SUPPLEMENTARY FIGURES AND TABLES

			Motif 1 Motif 2		
DAGKa DAGKζ CERK YegS DgkB SK1a SK2	$372 \\ 309 \\ 129 \\ 1 \\ 13 \\ 142$: : : : : : :	-NTHPL-LVFVNPKSGGKQGQRVLWKFQYILNPRQVFNLLKDGPEI-GLRLFKDVPDSR -LMKPL-LVFVNPKSGGNQGAKIIQSFLWYLNPRQVFDLSQGGPKE-ALEMYRKVHNLR -RPKHLLVFINPFGGKGQGKRIYERKVAPLFTLASITTDIIVTEHANQAKETLYEINIDKYDG MAEFPASLIILNGKSTDNLPLREAIMLLREEGMTIHVRVTWEKGDAARYVEEARKFGVATV -MRKRARIYNPTSGKEQFK-RELPDALIKLEKAGYETSAYATEKIGDATLEAERAMHENYDV -RPCRVLVLLNPRGGKGKALQLFRSHVQPLLAEAEISFTLMLTERRNHARELVRSEELGRWDAL -RPPRLLLVNPFGGRGLAWQWCKNHVLPMISEAGLSFNLIQTERQNHARELVQGLSLSEWDGI		429 365 191 61 62 75 205
DAGKα DAGKζ CERK YegS DgkB SK1a SK2	430 366 192 62 63 76 206	: : : : : : :	Motif 3 Motif 4 LVCCGDGTVGWILETIDKANLPVLPPVAULPLGTGNDLARCLRWGGGYEGQN LACGDGTVGWILSTLDQLRLPVLPPVAILPLGTGNDLARTLNWGGGYTDEP VCVGCDGMFSEVLHGLIGRTQRSAGVDQNHPRAVLVPSSLRIGIIPAGSTDCVCYSTVGTSD IAGGGDGTINEVSTALIQCEGDDIPALGILPLGTANDFATSVGIP-EA IAAGGDGTLNEVVNGIAEKPNRPKLGVIPLGTANDFATSVGIP-EA VVMSGDGLNEVVNGIAEKPNRPKLGVIPAGSGNALAASLNHYAGYEQVTNED VVMSGDGLLHEVLNGLLDRPDWETAIQKPLCSLPQGSGNALAGAVNQHGGFEPALGLD		481 417 253 108 107 133 263
DAGKα DAGKζ CERK YegS DgkB SK1a SK2	$\begin{array}{r} 4 & 8 & 2 \\ 4 & 1 & 8 \\ 2 & 5 & 4 \\ 1 & 0 & 9 \\ 1 & 0 & 8 \\ 1 & 3 & 4 \\ 2 & 6 & 4 \end{array}$: : : : : : :	LAKILKDLEMSKVVH-MDRWSVEVIPQQTEEKSDPVPFQIINNYFSIGVD VSKILSHVEEGNVVQ-LDRWDHAEPNPEAGPEDRDEGATDRLPLDVFNNYFSLGFD AETSALHIVVGDSLA-MDVSSVHHNSTLLRYSVSLLGYGFYGDIIKDSEKKRWLGLARYDFSGLK LDKALKLAIAGDAIA-IDMAQVNKQT		530 473 317 144 142 198 328
