Supplemental Information

Identification of a Non-Gatekeeper Hotspot for Drug-Resistant Mutations in mTOR Kinase Using a Novel *S. cerevisiae* System

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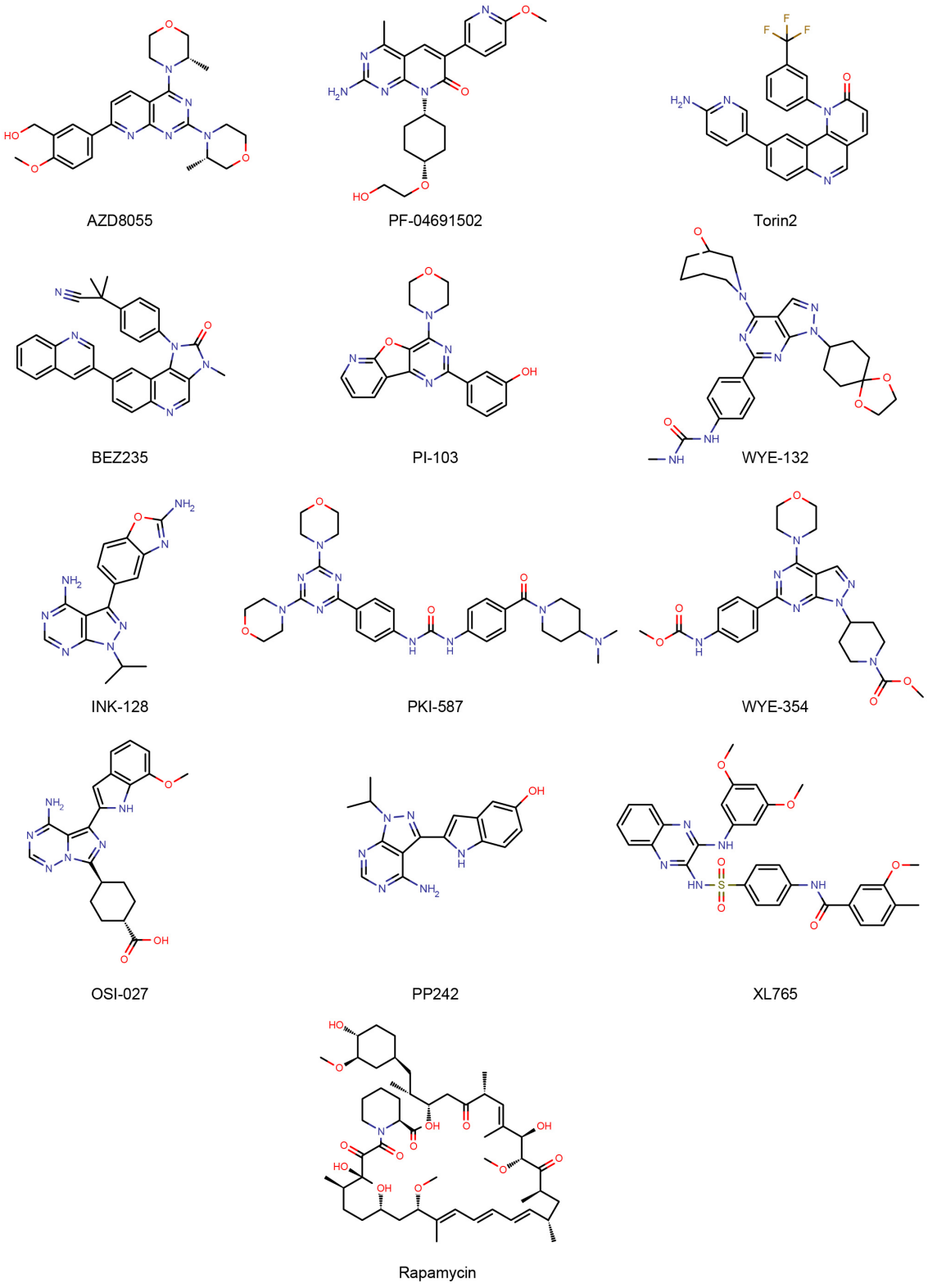


