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Published in:
Science

DOI:
[10.1126/science.1253397](https://doi.org/10.1126/science.1253397)

Publication date:
2014

Document Version
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Burke, J. E., Inglis, A. J., Perisic, O., Masson, G. R., McLaughlin, S. H., Rutaganira, F., Shokat, K. M., & Williams, R. L. (2014). Structures of PI4KIII complexes show simultaneous recruitment of Rab11 and its effectors. *Science*, *344*(6187), 1035-1038. <https://doi.org/10.1126/science.1253397>

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Published in final edited form as:

Science. 2014 May 30; 344(6187): 1035–1038. doi:10.1126/science.1253397.

Structures of PI4KIII β complexes show simultaneous recruitment of Rab11 and its effectors

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Abstract

Phosphatidylinositol 4-kinases (PI4Ks) and small guanosine triphosphatases (GTPases) are essential for processes that require expansion and remodeling of phosphatidylinositol 4-phosphate (PI4P)-containing membranes, including cytokinesis, intracellular development of malarial pathogens, and replication of a wide range of RNA viruses. However, the structural basis for coordination of PI4K, GTPases and their effectors is unknown. Here, we describe structures of PI4KB (PI4KIII β) bound to the small GTPase Rab11a without and with the Rab11 effector protein FIP3. The Rab11-PI4KIII β interface is unique compared with known structures of Rab complexes, and does not involve switch regions used by GTPase effectors. Our data provide a mechanism for how PI4KIII β coordinates Rab11 and its effectors on PI4P-enriched membranes, and also provide strategies for the design of specific inhibitors that could potentially target plasmodial PI4KIII β to combat malaria.

Intracellular compartments are essential to eukaryotic cell biology, and both small GTPases and lipids such as phosphoinositides are key components of compartment identity (1, 2). The phosphatidylinositol 4-kinases (PI4Ks) and the small G-protein Rab11 play prominent roles in compartment identity. PI4KIII β is one of four mammalian PI4K enzymes that phosphorylate phosphatidylinositol to generate phosphatidylinositol 4-phosphate (PI4P). PI4KIII β localizes primarily at the Golgi and is essential for Golgi formation and function (3-5). PI4P is recognized by protein modules including the PH domains of oxysterol-binding protein (OSBP), ceramide transfer protein (CERT) and four-phosphate-adaptor protein (FAPP) that are important for intra-Golgi transport (6-8). However, typically, lipid recognition alone is not sufficient for Golgi localization, which requires both PI4P and

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Author contributions J.E.B., O.P. and R.L.W. initiated the project and designed the experiments. J.E.B., A.J.I., G.R.M. and O.P. carried out the experiments. J.E.B. and R.L.W. carried out the structure determination and analysis. S.H.M. carried out SPR analysis. F.R. and K.M.S. synthesized PI4K inhibitors. J.E.B., O.P. and R.L.W. wrote the manuscript.

Supplementary Materials:

Materials and Methods

Figures S1-S9

Table S1

References (32-42)

specific small GTPases. In addition to its catalytic role in synthesizing PI4P, PI4KIII β also has non-catalytic roles that rely on the interactions with other proteins such as the small GTPase Rab11 (9). Rab11 is predominately located on recycling endosomes (10). However, Rab11 is also found associated with Golgi membranes, which requires an interaction with PI4KIII β (9).

PI4KIII β activity is essential for replication of a range of RNA viruses, including enteroviruses, SARS coronavirus and hepatitis C virus (11, 12). These RNA viruses hijack the activity of host cell PI4KIII β to generate replication organelles enriched in PI4P. There is no approved anti-viral therapy for enteroviruses. However, several compounds inhibit enteroviral replication by targeting cellular PI4KIII β (13, 14). PI4KIII β is also important in malaria, and inhibitors of *Plasmodium falciparum* PI4KIII β are potent anti-malarial agents. However, mutations in both PI4KIII β and Rab11 confer resistance to these compounds (15). Inhibition of the *P. falciparum* PI4KIII β prevents the membrane ingression that occurs during completion of the asexual erythrocytic stage of the plasmodial lifecycle. The role of *Plasmodium* Rab11 and PI4KIII β in membrane remodeling is similar to the role of Rab11 and PI4KIII β in cytokinesis in *Drosophila* spermactocytes (16). In *Drosophila*, PI4KIII β is required for the recruitment of both Rab11 and its downstream effectors.