DAGK¤ DAGKÇ CERK YegS DgkB SK1a SK2	531 474 318 145 143 199 329	: : : : : : :	ASIAHRFHI-MREKY EKFNSRMKNKLWYFEFATSESIFSTCKKLEESLTVEICGKPLDLSNL AHVTLEFHE-SREAN PEKFNSRFRNKMFYAGTAFSDFLMGSSKDLAKHIRVVCDGMDLTPKIQDL TFLSHHCYEGTVSFLPAQHTVGSP-RDRKPCRAGCFVCRQSKQQLEEEQKKALYGLEAAEDVEEW TRITTETPEKLKAALGSVSYIHG-LMRMDTLQPDRCEIRGEN-FHWQ TQVSYETPSKLKSIVGPFAYYIKG-FEMLPQMKAVDLRIEYDG-NVFQ RLAALRTYRGRLAYLPVGSKTPASPVVVQ-QGPVDAHLVPLEEPVPSHWTVVPD GLATLHTYRGRLSYLPATVEPAPTPARVGAST-CGPPDHLLPPLGTPLPPDWVTLEG		592 537 381 190 188 254 500
DAGKα DAGKζ CERK YegS DgkB SK1a SK2	593 538 382 191 189 255 501	: : : : : : : : : : : : : : : : : : : :	SLEGIAVLNIPSMHGGSNLWGDTRRPHGDIYGINQALGATAKVITDPDILKTCVPDLSDKRLEVV KPQCVVFLNIPRYCAGTMPWGHPGEHHDFEPQ-RHDDGYLEVI QVVCGKFLAINATNMSCACRRSPRGQLCPN	: : : : : : : :	657 579 423 226 224 290 536
DAGKa DAGKζ CERK YegS DgkB SK1a SK2	658 580 424 227 225 291 537	: : : : : : :	GLEGAIEMGQIYTKLKNAGRRLAKCSEITFHTTKTLPMQIDVBPWMQT GFTM-TSLAALQVGGHGERLTQCREVVLTTSKAIPVQVDGBPCKLA RKCSRFNFLRFLIRHTNQQDQFDFKRFGHICSSHPSCCCTVSNSSWNCDGBVLHSP TGDE-ILPALVSTLKSDEDNPNIIEGA-SSWFDIQAPHDITFNLDGBPLSGQ EKSNLAELGHIMTLASRGE-HTKHPKVIYEK-AKAINISSFTDLQLNVDGBYGGKLPANFLNL RAGVSRAMLLRLFLAMEKGRHMEYECPYLVVVPVVAFRLEPKDGKGVFAVDGELWVSE VSGISRAALLRLFLAMERGSHFSLGCPQLGYAAARAFRLEPLTPRGVLTVDGEQVEYG		705 624 509 276 285 348 593
DAGKα DAGKζ CERK YegS DgkB SK1a SK2	706 625 510 277 286 349 594	: : : : : : : :	PCTIKITHKNQMPMLMGPPPRSTNFFGFLS: 735ASRIRIALRNQATMVQKAKRRSAAPLHSDQQPVPEQL: 661AIEVRVHCQLVRLFARGIEENPKPDSHS: 537NFHIEILPAALRCRLPPDCPLLR: 299ERHIDVFAPNDIVNEELINNDHVDDNLIEE: 315AVQGQVHPNYFWMVSGCVEPPPSWKPQQMPPPEEPL-: 384PLOAOMHPGIGTLLTGPPGCPGREP: 618		

