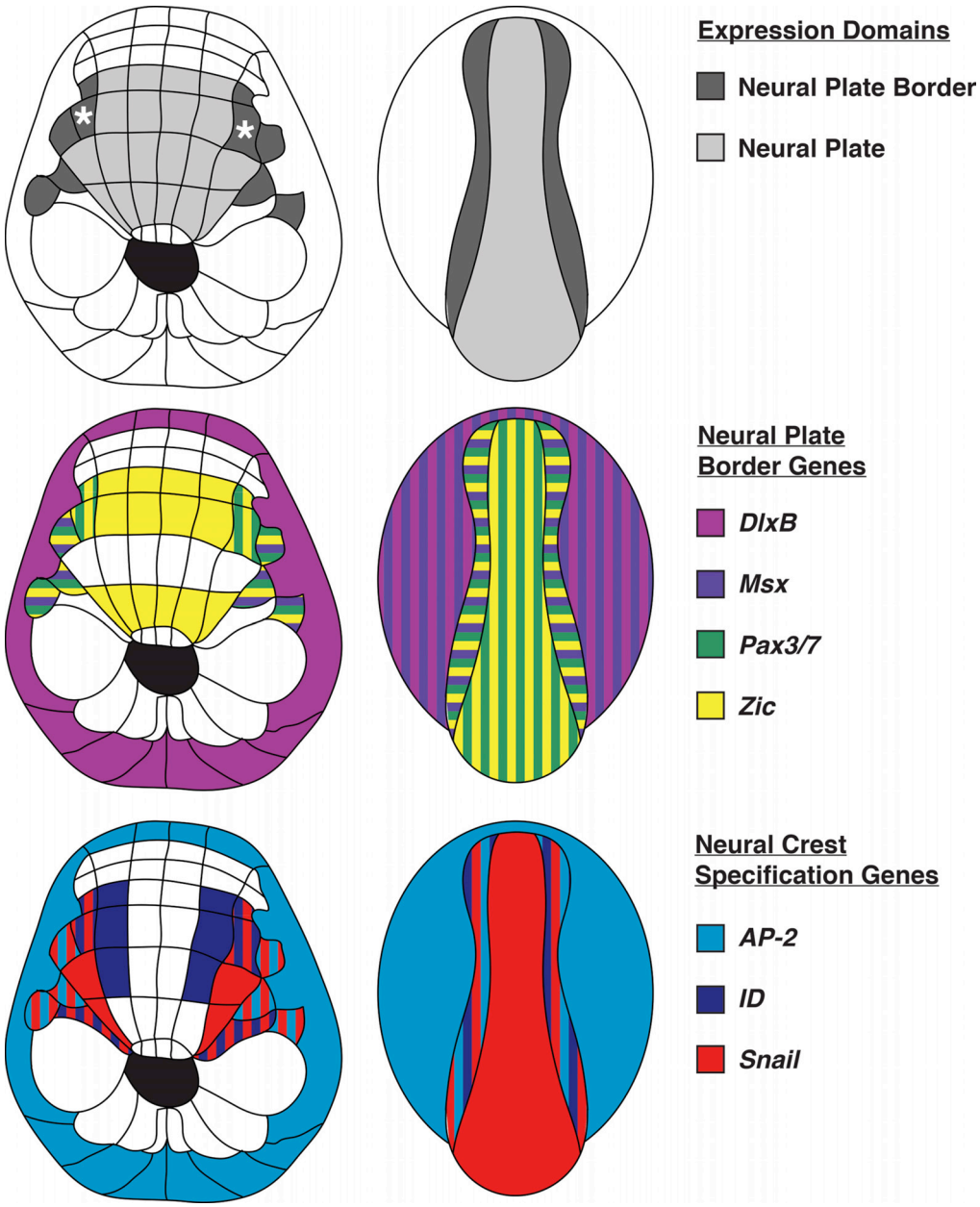
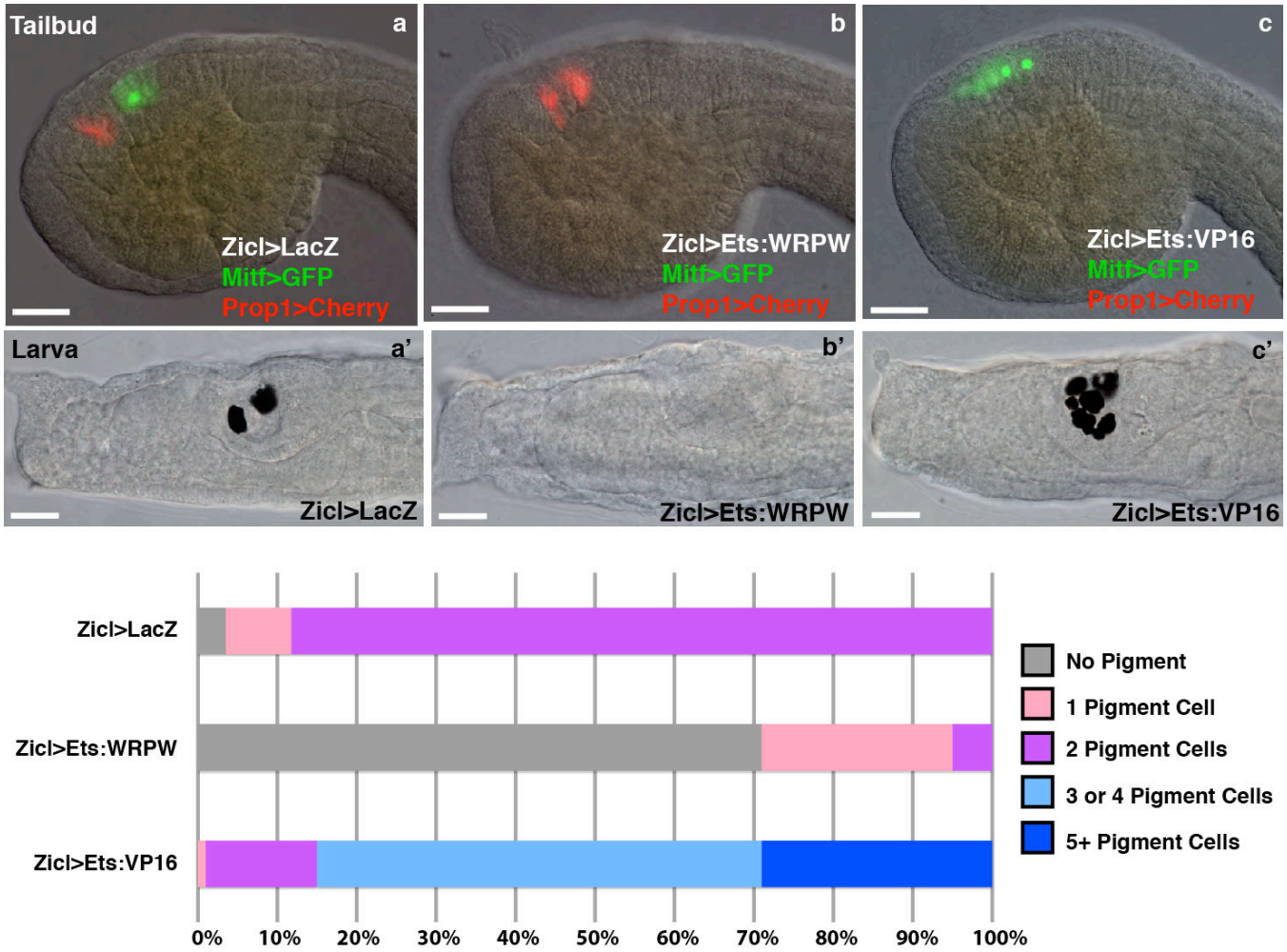
Supplementary Information

