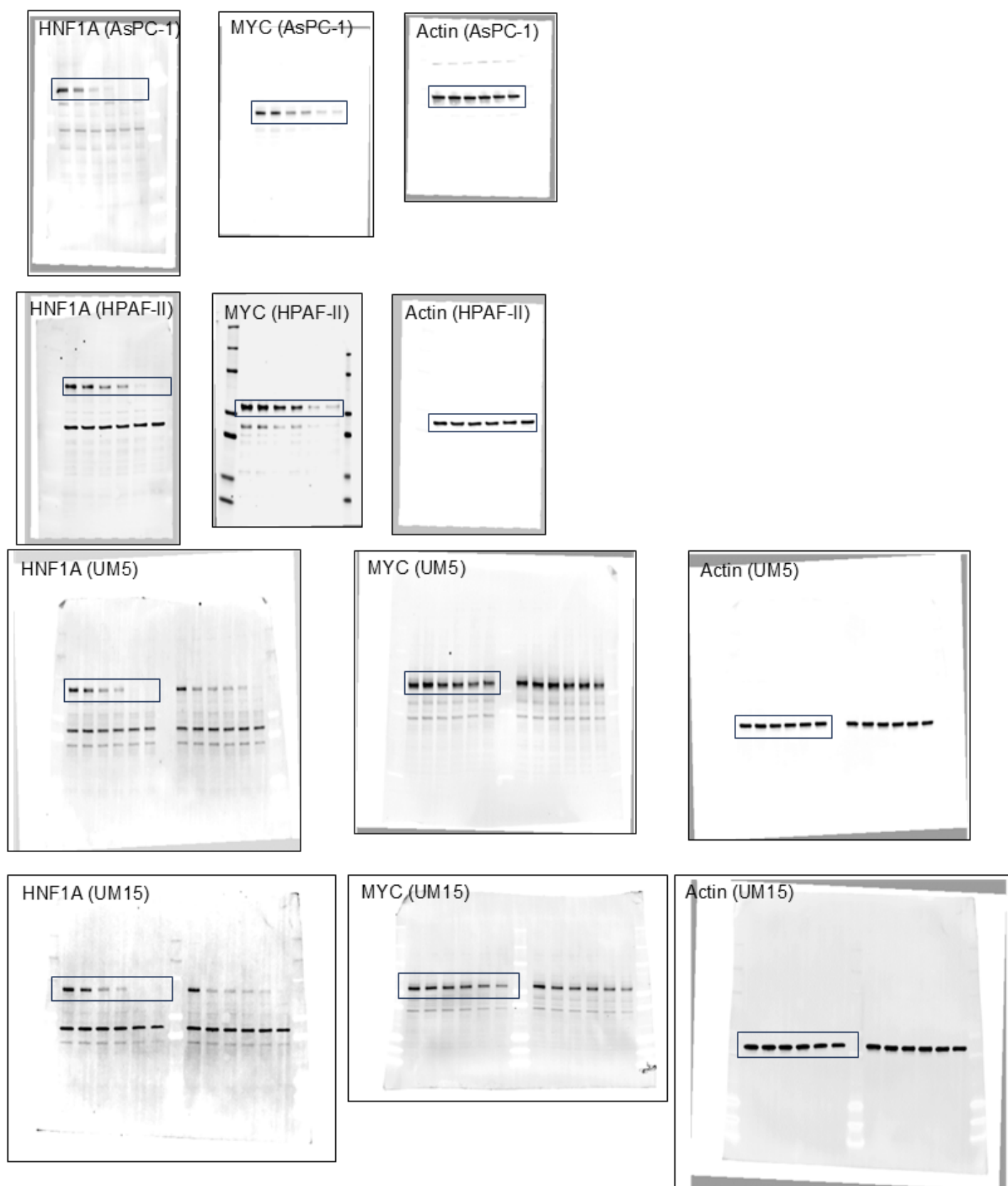
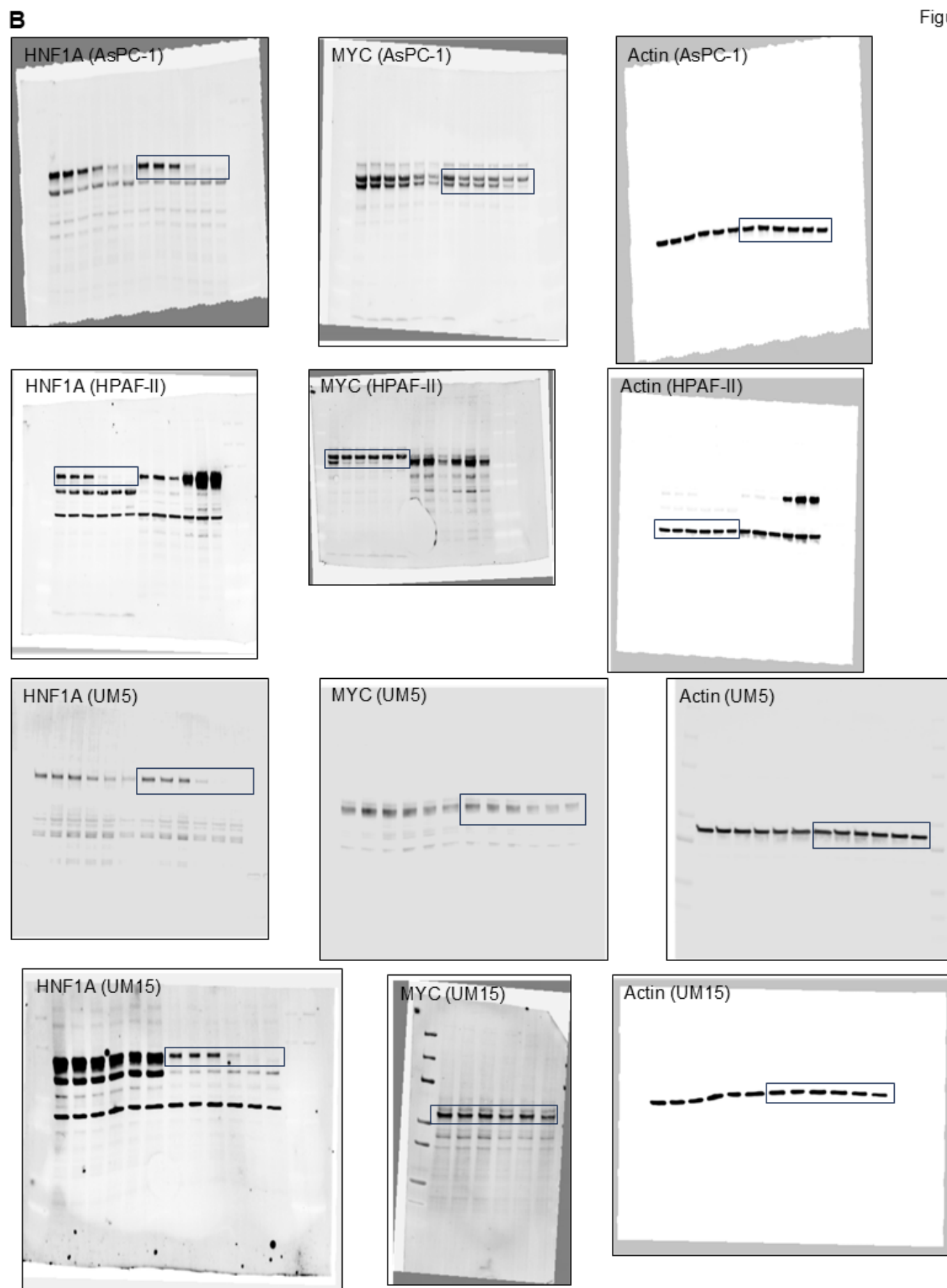
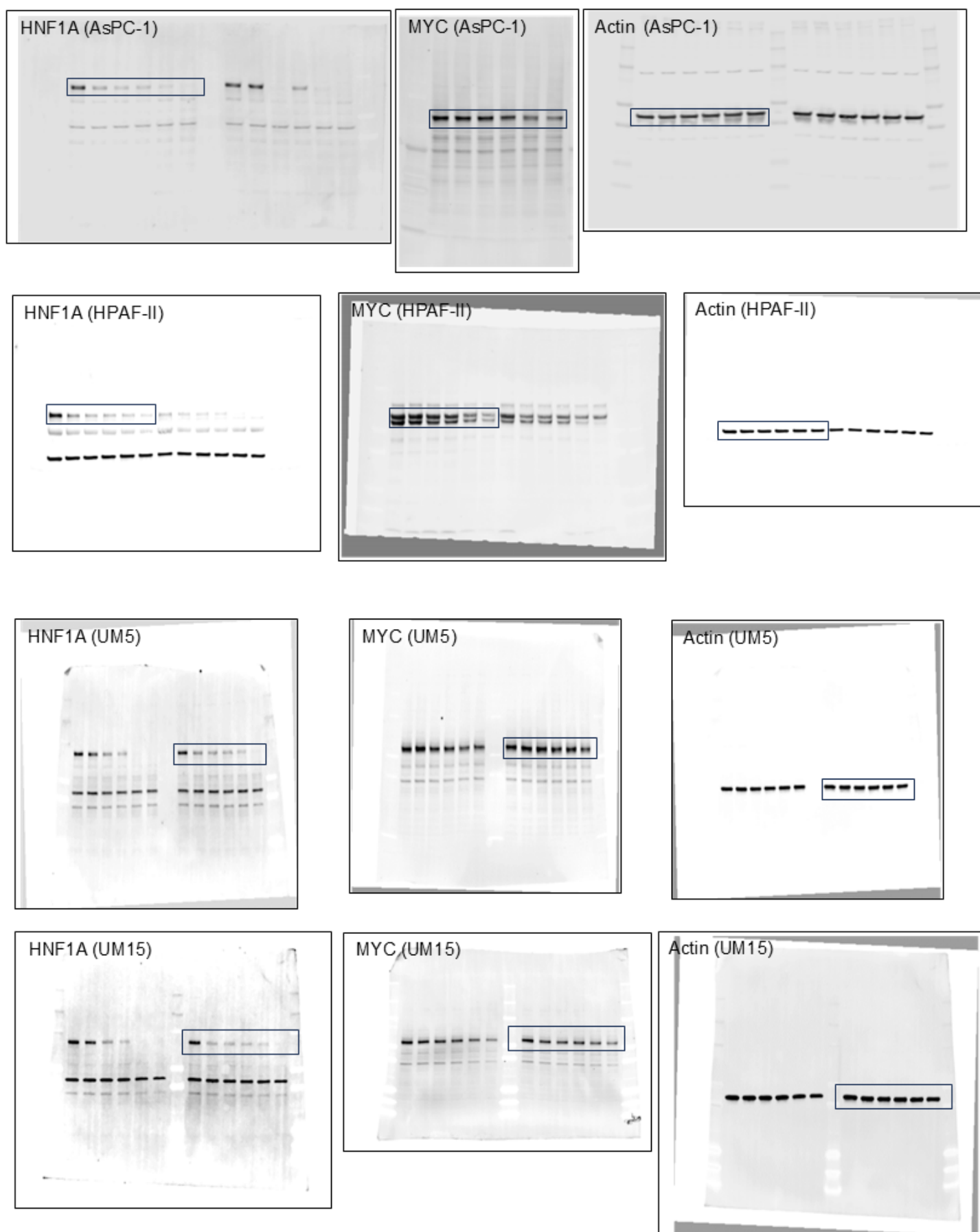


