

**Supplemental Table S1. List of 404 Kinases Analyzed by AlphaScreen**

#	Kinases	Value	#	Kinases	Value	#	Kinases	Value	#	Kinases	Value
1	PIM1	24020	36	MLKL	2880	71	DCAMKL1	1674	106	CDKL2	1184
2	LYN	22342	37	CK1g2	2602	72	MAPKAPK2	1668	107	CDK5	1180
3	ULK4	21784	38	CDKL3	2584	73	JAK3	1664	108	YANK2	1172
4	Fused	18910	39	CK1g1	2560	74	PAK1	1652	109	STLK6	1162
5	TGFbR1	15634	40	SgK495	2558	75	CK1d	1608	110	AMPKa2	1148
6	MLK1	12242	41	CK2a1	2534	76	FGR	1602	111	MNK2	1144
7	smMLCK	10084	42	PRPK	2528	77	CaMKK2	1578	112	TYRO3	1140
8	PDHK2	8868	43	GSK3B	2410	78	SYK	1576	113	RIOK2	1116
9	TSSK2	8310	44	PITSLRE	2304	79	LOK	1554	114	MST3	1114
10	HCK	8250	45	ULK3	2262	80	SgK223	1548	115	PKD2	1080
11	PIM2	7340	46	MAP2K2	2254	81	TSSK4	1546	116	MASTL	1066
12	DYRK3	5116	47	CK1g3	2218	82	SGK2	1530	117	MYO3A	1058
13	CK2a2	5068	48	ULK2	2212	83	SRPK1	1526	118	CASK	1056
14	JNK1	5048	49	MAP2K6	2112	84	RSK2	1496	119	RIPK1	1054
15	NEK7	5006	50	OSR1	2108	85	MNK1	1458	120	LATS2	1044
16	FGFR2	4930	51	NRBP1	2076	86	BMPR1A	1444	121	ILK	1040
17	TLK2	4716	52	LIMK2	2036	87	PINK1	1440	122	TIF1a	1038
18	Wee1B	4606	53	CHED	2018	88	Slob	1418	123	PKCa	1032
19	RSKL2	4552	54	CK1e	2012	89	SGK3	1400	124	DAPK1	1026
20	ROCK1	4502	55	RNAseL	1934	90	MAP2K1	1386	125	HIPK2	1022
21	HUNK	4448	56	FRAP	1932	91	FAK	1364	126	JAK1	1014
22	PKG2	4206	57	FER	1862	92	MAP3K2	1358	127	CaMK2g	992
23	FES	3900	58	PKACb	1854	93	CK1a	1354	128	JNK2	972
24	SGK	3782	59	DDR1	1846	94	ADCK1	1344	129	TIE1	966
25	HIPK1	3638	60	CaMK2b	1828	95	CDK8	1302	130	PKD3	954
26	MELK	3600	61	p70S6K	1824	96	ZC1/HGK	1262	131	Erk2	932
27	MARK3	3414	62	CaMK1a	1772	97	NIM1	1252	132	NIK	924
28	RET	3380	63	RSK1	1758	98	PHKg1	1248	133	NEK11	920
29	ADCK3	3380	64	Erk3	1744	99	PDGFRb	1244	134	Trb1	916
30	VRK1	3348	65	SCYL2	1740	100	ALK2	1238	135	MAP3K4	914
31	TAK1	3304	66	RIPK2	1692	101	MYT1	1236	136	KHS2	914
32	PLK1	3230	67	BUB1	1692	102	PKCd	1218	137	PDGFRa	912
33	PKN2	3152	68	p38a	1684	103	CLIK1	1218	138	NEK6	904
34	KIS	3126	69	skMLCK	1678	104	Trad	1204	139	FLT1	902
35	AurB	3102	70	ARAF	1676	105	AKT2	1198	140	AKT1	898

