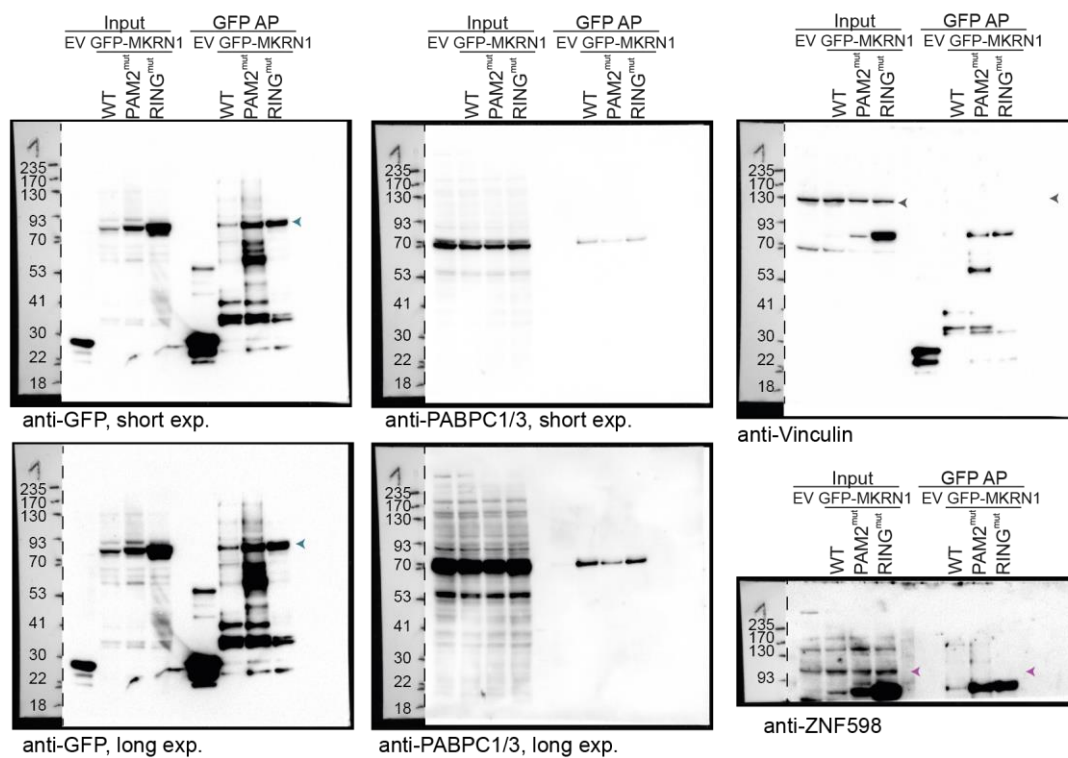


Fig. S10. Images of full membranes and different exposure times (exp.) for Western blot analyses in **Fig. 1B,D,F**, **Additional file 1: Fig. S1E**, and **S2C**. (A-C) Images of full membranes and replicate experiments for Western blot analyses of MKRN1 and PABPC1 in polysome profiling experiments in **Fig. 1B**. A 10%–50% sucrose gradient of cycloheximide-treated HEK293R cell extracts. Shown are the Western blot analyses of individual gradient fractions with antibodies against MKRN1 and PABPC1/3 ($n = 3$

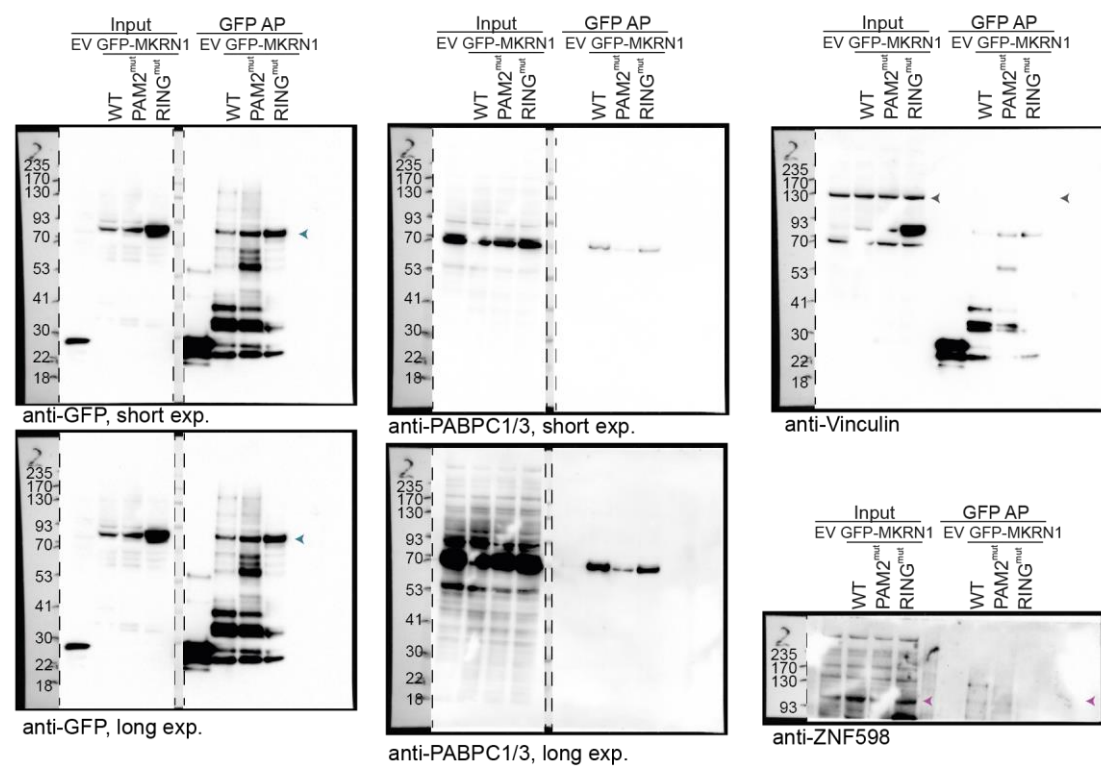
replicates, plus one technical replicate). UV absorbance was measured at $\lambda = 254$ nm. (A) Left, Images of full membranes for MKRN1 and PABPC1/3 antibodies are shown for replicate 1 (replicate 1.1, as in **Fig. 1B**). Right, Full membranes for both antibodies are shown for a technical replicate of replicate 1.1 (replicate 1.2). (B,C) Images of full membranes for both antibodies are shown for replicates 2 (B) and 3 (C).

