Expanded View Figures

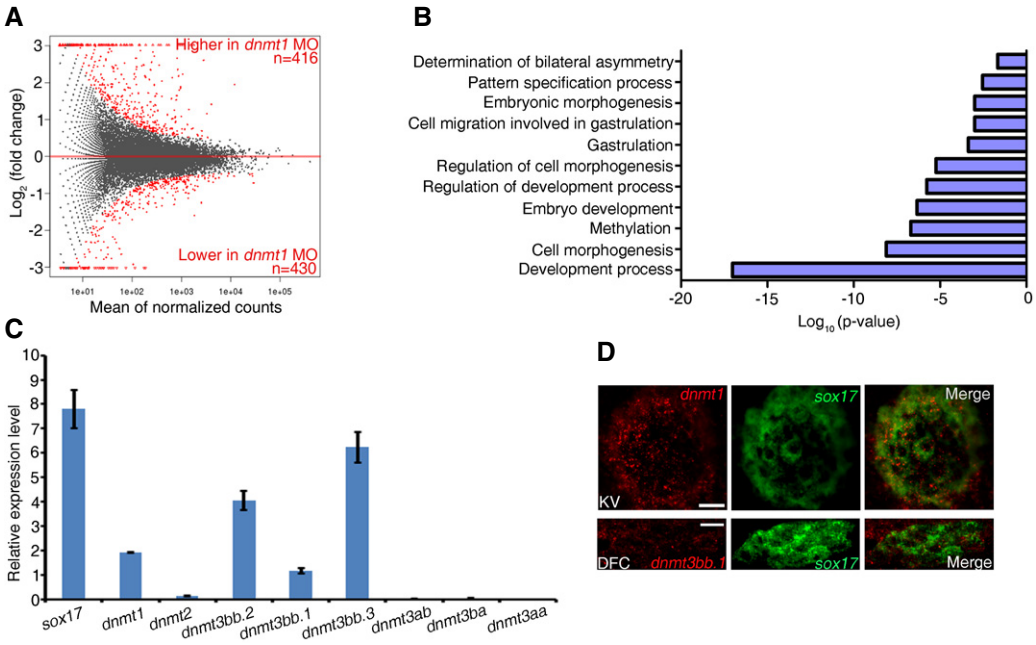

Figure EV1. DNA methyltransferases expressed in LR organizer.

A Differentially expressed genes in *dnmt1* morphants ($P < 0.05$).

B Representative GO terms enriched in differentially expressed genes in *dnmt1* morphants.

C Expression level of DNA methyltransferases in DFCs. Error bars, mean ± SD, $n = 3$ technical replicates. Student's *t*-test.

D Double FISH revealed that *dnmt1* and *dnmt3bb.1* are co-expressed with *sox17* in KV and DFC region. Scale bar, 20 μm.

