Nishimura et al., https://dx.doi.org/10.1083/jcb.201603107

A

MW	Protein fragments detected
>250	Filamin A, Filamin B, Spectrin-αII
200-250	Myosin9, Myosin14, Myosin10
150-200	Clathrin heavy chain
100-150	Myosin Ic, Na-K ATPase, E-cadherin
75-100	Actinin4, LRRC8C, Gelsorin, VCP1, α-catenin
50-75	α-1B tubulin, Coronin-1C
25-50	Tropomyosin α1, Tropomyosin β, Arp2/3 complex subunit 2
15-25	RhoA, RhoD, MLC-3, MRLC2, Rab25

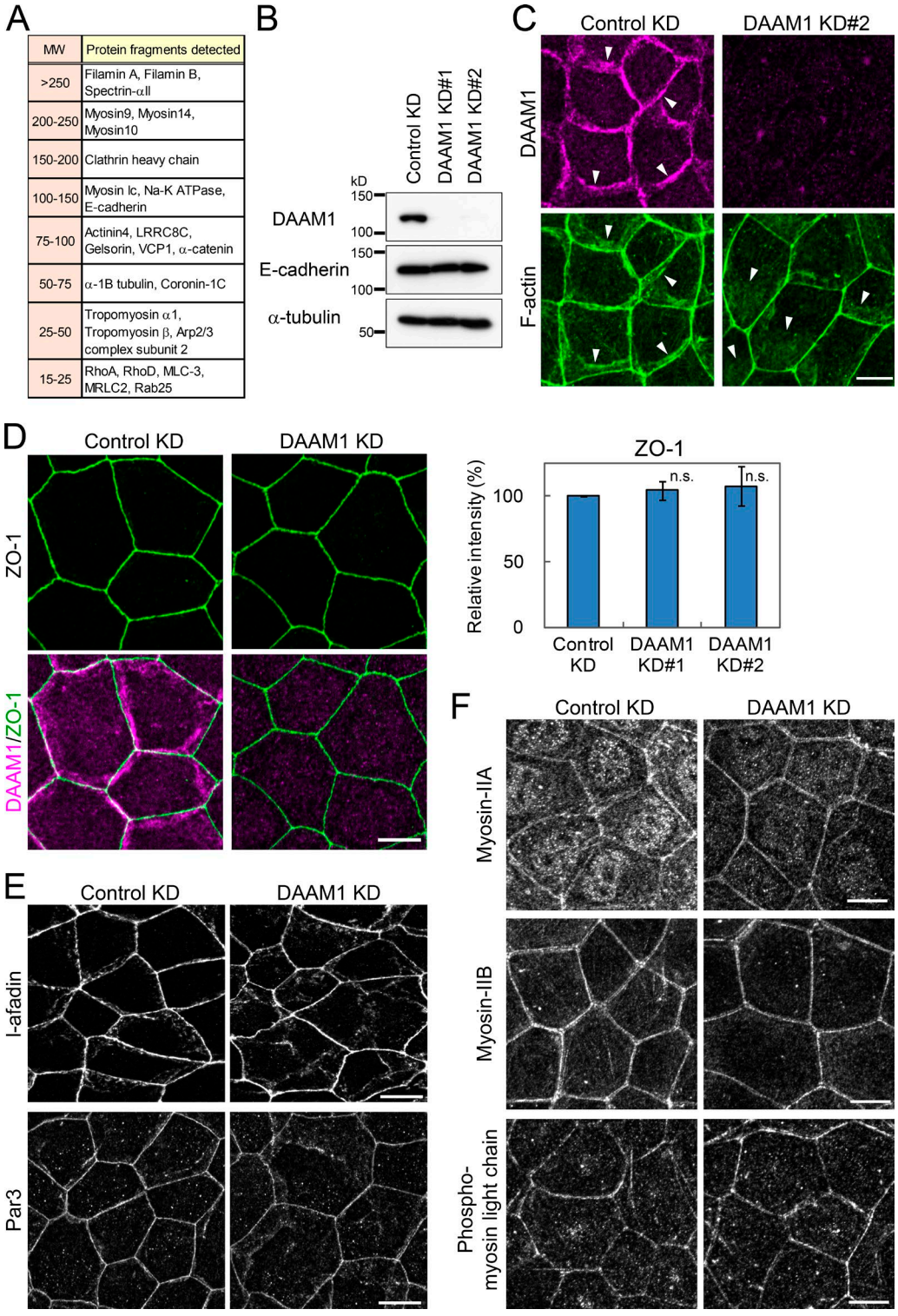


Figure S1. **Effects of DAAM1 depletion on the distribution of components of the AJC.** (A) Summary of protein fragments that interact with DAAM1-N, detected by mass spectrometry analysis. Lysates of EpH4 cells expressing HA-tagged DAAM1-N or parental EpH4 cells (control) were subjected to immunoprecipitation by anti-HA affinity gels. (B) Western blot analysis of DAAM1 and E-cadherin in EpH4 cells treated with two independent DAAM1-specific siRNAs. (C) Staining for DAAM and F-actin in EpH4 cells treated with DAAM1-specific siRNA#2. Arrowheads point to the basal edges of the junctions. (D–F) Immunostaining for ZO-1 (D), l-afadin and Par3 (E), and Myosin IIA, Myosin IIB, and phosphorylated myosin regulatory light chain (F) in control or DAAM1-depleted cells. Quantification of ZO-1 intensity is also shown in D. Error bars indicate SD. n.s., not significant. (C–F) Bars, 10 µm.

