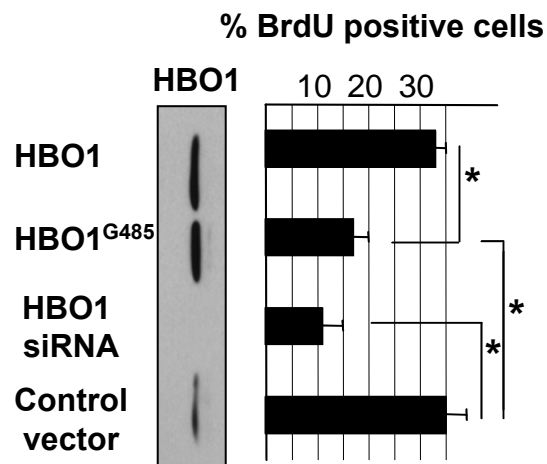


**HBO1 Histone Acetylase Activity Is Essential for DNA Replication Licensing and Inhibited by Geminin**

Benoit Miotto and Kevin Struhl

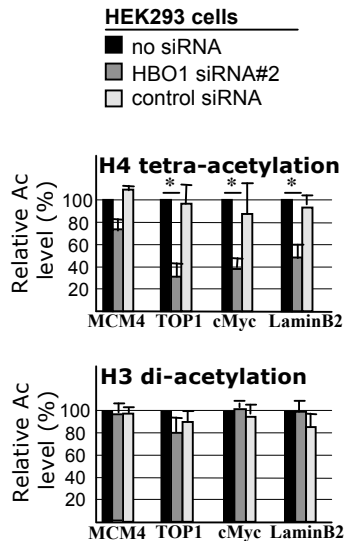
**Supplementary Figure S1, related to Figure 1**



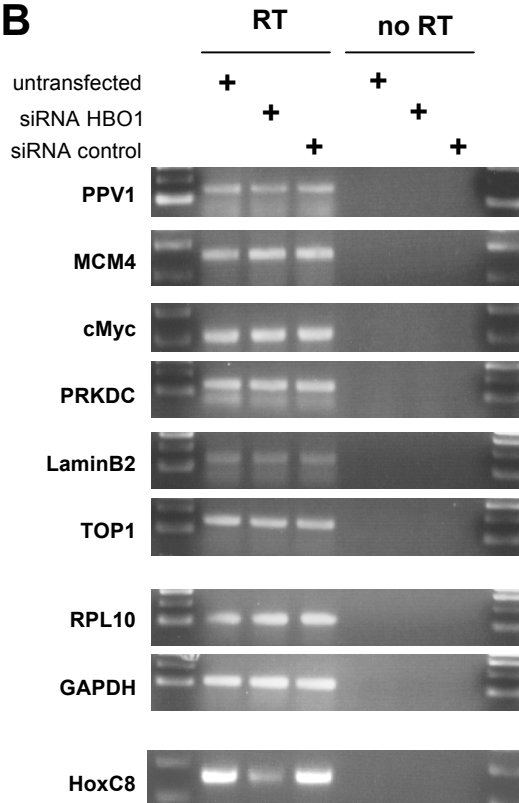
**HBO1 HAT activity is required for DNA replication and origin activity.**  
Comparison of HBO1, HBO1<sup>G485</sup> and HBO1 siRNA expression effect on BrdU incorporation (n=4) ( $P < 0.05$ , \*).

## Supplementary Figure S2, related to Figure 2

**A**



**B**

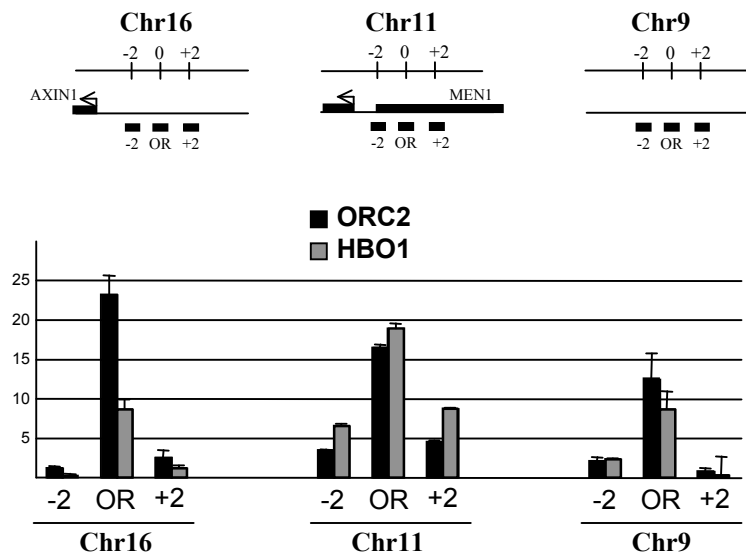


**HBO1 regulates H4 acetylation level at origins but does not affect expression level of genes surrounding replication origins.**

(A) Impact of HBO1 depletion on H4 and H3 acetylation level at replication origins in the human epithelial kidney 293 cell line (n=3). Data are presented as relative acetylation (Ac) level, normalized to the control condition without siRNA (n=3) (\*,  $P < 0.05$ ).

