Slevin, Marzluff – Supplemental Methods Table 1

Description	Primer
Preadenylated linker (Added nts)	5' Ap-CTGTAGGCACCATCAATCTCACTCCG-NH2
RT primer (linker complement)	5' CGGAGTGAGATTGATGGTGCCTACAG
Library primers - round 1	
V1.5-N5-RT primer	5' GGTTCAGAGTTCTACAGTCCGACGATC-NNNN-CGGAGTGAGATTGATGGTGCCTACAG
Rd2SP (Reverse)-N5-H2A consensus primer	5' GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-NNNNN-CTGGCGGGCAACGCGGC
Rd2SP (Reverse)-H2B consensus primer	5' GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-GGTCCACCCCGACACCGGCATCT
Library primers - round 2	
P5-V1.5	5' AATGATACGGCGACCACCGAGATCTACAC-CGACAGGTTCAGAGTTCTACAGTCCGAC
P7 (Reverse)-Index (Reverse)-RdSP2 (Reverse) *	5' CAAGCAGAAGACGGCATACGAGAT-CGTGAT-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
small RNA sequencing primer V1.5	5' CGACAGGTTCAGAGTTCTACAGTCCGACGATC
T7 Promoter-H2A-Luc primer	5' TAATACGACTCACTATAGGGAGA-CTGGCGGGCAACGCGGC-AACACCCCAACATCTTCGAC
pUC19 primer	5' GGCGAAAGGGGGATGTGCTG
T7 Promoter Reverse	5' TCTCCCTATAGTGAGTCGTATTA
Rd2SP (Reverse)-N5-Luc primer	5' GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-NNNNN-AACACCCCAACATCTTCGAC

*Note indices from Illumina's LT index list

	siRNA
C2 (control siRNA)	5' GGUCCGGCUCCCCCAAAUG
Pm/Scl-100	5' GCUGCAGCAGAACAGGCCA

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Low throughput methods used to detect oligouridylated intermediates, related to Figure 1.

Oligouridylated histone mRNA degradation intermediates were detected by different strategies, and individual clones sequenced.

(A) cDNA synthesis was primed with oligo$(dA)_7$ fused to a T7 primer, and histone cDNAs detected by PCR. Unlike the previous study (Mullen and Marzluff, 2008) where the PCR products were size-selected prior to cloning, the entire PCR reaction was cloned and the clones sequenced.

(B) Ligation mediated RT-PCR of oligouridylated histone mRNA using a preadenylated linker ligated to the 3' end followed by cDNA synthesis with a linker complement ending with $d(A)_3$.

(C) The sequences and position of non-templated tails found in histone H3C mRNA by cloning and sequencing clones obtained by the strategies outlined in panels A and B.

(D) The table summarizes three experiments done with synthetic RNAs added to total cell RNA to test for ligation bias: luciferase stemloop and a luciferase stemloop ending in 10 uridines.

Supplemental Figure 2. The 3' ends of histone mRNAs with no tails or 1 nt tails, related to Fig. 2.

The profile of the 3' ends of histone H2AA3, H2AC, and H2BC mRNAs analyzed in Fig. 2C-E containing no tails or a 1 nt tail categories are shown in panels A, B and C respectively. These are from the same sequencing reaction as the data in Fig.2C-E and are quantified in Fig. 2I

Supplmental Figure 3. Histone mRNA degradation intermediates are associated with polyribosomes, related to Fig. 3.

(A) The full polyribosome profile from the experiment shown in Fig. 3A is shown. The location of tRNA, 40S, 60S, 80S, and polysome fractions for control-HU and HU-pactamycin treated Jurkat cells are indicated.

(B) Ethidium stained agarose gel showing individual fractions for the HU-pactamycin gradient in panel A

(C-D) The complete histone H2a Northern blots for polyribosome analysis of HU-Control and HU-pactamycin treated Jurkat cells from Fig. 3A are shown. Note there are no histone mRNAs in postpolysomal fractions.

(E-F) Northern blots for histone H2a and 7SK show siRNA knockdown of DOM34 and Hbs1 stabilize histone mRNA from the experiment in Fig. 3F. The Western blot showing the Dom34 and Hbs1 protein levels relative to their siRNA C2 control is the same one as in Fig. 3F.

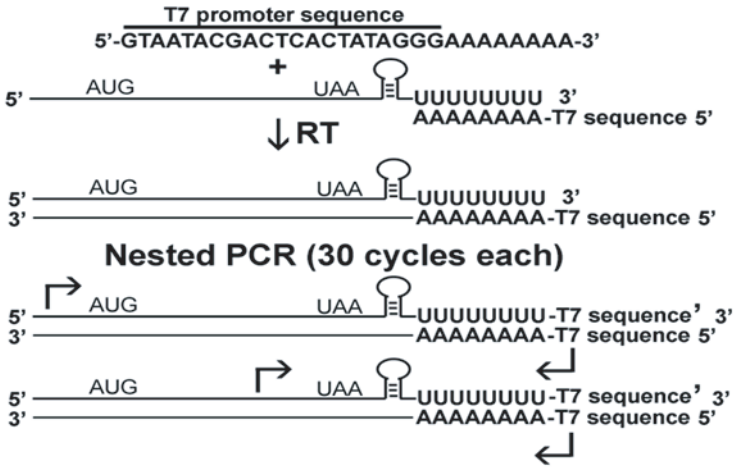
Supplemental Figure 4. ARCA and BTH capped luciferase stemloop and tetraloop degradation profiles, related to Fig. 4.

