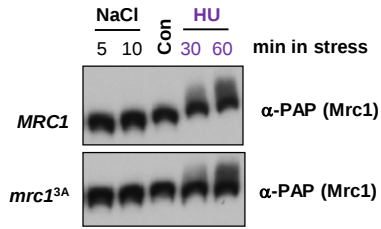
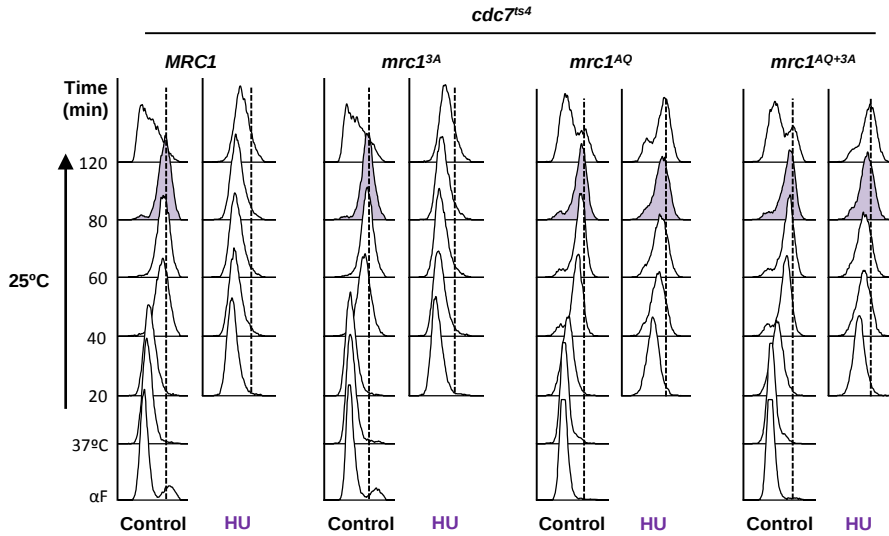


