Supplemental Methods

Cellular Phosphorylation Inhibition Assays

To assess pirtobrutinib activity on other C481 mutations, HEK293T cells were transiently transfected with expression constructs encoding full-length Bruton tyrosine kinase (BTK) and BTK mutants, C481S, C481T, C481R, and C481G using standard transfection methods. Cells were serum-starved for 18 hours after transfection, followed by treatment for 2 hours with a range of pirtobrutinib concentrations. Y223 autophosphorylation in BTK, BTK C481S, and BTK C481T was detected using standard Western blotting methodology and chemiluminescence, followed by signal quantification using a Pxi4 device (Syngene, Cambridge, UK). Y223 phosphorylation in BTK C481R and C481G was detected using the MesoScale Diagnostics (Rockville, MD). The BTK Y223 phosphorylation signal was normalized to total BTK, and IC_{50} values were calculated relative to percent dimethyl sulfoxide control using a 4-parameter fit in GraphPad Prism software.

Starved Ramos RA1 cells ($1.1x10^7$ cells/condition) were incubated for 2 hours with a range of pirtobrutinib and cBTKi concentrations, followed by the addition of sodium orthovanadate for 30 minutes. Cells were then stimulated with anti-IgM (Jackson ImmunoResearch, West Grove, PA) at 5 µg/mL for 5 minutes at room temperature and resuspended in lysis buffer. Lysates were analyzed by Simple Western for levels of phospho-PLCγ2 Y1217, total PLCγ2, and GAPDH as a loading control. Phosphorylation signals were normalized to the corresponding total protein and IC_{50} values were calculated relative to percent DMSO control using a 4-parameter fit.

REC-1 cells ($8x10^6$ cells/condition) were serum-starved for 4 hours, incubated with pirtobrutinib for 2 hours, and then stimulated with anti-IgM (5 µg/mL) for 10 minutes. Cells were harvested, washed, and lysed and then analyzed by Simple Western for levels of phosphorylated PLCγ2 Y1217 and total PLCγ2. Phosphorylation signals were normalized to total PLCγ2 protein.

T-cell isolation and expression of activation/exhaustion marker analysis

Whole blood samples were obtained from healthy donors after approved consent in accordance with ethical practice from the Lilly research biological donation program. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by Ficoll-Paque density gradient centrifugation (Cytiva, Marlborough, MA). T-cells were isolated from PBMCs using EasySep Human T-Cell Isolation Kit 17951RF on a Robosep-S fully automated cell separator (Stemcell, Vancouver, Canada). T-cells were cultured in RPMI-1640 with 10% FBS at 37°C. T-cell number, viability, and size were measured on a Vi-CELL XR Cell Viability Analyzer. On day 0, T-cells were plated in Falcon 6-well clear flat bottom tissue culture-treated plates at $4x10^5$ cells/ml and activated using Human T-Activator CD3/CD28 Dynabeads (Thermo Fisher, Waltham, MA). On day 3, fresh media was added and T-cells were treated with DMSO, pirtobrutinib, or ibrutinib. On day 6, fresh media with drug was added to cells. On day 8, Dynabeads were removed, cells were washed, and incubated with anti-CD279-eFluor450 (PD-1), anti-CD366-FITC (TIM3), anti-CD223-APC (LAG3), and anti-CD8a-PE for 30 minutes. The mean fluorescence intensity of PD-1, TIM3, LAG3, and CD127 for CD8+ cells was measured on BD FACSVerse and analyzed using BD FACSuite (BD Bioscience, Ashland, OR).

Cytokine Assay

REC-1 cells were seeded in a 6-well plate at $2x10^6$ cells/well in RPMI-1640 ATCC with 10% FBS. Cells were dosed with pirtobrutinib or ibrutinib and incubated for 24 hours. Supernatants were collected and run using a Human Cytokine/Chemokine Magnetic Bead Panel on a Magpix instrument (Luminex, Austin, TX). Data were normalized to vehicle control to calculate the percentage of inhibition for each cytokine.

Competitive binding chemoproteomics method

Pirtobrutinib binding to kinases in live human PBMCs was tested using competitive binding chemoproteomics.[1] Briefly, pirtobrutinib, at multiple concentrations, was incubated with PBMCs in media supplemented with 0.5% fetal bovine serum (FBS) for 1 hour at 37°C, and 5% CO_2 and

washed to remove unbound inhibitor. Biotinylated acyl phosphates of ATP and ADP, which irreversibly react with protein kinases at conserved lysine residues in the ATP-binding pockets, were added and allowed to covalently bind in the kinase ATP-binding sites not occupied by pirtobrutinib. The identity of 180 labeled kinases was determined by mass spectrometry (MS) analysis. Pirtobrutinib binding to the kinases was determined by comparing the ATP and ADP probe-labeled peptide signals from pirtobrutinib-treated to untreated PBMCs, and binding of the inhibitor was expressed as percent inhibition of probe binding to kinase.

Pharmacokinetics of pirtobrutinib in mice

Female Balb/c mice were dosed orally (po) with a suspension of pirtobrutinib in methylcellulose/ Tween 80/ water (0.6%/0.5%/98.9%; w/w/v) at 10 mg/kg and 30 mg/kg. Blood was collected at 0.5, 1, 2, 4, 6, 8 and 24 hours post dosing from the inferior vena cava directly into ethylenediaminetetraacetic acid tubes. Plasma was isolated and pirtobrutinib concentration was analyzed. Samples (40 µL) were mixed with 120 µL of the precipitant solution (acetonitrile + 0.02 µM trifluperidol as internal standard spiking solution). Supernatants were diluted 1/1 (v/v) in water and then analyzed by liquid chromatography (LC)-MS/MS following standard conditions.

Supplemental Tables and Figures

Supplemental Table 1. X-ray diffraction data collection, processing and refinement statistics.

PDB ID	**8FLL**	**8FLN**
Data collection and processing		
Wavelength (Å)	0.9793	0.9793
Collection temperature (K)	100	100
Resolution range	53.963 - 1.498 (1.524 - 1.498)	53.904 - 1.334 (1.357 - 1.334)
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell		
a, b, c (Å)	41.482, 67.269, 90.383	41.595 67.036 90.675
α, β, γ (°)	90, 90, 90	90, 90, 90
Total reflections	221767 (9816)	376211 (19150)
Unique reflections	40714 (2035)	58281 (2873)
Multiplicity	5.4 (4.8)	6.5 (6.7)
Completeness (%)	98.2 (99.7)	99.6 (99.9)
Mean I/σ(I)	16.0 (2.2)	14.1 (2.1)
R_{pim}	0.024 (0.424)	0.028 (0.387)
$CC_{1/2}$	0.999 (0.838)	0.997 (0.815)
Refinement		
Resolution range	21.42 - 1.498 (1.552 - 1.498)	19.24 - 1.334 (1.382 - 1.334)
Reflections used in refinement	40678 (4073)	58234 (5761)
R_{work}	0.1771 (0.2455)	0.1844 (0.2834)
R_{free}	0.1977 (0.2594)	0.1918 (0.2907)
Number of non-hydrogen atoms	2396	2403
macromolecules	2163	2157
ligands	65	65
solvent	168	181
R.m.s deviations		
bond lengths (Å)	0.015	0.016
bond angles (°)	1.66	1.70
Ramachandran plot (%)		
favored	98.85	98.85
allowed	1.15	1.15
outliers	0	0
Average B-factor	27.54	28.29
macromolecules	26.58	27.32
ligands	28.57	27.77
solvent	39.45	39.99

Statistics for the highest-resolution shell are shown in parentheses.

Supplemental Table 2. Antibodies used in this study.

Antibody	Vendor/Cat number	Assay
anti-β-actin	CST #4970	Western blots
anti-BTK	BD Biosciences #611117	MSD, capture antibody
anti-BTK	CST #8547	Western blots and Simple Western
anti-GAPDH	Novus #MAB5718-SP	Simple Western
anti-phospho-BTK (Y223)	CST #5082 or Ab68217	Western blots and Simple Western
anti-phospho-BTK (Y223)	R&D System #MAB7205	MSD, capture antibody
anti-phospho-BTK (Y551)	BD Biosciences #558034	Western blots and Simple Western
anti-phospho-p44/42 MAPK (ERK 1/2) (Thr202/Tyr204)	CST #9101	High content imaging
anti-PLCγ2	CST #3872	Simple Western
anti-rabbit IgG Alexa 488	ThermoFisher #A11008	High content imaging, secondary antibody
APC mouse anti-human CD69	BD Biosciences #555533	Flow cytometry
goat $F(ab')_2$ anti-human IgM	Jackson ImmunoResearch #109-006-129	Stimulation of cells
PE mouse anti-human CD19	BD Biosciences #555413	Flow cytometry
phospho-PLCγ2 (Y1217)	CST #3871	Simple Western
sulfo-tag anti-rabbit	MSD, #R32AB1	MSD, secondary antibody
eFluor™ 450 mouse anti-human CD279 (PD-1)	eBiosciences #48-9969-42	Flow cytometry
FITC mouse anti-human CD366 (TIM3)	eBiosciences #11-3109-42	Flow cytometry
APC mouse anti-human CD223 (LAG-3)	eBiosciences #17-2239-42	Flow cytometry
PE mouse anti-human CD8a	eBiosciences #12-0088-42	Flow cytometry

Supplemental Table 3. Inhibition of BTK autophosphorylation in HEK293T cells transiently expressing BTK C481 resistance mutants.

Kinase	Pirtobrutinib IC_{50} (Mean ± SD, nM)	N
BTK	3.9 ± 1.6	3
BTK C481S	8.1 ± 1.3	2
BTK C481T	7.1 ± 3.3	2
BTK C481G	13.9 ± 3.8	2
BTK C481R	12.6 ± 5.2	2

Supplemental Table 4. Pirtobrutinib and ibrutinib effects on cytokine/chemokine secretion in REC-1 cells.

	Pirtobrutinib	**Ibrutinib**
Cytokine	**Mean IC_{50} ± SD (n=2)**	**Mean IC_{50} ± SD (n=2)**
MIP-1β/CCL4	2.6 ± 1.8	0.6 ± 0.3
TNFα	3.1 ± 0.1	0.9 ± 0.1
IL-10	3.2 ± 0.9	0.6 ± 0.4
MDC/CCL22	3.4 ± 0.9	0.9 ± 0.1
MIP-1α/CCL3	4.3 ± 0.0	0.9 ± 0.2
IL-8	4.5 ± 1.6	1.0 ± 0.1
sCD40L	6.1 ± 0.1	>10
EGF	>100	>10
EOTAXIN	>100	>10
FGF-2	>100	>10
FLT-3L	>100	>10
FRACTALKINE	>100	>10
G-CSF	>100	>10
IFNα2	>100	>10
IL-4	>100	>10
MCP-3	>100	>10
PDGF-AA	>100	>10
VEGF	>100	>10

Supplemental Table 5. Selectivity of BTK inhibitors in biochemical assays.

Kinase	Percent Activity				
	1000 nM pirtobrutinib	100 nM pirtobrutinib	100 nM ibrutinib	100 nM acalabrutinib	100 nM zanubrutinib
	Mean ± SD, % (n=12)	Mean ± SD, % (n=6)	Mean ± SD, % (n=2)	Mean ± SD, % (n=4)	Mean ± SD, % (n=2)
ABL1	93.3 ± 2.6	88.3 ± 1.1	86.7 ± 1.3	102.5 ± 3.6	101.4 ± 0.1
ABL2/ARG	96.9 ± 1.5	88.2 ± 0.2	76.4 ± 0.4	95.55 ± 2.1	90.1 ± 0.4
ACK1	93.8 ± 3.4	84.1 ± 0.3	69.2 ± 0.4	98.2 ± 0.1	96.6 ± 0.4
AKT1	100.1 ± 2.8	88.3 ± 1.1	92.4 ± 1.1	100.1 ± 2.8	92.5 ± 0.3
AKT2	91.2 ± 3.7	83.4 ± 4.3	87.7 ± 1.9	84.4 ± 5.2	95.4 ± 1.3
AKT3	91.7 ± 0.9	106.4 ± 1.3	102.1 ± 0.2	117.5 ± 2.5	98.9 ± 1.5
ALK	86.1 ± 3.2	89.3 ± 0.6	88.5 ± 1	93.4 ± 3.1	98.3 ± 2
ALK1/ACVRL1	99.9 ± 5.2	94.5 ± 0.1	95.9 ± 0.5	91.2 ± 2.7	112.4 ± 4.7
ALK2/ACVR1	110.1 ± 6.2	100.7 ± 1.4	95.4 ± 1	97.2 ± 1.2	103.7 ± 4.4
ALK3/BMPR1A	108.6 ± 0.7	101.4 ± 1.7	102.3 ± 3.2	102.1 ± 0.3	90.5 ± 0.5
ALK4/ACVR1B	95.8 ± 1.3	88.9 ± 1.1	87.6 ± 0.6	91.05 ± 0.9	105.5 ± 6.1
ALK5/TGFBR1	106.9 ± 0.6	101.2 ± 0.8	102.2 ± 0.3	98.6 ± 0.3	103.1 ± 7
ALK6/BMPR1B	101.9 ± 2.9	108.6 ± 1.3	107.2 ± 0.4	105.25 ± 2.4	112.4 ± 2
ARAF	94.5 ± 1.2	98 ± 2.3	37 ± 0.8	93.9 ± 0.8	87.4 ± 1.4
ARK5/NUAK1	102.5 ± 1.7	88.8 ± 0.1	90.3 ± 1.2	119.75 ± 2	97.8 ± 0.2
ASK1/MAP3K5	107 ± 1.8	87.8 ± 7.9	94.7 ± 2.8	92.9 ± 0.3	90.8 ± 0.4
Aurora A	60.5 ± 3.5	80.4 ± 1.2	81.4 ± 2.5	98.3 ± 2.7	92.7 ± 0.3
Aurora B	87.1 ± 1.6	85.9 ± 2.2	78.2 ± 0.9	88.3 ± 2.7	101.4 ± 6.2
Aurora C	81.3 ± 1.8	89 ± 1	92.2 ± 1.6	94.65 ± 3.6	101.1 ± 1.3
AXL	89.7 ± 1.2	86.3 ± 0.6	84.1 ± 0.3	93.55 ± 0.4	97.2 ± 0.6
BLK	72.8 ± 2.2	81.7 ± 0.7	0.6 ± 0.2	94.95 ± 1.2	-0.4 ± 0
BMPR2	108.6 ± 8.4	101.6 ± 3	108.9 ± 2.5	86.85 ± 0.7	85.4 ± 0.5
BMX/ETK	70.2 ± 1.6	94.6 ± 0.8	-0.1 ± 0.2	55.05 ± 0.5	2.5 ± 0
BRAF	103 ± 1.4	93.3 ± 0.1	86.8 ± 0.5	90 ± 3.1	72.4 ± 5.2
BRK	10.3 ± 0.3	42 ± 0.7	3.1 ± 0.8	80.85 ± 1.1	40.6 ± 2.1
BRSK1	92.4 ± 1.7	96.8 ± 0	99.2 ± 1.5	99.6 ± 1.9	94.1 ± 5.7
BRSK2	88.7 ± 0.6	102.3 ± 0.8	100 ± 0.3	87.7 ± 2.7	87.3 ± 2.7
BTK	1.8 ± 0.4	3 ± 0.1	1.1 ± 0.1	24.35 ± 0.2	2.7 ± 0.1
c-Kit	89.4 ± 0.6	90.7 ± 1	87.5 ± 0.9	87.45 ± 1.1	99 ± 0.5
c-MER	91.4 ± 2.2	96.8 ± 1.2	94.6 ± 1.3	99.95 ± 0.7	93.2 ± 2.3
c-MET	83.8 ± 6	100 ± 1.9	88.6 ± 1.2	99.05 ± 6.7	66.2 ± 0
c-Src	90.5 ± 0.2	97.9 ± 0.1	44.7 ± 1.1	91.95 ± 0.1	83.9 ± 1.2
CAMK1a	96.4 ± 3.5	92.9 ± 0.9	78.4 ± 0.9	98.8 ± 3.7	97.4 ± 0.2
CAMK1b	95.6 ± 1	101.1 ± 1.6	97.8 ± 0.3	97.3 ± 0.1	99.5 ± 0.1
CAMK1d	91.8 ± 1.1	103.9 ± 0.8	98.6 ± 3.4	91.85 ± 1	99.6 ± 0.8
CAMK1g	97.9 ± 1	93.3 ± 0.1	92.8 ± 0.2	106.55 ± 8.6	95.3 ± 1.8
CAMK2a	94.9 ± 1.9	96.3 ± 0.9	93.1 ± 0.9	94.3 ± 2	100 ± 0.6

