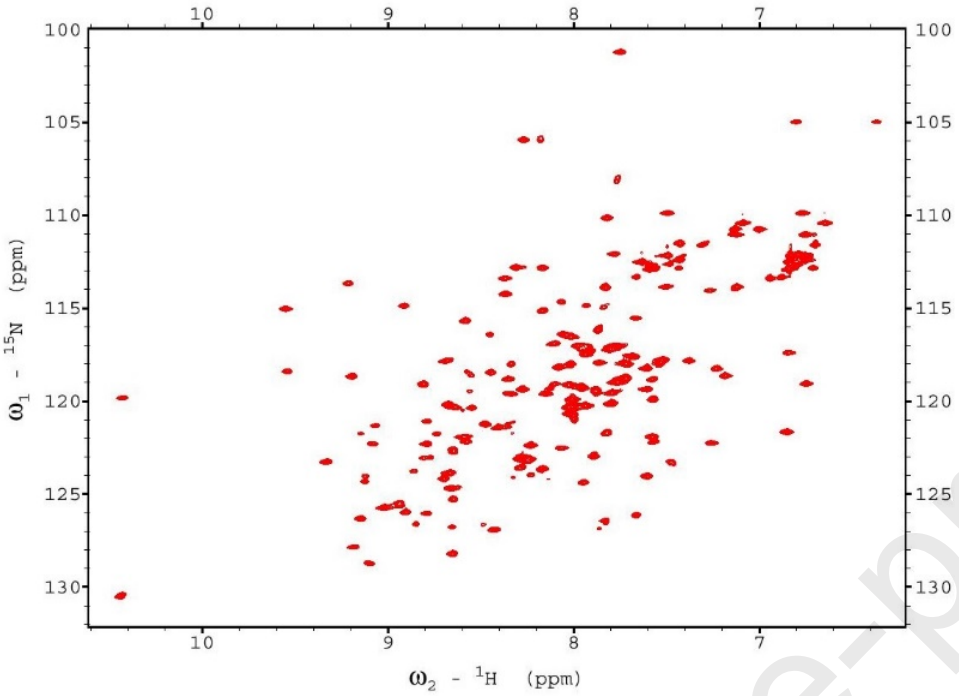
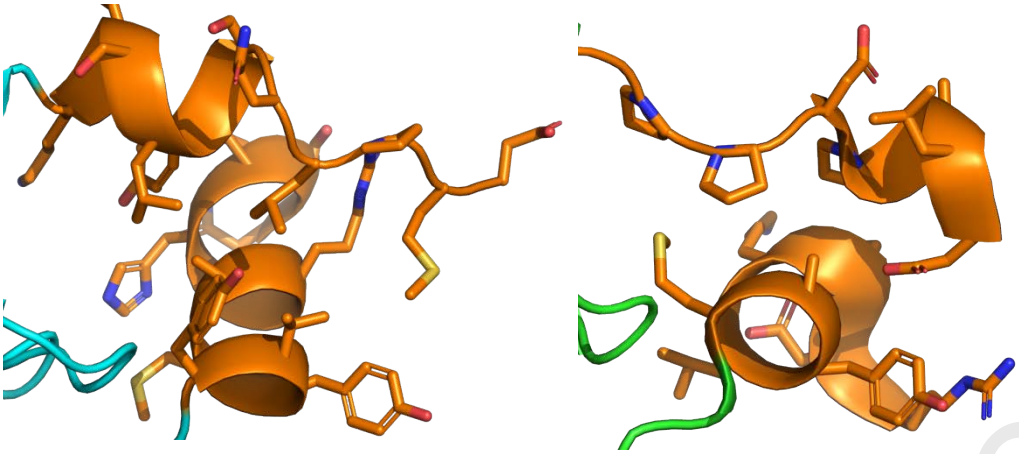
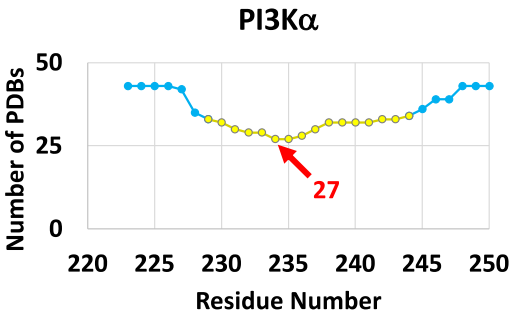
Supplementary Figures



Supp. Figure 1. ^{1}H-^{15}N 2D NMR HSQC spectrum of 157 µM ^{13}C/^{15}N-enriched PI3Kα-RBD (red) in 20 mM HEPES, 50 mM NaCl, 10 mM dithiothreitol, pH 6.8 acquired at 850 MHz and 25 °C. The spectrum is well dispersed indicative of a well folded protein with most NH resonances detectable.

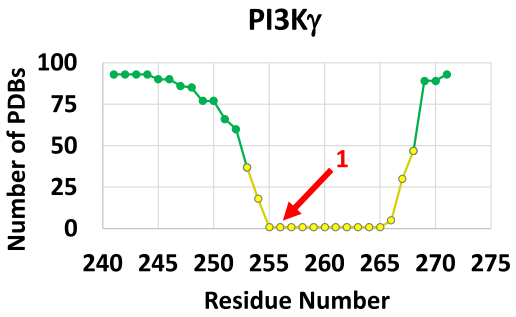


Supp. Figure 2. Amino acids are displayed for the termini of the PI3Kα (left) and PI3Kγ (right) RBD structures.



