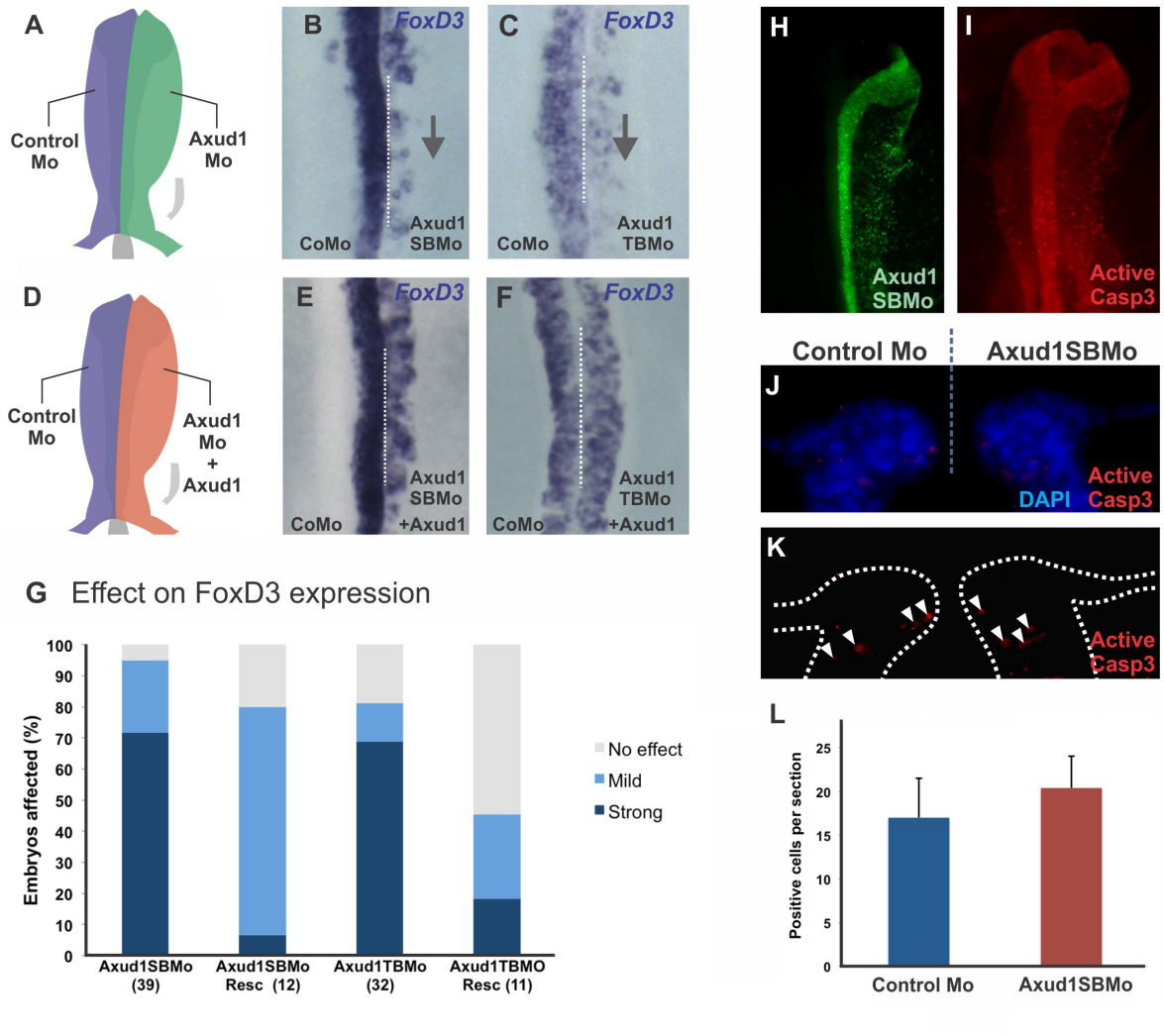

Figure S1, related to Figure 2. Specificity of Axud1 morpholinos. (A-C) Electroporation of both splice- (Axud1SBMo) and translation-blocking (Axud1TBMo) morpholinos into the right side of stage HH5 embryos resulted in strong down-regulation of *FoxD3*, when compared to the control side. (D-F) Co-electroporation of an Axud1 expression vector with either Axud1SBMo (E) or Axud1TBMo (F) resulted in recovery of phenotype associated with loss of FoxD3 expression. (G) Quantitation of loss-of-function and rescue experiments. The total number of embryos analyzed for each experiment is represented in parentheses. **(H-L) Axud1 loss-of-function does not cause cell death in dorsal neural folds.** (H-I) Whole mount views of embryo electroporated with Axud1 morpholino (green) and stained with an anti-active Caspase-3 antibody (red). (J-K) Histological analysis indicates that electroporation of Axud1 morpholino does not alter the number of cells positive to active Caspase-3 (shown with arrowheads) in the dorsal neural folds. (L) Average number of cells in the neural tube positive for active Caspase-3. Histological sections of six embryos electroporated with control and Axud1 morpholinos were used for this analysis (n = 6 embryos). Error bars represent standard deviation. Active Casp3: anti-active Caspase-3 antibody, CoMo: Control morpholino, Axud1SBMo: Axud1 splice-blocking morpholino, Axud1TBMo: Axud1 translation-blocking morpholino.

