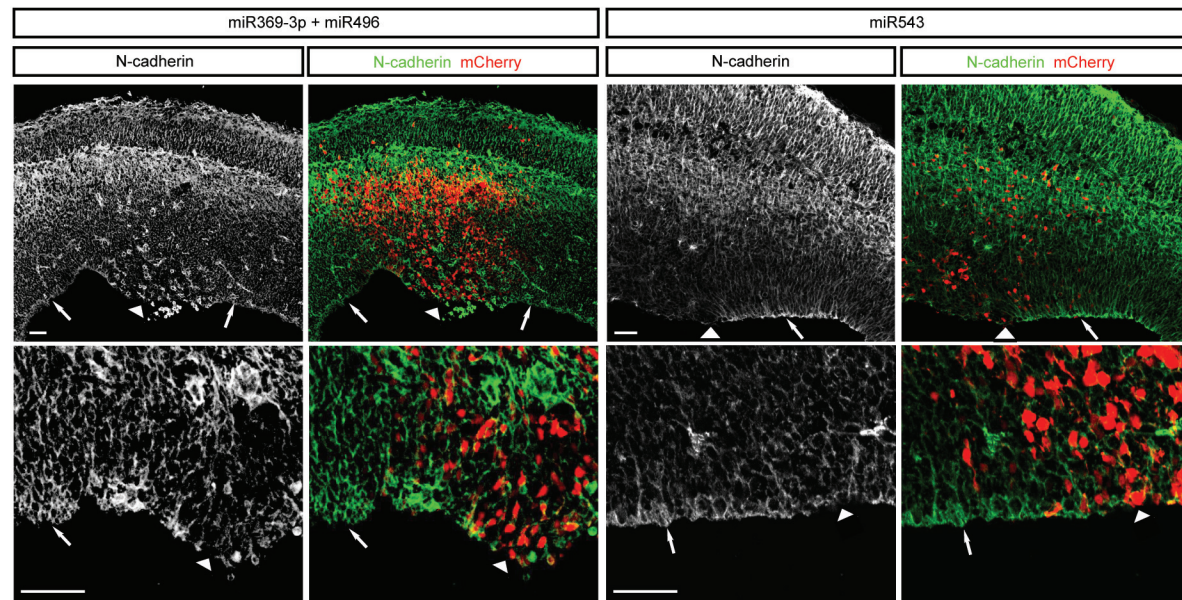
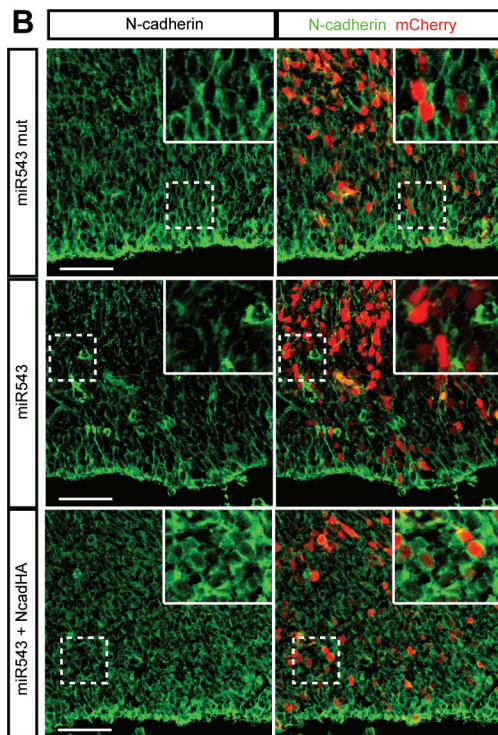


