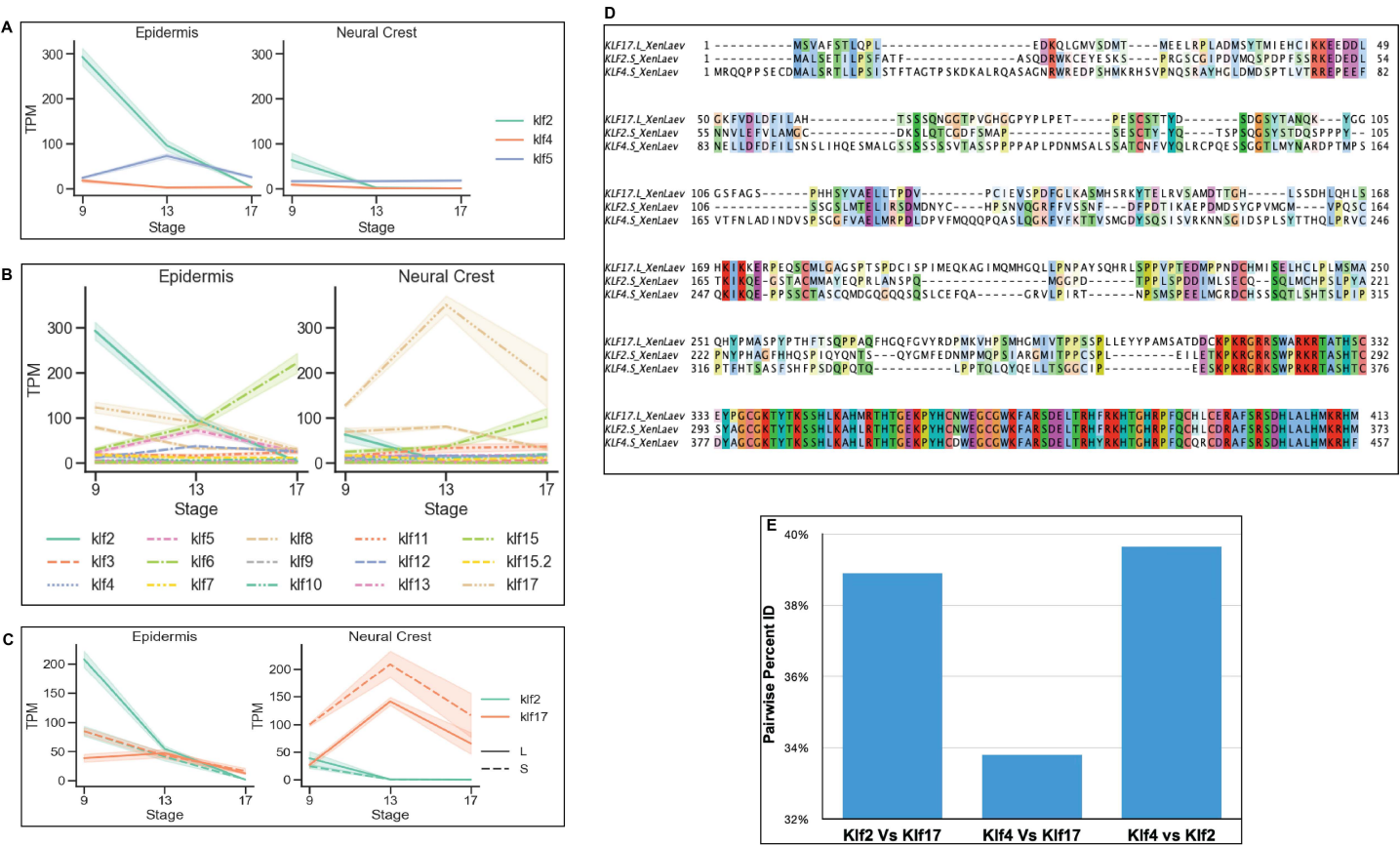
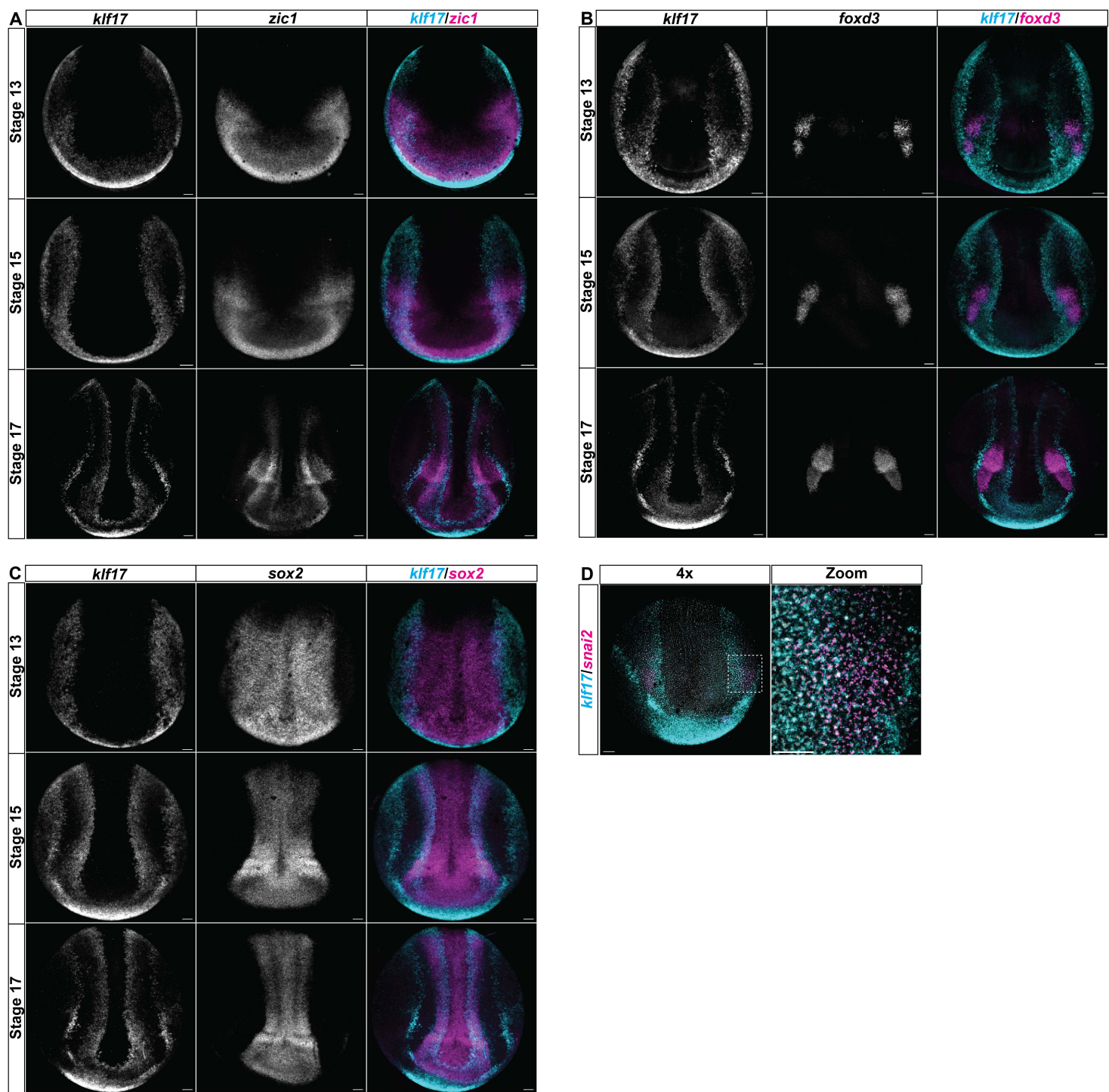
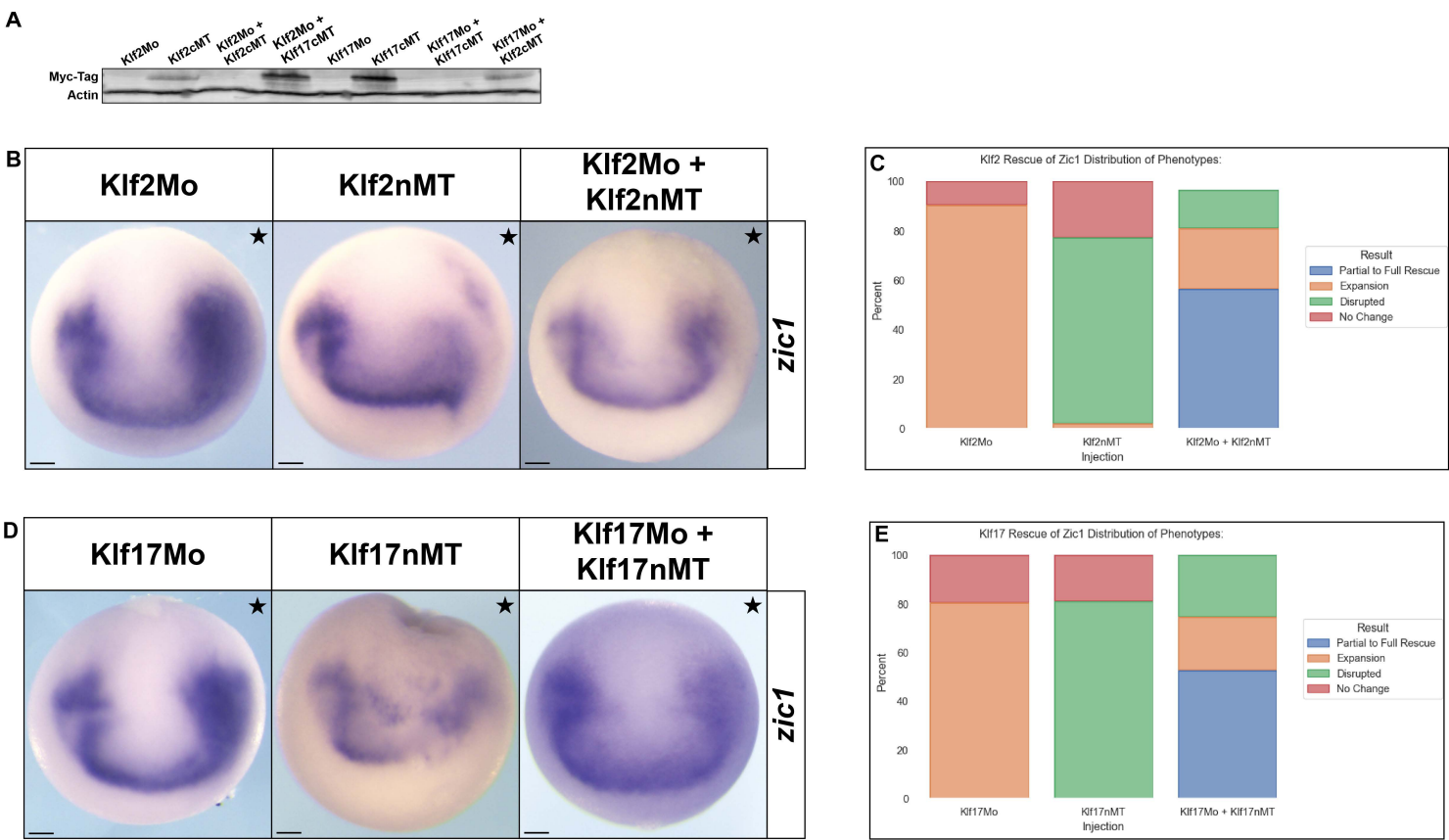




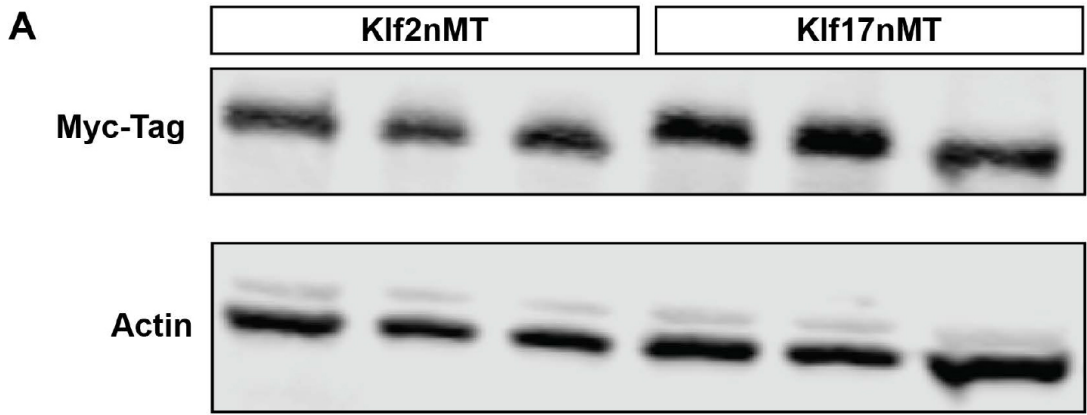
**Fig. S1. TPM plots of klf factors in epidermal and neural crest explants.** (A-B) Plots of the average TPMs of Klf factors in epidermis and neural crest explants at blastula (stage 9), early neurula (stage 13), and late neurula (stage 17) stages. Shading represents the standard error of mean. (A) Plots of klf2, klf4 and klf5 TPMs. (B) Plots