

Expanded View Figures

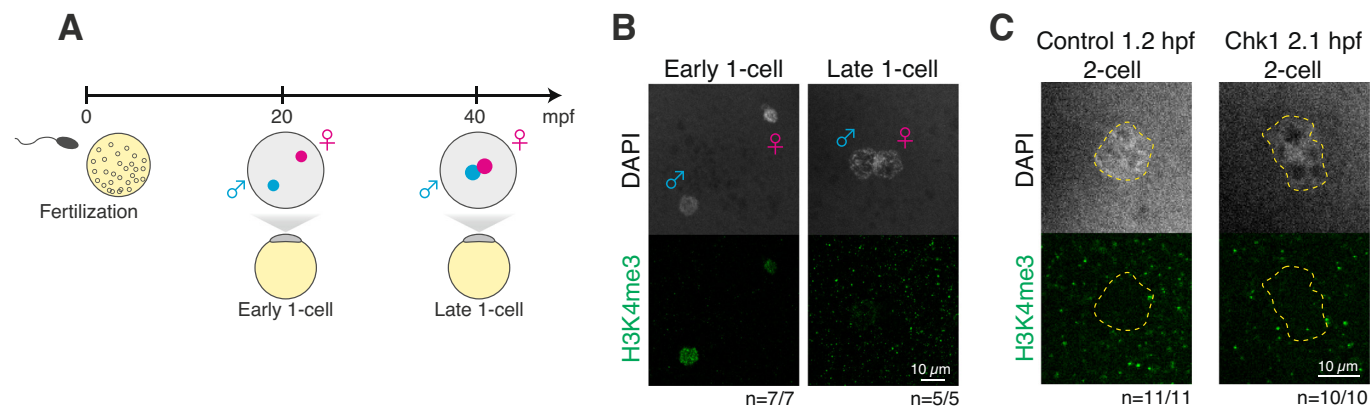
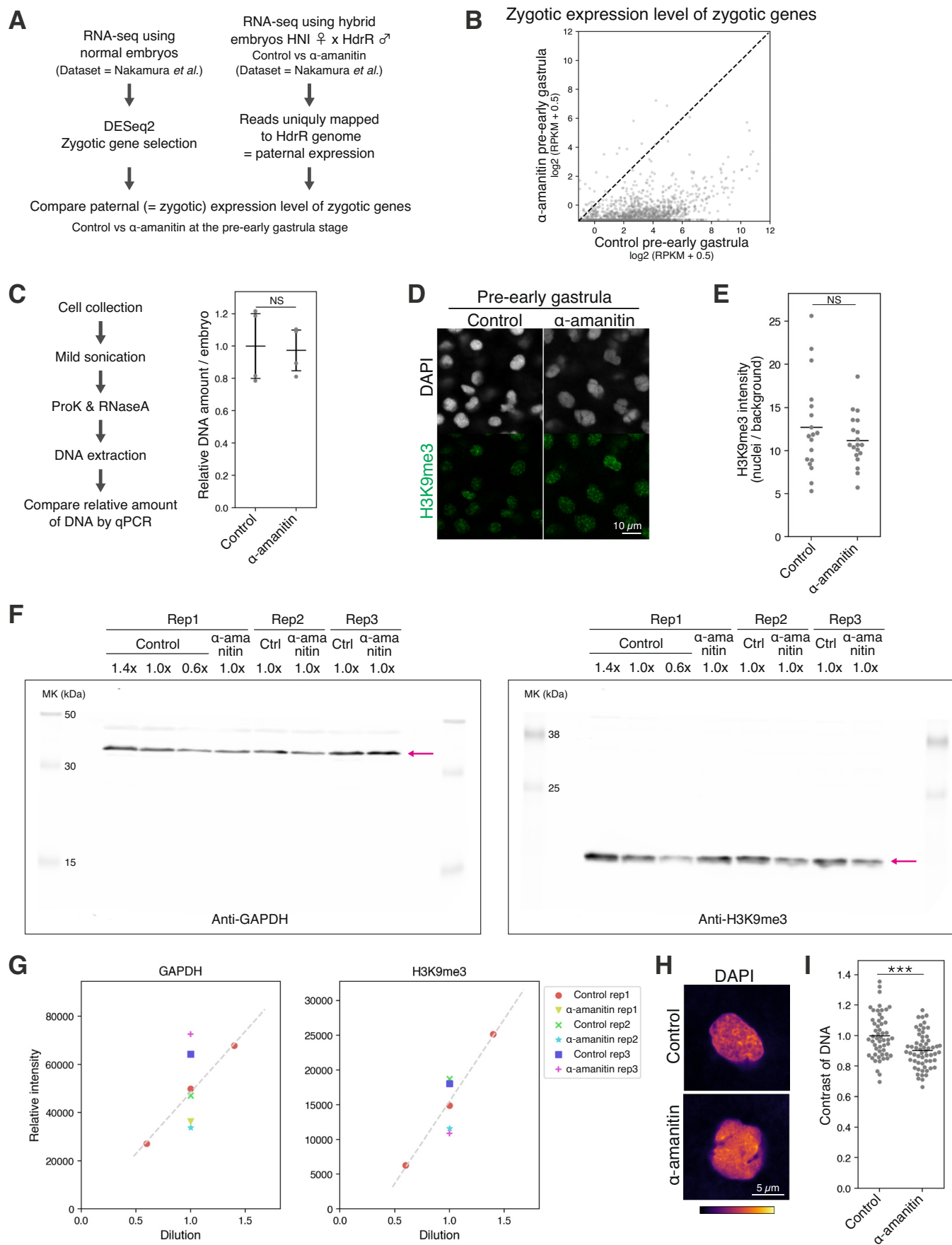


