
Figures and figure supplements

Tight Junction Protein 1a regulates pigment cell organisation during zebrafish colour patterning

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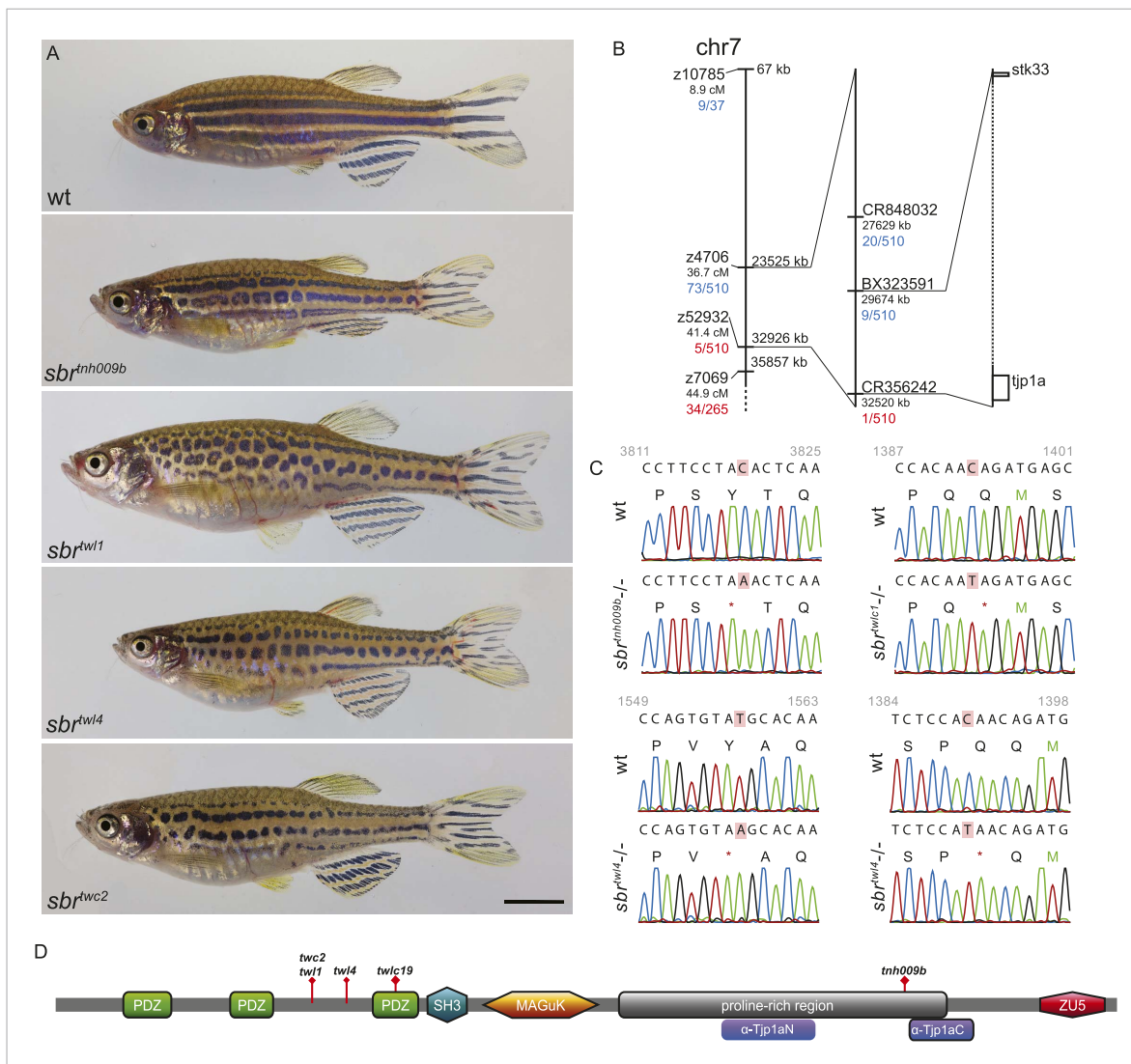


Figure 1. *schachbrett* encodes Tjp1a. **(A)** All alleles of *sbr* exhibit interrupted, undulating dark stripes of normal arrangement and width when compared to wild type, but no other obvious defects. Scale bar: 5 mm. **(B)** Scheme of meiotic mapping of *sbr*. Marked are z-markers and contigs on which SNPs were found with their genomic and genetic (where applicable) coordinates. The numbers of recombinants among all fish tested are given in red and blue. The right-most bar shows genes on the ends of the final mapped region. The dotted region is not to scale and contains multiple genes. **(C)** DNA sequence traces for four alleles of *sbr*. Red rectangles mark the mutated residues. Red asterisks stand for stop codons. **(D)** Scheme of Tjp1a protein. Purple rounded squares indicate regions corresponding to polypeptides used for antibody generation. Red diamonds show the positions of stop codons in the mutants.

