Supplemental Materials

Molecular Biology of the Cell

Qi et al.

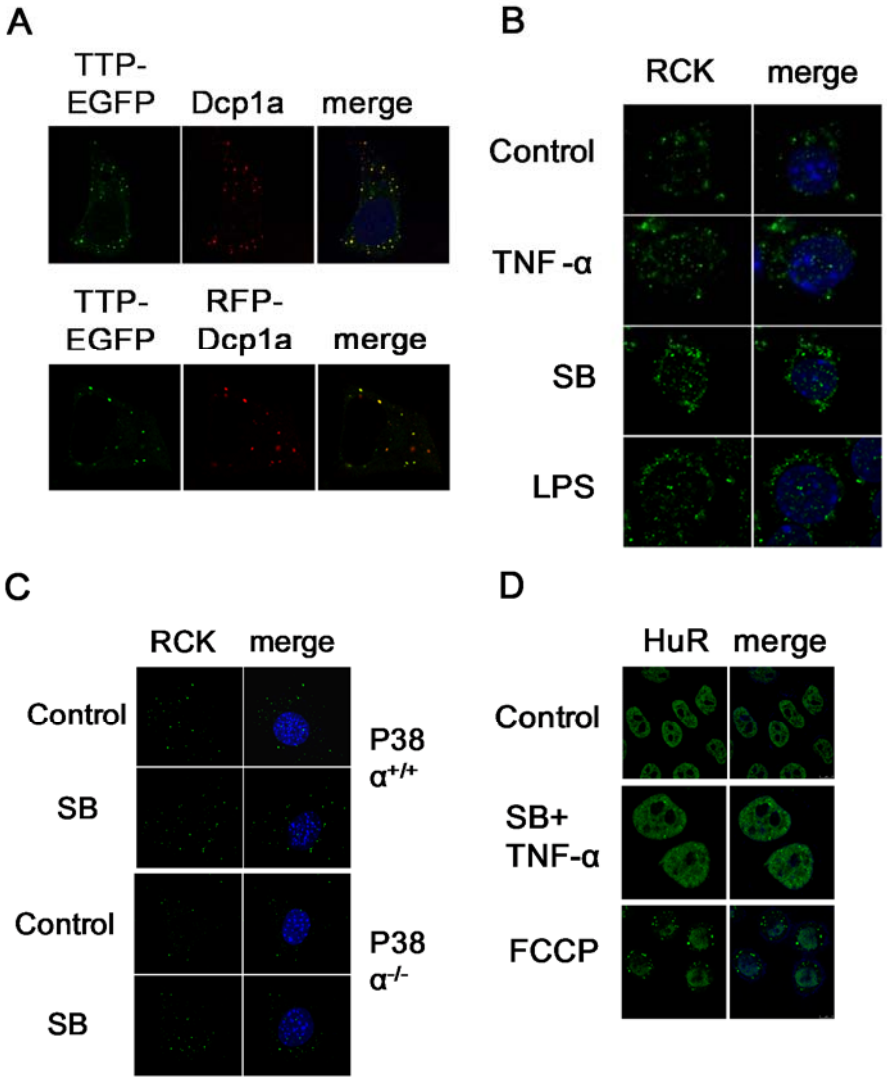

Fig. S1. P38 activity does not significantly affect PBs itself formation. (A) TTP-EGFP was expressed in Hela cells with or without DsRed-Dcp1a cotransfection. Showing TTP localized to discrete cytoplasmic foci and colocalized with the endogenous Dcp1a (upper panel) or Ds-Red-Dcp1a (lower panel), a signature component of the PBs. (B) RAW264.7 cells were treated with DMSO (10 uM) as a control, SB (10 uM), mouse TNF-α (10 ng/ml) or LPS (100 ng/ml) for 2h. PBs were visualized using the anti-RCK antibody staining. The right column represented the merge of RCK signal with DAPI. (C) P38 $\alpha^{+/+}$ and P38$\alpha^{-/-}$ MEF cells were treated with DMSO

(10 uM) as a control or SB (10 uM) for 2h. PBs were displayed using the anti-RCK antibody. (D) Hela cells were treated with DMSO (10 uM) as a control, with SB (10 uM) pretreatment for 1h and then with mouse TNF-α (10 ng/ml) for 2h, or with FCCP (30 uM) for 2h. Stress granules were visualized using the anti-HuR antibody staining.

