Supplementary Information

Table S1. List of XSSH peptides identified by mass spectrometry with no enrichment. Peptides and their start and end position in the protein are given. Note that the modifications that were identified on these peptides are also indicated; pyroglutamate (Q<Pyr>), methionine-sulfoxide (M<Mox>), phosphorylation (<P>). Further, the actual Mascot score and the corresponding identity threshold score at 99% confidence settings are indicated.

Table S2. List of XSSH peptides identified by mass spectrometry with TiO_2-enrichment. The format is the same as that in Table S1.

Fig. S1. Single or double mutations at phosphorylation sites in GST-N-tail caused slight decreases in the mobility shifts. Arrowheads indicate GST-N-tail phosphorylated in CSF-extracts (pGST-N-tail in the left lanes). Arrows represent the positions of nonphosphorylated GST-N-tail (the band is not shown).

Fig. S2. The specificity of anti-XSSH IgG used for the injection study. CSF-extracts were subjected to SDS-PAGE and staining with CBB (lane b) or immunoblot with anti-XSSH IgG (lane c). Lane a shows molecular size markers.

Mutagenesis procedures of phosphorylation site mutants of GST-N-tail

For single or double mutations, pGEX-N-tail was mutagenized with the forward primers and their complementary primers, as follows.

(1) S442G mutant

5’-GCCAGTAAGCAAAGACACGCGTACCTCTGGGACCCCAGTTC

(2) Y443A

5’-CAGTAAGCAAAGACACAGCGCTCTCTGGGACCCCAGTTCAG

(3) S452A

5’-GCTACCTCTGGGACCCGGGTGCAGCACCAGCTTTACCTCTGATGTCTCC

(4) S457A (P1)

5’-CCATCTTTACCCTGATGGCGCCTCCCCCCAAGAATTTTTC

(5) S464, 465AG

5’-CCCCCCAAGAATTTTGCAGGCCCCACAAC

(6) T467,468AG

5’-GAATTTTTCAAGCCCCGCGGCCTCACCTCTCACACC

(7) S469A (P2)

5’-CAAGCCCCACAACCGCACCTCTCACACCA

(8) T472A (P3)

5’-CCCCACAACCTCACCTCTGGCGCCAAGGCTGCAAAAGATG

(9) S486,488AA (P4)

5’-GAATCTGAGTACTCTAATGCGCGCCATCGCTGAGATGGAGGCAGCTGA

(10) T495, 497AA (P5)

5’-GAGATGGAGGCAGCTGATGCAATTGCAGAGGAGAAGGAAAGCAC

Double to quintuple mutations (from P1 to P5 sites) were generated as follows.

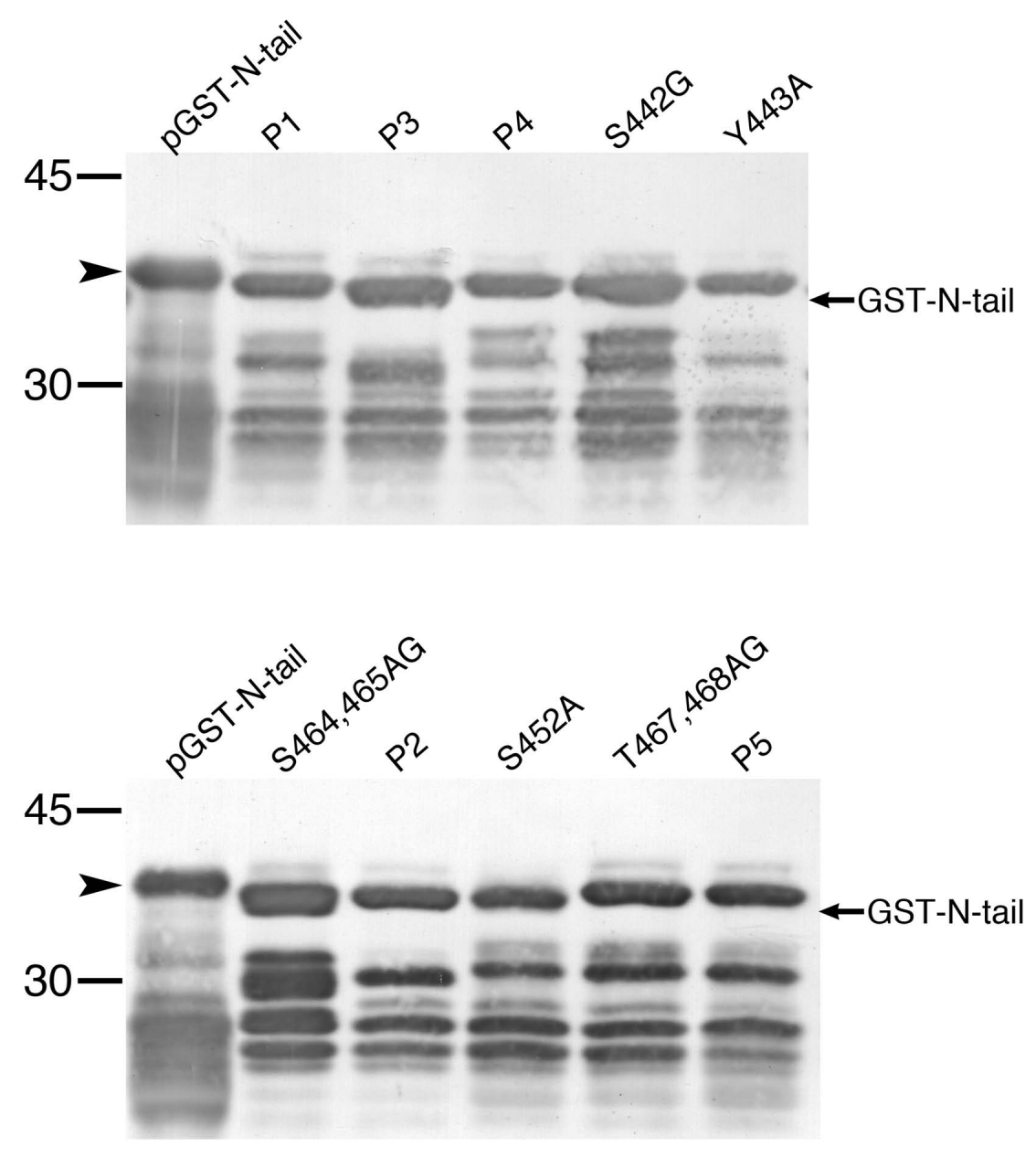
(11) P1, 3 mutant (pGEX-P1,3): pGEX-S457A (P1) was further mutagenized with the primers for T472A (P3).

