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Supplementary Materials for

The ufmylation cascade controls COPII recruitment, anterograde transport, and sorting of nascent GPCRs at ER

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The PDF file includes:

Figs. S1 to S9
Legends for movies S1 and S2
Uncropped gels

Other Supplementary Material for this manuscript includes the following:

Movies S1 and S2

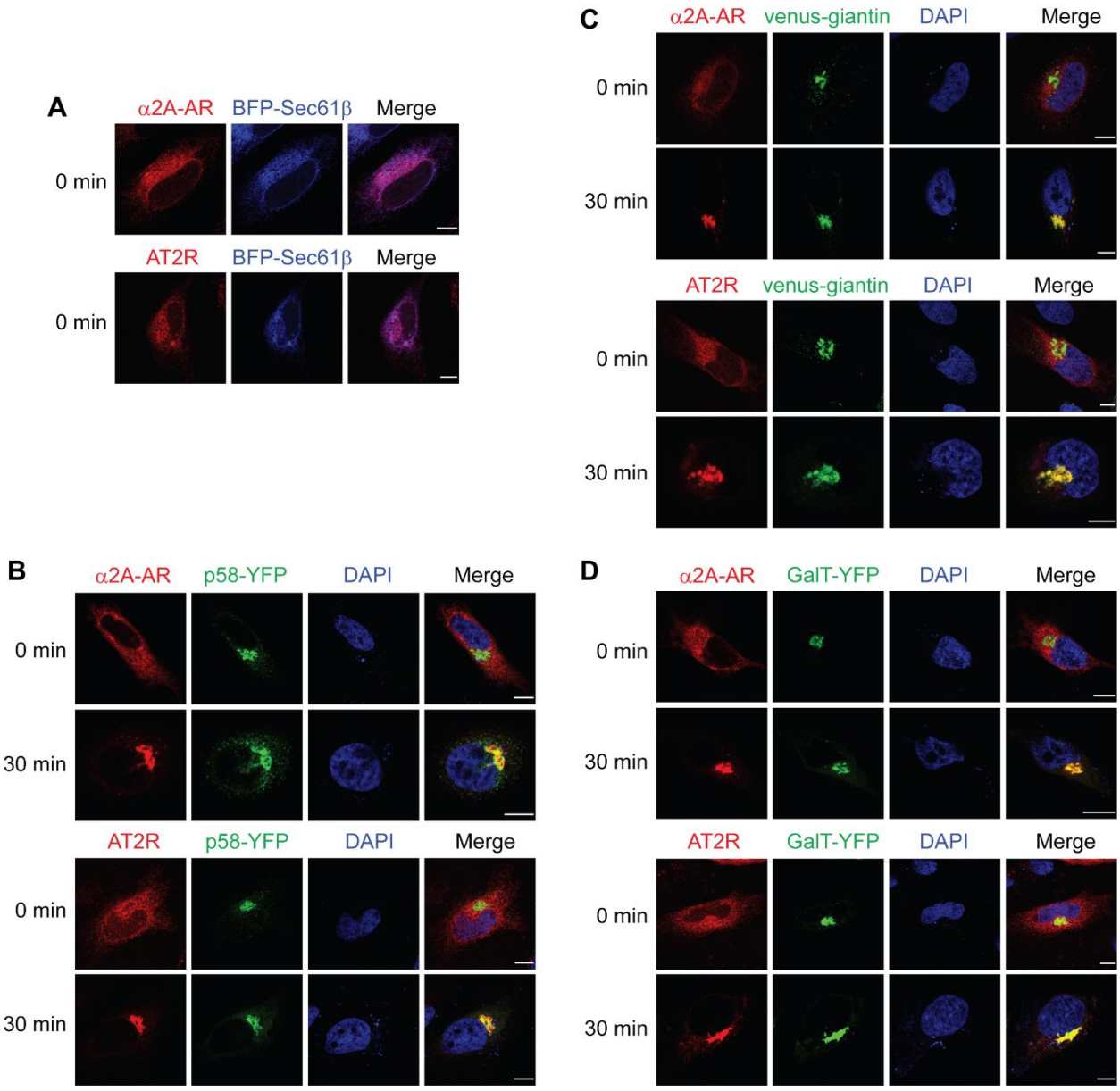


Fig. S1. Colocalization of α_{2A}-AR and AT2R with ER and Golgi markers in RUSH assays. Colocalization of α_{2A}-AR and AT2R with the ER marker Sec61β (**A**), the ERGIC marker p58 (**B**), the cis- and medial-Golgi marker giantin (**C**) and the trans-Golgi and trans-Golgi network marker Gal T (**D**). HeLa cells were transfected with RUSH plasmids expressing mCherry-tagged α_{2A}-AR or AT2R together with BFP-Sec61β (A), p58-YFP (B), Venus-giantin (C) or Gal T-YFP (D) for 24 h and then incubated with biotin for 30 min. Similar results were obtained in at least 3 repeats. Scale bars, 10 μm.

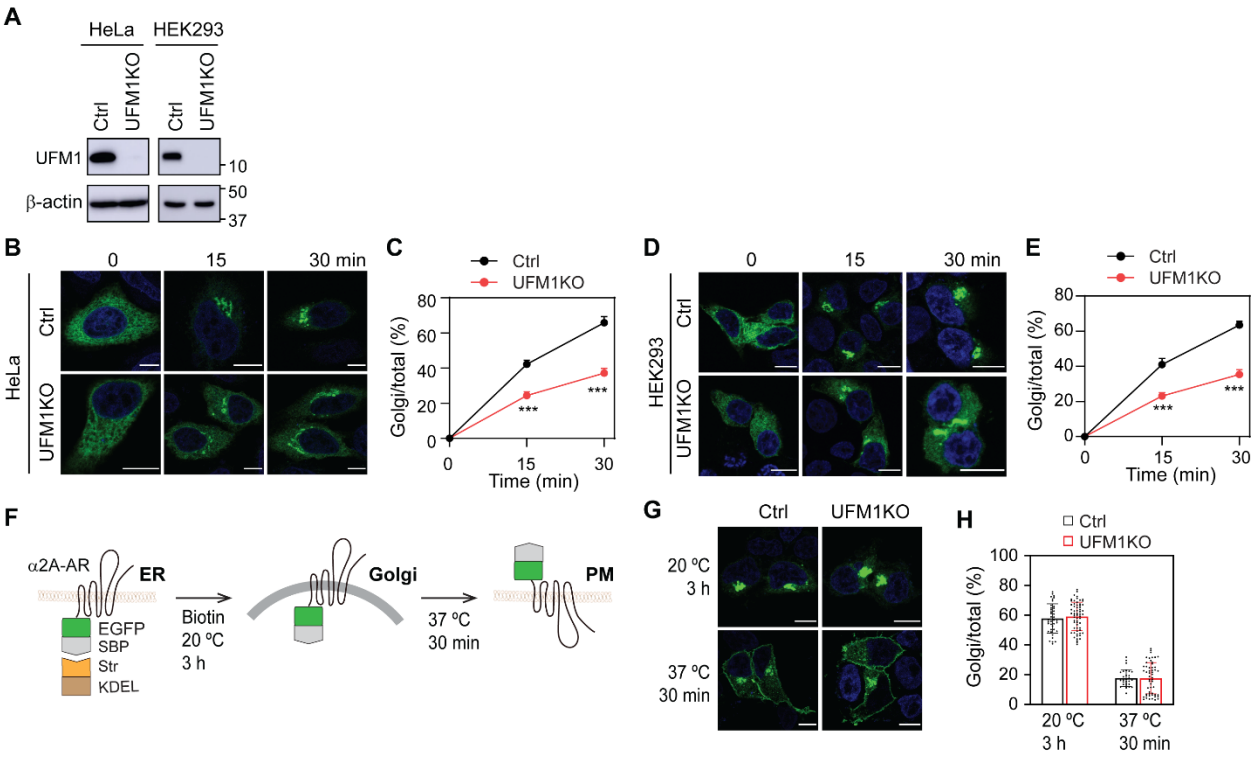


Fig. S2. UFM1 KO inhibits the ER-Golgi transport of α_{2A}-AR, but not its Golgi-PM transport, in HeLa and HEK293 cells. (**A**) Expression of UFM1 in control and KO cells. (**B** and **D**) Representative images showing the ER-Golgi transport of α_{2A}-AR in control and UFM1 KO HeLa (B) and HEK293 cells (D). The cells were transfected with α_{2A}-AR in RUSH plasmids and then treated with biotin for 15 and 30 min. (**C** and **E**) Quantitative data shown in (B) and (D). (**F**) The RUSH system in combination with the temperature-induced block to measure the Golgi-PM transport. (**G**) Images showing the Golgi-PM transport of α_{2A}-AR in control and UFM1 KO cells. HeLa cells transfected with α_{2A}-AR in RUSH plasmids were treated with biotin plus CHX at 20 °C for 3 h and then incubated at 37 °C for 30 min. (**H**) Quantitative data shown in (G). The quantitative data are mean ± SE; n = 35-51 cells in (C), 30-57 cells in (E) and 28-63 cells in (H), each from at least 3 separate experiments. ***$p < 0.001$ versus control. Scale bars, 10 μm.

