Shahbazi et al., http://www.jcb.org/cgi/content/full/jcb.201306019/DC1

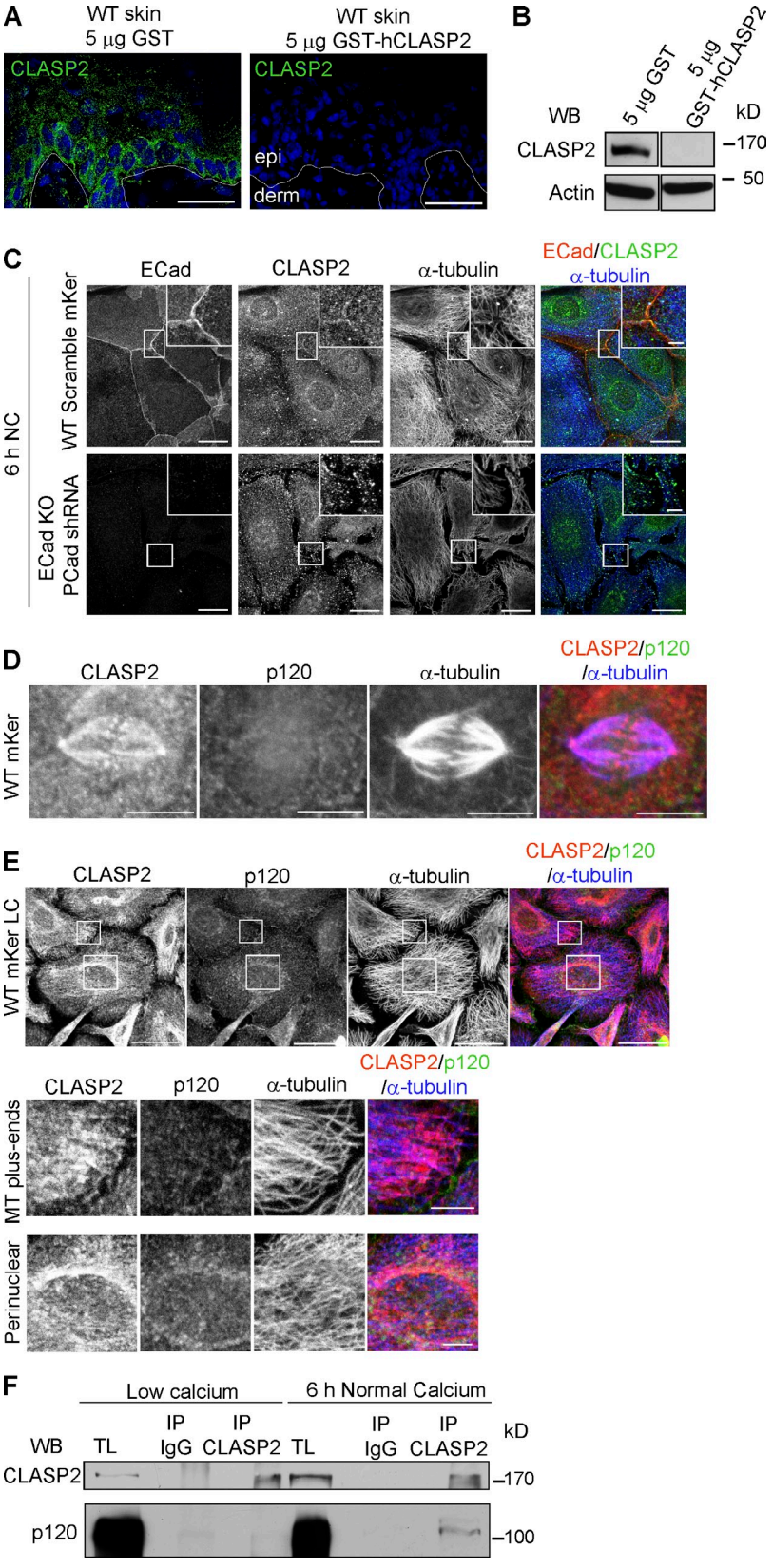


Figure S1. **Specificity of the CLASP2 association to junctional sites, validated in the absence of cadherins or under LC conditions.** (A) Skin sections from newborn WT mice were stained with the CLASP2 antibody in the presence of either 5 µg GST or 5 µg recombinant GST-hCLASP2. epi, epidermis; derm, dermis. (B) Peptide competition assay. Immunoblot for CLASP2 in mKer lysates using the CLASP2 antibody in the presence of either 5 µg GST or 5 µg recombinant GST-hCLASP2. (C) WT and ECad KO–PCad shRNA mKer immunostained for ECad, CLASP2, and α-tubulin in the presence of calcium. Insets are magnifications of the boxed regions. (D) WT mKer grown under LC conditions stained for CLASP2, p120, and α-tubulin. (E) WT mKer grown under LC conditions immunostained for CLASP2, p120, and α-tubulin. Insets corresponding to MT plus ends and the perinuclear region are shown. (F) Immunoprecipitation analysis of the interaction of CLASP2 with p120 under low calcium (LC) and normal calcium (NC) conditions. The immunoprecipitates (IP) were blotted for CLASP2 and p120. TL, total lysate; WB, Western blot. Bars: (A, C, main images, and E, main images) 25 µm; (C and E, insets) 5 µm; (D) 7.5 µm.

