DFG-1 residue controls inhibitor binding mode and affinity providing a basis for rational design of kinase inhibitor selectivity

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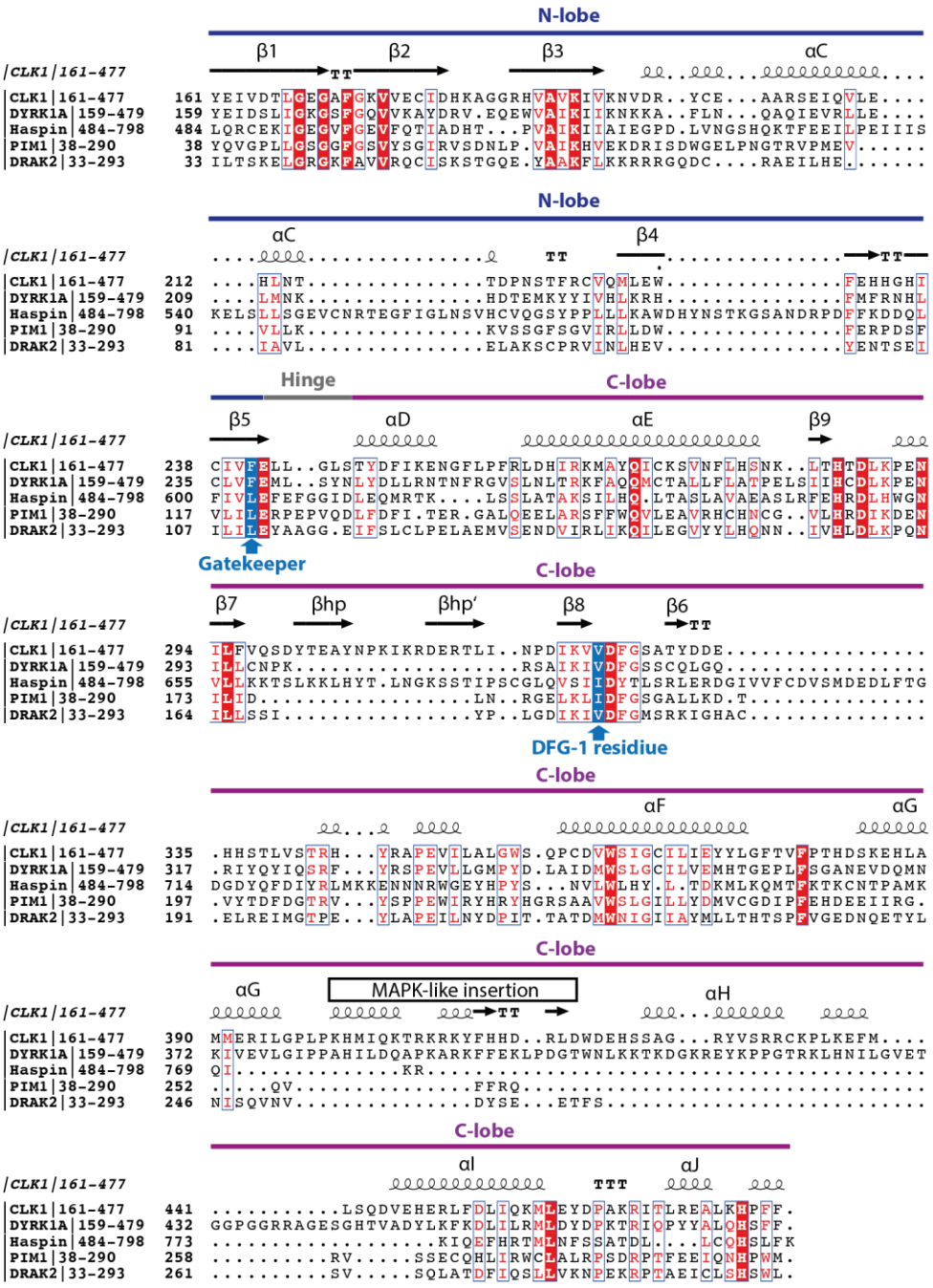
Supplementary information

