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).
Images of full membranes and different exposure times (exp.) for Western blot analyses in Fig. 1D. PABPC1 interacts strongly with MKRN1\(^{wt}\) and MKRN1\(^{RING\text{mut}}\) but only to a lesser extend with MKRN1\(^{PAM2\text{mut}}\). 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.
Recombinant His-MKRN1<sup>wt</sup> and His-MKRN1<sup>PAM2mut</sup> are capable of autoubiquitylation opposed to His-MKRN1<sup>RINGmut</sup>. Recombinant His-MKRN1<sup>wt</sup>, His-MKRN1<sup>PAM2mut</sup> and His-MKRN1<sup>RINGmut</sup> 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).
Image of the full gel for recombinantly expressed proteins, which were purified from *E. coli*, as shown in Additional file 1: Fig. S1E.
(J-L) Endogenous MKRN1 interacts with GFP-PABPC1 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 (J), shown in Additional file 1: Fig. S2C, 2 (K) and 3 (L). Black and petrol arrowheads indicate GFP-PABPC1 and MKRN1, respectively.
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 exposure times 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).
(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. 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).
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.
(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).
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), and RPS10.

**H**

**I** Fig. S12

**J**

**K**

**L**
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.