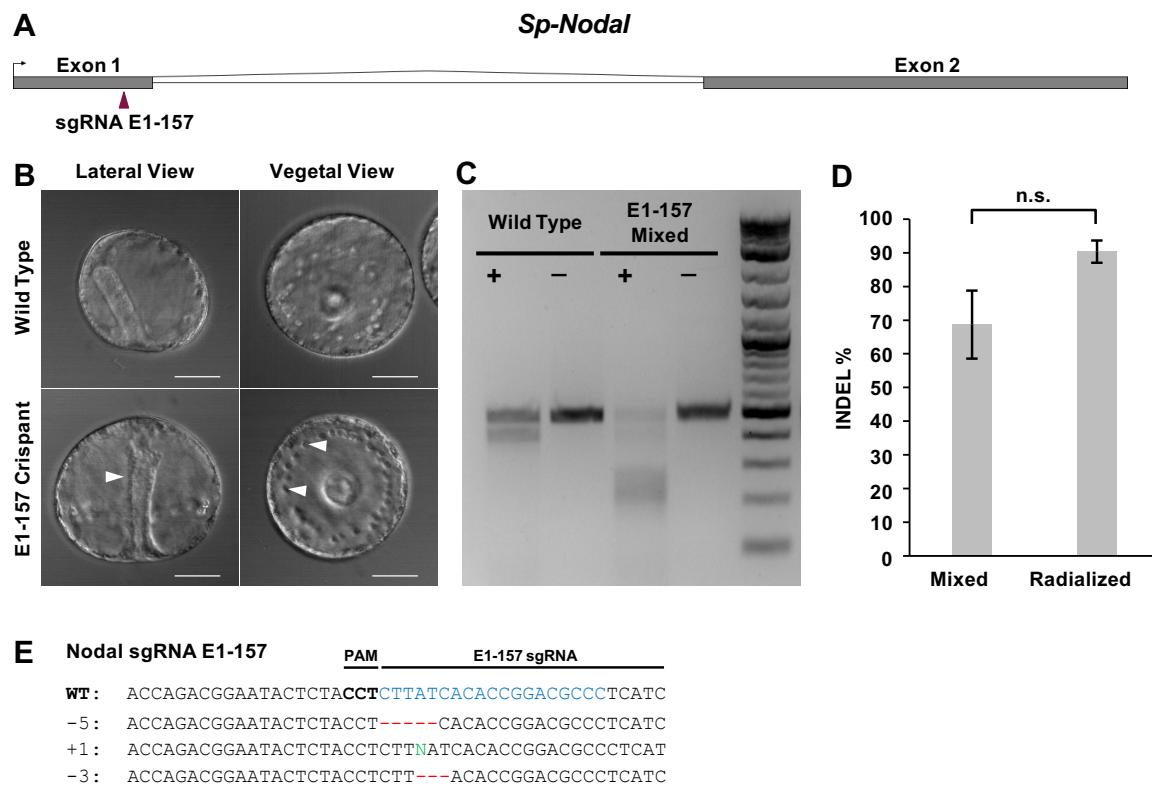
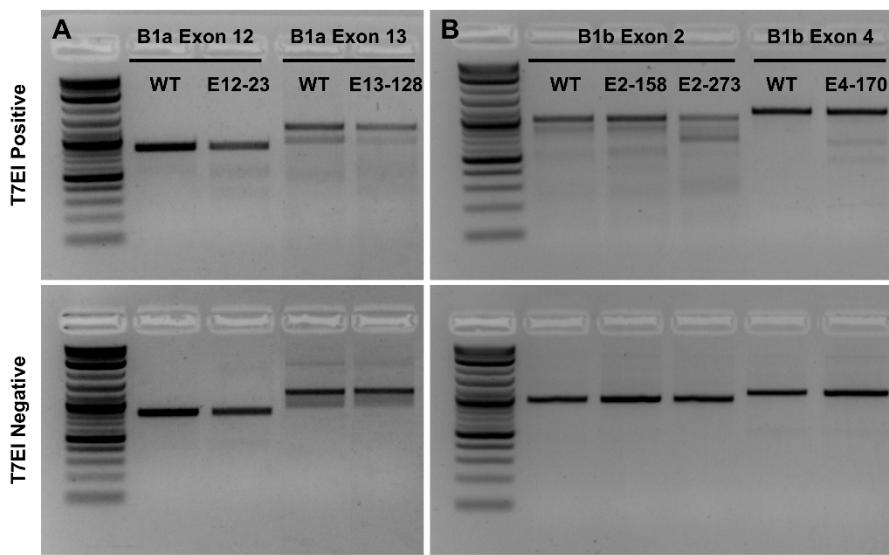


