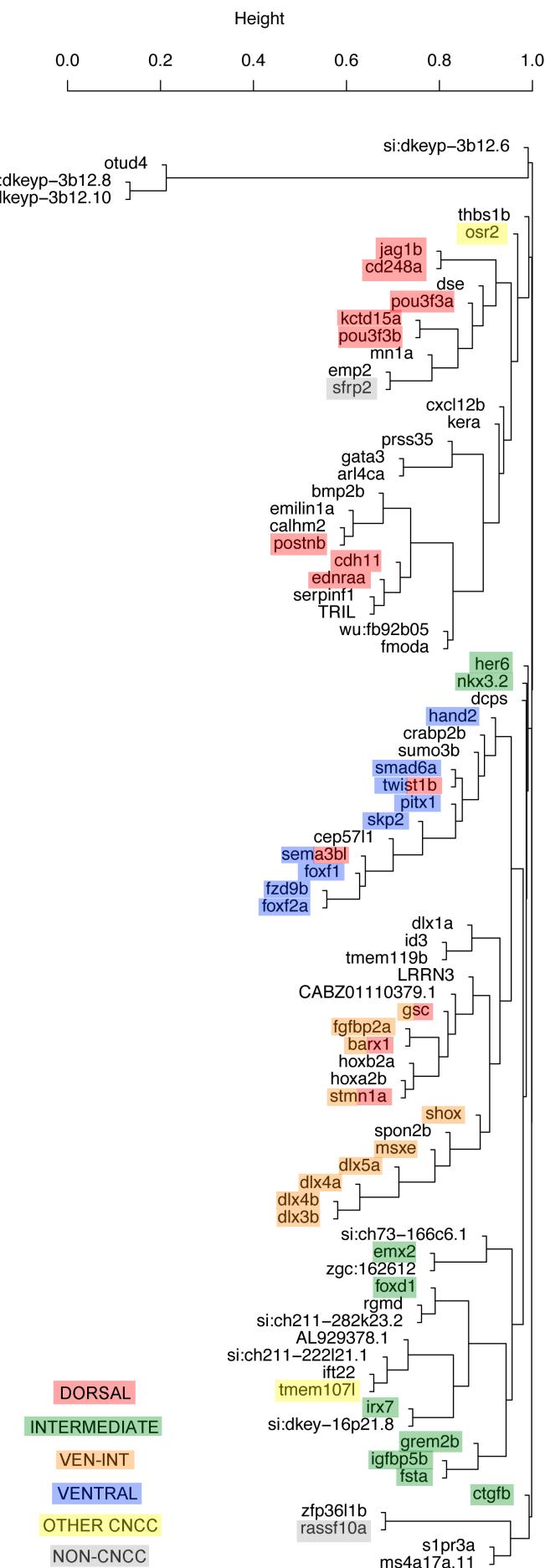
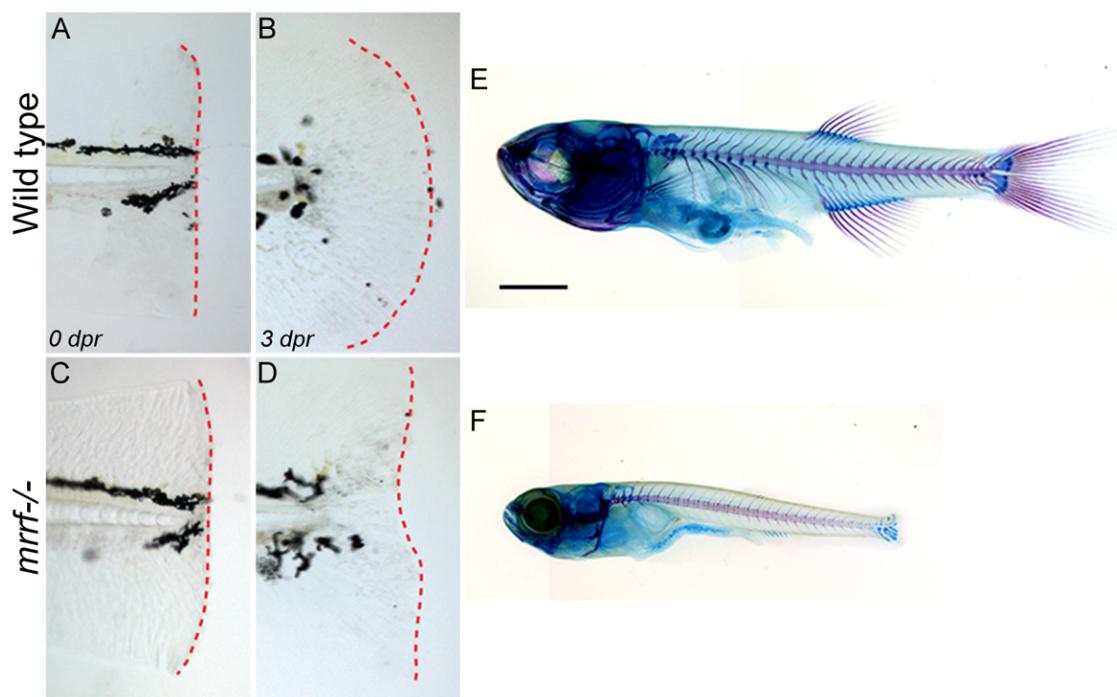


