Plutoni et al., http://www.jcb.org/cgi/content/full/jcb.201505105/DC1

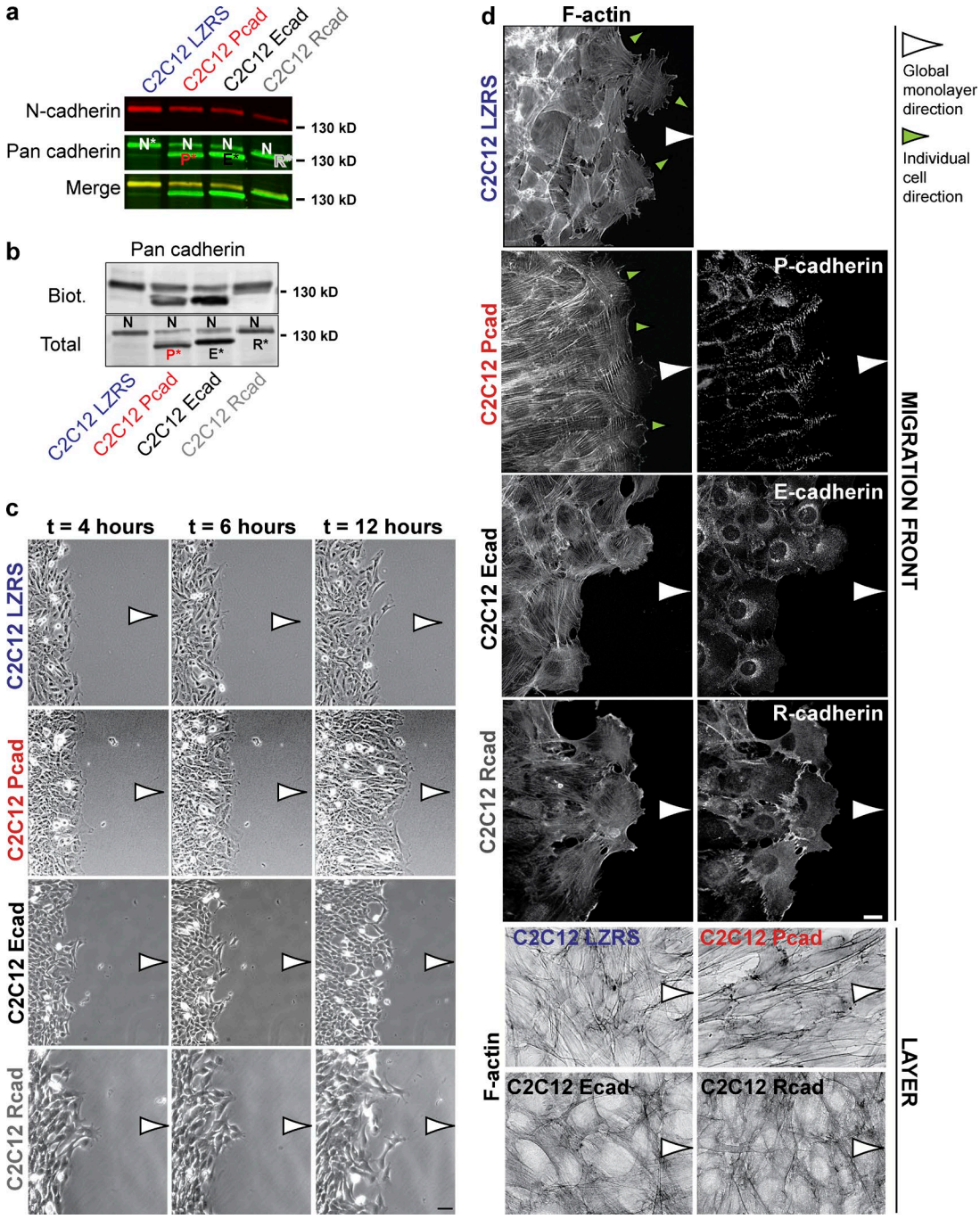


Figure S1. **Cell morphology during migration.** (a) Protein extracts (20 µg/well) from the indicated cell lines were immunoblotted using anti–N-cadherin (green) or anti–pan-cadherin (red) antibodies. The asterisks indicate the main endogenous (N in C2C12 LZRS cells) or ectopically expressed (P, E, and R) cadherin in each cell line. Ncad in C2C12 LZRS cells, Pcad in C2C12 Pcad cells, Ecad in C2C12 Ecad cells, and Rcad in C2C12 Rcad cells were expressed at the same level. (b) Cadherin content in total and biotinylated (Biot.) fractions analyzed by Western blotting. (c) Phase-contrast images at the indicated time after removal of the insert. Bar, 50 µm. (d) Images of F-actin and P-, E-, and R-cadherin staining 8 h after removal of the insert, illustrating the organization of the cells at the migration front and inside the layer (inverted contrast image). Bar, 15 µm.

