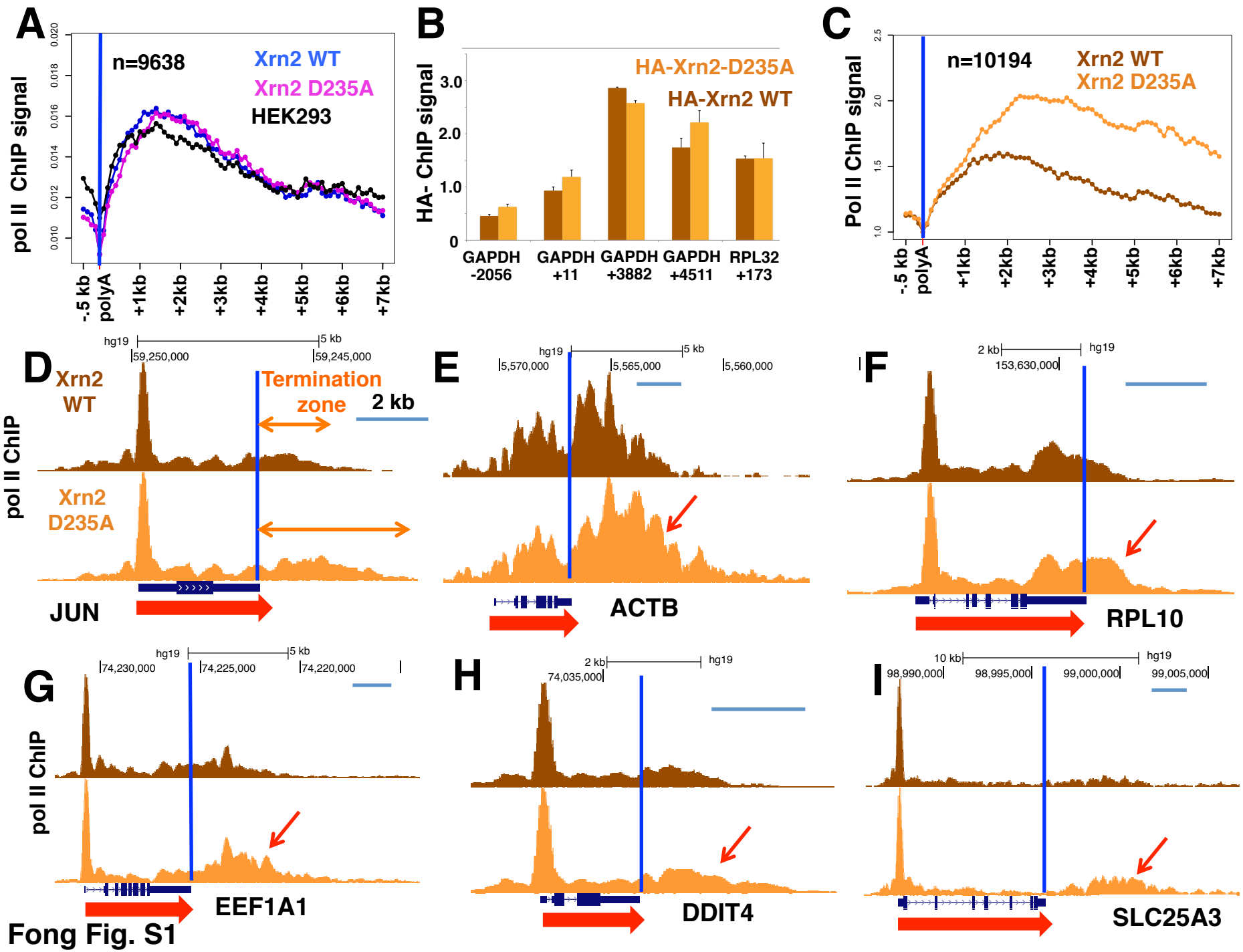

A
pol II ChIP signal
n=9638
Xrn2 WT
Xrn2 D235A
HEK293
-.5 kb polyA
+1kb
+2kb
+3kb
+4kb
+5kb
+6kb
+7kb
B
HA- ChIP signal
HA-Xrn2-D235A
HA-Xrn2 WT
GAPDH -2056
GAPDH +11
GAPDH +3882
GAPDH +4511
RPL32 +173
C
Pol II ChIP signal
n=10194
Xrn2 WT
Xrn2 D235A
D
pol II ChIP
Xrn2 WT
Xrn2 D235A
Termination zone
2 kb
JUN
E
ACTB
F
RPL10
G
EEF1A1
H
DDIT4
I
SLC25A3
Fong Fig. S1

