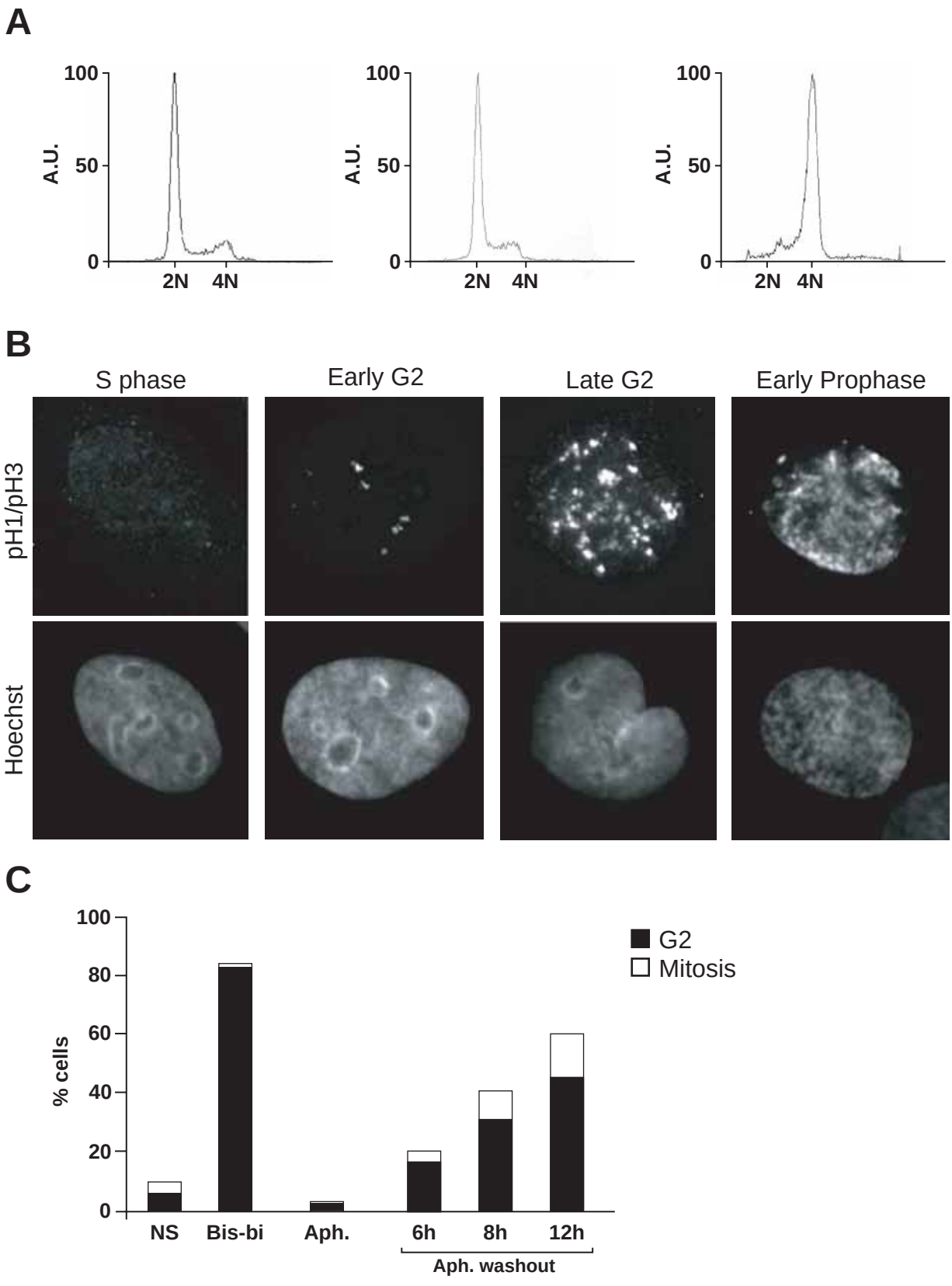
Supplementary Data

Supplementary Figure S1. FACS analysis of HeLa cells after synchronization in S and G2.

(A) Asynchronously growing HeLa cells (left panel) were arrested in S phase (central panel) or G2 (right panel); see Methods for details. DNA stained with propidium iodide was sorted using a coulter Elite FACscan. **(B)** HeLa cells were grown on coverslips, treated to be blocked in G2 or arrested in S-phase by double thymidine/aphidicoline block and, at various times after releasing the block, permeabilized by incubating for 2 min at room temperature in 0.1% Triton X-100 in PBS. The cells were then fixed and labelled with anti-phosphohistone-H1 and -H3 polyclonal antibodies (pH1/pH3), as markers of different cell-cycle phases (Hidalgo Carcedo *et al*, 2004), and with Hoechst to label DNA. Images were acquired using an inverted Axiophot equipped with a digital camera. Representative images of cells found in S phase (diffuse nuclear staining, uncondensed DNA), early G2 (faint staining of very few pericentromeric foci, uncondensed DNA), late G2 (strong staining at several centromeric foci, uncondensed DNA) and early prophase (strong staining extended to the chromosome arms, low level of chromosome condensation). **(C)** Relative percentages of mitotic (white) and G2 (black) populations in untreated HeLa cells (NS), and in HeLa cells treated with bisbenzimide (Bis-bi) and aphidicolin (Aph), and following aphidicolin washout, as indicated (see also Methods). More that 300 cells in three different experiments were evaluated as detailed in (B). The standard deviation never exceeded 7%.

Figure S1


A
100
50
0
A.U.
2N
4N
B
S phase
Early G2
Late G2
Early Prophase
pH1/pH3
Hoechst
C
100
80
60
40
20
0
% cells
G2
Mitosis
NS
Bis-bi
Aph.
6h
8h
12h
Aph. washout