Figure S1

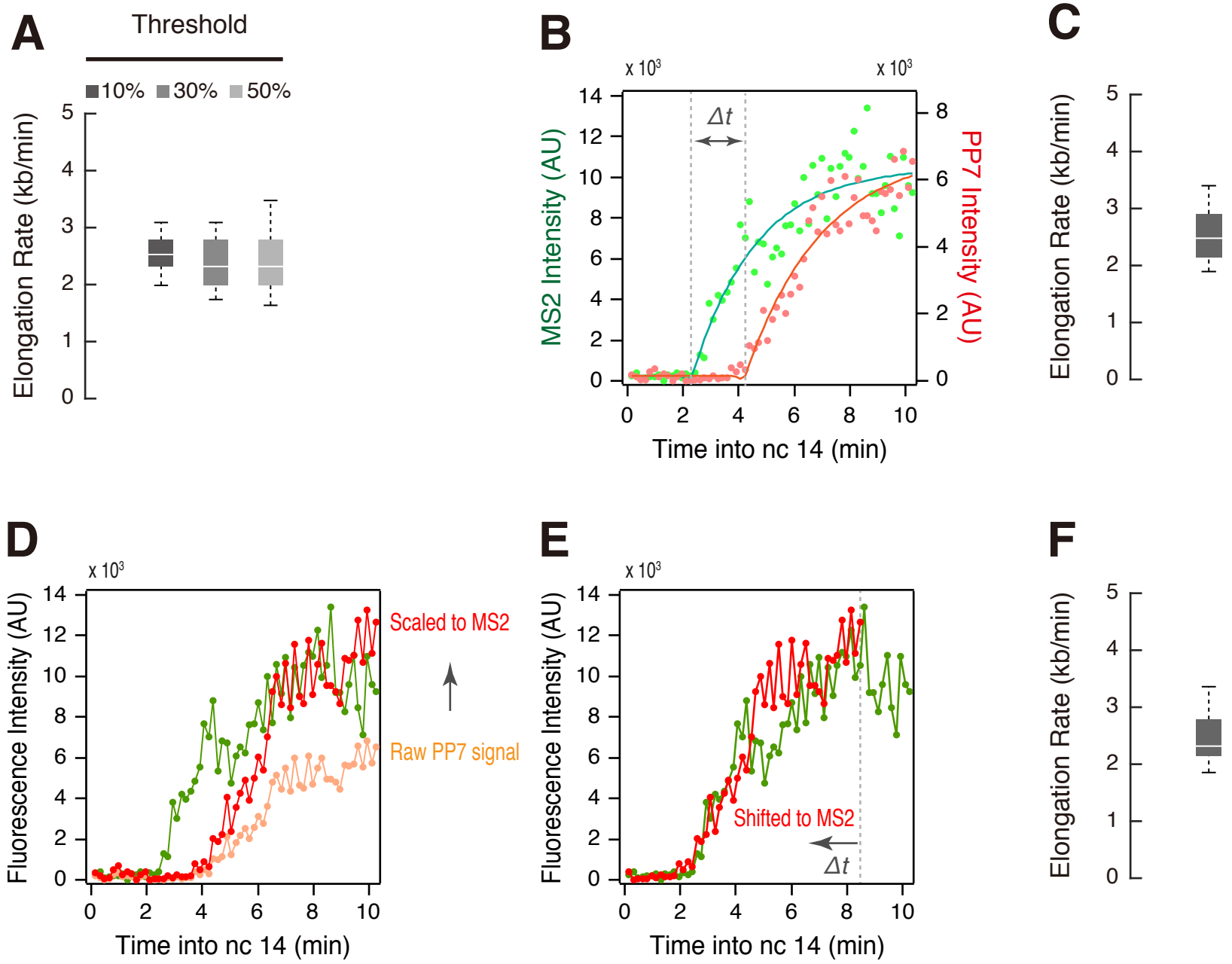

A
Threshold
10%
30%
50%
Elongation Rate (kb/min)
B
x 10^3
x 10^3
MS2 Intensity (AU)
PP7 Intensity (AU)
Δt
Time into nc 14 (min)
C
Elongation Rate (kb/min)
D
x 10^3
Fluorescence Intensity (AU)
Scaled to MS2
Raw PP7 signal
Time into nc 14 (min)
E
x 10^3
Fluorescence Intensity (AU)
Shifted to MS2
Δt
Time into nc 14 (min)
F
Elongation Rate (kb/min)