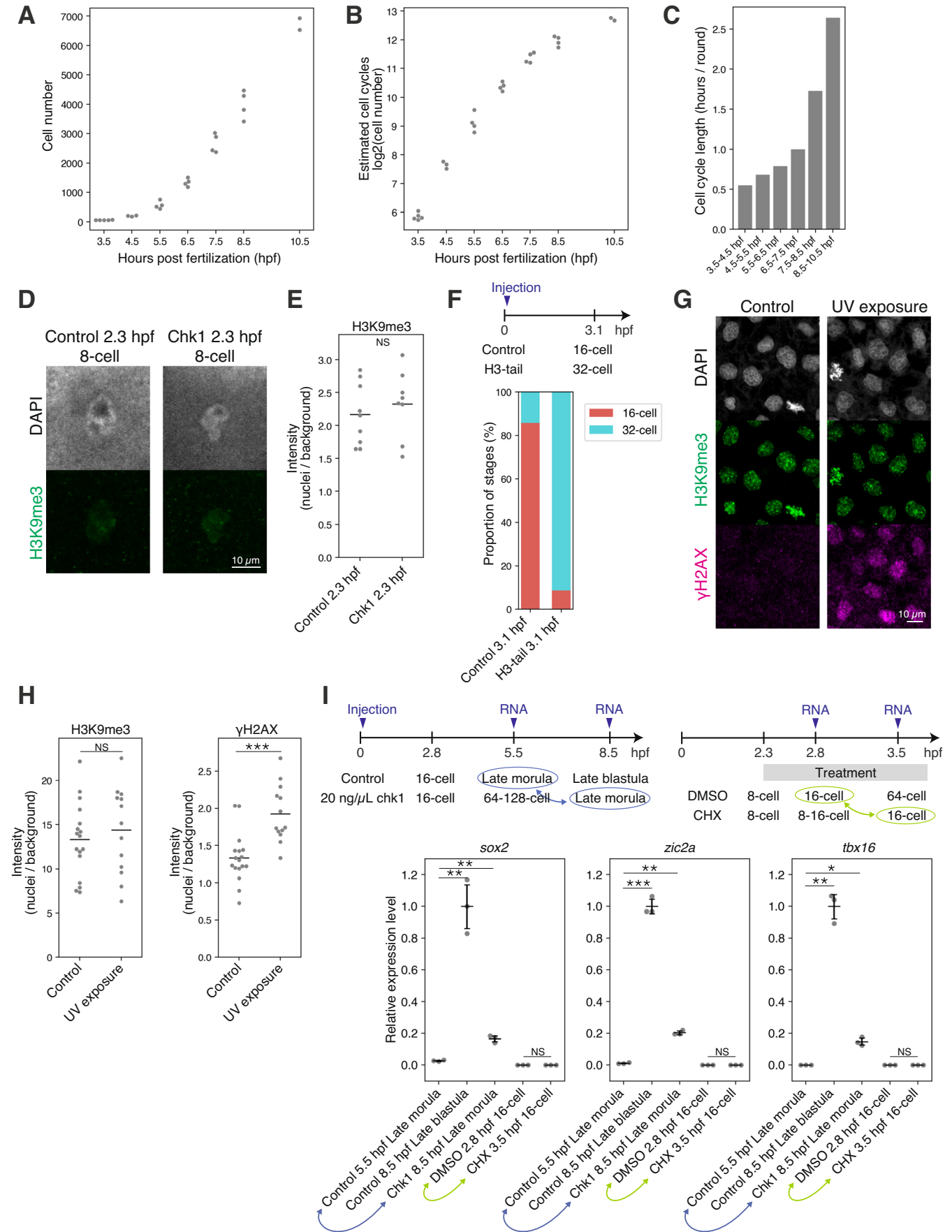
Figure EV1. Supportive data for Fig. 1.

