

# Testing the Promiscuity of Commercial Kinase Inhibitors Against the AGC Kinase Group Using a Split-luciferase Screen

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## Contents

Table S1: List of the kinases and other constructs used in this study.

Table S2: Full table of normalized % inhibition values for 72 inhibitors against 27 protein kinases.

Figure S1: Structures of the small molecules found to exhibit activity against luciferase.

Table S3: Pairwise percent identity scores for the kinase domain of all kinases tested.

Table S4: Kinase domain  $F$  values calculated for each kinase.

Table S5: Pairwise percent identity scores for active site pseudosequences of all kinases tested.

Table S6: Active site  $F$  values calculated for each kinase.

**Table S1.** Kinase and luciferase constructs

Construct	Reference
Nfluc	Porter, J. <i>et al</i> JACS, <b>130</b> , 6488 (2008).
Cfluc	Porter, J. <i>et al</i> JACS, <b>130</b> , 6488 (2008).
Fos-Nfluc	Porter, J. <i>et al</i> JACS, <b>130</b> , 6488 (2008).
Cfluc-Jun	Porter, J. <i>et al</i> JACS, <b>130</b> , 6488 (2008).

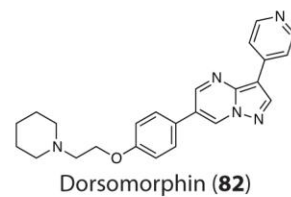
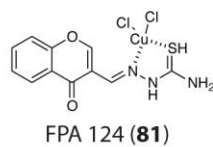
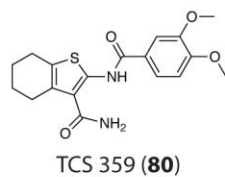
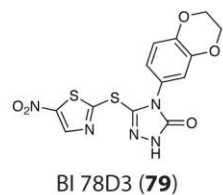
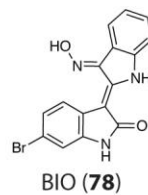
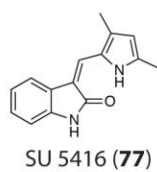
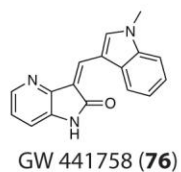
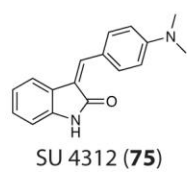
Kinase	NCBI Ref.Seq.
AKT1	NP_005154.2
AKT2	NP_001617.1
AKT3	NP_005456.1
DMPK	NP_004400.4
MSK1	NP_872198.1
MSK2	NP_003933.1
PDPK1	NP_002604.1
PKA	NP_032880.1
PKA $\beta$	NP_891993.1
PKC $\delta$	NP_997704.1
PKC $\epsilon$	NP_005391.1
PKC $\gamma$	NP_002730.1
PKC $\eta$	NP_006246.2
PKC $\theta$	NP_006248.1
PKG1	NP_006249.1
PKN1	NP_998725.1
PRKX	NP_005035.1
RSK1	NP_002944.2
RSK2	NP_004577.1
RSK3	NP_066958.2
RSK4	NP_055311.1
SGK2	NP_733794.1
SGK3	NP_037389.4
STK32B	NP_060871.1
AURKA	NP_060370.1
AURKB	NP_004208.2
AURKC	NP_003151.2