Figure S2

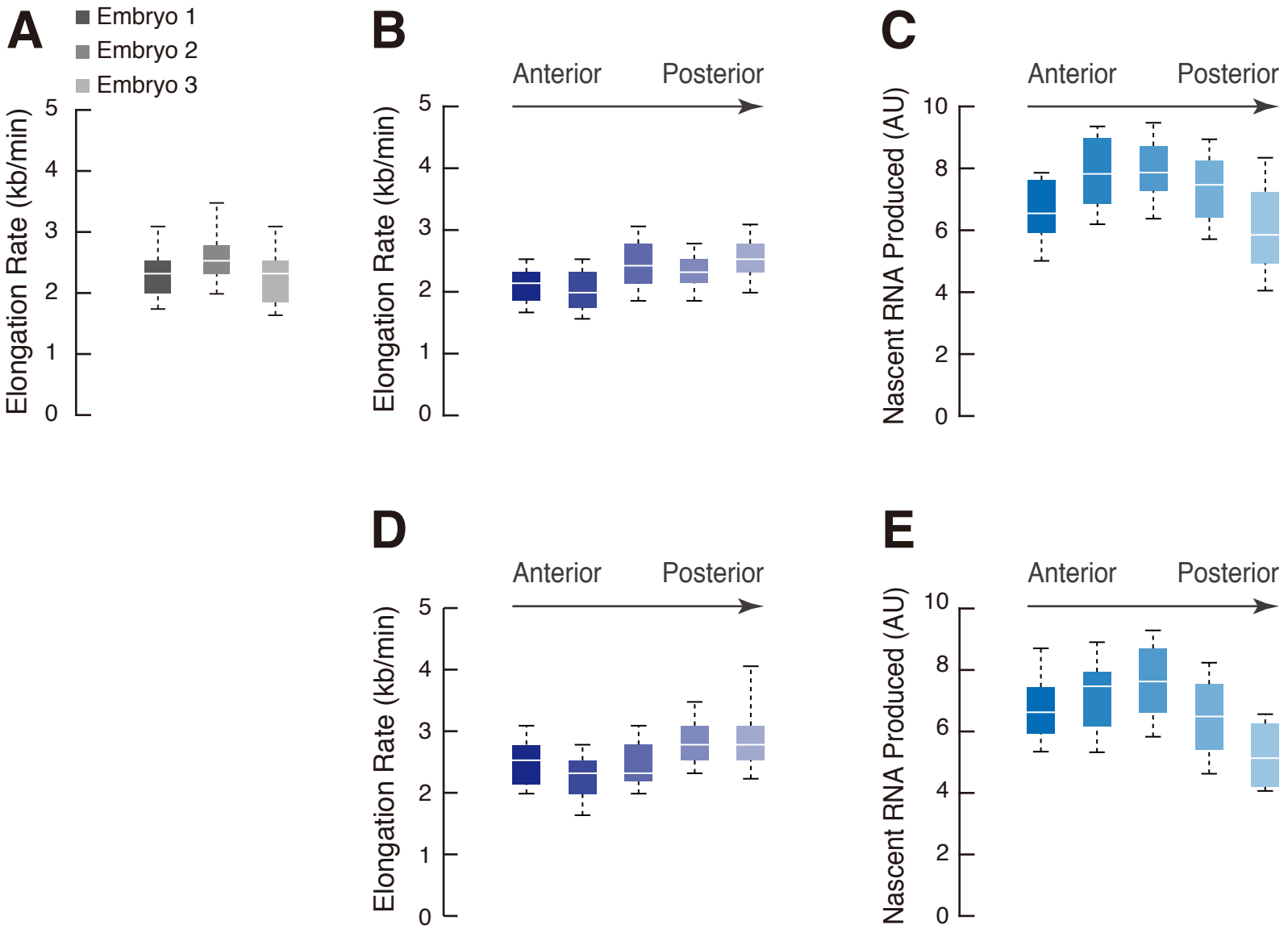

A
Embryo 1
Embryo 2
Embryo 3
Elongation Rate (kb/min)
0
1
2
3
4
5
B
Anterior
Posterior
Elongation Rate (kb/min)
C
Anterior
Posterior
Nascent RNA Produced (AU)
0
2
4
6
8
10
D
Anterior
Posterior
Elongation Rate (kb/min)
E
Anterior
Posterior
Nascent RNA Produced (AU)