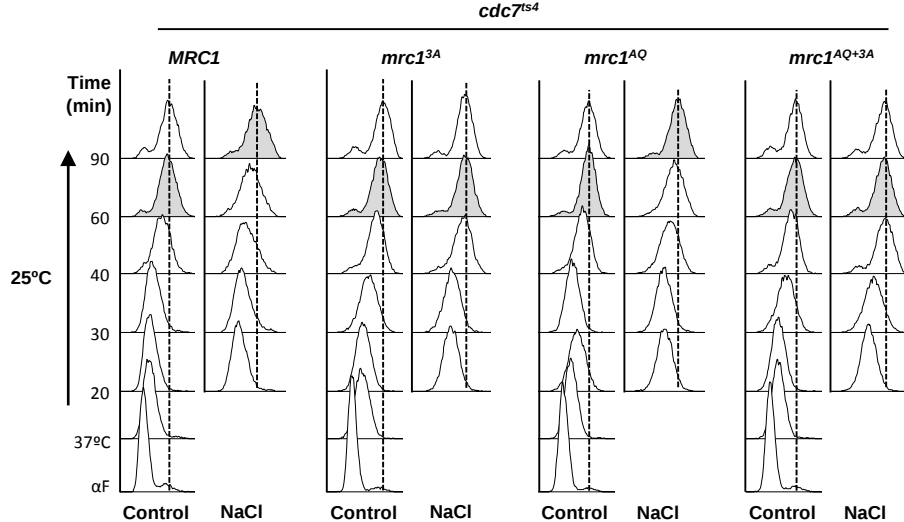
A



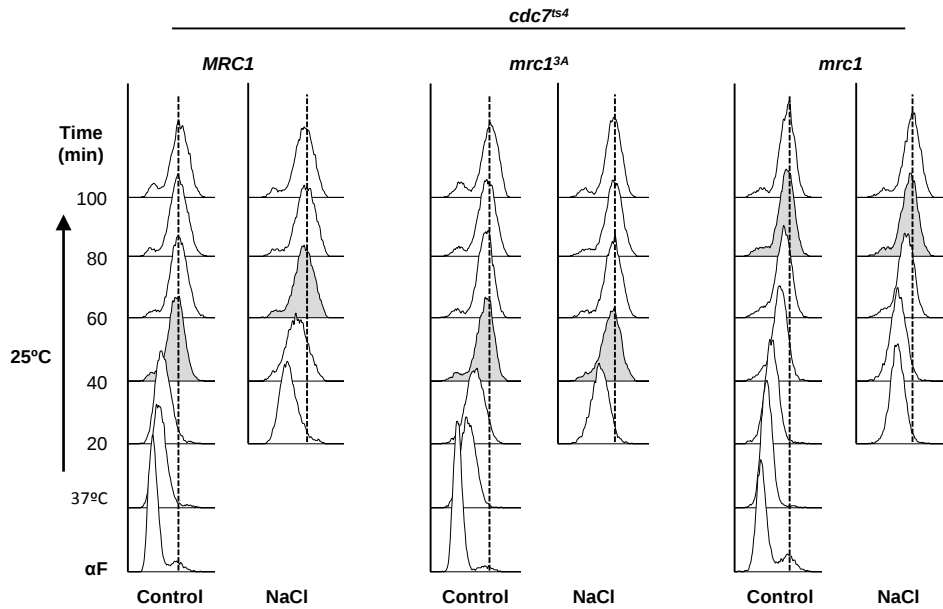
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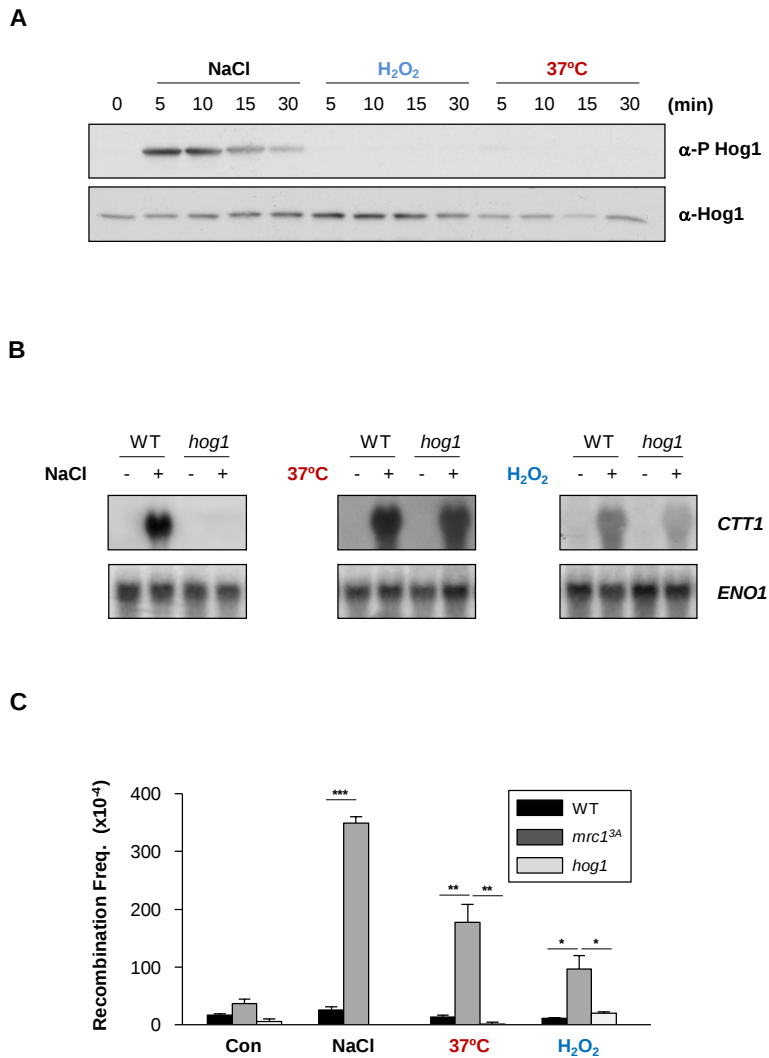
C



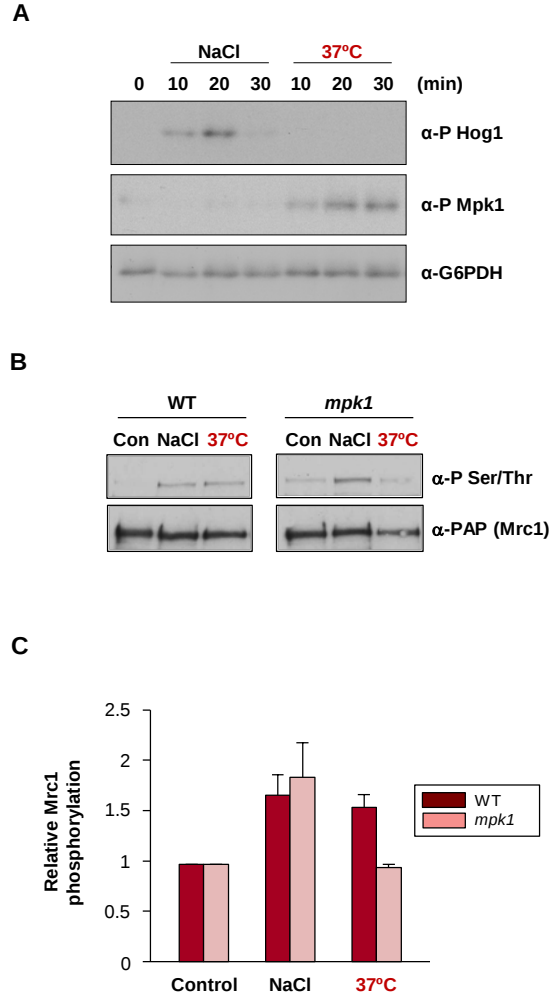
Supplementary Figure 1. *mrc1^{AQ}* and *mrc1^{3A}* alleles have genetically separable functions. (A) Mrc1 phosphorylation upon 0.2M HU induces an electrophoretic mobility shift that can be detected in phostag-containing gels (see Methods), while environmental stresses like NaCl don't provoke changes in Mrc1 mobility. (B,C) *mrc1^{AQ}* (with all putative Mec1 sites mutated to Ala) and *mrc1^{3A}* strains were synchronized as described (see Methods) and released upon replicative stress (0.05M HU, in D) or osmotic stress (0.4M NaCl, in E) and replication progression was followed by FACS.



