Supplementary Material

Hinge motif unveils the cryptic structural determinants of selective inhibitors towards PI3K α and VPS34

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P42336|PK3CA_HUMAN      MPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLITIKHELFKEARKYPLHQ      60
Q8NEB9|PK3C3_HUMAN      ------------------------------------------------------------      0

P42336|PK3CA_HUMAN      LLQDESSYIFVSVTQEAEREEFFDETRRLCDLRLFQPFLKVIEPVGNREEKILNREIGFA      120
Q8NEB9|PK3C3_HUMAN      ------------------------------------------------------------      0

P42336|PK3CA_HUMAN      IGMPVCEFDMVKDPEVQDFRRNILNVCKEAVDLRDLNSPHSRAMYVYPPNVESSPELPKH      180
Q8NEB9|PK3C3_HUMAN      ------------------------------------------------------------      0

P42336|PK3CA_HUMAN      IYNKLDKGQIIVVIWVIVSPNNDKQKYTLKINHDCVPEQVIAEAIRKKTRSMLLSSEQLK      240
Q8NEB9|PK3C3_HUMAN      ------------------------------------------------------------      0

P42336|PK3CA_HUMAN      LCVLEYQGKYILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNLMLMAKESLYSQLPMD      300
Q8NEB9|PK3C3_HUMAN      ------------------------MGEAEKFHYIYSCDLDINV--------------QL---      21
                                               .:::** ** :  .:              **

P42336|PK3CA_HUMAN      CFTMPSYSRRISTATPYMNGETSTKSLWVINSALRIKILCATYVNVNIRDIDKIYVRTGI      360
Q8NEB9|PK3C3_HUMAN      -------------KIGSLEGKREQKSYKAVLE-----DPMLKFSGLYQETCSDLYVTCQV      63
                                    ::*: . **  .: .          .: .:  .  ..:**   :

P42336|PK3CA_HUMAN      YHGGEPLCDNVNTQRV-PCSNPRWNEWLNYDIYIPDLPRAARLCLSICSVKGRKGAKEEH      419
Q8NEB9|PK3C3_HUMAN      FAEGKPLALPVRTSYKAFSTRWNWNEWLKLPVKYPDLPRNAQVALTIWDVYGPGKA----      119
                        :  *:**.  *.*.    .:. .*****:  :  ***** *::.*:* .* *   *

P42336|PK3CA_HUMAN      CPLAWGNINLFDYTDTLVSGKMALNLWPVPHGLEDLLNPIGVTGSNP-------------      466
Q8NEB9|PK3C3_HUMAN      VPVGGTTVSLFGKYGMFRQGMHDLKVWPNVEADGS--EPTKTPGRTSSTLSEDQMSRLAK      177
                         *:.  .:.**.  . : .*   *::**  ..  .  :*  . * .

P42336|PK3CA_HUMAN      --NKETPCLELEFDWFSSVVKFPDMSVIEEHANWS----------------VSREAGFSYS      509
Q8NEB9|PK3C3_HUMAN      LTKAHRQGHMVKVDWLDRL-TFREIEMINESEKRSSNFMYLMVEFRCVKCDDKEYGIVYY      236
                          : .     ::.**:. : .* ::.:*:*  : *               .:* *: *

P42336|PK3CA_HUMAN      ---------------------------------HAGLSNRL-----ARDNELRENDKEQL      531
Q8NEB9|PK3C3_HUMAN      EKDGDESSPILTSFELVKVPDPQMSMENLVESKHHKLARSLRSGPSDHDLKPNAATRDQL      296
                                                  *  *:. *      :* : .   ::**

P42336|PK3CA_HUMAN      KAISTRDPLSEITEQEKDFLWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQMYCLVKDWP      591
Q8NEB9|PK3C3_HUMAN      NIIVSYPPTKQLTYEEQDLVWKFRYYLTNQEKALTKFLKCVNWDLPQEAKQALELLGKWK      356
                        : * :  * .::* :*:*::*..*:* ..  : * *:* .*:*:  :*. *   *: .*

P42336|PK3CA_HUMAN      PIKPEQAMELLDCNYPDPMVRGFAVRCLEKYLTDDKLSQYLIQLVQVLKYEQYLDN-----      647
Q8NEB9|PK3C3_HUMAN      PMDVEDSLELLSSHYTNPTVRRYAVARLRQA-DDEDLLMYLLQLVQALKYENFDDIKNGL      415
                        *:. *::***..:* :* ** :**  *.:   *:.*  **:****.****:: *

P42336|PK3CA_HUMAN      ------------------------------------------------------------LL      649
Q8NEB9|PK3C3_HUMAN      EPTKKDSQSSVSENVSNSGINSAEIDSSQIITSPLPSVSSPPPASKTKEVPDGENLEQDL      475
                                                                                 *

P42336|PK3CA_HUMAN      VRFLLKKALTNQRIGHFFFWHLKSEMHNKTVSQRFGLLLESYCRACGMYLKHL---NRQV      706
Q8NEB9|PK3C3_HUMAN      CTFLISRACKNSTLANYLYWYVIVECEDQDTQQRDPKTHEMYLNVMRRFSQALLKGDKSV      535
                          **:.:* .*. :.::::*::  * .:: ..**     * * ..   : : *   ::.*

P42336|PK3CA_HUMAN      EAM-----------EKLINLTDILKQEKKDETQK-VQMKFLVEQMRRPDFMDALQGFLSP      754
Q8NEB9|PK3C3_HUMAN      RVMRSLLAAQQTFVDRLVHLMKAVQRESGNRKKKNERLQALLGDNEKMN-LSDVELIPLP      594
                        ..*           ::*::* . :::*. :..:*  ::: *: : .: : :. :: :  *

P42336|PK3CA_HUMAN      LNPAHQLGNLRLEECRIMSSAKRPLWLNWENPDIMSELLFQNNEIIFKNGDDLRQDMLTL      814
Q8NEB9|PK3C3_HUMAN      LEPQVKIRGIIPETATLFKSALMPAQLFFKTE------DGGKYPVIFKHGDDLRQDQLIL      648
                        *:*  :: .:  * . ::.**  *  * ::.          :  :***:******* * *

P42336|PK3CA_HUMAN      QIIRIMENIWQNQGLDLRMLPYGCLSIGDCVGLIEVVRNSHTIMQIQCKGGLKGALQFNS      874
Q8NEB9|PK3C3_HUMAN      QIISLMDKLLRKENLDLKLTPYKVLATSTKHGFMQFIQSVPVAEVLDTEGSIQNFFR---      705
                        *** :*::: :::.***:: **  *: .   *:::.::.  .   :: :*.::. ::

P42336|PK3CA_HUMAN      HTLHQWL-KDKNKGEIYDAAIDLFTRSCAGYCVATFILGIGDRHNSNIMVKDDGQLFHID      933
Q8NEB9|PK3C3_HUMAN      ----KYAPSENGPNGISAEVMDTYVKSCAGYCVITYILGVGDRHLDNLLLTKTGKLFHID      761
                            ::  .::. . *   .:* :.:******* *:***:**** .*:::.. *:*****

P42336|PK3CA_HUMAN      FGHFLDHKKKKFGYKRERVPFVLTQDFLIVISKGAQECTKTREFERFQEMCYKAYLAIRQ      993
Q8NEB9|PK3C3_HUMAN      FGYILGRDPKPLPP------PMKLNKEM--VE---GMGGTQSEQYQEFRKQCYTAFLHLRR      811
                        **::*.:. * :       *: *.:::  *    .   *::.:::.*:: **.*:* :*:

P42336|PK3CA_HUMAN      HANLFINLFSMMLGSGMPELQSFDDI--AYIRKTLALDKTEQEALEYFMKQMNDAHHGGW      1051
Q8NEB9|PK3C3_HUMAN      YSNLILNLFSLMVDANIPDIALEPDKTVKKVQDKFRLDLSDEEAVHYMQSLIDESVHALF      871
                        ::**::****:*:.:.:*::    *     ::..: ** :::**:.*: . :::: *. :

P42336|PK3CA_HUMAN      TTKMDWIFHTIKQHALN     1068
Q8NEB9|PK3C3_HUMAN      AAVVEQIHKFAQYWRK-     887
                        :: :: *.:  :
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Identical positions	203
Identity	27.51%
Similar positions	302
Program	CLUSTALO

Figure S1. UniProt Align results of human PI3Kα and mouse PI3Kβ protein sequence.

