Supporting Information

Bethoney et al. 10.1073/pnas.0906945106

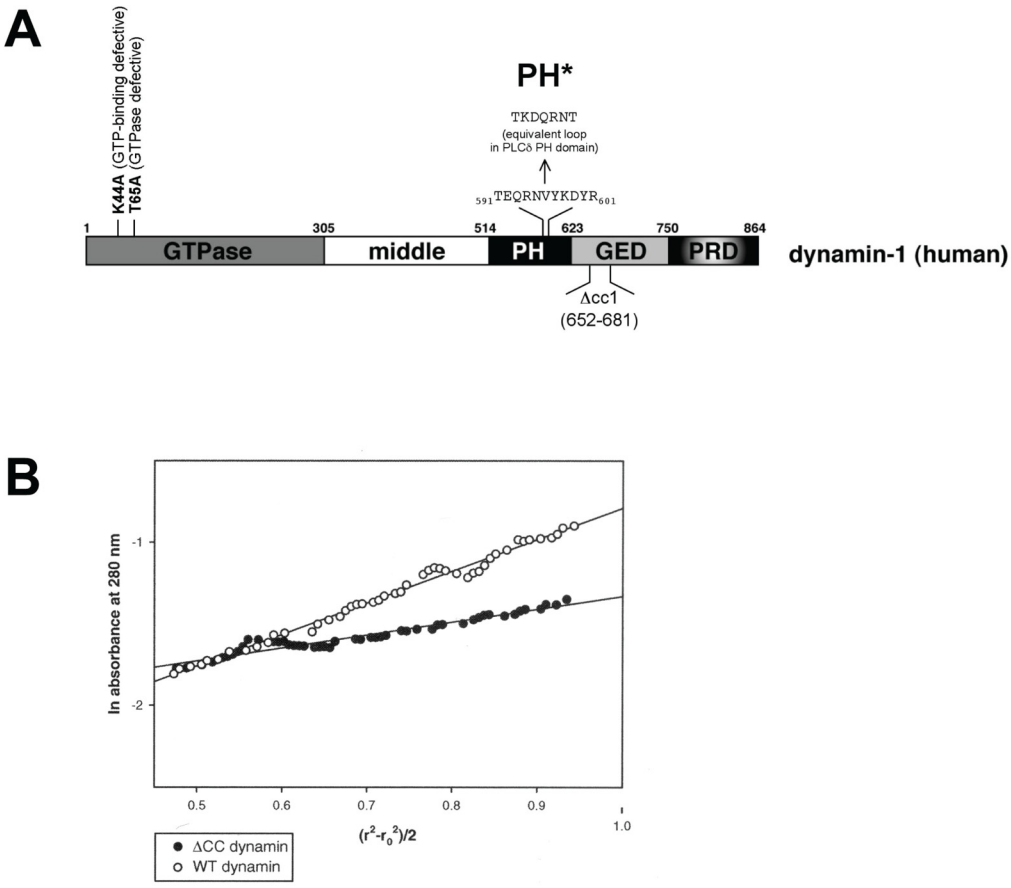

Fig. S1. (*A*) Schematic of human dynamin, showing domain structure and locations of mutations. (*B*) Deletion of the cc_1 sequence in the GED domain to give Δcc_1 dynamin (which lacks residues 652–681) disrupts dynamin tetramerization as analyzed by sedimentation equilibrium ultracentrifugation, resulting in monomeric dynamin with an average molecular mass of 93.5 ± 6.5 kDa compared with an average molecular mass of 347 ± 22 kDa for WT dynamin. Representative log-plots of sedimentation equilibrium data are presented, in which the slopes of the lines are proportional to molecular mass. Mean molecular masses quoted above are calculated from global fits of at least six datasets.

