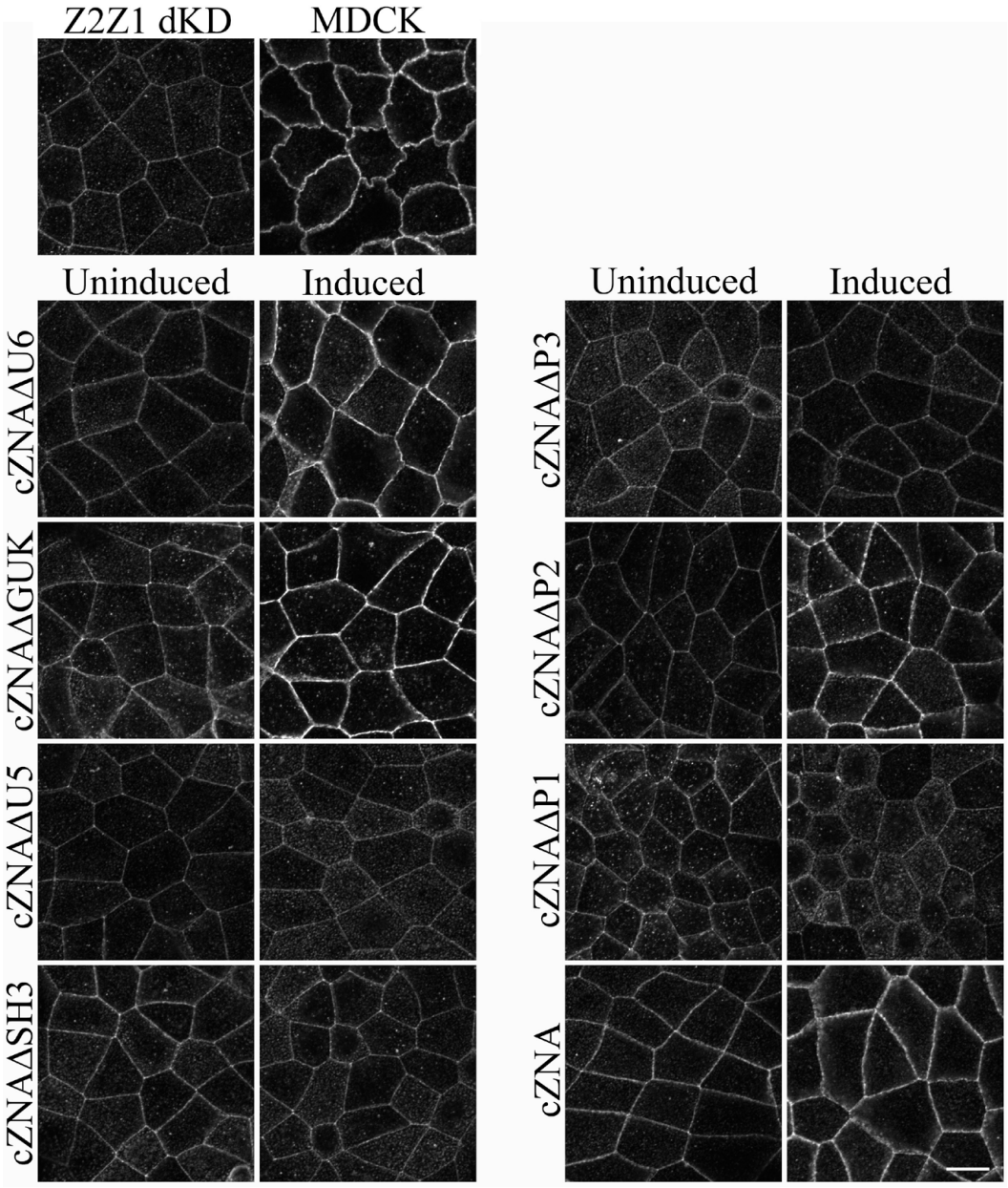

Fig. S1. PDZ1 and PDZ3 are required for localization of claudin-1 to the AJC. Induced and uninduced Z2Z1dKD cells were fixed and stained with antibodies against claudin-2. Images are 1.3 μm maximum density projections of Z-stacks taken through the apical most aspect of the cells. Scale bar: 10 μm.