## Supplemental Figures

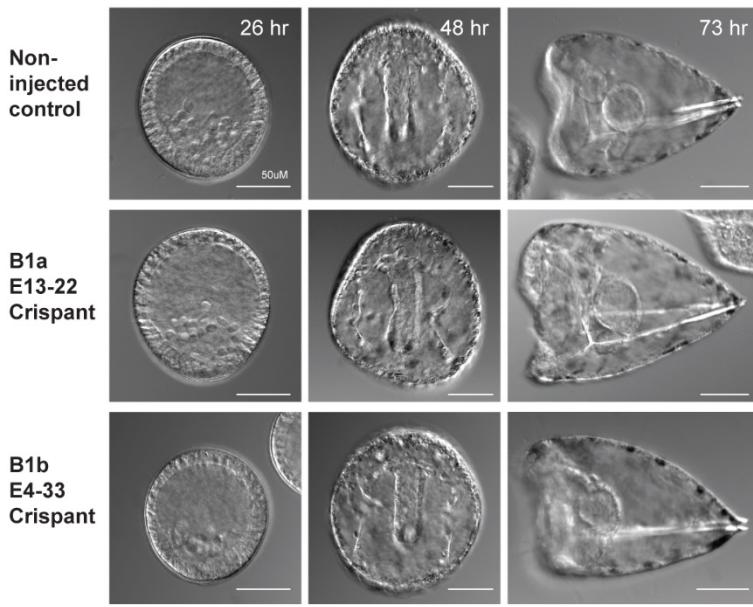


**Figure S1. CRISPR/Cas9 mutagenesis efficiency in *Sp-nodal* sgRNA E1-157 crisprants.** (A) Gene structure of *Sp-Nodal*. Red arrowhead indicates the synthetic sgRNAEx1-157 target site. (B) Lateral and vegetal views of WT and Nodal E1-157 Crispants. White arrowheads indicate the Nodal E1-157 crispant archenteron lack of bending toward the embryos' ventral side (left panels) and the altered position of primary mesenchyme cells (PMCs) (right panels). These characteristics are indicative of a radialized embryo. (C) T7EI assay of the PCR-amplified *Sp-Nodal*. Products were run on a 2% agarose gel. (+) represents T7EI-positive and (-) represents T7EI-negative reactions. (D) ICE analysis of CRISPR efficiency. Indel percentages are shown, (n = 4 batches). 'Mixed' and 'Radialized' correspond to ICE analyses of PCR amplicons from all injected embryos (a mix of WT-like and radialized embryos) and only injected embryos exhibiting the radialized phenotype, respectively. (E) ICE-predicted indels. ICE accurately predicts the identity and distribution of mutant alleles across populations of edited cells. The top

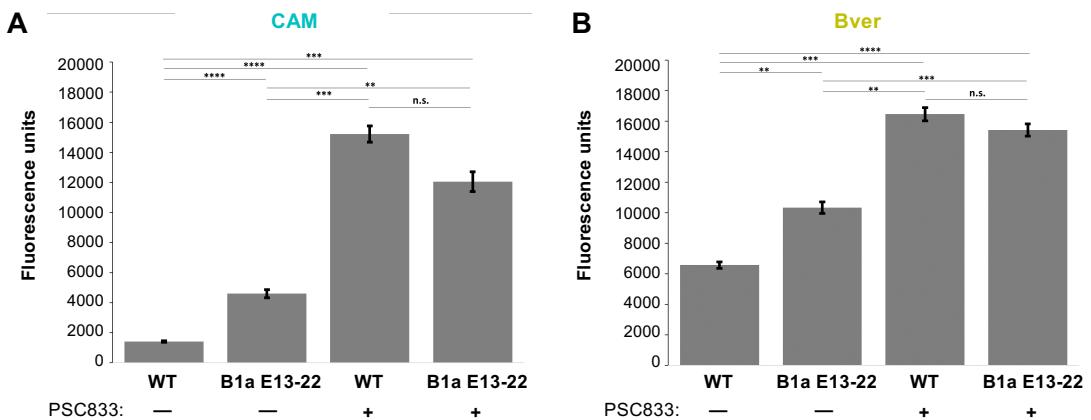
sequence for *Sp-Nodal* shows the wild type allele annotated to show the PAM site (bold) and sgRNA target sequence (blue text). The numbers on the left indicate the corresponding indel size for each mutant allele. Scale bars = 50 µM.



