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Supplementary Materials for

Tyrosine Kinase BMX Phosphorylates Phosphotyrosine-Primed Motif Mediating the Activation of Multiple Receptor Tyrosine Kinases

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The PDF file includes:

Supplementary Materials

Figure S1

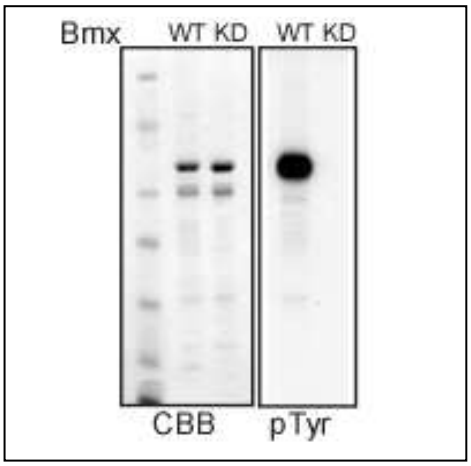


Figure S1. In vitro kinase reactions on purified 3XFlag-tagged wild-type and kinase-deficient BMX. Left panel, Coomassie Briliant Blue (CBB) staining; right panel, Western blot for phosphorylated Tyr (pTyr) after an in vitro kinase assay in LNCaP cells overexpressing wild-type (WT) or kinase-deficient (KD) Bmx.

Figure S2

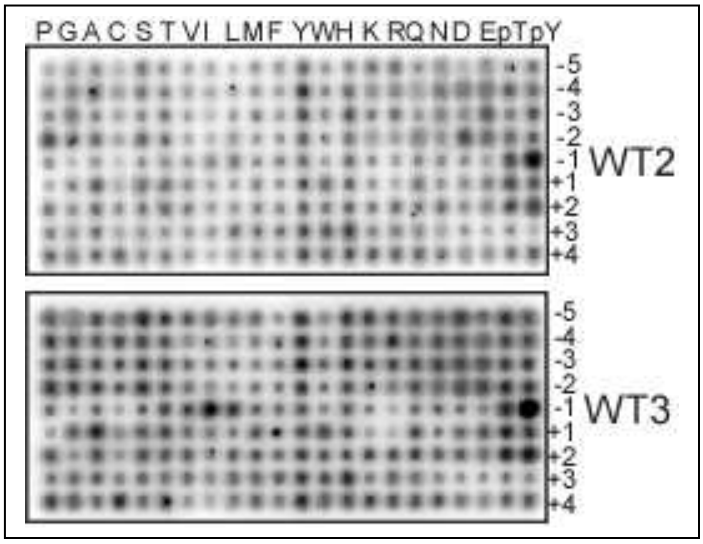
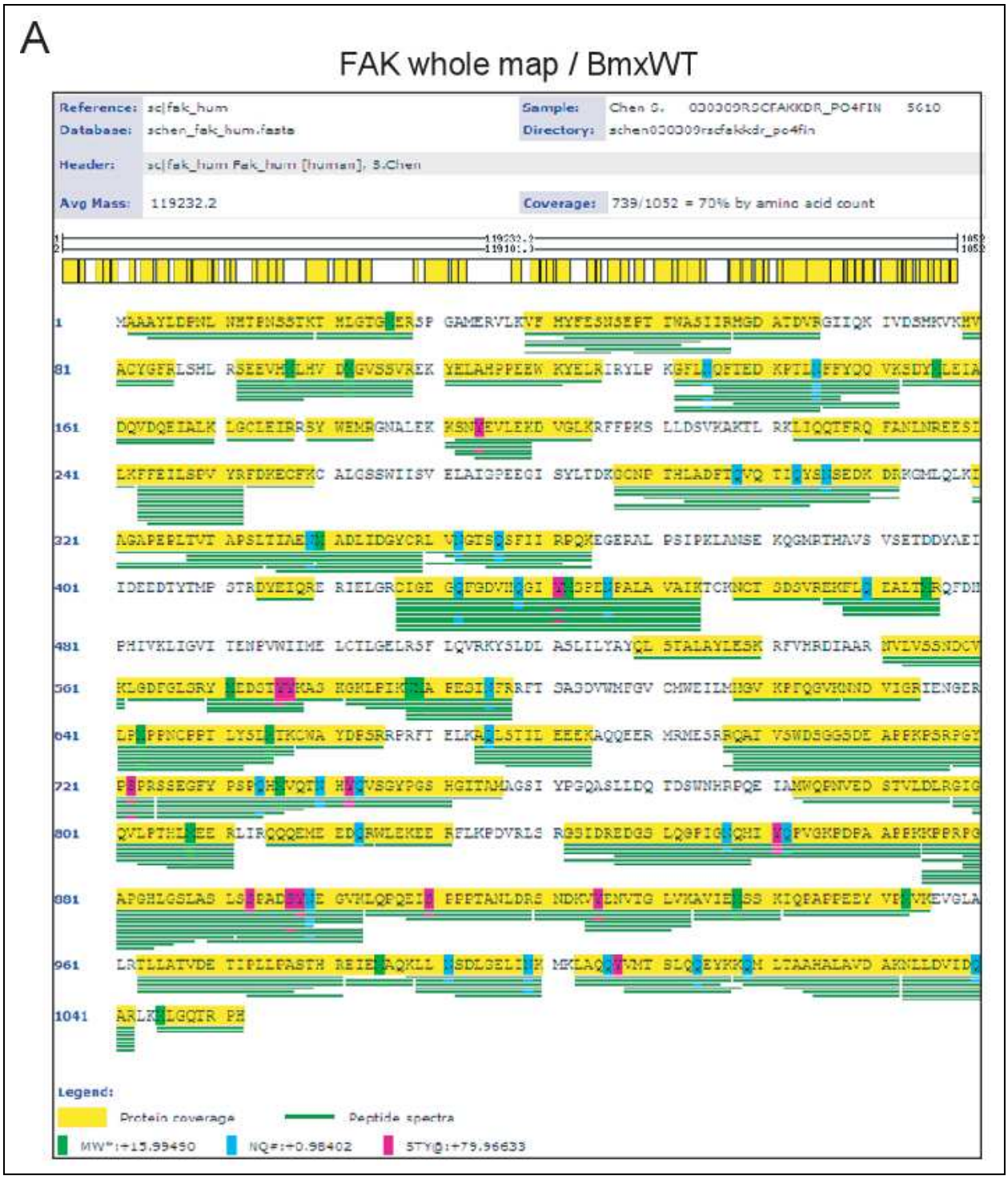