Supplementary Information



Supplementary Figure 1. WGCNA analysis of the dorsoventral gene list.

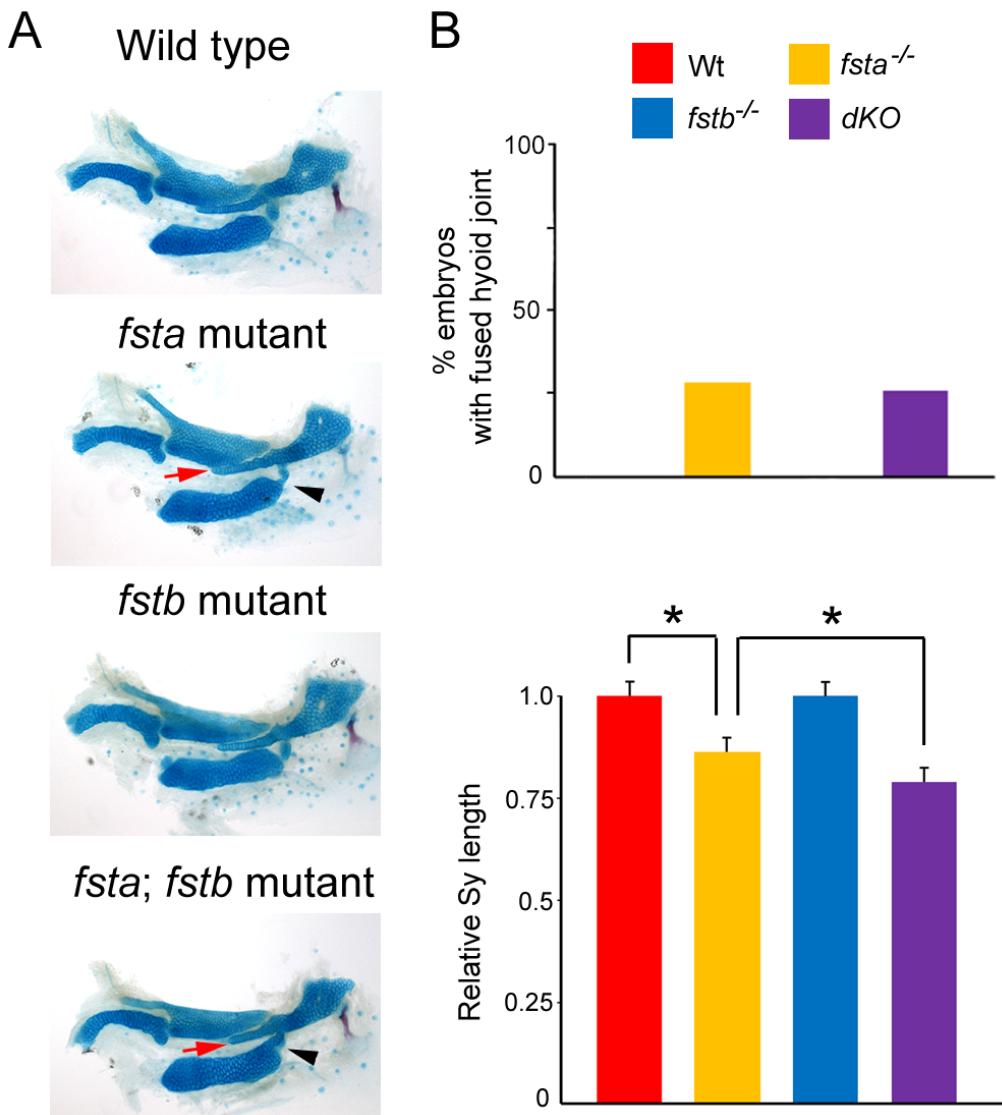
WGCNA analysis (see also Figure 5 and Methods) was applied to the more restricted dorsoventral gene list from Table 1 to reveal clusters of co-varying genes, which are color-coded based on known and/or validated *in situ* expression in the zebrafish pharyngeal arches at 36 hpf. Two-color shading denotes expression in both domains. Height refers to TOM dissimilarity – 0 indicates perfect co-variation between genes and 1 indicates complete lack of co-variation. This analysis confirms *in situ* validation showing that *rassf10a* and *osr2* were incorrectly assigned to the dorsal list, and *tmem107l* and *her6* incorrectly assigned to the ventral-intermediate (ven-int) list.