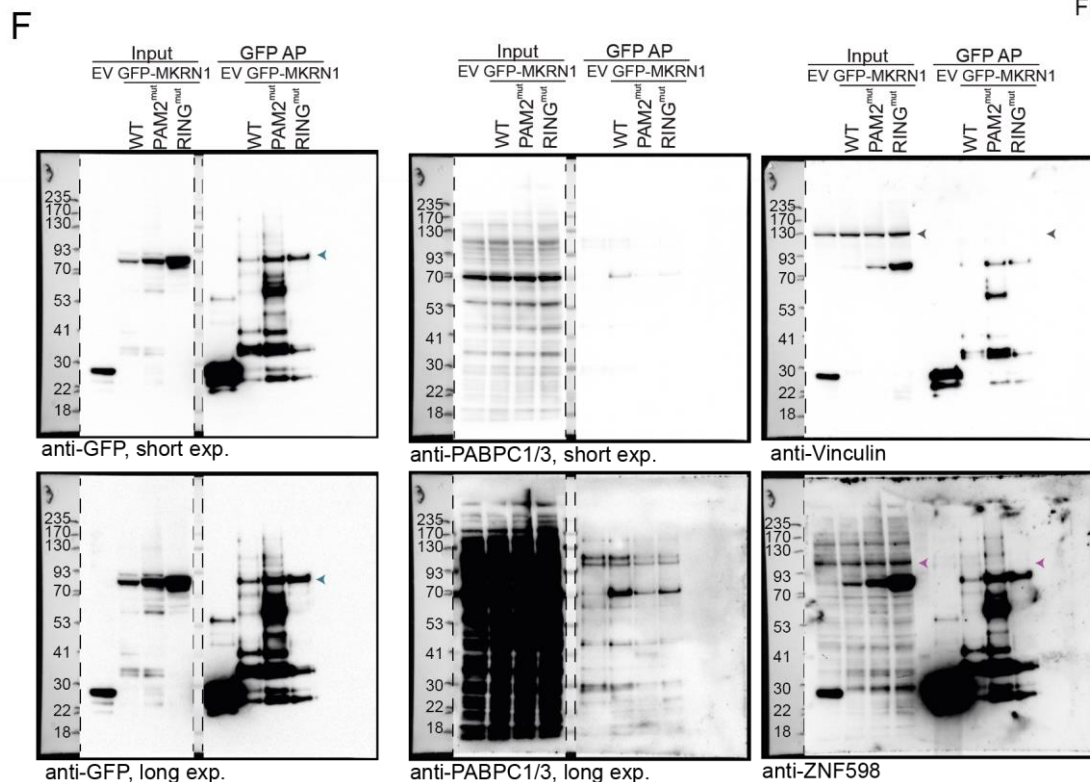
Fig. S10

D

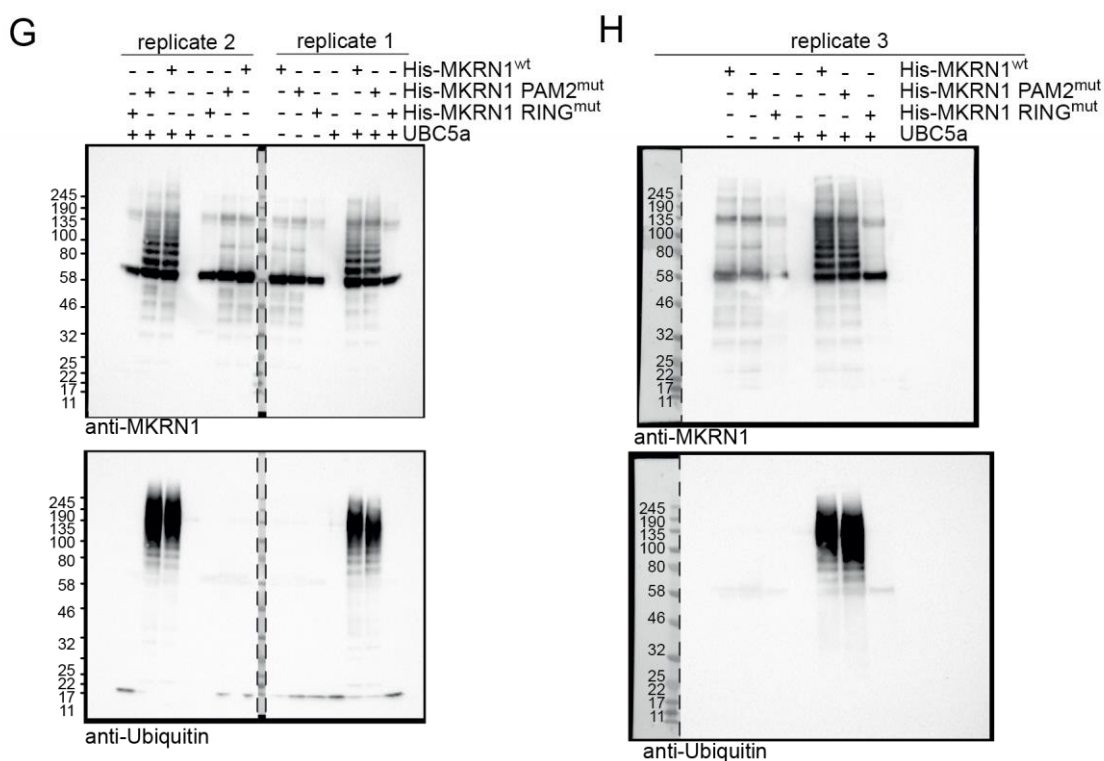


E



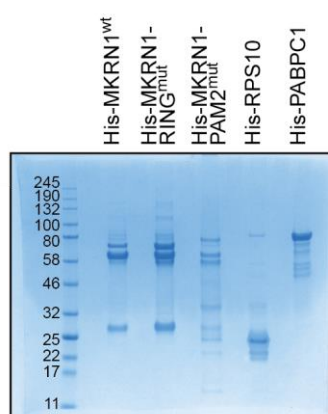


(D-F) Images of full membranes and different exposure times (exp.) for Western blot analyses in **Fig. 1D**. PABPC1 interacts strongly with MKRN1^{wt} and MKRN1^{RINGmut} but only to a lesser extent with MKRN1^{PAM2mut}. Western blot analysis was performed with antibodies against PABPC1/3 and GFP. Images of full membranes and different exposure (exp.) times for both antibodies are shown for replicate 1 (D), which is presented in **Fig. 1D**, as well as replicates 2 (E) and 3 (F). Petrol arrowheads indicate GFP-MKRN1. Grey and pink arrowheads indicate vinculin and ZNF598, respectively.



