

Figure S1: Morpholinos affect *cdh6* mRNA splicing and protein levels. (A) Schematic of zebrafish *cdh6*. Exons are numbered; open boxes represent untranslated regions. Exon 2 (red) is targeted for deletion by a splice-blocking morpholino (*cdh6SplMO*). Primer sites for PCR from cDNA are marked. (B) PCR amplified *cdh6* cDNA from uninjected, scrambled control morpholino (*controlMO*), and *cdh6SplMO* injected embryos. The boxes mark bands whose sequences and are shown in (C)-blue box, (D)-orange box, and (E)-green box. (C) *cdh6* cDNA from *controlMO* injected embryos shows wildtype splicing. (D) The larger *cdh6* cDNA fragment from *cdh6SplMO* injected embryos excludes a portion of exon 2, indicating a cryptic splice site was found. The mis-splice introduced a frame shift (highlighted in yellow) and a premature stop (red). (E) The smaller *cdh6* cDNA fragment from *cdh6SplMO* injected embryos excludes all of exon 2. This splicing defect also introduced a frame shift (yellow) and premature stop (red). (F) Western blot with antibody against Cdh6 on protein extracted from control or morphant embryos. Blot for anti-tubulin shown as a loading control.

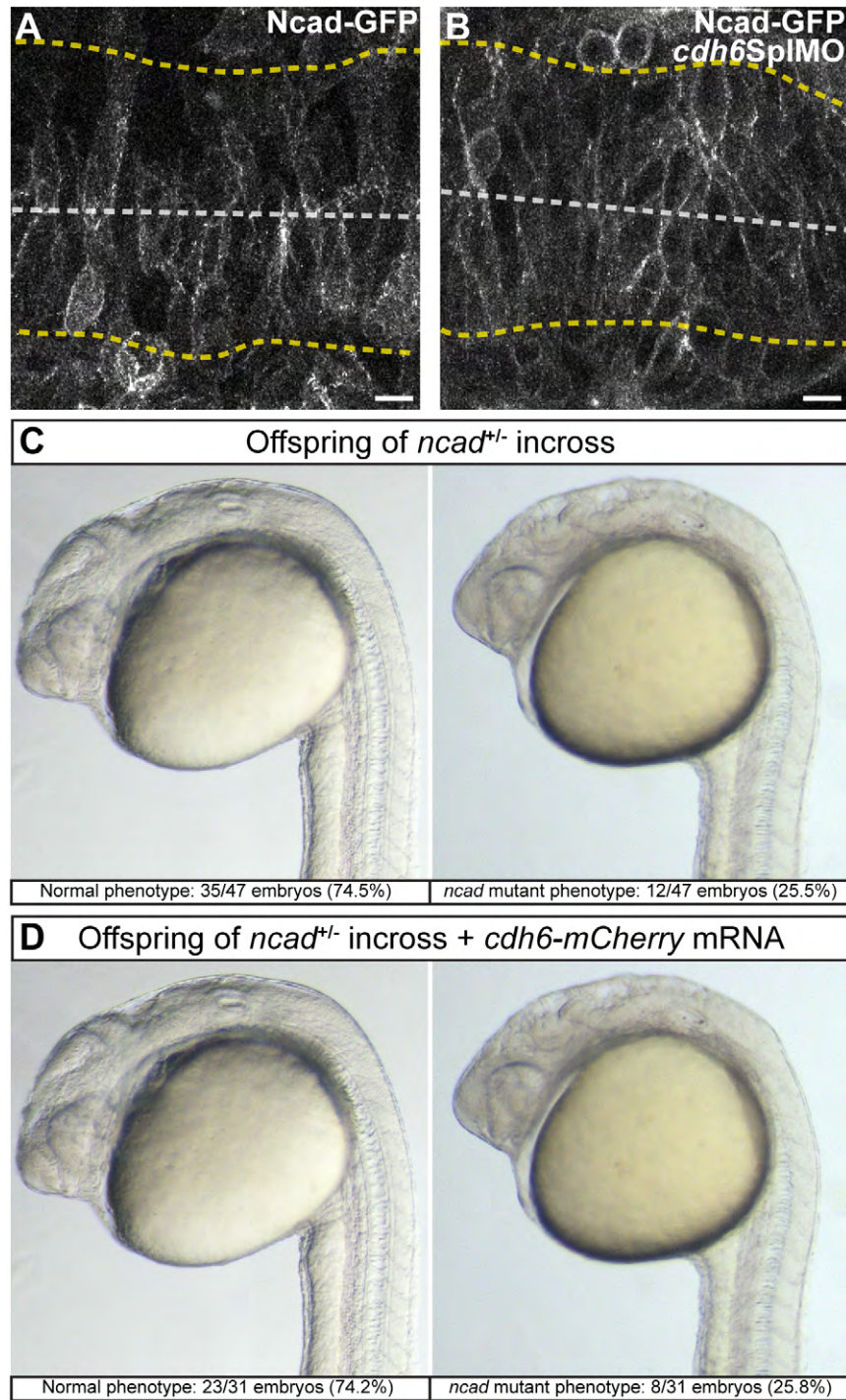
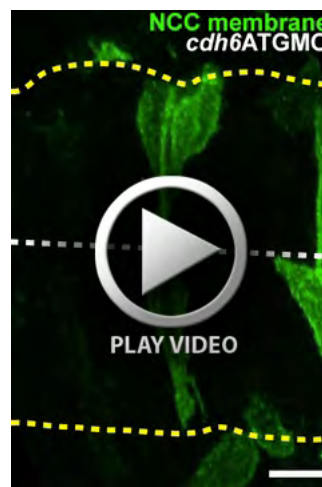