**Table S2.** Normalized % inhibition values for 72 inhibitors, each tested at 10  $\mu$ M, against 27 protein kinases.

Inhibitor Name	Cmpd #	AKT1	AKT2	AKT3	AURKA	AURKB	AURKC	DMPK	MSK1	MSK2	PDPK1	PKA	PKA $\beta$	PKC $\delta$	PKC $\epsilon$
SB 218078	3	78%	56%	45%	12%	60%	45%	37%	73%	77%	29%	65%	71%	58%	59%
PKC-412	4	55%	41%	31%	26%	44%	38%	29%	66%	70%	10%	63%	81%	52%	57%
Ro 31-8220	5	62%	28%	78%	2%	13%	10%	76%	64%	56%	2%	62%	73%	91%	71%
GF 109203X	6	5%	0%	37%	2%	0%	0%	34%	39%	30%	0%	26%	34%	52%	37%
Arctriaflavin A	7	22%	0%	0%	1%	50%	35%	30%	47%	55%	22%	27%	20%	17%	25%
PD 407824	8	0%	0%	0%	0%	34%	24%	15%	45%	62%	48%	10%	3%	12%	5%
CGP 53353	9	15%	0%	0%	0%	3%	0%	30%	0%	0%	10%	13%	8%	0%	0%
Sunitinib	10	28%	36%	26%	0%	22%	30%	0%	76%	86%	10%	2%	0%	0%	22%
H-89	11	21%	0%	15%	0%	2%	12%	0%	0%	10%	0%	67%	75%	10%	9%
Fasudil	12	0%	0%	0%	0%	0%	6%	0%	0%	0%	0%	35%	16%	1%	0%
HA-1100	13	0%	0%	0%	0%	14%	5%	0%	0%	0%	0%	19%	0%	0%	0%
ML-9	14	2%	0%	0%	0%	13%	0%	0%	0%	0%	0%	3%	0%	0%	0%
Y-27632	15	0%	0%	0%	0%	0%	5%	0%	0%	0%	0%	2%	0%	2%	1%
PP1	16	2%	0%	0%	0%	0%	0%	28%	0%	1%	0%	19%	0%	0%	8%
PP2	17	0%	0%	0%	0%	0%	6%	26%	1%	0%	0%	30%	8%	0%	0%
1-naphthyl PP1	18	18%	0%	0%	0%	12%	17%	27%	0%	0%	0%	32%	21%	0%	14%
CGP 57380	19	3%	0%	0%	0%	14%	16%	2%	0%	0%	0%	9%	1%	0%	6%
IKK 16	20	0%	0%	0%	19%	32%	15%	18%	0%	0%	9%	12%	12%	4%	40%
Purvalanol A	21	0%	0%	0%	0%	0%	0%	11%	2%	12%	0%	7%	0%	0%	2%
Purvalanol B	22	0%	0%	0%	3%	15%	7%	0%	0%	44%	0%	21%	3%	5%	2%
Aminopurvalanol A	23	0%	0%	0%	0%	0%	0%	7%	18%	39%	0%	13%	1%	6%	6%
Ki 8751	24	6%	0%	0%	41%	71%	80%	13%	43%	23%	0%	16%	5%	0%	0%
ZM 447439	25	9%	0%	0%	13%	55%	66%	9%	0%	0%	0%	7%	0%	0%	0%
SD 208	26	39%	26%	35%	0%	12%	8%	18%	24%	8%	12%	21%	19%	10%	27%
TPCA-1	27	0%	0%	0%	1%	56%	31%	0%	0%	16%	0%	4%	0%	0%	19%
PHA 665752	28	8%	0%	0%	19%	33%	39%	9%	22%	12%	7%	1%	13%	0%	7%
GW 843682X	29	12%	0%	0%	2%	39%	28%	18%	0%	14%	2%	14%	12%	4%	12%
Flavopiridol	30	1%	0%	0%	12%	0%	28%	6%	16%	23%	0%	5%	0%	15%	0%
GW 5074	31	19%	0%	0%	0%	26%	0%	11%	13%	4%	0%	9%	11%	2%	10%
LY 364947	32	0%	0%	0%	0%	25%	6%	3%	0%	0%	0%	1%	0%	0%	5%
Gefitinib	33	0%	0%	0%	0%	27%	19%	13%	0%	0%	2%	1%	0%	0%	7%
(-)-terreic acid	34	0%	0%	0%	0%	0%	0%	0%	2%	0%	2%	0%	0%	0%	0%
SB 239063	35	0%	0%	0%	4%	6%	0%	0%	0%	0%	0%	0%	1%	6%	0%
SB 203580	36	11%	0%	0%	6%	0%	0%	0%	0%	0%	3%	0%	0%	0%	0%