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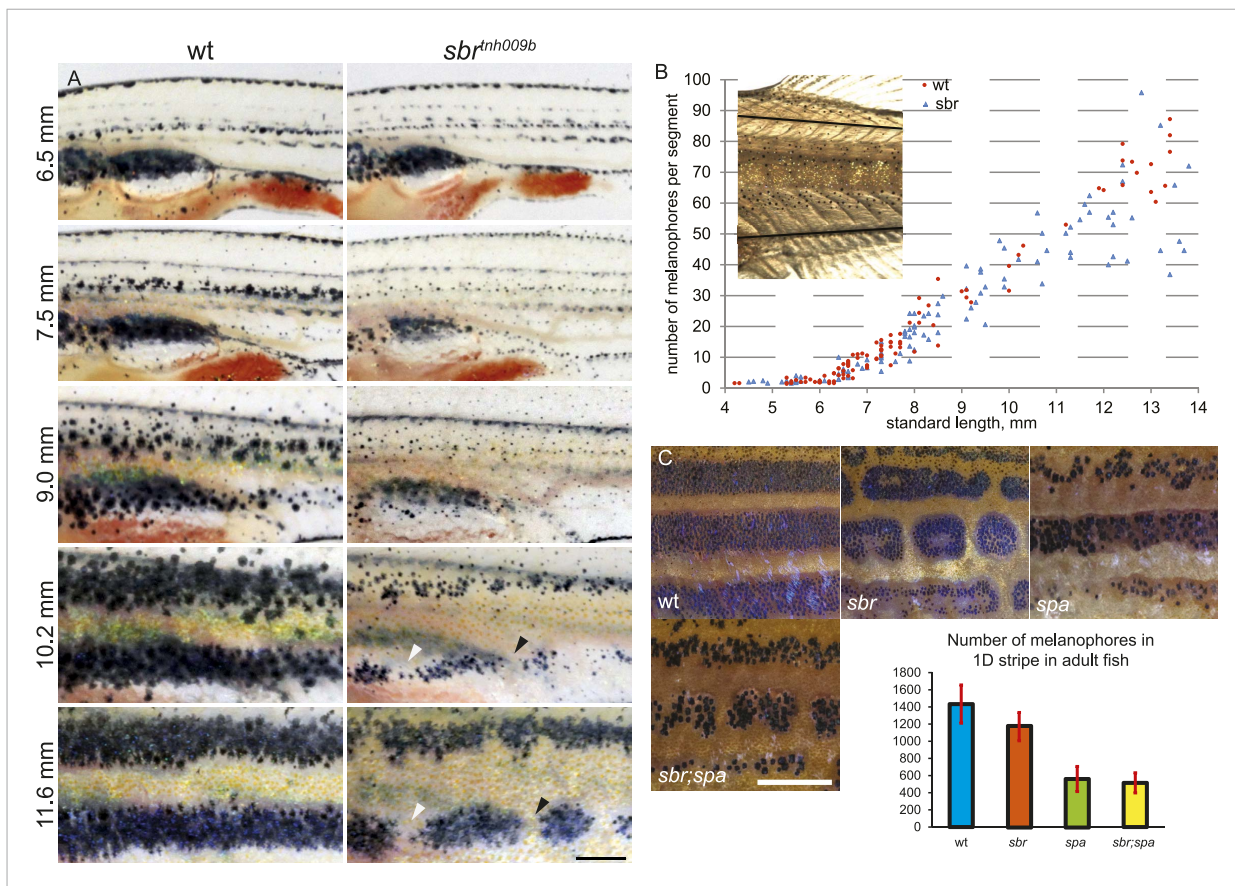


Figure 2. Abnormal behaviour of *sbr* mutant melanophores. **(A)** Pigment pattern during metamorphosis in the mid-trunk of individual wild type and *sbr* mutant fish. Arrowheads: forming interruptions. White arrowheads: disappearing melanophores ($N = 6$). Scale bar: 1 mm. **(B)** Average number of melanophores per segment in the first two dark stripes in wild type and mutant fish plotted against standard length. Red circles—individual wild type fish; blue squares—individual *sbr* fish. Inset shows the area where melanophores were counted. Distributions of melanophore numbers in mutants and wild type fish do not differ significantly until the 10 mm stage as shown by Kolmogorov–Smirnov statistics. At 10–14 mm stages the distributions are different with p -values < 0.05 . **(C)** Close-ups of mid-trunk regions of adult wild type, *sbr*, *spa* and *spa;sbr* and melanophore numbers in a dark stripe dorsal to the first light stripe of adult fish. Red lines—standard deviation. Scale bar: 2 mm.

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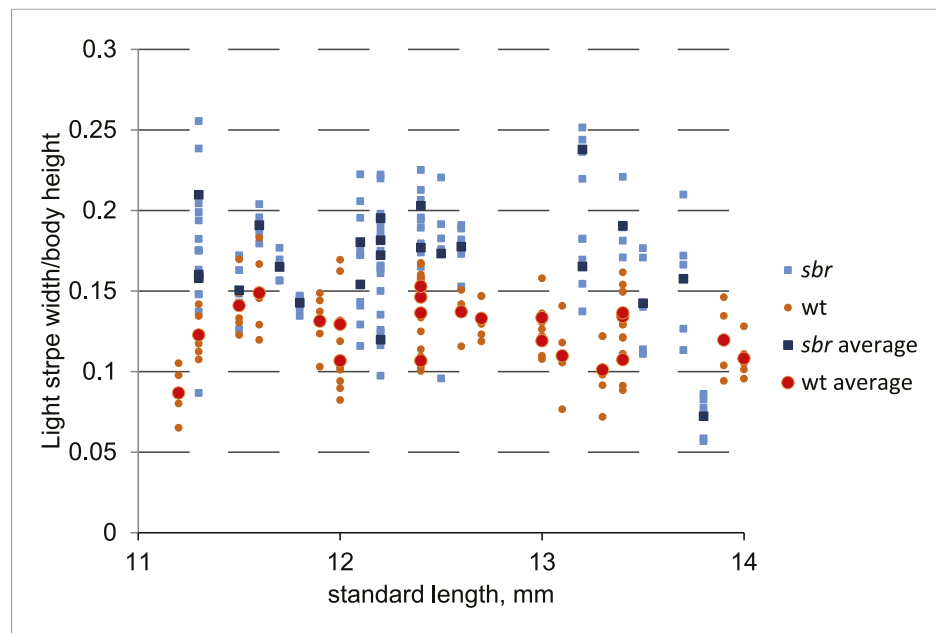


