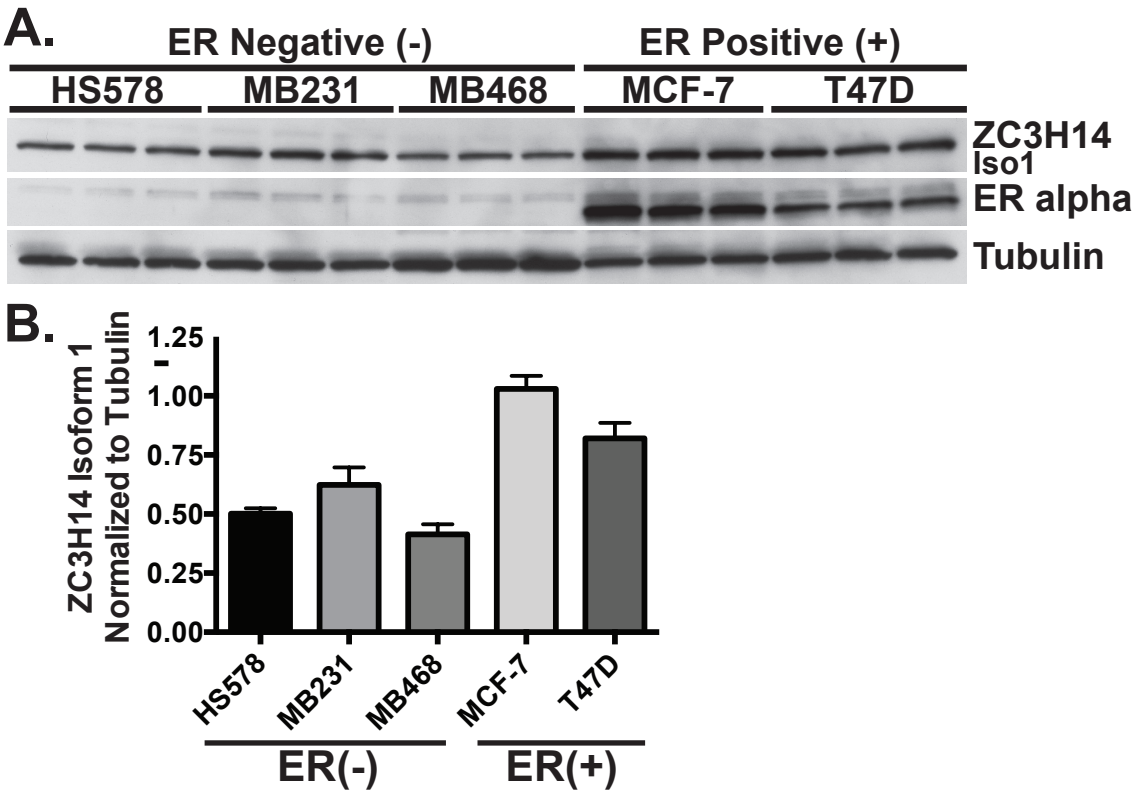

FIGURE 3.1. ZC3H14 steady-state protein levels correlate with Estrogen Receptor (ER) status across a panel of breast cancer cell lines. *A*) Three Estrogen Receptor negative (ER[-]; HS578, MB231 and MB468) and two ER(+) breast cancer cell lines (MCF7 and T47D) were collected in triplicate and subjected to immunoblot analysis with ZC3H14, ER alpha and Tubulin antibodies. *B*) Quantification of ZC3H14 Isoform 1 (Iso1) steady-state levels reveals a correlation between Estrogen Receptor status and ZC3H14 protein levels in these cell lines tested.