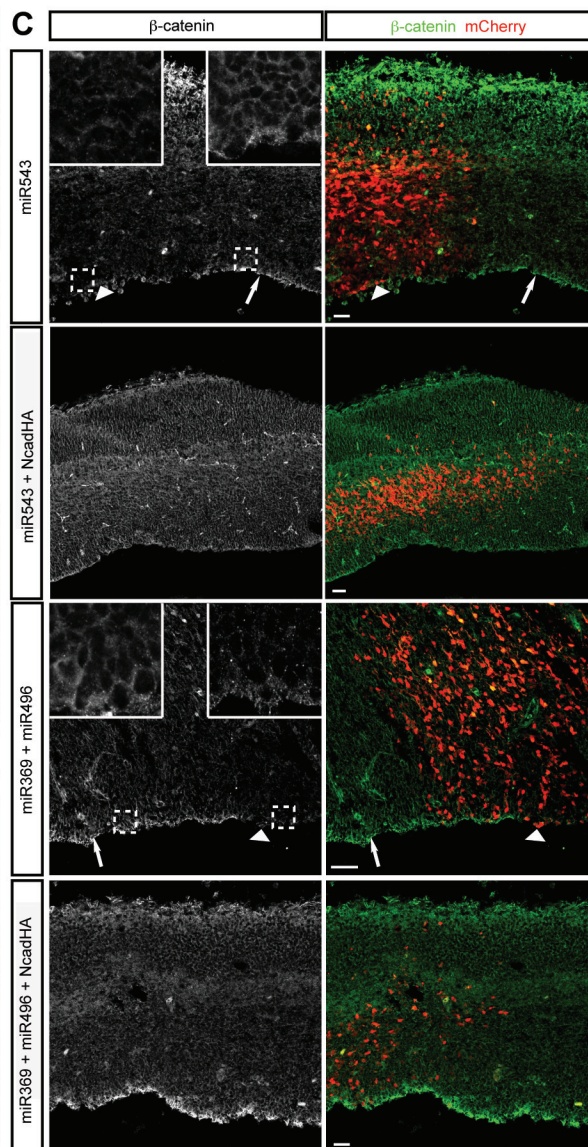
A



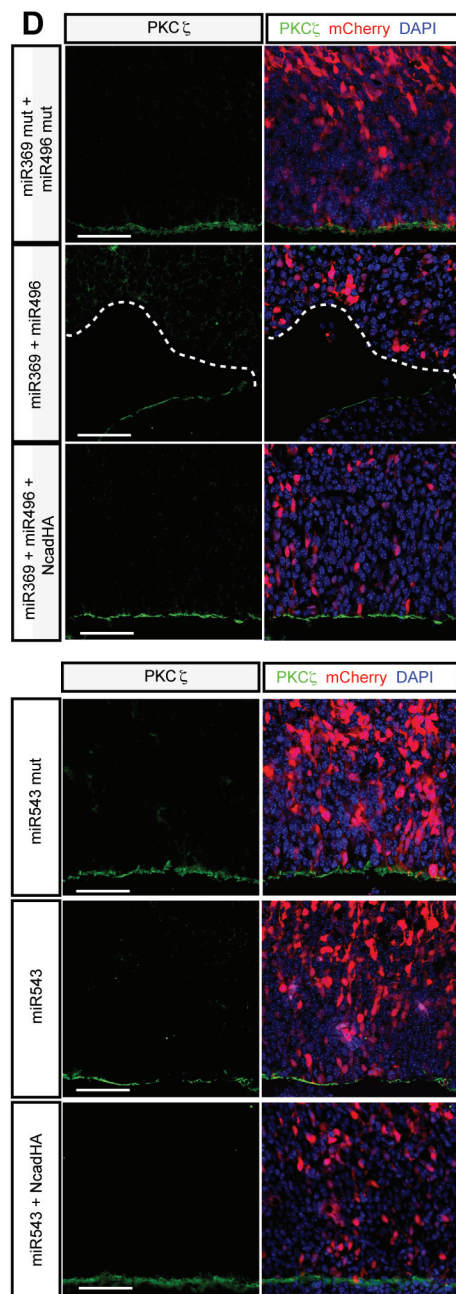
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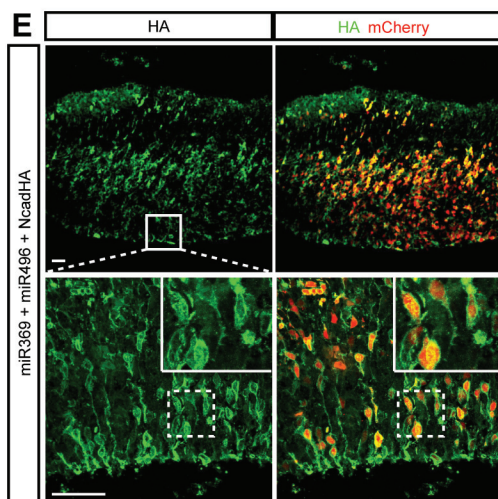
C



D



E



Supplementary Figure 3. Related to Figure 3. The overexpression of either miR543 or miR369-3p with miR496 results in the downregulation of the N-cadherin expression level and disrupts the AJs in the ventricular zone. (A) The miR543 construct or the miR369-3p together with miR496 constructs were electroporated with pCAG-mCherry at E13.5, and the brains were analysed at E15.5. mCherry is depicted in red. Immunostaining for N-cadherin (green) illustrates that N-cadherin expression is downregulated in the VZ when miRNAs are overexpressed only in the electroporated areas (arrowheads) but is maintained in non-electroporated areas (arrows). Scale bars: 50 μ m. (B) The overexpression of miR543 downregulates the Ncad protein level, and the co-expression of an HA-tagged Ncad lacking its endogenous 3'UTR rescues this effect. Ventricular surfaces are shown at 40X magnification. Scale bars: 50 μ m. Insets are indicated in dashed boxes on the original pictures. (C, D) The AJs are disrupted at the ventricular surface when miR543 or miR369-3p together with miR496 are overexpressed. This phenotype can be rescued by co-expressing the NcadHA lacking the N-cadherin 3'UTR (miR369-3p + miR496 + NcadHA). (C) Immunostaining specific for β -catenin is lost in electroporated areas (arrowheads) and maintained in non-electroporated areas (arrows) of brain cortices overexpressing the indicated miRNAs. Effective rescue of the phenotype is achieved when co-expressing NcadHA. Scale bars: 50 μ m. Insets are indicated in dashed boxes on the original pictures. (D) Immunostaining for atypical PKC ζ , a member of the polarity complex was performed on brain cortices overexpressing miR543 or miR369-3p together with miR496. Atypical PKC ζ is present at the AJs when miRNA constructs containing mutations in the seed sequences are electroporated and absent from the AJs when wt miR369-3p and miR496 are co-expressed. The co-expression of N-cadherin rescues this phenotype. Scale bars: 50 μ m. (E) Immunostaining for HA reveals that all the electroporated cells (mCherry⁺, in red) express the NcadHA construct (HA⁺, in green). Insets are indicated in dashed boxes on the original pictures. Scale bars: 50 μ m.