

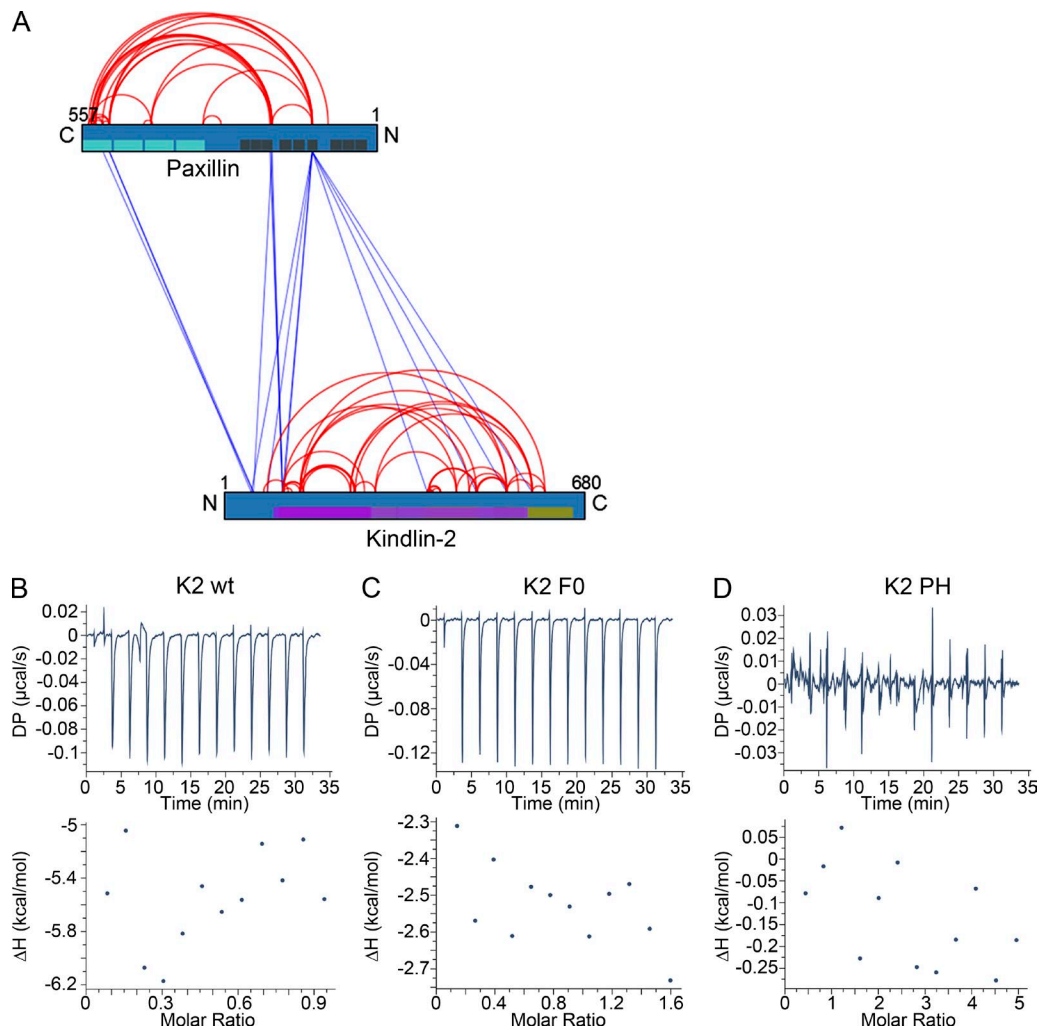
Böttcher et al., <https://doi.org/10.1083/jcb.201701176>

Figure S1. **Zn<sup>2+</sup> dependence of kindlin-paxillin binding.** (A) Cross-link map of kindlin-2-paxillin with intraprotein cross-links in red and interprotein cross-links in blue. (B–D) ITC measurements of full-length paxillin bound to recombinant K2 wild type (B), K2 F0 (C), and K2 PH (D) in the presence of 5 mM EDTA. C, C terminus; DP, differential power; N, N terminus.