(G,H) Recombinant His-MKRN1^{wt} and His-MKRN1^{PAM2mut} are capable of autoubiquitylation opposed to His-MKRN1^{RINGmut}. Recombinant His-MKRN1^{wt}, His-MKRN1^{PAM2mut} and His-MKRN1^{RINGmut} proteins were incubated with or without the E2 enzyme UBC5a. UBC5a without an E3 enzyme was used as a control. Autoubiquitylation of MKRN1 was assessed by Western blot. Western blot analysis was performed with antibodies against MKRN1 and Ubiquitin. Images of full membranes for both antibodies are shown for replicate 1 (G, right), which is presented in **Fig. 1F**, and 2 (G, left), as well as for replicate 3 (H). Note the opposite order of replicates 1 and 2 (2 left, 1 right) in (G).

Fig. S10



(I) Image of the full gel for recombinantly expressed proteins, which were purified from *E. coli*, as shown in **Additional file 1: Fig. S1E**.

Figure 1: Western blot analysis of MKRN1 and GFP-AP interaction. The figure consists of three panels. The left panel is a Ponceau stain showing total protein loading. The middle panel is an anti-GFP blot showing the interaction between MKRN1 and GFP-AP. The right panel is an anti-MKRN1 blot showing the interaction between MKRN1 and GFP-AP. Each panel has two main sections: 'Input' and 'GFP AP'. Each section has three lanes: 'EV', 'GFP-PABPC1', and 'EV GFP-PABPC1'. The 'EV' and 'GFP-PABPC1' lanes are further divided into '-' and '+' RNase conditions. Molecular weight markers are indicated on the left of each blot.



Figure 3 shows three Western blot panels. The first panel is stained with Ponceau, showing total protein loading. The second panel is probed with anti-GFP antibody, showing GFP-PABPC1 levels. The third panel is probed with anti-MKRN1 (short exposure), showing MKRN1 levels. In all panels, the Input lanes show consistent protein levels across EV, GFP-PABPC1, and RNase conditions. The GFP AP lanes show a significant increase in MKRN1 levels when co-expressed with GFP-PABPC1, which is partially resistant to RNase treatment.

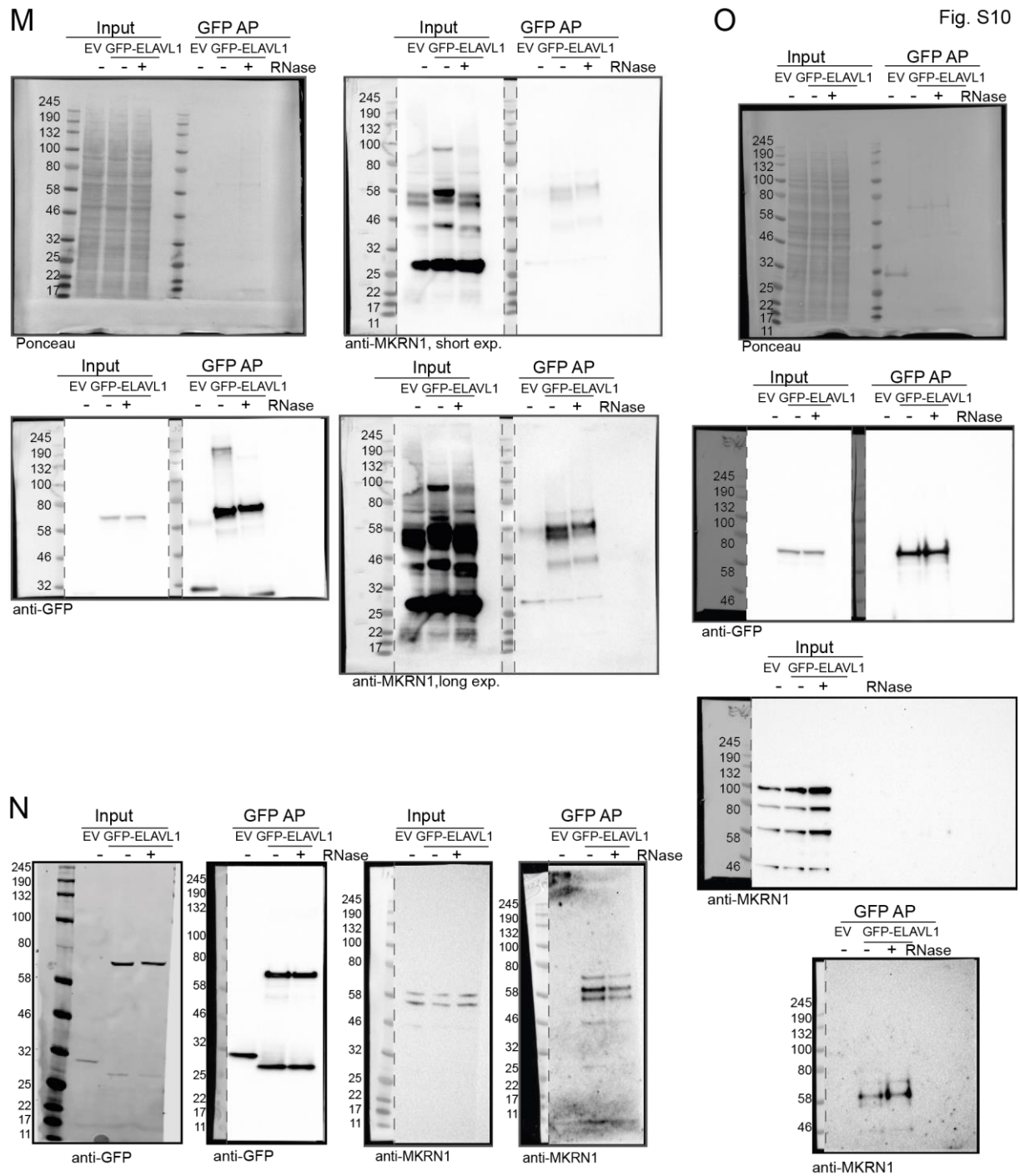
Western blot analysis of anti-MKRN1 immunoprecipitation. The blot shows Input and GFP-AP fractions, each with EV, GFP-PABPC1, and RNase treatments. Molecular weight markers are on the left. A band at ~58 kDa is visible in the GFP-PABPC1 + RNase lane.