Figure S1. Related to Figure 1.

Exonuclease dead Xrn2 causes widespread delays in poly(A) site dependent termination.

A. Metaplots of mean pol II ChIP signals for well-separated genes (>5kb apart) in HEK293 cells expressing WT HA-Xrn2 or HA-Xrn2 D235A without knockdown of endogenous Xrn2 and HEK293 cells expressing WT Am^r Rpb1 without Xrn2 overexpression (from Fig. 5). Mean ChIP signals are normalized to the poly(A) site marked by the blue line. Note a small delay in termination in cells expressing mutant Xrn2.

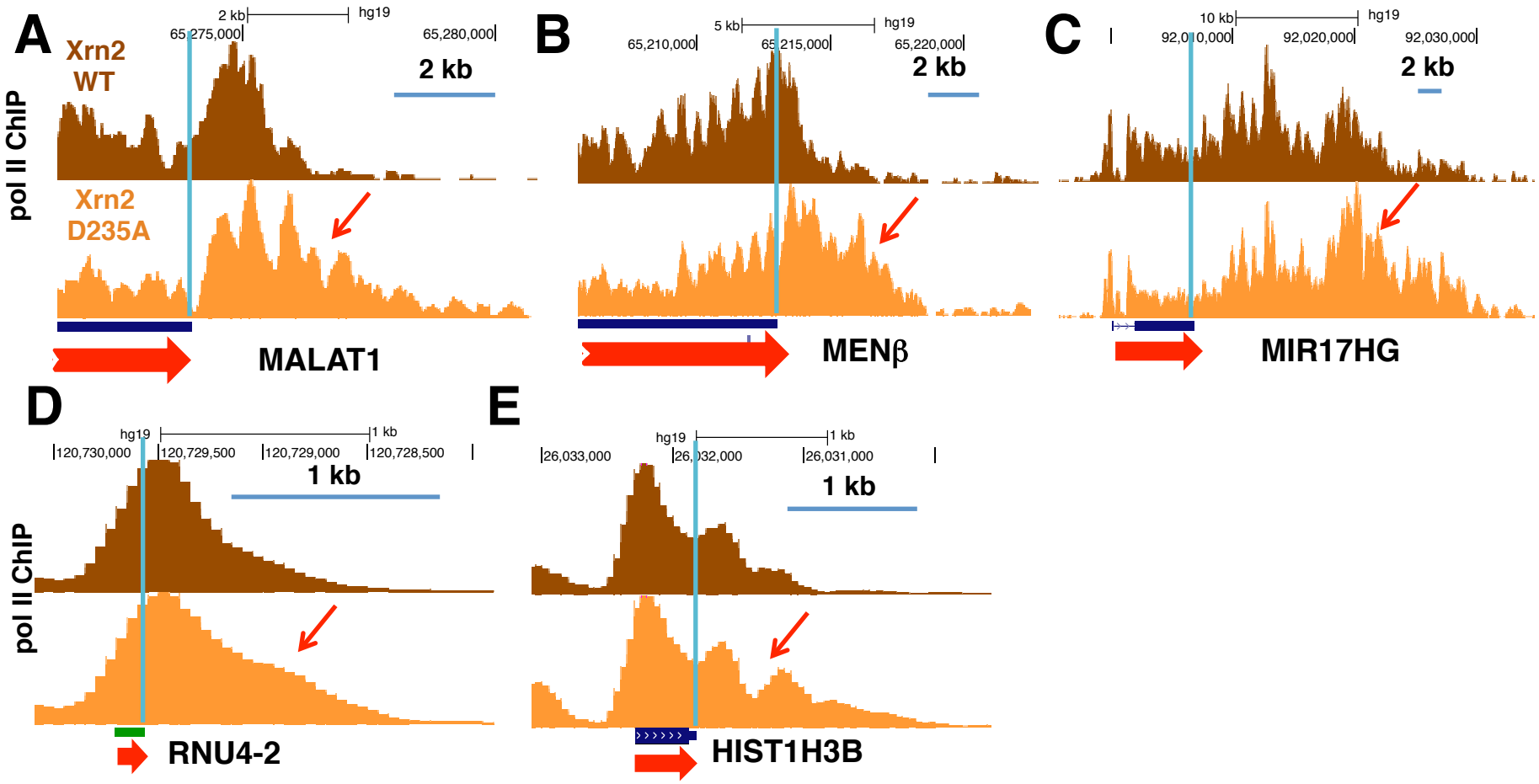
B. WT and Xrn2(D235A) are recruited equivalently to genes. Anti-HA ChIP was performed on doxycycline induced (24hr) HEK293 cells expressing WT HA-Xrn2 or HA-Xrn2 D235A and quantified at amplicons on the GAPDH and RPL32 genes relative to input. Positions refer to the center of the amplicon relative to the transcription start site. GAPDH +3882 and +4511 are in the termination zone. Note the signals for WT and D235A Xrn2 proteins are similar to one another. Means and SEM for 3 PCR reactions are shown.

