MS ID#: JBC/2011/234955

MS TITLE: Distinct docking mechanisms mediate interactions between Msg5 phosphatase and mating or cell integrity MAPKs in *S. cerevisiae*

Supplemental Table I. Oligonucleotides used for amplifying DNA fragments by PCR:

Plasmid	Sense oligonucleotide	Antisense oligonucleotide
pGBKT7-MSG5	5'GGAATTCCATATGATGCAATTTCACT CAGATAAGC 3'	5'CCGCTCGAGTTAAGGAAGAAACATC ATCTG 3'
pGBKT7-Msg5$^{1-245}$	5'GGAATTCCATATGATGCAATTTCACT CAGATAAGC 3'	5'CCGCTCGAGATATAGATACAAATTAG GCGGC 3'
pGBKT7-Msg5$^{246-489}$	5'GGATTCCATATGTATTCAGAACCCAA ACTAGAAG 3'	5'CCGCTCGAGTTAAGGAAGAAACATC ATCTG 3'
pGBKT7-Msg5$^{124-489}$	5'GGAATTCCATATGACAGTATCGTCTA TCAGTAAG 3'	5'CCGCTCGAGTTAAGGAAGAAACATC ATCTG 3'
pGBKT7-Msg5$^{1-123}$	5'GGAATTCCATATGATGCAATTTCACT CAGATAAGC 3'	5'CCGCTCGAGGGATGATTTTGTATACA CGCTTGG 3'
pGBKT7-Msg5$^{90-489}$	5'GGATTCCATATGCCACCATCGCTGTC GATGAGG 3'	5'CCGCTCGAGTTAAGGAAGAAACATC ATCTG 3'
pGBKT7-Msg5$^{1-45}$	5'GGAATTCCATATGATGCAATTTCACT CAGATAAGC 3'	5'CCGCTCGAGTAATGGATGGAGTGCTG CTATATC 3'
pGBKT7-Msg5$^{46-489}$	5'GGAATTCCATATGGAATTCTCATCGC CAAGC 3'	5'CCGCTCGAGTTAAGGAAGAAACATC ATCTG 3'
pGADT7-Kss1	5'GGAATTCCATATGGCTAGAACCATAA CTTTTGAT 3'	5'CCGCTCGAGCTATTCCATGGTCTTCA TTAGTTC 3';
pGADT7-Fus3	5'GGAATTCCATATGCCAAAGAGAATTG TATACAAT 3'	5'CGCGGATCCCTAACTAAATATTTCGT TCCAAATGAG 3'
pGADT7-Smk1	5'GGAATTCCATATGAATTGCACACTTA CAGATAAT 3'	5'CCGCTCGAGCTATAAAGACGAGGAG GACAATCGGT 3'
pGADT7-Slt2	5'GGAATTCCATATGGCTGATAAGATAG AGAGGCAT 3'	5'CCGCTCGAGCTAAAAATATTTTCTAT CTAATCC 3'
pGADT7-Mlp1	5'GGAATTCCATATGGCGACTGACACCG AGAGGTGT 3'	5'CCGCTCGAGTTAGTTAACACCCTGAA ATGAATTAG 3'
pGADT7-Hog1	5'GGAATTCCATATGACCACTAACGAGG AATTCATTAGG 3'	5'CCGCTCGAGTCATTTGCAGCTACATG ATCGCTG 3'
pEG(KG)-Slt2$^{274-373}$	5´CCCCCCGGGATCCTTCATTCCAAAAG TACC 3´	5´CCCCCCGGGATCCCTAAAAATATTTT CTATC 3´
pET15B- Slt2-His	5´ACGCGTCGACTTCATATGATGGCTGA TAAGATAGAGAG 3´	5´TCGCGTCGACCTCGAGCTAAAAATAT TTTCTATCTAATCCAAAC 3´
pET15B- Slt2$^{1-273}$-His	5´ACGCGTCGACTTCATATGATGGCTGA TAAGATAGAGAG 3´	5´TCGCGTCGACCTCGAGCTAACCTAAT TGATGTATGTAGTCC 3´
pET15B- Slt2$^{1-373}$-His	5´ACGCGTCGACTTCATATGATGGCTGA TAAGATAGAGAG 3´	5´TCGCGTCGACCTCGAGCTATAATTGC CTTTGCTCTTCTAATAG3´
pET15B- Slt2$^{274-373}$-His	5´ACGCGTCGACCCCATATGTTCATTCC AAAAGTACCTTTTGTC 3´	5´TCGCGTCGACCTCGAGCTATAATTGC CTTTGCTCTTCTAATAG 3´
pET15B- Slt2$^{274-373(323N,326N,327N)}$-His	5´ACGCGTCGACCCCATATGTTCATTCC AAAAGTACCTTTTGTC 3´	5´TCGCGTCGACCTCGAGCTATAATTGC CTTTGCTCTTCTAATAG 3´
pET15B- Mlp1$^{274-373}$-His	5´ACGCGTCGACCCCATATGAATATCCC GGGAAGATCGTTTG 3´	5´TCGCGTCGACCTCGAGTTATGATGGG GAATCACCGC 3´
pET15B- Mlp1$^{274-373(326N)}$-His	5´ACGCGTCGACCCCATATGAATATCCC GGGAAGATCGTTTG 3´	5´TCGCGTCGACCTCGAGTTATGATGGG GAATCACCGC 3´

