Supplementary data

Supplementary Materials and Methods

Targeted genome mutagenesis in zebrafish by CRISPR/cas9 system

We identified the *nlz1* specific CRISPR target sequence 5′GGGATCACGGTACGGCAGTG3′ from UCSC Genome Browser in "CRISPRS" track of the "Zebrafish Genomics" data hub of "Burgess Lab Zebrafish Genomic Resources" (http://genome.ucsc.edu/cgi-bin/hg HubConnect) (LaFave et al., 2014). The Sg RNA template assembly, Single –guide RNA (Sg RNA) synthesis and *in-vitro* Cas-9 (Jao et al., 2013) mRNA synthesis were performed as described in (Varshney et al., 2015). Embryos at 1-cell stage were co-injected with 250 pico-gram (pg) cas9 mRNA and 25 pg *nlz1* Sg RNA. The injected embryos were raised to adulthood (putative F0 founders) to screen for germline transmission of CRISPR/cas9induced mutation. Three pairs of putative F0 founder males and females were inter-crossed in one tank to generate F1 embryos and to observe any visible phenotypic changes at 48h of development. F1 embryos with visible *nlz1* morphant like phenotype were selected and imaged using Leica Sp2 (Leica, Switzerland) microscope. The genomic target region (250bp) in F1 embryos were PCR amplified using forward and reverse primers (5′GTCGTACCTCAATGCTCACG3′, 5′CCTGGATTTGCAGTCAGTCC3′) flanking the *nlz1* target site in the genome. The PCR product was cloned into PCR2.1-TOPO vector and recombinant clones were sequenced as described in (Jao et al., 2013).

Plasmid Constructs

The ORF of human (H) *NLZ1*, and H*FOXJ1* were PCR amplified and sub-cloned into into pCDNA3 vector. The genomic region containing ~2 kb upstream of the *HNLZ1* translation start site was PCR amplified and the fragment was cloned into pGL4 luciferase reporter vector (Promega), designated as 2kb*NLZ1 –luc*.

ChIP assay

ChIP was performed with the Active Motif ChIP-IT Express kit and ChIP-IT Control Kits. Cells were transfected with the H*FOXJ1* construct. Chromatin was extracted and sheared by sonication following the Active Motif ChIP-IT protocol, then immunoprecipitated using a mouse monoclonal IgG anti-Foxj1 antibody (Santa Cruz Biotechnology), a mouse monoclonal RNA Polymerase II antibody as a positive control, and a mouse monoclonal IgG antibody as a negative control. DNA was de-crosslinked, purified, and analyzed by PCR.

Supplementary References

Jao, L.E., Wente, S.R., Chen, W., 2013. Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. Proceedings of the National Academy of Sciences of the United States of America 110, 13904-13909.

LaFave, M.C., Varshney, G.K., Vemulapalli, M., Mullikin, J.C., Burgess, S.M., 2014. A defined zebrafish line for high-throughput genetics and genomics: NHGRI-1. Genetics 198, 167-170.

Varshney, G.K., Pei, W., LaFave, M.C., Idol, J., Xu, L., Gallardo, V., Carrington, B., Bishop, K., Jones, M., Li, M., Harper, U., Huang, S.C., Prakash, A., Chen, W., Sood, R., Ledin, J., Burgess, S.M., 2015. High-throughput gene targeting and phenotyping in zebrafish using CRISPR/Cas9. Genome research 25, 1030-1042.

Supplementary Figure Legends

Fig. S1 Classification of gross morphology of *nlz1* morphant fish at 24h

(A) Class I, normal fish; Class II, embryos with abnormal phenotype, and Class III, embryos with very severe defects and dead embryos. (B-D) Bar diagrams summarize the phenotypes

resulted from injection of (B) different doses of morpholino(s), (C) rescue of morphant phenotype by RNA injection, where very low dose of MO1 and MO2 coinjection resulted in predominantly ClassII like phenotype. Coinjection dose is stated for total MO. (D) Approximately 200 embryos were injected with Nlz1 MO1 and divided into three groups, the embryos from two of these three groups were separately injected with wild-type *nlz1* mRNA and Mut *nlz1* mRNA (containing an engineered stop codon.) The phenotypes of embryos from all three groups were observed at 24h. Morphants injected with *nlz1* mRNA—but not those with Mut *nlz1* mRNA were rescued. Scale bar, 100μm.

