Supplemental Fig. legends

Supplemental Fig. 1 Characterization of the anti-P-S1078/1096-Upf1 monoclonal antibodies.

The anti-P-S1078/1096-Upf1 monoclonal antibodies (clone 7D5 and 8E6) recognize S1078/S1096 phosphorylation of Upf1. Cells were transfected with the HA-Upf1 plasmid indicated above the blot, and were treated with (+) or without (-) 50 nM Okadaic acid for 4 hr. Cell lysates were immunoprecipitated with anti-HA antibody, followed by Western blot analysis using antibody probes shown to the left of each blot.

Supplemental Fig. 2 Identification of Upf1 and EJC binding sites in SMG-6.

(A) Alignment of the Upf3 homology region with SMG-6. Arrowhead and asterisk indicate amino acid residues replaced in this study and in the study of Kashima et al. 2010, respectively. T **(B)** Schematic representation of the SMG-6 mutant. The Upf3 like domain, RNA binding domain, 14-3-3-like domain and PIN domain are shown in gray boxes. In SMG-6-mtEJC, RRP45-47 and KKP139-141 are substituted with AAA and AEA, respectively. **(C)** HeLaTetOff cells were transfected with the plasmids shown above or with an empty vector (“vector”). The cell lysates were purified with streptavidin sephorose. The cell lysates (Input) and purified fractions were analyzed by western blotting with the antibodies shown on the left of the panels. Although SMG-6-mtEJC

abolished EJC binding, it supports NMD, making a contrast to previous report (Kashima et al, 2010). It might be caused by the difference in the mutation site (Supplemental Fig. 1A).

Supplemental Fig. 3 S/TQ motifs in the N- and C-terminal regions of Upf1 are conserved in multicellular organisms.

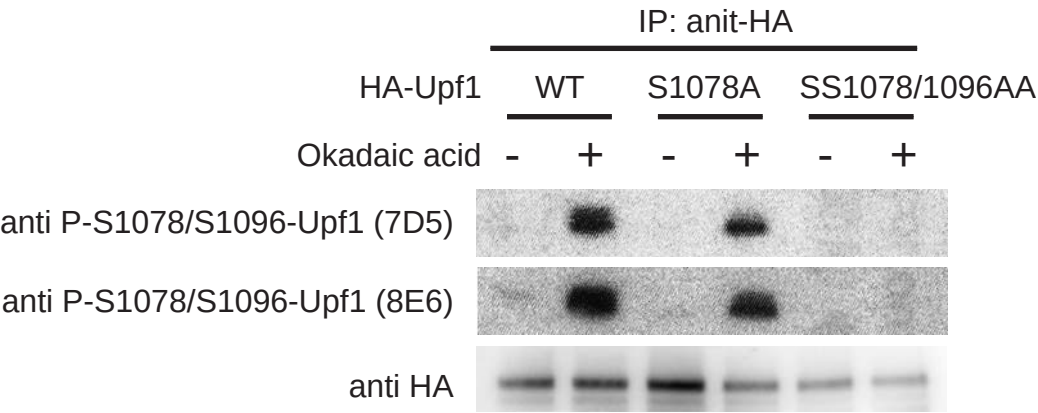
(A) Schematic representation of Upf1. The N-terminal conserved region (NCR), cystein rich region, helicase domains, and SQ-rich region are shown by gray boxes. **(B, C)** Alignments of S/TQ motifs in the N-terminal (B) and C-terminal (C) regions of Upf1 among multicellular organisms. Arrowhead and asterisk indicate amino acid residues replaced in this study.

Supplemental Fig. 4 The Upf1 complex containing SMG-5-dCT or SMG-6-dCT accumulates on mRNP with CBP80 and PABPC1/4.

