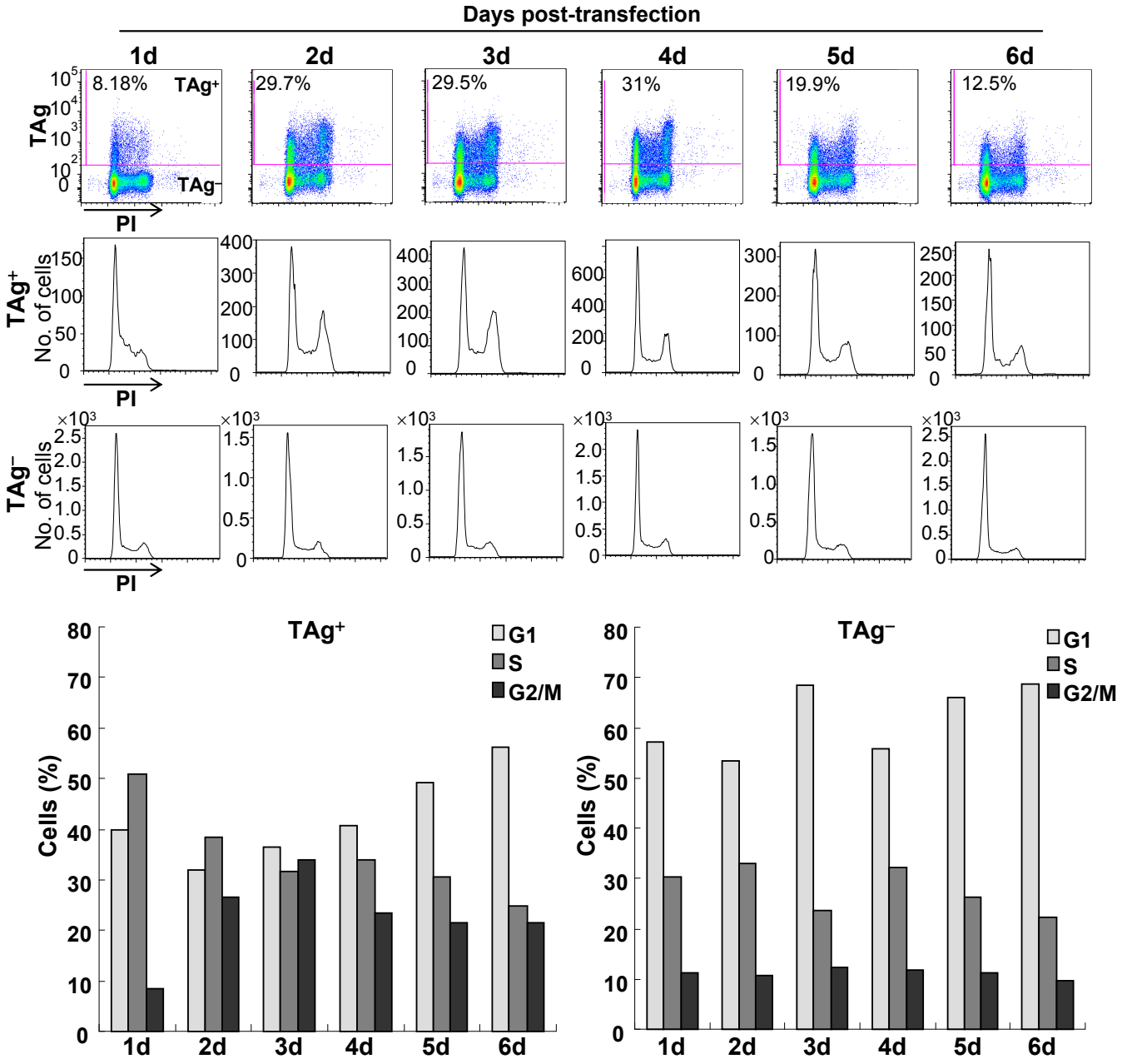
