Figure S1

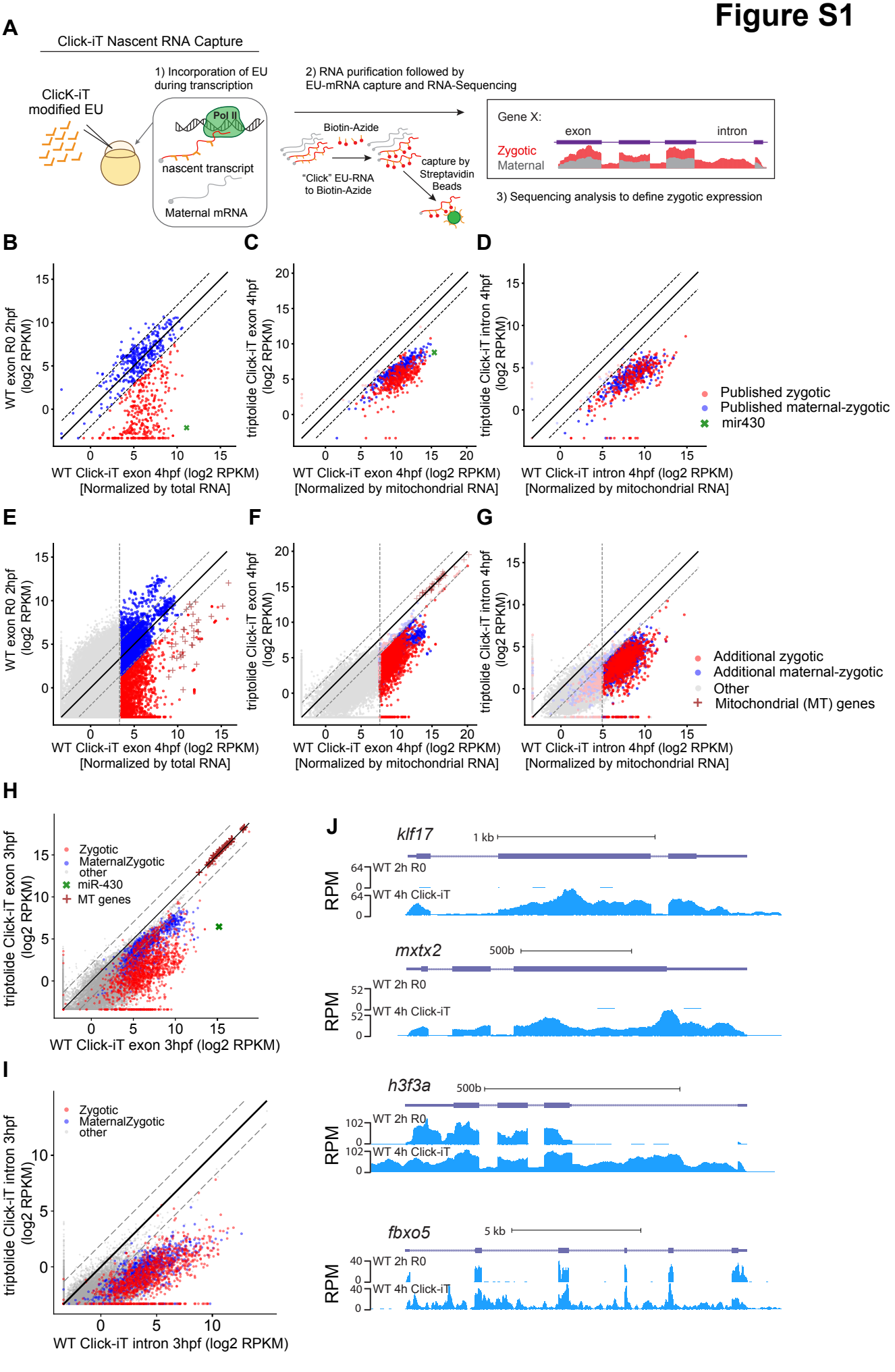

A
Click-iT Nascent RNA Capture
ClicK-iT modified EU
1) Incorporation of EU during transcription
Pol II
nascent transcript
Maternal mRNA
2) RNA purification followed by EU-mRNA capture and RNA-Sequencing
Biotin-Azide
"Click" EU-RNA to Biotin-Azide
capture by Streptavidin Beads
Gene X:
exon
intron
Zygotic
Maternal
3) Sequencing analysis to define zygotic expression
B
C
D
WT exon R0 2hpf (log2 RPKM)
triptolide Click-iT exon 4hpf (log2 RPKM)
triptolide Click-iT intron 4hpf (log2 RPKM)
WT Click-iT exon 4hpf (log2 RPKM) [Normalized by total RNA]
WT Click-iT exon 4hpf (log2 RPKM) [Normalized by mitochondrial RNA]
WT Click-iT intron 4hpf (log2 RPKM) [Normalized by mitochondrial RNA]
Published zygotic
Published maternal-zygotic
mir430
E
F
G
Additional zygotic
Additional maternal-zygotic
Other
Mitochondrial (MT) genes
H
triptolide Click-iT exon 3hpf (log2 RPKM)
WT Click-iT exon 3hpf (log2 RPKM)
Zygotic
MaternalZygotic
other
miR-430
MT genes
I
triptolide Click-iT intron 3hpf (log2 RPKM)
WT Click-iT intron 3hpf (log2 RPKM)
J
klf17
1 kb
mxtx2
500b
h3f3a
500b
fbxo5
5 kb
RPM
WT 2h R0
WT 4h Click-iT

Figure S1. Identifying de novo zygotic transcription by Click-iT-seq; related to Figure 1 and STAR Methods.

(A) Schematic illustrating the workflow of Click-iT pulldown assay to capture nascent RNA during ZGA for RNA-sequencing (Click-iT-seq). **(B)** Biplot comparing exon expression levels of zygotic and maternal-zygotic genes defined in published studies (Materials and Methods) from Click-iT-seq experiment at 4hpf and mRNA-seq experiment at 2hpf in wild-type embryos. **(C-D)** Biplot comparing expression levels of exons (C) and introns (D) of zygotic and maternal-zygotic genes defined in published studies (Materials and Methods) in wild-type (WT) and triptolide treated embryos (triptolide) from Click-iT-seq experiment at 4hpf. **(E)** Biplot comparing exon expression levels of additional zygotic and maternal-zygotic genes (Materials and Methods) between Click-iT-seq experiment at 4hpf and mRNA-seq experiment at 2hpf in wild-type embryos. **(F-G)** Biplot comparing expression levels of exons (F) and introns (G) of additional zygotic and maternal-zygotic genes (Materials and Methods) between wild-type (WT) and triptolide treated embryos (triptolide) from Click-iT-seq experiment at 4hpf. **(H-I)** Biplot comparing expression levels of exons (H) and introns (I) of genes between wild-type (WT) and triptolide treated embryos (triptolide) from Click-iT-seq experiment at 3hpf. **(J)** Genome tracks representing normalized mRNA-seq and Click-iT-seq signal measured at 2hpf and 4hpf in wild-type embryos for 2 examples of zygotic genes (*klf17* and *mxtx2*) and 2 examples of maternal-zygotic genes (*h3f3a* and *fbxo5*). RPM = Reads per million mapped reads on mitochondrial protein coding genes. In (B-I), dashed lines represent 4-fold change. Here, 4-fold change in (B) and (E) serves as a criteria separating zygotic and maternal-zygotic genes, and 4-fold change in (C), (D), (F), and (G) serves as criteria for defining zygotically expressed genes (Materials and Methods). In (B) and (E), the RPKM from the Click-iT-seq experiment is derived from total read number mapped to the exons on the gene normalized by the total exonic reads in the same sample. In (C), (D), (F), and (G), the RPKM from the Click-iT-seq experiment is derived from total number of reads mapped to the gene normalized by the total number of reads mapped to mitochondrial protein-coding genes in the same sample.

