

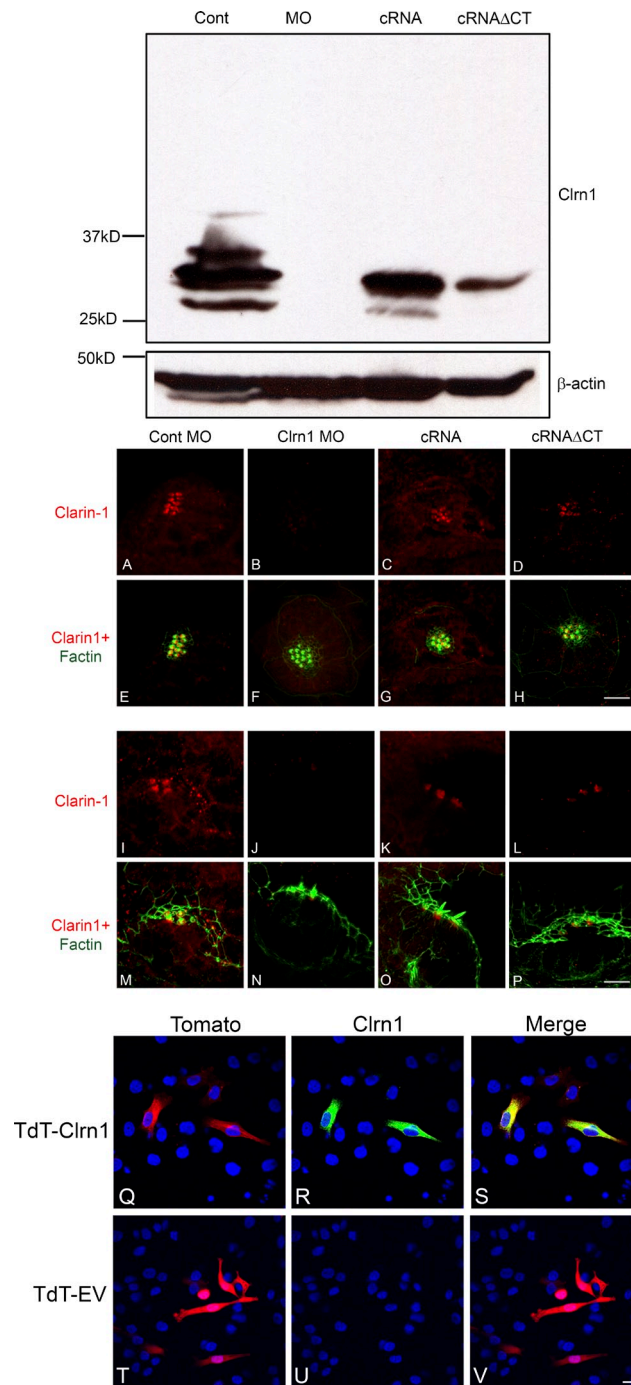
Ogun and Zallochi, <http://www.jcb.org/cgi/content/full/jcb.201404016/DC1>

Figure S1. **Anti-clarin-1 specifically detects the zebrafish clarin-1 protein.** (top) Representative Western blot image of three independent experiments. Lysates from 2 dpf larvae injected with control (Cont) morpholinos, clarin-1 (Cln1) morpholinos (MO), clarin-1 morpholinos + cRNA, or cRNA Δ CT and immunoblotted for clarin-1. β -Actin was used as a loading control. (A–P) Representative images of at least six independent experiments. 3 dpf larvae were injected with control morpholinos (A, E, I, and M), clarin-1 morpholinos (B, F, J, and N), clarin-1 morpholinos + cRNA (C, G, K, and O), or clarin-1 morpholinos + cRNA Δ CT (D, H, L, and P). Clarin-1 protein was assessed in neuromast (A–H) or ear (I–P) hair cells. Hair cell bundle was counterstained for F-actin. (Q–V) HeLa cells transiently transfected with tdTomato (TdT)–clarin-1 (Q–S) or tdTomato empty vector (EV; T–V). Bars: (A–H) 8 μ m; (I–P) 7 μ m; (Q–V) 14 μ m.