A

Comparison of Vps34 structure versus the other classes of PI3Ks								
	Vps34 (4OYS)	PI3K Class I				PI3K Class II		
		α (4JPS)	β	δ	γ	α	β	γ
P-loop	F612	M772	M	M	M	F	F	F
	K613	S773	D	D	A	S	N	T
	L616	K776	M	M	K	A	A	A
Induced pocket of PI3KC1	Q620	W780	W	W	W	K	K	K
Top	I634	I800	I	I	I	M	I	I
Bottom	Y670	Y836	Y	Y	Y	F	F	Y
Gate Keeper	M682	I848	I	I	I	V	V	V
Hinge	Q683	E849	E	E	E	E	E	Q
	F684	V850	V	V	I	L	M	M
	I685	V851	V	V	V	V	I	V
	Q686	R852-N853	S-T	L-H	K-D	P	P	P
	S687	S854	S	S	A	A	N	D
	V688	H855	E	D	T	S	A	A
Entrance	P689	T856	T	T	T	D	E	V
	D747	S919	D	D	D	D	D	D
Floor	L750	M922	M	M	M	M	M	M
	F758	F930	F	F	F	F	F	F

B

Sequence alignment of hinge region														
VPS34	(4OYS)	684	F	I	Q		S	V	P	V	A	E	V	L
PI3Kα	(4JPS)	850	V	**V**	R	N	S	H	**T**	**I**	M	Q	**I**	**Q**
PI3Kβ	(4BFR)	847	V	**V**	S	T	S	E	**T**	**I**	A	D	**I**	**Q**
PI3Kδ	(5O83)	827	V	**V**	L	H	S	D	**T**	**I**	A	N	**I**	**Q**
PI3Kγ	(5JHB)	881	I	**V**	K	D	A	T	**T**	**I**	A	K	**I**	**Q**

Figure S2. Comparison of VPS34 structure versus the other classes of PI3Ks. Residues surrounding the ligand are listed, with their equivalence in the other kinases (only non-conserved residues are listed). The color in orange corresponds to identical residues with VPS34.

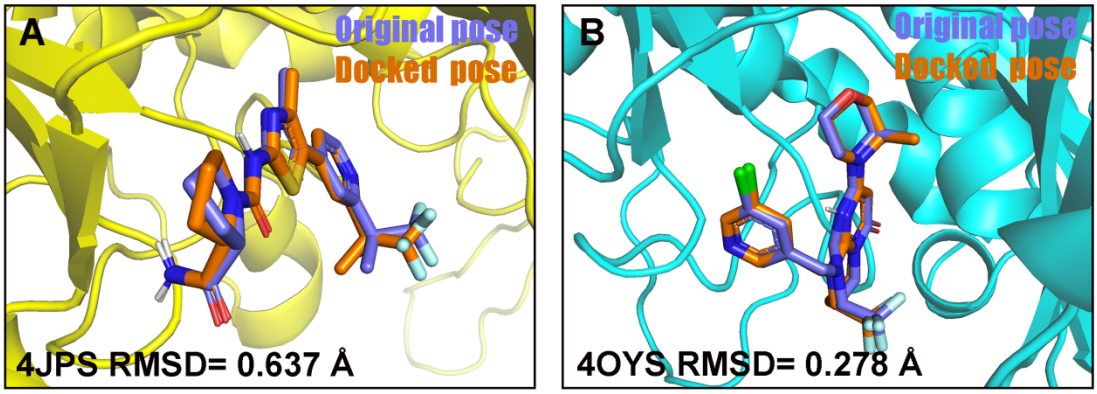

Figure S3. The comparisons between the predicted and experimental poses for PI3Kα (A), and VPS34 (B). The purple sticks represented the experimental pose extracted from X-ray structure, and the orange sticks represented the docked pose.

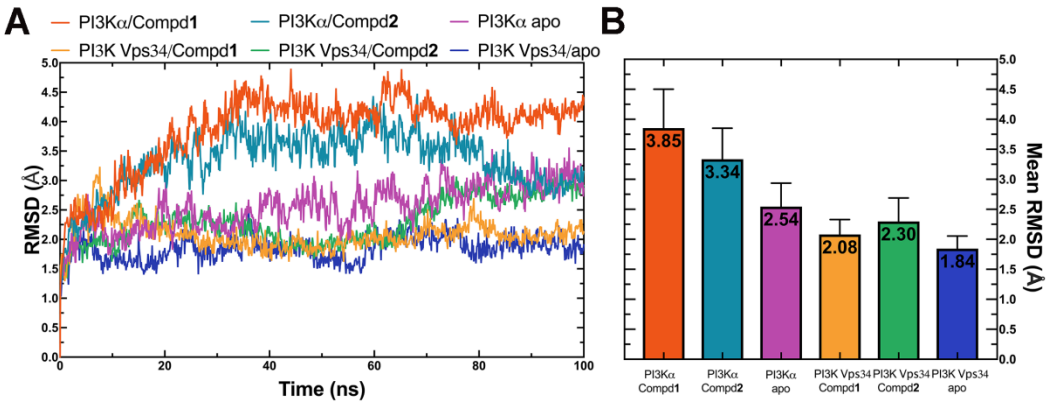

Figure S4. RMSD plots of the protein backbones throughout MD simulations process. (A) The RMSD line chart for the protein backbones of PI3Kα and VPS34. (B) The average bar chart for the protein backbones of PI3Kα and VPS34. The average value was labeled on top of each bar, and the error bar indicated the highest value of RMSD during the simulation.

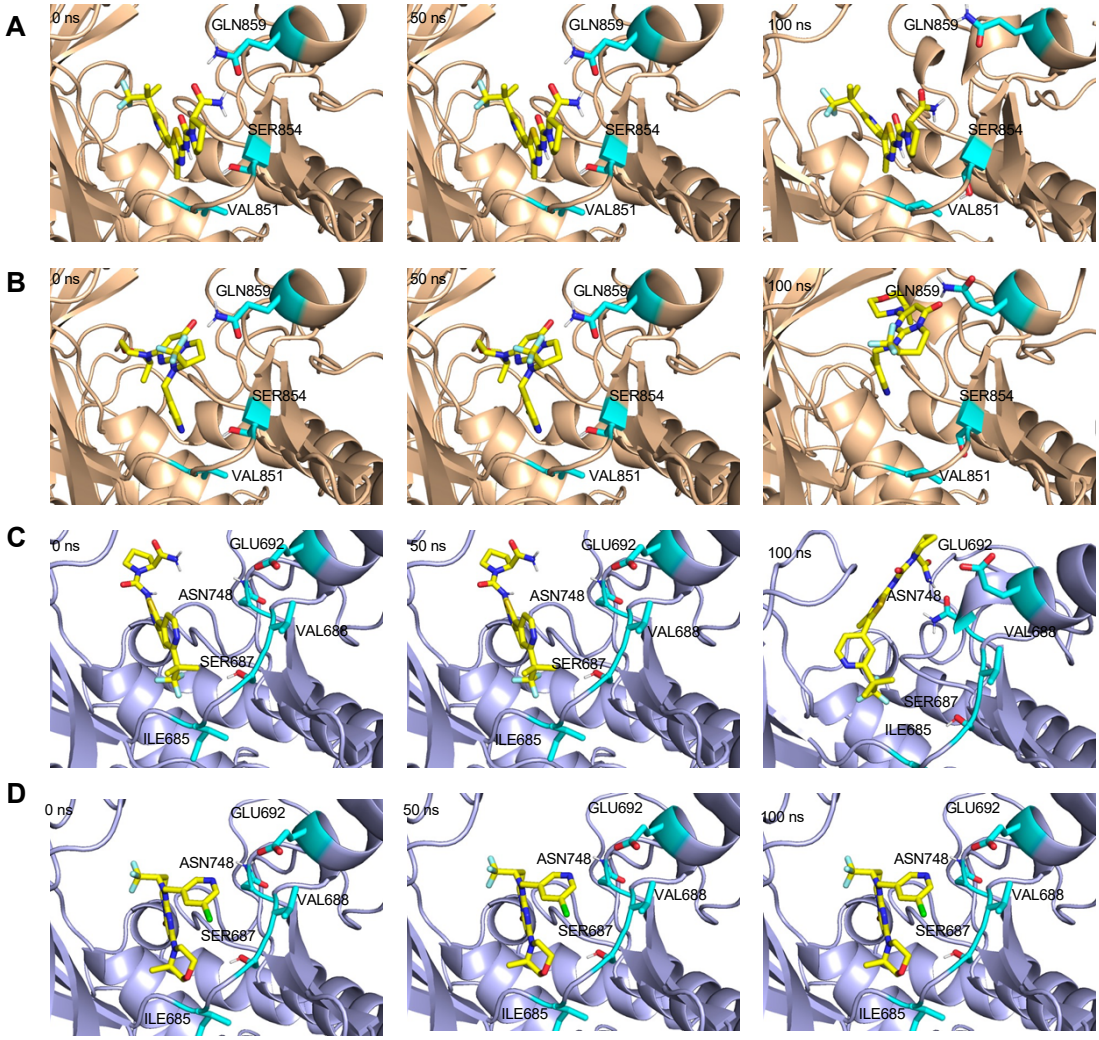

Figure S5. Snapshoots of the MD simulated ligand-protein complex at different time intervals. (A) PI3Kα/Compd**1**. (B) PI3Kα/Compd**2**. (C) VPS34/Compd**1**. (D) VPS34/Compd**2**.