of all 15 klfs in the *Xenopus laevis* genome. (C) Plots of Klf2 and Klf17 L & S alleles (D) Multi-Sequence Alignments of human, mouse, and *Xenopus* Klf2, Klf4, & Klf17 (D) Mult-Sequence Alignments *Xenopus* Klf2, Klf4, and Klf17. (E) Plot comparing percent identities calculated from pairwise alignments Between Klf2, Klf4, and Klf17.



**Fig. S2. Additional HCR of overlapping expression patterns of Klf17 with neural plate, neural plate border and neural crest changes during neurulation.** (A-C)Whole embryos probed with HCR oligos examining the expression patterns of (A) *klf17* (cyan) and the neural crest marker *foxd3* (magenta) ; (B) *klf17* (cyan) and the neural plate border marker *zic1* (magenta); or (C) *klf17* (cyan) and the neural plate marker *sox2* (magenta) at early (stage 13), mid (stage 15), and late (stage 17) neurulation. Scale bars: 150 μm. (D) Zoom of 4x HCR images from Fig. 2C showing co expression of *klf17* with the neural crest marker *snai2* at stage 13. Dashed box: zoom area; Scale bars: 150μm (4x) or 50μm (zoom).

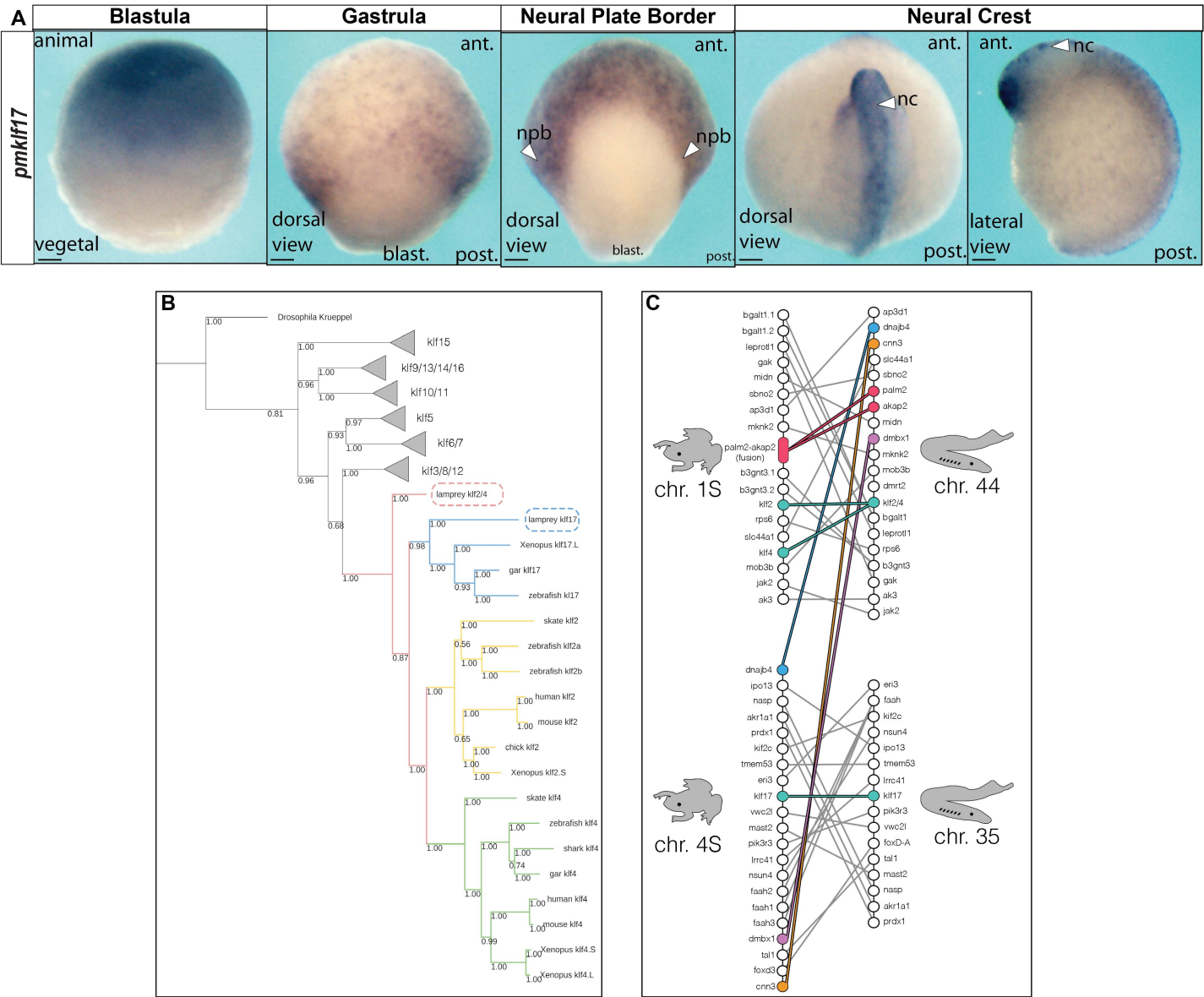


**Fig. S3. Klf2 and Klf17 Morpholino Validation.** (A) Western blot validation of Klf2 and Klf17 morpholinos. Lanes 3 & 7 show each morpholino blocks the translation of their respective Klf. Lanes 4 & 8 show each morpholino does not block translation of the other Klf. (B) In situ hybridizations showing the rescue of *zic1* expression in *klf2*-morphant embryos co-injected with epitope tagged *klf2* mRNA (C) Stacked bar graphs of the percent distribution of phenotypes for the *klf2* rescue of *zic1* expression (Klf2Mo n=52; *klf2*-nMT(n=92; Klf2Mo + *klf2*-nMT n= 90). (D) In situ hybridizations showing the rescue of *zic1* in *klf17*-morphant embryos co-injected with epitope tagged *klf17* mRNA. (E) Stacked bar graphs of the percent distribution of phenotypes for the *klf17* rescue of *zic1* expression (Klf17Mo n=62; *klf17*-nMT n=63; Klf17Mo + *klf17*-nMT n=55) . cMT, C-terminal myc tag; nMT, N-terminal myc tag; MO, morpholino.