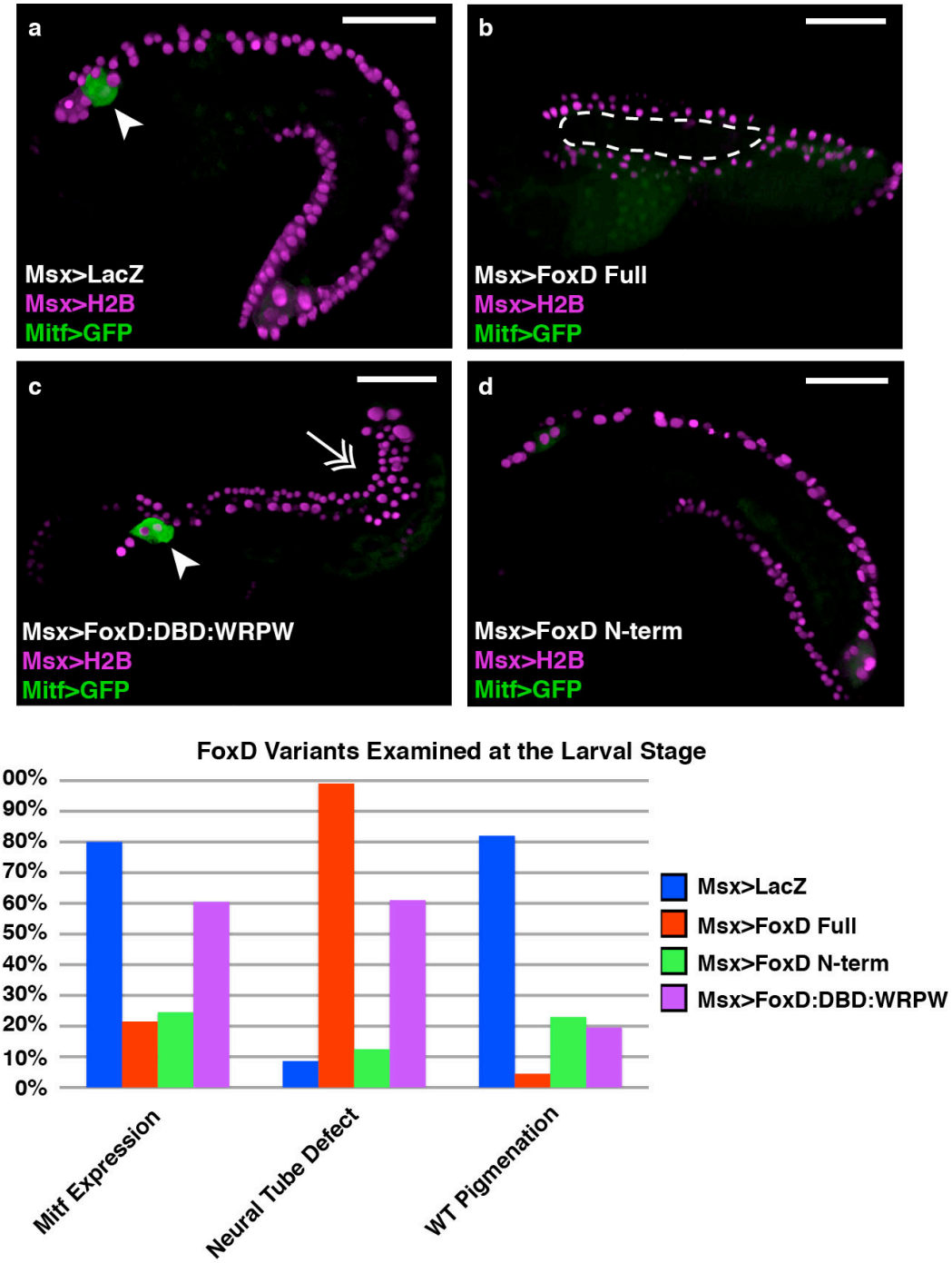
Supplementary Figures and Legends



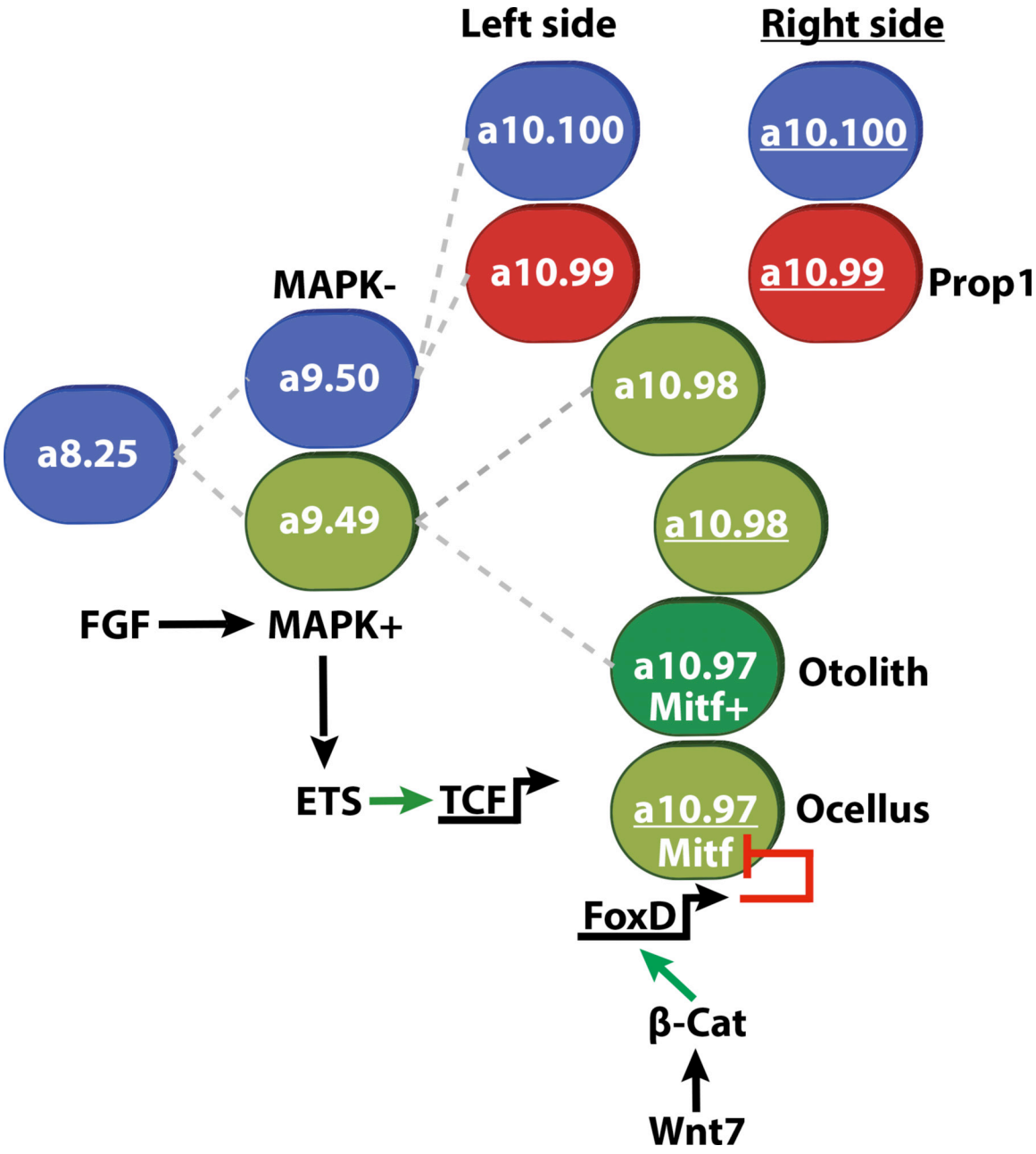
Supplementary Figure 1 | *Ciona intestinalis* and *Petromyzon marinus* neural crest expression domain comparison. Cartoon shows dorsal views of *Ciona* mid gastrula (left) and *Petromyzon* early neurula (right). The a9.49 lineage is marked by asterisks and in all images anterior is up. Initially in Ciona *DlxB* and *AP-2* are broadly expressed in the epidermis (including the mother of a9.49). Later *DlxB* is cleared from the neural ectoderm, whereas *AP-2* is maintained in the border as shown. During Ciona neurulation *Msx* expands medially into the a9.49 lineage and dorsal neural tube precursors. In both species *FoxD* is expressed only after neural tube formation is complete.