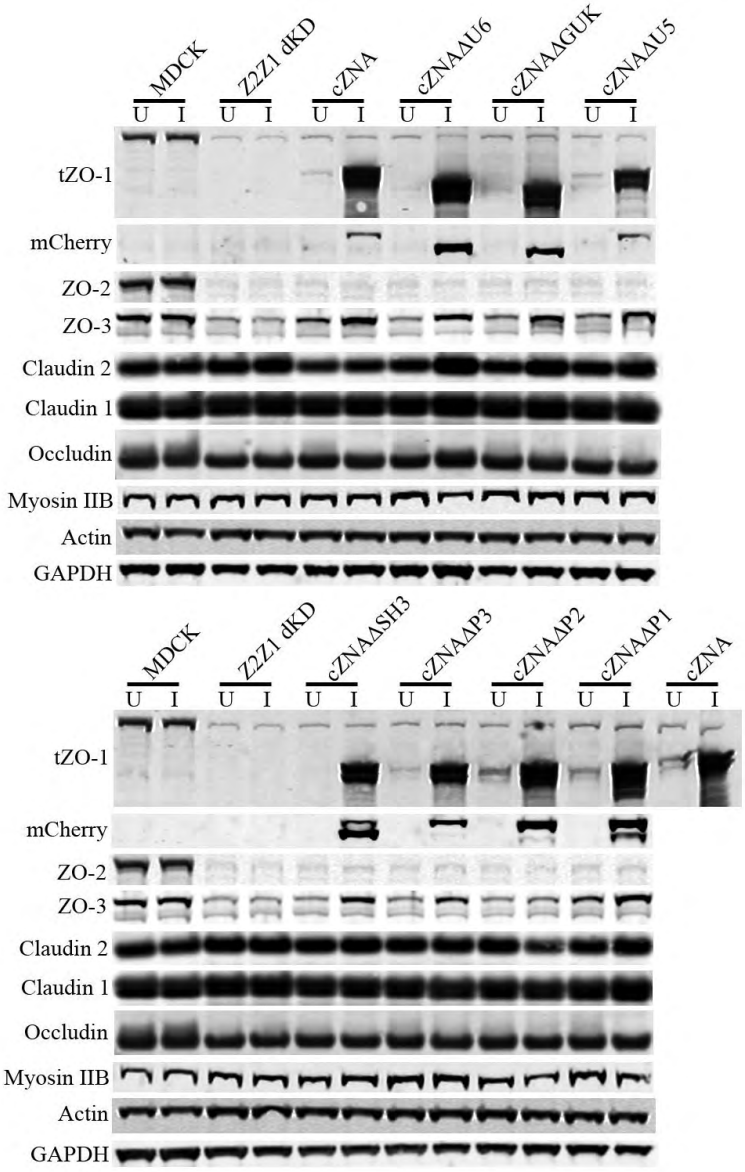

Fig. S2. Expression of cZNA deletion transgenes and other proteins of interest. Stoichiometric volumes of uninduced and induced cell lysates were resolved by SDS-PAGE, transferred to nitrocellulose and probed with the indicated antisera as described in the Materials and Methods. U, uninduced; I, induced; ZO1, endogenous ZO1 protein; Cherry, transgene product; GAPDH, loading control.

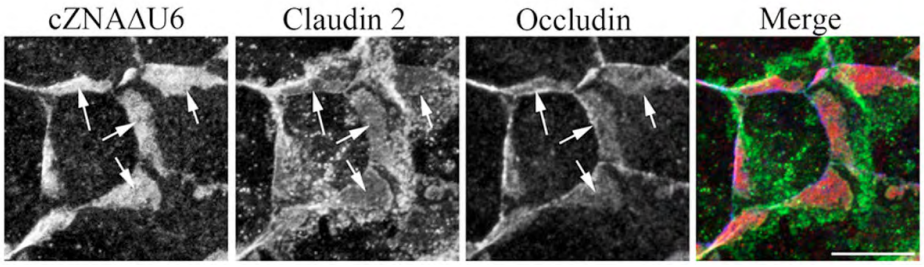

Fig. S3. Claudin-2 and occludin are colocalized with cZNAΔU6 along the lateral membrane. Z2Z1dKD cells expressing cZNAΔU6 were fixed and stained with antibodies against the mCherry epitope tag (red), claudin-2 (green) and occludin (blue). Images are maximum density projections of Z-stacks taken through the apical-most aspect of the cells. Arrows indicate areas of lateral membrane colocalization. Scale bar: 10 μm.