Figure 1



C

Figure 1



D

Figure 1

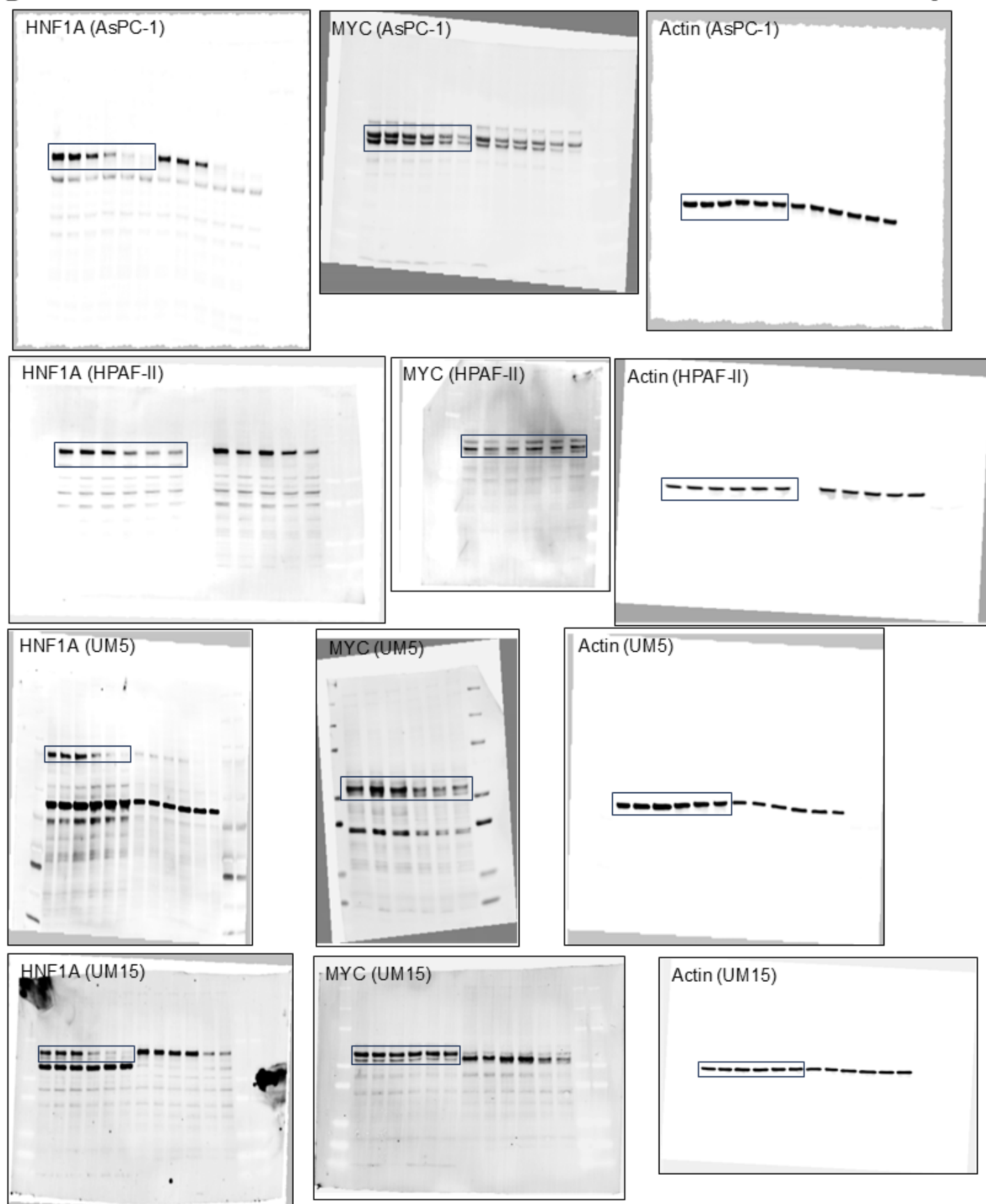


Figure 2

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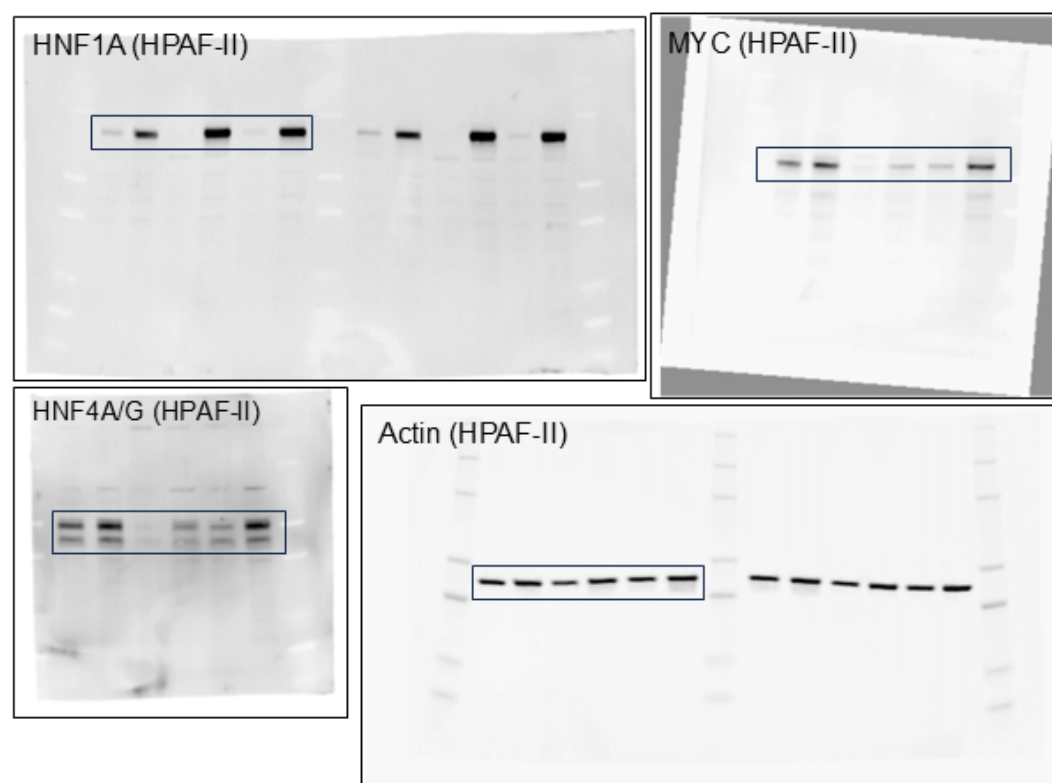
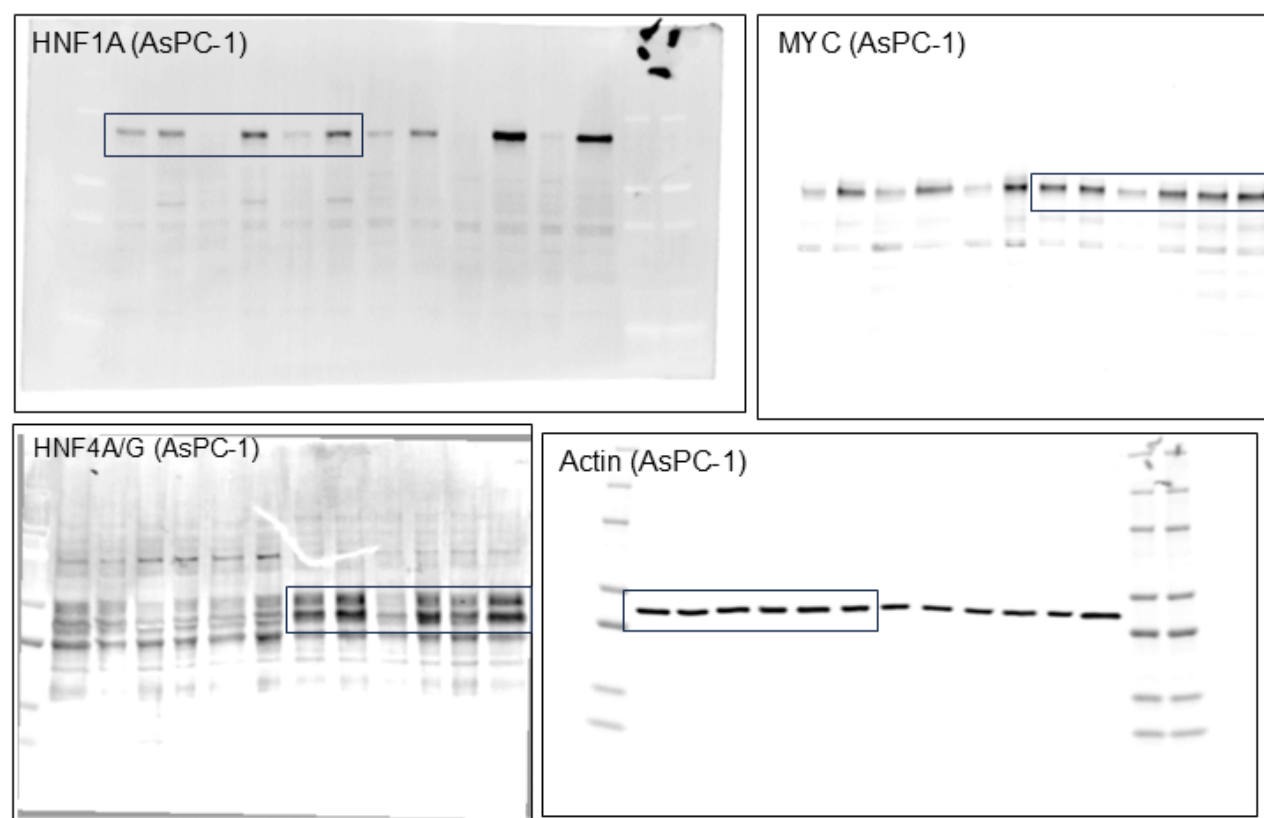


Figure 2

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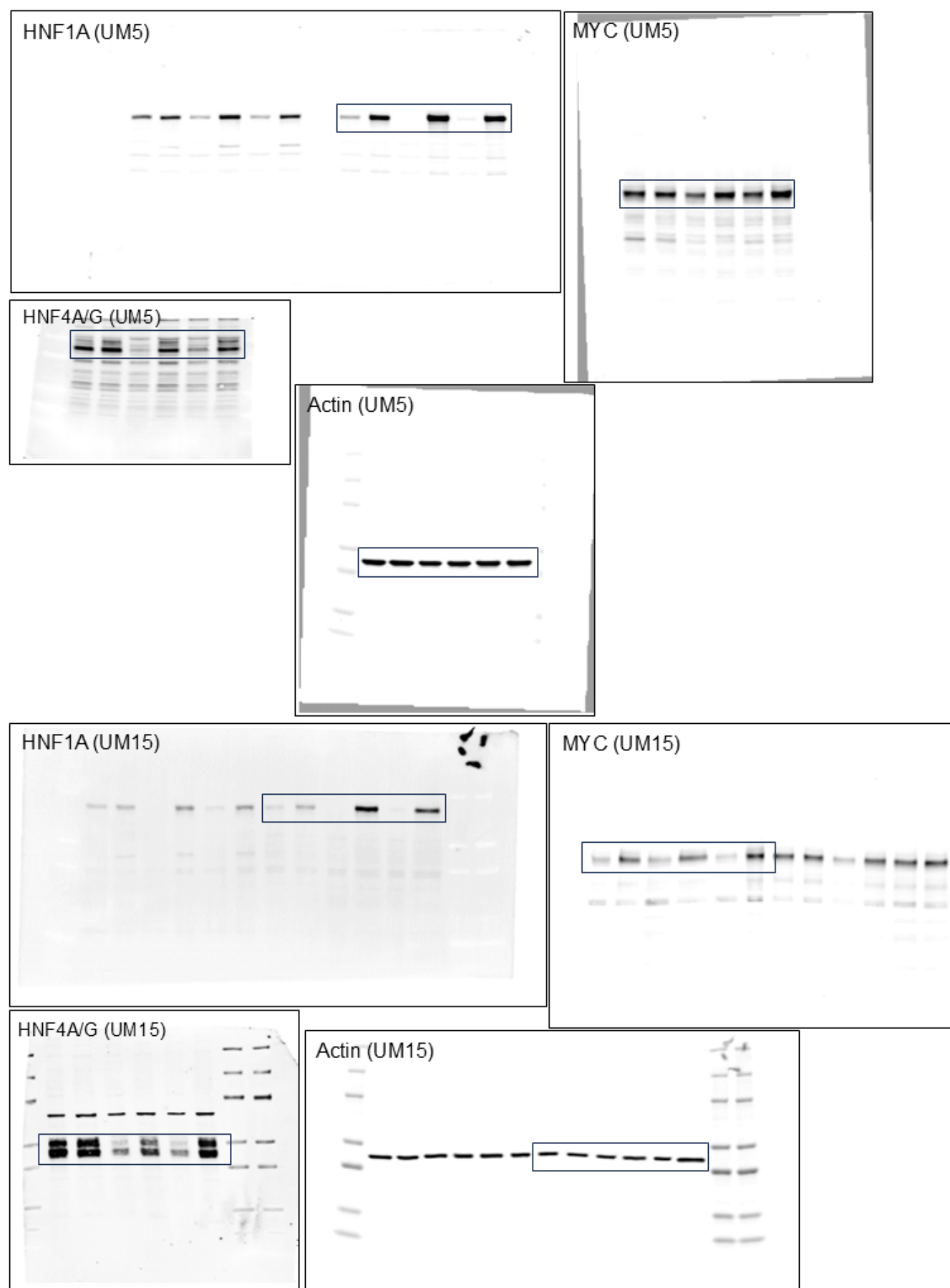
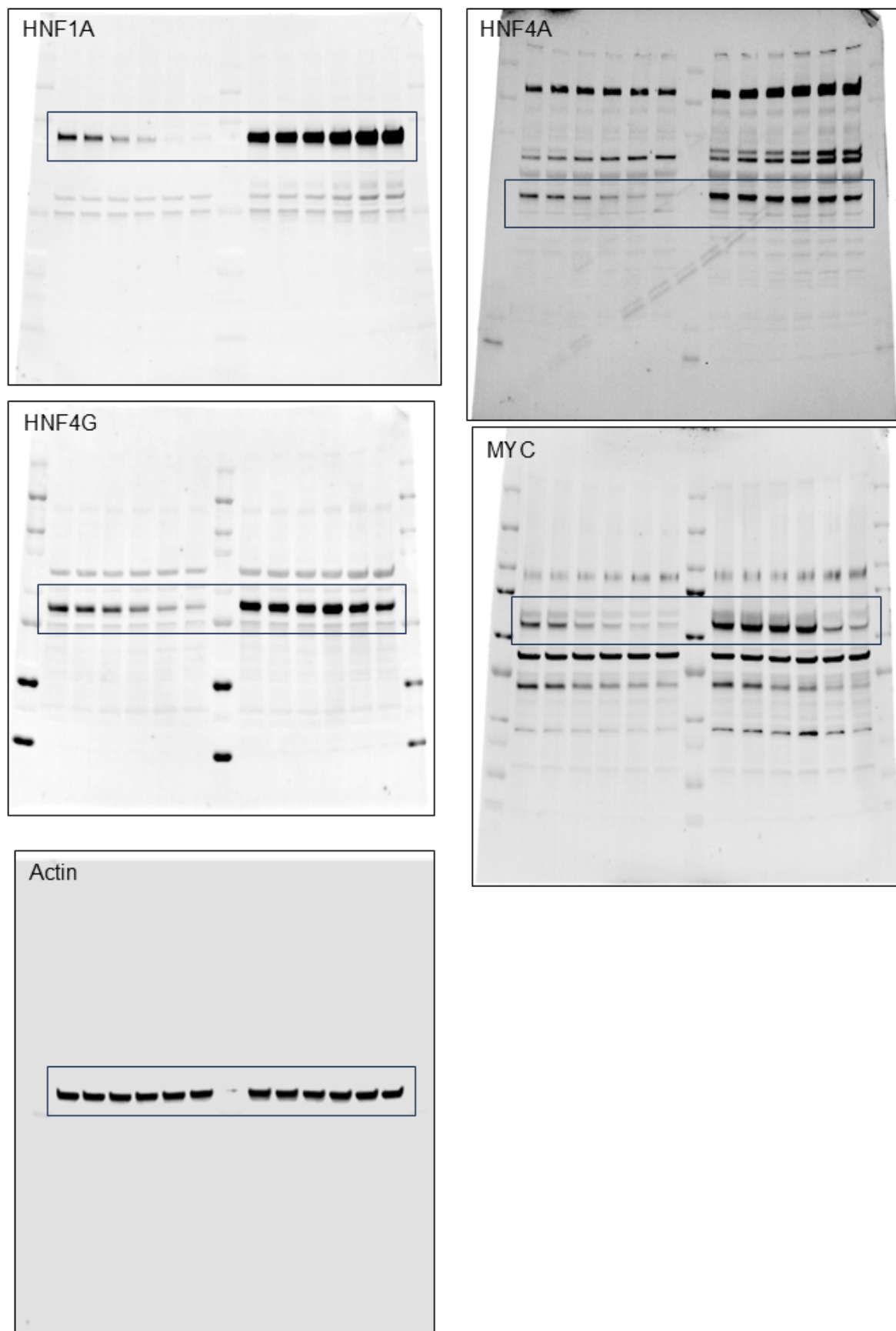


