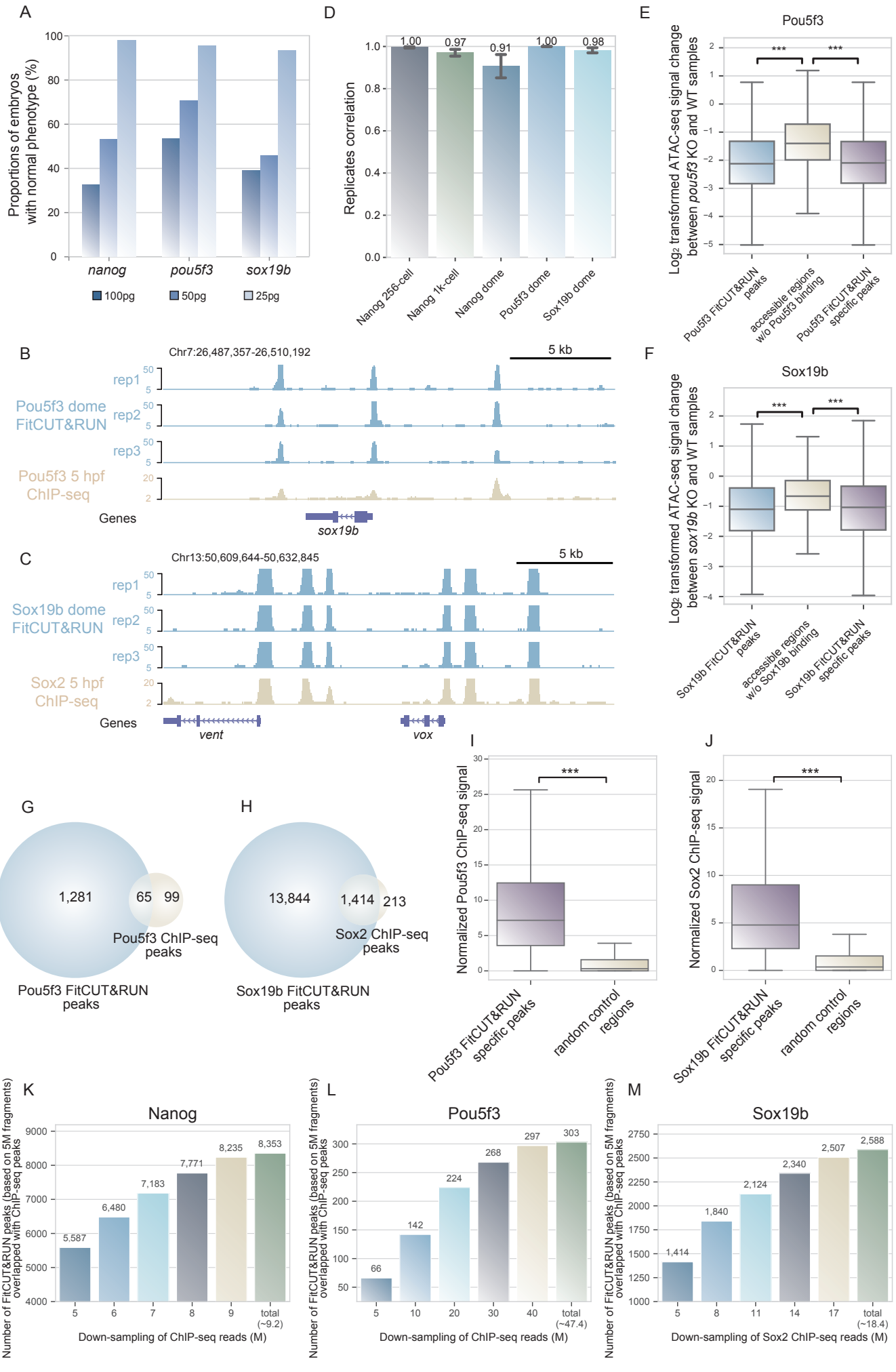
A
Proportions of embryos with normal phenotype (%)
nanog
pou5f3
sox19b
100pg
50pg
25pg
D
Replicates correlation
1.00
0.97
0.91
1.00
0.98
Nanog 256-cell
Nanog 1k-cell
Nanog dome
Pou5f3 dome
Sox19b dome
E
Pou5f3
Log2 transformed ATAC-seq signal change between pou5f3 KO and WT samples
***
***
Pou5f3 FitCUT&RUN peaks
accessible regions w/o Pou5f3 binding
Pou5f3 FitCUT&RUN specific peaks
B
Chr7:26,487,357-26,510,192
5 kb
rep1
rep2
rep3
Pou5f3 dome FitCUT&RUN
Pou5f3 5 hpf ChIP-seq
Genes
sox19b
C
Chr13:50,609,644-50,632,845
5 kb
rep1
rep2
rep3
Sox19b dome FitCUT&RUN
Sox2 5 hpf ChIP-seq
Genes
vent
vox
F
Sox19b
Log2 transformed ATAC-seq signal change between sox19b KO and WT samples
***
***
Sox19b FitCUT&RUN peaks
accessible regions w/o Sox19b binding
Sox19b FitCUT&RUN specific peaks
G
1,281
65
99
Pou5f3 ChIP-seq peaks
Pou5f3 FitCUT&RUN peaks
H
13,844
1,414
213
Sox2 ChIP-seq peaks
Sox19b FitCUT&RUN peaks
I
Normalized Pou5f3 ChIP-seq signal
***
Pou5f3 FitCUT&RUN specific peaks
random control regions
J
Normalized Sox2 ChIP-seq signal
***
Sox19b FitCUT&RUN specific peaks
random control regions
K
Nanog
Number of FitCUT&RUN peaks (based on 5M fragments) overlapped with ChIP-seq peaks
5,587
6,480
7,183
7,771
8,235
8,353
Down-sampling of ChIP-seq reads (M)
total (~9.2)
L
Pou5f3
Number of FitCUT&RUN peaks (based on 5M fragments) overlapped with ChIP-seq peaks
66
142
224
268
297
303
Down-sampling of ChIP-seq reads (M)
total (~47.4)
M
Sox19b
Number of FitCUT&RUN peaks (based on 5M fragments) overlapped with ChIP-seq peaks
1,414
1,840
2,124
2,340
2,507
2,588
Down-sampling of Sox2 ChIP-seq reads (M)
total (~18.4)

Supplemental Figure 4. Application of FitCUT&RUN in early zebrafish embryos. (*A*) Proportions of embryos with a normal phenotype after microinjection of TF-rFc mRNA. (*B*) The genome browser view shows Pou5f3 FitCUT&RUN (three replicates) and ChIP-seq (one replicate) signals around *sox19b* loci as a representative example of the consistency between these two methods. (*C*) The genome