Figure 2—figure supplement 1. Width of the first light stripe in *sbr* and wild type fish. The width was measured at the anal fin area at five points for every individual. The obtained data measurements were divided by fish body height. These ratios were plotted against SL of metamorphic fish. Orange circles represent individual measurements for wild type. Red circles show the average of five measurements for each wild type fish. Light blue squares—individual measurements for *sbr*. Dark blue squares—average of five measurements of *sbr*.
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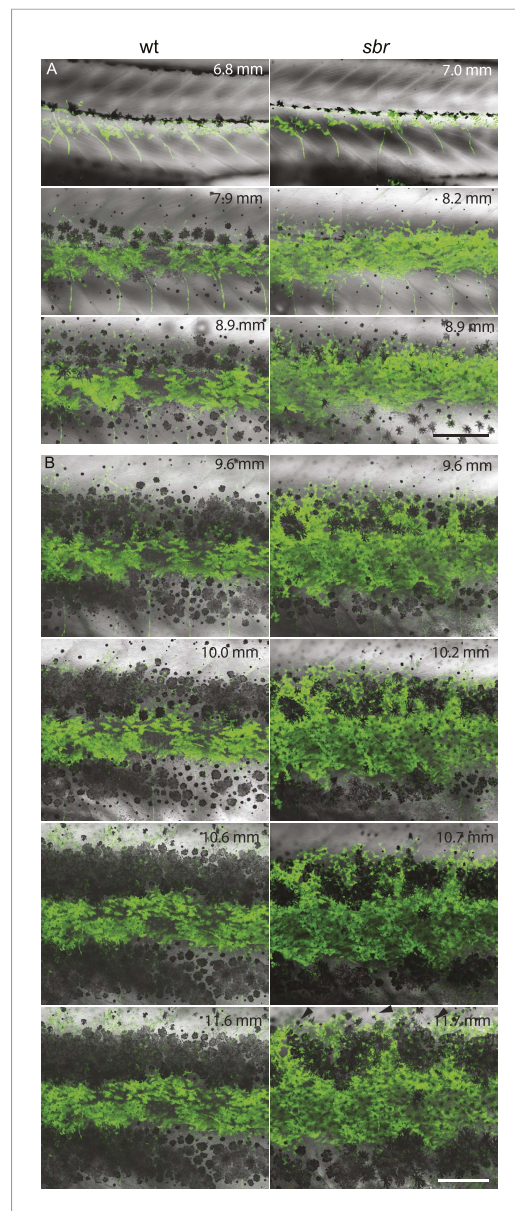


Figure 3. Behaviour of *sbr* mutant iridophores during metamorphosis. **(A)** Repeated imaging of *Tg(TDL358:GFP)* wild type and mutant metamorphic individual ($N = 5$ each, one shown). Scale bar: 300 μm . **(B)** Same individuals with another magnification. Empty patches in the light stripe of wild type fish are caused by variegation of the transgene expression. Arrowheads: loose iridophores. Scale bar: 300 μm .

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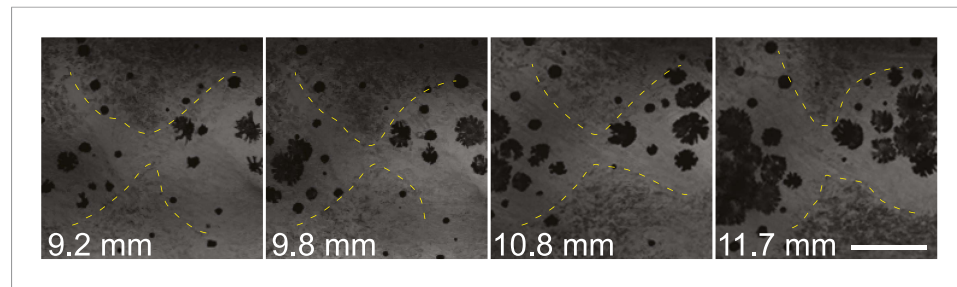


Figure 3—figure supplement 1. Invading *sbr* iridophores occasionally retreat. Iridophores (marked with the yellow outline) are retreating from the area between two groups of melanophores. Scale bar: 150 μ m.

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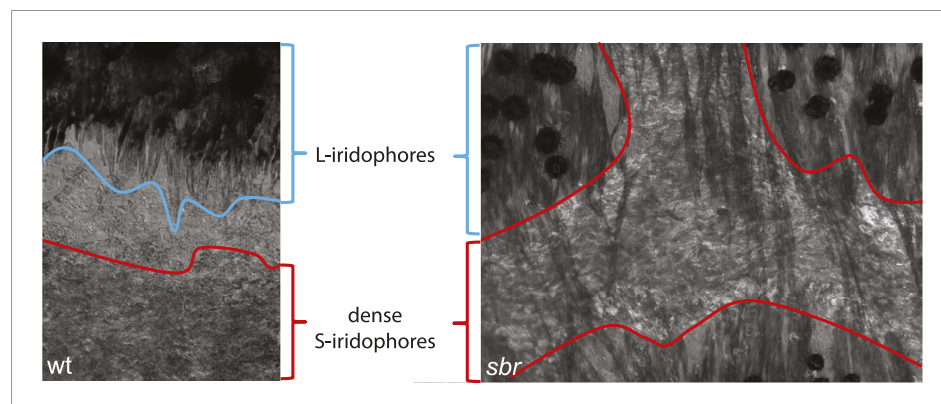


Figure 3—figure supplement 2. L-iridophores in wt and *sbr*. In wild type dense S-iridophores and L-iridophores are separated, but in *sbr* L-iridophores can be observed in S-iridophore area of light stripes. Pigment assemblies in the centres of melanophores due to prolonged light exposure prior to fixation.