Resolution of crystal structures of PI3Kα

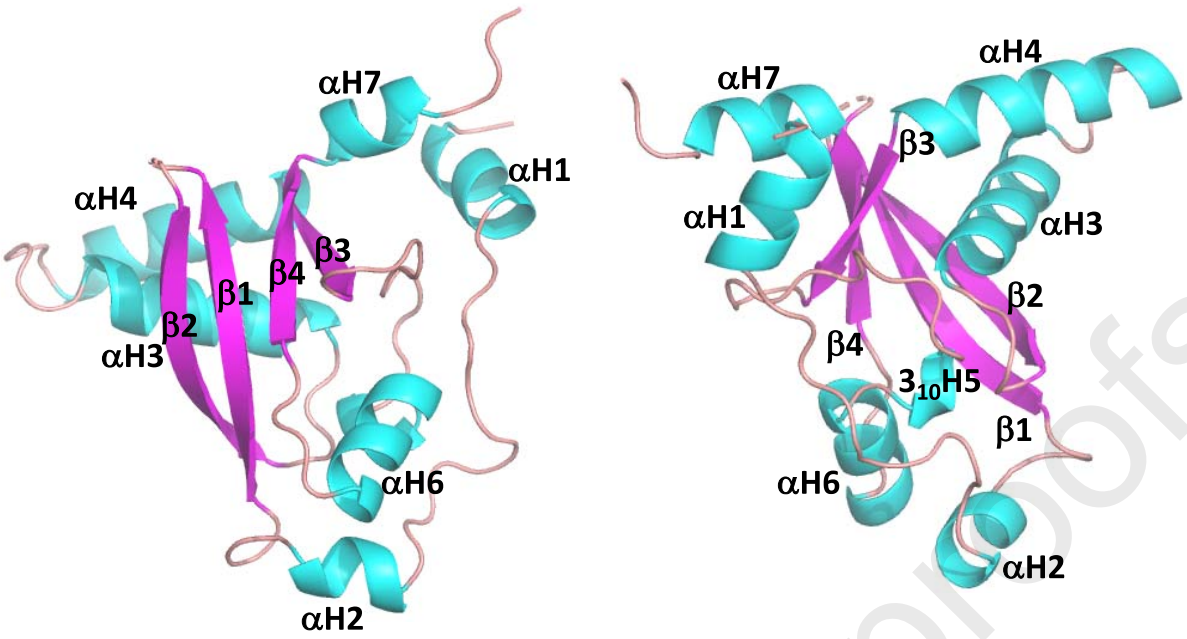
Resolution (Å)	Count
2.0-2.5	4
2.5-3.0	16
> 3.0	24
Total	44



Resolution of crystal structures of PI3Kγ

Resolution (Å)	Count
2.0-2.5	16
2.5-3.0	65
> 3.0	12
Total	93

Supp. Figure 3. A count of the number of PDB files that contain coordinates for residues within the activation loop of PI3Kα (223-250) and PI3Kγ (241-271) are depicted on the left and right, respectively. The minimum PDB count is indicated in red.



Supp. Figure 4. Labeling and representation of the PI3Kα RBD construct crystal structure in the context of secondary structure. Helical regions are in cyan, beta sheet regions are in magenta and loop and connecting regions are colored beige. Helices and beta sheets are numbered separately from N-terminus to C-terminus.

Description	PDB ID	Start Residue	End Residue	Total No. Residues*	No. Helical	% Helical
PI3Kγ, HRAS-bound	1HE8	191	316	126	36	29%
PI3Kγ, unbound	6T3B	191	316	126	25	20%
PI3Kγ, unbound, exclude N-term α-helix	6T3B	199	316	126	19	15%
PI3Kα, unbound	3HHM	157	300	144	55	38%
PI3Kα, unbound, exclude N-term α-helix	3HHM	167	316	144	46	32%
PI3Kα, exclude both helices at termini	3HHM	167	289	144	40	28%

Supp. Table 1. The secondary structure assignment for each crystal structure was calculated by the STRIDE webserver [54]. Each PDB structure of the full-length PI3K was truncated to the specified residue before upload. Residues designated as alpha helical were counted and divided by the total number of residues in the RBD construct expressed *in vitro*. The unbound PI3Kγ construct was chosen based on similar resolution to 1HE8 (~3.0 Å) [55].

	ΔG (REU*)	ΔG/ΔSASA (REU/A^2)	ΔSASA_int (Å^2)
PI3Kα	20	0.7	2986
PI3Kγ	66	2.5	2701

* Rosetta Energy Units (REU)

Supp. Table 2. Analysis of RBD Intramolecular Contacts. The p110 subunit of PI3Kα and PI3Kγ were extracted from PDB ID: 3HHM and 1HE8, respectively. Whereas most of the p110 subunit was classified as chain A, the RBD domain was renamed chain B. The interface between the two chains was minimized and repacked in order to reach a lower energy structure before calculating the ΔSASA int (SASA at interface), ΔG (difference in total energy between bound and unbound, ΔG_separated), and ΔG/ΔSASA (quality of interface) with RosettaScripts (see Supplemental Method for example script). The lowest energy structure (ΔG/ΔSASA) of 10 independent runs is presented.