Expanded View Figures

Figure EV1. Generation and characterization of *chd7* mutants.

A Translation following genome editing resulted in a premature stop codon (*) in $chd7^{-/-}$ fish.

B qPCR analysis of RNAs from 3dpf larvae shows a significant reduction of *chd7* mRNA expression in both $chd7^{+/-}$ and $chd7^{-/-}$ compared with wild-type ($N = 5$). ****$P < 0.0001$, one-way ANOVA.

C Examination of brain tissues in $chd7^{+/+}$ (left images) and $chd7^{-/-}$ (right images) by H&E staining at 5 dpf. Zebrafish brains were sectioned at telencephalic (1,1'), diencephalic (2, 2'), mesencephalic (3,3') and rhombencephalic levels (4,4'). Levels of sections are indicated in the sketch of a sagittal view of a 5 dpf zebrafish brain (top image). The scale bar is 0.12 mm. P: pallium, S: subpallium, Po:preoptic region, Tel: Telencephalon; TeO: tectum opticum (or OT: optic tectum), m: medial tectal proliferating zone, DT: dorsal thalamus, PTd: dorsal part of posterior tuberculum, PTv: ventral part of posterior tuberculum, MO: medulla oblongata, Hyp: hypothalamus, CeP: cerebellar plate.

D $chd7^{-/-}$ mutant fish displayed features of CS such as heart defects (red arrow) at a low penetrance ($N = 3$). ****$P < 0.0001$; Student's *t*-test.

E Alcian blue staining of 6dpf larvae showing craniofacial defects at Meckel's cartilage (red arrow).

Data information: Data are presented as mean ± SEM. *n* is the number of fish used. *N* is the number of experimental repeats.

