

TABLE S1. Yeast strains used in this study.		
Strain	Genotype	Source
JRY2726	<i>MATa his4</i>	P. Schatz
BY4741	<i>S288C MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	(BRACHMANN <i>et al.</i> 1998)
W303	<i>MATα ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100</i>	R. Rothstein
AKY1677	<i>MATα HMRae** ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100</i>	(MILLER <i>et al.</i> 2010) ¹
AKY2399	<i>MATα HMRae** ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 cac1Δ::KanMX</i>	(MILLER <i>et al.</i> 2010) ¹
AKY8786	<i>MATα HMRae** ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 cac1Δ::KanMX sir2Δ::TRP1</i>	This Study
AEY2363	<i>MATα HMRae** ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 asf1Δ::KanMX</i>	(MEIJSING AND EHRENHOFER-MURRAY 2001) ¹
AKY1664	<i>MATα HMRae** ade2-1 his3 leu2-3, 112 ura3 can1-100 cdc7-90</i>	This Study ¹
AKY1912	<i>MATα HMRae** ade2-1 his3 leu2-3, 112 ura3-1 can1-100 cdc7-90 SIR2Δ::HIS3</i>	This Study
AKY5478	<i>MATα HMRae** ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 SIR2Δ::URA3</i>	This Study
AKY1744	<i>MATα HMRae** ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3 pPK189</i>	(MILLER <i>et al.</i> 2008) ¹
AKY1692	<i>MATa HMR::ADE2 ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3 pPK189</i>	(MILLER <i>et al.</i> 2008) ¹
AKY2809	<i>MATa HMR::ADE2 ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3 cac1Δ::KanMX pPK189</i>	(MILLER <i>et al.</i> 2008) ¹
AEY474	<i>MATα HMRae** ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 sas2Δ::TRP1</i>	(MEIJSING AND EHRENHOFER-MURRAY 2001)
AKY1665	<i>MATα HMRae** ade2-1 his3 leu2-3, 112 ura3 can1-100 cdc7-90 bob1-1</i>	This Study
AKY6219	<i>MATα HMRae** ade2-1 his3 leu2-3, 112 ura3 can1-100 bob1-1</i>	This Study
AKY6382	<i>MATα HMRae** ade2-1 his3 leu2-3, 112 ura3 can1-100 bob1-1 cdc7Δ::NatMX</i>	This Study
AKY1785	<i>MATa HMR::ADE2 ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 pol30Δ::hisG pPK169</i>	(MILLER <i>et al.</i> 2008) ¹
AKY2821	<i>MATa HMR::ADE2 ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 pol30Δ::hisG cac1Δ::KanMX pPK169</i>	This Study
AKY6019	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 CDC7-GFP(S65T)-HIS3MX</i>	(HUH <i>et al.</i> 2003) ¹
AKY6236	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 CDC7-GFP(S65T)-HIS3MX pAK1185</i>	This Study
AKY6238	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 CDC7-GFP(S65T)-HIS3MX pAK1166</i>	This Study

AKY6248	<i>MATa HMRae** ade2-1 his3 leu2-3,112 ura3 trp1-1 can1-100 bar1Δ::KanMX CAC1-3x FLAG</i>	This Study
AKY6254	<i>MATa HMRae** ade2-1 his3 leu2-3,112 ura3 trp1-1 can1-100 bar1Δ::KanMX cdc7-90 bob1-1 CAC1-3xFLAG</i>	This Study
AKY6159	<i>MATa HMRae** ade2-1 his3 leu2-3,112 ura3 can1-100 cdc7-90 cac1Δ::KanMX</i>	This Study
AKY6158	<i>MATα HMRae** ade2-1 his3 leu2-3,112 ura3 can1-100 cdc7-90 cac1Δ::KanMX</i>	This Study
¹ Parental Strains used for this study. See Supplementary Table 2 for description of plasmids that were introduced into the above strains during strain construction or for experiments described in text.		