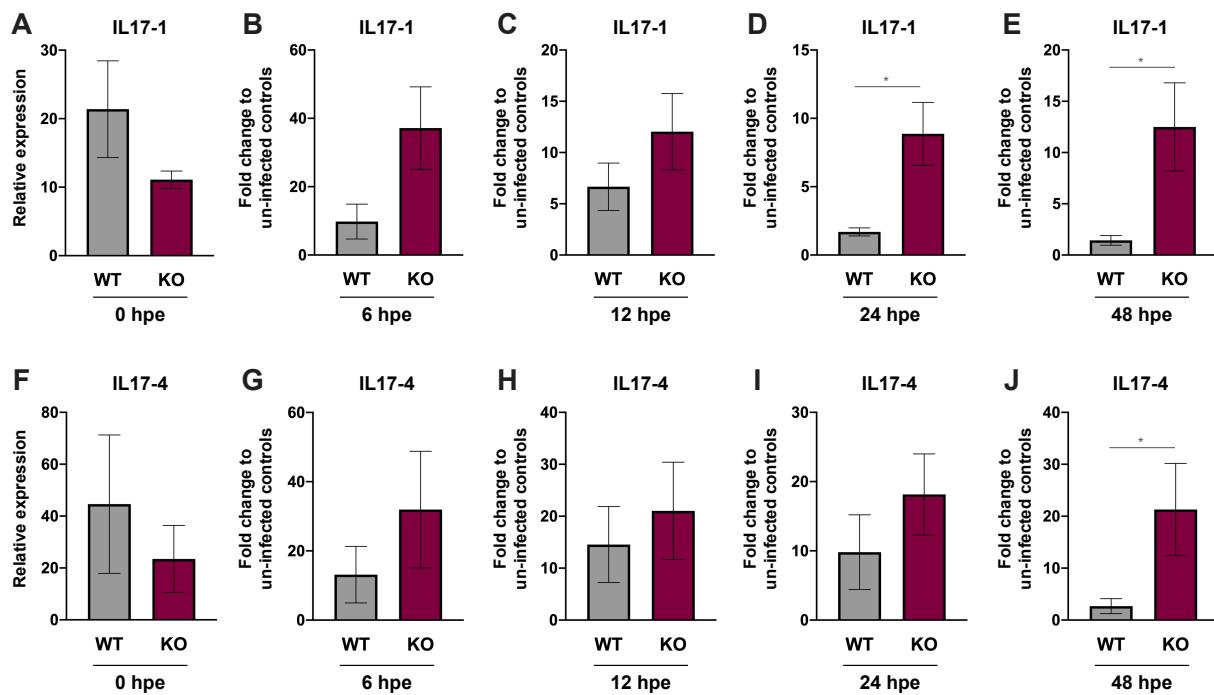
**Figure S2. T7E1 assays of inefficient sgRNAs targeting *Sp-ABCB1a* and *Sp-ABCB1b*.** T7E1 assays of PCR-amplified regions of the sgRNA target sites for *Sp-ABCB1a* (**A**) and *Sp-ABCB1b* (**B**). Top panels show products incubated with T7E1. Bottom panels show denatured and reannealed heteroduplexes without the addition of T7E1.



**Figure S3. *Sp-ABCB1a* crispants exhibit normal development.** Crispant and control embryos imaged at mesenchyme blastula (24–26 hr), gastrula (48 hr) and larval (73 hr) stages. Embryos did not exhibit morphological or developmental timing phenotypes ( $n=20$  for each time point and condition). Representative DIC images are shown from one of two independent mate pairs. Scale bars = 50  $\mu$ M.



**Figure S4. Bver and CAM accumulation in *Sp-ABCB1a* crispants.** Bar graphs representing raw mean fluorescence values for Bver (A) and CAM (B) accumulation in control (WT) and B1aE13-22 crispants with and without 1  $\mu$ M PSC833 inhibitor. n=30-40 embryos from 3-4 biological repeats. \*\*,  $P<0.01$ ; \*\*\*,  $P<0.001$ ; \*\*\*\*,  $P<0.0001$ ; n.s., non-significant  $P>0.01$ .



