

## Expanded View Figures

### Figure EV1. H4K20 methylation promotes the replication of EBV-derived episomes.

- A Expression levels of GAL4, GAL4-PR-Set7, and GAL4-PR-Set7<sup>SETmut</sup> in EBNA1-expressing HEK293 cell lines. Immunoblot analysis with GAL4 antibody.
- B ChIP-qPCR analysis of the FR-UAS plasmid with anti-GAL4, anti-H4K20me1, and anti-H4K20me3 antibodies. Values are depicted relative to isotype control. Data are means  $\pm$  SEM ( $n = 3$ ).
- C Replication efficiency of FR-DS and FR-UAS-DS plasmids in GAL4, GAL4-PR-Set7, and Gal4-PRset7<sup>SETmut</sup> cells. Replication efficiencies are depicted relative to the FR-DS plasmid arbitrarily set as 100% in every cell line. Data are means  $\pm$  SEM ( $n = 4$ ).
- D ChIP-qPCR analysis at the FR, UAS, and DS sites with anti-GAL4, anti-MCM3, anti-H4K20me1, and anti-H4K20me3 using chromatin from GAL4, GAL4-PR-Set7, and GAL4-PR-Set7<sup>SETmut</sup> cells expressing EBNA1 and transfected with similar amounts of FR-DS or FR-UAS-DS plasmids. The y-axis represents the relative fold enrichment of the specific antibody versus isotype control. Data are means  $\pm$  SEM ( $n = 5$ ).

