Supplemental material

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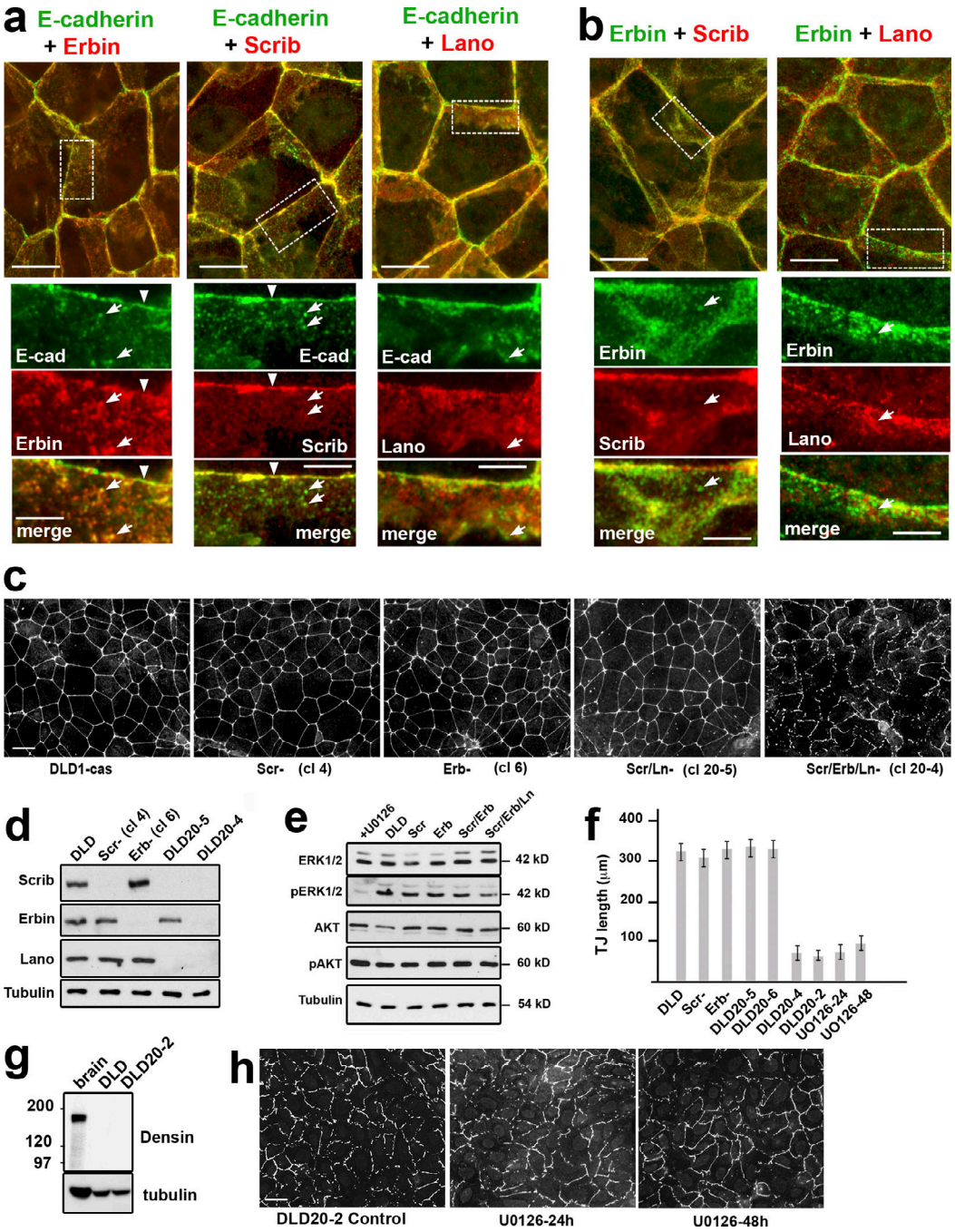

