Donated Chemical Probe

PIP4K2A Inhibitor BAY-091

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PIP4K2A Probe BAY-091

Target rationale

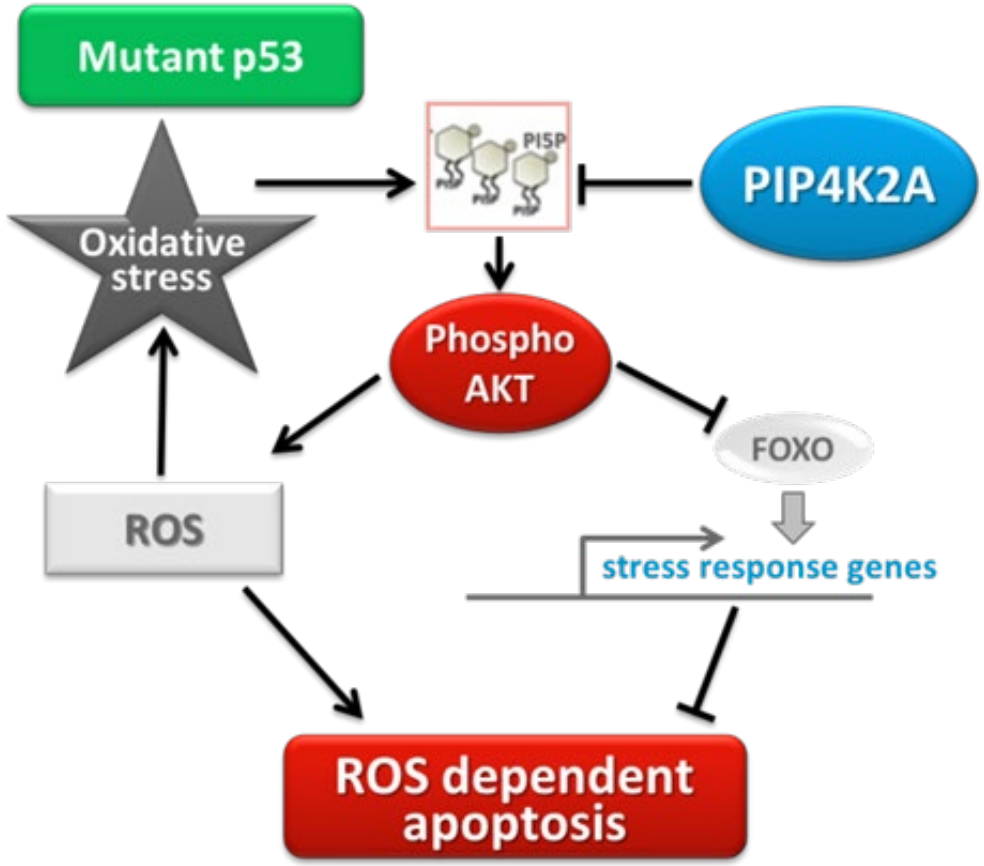
Working Hypothesis:

PIP4K2A inhibition induces cell death in p53 mutant tumors by hyperactivating AKT*

- p53 mutant cells have increased redox levels
- maintaining high levels of PIP4K2A is critical to prevent over-activation of AKT by PI5P
- PIP4K2A inhibitors are expected to induce ROS dependent apoptosis in p53 mutant tumor cells

Target Validation:

- PIP4K2A silencing significantly inhibits proliferation of p53 mutant cells (but not of p53 WT cells)
- PIP4K2A silencing induces AKT phosphorylation

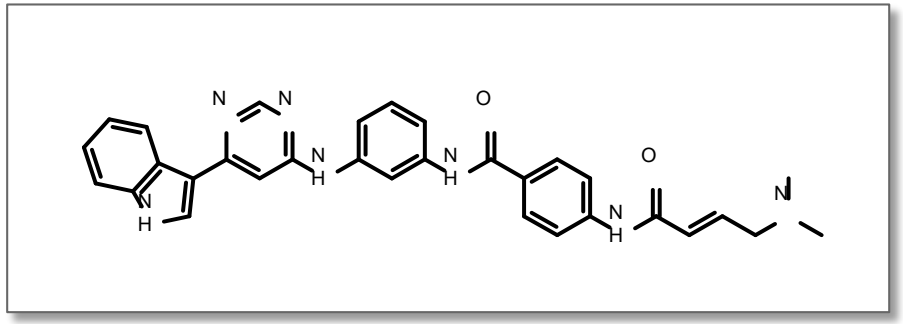


- **PIP4K2A inhibition expected to induce cell death in p53 mutant tumors by hyperactivating AKT**

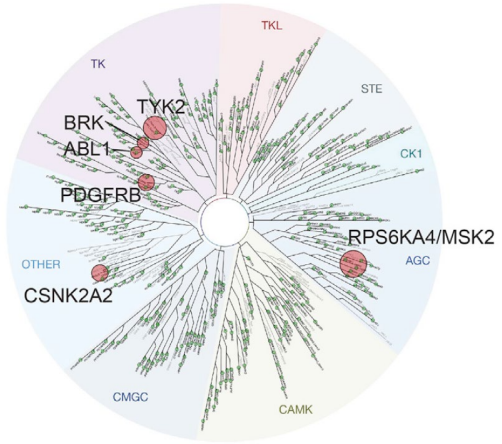
** Emerling et al. Cell 2013; Jude et al. Oncogene 2015; Nogueira et al. Cancer Cell 2008*

PIP4K2A Probe BAY-091

Literature known compound: THZ-P1-2



- Inhibitor THZ-P1-2 shows PI5P4K enzyme inhibition and target engagement in cells
- THZ-P1-2 covalently targets unannotated cysteines outside the PI5P4K active site
- AML/ALL cell lines are broadly sensitive to THZ-P1-2's covalent effects
- PI5P4K inhibition causes autophagy disruption and upregulates TFEB signaling



Summary of THZ-P1-2 activity on off-targets	
Kinase	**Secondary assays**
ABL1	21.3 nM (Invitrogen); >8000 nM on ABL1-positive cell lines; negative by streptavidin pulldown in lysate
CSNK2A2	1340 nM (Invitrogen)
KIT	>10000 nM (Invitrogen)
PDGFRB	3050 nM (Invitrogen)
RPS6KA4/MSK2	>10000 nM (Invitrogen)
TYK2	1600 nM (Invitrogen)
BRK	313 nM (Invitrogen); negative by streptavidin pulldown in lysate
PIKfyve	40 nM (Carna); ≥10000 nM in vacuolar assay; negative by streptavidin pulldown in lysate

Remarks:

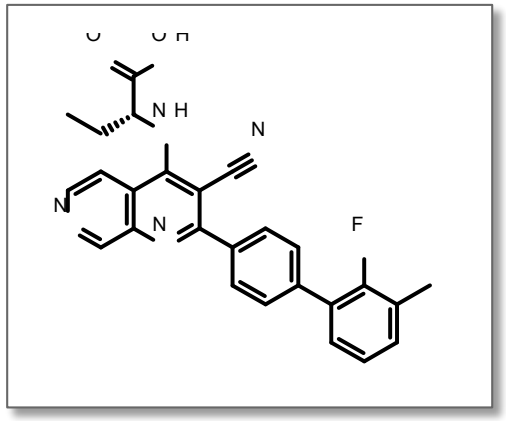
- moderate antiproliferative activity in AML and ALL cells, including THP-1 **but not p53 mutant selective**
- antiproliferative activity of THZ-P1-2 is in part due to its covalent binding

