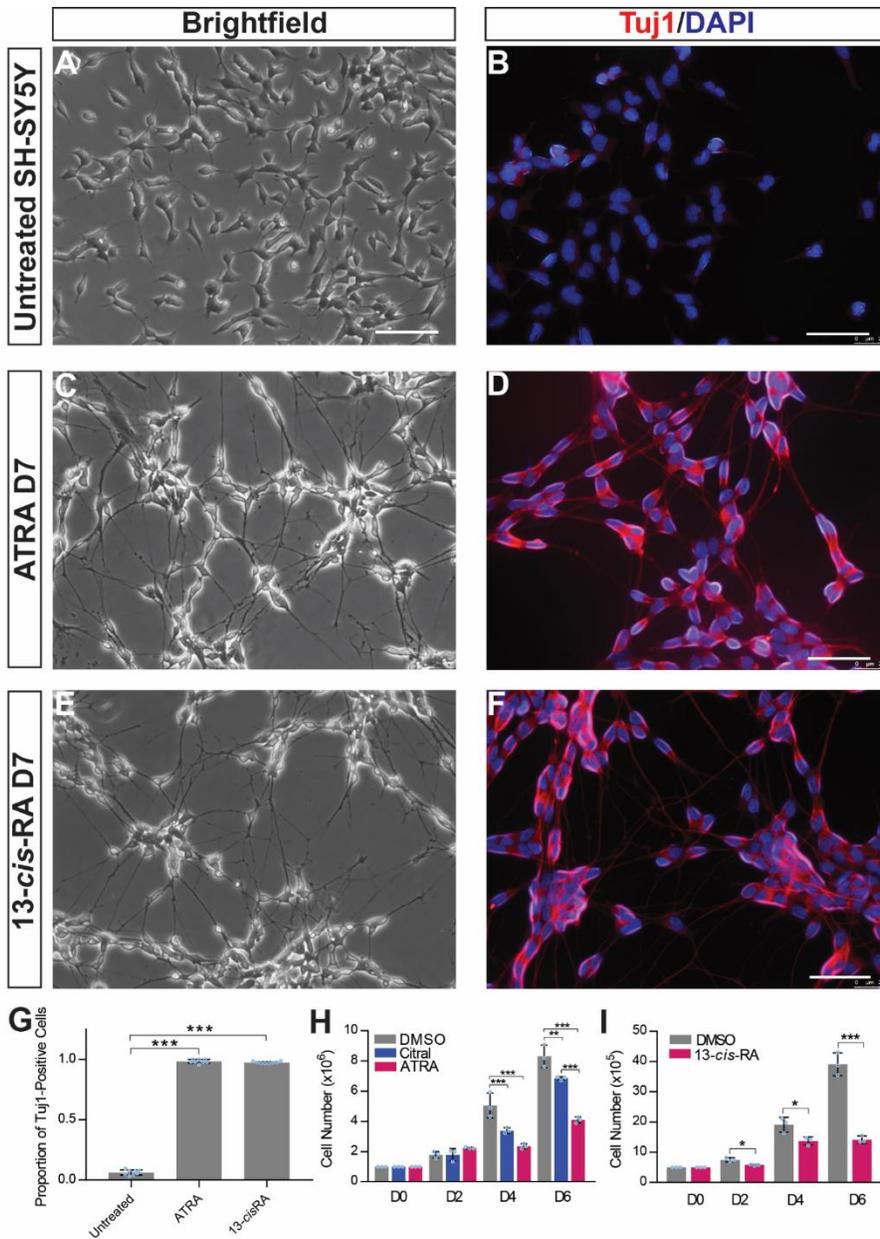
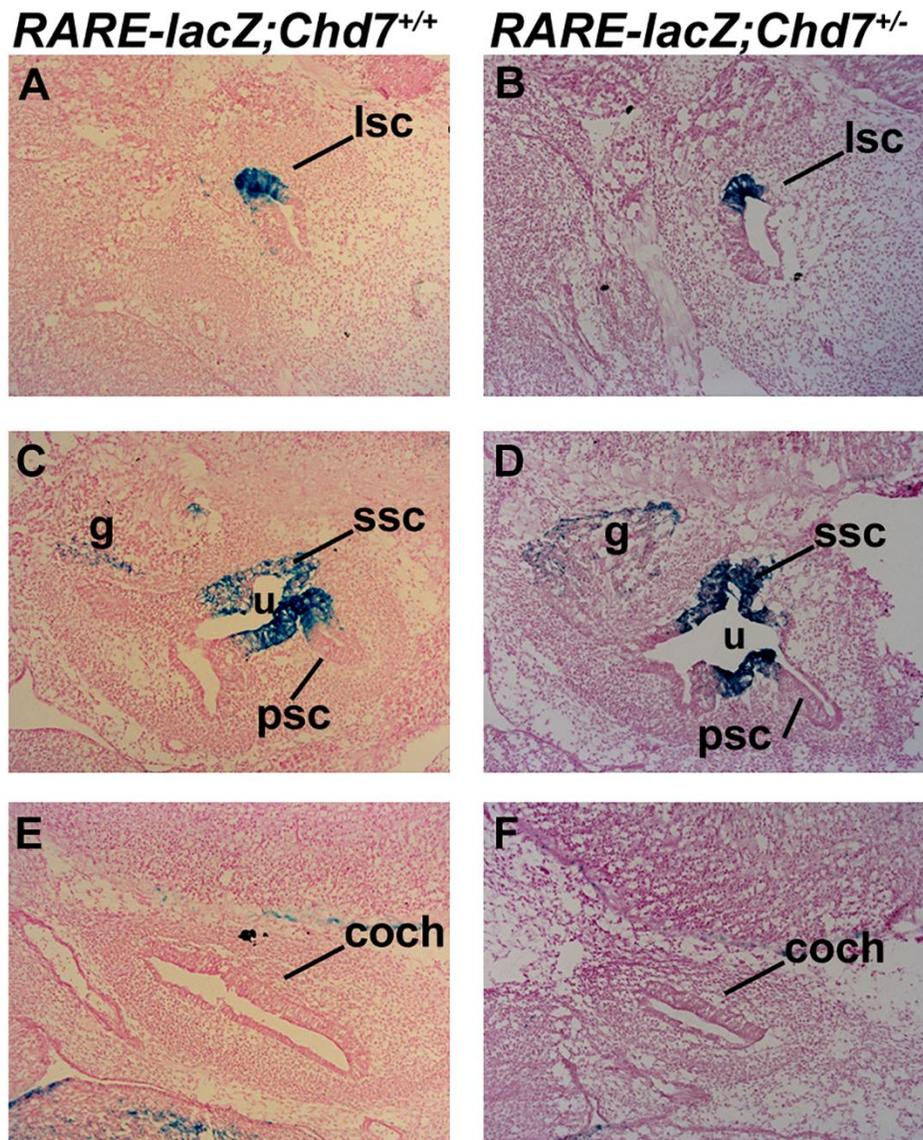