browser view shows Sox19b FitCUT&RUN (three replicates) and Sox2 ChIP-seq (one replicate) signals around *vent* loci as a representative example of the consistency between these two methods. (*D*) Bar plots showing the correlation between FitCUT&RUN replicates. The high correlation confirms the reproducibility of the FitCUT&RUN data. (*E, F*) Boxplots showing the ATAC-seq signal (oblong stage, Supplemental Table S3) (Palfy et al. 2020) change between *pou5f3*-KO (E), *sox19b*-KO (F) and wild-type samples of three different regions. Significant decreases in Pou5f3 (E) and Sox19b (F) FitCUT&RUN peaks compared to those of accessible regions (oblong stage, Supplemental Table S3) (Liu et al. 2018) without overlap with Pou5f3 (E) or Sox19b (F) FitCUT&RUN peaks indicate that Pou5f3 (E) and Sox19b (F) are critical for the establishment of accessible chromatin in these Pou5f3 (E) and Sox19b (F) FitCUT&RUN peaks. Significant decreases in Pou5f3 (E) and Sox19b (F) FitCUT&RUN specific peaks compared to those of accessible regions (oblong stage, Supplemental Table S3) (Liu et al. 2018) without overlap with Pou5f3 (E) or Sox19b (F) FitCUT&RUN peaks show the reliability of FitCUT&RUN specific peaks, indicating that FitCUT&RUN can produce reliable TF occupancy profiles during zebrafish ZGA. (*G*) Venn diagram showing the overlap status between Pou5f3 FitCUT&RUN and ChIP-seq peaks. FitCUT&RUN could detect much more peaks than ChIP-seq (Supplemental Table S4). (*H*) Venn diagram showing the overlap status between Sox19b FitCUT&RUN and Sox2 ChIP-seq peaks. FitCUT&RUN could detect much more peaks than ChIP-seq (Supplemental Table S4). (*I, J*) Boxplots showing that the normalized Pou5f3 (I) and Sox2 (J) ChIP-seq signals at Pou5f3 (I) and Sox19b (J) FitCUT&RUN-specific peaks were significantly greater than those of random control regions. (*K, L, M*) Barplots showing that more Nanog (K), Pou5f3 (L) and Sox19b (M) FitCUT&RUN peaks are detected by Nanog (K), Pou5f3 (L) and Sox2 (M) ChIP-seq when greater sequencing depth of ChIP-seq been applied.