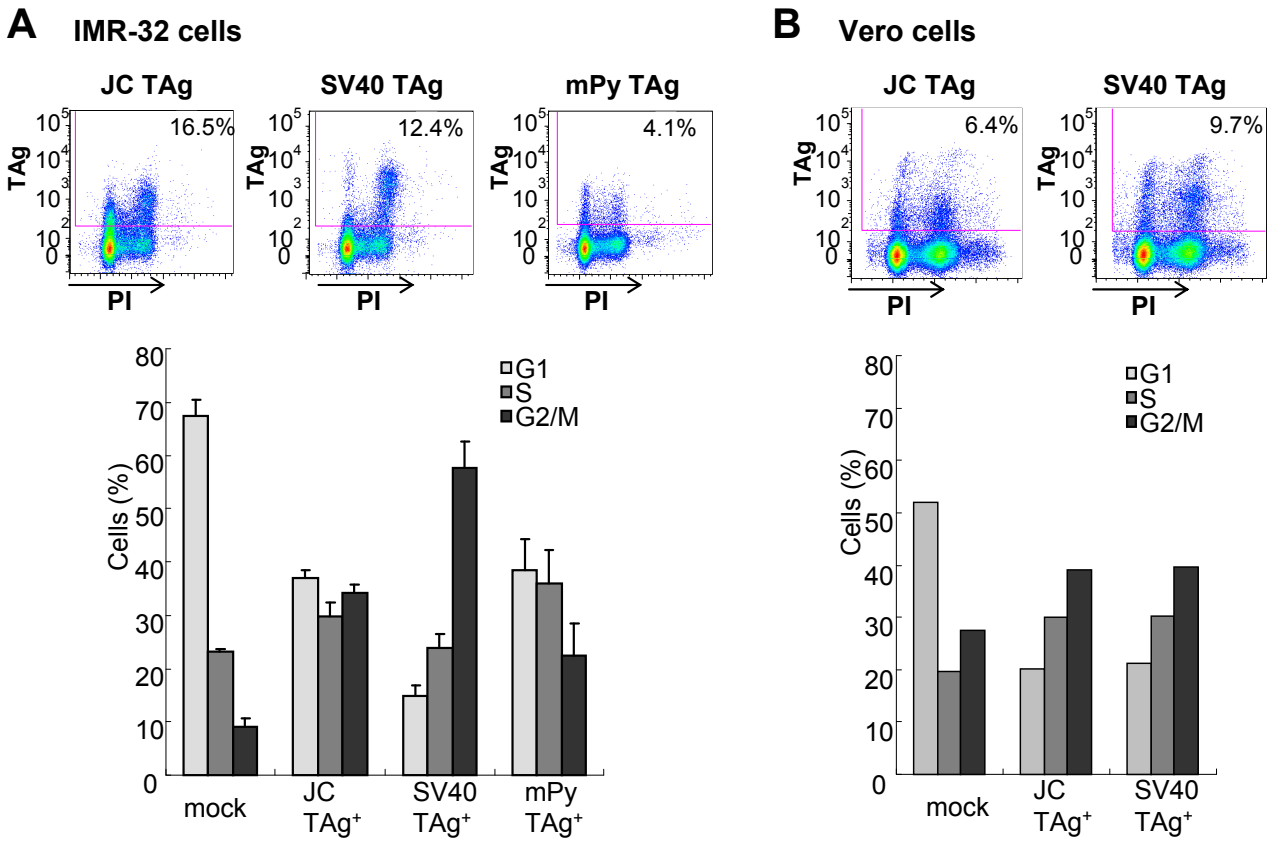
Supplementary Figure 1