Figure S2. Peptide array images of phosphorylation by wild-type BMX. In vitro kinase assays on biotinylated peptide libraries were performed with wild-type (WT) or kinase-deficient BMX. Images are from two independent assays performed with wild-type BMX additional to that shown in Fig. 1B.

Figure S3


A
FAK whole map / BmxWT
Reference: sc|fak_hum
Database: schen_fak_hum.fasta
Sample: Chen G. 000309RSCFAKKDR_PO4FIN 5610
Directory: schen000309rscfakkdr_po4fin
Header: sc|fak_hum Fak_hum [human]; S.Chen
Avg Mass: 119232.2
Coverage: 739/1052 = 70% by amino acid count
Legend:
Protein coverage
Peptide spectra
MW*:+15.99490
NQ#:+0.98402
STY@:+79.96633

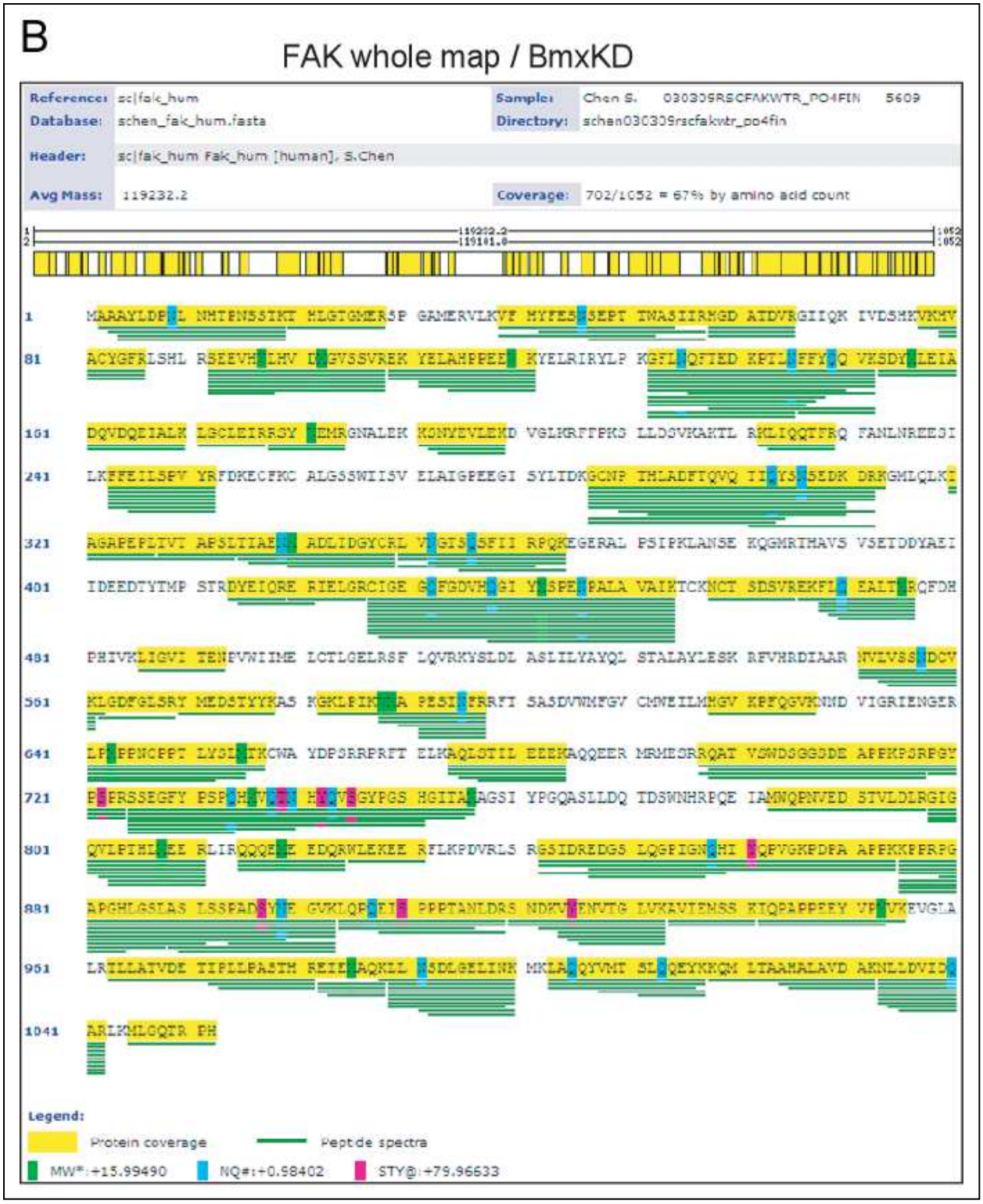


Figure 3. MS results for FAK tyrosine phosphorylation. Phosphopeptide maps for SRC-induced phosphoryated FAK after in vitro kinase reactions with wild-type BMX (BmxWT; A) or kinase-deficient BMX (BmxKD; B). Note that pTyr^{576} was not found in (B) due to limited peptide coverage.

