Tsuboi *et al.*, Supplementary Fig. 1

ATG*gactacaaggacgacgacgacaag*CTTGCTGAAATTGCTGAAGACGCTGC
AGAAGCTGAAGACGCTGGTAAGCCAAAGACCGCTCAATTGTCTTTGCAAGACT
ACTTGAACCAACAAGCTAACAACCAGTTCAACAAGGTCCCAGAAGCTAAGAAG
GTTGAATTAGACGCTGAAAGAATTGAAACTGCTGAAAAGGAAGCTTACGTTCC
AGCAACCAAGGTCAAGAACGTCAAATCTAAGCAATTGAAGACCAAGGAGTACT
TGGAATTTG**c**actagtCACTTTTGTTGAATCTAACACTAGAAAGAACTTCGGT
GACAGAAACAACAACAGCAGAAACAACTTCAACAACCGTCGTGGTGGTAGAGG
CGCTAGAAAGGGTAACAACACTGCTAACGCTACTAACTCTGCTAACACCGTTC
AAAAGAACCGTAACATTGACGTTTCTAACTTGCCATCTTTGGCTTAA

Supplementary Fig. 1
Sequence of the reporter genes. The reporter genes were constructed using the C-terminal region of the *MPT4* ORF (337-822 nts, Upper case). The