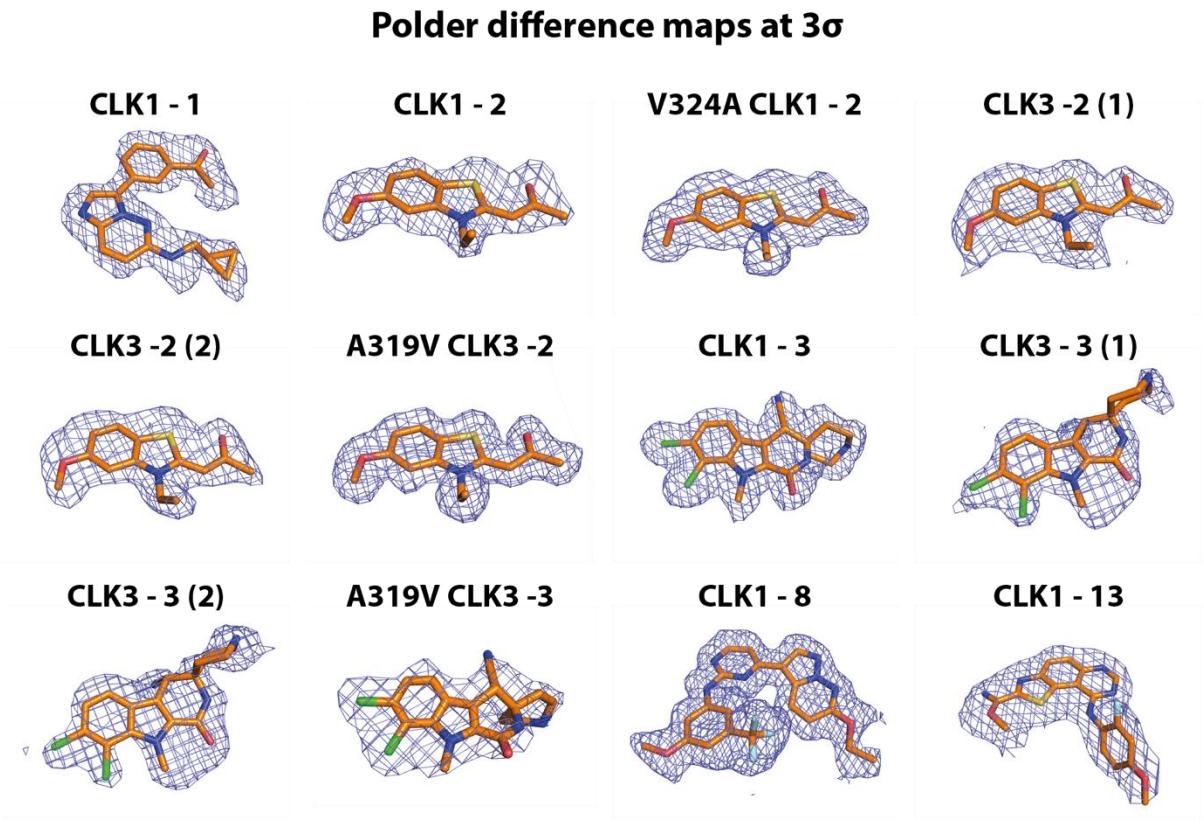
A



B

Percent Identity	CLK1 161-477	DYRK1A 159-479	Haspin 484-798	PIM1 38-290	DRAK2 33-293
CLK1 161-477	100				
DYRK1A 159-479	33	100			
Haspin 484-798	17	27	100		
PIM1 38-290	26	24	22	100	
DRAK2 33-293	26	26	17	26	100

Supplementary figure 1. Comparative sequence analyses of the most common kinase targets of 1-5. Sequence alignment of the kinase domains of CLK1, DYRK1A, haspin, PIM1 and DRAK2 is shown in panel **A** and sequence identities in panel **B**.



Supplementary figure 2. Polder difference maps at 3σ for the bound compounds.

Supplementary table 1. Thermal shift results of compounds 1-4 against various kinases.