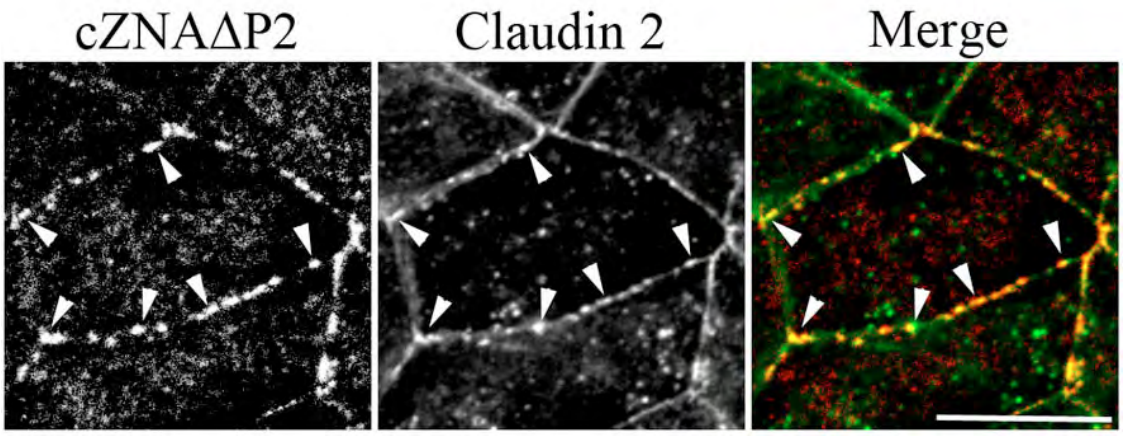

Fig. S4. Claudin-2 colocalizes with cZNAΔP2 in a discontinuous pattern at sights of cell-cell contact. Z2Z1dKD cells expressing cZNAΔP2 were fixed and stained with antibodies against the mCherry epitope tag (red) and claudin-2 (green). Images are 1.3 μm maximum density projections of Z-stacks taken through the apical-most aspect of the cells. Arrows indicate areas of co--localization. Scale bar: 10 μm.

