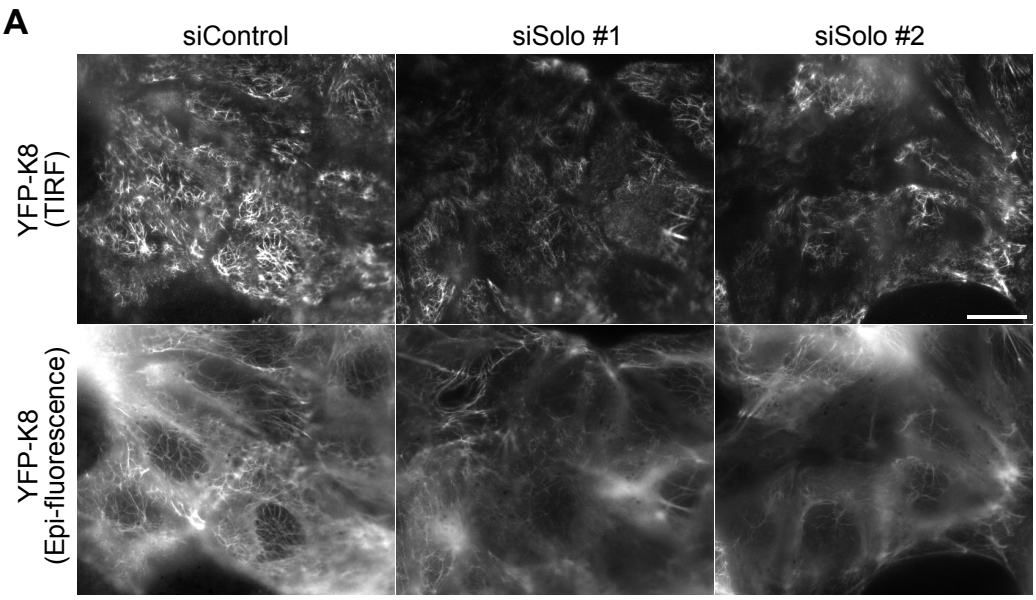
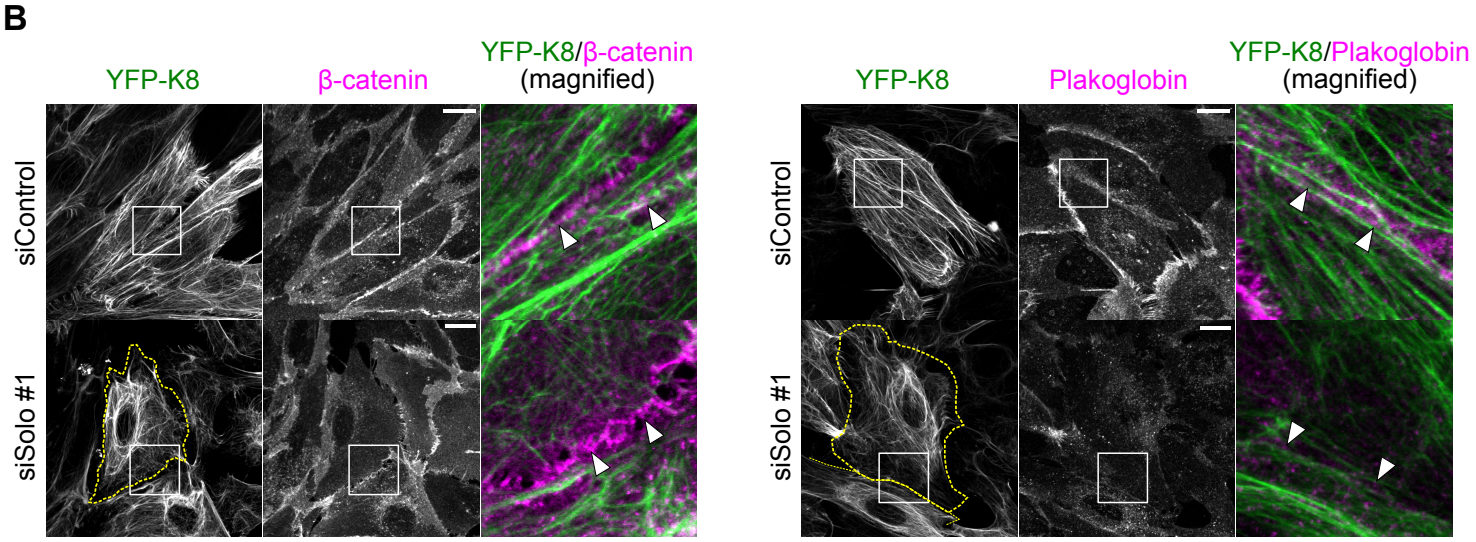
# Supplemental Materials