Sivakumaren et al., Cell Chemical Biology 2020, 27, 1–13

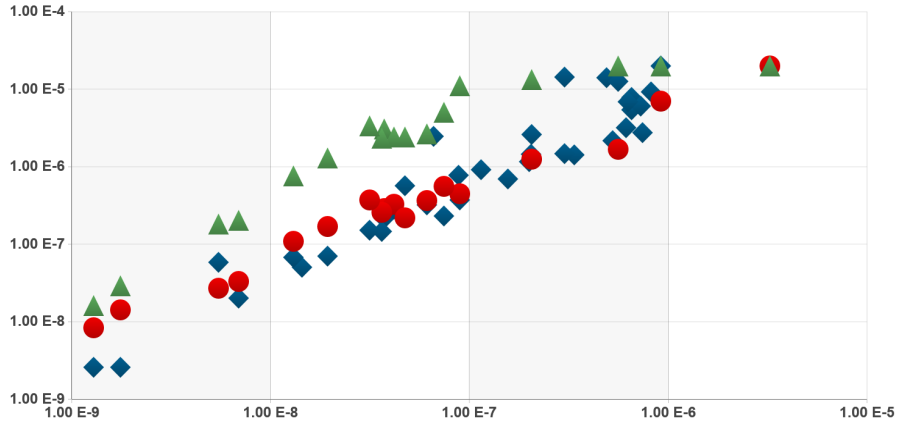
- **The availability of complementary inhibitors BAY-091 (non-covalent) and THZ-P1-2 (covalent) will help to better understand PIP4K2 pharmacology**

PIP4K2A Probe BAY-091

Biochemical activity



Pharmacological *in vitro* Properties	
PIP4K2A 10µM ATP IC_{50}	1 nM
PIP4K2A 250 µM ATP IC_{50}	3 nM
PIP4K2A HTRF 10 µM ATP IC_{50}	9 nM
PIP4K2A HTRF 2 mM ATP IC_{50}	16 nM



In vitro biochemical assay correlation

X-axis: PIP4K2A ADP Glo 10 µM ATP IC_{50} [M];

Y-axis: PIP4K2A ADP Glo 250 µM ATP IC_{50} [M] (blue diamonds), PIP4K2A HTRF 10 µM ATP IC_{50} [M] (red circles), PIP4K2A HTRF 2 mM ATP IC_{50} [M] (green triangles)

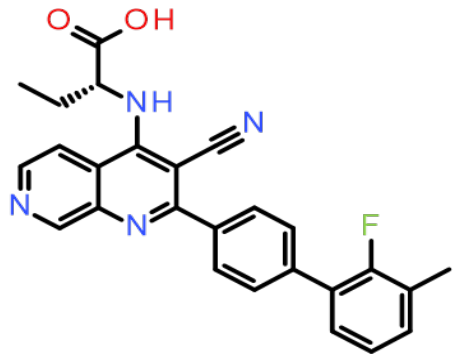
- **BAY-091 is a potent PIP4K2A inhibitor at low and high ATP concentrations in two different assay formats (for details see backup).**
- **All 4 biochemical PIP4K2A assay formats were shown to correlate well**

PIP4K2A Probe BAY-091

In vitro profile

Pharmacological *in vitro* Properties	
PIP4K2A 10µM ATP IC_{50}	1 nM
PIP4K2A 250 µM ATP IC_{50}	3 nM
PIP4K2A HTRF 10 µM ATP IC_{50}	9 nM
PIP4K2A HTRF 2 mM ATP IC_{50}	16 nM

Physicochemical Properties	
MW corr [g*mol]	427
TPSA [Å²]	99
LogD @pH 7.5	2.1
Sw pH 6.5 [mg/L]	> 1000
Stability, pH	stable



BAY-091
PIP4K2A Inhibitor

Safety Properties	
hERG [µM]	> 10

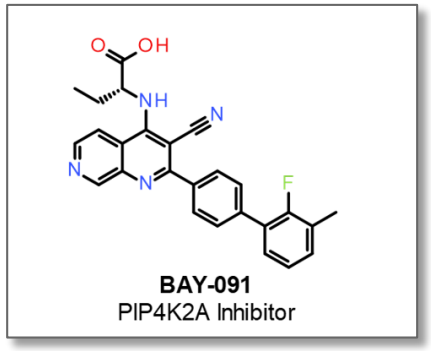
In vitro DMPK Properties						
Caco-2 permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	0		257		-	
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	liver microsomes (h /r)		0 / 2.8		100 / 33	
	hepatocytes (r)		2.3		44	
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	> 20	3.1	16	> 20	> 20	> 20

→ $PAMPA_{(A-B)}$ = 14 nm/s

- **Overall BAY-091 shows a good *in vitro* DMPK & PhysChem profile**

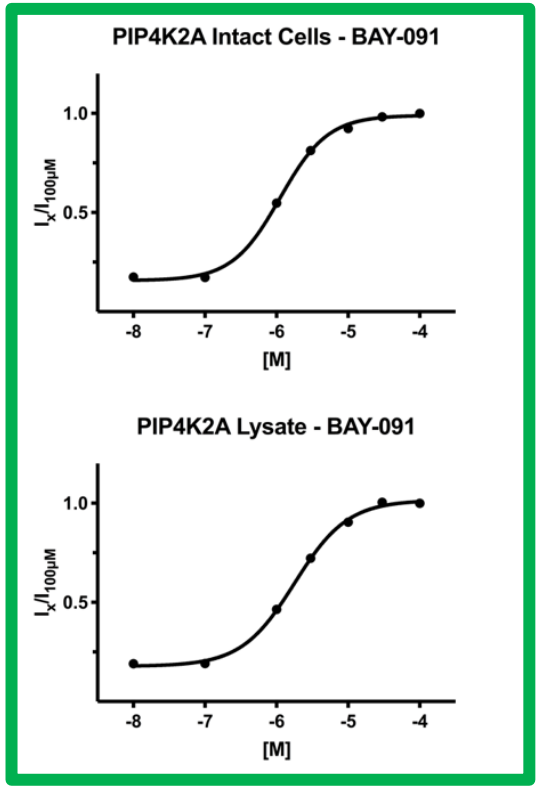
PIP4K2A Probe BAY-091

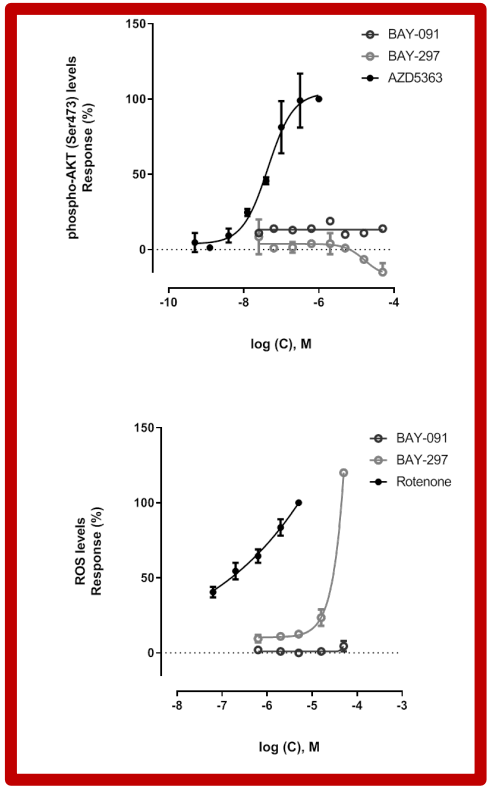
Cellular assays and target Engagement proven by CETSA



