Supporting Information

SI Table 1. Primers for BIK1 and BAK1 point mutation

Primers	Sequences
BIK1-S71A-upstream	AAACTTCAGACCTGATGCTGTGATCGGAGA
BIK1-S71A-downstream	GCATCAGGTCTGAAGTTTCTTGTGGCGA
BIK1-D202A-upstream	CAAAGTGATATACCGAGCCATTAAAGCCTC
BIK1-D202A-downstream	GCTCGGTATATCACTTTGACGGGATCGCTG
BIK1-S206A-upstream	ATACCGAGACATTAAAGCCGCGAACATCTTA
BIK1-S206A-downstream	CGGCTTTAATGTCTCGGTATATCACTTTGAC
BIK1-S233A-upstream	TCCAATGGGTGATTTGGCTTATGTTAGTAC
BIK1-S233A-downstream	GCCAAATCACCCATTGGACCGTCTCTAG
BIK1-S236A-upstream	GGTGATTTGAGTTATGTTGCTACAAGGGTCAT
BIK1-S236A-downstream	GCAACATAACTCAAATCACCCATTGGACCG
BIK1-S237A-upstream	TTTGAGTTATGTTAGTGCAAGGGTCATGG
BIK1-S237A-downstream	CACTAACATAACTCAAATCACCCATTGGAC
BIK1-S237D-upstream	TTTGAGTTATGTTAGTGACAGGGTCATGGG
BIK1-S237D-downstream	GTCACTAACATAACTCAAATCACCCATTGGAC
BIK1-S242A-upstream	TACAAGGGTCATGGGTGCTTATGGGTAC
BIK1-S242A-downstream	CACCCATGACCCTTGTACTAACATAACTCA
BIK1-S242D-upstream	TACAAGGGTCATGGGTGATTATGGGTAC
BIK1-S242D-downstream	TCACCCATGACCCTTGTACTAACATAACTCA
BIK1-Y168F-upstream	ATTCAGAAGAGGTGCATTTTTTAAGCCAC
BIK1-Y168F-downstream	AATGCACCTCTTCTGAATAGATGATTCTCA
BIK1-Y214F-upstream	CTTACTTGATGCGGACTTCAACGCAAAAC
BIK1-Y214F-downstream	AAGTCCGCATCAAGTAAGATGTTCGAGGCT
BIK1-Y250F-upstream	TACGCCGCGCCTGAGTTCATGTCATCA
BIK1-Y250F-downstream	AACTCAGGCGCGGCGTACCCATAAGTACC
BAK1-N421A-upstream	AGATGTGAAAGCTGCAGCTATTTTGTTGGA
BAK1-N421A-downstream	GCTGCAGCTTTCACATCTCGATGAATAATC

SI Table 2. Comparison of phosphopeptide quantitation using one-charge-state *vs* multi-charge-state method [a]

BIK1 phosphorylation site	S71	S206	S236	S236+T237	T368
BIK1-S233A (multi-charge state)	168.6%±20.3%	128.0%±9.2%	13.2%±1.0%	8.2%±0.8%	141.4%±14.2%
BIK1-S233A (single-charge state)	145.6%±1.5%	122.2%±7.4%	11.7%±0.6%	8.3%±0.3%	122.1%±2.0%
Variation between two ratios [b]	10.4%	3.3%	8.9%	0.4%	10.4%

[a]Relative quantitation of phosphopeptides was determined using either multiple peptide variants with different charge states or a single peptide variant of the highest abundance. The ratio represents relative change of phosphopeptide levels from S233A *vs* that from the wide-type. STD represents quantitation variation from three experimental replicates.

[b]Relative STD between the two ratios determined using different methods reflect quantitative consistency.