To understand how PI4KIII β can both recruit Rab11 and enable its interactions with Rab11 effectors, we used a hydrogen deuterium exchange mass spectrometry (HDX-MS) to facilitate the x-ray crystal structure determination of human PI4KIII β in complex with Rab11a-GTP γ S at 2.9 Å resolution (see supplementary materials and methods). To form crystals, highly flexible regions of PI4KIII β identified by HDX-MS were truncated (residues 1-120, 408-507, and 785-801, Fig. 1A). This included a C-terminal region necessary for catalytic activity (fig. S2A). The PI4KIII β structure consists of two domains, a right-handed helical solenoid (residues 128-243) and a kinase domain (residues 306-801) (Fig. 1B) that are related to the PI3Ks (fig. S3). The kinase domain has two lobes, an N-terminal lobe dominated by a five-stranded anti-parallel β -sheet and a C-terminal lobe that is largely helical, with the ATP-binding site located in a cleft between the lobes. The N-lobe of PI4KIII β has a PI4KIII β -unique, large insertion (residues 391-539) (Fig. 1B).

PI4KIII β makes a unique interaction with Rab11a that is not characteristic of any Rab or Arf effector (17). This interaction involves primarily helix α 3 of the PI4KIII β helical domain (Fig. 1B and 2A). Because a previous study of human PI4KIII β mapped the epitope interacting with Rab11a to the insertion in the N-lobe of the kinase domain (9), we also used HDX-MS to determine the Rab11 binding site on full-length PI4KIII β in solution (figs. S4 and S5). The HDX-MS measurements confirmed the interaction between the N-terminal part of the PI4KIII β helical domain and Rab11a (Fig. S5). Soluble Rab11 had no effect on PI4KIII β activity (fig. S2B).

The PI4KIII β -Rab11 interface is predominately hydrophobic with no hydrogen bonds or salt links (Fig. 2A). Surprisingly, PI4KIII β makes only a single, peripheral contact with the Rab11 switch I (only with the edge residues 38-LES-40 of switch I) and no contact with switch II (Fig. 2A and B). The “switch” regions change conformation depending on whether Rab11 is bound to GTP or GDP. Consistent with the structure, PI4KIII β bound both GTP-

and GDP-loaded Rab11, with a slight preference for GTP (Fig. 2D and fig. S6). Within the PI4KIII β -Rab11 interface, G155 (18) of PI4KIII β makes a direct contact with the ribose moiety of GTP γ S bound to the Rab11 (Fig. 2A). The small residue at position 155 is completely conserved among PI4KIII β homologs, from yeast to mammals, and might be required to maintain the contact with Rab11. Indeed, a PI4KIII β -G155D mutation decreased binding to Rab11 (Fig. 2C and D). Consistent with PI4KIII β directly contacting the nucleotide, PI4KIII β decreased the rate of EDTA-mediated Rab11 nucleotide exchange (Fig. S2C). PI4KIII β residues Y159, N162 and F165 also form prominent contacts with the Rab11. The mutants PI4KIII β -Y159A, N162A, F165A and the double mutant Y159A/N162A all eliminated measurable binding to Rab11 (Fig. 2C and D). The Rab11 residues interacting with PI4KIII β are all conserved among Rab11a and Rab11b, but not among other Rabs that do not bind PI4KIII β (Fig. 2B). The PI4KIII β residues interacting with Rab11 are also strongly conserved among PI4KIII β orthologs (Fig. 2B, fig. S3).

The structure of the PI4KIII β -Rab11a complex shows that the Rab11a switch regions remained available to contact Rab11a effectors. Among the Rab11 effectors is a family of related proteins known as the Rab11 family interacting proteins or FIPs (19). In *Drosophila*, both catalytic and non-catalytic functions of Four wheel drive (PI4KIII β ortholog in *Drosophila*) are required for the proper localization of both Rab11a and its downstream effector Nuf (ortholog of human FIP3) at the cleavage furrow, which is essential for cytokinesis in spermatocytes (16). During interphase, FIP3 is required for the structural integrity of the pericentriolar recycling endosome compartment (20), whereas during cytokinesis it is involved in delivering material from recycling endosomes to the cleavage furrow (21). The FIP proteins interact with Rab11 using a conserved C-terminal region known as the Rab-binding domain (RBD). Two FIPs form a parallel coiled-coil dimer with two Rab11 binding sites (22-24) that engage both switch I and switch II of Rab11.

