PNAS
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Supplementary Information for

The nuclear to cytoplasmic ratio directly regulates zygotic transcription in *Drosophila* through multiple modalities

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This PDF file includes:

Other supplementary material for this manuscript include the following:

Movies S1 to S3

Supplementary Information Text

Materials and Methods

Generation of wild-type, diploid embryos

Wild-type embryos were produced by crossing *y*,*w*;;MCP:GFP,His2Av-mRFP virgin females to *y*,*w*;; males. The resulting embryos were provided with one maternal copy of the MCP:GFP,His2Av-mRFP while the desired MS2 was provided paternally. Crosses were conducted in collection cups at 25°C. We observed no difference in MS2 signal between wild-type embryos that had MS2 provided maternally and from those provided paternally.

Generation of haploid embryos

Haploid embryos were derived from the *sesame/Hira*^{185b}(*ssm*) line. Haploid embryos were made by crossing hemizygous *ssm* mutant males in the desired MS2 background to heterozygous *ssm*/FM7c virgin females in the MCP:GFP,His2Av-mRFP background to produce *ssm* homozygous females that were heterozygous for both MCP:GFP and the desired MS2. These females, in turn lay embryos that do not include the male contribution and are thus haploid. For example, to observe haploid *sna* transcription: *w,ssm*;;*sna*-Promoter-Distal-Enhancer-MS2 males were crossed to *w,ssm*/FM7c;;MCP:GFP,His2Av-mRFP virgins. Homozygous *ssm* females were collected, crossed with *y*,*w*;; males and placed in collection cups at 25°C. Embryos laid by the *ssm* homozygous mothers with one copy of the MCP:GFP,His2Av-mRFP and one copy of the desired MS2 were used for haploid. Only embryos that inherited the desired MS2 were analyzed.

Generation of short-cycle diploid (*grp*) embryos

Short-cycle diploid embryos were derived from *w*; *grp*^{1}/ CyO; *ry*^{506}*.* Short-cycle diploid embryos were produced by crossing *w*; *grp*^{1}/ CyO; *ry*^{506} virgins to *w*; *grp*^{1} / CyO; MCP:GFP,His2Av-mRFP males. Resulting *grp*^{1} homozygous virgins were collected, crossed to desired MS2 males, and placed in collection cups at 25°C. Embryos laid by these mothers were used for short-cycle diploid embryos.

MS2 constructs

kni-vk33

The *knirps>MS2-yellow* reporter was constructed by amplifying the *knirps* regulatory region from 5.5 kb upstream to 1kb downstream of the promoter, including the *knirps* promoter. This sequence was cloned in the pBphi-*MS2-yellow* vector (1) using the NotI and BamHI restriction sites. The plasmid was integrated into a specific landing site on the 3rd chromosome (Bloomington *Drosophila* Stock Center, #9750) using the phiC31-mediated integration.

gt-vk33

The *giant>MS2-yellow* reporter was generated using the same method as *knirps* to clone the *giant* regulatory region from 10kb upstream of the promoter including the *giant*

promoter. The plasmid was integrated into a specific landing site on the 3rd chromosome (Bloomington *Drosophila* Stock Center, #9750) using the phiC31-mediated integration.

sna-distal-vk33
The *sna-distal>MS2-yellow* reporter was generated by amplifying the *snail* distal regulatory region from 7kb to 8.5kb upstream of the promoter and the 100bp core *snail* promoter. This sequence was cloned to the pBphi-*MS2-yellow* vector using the HindIII and BamHI restriction sites. The plasmid was integrated into a specific landing site on the 3rd chromosome (Bloomington *Drosophila* Stock Center, #9750) using the phiC31-mediated integration.

KrCD2-vk33
The *KrCD2>MS2-yellow* reporter was generated by amplifying the *Kr* CD2 regulatory region 1.6kb upstream of the promoter and the 100bp core *eve* promoter. This sequence was cloned to the pBphi-*MS2-yellow* vector using the HindIII and BamHI restriction sites. The plasmid was integrated into a specific landing site on the 3rd chromosome (Bloomington *Drosophila* Stock Center, #9750) using the phiC31-mediated integration.

gt-vk33
The *giant>MS2-yellow* reporter was generated using the same method as *knirps* to clone the *giant* regulatory region from 10kb upstream of the promoter including the *giant* promoter. The plasmid was integrated into a specific landing site on the 3rd chromosome (Bloomington *Drosophila* Stock Center, #9750) using the phiC31-mediated integration.

bnk-vk18
The *bottleneck>MS2-yellow* reporter was generated using the same method as *knirps* to clone the *bottleneck* regulatory region 206 bp upstream and 48 bp downstream of the *bottleneck* transcription start site (2). The plasmid was integrated into a specific landing site on the 2nd chromosome (Bloomington *Drosophila* Stock Center, #9736) using the phiC31-mediated integration.

Endogenous *ftz-MS2*
ftz-MS2 line from (3) was used in this study.

Endogenous *frs*-MS2
frs-MS2 was generated using CRISPR-cas9 homology-directed repair to insert 24x MS2 loops into the 5′UTR of the endogenous *frs* locus. A single target site within the *frs* 5′UTR was selected using the Target Finder (http://targetfinder.flycrispr.neuro.brown.edu) tool (4).

gRNA oligo sequences
Sense: CTTCGCGACATAATAACTGCTAGGC
Antisense: AAACGCCTAGCAGTTATTATGTCGC

The annealed gRNA oligo was subcloned into the pU6-BbsI-chiRNA vector (a gift from Melissa Harrison & Kate O'Connor-Giles & Jill Wildonger, Addgene plasmid #45946) via BbsI restriction sites. Approximately 1-kb fragments of *frs* homology arm sequences were synthesized and inserted into the pHD-MS2-loxP-dsRed-loxP plasmid (3) (GeneWiz,Inc.). The gRNA and *frs* homology arm MS2 plasmids were co-injected into *nos*-Cas9 embryos (TH00787.N), and DsRed+ progeny were screened (BestGene). The resulting endogenous *frs-MS2* fly strains were homozygous viable.

Live imaging of transcription

All fly stocks were maintained by standard method at 25°C and were grown on standard cornmeal media. All embryos were collected on apple juice agar plates. Sex of the embryo was not considered in this study. Embryos were collected after laying for 1.5 hours at 25°C then staged as pre-blastoderm with halocarbon oil. Staged embryos were then washed with DI H_2O to remove any halocarbon oil, dechorionated with 4% sodium hypochlorite for 1-1.5 minutes, mounted on a 35mm coverslip dish (MatTek), and covered with water.

All movies except for the *KrCD2>MS2* and *ftz-MS2* movies were acquired at 23-24°C using a Nikon Ti-E confocal microscope with a Yokogowa CSU-21 spinning disk module. Movies for the *KrCD2>MS2* and *ftz-MS2* were acquired at ~23°C using a Zeiss LSM800 confocal laser scanning microscope. Images were acquired with a Plan-Apochromat 40x1.3 NA oil objective using a 488 and 561 laser to visualize MCP:GFP and His2Av-mRFP, respectively, at a time resolution of 24s/frame for *KrCD2>MS2 and ftz-MS2* and 30s/frame for all other movies. At each time point, a stack of 13 images with 0.5 μm steps (Nikon) or a stack of 14 images with 0.75 μm steps (Zeiss) was taken. The same exposure and laser settings were used for all samples at each respective microscope and controls and experiments were acquired under identical conditions for each genotype. All images were acquired in 16-bits.

Imaging began when nuclei first emerged onto the surface of the embryo at NC10 until gastrulation (approximately 60 minutes after entry into NC14 or NC15 in haploids). Only the first 30 minutes of NC14 (wild-type) or NC15 (haploid) were analyzed due to the greater z-resolution needed to capture the total MS2 signal during cellularization. Imaging of short-cycle diploid embryos ended after the catastrophic 13^{th} mitosis.