Supplementary Figure 1: Multiple sequence alignment used as input for SK homology modelling. A multiple sequence alignment of human DAGK α (Genbank Accession number: NP_963848), human DAGK ζ (NP_963290), human CERK (NP_073603), *Escherichia coli* YEGs (P76407), *Staphylococcus aureus* DgkB (Sequence from PDB code: 2QV7), human SK1a (Q9NYA1) and SK2 (AAH10671) was performed using ClustalW [58]. This alignment was used as input for Modeller 9v6 [59]. DgkB (2QV7) and YegS (2JGR) crystal structures were used as templates for homology modelling. A large loop section (residues 447–477, denoted by \blacktriangle) was removed from CERK and the first 142 and a large internal loop section (residues 355–370, denoted by Δ) was removed from the SK2 sequence to aid the alignment. SK1 and SK2 secondary structure is represented with symbols: open rectangles are helices, black arrows are beta sheets. Motifs 1–5 and important arginines are showed in coloured boxes and highlight residues that constitute the ATP-binding pocket. SK1 shares 58%, 46% and 85% amino acid identity in motifs 1–5 with DgkB, YEGs and SK2, respectively. SK1 Arg185 and Arg191 are only conserved in SK2 and CERK. Residues that were mutated in the study are represented in bold.



Supplementary Figure 2: Comparison of SK1 structural model with the recently solved SK1 structure. The SK1 structural model with ATP docked in ribbon (**A**) and surface view (**B**) The SK1 crystal structure co-crystallised with ADP (3VZD) in ribbon (**C**) and surface view (**D**) Each of the regions highly conserved in all SKs (see Supplementary Figure 1) that comprise the ATP-binding pocket are coloured separately; Motif 1 (²¹LNPRGG²⁶) in teal, Motif 2 (⁵⁴TERR⁵⁷) in orange, Motif 3 (⁷⁹SGDGLMHE⁸⁶) in blue, Motif 4 (¹¹⁰GSGN¹¹⁴) in purple, and Motif 5 (³⁴⁰VDGE³⁴³) in yellow. Arg185 and Arg191 are coloured in light pink in the crystal structure. The atoms in ATP are coloured according to chemicals elements; oxygen in red, and nitrogen in blue. Predicted hydrogen bonding is depicted by pink dashes.



Supplementary Figure 3: Comparison of MP-A08 docking into the SK1 crystal structure and the SK2 structural model. (A) The SK1 crystal structure (pdbcode 3VZD) with MP-A08 docked in surface view (B) The SK2 model docked with MP-A08 in surface view. Each of the motifs that comprise the ATP-binding pocket are coloured using the same scheme described in Supplementary Figure 2. SK1^{Arg185}/SK2^{Arg315} and SK1^{Arg191}/SK2^{Arg321} are coloured in light pink in the crystal structure. The atoms in MP-A08 are coloured according to chemical elements: oxygen in red, nitrogen in blue and sulphur in yellow.



Supplementary Figure 4: Tolerance of MP-A08 in NOD/SCID mice. NOD/SCID mice were treated with 50, 75 or 100 mg/kg of MP-A08 or vehicle control (70% PEG400) by i.p. injection daily for 14 days. Animals were assessed for weight (A) as well as white blood cell count (B) blood haemoglobin (C) and blood platelet count (D).

Supplementary Table 1: *In vitro* screening for the effects of MP-A08 against a panel of protein kinases. MP-A08 (25 μ M) was tested against a panel of 140 protein kinases by the International Centre for Kinase Profiling (University of Dundee). Enzymes that MP-A08 showed a >30% increase or decrease at 25 μ M were re-analysed with 250 μ M MP-A08. All data represent mean \pm range from duplicate determinations.

Kinase	Residual activity (% vehicle)			
	25 μΜ	250 μΜ		
Abelson murine leukaemia viral oncogene homolog 1 (ABL)	103 ± 12			
AMP-activated protein kinase (AMPK)	91 ± 2			
Apoptosis Signal Regulating Kinase 1 (ASK1)	129 ± 10			
Aurora A	110 ± 10			
Aurora B	146 ± 11	63 ± 14		
Breast tumour kinase (BRK)	85 ± 6			
Brain-specific kinase 1 (BRSK1)	69 ± 19	80 ± 5		
BR serine/threonine kinase 2 (BRSK2)	96 ± 0			
Bruton agammaglobulinemia Tyrosine Kinase (BTK)	120 ± 15			
Calmodulin dependent kinase 1 (CAMK1)	74 ± 7			
Calmodulin dependent kinase kinase ß (CAMKKb)	76 ± 4			
Cyclin-dependent kinase 2-cyclin A complex (CDK2-Cyclin A)	104 ± 10			
Cyclin-dependent kinase 9 (CDK9-Cyclin T1)	118 ± 9			
Checkpoint kinase-1 (CHK1)	140 ± 14	129 ± 39		
Checkpoint kinase-2 (CHK2)	92 ± 17			
Casein kinase 1y2 (CK1y2)	101 ± 9			
Casein kinase-16 (CK16)	131 ± 26	76 ± 1		
Casein kinase-2α (CK2)	111 ± 8			
CDC-like Kinase 2 (CLK2)	88 ± 7			
C-terminal Src kinase (CSK)	147 ± 36	84 ± 9		
Death-associated protein kinase 1 (DAPK1)	122 ± 39			
Discoidin domain receptor tyrosine kinase 2 (DDR2)	101 ± 0			
Dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A)	84 ± 4			
Dual specificity tyrosine phosphorylation regulated kinase 2 (DYRK2)	105 ± 11			
Dual specificity tyrosine phosphorylation regulated kinase 3 (DYRK3)	110 ± 12			
Elongation factor kinase (EF2K)	89 ± 6			
Eukaryotic translation initiation factor 2-alpha kinase 3 (EIF2AK3)	59 ± 6	82 ± 3		
Ephrin receptor A2 (EPH-A2)	102 ± 0			
Ephrin receptor A4 (EPH-A4)	113 ± 4			
Ephrin receptor B1 (EPH-B1)	112 ± 11			
Ephrin receptor B2 (EPH-B2)	106 ± 6			
Ephrin receptor B3 (EPH-B3)	107 ± 14			
Ephrin receptor B4 (EPH-B4)	$1\overline{05\pm0}$			