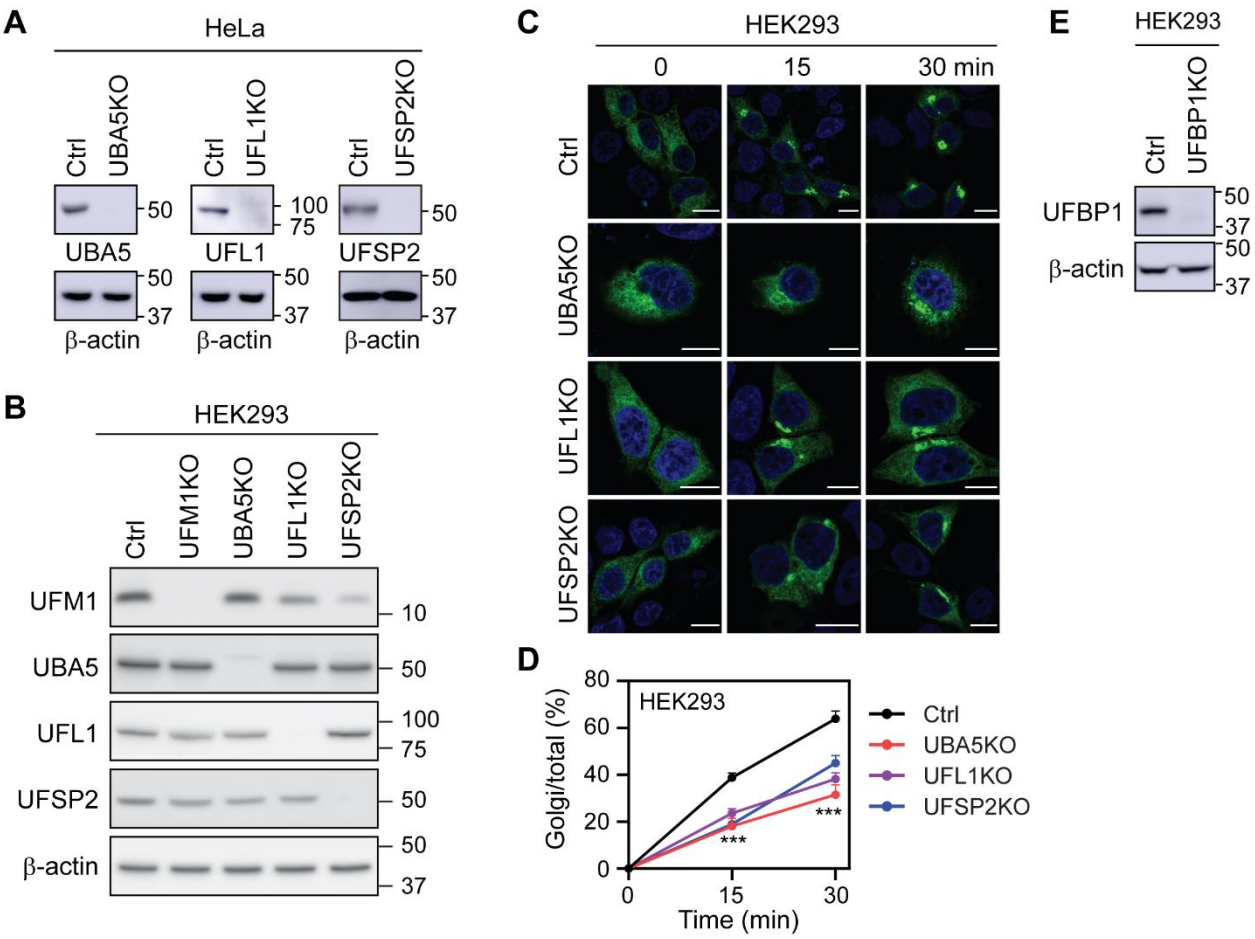


Fig. S3. Depletion of UBA5, UFL1 and UFSP2 attenuates the ER-Golgi transport of α_{2A}-AR in HEK293 cells. (**A** and **B**) Expression of UBA5, UFL1 and UFSP2 in control and KO HeLa (A) and HEK293 cells (B). (**C**) Representative images showing the ER-Golgi transport of α_{2A}-AR in control and KO cells. HEK293 cells transfected with α_{2A}-AR in RUSH plasmids were treated with biotin for 15 and 30 min. (**D**) Quantitative data shown in (C). (**E**) Expression of UFBP1 in control and KO HEK293 cells. The quantitative data are mean ± SE; n = 21-43 cells from 3 experiments. ***p < 0.001 between individual KO and control. Scale bars, 10 μm.

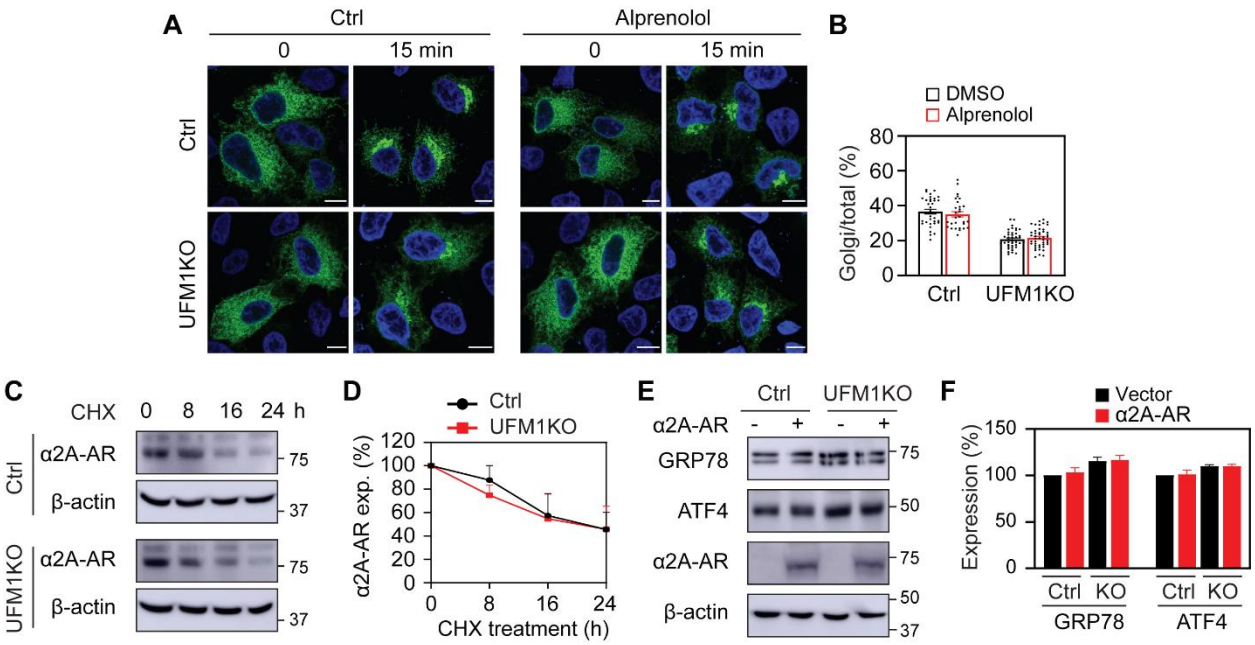


Fig. S4. UFM1 KO does not affect the folding and degradation of GPCRs and UPR. (**A**) Images showing the effect of alprenolol treatment on the ER-Golgi transport of β_2-AR in RUSH assays. Control and UFM1 KO HeLa cells were transfected with β_2-AR in RUSH plasmids for 6 h and treated with alprenolol at 10 μM for 18 h before incubation with biotin for 15 min. (**B**) Quantitative data shown in (A). (**C**) Degradation of α_{2A}-AR in control and UFM1 KO HEK293 cells. The cells were transfected with α_{2A}-AR-GFP in RUSH plasmids for 24 h and then treated with CHX for 8, 16 and 24 h. α_{2A}-AR expression was measured by immunoblotting using GFP antibodies. (**D**) Quantitative data shown in (C). (**E**) Effects of α_{2A}-AR on the expression of GRP78 and ATF4 in control and UFM1 KO HEK293 cells. The cells were transfected with α_{2A}-AR-GFP in RUSH plasmids for 24 h and the expression of GRP78 and ATF4 was measured by immunoblotting. (**F**) Quantitative data shown in (E). The quantitative data are mean ± SE; n = 22-37 cells in (B) from 3 repeats and 3-4 in (D) and (F). Scale bars, 10 μm.

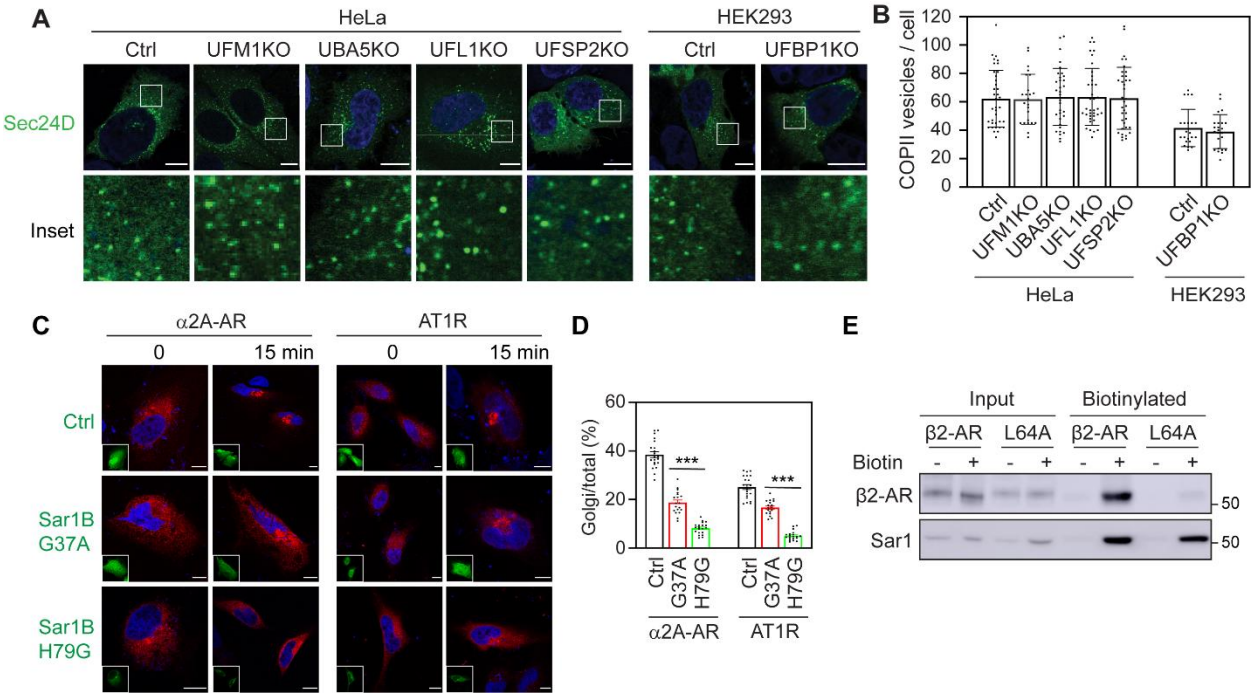


Fig. S5. COPII formation in ufmylation KO cells and COPII-mediated ER-Golgi transport of GPCRs. (**A**) Representative images showing the punctate structures containing GFP-Sec24D in control and KO cells after transfection for 24 h. (**B**) Quantitative data shown in (A). (**C**) Images showing the effect of Sar1B mutants, G37A and H79G, on the ER-Golgi transport of α_{2A}-AR and AT1R in RUSH assays. HeLa cells were transfected with mCherry-tagged α_{2A}-AR or AT1R in RUSH plasmids together with GFP (Ctrl) or GFP-tagged Sar1B mutants for 20 h and then incubated with biotin for 15 min. Insets show expression of GFP or GFP-Sar1B. (**D**) Quantitative data shown in (C). (**E**) Sar1B-BirA*-mediated biotinylation of β_2-AR and its mutant L64. HEK293 cells were transfected with HA-tagged β_2-AR or L64 plus Myc-tagged Sar1B-BirA* and then incubated with (+) or without (-) biotin. β_2-AR and Sar1 were measured by using HA and Myc antibodies, respectively. Similar results were obtained in 2 experiments. Input contains 3% of the total proteins used in the experiments. The quantitative data are mean ± SE; n = 29-45 cells in (B) and 18-22 cells in (D), each from 3 separate experiments. ***p < 0.001 versus control. Scale bars, 10 μm..