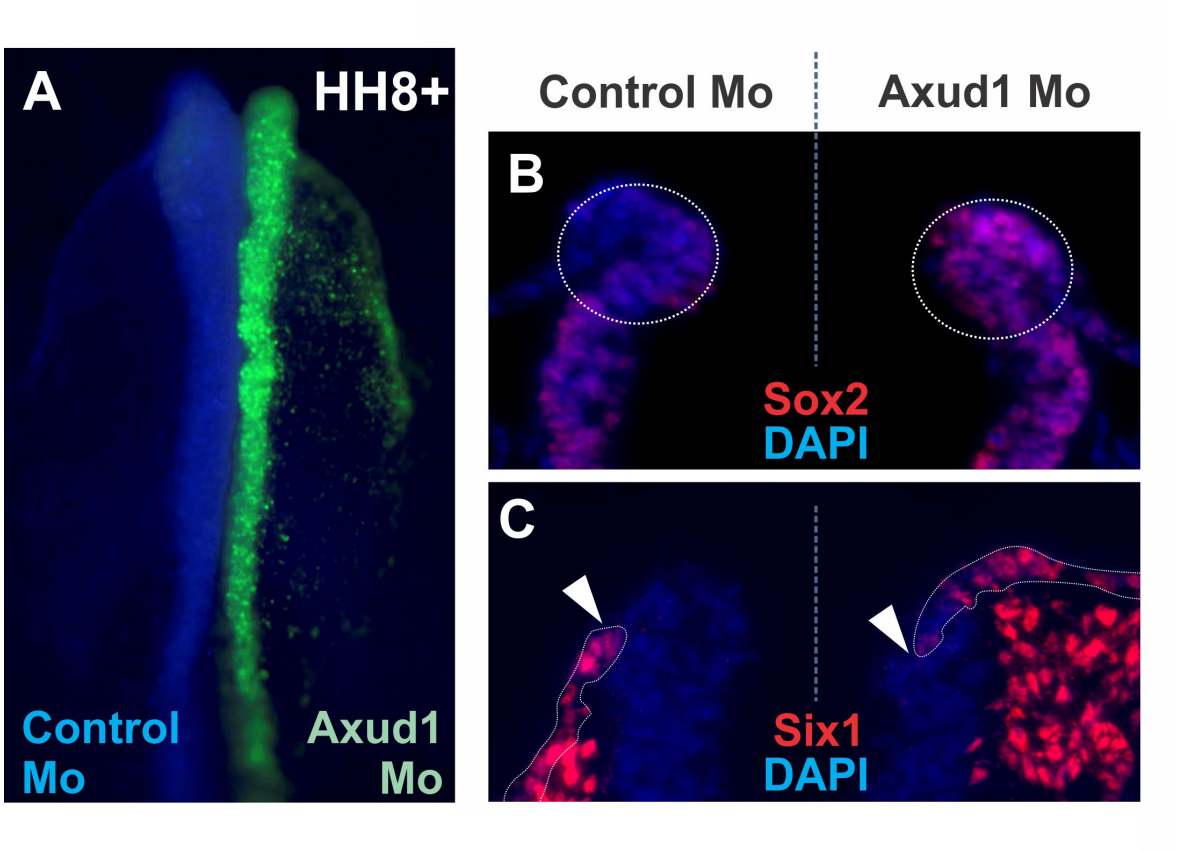

Figure S2, related to Figure 4. Loss of Axud1 results in upregulation of neuronal and placodal markers in the dorsal neural folds. (A) Whole mount views of embryo electroporated with Axud1 morpholino (green) and control morpholino (blue). (B) Cross section of the cephalic region of an embryo electroporated with control and Axud1 morpholinos. Immunohistochemistry with the neuronal marker Sox2 antibody reveals Axud1 knockdown results in upregulation of this protein in the dorsal neural folds (n = 6/6). (C) Consistent with the results of Nanostring analysis (Fig. 4), Axud1 loss-of-function also causes a medial expansion of the Six1 positive domain (n = 5/6). The arrowhead shows the medial limit of Six1 expression, at hindbrain-level sections. Mo: Morpholino.

