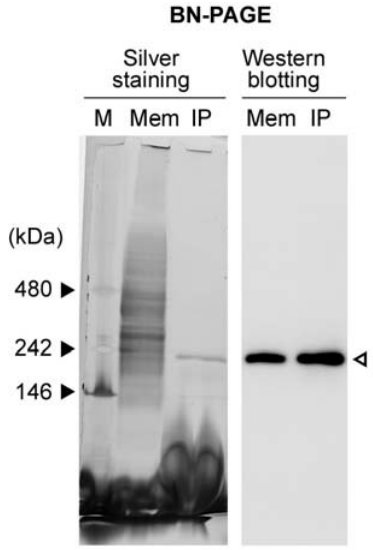
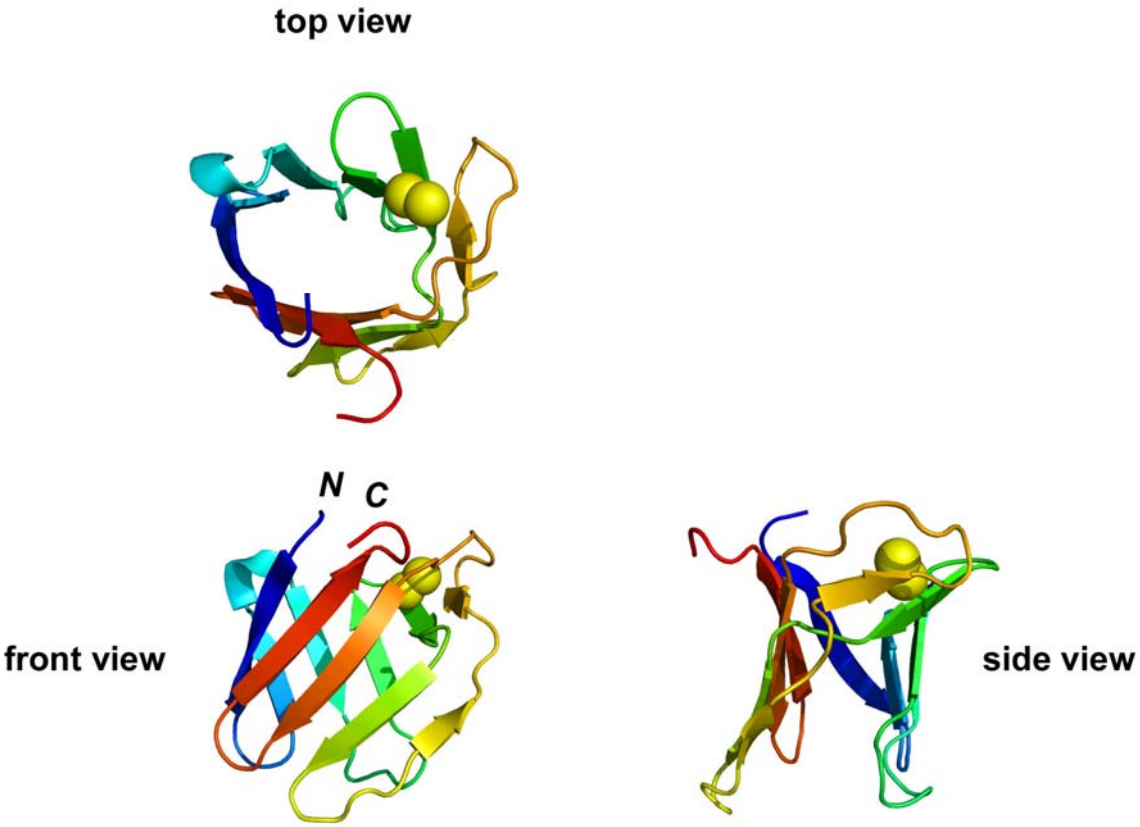
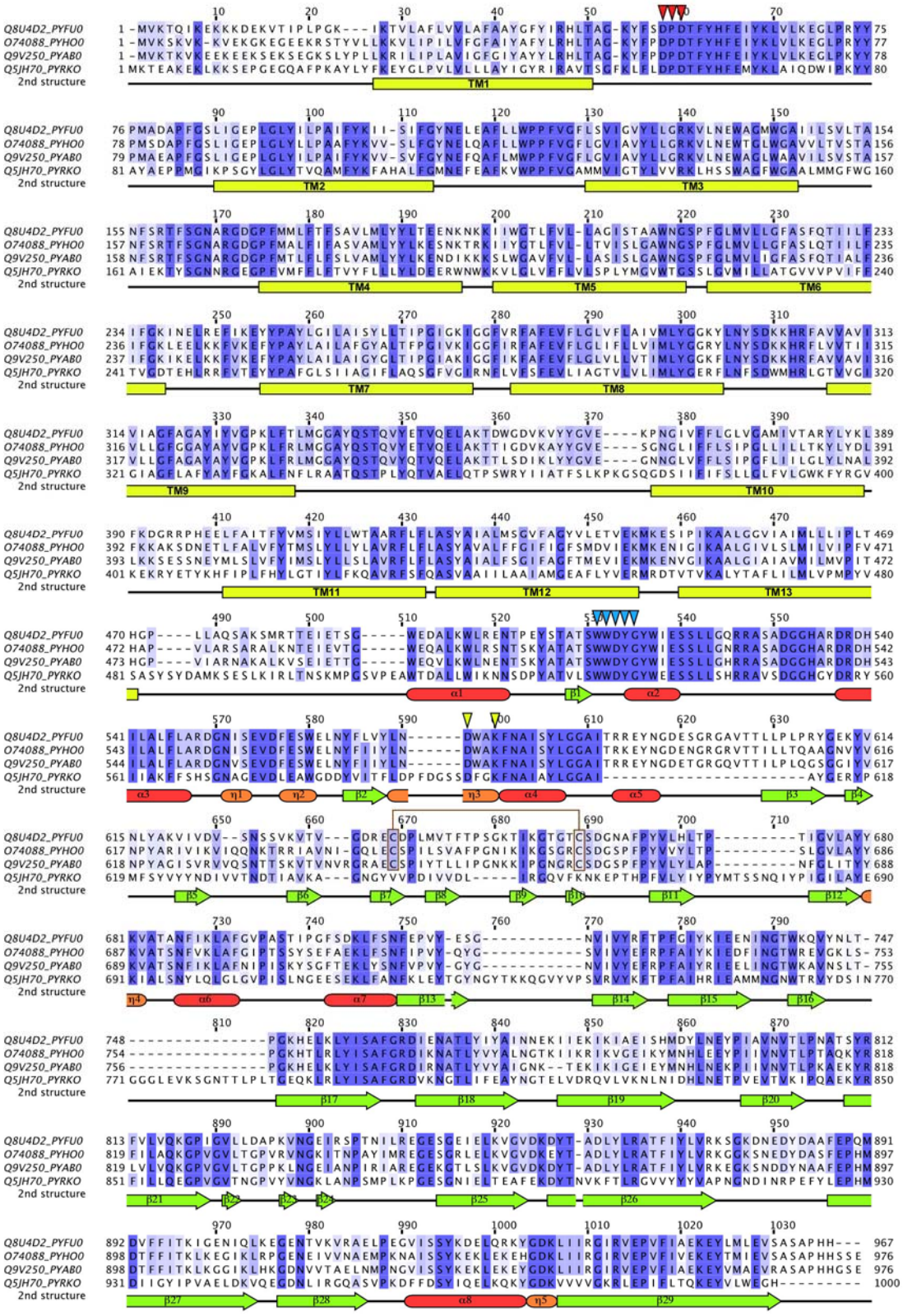