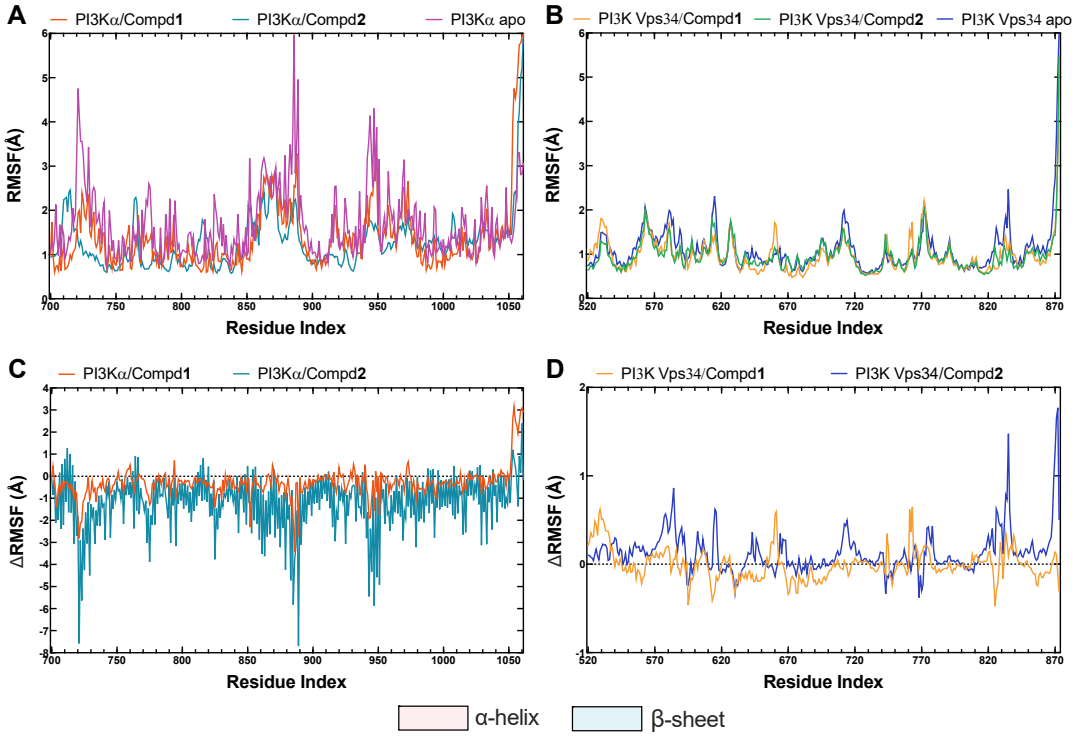

Figure S6. RMSF plots of each residue of PI3Kα and VPS34 obtained from the equilibrium states of MD simulations. (A) RMSF chart of PI3Kα. (B) RMSF chart of VPS34. (C) ΔRMSF chart of PI3Kα. (D) ΔRMSF chart of VPS34.

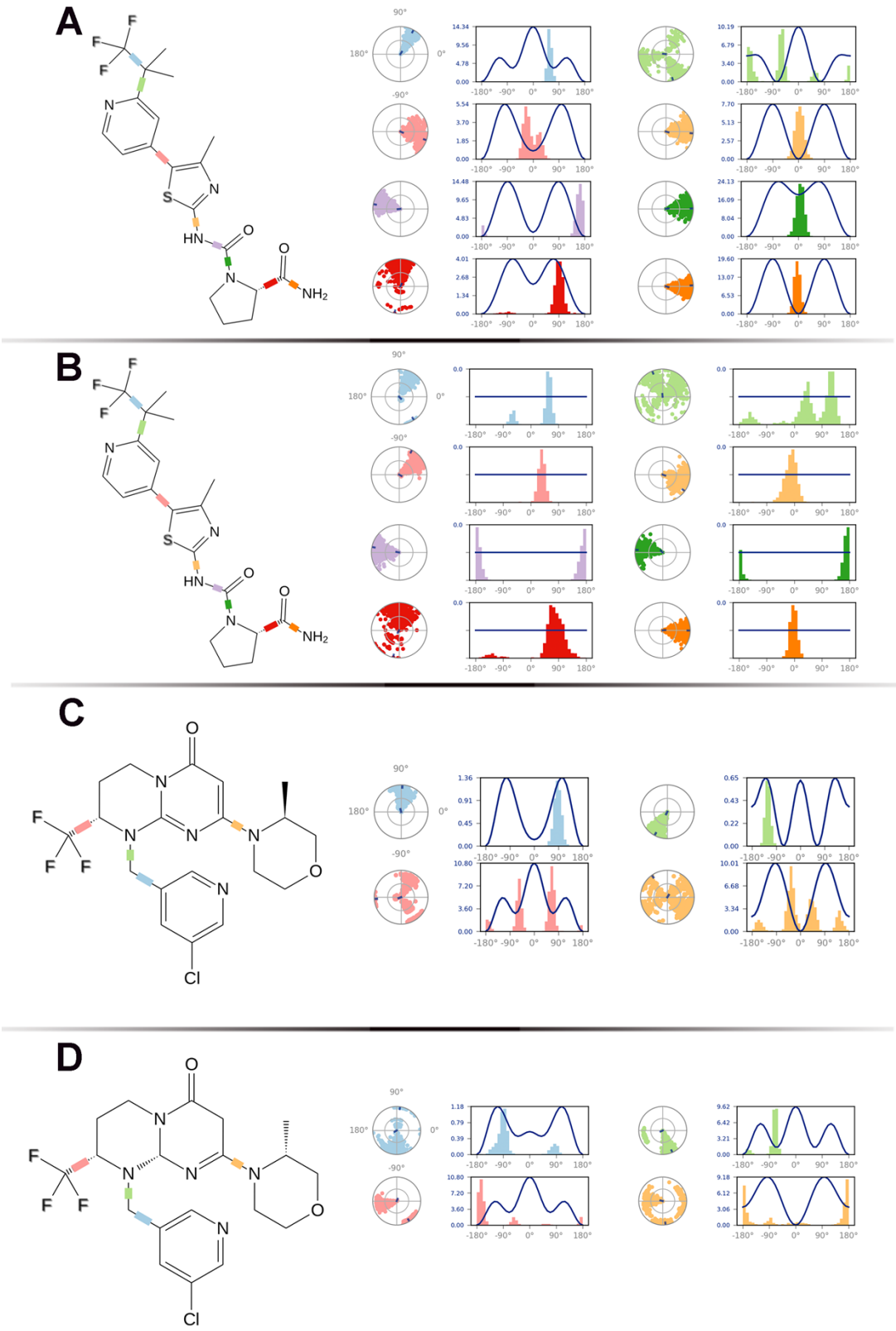

Figure S7. Ligand Torsion Profile of four complexes. (A) PI3Kα/Compd**1**. (B) VPS34/Compd**1**. (C) PI3Kα/Compd**2**. (D) VPS34/Compd**2**.