SI Table 3. Summary of BIK1 Ser/Thr autophosphorylation sites *in vitro* identified by high-resolution MS analysis

Phosphorylation site	Peptide sequence	Mass error (ppm)	PLGS score
S71	NFRPD**pS**VIGEGGFGCVFK	3.85	9.6
S360+T368	ALQQLQDNLGKP**pS**QTNPVKD**pT**K	0.16	8.4
T362+T368	ALQQLQDNLGKP**pS**QTNPVKD**pT**K	0.61	9.4
S233+S236	DGPMGDL**pS**YV**pS**TR	4.45	7.9
S236+T237	DGPMGDLSYV**pSpT**R	0.41	7.5
S206	A**pS**NILLDADYNAK	1.32	9.1
S54+T56	**pS**F**pT**FNELK	3.73	7.2
S253	VMGTYGYAAPEYMS**pS**GHLNAR	6.85	8.6
S252+S253	VMGTYGYAAPEYM**pSpS**GHLNAR	8.34	9.0
T242	VMG**pT**YGYAAPEYMSSGHLNAR	0.85	8.3
S129	EWLTEINYLGQL**pS**HPNLVK	3.23	7.9
T314	LD**pT**QYLPEEAVR	2.71	7.1
S48	TEGEIL**pS**STPVK	2.55	7.8
S49	TEGEILS**pS**TPVK	4.55	7.4

*All the phosphosites were confirmed by manual inspection of their MS/MS spectra (shown in SI Fig. S2). A plus sign indicates both sites are phosphorylated.

SI Table 4. Summary of BIK1 KM transphosphorylation sites installed by BAK1-CD identified using high-resolution MS analysis

Phosphorylation site[a]	Peptide sequence	Mass error (ppm)	PLGS score
S360/T362+T368	ALQQLQDNLGKPSQTNPVKD**pT**K	10.01	6.2
S236	DGPMGDLpSYV**pS**TR	-3.69	7.5
S236+T237	DGPMGDLSYV**pSpT**R	-2.02	7.1
S71	NFRPD**pS**VIGEGGFGCVFK	-0.22	6.7
S48	TEGEIL**pS**STPVK	-5.42	6.4
S206	A**pS**NILLDADYNAK	2.12	7.2
T242/S252	VMG**pT**YGYAAPEYMS**pS**GHLNAR	5.24	7.3

[a]A slash indicates either site can be phosphorylated, which is not ambiguously determined by MS/MS analysis. A plus sign indicates both sites can be phosphorylated.

SI Table 5. Relative quantitation of site-specific autophosphorylation in BIK1 Ser/Thr mutants *vs* wild-type (WT)

	BIK1 phospho site	S71	S206	T368	T362/S360 +T368	S233/S236	S236+T237	T242
	WT	100.0%±11.3%	100.0%±1.8%	100.0%±3.7%	100.0%±1.3%	100.0%±4.4%	100.0%±5.6%	100.0%±8.3%
	KM	5.0%±0.3%	6.3%±0.6%	4.4%±0.1%	13.5%±0.2%	1.9%±0.2%	1.4%±0.1%	5.0%±0.3%
Group 1	T237A	12.7%±1.5%	7.7%±0.9%	28.4%±1.9%	12.1%±1.3%	1.1%±0.1%	ND	24.4%±7.7%
	T237D	102.7%±12.5%	52.0%±2.9%	57.1%±6.1%	37.9%±4.5%	1.2%±0.2%	ND	55.1%±3.2%
	T242A	3.7%±0.7%	8.5%±0.7%	9.4%±0.4%	28.2%±1.8%	1.4%±0.1%	ND	ND
	T242D	1.6%±0.1%	1.0%±0.1%	1.1%±0.1%	0.8%±0.0%	2.1%±0.2%	ND	ND
Group 2	S233A	145.6%±1.5%	122.2%±7.4%	122.1%±2.0%	74.6%±2.0%	11.7%±0.6%	8.3%±0.3%	40.3%±3.0%
	S236A	103.5%±5.2%	178.7%±8.8%	109.0%±5.8%	172.2%±4.8%	2.6%±0.1%	ND	39.7%±3.6%
Group 3	S71A	ND	120.8%±10.2%	92.7%±5.7%	129.4%±10.9%	74.4%±5.7%	98.7%±12.6%	62.7%±8.1%
	S206A	85.8%±10.7%	ND	68.4%±2.0%	72.2%±10.8%	104.3%±7.8%	52.6%±9.4%	81.2%±4.4%

*Standard deviations of relative percentages represent variation from experimental triplicates. “ND” indicates no detection of the peptide signal in MS analysis.