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#	Kinases	Value	#	Kinases	Value	#	Kinases	Value	#	Kinases	Value
141	BRD2	886	176	TESK1	740	211	SPEG	610	246	SRPK2	532
142	MOK	874	177	CSK	740	212	CaMK2d	610	247	PKCh	530
143	ROR2	870	178	SgK288	726	213	YES	608	248	IRR	530
144	GCN2	864	179	FLT3	710	214	PCTAIRE2	606	249	IRAK2	530
145	SCYL3	858	180	CDK3	710	215	LCK	600	250	TXK	528
146	CDKL1	854	181	IKKe	706	216	EphA7	598	251	AlphaK3	526
147	SRC	840	182	CDK4	706	217	CDK2	598	252	BARK2	516
148	DYRK1B	836	183	PDHK3	704	218	GPRK4	596	253	JAK2	510
149	CaMK1g	834	184	PIK3R4	702	219	PKCb	594	254	p38b	508
150	PAK2	822	185	TRKB	700	220	BLK	594	255	FYN	508
151	PKR	812	186	CDK10	700	221	STLK5	590	256	NEK1	506
152	PDHK4	812	187	IRAK3	696	222	TIF1b	584	257	NDR1	506
153	caMLCK	812	188	CLK4	694	223	LRRK2	584	258	PHKg2	500
154	IRAK1	806	189	MAK	690	224	DAPK3	584	259	CLK2	500
155	CTK	806	190	BMPR1B	690	225	VRK3	582	260	DDR2	498
156	JNK3	802	191	MUSK	688	226	TSSK1	576	261	TNK1	496
157	RYK	798	192	KDR	688	227	CRIK	576	262	p70S6Kb	496
158	HER4/ErbB4	794	193	PKCe	678	228	ULK1	572	263	TAO3	494
159	MST2	788	194	BARK1	678	229	TESK2	572	264	MLK2	494
160	MAP3K3	788	195	SgK196	668	230	PKN1	572	265	NEK5	492
161	DYRK1A	784	196	ACTR2	664	231	PKCg	570	266	CCRK	492
162	eEF2K	778	197	PRKX	658	232	EphA3	570	267	CaMKK1	490
163	CHK1	774	198	p38g	658	233	ALK4	564	268	BMX	484
164	DAPK2	772	199	MSK2	652	234	A6r	564	269	PAK5	474
165	RIPK3	770	200	INSR	652	235	EphA1	562	270	TTBK1	470
166	DCAMKL3	766	201	MAPKAPK5	644	236	EphB4	558	271	Trb3	470
167	SgK269	762	202	TBCK	642	237	ZAP70	556	272	G11	466
168	CDKL5	762	203	HER3/ErbB3	640	238	H11	552	273	IKKb	464
169	TYK2	760	204	TTK	638	239	GPRK5	548	274	CaMK2a	464
170	NuaK2	760	205	TAO2	638	240	COT	546	275	Trb2	462
171	IKKa	758	206	STK33	628	241	NDR2	542	276	EphA4	462
172	RSK4	756	207	SNRK	622	242	DRAK1	542	277	DYRK2	460
173	DNAPK	750	208	MST1	620	243	HRI	538	278	TRKA	458
174	YSK1	748	209	TRKC	614	244	MAPKAPK3	536	279	FGFR1	458
175	RSK3	742	210	FRK	612	245	AurC	534	280	MAP3K6	456

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#	Kinases	Value	#	Kinases	Value	#	Kinases	Value	#	Kinases	Value
281	SgK496	454	312	ANKRD3	396	343	PIM3	352	374	DCAMKL2	284
282	KSR1	454	313	BCKDK	394	344	ITK	352	375	GCK	282
283	CLK1	454	314	CDK6	392	345	CDK11	350	376	PRP4	280
284	IRE2	452	315	ADCK4	390	346	CDC2	348	377	MLK4	274
285	TSSK3	446	316	TBK1	388	347	ABL	346	378	GPRK6	274
286	CYGD	438	317	CaMK1b	388	348	PAK6	344	379	DLK	272
287	PKCt	436	318	SuRTK106	386	349	MARK1	344	380	PSKH1	270
288	PCTAIRE3	432	319	PKG1	386	350	FGFR3	342	381	NEK2	268
289	AlphaK1	432	320	PFTAIRE1	386	351	BUBR1	342	382	AXL	268
290	EphB1	430	321	NEK8	386	352	p38d	340	383	CDK9	266
291	MET	428	322	ROR1	382	353	MST4	338	384	Erk4	264
292	IRE1	428	323	LMR1	382	354	CaMK4	336	385	PYK2	262
293	MARK2	424	324	GAK	382	355	AMPKa1	334	386	ALK7	260
294	CCK4	424	325	SCYL1	380	356	NEK4	332	387	PDK1	256
295	ZAK	422	326	MAP3K7	378	357	BRK	332	388	PASK	256
296	HER2/ErbB2	420	327	IGF1R	372	358	EphA6	330	389	EphA2	256
297	EphB6	420	328	AurA	370	359	TIE2	324	390	MAST2	254
298	BIKE	420	329	SSTK	368	360	PAK3	324	391	MPSK1	252
299	FASTK	418	330	RSKL1	368	361	MARK4	324	392	CDK7	252
300	BRD3	416	331	GSK3A	366	362	PCTAIRE1	320	393	SIK	250
301	LIMK1	414	332	MAP2K5	364	363	MAP2K3	320	394	DRAK2	250
302	HIPK3	414	333	FMS	364	364	EphA5	320	395	YANK3	244
303	VRK2	408	334	MAST4	362	365	PKCz	318	396	PAK4	244
304	HSER	408	335	FGFR4	362	366	TEC	312	397	KHS1	244
305	QIK	406	336	EphB3	360	367	A6	310	398	ZC2/TNIK	240
306	CaMK1d	406	337	TLK1	358	368	RIOK3	304	399	RIOK1	240
307	RON	404	338	CHK2	358	369	STLK3	298	400	MAP2K7	240
308	FLT4	402	339	ACTR2B	358	370	Erk5	296	401	PFTAIRE2	236
309	CLK3	402	340	PEK	356	371	PKCi	290	402	MYO3B	224
310	RAF1	400	341	PDHK1	356	372	PBK	284	403	MAP2K4	218
311	ICK	398	342	TGFbR2	352	373	IRAK4	284	404	KIT	216

