Supplemental material

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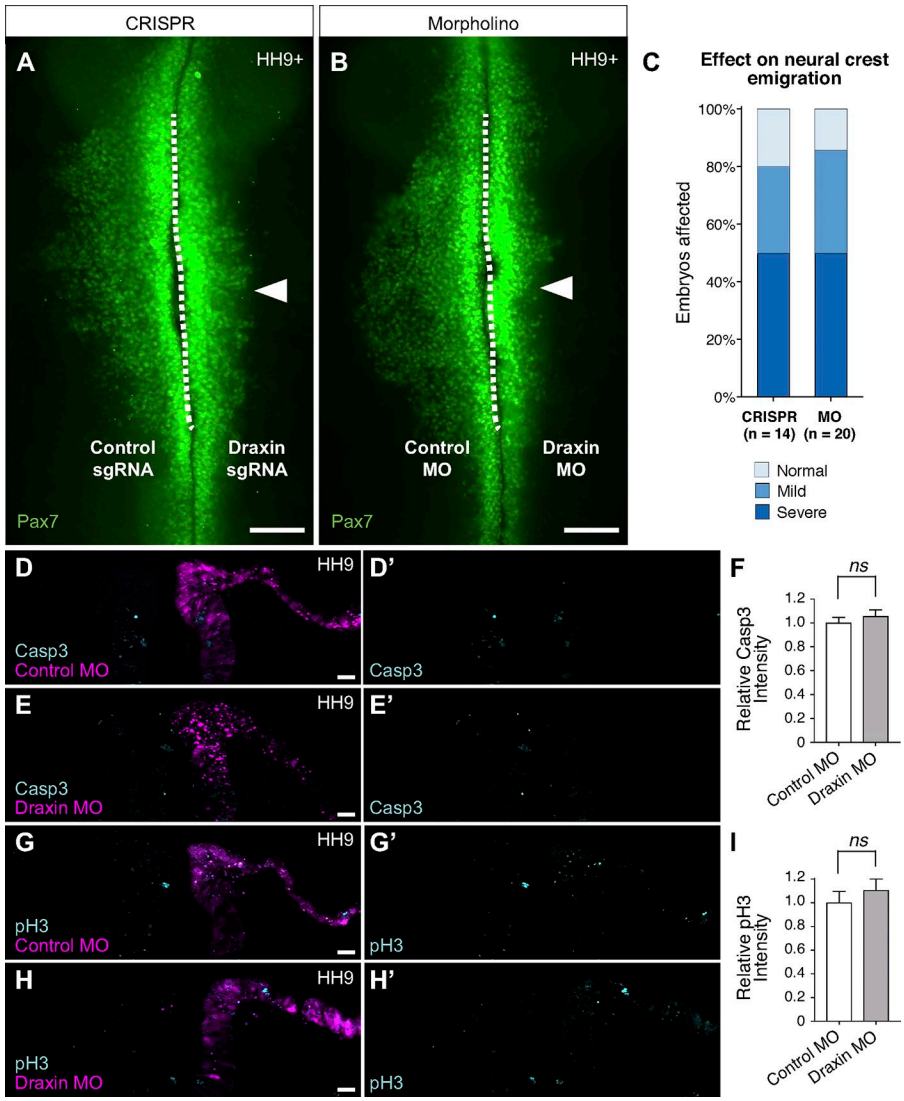

Figure S1. **CRISPR/Cas9-mediated knockdown of Draxin phenocopied MO-mediated Draxin knockdown. (A and B)** Representative images for Pax7 immunostaining (green) in embryos knocked down for Draxin using CRISPR/Cas9 (A) or translation-blocking MO (B). Embryos were bilaterally electroporated with recombinant Cas9/control sgRNA (A, left) and recombinant Cas9/Draxin sgRNA (A, right), or control MO (B, left) and Draxin MO (B, right). Bars, 100 µm. Arrowhead highlights defects in neural crest emigration resulting from perturbation. **(C)** Quantitation of phenotype penetrance for knockdown embryos analyzed at HH9+ for severe, mild, or no defects in cranial neural crest emigration from the midline. Data are presented as fraction of the total number of CRISPR/Cas9- and MO-mediated knockdown embryos (n = 14 and n = 20, respectively; pooled from more than three independent experiments). "Normal" describes migration distances within the SEM for control MO; "mild" describes migration distances between the lower limit of the control MO SEM and the upper limit of Draxin MO SEM; "severe" describes migration distances lower than the upper limit of Draxin MO SEM. **(D–I)** Representative images of embryos unilaterally electroporated with control MO (D and G) or Draxin MO (E and H) and immunostained for cleaved caspase 3 (D and E; Casp3, cyan) or phosphohistone H3 (G and H; pH3, cyan). Quantitation of the relative fluorescence intensity for Casp3 (F) and pH3 (I) found no difference in the levels of cell death or proliferation, respectively, between control and Draxin MO–electroporated embryos. Bars, 20 µm. Data are presented as mean ± SEM (three sections/embryo; n = 3 embryos/condition); $P > 0.4$, two-tailed t test.

