Supplemental Materials

Molecular Biology of the Cell

Dar and Pucadyil

Supplementary material

Fig. S1

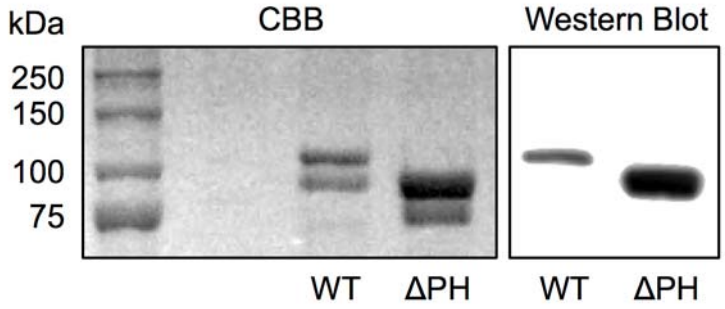

Fig. S1. Validation of partial proteolysis of dynamin constructs. Shown is a 10% SDS-PAGE of WT and ΔPH stained with CBB and developed with streptavidin-HRP, which binds an engineered StrepII tag at the C-terminus.

Fig. S2

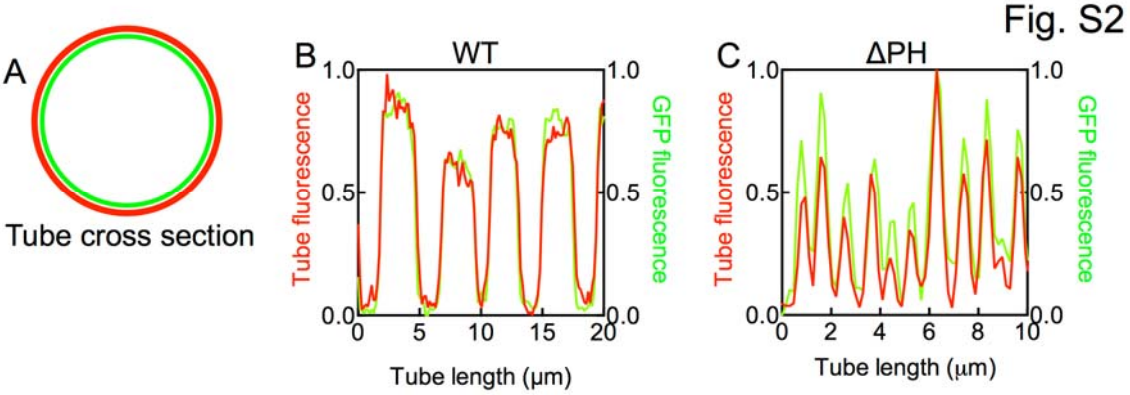

Fig. S2. Validation of tube constriction. (A) Schematic cross-sectional view of a membrane tube labeled with *p*-Texas Red DHPE (red) and containing 6xHis-mEGFP (green) recruited to the inner monolayer. Line profiles of tube (red) and mEGFP (green) fluorescence after scaffold assembly with WT (B) and ΔPH (C). The coincidence in fluorescence indicates that the drop seen in tube fluorescence is due to tube constriction.