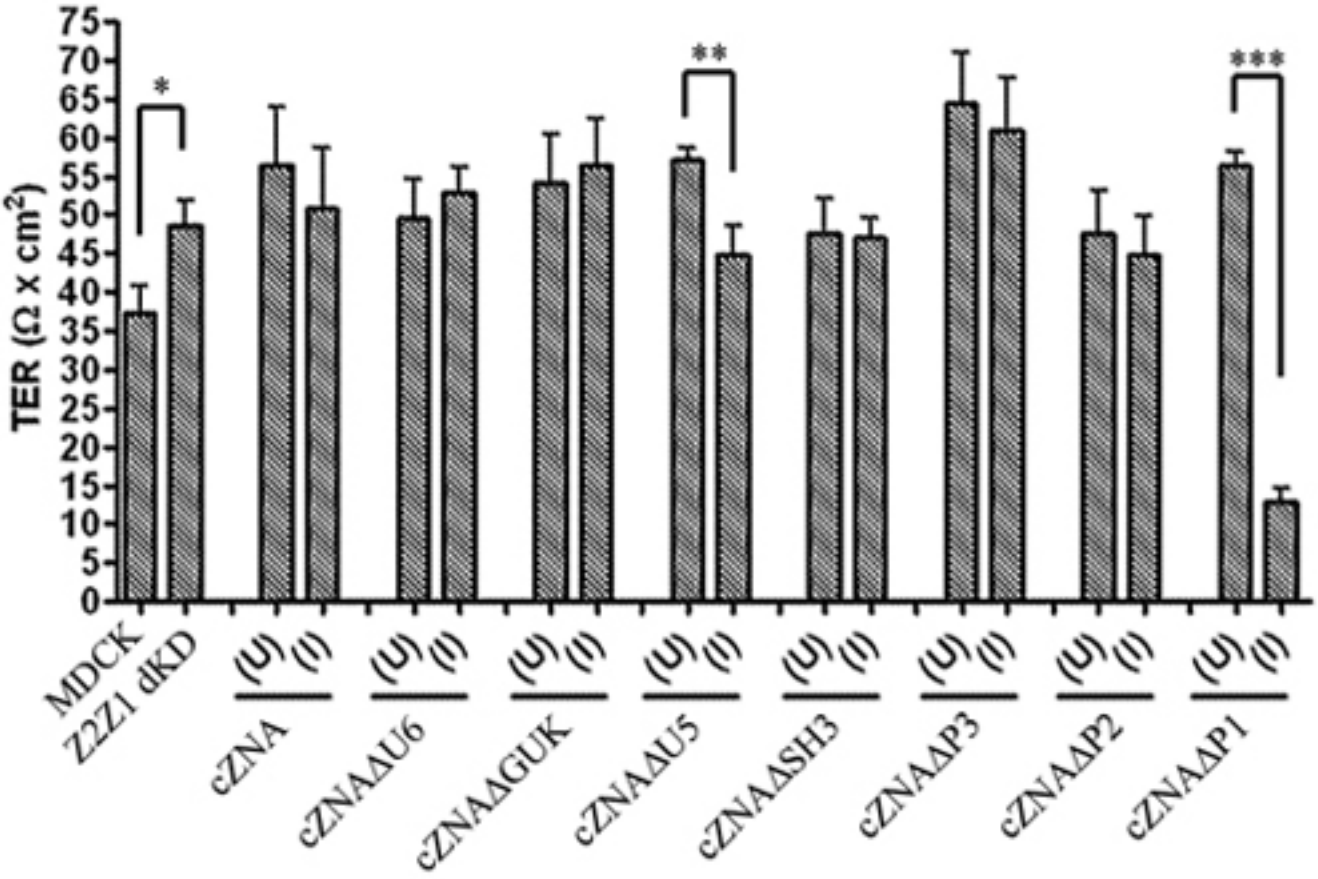

Fig. S5. Expression of cZNAΔP1 in Z2Z1 dKD cells significantly reduces trans-epithelial resistance (TER). TER was measure on fully confluent cells cultured for 7 days on filters uninduced or induced to express rescue constructs. Each bar depicts one experiment that is representative of three separate experiments. *N*=4 for each experiment. Statistics were calculated using a two-tailed unpaired Student's *t*-test. **P*=less than 0.001, ***P*=0.006, ****P*=less than 0.001.