(A) Development of medaka embryos at the one-cell stage. Blue and magenta indicate paternal and maternal pronuclei, respectively. mpf: minutes post fertilization. (B) Immunofluorescence staining of H3K4me3 at the one-cell stage. The number of embryos with the representative pattern is indicated at the bottom. (C) Immunofluorescence staining of H3K4me3 at the 2-cell stage in the Chk1 experiment. Yellow dashed line indicates nuclei. The number of embryos with the representative pattern is indicated at the bottom.



◀ **Figure EV2. Supportive data for Fig. 2.**

(A) Procedure of analyzing zygotic expression level in α -amanitin-injected embryos using previous dataset (Nakamura et al, 2021). The number of RNA-seq biological replicate is $n = 2$ and 1 for normal embryos and hybrid embryos, respectively. See Methods for the detail. (B) Scatterplot indicates that α -amanitin injection impaired zygotic expression at the pre-early gastrula stage in medaka. Previous dataset (Nakamura et al, 2021) was analyzed as shown in (A) and Methods. (C) Procedure of quantification of relative amount of DNA per embryo (left) and the results (right) at the late blastula stage. Two-sided unpaired Student's t-test. Error bars indicate the mean $\pm$ s.d. $n = 4$ biological replicates. (D) Immunofluorescence staining of H3K9me3 in α -amanitin-injected medaka embryos at the pre-early gastrula stage. (E) Quantification of (D). Each dot indicates the average intensity of ~ 50 cells in a single broad field slice image of single embryo. Two-sided Welch's t-test. Bars indicate the means. $n = 18$ and 19 embryos for the control and α -amanitin, respectively. Data were pooled from two independent experiments. (F) Uncropped results of quantitative western blot at the late blastula stage using anti-GAPDH and anti-H3K9me3 antibodies. Magenta arrows indicate the specific bands. (G) Quantification of western blot signal intensities in Figs. 1F and EV2F. Scatter plots show that all signal intensities of western blots were within the linear range. (H) DAPI staining of control or α -amanitin-injected embryos at the late blastula stage. (I) Quantification of (H). Each dot indicates the DNA contrast of a single nucleus. Ten embryos were analyzed. Two-sided unpaired Student's t-test. Bars indicate the means. $n = 53$ and 59 nuclei for the late morula and late blastula, respectively. Data were pooled from two independent experiments. *** $p < 0.001$, NS: not significant.