Supplementary Figure 2 | Ets1/2 activity controls the number of pigmented cells. **a**,**a**' Tailbud embryo co-electroporated with *ZicL>LacZ*, *Prop1>mCherry*, *Mitf>GFP* (**a**) larva stage reveals two pigmented cells (**a'**) (n=85). **b**,**b**' Tailbud embryo co-electroporated with *ZicL>Ets:WRPW*, *Prop1>mCherry*, *Mitf>GFP* does not express *Mitf* reporter (**b**) resulting larva is not pigmented (**b'**) (n=100). **c**,**c**' Tailbud embryo co-electroporated with *ZicL>Ets:VP16*, *Prop1>mCherry*, *Mitf>GFP* shows expanded *Mitf* reporter at the expense of *Prop1* expression in the a10.99 lineage (**c**) resulting larva has supernumerary otoliths (**c'**) (n=100). In all images anterior is to the left and the scale bar represents 25 μm.

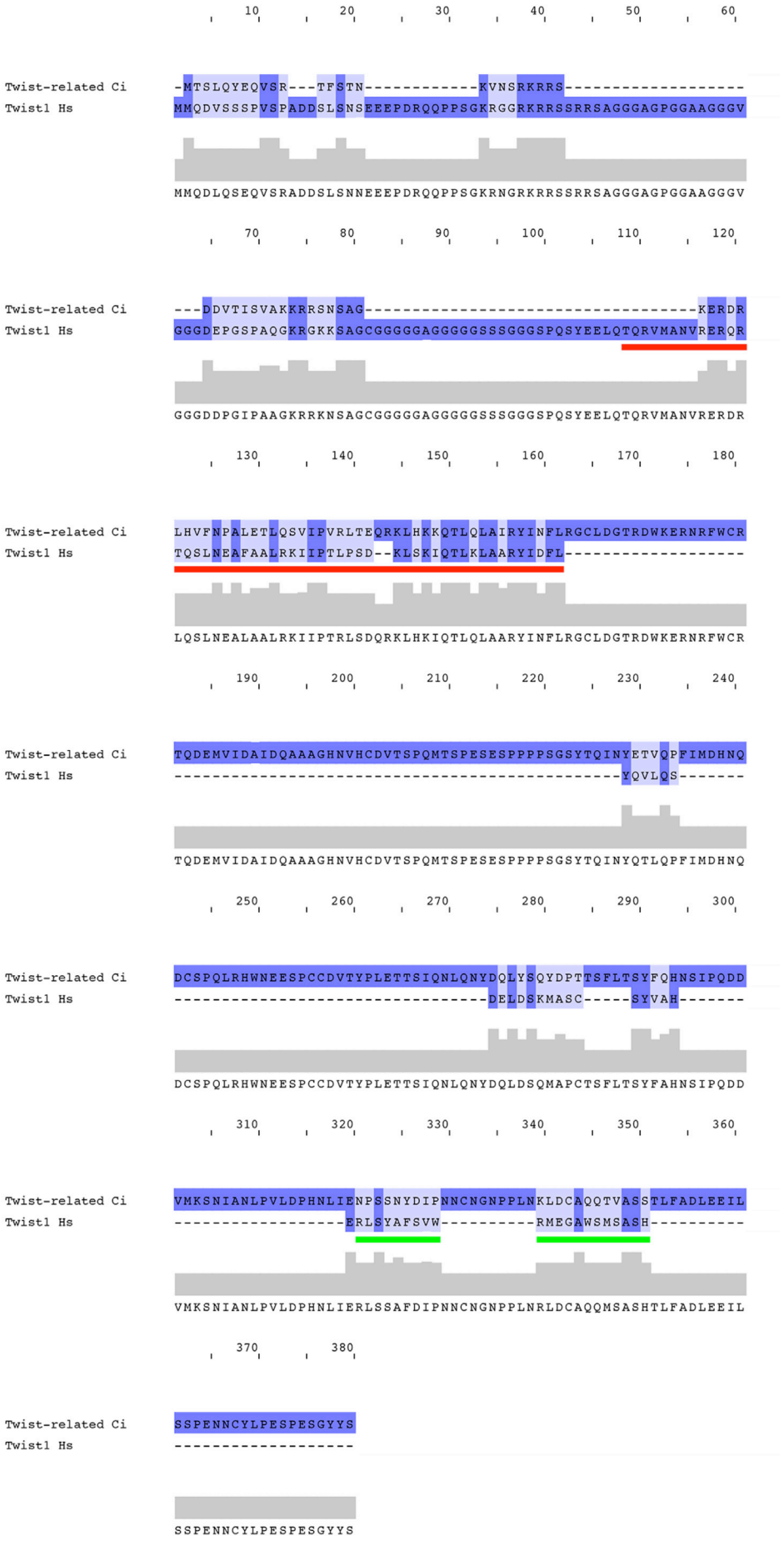


Supplementary Figure 3 | Misexpression of FoxD constructs in the midline. a, Tailbud embryo co-electroporated with *Msx>LacZ*, *Msx>H2B mCherry*, and *Mitf>GFP*. Msx expression marks the midline and arrowheads show GFP expression in the a9.49 lineage in the series (n=176). **b**, Tailbud embryo co-electroporated with *Msx>FoxD* full length, *Msx>H2B mCherry*, and *Mitf>GFP*. Dotted line denotes the space between the neural folds that failed to intercalate (n=200). **c**, Tailbud embryo co-electroporated with *Msx>FoxD:DBD:WRPW*, *Msx>H2B mCherry*, and *Mitf>GFP*. Double arrow shows kinked tail resulting from a neural tube closure defect (n=200). **d**, Tailbud embryo co-electroporated with *Msx>FoxD* N-terminal, *Msx>H2B mCherry*, and *Mitf>GFP* (n=200). In all images anterior is to the left and the scale bar represents 50 μm. Graph indicates phenotypes observed at the larval stage.