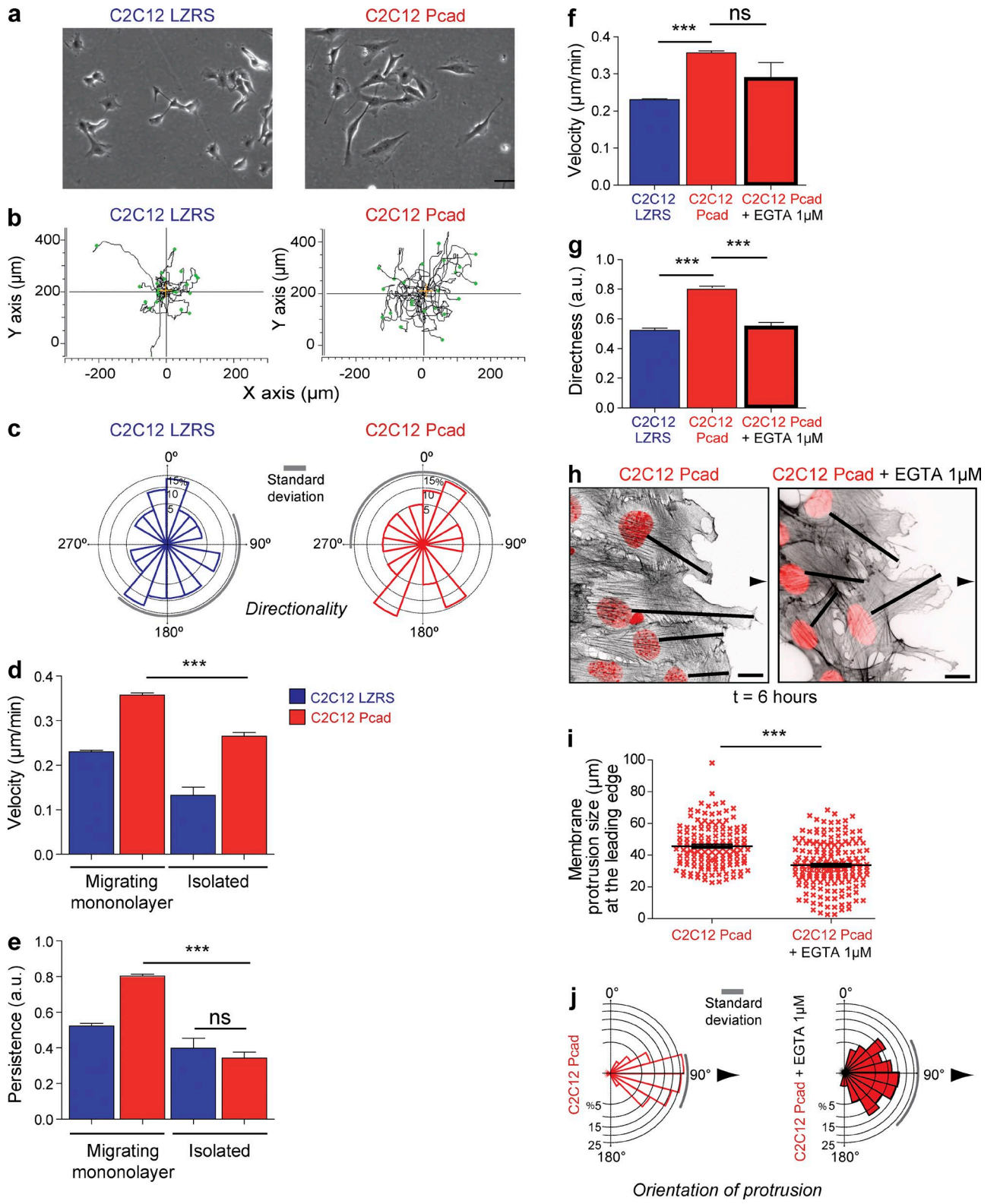


Figure S2. **P-cadherin–induced CCM requires cell–cell interactions.** (a–c) Phase-contrast images of isolated cells (a), trajectories covered over 24 h for 22 representative cells (b), and rose plots of the angle trajectories (i.e., directionality; c). Bar, 40 µm. (d–g) Velocity and persistence from 4 to 24 h in isolated cells (d and e) and contacting cells treated or not with EGTA (f and g). (h–j) F-actin staining and nuclei visualization indicate that the formation and orientation of the large protrusion in P-cadherin–expressing cells is perturbed after incubation with EGTA. 384 (C2C12 LZRS), 373 (C2C12 Pcad), 115 (C2C12 Ecad), and 263 (C2C12Rcad) cells from four independent experiments were analyzed. Bar, 10 µm. $n = 80$ for each point. ***, $P < 0.001$; ns, nonsignificant; a.u., arbitrary units.