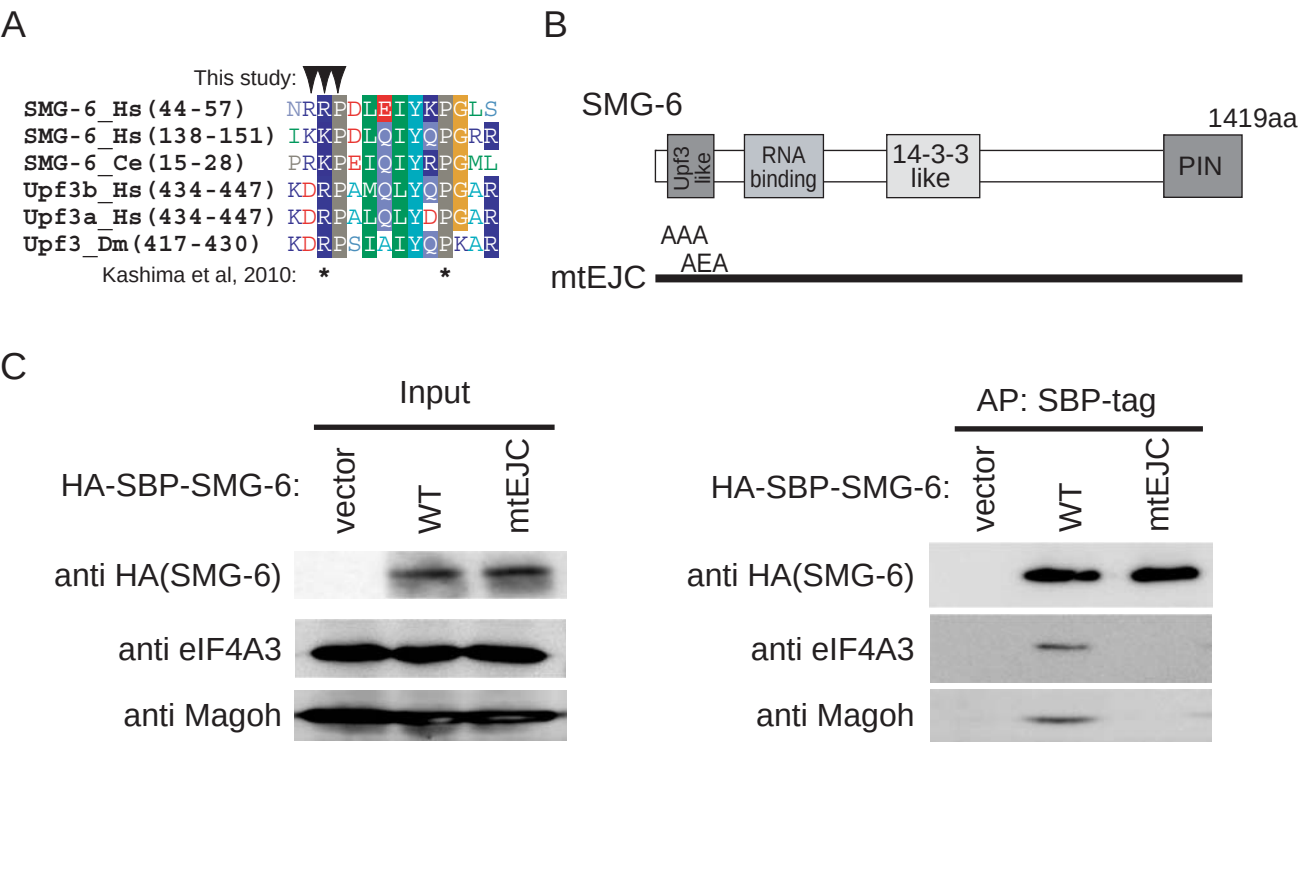
HeLa TetOff cells were transfected with the indicated plasmids. The cell extracts were immunoprecipitated with anti-Upf1 (5C3) antibody in the presence or absence of RNaseA. Immunoprecipitates (IP) were analyzed by western blotting with the antibodies shown on the left.

Supplemental Fig. 5 ATPase deficient Upf1 accumulates on mRNP with CBP80 and PABPC1/4.

(A) Schematic representation of Upf1 mutants. The NCR, cystein rich region, helicase domains and SQ-rich region are depicted by gray boxes. In Upf1-K498Q, K498 is substituted for Q. (B) HeLa TetOff cells were transfected with the indicated plasmids. The cell extracts were immunoprecipitated with anti-HA-sepharose in the absence or presence of RNaseA. The cell extracts and immunoprecipitates were analyzed by western blotting with the antibodies shown on the left of the panels. (C) Schematic presentation of the Upf1-K498Q complex. The presence of SMG1C is not determined.

