Supplemental Materials

Molecular Biology of the Cell

Mellado et al.

Supplemental Table S1. List of strains used in this work

Strain	Genotype	Reference
MAD1425	*pyrG89, pyroA4, nkuAΔ::argB (argB2)*	(TN02A3, Nayak et al., (2006))
MAD1427	*pyrG89, pabaB22, nkuAΔ::argB (argB2), riboB2*	Markina-Iñarrairaegui et al., (2011)
MAD2457	*pyrG89, wA3, cnaAΔ::pyroA, pyroA4*	CNA1, Soriani et al., (2008)
MAD2733	*pabaB22, nkuAΔ::argB (argB2)*	Markina-Iñarrairaegui *et al*. 2011
MAD2743	*yA2, pyroA4, pantoB100*	Zhang et al., (2011)
MAD3625	*pyrG89, sltB::gfp::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2*	This work
MAD3626	*pyrG89, pabaB22, nkuAΔ::argB (argB2), sltAΔ::pyrG^{Af}, riboB2*	This work
MAD3650	*pyrG89, sltB::gfp::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2)*	This work
MAD3651	*pyrG89, pabaB22, nkuAΔ::argB (argB2), sltAΔ::pyrG^{Af}*	Mellado et al. (2015)
MAD3652	*pyrG89, pabaB22, nkuAΔ::argB (argB2), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD3669	*pyrG89, sltBΔ::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2*	Mellado et al., (2015)
MAD3682	*sltBΔ::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2*	Mellado et al., (2015)
MAD3693	*pyrG89, sltBΔ::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD3694	*pyrG89, sltB$^{1-590}$::ha$_3$::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2*	This work
MAD3705	*pyrG89, sltB::gfp::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2), sltAΔ::riboB^{Af}, riboB2*	This work
MAD3710	*pyrG89, sltB$^{692-1272}$::myc$_3$::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2*	This work
MAD3711	*pyrG89, sltB$^{692-1272}$::gfp::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2*	This work
MAD3734	*pyrG89, sltB::myc$_3$::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2*	This work
MAD3750	*pyrG89, sltB$^{692-1272}$::gfp::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD3751	*pyrG89, sltB::gfp::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD3770	*sltB53, pabaA1, yA2, pyroA4*	Mellado et al., (2015)
MAD3815	*pyrG89, sltB$^{1-590}$::gfp::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD3816	*pyrG89, pyroA4, nkuAΔ::argB (argB2), sltAΔ::pyrG^{Af}*	This work
MAD3915	*pabaB22, nkuAΔ::argB (?), cnaAΔ::pyroA^{Af}, sltA::ha$_3$::pyrG^{Af}*	This work

MAD3919	*pyrG89, pabaB22, nkuAΔ::argB (argB2), sltAΔ::riboB^{Af}, riboB2*	Mellado et al., (2015)
MAD3934	*sltB56, nkuAΔ::BAR(?), nkuAΔ::argB(?), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD4013	*pyrG89, sltB53::gfp::pyrG^{Af}, biA1, nkuAΔ::BAR(?), pantoB100*	This work
MAD4015	*nkuAΔ::argB (argB2), pyroA-[alcA^{P}::sltB::myc$_3$]$_{1x}$, sltAΔ::pyrG$^{A.f}$, riboB2*	This work
MAD4016	*nkuAΔ::argB (argB2), pyroA-[alcA^{P}::sltB::myc$_3$]$_{nx}$, sltAΔ::pyrG$^{A.f}$, riboB2*	This work
MAD4025	*pyrG89, pabaB22, nkuAΔ::argB (argB2), sltA$^{200-698}$::ha$_3$:: pyrG^{Af}, riboB2*	This work
MAD4026	*pyrG89, pabaB22, nkuAΔ::argB (argB2), sltA$^{331-698}$::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD4048	*pyrG89, pabaA1, sltA1*	This work
MAD4080	*pyrG89, sltB::gfp::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), sltA$^{331-698}$::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD4081	*pyrG89, pabaB22, nkuAΔ::argB (argB2), sltA$^{331-698}$::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD4096	*Prototrophic wild type*	HHF27a, Findon et al., (2010)
MAD4097	*sltAΔ::riboB^{Af}*	HHF27b, Findon et al., (2010)
MAD4247	*pyrG89, sltB::gfp::pyrG^{Af}, pabaA1, sltA1*	This work
MAD4294	*pyrG89, pabaB22, nkuAΔ::argB (argB2), thiA^{P}::sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD4362	*pyrG89, sltB$^{1-477}$::gfp::riboB^{Af}, pabaB2, nkuAΔ::argB (argB2), riboB2*	This work
MAD4443	*pyrG89, sltB57::gfp::riboB^{Af}, pabaB22, sltA::ha$_3$::pyrG^{Af}, nkuAΔ::argB^{+}(argB2), riboB2*	This work
MAD4487	*pyrG89, sltB53, biA1, nkuAΔ::BAR(?), sltA::ha$_3$::pyrG^{Af}, pantoB100*	This work
MAD4510	*pyrG89, sltB$^{692-1272}$::myc$_3$::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2/ pyrG89, sltB53::gfp::pyrG^{Af}, biA1, nkuAΔ::BAR(?), pantoB100*	This work
MAD4511	*pyrG89, sltB$^{692-1272}$::myc$_3$::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2/ pyrG89, sltB53::gfp::pyrG^{Af}, biA1, nkuAΔ::BAR(?), pantoB100*	This work
MAD4519	*yA2, pyroA4, pantoB100/ pyrG89, sltB53::gfp::pyrG^{Af}, biA1, nkuAΔ::BAR(?), pantoB100*	This work
MAD4520	*yA2, pyroA4, pantoB100/ pyrG89, sltB53::gfp::pyrG^{Af}, biA1, nkuAΔ::BAR(?), pantoB100*	This work
MAD4581	*pyrG89, sltB$^{1-590+TGA}$::gfp::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD4661	*pyrG89, thiA^{P}::sltA$^{400-698}$::ha$_3$::pyrG^{Af}, pabaB22, nkuAΔ::argB*	This work