(Continued)

Kinase	Residual activ	Residual activity (% vehicle)			
	25 μΜ	250 μΜ			
Extracellular signal-regulated kinase 1 (ERK1)	98 ± 10				
Extracellular signal-regulated kinase 2 (ERK2)	164 ± 24	82 ± 4			
Extracellular signal-regulated kinase 7 (ERK5)	112 ± 4				
Extracellular signal-regulated kinase 8 (ERK8)	79 ± 9				
Fibroblast growth factor receptor 1 (FGF-R1)	112 ± 7				
Germinal centre kinase (GCK)	102 ± 1				
Glycogen synthase kinase-3β (GSK3β)	100 ± 0				
V-erb a erythroblastic leukaemia viral oncogene homolog 1 (HER4)	107 ± 5				
Homeodomain-interacting protein kinase 1 (HIPK1)	91 ± 10				
Homeodomain-interacting protein kinase 2 (HIPK2)	80 ± 4				
Homeodomain-interacting protein kinase 3 (HIPK3)	77 ± 2				
Insulin-like growth factor 1 receptor (IGF-1R)	60 ± 8	103 ± 3			
Inhibitory κB kinase β (IKK β)	101 ± 12				
Inhibitory κB kinase ϵ (IKK ϵ)	108 ± 22				
Insulin receptor (IR)	96 ± 0				
Interleukin-1 receptor-associated kinase 1 (IRAK1)	107 ± 1				
Interleukin-1 receptor-associated kinase 4 (IRAK4)	113 ± 25				
Insulin receptor related receptor 1 (IRR)	110 ± 3				
Janus kinase 2 (JAK2)	115 ± 17				
c-Jun N-terminal kinase 1 (JNK1)	107 ± 4				
c-Jun N-terminal kinase 2 (JNK2)	111 ± 17				
c-Jun N-terminal kinase 3 (JNK3)	89 ± 18				
Lymphocyte kinase (Lck)	70 ± 13	105 ± 9			
Ser/Thr kinase 11 (LKB1)	126 ± 10				
MAPK kinase kinase 3 (MAP4K3)	92 ± 8				
MAPK kinase kinase 5 (MAP4K5)	83 ± 1				
MAPK-activated protein kinase-2 (MAPKAP-K2)	119 ± 4				
MAPK-activated protein kinase 3 (MAPKAP-K3)	61 ± 11	70 ± 3			
Microtubule-affinity-regulating kinase 1 (MARK1)	94 ± 12				
Microtubule-affinity-regulating kinase 2 (MARK2)	94 ± 8				
Microtubule affinity regulating kinase 3 (MARK3)	73 ± 3				
Microtubule-affinity-regulating kinase 4 (MARK4)	102 ± 18				
MAPK kinase kinase 1 (MAP3K1/MEKK1)	111 ± 9				
Maternal embryonic leucine zipper kinase (MELK)	131 ± 21	76 ± 15			
Misshapen-like kinase 1 (MAP4K6)	124 ± 2	117 ± 7			

(*Continued*)

