SUPPLEMENTAL MATERIALS

PICK1 is implicated in organelle motility in an Arp2/3 complex-independent manner

Yadaiah Madasu[a], Changsong Yang[b], Malgorzata Boczkowska[a], Kelley A. Bethoney[a], Adam Zwolak[a], Grzegorz Rebowski[a], Tatyana Svitkina[b], and Roberto Dominguez[a]

[a] Department of Physiology, Perelman School of Medicine, and [b] Department of Biology, University of Pennsylvania, Philadelphia, PA 19104*

Address correspondence to: Roberto Dominguez (droberto@mail.med.upenn.edu)

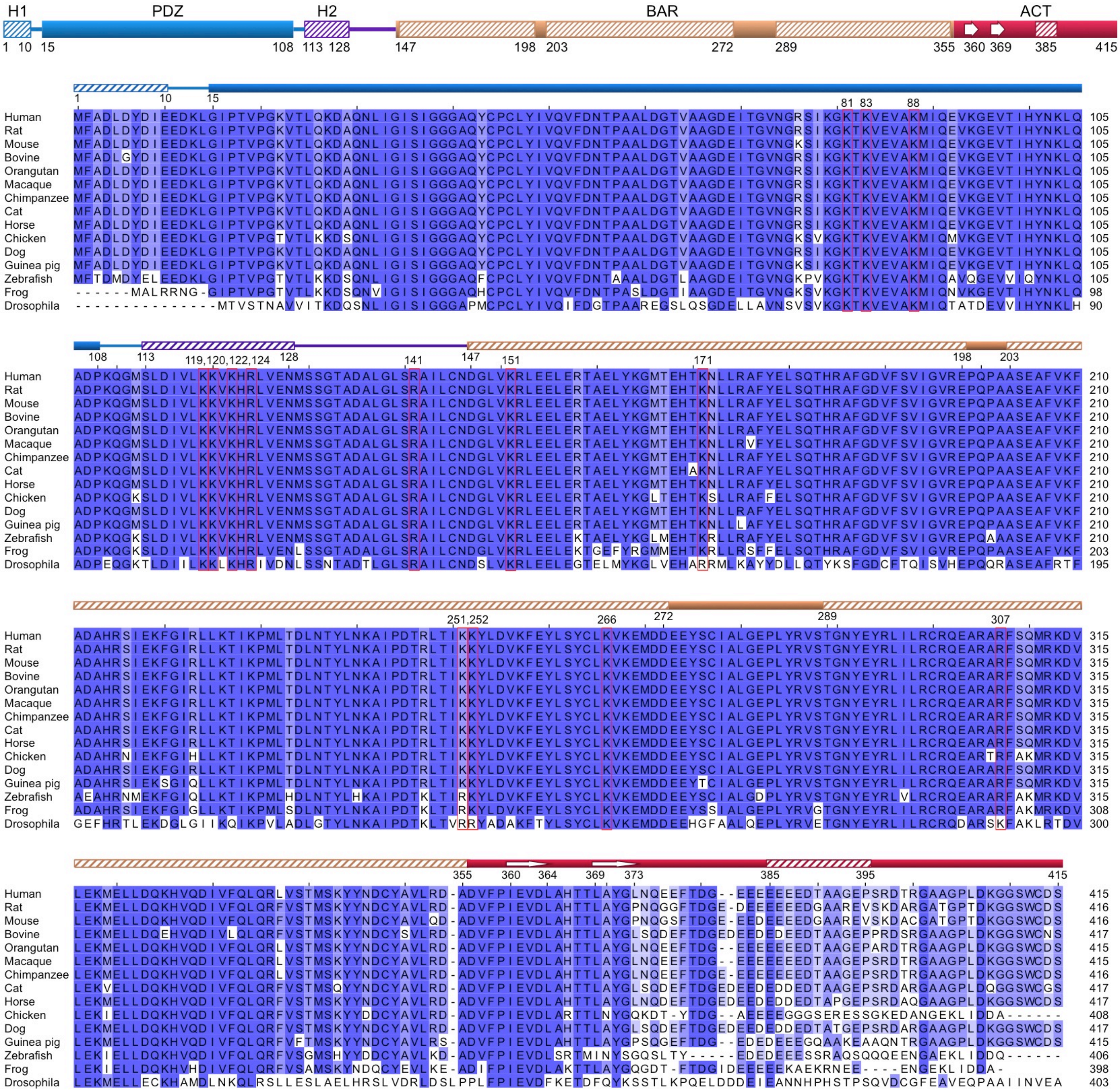

Figure S1. Alignment of PICK1 sequences from different organisms. A diagram above the sequences indicates the boundaries of domains and helices of the BAR domain (according to Figure 2A in main text). Highlighted inside red boxes are positively charged amino acids that the atomic model of PICK1 suggests could be implicated in membrane interactions. Sequence conservation decreases from dark to light blue shading. The UniProt accession codes of the sequences are: Human (Q9NRD5), Rat (Q9EP80), Mouse (Q80VC8), Bovine (Q2T9M1), Orangutan (Q5REH1), Macaque (Q4R7Q5), Chimpanzee (H2QLN1), Cat (M3VZ64), Horse (F7DB33), Chicken (E1BQ62), Dog (E2RPE0), Guinea pig (H0VHQ6), Zebrafish (F1Q8Q4), Frog (Q28BS9) and Drosophila (Q86PF5). Figure related to Figure 2A in main text.