Fig. S3

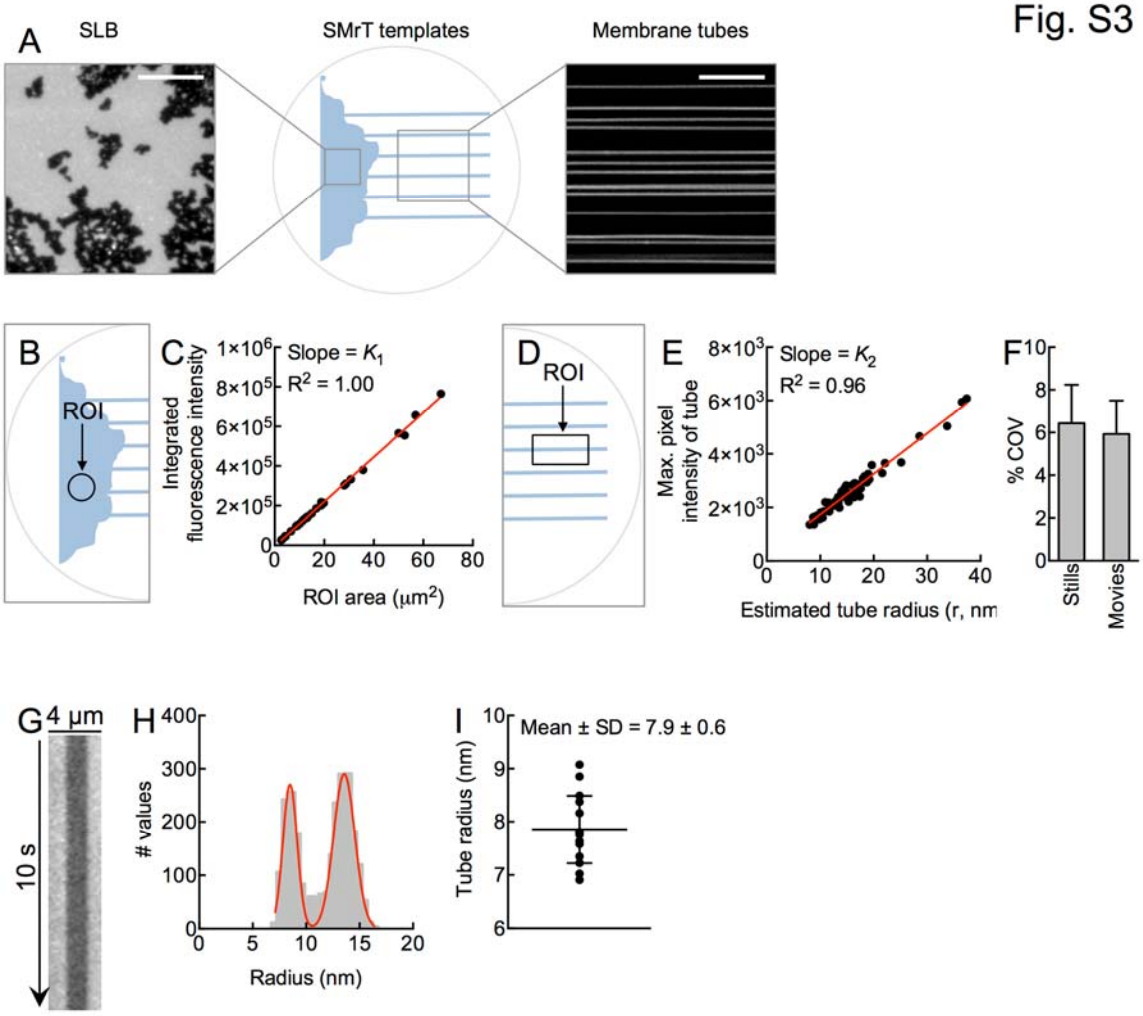

Fig. S3. Procedure for converting tube fluorescence to radius. (A) Schematic of SMrT templates showing the supported lipid bilayer (SLB) at source and membrane tubes. Scale bar = 10 μm. (B) Schematic of the SLB and the ROI position. (C) Plot of integrated fluorescence intensity in ROI against the area of ROI. (D) Schematic of membrane tubes and the ROI position. (E) Plot of maximum pixel intensity in ROI to tube radius. (F) Plot showing the coefficient-of-variation (COV) about the mean fluorescence intensity for still and time-lapse images of membrane tubes. Data represents the mean ± SD ($n \geq 20$, N=1). (G) Kymograph of tube fluorescence about a dynamin scaffold. Dark and light portions reflect regions under and adjacent to the scaffold on the tube, respectively. (H) Histogram of tube radii calculated from pixels of the kymograph shown in (G) and fitted to a sum of 2 gaussian. (I) Estimated tube radius under the dynamin scaffold.

