Supplemental Materials

Molecular Biology of the Cell

Edwards et al.

Legends for Supplemental Figures and Movies

Figure S1. Immunoblot for CP documenting the efficacy of knockdown of endogenous CP. The level of actin was increased, consistent with previous studies (Canton et al., 2005). The levels of the Arp2/3 regulators WAVE2 and cortactin did not appear to change.

Figure S2. CARMIL1 CP-binding mutant fails to rescue the lamellipodial dynamics phenotype of CARMIL1-knockdown cells. A) Frames from movies S1-S4. White bars indicate regions of the cells analyzed in the kymographs below. B) Quantitative kymograph analysis of protrusion rates. The rate of protrusion formation at the leading edge per cell is plotted in a box-and-whisker format showing the median, the interquartile range, and the extremes. n=10 cells. All differences were significant, $P<0.01$, unless otherwise indicated.

Figure S3. CP fails to localize to the leading edge in CARMIL1-knockdown cells. Representative images are shown, n=10 cells.

Figure S4 CARMIL1 CP-binding mutant fails to rescue the actin assembly defect of CARMIL1-knockdown in HEK-293 cells. Representative images are shown, n=13 cells. Cells on fibronectin-coated coverslips were fixed and stained for F-actin (phalloidin). Cell edges in knockdown and mutant rescue cells show decreased staining for F-actin (arrowheads), compared to control (scrambled) and wild-type rescue cells.

Movies S1–S8. Rescue of CARMIL1-knockdown phenotypes by the CARMIL1 mutant defective in binding CP. Phase-contrast time-lapse movies of HT-1080 cells on fibronectin are shown. Movies S1 and S5 show a CARMIL1-knockdown cell. Movies S2 and S6 show a cell expressing control shRNA. Movies S3 and S7 show a CARMIL1-knockdown cell expressing shRNA-resistant wild-type full-

length CARMIL1. Movies S4 and S8 show a CARMIL1-knockdown cell expressing shRNA-resistant mutant CARMIL1. Images were captured every 6 seconds. Movies are designed to play at 25 frames per sec. Cells are imaged at 40X magnification.

Fig. S1 Edwards et. al.