The structure of Rab11a bound to the RBD domain of FIP3 (22-24) and our structure of the PI4KIII β -Rab11a complex suggest that a ternary complex might be formed. Indeed, GST pull-downs carried out with the GST-tagged RBD domain from FIP3 (residues 713-756) revealed that the complex of the FIP3-RBD with GTP γ S-loaded Rab11a (Q70L) was able to bind PI4KIII β (Fig. 3A). To understand how this occurs, we crystallized a ternary PI4KIII β /Rab11a/FIP3-RBD complex. Although this is a low-resolution (6 Å) structure with a very large asymmetric unit (~ 1 MDa, containing 12 ternary complexes), both FIP3 and PI4KIII β were bound simultaneously to Rab11a-GTP γ S (Fig. 3B, fig. S7). The ability of PI4KIII β to recruit both Rab11a and its downstream effectors is unlikely to be limited to only the FIP family of proteins. The production of PI4P by Pik1, the yeast ortholog of PI4KIII β , is required for the Ypt32 (yeast ortholog of Rab11) dependent recruitment of the Rab guanine nucleotide-exchange factor Sec2 (25), in a phosphoinositide-dependent regulation of Rab activation cascade. The conservation of the PI4KIII β -Rab11a interface in Pik1 and Ypt32 indicates the possibility of a ternary Pik1-Ypt32-Sec2 complex. Furthermore, the switch-independent interaction of PI4KIII β with Rab11 suggests that ternary PI4KIII β /Rab11/RabGAP and PI4KIII β /Rab11/Rab Escort complexes could also be formed (fig. S8).

The inhibitor PIK93 shows a clear selectivity for PI4KIII β over PI4KIII α , with partial selectivity over PI3Ks (26). This inhibitor has been used to decipher PI4K-specific functions

(27), for example in demonstrating the role of PI4KIII β in viral replication (11). PIK93 bound in the ATP-binding pocket, using its thiazol and acetamide moieties to make a pair of hydrogen bonds with the backbone of V598 in the hinge between the N- and C-lobes (Fig. 4A, fig. S9A). The PI4KIII β ATP-binding pocket and those of PI3Ks and phosphatidylinositol 3-kinase-related kinases (PIKKs) show considerable consistency. Most of the contacts that PIK93 makes with PI4KIII β involve residues that are conserved among both the PI3Ks and PI4Ks (Fig. 4A and C). The PIK93 sulfamoyl moiety makes a hydrogen bond with K549 of PI4KIII β . Although this lysine is conserved in the PI3Ks, its conformation is variable and in the structures of PIK93 in complex with either p110 γ (26) or Vps34 (28), the equivalent lysine does not make this hydrogen bond (fig. S9B). There is more sequence divergence in the residues near the hinge. Though the class III PI3K Vps34 is more closely related to PI4KIII β than other PI3Ks, PIK93 is a poor inhibitor of Vps34. The PI4KIII β has a great deal of space near the hinge that allows PIK93 to form hydrogen bonds with the hinge and simultaneously optimize contacts elsewhere (fig. S9B). Y583 packs against the hinge to form one wall of the pocket contacting both the Cl substituent of the thiazole and the phenyl moiety. The structure helps rationalize why a Y583M mutation makes PI4KIII β insensitive to wortmannin and PIK93 (29) (fig. S9C).

Inhibitors specific against plasmodial PI4KIII β are potent anti-malarial agents and resistance mutations in either Rab11 α or PI4KIII β can evade the anti-malarial activity of these compounds (15). The plasmodial Y1356F resistance mutation involves the hinge residue, which is equivalent to human P597. It may be that Y1356 makes an additional interaction with the inhibitor (Fig. 4B and D). The S1320L resistance mutation corresponds to F561 in human PI4KIII β . This residue packs against Y583 (Y1342 in *P. falciparum*), which forms one wall of the inhibitor-binding pocket, and the mutation may alter the conformation of the pocket. The third mutation, H1484Y is in a bend between α 8 and α 9 and is equivalent to H728 in human PI4KIII β . Several somatic mutations associated with cancer have been detected for human p110 α PI3K in the equivalent bend, and the most common of these mutations increases enzymatic activity and membrane binding (30). It is plausible that the H1484Y resistance mutation is making PI4KIII β more active.

