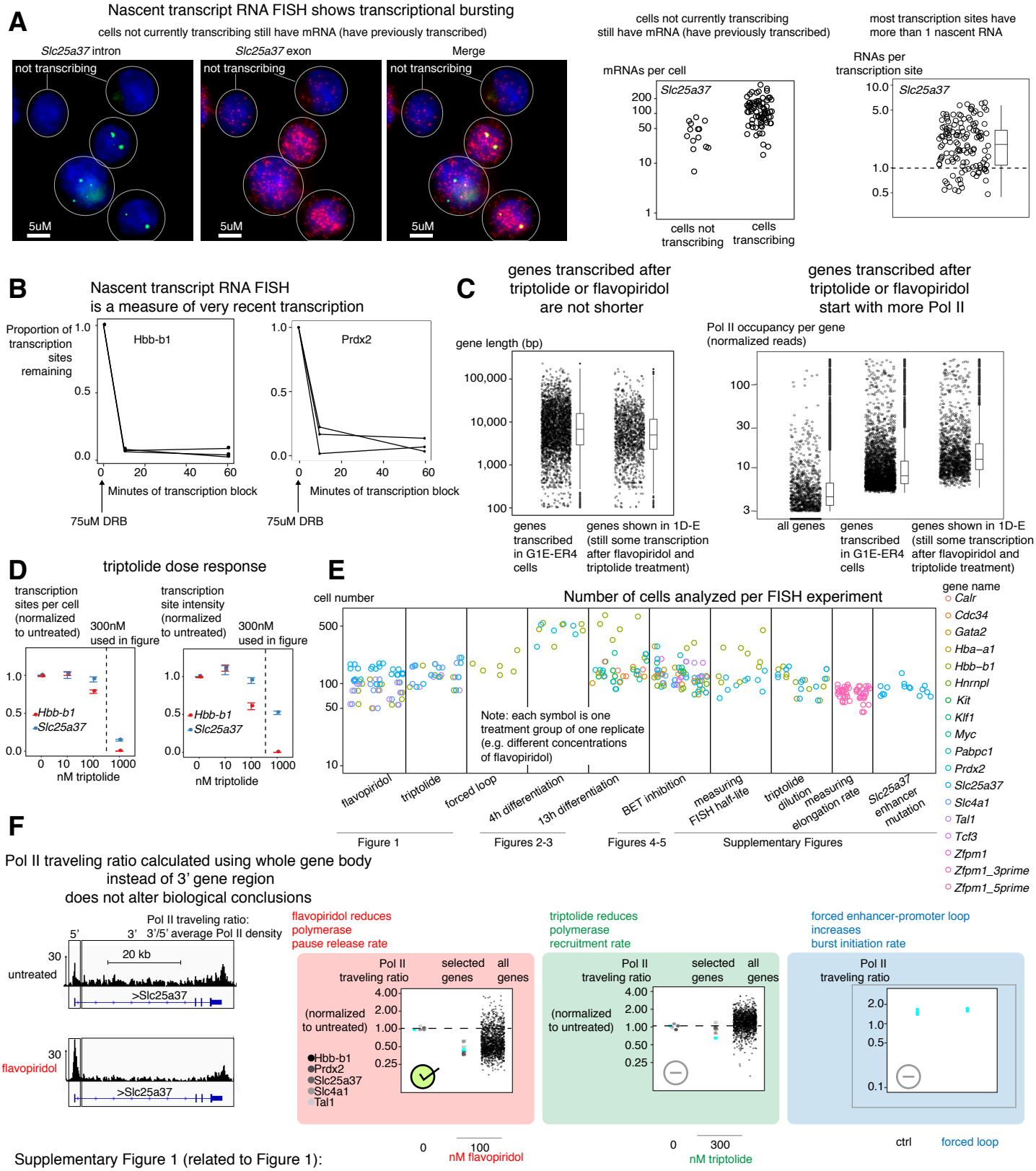
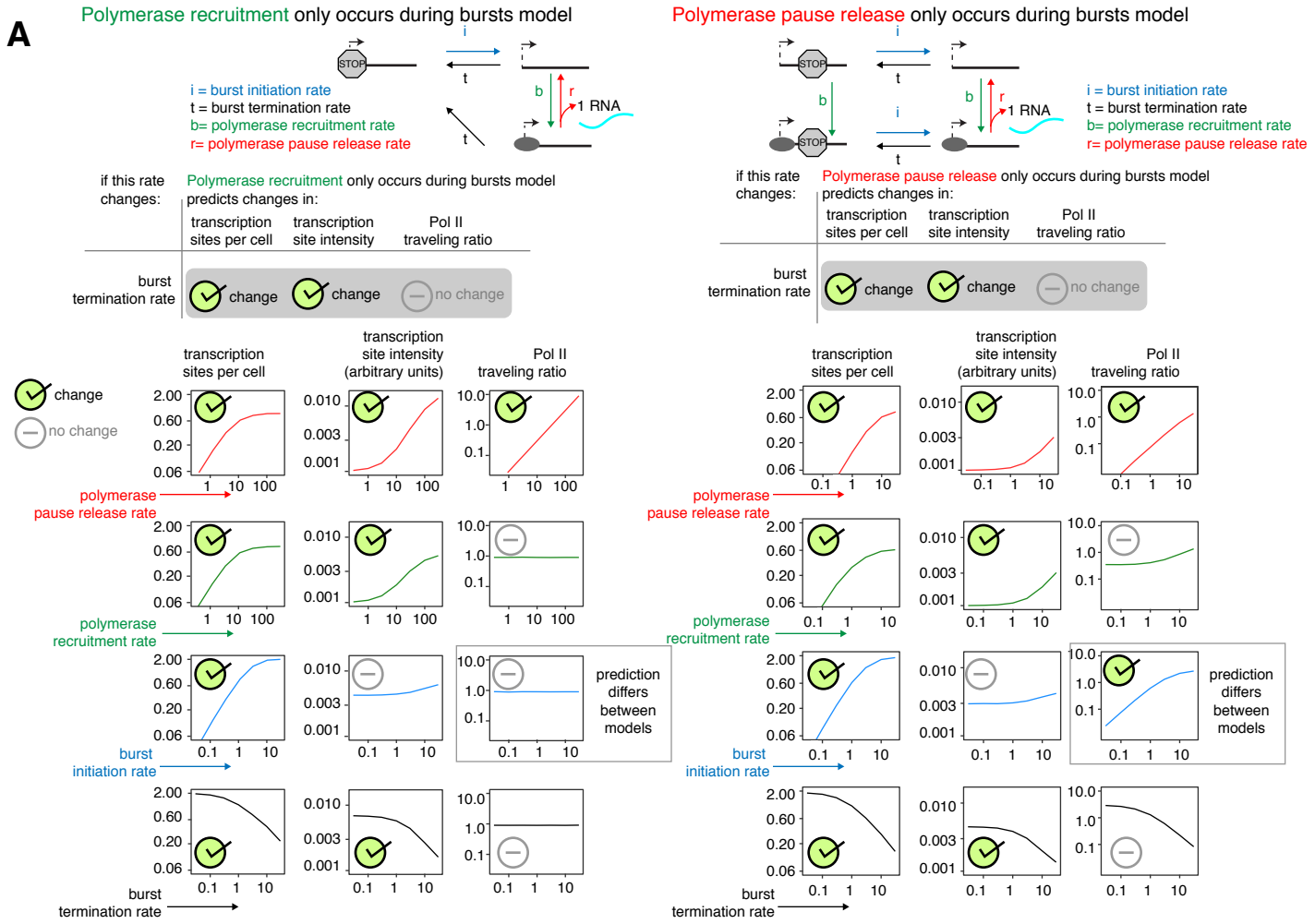