TABLE S2. Plasmids used in this study.		
Plasmid	Description	Source
AE778	<i>6myc-SAS2 ARS/CEN/LEU2</i>	(MEIJSSING AND EHRENHOFER-MURRAY 2001)
pAK1347	<i>6myc-SAS2-M1 ARS/CEN/LEU2</i>	This Study
pRS415	<i>ARS4/CEN6/LEU2</i>	(CHRISTIANSON <i>et al.</i> 1992)
pPK189	<i>HHT2-HHF2 ARS/CEN/URA3</i>	P. Kaufman
pMP3	<i>HHT2-HHF2 ARS/CEN/TRP1</i>	(KELLY <i>et al.</i> 2000)
pAK1156	<i>H3 T45A H4 ARS/CEN/TRP1</i>	This Study
pAK972	<i>H3 H4 K16R ARS/CEN/TRP1</i>	(MILLER <i>et al.</i> 2008)
pRS303	<i>HIS3</i>	(SIKORSKI AND HIETER 1989)
pRS306	<i>URA3</i>	(SIKORSKI AND HIETER 1989)
pRS416	<i>ARS4/CEN6/URA3</i>	(CHRISTIANSON <i>et al.</i> 1992)
pAK1223	<i>CAC1 ARS/CEN/URA3</i>	This Study
pAK1224	<i>CAC1-3xFLAG ARS/CEN/URA3</i>	This Study
pAK1225	<i>cac1 S503A ARS/CEN/URA3</i>	This Study
pAK1226	<i>cac1 S503D ARS/CEN/URA3</i>	This Study
pAK1228	<i>cac1 S238D ARS/CEN/URA3</i>	This Study
pAK1229	<i>cac1 S238A ARS/CEN/URA3</i>	This Study
pAK1308	<i>cac1 S238, 503D ARS/CEN/URA3</i>	This Study
pAK1309	<i>cac1 S238, 503A ARS/CEN/URA3</i>	This Study
pAK1328	<i>cac1 S501, 503D ARS/CEN/URA3</i>	This Study
pAK1329	<i>cac1 S501, S503A ARS/CEN/URA3</i>	This Study
pAK1333	<i>cac1 S503A-3xFLAG ARS/CEN/URA3</i>	This Study
pAK1334	<i>cac1 S503D-3xFLAG ARS/CEN/URA3</i>	This Study
pAK1335	<i>cac1 S238D-3xFLAG ARS/CEN/URA3</i>	This Study
pAK1337	<i>cac1 S238A-3xFLAG ARS/CEN/URA3</i>	This Study
pPK169	<i>POL30 ARS/CEN/URA3</i>	P. Kaufman
pAK1185	<i>CAC1-mCherry ARS/CEN/URA3</i>	This Study
pAK1128	<i>mCherry-KanMX cassette</i>	This Study
pAK1171	<i>CAC1 no stop codon ARS/CEN/URA3</i>	This Study
pAK1166	<i>SPC29-mCherry ARS/CEN/LEU2</i>	This Study
pAK728	<i>POL30 ARS/CEN/TRP1</i>	(HOEGE <i>et al.</i> 2002)
pAK730	<i>pol30-K127R ARS/CEN/TRP1</i>	(HOEGE <i>et al.</i> 2002)
pAK731	<i>pol30-K164R ARS/CEN/TRP1</i>	(HOEGE <i>et al.</i> 2002)
pAK732	<i>pol30-K127, 164R ARS/CEN/TRP1</i>	(HOEGE <i>et al.</i> 2002)
pFA6::kanMX4	<i>KanMX cassette</i>	(WACH <i>et al.</i> 1994)
pFA6::natMX4	<i>NatMX cassette</i>	(GOLDSTEIN AND MCCUSKER 1999)