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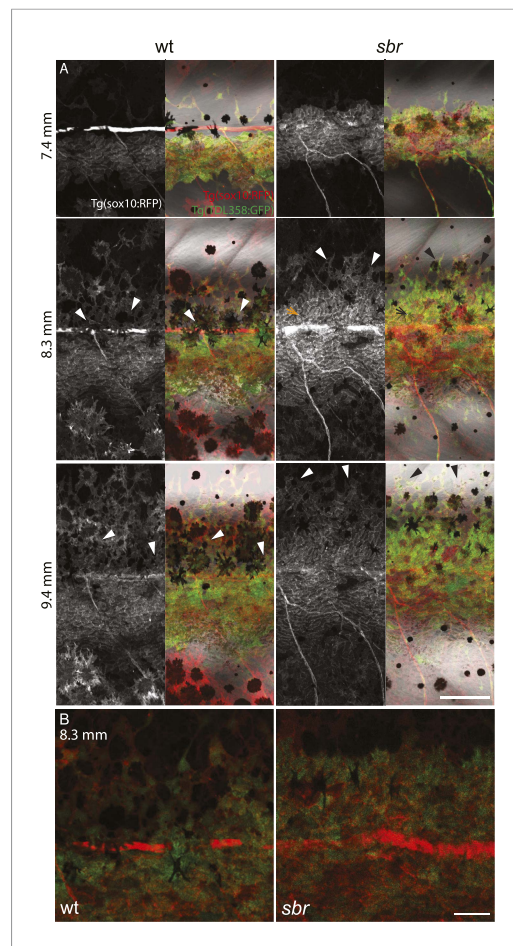


Figure 4. Behaviour of *sbr* mutant iridophores during establishment of the first dark stripes. **(A)** *Tg(TDL358:GFP)*; *Tg(sox10:mRFP)* wild type and *sbr* metamorphic fish (N = 4 each, one shown). Arrowheads point to delaminating loose iridophores. Arrow shows dense iridophores failing to delaminate. Scale bar: 150 μ m. **(B)** Close-ups of *Tg(TDL358:GFP)*; *Tg(sox10:mRFP)* wild type and *sbr* metamorphic fish 8.3 SL. Note difference in iridophore shapes in wild-type. Scale bar: 50 μ m.

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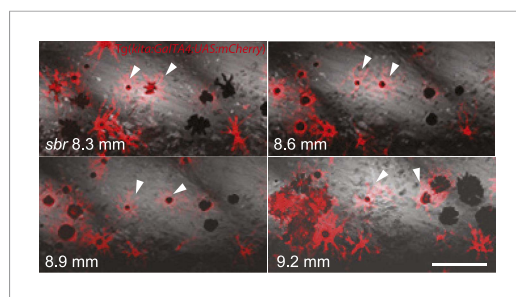


Figure 5. Two closely positioned melanophores in *sbr* (arrowheads), are migrating away from the iridophores in posterior and anterior directions. Scale bar: 100 μ m.

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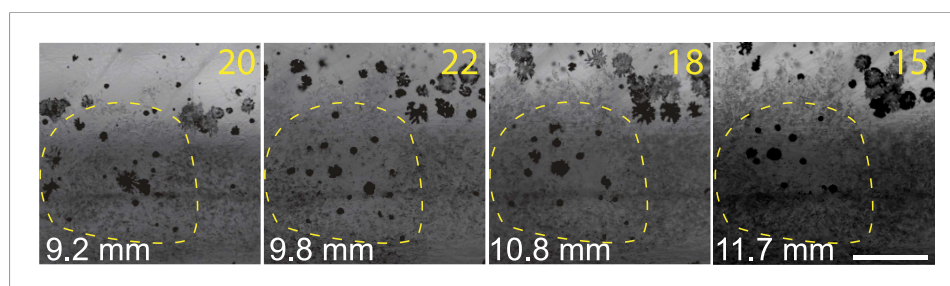


Figure 5—figure supplement 1. Melanophores trapped in the mass of iridophores are disappearing in *sbr*. Number of melanophores in the marked light stripe area is shown in the upper right corner. Scale bar: 200 μ m.

