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Supplemental Information

Conserved Herpesvirus Protein Kinases Target SAMHD1 to Facilitate Virus Replication

Kun Zhang, Dong-Wen Lv, and Renfeng Li

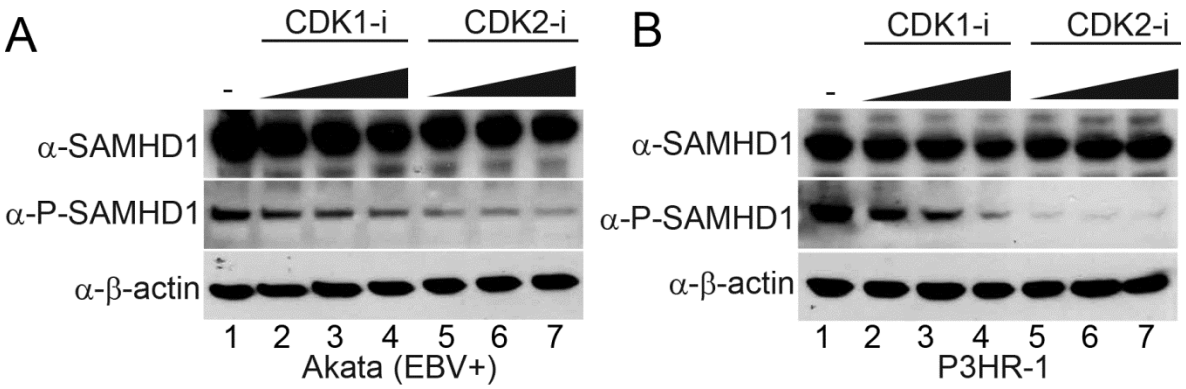

Figure S1. SAMHD1 phosphorylation is regulated by CDK1 and CDK2. Related to Figures 1-3.
Western blot showing that SAMHD1 phosphorylation is reduced with CDK1 and CDK2 inhibition. The EBV-positive Akata (A) and P3HR-1 (B) cells were treated with vehicle control or increasing amount (10, 20 and 50 μM) of CDK1 inhibitor (CDK1-i) or CDK2 inhibitor (CDK2-i) for 48 hrs as indicated.