TABLE S3. Oligos used in this study.		
Region	Oligos	Application
SAS2-M1	oALK1687 5' CCCCATATCAACGACGTGCAGCAGCCCTTTTACTTATCGAGTTTTC	Site-directed mutagenesis
SAS2-M1	oALK1688 5' GAAAACTCGATAAGTAAAAGGGCTGCTGCACGTCGTTGATATGGGG	Site-directed mutagenesis
SAS2-M1	oALK1689 5' CCAGGGTCACTTTATCATAATGG	Sequencing for SAS2-M1 mutation
<i>cac1</i> S238D	oALK1355 5' AACTTCTTTAAAAAACTAGACGATTCTAATACGCCTGTGG oALK1356 5' CCACAGGCGTATTAGAATCGTCTAGTTTTTTAAAGAAGTT	Site-directed mutagenesis
<i>cac1</i> S238A	oALK1357 5' AACTTCTTTAAAAAACTAGCCGATTCTAATACGCCTGTGG oALK1358 5' CCACAGGCGTATTAGAATCGGCTAGTTTTTTAAAGAAGTT	Site-directed mutagenesis
<i>cac1</i> S503D	oALK1359 5' CCATCCAAAAGAAGTAATGACGACTTACAGGCACAGACCG oALK1360 5' CGGTCTGTGCCTGTAAGTCGTCATTACTTCTTTTGGATGG	Site-directed mutagenesis
<i>cac1</i> S503A	oALK1361 5' CCATCCAAAAGAAGTAATGCCGACTTACAGGCACAGACCG oALK1362 5' CGGTCTGTGCCTGTAAGTCGGCATTACTTCTTTTGGATGG	Site-directed mutagenesis
<i>cac1</i> S501D	oALK1624 5' CGTCTCTACCATCCAAAAGAGATAATGACGACTTACAGGCAC oALK1625 5' GTGCCTGTAAGTCGTCATTATCTCTTTTGGATGGTAGAGACG	Site-directed mutagenesis
<i>cac1</i> S501A	oALK1626 5' CGTCTCTACCATCCAAAAGAGCTAATGCCGACTTACAGGCAC oALK1627 5' GTGCCTGTAAGTCGGCATTAGCTCTTTTGGATGGTAGAGACG	Site-directed mutagenesis
H3 T45A	oALK1242 5' TCACAGATATAAGCCAGGTGCTGTTGCTTTGAGAGAAATC oALK1244 5' GATTTCTCTCAAAGCAACAGCACCTGGCTTATATCTGTGA	Site-directed mutagenesis
H3	oALK706 5' GTTTTGTGACTTCCACTTTGGCCCT	Sequencing H3 T45A
C-term <i>CAC1</i> -3XFLAG	oALK1271 5' GAATCTTCGAGCCAATGCAAATATGCCAACCCCGTCTTTGAGGGAACAAAAGCTGGAG oALK1272 5' TATCTGTATGTTTCTATATACTAAAGATCCGTTCAAGTTACTATAGGGCGAATTGGGT	C-terminally tag genomic <i>CAC1</i> with 3XFLAG
<i>cac1</i> Δ::KanMX	oALK507 5' TAGTGAACCTCAAGACAGAAGAGAATCGAAAGGAAAAGGGAAACGTACGCTGCAGGTCGAC oALK508 5' CAGTTTATCTGTATGTTTCTATATACTAAAGATCCGTTCAAGATCGATGAATTCGAGCTCG	Gene Disruption
<i>cac1</i> Δ::KanMX	oALK72 5' CTGCAGCGAGGAGCCGTAAT oALK509 5' GGACGCGTGAAAGGCTTCAA	Screen for Knockout of <i>CAC1</i>
<i>cdc7</i> Δ::NatMX	oALK1365 5' AATTTTCTTAGGAAAGAGGCAGTTTCAAGTAGAACAATCATACGTACGCTGCAGGTCGAC oALK1366 5' GAAAAGGAAATTATTTTCAGACTAGCAGTAATTTATCACTTTGATCGATGAATTCGAGCTCG	Gene Disruption
<i>cdc7</i> Δ::NatMX	oALK217 5' TTCGTCGTCGGGGAACACCTT oALK1367 5' CTCCTTTCGACCTTTATTCA	Screen for Knockout of <i>CDC7</i>
<i>HMRae</i> **	oALK1299 5' ATTATATTGCACAAACA oALK1300 5' TCGCCTACCTTCTTGAA	Screen for <i>HMRae</i> ** mutation
<i>bob1-1</i>	oALK1301 5' AAACTATCAGACGAACT oALK1302 5' TCCACAATTTTCTTGG	Screen for <i>bob1-1</i> mutation
<i>CAC1</i>	oALK1255 5' GGAGAGTTTGACGGGTTTCTAGAC	Sequencing <i>CAC1</i> for S501 and S503 mutations