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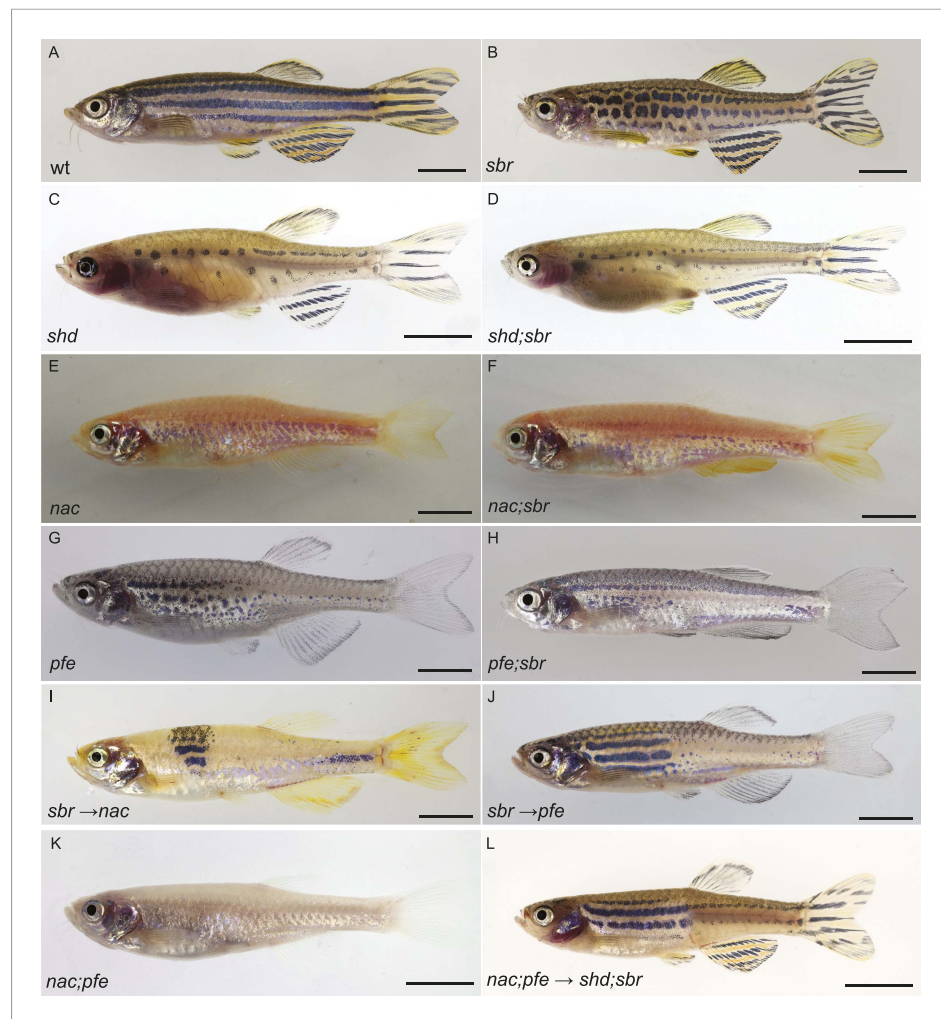


Figure 6. *tjp1a* is required in iridophores, but not melanophores or xanthophores. (A) Wild type fish. (B) *sbr* fish. (C) *shady* (*shd*) mutant, which lacks iridophores. (D) *shd;sbr* mutant is indistinguishable from *shd*. (E) *nacre* (*nac*) mutant, which lacks melanophores. (F) *nac;sbr* double mutant exhibiting expanded dense iridophore areas in comparison to *nac* alone. (G) *pfeffer* (*pfe*) mutant, which has no xanthophores. (H) *pfe;sbr* double mutant exhibiting expanded dense iridophore areas in comparison to *pfe* alone. (I) Chimeras, obtained from transplantation of *sbr* blastomeres into *nac* recipient blastulas, show clonal rescue. (J) Chimeras obtained from transplantation of *sbr* blastomeres into *pfe* recipient blastulas, show clonal rescue. (K) *nac;pfe* fish have only one type of pigment cells—iridophores. (L) Chimeras obtained from transplantation of *nac;pfe* blastomeres into *shd;sbr* recipient blastulas, show clonal rescue. Scale bars: 5 mm.

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Figure 6—figure supplement 1. Phenotypes of *shd* and *shd;sbr* mutants. Shown are (A) *shd* and (B) *shd;sbr*. Scale bars 5 mm.

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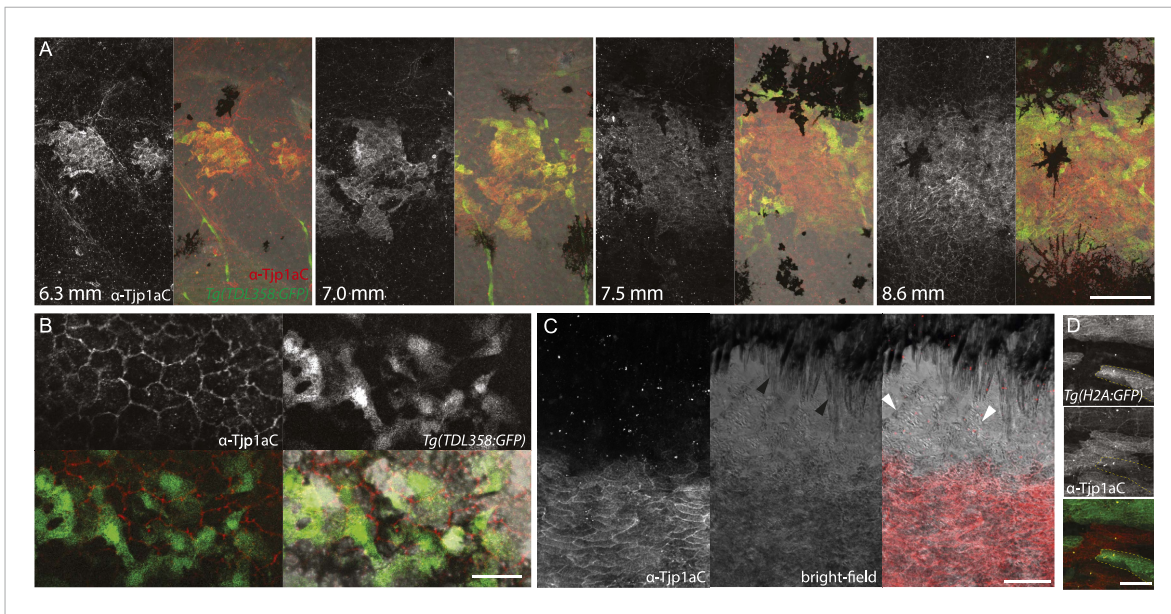


