Figure S1. Toe-print analysis of termination complexes on MVHL mRNA. (A) Examples of raw data from capillary electrophoresis of cDNA products obtained using fluorescently labeled primers for toe-print analysis. Elongation complexes on MVHL mRNA, preTCs after SDG purification, TC formation induced by addition of eRF1 and eRF3a to the preTCs, TC formation induced by addition of eRF1, eRF3a and PABP to the preTCs. **(B)** Toe-print analysis of termination complexes in the presence of high concentrations of eRFs and PABP. Positions of preTCs and TCs are labeled by white and black triangles respectively. Full-length mRNA corresponds the 219 nt peak.

Figure S2. PABP increases the recognition of stop codons on all +4 contexts. Toe-print analysis of termination complexes formed by preTCs containing MVHL-UAAC (left), MVHL-UAAG (middle) and MVHL-UAAA (right) mRNAs in the presence of eRF1•eRF3a•GTP (above and below) and PABP (below). Rfu – relative fluorescence unit. Positions of preTCs and TCs are labeled by white and black triangles respectively. The MVHL mRNA used in this study has a MVHL-UAAU context (Figure 1).

Figure S3. PABP increases stop codon recognition by release factors in *cis* and *trans*. Negative controls: Toe-print analysis of termination complexes formed by addition to the preTCs of eRF1(AGQ)•eRF3a•GTP (middle) or eRF1(AGQ)•eRF3c•GTP (right). Complexes were assembled utilizing mixtures of MVHC-polyA and MVHL mRNAs in the absence of PABP. Different toe-printing primers were used to monitor stop codon recognition for complexes bound to MVHC-polyA mRNA (above) or to MVHL mRNA (below). Rfu – relative fluorescence unit. Positions of preTCs and TCs are labeled by white and black triangles respectively.

Figure S4. PABP increases the efficiency of peptidyl-tRNA hydrolysis in the presence of limiting amounts of release factors. (A) Minimal and maximal level of peptidyl-tRNA hydrolysis in the TCs induced by addition of eRF1 and eRF3. The minimal level of peptide release was determined by incubation of 0.125 pmol of either eRF1 or eRF3a with preTCs for 3 minutes. The maximal level was determined by incubation of 4 pmol of both factors eRF1 and eRF3a with the preTCs for 3 minutes. **(B**) Hydrolysis of peptidyl-tRNA in the TCs induced by different amounts of individual release factors eRF1 and eRF3a in the presence of PABP. The star (*) marks a significant difference from the respective control $p<0,05$.

Figure S5. Additional Western blot analyses of eRFs and PABP binding to TCs. (A) Western blot analysis of preTCs separated via SDG centrifugation using antibodies raised against ribosomal proteins S15 and L9. **(B)** Distribution of eRF3c, eRF3a•PABP and eRF1•eRF3a•PABP in absence of ribosomes in SDG. **(C)** Western blot analysis of preTCs incubated with PABP using antibodies against PABP. The fractions of the SDG are indicated above the Western blots; 1 corresponds to the top and 14 to the bottom of the gradient.