Supplemental Materials

Molecular Biology of the Cell

Grézy et al.

Supplementary Figures Legends

Figure S1 : Typical experiments monitoring the efficiency of the various siRNAs in the various experimental settings

A) Suv39H-/- cells were transfected using Tip60-1, Tip60-2 or control siRNA. These siRNAs were described previously and shown to inhibit Tip60 RNA and protein expression (Chevillard-Briet *et al.*, 2014). After 72h, RNA was extracted and the presence of Tip60 and β2m mRNAs were quantified by reverse transcription followed by qPCR. The amount of Tip60 mRNAs were divided by the amount of β2m. Representative experiment is shown. Error bars represent standard deviation within technical triplicates. B) Suv39H -/- cells were transfected using BRD2 or control siRNA. 72h later, cells were harvested and after nuclear extraction, western-blot analysis was realised with anti-BRD2 and anti-Actin antibodies. C) Suv39H-/- cells or cells rescued for Suv39H1 expression were transfected by the indicated siRNA. 72 hours later, cells were harvested and the amount of Tip60, Suv39H1 and β2m mRNAs were quantified by reverse transcription followed by qPCR. The amount of Tip60 or Suv39H1 mRNAs were divided by the amount of β2m. Representative experiment is shown. Error bars represent standard deviation within technical triplicates.

Figure S2 : Tip60 depletion does not induce satellite expression upon 5-Aza treatment

NIH3T3 transfected using indicated siRNA were treated or not with 5-Aza 48h after transfection for 24 hours. RNA was extracted and the presence of satellite RNAs and β2m mRNAs were quantified by reverse transcription followed by qPCR. The amount of satellite RNAs were divided by the amount of β2m. Representative experiment is shown. Error bars represent standard deviation within technical triplicates.

Figure S3 : Increased number of cells harbouring micronuclei upon Tip60 knockdown using a different siRNA

Suv39H-/- cells were transfected by the indicated siRNAs. 48 hours later, cells were harvested and the amount of cells harbouring micronuclei was quantified after DAPI staining and microscopy analysis. Two siRNAs (Ctrl, used elsewhere in this work, and Ctrl-2, described in supplemental procedures) targeting no messenger were used as control. Representative experiment is shown.

Figure S4 : Tip60 depletion stimulates the expression of LINE sequences in a Suv39H-dependent manner

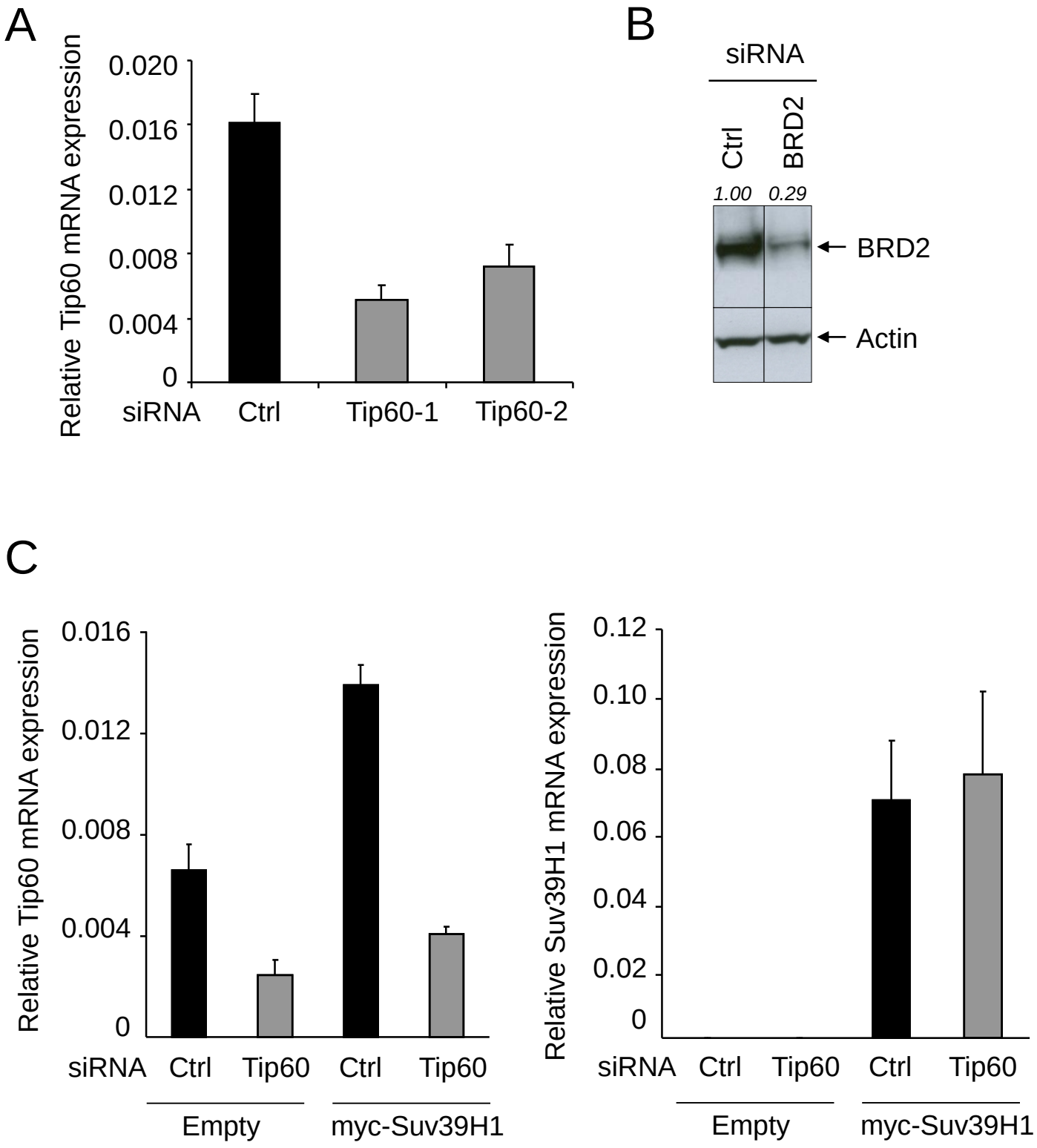
Suv39H-/- cells or cells rescued for Suv39H1 expression were transfected by the indicated siRNA. 72 hours later, cells were harvested and the amount of LINEs and β2m mRNA were quantified by reverse transcription followed by qPCR. The amount of LINEs were divided by the amount of β2m. Representative experiment is shown. Error bars represent standard deviation within technical triplicates.