Figure 7. Tjp1a is expressed in dense iridophores. **(A)** Double antibody staining of metamorphic *Tg(TDL358:GFP)* fish with α -Tjp1aC and α -GFP antibodies. Note: not all iridophores are expressing GFP due to transgenic line variegation. Scale bar: 100 μ m. **(B)** Loose iridophores migrating over the dark stripe in 8.3 mm metamorphic *Tg(TDL358:GFP)* fish express GFP, but not Tjp1a, although the epithelial staining is still visible. Scale bar: 30 μ m. **(C)** α -Tjp1aC staining in skin of adult wild type fish. The protein is detected in the sheet of dense S-iridophores of the light stripe, but not in L-iridophores (black arrowheads), loose iridophores (white arrowheads), melanophores or xanthophores. Scale bar: 100 μ m. **(D)** Double antibody staining with α -Tjp1aC and α -GFP of skin of adult chimera, obtained by transplanting *sbr;Tg(H2A:GFP)* blastomeres into wild type blastula. Either GFP or Tjp1a was detected in cells, never both. Some *sbr* cells express no GFP due to variegation of the transgene expression. Scale bar: 30 μ m.

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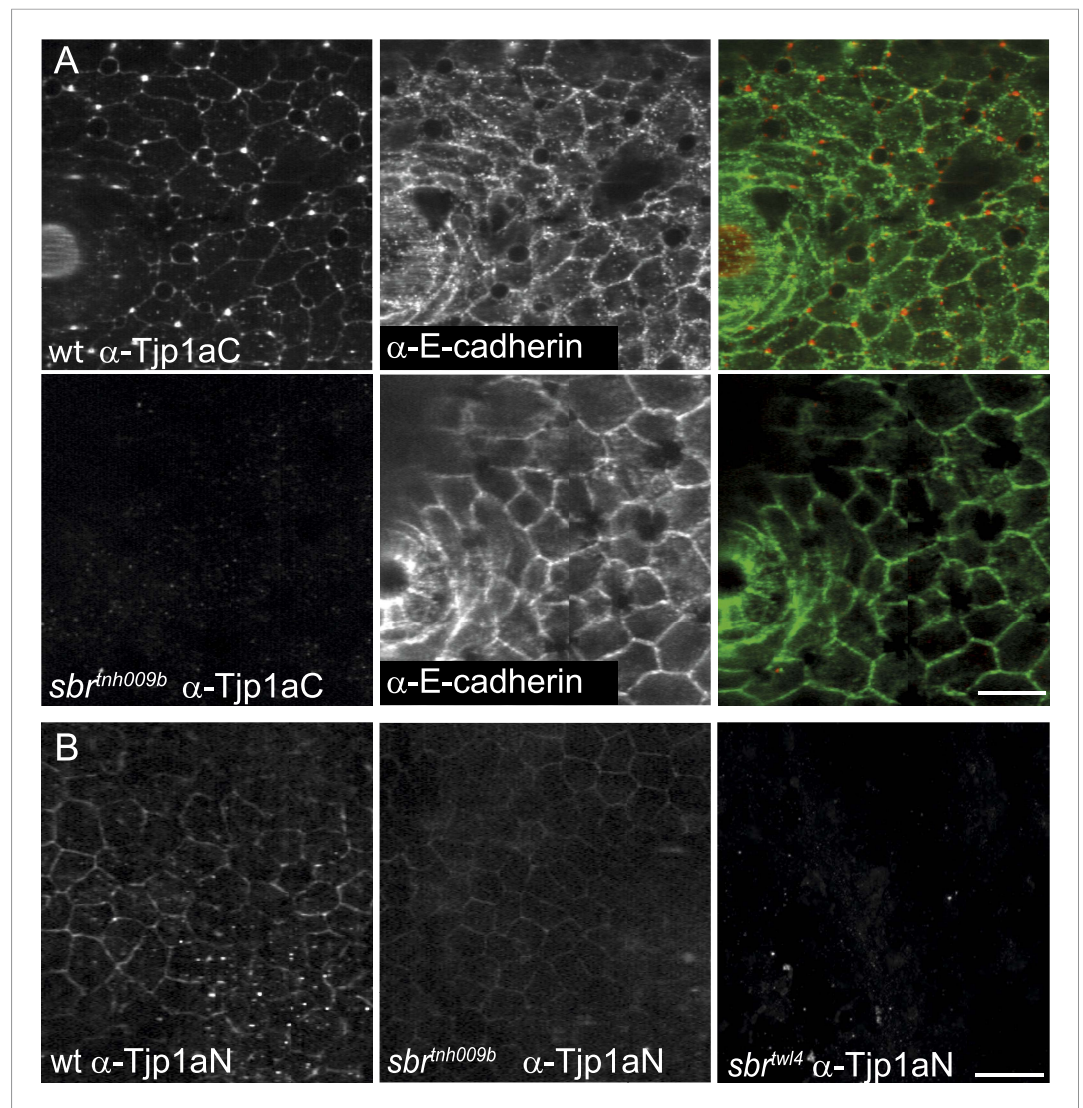


Figure 7—figure supplement 1. Tjp1a stainings in wild type and *sbr*. **(A)** α -Tjp1aC staining in adult wild type fish skin sample shows signal colocalizing with E-cadherin, expressed in epithelial cells. In adult *sbr^{tnh009b}* skin samples the Tjp1a staining is not observed, but E-cadherin is detected. Scale bar: 20 μ m. **(B)** α -Tjp1aN antibody stains skin epithelium of both wild type and *sbr^{tnh009b}* adult fish but not *sbr^{twl4}*. Scale bar: 20 μ m.

