Supporting Information

Lees et al. 10.1073/pnas.1718471115

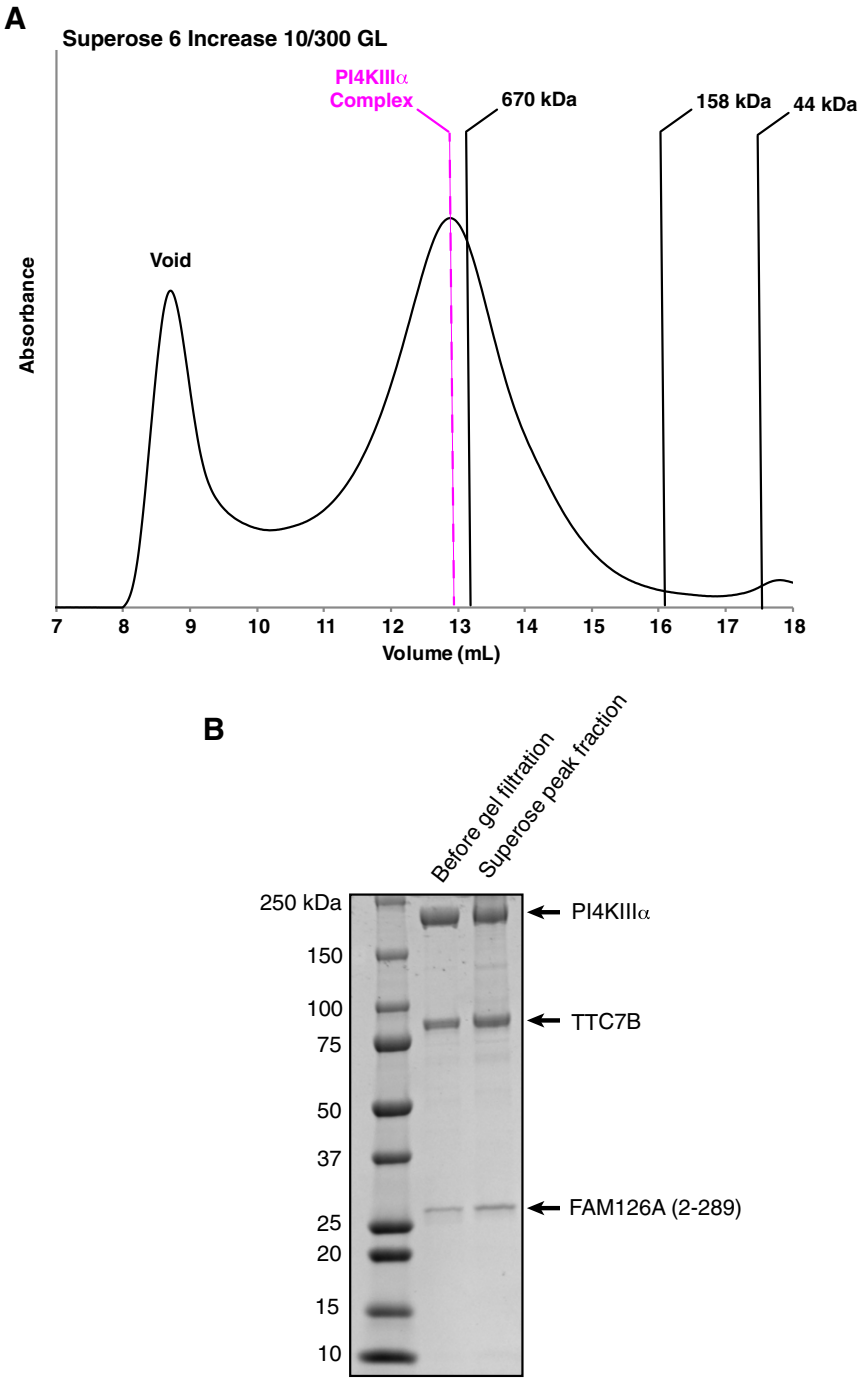

Fig. S1. The PI4KIIIα/TTC7B/FAM126A complex behaves as a homodimer in solution. (*A*) Purified PI4KIIIα/TTC7/FAM126 complex was analyzed on a Superose 6 Increase 10/300 GL size exclusion column. Peak elution volumes for the complex, as well as molecular weight standards, are indicated. The estimated molecular weight of the complex, based on this analysis, is ~700 kDa. (*B*) Superose 6 peak fractions contain all three complex components in equal stoichiometry. Anti-FLAG-purified PI4KIIIα/TTC7/FAM126 complex was analyzed by SDS/PAGE before and after gel filtration on the Superose 6 Increase column. The complex components are present in approximately equal amounts after gel filtration. Note slight enrichment of TTC7 and FAM126 due to removal of excess PI4KIIIα.