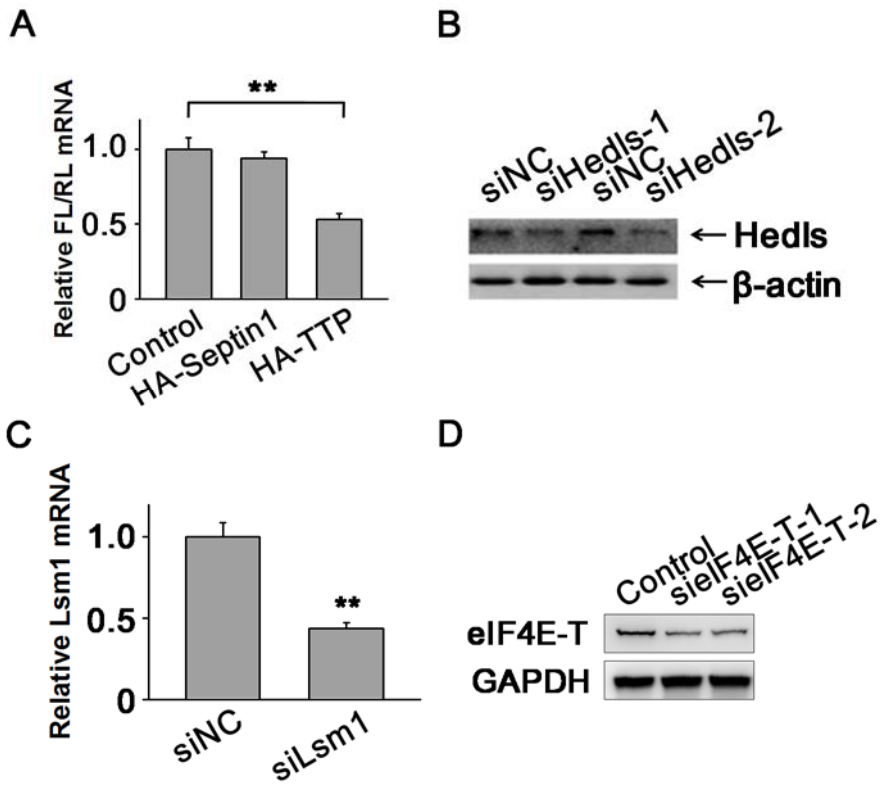

Fig. S2. The knockdowns of PBs component Hedls, Lsm1 and eIF4E-T effectively reduce their protein or mRNA expression. (A) 293T cells were transfected with FL-GM-CSF reporter plasmid and plasmids expressing HA-tagged TTP or Septin1. The corresponding vector served as control. Samples were analyzed as described above. The normalized value of FL mRNA level was set to 1 for cells transfected with empty vector. Means and SD from three independent experiments are shown. **, $P < 0.01$. (B) Knockdown effect of Hedls siRNAs. 293T cells were

transfected twice with the indicated siRNAs. Endogenous Hedls was analyzed by Western blotting with Hedls polyclonal antibody. β-actin served as a loading control. (C) Knockdown effect of Lsm1 siRNA. 293T cells were transfected twice with the Lsm1 siRNAs. The effectiveness of Lsm1 siRNA was analyzed by qRT-PCR. GAPDH mRNA served as an internal control. **, $P < 0.01$. (D) Knockdown effect of eIF4E-T siRNAs. 293T cells were transfected twice with the indicated siRNAs. Endogenous eIF4E-T was analyzed by Western blotting with eIF4E-T polyclonal antibody. GAPDH served as a loading control.