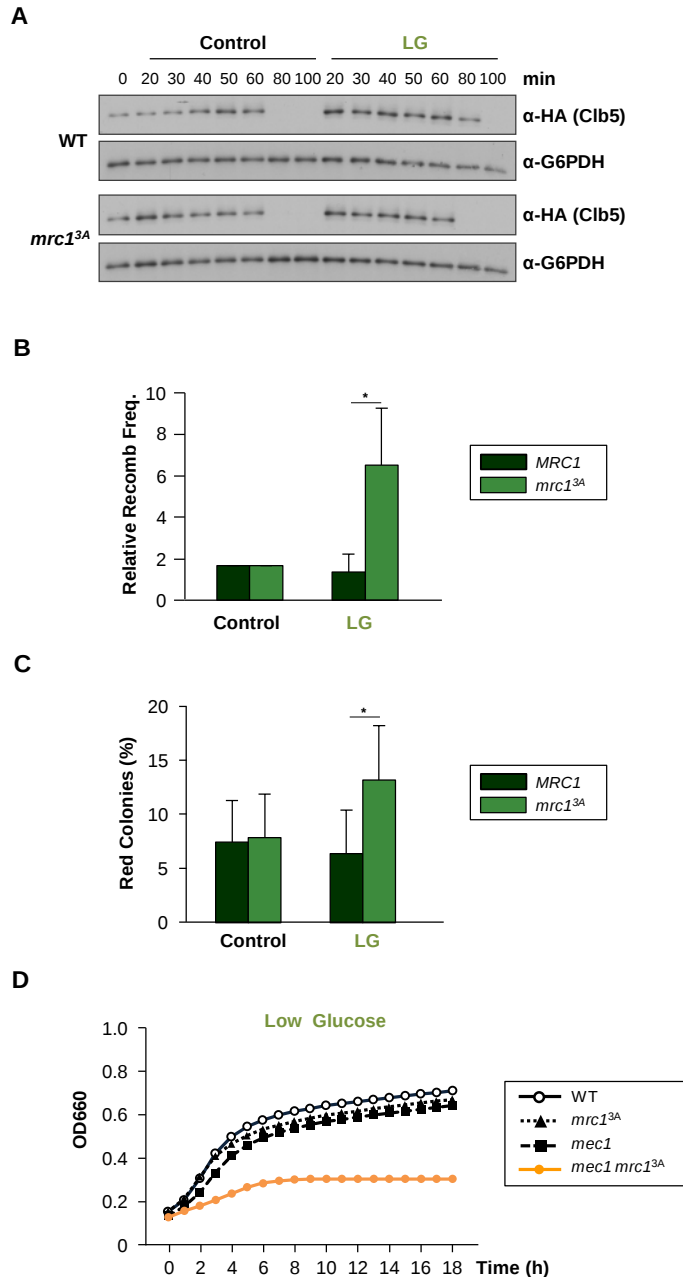
Supplementary Figure 2. *mrc1* shows a delay in DNA replication under control conditions when compared to *MRC1* and *mrc1^{3A}* cells. The indicated strains were synchronized as described (see Methods) and released in control conditions or in osmostress (0.4M NaCl) and replication progression was followed by FACS.



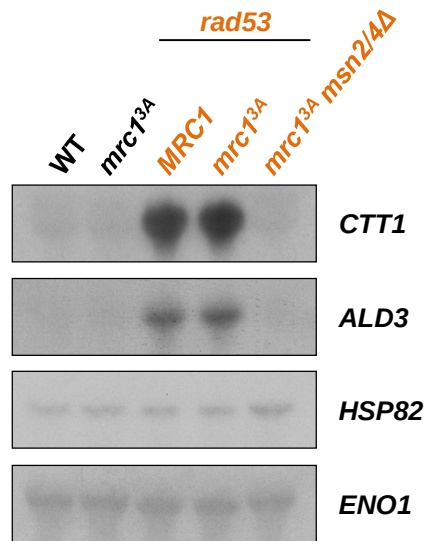
Supplementary Figure 3. Hog1 does not mediate the regulation of Mrc1 upon heat and oxidative stresses. (A) Hog1 is not phosphorylated upon oxidative and heat stresses. Western blotting of phosphorylated and total Hog1 protein is shown. (B) Hog1 is not required for induction of *CTT1* expression upon heat or oxidative stress. *CTT1* expression was analyzed in wild type (WT) and *hog1* cells upon the indicated stress conditions using Northern blotting. *ENO1* was blotted as a control. (C) *mrc1*^{3A} but not *hog1* cells display associated recombination (TAR) upon oxidative and heat stresses. TAR was not determined in *hog1* cells upon osmotic stress due to their osmosensitivity. Data represent the mean and standard deviation of three independent experiments. Values significantly different (**p*<0.05, ***p*<0.01, ****p*<0.005) by t-student test are annotated.



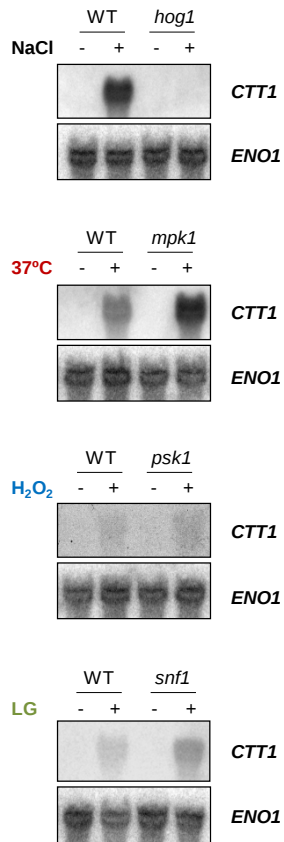
Supplementary Figure 4. Mpk1 phosphorylates Mrc1 upon heat stress. (A) Hog1 and Mpk1 are specifically activated upon osmostress or heat stress respectively. Hog1 phosphorylation or Mpk1 phosphorylation was assessed using specific phospho-antibody. G6PDH was monitored as a loading control. (B) Mrc1 is phosphorylated *in vivo* by Mpk1 upon heat stress but not upon osmostress. Mrc1 phosphorylation over time was followed by western blotting as in Figure 1A. (C) Quantification of phosphorylation experiments with the indicated strains. Data represent mean and standard deviation.