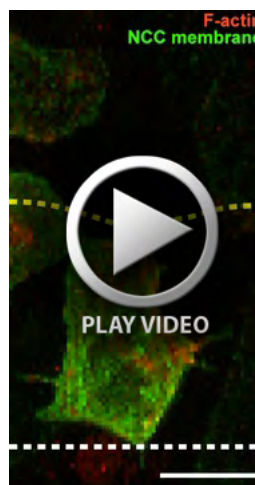
Figure S2: Cdh6 does not modulate Ncad-GFP and cannot compensate for Ncad loss. (A, B) Individual confocal z-planes (dorsal views, anterior left) of living 14 hpf embryos injected with *ncad-gfp* mRNA. (A) Ncad-GFP is localized to the cell membrane and enriched in many apical neuroepithelium cell regions. (B) Cdh6 knockdown (*cdh6*SplMO) does not alter Ncad-GFP localization. (C, D) Examples of normal and *ncad* mutant phenotypes in offspring of *ncad*^{tm101+/-} adults. Embryos were uninjected (C) or injected with *cdh6-mcherry* mRNA (D). Cdh6-mCherry does not rescue Ncad mutation.



Movie S1: NCCs exhibit normal behaviors and undergo EMT in embryos injected with *controlMO*. Movie of NCCs labeled with membrane GFP (GFP-CAAX) in an embryo injected with *controlATGMO*. Images were acquired every 30 seconds; movie shows 38 minutes. In the first frame the yellow dashed lines marks the basal surfaces of the neuroepithelium, the white dashed line marks the midline, and the scale bar = 10 μ M.



Movie S2: Cdh6 knockdown disrupts apical NCC tail detachment and EMT. Movie of NCCs labeled with membrane GFP (GFP-CAAX) in an embryo injected with *cdh6ATGMO*. Images were acquired every 35 seconds; movie shows 119 minutes. In the first frame the yellow dashed lines marks the basal surfaces of the neuroepithelium, the white dashed line marks the midline, and the scale bar = 10 μ M.



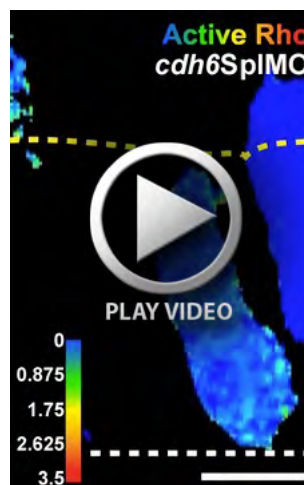
Movie S3: F-actin accumulates in apical NCC tails prior to detachment in a wildtype embryo. Movie of NCCs expressing membrane GFP and an F-actin biosensor (red). Images were acquired every 30 seconds; movie shows 15.5 minutes. In the first frame the yellow dashed line marks the basal surface of the neuroepithelium, the white dashed line marks the midline, and the scale bar = 10 μ M.



Movie S4: Knockdown of Cdh6 disrupts F-actin accumulation in apical NCC tails. Movie of NCCs expressing membrane GFP and an F-actin biosensor (red) in an embryo injected with *cdh6*SpIMO. Images were acquired every 30 seconds; movie shows 15 minutes. Note that F-actin in the lamellipodial protrusions along the lateral (right) side of the NCC are not affected by Cdh6 knockdown. In the first frame the yellow dashed line marks the basal surface of the neuroepithelium, the white dashed line marks the midline, and the scale bar = 10 μ M.



Movie S5: Rho is activated in apical tails during NCC EMT in wildtype embryo. Movie showing ratiometric imaging of NCC expressing an active Rho biosensor (GFPrGBD) and mCherry. Images were acquired every 30 seconds; movie shows 30 minutes. In the first frame the yellow dashed line marks the basal surface of the neuroepithelium, the white dashed line marks the midline, the look up table shows the GFP:mCherry ratio, and the scale bar = 10 μ M.



Movie S6: Active Rho occupies an expanded area after Cdh6 knockdown. Movie showing ratiometric imaging of NCCs expressing an active Rho biosensor (GFPrGBD) and mCherry in an embryo injected with *cdh6*ATGMO. Images were acquired every 30 seconds; movie shows 100 minutes. In the first frame the yellow dashed line marks the basal surface of the neuroepithelium, the white dashed line marks the midline, the look up table shows the GFP:mCherry ratio, and the scale bar = 10 μ M.