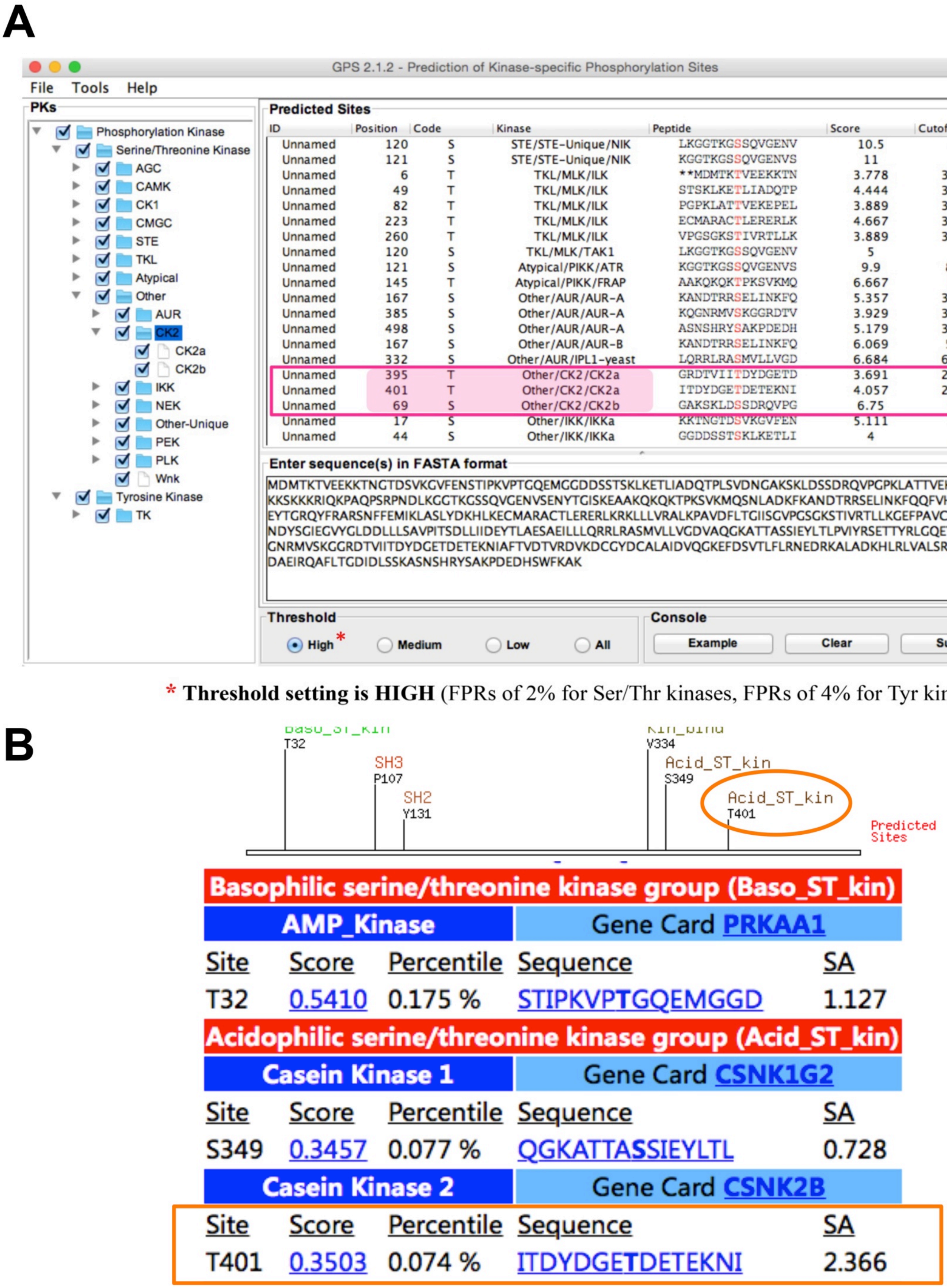

Predicted Sites

ID	Position	Code	Kinase	Peptide	Score	Cutoff
Unnamed	120	S	STE/STE-Unique/NIK	LKGGTKGSSQVGENV	10.5	8
Unnamed	121	S	STE/STE-Unique/NIK	KGGTKGSSQVGENVS	11	8
Unnamed	6	T	TKL/MLK/ILK	**MDMTKTVEEKKTN	3.778	3.556
Unnamed	49	T	TKL/MLK/ILK	STSKLKETLIADQTP	4.444	3.556
Unnamed	82	T	TKL/MLK/ILK	PGPKLATTVEKEPEL	3.889	3.556
Unnamed	223	T	TKL/MLK/ILK	ECMARACTLERERLK	4.667	3.556
Unnamed	260	T	TKL/MLK/ILK	VPGSGKSTIVRTLLK	3.889	3.556
Unnamed	120	S	TKL/MLK/TAK1	LKGGTKGSSQVGENV	5	5
Unnamed	121	S	Atypical/PIKK/ATR	KGGTKGSSQVGENVS	9.9	8.11
Unnamed	145	T	Atypical/PIKK/FRAP	AAKQKQKTPKSVKMQ	6.667	5.8
Unnamed	167	S	Other/AUR/AUR-A	KANDTRRSELINKFQ	5.357	3.536
Unnamed	385	S	Other/AUR/AUR-A	KQGNRMVSKGGRDTV	3.929	3.536
Unnamed	498	S	Other/AUR/AUR-A	ASNSHRYSAKPDEDH	5.179	3.536
Unnamed	167	S	Other/AUR/AUR-B	KANDTRRSELINKFQ	6.069	5.69
Unnamed	332	S	Other/AUR/IPL1-yeast	LQRRLRASMVLLVGD	6.684	6.211
Unnamed	395	T	Other/CK2/CK2a	GRDTVIITDYDGETD	3.691	2.927
Unnamed	401	T	Other/CK2/CK2a	ITDYDGETDETEKNI	4.057	2.927
Unnamed	69	S	Other/CK2/CK2b	GAKSKLDSSDRQVPG	6.75	6.5
Unnamed	17	S	Other/IKK/IKKa	KKTNGTDSVKGVFEN	5.111	4
Unnamed	44	S	Other/IKK/IKKa	GGDDSSTSKLKETLI	4	4