CAMK2b	93 ± 1.5	91.4 ± 0.3	95.4 ± 0.8	93.75 ± 0.3	103.7 ± 2.3
CAMK2d	95.7 ± 3.9	94.9 ± 0.3	92.7 ± 1	95.9 ± 1.6	99.6 ± 0.3
CAMK2g	100.6 ± 1.7	98 ± 1.1	99.7 ± 1.3	97.75 ± 1.2	101.7 ± 1.6
CAMK4	95.7 ± 0.8	87.4 ± 0.3	88.4 ± 0.7	93.25 ± 5.5	107.3 ± 2.7
CAMKK1	96.8 ± 0.4	91.9 ± 11.5	88.4 ± 0.1	95.2 ± 1	91.2 ± 4.3
CAMKK2	86 ± 3.4	105.7 ± 1.9	102.9 ± 0.6	102.9 ± 0.8	76.9 ± 1.7
CDC7/DBF4	113.1 ± 6.9	104 ± 0.2	102.8 ± 3.7	94.8 ± 2.9	89.9 ± 0.8
CDK1/cyclin A	100 ± 1.3	88.7 ± 0.4	87.8 ± 0.2	101.35 ± 0.6	96.8 ± 0
CDK1/cyclin B	96.8 ± 0.3	93.7 ± 0.6	95.2 ± 0.7	94.05 ± 1.1	89.8 ± 0.7
CDK1/cyclin E	89.8 ± 1.6	95 ± 0.7	92.5 ± 0.7	95.2 ± 2	105.9 ± 1.3
CDK14/cyclin Y (PFTK1)	101.5 ± 0.7	105.5 ± 0.3	100.2 ± 1.8	97.25 ± 3.1	98 ± 0.2
CDK16/cyclin Y (PCTAIRE)	102.6 ± 1	92.3 ± 1.5	91.7 ± 5.2	97.35 ± 3.4	77.7 ± 0.4
CDK17/cyclin Y (PCTK2)	101.7 ± 2.2	99.8 ± 1.2	94.8 ± 0.2	106.15 ± 0.8	87.1 ± 0.1
CDK18/cyclin Y (PCTK3)	99.7 ± 1.9	102.1 ± 0	104 ± 1.9	109.5 ± 2.4	100.1 ± 0.1
CDK19/cyclin C	105.5 ± 5.4	101 ± 1.3	96.6 ± 0.7	47.8 ± 0.4	ND
CDK2/cyclin A	102.8 ± 3.5	102.8 ± 1.7	95.1 ± 0.6	101.85 ± 8.7	100.6 ± 1.3
CDK2/cyclin A1	95.4 ± 1.8	79.9 ± 2.1	86.4 ± 1.1	88.75 ± 3.3	96.5 ± 2.6
CDK2/cyclin E	98.7 ± 1.1	96.2 ± 0.4	101 ± 0.2	93.8 ± 2.1	124.9 ± 1.5
CDK2/cyclin E2	95.3 ± 2.3	93.3 ± 0.3	95.1 ± 0.6	100.9 ± 0.9	143.9 ± 1.7
CDK2/cyclin O	97.4 ± 3.2	97.2 ± 1	98.7 ± 0.2	107.15 ± 0.6	97.5 ± 1.9
CDK3/cyclin E	106.3 ± 3.7	95.9 ± 1.3	96.2 ± 0.3	95.5 ± 0.7	94.5 ± 0.9
CDK3/cyclin E2	107.4 ± 2.6	93.6 ± 0.1	87.7 ± 1.7	90.5 ± 1.8	93.3 ± 0.3
CDK4/cyclin D1	96.5 ± 0.6	98.6 ± 0.7	92.3 ± 0.1	95.9 ± 2.5	114.5 ± 0.4
CDK4/cyclin D3	96.9 ± 1.2	92.6 ± 0.3	95.4 ± 0.1	98.4 ± 3.8	88.6 ± 0.2
CDK5/p25	87.2 ± 0.6	69.4 ± 1.9	73.2 ± 0	89.55 ± 1.4	93.3 ± 0.9
CDK5/p35	102.3 ± 1	103.5 ± 0.1	100.7 ± 0.6	103.05 ± 1	99.6 ± 2
CDK6/cyclin D1	95.7 ± 2.3	90.9 ± 0.4	90 ± 0.5	100.55 ± 0.7	84.9 ± 1.1
CDK6/cyclin D3	100.2 ± 0.8	93 ± 1.8	86.6 ± 0.4	94.7 ± 1.5	99.7 ± 0.6
CDK7/cyclin H	106.5 ± 4	90.8 ± 0.4	99.5 ± 1.6	96.3 ± 0.6	100.8 ± 0.1
CDK9/cyclin K	81.6 ± 2.3	100.3 ± 4.4	109.5 ± 2.7	89.25 ± 6.4	100.3 ± 0.8
CDK9/cyclin T1	104.4 ± 5.6	141.7 ± 1.7	107.3 ± 5.4	104.7 ± 2.8	100.7 ± 0
CDK9/cyclin T2	99 ± 1.6	96.9 ± 0.1	95 ± 0.5	85.35 ± 1.4	99.2 ± 0.1
CHK1	91.3 ± 2.3	97.5 ± 0.3	107.9 ± 0.2	98.25 ± 2.3	95 ± 0.9
CHK2	92.4 ± 2.1	97.7 ± 0.2	97.3 ± 0.5	94.35 ± 1.6	98.8 ± 2.6
CK1a1	98.8 ± 4.1	69.2 ± 2.7	78.8 ± 2.6	102.45 ± 2.3	105.8 ± 0
CK1a1L	96.4 ± 2.2	113.8 ± 0	101.3 ± 1	94.7 ± 4.5	90.5 ± 1
CK1d	105 ± 1.9	109.2 ± 1.6	97.6 ± 1.8	88.05 ± 2.5	116.4 ± 1.5
CK1epsilon	68.4 ± 0.3	85.7 ± 0.6	82.9 ± 2.2	106.3 ± 2.4	107.9 ± 1.1
CK1g1	91.7 ± 1.1	103.4 ± 4.6	97.7 ± 1.2	97.95 ± 0.9	107.7 ± 1.3
CK1g2	97.8 ± 2	97.7 ± 1	102.5 ± 1.4	90.75 ± 1.8	107 ± 1
CK1g3	95.5 ± 2.5	96.6 ± 0.9	94.2 ± 2	96.2 ± 1.1	99.2 ± 1.6

CK2a	98.1 ± 0.7	89.3 ± 0.8	88.8 ± 1	95.2 ± 2.6	97.4 ± 4.9
CK2a2	81 ± 4.4	80.9 ± 1.4	84.4 ± 1.2	109.95 ± 7.3	87.2 ± 1.3
CLK1	102.5 ± 3.3	113.4 ± 1.1	110.7 ± 0.2	100.35 ± 3.5	99.7 ± 3.1
CLK2	102.3 ± 0.8	99.3 ± 0.2	104.7 ± 1	102 ± 3	87.2 ± 0.3
CLK3	99 ± 4.4	113 ± 2	105.1 ± 1.4	87.85 ± 1.1	102.2 ± 3.7
CLK4	89.4 ± 1.3	90.3 ± 0.4	94.6 ± 2.3	88.8 ± 1.6	93.9 ± 3.1
COT1/MAP3K8	103.2 ± 1	96.3 ± 0	93.7 ± 0	99.3 ± 1.1	84.5 ± 2.7
CSK	34.3 ± 0.5	79.7 ± 0.9	14.2 ± 0.1	97.75 ± 0.2	88.9 ± 0.4
CTK/MATK	92.3 ± 4.1	95.6 ± 0.9	102.4 ± 1.4	99.85 ± 0.8	94.9 ± 0.1
DAPK1	116.4 ± 6.5	88.8 ± 0.8	96.8 ± 0.1	95.55 ± 1	101.3 ± 0.3
DAPK2	100.1 ± 4.6	87.7 ± 1.1	90.9 ± 1.2	98.35 ± 2.1	96.4 ± 0.1
DCAMKL1	101.4 ± 2.6	92.5 ± 0.6	96.5 ± 1	87.75 ± 2.5	99.3 ± 1.8
DCAMKL2	91.9 ± 1.1	98.5 ± 0.1	95.9 ± 0.3	94.05 ± 2.5	100.6 ± 0.3
DDR1	87.8 ± 3.7	75 ± 0.4	70.9 ± 1.6	87.05 ± 0.6	108 ± 0.8
DDR2	107.7 ± 1.3	98.1 ± 0.9	95.8 ± 0.2	99.3 ± 5	98.8 ± 0.9
DLK/MAP3K12	91.9 ± 3.2	107.7 ± 1.2	105.4 ± 5.2	ND	ND
DMPK	93.4 ± 4.2	99.9 ± 0.8	97.9 ± 0.9	96.3 ± 0.5	106.9 ± 0.5
DMPK2	92.3 ± 1.6	110.4 ± 1	100.6 ± 0	91.05 ± 1.3	97.2 ± 0.5
DRAK1/STK17A	106.5 ± 1.8	96.4 ± 0.1	95.1 ± 0.7	105.95 ± 1.8	120.9 ± 0.2
DYRK1/DYRK1A	97.9 ± 0.8	97.2 ± 1.1	99.3 ± 0.6	98.65 ± 0.9	147.5 ± 5.4
DYRK1B	96.9 ± 4.9	95.9 ± 0	92.4 ± 0.6	99.15 ± 1.4	96.5 ± 0.9
DYRK2	86.9 ± 6.1	94.8 ± 0.1	94 ± 0.1	95.35 ± 1	102.2 ± 0.1
DYRK3	100.3 ± 1.6	96.3 ± 0.5	91.5 ± 0.4	88.8 ± 0.8	100.5 ± 3.2
DYRK4	98 ± 2	87.9 ± 0.4	89.1 ± 1.7	103.2 ± 2	96.4 ± 0.5
EGFR	60.6 ± 0.6	105.5 ± 1.6	13.6 ± 1.7	95.9 ± 1.2	65.5 ± 0.9
EPHA1	103 ± 1.9	88.2 ± 3.2	84.9 ± 2	90.1 ± 3.9	97.5 ± 2.4
EPHA2	101.5 ± 1.6	87.8 ± 0.2	87.8 ± 1.3	95.9 ± 1.1	91 ± 0.6
EPHA3	95.3 ± 0.9	103.6 ± 1.5	106.3 ± 1.1	96.05 ± 1.9	84 ± 0.3
EPHA4	99.3 ± 3.2	88.5 ± 0	90.3 ± 0	93.45 ± 1.9	99.4 ± 0
EPHA5	96.9 ± 1	97.4 ± 0.3	99 ± 1.1	97.9 ± 0.5	102 ± 0.9
EPHA6	92.7 ± 0.7	95.6 ± 0.2	80.7 ± 0.1	94.55 ± 1.4	101.3 ± 0.9
EPHA7	96.6 ± 1	98 ± 0.6	96.9 ± 0	97.25 ± 2.5	96.1 ± 0.3
EPHA8	99.9 ± 0.5	90.2 ± 1	88.1 ± 1.4	93.2 ± 2.5	100.2 ± 0.2
EPHB1	108.6 ± 7	104.1 ± 0.1	99.6 ± 0.5	97.6 ± 0.5	94.4 ± 0.4
EPHB2	96.4 ± 4.4	88.5 ± 0.5	86.4 ± 0.4	95.9 ± 1.7	89.9 ± 2.8
EPHB3	80.4 ± 3	100.4 ± 0.4	99.9 ± 0	93.45 ± 1.3	99.3 ± 0.5
EPHB4	85.6 ± 1.4	84.9 ± 0.6	81.4 ± 1.1	106.95 ± 4.1	93.6 ± 1.2
ERBB2/HER2	66.2 ± 1.3	97.3 ± 0.2	8.1 ± 0.4	99.5 ± 1.3	102.6 ± 0
ERBB4/HER4	2.6 ± 0.6	43 ± 0	1.9 ± 0.3	17.7 ± 0.6	0.1 ± 0.1
ERK1	106.8 ± 0.9	91.5 ± 0.4	91.5 ± 0.9	102.4 ± 0.6	76.1 ± 2.5
ERK2/MAPK1	96.9 ± 2.3	101.9 ± 0.1	102.7 ± 1.3	97.5 ± 0.4	98.8 ± 2.5
ERK5/MAPK7	94.8 ± 1.1	92.5 ± 0.6	97.1 ± 1.1	100.6 ± 1	100.9 ± 0.3
ERK7/MAPK15	98.5 ± 4	112.2 ± 2.3	101.3 ± 0.3	107.5 ± 3.3	85.3 ± 0.8