Figure S3

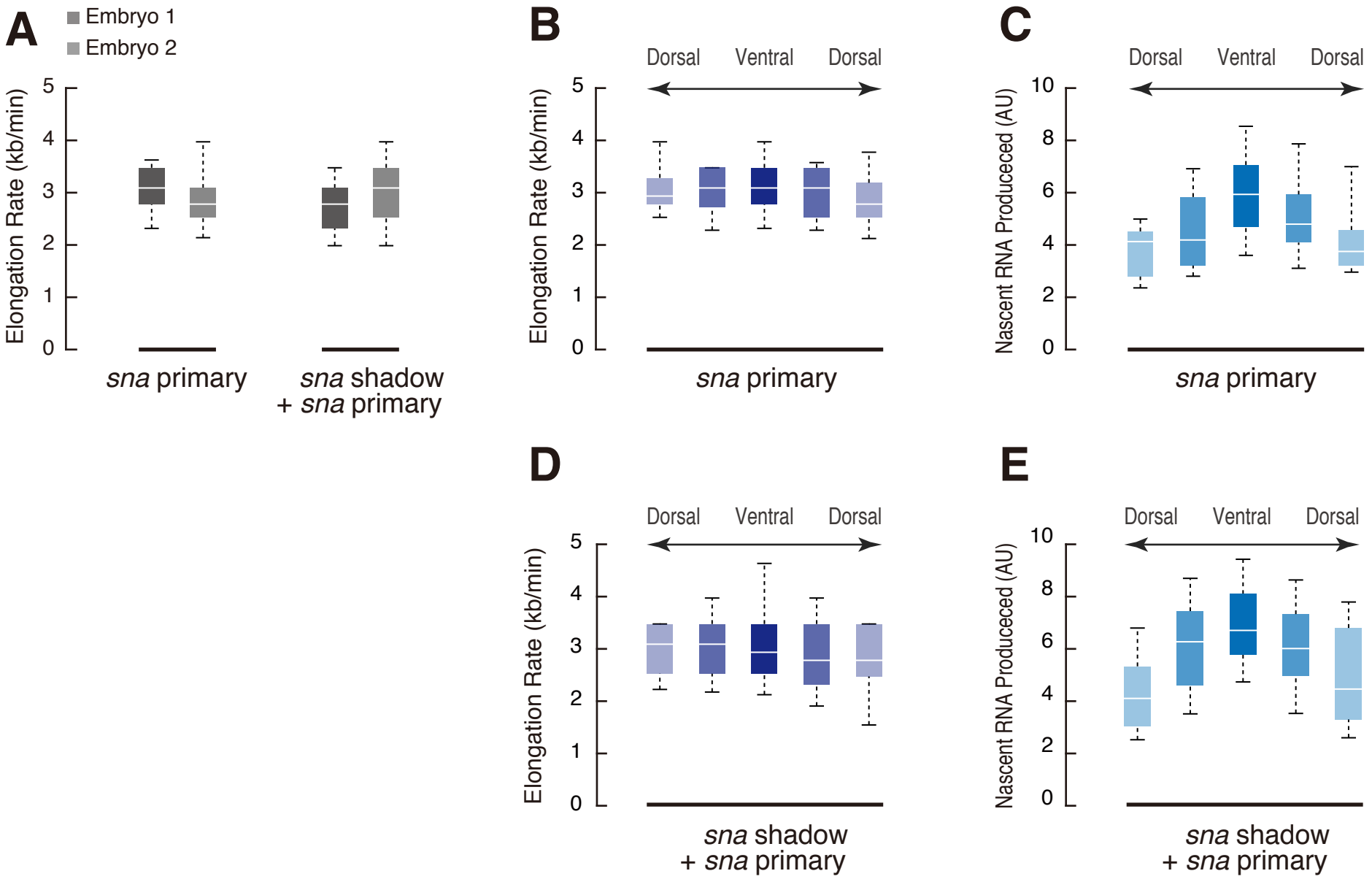
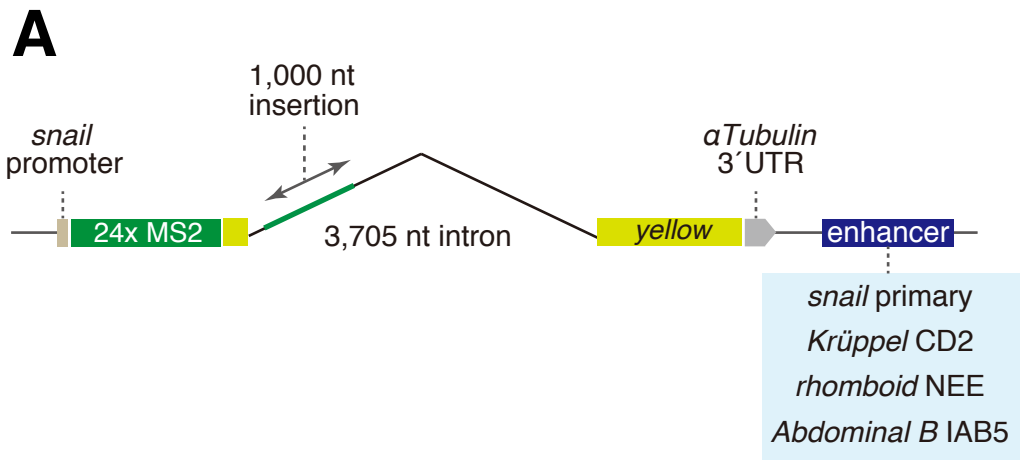
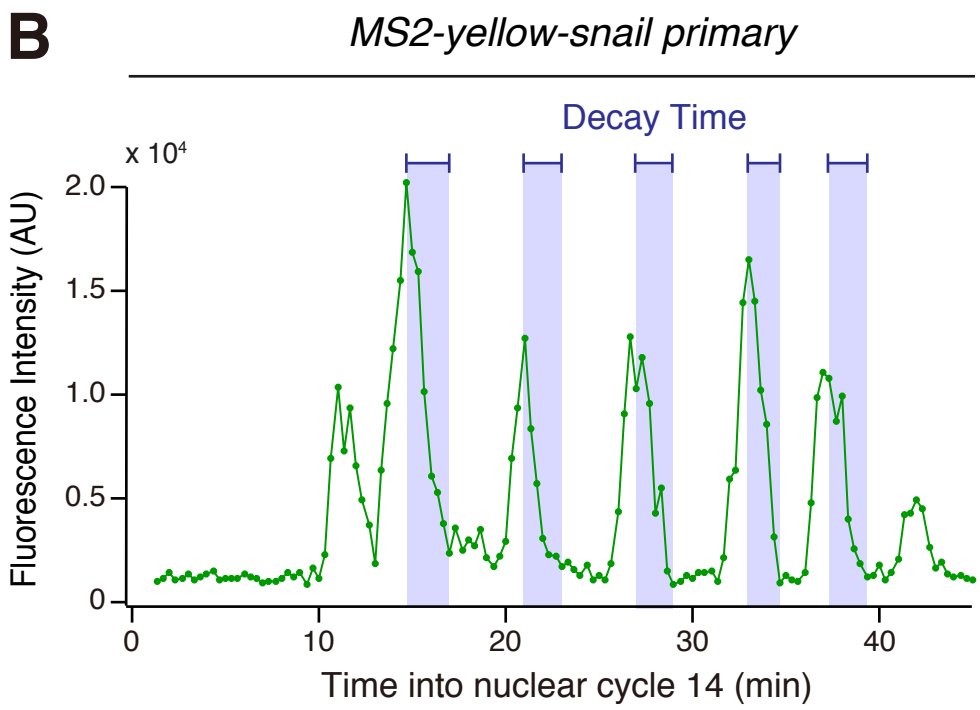

A
Embryo 1
Embryo 2
Elongation Rate (kb/min)
0
1
2
3
4
5
sna primary
sna shadow
+ sna primary
B
Dorsal
Ventral
Dorsal
Elongation Rate (kb/min)
sna primary
C
Dorsal
Ventral
Dorsal
Nascent RNA Produceced (AU)
0
2
4
6
8
10
sna primary
D
Dorsal
Ventral
Dorsal
Elongation Rate (kb/min)
sna shadow
+ sna primary
E
Dorsal
Ventral
Dorsal
Nascent RNA Produceced (AU)
sna shadow
+ sna primary

