## Supplementary Figures

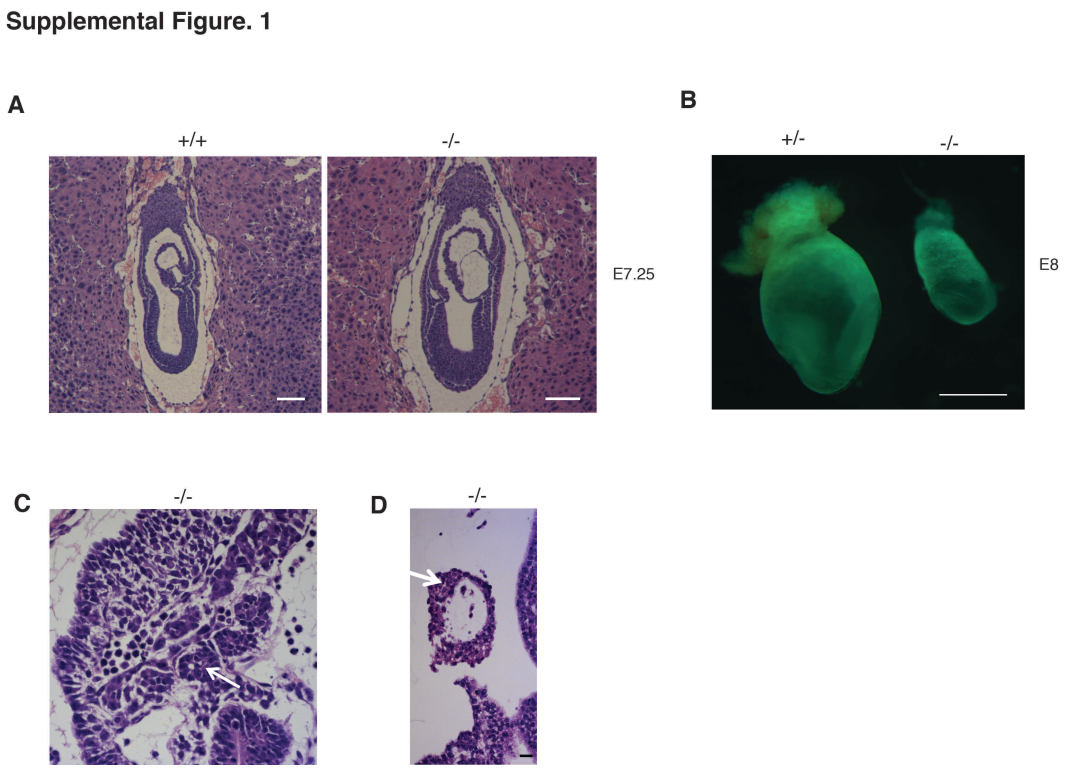



**Figure. 1. Phenotype of *Rnh1*$^{-/-}$ embryos**

(**A**) H&E stained para sagittal section of E7.25 embryo. Scale bar 100 µm. (**B**) Morphology of E8.5 embryos. Scale bar 500 µm. (**C** and **D**) H&E stained para sagittal section of *Rnh1*$^{-/-}$ embryo somites (C) and heart (D) (scale bar 20 µm).

**Supplemental Figure. 2**

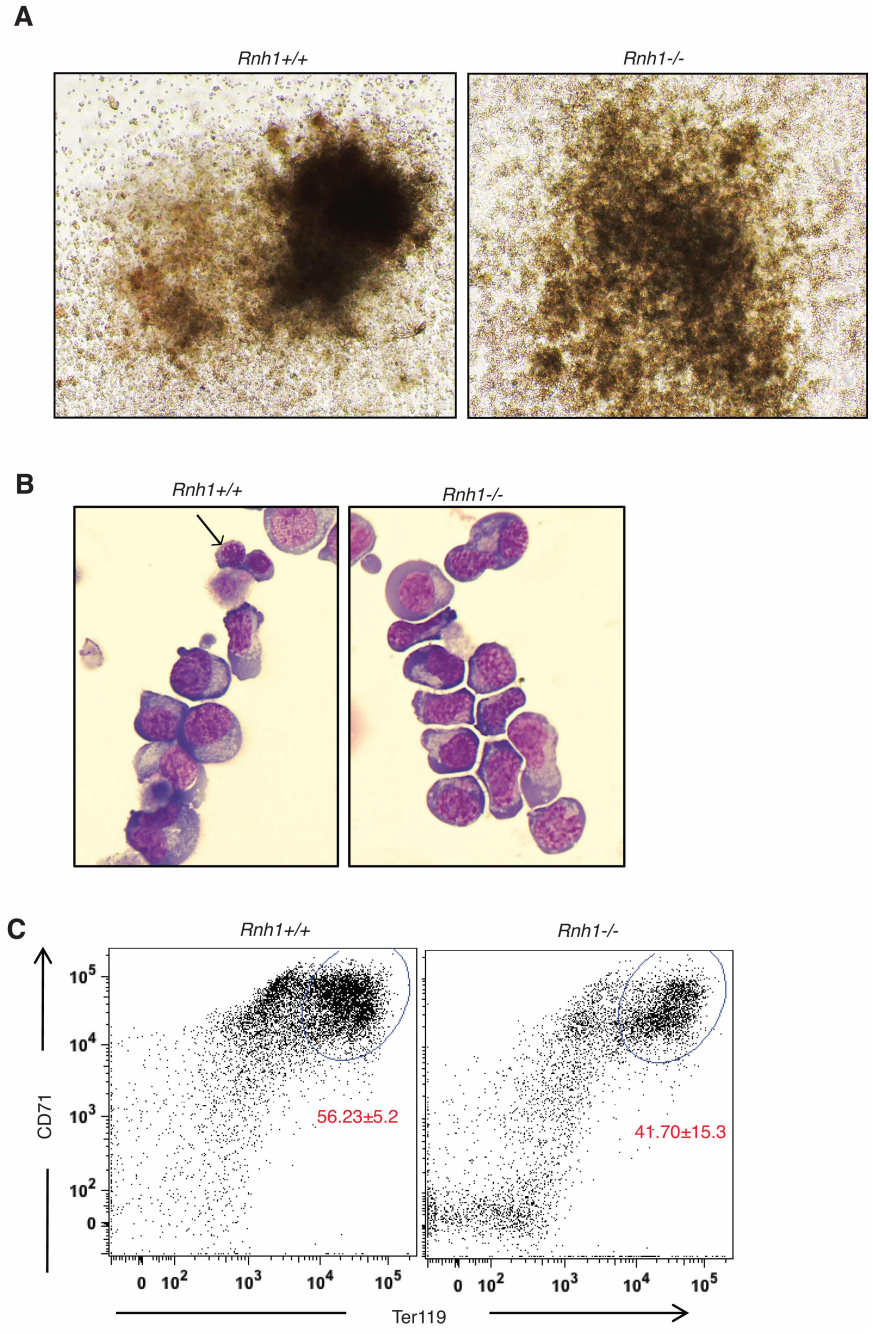



**Figure 2. Decreased erythroid cells in yolk sac hematopoietic colonies of Rnh1 deficient embryo**

(**A**) Morphology of colonies derived from *Rnh1*$^{+/+}$ and *Rnh1*$^{-/-}$ E8.5 yolk sac cells cultured for 7 days in methyl cellulose medium that support growth of erythroid cells (n = 3). (**B**) Cytospin images of *Rnh1*$^{+/+}$ and *Rnh1*$^{-/-}$ erythroid cells derived from methylcellulose cultures. Arrow shows mature erythroid cells. (**C**) Flow cytometry analysis for erythroid cells in hematopoietic colonies derived from E8.5 yolk sac cells cultured for 7 days in methyl cellulose medium that support growth of erythroid and myeloid colonies (n = 3). Data are means ±SEM.

**Supplemental Figure. 3**

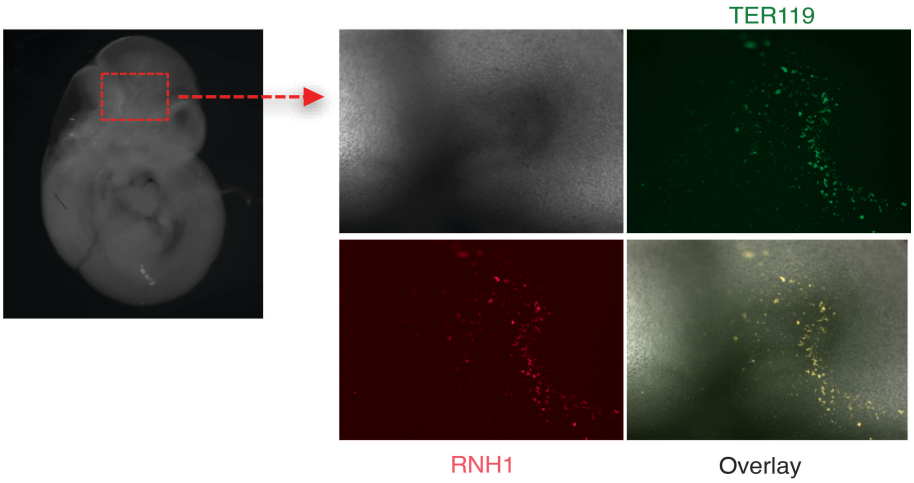



**Figure 3. Rnh1 expression colocalized to Ter119-positive cells**

Immunostaining of Rnh1 and erythroid marker Ter119 on wild type E10.5 Embryos. Experiments were performed minimum three times.