ERN1/IRE1	99.3 ± 3.4	91.4 ± 1.3	93.2 ± 0.1	91.45 ± 0.8	92.2 ± 0.9
ERN2/IRE2	102.1 ± 1.8	102.4 ± 0.3	99.2 ± 0.1	90.05 ± 1.1	103.6 ± 0.1
FAK/PTK2	95.3 ± 1.3	92.9 ± 0.4	94.4 ± 0.1	100.45 ± 16.9	95.6 ± 1
FER	101.7 ± 4	96.5 ± 0	91.1 ± 0.8	103.2 ± 0.3	101.9 ± 0.8
FES/FPS	96.7 ± 0.8	95.2 ± 0.3	97.1 ± 1.6	97.4 ± 0.1	98.5 ± 1.1
FGFR1	93.8 ± 2.1	92.7 ± 1.2	75.4 ± 1.2	103 ± 1.5	102.1 ± 1.4
FGFR2	78.7 ± 11.7	99.2 ± 0.6	82.6 ± 0.7	99.3 ± 1.5	94.3 ± 0.3
FGFR3	97.6 ± 0.8	93.8 ± 1.1	74.4 ± 0.8	93 ± 0.3	101.2 ± 0.2
FGFR4	96.6 ± 1.4	94 ± 0.8	87.4 ± 0.6	97.55 ± 0.4	95.7 ± 2.1
FGR	67.1 ± 0.5	81 ± 0.5	6.7 ± 0.1	99 ± 2.5	64.2 ± 0.3
FLT1/VEGFR1	91.6 ± 4.2	87.2 ± 2.2	81.8 ± 0.7	88.65 ± 3.9	119.4 ± 0.8
FLT3	89.6 ± 0.6	83.2 ± 0.5	35.9 ± 0.5	100.3 ± 0.4	84.9 ± 3.4
FLT4/VEGFR3	94.7 ± 1.6	96.7 ± 0.3	87.1 ± 1.1	89.7 ± 0.4	94.7 ± 2
FMS	100.5 ± 1.1	94 ± 2.5	89.4 ± 0.3	89.5 ± 5.6	100.8 ± 1.4
FRK/PTK5	98.4 ± 2.8	98.2 ± 0.1	73.1 ± 1.1	101.35 ± 1	91 ± 1.2
FYN	48.8 ± 1.8	66.9 ± 1.2	17 ± 0.9	100.35 ± 2	76.3 ± 3.2
GCK/MAP4K2	95.8 ± 2.1	84.2 ± 0.7	94.4 ± 0.4	94.25 ± 2.1	104.4 ± 1.3
GLK/MAP4K3	98.3 ± 3.4	88.1 ± 0.4	91.9 ± 0.1	93.55 ± 1.7	96.4 ± 0.7
GRK1	111 ± 6.2	100.8 ± 1.6	100.3 ± 0.8	104.15 ± 0.9	103.3 ± 2.3
GRK2	97.7 ± 0.3	107.4 ± 0.1	108.1 ± 0.1	98.3 ± 1.3	101.3 ± 0.6
GRK3	105.2 ± 1.8	86.6 ± 1	94.7 ± 1.5	102.7 ± 0.6	91.2 ± 1.4
GRK4	106.9 ± 1.2	98.8 ± 0.4	102.1 ± 0.6	95.15 ± 1.8	96.9 ± 4.6
GRK5	100.6 ± 4.1	103 ± 0.2	104.4 ± 0.6	106.25 ± 1.9	103.2 ± 0.7
GRK6	100.6 ± 1.8	30.5 ± 17.1	95.3 ± 1	105.05 ± 1.7	ND
GRK7	97.9 ± 4	96.5 ± 1.1	99.8 ± 0.8	92.85 ± 2.5	86.4 ± 3.7
GSK3a	96.9 ± 3.3	97.2 ± 0.3	99.2 ± 1.6	99.75 ± 1.7	91.6 ± 1.5
GSK3b	94.7 ± 0.6	89.8 ± 1.3	90.8 ± 0.1	89.65 ± 1.1	101.8 ± 1.3
Haspin	99.9 ± 1	89.9 ± 0.1	84.8 ± 0.9	94.6 ± 0.9	107.5 ± 0.7
HCK	58.1 ± 0.7	85 ± 1	15 ± 0.2	101.95 ± 1.5	83.3 ± 0
HGK/MAP4K4	95.7 ± 2.6	105.5 ± 0.4	101.8 ± 2.1	93.65 ± 0.4	94.9 ± 0.6
HIPK1	109 ± 7.7	106.3 ± 0	97.1 ± 0	101.2 ± 2.3	105.2 ± 0.4
HIPK2	99 ± 1.1	100.5 ± 1.3	95.3 ± 0.3	96.75 ± 2.4	96.5 ± 0.1
HIPK3	95.6 ± 2.1	87.5 ± 3.7	95 ± 0.3	99.55 ± 2.5	87.6 ± 5.4
HIPK4	106.7 ± 4.3	102.3 ± 2.1	102.3 ± 1.2	88 ± 1	100.9 ± 0.1
HPK1/MAP4K1	103.1 ± 1.4	79.7 ± 1.3	87.3 ± 0.8	95.65 ± 2	86.9 ± 2.1
IGF1R	98.3 ± 6.6	86.4 ± 0.5	89.7 ± 0.9	92 ± 1.2	98.7 ± 4.4
IKKa/CHUK	89.7 ± 2.6	94.2 ± 0.9	91.5 ± 2.3	104.15 ± 2.8	102.5 ± 0.8
IKKb/IKBKB	96.3 ± 1	97.9 ± 2.1	97.3 ± 1.9	98.95 ± 1.7	100.7 ± 2.8
IKKe/IKBKE	97.6 ± 3	78.7 ± 0.2	79.8 ± 0.3	105.95 ± 4.1	93.3 ± 0.4
IR	98.1 ± 2.1	94.6 ± 0.9	95.6 ± 1.2	100.65 ± 0.8	90.9 ± 3.6
IRAK1	98.1 ± 0.7	96.4 ± 0.8	91.5 ± 0.8	100.4 ± 1.7	91 ± 0.4
IRAK4	80 ± 7.3	103.6 ± 0.1	95.9 ± 0.1	96.3 ± 1.9	93.7 ± 0.6
IRR/INSRR	88.1 ± 2.9	97.5 ± 0.6	92 ± 0.5	102.6 ± 1.7	105.4 ± 1.3

ITK	103.4 ± 8.3	106.1 ± 3.9	2.3 ± 0.2	97.45 ± 1.3	85.2 ± 0
JAK1	96.4 ± 1.1	103.2 ± 0.7	100.6 ± 0.2	105.4 ± 1.7	90.2 ± 1.5
JAK2	94.5 ± 1.5	97 ± 1	96.4 ± 0.1	93.7 ± 4.1	95.4 ± 1.5
JAK3	97 ± 1.7	97.4 ± 0.3	7.8 ± 0.1	96.1 ± 1.5	97.1 ± 4
JNK1	99.7 ± 1.8	99 ± 0.4	99.2 ± 2.8	95.7 ± 0.6	89.7 ± 0.7
JNK2	104 ± 4.8	98.9 ± 0.7	101.3 ± 1	95.4 ± 2.2	96.7 ± 0.9
JNK3	88.5 ± 1.7	102.9 ± 0.9	96.4 ± 2.7	99 ± 5.3	102.2 ± 1.2
KDR/VEGFR2	91.7 ± 4.6	103 ± 0	92.5 ± 0	112.65 ± 5	96.9 ± 0.5
KHS/MAP4K5	82.7 ± 1	98.5 ± 0.1	98.6 ± 0.3	106.55 ± 0.2	93.7 ± 1.1
KSR1	98.9 ± 1.1	92.8 ± 0	89.8 ± 1.5	96.75 ± 2.8	107.6 ± 1
KSR2	98.3 ± 0.9	97.9 ± 0.6	95.4 ± 0.9	102.15 ± 0.6	97.4 ± 0
LATS1	105.8 ± 1.4	89.6 ± 0.4	88 ± 0.1	100.6 ± 0.3	100.2 ± 0.2
LATS2	89.2 ± 6.8	94.4 ± 0.2	91.5 ± 0.9	100.3 ± 1.1	99.2 ± 0.1
LCK	63.2 ± 3.8	76.7 ± 0.1	9.5 ± 0.4	97.25 ± 0.8	56.7 ± 0.5
LCK2/ICK	98.1 ± 3.7	99.8 ± 1.4	103.2 ± 1	94.4 ± 2.2	95.4 ± 3.4
LIMK1	96.1 ± 1.1	95.9 ± 0.1	94.5 ± 1.6	89.2 ± 2.4	87.6 ± 0.2
LIMK2	98 ± 0.5	89.9 ± 0.8	87.1 ± 2.9	102.1 ± 1.2	98 ± 0.2
LKB1	100.4 ± 9.8	112 ± 0.9	116.3 ± 0.8	89.2 ± 0.4	108.8 ± 1.6
LOK/STK10	102.3 ± 1.7	102.7 ± 1.3	100.7 ± 2.3	96.95 ± 0.9	104 ± 0.1
LRRK2	95.1 ± 0.5	101.5 ± 0.3	99 ± 0.3	104.35 ± 2.5	98.3 ± 0.3
LYN	77.1 ± 2.6	87.2 ± 0.1	25.3 ± 0.1	103.9 ± 0.5	89.9 ± 1.8
LYN B	90 ± 1.9	101.4 ± 0	52.1 ± 0.4	99.4 ± 1.8	90.1 ± 3.6
MAK	97.1 ± 3.3	105.1 ± 1.6	101.6 ± 0.1	95.2 ± 2.6	89.5 ± 2.5
MAPKAPK2	97.5 ± 0.8	90.7 ± 0.4	91.1 ± 0.7	95.45 ± 0.9	97.4 ± 2
MAPKAPK3	104.6 ± 6.3	96 ± 0.7	98.7 ± 0.7	99.9 ± 1.5	108.4 ± 0.6
MAPKAPK5/PRAK	93.1 ± 3.1	93 ± 0.2	90.7 ± 1	97.85 ± 2.3	93 ± 0.6
MARK1	97.3 ± 1.6	90.7 ± 0.5	105.5 ± 0.8	97.1 ± 0.3	98.4 ± 1.2
MARK2/PAR-1Ba	95.8 ± 1.3	92.2 ± 0.9	100 ± 1.9	91.5 ± 1.7	91.5 ± 2.4
MARK3	99.1 ± 0.8	95.3 ± 0.4	93.8 ± 1.6	97.15 ± 1.9	93.8 ± 0.7
MARK4	90 ± 1.9	96.5 ± 0.1	98.6 ± 1.9	94.35 ± 1.2	100 ± 1.4
MEK1	12.2 ± 1.4	69.6 ± 0.2	88.9 ± 0.8	97.7 ± 2.4	110.7 ± 1.9
MEK2	7.6 ± 0.6	67.8 ± 0.2	91 ± 0.4	94.45 ± 1.7	55.1 ± 0.4
MEK3	86.1 ± 3.7	99.7 ± 0.2	99 ± 0.8	95.95 ± 0.2	99.6 ± 0.3
MEK5	113 ± 2.5	106.8 ± 1.3	110.9 ± 0.1	102.15 ± 1.2	88.6 ± 0.6
MEKK1	102.8 ± 3.4	98.7 ± 0.7	97.8 ± 0.7	94.95 ± 2.6	96.5 ± 0.4
MEKK2	96.9 ± 7.6	95.1 ± 0.3	86 ± 4.3	95.3 ± 2	98.7 ± 6.2
MEKK3	82.2 ± 2.5	81.8 ± 0.2	83.3 ± 0.2	91.5 ± 5.9	69.6 ± 1
MEKK6	103.6 ± 4	89.8 ± 0.5	92 ± 0.5	99.55 ± 5	101 ± 0.1
MELK	94 ± 1.5	81.9 ± 1.2	85.5 ± 0.2	103.15 ± 1.8	88.2 ± 2
MINK/MINK1	102.1 ± 2	82.7 ± 0.1	89.8 ± 0.7	100.8 ± 1	101 ± 0.3
MKK4	102.2 ± 1.8	98 ± 0.3	96 ± 0.3	98.8 ± 0.6	103.8 ± 0.7
MKK6	69.4 ± 4.7	91.1 ± 1.3	91.1 ± 0.1	91.65 ± 4.1	99.6 ± 1.4
MKK7	103.3 ± 7.9	97.6 ± 2	62.7 ± 0.6	97.75 ± 1	105.1 ± 0.2