Supplementary Figure 5. N-terminal Mrc1 phosphorylation is required for cell cycle delay and to prevent TAR and genomic instability in response to nutrient deprivation (low glucose). (A) Wild type cells delay Clb5 degradation when subjected to low glucose in contrast to *mrc1*^{3A} cells. Cells were synchronized at the beginning of S phase as described (see STAR methods) and released into S phase at 25 °C in YPD (Control) or in low glucose (YP-0.05% Dextrose)(LG). Clb5 degradation was assessed using western blotting. (B) *mrc1*^{3A} cells display higher levels of recombination than wild type cells in TAR assays upon LG stress (see STAR methods). (C) *mrc1*^{3A} cells show higher frequency of plasmid loss upon LG stress in a red sectoring assay (see STAR methods). Data in (B) and (C) represent the mean and standard deviation of three independent experiments. Values significantly different (*p<0.05, **p<0.01, ***p<0.005) by t-student test are annotated. (D) *mrc1*^{3A} cells are sensitive to LG when the DNA damage checkpoint pathway is impaired. The indicated strains were grown to log phase and incubated in LG.



Supplementary Figure 6. Mutation of the *RAD53* gene triggers the canonical ESR response. *rad53* cells (wild type or containing the *mrc1^{3A}* mutation) trigger the transcription of *CTT1* and *ALD3* ESR genes in a Msn2 and Msn4-dependent manner. Northern blot analysis is shown. *HSP82* and *ENO1* are loading controls.



Supplementary Figure 7. Mpk1, Psk1 and Snf1 are dispensable for the expression of the prototypical ESR gene *CTT1* upon heat, oxidative and low glucose stress. *CTT1* expression was analyzed by Northern Blot in wild type (WT) and *mpk1*, *psk1* and *snf1* cells upon the indicated stresses. *ENO1* was assessed as a control of total RNA levels.