Supplementary Figure 2. Growth delay and defective fin regeneration in *mrrf* mutants.

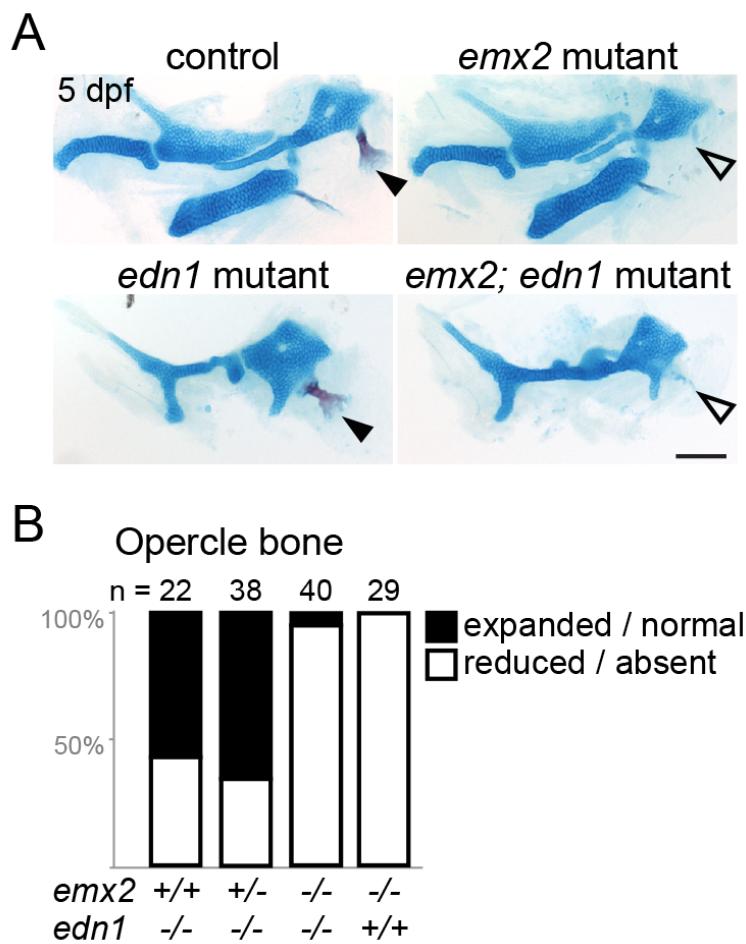
(A-D) Wild-type and *mrrf*-/- embryos had their tail fins amputated at 3 dpf. The dotted red lines show the caudal extent of the tail fin and the lack of regeneration in mutants 3 days post-resection (dpr). (E and F) Alcian blue and Alizarin red staining of cartilage and bone, respectively, show a severe growth defect in *mrrf* mutants at one month of age.