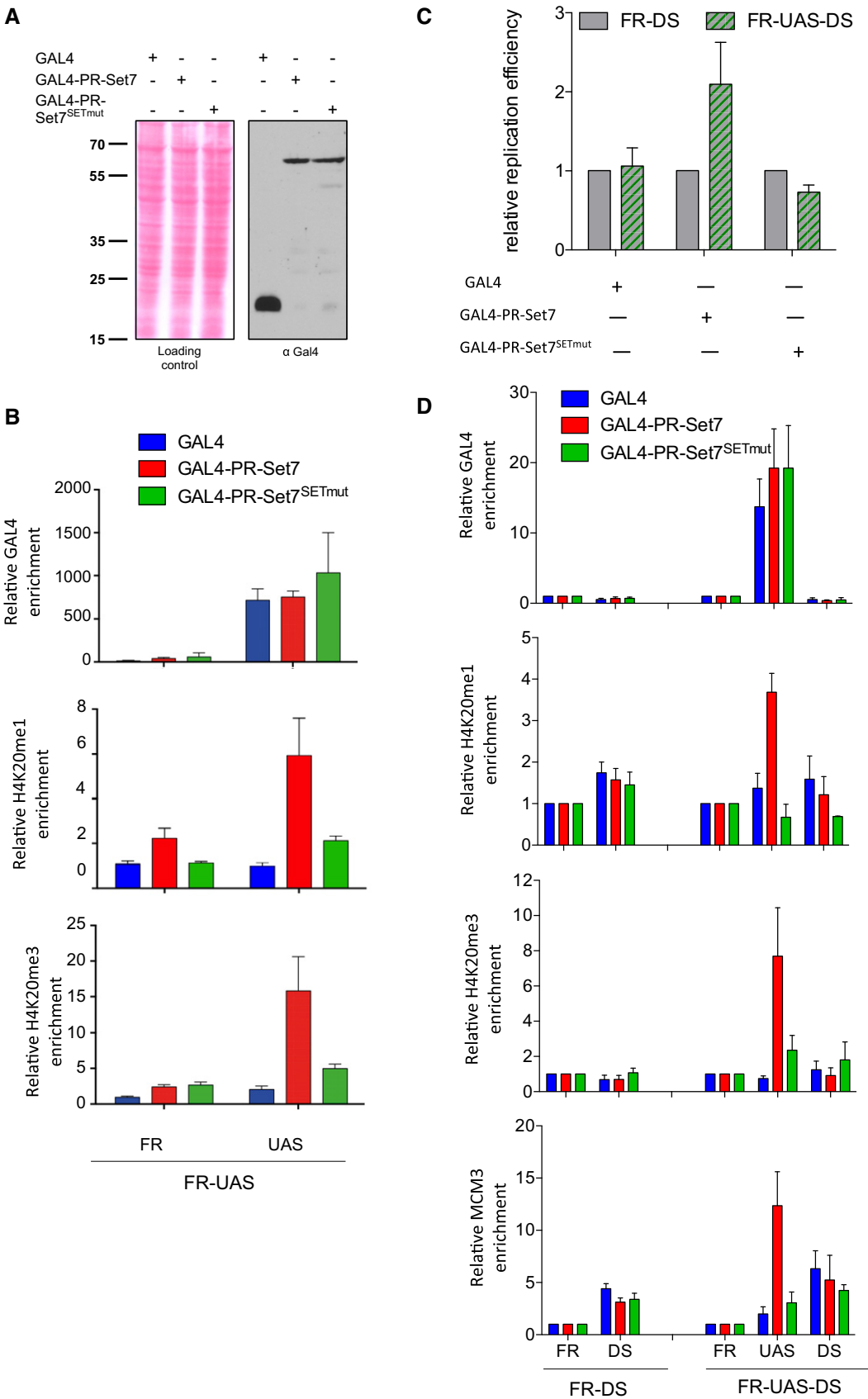


Figure EV1.

**Figure EV2. Loss of Suv4-20h in MEFs affects the replication of late S-phase.**

- A Proliferation rates of mouse embryonic fibroblasts MEFs<sup>364.2</sup> (*SUV4-20H2*<sup>-/-</sup>, *SUV4-20H1*<sup>-lox</sup>, Cre-ER cells) untreated (blue curve) or treated with 4OHT (red curve) to induce the loss of H4K20me2/3. Data are means  $\pm$  SD ( $n = 3$ ).
- B FACS analysis of MEFs<sup>364.2</sup> (*SUV4-20H1*<sup>-lox/-</sup>; *SUV4-20H2*<sup>-/-</sup>) treated or not with 4OHT at the time of the replication-timing analysis. The black arrow points to the accumulation of replicating (BrdU-positive) 4OHT-treated cells in late S-phase.
- C Replication-timing profiles of the whole chromosome 17 and at higher magnitude of the chromatin region 35979972-44013079 (8 Mb, mm9) in wild-type (black line) and constitutive *SUV4-20H1/H2*-null MEFs (gray line). Arrows point to delayed mid/late domains. Lower panel is the replication-timing profile of the same chromatin region of chromosome 17 in untreated and 4OHT-treated (*SUV4-20H* null) MEFs<sup>364.2</sup> (*SUV4-20H1*<sup>-lox/-</sup>; *SUV4-20H2*<sup>-/-</sup>; CRE-ER). The blue line indicates the same nucleotide position in all replication-timing profiles.