**Supplemental Figure. 4**

**A**

| Rank | Phenotype | BH p-value |
|---|---|---|
| 15 | Abnormal blood coagulation | 2.21E-06 |
| 17 | Abnormal hemostasis | 2.37E-06 |
| 27 | Hematopoietic system phenotype | 4.59E-05 |
| 38 | Abnormal erythrocyte physiology | 0.000113 |
| 40 | Abnormal inflammatory response | 0.000149 |
| 42 | Decreased hemoglobin content | 0.000218 |
| 44 | Increased inflammatory response | 0.000229 |
| 52 | Decreased hematocrit | 0.000469 |
| 54 | Abnormal reticulocyte cell number | 0.000513 |
| 55 | Abnormal iron level | 0.000551 |
| 57 | Abnormal hematocrit | 0.000603 |
| 61 | Abnormal spleen iron level | 0.000654 |
| 66 | Abnormal reticulocyte morphology | 0.000788 |
| 67 | Abnormal iron homeostasis | 0.000817 |

**B**

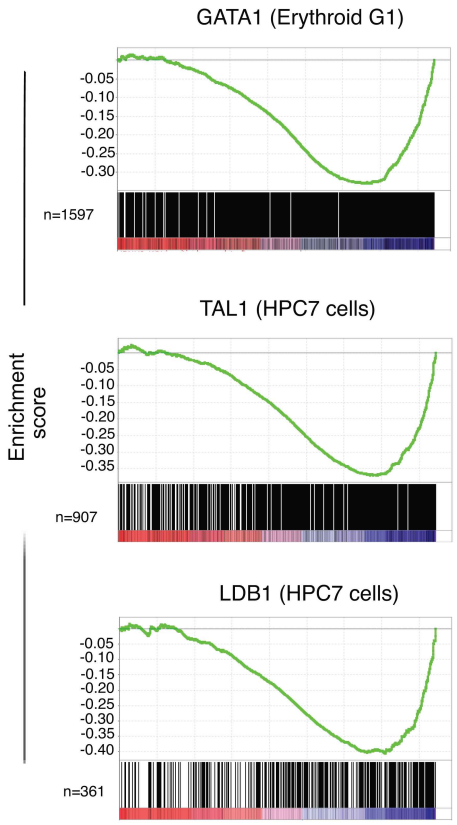



**Figure 4. Gene expression analysis on E9.5 yolk sac**

**(A)** Enriched hematopoietic defect phenotypes in *Rnh1*-deficient mice according to the MouseMine tool (see also **Supplementary Table. 2**). **(B)** GSEA analysis identified a down-regulation signature of haematopoietic transcription factor targets in E9.5 yolk sacs from *Rnh1*$^{-/-}$ mice. The lists of hematopoietic transcription factor targets were obtained from publicly available ChIP-seq experiments (see Methods).

**Supplemental Figure. 5**

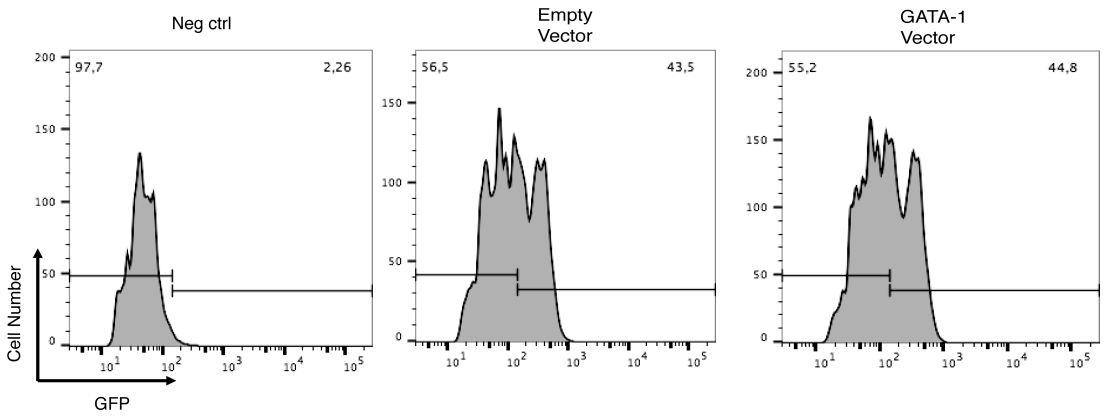



**Figure 5. GATA1 overexpression in Rnh1-deficient yolk sac cells**

E9.5 Rnh1-deficient yolk sac cells were infected with lentiviral control (empty) and GATA1- expressing plasmids with GFP expression. After 48 hours of infection GFP-positive cells were selected by FACS sorting. Histogram plot showing GFP expression in infected cells (N=3).

**Supplemental Figure. 6**

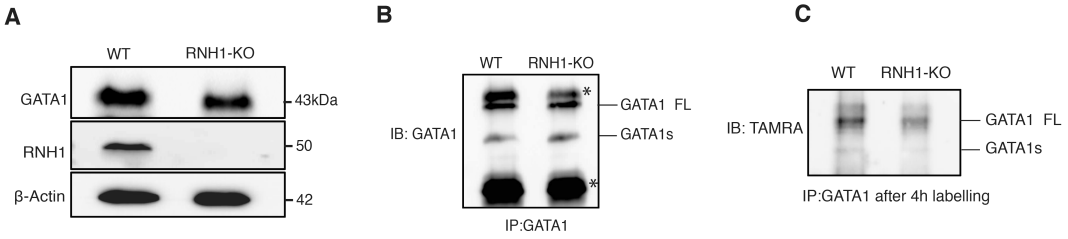



**Figure 6. RNH1-deficiency reduced GATA1 translation.**

(**A**) Total protein lysates of wild type and RNH1-KO K562 cells were analyzed by western blot with the indicated antibodies. GATA1 protein levels were reduced in RNH1-KO cells. (**B**) Western blot of GATA1 protein levels after immunoprecipitation of GATA1 using GATA1 antibody. Even though RNH1-KO cells have reduced total GATA1 proteins levels, comparable GATA1 protein levels were immunoprecipitated in wildtype and RNH1-KO cells. * non-specific band corresponded to antibody heavy chain. (**C**) Western blot of TAMRA showing detection of L-azidohomoalanine levels after 4 hours of labelling and GATA1 immunoprecipitation. Newly synthesized GATA1 protein is decreased in RNH1-KO cells. All blots are representative of three independent experiments.

## Supplemental Figure. 7

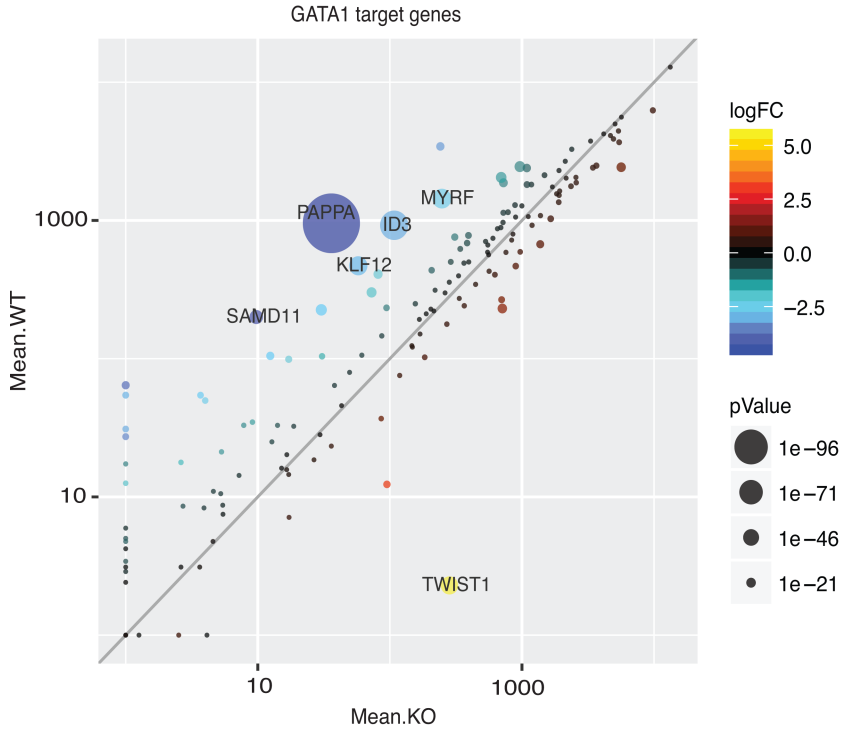



**Figure 7. Decreased GATA1 target genes in *RNH1*-KO K562 cells**

LogFC plots show the up and down regulated genes in *RNH1*-KO cells compared to the WT cells for GATA1 target genes. The x and y axes show the expression levels for each gene as normalized read counts in the KO and WT cells respectively. The colour of each dot reflects the log2 fold change and the size of a dot reflects the adjusted P-value of a gene.

**Supplemental Figure. 8**

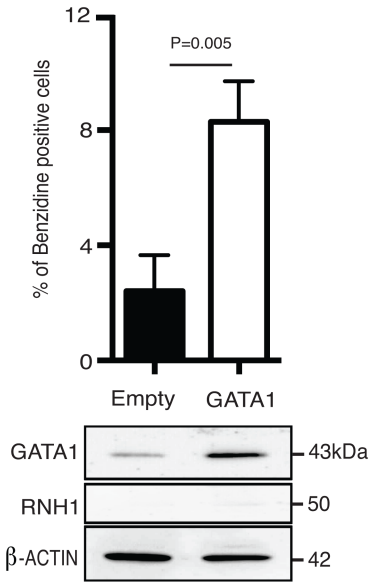
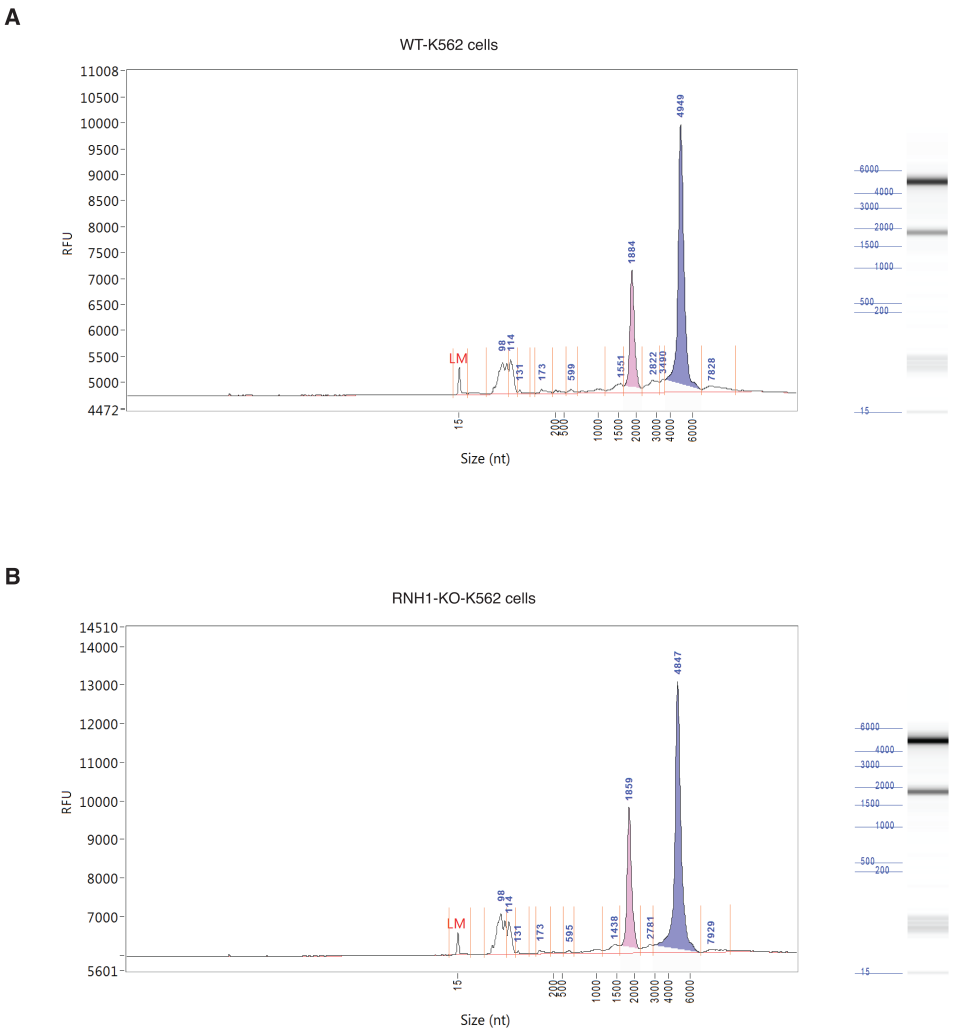



**Figure 8. GATA1 overexpression rescue erythroid phenotype in *RNH1*-KO K562 cells**

Percentage of benzidine-positive cells in GATA1- or HMD (empty control)-transfected *RNH1*-KO K562 cells (upper part). Data are means ±SEM and are representative of three independent experiments. Western blot analysis for GATA1 in transfected cells (lower part). Blots were representative of three independent experiments. *P* values determined by two-tailed *t*-test.