Fig. S2 CRISPR/Cas9 mediated *nlz1* knockout alters heart laterality and motile cilia formation

(A) Wild-type embryo at 48h. After putative F0 founders (3 male and 3 female) were bred in one tank, the F1 *nlz1* mutants (B, n=20/150) had bent tail, hydrocephaly, ocular coloboma, and three otoliths in the otic vesicle. (C) *in situ* hybridization using *cmlc2* ribo probe showed different heart looping (normal looping, reverse looping, no looing) phenotypes in zebrafish embryos at 48h. Bar graph represent distribution of different heart looping phenotypes in wild-type and in *nlz1*$^{-/-}$ mutant embryos. (D) Motile cilia in the KV were labelled with acetylated -α-tubulin antibody (green), nuclei were counterstained with DAPI (blue). In nlz1$^{-/-}$ mutant embryos (n=5/5) the number of KV cilia were dramatically reduced compared to nlz1$^{+/+}$ wild-type embryos (n=10/10). (E) Sequences of the *nlz1* target region in eight F1 putative mutant progeny. All sequences had indels close to *nlz1* target region (highlighted in yellow), and 5 different nlz1 mutant alleles were recovered. The sequence variations are indicated in the right (+ insertion, - deletion), the wild-type reference allele sequence is underlined, and the protospacer- adjacent motif (PAM) is highlighted in blue. (A,B) Lateral view, anterior to left; (C) anterior view; (D)

flat mount of posterior region; a, atrium; v, ventricle; hy, hydrocephaly; ov, otic vesicle;KV, Kupffer's Vesicle; scale bar 100 μm.

Fig. S3 Nlz1 MO dose response in LR asymmetry

Bar graph represents *lefty2* expression as a measure of laterality defects with various doses of Nlz1 MO injection. Ab: absent; Bi: bilateral; R: right; L: left.

Fig. S4 *nlz1* expression pattern in zebrafish embryos

(A-F')Whole mount *in situ* hybridization of zebrafish embryos with *nlz1* riboprobe at different developmental stages as indicated at the lower bottom of each panel. mhb, mid-hindbrain boundary; e,eye; pd, pronephric duct. (C', D', E') dorsal view; (E, F, F') lateral view.

Fig.S5 *foxj1a* expression is unaltered in *nlz1* morphant embryos

(A-C) Control MO injected, and (D-F) Nlz1 MO injected embryos were *in situ* hybridized with *foxj1a* antisense RNA probe. The stages of embryos are indicated on the top. Scale bar 50μm. (A,B,D,E) dorsal view; (C,F) lateral view.

Fig. S6 Nlz1 is potentially a direct target of Foxj1a

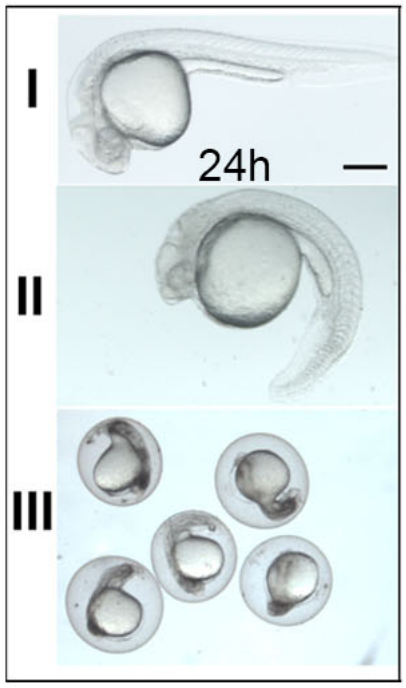
(A) Luciferase activity of transiently transfected 2kb-H*NLZ1-luc* construct in HEK293T cells. (B) ChIP assay using anti-Foxj1 antibody. IgG was used as a negative control.