Quantification and Statistical Analysis

All the image processing methods and analyses were implemented in MATLAB (R2018b, MathWorks). Histograms of all the snapshots and movies shown in Figure 1 and in Supplemental Movies were adjusted for visualization purposes only. All the analyses were performed with raw images.

Nuclei Segmentation and tracking

At each time frame, maximum projections for all z-sections per image were obtained. His2Av-mRFP channel was used to segment nuclei. Nuclei-labeled channels were first filtered with Gaussian filtering to minimize signal noise, and then were converted into

binary images using a threshold value using Otsu's method. Frames were manually corrected as needed to ensure proper segmentation. The number of nuclei that were segmented from each frame was obtained and the center of mass of each nucleus was assigned x and y coordinates for tracking. Nuclei tracking within each nuclear cycle was obtained by finding the nucleus with minimal movement across the segmented frames. In all frames, the nuclei located at the edge of the frame were excluded from the analysis.

MS2 signal extraction

MS2 fluorescent intensities were recorded using maximum projections of raw images and were extracted from within each nucleus after nuclei segmentation. After subtracting the background signal, the MS2 signal in a given nucleus was determined by averaging the top two pixels with the highest fluorescence intensity within each nucleus.

Defining active nuclei for various metrics

For all genotypes, active nuclei were defined as the nuclei that exceed an MS2 fluorescence intensity threshold for a percentage of the total duration of a given cell cycle. This criterion was used to compute the various metrics and properties, including total mRNA output, duration of active transcription, and average trajectories of all cell cycles. When obtaining the number of active nuclei as a fraction of the total nuclei in a cell cycle, all nuclei that show an MS2 signal above the predetermined threshold at any time point in a given cell cycle were considered.

Plots

Since the variability between individual nuclei within an embryo was determined to be greater than embryo to embryo variability according to T-test values, nuclei from all replicates were merged to obtain the figures. In all boxplots, the box indicates the 25% quantile and 75% quantile, and the solid line indicates the median. The top and bottom whiskers correspond to the 10th and 90th percentiles of each distribution. The error bars represent the standard error of the mean (SEM) of all replicates. Wild-type NC14 and haploid NC15 were analyzed for only 30 minutes (60 (Nikon) or 75 (Zeiss) frames).

Trajectories of cell cycles

Transcriptional activity of each MS2-reporter gene and endogenous gene can be plotted over time at single-cell resolution. Each cycle was normalized to start at frame 1, or the first frame after mitosis. Segmentation and further analysis were performed 3~4 frames after mitosis to minimize nuclei movement and obtain nuclei lineages. To obtain an average transcriptional trajectory per cell cycle, data from all active nuclei of a particular genotype in a given cell cycle was combined.

Total mRNA output

The fluorescence intensity at a given time frame in each nucleus was used as a proxy to measure instantaneous amplitude of a given transcript. Cumulative mRNA output of a nucleus was computed by integrating an active nucleus trajectory of fluorescence intensity over time.

Median signal of cell cycles
The trajectories were smoothened using the local regression (LOESS) method. The maximum amplitude for each nucleus was obtained using the smooth curve. The median signal refers to the median fluorescence intensity an active nucleus exhibits during a given cell cycle.

Duration of cell cycles
Duration of a cell cycle was determined to be the time in between two mitosis. The duration of active transcription in a given cell cycle was the time during which the MS2 signal exceeded the predetermined threshold.

Transcription slopes
The transcription slope of an active nucleus was obtained by measuring the initial slope of the nucleus's smoothened fluorescence trajectory. The smoothened curve was interpolated by a factor of 10. The transcription slope was determined to be the slope of the best-fit line after linear regression on the first 30 points above the threshold.

Statistical tests
Two different statistical tests were performed to verify the significance among pairs of results. Student's t-test was used to calculate p-values, and the significance at 2 different thresholds is shown in each figure. * indicates $p<0.05$, while ** represents $p<0.005$. Bootstrapping was performed to confirm statistical significance from the Student's t-test p-value results. The mean of a parameter (i.e. transcription slope or median signal) was computed for a single genotype after resampling 10,000 times. The distribution of the difference in means of the parameter between two genotypes was used to generate 95% confidence intervals. Bootstrapping was performed both by sampling the entire sample size and by sub-sampling. Both methods yielded the same statistical results.

Calculations for parameter contribution
An estimate of mRNA output of a wildtype embryo was calculated by multiplying the average median signal, duration of the cell cycle, and the fraction of active nuclei. To determine the contribution of each parameter (i.e. duration, median signal, or fraction of active nuclei), we calculated the mRNA output of the haploid embryo by changing only one parameter at a time while keeping the other two parameters as the wildtype values. As an example, to determine the effect of haploid duration on *knirps* mRNA output, the duration of the haploid embryo was multiplied by the wildtype value of median signal and fraction of active nuclei. This value is used as an estimate of the contribution of the duration in the total mRNA output by measuring the deviation and percent difference from the wildtype embryo. To calculate the relative contribution of each parameter, the percent differences between the mRNA outputs were normalized by the sum of all percent differences obtained when changing one parameter at a time.