	(argB2), riboB2	
MAD4731	*pyrG89, sltB^{H1033A}::gfp::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD4733	*pyrG89, sltB::gfp::riboB^{Af}, thiA^{P}::sltA$^{400-698}$::ha$_3$::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2*	This work
MAD4760	*pyrG89, sltB^{S1033A}::gfp::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD4784	*hypA::ha$_3$::pyrG^{Af}, pyrG89, pyroA4, nkuAΔ::BAR,*	Pinar et al., (2015)
MAD4819	*pyrG89, sltBΔ::riboB^{Af}, pabaB22, thiA^{P}::sltA$^{400-698}$::ha$_3$::pyrG^{Af}, nkuAΔ::argB (argB2), riboB2*	This work
MAD4899	*pyrG89, gfp::sltB::myc$_3$::pyrG^{Af}, pabaB22, nkuAΔ::argB (argB2), riboB2*	This work
MAD4965	*nkuAΔ::argB (argB2), sltAΔ::pyrG^{Af}, riboB2*	This work
MAD5268	*pyrG89, sltB$^{1-477+TGA}$::gfp::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), sltA::ha$_3$::pyrG$^{A.f}$, riboB2*	This work
MAD5269	*pyrG89, sltB$^{1-536+TGA}$::gfp::riboB^{Af}, pabaB22, nkuAΔ::argB (argB2), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD5355	*sltB56::gfp::riboB^{Af}, nkuAΔ::BAR(?), nkuAΔ::argB+(?), sltA::ha$_3$::pyrG^{Af}, riboB2*	This work
MAD5384	*sltB56^{TGA}::gfp::riboB^{Af}, nkuAΔ::BAR(?),nkuAΔ::argB+(?), sltA:: ha$_3$::pyrG^{Af}, riboB2*	This work
MAD5411	*pyrG89, pyroA4, pyroA^{Af}::alcA^{p}::sltA, nkuAΔ::argB (argB2)*	This work
MAD5419	*pyrG89, sltBΔ::pyrG^{Af},pyroA4, pyroA^{Af}::alcA^{p}::sltA, nkuAΔ::argB (argB2)*	This work
MAD5423	*inoB2, nkuAΔ::BAR?, sltA114, riboB2*	This work

BAR, herbicide bialaphos resistance gene from *Streptomyces hygroscopicus*.