Enter sequence(s) in FASTA format

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MDMTKTVEEKKTNGTDSVKGVFENSTIPKVPTGQEMGGDDSSTSKLKETLIADQTPLSVDNGAKSKLDSSDRQVPGPKLATTVEKEPELKPNV
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NDYSGIEGVYGLDDLLLSAVPITSDLLIIDEYTLAESAEILLLQRRLRASMVLLVGDVAQGKATTASSIEYLTLPVIYRSETTYRLGQETASLCSKQ
GNRMVSKGGRDTVIITDYDGETDETEKNIAFTVDTVRDVKDCGYDCALAIDVQGKEFDSVTLFLRNEDRKALADKHLRLVALSRHKSKLIIRA
DAEIRQAFLTGDIDLSSKASNSHRYSAKPDEDHSWFKAK
```

Threshold ● High* ○ Medium ○ Low ○ All

Console

* **Threshold setting is HIGH** (FPRs of 2% for Ser/Thr kinases, FPRs of 4% for Tyr kinases)

Basophilic serine/threonine kinase group (Baso_ST_kin)

AMP_Kinase — Gene Card PRKAA1

Site	Score	Percentile	Sequence	SA
T32	0.5410	0.175 %	STIPKVPTGQEMGGD	1.127

Acidophilic serine/threonine kinase group (Acid_ST_kin)

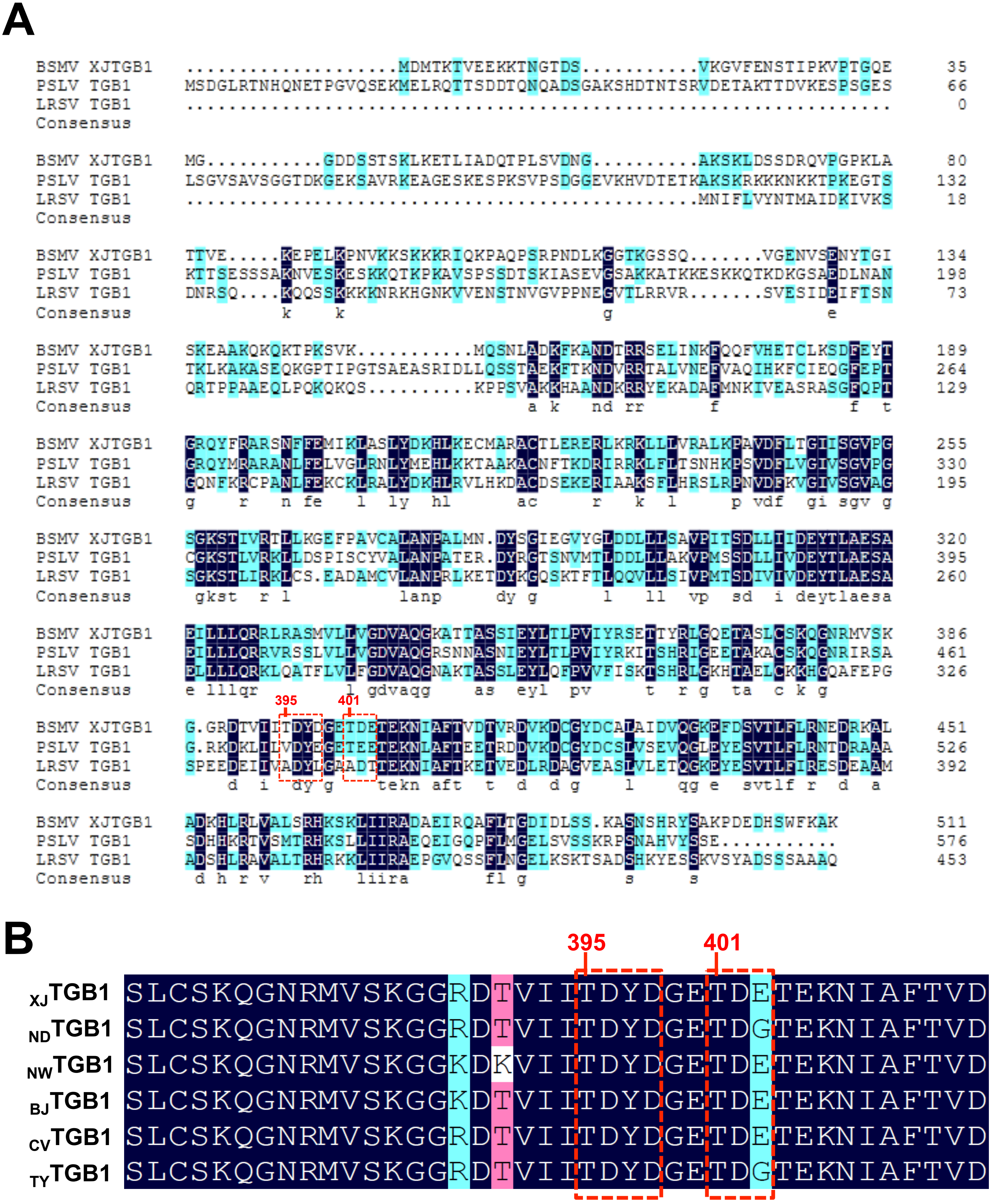
Casein Kinase 1 — Gene Card CSNK1G2