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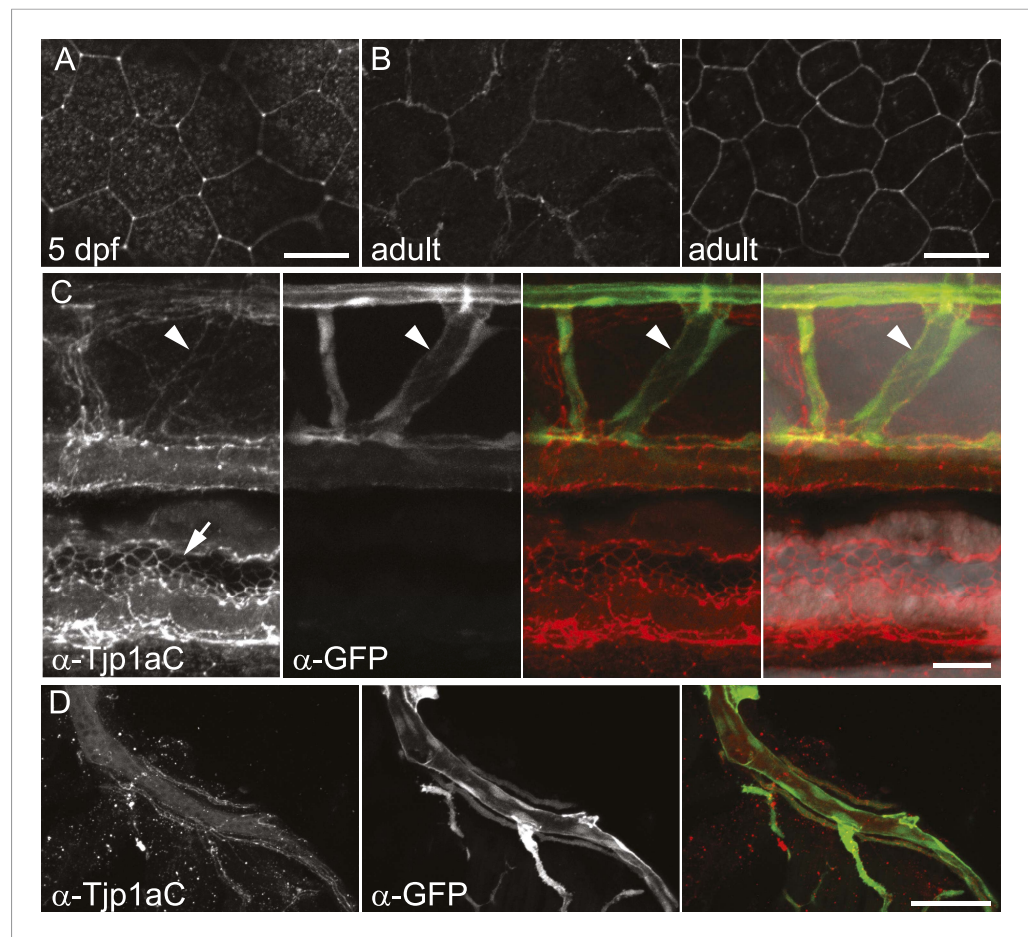


Figure 7—figure supplement 2. Characterization of the Tjp1a expression domain. (A) Signal in epithelium of wild type 5 dpf larva stained with α-Tjp1aC. Scale bar: 20 μm. (B) Signal in two layers of adult wild type epithelium (about 7 μm apart) stained with α-Tjp1aC st. Scale bar: 20 μm. (C) Double staining of whole-mount *Tg(kdrl:GFP)* 5 dpf larvae with α-Tjp1aC and α-GFP demonstrates the expression of Tjp1a in blood vessels (arrowheads) and intestinal epithelium (arrows). Scale bar: 20 μm. (D) α-Tjp1aC staining shows expression of Tjp1a in vasculature of adult *Tg(kdrl:GFP)* animal. Scale bar: 50 μm.

