Supporting Information

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SI Materials and Methods

Reagents. Chemical cross-linker BS3 was from Pierce/Thermo Scientific. Ni^{2+}-NTA Superflow was supplied by Qiagen, and Glutathione Sepharose 4B as well as Superdex 200 HR 10/30 by GE Healthcare Europe GmbH.

Cloning of FAPP2 and Plasmid Construction. Total RNA was isolated from Madin-Darby canine kidney II (MDCK II) cells using RNAeasy Qiagen kit. RT-PCR was performed using SuperScript reverse transcriptase (Invitrogen) with a poly dT oligo as primer. The cDNA was used as template for PCR. According to FAPP2 sequence predicted by Bioinformatics (provided by MPI-CBG Bioinformatics Facility), primers for FAPP2 were designed as following: forward primer5′-ATG GAG GGG GTG CTG TAC AAG T-3′ and reverse primer 5′-TAC CAC CTC ATC AGA CTC CAG-3′. The PCR products were cloned into pCR4-TOPO vector using TOPO TA Cloning kit (Invitrogen), and processed for sequencing. FAPP2 was then cloned into site SalI/NotI of pET-24d (Novagen), which was modified with a 3myc-tag at the N terminus, or into the EcoRI/SalI site of pGEX-6P-1 (GE Healthcare). For the construction of mCherry-FAPP2, FAPP2 was inserted into the SalI/XhoI site of a modified pGEX-6P-1, in which mCherry was in frame with the EcoRI/SalI inserted mCherry. GST-tagged FAPP2 mutants R18L, W407A were created using the QuikChange XL site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's manual using primers as described in Table S1. All constructs were sequenced in full.

Protein Expression and Purification. Transfected BL 21(DE3) cells were grown in LB medium at 37 °C, induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and grown for another 16–20 h at 18 °C. Purification of recombinant proteins from *E. coli* lysate protein was accomplished by Ni^{2+} and glutathione affinity chromatography, respectively, following the manufacturer's instructions. PBS containing 9.6 mM β-mercaptoethanol was used for all purification steps. For the elution of His-tagged protein, an imidazole step gradient (40 mM, 60 mM, 80 mM, 100 mM, 120 mM, and 200 mM) in elution buffer was applied with five column volumes in each step. Fractions containing FAPP2 were pooled and subsequently applied on a Superdex 200 HR 10/300 column (GE Healthcare) equilibrated in PBS with 9.6 mM β-mercaptoethanol. Protein containing fractions were pooled and concentrated by ultrafiltration in YM-100 units (Millipore). In case of GST-FAPP2, the size exclusion chromatography was optionally performed.

Lentivirus Production. EGFP-FAPP2 was cloned into the lentiviral pRRLSIN. cPPT. SFFV/GFP. WRPR (provided by Marino Zerial) using the restriction sites BamHI and SalI. The intrinsic GFP fusogenic site of the aforementioned lentiviral vector was removed through this insertion. The construct was further transfected into HEK 293T cells using helper plasmids pVSVG, pSPAX2 and Lipofectamine 2000 transfection reagent (Invitrogen). After 48 h of infection, supernatants bearing lentiviral particles were used to infect MDCK II epithelial cells. EGFP-FAPP2 expression was visible in 3 days, and the maximum expression was achieved within 5–6 days.

Immunostaining and Confocal Microscopy. Wild-type and TGN38-myc stable MDCK II cell lines were grown over night on coverslips, fixed, and immunostained as described elsewhere (1). Images were analyzed using LSM-510 META laser scanning confocal microscope with a plan-Apochromat 100x immersion oil objective (Carl Zeiss Jena). The antibodies were used in following dilutions: rabbit anti-furin (Affinity Bioreagents) 1:500, mouse anti-FAPP2 (clone A655-A08–2, prepared by the Antibody Facility, MPI-CBG Dresden) 1:100 and rabbit anti-myc (Santa Cruz) 1:500.

