Supplementary Data

The genome maintenance factor Mgs1 is targeted to sites of replication stress by ubiquitylated PCNA

Irene Saugar, Joanne L. Parker, Shengkai Zhao and Helle D. Ulrich

Supplementary Figures

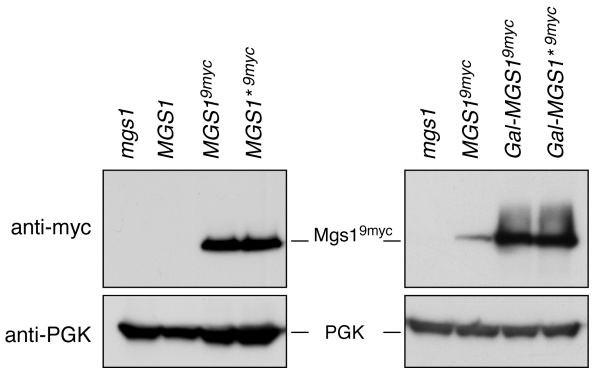

Figure S1. Protein levels of Mgs1 and Mgs1*

Western blots of total cell extracts from the indicated strains show equal protein levels of Mgs1 and Mgs1* at native levels (left) and when overexpressed (right). Proteins were detected by means of a 9myc-epitope appended to the open reading frame. Anti-phosphoglycerate kinase (PGK) blots served as loading control.

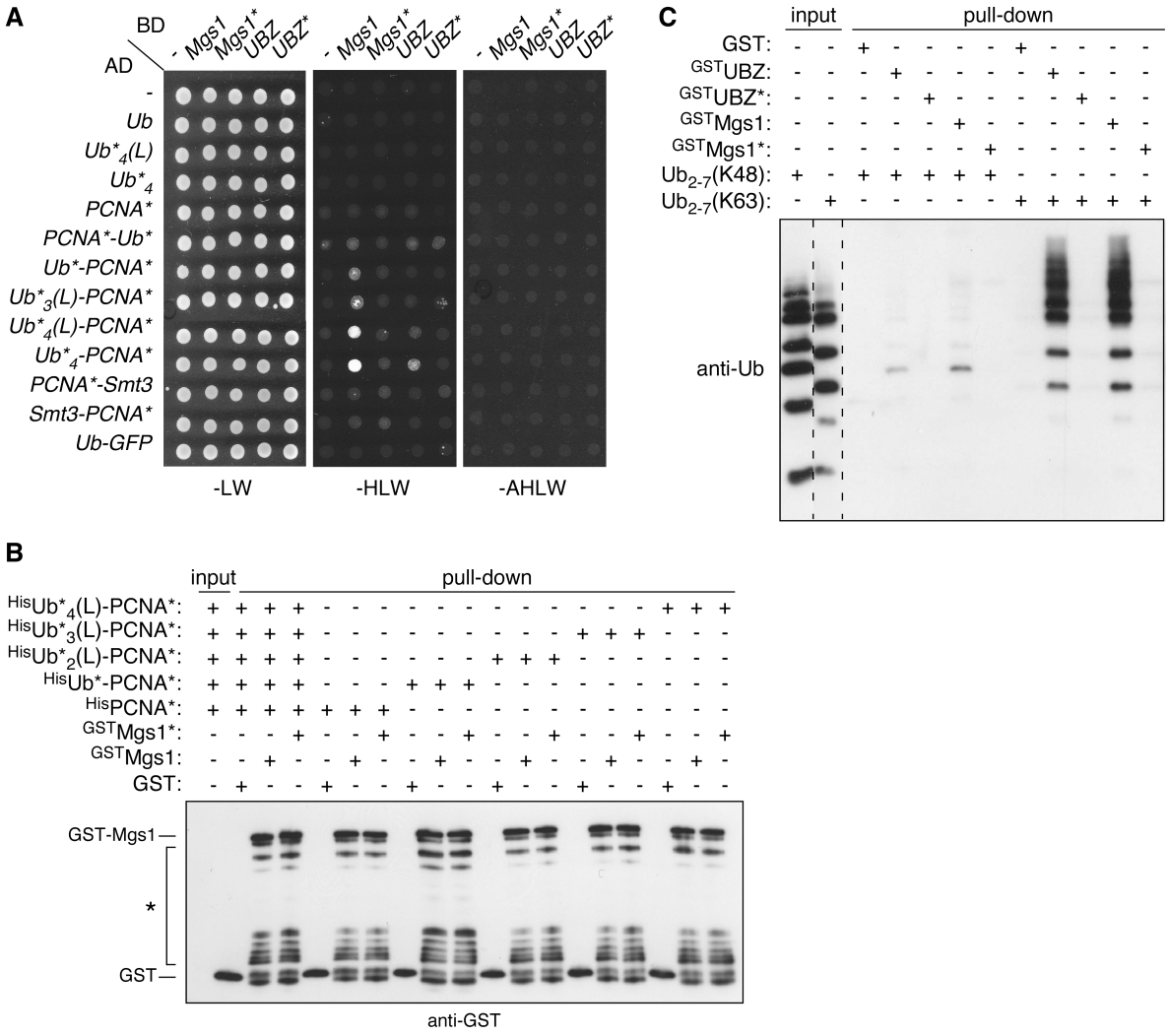

Figure S2. UBZ-dependent interactions of Mgs1 with ubiquitin and ubiquitylated PCNA

(**A**) Protein-protein interactions with full-length Mgs1 and the isolated UBZ domain were analysed in the two-hybrid system using fusions to the Gal4 activation (AD) and DNA-binding (BD) domains as described in Figure 3A, but reversing AD and BD fusions.