MLCK/MYLK	94.1 ± 1.6	89.2 ± 0.9	89.4 ± 0.6	102.45 ± 1.1	117.2 ± 1.5
MLCK2/MYLK2	92.4 ± 2.8	101.1 ± 0.6	102.7 ± 1.2	96.25 ± 0.5	94.5 ± 2.4
MLK1/MAP3K9	99.9 ± 1.1	88.9 ± 1.1	90.6 ± 0.1	96.45 ± 0.6	105.8 ± 0.3
MLK2/MAP3K10	115.7 ± 8	93.7 ± 1.1	96.8 ± 1.7	104.15 ± 6.6	93.3 ± 1.4
MLK3/MAP3K11	91.4 ± 1.6	91 ± 0.5	89.1 ± 0.6	100.4 ± 2.7	78.4 ± 0.8
MLK4	103.6 ± 3.4	97.2 ± 0.9	96 ± 0.7	97.7 ± 1.5	102.5 ± 0.4
MNK1	88 ± 3.5	115 ± 3	113.5 ± 2.6	105.15 ± 1.1	103.8 ± 1.5
MNK2	98.6 ± 7.3	90.4 ± 1	96.2 ± 0.4	101.45 ± 1.7	97.4 ± 0.6
MRCKa/CDC42BPA	94.1 ± 1.2	109.8 ± 1.6	108.8 ± 1.9	95.45 ± 1.2	97.2 ± 0.1
MRCKb/CDC42BPB	96.4 ± 0.7	105.7 ± 0.1	105 ± 0.4	96.8 ± 1.3	100.6 ± 1.4
MSK1/RPS6KA5	92.5 ± 1.4	114.2 ± 0.8	118.7 ± 1.4	107.45 ± 1.9	95.8 ± 0.9
MSK2/RPS6KA4	97.1 ± 1.6	77.7 ± 0.1	78.4 ± 1.4	95.05 ± 1.3	94 ± 0
MSSK1/STK23	101.6 ± 2.1	98.9 ± 1.1	94.2 ± 0.1	101.55 ± 1.8	96.7 ± 3.1
MST1/STK4	90.6 ± 2.4	90.9 ± 0.7	91.1 ± 1.3	105.75 ± 1.1	92.7 ± 0.2
MST2/STK3	92.6 ± 3.1	108.3 ± 1	105.7 ± 1	94.2 ± 4.5	94.9 ± 0.9
MST3/STK24	98.8 ± 2.9	92.4 ± 0.3	95.7 ± 1.3	90.95 ± 6.3	66.1 ± 0.5
MST4	74.9 ± 2.7	81 ± 0	76.5 ± 1	88.4 ± 1.3	79.3 ± 0.3
MUSK	98.6 ± 2	99.8 ± 2.8	114.3 ± 0.6	94.7 ± 0.2	81.3 ± 1.9
MYLK3	101.2 ± 1.8	101.8 ± 1.7	105.3 ± 0.1	98.05 ± 1.1	97.6 ± 0.1
MYLK4	95.6 ± 3	96.6 ± 2.4	100.6 ± 0	97.75 ± 2	108.2 ± 4.5
MYO3A	98.1 ± 1.1	93.4 ± 1.1	97.2 ± 1	100.15 ± 0.7	93.3 ± 4.3
MYO3b	88.3 ± 2.2	85.7 ± 1.5	95.4 ± 2.3	83.25 ± 3.9	102.1 ± 2.8
NEK1	119.8 ± 6.4	106.5 ± 0.9	103.6 ± 1.5	99.95 ± 1	94.1 ± 1.4
NEK11	93.9 ± 2.5	100.1 ± 2.9	97.7 ± 1.9	90.2 ± 5.1	117.6 ± 1.7
NEK2	107.7 ± 5.8	95.8 ± 1.2	94.7 ± 1	102.15 ± 0.7	110.6 ± 2.4
NEK3	102.7 ± 2	85 ± 0.4	89.5 ± 1.5	107.5 ± 2.5	100.1 ± 0.4
NEK4	100.5 ± 4.8	101 ± 4	91.4 ± 3.9	91.95 ± 1.3	101.5 ± 0.6
NEK5	91.9 ± 3.9	90.3 ± 0.5	94.2 ± 0.8	90.35 ± 3.2	99.4 ± 4
NEK6	101.3 ± 2.4	95.4 ± 0.2	96.8 ± 0.1	97.4 ± 3.2	94.5 ± 1.9
NEK7	102.8 ± 1.4	94.2 ± 0.3	95 ± 0.2	94.65 ± 2.2	92.5 ± 0.3
NEK8	97.7 ± 2.2	92.2 ± 1.1	91 ± 2.1	ND	ND
NEK9	95.9 ± 2.3	98.9 ± 0.3	95.7 ± 1.4	93.2 ± 2.2	91.7 ± 0
NIM1	96.2 ± 6.5	91.8 ± 0.3	92 ± 0	101 ± 4.3	97.8 ± 0.6
NLK	90.1 ± 2	93.4 ± 2.2	86.8 ± 2.6	109.7 ± 2.6	100.4 ± 1.5
OSR1/OXSR1	98.5 ± 0.9	97.3 ± 0.1	95 ± 0.8	101.25 ± 1.7	106.7 ± 0.3
P38a/MAPK14	95.7 ± 2.4	116.7 ± 2.4	123.4 ± 2.8	80.7 ± 0.7	103.2 ± 0.3
P38b/MAPK11	93 ± 2.6	82.2 ± 0.4	94 ± 0.1	87.9 ± 0.4	98.4 ± 3.2
P38d/MAPK13	102.2 ± 2.1	92.6 ± 0.3	97.5 ± 0.6	96.85 ± 2	99.9 ± 1.7
P38g	115.8 ± 3.1	104 ± 0.9	101.7 ± 0.4	100.95 ± 0.5	105.5 ± 2.3
p70S6K/RPS6KB1	92.4 ± 3.1	100.1 ± 0	95.4 ± 1.3	99.9 ± 2	92.8 ± 0.1
p70S6Kb/RPS6KB2	93.5 ± 1.3	96.8 ± 1.3	97.9 ± 0	101.8 ± 1.8	99.3 ± 1.9
PAK1	98.5 ± 0.8	98.1 ± 0.1	95.8 ± 0.8	106.6 ± 1.1	95.3 ± 2.5
PAK2	92.5 ± 0.7	90.4 ± 2.1	99 ± 0.1	99.1 ± 1	106.3 ± 0.6

PAK3	94.1 ± 4.1	86.7 ± 0.8	88 ± 0.3	106.85 ± 1	ND
PAK4	110.9 ± 4.2	97.8 ± 1.8	94.3 ± 1.8	94.3 ± 0.7	89.8 ± 0.3
PAK5	98 ± 2.4	101.9 ± 0.3	100.8 ± 0.8	103.7 ± 2.3	102.7 ± 2.4
PAK6	92 ± 0.6	89.4 ± 0.5	89.9 ± 0.7	96.95 ± 1.8	106.1 ± 1.2
PASK	93.7 ± 4.2	94.6 ± 1.6	97.5 ± 1.6	96.7 ± 3.3	95.5 ± 2
PBK/TOPK	102.1 ± 1.9	96.7 ± 1.2	92.7 ± 0.5	92 ± 1.6	102 ± 3
PDGFRa	101.4 ± 2.3	100.9 ± 1.3	87.7 ± 0.2	95.85 ± 0.8	98.6 ± 1.5
PDGFRb	87 ± 0.7	87.3 ± 1	84.1 ± 0.6	96.8 ± 0.8	94.4 ± 1.5
PDK1/PDPK1	107.1 ± 2.9	97.6 ± 1.3	94.1 ± 2	103.4 ± 1.1	99.4 ± 1.4
PEAK1	95.4 ± 0.7	89 ± 0	73.9 ± 0.3	ND	ND
PHKg1	98.7 ± 3.3	104.8 ± 0.5	99.9 ± 0.6	98.85 ± 1.8	104.2 ± 1.7
PHKg2	94.2 ± 1.8	100.9 ± 0.7	93 ± 1.9	88.25 ± 1.2	100.5 ± 1.7
PIM1	103.1 ± 0.5	99.1 ± 0.1	95.6 ± 0.3	107.95 ± 1.8	101.8 ± 1
PIM2	93.6 ± 2.5	90.8 ± 0.7	89.6 ± 1.8	103.7 ± 0.3	103 ± 2.5
PIM3	105.5 ± 8.5	98.7 ± 1.7	94 ± 3.3	94.3 ± 1	82.2 ± 1.1
PKA	97 ± 1.4	98.9 ± 0.6	100.8 ± 0	92.6 ± 3.3	98.3 ± 1.4
PKAcb	95.5 ± 2.8	84.8 ± 0.4	79.4 ± 0.5	101.7 ± 1	93.9 ± 0
PKAcg	97.8 ± 4.1	92 ± 0.1	89.3 ± 2.6	102.8 ± 2.6	99.4 ± 1.3
PKCa	98.1 ± 3	90.2 ± 0.7	95 ± 3.5	93.25 ± 1.7	93 ± 0.6
PKCb1	86.3 ± 1.6	91.7 ± 1.3	98 ± 1.2	103.45 ± 1.2	97.3 ± 1.3
PKCb2	93.6 ± 0.5	99.8 ± 0.2	96.3 ± 0.5	99.35 ± 1.2	97.5 ± 1.2
PKCd	93.7 ± 1.4	95.6 ± 0.1	89.9 ± 1.5	92.6 ± 1.3	92.8 ± 0.4
PKCepsilon	96.6 ± 1.1	96 ± 0.7	90.8 ± 1.9	101.15 ± 1.6	95.8 ± 0.5
PKCeta	93.7 ± 1.9	93.8 ± 1.1	84.5 ± 1.7	88.65 ± 2.4	95.7 ± 0.6
PKCg	96.8 ± 3.5	95.8 ± 0.3	111.4 ± 1.1	106.7 ± 0.5	100.2 ± 1.3
PKCiota	106.8 ± 2	117 ± 3.9	126.5 ± 6.2	94.1 ± 0.9	99.7 ± 0.5
PKCmu/PRKD1	94.9 ± 2.5	94.7 ± 0.5	99.4 ± 1	99.5 ± 0.6	101.9 ± 0.9
PKCnu/PRKD3	96.3 ± 1.5	86.7 ± 0.4	87.2 ± 0.4	102.25 ± 0.8	97.4 ± 0.7
PKCtheta	98.6 ± 1.1	88.5 ± 2.4	90.4 ± 0.3	90.65 ± 1.1	96.9 ± 0
PKCzeta	99.9 ± 2.4	93.4 ± 0.2	92.5 ± 0.4	95.1 ± 1.7	100.7 ± 2.5
PKD2/PRKD2	93.4 ± 2.5	91 ± 0.2	91 ± 0.1	102.2 ± 3.1	143 ± 2.8
PKG1a	98 ± 2.4	96.5 ± 0.3	94.2 ± 1.3	99.9 ± 0.6	95.4 ± 1.3
PKG1b	103.6 ± 1.1	96.5 ± 0.6	92.9 ± 0.7	93.55 ± 8.1	83 ± 3.4
PKG2/PRKG2	83.9 ± 1.9	86.9 ± 0.8	88.5 ± 0.3	101.6 ± 1.6	95.8 ± 1.2
PKN1/PRK1	93.2 ± 1.6	87.8 ± 9	97.9 ± 2.4	91.25 ± 0.8	101.5 ± 0
PKN2/PRK2	103.3 ± 0.9	96.6 ± 0.4	92.5 ± 0.4	109.05 ± 2.9	97.5 ± 1.3
PKN3/PRK3	98.2 ± 1	84.5 ± 1.6	79.3 ± 0.3	98.25 ± 1.1	88 ± 1.3
PLK1	97.2 ± 4.3	97.6 ± 1	94.3 ± 1	100.6 ± 2.2	100.6 ± 2.3
PLK2	98.3 ± 0.8	90.1 ± 1.8	91.9 ± 1.6	106.15 ± 1.8	97.7 ± 1.3
PLK3	101.9 ± 3.2	95.6 ± 0.6	95 ± 0.8	108.65 ± 0.8	100.8 ± 2.8
PLK4/SAK	95 ± 4	96.7 ± 3	104 ± 0.7	93 ± 3.2	101.5 ± 7.9
PRKX	99.4 ± 1.6	84.2 ± 1.1	80.2 ± 0.4	108.25 ± 1.6	80.2 ± 0.4
PYK2	89.9 ± 1.2	102.1 ± 0.3	99.7 ± 0.2	93.05 ± 2.7	95.6 ± 2.4

RAF1	100.4 ± 1.8	94 ± 0.7	85.4 ± 0.1	106.4 ± 1.3	125.3 ± 1.1
RET	53.9 ± 1.2	90.4 ± 0.6	66.9 ± 1.5	93.6 ± 1	103.4 ± 1
RIPK2	94.5 ± 12.4	92.9 ± 0.4	106 ± 7.1	80.95 ± 0.7	92.2 ± 0.7
RIPK3	68.3 ± 1.9	122.6 ± 1.1	44.9 ± 0.3	ND	ND
RIPK4	91 ± 1.9	101.7 ± 0.8	93.3 ± 0.6	98.95 ± 0.6	102.6 ± 0.6
RIPK5	98.6 ± 3.6	103.8 ± 0.2	114.4 ± 1.1	98.6 ± 2.4	92.9 ± 0.5
ROCK1	97.2 ± 1.4	97.2 ± 1.6	93.8 ± 0.3	98.4 ± 1.8	101.1 ± 0.1
ROCK2	96.8 ± 6	101.4 ± 6.8	99.7 ± 1.9	104.3 ± 0.7	98.4 ± 1.2
RON/MST1R	103.2 ± 0.4	89 ± 1.3	91.1 ± 0.1	103.05 ± 0.8	106.2 ± 0
ROS/ROS1	96 ± 1.2	92.2 ± 0.1	91.8 ± 1.3	98.1 ± 0.4	80.3 ± 2.9
RSK1	93.9 ± 1.3	97.1 ± 0.4	91.8 ± 0.8	94.3 ± 0.5	101.5 ± 1
RSK2	96.5 ± 1.1	89.4 ± 0.4	91.2 ± 1.4	96.15 ± 4.6	97.3 ± 0.5
RSK3	112.7 ± 4.1	101.9 ± 0.6	97.3 ± 0.2	96.65 ± 1	91.8 ± 0.6
RSK4	94.6 ± 2	90.3 ± 0.1	88.1 ± 1	96 ± 1.1	121.7 ± 0.3
SBK1	101 ± 0.6	105.5 ± 0.5	105.6 ± 0.3	96.1 ± 0.7	83.4 ± 6.6
SGK1	99.7 ± 4.8	83.9 ± 8.8	89.6 ± 0.8	94.6 ± 5.2	106.1 ± 3.3
SGK2	93.5 ± 0.8	102.1 ± 0.5	81.2 ± 5.3	110.05 ± 6	95.2 ± 0.2
SGK3/SGKL	95.5 ± 2	94.6 ± 0.9	90.7 ± 1.1	103.9 ± 0.2	98.8 ± 0.9
SIK1	94.9 ± 1.7	100 ± 0.6	94.5 ± 0.5	93.35 ± 2.6	81.7 ± 3.9
SIK2	93.9 ± 0.7	106.3 ± 0.7	102.5 ± 1	105.8 ± 6.7	95.3 ± 1
SIK3	99.1 ± 2	102.3 ± 0.3	95 ± 1.4	96.75 ± 1.3	90.2 ± 0.2
SLK/STK2	90.6 ± 0.8	92.9 ± 0.9	96.2 ± 1.2	96.65 ± 1.3	91.9 ± 1.4
SNARK/NUAK2	97.8 ± 4.4	105.7 ± 0.2	100.6 ± 0.4	99.95 ± 3.5	100.4 ± 0.6
SNRK	106.7 ± 1.8	105.3 ± 0.2	102.6 ± 0.8	103.3 ± 0.8	98.8 ± 5
SRMS	101.5 ± 2.6	89.3 ± 0.1	28.9 ± 0.2	92.7 ± 1.8	94.9 ± 0
SRPK1	107.4 ± 11.9	94.8 ± 1.1	92.3 ± 2.4	95.9 ± 0.3	90.5 ± 0.6
SRPK2	101.8 ± 0.4	98.3 ± 0.1	96.7 ± 1.2	102.9 ± 2.3	97.5 ± 0.1
SSTK/TSSK6	108.9 ± 1.4	96.1 ± 1.3	101 ± 0.5	102.4 ± 2.6	100.7 ± 0.7
STK16	106.8 ± 2.7	99.8 ± 0.5	98.6 ± 1.7	95.35 ± 0.7	98.7 ± 1.5
STK21/CIT	96.4 ± 1.9	112.2 ± 0.3	117.2 ± 0.3	97 ± 2.4	99 ± 2.7
STK22D/TSSK1	97.2 ± 2.5	98.3 ± 1.5	97.3 ± 0	93.9 ± 0.7	110.6 ± 0.1
STK25/YSK1	88.8 ± 1.6	109.6 ± 0	103.4 ± 2.2	99.35 ± 0.5	79.1 ± 0.8
STK32B/YANK2	121.2 ± 16.8	98.3 ± 0	98.5 ± 0.4	110.25 ± 2.6	122.9 ± 0.5
STK32C/YANK3	81 ± 4.5	100.4 ± 0.4	93.8 ± 1.3	101.45 ± 7.9	98.9 ± 1.4
STK33	118.2 ± 7.1	87.3 ± 0.3	92.6 ± 1.8	107.05 ± 3	90.9 ± 1.5
STK38/NDR1	97.2 ± 1.5	96.2 ± 0.6	95.7 ± 0.4	107.4 ± 0.3	99.3 ± 1
STK38L/NDR2	101.3 ± 4	92.7 ± 0.4	94.2 ± 0.5	105.15 ± 1.6	93 ± 0.6
STK39/STLK3	99.2 ± 1.2	105.9 ± 1.2	109.6 ± 0.3	89.9 ± 2.3	97.3 ± 1.4
SYK	115.5 ± 6.8	93.7 ± 0.4	91.8 ± 0.8	97.5 ± 3.6	99 ± 0.9
TAK1	105.3 ± 1.1	97.4 ± 0.3	91.7 ± 0.2	103 ± 1.9	92.9 ± 0.7
TAOK1	97.5 ± 2.1	105 ± 1.8	98.5 ± 0.9	98.55 ± 0.6	105 ± 0.1
TAOK2/TAO1	91.4 ± 3.4	92.3 ± 2.2	98.5 ± 1.2	97.25 ± 0.8	104.7 ± 5.5
TAOK3/JIK	111.6 ± 2.7	91.6 ± 1.1	87 ± 0.5	99.85 ± 0.3	86.5 ± 0