Figure S1, related to Figure 1: Chemical Structures of mTOR inhibitors

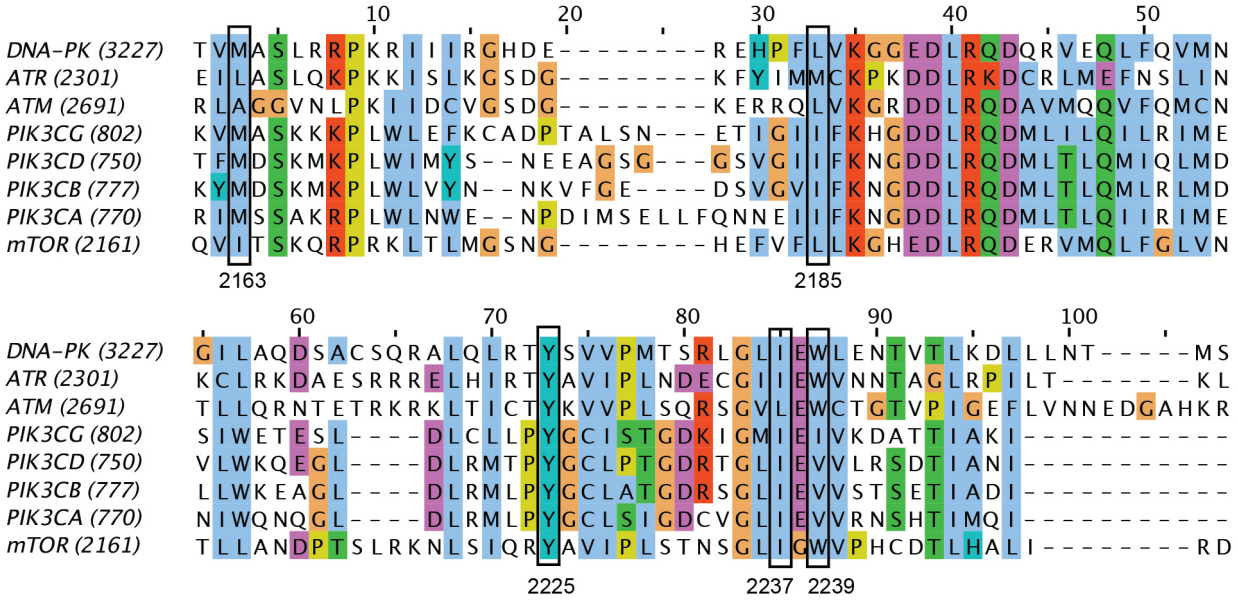


Figure S2, related to Figure 3: Alignment of hydrophobic pocket sequences of PI3Ks and PIKKs