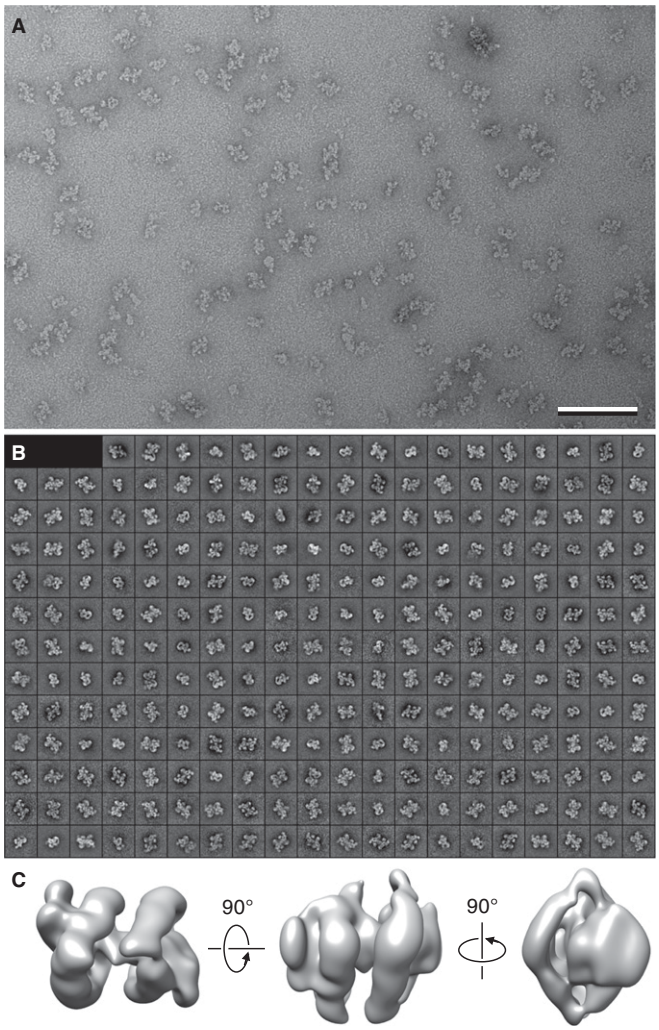

Fig. S2. Initial model generation using images of negatively stained PI4KIIIα/TTC7/FAM126 complex. (*A*) Representative image of negatively stained PI4KIIIα/TTC7/FAM126 complex. (Scale bar, 100 nm.) (*B*) The 257 2D class averages obtained with the iterative stable alignment and classification (ISAC) algorithm. Side length of individual averages is 42 nm. (*C*) Different views of the initial model generated with the validation of individual parameter reproducibility (VIPER) algorithm using the averages shown in *B*.