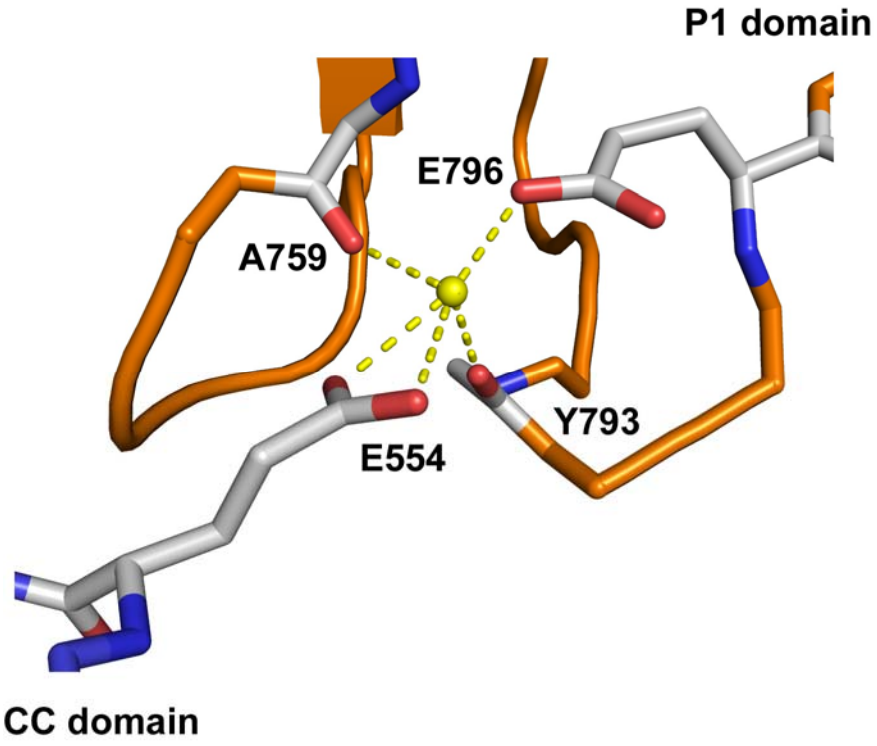
Supplementary Figure 1 BN-PAGE analysis of *Pyrococcus furiosus* oligosaccharyltransferase before and after immunoaffinity purification. Oligosaccharyltransferase was purified from *P. furiosus* cells by immunoaffinity, using an anti-*P. furiosus* sSTT3 antibody. The membrane fraction (Mem) and the immunoaffinity purified sample (IP) were subjected to BN-PAGE, and the proteins on the gels were visualized by silver staining and western blotting, using an anti-*P. furiosus* sSTT3 antibody as the primary antibody.