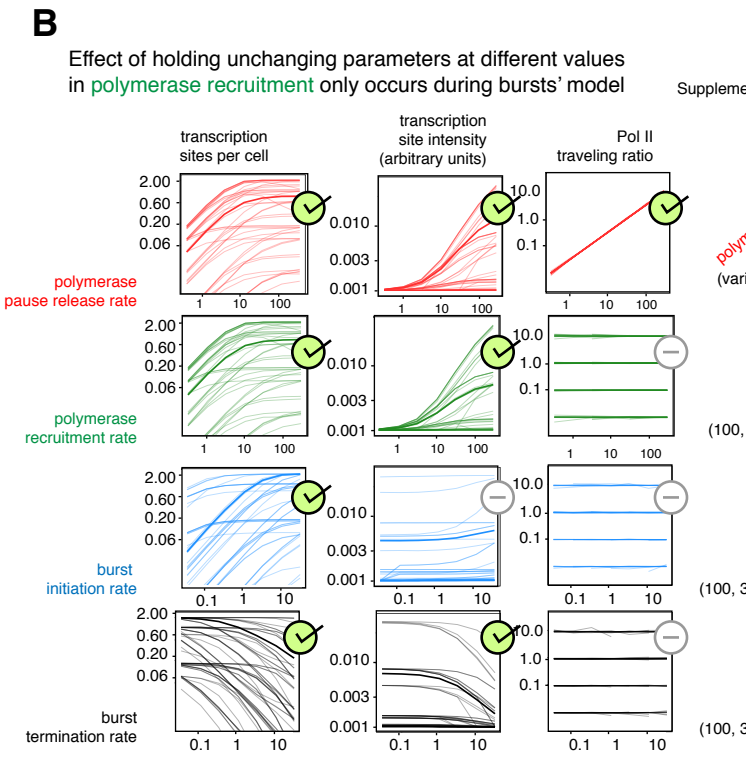
# Supplementary Figure 1