SI Table 6. Relative quantitation of site-specific transphosphorylation of BIK1 mutants by BAK1-CD *vs* autophosphorylation on BIK1 WT

BIK1 phosphosite	S71	T368	S206	S360/T362+T368	S236	S236+T237	T242
BIK1 WT	100.0%±14.8%	100.0%±3.7%	100.0%±0.4%	100.0%±10.4%	100.0%±3.4%	100.0%±2.2%	100.0%±3.5%
BIK1 T242A + BAK1 KM	1.0%±0.1%	2.2%±0.2%	8.2%±0.2%	0.4%±0.1%	8.9%±0.9%	ND	ND
BIK1 T242A + BAK1 WT	1.2%±0.1%	2.2%±0.4%	94.0%±10.6%	5.7%±1.0%	89.2%±7.4%	4.6%±1.0%	ND
BIK1 T242D + BAK1 KM	0.7%±0.1%	1.5%±0.1%	0.9%±0.2%	1.0%±0.1%	30.0%±1.8%	1.2%±0.1%	ND
BIK1 T242D + BAK1 WT	61.7%±6.1%	1.6%±0.2%	1.2%±0.1%	1.0%±0.1%	106.3%±2.3%	3.9%±0.5%	ND

*Standard deviations of relative percentages represent variation from experimental triplicates. “ND” indicates no detection of the peptide signal in MS analysis.

SI Table 7. Relative quantitation of site-specific transphosphorylation of BAK1-CD KM installed by BIK1 Ser/Thr mutants *vs* BIK1 WT

BAK1-CD phosphosite	T446	T446-450	S290	T333+S339	T455
WT^{BIK1}	100.0%±7.6%	100.0%±18.7%	100.0%±4.7%	100.0%±18.5%	100.0%±3.7%
$S71A^{BIK1}$	85.8%±10.6%	48.4%±3.5%	91.9%±2.5%	ND	102.8%±4.5%
$S206A^{BIK1}$	94.0%±5.8%	81.5%±8.4%	115.5%±12.5%	72.2%±4.5%	118.4%±4.8%
$S233A^{BIK1}$	100.5%±14.0%	118.6%±2.2%	44.2%±1.2%	49.7%±6.3%	84.0%±4.4%
$S236A^{BIK1}$	74.2%±13.8%	52.1%±4.7%	46.4%±2.4%	18.0%±1.9%	76.7%±1.4%
$T237A^{BIK1}$	28.6%±0.5%	43.5%±2.3%	33.7%±2.3%	20.2%±4.7%	37.4%±2.7%
$T237D^{BIK1}$	50.0%±1.8%	42.1%±2.1%	125.3%±9.0%	25.4%±1.5%	110.6%±6.1%
$T242A^{BIK1}$	16.6%±0.2%	66.2%±5.4%	19.3%±1.3%	16.1%±0.2%	20.2%±0.3%
$T242D^{BIK1}$	26.1%±4.9%	72.9%±4.5%	47.6%±1.1%	8.7%±0.5%	29.7%±2.3%
$D202A^{BIK1}$	20.8%±2.9%	21.7%±3.0%	24.2%±3.3%	35.9%±4.1%	15.5%±2.3%

*Standard deviations of relative percentages represent variation from experimental triplicates. “ND” indicates no detection of the peptide signal in MS analysis. D202A is the kinase dead mutant of BIK1.