BAY-091
PIP4K2A Inhibitor

PIP4K2A kinase assay, 10µM / 250µM ATP [nM]	1.3 / 2.6
PIP4K2A HTRF, 10µM / 2mM ATP [nM]	8.5 / **16.4**
CETSA lysates, 60°C EC_{50} (95% CI) [µM]	**1.8** (1.4-2.2)
CETSA int. cells, 56°C EC_{50} (95% CI) [µM]	**1.1** (09-1.4)
Cellular mechanistic assay p-AKT HTRF, THP-1, EC_{50} [µM]	> 50
Cellular mechanistic assay ROS levels, THP-1, EC_{50} [µM]	> 50
2D proli, THP-1 (p53 mut) [µM]	> 30
2D proli, EBC-1 (p53 mut) [µM]	≈ 30
2D proli, NCI-H460 (p53 WT) [µM]	> 30

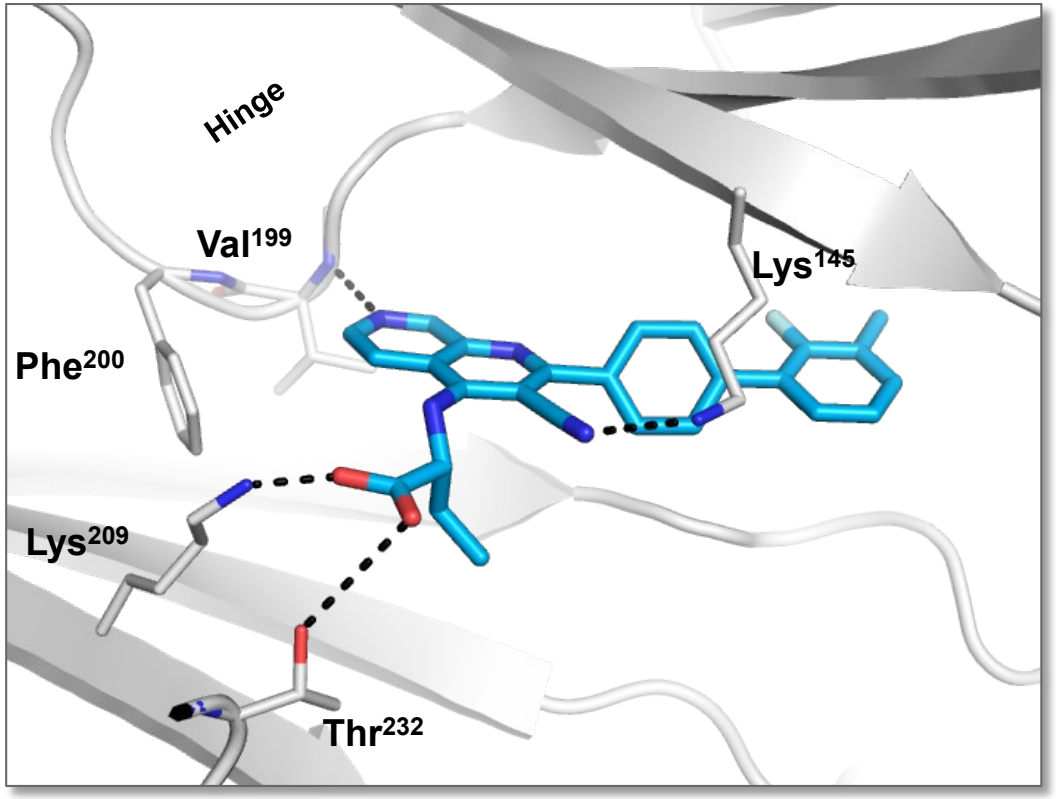


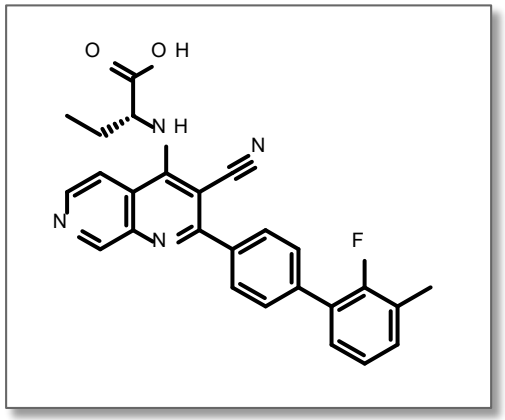


- **Cellular PIP4K2A target engagement with BAY-091 was proven by CETSA technology (IC_{50} ~ 1 µM)**
- **No effect in cellular mechanistic (pAKT, ROS) or functional assays (p53 mutant proliferation) → *Working Hypothesis to be questioned!***

PIP4K2A Probe BAY-091

X-ray Structure in complex with BAY-091





Key Interactions include

- Single hydrogen bond between naphythridine nitrogen and kinase hinge motif (Val^{199})
- Electronic interaction between the terminal naphythridine ring system and the adjacent Phe^{200} side chain
- The R-configuration provides an optimal exit vector for the carboxylic acid that allows hydrogen-bonding and salt-bridge interactions with Thr^{232} and Lys^{209}

- **X-ray of a BAY-091 in complex with PIP4K2A available**

PIP4K2A Probe BAY-091

BAY-091 was investigated in Eurofins Kinase Panel (373 kinases, 1 µM compound)

Kinase	Inhibition [%]
PIP4K2a(h)	142.02
Flt1(h)	56.81
KDR(h)	50.07
c-RAF(h)	35.96
NEK4(h)	32.07
PI3 Kinase (	30.93
SRMS(h)	26.31
PI3KC2g(h)	25.37
PAK2(h)	24.95
NEK1(h)	24.39
TSSK4(h)	23.97
PAK1(h)	23.77
PRAK(h)	23.56
B-Raf(h)	23.47
cKit(h)	22.32
MEK1(h)	21.54
RIPK1(h)	21.25
MLK3(h)	21.13
MST1(h)	20.61
SLK(h)	19.74
CDKL2(h)	19.42
TGFBR2(h)	19.28
DYRK2(h)	19.11
PRP4(h)	18.88
CLK4(h)	18.82
MYO3B(h)	18.15
CDK14/cycl	18.05
MLK2(h)	18.01
PKCbetaI(h)	16.52
DDR2(h)	16.27
PKCalpha(h)	16.24
ATR/ATRIP	16.22
STK32A(h)	15.99
NEK2(h)	15.9
EphA5(h)	15.8
HIPK4(h)	15.73
DMPK(h)	15.1
STK32B(h)	14.91

