

Figure S2. DNA editing by different *lhx1* sgRNAs and Cas9 protein. All data shown is from stage 10-12 embryos injected with *lhx1* sgRNA and Cas9 protein at the 1-cell stage. A,B,C) DNA editing results from a single representative embryo using a sgRNA against exon 1 of *lhx1*. D,E,F) DNA editing results from a single representative embryo using a sgRNA against exon 2 of *lhx1*. G,H,I) DNA editing results from a single representative embryo using a sgRNA against exon 3 of *lhx1*. A,D,E) TIDE analysis graph showing the showing the difference in aberrant sequences between an uninjected control embryo and an *slc45a2* knockout embryo. The percent of aberrant sequences in the knockout embryo increases in comparison to

the control embryo near the expected Cas9 cut site only for the *lhx1* exon 3 sgRNA. B,E,H) *lhx1* editing efficiency in single embryos as measured by TIDE. *lhx1* exon 3 sgRNA is the most efficient of the three sgRNAs tested. C,F,I) Chart showing the percent of sequences that result in in frame and out of frame insertion and deletion events. *lhx1* exon 3 sgRNA results in mostly out of frame deletions, while the sgRNAs against exons 1 and 2 result in mostly unedited DNA.