SI Table 8A. Summary of FLS2-CD transphosphorylation sites installed by BIK1 identified using high-resolution MS analysis

Phosphorylation site	Peptide sequence	Mass error (ppm)	PLGS score
T941	ILGFAWESGK**pT**K	2.28	7.4
S1084	QRPTSLNDED**pS**QDMTLR	-0.07	7.8
S938	ILGFAWE**pS**GKpTK	-2.58	7.4
S1115	VLDMELGD**pS**IVSLK	2.89	7.5
S906	EF**pS**AESDKWFYTEAK	4.52	6.4

*All the phosphosites were confirmed by manual inspection of their MS/MS spectra (shown in SI Fig. S3).

SI Table 8B. Summary of FLS2-CD transphosphorylation sites installed by BAK1 identified by high-resolution MS analysis (cited from reference 14 in the text)

Phosphorylation site	Peptide sequence	Mass error for peptide identification
S869	ELEQATD**pS**FNSANIIGSSSLSTVYK	2.6 ppm
S906	EF**pS**AESDKWFYTEAK	4.3 ppm
S961	ALVLPFMENGNLEDTIHG**pS**AAPIGSLLEK	1.2 ppm
S1115	VLDMELGD**pS**IVSLK	3.8 ppm

SI Table 9. BIK1 *in vitro* phosphotyrosine sites identified by high-resolution MS analysis

Phosphorylation site	Peptide sequence	Mass error (ppm)	PLGS score
Y168	GA**pY**FKPLPWFLR	0.62	8.4
Y214	ASNILLDAD**pY**NAK	1.72	7.9
Y250	VMGTYGYAAPE**pY**MSSGHLNAR	3.56	7.7

*Y168 and Y214 phosphorylation were verified by manual inspection of their MS/MS spectra (shown in SI Fig. S4). Y250 phosphorylation is automatically assigned yet its MS/MS spectrum is not conclusive

SI Table 10. Relative quantitation of site-specific autophosphorylation in BIK1 Tyr mutants *vs* WT

BIK1 phospho site	S71	S206	T368	T362/S360+T368	S236/S233	S236+T237	T242
Y168F	62.9%±5.6%	49.2%±5.5%	68.0%±7.1%	68.9%±4.1%	100.5%±8.0%	71.1%±10.5%	102.8%±13.8%
Y250F	22.5%±4.2%	9.3%±1.6%	27.3%±0.4%	14.4%±1.9%	51.7%±1.7%	17.5%±1.1%	97.5%±2.9%
Y214F	75.4%±6.6%	5.5%±0.8%	79.3%±3.3%	71.6%±2.6%	11.3%±1.1%	54.0%±3.6%	ND

*Standard deviations of relative percentages represent variation from experimental triplicates. “ND” indicates no detection of the peptide signal in MS analysis.

SI Table 11. Relative quantitation of site-specific transphosphorylation in BAK1-CD KM installed by BIK1 Tyr mutants *vs* WT

BAK1 phosphosite	T446	T446-450	S290	T333+S339	T455
WT^{BIK1}	100.0%±4.6%	100.0%±15.9%	100.0%±1.9%	100.0%±16.5%	100.0%±14.9%
$Y168F^{BIK1}$	97.8%±15.2%	116.9%±22.4%	88.2%±2.6%	65.9%±6.3%	100.0%±8.3%
$Y214F^{BIK1}$	47.2%±6.9%	54.2%±7.2%	73.0%±12.3%	44.6%±3.9%	66.6%±3.7%
$Y250F^{BIK1}$	20.1±1.2%	49.7%±3.8%	50.7%±3.3%	29.8%±1.9%	94.4%±10.6%
$D202A^{BIK1}$	20.8%±2.9%	21.7%±3.0%	24.2%±3.3%	43.7%±4.1%	15.5%±2.3%

*Standard deviations of relative percentages represent variation from experimental triplicates. D202A is the kinase dead mutant of BIK1.