Figure S2

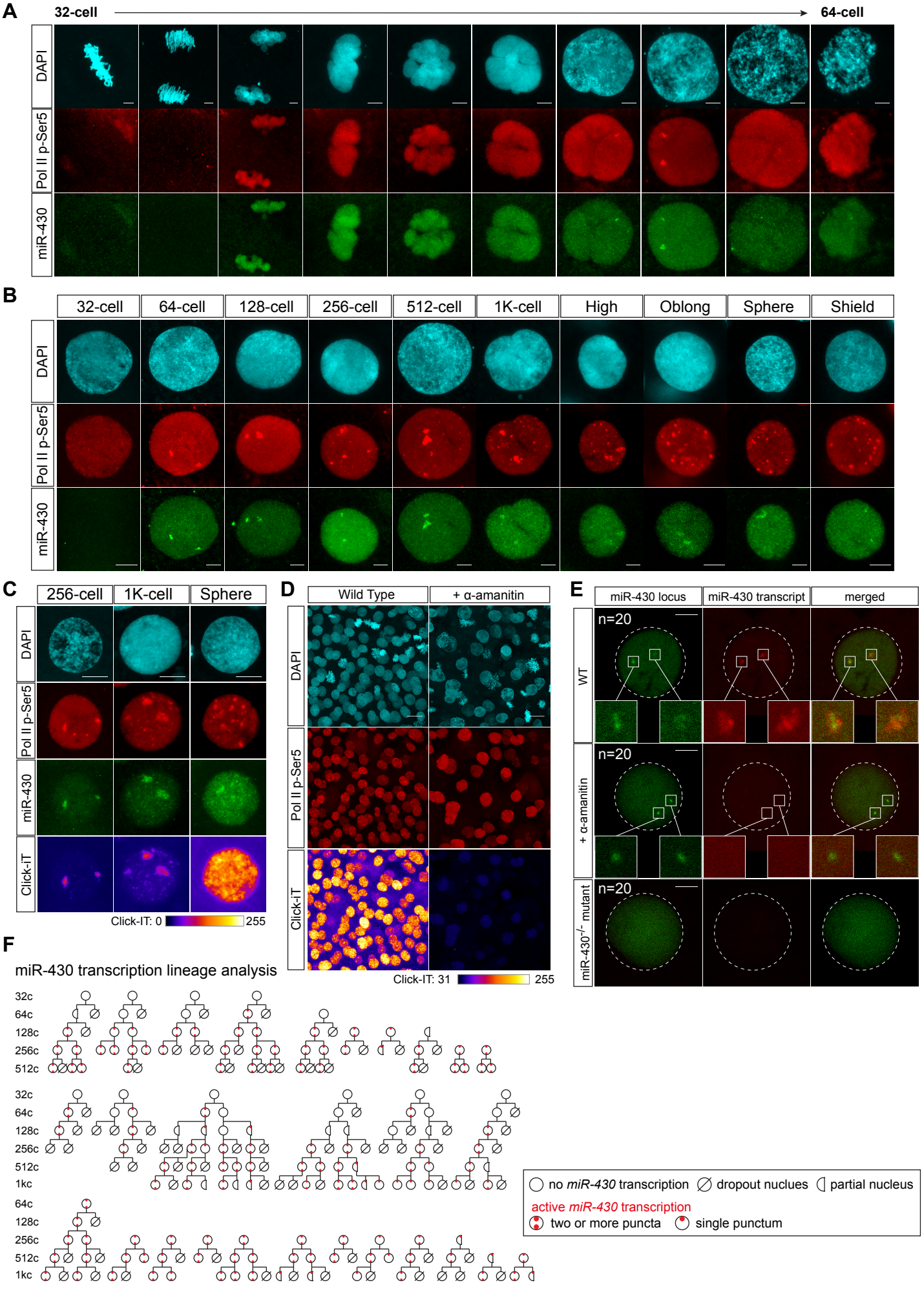

A
32-cell
64-cell
DAPI
Pol II p-Ser5
miR-430
B
32-cell
64-cell
128-cell
256-cell
512-cell
1K-cell
High
Oblong
Sphere
Shield
C
256-cell
1K-cell
Sphere
Click-iT
Click-IT: 0
255
D
Wild Type
+ α-amanitin
Click-IT: 31
255
E
miR-430 locus
miR-430 transcript
merged
WT
+ α-amanitin
miR-430−/− mutant
n=20
F
miR-430 transcription lineage analysis
32c
64c
128c
256c
512c
1kc
no miR-430 transcription
dropout nuclues
partial nucleus
active miR-430 transcription
two or more puncta
single punctum

Figure S2. Temporal analysis of zygotic transcription by single nucleus imaging and Cell-lineage tracing for miR-430 transcription with molecular beacon by live imaging; related to Figure 1.

(A) Time-resolved confocal images labeled for DAPI, Pol II p-Ser5, miR-430 loci. Displayed nuclei are collected from embryos between 32- and 64-cell stages that are fixed in 2 minutes intervals throughout the cell cycle. Scale bar represents 5 μm. Embryos with different temporal stages within the cell cycle was organized in a presumptive chronological order based on DAPI staining to illustrate the subset of nuclei stage when Pol II p-Ser5 can be detected throughout the rapid cell cycle. Note that Pol II p-Ser5 signal emerges as bright foci in late interphase and early prophase. **(B)** Time-course analysis of single nuclei labeled for Pol II p-Ser5 and dCas9 targeting the miR-430 locus from 32-cells to shield stage. Scale bar represents 5 μm. Note that the first detectable Pol II p-Ser5 foci signal co-localizes with miR-430 loci at 64-cell stage. As transcription is globally activated, multiple Pol II p-Ser5 foci appear across the nucleus. DAPI staining is used to select temporally comparable stages of nucleus within each cell cycle as rationalized above. **(C)** Representative single nucleus confocal images labeled with DAPI, Pol II p-Ser5, miR-430 loci and Click-iT at 256-, 1K-cell and Sphere stage. Note the co-localization of miR-430 loci with Pol II p-Ser5 signal as well as Click-iT signal. Scale bar represents 5 μm. Click-iT signal intensity is presented in a heatmap color scale**. (D)** Confocal images labeled with DAPI, Pol II p-Ser5 and Click-iT at Sphere stage embryos at a zoom-out field of view. Click-iT signal intensity is presented in a heatmap color scale**.** Scale bar, 15 μm. **(E)** Confocal imaging of wild-type (WT), α-amanitin treated (+α-amanitin), and miR-430$^{-/-}$ mutant embryos between 512- and 1K-cell stage labeled with MB^{miR430} (red) and dCas9-miR-430 (green). Scale bar represents 5 μm. Note the absence of miR-430 transcription in miR-430$^{-/-}$ deletion mutants and after Pol II inhibition. Also note the co-localization of MB^{miR430} signal with dCas9-miR-430 signal in wild-type embryos from the merged channel confocal image. (n=number of nuclei from three independent embryos examined using confocal microscopy) **(F)** Cell-lineage tracing for miR-430 transcription. Nuclei from two independent developing live zebrafish embryos are imaged. Transcription competency is acquired in a stochastic manner at 64c, and once gained is maintained in the daughter cells after cell division. Transcription of miR-430 is scored with the detection of puncta signal from the molecular beacon (MB^{miR430}). Nuclei dropped

out from the field of view during live imaging were noted as either partially visualized (partial nucleus) or untraceable (dropout nucleus).