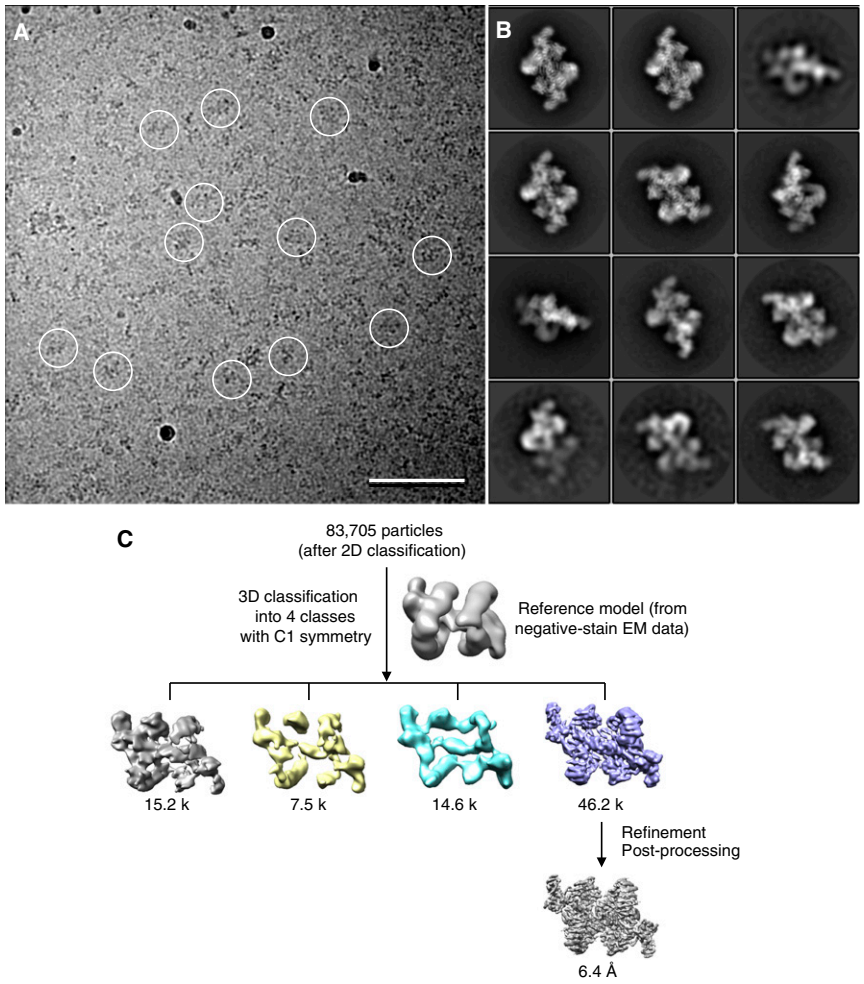

Fig. S3. Intermediate-resolution cryo-EM density map obtained with a small dataset recorded with an FEI Talos Arctica electron microscope. (*A*) Representative cryo-EM image of vitrified PI4KIIIα/TTC7/FAM126 complex recorded with a Talos Arctica electron microscope. Some particles are circled. (Scale bar, 100 nm.) (*B*) Selected 2D averages generated by 2D classification in Relion. Side length of individual averages is 42 nm. (*C*) Image processing workflow that yielded a density map at 6.4-Å resolution. See *Materials and Methods* for details.