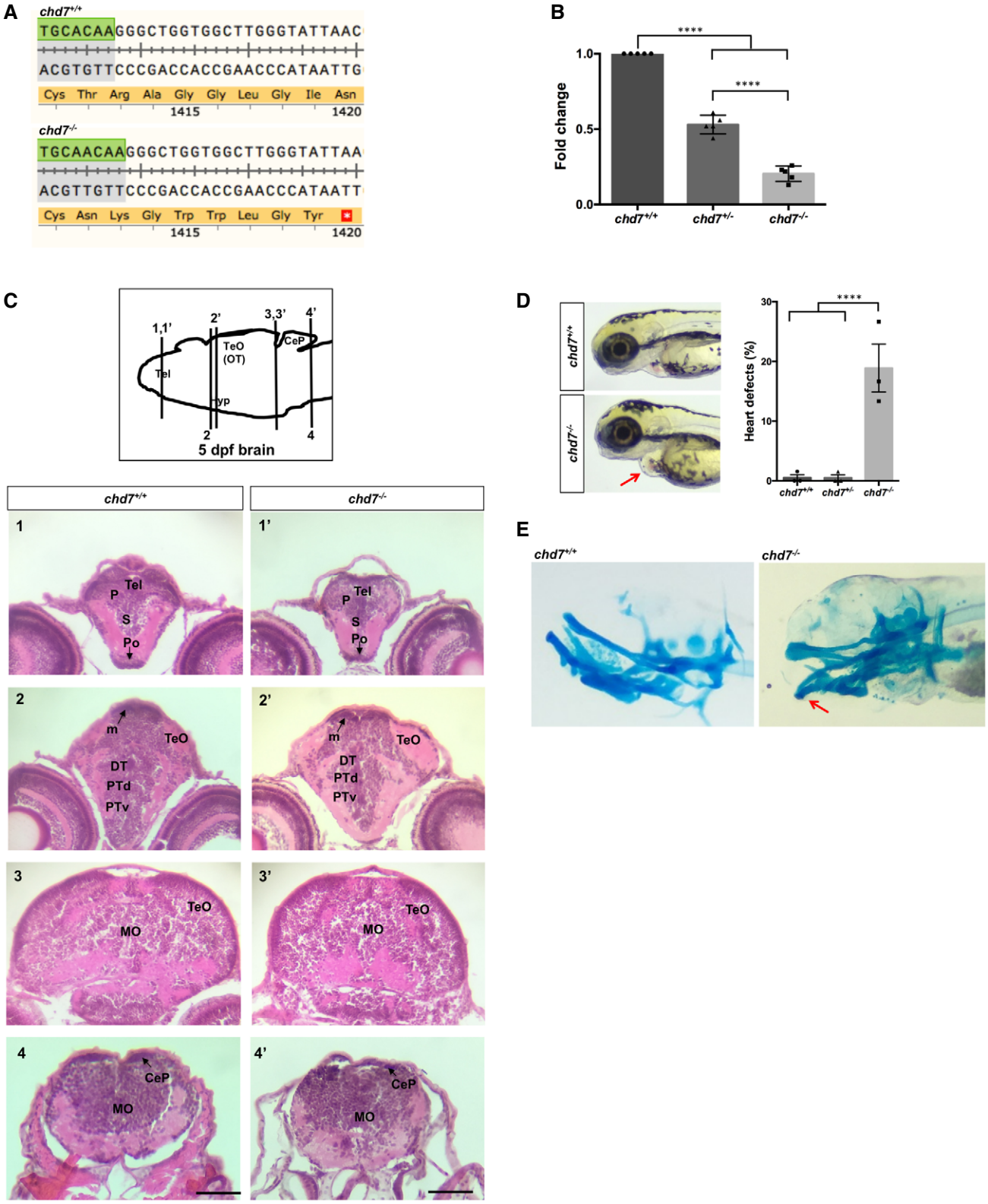

Figure EV1.

Figure EV2. Proliferation and apoptosis analyses in wild-type and *chd7* mutant zebrafish.

A Analysis of GABAergic neurons network development between 1 dpf and 5 dpf between control (top row) and *chd7* mutants (bottom row) ($N = 3$).

B Proliferation analysis by pH3 staining at 2 dpf in control and *chd7* mutants.

C Bar graph showing no difference in pH3-positive cells in zebrafish brain at 2 dpf between control and mutants ($n = 11$; ns, $P = 0.1816$; Student's *t*-test).

D Cell death analysis by TUNEL assay in $chd7^{-/-}$ and $chd7^{+/+}$ brains shows no change in apoptotic cells at 2 dpf ($N = 3$, $n = 8$; ns, $P = 0.464$; Student's *t*-test).

E Transverse sections of 2 dpf larvae after immunostaining with pH3 (red) and NeuroD1 (green). Bar graph showing no difference in pH3 and NeuroD1 double-positive cells (arrows) in zebrafish brain at 2 dpf between control and mutants ($N = 3$, $n = 5$; ns, $P = 0.124$; Student's *t*-test). P: pallium, S: subpallium, TeO: tectum opticum, m: medial tectal proliferating zone, DT: dorsal thalamus R: retina.

F Proliferation analysis by pH3 staining at 5 dpf in control and *chd7* mutants.

G An increase in pH3-positive cells was noted in brains of mutant fish compared with controls at 5 dpf ($N = 4$, $n = 10$; $^{****}P < 0.0001$; Student's *t*-test).

H Transverse sections of 5 dpf larvae after immunostaining with pH3 (red) and HuC/D (green). pH3-positive cells (arrows) were observed at 5 dpf in the medial tectal proliferating zone of mutant fish brains but none in controls ($N = 3$). *P*: pallium, S: subpallium, TeO: tectum opticum, m: medial tectal proliferating zone, DT: dorsal thalamus.

Data information: Data are presented as mean ± SEM. Scale bar = 50 μm and 10 μm for 2 dpf NeuroD1 and pH3 co-stain.