Scale bar = 100 μ m.



Supplementary Figure 3. Redundancy of *fsta* and *fstb* in hyoid skeletal development.

(A) Alcian blue and Alizarin red staining of control, *fsta*, *fstb*, and *fsta*; *fstb* double mutant embryos at 5 dpf. In *fsta* but not *fstb* single mutants, the Sy is shortened (red arrow), and the hyoid joint is fused (black arrowhead). *fsta*; *fstb* double mutants display a slight enhancement of craniofacial defects compared to *fsta* mutants. (B) Quantification of fused hyoid joints and Sy length (number of sides examined for *fsta*, *fstb*, and *fsta*; *fstb* mutants = 14, 10 and 23, respectively). * $p < 0.05$ in student's *t*-test.



Supplementary Figure 4. Analysis of *emx2*; *edn1* compound mutants.

(A) Alcian blue and Alizarin red staining of control, *emx2*, *edn1*, and *emx2*; *edn1* double mutant embryos at 5 dpf. In *edn1* mutants, the opercle bone is variably expanded / normal (closed arrowhead) or reduced / absent (open arrowhead). In *emx2* and *emx2*; *edn1* mutants, the opercle is almost always reduced or absent. In addition, loss of *emx2* fails to rescue the reduced ventral cartilage in *edn1* mutants. Scale bar = 100 μ m.

(B) Quantification of the percentage of opercle bone defects in single and double mutants. The number of sides examined is listed above each bar. The decrease in the proportion of larvae with expanded / normal opercle bone in *emx2*^{-/-}; *edn1*^{-/-} mutants compared with *emx2*^{+/+}; *edn1*^{-/-} was significant at $p < 0.0001$ (two-tailed Chi-square).

Supplementary Figure 5. Searchable dendrogram of co-varying arch genes.

[Click here to Download Figure 5](#)

Supplementary Table 1. Additional information about the dorsoventral gene list.

Gene Name	Description	Arch expression pattern
Ventral		
<i>hand2</i>	Transcription factor	Ventral (Miller et al., 2000)
<i>sema3bl</i>	Hydrolase of Histidine triad (HIT) family	Validated here
<i>fzd9b</i>	G-protein coupled receptor	Validated here
<i>foxf2a</i>	Transcription factor	Validated here
<i>dcps</i>	Bmp signaling pathway transducer	Not tested
<i>smad6a</i>	Centrosomal protein	Validated here
<i>cep57II</i>	Transcription factor	Not tested
<i>skp2</i>	Ubiquitin protein ligase	Validated here
<i>crabp2b</i>	Retinoic acid binding protein	Ventral (Sharma et al., 2005)
<i>pitx1</i>	Transcription factor	Validated here
<i>twist1b</i>	Transcription factor	Validated here
<i>LRRN3</i>	Transmembrane protein	Not tested
<i>sumo3b</i>	Polypeptide similar to ubiquitin	Not tested
<i>barxl</i>	Transcription factor	Dorsal and ventral (Barske et al., 2016)
Ventral-Intermediate		
<i>fgfbp2a</i>	Fgf binding protein	Validated here
<i>dlx4b</i>	Transcription factor	Ventral- intermediate (Talbot et al., 2010)
<i>dlx3b</i>	Transcription factor	Ventral- intermediate (Talbot et al., 2010)
<i>si:dkey-16p21.8</i>	Not tested	
<i>hoxa2b</i>	Transcription factor	Arch-wide (Hunter and Prince, 2002)
<i>shox</i>	Transcription factor	Ventral- intermediate (Sawada et al., 2015)
<i>dlx5a</i>	Transcription factor	Ventral- intermediate (Talbot et al., 2010)
<i>gsc</i>	Transcription factor	Ventral and Dorsal (Miller et al., 2000)
<i>tmem107I</i>	Transmembrane protein 107	Validated here
<i>stmn1a</i>	Oncoprotein	Validated here
<i>si:ch211-222l21.1</i>	Not tested	
<i>CABZ0111</i>	Regulator of Bmp signaling	Not tested
<i>0379.1</i>		
<i>her6</i>	Notch-dependent transcription factor	Validated here
<i>msxe</i>	Transcription factor	Ventral-intermediate (Zuniga et al., 2011)
<i>si:ch211-282k23.2</i>	Not tested	
<i>otud4</i>	Regulator of DNA alkylation repair	Not tested
<i>hoxb2a</i>	Transcription factor	Arch-wide (Hunter and Prince, 2002)
<i>ift22</i>	Intraflagellar transport particle protein	Not tested
<i>dlx1a</i>	Transcription factor	Ventral-intermediate (Sperber et al., 2008)
<i>id3</i>	Transcriptional regulator	Not tested