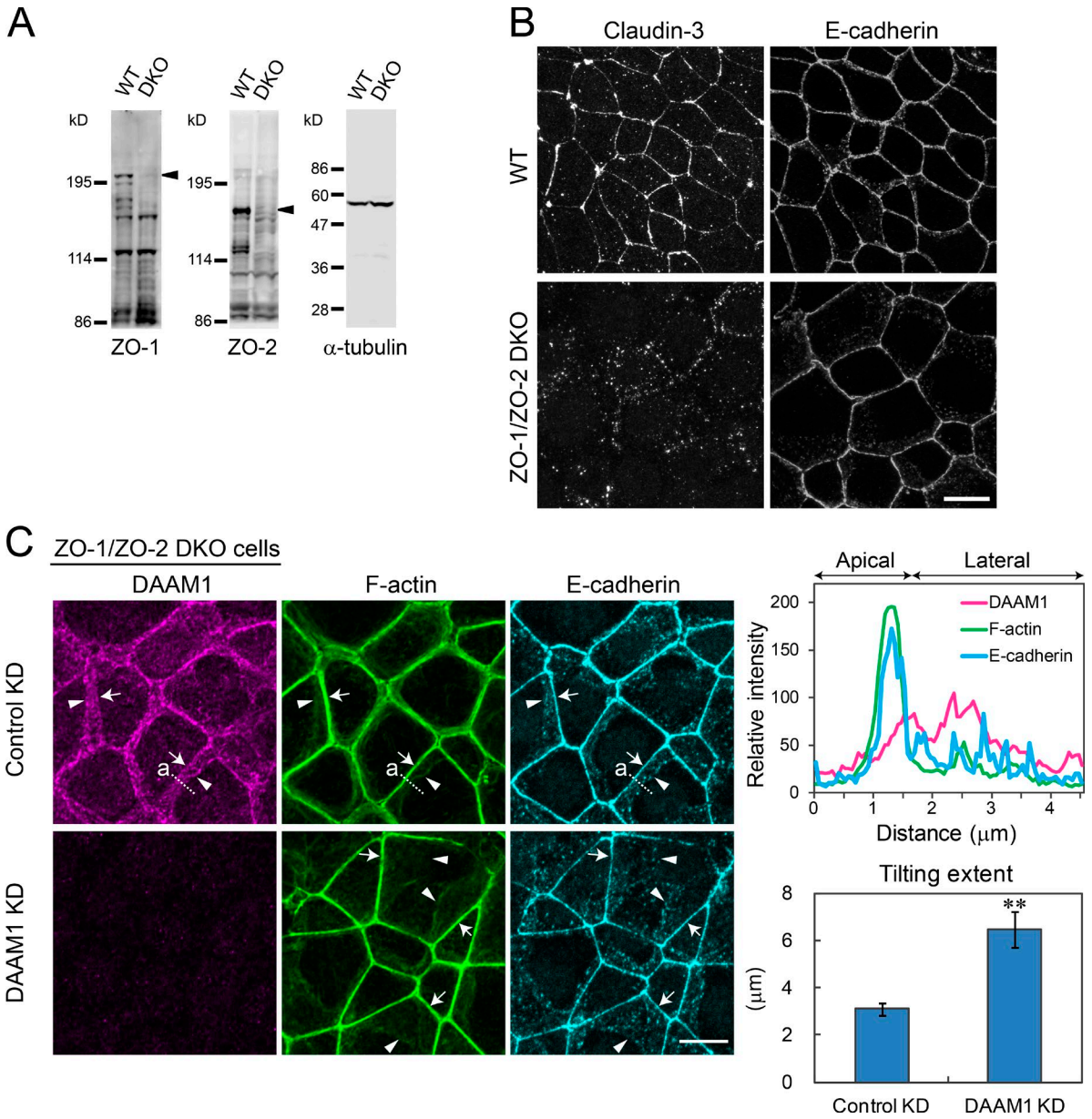


Figure S2. **Effect of DAAM1 KD on EpH4 cells without ZO-1 and ZO-2.** (A) Western blot analysis for ZO-1 and ZO-2 in cells whose genes were doubly removed (ZO-1/ZO-2 DKO cells) and in wild-type (WT) cells. (B) Immunostaining for Claudin-3 and E-cadherin in ZO-1/ZO-2 DKO cells. Claudin-3 became diffuse in the DKO cells. (C) Effect of DAAM1 depletion on the distribution of F-actin and E-cadherin in ZO-1/ZO-2 DKO cells. In ZO-1/ZO-2 DKO cells, the levels of F-actin and E-cadherin at the AJC increased relative to those in LCs (top, compare the images with those in Fig. 1 A). Nevertheless, DAAM1 levels at the AJC did not proportionally increase, as shown by densitometric traces along the dotted line (a), which start from the apical side (top, right). After DAAM1 depletion, the lateral E-cadherin signals significantly expanded (bottom), as shown by histograms (bottom, right). Arrows and arrowheads indicate the apical and basal edge, respectively, of cell junctions. Error bars indicate SD. **, $P < 0.01$. (B and C) Bars, 10 μm.

