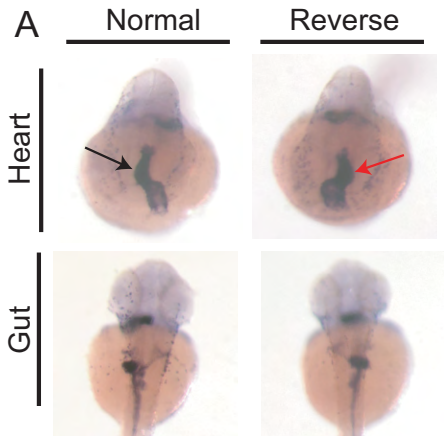
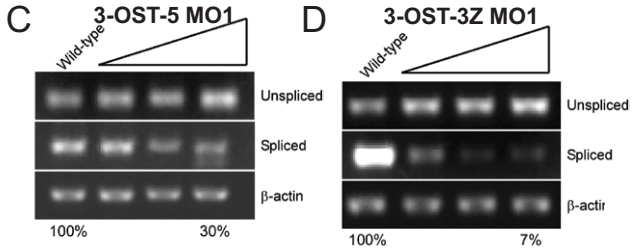


B

Gene	Splice State	Primer
3-OST-5	spliced	5'-GGAATAGAGAAGAATGAAAGTCCC-3'
		5'-GTTCATGAAGACAAACAAGCAGC-3'
	Unspliced	5'-AATGATTCGCTTGATGTTTTTTGGGG-3'
		5'-GTTCATGAAGACAAACAAGCAGC-3'
3-OST-3Z	Spliced	5'-GCTTCCTCAGGCAATCATCATCG-3'
		5'-GTCCCGGACAACCACTATCAGC-3'
	Unspliced	5'-GCTTCCTCAGGCAATCATCATCG-3'
		5'-GTCGTCAGGCTCGTGAGAAAATG-3'
B-actin	Load control	5'-GGTATGGGACAGAAAGACAG-3'
		5'-AGAGTCCATCACGATACCAG-3'



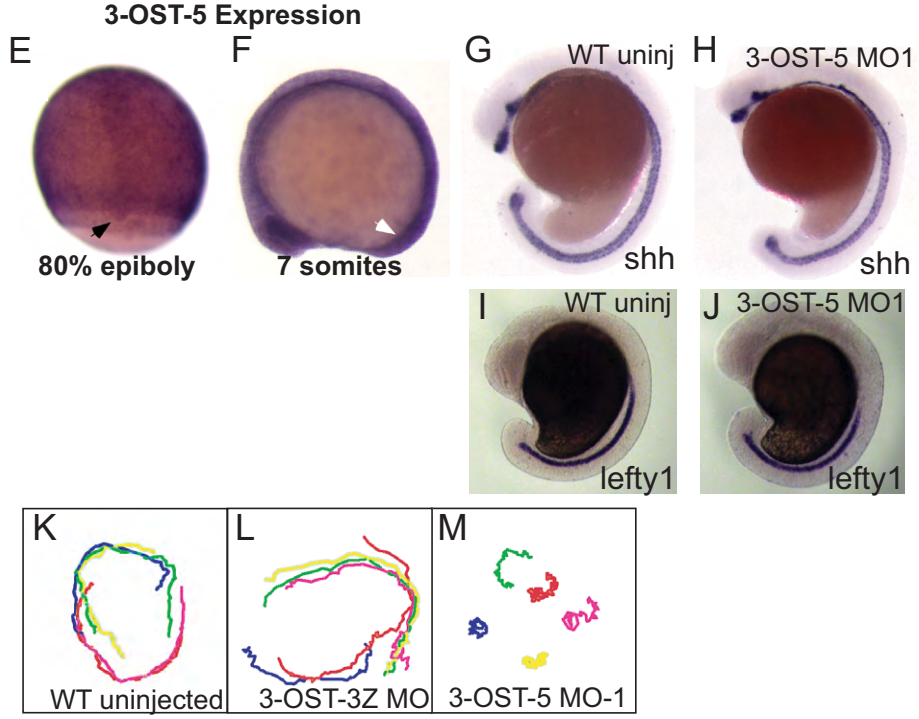
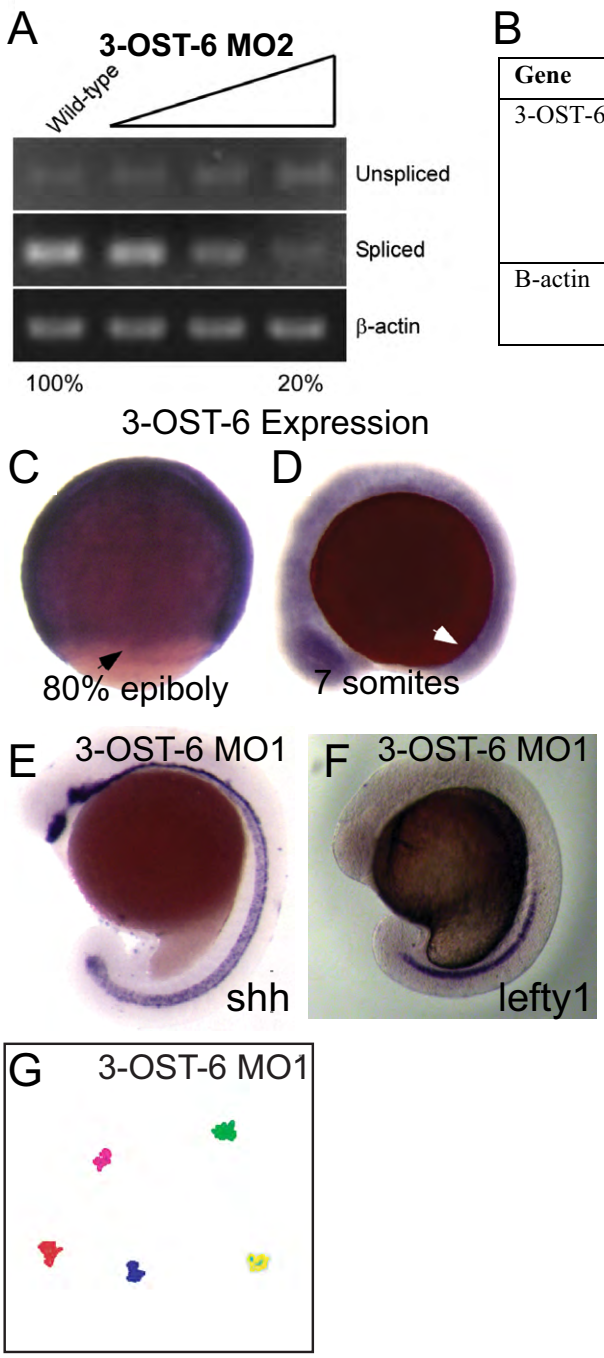