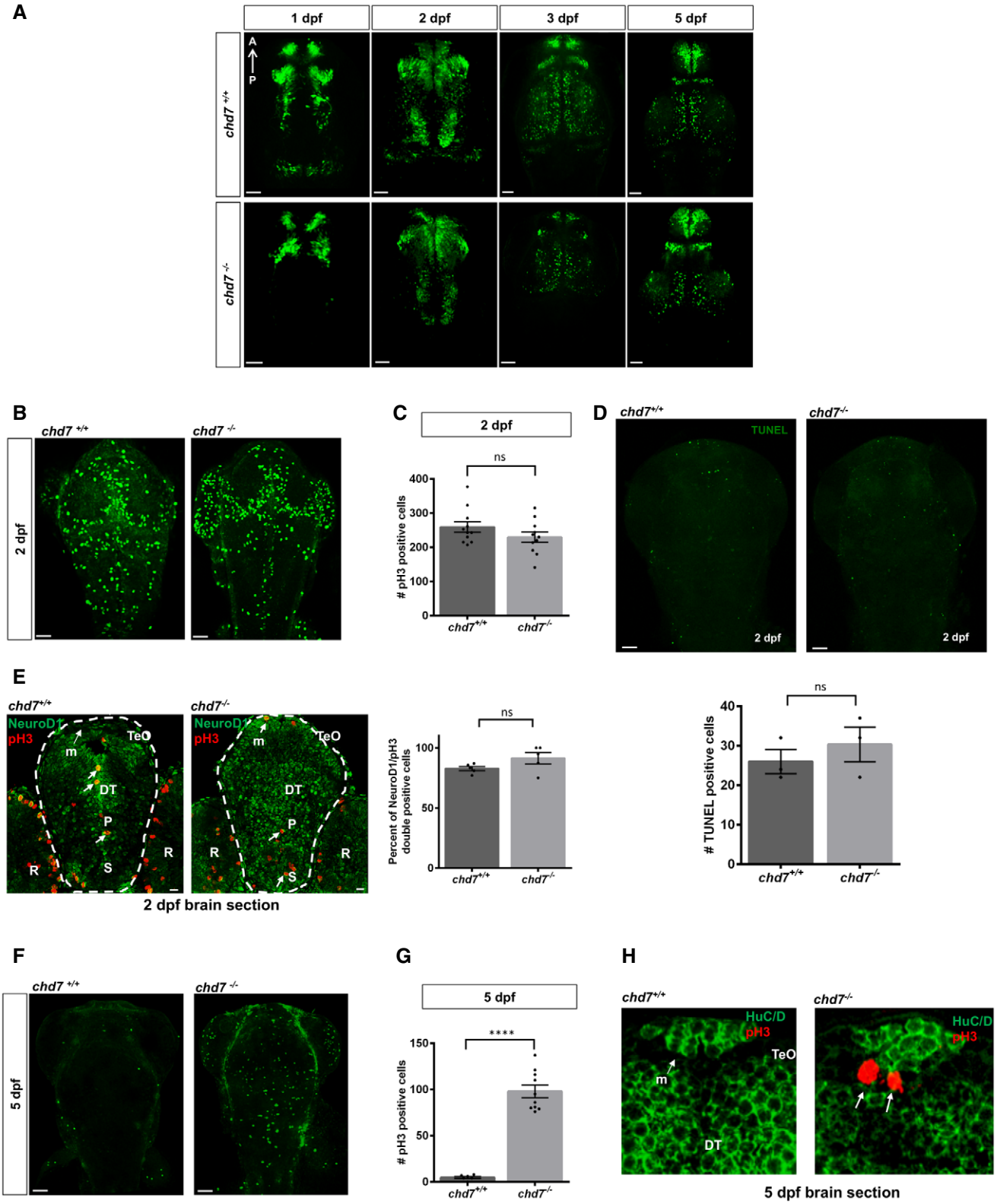

Figure EV2.