Supplemental Table II: Oligonucleotides used for directed mutagenesis by PCR:

Plasmid	Sense oligonucleotide*	Antisense oligonucleotide*
pGBKT7-Msg5^{MD1}	5'tccttacaaaatGCgaataccGCaaatGCatct GCagatatagcagca 3'	5'tgctgctatatctGCagatGCatttGCggtattcGC attttgtaagga 3'
pGBKT7-Msg5^{MD2}	5'tcgctctcgatgGCCGCCagcgaggcctct 3'	5'agaggcctcgctGGCGGCcatcgacagcga 3'
pGADT7-Kss1^{CD}	5'catgacccaagtAatgagccggaatat 3'	5'atattccggctcatTacttgggtcatg 3'
pGADT7-Fus3^{CD}	5'cacgatccaaatAacgaacctgaaggc 3'	5'-gccttcaggttcgtTatttggatcgtg 3'
pGADT7-Slt2^{CD}	5'tggcatgatccagctAacgaacttgtgtgt 3'	5'acacacaggttcgtTagctggatcatgcca 3'
pGADT7-Slt2^{CD3}	5'ttgtctatatggcatAatccagctAacAaTcctgt gtgtagtgaa 3'	5'ttcactacacacaggAtTgtTagctggatTatgccat atagacaa 3'
pGADT7-Mlp1^{CD}	5'tgtggcatAatataAatgaggaattctcatgtc 3'	5'cctcatTtatatTatgccacattgacaaatatgg 3'
YCplac22MSG5^{MD1}	5´ggaataccaaaaatGCatctGCagatatagcag cactccatcc 3´	5´ggatggagtgctGCtatatctGCagatgcatttttggt attcc 3´
YCplac22MSG5^{MD2}	5´ccaccatcgctgtcgatgGCGGCaagcgaggc ctc 3´	5´gaggcctcgcttGCCGCcatcgacagcgatggtg g 3´
YCplac22MSG5^{MD3}	5'agcgaggcctctGCaGCTGcactaccaa catctttgaagaaccgaactg 3'	5'agatgttggtagtgCAGCtGCagaggcctcg cttcgcctcatcgacagc 3'
YCplac111-Slt2$^{1-373}$	5´gctattagaagaccaaaggcaattaTGATGAc agcagcagcaacagcagc 3´	5´gctgctgttgctgctgctgTCATCAtaattgcctttg gtcttctaatagc 3´
pGBKT7-Msg5^{MD3}	5'agcgaggcctctGCaGCTGcactaccaa catctttgaagaaccgaactg 3'	5'agatgttggtagtgCAGCtGCagaggcctcg cttcgcctcatcgacagc 3'

