Binding the atypical RA domain of Ste50p to the unfolded Opy2p cytoplasmic tail is essential for HOG pathway

Irena Ekiel[*#], Traian Sulea[*], Gregor Jansen[†], Maria Kowalik[*], Ovidiu Minailiuc[*#], Jing Cheng[*#], Doreen Harcus[*], Miroslaw Cygler[*], Malcolm Whiteway[*‡] and Cunle Wu[*§¶]

Supplementary data

Figure S1. Sequence alignment of the RA-like domains of various fungal Ste50p orthologs. Conserved residues are highlighted in red.

Figure S2. **A)** Prediction of an intrinsically unstructured region in the cytoplasmic tail of Opy2p using metaPrDOS server [1]. **B)** HSQC spectrum of a recombinant ^{15}N-enriched fragment of Opy2p encompassing amino acid residues 194-333. Expanded region in the insert indicates high resolution of signals typical for mobile unstructured protein regions. **C)** Sequence alignment showing the conservation of the f-peptide among fungal Opy2 homologs.

Figure S3. **A)** The changes in the HSQC spectra of ^{15}N-labeled Ste50p-RA domain (black) in the presence of the synthetic s^{Opy2p} peptide (red).

Figure S4. Mutagenesis analysis of Ste50p-RA domain interaction surface with Opy2p. **A)** Yeast cells (*ste50Δ opy2Δ ssk1Δ*) transformed with *STE50* plasmids with indicated mutations in combination with indicated *OPY2* alleles were assayed for their ability to grow on hyperosmolarity media by serial dilutions. The mutations of Ste50p were indicated on the left, and the Opy2p mutants indicated on the top. The growth media selecting for both the *STE50* and *OPY2* plasmids was –ura –his (left panel of each combination);

and hyperosmotic media was 1.25 M sorbitol (right panel of each combination). **B)** Yeast cells (*ste50Δ FUS1-LacZ*) transformed with *STE50* plasmids with indicated mutations were assayed for their ability to response to pheromone-induced cell cycle arrest by halo assay (left panel), and pheromone-induced transcriptional activation of a *FUS1-LacZ* reporter (right panel). **C)** Ste50p mutant specifically defective in HOG pathway signaling. Yeast cells (*ste50Δ ssk1Δ*) transformed with *STE50* plasmids with indicated mutations were assayed for their ability to grow on hyperosmolarity media by serial dilutions (left panels), and assayed for their ability to response to pheromone-induced cell cycle arrest by halo assay (right panel).

Table S1. NMR and refinement statistics for the RA domain[a,b]

NMR distance and dihedral constraints	
Distance constraints	
Total NOE	1094
Intra-residue	264
Sequential (|i-j| = 1)	312
Medium-range (|i-j| <= 4) ≤	208
Long range (|i-j| >= 5) ≥	310
Total dihedral angle restraints	
ϕ	48
ψ	48
Hydrogen bonds	33
Deviations from idealized geometry	
Bond lengths (Å)	0.0130±0.00014
Bond angles (deg)	2.83±0.03
Average pairwise r.m.s. deviation (Å)	
Heavy atoms	1.15±0.15
Backbone	0.57±0.18
Ramachandran statistics[c] (%)	
Residues in most favorable regions	83.5
Residues in additionally allowed regions	15.6
Residues in generously allowed regions	0.9
Residues in disallowed regions	0.0