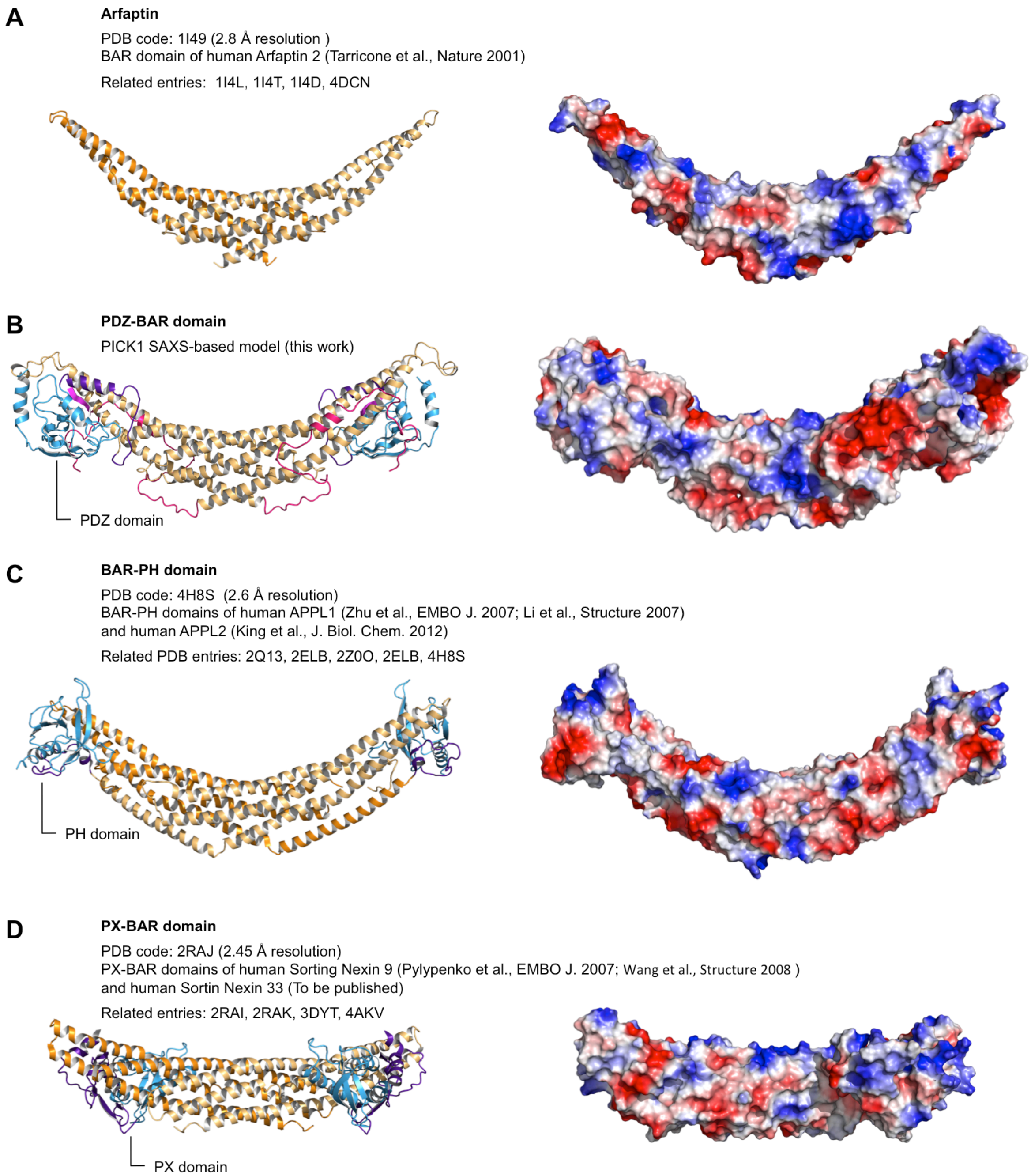

Figure S2. Comparison of the atomic model of PICK1 with other BAR domain proteins. (**A**) Structure of the BAR domain of arfaptin (Tarricone *et al.*, 2001), which served as a model for the BAR domain of PICK1, showing both a ribbon diagram and an electrostatic surface representation (red: negatively charged, blue: positively charged). (**B**). Model of PICK1 (according to Figure 2 in main text). (**C**) Structure of the BAR-PH domain of APPL1 (Zhu *et al.*, 2007). (**D**) Structure of the PX-BAR domain of sorting nexin 9 (Pylypenko *et al.*, 2007). Indicated above each structure are the references and accession codes of related structures in the Protein Data Bank (PDB). Note that the curvature of the BAR domain of APPL1 is similar to that of arfaptin and PICK1, whereas the curvature of the BAR domain of sorting nexin 9 is significantly less pronounced. Note also that the BAR domains of APPL1 and sorting nexin 9 are tightly associated with the PH and PX domains, respectively, through interactions that also involve accessory sequences N- and C-terminal to these domains. This provides the structural bases for association of the BAR domain with a secondary membrane-binding module, such that membrane interactions are the sum of weak interactions of the two folds, further enhanced by dimerization. This mechanism is referred to as “coincidence detection” (Moravcevic *et al.*, 2012). The model of PICK1 predicts a similar arrangement of the BAR and PDZ domains. Figure related to Figure 2 in main text.