Gene Name	Description	Arch expression pattern	
<i>tmem119b</i>	<i>transmembrane protein 119b</i>	Not tested	
<i>AL929378.1</i>	<i>SRY (sex determining region Y)-box 11a, sox11a</i>	Transcription factor	Not tested
Intermediate			
<i>emx2</i>	<i>empty spiracles homeobox 2</i>	Transcription factor	Validated here
<i>irx7</i>	<i>iroquois homeobox 7</i>	Transcription factor	Intermediate (Askary et al., 2015)
<i>si:ch7a-166c6.1</i>			Not tested
<i>fsta</i>	<i>follistatin a</i>	Activin/Bmp antagonist	Validated here
<i>zgc:162612</i>			Not tested
<i>igfbp5b</i>	<i>insulin-like growth factor binding protein 5b</i>	Secreted protein modulating tissue distribution of IGF	Validated here
<i>ctgfβ</i>	<i>connective tissue growth factor b</i>	Secreted protein regulating Vegf, Tgf- β and Bmp signaling	Validated here
<i>grem2b</i>	<i>gremlin 2, DAN family BMP antagonist b</i>	Bmp antagonist	Intermediate (Zuniga et al., 2011)
<i>ms4a17a.11</i>	<i>membrane-spanning 4-domains, subfamily A, member 17A.11</i>		Not tested
<i>foxd1</i>	<i>forkhead box D1</i>	Transcription factor	Validated here
<i>nkx3.2</i>	<i>NK3 homeobox 2 (bapx1)</i>	Transcription factor	Intermediate (Miller et al., 2003)
<i>dlx4a</i>	<i>distal-less homeobox 4a</i>	Transcription factor	Ventral-intermediate (Talbot et al., 2010)
<i>spon2b</i>	<i>spondin 2b, extracellular matrix protein</i>	Extracellular matrix protein	Not tested
<i>si:dkeyp-3b12.10</i>			Not tested
<i>si:dkeyp-3b12.8</i>			Not tested
<i>rgmd</i>	<i>RGM domain family, member D</i>	Activator of Bmp signaling pathway	Not tested
Dorsal			
<i>pou3f3a</i>	<i>POU class 3 homeobox 3a</i>	Transcription factor	Validated here
<i>prss35</i>	<i>protease, serine, 35</i>	Serine proteases	Not tested
<i>fmoda</i>	<i>fibromodulin a</i>	Interstitial proteoglycan interacts with collagen type I and II	Not tested
<i>si:dkeyp-3b12.6</i>			Not tested
<i>sfrp2</i>	<i>secreted frizzled-related protein 2</i>	Modulator of Wnt signaling	Validated here
<i>kera</i>	<i>keratanocan</i>	Cornea-specific keratan sulfate proteoglycan	Not tested
<i>emp2</i>	<i>epithelial membrane protein 2</i>	Regulator of cell membrane composition	Not tested
<i>calhm2</i>	<i>calcium homeostasis modulator 2</i>	Cation channel activity	Not tested
<i>cdh11</i>	<i>cadherin 11</i>	Membrane proteins mediating cell-cell adhesion	Validated here
<i>pou3f3b</i>	<i>POU class 3 homeobox 3b</i>	Transcription factor	Validated here
<i>gata3</i>	<i>GATA binding protein 3</i>	transcription factor	Maxillary (Sheehan-Rooney et al., 2013)
<i>serpinf1</i>	<i>serpin peptidase inhibitor, clade F, member 1</i>	Secreted collagen-binding glycoprotein	Not tested
<i>ednraa</i>	<i>endothelin receptor type Aa</i>	G-protein coupled receptor mediating Edn1 signaling	Dorsal (Nair et al., 2007)
<i>emilin1a</i>	<i>lastin microfibril interfacer 1a</i>	Extracellular matrix protein	Not tested
<i>postnb</i>	<i>periostin, osteoblast specific factor b</i>	Cell adhesion molecule	Validated here
<i>cd248a</i>	<i>endosialin a</i>	Transmembrane glycoprotein	Validated here
<i>arl4ca</i>	<i>ADP-ribosylation factor-like 4Ca</i>	Small GTPase	Not tested
<i>jag1b</i>	<i>jagged 1b</i>	Notch ligand	Dorsal (Zuniga et al., 2010)
<i>zfp36Ilb</i>	<i>zinc finger protein 36, C3H type-like 1b</i>		Not tested
<i>thbs1b</i>	<i>thrombospondin 1b</i>	Matrix protein	Not tested