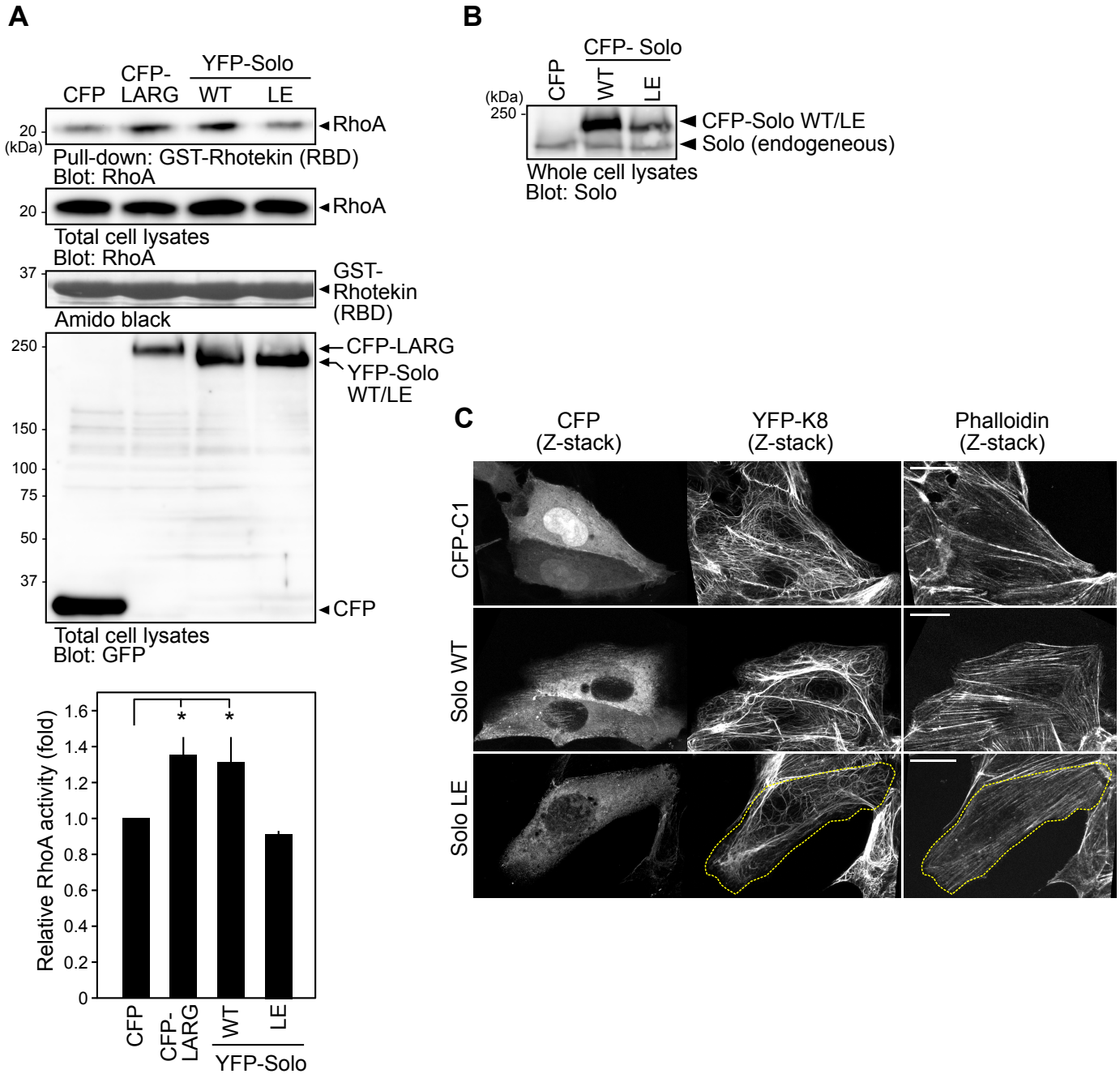
***Molecular Biology of the Cell***

Fujiwara et al.