(A and B). The mRNAs from the transfected RNAs containing either the stemloop or tetraloop versions of ARCA luciferase, 20 min after treatment with HU are shown for no tails and $\geq$ 2 nt U-tails categories The ARCA cap is cleaved normally by dcp2 (Su et al., 2013).

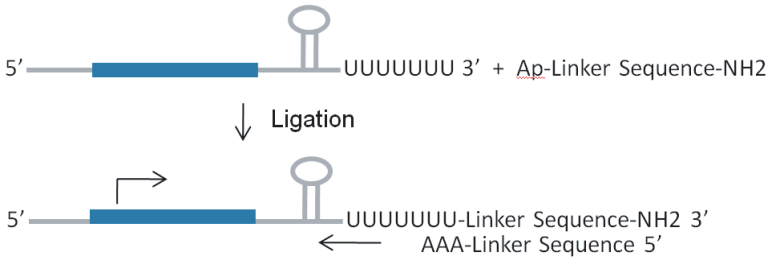
(C). The BTH-SL and BTH-TL transcripts with no untemplated tails from the same libraries analyzed in Fig. 4A and B.

Supplemental Figure 1

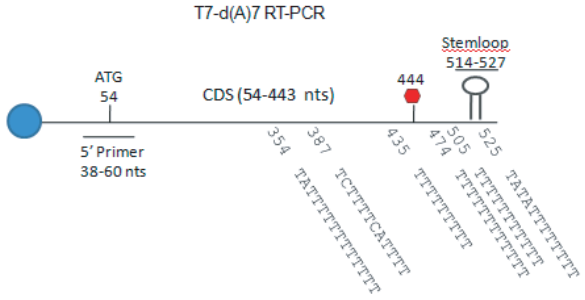
A

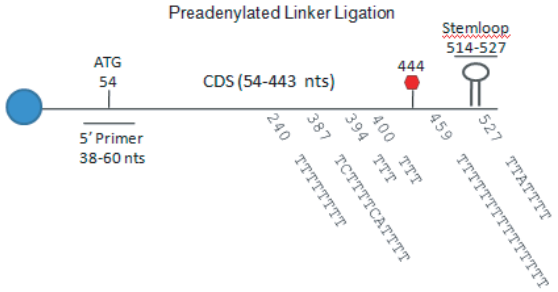


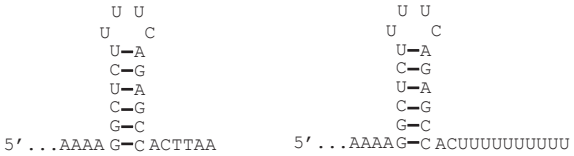