Gene Name	Description	Arch expression pattern
<i>s1pr3a</i>	sphingosine-1-phosphate receptor 3a	G-protein coupled receptor Not tested
<i>rassf10a</i>	Ras association domain family (N-terminal) member 10a	Ras effector Validated here
<i>wu:fu92b05</i>		Not tested
<i>osr2</i>	odd-skipped related transcription factor 2	Transcription factor Frontonasal and maxillary (Swartz et al., 2011)
<i>TRIL</i>	TLR4 interactor with leucine-rich repeats	Component of toll-like receptor 4 signaling complex Not tested
<i>cxcl12b</i>	Chemokine ligand 12b	Ligand of chemokine signaling (Ventral Hess and Boehm, 2012)
<i>dse</i>	dermatan sulfate epimerase	Epimerase Not tested
<i>mn1a</i>	meningioma 1a	Protooncogene Not tested
<i>bmp2b</i>	bone morphogenetic protein 2b	Ligand of bmp signaling Oral ectoderm (Swartz et al., 2011)
<i>kctd15a</i>	potassium channel tetramerization domain containing 15a	Adaptors for Cullin E3 ubiquitin ligases Dorsal (Dutta and Dawid, 2010)

Supplementary Table 2. TALEN/CRISPR target sites and genotyping conditions.