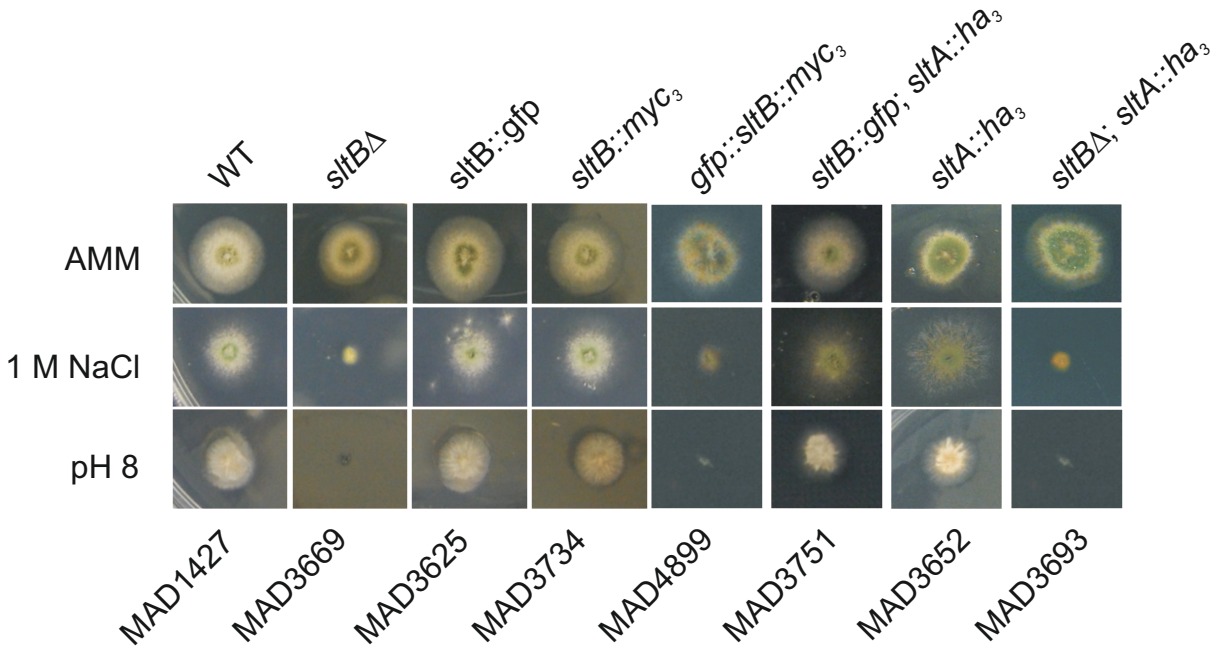
Supplemental Table S2

Primer	Sequence (5´ → 3´)	Target
sltB SMP1	CGAGTCGTCTCCGGTGTCGCCAAGA CCGGTCGCCTCAAACAATGCTCT	Amplification of *pyrG*^{Af}/*riboB*^{Af} SM*
sltB GFP2	GCACAGTGGACGGGGTAAGGTGAG TCTGAGAGGAGGCACTGATGC	Amplification of *pyrG*^{Af}/*riboB*^{Af} SM
sltB GSP3	TCACCTTACCCCGTCCACTGTGC	*sltB* terminator
sltB GSP4	CGAAAGAAGCTCTACATTGTCCACG AGGAT	*sltB* terminator
sltB gfp GSP1	GCCTTCATGAATGAAGAACATCTCCC TC	*sltB* ORF for epitope tagging at C-terminus
sltB gfp GSP2	AGCAAGCTGTCTTTCCAGACTGCGAC C	*sltB* ORF for epitope tagging at C-terminus
sltB gfp GFP1	GGTCGCAGTCTGGAAAGACAGCTTG CTGGAGCTGGTGCAGGCGCTGGAGC C	Amplification of epitopes for tagging at C-terminus of *sltB*
sltA SMP1	CGGACATTAGGGACCGTCCATCACC GGTCGCCTCAAACAATGC	Amplification of *pyrG*^{Af} or *riboB*^{Af} SM
SltB met	CCAAGATGTCCGTACTCCCGCACCAT GG	*sltB* ORF at initiation Met to construct truncated forms
sltB delec COOH GSP2	GTCATCAAATGGCTGGGTCGAAAGG	*sltB* ORF to construct truncated forms
sltB delec COOH GFP1	CCTTTCGACCCAGCCATTTGATGACG GAGCTGGTGCAGGCGCTGGAGC	Amplification of epitopes for tagging SltB truncated forms at C-terminus
sltB delec NH met compl	CGTCTCCGGTGTCGCCAAGATGAAG ATCGGGCTTGAAGAAAAAAGGCTGA GG	*sltB* ORF to construct truncated forms
sltB delec NH met	TTCTTCAAGCCCGATCTTCATCTTGG CGACACCGGAGACG	*sltB* ORF to construct truncated forms
sltA met200	CGGACATTAGGGACCGTCCATCATG GATGCCCAAAGCAC	*sltA* ORF to construct truncated form starting at Met200
sltA arg331	CGGACATTAGGGACCGTCCATCATG CACGAGGCTGACG	*sltA* ORF to construct truncated form starting at Arg331
SltBmyc BamHI sense	GCGGATCCATGTCCGTACTCCC	alcA^{P}-SltB-Myc plasmid, harbors BamHI restriction site
SltBmyc XmaI antisense	CCCCCCGGGTCAATTAAGATCCTCCT CGG	alcA^{P}-SltB-Myc plasmid, harbors XmaI restriction site
sltA met	ATGAGTCCAGCACAAGACTCTG	Fusion of *sltA* ORF to *thiA*^{P}
sltA stop	GCATTGTTTGAGGCGACCGGTTTAG AGACCACCAGGGCCGGGGT	Fusion of *sltA* ORF to *thiA*^{P}
sltA SMP1b	ACCGGTCGCCTCAAACAATGC	Amplification of SM for tagging SltA in *thiA*^{P}*::sltA* construct
thiA-SltAprom	CGGACATTAGGGACCGTCCATCACC TGGCACCTACAGAAGAATCC	Fusion of *sltA* and *thiA* promoters
thiA-SltAgen	CAGAGTCTTGTGCTGGACTCATATG GACTCAGTTCAATGGTTCG	Fusion of *sltA* ORF to *thiA*^{P}
thiA-sltAgen comp	CGAACCATTGAACTGAGTCCATATG AGTCCAGCACAAGACTCTG	Fusion of *sltA* ORF to *thiA*^{P}
sltA gsp1	GCATCGTAGTGTAGCTGTGC	Amplification of *sltA* promoter

