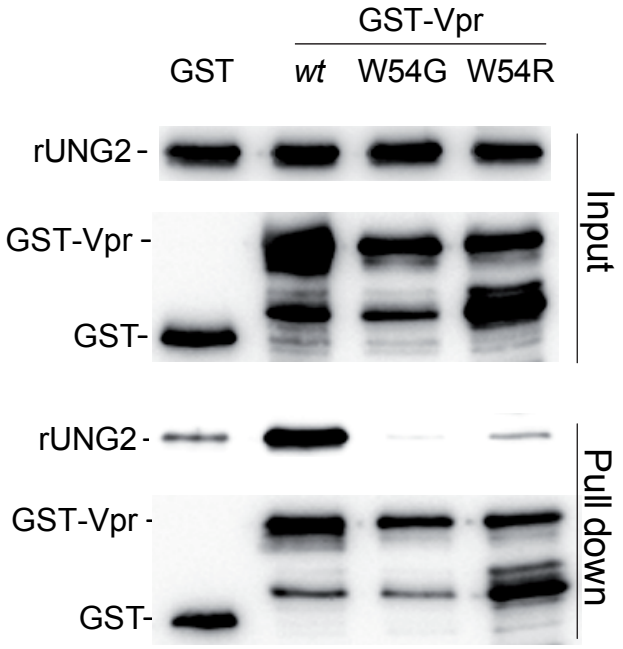
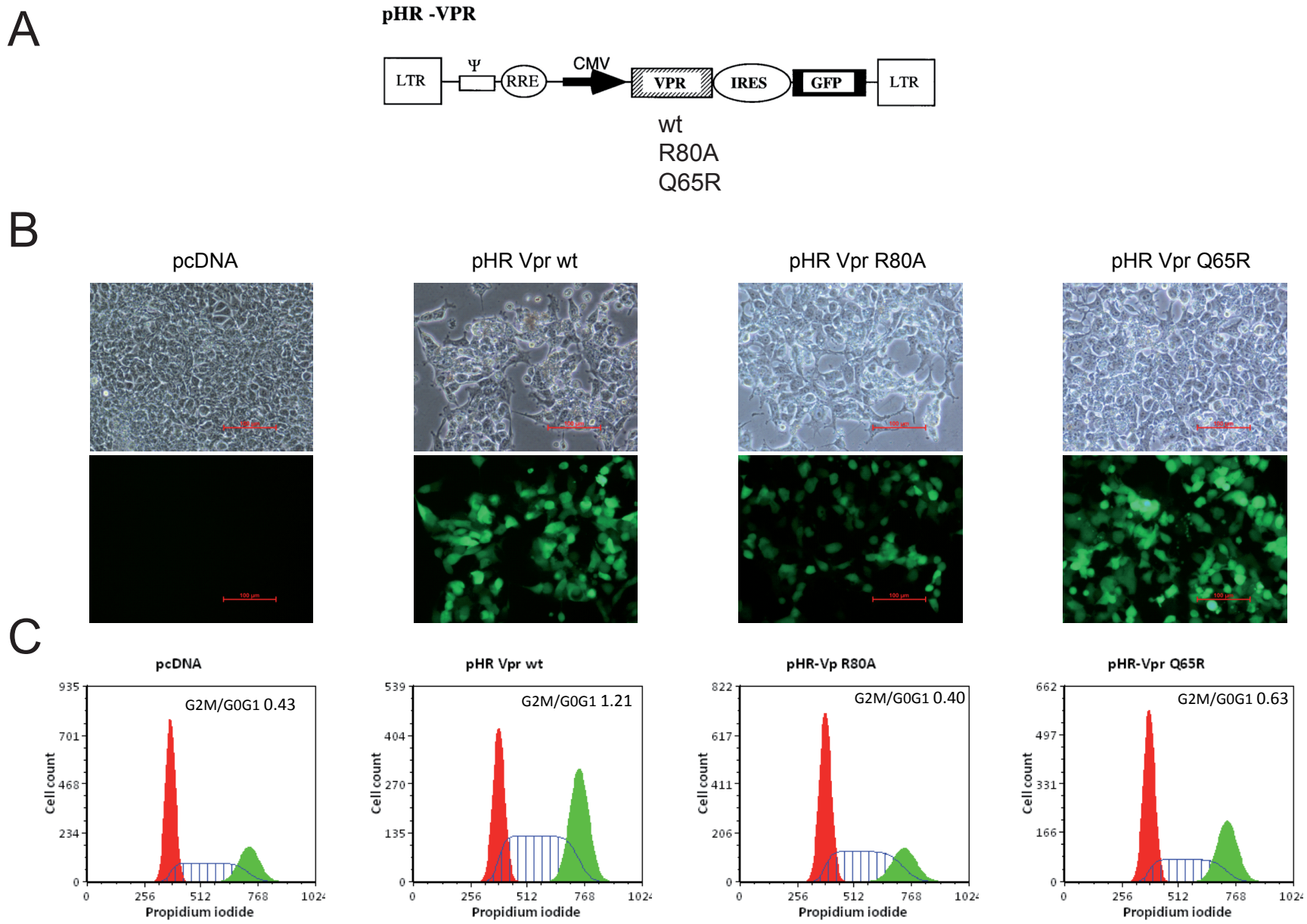