B



C



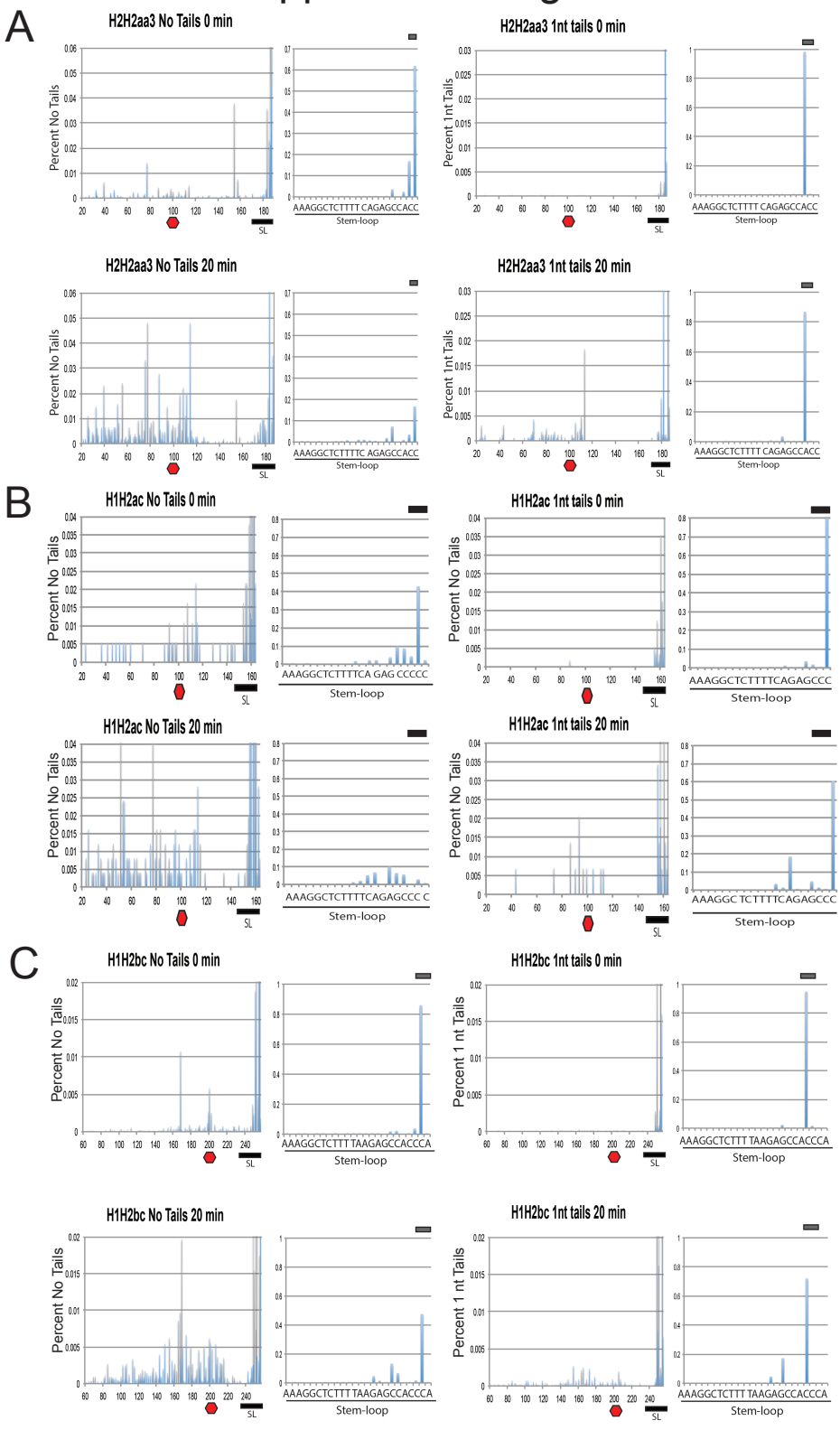




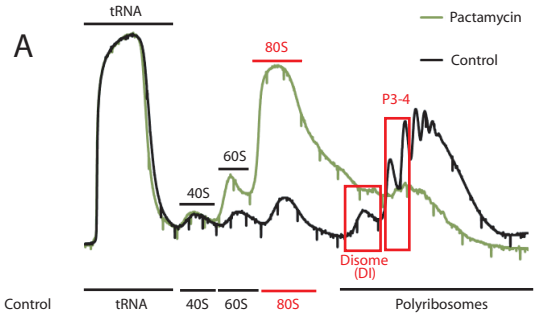
D

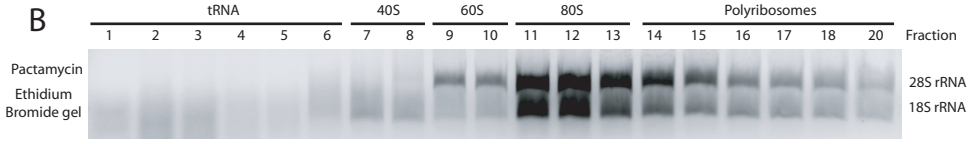
	Total	Luc-SL (% of Total)	Luc-SL U(10) (% of Total)
Experiment 1	257,941	150,344 (41.7 %)	107,597 (58.3 %)
Experiment 2	391,596	226,225 (42.2 %)	165,371 (57.7 %)
Experiment 3	184,535	107,382 (41.8 %)	77,153 (58.2 %)
Mean		41.9 %	58.1 %
SD		0.28	0.32

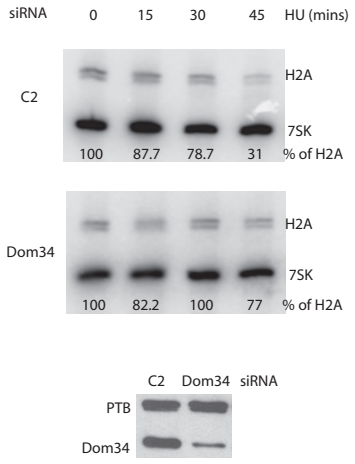
Supplemental Figure 2

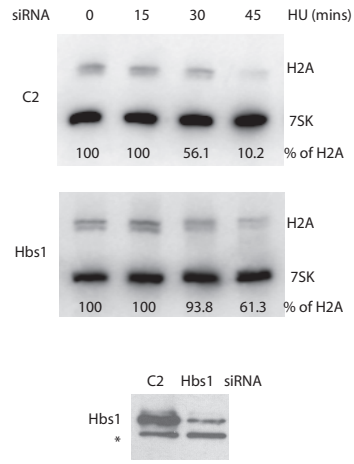


Supplemental Figure 3









Supplemental Figure 4