**Supplemental Figure. 9**




## Figure 9. Qualitative assessment of total RNA integrity from K562 cells

Total RNA was isolated from WT and RNH1-KO K562 cells and analyzed using Fragment Analyzer. The data are presented as electropherograms (left) and digital gels (right). The blue peak corresponds to the 28S region and the pink peak to the 18S region. Data are representative of three independent experiments.

**Supplemental Figure. 10**

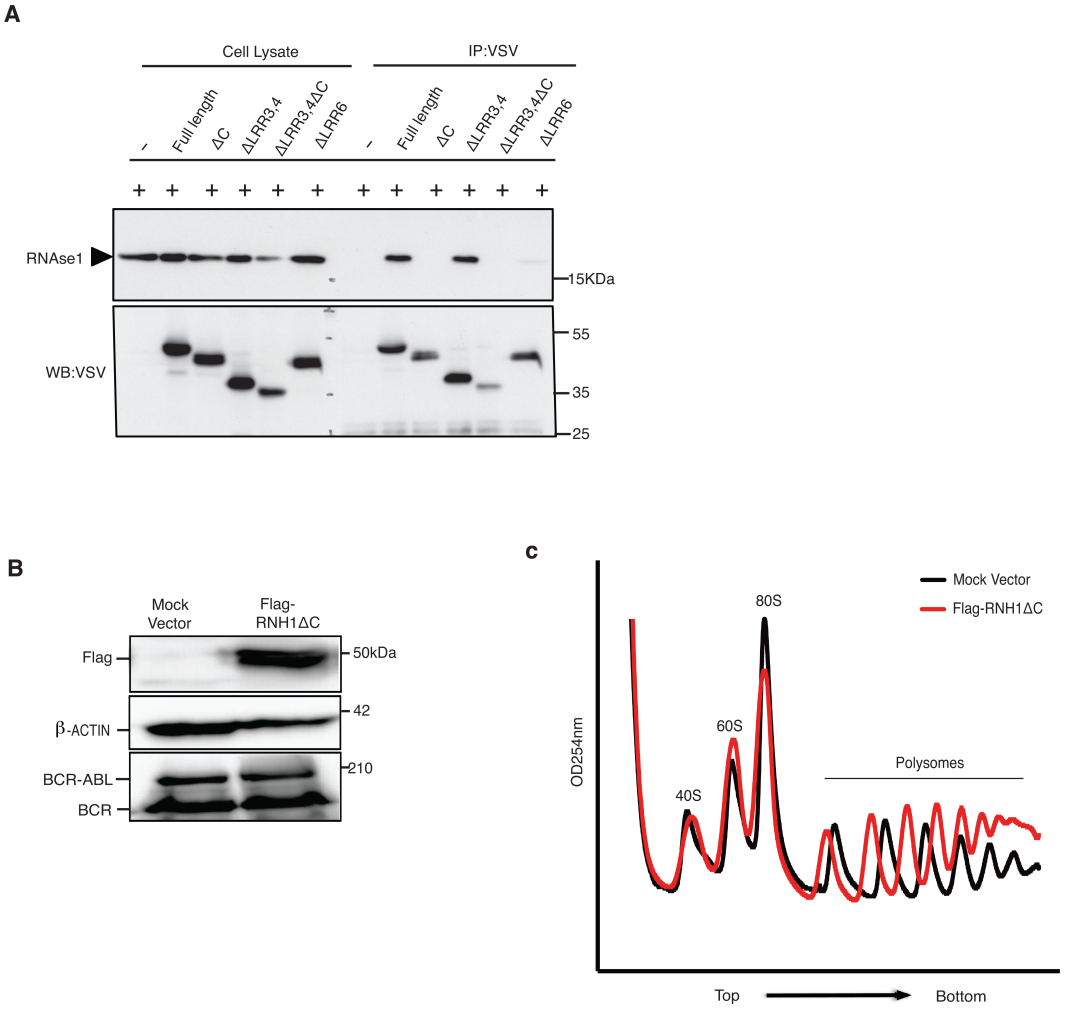



**Figure 10. RNH1 stabilizes polysomes independent of RNAse inhibitor function.**

**(A)** Screening of RNH1 mutants for binding to RNase1. ΔC is C-terminal deletion mutant (435-460). Δ LRR3,4 (144-257) and Δ LRR 6 (315-371) are internal deletion mutants. Co-immunoprecitation experiments were performed with extracts of HEK293T cells transiently expressing full length Flag-RNase1 and VSV-RNH1 mutants, followed by immunoblotting. Blots are representative of three independent experiments. **(B)** Western blot analysis of RNH1(Flag) expression in K562 cells stably transfected with an empty control vector or with Flag-RNH1ΔC. Blots are representative of three independent experiments. **(C)** Sucrose gradient polysome profiles for mock- or RNH1ΔC -expressing stable K562 cells. Arrows show direction of the sucrose gradient from less to more dense. Data are representative of three independent experiments.

**Supplemental Figure. 11**

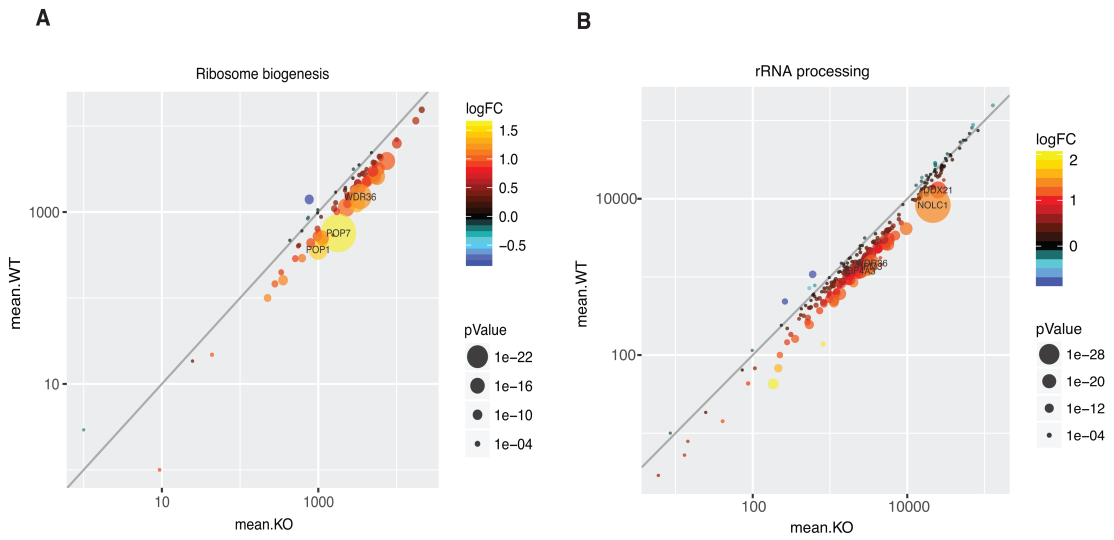



**Figure 11. No decrease of ribosome biogenesis and rRNA processing related gene expression in RNH1- deficient K562 cells**

**(A** and **B**) LogFC plots show the up and down regulated ribosome biogenesis (A) and rRNA processing genes (B) in RNH1-KO cells compared to the WT cells. The x and y axes show the expression levels for each gene as normalized read counts in the KO and WT cells respectively. The colour of each dot reflects the log2 fold change and the size of a dot reflects the adjusted P-value of a gene.

**Supplemental Figure. 12**

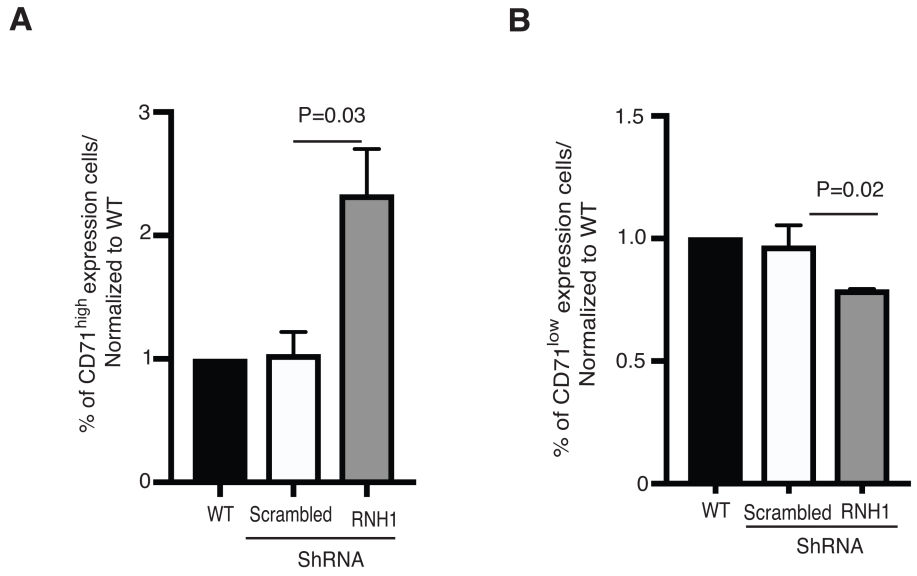



**Figure 12. Decreased erythroid maturation in RNH1-knodown CD34+ HSPCs**

**(A** and **B)** Percentage of CD71 high and low expressing cells on day 12 of erythroid differentiation (N=3). Values were normalized to WT. Data were expressed as mean ± SD (right). *P* values determined by two-tailed *t*-test.

