Supplementary data Figure S1

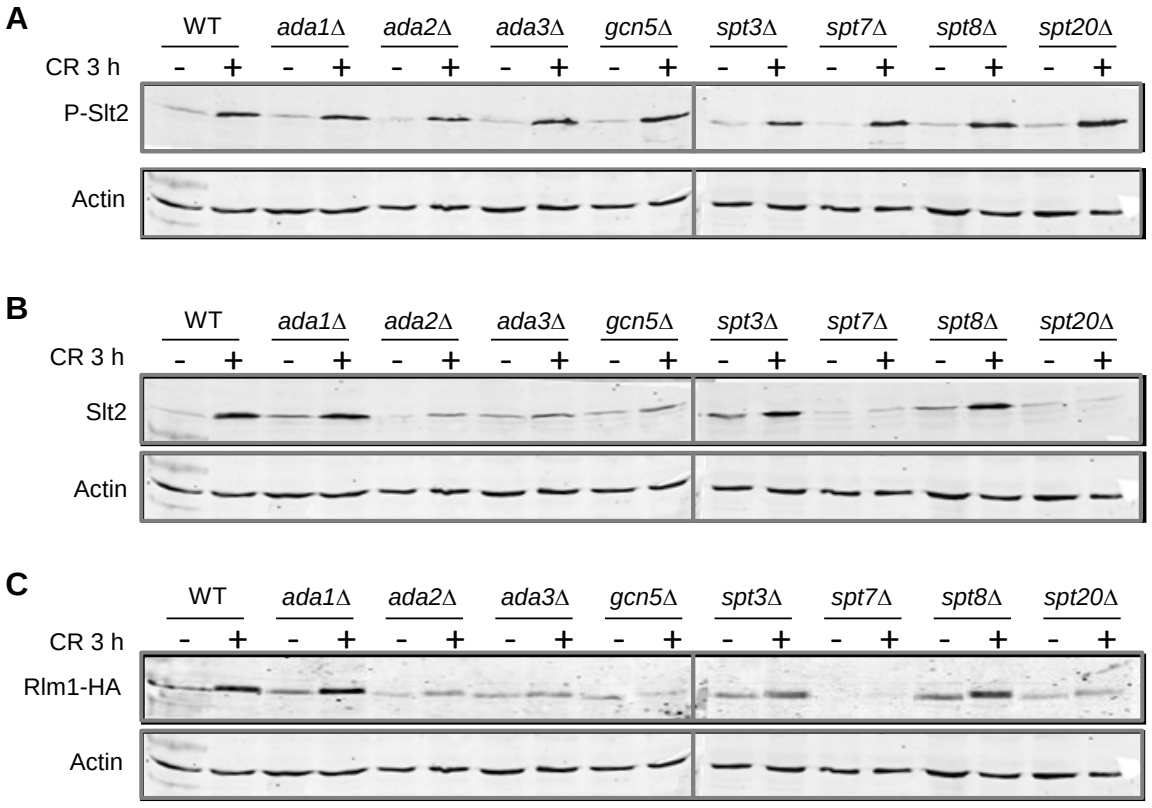

Figure S1. Activation of the CWI pathway in several mutants of the SAGA complex. (A) Slt2 activation was analyzed by western blotting using anti-phospho-p44/42 MAPK Ab (Thr202/Tyr204; Cell Signalling Technology, Beverly, MA) in WT and in the indicated *saga* mutants exposed to CR during 3 hours. **(B)** Total Slt2 protein was analyzed using an anti-Slt2 mAb (E-9 clone, Santa Cruz Biotechnology, Inc.), in the same strains and conditions. **(C)** Levels of Rlm1 were analyzed in WT and indicated *saga* mutants expressing Rlm1-HA from a centromeric plasmid (pRS416-RLM1HA) after CR treatment (3 hours), using an anti-HA mAb (HA.11 16B12 clone Covance Research Products, Inc.). The protein load was monitored using a mouse anti-actin mAb C4 (ICN Biomedicals, Aurora, OH).