S1

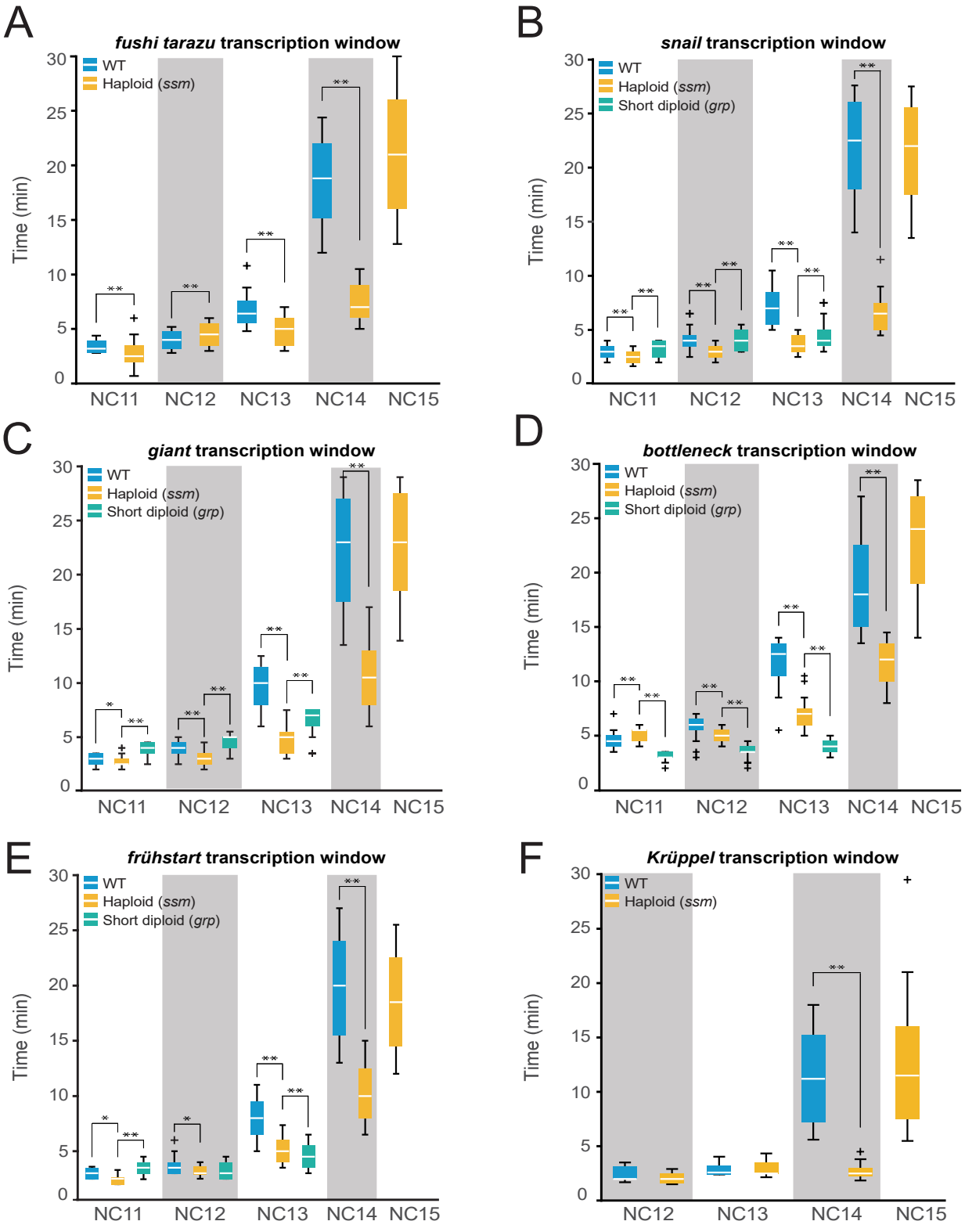

A
fushi tarazu transcription window
WT
Haploid (ssm)
Time (min)
NC11
NC12
NC13
NC14
NC15
B
snail transcription window
WT
Haploid (ssm)
Short diploid (grp)
Time (min)
C
giant transcription window
D
bottleneck transcription window
E
frühstart transcription window
F
Krüppel transcription window

Fig S1. Duration of active transcription scales directly with cell cycle length

Boxplots of transcription duration for (A) *ftz-MS2, (B) sna_distalE>MS2,* (C) *gt>MS2,* (D) *bnk>MS2*, (E) *frs-MS2*, (F) *KrCD2>MS2* and per nucleus for WT (blue), haploid (yellow), and short-cycle diploid (green) embryos throughout the syncytial blastoderm stage. Boxplot shows minimum (10%), lower (25%), median, upper (75%), and maximum (90%) quantiles. Outliers are shown as '+'. * $p<0.05$, ** $p<0.005$ from Student's t-test.

The number of nuclei examined for *gt>MS2, frs-MS2*, and *KrCD2>MS2* is described in the legends of Figure 3E, 4E, and 4J respectively.

For *ftz-MS2,* the number of nuclei analyzed is as follows: 51 NC11, 102 NC12, 192 NC13, and 518 NC14 nuclei from 3 replicate WT, and 59 NC11, 176 NC12, 344 NC13, 528 NC14, and 1041 NC15 nuclei from 3 replicate haploid embryos.

For *sna_distalE>MS2,* the number of nuclei analyzed is as follows: 109 NC11, 163 NC12, 272 NC13, and 1283 NC14 nuclei from 4 replicate WT, 58 NC11, 153 NC12, 239 NC13, 375 NC14, and 1259 NC15 nuclei from 3 replicate haploid, and 146 NC11, 175 NC12, and 139 NC13 nuclei from 4 replicate short-cycle diploid embryos.

For *bnk>MS2,* the number of nuclei analyzed is as follows: 137 NC11, 272 NC12, 497 NC13, and 726 NC14 nuclei from 3 replicate WT, 89 NC11, 333 NC12, 662 NC13, 1062 NC14, and 1196 NC15 nuclei from 4 replicate haploid, and 107 NC11, 186 NC12, and 327 NC13 nuclei from 2 replicate short-cycle diploid embryos.

S2

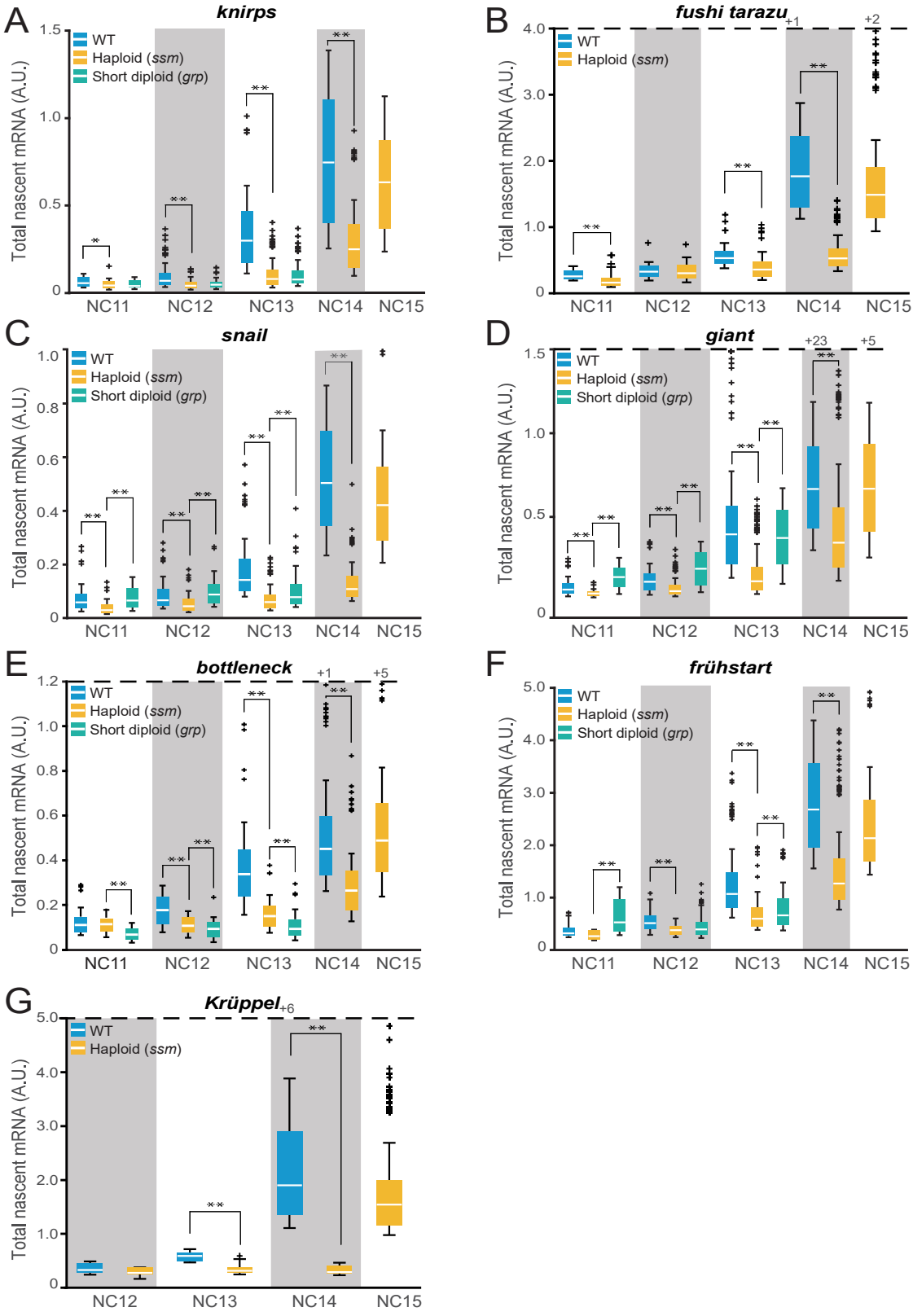

A
knirps
B
fushi tarazu
C
snail
D
giant
E
bottleneck
F
frühstart
G
Krüppel
WT
Haploid (ssm)
Short diploid (grp)
Total nascent mRNA (A.U.)
NC11
NC12
NC13
NC14
NC15

Fig S2. Total RNA output depends on cell cycle duration

Boxplots of total RNA output for (A) *kni*(5'+int)*>MS2,* (B) *ftz-MS2,* (C) *sna_distalE>MS2*, (D) *gt>MS2*, (E) *bnk>MS2*, (F) *frs-MS2*, and (G) *KrCD2>MS2* per nucleus for WT (blue), haploid (yellow), and short-cycle diploid (green) embryos throughout the syncytial blastoderm stage. Dashed line represents a cut-off for outlier values. Number of outlier values above the cut-off are given after '+'.
* $p<0.05$, ** $p<0.005$ from Student's t-test.
The number of analyzed nuclei is the same as in Figure S1.