Fig.S7 *foxj1a* induce ectopic *nlz1* expression

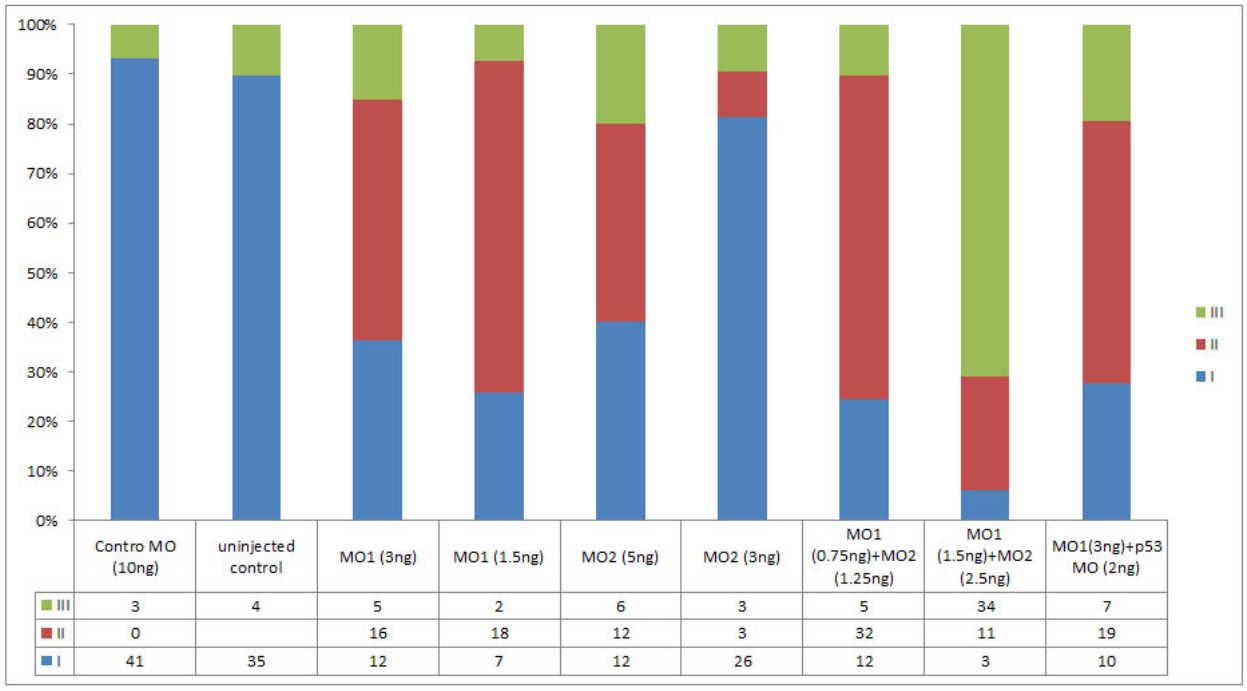
(A, A′) control embryos and (B, B′) *foxj1* mRNA injected embryos were fixed in PFA at 8-somite stage and *nlz1* expression was assayed by whole mount in-situ hybridization . Arrows indicate ectopic expression of *nlz1* (B, B′, n=28/35) in the posterior region of the embryo. (A, B) lateral view, anterior to top; (A′, B′) posterior view.