Figure 2

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Figure 2

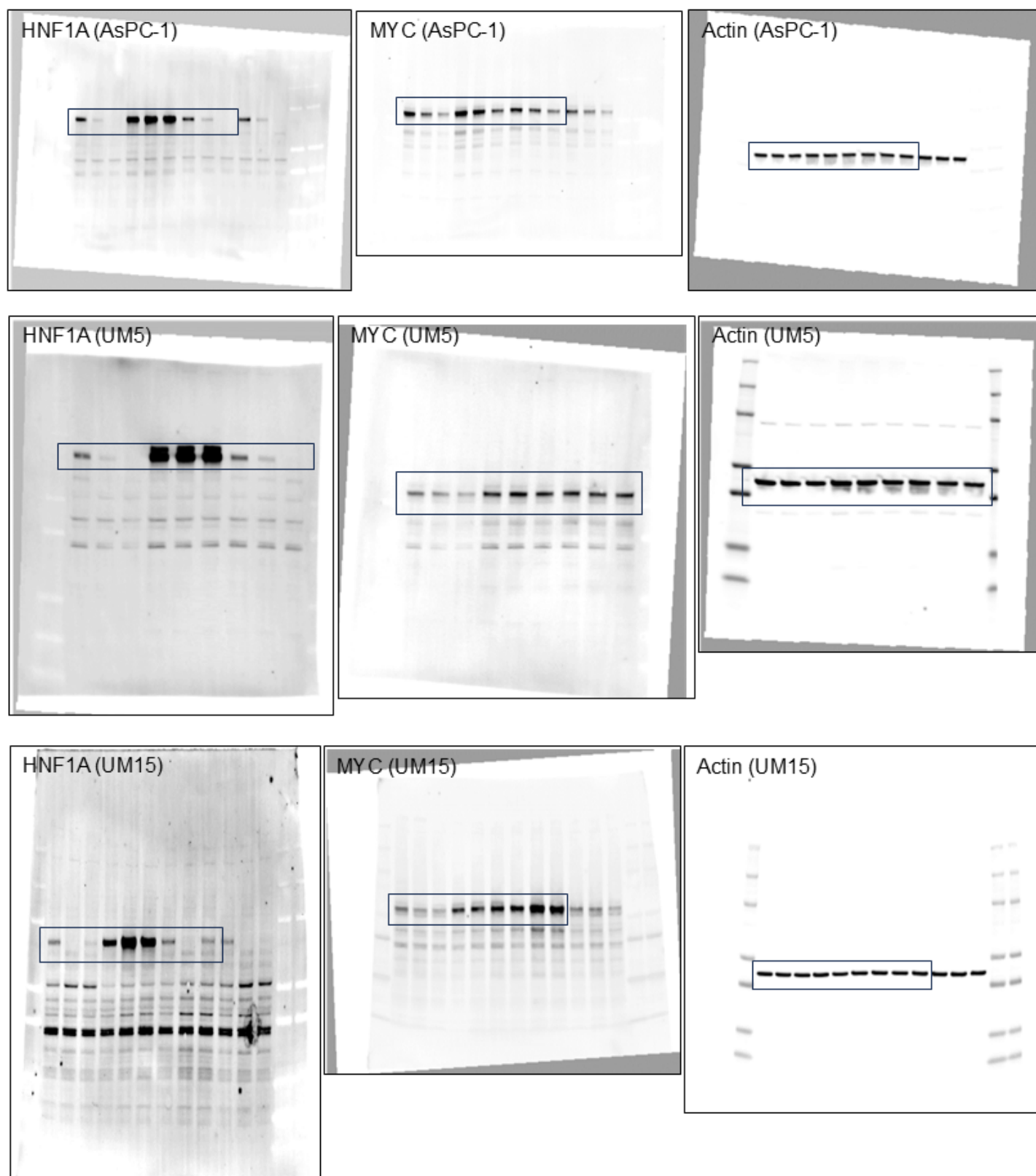


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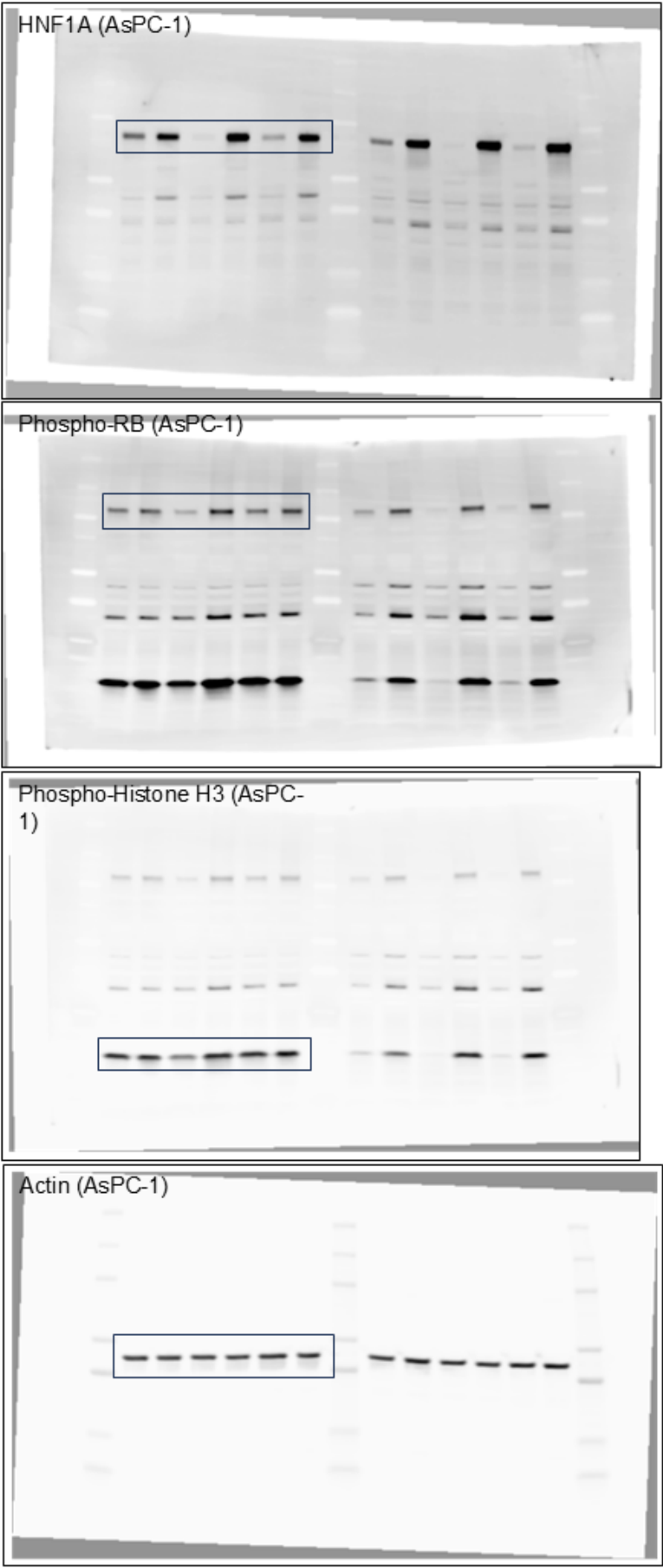


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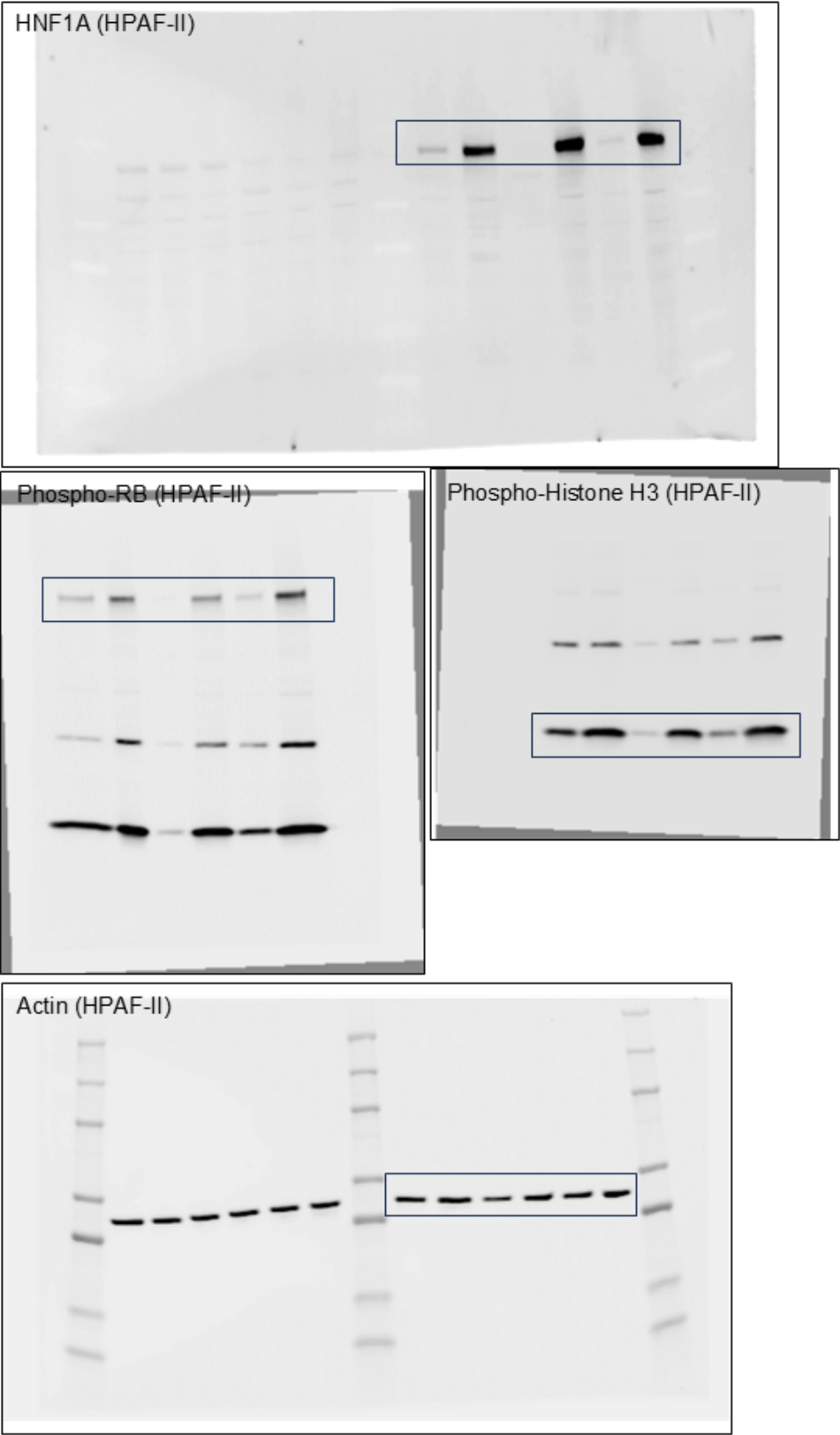
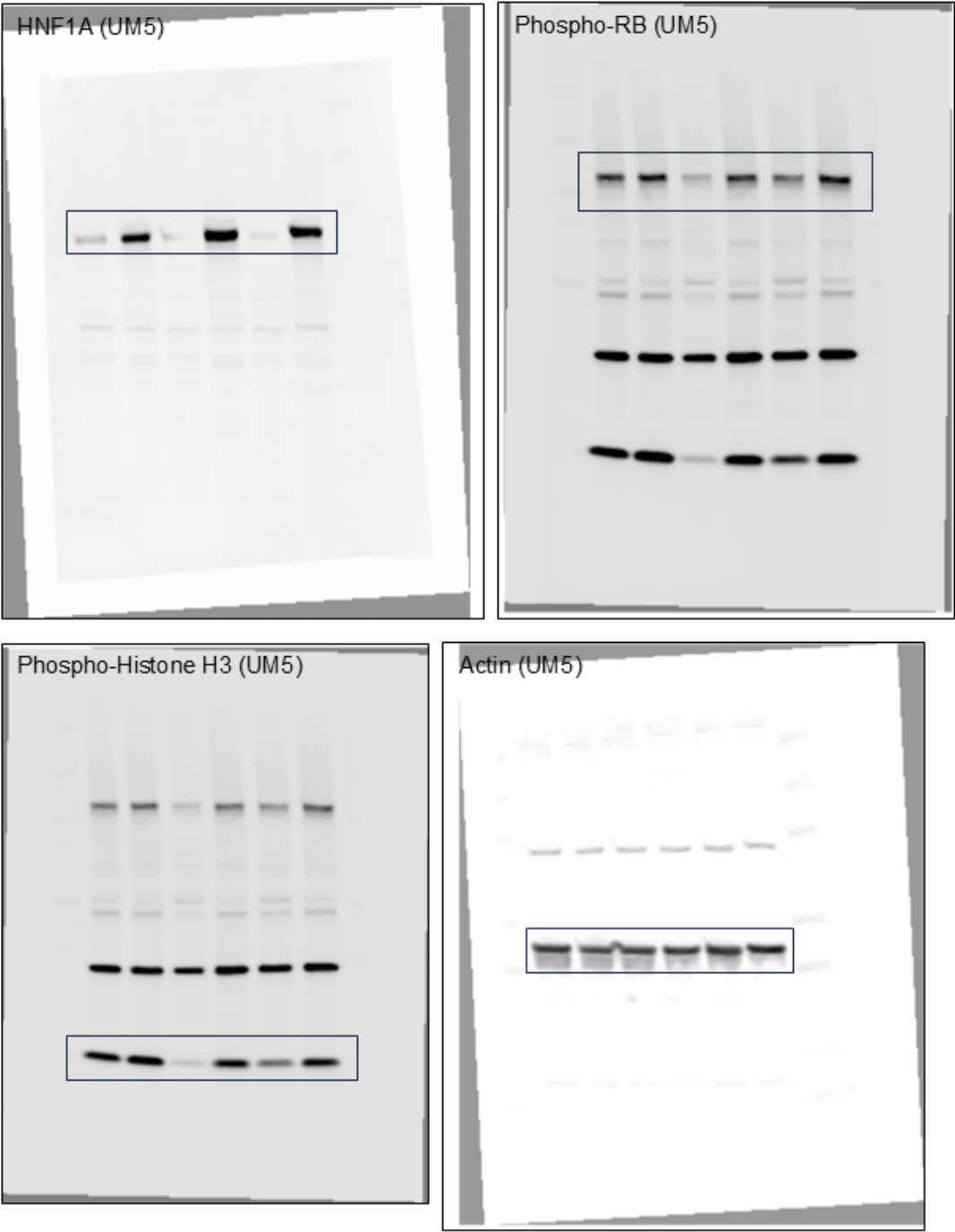


Figure 3

A



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Figure 3

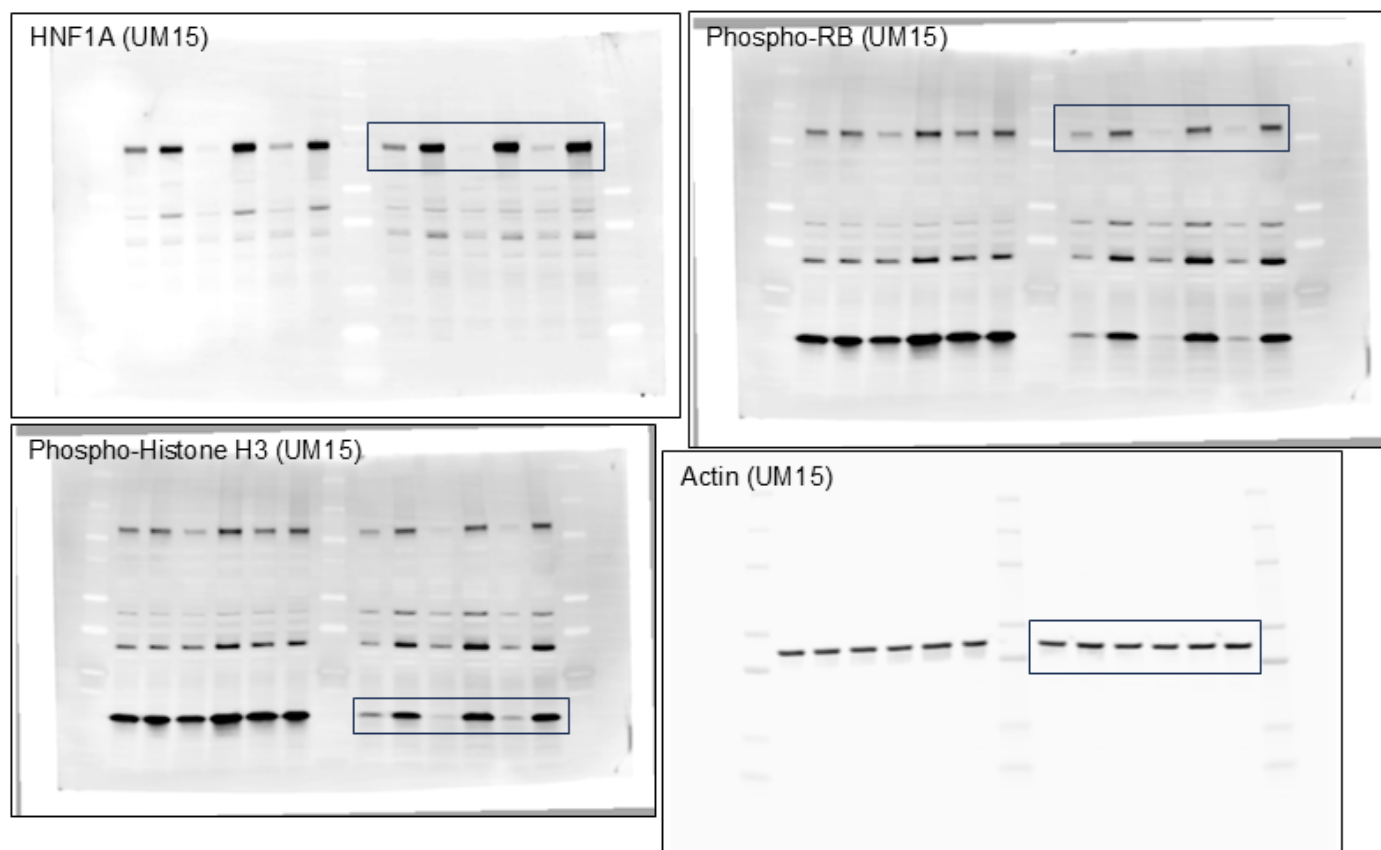
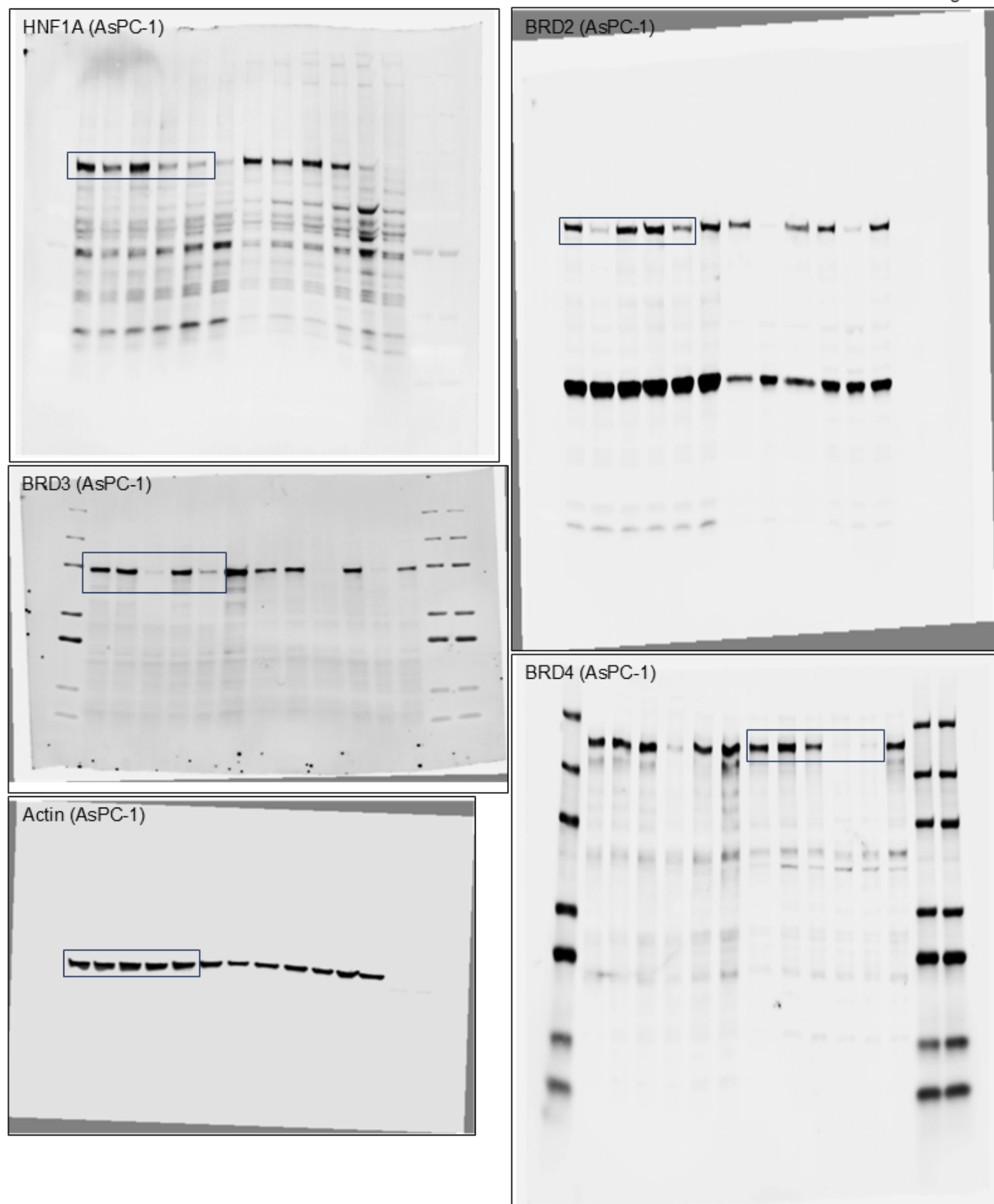


