Supplementary Figure S1

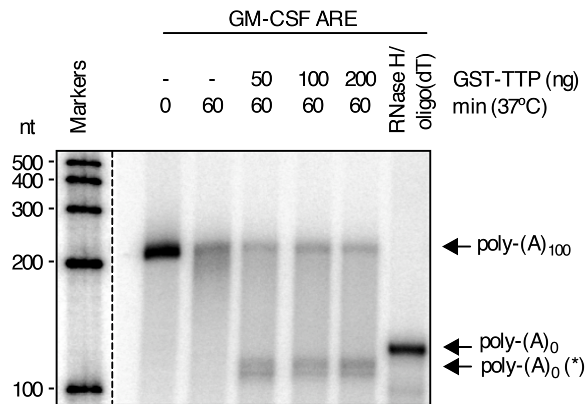

Figure S1 The intermediate seen in IVDA lacks a poly-(A) tail and additional ≈5 nt. IVDA of HeLa cell S100 (5 μg) incubated with ^{32}P-labeled GM-CSF ARE RNA substrate in the absence or presence of different amounts of GST-TTP at 37°C for 60 min. Where indicated the RNA was incubated with oligo(dT) and RNase H. Markers are shown and molecular size is given in nucleotides (nt). The positions of polyadenylated substrate and TTP-mediated deadenylated product bands are indicated as poly-$(A)_{100}$ and poly-$(A)_0$ (*), respectively. Dashed line indicates lanes removed from phosphorimage.

Supplementary Figure S2

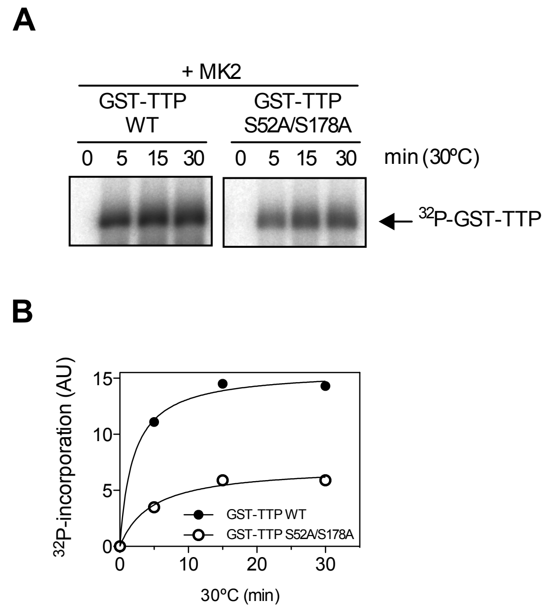

Figure S2 Time course of phosphorylation of wild-type and S52A/S178A GST-TTP by MK2. (A) Recombinant active MK2 was used to phosphorylate GST-TTP (wild-type or S52A/S178A) for the times indicated at 30ºC in the presence of [γ-^{32}P]-ATP. Proteins were separated by SDS-PAGE and ^{32}P incorporation was visualised by phosphorimaging. Analysis of GST-TTP phosphorylated for different times in the presence of [γ-^{32}P]-ATP showed that ^{32}P incorporation was essentially complete at 15 min. Some phosphorylation of S52A/S178A GST-TTP occurred, consistent with the observation that MK2 phosphorylates additional residues in TTP (5). Different portions of the same gel are shown. A graph of ^{32}P incorporation (AU, arbitrary unit) in wild-type and S52A/S178A GST-TTP is shown (B).