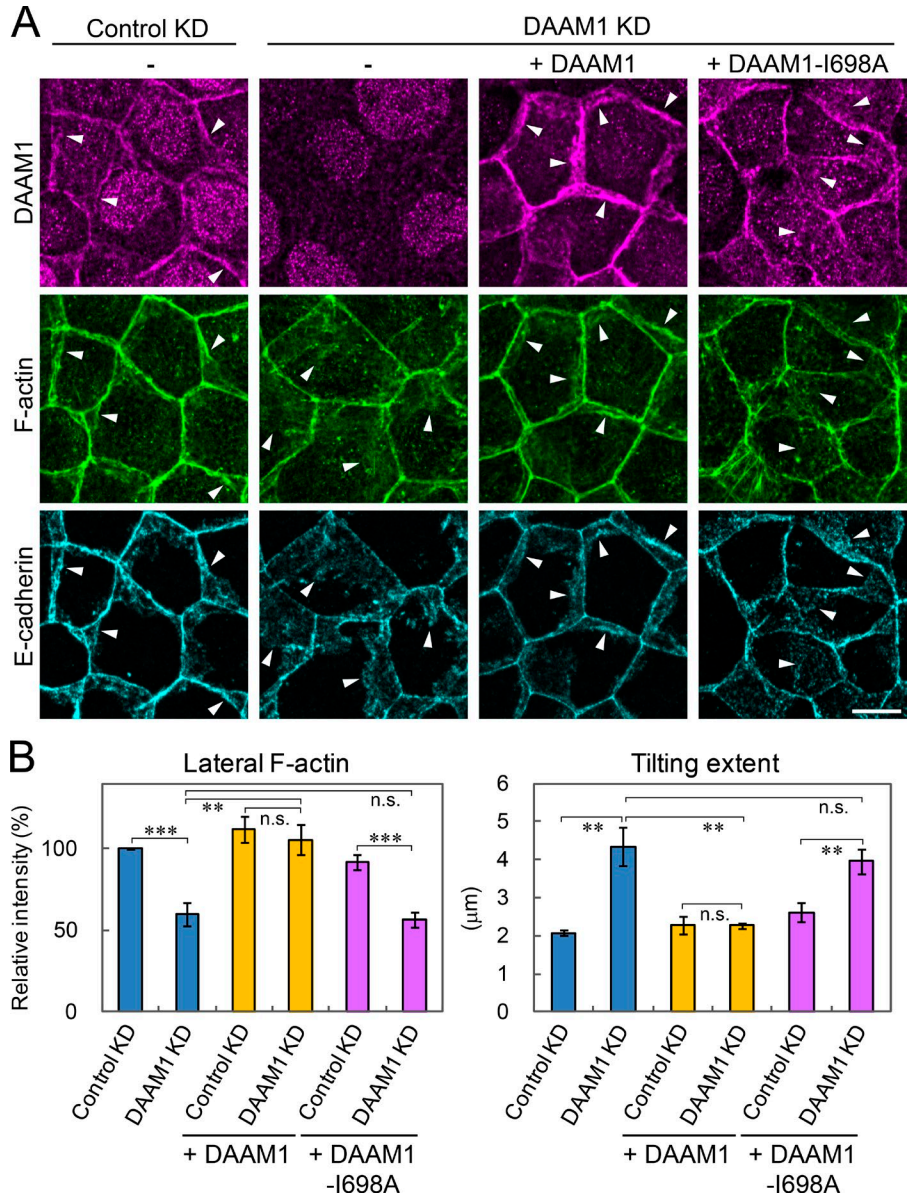


Figure S3. **DAAM1 mutant that is unable to polymerize actin cannot rescue the DAAM1 depletion phenotypes.** (A) DAAM1 was depleted in EpH4 cells, EpH4 cells expressing an siRNA-resistant DAAM1 (+DAAM1), and EpH4 cells expressing a siRNA-resistant DAAM1 carrying the I698A mutation (+DAAM1-1698A), followed by immunostaining for E-cadherin and F-actin. The diffused lateral F-actin and E-cadherin of DAAM1 KD cells were rescued by siRNA-resistant DAAM1, but not by its actin polymerization–deficient mutant (I698A). Nuclear signals are caused by nonspecific reaction of the primary antibody. Arrowheads point to the basal edges of the junctions. Bar, 10 μm. (B) Quantification of F-actin intensity and tilting extent of LCs in the experiments shown in A. Histograms represent the mean of three experiments. Error bars indicate SD. **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant.

