Epigenetic regulation of left-right asymmetry by DNA methylation

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Appendix Table of Contents

Appendix Materials and Methods

ELISA assay for 5mC level

Genomic DNA was extracted from embryos and the 5mC levels were quantitated by following manual instruction (5-mC DNA ELISA Kit, Zymo Research). Then the absorbance was measured at 405-450 nm using a luminometer (BioTek).

TUNEL assay

Embryos at 75%epi stage were fixed in 4% PFA at 4℃ overnight and dehydrated with methanol at -20℃ for 2 hours. After washing 5 times with PBST, the embryos were incubated with solution mixture containing labeling solution (45 μl) and enzyme solution (5 μl) ((In Situ Cell Death Detection Kit, Roche). For imaging, the DFC regions were dissected and embedded in the mounting medium and observed using Nikon A1 confocal microscope.

Appendix Figure Legends

Appendix Figure S1. 5mC level in control-, dnmt1 MO-injected and 5-AZA-treated embryos.

5mC levels were decreased in *dnmt1* morphants as well as in 5-AZA treated embryos, compared to controls.

Appendix Figure S2. Validation of *dnmt3bb.1* atgMO.

The efficacy of *dnmt3bb.1* atgMO was evaluated by co-injection of pEGFPN1-*dnmt3bb.1* and *dnmt3bb.1* atgMO into zebrafish embryos. Scale bar, 100μm

Appendix Figure S3. Deficiency of *dnmt1* causes defects of asymmetric genes.

Representative images showing *spaw* expression at 18-somite stage (upper panel) and *lefty2* at 26 hpf (lower panel). Statistical data were shown in the right panel with the total observed number as indicated.

Appendix Figure S4. Apoptosis and cell proliferation of DFCs in control and *dnmt1* morphants

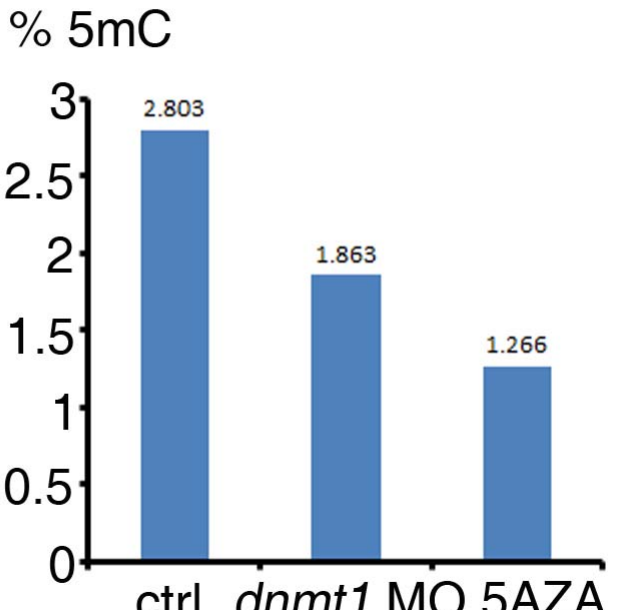
A, Confocal images of TUNEL signal in DFCs in control and *dnmt1*-deficient embryos. The white arrowheads show the TUNEL signal. Scale bar, 20μm.

B, Confocal images of anti-pH3 staining in DFCs at 80%epi in control and *dnmt1*-deficient embryos. The white arrowheads show the pH3 signal. Scale bar, 20μm.