Figure 1: Western blot analysis of GFP-AP and anti-MKR1.

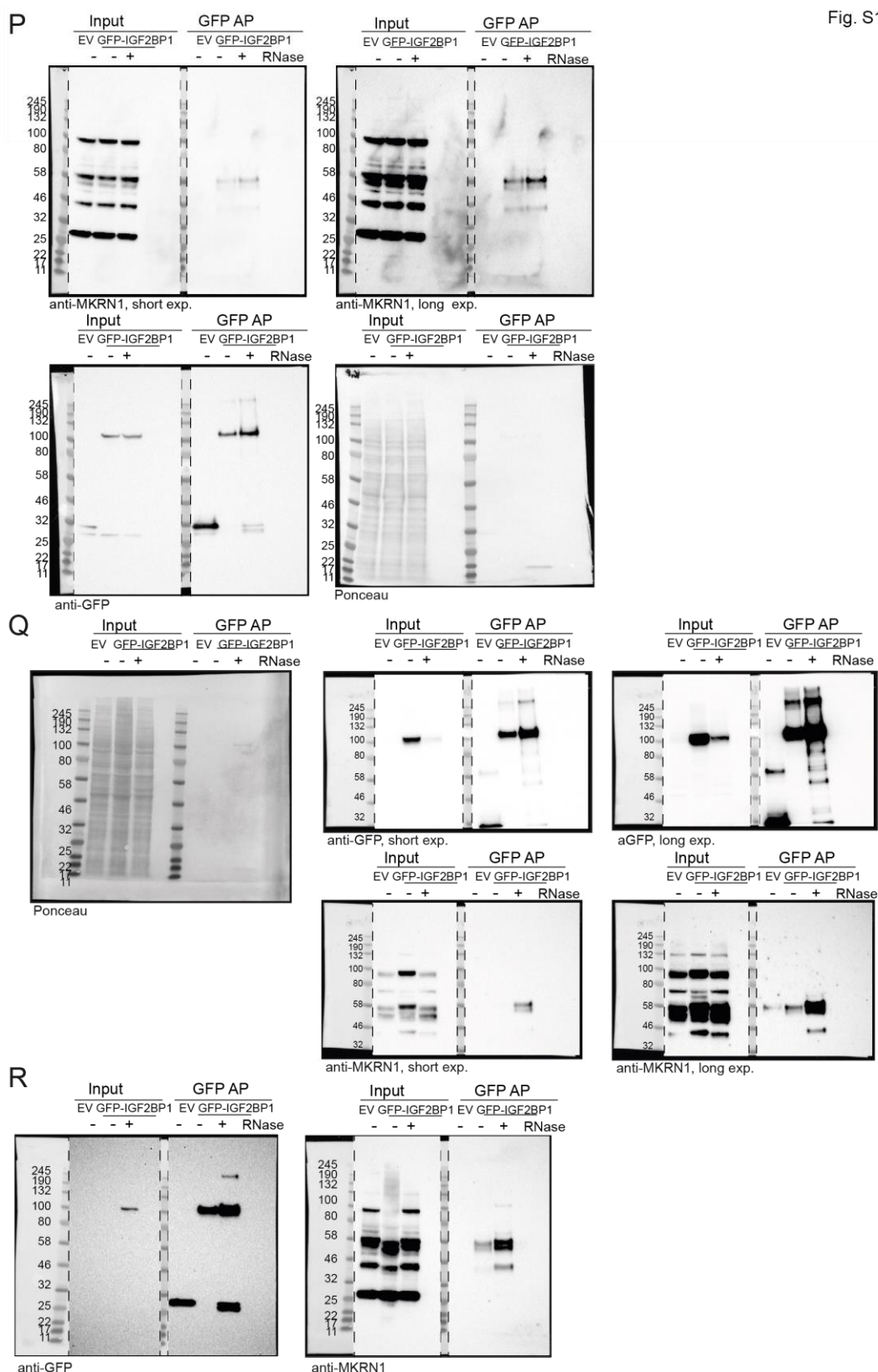
The figure displays four Western blot panels. Each panel has two main sections: **Input** and **GFP AP**. Under **Input**, there are lanes for **EV** and **GFP-PABPC1**, each with **-** and **+** RNase treatments. Under **GFP AP**, there are lanes for **EV** and **GFP-PABPC1**, each with **-** and **+** RNase treatments. Molecular weight markers (245, 190, 132, 100, 80, 58, 46, 32, 25, 22, 17, 11 kDa) are indicated on the left of each blot.

- Top-left (Ponceau):** Shows total protein loading. A strong band is visible at ~25 kDa in the EV lanes, and a weaker band at ~100 kDa in the GFP-PABPC1 lanes.
- Top-right (anti-GFP):** Shows GFP-AP expression. A strong band is visible at ~100 kDa in the GFP-PABPC1 lanes, and a weaker band at ~25 kDa in the EV lanes.
- Bottom-left (anti-MKR1, short exp.):** Shows MKR1 expression. A strong band is visible at ~25 kDa in the EV lanes, and a weaker band at ~100 kDa in the GFP-PABPC1 lanes.
- Bottom-right (anti-MKR1, long exp.):** Shows MKR1 expression. A strong band is visible at ~25 kDa in the EV lanes, and a weaker band at ~100 kDa in the GFP-PABPC1 lanes.



