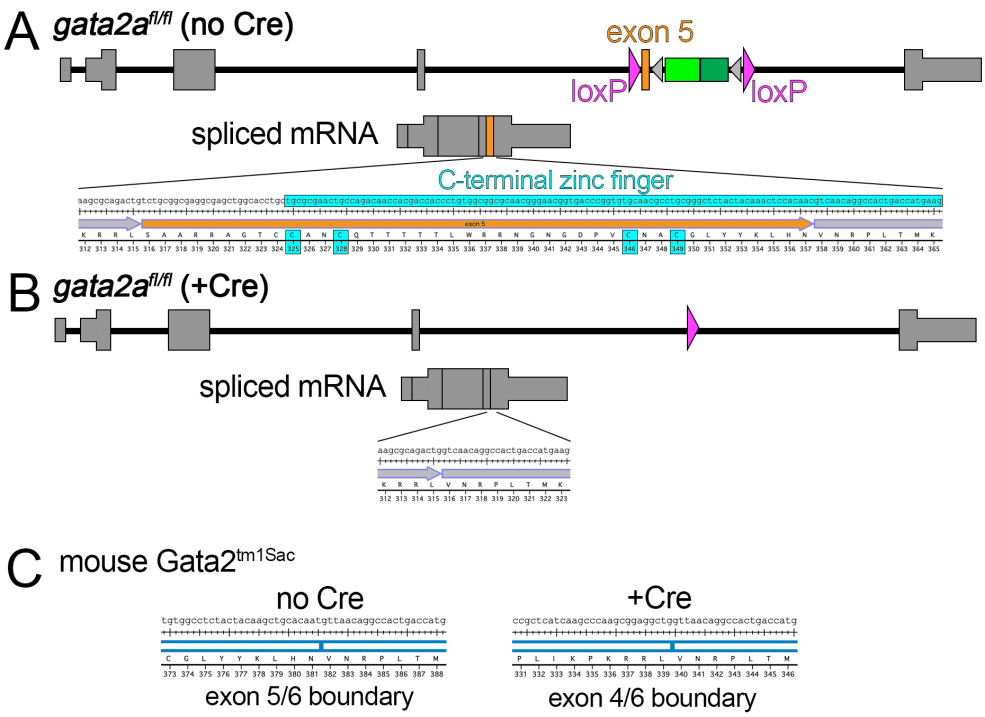

Figure S1. Schematic description of Cre-mediated exon 5 deletion at the *gata2a*$^{fl/fl}$ locus, related to Figure 1. (**A**) Zebrafish *gata2a*$^{fl/fl}$ locus in the absence of Cre. Position of loxP sites flanking exon 5 are indicated. Structure of spliced mRNA is indicated with position of exon 5 denoted in orange. Sequence of exon 5 and flanking exon boundaries in *gata2a* transcript is shown. Coding sequence for C-terminal zinc finger is highlighted in cyan, as are individual cysteine residues within the zinc finger that are responsible for zinc binding. (**B**) Zebrafish *gata2a*$^{fl/fl}$ locus following Cre-mediated recombination. Spliced mRNA is indicated with highlight showing exon 4/6 boundary and in-frame coding sequence. (**C**) Sequence of indicated exon boundary in *Gata2* transcript expected from the mouse *Gata2*^{tm1Sac} allele in the absence of presence or Cre.