Supplementary data Figure S2

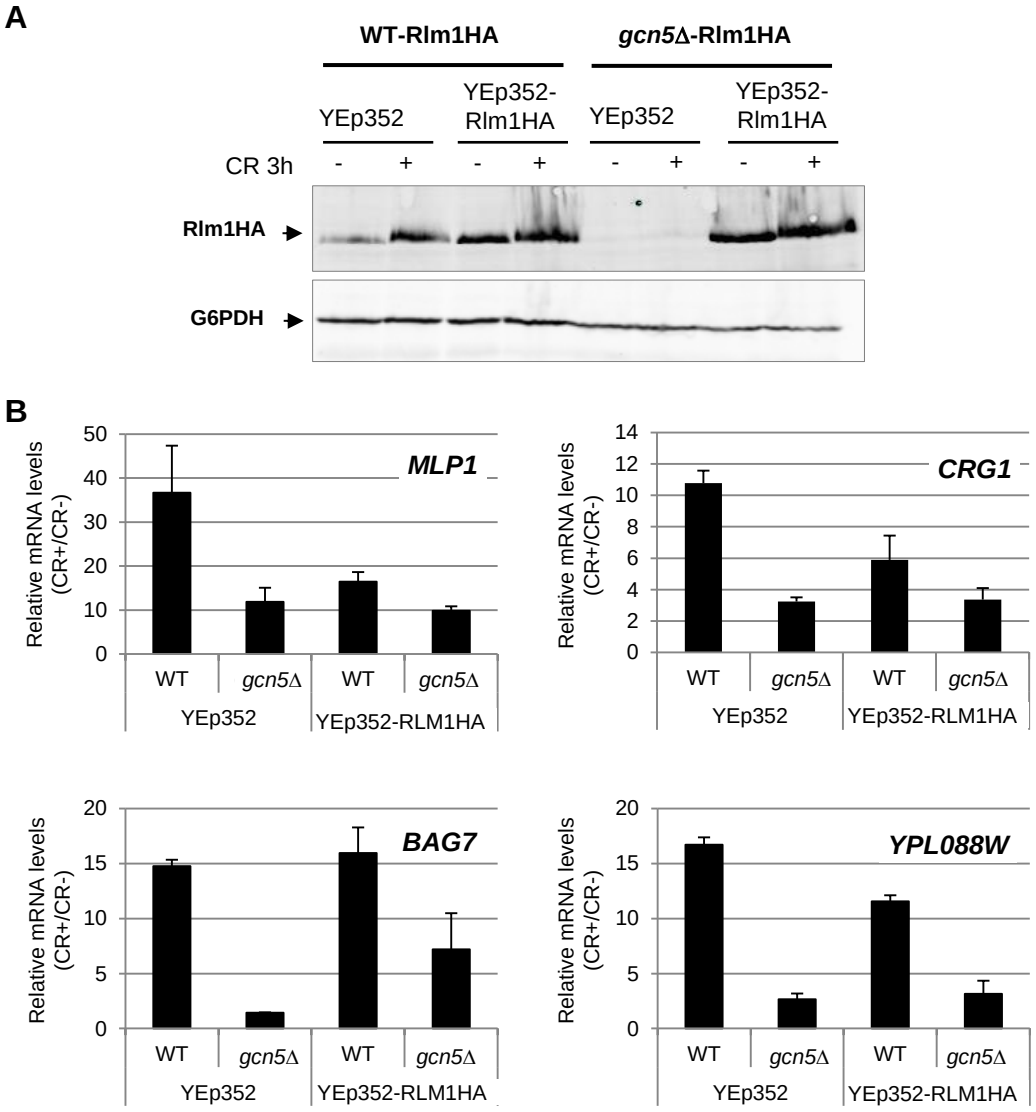

Figure S2: Rlm1 overexpression does not suppress the transcriptional defects of a *gcn5*Δ mutant. (A) Expression of Rlm1-HA under the control of native *RLM1* promoter from an episomal plasmid restores levels of Rlm1 similar to those found in a WT strain both under basal and stress conditions. WT and *gcn5*Δ strains expressing the Rlm1-HA tagged protein were transformed with YEp352-RLM1HA and the corresponding empty vector YEp352 and exposed to cell wall stress (CR 30 µg/ml, 3 hours). The amount of Rlm1-HA was determined by western blotting using an anti-HA mAb. Protein load was monitored using an anti-G6PDH Ab. **(B)** Overexpression of Rlm1 in a *gcn5*Δ mutant strain does not restore levels of transcriptional induction of CWI-responsive genes of a WT strain under cell wall stress conditions. mRNA levels of *MLP1, CRG1, BAG7* and *YPL088W* were analyzed by RT-qPCR in WT and *gcn5*Δ cells transformed with YEp352-RLM1HA