**Footnotes.**

- Kinases are sorted in descending order (largest value first) according to AlphaScreen values.
- Data shown are mean values of duplicate experiments.

**Supplemental Table S2.**

**Serine/Threonine Kinases Analyzed by *In Vitro* Phosphorylation Assay**

Kinases	Relative Kinase Activity			Kinases	Relative Kinase Activity		
	LU	FL	D3		LU	FL	D3
PIM1	24020	1.77	4.06	CKI- $\gamma$ 1	2560	n/a*	138.69
ULK4	21784	1.02	0.79	PRPK	2528	1.57	3.04
Fused	18910	0.84	0.92	GSK3B	2410	1.53	1.58
TGF $\beta$ R1	15634	1.10	2.12	PITSLRE	2304	2.26	2.94
MLK1	12242	2.69	0.64	CKI- $\gamma$ 3	2218	0.23	52.51
smMLCK	10084	1.41	0.92	MAP2K6	2112	n/a	0.14
PDHK2	8868	0.91	2.54	NRBP1	2076	n/a	0.97
TSSK2	8310	4.24	14.94	LIMK2	2036	0.80	0.47
PIM2	7340	0.95	1.00	CHED	2018	n/a	1.50
DYRK3	5116	1.62	0.99	CKI- $\epsilon$	2012	7.95	9.48
CKII- $\alpha'$	5068	2.30	8.20	RNaseL	1934	n/a	1.15
JNK1	5048	0.64	1.95	FRAP	1932	n/a	1.30
NEK7	5006	1.69	1.52	PKAC $\beta$	1854	0.15	69.54
TLK2	4716	1.27	0.96	CaMK2 $\beta$	1828	1.53	0.97
Wee1B	4606	1.27	2.62	RSK1	1758	0.78	0.55
RSKL2	4552	n/a	1.27	Erk3	1744	n/a	1.63
ROCK1	4502	n/a*	1.26	BUB1	1692	1.02	0.95
HUNK	4448	1.67	1.00	p38 $\alpha$	1684	n/a	1.01
PKG2	4206	0.22	2.44	skMLCK	1678	n/a	1.45
SGK	3782	2.28	1.19	DCAMKL1	1674	n/a	5.55
MARK3	3414	n/a	4.48	MAPKAPK2	1668	1.39	1.63
ADCK3	3380	n/a	1.49	CaMKK2	1578	n/a	3.49
VRK1	3348	2.41	1.75	SGK2	1530	n/a	1.26
Plk1	3230	2.70	44.65	MNK1	1458	1.69	2.84
PKN2	3152	0.80	1.99	Slob	1418	n/a	0.63
KIS	3126	3.36	1.11	MAP3K2	1358	n/a	1.22
CKI- $\gamma$ 2	2602	4.31	3.15	CKI- $\alpha$	1354	4.66	1.00
CDKL3	2584	0.98	3.09				

**Footnotes.** LU: light unit in AlphaScreen; FL: full-length NS5A; D3: domain III of NS5A; n/a: not assessed due to low amounts of full-length NS5A; n/a\*: not assessed due to overlap between purified kinases and NS5A on the gel; Relative Kinase Activity: fold increase of the *in vitro* kinase activity of each kinase relative to that of DHFR.