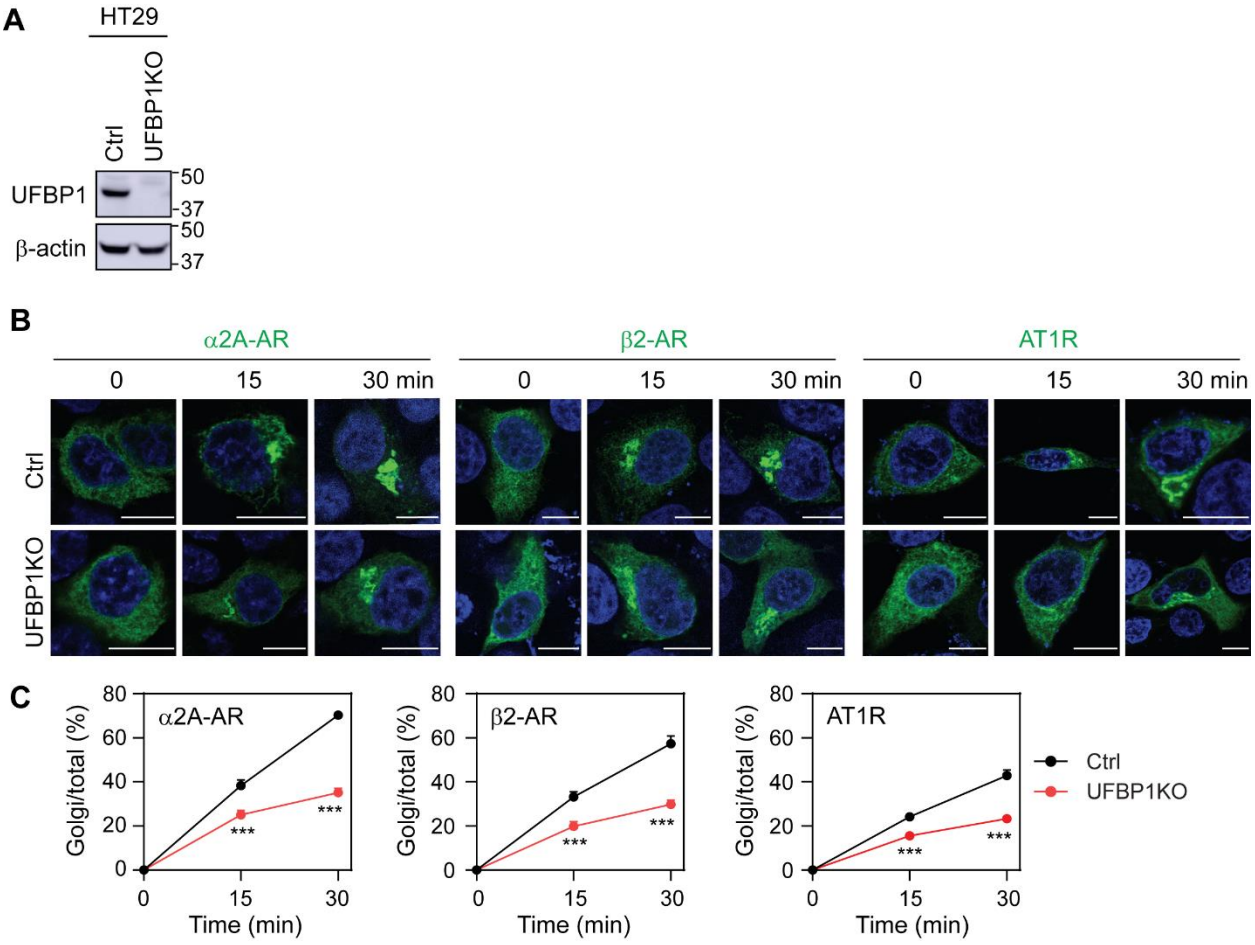


Fig. S6. UFBP1 KO impedes the ER-Golgi transport of α_{2A}-AR, β_2-AR and AT1R in HT29 cells. (**A**) Expression of UFBP1 in control and KO HT29 cells. (**B**) Images showing the ER-Golgi transport of the receptors in control and KO HT29 cells. The cells were transfected with individual GPCRs in RUSH plasmids and treated with biotin for 15 and 30 min. (**C**) Quantitative data shown in (B). The quantitative data are mean ± SE; n = 26-42 cells from 3 separate experiments. ***p < 0.001 versus control. Scale bars, 10 μm.

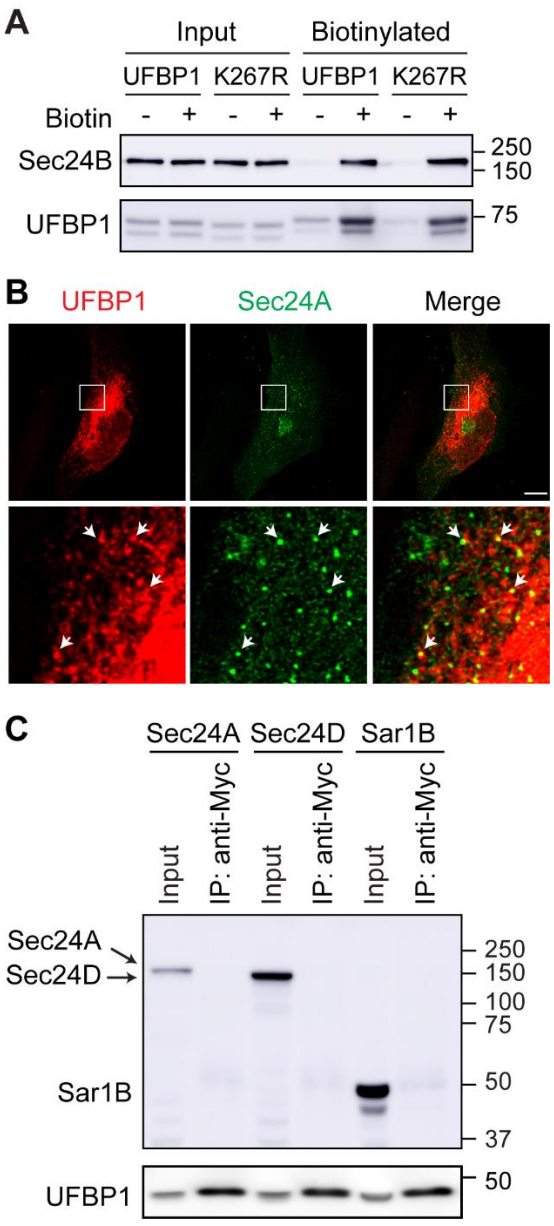


Fig. S7. UFBP1 localizes at COPII, but does not interact with COPII components. (**A**) Biotinylation of Sec24B by UFBP1-BirA* and UFBP1 K267R-BirA*. HEK293 cells were transfected with GFP-Sec24B together with UFBP1-BirA* or K267R-BirA* and then incubated with (+) or without (-) biotin. (**B**) Colocalization of UFBP1 and Sec24A. HeLa cells were transfected with UFBP1-RFP and GFP-Sec24A. Arrows indicate the colocalization of UFBP1 and Sec24A in COPII vesicles. (**C**) Co-IP of UFBP1 with COPII components. HEK293 cells were transfected with Myc-UFBP1 together with GFP-tagged Sec24A, Sec24D or Sar1B and subjected to IP with Myc antibodies. Sec24 and Sar1 were measured by using GFP antibodies and UFBP1 was detected by UFBP1 antibodies. Input contains 3% of the total proteins used in the experiments. Similar results were obtained in 2 experiments. Scale bar, 10 μm.

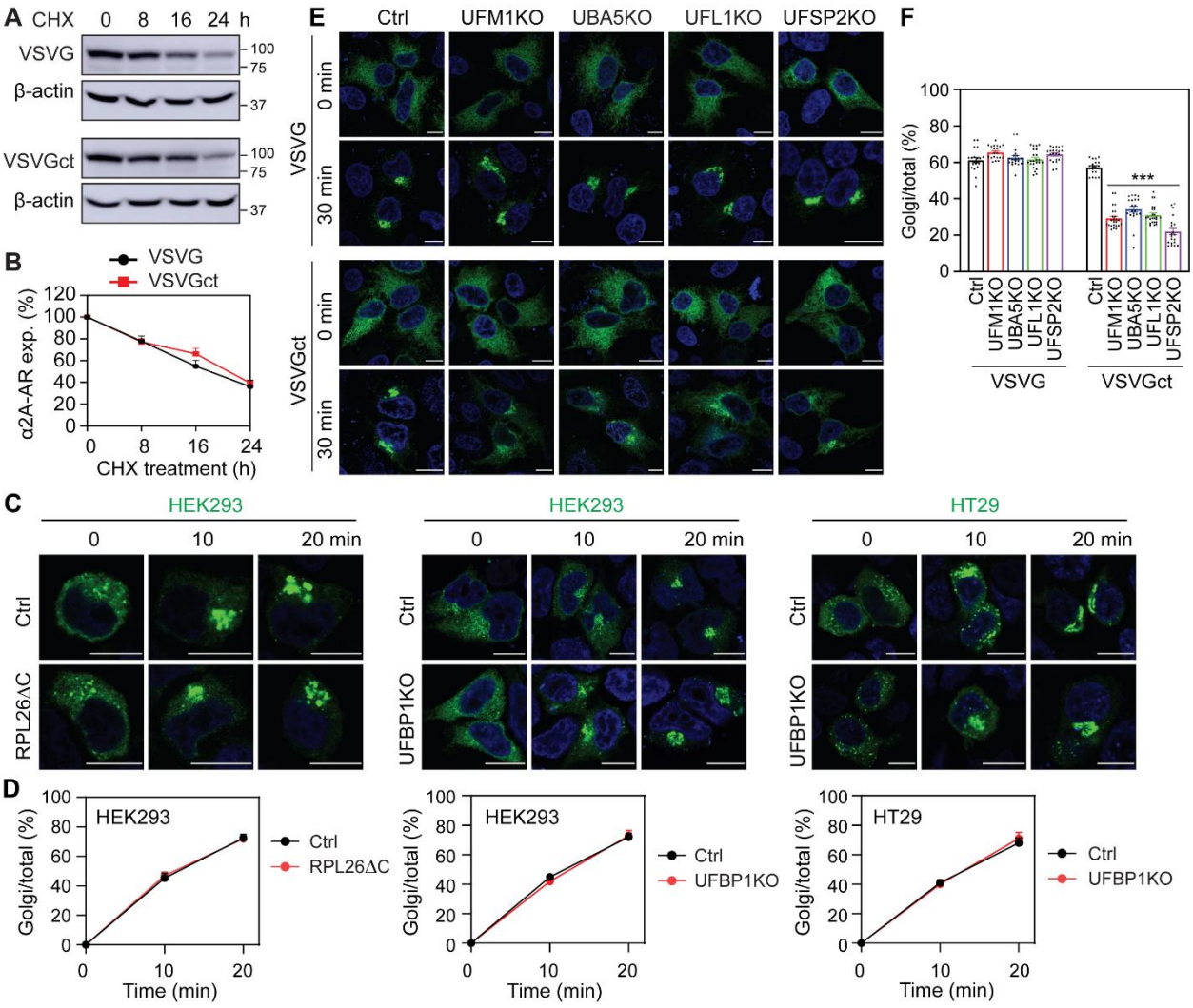


Fig. S8. The effects of the AT1R CT fusion, RPL26 knock-in and UFBP1 KO on the degradation and ER-Golgi transport of VSVG. (**A**) Degradation of VSVG and VSVGct. HEK293 cells were transfected with GFP-tagged VSVG and VSVGct in RUSH plasmids for 24 h and then treated with CHX for 8, 16 and 24 h. The expression of VSVG and VSVGct was measured by immunoblotting using GFP antibodies. (**B**) Quantitative data shown in (A). (**C**) Representative images showing the ER-Golgi transport of VSVG in RPL26 knock-in and UFBP1 KO cells. The cells were transfected with VSVG in RUSH plasmids and then treated with biotin for 10 and 20 min. (**D**) Quantitative data shown in (C). (**E**) Images showing the ER-Golgi transport of temperature-sensitive VSVG and VSVGct in control and ufmylation component KO cells. HeLa cells transfected with GFP-tagged VSVG or VSVGct were incubated at 40°C for 24 h (0 min) and then 32°C for 30 min. (**F**) Quantitative data shown in (E). The ER-Golgi transport of VSVG and VSVGct was expressed as the Golgi/total expression ratio. The quantitative data are mean ± SE; n = 3 in (B), 35-46 cells from 3 repeats in (D), and 20-24 cells from 3 experiments in (F). ***p < 0.001 versus control. Scale bars, 10 μm.