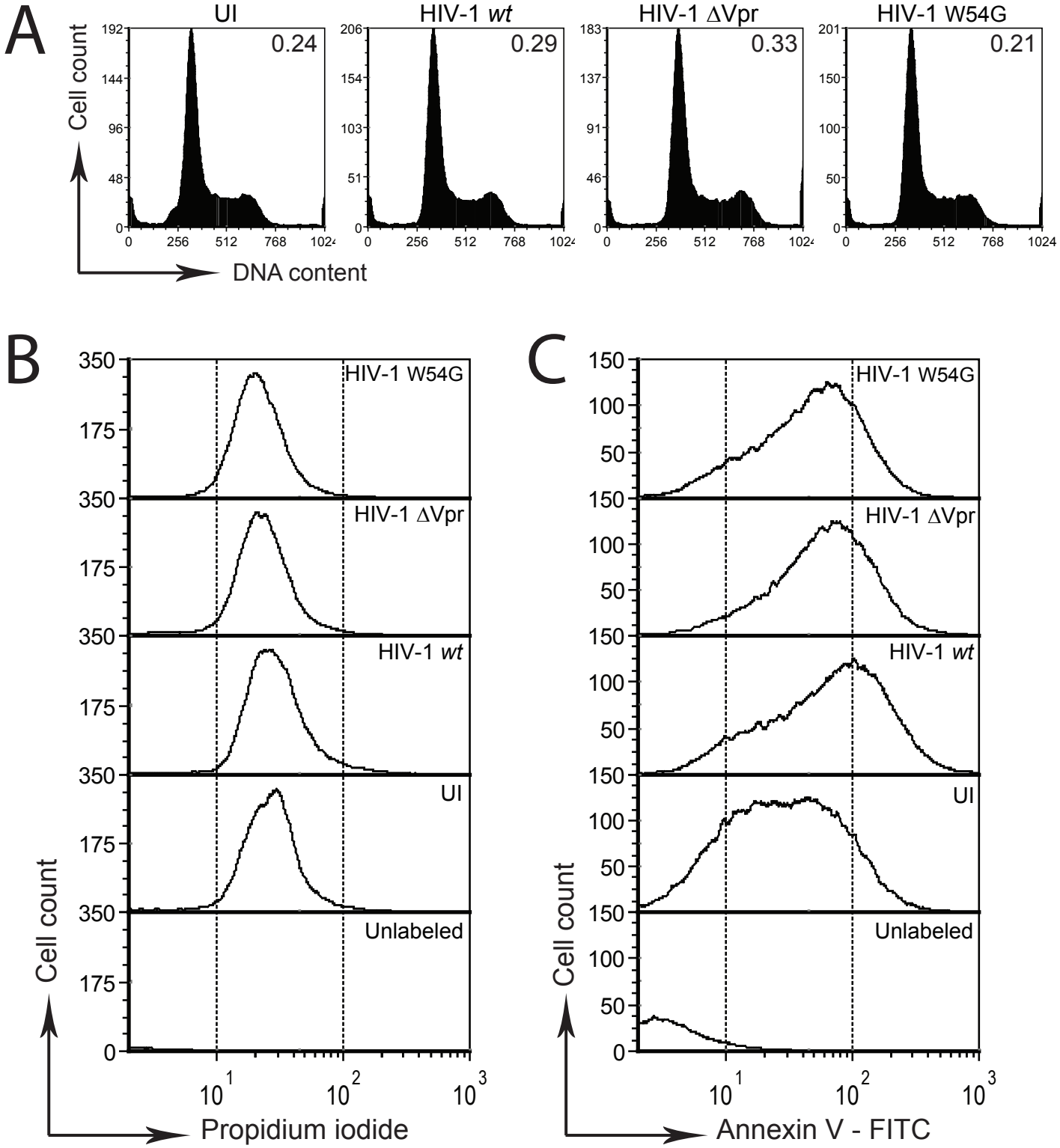
Supplementary data S1. Eldin et al.