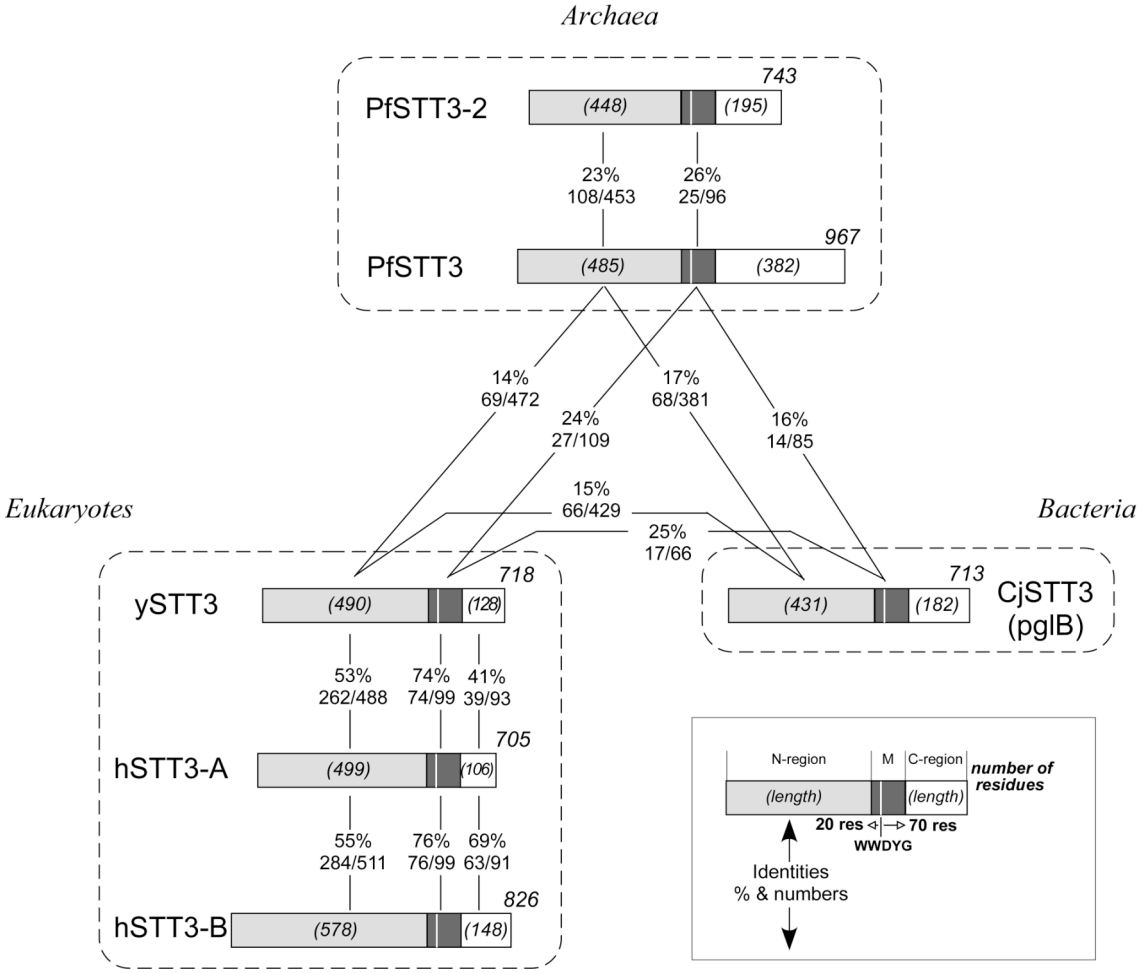
Supplementary Figure 2 Architecture of the IS domain. The IS (insertion) domain (residues 601-682) is shown in a ribbon representation. The sulfur atoms that form a disulfide bond are depicted as yellow spheres.