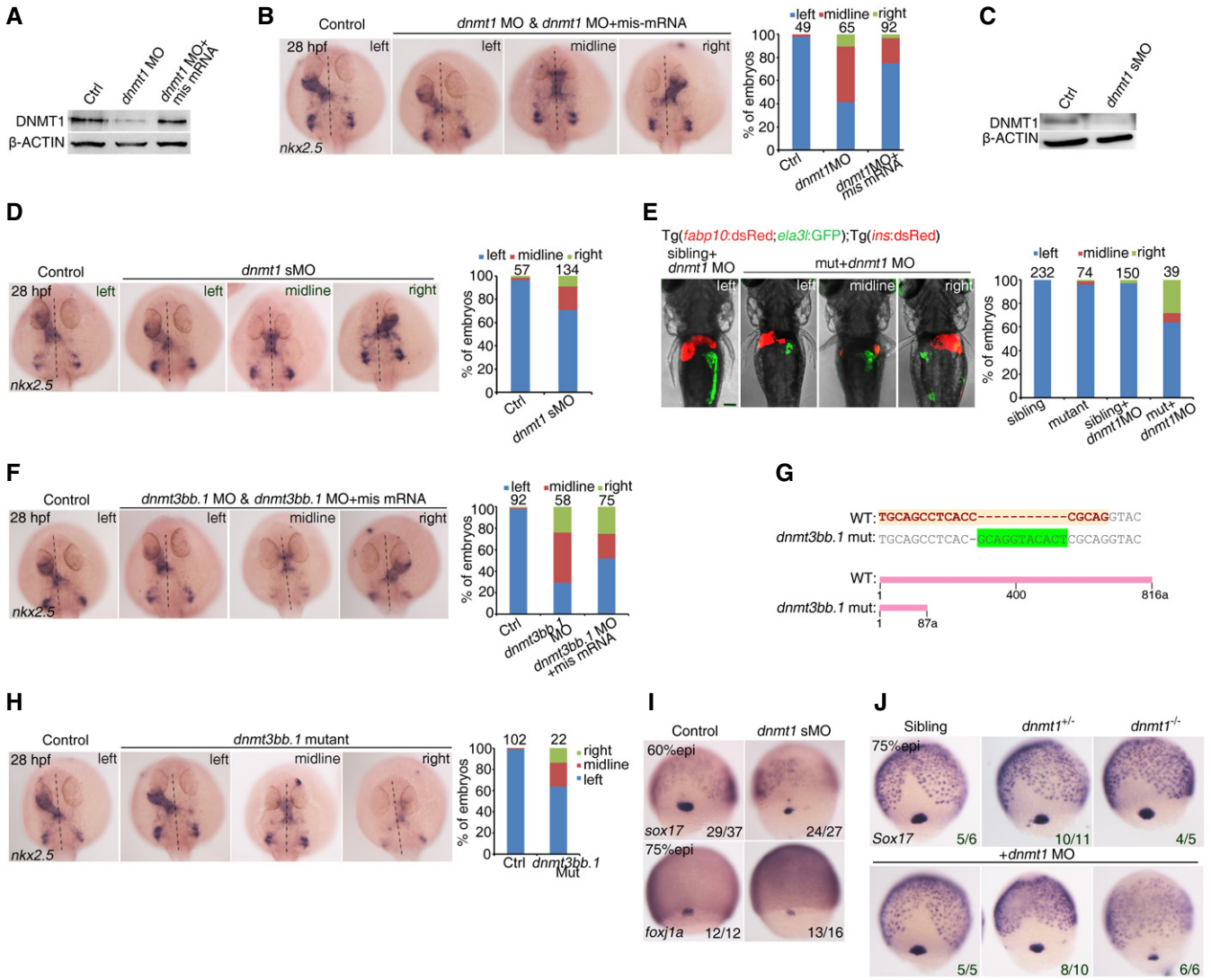

Figure EV2. Deficiency of *dnmt1* or *dnmt3bb.1* causes defects of organ patterning and DFC development.

A Protein level of Dnmt1 in the control, *dnmt1* morphants, *dnmt1* MO and mis-mRNA-co-injected embryos at 75% epi stage by Western blotting.

B Representative images showing heart asymmetry labeled by *nkx2.5* at 30 hpf (left panel) with quantification (right panel).

C Western blotting analysis of Dnmt1 in the control and *dnmt1* splice MO-injected embryos at 75% epi stage.

D Representative images showing heart asymmetry labeled by *nkx2.5* at 30 hpf (left panel) with quantification (right panel).

E Representative images showing pattern of liver and pancreas in Tg(*fabp10*:dsRed; *ela3l*:GFP); Tg(*ins*:dsRED) embryos at 4 dpf (left panel) with quantification (right panel). Scale bar, 100 μm.

F Representative images showing *nkx2.5* expression at 30 hpf in control, *dnmt3bb.1* morphants and *dnmt3bb.1* mis-mRNA-rescued embryos (left panel) with quantification (right panel).

G Generation of *dnmt3bb.1* mutant using the CRISPR/Cas9 technique.

H Representative images showing *nkx2.5* expression at 30 hpf in control, *dnmt3bb.1* mutants (left panel) with quantification (right panel).

I The expression of *foxj1a* and *sox17* in DFCs at 75% epi stage in control and embryos injected with *dnmt1* splice MO.

J The expression of *sox17* in DFCs at 75% epi stage was reduced in *dnmt1* homozygous mutant injected with a lose-dose *dnmt1* MO.