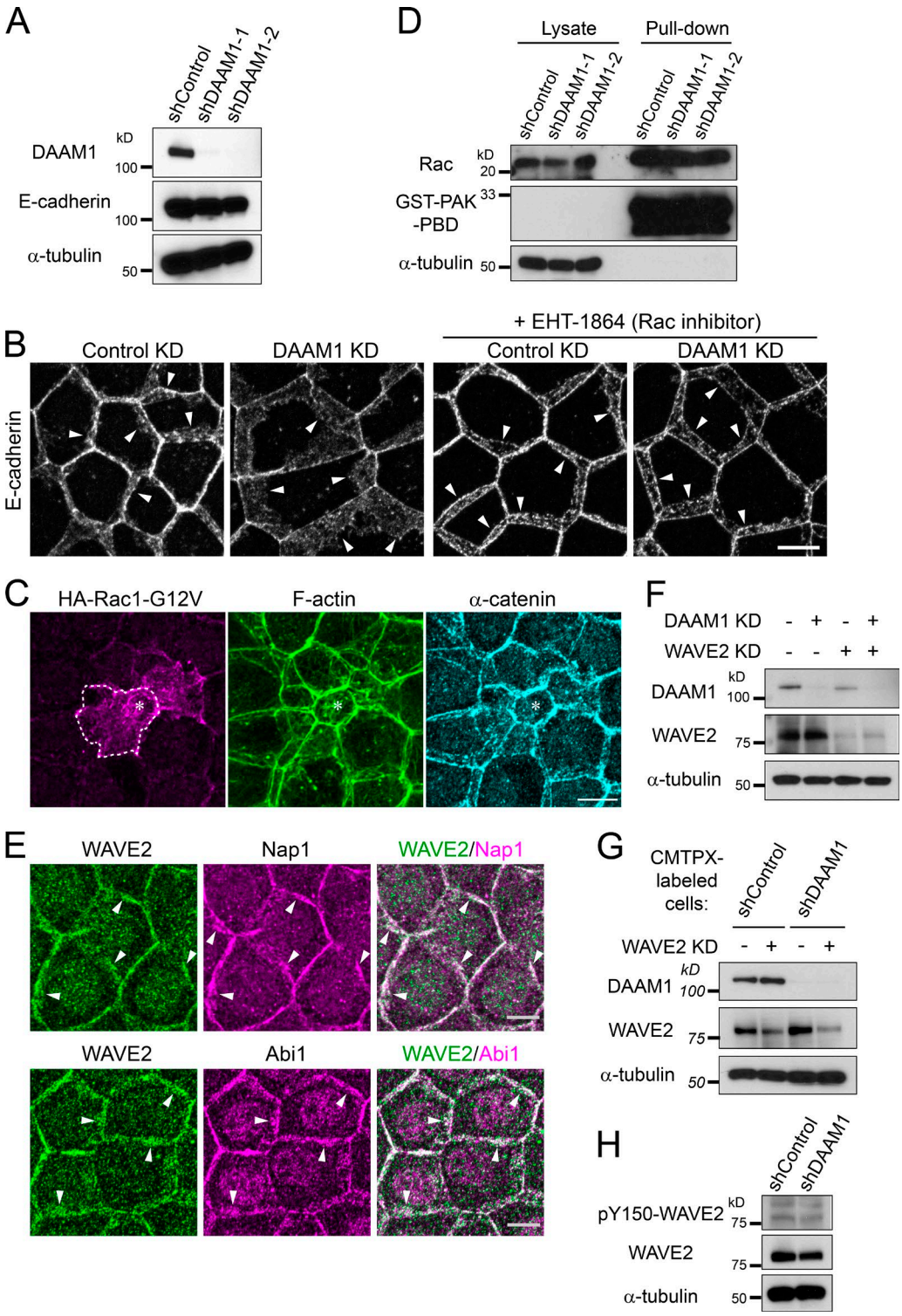


Figure S4. **Depletion of DAAM1 with shRNAs and analysis of WAVE complex components.** (A) Stable KD of DAAM1 in EpH4 cells. EpH4 cells were transfected with control or DAAM1-specific shRNA plasmids; and stable transfectant lines (shControl, shDAAM1-1, and shDAAM-2) were isolated. Western blotting for DAAM1, E-cadherin, and α-tubulin are shown. (B) EpH4 cells, which were transfected with control or DAAM1 siRNA, were treated with 100 µM EHT-1864 (Rac inhibitor) in DMSO or DMSO only (0.1% in final concentration) for 