Ro 08-2750	37	24%	0%	0%	5%	3%	12%	0%	8%	0%	0%	11%	21%	31%	13%
Rottlerin	38	24%	0%	0%	5%	10%	8%	23%	0%	0%	0%	16%	22%	6%	14%
SB 415286	39	0%	0%	0%	6%	12%	23%	0%	0%	0%	0%	0%	5%	9%	4%
SB 216763	40	3%	0%	0%	3%	0%	0%	8%	0%	0%	0%	5%	8%	5%	3%
Roscovitine	41	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%
Olomoucine	42	5%	0%	0%	0%	4%	0%	0%	0%	0%	0%	5%	0%	3%	0%
API-2	43	0%	0%	0%	9%	3%	0%	0%	0%	0%	0%	0%	0%	4%	0%
TBB	44	0%	0%	0%	6%	2%	1%	0%	0%	0%	0%	0%	1%	0%	0%
Arctigenin	45	0%	0%	0%	11%	0%	6%	0%	0%	0%	0%	0%	1%	0%	0%
ER 27319	46	3%	0%	0%	7%	9%	6%	0%	0%	0%	0%	0%	0%	0%	0%
10-DEBC	47	0%	0%	0%	0%	8%	16%	7%	0%	0%	0%	0%	0%	0%	3%
SP 600125	48	0%	0%	0%	0%	6%	0%	0%	0%	0%	0%	0%	0%	0%	0%
ZM 336372	49	0%	0%	0%	3%	0%	8%	0%	0%	0%	0%	7%	0%	1%	0%
ZM 39923	50	0%	0%	0%	0%	0%	0%	0%	2%	0%	1%	1%	0%	0%	0%
ZM 449829	51	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
GW 583340	52	16%	0%	0%	13%	0%	21%	5%	0%	6%	0%	8%	5%	0%	14%
hexabromocyclohexane	53	0%	0%	0%	4%	0%	0%	0%	0%	0%	0%	12%	0%	1%	0%
U0126	54	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
AG 213	55	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	4%	0%	0%	0%
AG 490	56	0%	0%	0%	0%	0%	10%	0%	0%	0%	0%	3%	0%	2%	0%
LFM-A13	57	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	7%	0%	0%	0%
SL 327	58	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	2%	0%	1%	0%
IMD 0354	59	0%	0%	0%	0%	9%	0%	3%	0%	0%	0%	0%	1%	0%	11%
D 4476	60	2%	0%	0%	0%	0%	2%	7%	2%	0%	0%	0%	3%	0%	1%
SB 431542	61	1%	0%	0%	11%	0%	0%	4%	8%	0%	0%	0%	0%	0%	0%
SB 202190	62	5%	0%	0%	0%	0%	21%	0%	0%	0%	0%	11%	0%	7%	0%
SC 514	63	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	0%
PQ 401	64	0%	0%	0%	0%	14%	16%	6%	3%	0%	0%	5%	0%	0%	0%
CGK 733	65	5%	0%	0%	0%	0%	3%	0%	2%	0%	0%	0%	7%	0%	16%
ZM 323881	66	0%	0%	0%	0%	1%	0%	0%	0%	0%	2%	2%	0%	0%	6%
ZM 306416	67	0%	0%	0%	7%	9%	18%	0%	0%	2%	0%	2%	4%	0%	2%
BIBX 1382	68	0%	0%	0%	0%	8%	10%	3%	0%	0%	0%	6%	0%	0%	0%
LY 294002	69	0%	0%	0%	0%	0%	0%	0%	4%	0%	0%	0%	0%	0%	0%
NU 7026	70	0%	0%	0%	0%	7%	14%	0%	1%	0%	0%	0%	0%	0%	6%
PI 828	71	2%	0%	0%	2%	7%	14%	4%	2%	3%	0%	0%	0%	0%	4%
Compound 401	72	0%	0%	0%	0%	0%	5%	0%	0%	0%	0%	0%	0%	0%	6%
EO 1428	73	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	4%	0%	0%	12%
PD 198306	74	0%	0%	0%	0%	4%	0%	20%	0%	0%	0%	0%	0%	0%	9%