	ΔTm (°C) for inhibitor						
Kinase	1	2	3	4	Gatekeeper	DFG-1 residue	subfamily
AAK1	6.9	1.5	1.5	3.4	M	C	Other
ABL2		-0.5			T	A	TK
ACVR2A	4.0			0.3	T	A	TKL
ACVR2B	7.4			0.0	T	A	TKL
AKT3	0.4	0.0	0.4		M	T	AGC
ACVRL1	11.1			1.2	T	A	TKL
ACVR1	11.0			0.7	T	A	TKL
ACVR1B	9.1			1.1	S	A	TKL
PRKAA1	1.3	-0.3		0.5	M	A	CAMK
PRKAA2	1.1	-0.1			M	A	CAMK
ADRBK1		0.1			L	S	AGC
ADRBK2		-0.2	1.1		L	S	AGC
BMP2K	10.7	0.2	1.0		M	C	Other
BMPR1A	10.9				T	A	TKL
BMPR1B	11.5			0.2	T	A	TKL
BMPR2	7.8		0.3		M	S	TKL
BMX	0.4	-0.1		0.4	T	S	TK
PTK6	0.4				T	G	TK
CAMK1D	0.6	0.0	1.2	0.2	M	S	CAMK
CAMK1G	0.6	-0.1		0.3	M	T	CAMK
CAMK2A	0.5	-0.1	1.2	0.1	F	A	CAMK
CAMK2B	0.2	0.3			F	A	CAMK
CAMK2D		-0.6	1.8		F	A	CAMK
CAMK2G		0.1			F	A	CAMK
CAMK4		-0.2			L	A	CAMK
CAMKK1		0.0	1.0		F	A	Other
CAMKK2	2.5	0.4			F	A	Other
CDK2	1.5	-0.1	0.4	0.3	F	A	CMGC
CDK4		-0.6		1.5	F	A	CMGC
CDK6	0.1	0.4			F	A	CMGC
CDK8	2.7	0.1	2.2	1.9	F	A	CMGC
CDKL1	0.2	0.4	0.9	0.8	F	C	CMGC
CDKL2		-0.5		0.1	F	C	CMGC
CDKL3		0.1			F	C	CMGC
CDKL5		1.1		0.3	F	C	CMGC
CHEK2	0.8	0.1	0.3	0.5	L	T	CAMK
CSNK1E	4.4	5.1	1.0		M	I	CK1
CSNK1G1	1.7	3.2		1.0	L	I	CK1
CSNK1G2	2.5	2.7			L	I	CK1
CSNK1G3	0.3	3.3	2.9	1.2	L	I	CK1
CSNK2A1	3.3	2.6	3.5	0.2	F	I	CMGC
CSNK2A2	3.3	2.6	4.2	0.9	F	I	CMGC
CLK1	5.9	6.7	7.0	7.7	F	V	CMGC
CLK2	5.5	5.0	3.5	7.2	F	V	CMGC
CLK3	1.9	2.2	2.2	1.0	F	A	CMGC
CLK4	8.5	9.8	4.6	0.9	F	V	CMGC
DAPK3	2.8	0.9	10.6		L	I	CAMK
DCLK1	0.6	-0.4	0.9	0.2	M	G	CAMK
DDR1		0.4			T	A	TK
DMPK	1.0	1.0		0.8	M	A	AGC
CDC42BPG	0.6	-1.3			M	A	AGC
STK17A	3.4	1.7	4.3	2.2	L	V	CAMK
STK17B	4.2	1.0	3.4	6.9	L	V	CAMK
DYRK1A	3.6	6.1	2.5		F	V	CMGC

Supplementary table 1 (cont.). Thermal shift results of compounds 1-4 against various kinases.

	ΔTm (°C) for inhibitor						
Kinase	1	2	3	4	Gatekeeper	DFG-1 residue	subfamily
DYRK2	4.1	7.3	3.6	8.9	F	I	CMGC
MAPK3	0.5	-0.1	0.5		Q	C	CMGC
MAPK6	0.4	0.0			Q	G	CMGC
MAPK7		0.2			L	G	CMGC
FES	0.6	-0.1	4.1	0.3	M	S	TK
FGFR4				0.2	V	A	TK
FGR		-0.2	0.1		T	A	TK
GAK	7.3	0.2	1.4	3.3	T	C	Other
GRK5		0.8	1.4		L	S	AGC
GSK3B	4.1	1.4	3.9		L	C	CMGC
GSG2	6.5		5.6	4.0	F	I	Other
MAP4K4		-0.1		0.2	M	V	STE
IGF1R				0.2	M	G	TK
IKBKB		0.1	0.6	1.5	M	I	Other
ERN2				0.4	L	S	Other
ITK		1.1			F	S	TK
JAK1	6.4	0.1	0.9		E	S	TK
MAPK8		0.7	0.2	1.0	M	L	CMGC
MAPK9	0.8	0.4	0.2	0.9	M	L	CMGC
LATS1		0.1			M	T	AGC
LIMK1				0.5	T	A	TKL
STK10	3.3	0.8	0.1	1.9	I	A	STE
LYN	2.4	0.1	0.2	0.3	T	A	TK
MAP2K1		-0.1		0.2	M	C	STE
MAP2K2	2	0.5		0.1	M	C	STE
MAP2K6	1.1	0.0	0.3		M	C	STE
MAP3K5	2.2	0.4	0.2		M	S	STE
MERTK	1.3	0.0	0.5		L	A	TK
STK16	2.3	0.2	1.0	3.5	L	M	Other
CDC42BPA		-0.4		0.6	M	A	AGC
CDC42BPB		0.3		0.2	M	A	AGC
SRPK3	0.4	0.0	1.9	0.2	L	A	CMGC
STK4	2.6	0.7			M	A	STE
STK3	2.9		0.3	1.6	M	A	STE
STK24		-0.2		0.8	M	A	STE
STK26	0.3	-0.3		0.1	M	A	STE
MYO3A				0.2	L	V	STE
PKMYT1	0.9	-0.6		0.1	T	G	Other
STK38	1.1	0.4	0.4		M	S	AGC
STK38L	0.4	0.3		0.9	M	S	AGC
NEK1		-0.3		0.6	M	G	Other
NEK11		0.6			T	G	Other
NEK2		0.8		0.1	M	G	Other
NEK6		-1.0			L	G	Other
NEK9		0.5			L	G	Other
NLK		-0.2			T	C	CMGC
OXSR1	0.5				M	A	STE
MAPK11	0.3	0.0	0.2		T	L	CMGC
MAPK13		0.4	0.5	0.5	M	L	CMGC
PAK1		0.0			M	T	STE
PAK2		-0.6			M	T	STE
PAK4	1.5	1.7		1.5	M	S	STE
PAK7	0.4	-0.1		0.6	M	S	STE
PAK6	1.1	-0.9			M	S	STE
PBK		0.5			M	C	Other

