Supplementary data

Material and Methods

Purification of GST-FHA fusion proteins and pull-down assays

E. coli cultures containing GST-FHA1, GST-FHA2, GST-FHA1^{R70A} or GST-FHA2^{R605A} (Durocher et al., 1999) were induced for 3h by 1mM IPTG after culturing in LB to OD_{600}=0.8. Cells were resuspended in PBS, sonicated, adjusted to 1% Triton X-100, and the supernatant was recovered after centrifugation at 13,000 rpm for 20 min at 4^{o}C. Aliquots were stored in 15% glycerol at –80^{o}C. GST-FHA fusions from the *E. coli* extracts were bound to glutathione agarose beads (Sigma) in 1 x PBS with 1% Trition X-100 for 45 min at 4^{o}C. Beads were washed three times with PBS/Triton and resuspended in the same buffer. One volume of chromatin extract (see below) was bound to beads for 90 min at 4^{o}C. The beads were washed for 10 min three times in 0.5x PBS with 50 mM Tris-Cl pH 7.4, 1%Triton X-100 and 2 mM EDTA. Proteins were eluted in SDS containing sample buffer by heating for 5 min, followed by SDS-PAGE and Western blotting with anti-Myc mAb 9E10 for Myc-Sgs1 and Myc-Top3.

Chromatin fractionation

Chromatin fractionation was performed as described (Frei and Gasser, 2000), except that the first chromatin fraction was resuspended in 50 mM Tris-Cl pH 7.4, 5 mM $MgCl_2$, 0.5% Triton X-100, with 50 U Dnase I (Roche Molecular Biology), protease inhibitors: 300 μg/ml benzamidine, 1μg /ml pepstatin, 2 μg /ml antipain, 0.5 μg /ml leupeptin, 100 μg /ml TPCK and 50 μg /ml TLCK. After 10 min at 4^{o}C, EDTA was added to 10mM. After centrifugation at 13,000 rpm at 4^{o}C for 15 min, the supernatant was used as the extract in the GST pull-down assay.

Reference

Durocher, D., Henckel, J., Fersht, A.R. and Jackson, S.P. (1999) The FHA domain is a modular phosphopeptide recognition motif. *Mol Cell*, 4, 387-394.