Kinase	Residual activ	Residual activity (% vehicle)			
	25 μΜ	250 μΜ			
MAPK kinase (MKK1)	81 ± 7				
MAPK kinase 2 (MKK2)	155 ± 4	98 ± 9			
MAPK kinase 6 (MKK6)	130 ± 32	92 ± 11			
Mixed lineage kinase 1 (MLK1)	90 ± 7				
Mixed lineage kinase 3 (MLK3)	97 ± 18				
MAPK interacting kinase 1 (MNK1)	119 ± 5				
MAPK interacting Kinase 2a (MNK2)	110 ± 9				
Myristoylated and palmitoylated serine/threonine protein kinase (MPSK1)	99 ± 12				
Mitogen and stress-activated protein kinase-1 (MSK1)	93 ± 10				
Mammalian sterile 20-like 2 (MST2)	106 ± 14				
Mammalian homologue Ste20-like kinase (MST3)	100 ± 6				
Mammalian sterile 20-like 4 (MST4)	119 ± 16				
NIMA related protein kinase 2a (NEK2a)	104 ± 3				
NIMA related protein kinase 6 (NEK6)	92 ± 5				
SnF1-like kinase 1 (NUAK1)	68 ± 9	91 ± 15			
Oxidative stress responsive 1 (OSR1)	125 ± 2				
Stress-activated protein kinase-2a (p38a MAPK)	100 ± 6				
Stress-activated protein kinase-2ß (p38ß MAPK)	118 ± 9				
Stress-activated protein kinase-4 (p386 MAPK)	113 ± 13				
Stress-activated protein kinase-3 (p38y MAPK)	147 ± 2	114 ± 1			
p21-activated protein kinase 2 (PAK2)	94 ± 5				
p21 activated kinase 4 (PAK4)	74 ± 42				
p21 activated kinase 5 (PAK5)	105 ± 13				
p21 activated kinase 6 (PAK6)	128 ± 13				
Platelet-derived growth factor receptor (PDGFRA)	135 ± 0	74 ± 4			
3-phosphoinositide-dependent protein kinase-1 (PDK1)	117 ± 6				
Phosphorylase B kinase (PhKy1)	137 ± 4	94 ± 0			
Provirus integration site for Moloney murine leukaemia virus (PIM1)	95 ± 9				
Provirus integration site for Moloney murine leukaemia virus (PIM2)	106 ± 7				
Provirus integration site for Moloney murine leukaemia virus (PIM3)	103 ± 2				
Cyclic AMP-dependent protein kinase (PKA)	100 ± 3				
Protein kinase B-α (PKBα)	95 ± 3				
Protein kinase B-β (PKBβ)	93 ± 8				
Protein kinase C-a (PKCa)	112 ± 16				
Protein kinase C-ζ (PKCζ)	139 ± 4	121 ± 29			

(*Continued*)

Kinase	Residual activity (% vehicle)			
	25 μΜ	250 μΜ		
Protein kinase C-γ (PKCγ)	93 ± 2			
Protein kinase D 1 (PKD1)	96 ± 10			
Polo like kinase 1 (PLK1)	107 ± 8			
p38-regulated/activated kinase (PRAK)	96 ± 2			
PKC like kinase 2 (PRK2)	127 ± 25			
Receptor interacting protein kinase 2 (RIPK2)	116 ± 5			
Rho-dependent protein kinase 2 (ROCK2)	109 ± 5			
p70 ribosomal protein S6 kinase (S6K1)	98 ± 2			
Serum and glucocorticoid-induced kinase (SGK1)	104 ± 10			
Salt inducible protein kinase 2 (SIK2)	87 ± 1			
Salt inducible protein kinase 3 (SIK3)	98 ± 9			
Smooth muscle myosin light chain kinase (SmMLCK)	76 ± 2			
Sarcoma kinase (Src)	117 ± 4			
Serine arginine protein kinase (SRPK1)	94 ± 9			
Serine/threonine kinase 33 (STK33)	151 ± 8	97 ± 4		
Spleen tyrosine kinase (SYK)	100 ± 13			
Transforming growth factor-β activated kinase 1 (TAK1)	103 ± 2			
Thousand and one amino acid protein kinase (TAO1)	119 ± 2			
TANK binding kinase 1 (TBK1)	106 ± 17			
Testis-specific kinase 1 (TESK1)	106 ± 7			
Transforming growth factor-β receptor 1 (TGFβR1)	123 ± 5			
Tunica interna endothelial cell kinase (TIE2)	78 ± 8			
Tousled-like kinase 1 (TLK1)	103 ± 9			
Neurotrophic tyrosine kinase receptor, type 1 (TrkA)	91 ± 2			
Testis-specific serine kinase 1 (TSSK1)	47 ± 3	43 ± 8		
Tau tubulin kinase 1 (TTBK1)	90 ± 1			
Tau tubulin kinase 2 (TTBK2)	103 ± 17			
Phosphotyrosine picked threonine kinase (TTK)	99±6			
Unc-51-like kinase 1 (ULK1)	114 ± 2			
Unc-51-like kinase 2 (ULK2)	96 ± 9			
Vascular endothelial growth factor receptor 1 (VEGFR)	85 ± 7			
With no lysine deficient protein kinase 1 (WNK1)	101 ± 21			
Yamaguchi sarcoma viral oncogene homolog 1 (YES1)	106 ± 15			
Zeta chain (TCR) associated protein kinase 70 (ZAP70)	122 ± 18			

