SUPPLEMENTARY FIGURE LEGENDS

Supplementary figure 1. Protein sequence alignment of human TOB1 and TOB2 proteins. Different symbols denote the specific mutations introduced into wild-type proteins. Red line

5 underlines PAM2 motifs.

Supplementary figure 2. Secondary structure prediction of TOB1 wild-type vs. TM mutant. The analysis indicates no apparent change in the predicted secondary structures between wild-type and TM mutant.

10

Supplementary figure 3. The ability of TOBs to promote deadenylation and decay is not affected by the phosphorylation status of three conserved serines during G0 to G1 transition.
(A) Northern blot analyses of deadenylation and decay of reporter transcript (BBB+12bs mRNA) in cells expressing MS2, MS2-TOB1, MS2-TOB2, or the 3SA and 3SE mutants of the

- 15 MS2-TOBs. Cell transfection and poly(A)⁻ mRNA (A-) preparation were as described in the Fig. 1 legend. Cells were cultured in medium with 0.5% serum for 24 hours prior to serum stimulation. Following serum stimulation, samples were taken at the indicated times after tetracycline addition. (B) Western blot analysis of protein levels for MS2-TOBs and their mutant derivatives, with GAPDH served as loading control. mRNA half-life (t1/2)
- 20 determination is described under Materials and Methods section.

hTOB1 hTOB2	1 1	MQLEIQVALNFIISYLYNKLPRRR <mark>VNIFGEELERLLKKKYEGF</mark> MQL <mark>EIKVALNFIISYLYNKLPRRRAD</mark> LFG <mark>EELERLLKKKYE</mark> GF	WYPEKPYKGSGFRCIHI WY <mark>PEK</mark> Plkgsgfrcvhi	60 60
hTOB1 hTOB2	61 61	GEKVDPVIEQASKESGLDIDDVRGNLPQDLSVWIDPFEVSYQI GEMVDPVVELAAKRSGLAVEDVRANVPEELSVWIDPFEVSYQI	I <mark>GEKG</mark> PVKVLYVDDNNEN IG <mark>EKG</mark> AVKVLYLDDSEGC	120 120
hTOB1 hTOB2	121 121	* GC-ELDKEIKNSFNPEAQVFMPISDPASSVSSSPSPPFGHSAA GAP <mark>ELDKEIKSSFNPD</mark> AQVFVP <mark>I</mark> GSQD <mark>SSLSNSPSP</mark> SFG <mark>QS</mark> -F	V <mark>SPTFMPRSTQPLTF</mark> TT 2- <mark>SPTFIPRS</mark> AQ <mark>PITF</mark> TT	179 178
hTOB1 hTOB2	180 179	ATFAATKFGSTKMK <mark>NSG</mark> R <mark>S</mark> NK-VA	RT <mark>SP</mark> INLGLNV <mark>N</mark> DLLKQK R- <mark>SP</mark> T <mark>N</mark> SLLKHK	220 231
hTOB1 hTOB2	221 232	AISSSMHSL-YGLGLGSQQQPQQQQQ <mark>P</mark> AQPPPPPPPPPPPQQQQQQ SLSLSMHSLNF-ITANPAPQSQ	* QKTSA <mark>LSPNAKEF</mark> L <mark>SPNAKE</mark> FVYNGG	274 265
hTOB1 hTOB2	274 266	<mark>I-FPNMQGQGSSTNGMFPG</mark> DSPL <mark>NLS</mark> PLQY <mark>SNAFD</mark> - GSPSLFFDAADGQGSGTPGPF-GGSGAGTC <mark>NSSSFD</mark> N	<mark>VFAAY</mark> GGLN <mark>EK</mark> 1AQ <mark>VF</mark> G <mark>GG</mark> ANSLFL <mark>E</mark> K	319 316
hTOB1 hTOB2	320 317	<mark>S-FVDGLNFSLNN</mark> MQY- <mark>S</mark> NQQFQ <mark>PVM-AN</mark> 345 T <mark>PFVEGLSYNLN</mark> IMQY <mark>PS-QQFQPVVLAN</mark> 344	TOB1: S(152,154,164) -> A (3SA) S(152 154 164) -> F (3SF)	
: amino acids mutated to abolish CAF1 interaction			L(32,35,36) -> G(3LG)	
↓ : serines mutated to alter phosphorylation state				
*: F to A change to abolish PABP interaction TOB2: S(153,155,163) -> A (TOB2: S(153,155,163) -> A (3SA)	
: PAM2 motifs			S(153,155,163) -> F (3SE)	

Shyu Supplementary Figure 1

S(153,155,163) -> E (3SE) L(32,35,36) -> G (3LG) E62,M63,D65 -> A (TM)

Secondary structure prediction of TOB1 wild-type vs. TM mutant



TM MUTATIONS (E62,K63,D65 -> A) Pred: AA: MQLEIQVALNFIISYLYNKLPRRRVNIFGEELERLLKKKY 30 10 20 40 Conf: Pred: AA: EGHWYPEKPYKGSGFRCIHIGAAVAPVIEQASKESGLDID 50 70 80 Pred: Pred: HHHHHCCCCEEEEEECCCCEEEEEECCCCCCE AA: DVRGNLPQDLSVWIDPFEVSYQIGEKGPVKVLYVDDNNEN 90 100 110 120 Pred: AA: GCELDKEIKNSFNPEAQVFMPISDPASSVSSSPSPPFGHS 130 140 150 160 Pred: _ AA: AAVSPTFMPRSTQPLTFTTATFAATKFGSTKMKNSGRSNK 170 180 190 200 Pred: Pred: CCCCCHHHHCCCCCCCHHCCHHHHHHHCCCCCCCCCHHHCC AA: VARTSPINLGLNVNDLLKQKAISSSMHSLYGLGLGSQQQP 210 220 230 240 Pred: AA: QQQQQPAQPPPPPPPQQQQQQKTSALSPNAKEFIFPNMQ 250 260 270 280

Shyu Supplementary Figure 2





Shyu Supplementary Figure 3