Supplementary table 1 (cont.). Thermal shift results of compounds 1-4 against various kinases.

	ΔTm (°C) for inhibitor						
Kinase	1	2	3	4	Gatekeeper	DFG-1 residue	subfamily
CDK16	0.5	-0.4	2.6	1.2	F	A	CMGC
CDK17		0.0		0.4	F	A	CMGC
PDPK1		0.2		0.3	L	T	AGC
PHKG2	1.7	0.2		1.7	F	S	CAMK
PIM1	6.3	5.9	10.7	5.9	L	I	CAMK
PIM2	4.7	3.6	6.5	2.1	L	I	CAMK
PIM3	6.8	4.9	9.6	5.4	L	I	CAMK
PRKACA		-0.2	3.3		M	T	AGC
PRKCZ		-0.2	0.9		I	T	AGC
PRKD2		0.7	0.9		M	C	CAMK
PRKD3	0.8	2.7	2.9		M	C	CAMK
PRKG1		1.6	6.1	0.5	M	V	AGC
PRKG2		-0.1			L	V	AGC
PKN1	1.7	-0.5		0.4	M	A	AGC
PKN2	3.7	-0.5	2.9		M	A	AGC
PLK1	2.0	0.3	1.4	0.4	L	G	Other
PLK4	2.6	0.2	6.8	0.4	L	A	Other
PRKX		-0.2	5.8		M	T	AGC
SIK2		-0.2		0.4	T	A	CAMK
GRK1	1.1	0.4	2.4		M	S	AGC
RIOK2	0.3	-0.2			M	I	Atypical
RIPK2	6.0				T	A	TKL
RIPK3		-0.6			T	A	TKL
RPS6KA1	5.2	0.6	1.0	0.6	L	T	CAMK
RPS6KA3	3.4	0.7			L	T	CAMK
RPS6KA2	2.7	0.4	2.2	1.0	L	T	CAMK
RPS6KA6	4.2	0.4	0.8	2.1	L	T	CAMK
MYLK4	5.8	0.9	3.5	3.9	M	I	CAMK
SgK223				0.4	T	S	Other
SGK3		-0.1			L	T	AGC
STK40		0.0			L	T	CAMK
SIK1		1.5			T	A	CAMK
MYLK2		-1.4		0.0	M	I	CAMK
SLK	2.3	-0.5		0.5	I	A	STE
MYLK		-0.2	3.4	0.1	L	I	CAMK
SRPK1		0.0			F	A	CMGC
SRPK2		-0.2	2.4	0.4	F	A	CMGC
STK33	3.1	0.6	5.9	2.4	M	T	CAMK
STK39	0.8	0.5		0.0	M	A	STE
TEC		1.0			T	S	TK
TGFBR1	9.5			0.9	S	A	TKL
TGFBR2	13.7			2.0	T	C	TKL
TLK1		-0.1			L	T	Other
TNIK	1.2	0.1		1.2	M	V	STE
TRIB1		0.2		0.1	L	E	CAMK
TTK	2.7	0.5	0.7	1.7	M	I	Other
TYK2		0.6			T	S	TK
TYRO3	0.4	0.1			L	A	TK
VRK1	0.7	1.1	0.3		M	V	CK1
VRK2	1.5	0.2	0.3	0.0	M	A	CK1
VRK3		0.5	0.1		L	A	CK1
WNK3	0.0	-0.3			T	G	Other
STK32A		-0.2	0.8	0.2	V	T	AGC
STK32B		-0.2			V	T	AGC
STK32C		-0.9	0.7		V	T	AGC
STK25	0.5	0.7	1.1		M	A	STE
ZAK	1.4	-0.3		0.3	T	C	TKL

Supplementary table 2. The gatekeeper and DFG-1 amino acid compositions of the kinases that interact with inhibitor **1-5.** The kinases that were test are indicated with T, while those that showed inhibitor binding, either in thermal shift assays or KINOMEscan [1-3], are marked with X. The percentage of the occurrence of each amino acid are shown in Figure 2.