Figure S4

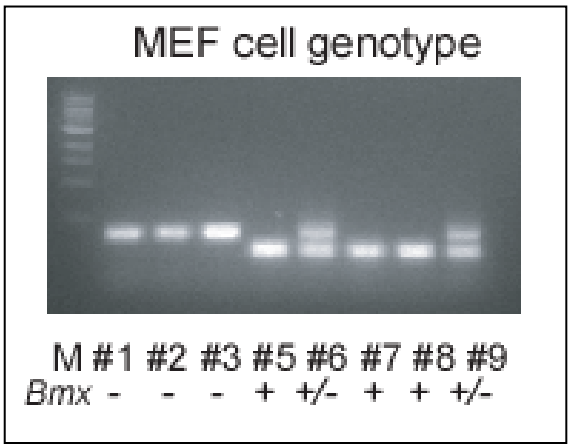


Figure S4. Genotyping of MEFs from embryos derived from *Bmx*$^{-}$ and *Bmx*$^{+/-}$ breeding. Male littermates that were wild-type for *Bmx* (*Bmx*$^{+}$) or *Bmx* negative (*Bmx*$^{-}$), and females that were heterozygous for *Bmx* (*Bmx*$^{+/-}$) were identified by genotyping and used to generate short-term MEF lines. *Bmx* is on the X chromosome, hence the single allele in male mice. MEFs #5, #7 and #8 were *Bmx*$^{+}$ (+), MEFs #1, #2 and #3 were *Bmx*$^{-}$ (-), and MEFs #6 and #9 were *Bmx*$^{+/-}$.