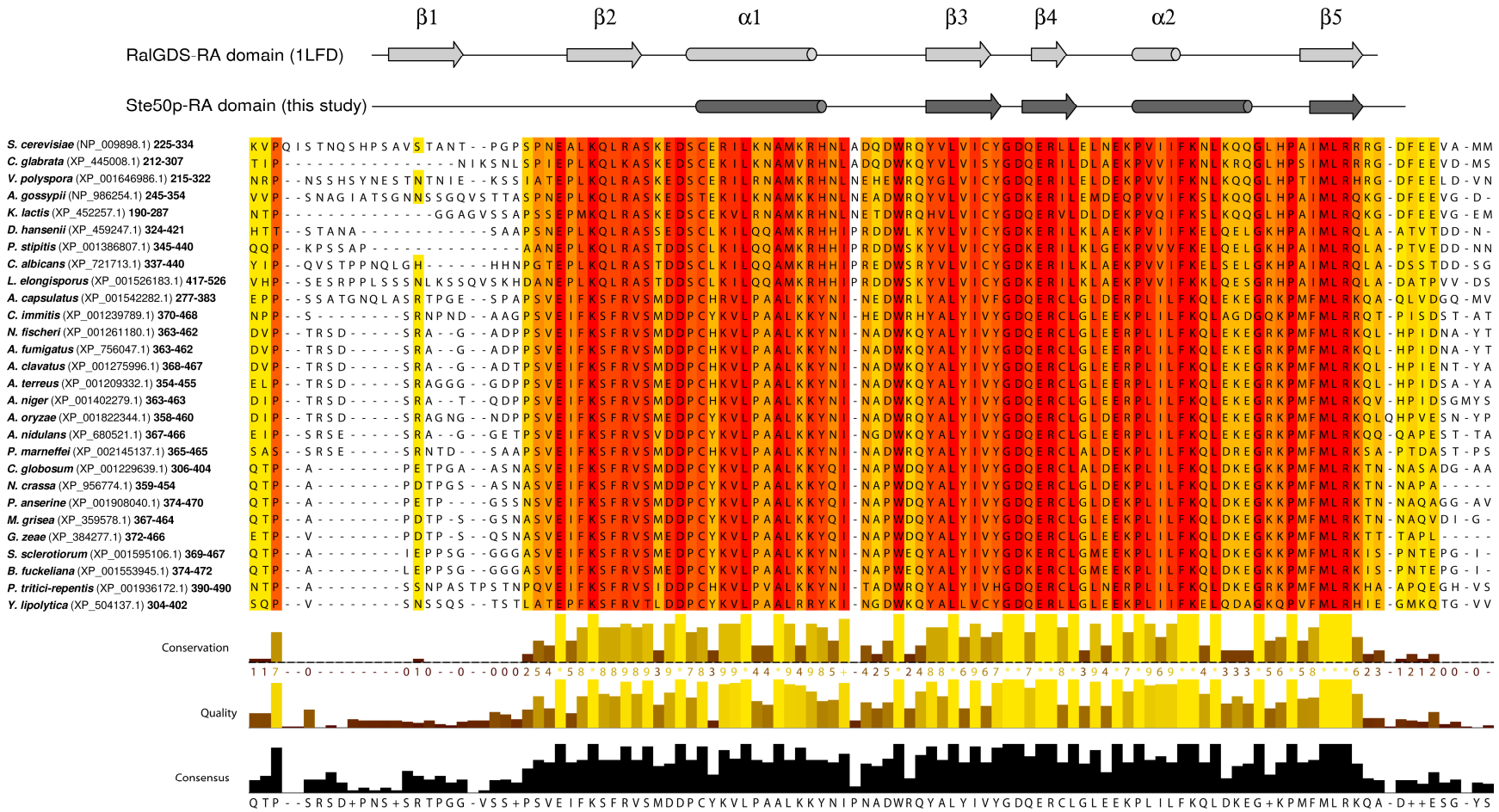
[a] Protein residues 26-93; [b] Twenty final structures were used in r.m.s. deviation calculations; [c] PROCHECK nomenclature

Table S2. Yeast strains used in this study

Strain	Relevant genotype	Source
BY4741	*MAT***a** *ura3 his3 leu2 trp1 met15*	ATCC
BY4742	*MATα ura3 his3 leu2 trp1 lys2*	ATCC
YCW365	*MAT***a** *ura3 leu2 his3 ssk2Δ::LEU2 ssk22Δ::LEU2 ste50Δ::TRP1*	Wu et al, 1999
YCW393	*MAT***a** *ura3 leu2 his3 trp1 ste50Δ::TRP1 sst1::hisG Fus1::LacZ::LEU2*	Wu et al., 1999
YCW888	*MAT***a** *ssk1Δ::Kan^R ura3 his3 leu2 met15*	ATCC
YCW1299	*MAT***a** *ste50Δ::Kan^R ura3 his3 leu2 met15*	ATCC
YCW1301	*MATα ssk1Δ::Kan^R ura3 his3 leu2 lys2*	ATCC
YCW1313	*MAT***a** *ste50Δ::Kan^R ssk1Δ::Kan^R ura3 his3 leu2*	This study
YCW1323	*MATα ssk1Δ::Nat^R ura3 his3 leu2 lys2*	Wu et al., 2006
YCW1363	*MAT***a** *ssk1Δ::Nat^R ura3 his3 leu2 lys2 met15*	Wu et al., 2006
YCW1377	*MAT***a** *opy2Δ::Kan^R ura3 his3 leu2 met15*	ATCC
YCW1380	*MAT***a** *opy2Δ::Kan^R ssk1Δ::Nat^R ura3 his3 leu2*	Wu et al., 2006
YCW1381	*MATα opy2Δ::Kan^R ssk1Δ::Nat^R ura3 his3 leu2*	This study
YCW1642	*MAT***a** *opy2Δ::Kan^R ste50Δ::Kan^R ssk1Δ::Nat^R ura3 his3 leu2*	This Study
YCW1643	*MATα opy2Δ::Kan^R ste50Δ::Kan^R ssk1Δ::Nat^R ura3 his3 leu2*	This Study
DC17	*MATα his1*	J. Hicks
DC16	*MAT***a** *his1*	J. Hicks