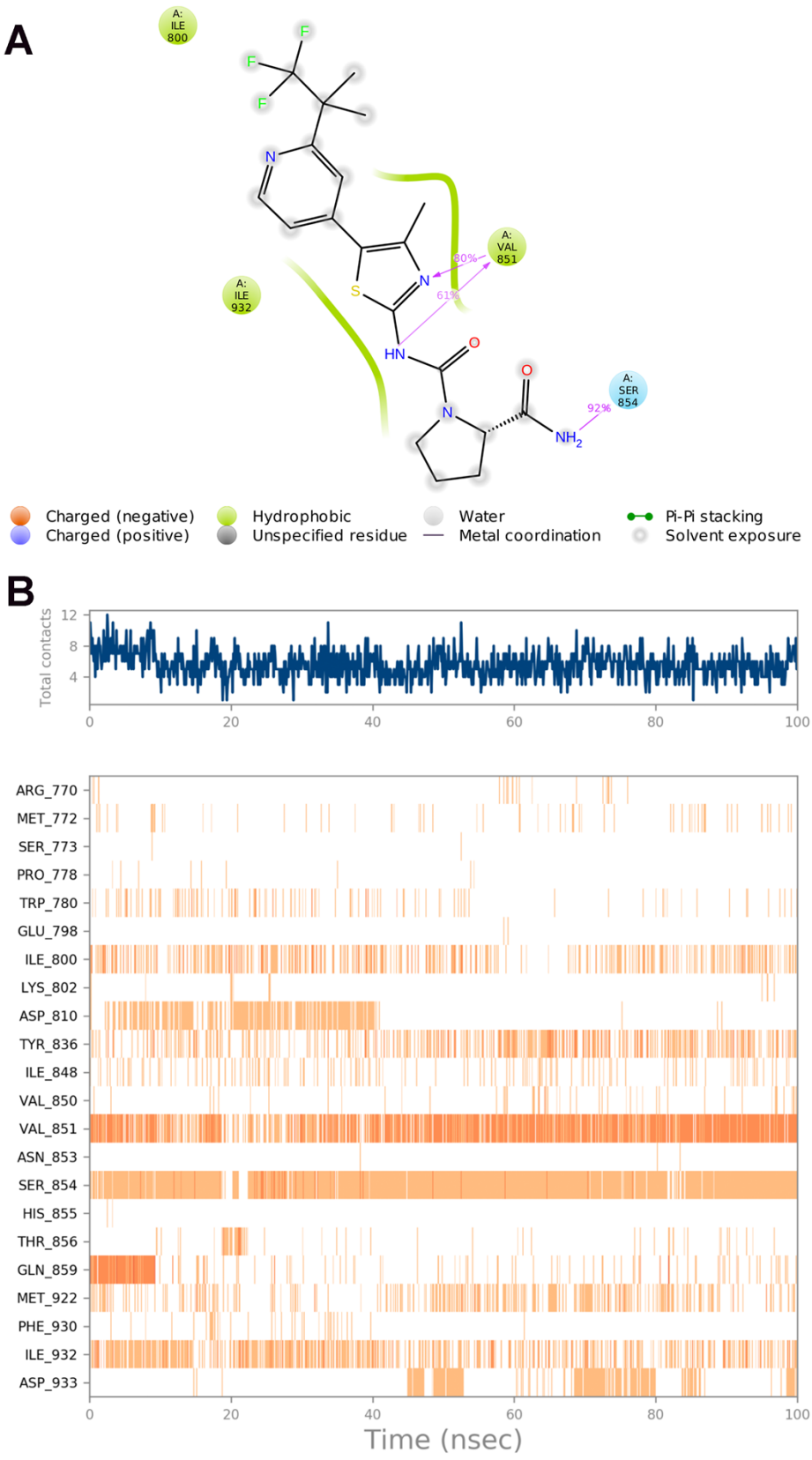

Figure S8. Ligand-Protein contacts of PI3Kα/Compd**1** complex.

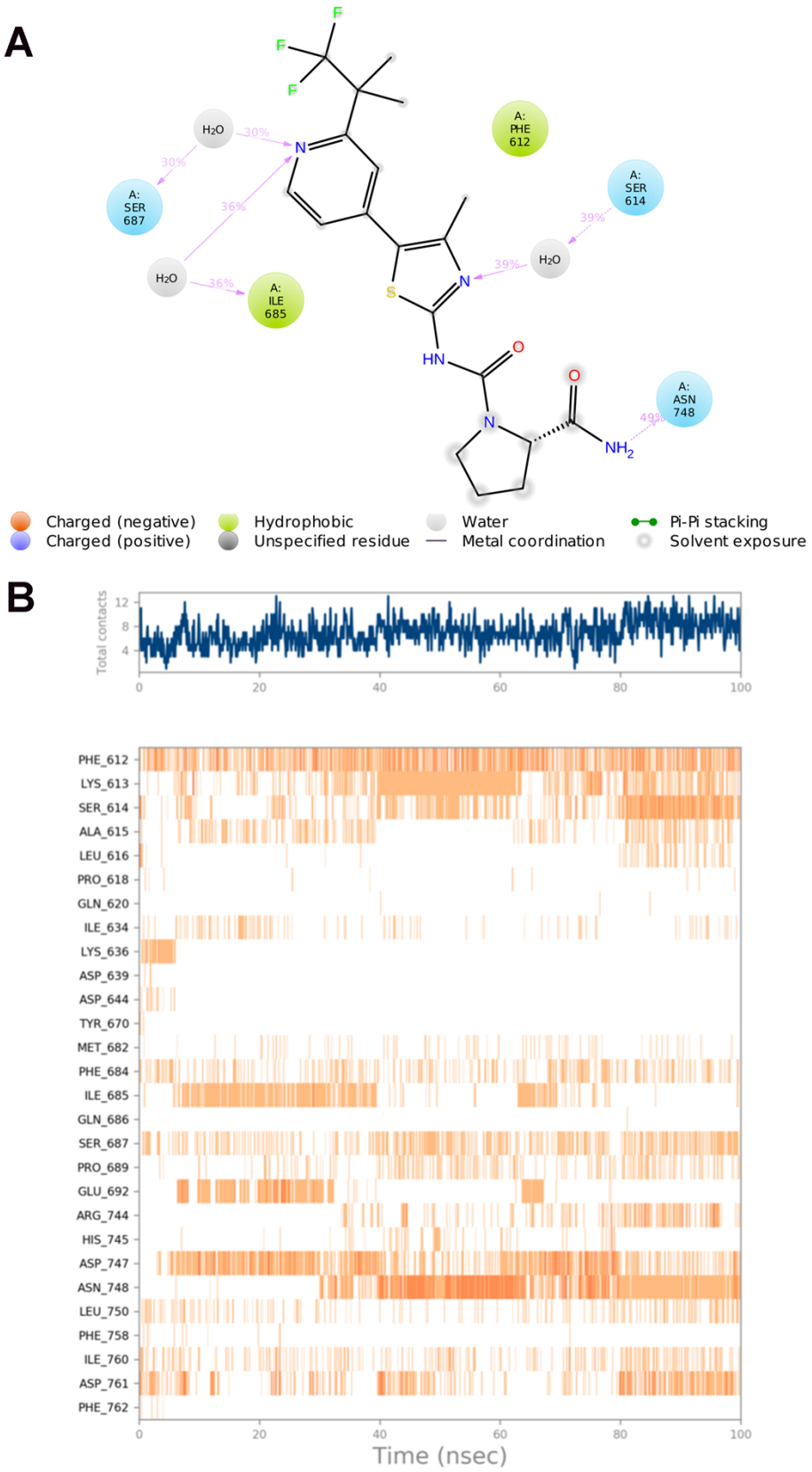

Figure S9. Ligand-Protein contacts of VPS34/Compd**1** complex.