Supplementary Figure 4 | Summary of the Mitf regulatory network. An FGF signal activates the MAPK pathway leading to the phosphorylation of Ets1/2 in a9.49 whose descendants are initially Mitf+ (green). The sister cell, a9.50, remains MAPK- and gives rise to the Prop1+ cell (red). Ets1/2 activation causes the transcription of *TCF* in the a10.97 pair allowing for Wnt competence. The left a10.97 and right a10.97 intercalate at the midline and the posterior cell receives the Wnt7 ligand leading to the expression of *FoxD* only in a10.97. FoxD represses *Mitf* in the presumptive ocellus while *Mitf* remains active in the otolith (dark green).

a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70 80 90 100 110 120
Twist-related Ci ---DDVTISVAKKRRSNSAG----------KERDR
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GGGDDPGIPAAGKRRKNSAGCGGGGGAGGGGGSSSGGGSPQSYEELQTQRVMANVRERDR
130 140 150 160 170 180
Twist-related Ci LHVFNPALETLQSVIPVRLTEQRKLHKKQTLQLAIRYINFLRGCLDGTRDWKERNRFWCR
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190 200 210 220 230 240
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Twist1 Hs ----------ERLSYAFSVW----------RMEGAWSMSASH----------
VMKSNIANLPVLDPHNLIERLSSAFDIPNNCNGNPPLNRLDCAQQMSASHTLFADLEEIL
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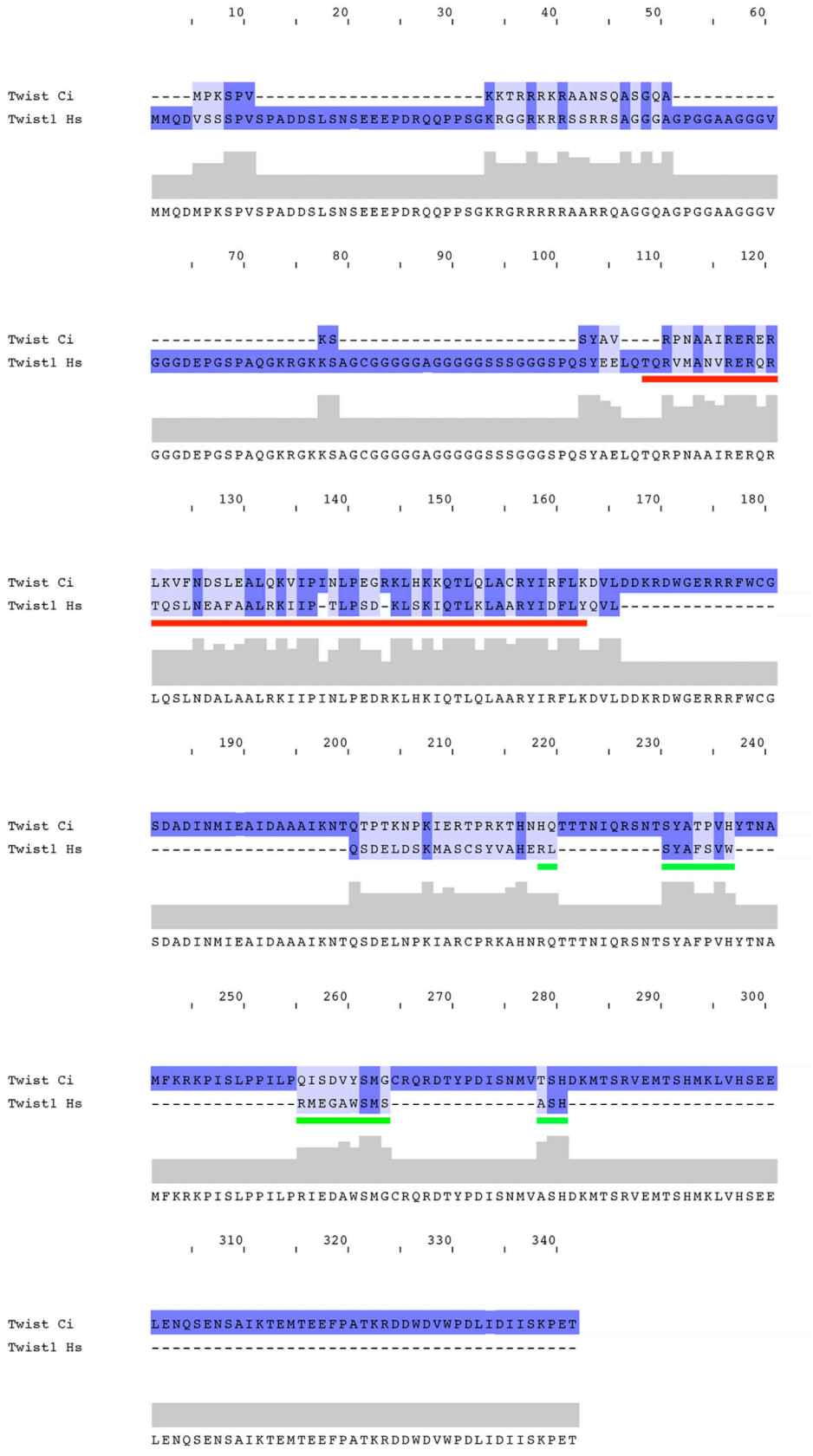
Twist1 Hs ----------
SSPENNCYLPESPESGYYS