Referennce:

1. Ishida, T. & Kinoshita, K. Prediction of disordered regions in proteins based on the meta approach. *Bioinformatics* **24**, 1344-8 (2008).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β2
α1
β3
β4
α2
β5
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Ste50p-RA domain (this study)
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C. glabrata (XP_445008.1) 212-307
V. polyspora (XP_001646986.1) 215-322
A. gossypii (NP_986254.1) 245-354
K. lactis (XP_452257.1) 190-287
D. hansenii (XP_459247.1) 324-421
P. stipitis (XP_001386807.1) 345-440
C. albicans (XP_721713.1) 337-440
L. elongisporus (XP_001526183.1) 417-526
A. capsulatus (XP_001542282.1) 277-383
C. immitis (XP_001239789.1) 370-468
N. fischeri (XP_001261180.1) 363-462
A. fumigatus (XP_756047.1) 363-462
A. clavatus (XP_001275996.1) 368-467
A. terreus (XP_001209332.1) 354-455
A. niger (XP_001402279.1) 363-463
A. oryzae (XP_001822344.1) 358-460
A. nidulans (XP_680521.1) 367-466
P. marneffei (XP_002145137.1) 365-465
C. globosum (XP_001229639.1) 306-404
N. crassa (XP_956774.1) 359-454
P. anserine (XP_001908040.1) 374-470
M. grisea (XP_359578.1) 367-464
G. zeae (XP_384277.1) 372-466
S. sclerotiorum (XP_001595106.1) 369-467
B. fuckeliana (XP_001553945.1) 374-472
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Conservation
Quality
Consensus

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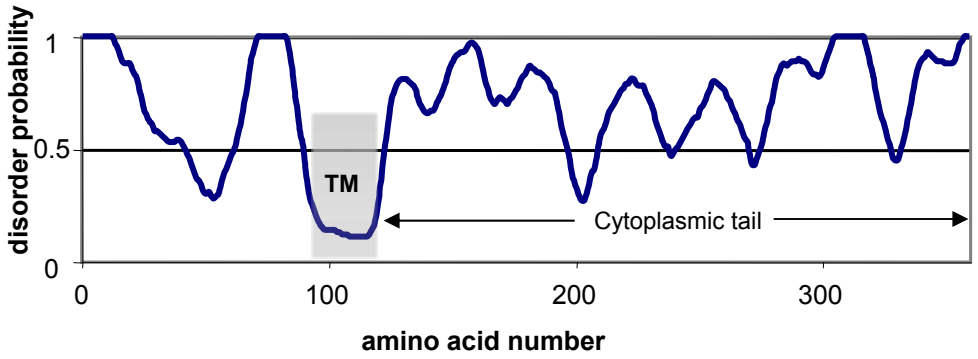