Supplementary Figure 1 (related to Figure 1):
A. G1E-ER4 cells transcribe in bursts: cells not currently transcribing still have mRNA for *Slc25a37*, and transcription sites on average have transcribed more than 1 RNA (as measured by normalizing fluorescence intensity of transcription sites to mRNA intensity.
B. Active-transcribing fraction in response to 75uM DRB, measuring half-life of transcription sites (n=3 biological replicates per gene).
C. Characteristics of genes displayed in traveling ratio analysis in Figures 1D-E: genes are not shorter than average, but do tend to have more Pol II occupancy in the untreated setting, so moderate drug dose does not fully block transcription (n=3 biological replicates).
D. Dose titration of triptolide using nascent transcript RNA FISH: 300nM reduced transcription sites per cell and transcription site intensity somewhat but not completely (n=3 biological replicates).
E. Numbers of cells counted per experimental condition per replicate for all FISH experiments in the paper (total= 327 gene-replicates performed, 43790 cells examined).
F. Using gene body instead of 3' end to calculate traveling ratio does not alter biological conclusions: traveling ratios from 1D-F right-side panels recalculated using whole gene body.

# Supplementary Figure 2



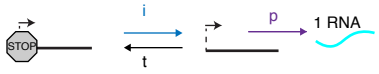




Supplementary Figure 2 (related to Figure 1):
A.Structure and quantitative predictions of the polymerase recruitment only occurs during bursts and the polymerase pause release only occurs during bursts models. Rate parameters not labeled as changing in each graph held at the values of burst initiation rate=1, burst termination rate=3, polymerase recruitment rate= 100, polymerase pause release rate=30. These trends are representative of all parameter values.
B. Model predictions for all parameter values for polymerase recruitment only occurs during bursts model.

# Supplementary Figure 3

**A** Alternative models of transcriptional regulation:

Telegraph model



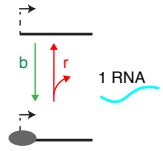

i = burst initiation rate
t = burst termination rate
p= RNA production rate

| if this rate changes: | transcription sites per cell | transcription site intensity | Pol II traveling ratio |
|---|---|---|---|
| RNA production rate | change | change | no change |
| burst termination rate | change | change | no change |
| burst initiation rate | change | no change | no change |

Consistent with experimental data?