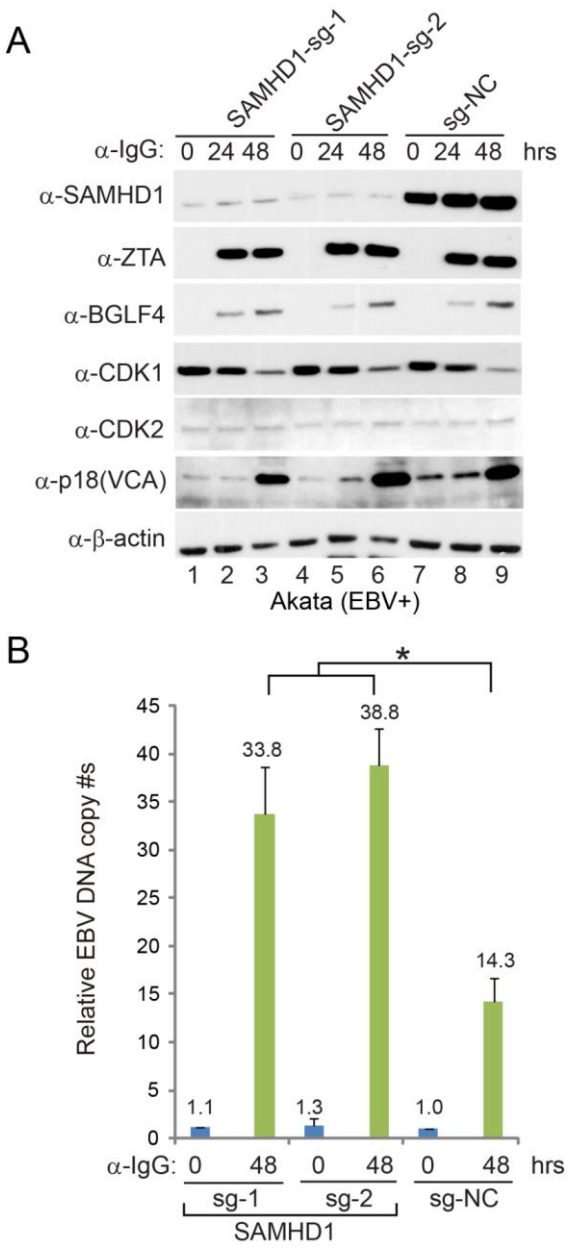

Figure S2. SAMHD1 depletion facilitates EBV lytic replication in Akata (EBV+) cells. Related to Figure 3.
(A) SAMHD1 depletion doesn't affect EBV protein expression. SAMHD1-depleted and control Akata (EBV+) cells were treated with IgG cross-linking to induce EBV lytic reactivation. SAMHD1, the accumulation of EBV ZTA, BGLF4 and p18, and the CDK1 and CDK2 protein levels were analyzed by immunoblotting at the indicated time points. β-actin served as a loading control
(B) EBV DNA replication is enhanced in SAMHD1-depleted cells. DNA samples were extracted from Akata (EBV+) cells treated as indicated. The relative viral genome copy numbers were determined by quantitative PCR using primer to *BALF5* gene normalized by *β-actin*. Representative results from three biological replicates are presented. Data are represented as mean ± SD of technical replicates (n=3). * $p < 0.05$.

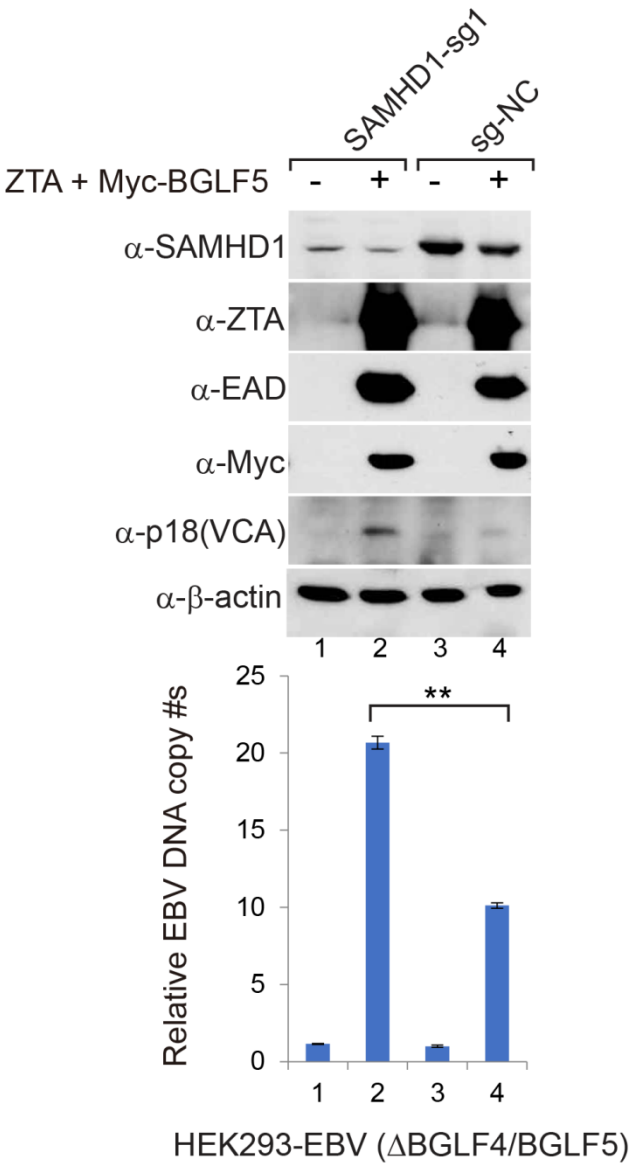

Figure S3. SAMHD1 depletion facilitates EBV DNA replication in BGLF4-knockout cells. Related to Figure 3.

HEK293 (EBV+) cells carrying a BGLF4/BGLF5 deletion were used to establish control (sg-NC) and SAMHD1-depleted (sg1) cell lines. The cells were transfected with ZTA plus BGLF5 to induce lytic replication. Western blot analysis was performed using antibodies as indicated. β-actin served as a loading control. The EBV genome copy numbers were measured by qPCR using primers specific to EBV *BALF5* gene normalized by *β-actin*. Representative results from three biological replicates are presented. Data are represented as mean ± SD of technical replicates (n=3). ** $p < 0.01$.