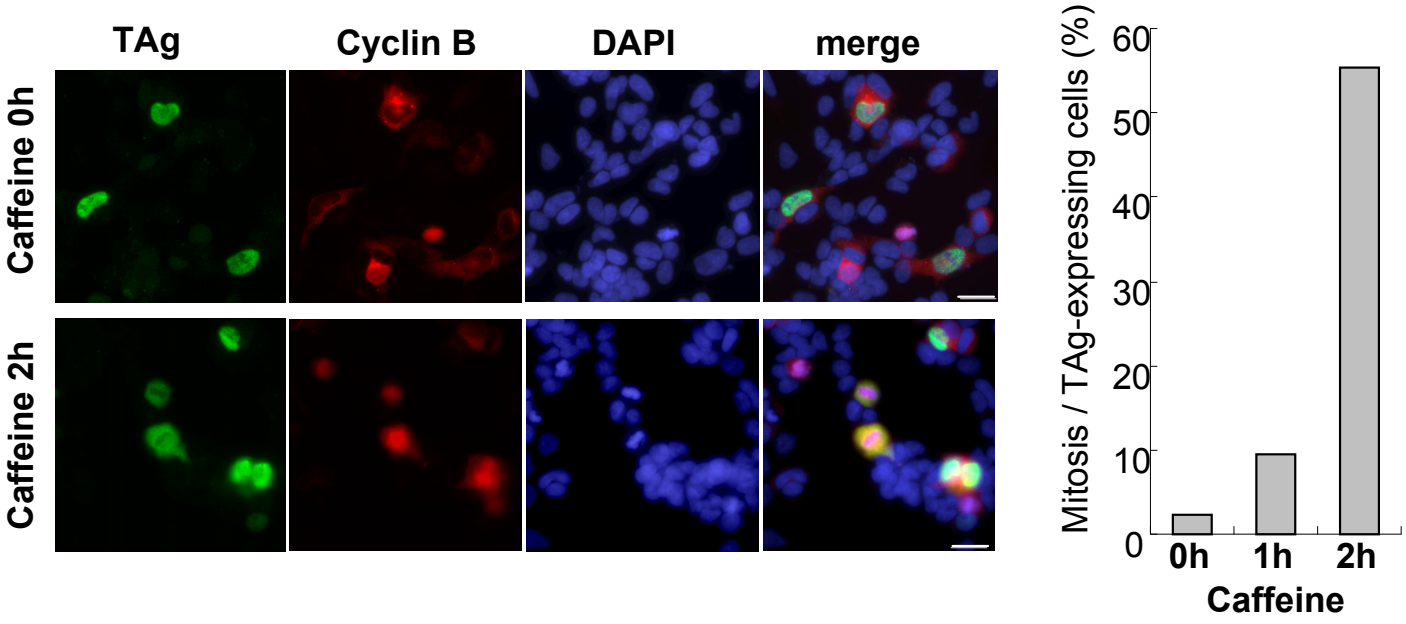
Supplementary Figure 1. Time course analysis of cell cycle in TAg-expressing cells after transfection. IMR-32 cells were transfected with an expression vector for Flag-TAg. At 1 to 6 days post-transfection, the cells were subjected to flow cytometric analysis of cell cycle distribution as in Figure 1C. The TAg^+ and TAg^- cell subsets indicated in the dot plot are shown in the histograms of PI fluorescence intensity. The bar graph indicates the percentage of cells in each phase of the cell cycle for the TAg^+ and TAg^- cell subsets.