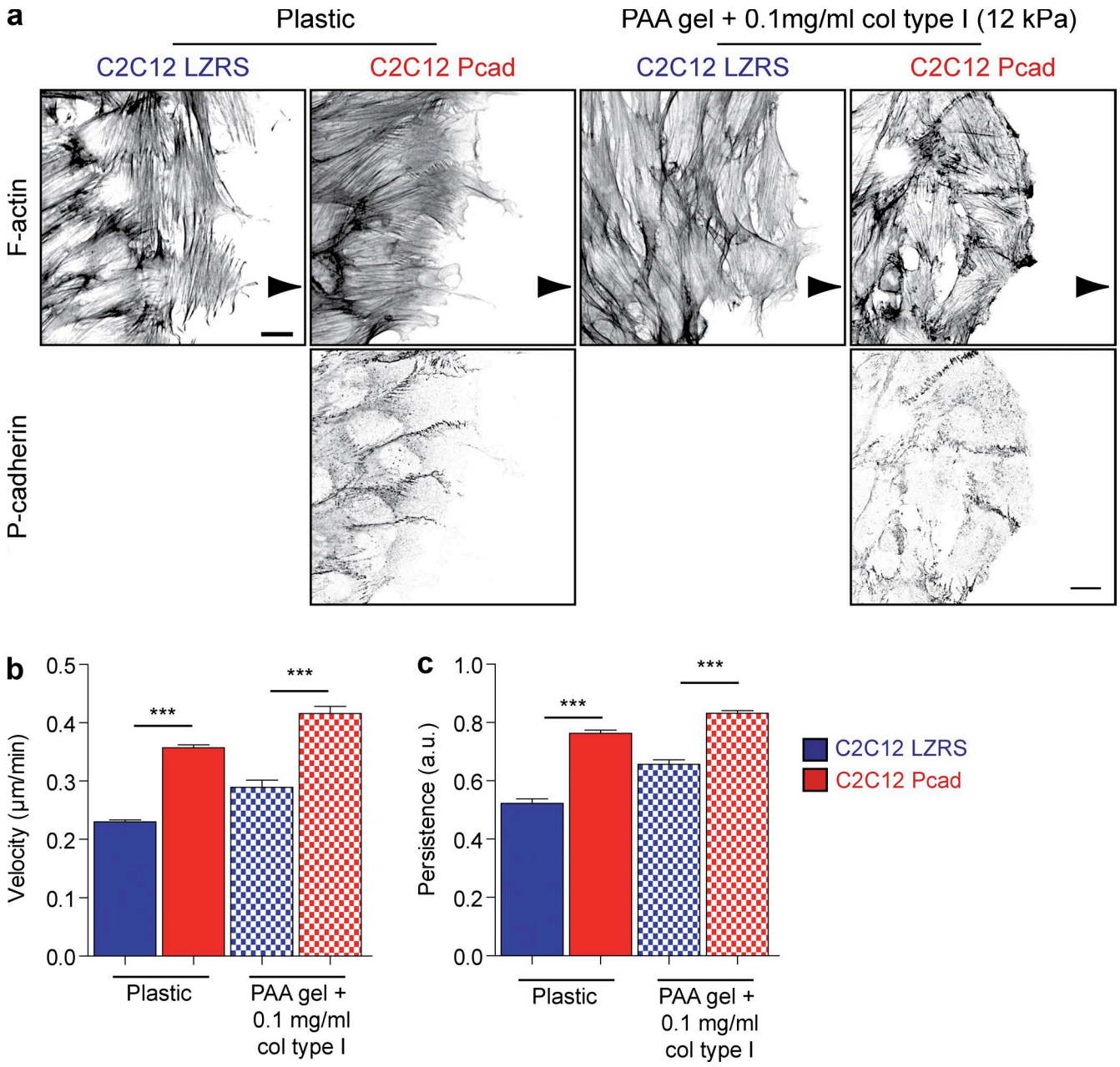


Figure S3. **Morphology and migration comparison on plastic versus polyacrylamide gels.** (a) Morphology of cells cultured on a rigid (plastic) substrate and softer (12 kPa) collagen-coated polyacrylamide (PAA) gels. Shown are inverted contrast images of F-actin and P-cadherin staining of migrating cells at 8 h after insert removal. Bar, 10 µm. (b and c) Velocity and persistence of cells from 4 to 15 h after removal of the insert or the pencil. Shown is the mean ± SEM: ***, $P < 0.0005$. col, collagen; a.u., arbitrary units.