Suppler	nentary	Table 2:	Primers	used for	r generation	of Sk	K1 and	SK2	mutants
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	Mutant	Forward Primer	Reverse Primer			
SK1	N22A	5'-GTGCTGGTGCTGCTGGCGCCGCGCGGC GGCAAG-3'	5'-CTTGCCGCCGCGCGCGCGCCAGCAGCA CCAGCAC-3'			
	R24A	5'-GTGCTGCTGAACCCGGCCGGCGGCAAG GGCAGG-3'	5'-CCTGCCCTTGCCGCCGGCCGGGTTCAG CAGCAC-3'			
	T54A	5'-CTTCACGCTGATGCTAGCTGAGCGGCG GAACC-3'	5'-GGTTCCGCCGCTCAGCTAGCATCAGCG TGAAG-3'			
	E55A	5'-CGCTGATGCTCACTGCGCGCCGGAACC ACGCGCGG-3'	5'-CCGCGCGTGGTTCCGGCGCGCAGTGA GCATCAGCG-3'			
	D81A	5'-GTGGTCATGTCCGGAGCCGGCCTGATG CACGAGGTG-3'	5'-CACCTCGTGCATCAGGCCGGCTCCGG ACATGACCAC-3'			
	L83A	5'-CATGTCTGGAGACGGCGCCATGCACGA GGTGGTG-3'	5'-AGCCCGTTCACCACCGCATGCATCAG CCCGTCT-3'			
	E86A	5'-AGACGGGCTGATGCATGCGGTGGTGAA CGGGCT-3'	5'-AGCCCGTTCACCACCGCATGCATCAG CCCGTCT-3'			
	S112A	5'-AGCCTCCCAGCAGGCGCCGGCAACGC GCTGGCA-3'	5'-TGCCAGCGCGTTGCCGGCGCCTGCTGG GAGGCT-3'			
	D341A	5'-GTGTGTTTGCAGTCGCCGGGGAATTGA TGGT-3'	5'-ACCATCAATTCCCCGGCGACTGCAAA CACAC-3'			
	E343A	5'-GTTTGCAGTCGACGGCGCCTTGATGGT TAGCGAG-3'	5'-CTCGCTAACCATCAAGGCGCCGTCGA CTGCAAAC-3'			
SK2	N152A	5'-GTTGCTTCTATTGGTGGCGCCCTTTGGG GGTCGG-3'	5'-CCGACCCCCAAAGGGCGCCACCAATA GAAGCAAC-3'			
	F154A	5'-CTATTGGTCAATCCCGCGGGGGGGTCGG GGCCTG-3'	5'-CAGGCCCCGACCCCCGCGGGATTGA CCAATAG-3'			
	T184A	5'-TTCAACCTCATCCAGGCAGAACGACAG AACCAC-3'	5'-GTGGTTCTGTCGTTCTGCCTGGATGAG GTTGAA-3'			
	L213A	5'-GGTCTCGGGAGACGGCGCCCTCCATGA GGTGCTG-3'	5'-CAGCACCTCATGGAGGGGGCGCCGTCTC CCGAGACC-3'			
	S242A	5'-ATCCTCCCCTGCGGCGCCGGCAACGCG CTGGCC-3'	5'-GGCCAGCGCGTTGCCGGCGCGCAGG GGAGGAT-3'			
	R315A	5'-TATCCAGAGCGAGCGATTCGCGGCCTT GGGCAGTGC-3'	5'-GCACTGCCCAAGGCCGCGAATCGCTC GCTCTGGATA-3'			
	R321A	5'-TTCAGGGCCTTGGGCTCCGCGGCCTTC ACACTGGGCAC-3'	5'-GTGCCCAGTGTGAAGGCCGCGGAGCC CAAGGCCCTGAA-3'			