Fig S1

A

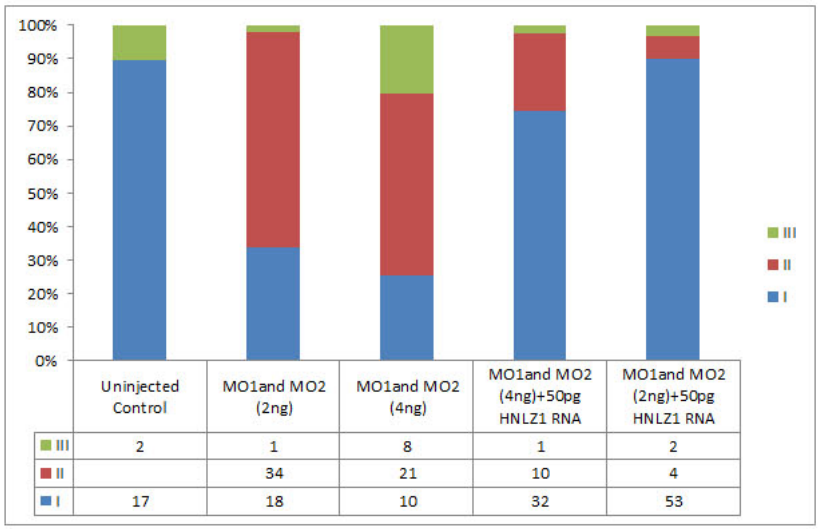


B



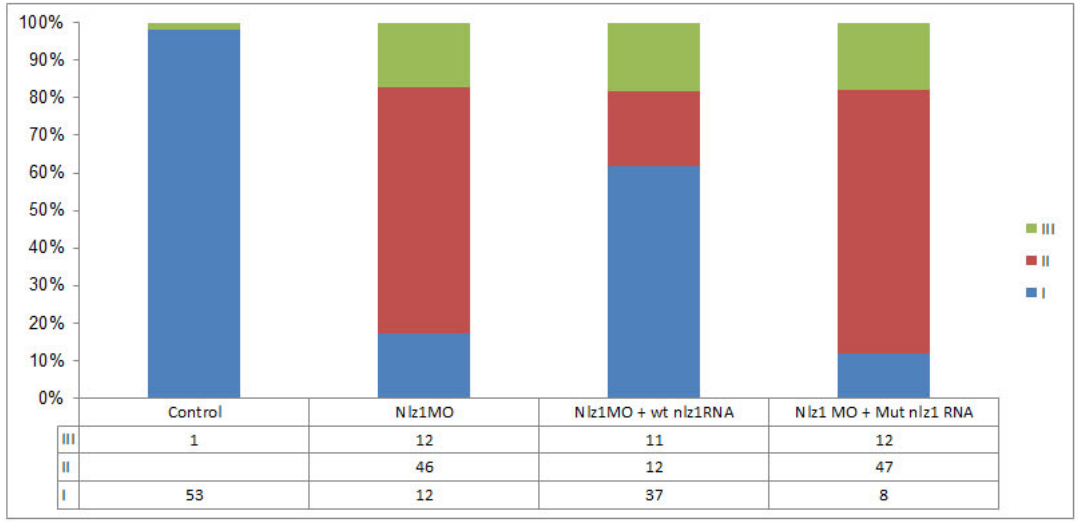
	Contro MO (10ng)	uninjected control	MO1 (3ng)	MO1 (1.5ng)	MO2 (5ng)	MO2 (3ng)	MO1 (0.75ng)+MO2 (1.25ng)	MO1 (1.5ng)+MO2 (2.5ng)	MO1(3ng)+p53 MO (2ng)
III	3	4	5	2	6	3	5	34	7
II	0		16	18	12	3	32	11	19
I	41	35	12	7	12	26	12	3	10

C



	Uninjected Control	MO1and MO2 (2ng)	MO1and MO2 (4ng)	MO1and MO2 (4ng)+50pg HNLZ1 RNA	MO1and MO2 (2ng)+50pg HNLZ1 RNA
III	2	1	8	1	2
II		34	21	10	4
I	17	18	10	32	53

D



	Control	Nlz1MO	Nlz1MO + wt nlz1RNA	Nlz1 MO + Mut nlz1 RNA
III	1	12	11	12
II		46	12	47
I	53	12	37	8