C.Metaplot of mean pol II ChIP signals normalized to the poly(A) site for over 10,000 well separated genes (>5kb apart) in cells expressing WT and dominant negative Xrn2 (D235A). Note the downstream shift in average pol II density caused by expression of mutant Xrn2.

D-I. Anti-Pol II ChIP-seq shows delayed termination downstream of most genes with poly(A) sites in cells expressing D235A dominant negative mutant Xrn2 relative to WT. Endogenous Xrn2 is stably knocked down with an shRNA against the 3′ UTR. UCSC genome browser screen shots are shown with arrows indicating 3′ extension of the

termination zone caused by Xrn2 (D235A). Poly(A) sites are marked by vertical blue lines. Scale bars represent 2kb.

All data are from a biological replicate of the experiment in Figure 1.


A
Xrn2
WT
Xrn2
D235A
pol II ChIP
2 kb
hg19
65,275,000
65,280,000
2 kb
MALAT1
B
5 kb
hg19
65,210,000
65,215,000
65,220,000
2 kb
MENβ
C
10 kb
hg19
92,010,000
92,020,000
92,030,000
2 kb
MIR17HG
D
hg19
1 kb
120,730,000
120,729,500
120,729,000
120,728,500
1 kb
RNU4-2
E
hg19
1 kb
26,033,000
26,032,000
26,031,000
1 kb
HIST1H3B