**Supplementary Tables were included in separate file**

**Supplementary Table. 1**

List of genes that were up regulated and down regulated in the gene array experiments.

**Supplementary Table. 2**

A complete list of significant phenotypes enriched among down-regulated genes in *Rnh1*$^{-/-}$ mice using MouseMine tool (http://www.mousemine.org)

**Supplementary Table. 3**

Complete list of GSEA results and target gene sets

**Supplementary Table. 4**

List of genes that were regulated in RNA-seq experiments.

**Supplementary Table. 5**

Complete list of proteins specific to Flag-RNH1that were identified in mass spectrometry experiments.

**Supplementary Table. 6**

List of primer sequences used for real-time PCR

**Figure 5 C**

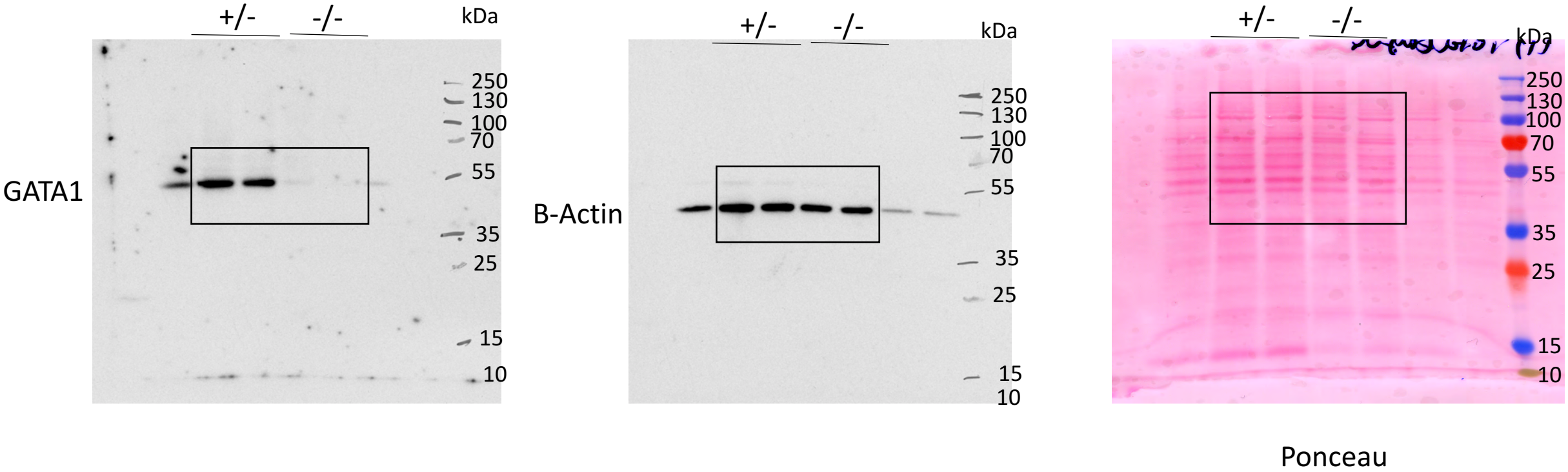

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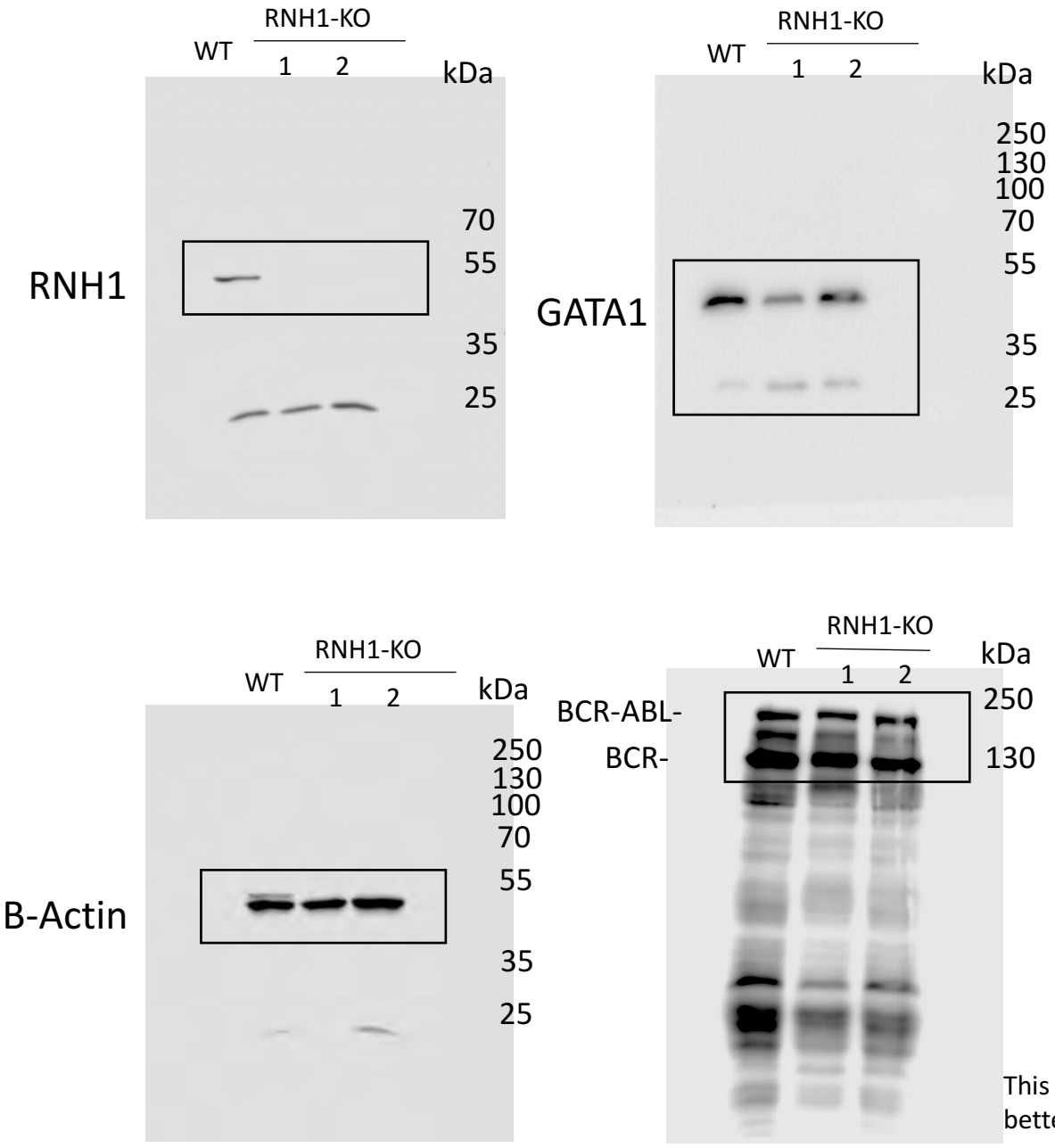
**Figure 7A**




This is separate blot with same samples run in 10% gel to get better resolution for high molecular weight proteins