The structures of a binary complex of PI4KIII β with Rab11 α and a ternary complex of PI4KIII β with Rab11 α and Rab11-effector FIP3 revealed a novel Rab11 interface that is compatible with the PI4KIII β -driven recruitment of Rab11 and its effectors to PI4P-enriched membranes. PI4KIII β plays key roles in regulating Rab11 α in cytokinesis of spermatocytes, recruitment of Rab-guanine nucleotide exchange factors in yeast, and membrane remodeling in *Plasmodium* development. The PI4KIII β -Rab11 interface is conserved and demonstrates that PI4K, in addition to its kinase activity, plays key kinase-independent roles in mediating these membrane trafficking events. This structure opens up exciting prospects for the development of highly specific inhibitors, which may act as potent anti-malarial and anti-viral therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank C. Mueller-Dieckmann and D. De Sanctis at the European Synchrotron Radiation Facility and C. Lobley at Diamond Light Source for assistance with beamlines ID23-EH2, ID29 and I04-1; S. Maslen, M. Skehel and S.Y. Peak-Chew for help with HDX-MS setup and M. Yu for help with x-ray data collection. J. Burke was supported by a grant from the British Heart Foundation (PG11/109/29247) and F. Rutaganira by an NSF-predocorral fellowship. This work was funded by the NIH (RO1AI099245 to KMS) and UK Medical Research Council (MC_U105184308 to RLW). KMS has filed for patent protection on PIK93 analogs in the following application between UCSF and Stanford University, PCT/US2012/059023. Coordinates and structure factors have been deposited in the Protein Data Bank with accession numbers 4D0L and 4D0M, for PI4KIII β /Rab11a and PI4KIII β /Rab11a/FIP3 complexes, respectively.

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18. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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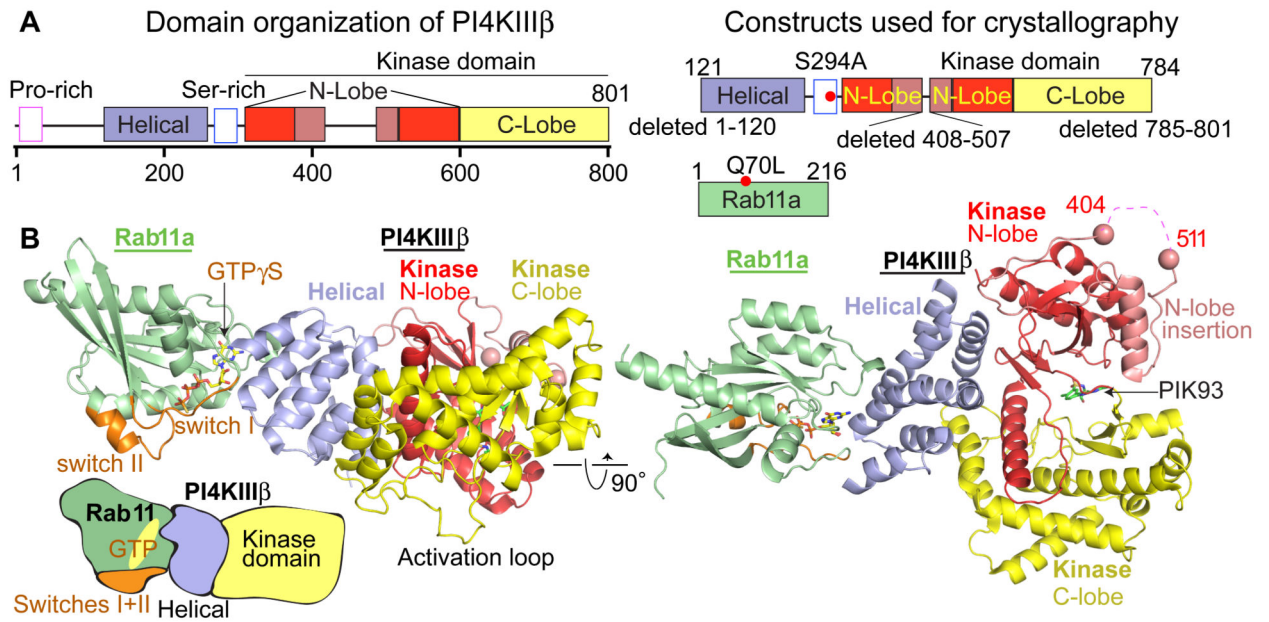


Fig. 1. Crystal structure of human PI4KIII β complex with Rab11a(Q70L)-GTP γ S
(A) Full-length PI4KIII β (isoform 2) (left) and constructs of PI4KIII β and Rab11a(Q70L) used for crystallization (right). The PI4KIII β crystallization construct contained three deletions as well as an S294A mutation. **(B)** Overall architecture of the complex. The PI4K-specific insertion (residues 391-540) in the N-lobe is salmon colored. The large spheres mark a disordered region within which residues 408-507 are deleted. The switch I and switch II regions of Rab11a(Q70L) are represented in orange.