Figure S5

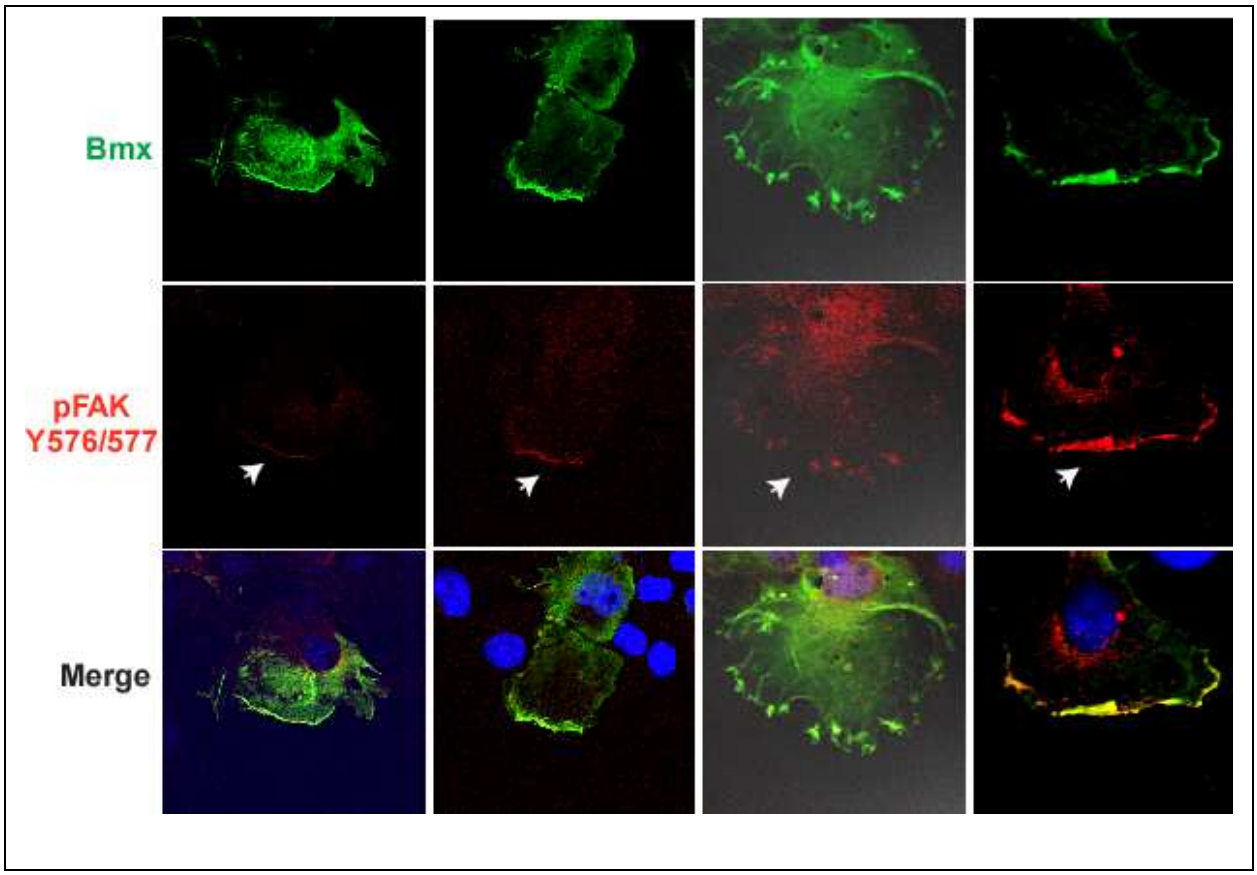


Figure S5. Immunofluorescence for BMX and pTyr$^{576/577}$ FAK in BMX-transfected COS7 cells.

COS7 cells were transiently transfected with wildtype BMX for two days. Scratch wounds were then introduced, and BMX and dual-phosphorylated FAK at Tyr$^{576/577}$ were assessed 3 hours later by immunofluorescence and confocal microscopy. Arrows in the middle panels indicate the leading edge.

Figure S6

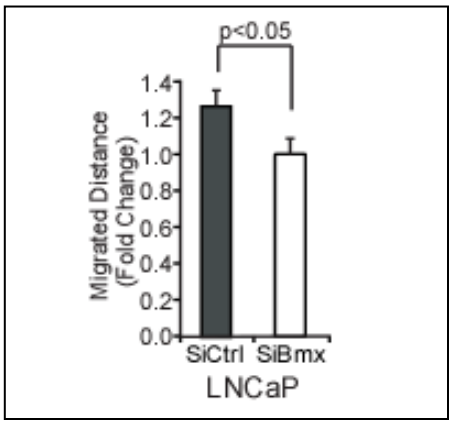


Figure S6. BMX knockdown impairs wound healing in LNCaP cells. Scratch wounds were introduced in LNCaP cells transfected with BMX-targeted or control siRNA, and light microscopy images were taken at 0 and 24 hours. The migrated distance was measured and normalized to the BMX -knockdown group. Data are means ± SEM of three independent experiments.

Table S1

A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	*	
1	1.2	1.2	1.2	1	1.2	1.2	1.2	1.2	1	1	1	1	1	1.2	1.2	1.2	1	1.2	1	0.1	
