Figure S1

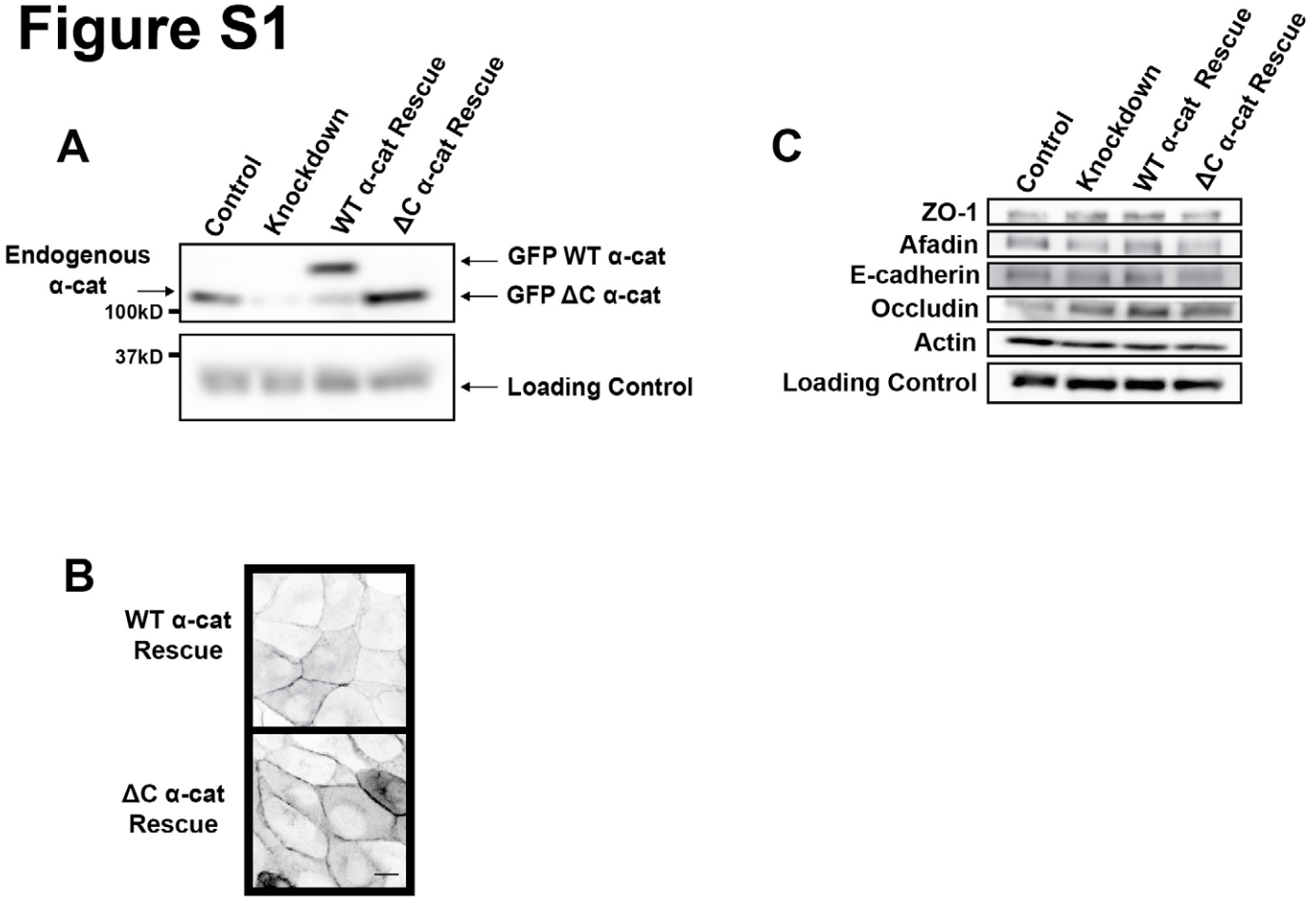

Fig. S1. GFP fused WT α-catenin or ΔC α-catenin are expressed in MDCKII cells and localize properly. (A) Expression levels of α-catenin using the knockdown/add-back approach. MDCKII cells were infected with GFP, GFP-tagged human α-catenin (WT α-cat Rescue) or a GFP-tagged mutant version of α-catenin lacking the C-terminal 209 amino acids (ΔC α-cat Rescue). These cells were infected a second time with a shRNA against canine α-catenin (Knockdown) or an empty vector (Control). Cells were lysed and expression of endogenous and exogenous α-catenin was analyzed via immunoblot using an antibody against α-catenin. p34-Arc serves as a loading control. (B) Loss of the α-catenin C-terminus does not affect α-catenin localization. WT α-cat Rescue and ΔC α-cat Rescue cells were grown to confluence, incubated in calcium free medium overnight, and was placed in normal calcium medium for 4 hours. The cells were then fixed, prepared for immunofluorescence, and analyzed using a Zeiss 510 confocal microscope. Images are shown in inverted grayscale. Bar=10 μm. (C) Expression of adherens junction and tight junction proteins is unaffected by knockdown and reexpression of α-catenin. The levels of ZO-1, occludin, afadin, E-cadherin, and actin were analyzed in Control, Knockdown, WT α-cat Rescue and ΔC α-cat Rescue cells. No significant changes were noted in the expression of these proteins.