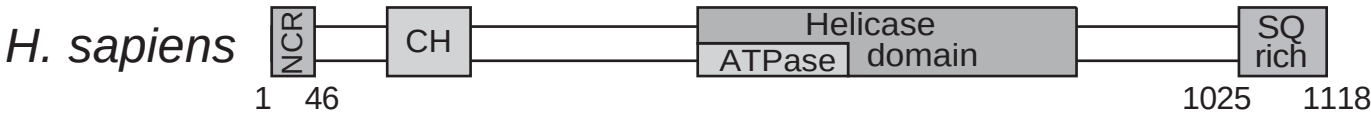


Okada-Katsuhata et al. Supplemental Figure 1



Okada-Katsuhata et al. Supplemental Figure 2

A



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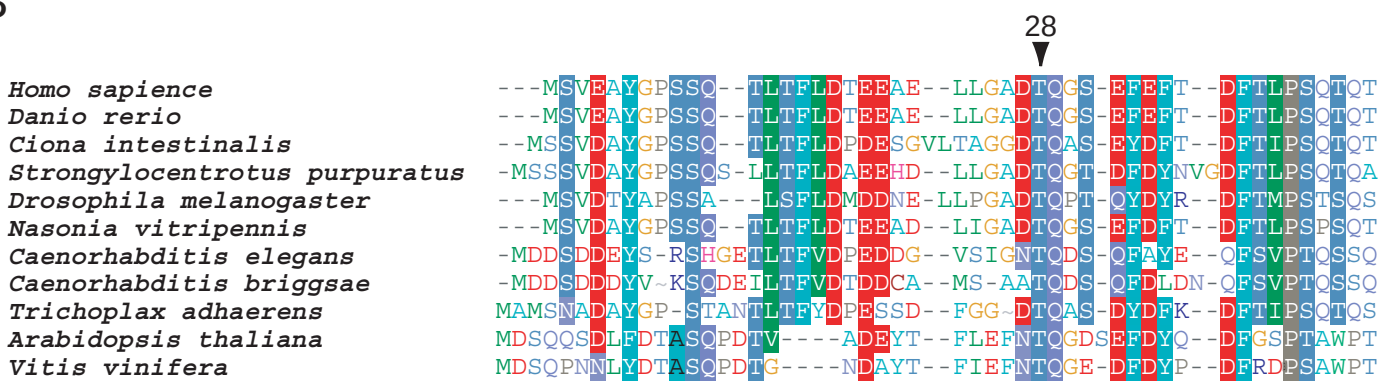