Figure S3

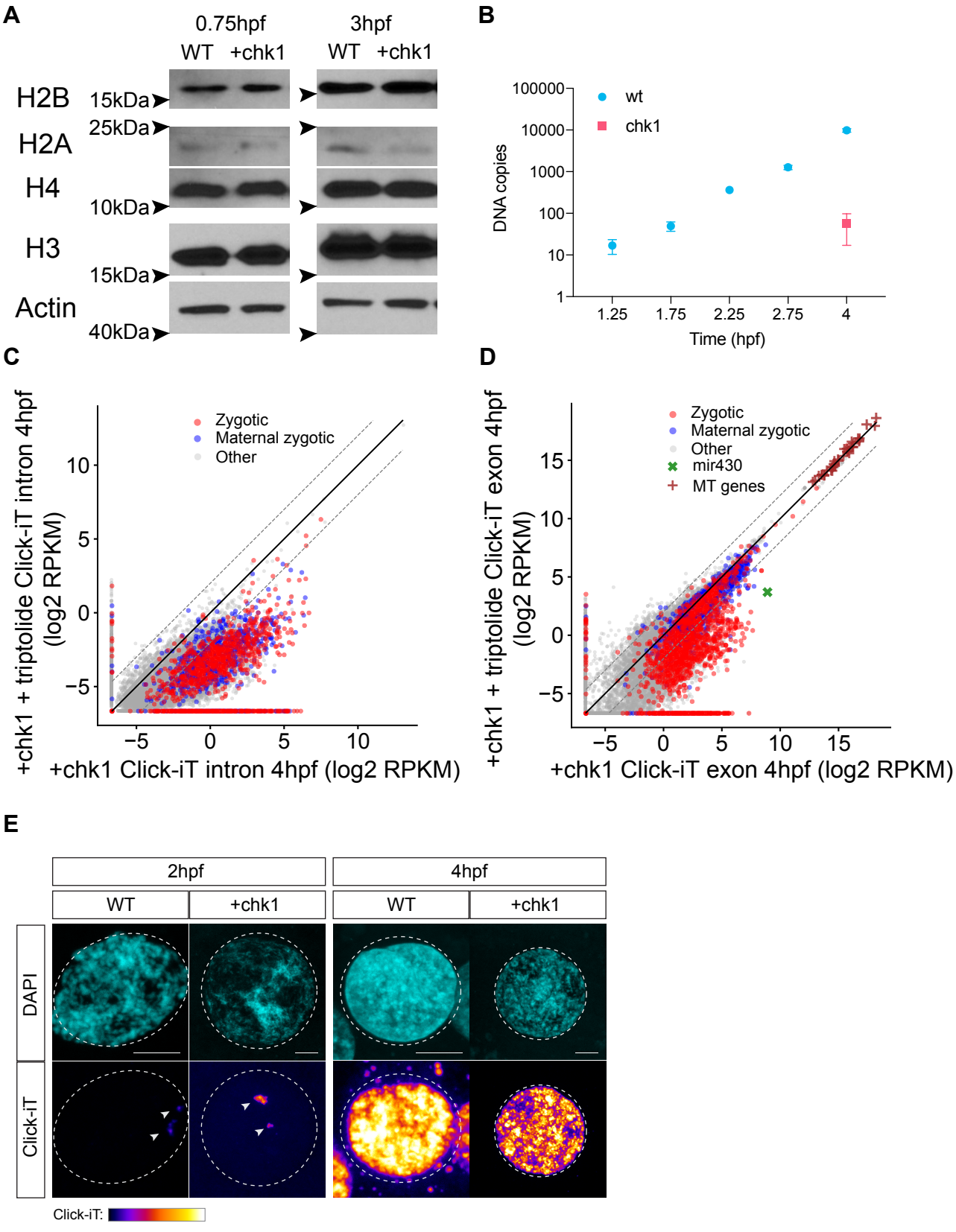
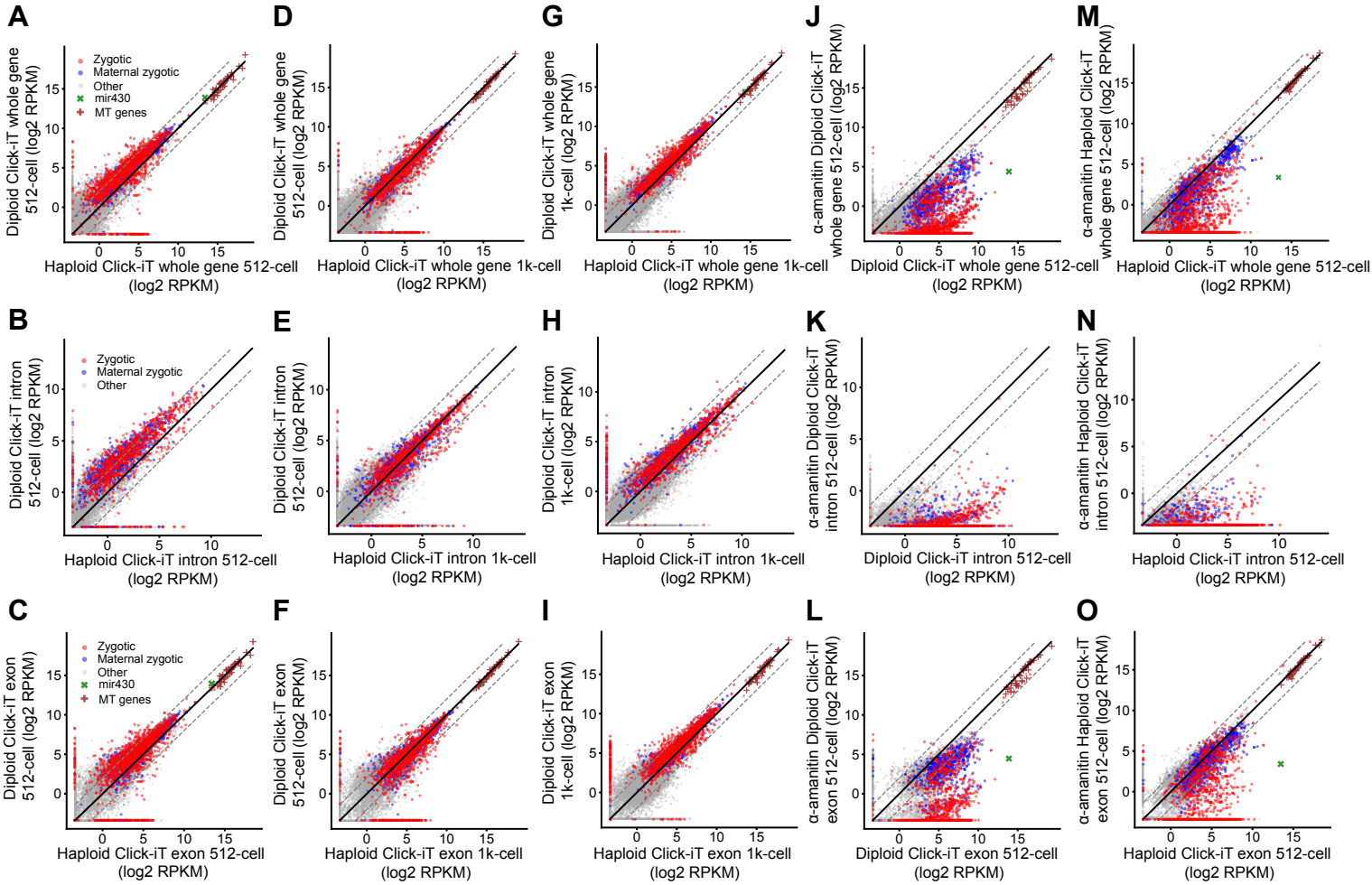

A
0.75hpf
3hpf
WT
+chk1
WT
+chk1
H2B
15kDa
H2A
25kDa
H4
10kDa
H3
15kDa
Actin
40kDa
B
wt
chk1
DNA copies
Time (hpf)
C
Zygotic
Maternal zygotic
Other
+chk1 + triptolide Click-iT intron 4hpf (log2 RPKM)
+chk1 Click-iT intron 4hpf (log2 RPKM)
D
Zygotic
Maternal zygotic
Other
mir430
MT genes
+chk1 + triptolide Click-iT exon 4hpf (log2 RPKM)
+chk1 Click-iT exon 4hpf (log2 RPKM)
E
2hpf
4hpf
WT
+chk1
WT
+chk1
DAPI
Click-iT
Click-iT:

Figure S3. Zygotic genome activation occurs in chk1-injected embryos despite their lower N/C ratio; related to Figure 2.