S3

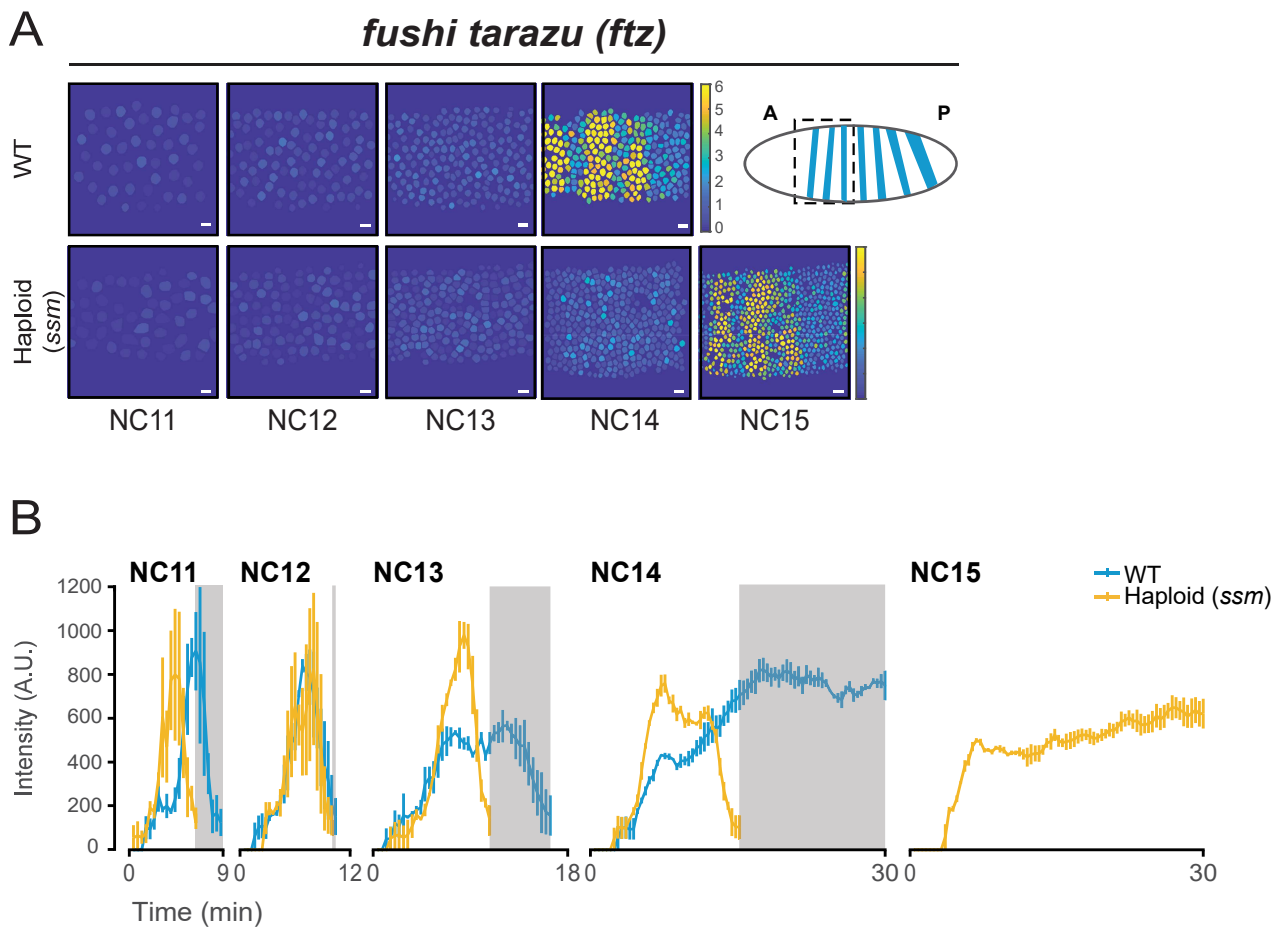

A
fushi tarazu (ftz)
WT
Haploid (ssm)
A
P
6
5
4
3
2
1
0
NC11
NC12
NC13
NC14
NC15
B
NC11
NC12
NC13
NC14
NC15
WT
Haploid (ssm)
Intensity (A.U.)
1200
1000
800
600
400
200
0
0
90
120
180
300
30
Time (min)
C
ftz transcription activation
WT
Haploid (ssm)
Total nuclei
Nuclei
700
600
500
400
300
200
100
0
NC11
NC12
NC13
NC14
NC15
D
ftz transcription slope
+1
+1
+2
WT
Haploid
**
**
Intensity (A.U.) / Time (min)
150
100
50
0
NC11
NC12
NC13
NC14
NC15

Fig S3. *ftz* transcription is mediated mainly by cell cycle duration
(A) Heat map showing that total RNA output is greater in representative WT embryos expressing *ftz-MS2*, in NC11-NC14, than haploid (*ssm*). Haploids catch up as the cell cycle slows in NC15. Color bar represents total cumulative output per nucleus per NC (A.U.). The cartoon shows an endogenous *ftz* pattern at NC14 and the dashed box indicates the area under analysis. Scale bar represents 10 μm.

(B) Average *ftz-MS2* transcriptional trajectory over time for all transcribing nuclei per nuclear cycle. Gray boxes represent haploid mitoses.

(C) Bar chart showing that the number of nuclei transcribing *ftz-MS2* in a given nuclear cycle is similar between WT (blue) and haploid (yellow) embryos. Gray bars represent the total number of nuclei analyzed in each cycle and colored bars represent the number of active nuclei. 96 NC11, 196 NC12, 420 NC13, and 761 NC14 nuclei from 3 replicate *ftz-MS2* WT, and 79 NC11, 213 NC12, 404 NC13, 762 NC14, and 1523 NC15 from 3 replicate *ftz-MS2* haploid embryos were analyzed.

Data in (B) and (C) are represented as mean ± SEM of 3 replicate WT and 3 haploid, biologically replicate *ftz-MS2* embryos.

(D) Boxplots showing comparable rates of transcriptional activation of *ftz-MS2* for all actively transcribing nuclei for WT and haploid (*ssm*) embryos.

(E) Boxplots showing similar median transcriptional activity of *ftz-MS2* from all transcribing nuclei.