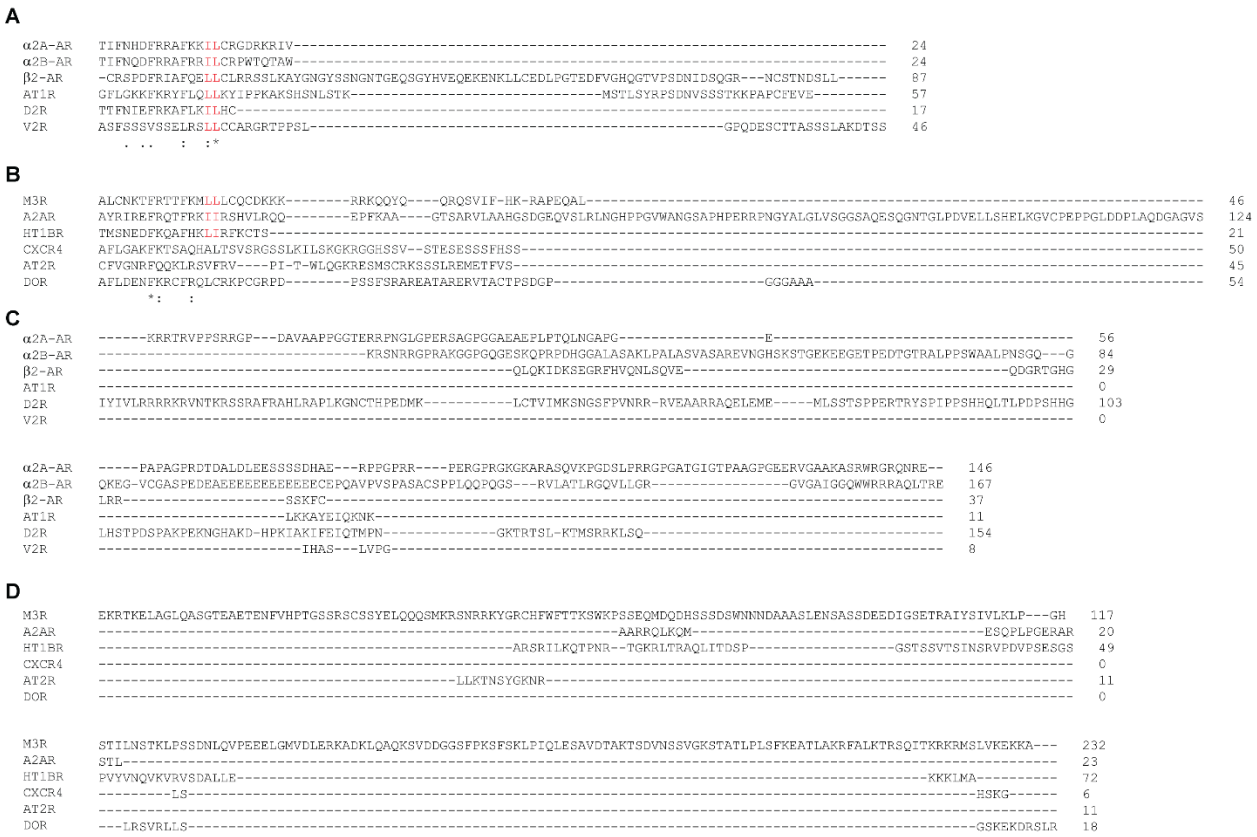


Fig. S9. Alignments of the CT and the ICL3 of GPCRs. Amino acid sequence alignments of the CT (A and B) and the ICL3 (C and D) of UFM1-regulated (A and C) and UFM1-independent GPCRs (B and D) by using Clustal Omega Multiple Sequences Alignment (https://www.ebi.ac.uk/jdispatcher/msa/clustalo). The di-leucine motifs in helix 8 are marked in red.

Movie S1.

α_{2A}-AR export in control cells. HeLa cells were transfected with α_{2A}-AR-GFP in RUSH plasmids for 20 h and then incubated with biotin. Images were captured for 60 min with 10 sec intervals.

Movie S2.

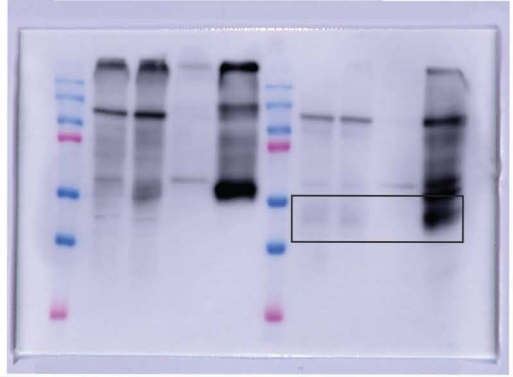
α_{2A}-AR export in UFM1 KO cells. HeLa cells were transfected with α_{2A}-AR-GFP in RUSH plasmids for 20 h and then incubated with biotin. Images were captured for 60 min with 10 sec intervals.

Uncropped gels

Fig. 4B

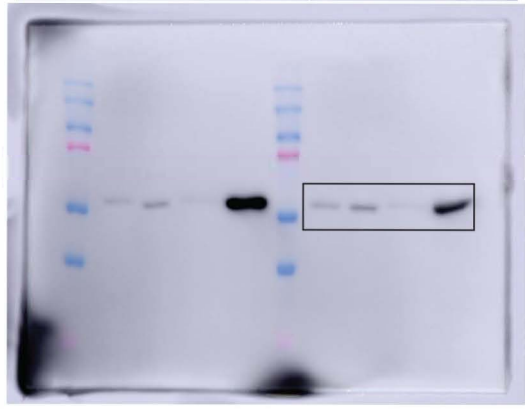
a2A-AR

AT1R

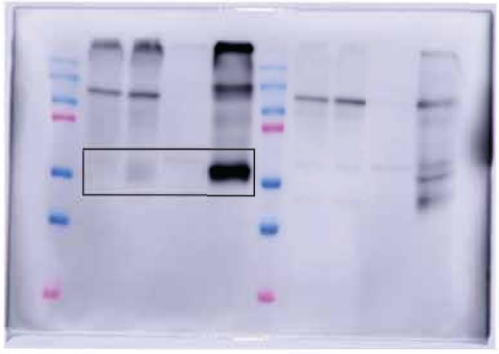


Sar1

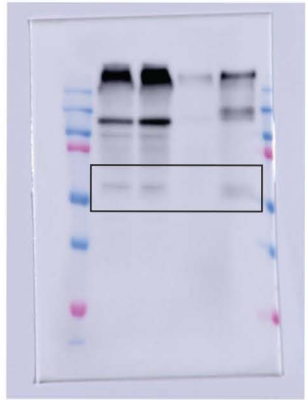
Sar1



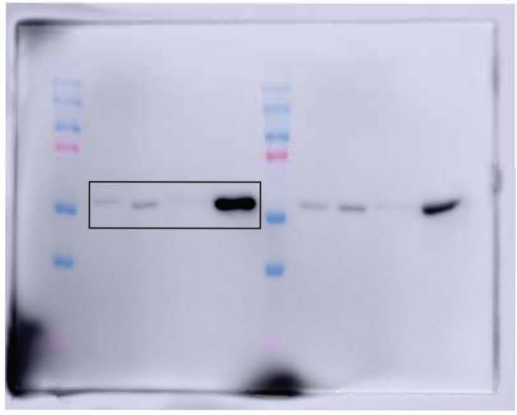
b2-AR



b2-AR



Sar1



Sar1G37A

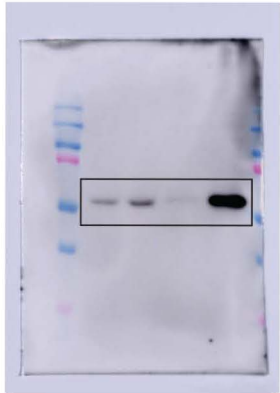


Fig. 4C

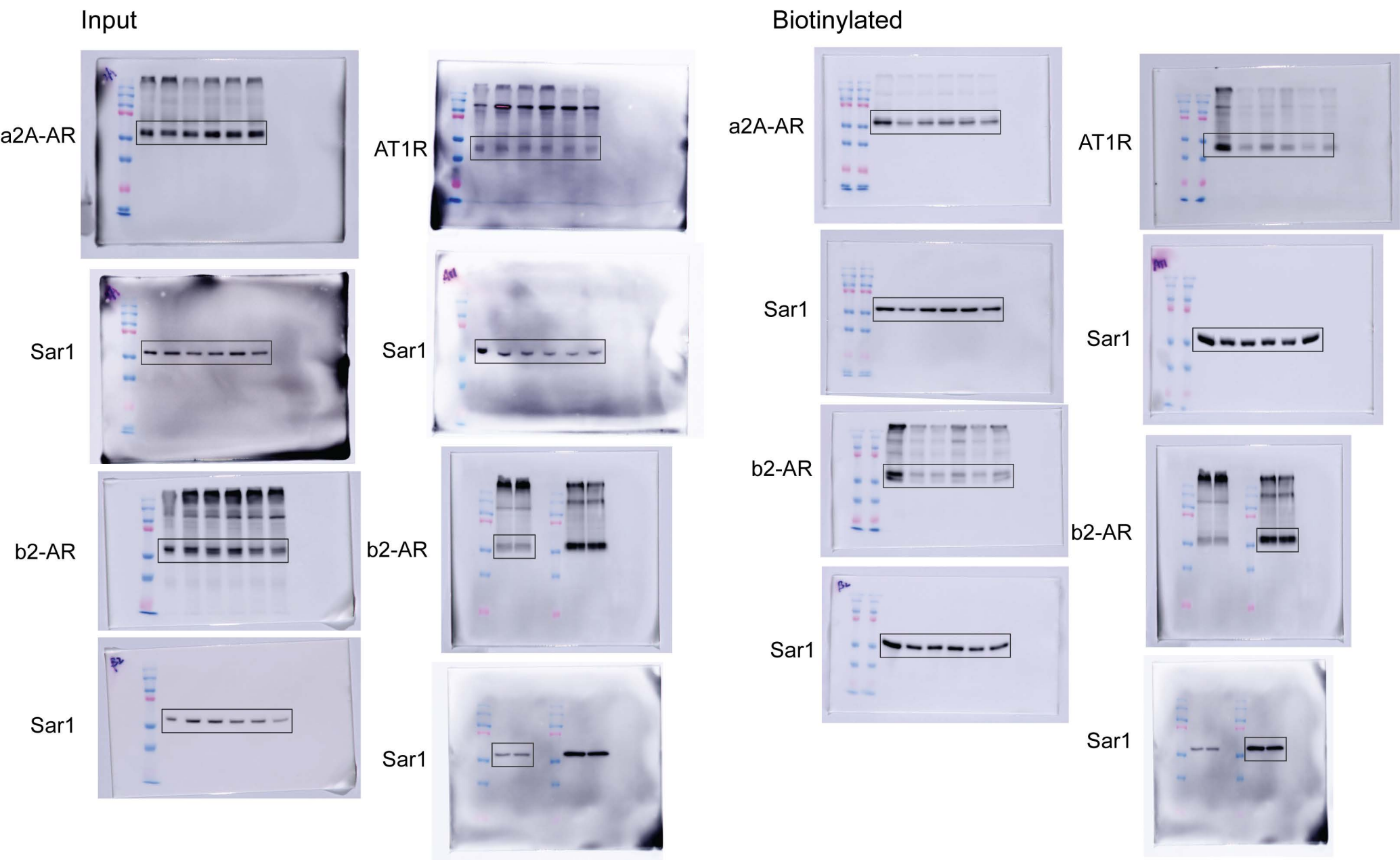
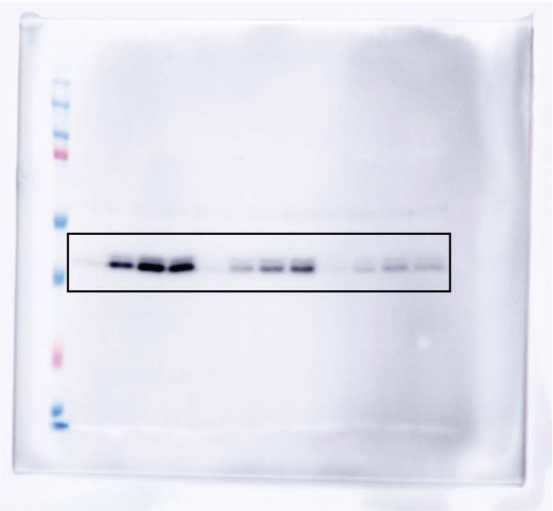