Figure S1. **AJs, TJs, and LAPPs in DLD1 and its clones. (a)** DLD1 cells were double stained for E-cadherin (E-cad, green) and LAP proteins Erbin, hScrib (Scrib), and Lano (red). E-cadherin and LAPP staining is merged in the general view (top image), and the magnified area indicated by the dashed line stained separately for each marker is presented at the bottom. Note that slAJs (some are indicated by arrows) are brightly stained for Erbin but not for Lano or hScrib. By contrast, apical AJC (arrowheads) specifically recruits hScrib, but much less Erbin or Lano. **(b)** To confirm differences in LAPP distribution at the lateral membrane, the cells were double-stained for Erbin (green) and Scrib or Lano (red). The selected areas are zoomed at the bottom as in a. Note that numerous Erbin-enriched clusters (some of which are marked by arrows) are completely devoid of hScrib or Lano. Also note that hScrib is predominantly localized in the straight apical band, corresponding to AJC. Bars, 10 µm (major images) or 4 µm (zoomed images). **(c)** TJs of the control DLD1cas cells and their clones (cl) lacking hScrib (Scr-, cl 4), Erbin (Erb-, cl 6), hScrib, and Lano (Scr/Ln-, cl 20-5), or hScrib, Erbin, and Lano (Scr/Erb/Ln-, cl 20-4) were imaged using wide-field microscopy after staining for cingulin. Bar, 20 µm. **(d)** Western blot of the total lysates obtained from cells shown in c, probed for hScrib (Scrib), Erbin, Lano, and Tubulin. **(e)** Representative Western blot of the cell clones shown above, as well as control DLD cells cultured for 10 h in the presence of 10 mm MEKK inhibitor U0126 (+U0126), were probed for ERK1/2, phosphorylated ERK1/2 (pERK1/2), AKT, phosphorylated AKT (pAKT), and tubulin. Note the same level of ERK and AKT phosphorylation in all cells and a strong reduction of ERK1/2 phosphorylation after U0-126 treatment. **(f)** The continuous length of TJs in these cultures assessed as described in Materials and methods. Note a dramatic fragmentation of TJs in cells lacking all three LAPPs, which cannot be blocked by inhibition of the MEKK pathway by U0126 during 24 h (U0126-24) or 48 h (U0126-48). The error bars represent standard error (n = 10). **(g)** Western blot of human hippocampus extract (brain) and total lysates of DLD1 and DLD20-2 cells probed for human Densin and tubulin. **(h)** 3-d-old culture of DLD20-2 cells cultured in control conditions and in the presence of U0126 for 24 and 48 h. Cells were stained for cingulin. Bar, 20 µm.

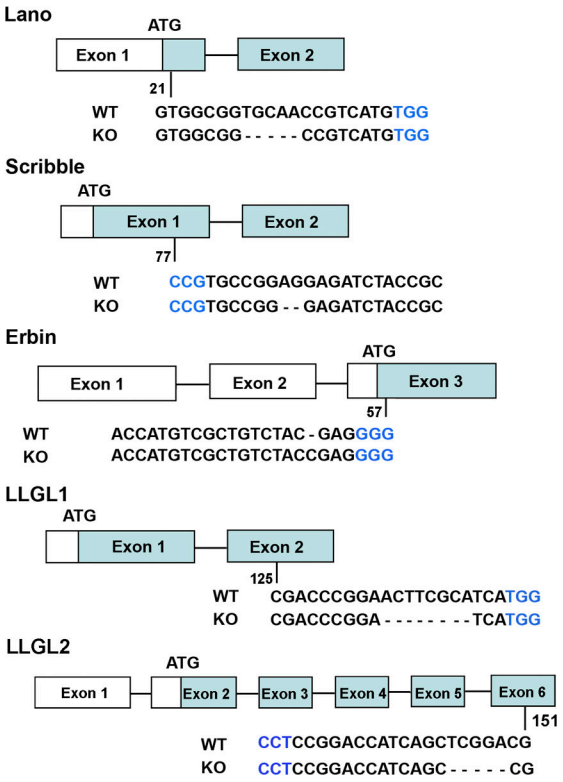

Figure S2. **Knockout of LAPP (Lano, Scrib, and Erbin), Llgl1, and Llgl2 genes in DLD1 cells.** For each gene, the upper panel schematically represents the position of the target sites. The line and a number indicate the nucleotide in the target sequence presented in the WT line (PAM sites are blue). The corresponding cDNA sequences in the KO cell lines show homogeneous frameshift mutations.