The W54G mutation inhibits Vpr binding to UNG2. GST and GST-Vpr (*wt* or mutants) were produced in *E. coli* and purified by Gluthatione-Sepharose affinity according to standard protocols. The pET-TEV-UNG2 construct was expressed in BL21(DE3)RIL cells (Stratagene) and purified on a Ni-NTA Superflow column, as recommended (Qiagen). After incubation with recombinant UNG2, GST-fused proteins were pulled down using standard methods. Bead-bound proteins were then analyzed by immunoblotting with anti-UNG2 serum or anti-GST antibodies.



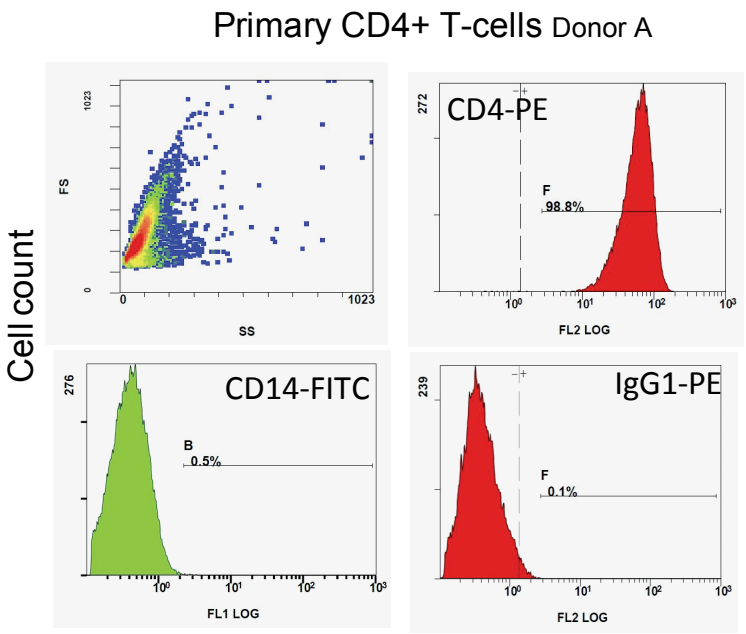
Supplementary data S2. Eldin et al.

Phenotypic analysis of Vpr proteins. (A) Vpr coding sequence (wild type and mutants) were cloned in a LTR-Vpr-IRES-GFP vector for transfection in 293T cells. (B) Forty-eight hours post-transfection, transfection efficiency was controlled by visualizing GFP expression in the cell cultures using a Nikon inverted fluorescent microscope. (C) Cells were permeabilized and labeled with propidium iodide. DNA content was determined by flow cytometry analysis. Wild type Vpr overexpression results in accumulation of cells in the G2/M stage. As expected, this phenotype is not observed when cells express the VprR80A mutant that cannot inhibit cell cycle progression or the VprQ65R mutant that cannot recruit DCAF-1 (84).