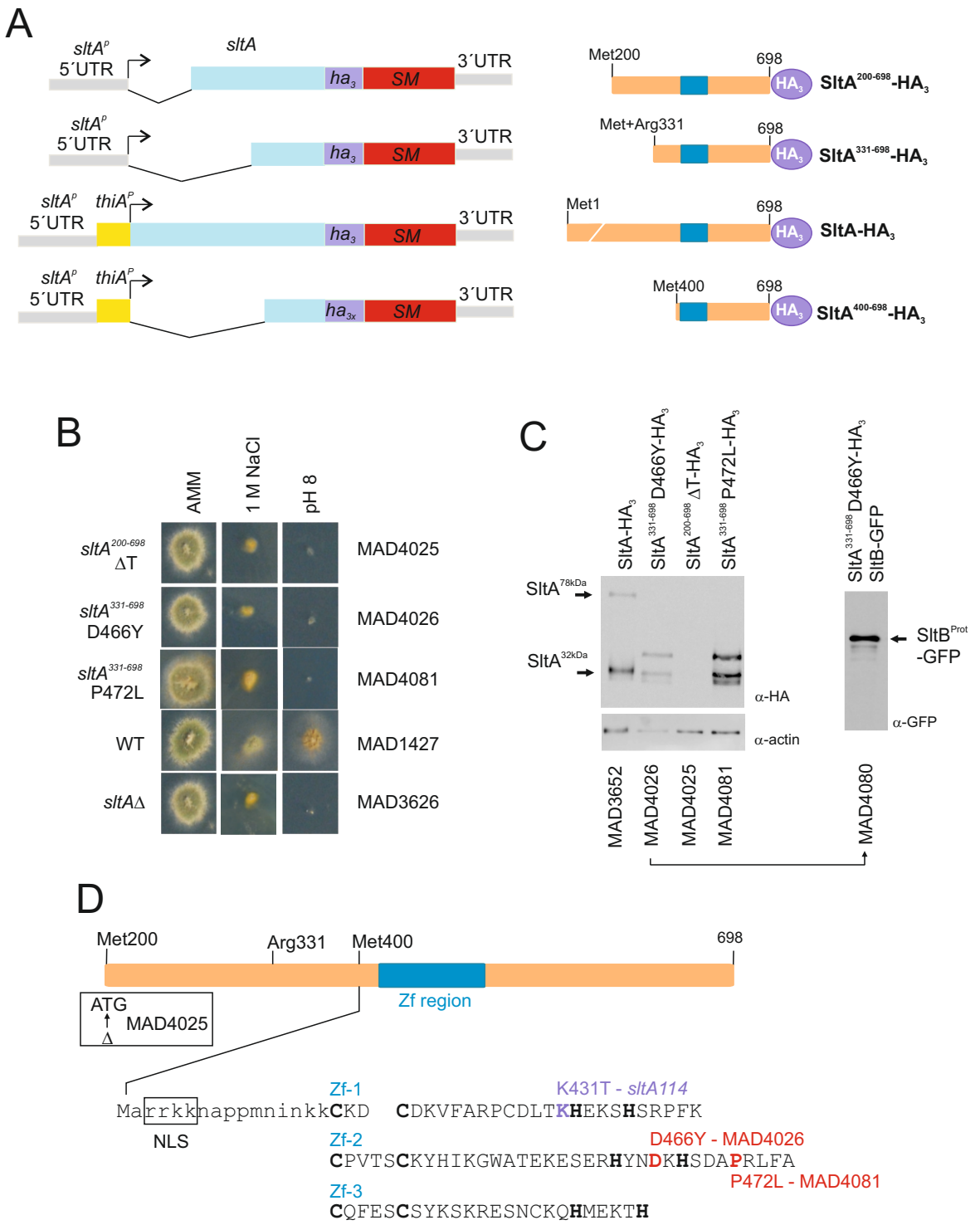
Primer	Sequence (5´ → 3´)	Target
sltA gsp5	GCTCAGAGAGTCCATTGTCATGC	*sltA* ORF, epitope tagging at C-terminus, fusion cassette
sltA gsp6	GAGACCACCAGGGCCGG	*sltA* ORF, epitope tagging at C-terminus, fusion cassette
sltA gsp6´	CCGGCCCTGGTGGTCTCGGAGCTGG TGCAGGCGCTGGAGC	Amplification of epitopes for tagging SltA at C-terminus, fusion cassette
sltA gsp3´	CCACCAAATGTGACGAGACTGTCTG AGAGGAGGCACTGATGCG	Amplification of epitopes for tagging SltA at C-terminus, fusion cassette
sltA gsp3	TCTCGTCACATTTGGTGG	*sltA* terminator, epitope tagging at C-terminus, fusion cassette
sltA gsp4	GCTACGGATGCTGACTCC	*sltA* terminator, epitope tagging at C-terminus, fusion cassette
sltB 477 gsp2	AGGACGACGGAGGTTTTCAGTCAC	*sltB* ORF, to construct $SltB^{1-477}$-GFP
sltB 477 gfp1	GTGACTGAAAACCTCCGTCGTCCTG GAGCTGGTGCAGGCGCTGGAGC	*sltB* ORF, to construct $SltB^{1-477}$-GFP
sltAmet400	CGAACCATTGAACTGAGTCCATATG GCCCGTCGCAAGAAGAATGC	*sltA* ORF, to construct $SltA^{400-698}$ truncated form
sltB-R2	TGAGAAAGGGTCGTCGTGGGG	*sltB,* PCR amplification and sequencing
sltB-R1	CATGTGATGTACTGTCATACCG	*sltB,* PCR amplification and sequencing
sltB-F1	AGTCCTGGCGGTGATCTGGGC	*sltB,* PCR amplification and sequencing
sltB 590 stop	CCTTTCGACCCAGCCATTTGATGACT GAGGAGCTGGTGCAGGCGCTGGAG C	*sltB* ORF, to construct $SltB^{1-590}$ truncated form
sltB mut His1033	CACGACAGGGCATCAAGGCTGAAAT CGACTGGGC	*sltB,* mutagenesis of His1033 codon to Ala codon
sltB mut His31033 compl	AGTCGATTTCAGCCTTGATGCCCTGT CGTGTCCAGC	*sltB,* mutagenesis of His1033 codon to Ala codon
sltB mut Ser1142	GAGTTCCCGGTGATGCTGGCGCTTG GGTCTTTG	*sltB,* mutagenesis of S1142 codon to Ala codon
sltB mut Ser1142 compl	AAGACCCAAGCGCCAGCATCACCGG GAACTCCG	*sltB,* mutagenesis of S1142 codon to Ala codon
sltB gfp SMP2	CCCCATGGTGCGGGAGTACGGACAT TTTGTATAGTTCATCCATGCCATGT	*sltB* ORF, for epitope tagging at N-terminus
sltB inicio	ATGTCCGTACTCCCGCACCATGGGG	*sltB* ORF, for epitope tagging at N-terminus
sltB gfp SMP1*	CGAGTCGTCTCCGGTGTCGCCAAGA TGGGAGCTGGTGCAGGCGCTGGAG CC	*sltB* ORF, for epitope tagging at N-terminus
sltB477 stop	GAAAACCTCCGTCGTCCTTGAGGAG CTGGTGCAG	*sltB* ORF, to construct $SltB^{1-477+TGA}$-GFP fusion
sltB56 mutag	GAAAACCTCCGTCGTCCTT**GA**CTTAC CTCCTTCGCTCAGT	Mutagenesis of *sltB56* TAA codon to TGA codon
sltB56 mutag compl	ACTGAGCGAAGGAGGTAAG**TC**AAG GACGACGGAGGTTTTC	Mutagenesis of *sltB56* TAA codon to TGA codon
sltA prom pyro	CGGACATTAGGGACCGTCCATCTGG	Fusion of *sltA* promoter to *A.*