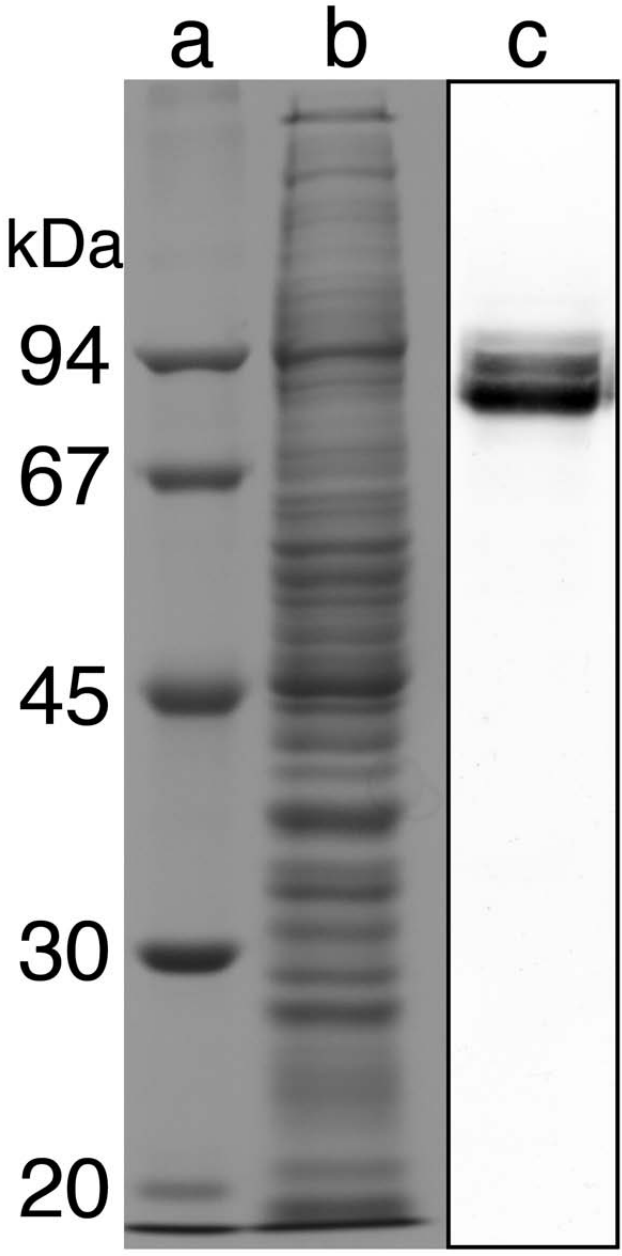
(12) P1, 3, 4 mutant (pGEX-P1, 3, 4): pGEX-P1, 3 was mutagenized with S486, 488AA (P4) primers.

(13) P1, 2, 3, 4 mutant (pGEX-P1, 2, 3, 4): pGEX-P1, 3, 4 was mutagenized with new primers as follows, since the P3 site had been substituted.

5’- CAAGCCCCACAACCGCACCTCTGGCGCCA and its complementary primer

(14) P1, 2, 3, 4, 5 mutant (pGEX-P1, 2, 3, 4, 5): pGEX-P1, 2, 3, 4 was mutagenized with T495, 497AA (P5) primers.


pGST-N-tail
P1
P3
P4
S442G
Y443A
45
30
GST-N-tail
pGST-N-tail
S464,465AG
P2
S452A
T467,468AG
P5
45
30
GST-N-tail


a
b
c
kDa
94
67
45
30
20