1A



***mrc1*^{3A}**

1E

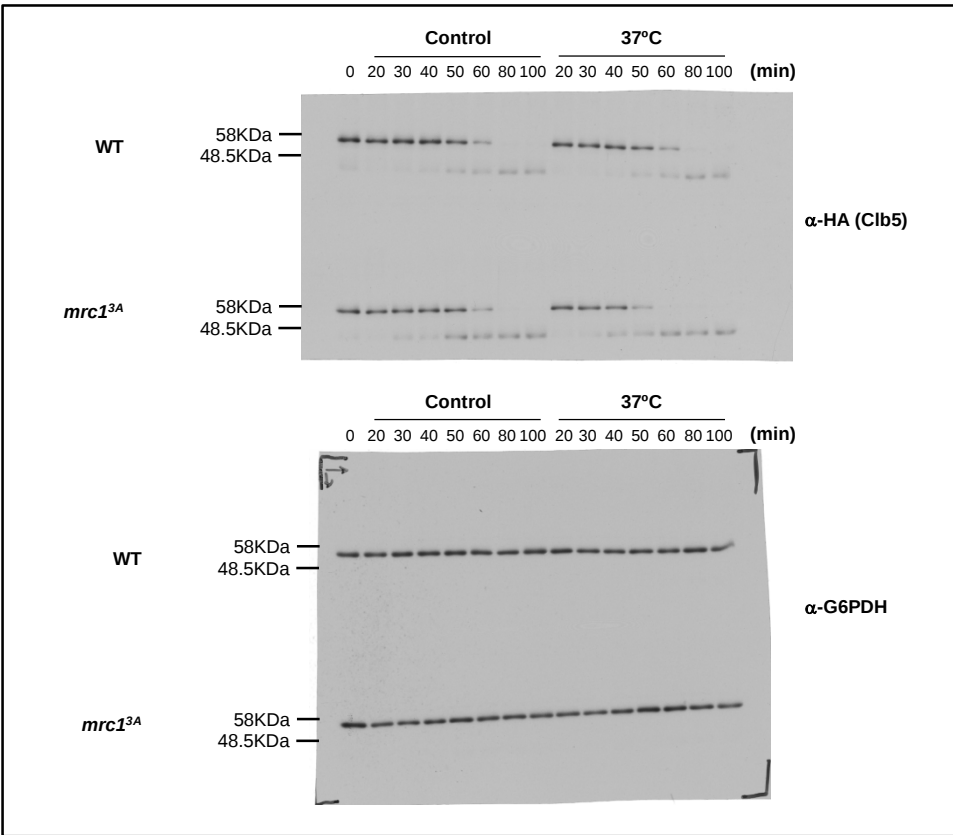


mrc1^{3A}

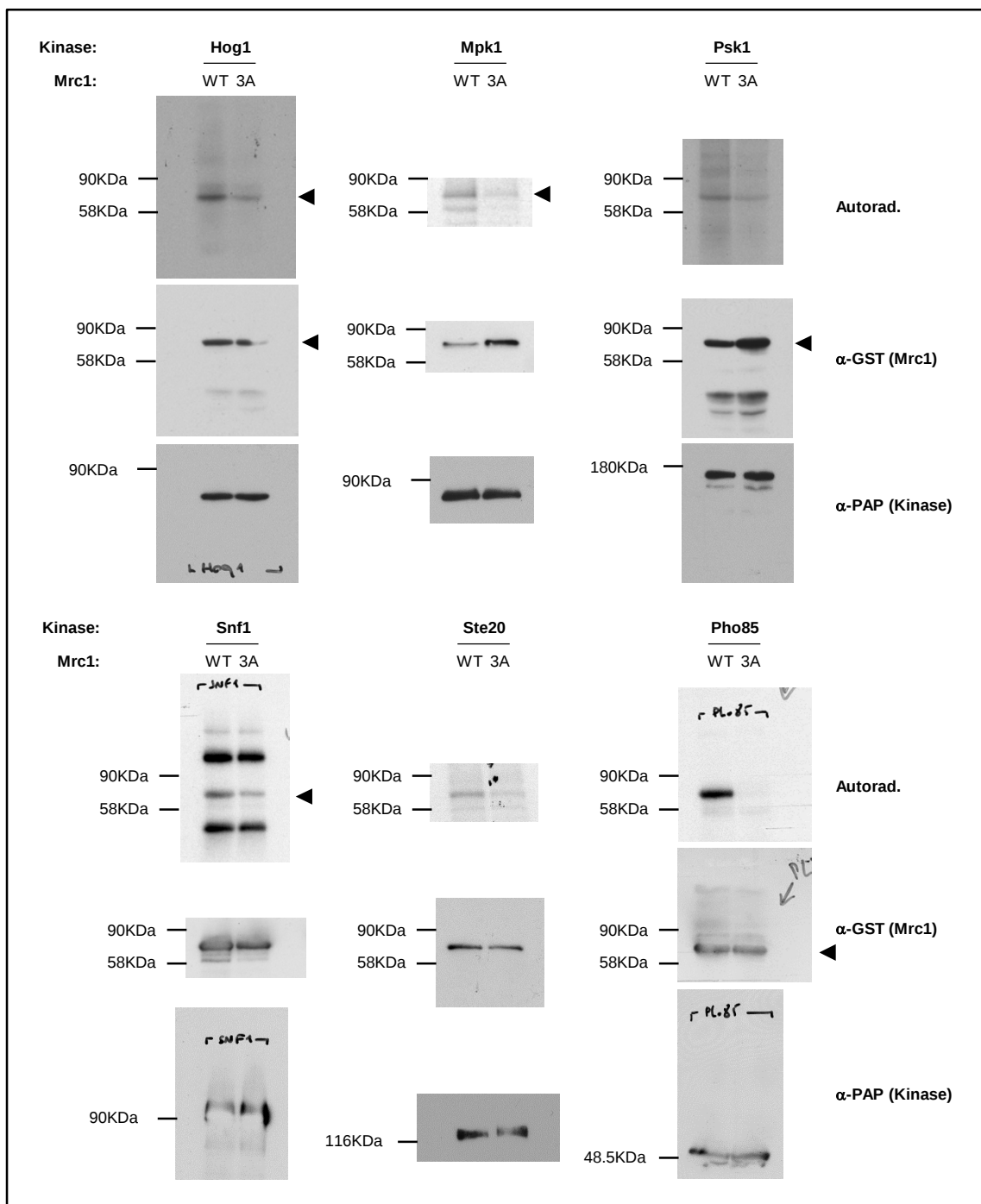
WT

mrc1^{3A}

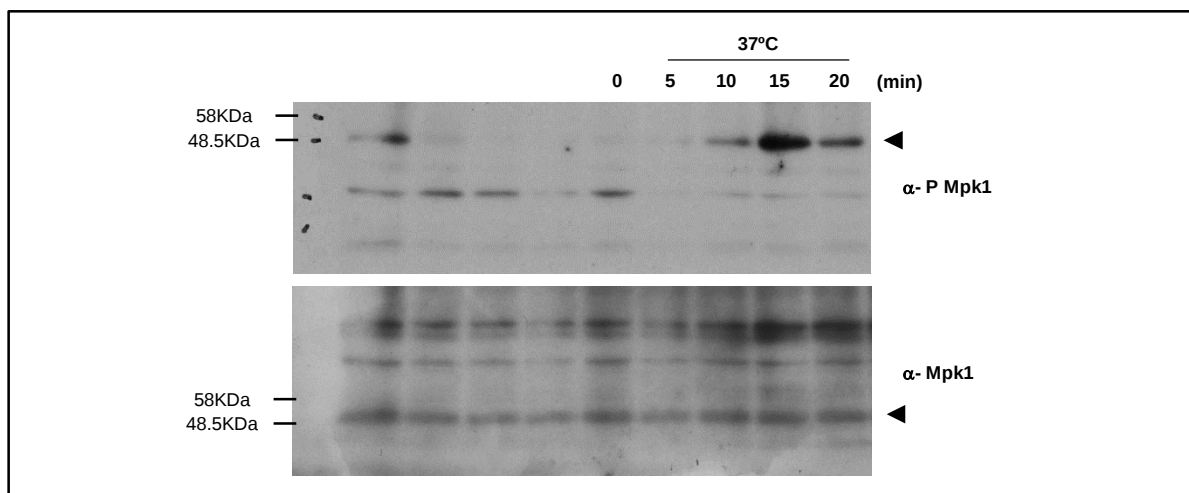
1F



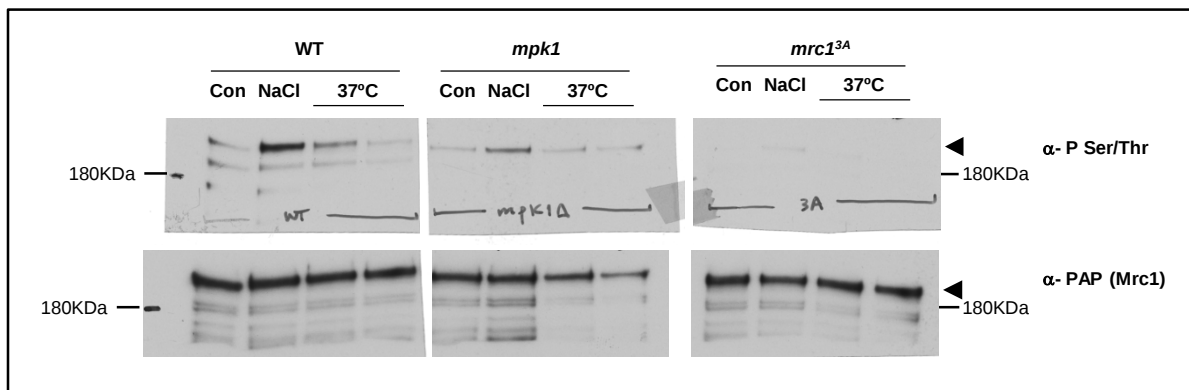