Inhibitor Name	Cmpd #	PKC $\gamma$	PKC $\eta$	PKC $\theta$	PKG1	PKN1	PRKX	RSK1	RSK2	RSK3	RSK4	SGK2	SGK3	STK32B
SB 218078	3	45%	78%	82%	28%	10%	79%	81%	77%	81%	80%	87%	85%	12%
PKC-412	4	52%	60%	55%	65%	23%	71%	56%	79%	70%	75%	22%	43%	56%
Ro 31-8220	5	47%	81%	93%	63%	36%	32%	71%	87%	89%	80%	34%	65%	63%
GF 109203X	6	11%	77%	69%	35%	5%	10%	40%	33%	59%	32%	0%	2%	29%
Arctriafavin A	7	7%	65%	45%	0%	0%	62%	51%	23%	42%	36%	70%	81%	9%
PD 407824	8	0%	44%	42%	0%	0%	22%	49%	28%	78%	27%	26%	37%	0%
CGP 53353	9	22%	1%	12%	0%	0%	8%	0%	3%	0%	0%	0%	12%	19%
Sumitinib	10	0%	39%	7%	22%	0%	25%	86%	59%	69%	69%	44%	42%	0%
H-89	11	0%	34%	40%	80%	0%	87%	0%	5%	0%	5%	20%	41%	17%
Fasudil	12	0%	17%	1%	38%	0%	77%	2%	0%	0%	0%	1%	0%	0%
HA-1100	13	0%	0%	15%	37%	6%	53%	0%	0%	0%	0%	0%	0%	0%
ML-9	14	0%	0%	0%	1%	1%	25%	0%	0%	1%	0%	0%	0%	0%
Y-27632	15	0%	18%	33%	31%	0%	51%	0%	0%	0%	0%	1%	0%	0%
PP1	16	0%	8%	0%	0%	0%	0%	27%	8%	1%	14%	0%	0%	58%
PP2	17	0%	4%	0%	0%	0%	0%	7%	6%	4%	7%	0%	0%	43%
1-naphthyl PP1	18	5%	8%	31%	0%	0%	9%	24%	30%	10%	4%	0%	0%	66%
CGP 57380	19	0%	13%	17%	0%	1%	24%	0%	0%	0%	0%	9%	23%	42%
IKK 16	20	13%	83%	38%	0%	0%	29%	4%	2%	0%	10%	69%	62%	8%
Purvalanol A	21	0%	0%	0%	0%	0%	0%	65%	41%	48%	19%	0%	0%	4%
Purvalanol B	22	0%	2%	11%	0%	0%	3%	81%	66%	79%	50%	0%	3%	9%
Aminopurvalanol A	23	0%	10%	29%	0%	0%	6%	83%	77%	79%	55%	2%	5%	20%
Ki 8751	24	3%	0%	20%	0%	10%	12%	0%	12%	32%	21%	17%	12%	0%
ZM 447439	25	6%	0%	0%	0%	2%	7%	5%	0%	2%	3%	0%	0%	1%
SD 208	26	10%	43%	51%	8%	0%	12%	18%	12%	5%	13%	22%	14%	15%
TPCA-1	27	0%	56%	18%	0%	0%	9%	44%	7%	20%	37%	0%	0%	0%
PHA 665752	28	12%	12%	0%	7%	0%	7%	32%	13%	14%	25%	2%	0%	23%
GW 843682X	29	10%	0%	23%	0%	4%	11%	10%	27%	30%	59%	4%	0%	15%
Flavopiridol	30	0%	0%	35%	0%	1%	8%	19%	8%	2%	2%	0%	0%	11%
GW 5074	31	5%	10%	0%	0%	4%	11%	23%	12%	14%	19%	10%	0%	0%
LY 364947	32	4%	0%	1%	0%	7%	5%	0%	0%	0%	0%	0%	0%	8%
Gefitinib	33	9%	0%	2%	0%	0%	7%	0%	0%	0%	0%	0%	3%	0%
(-)-terreic acid	34	0%	7%	0%	0%	0%	0%	5%	0%	0%	5%	0%	1%	36%
SB 239063	35	9%	0%	6%	0%	12%	11%	0%	0%	0%	0%	0%	0%	40%
SB 203580	36	0%	5%	0%	0%	0%	0%	0%	2%	3%	0%	0%	0%	36%
Ro 08-2750	37	9%	8%	5%	0%	0%	2%	0%	0%	8%	1%	3%	0%	18%