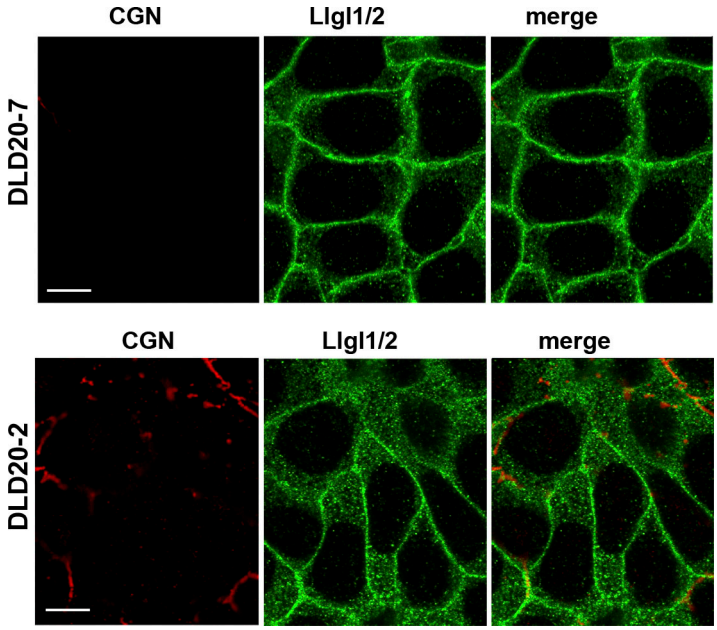

Figure S3. **Localization of Llgl1/2 in Erbin expressing DLD20-7 cells and LAPP-deficient DLD20-2 counterparts.** The cells were stained for the TJ marker CGN (red) and Llgl1/2 (green). Only x-y 0.4-µm projection crossing the center of the cells is presented. Note the localization of Llgl1/2 only within the cortex of the lateral plasma membrane, which is completely deprived with TJs in the DLD20-7 cells. In contrast, Llgl1/2 is localized at both the lateral membrane (which shows numerous TJs) and the cytosol in LAPP-deficient DLD20-2 cells. Bars, 10 µm.