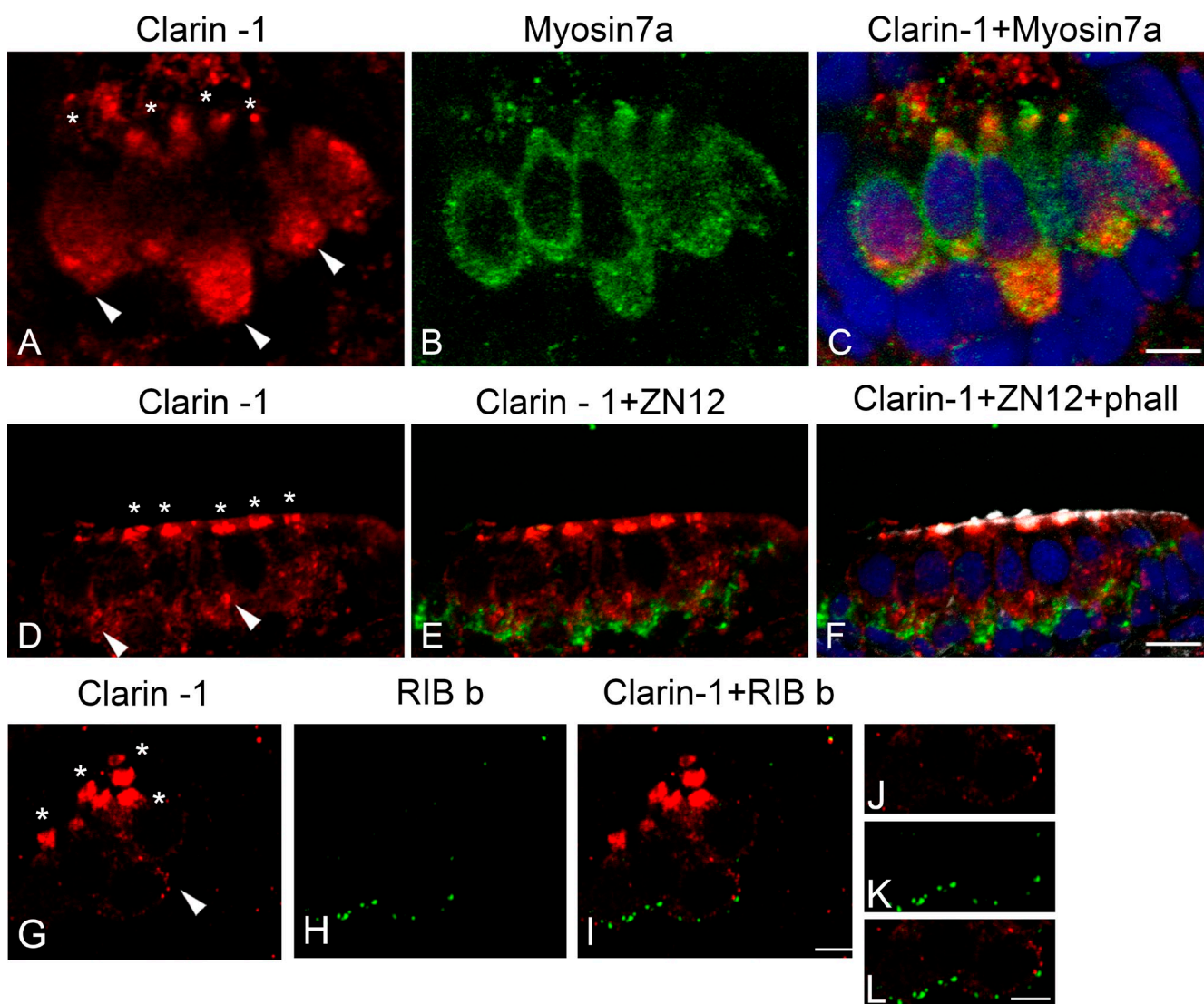


Figure S2. **Clarin-1 is expressed at the basal aspect of hair cells.** (A–L) Representative images of at least six independent experiments. Cross sections of a 3 dpf neuromast (A–C, oblique cut), anterior macula (D–F), and lateral crista (G–L), immunostained for clarin-1 (red; A, C, D, F, G, and I) and the hair cell marker myosin7a (green; B and C), the afferent marker ZN12 (green; E and F), or the presynaptic marker ribeye b (RIB b; green; H, I, K, and L). DAPI was used to counterstain the nucleus. Arrowheads denote clarin-1 basal expression. Asterisks denote apical expression. Bars: (A–C) 3 μ m; (D–L) 5 μ m. phall, phalloidin.

