

Supplementary Material

Supplementary Figure S1: Identified proteins overlap among the replicates.

Protein factors identified after the GFP-H2A variants chromatin purification using the GFP nanotrap beads are displayed in Venn diagrams showing the identified proteins overlap in three independent biological replicates (e.g. H2A.Bbd exp.#1, #2 and #3).

Supplementary Figure S2: GFP-H2A.Bbd co-localizes with DNA-dense regions

in a subpopulation of cells. Single confocal slice of asynchronous population of female MEF cells that express GFP-H2A.Bbd. To-PRO: DNA (blue); GFP: GFP-H2A.Bbd (green). The arrow indicates GFP-H2A.Bbd co-localizing with DNA dense regions. The two panels represent different areas of acquisition. Scale bar upper panel: 5 μm ; lower panel: 10 μm .

Supplementary Figure S3: GFP-H2A.Bbd localizes to sites of replication during

all stages of S-phase. Single confocal slice of female MEFs expressing H2A.Bbd labeled with EdU and stained with anti-GFP at different time of replication. EdU: replication foci (red); GFP-H2A.Bbd: GFP (green); DAPI: DNA (blue). Early S-phase: eS; late S-phase: lS. Scale bar: 5 μm .

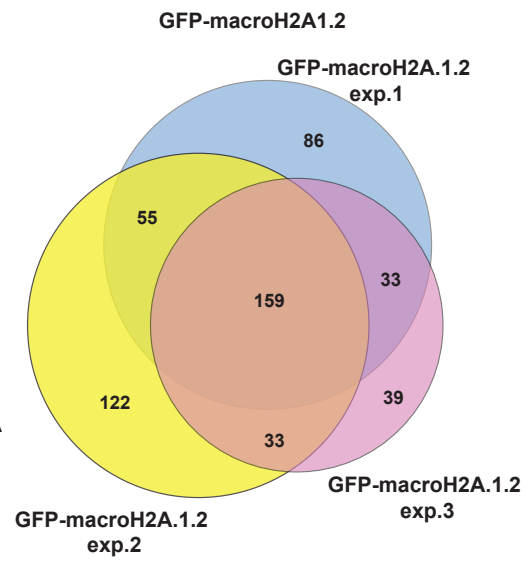
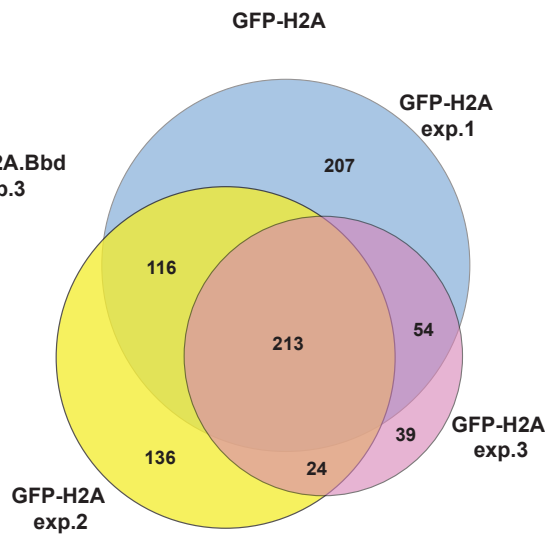
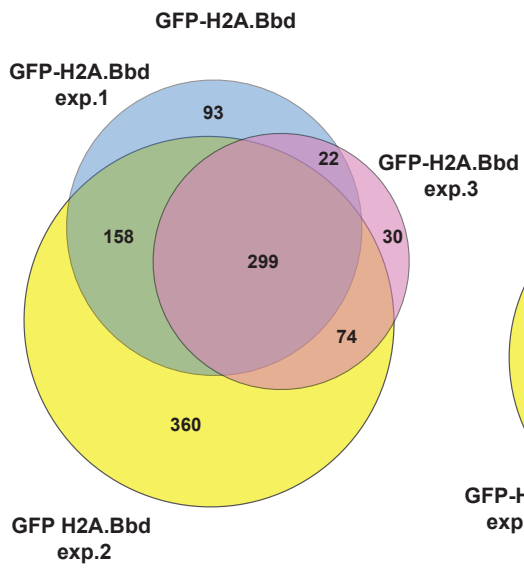
Supplementary Figure S4: GFP-H2A.Bbd has different resident time in early-

and late- replicating chromatin. Optical sections of confocal images of replicating MEF cells stably expressing GFP-H2A.Bbd and pulsed labeled with BrdU (red) for 5 min and fixed 0 / 5 / 10 min thereafter as indicated. Scale bars: 5 μm .