b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GGGDEPGSPAQGKRGKKSAGCGGGGGAGGGGGSSSGGGSPQSYAELQTQRPNAAIRERQR
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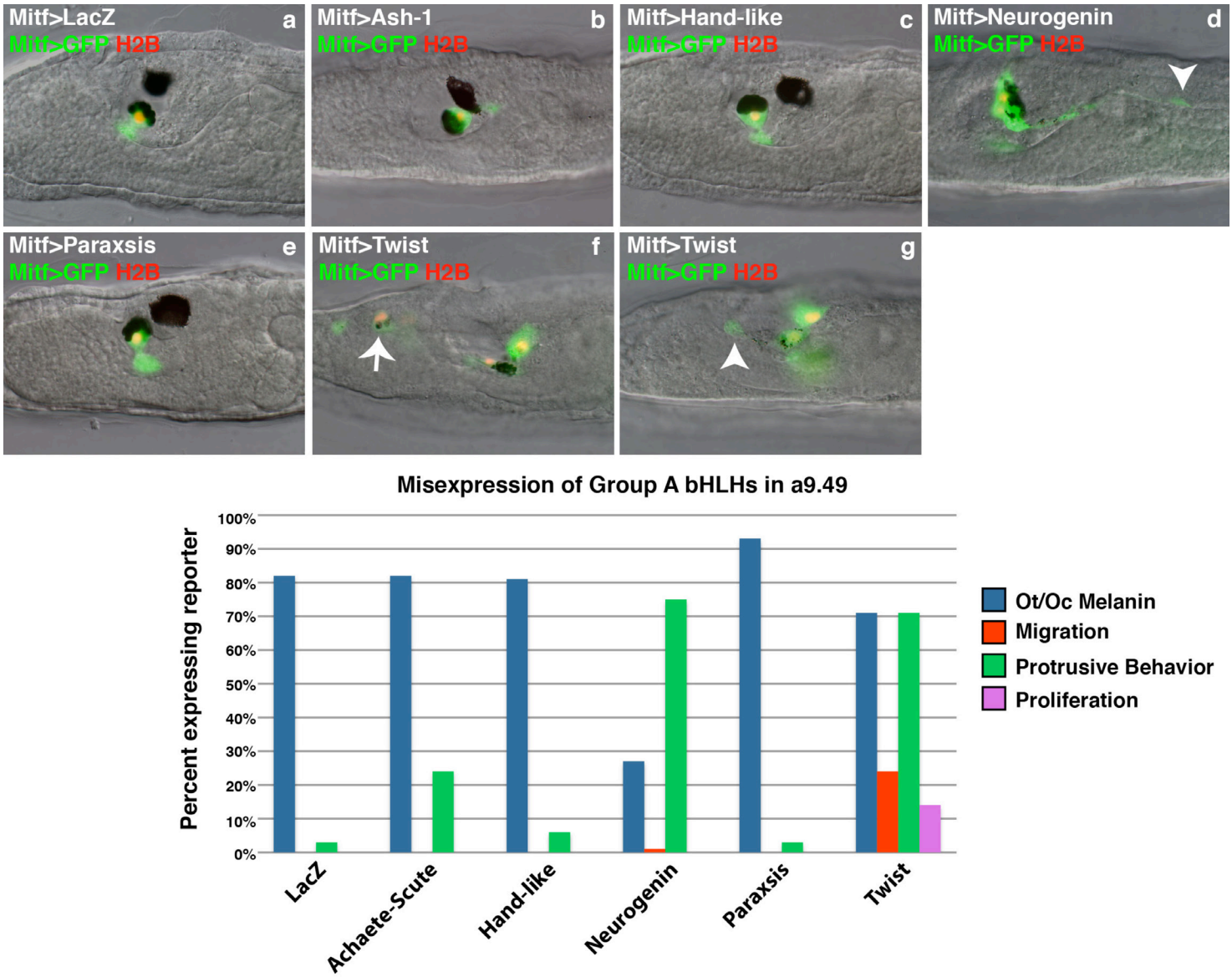
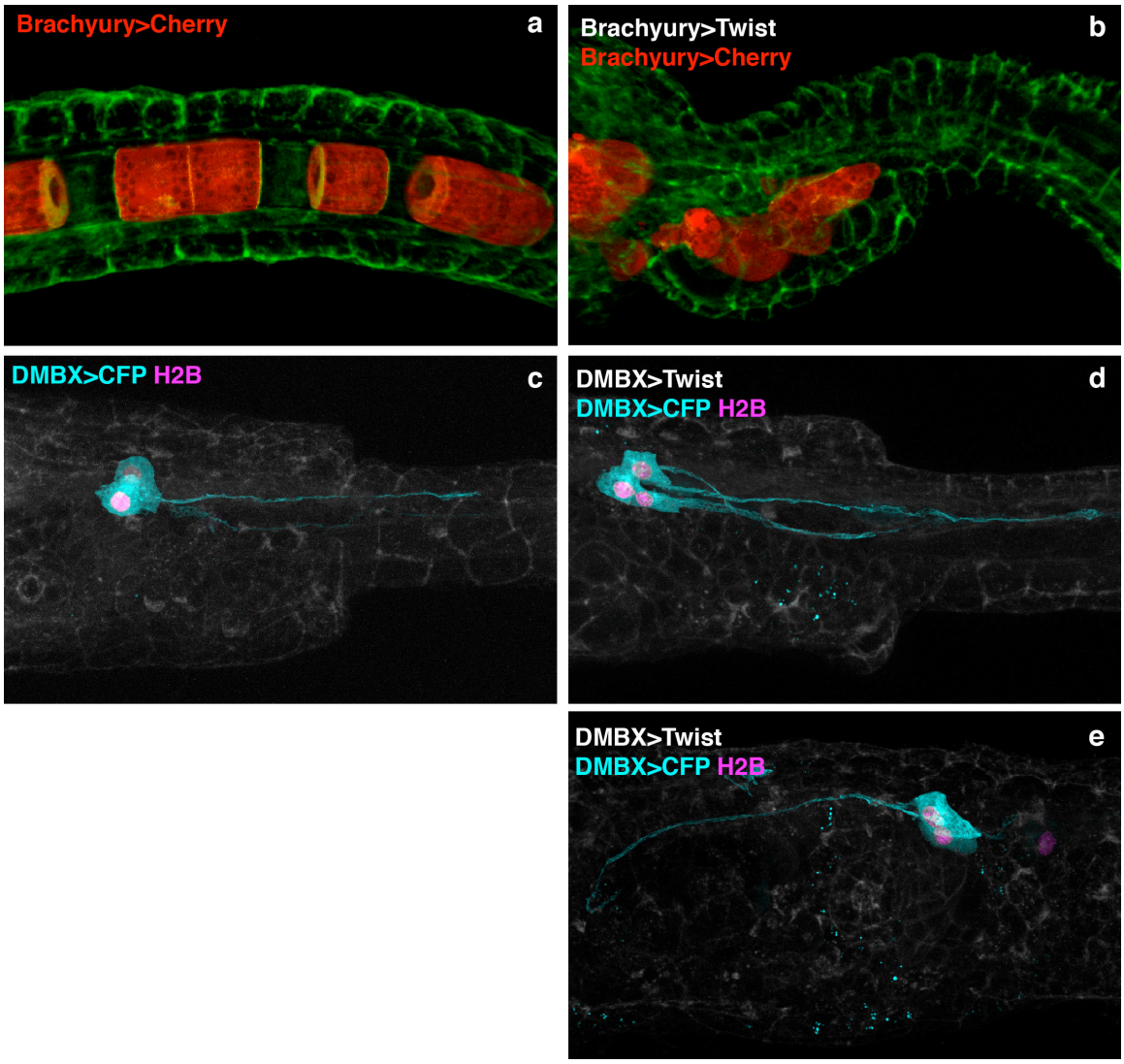
LQSLNDALAALRKIIPINLPEDRKLHKIQTLQLAARYIRFLKDVLDDKRDWGERRRFWCG
190 200 210 220 230 240
Twist Ci SDADINMIEAIDAAAIKNTQTPTKNPKIERTPRKTHNHQTTTNIQRSNTSYATPVHYTNA
Twist1 Hs ----------QSDELDSKMASCSYVAHERL----------SYAFSVW----
SDADINMIEAIDAAAIKNTQSDELNPKIARCPRKAHNRQTTTNIQRSNTSYAFPVHYTNA
250 260 270 280 290 300
Twist Ci MFKRKPISLPPILPQISDVYSMGCRQRDTYPDISNMVTSHDKMTSRVEMTSHMKLVHSEE
Twist1 Hs ----------RMEGAWSMS----------ASH----------
MFKRKPISLPPILPRIEDAWSMGCRQRDTYPDISNMVASHDKMTSRVEMTSHMKLVHSEE
310 320 330 340
Twist Ci LENQSENSAIKTEMTEEFPATKRDDWDVWPDLIDIISKPET
Twist1 Hs ----------
LENQSENSAIKTEMTEEFPATKRDDWDVWPDLIDIISKPET