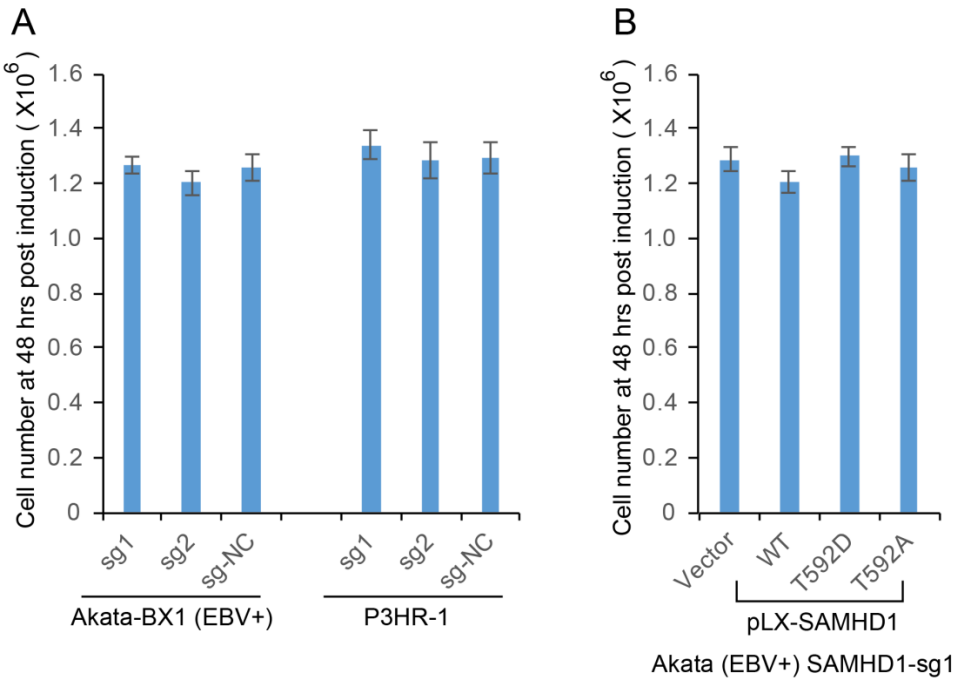

Figure S4. SAMHD1 depletion and overexpression does not affect cell growth. Related to Figures 3-4. Control and SAMHD1-depleted Akata-BX1 (EBV+) and P3HR-1 cells (A) and SAMHD1-reconstituted Akata (EBV+) cells (B) were lytically induced for 48 hrs with the same methods used in Figures 3 and 4. The total live cell numbers are counted by trypan-blue staining methods. Data are represented as mean ± SD of biological replicates (n=3).