(M-O) Endogenous MKRN1 interacts with GFP-ELAVL1 independent of RNA. Western blot analysis was performed with antibodies against MKRN1 and GFP. Images of full membranes and different exposures for both antibodies are shown for replicate 1 (M) which is presented in **Additional file 1: Fig. S2C**, as well as replicates 2 (N) and 3 (O).

Fig. S10



(P-R) Endogenous MKRN1 interacts with GFP-IGF2BP1 independent of RNA. Western blot analysis was performed with antibodies against MKRN1 and GFP. Images of full membranes and different exposure times for both antibodies are shown for replicate 1 (P) which is presented in **Additional file 1: Fig. S2C**, as well as replicates 2 (Q) and 3 (R).

Fig. S11

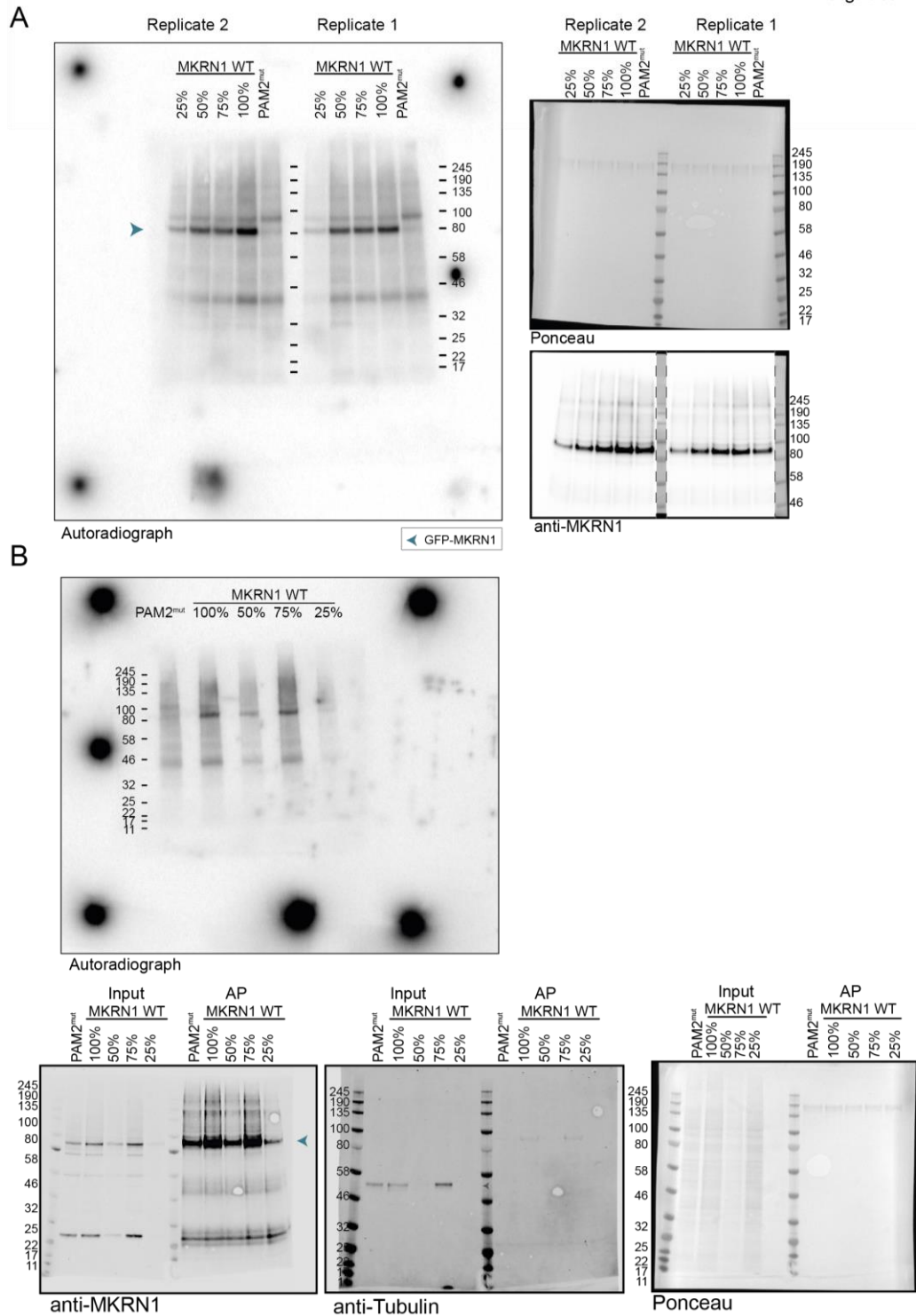
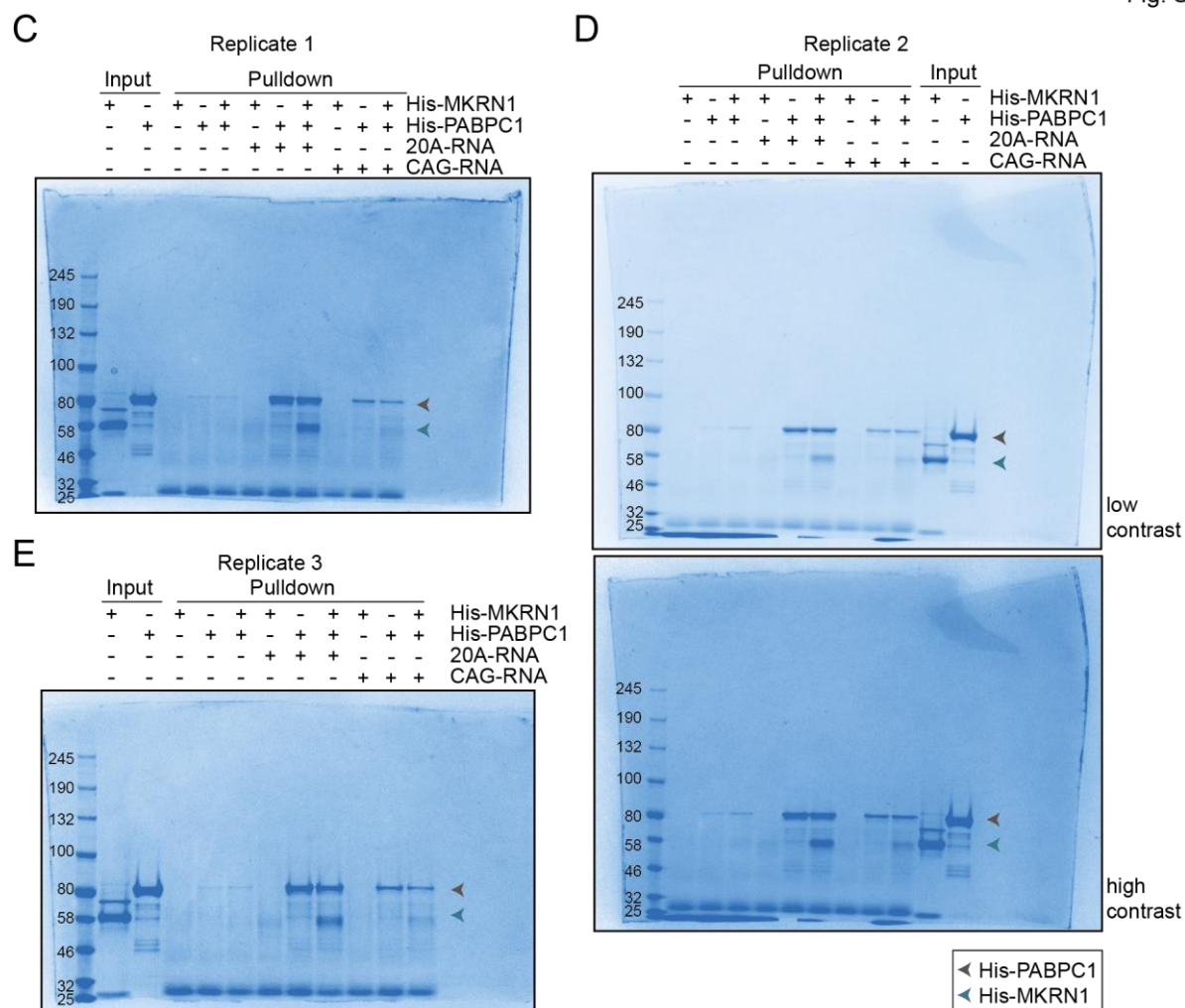