Supplemental Table S3. NS5A Peptides Identified by LC-MS/MS

m/z	Mass	Charge State	Peptide Score	Peptide Sequence	Location in NS5A (aa)	Modification (Residue)	Knockdown	No. of Peptide
1196.5	3586.6	3	25.1	ATCTTHSNTYDVMVDANLLMEGGVAGTEPESR	241 - 273	Oxidation (M)	Ctrl	1
1182.6	2363.2	2	31.5	LARGSPPEASSSVSLSAPSLR	218 - 240	Phosphorylation (S/T)	Ctrl	1
1182.6	2363.2	2	27.6	LARGSPPEASSSVSLSAPSLR	218 - 240	Phosphorylation (S/T)	CKI-α	1
1119.6	2237.1	2	27.3	ALPAWARPDYNPPLVESWR	308 - 326		Ctrl	1
1072.6	2143.2	2	63.4	RTVGLSESTISEALQQLAIK	355 - 374		Ctrl	5
1072.6	2143.2	2	99.2	RTVGLSESTISEALQQLAIK	355 - 374		CKI-α	3
1052.5	2102.9	2	25.0	GSPPEASSSVSLSAPSLR	221 - 240	Phosphorylation (S/T)	Ctrl	1
1034.5	2067.0	2	34.0	TVGLSESTISEALQQLAIK	356 - 374	Phosphorylation (S/T)	Ctrl	1
1012.5	2022.9	2	99.4	GSPPEASSSVSLSAPSLR	221 - 240	Phosphorylation (S/T)	Ctrl	8
1012.5	2022.9	2	96.2	GSPPEASSSVSLSAPSLR	221 - 240	Phosphorylation (S/T)	CKI-α	13
1012.5	2022.9	2	72.5	GSPPEASSSVSLSAPSLR	221 - 240	Phosphorylation (S/T)	Ctrl	2
1012.5	2022.9	2	70.8	GSPPEASSSVSLSAPSLR	221 - 240	Phosphorylation (S/T)	CKI-α	2
994.5	1987.1	2	123.0	TVGLSESTISEALQQLAIK	356 - 374		Ctrl	10
994.5	1987.1	2	107.0	TVGLSESTISEALQQLAIK	356 - 374		CKI-α	10
989.5	1977.0	2	65.0	RPDYQPPTVAGCALPPPK	327 - 344	Propionamide (C)	CKI-α	8
972.5	1943.0	2	122.3	GSPPEASSSVSLSAPSLR	221 - 240		Ctrl	48
972.5	1943.0	2	132.3	GSPPEASSSVSLSAPSLR	221 - 240		CKI-α	73
968.8	2903.4	3	60.7	VAASEYAEVTQHGSYSYVTLTDDNLK	113 - 139		Ctrl	5
968.8	2903.4	3	70.2	VAASEYAEVTQHGSYSYVTLTDDNLK	113 - 139		CKI-α	12
954.0	1906.0	2	59.0	RPDYQPPTVAGCALPPPK	327 - 344		CKI-α	8
863.9	1725.8	2	81.8	SMLTDPPIHATAAAR	201 - 216	Oxidation (M)	Ctrl	20
863.9	1725.8	2	87.0	SMLTDPPIHATAAAR	201 - 216	Oxidation (M)	CKI-α	75
855.9	1709.8	2	86.6	SMLTDPPIHATAAAR	201 - 216		Ctrl	23
855.9	1709.8	2	86.4	SMLTDPPIHATAAAR	201 - 216		CKI-α	17
842.8	2525.3	3	58.5	IPCQLPSPEFFSWVDGVQIHR	140 - 160	Propionamide (C)	Ctrl	2
842.8	2525.3	3	53.9	IPCQLPSPEFFSWVDGVQIHR	140 - 160	Propionamide (C)	CKI-α	1
819.1	2454.2	3	68.6	IPCQLPSPEFFSWVDGVQIHR	140 - 160		Ctrl	2
819.1	2454.2	3	53.2	IPCQLPSPEFFSWVDGVQIHR	140 - 160		CKI-α	1
799.4	1596.8	2	60.8	GKGVWAGTGIMTTR	42 - 56		Ctrl	2
799.4	1596.8	2	72.0	GKGVWAGTGIMTTR	42 - 56		CKI-α	2
794.1	2379.3	3	59.0	RRTVGLSESTISEALQQLAIK	354 - 374	Phosphorylation (S/T)	CKI-α	1
788.7	2363.1	3	46.0	LARGSPPEASSSVSLSAPSLR	218 - 240	Phosphorylation (S/T)	Ctrl	3
788.7	2363.1	3	54.4	LARGSPPEASSSVSLSAPSLR	218 - 240	Phosphorylation (S/T)	CKI-α	2
679.0	2034.1	3	75.4	RPDYQPPTVAGCALPPPK	327 - 345		Ctrl	6
