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Supporting information for article:

High-resolution crystal structure of phosphatidylinositol 4-kinase II β and crystal structure of phosphatidylinositol 4-kinase II α containing a nucleoside analogue provide the structural basis for isoform-specific inhibitor design

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S1. Materials and Methods

S1.1. Chemical synthesis of AdeN3

Derivative AdeN3 was obtained by heating of 5'-chloro-5'-deoxyadenosine (1) with sodium azide in DMF. Sodium azide (300 mg, 4.61 mmol) was added to a solution of 5'-chloro-5'-deoxyadenosine (1) in DMF (7 ml) and the mixture heated to 150 °C under argon for 1 h. After evaporation of the solvent *in vacuo* and the residue was purified by flash chromatography on silica gel (80 g) in 0-80% of solvent B in solvent A (A: EtOAc-THF-EtOH-H₂O (60:6:3:1), B: ethyl acetate-THF-EtOH-H₂O (36:6:5:3)). The product, 5'-azido-5'-deoxyadenosine, was obtained after crystallization from ethanol (698 mg, 68% as a white powder), m.p. 169-170 °C (decomp.). For C₁₀H₁₂N₈O₃.1/3CH₃CH₂OH (307.62) calculated: 41.65% C, 4.59% H, 36.43% N; found: 41.26% C, 4.59% H, 36.08% N. 1H NMR (400 MHz, DMSO-d6) δ 8.36 (s, 1H, H-8), 8.16 (s, 1H, H-2), 7.31 (s, 2H, NH₂), 5.93 (d, J1',2' = 5.5 Hz, 1H, H-1'), 5.59 (d, J = 5.5 Hz, 1H, OH), 5.39 (d, J = 5.5 Hz, 1H, OH), 4.75 (q, 1H, J2',1' = J2'3' = J2',OH = 5.5 Hz, H-2'), 4.36 (t, J = 5.1 Hz, 0.33H, OH of EtOH), 4.20 (q, J3',2' = J3'4' = J3',OH = 5.5 Hz, 1H, H-3'), 4.04 (ddd, J4',3' = 5.5, J4',5'a = 7.1, J4',5'b = 3.7 Hz, 1H, H-4'), 3.69 (dd, Jgem = 13.1, J5'a,4' = 7.1 Hz, 1H, H-5'a), 3.55 (dd, Jgem = 13.1, J5'a,4' = 3.7 Hz, 1H, H-5'b), 3.44 (qd, J = 7.0, 5.1 Hz, 0.66H, CH₂ of EtOH), 1.05 (t, J = 7.0 Hz, 1H, CH₃ of EtOH). [α]_D²⁰ +51.5 (c = 0.456, CHCl₃). HRMS (EI): calcd. for C₁₀H₁₂N₈O₃ [M] 292.1032; found: 292.1029.

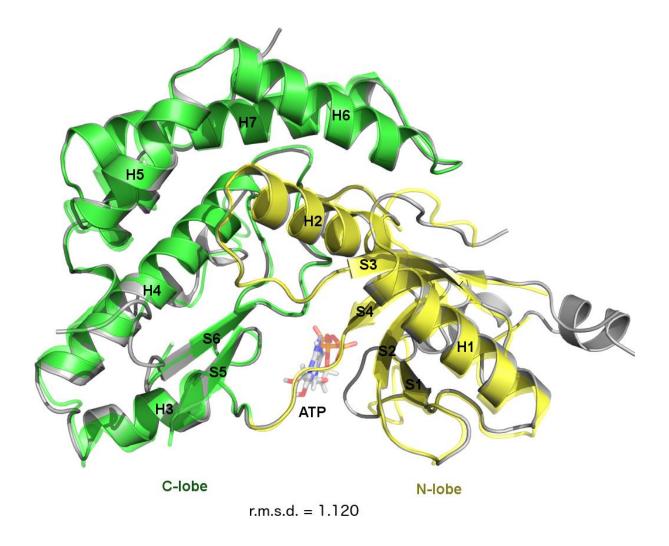


Figure S1 Structural superposition of PI4K II α and II β - The protein backbones are shown in the cartoon representation. The N-lobe of PI4K II β is depicted in yellow, the C-lobe of PI4K II β in green, both N- and C-lobes of PI4K II α are coloured in grey.