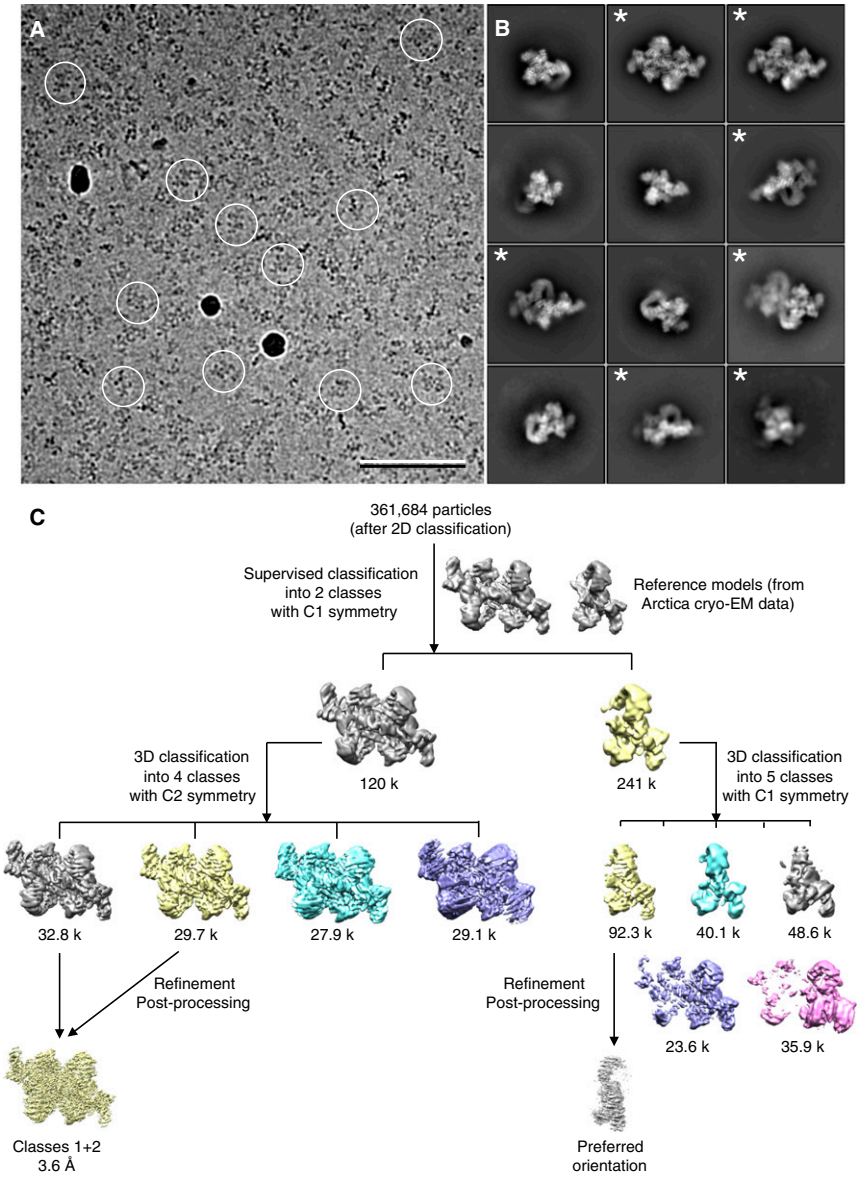

Fig. S4. High-resolution cryo-EM density map obtained with a dataset recorded with an FEI Titan Krios electron microscope. (*A*) Representative cryo-EM image of vitrified PI4KIIIα/TTC7/FAM126 complex recorded with a Titan Krios electron microscope. Some particles are circled. (Scale bar, 100 nm.) (*B*) Selected 2D averages generated by 2D classification in Relion. Side length of individual averages is 41.6 nm. Asterisks indicate classes representing PI4KIIIα complex dimers. (*C*) Image processing workflow that yielded a density map at 3.6-Å resolution. See *Materials and Methods* for details.