TBK1	92.3 ± 1.8	87.2 ± 0.3	94.7 ± 0.5	99.65 ± 3.1	107.9 ± 8.2
TEC	64.6 ± 1	97.2 ± 0	3 ± 0.4	43.45 ± 0.9	8.9 ± 0.1
TESK1	102.9 ± 0.6	110.2 ± 1.2	99.3 ± 3.5	104.4 ± 1.7	105.6 ± 1.1
TESK2	99.5 ± 2.6	103.7 ± 1.4	96.7 ± 0.2	108.25 ± 2.3	91.9 ± 0.7
TGFBR2	99.5 ± 1.8	114.8 ± 2.9	121.5 ± 1.3	97.05 ± 2.5	ND
TIE2/TEK	98.9 ± 1.8	95.6 ± 1.2	95.2 ± 0.1	91.85 ± 5.6	88 ± 0.3
TLK1	126.8 ± 10	104.5 ± 1.2	95.7 ± 0.8	92.95 ± 1.9	95.9 ± 5.3
TLK2	97.2 ± 2.2	102.6 ± 0.2	98.7 ± 1.4	98.75 ± 2.8	106.8 ± 2.7
TNIK	64.4 ± 2.8	73.1 ± 0.1	72.4 ± 0.9	91.25 ± 1.7	101.4 ± 0.2
TNK1	98.2 ± 2.5	92.6 ± 0	102.6 ± 2.9	90.2 ± 1.2	93.8 ± 0.7
TRKA	86.1 ± 3.4	83 ± 0.8	82.3 ± 0.7	101.45 ± 1.2	90.3 ± 0.3
TRKB	92 ± 1.9	93.5 ± 0.1	89.5 ± 1.6	97.55 ± 1.2	99.7 ± 0.4
TRKC	115.1 ± 7.2	99.3 ± 0.3	99.6 ± 0.7	94.25 ± 4	93.5 ± 1
TSSK2	93.9 ± 5.3	92.3 ± 0.5	94.2 ± 0	96.15 ± 1.1	100 ± 1.3
TSSK3/STK22C	95.7 ± 4.1	86.1 ± 2.1	82.2 ± 0.9	96.2 ± 4.7	104.3 ± 4.3
TTBK1	84.1 ± 6.4	97.8 ± 0.1	92.4 ± 4.3	104.85 ± 2.1	114.1 ± 2.4
TTBK2	103.5 ± 9	93.2 ± 4.2	95.4 ± 2.7	92.25 ± 0.8	97.7 ± 5.5
TXK	19.6 ± 0.6	68.4 ± 0.4	-0.1 ± 0.1	86.75 ± 2	0.4 ± 0.1
TYK1/LTK	112.1 ± 1.4	103.2 ± 1.4	96.4 ± 0.6	95.8 ± 2.9	102.8 ± 0.9
TYK2	94.3 ± 2.1	88.4 ± 0.2	95.1 ± 0.1	86.45 ± 1.7	102.5 ± 0.2
TYRO3/SKY	101.3 ± 2.2	94.3 ± 1.6	97.1 ± 0.1	110.4 ± 4.8	109.8 ± 0.6
ULK1	92 ± 2.8	100.5 ± 1.3	105 ± 0.2	99.6 ± 1.4	99.5 ± 1.1
ULK2	97.1 ± 2	117.9 ± 0.7	126 ± 0.7	94.2 ± 5.1	104.9 ± 5.8
ULK3	103.4 ± 2	94.5 ± 0.1	93.4 ± 0.1	102.45 ± 2.7	98.9 ± 4.9
VRK1	92.3 ± 3.3	104.5 ± 0.7	100.5 ± 0.8	96.8 ± 0.7	80.5 ± 10.1
VRK2	97.6 ± 1.6	96.3 ± 0.3	96.6 ± 0.4	94.1 ± 4.4	114.5 ± 2.9
WEE1	101.9 ± 7	94.5 ± 0.4	91.8 ± 0.2	92.9 ± 1.4	ND
WNK1	91.1 ± 4.7	102.5 ± 1.5	94.4 ± 2.2	96.95 ± 2.9	96 ± 0.2
WNK2	93.9 ± 1.9	91.8 ± 1.5	95.4 ± 0.1	94.05 ± 3.4	95 ± 0.5
WNK3	100.8 ± 1.1	94.8 ± 2.6	100.7 ± 1.5	106.15 ± 1	99 ± 0
YES/YES1	38.6 ± 0.5	47.6 ± 0.2	6.6 ± 0.9	88.55 ± 0.5	67 ± 0.4
YSK4/MAP3K19	102.4 ± 2.5	97 ± 1.9	91.1 ± 1.6	102.1 ± 1.5	100.1 ± 1.7
ZAK/MLTK	89.9 ± 2.9	93.3 ± 0.5	79.3 ± 0.2	105.15 ± 3.5	100.6 ± 0.5
ZAP70	110.9 ± 1.9	94.4 ± 1	87.8 ± 0.2	101.25 ± 0.6	100.5 ± 1.1
ZIPK/DAPK3	102.2 ± 3.4	93.5 ± 1.2	92.3 ± 1.2	93 ± 2.9	85.2 ± 1.9

Supplemental Table 6. Biochemical potency of pirtobrutinib on selected kinases.

Kinase	Pirtobrutinib IC_{50}		
	Mean ± SD, nM	**N**	**Fold selectivity vs BTK**
BTK	3.15 ± 1.32	12	1
ERBB4/HER4	13.25 ± 2.47	2	4.2
BRK	54.25 ± 3.04	2	17.2
MEK2	82.70 ± 5.37	2	26.3
MEK1	147.00 ± 5.66	2	46.7
YES/YES1	157.00 ± 4.24	2	49.8
TXK	209.00 ± 12.73	2	66.3

Supplemental Table 7. Percent inhibition of probe binding to kinases in live PBMCs.

		Percent inhibition			
Kinase	**Labeled sequence**	**Pirtobrutinib 10μM**	**Pirtobrutinib 1μM**	**Pirtobrutinib 0.1μM**	**Pirtobrutinib 0.01μM**
BTK	GQYDVAIKMIK	**>90**	**>90**	**>90**	45.4
BTK	YVLDDEYTSSVGSKFPVR	**98.3**	**96.0**	**91.8**	41.0
TEC	YVLDDQYTSSSGAKFPVK	**>90**	**53.1**	29.9	23.0
MEK1/MEK2	KLIHLEIKPAIR	**60.2**	11.4	1.4	0.2
MEK1/MEK2	DVKPSNILVNSR	**56.0**	11.5	6.0	4.5
MAP2K1	IMHRDVKPSNILVNSR	35.9	3.8	-14.1	-5.7
HCK	EGAKFPIKWTAPEAINFGSFTIK	33.7	-8.5	0.1	-10.9
CSK	VAVKCIK	30.1	7.1	16.1	-2.4
STRADB	SIKASHILISGDGLVTLSGLSHLHSLVK	26.6	19.9	8.1	10.8
STRADB	HTPTGTLVTIKITNLENCNEER	25.2	23.2	15.3	30.7
MARK2, MARK3	DLKAENLLLDADMNIK	25.0	17.2	8.5	21.9
LYN	EGAKFPIKWTAPEAINFGCFTIK	22.7	-17.6	3.0	-1.6
CAMK2A, CAMK2B, CAMK2D, CAMK2G	DLKPENLLLASK	21.0	7.1	7.5	-0.2
CSK	VSDFGLTKEASSTQDTGKLPVK	20.3	0.5	1.2	-2.2
CTK	VSDFGLAKAER	17.7	17.7	7.2	3.0
MST1	ETGQIVAIKQVPVESDLQEIIK	16.5	3.5	6.2	13.4
BLK	VAIKTLK	16.2	9.9	10.7	-7.0
MAP2K5	DVKPSNMLVNTR	15.7	-28.2	-23.2	-16.1
CaMK4	GTQKPYALKVLK	15.6	17.3	12.7	13.7
CK1a	DIKPDNFLMGIGR	15.5	0.1	-5.7	7.2
RSK2 domain1	LTDFGLSKESIDHEKK	15.1	19.6	18.1	22.2
SNRK	VAVKVIDK	14.9	-5.6	18.8	19.9
MSK1 domain1	DIKLENILLDSNGHVVLTDFGLSK	14.4	6.0	-4.6	3.6
FGR	LIKDDEYNPCQGSKFPIK	14.0	2.2	2.6	-2.2

IRAK3	VEIQNLTYAVKLFK	14.0	-5.5	8.5	9.2
PKR	DLKPSNIFLVDTK	13.9	1.2	3.3	-13.7
JAK3 domain2	IADFGLAKLLPLDKDYYVVR	13.6	-14.0	-0.1	-0.2
LRRK2	AAYEGEEVAVKIFNK	13.5	-7.1	-5.1	-0.8
eEF2K	YIKYNSNSGFVR	13.5	-92.3	-84.0	27.4
RSK1 domain1, RSK2 domain1, RSK3 domain1	DLKPENILLDEEGHIK	13.5	14.9	8.8	0.2
JAK2 domain2	IGDFGLTKVLPQDKEYYK	13.1	13.6	9.6	0.2
MLKL	LHHSEAPELHGKIR	13.0	12.2	9.2	5.3
CASK	ETGQQFAVKIVDVAK	12.7	22.0	10.9	-4.8
RSK2 domain2	DLKPSNILYVDESGNPESIR	12.4	13.6	15.2	19.3
ROCK1, ROCK2	DVKPDNMLLDK	12.3	21.9	18.2	19.4
SMG1	SYPYLFKGLEDLHLDER	12.3	2.2	-6.7	0.4
LCK	EGAKFPIKWTAPEAINYGTFTIK	11.9	-38.8	-16.8	4.1
MAP3K2	ELAVKQVQFDPDSPETSKEVNALECEIQLLK	11.9	-12.8	-4.3	6.1
SNRK	DLKPENVVFFEK	11.4	-4.1	-16.1	8.5
TAO2	DVKAGNILLSEPGLVK	11.4	-11.0	-12.0	14.8
MLKL	APVAIKVFK	10.9	9.8	-0.4	2.6
p38b	QELNKTVWEVPQR	10.8	-47.0	-19.7	-3.9
CaMKK2	DIKPSNLLVGEDGHIK	10.1	-1.3	5.0	7.0
PRP4	CNILHADIKPDNILVNESK	10.0	9.0	-7.5	15.0
GSK3B	DIKPQNLLLDPDTAVLK	10.0	-76.0	-62.8	26.1
JAK3 domain2	YDPLGDNTGALVAVKQLQHSGPDQQR	9.8	9.6	8.2	8.5
HCK	VAVKTMKPGSMSVEAFLAEANVMK	9.8	-21.7	-16.5	-25.1
STLK3	DLKAGNILLGEDGSVQIADFGVSAFLATGGDVTR	9.6	11.3	17.3	34.0

TAO1, TAO3	DIKAGNILLTEPGQVK	9.4	-5.4	-11.3	-2.5
PIP5K3	GGKSGAAFYATEDDRFILK	9.2	-9.8	-12.8	15.6
LKB1	DIKPGNLLLTTGGTLK	9.1	-2.1	2.4	1.8
PIK3CB	VFGEDSVGVIFKNGDDLRQDMLTLQMLR	9.1	31.5	-4.7	7.3
JAK1 domain2	IGDFGLTKAIETDKEYYTVK	9.1	6.5	10.7	-7.4
LATS2	VDTHALYAMKTLR	8.8	8.5	-1.4	0.0
LRRK2	DLKPHNVLLFTLYPNAAIIAK	8.6	-0.5	5.1	-5.5
MAP2K6	DVKPSNVLINALGQVK	8.4	6.2	7.4	2.1
HPK1	DIKGANILINDAGEVR	8.0	1.3	10.7	15.2
YANK3	DVKPDNILLDER	7.8	15.5	4.4	-12.8
CDK5	NRETHEIVALKR	7.7	-12.2	-1.2	6.2
PRPK	FLSGLELVKQGAEAR	7.6	-10.7	-6.7	-9.8
CDK11, CDK8	DLKPANILVMGEGPER	7.4	1.9	-6.9	-12.6
PKN1	DLKLDNLLLDTEGYVK	7.4	-17.5	-2.4	-5.2
PKR	TYVIKR	7.4	7.8	-2.6	-1.2
GCK	DIKGANLLLTLQGDVK	7.3	10.4	13.6	13.5
ULK1	DLKPQNILLSNPAGR	7.2	-4.6	-2.5	15.1
GCK	DTVTSELAAVKIVK	7.0	5.2	6.0	-12.7
CaMK2G	TSTQEYAAKIINTK	6.9	3.2	5.3	1.6
IRE1	DLKPHNILISMPNAHGK	6.9	4.4	-3.7	0.8
TYK2 domain2	IGDFGLAKAVPEGHEYYR	6.6	11.9	9.3	-6.6
SYK	ISDFGLSKALR	6.2	7.3	10.2	-2.8
DNAPK	EHPFLVKGGEDLR	6.0	-34.7	-31.7	4.0
PI4KB	VPHTQAVVLNSKDK	5.8	-8.5	4.6	7.7
MAP3K4	VYTCISVDTGELMAMKEIR	5.8	14.8	4.6	9.7
MST4, YSK1	DIKAANVLLSEQGDVK	5.8	3.8	11.8	18.4
ULK3	NISHLDLKPQNILLSSLEKPHLK	5.7	-5.5	-6.1	-4.5
MAP3K6	DIKGDNVLINTFSGLLK	5.7	11.8	10.5	0.4
LATS1	ALYATKTLR	5.4	14.3	7.9	4.7
MST3	DIKAANVLLSEHGEVK	5.4	6.2	15.2	14.9