Fig S2

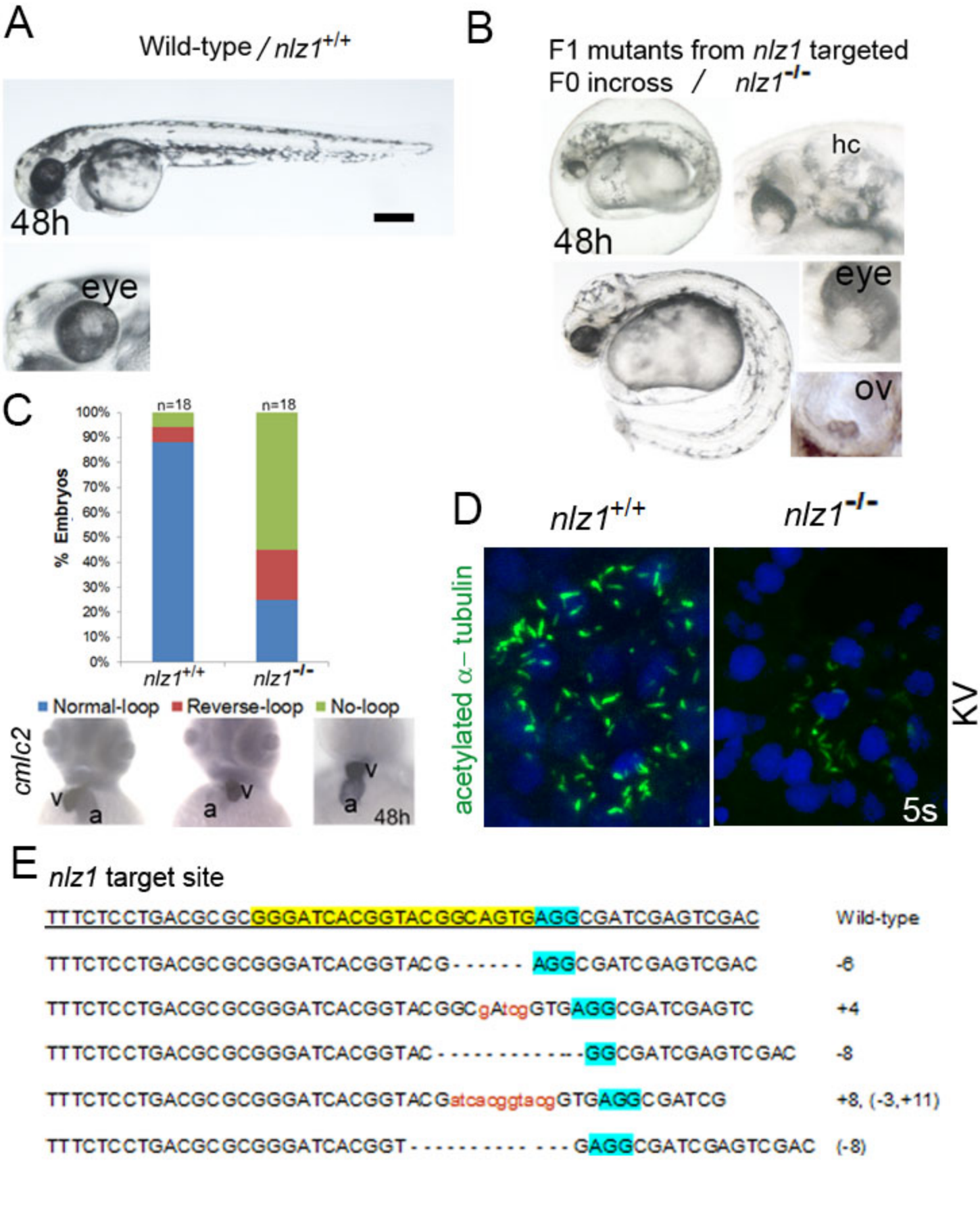
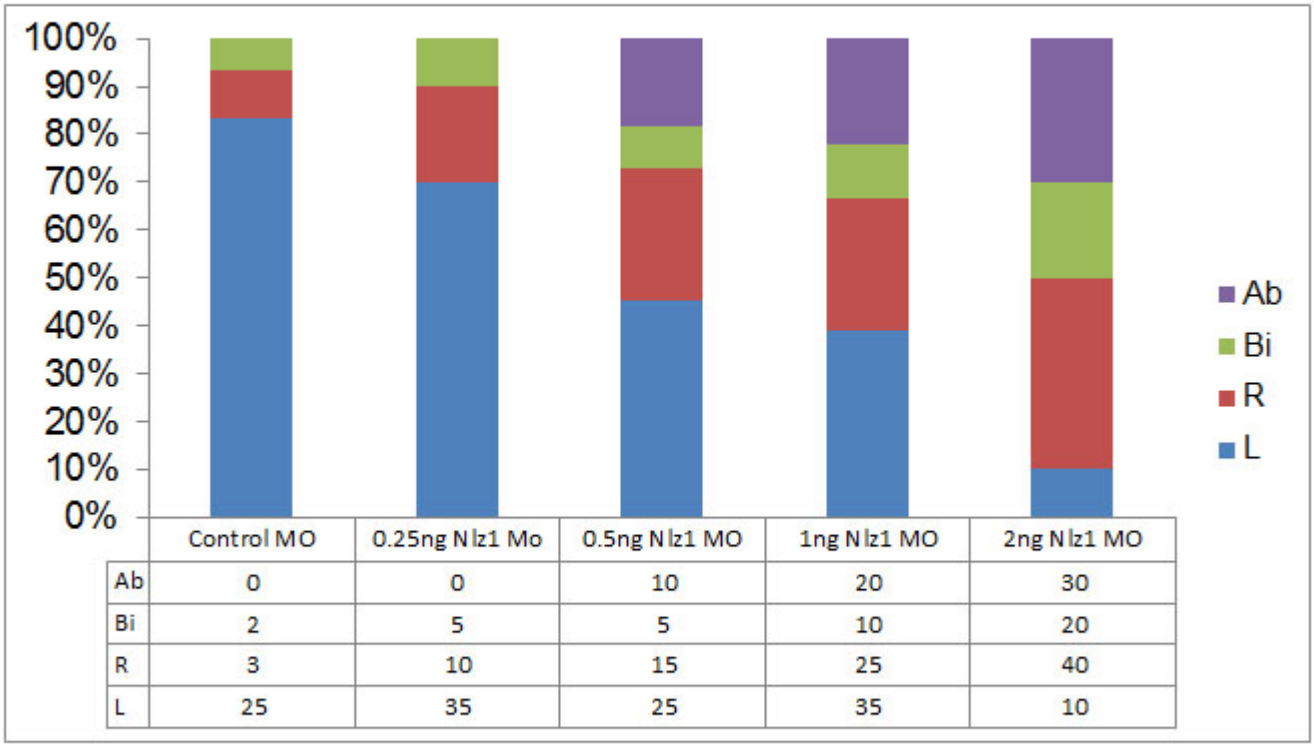