TrkC(h)	14.82
mTOR(h)	14.8
ICK(h)	14.79
WNK1(h)	14.66
PI3 Kinase (	14.4
CaMKIgamr	14.37
TSSK1(h)	14.25
Haspin(h)	14.18
ULK1(h)	14.16
PKR(h)	13.92
Aurora-A(h)	13.76
SIK(h)	13.36
MAP4K5(h)	13.35
FAK(h)	13.32
DCAMKL1(	12.96
Flt4(h)	12.93
LRRK2(h)	12.9
BTK(h)	12.82
MEKK2(h)	12.82
MKK3(h)	12.82
Tie2(h)	12.81
PRKG2(h)	12.72
PKCmu(h)	12.7
PKD3(h)	12.64
Axl(h)	12.63
CDKL4(h)	12.6
Lyn(h)	12.49
PDHK2(h)	12.45
CK1gamma2	12.38
Abl(h)	12.1
DYRK1B(h)	12.05
IGF-1R(h), a	12.02
NIM1(h)	12.01
CDK9/cyclin	11.83
EphB4(h)	11.81
DDR1(h)	11.7
CDK4/cyclin	11.31
CaMKK2(h)	11.28
PASK(h)	11.07
WNK4(h)	11.06

PI3 Kinase (	11.03
PKA(h)	11
Rsk3(h)	10.89
EGFR(h)	10.85
Pyk2(h)	10.72
CDK18/cycl	10.59
ACK1(h)	10.5
Syk(h)	10.42
NUAK2(h)	10.39
CDK12/cycl	10.3
TAO2(h)	10.27
SNRK(h)	10.26
IKKepsilon(h	10.23
Mer(h)	10.21
PIP5K1a(h)	10
PKBgamma(	9.92
PDHK4(h)	9.88
DRAK1(h)	9.65
Wee1B(h)	9.64
FGFR2(h)	9.63
MLK4(h)	9.6
CLIK1(h)	9.55
STK16(h)	9.54
IR(h), activat	9.43
PDGFRalpha	9.33
PDGFRbeta(	9.33
NEK6(h)	9.31
MAK(h)	9.23
EphA1(h)	9.2
Itk(h)	9.18
CK1delta(h)	9.07
TSSK3(h)	8.88
CK1epsilon(h	8.85
CDK1/cyclin	8.82
CaMKIIbeta	8.65
LIMK1(h)	8.64
NLK (h)	8.59
ErbB2(h)	8.53
TBK1(h)	8.44
NEK3(h)	8.41

LATS1(h)	8.31
MAP4K3(h)	8.29
CDK6/cyclin	8.28
TLK2(h)	8.27
GRK3(h)	8.23
PKG1beta(h	8.22
CDKL3(h)	8.18
CK1gamma1	8.17
LOK(h)	8.13
BRK(h)	8.07
TAK1(h)	7.89
MYLK2(h)	7.84
Arg(h)	7.75
MAPK2(h)	7.74
DYRK3(h)	7.66
cSRC(h)	7.51
Snk(h)	7.48
AAK1(h)	7.44
CaMKI(h)	7.33
CaMKIV(h)	7.26
CDK2/cyclin	7.13
NEK11(h)	7.12
DRAK2(h)	7.08
CSK(h)	6.94
PKG1alpha(h	6.77
PAR-1Balph	6.75
PKACbeta(h	6.73
CDK5/p25(h	6.65
NEK9(h)	6.61
FGFR1(h)	6.44
MAPK1(h)	6.43
Pim-2(h)	6.42
IRR(h)	6.25
IRAK4(h)	6.22
SGK3(h)	6.09
NDR2(h)	5.88
PKCiota(h)	5.85
MARK4(h)	5.8
LKB1(h)	5.7
MST4(h)	5.69

PRK1(h)	5.64
STK39(h)	5.6
eEF-2K(h)	5.5
NEK7(h)	5.49
STK32C(h)	5.49
GSK3alpha(h	5.47
MRCKgamm	5.46
ULK2(h)	5.46
MOK(h)	5.44
CHK2(h)	5.38
DCAMKL3(	5.32
MAPKAP-K	5.32
TrkA(h)	5.32
WEE1(h)	5.28
Fer(h)	5.23
BIKe(h)	5.17
ALK2(h)	5.14
SIK3(h)	5.14
MuSK(h)	5.1
Fgr(h)	5.05
MAPKAP-K	5.05
CaMKK1(h)	5.03
PRK2(h)	5.02
EphA3(h)	4.71
CaMKIdelta	4.68
CK2(h)	4.66
Aurora-C(h)	4.64
Tec(h) activa	4.62
CHK1(h)	4.56
ARK5(h)	4.55
MSSK1(h)	4.51
BMPR2(h)	4.45
PKD2(h)	4.37
HIPK3(h)	4.32
p70S6K(h)	4.3
Plk1(h)	4.29
JNK1alpha1(	4.24
DYRK1A(h)	4.22
PI3KC2a(h)	4.18
PAK3(h)	4.13

VRK2(h)	4.1
CDKL1(h)	4.02
SBK1(h)	4.02
IKKalpha(h)	3.94
ErbB4(h)	3.85
BrSK2(h)	3.83
PKCeta(h)	3.77
CLK1(h)	3.74
Bmx(h)	3.69
SIK2(h)	3.68
HIPK1(h)	3.6
Cdc7/cyclinE	3.59
PKBbeta(h)	3.52
SRPK1(h)	3.4
Mnk2(h)	3.39
CK2alpha1(h	3.34
WNK2(h)	3.31
PEK(h)	3.26
CK1alpha(h)	3.17
MINK(h)	3.07
VRK1(h)	3.04
LIMK2(h)	3.03
STK25(h)	3.01
ZAP-70(h)	2.99
IKKbeta(h)	2.85
CLK3(h)	2.84
STK33(h)	2.82
SAPK2a(h)	2.76
GRK7(h)	2.75
IRE1(h)	2.72
PrKX(h)	2.66
MARK1(h)	2.52
A-Raf(h)	2.41
CDK3/cyclin	2.41
Met(h)	2.36
TAO3(h)	2.25
EphA7(h)	2.1
TrkB(h)	2.08
TAO1(h)	1.92
GRK2(h)	1.9