Input
Biotinylated
a2A-AR
AT1R
Sar1
Sar1
b2-AR
b2-AR
Sar1
Sar1
a2A-AR
AT1R
Sar1
Sar1
b2-AR
b2-AR
Sar1
Sar1

Fig. 5E



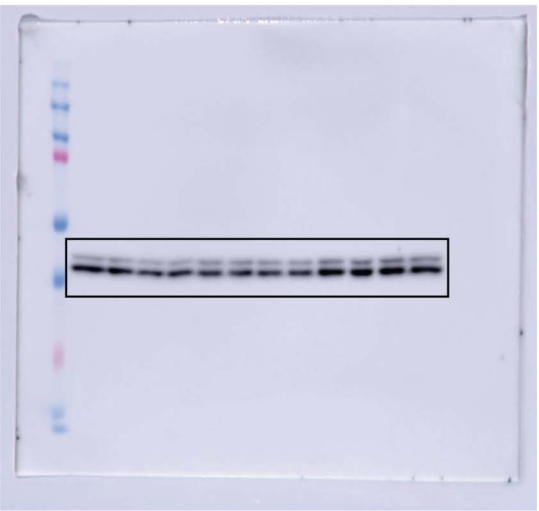


Fig. 5G

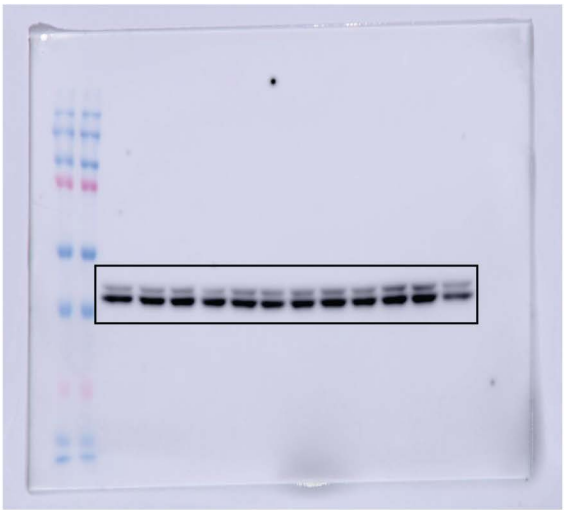


Fig. 6A

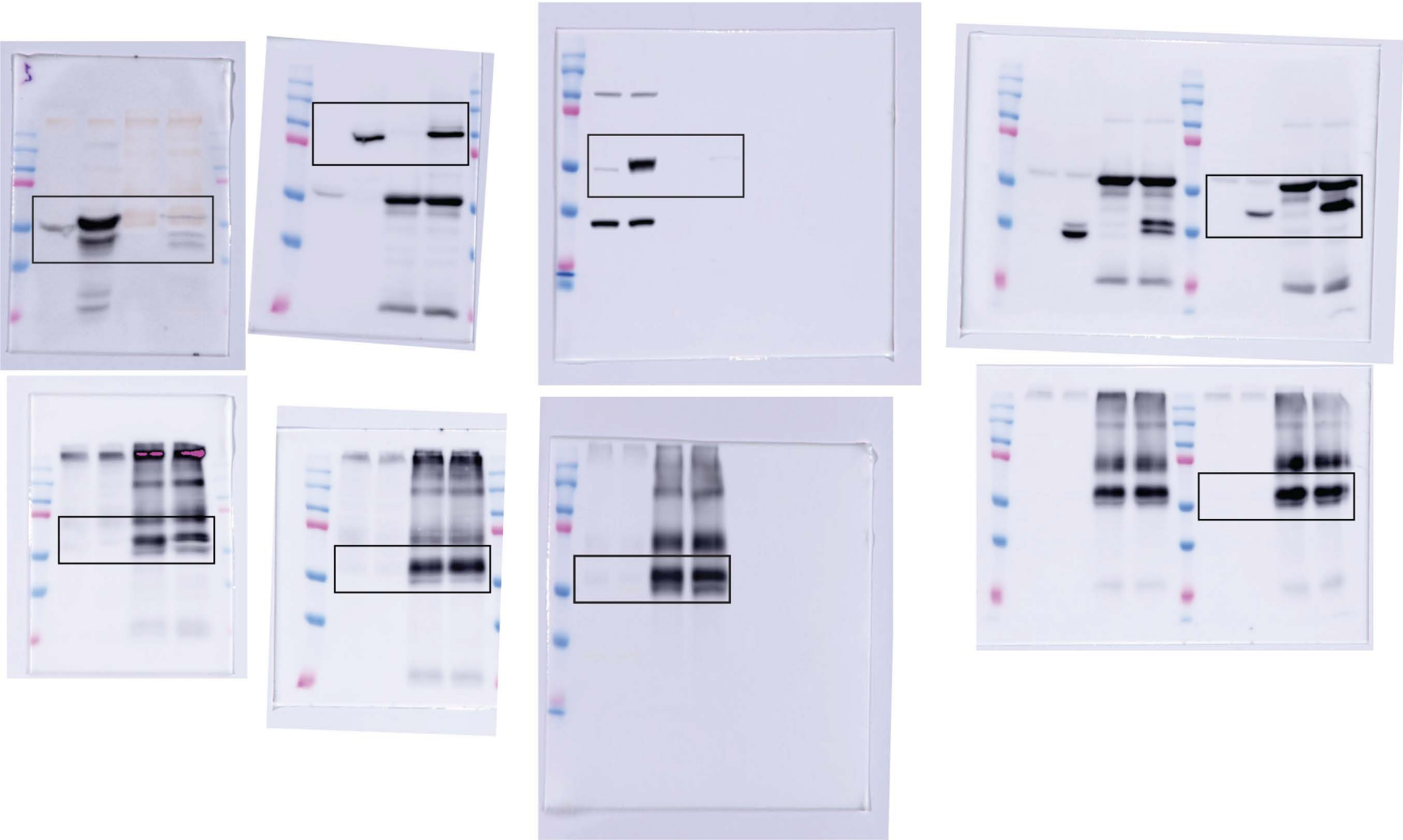
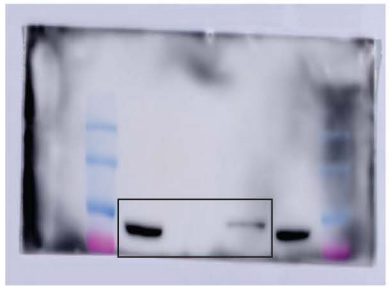
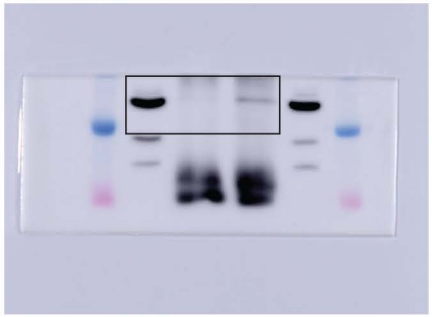
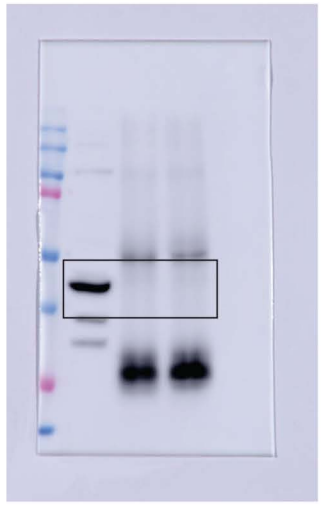
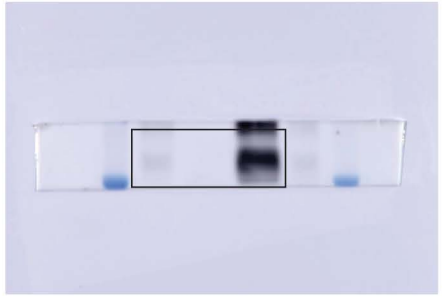


Fig. 6B









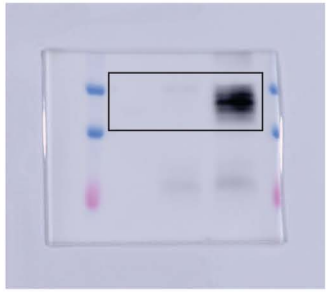
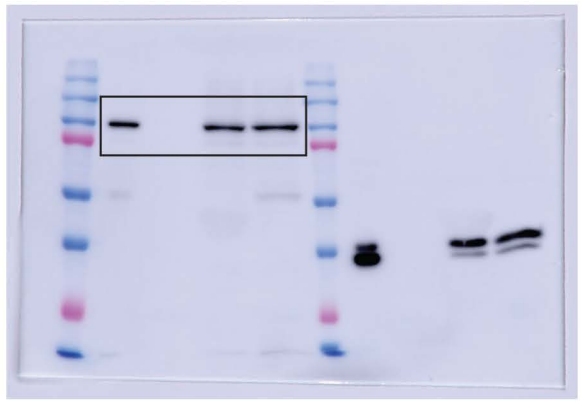
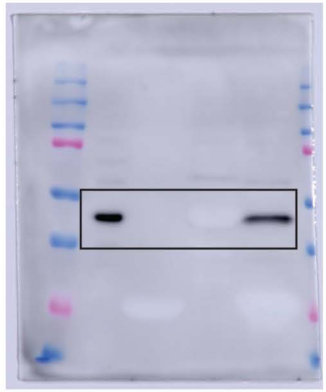