No:
Pol II traveling ratio never predicted to change

**B** Recruitment-release (no bursting)

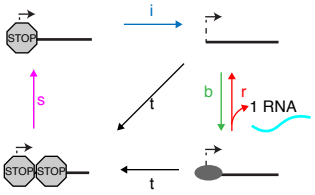



b= polymerase recruitment rate
r= polymerase pause release rate

| if this rate changes: | transcription sites per cell | transcription site intensity | Pol II traveling ratio |
|---|---|---|---|
| polymerase pause release rate | change | change | change |
| polymerase recruitment rate | change | change | no change |

No:
Transcription sites per cell never predicted to change without transcription site intensity change

**C** Polymerase recruitment only occurs during bursts with refractory period

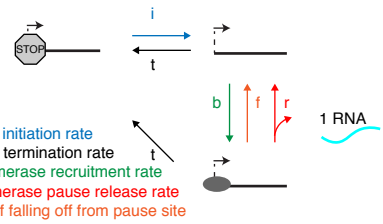



i = burst initiation rate
t = burst termination rate
b= polymerase recruitment rate
r= polymerase pause release rate
s=escape refractory period

| if this rate changes: | transcription sites per cell | transcription site intensity | Pol II traveling ratio |
|---|---|---|---|
| polymerase pause release rate | change | change | change |
| polymerase recruitment rate | change | change | no change |
| burst termination rate | change | change | no change |
| burst initiation rate | change | no change | no change |
| escape from refractory period rate | change | no change | no change |

Yes:
could suggest that escape from refractory period rate also could be a key regulatory rate, our data shows no evidence of refractory periods

**D** Polymerase recruitment only occurs during bursts with possibility of termination from pause site



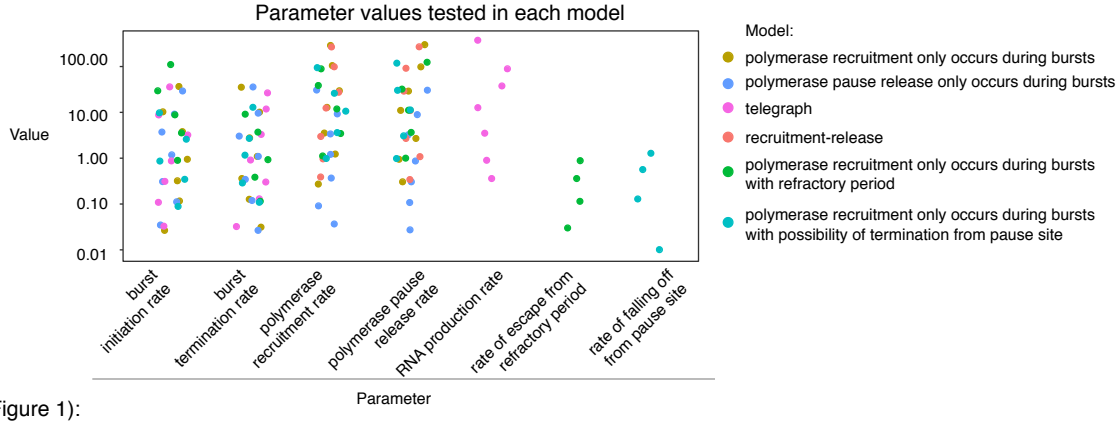

i = burst initiation rate
t = burst termination rate
b= polymerase recruitment rate
r= polymerase pause release rate
f= rate of falling off from pause site

| if this rate changes: | transcription sites per cell | transcription site intensity | Pol II traveling ratio |
|---|---|---|---|
| polymerase pause release rate | change | change | change |
| polymerase recruitment rate | change | change | no change |
| burst initiation rate | change | no change | no change |
| burst termination rate | change | change | no change |

for any proportion of paused polymerase falling off (0% falling off to 100%)