Surface Pressure Measurements of Lipid Monolayers. Monolayer tests were performed via the surface pressure measurement by the Wihelmy plate (Whatman Chr1), using a trough with a constant surface area 24 cm^2 (volume 35 ml) and a Nima tensiometer (Nima Technology) at room temperature. The monolayer was formed in a dropwise manner by the injection of a chloroform solution of a mixture of POPC:PI (4)P 98:2 mol% (1 mg/ml) onto the subphase. The measurements were performed after evaporation of the organic solvent (15 min), at an initial surface pressure of 30 mN/m (± 1 mN/m). WT-FAPP2 was dialyzed against assay buffer (25 mM Hepes, pH 7.25, 150 mM NaCl, 9.6 mM β-mercaptoethanol) and injected into the subphase to obtain the indicated concentration. The changes in the surface pressure were recorded after the system reached equilibrium (usually after 3 min). The protein concentration in the subphase was increased by stepwise injection after 5 min. As a control the respective volume of buffer was injected into the subphase.

Analytical Ultracentrifugation. A Beckman XL-I analytical ultracentrifuge (Beckman Coulter) using an eight-cell 50Ti rotor was used for the analytical utracentrifugation studies. For both velocity and equilibrium sedimentation experiments, samples of FAPP2 protein were prepared in Dulbecco's PBS including 9.6 mM β-mercaptoethanol. Sedimentation velocity experiments were carried out by centrifuging a two-sector cell at 40,000 rpm for 17 h at 4 °C. The absorbance of the sample was measured at a wavelength of 280 nm throughout the cell. A total of 126 measurements were taken of each sample during the run. These data were then analyzed by applying the c(s) routine in SEDFIT (2), with values for the partial specific volume of the protein, viscosity, and density of the buffer being calculated using SEDNTERP (3). The resulting values were then converted to $S^{o}_{20,W}$ using SEDNTERP.

Sedimentation equilibrium experiments were carried out by centrifuging a six-sector cell at 8,000, 11,000, and 14,000 rpm at 4 °C. Each speed was maintained for 20 h, and readings were then taken once per hour for the next 4 h. These scans were then compared to ensure that the sample had reached equilibrium. These data were analyzed using SEDPHAT (4), applying a single-species model with mass free to vary for the 3myc-FAPP2-His_6 and a two-species model with mass free to vary for the GST-FAPP2. The latter model was used to take into account a low-molecular-weight contaminant $<$1000 Da that made fitting with a single species model impossible. The prolate frictional ratios [*f/fp*] for both FAPP2 constructs were calculated using SEDNTERP.

1. Füllekrug J, Scheiffele P, Simons K (1999) VIP36 localisation to the early secretory pathway. *J Cell Sci* 112(Pt 17):2813–2821.
2. Schuck P (2000) Size-distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and lamm equation modeling. *Biophys J* 78:1606–1619.
3. Laue TM, Shah BD, Ridgeway TM, Pelletier SL (1992) in *Analytical Ultracentrifugation in Biochemistry and Polymer Science*, eds. Harding SE, Roew AJ, Horton JC (The Royal Society of Chemistry, Cambridge, UK), pp. 90–125.
4. Vistica J, et al. (2004) Sedimentation equilibrium analysis of protein interactions with global implicit mass conservation constraints and systematic noise decomposition. *Anal Biochem* 326:234–256.