Fig. 6C





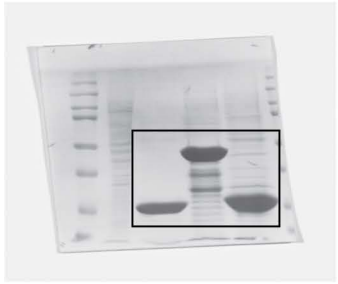
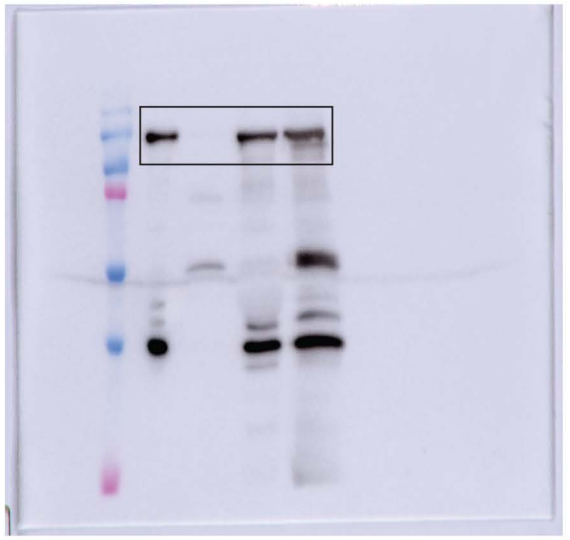
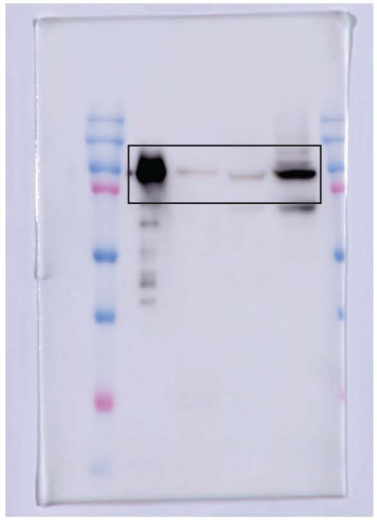


Fig. 6D





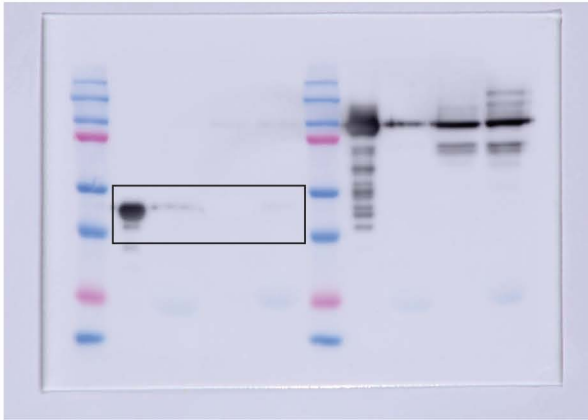
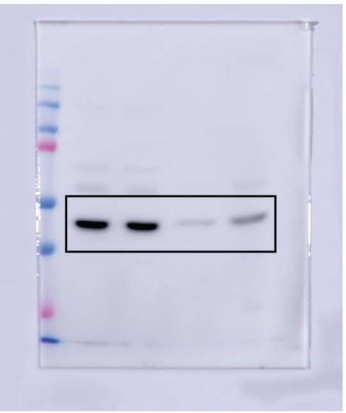


Fig. 6H



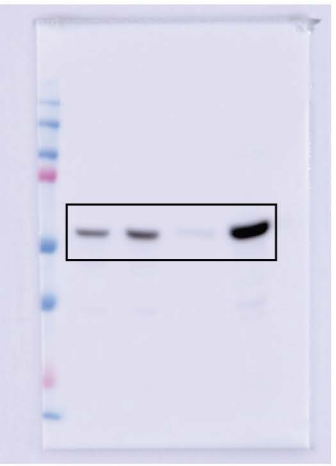
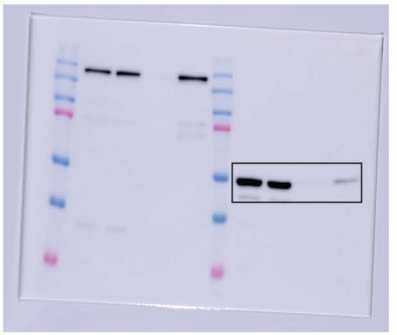


Fig. 6I

Sar1B



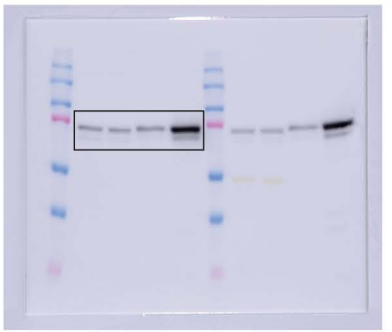
UFBP1

Sec24A

UFBP1

Sec24B

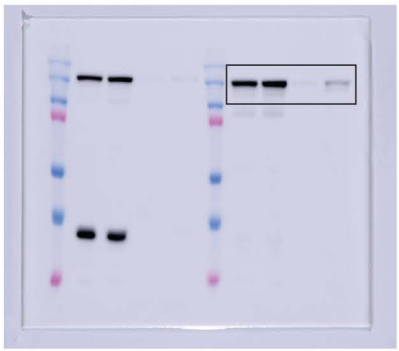
UFBP1



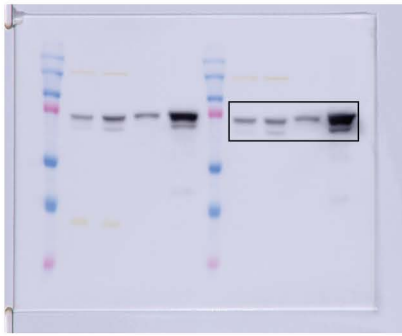
Sec24C

UFBP1

Sec24D



UFBP1



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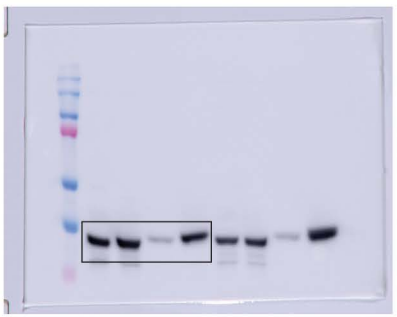
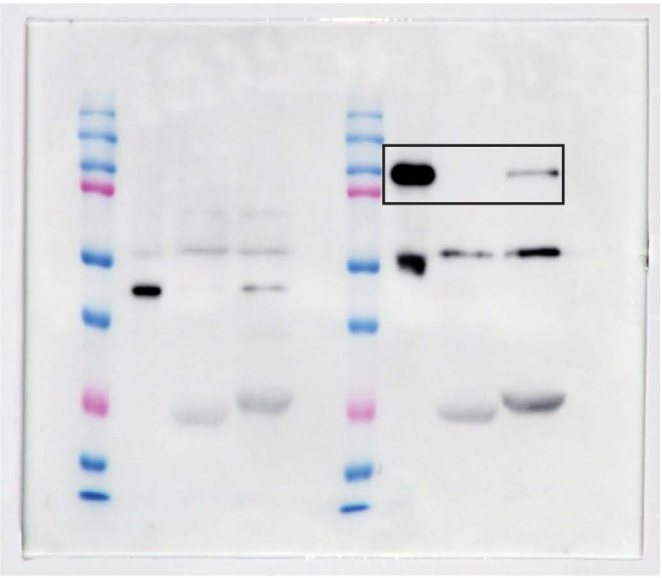
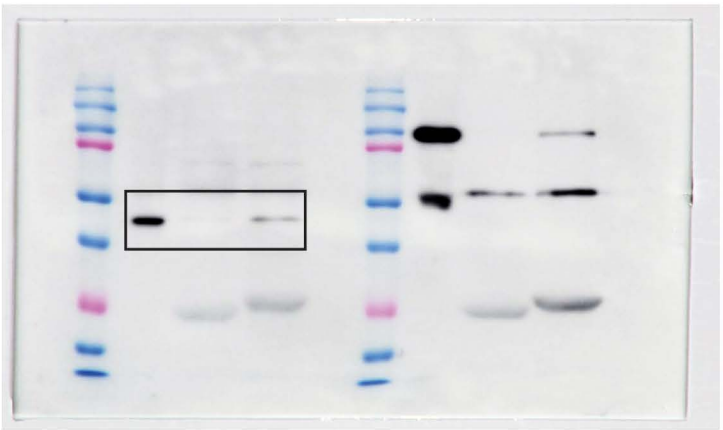


Fig. 7A





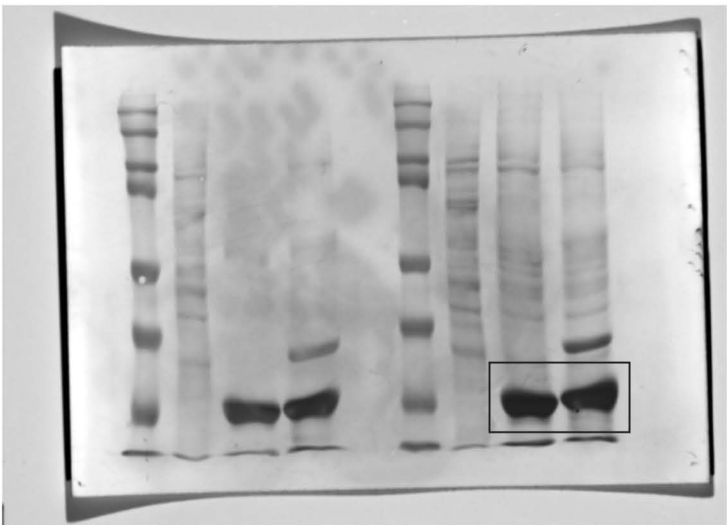
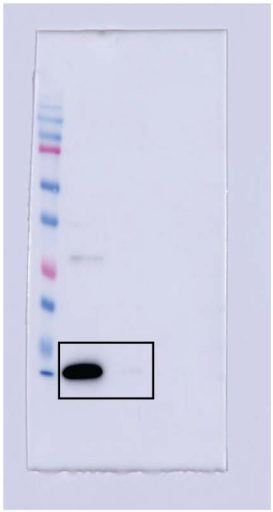