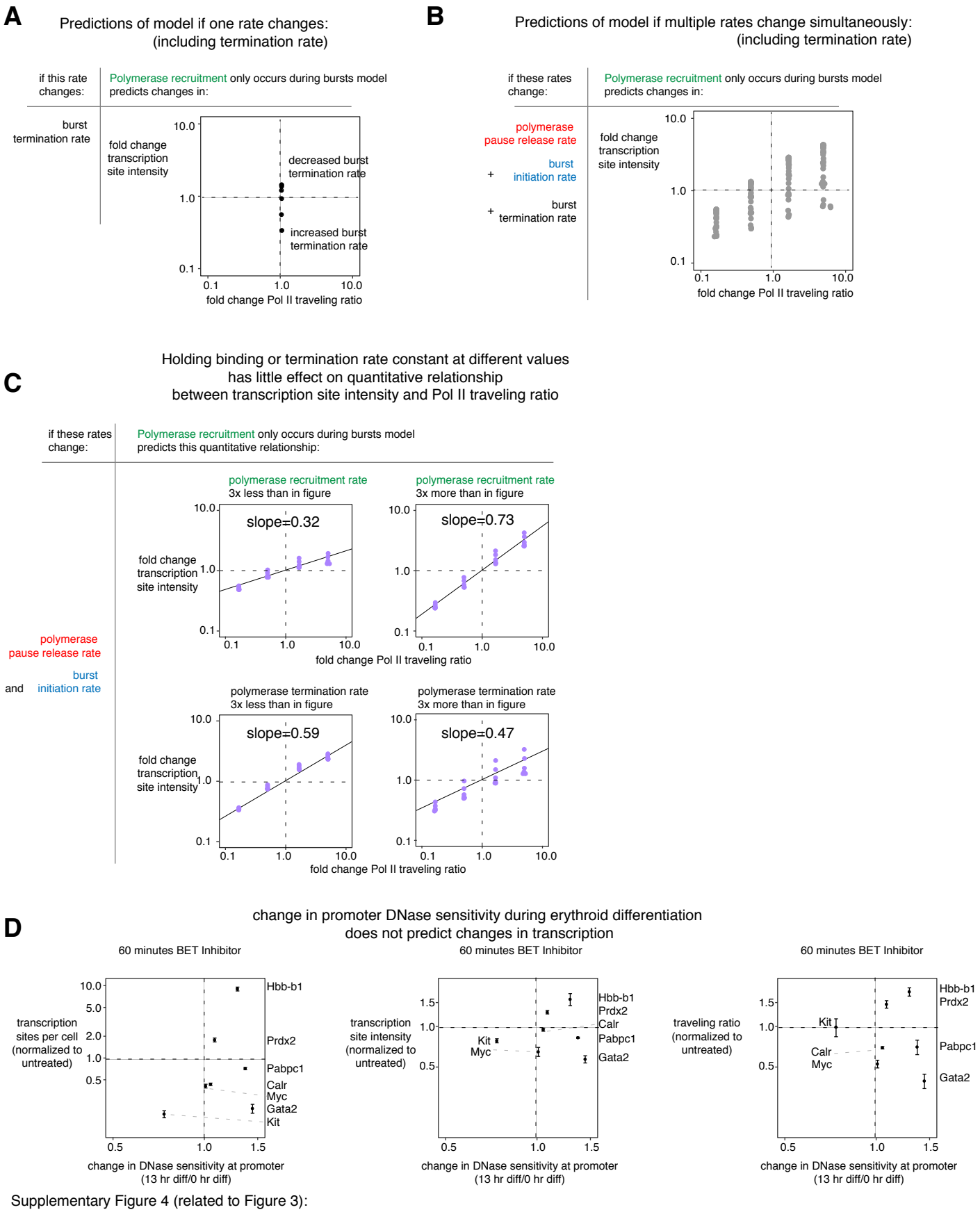
Yes:
does not change conclusions

**E**




Supplementary Figure 3 (related to Figure 1):
A. Predictions of telegraph model for RNA FISH and Pol II ChIP-seq are not consistent with experimental data.
B. Predictions of recruitment-release model without bursting for RNA FISH and Pol II ChIP-seq are not consistent with experimental data.
C. Predictions of polymerase recruitment only occurs during bursts model with refractory period are consistent with experimental data, and could suggest that rate of escape from refractory period is a regulated rate.