Figure S4


A
1,000 nt
insertion
snail
promoter
αTubulin
3´UTR
24x MS2
3,705 nt intron
yellow
enhancer
snail primary
Krüppel CD2
rhomboid NEE
Abdominal B IAB5


B
MS2-yellow-snail primary
Decay Time
x 10^4
Fluorescence Intensity (AU)
2.0
1.5
1.0
0.5
0
0
10
20
30
40
Time into nuclear cycle 14 (min)

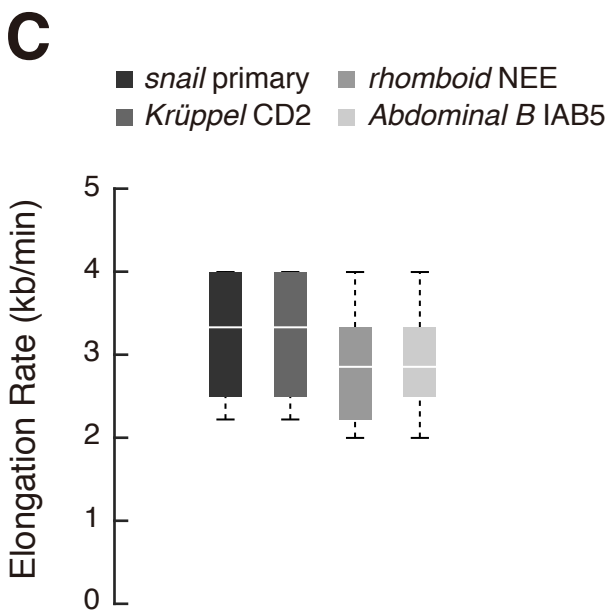

C
snail primary
rhomboid NEE
Krüppel CD2
Abdominal B IAB5
Elongation Rate (kb/min)
5
4
3
2
1
0

Figure S1. Measurement of Pol II elongation rate, Related to Figure 2.

(A) Boxplots showing the distribution of elongation rate per nucleus. 10%, 30% and 50% of the peak intensity were used as a threshold to measure elongation rate. 877 nuclei from three independent embryos at nc 14 were analyzed. The plot shown in Figure 2B is identical to the plot of 30% threshold.

(B) Raw MS2 and PP7 intensities (circle) and a fit to the equation described in the Experimental Procedures (line) in an individual nucleus expressing *hb-MS2-lacZ-PP7*. The time point at which the MS2 or PP7 signal starts to appear (t_0) was determined and used to calculate the Δt at individual nuclei.

(C) Boxplot showing the distribution of elongation rate per nucleus. Δt values obtained by the method of data fitting to an equation (B) were used to calculate the elongation rate.

(D) Raw PP7 trajectory was scaled to match the amplitude of MS2 trajectory.

(E) A scaled PP7 trajectory (D) was shifted in time to minimize the distance from the MS2 trajectory. Δt that minimizes the squared sum between shifted PP7 and the corresponding MS2 trajectory was determined for individual nuclei.

(F) Boxplot showing the distribution of elongation rate per nucleus. Δt values obtained by the method of least square optimization (D, E) were used to calculate the elongation rate.

Figure S2. Constant rates of Pol II elongation along the AP axis, Related to Figure 2.

(A) Boxplot showing the distribution of elongation rate per nucleus in three independent embryos expressing *hb-MS2-lacZ-PP7*. 340, 245 and 292 nuclei at nc 14 were analyzed.
(B-E) Boxplot showing the distribution of elongation rate (B, D) and output (C, E) in AP binned nuclei in two individual embryos. 28, 47, 76, 96 and 80 nuclei from the first replicate were analyzed in (B, C). 25, 43, 63, 73 and 40 nuclei from the second replicate were analyzed in (D, E). The third replicate is shown in Figure 2C and D.

Figure S3. Constant rates of Pol II elongation along the DV axis, Related to Figure 3.