◀ **Figure EV3. Supportive data for Fig. 3.**

(A) Number of cells per embryo at 3.5–10.5 hpf counted by Imaris software using DAPI staining data. $n = 5, 3, 4, 4, 4, 2$ embryos for each stage. (B) Number of post fertilization cell-cycles estimated by total number of cells per embryo in (A). $n = 5, 3, 4, 4, 4, 2$ embryos for each stage. (C) Cell cycle length estimated by cell cycle number and time line in (B). (D) Immunofluorescence staining of H3K9me3 in the *chk1* injection experiment at the 8-cell stage (2.3 hpf). (E) Quantification of (D). Each dot indicates the average of single cells in a single broad field slice image of single embryo. Two-sided unpaired Student's t-test was performed. Bars indicate the means. $n = 9$ and 8 embryos for the control 2.3 hpf and *chk1* 2.3 hpf, respectively. Data were pooled from two independent experiments. (F) Schematic summarizing H3-tail injection (top) and proportion of stages of H3-tail-injected embryos in the cleavage stages (bottom). (G) Immunofluorescence staining of H3K9me3 and γ H2AX in control and UV-treated embryos at the late blastula stage. (H) Quantification of (G). Each dot indicates the average of ~100 cells in a single broad field slice image of single embryo. Two-sided unpaired Student's t-test. Bars indicate the means. $n = 17$ and 13 embryos for the control and UV exposure, respectively. Data were pooled from two independent experiments. (I) Schematics summarizing RNA sampling (top) and RT-qPCR of zygotic genes (bottom). Expression level was first normalized by that of *actb* in each sample and subsequently normalized by the average expression level in control 8.5 hpf. Stages highlighted in blue or green were compared. Two-sided Welch's t-test. Error bars indicate the mean $\pm$ s.d. $n = 3$ biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: not significant.