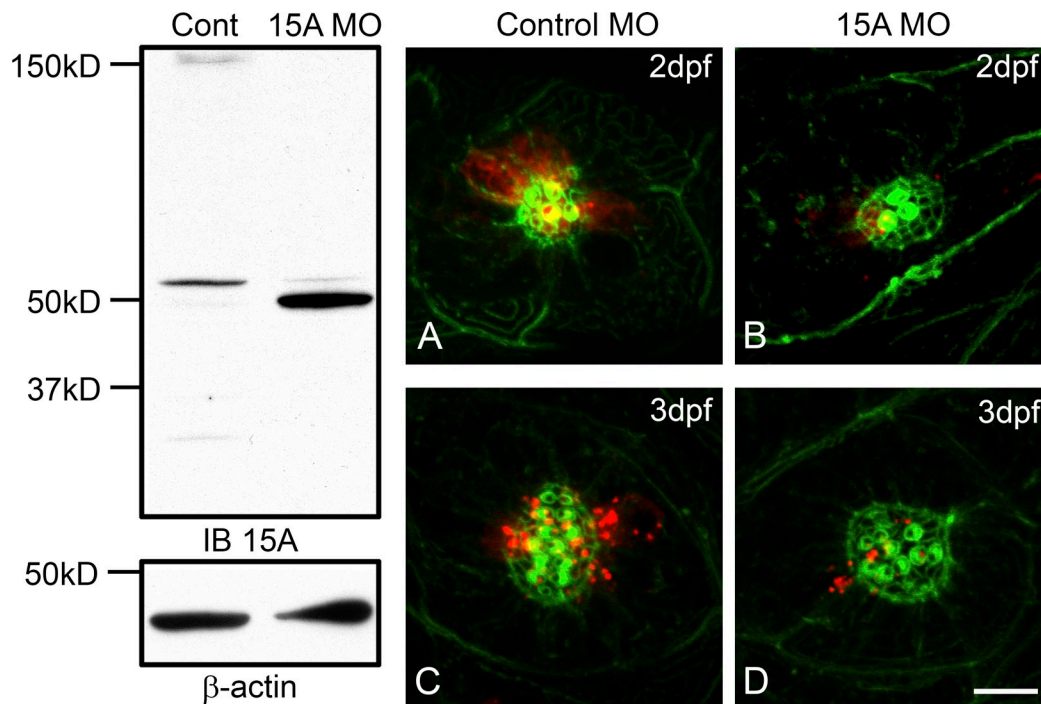


Figure S3. **Pcdh15a protein and FM1-43 uptake are diminished in Pcdh15a MOs.** (left) Representative Western blot of at least three independent experiments. Lysates from 3 dpf control (Cont) MOs and Pcdh15a MOs (15A MO) were immunoblotted with anti-Pcdh15a. Three bands can be detected in the control injected animals: the full-length Pcdh15a around 150 kD and two small variants at 50 and 30 kD. 15A MOs show a prominent band <50 kD that probably corresponds to a proteolytic product. β -Actin was used as a loading control. IB, immunoblot. (A–D) Representative images of at least six independent experiments. Control MOs (A and C) or Pcdh15a MOs (B and D) were assessed for FM1-43 uptake in neuromast hair cells at 2 dpf (A and B) and 3 dpf (C and D). Bar, 7 μ m.