Figure S5 : BRD2 depletion does not affect satellite expression in Suv39H cells

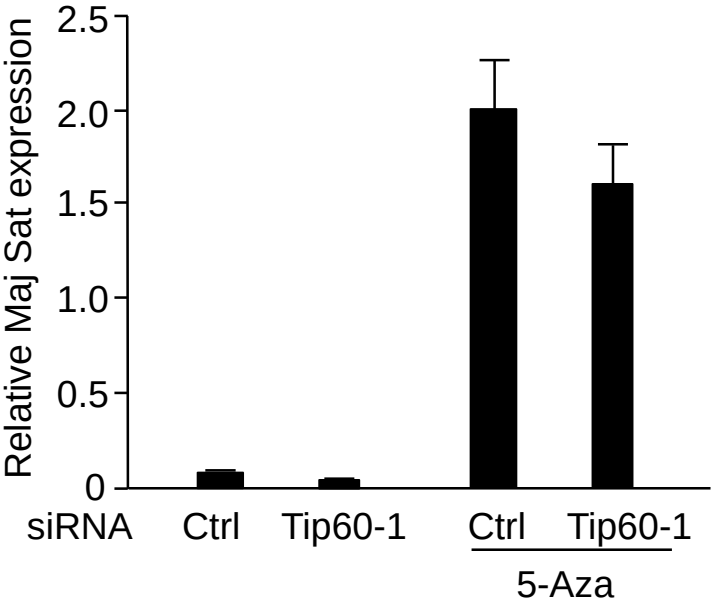
Suv39H -/- cells were transfected using BRD2 or control siRNA. 72h later, cells were harvested and the presence of satellite RNAs and β2m mRNA were quantified by reverse transcription followed by qPCR. The amount of satellite RNAs were divided by the amount of β2m. The mean and standard deviation for 3 experiments are shown.

Figure S6 : H4K12 becomes hyperacetylated during late S phase

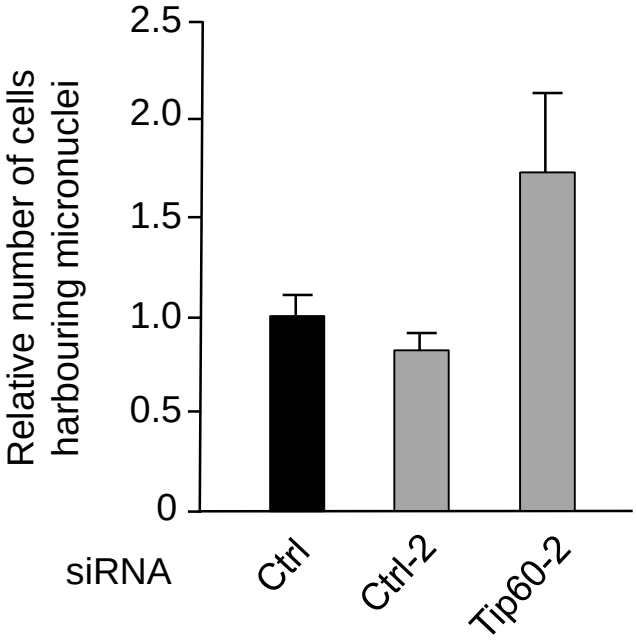
NIH3T3 cells were harvested by serum deprivation for 48h, then released in the cell cycle by serum addition, fixed 4, 9, 11 or 15h after serum addition and subjected to a ChIP assay using anti-H4K12Ac antibodies, anti-histone H3 antibody or no antibody (No Ab) as a control. The amounts of major satellite and β2m sequences were quantified by qPCR in the immunoprecipitates and the inputs. The mean and standard deviation from two independent immunoprecipitations are shown.