4A



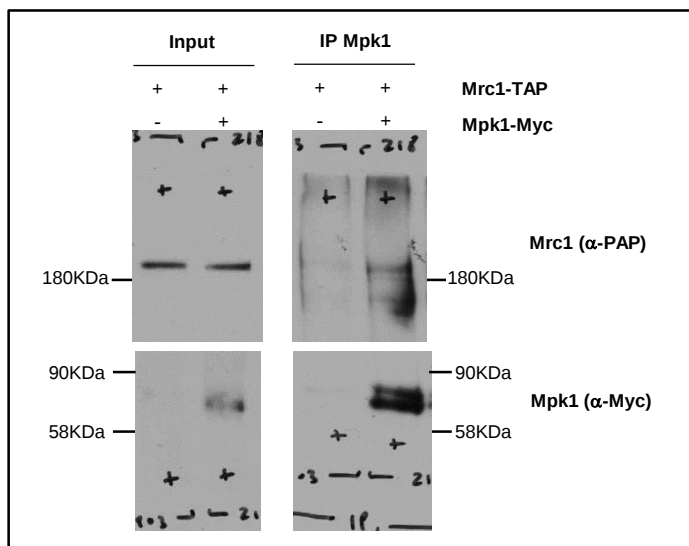
4B



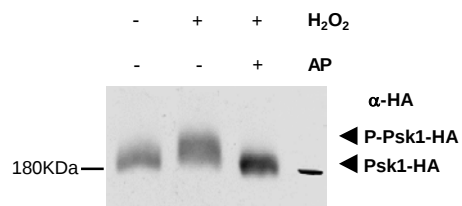
4C



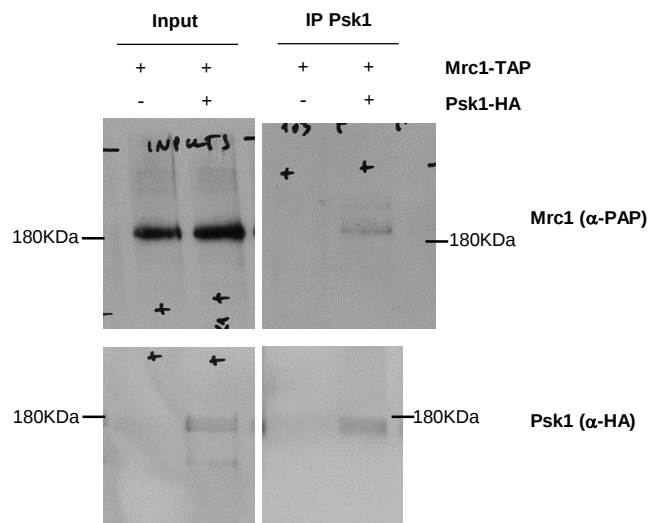
4D



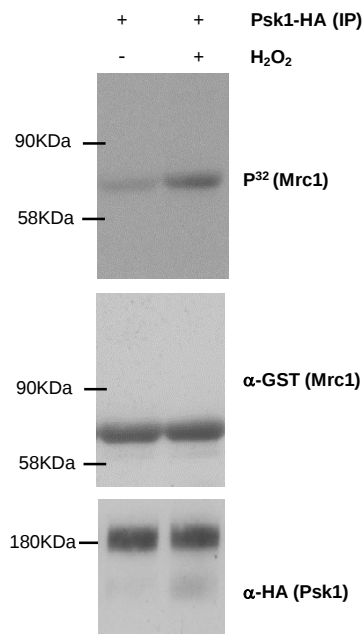
5A