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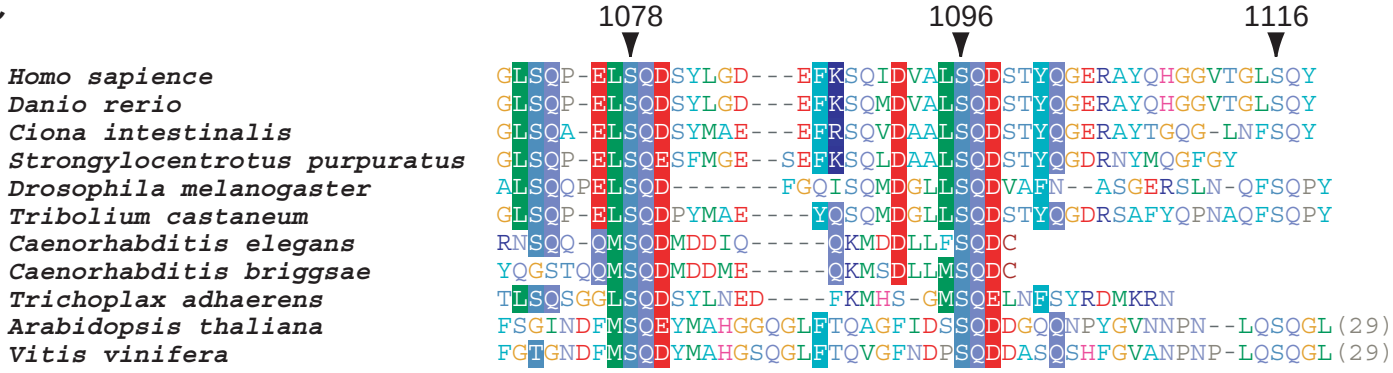
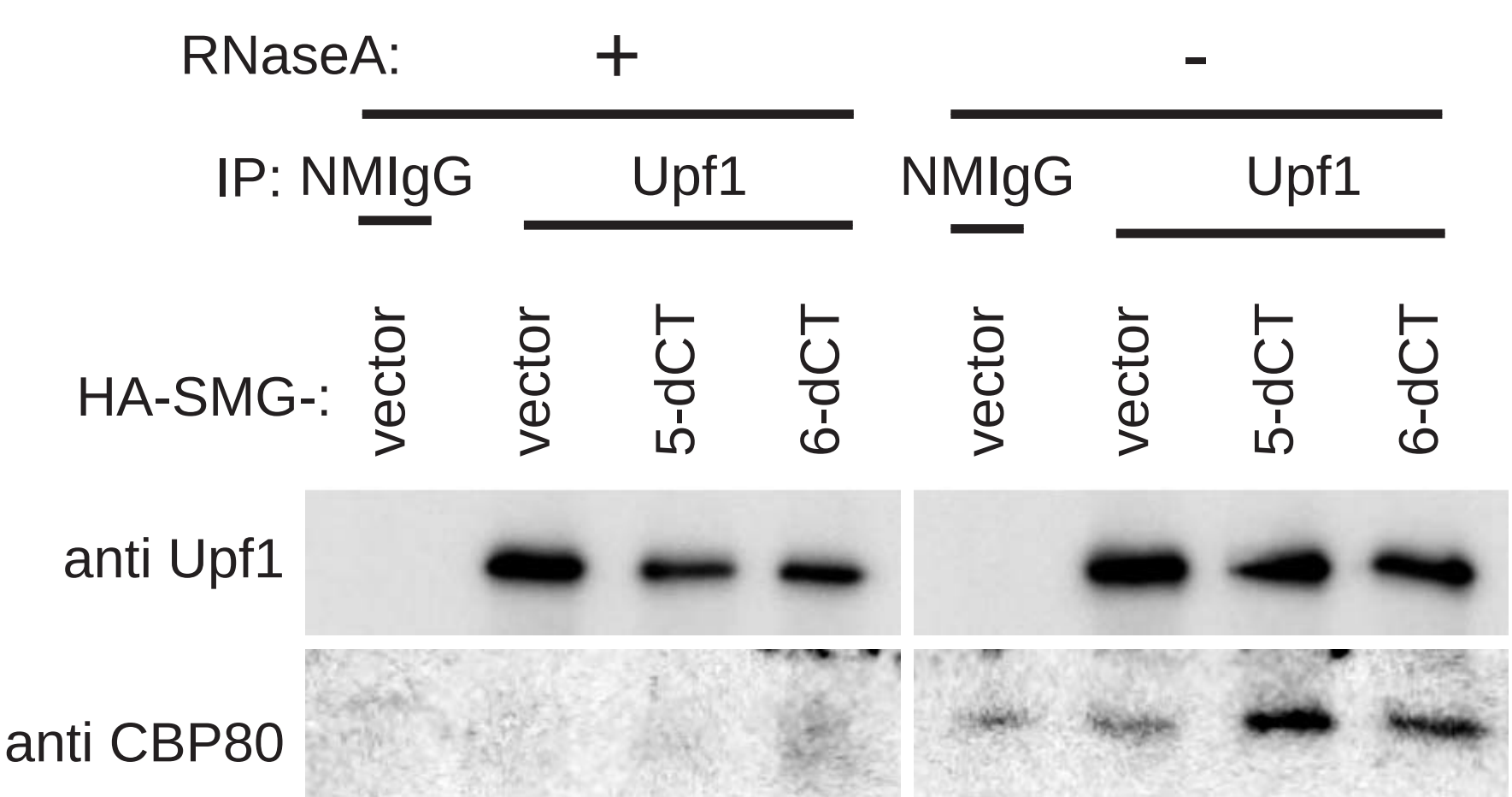
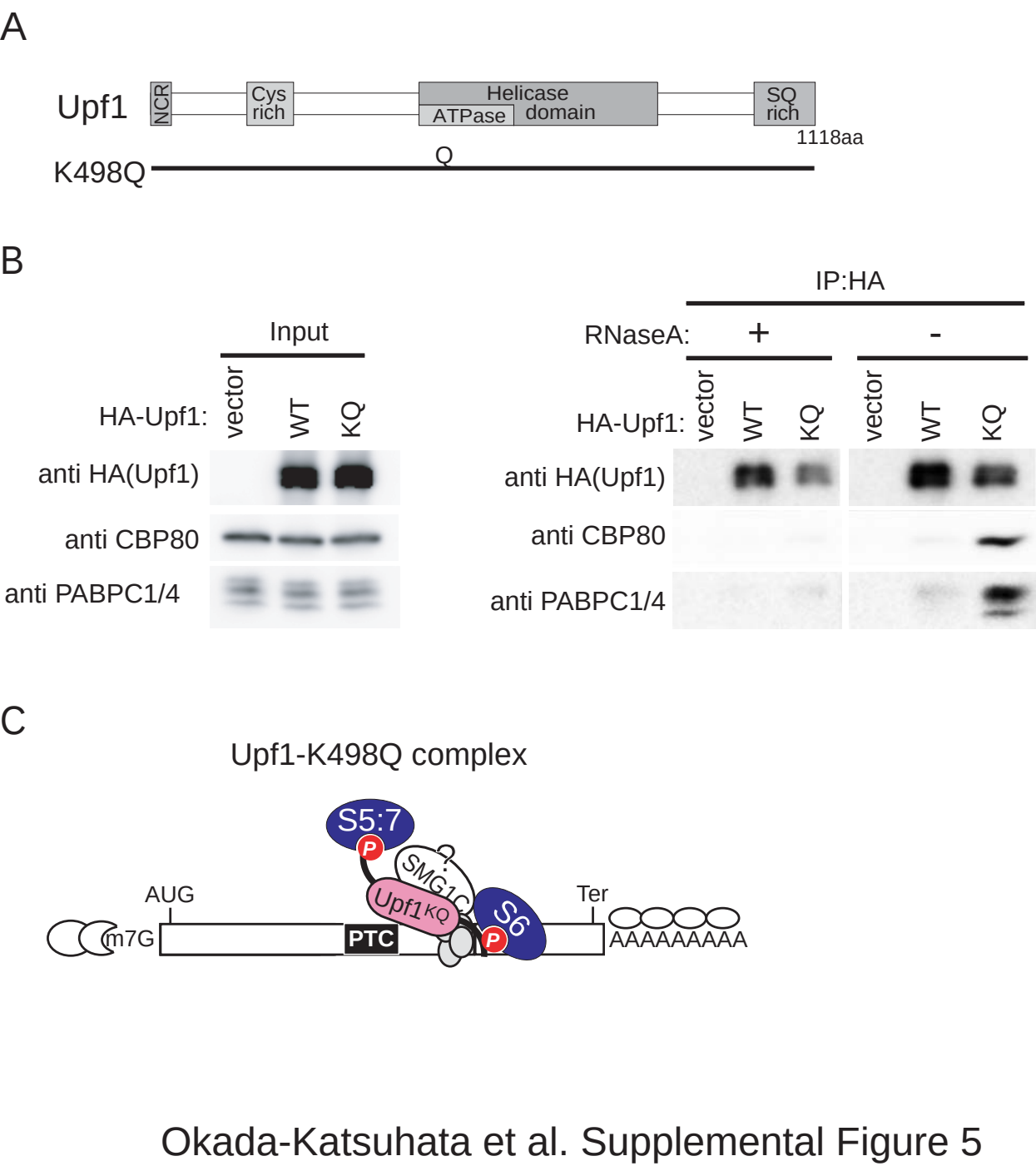


Okada-Katsuhata et al. Supplemental Figure 3



Okada-Katsuhata et al. Supplemental Figure 4



Okada-Katsuhata et al. Supplemental Figure 5