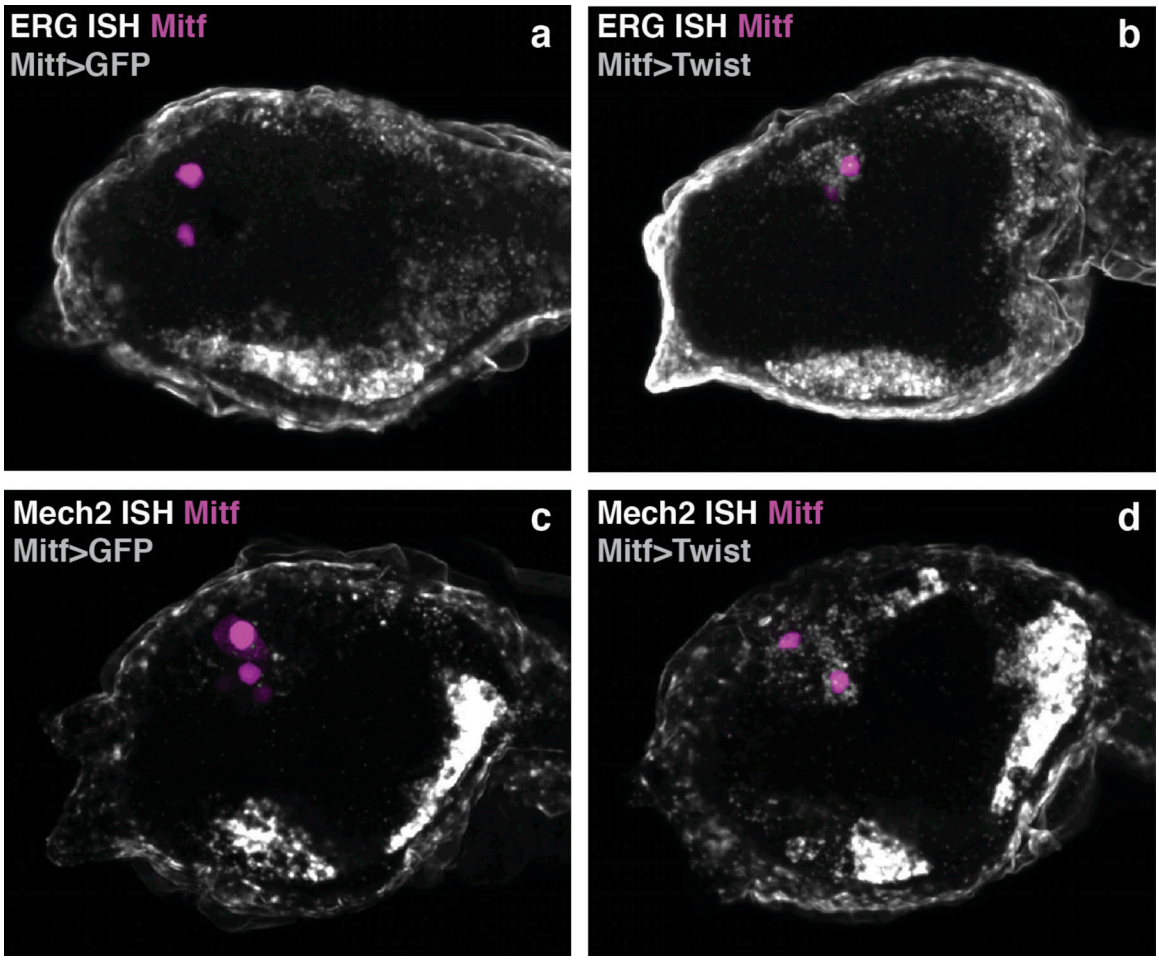
Supplementary Figure 5 | *Ciona intestinalis* Twist is the closest homolog to human Twist1. **a**, *Ciona Twist*-related (model ID: KH.C5.554.v1.A.nonSL1-1) aligned to Human *Twist1*. The best blastp hit for *Twist*-related in the vertebrate database is atonal homolog 1a (*Danio rerio*). **b**, *Ciona Twist* (model ID: KH.C5.202.v1.A.ND1-1) aligned to Human *Twist1*. The best blastp hit for *Twist* in the vertebrate database is twist-related protein (*Xenopus laevis*), expressed in cranial neural crest. Alignment generated using webPRANK. The red underline indicates the annotated Human bHLH and the green underline shows the C-terminal Twist Box.