Q8U4D2_PYFU0
O74088_PYHO0
Q9V250_PYAB0
Q5JH70_PYRKO
2nd structure
TM1
TM2
TM3
TM4
TM5
TM6
TM7
TM8
TM9
TM10
TM11
TM12
TM13
α1
β1
α2
α3
η1
η2
β2
η3
α4
α5
β3
β4
β5
β6
β7
β8
β9
β10
β11
β12
η4
α6
α7
β13
β14
β15
β16
β17
β18
β19
β20
β21
β22
β23
β24
β25
β26
β27
β28
α8
η5
β29

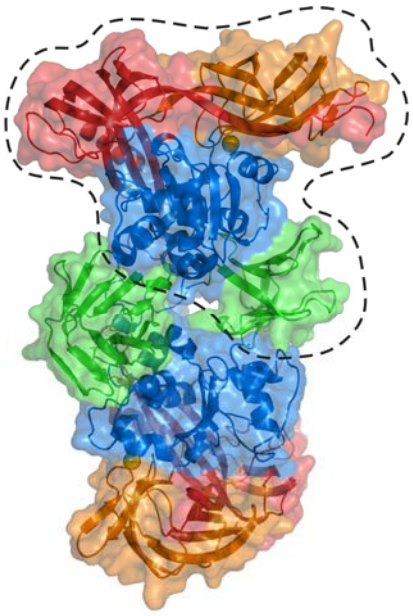
Supplementary Figure 3 Sequence alignment and secondary structure elements of STT3s from four *Thermococcales* genomes. The multiple sequence alignment of *Thermococcales* STT3 proteins was retrieved from the HOGENOM database (release 03, October, 2005, http://pbil.univ-lyon1.fr/databases/hogenom.html). The gene family alignment HBG230711 contains three sequences belonging to *Pyrococcus: P. furiosus* (Q8U4D2_PYFU0), *P. horikoshii* (O74088_PYHO0), and *P. abyssi* (Q9V250_PYAB0), and one sequence belonging to *Thermococcus: T. kodakaraensis* (Q5JH70_PYRKO). *T. kodakaraensis* was reported as Pyrococcus sp., but has been re-classified as Thermococcus sp. (Atomi *et al*, 2004). The positions of the transmembrane helices were estimated using the program SOSUI (http://bp.nuap.nagoya-u.ac.jp/sosui/) as TM1 - TM13 below the N-terminal half of the alignment, and the experimentally determined secondary structure elements are shown below the C-terminal half of the alignment. β, α, η represent a β-strand, an α-helix, and a 3_{10} helix, respectively. The secondary structure assignment was carried out by the program DSSP implemented in ESPript 2.2 (http://espript.ibcp.fr/ESPript/ESPript/index.php). The triangles indicate the amino acid residues belonging to the DXD motif (red), the WWDYG motif (cyan), and the DK motif (yellow). The figure of the alignment was generated with the program Jalview (http://www.jalview.org/).