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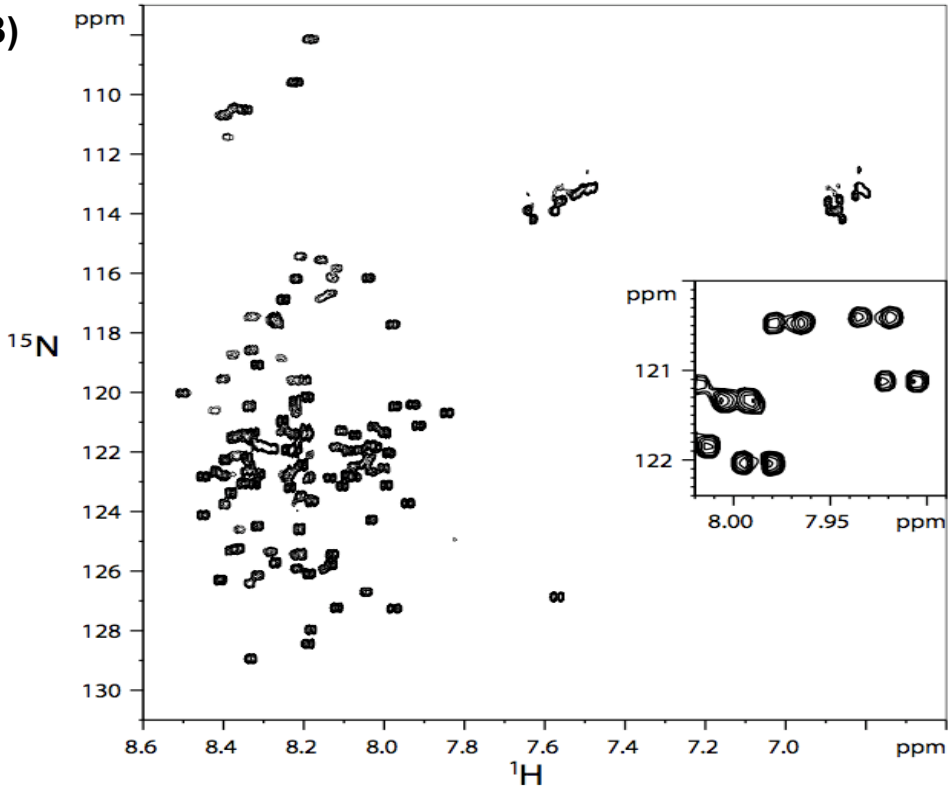

C)