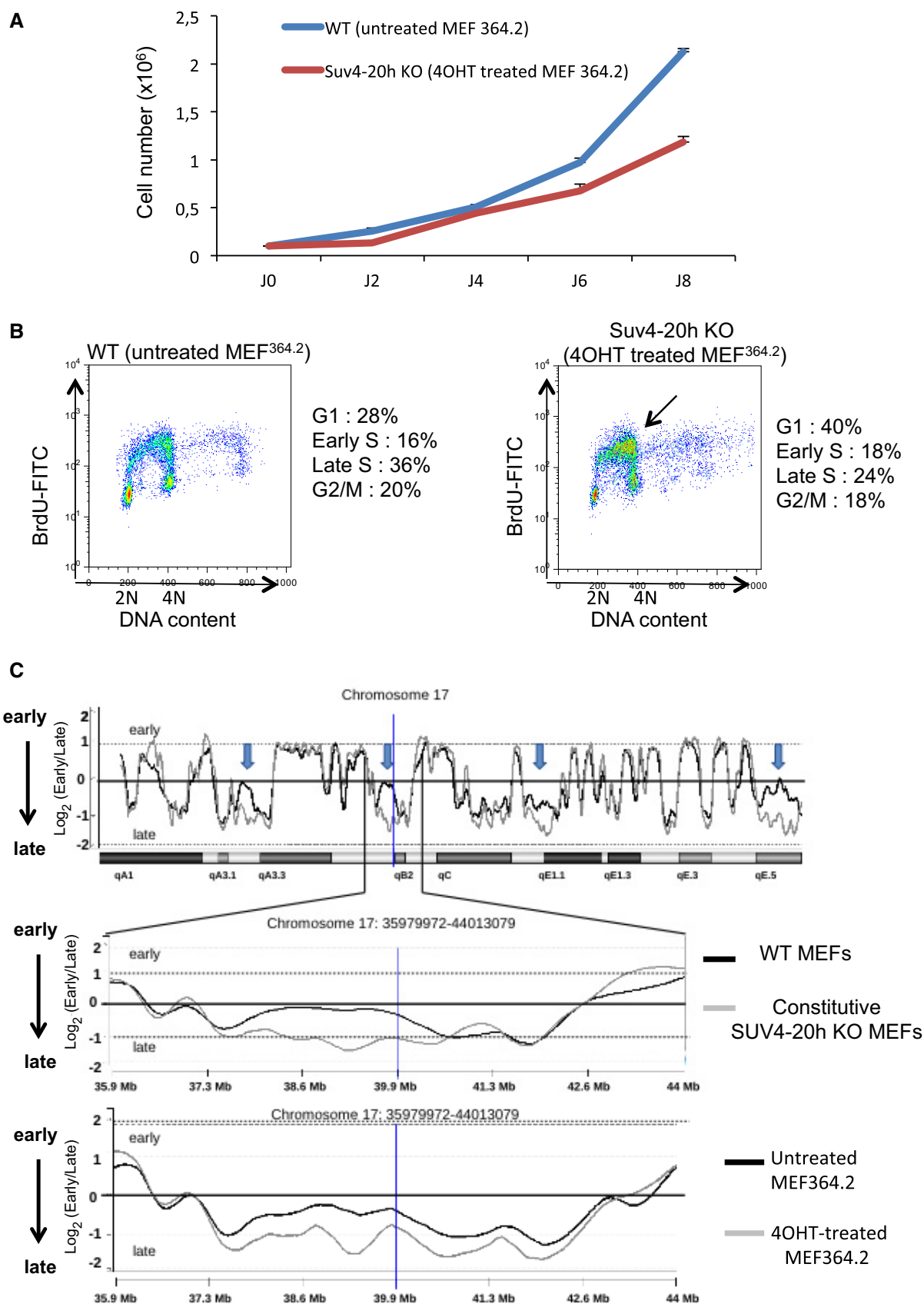


Figure EV2.

**Figure EV3. Replication-timing alterations in Suv4-20h-null cells occur in late-replication domains harboring high levels of H4K20me3 at origins.**

- A ChIP-qPCR analysis in untreated and 4OHT-treated MEFs<sup>364,2</sup> of H4K20me3 levels at the control early-firing MYC origin and at late-firing origins located in delayed late-replication domains (ORI-5 to ORI-14) and in non-delayed late-replication domains (ORI5-ND to ORI14-ND) using anti-H4K20me3 or isotype non-relevant antisera (IgG) as a control. Data are means  $\pm$  SD ( $n = 3$ ). Coordinates of origins are indicated in Appendix Table S2.
- B ChIP-qPCR analysis in untreated and 4OHT-treated MEFs<sup>364,2</sup> of H4K20me1 levels at the control early-firing MYC origin and at late-firing origins located in delayed late-replication domains (ORI-1 to ORI-4) of chromosome 11 with anti-H4K20me1 antibody showing four late-replicating heterochromatin origins (ORI-1 to ORI-4) using anti-H4K20me1 or isotype non-relevant antisera (IgG) as a control. Data are means  $\pm$  SD ( $n = 3$ ). Coordinates of origins are indicated in Appendix Table S2.