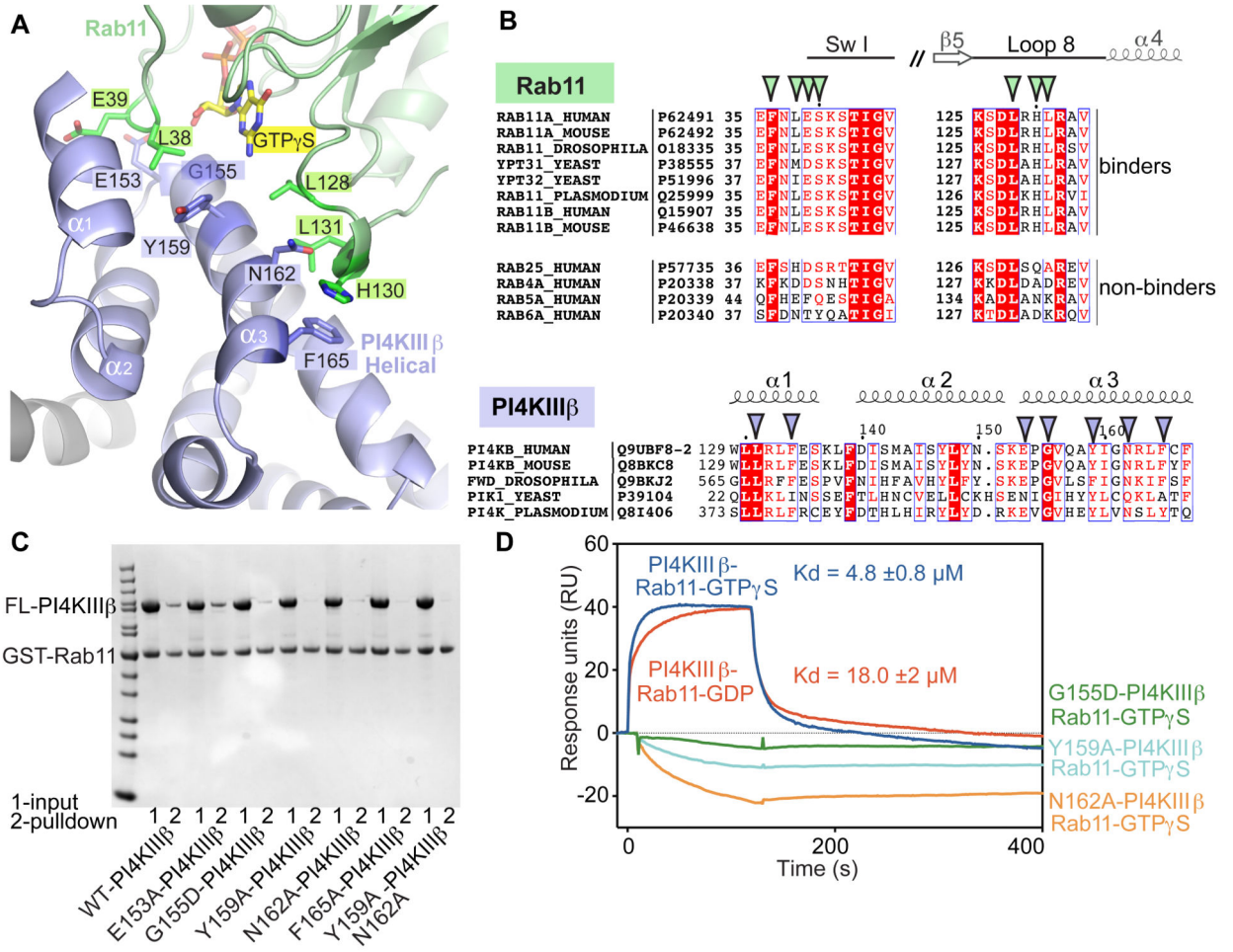


Fig. 2. The PI4KIIIβ/Rab11a interface

(A) Close-up view of the PI4KIIIβ/Rab11a interface. (B) Sequence alignment of interacting regions in Rabs and PI4KIIIβs. Conserved and similar residues are highlighted (red shading and red letters, respectively). Rab11a residues that interact with PI4KIIIβ are indicated with green arrowheads; light blue arrowheads denote PI4KIIIβ residues interacting with Rab11. Rab sequences are grouped into Rabs previously shown to either bind PI4KIIIβ (binders) or not bind PI4KIIIβ (non-binders). (C) Pull-down assays with GST-tagged Rab11A(Q70L)-GTPγS and either wild-type or mutant full-length PI4KIIIβ. The inputs and the bound proteins (lanes 1 and 2, respectively) were analysed on SDS gels stained with InstantBlue. (D) Surface plasmon resonance (SPR) analysis of the