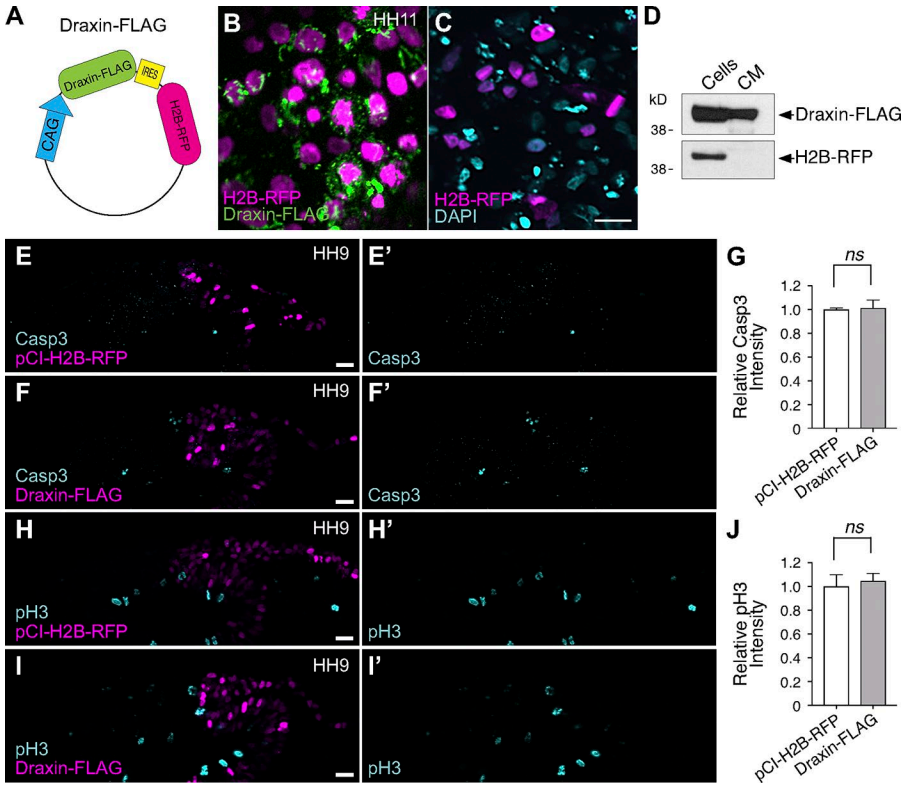

Figure S2. **Validation of the Draxin overexpression construct Draxin-FLAG. (A)** Schematic of the Draxin-FLAG expression vector. The coding region for full-length chick Draxin with a C-terminal FLAG tag (Draxin-FLAG) was cloned into pCI-H2B-RFP, which contains a chick β-actin promoter (CAG) and an IRES followed by coding for an RFP-tagged histone H2B (H2B-RFP). **(B)** Electroporation of Draxin-FLAG in HH4 chick embryos yielded expression of H2B-RFP (magenta), which allows easy validation of electroporation efficiency. Immunostaining with an antibody against FLAG (green) at HH11 revealed efficient, full-length expression of Draxin-FLAG in vivo. **(C and D)** Chick DF1 fibroblast cells were transfected with Draxin-FLAG and assessed for successful transfection by expression of H2B-RFP (magenta) and DAPI (cyan) staining (C). Western blots were performed on the retrieved conditioned media as well as Draxin-FLAG–transfected DF1 cells, and blots were probed for anti-FLAG and anti-RFP (D). **(E–J)** Representative images of embryos unilaterally electroporated with pCI-H2B-RFP (E and H; control) or Draxin-FLAG (F and I) and immunostained for cleaved caspase 3 (E and F; Casp3, cyan) or phosphohistone H3 (H and I; pH3, cyan). Quantitation of the relative fluorescence intensity for Casp3 (G) and pH3 (J) found no difference in the levels of cell death or proliferation, respectively, between pCI-H2B-RFP and Draxin-FLAG–electroporated embryos. Bars, 20 µm. Data are presented as mean ± SEM (three sections/embryo; *n* = 3 embryos/condition); P > 0.7, two-tailed *t* test.