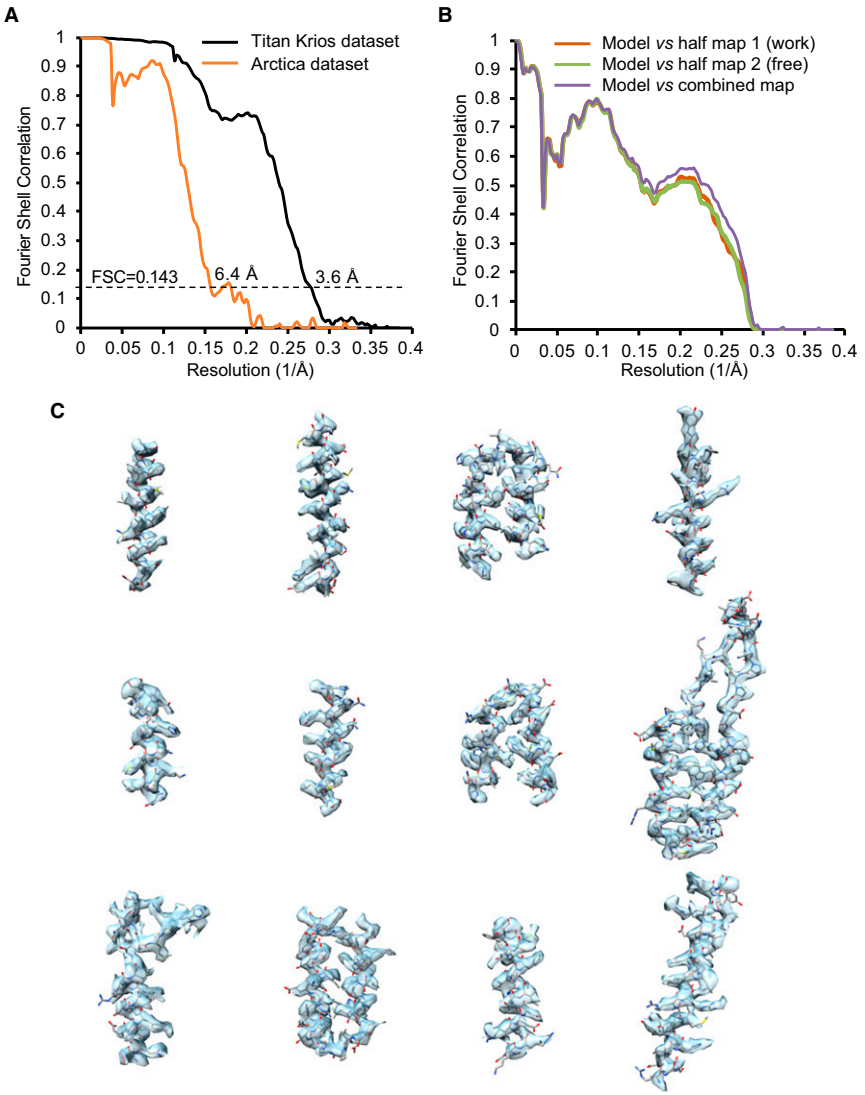

Fig. S5. FSC curves and model-density fit. (*A*) Gold-standard FSC curve calculated between independently refined half maps. (*B*) Cross-validation FSC curves: red (work), refined model versus half map 1 (used for test refinement); green (free), refined model versus half map 2 (not used for test refinement); purple, refined model versus the combined final map. The similarity of the "work" and "free" curves suggests no substantial overfitting. (*C*) Example map density and segments of the fitted model are shown from several regions of the cryo-EM map into which PI4KIIIα was built de novo.