<i>CAC1</i>	oALK1252 5' CAATAAAGAGTGTGCAGATGAAATGA	Sequencing <i>CAC1</i> for S238 mutations
<i>CAC1-3XFLAG</i>	oALK1176 5' TTTTTTTTGAATTCGTTTCAGTACGACAATGACGATATTCACAGTACTACTAACTAGCG oALK1180 5' TTTTTTGGTACCGTTGGAGAGCTAGACTGTTGAGACATTTTAAGTTCTTGAGGTTTCTTT	Cloning <i>CAC1</i> , <i>CAC1-3XFLAG</i> into EcoRI/KpnI of pRS416
<i>sir2Δ::HIS3</i> <i>sir2Δ::URA3</i>	oALK56 5' CAAACCATTTTTCCCTCATCGGCACATTAAAGCTGGGATTGTAAGTACTGAGAGTGCACC oALK57 5' AATTTGGCACTTTTAAATTATTAAATTGCCTTCTACCTGTGCGGTATTTACACCG	Gene Disruption
<i>sir2Δ::HIS3</i>	oALK66 5' GTCCTCTTTGGCCGCCAGT oALK180 5' CTATGTGTAAGTCACCAATGCACTC	Screening for Knockout of <i>SIR2</i>
<i>sir2Δ::URA3</i>	oALK66 5' GTCCTCTTTGGCCGCCAGT oALK183 5' CTGTTGACCCAATGCGTCTCCCTTG	Screening for Knockout of <i>SIR2</i>
<i>e**</i>	oALK261 5' CCCGTCCAAGTTATGAGCTTAATCT oALK262 5' GGAGTCTTAATTTCCCTGATTTTAGTTTAG	ChIP
<i>a1</i>	oALK270 5' TTTAGAAGAAAGCAAAGCCTTAATTCC oALK271 5' CTTGAAGTGGAGTAATGCCACATT	ChIP

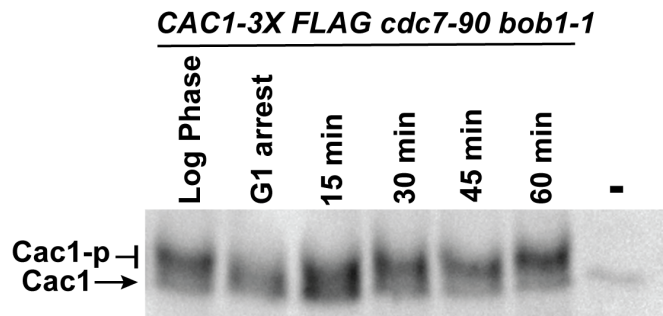


Figure S1. Cell cycle-dependent phosphorylation of Cac1p does not exclusively require Cdc7p. Whole cell extracts prepared from *CAC1-3X FLAG cdc7-90 bob1-1* cells collected during logarithmic growth, G1 arrest, and every 15 min for one hour after release into S phase were separated on a 7% SDS-PAGE, 30 μ M PhosTag gel and analyzed by immunoblotting with anti-FLAG antibodies. Untagged *CAC1* cells were collected during logarithmic growth (negative control).

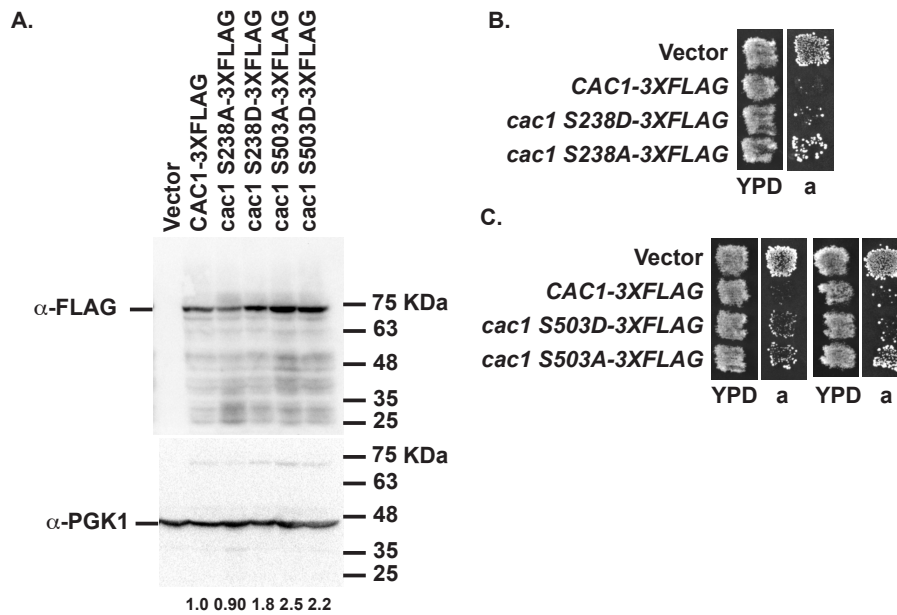
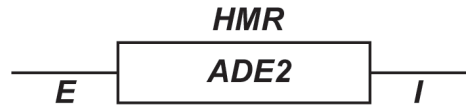