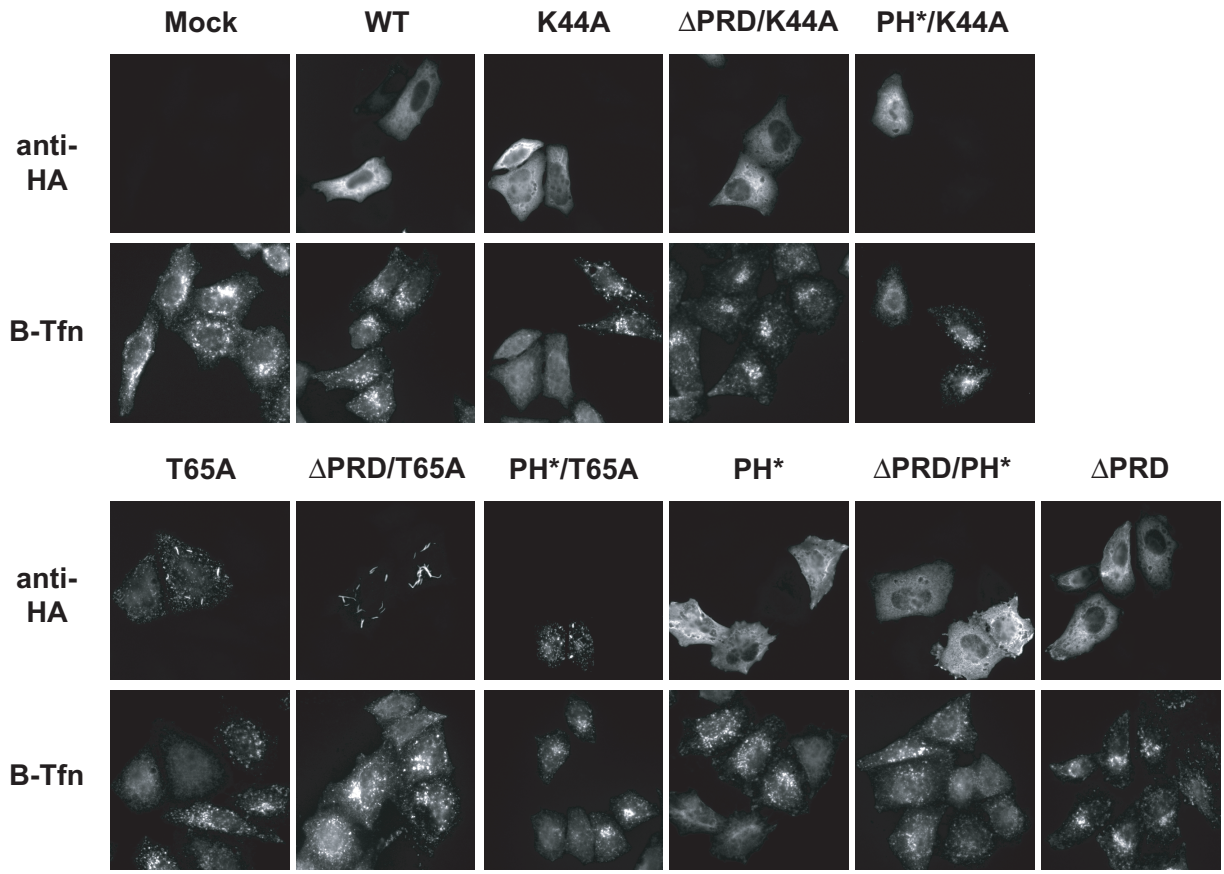

Fig. S2. Transferrin internalization in cells expressing different dynamin mutants. Representative images of HeLa cells transfected with control vectors or vectors directing expression of HA-tagged dynamin variants. Dynamin expression is detected by immunofluorescence staining with anti-HA, and internalized transferrin is detected by staining fixed cells with Texas Red-labeled streptavidin, as described in ref. 1. Internalization was scored as described in Methods for at least 100 cells expressing each dynamin variant, and plotted in Fig. 4.

1. Lee A, Frank DW, Marks MS, Lemmon MA (1999) Dominant-negative inhibition of receptor-mediated endocytosis by a dynamin-1 mutant with a defective pleckstrin homology domain. *Curr Biol* 9:261–264.