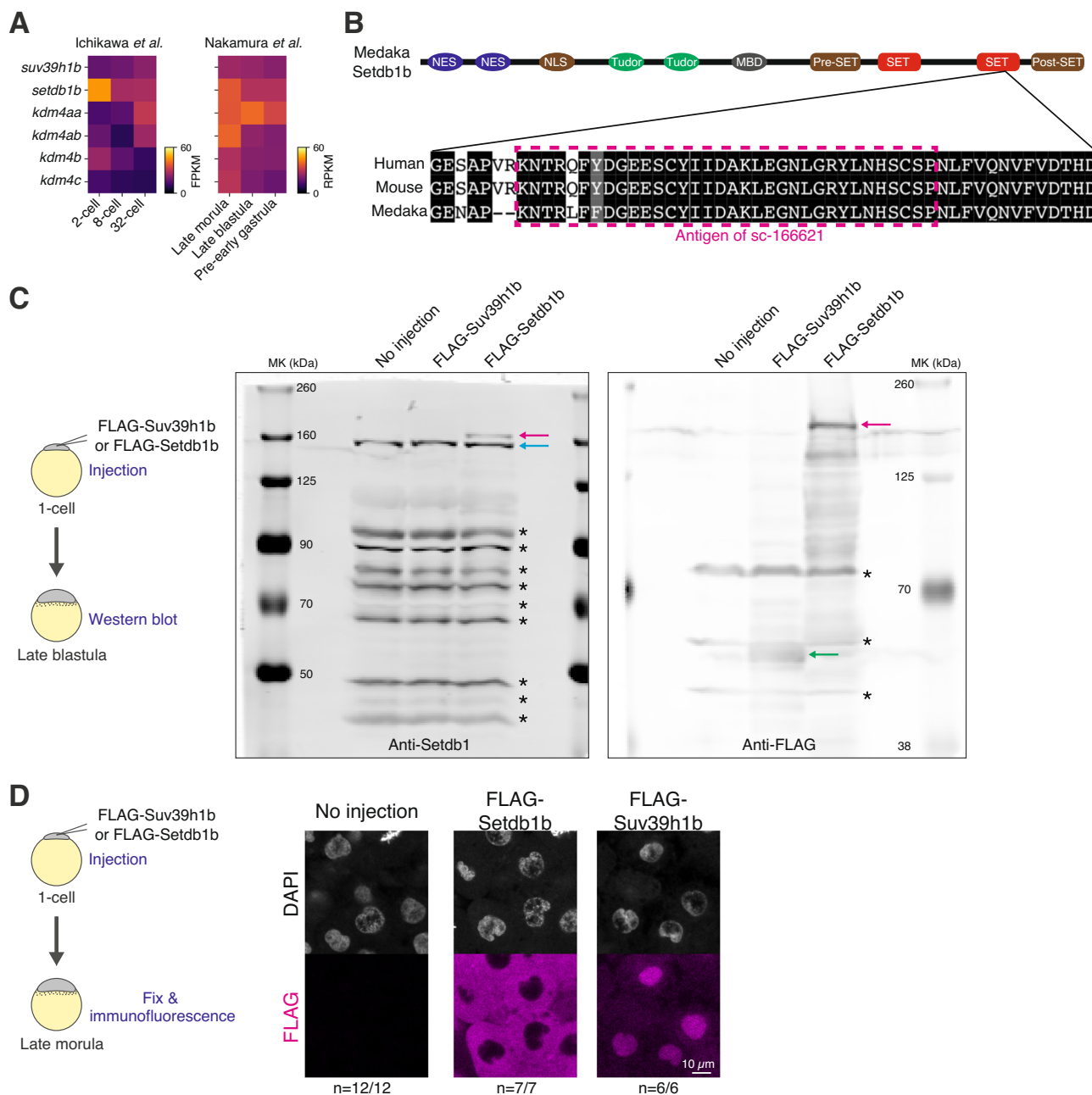
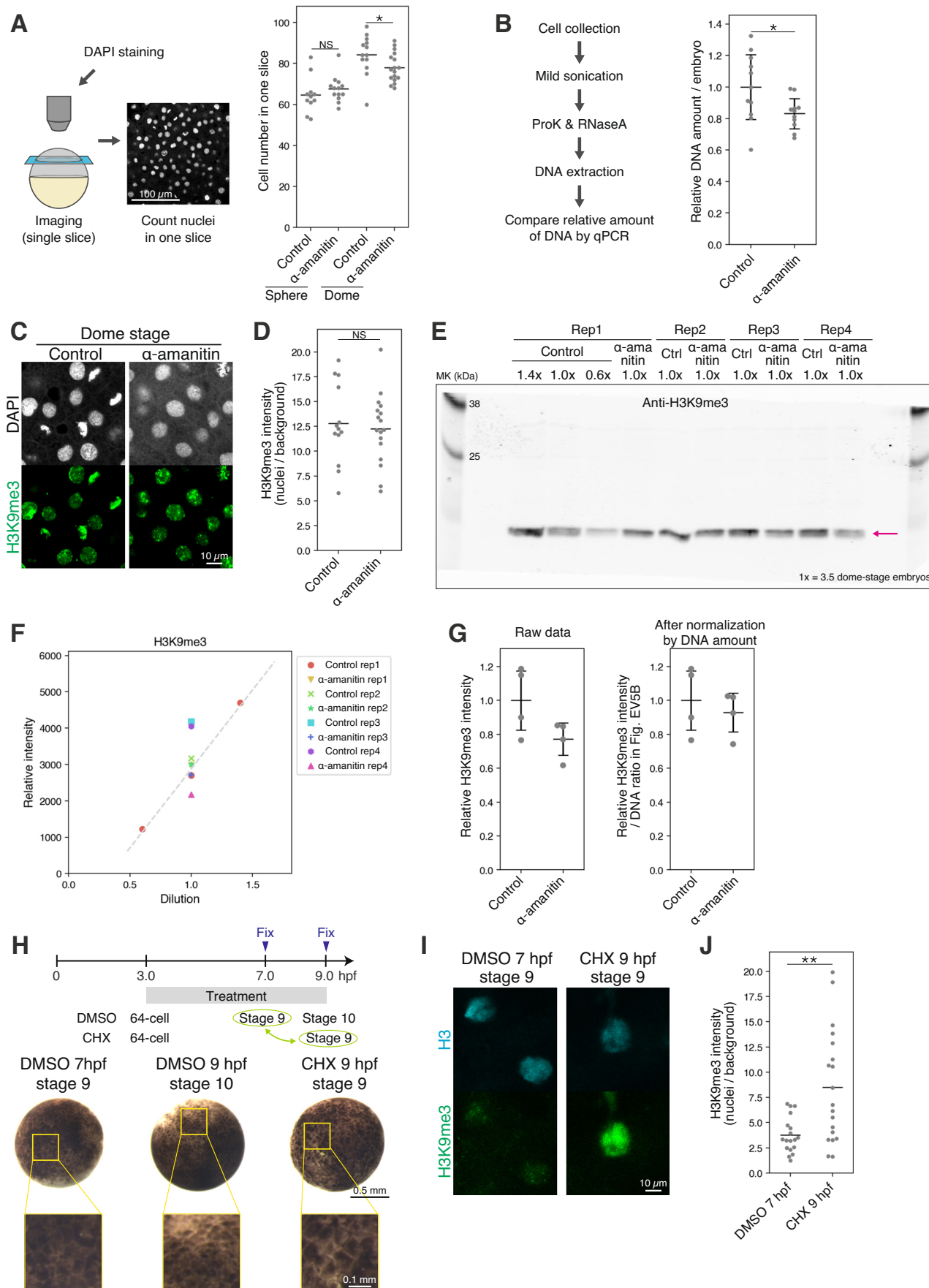


Figure EV4. Supportive data for Fig. 4.