Supplementary Figure S3

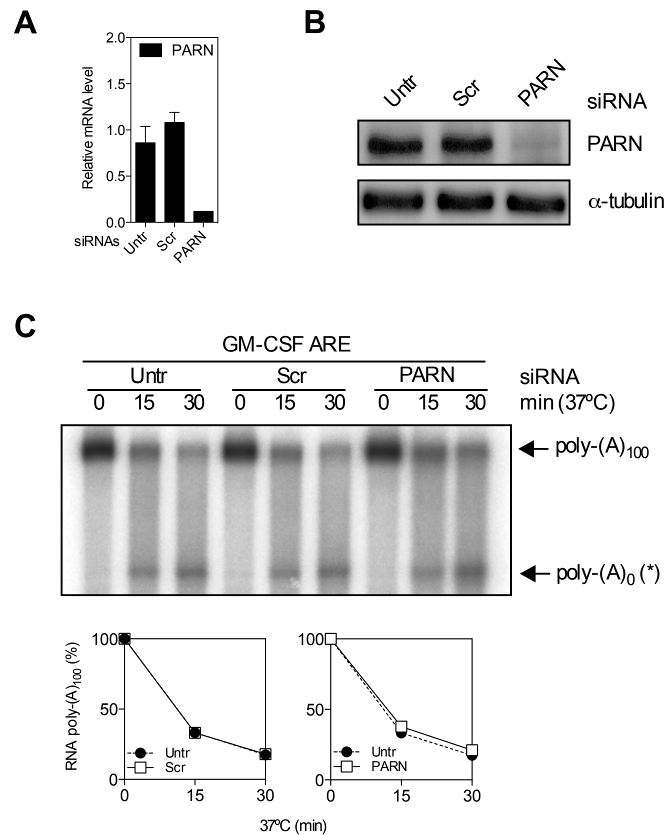

Figure S3 Depletion of PARN in HeLa cells has no effect on TTP-directed deadenylation. PARN expression was suppressed in HeLa cells by RNAi as in Fig. 6. Depletion efficiency was determined by Q-RT-PCR of RNA from knockdown cells (A) and by western blotting (B). IVDAs were performed in the presence of GST-TTP as in Fig. 2 using PARN siRNA-depleted S100 (C). Graphs show deadenylation of poly-$(A)_{100}$ RNA substrate against time as in Fig. 2.

Supplementary Figure S4

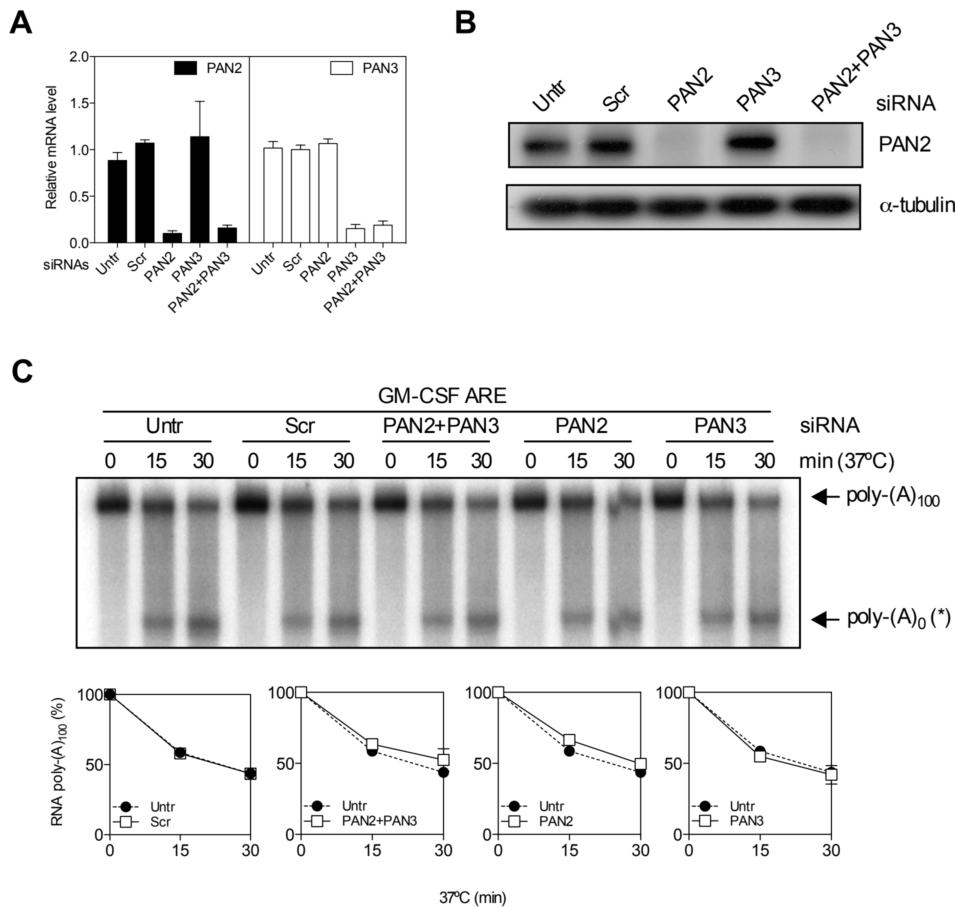

Figure S4 Depletion of PAN2-PAN3 in HeLa cells has no effect on TTP-directed deadenylation. PAN2 and PAN3 expression was suppressed, separately or in combination (PAN2 + PAN3), in HeLa cells by RNAi as in Fig. 6. Depletion efficiency was determined by Q-RT-PCR of RNA from knockdown cells (A) and by western blot for PAN2 (B). IVDAs were performed as in Fig. 2 using PAN2-PAN3 siRNA-depleted S100 (C). Graphs show deadenylation of poly-$(A)_{100}$ RNA substrate against time as in Fig. 2.