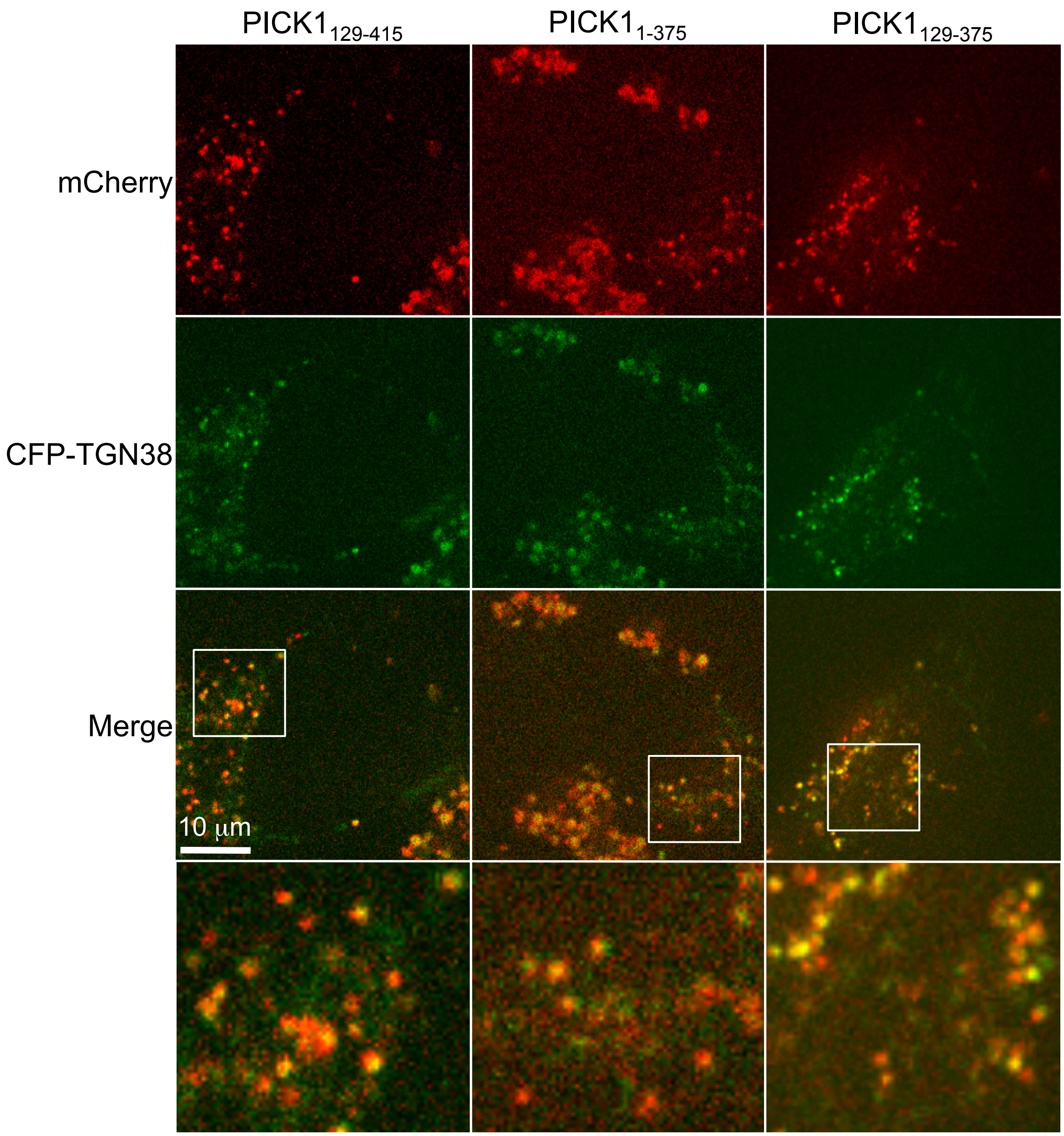

Figure S3. Colocalization of PICK1 constructs with the *trans*-Golgi network marker TGN38.
From left to right, confocal fluorescence microscopy analysis of HeLa cells coexpressing constructs mCherry-PICK1$_{129\text{-}415}$, mCherry-PICK1$_{1\text{-}375}$, and mCherry-PICK1$_{129\text{-}375}$ (red, top row) with CFP-TGN38 (green, second row). The third row shows an overlay of the first two rows (see also Movie S5). Fourth row - zooms of the regions boxed in the third row.