Fig. S1. Splice-blocking efficacy, primers used to assay splicing, left-right markers examined in 3-OST-5 morphants, tracks of fluorescent beads injected into the KV at 6 SS, 3-OST-5 RNA expression, midline marker expression. (**A**) Representative *in situ* images illustrating normal (black arrows) and reversed (red arrows) heart (*cmlc2*) and gut (*fkd2*) looping in 3-OST-5 morphants. (**B**) Table containing primers used for detecting spliced versus unspliced transcript when embryos were injected with translational splice blocking morpholinos. (**C**) Dose-dependent splice blocking efficacy of 3-OST-5 MO1. Increasing amounts of MO injected resulted in a 70±12% decrease of spliced mRNA. (**D**) Dose-dependent splice blocking efficacy of control 3-OST-3Z MO1. Increasing MO dose resulted in a 93±4% decrease of spliced mRNA. ImageQuant TL software was used to semi-quantitatively measure band intensity. The values listed are the percentage of spliced product compared with uninjected WT. (**E**,**F**) Whole-mount *in situ* analysis of 3-OST-5 mRNA, which was expressed ubiquitously throughout the embryo during epiboly (E) and early somitogenesis (F). Black arrow indicates staining in DFCs, white arrow indicates the position of KV. (**G**,**H**) Whole-mount *in situ* analysis of *shh* expression in uninjected (G) and 3-OST-5 morphants (H). (**I**,**J**) Whole-mount *in situ* analysis of *lefty1* expression in the midline of uninjected (I, *n*=113) and 3-OST-5 morphants (J, *n*=82), 3-OST-5 morphants had normal expression of *lefty1* expression in the midline. (**K-M**) Tracks of fluorescent beads injected into KV of uninjected (K) and 3-OST-3Z MO1 embryos (L) with normal bead flow, and 3-OST-5 MO1 embryos (M), displaying uncoordinated bead movement due to short but motile cilia.



B

Gene	Splice State	Primer
3-OST-6	Spliced	5'-GCTTCCACACGCGCTGATCATC-3'
		5'-ATGCGGCCTGGAGTTTCTGGAGTC-3'
	Unspliced	5'-GCTTCCACACGCGCTGATCATC-3'
		5'-AGCCCATATACATTGTTTGCAACC-3'
B-actin	Load control	5'-GGTATGGGACAGAAAGACAG-3'
		5'-AGAGTCCATCACGATACCAG-3'

Fig. S2. Splice-blocking efficacy, primers used to assay splicing, tracks of fluorescent beads injected into the KV at 6 SS, 3-OST-6 RNA expression, midline marker expression. (**A**) Analysis of 3-OST-6 MO2 splice-blocking efficacy. Increasing amounts of MO injected resulted in an 80±20% decrease of spliced mRNA. Analysis was performed as described in Fig. S1. (**B**) Table containing primers used for detecting spliced versus unspliced transcript when embryos were injected with translational splice blocking morpholinos. (**C**,**D**) Whole mount *in situ* analysis of 3-OST-6 mRNA, which was expressed ubiquitously throughout the embryo during epiboly (C) and early somitogenesis (D). Black arrow indicates staining in DFCs, white arrow indicates the position of KV. (**E**) Whole-mount *in situ* analysis of *shh* expression, which was normal in 3-OST-6 morphants (*n*=109). (**F**) Whole-mount *in situ* analysis of *lefty1* expression in the midline, was normal in 3-OST6 morphants (*n*=49). (**G**) Bead tracks of fluorescent beads injected into 3-OST-6 MO1 embryos showing a lack of bead movement, indicative of non-motile cilia.

Movie 1. Uninjected control embryos, normal KV flow. Embryos were injected at 8 SS with fluorescent beads and filmed by DIC microscopy (to show orientation of notochord, to the left of each panel) and then by fluorescent imaging to capture bead movement.

Movie 2. 3-OST-3Z MO, normal KV flow. Embryos were injected at 8 SS with fluorescent beads and filmed by DIC microscopy (to show orientation of notochord, to the left of each panel) and then by fluorescent imaging to capture bead movement.

Movie 3. 3-OST-5 MO, aberrant KV flow. Embryos were injected at 8 SS with fluorescent beads and filmed by DIC microscopy (to show orientation of notochord, to the left of each panel) and then by fluorescent imaging to capture bead movement.

Movie 4. 3-OST-6 MO, absent KV flow. Embryos were injected at 8 SS with fluorescent beads and filmed by DIC microscopy (to show orientation of notochord, to the left of each panel) and then by fluorescent imaging to capture bead movement.