(A) Western blot of lysate from wild-type (WT) and chk1-injected (+chk1) zebrafish embryos collected at 0.75hpf and 3hpf respectively. Note the comparable histone (H2B, H2A, H3, H4) protein levels between the time-matched wild-type and chk1-injected embryos. Beta-Actin was used as loading control. The molecular weight size of the closet Protein Ladder bands are highlighted respectively by black arrows. **(B)** Quantification of DNA copies by qPCR analysis of miR-430 locus in 8-cell (1.25hpf), 32-cell (1.75hpf), 128-cell (2.25hpf), 512-cell (2.75hpf) and sphere stage (4hpf) wild-type embryos v.s. chk1-injected embryos collected at 4hpf. Note that the number of DNA templates for miR-430 in chk1-injected embryos at 4 hpf resembles that from embryos between 8- and 32-cell stages. 4 replicates were analyzed. **(C-D)** Biplot comparing intron (C) and exon (D) expression levels of genes in chk1-injected embryos with (+chk1+triptolide) and without triptolide (+chk1). Note that zygotic genome activation occurs in chk1-injected embryos at 4hpf. Dashed lines represent 4-fold change. **(E)** Single nucleus confocal image of DAPI and Click-iT in wild-type (WT) and chk1-injected (+chk1) embryo at 2hpf and 4hpf. Click-iT signals are highlighted by white arrows in the confocal images for 2hpf embryos. Click-iT signal intensity is presented in a heatmap color scale (2hpf: 0-255; 4hpf: 20-255). Scale bar represents 5 μm. Note the increase in Click-iT signal intensity in chk1-injected (+chk1) embryos than wild-type (WT) embryos at 2hpf. Genome activation takes place in a time-dependent manner at 4hpf despite the lengthening of cell cycle during early development.

Figure S4


A
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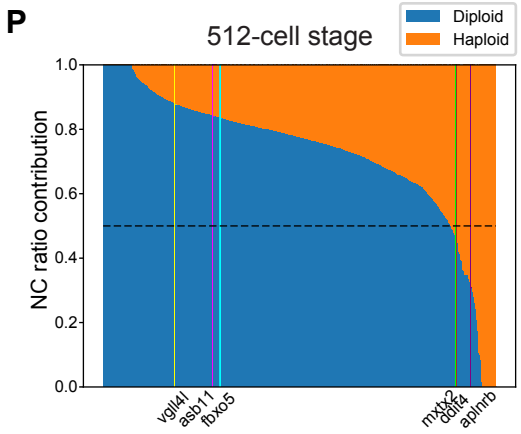
I
J
K
L
M
N
O
Zygotic
Maternal zygotic
Other
mir430
MT genes
Diploid Click-iT whole gene 512-cell (log2 RPKM)
Haploid Click-iT whole gene 512-cell (log2 RPKM)
Diploid Click-iT whole gene 1k-cell (log2 RPKM)
Haploid Click-iT whole gene 1k-cell (log2 RPKM)
α-amanitin Diploid Click-iT whole gene 512-cell (log2 RPKM)
Diploid Click-iT whole gene 512-cell (log2 RPKM)
α-amanitin Haploid Click-iT whole gene 512-cell (log2 RPKM)
Haploid Click-iT whole gene 512-cell (log2 RPKM)
Diploid Click-iT intron 512-cell (log2 RPKM)
Haploid Click-iT intron 512-cell (log2 RPKM)
Diploid Click-iT intron 512-cell (log2 RPKM)
Haploid Click-iT intron 1k-cell (log2 RPKM)
Diploid Click-iT intron 1k-cell (log2 RPKM)
Haploid Click-iT intron 1k-cell (log2 RPKM)
α-amanitin Diploid Click-iT intron 512-cell (log2 RPKM)
Diploid Click-iT intron 512-cell (log2 RPKM)
α-amanitin Haploid Click-iT intron 512-cell (log2 RPKM)
Haploid Click-iT intron 512-cell (log2 RPKM)
Diploid Click-iT exon 512-cell (log2 RPKM)
Haploid Click-iT exon 512-cell (log2 RPKM)
Diploid Click-iT exon 1k-cell (log2 RPKM)
Haploid Click-iT exon 1k-cell (log2 RPKM)
α-amanitin Diploid Click-iT exon 512-cell (log2 RPKM)
Diploid Click-iT exon 512-cell (log2 RPKM)
α-amanitin Haploid Click-iT exon 512-cell (log2 RPKM)
Haploid Click-iT exon 512-cell (log2 RPKM)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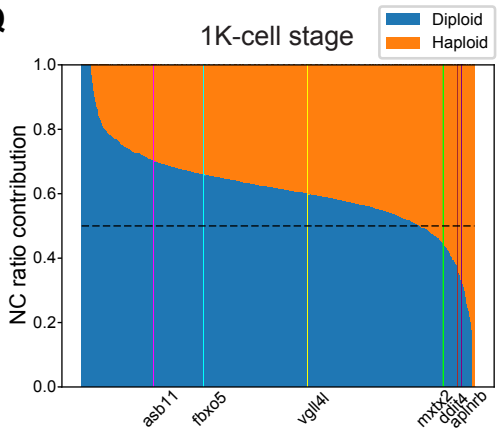
512-cell stage
Diploid
Haploid
NC ratio contribution
vgll4l
asb11
fbxo5
mxtx2
ddit4
aplnrb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1K-cell stage
Diploid
Haploid
NC ratio contribution
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fbxo5
vgll4l
mxtx2
ddit4
aplnrb