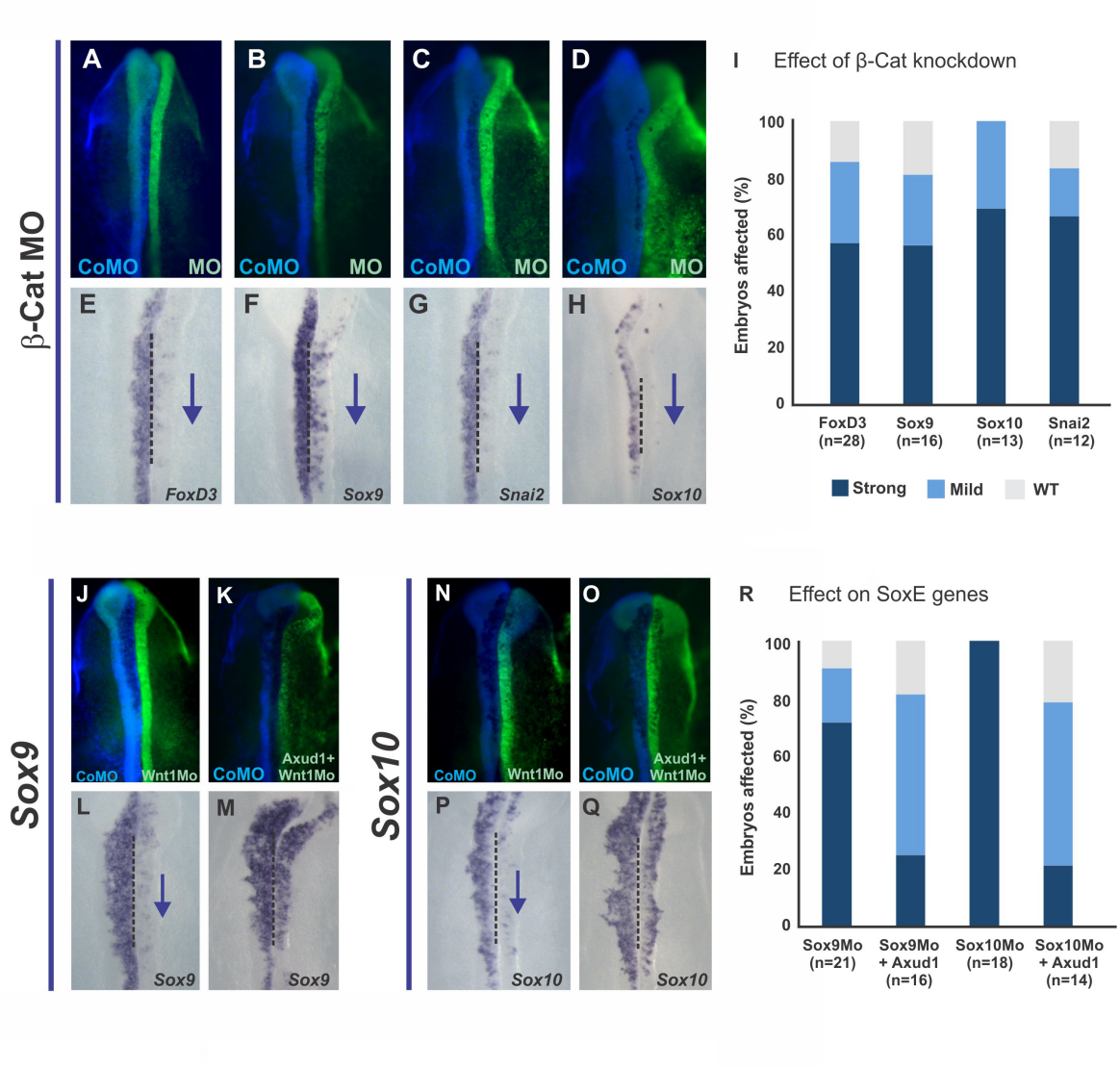

Figure S3, related to Figure 5. The Wnt/Axud1 pathway is required for neural crest specification. Whole mount views of embryos (A-D) after β-catenin morpholino (green) and control morpholino (blue) were electroporated into the right and left neural folds, respectively. (E-H) Expression of neural crest specifiers in the same embryo represented in A-D. Knockdown of β-catenin caused loss of multiple neural crest specifier genes, including *FoxD3* (A, E), *Sox9* (B, F), *Snai2* (C, G) and *Sox10* (D-H). (I) Quantitation of β-catenin loss-of-function experiments. The total number of embryos analyzed for each experiment is represented in parentheses. **(J-R) Axud1 is sufficient to rescue expression of *SoxE* genes, *Sox9* and *Sox10*, following Wnt1 knockdown**. Morpholino-mediated Wnt1 loss-of-function results in loss of neural crest specification, as assayed by *FoxD3* (Fig. 5). Neural crest specifiers *Sox9* (J, L), *Sox10* (N, P) are also strongly downregulated following Wnt1 knockdown. Co-electroporation of an Axud1 expression vector with the Wnt1 morpholino resulted in recovery of *Sox9* (K, M) and *Sox10* (O, Q) expression. (R) Quantitation of loss-of-function and rescue experiments. The total number of embryos analyzed for each experiment is represented in parentheses. β-cat Mo: β-catenin morpholino. CoMo: Control morpholino, Wnt1Mo: Wnt1 morpholino.