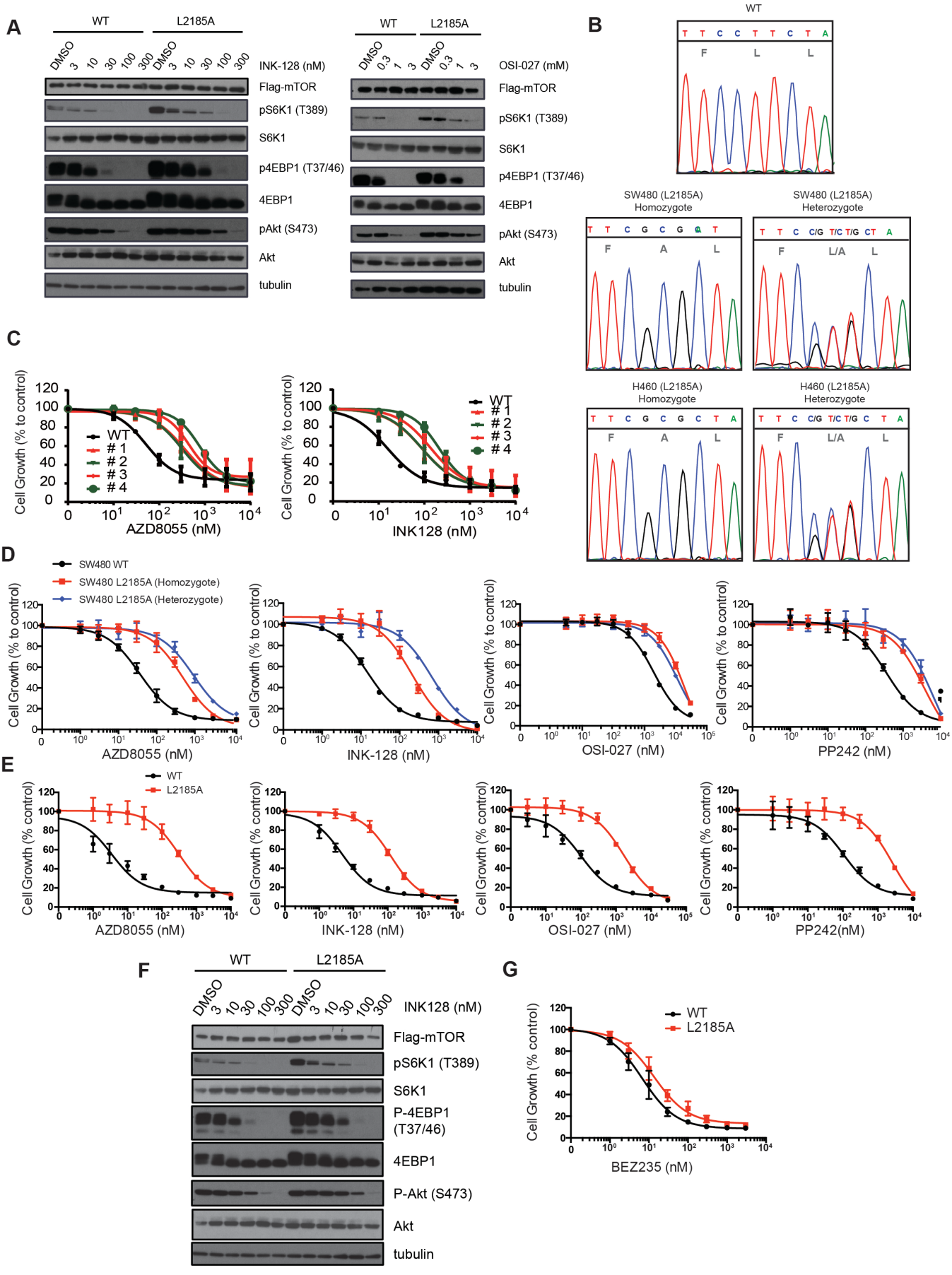

A
WT
L2185A
DMSO
3
10
30
100
300
INK-128 (nM)
Flag-mTOR
pS6K1 (T389)
S6K1
p4EBP1 (T37/46)
4EBP1
pAkt (S473)
Akt
tubulin
OSI-027 (mM)
0.3
1
3
B
WT
T T C C T T C T A
F L L
SW480 (L2185A)
Homozygote
Heterozygote
H460 (L2185A)
F A L
F L/A L
C
Cell Growth (% to control)
AZD8055 (nM)
INK128 (nM)
WT
1
2
3
4
D
SW480 WT
SW480 L2185A (Homozygote)
SW480 L2185A (Heterozygote)
INK-128 (nM)
OSI-027 (nM)
PP242 (nM)
E
WT
L2185A
AZD8055 (nM)
INK-128 (nM)
OSI-027 (nM)
PP242(nM)
F
INK128 (nM)
Flag-mTOR
pS6K1 (T389)
S6K1
P-4EBP1
(T37/46)
4EBP1
P-Akt (S473)
Akt
tubulin
G
WT
L2185A
BEZ235 (nM)

Figure S3, related to Figure 5: L2185A mutation confers resistance to mTOR kinase inhibitors in lung cancer cells

(A) Flag-mTOR(L2185A), when transiently expressed in HEK293T cells, confers drug resistance to mTOR kinase inhibitors. HEK293T cells transiently expressing WT and L2185A mutant Flag-mTOR were treated with various concentrations of INK-128, OSI-027 for 1 hr. The effect on the level of P-S6K, S6K, P-4E-BP1, 4EB-P1, P-AKT and AKT was analyzed by immunoblot.

(B) Sequencing verification of genome-engineered SW480 and H460 cells carrying homozygous and heterozygous L2185A mutations in mTOR locus.

(C) Independent clones of engineered SW480 cells confer similar drug resistance. Cells were treated with various concentrations of AZD8055 and INK-128 for 2 days. Cell growth was measured by SRB assay. Clones #1, 3 carry heterozygous L2185A mutation. Clones #2, 4 carry homozygous L2185A mutation. Data represent means ± SD in three independent experiments.

