Expanded View Figures

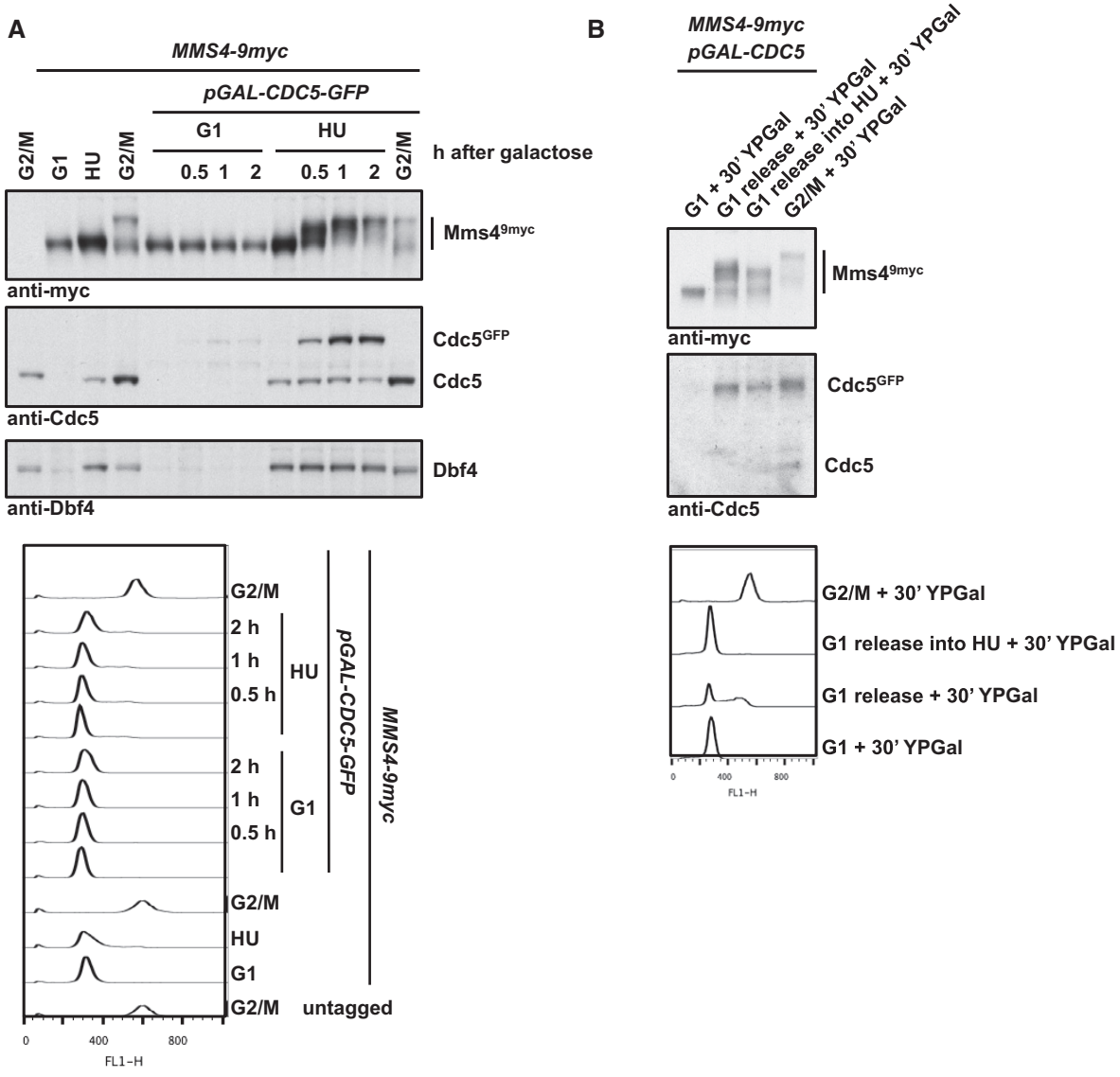

Figure EV1. Cdc5 restricts Mms4 hyperphosphorylation to mitosis.

