In situ chromatin binding assay:
Swi1-GFP fluorescence after triton-X100 extraction and DNaseI treatment method (Kearsey et al, EMBO.J, 19, 1681-1690, 2000)

Y171 (swi1-GFP) was cultured in EMM-LUAH media at 25 ˚C in the absence or presence of HU

Samples (4 x 10^6 cells/ml : 50mL for each sample)
Non-treated cells
HU : 12mM : 6h

Buffers
ZM (50mM Sodium citrate pH5.6, 1.2M Sorbitol, 0.5mM MgOAc, 10mM DTT)
STOP (0.1M Mes pH6.4, 1.2M Sorbitol, 1mM EDTA, 0.5mM MgOAc)
EB (20mM Pipes-KOH pH6.8, 0.4M Sorbitol, 2mM MgOAc, 150mM Kac)
EBM (20mM Pipes-KOH pH6.8, 0.4M Sorbitol, 7.5mM MgOAc, 150mM Kac)
EBMT (EBM + 10% Triton X-100)

Preparation of samples for microscopy
Add NaN3 (0.01% final) to culture to stop cell growth
Wash cells in 5mL of ZM buffer
(Centrifuge, discard sup, add 5ml ZM to the pellet, vortex, centrifuge, discard sup)
Suspend cells in 1ml of ZM + 2mg/mL Zymolyase
Incubate at 32C for 10-20min
Check cells under the microscope (1:1 ratio with 2% SDS, 95% cell should be dark)
after this step, perform experiments **on ice**
Add 3 volumes of cold stop buffer (3mL) and suspend cells **genly**
Wash cells in cold stop buffer twice (5mL x 2)
(Centrifuge, discard sup, add 5ml STOP to the pellet, suspend cells **by pipetting**, centrifuge, discard sup)
Wash cells in cold EB buffer (5mL)
(Centrifuge, discard sup, add 5ml EB to the pellet, suspend cells **by pipetting**, centrifuge, discard sup)

Suspend (**by pepetting**) cells in 1mL of cold EBM + 1mM pAPMSF + completeMiniEDTAfree
Split cell suspension into 4tube (250uL each sample A, B, C, D)

Sample A (non-extraction)
Add 25uL of EBM and incubate at 0 ˚C for 30min
Sample B (1% triton extraction)
Add 25uL of EBMT and incubate at 0 ˚C for 30min
Sample C (DNase extraxtion, 1% triton extraxtion)
Add 25uL of EBMT and incubate at 0 ˚C for 30min
Add 3uL of DNaseI(450U): Sigma D-7291
Incubate at 0C for 30min
Add NaCl (final 250mM) to stop DNase reaction

After extraction, spin cells down and suspend (**by pipetting**) cells in 1mL of MetOH (-80C)
Spin down cells and re-suspend (**by pipetting**) cells in 300uL of Acetone (-80C)
Keep cell suspension at –80C

Microscopic analysis
Have 2uL of mounting solution (0.4ug/mL DAPI, 50% glycerol, 0.5 x PBS) on a slide glass
Spread 5uL of cell suspension just next to the mounting solution
Place a cover slip (poly-L-lysine coated) over the mounting solution.
* do not dry up cells on the slide glass.
* let mounting solution cover the cells