Rotlerin	38	26%	0%	9%	0%	0%	16%	0%	4%	9%	13%	20%	0%	23%
SB 415286	39	0%	2%	0%	0%	0%	2%	7%	0%	0%	9%	0%	0%	0%
SB 216763	40	2%	0%	3%	11%	0%	1%	21%	0%	1%	0%	0%	0%	0%
Roscovitine	41	0%	0%	0%	0%	0%	0%	20%	0%	0%	0%	0%	0%	0%
Olomoucine	42	0%	0%	0%	0%	6%	7%	0%	0%	0%	0%	0%	0%	0%
API-2	43	4%	0%	0%	0%	0%	6%	0%	0%	0%	1%	0%	0%	0%
TBB	44	0%	0%	0%	0%	0%	2%	0%	3%	4%	6%	0%	0%	4%
Arctigenin	45	0%	0%	0%	0%	0%	0%	2%	6%	0%	12%	7%	1%	15%
ER 27319	46	0%	0%	0%	0%	1%	5%	3%	0%	0%	0%	0%	3%	12%
10-DEBC	47	0%	0%	4%	2%	6%	7%	0%	0%	7%	0%	0%	0%	0%
SP 600125	48	0%	0%	0%	0%	4%	0%	0%	0%	0%	0%	2%	0%	0%
ZM 336372	49	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
ZM 39923	50	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
ZM 449829	51	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%
GW 583340	52	10%	6%	0%	0%	0%	7%	14%	0%	0%	5%	11%	6%	9%
hexabromocyclohexane	53	6%	0%	0%	0%	7%	6%	0%	0%	0%	0%	0%	0%	3%
U0126	54	0%	0%	0%	3%	0%	0%	0%	0%	0%	0%	0%	0%	0%
AG 213	55	0%	0%	0%	0%	5%	0%	0%	0%	0%	0%	0%	0%	1%
AG 490	56	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
LFM-A13	57	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
SL 327	58	0%	0%	0%	0%	6%	11%	0%	0%	0%	0%	0%	4%	0%
IMD 0354	59	6%	8%	2%	0%	0%	4%	1%	0%	0%	0%	0%	1%	11%
D 4476	60	0%	0%	1%	1%	0%	0%	0%	0%	0%	5%	3%	1%	0%
SB 431542	61	0%	0%	0%	0%	0%	1%	0%	0%	3%	0%	0%	0%	0%
SB 202190	62	0%	0%	0%	0%	0%	13%	0%	0%	0%	0%	0%	0%	11%
SC 514	63	0%	11%	0%	0%	0%	0%	0%	0%	0%	24%	0%	0%	0%
PQ 401	64	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%
CGK 733	65	15%	7%	0%	0%	0%	4%	2%	0%	2%	4%	3%	0%	8%
ZM 323881	66	1%	0%	0%	5%	0%	1%	0%	0%	0%	0%	0%	0%	16%
ZM 306416	67	2%	11%	7%	0%	1%	7%	0%	8%	0%	0%	7%	7%	14%
BIBX 1382	68	0%	0%	0%	0%	3%	6%	0%	0%	4%	0%	0%	0%	0%
LY 294002	69	0%	0%	0%	0%	0%	0%	16%	0%	0%	0%	0%	0%	0%
NU 7026	70	3%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
PI 828	71	0%	1%	0%	0%	0%	2%	0%	0%	0%	0%	0%	0%	0%
Compound 401	72	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
EO 1428	73	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
PD 198306	74	12%	0%	1%	0%	0%	6%	6%	0%	0%	0%	0%	2%	0%