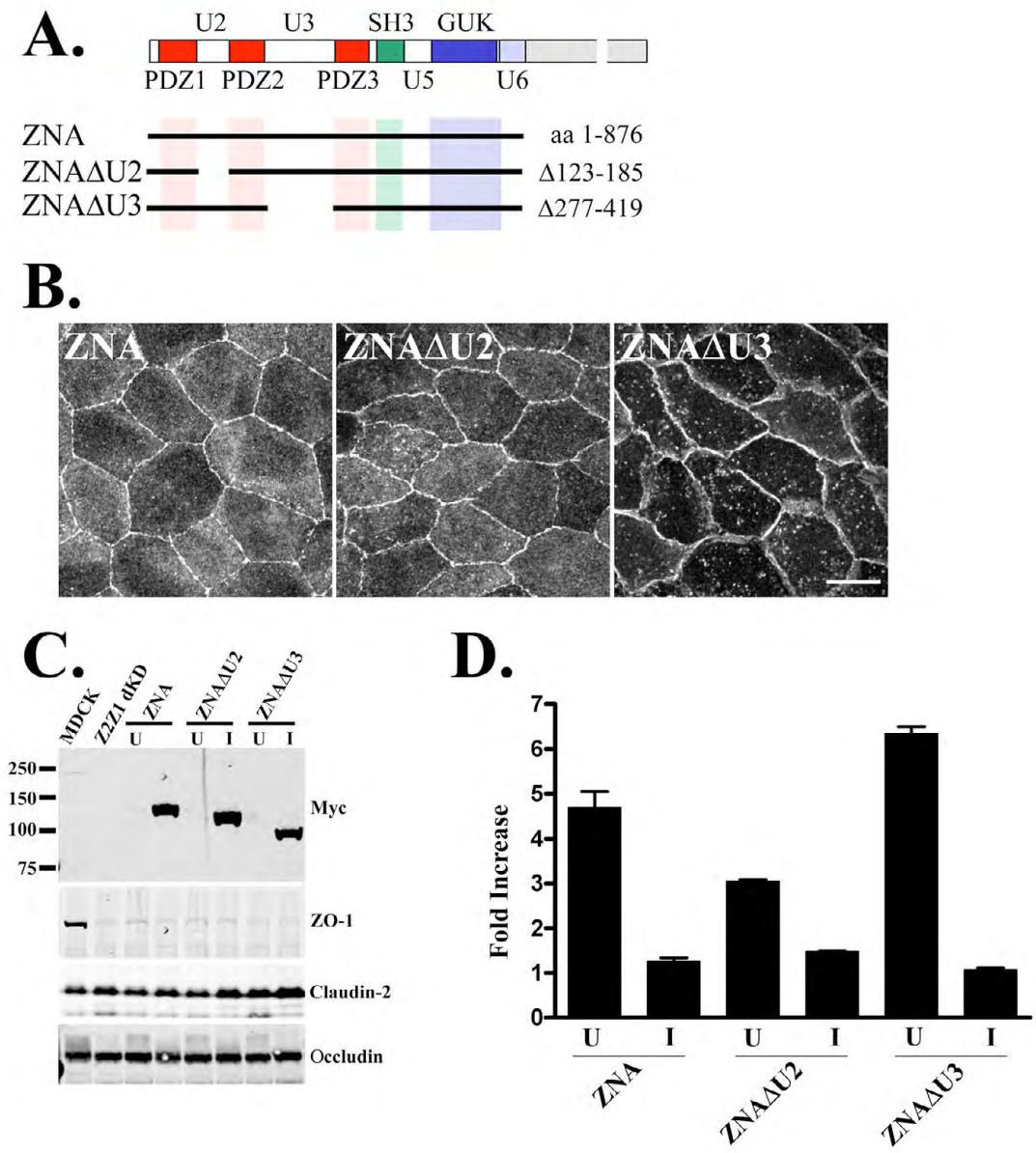

Fig. S6. The Unique 2 (U2) and U3 motifs are not required for TJ localization or normal paracellular permeability. (A) Schematic diagram of the ZNA ΔU2 and ΔU3 transgenes. All transgenes were tagged at the C-terminus with the c-myc epitope. (B) Western blot of transgene expression in control MDCK, Z2Z1 dKD, and uninduced (U) or induced (I) dKD cells stably transfected with the Tet-responsive ZNA ΔU2 and ΔU3 transgenes. The steady state levels of claudin-2 and occludin are not altered by transgene induction (bar: 10 μm). (C) Cell lines expressing ZNA, ZNA ΔU2 and ΔU3 were grown on filters, fixed, and stained with antibodies against the myc epitope tag. Images are 1.05 μm maximum density projections through the AJC . (D) Induction (I) of ZNA ΔU2 and ΔU3 can restore the flux of 3-kDa FITC-dextran to near normal levels relative to uninduced (U) or Z2Z1 dKD cells. The movement of dextran across filter-grown cells is shown as fold increase over that of control MDCK cells.