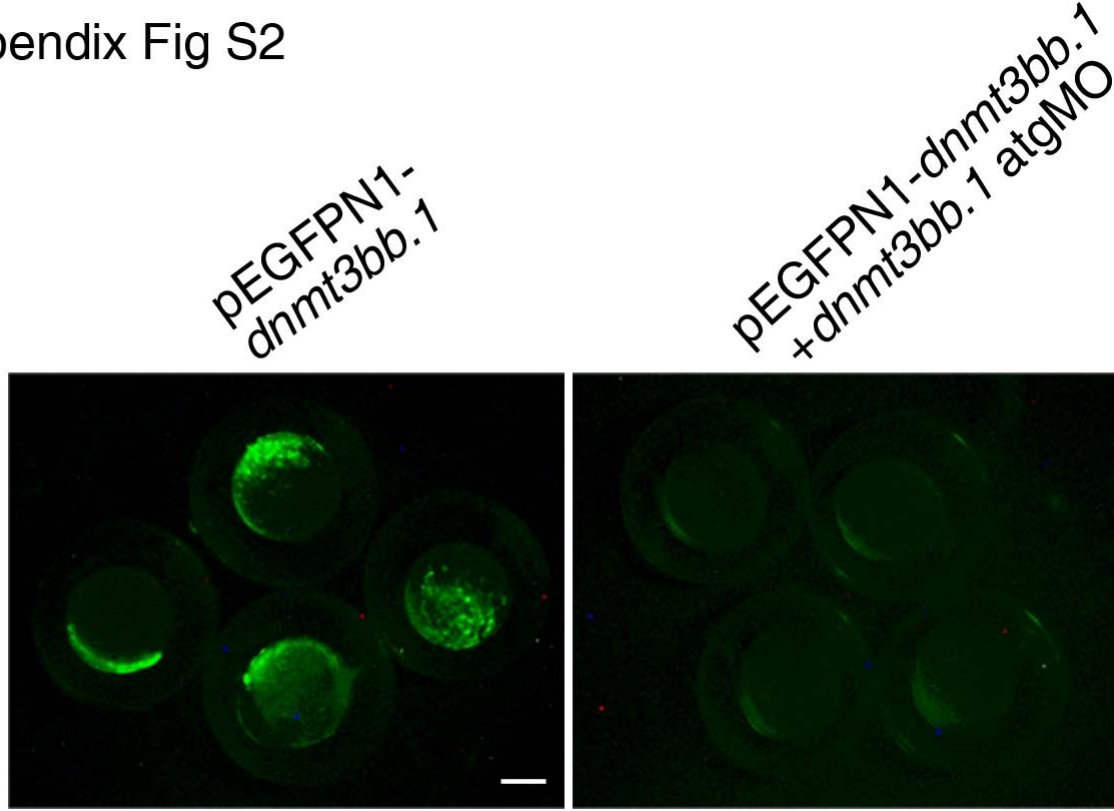
Appendix Figure S5. 5-AZA-treatment disrupts DFC specification and clustering.

A-B, Embryos were treated with 5-AZA from sphere to shield stage, and examined for *sox17* and *foxj1a* expression at shield and 90% epi stage, respectively. *Sox17* expression (**A**) and *foxj1a* expression (**B**) were disrupted in 5AZA-treated embryos. **C,** q-PCR results showed that increased *lefty2* and decreased *cdh1* expression in 5AZA-treated embryos.