A
Wild-type / nlz1+/+
48h
eye
B
F1 mutants from nlz1 targeted
F0 incross / nlz1-/-
48h
hc
eye
ov
C
n=18
n=18
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Embryos
nlz1+/+
nlz1-/-
Normal-loop
Reverse-loop
No-loop
cmlc2
v
a
v
a
v
a
48h
D
nlz1+/+
nlz1-/-
acetylated α– tubulin
KV
5s
E
nlz1 target site
TTTCTCCTGACGCGCGGGATCACGGTACGGCAGTGAGGCGATCGAGTCGAC
Wild-type
TTTCTCCTGACGCGCGGGATCACGGTACG------AGGCGATCGAGTCGAC
-6
TTTCTCCTGACGCGCGGGATCACGGTACGGCgAtcgGTGAGGCGATCGAGTC
+4
TTTCTCCTGACGCGCGGGATCACGGTAC----------GGCGATCGAGTCGAC
-8
TTTCTCCTGACGCGCGGGATCACGGTACGatcacggtacgGTGAGGCGATCG
+8, (-3,+11)
TTTCTCCTGACGCGCGGGATCACGGT----------GAGGCGATCGAGTCGAC
(-8)

Fig S3



	Control MO	0.25ng Nlz1 Mo	0.5ng Nlz1 MO	1ng Nlz1 MO	2ng Nlz1 MO
Ab	0	0	10	20	30
Bi	2	5	5	10	20
R	3	10	15	25	40
L	25	35	25	35	10