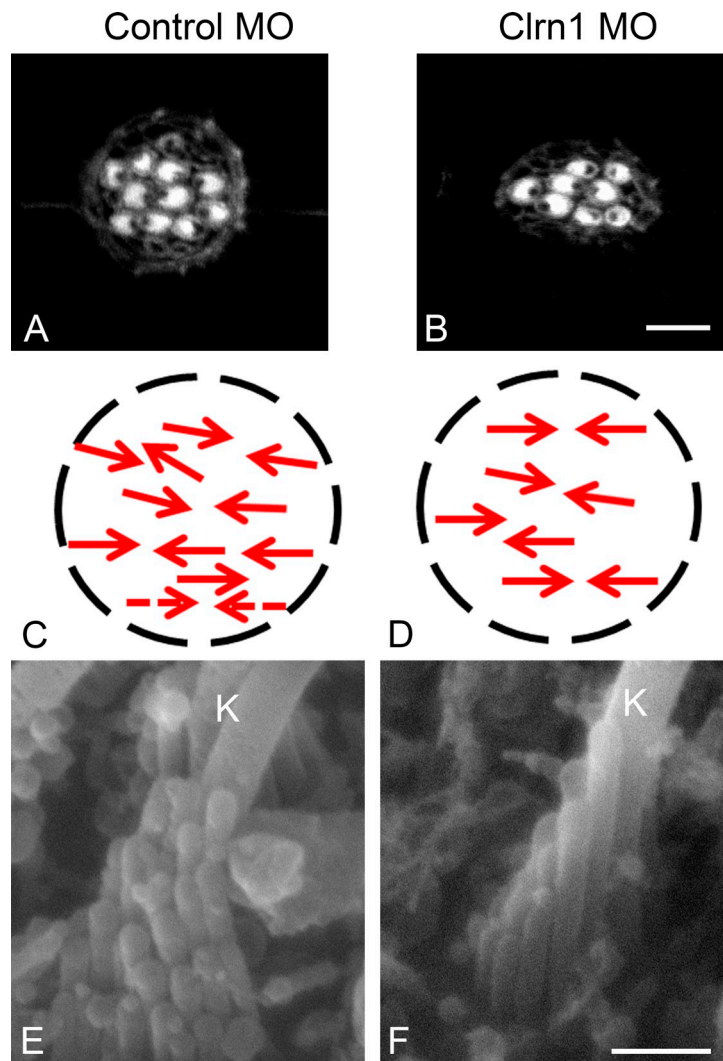


Figure S4. **Clarin-1 MOs have normal PCP and hair cell bundle morphology.** (A–D) Representative images of ≥ 10 independent experiments. PCP in 3 dpf control and MOs was determined by labeling of the hair cell bundle with phalloidin. *Cln1*, *clarin-1*. (C and D) Cartoons depicting the opposite polarization for each sister hair cell pair observed in A and B, respectively. (E and F) Representative images of eight independent experiments. Ultrastructural analysis of the hair cell bundle in 3 dpf control and MOs showing normal staircase shape for both treatments. K, kinocilium. Bars: (A and B) 5 μm ; (E and F) 350 nm.

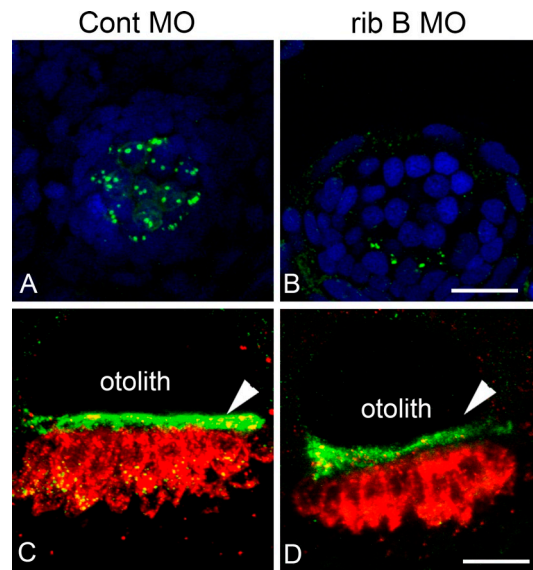


Figure S5. **Microinjection of ribeye b morpholinos produces reduction of ribeye puncta.** (A–D) Representative images of six independent experiments. 5 dpf neuromasts (A and B) and 4 dpf ears (C and D) from control (Cont; A and C) or ribeye b MOs (B and D), immunostained for ribeye b (rib B; green) and HCS-1 (red; C and D). DAPI was used to counterstain the nucleus (A and B). Ribeye b characteristic puncta is reduced from the basal aspect of hair cells in ribeye b MOs. Arrowheads denote nonspecific fluorescence from the otolith interface. Bar, 15 μ m.