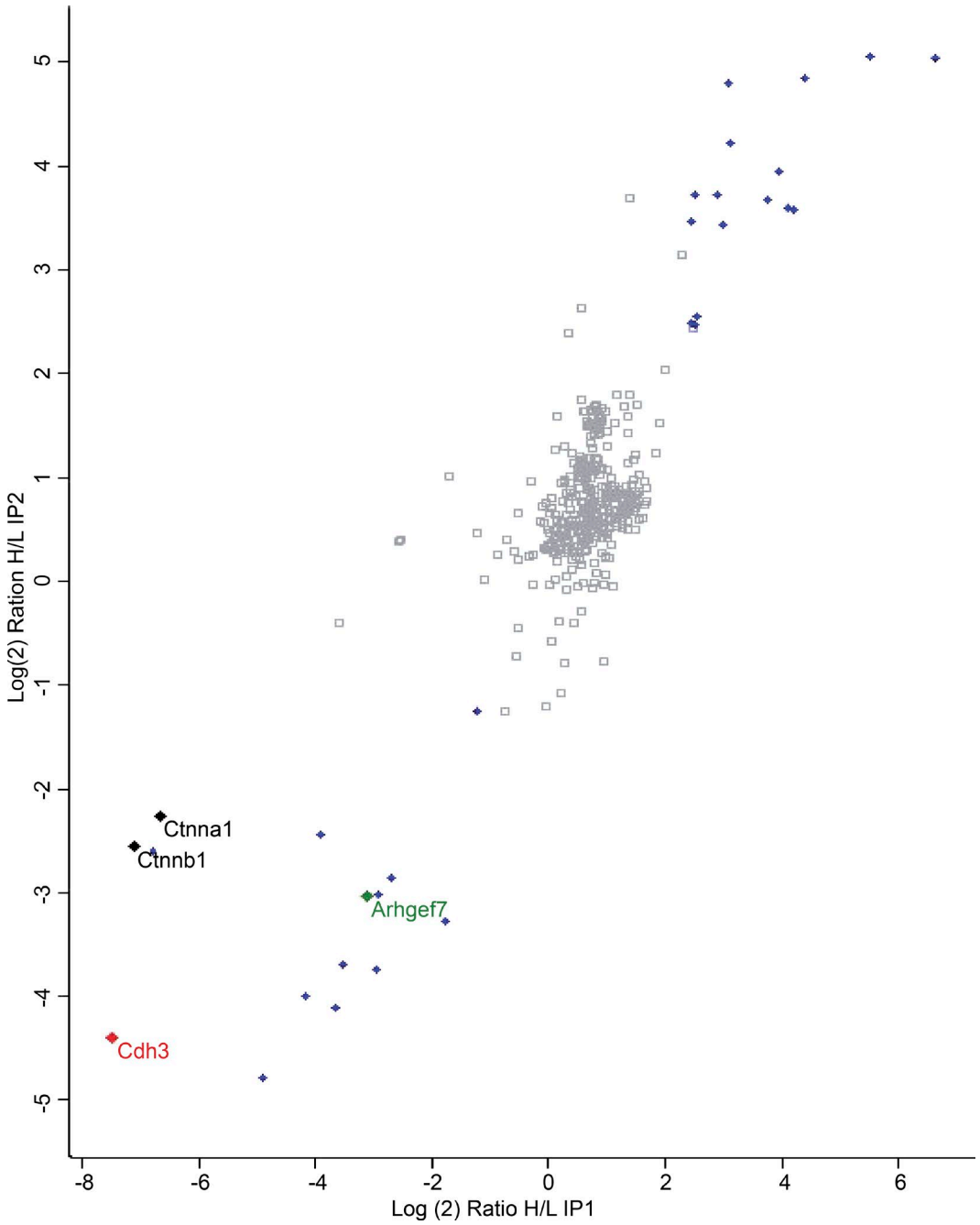


Figure S4. **β-PIX identification as a P-cadherin partner by proteomic analysis.** MaxQuant and Perseus were used to identify and quantify proteins with statistically significant changes in P-cadherin interaction in two conditions (IP1, confluent cells, and IP2, collectively migrating cells). The graph illustrates the log2 (H/L) SILAC ratio values for proteins from IP1 (*x* axis) versus