

Fig. S1. Eya1 and TJ proteins are expressed in the lung. Representative x-y sections and transverse x-z views obtained in E14.5 distal lung epithelial cells labeled with either Eya1 (A), ZO-1 (B), occludin (C) or claudin1 (D) antibodies. Note the localization of Eya1 at areas of intercellular contact (A; arrowheads) similar to TJ proteins that had a belt-like pattern of membrane immunostaining (B,C,D; arrowheads). Scale bars: 50 μ m. Arrows in x-z images indicate the thickness of the epithelium. Bars: 10 μ m. Broken line represents the collagen IV-stained basement membrane.

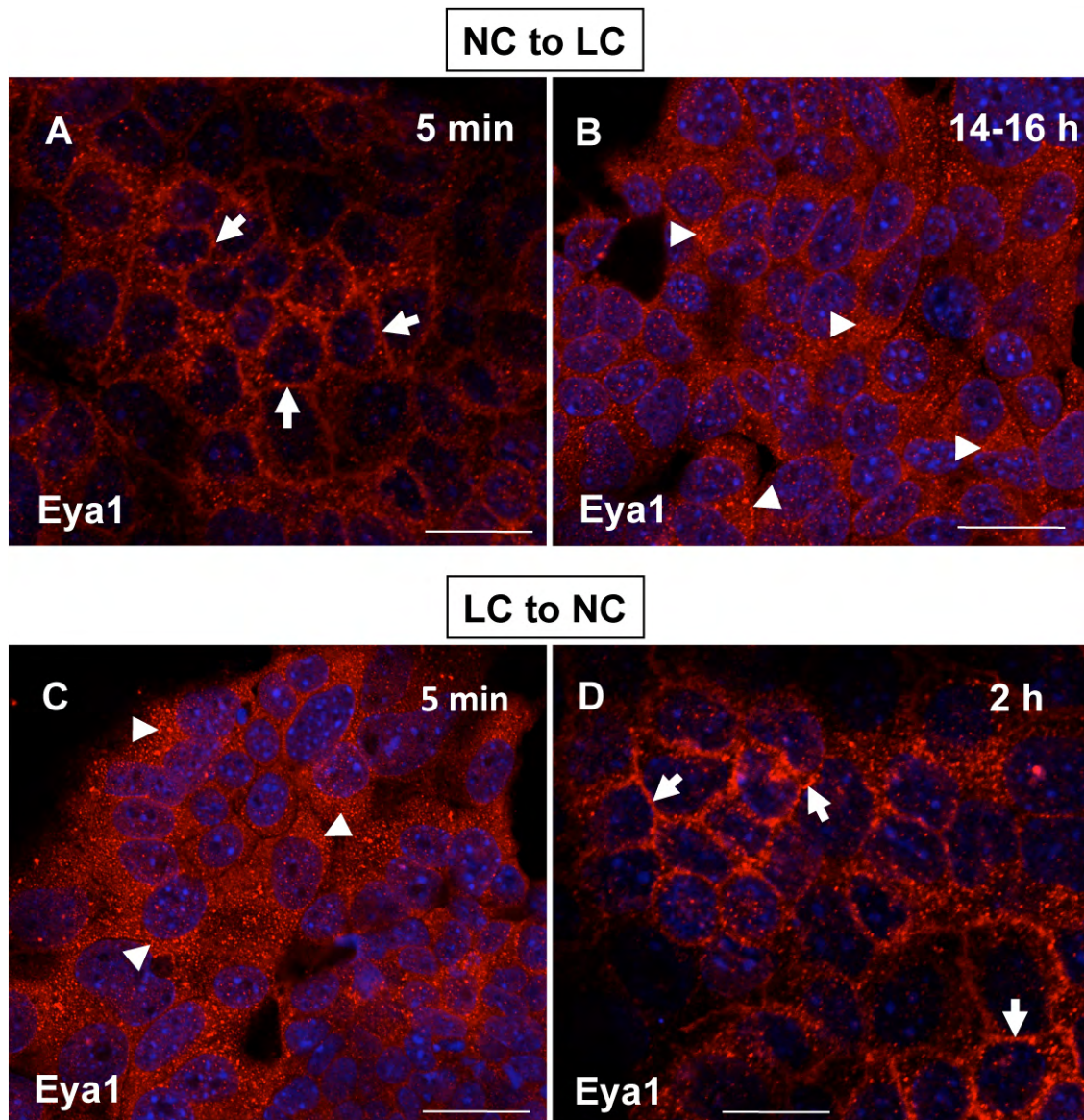


Fig. S2. (A-D) Immunocytochemistry with Eya1 antibody shows that the sub-cellular localization of Eya1 protein in MLE15 cells is Ca^{2+} dependent. (A) The Eya1 expression domain (arrows) is