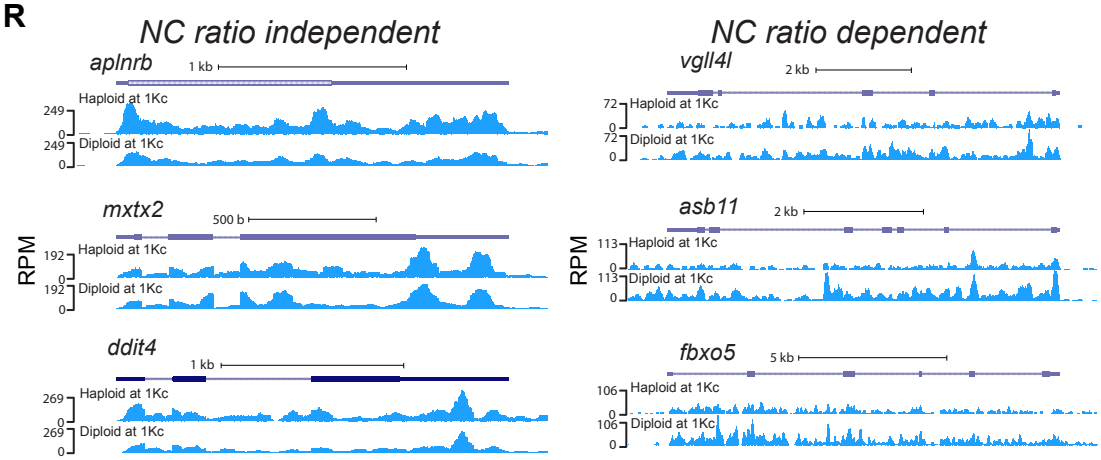
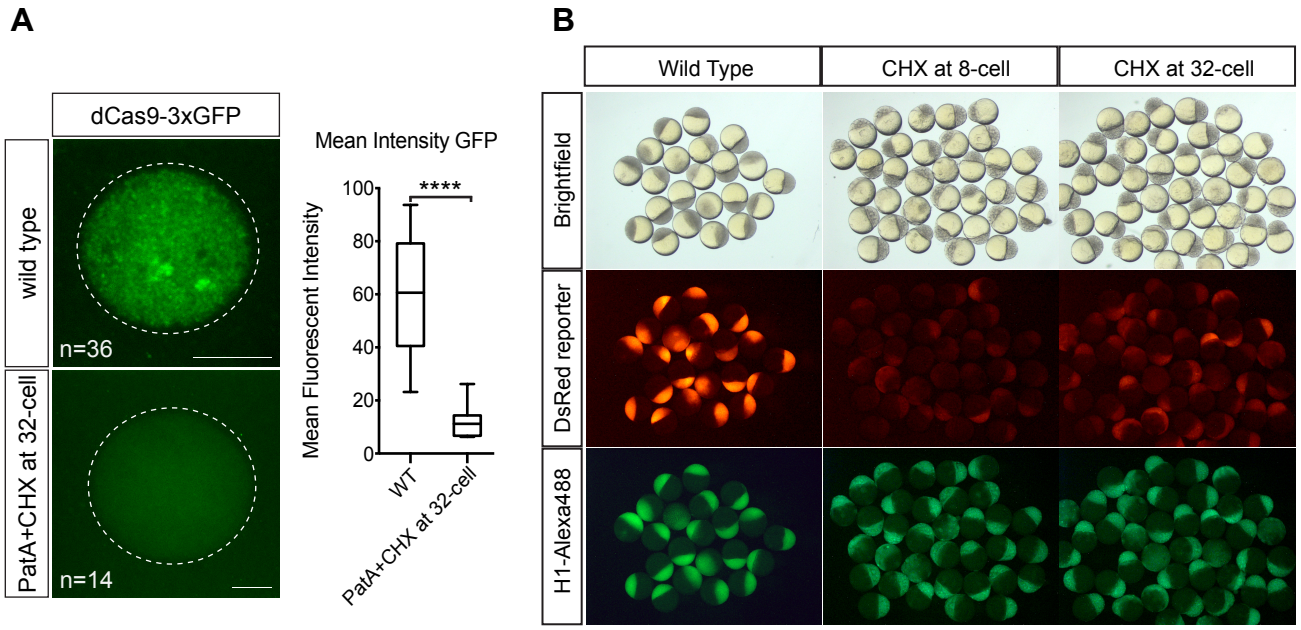

R
NC ratio independent
NC ratio dependent
aplnrb
vgll4l
mxtx2
asb11
ddit4
fbxo5
Haploid at 1Kc
Diploid at 1Kc
RPM

Figure S4. Transcription levels are modulated by the N/C ratio; related to Figure 3.

(A-C) Biplot comparing whole gene (A), intron (B) and exon (C) expression levels of genes measured by Click-iT-seq in haploid and diploid embryos at 512-cell stage. Dashed lines represent 4-fold change. **(D-F)** Biplot comparing whole gene (D) and intron (E) and exon (F) expression levels of genes measured by Click-iT-seq in haploid embryos at 1K-cell stage with diploid embryos at 512-cell stage. Despite less DNA template for transcription in diploid embryos at 512-cell stage (as half of the diploid embryos are treated with α-amanitin) compared to haploid embryos at 1K-cell stage, most of the genes have similar expression level measured by Click-iT-seq in these two types of embryos, suggesting they are N/C ratio dependent. **(G-I)** Biplot comparing whole gene (G) and intron (H) and exon (I) expression levels of genes measured by Click-iT-seq in haploid and diploid embryos at 1K-cell stage. **(J-L)** Biplot comparing whole gene (J) and intron (K) and exon (L) expression levels of genes measured by Click-iT-seq in diploid 512-cell stage embryos treated with and without α-amanitin. **(M-O)** Biplot comparing whole gene (M) and intron (N) and exon (O) expression levels of genes measured by Click-iT-seq in haploid 512-cell stage embryos treated with and without α-amanitin. Dashed lines represent 4-fold change. The solid black line represents the diagonal and the dashed lines represent 4-fold change. **(P-Q)** Stacked bar plot comparing the normalized expression levels of genes (Materials and Methods) in diploid embryos with haploid embryos at 512-cell stage (P) and 1K-cell stage (Q) on all zygotic (512-cell: 1,571 genes; 1K-cell: 1,640 genes) and maternal zygotic (512-cell: 987 genes; 1K-cell: 999 genes) genes. Genes are ranked by the normalized expression level in diploid embryos. Dash line represents N/C ratio contribution of 0.5, which is used as a reference threshold to distinguish between N/C ratio dependent and independent genes. Example N/C ratio dependent and independent genes are highlighted. **(R)** Genome tracks representing normalized Click-iT-seq signal measured at 1K-cell stage in diploid and haploid embryos of N/C ratio independent and dependent genes. Here diploid embryos are WT embryos with half of the embryos treated with α-amanitin (STAR Methods).

Figure S5


A
dCas9-3xGFP
wild type
n=36
PatA+CHX at 32-cell
n=14
Mean Intensity GFP
Mean Fluorescent Intensity
****
WT
PatA+CHX at 32-cell
B
Wild Type
CHX at 8-cell
CHX at 32-cell
Brightfield
DsRed reporter
H1-Alexa488