IP2 (*y* axis). The bait protein (P-cadherin = Cdh3), α- and β-catenin (CTNNA1 and CTNNB1, two known cadherin-associated proteins) and β-PIX (Arhgef7) are highlighted and labeled in red, black, and green, respectively. Blue diamonds show the other significant proteins in both IPs (identified using Perseus).

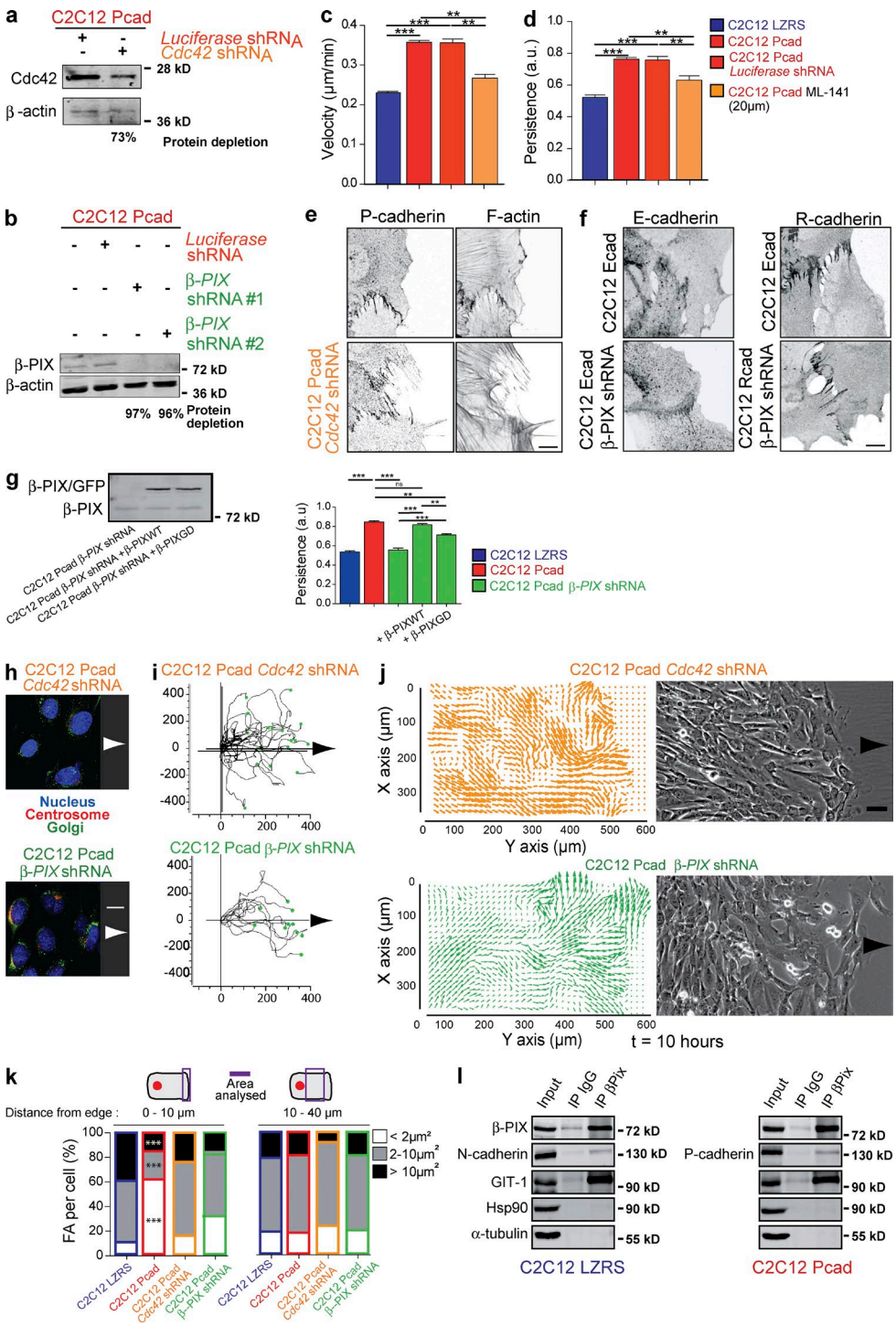
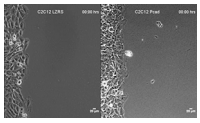
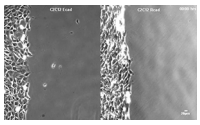