PIP4K2A	AKELPTLKDNDFINEGQK	5.3	9.3	10.7	-2.7
DNAPK	KGGSWIQEINVAEK	5.2	-16.2	6.2	-19.0
EphA1	LLDDFDGTYETQGGKIPIR	5.1	7.7	14.6	0.3
MAP2K4	DIKPSNILLDR	5.0	5.4	7.3	-0.7
PIP4K2C	VKELPTLKDMDFLNK	5.0	3.3	-1.5	-4.0
MST1, MST2	DIKAGNILLNTEGHAK	4.8	-1.9	2.1	1.5
p38a	DLKPSNLAVNEDCELK	4.7	3.8	8.5	-4.0
COT	GAFGKVYLAQDIK	4.6	-11.6	5.0	-6.2
MAST3	DLKPDNLLITSLGHIK	4.6	9.8	10.7	7.8
PAK2	IGQGASGTVFTATDVALGQEVAIKQINLQK	4.5	7.1	16.7	13.3
MAP2K3	DVKPSNVLINK	4.4	13.6	15.5	3.3
PLK1	CFEISDADTKEVFAGKIVPK	4.2	-10.5	-0.1	12.0
MAP4K5	DIKGANILLTDHGDVK	4.2	2.7	11.8	18.5
MAPKAPK2, MAPKAPK3	DVKPENLLYTSK	4.1	7.2	-5.1	2.8
MST2	ESGQVVAIKQVPVESDLQEIIK	4.1	-26.2	-6.3	-9.6
MARK3, MARK4	EVAIKIIDK	4.1	-25.3	-21.4	-31.1
ZAP70	QIDVAIKVLK	3.9	1.3	7.4	2.5
CDK2	DLKPQNLLINTEGAIK	3.8	-15.0	-4.9	10.4
LATS1	DIKPDNILIDR	3.3	5.4	-4.6	-10.4
SMG1	DTVTIHSVGGTITILPTKTKPK	3.2	7.6	-0.2	2.2
IKKa	DLKPENIVLQDVGGK	2.9	1.5	4.9	8.4
ZAP70	ISDFGLSKALGADDSYYTAR	2.8	7.3	10.6	1.2
NDR1, NDR2	LSDFGLCTGLKK	2.7	1.7	6.2	-15.5
JAK2 domain1	QLAWAMHFLEENTLIHGNVCAKNILLIR	2.6	-3.8	-16.7	-6.8
NDR2	DIKPDNLLLDAK	2.6	-9.1	-3.6	-3.3
AKT2, AKT3	GTFGKVILVR	2.4	-9.8	-3.1	-9.5
GCN2 domain 2	LDGCCYAVKR	2.2	-11.4	-11.8	-2.3
PFTAIRE1	LVALKVIR	2.2	2.0	12.2	-14.7
Erk5	DLKPSNLLVNENCELK	2.2	9.0	9.7	9.6

CHK2	VAIKIISK	2.2	-20.8	-5.9	6.6
MAP3K5	DIKGDNVLINTYSGVLK	2.1	6.9	-1.9	3.7
RSKL1	VLGVIDKVLLVMDTR	2.1	-9.4	-14.7	-13.8
LYN	VAVKTLKPGTMSVQAFLEEANLMK	2.1	-10.8	-26.3	-18.0
NEK4	DLKTQNVFLTR	2.0	7.2	-3.0	-19.8
NEK3	SKNIFLTQNGK	2.0	3.1	-4.3	-2.3
RIPK3	DLKPSNVLLDPELHVK	2.0	0.5	4.0	-5.7
IRAK4	DIKSANILLDEAFTAK	1.7	-1.4	7.0	-4.1
HPK1	DKVSGDLVALKMVK	1.7	-1.2	-1.8	7.1
MAP4K5	NVHTGELAAVKIIK	1.6	-14.1	-25.5	-1.1
RSK1 domain1	KVTRPDSGHLYAMKVLK	1.6	4.3	-10.5	-14.0
Wnk1, Wnk2, Wnk4	IGDLGLATLKR	1.6	13.7	6.7	3.5
p70S6K	DLKPENIMLNHQGHVK	1.6	12.2	-0.3	-5.8
CAMK2D	IPTGQEYAAKIINTKK	1.6	3.4	10.2	5.2
SYK	TVAVKILK	1.5	1.0	7.0	-12.8
MAP3K1	DVKGANLLIDSTGQR	1.4	14.4	5.8	6.8
MAP3K2, MAP3K3	DIKGANILR	1.3	0.9	13.3	5.5
ULK2	DLKPQNILLSYANR	1.3	1.4	-4.1	-4.7
PI4KB	LLSVIVKCGDDLRQELLAFQVLK	1.2	-29.4	1.1	-10.9
SGK3	DLKPENILLDSVGHVVLTDFGLCK	1.1	-10.3	-8.1	-25.6
OSR1	DVKAGNILLGEDGSVQIADFGVSAFLATGGDITR	1.1	13.7	9.4	28.2
AMPKa1, AMPKa2	VAVKILNR	1.1	4.4	1.5	8.3
NEK8	DLKTQNILLDK	1.1	6.3	-6.7	-5.5
GRK2	DLKPANILLDEHGHVR	1.0	-4.7	2.1	1.5
NEK1	DIKSQNIFLTK	0.8	2.8	3.3	-2.4
PITSLRE	DLKTSNLLLSHAGILK	0.8	-4.1	-8.6	1.1
NDR1	DTGHVYAMKILR	0.7	11.6	0.1	-2.2
IKKb	DLKPENIVLQQGEQR	0.7	6.9	7.5	14.9
NEK9	DIKTLNIFLTK	0.7	12.8	8.6	1.7
PKR	IGDFGLVTSLKNDGKR	0.7	8.6	5.1	-7.3
MAP2K3	MCDFGISGYLVDSVAKTMDAGCKPYMAPER	0.6	24.4	9.7	4.5

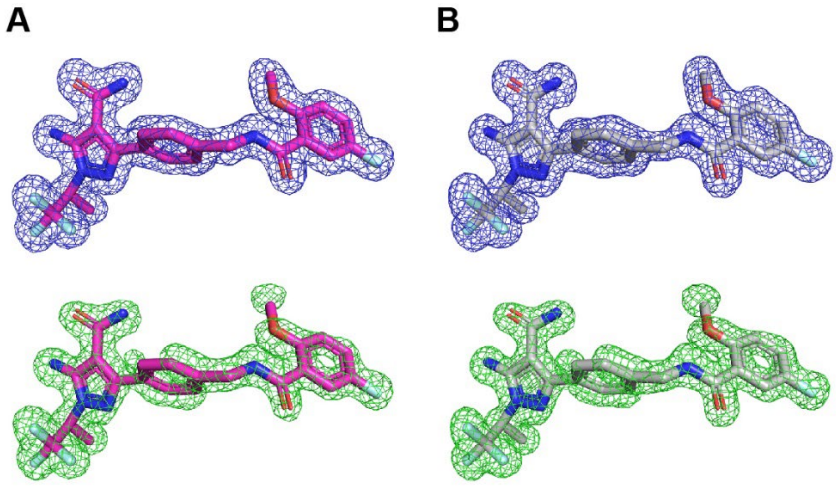
IKKe	SGELVAVKVFNTTSYLRPR	0.2	1.4	6.2	-4.7
CaMK4	DLKPENLLYATPAPDAPLK	0.1	3.8	8.5	23.1
PCTAIRE2	DLKPQNLLINEK	-0.1	-5.1	0.9	0.0
Erk2	DLKPSNLLLNTTCDLK	-0.2	5.5	16.0	2.8
MST3, MST4, YSK1	LADFGVAGQLTDTQIKR	-0.3	-1.7	6.8	0.8
NEK6, NEK7	DIKPANVFITATGVVK	-0.4	-1.7	11.3	16.5
NDR2	DTGHIYAMKILR	-0.5	-13.3	-29.8	1.8
FRAP	IQSIAPSLQVITSKQRPR	-0.5	11.3	4.5	2.9
AKT1	GTFGKVILVK	-0.7	9.3	4.6	-8.9
RSK1 domain1	LTDFGLSKEAIDHEKK	-0.8	4.6	-1.5	4.5
ULK3	EVVAIKCVAK	-1.0	15.2	25.6	18.3
CDK7	DLKPNNLLLDENGVLK	-1.2	-23.8	-27.2	-14.8
MAP2K4	LCDFGISGQLVDSIAKTR	-1.4	-2.6	9.6	0.6
FES	LRADNTLVAVKSCR	-1.5	2.8	10.7	-5.0
MSK2 domain1	LYAMKVLR	-1.6	4.8	-6.5	-11.9
CAMK1	DLKPENLLYYSLDEDSK	-1.6	12.9	18.7	8.3
TNIK	TGQLAAIKVMDVTGDEEEEIKQEINMLKK	-1.8	0.0	-7.2	-2.3
GCN2 domain 2	DLKPVNIFLDSDDHVK	-2.2	-2.8	-5.9	3.5
PCTAIRE2, PCTAIRE3	SKLTENLVALKEIR	-2.2	5.2	4.5	-16.9
PKCa, PKCb	DLKLDNVMLDSEGHIK	-2.2	11.2	-11.5	-5.6
PEK	DLKPSNIFFTMDDVVK	-2.3	-2.7	-12.7	-14.0
STLK5	SVKASHILISVDGK	-2.4	4.2	4.9	13.6
ABL1, ABL2	LMTGDTYTAHAGAKFPIK	-2.4	-4.5	2.6	-0.3
MAP4K4, TNIK, MINK1	DIKGQNVLLTENAEVK	-2.6	-14.0	-7.8	2.5
LATS2	DIKPDNILIDLDGHIK	-2.6	-2.6	15.1	-5.6

p70S6Kb	IYAMKVLR	-2.6	-4.1	-13.9	-13.7
ZAK	WISQDKEVAVKK	-2.7	-1.0	6.2	-2.4
MAP3K15, MAP3K5, MAP3K6	IAIKEIPER	-2.8	0.9	-0.2	-10.0
ITPK1	ESIFFNSHNVSKPESSSVLTELDKIEGVFERPSDEVIR	-2.8	-59.1	-23.5	-72.6
JNK1, JNK2, JNK3	DLKPSNIVVK	-2.9	-1.2	8.9	0.2
LOK	NKETGALAAAKVIETK	-3.0	11.3	8.6	3.8
Erk1	DLKPSNLLINTTCDLK	-3.3	1.5	0.4	5.0
ILK	WQGNDIVVKVLK	-3.5	-0.9	-4.0	-25.0
RSK1 domain2	DLKPSNILYVDESGNPECLR	-3.6	-1.1	11.2	2.2
MARK3	EVAIKIIDKTQLNPTSLQK	-3.8	-42.3	-32.0	6.8
MLK3	DLKSNNILLLQPIESDDMEHK	-3.9	2.5	-4.9	-4.5
PKN1	VLLSEFRPSGELFAIKALK	-3.9	-25.5	-9.5	-7.8
KSR1	SKNVFYDNGKVVITDFGLFGISGVVR	-3.9	-6.8	1.7	4.8
NEK7	AACLLDGVPVALKK	-4.0	-3.2	2.9	-7.0
FER	TSVAVKTCKEDLPQELK	-4.2	-7.5	-5.7	-4.6
SLK	DLKAGNILFTLDGDIK	-4.2	-4.5	6.3	17.7
MAP3K4	DIKGANIFLTSSGLIK	-4.3	-0.2	4.2	11.6
ATR	FYIMMCKPK	-4.5	-4.9	-11.8	-12.7
MAP2K3	HAQSGTIMAVKR	-4.6	-6.8	3.1	-6.8
SLK	AQNKETSVLAAAKVIDTK	-4.6	-39.7	-24.3	-7.1
CHK2	DLKPENVLLSSQEEDCLIK	-4.9	-2.9	-6.1	-16.5
MAPKAPK3	QVLGLGVNGKVLECFHR	-4.9	6.6	17.4	-0.7
PIK3C3	TEDGGKYPVIFKHGDDLR	-4.9	8.1	-1.3	-6.6
ILK	ISMADVKFSFQCPGR	-5.1	-13.4	-11.5	-16.2
p70S6Kb	DLKPENIMLSSQGHIK	-5.1	8.8	-5.2	-1.4
DGKA	IDPVPNTHPLLVFVNPKSGGK	-5.1	11.0	12.0	-16.1
LCK	VAVKSLK	-5.2	-29.3	-32.8	-10.8
Wnk1, Wnk2, Wnk3	DLKCDNIFITGPTGSVK	-5.6	3.1	-5.6	-9.2
RAF1	DMKSNNIFLHEGLTVK	-5.8	1.0	-15.1	-16.9
MAP2K6	HVPSGQIMAVKR	-5.9	3.6	2.5	-6.8

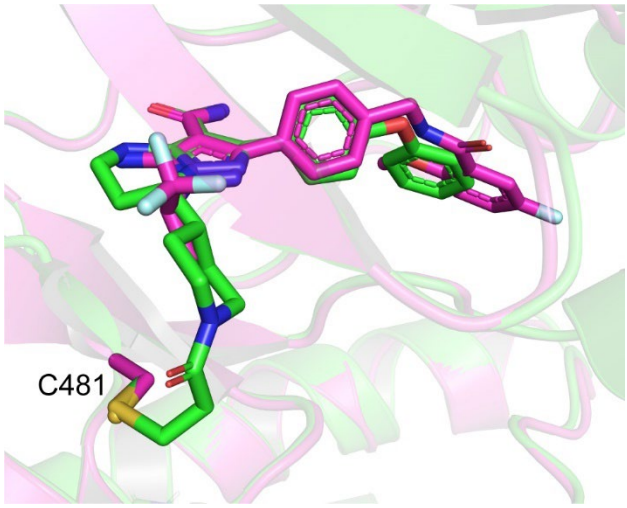
NDR1	DIKPDNLLLDSK	-6.1	-2.4	-3.3	-21.1
p38a	QELNKTIWEVPER	-6.6	-17.9	-2.6	-23.8
NEK9	LGDYGLAKK	-6.6	7.5	6.6	6.1
IRAK2	HGKPFVFKK	-6.7	6.6	0.2	-5.7
p38d, p38g	DLKPGNLAVNEDCELK	-6.9	2.4	14.4	-3.4
CDK5	DLKPQNLLINR	-6.9	-15.2	-8.9	12.3
PCTAIRE1, PCTAIRE3	DLKPQNLLINER	-6.9	-9.8	14.4	37.0 NS
IRAK3	SHLEHQSCTINMTSSSSKHLWYMPEEYIR	-7.5	19.1	3.4	-6.1
FER	QEDGGVYSSSGLKQIPIK	-7.5	-28.0	-30.7	-0.7
GSK3A	DIKPQNLLVDPDTAVLK	-8.1	-37.3	-25.4	-12.7
PYK2	INVAVKTCK	-8.3	4.8	-9.2	-2.9
IKBKE, TBK1	DIKPGNIMR	-8.7	1.9	-6.7	-8.9
IRAK1	AIQFLHQDSPSLIHGDIKSSNVLLDER	-9.0	-1.1	8.0	-5.0
PRKAA1, PRKAA2	DLKPENVLLDAHMNAK	-9.1	1.4	-7.6	-4.6
PAN3	IQKSSNFGYITSCYK	-9.1	2.8	1.6	-5.0
IRAK4	GYVNNTTVAVKK	-9.2	6.2	-4.0	-19.1
PKD2	DVAVKVIDK	-9.3	-14.4	-31.4	-10.6
FYN, SRC, YES	QGAKFPIKWTAPEAALYGR	-9.4	0.5	15.0	-4.4
PIP4K2B	AKDLPTFKDNDFLNEGQK	-9.5	2.0	8.7	-5.7
CDK2	LTGEVVALKK	-9.5	-11.6	-14.0	6.1
PYK2	YIEDEDYYKASVTR	-10.1	17.2	-4.9	18.1
TLK2	YVAVKIHQLNK	-10.2	-8.8	-6.8	-6.6
CaMK1d	DLKPENLLYYSQDEESK	-10.3	12.8	-5.3	-1.9
SGK3	FYAVKVLQK	-10.5	8.6	3.9	-0.7
PIP4K2C	TLVIKEVSSEDIADMHSNLSNYHQYIVK	-11.5	2.3	-9.6	-12.0
MSK2 domain1	DLKLENVLLDSEGHIVLTDFGLSK	-11.6	-5.1	3.1	-13.9
NuaK2	LVAIKSIR	-12.0	-3.0	-12.4	-9.0
JAK1 domain2	YDPEGDNTGEQVAVKSLKPESGGNHIADLKK	-12.0	3.2	7.5	-15.0
STLK5	YSVKVLPWLSPEVLQQNLQGYDAK	-12.1	-11.6	-5.5	-13.3