Kinase	1	2	3	4	5	Gatekeeper	DFG-1 residue	DFG	subfamily
AAK1	X	T	T	T		M	C	DFG	Other
ACVR1	X	T		T		T	A	DLG	TKL
ACVR1B	X	T		T	T	S	A	DLG	TKL
ACVR2A	X	T		T		T	A	DFG	TKL
ACVR2B	X	T		T		T	A	DFG	TKL
ACVRL1	X	T	T	T		T	A	DLG	TKL
BMP2K	X	T	T			M	C	DFG	Other
BMPR1A	X	T				T	A	DLG	TKL
BMPR1B	X	T		T		T	A	DLG	TKL
BMPR2	X	T	T			M	S	DFG	TKL
CLK1	X	X	X	X	X	F	V	DFG	CMGC
CLK2	X	X		X	X	F	V	DFG	CMGC
CLK4	X	X	X		X	F	V	DFG	CMGC
CSNK1E	X	X	T			M	I	DFG	CK1
CSNK1G2	T	X			T	L	I	DFG	CK1
CSNK1G3	T	X	T	T	T	L	I	DFG	CK1
CSNK2A2	T	T	X	T	T	F	I	DWG	CMGC
DAPK3	T	T	X		T	L	I	DFG	CAMK
DRAK1	T	T	X	T	T	L	V	DFG	CAMK
DRAK2	X	T	T	X		L	V	DFG	CAMK
DYRK1A	T	X	T		X	F	V	DFG	CMGC
DYRK1B		X			X	F	V	DFG	CMGC
DYRK2	X	X	T	X	X	F	I	DFG	CMGC
GAK	X	T	T	T		T	C	DFG	Other
GSK3A		T			X	L	C	DFG	CMGC
GSK3B	X	T	T		X	L	C	DFG	CMGC
Haspin	X	T	X	X	X	F	I	DYT	Other
HIPK2		T			X	F	I	DFG	CMGC
HIPK3		T			X	F	I	DFG	CMGC
IRAK4		T			X	Y	S	DFG	TKL
JAK1	X	T	T		T	E	S	DPG	TK
MAP3K19		X				M	I	DFG	STE
MYLK4	X	T	T	T		M	I	DFG	CAMK
NTRK1		T			X	F	G	DFG	TK
PIM1	X	X	X	X	X	L	I	DFG	CAMK
PIM2	X	T	X	T	X	L	I	DFG	CAMK
PIM3	X	X	X	X	T	L	I	DFG	CAMK
PLK4	T	T	X	T		L	A	DFG	Other
PRKG1		T	X	T	T	M	V	DFG	AGC
PRKX		T	X		T	M	T	DFG	AGC
RIPK2	X	T				T	A	DFG	TKL
RPS6KA1	X	T	T	T	T	L	T	DFG	CAMK
RPS6KA6	X	T	T	T	T	L	T	DFG	CAMK
STK33	T	T	X	T	T	M	T	DFG	CAMK
TGFbR1	X	T		T	T	S	A	DLG	TKL
TGFbR2	X	T		T		T	C	DFG	TKL

Supplementary table 3. ΔTm data for wild type and DFG-1-mutated CLK1 and CLK3.