Gene	TALEN/CRISPR target site	Mutation type	Genotyping primers (5'-3')	Restriction enzyme
<i>cd248a</i>	GGCTACCACATCAGACATCCAA	11-bp deletion induces frameshift after aa 63 (of 719)	F: GCAATGAGGATGGGTGCTAT R: CGTCTTGAAGCCAGTTGTG	BccI
<i>ctgfa</i>	GGACACCTGTGGGTGCTGCC	10-bp deletion induces frameshift after aa 52 (of 345)	F: GCTCAAGAGTCAGTGGACA R: GCCAAGATCCTTACCTGTGC	HpaII
<i>cdh11</i>	L: TTAAATACATCCTTCAGGG; R: TTGTCATCGATGACAAATAT	4-bp deletion induces frameshift after aa 104 (of 800)	F: ATAGGCAGCACCCACAGTTC R: TGCAAGAGAACAAAGCTGTTC	BfuAI
<i>ctgfb</i>	GGAGCCATGCGACCATCATA	4-bp deletion induces frameshift after aa 70 (of 347)	F: GACGAGAGCCCATTATGTCC R: AAAATGTTGGGCTACCTTCA	BccI
<i>emx2</i>	L: TTAGTGTCCCTGAGGCTGT; R: TGAATGGACTGGACGGCGG	10-bp deletion induces frameshift after aa 74 (of 247)	F: ACTATGTTCAACCCACACCG R: TTGCGAAAAGAGGGTGC	BtsCI
<i>fsta</i>	GGTGGATGATCTTCAATGGC	14-bp deletion induces frameshift after aa 84 (of 322)	F: GGTAACTGCTGGCTTCAA R: TCATTCAATACTGACGGGA	AciI
<i>fstb</i>	GAATGAGTGTGCCCTCCTTA	14-bp deletion induces frameshift after aa 142 (of 344)	F: GGTGGCTGATCTTCAATGGT R: TTTGCATTTCCCTGGTACTG	Bsu36I
<i>her6</i>	L: TGCGATATCATGGAAAAAA; R: TGCTCGCCGGAGTCGCGCG	8-bp deletion induces frameshift after aa 10 (of 270)	F: GCGTACTTGACAGCGTTACT R: CAAGCTTCGTTGATTCTCGC	EarI
<i>mrrf</i>	GGAGGATATTATCAGCCTGG	4-bp deletion induces frameshift after aa 78 (of 257)	F: AAGGGCAGACAGCTAAAG R: ATAACGTGGCTTCAGGTT	EcoNI
<i>sfrp2</i>	I: GGTGCAGAAACAGTGTCACTC; II: GGCGGAGATGCTGGACTGC	161-bp deletion induces frameshift after aa 85 (of 249)	F: AAACATGCGTCTGCCTAACCC R: AAATGAATTGATCGCGCATT	n/a
<i>osr1</i>	L: TGCCTGGCCTGGATGCC; R: TGGGGAACAGTGGATTGA	7-bp deletion induces frameshift after aa 80 (of 264)	F: GGCAGAACTATTCTTCAGACC R: GGTTTACTTTGCTGGATGCTCC	EarI
<i>osr2</i>	L: TGGACGGTGGCTCCCGCA; R: TCGGCCGGGAAGCGCGGGT	23-bp deletion induces frameshift after aa 57 (of 238)	F: ACACTGAACCGCGTTCCCG R: GATTGGCGAAGTCGAAGCG	BstNI

TALEN pairs (L,R) were used to generate mutants for *cdh11*, *emx2*, *her6*, *osr1*, and *osr2*. CRISPR gRNAs were used to generate mutants for *cd248a*, *ctgfa*, *ctgfb*, *fsta*, *fstb*, *mrrf*, and *sfrp2*. For *sfrp2*, two gRNAs were used to make a larger deletion of the coding region.

Supplementary Table 3. Cloning and synthesis of in situ probes.