**Figure 8A**

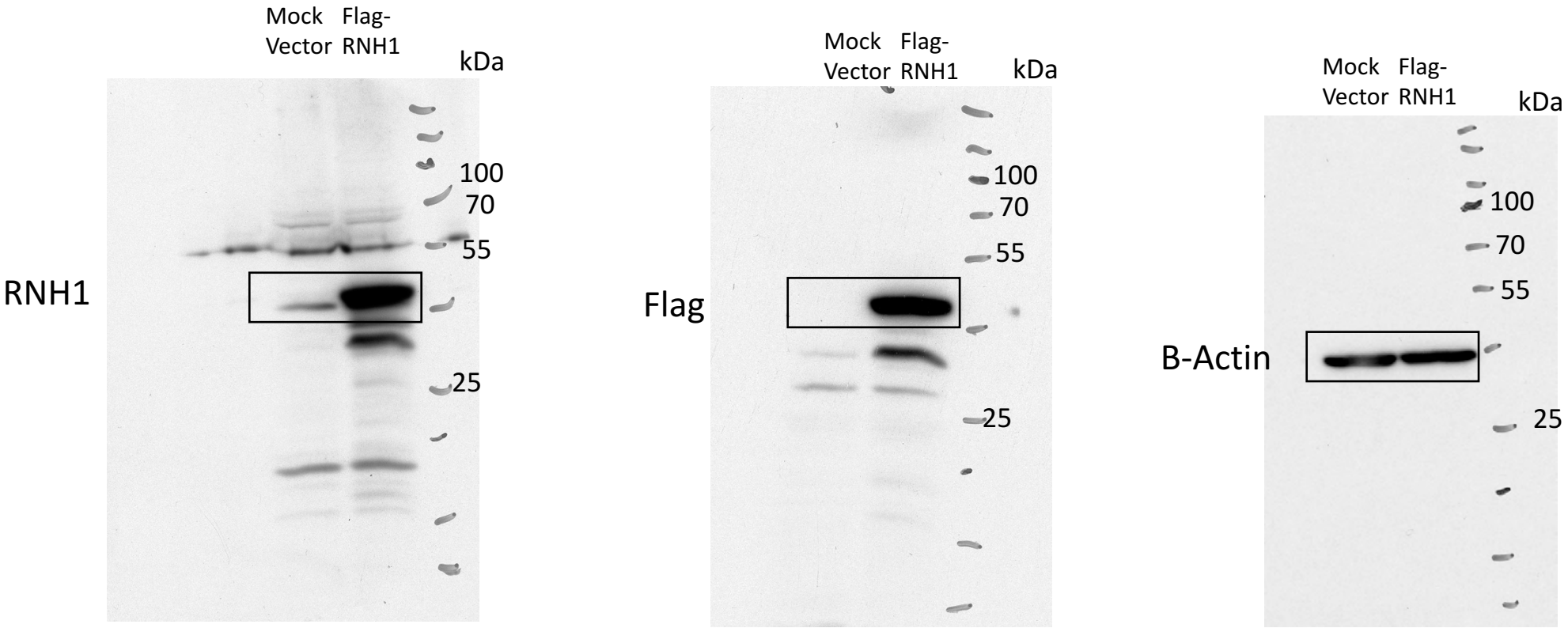
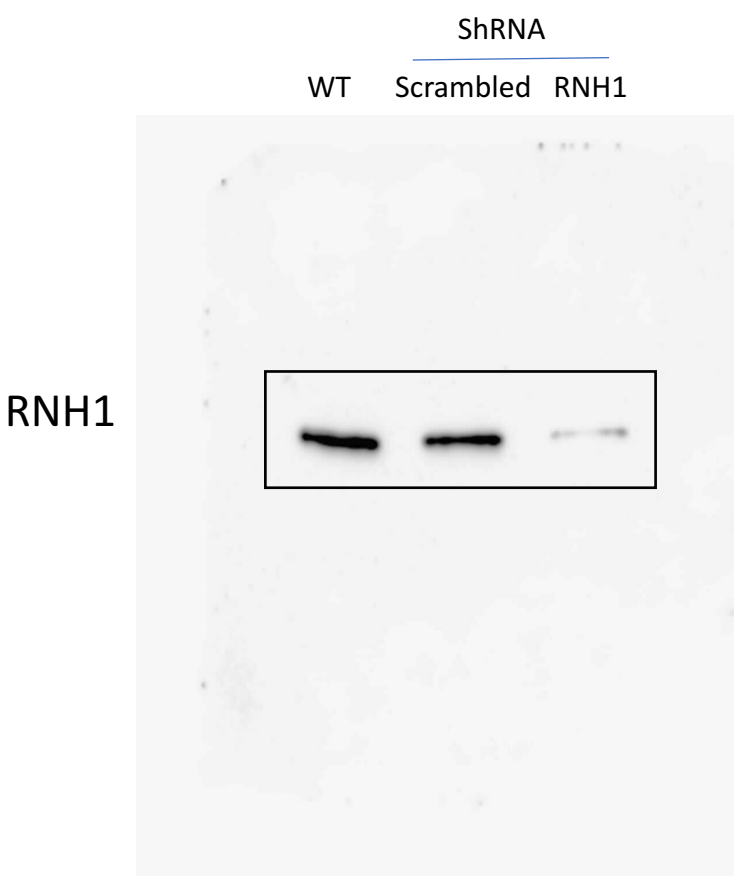
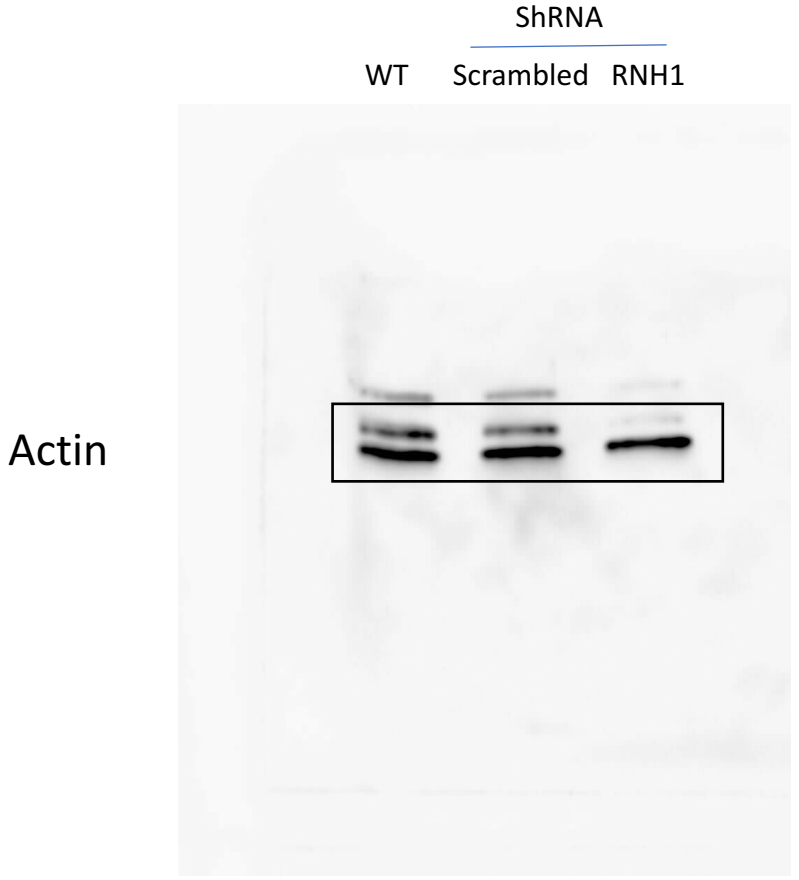


**Figure 9B**



# Figure 9H

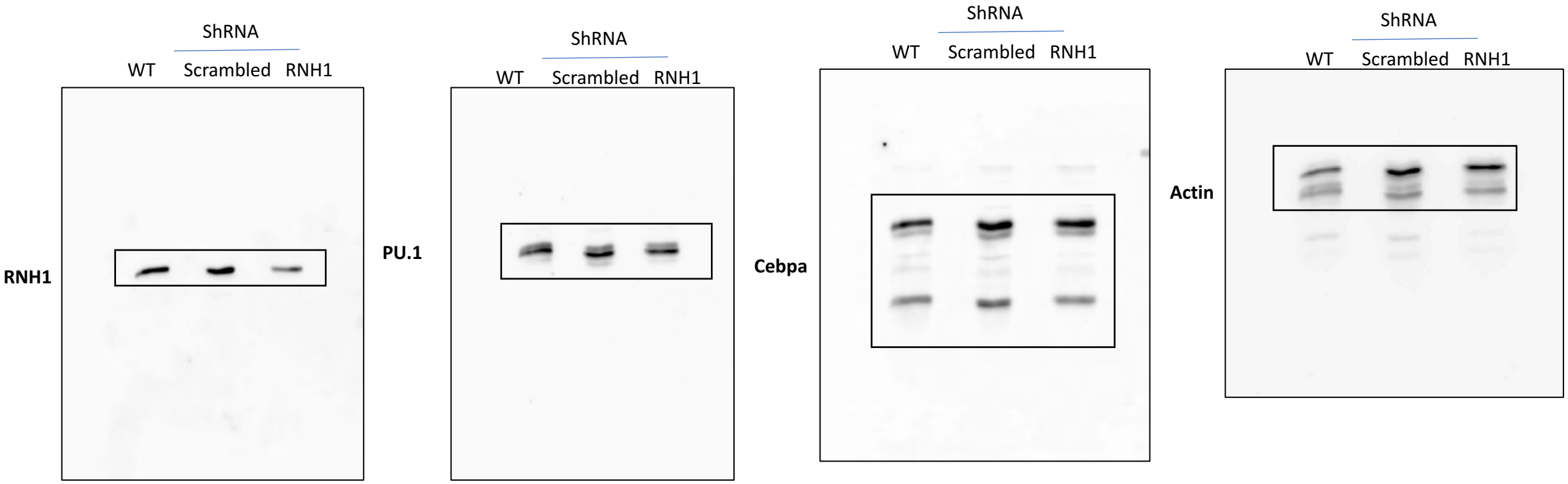
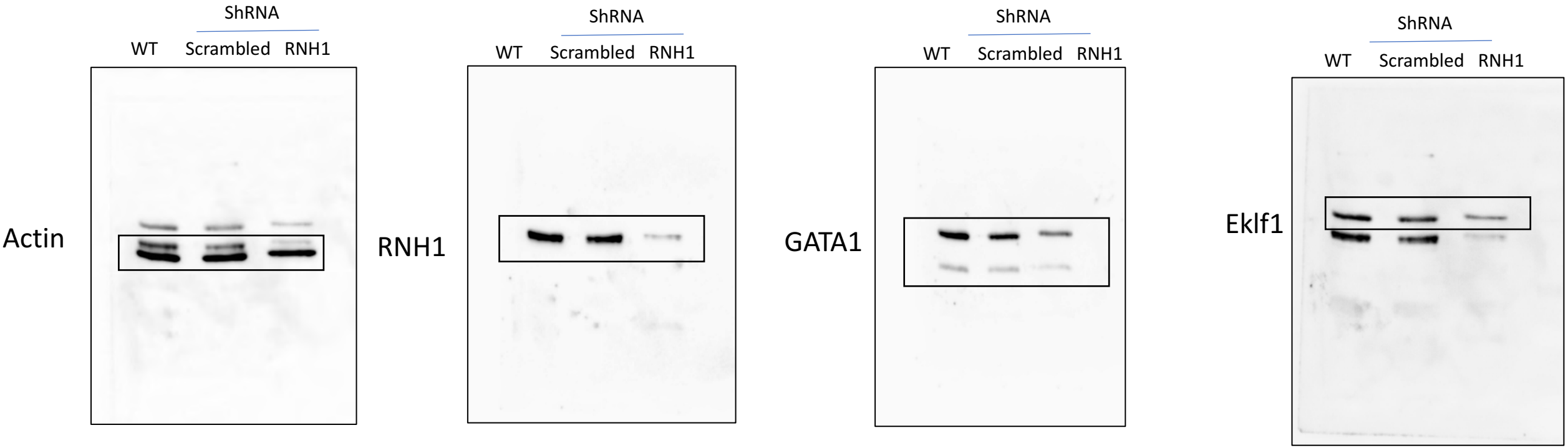


**Figure 9J**



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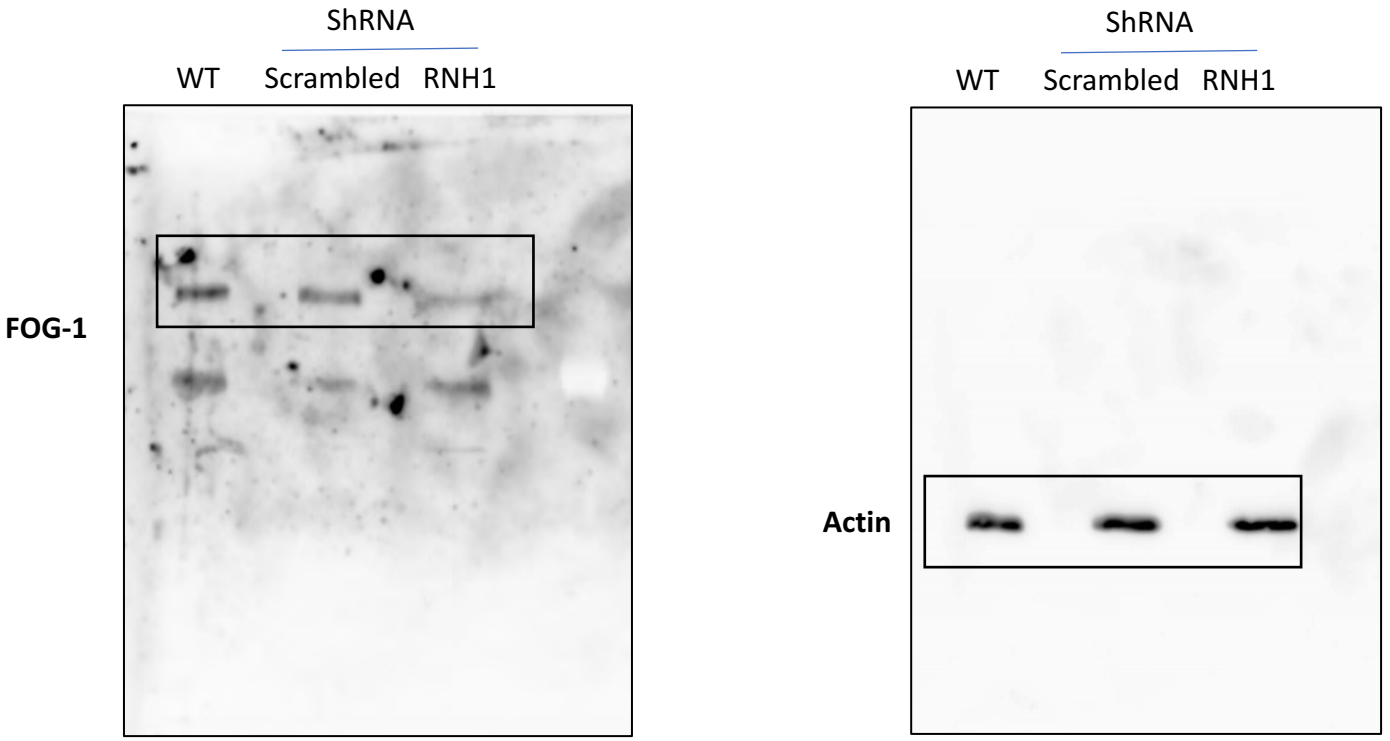