A Overexpression of *CDC5* in S phase results in premature Mms4 hyperphosphorylation. Western blot analysis of Mms4^{9myc}, Cdc5 and Dbf4 from whole-cell extracts (upper panel) and FACS data (lower panel). Cells were arrested in G1 (with alpha-factor), S phase (with HU) or G2/M phase (with nocodazole). After arrest, *CDC5*^{GFP} overexpression was induced by addition of 2% galactose for the indicated time to cells harbouring an additional copy of GFP-tagged *CDC5* under the *GAL1* promoter. Samples were run in 7% Tris-acetate gels.

B Mms4 hyperphosphorylation by *CDC5* overexpression in S phase is reduced in HU-treated cells. Western blot analysis of Mms4^{9myc} and Cdc5 from precipitated whole-cell extracts (upper panel) and FACS data (lower panel) of cells arrested in G1 (with alpha factor) or G2/M phase (with nocodazole), or released to S phase (with or without HU). *CDC5*^{GFP} overexpression was induced for 30 min by addition of 2% galactose to cells harbouring an additional copy of GFP-tagged *CDC5* under the *GAL1* promoter. Note that upon *CDC5* overexpression cells are partially defective in bulk replication. Samples were run in 7% Tris-acetate gels.

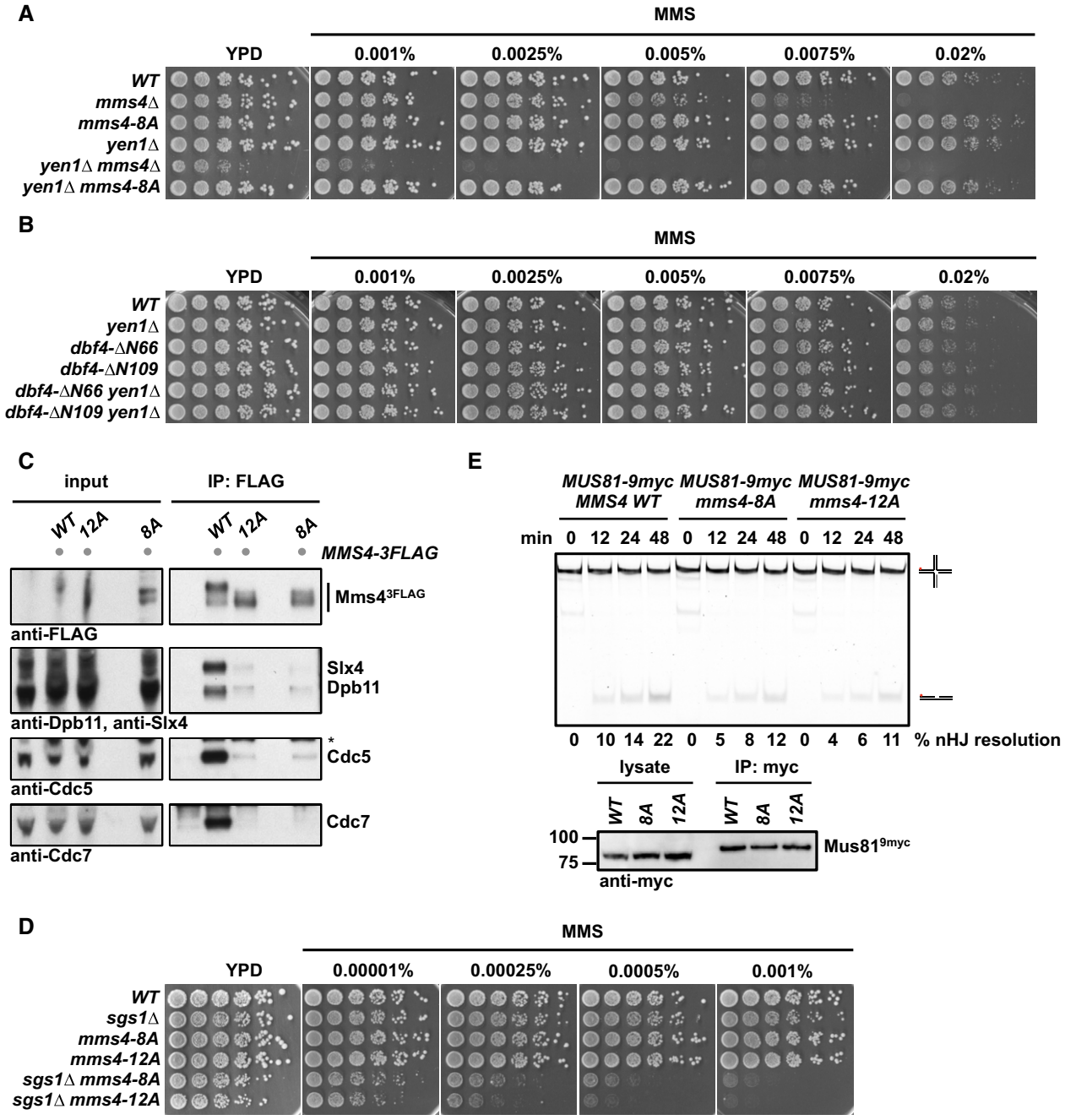

Figure EV2. Phenotypic analysis of Mms4 variants deficient in (S/T)(S/T) phosphorylation sites.