or YEp352 and subjected or not to cell wall stress (CR 30 µg/ml, 3 hours). Levels of mRNA are expressed relative to the *ACT1* control, representing the ratio between CR-treated and non-treated cells. Data represent the mean and standard deviation of at least three independent experiments.

Supplementary data Figure S3

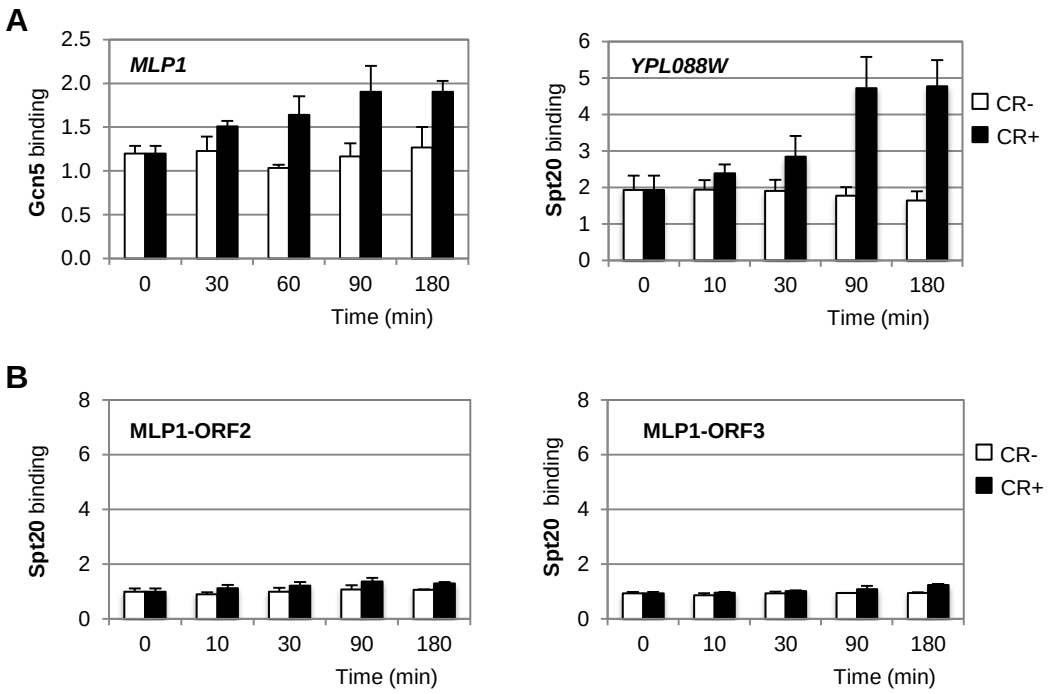

Figure S3: SAGA complex binds to *MLP1* and *YPL088W* promoters but not to *MLP1* coding regions after CR treatment . (A) The recruitment of Gcn5 (left panel) and Spt20 (right panel) was analyzed by ChIP in WT strains expressing Gcn5-myc and Spt20-myc, at MLP1-BOX1 and YPL088W-BOX1 regions respectively, in the absence and presence of CR at the indicated times. **(B)** Recruitment of Spt20 was determined by ChIP in a WT strain expressing Spt20-myc, at MLP1-ORF2 and MLP1-ORF3 coding regions, in the absence and presence of CR at the indicated times. Data represent the mean and standard deviation of at least three independent experiments.