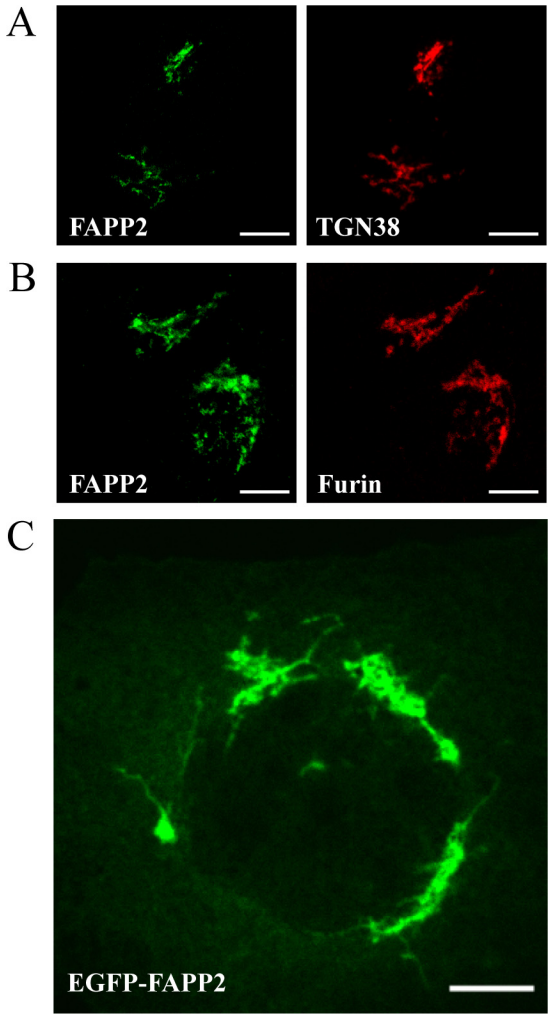


Fig. S1. Localization of FAPP2 at the TGN and its tubules. TGN38-myc stable cells were double-labeled with monoclonal A655-A08–2 anti-FAPP2 (green) and rabbit anti-myc (red) (*A*) and rabbit anti-furin (*B*) antibodies. (Scale bar, 10 μm.) (*C*) EGFP-FAPP2 lentiviral overexpression induced Golgi tubulation. (Scale bar, 5 μm.)