Primer	Sequence (5´ → 3´)	Target
	TATCATGGTTGTTGGGTC	*fumigatus pyroA*^{Af} SM
sltA prom alcA	CAGAGTCTTGTGCTGGACTCATCCCG GTACCGCTAATTAACTGAGTA	Fusion of *sltA* promoter to *alcA* promoter

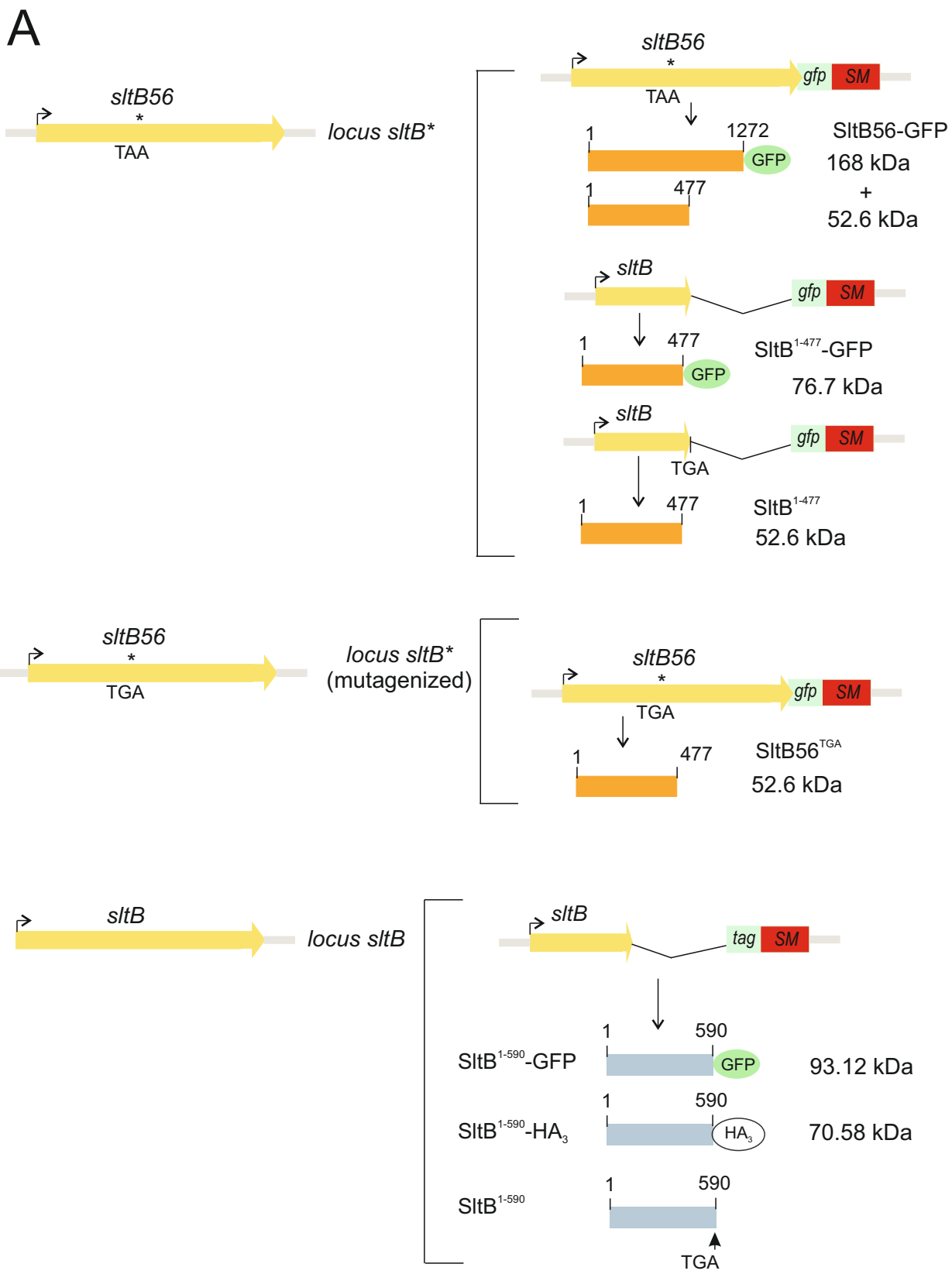
* SM: Selectable Marker

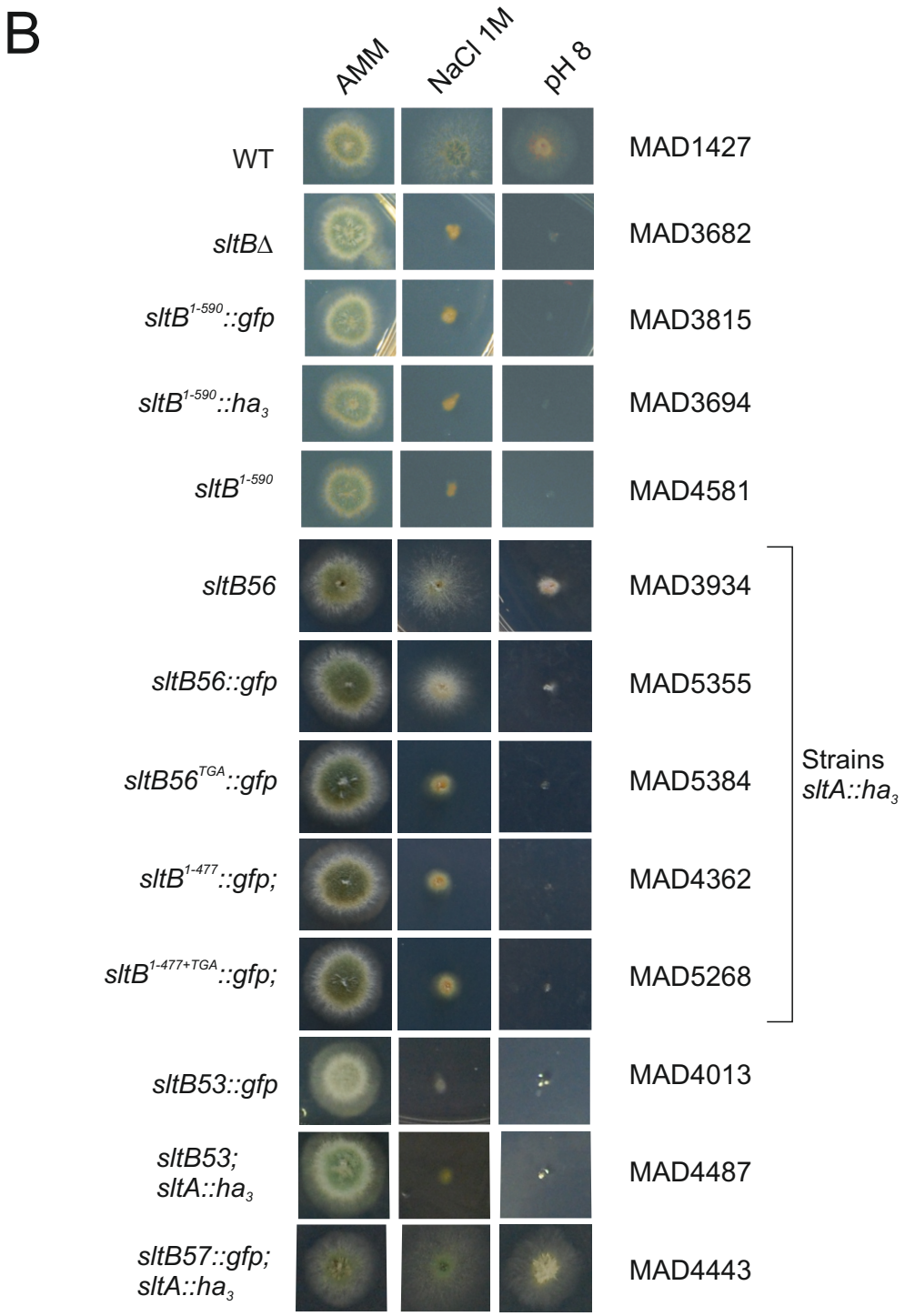


Supplemental Figure S1. Growth tests of strains expressing tagged versions of SltB and/or SltA. AMM is the control minimal medium. Tolerance to sodium stress (addition of 1 M NaCl to AMM) or to alkaline pH (pH 8, containing additionally 100 mM Na_2HPO_4) was tested. Conidiospores were point inoculated and colonies grown for two days at 37° C. Note that the sensitivity to high sodium concentrations (1 M Na^+) or alkaline pH (pH 8) of null *sltB* strains is independent of HA_3-tagging of SltA.