Figure S2. *HMRae* is silenced in *cac1* mutants that mimic the unphosphorylated state at Cdc7p consensus sites. A)** Expression of *cac1* mutants. Whole cell extracts prepared from the indicated *MAT α* *HMRae*** *cac1* Δ ::*KanmX* strains during logarithmic growth were separated on a 10% SDS-PAGE, and analyzed by immunoblotting with anti-FLAG antibodies (top panel), then stripped, and reprobed with anti-PGK1 antibodies (bottom panel; loading control). Protein expression levels noted below blots were quantified as follows and normalized to WT, which was set to 1.0: (FLAG/PGK1)_{mutant}/(FLAG/PGK1)_{wild-type}, where mutant=indicated strain. **B)** and **C)** Patch mating assays were performed with indicated strains as described in Figure 1. Two independent clones of *cac1* S503D/A mutants are shown in **C** to illustrate variegation.

A.



B.

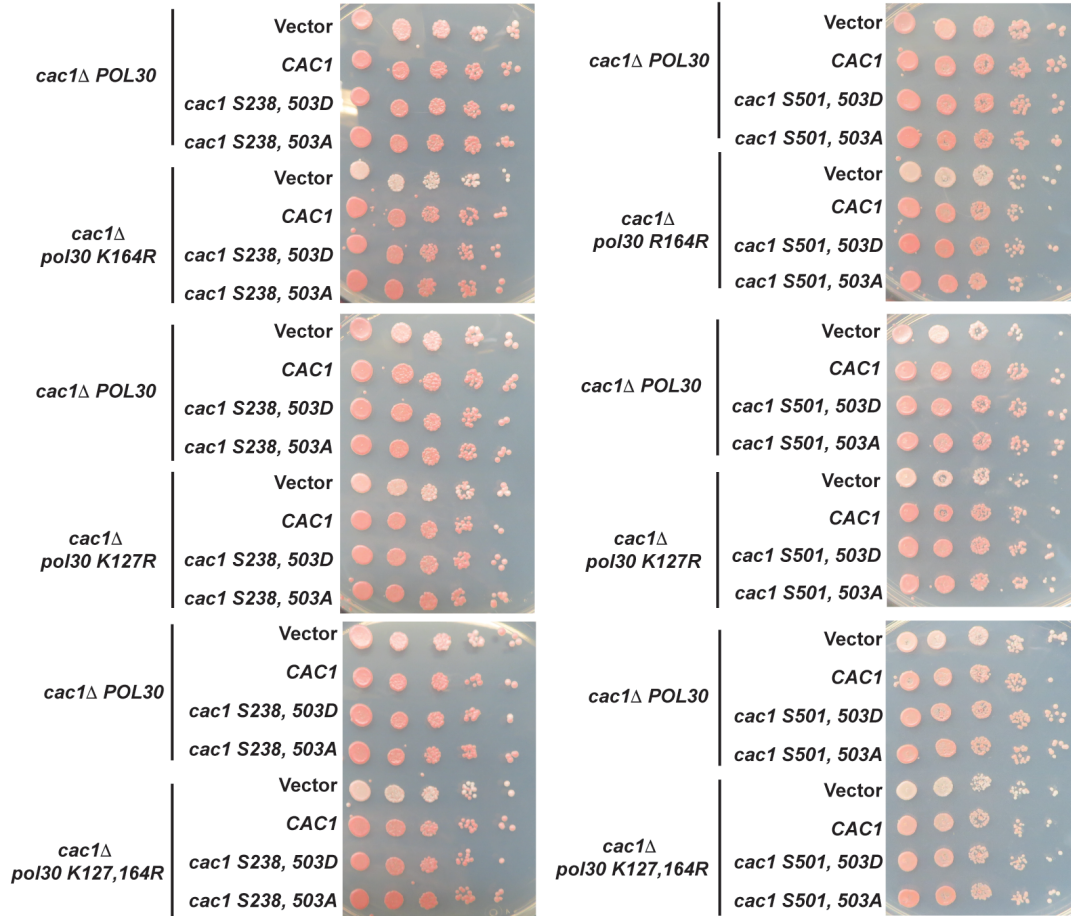


Figure S3. Phosphorylation of Cac1p at S238, S503 and S501, S503 are not required for silencing at *HMR::ADE2*. **A)** Map of *HMR::ADE2*. **B)** Colony color assay. Yeast with genotypes as indicated were grown overnight in YPD and 2.5 μ l of 10-fold serial dilutions were spotted onto a CSM-URA plate and incubated for two days at 30°C and then incubated for four days at 4°C prior to imaging.