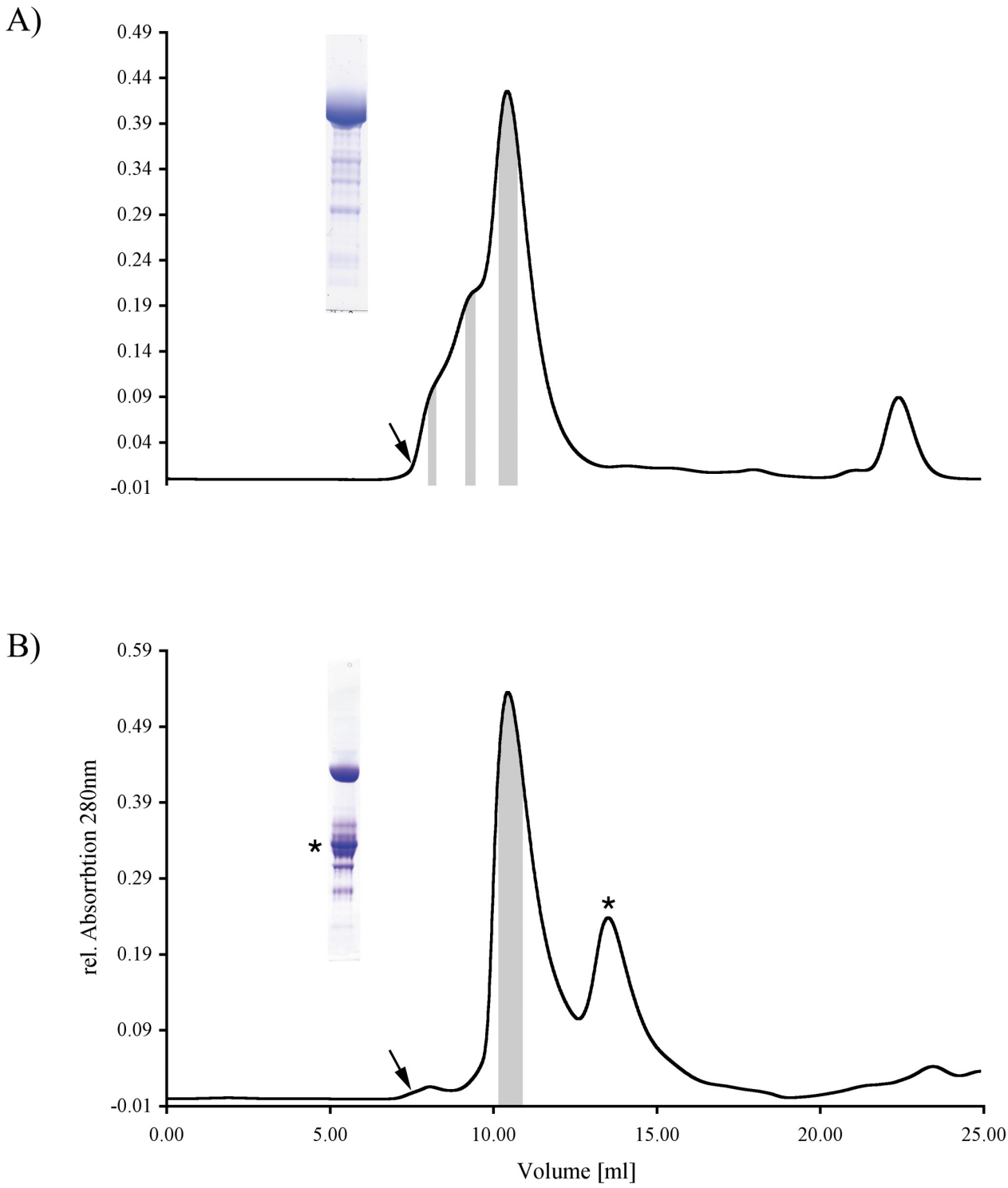


Fig. S2. Size exclusion chromatography profiles. Affinity purified proteins were loaded on Superdex-200 HR10/30 column after equilibrating with PBS and 9.6 mM β-mercaptoethanol. SDS/PAGE of GST-FAPP2 (*A*) or 3myc-FAPP2-His_6 (*B*) protein samples before SEC is depicted in *insets.* FAPP2 containing fractions are indicated by gray-shaded areas. For biochemical and structural experiments, only fractions after a retention volume of $\approx$10 ml were used. The GST-FAPP2 protein eluted as polydispersed protein indicated by additional shoulders at early retention volumes, whereas 3myc-FAPP2-His_6 eluted in a single peak. Second peak in the 3myc-FAPP2-His_6 profile represents low-molecular-weight impurities, labeled with asterisk. Black arrows indicate the column void volume. For SDS/PAGE of the purified proteins after SEC, see Fig. S3.