Fong Fig. S2

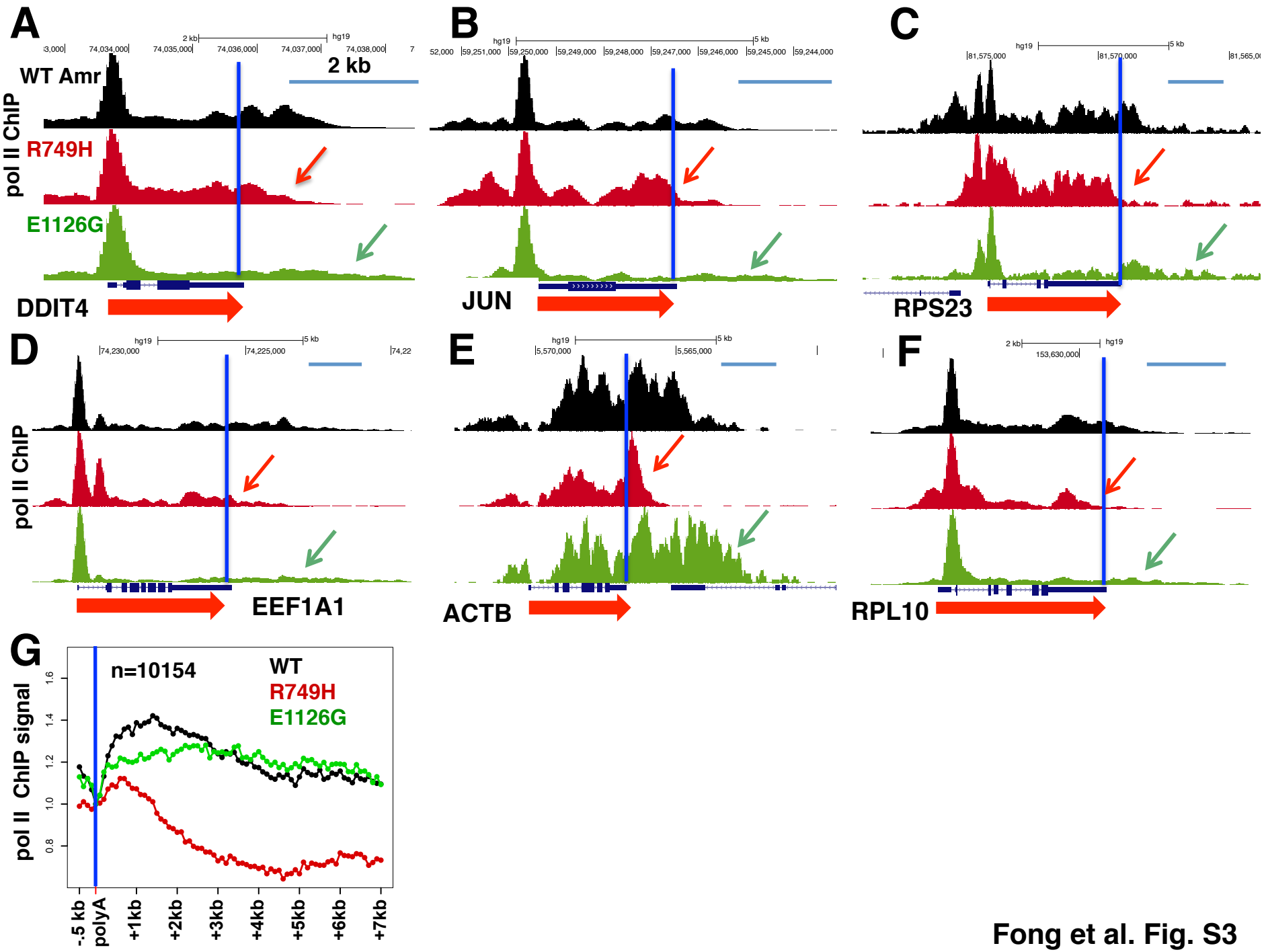
Figure S2. Related to Figure 3.

Mutant Xrn2 inhibits termination at a subset of genes with alternative modes of 3′ end formation.

A-C. Xrn2(D235A) impairs termination at lnc RNA genes with RNAseP- (A, B) and microprocessor-mediated (C) 3′ end cleavage. UCSC genome browser screen shots of pol II ChIP-seq results as in Fig. 1C. Arrows indicate 3′ extension of the termination zone caused by Xrn2 inactivation. 3′ ends are marked by vertical pale blue lines.

D, E. Xrn2 inactivation delays termination at a U4 snRNA gene and a replication-coupled histone H3 gene.

All data are from biological replicates of the experiments in Figure 3.