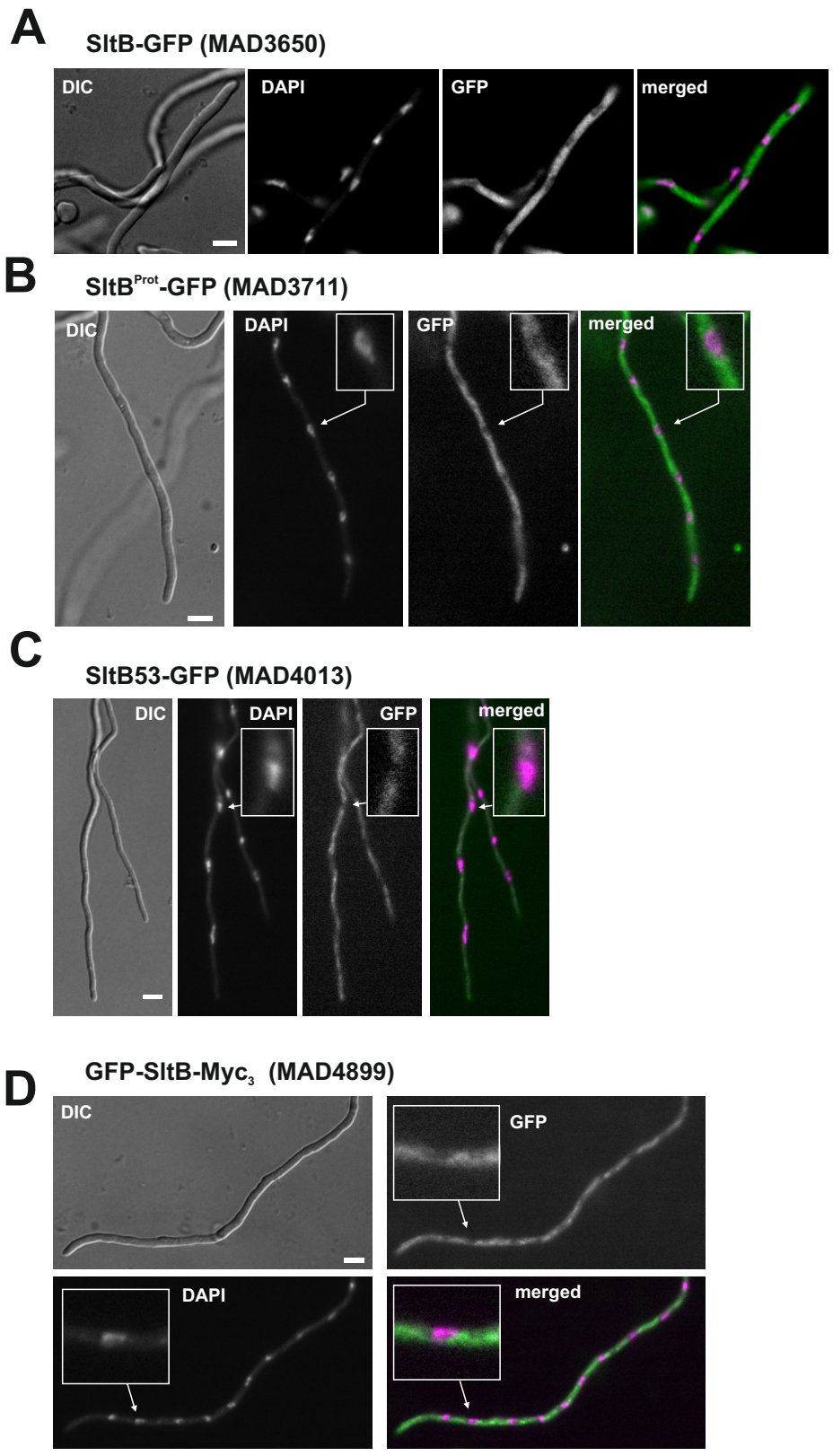


Supplemental Figure S2. Construction of truncated SltA versions putatively mimicking SltA32kDa. (A) Schematic representation of the transcription and translation products generated by gene replacement at the *sltA* locus and allowing expression by the endogenous *sltA* promoter or the conditional *thiA*^{p} promoter. As control, a conditional allele expressing the full length SltA-HA$_3$ fusion was constructed. SM indicates selectable marker. (B) Growth tests of selected transformants obtained with DNA cassettes for the generation of alleles expressing SltA$^{200\text{-}698}$ or SltA$^{331\text{-}698}$. Transformants have a loss of function phenotype comparable to a null *sltA* strain. (C) Western blot showing the detectable fragments of SltA from transformants shown in panel B. Immuno-detection of actin is shown as a loading control. (D) Diagram representing the expressed SltA fusions and the mutations occurring in transformants. Limits of the DNA binding domain consisting of three classical C_2H_2 zinc fingers are shown. Mutational substitutions are indicated: those occurring during the construction of strains expressing the SltA$^{331\text{-}698}$ form are shown in red and the change due to *sltA114* mutation, selected as a suppressor of the null *vps3* allele ((Mellado et al., 2015), see Fig 10) is shown in blue. A putative nuclear localization signal, NLS, is indicated.

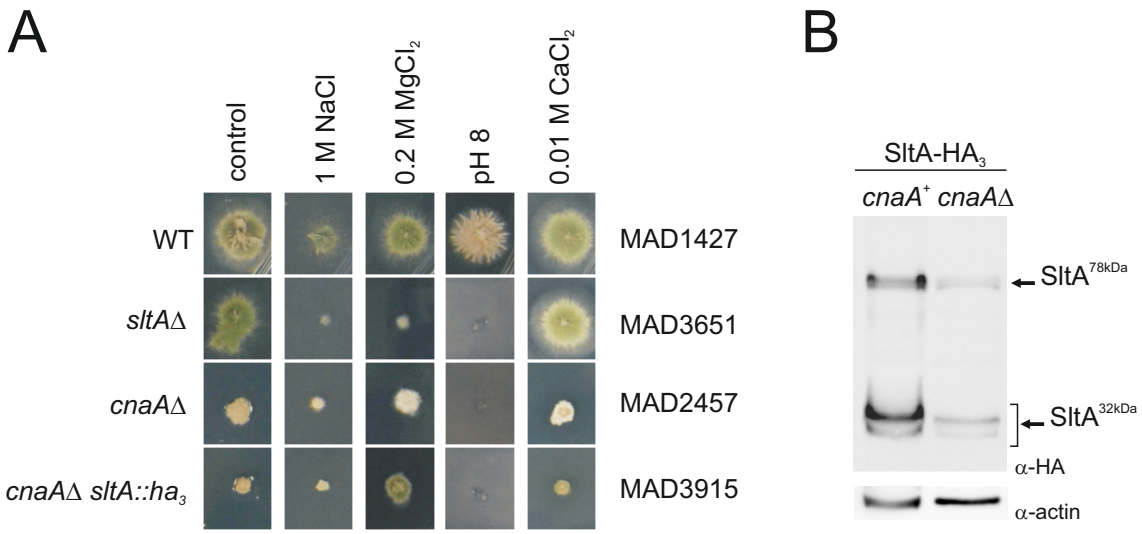

A
sltB56
*
TAA
locus sltB*
sltB56
*
TAA
gfp
SM
1
1272
GFP
SltB56-GFP
168 kDa
+
1
477
52.6 kDa
sltB
gfp
SM
1
477
GFP
SltB1-477-GFP
76.7 kDa
sltB
gfp
SM
TGA
1
477
SltB1-477
52.6 kDa
sltB56
*
TGA
locus sltB*
(mutagenized)
sltB56
*
TGA
gfp
SM
1
477
SltB56TGA
52.6 kDa
sltB
locus sltB
sltB
tag
SM
1
590
SltB1-590-GFP
GFP
93.12 kDa
1
590
SltB1-590-HA3
HA3
70.58 kDa
1
590
SltB1-590
TGA