Individual data points (up to 200 points) in each nuclear cycle are overlaid on the respective boxplots. Dashed line represents a cut-off for outlier values. Number of outlier values above the cut-off are given after '+'. ** indicates $p < 0.005$. The number of analyzed nuclei in (B, D, E) is the same as in Figure S1 for *ftz-MS2.*

S4

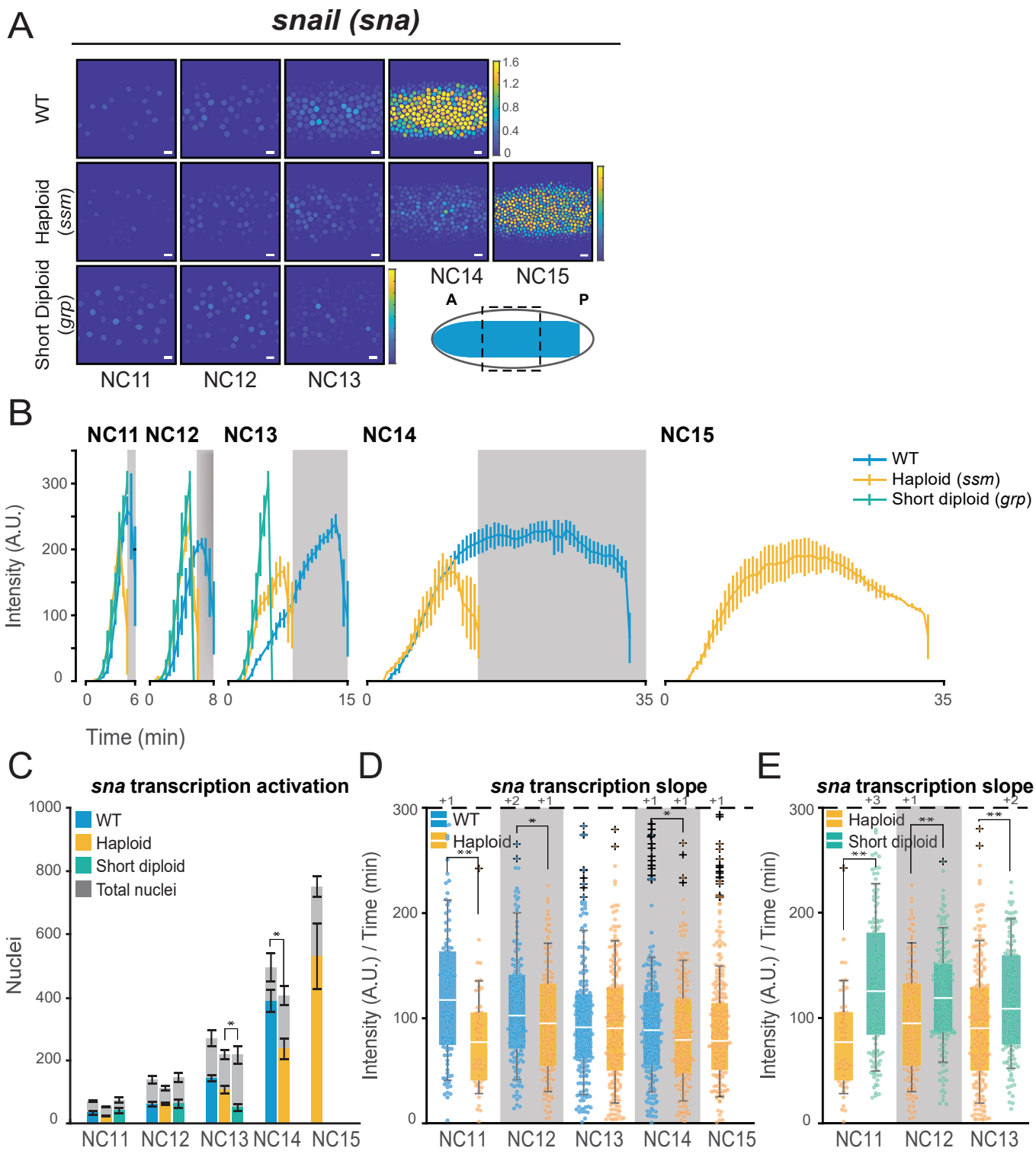

A
snail (sna)
WT
Haploid (ssm)
Short Diploid (grp)
1.6
1.2
0.8
0.4
0
NC11
NC12
NC13
NC14
NC15
A
P
B
NC11 NC12
NC13
NC14
NC15
Intensity (A.U.)
300
200
100
0
0
6
0
8
0
15
0
35
0
35
Time (min)
WT
Haploid (ssm)
Short diploid (grp)
C
sna transcription activation
Nuclei
1000
800
600
400
200
0
WT
Haploid
Short diploid
Total nuclei
NC11
NC12
NC13
NC14
NC15
D
sna transcription slope
Intensity (A.U.) / Time (min)
300
200
100
0
WT
Haploid
+1
+2
+1
+1
+1
+1
NC11
NC12
NC13
NC14
NC15
E
sna transcription slope
Intensity (A.U.) / Time (min)
300
200
100
0
Haploid
Short diploid
+3
+1
+2
NC11
NC12
NC13

Fig S4. *sna* transcription is largely mediated by cell cycle duration
(A) Heat map showing that total RNA output is greater in representative WT embryos expressing *sna_distalE>MS2*, in NC11-NC14, than haploid (*ssm*) or short-cycle diploid embryos (*grp*). The cartoon shows ah endogenous *sna* pattern at NC14 and the dotted box indicates the area under analysis. Scale bar represents 10 μm.

(B) Average *sna_distalE>MS2* transcriptional trajectory over time for all transcribing nuclei per nuclear cycle. Gray boxes represent haploid mitoses.

(C) Bar chart showing that the number of nuclei transcribing *sna_distalE>MS2* in a given nuclear cycle is similar between WT (blue), haploid (yellow), and short-cycle diploid (green) embryos. Gray bars represent the total number of nuclei analyzed in each cycle and colored bars represent the number of active nuclei. 132 NC11, 243 NC12, 573 NC13, and 1550 NC14 from 4 replicate *sna_distalE>MS2* WT embryos, 68 NC11, 187 NC12, 321 NC13, 711 NC14, and 1590 NC15 from 3 replicate *sna_distalE>MS2* haploid embryos, and 165 NC11, 251 NC12, and 201 NC13 from 4 replicate *sna_distalE>MS2* short-cycle diploid embryos were analyzed.

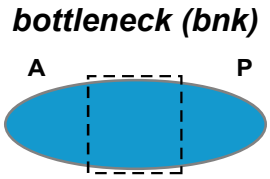
Data in (B) and (C) are represented as mean ± SEM of 4 WT, 3 haploid (*ssm*), and 4 short-cycle diploid (*grp*) biologically replicate *sna_distalE>MS2* embryos.

(D-E) Boxplots showing the initial slope of transcriptional activation of *sna_distalE>MS2* for all actively transcribing nuclei for (D) WT vs. haploid (*ssm*), and (E) haploid vs. short-cycle diploid (*grp*) embryos. The initial slope is lower in haploids compared to WT and short-cycle diploids.

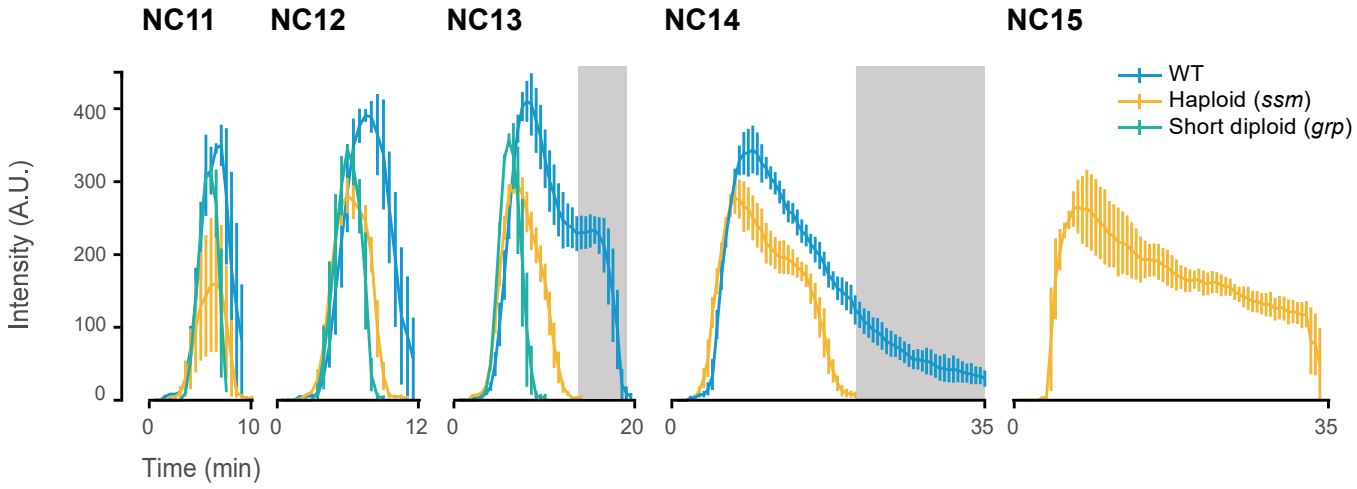
Individual data points (up to 200 points) in each nuclear cycle are overlaid on the respective boxplots. Dashed line represents a cut-off for outlier values. Number of outlier values above the cut-off are given after '+'. * $p<0.05$, ** $p<0.005$ from Student's t-test. The number of analyzed nuclei in (B, D, E) is the same as in Figure S1 for *sna_distalE >MS2.*