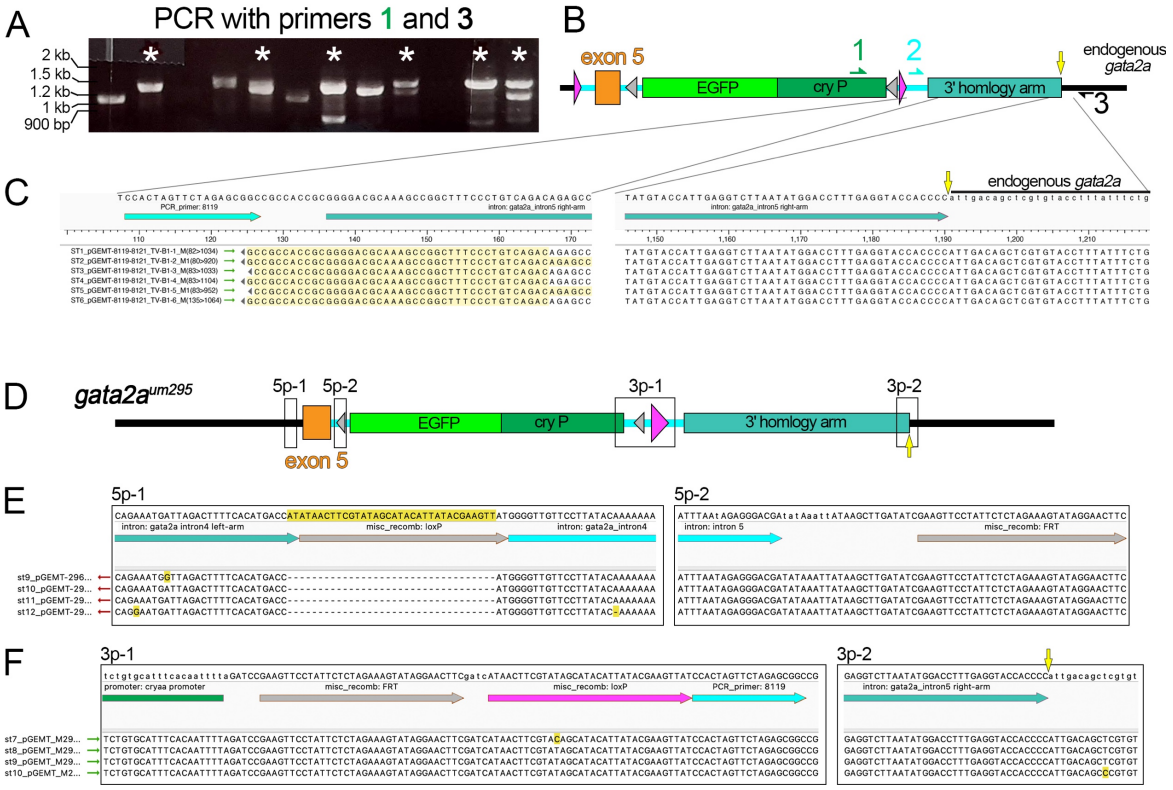

Figure S2. Junction sequencing at *gata2a*^{um295} locus confirms failure to insert 5' loxP site, related to Figure 1. (A) PCR of individual *cryaa:egfp*-positive embryos injected with *gata2a* targeting construct, Cas9 RNP and ISce-I. Lanes with amplification of expected product size are indicated by an asterisk. PCR primers are indicated and their location is shown in (B). (B) Schematic of 3' junction PCR at *gata2a* exon 5 target. (C) Alignment of cloned fragments spanning the 3' homology arm and junction. Each sequence is a contiguous cloned fragment, of which only the 5' and 3' ends are shown. (B, C) Yellow arrow denotes junction between endogenous sequence and targeting construct homology arm sequence. (D) Exon 5 in *gata2a*^{um295}. Labeled boxes denote regions for which sequence is shown in (E, F). (E). 5' and 3' sequence from cloned fragments spanning exon 5 aligned to *gata2a*$^{fl/fl}$ reference sequence. Note absence of 5' loxP site from all 4 cloned fragments. (F) 5' and 3' sequence from fragments spanning the 3' loxP site, across the homology arm sequence and into the endogenous *gata2a* locus. (D, F) Yellow arrow denotes junction between endogenous sequence and targeting construct homology arm sequence.