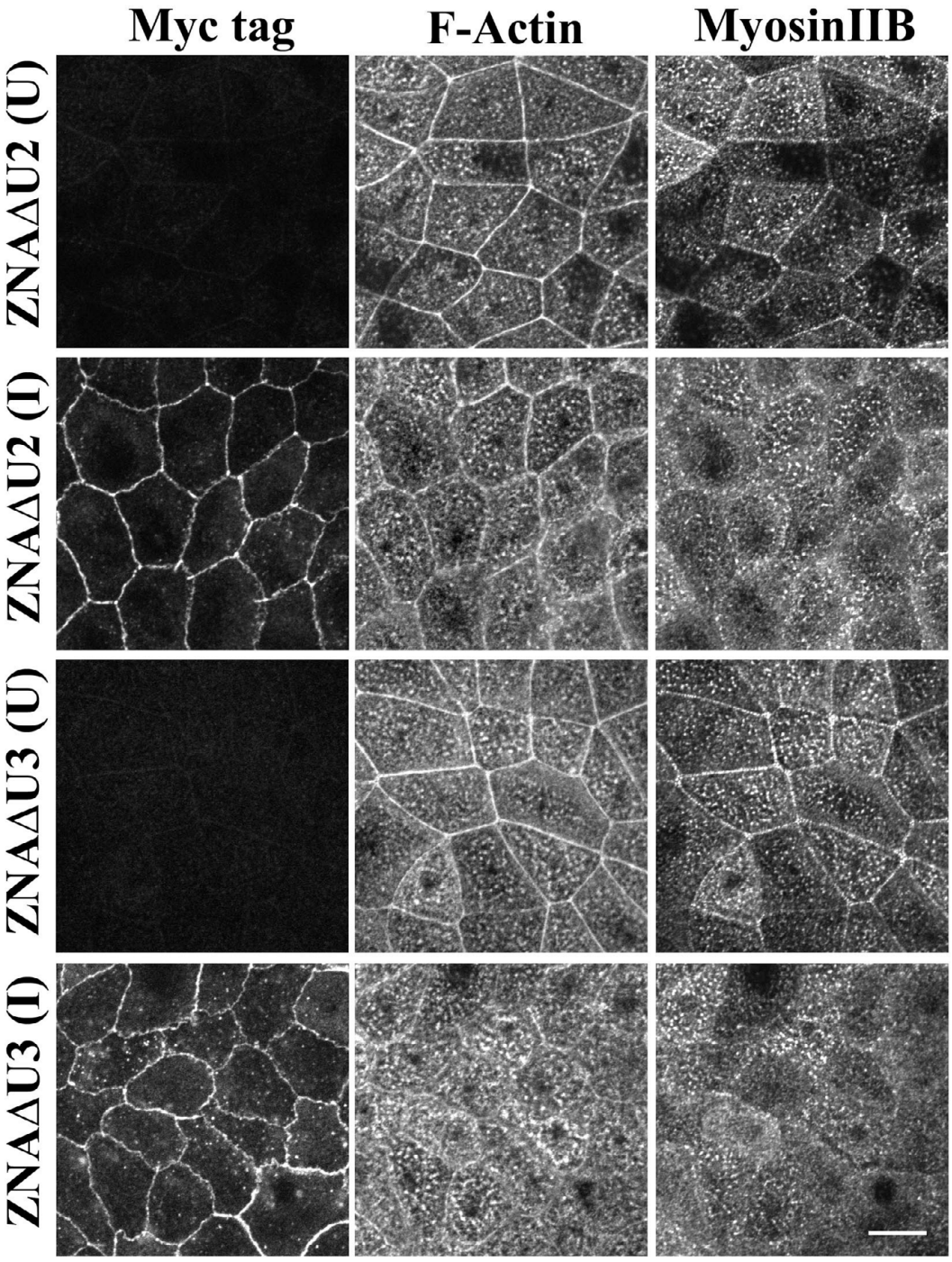

Fig. S7. The Unique 2 (U2) and U3 motifs are not required for normal actomyosin architecture of the AJC. MDCK, Z2Z1 or Z2Z1 cells transfected with the indicated transgene were grown on filter inserts for 7 days in presence (U=uninduced) or absence (I=Induced) of 1.0 μg/ml doxycycline, and subsequently fixed and stained with TRITC-phalloidin and antibodies against myc and myosin IIB. Images are 1.05 μm maximum density projections of the AJC (bar: 10 μm).

