

Supplementary Figure S4. (A) Replication kinetics of RRV-GFP carrying various length of A's in the A bulge was measured by MFI in infected U87-MG cells (MOI of 0.01) at indicated time points during the course of infection. The percentage of GFP-positive cells was determined by flow cytometry using proper gating to exclude GFP-negative cells. (B) The average vector copy number (VCN) per cell in maximally infected U87-MG cells (day 14 post infection) relative to the GFP-6A vector. (C) RNA and GFP expression levels relative to the GFP-6A vector. Graph represents the RNA and protein expression levels relative to the GFP-6A vector. (D) GFP expression level relative to GFP-6A in maximally infected U87-MG ells. GFP expression was normalized to vector copy number. T-test was performed for statistical analysis. \*, (p = 0.0005); \*\*, (p = 0.0002) (E) Stability of proviral DNA of IRES-GFP cassette in RRV-IRES-GFP variants from one round infection showed no detection of deletion mutants. Arrow indicates the expected 1.4 kb PCR product containing the IRES-GFP cassette. NTC, no template control. Experiements from 4A, 4B and 4D were performed in triplicate (means  $\pm$  SD). Data shown represent one set of multiple independent experiments.