



Fig. S1. Rust coloration is affected by a nonsynonymous polymorphism in *CYS4*.
A. QTL mapping of rust coloration using 45 M22xYP163 (MY) and 45 M22xS2188C (MS) segregants genotyped at 198 markers and the Haley-Knott regression algorithm implemented in R/QTL [Broman KW, Wu H, Sen S & Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. *Bioinformatics* **19**: 889-890]. Fine mapping to six genes was performed in parallel to drug-sensitivity [Kim HS & Fay JC (2007) Genetic variation in the cysteine biosynthesis pathway causes sensitivity to pharmacological compounds. *P Natl Acad Sci USA* **104**: 19387-19391].
B. The YPS163 (Y) and S288c (S) but not the M22 (M) allele of *CYS4* complements the rust coloration phenotype in a M22xS288c recombinant strain of intermediate phenotype. Complementation tests were carried out using a CEN based plasmid, pRS316, for the 6 protein coding genes within the 9.7kb interval. C. An allele-replacement of the nonsynonymous polymorphism in M22 causes near-white coloration on rich medium with copper sulfate. The allele-replacement was generated by transforming PCR products containing the desired allele into M22 lacking *CYS4* (*CYS4::kanMX*) and selection on complete medium [Kim HS & Fay JC (2007) Genetic variation in the cysteine biosynthesis pathway causes sensitivity to pharmacological compounds. *P Natl Acad Sci USA* **104**: 19387-19391].