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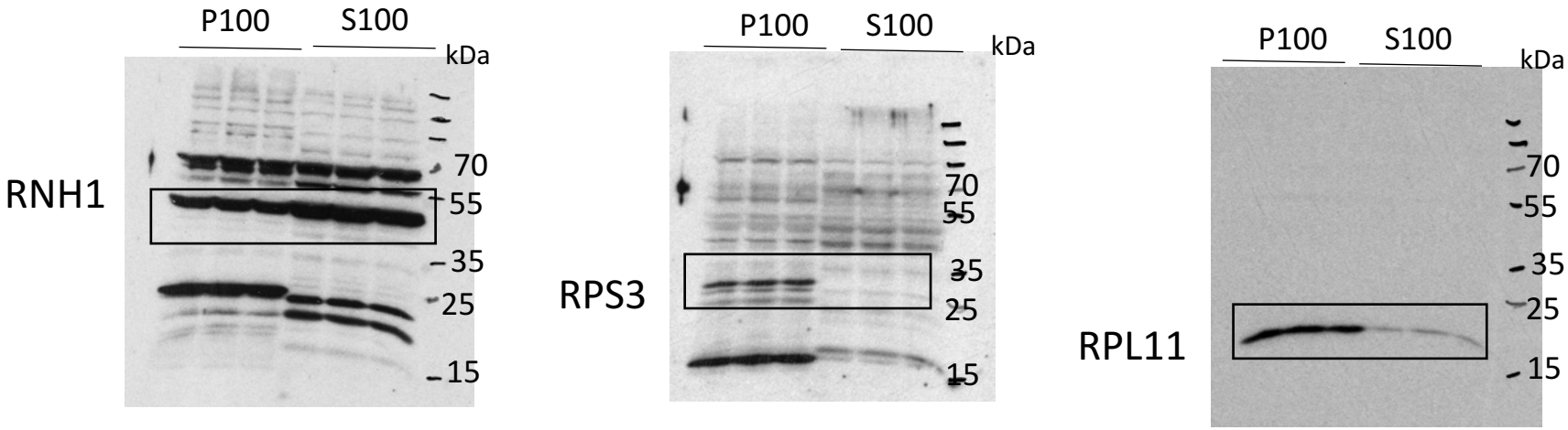
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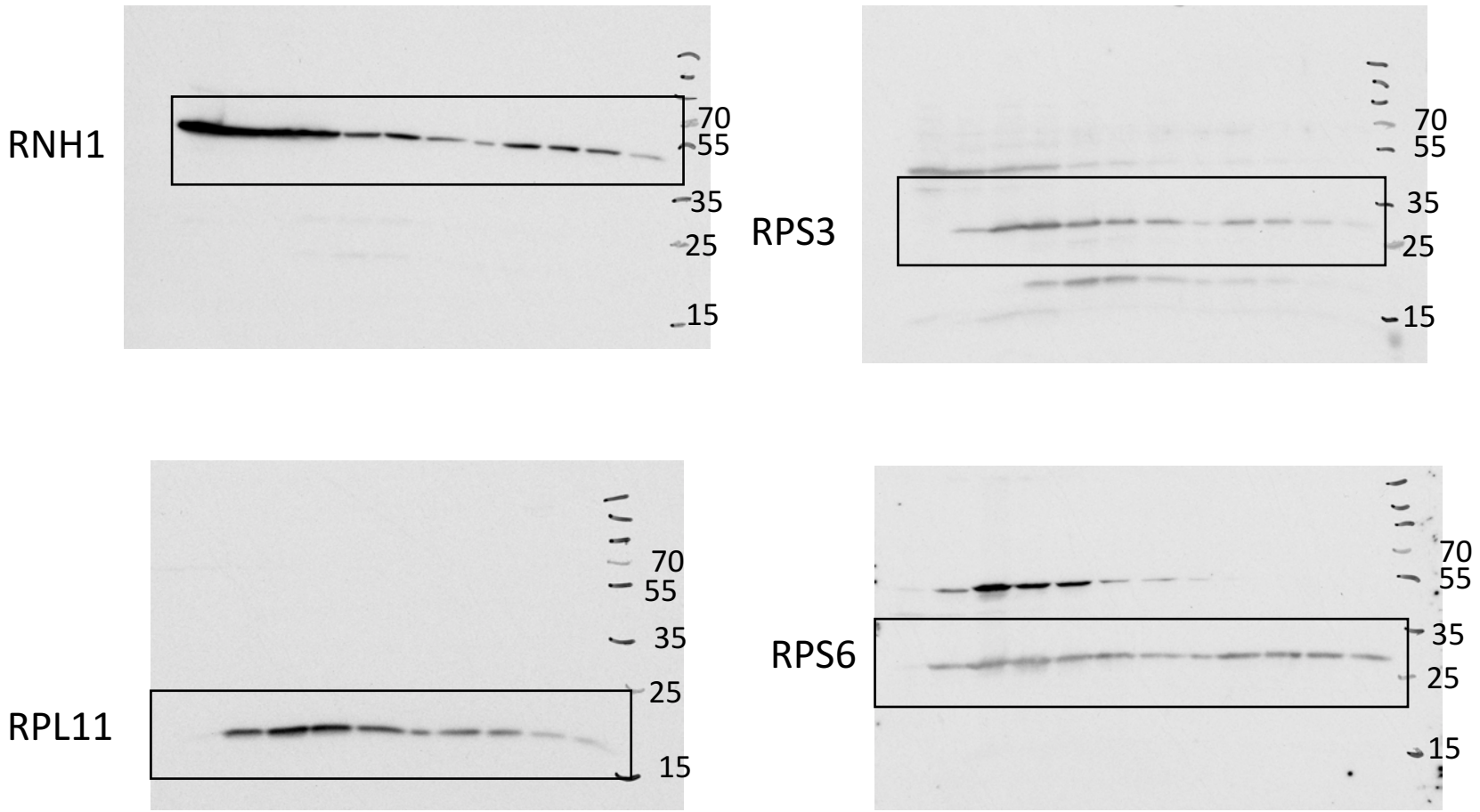
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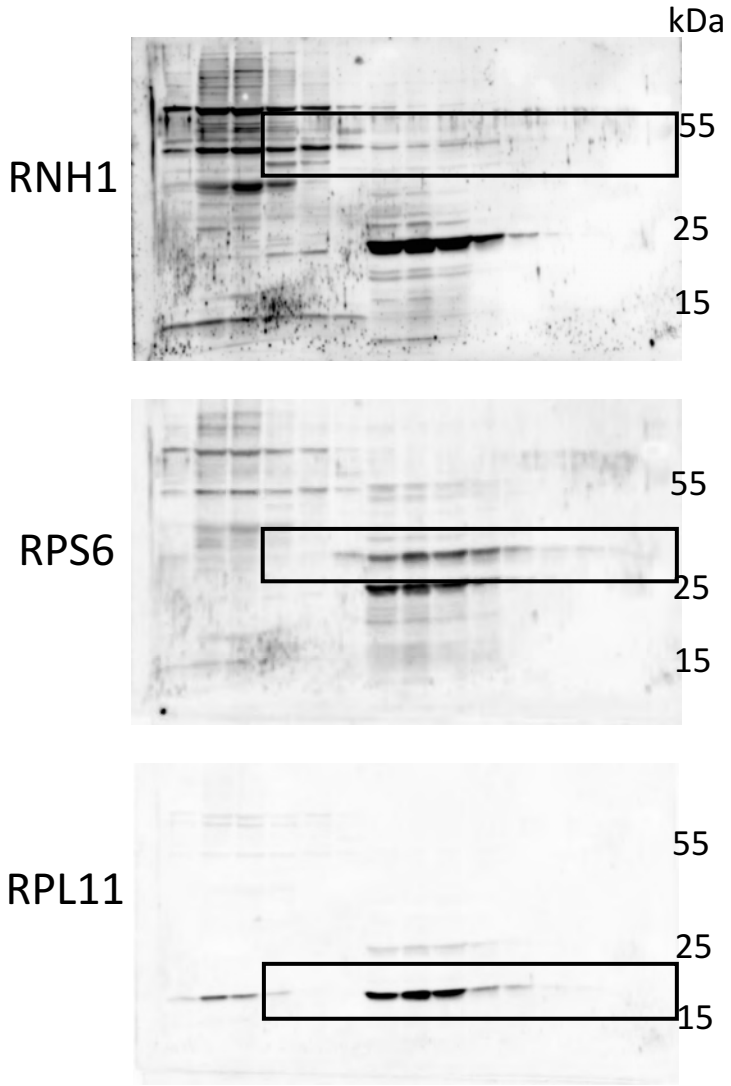
**Figure 10C**