**Figure S1.** CHD7 and ALDH1A3 are expressed in complementary regions of the embryonic inner ear.

Adjacent transverse sections of E12.5 wild type embryos stained with anti-CHD7 (A, B) or anti-ALDH1A3 (C, D) antibodies. CHD7 is enriched in the vestibular (vg) and spiral (sg) ganglia, and in the epithelia of the anterior semicircular canal (asc) and cochlea (c). In contrast, ALDH1A3 is present in all three (posterior, lateral, and anterior) semicircular canals, and less abundant in the vestibular and spiral ganglia and cochlea. Scale bars are 100 $\mu$ m. *N*=6 ears for each antibody.



**Figure S2.** Retinoic acid induces differentiation of SH-SY5Y neuroblastoma cells. (A-F) Brightfield images (A, C, E) and anti-Tuj1 immunostaining with DAPI counterstain (B, D, F) of SH-SY5Y cells that were untreated (A, B), treated with all-*trans*-retinoic-acid (ATRA) (C, D), or treated with 13-*cis*-retinoic-acid (13-*cis*-RA) (E, F) for 7 days. ATRA and 13-*cis*-RA treated SH-SY5Y cells extend neurites in culture. (G) The percentage of TuJ1-positive neurons increased after 7 days of treatment with ATRA and 13-*cis*-RA. (H, I) Counts of SH-SY5Y cells treated over a 6-day period with DMSO (vehicle), ATRA, Citral (an inhibitor of RA synthesis) or 13-*cis*-RA. Scale bars show 50  $\mu$ m. Student's *t*-tests were used to determine significance. \* =  $p \leq 0.05$ . \*\* =  $p \leq 0.01$ . \*\*\* =  $p \leq 0.001$ .