Supplementary data Table S1

Table S1: Primers used in this work

ChIP (locations are indicated by the distance from the respective ATG initiation codon)

Primer name	Sequence (5´-3´)	Position
MLP1		
MLP1-BOX1 UP	AATTTCACTGTTTCCGTTAACTGTTG	-453
MLP1-BOX1 DW	TCCTACGGTGCTTCCTGCAT	-313
MLP1-POL II UP	GCAAAAAGCCTATTTAATGTAAGTCC	-143
MLP1-POL II DW	AGGATAAAATCTTGGCCGAATG	56
MLP1-ORF2 UP	CGACGGCTTCATTAAGGGTTATA	555
MLP1-ORF2 DW	CCTACCAAGTAGTTCGGCCAAG	687
MLP1-ORF3 UP	TCCTATGTAAGCCAACTTCCATCA	1210
MLP1-ORF3 DW	TCCGTTCAGTAGTTCACTATTTTCATG	1359
YLR194C		
YLR194C-BOX UP	AATGGCTGGCTTTAGAGTGTCAG	-254
YLR194C-BOX DW	TTCAGCTACCTCAGTCGCTAAAAAT	-123
YLR194C-POL II UP	TGAAAAACGCCTACTTTCTCAGAA	-130
YLR194C-POL II DW	GTCTTTTTGAGCAGCGGCTAGA	60
YLR194C-ORF2 UP	GAGAGACGTCAAGCGGAATC	359
YLR194C-ORF2 DW	AAGCCGTGTTGTCAGTTGTG	520
YLR194C-ORF3 UP	GGTAGCGTCCGTAATGTCCAA	666
YLR194C-ORF3 DW	TGCAGAACTATGTGGTGGTATACAGA	805
YPL088W		
YPL088W-BOX1 UP	ACGTTTCTTTTTGTCTGTCCTTT	-573
YPL088W-BOX1 DW	CGGGTGATGAATGCCAAGC	-423
YPL088W-POL II UP	ATTAGGACCCAGAGAACAAGGT	-91
YPL088W-POL II DW	TCGGTGATATCTTAAGACCTGAGT	55
YPL088W-ORF2 UP	CTGCAGTTCACAGCCGATAA	562
YPL088W-ORF2 DW	CTCGTGCGTTAGGAGACCAT	712
YPL088W-ORF3 UP	TGGGATTGAACACTACAGCAAGA	911
YPL088W-ORF3 DW	CCTTCTATATATCAGTGGGCCCATG	1064
VMA8		
VMA8 UP	CACAGATGAGCTCCACCGATT	-321
VMA8 DW	GGTGTAACTGCGGCAATTGTT	-191

RT-qPCR

Gene	Primer name	Sequence (5´-3´)
MLP1	MLP1-UP	TGAATTATCAAGAATGCACAAAAGC
	MLP1-DW	TCCTTCCCTTCAAACATTGGTT
YPL088W	YPL088W-UP	ATGGCTTCCTAACGCACGAG
	YPL088W-DW	GTCCTTCGACACCTTTTCCA
CRG1	YHR209W-UP	TCGATTTGGAGATATTGAAGTCACA
	YHR209W-DW	GCATTTGGGTCCGAAGGA
BAG7	YOR134W-UP	GGCATCAAAGACCCTACAGAAAGA
	YOR134W-DW	TGACTGTCGTTGTTTGTGTTTGG
YLR194C	YLR194C-UP	GGTAGCGTCCGTAATGTCCAA
	YLR194C-DW	CCCGCACCATAAGCTATGTGA
SRL3	YKR091W-UP	TTCGTCCAAACATGCCAAAA
	YKR091W-DW	GACCAACGTAACGGCGAAA
PRM5	YIL117C-UP	AGACATAAGGAAACCCGCAAAAA
	YIL117C-DW	ACGATTTACGCTACCATCACTTTCT
ACT1	ACT-UP	ACGAAAGATTCAGAGCCCCA
	ACT-DW	GCAGATTCCAAACCCAAAACA