Fig. S2A



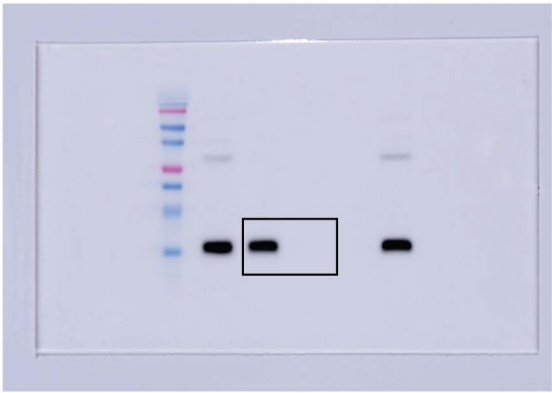


Fig. S3A

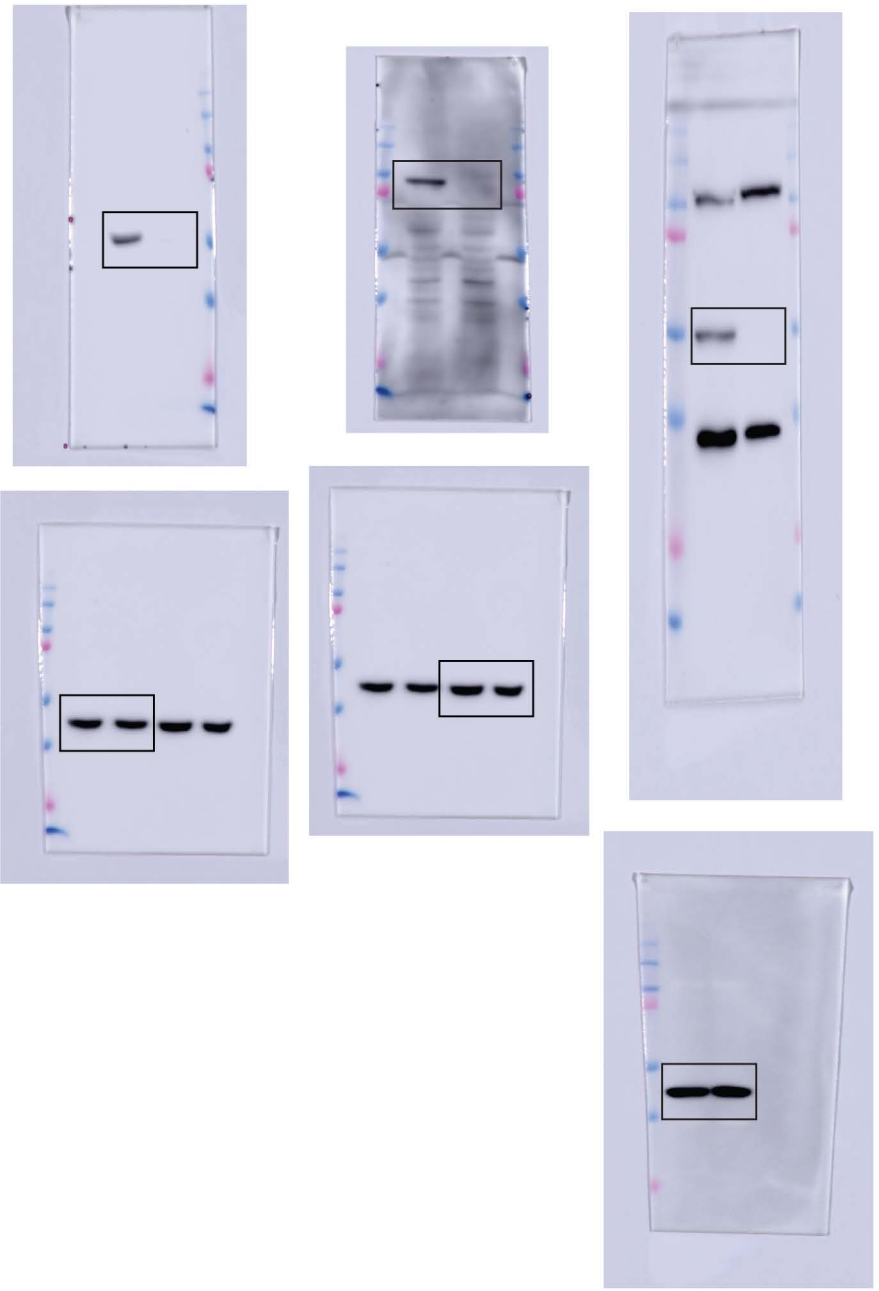


Fig. S3B

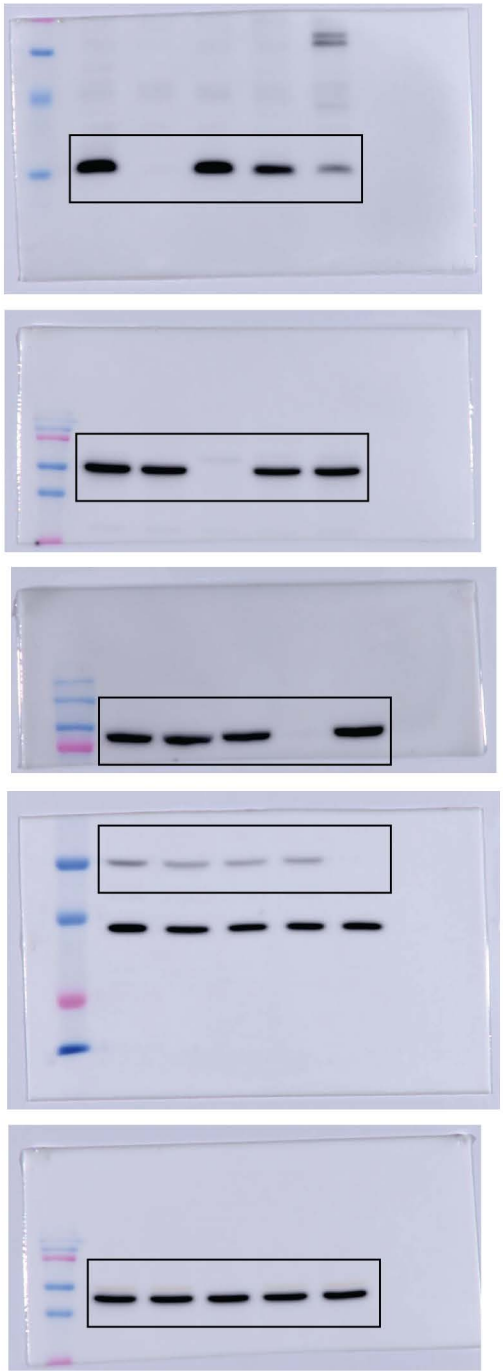


Fig. S3E

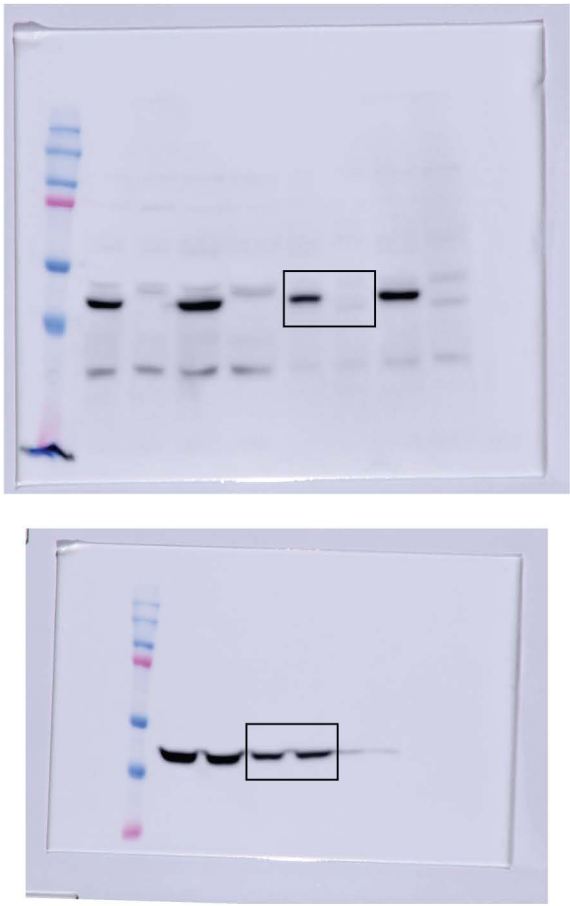
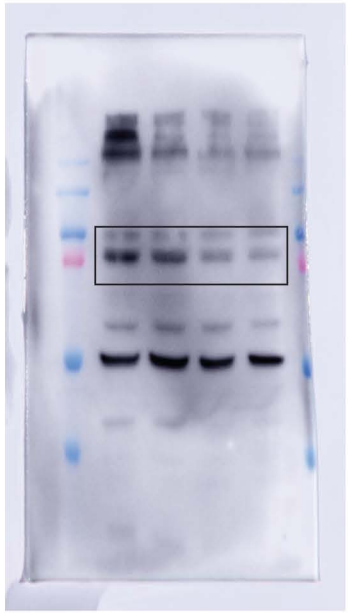
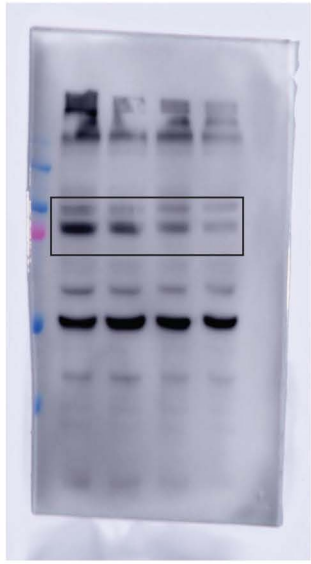
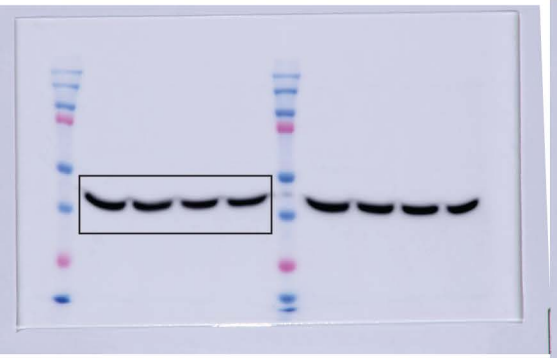


Fig S4C







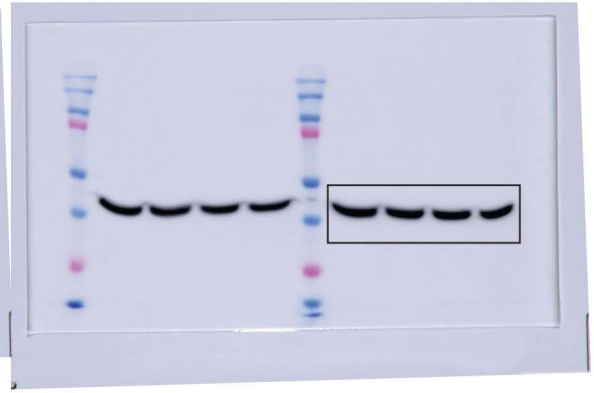
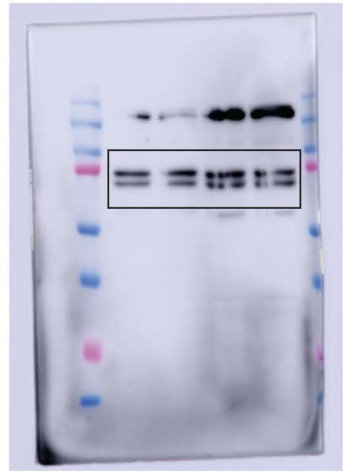
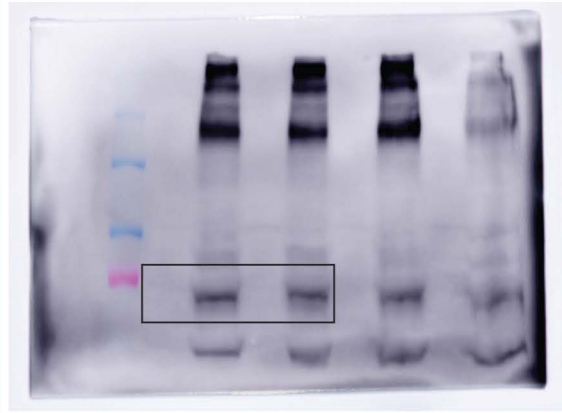