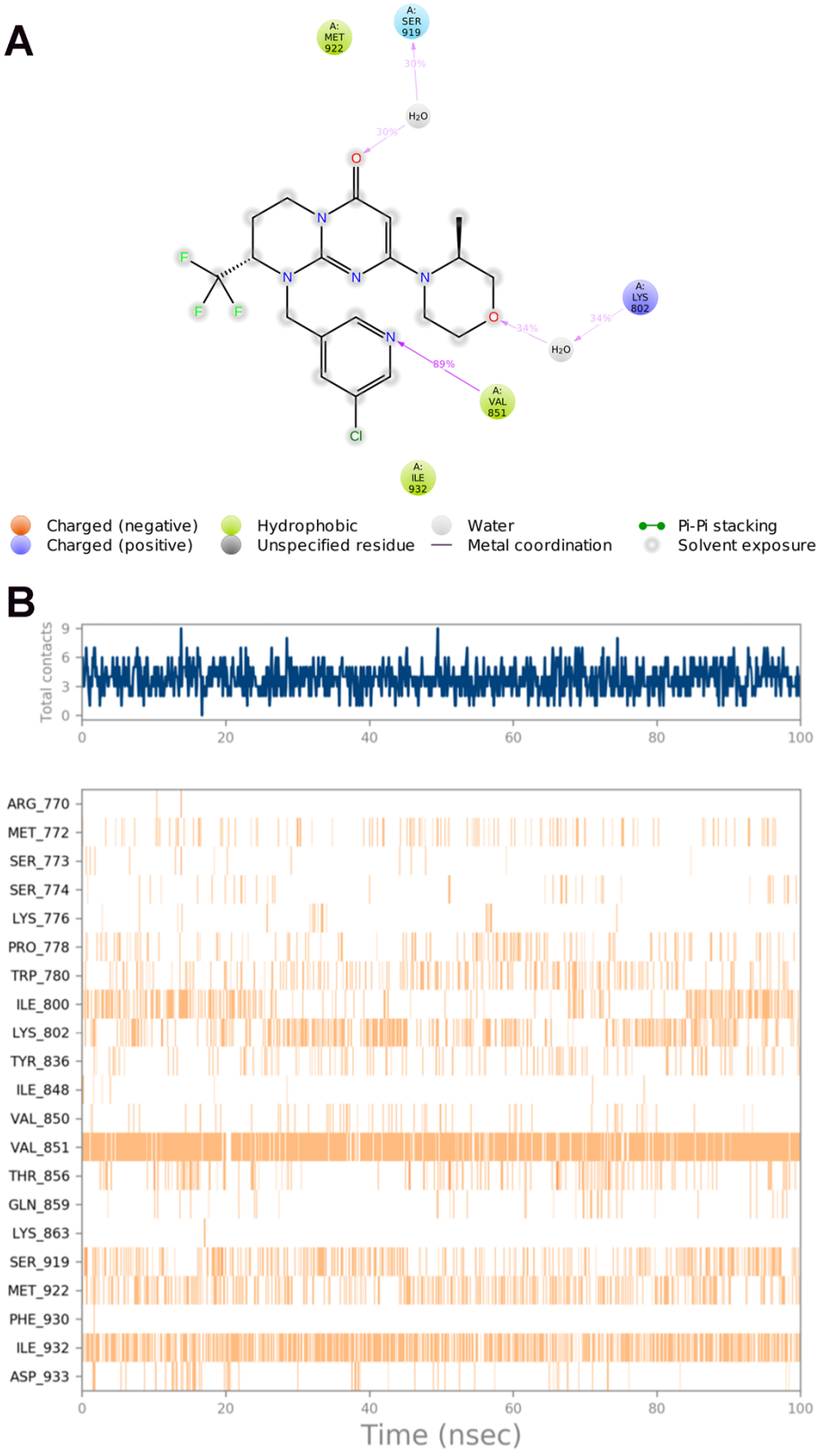

Figure S10. Ligand-Protein contacts of PI3Kα/Compd**2** complex.

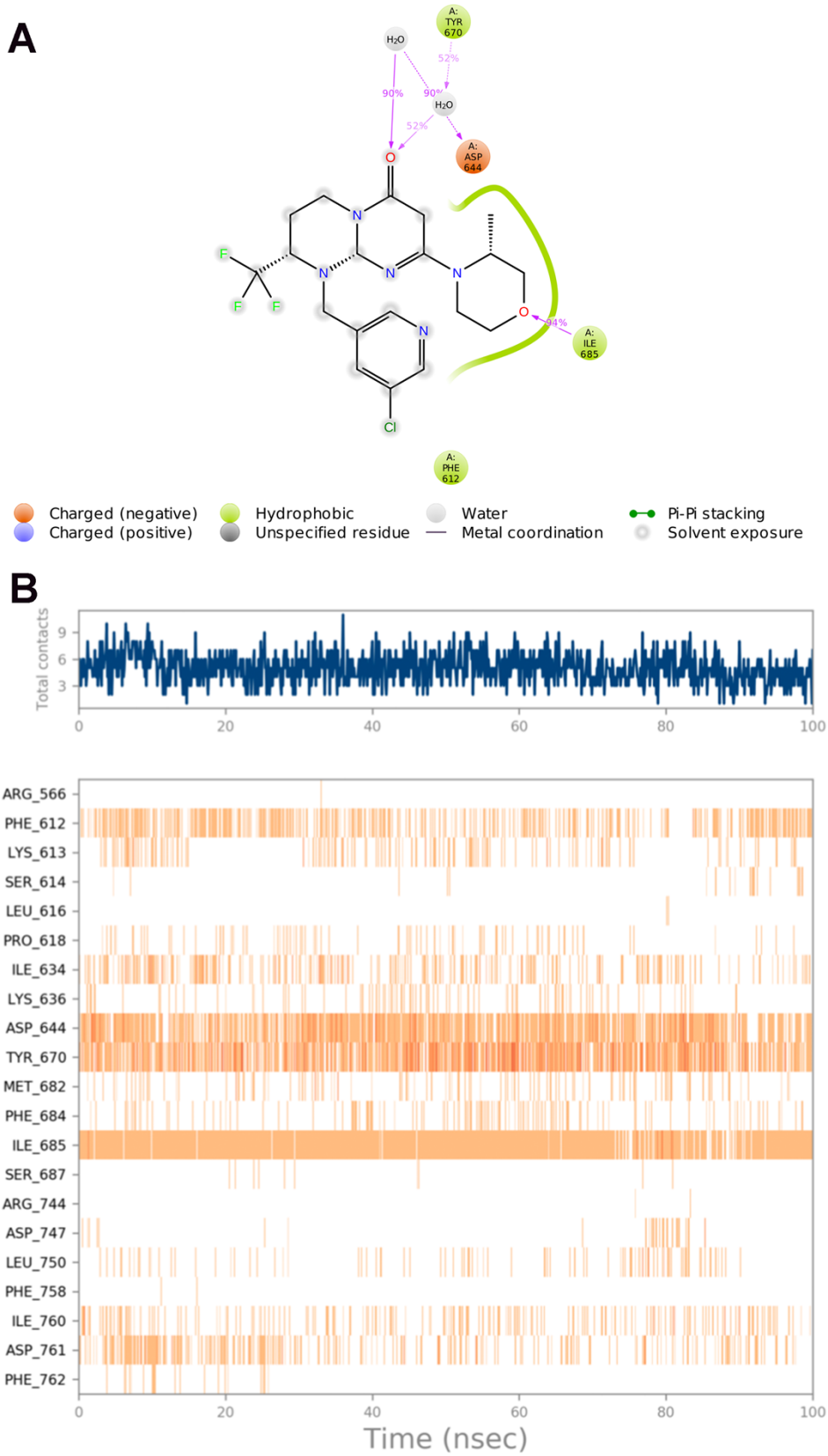

Figure S11. Ligand-Protein contacts of VPS34/Compd**2** complex.