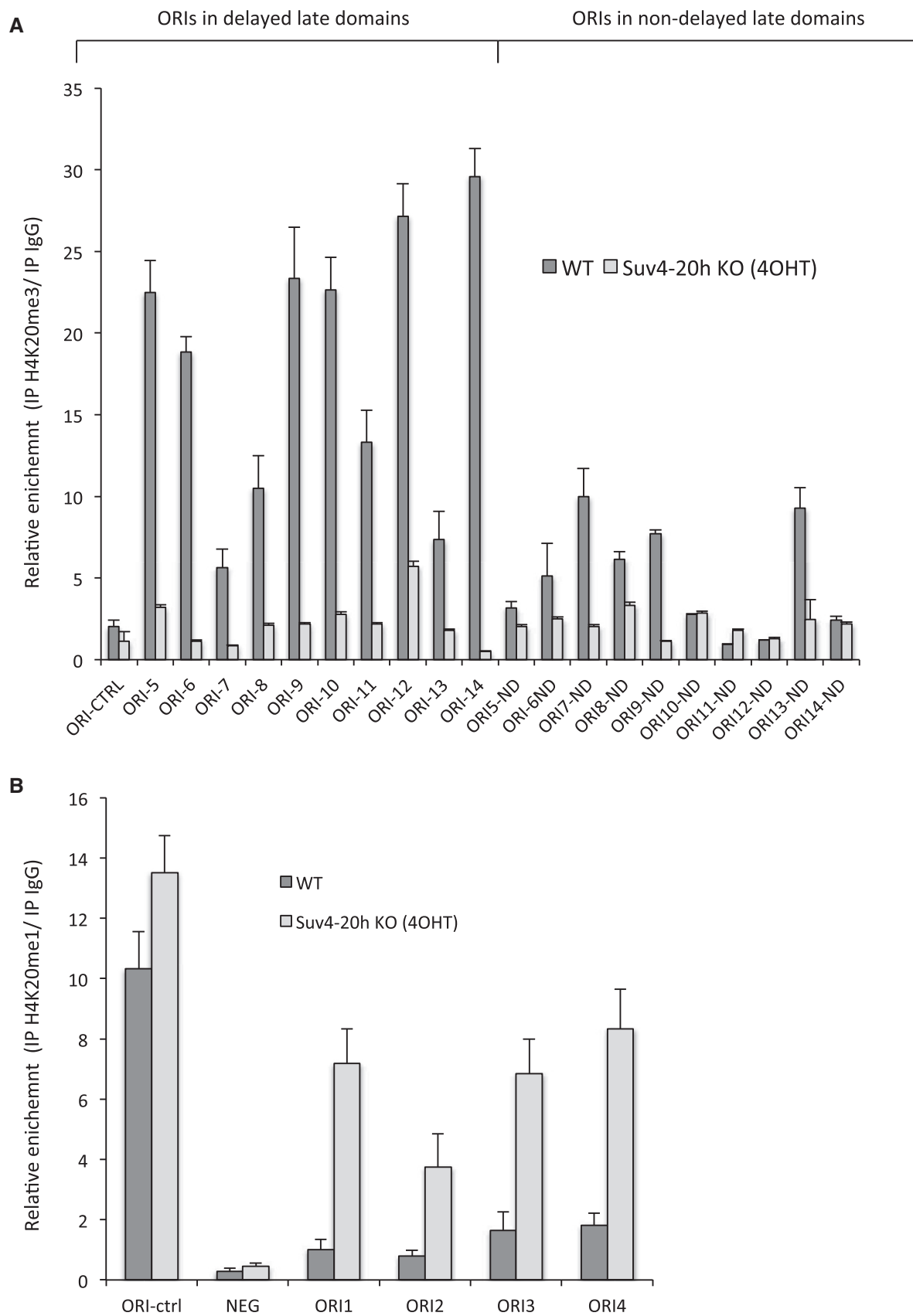


Figure EV3.

**Figure EV4. Expression of histone H4K20A mutant impairs the licensing and activation efficiency of H4K20me3-associated origins.**