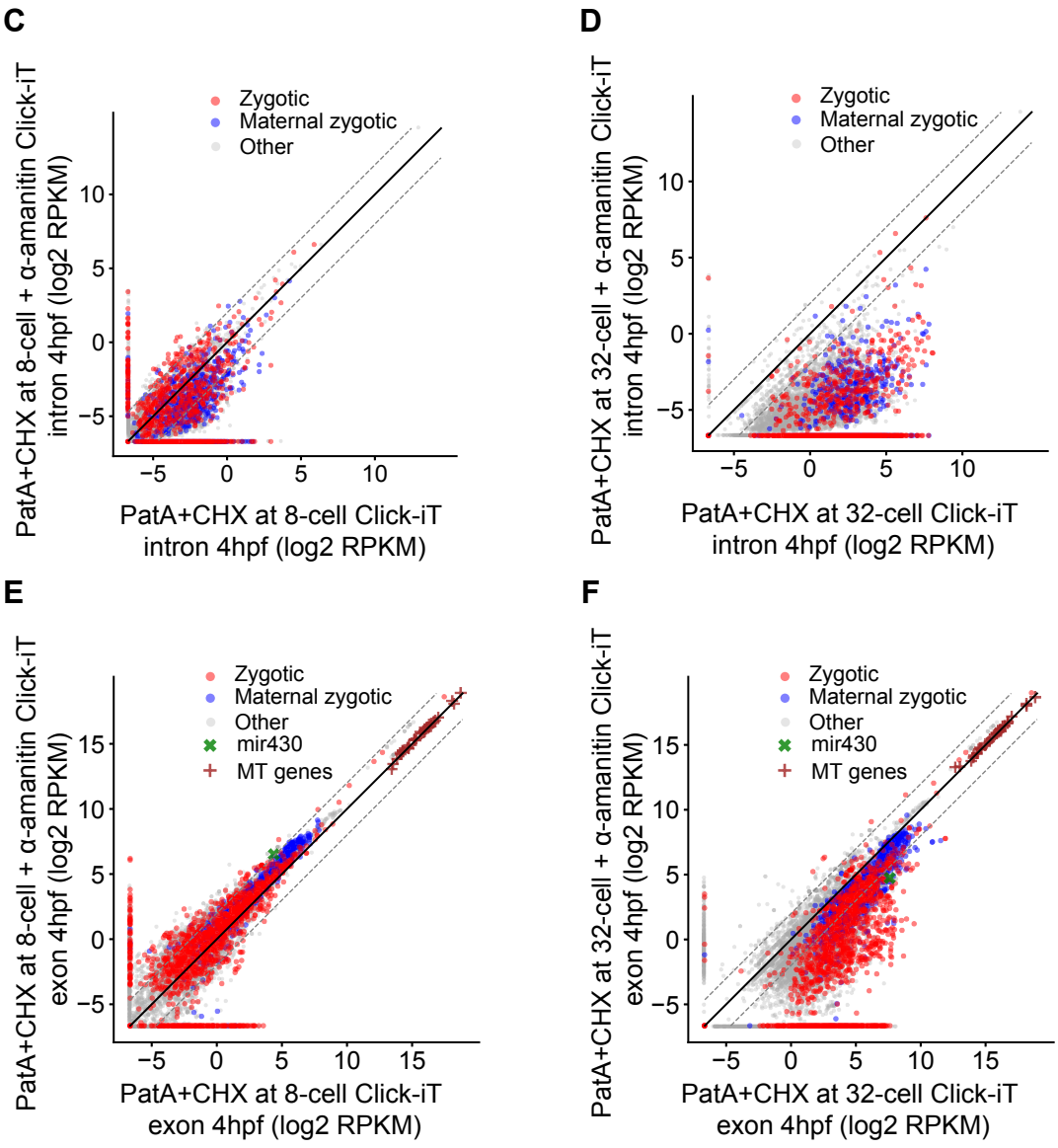

C
D
E
F
Zygotic
Maternal zygotic
Other
mir430
MT genes
PatA+CHX at 8-cell + α-amanitin Click-iT intron 4hpf (log2 RPKM)
PatA+CHX at 8-cell Click-iT intron 4hpf (log2 RPKM)
PatA+CHX at 32-cell + α-amanitin Click-iT intron 4hpf (log2 RPKM)
PatA+CHX at 32-cell Click-iT intron 4hpf (log2 RPKM)
PatA+CHX at 8-cell + α-amanitin Click-iT exon 4hpf (log2 RPKM)
PatA+CHX at 8-cell Click-iT exon 4hpf (log2 RPKM)
PatA+CHX at 32-cell + α-amanitin Click-iT exon 4hpf (log2 RPKM)
PatA+CHX at 32-cell Click-iT exon 4hpf (log2 RPKM)

Figure S5. Embryos treated with PatA and CHX at 8- and 32-cell stages demonstrate different transcriptional competency at 4hpf; related to Figure 4.

(A) Single nucleus imaging analysis of dCas9-3XGFP signal at 4hpf in wild type embryos and embryos treated with PatA + CHX at 32-cell stage (left). Scale bar represents 5 μm. Box and whisker plots (right) showing the mean fluorescence intensity for dCas9-3xGFP signal in the conditions shown on left (****P<0.0001, two-sample t-test: WT (n=36); PatA + CHX at 32-cell (n=14)) **(B)** Brightfield and fluorescent microscopy images of wild type embryos and embryos treated with CHX at 8-cell and 32-cell stage respectively, expressing dsRed reporter and H1-Alexa488 control 4 hours post-injection. Note the reduction of DsRed reporter signal in embryos treated with CHX at 8-cell and 32-cell stage respectively when compared to wild type embryos. **(C-D)** Biplot of Click-iT-seq RNA levels at 4hpf comparing the level of transcription using intron signal. Embryos were treated with PatA + CHX at 8-cell stage (C) or at 32-cell stage (D), with and without α-amanitin. **(E-F)** Biplot of Click-iT-seq RNA levels at 4hpf comparing the level of transcription using exon signal. Embryos were treated with PatA + CHX at 8-cell stage (E) or at 32-cell stage (F), with and without α-amanitin. Note that embryos with PatA and CHX treatment at 32-cell stage demonstrate higher level of transcription competency than embryos with PatA and CHX treatment at 8-cell stage, indicating the additional time for translation of maternal RNAs is required for zygotic genome activation. Dashed lines represent 4-fold change**.** Intron expression comparison allows more sensitive measurement of zygotic RNA expression from maternal RNA contribution.