Site	Score	Percentile	Sequence	SA
S349	0.3457	0.077 %	QGKATTASSIEYLTL	0.728

Casein Kinase 2 — Gene Card CSNK2B

Site	Score	Percentile	Sequence	SA
T401	0.3503	0.074 %	ITDYDGETDETEKNI	2.366

Fig. S1 Phosphorylation predictions of the $_{XJ}$TGB1 protein by the GPS 2.1 program (A) and the Scansite Motif Scanner online server (B).



```
BSMV XJTGB1   ....................MDMTKTVEEKKTNGTDS...........VKGVFENSTIPKVPTGQE     35
PSLV TGB1     MSDGLRTNHQNETPGVQSEKMELRQTTSDDTQNQADSGAKSHDTNTSRVDETAKTTDVKESPSGES     66
LRSV TGB1     ..................................................................      0
Consensus

BSMV XJTGB1   MG...........GDDSSTSKLKETLIADQTPLSVDNG..........AKSKLDSSDRQVPGPKLA     80
PSLV TGB1     LSGVSAVSGGTDKGEKSAVRKEAGESKESPKSVPSDGGEVKHVDTETKAKSKRKKKNKKTPKEGTS    132
LRSV TGB1     ................................................MNIFLVYNTMAIDKIVKS     18
Consensus