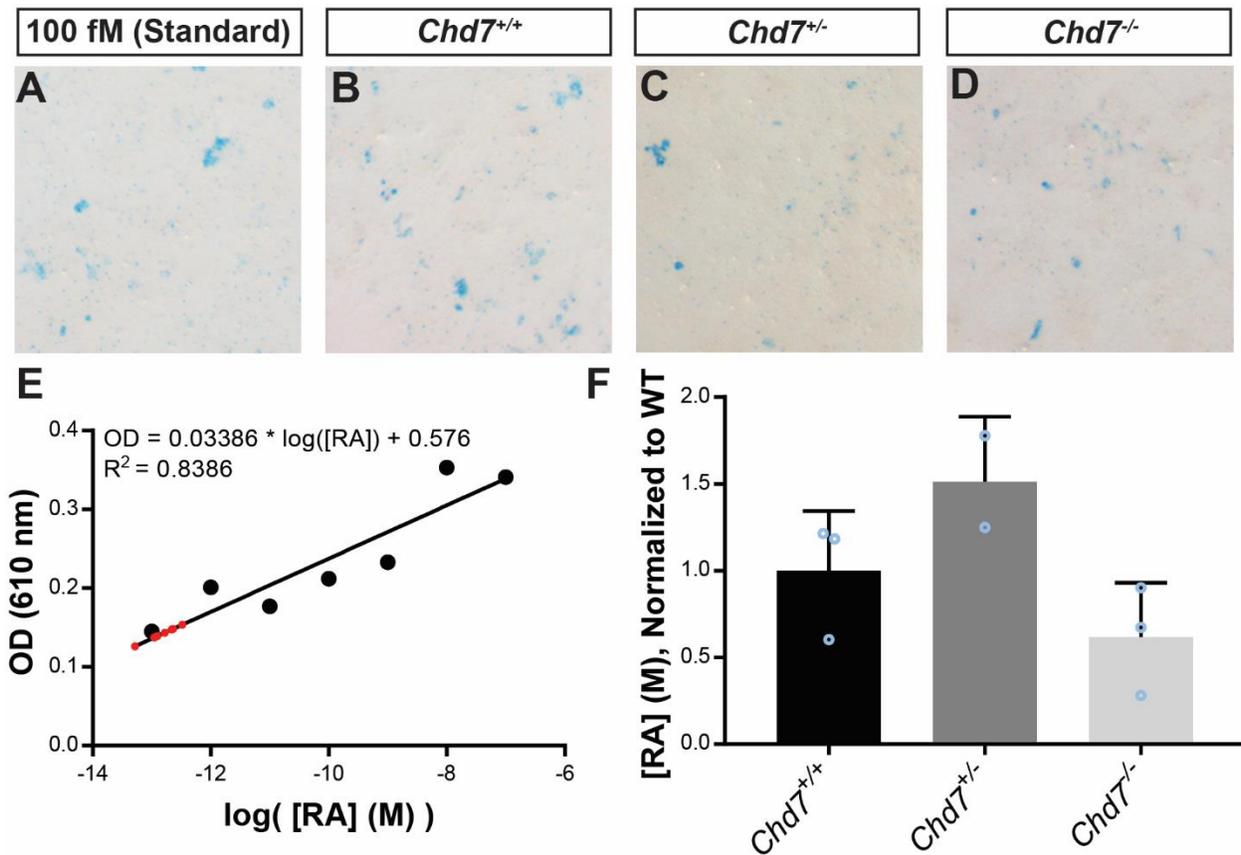


**Figure S3.** *Chd7* dosage does not impact retinoic acid reporter activity in the E13.5 inner ear.

E13.5 embryos sectioned in the sagittal plane and stained with X-gal to reflect RA activity.

(A-D) Sectioned inner ears from RARE-*lacZ*; *Chd7*<sup>+/+</sup> (*N*=2) and RARE-*lacZ* *Chd7*<sup>+/-</sup> (*N*=2) embryos exhibit high  $\beta$ -galactosidase activity in the lateral semicircular canal (lsc), superior semicircular canal (ssc), at the base of the posterior semicircular canal (psc), and in the utricle (u).

(E-F) Low level  $\beta$ -galactosidase activity is present in the VIII nerve ganglion (g), and not in the cochlea (coch) in both RARE-*lacZ*; *Chd7*<sup>+/+</sup> and RARE-*lacZ* *Chd7*<sup>+/-</sup> ears.



**Figure S4.** *Chd7* dosage does not affect embryonic retinoic acid activity.

E8.5 *Chd7*<sup>+/+</sup>, *Chd7*<sup>+/-</sup>, and *Chd7*<sup>-/-</sup> embryos were digested with trypsin and co-cultured with RARE-*lacZ* F9 cells and stained with X-gal.

(A) F9 cells treated with 100 fM RA and stained with X-gal are shown for reference.

(B-D) Representative images of cells from each genotype are shown.

(E) A linear best-fit line showing the OD 610 reading of each well as a function of the log-transformation of the concentration of RA (M) was used to determine the concentration of RA in each embryo. Individual readings are depicted as black dots, while wells containing embryos with their extrapolated concentrations of RA are shown in red.

(F) Extrapolated concentrations of RA, normalized to the average wild type concentration, were grouped by genotype. Each point represents one embryo.

This experiment was repeated on five separate litters of embryos, and data from one representative litter are shown. Significance was determined using an ordinary one-way ANOVA.