PI4K2B PI4K2A LSB6	H.sapiens H.sapiens S.cerevisiae	57	EAGDEELPLPPGDVG-VSRSSSAELDRSRPAVSVTIGTSEMNAFLDDPEFADIMLRAEQAIEVGIFPERISQGSSGSYFVK- ERQPLLDRARGAAAQGQTQTVAAQAQALAAQAAAAAHAAQAHRERNEFPEDPEFEAVVRQAELAIERCIFPERIYQGSSGSYFVK- AVGLLHNAEDKASGQEEEGSQYEIQYSVFRPLHAYPTKGLAYEQLRRKEEQEQRENFNHLVSDCIEAVETFGRELERIQTGSSGSYFVYG	141
PI4K2B PI4K2A LSB6	H.sapiens H.sapiens S.cerevisiae	142	DPKRKIIGVEKPKSEEPYGQLNPKWTKVVHKVCCPCCFGRGCLIPNGYLSEAGAYLVDNKLHLSIVPKTKVVWLVSETFNYNAIDRA DPQCRIIAVEKPKNEEPYGHLNPKWTKWLQKLCCPCCFGRDCLVLNQGYLSEAGASLVDQKLELNIVPRTKVVYLASETFNYSAIDRV TRADESVPVGVFKPKDEEPYGPFSPKWTKWAHRTFFPCLFGRSCLIPNLGYICESAASLLDRRLETHLVPYTDTASIESFNFYDNRKKWV	229
PI4K2B PI4K2A LSB6	H.sapiens H.sapiens S.cerevisiae	230	KSRGKKYALEKVPKVGRKFHRIGLPPKIGSFQLFVEGYKEAEYWLRKFEADPLPENIR KSRGKRLALEKVP	287
PI4K2B PI4K2A LSB6	H.sapiens H.sapiens S.cerevisiae	288	ROFQSOFERLVILDYIIRNTDRGNDNWLVRYEKQKCEKEIDHKESKWIDDEEFLIKIAAIDNGLAFPFKHPDEWRAYPFHWAW ROLLLQFERLVVLDYIIRNTDRGNDNWLIKYDCPMDSSSSRDTDWVVVKEPVIKVAAIDNGLAFPLKHPDSWRAYPFYWAW EWTESSLSQFRLELEKLIILDYIMRNTDRGLDNWMVKLIKLSNNKWRLKLAAIDNGLSFPWKHPDEWRLYPYGWLY	368
PI4K2B PI4K2A LSB6	H.sapiens H.sapiens S.cerevisiae		LPQAKVPFSEEIRNLILPYISDMNFVQDLCEDLYELFKTDKGFDKATFESQMSVMRGQILNLTQALRDGK	438
PI4K2B PI4K2A LSB6	H.sapiens H.sapiens S.cerevisiae	439		

Figure S2 Multiple alignment of type II PI4 kinases – Sequences were obtained from Genbank and aligned using the ClustalX algorithm as follows: human PI4K II α (NP_060895.1), PI4K II β (NP_060793.2), and yeast LSB6 (NP_012435.1). Blue areas represent conserved amino acids; the numbers indicate amino acids positions. The secondary structures present in the crystal structure of the N-lobe and the C-lobe of the human PI4K II β are indicated in yellow and green, respectively. The disordered regions and the regions omitted in the crystallization construct of PI4K II β are indicated by dotted line.

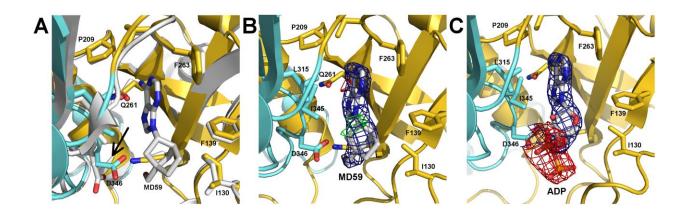


Figure S3 Structural superposition of PI4K II α bound to MD59 and ADP – A) Superposition of PI4K II α bound to MD59 and PI4K II α bound to ADP reveals a conformational change of the D³⁴⁶ sidechain (arrow). It moves to contact the hydroxyl group of the MD59. B) MD59 shown in 2F₀-F_c and F₀-F_c densities. C) ADP built in place of MD59 shown in 2F₀-F_c and F₀-F_c densities. For all panels, the protein backbones are shown in the cartoon representation. The N-lobe of PI4K II α bound to MD59 is depicted in yellow, the C-lobe of PI4K II α in cyan, both N- and C-lobes of PI4K II α bound to ADP are coloured in grey. The 2F₀-F_c density is colored blue and contoured at 1.5 σ . The F₀-F_c density is colored green when contoured at 3 σ and red when contoured at -3 σ .

Table S1 Crystallization

Crystal	ΡΙ4Κ ΙΙβ	PI4K IIα + MD59
Method	Vapor diffusion, sitting drop	Vapor diffusion, sitting drop
Plate type	INTELLI-PLATE 96-2	INTELLI-PLATE 96-2
Temperature (K)	291	291
Protein concentration	5.4 mg/ml	7 mg/ml
Buffer composition of protein solution	10 mM MES pH = 6.5, 200 mM NaCl, 3 mM β -ME, 5 mM ATP, 2 mM MgCl ₂	20 mM Citrate pH =5 .5, 200 mM NaCl, 3 mM β-ME, 2 mM MD59, 0.5 mM ADP
Composition of reservoir solution	100 mM MES/Imidazole pH = 6.5, 10% w/v PEG 4000, 20% v/v glycerol, 20 mM 1,6- hexanediol, 20 mM 1-butanol, 20 mM 1,2-propanediol, 20 mM 2-propanol, 20 mM 1,4- butanediol, 20 mM 1,3- propanediol	100 mM HEPES pH = 7.0, 10% w/v PEG 4000, 10% v/v 2-propanol
Volume and ratio of drop	1 μl, 1:1	1 μl, 1:1
Volume of reservoir	70 µl	70 µl