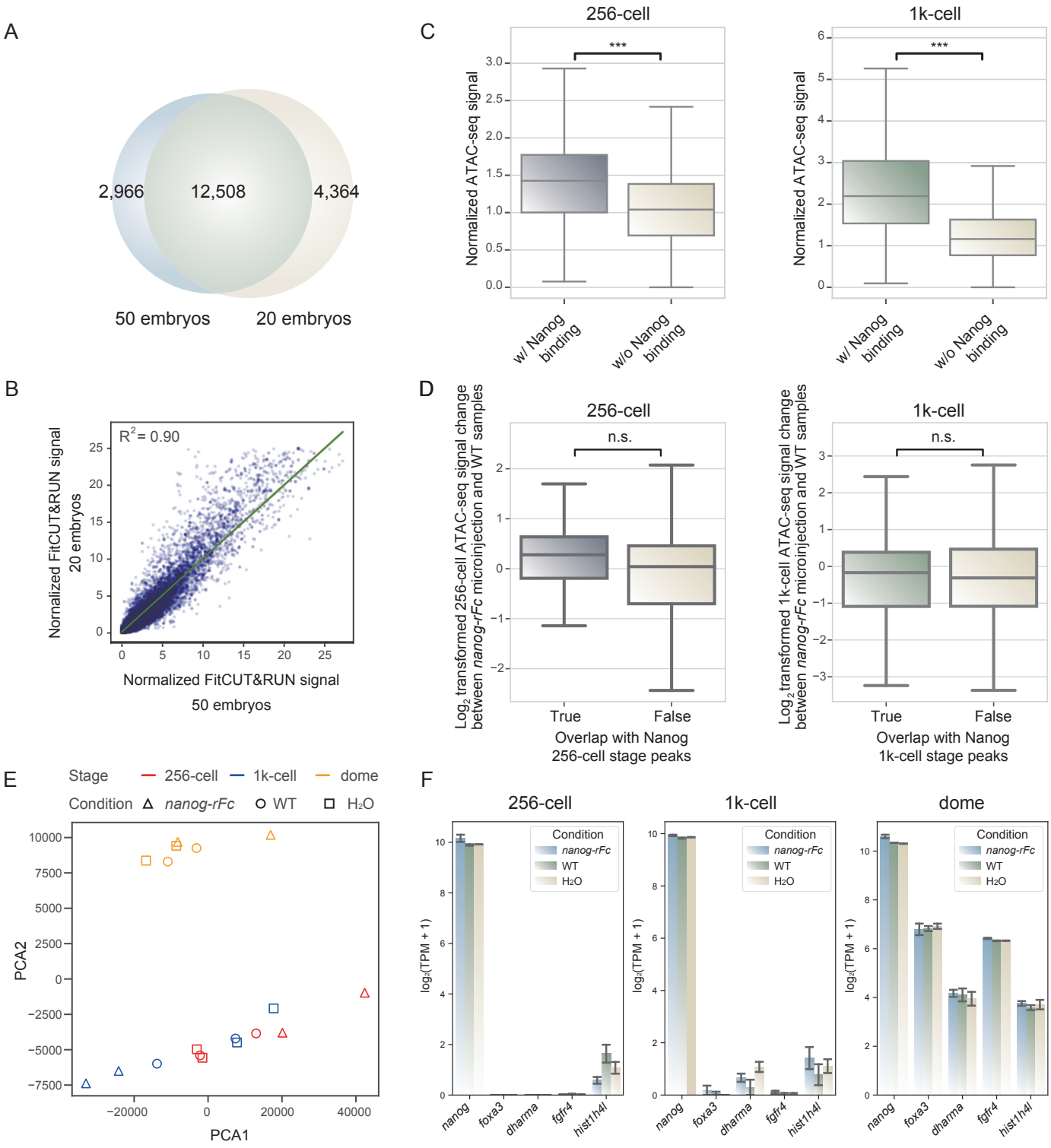

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Stage	Gene regulation state	pre-MBT targets (1,317)	MBT new targets (1,891)	post-MBT new targets (4,047)	Other genes (24,718)
256-cell stage	up	3 (0.23%)	9 (0.48%)	9 (0.22%)	45 (0.18%)
	down	1 (0.08%)	6 (0.32%)	10 (0.25%)	87 (0.35%)
1k-cell stage	up	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (0.01%)
	down	0 (0.00%)	1 (0.05%)	0 (0.00%)	2 (0.01%)
dome stage	up	9 (0.68%)	5 (0.26%)	25 (0.62%)	94 (0.38%)
	down	2 (0.15%)	6 (0.32%)	11 (0.27%)	53 (0.21%)

Supplemental Figure 5. Nanog profiling by FitCUT&RUN during zebrafish ZGA. *(A)* Comparison of Nanog FitCUT&RUN at dome stage using 20 embryos and 50 embryos. Venn diagram showing the overlap between Nanog binding sites derived from 20 embryos and 50 embryos. *(B)* Scatterplot presenting the high correlation of the Nanog FitCUT&RUN peak signals between experiments with different input embryos (x-axis: 50 embryos; y-axis: 20 embryos). *(C)* Boxplots showing the normalized 256-cell (left) and 1k-cell (right) wild-type ATAC-seq signals (Supplemental Table S3) (Liu et al. 2018) on promoters. Nanog target promoters show a higher ATAC-seq signal, indicating that Nanog plays a role in the establishment of accessible chromatin at these promoters. *(D)* Boxplots showing the ATAC-seq signal (Supplemental Table S2) change after *nanog-rFc* mRNA microinjection into embryos in the 256-cell (left) and 1k-cell (right) stages. No obvious difference between oblong accessible regions (Supplemental Table S3) (Liu et al. 2018) that overlap or not with Nanog 256-cell FitCUT&RUN peaks, proving that the microinjection is not likely to generate artificial FitCUT&RUN peaks. *(E)* PCA analysis of RNA-seq data at 256-cell, 1k-cell and dome stages for WT, H_2O injection and *nanog-rFc* injection embryos. Circle: WT sample; square: H_2O injection sample; triangle: *nanog-rFc* injection sample; red: 256-cell stage; blue: 1k-cell stage; yellow: dome stage. *(F)* Expression of *nanog* and genes in Fig 4F in WT, H_2O injection and *nanog-rFc* injection sample. *(G)* Numbers of differentially expressed genes between *nanog-rFc* injection samples and WT/H_2O injection samples.