LATS2(h)	1.83
Rsk2(h)	1.78
Pim-3(h)	1.67
GRK1(h)	1.52
PIP5K1g(h)	1.51
MSK1(h)	1.48
PAK5(h)	1.42
EphB2(h)	1.4
mTOR FKB	1.38
PAK4(h)	1.36
TYK2(h)	1.22
ACTR2(h)	1.17
TTBK2(h)	1.17
CDK13/cycl	1.16
MST3(h)	1.03
ZIPK(h)	0.91
CDK16/cycl	0.85
GCK (h)	0.85
Ron(h)	0.76
TRB2(h)	0.49
Hck(h)	0.45
PI3 Kinase (	0.43
ULK3(h)	0.33
ATM(h)	0.32
CDK7/cyclin	0.3
HIPK2(h)	0.19
GRK6(h)	0.16
CDK17/cycl	0.09
ChaK1(h)	0.08
MST2(h)	0.08
CLK2(h)	0.07
CDK5/p35(h	-0.03
GSK3beta(h	-0.05
PhKgamma2	-0.34
PKCgamma(	-0.39
JAK1(h)	-0.44
EphA2(h)	-0.55
ROCK-II(h)	-0.6
Lck(h)	-0.68
TGFBR1(h)	-0.85

TLK1(h)	-0.87
FGFR3(h)	-1
PKBalpha(h	-1.04
JNK2alpha2(	-1.41
CDK2/cyclin	-1.5
PKCbetaII(h	-1.59
WNK3(h)	-1.89
AMPKalpha	-1.91
TSSK2(h)	-1.97
DCAMKL2(	-2.01
TTBK1(h)	-2.05
SAPK2b(h)	-2.08
PKCzeta(h)	-2.41
Pim-1(h)	-2.53
ROCK-I(h)	-2.77
Txk(h)	-2.9
IR(h)	-2.92
GCN2(h)	-3.21
DAPK1(h)	-3.29
IRAK1(h)	-3.31
GRK5(h)	-3.49
TAF1L(h)	-3.49
Ret(h)	-3.56
MEKK3(h)	-3.58
SAPK4(h)	-3.58
Lck(h) activa	-3.67
ALK4(h)	-3.68
Yes(h)	-3.76
OSR1(h)	-3.86
MSK2(h)	-3.96
PKCepsilon(	-4.26
CK2alpha2(h	-4.36
BrSK1(h)	-4.39
MARK3(h)	-4.67
ZAK(h)	-4.94
MRCKalpha	-4.99
Fms(h)	-5.04
MLCK(h)	-5.27
AMPKalpha	-5.39
EphB3(h)	-5.61

JAK2(h)	-5.62
PAK6(h)	-5.79
NDR1(h)	-6
Rsk1(h)	-6.03
ALK1(h)	-6.17
Rsk4(h)	-6.19
Hck(h) activ	-6.21
HRI(h)	-6.26
SGK(h)	-6.56
EphA8(h)	-6.72
DAPK2(h)	-6.79
Plk3(h)	-7.76
CaMKIbeta(	-7.99
SRPK2(h)	-8.51
PDK1(h)	-8.72
Plk4(h)	-8.83
PhKgamma1	-9.06
TTK(h)	-9.1
MEK2(h)	-9.56
ASK1(h)	-9.98
IGF-1R(h)	-10.09
PKCtheta(h)	-10.11
EphB1(h)	-10.48
CRIK(h)	-10.67
DNA-PK(h)	-10.68
Ros(h)	-11.32
ALK6(h)	-11.34
SGK2(h)	-11.48
MELK(h)	-11.67
Fes(h)	-11.86
Flt3(h)	-12.01
JNK3(h)	-12.1
MAP4K4(h)	-12.23
SAPK3(h)	-12.55
CaMKIIalph	-13.29
JAK3(h)	-14.21
PTK5(h)	-14.53
TNIK(h)	-14.85
FGFR4(h)	-15.75
MRCKbeta(	-15.79

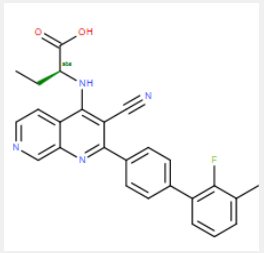
CaMKIIgam	-16.51
LTK(h)	-16.81
EphA4(h)	-16.84
Fyn(h)	-17.31
MKK6(h)	-18.97
PKCdelta(h)	-20.86
CaMKIIdelta	-21.46
Aurora-B(h)	-23.74
MLK1(h)	-23.82
Blk(h)	-26.86
CK1gamma3	-28.46
RIPK2(h)	-29.87
Rse(h)	-32.02
ALK(h)	-33.12
HPK1(h)	-34.19

- **BAY-091 does not inhibit any off-target kinases > 60% @ 1 µM compound concentration**

PIP4K2A Probe BAY-091

In vitro technical profile of Negative Control BAY-0361

*For accurancy, the probe candidate BAY-091 was also tested at Eurofins: IC_{50} (BAY-91): 21 nM



BAY-0361

POTENCY (IC_{50} [nM])	
PIP4K2A IC_{50} eurofins	371 nM* (18 fold)

Properties & Physchem	
LogD @ pH 7.5	2.0
fu [%] Williams_E / rat / Mouse	-
Sw @ pH 6.5 [mg/L]	tbd
MW / TPSA [g*mol / Å^2]	440 / 99
Stability (r /h plasma, 4h) [%]	-

in vitro DMPK Properties						
Caco2 Permeability	P_{app}(A-B) [nm/s]		P_{app}(B-A) [nm/s]		efflux ratio	
	tbd					
metabolic stability			CL [L/h/kg]		F_{max} [%]	
	Human liver mics		tbd			
	rat hepatocytes					
	human hepatocytes					
CYP inhibition IC_{50} [µM]	1A2	2C8	2C9	2D6	3A4	3A4 preinc.
	-	-	-	-	-	
PXR	-					

Selectivity	
In-house kinase panel (#)	tbd
Eurofins safety panel	Not available

SAFETY	
Cytotox	Not available
hERG IC_{50} [µM]	Not available

- **BAY-0361 was suggested as negative control**

PIP4K2A Probe BAY-091

Summary / Conclusion

Probe criteria	
Inhibitor/agonist potency: goal is < 50 nM (IC_{50}, Kd)	**BAY-091** meets criteria
Selectivity within target family: goal is > 30-fold	**Surpasses criteria** **BAY-091** was investigated in Eurofins Kinase Panel: No off-target kinase inhibition > 60% at 1 µM compound concentration.
Selectivity outside target family: describe the off-targets (which may include both binding and functional data)	**BAY-091** was investigated in Bayer-Eurofins Safety Panel. For results please see backup slide
On target cell activity for cell-based targets: goal is < 1 µM IC_{50}/EC_{50}	**Surpasses criteria** Cellular target engagement demonstrated by CETSA technology: IC_{50} (intact cells) ~ 1 µM.
Suitability as in vivo chemical probe	No
Neg ctrl: in vitro potency – > 100 times less; Cell activity – >100 times less potent than the probe	BAY-0361 (18 fold less active)