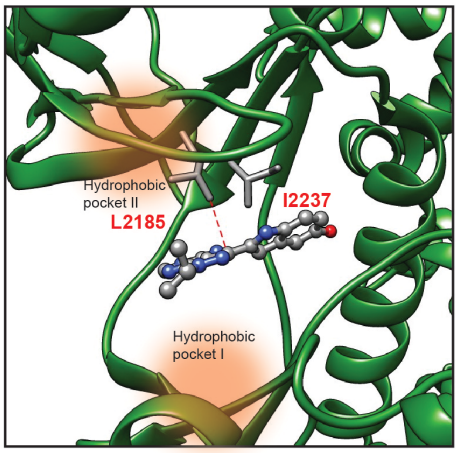
(D) SW480 cells carrying heterozygous and homozygous mTOR(L2185A) mutant alleles have similar drug resistance to mTOR kinase inhibitors in. SW480 cells carrying homozygous WT and L2185A mutant mTOR alleles were treated with various concentrations of AZD8055, INK-128, OSI-027, and PP242 for 2 days. Cell growth was measured by SRB assay. The drug carrier DMSO was used as a control. Data represent means ± SD in three independent experiments.

(E) H460 cells carrying homozygous WT and L2185A mutant mTOR alleles were treated with various concentrations of AZD8055, INK-128, OSI-027, and PP242 for 2 days. Cell growth was measured by SRB assay. The drug carrier DMSO was used as a control. Data represent means ± SD in three independent experiments.

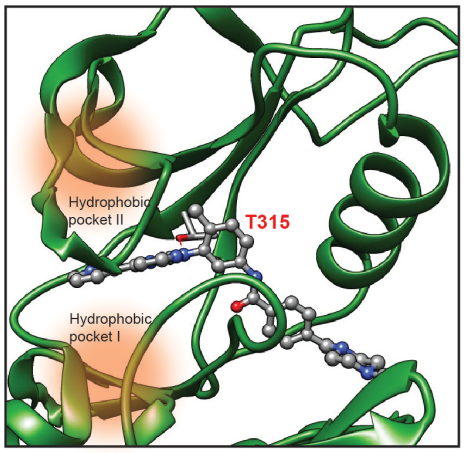
(F) H460 cells carrying homozygous WT and L2185A mutant mTOR alleles were treated with various concentrations of INK-128, OSI-027, AZD8055 and PP242 for 1 hr. The effect on the level of P-S6K, S6K, P-4E-BP1, 4EB-P1, P-AKT and AKT was analyzed by immunoblot.

(G) H460 cells carrying the WT and L2185A mutant mTOR allele were treated with various concentrations of BEZ235 for 2 day. The growth of H460 cells was measured by SRB assay. Data represent means ± SD in three independent experiments.