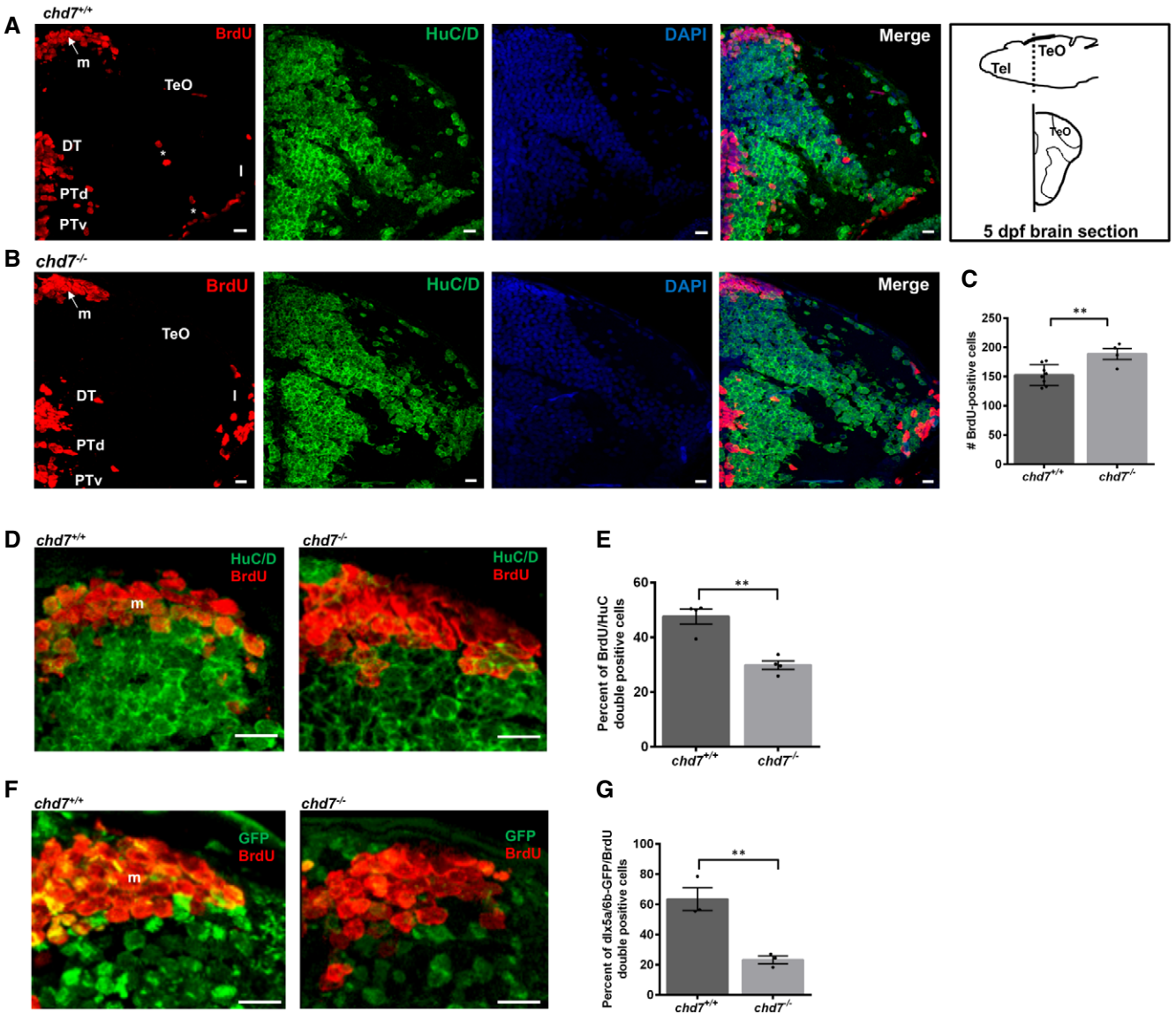

Figure EV3. Aberrant GABAergic neuronal differentiation in *chd7* mutant zebrafish.