PIP4K2A Probe BAY-091

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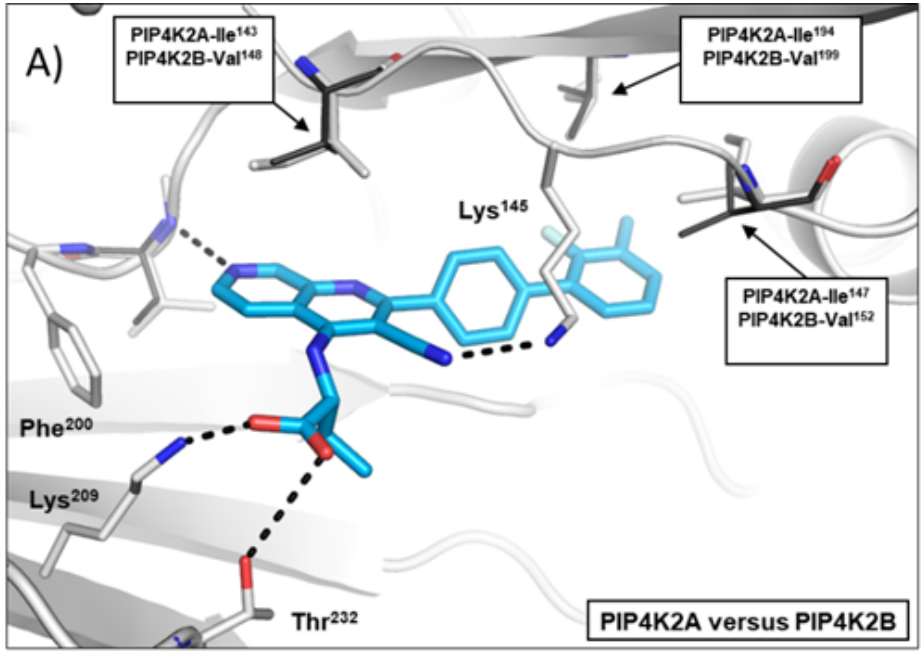
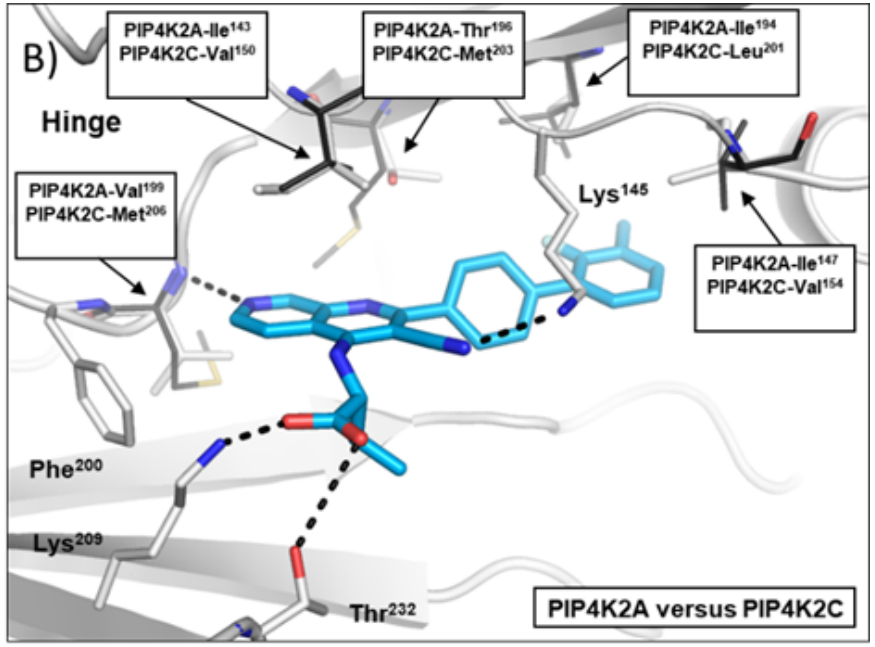
Thank you to the whole team!

Thank You

PIP4K2A Probe BAY-091

Selectivity versus PIP4K2B and PIP4K2C





- Key PIP4K2A residues interacting with BAY-091 are conserved in PIP4K2B. Other residues differences are conservative changes that are not expected to sterically disrupt BAY-019 binding

- PIP4K2A gatekeeper threonine residue is replaced by a methionine in the PIP4K2C isoform.

➢ BAY-091 may have reduced activity against the PIP4K2C isoform

PIP4K2A Probe BAY-091

Selectivity Profile in more detail: safety screen (Eurofins, # 77 targets)

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
Compound: CHH004-2020, PT #: 1234752						
107000	Aldose Reductase	449834	rat	2	10 µM	20
107710	ATPase, Na^+/K^+, Heart, Pig	449916	pig	2	10 µM	-22
112020	Carbonic Anhydrase II	449666	hum	2	10 µM	-6
104010	Cholinesterase, Acetyl, ACES	449747	hum	2	10 µM	27
116030	Cyclooxygenase COX-1	449646	hum	2	10 µM	19
118030	Cyclooxygenase COX-2	449647	hum	2	10 µM	29
124010	HMG-CoA Reductase	449836	hum	2	10 µM	-7
132000	Leukotriene LTC_4 Synthase	449838	gp	2	10 µM	-7
199017	Lipoxygenase 15-LO	449987	hum	2	10 µM	20
140010	Monoamine Oxidase MAO-A	449648	hum	2	10 µM	7
140120	Monoamine Oxidase MAO-B	449649	hum	2	10 µM	81
142000	Nitric Oxide Synthase, Neuronal (nNOS)	449840	rat	2	10 µM	13
199010	Nitric Oxide Synthetase, Inducible (iNOS)	449841	mouse	2	10 µM	-12
107300	Peptidase, Angiotensin Converting Enzyme	449664	rabbit	2	10 µM	0
152000	Phosphodiesterase PDE3	449848	hum	2	10 µM	74
154420	Phosphodiesterase PDE4D2	449650	hum	2	10 µM	38
156000	Phosphodiesterase PDE5	449849	hum	2	10 µM	62
194020	Thromboxane Synthase	449842	hum	2	10 µM	98
200510	Adenosine A_1	449707	hum	2	10 µM	13
200610	Adenosine A_{2A}	449708	hum	2	10 µM	7
200720	Adenosine A_3	449787	hum	2	10 µM	21
203110	Adrenergic α_{1A}	449651	hum	2	10 µM	-4
203630	Adrenergic α_{2A}	449652	hum	2	10 µM	-15
203710	Adrenergic α_{2B}	449653	hum	2	10 µM	-2
203810	Adrenergic α_{2C}	449691	hum	2	10 µM	4
204010	Adrenergic β_1	449702	hum	2	10 µM	2
204110	Adrenergic β_2	449761	hum	2	10 µM	-12
204200	Adrenergic β_3	449875	hum	2	10 µM	16
206000	Androgen (Testosterone)	449771	hum	2	10 µM	14
210030	Angiotensin AT_1	449654	hum	2	10 µM	22
210120	Angiotensin AT_2	449655	hum	2	10 µM	11
212520	Bradykinin B_1	449852	hum	2	10 µM	0
212620	Bradykinin B_2	449762	hum	2	10 µM	1
217050	Cannabinoid CB_1	449714	hum	2	10 µM	-11
217100	Cannabinoid CB_2	449716	hum	2	10 µM	1

