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Supporting information for article:

High-resolution crystal structure of phosphatidylinositol 4-kinase IIβ and crystal structure of phosphatidylinositol 4-kinase IIα 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-H_2O (60:6:3:1), B: ethyl acetate-THF-EtOH-H_2O (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 $C_{10}H_{12}N_8O_3$.1/3CH_3CH_2OH (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, NH_2), 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´b,4´= 3.7 Hz, 1H, H-5´b), 3.44 (qd, J = 7.0, 5.1 Hz, 0.66H, CH_2 of EtOH), 1.05 (t, J = 7.0 Hz, 1H, CH_3 of EtOH). $[\alpha]_D^{20}$ +51.5 (c = 0.456, $CHCl_3$). HRMS (EI): calcd. for $C_{10}H_{12}N_8O_3$ [M] 292.1032; found: 292.1029.

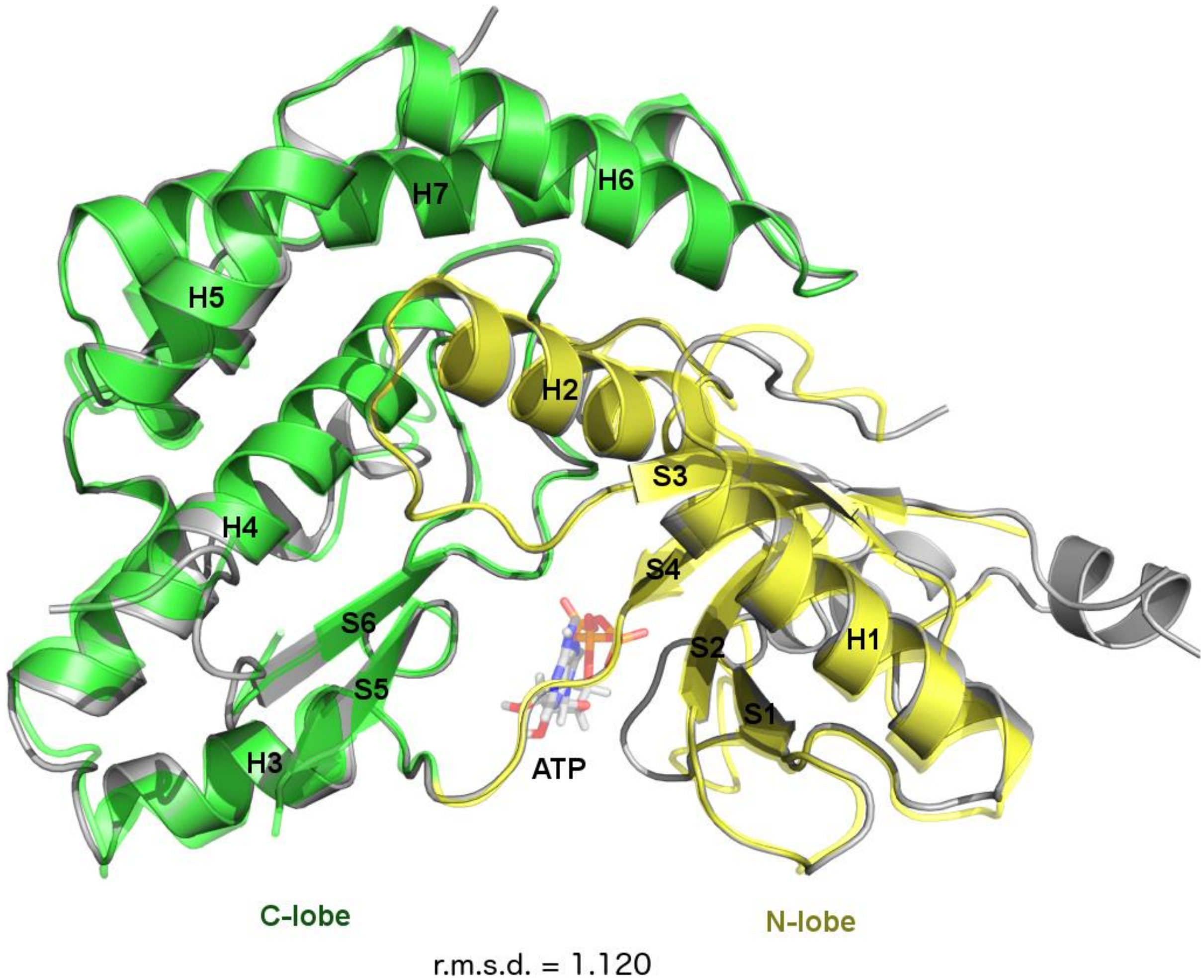

Figure S1 Structural superposition of PI4K IIα and IIβ - The protein backbones are shown in the cartoon representation. The N-lobe of PI4K IIβ is depicted in yellow, the C-lobe of PI4K IIβ in green, both N- and C-lobes of PI4K IIα are coloured in grey.