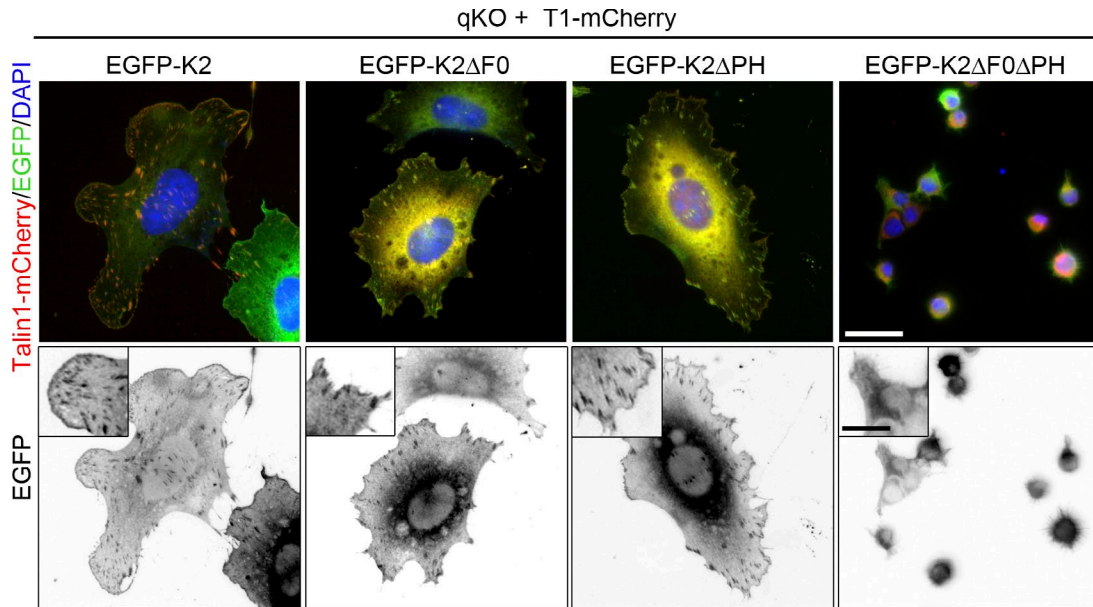


Figure S2. **FA recruitment of kindlin-2 deletions.** Localization of T1-mCherry and EGFP-tagged kindlin-2 mutants in T1-mCherry-expressing qKO cells seeded for 2 h on FN. DAPI was used to stain nuclei. Bar: 20  $\mu\text{m}$ ; (inset) 10  $\mu\text{m}$ .

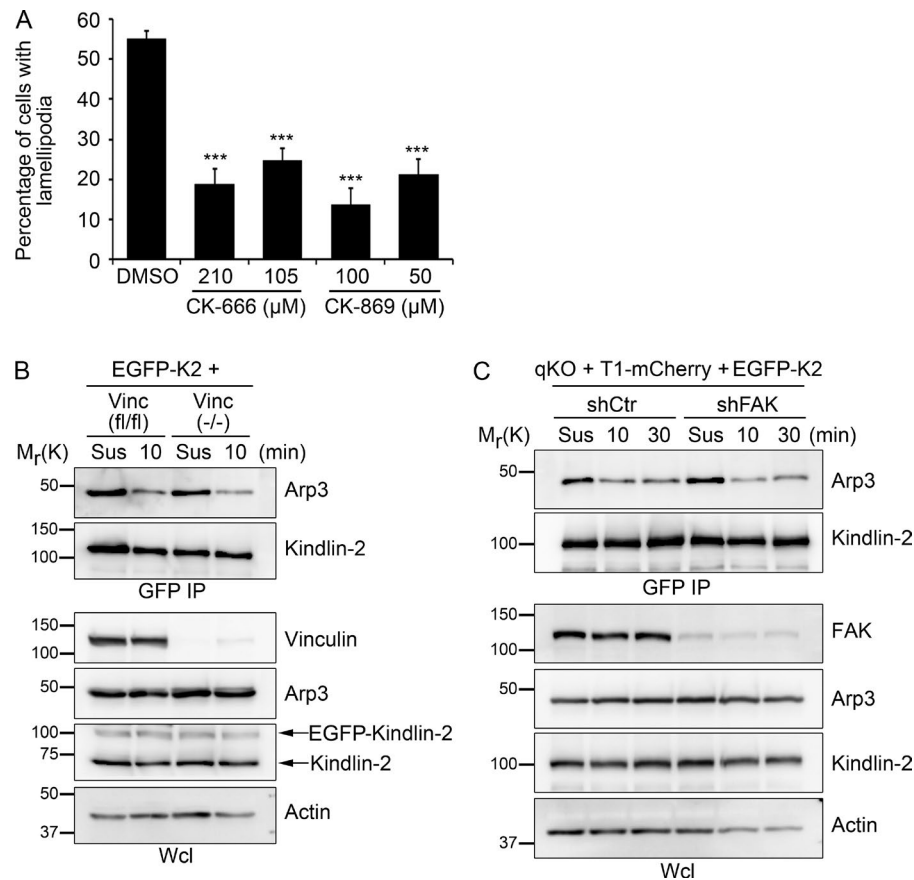


Figure S3. **Kindlin-2-Arp2/3 interaction.** (A) Quantification of membrane protrusions of FN-seeded EGFP-K2 cells 30 min after plating treated with 5 mM  $\text{Mn}^{2+}$  and either DMSO or the Arp2/3 inhibitors CS-666 and CD-869 ( $n = 6$  independent repeats;  $>100$  cells/condition; error bars indicate SEM,  $t$  test significances are calculated relative to DMSO-treated cells). \*\*\*,  $P < 0.001$ . (B and C) Western blot analysis of GFP-K2 immunoprecipitations from vinculin flox ( $\text{Vinc}^{\text{fl/fl}}$ ) and knockout ( $\text{Vinc}^{-/-}$ ) fibroblasts (B) or FAK-depleted cells (shFAK; C) kept in suspension (Sus) or seeded for the indicated times (in minutes) on FN.