30 min and immunostained for E-cadherin. (C) Effect of expression of a constitutively active Rac1 (Rac1-G12V) on cell junctions. A cell marked with an asterisk at its apical portion expressed Rac1-G12V and extended its lateral edges, which are outlined by a dashed line, under adjacent cells. (D) Rac1 activation level did not change between shControl and shDAAM1 lines, as assessed by the pull-down assay using GST-PAK-PBD. (E) Co-immunostaining for WAVE2 with Nap1 or Abi1. WAVE2 codistributed with Nap1 and Abi1 at LCs. (B, C, and E) Arrowheads point to the basal edges of the junctions. Bars, 10 µm. (F) Western blots for DAAM1 and WAVE2 in EpH4 cells treated with siRNAs specific for DAAM1 and/or WAVE2. (G) shControl and shDAAM1 cell lines, transfected with WAVE2-specific or control siRNA for 1 d and then labeled with CMTPX, were cultured for further 2 d and subjected to Western blotting. (H) Western blots for phospho-Y150 WAVE2 and WAVE2 in the lysates of shControl and shDAAM1 cells.

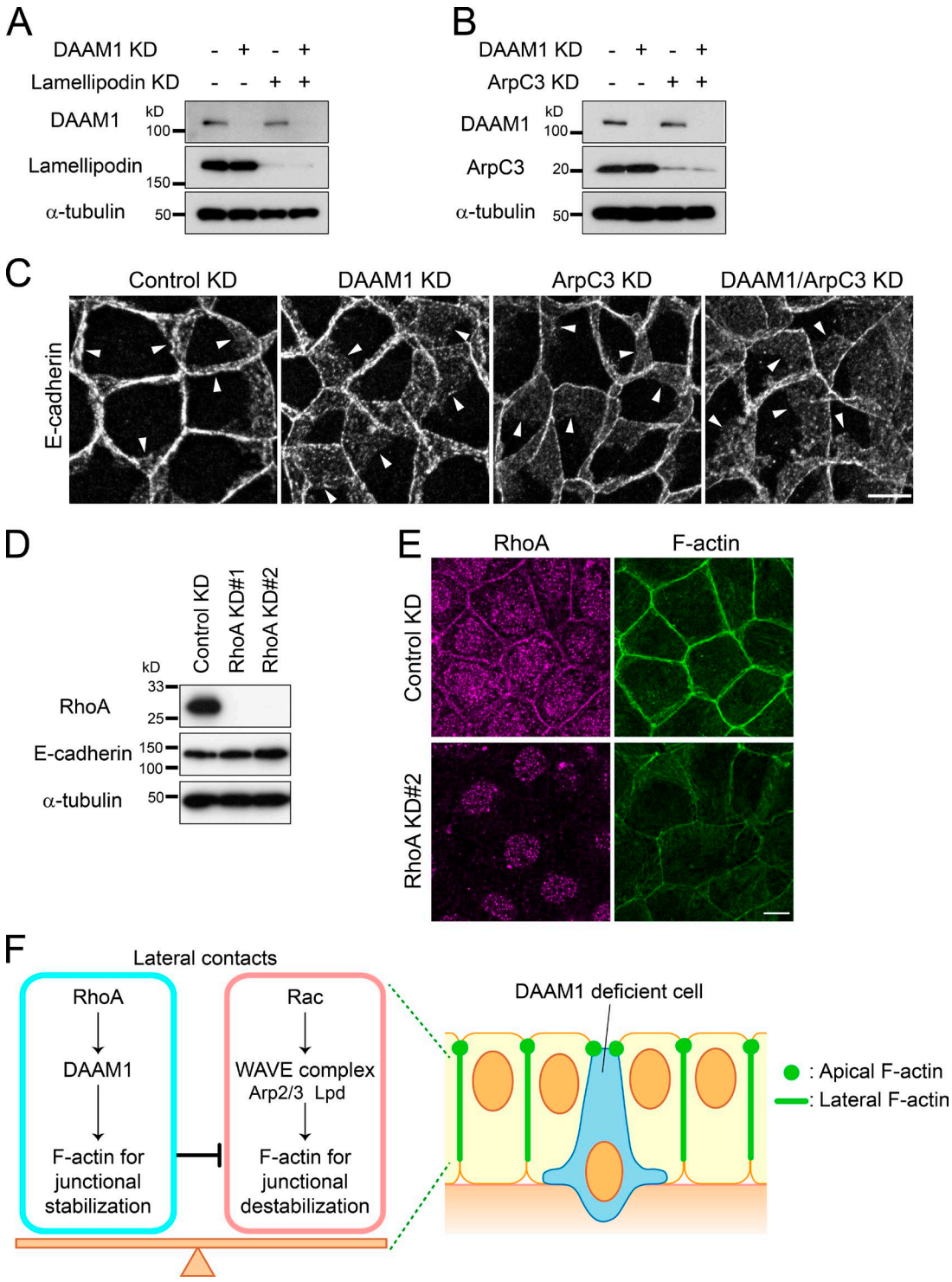
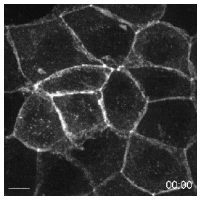
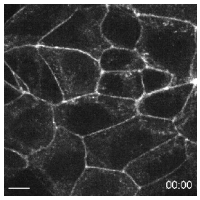