A, B The *mms4-8A* mutation or lack of Cdc5-DDK interaction does not lead to a synthetic hypersensitivity towards MMS in the *yen1Δ* background. Spotting assay as in Fig 4D and E.

C–E Additional mutation of 4 additional (S/T)(S/T) motifs in the background of the *mms4-8A* mutant (*mms4-12A*) leads to a reduction in the Mms4 phosphorylation shift (C), increases the hypersensitivity to MMS in the *sgs1Δ* background (D) and shows a slightly but not significantly decreased activity of Mus81-Mms4 (E). (C) $Mms4^{3FLAG}$ pull down as in Fig 1A, but in G2/M-arrested cells in untagged, WT, *mms4-12A* and *mms4-8A* backgrounds. Asterisk marks a cross-reactive band. (D) Spotting assay as in Fig 4D and E. (E) Resolution assay using a nHJ substrate and $Mus81^{9myc}$-$Mms4^{3FLAG}$ purified from mitotically arrested WT, *mms4-8A* or *mms4-12A* cells. Lower panel: Western blot samples of anti-myc IPs.

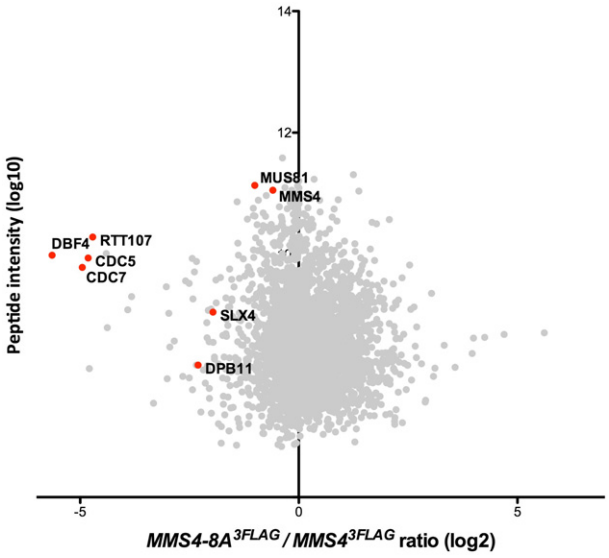

Figure EV3. A defect in the phosphorylation of Mms4 (S/T)(S/T) sites (*mms4-8A*) causes reduced association of Cdc5, DDK and Rtt107 with Mus81-Mms4.

SILAC-based quantification of $Mms4^{3FLAG}$ pull downs in WT vs. *mms4-8A* cells. Plotted are the H/L ratios against peptide intensity