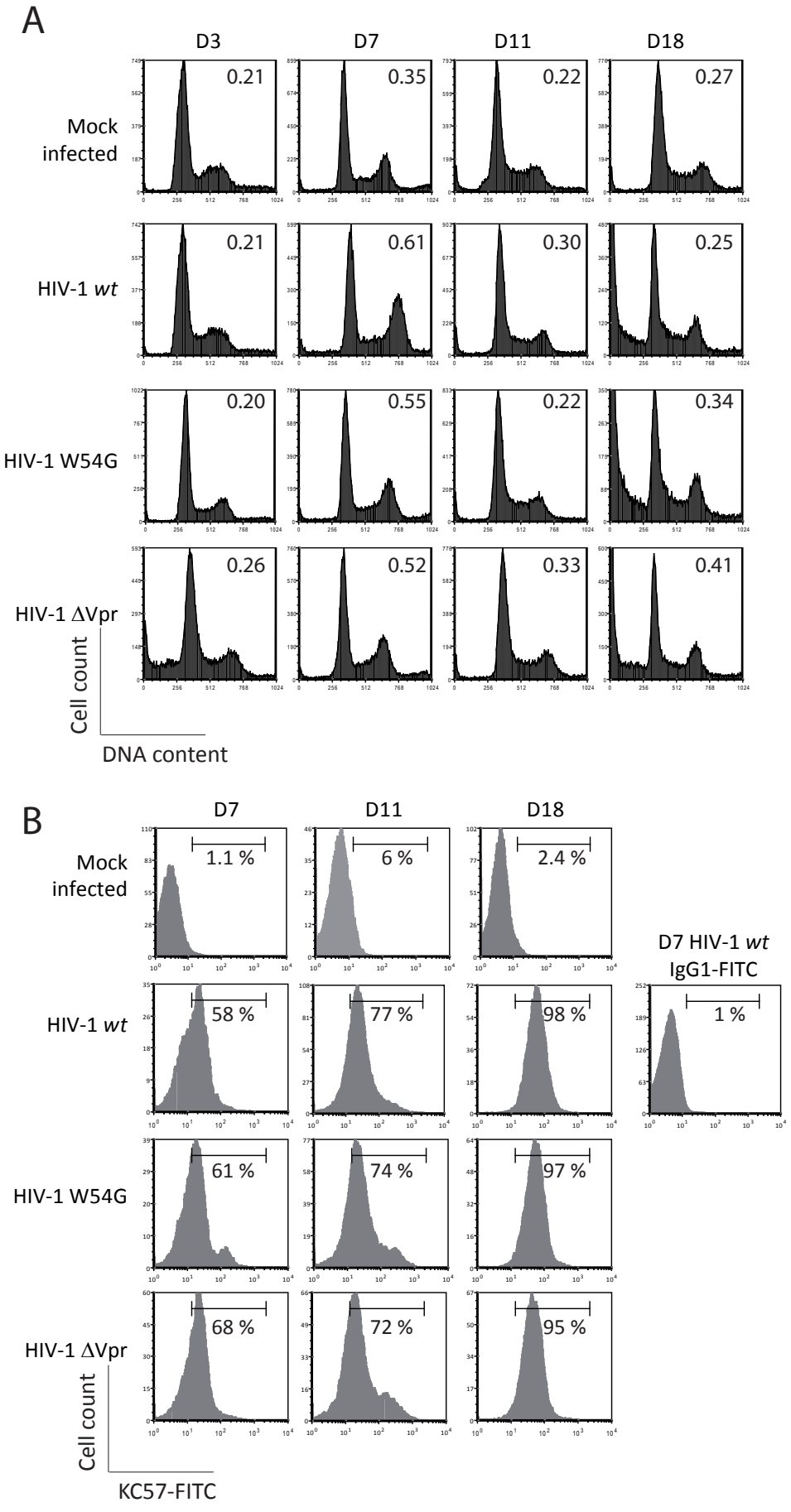
Supplementary S3. Eldin et al.

Depletion of uracil DNA glycosylase activity in HIV-1-infected T cells does not result from Vpr cytostatic and pro-apoptotic properties. (A) H9 cells were collected at day 3 post-infection with wild type or mutant HIV-1 strains and cell cycle progression analyzed as described in Material and Methods. The ratio of G2/M to G0/G1 cells in each condition is indicated. Cell viability (B) and apoptosis (C) were determined in the corresponding cell cultures by flow cytometry after propidium iodide permeability assay or Annexin V-FITC labeling, as previously described (85).



Supplementary data S4 Eldin et al.

Characterization of CD4$^+$ primary T lymphocytes purified from PBMCs. After separation, cells were characterized by using anti-CD4 and anti-CD14 monoclonal antibodies and flow cytometry analysis.



Supplementary S5. Eldin et al.

Cell cycle analysis and infection monitoring in T cells infected with wild type HIV-1 or with mutant Vpr. (A) H9 cells from Figure 4 were collected at day 3, 7, 11 or 18 post-infection and cell cycle analyzed as described in Material and Methods. The ratio of G2/M to G0/G1 in each condition is indicated in the upper right corner. (B) The percentage of HIV-1 expressing cells in the cultures was determined at day 7, 11, 18 post-infection by intracellular labelling of the Gag antigens with KC57-FITC, as recommended by the manufacturer (Beckman Coulter).