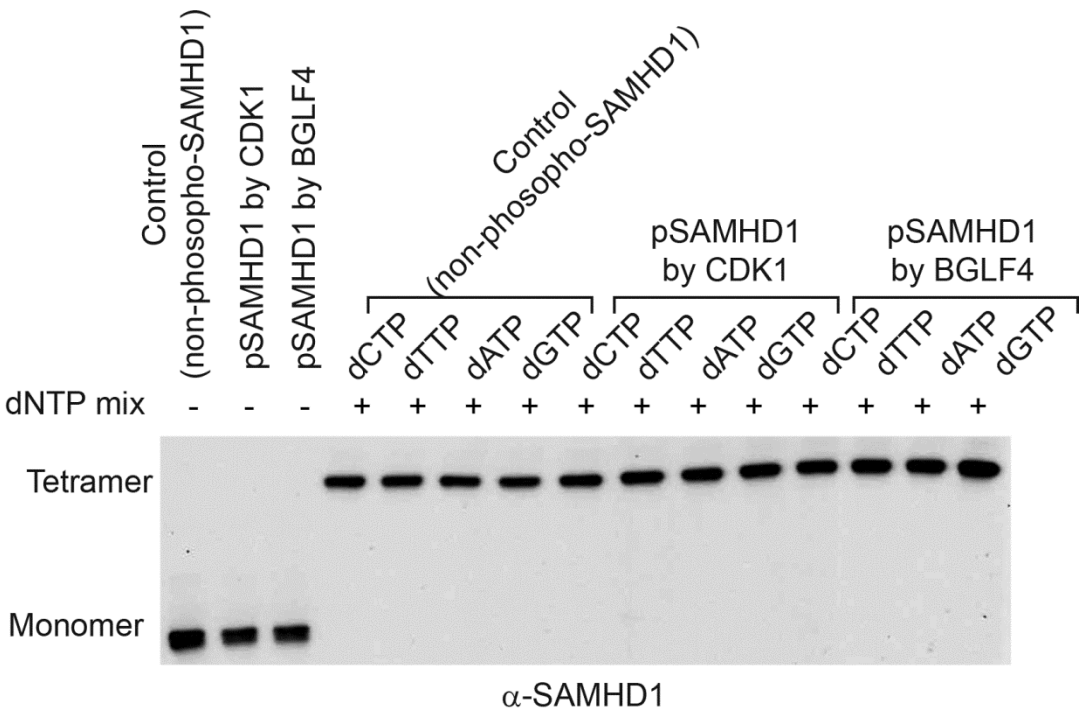

Figure S5. SAMHD1 phosphorylation does not affect its tetramerization. Related to Figure 5.
Sequential activation of SAMHD1 was performed as described in Figure 5. After 30 min of adding individual dNTPs for catalysis, the reaction mixture was subjected to cross-linking with 2.5 mM glutaraldehyde for 10 min and then quenched with 1 M Tris-HCl, pH 8.0. The mixtures were separated by SDS-PAGE and analyzed by immunoblotting with anti-SAMHD1 antibody.

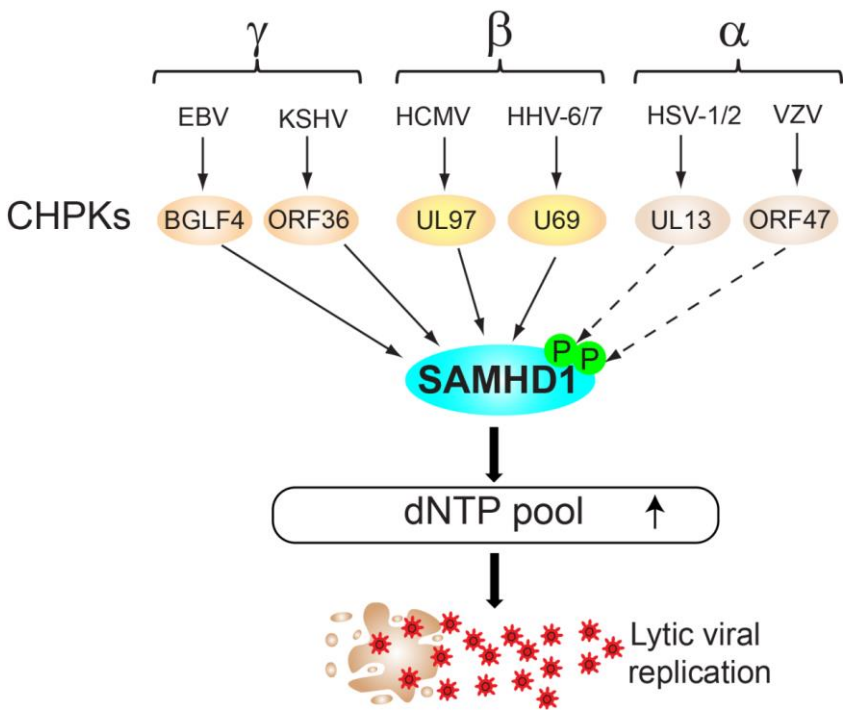

Figure S6. The model of SAMHD1 regulation by CHPKs in viral replication. Related to Figures 6 and 7. Beta- and gamma- herpevirus protein kinases trigger the phosphorylation of SAMHD1, which leads to the increase of cellular dNTP pool for efficient viral DNA replication. The phosphorylation of SAMHD1 by beta- and gamma-herpevirus protein kinases is indicated by solid arrows. The possible regulation of SAMHD1 by alpha-herpevirus protein kinases is indicated by dashed arrows.