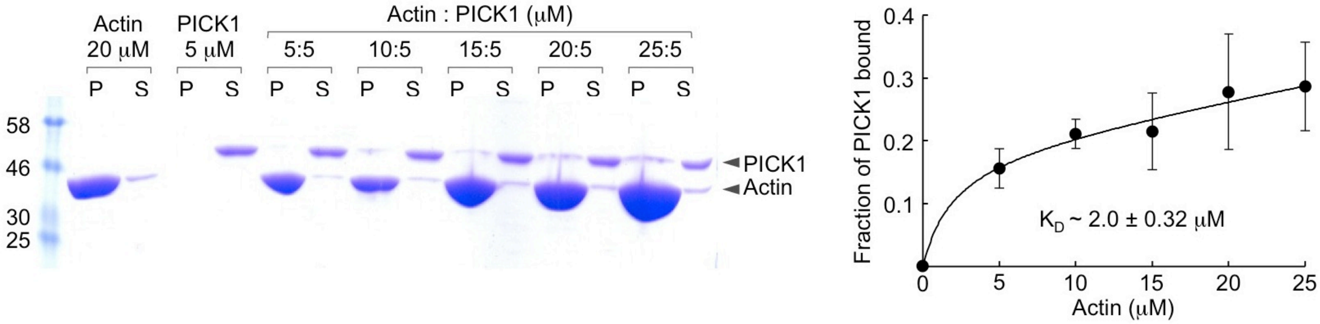

Figure S4. PICK1 binds F-actin *in vitro*. SDS–PAGE analysis of the pellet (P) and supernatant (S) fractions of high-speed sedimentations performed at a fixed PICK1 concentration (5 μM) and varying F-actin concentrations (0–25 μM). The graph on the right shows the fraction of F-actin-bound PICK1 as a function of PICK1 concentration, determined from densitometric analysis of the gels. The solid line represents the global fit of the data from three independent experiments, resulting in a K_D estimate of ~2.0 μM. Yet, it must be noted that the affinity measurement from this experiment is only approximate, because the actual concentration of actin filaments vs. monomers is not precisely determined and the titration did not reach saturation. Figure related to Figure 4A in main text.

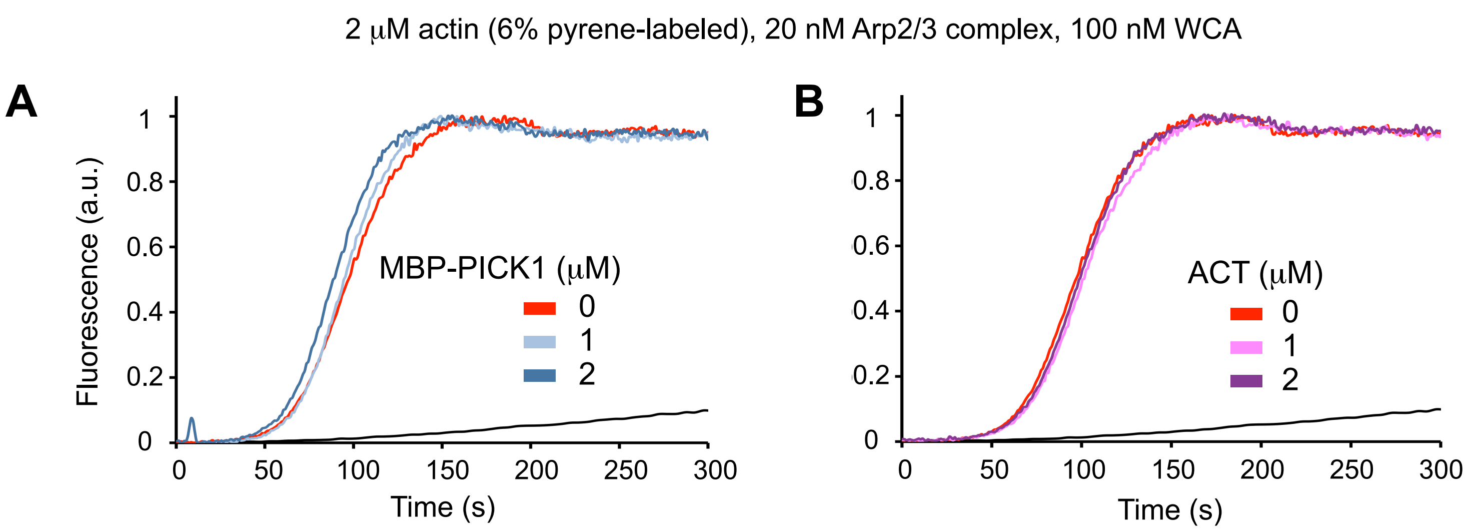

Figure S5. Lack of inhibition of Arp2/3 complex polymerization by MBP-PICK1 or ACT. (**A** and **B**) Time course of the fluorescence increase upon polymerization of 2 μM actin (6% pyrene-labeled) alone (black line) and with addition of the indicated proteins at the indicated protein concentrations. Experimental conditions are given on top, and each curve is color-coded. Each measurement was performed three times and one representative curve is shown. Figure related to Figure 5 in main text.