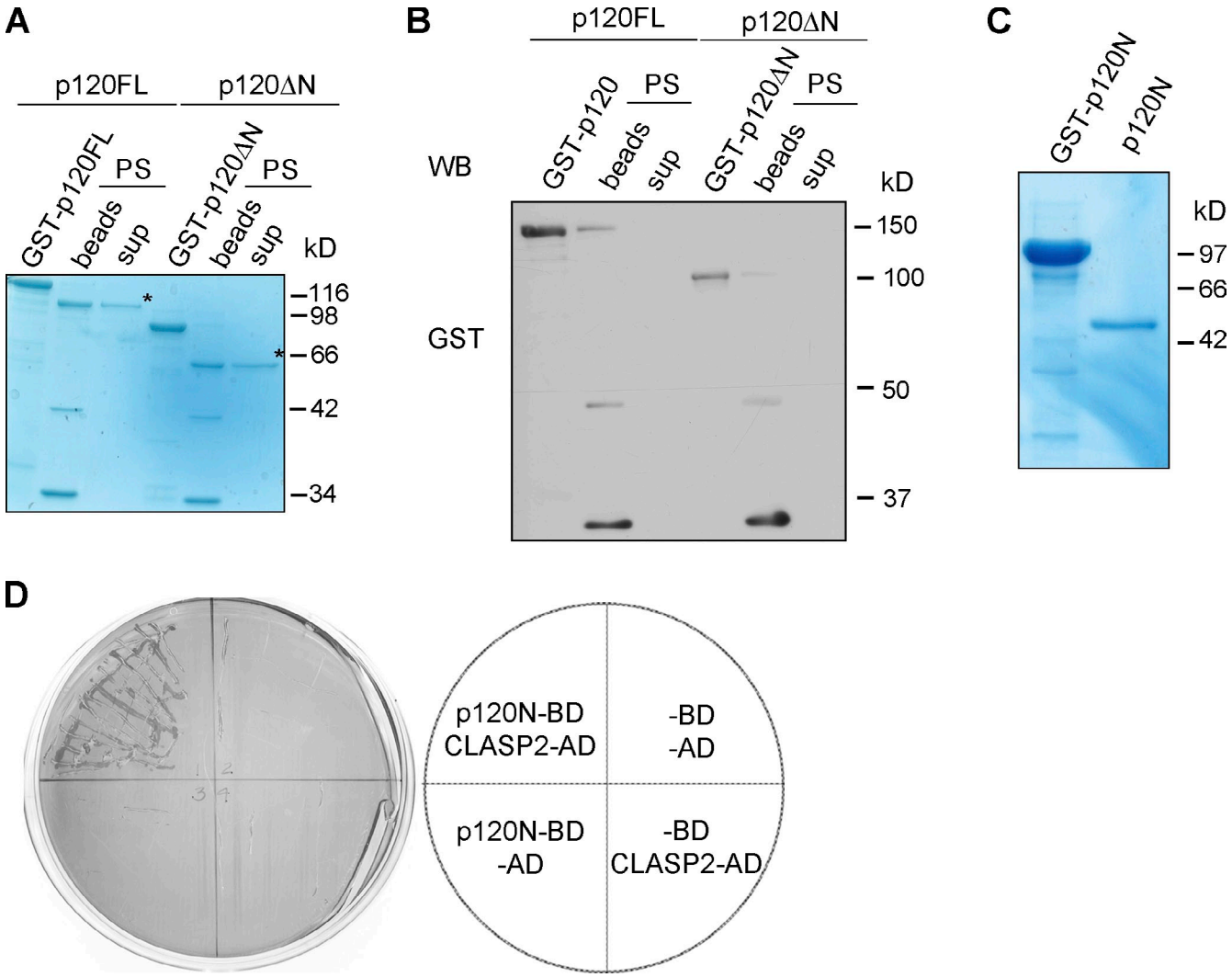


Figure S2. **Purification of recombinant GST-tagged p120 proteins and the interaction of the recombinant proteins CLASP2 and p120N in yeast.** (A) GST-p120FL and GST-p120ΔN expressed in ArticExpress bacteria were purified with glutathione–Sepharose beads after cleavage from GST with PreScission protease (PS). The untagged proteins were recovered in the supernatant (sup), and the GST tag remained associated to the beads. The constructs were run on a SDS-PAGE and stained with Coomassie. Asterisks point to p120FL and p120ΔN, respectively, after removal of the GST tag. (B) GST immunoblots of purified constructs from A. (C) Purified GST-p120N was digested with PreScission Protease, run on an SDS-PAGE, and stained with Coomassie. (D) Yeast expressing CLASP2–activation domain (AD) and p120–DNA binding domain (BD) recombinant proteins were grown in the absence of leucine and tryptophan to validate their interaction. WB, Western blot.