BSMV XJTGB1   TTVE.....KEPELKPNVKKSKKKRIQKPAQPSRPNDLKGGTKGSSQ.......VGENVSENYTGI    134
PSLV TGB1     KTTSESSSAKNVESKESKKQTKPKAVSPSSDTSKIASEVGSAKKATKKESKKQTKDKGSAEDLNAN    198
LRSV TGB1     DNRSQ....KQQSSKKKKNRKHGNKVVENSTNVGVPPNEGVTLRRVR.......SVESIDEIFTSN     73
Consensus              k    k                        g                    e

BSMV XJTGB1   SKEAAKQKQKTPKSVK...........MQSNLADKFKANDTRRSELINKFQQFVHETCLKSDFEYT    189
PSLV TGB1     TKLKAKASEQKGPTIPGTSAEASRIDLLQSSTAEKFTKNDVRRTALVNEFVAQIHKFCIEQGFEPT    264
LRSV TGB1     QRTPPAAEQLPQKQKQS..........KPPSVAKKHAANDKRRYEKADAFMNKIVEASRASGFQPT    129
Consensus                                     a k   nd rr      f            f  t

BSMV XJTGB1   GRQYFRARSNFFEMIKLASLYDKHLKECMARACTLERERLKRKLLLVRALKPAVDFLTGIISGVPG    255
PSLV TGB1     GRQYMRARANLFELVGLRNLYMEHLKKTAAKACNFTKDRIRRKLFLTSNHKPSVDFLVGIVSGVPG    330
LRSV TGB1     GQNFKRCPANLFEKCKLRALYDKHLRVLHKDACDSEKERIAAKSFLHRSLRPNVDFKVGIVSGVAG    195
Consensus     g    r   n fe   l  ly  hl      ac     r   k  l     p vdf  gi sgv g

BSMV XJTGB1   SGKSTIVRTLLKGEFPAVCALANPALMN.DYSGIEGVYGLDDLLLSAVPITSDLLIIDEYTLAESA    320
PSLV TGB1     CGKSTLVRKLLDSPISCYVALANPATER.DYRGTSNVMTLDDLLLAKVPMSSDLLIVDEYTLAESA    395
LRSV TGB1     SGKSTLIRKLCS.EADAMCVLANPRLKETDYKGQSKTFTLQQVLLSIVPMTSDIVIVDEYTLAESA    260
Consensus      gkst  r l          lanp     dy g      l   ll  vp  sd  i deytlaesa

BSMV XJTGB1   EILLLQRRLRASMVLLVGDVAQGKATTASSIEYLTLPVIYRSETTYRLGQETASLCSKQGNRMVSK    386
PSLV TGB1     EILLLQRRVRSSLVLLVGDVAQGRSNNASNIEYLTLPVIYRKITSHRIGEETAKACSKQGNRIRSA    461
LRSV TGB1     ELLLLQRKLQATFLVLFGDVAQGNAKTASSLEYLQFPVVFISKTSHRLGKHTAELCKKHGQAFEPG    326
Consensus     e lllqr        l gdvaqg    as  eyl  pv     t  r g  ta  c k g

BSMV XJTGB1   G.GRDTVIITDYDGETDETEKNIAFTVDTVRDVKDCGYDCALAIDVQGKEFDSVTLFLRNEDRKAL    451
PSLV TGB1     G.RKDKLILVDYEGETEETEKNLAFTEETRDDVKDCGYDCSLVSEVQGLEYESVTLFLRNTDRAAA    526
LRSV TGB1     SPEEDEIIVADYLGAADTTEKNIAFTKETVEDLRDAGVEASLVLETQGKEYESVTLFIRESDEAAM    392
Consensus         d  i  dy g    tekn aft  t  d  d g    l    qg e  svtlf r  d  a