679.0	2034.1	3	83.9	RPDYQPPTVAGCALPPPK	327 - 345		CKI-α	4
675.3	2022.9	3	36.9	GSPPEASSSVSLSAPSLR	221 - 240	Phosphorylation (S/T)	Ctrl	1
675.3	2022.9	3	25.0	GSPPEASSSVSLSAPSLR	221 - 240	Phosphorylation (S/T)	Ctrl	1
666.8	1331.6	2	67.7	CPCGANISGNVR	57 - 68	Propionamide (C)	Ctrl	2
666.8	1331.6	2	64.1	CPCGANISGNVR	57 - 68	Propionamide (C)	CKI-α	2
660.0	1977.0	3	74.2	RPDYQPPTVAGCALPPPK	327 - 344	Propionamide (C)	Ctrl	8
637.4	1272.7	2	44.4	LPGLPFISQCK	31 - 41	Propionamide (C)	Ctrl	4
637.4	1272.7	2	53.0	LPGLPFISQCK	31 - 41	Propionamide (C)	CKI-α	4
636.3	1906.0	3	68.9	RPDYQPPTVAGCALPPPK	327 - 344		Ctrl	13
633.3	1264.6	2	66.6	GWAGTGIMTTR	45 - 56	Oxidation (M)	Ctrl	6
633.3	1264.6	2	64.4	GWAGTGIMTTR	45 - 56	Oxidation (M)	CKI-α	8
631.3	1260.6	2	74.9	CPCGANISGNVR	57 - 68	Propionamide (C)	Ctrl	1
631.3	1260.6	2	55.5	CPCGANISGNVR	57 - 68	Propionamide (C)	CKI-α	1
631.3	1260.6	2	72.7	CPCGANISGNVR	57 - 68	Propionamide (C)	Ctrl	2
631.3	1260.6	2	61.2	CPCGANISGNVR	57 - 68	Propionamide (C)	CKI-α	2
625.3	1248.6	2	75.5	GWAGTGIMTTR	45 - 56		Ctrl	71
625.3	1248.6	2	69.6	GWAGTGIMTTR	45 - 56		CKI-α	42
604.3	1206.7	2	27.3	FAPTPKPFRR	161 - 170		CKI-α	2
601.8	1201.7	2	45.3	LPGLPFISQCK	31 - 41		Ctrl	6
601.8	1201.7	2	51.6	LPGLPFISQCK	31 - 41		CKI-α	5
595.8	1189.5	2	79.8	CPCGANISGNVR	57 - 68		Ctrl	9
595.8	1189.5	2	74.6	CPCGANISGNVR	57 - 68		CKI-α	11
550.3	1098.6	2	39.7	KAPTTPRRR	345 - 353	Phosphorylation (S/T)	Ctrl	10
550.3	1098.6	2	33.6	KAPTTPRRR	345 - 353	Phosphorylation (S/T)	CKI-α	8
510.3	1018.6	2	40.4	KAPTTPRRR	345 - 353		Ctrl	9
510.3	1018.6	2	28.8	KAPTTPRRR	345 - 353		CKI-α	4
472.2	942.5	2	45.1	KAPTTPRRR	345 - 352	Phosphorylation (S/T)	Ctrl	6
472.2	942.5	2	30.7	KAPTTPRRR	345 - 352	Phosphorylation (S/T)	CKI-α	6
446.3	890.5	2	31.4	APTTPRRR	346 - 353		CKI-α	1
432.3	862.5	2	35.3	KAPTTPRRR	345 - 352		Ctrl	3
432.3	862.5	2	35.3	KAPTTPRRR	345 - 352		CKI-α	2
374.7	747.4	2	25.8	NWLTSK	21 - 26		CKI-α	1
353.2	704.4	2	36.0	SGSWLR	1 - 6		Ctrl	2
353.2	704.4	2	43.0	SGSWLR	1 - 6		CKI-α	2
Total								629

**SUPPLEMENTAL TABLE S3. NS5A Peptides Identified by LC-MS/MS**

**Footnotes.** *m/z*: mass-to-charge ratio; Mass: molecular weight of a peptide; Charge State: number of charges of peptide ions; Peptide Score: parameter for peptide selection, and a minimum peptide score of 25 is used in this study; No. of Peptide: number of peptides identified. Peptides #1, #2, and #3 are highlighted in green, blue, and yellow, respectively. Phosphopeptides are indicated in bold red letters. Modified amino acids are underlined.