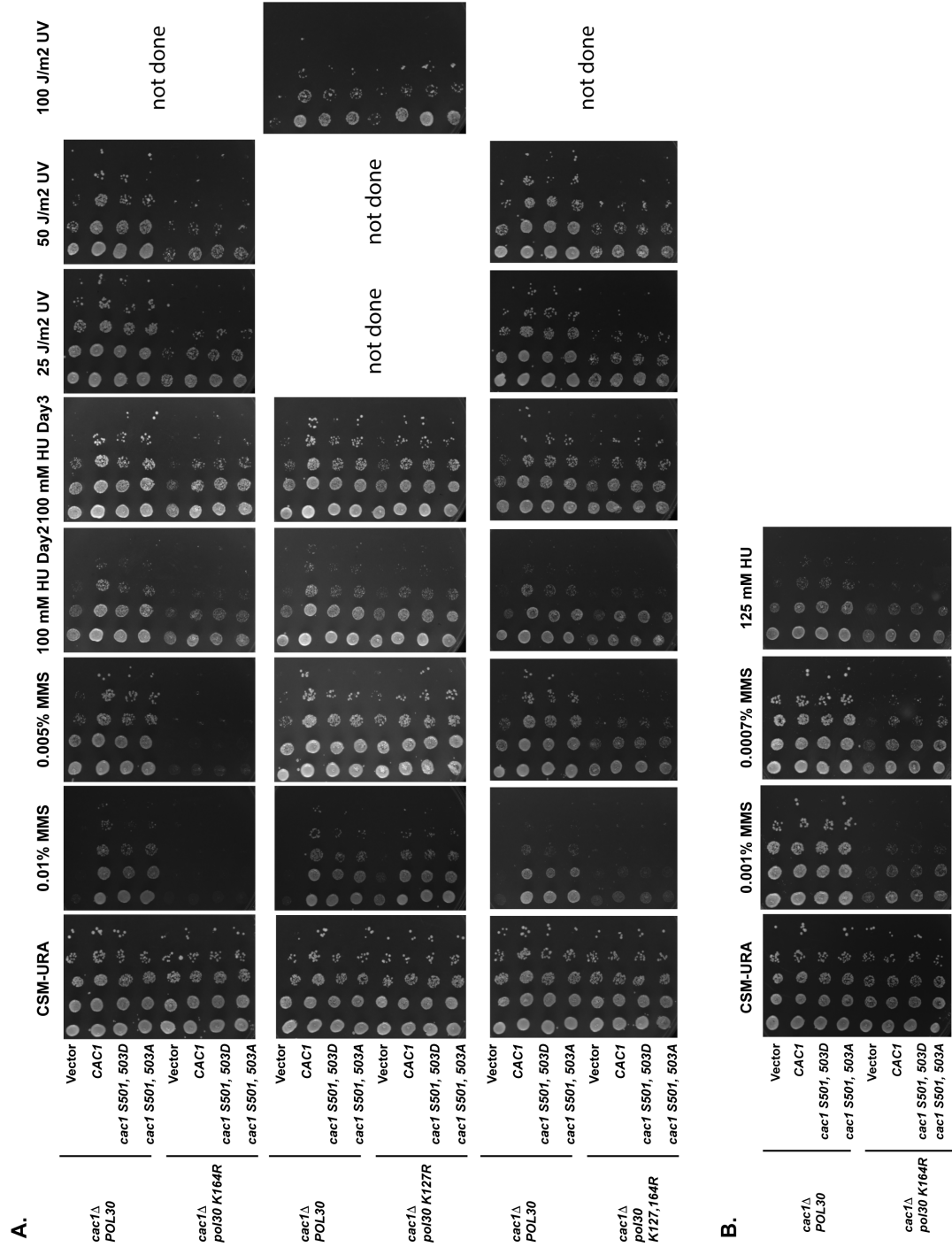


Figure S4. Synthetic interaction analyses of DNA damage sensitivity of *cac1 S501*, *503* and *pol30* mutants. A) and B) Yeast with genotypes as indicated were grown overnight in CSM-URA, and 2.5 μ l of ten-fold serial dilutions were spotted onto either CSM-URA plates or CSM-URA plates containing the indicated dosages of DNA damaging