LOK	DLKAGNVLMTLEGDIR	-12.4	-11.4	-4.3	-6.2
JAK1 domain1	QLASALSYLEDKDLVHGNVCTKNLLLAR	-12.6	-7.5	-16.2	-2.8
CAMK1D	LFAVKCIPK	-12.7	-1.5	1.6	-8.4
PIK3CG	KKPLWLEFK	-12.8	-8.6	0.4	-8.2
ARAF	DLKSNNIFLHEGLTVK	-13.1	-0.7	5.3	-5.9
CDK9	DMKAANVLITR	-13.1	-30.6	-38.8	-19.3
PAN3	VMDPTKILITGK	-13.6	1.0	-6.7	-6.2
PIK3C3	TEDGGKYPVIFKHGDDLRQDQLILQIISLMDK	-14.8	-41.1	2.7	-15.9
MRCKb	NHHVHLYPWSSLDGAEGSFDIKLPETK	-15.2	11.5	3.2	-25.3
MAP2K7	DVKPSNILLDER	-15.9	-16.3	6.8	-6.8
NEK9	RTEDDSLVVWKEVDLTR	-16.2	-4.6	3.3	-11.8
KSR1, KSR2	SKNVFYDNGK	-16.6	5.8	3.9	-4.8
TLK1	YLNEIKPPIIHYDLKPGNILLVDGTACGEIK	-16.6	-19.8	-2.7	-11.0
PIK3CD	VNWLAHNVSKDNRQ	-17.0	-6.3	6.0	-16.1
ACK	TVSVAVKCLKPDVLSQPEAMDDFIR	-17.3	-17.9	-55.4	-26.9
CAMK1	LVAIKCIAK	-17.3	-3.4	5.0	-8.5
ROCK1	KLQLELNQER	-17.7	-52.9	-33.2	1.5
TBK1	TGDLFAIKVFNNISFLRPVDVQMR	-19.5	-3.0	-9.9	-12.9
PI4KA, PI4KAP2	SGTPMQSAAKAPYLAK	-20.6	-19.0	4.4	-25.8
TLK1	YAAVKIHQLNK	-21.0	0.4	-15.2	-1.5
ZAP70	SAGKWPLK	-21.3	-14.0	5.3	-8.1
MARK2	EVAVKIIDKTQLNSSSLQK	-22.7	-13.1	2.4	-2.6
ACK	KVPFAWCAPESLK	-24.3	-3.1	-14.3	-15.4
BRAF	DLKSNNIFLHEDLTVK	-34.4	-11.4	-3.2	-7.7
CDC2	DLKPQNLLIDDK	-35.8	-13.0	-47.1	-7.3
MAP3K3	ELASKQVQFDPDSPETSKEVSALECEIQLLK	-38.5	-38.6	26.3	4.7
TLK2	YLNEIKPPIIHYDLKPGNILLVNGTACGEIK	-60.4	-28.4	-18.6	-46.8
TAK1	DLKPPNLLLVAGGTVLK	-61.8	8.0	-10.8	22.9

Supplemental Figure 1. Pirtobrutinib omit electron density. (A) BTK – pirtobrutinib *2mF$_o$-DF$_c$* omit electron density map contoured at 1σ (top), and *mF$_o$-DF$_c$* omit electron density map contoured at 4σ (bottom). (B) BTK C481S – pirtobrutinib *2mF$_o$-DF$_c$* omit electron density map contoured at 1σ (top), and *mF$_o$-DF$_c$* omit electron density map contoured at 4σ (bottom). The maps were generated with the *Composite omit map* program within the *Phenix* package.[3]

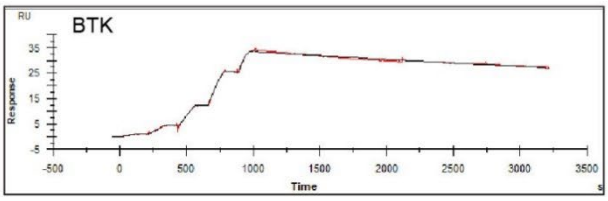


Supplemental Figure 2. Superposition of the crystal structures of BTK in complex with pirtobrutinib (magenta cartoon/stick representation) and BTK covalently bound to zanubrutinib (green cartoon/stick representation; PDB ID 6J6M).

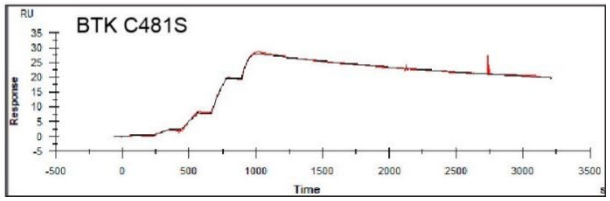


Supplemental Figure 3. Representative sensorgrams for pirtobrutinib binding to (A) wildtype BTK and (B) BTK C481S. The biotinylated BTK proteins were immobilized on streptavidin sensor chip followed by 5 consecutive injections of increasing concentrations of pirtobrutinib during the association phase followed by a long dissociation phase. The red line shows the raw data. The superimposed black line represents the data fit that was used to generate the affinity constants using the Biacore's single cycle kinetics data analysis program.

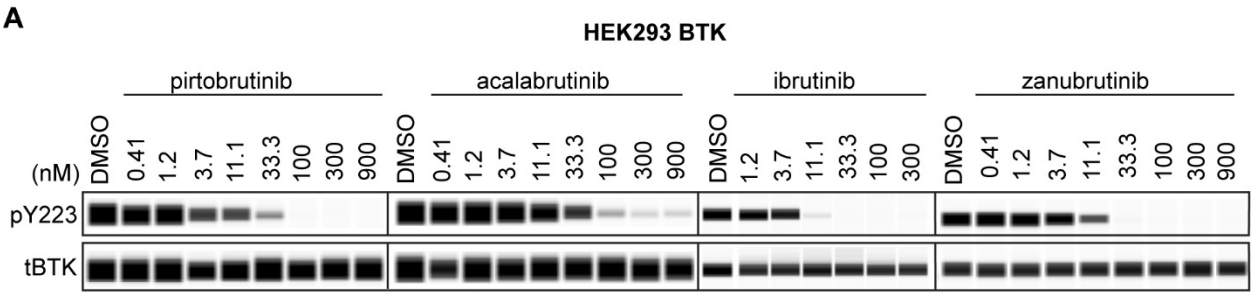
A

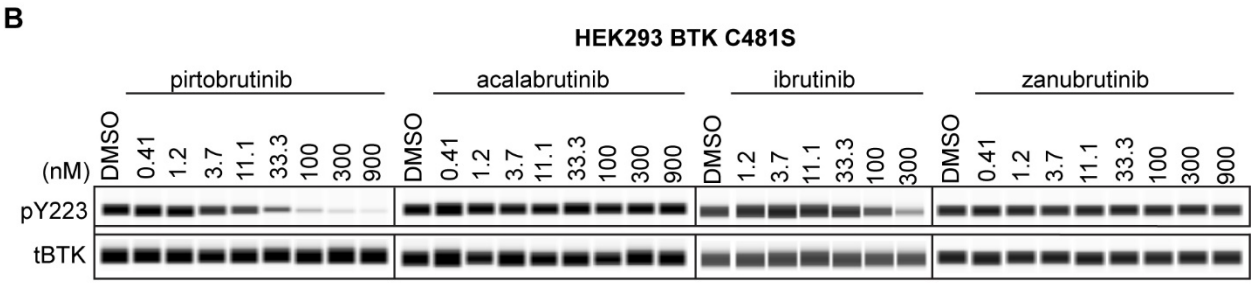


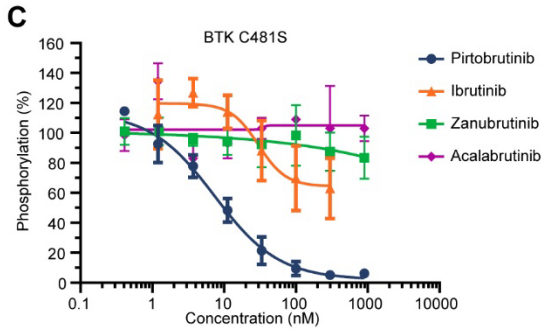
B



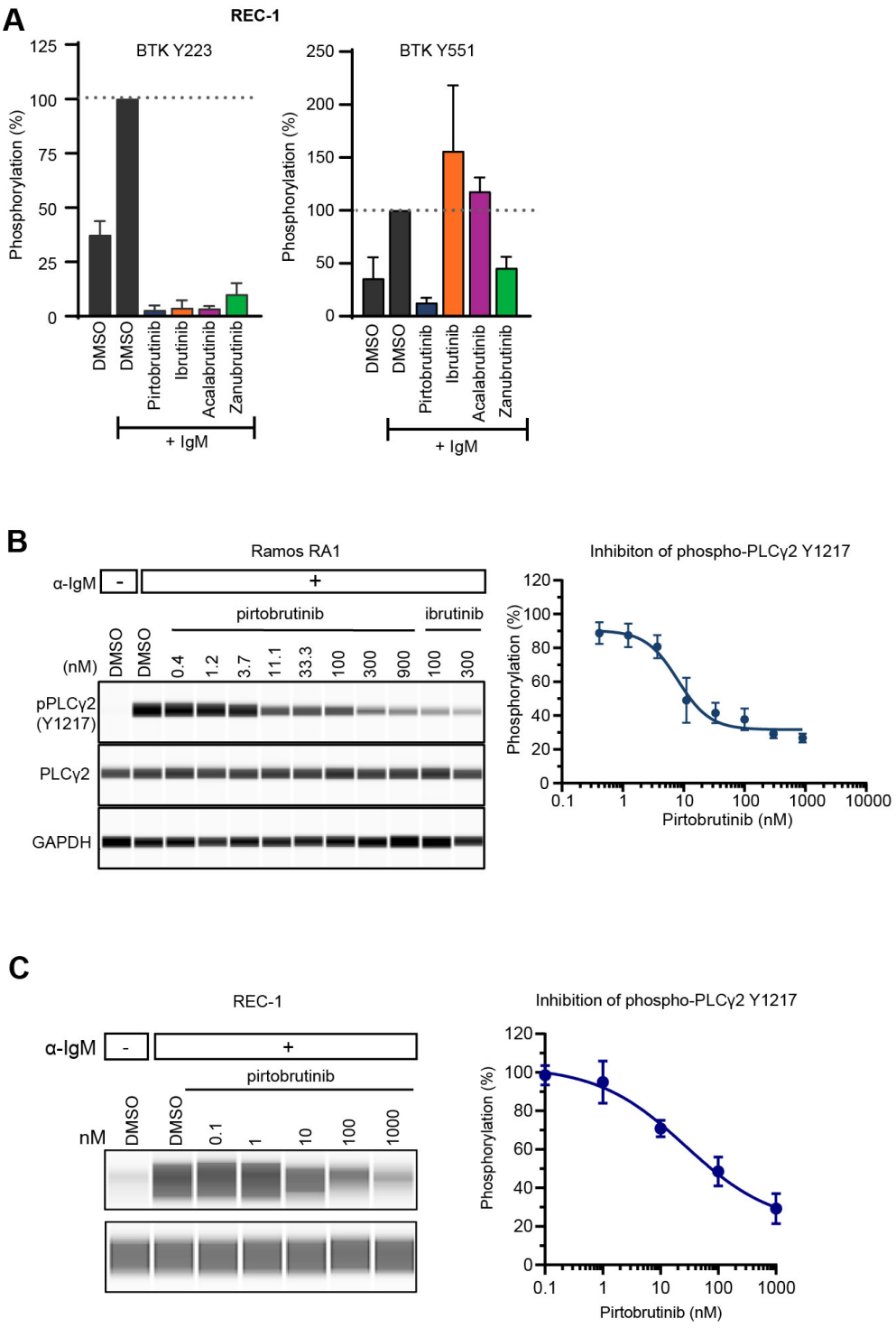
Supplemental Figure 4. Pirtobrutinib inhibits BTK autophosphorylation in HEK293 cells expressing BTK and BTK C481S. Autophosphorylation of BTK Y223 was measured by Simple Western in HEK293 expressing (A) BTK and (B) C481S mutant BTK treated with pirtobrutinib, ibrutinib, acalabrutinib, and zanubrutinib. (C) Dose-response curves generated from the Simple Western data shown in (B). Data are presented as mean ± standard deviation. Data are representative of at least 2-3 experiments.



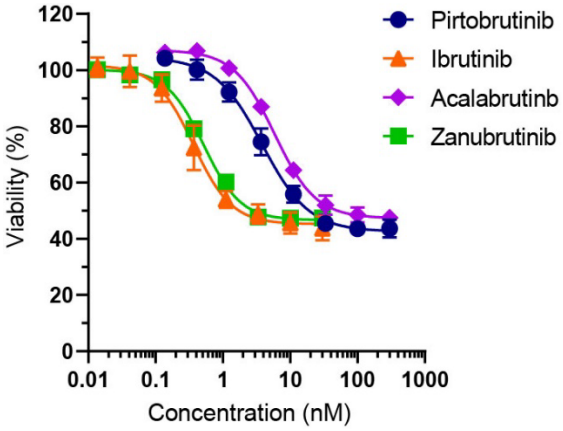




Supplemental Figure 5. Pirtobrutinib inhibited BTK signaling in human B-cell lymphoma cell lines. (A) Pirtobrutinib inhibited phosphorylation of BTK Y223 and Y551 in REC-1 cells. Autophosphorylation of BTK at Y223 and phosphorylation at Y551 were analyzed by Simple Western or Western blotting in cells treated with 100 nM of pirtobrutinib, ibrutinib, acalabrutinib and zanubrutinib. Percentage of phosphorylation relative to DMSO control was graphed (n = 2-to-5). (B) PLCγ2 phosphorylation was inhibited by pirtobrutinib treatment in Ramos RA1 cells and (C) REC-1 cells. Representative Simple Western images are shown (left), and dose-response curves generated from Simple Western results are shown (right). Data are presented as mean ± standard deviation.