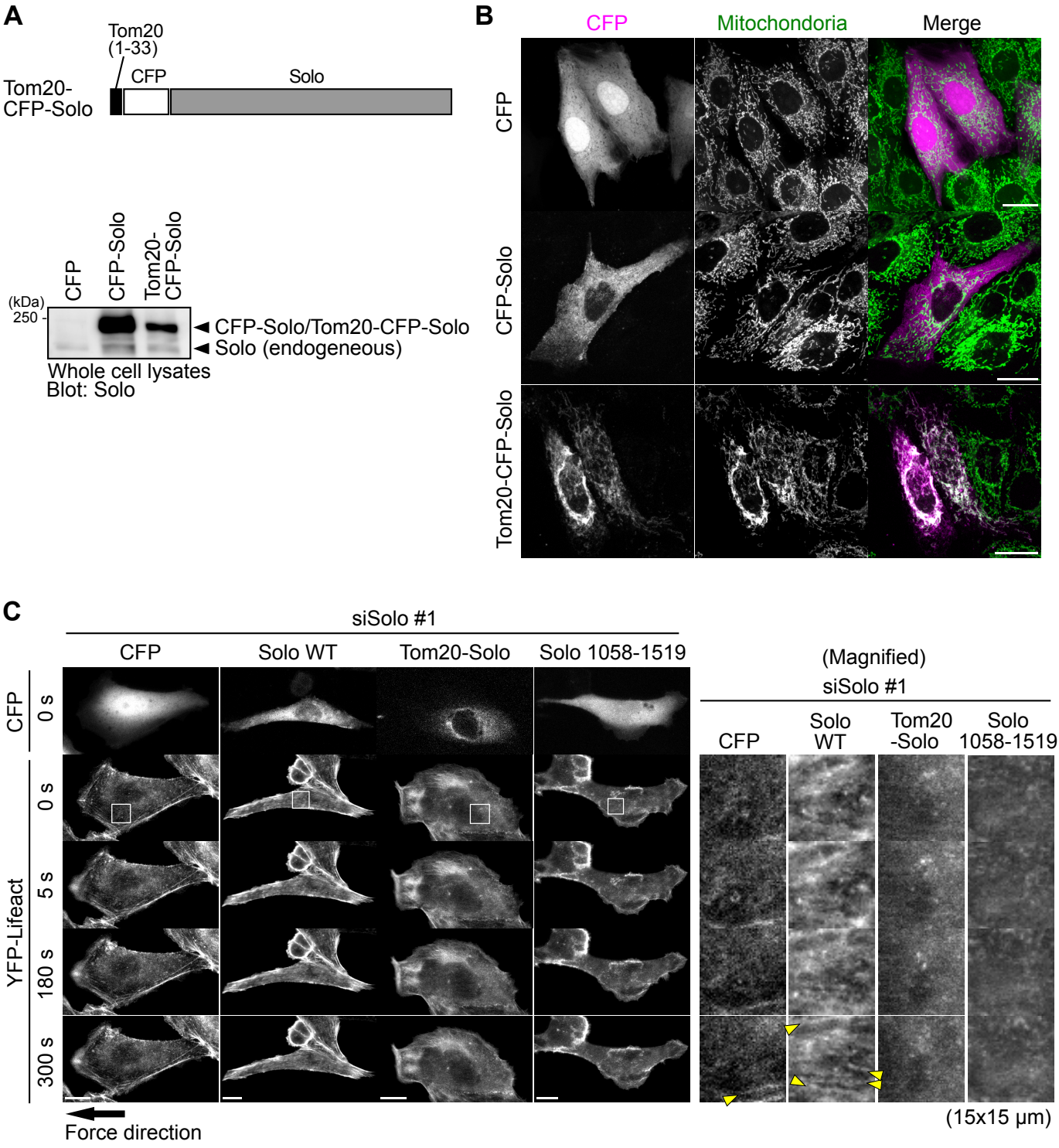


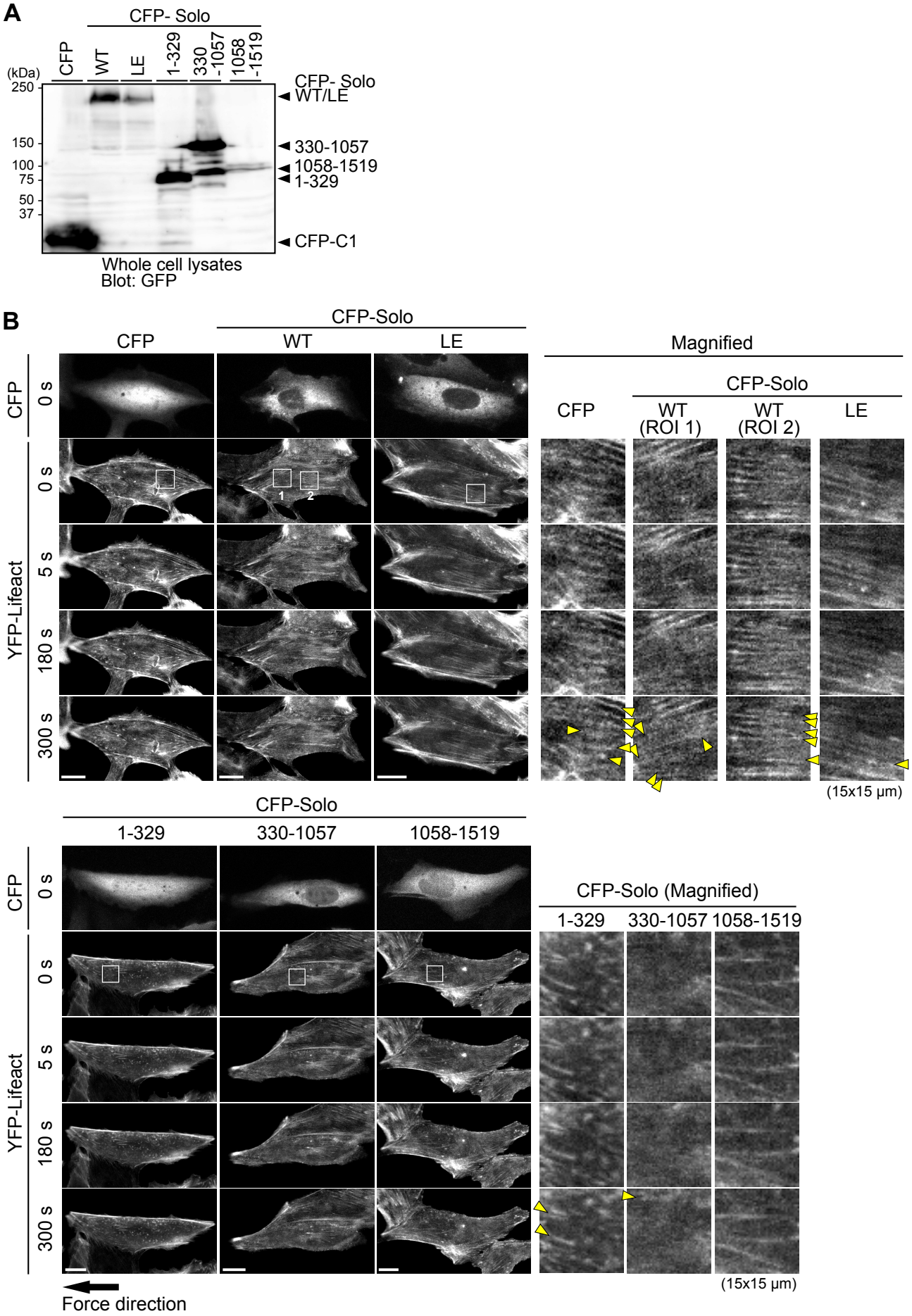

**Supplemental Figure S1. Effects of Solo knockdown on K8 filaments at cell-substrate adhesion sites and localizations of β-catenin and plakoglobin. (A)** TIRF images of K8 filaments in control and Solo knockdown cells. MDCK/YFP-K8 cells were transfected with control or Solo-targeting siRNAs, cultured for 48 h, and fixed. TIRF (90 nm range) and epi-fluorescence images were obtained. **(B)** Effects of Solo knockdown on the localizations of β-catenin and plakoglobin. MDCK/YFP-K8 cells were transfected with control or Solo-targeting siRNAs, cultured for 48 h, and stained with antibodies against β-catenin (upper