D. Predictions of polymerase recruitment only occurs during bursts model with possibility of termination from pause site are consistent with experimental data, but adding this possibility gives the same predictions as the polymerase recruitment only occurs during bursts model.
E. Parameter values tested for each rate in each model. Each combination of parameters was tested, for example recruitment-release model, 2 steps, 7 values each= 7^2=49 different simulations.

# Supplementary Figure 4




Supplementary Figure 4 (related to Figure 3):

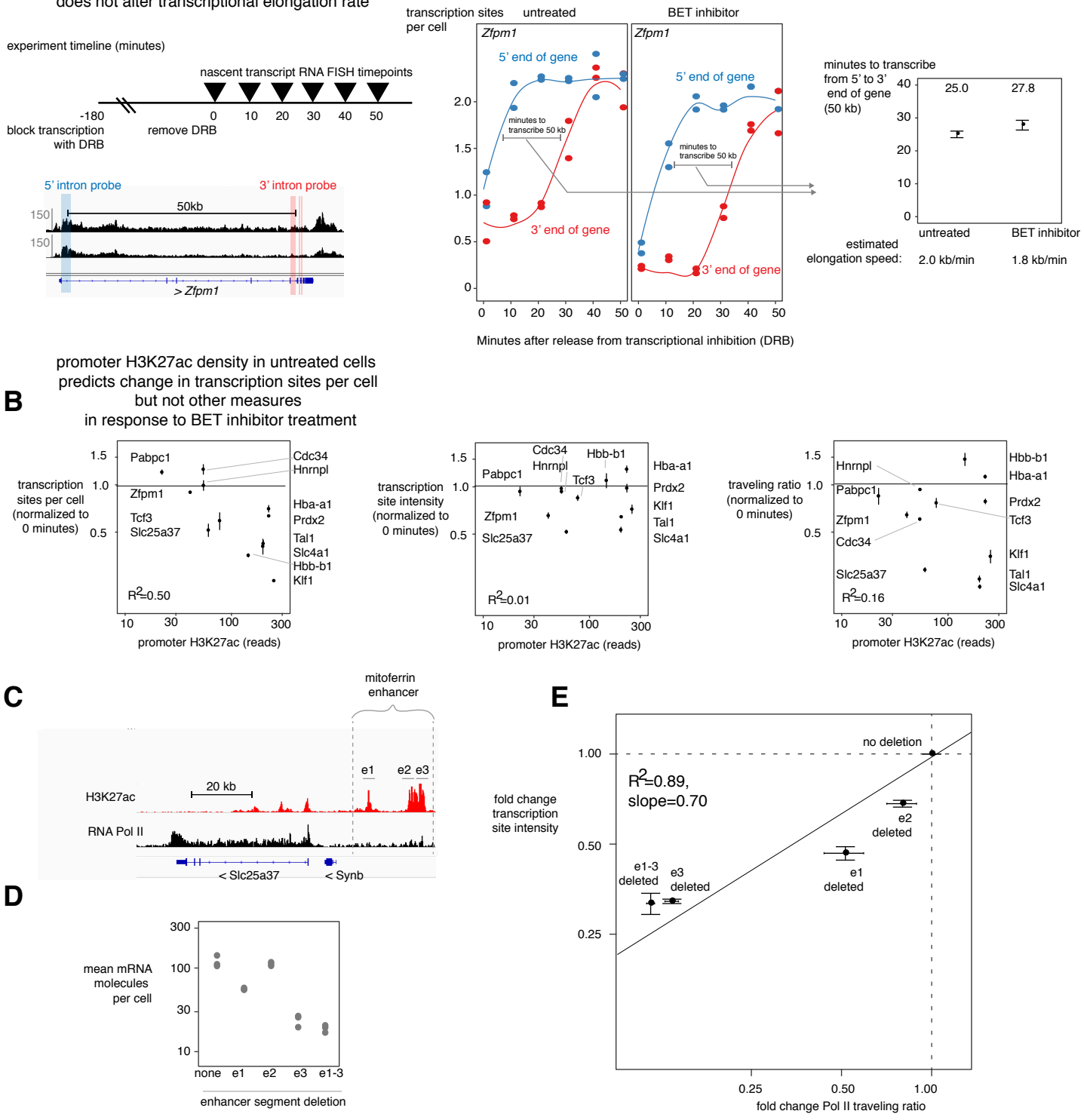
A. Effect of changing individual rates on the relationship between transcription site intensity and Pol II traveling ratio, including burst termination rate.