**Figure S1.** Structures of 8 compounds found to exhibit activity against a Fos-Nfluc/Cfluc-Jun control and excluded from the final inhibitor analysis.





**Table S4.** Kinase domain *F* values calculated for each kinase. The averages and corresponding group numbers were used to plot Figure 7A. The percent identity cutoffs used to achieve each number of groups is also listed, with the “9” group data highlighted in green.

Number Identity of Groups	Cutoff	MSK1	MSK2	RSK1	RSK2	RSK3	RSK4	AKT1	AKT2	AKT3	SGK2	SGK3	PKCδ	PKCθ	PKCε	PKCη	PKCγ	PKN1
27	100	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
26	95	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
25	92	100%	100%	100%	88%	88%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
23	91	100%	100%	100%	78%	78%	78%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
22	90	100%	100%	75%	75%	75%	75%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
21	89	100%	100%	75%	75%	75%	75%	89%	89%	89%	100%	100%	100%	100%	100%	100%	100%	100%
20	85	100%	100%	75%	75%	75%	75%	89%	89%	89%	100%	100%	100%	100%	100%	100%	100%	100%
17	76	100%	100%	75%	75%	75%	75%	89%	89%	89%	88%	88%	64%	64%	77%	77%	100%	100%
15	74	85%	85%	75%	75%	75%	75%	89%	89%	89%	88%	88%	64%	64%	77%	77%	100%	100%
14	63	85%	85%	75%	75%	75%	75%	89%	89%	89%	88%	88%	64%	64%	58%	58%	100%	100%
13	60	85%	85%	75%	75%	75%	75%	89%	89%	89%	88%	88%	46%	46%	46%	46%	100%	100%
12	57	68%	68%	68%	68%	68%	68%	89%	89%	89%	88%	88%	46%	46%	46%	46%	100%	100%
11	56	68%	68%	68%	68%	68%	68%	89%	89%	89%	88%	88%	46%	46%	46%	46%	100%	100%
10	55	68%	68%	68%	68%	68%	68%	60%	60%	60%	60%	60%	46%	46%	46%	46%	100%	100%
9	54	68%	68%	68%	68%	68%	68%	60%	60%	60%	60%	60%	39%	39%	39%	39%	39%	39%
8	53	68%	68%	68%	68%	68%	68%	37%	37%	37%	37%	37%	37%	37%	37%	37%	37%	37%
6	49	35%	35%	35%	35%	35%	35%	35%	35%	35%	35%	35%	35%	35%	35%	35%	35%	35%
5	47	30%	30%	30%	30%	30%	30%	30%	30%	30%	30%	30%	30%	30%	30%	30%	30%	30%
4	39	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%
2	38	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%
1	36	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%