Fig. S11. Images of full membranes for Western blot and SDS-PAGE (Coomassie staining) analyses as in **Fig. 3D** and **Additional file 1: Fig. S6**. (A,B) Images of full membranes of autoradiographs and Western blot analyses in **Fig. 3D** (replicate 1) and **Additional file 1: Fig. S6A,B** (replicates 2 and 3). UV crosslinking experiments to measure the RNA binding capacity of GFP-MKRN1^{wt} and GFP-MKRN1^{PAM2mut}. Autoradiographs (A, left; B, top) and Western blots (A, right; B, bottom) show GFP-MKRN1/RNA complexes and GFP-MKRN1 protein, respectively, in the eluates from replicates 1 and 2 (with 4SU and UV crosslinking at 365 nm) (A) and 3 (with conventional UV crosslinking at 254 nm) (B). (B) Images of full membranes of Western

blot analyses with both antibodies are shown for replicate 3 (*B*). Petrol arrowheads indicate GFP-MKRN1-RNA complexes (*A*) or GFP-MKRN1 protein (*B*).



(C-E) Images of full gels of Coomassie SDS-PAGE analyses as presented in **Fig. 3E** (replicate 1; C), and **Additional file 1: Fig. S6C** (replicate 2; D) and **Additional file 1: Fig. S6D** (replicate 3, E) are shown. Petrol and grey arrowheads indicate His-MKRN1 and His-PABPC1, respectively.