full length wild-type PI4KIIIβ binding to the immobilized GST-tagged Rab11a(Q70L) loaded with either GDP (red sensogram) or GTPγS (blue). The affinity of PI4KIIIβ for GST-Rab11a is indicated next to the graphs (data are mean ± SEM based on five independent experiments). Full details are shown in Fig. S6. Also shown are the sensograms for several PI4KIIIβ mutants binding to Rab11a(Q70L)-GTPγS.

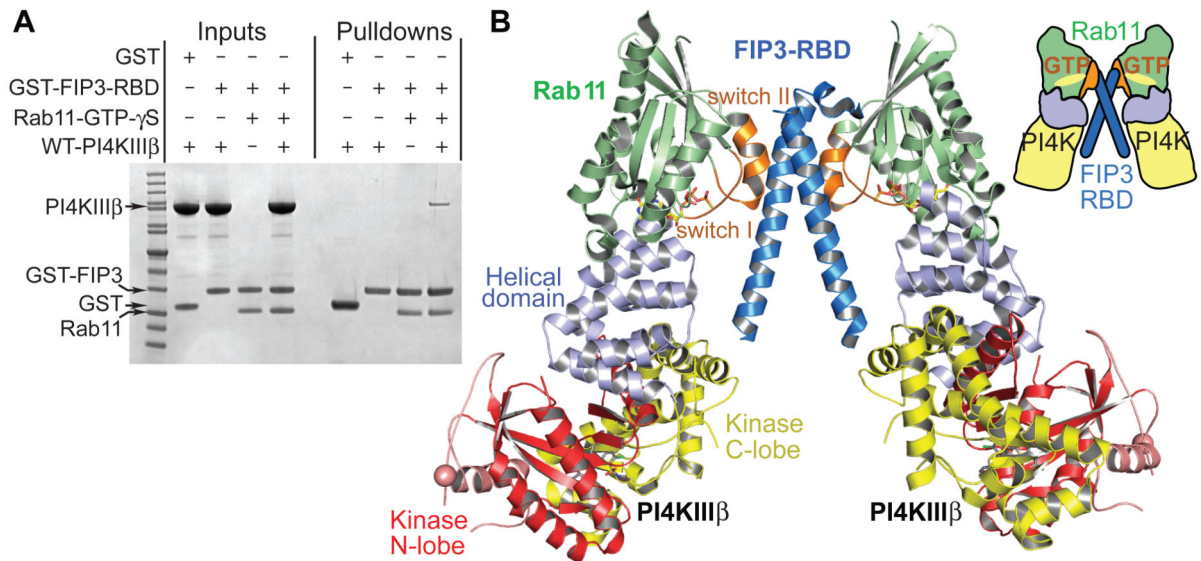


Fig. 3. Ternary complex of PI4KIII β with Rab11a-GTP and effector protein FIP3
(A) Pulldown assays with GST-tagged-FIP3 fragment (residues 713-756) and full length PI4KIII β either with or without Rab11a(Q70L)-GTP γ S. The inputs and the bound proteins were analyzed on SDS gels stained with InstantBlue. **(B)** Crystal structure of the ternary complex of PI4KIII β , Rab11a-GTP γ S and the FIP3 RBD domain.