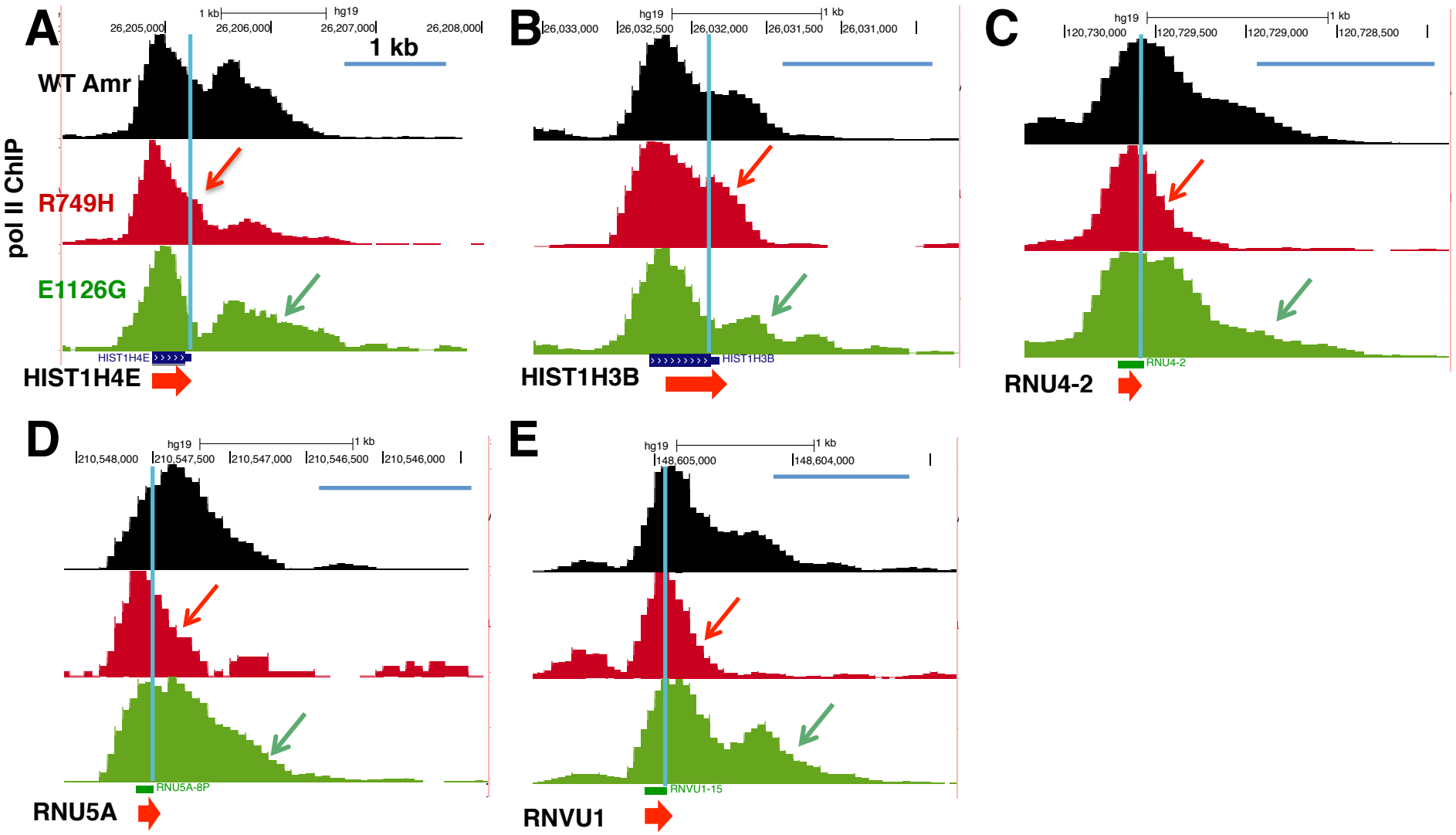
Fong et al. Fig. S3

Figure S3. Related to Figure 5.

Pol II elongation rate has widespread effects on poly(A) site dependent termination.

A-F. Anti-Pol II ChIP-seq in HEK 293 Flp-in TREX cells expressing WT, slow (R749H, red) and fast (E1126G, green) α-amanitin resistant (Am^r) pol II large subunits. Cells were treated with doxycycline to induce expression of Amr large subunits, then with α-amanitin for 42 hrs to inhibit and degrade endogenous pol II prior to ChIP with anti-pol II antibody. UCSC genome browser screen shots are shown with red and green arrows indicating shortening and extension of the termination zone caused by slow and fast mutants. Poly(A) sites are marked by vertical blue lines. Scale bars represent 2kb. These data are from a biological replicate of the experiment in Figure 5.