(A) Boxplot showing the distribution of elongation rate per nucleus in two independent embryos. 182 and 197 nuclei were analyzed for *sna primary-MS2-lacZ-PP7*. 315 and 258 nucleis were analyzed for *sna shadow-sna primary-MS2-lacZ-PP7.*

(B-E) Boxplot showing the distribution of elongation rate (B, D) and output (C, E) in DV binned nuclei. 20, 53, 50, 43 and 9 nuclei from the same embryo expressing *sna primary-MS2-lacZ-PP7* were analyzed (B, C). 23, 67, 74, 69 and 25 nuclei from the same embryo expressing *sna shadow-sna primary-MS2-lacZ-PP7* were analyzed (D, E). The other replicate is shown in Figure 3C-F.

Figure S4. Rapid rates of Pol II elongation foster dynamic transcriptional bursting, Related to Figure 4.

(A) Schematic representation of *yellow* reporter gene containing the 100 bp minimal *sna* promoter and 24x MS2 RNA stem loops within the 5´ UTR. The enhancer was placed 7.5 kb downstream of the promoter. This reporter gene also contains a linked *PP7-yellow* in symmetric location [S1].

(B) Representative trajectory of MS2 intensity in an individual nucleus expressing *MS2-yellow* under the control of a *sna* primary enhancer. The time delay from the peak to the baseline (decay time) was measured for individual bursts to estimate Pol II elongation rate.

(C) Boxplot showing the distribution of elongation rate measured from the decay time of individual transcriptional bursts. 364, 369, 300 and 372 nuclei from two independent embryos were analyzed for the reporter genes containing the *sna* primary, *Kr* CD2, *rho* NEE and *Abd-B* IAB5 enhancers, respectively.

Supplemental Experimental Procedures

Expression plasmid

pbphi-SV40NLS-mCherry-PCP

A DNA fragment containing *SV40NLS-mCherry* was amplified using primers (5′-GGG GGC TCG AGA TGC CTA AGA AAA AGA GGA AGG TTG GAT CAG GCT CGG GAT CAA TGG TGA GCA AGG GCG AGG A-3′) and (5′-CCC CCA AGC TTC TTG TAC AGC TCG TCC ATG C-3′) and digested with XhoI and HindIII. The resulting fragment was inserted between the XhoI and HindIII sites in the pbphi-nanos>PCP-αTubulin 3′UTR expression vector [S1].

pbphi-His2Av

A DNA fragment containing 3′ UTR of *His2Av* was amplified from genomic DNA using primers (5′-GGG GGG CTA GCG CCA GTC GGC AAT CGG ACG C-3′) and (5′-CCC CCT CTA GAA CGA CGC AGC GCA CAC GGT A-3′) and digested with NheI and XbaI. The resulting fragment was inserted between the NheI and XbaI sites in the pbphi-multi cloning site [S1]. Subsequently, a DNA fragment containing the coding sequence of *His2A* was amplified from genomic DNA using primers (5′-GGG GGG CGG CCG CTC TAG ACC ACG GAA CCA TTA GCC AGT-3′) and (5′-CCC CCA AGC TTG TAG GCC TGC GAC AGA ATG A-3′) and digested with NOTI and HindIII. The resulting fragment was inserted between the NOTI and HindIII sites in the plasmid.

pbphi-His2Av-eBFP2

A DNA fragment containing *eBFP2* was amplified from pBad-EBFP2 [S2] using primers (5′-GGG GGA AGC TTG GCG GAT CAG GCT CGG GAT CAT CGA TGG TGA GCA AGG GCG AGG A-3′) and (5′-CCC CCG CTA GCT TAC TTG TAC AGC TCG TCC A-3′) and digested with HindIII and NheI. The resulting fragment was inserted between the HindIII and NheI sites in the pbphi-His2Av.

pbphi-SV40NLS-mCherry-PCP, His2AV-eBFP2

A DNA fragment containing *His2Av-eBFP2* was purified from pbphi-His2Av-eBFP2 by digesting with XbaI. The resulting fragment was inserted into the unique XbaI site in the pbphi-SV40NLS-mCherry-PCP.