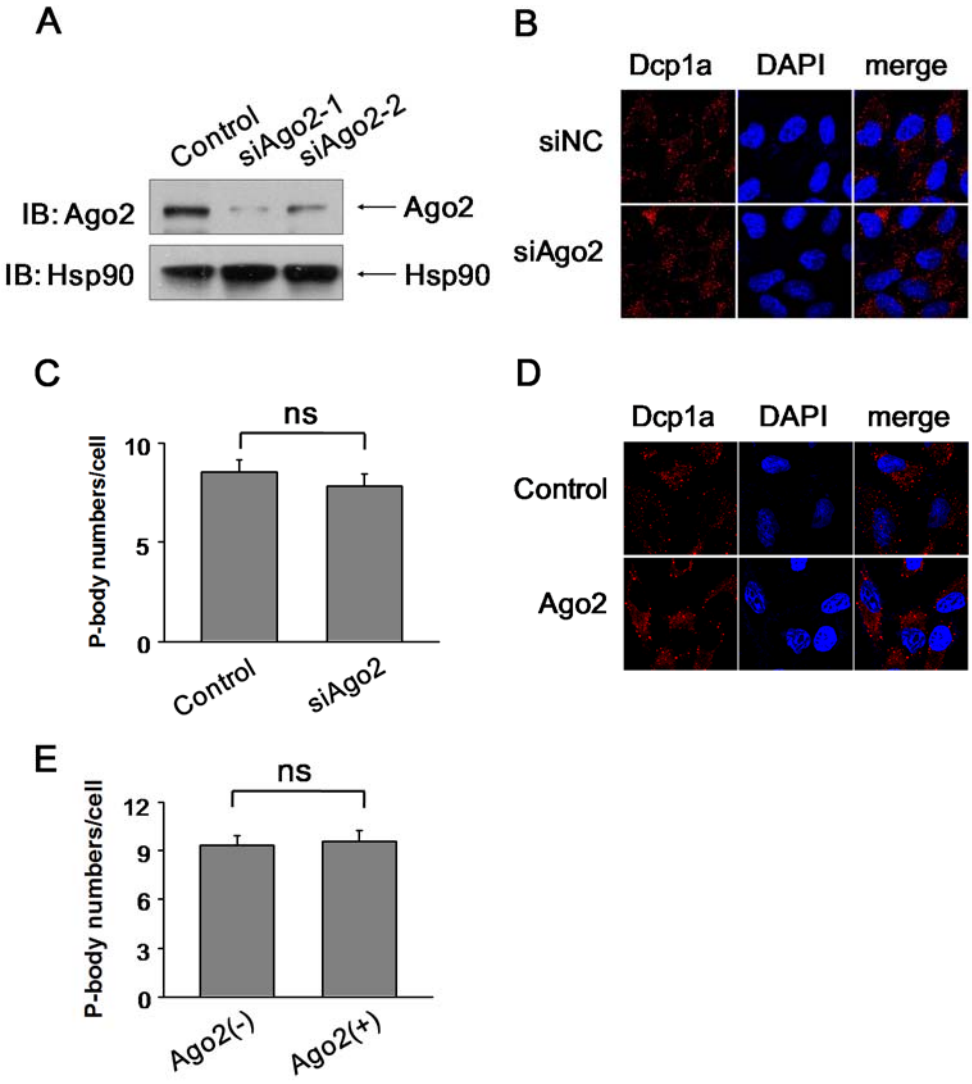

Fig. S3. Ago2 does not significantly affect PBs itself formation. (A) Knockdown effect of Ago2 siRNAs. 293T cells were transfected twice with the indicated siRNAs. Endogenous Ago2 was analyzed by Western blotting with Ago2 monoclonal antibody. Hsp90 served as a loading control. SiAgo2-1, but not siAgo2-2 was used in subsequent assays. (B) Ago2 knockdown did not affect the PBs formation. Hela cells were treated with siAgo2 or siNC twice. PBs were showed by anti-Dcp1a staining. (C) Graph showing the P-body numbers per cell. Error bars represent standard error calculations obtained from averaging the PB number for 50 cells. ns, no significance. (D) Ago2 overexpression did not affect the PBs formation. Hela cells were transfected with Myc-Ago2 or corresponding empty vector. PBs were displayed with anti-Dcp1a staining. (E) Graph showing the P-body numbers per cell. Error bars represent standard error calculations obtained from averaging the PB number for 50 cells. ns, no significance.

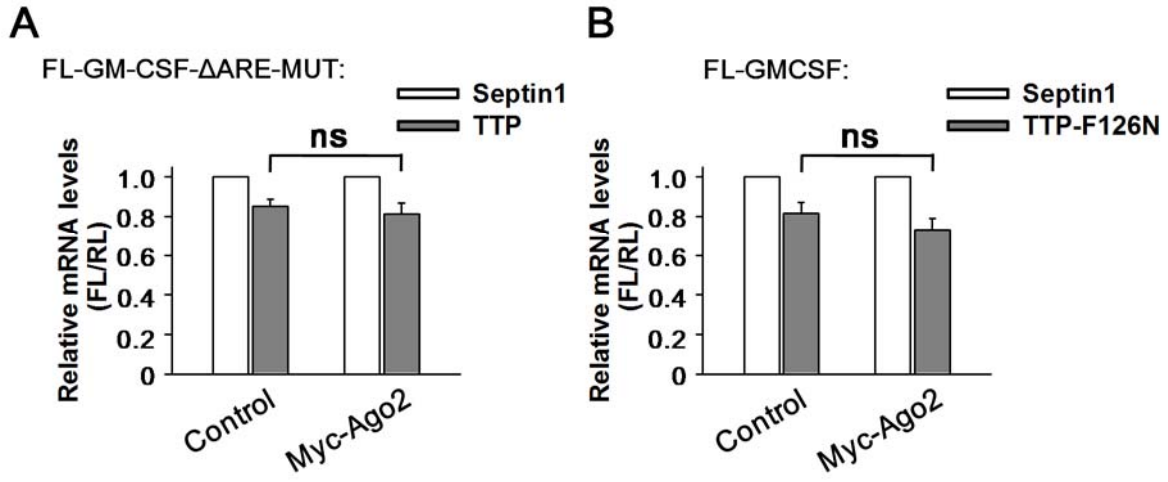

Fig. S4. Ago2 mediates ARE-mRNA degradation requires functional ARE motif or TTP. (A) 293T cells were transfected with the FL-GM-CSF-ΔARE reporter and RL plasmids, together with two plasmids, one expressing either HA-tagged TTP or HA-Septin1 and another expressing either Myc-tagged Ago2 or Myc-Septin1 (control). The relative value of FL mRNA level was set to 1 for cells transfected with plasmid expressing HA-tagged Septin1 in each condition. Means

and SD from three independent experiments are shown. ns, no significance. (B) Results of an experiment similar to that for panel A, except that the reporter FL-GM-CSF-ΔARE was replaced with FL-GM-CSF, HA-tagged TTP was replaced with HA-tagged TTP-F126N. ns, no significance.