(**B**) Anti-GST blot as control for Figure 3B. The asterisk indicates degradation products.

(**C**) Interactions of Mgs1 with polyubiquitin chains were analysed in vitro. GST-tagged versions of Mgs1 and Mgs1* or the corresponding UBZ domains only (amino acids 1-47) were immobilised on glutathione Sepharose and assayed for binding to purified Ub_{2-7} of K48- or K63-linkage.

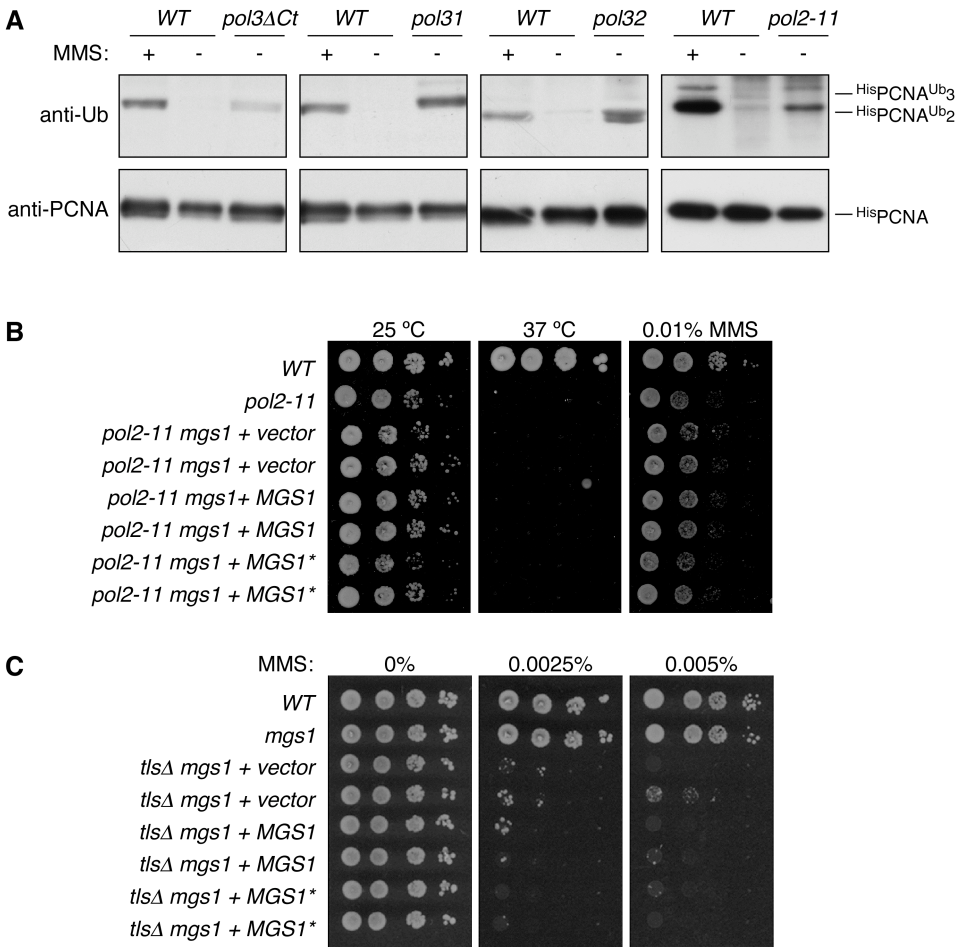

Figure S3. PCNA ubiquitylation and effects of Mgs1 in DNA polymerase mutants

(**A**) PCNA undergoes damage-independent ubiquitylation in polymerase mutants. PCNA modification was assessed by denaturing Ni-NTA chromatography and Western blotting as described in Figure 2C in the indicated strains harboring the ^{His}*POL30* allele.

(**B**) Deletion of *MGS1* or inactivation of its UBZ domain has no effect on the temperature or DNA damage sensitivity of a *pol2-11* mutant. MMS and temperature sensitivities were determined by spot assays in the indicated strains. Two colonies each were analysed for strains complemented with integrative vectors.

(**C**) Deletion of *MGS1* or inactivation of its UBZ domain has no effect on the DNA damage sensitivity of a TLS-deficient mutant. MMS sensitivities were determined as in panel B.