A, B Immunostaining with BrdU and HuC/D in brain sections of the zebrafish tectal region in *chd7*$^{+/+}$ (A) and *chd7*$^{-/-}$ (B). Level of the sections is indicated in the sketch of a 5 dpf zebrafish brain (top right image in (A)). The scale bar is 10 μm. Tel: Telencephalon; TeO: tectum opticum, m: medial tectal proliferating zone, DT: dorsal thalamus, PTd: dorsal part of posterior tuberculum, PTv: ventral part of posterior tuberculum, l: lateral tectal proliferation zone. Asterisks (*) marks early migrated region of pretectum and proglomerular.

C The number of BrdU-positive cells in transverse sections of the zebrafish brain in *chd7*$^{+/+}$ and *chd7*$^{-/-}$ ($N = 3$, *chd7*$^{+/+}$: $n = 8$; *chd7*$^{-/-}$: $n = 4$; $^{**}P < 0.05$; Student's *t*-test).

D Immunostaining with BrdU and HuC/D in brain sections of the zebrafish medial tectal region. Scale bar = 10 μm. m: medial tectal.

E The percentage of BrdU and HuC/D-double positive cells among the BrdU-positive cells in the medial tectal zone ($N = 3$, $n = 4$; $^{**}P < 0.05$; Student's *t*-test).

F Immunostaining with BrdU and GFP (to label *dlx5a/6a-GFP* + GABAergic neurons) in brain sections of the zebrafish medial tectal region. Scale bar = 10 μm. m: medial tectal.

G The percentage of BrdU and *dlx5a/6a-GFP*-double positive cells among the BrdU-positive cells in the medial tectal zone ($N = 3$, $n = 3$; $^{**}P < 0.05$; Student's *t*-test).

Data information: Data are presented as mean ± SEM. *n* is the number of fish used. *N* is the number of experimental repeats.