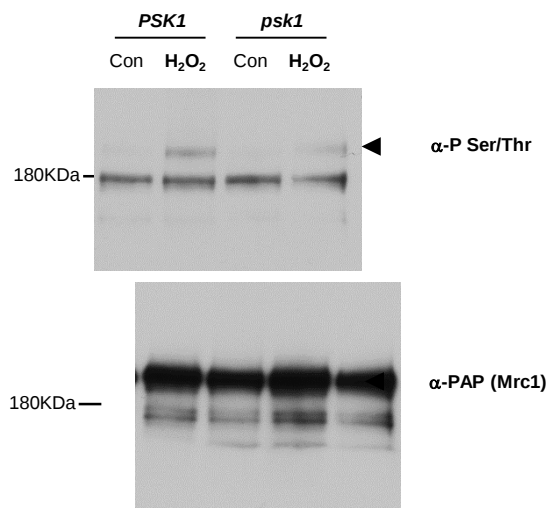
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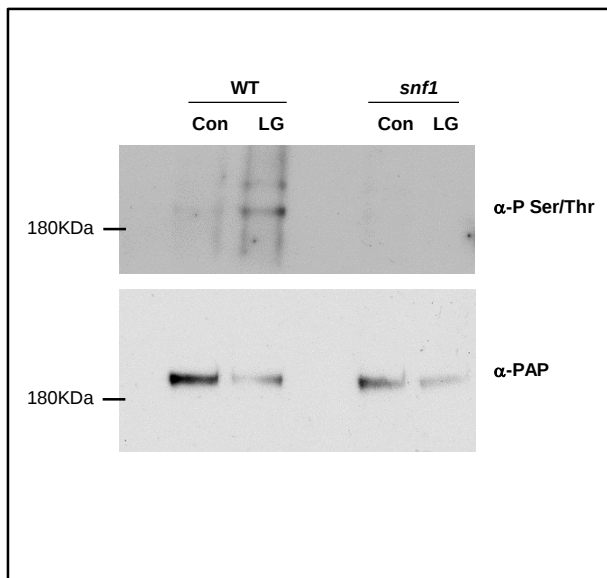
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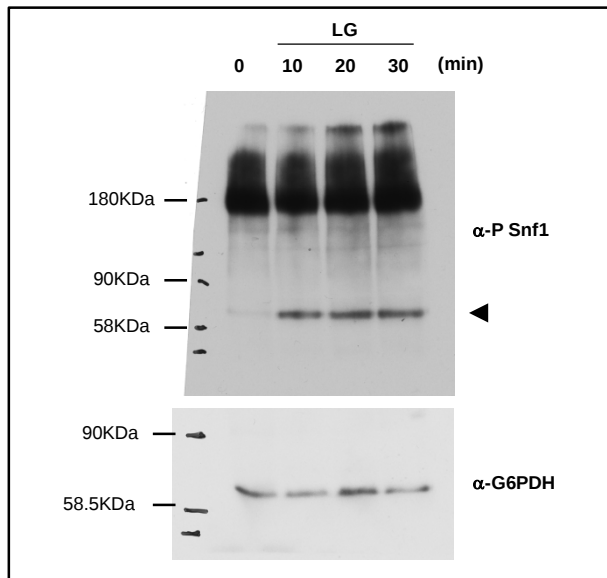
5D