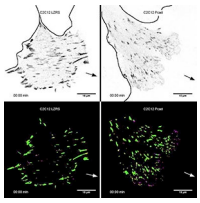
Figure S5. **Generation of P-cadherin–expressing cells knocked down for Cdc42 and β-PIX.** (a and b) C2C12 Pcad cells were infected with viruses expressing control *Luciferase* or Cdc42 shRNA (a), and 48 h later, the cells were lysed and analyzed by immunoblotting for Cdc42 and β-actin (b) *Luciferase* shRNA or β-PIX shRNA and selected to generate a stable cell line analyzed for β-PIX and β-actin expression. (c and d) Velocity and persistence of cells measured 4–15 h after removal of the insert. The drug was added 2–4 h before insert removal, and free medium with the drug was added after insert removal. Shown is the mean ± SEM. **, P < 0.005; ***, P < 0.0005. (e) Inverted contrast images of P-cadherin and F-actin (e) or E- and R-cadherin staining (f) of the indicated cell lines 8 h after removal of the insert. Bar, 10 μm. (g) Western blotting showing β-PIXWT or β-PIXGD expression in C2C12 Pcad *β-PIX* shRNA cells. Persistence of the indicated cells 4–15 h after removal of the insert. Shown is the mean ± SEM of three independent experiments. **, P < 0.005; ***, P < 0.0005. (h) Migrating cells (8 h after insert removal) stained for nucleus, centrosome, and Golgi distribution. Arrows indicate the migration direction. (i) Trajectories over 15 h of 17 C2C12 Pcad *Cdc42* shRNA cells and 10 C2C12 Pcad *β-PIX* shRNA cells. The corresponding rose plots of angle trajectories (i.e., directionality) are shown in Fig. 6 e. (j) Velocity fields and the corresponding phase-contrast images measured using the MatPIV software 10 h after insert removal. *n* = 1006 cells for C2C12 Pcad *Cdc42* shRNA cells; 998 cells for C2C12 Pcad *β-PIX* shRNA cells. Compare with Fig. 1 h. Bar, 40 μm. (k) Quantification of the FA number measured 0–10 or 10–40 μm from the leading edge. More than 700 FAs were analyzed from 50 cells. Data represent the mean ± SEM of five independent experiments. (l) C2C12 LZRS or C2C12 Pcad cell lysates were immunoprecipitated using an anti–β-PIX antibody and immunoblotted to assess the expression of β-PIX, GIT-1, α-tubulin, Hsp90, and the indicated cadherins. a.u., arbitrary units.