Fig S4

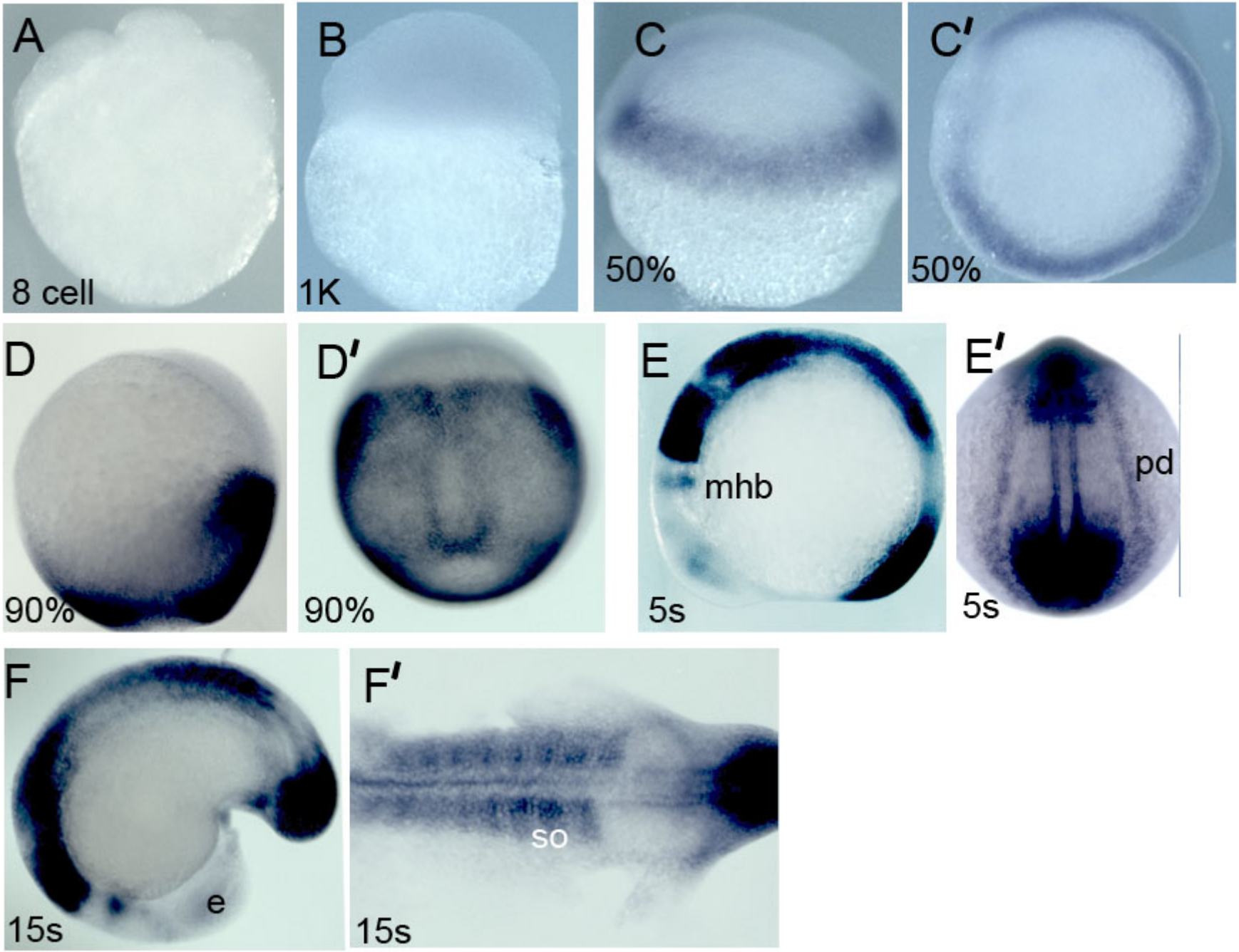


Fig S5

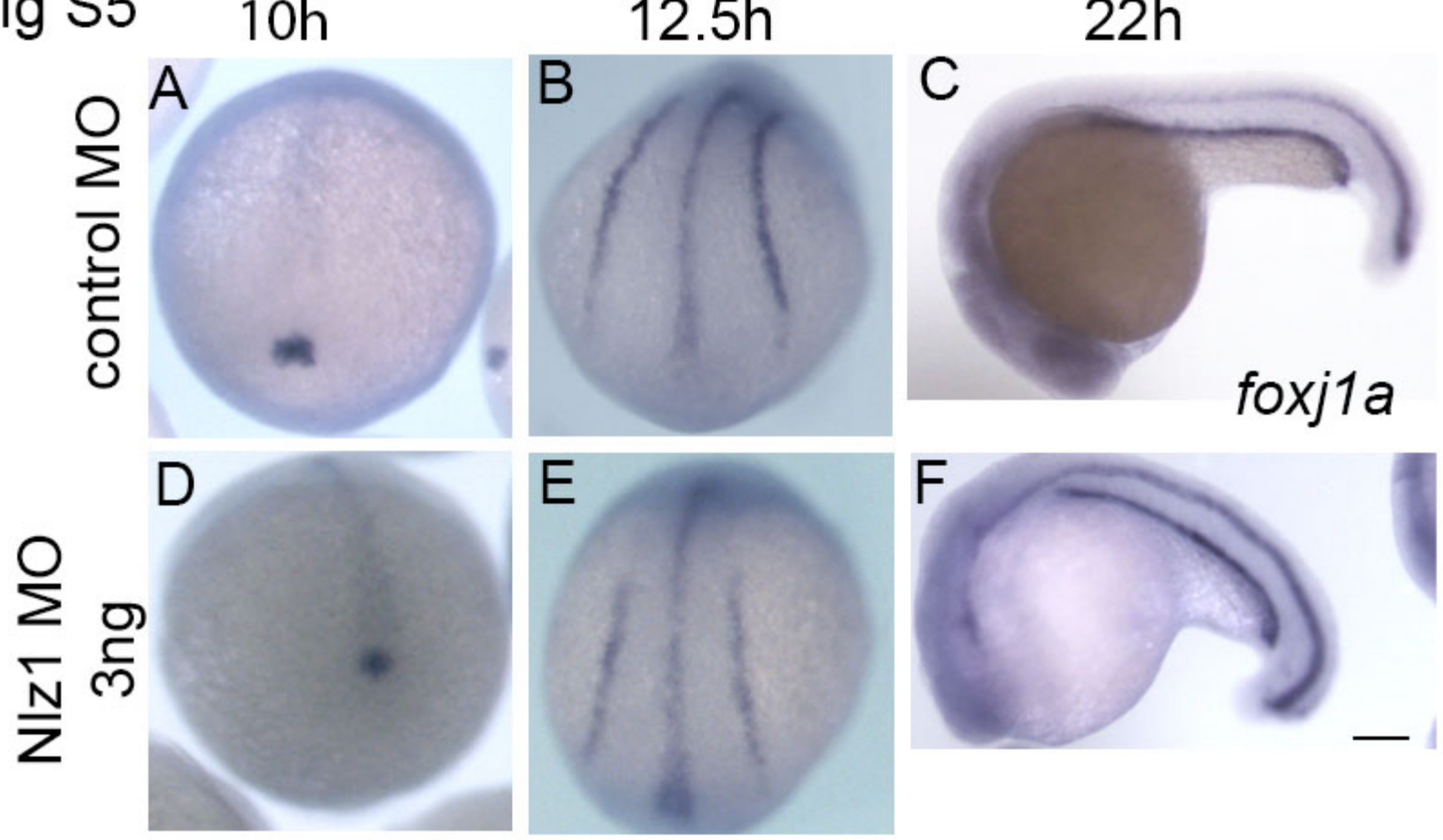