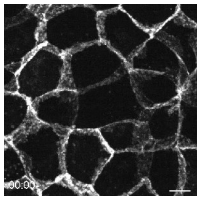
Figure S5. **Analyses of lamellipodin, ArpC3, and RhoA depletion.** (A) Western blots for DAAM1 and lamellipodin in EpH4 cells treated with siRNAs specific for DAAM1 and/or lamellipodin. (B) Western blots for DAAM1 and ArpC3 in EpH4 cells treated with siRNAs specific for DAAM1 and/or ArpC3. (C) Immunostaining for E-cadherin in EpH4 cells treated as explained in B. Note that ArpC3 depletion affected E-cadherin distribution in control cells and did not rescue the diffused distribution of E-cadherin caused by DAAM1 depletion. Arrowheads point to the basal edges of the junctions. (D) Western blots for RhoA and E-cadherin in EpH4 cells treated with two independent siRNAs (#1 and #2) specific for RhoA. (E) Immunostaining for RhoA in EpH4 cells treated with RhoA siRNA#2. (C and E) Bars, 10 µm. (F) Schematic summary of the lateral contact regulation by DAAM1 and WRC.



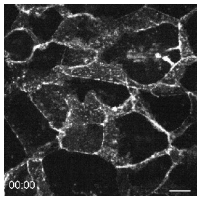
Video 1. **F-actin in control EpH4 cells.** EpH4 cells stably expressing Lifeact-EGFP were transfected with control siRNA for 3 d and imaged by a spinning-disc laser confocal microscope (IX71; Olympus) equipped with CSU-X1 (Yokogawa Electric Corporation) and an UplanSApo 60×/1.35 oil lens (Olympus). Images were taken every 2 min for 36 min with Z-stacks of 0.25-µm thickness. Pictures at each time point were Z-projected. Frame rate of the video is 6 frames/s. Bar, 10 µm. Related to Fig. 3 C.



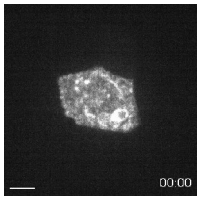
Video 2. **F-actin in DAAM1-depleted EpH4 cells.** EpH4 cells stably expressing Lifeact-EGFP were transfected with DAAM1 siRNA and imaged. Images were collected as explained in the legend for Video 1. Note that lateral F-actin diffuses or is fragmented without loss of apical F-actin. Bar, 10 µm. Related to Fig. 3 C.



