Supporting Information

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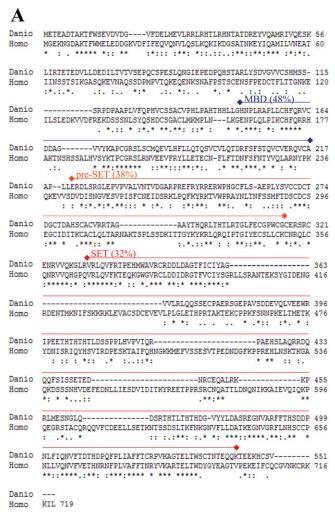


Fig. S1. Alignment of the amino acid sequences of zebrafish and human setdb2. Sequence alignment was performed with ClustalW program. The percentages in the brackets denote the amino acid identities of each domain.

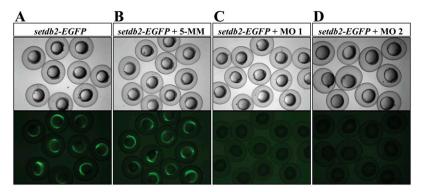


Fig. S2. Efficiency and specificity of setdb2 morpholino oligonucleotides. (A–D) Light and fluorescent images of 12-hpf embryos injected with a reporter EGFP mRNA alone (A), a reporter EGFP mRNA plus 5-MM control morpholino (B), a reporter EFGP mRNA plus either setdb2 MO1 (C), or setdb2 MO2 (D). The sequence of partial 5'-UTR plus 177 bp immediately downstream of setdb2 ATG was fused to the ATG-deleted ORF of the EGFP gene as a reporter to test the knockdown efficiency and specificity of setdb2 morpholinos.

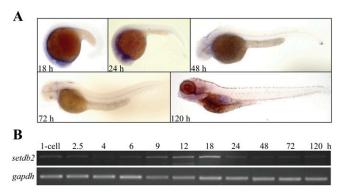


Fig. S3. Spatial and temporary expression of setdb2 during embryogenesis. (A) Whole-mount in situ hybridization analyses of setdb2 expression patterns at the indicated developmental stages. Embryos are lateral view with head to the left. (B) Semiquantitative RT-PCR analyses of setdb2 expression at the indicted developmental stages with gapdh as an internal control.

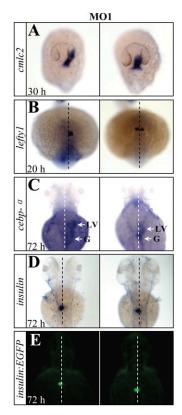


Fig. S4. Organ laterality is randomized in setdb2-knockdown embryos with MO1. (A–D) Whole-mount mRNA in situ hybridization of visceral and diencephalic laterality in the setdb2 MO1-injected embryos at the indicated developmental stages with probes cmlc2 (A), lefty1 (B), cebpα (C, arrows), and insulin (D). (E) Determination of pancreatic laterality in 72-hpf Tg(insulin:EGFP) transgenic embryos injected with setdb2 MO1. Dashed lines denote the embryonic midline. LV, liver; G, gut. All embryos are dorsal views with head to the top.

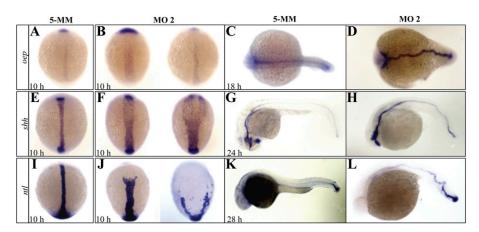


Fig. S5. Defective midline development in setdb2-knockdown embryos. (A–L) Whole-mount in situ hybridization analyses of midline markers oep (A–D), shh (E–H), and ntl (I–L) in the 5-MM control and setdb2 MO2-injected embryos at the indicated developmental stages. Embryos are dorsal views with head to the top in panels A, B, E, F, I, and I and to the left in panels I0 and I1.

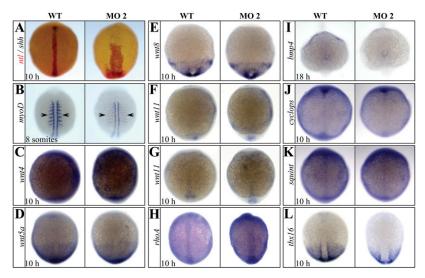


Fig. S6. The expression of components of noncanonical Wnt and TGF- β signaling transduction pathway and T-box gene family were not affected in setdb2-knockdown embryos. (A) Double in situ hybridization analyses of ntl (red) and shh (black) in the 10-hpf embryos injected with 5-MM control and setdb2 MO2. (B–L) Whole-mount in situ hybridization analyses of myod (B), wnt4 (C), wnt5a (D), wnt8 (E), wnt11 (F, lateral view), wnt11 (G, dorsal view), rhoA (H), bmp4 (I), cyclops (J), squint (K), and tbx16 (L) in the 10-hpf embryos injected with 5-MM control and setdb2 MO2. Embyros are dorsal views in panels A–E and G–L, and lateral views in panel F.

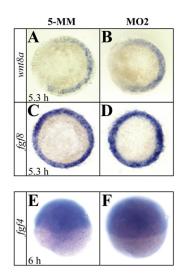


Fig. 57. Earlier stages of gastrulation and expression of fgf4 is not affected in setdb2-knockdown embryos. (A–D) Whole-mount in situ hybridization analyses of the expression of wnt8a and fgf8 in the 5-MM control and setdb2 MO2-injected embryos at 5.3 hpf. Embryos are shown as animal views. (E and F) Whole-mount in situ hybridization analyses of the expression of fgf4 in the 5-MM control and setdb2 MO2-injected embryos at 6 hpf. Embryos are lateral views with animal pole to the top.

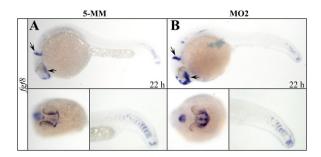


Fig. S8. Expression of *fgf8* is increased in *setdb2*-knockdown embryos at 22 hpf. (A and B) Whole-mount in situ hybridization analyses of *fgf8* expression in the 22-hpf embryos injected with 5-MM control and *setdb2* MO2. Embryos are lateral views with head to the left in the *Upper Panels*, anterior-ventral views in the *Lower Left Panels*, and posterior-lateral views in the *Lower Right Panels*.