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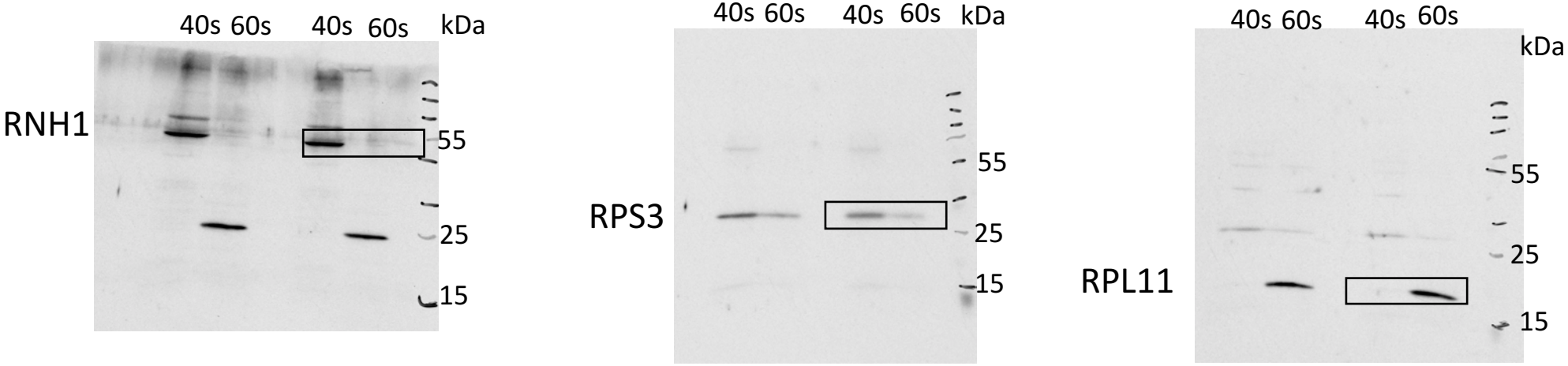
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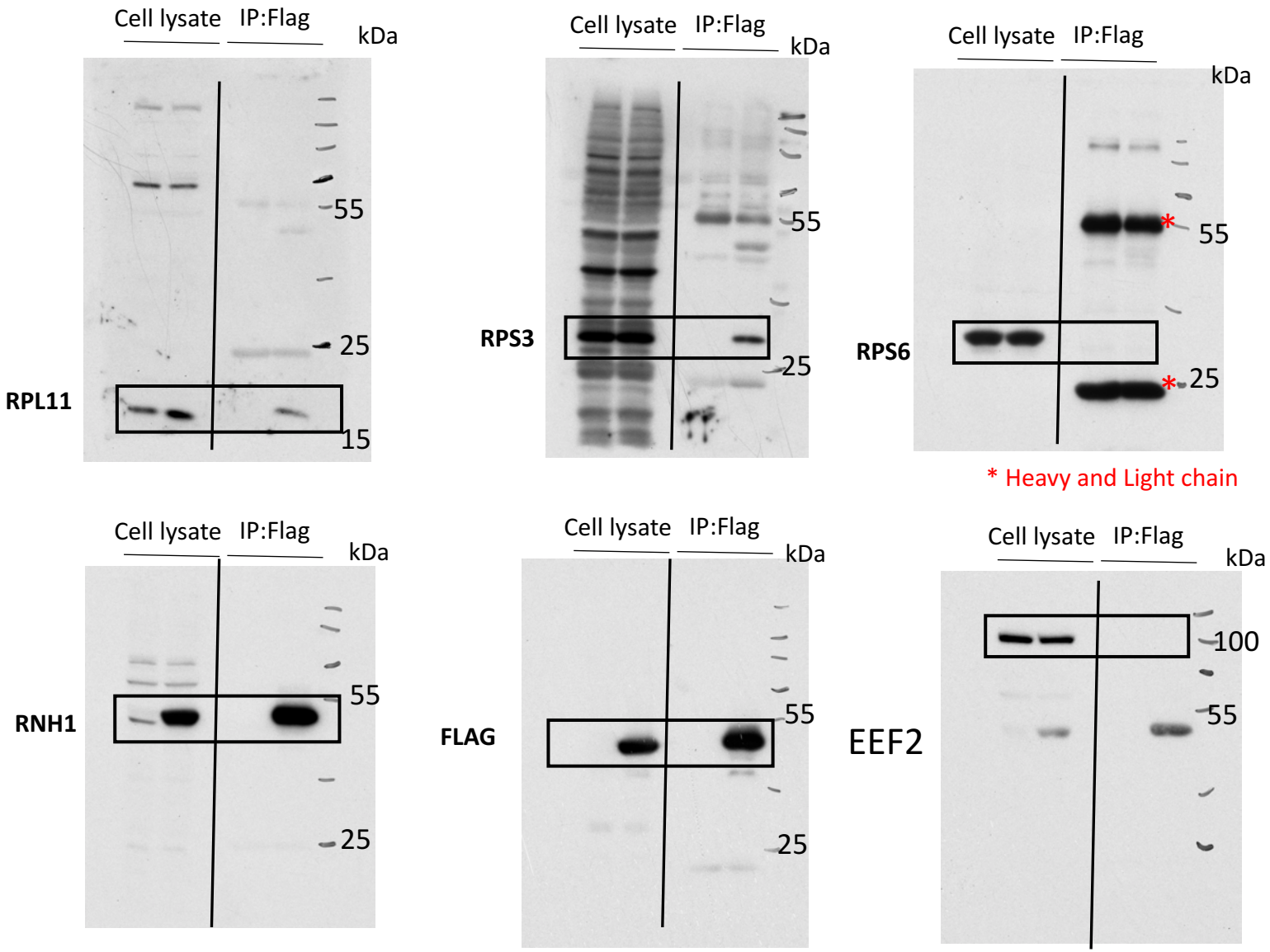
# Figure 11B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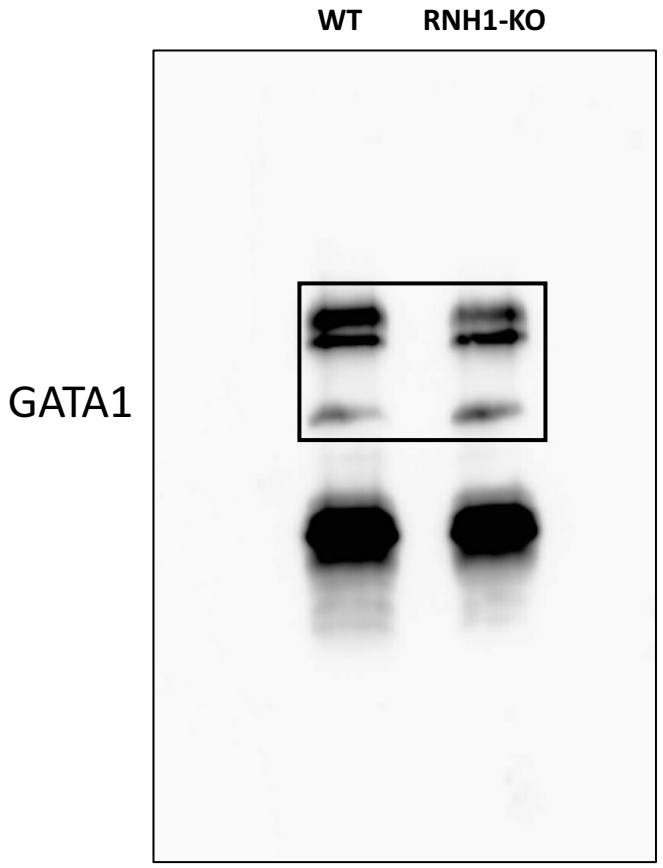
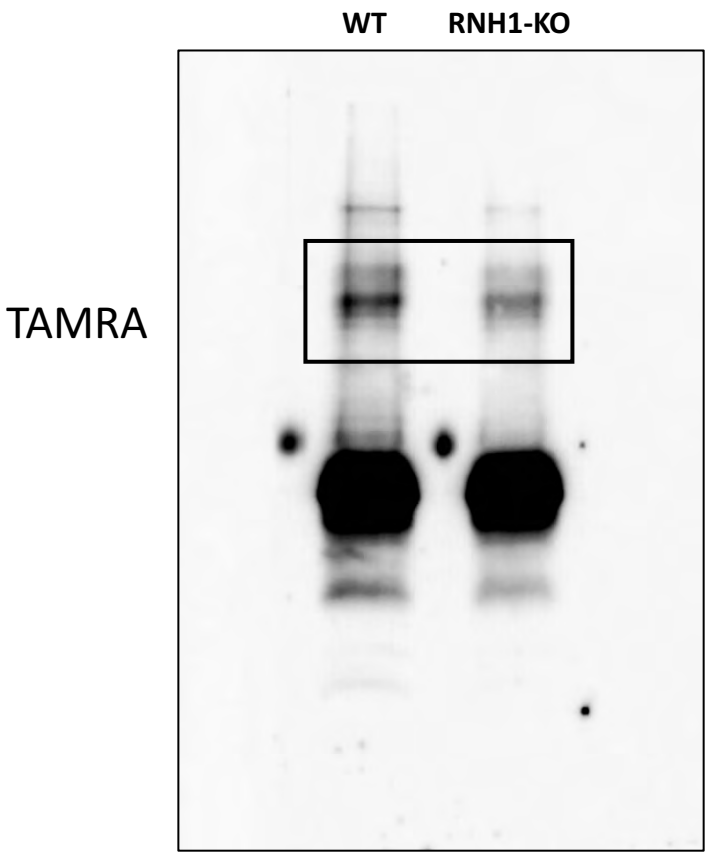
Supplemental Figure 6 A