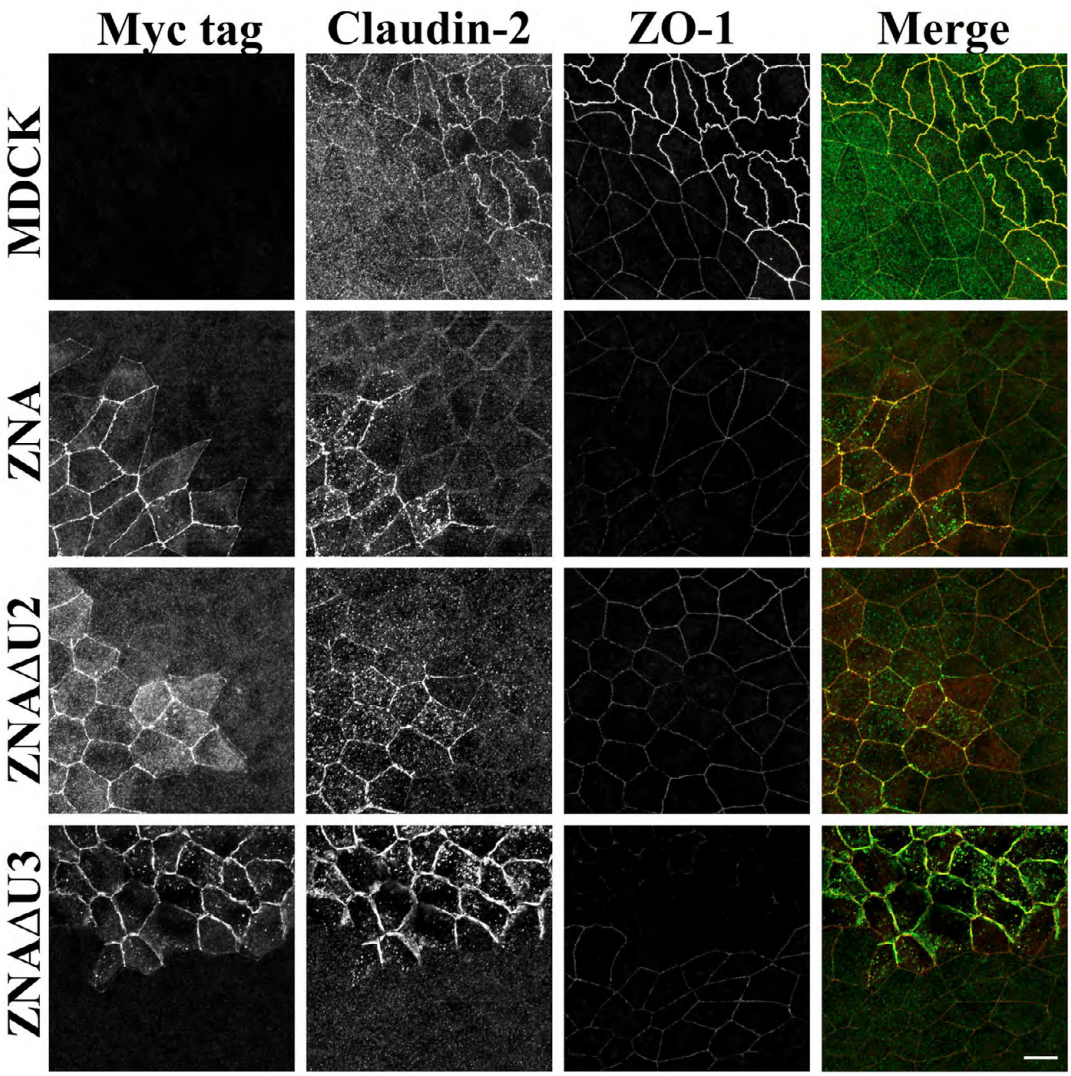

Fig. S8. The Unique 2 (U2) and U3 motifs are not required for localization of claudin-2 to cell-cell contacts. MDCK cells or Z2Z1dKD cells expressing ZNA, ZNAΔU2 or ΔU3 transgenes were mixed at a 1:5 ratio with ZO-depleted cells and grown on filter inserts for 7 days, fixed and stained with antibodies against myc, claudin-2, and the endogenous canine ZO-1. Note that claudin-2 localization to cell-cell contacts is greatly reduced in ZO-depleted Z2Z1 dKD cells relative to MDCK control cells or transgene-expressing cells on the same filter. Images are 1.75 μm maximum density projections (bar: 10 μm).