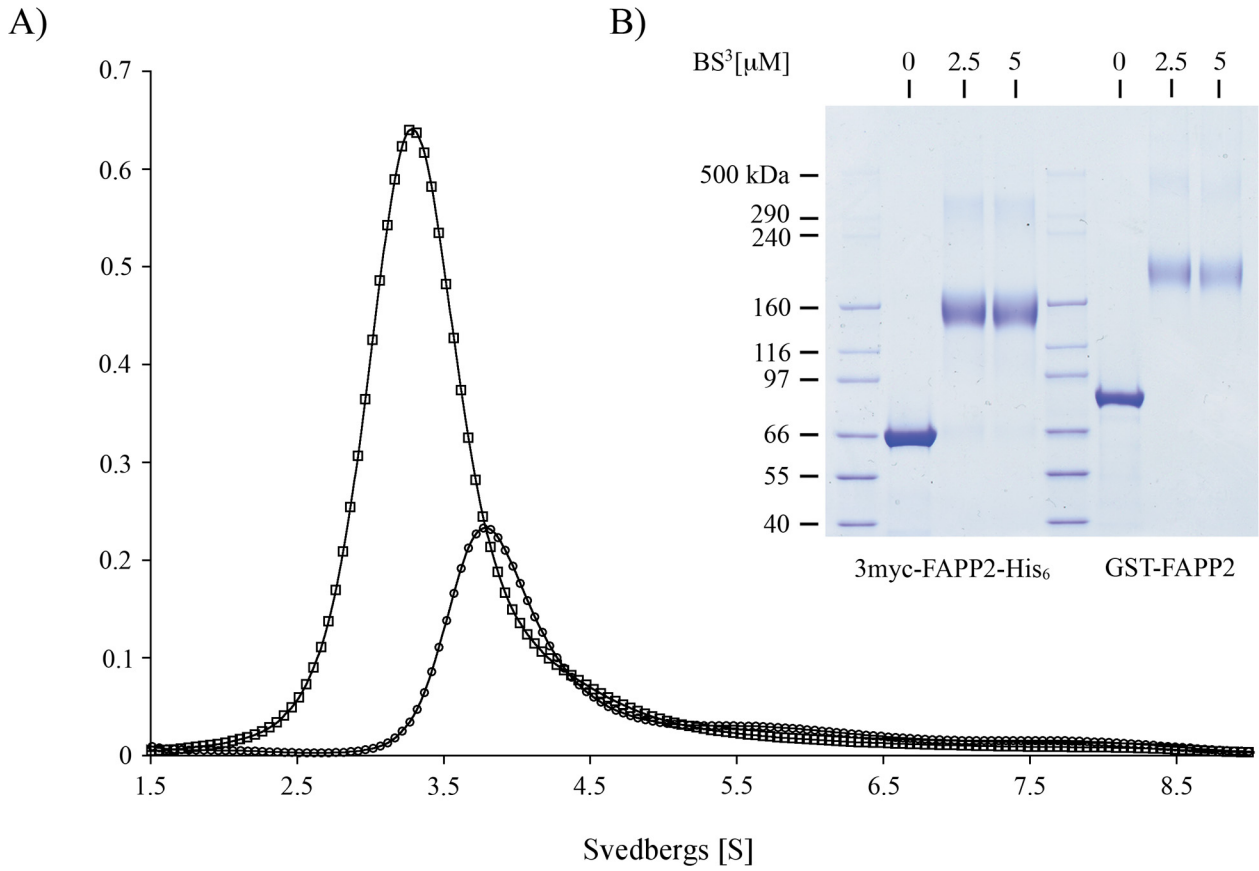


Fig. S3. 3mycFapp2-His6 and GST-FAPP2 are both dimeric in solution. (*A*) Sedimentation velocity reveals that both proteins are monodispersed in solution with S values of 3.31(3myc-FAPP2-His_6, squares) and 3.84 (GST-FAPP2, circles) respectively (see SI Materials and Methods). (*B*) GST-FAPP2 and 3myc-FAPP2-His_6 were incubated with the chemical cross-linker BS^3 at indicated concentrations at room temperature for 30 min. Cross-linking products were resolved on 3–8% NuPAGE Tris-Acetate Gradient gels.

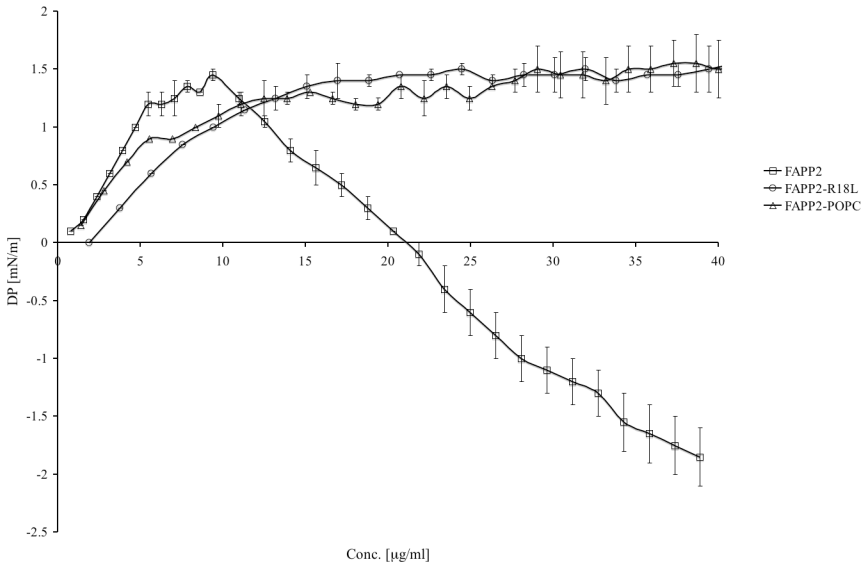
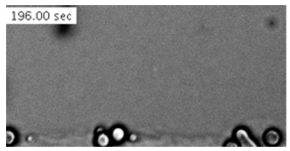
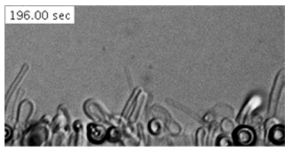