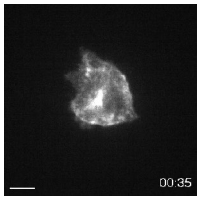
Video 3. **E-cadherin in control-depleted EpH4 cells.** EpH4 cells stably expressing E-cadherin–EGFP were transfected with control siRNA and imaged. Images were collected as explained in the legend for Video 1. Bar, 10 µm. Related to Fig. 3 D.



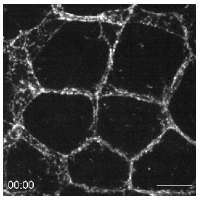
Video 4. **E-cadherin in DAAM1-depleted EpH4 cells.** EpH4 cells stably expressing E-cadherin–EGFP were transfected with DAAM1 siRNA and imaged. Images were collected as explained in the legend for Video 1. Note the deformed edges of lateral contacts, which actively move. Bar, 10 µm. Related to Fig. 3 D.



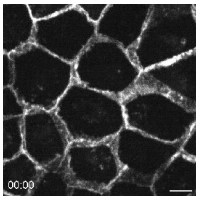
Video 5. **Movement of cell membranes in control EpH4 cells.** shControl EpH4 cells, which were transfected with membrane-EGFP. See Fig. 6 A legend for culture conditions. Images were taken by spinning-disc laser confocal microscope (IX71; Olympus) equipped with CSU-X1 (Yokogawa Electric Corporation) and LUCPlanFLN a 60×/0.70 lens (Olympus). Images were collected every 5 min for 40 min with Z-stacks of 1-µm thick. Pictures at each time point were Z-projected. Frame rate of the video is 4 frames/s. Bar, 10 µm.



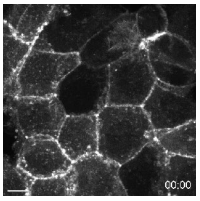
Video 6. **Movement of cell membranes in DAAM1-depleted EpH4 cells.** shDAAM1 EpH4 cells, which were transfected with membrane-EGFP. See Fig. 6 A legend for culture conditions. Images were collected as explained in the legend for Video 5, except that the total time was 65 min. Bar, 10 µm.



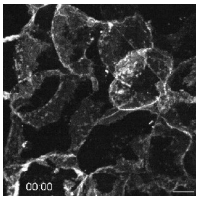
Video 7. **E-cadherin in DAAM1-depleted EpH4 cells treated with a Rac inhibitor.** EpH4 cells stably expressing E-cadherin–EGFP were transfected with DAAM1 siRNA, treated with 100 µM EHT-1864 for 30 min, and then subjected to time-lapse imaging. Images were collected as explained in the legend for Video 1, except that the total time was 40 min. Bar, 10 µm. Related to Fig. S4 B.



Video 8. **E-cadherin in EpH4 cells simultaneously transfected with DAAM1 and WAVE2 siRNAs.** EpH4 cells stably expressing E-cadherin–EGFP were cotransfected with DAAM1 and WAVE2 siRNAs and subjected to time-lapse imaging. Images were collected as explained in the legend for Video 1, except that the total time was 30 min. Bar, 10 µm. Related to Fig. 7 B.



Video 9. **F-actin in RhoA-depleted EpH4 cells.** EpH4 cells stably expressing Lifeact-EGFP were transfected with RhoA siRNA and imaged. Images were collected as explained in the legend for Video 1. Note that the distribution of both apical and lateral F-actin was affected. Bar, 10 µm. Related to Fig. 9 E.



Video 10. **E-cadherin in RhoA-depleted EpH4 cells.** EpH4 cells stably expressing E-cadherin–EGFP were transfected with RhoA siRNA and imaged. Images were collected as explained in the legend for Video 1. Note the abnormalities similar to but more severe than those observed in DAAM1-depleted cells. Bar, 10 µm. Related to Fig. 9 B.