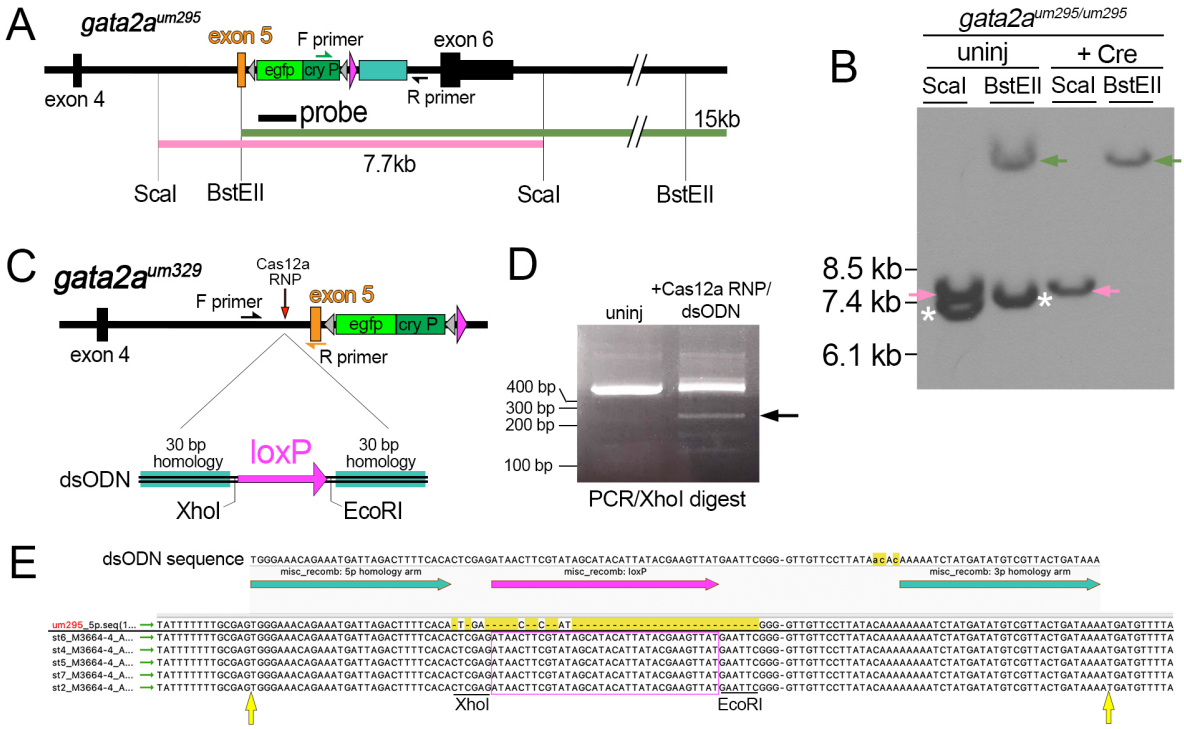

Figure S3. Removing off-target cassette and inserting a 5' loxP site to generate a floxed *gata2a* allele, related to Figure 1. (A) *gata2a*^{um295} locus. (B) Southern analysis with genomic DNA from homozygous *gata2a*^{um295} embryos left uninjected or injected with *cre* mRNA. Blot was hybridized to a DIG-labeled probe for EGFP. (C) *gata2a*^{um329} locus showing location of Cas12a RNP and dsODN structure used to insert the 5' loxP site. (D) PCR product across insertion point for 5' loxP site in embryos left uninjected or those injected with Cas12a RNP and loxP dsODN shown in (C). PCR products were digested with XhoI. Only products from embryos injected with Cas12a and dsODN show evidence of cutting, consistent with insertion of the exogenous sequence at the target site. (E) Sequence validation of the 5' loxP insertion in *gata2a*$^{fl/fl}$. Sequence of cloned fragments spanning the loxP insertion in F1 embryos from a P0 founder. Sequences are aligned to the *gata2a*^{um295} as reference and the ODN sequence is shown. Yellow arrows denote junction points between ODN homology and endogenous sequences.