B. Effect of changing termination, initiation and pause release rates simultaneously on the relationship between transcription site intensity and Pol II traveling ratio.

C. Predicted effect of holding polymerase recruitment rate, or burst termination rate, constant at different levels on the relationship between nascent RNA intensity and traveling ratio when both polymerase pause release rate and burst initiation rate are changed.

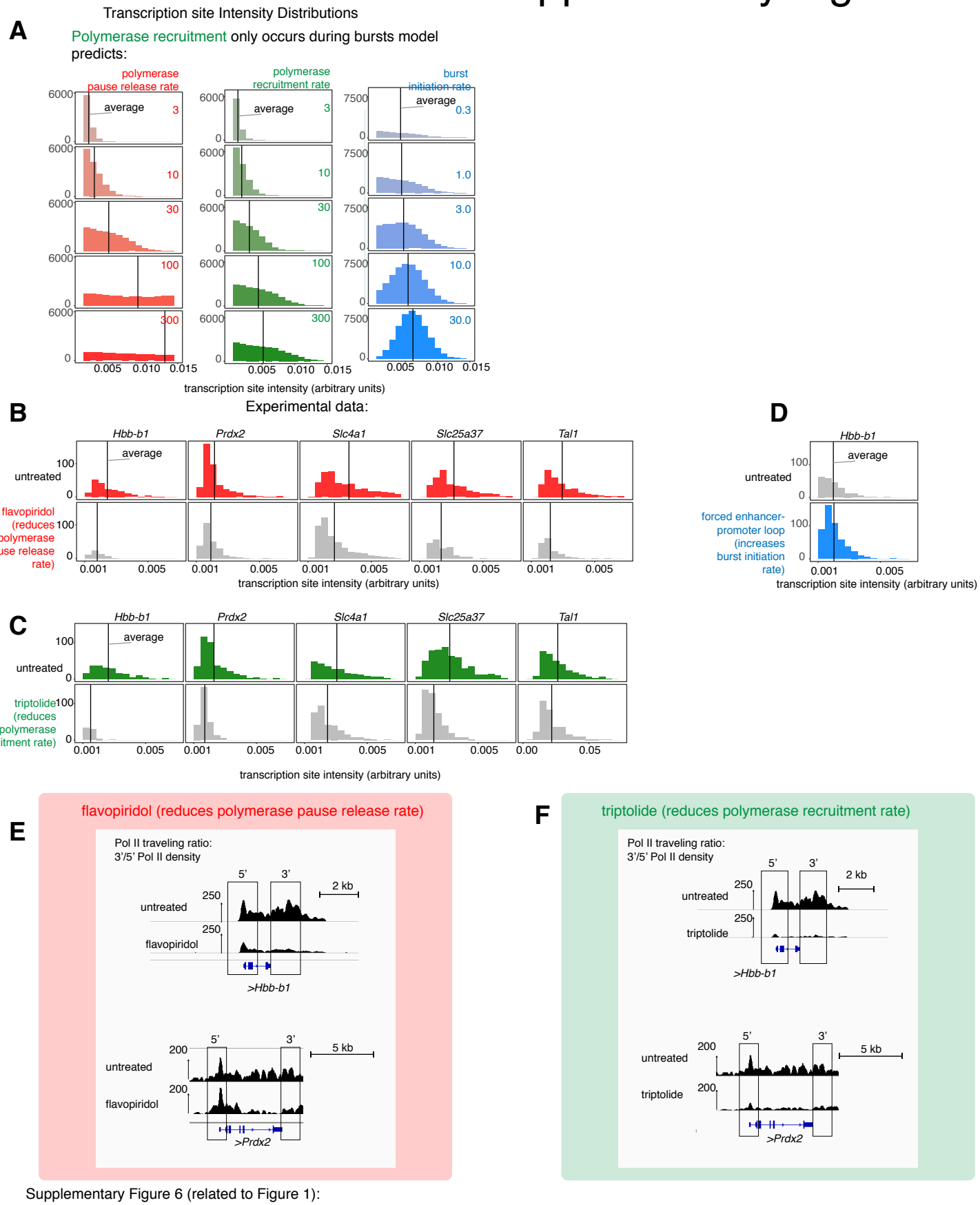
D. Change in promoter DNase sensitivity during erythroid differentiation does not predict changes in transcription.