		ΔTm (°C) for kinases				
Cpd	name	wild type CLK1	V324A CLK1	wild type CLK3	A319V CLK3	SMILES
1	K00135[4]	7.2	4.9	1.4	5.8	N(C(=C1)C(=CC2C(=O)C)C=CC=2)(N=C2NCC(C3)C3)C(=N1)C=C2
2	Tg003[5]	7.1	3.6	1.1	5.8	O=C(C)/C=C1SC2=CC=C(OC)C=C2N\1CC
3	KH-CARB13[6]	7.4	2.8	1.2	5.8	[Cl-].CN1C2C(C(C#N)C3(CC[NH2+]CC3)NC2=O)c4ccc(Cl)c(Cl)c14
4	K00972[7]	5.7	2.4	0.1	0.6	C(C(C=CC1C(=NC(=NC2)N)C=2)=N2)(C=1)=C(O2)C(=CC=C(C1)[Cl])C=1
5	MU1210[2]	9.3	5.94	1.5	8.27	Cn1cc(cn1)c2ccc3occ(c4cccc(c4)c5ccncc5)c3n2
6	staurosporine	13.4	11.8	4.7	8.1	CC12C(C(CC(O1)N3C4=CC=CC=C4C5=C6C(=C7C8=CC=CC=C8N2C7=C53)CNC6=O)NC)OC
7	KH-CB19[8]	15.0	7.5	8.6	11	C(=C1C=C2)(C(=C2[Cl])[Cl])[Cl])N(C(=C1C(C#N)=CN)C(OCC)=O)C
8	GW807982X[9]	9	2.5	2.0	5.5	CCOc1ccc2c(cnn2n1)c3ccnc(Nc4cc(OC)cc(c4)C(F)(F)F)n3
9	K00518 (biofocus)	6.8	3.9	1.8	8.6	N(C(=C1)C(=CC2C(=O)C)C=CC=2)(N=C2NC(C(C)C)CO)C(=N1)C=C2
10	T3-CLK[10]	18.7	13.7	13.7	17.3	CN1CCN(C(C(C)(C)C2=CC=C(C(NC3=CN(C=C(C4=CC=NC=C4)C=C5)C5=N3)=O)C=C2)=O)CC1
11	KuWal151[11]	9.4	5.1	1.7	4.7	Clc1cccc(c1)c2c[nH]c3c4C(=O)NCc4ccc23
12	FC162[12]	9.5	3.0	3.6	5.5	O=C1N(C=Nc2ccc3nc(sc3c12)c4cccnc4)C5CC5
13	ETH1610[13]	10.3	3.8	3.4	9.2	COC(=N)c1nc2ccc3ncnc(Nc4ccc(OC)cc4F)c3c2s1
14	VN412[2]	12.3	7.8	4.8	10.1	Cn1cc(cn1)c2ccc3occ(c4cccc(Oc5ccccc5)c4)c3n2
15	GW779439X[9]	15.0	9.8	5.7	10.2	CN1CCN(CC1)C2=C(C=C(C=C2)NC3=NC=CC(=N3)C4=C5C=CC=NN5N=C4)C(F)(F)F
16	KH-CARB10[6]	6.1	1.8	1.1	5.0	CN1CCC2(CC1)NC(=O)C3C(C2C#N)c4ccc(Cl)c(Cl)c4N3C
17	KH-CARB11[6]	4.8	1.0	0.6	3.8	CCN1CCC2(CC1)NC(=O)C3C(C2C#N)c4ccc(Cl)c(Cl)c4N3C
18	iodotubercidin	13.1	9.7	7.9	14.1	C1=C(C2=C(N=CN=C2N1C3C(C(C(O3)CO)O)O)N)I

Supplementary table 4. Inhibition constant (K_i) from nanoBRET assays for CLK1 wild type and V324A mutant.

	Ki (µM)		
Compound	**wild type CLK1**	**V324A CLK1**	**ratio mutant/wild type**
1	0.229 ± 0.10	0.512 ± 0.10	2.2
2	0.21 ± 0.12	0.671 ± 0.23	3.2
3	0.136 ± 0.09	2.81 ± 0.67	20.6
6	0.009 ± 0.002	0.006 ± 0.002	0.7
7	0.018 ± 0.008	0.166 ± 0.031	9.2
8	0.036 ± 0.02	3.93 ± 1.1	109.2
9	0.943 ± 0.64	2.64 ± 1.80	2.8
10	0.001 ± 0.0001	0.0023 ± 0.0001	2.4
11	0.228 ± 0.08	0.878 ± 0.046	3.9
12	1.86 ± 1.2	5.45 ± 0.79	2.9

Supplementary table 5. Data collection and refinement statistics.