Fig. S4E





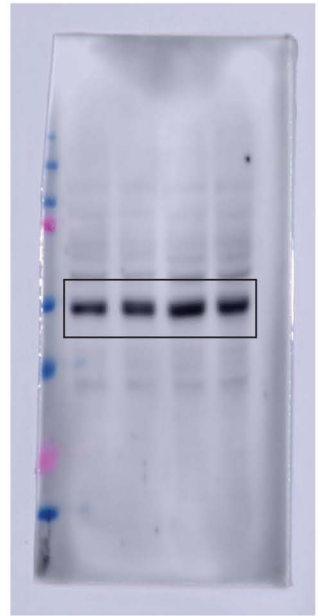


Fig. S5E

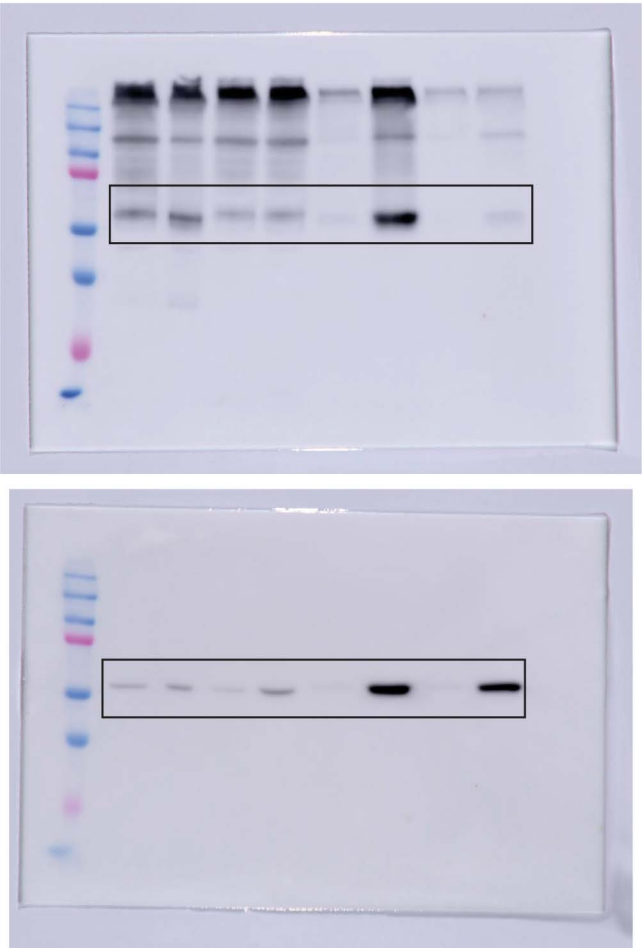
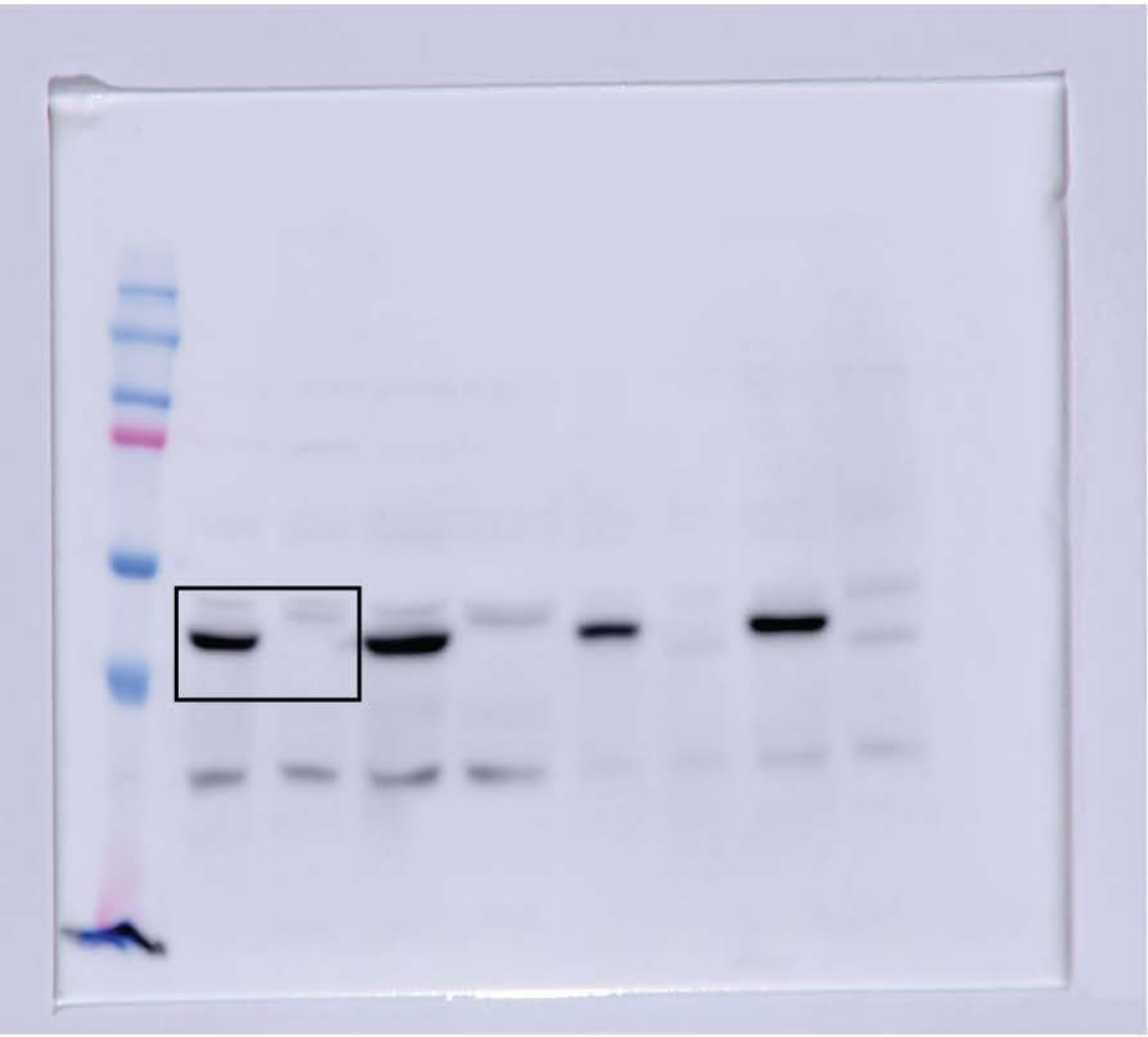


Fig. S6A



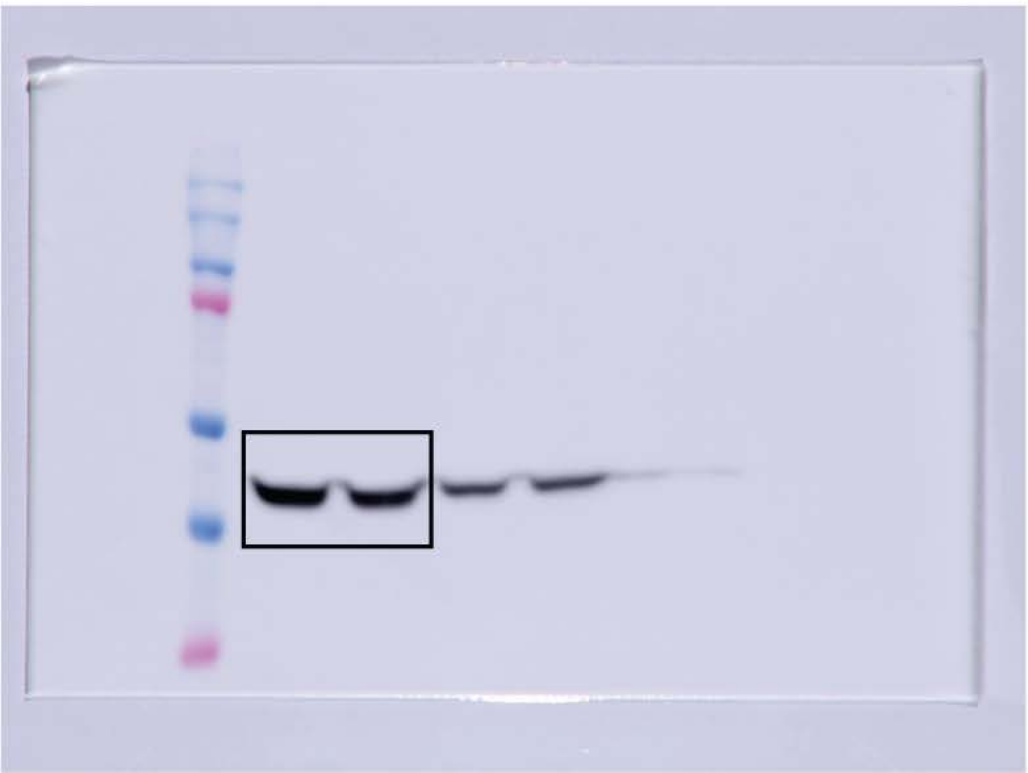


Fig. S7A

Fig. S7C

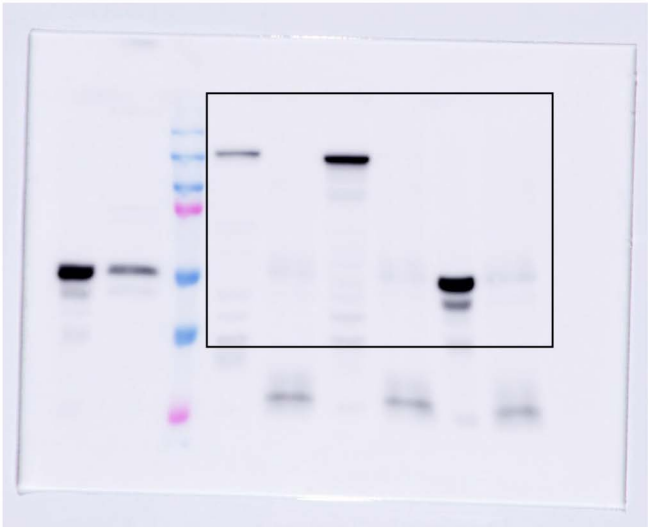


Fig. S8A