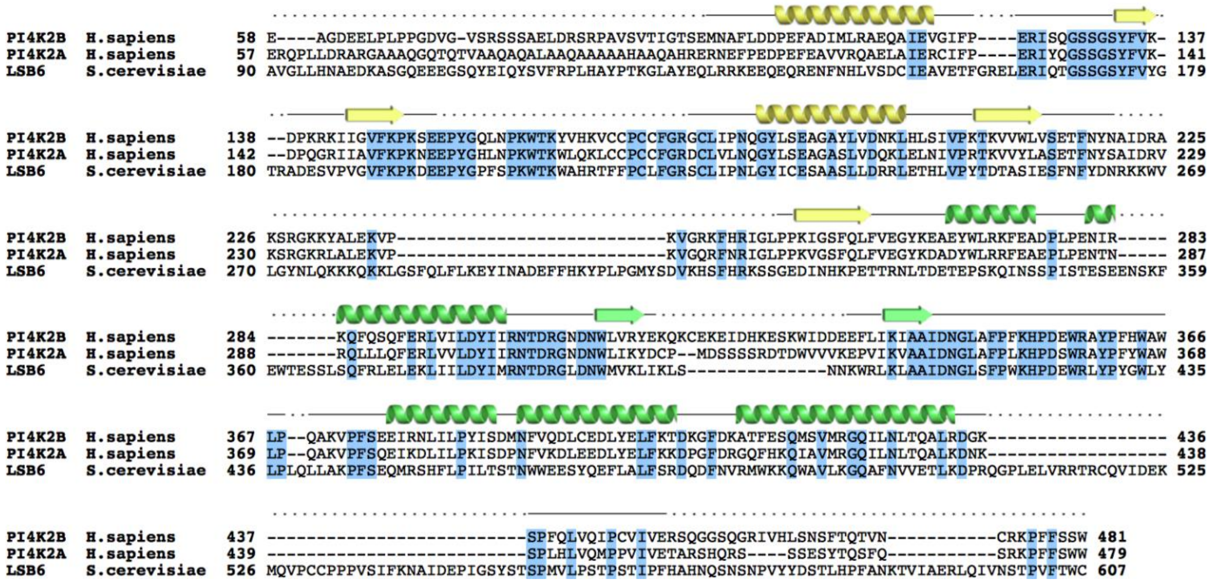

Figure S2 Multiple alignment of type II PI4 kinases – Sequences were obtained from Genbank and aligned using the ClustalX algorithm as follows: human PI4K IIα (NP_060895.1), PI4K IIβ (NP_060793.2), and yeast LSB6 (NP_012435.1). Blue areas represent conserved amino acids; the numbers indicate amino acids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

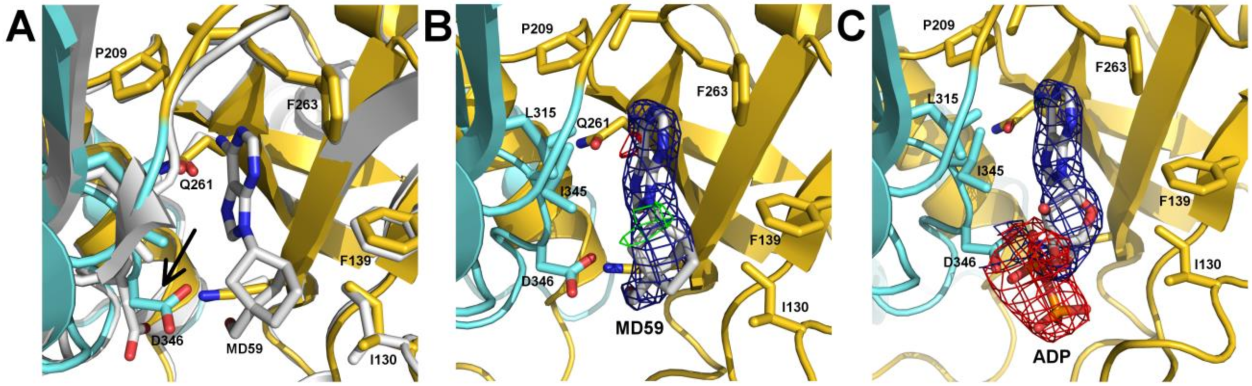

Figure S3 Structural superposition of PI4K IIα bound to MD59 and ADP – A) Superposition of PI4K IIα bound to MD59 and PI4K IIα bound to ADP reveals a conformational change of the D^{346} sidechain (arrow). It moves to contact the hydroxyl group of the MD59. B) MD59 shown in $2F_0$-F_c and F_0-F_c densities. C) ADP built in place of MD59 shown in $2F_0$-F_c and F_0-F_c densities. For all panels, the protein backbones are shown in the cartoon representation. The N-lobe of PI4K IIα bound to MD59 is depicted in yellow, the C-lobe of PI4K IIα in cyan, both N- and C-lobes of PI4K IIα bound to ADP are coloured in grey. The $2F_0$-F_c density is colored blue and contoured at 1.5 σ. The F_0-F_c density is colored green when contoured at 3 σ and red when contoured at -3 σ.

Table S1 Crystallization

Crystal	PI4K IIβ	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