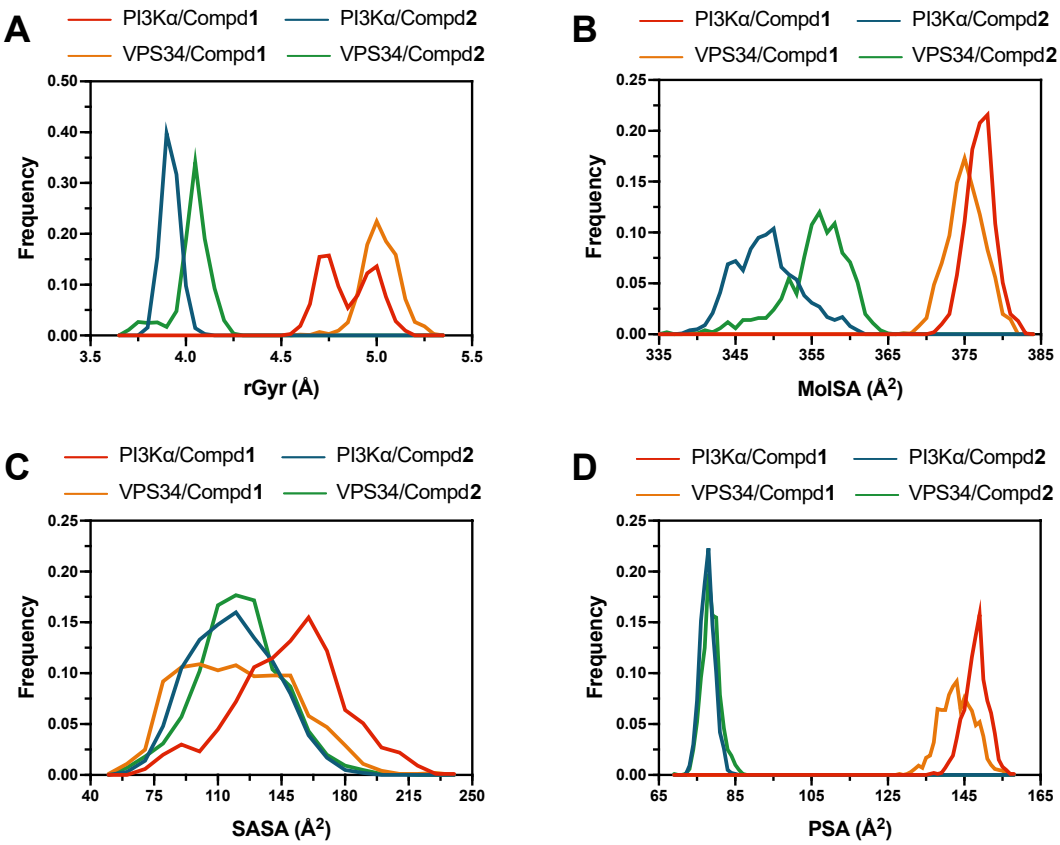

Figure S12. Variation in the ligand's properties w.r.t time during the 100 ns MD simulation. (A) PI3Kα/Compd**1**. (B) VPS34/Compd**1**. (C) PI3Kα/Compd**2**. (D) VPS34/Compd**2**.

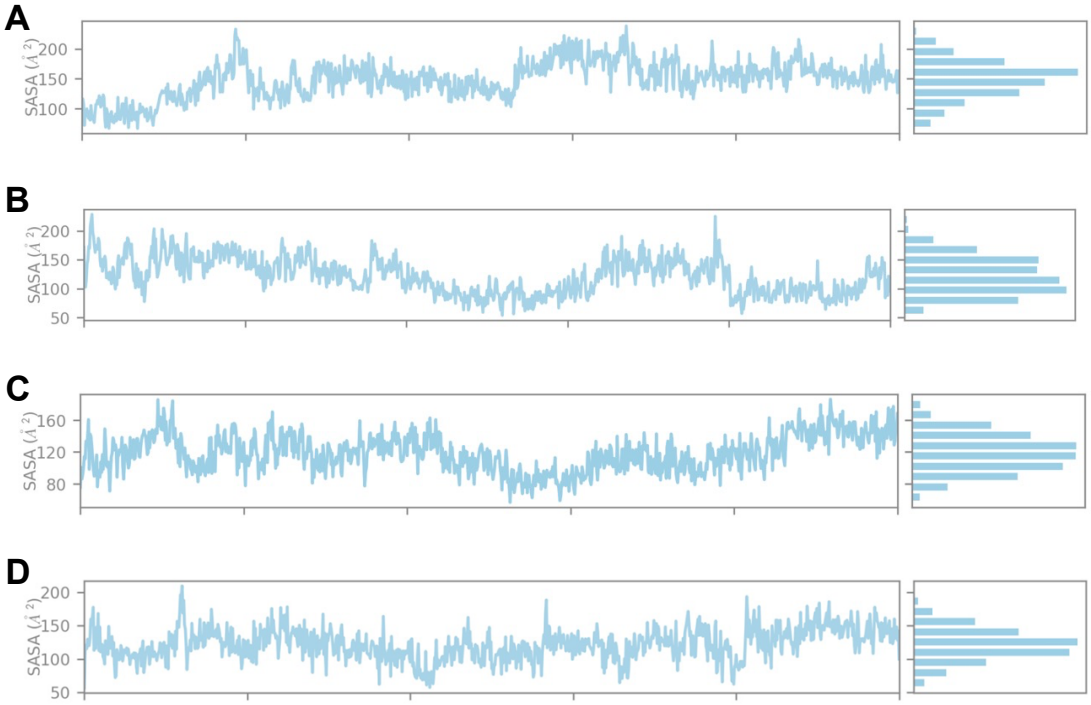

Figure S13. SASA analysis of four complexes. (A) PI3Kα/Compd**1**. (B) VPS34/Compd**1**. (C) PI3Kα/Compd**2**. (D) VPS34/Compd**2**.

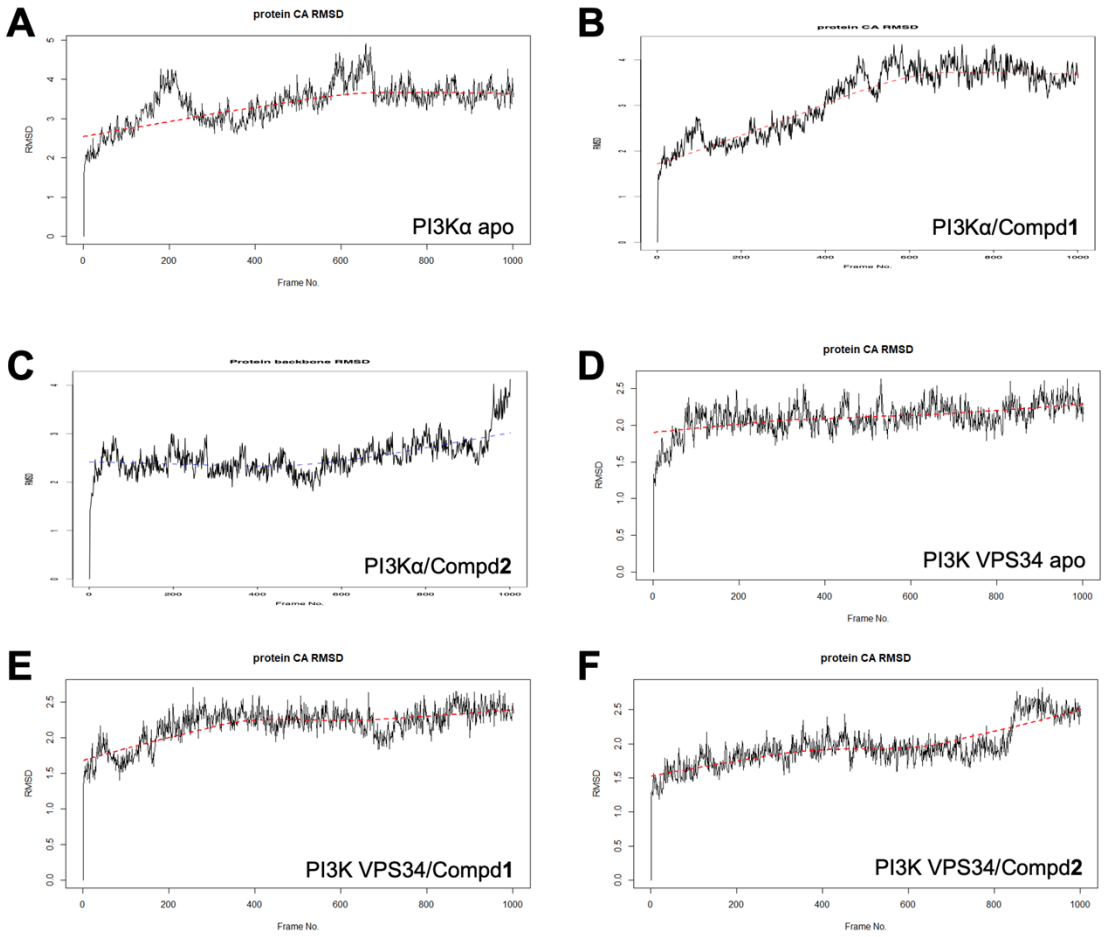

Figure S14. RMSD plots of α-C atoms during the 100 ns MD simulation from GROMACS. (A) PI3Kα apo. (B) PI3Kα/Compd**1**. (C) PI3Kα/Compd**2**. (D) VPS34 apo. (E) VPS34/Compd**1**. (F) VPS34/Compd**2**.