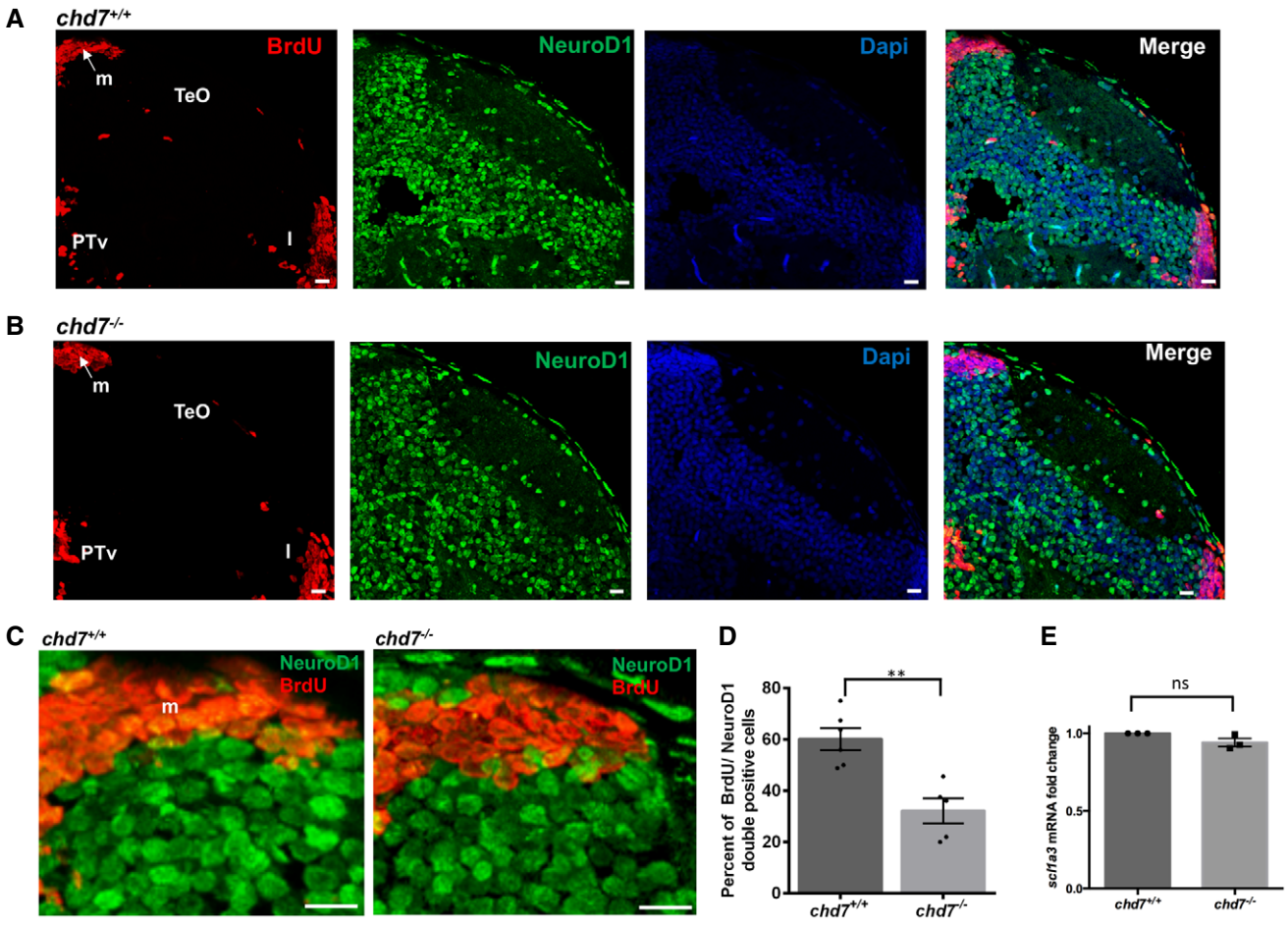

Figure EV4. Impaired neurogenesis in *chd7* mutant zebrafish.

A, B Immunostaining with BrdU and NeuroD1 in brain sections of the zebrafish tectal region in $chd7^{+/+}$ (A) and $chd7^{-/-}$ (B). The scale bar is 10 μm. Tel: Telencephalon; TeO: tectum opticum, m: medial tectal, DT: dorsal thalamus, PTd: dorsal part of posterior tuberculum, PTv: ventral part of posterior tuberculum, l: lateral tectal proliferation zone.

C Immunostaining with BrdU and NeuroD1 in brain sections of the zebrafish medial tectal region. Scale bar = 10 μm. m: medial tectal.

D The percentage of BrdU and NeuroD1-double positive cells among BrdU-positive cells in the medial tectal zone ($N = 3$, $chd7^{+/+}$: $n = 6$; $chd7^{-/-}$: $n = 6$; $^{**}P < 0.05$; Student's *t*-test).

E Expression level of *scl1a3* mRNA in $chd7^{-/-}$ relative to $chd7^{+/+}$ ($N = 4$). ns, not significant; Student's *t*-test.

Data information: Data are presented as mean ± SEM. *n* is the number of fish used. *N* is the number of experimental repeats.