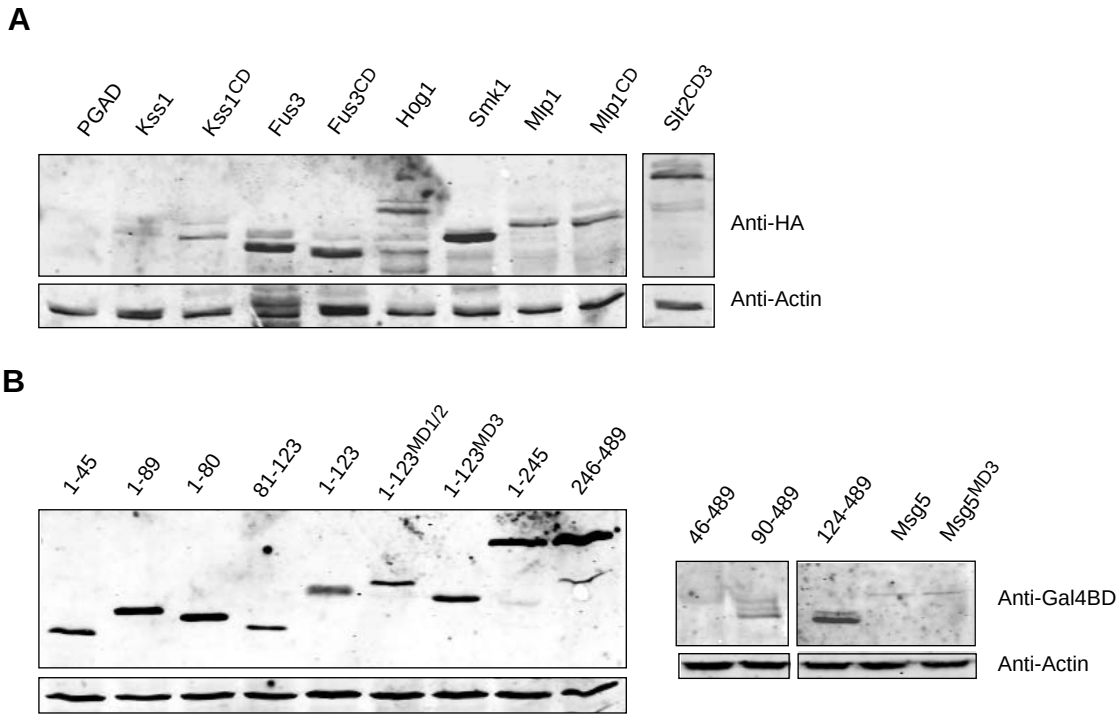
* Nucleotide changes are indicated in upper case

Supplemental Figure Legends

FIGURE S1: **Expression of hybrid proteins used in the two-hybrid assays**. *A*, western blot analysis showing expression of fusion proteins of each of the MAPKs or the indicated mutant versions to the Gal4 activation domain. *B,* western blot analysis showing expression of fusion proteins of Msg5 or the indicated Msg5 fragments or mutated versions to the Gal4 DNA-binding domain. PJ69-4A and PJ69-4α cells were transformed with pGADT7 or pGBKT7-derived plasmids and the amount of the respective proteins in whole extracts monitored using an anti-HA antibody in *A*, or an antibody against the Gal4p DNA-binding domain (anti-Gal4BD) in *B*. Actin (as a loading control) was immunodetected by using anti-actin antibodies. Reproducible results were obtained in different experiments and selected images correspond to representative blots.