Fig. S4. FAPP2 removes lipids on a monolayer lipid surface. Surface pressure changes ($\Delta\Pi$) after injection of WT-FAPP2 and mutants in the subphase of POPC and PI (4)P (98:2 mol%) lipid monolayers. Isotherm was normalized to the initial established Π ($\approx$3 mN/m).



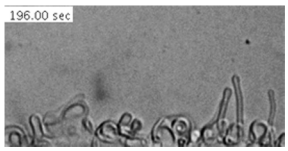
Movie S1. The PH domain of FAPP2 is involved in tubulation activity. PI(4)P binding deficient FAPP2-R18L lacks tubulation activity. Lipid mixture was consisting of POPC:PI(4)P:GlcCer (96:2:2 mol%). Tubulation was initiated by injection of 5 μl FAPP2 (1 mg/ml) into the reaction chamber.

Movie S1



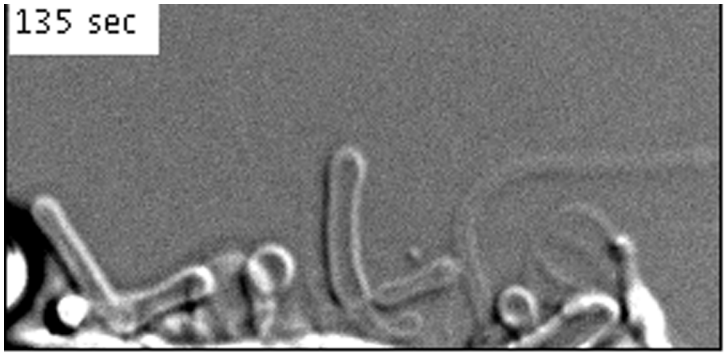
Movie S2. Addition of WT-FAPP2 into the same reaction chamber as in Movie S1 rescues membrane tubulation.

Movie S2



Movie S3. The glycolipid binding domain GLTP is not involved in the formation of membrane tubules. FAPP2-W407A, lacking GlcCer binding, displays tubulation activity as WT-FAPP2.

Movie S3



Movie S4. Tubulation activity of 3myc-FAPP2-His6 protein, which was used for AUC and SAXS analysis.

Movie S4

Table S1. Primers for GST-FAPP2 mutants

Mutant	Primer
R18L	Forward 5′- CTG AGC GGT TGG CAG CCT CTA TGG TTC CTA CTC TG −3′
	Reverse 5′- CAG AGT AGG AAC CAT AGA GGC TGC CAA CCG CTC AG −3′
W407A	Forward 5′- GCT ACA GAA GCC CTC TTG GCG CTG AAG AGA GGT CTC −3′

Table S2. Analytical ultracentrifugation results

	3myc-FAPP2-His_6	GST-FAPP2
S	3.31 ± 0.258	3.84 ± 0.184
$S^{o}_{20,w}$	5.25	5.97
MW from equilibrium	129 kDa ± 1.6	166 kDa ± 3.3
Prolate frictional ratio *(f/fp)*	1.71	1.76