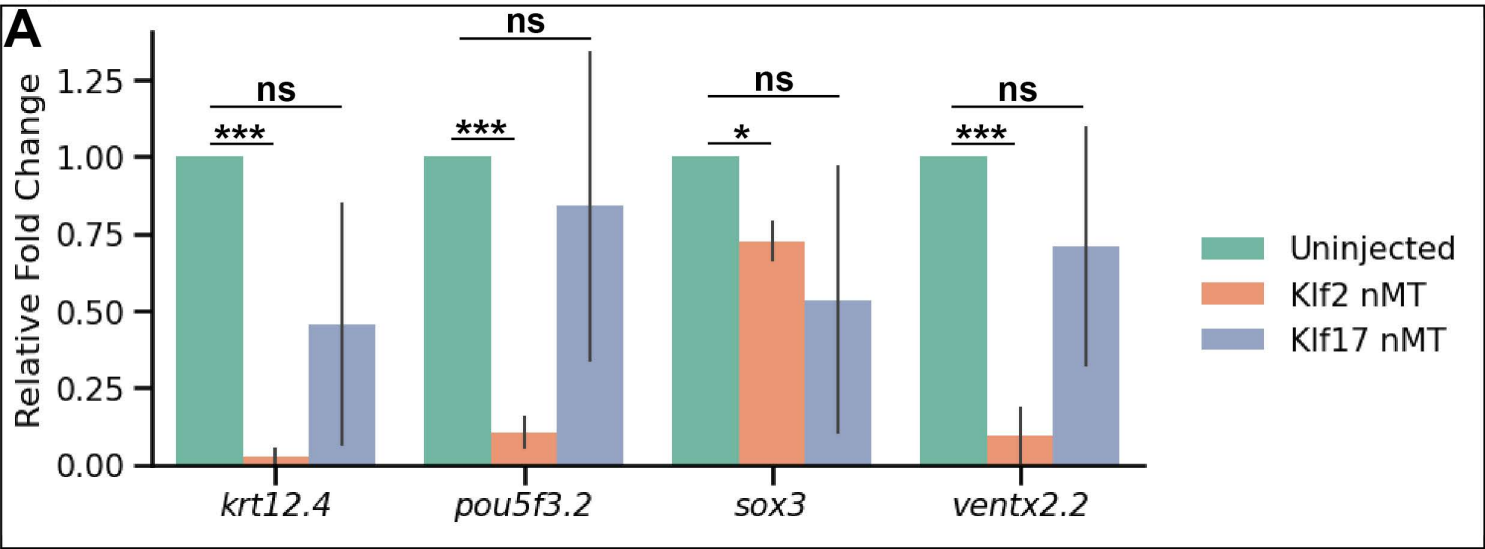


**Fig. S4. Western Blot to Validate Protein Levels.** (A) Western blot of three replicates of epitope tagged *klf2* and *klf17* used in gain of function experiments at consistent levels. nMT, N-terminal myc tag.





**Fig. S5. Evolution and Conservation of Klf factors.** (A) In situ hybridizations of *klf17* expression in the blastula, gastrula, neural plate border and neural crest of wild type sea lamprey embryos. (B) Phylogenetic tree of Klf factors across several species. (C) Synteny analysis of *klf2* and *klf17* in *Petromyzon marinus* (sea lamprey) and *Xenopus laevis*. pm, *Petromyzon marinus*; ant, anterior; post., posterior; blast., blastopore; npb, neural plate border; nc, neural crest



**Fig. S6. qPCR of Explants with Ectopic Klf2 and Klf17**(A) Plots of relative fold change of uninjected, ectopic Klf2 or ectopic Klf17 explants from qPCR. Normalized to uninjected explants (stage13) for *krt12.4*, *pou5f3.2*, *sox3*, and *ventx2.2*. \* $p \leq 0.05$ ; \*\*\* $p \leq 0.001$ , ns not significant; error bars: standard error of the mean

**Table S1. qPCR primers**

| Gene            | Forward               | Reverse                |
|-----------------|-----------------------|------------------------|
| <i>ODC</i>      | TGAAAACATGGGTGCCTACA  | TGCCAGTGTGGTCTTGACAT   |
| <i>sox3</i>     | CACAACTCGGAGATCAGCAA  | TCGTGATGAAGGGTCTTTT    |
| <i>pou5f3.2</i> | AATGGGGCAATTAATGAACG  | GGGAACCTCCTCCTCATTGT   |
| <i>ventx2.2</i> | GCTACACAGGGACACAACCTC | GCCTGAGTCAGTGCTAGTGC   |
| <i>krt12.4</i>  | TTGGTGCTGGGTCTAAAGATT | TTTGCAGAGTCACTGTAGCATT |