pbphi-αTubulin 3′UTR

A DNA fragment containing the 3′UTR of *αTubulin* was amplified from genomic DNA using primers (5′-GGG GGC TCG AGG GAT CCG CGT CAC GCC ACT TCA ACG C-3′) and (5′-CCC CCT CTA GAG AGC TTC GCA TGG TTT TGC C-3′) and digested with XhoI and XbaI. The resulting fragment was inserted between the XhoI and XbaI sites in the pbphi-multi cloning site [S1].

pbphi-24x PP7-αTubulin 3′UTR

A DNA fragment containing 24x PP7 was purified from pBlueScript-24x PP7 [S1] by digesting with BamHI and BglII. The resulting fragment was inserted into the unique BamHI site in the pbphi-αTubulin 3′UTR. Subsequently, a DNA fragment containing HindIII and NheI sites was amplified from genomic DNA using primers (5′-GGG GGG CGG CCG CAA GCT TTC CGA ATT TAA CTA CGA GTC-3′) and (5′-CCC CCG CGG CCG CGC TAG CGC CTG CAC AAA GTA CAT ACA-3′) and digested with NOTI. The resulting fragment was inserted into the unique NOTI site in the plasmid to create additional cloning sites.

pbphi-lacZ-24x PP7-αTubulin 3′UTR

A DNA fragment containing *lacZ* was amplified using primers (5′-GGG GGA GAT CTA TGC AGA ACT GGG AGA CGA C-3′) and (5′-CCC CCC TCG AGT TAT TTT TGA CAC CAG ACC A-3′) and digested with BglII and XhoI. The resulting fragment was inserted between the BglII and XhoI sites in the pbphi-24x PP7-αTubulin 3′UTR.

pbphi-hb proximal enhancer-P2 promoter-lacZ-24x PP7-αTubulin 3′UTR

A DNA fragment containing *hunchback* proximal enhancer and P2 promoter was amplified from genomic DNA using primers (5′-GGG GGA AGC TTC ACG CTA GCT GCC TAC TCC T-3′) and (5′-CCC CCA GAT CTG TCG TCT CCC AGT TCT GCA T-

3′) and digested with HindIII and BglII. The resulting fragment was inserted between the HindIII and BglII sites in the pbphi-lacZ-24x PP7-αTubulin 3′UTR.

pbphi-hb proximal enhancer-P2 promoter-24x MS2-lacZ-24x PP7-αTubulin 3′UTR

A DNA fragment containing 24x MS2 stem loops was purified from pCR4-24xMS2SL-stable [S3] by digesting with BamHI and BglII. The resulting fragment was inserted into the unique BglII site in the pbphi-hb proximal enhancer-P2 promoter-lacZ-24x PP7-αTubulin 3′UTR.

pBlueScript-12x PP7-MS2

A synthesized DNA oligonucleotide (5′-CTG CAG CCC GGG GGA TCC TAC GGT ACT TAT TGC CAA GAA AGC ACG AGA CGA TAT GGC GTC CGT GCC TCC AGG TCG AAT CTT CAA ACG ACG ACG ATC ACG CGT CGC TCC AGT ATT CCA GGG TTC ATC AGA TCT GCG GCC GCC ACC GCG-3′) was digested with BamHI and NOTI and inserted into the pBlueScript SK vector (pBlueScript-PP7-MS2). Subsequently, a DNA fragment containing PP7-MS2 was purified from the plasmid using BamHI and NotI and cloned into pBlueScript-MS2-PP7 using BglII and NotI restriction sites (pBlueScript-2x PP7-MS2). By repeating this procedure, a series of vectors containing different copy numbers of PP7-MS2 tandem stem loops were created (pBlueScript-4x PP7-MS2, pBlueScript-8x PP7-MS2 and pBlueScript-12x PP7-MS2).

pbphi-hb proximal enhancer-P2 promoter-lacZ-αTubulin 3′UTR

A DNA fragment containing *lacZ* was purified from pbphi-lacZ-24x PP7-αTubulin 3′UTR by digesting with BglII and XhoI. The resulting fragment was inserted between the BglII and XhoI sites in the pbphi-αTubulin 3′UTR (pbphi-lacZ-αTubulin 3′UTR). Subsequently, a DNA fragment containing *hunchback* proximal enhancer and P2 promoter was purified from pbphi-hb proximal enhancer-P2 promoter-lacZ-24x PP7-αTubulin 3′UTR by digesting NOTI and BglII. The resulting fragment was inserted between the NOTI and BglII sites in pbphi-lacZ-αTubulin 3′UTR.