Movie S1. 360° rotation of the MBP-PICK1 model inside the SAXS envelope. The PICK1 domains are color-coded according to Figure 2A, and MBP is shown in green. Movie related to Figure 2B.

Movie S2. 360° rotation of the atomic model of PICK1. The PICK1 domains are color-coded according to Figure 2A. The side chains of positively-charged amino acids predicted to participate in membrane binding are shown. Movie related to Figure 2C.

Movie S3. 360° rotation of an electrostatic surface representation of the PICK1 model. Red: negatively charged, blue: positively charged. Movie related to Figure 2E.

Movie S4. HeLa cells expressing GFP-PICK1 constructs. From left to right the movies show cells expressing constructs GFP-PICK1, GFP-PICK1$_{129-415}$, GFP-PICK1$_{1-375}$, and GFP-PICK1$_{129-375}$ (as indicated). Each movie consists of 20 frames, taken at 3 s intervals, for a total of 60 s, and played at 10x their actual speeds. Movies related to Figure 3B.

Movie S5. HeLa cells coexpressing PICK1 constructs and the *trans*-Golgi marker TGN38. From left to right the movies show cells coexpressing constructs mCherry-PICK1$_{129-415}$, mCherry-PICK1$_{1-375}$, and mCherry-PICK1$_{129-375}$ (red) with CFP-TGN38 (green). Each movie consists of 20 frames, taken at 2 s intervals, for a total of 40 s, and played at 5.7x their actual speeds. Movies related to Supplemental Figure S3.

Movie S6. HeLa cells coexpressing GFP-PICK1$_{129-415}$ (green) and RFP-actin (red). The movie consists of 20 frames, taken at 3 s intervals, for a total of 60 s, and is played at 10x its actual speed. Movie related to Figure 4B.

Movie S7. B16F1 cells expressing GFP-PICK1$_{129-415}$ with or without treatment with latrunculin B. Each movie consists of 15 frames, taken at 4 s intervals, for a total of 60 s, and played at 10x their actual speeds. Movies related to Figure 4C.

Movie S8. HeLa cells expressing GFP-PICK1$_{129-415}$ with or without treatment with CK-666. Each movie consists of 20 frames, taken at 3 s intervals, for a total of 60 s, and played at 10x their actual speeds. Control cells were treated with DMSO. Movies related to Figure 6.

SUPPLEMENTAL REFERENCES

Moravcevic, K., Oxley, C.L., and Lemmon, M.A. (2012). Conditional peripheral membrane proteins: facing up to limited specificity. Structure *20*, 15-27.

Pylypenko, O., Lundmark, R., Rasmuson, E., Carlsson, S.R., and Rak, A. (2007). The PX-BAR membrane-remodeling unit of sorting nexin 9. Embo J *26*, 4788-4800.

Tarricone, C., Xiao, B., Justin, N., Walker, P.A., Rittinger, K., Gamblin, S.J., and Smerdon, S.J. (2001). The structural basis of Arfaptin-mediated cross-talk between Rac and Arf signalling pathways. Nature *411*, 215-219.

Zhu, G., Chen, J., Liu, J., Brunzelle, J.S., Huang, B., Wakeham, N., Terzyan, S., Li, X., Rao, Z., Li, G., and Zhang, X.C. (2007). Structure of the APPL1 BAR-PH domain and characterization of its interaction with Rab5. Embo J *26*, 3484-3493.