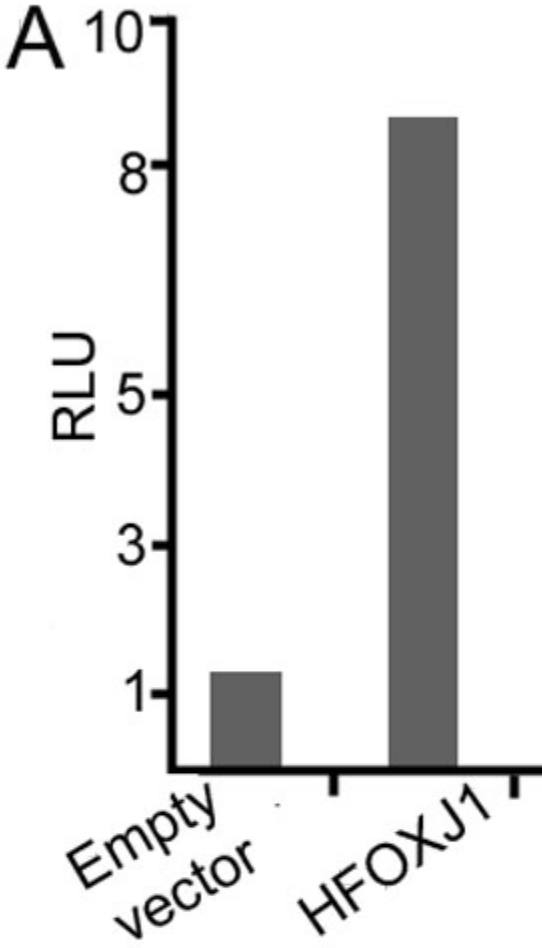


Fig S6



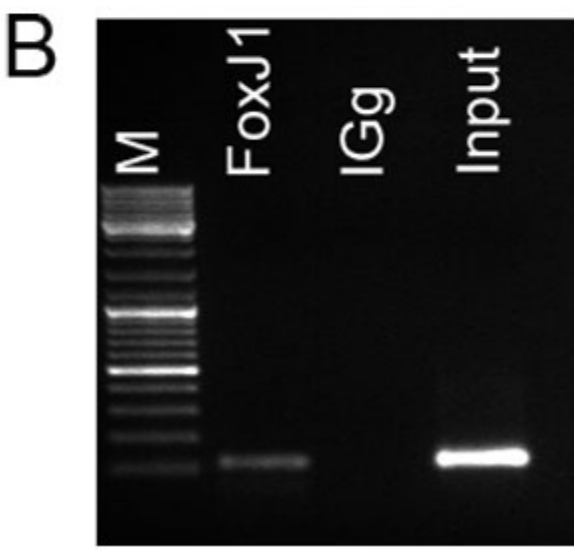


Fig S7