Fig. S4

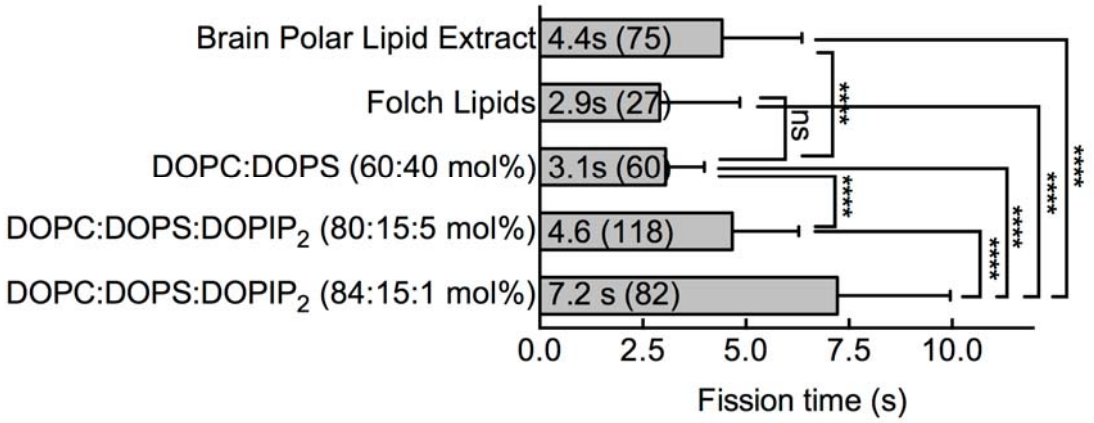

Fig. S4. Comparison of dynamin-catalyzed membrane fission reconstituted on native and synthetic lipids. Comparison of fission time with preassembled dynamin on SMrT templates prepared of the indicated lipid mix. Data represent mean ± SD. Numbers within bars indicate the mean (number of events analyzed). **** indicates $P < 0.0001$ (Student's t-test) and ns indicates not significant.

Fig. S5

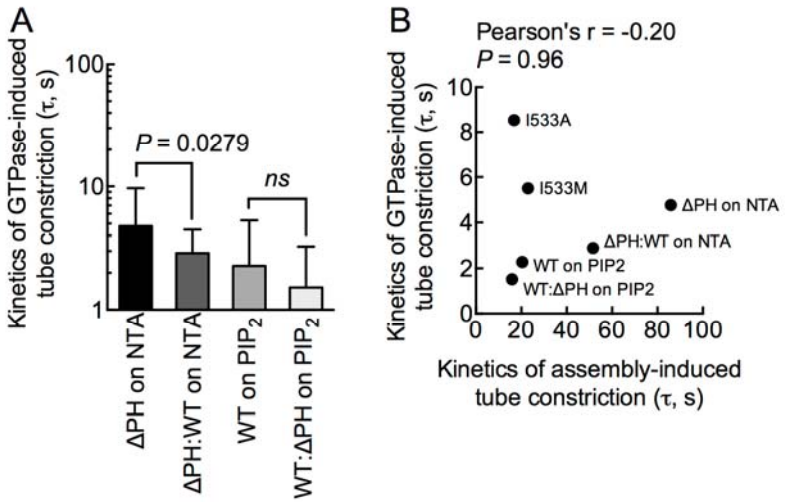

Fig. S5. Role of the PHD in GTPase-induced membrane constriction. (A) Time constants of GTPase-induced tube constriction for pure (ΔPH or WT) and mixed (ΔPH:WT) scaffolds. Data represent the mean ± SD ($n \geq 20$, N=3, Student's t-test). (B) Lack of correlation between time constant (τ) of GTPase-induced tube constriction and fission time.