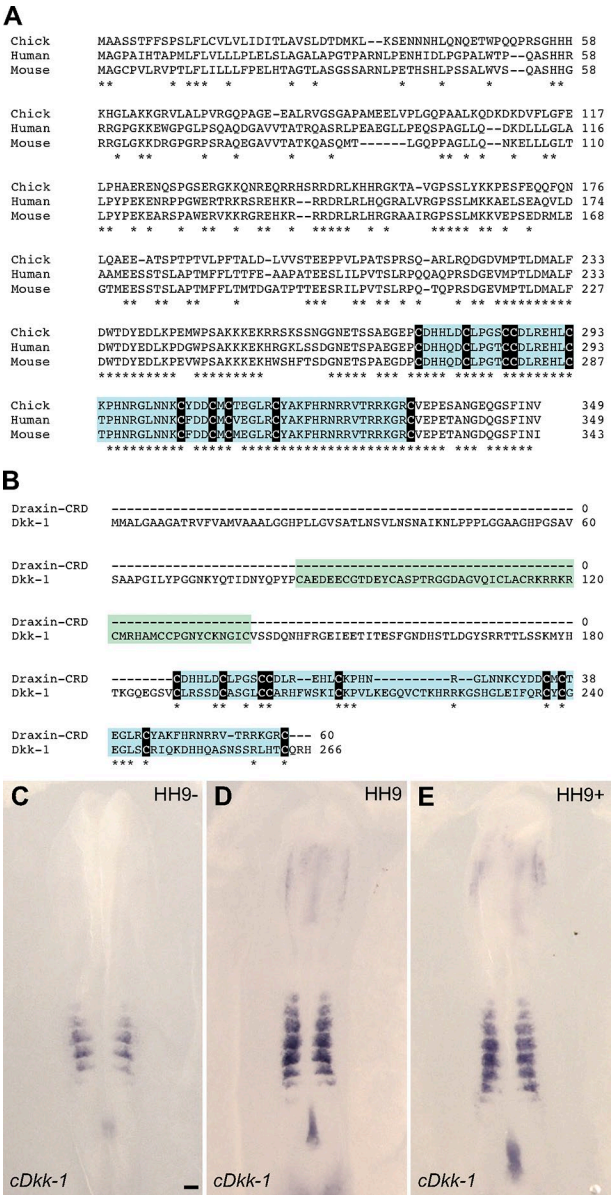

Figure S3. **Draxin's CRD is highly conserved and homologous to the colipase fold domain of Dkk-1. (A)** Multiple sequence alignment of Draxin amino acid sequences for multiple species (GenBank accession nos.: chick, NP_001136320.1; human, NP_940947.3; mouse, NP_081702.2) shows high conservation of the CRD. **(B)** Amino acid alignment of the chick Draxin CRD against human Dkk-1 (GenBank accession no.: NP_036374.1) shows conservation of 9 of 10 cysteines within the colipase fold. Asterisks indicate 100% conservation among all species examined. The CRD of Draxin and colipase fold domain of Dkk-1 are indicated by blue shading, and the conserved cysteines within these domains are highlighted in black. The DKK_N domain of Dkk-1 is indicated by green shading. **(C–E)** Representative images of chromogenic wholemount in situ hybridization for chick *Dkk-1* (*cDkk-1*) in stage HH9- (C), HH9 (D), and HH9+ (E) chick embryos ($n > 3$ embryos per stage). Bars, 100 µm.