Complex	**CLK1-1**	**CLK1-2**	**V324A CLK1-2**	**CLK3-2**	**A319V CLK3-2**
PDB accession code	**6YTA**	**6YTE**	**6YTD**	**6YTW**	**6YTY**
Beamline	**SLS PXIII-X06DA**	**BESSY 14.2**	**BESSY 14.2**	**BESSY 14.2**	**BESSY 14.2**
Data Collection					
Resolution[a] (Å)	67.46-1.95 (2.00-1.95)	64.70-2.30 (2.38-2.30)	71.30-2.00 (2.05-2.00)	79.52-2.00 (2.05-2.00)	53.10-1.76 (1.80-1.76)
Spacegroup	*C*2	*C*2	*I*2	*C*2	*I*2
Cell dimensions	*a* = 92.9, *b* = 64.2, *c* = 80.8 Å $\alpha = \gamma = 90.0^{\circ}$; $\beta = 123.4^{\circ}$	*a* = 91.7, *b* = 64.3, *c* = 73.1 Å $\alpha = \gamma = 90.0^{\circ}$; $\beta = 117.7^{\circ}$	*a* = 80.9, *b* = 64.7, *c* = 89.3 Å $\alpha = \gamma = 90.0^{\circ}$; $\beta = 114.7^{\circ}$	*a* = 96.1, *b* = 131.6, *c* = 83.6 Å $\alpha = \gamma = 90.0^{\circ}$; $\beta = 108.0^{\circ}$	*a* = 84.2, *b* = 45.0, *c* = 106.3 Å $\alpha = \gamma = 90.0^{\circ}$; $\beta = 111.1^{\circ}$
No. unique reflections[a]	29,054 (2,009)	16,851 (1,648)	27,570 (2,045)	66,435 (4,475)	36,800 (2,104)
Completeness[a] (%)	100.0 (100)	100.0 (100.0)	97.1 (98.0)	99.8 (100.0)	99.9 (99.8)
I/σI[a]	12.8 (3.7)	8.0 (3.7)	10.2 (2.8)	11.1 (2.8)	10.7 (3.1)
R_{merge}[a]	0.077 (0.413)	0.131 (0.383)	0.097 (0.618)	0.104 (0.653)	0.097 (0.553)
CC (1/2)	0.998 (0.909)	0.969 (0.893)	0.996 (0.851)	0.998 (0.839)	0.995 (0.793)
Redundancy[a]	6.3 (5.9)	4.8 (4.9)	6.0 (6.2)	6.3 (6.7)	5.2 (5.3)
Refinement					
No. atoms in refinement (P/L/O)[b]	2,716/23/220	2,810/ 17/ 233	2,779/ 17/ 254	5,686/ 34/ 623	2,912/ 17/ 186
B factor (P/L/O)[b] (Å^2)	24/23/26	26/ 16/ 29	31/ 20/ 39	36/ 52/ 40	18/ 13/ 20
R_{fact} (%)	19.0	18.1	17.9	18.4	18.6
R_{free} (%)	27.5	25.8	22.8	22.7	22.2
rms deviation bond[c] (Å)	0.013	0.013	0.013	0.012	0.014
rms deviation angle[c] (°)	1.9	1.6	1.6	1.7	1.7
Molprobity Ramachandran					
Favour (%)	94.05	94.05	95.25	95.00	96.56
Outlier (%)	0	0	0	0.15	0
Crystallization conditions	14% PEG 6k, 0.1M bicine 8.0	26% PEG 6k, 0.1M bicine 9.0	17% PEG 3350, 0,2M Na malonate pH 7	21% PEG 3350, 0,2M Na/K PO4, 10% Ethylene Glycol	17% PEG 3350, 0,2M NaBr, 10% Ethylene Glycol, 0.1M bis-tris propane 7.0

[a] Values in brackets show the statistics for the highest resolution shells.

[b] P/L/O indicate protein, ligand molecules presented in the active sites, and other (water and solvent molecules), respectively.

[c] rms indicates root-mean-square.

Supplementary table 5 (cont.). Data collection and refinement statistics.

