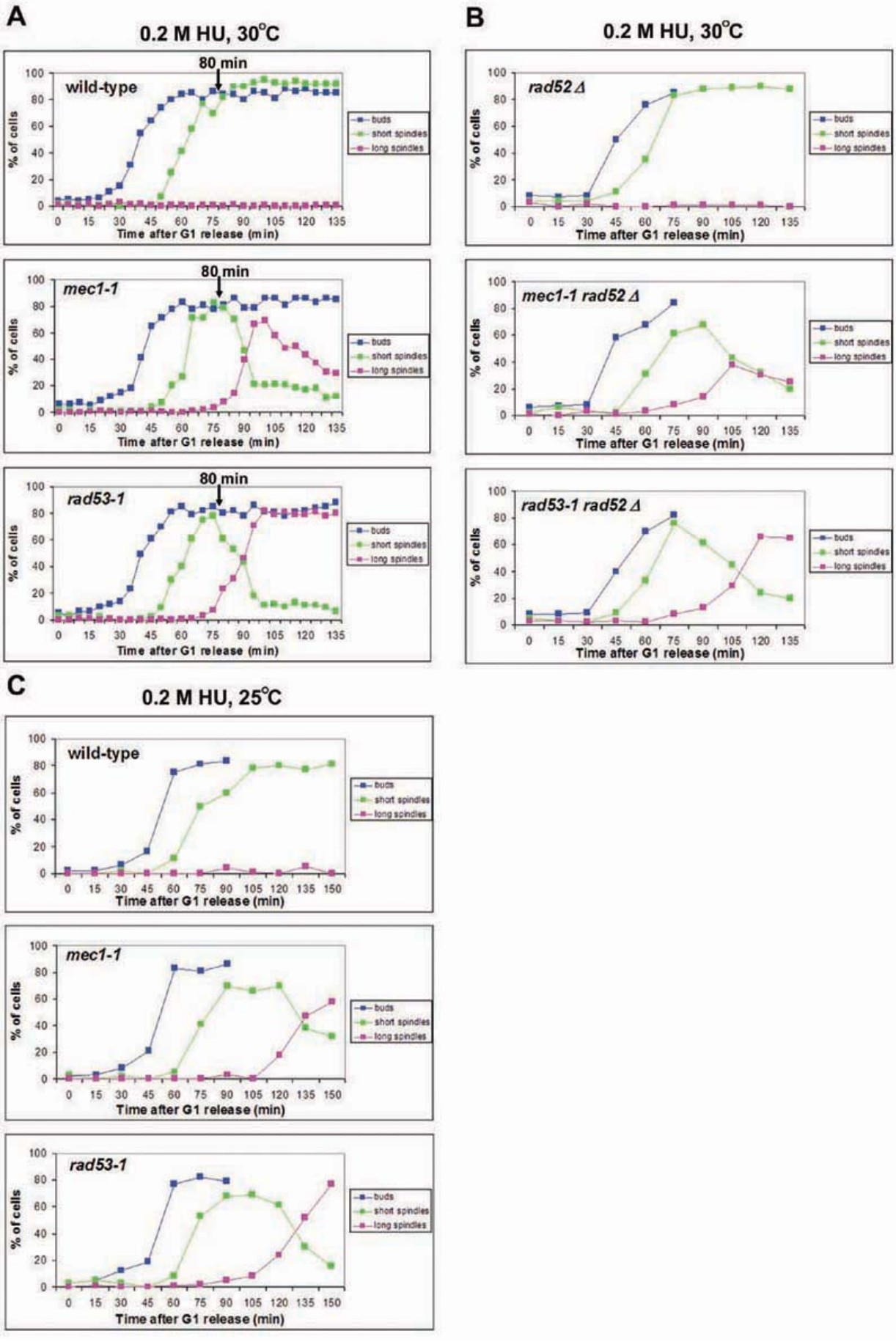
Supplementary Figure S2. Cell cycle dynamics of wild-type cells and S phase checkpoint mutants. Cells arrested in G1 phase (α factor) were released into S phase in the presence of 0.2 M HU at 30°C (A, B) and in the presence of 0.2 M HU at 25°C (C). Samples were taken at the indicated time points and spindles were observed using a *GFP-TUB1* construct (A-C). Spindles were classified as short (1-3 μm) or long (>3 μm). For all origin activation experiments, G1-arrested cells were released into HU until the budding index and the number of short spindles reached their maximum (approximately 80 min) as indicated by the arrows on the graphs (A). (D) FACS analysis of wild-type cells, released from α factor in the absence of HU at 30°C. The budding profile of the same experiment is shown as well.

S phase dynamics in wild-type, *mec1-1* and *rad53-1* cells

To provide a meaningful comparison among wild-type and mutant strains with regard to origin activation, we wanted to ensure that all cells were analyzed at identical points in S phase. Thus, we arrested cells in G1, released them into HU and measured bud formation, spindle assembly and spindle elongation in 5-min intervals (Sup. Fig. S2A). To perform the latter, we utilized strains that express green fluorescent protein (GFP) tagged α-tubulin (Clarke *et al*, 2001). Spindle length was determined by fluorescence microscopy and spindles were classified as short (1-3 μm) or long (> 3 μm). As expected, wild-type cells arrested in S phase with short spindles over a period of 130 min after release from α-factor (Sup. Fig. S2A, top panel). In contrast, *mec1-1* and *rad53-1* mutants failed to inhibit spindle elongation. While *rad53-1* cells arrested with long spindles (Sup. Fig. S2A, bottom panel), *mec1-1* cells progressed further through the cell cycle, as spindle disassembly began to occur beyond the 110-min time point (Sup. Fig. S2A, middle panel). These results confirm previous studies (Clarke *et al*, 2001; Shimada *et al*, 2002). Importantly however, they allowed us to define the time window of zero to 80 min during which cell cycle dynamics appeared to be uniform for the three strains.

Supplementary Figure S2



Supplementary Figure S2

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