agents or exposure to UV and incubated for two days at 30°C prior to imaging.

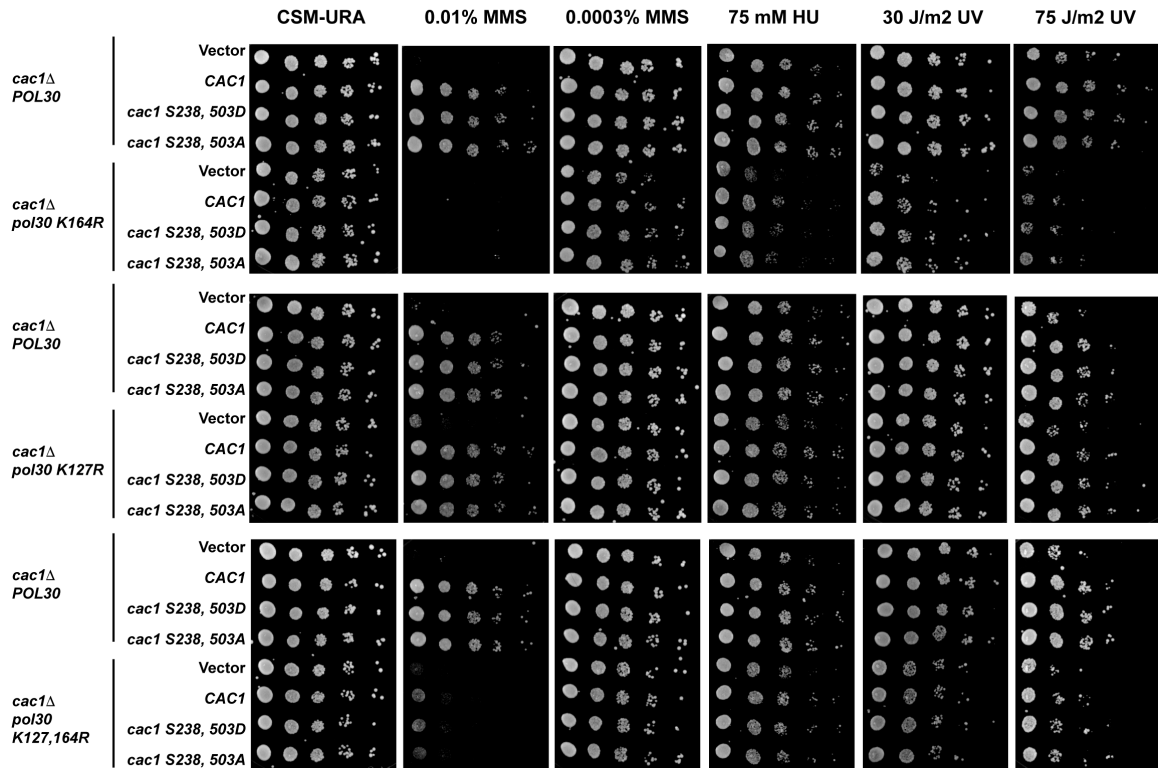


Figure S5. Synthetic interaction analyses of DNA damage sensitivity of *cac1 S238, 503* and *pol30* mutants. A) and B) Yeast with genotypes as indicated were analyzed as in Figure S4.

SUPPLEMENTARY REFERENCES

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