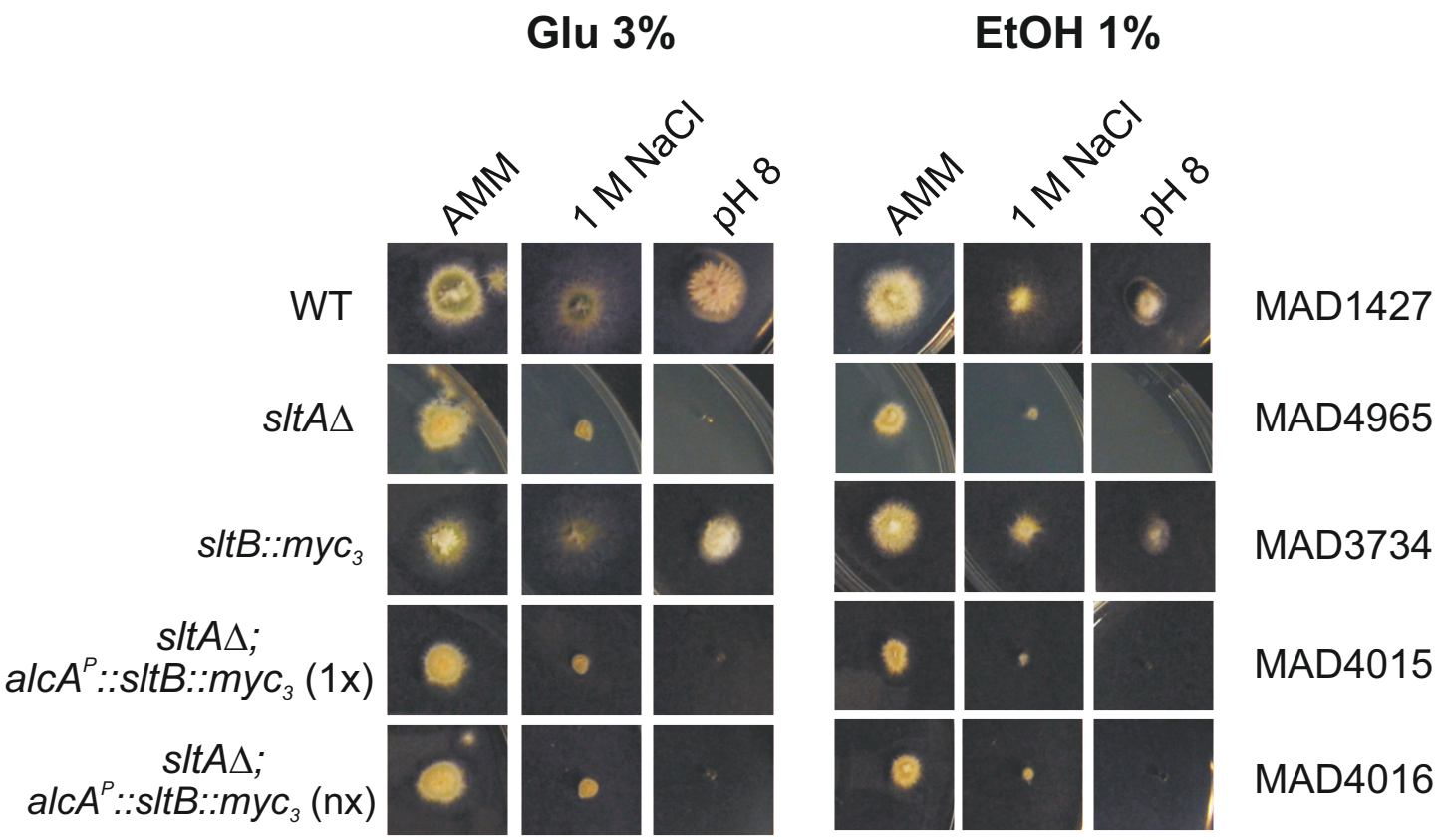
Supplemental Figure S3. Construction and phenotypic analysis of strains expressing mutant forms of SltB. (A) Schematic representation of the alleles generated by reverse genetics to study the roles of the Psk and protease domains of SltB. SM indicates selectable marker. (B) Growth tests on AMM to determine the responses of various *sltB* mutant strains to an elevated concentration of sodium (1 M NaCl) or alkalinity (pH 8). Colonies were photographed after two days' incubation at 37ºC.



Supplemental Figure S4. Absence of nuclear SltB protein. DAPI staining of nuclei required fixing of cells and this process reduced detection of fluorescence of SltB-GFP chimaeras (compare with Fig. 7). In all cases SltB-GFP fusions were cytoplasmic and excluded from nuclei. A representative hypha from each strain is shown, A) MAD3650, B) MAD3711, C) MAD4013 and D) MAD4899. Merged images of GFP (green) and DAPI (magenta) fluorescence are shown. Magnification of a nucleus is shown for each hypha. Bar = 5 μm



Supplemental Figure S5: Absence of calcineurin activity reduces levels of all forms of SltA. A) Phenotypic analysis of null *cnaA* strain expressing the SltA-HA_3 tagged protein. Strain MAD3915 was selected from progeny of a cross between strains MAD3652 and MAD2457, (see Supplemental Table S1). Strain MAD3915 showed identical morphology phenotype, compact morphology and sensitivity to sodium (1 M NaCl) and alkalinity (pH 8), to that displayed by parental strain MAD2457 (Soriani et al., 2008), both lacking the activity of calcineurin catalytic subunit. B) Detection of SltA-HA_3 forms in *cnaA* null and WT backgrounds. Notably, all SltA forms were detected in both strains without alteration in their electrophoretic mobilities. Relative to actin, levels were reduced for the three SltA-HA_3 forms.



Supplemental Figure S6. Phenotypic analyses of strains carrying the *alcA*-promoter-driven *sltB* coding region. Growth tests of gene replacement strains carrying a single copy (1x) or more than three copies (nx) of the *alcA*^{P}*::sltB::myc*$_3$ fusion construct. 3% glucose as carbon source is a non-inducing, repressing condition for expression for the *alcA* promoter whereas 1% ethanol as carbon source is an inducing, derepressing condition. Point inoculated colonies were grown for 48 h at 37ºC.