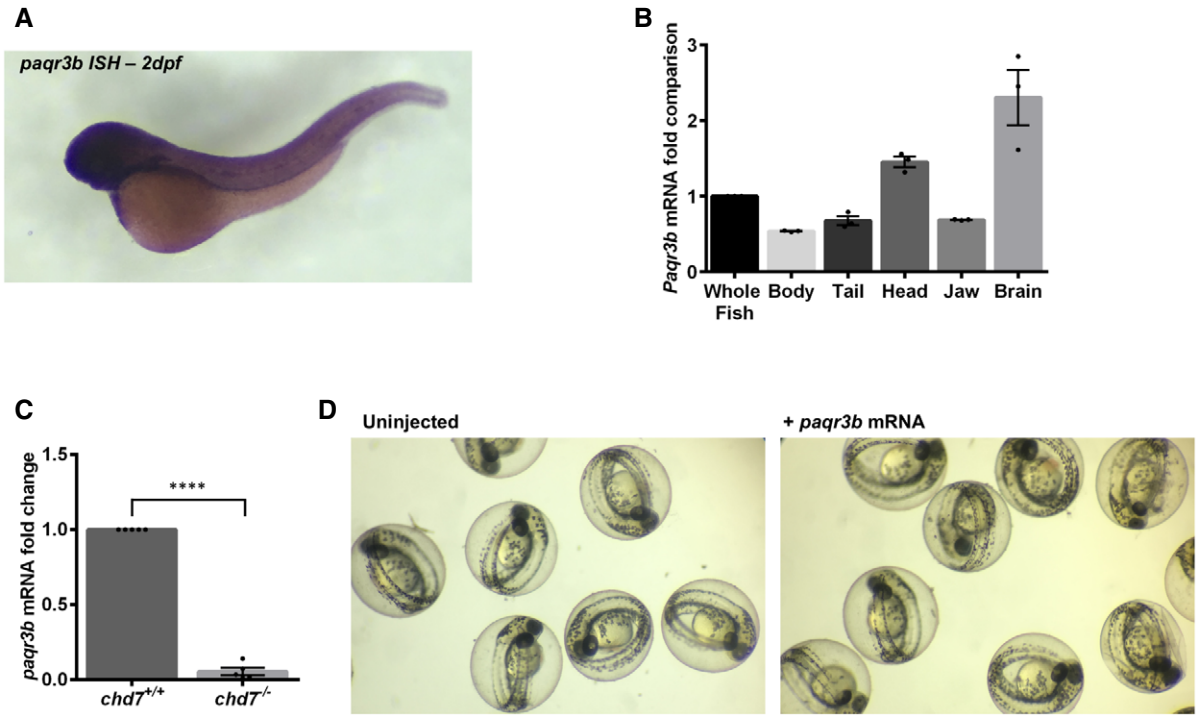

Figure EV5. Expression of *paqr3b* in wild-type and *chd7* mutant zebrafish.

A, B Expression profile of *paqr3b* in whole-mount zebrafish by *in situ* hybridization (A) and in tissues by qRT–PCR (B). $N = 4$.

C qRT–PCR validation of the downregulation of *paqr3b* ($N = 4$; ****$P < 0.0001$, Student's *t*-test).

D Images of gross morphology of 2 dpf zebrafish embryos with or without overexpression of *paqr3b* mRNA. Of note, neither abnormalities nor death were observed in zebrafish embryos upon overexpression of *paqr3b* mRNA.

Data information: Data are presented as mean ± SEM. *n* is the number of fish used. *N* is the number of experimental repeats.