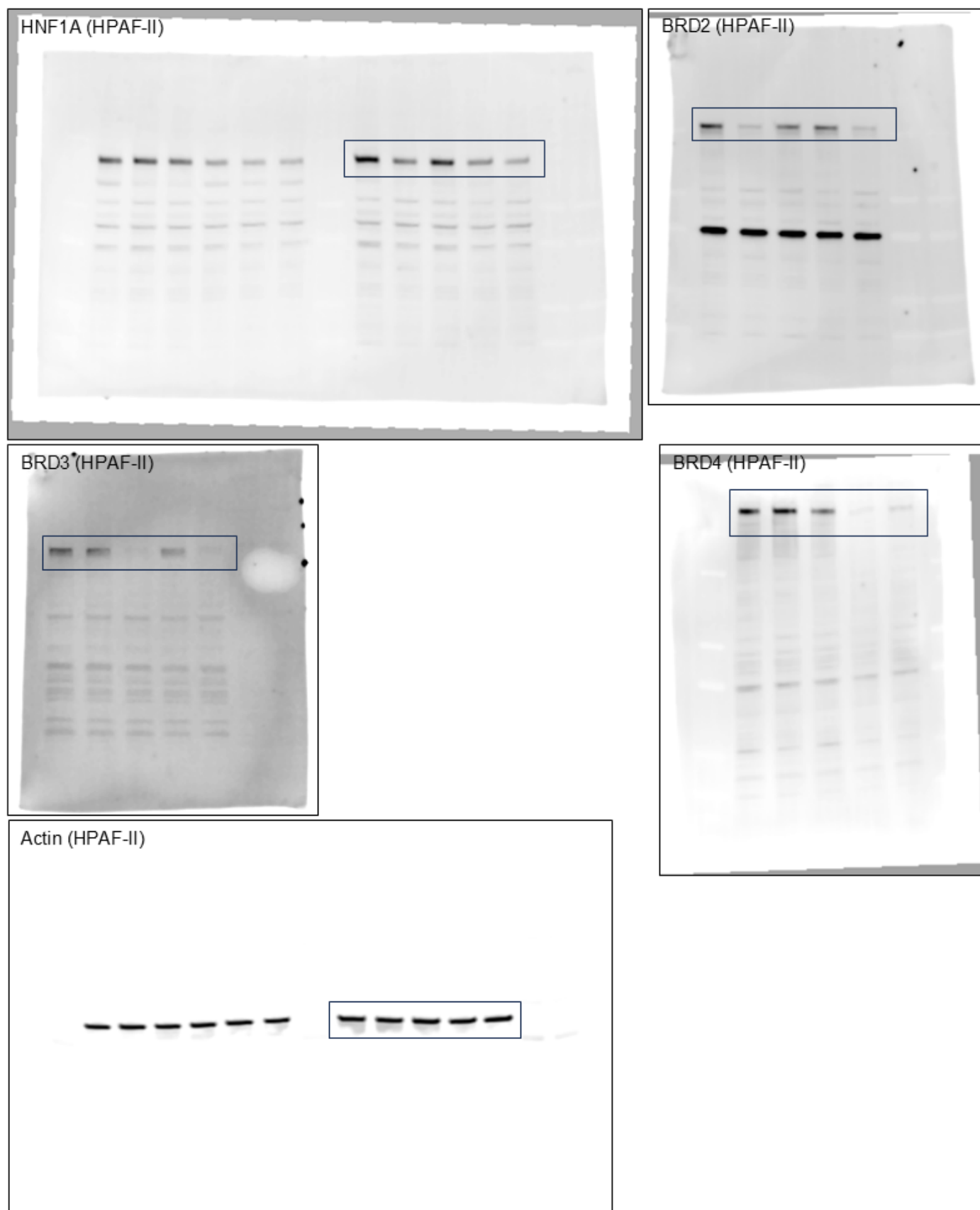
Figure 4

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Figure 4



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Figure 4

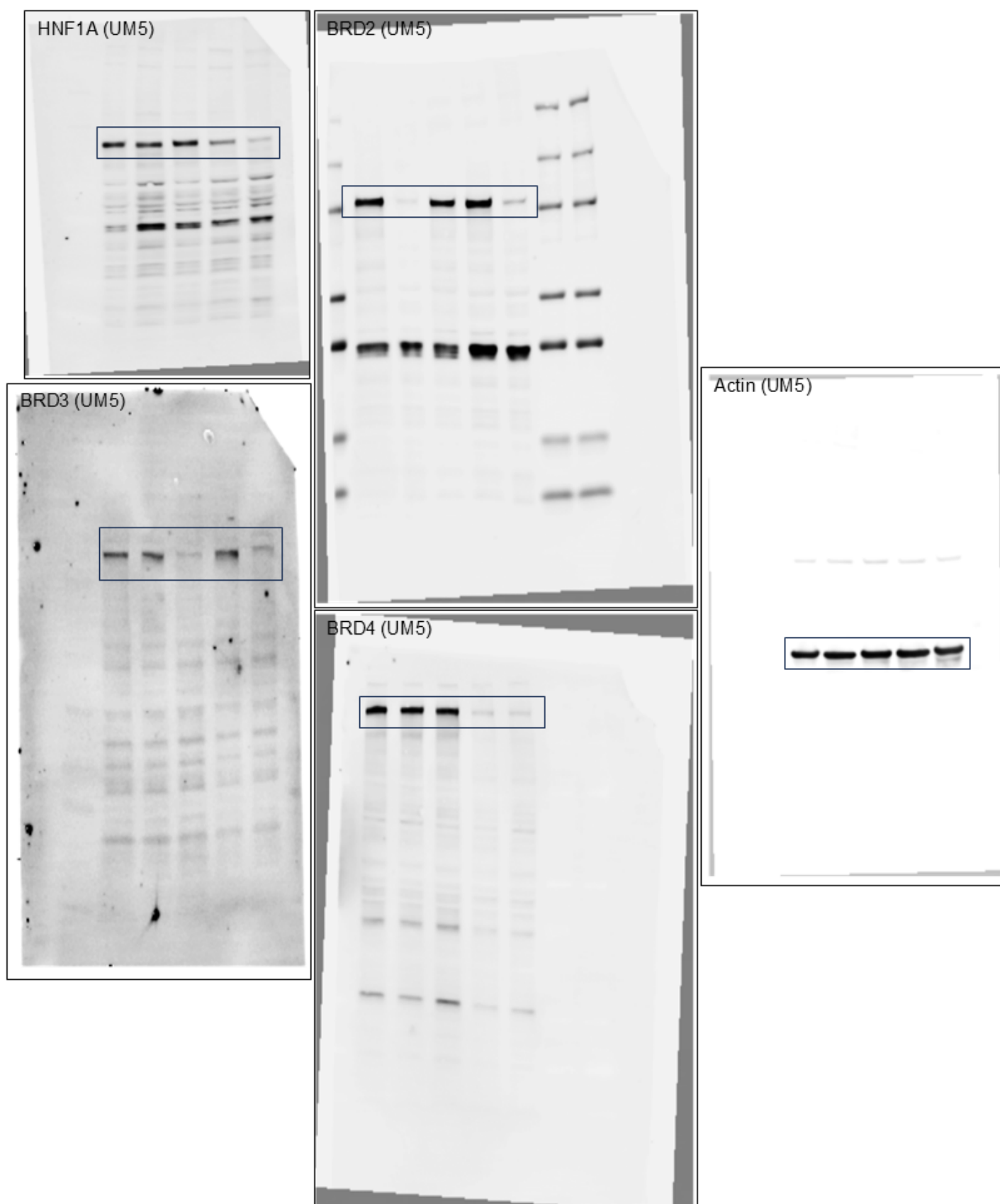


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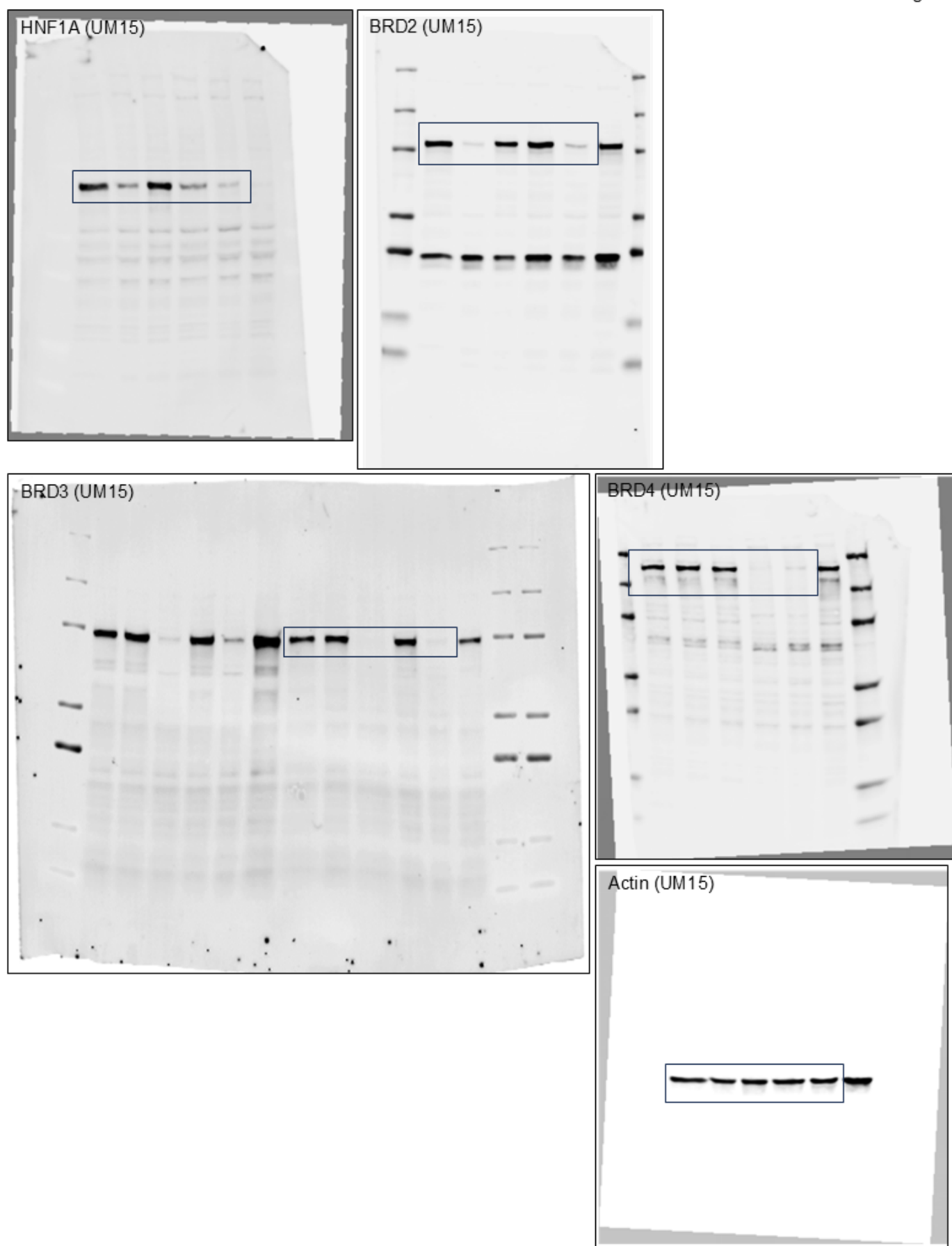


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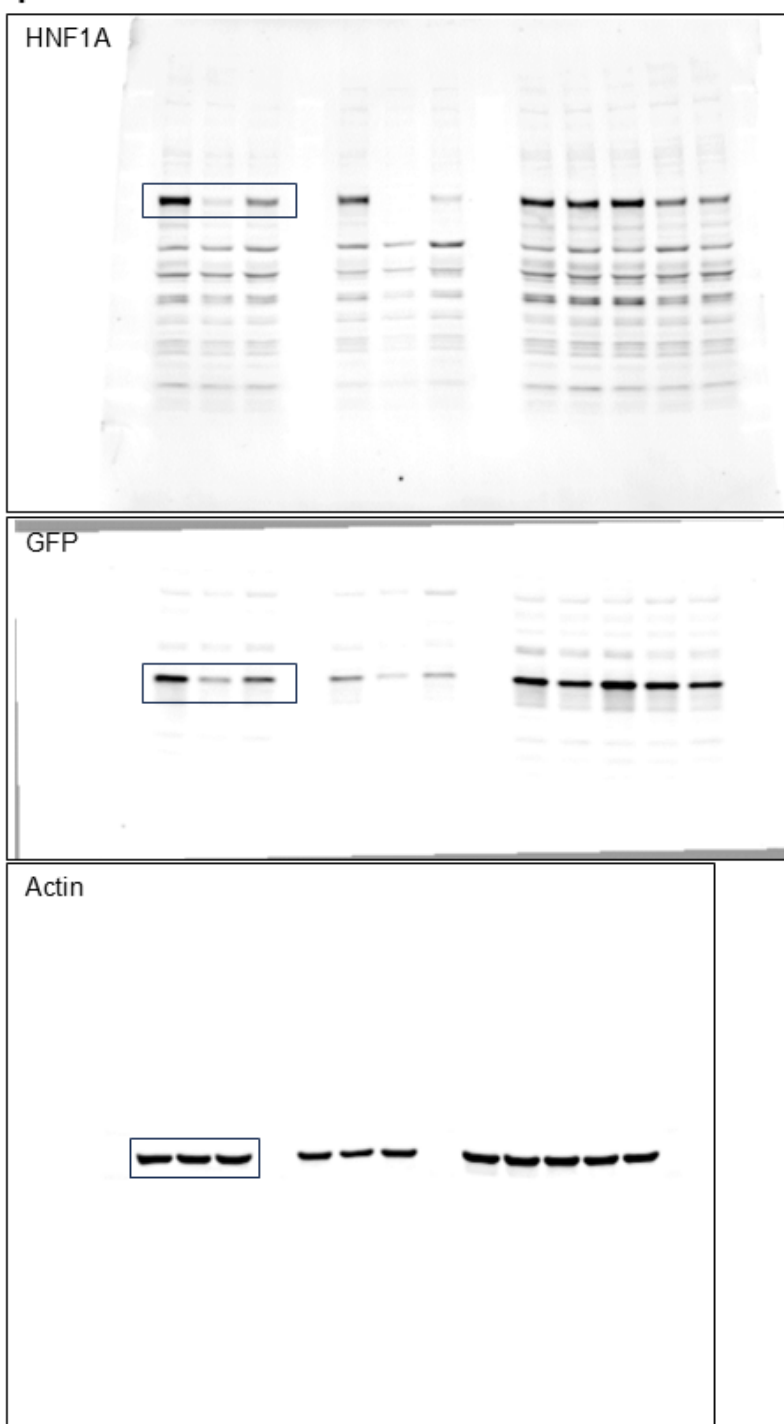