S5

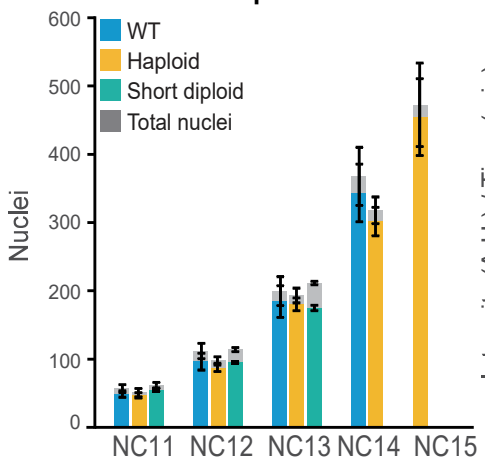
A


bottleneck (bnk)
A
P

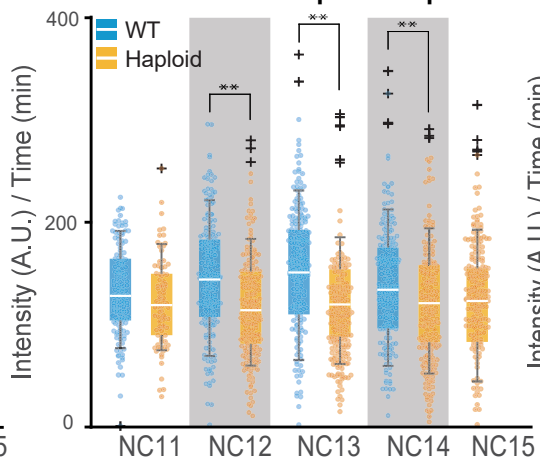
B


NC11
NC12
NC13
NC14
NC15
WT
Haploid (ssm)
Short diploid (grp)
Intensity (A.U.)
400
300
200
100
0
0
10
0
12
0
20
0
35
0
35
Time (min)

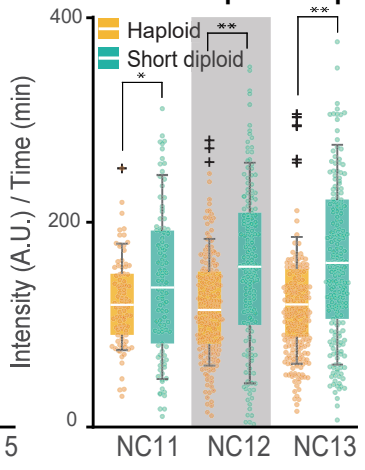
C


bnk transcription activation
WT
Haploid
Short diploid
Total nuclei
Nuclei
600
500
400
300
200
100
0
NC11
NC12
NC13
NC14
NC15

D


bnk transcription slope
WT
Haploid
Intensity (A.U.) / Time (min)
400
200
0
**
**
**
NC11
NC12
NC13
NC14
NC15

E


bnk transcription slope
Haploid
Short diploid
Intensity (A.U.) / Time (min)
400
200
0
*
**
**
NC11
NC12
NC13

FigS5. *bnk* responds to N/C ratio in a kinetic-dependent manner
(A) A cartoon that shows endogenous *bnk* pattern at NC14 and dotted box indicates the area under analysis.

(B) Average *bnk>MS2* transcriptional trajectory over time for all transcribing nuclei per nuclear cycle. Gray boxes represent haploid mitoses.

(C) Bar chart showing that the number of nuclei transcribing *bnk>MS2* in a given nuclear cycle is similar between WT (blue), haploid (yellow), and short-cycle diploid (green) embryos. Gray bars represent the total number of nuclei analyzed in each cycle and colored bars represent the number of active nuclei. 147 NC11, 289 NC12, 553 NC13, and 1030 NC14 nuclei from 3 replicate *bnk>MS2* WT embryos, 94 NC11, 347 NC12, 721 NC13, 1206 NC14, and 1363 NC15 nuclei from 4 replicate *bnk>MS2* haploid embryos, and 110 NC11, 190 NC12, and 350 NC13 nuclei from 2 replicate *bnk>MS2* short-cycle diploid embryos were analyzed.

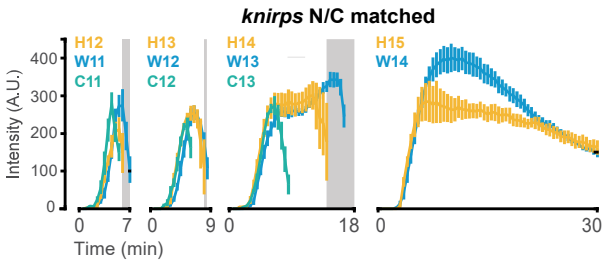
Data in (B) and (C) are represented as mean ± SEM of 3 WT, 4 haploid (*ssm*), and 2 short-cycle diploid (*grp*) biologically replicate *bnk>MS2* embryos.

(D-E) Boxplots showing the initial slope of transcriptional activation of *bnk>MS2* for all actively transcribing nuclei for (D) WT vs. haploid (*ssm*), and (E) haploid vs. short-cycle diploid (*grp*) embryos. The initial slope is lower in haploids compared to WT and short-cycle diploid embryos.

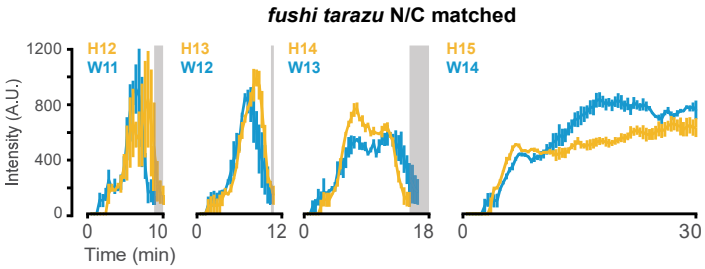
Individual data points (up to 200 points) in each nuclear cycle are overlaid on the respective boxplots. Dashed line represents a cut-off for outlier values. Number of outlier values above the cut-off are given after '+'. * $p<0.05$, ** $p<0.005$ from Student's t-test. The number of analyzed nuclei in (B, D, E) is the same as in Figure S1 for *bnk>MS2*.