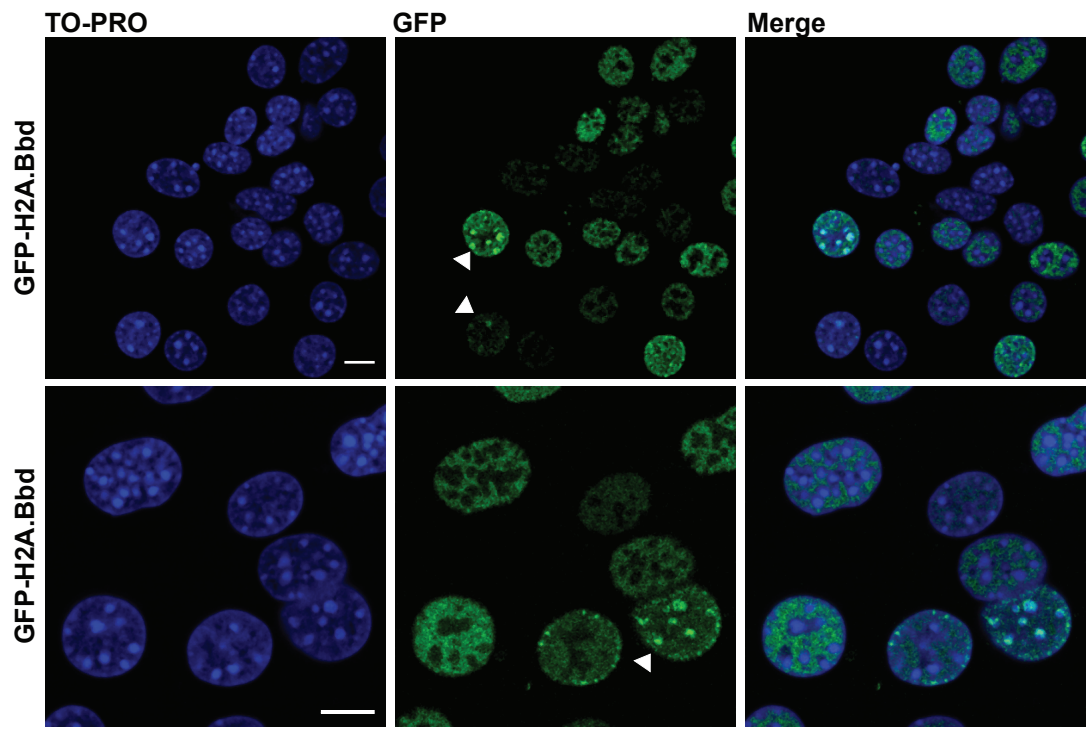
Supplementary Movie. Localization of H2A.Bbd to sites of DNA synthesis *in*

***vivo*.** Live cell imaging of MEF cells stably expressing GFP-H2A.Bbd (green) transfected with mRFP-PCNA (red). 3D-stacks were acquired at 20-minute intervals