Supplemental Figure 6 B




Supplemental Figure 6 C



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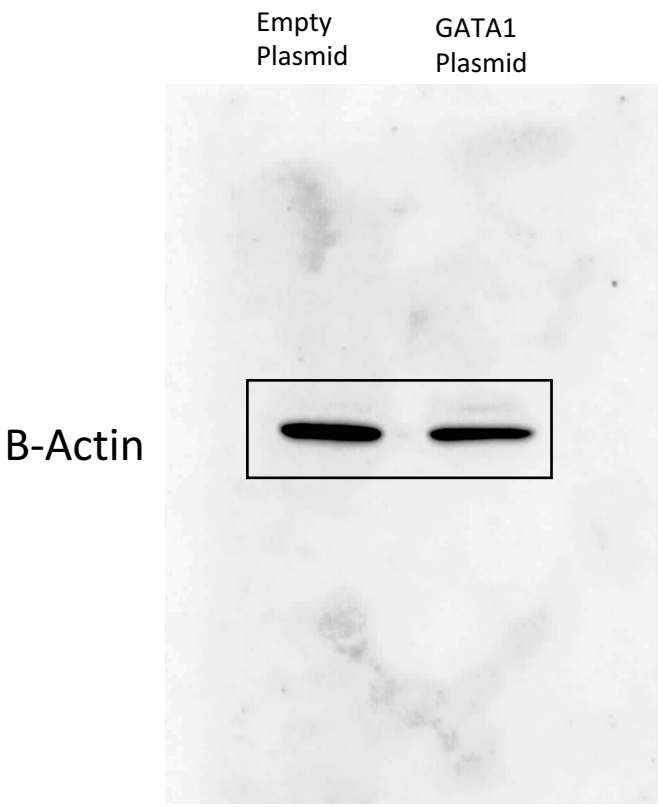
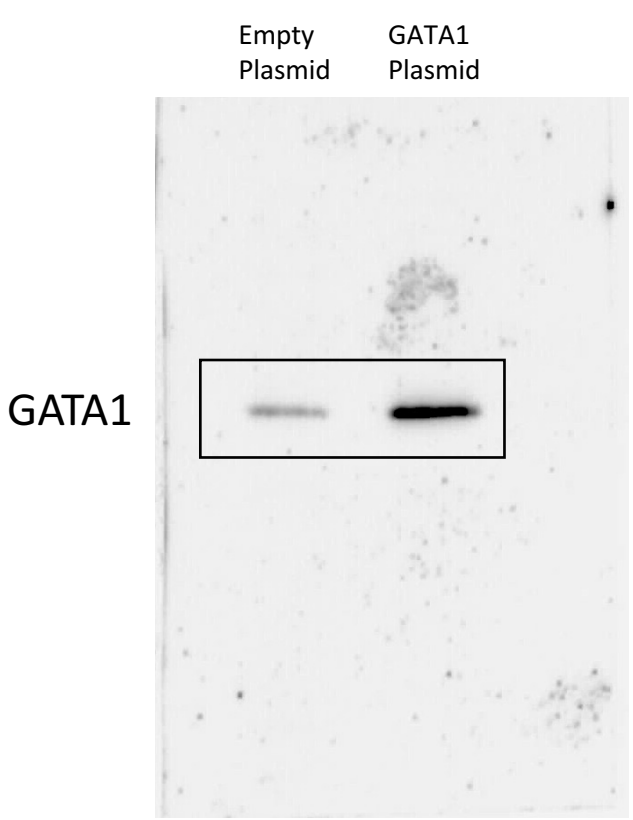
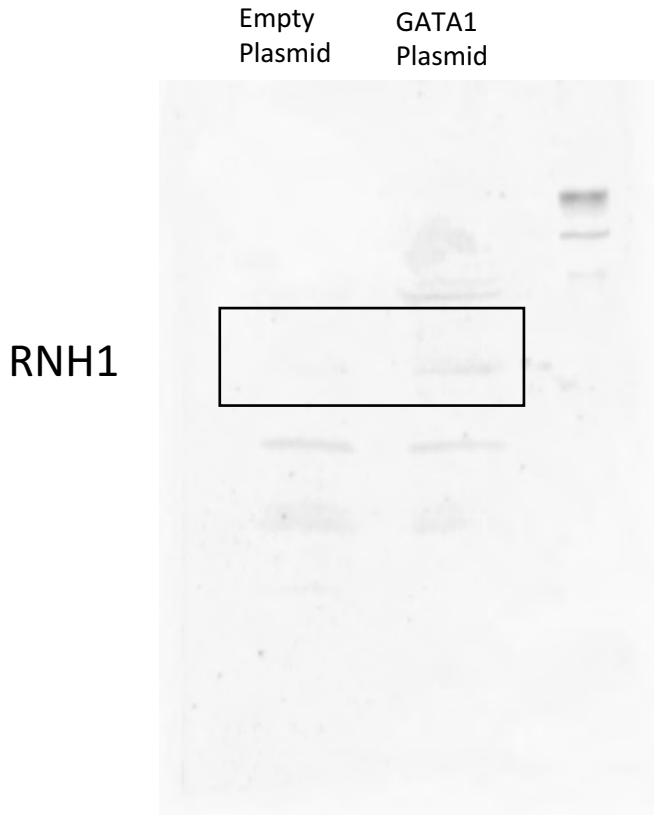
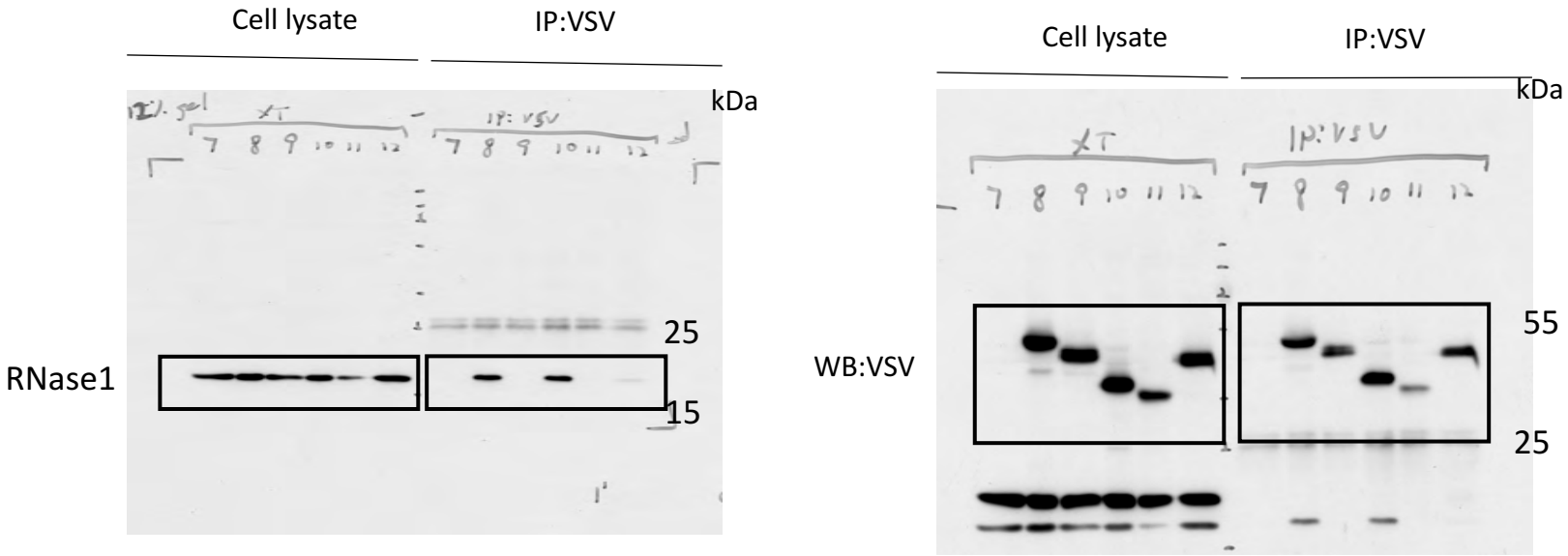


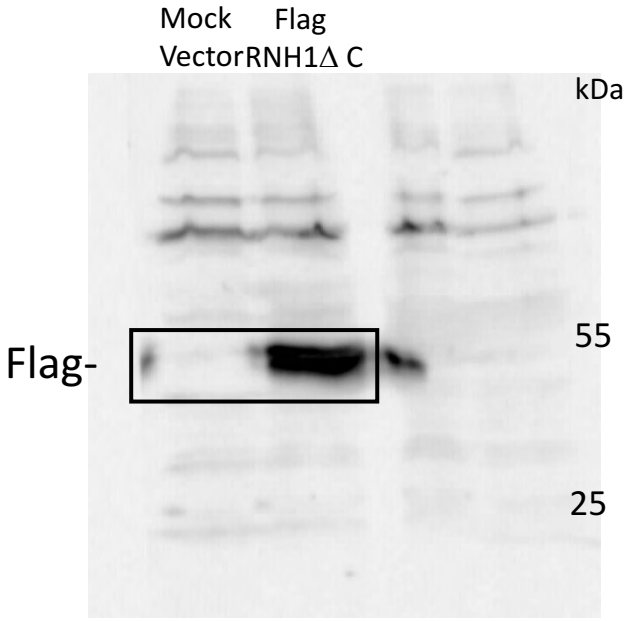



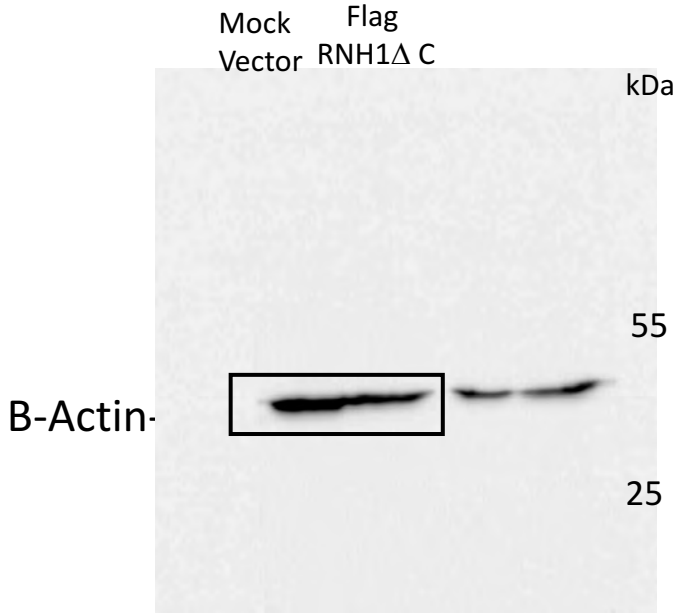



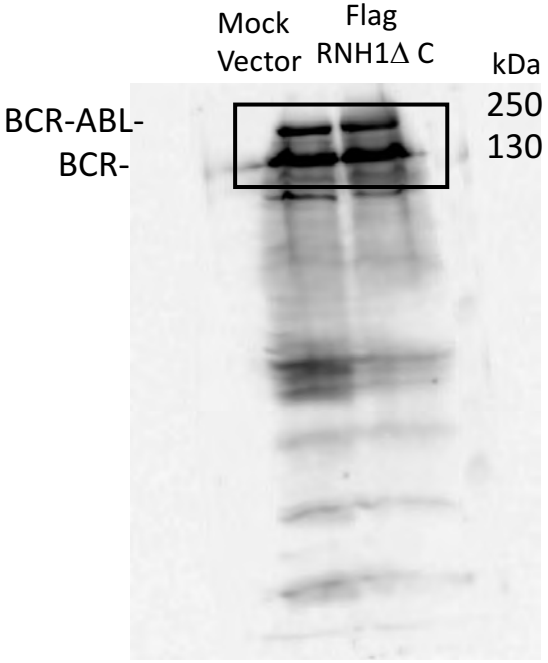
Supplemental Figure 10A



Supplemental Figure 10 B




This is separate blot with same samples run in 10% gel to get better resolution for high molecular weight proteins