panels) or plakoglobin (bottom panels). The dashed lines indicate the outline of Solo knockdown cells. Magnified images of the white boxes are shown in right columns. Arrowheads in magnified images indicate cell-cell adhesion sites. The z-stack images were obtained by confocal microscopy. Scale bars, 20 μm.




**Supplemental Figure S2. Effects of expression of Solo-WT or Solo-LE on actin and keratin cytoskeletal organization. (A)** Effects of expression of Solo (WT or LE) or LARG on RhoA activity. CHO-K1 cells were transfected with CFP, CFP-LARG, or YFP-Solo (WT or LE), cultured for 14 h, and serum-starved for 10 h. CFP-LARG was used as a positive control of the RhoA-targeting GEF. RhoA activity was measured by GST-RBD pull-down assays. Relative RhoA activity is shown as the means ± *SD* of three independent experiments, with the activity in control CFP-expressing cells set as 1.0. *, $P < 0.05$ (one-way ANOVA followed by Dunnett's test). (B) Immunoblot analysis of expression of CFP-Solo-WT and its LE mutant. MDCK cells were transfected with CFP, CFP-Solo-WT, or CFP-Solo-LE, and cell lysates were analyzed by immunoblotting with an anti-Solo antibody. (C) The z-stack images of the cells shown in Figure 3A. MDCK/YFP-K8 cells were transfected with CFP or CFP-Solo (WT or LE) and analyzed as in Figure 3A. The z-stack images were obtained by confocal microscopy. Scale bars, 20 μm.