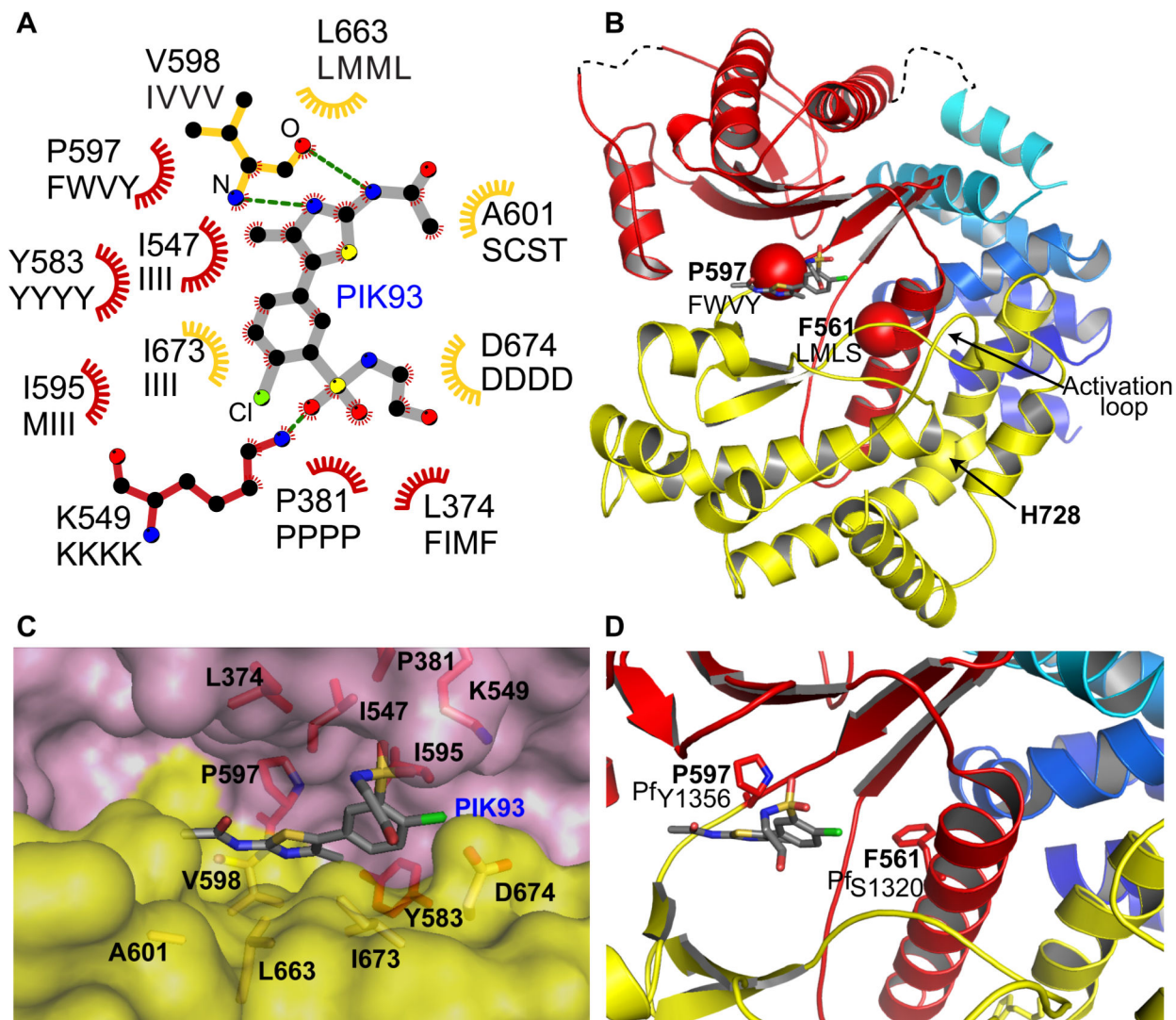


Fig. 4. Inhibitor binding to PI4KIII β

(A) Interactions of PI4KIII β with PIK93. Dotted lines represent putative hydrogen bonds (prepared by LIGPLOT (31)). For each PI4KIII β residue, the equivalent residues are shown below for human Vps34, human mTOR, human p110 α and *P. falciparum* PI4KIII β (left to right). (B) Ribbon diagram of PI4KIII β illustrating sites of *P. falciparum* resistance mutations (spheres). The helical domain is colored from dark to light blue from N-terminus to the C-terminus. (C) PIK93 bound to PI4KIII β . (D) Close-up of the active site, illustrating positions of *P. falciparum* resistance mutations.