A

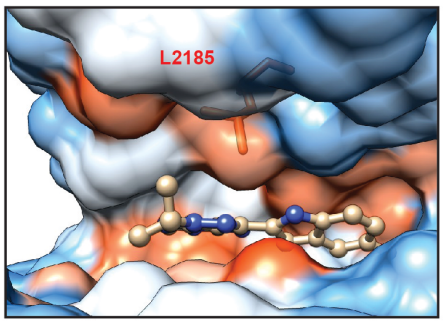


mTOR-PP242

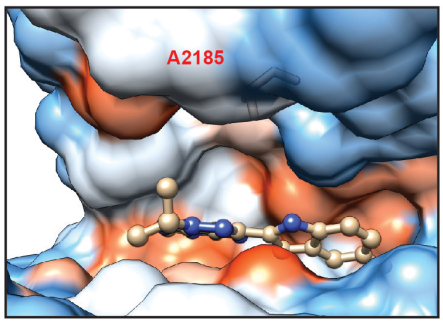


c-ABL-imatinib

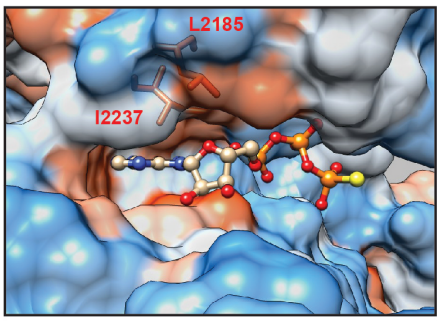
B



mTOR-PP242

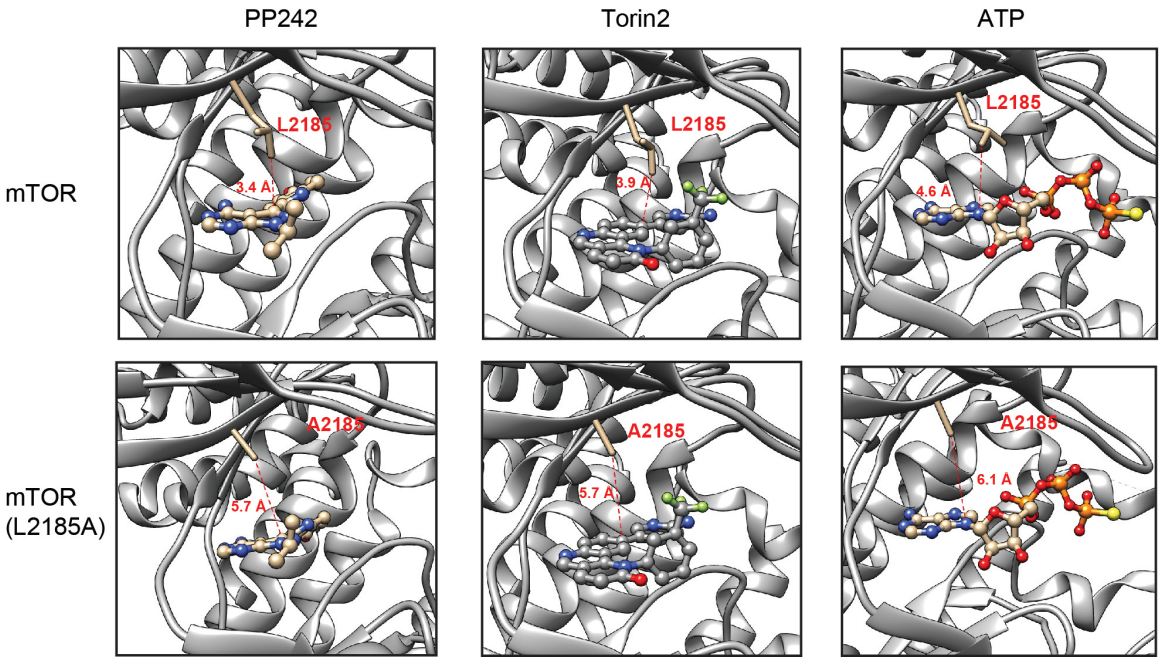


mTOR (L2185A)-PP242

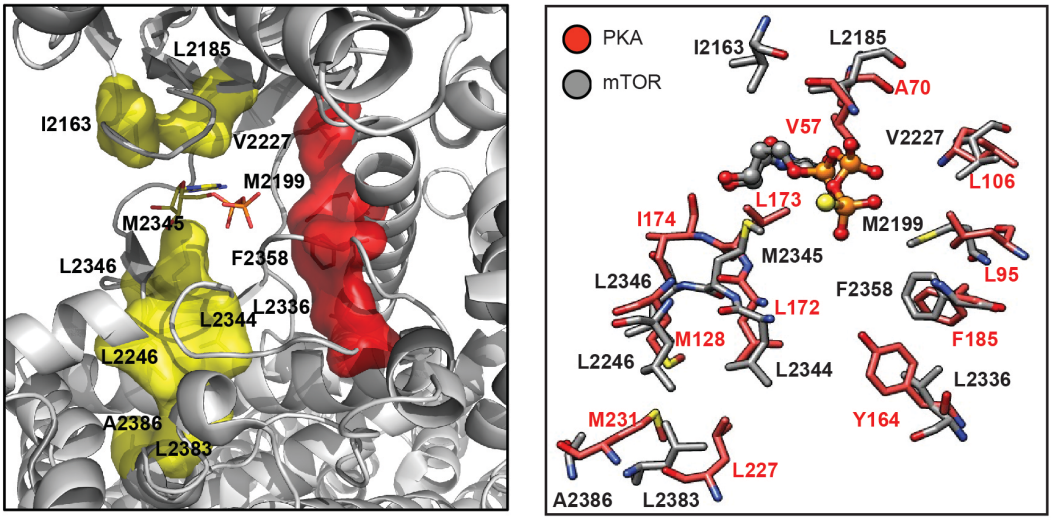


mTOR-ATP

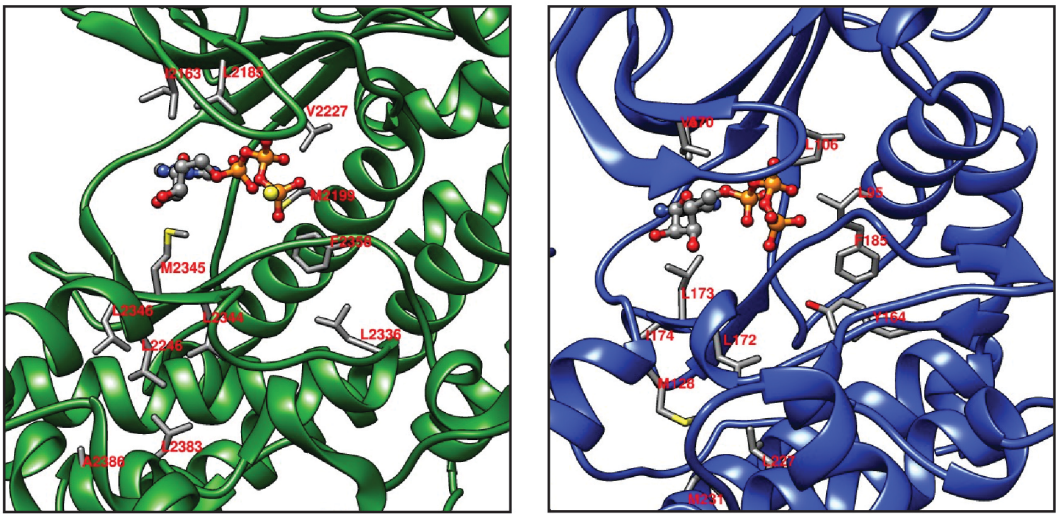
C



D


L2185
I2163
V2227
M2199
M2345
L2346
F2358
L2344
L2336
L2246
A2386
L2383
PKA
mTOR
I2163
L2185
A70
V57
V2227
L106
L173
I174
M2199
M2345
L95
L2346
F2358
L172
M128
F185
L2246
L2344
L2336
Y164
M231
L227
A2386
L2383

E


V2227
M2345
L2336
mTOR
PKA

F

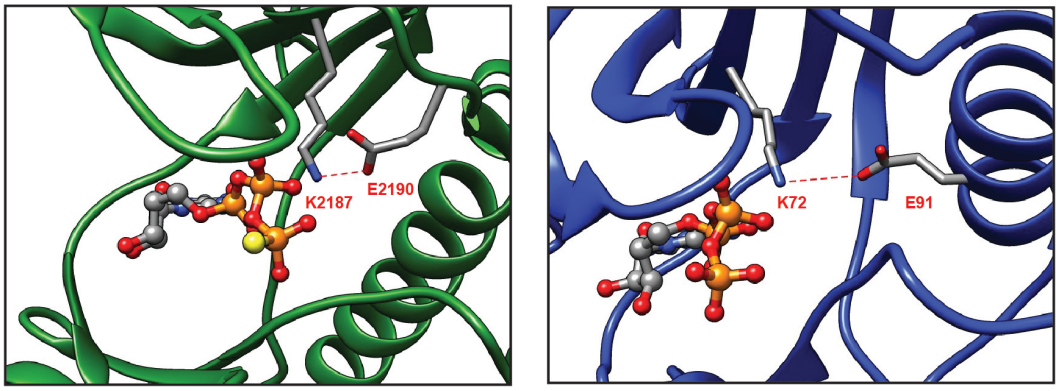

K2187
E2190
K72
E91
mTOR
PKA

Figure S4, related Figure 7: Structure analysis of mTOR L2185 residue in drug-binding pocket

(A) The location of L2185 and I2237 residue is shown within the structure of PP242-bound mTOR (PDB ID code 4JT5). Gatekeeper residue in the structure of imatinib-bound c-ABL kinase domain (PDB ID code 1IEP) is also shown for comparison. Atom N is labeled in blue and O labeled in red.

(B) Surface representation of the location of L2185 and modeled A2185 within the structures of ATP- and PP242-bound mTOR kinase domain (PDB ID codes 4JSP and 4JT5). Atom is colored as follows: N, blue; O, red; P, orange; S, yellow; Mg^{+2}, green. Surface representation is as follows: hydrophobic residue, red; neutral residue, white; hydrophilic residue, blue.

(C) Comparing the drug-binding pocket of PP242-bound (PDB ID code 4JT5), Torin2-bound (PDB ID code 4JSX), ATP-bound (PDB ID code 4JSP) WT, and modeled L2185A mutant mTOR kinase domain. Measured and estimated distances between WT or L2185A mutant mTOR and PP242, Torin2 or ATP are shown. Atom N is labeled in blue and O labeled in red.

(D) Two hydrophobic spines of mTOR compared with PKA. Left panel, the hydrophobic residues the R-spine are shown in red surface, and the C-spine residues are in yellow. Right panel shows only R- and C-spine residues (mTOR numbering is shown in black and PKA is in red).

(E) Shown are separate views of the ATP-binding pockets of mTOR and PKA, and the location of key residues in R- and C-spines.

(F) Shown are the salt bridge between K2187 and E2190 (mTOR), and K72 and E91 (PKA).