Supplementary Figure 2



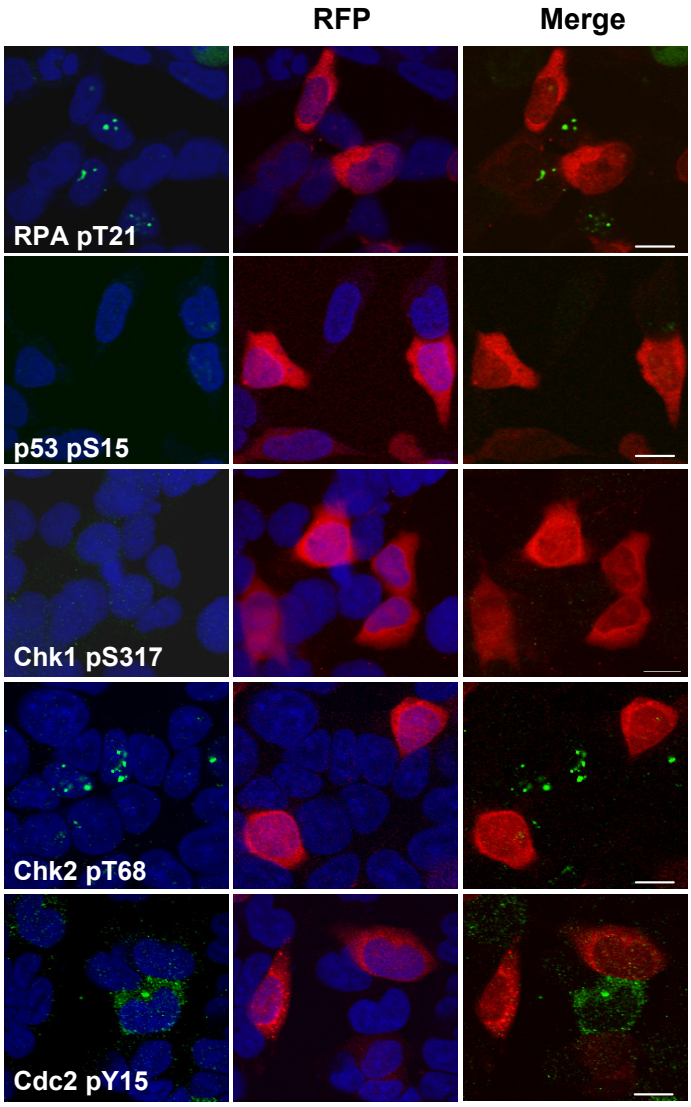
Supplementary Figure 2. Comparison of cell cycle profile among cells expressing TAg of other polyomaviruses. IMR-32 cells (A) or Vero cells (B) were transfected with an expression vector for JC TAg, SV40 TAg or murine polyomavirus (mPy) TAg, or with the corresponding empty vector (mock) 3 days before flow cytometric analysis of cell cycle distribution. The percentage of the TAg+ cell subsets indicated in the dot plot is shown in the histograms of PI fluorescence intensity. The bar graph indicates the percentage of cells in each phase of the cell cycle for mock-transfected cells and for the TAg+ cell subsets.