BSMV XJTGB1   ADKHLRLVALSRHKSKLIIRADAEIRQAFLTGDIDLSS.KASNSHRYSAKPDEDHSWFKAK         511
PSLV TGB1     SDHHKRTVSMTRHKSLLIIRAEQEIGQPFLMGELSVSSKRPSNAHVYSSE...........         576
LRSV TGB1     ADSHLRAVALTRHRKKLIIRAEPGVQSSFLNGELKSKTSADSHKYESSKVSYADSSSAAAQ         453
Consensus      d h r v   rh   liira       fl g         s     s
```

$_{XJ}$TGB1 SLCSKQGNRMVSKGGRDTVIITDYDGETDETEKNIAFTVD

$_{ND}$TGB1 SLCSKQGNRMVSKGGRDTVIITDYDGETDGTEKNIAFTVD

$_{NW}$TGB1 SLCSKQGNRMVSKGGKDKVIITDYDGETDETEKNIAFTVD

$_{BJ}$TGB1 SLCSKQGNRMVSKGGKDTVIITDYDGETDETEKNIAFTVD

$_{CV}$TGB1 SLCSKQGNRMVSKGGRDTVIITDYDGETDETEKNIAFTVD

$_{TY}$TGB1 SLCSKQGNRMVSKGGRDTVIITDYDGETDGTEKNIAFTVD

Fig. S2 Alignment of the TGB1 proteins of the hordeiviruses (A) and amongst six sequenced BSMV strains (B).

Table S1 Primers used in construction and analysis of biologically active BSMV Xinjiang cDNA clones

Primer	Primer sequence (5'-3') [a]	Position and description [b]	Purpose
XJ-1	TGTAATACGACTCACTATAGGTATGTAAGTTGCCTTTGGGTG	$_{XJ}$BSMV RNAα nt 1-22	pT7α_{XJ} construction in combination with BS-32
XJ-2	TGTAATACGACTCACTATAGGTAAAAGAAAAGGAACAACCCTG	$_{XJ}$BSMV RNAβ nt 1-23	pT7β_{XJ} construction in combination with BS-32
XJ-3	TGTAATACGACTCACTATAGGTATAGCTTGAGCATTACCGTC	$_{XJ}$BSMV RNAγ nt 1-22	pT7γ_{XJ} construction in combination with BS-32
CH-10	GTATGTAAGTTGCCTTTGGGTGTA	$_{XJ}$BSMV RNAα nt 1-24	pCaBS-α_{XJ} construction in combination with BS-26
CH-11	GTAAAAGAAAAGGAACAACCCTGT	$_{XJ}$BSMV RNAβ nt 1-24	pCaBS-β_{XJ} and its mutants construction in combination with BS-26
CH-12	GTATAGCTTGAGCATTACCGTCGT	$_{XJ}$BSMV RNAγ nt 1-24	pCaBS-γ_{XJ} construction in combination with BS-26
BS-26	CGGGATCCTGGTCTTCCCTTGGGGGAC	$_{XJ}$BSMV RNAγ nt 2793-2775 (common to the 3' termini of BSMV gRNAs)	pCaBS-α_{XJ}, pCaBS-β_{XJ}, and pCaBS-γ_{XJ} construction
BS-10	GGTGCTTGATGCTTTGGATAAGG	$_{XJ}$BSMV RNAγ nt 1864-1886	verification of BSMV genome by RT-PCR
BS-32	TGGTCTTCCCTTGGGGGAC	$_{XJ}$BSMV RNAγ nt 2790-2772	
TGB1-XhoIF	CCGCTCGAGCTATGGACATGACGAAAACTGTTGAGG	$_{XJ}$TGB1 nt 1-25	pGDG-$_{XJ}$TGB1and its mutants construction
TGB1-BamHIR	CGGGATCCTTATTTGGCCTTGAACCAACTGTGG	$_{XJ}$TGB1 nt 1536-1512	
TGB2-XhoIF	CCGCTCGAGCTATGAAGACCACAGTTG	$_{XJ}$TGB2 nt 1-16	pGD-$_{XJ}$TGB2 construction
TGB2-BamHIR	CGGGATCCCTAGCCAATATCGCATAG	$_{XJ}$TGB2 nt 395-379	

Primer	Primer sequence (5'-3') [a]	Position and description [b]	Purpose
TGB3-XhoIF	CCGCTCGAGCTATGGCAATGCCTCATC	$_{\mathrm{XJ}}$TGB3 nt 1-16	pGDG-$_{\mathrm{XJ}}$TGB3 and pGDR-$_{\mathrm{XJ}}$TGB3 construction
TGB3-BamHIR	CGGGATCCTTACCTTTTTGAAGAAAG	$_{\mathrm{XJ}}$TGB3 nt 469-451	
TGB1-NdeIF	GGAATTCCATATGATGGACATGACGAAAACTG	$_{\mathrm{XJ}}$TGB1 nt 1-19	pET-$_{\mathrm{XJ}}$TGB1 construction