Figure S6

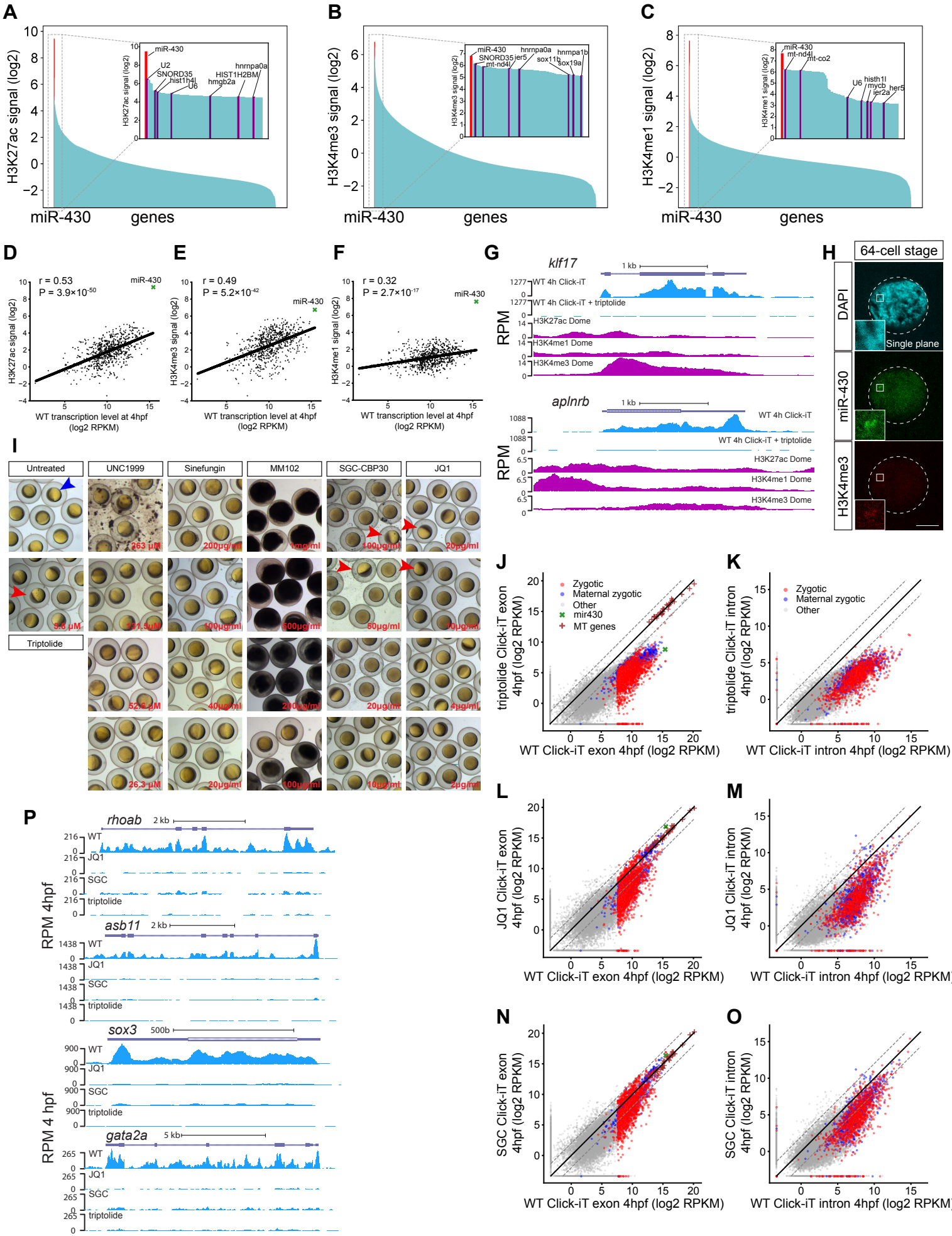

A
H3K27ac signal (log2)
miR-430
genes
B
H3K4me3 signal (log2)
C
H3K4me1 signal (log2)
D
r = 0.53
P = 3.9×10-50
WT transcription level at 4hpf (log2 RPKM)
E
r = 0.49
P = 5.2×10-42
F
r = 0.32
P = 2.7×10-17
G
klf17
aplnrb
RPM
WT 4h Click-iT
WT 4h Click-iT + triptolide
H3K27ac Dome
H3K4me1 Dome
H3K4me3 Dome
H
64-cell stage
DAPI
Single plane
miR-430
H3K4me3
I
Untreated
UNC1999
Sinefungin
MM102
SGC-CBP30
JQ1
Triptolide
J
triptolide Click-iT exon 4hpf (log2 RPKM)
WT Click-iT exon 4hpf (log2 RPKM)
Zygotic
Maternal zygotic
Other
mir430
MT genes
K
triptolide Click-iT intron 4hpf (log2 RPKM)
WT Click-iT intron 4hpf (log2 RPKM)
L
JQ1 Click-iT exon 4hpf (log2 RPKM)
M
JQ1 Click-iT intron 4hpf (log2 RPKM)
N
SGC Click-iT exon 4hpf (log2 RPKM)
O
SGC Click-iT intron 4hpf (log2 RPKM)
P
rhoab
asb11
sox3
gata2a
RPM 4hpf
RPM 4 hpf
WT
JQ1
SGC
triptolide

Figure S6. miR-430 is among the top labeled loci with H3K27ac, H3K4me3, and H3K4me1 marks, and *small molecule inhibitors treatment (JQ1 and SGC) perturbs zygotic gene activation and gastrulation similar to triptolide treatment*; related to Figure 5.

(A-C) Barplot showing level of H3K27ac (A), H3K4me3 (B) and H3K4me1 (C) on all zebrafish genes. Inset barplot represents the top-100 genes. MiR-430 locus is represented by a red bar. Note miR-430 is among the top labeled loci with H3K27ac, H3K4me3 and H3K4me1. **(D)** Biplot showing significant correlation between levels of H3K27ac (r = 0.53 (Spearman correlation), $P = 3.9\times10^{-50}$, rank correlation independence test) and gene transcription level (difference in gene expression (exon RPKM) between wild-type embryos and triptolide treated embryos) on published zygotic and maternal-zygotic genes (Materials and Methods). **(E)** Biplot showing significant correlation between levels of H3K4me3 (r = 0.49 (Spearman correlation), $P = 5.2\times10^{-42}$, rank correlation independence test) and gene transcription level. **(F)** Biplot showing significant correlation between levels of H3K4me1 (r = 0.32 (Spearman correlation), $P = 2.7\times10^{-17}$, rank correlation independence test) and gene transcription level. In (d-f), trend line is shown in the scatter plots. **(G)** Genome tracks representing normalized Click-iT-seq signal and histone mark level at the *klf17* and *aplnrb* locus. RPM = Reads per million mapped reads on mitochondrial protein coding genes. **(H)** Single plane stimulated emission depletion microscopy (STED) image labeled for H3K4me3, dCas9-miR-430 and DAPI (confocal). Scale bar represents 5 μm. Note the co-localization of H3K4me3 with individual miR-430 locus. **(I)** The effect of titrated treatments of different small molecule inhibitors targeting readers or writers for H3K4me3/H3K4me1 and H3K27Ac on gastrulation at 6hpf. UNC1999 (Sigma Aldrich) is a selective inhibitor of both EZH2 and EZH1 lysine methyltransferases; Sinefungin (Abcam) is a competitive methyltransferase inhibitor; MM-102 (Selleck Chem.) is a selective WDR5/MLL (WD-repeat protein 5/mixed-lineage leukemia methyltransferase complex) interaction inhibitor; SGC-CBP30 (SGC) is a potent inhibitor for CREBP (cAMP-responsive element-binding protein binding protein) and EP300 (E1A-associated protein p300) bromodomains; JQ1 (Sigma Aldrich) is a selective bromodomain inhibitor targeting the BET (bromodomain and extra terminal domain) family of proteins, including BRD2, BRD3 and BRD4. Blue arrow denotes a gastrulating wild-type embryo at 6hpf; red arrows denote embryos with gastrulation arrest phenotype at

6hpf. Note that embryos with JQ1 and SGC-CBP30 treatment fail to gastrulate similar to the phenotype shown in triptolide treated embryos (Triptolide). **(J-K)** Biplot comparing exon (J) and intron (K) expression levels of genes measured by Click-iT-seq in triptolide treated embryos (triptolide) with wild-type embryos (WT) at 4hpf. Dashed lines represent 4-fold change. **(L-M)** Biplot comparing exon (L) and intron (M) expression levels of genes measured by Click-iT-seq in JQ1 treated embryos (JQ1) with wild-type embryos (WT) at 4hpf. **(N-O)** Biplot comparing exon (N) and intron (O) expression levels of genes measured by Click-iT-seq in SGC treated embryos (SGC) with wild-type embryos (WT) at 4hpf. **(P)** Genome tracks representing normalized Click-iT-seq signal measured at 4hpf in wild-type, triptolide, JQ1 and SGC treated embryos for examples of house keeping (*rhoab*, *asb11*) and developmental (*sox3*, *gata2a*) genes. Note the marked reduction in gene expression in both JQ1 and SGC treated embryos respectively when compared to wild-type embryos. RPM = Reads per million mapped reads on mitochondrial protein coding genes.