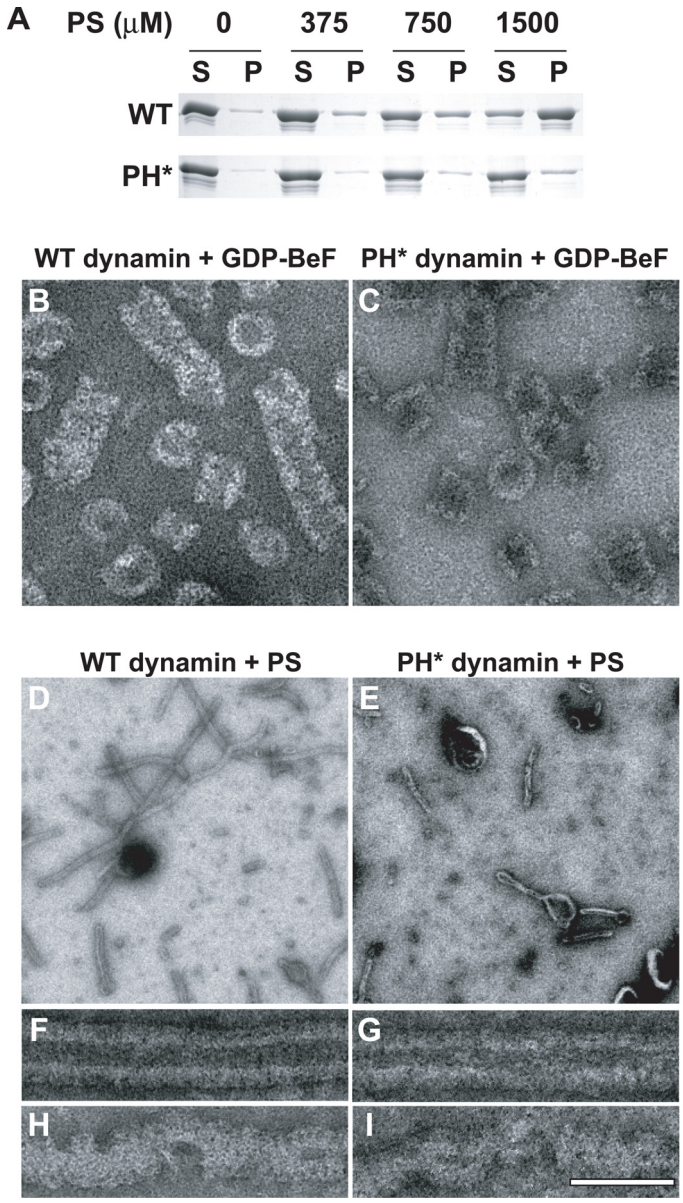

Fig. S3. PH* dynamin can tubulate and constrict lipid vesicles in vitro. (*A*) PH* dynamin sediments less readily with phosphatidylserine (PS) vesicles than WT protein. Mixtures of dynamin (WT or PH*) at 2.5 μM and PS vesicles (at the indicated concentration) were incubated for 30 min at room temperature and centrifuged at 426,000 $\times$ *g* for 15 min. The resulting supernatant and pellet fractions were analyzed by SDS/PAGE, staining for protein with Coomassie Blue. Both proteins sediment with PS vesicles, but PH* dynamin does so less readily—consistent with the appearance of fewer tubules in *E*. (*B* and *C*) PH* dynamin forms rings and helices indistinguishable from those formed by WT dynamin when incubated with a nucleotide analogue at physiological salt concentrations. WT and PH* dynamin were dialyzed at 0.25 mg/mL into 20 mM Hepes, 100 mM NaCl, 1 mM $MgCl_2$, 2 mM EGTA, 1 mM DTT, and 1 mM PMSF, pH 7.2, containing 0.5 μM GDP-BeF for 5h at 4 °C, and processed for negative stain EM as described in ref. 2. Both WT (*B*) and PH* (*C*) proteins assemble into rings and helices. (*D* and *E*) Negative stain EM studies show that both WT (*D*) and PH* (*E*) dynamin can induce tubulation of PS vesicles and form ordered helices on these substrates. Mixtures of 0.25 mg/mL dynamin (in 20 mM Hepes, 150 mM NaCl, 1 mM $MgCl_2$, 2 mM EGTA, 1 mM DTT, and 1 mM PMSF, pH 7.2), and 2.5 mg/mL DOPS vesicles (extruded through 1-μm membranes) were mixed in a volume ratio of 49:1, incubated at room temperature for 1 h, and then absorbed onto carbon-coated cooper EM grids and stained with 2% uranyl acetate. Examples of vesicles that have been deformed into $\approx$50-nm tubules are shown. Although fewer tubules are formed by PH* dynamin than with WT dynamin, those that do form appear identical to those seen with WT protein (*F* and *G*). (*H* and *I*) Both PH* and WT dynamin constrict PS tubules when 1 mM GTP is added to the protein/lipid complex absorbed to grids. (Scale bar, 100 nm.)

2. Carr JF, Hinshaw JE (1997) Dynamin assembles into spirals under physiological salt conditions upon the addition of GDP and gamma-phosphate analogues. *J Biol Chem* 272:28030–28035.