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Vanderwaltozyma polyspora (XP_001642205.1)      191 QPGNRSSSS.TLHTK.ASNIIPIAYIPGVTTVDG 222
Candida glabrata (XP_445470.1)                  269 DAQSIYSNN.TVHTKAASNVLPIAYIPGVTINQM 301
Ashbya gossypii (NP_983729.1)                   185 QPRNRSSAA.TMATR.ASNVLPVAYIPGVTAVGK 216
Candida albicans (XP_717646.1)                  277 QPSNRNSVATSFS..NASNIIPIAYIPGVTVRPT 308
Lodderomyces elongisporus (XP_001527855.1)      362 QPSNRNSVATSFS..NASNIIPIAYIAGVTVRPT 393
Pichia guilliermondii (XP_001485612.1)          211 NPSHRNSVATTVSTTNASNIIPIAYIPGVTVRPT 244
Pichia stipitis (XP_001382684.2)                245 NNSNRNSVATSISTTNASNIIPIAYIPGVTVRPT 278
Debaryomyces hansenii (XP_460539.1)             198 NNSSRHSVATTMSTTNASNIIPIAYIPGVTVRPT 231
Gibberella zeae (XP_390291.1)                   141 STHTVHSIASTVLTR.ASNIIQIAYIPGVTNRAT 173
Botryotinia fuckeliana (XP_001557189.1)         132 STHTVGSIASTVLTR.ASNIIQIAYIPGVTNRST 164
Sclerotinia sclerotiorum (XP_001595769.1)        65 STHTVGSIASTVLTR.ASNIIQIAYIPGVTNRST  97
Neurospora crassa (XP_961340.2)                 143 STHTVGSVADTVFTR.ASNIIQIAYIPGVTNR.T 174
Podospora anserina (XP_460539.1)                143 STHTVHSIASTVLTR.ASNIIQIAYIPGVTNRAT 175
Chaetomium globosum (XP_001219819.1)            108 STHTTHSIASTVLTR.ASNIIQIAYIPGVTNRAT 140
Magnaporthe grisea (XP_364652.2)                149 STHTSHSVASTVLTR.ASNIIQIAYIPGVTNRAT 181