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
219500	Dopamine D_1	449770	hum	2	10 µM	15
219600	Dopamine D_{2L}	449693	hum	2	10 µM	8
219700	Dopamine D_{2S}	449695	hum	2	10 µM	10
219800	Dopamine D_3	449694	hum	2	10 µM	27
224010	Endothelin ET_A	449763	hum	2	10 µM	-11
224110	Endothelin ET_B	449880	hum	2	10 µM	1
226010	Estrogen ERα	449769	hum	2	10 µM	10
226810	$GABA_A$, Chloride Channel, TBOB	449780	rat	2	10 µM	12
226600	$GABA_A$, Flunitrazepam, Central	449764	rat	2	10 µM	3
228510	$GABA_B$, Non-Selective	449712	rat	2	10 µM	5
232030	Glucocorticoid	449659	hum	2	10 µM	32
232600	Glutamate, AMPA	449720	rat	2	10 µM	-4
232710	Glutamate, Kainate	449699	rat	2	10 µM	-2
232810	Glutamate, NMDA, Agonism	449697	rat	2	10 µM	-3
232910	Glutamate, NMDA, Glycine	449768	rat	2	10 µM	-1
239300	Growth Hormone Secretagogue (GHS, Ghrelin)	449787	hum	2	10 µM	6
239610	Histamine H_1	449767	hum	2	10 µM	27
239710	Histamine H_2	449702	hum	2	10 µM	-26
239820	Histamine H_3	449877	hum	2	10 µM	3
243000	Insulin	449792	rat	2	10 µM	-1
252200	Motilin	449823	hum	2	10 µM	-2
252610	Muscarinic M_1	449721	hum	2	10 µM	13
252710	Muscarinic M_2	449722	hum	2	10 µM	8
252810	Muscarinic M_3	449722	hum	2	10 µM	0
252910	Muscarinic M_4	449721	hum	2	10 µM	6
258730	Nicotinic Acetylcholine α3β4	449781	hum	2	10 µM	-8
260130	Opiate δ_1 (OP1, DOP)	449723	hum	2	10 µM	25
260210	Opiate κ (OP2, KOP)	449724	hum	2	10 µM	-2
260410	Opiate µ (OP3, MOP)	449725	hum	2	10 µM	13
299005	Progesterone PR-B	449766	hum	2	10 µM	29
299036	Purinergic P2X	449925	rat	2	10 µM	-30
268820	Purinergic P2Y, Non-Selective	449883	rat	2	10 µM	31
271110	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{1A}$	449728	hum	2	10 µM	27
271650	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2A}$	449704	hum	2	10 µM	19
271700	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	449815	hum	2	10 µM	98
271800	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2C}$	449759	hum	2	10 µM	29

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
202020	Transporter, Adenosine	449765	hum	2	10 µM	42
220320	Transporter, Dopamine (DAT)	449782	hum	2	10 µM	-12
226400	Transporter, GABA	449786	rat	2	10 µM	1
204410	Transporter, Norepinephrine (NET)	449790	hum	2	10 µM	25
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	449729	hum	2	10 µM	10
287530	Vasopressin V_{1A}	449874	hum	2	10 µM	-3

Significant inhibition at 10 µM compound concentration for the following targets:

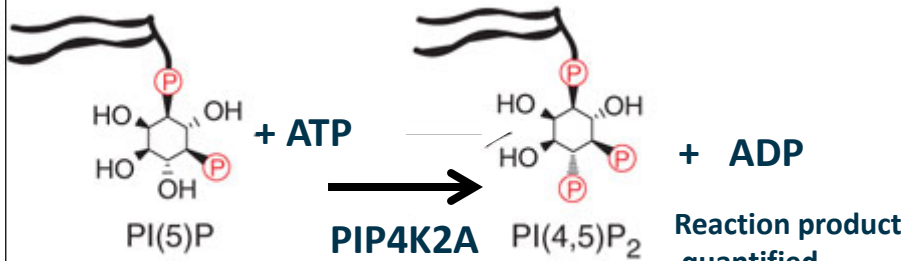
Cat #	Assay Name	Species	Conc.	% Inh.
140120	Monoamine Oxidase MAO-B	hum	10 µM	81
152000	Phosphodiesterase PDE3	hum	10 µM	74
156000	Phosphodiesterase PDE5	hum	10 µM	62
194020	Thromboxane Synthase	hum	10 µM	98
271700	Serotonin (5-Hydroxytryptamine) $5\text{-}HT_{2B}$	hum	10 µM	98

- BAY-091 shows good selectivity beyond kinases. Significant inhibition @ 10 µM: MAO-B, PDE3, PDE5, 5-HT2B, Thromboxane Synthase

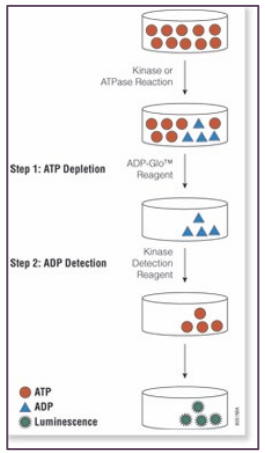
PIP4K2A Probe BAY-091

Biochemical Assay Formats

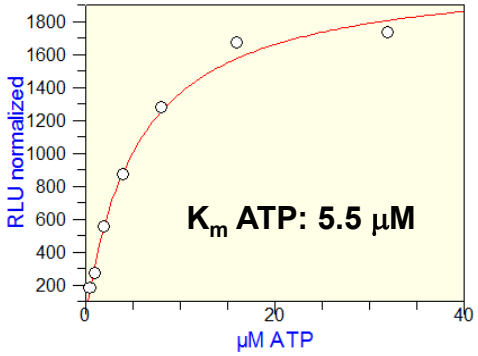
Assay 1: Quantification of reaction product **ADP**

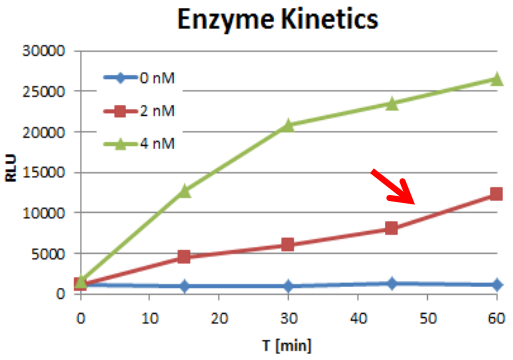


Luminescence readout: ADP-Glo (Promega)

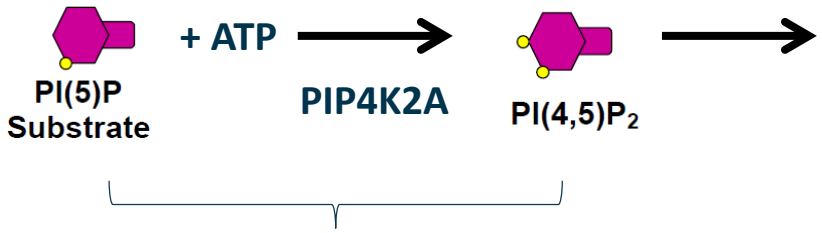


Buffer:
25mM HEPES pH 7.8
0.3mM EGTA
0.1% CHAPS
5mM MgCl2
12.5mM NaCl
1mM DTT
2nM PIP4K2A
15uM diC8 (PI(5)P-diC8)
10uM ATP