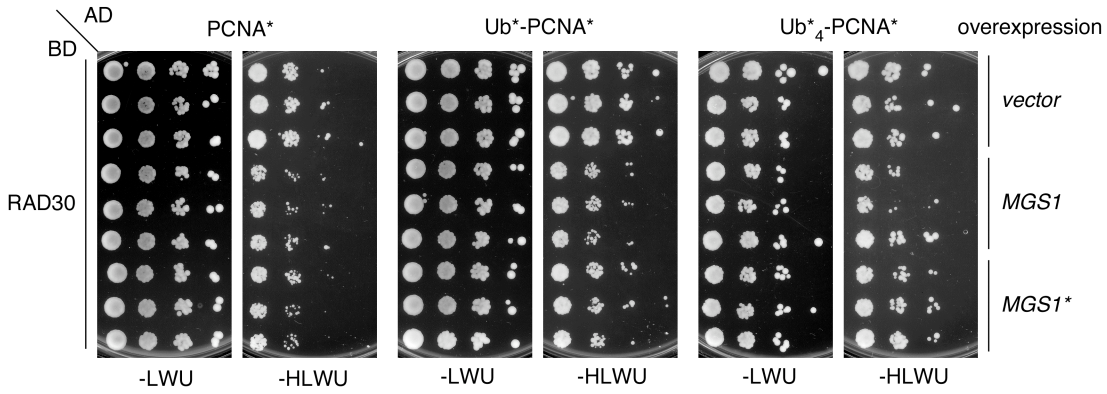

Figure S4. Effects of *MGS1* overexpression on interactions of PCNA with polymerase η

Three-hybrid experiments were carried out as described in Figure 6, but using Rad30 (polymerase η) as a fusion to the Gal4 DNA-binding domain.

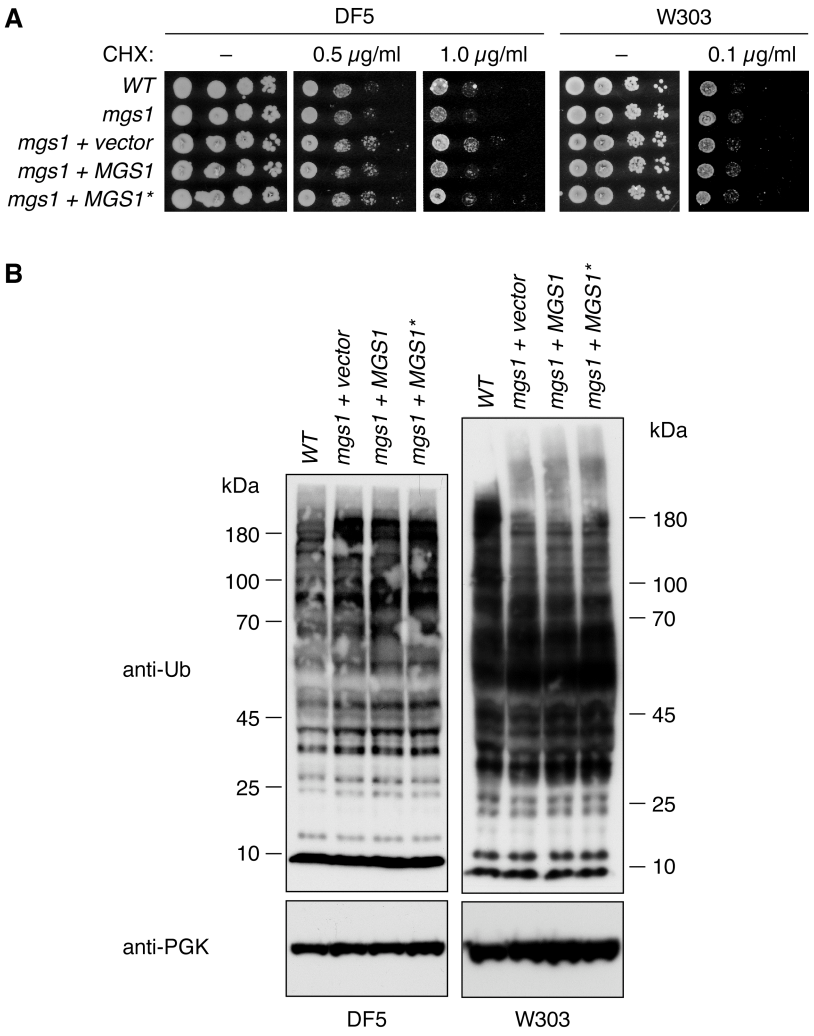

Figure S5. Lack of effects of Mgs1 on the general turnover of ubiquitin conjugates

(**A**) Deletion of *MGS1* or mutation of its UBZ domain does not lead to an increased resistance to cycloheximide. Spot assays were performed in the presence of the indicated cycloheximide concentrations in two different strain backgrounds, DF5 and W303.

(**B**) Steady-state levels of total ubiquitin conjugates are unaffected by deletion of *MGS1* or mutation of its UBZ domain. Total cell extracts from the indicated strains (DF5 and W303 background) were analysed by Western blotting with an anti-ubiquitin antibody. Detection of PGK served as a loading control.