- A ChIP-qPCR analysis of H4K20me3 levels at the control early-firing MYC origin and at late-firing origins in delayed and non-delayed late domains of chromosome 11 in histone H4<sup>WT</sup>- and H4<sup>K20A</sup>-expressing MEFs<sup>364.2</sup> using anti-H4K20me3 and an isotype non-relevant antisera (IgG) as control. The y-axis represents the relative enrichment between H4K20me3 and control IgG immunoprecipitate DNA. NEG corresponds to a negative control region at 5 kb downstream of ORI-1. Coordinates of ORIs are indicated in Appendix Table S2. Errors bars represent SD ( $n = 3$ ). (\*) Statistical significance (unpaired t-test) with  $P < 0.05$ .
- B ChIP-qPCR analysis of FLAG-MCM5 levels at the same origins as above in histone H4<sup>WT</sup>- and H4<sup>K20A</sup>-expressing MEFs<sup>364.2</sup> using anti-FLAG antibody and an isotype non-relevant antisera (IgG) as control. Errors bars represent SD ( $n = 3$ ). (\*) Statistical significance (unpaired t-test) with  $P < 0.05$ . Noted that ectopically expressed histone H4 proteins contained a C-terminal epitope FLAG, which was not recognized by FLAG antibody in the context of formaldehyde-crosslinked chromatin (control data not shown).
- C Quantitation of the relative SNS enrichment of the same origins as above in histone H4<sup>WT</sup>- and H4<sup>K20A</sup>-expressing MEFs<sup>364.2</sup>. SNS enrichment was arbitrarily normalized with respect to the control origin. Data are means  $\pm$  SD ( $n = 3$ ). (\*) Statistical significance (unpaired t-test) with  $P < 0.05$ .
- D ChIP-qPCR analysis of FLAG-MCM5 levels at the same origins as above in untreated and 4OHT-treated MEFs<sup>364.2</sup> using anti-FLAG antibody and an isotype non-relevant antisera (IgG) as control. Errors bars represent SD ( $n = 3$ ). (\*) Statistical significance (unpaired t-test) with  $P < 0.01$ .