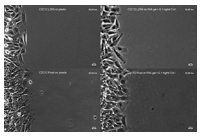
Video 1. **P-cadherin expression induces CCM.** Phase-contrast images of migrating C2C12 LZRS (left) and C2C12 Pcad cells (right); 1 frame/10 min for 15 h.



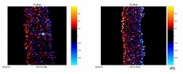
Video 2. **E- or R-cadherin expression does not promote CCM.** Phase-contrast images of migrating C2C12 Ecad (left) and C2C12 Rcad (right) cells; 1 frame/10 min for 15 h.



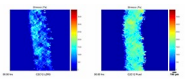
Video 3. **P-cadherin expression modifies FA organization and dynamics.** Top, inverted confocal images of paxillin-GFP in migrating C2C12 LZRS (left) and C2C12 Pcad cells (right) located at the first multicellular row; 1 frame/5 s for 15 min. Arrows indicate the migration direction. Lines in the first frame indicate nontransfected surrounding cells. Bottom, ratio images generated from the time-lapse shown at top to illustrate FA dynamics, with magenta showing the extension and yellow the loss of FAs. Green represents the FA area maintained during the analyzed period.



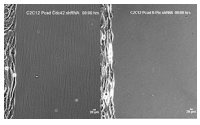
Video 4. **Migration of cells on plastic versus collagen-coated polyacrylamide gels.** Phase-contrast images of migrating C2C12 LZRS and C2C12 Pcad cells cultured on plastic (left) or collagen-coated polyacrylamide gels (right); 1 frame/45 min for 15 h.



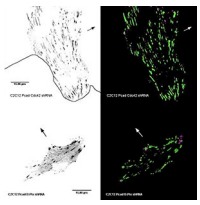
Video 5. **P-cadherin expression increases traction forces at the multicellular leading row.** Evolution of Tx of migrating C2C12 LZRS (left) and C2C12 Pcad cells (right) during a 10-h time course; 1 frame/30 min. Maximum Tx of cells moving to the left appear in yellow; maximum Tx of cells moving to the right appear in blue.



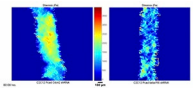
Video 6. **P-cadherin expression increases intercellular stresses.** Monolayer stress component σxx of migrating C2C12 LZRS (left) and C2C12 Pcad cells (right); 1 frame/30 min for 10 h.



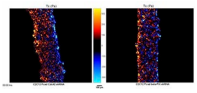
Video 7. **P-cadherin expression induction of CCM requires Cdc42 and β-PIX.** Phase-contrast images of migrating C2C12 Pcad Cdc42 shRNA (left) and C2C12 Pcad β-PIX shRNA cells (right); 1 frame/10 min for 15 h.



Video 8. **FA dynamics.** Left, inverted confocal images of paxillin-GFP in migrating C2C12 Pcad Cdc42 shRNA (top) and C2C12 Pcad β-PIX shRNA (bottom) cells located at the first multicellular row; 1 frame/5 s for 15 min. Arrows indicate the migration direction. Lines in the first frame indicate nontransfected surrounding cells. Right, ratio images generated from the time lapse shown on the left to illustrate FA dynamics, with magenta showing the extension and yellow the loss of FAs. Green represents the FA area maintained during the analyzed period.



Video 9. **Cdc42 and β-PIX are required for the P-cadherin–dependent increase in intercellular stresses.** Monolayer stress component σxx of migrating C2C12 Pcad Cdc42 shRNA (left) and C2C12 Pcad β-PIX shRNA (right) cells; 1 frame/30 min for 10 h.



Video 10. **Cdc42 or β-PIX inhibition perturbs P-cadherin–dependent traction-force generation of migrating cells.** Evolution of Tx of migrating C2C12 Pcad Cdc42 shRNA (left) and C2C12 Pcad *β-PIX* shRNA (right) cells during a 10-h time course; 1 frame/30 min.

Table S1 is provided as an Excel file and shows SILAC MaxQuant and Perseus data.
Three datasets are also provided for the MatPIV toolbox used to analyze sequential time frames for velocity and angle measurements, the method for FA detection using Cell Profiler software, and the ImageJ macro to measure FA orientation.