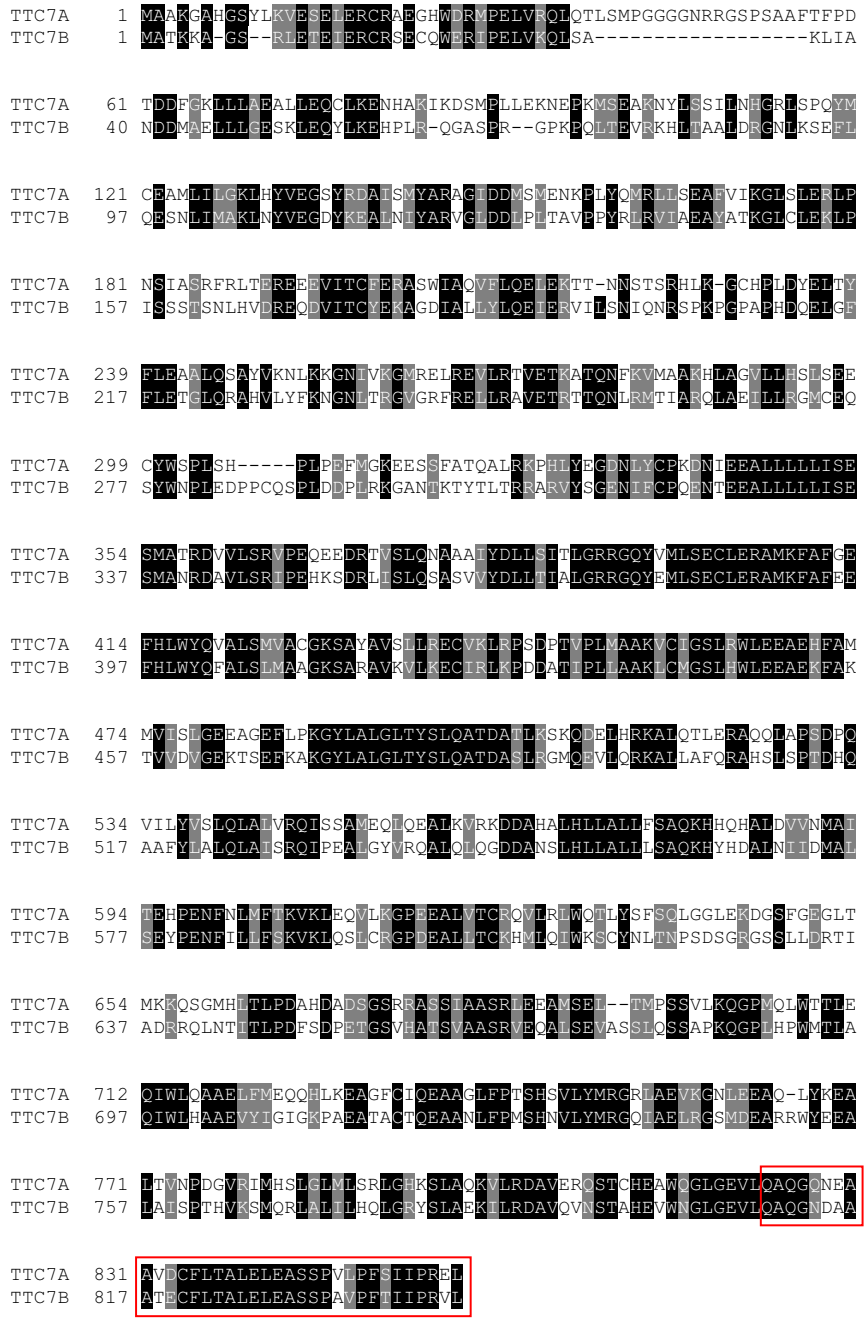

Fig. S6. Sequence alignment between A and B isoforms of TTC7. Identical residues are highlighted in black and similar residues in gray. The region of TTC7A deleted in patient 4 and the corresponding aligned region of TTC7B are indicated by a red box.