Complex	**CLK1-3**	**CLK3-3**	**A319V CLK3-3**	**CLK1-8**	**CLK1-13**
PDB accession code	**6YTG**	**6YU1**	**6Z2V**	**6ZLN**	**6YTI**
Beamline	**SLS PXIII-X06DA**	**BESSY 14.1**	**BESSY 14.2**	**SLS PXI-X06SA**	**SLS PXIII-X06DA**
Data Collection					
Resolution[a] (Å)	64.30-1.95 (2.00-1.95)	79.66-1.90 (1.94-1.90)	76.54-2.60 (2.72-2.60)	45.53-1.70 (1.73-1.70)	69.57-2.40 (2.49-2.40)
Spacegroup	*C*2	*C*2	*I*2	*C*2	*C*2
Cell dimensions	*a* = 92.49, *b* = 64.1, *c* = 80.8 Å $\alpha = \gamma = 90.0^{\circ}$; $\beta = 123.4^{\circ}$	*a* = 96.3, *b* = 131.0, *c* = 83.6 Å $\alpha = \gamma = 90.0^{\circ}$; $\beta = 107.7^{\circ}$	*a* = 84.4, *b* = 45.4, *c* = 106.3 Å $\alpha = \gamma = 90.0^{\circ}$; $\beta = 111.2^{\circ}$	*a* = 91.6, *b* = 63.6, *c* = 80.1 Å $\alpha = \gamma = 90.0^{\circ}$; $\beta = 118.4^{\circ}$	*a* = 91.5, *b* = 63.9, *c* = 79.4 Å $\alpha = \gamma = 90.0^{\circ}$; $\beta = 118.8^{\circ}$
No. unique reflections[a]	29,054 (2,009)	76,479 (4,232)	11,812 (1,441)	44,218 (2,200)	15,727 (1,633)
Completeness[a] (%)	100 (100)	98.7 (91.8)	100.0 (100.0)	99.42(93.8)	99.4 (98.9)
I/σI[a]	26.8 (3.7)	10.7 (2.7)	6.4 (1.6)	11.1 (2.4)	10.1 (1.9)
R_{merge}[a]	0.071 (0.374)	0.073 (0.402)	0.246 (1.319)	0.065 (0.417)	0.153 (1.064)
CC (1/2)	0.998 (0.909)	0.997 (0.838)	0.985 (0.597)	0.997 (0.839)	0.996(0.707
Redundancy[a]	6.3 (5.9)	4.0 (3.4)	6.9 (7.2)	3.9 (3.6)	7.0 (6.9)
Refinement					
No. atoms in refinement (P/L/O)[b]	2,685/48/233	5,707/48/579	2,761/ 24/ 66	2,783/62/415	2,665/27/103
B factor (P/L/O)[b] (Å^2)	29/24/32	29/60/40	39/ 61/ 29	23/21/35	49/48/46
R_{fact} (%)	20.4	17.1	20.2	17.0	19.5
R_{free} (%)	27.0	20.7	26.4	19.9	25.6
rms deviation bond[c] (Å)	0.015	0.011	0.017	0.015	0.012
rms deviation angle[c] (°)	1.7	1.6	1.8	1.7	1.7
Molprobity Ramachandran					
Favour (%)	94.20	96.04	91.04	96.73	94.12
Outlier (%)	0	0.15	0.30	0.30	0.31
Crystallization conditions	17% PEG 6k, 0.1M bicine 8.0	18% PEG 3350, 0,2M Na/K PO4, 10% Ethylene Glycol - -	24% PEG 3350, 0,2M KSCN, 10% Ethylene Glycol, 0.1M bis-tris propane 6.5	14% PEG 6k, 0.1M bicine 9.0	29% PEG 6k, 0.1M bicine 9.3

[a] Values in brackets show the statistics for the highest resolution shells.

[b] P/L/O indicate protein, ligand molecules presented in the active sites, and other (water and solvent molecules), respectively.

[c] rms indicates root-mean-square.

Supplementary table 5 (cont.). Data collection and refinement statistics.

Complex	**ACVR1-1**	**CLK2-Ro-3306**
PDB accession code	**4DYM**	**3NR9**
Beamline	**Diamond I02**	**Diamond I24**
Data Collection		
Resolution[a] (Å)	44.73-2.42 (2.55-2.42)	55.90-2.89 (3.04-2.89)
Spacegroup	$C222_1$	$P322_1$
Cell dimensions	a = 57.8, b = 81.86, c = 140.39 Å	a = b = 97.7, c = 223.0 Å
	$\alpha = \gamma = \beta = 90.0^{\circ}$	$\alpha = \gamma = 90.0^{\circ}$; $\beta = 120^{\circ}$
No. unique reflections[a]	13,074 (1,858)	28,133 (4,062)
Completeness[a] (%)	99.8 (100.0)	99.3 (99.2)
I/σI[a]	8.1 (2.0)	8.5 (2.0)
R_{merge}[a]	0.146 (0.75)	0.173 (0.989)
CC (1/2)		
Redundancy[a]	4.5 (4.7)	4.9 (5.0)
Refinement		
No. atoms in refinement (P/L/O)[b]	2,306/23/158	8,427/72/63
B factor (P/L/O)[b] (Å^2)	43/29/40	45/39/26
R_{fact} (%)	22.0	19.4
R_{free} (%)	28.0	25.2
rms deviation bond[c] (Å)	0.012	0.013
rms deviation angle[c] (°)	1.5	1.4
Molprobity Ramachandran		
Favour (%)	96.91	95.13
Outlier (%)	0.69	0.19
Crystallization conditions	1.60M MgSO4; 0.1M MES pH 6.5	1.60M MgSO4; 0.1M MES pH 6.5

[a] Values in brackets show the statistics for the highest resolution shells.

[b] P/L/O indicate protein, ligand molecules presented in the active sites, and other (water and solvent molecules), respectively.

[c] rms indicates root-mean-square.

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