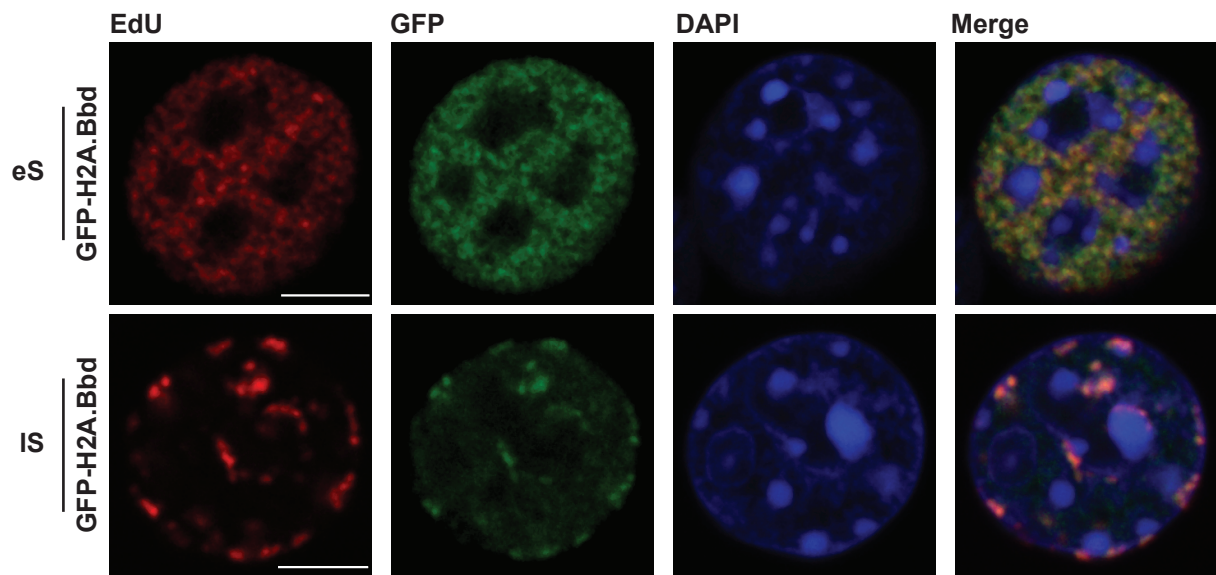
over night using a spinning disk microscope equipped with temperature, humidity and CO₂ control. Shown are maximum intensity projections of each time point. Scale bar: 5 μm.



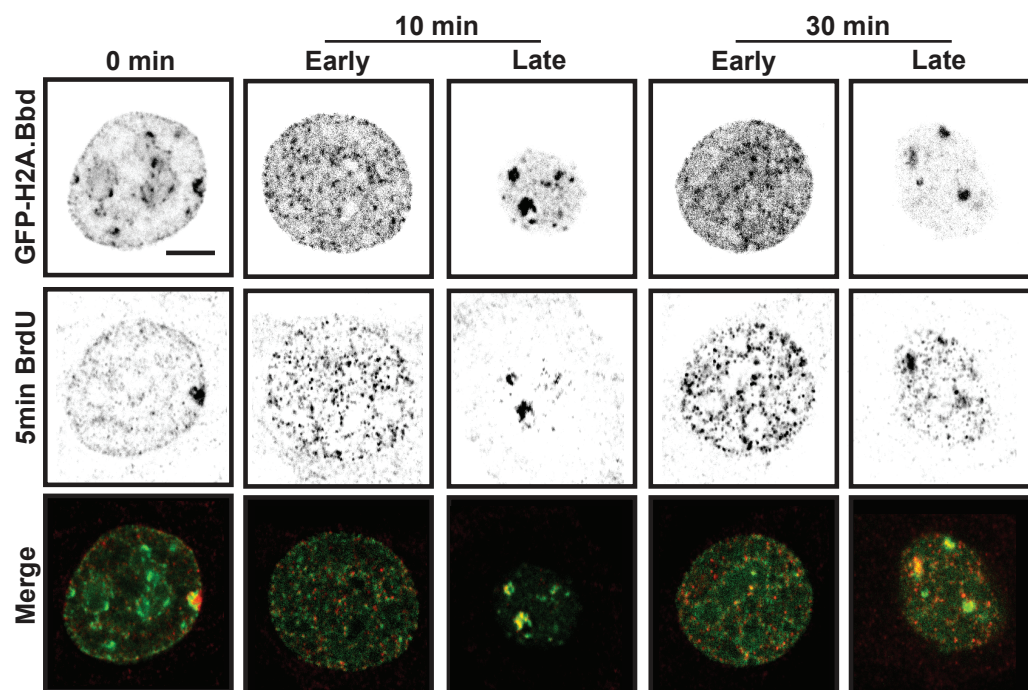
Supplementary figure S1, Sansoni et al.



Supplementary figure S2, Sansoni et al.



Supplementary figure S4, Sansoni et al.



Supplementary figure S4, Sansoni et al.