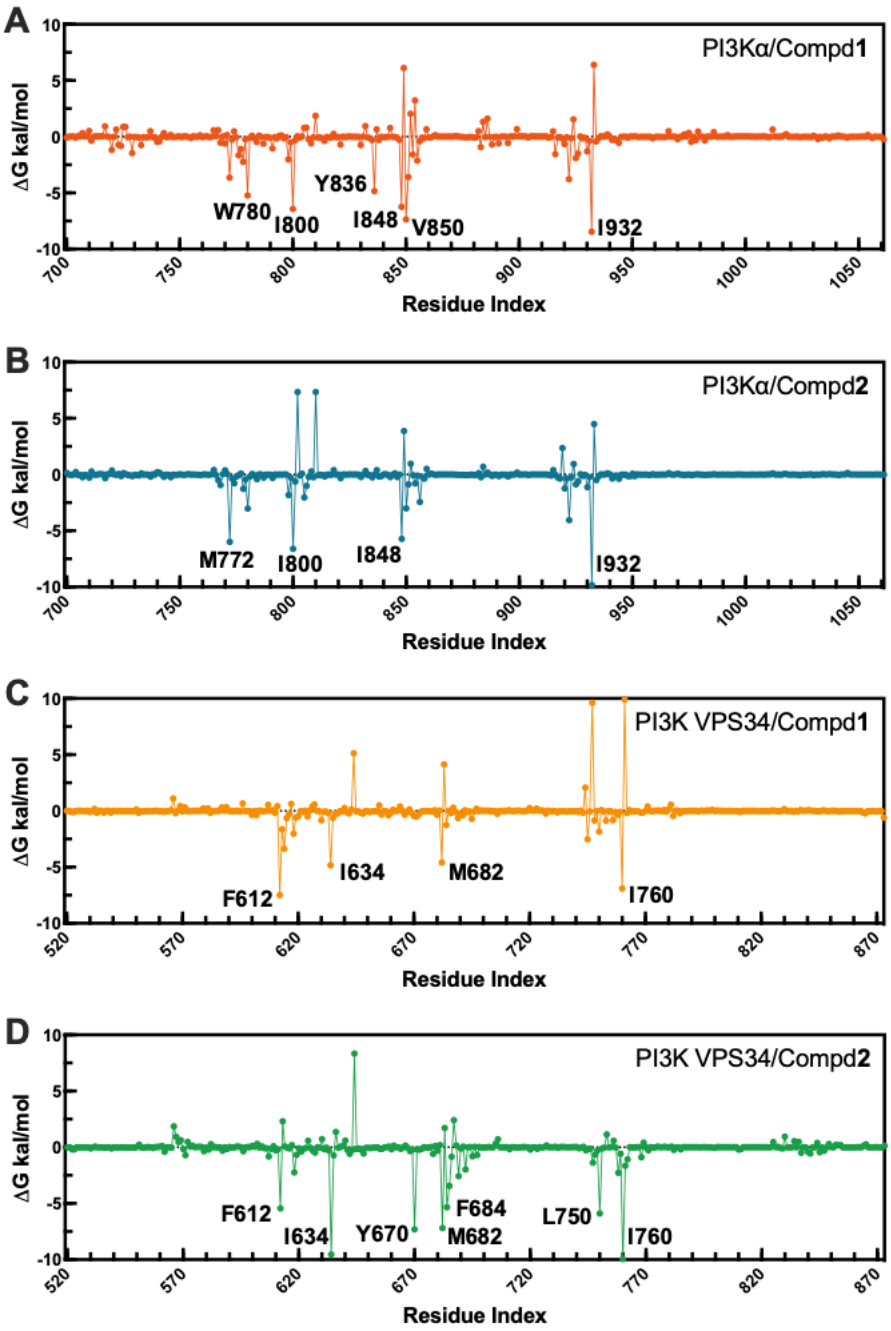

Figure S15. The calculated MM/PBSA binding energy on a per-residue basis in the binding pocket of each complex. (A) PI3Kα/Compd**1**. (B) (A) PI3Kα/Compd**2**. (C) VPS34/Compd**1**. (D) VPS34/Compd**2**.

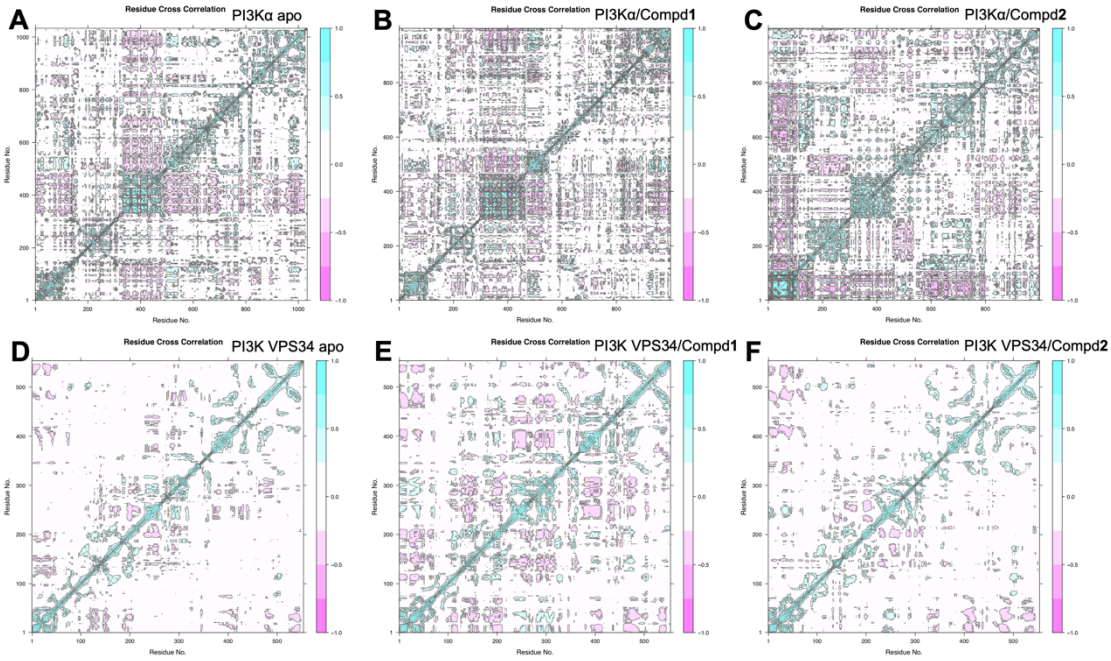

Figure S16. The results of DCCM analysis. (A) PI3Kα apo. (B) PI3Kα/Compd**1**. (C) PI3Kα/Compd**2**. (D) VPS34 apo. (E) VPS34/Compd**1**. (F) VPS34/Compd**2**. The residue number was rearranged according to the original sequence.