Number Identity of Groups	Cutoff	PKA	PKAβ	PRKX	PKG1	PDK1	DMPK	AURKA	AURKB	AURKC	STK32B	Average
27	100	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
26	95	78%	78%	100%	100%	100%	100%	100%	100%	100%	100%	98%
25	92	78%	78%	100%	100%	100%	100%	100%	100%	100%	100%	97%
23	91	78%	78%	100%	100%	100%	100%	100%	100%	100%	100%	96%
22	90	78%	78%	100%	100%	100%	100%	100%	100%	100%	100%	95%
21	89	78%	78%	100%	100%	100%	100%	100%	100%	100%	100%	93%
20	85	78%	78%	100%	100%	100%	100%	100%	79%	79%	100%	92%
17	76	78%	78%	100%	100%	100%	100%	100%	79%	79%	100%	87%
15	74	78%	78%	100%	100%	100%	100%	57%	57%	57%	100%	82%
14	63	78%	78%	100%	100%	100%	100%	57%	57%	57%	100%	79%
13	60	78%	78%	100%	100%	100%	100%	57%	57%	57%	100%	77%
12	57	78%	78%	100%	100%	100%	100%	57%	57%	57%	100%	74%
11	56	69%	69%	69%	100%	100%	100%	57%	57%	57%	100%	72%
10	55	69%	69%	69%	100%	100%	100%	57%	57%	57%	100%	67%
9	54	69%	69%	69%	100%	100%	100%	57%	57%	57%	100%	64%
8	53	69%	69%	69%	100%	100%	100%	57%	57%	57%	100%	59%
6	49	59%	59%	59%	100%	100%	100%	57%	57%	57%	100%	48%
5	47	30%	30%	30%	100%	100%	100%	57%	57%	57%	100%	41%
4	39	29%	29%	29%	100%	100%	29%	57%	57%	57%	100%	37%
2	38	25%	25%	25%	100%	25%	25%	57%	57%	57%	100%	28%
1	36	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%





**Table S6.** Active site *F* values calculated for each kinase. The averages and corresponding group numbers were used to plot Figure 7A. The percent identity cutoffs used to achieve each number of groups is also listed, with the “9” group data highlighted in green.

Number Identity of Groups	Cutoff	MSK1	MSK2	RSK1	RSK2	RSK3	RSK4	AKT1	AKT2	AKT3	SGK2	SGK3	PKCδ	PKCθ	PKCε	PKCη	PKCγ	PKN1
27	N/A	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
23	100	100%	100%	80%	80%	100%	80%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
19	97	100%	100%	75%	75%	75%	75%	100%	100%	100%	100%	100%	64%	64%	77%	77%	100%	100%
16	94	85%	85%	75%	75%	75%	75%	89%	89%	89%	100%	100%	64%	64%	77%	77%	100%	100%
13	91	85%	85%	75%	75%	75%	75%	89%	89%	89%	88%	88%	46%	46%	46%	46%	46%	100%
11	85	65%	65%	75%	75%	75%	75%	89%	89%	89%	65%	65%	46%	46%	46%	46%	46%	100%
9	82	55%	55%	55%	55%	55%	55%	49%	49%	49%	55%	55%	49%	49%	49%	49%	49%	100%
4	76	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%	29%
2	73	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%	25%
1	70	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%

Number Identity of Groups	Cutoff	PKA	PKAβ	PRKX	PKG1	PDK1	DMPK	AURKA	AURKB	AURKC	STK32B	Average
27	N/A	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
23	100	100%	100%	100%	100%	100%	100%	100%	79%	79%	100%	96%
19	97	78%	78%	100%	100%	100%	100%	100%	79%	79%	100%	89%
16	94	78%	78%	100%	100%	100%	100%	57%	57%	57%	100%	83%
13	91	78%	78%	100%	100%	100%	100%	57%	57%	57%	100%	77%
11	85	69%	69%	69%	100%	100%	100%	57%	57%	57%	100%	72%
9	82	69%	69%	69%	100%	100%	100%	57%	57%	57%	100%	63%
4	76	29%	29%	29%	29%	29%	100%	57%	57%	57%	100%	37%
2	73	25%	25%	25%	25%	25%	25%	25%	25%	25%	100%	28%
1	70	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%	22%