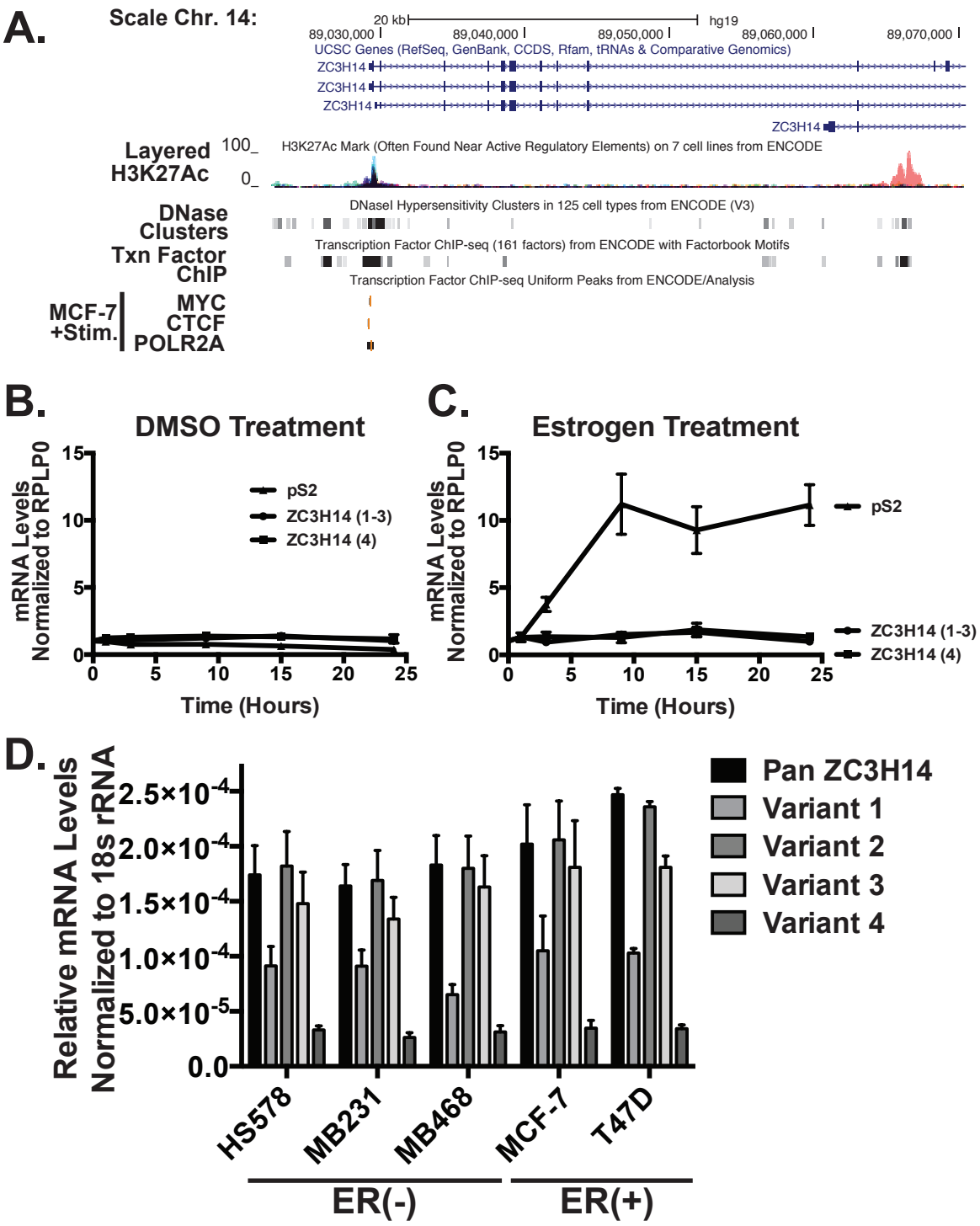

FIGURE 3.2. *ZC3H14* is not transcriptionally activated by Estrogen. *A*) UCSC genome browser view of the 5' region of the ZC3H14 locus, including four confirmed splice variants of ZC3H14. Gene positions are shown at the top, followed by H3K27Ac marks, a sign of active enhancer regions that are commonly located near active regulatory elements, and DNase I hypersensitive clusters, highlighting regions of uncondensed, or exposed chromatin. Transcription factor ChIP-Seq data are shown in the tracks below and reveal confirmed transcription factor binding sites from various human cell lines,