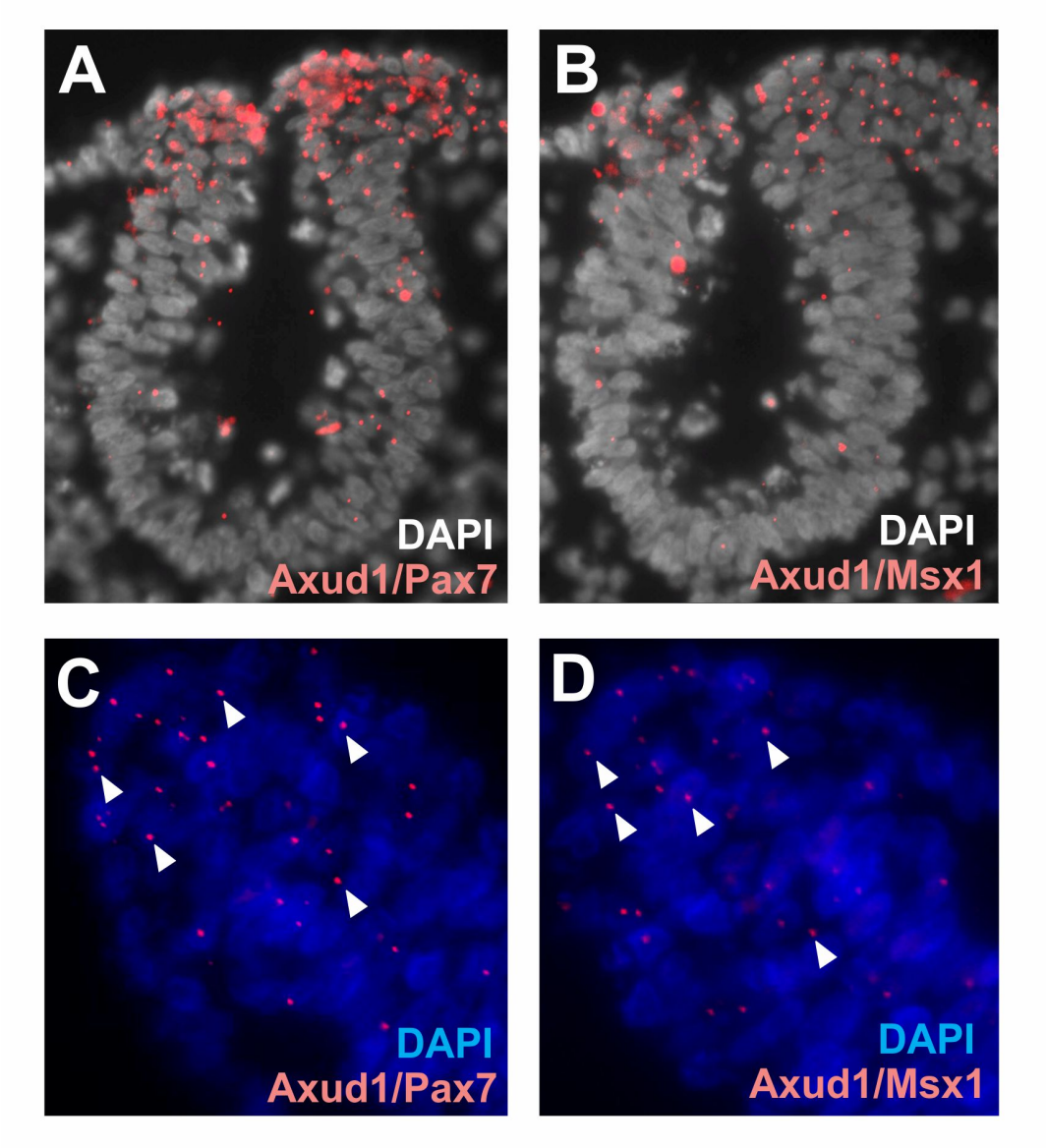

Figure S4, related to Figure 6. Proximity ligation assay indicates putative interactions between Axud1 and neural plate border specifiers in dorsal neural tube. (A, B) Proximity ligation assays (PLA) puncta visualized in transverse sections through the neural tube of HH9 embryos indicate a large number of putative interactions between Axud1/Pax7 and Axud1/Msx1 in the region where the neural crest becomes specified. (B) High magnification images of dorsal neural folds highlight the presence of PLA puncta in the nucleus of the cells residing in the dorsal neural tube (white arrowheads).