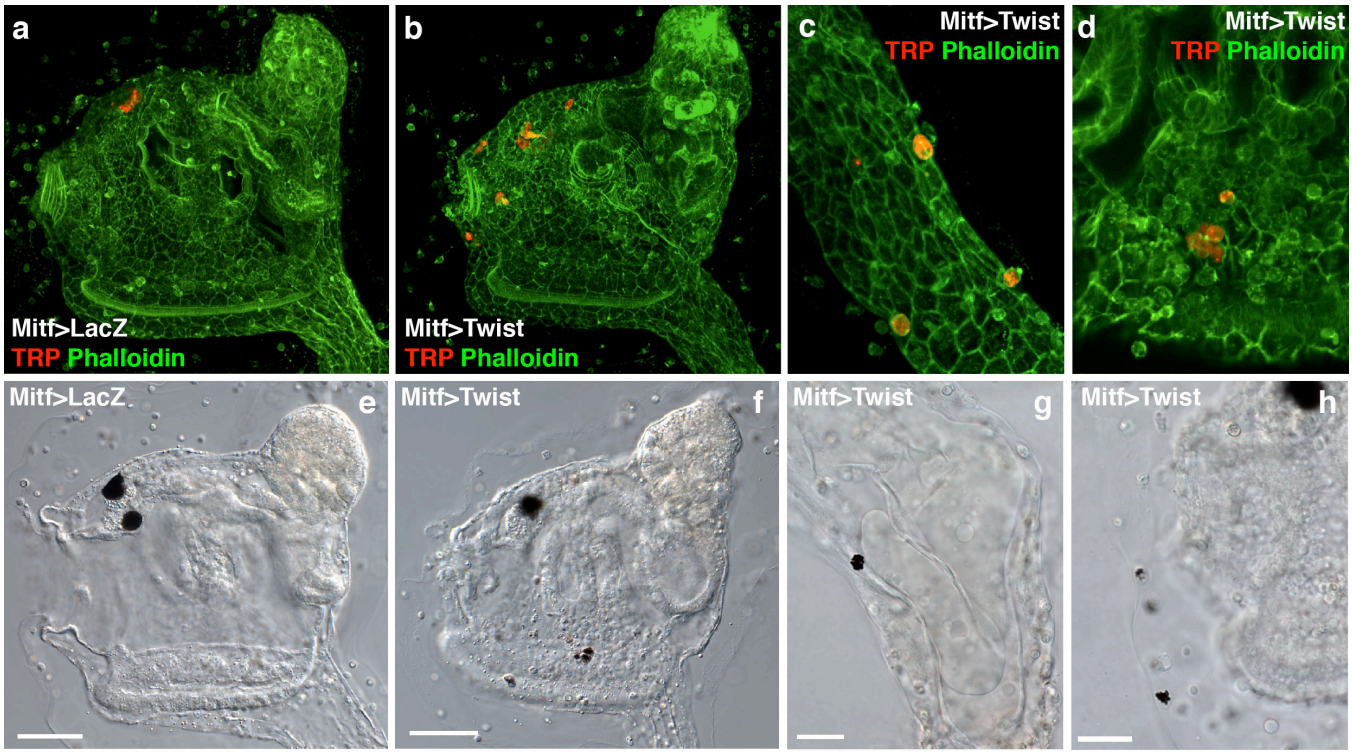
Supplementary Figure 6 | Related group A bHLH genes do not cause the same phenotype as Twist when misexpressed in a9.49. All larvae electroporated with *Mitf>GFP* and *Mitf>H2B mCherry* and 100 embryos were scored in each condition. **a**, Larva electroporated with *Mitf>LacZ*. **b**, Larva electroporated with *Mitf>Ash-1*. **c**, Larva electroporated with *Mitf>Hand-like*. **d**, Larva electroporated with *Mitf>Neurogenin*. **e**, Larva electroporated with *Mitf>Paraxsis*. **f**,**g**, Larvae electroporated with *Mitf>Twist*. Graph summarizes the phenotypes for each condition. Larvae were scored positive for ot/oc melanin if both otolith and ocellus pigmentation were observed. H2B mCherry fluorescence found outside of the sensory vesicle was marked positive for migration (arrow). Larvae with irregular or elongated cell shapes were marked for protrusive behavior (arrowheads). Five or more Mitf+ cells seen in a larva was scored as positive for proliferation. In all images anterior is to the left.

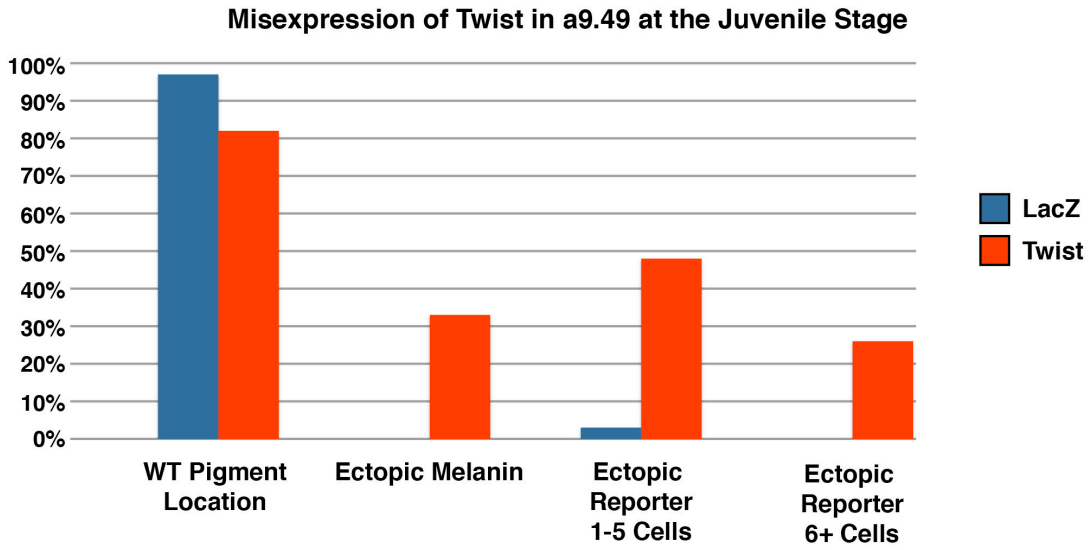


Supplementary Figure 7 | Misexpression of Twist in other lineages does not induce migratory cells. a, Tailbud embryo electroporated with *Brachyury>mCherry* labels the notochord. **b**, Tailbud embryo co-electroporated with *Brachyury>mCherry* and *Brachyury>Twist* displays a misshapen notochord causing a truncated tail. **c**, Larva electroporated with *DMBX>CFP* and *DMBX>H2B YFP* labels the A12.239 lineage within the motor ganglion. **d**, Larva co-electroporated with *DMBX>CFP, DMBX>H2B YFP, and DMBX>Twist.* An extra cell is seen but the lineage still projects axons posteriorly. **e**, Twist misexpression sometimes results in anteriorly projecting axons, but the cell morphology and position of cell body remain largely unaffected. In all images anterior is to the left.