including MCF-7. To determine whether ZC3H14 is transcriptionally activated by Estrogen, MCF-7 cells were hormone starved for 72 hours and then introduced to media containing DMSO (*B*) or 10 nM Estrogen (*C*) and collected at the indicated time points. Total cellular RNA isolated from treated cells was subjected to cDNA generation and subsequent qRT-PCR analysis with primers to detect the known Estrogen-responsive transcript, *pS2*, *ZC3H14 Variants 1-3* or *Variant 4* and *RPLP0*. Treatment with DMSO (*B*) did not result in transcriptional activation of any of the tested transcripts. However, treatment with Estrogen (*C*) resulted in a striking induction of *pS2* transcript levels without impacting *ZC3H14* mRNA levels, suggesting that *ZC3H14* is not an Estrogen-responsive gene. *D*) Total cellular RNA isolated from cells in Figure 3.1A was subjected to cDNA generation and subsequent qRT-PCR analysis with primers to detect *ZC3H14 Variants 1-4* individually or collectively (*Pan ZC3H14*) and *18s rRNA*. Although each variant is expressed at different levels relative to one another across all cell lines tested, the steady-state level of each variant is consistent between cell lines.

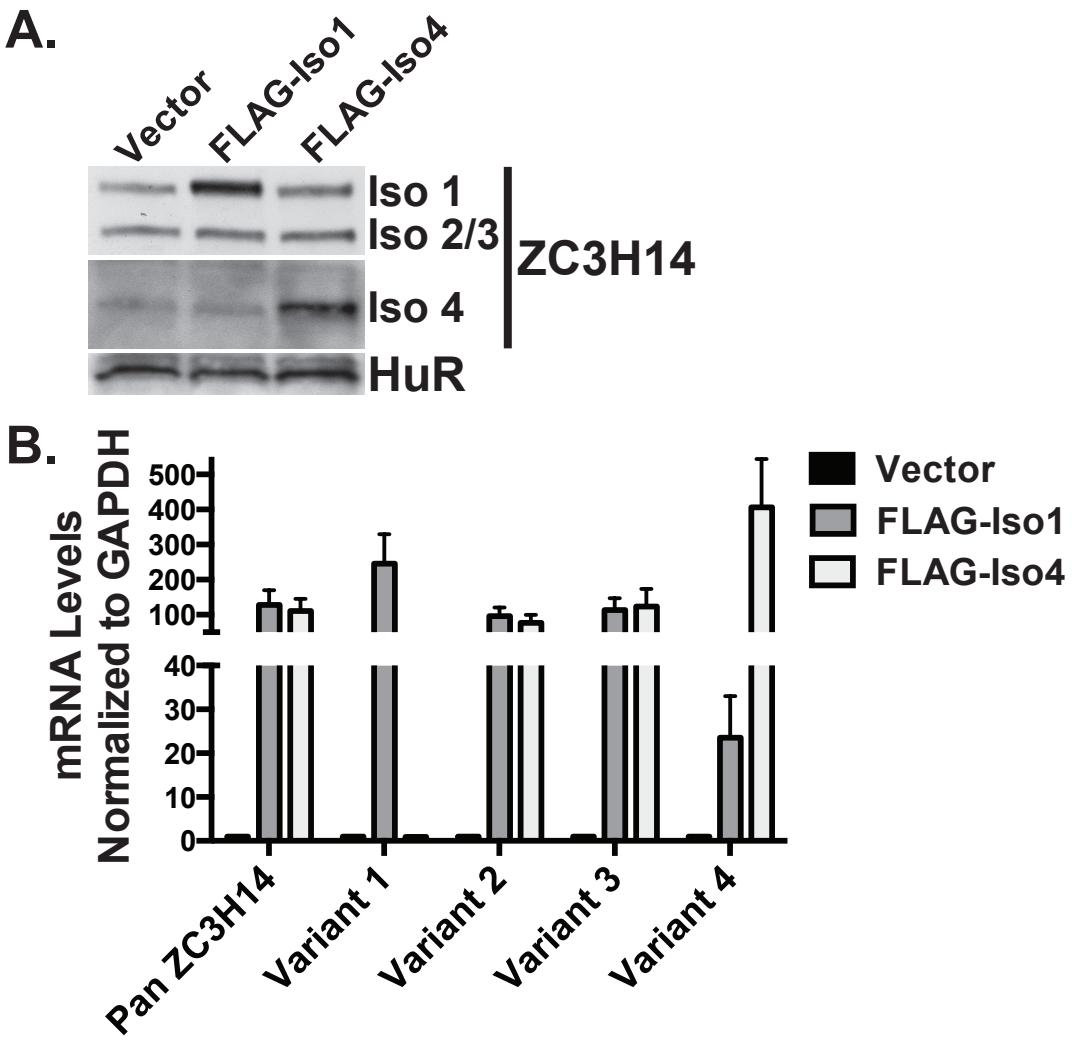

FIGURE 3.3. Overexpression of FLAG-tagged ZC3H14 isoforms 1 and 4 results in selective upregulation of *ZC3H14* splice variants. *A*) MCF-7 cells were transfected with plasmids encoding FLAG-tagged Isoforms 1 and 4 (Iso1 and Iso4, respectively) of ZC3H14 and subjected to immunoblot analysis with ZC3H14 (N-terminal to detect Isoforms 1-3 and Isoform 4-specific) and HuR (loading control) antibodies. We achieve robust expression of FLAG-Iso1 and -Iso4 as evidenced by the ZC3H14 blots. *B*) Total RNA isolated from MCF-7 cells in (*A*) was used for cDNA generation and subsequent qRT-PCR analysis with primers to detect *ZC3H14 Variants 1-4* individually or collectively (*Pan ZC3H14*) and *GAPDH*.