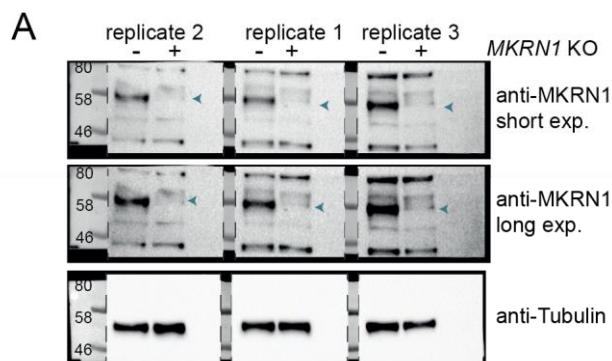
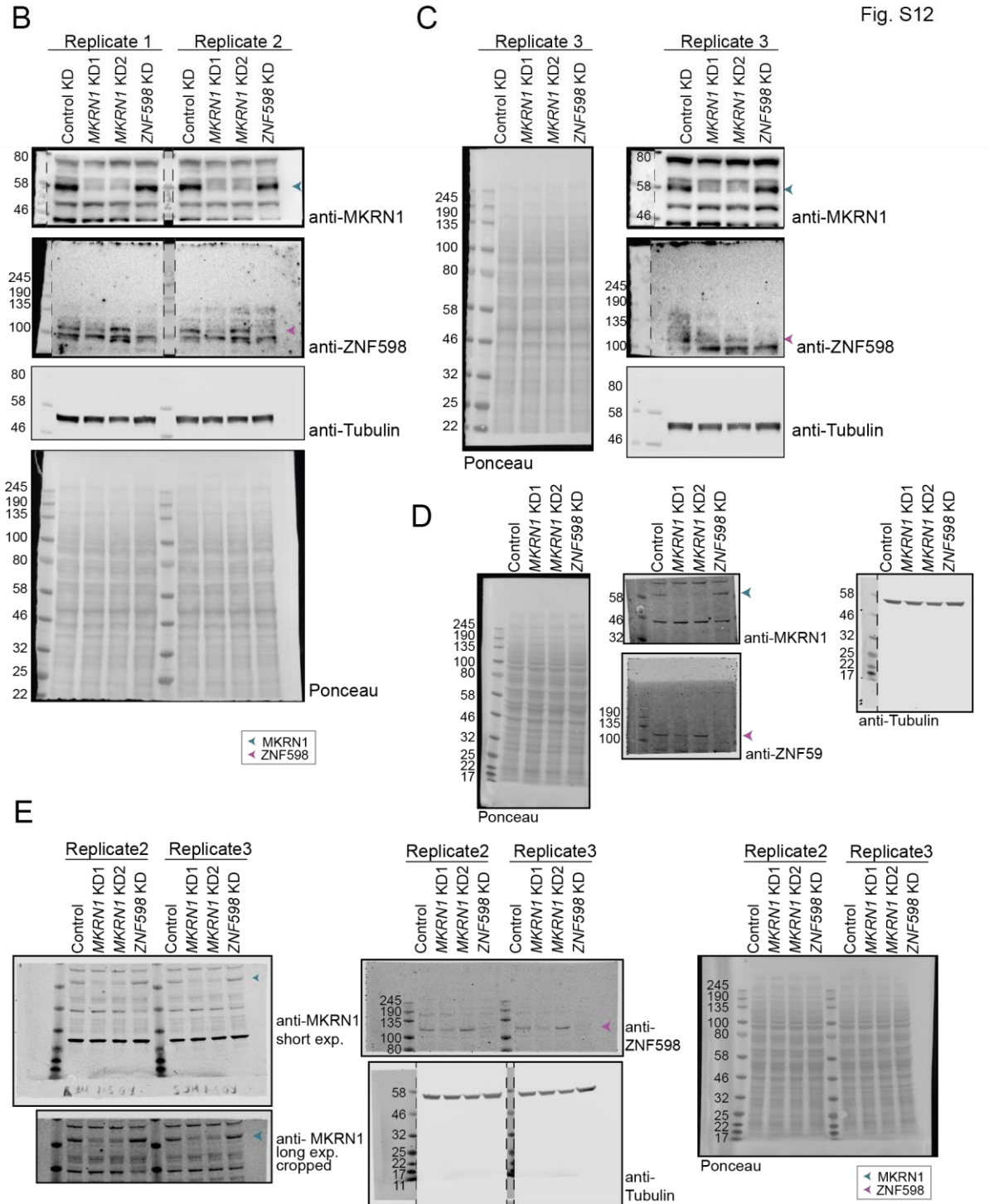
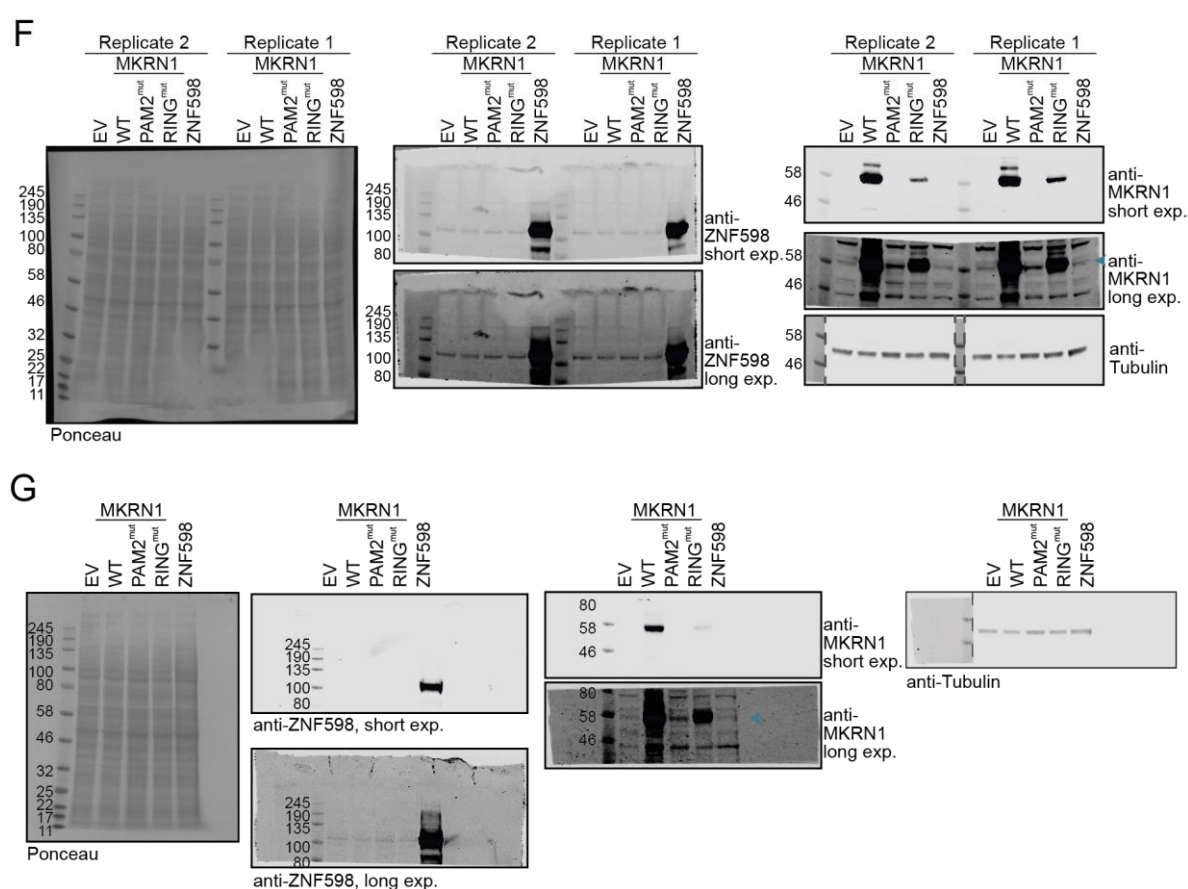


Fig. S12. Images of full membranes for Western blot analyses in **Additional file 1: Fig. S8A,C,E,F, Fig. 5G**, and **Additional file 1: Fig. 9B**. (A) Images of full membranes for Western blot analyses in **Additional file 1: Fig. S8C**. Images of full membranes are presented for replicates 1, 2 and 3. MKRN1 protein levels were assessed by Western blot in *MKRN1* KO HEK293T (*MKRN1* KO) and HEK293T wild type (WT) cells. Western blot analysis was performed with antibodies against MKRN1 and tubulin. Petrol arrowheads indicate MKRN1.