(A) Expression level of H3K9me3 methyltransferases and demethylases during early development. Data was obtained from previous RNA-seq data (Nakamura et al, 2021; Ichikawa et al, 2017). (B) Domains of Setdb1 and amino acid sequence in the SET domain. The antigen sequence of the anti-Setdb1 antibody (sc-166621) is highlighted in magenta. (C) Schematic of the experiments to validate the specificity of the anti-Setdb1 antibody (sc-166621) (left) and the results of western blot at the late blastula stage (right). Blue, magenta, and green arrows indicate endogenous Setdb1b, exogenously expressed FLAG-Setdb1b, and exogenously expressed FLAG-Suv39h1b, respectively. Asterisks (*) indicate non-specific bands. (D) Schematic of the experiments to validate the localization of exogenously expressed FLAG-Suv39h1 and FLAG-Setdb1 (left) and immunofluorescence staining against anti-FLAG at the late morula stage (right). Consistent with the Fig. 4, exogenously overexpressed FLAG-Setdb1 localized to cytoplasm, while FLAG-Suv39h1 mainly accumulated in nuclei. The number of embryos with the representative pattern is indicated at the bottom.



◀ **Figure EV5. Supportive data for Figs. 5 and 6.**

(A) Schematic of counting nuclei in an embryo using a single slice (left) and the cell number in α -amanitin-injected zebrafish embryos at the sphere or dome stage (right). Two-sided unpaired Student's t-test. Bars indicate the means. $n = 11, 13, 13$, and 17 embryos for the sphere control, sphere α -amanitin, dome control, and dome α -amanitin, respectively. Data were pooled from two independent experiments. (B) Procedure of quantification of the relative amount of DNA per embryo (left) and the results (right) in α -amanitin-injected zebrafish embryos at the dome stage. Two-sided Welch's t-test. Error bars indicate the mean $\pm$ s.d. $n = 11$ biological replicates. (C) Immunofluorescence staining of H3K9me3 in α -amanitin injection experiment at the dome stage. (D) Quantification of (C). Each dot indicates the average of ~ 80 cells in a single broad field slice image of single embryo. Two-sided unpaired Student's t-test. Bars indicate the means. $n = 13$ and 17 embryos for the control and α -amanitin, respectively. Data were pooled from two independent experiments. (E) Uncropped results of quantitative western blot at the dome stage using anti-H3K9me3 antibody. The Magenta arrow indicates the specific bands. The same number of dome-stage embryos ($1\times = \sim 3.5$ embryos/lane) were loaded into each lane to compare total H3K9me3 levels per embryo. (F) Quantification of western blot signal intensities in (E). Scatter plots show that all western blot signal intensities were within the linear range. (G) Quantification of (E). On the right, data after normalization by the DNA ratio measured in Fig EV5B. Error bars indicate the mean $\pm$ s.d. $n = 4$ biological replicates. (H) Schematic summarizing the CHX treatment (top) and animal view of CHX-treated *X. laevis* embryos (bottom). Stages highlighted in green were compared in (I) and (J). To compare developmental stages, cells at the animal poles are magnified (yellow squares). (I) Immunofluorescence staining of H3 and H3K9me3 in CHX treatment at the stage 9. (J) Quantification of (I). Each dot indicates the average of ~ 5 – 10 cells in a single broad field slice image of single embryo. Two-sided Welch's t-test. Bars indicate the means. $n = 18$ and 19 embryos for the DMSO 7 hpf and CHX 9 hpf, respectively. Data were pooled from two independent experiments. * $p < 0.05$, ** $p < 0.01$, NS: not significant.