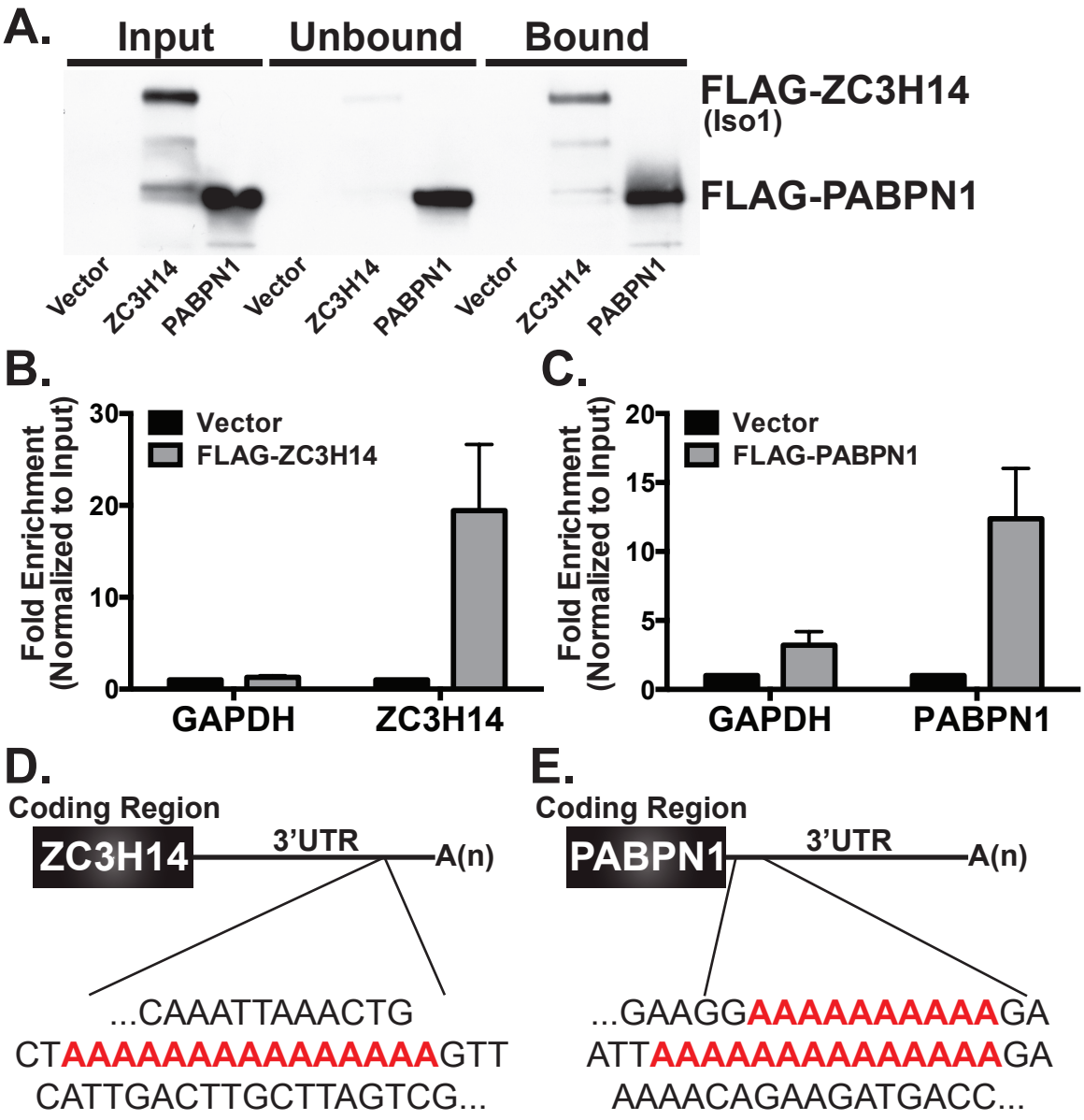

FIGURE 3.4. The nuclear Pabs ZC3H14 and PABPN1 bind their own mRNA transcripts. *A*) HeLa cells were transfected with plasmids encoding FLAG-tagged ZC3H14 Isoform 1 (Iso1), FLAG-PABPN1 or pcDNA3 (Vector control). Cells expressing vector control or FLAG-tagged proteins were subjected to RNA-IP using FLAG antibody-conjugated beads. Immunoblot analysis of IP samples demonstrates specific enrichment of FLAG-ZC3H14 Iso1 and -PABPN1 in the bound fractions. *B*) and *C*) RNA that co-precipitated with FLAG-tagged proteins was subjected to qRT-PCR analyses with *GAPDH*, *ZC3H14* and *PABPN1* primers to detect bound transcripts. *ZC3H14* and *PABPN1* transcripts were significantly enriched upon purification of their respective FLAG-tagged proteins whereas *GAPDH* mRNA did not co-precipitate with either protein. Schematics of the *ZC3H14* (*D*) and *PABPN1* (*E*) transcripts highlight (in red) the striking polyadenosine stretches located within the 3'UTRs of these mRNAs that may represent the cis-elements responsible for the self-enrichment observed in (*B*) and (*C*).