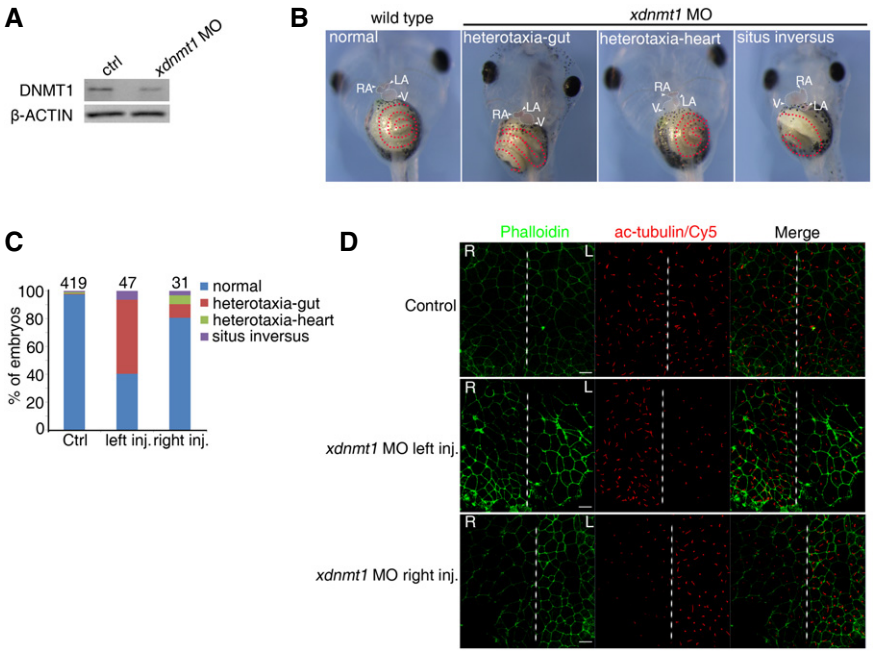

Figure EV3. Knockdown of *dnmt1* in *Xenopus* disrupts left–right asymmetry and ciliogenesis.

A Western blotting analysis showing the protein level of DNMT1 in the control and *xdnmt1* morphants at stage 45.

B, C Representative imaging showing heart and gut looping in *Xenopus* embryos at stage 45 (B) with quantification (C). (B) The white and red dashed lines mark heart and gut, respectively.

D Visualization of cilia in GRP using anti-acetylated tubulin immunofluorescence with gastrocoel roof cells labeled by Alexa488-phalloidin staining, showing that the cilia length and number were decreased on the side that received the *xdnmt1* MO injection, but not on the uninjected side. Scale bar, 20 μm.

Source data are available online for this figure.