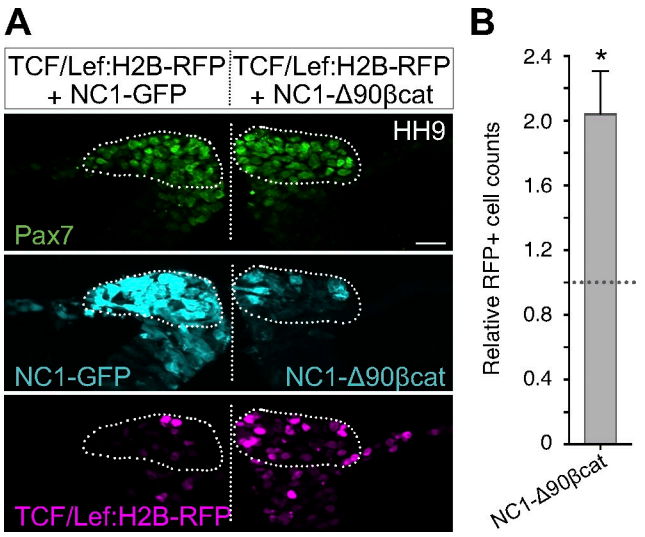

Figure S4. **The NC1-Δ90βcat construct increased canonical Wnt signaling in vivo. (A)** Representative cross section of embryo coelectroporated with TCF/Lef:H2B-RFP (magenta) and NC1-GFP (left, cyan; control) or NC1-Δ90βcat (right, cyan) immunostained for Pax7 (green). To visually compare TCF/Lef:H2B-RFP reporter expression levels in neural crest cells undergoing EMT, Pax7$^+$ cells that have delaminated from the neural tube are circled. Straight dotted line indicates the midline. Bar, 20 µm. **(B)** Quantitation of RFP$^+$ cells on NC1-GFP versus NC1-Δ90βcat sides of embryo (three sections/embryo; *n* = 3 embryos/condition) yielded a significant increase (*, P = 0.03, two-tailed *t* test) in total RFP$^+$ cells on the NC1-Δ90βcat–electroporated side compared with the control side. Data are presented as mean ± SEM.

Table S1. **List of reagents**

Antibody	Species	Source	Identifier
HNK-1 carbohydrate epitope	Mouse IgM	Developmental Studies Hybridoma Bank	3H5
Pax7	Mouse IgG1	Developmental Studies Hybridoma Bank	pax7, RRID:AB_528428
Snail2 (Slug)	Rabbit	Cell Signaling Technology	9585
Sox9	Rabbit	EMDMillipore	AB5535
Cadherin6B (Cad6B)	Mouse IgG1	Developmental Studies Hybridoma Bank	CCD6B-1
RFP	Mouse IgG1	Abcam	ab125244
GFP (Western blots)	Goat	Rockland	600-101-215
GFP (Immunohistochemistry)	Rabbit	Abcam	ab290
S6 ribosomal protein (5G10)	Rabbit mAb	Cell Signaling Technology	2217
LRP5 (D80F2)	Rabbit mAb	Cell Signaling Technology	5731
FLAG M2	Mouse IgG1	Sigma-Aldrich	F3165
Fluorescein	Goat	Novus Biologicals	NB600-493
Cleaved caspase 3 (Casp3)	Rabbit	R&D Systems	AF835
Phosphohistone H3	Rabbit	EMD Millipore	06-570

Table S2. **List of primers**

Oligonucleotide/primer name	Sequence (5′–3′)
Primers for CRISPR sgRNA	
Draxin short guide oligonucleotide	GCGTAATACGACTCACTATAGGACGCAGAGGCCCCAGGATTAGTTTTAGAGCTAGAAATAGC
Control short guide oligonucleotide	GCGTAATACGACTCACTATAGGCACTGCTACGATCTACACCGTTTTAGAGCTAGAAATAGC
gRNA Primer 1	CACGCGTAATACGACTCACTATAG
gRNA Primer 2	AAAGCACCGACTCGGTGCCAC
Guide-constant oligonucleotide	AAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCT CTAAAAC
MOs	
Draxin MO with 3′ fluorescein	AAGGTGGAAGAAGCTGCCATAATCC
Standard control MO with 3′ fluorescein	CCTCTTACCTCAGTTACAATTTATA