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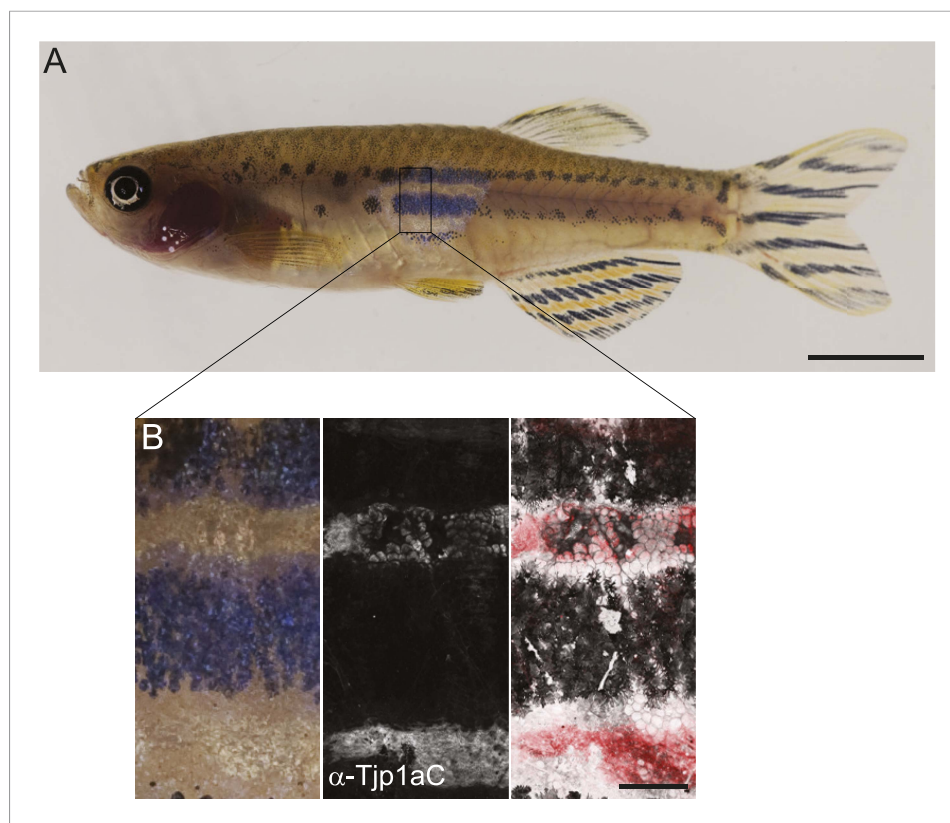


Figure 7—figure supplement 3. Correlation between clonal rescue of *sbr* phenotype and Tjp1a expression. (A) *shd;sbr* with *nac;pfe* clones show rescue of the wild type phenotype. Scale bar: 5 mm. (B) Immunostaining of *shd;sbr* with *nac;pfe* clone with α -Tjp1aC antibody demonstrates the presence of the protein in dense iridophores but not in the epithelium. Scale bar: 500 μ m.

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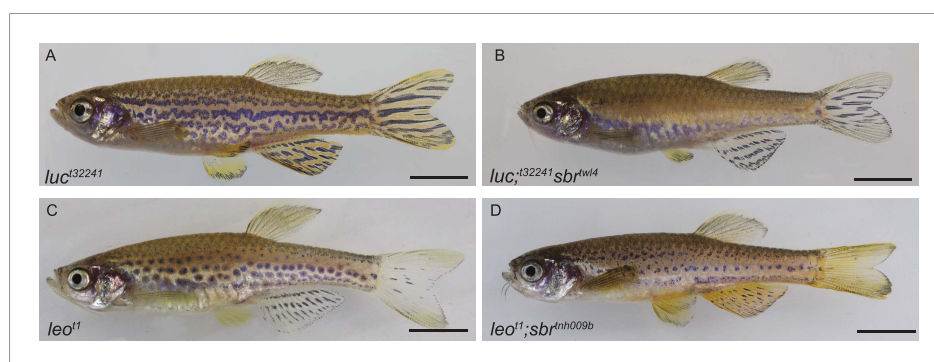


Figure 8. Genetic interactions between *luc*, *leo* and *sbr*. (A) *luc*^{t32241} (*luc*) mutant affects Cx39.4 and results in meandering and broken stripes. (B) *luc*^{t32241};*sbr*^{tw4} mutant exhibits complete loss of stripes and expansion of dense iridophore area. (C) leopard^{t1} (*leo*, cx41.8) stripes are broken into spots. (D) *leo*^{t1};*sbr*^{tnh009b} double mutant displays decrease in the size of the spots. Scale bars: 5 mm.

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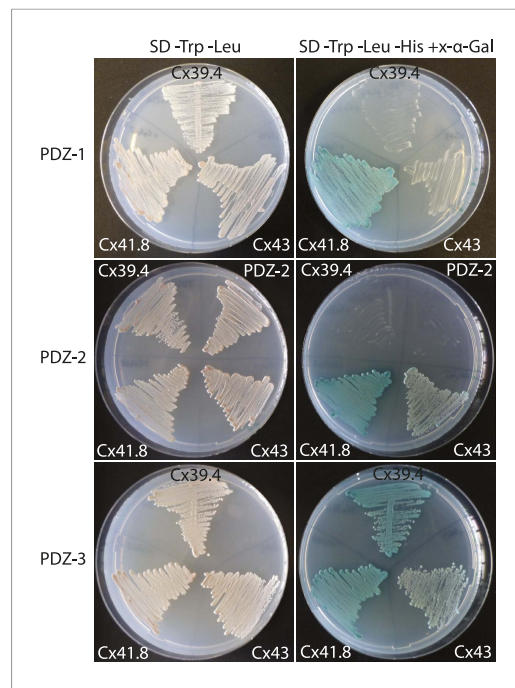


Figure 8—figure supplement 1. Interaction of PDZ domains of Tjp1a with connexins. Shown are the results of a yeast two-hybrid experiment. Left column: growth on SD plates lacking Trp and Leu indicates the presence of both plasmids (bait and prey); right column: growth and blue colour on SD plates lacking Trp, Leu and His, supplemented with x- α -Gal, indicates interaction of the two proteins in the yeast cell. All three PDZ domains of Tjp1a strongly interact with Cx41.8 (*leo*), PDZ-2 and PDZ-3 strongly interact with Cx43 as well. Only PDZ-3 shows strong interaction with Cx39.4 (*luchs*).

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