strongly visualized at the periphery of MLE15 cells grown in NC medium. (B) Ca^{2+} -deprived MLE15 cells show an apparent disappearance of the peripheral membrane staining for Eya1 that localizes to the cytosol (arrowheads). (C,D) Ca^{2+} starvation of cells overnight before switching to NC medium for 5 min (C) or 2 h (D) in order to induce junction biogenesis results in gradual re-concentration of Eya1 protein at sites of cell-cell contact (arrows in D). Scale bars: 50 μm .

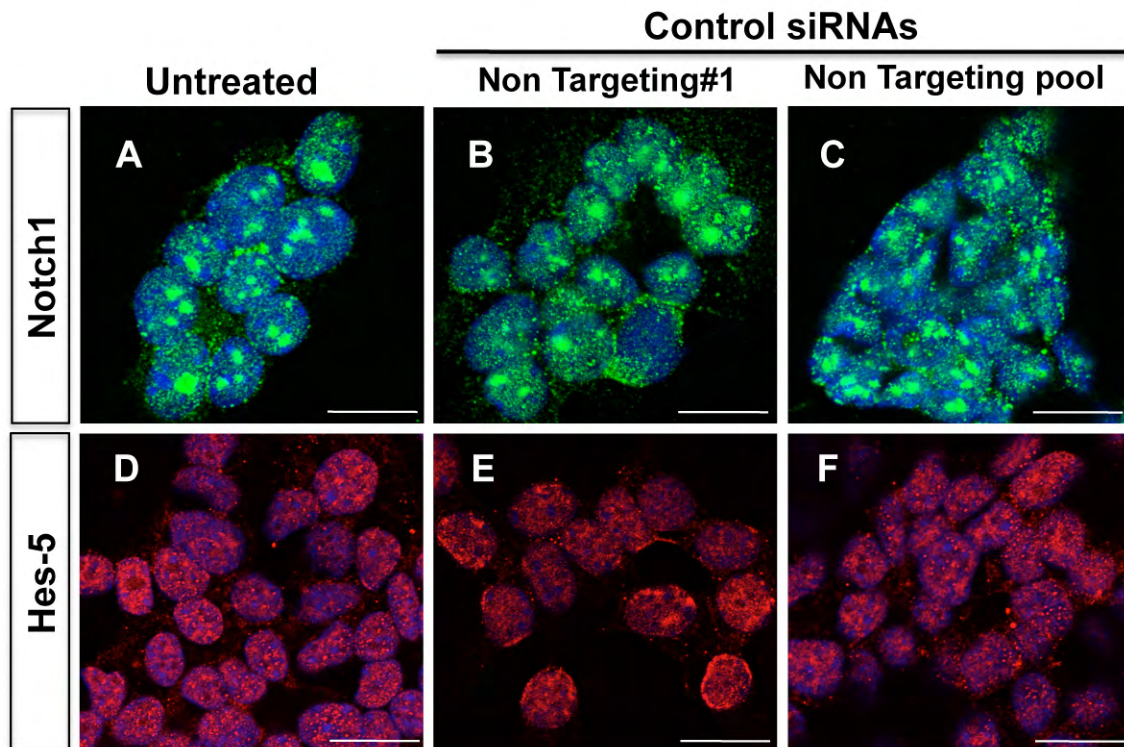


Fig. S3. (A-F) Immunostaining of MLE15 cells grown in culture with specific antibodies after transfection for 3 days with the indicated control siRNAs, used at 20 nM. Note that nonspecific siRNAs displayed no apparent effect on Notch1 and Hes-5 expression levels. Scale bars: 50 μ m.