G. Metaplots for cells expressing WT (black) slow (R749H, red) and fast (E1126G, green) α-amanitin resistant (Am^r) pol II large subunits. Note more proximal termination in the slow mutant relative to WT and delayed termination in the fast mutant and a less prominent peak of pol II density 3′ of the poly(A) site. These data are from replicates of the experiments in Figure 5.


A
B
C
D
E
pol II ChIP
WT Amr
R749H
E1126G
1 kb
hg19
HIST1H4E
HIST1H3B
RNU4-2
RNU5A-8P
RNVU1-15
HIST1H4E
HIST1H3B
RNU4-2
RNU5A
RNVU1

Fong Fig. S4

Figure S4. Related to Figure 6.

Pol II elongation rate effects on termination at histone and U snRNA genes.

A-E. UCSC genome browser screen shots of pol II ChIP-seq results for selected histone (A, B) and U snRNA genes (C-F) are shown with red and green arrows indicating shortening and extension of the termination zone caused by slow and fast mutants. Vertical blue lines mark RNA 3′ ends. Blue scale bars represent 1kb. These data are from replicates of the experiments in Figure 6.

Table S1. Related to Figure 2. Gene lists for Group I and Group II genes with proximal and distal termination sites. Positions of relevant poly(A) sites are given. See attached Excel file.

Table S2. Related to Figures 1-7. Summary of ChIP-seq experiments from this study. Hela pol II data using the same antibody is from (Kim et al., 2011) GEO Accession GSE30895.

Fong et al Table S2 summary of ChIP-seq experiments

Cell line	Antibody	Reads Mapped (% mapped)
21NT	Rabbit anti-pan pol II CTD	5244252 (79.25%)
Hap1	Rabbit anti-pan pol II CTD	6495449 (75.95%)
HeLa GSE 30895	Rabbit anti-pan pol II CTD	
HEK293 shXrn2 pcDNA5 HA-Xrn2 WT R1	Rabbit anti-pan pol II CTD	9552763 (82.49%)
HEK293 shXrn2 pcDNA5 HA-Xrn2 D235A R1	Rabbit anti-pan pol II CTD	9879859 (82.28%)
HEK293 shXrn2 pcDNA5 HA-Xrn2 WT R2	Rabbit anti-pan pol II CTD	13208471 (73.27%)
HEK293 shXrn2 pcDNA5 HA-Xrn2 D235A R2	Rabbit anti-pan pol II CTD	15945430 (91.66%)
HEK293 pcDNA5 HA-Xrn2 WT	Rabbit anti-pan pol II CTD	17468028 (95.02%)
HEK293 pcDNA5 HA-Xrn2 D235A	Rabbit anti-pan pol II CTD	18802999 (96.37%)
HEK293 pcDNA5 Rpb1 WT Amr R1	Rabbit anti-pan pol II CTD	5771993 (93.20%)
HEK293 pcDNA5 Rpb1 R749H Amr R1	Rabbit anti-pan pol II CTD	7850642 (92.32%)
HEK293 pcDNA5 Rpb1 E1126G Amr R1	Rabbit anti-pan pol II CTD	9915615 (92.71%)
HEK293 pcDNA5 Rpb1 WT Amr R2	Rabbit anti-pan pol II CTD	10236031 (89.24%)
HEK293 pcDNA5 Rpb1 R749H Amr R2	Rabbit anti-pan pol II CTD	8508683 (88.41%)
HEK293 pcDNA5 Rpb1 E1126G Amr R2	Rabbit anti-pan pol II CTD	6799605 (64.27%)
HEK293 shXrn2 pcDNA5 HA-Xrn2 WT	Rabbit anti-Xrn2	12702001 (86.11%)