S6

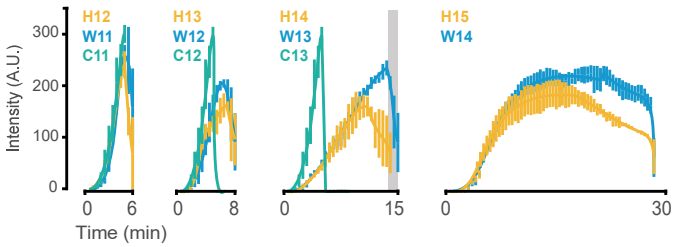
A


knirps N/C matched
H12
W11
C11
H13
W12
C12
H14
W13
C13
H15
W14
Intensity (A.U.)
Time (min)

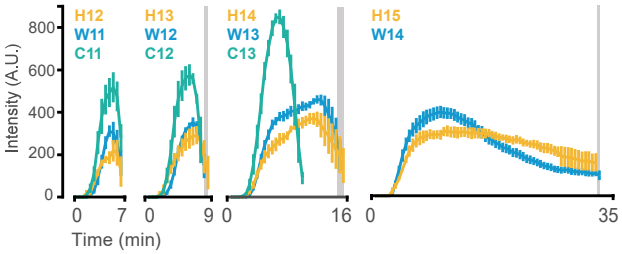
B


fushi tarazu N/C matched
H12
W11
H13
W12
H14
W13
H15
W14
Intensity (A.U.)
Time (min)

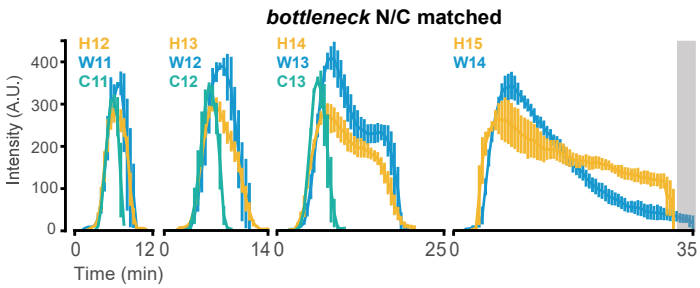
C


snail N/C matched
H12
W11
C11
H13
W12
C12
H14
W13
C13
H15
W14
Intensity (A.U.)
Time (min)

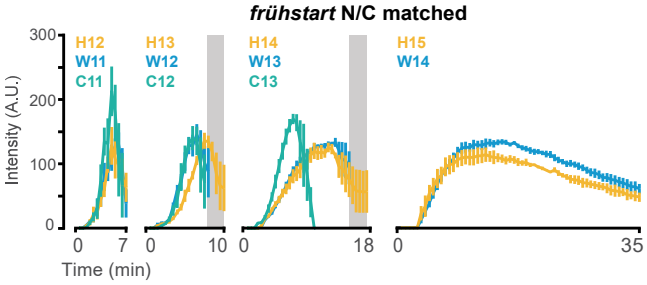
D


giant N/C matched
H12
W11
C11
H13
W12
C12
H14
W13
C13
H15
W14
Intensity (A.U.)
Time (min)

E


bottleneck N/C matched
H12
W11
C11
H13
W12
C12
H14
W13
C13
H15
W14
Intensity (A.U.)
Time (min)

F


frühstart N/C matched
H12
W11
C11
H13
W12
C12
H14
W13
C13
H15
W14
Intensity (A.U.)
Time (min)

G

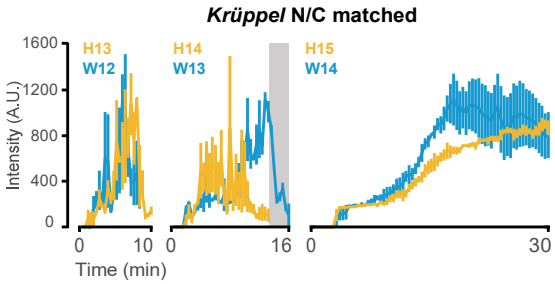

Krüppel N/C matched
H13
W12
H14
W13
H15
W14
Intensity (A.U.)
Time (min)

Fig S6. N/C ratio-matched trajectories
Average transcriptional trajectory over time for all transcribing nuclei aligned by the N/C ratio for (A) *kni*(5'+int)>*MS2,* (B) *ftz-MS2,* (C) *sna_distalE >MS2*, (D) *gt>MS2,* (E) *bnk>MS2*, (F) *frs-MS2,* and (G) *KrCD2>MS2*. Data represented as mean ± SEM of biologically replicate embryos (number of embryos is shown in Figure S1). Gray boxes represent haploid mitoses.

The number of nuclei analyzed in Figure S6 is the same as in Figure S1.

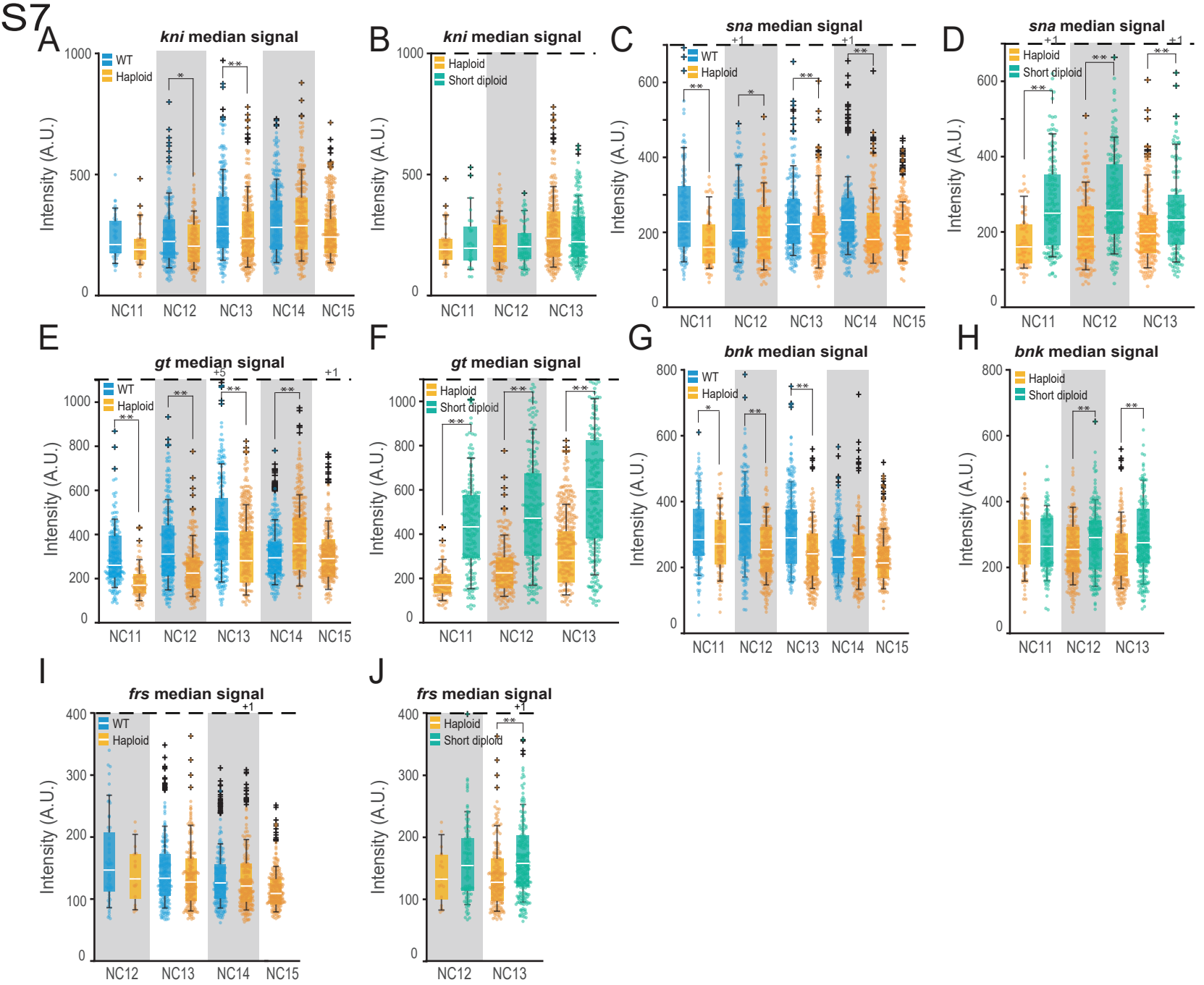

S7
A
kni median signal
WT
Haploid
Intensity (A.U.)
NC11
NC12
NC13
NC14
NC15
B
kni median signal
Haploid
Short diploid
C
sna median signal
D
sna median signal
E
gt median signal
F
gt median signal
G
bnk median signal
H
bnk median signal
I
frs median signal
J
frs median signal

Fig S7. Median transcriptional activities
Boxplots showing median transcriptional activity from all transcribing nuclei.
(A-B) Comparable levels of median signal for *kni*(5'+int)>*MS2* between (A) WT vs. haploid (*ssm*) and (B) haploid vs. short-cycle diploid (*grp*) embryos.