DNA sequence of the *AUG-FLAG-MS2* reporter gene is shown. The ATG translation initiation codon of this reporter is indicated in bold and is followed by the FLAG tag sequence (Italics, lower case). An internal ATG codon at 629-631 nts was eliminated by the introduction of an SpeI restriction site (underline). The ATG initiation codon was converted to a TAC codon in the *No-AUGFLAG-MS2* reporter gene. An MS2 site (not shown) was inserted into the 3' -UTR region to tether FLAG-MS2-Pab1p fusion proteins.
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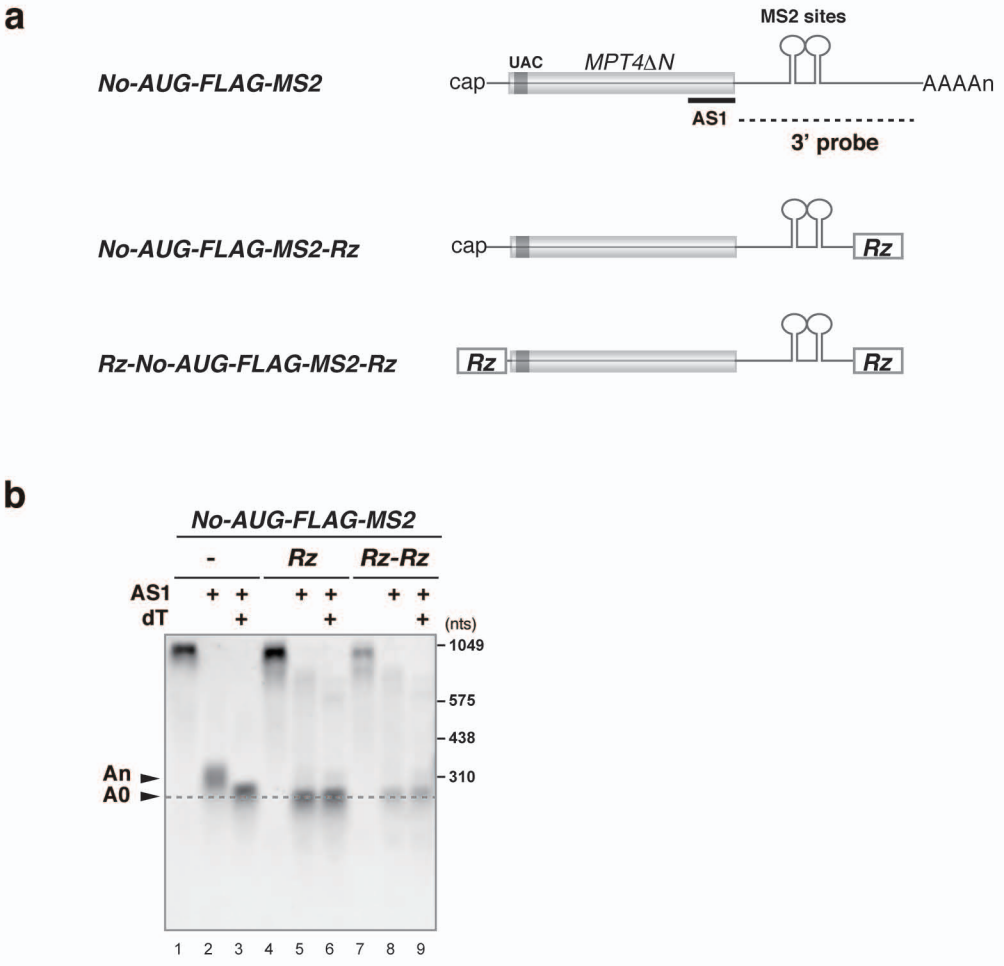
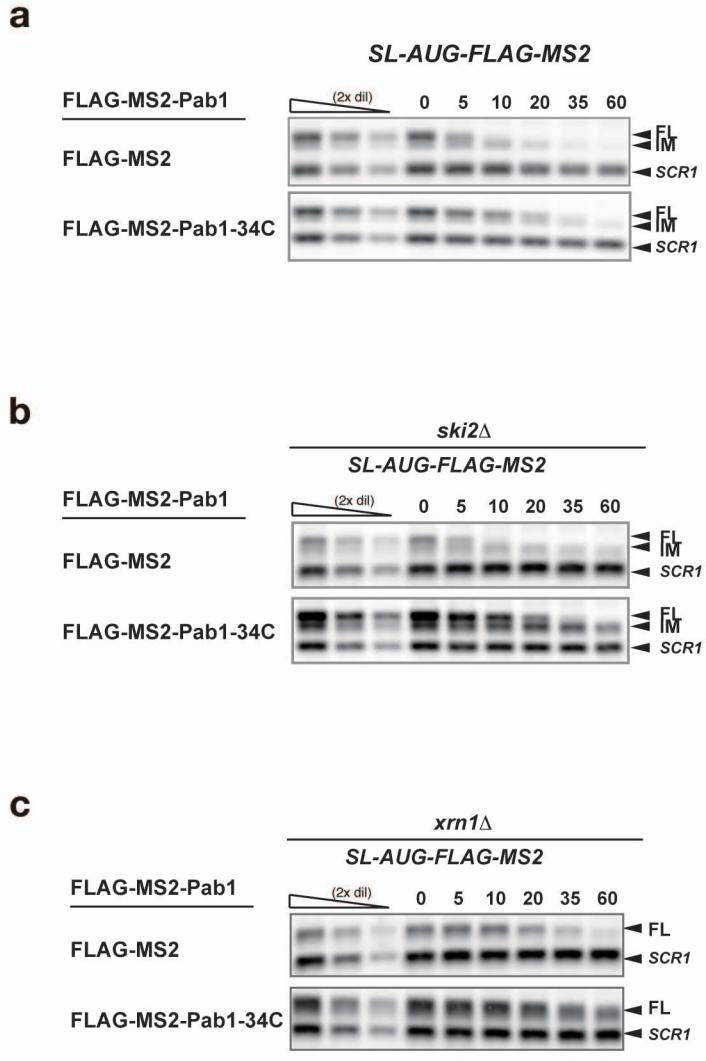
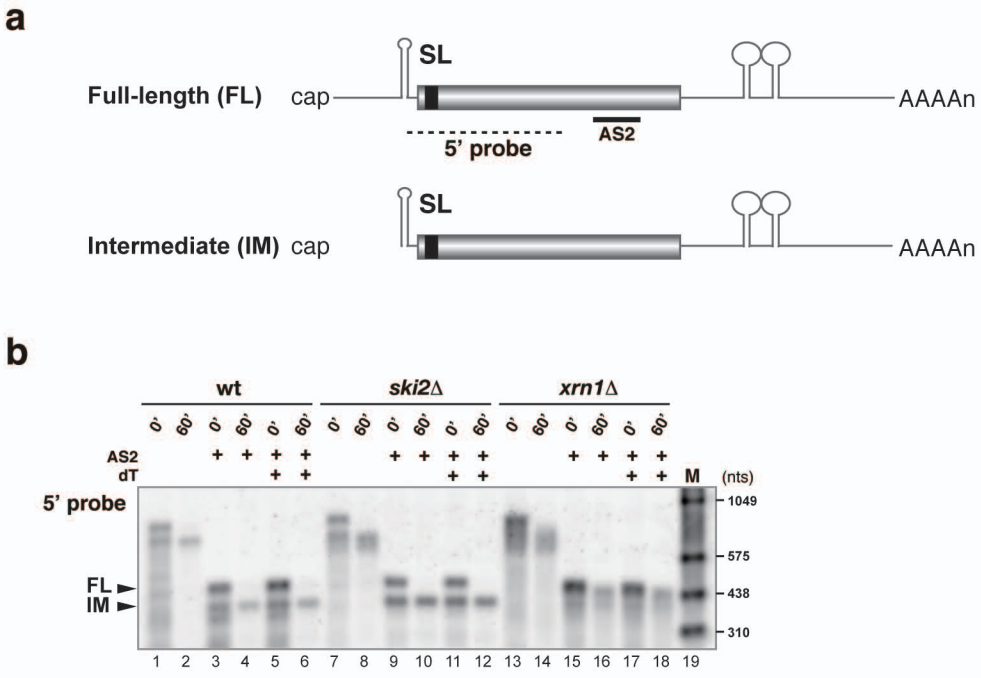