1	1.2	1.2	1.2	1	1.2	1.2	1.2	1.2	1	1	1	1	1	1.2	1.2	1.2	1	1.2	1	0.1	
0.7	1.2	1.4	1.2	0.6	1	1.6	1.1	1	1.4	0.7	0.8	1.2	0.5	0.8	0.9	1	1	1.2	0.7	0.1	-5
1	1.7	1.7	1.4	1.1	2.4	1.6	1.9	1	1	1	1.1	1.3	1	1.5	1.3	1.7	1.2	2.8	0.9	0.1	-4
0.7	0.7	0.9	1	0.6	1.3	0.8	1.5	0.4	0.5	0.6	0.5	1	1	0.9	1	1.2	0.8	1.3	0.8	0.1	-3
0.8	0.4	1.8	1.4	0.7	2.3	1.3	1.6	0.6	0.8	0.8	0.4	0.7	0.5	1.7	1.4	1.4	0.3	0.8	0.9	0.1	-2
0.3	0.5	0.8	0.5	0.2	1.3	0.9	0.5	0.8	2.7	1.6	0.4	1	0.8	0.5	0.4	1.2	0.5	7.6	1.3	0.1	-1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0	
1.4	0.3	0.6	1	0.7	1.6	1.7	1.3	0.8	1	0.8	0.6	1.7	0.8	0.4	1.7	1.8	1.9	1.5	1.4	0.1	+1
0.7	1.1	0.8	0.7	0.4	0.8	1	0.7	0.8	1	0.8	0.8	0.8	1.1	0.9	1	1.3	0.8	1.6	1.2	0.1	+2
0.5	1.3	0.6	0.2	0.6	0.3	1	1.5	1.8	1.1	1.2	0.6	1.7	1.8	0.7	0.8	1.1	1.7	2.7	1.3	0.1	+3
0.6	1.4	0.8	1	1.6	0.9	0.8	1.6	1.3	0.9	1	1	1.2	1	1	1	1.2	2	1.3	1.4	0.1	+4
1	1.2	1.2	1.2	1	1.2	1.2	1.2	1	1	1	1	1	1	1.2	1.2	1.2	1	1.2	1	1	
1	1.2	1.2	1.2	1	1.2	1.2	1.2	1	1	1	1	1	1	1.2	1.2	1.2	1	1.2	1	1	
1	1.2	1.2	1.2	1	1.2	1.2	1.2	1	1	1	1	1	1	1.2	1.2	1.2	1	1.2	1	1	