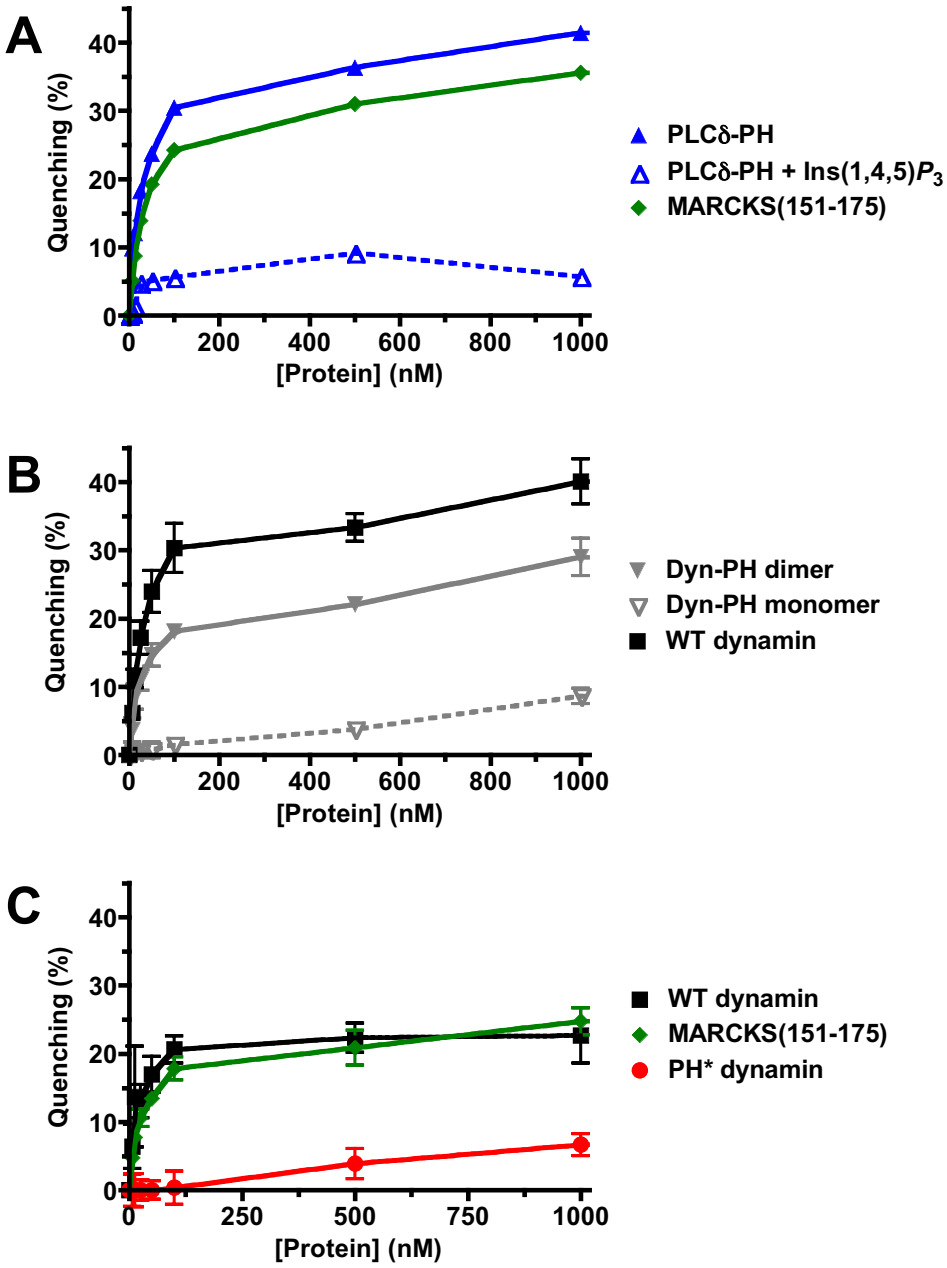

Fig. S4. Fluorescence self-quenching of BODIPY-TMR labeled lipids. (*A*) The isolated PH domain from PLC-δ_1 is as effective at promoting self-quenching of BODIPY-TMR-PtdIns(4,5)P_2 (at 1% mole/mole in DOPC) as the MARCKS (151–175) peptide. Addition of excess (10 μM) Ins(1,4, 5)P_3, which displaces PLCδ-PH from PtdIns(4,5)P_2-containing membranes (3), reversed the ability of PLCδ-PH to promote quenching of the labeled PtdIns(4,5)P_2. (*B*) A dimeric form of the dynamin PH domain, generated as described in ref. 4, also promotes quenching of BODIPY-TMR-PtdIns(4,5)P_2 fluorescence. (*C*) MARCKS (151–175) and WT dynamin also promote quenching of BODIPY-TMR-labeled phosphatidylinositol (PtdIns) when incorporated at 1% (mole/mole) in DOPC vesicles that also contain PtdIns(4,5)P_2 at 1% (mole/mole). The extent of fluorescence quenching is less than that seen with labeled PtdIns(4,5)P_2. It is not clear whether this result reflects a change in the environment of the fluorescent label on the lipid's acyl chain or some degree of PtdIns clustering. PH* dynamin had no influence.

3. Lemmon MA, Ferguson KM, O'Brien R, Sigler PB, Schlessinger J (1995) Specific and high-affinity binding of inositol phosphates to an isolated pleckstrin homology domain. *Proc Natl Acad Sci USA* 92:10472–10476.
4. Klein DE, Lee A, Frank DW, Marks MS, Lemmon MA (1998) The pleckstrin homology domains of dynamin isoforms require oligomerization for high affinity phosphoinositide binding. *J Biol Chem* 273:27725–27733.