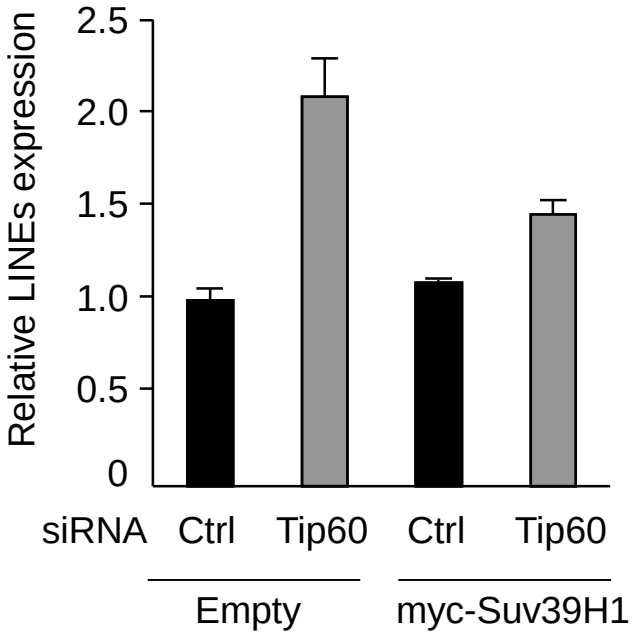
Grézy et al., SuppFig. S1



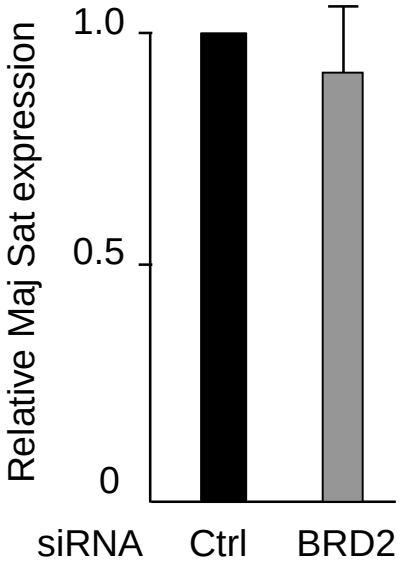
Grézy et al., SuppFig. S2



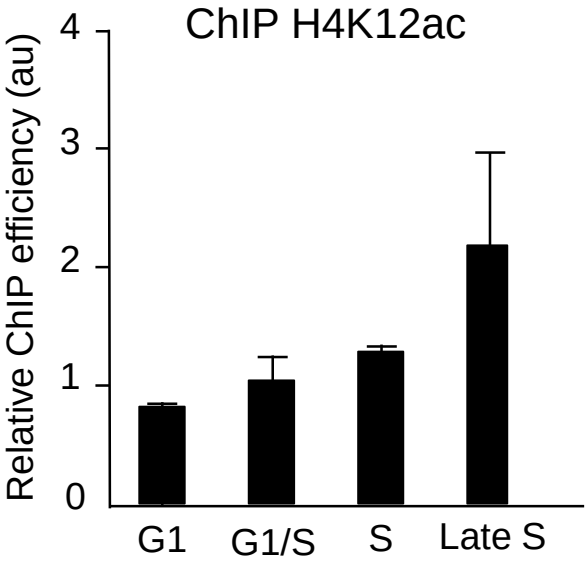
Grézy et al., SuppFig. S3



Grézy et al., SuppFig. S4



Grézy et al., SuppFig. S5



Grézy et al., SuppFig. S6

Supplemental Procedures

Western-blot analysis

Total cell lysates were prepared as previously described (Mattera *et al.*, 2009) and analysed in western-blot experiments using an anti-BRD2 antibody from Bethyl Laboratories.

Additional siRNA

The following additional siRNA was used in the micronuclei analysis :
Ctrl-2: CGUACGCGGAAUACUUCGA-dTdT

Expression des LINEs

Expression of LINEs was measured by RT-qPCR using the following primers :
5'-CACTCCCACCCCACCTAGT-3' and 5'-TAACTCTTTAGCAGTGCTCTCCTGT-3'

Synchronisation of cells

Cells were synchronised by serum deprivation (medium complemented with 0.25% serum) during 48h, then released in the cell cycle by addition of complete medium containing 10% FBS.

Mattera, L., Escaffit, F., Pillaire, M.J., et al. The p400/Tip60 ratio is critical for colorectal cancer cell proliferation through DNA damage response pathways. (2009). ***Oncogene****, 28, 1506-1517.*