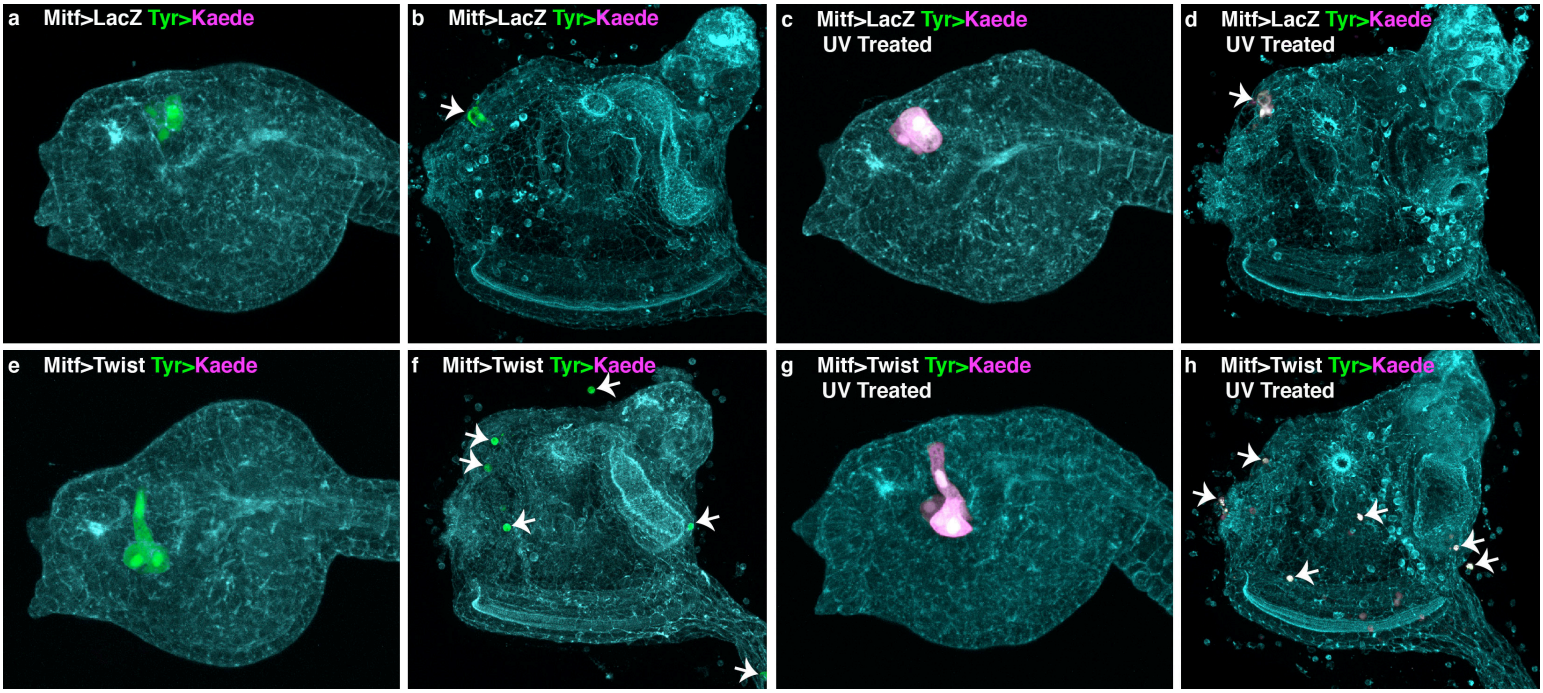


Supplementary Figure 8 | Misexpression of Twist in a9.49 causes the ectopic activation of mesenchyme genes. a, Late tailbud embryo hybridized with an *ERG* probe (white) and electroporated with *Mitf>GFP* (plasmid control) and *Mitf>LacZ*, which was detected with an antibody for β-gal (magenta). **b**, Late tailbud embryo hybridized with an *ERG* probe and electroporated with *Mitf>Twist* and *Mitf>LacZ*. Note the ectopic expression of ERG in a9.49. **c**, Late tailbud embryo hybridized with a *Mech2* probe and electroporated with *Mitf>GFP and Mitf>LacZ*. **d**, Late tailbud embryo hybridized with a *Mech2* probe and electroporated with *Mitf>Twist* and *Mitf>LacZ*. Note the ectopic expression of *Mech2* in a9.49.





Supplementary Figure 9 | Twist misexpression causes ectopic a9.49 cells in juveniles. **a**, Juvenile electroporated with *Mitf>LacZ* and *TRP>mCherry* (n=100). **b**, Juvenile electroporated with *Mitf>Twist* and *TRP>mCherry* (n=100). **c**, Stalk of a juvenile electroporated with *Mitf>Twist* and *TRP>mCherry*. **d**, View of internal mesenchyme electroporated with *Mitf>Twist* and *TRP>mCherry*. **e**, DIC image of juvenile electroporated with *Mitf>LacZ* shows normal pigmentation. **f**, DIC image of juvenile electroporated with *Mitf>Twist* displays ventral ectopic pigment. **g**, DIC image of juvenile stalk electroporated with *Mitf>Twist* shows ectopic pigment. **h**, DIC image of juvenile tunic shows melanized tunic cells. In all images anterior is to the left. Scale bar represents 50 μm in e and f, and 25 μm in g and h.



Supplementary Figure 10 | Lineage tracing of Mitf>Twist juveniles confirms ectopic cells arose from a9.49. **a**, Tailbud electroporated with *Mitf>LacZ* and *Tyr>Kaede* labeled the a9.49 lineage (green). **b**, Non-converted Kaede traced through the juvenile stage (arrow). **c**, Tailbud electroporated with *Mitf>LacZ* and *Tyr>Kaede* in which UV treatment converted the fluorescence (red). **d**, Photoconverted Kaede traced through the juvenile stage (arrow). **e**, Tailbud electroporated with *Mitf>Twist* and *Tyr>Kaede* labeled the a9.49 lineage (green). **f**, Non-converted Kaede traced through the juvenile stage (arrows). **g**, Tailbud electroporated with *Mitf>Twist* and *Tyr>Kaede* in which UV treatment converted the fluorescence (red). **h**, Photoconverted Kaede traced through the juvenile stage (arrows).

Supplementary Table

PCR Product Name	Forward Primer	Reverse Primer
Mitf 963 bp	ctgctaaaacaggctaacag	cgaatatctagtttacgagac
Prop1 1474 bp	tgttgctcatgctcgctgtc	aaaccaaacctaaacgcaaaggc
βγ-crystallin 1110 bp	gtccttacgtcataataaac	gccattgttccaccagcaac
Twist 1526 bp	accacagcttctattatatattaacctc	catcgtgtgttgattgatttgaaag
Ash-1 CDS	atggcgaccggaagtgacgaac	gtcacgtggtgatcagaaatg
Hand-like CDS	atgacaacagtagttatgcg	ttaataactttcgttgcgatg
Paraxsis CDS	atgccagatcacgtggttcatacg	tcacaatcgaaccgtcgaagc
Neurogenin CDS	atgttggatttttcttcaaataagtcgg	ttatcggagtaaatgcatggagtag
Wnt7 CDS	atgtacaattggagcagctt	gcacttgtcagttgcaggta
Stabilized β-catenin CDS	acgatgggaacttccccgatac	ttagaggtcagtatcgagccatg
Twist CDS	atgccaaaatcaccagtcaag	ccacacattaagtttctggc
Foxd Full length CDS	atgatgacagtgcagtgttg	ttaagttctgccaaaacaaggcc
Foxd DBD CDS	cgaatcaaagaagaatgtaaaacctccg	ccggaacggatctctctg
Foxd N-term CDS	atgatgacagtgcagtgttg	cggtgatttcggcaaccag

Supplementary Table 1 | Primer pairs used as described in the Methods

Supplementary Movies

Supplementary Movie 1
This movie shows the lateral view of a *Mitf>Twist* expressing larva labeled with *Tyr>mCherry*. The time-lapse covers a period of about 168 minutes, during which time the protrusive behavior of the a9.49 lineage is seen. The a9.49 lineage misexpressing *Twist* commonly forms filopodia, which is indicative of cellular reprogramming to mesenchymal fate (QuickTime; 2.4 MB).

Supplementary Movie 2
This movie shows the lateral view of a *Mitf>Twist* expressing early juvenile labeled with *Tyr>mCherry* (grey is UV autofluorescence). The time-lapse covers about 6 hours of development, during which time the ectopic a9.49 lineage can be seen migrating around the periphery like the endogenous migrating tunic cells (autofluorescence). The movie loops twice and during the second time the migrating descendants of a9.49 are labeled with asterisks. Other *Tyr>mCherry* that have been incorporated into the mesenchyme remain stationary (QuickTime; 20.3 MB).