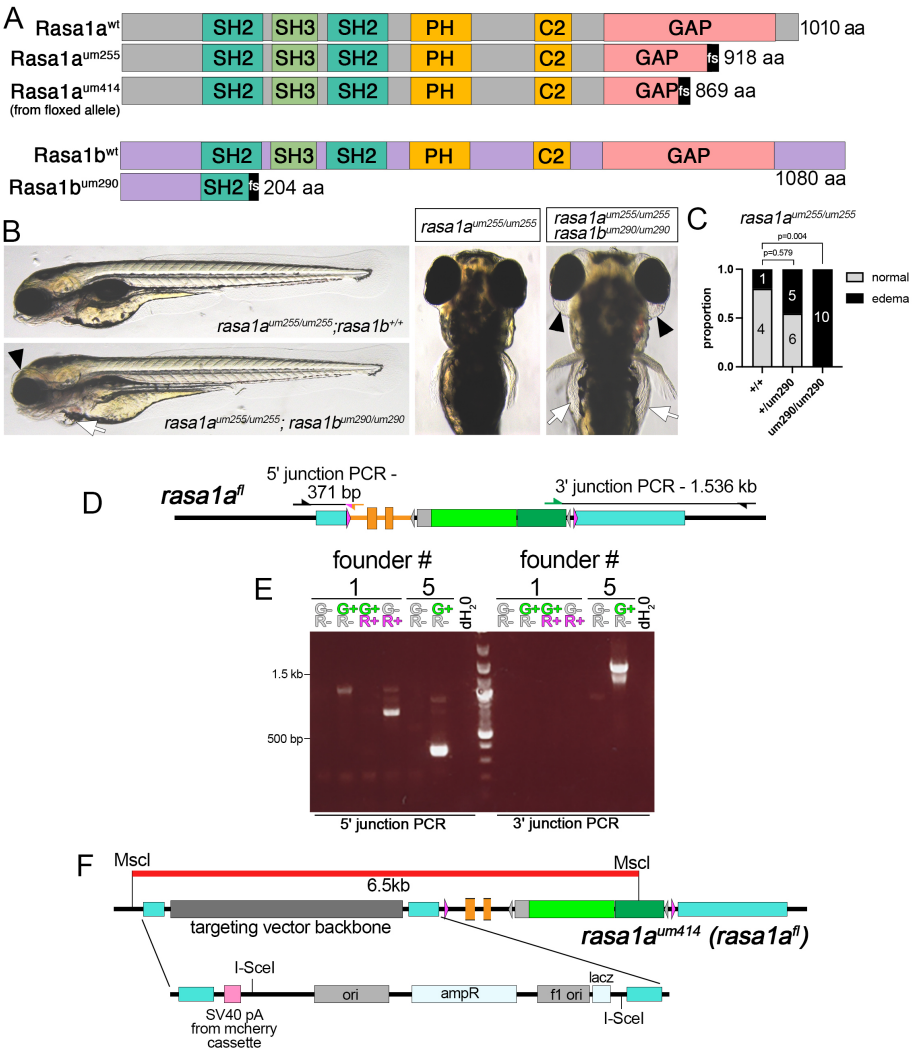

Figure S4. *Rasa1a/b* deletion alleles and phenotypes, related to Figure 6. (**A**) Rasa1a and Rasa1b proteins in deletion mutants. Rasa1a^{um255} and Rasa1b^{um290} are deletions causing frameshift and truncation as indicated. Rasa1a^{um414} shows consequence of Cre-mediated deletion of exons 20 and 21 at the floxed *rasa1a* allele leading to frameshift and truncation in the GAP domain. "fs" – denotes frameshift. (**B**) *Left panels*, 4 dpf larvae of indicated genotype with ocular and cardiac edema, arrowhead and arrow, respectively. Lateral view, anterior to the left, dorsal is up. *Right panels*, same larvae as on right, dorsal view, anterior is up. Gut and ocular edema indicated by arrows and arrowheads, respectively. (**C**) Penetrance of edema in 4 dpf larvae of indicated genotype. Fisher's exact test, p-values shown. (**D**) Schematic of expected *rasa1a^{fl}* locus with location of PCR primers and products used for screening. (**E**) 5' and 3' junction PCRs in embryos from P0 adults. Founder #5 gave rise to *cryaa:venus*-positive embryos with positive PCR for both junctions. (**F**) *rasa1a^{um414}* knock-in allele derived from founder #5. Southern analysis using MscI for restriction digest revealed a larger than expected fragment (see **Figure 6D**). PCR and sequencing revealed insertion of vector backbone upstream of the floxed exon, explaining the increased size of the MscI fragment (see **Supplementary File 5**).