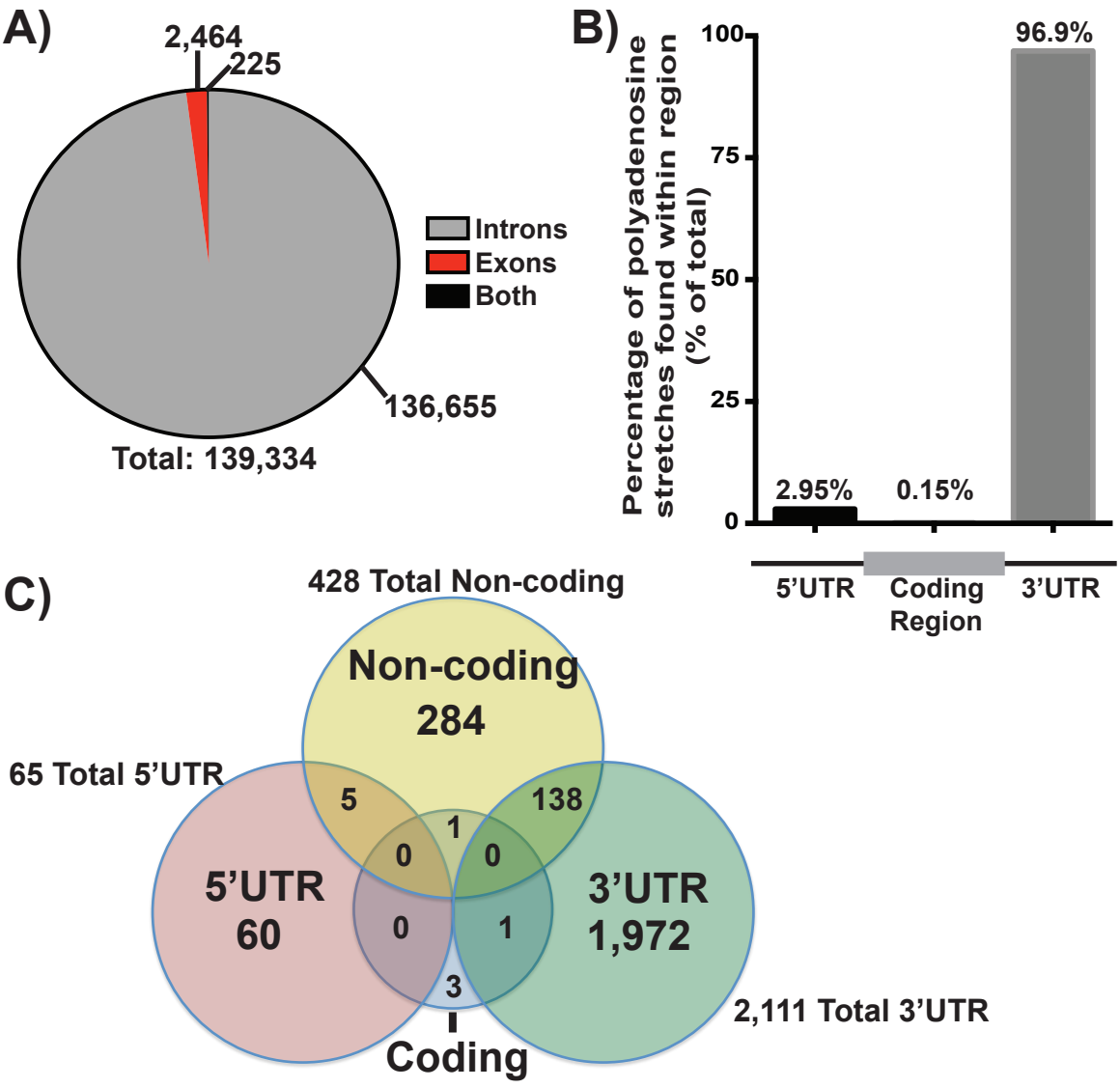

FIGURE 3.5. Prevalence and location of templated, internal polyadenosine stretches within the human transcriptome. *A*) Analysis of the human transcriptome for the frequency and enrichment of internal polyadenosine stretches containing at least 12 consecutive adenosines reveals that the vast majority (136,655 out of 139,334) of these sequences are located in the introns of mRNAs whereas a much smaller fraction are located in exonic regions (2,464 out of 139,334), which includes 5' and 3'UTR regions. A much smaller number of these internal adenosine stretches are found in sequences that can be either introns or exons as a result of alternative splice variants. *B*) Internal polyadenosine stretches found in exonic sequences are almost exclusively located in untranslated regions. Further analysis of the ≥12nt polyadenosine sequences that occur in mature mRNA transcripts reveals that almost all (99.9%) of these instances are located in the UTRs, with an extremely large percentage present in the 3'UTRs (96.9%) of mRNA transcripts. *C*) Non-coding RNAs also include templated stretches of polyadenosine. A number (428) of noncoding RNAs (yellow circle) contain templated polyadenosine sequences, suggesting that Pabs could modulate non-coding RNAs through this

templated binding site. Consistent with a model of 3'UTR regulation, a large number of the internal polyadenosine stretches identified in ncRNAs (138) are found in 3'UTRs (yellow and green overlap).