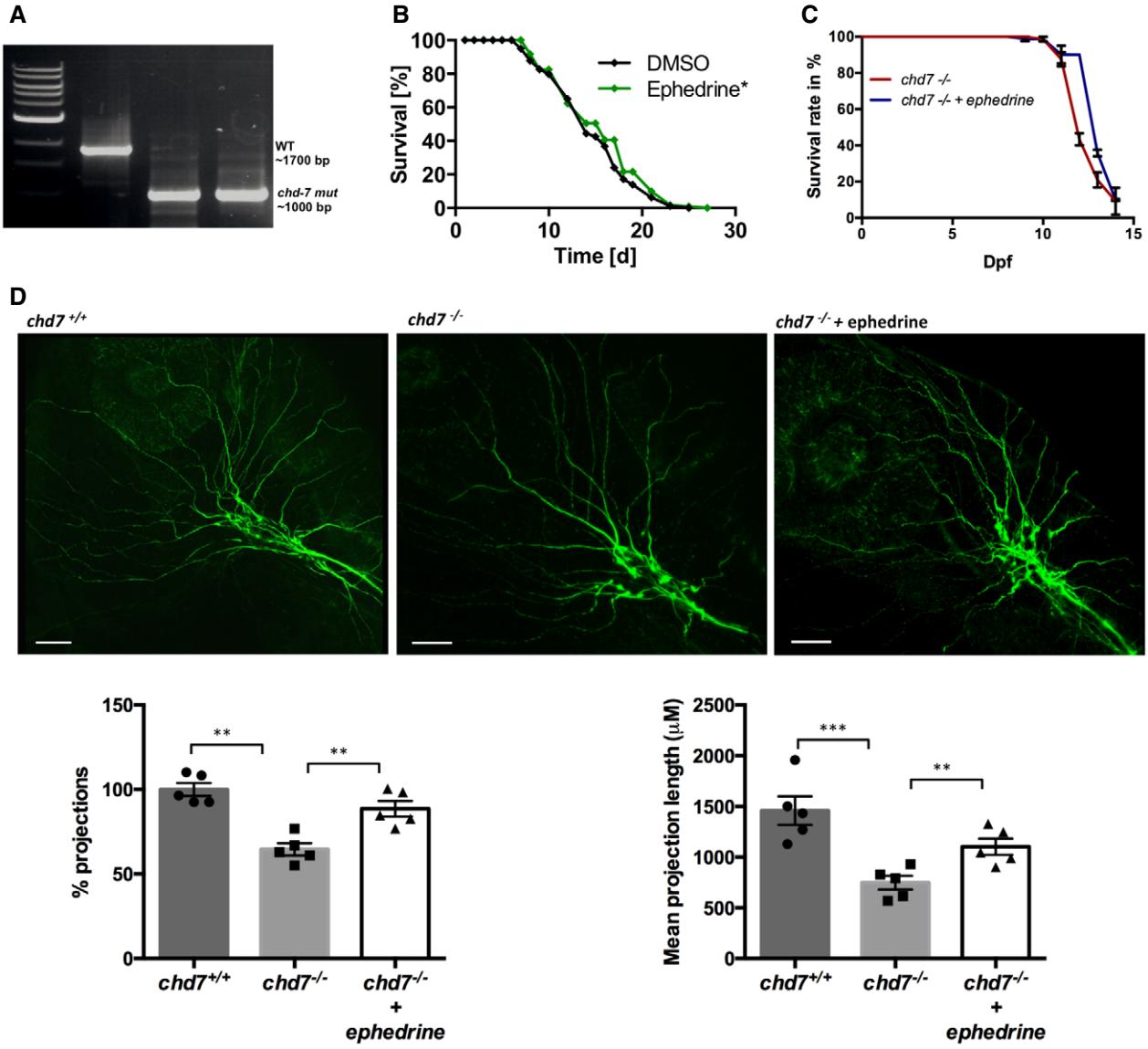

Figure EV6. Pharmacological responses of *chd7* mutants and amelioration of neuronal network development by ephedrine.

A PCR proof of a 700 bp deletion in the *chd-7* gene in *chd-7(gk290)* mutant worms.

B Lifespan analyses of *chd-7(gk290) Caenorhabditis elegans* mutants treated with ephedrine (green) compared with control DMSO (black). Log-rank test was performed for statistical analyses. ($N = 3$, $n = 50$; $^{*}P < 0.05$).

C Survival rate of $chd7^{-/-}$ zebrafish mutants treated with ephedrine (blue) compared with untreated mutants (red). $N = 3$, $n = 60$.

D Acetylated tubulin staining in non-treated and ephedrine-treated $chd7^{-/-}$ zebrafish mutants showing rescue of the severely affected outbranching structure of V^{th} cranial nerves. Graphs showing quantitative analyses of percentage and mean total length of peripheral projections per zebrafish in controls and mutants without and with ephedrine treatment ($n = 5$; $^{***}P < 0.001$; $^{**}P < 0.005$; one-way ANOVA).

Data information: Data are presented as mean ± SEM. Scale bar = 50 µm. *n* is the number of fish or worms used. *N* is the number of experimental repeats.