TGB1-XhoIR	CCGCTCGAGTTTGGCCTTGAACCAAC	$_{\mathrm{XJ}}$TGB1 nt 1533-1517	
TGB3-BamHIF	CGGGATCCATGGCAATGCCTCATC	$_{\mathrm{XJ}}$TGB3 nt 1-16	pGEX-$_{\mathrm{XJ}}$TGB3 construction
TGB3-XhoIR	CCGCTCGAGTTACCTTTTTGAAGAAAG	$_{\mathrm{XJ}}$TGB3 nt 468-451	
NbCK2α-NdeIF	GGAATTCCATATGATGTCAAAAGCTCGTG	NbCK2α nt 1-16	pET- NbCK2α construction
NbCK2α-XhoIR	CAACTCGAGCTGCGTCCTCATCC	NbCK2α nt 999-986	
HvCK2α-NdeIF	GGAATTCCATATGATGTCGAAGGCGAGGGTC	HvCK2α nt 148-165	pET- HvCK2α construction
HvCK2α-SalIR	GCGTCGACTTGCGGTCGTGC	HvCK2α nt 1146-1135	

[a] Underlined letters indicate restriction enzyme sites, gray covered letters show T7 promoter sequence.

[b] Numbers correspond to target nucleotide positions; a reverse order of numbers indicates that the primer is complementary to the targeted sequences.

Table S2 Primers used for site-specific mutagenesis of Xinjiang RNAβ clones

Primer	Primer sequence (5'-3') [a]	Position and description [b]	Purpose
T395A-F	GGGACACAGTGATCATT**G**CTGATTACGATGGCG	$_{XJ}$BSMV RNAβ nt 1958-1990	pT7β$_{XJ}$TGB1$_{T395A}$ and pET-$_{XJ}$TGB1$_{T395A}$ construction
T395A-R	CGCCATCGTAATCAG**C**AATGATCACTGTGTCCC	$_{XJ}$BSMV RNAβ nt 1990-1958	
T395D-F	GGAAGGGACACAGTGATCATT**GA**TGATTACGATGGCGAAACAG	$_{XJ}$BSMV RNAβ nt 1954-1996	pT7β$_{XJ}$TGB1$_{T395D}$ and pET-$_{XJ}$TGB1$_{T395D}$ construction
T395D-R	CTGTTTCGCCATCGTAATCA**TC**AATGATCACTGTGTCCCTTCC	$_{XJ}$BSMV RNAβ nt 1996-1954	
T395E-F	GGAAGGGACACAGTGATCATT**GAA**GATTACGATGGCGAAACAG	$_{XJ}$BSMV RNAβ nt 1954-1996	pT7β$_{XJ}$TGB1$_{T395E}$ and pET-$_{XJ}$TGB1$_{T395E}$ construction
T395E-R	CTGTTTCGCCATCGTAATC**TTC**AATGATCACTGTGTCCCTTCC	$_{XJ}$BSMV RNAβ nt 1996-1954	
T401A-F	GATTACGATGGCGAA**G**CAGATGAAACGGAGAAAAATATCG	$_{XJ}$BSMV RNAβ nt 1978-2017	pT7β$_{XJ}$TGB1$_{T401A}$ and pET-$_{XJ}$TGB1$_{T401A}$ construction
T401A-R	CGATATTTTTCTCCGTTTCATCTG**C**TTCGCCATCGTAATC	$_{XJ}$BSMV RNAβ nt 2017-1978	
T401D-F	GATTACGATGGCGAA**GAC**GATGAAACGGAGAAAAATATCG	$_{XJ}$BSMV RNAβ nt 1978-2017	pT7β$_{XJ}$TGB1$_{T401D}$ and pET-$_{XJ}$TGB1$_{T401D}$ construction
T401D-R	CGATATTTTTCTCCGTTTCATC**GTC**TTCGCCATCGTAATC	$_{XJ}$BSMV RNAβ nt 2017-1978	
T401E-F	GATTACGATGGCGAA**GA**AGATGAAACGGAGAAAAATATCG	$_{XJ}$BSMV RNAβ nt 1978-2017	pT7β$_{XJ}$TGB1$_{T401E}$ and pET-$_{XJ}$TGB1$_{T401E}$ construction
T401E-R	CGATATTTTTCTCCGTTTCAT**CT**TCTTCGCCATCGTAATC	$_{XJ}$BSMV RNAβ nt 2017-1978	
T395A/ T401A -F	CACAGTGATCATT**G**CTGATTACGATGGCGAA**G**CAGATGAAACGG	$_{XJ}$BSMV RNAβ nt 1962-2005	pT7β$_{XJ}$TGB1$_{T395A/T401A}$ and pET-$_{XJ}$TGB1$_{T395A/T401A}$ construction
T395A/ T401A -R	CCGTTTCATCTG**C**TTCGCCATCGTAATCAG**C**AATGATCACTGTG	$_{XJ}$BSMV RNAβ nt 2005-1962	