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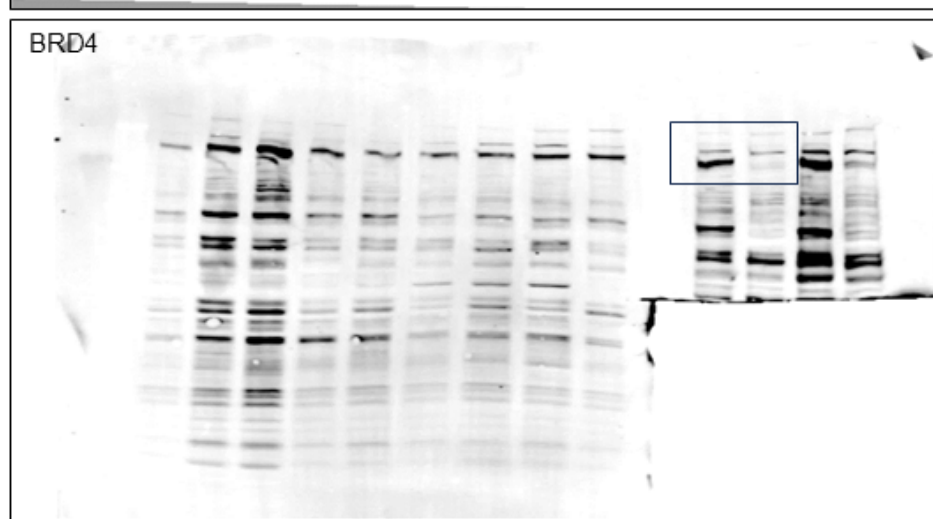
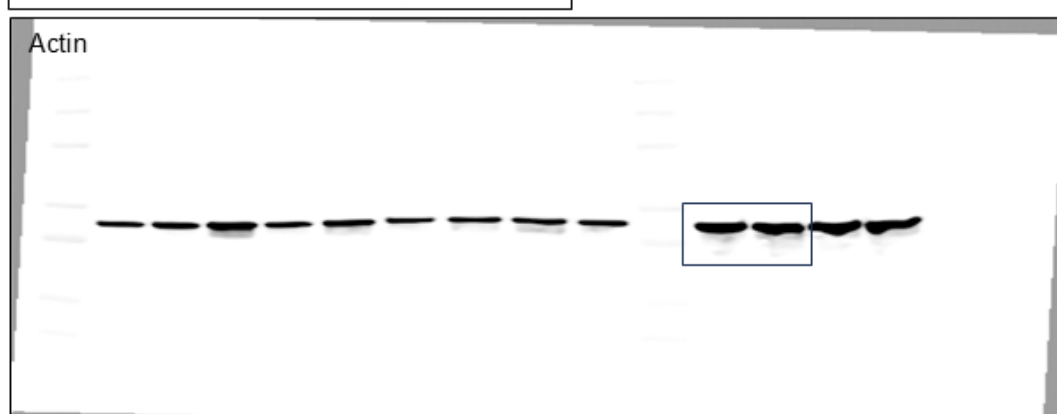
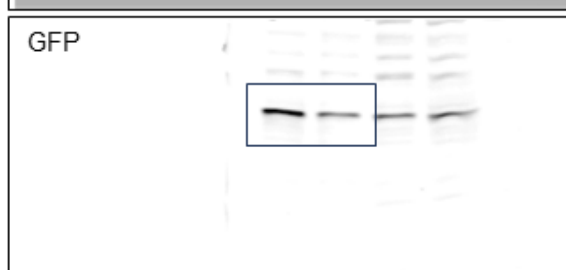
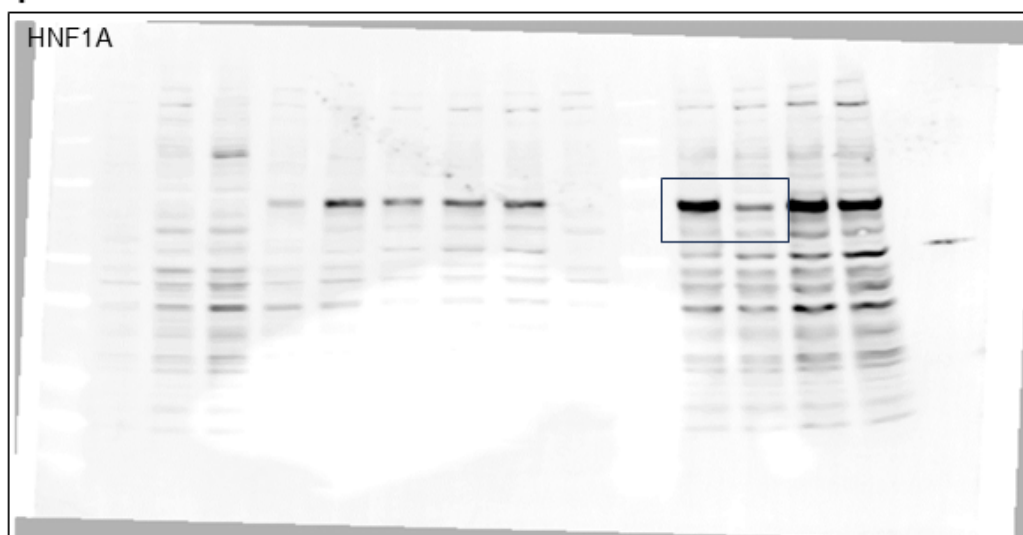


Figure 5

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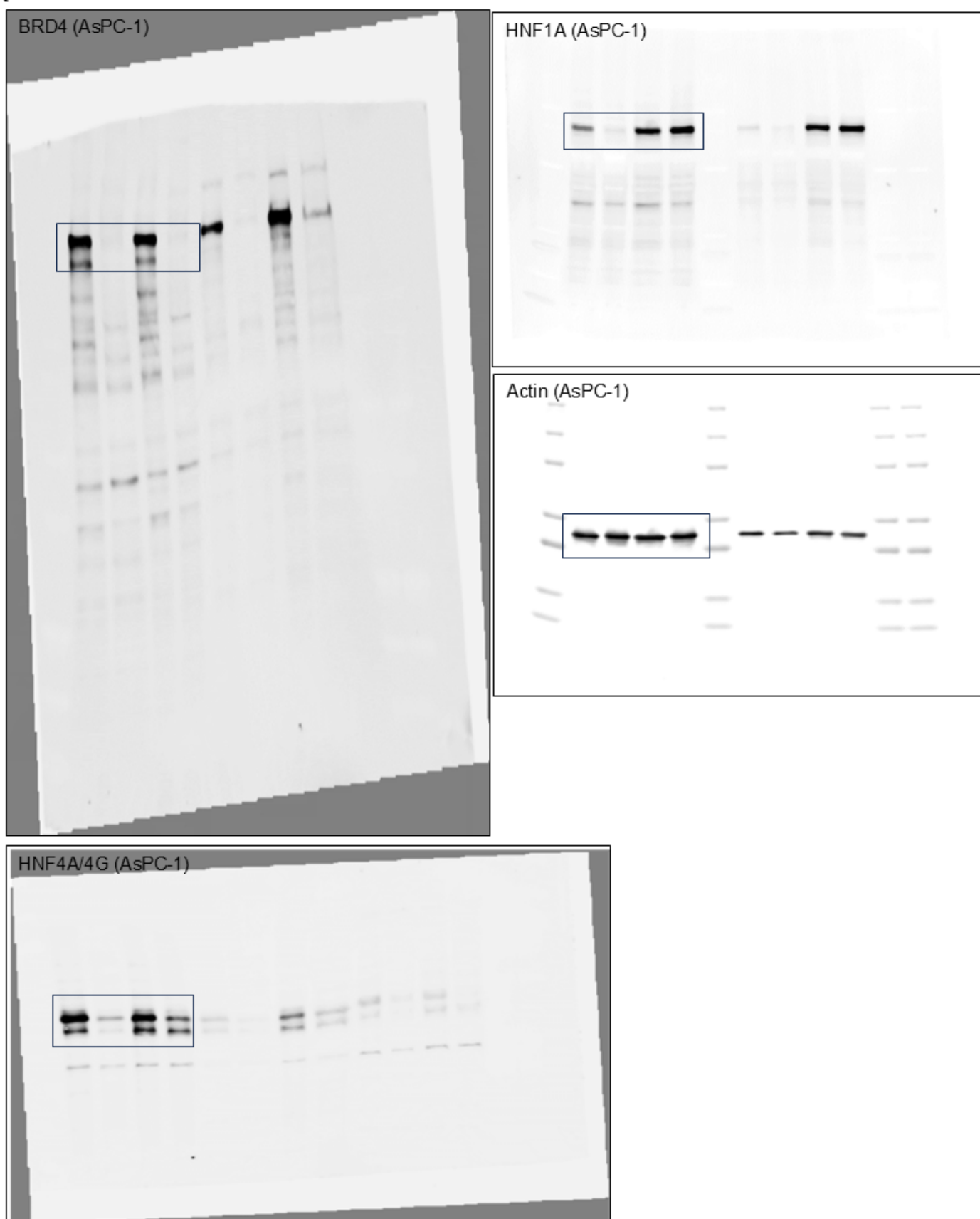


Figure 5

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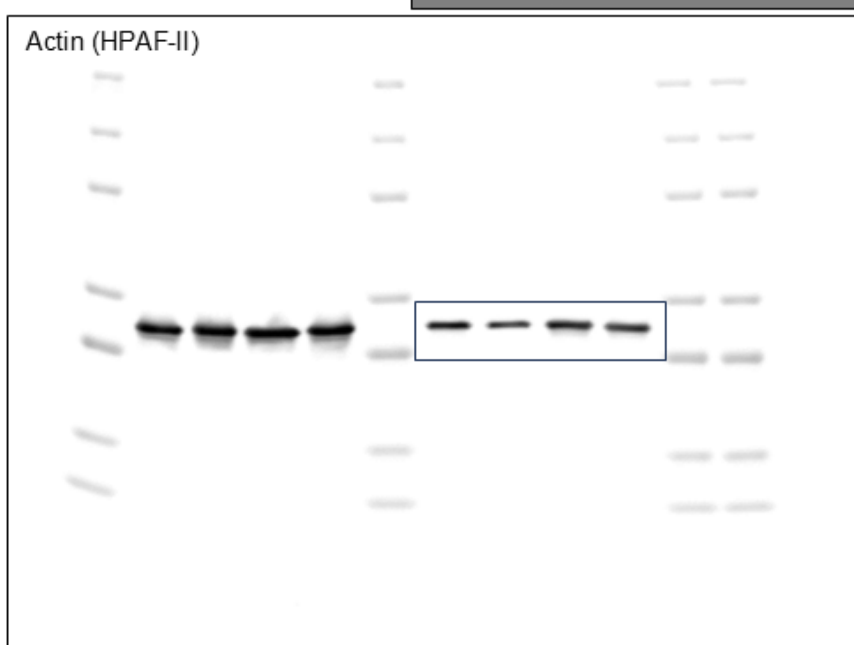
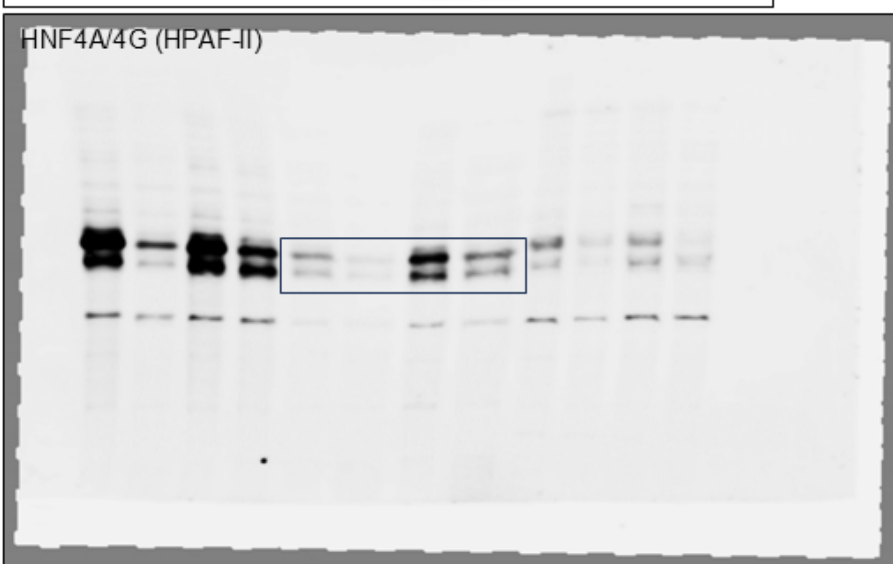
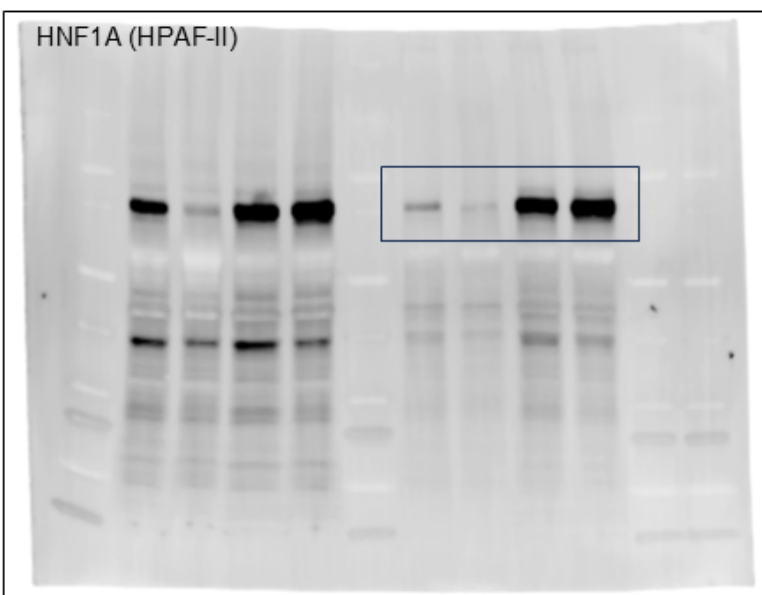
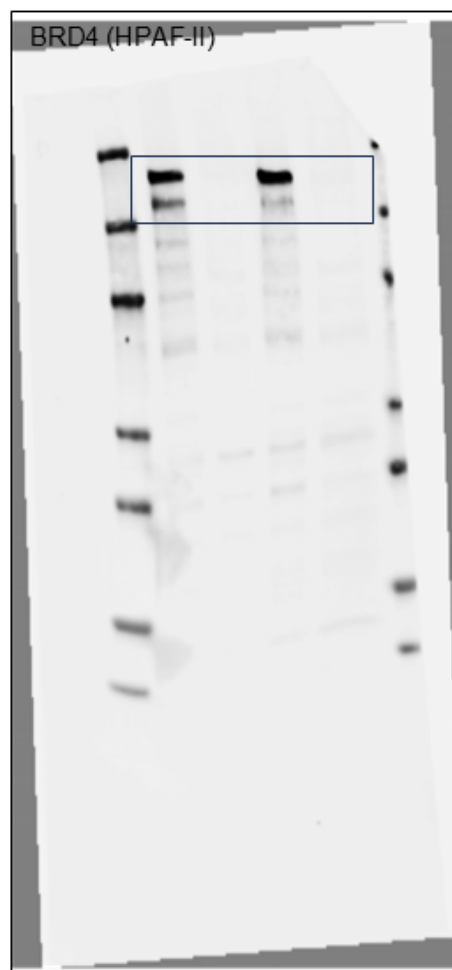
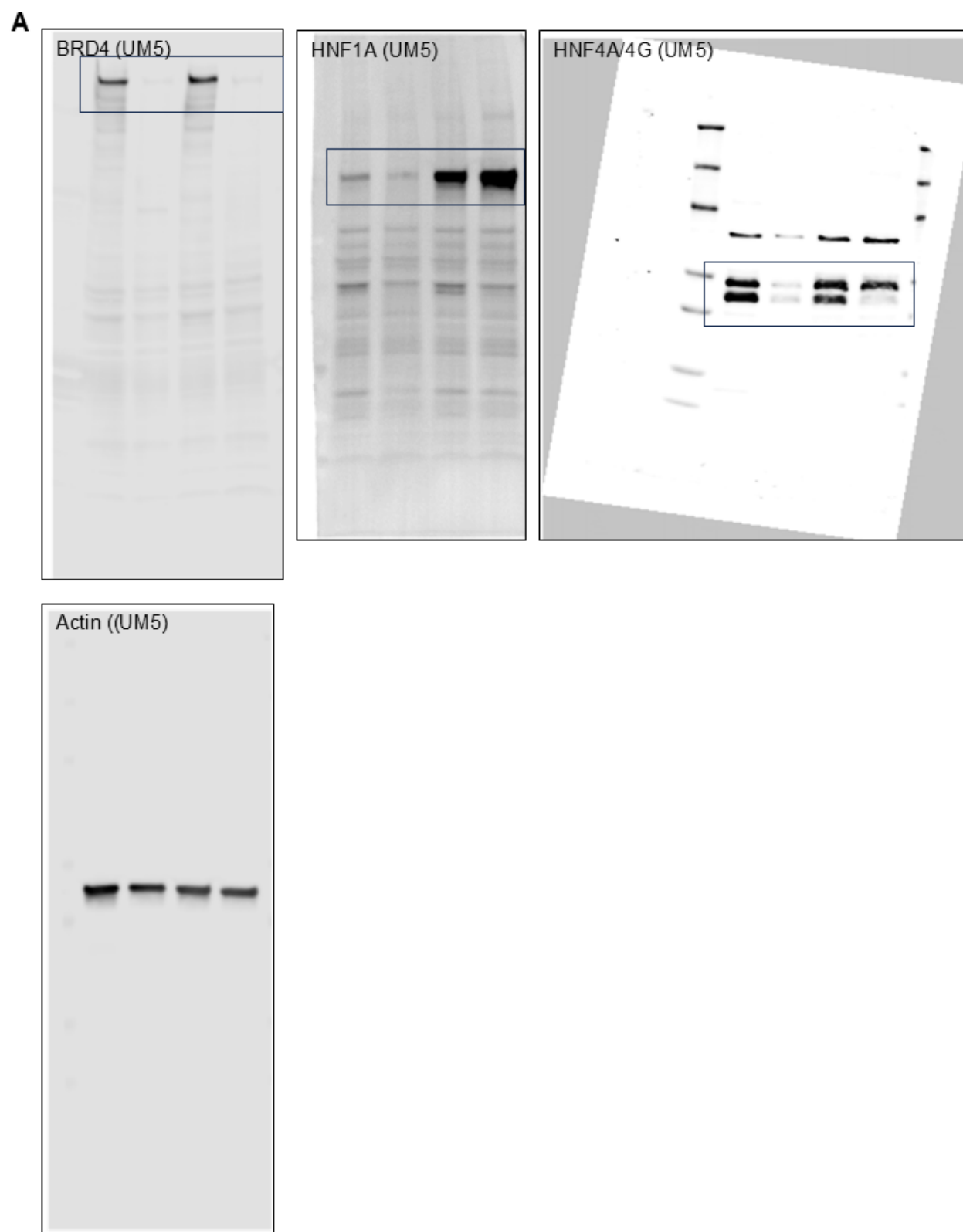