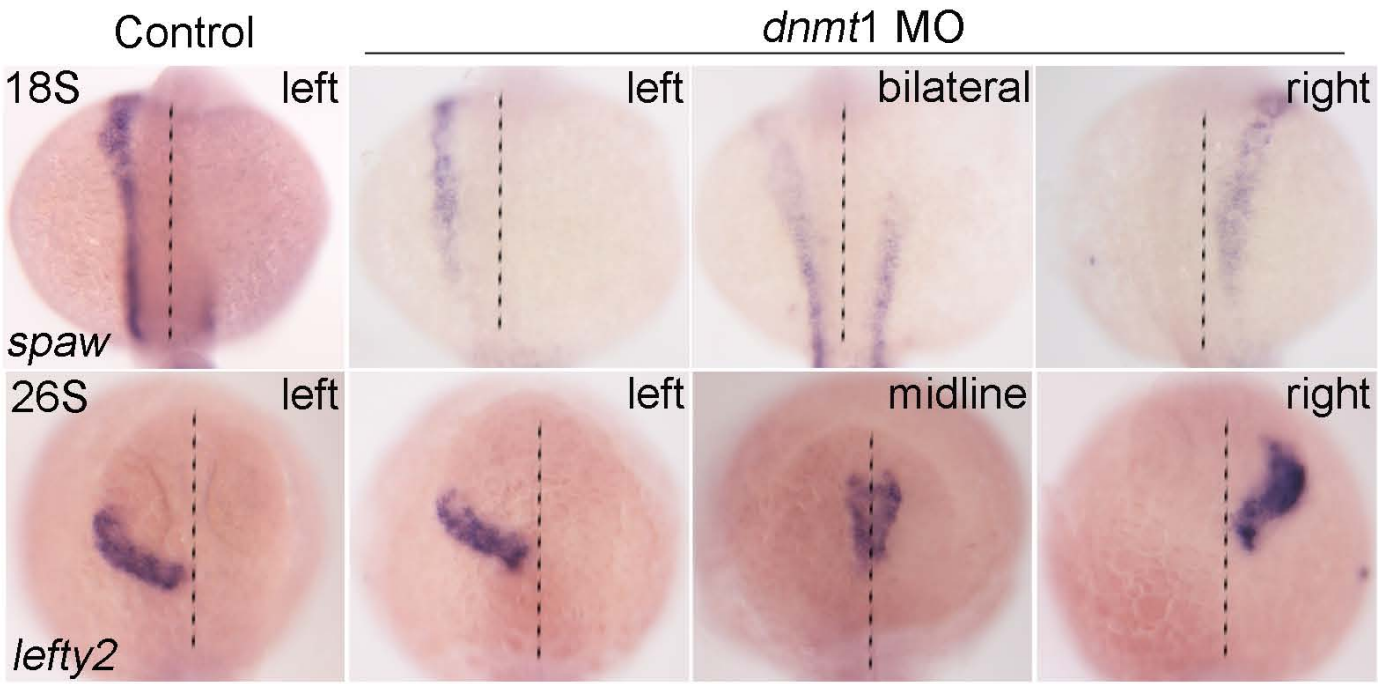
Appendix Fig S1

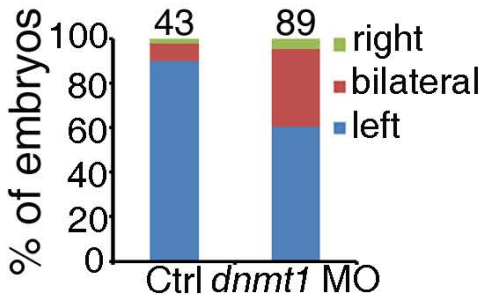


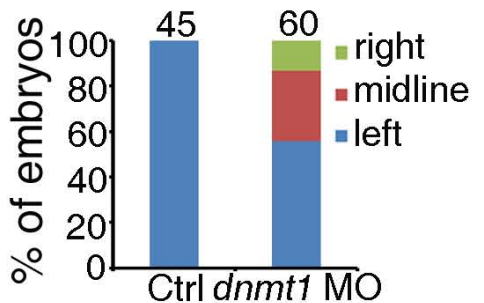
Appendix Fig S2



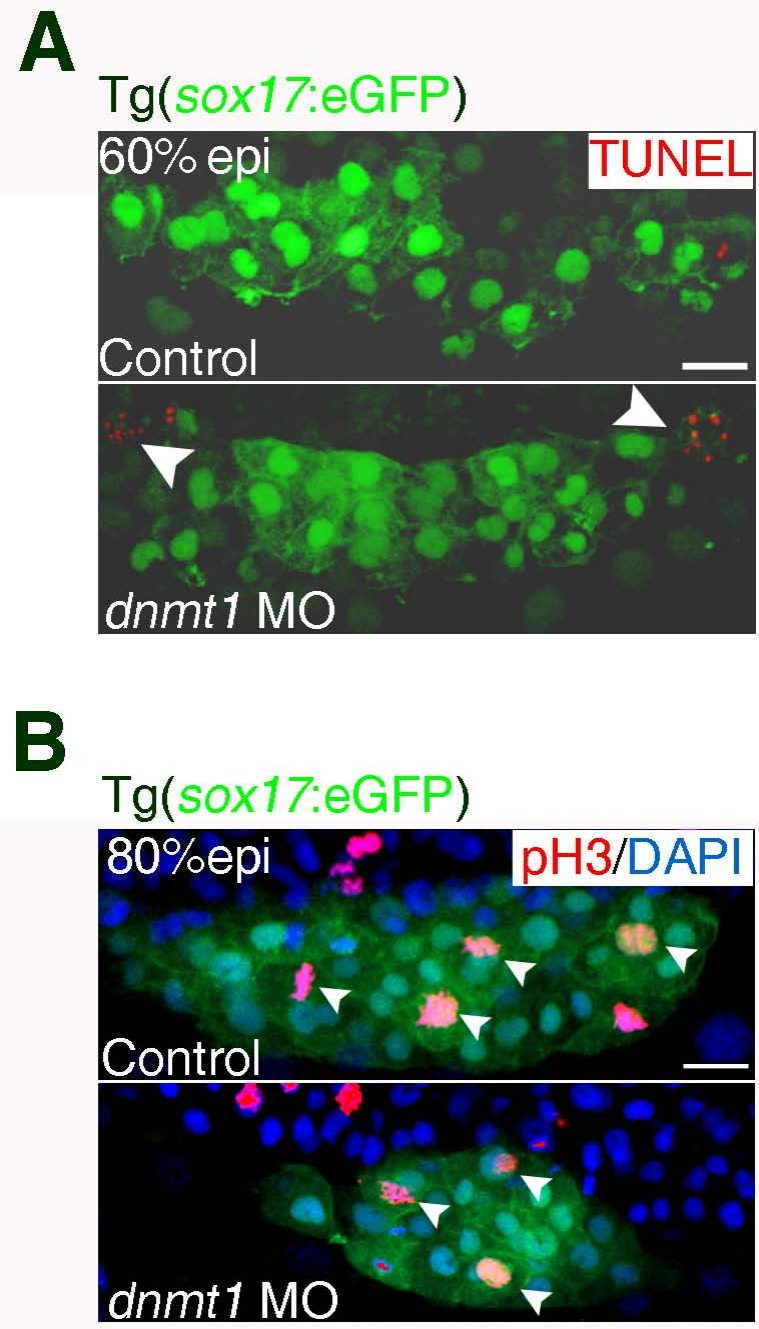
Appendix Fig S3







Appendix Fig S4



Appendix Fig S5