pbphi-hb proximal enhancer-P2 promoter-12x PP7-MS2-lacZ-αTubulin 3′UTR

A DNA fragment containing 12x PP7-MS2 stem loops was purified from pBlueScript-12x PP7-MS2 by digesting with BamHI and BglII. The resulting fragment was inserted into the unique BglII site in the pbphi-hb proximal enhancer-P2 promoter-lacZ-αTubulin 3′UTR.

pbphi-sna promoter-24x MS2-lacZ-24x PP7-αTubulin 3′UTR

A DNA fragment containing *snail* promoter was amplified from genomic DNA using primers (5′-GGG GGG CTA GCG ACA GCG GCG TCG GCA GAG G-3′) and (5′-CCC CCA GAT CTT GGT TGC GTT CTC AAC GAG A-3′) and digested with NheI and BglII. The resulting fragment was inserted between the NheI and BglII sites in the pbphi-lacZ-24x PP7-αTubulin 3′UTR. Subsequently, a DNA fragment containing 24x MS2 stem loops was purified from pCR4-24xMS2SL-stable by digesting with BamHI and BglII. The resulting fragment was inserted into the unique BglII site in the plasmid.

pbphi-sna primary enhancer-sna promoter-24x MS2-lacZ-24x PP7-αTubulin 3′UTR

A DNA fragment containing the *snail* primary enhancer was amplified from genomic DNA using primers (5′-GGG GGA GCT TCC CGC CGA AGG ATT CCG AGA-3′) and (5′-CCC CCA CTA GTG CCA TTT GGT GGT GGT TCT TCT TC-3′) and digested with HindIII and SpeI. The resulting fragment was inserted between the HindIII and NheI sites in the pbphi-sna promoter-24x MS2-lacZ-24x PP7-αTubulin 3′UTR.

pbphi-sna shadow enhancer-sna primary enhancer-sna promoter-24x MS2-lacZ-24x PP7-αTubulin 3′UTR

A DNA fragment containing the *snail* shadow enhancer was amplified from genomic DNA using primers (5′-GGG GGG CGG CCG CGC ATT GAG GTG TTT TGT TGG-3′) and (5′-CCC CCG CGG CCG CTA AAT TCC GAT TTT TCT TGT CTG GG-3′) and digested with NOTI. The resulting fragment was inserted into the unique NOTI site in the pbphi-sna primary enhancer-sna promoter-24x MS2-lacZ-24x PP7-αTubulin 3′UTR.

pbphi-yellow-24x PP7-αTubulin 3′UTR

A DNA fragment containing *yellow* was amplified from genomic DNA using primers (5′-GGG GGA GAT CTC ATG TTC CAG GAC AAA GGG-3′) and (5′-CCC CCC TCG AGT TAA CCT TGA TGC TGA TGA TGC CAC-3′) and digested with BglII and XhoI. The resulting fragment was inserted between the BglII and XhoI sites in the pbphi-24x PP7-αTubulin 3′UTR.

pbphi-hb proximal enhancer-P2 promoter-24x MS2-yellow-24x PP7-αTubulin 3′UTR

A DNA fragment containing *hunchback* proximal enhancer, proximal P2 promoter and 24x MS2 was purified from pbphi-hb proximal enhancer-P2 promoter-MS2-lacZ-24x PP7-αTubulin 3′UTR by digesting with NOTI and BglII. The resulting fragment was inserted between the NOTI and BglII sites in the pbphi-yellow-24x PP7-αTubulin 3′UTR.

Supplemental References

S1. Fukaya T., Lim B., and Levine M. (2016). Enhancer Control of Transcriptional Bursting. Cell. *166*, 358-368.

S2. Ai H.W., Shaner N.C., Cheng Z., Tsien R.Y., and Campbell R.E. (2007). Exploration of new chromophore structures leads to the identification of improved blue fluorescent proteins. Biochemistry. *46*, 5904-5910.

S3. Bertrand E., Chartrand P., Schaefer M., Shenoy S.M., Singer R.H., and Long R.M. (1998). Localization of *ASH1* mRNA particles in living yeast. Mol Cell. *2*, 437-445.