Supplementary Figure 2. The poly(A) tail by insertion of a hammerhead ribozyme sequence downstream of the MS2 binding sites.
(a) Schematic drawing of the *No-AUG-FLAG-MS2*, *No-AUG-FLAG-MS2-Rz* and *Rz-No-AUG-FLAG-MS2-Rz* mRNAs. The filled box indicates the open reading frame of *MPT4ΔN* and the dark grey box indicates the FLAG tag. The lines represent non-translated regions, and the tract of As denotes the poly(A) tail. MS2 binding sites are indicated as stem-loop structures in the 3' -UTR. Rz indicates the hammerhead ribozyme sequence. The probe used for Northern hybridization of the constructs shown in b is indicated as dashed lines. The antisense oligonucleotide (AS1: 5' -CCAAAGATGGCAAGTTAGAAACGTCAATGT-3 '), used for RNase H digestion, is indicated as a bold line. (b) W303 cells harboring pIT2071 (*No-AUG-FLAG-MS*), pIT2085 (*No-AUG-FLAG-MS2-Rz*) or pIT2087 *(Rz-No-AUG-FLAG-MS2-Rz*) were grown in SC-Gal UraLeu medium, and RNA samples were prepared as in Figure 3a. RNA samples were digested with RNase H after hybridization with the AS1 oligonucleotide (AS1) with or without oligo dT (dT), and Northern blot analysis was performed with the DIG-labeled 3' probe.



Supplementary Figure 3. Tethering of Pab1p-34C stabilizes mRNAs that contained a stable stem-loop structure at the 5' -UTR. W303 (a) or W303*ski2*Δ (b), W303*xrn1*Δ (c) cells harboring the pIT2073 (*SL-AUG-FLAG-MS2*) plasmid were transformed with the control p415*TEF1p* or p415*TEF1p-FLAG-MS2-Pab1-34C* plasmid. Cells were grown in SC-Gal UraLeu medium, and RNA samples were prepared to measure the mRNA stability as described in Fig. 3a.

FL; full length, IM; intermediate form that arises during degradation, *SCR1*; RNA subunit of the Signal Recognition Particle.



Supplementary Figure 4. The shorter mRNA corresponds to an intermediate mRNA form that lacks a 5' region upstream of the stem-loop structure. (a) Schematic drawing of the full length (FL) and intermediate (IM) forms of the *SL-AUG-FLAG-MS2* gene. The filled box indicates the black reading frame of MPT4ΔN and the open box indicates the FLAG tag; the lines represent non-translated regions, and the tract of As denotes the poly(A) tail. MS2 binding sites are indicated as stem-loop structures in the 3' -UTR. A stable stem-loop structure for inhibition of translation (SL) was introduced into the 5' -UTR of the *AUG-FLAG-MS2*. The probes used for Northern hybridization in b are shown as dashed lines. The antisense oligonucleotide (AS2: 5' -GCTCTAGACCTCTACCACCACGACGGTTGT-3'), used for RNase H digestion, is indicated as a bold line. (b) Wild type (wt), *ski2*Δ or *xrn1*Δ cells harboring p416*GAL1p-SL-AUG-FLAG-MS2* were grown in SC-Gal UraLeu medium, and RNA samples were prepared as shown in Figure 3a. RNA samples were prepared before (0') or 60 min (60') after the addition of glucose. RNA samples were digested with RNase H after hybridization with the AS2 oligonucleotide (AS2) with or without oligo dT (dT), and Northern blot analysis was performed using a DIG-labeled 5' probe.