Supplementary Figure 3



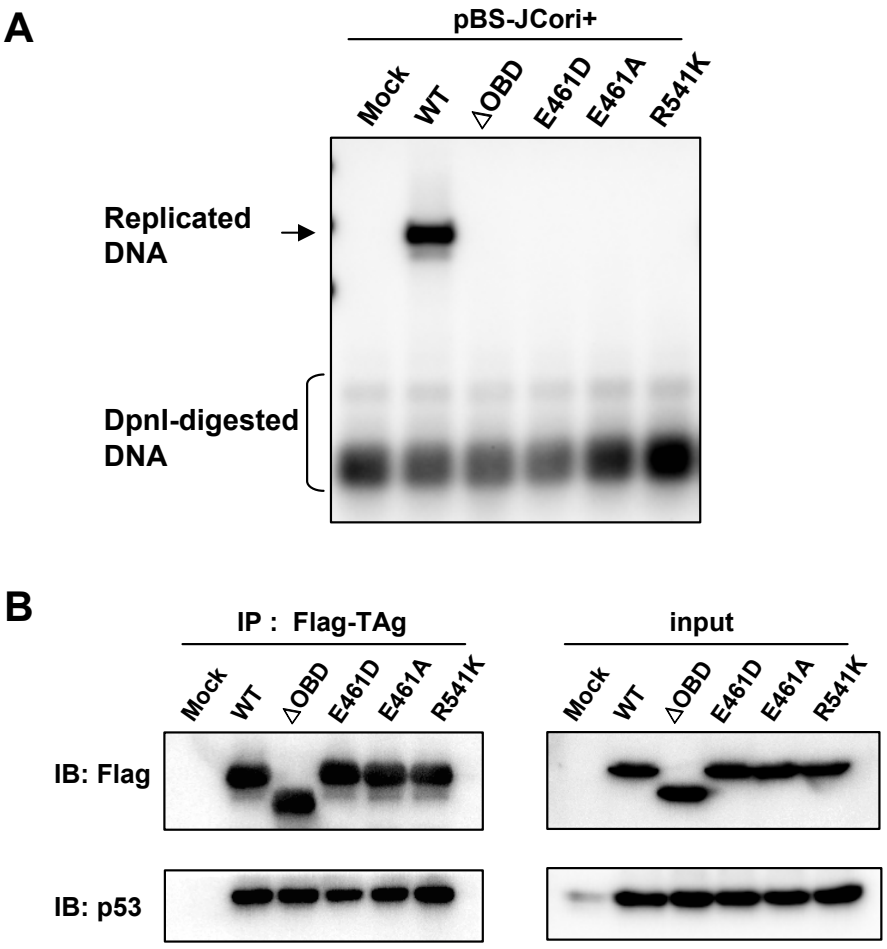
Supplementary Figure 3. TAg-expressing cells enter mitosis after inhibition of G2 checkpoint signaling. JCV-infected IMR-32 cells (14 d.p.i.) were incubated in the presence of caffeine for 0, 1, or 2 h before immunofluorescence staining with antibodies to TAg (green) and cyclin B (red). Cell nuclei were stained with DAPI (blue). Scale bars, 20 mm. The number of mitotic cells that show a diffuse pattern of Cyclin B1 staining and chromatin condensation among TAg-expressing cells was counted under a fluorescence microscope. The bar graph indicates the percentage of mitotic cells among TAg-expressing cells.

Supplementary Figure 4



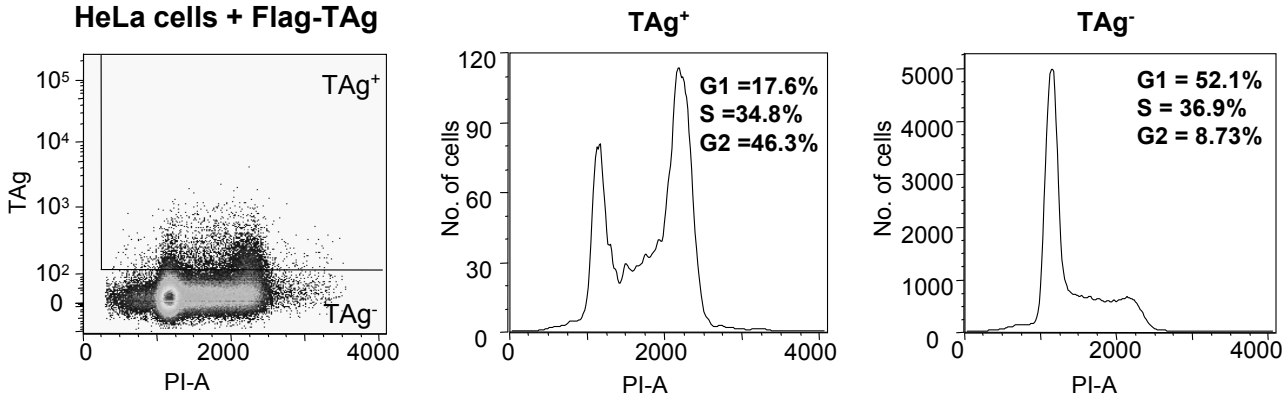
Supplementary Figure 4. Immunofluorescence detection of phosphorylated checkpoint proteins in RFP-expressing IMR-32 cells. Cells were transfected with an expression vector for Flag-RFP (red) 3 days before immunofluorescence staining with antibodies to the indicated phosphorylated forms of checkpoint proteins (green). Cell nuclei were stained with DAPI (blue). Scale bars, 10 mm.