# Supplementary Figure 5




Supplementary Figure 5 (related to Figures 4 and 5):
A. BET inhibitor treatment does not change elongation rate for *Zfpm1*, as measured by nascent transcript RNA FISH measurements at 5' and 3' ends of gene at timepoints after release of DRB transcriptional block.
B. Promoter H3K27ac density in untreated cells predicts change in transcription sites per cell but not other measures in response to BET inhibitor treatment.
C. Schematic of *Slc25a37* locus with labeled enhancer segments.
D. *Slc25a37* mRNA counts measured by RNA FISH in unmutated or enhancer-mutant G1E-ER4 cells differentiated for 24 hours (n=3 biological replicates of exon RNA FISH).
E. Effects of *Slc25a37* enhancer mutation on transcription site intensity and Pol II traveling ratio of *Slc25a37* (n=3 biological replicates each of nascent transcript RNA FISH and Pol II ChIP-qPCR).

# Supplementary Figure 6




Supplementary Figure 6 (related to Figure 1):
A. Distributions of transcription site intensities predicted by the polymerase recruitment only occurs during bursts model.
B. Distributions of transcription site intensities in cells treated with 100nM flavopiridol (n=3 biological replicates pooled).
C. Distributions of transcription site intensities in cells treated with 300nM triptolide (n=3 biological replicates pooled).
D. Distributions of transcription site intensities in cells expressing looping factor (n=3 biological replicates pooled).
E. Representative Pol II ChIP-seq tracks from G1E-ER4 cells treated with flavopiridol. Pol II traveling ratio for these experiments quantified in Figure 1D.
F. Representative Pol II ChIP-seq tracks from G1E-ER4 cells treated with triptolide. Pol II traveling ratio for these experiments quantified in Figure 1E.

**Table S2, related to STAR Methods.**

| Primer name | Sequence |
|---|---|
| Hbbb1_TSS_F | CAGGGAGAAATATGCTTGTCATCA |
| Hbbb1_TSS_R | GTGAGCAGATTGGCCCTTACC |
| Hbbb1_TES_F | GCCCTGGCTCACAAGTACCA |
| Hbbb1_TES_R | TTCACAGGCAAGAGCAGGAA |
| Slc25a37_TSS_F | TGGTCGGTAGGTTCTCGTAGTC |
| Slc25a37_TSS_R | GAGGATGGATGGGGACTG |
| Slc25a37_TES_F | CCAGCGTTCTCAAAGCAAAC |
| Slc25a37_TES_R | TATCACAGCCAAAGCCAGAG |
| CD4_F (negative control) | CCAGAACATTCCGGCACATT |
| CD4_R (negative control) | GGTAAGAGGGACGTGTTCAACTTT |