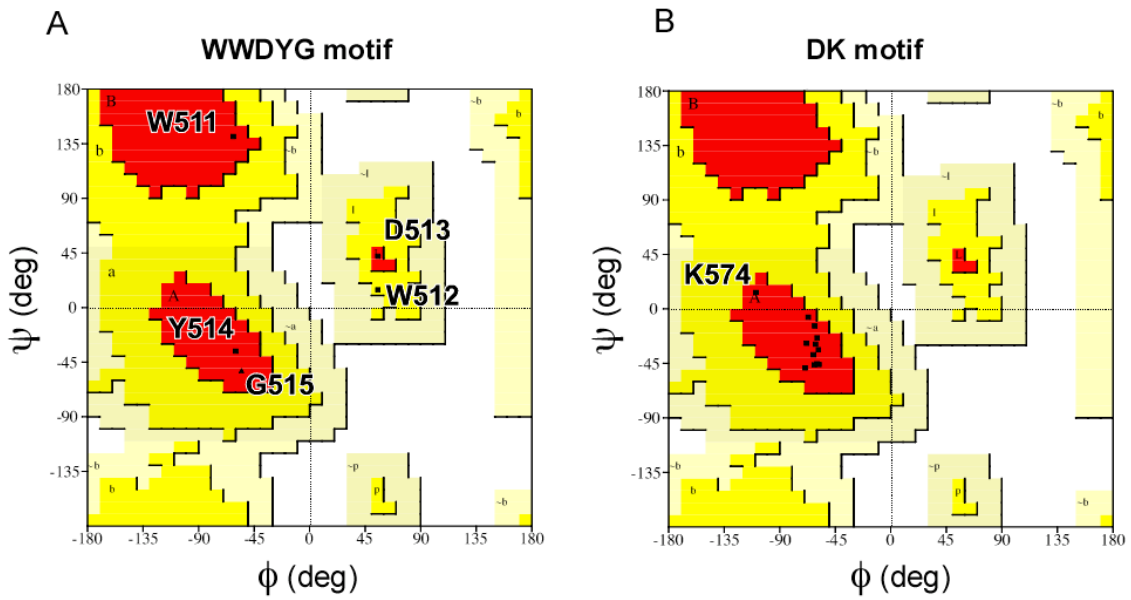
Supplementary Figure 4 Metal binding site. Close-up view of the metal binding site, with the side chains of E554 and E796, and the main chains of A759 and Y793. The metal ion is estimated to be a Ca^{2+} ion. The distances shorter than 3 Å are shown as yellow dotted lines.