Figure 5



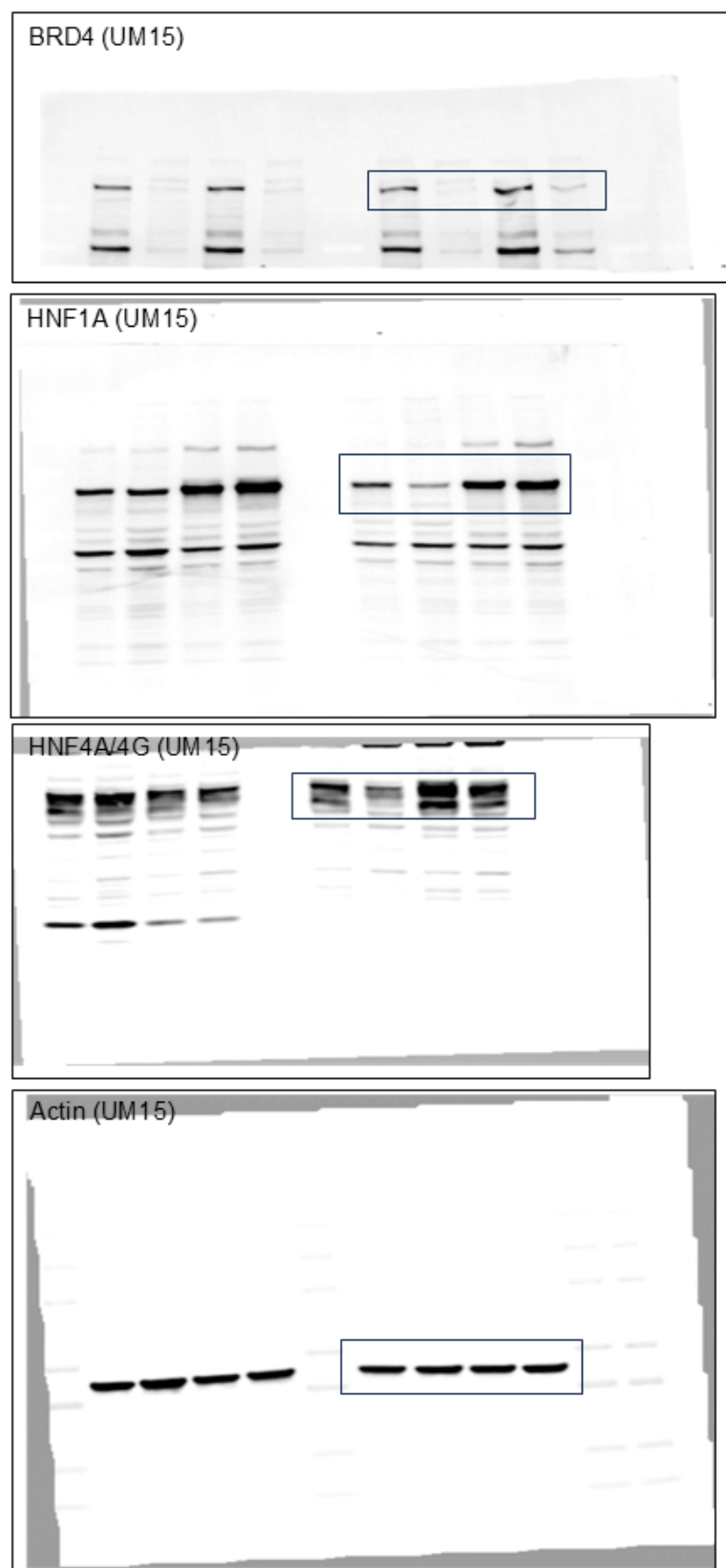
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Figure 5