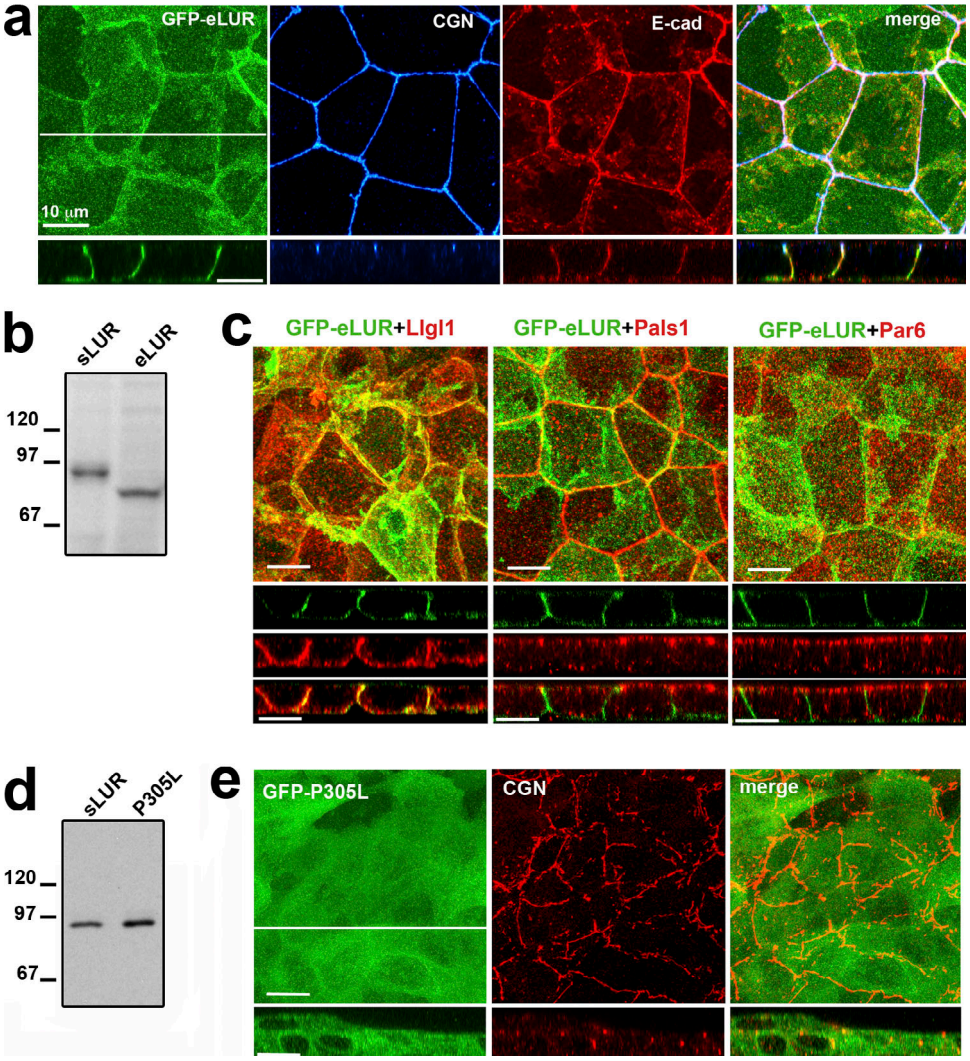

Figure S4. **GFP-tagged LUR of Erbin (GFP-eLUR), but not a P305L mutant of GFP-sLUR, is localized at the basolateral membrane and rescues DLD20-2 cell polarity. (a)** Projections of all x-y optical slices of DLD20-2 cells expressing GFP-eLUR. Cells were stained for CGN (blue) and E-cad (red) and also imaged for GFP fluorescence (green). The optical z-sections along the middle of the image (white line) are shown at the bottom. Bar, 10 µm. **(b)** Western blot of the total lysates of DLD20-2 cells expressing GFP-sLUR (sLUR) or GFP-eLUR (eLUR). The blots were probed by anti-GFP. Molecular weight markers (in kD) are indicated. **(c)** Western blot probed for GFP of the DLD20-2 cells expressing GFP-sLUR (sLUR) or GFP-sLUR bearing P305L mutation. Bar, 10 µm. **(d)** Projections of all optical slices of DLD20-2 cells expressing GFP-eLUR. Cells were imaged for GFP fluorescence (green) in conjunctions with immunostaining for Llgl1, Pals1, or Par6B (all red). The corresponding cross-sections through the middle of each image are shown at the bottom. **(e)** Projections of all x-y optical slices of DLD20-2 cells expressing GFP-sLUR-P305L mutant imaged for GFP (GFP-P305L, green) and CGN (red). The optical z-sections along the white line are shown at the bottom. Note that the mutant is unable to rescue TJ integrity and is cytosolic. Bars, 10 µm.

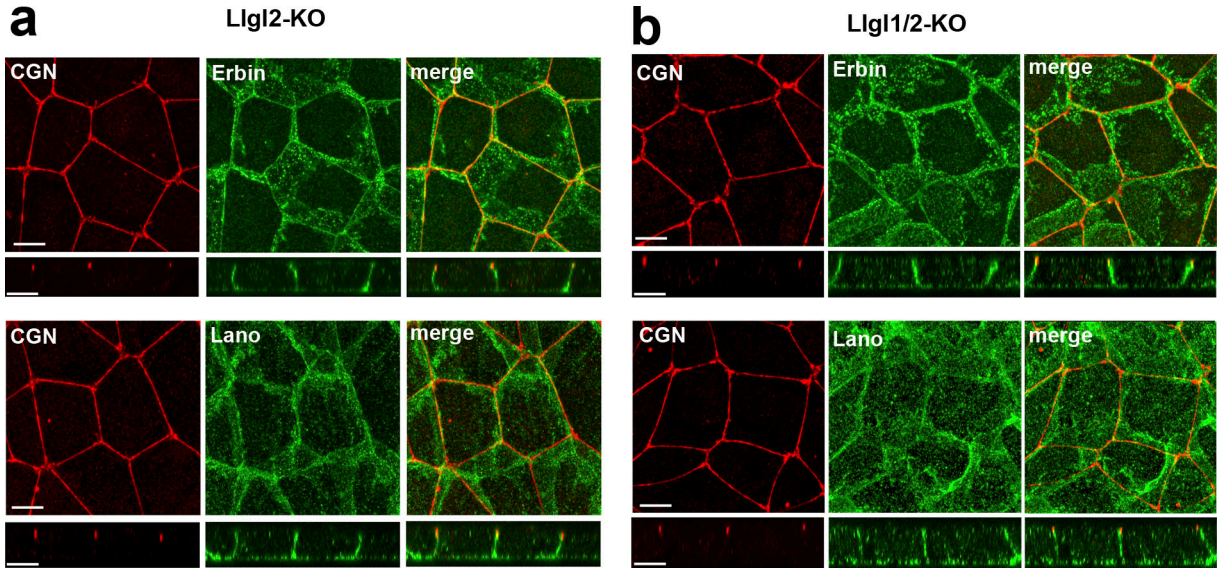

Figure S5. **Llgl1/2 deficiency had no effect on Erbin or Lano localization. (a and b)** Immunofluorescent microscopy mapping of Erbin and Lano localization in the Llgl2-deficient (Llgl2-KO; a) and Llgl1/2-deficient (Llgl1/2-KO; b) DLD1 cells. The confluent 3-d-old cultures were stained with anti-cingulin antibody (CNG, red) and with antibodies against LAP proteins Erbin or Lano (green). Projections of all x-y optical slices for each antibody staining are shown. The corresponding optical cross-sections for each image are provided at the bottom. Bars, 10 µm.