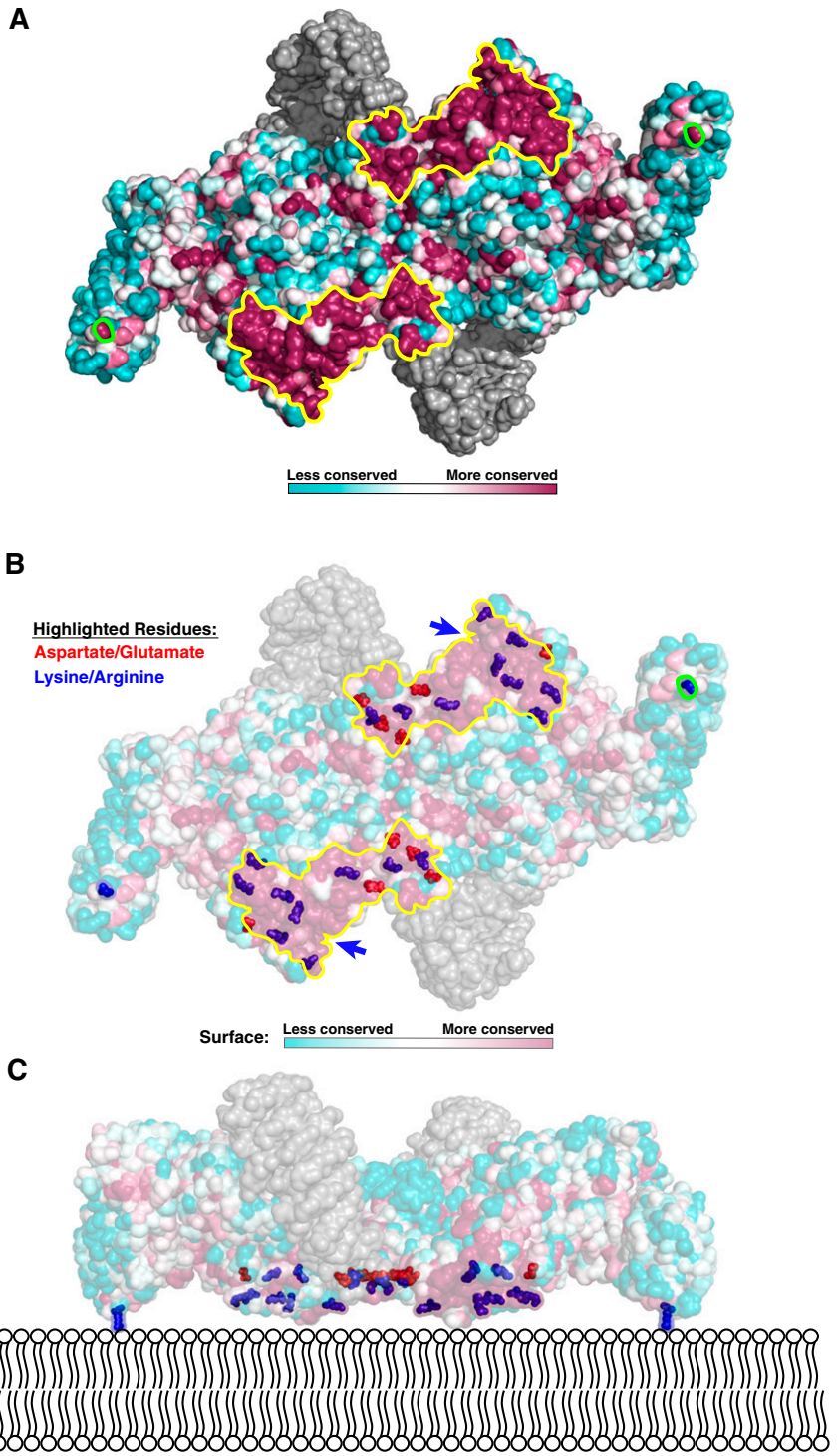

Fig. S7. The conserved membrane-proximal surface of PI4KIIIα complex carries an excess of basic residues. (*A*) Solvent-accessible surface representation of the PI4KIIIα complex, colored by sequence conservation. Hotspots of conservation in close proximity to the membrane are outlined on PI4KIIIα and TTC7B in yellow and green, respectively. (*B*) Positively and negatively charged amino acids occurring in the highlighted areas, identical to those in *A*, are indicated in sphere representation, colored blue and red, respectively. The overall structure of the complex, colored by conservation, is overlaid as a semitransparent surface. The active site cleft of each catalytic domain is indicated by a blue arrow. (*C*) Same as in *B*, but rotated by 90°, juxtaposed with a cartoon representation of the membrane, approximately to scale, to indicate relative distance of the highlighted residues from the membrane.

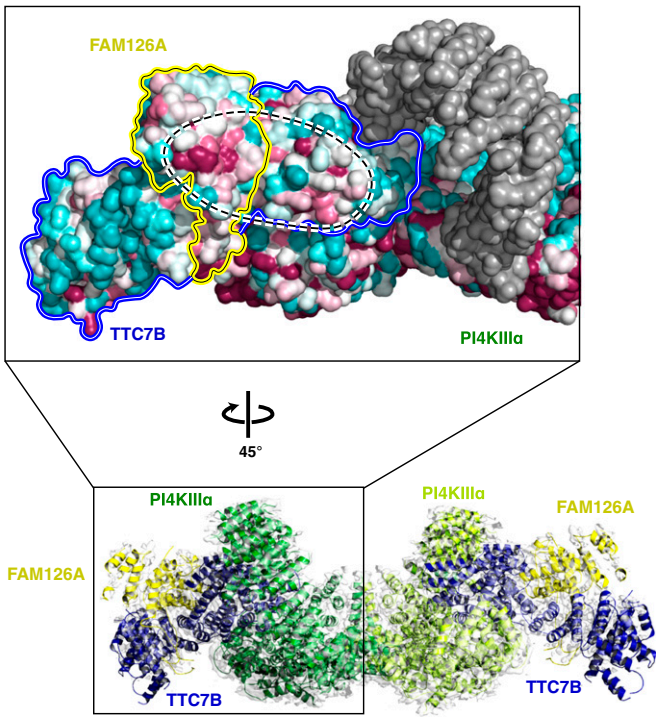

Fig. S8. A candidate EFR3-binding surface on TTC7/FAM126. FAM126 and the C-terminal half of TTC7 form a conserved surface, enclosed in the *Inset* by a black and white dotted line, which is accessible and a candidate for an EFR3-binding site. FAM126 and TTC7 are outlined in yellow and blue, respectively.