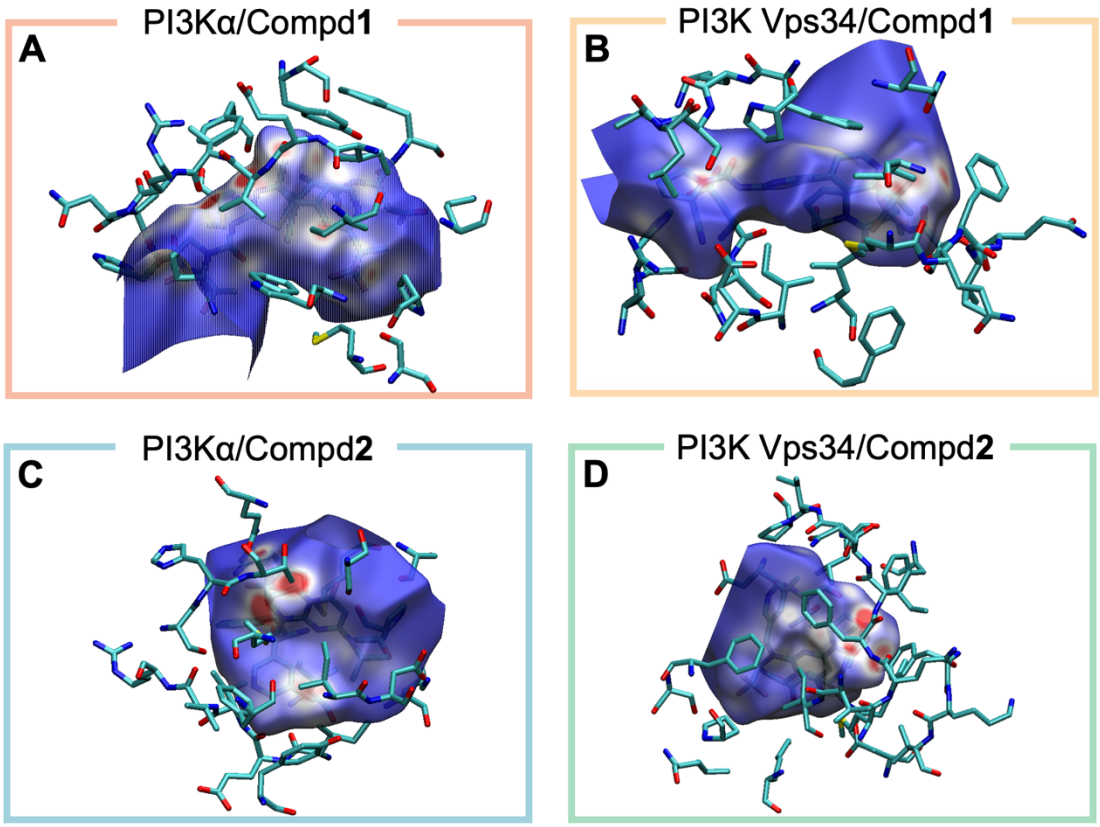

Figure S17. Hirshfeld surfaces map for the four complexes. (A) PI3Kα/Compd**1**. (B) VPS34/Compd**1**. (C) PI3Kα/Compd**2**. (D) VPS34/Compd**2**.

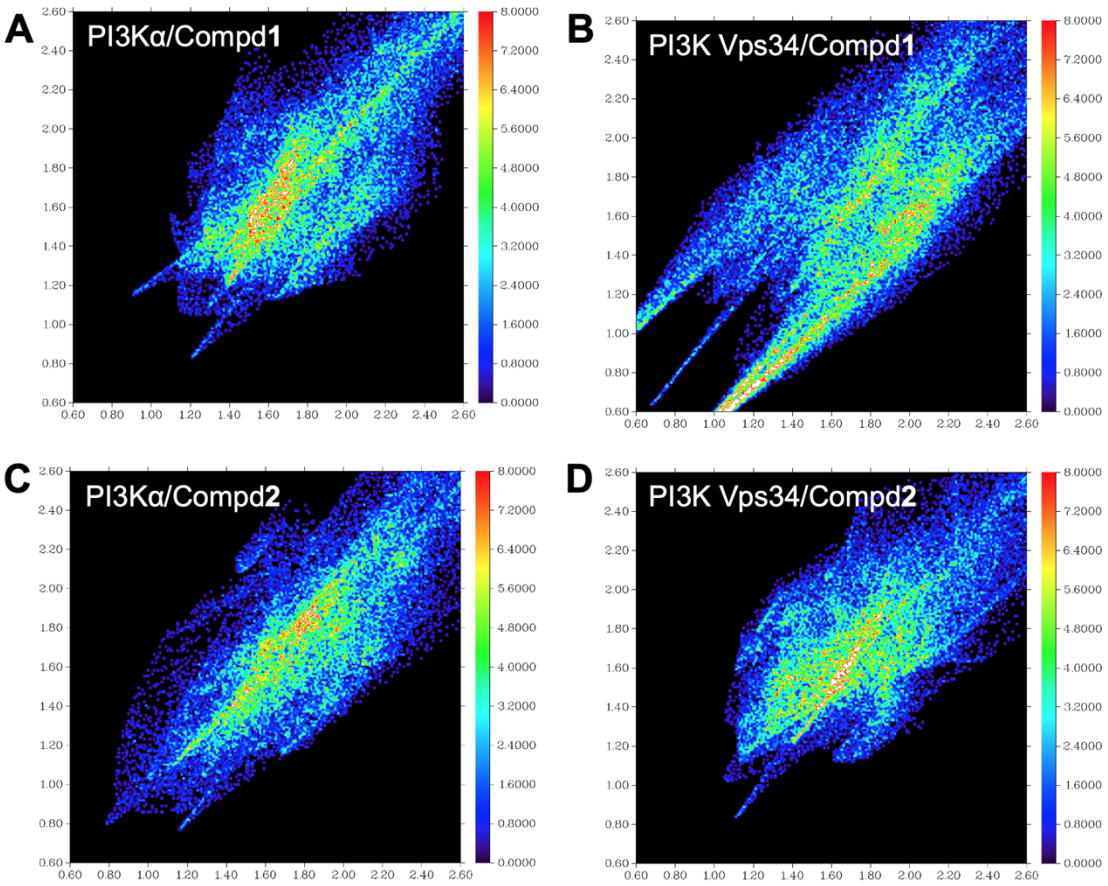

Figure S18. Fingerprint plots of intermolecular interaction from Hirshfeld surface analysis. (A) PI3Kα/Compd**1**. (B) VPS34/Compd**1**. (C) PI3Kα/Compd**2**. (D) VPS34/Compd**2**.

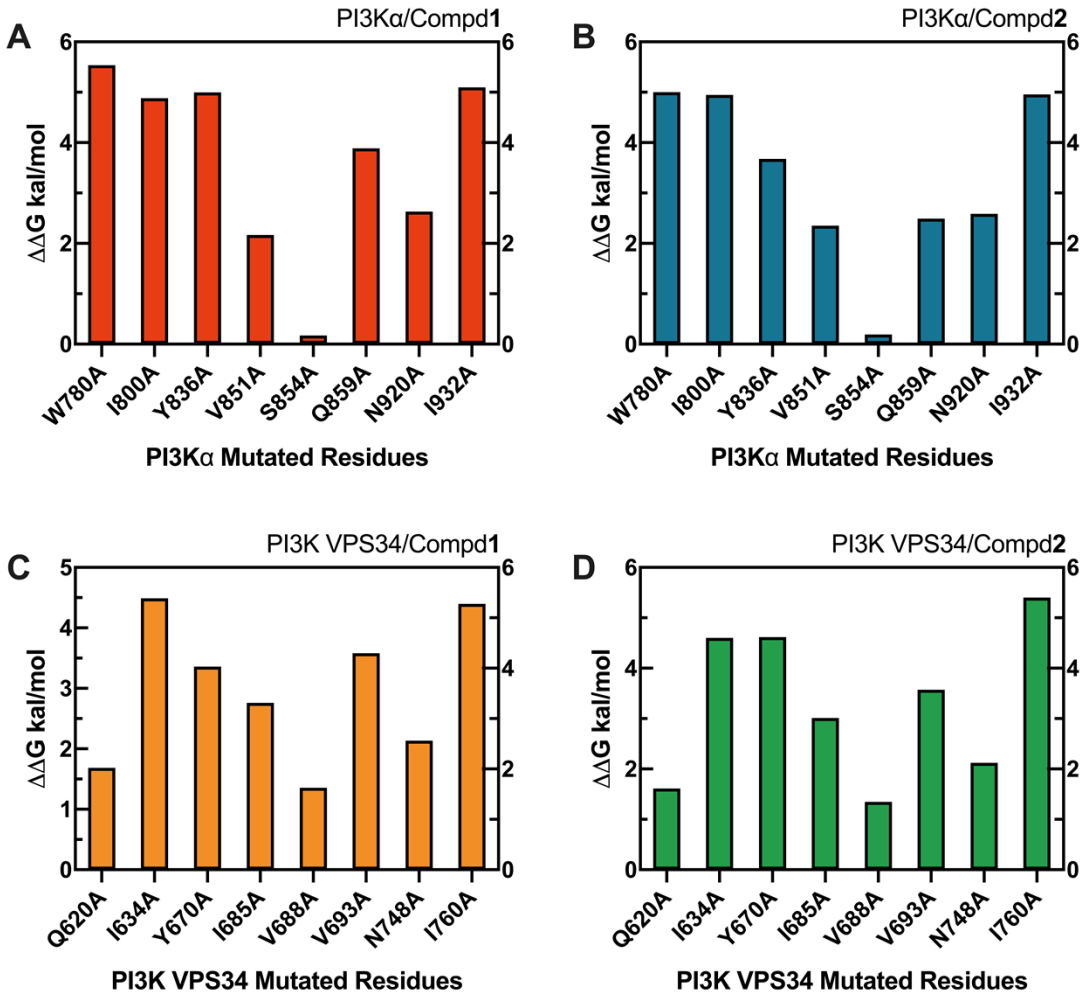

Figure S19. Alanine scanning mutagenesis analysis of PI3Kα/Compd**1** complex (A), PI3Kα/Compd**2** complex (B), VPS34/Compd**1** complex (C), and VPS34/Compd**2** complex (D).