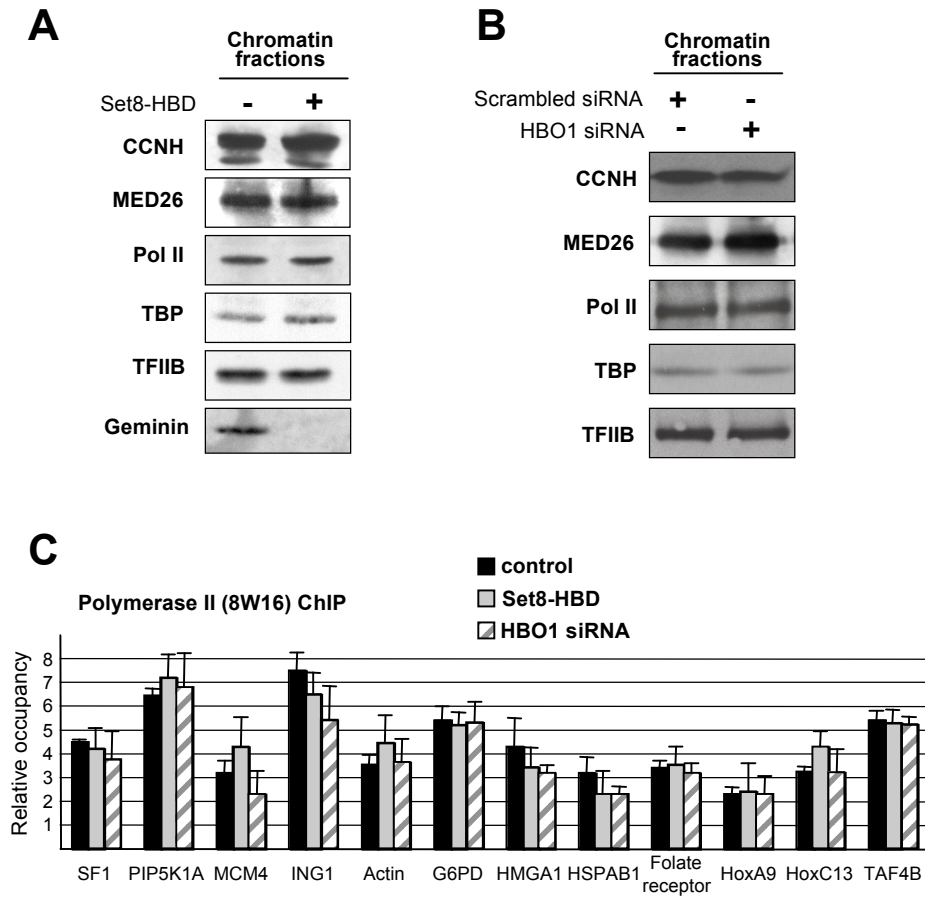
(B) Levels of expression of genes surrounding origins as determined by semi-quantitative RT-PCR from HeLa cells treated with HBO1 or control siRNA constructs. RPL10 and GAPDH are used as internal control. HoxC8 is shown as a positive control of the experiment. The figure is representative of 4 independent experiments.

## Supplementary Figure S3, related to Figure 3



**Characterization of “new” human replication origins studied in this manuscript.** Occupancy level of ORC2 (black) and HBO1 acetylase (grey) at new replication origins on Chromosome 9, 11 and 16 identified by nascent strand DNA accumulation in Cadoret et al. (2008) (n=3). Distances are in kilobases.

## Supplementary Figure S4, related to Figure 5



### Set8-HBD expression and HBO1 depletion do not have a global effect on polymerase II function.

(A) Analysis of chromatin binding of CCNH, MED26, Polymerase II, TBP and TFIIB in cells expressing or not Set8-HBD. Geminin is displayed as a control of G1-accumulation induced by Set8-HBD.

(B) Same analysis in HBO1 depleted cells compared to cell treated with a scrambled siRNA.

(C) ChIP analysis of Polymerase II occupancy in the coding region of different genes in cells expressing Set8-HBD, depleted of HBO1 or untreated (n=2). Relative occupancy is expressed as a ratio binding in coding sequence over intergenic regions (average of 3 intergenic primer pairs).