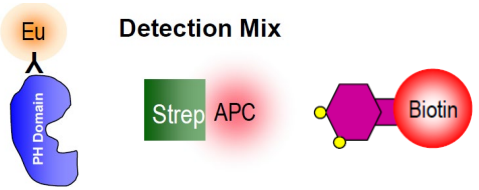




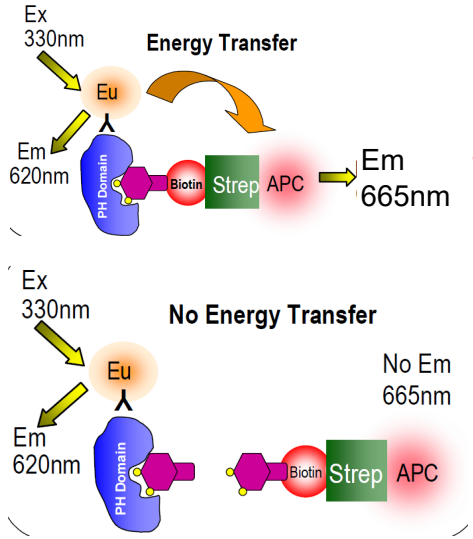
Assay 2: Quantification of reaction product **PI(4,5)P2** (HTRF)



Addition of detection mix

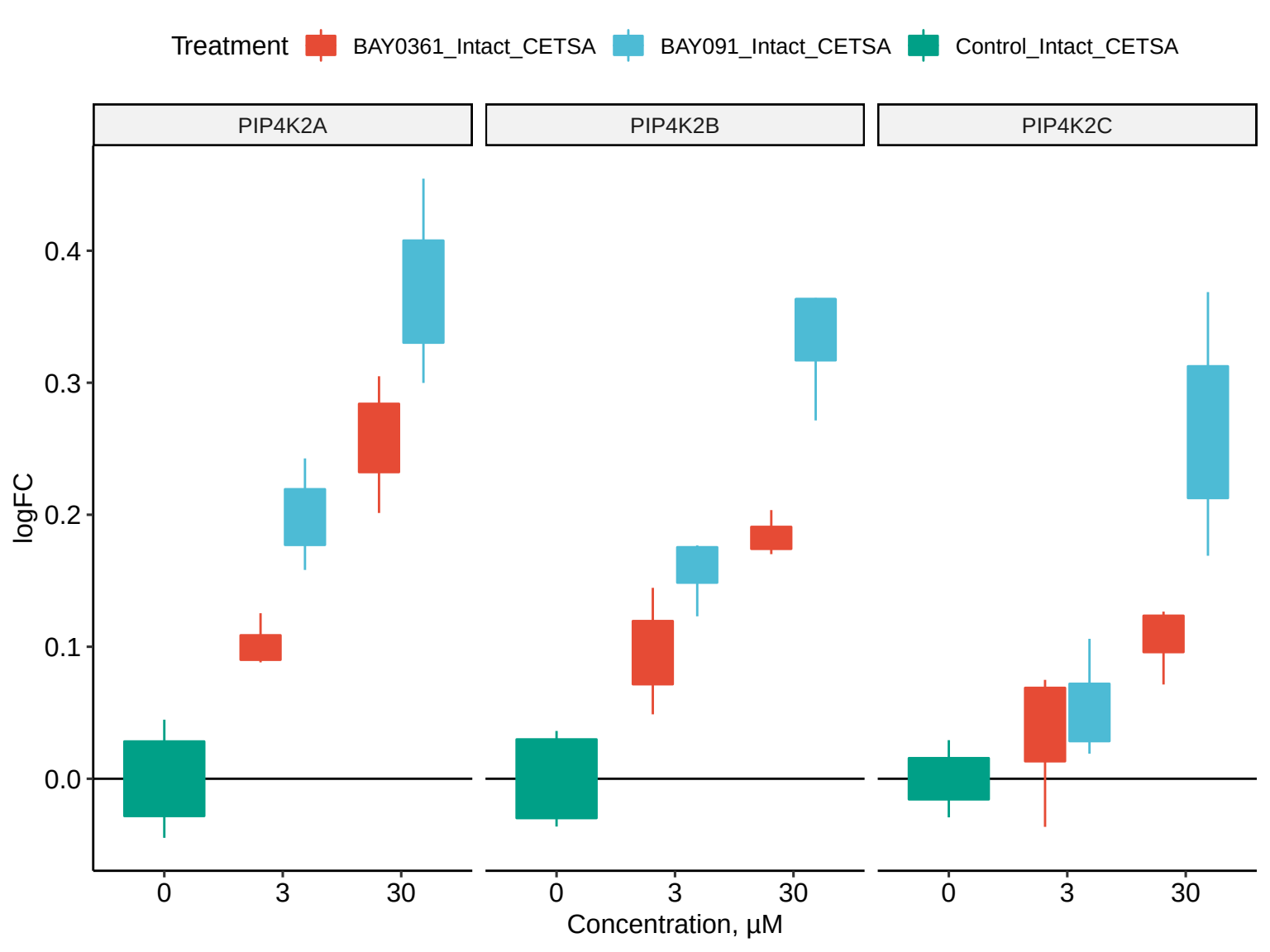


- Purified PH domain
- Biotinylated PI(4,5)P2
- Fluorophore labelled Streptavidin
- Stop Solution



FRET complex built
if no PI(4,5)P2 present
Signal ON.

FRET complex disrupted
If PI(4,5)P2 is present
Signal OFF


Treatment
BAY0361_Intact_CETSA
BAY091_Intact_CETSA
Control_Intact_CETSA
PIP4K2A
PIP4K2B
PIP4K2C
logFC
0.4
0.3
0.2
0.1
0.0
0
3
30
Concentration, µM

BAY0361_Intact_CETSA_3
BAY0361_Intact_CETSA_30
BAY091_Intact_CETSA_3
BAY091_Intact_CETSA_30
−log10(p_adj)
20
15
10
5
0
FECH
SULT1A1
SOD2
HBZ
CS
TNPO1
SULT1A4
HNRNPL
RBM7
SCP2
FECH
SULT1A1
CPOX
HSD17B4
P4HB
ACOX1
HNRNPM
CCAR2
DCUN1D5
ECPAS
NIT2
FANCI
KNTC1
XPO5
NUCB2
XPO1
CDC73
FNTB
UBE4A
NCAPD3
NDC80
USP9X
GLO1
RPRD1A
EIF4G2
FNTA
SULT1A4
GCN1
BZW1
XPO1
CPSF2
PES1
PRRC1
NUCB1
NUF2
TXNDC5
ACAA2
HIBCH
HSP90B1
HACL1
ADSS2
NDUFA3
ERP29
PWP1
NDUFAF2
PIP4K2A
FECH
PIP4K2B
HNRNPL
HNRNPA2B1
FECH
CPOX
PIP4K2B
SCP2
PIP4K2A
HSD17B4
APOB
BCAP31
GLO1
ACOX1
PIP4K2C
WDFY1
GALNT5
NT5DC1
HNRNPM
NUDT2
−1.0
−0.5
0.0
0.5
1.0
Amplitude