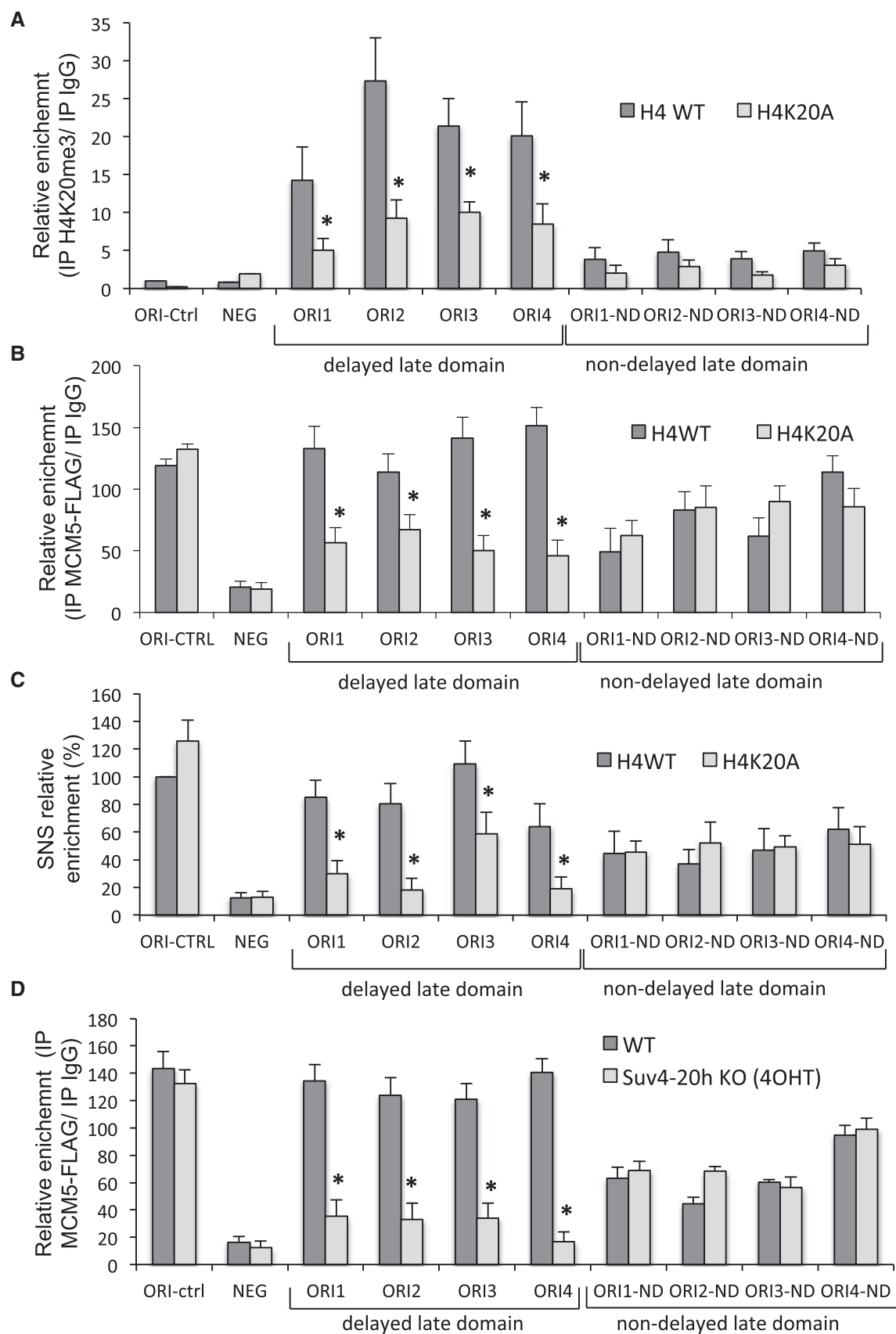
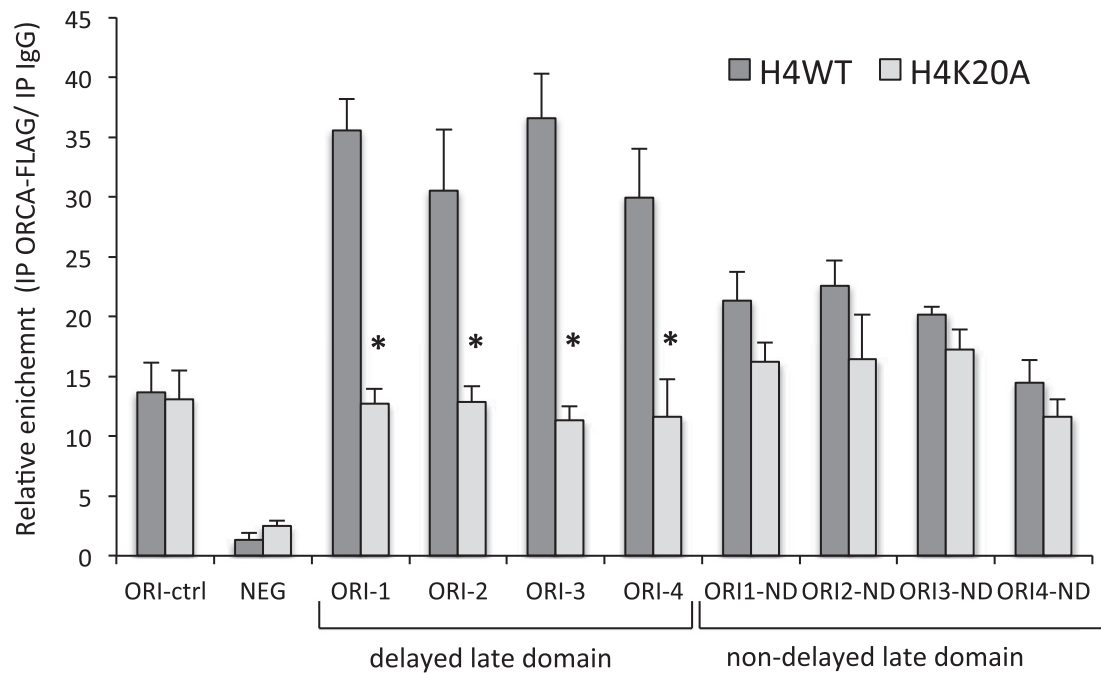


Figure EV4.





**Figure EV5. ORCA binding to H4K20me3-associated origins is reduced in histone H4<sup>K20A</sup>-expressing cells.**

ChIP-qPCR analysis of ORCA levels at the control early-firing MYC origin and at late-firing origins in delayed and non-delayed late domains of chromosome 11 in histone H4<sup>WT</sup>- and H4<sup>K20A</sup>-expressing MEFs<sup>364,2</sup> using anti-FLAG antibody and an isotype non-relevant antisera (IgG) as control. NEG corresponds to a negative control region at 5 kb downstream of ORI-1. Coordinates of ORIs are indicated in Appendix Table S2. Errors bars represent SD ( $n = 3$ ). (\*) Statistical significance (unpaired t-test) with  $P < 0.05$ . Noted that ectopically expressed histone H4 proteins contained a C-terminal epitope FLAG, which was not recognized by FLAG antibody in the context of formaldehyde-crosslinked chromatin (control data not shown).