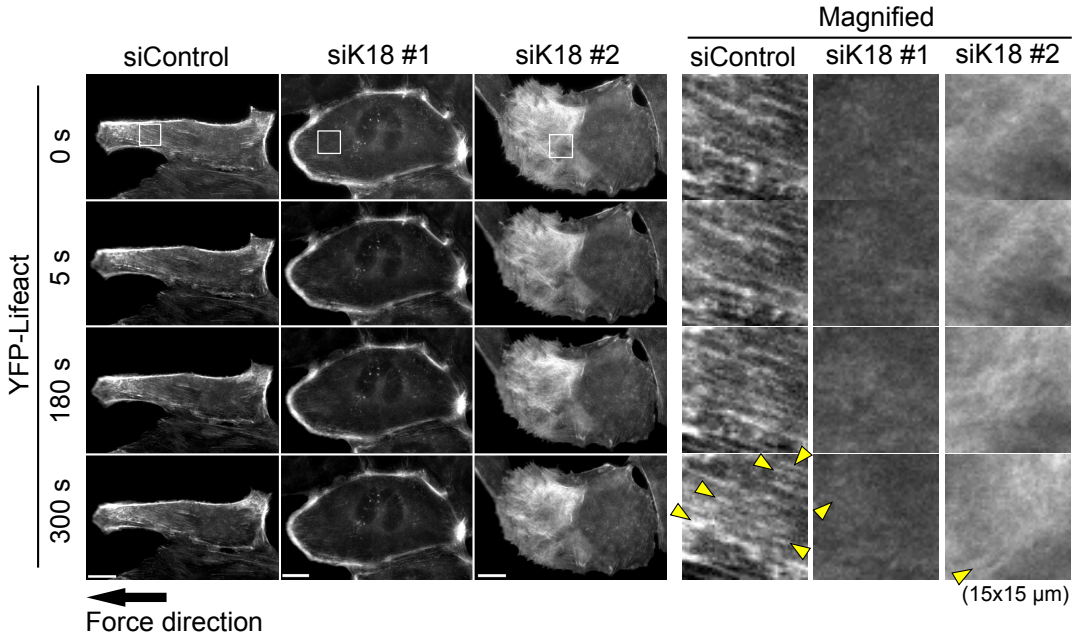



**Supplemental Figure S3. Proper localization of Solo is required for tensional force-induced stress fiber formation. (A)** Immunoblot analysis of expression of CFP-Solo-WT and Tom20(1-33)-CFP-Solo. MDCK cells were transfected with CFP, CFP-Solo-WT, or Tom20(1-33)-CFP-Solo, and cell lysates were analyzed by immunoblotting with an anti-Solo antibody. **(B)** Localization of Tom20(1-33)-CFP-Solo to mitochondria. MDCK cells were transfected with CFP, CFP-Solo-WT, or Tom20(1-33)-CFP-Solo and treated with Cytopainter MitoRed Indicator to visualize mitochondria. Cells were fixed and analyzed by a confocal microscopy. Scale bars, 20 μm. **(C)** Effects of expression of Solo-WT or its mutants on tensional force-induced stress fiber formation in Solo-knockdown cells. MDCK/YFP-Lifeact cells were transfected with control or Solo-targeting siRNAs and cultured for 24 h. Cells were transferred to a silicone membrane, transfected with CFP, CFP-Solo, or its mutants, and cultured for 24 h, and then tensile force was applied as in Figure 5A. Time-lapse fluorescence images of YFP-Lifeact near the ventral surface of the cell are shown. Magnified images of the white boxes are shown in the right-hand panels. Yellow arrowheads indicate stress fibers that were strengthened or generated after force application. Scale bars, 20 μm. See also Supplemental Movie 2.