Pyrenophora tritici-repentis (XP_001931691.1)   135 STHTVASMASSVLTR.ASNIIQIAYIPGVTNRSG 167
Phaeosphaeria nodorum (XP_001796076.1)          135 STHTVASMASSVLTR.ASNIIQIAYIPGVTNRSG 167
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Aspergillus terreus (XP_001212680.1             136 SRKSTHSIASTVLTR.ASNVIQIAYIPGVTNRSP 168
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Ajellomyces capsulatus (XP_001536244.1)         133 RQSTVGSIASTVLTR.ASNVIQIAYIPGVTNRSP 165
Coccidioides immitis (XP_001246197.1)           126 RMSAVGSIASTVLTR.ASNVIQIAYIPGVTNRSP 158
Yarrowia lipolytica (XP_503709.2)               119 SSSTFASIASSALSR.ASNVIPIAYVPGVTSRGQ 151
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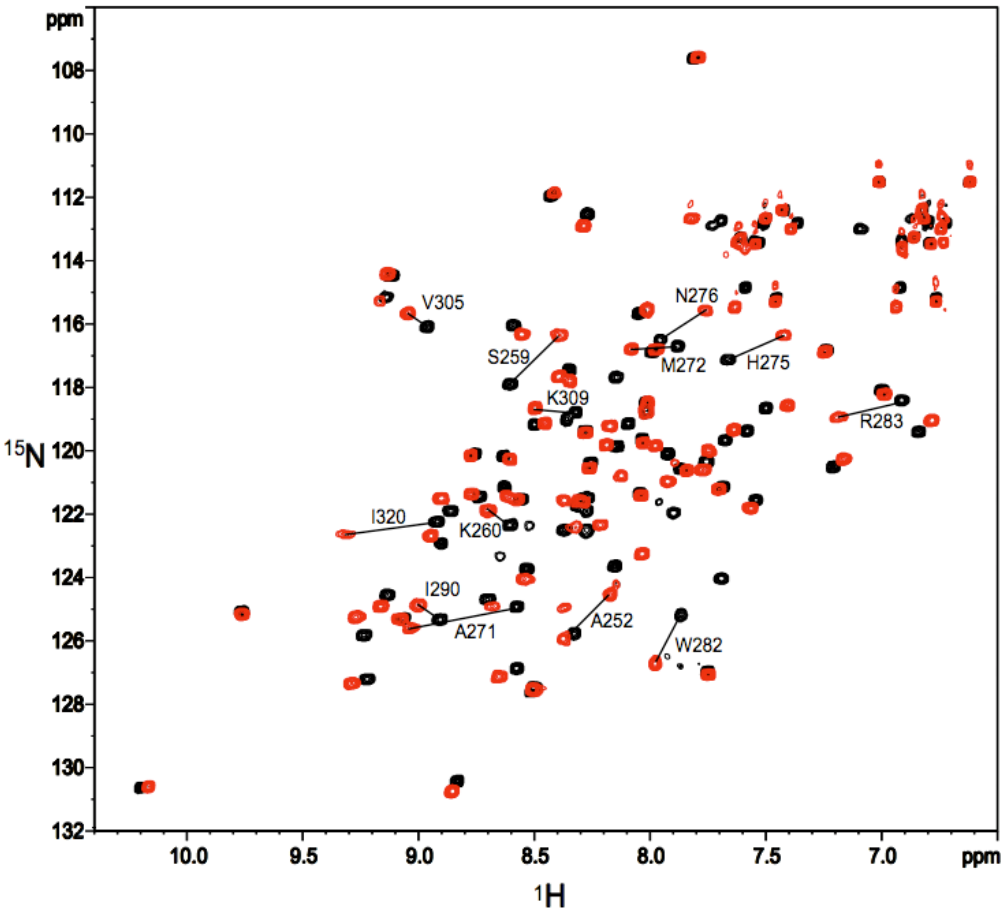
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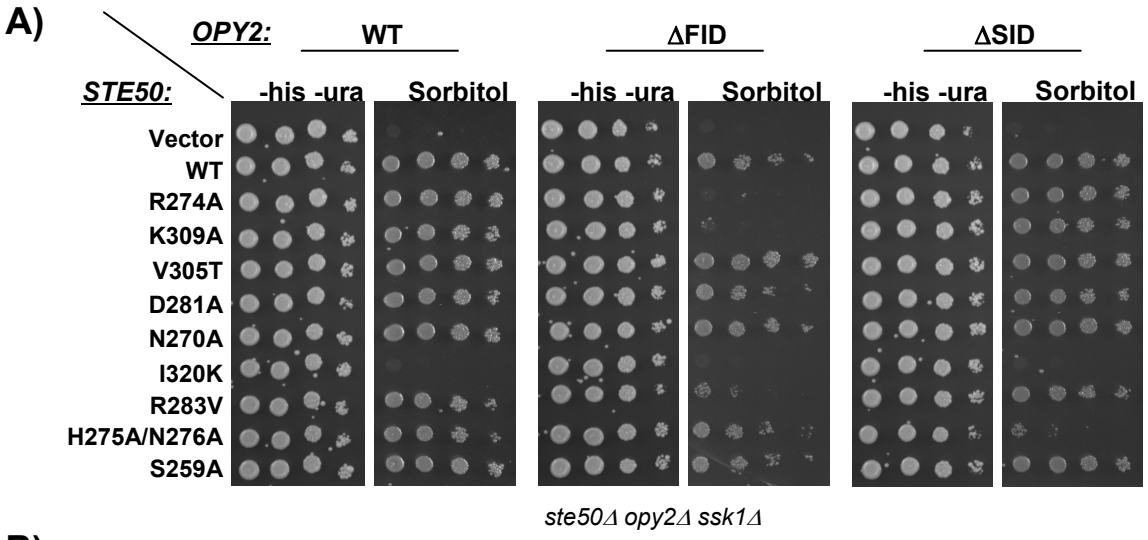
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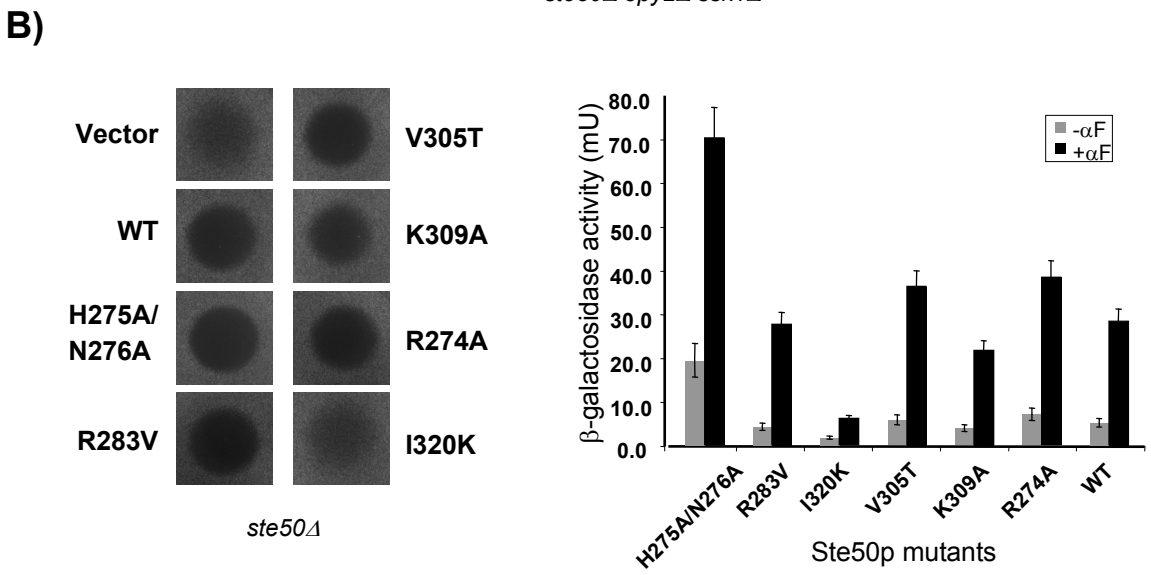
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60.0
50.0
40.0
30.0
20.0
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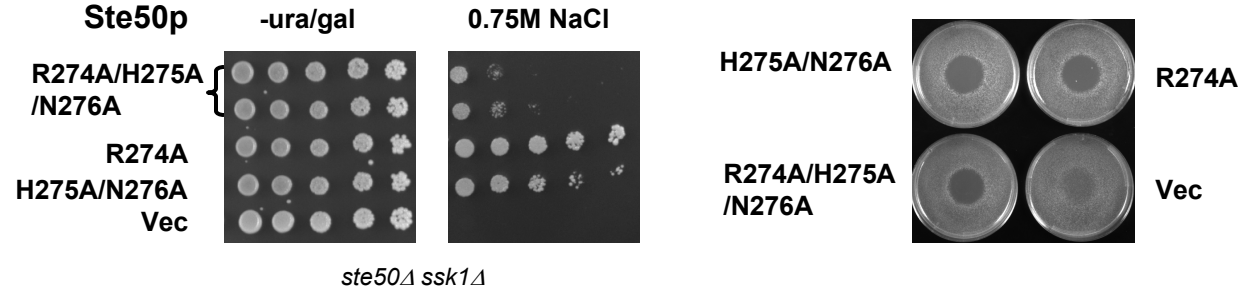
WT
Ste50p mutants


C)
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/N276A
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H275A/N276A
Vec
ste50Δ ssk1Δ
H275A/N276A
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