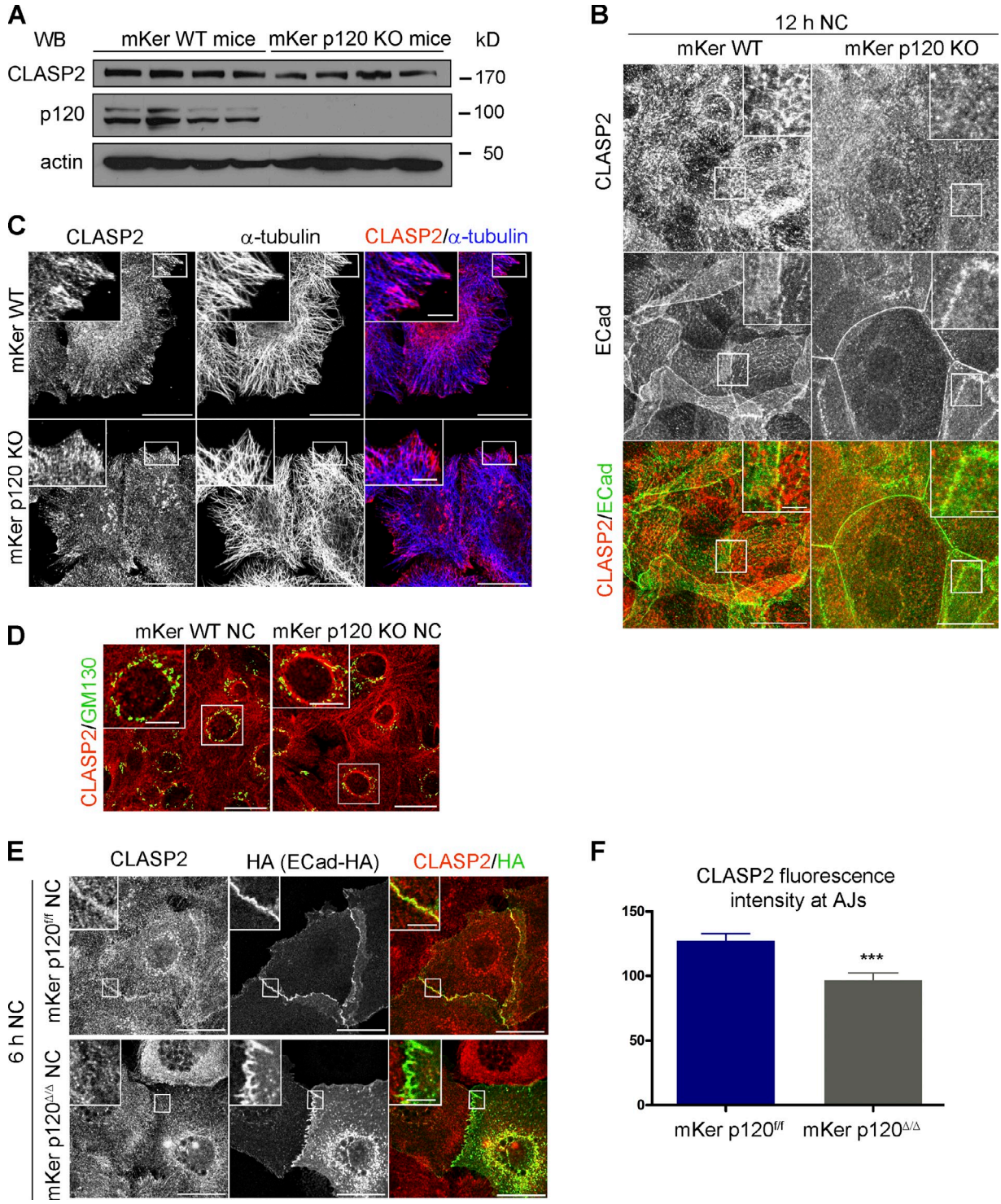


Figure S3. **p120 deficiency does not disrupt CLASP2 localization to MT plus ends but alters its recruitment to AJs at early time points of contact formation.** (A) Immunoblot showing the levels of CLASP2 in WT and p120 KO mKer directly isolated from the epidermis of newborn mice. A Western blot (WB) for p120 is shown as a control. (B) WT and p120 KO mKer were treated with calcium for 12 h and immunostained for ECad and CLASP2. (C) WT and p120-null mKer, grown in low confluency to allow the formation of small colonies, were subjected to a calcium switch and immunostained for CLASP2 and α-tubulin. (D) WT and p120 KO mKer stained for CLASP2 and GM130. (E) ECad-HA was overexpressed in control ($p120^{f/f}$) and p120-null ($p120^{\Delta/\Delta}$) mKer. Cells were switched to calcium-containing media for 6 h and immunostained for CLASP2 and HA. (F) Random individual plot profiles were generated in both $p120^{f/f}$ and $p120^{\Delta/\Delta}$ mKer. The point of maximum ECad-HA fluorescence intensity was identified and the CLASP2 fluorescence intensity associated to this value was quantified and normalized to the cytoplasmic CLASP2 fluorescence intensity. (n = 16 cells, 5 profiles per cell, 3 independent experiments). Data are normalized to control values and represented as means ± SEM; ***, $P < 0.0003$, Student's t test. Insets show magnifications of the boxed regions. Bars: (B–E, main images) 25 µm; (B–E, insets) 5 µm.