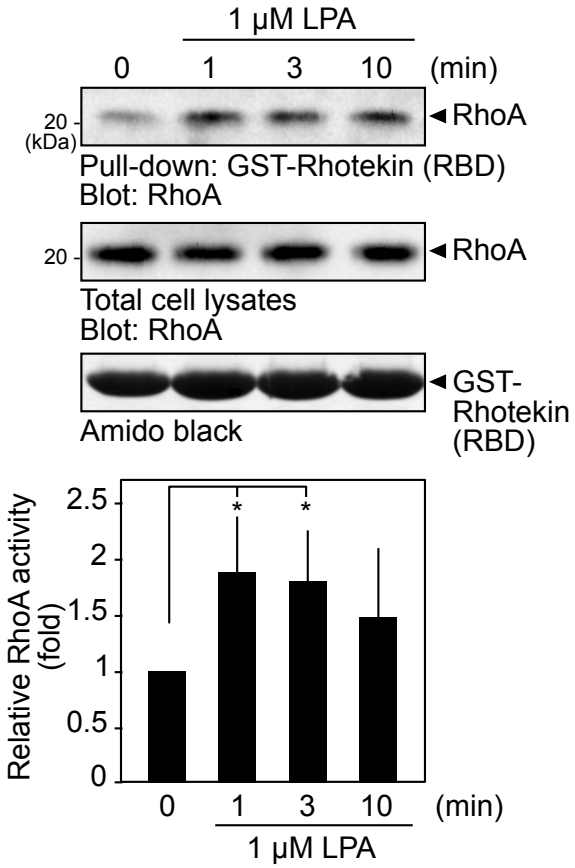



See next page for legend.

**Supplemental Figure S4. Effects of expression of Solo or its mutants on tensional force-induced stress fiber formation. (A)** Immunoblot analysis of expression of CFP-tagged Solo-WT, Solo-LE, and deletion mutants. MDCK cells were transfected with CFP, CFP-Solo-WT, CFP-Solo-LE, or deletion mutants, and cell lysates were analyzed by immunoblotting with an anti-GFP antibody. **(B)** Expression of Solo-LE or Solo deletion mutants suppresses force-induced stress fiber formation. MDCK /YFP-Lifeact cells were cultured on a silicone membrane, transfected with CFP, CFP-Solo, or its mutants, cultured for 24 h, and tensile force was applied as in Figure 5A. Time-lapse fluorescence images of YFP-Lifeact near the ventral surface of the cell are shown. Magnified images of the white boxes are shown in the right-hand panels. Yellow arrowheads indicate stress fibers that were strengthened or generated after force application. Scale bars, 20 μm. See also Supplemental Movie 3.




**Supplemental Figure S5. Knockdown of K18 suppresses tensional force-induced stress fiber formation.** MDCK/YFP-Lifeact cells were cultured on a silicone membrane, transfected with control or K18-targeting siRNAs, cultured for 48 h, and tensile force was applied as in Figure 5A. Time-lapse fluorescence images of YFP-Lifeact near the ventral surface of the cell are shown. Magnified images of the white boxes are shown in the right-hand panels. Yellow arrowheads indicate stress fibers that were strengthened or generated after force application. Scale bars, 20 μm. See also Supplemental Movie 5.




**Supplemental Figure S6. Effect of LPA treatment on RhoA activity.** MDCK cells were cultured in growth medium for 10 h on 35-mm dishes, serum-starved for 18 h, and treated with LPA at a final concentration of 1 μM. RhoA activity was measured by GST-RBD pull-down assays. Relative RhoA activity is shown as the mean ± *SD* of five independent experiments, with the activity at zero time set as 1.0. *, $P < 0.05$ (one-way ANOVA followed by Dunnett's test).

**Supplemental Table S1. The siRNA sequences used in this study.**

| siRNAs | Ref Seq No. | Target site, target sequence or catalog No. (Sigma-Aldrich) | |
|---|---|---|---|
| Control | | MISSON siRNA Universal Negative Control, SIC-001 | |
| Solo #1 | XM_532621.6 | 2029 bp - 2051 bp | GAGCTGAAAGAGGAACTCAAACC |
| Solo #2 | | 4626 bp - 4648 bp | GGGATCAGAGACCTTTGTTTACA |
| Solo #3 | | 2509 bp - 2531 bp | GCCCTCATTCCTGTCCTAAGTCA |
| K18 #1 | XM_534794.4 | 794 bp - 816 bp | GAGTTGGATGCCCCCAAATCTCA |
| K18 #2 | | 1373 bp - 1395 bp | AGGCGTTGAGGCAGCAAAACAGG |
| K18 #3 | | 532 bp - 554 bp | CCGCATCGTTCTGCAGATTGACA |