# Supplemental material

**Wang and Brieher,** https://doi.org/10.1083/jcb.201812087

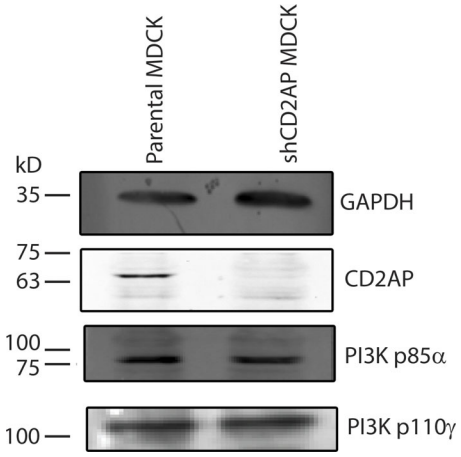


Figure S1. **Western blots of total cell extracts showing that CD2AP knockdown does not affect total amounts of PI3K p85α and p110γ in shCD2AP-MDCK cells.**

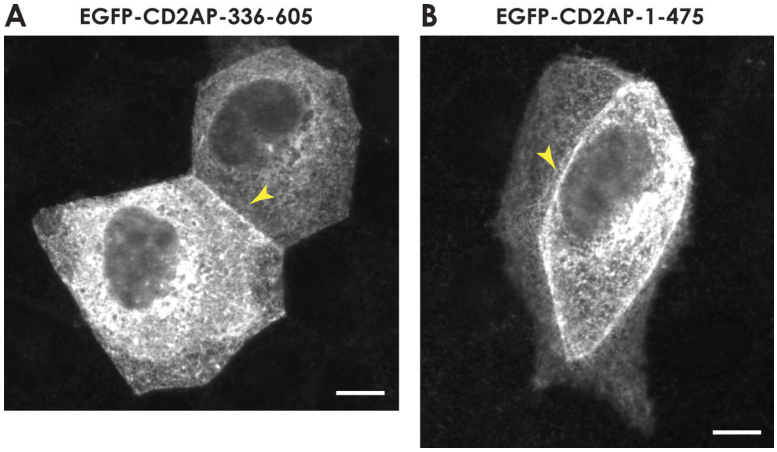


Figure S2. **C-terminal and N-terminal fragments of CD2AP target to the cell membrane.** shCD2AP-MDCK cells were transfected with GFP-CD2AP-336–605 (A) or GFP-CD2AP-1–475 (B) and plated onto glass coverslips. Arrowheads indicate cell–cell boundaries. Wide-field fluorescence images were taken 4 d after transfection. Scale bars, 8 µm.

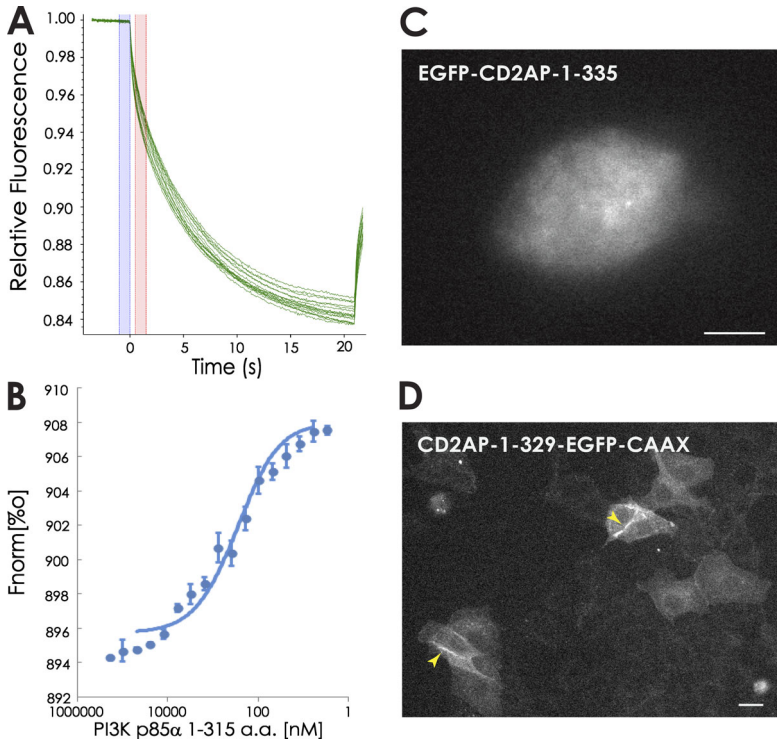


Figure S3. **CD2AP SH3 domains do not localize at the cell membrane, but associate with PI3K p85α. (A)** The association of GFP-CD2AP-1-335 aa with PI3K p85α 1–315 aa was measured by MST. PI3K p85α 1–315 aa (from 3.05 nM to 200 µM) was titrated into GFP-CD2AP-1–335 aa (40 nM). Thermophoresis trace shown from one representative experiment is shown. **(B)** Binding isotherm derived from the raw data and fitted to yield a $K_d$ of 0.35 ± 0.31 µM ($n$ = 3). Error bars represent SD; $n$ = 3. **(C)** shCD2AP-MDCK cells transiently transfected with EGFP-CD2AP-1–335 aa Wide-field microscopy image showing defective CD2AP 1–335 aa recruitment to cell borders. Scale bar, 16 µm. **(D)** shCD2AP-MDCK cells stably transfected with CD2AP-1–329-EGFP-CAAX constructs, which were clearly located at cell borders as indicated by the fluorescence microscopy imaging (arrowheads). Scale bar, 16 µm.

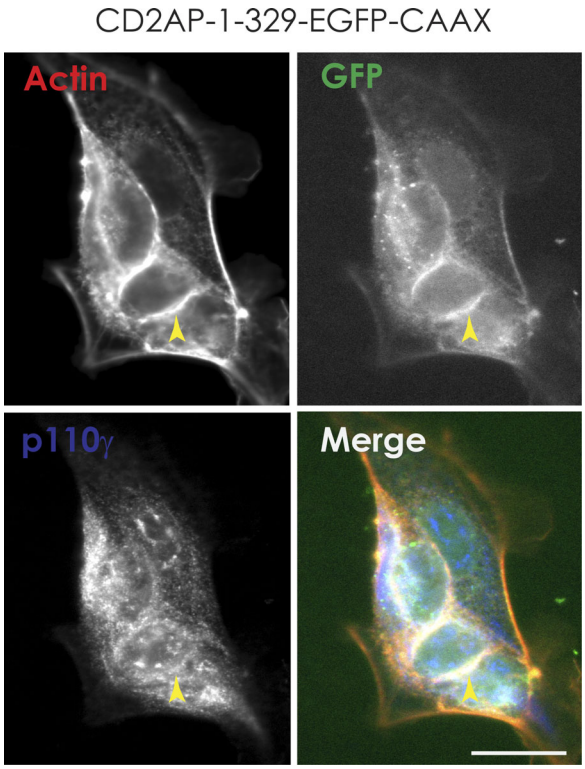


Figure S4. **shCD2AP-MDCK cells were stably transfected with EGFP-CD2AP-1–329-EGFP-CAAX, paraformaldehyde fixed, and stained for actin (phalloidin) and p110γ.** Arrowheads indicate cell–cell boundaries. Scale bar, 16 µm.