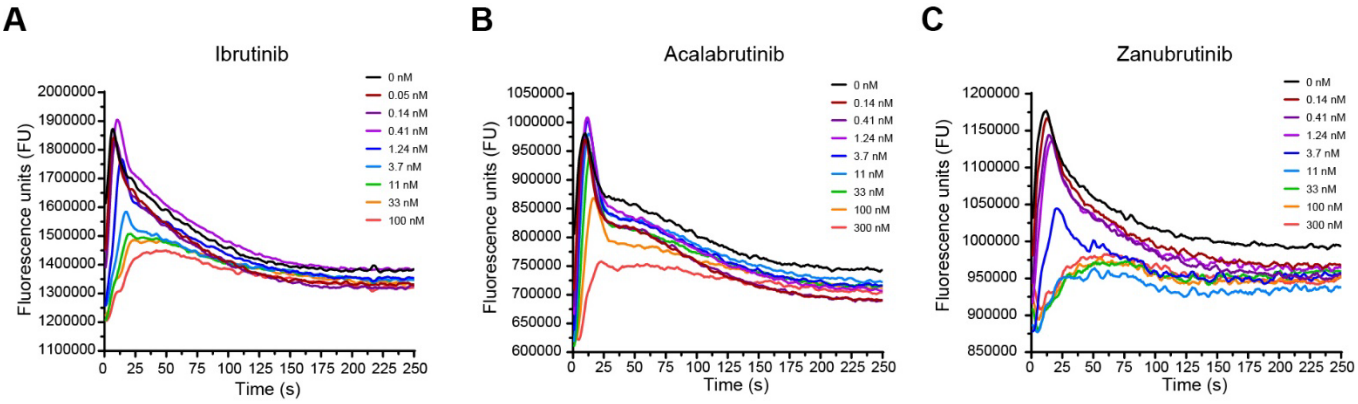

A
REC-1
BTK Y223
BTK Y551
Phosphorylation (%)
DMSO
DMSO
Pirtobrutinib
Ibrutinib
Acalabrutinib
Zanubrutinib
+ IgM
B
Ramos RA1
α-IgM
pirtobrutinib
ibrutinib
(nM)
pPLCγ2 (Y1217)
PLCγ2
GAPDH
Inhibiton of phospho-PLCγ2 Y1217
Pirtobrutinib (nM)
C
REC-1
nM
Inhibition of phospho-PLCγ2 Y1217

Supplemental Figure 6. Effects of Pirtobrutinib and covalent BTK inhibitors on REC-1 MCL cell proliferation. Pirtobrutinib inhibited the proliferation of REC-1 MCL cells in a dose-dependent manner. Data are presented as mean ± standard deviation.



Compound	n	IC_{50} (nM)
Pirtobrutinib	5	4.0 ± 1.2
Ibrutinib	5	0.4 ± 0.1
Zanubrutinib	1	0.5
Acalabrutinib	2	5.9 ± 0.2

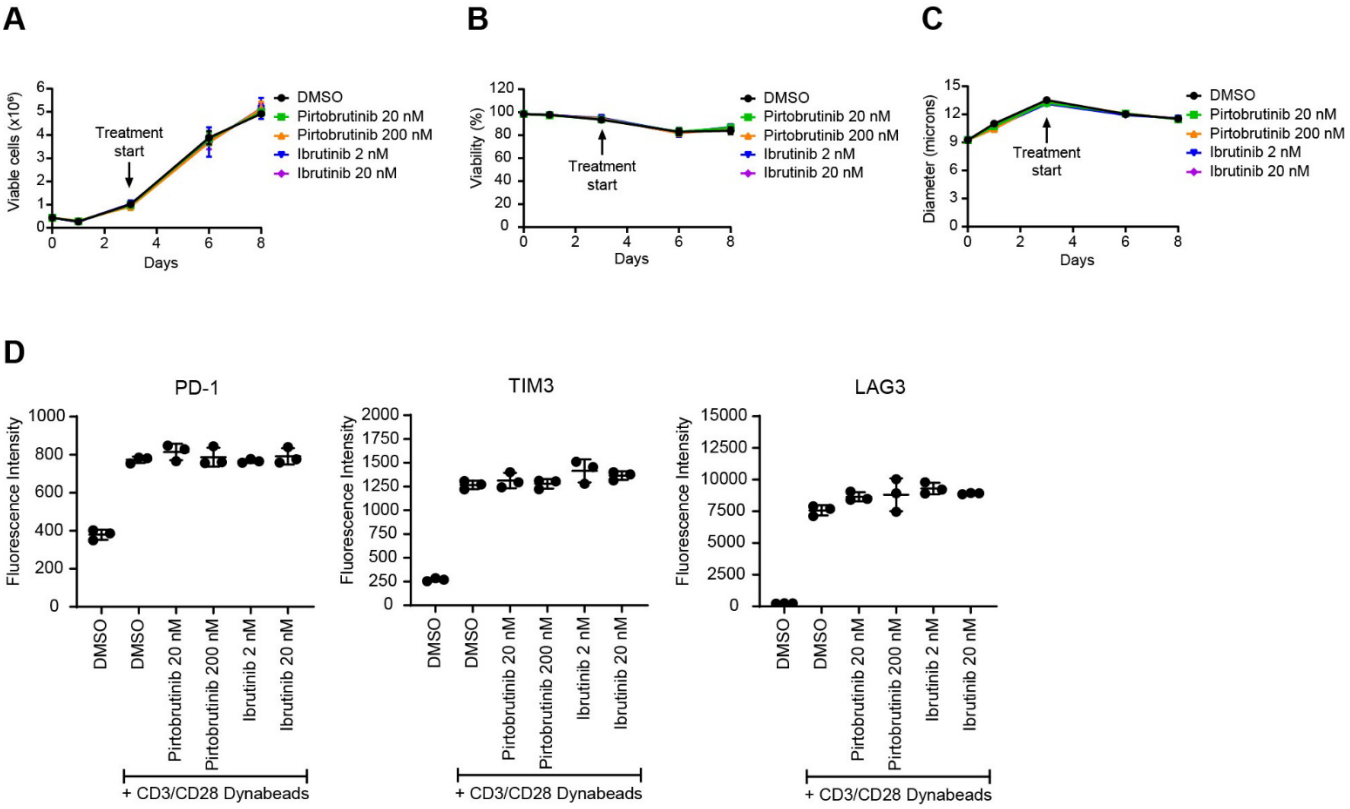
Supplemental Figure 7. Calcium flux measurements for REC-1 cells treated with covalent BTK inhibitors. Representative traces of α-IgM-stimulated REC-1 cells treated with (A) ibrutinib, (B) acalabrutinib, and (C) zanubrutinib. (D) Calcium flux half maximal inhibitory concentrations (IC_{50}) calculated from the maximum value of individual traces. Data are presented as mean ± standard deviation.



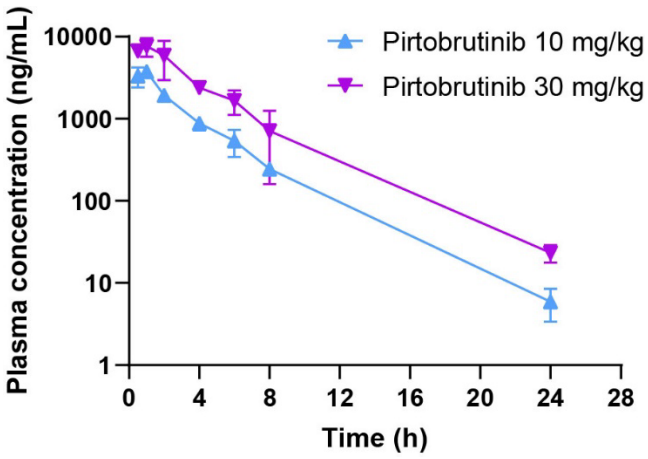
D

Compound	n	IC_{50} nM
Ibrutinib	3	1.7 ± 0.6
Zanubrutinib	2	4.3 ± 1.7
Acalabrutinib	2	72.7 ± 34.9

Supplemental Figure 8. Effects of pirtobrutinib and ibrutinib on in vitro human T-cell viability, proliferation and expression of T-cell activation/exhaustion markers. T-cells isolated from healthy donor PBMCs were treated with Human T-Activator CD3/CD28 Dynabeads. Pirtobrutinib, ibrutinib or DMSO vehicle control was added on day 3 and (A) cell number, (B) cell viability, and (C) cell size was measured. Data presented as mean ± standard deviation. (D) On day 8, expression of PD-1, TIM3, and LAG3 was measured by flow cytometry. Data are presented as mean fluorescence intensity ± standard deviation (n=2-to-3 experiments).

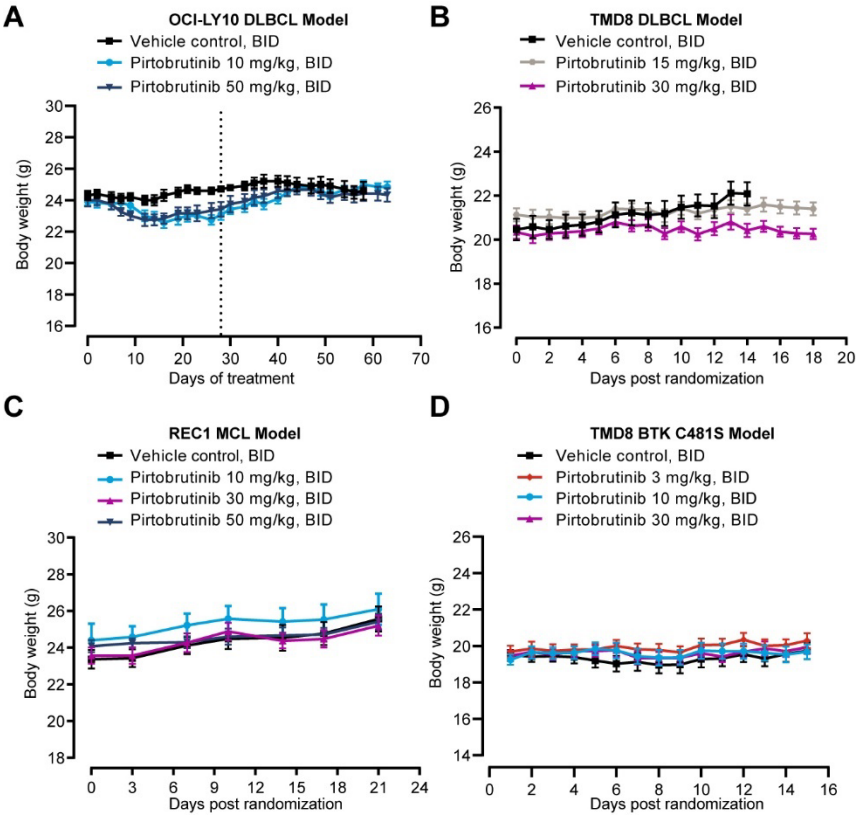


Supplemental Figure 9. Pharmacokinetic profile of single dose pirtobrutinib in Balb/c female mice. Pirtobrutinib was dosed orally at 10 mg/kg and 30 mg/kg and plasma concentrations were measured over time. Data presented as mean ± standard deviation.



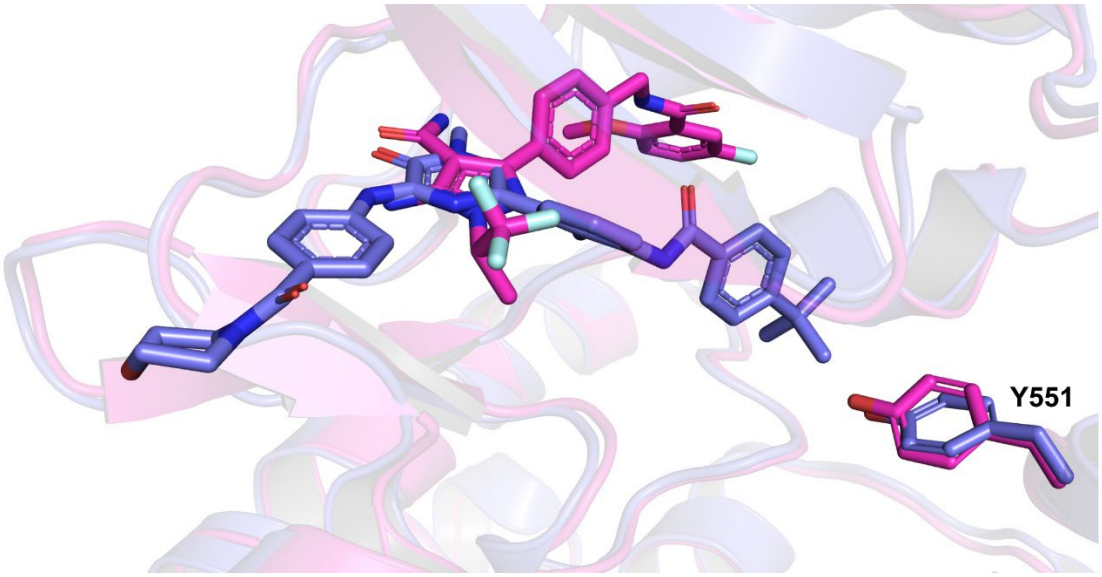
Supplemental Figure 10. In vivo tolerability of pirtobrutinib in DLBCL and MCL lymphoma xenograft models. Tumor-bearing mice were treated with pirtobrutinib with the indicated doses and time. The mean body weight and SEM were plotted for each treatment group versus days of treatment (A) or days post-randomization (B-D). For OCI-LY10 tumor-bearing mice (A) body

weight was measured daily, and the body weight was recorded for an additional 35 days post-last dose. For TMD8 tumor-bearing mice (B) body weight was measured daily, and the experiment was stopped on Day 14 post-randomization for the vehicle control group and on Day 18 post-randomization for the other groups. For REC-1 tumor bearing mice (C) body weight was measured bi-weekly. For TMD8 BTK C481S tumor-bearing mice (D) body weight was measured daily. Abbreviations: BID, twice daily; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; SEM, standard error of the means.



Supplemental Figure 11. Superposition of the crystal structure of BTK in complex with pirtobrutinib (magenta cartoon/stick representation) and BTK in complex with CGI1746 (slate cartoon/stick representation; PDB ID 3OCS). The binding mode of CGI1746 has been reported to inhibit phosphorylation of Y551 by occupying the nearby H3 selectivity pocket.[2]

Pirtobrutinib was also observed to inhibit Y551 phosphorylation without occupying the H3 pocket.



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