Supplementary Figure 5



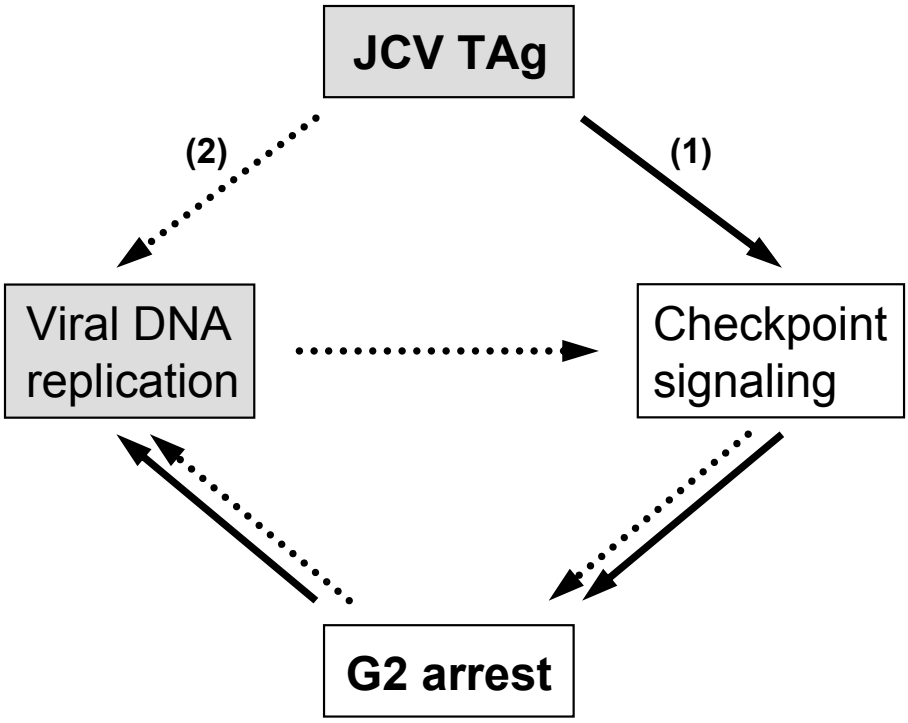
Supplementary Figure 5. (A) Replication activity of mutant forms of TAg was determined by Dpn I replication assay. Low-molecular-weight DNA extracted from the cells transfected with pBS-JCori and expression vectors for Flag-tagged WT, or mutant forms of Tag, were digested with Dpn I and Eco RI, and were then detected by Southern blot analysis with a DNA probe specific for pBS-JCori. (B) IMR-32 cells expressing Flag-tagged WT or mutant forms of JCV TAg were subjected to immunoprecipitation (IP) with antibody to Flag. The presence of p53 in the precipitate was probed by immunoblotting with the antibody to p53. The expression levels of the WT or TAg mutants and endogenous p53 were also determined (input). These results were summarized in Figure 4A.

Supplementary Figure 6



Supplementary Figure 6. Cell cycle profile of TAg-expressing HeLa cells. HeLa cells were transfected with an expression vector for Flag-TAg 3 days before flow cytometric analysis of cell cycle distribution. The TAg^+ and TAg^- cell subsets indicated in the dot plot are shown in the histograms of PI fluorescence intensity. The percentage of cells in each phase of the cell cycle is indicated in the histogram.

Supplementary Figure 7



Supplementary Figure 7. Possible mechanism for promotion of viral DNA replication by JCV TAg in host cells. TAg induces at least two independent pathways to arrest cells at G2 phase: (1) JCV TAg triggers G2 checkpoint signaling without viral DNA replication. The association of TAg with cellular DNA in the nuclei is involved in this pathway (represented as the black arrow). (2) In addition, the viral DNA replication caused by TAg leads to further accumulation of the cells at G2 phase (represented as the dotted arrow). Viral DNA replication is facilitated by this TAg-induced G2 arrest, but not by the kinase activity involved in G2 checkpoint signaling.