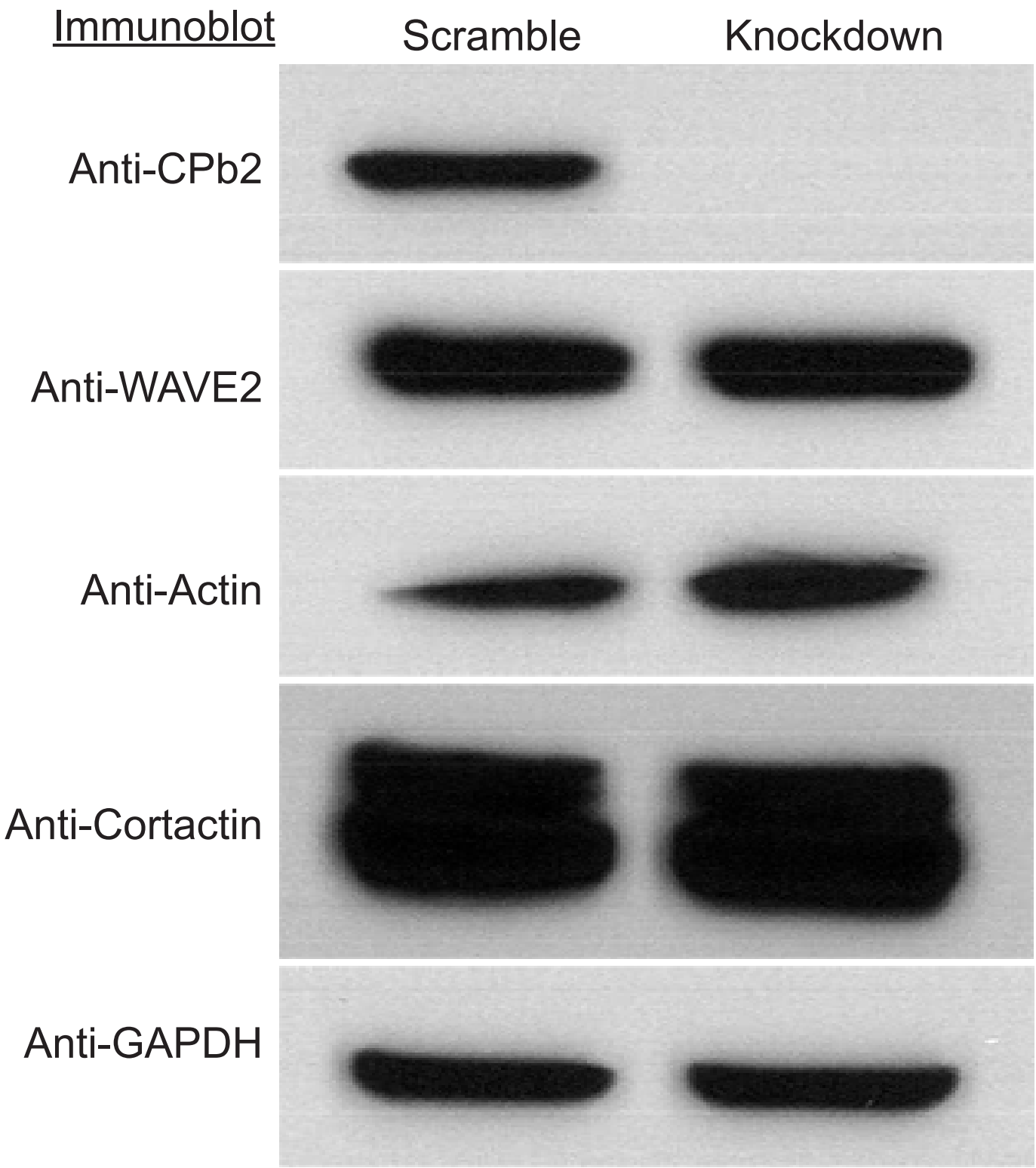
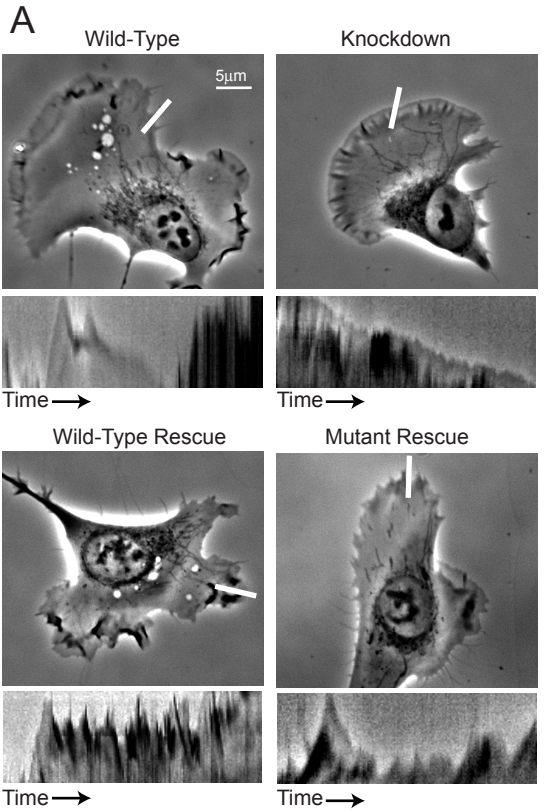


Fig. S2. Edwards et. al.



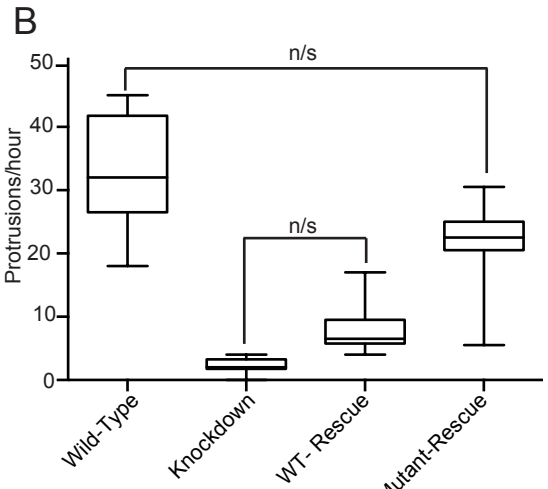


Fig. S3. Edwards et. al.

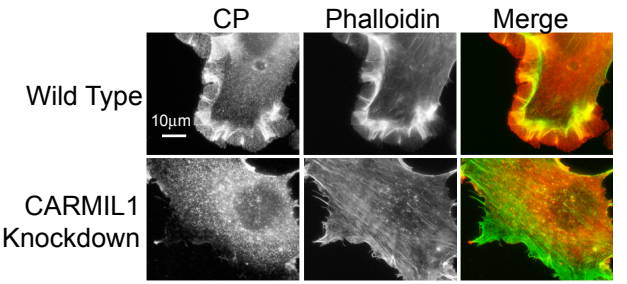


Fig. S4 Edwards et. al.