Gene	Forward cloning primer (5'-3')	Reverse cloning primer (5'-3')	Enzymes
<i>cd248a</i>	CCTCTTGACTTCCCTGGAGA	GCGAACCTCATGAAACACAA	BamHI, T7
<i>cdh11</i>	TGATGAACAACCCCATTCAAGA	GCTAAATACGACTCACTATAGGTGTTCTCC	EcoRI, T7
<i>ctgfb</i>	GGCTACACCCCCATTCTGCTA	CGAATGTCTTC	
<i>emx2</i>	AACTGGAGGAAGAAGGGTCG	GGCATCCAGACAACCTCGAAA	EcoRV, Sp6
<i>fgf20b</i>	CAGCTATGGGAGAGATCGGG	AAAACACTATAACGCAGCACTG	PstI, Sp6
<i>fgfbp2a</i>	TGTGGACAATCTCAAGCACAC	GGTCCACAGGTCTTGGAAAGA	EcoRV, Sp6
<i>fgfbp2b</i>	AGTCCATTACACCTTCGCCA	TGACGAAGGAGCATGCACTA	BamHI, T7
<i>foxd1</i>	GAGATCCTGCTCAACGGTTC	ACTGATTGACTCTCCTCCGC	BamHI, T7
<i>foxf1</i>	GATCGTATCAGGGCTGGAAA	GGACTTAGAGGGGGTAGAA	EcoRI, T7
<i>foxf2a</i>	GTCACTACTGGACCATAGAT	TGTCACACATGCTGGGAGAT	EcoRI, T7
<i>fsta</i>	GGAAAGACCAGGAGGATGACGATG	AGAGAATACGGAGGCATACT	BamHI, T7
<i>fzd9b</i>	TATCATCCGATCAGTCGCCG	TCGGTGACCTTGTGTCGC	EcoRV, Sp6
<i>her6</i>	ACCAGTTGAACCTGGGACAC	TACGGTGCATCCCAGATGAGG	NotI, Sp6
<i>igfbp5b</i>	GCTGGGTACATTCTGACGG	TTGAACCATGGGTTGACTGA	EcoRV, Sp6
<i>kctd15a</i>	GCACTCTCACGCTTCAACAA	TGGATGTTACCGCCACTGTA	PstI, Sp6
<i>mrrf</i>	GTTCCCAAGGTAACTCGTG	AATAACATGGTCGGGTCCCT	SpeI, T7
<i>osr2</i>	CAGCTCAACTACTCGCTCCTG	TCGTTGTTCACCCCTGTGTG	BamHI, T7
<i>pitx1</i>	CCCGAAGAAGAAGAACGAC	ATTGAGGGGTGAGTGTGTCC	BamHI, T7
<i>postnb</i>	AGTGACCCGAGTTATCCAGG	TATGCTCGTCTGTGCTCCAG	HindIII, T7
<i>pou3f3a</i>	AAACAAACTCCATGCACAATG	GTGCCCAGTGAGGTCTTC	EcoRV, Sp6
<i>pou3f3b</i>	GCATTCTTTGCCCTTAC	TCATCTTACGGAATCACTACTGAAA	BamHI, T7
<i>rassf10a</i>	CAGGAAAAACAGCGGAGGATTG	CCCAAACATTGTCTACTGAAGGCAG	NotI, Sp6
<i>sema3bl</i>	TGCTGTTGGCTCGAGATCC	GTCTACTGTGAGGCGATGCG	EcoRV, Sp6
<i>sfrp2</i>	TACAAACCTGCTCTGTGCC	TGAAGTTGCGAATGCTGCG	HindIII, T7
<i>shox</i>	AACAGCAGGAGTGTAAACCC	TCTTGCTTGAGTCGCAGGTCA	EcoRV, Sp6
<i>skp2</i>	GACATCTGGACTGAGCCG	TCCATCAGGTCAAGGTACTGC	HindIII, T7
<i>smad6a</i>	TATGTCTCCCTCCTCGCTGG	TCATTTGGGGTTAGTCGTGG	HindIII, T7
<i>snaila</i>	AGCTTGCTACCTTCCCTCA	ACCCCTACCATAGTCAACCCAC	EcoRV, Sp6
<i>stmn1a</i>	GTGAGAAAACGGCGCGAGG	ACACAAGTCCCCACCAAGTCC	NotI, Sp6
<i>tmem107I</i>	AATGTCGGTGGTCAGCAGTCTG	GAGTTTATTGTGCCAGTGAGTGAATG	EcoRV, Sp6
<i>twist1b</i>	CTCCCTCCTCTCAAACACTTT	GCACACTAGTGAGATGCAGAC	BamHI, T7

Restriction enzymes and type of RNA polymerase for synthesis are shown in the last column.

Table S4. TPM values for all RNA sequencing experiments.

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Table S5. TPM values and filtering of dorsoventral gene list.

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Supplementary References

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