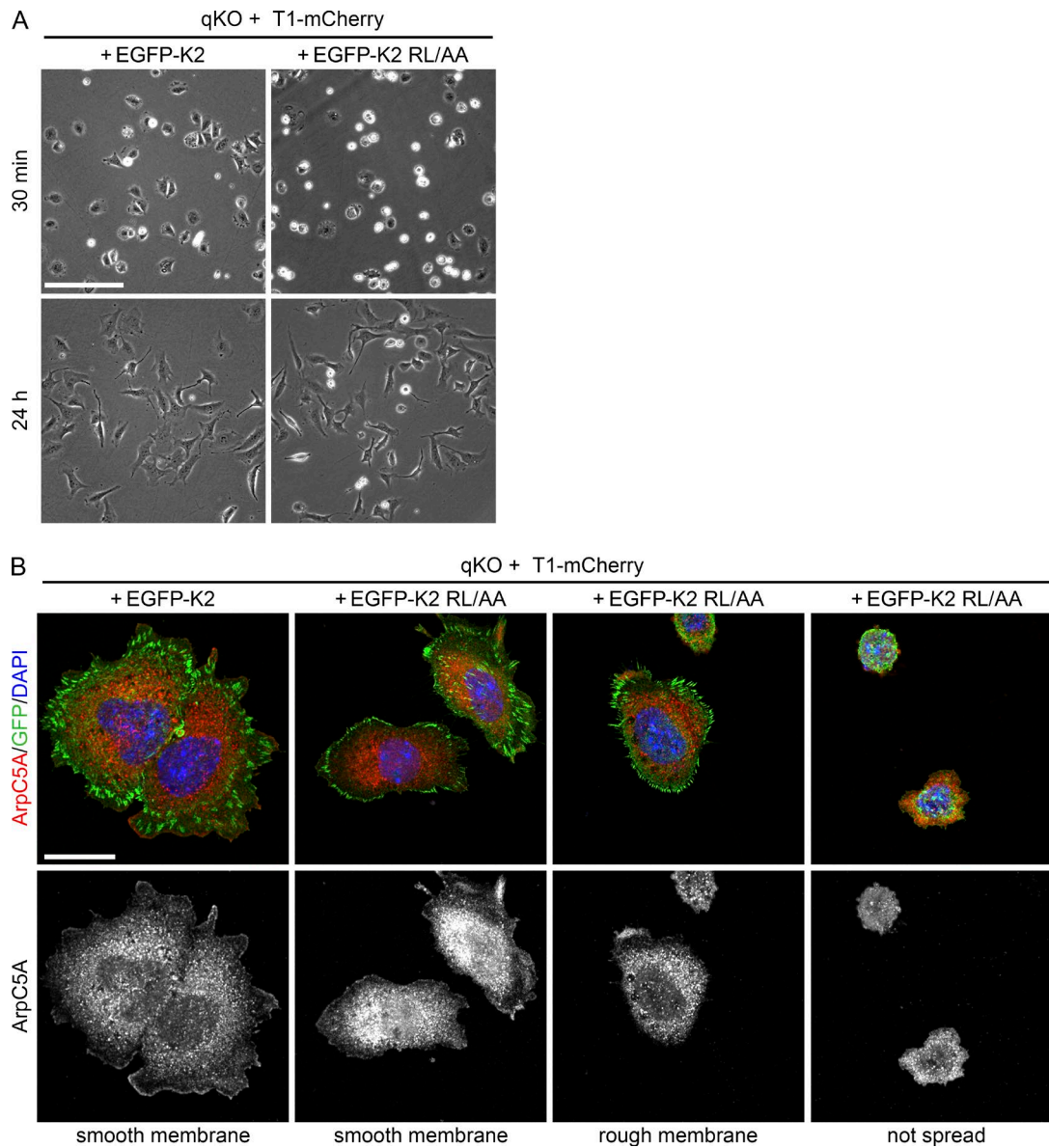


Figure S4. **Kindlin-2 RL/AA-expressing cells.** (A) Bright-field images of the indicated cell lines 30 min and 24 h after seeding on FN. Bar, 200  $\mu$ m. (B) Localization of ArpC5A and EGFP-tagged kindlin-2 and kindlin-2 RL/AA in T1-mCherry cells seeded for 30 on FN. Note the different cell morphologies with smooth membranes to rough or spiky membrane protrusions and nonspreading cells. DAPI was used to stain nuclei. Bar, 20  $\mu$ m.

**Provided online is supplemental data in Excel, showing high-confidence lysine-lysine cross-links of the kindlin-2-paxillin complex.**