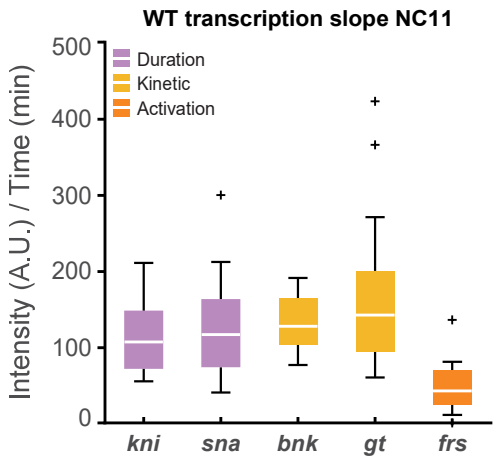
(C-H) Reduced median amplitude in haploid (*ssm*) embryos compared to WT and short-cycle diploid (*grp*) embryos for (C,D) *sna_distalE >MS2* (E,F) *gt>MS2* and (G,H) *bnk>MS2*.

(I-J) Similar median transcriptional activity for *frs-MS2* between (I) WT vs. haploid (*ssm*) and (J) haploid vs. short-cycle diploid (*grp*) embryos.

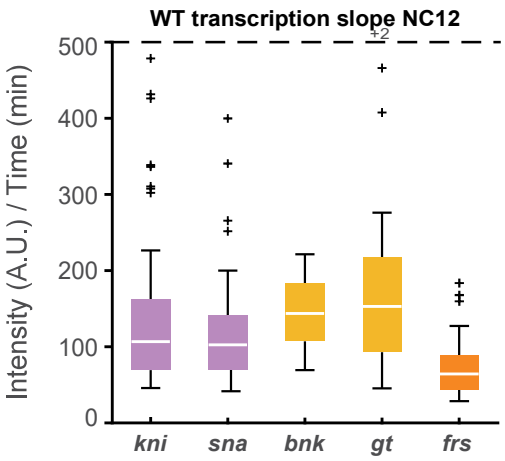
Individual data points (up to 200 points) in each nuclear cycle are overlaid on the respective boxplots. Dashed line represents a cut-off for outlier values. Number of outlier values above the cut-off are given after '+'. * $p<0.05$, ** $p<0.005$ from Student's t-test. The number of nuclei analyzed in Figure S7 is the same as in Figure S1.

S8

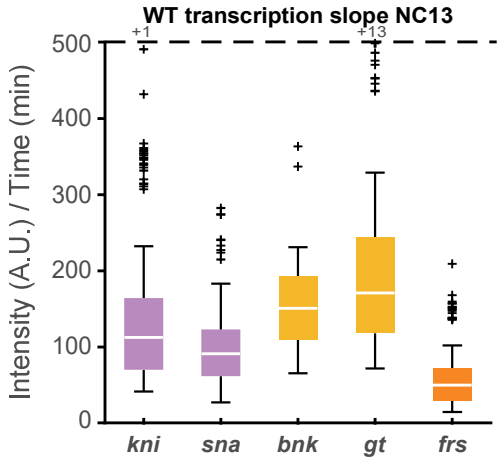
A


WT transcription slope NC11
Duration
Kinetic
Activation
Intensity (A.U.) / Time (min)
0
100
200
300
400
500
kni
sna
bnk
gt
frs

B


WT transcription slope NC12
+2
Intensity (A.U.) / Time (min)
0
100
200
300
400
500
kni
sna
bnk
gt
frs

C


WT transcription slope NC13
+1
+13
Intensity (A.U.) / Time (min)
0
100
200
300
400
500
kni
sna
bnk
gt
frs

D

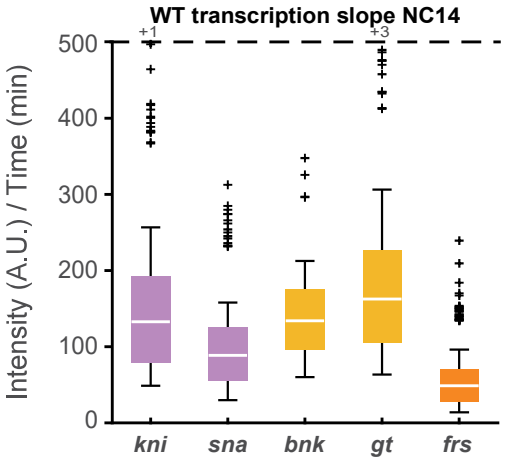

WT transcription slope NC14
+1
+3
Intensity (A.U.) / Time (min)
0
100
200
300
400
500
kni
sna
bnk
gt
frs

Fig S8. N/C-kinetic mediated genes display higher transcription slopes
Boxplots showing the initial slopes of transcriptional activation of *kni*(5'+int)*>MS2, sna_distalE>MS2, bnk>MS2, gt>MS2, and frs-MS2* for all actively transcribing nuclei in (A) NC11, (B) NC12, (C) NC13, and (D) NC14. The genes are characterized based on the three categories: N/C independent, N/C kinetic-mediated, and N/C activation-mediated. Dashed line represents a cut-off for outlier values. Number of outlier values above the cut-off are given after '+'.
The number of nuclei analyzed in (A-D) is the same as in Figure S1.

Supplemental Movie Legends

Movie S1. Live imaging of *kni*(5'+int)*>MS2*

(left) WT, NC11-NC14; (middle) haploid, NC11-NC15; (right) short-cycle diploid NC11-NC13. *MS2* signal is shown in green. Nuclei are marked with His2Av-mRFP. Histogram was adjusted for visualization purposes. Embryos are oriented top-anterior, bottom-posterior.

Movie S2. Live imaging of *gt>MS2*

(left) WT, NC11-NC14; (middle) haploid, NC11-NC15; (right) short-cycle diploid NC11-NC13. *MS2* signal is shown in green. Nuclei are marked with His2Av-mRFP. Histogram was adjusted for visualization purposes. Embryos are oriented top-posterior, bottom-anterior.

Movie S3. Live imaging of *frs-MS2*

(left) WT, NC11-NC14; (middle) haploid, NC11-NC15; (right) short-cycle diploid NC11-NC13. *MS2* signal is shown in green. Nuclei are marked with His2Av-mRFP. Histogram was adjusted for visualization purposes.

SI References

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2. N. J. V. Djabrayan, *et al.*, Metabolic Regulation of Developmental Cell Cycles and Zygotic Transcription. *Curr. Biol.* **29**, 1193-1198.e5 (2019).
3. B. Lim, T. Fukaya, T. Heist, M. Levine, Temporal dynamics of pair-rule stripes in living Drosophila embryos. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 8376–8381 (2018).
4. S. J. Gratz, *et al.*, Highly Specific and Efficient CRISPR/Cas9-Catalyzed Homology-Directed Repair in Drosophila. *Genetics* **196**, 961–971 (2014).