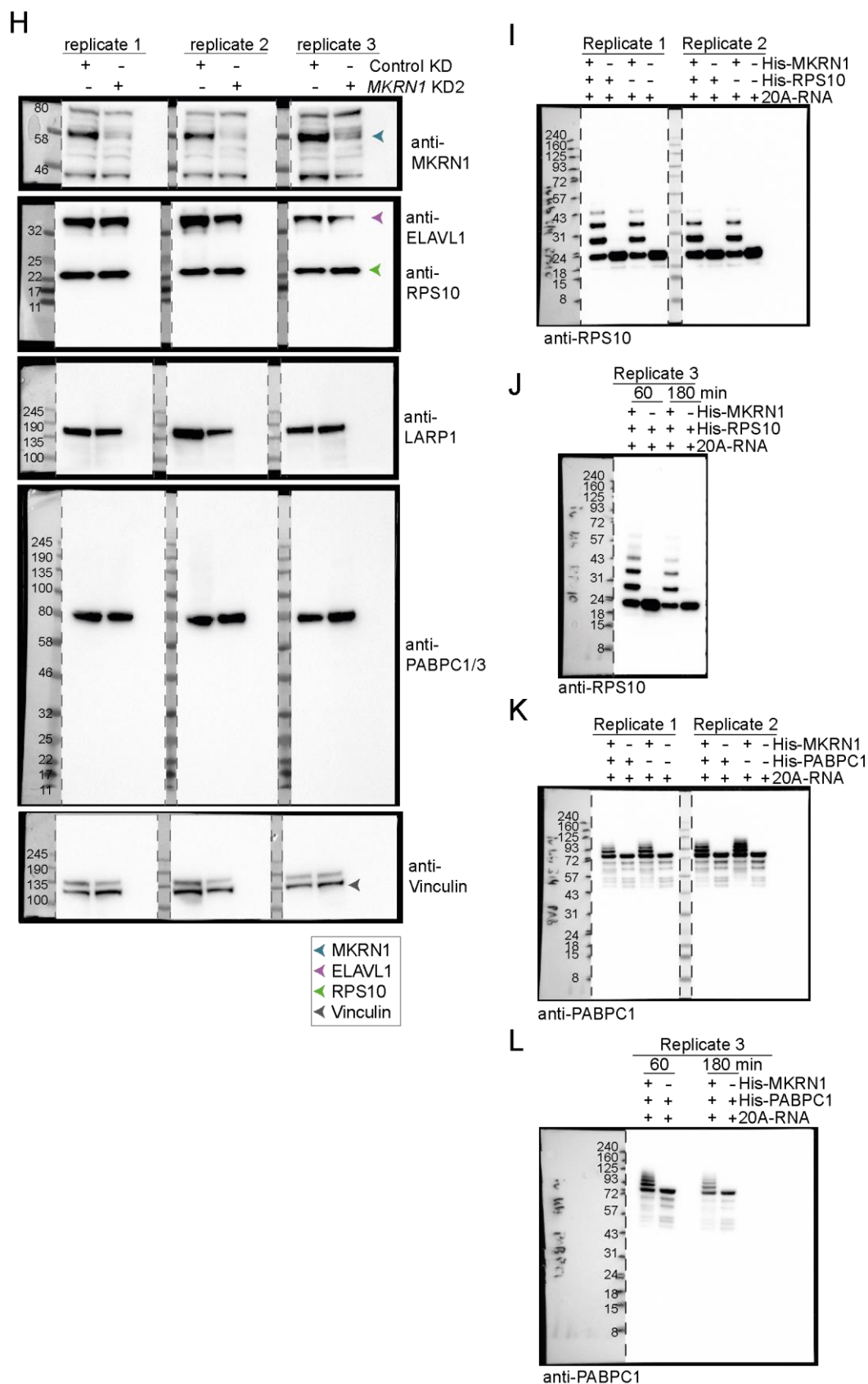
Fig. S12





(B-G) Images of full membranes and different exposure (exp.) times for Western blot analyses in **Additional file 1: Fig. S8A,E,F**. (B,C) KDs of *MKRN1* and *ZNF598* assessed by Western blot (n=3 replicates) from **Additional file 1: Fig. S8A**. Western blot analysis was performed with antibodies against *MKRN1*, *ZNF598*, and tubulin. Petrol and pink arrowheads indicate *MKRN1* (53 kDa) and *ZNF598* (99 kDa), respectively. Uncropped gel images of replicates 1 and 2 (B) and 3 (C). (D,E) Images of full membranes are shown for cross-regulation between *MKRN1* and *ZNF598* KD from **Additional file 1: Fig. S8E**. *MKRN1* KD1 reduces endogenous *ZNF598* protein levels. Western blot analysis was performed with antibodies against *MKRN1*, *ZNF598*, and tubulin. Coloured arrowheads as in (B). Uncropped gel images of replicate 1 (D) and replicates 2 and 3 (E). (F,G) Images of full membranes are shown for cross-regulation of *MKRN1* and *ZNF598* overexpression (OE) from **Additional file 1: Fig. S8F**. *ZNF598* OE reduces *MKRN1* protein levels. Western blot analysis was performed with antibodies against *MKRN1*, *ZNF598*, and tubulin. Black arrowheads indicate *MKRN1*. Images of full membranes and different exposure times (exp.) for both antibodies are shown for replicates 1, 2 (F), and 3 (G). Note the opposite order of replicates 1 and 2 (2 left, 1 right) in (F). Coloured arrowheads as in (B).

Fig. S12



(H) Images of full membranes for Western blot analyses for replicate 1, as presented in **Additional file 1: Fig. S9B**, replicate 2, and 3 are shown. Western blot analyses were performed with antibodies against MKRN1 (petrol arrowhead), ELAVL1 (pink

arrowhead), RPS10 (green arrowhead), LARP1, PABPC1/3, and vinculin (grey arrowhead). (I,J) His-RPS10 was incubated with or without His-MKRN1 and with or without A₂₀ RNA oligonucleotides. Images of full membranes for Western blot analyses for replicate 1, as presented in **Fig. 5G** (left), replicate 2 (both I) and replicate 3 (J) are shown. Western blot analysis was performed with an antibody against RPS10. (K,L) His-PABPC1 was incubated with or without His-MKRN1 and with or without A₂₀ RNA oligonucleotides. Images of full membranes for Western blot analyses for replicate 1, as presented in **Fig. 5G** (right), replicate 2 (both K), and 3 (J) are shown. Western blot analysis was performed with antibody against PABPC1/3.