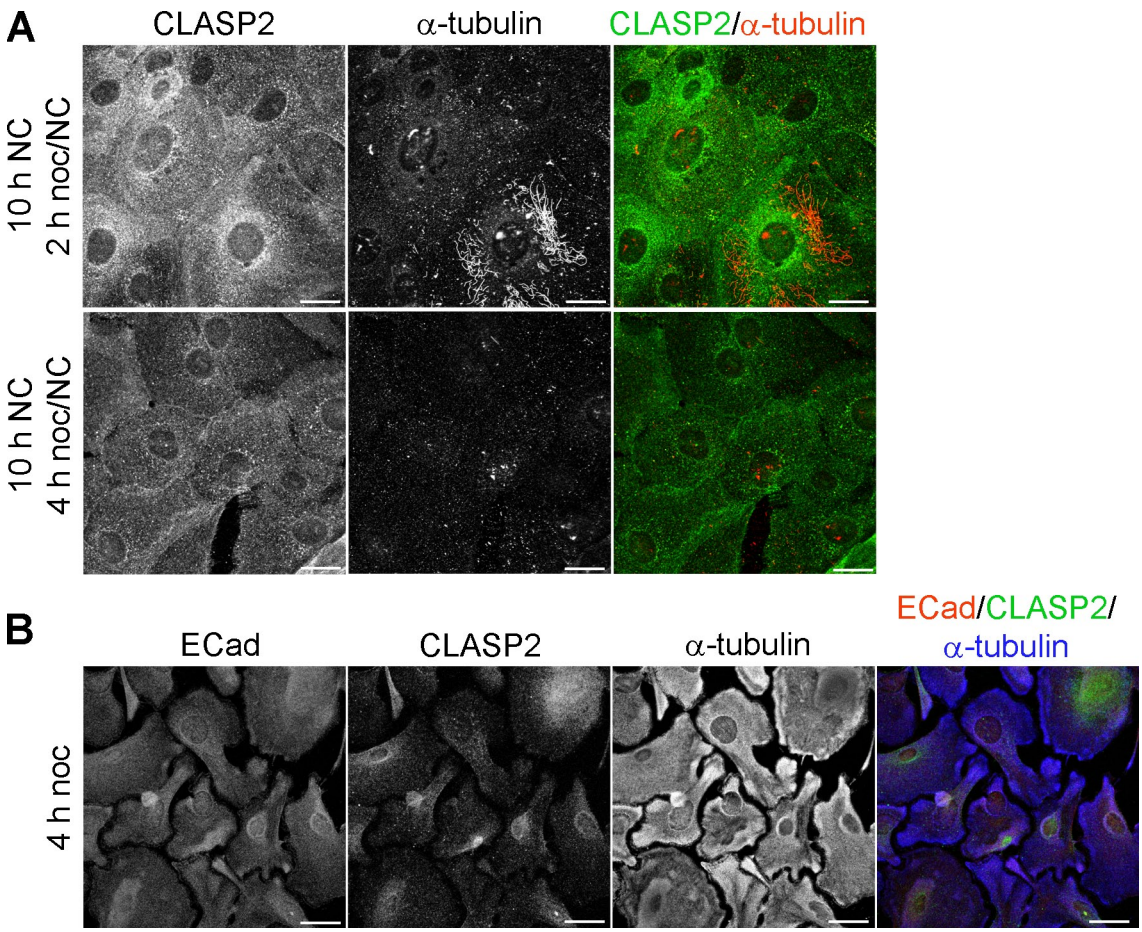


Figure S4. **Treatment of mKer with nocodazole does not induce AJ formation.** (A) mKer were treated with calcium followed by the addition of 30 µM nocodazole (noc) for either 2 or 4 h and extraction of monomeric tubulin with saponin. Cells were immunostained for CLASP2 and α-tubulin. (B) mKer were treated with 30 µM nocodazole in the absence of calcium and immunostained for ECad, CLASP2, and α-tubulin. Bars, 25 µm.