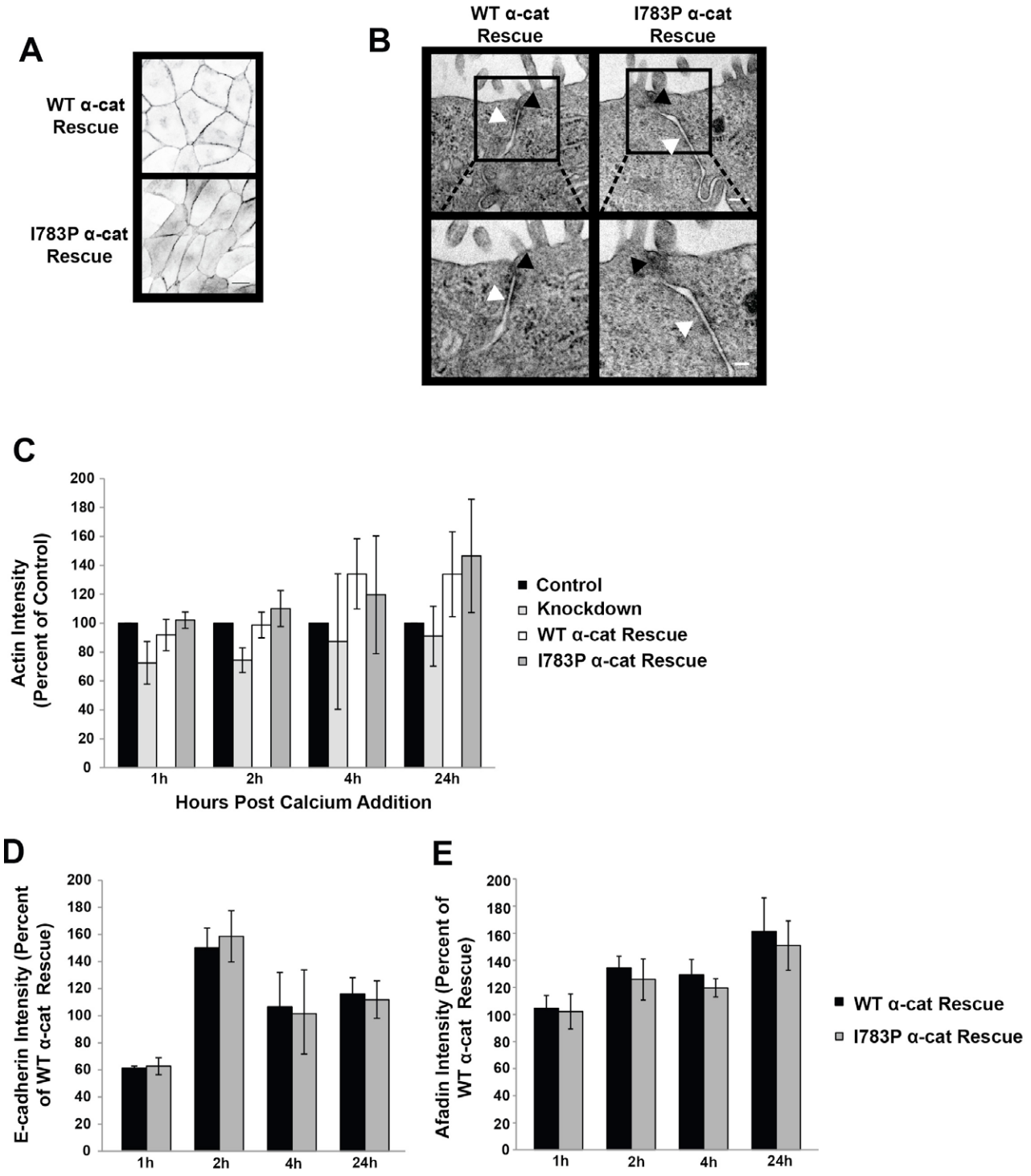

Fig. S2. Proline substitution at residue 783 does not affect localization of α-catenin. (A) α-Catenin I783P localizes to sites of cell-cell contact. WT α-cat Rescue and I783P α-cat Rescue cells were plated on glass coverslips and grown to confluence. GFP localization was examined as described in Figure S1B. Images are shown in inverted grayscale. Bar=10 μm. (B) I783P α-cat Rescue cells display no gross structural alterations. Cells were analyzed using TEM to visualize structure of tight junctions and adherens junctions 4 hours post calcium addition. The boxed areas within the top pictures are magnified and shown in the bottom micrographs. Top pictures: Bar = 0.2 μm, bottom pictures: Bar: 0.1μM. (C). Expression of I783P α-catenin does not affect localization of actin at cell-cell contacts. Control, Knockdown, WT α-cat Rescue and I783P α-cat Rescue cells were plated on glass coverslips, grown to confluence, underwent calcium switch, and stained for actin at the indicated times. Representative images are presented in Figure 4B. The intensity of staining at cell-cell contacts was measured using ImageJ and displayed as percent intensity compared to Control cells. (D). E-cadherin localization is unaffected by proline substitution at I783. E-cadherin localization at cell-cell contacts was analyzed as described in Figure 5B. Pixel intensity at cell-cell contacts was quantified using ImageJ and is presented as percent intensity compared to WT α-cat Rescue. (E). Afadin localization is unaltered in I783P α-cat Rescue cells. Afadin localization during junction assembly was examined as described in Figure 6B. Intensity of afadin staining at cell-cell contacts, as shown in Figure 6B, was quantified using ImageJ and is presented as percent intensity of WT α-cat Rescue.