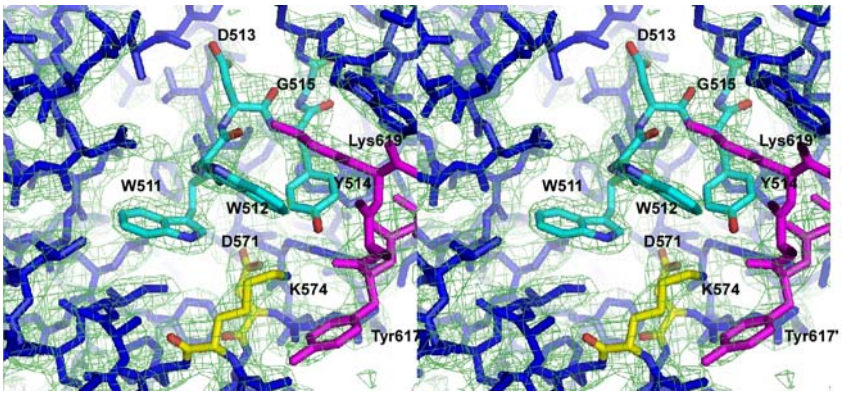
Supplementary Figure 5 Sequence identities between STT3 proteins. The entire sequence of the STT3 proteins is divided into three regions. The M-region (95 residues) was defined as extending 20 residues toward the N-terminus from the WWDYG motif and 70 residues toward the C-terminus. The N-region and C-region were defined as the N-terminal and C-terminal segments that frank the M-region. Percentage sequence identities were calculated using Blast2p (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi). If Blast2p failed to calculate a sequence identity that covered the majority of the region, then PSI-BLAST was used (http://www.ncbi.nlm.nih.gov/BLAST). The numerators are the number of identical residues, and the denominators are the number of aligned positions including gaps. h, human; y, yeast; Pf, *Pyrococcus furiosus*; Cj, *Campylobacter jejuni*. The human and *P. furiosus* genomes each contain two paralogs of STT3.



Supplementary Figure 6 Dimer interaction in crystal structure. Two sSTT3 molecules in the crystal form a dimer, with a contact surface area of 1,300 $Å^2$ per molecule. Since the asymmetric unit contains four sSTT3 molecules, two pairs of the sSTT3 dimers exist in the asymmetric unit. A gel filtration experiment suggested that sSTT3 is monomeric in solution (Igura *et al*, 2007).



Supplementary Figure 7 Ramachandran plot. (**A**) The N-terminal half segment of the WWDYG motif, $W^{511}WD^{513}$, adopts a 'left-handed' 3_{10} conformation. The program DSSP was used for secondary structure element assignment. The assignment to a left-handed 3_{10} conformation is attributed to the unusual location of W512 and D513 in the Ramachandran plot. (**B**) The segment containing the DK motif, $L^{569}NDWAK^{574}$, forms a 6-residue 3_{10} helix and the subsequent segment, $F^{575}NAISYL^{581}$, adopts an α-helical conformation. The Ramachandran plots were generated by the program PROCHECK (Laskowski *et al*, 1993) in the CCP4 suite.



Supplementary Figure 8 Close-up Stereoview of the Putative Active Site of OST/STT3. The electron density from the sigmaA weighted $2F_o$-F_c map calculated from the final coordinates, contoured at 1.5σ (green), is superimposed on the final refined structure. The WWDYG and DK motifs are drawn in cyan and yellow, respectively. The residues belonging to the IS domain of the other molecule of the STT3 dimer in the crystal (Supplementary Figure 6) are drawn in magenta, and their numbering is marked by an apostrophe.

Supplementary Table 1 Growth phenotype of yeast STT3 point mutations

Mutant[a]	Growth phenotype[b]	reference[c]
WT	normal	-
WWDYG motif		
W516A	t.s.	1
W516Y	t.s.	1
W517Y	lethal	1
D518E	lethal	1, this study
Y519A	t.s.	1, this study
G520A	t.s.	1
G520S	t.s.	2
G520D	t.s.	1
DK motif		
D582A	t.s.	this study
D583A	lethal	this study
D583E	normal	this study
I584A	normal	this study
N585A	normal	this study
K586A	lethal	this study
K586R	lethal	this study
F587A	normal	this study
L588A	normal	this study
W589A	t.s.	this study
M590A	normal	this study
I591A	normal	this study
R592A	normal	1
I593A	t.s.	1
I593D	t.s.	1

[a] The position of replaced amino acid residues in yeast STT3 protein.

[b] Growth phenotype was determined by the plasmid shuffling procedure using 5-FOA selection. t.s., temperature-sensitive.

[c] Reference 1, Yan and Lennarz (2002); reference 2, Spirig et al. (1997).