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BRD4



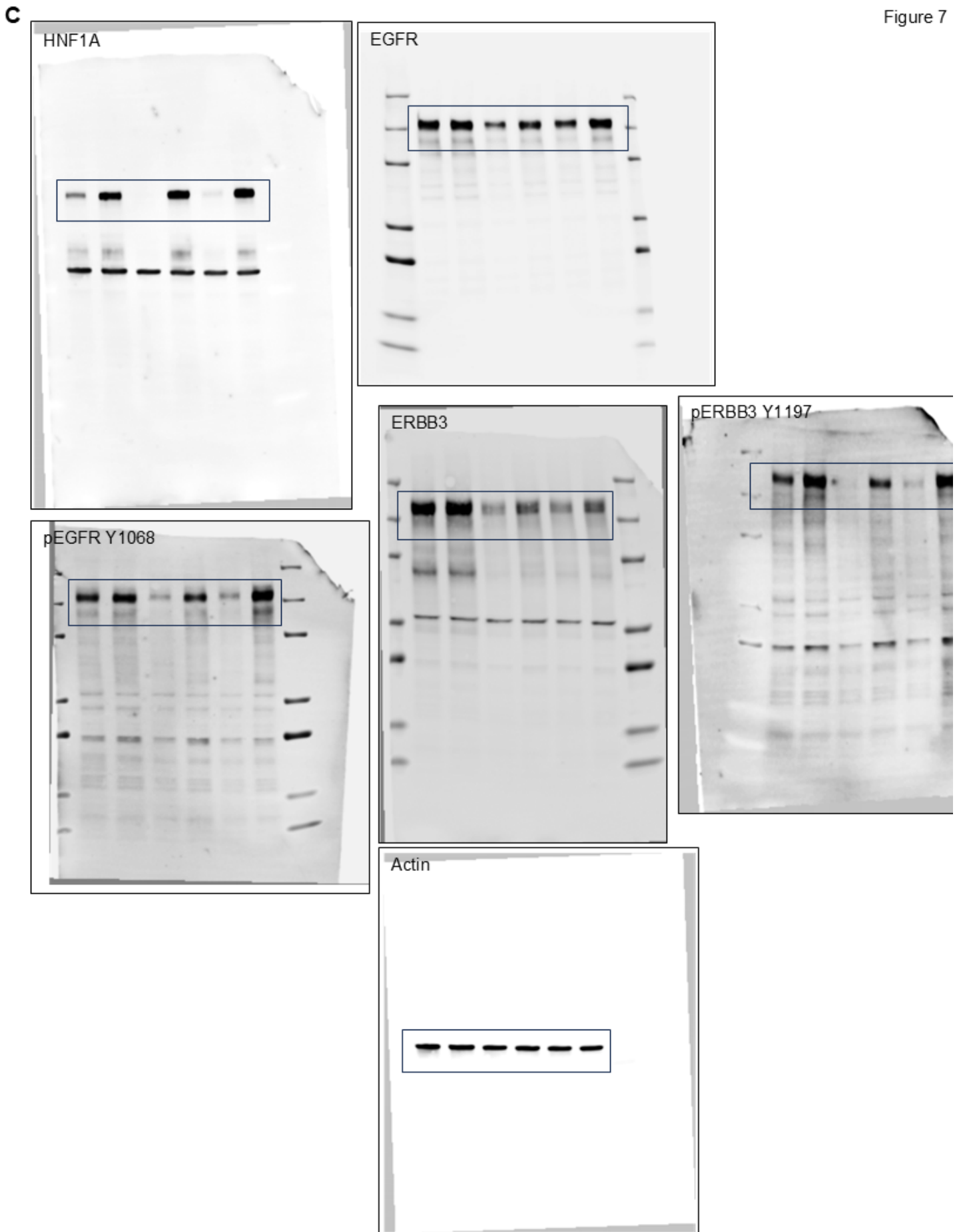
HNF1A



Actin

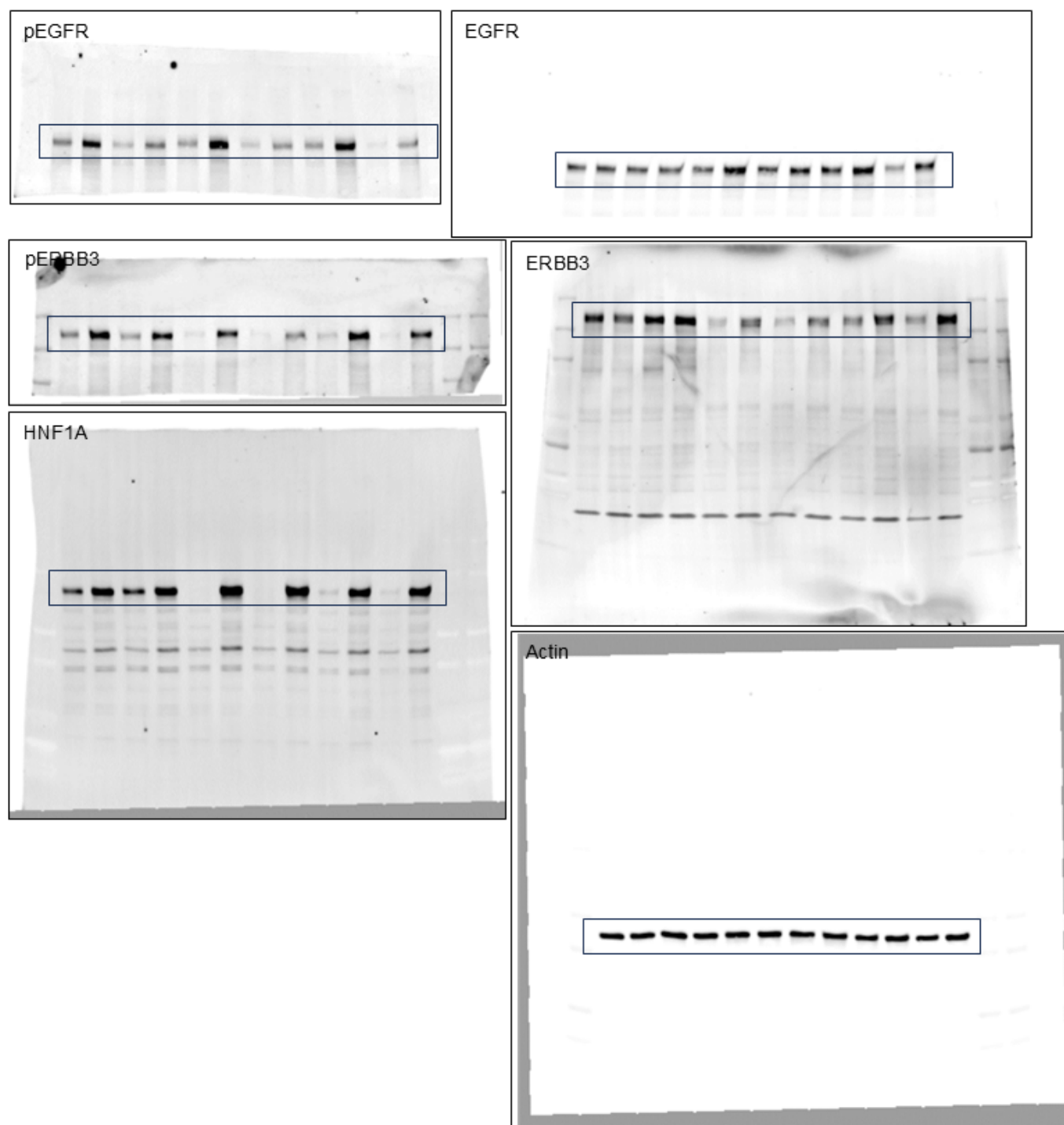


Figure 7



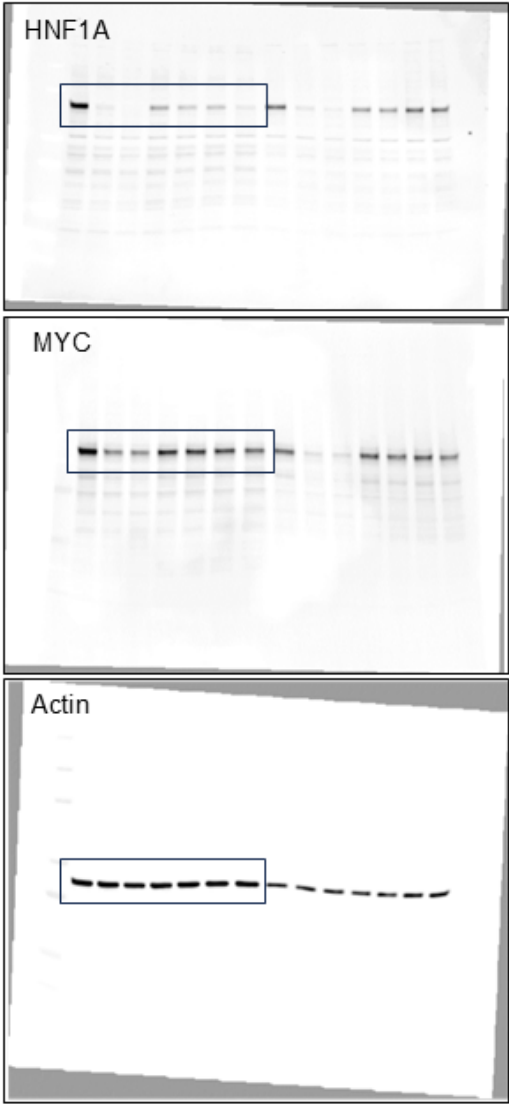
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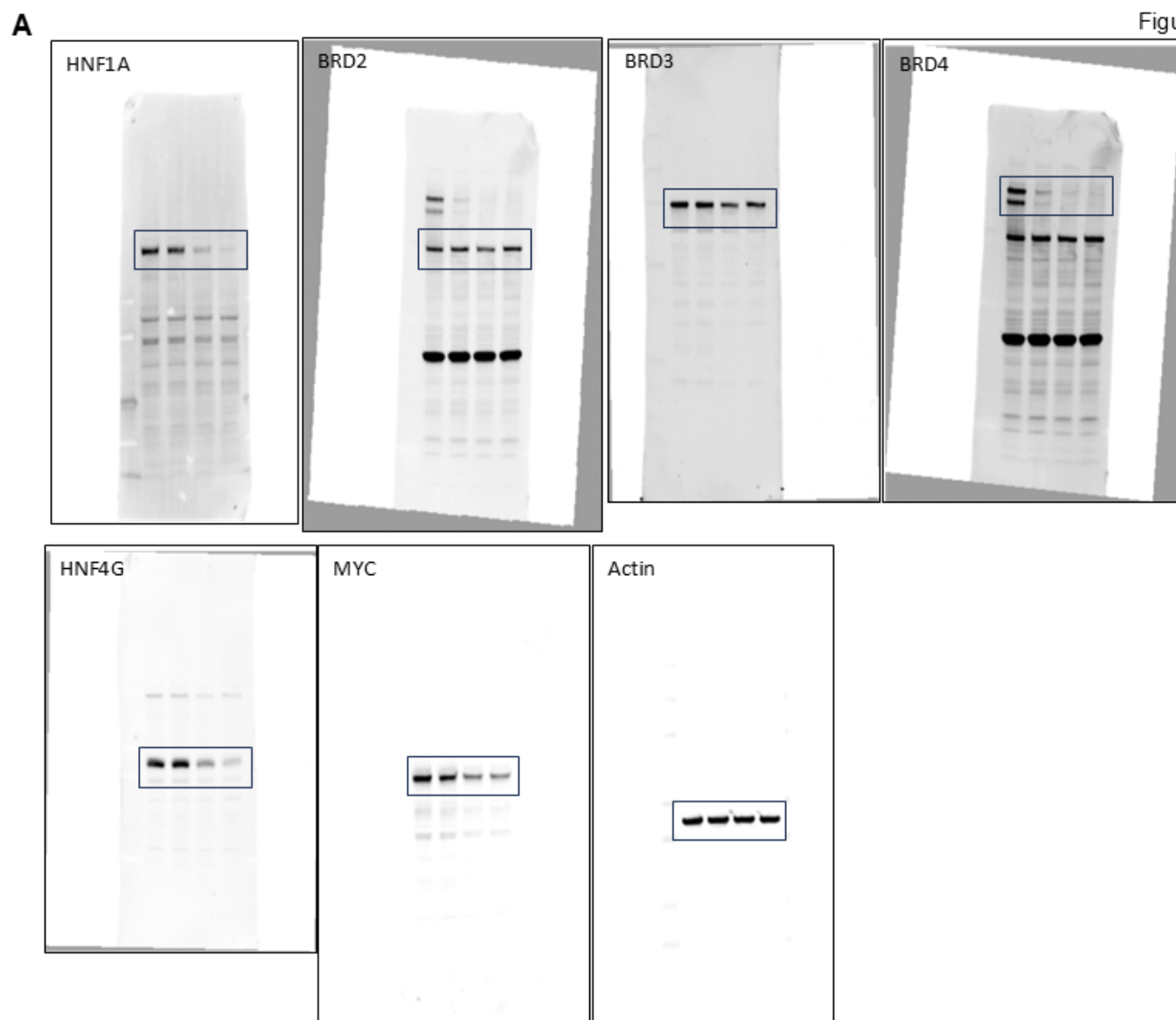
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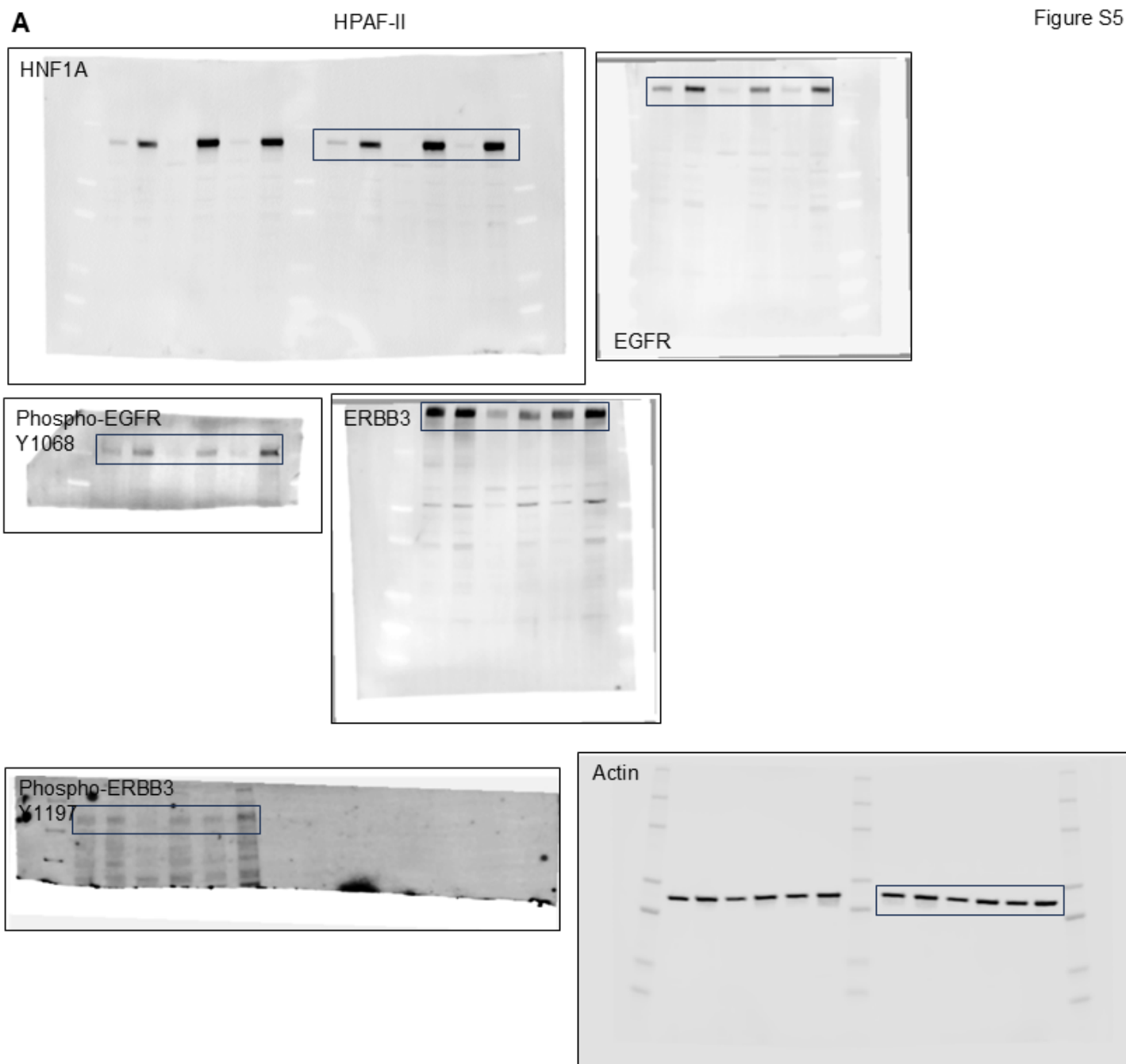


A

Figure S1



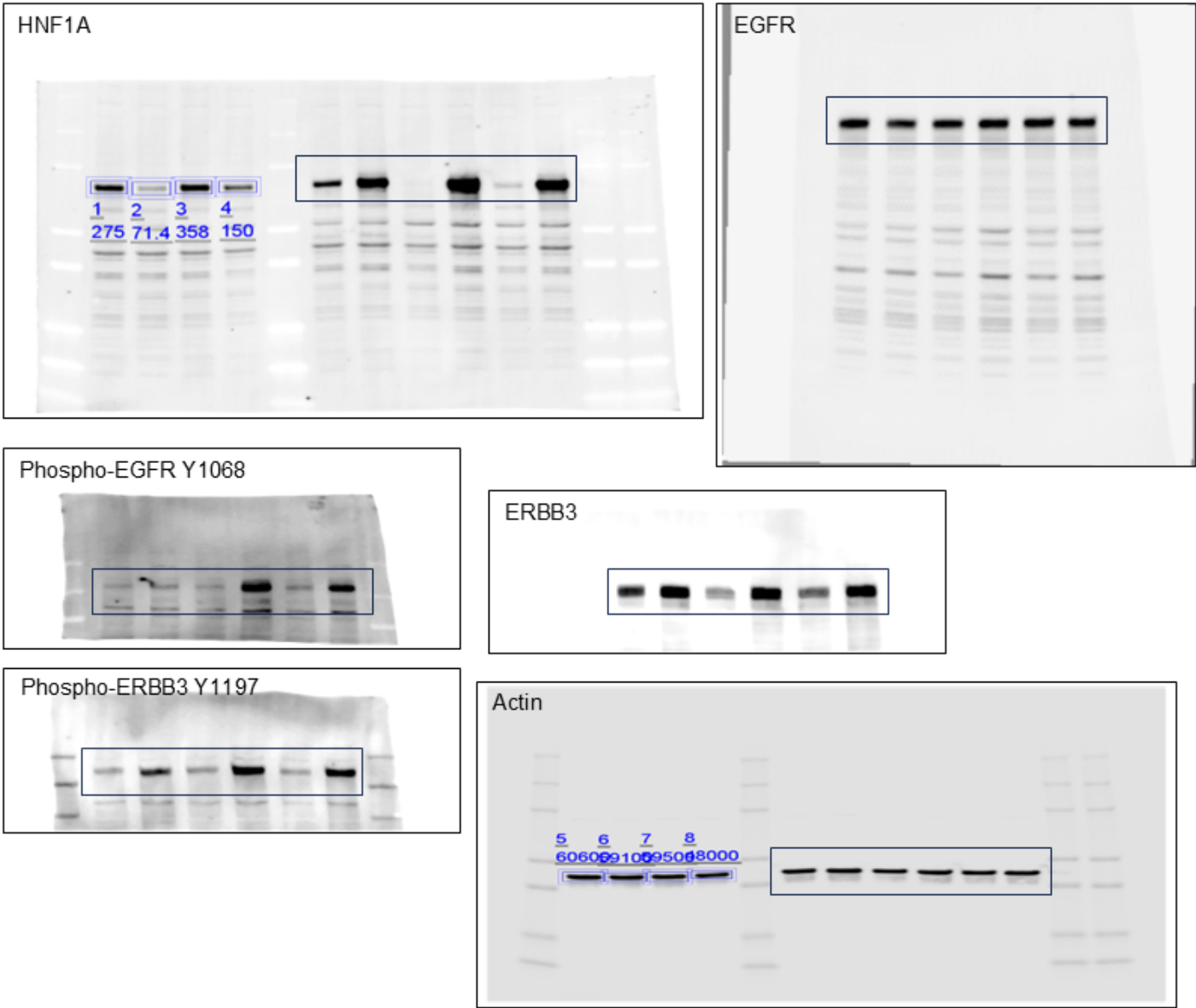


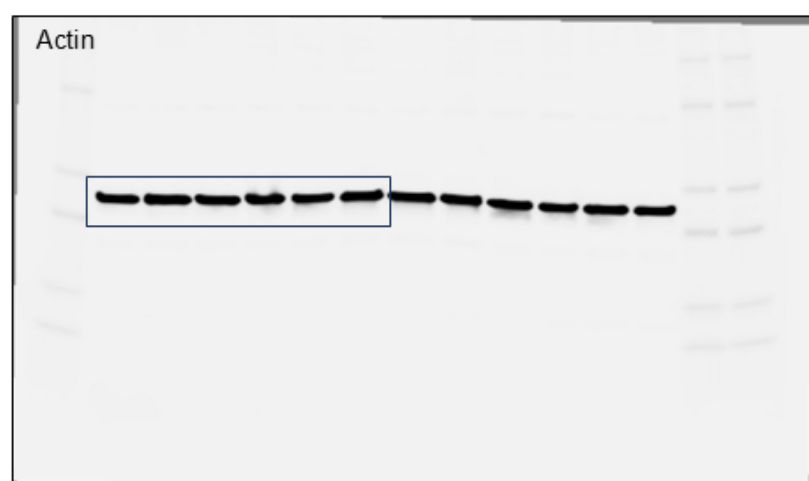
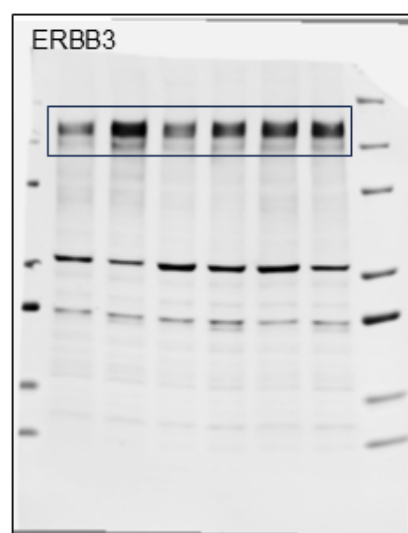
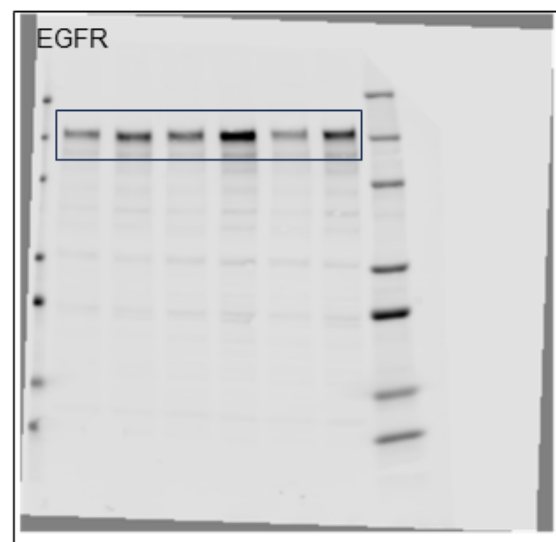
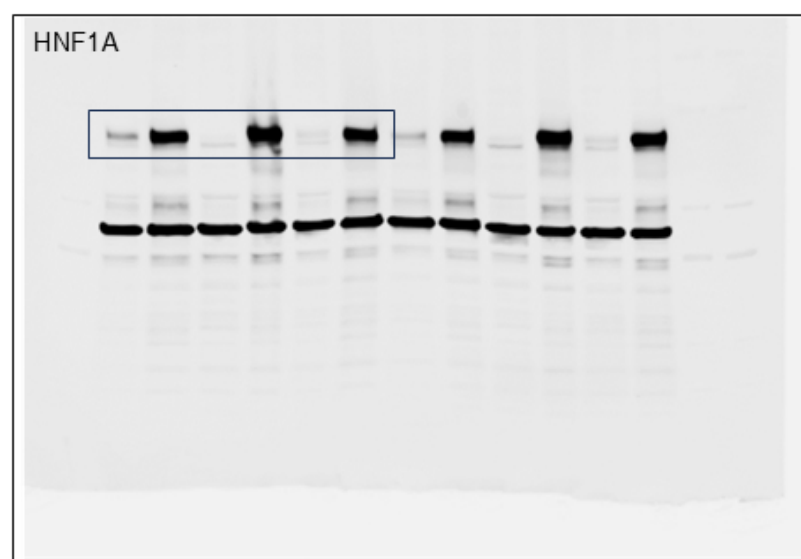