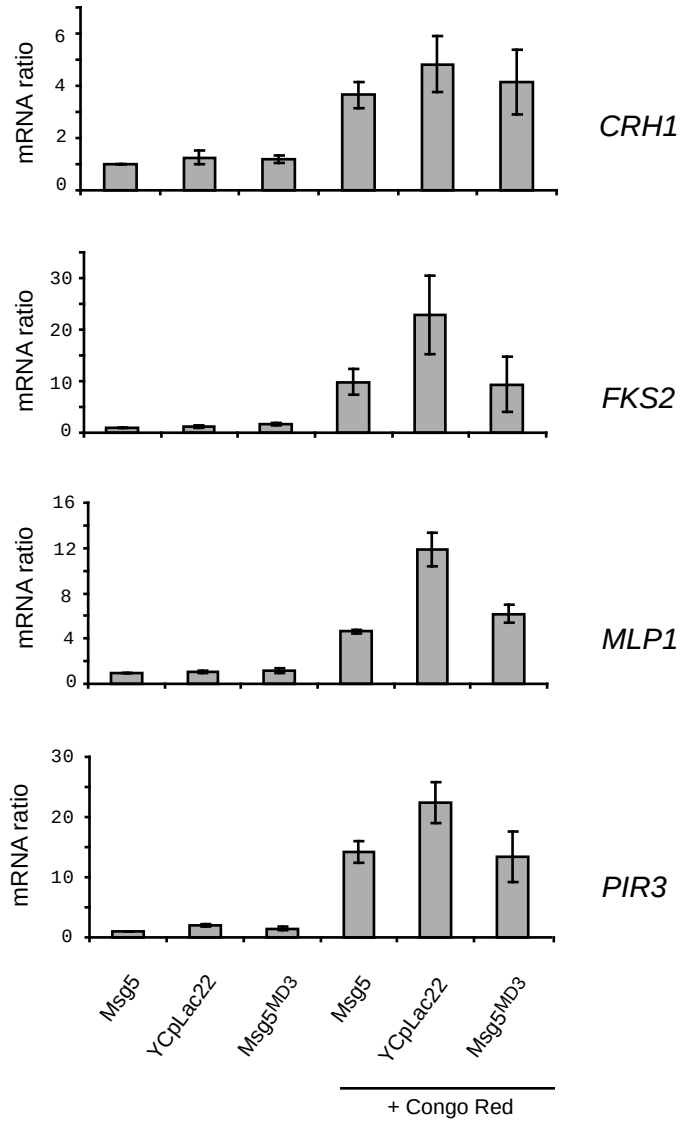
FIGURE S2: **Expression analysis of CWI-regulated genes**. Analysis by real time Q-RT-PCR of the expression level of the indicated genes in the *msg5Δ* DD1–2D mutant strain transformed with the vector YCplac22m, or plasmids YCplac22MSG5m (Msg5) or YCplac22MSG5^{MD3} (Msg5^{MD3}) The expression of *YGR189C* (*CRH1*), *YGR032W* (*FKS2*), *YKL161C* (*MLP1*), and *YKL163W* (*PIR3*) genes was determined in the absence or the presence of Congo red (30μg/ml). The results are expressed as the ratios of the values from the different strains or conditions versus values from untreated *msg5Δ* cells transformed with YCplac22MSG5m. Each cDNA was assayed in at least duplicate PCR reactions. Results are the mean from three different transformants. Error bars represent standard deviation.

FIG. S1


A
PGAD
Kss1
Kss1CD
Fus3
Fus3CD
Hog1
Smk1
Mlp1
Mlp1CD
Slt2CD3
Anti-HA
Anti-Actin
B
1-45
1-89
1-80
81-123
1-123
1-123MD1/2
1-123MD3
1-245
246-489
46-489
90-489
124-489
Msg5
Msg5MD3
Anti-Gal4BD
Anti-Actin

-

FIG. S2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2
4
6
CRH1
mRNA ratio
0
10
20
30
FKS2
mRNA ratio
0
4
8
12
16
MLP1
mRNA ratio
0
10
20
30
PIR3
Msg5
YCpLac22
Msg5MD3
Msg5
YCpLac22
Msg5MD3
+ Congo Red