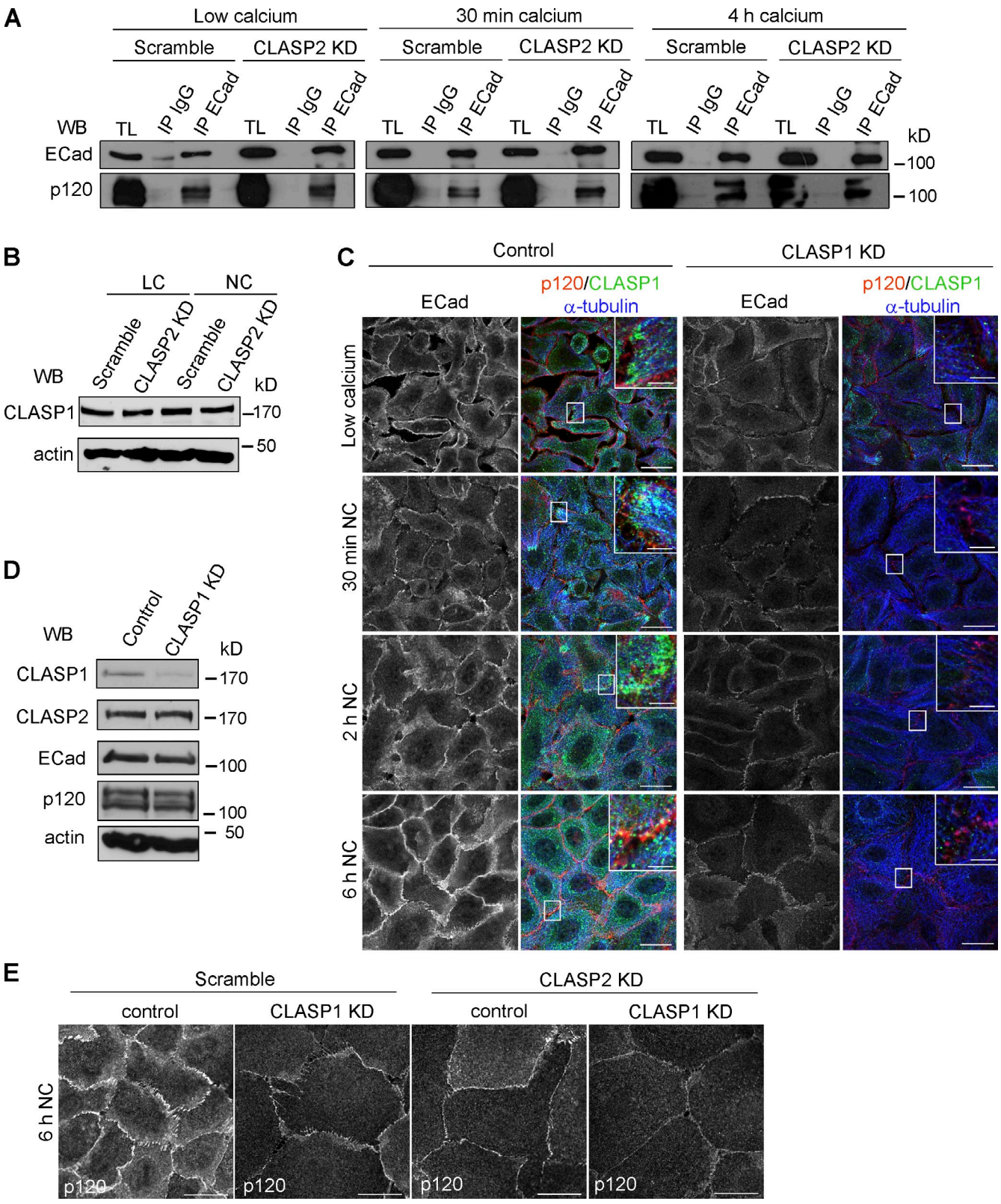
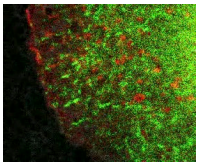


Figure S5. **Interaction of ECad and p120 in absence of CLASP2 and analysis of possible functional redundancy between CLASP2 and CLASP1.** (A) Scramble control and CLASP2-deficient (CLASP2 knockdown [KD]) mKer were immunoprecipitated for ECad at different time points of a calcium switch. The immunoprecipitates (IP) were blotted for ECad and p120. TL, total lysate. (B) Western blot (WB) showing the levels of CLASP1 after infection with the corresponding shRNA-expressing lentiviruses. (C) Control and CLASP1-deficient mKer subjected to a calcium-switch time course and stained for p120, CLASP1, and α-tubulin. Insets show magnifications of the boxed regions. (D) Western blot showing the levels of CLASP1, CLASP2, ECad, and p120 in control and CLASP1-deficient mKer. (E) ECad immunofluorescence analysis of CLASP1/2 double-deficient mKer with the corresponding controls. Bars: (C, main images, and E) 25 µm; (C, insets) 5 µm.



Video 1. **CLASP2 dynamics at cell–cell adhesion sites.** WT mKer expressing GFP-CLASP2 (green) and p120-cherry (red) were switched to a calcium-containing medium for 4–6 h. The dynamic behavior of both proteins was monitored at cell–cell adhesion sites using a laser-scanning confocal microscope (TCS-SP5 Acousto-Optical Beam Splitter; Leica). Frames were taken every 2 s for 1 min and 30 s.

Table S1. **FRAP analysis of p120-cherry in scramble control mKer and CLASP2-deficient (CLASP2 knockdown) mKer**

Parameters	Scramble	CLASP2 KD
Half-life (s)	8.674	7.523*
Mobile fraction	0.6545	0.3824***

Data were normalized to prebleach and postbleach values as described in the Materials and methods section. n = 13 cells, 4 independent experiments. KD, knockdown. ***, $P < 0.0001$; *, $P < 0.05$, Student's t test.