**Figure S5. Changes in *Sp-IL-17* expression between ABCB1 crissants and control (WT) larvae during *Vibrio diazotrophicus* infection.** Transcript prevalence was measured using qPCR. Expression values were normalized to 18S rRNA. Bar graphs representing relative expression at 0 hpe (A, F) and the fold-change in expression compared to un-infected 0 hpe larvae for *Sp-IL-17-1* (B-E) and *Sp-IL-17-4* (G-J). Crispant *Sp-IL-17* expression trends higher than control WTs at most timepoints and is significantly different at 24 and 48 hpe for *Sp-IL-17-1* (D, \*P=0.0262. E, \*P=0.0500) and 48 hpe for *Sp-IL-17-4* (J, \*P=0.0411). Unpaired t-tests with Welch's correction (A, D, E) or Mann-Whitney tests (B, C, F-J). n=6 internal replicates pooled from three biological replicates.

## Supplemental Tables

**Table S1. Primers and MASO sequences used for genomic PCR amplification of target loci, *in-situ* hybridization probe cloning, quantitative PCR, and antisense knockdown.**

Gene locus	Forward primers (5'-3')	Reverse primers (5'-3')
<i>Sp-Nodal</i> Ex1 for both T7EI and ICE	CTGAGACATCCCAGTGACGA	TGATTTACCCACACACAGCA
<i>Sp-ABCB1a</i> Ex12 for T7EI	GCAGCTGAAGAGAAAGCTCAAATAA	CGAGAAAGGGACATACAGACG
<i>Sp-ABCB1a</i> Ex12 for ICE	TTTAGGTGACACTATAG <u>TGTAAAACGA</u> <u>CGGCCAGT</u> GCAGCTGAAGAGAAAGCTC AAATAA	TAATACGACTCACTATAGGG <u>AGGAAACAGCT</u> <u>ATGAC</u> CGAGAAAGGGACATACAGACG
<i>Sp-ABCB1a</i> Ex13 for T7EI	CAGGTGGTTATCTCATCAAATCAG	GTCAGAGTTGAAGATGGTTGAGAGAC
<i>Sp-ABCB1a</i> Ex13 for ICE	ATTTAGGTGACACTATAG <u>TGTAAAACG</u> <u>ACGGCCAGT</u> CAGGTGGTTATCTCATCA AATCAG	TAATACGACTCACTATAGGG <u>AGGAAACAGC</u> <u>TATGAC</u> TACAGTTGGAAAGCTGGGAAAA
<i>Sp-ABCB1b</i> Ex2 for T7EI	TTTCATGCCCGGGATTATGTTCACCC	GTCGGTTAGTTCAAATTAGACCTTGCAG
<i>Sp-ABCB1b</i> Ex4 for T7EI	GGTACAGTTGAATTCTTGACGTGTGAT	GTACTAATCATTCTAGGCCATTG
<i>Sp-ABCB1b</i> Ex4 for ICE	TTTAGGTGACACTATAG <u>TGTAAAACGA</u> <u>CGGCCAGT</u> AGTAATCTTGCTGAAACT GCACAC	GACTCACTATAGGG <u>AGGAAACAGCTATGAC</u> GTACTAATCATTCTAGGCCATTG
<i>Sp-ABCB1a</i> ISH Antisense probe cloning	CCCACCTGCTGGGTTCTCCAAT	AAGTAATACGACTCACTATAGGGAGAGCCCG CATCCACTGCTGCCAACCATG
<i>Sp-ABCB1a</i> ISH Sense probe cloning	AAGTAATACGACTCACTATAGGGAGAG CCCACCTGCTGGGTTCTCCAAT	CCCGCATCCACTGCTGCCAACCATG
<i>Sp-18S</i> qPCR	CAGGGTCGATTCCGTAGAG	CCTCCAGTGGATCCTCGTTA
<i>Sp-IL17-1</i> qPCR	CATCAAGCTGCCATACGAT	GCTGATCGACATCGGGATAC
<i>Sp-IL17-4</i> qPCR	CTCTGTCCCAGGAAGCAATA	GGTGGCCAGTGGGTCTTC
Control MASO	CCTCTTACCTCAGTTACAATTATA	
<i>Sp-ABCB1a</i> MASO	TCTCTTATCTGGAGGCCAGGC	

Forward primers used to prepare amplicons for Sanger sequencing/ICE are 60mers containing part of the SP6 promoter sequence, **M13 forward sequence** and the corresponding **gene-specific primer** sequence used for amplification for T7E1 assays. Reverse primers are similarly designed but the SP6 promoter sequence is replaced with a portion of the T7 promoter.