Table S1. BMX matrix for pTyr at the −1 position. The images from the positional peptide library assays were quantified. Each score represents the preference of BMX toward the fixed peptide in each well.

Table S2

Name	Site	Domain	Sequence
InsR Family			
InsR_Human	1189,1190	Kinase domain	FGMTRDIYETDYYRKGGKGL
IGF1R_Human	1165,1166	Kinase domain	FGMTRDIYETDYYRKGGKGL
FGFR Family			
FGFR1_Human	653,654	Kinase domain	RDIHHIDYYKKTTNG
FGFR2_Human	656,657	Kinase domain	RDINNIDYYKKTTNGR
FGFR3_Human	647,648	Kinase domain	RDVHNLDYYKKTTNGR
FGFR4_Human	642,643	Kinase domain	RGVHHIDYYKKTSNGR
Met Family/related			
Met_Human	1234,1235	Kinase domain	FGLARDMYDKEYYSVHNKTG
RON_Human	1238,1239	Kinase domain	RDILDREYYSVQQHRH
MER_Human	753,754	Kinase domain	FGLSKKIYSGDYYRQGRIAK
Axl_Human	695,696	Kinase domain	FGLSKKIYNGDYYRQGRIAK
Tyro3/SKY_Human	685,686	Kinase domain	FGLSRKIYSGDYYRQGCASK
Trk Family/related			
TrkA_Human	680,681	Kinase domain	FGMSRDIYSTDYYRVGGRT
TrkB_Human	706,707	Kinase domain	FGMSRDVYSTDYYRVGGHT
TrkC_Human	709,710	Kinase domain	FGMSRDVYSTDYYRLFNPS
MuSK_Human	755,756	Kinase domain	FGLSRNIYSADYYKANEND
DDR2_Human	740,741	Kinase domain	FGMSRNLYSGDYYRIQGRA
DDR1_Human	796,797	Kinase domain	FGMSRNLYAGDYYRVQGRAV
FAK, Syk Family			
FAK_Human	576,577	Kinase domain	RYMEDSTYYKASKGK
Pyk2_Human	579,580	Kinase domain	RYIEDEDYYKASVTRL
Syk_Human	525,526	Kinase domain	ALRADENYYKAQTHGK
ZAP70_Human	429,493	Kinase domain	ALGADDSYYTARSAGK
Jak Family/related			
Jak1_Human	1034,1035	Kinase domain	AIETDKEYYTVKDDR
Jak2_Human	1007,1008	Kinase domain	VLPQDKEYYKVKEPG
Jak3_Human	980,981	Kinase domain	LLPLDKDYYVVREPG
Tyk2_Human	1054,1055	Kinase domain	AVPEGHEYYRVREDG
Src Family			
Fgr_Human	208,209	SH2domain	RKLDMGGYYITTRVQ
Fyn_Human	213,214	SH2domain	RKLDNGGYYITTRAQF
Yes_Human	221,222	SH2domain	RKLDNGGYYITTRAQF
Lyn_Human	192,193	SH2domain	RSLDNGGYYISPRITF
Blk_Human	187,188	SH2domain	RCLDEGGYYISPRITF
ACK			
Ack_Human	859,860		KKVSSTHYYLLPERP

Table S2. List of proteins with dual tyrosine (pYpY) phosphorylation. Data are based on mass spectrometry data in the PhosphoSite Plus database from Cell Signaling Technologies (http://www.phosphosite.org).