T395D/ T401A -F	CACAGTGATCATT**GA**TGATTACGATGGCGAA**G**CAGATGAAACGG	$_{XJ}$BSMV RNAβ nt 1962-2005	pT7β$_{XJ}$TGB1$_{T395D/T401A}$ and pET-$_{XJ}$TGB1$_{T395D/T401A}$ construction
T395D/ T401A -R	CCGTTTCATCTG**C**TTCGCCATCGTAATCA**TC**AATGATCACTGTG	$_{XJ}$BSMV RNAβ nt 2005-1962	

T395E/ T401A -F	CACAGTGATCATT**GAA**GATTACGATGGCGAA**G**CAGATGAAACGG	${}_{XJ}$BSMV RNAβ nt 1962-2005	pT7β$_{XJ}$TGB1$_{T395E/T401A}$ and pET-$_{XJ}$TGB1$_{T395E/T401A}$ construction
T395E/ T401A -R	CCGTTTCATCTG**C**TTCGCCATCGTAATC**TTC**AATGATCACTGTG	${}_{XJ}$BSMV RNAβ nt 2005-1962	

[a] Underlined and bold letters indicate sequences altered by site-specific mutagenesis.

[b] Numbers correspond to target nucleotide positions; a reverse order of numbers indicates that the primer is complementary to the targeted sequences.

Table S3 Sequence alignment of Xinjiang strain RNAα with different BSMV strains

Identity of sequences (%)			
Strain	Full length	αa	
	nt	nt	aa
ND18	99.82	99.85	99.65
Type	99.66	99.82	99.56
Norwich	98.52	98.51	98.77
Beijing	95.18	94.96	96.66

Table S4 Sequence alignment of Xinjiang strain RNAβ with different BSMV strains

Identity of sequences (%)								
Strain	Full length	βa		TGB1		TGB2		TGB3	
	nt	nt	aa	nt	aa	nt	aa	nt	aa
ND18	99.23	99.83	99.49	99.67	99.41	100	100	99.78	100
Type	99.41	100	100	99.67	99.22	99.75	100	100	100
Norwich	95.85	99.16	100	95.72	93	97.2	99.24	95.27	93.55
Beijing	94.08	92.42	92.42	95.39	93.76	96.69	99.24	96.13	94.19

Table S5 Sequence alignment of Xinjiang strain RNAγ with different BSMV strains

Identity of sequences (%)					
Strain	Full length	γa		γb	
	nt	nt	aa	nt	aa
ND18	98.78	98.92	99.38	97.6	96.73
Type	87.82	83.74	83.83	99.35	99.35
Norwich	98.5	98.77	99.54	97.42	98.69
Beijing	94.36	95.02	96.92	94.55	94.12

Table S6 Systemic infectivity efficiency of $_{XJ}$BSMV phosphorylation related mutants on hosts

	Mock	WT	T395A	T395D	T395E	T401A	T401D	T401E	T395A T401A	T395D T401A	T395E T401A
Systemic infectivity efficiency on *Nicotiana benthamiana*											
Exp. 1	0[a]/8[b]	7/8	6/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Exp. 2	0/8	7/8	6/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Exp. 3	0/8	7/8	5/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8	0/8
Systemic infectivity efficiency on barley											
Exp. 1	0/8	6/8	5/8	0/8	4/8	1/8	0/8	4/8	0/8	0/8	0/8
Exp. 2	0/8	6/8	3/8	0/8	2/8	2/8	0/8	1/8	0/8	0/8	0/8
Exp. 3	0/8	6/8	4/8	0/8	4/8	1/8	0/8	4/8	0/8	0/8	0/8
Systemic infectivity efficiency on wheat											
Exp. 1	0/8	7/8	6/8	0/8	6/8	4/8	0/8	5/8	0/8	0/8	0/8
Exp. 2	0/8	7/8	5/8	0/8	5/8	3/8	0/8	5/8	0/8	0/8	0/8
Exp. 3	0/8	8/8	6/8	0/8	5/8	2/8	0/8	4/8	0/8	0/8	0/8

[a] numbers of systemically infected plants
[b] numbers of the total inoculated plants