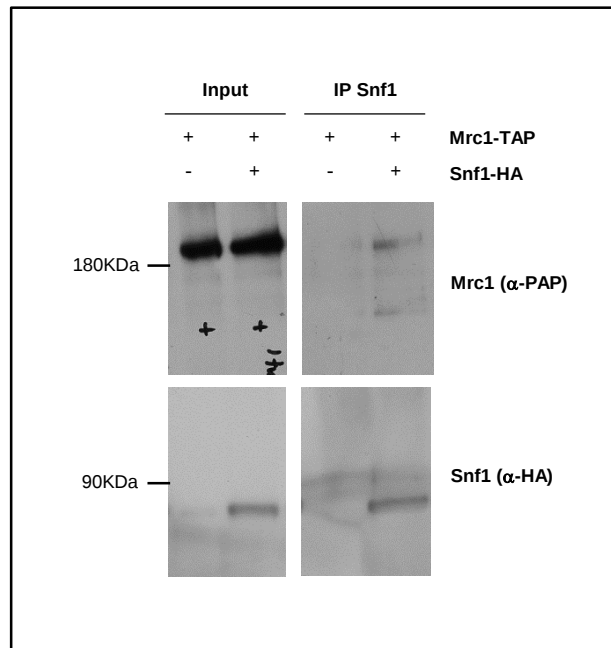
6A



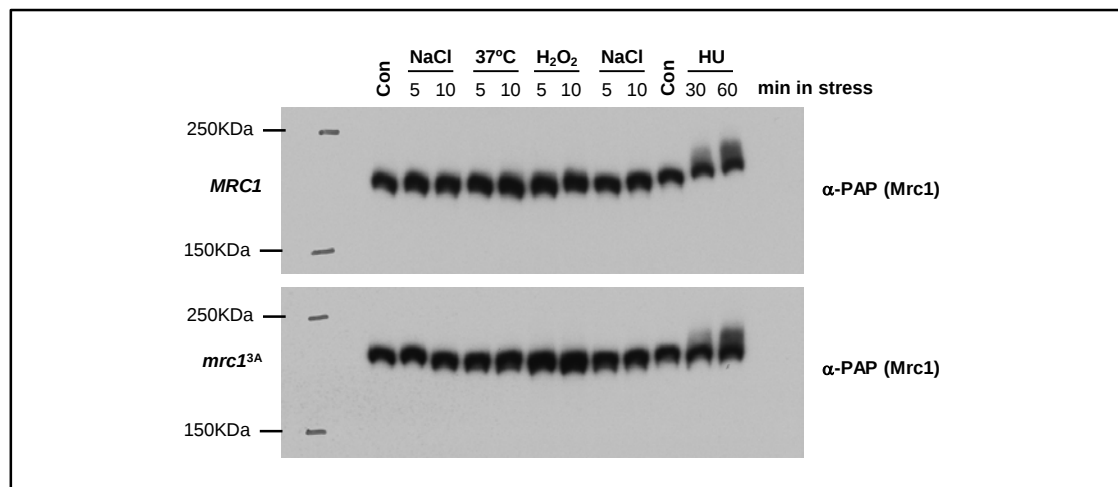
6E



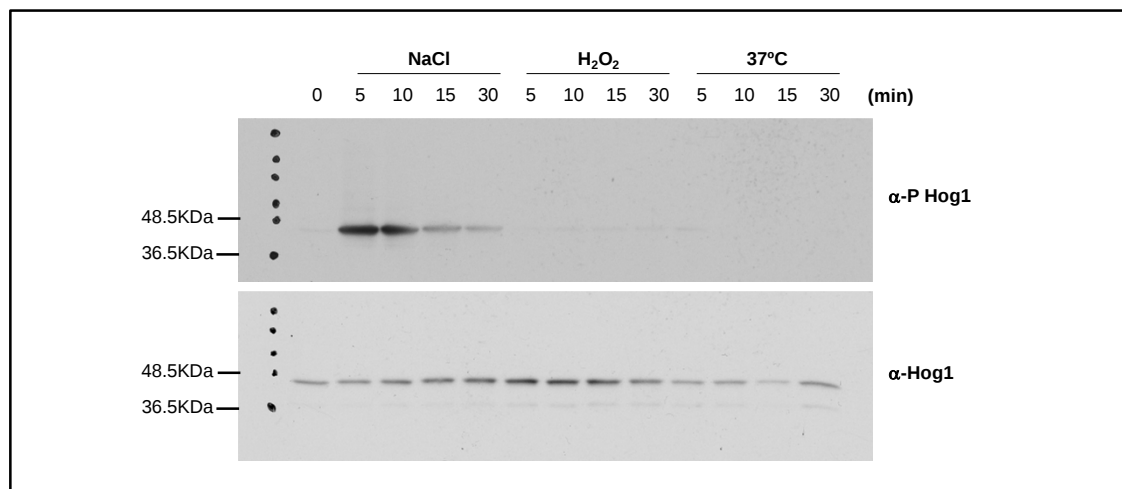
6F



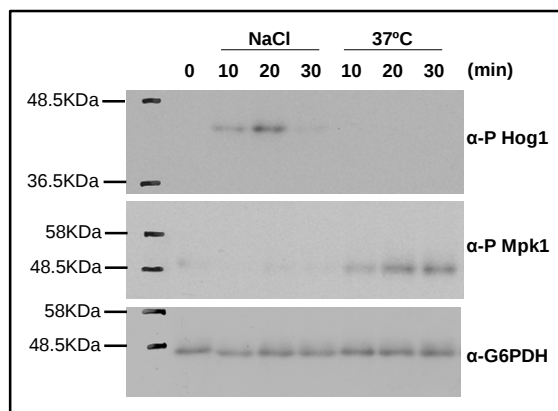
S1A



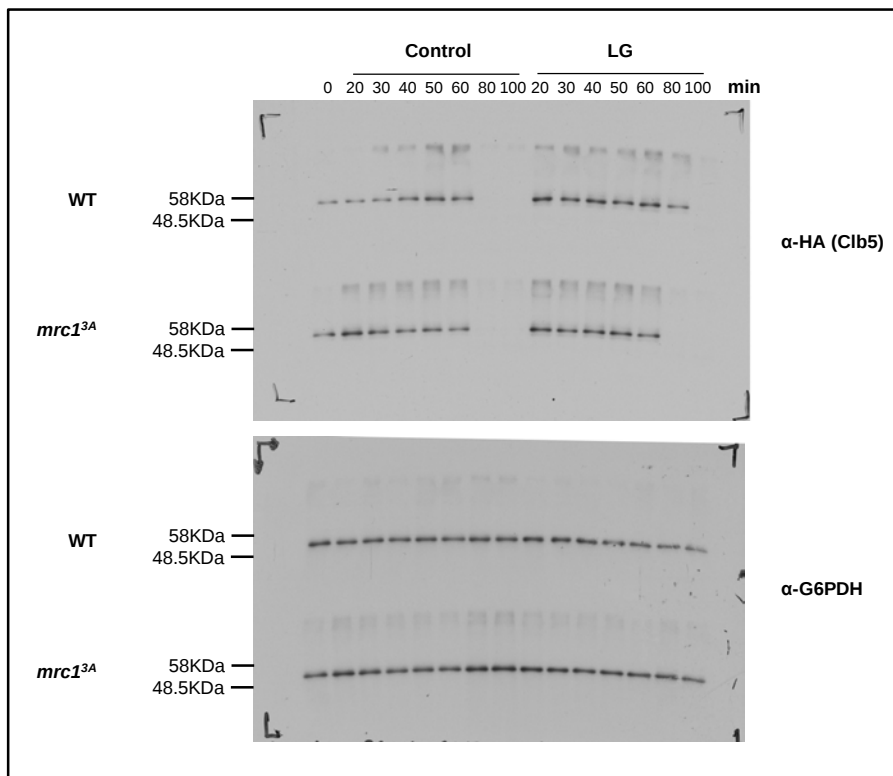
S3A



S4A



S5A



Supplementary Figure 8. Uncropped scans of the blots displayed in the main and supplementary figures.