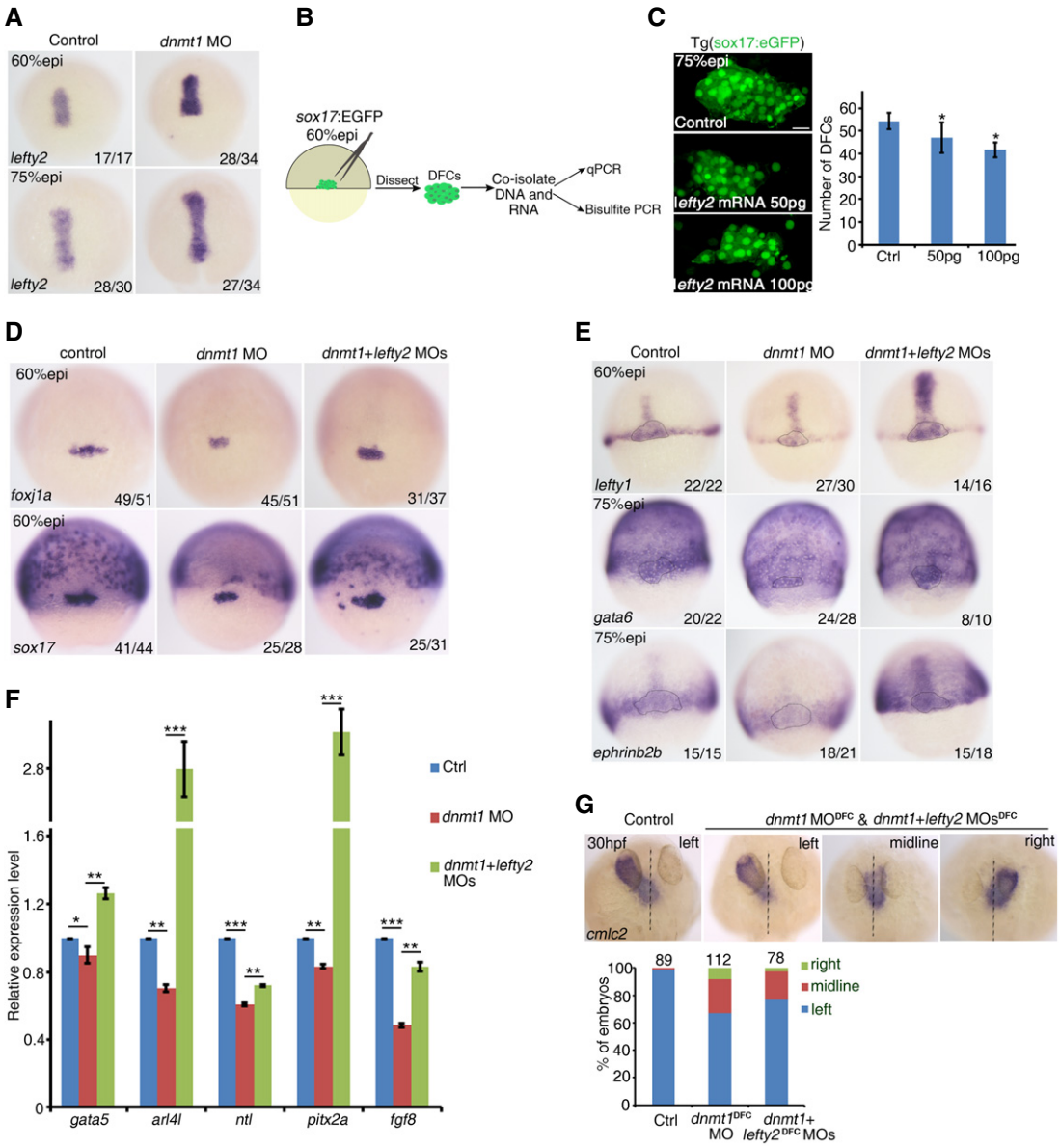

Figure EV4. *lefty2* deficiency rescues DFC specification defects through restoration of Nodal signaling.

A The expression of *lefty2* at 60% epi and 75% epi stage in *dnmt1*-deficient embryos was upregulated, compared to control embryos.

B Schematic diagram showing the experimental procedure for the isolation of GFP-positive DFCs from *sox17*:GFP transgenic zebrafish and using DNA and RNA extracted from these DFCs for bisulfite PCR or qPCR.

C *lefty2* mRNA overexpression decreased the number of *sox17*$^{+}$ DFCs at 75% epi stage. Scale bar, 20 μm.

D The reduced expression of *foxj1a* and *sox17* at 60% epi in *dnmt1* morphants was restored by *lefty2*-MO co-injection.

E *lefty2* MO partially rescued the reduced expression of *lefty1*, *gata6* and *ephrinb2b* in DFCs of *dnmt1* morphants. The dotted black outlines denote DFC region.

F qPCR analysis of Nodal target genes in control embryos, *dnmt1* morphants, and *dnmt1* morphants co-injected with *lefty2* MO.

G Representative images showing heart pattern labeled by *cmlc2* at 30 hpf (upper panel) with quantification (lower panel).

Data information: Error bars, mean ± SD, $n \geq 5$ embryos per experiment, $n \geq 2$ technical replicates (C) and $n = 3$ technical replicates (F). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Student's *t*-test. (A, D and E) Numbers indicate the number of embryos with the respective phenotype/total number of embryos analyzed in each experiment.

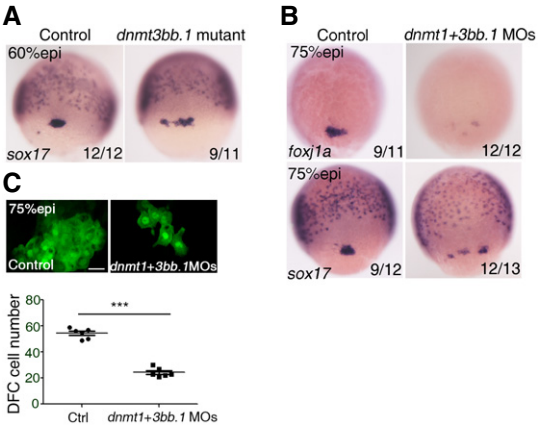

Figure EV5. Deficiency of DFC development in *dnmt3bb.1* mutant and *dnmt1* + *dnmt3bb.1* double-deficient embryos.

A The expression of *sox17* in DFCs at 75% epi stage was disrupted in *dnmt3bb.1* mutant embryos.

B The expression of *foxj1a* and *sox17* in DFCs in *dnmt1* + *3bb.1* MO-injected embryos at 75% epi stage.

C DFCs were visualized at 60% epi stage in Tg(*sox17*:eGFP) zebrafish embryos injected with control MO or *dnmt1* + *3bb.1* MOs (upper panel) with quantification (lower panel). Data are shown as mean ± SD. ***$P < 0.001$. Scale bar, 20 μm. $n \geq 5$ embryos per experiment and $n \geq 2$ technical replicates. Student's *t*-test.