Figure S7

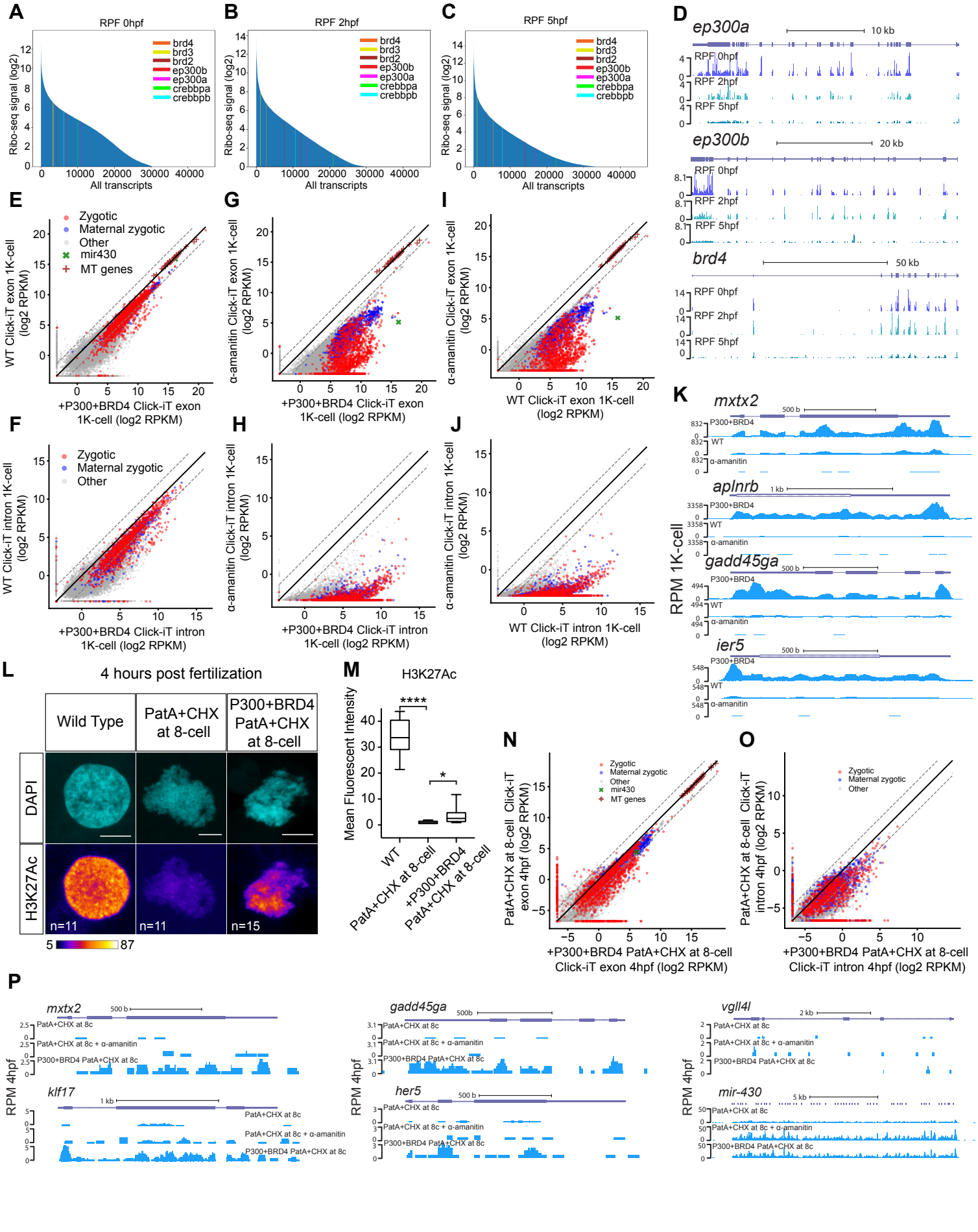

A
RPF 0hpf
B
RPF 2hpf
C
RPF 5hpf
brd4
brd3
brd2
ep300b
ep300a
crebbpa
crebbpb
Ribo-seq signal (log2)
All transcripts
D
ep300a
10 kb
ep300b
20 kb
brd4
50 kb
RPF 0hpf
RPF 2hpf
RPF 5hpf
E
Zygotic
Maternal zygotic
Other
mir430
MT genes
WT Click-iT exon 1K-cell (log2 RPKM)
+P300+BRD4 Click-iT exon 1K-cell (log2 RPKM)
G
α-amanitin Click-iT exon 1K-cell (log2 RPKM)
+P300+BRD4 Click-iT exon 1K-cell (log2 RPKM)
I
α-amanitin Click-iT exon 1K-cell (log2 RPKM)
WT Click-iT exon 1K-cell (log2 RPKM)
F
WT Click-iT intron 1K-cell (log2 RPKM)
+P300+BRD4 Click-iT intron 1K-cell (log2 RPKM)
H
α-amanitin Click-iT intron 1K-cell (log2 RPKM)
+P300+BRD4 Click-iT intron 1K-cell (log2 RPKM)
J
α-amanitin Click-iT intron 1K-cell (log2 RPKM)
WT Click-iT intron 1K-cell (log2 RPKM)
K
mxtx2
aplnrb
gadd45ga
ier5
500 b
1 kb
P300+BRD4
WT
α-amanitin
RPM 1K-cell
L
4 hours post fertilization
Wild Type
PatA+CHX at 8-cell
P300+BRD4 PatA+CHX at 8-cell
DAPI
H3K27Ac
n=11
n=11
n=15
5
87
M
H3K27Ac
Mean Fluorescent Intensity
****
*
WT
PatA+CHX at 8-cell
+P300+BRD4 PatA+CHX at 8-cell
N
PatA+CHX at 8-cell Click-iT exon 4hpf (log2 RPKM)
+P300+BRD4 PatA+CHX at 8-cell Click-iT exon 4hpf (log2 RPKM)
O
PatA+CHX at 8-cell Click-iT intron 4hpf (log2 RPKM)
+P300+BRD4 PatA+CHX at 8-cell Click-iT intron 4hpf (log2 RPKM)
P
mxtx2
klf17
gadd45ga
her5
vgll4l
mir-430
PatA+CHX at 8c
PatA+CHX at 8c + α-amanitin
P300+BRD4 PatA+CHX at 8c
RPM 4hpf

Figure S7. Early expression of P300 and BRD4 proteins leads to premature activation of the zygotic genome and restores transcription competency in embryos treated with PatA and CHX at 8-cell stage; related to Figure 6.

(A-C) Barplot showing ribosome profiling level of brd2-4, p300 and CREBBP (CBP) at 0hpf (A), 2hpf (B), 5hpf (C) on all zebrafish transcripts. **(D)** Genome tracks representing ribosome profiling signal of brd4 and ep300a and ep300b measured at 0hpf, 2hpf, 5hpf. **(E-F)** Biplot comparing exon (E) and intron (F) expression levels of genes measured by Click-iT-seq in wild-type (WT) embryos and embryos with early expression of P300 and BRD4 proteins (+P300+BRD4) at 1K-cell stage. Dashed lines represent 4-fold change. **(G-H)** Biplot comparing exon (G) and intron (H) expression levels of genes measured by Click-iT-seq in embryos with early expression of P300 and BRD4 proteins (+P300+BRD4) and α-amanitin treated embryos (α-amanitin) at 1K-cell stage. **(I-J)** Biplot comparing exon (I) and intron (J) expression levels of genes measured by Click-iT-seq in wild-type embryos (WT) and α-amanitin treated embryos (α-amanitin) at 1K-cell stage. Note the marked increase in gene expression in embryos with early expression of P300 and BRD4 proteins when compared to wild-type embryos (WT). **(K)** Genome tracks representing normalized Click-iT-seq signal measured at 1K-cell stage in embryos with early expression of P300 and BRD4 (P300+BRD4), wild-type (WT) and α-amanitin treated (α-amanitin) embryos for examples of zygotic genes. RPM = Reads per million mapped reads on mitochondrial protein coding genes. **(L)** Single nucleus imaging analysis of H3K27Ac signal at 4hpf comparing wild type embryos (Wild Type) and embryos treated with PatA + CHX at 8-cell stage injected without (PatA+CHX at 8-cell) and with P300 and BRD4 (P300+BRD4 PatA+CHX at 8-cell) (n=number of nucleus imaged from independent embryos). H3K27Ac signal intensity is presented in a heatmap color scale. Scale bar, 5 μm. Note the decrease in acetylation signal by the treatment of PatA + CHX at 8-cell stage; and the increase in the acetylation signal when PatA+CHX at 8-cell embryos are expressed with P300 and BRD4. **(M)** Box and whisker plots quantifying the mean fluorescence intensity for H3K27Ac signal in the conditions described in (A) (Two sample t-test of H3K27Ac signal: WT > PatA+CHX at 8-cell $P<0.0001$; P300+BRD4 PatA+CHX at 8-cell > PatA+CHX at 8-cell $P=0.0123$). **(N-O)** Biplot comparing exon (N) and intron (O) expression levels of genes measured by Click-iT-seq in embryos treated with PatA and CHX at 8-cell stage with

(+P300+BRD4 PatA+CHX at 8-cell) and without early expression of P300 and BRD4 (PatA+CHX at 8-cell) at 4hpf. Dashed lines represent 4-fold change. Note the marked increase in gene expression in embryos treated with PatA and CHX at 8-cell stage with early expression of P300 and BRD4 when compared to embryos treated with PatA and CHX at 8-cell stage at 4hpf. **(P)** Genome tracks representing normalized Click-iT-seq signal in the conditions described in (L-O). Normalized Click-iT-seq signal for embryos treated with PatA + CHX at 8-cell stage in the presence of α-amanitin is shown as control. RPM = Reads per million mapped reads on mitochondrial protein coding genes.