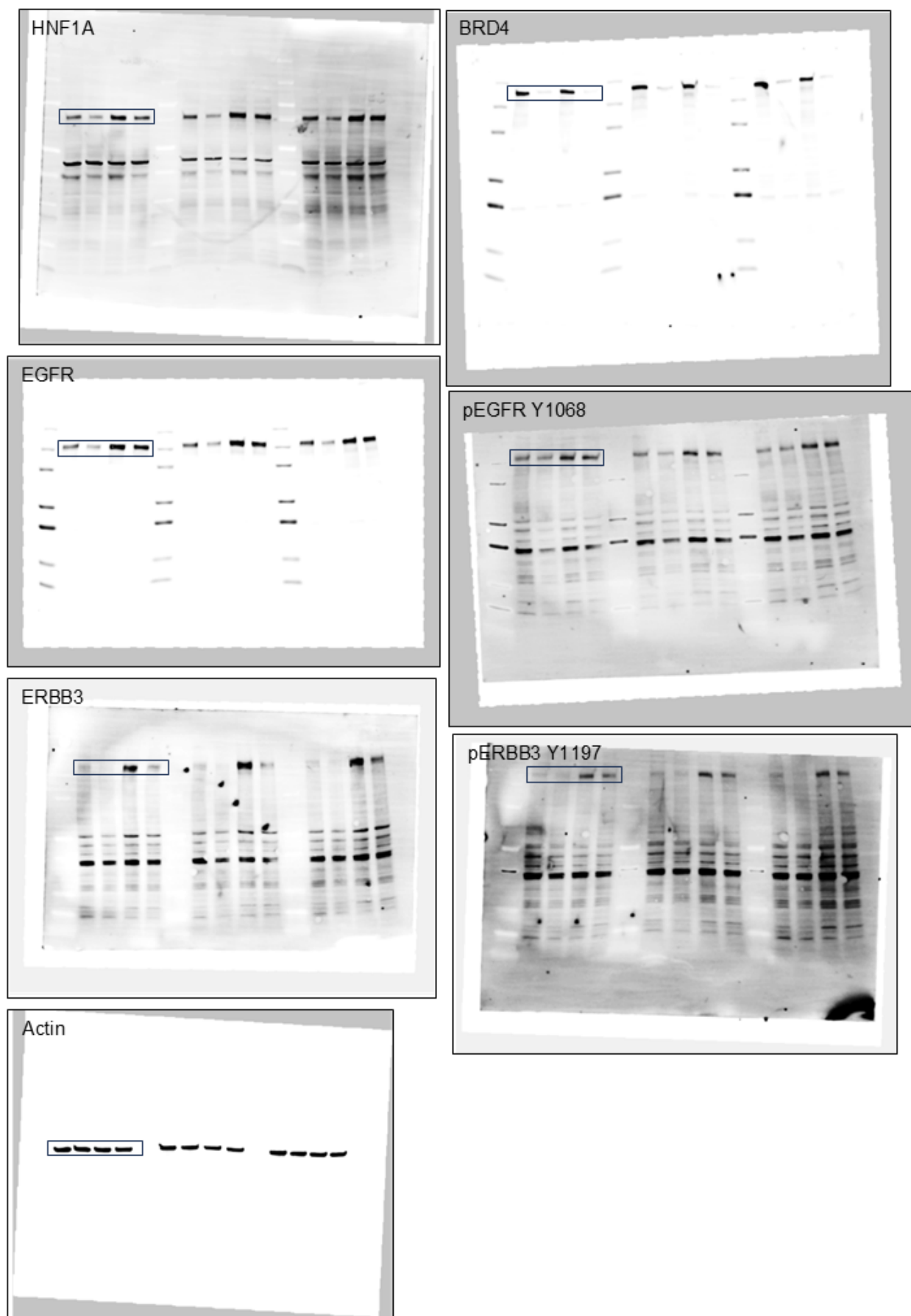
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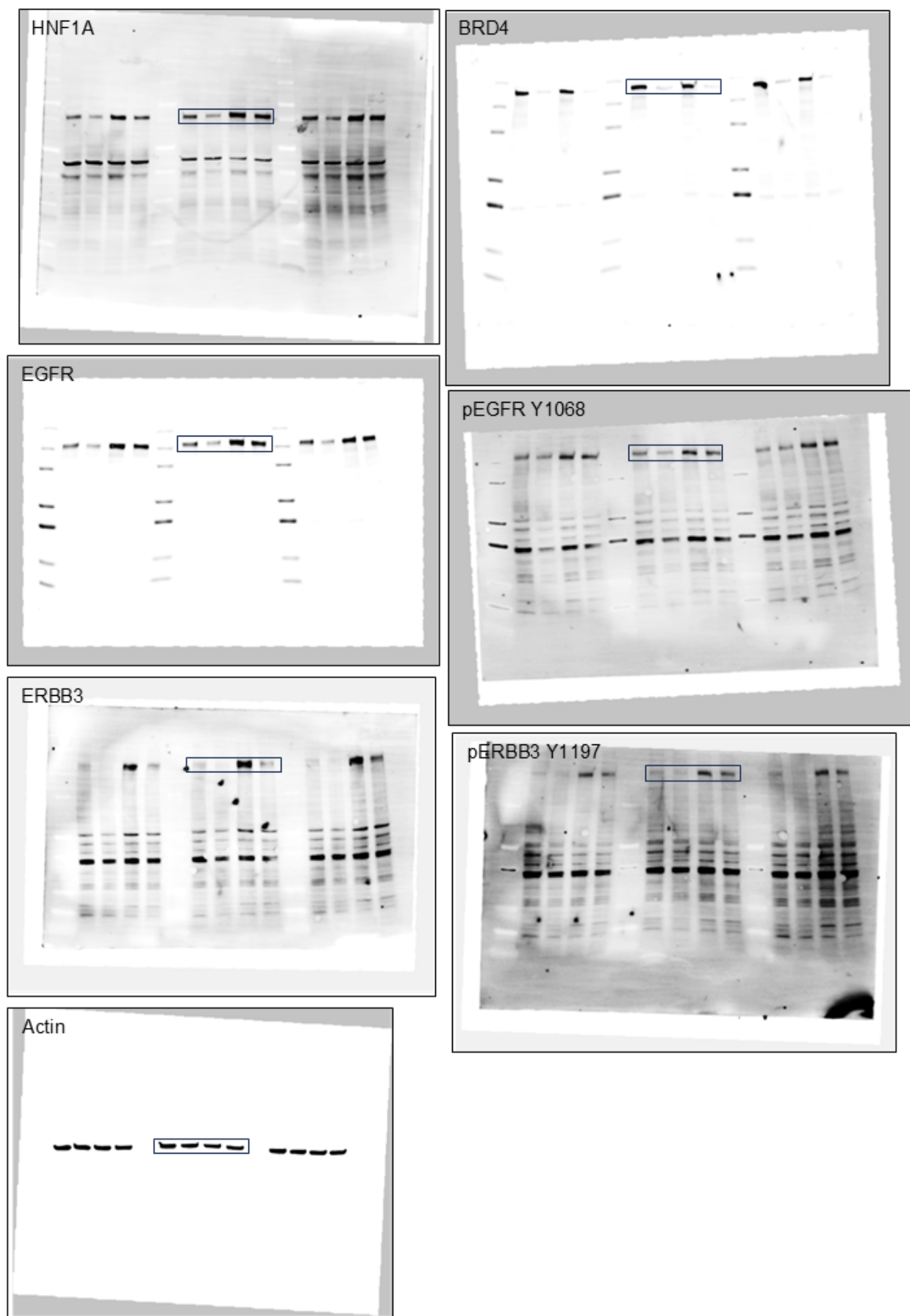
UM5

Figure S5





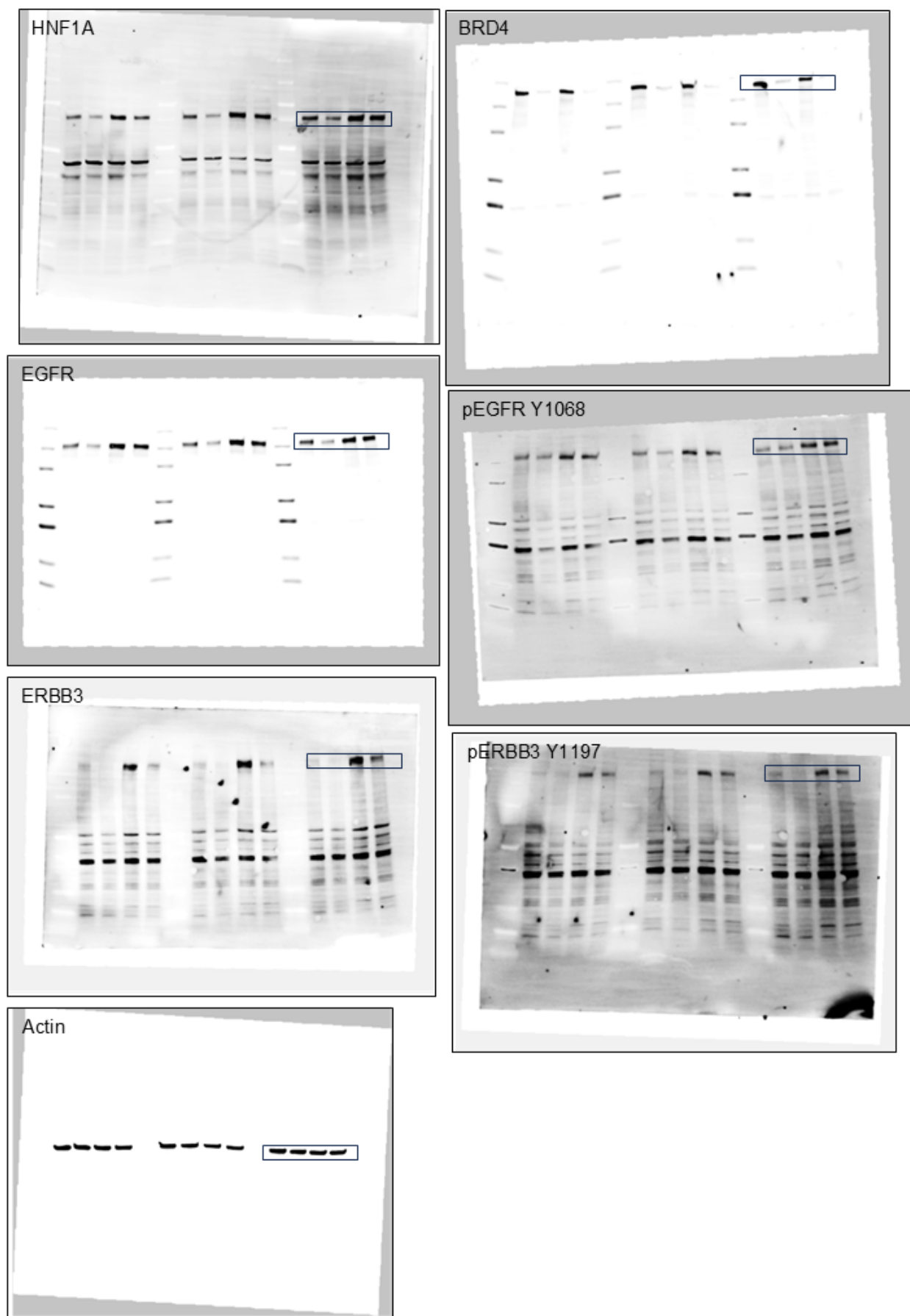




B

UM5

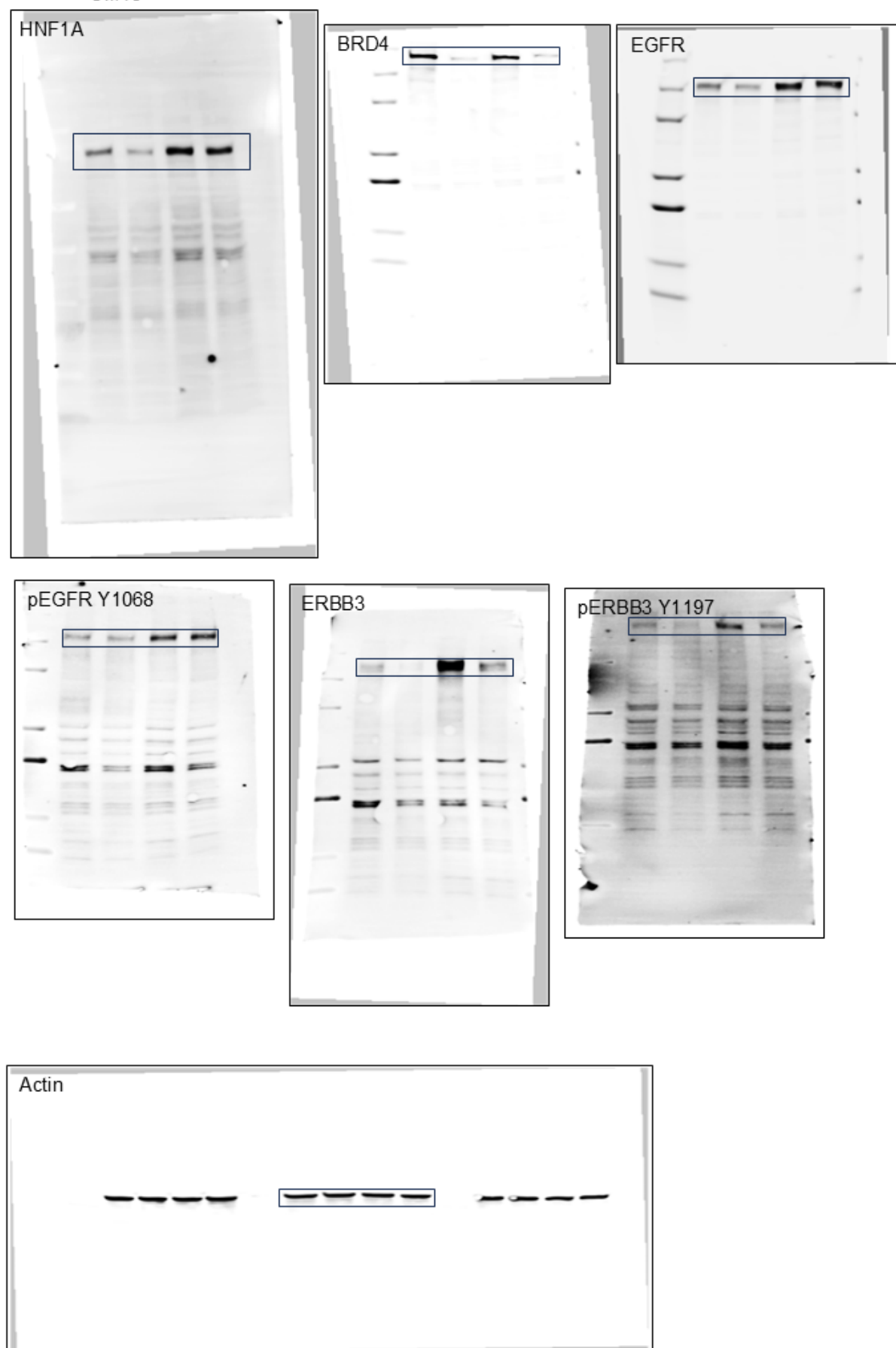
Figure S5



B

UM15

Figure S5



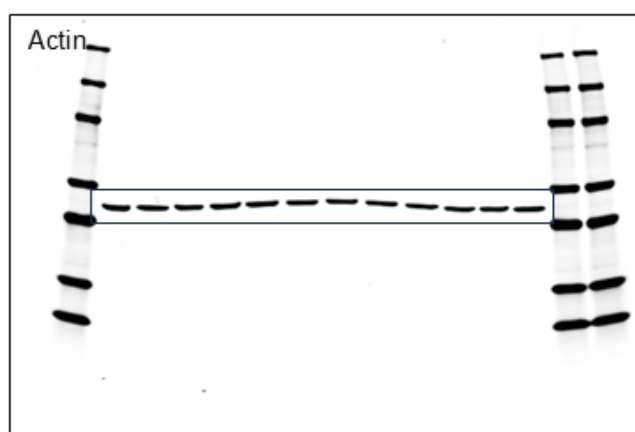
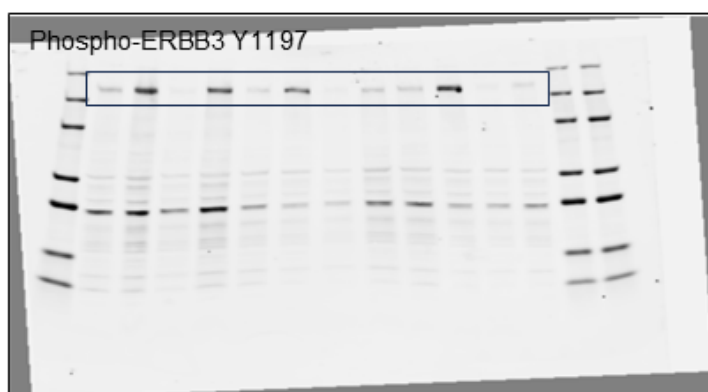
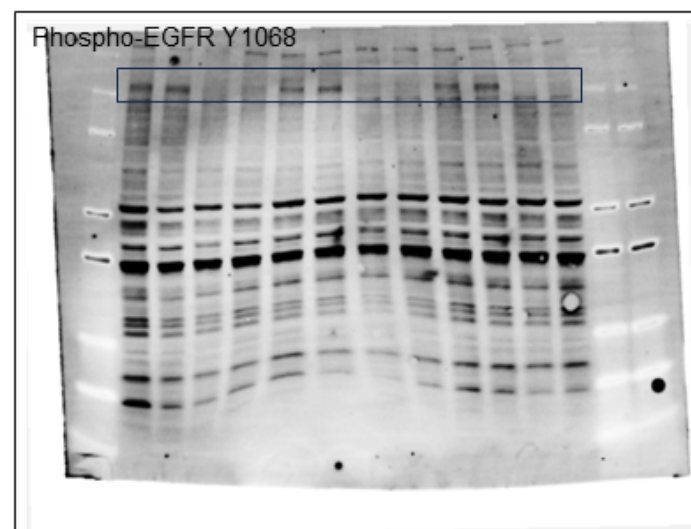
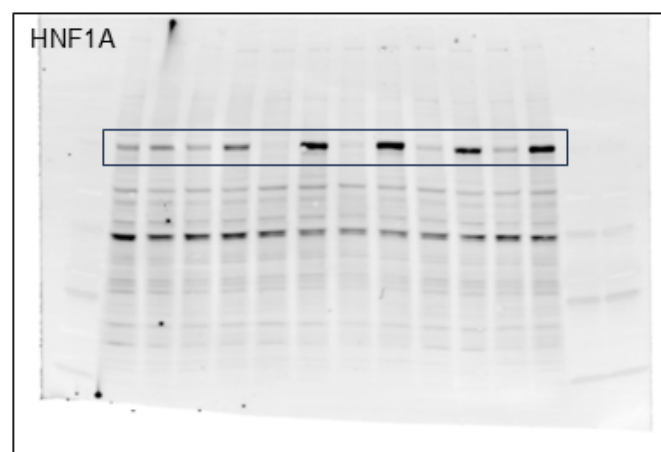
C

Figure S6

