EBV BORF2 is a Novel Inhibitor of the Virus Restriction Enzyme APOBEC3B

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Introduction

Human cells express up to seven different APOBEC3 (A3) enzymes, which belong to a family of DNA cytosine deaminases that function in innate antiviral immunity. At least two family members including APOBEC3B (A3B) also contribute to cancer mutagenesis. APOBEC3G (A3G) and other A3s have been well characterized in their ability to inhibit HIV-1 replication by converting cytosines to uracils in retroviral single-stranded cDNA replication intermediates, thereby generating hypermutated viral genomes. To overcome A3-mediated restriction, viruses have evolved counteraction measures such as the HIV-1 Vif protein, which nucleates the formation of a ubiquitin ligase complex and targets A3s for degradation. We hypothesize that, like HIV-1 and other lentiviruses, Epstein-Barr virus (EBV) and related γ-herpesviruses have evolved mechanisms to counteract the potent mutagenic capacity of A3 enzymes.

Our studies have identified EBV BORF2 as a novel inhibitor of A3B. Biochemical studies reveal a robust direct interaction and identify the responsible domains involved. Additionally, BORF2 disrupts the physiologic localization of A3B from the nucleus to cytoplasmic punctate structures in a cell-cycle dependent manner. Preliminary data also shows that deletion of BORF2 by CRISPR-Cas9 leads to increased hypermutation of the viral genome. These results combine to suggest a model in which EBV BORF2 has evolved complementary strategies to target A3B and preserve the genetic integrity of the large DNA tumor virus.

BORF2 interacts with A3B

Figure 1. Verification of AP-MS results show that BORF2 interacts with APOBEC3B loop 7.

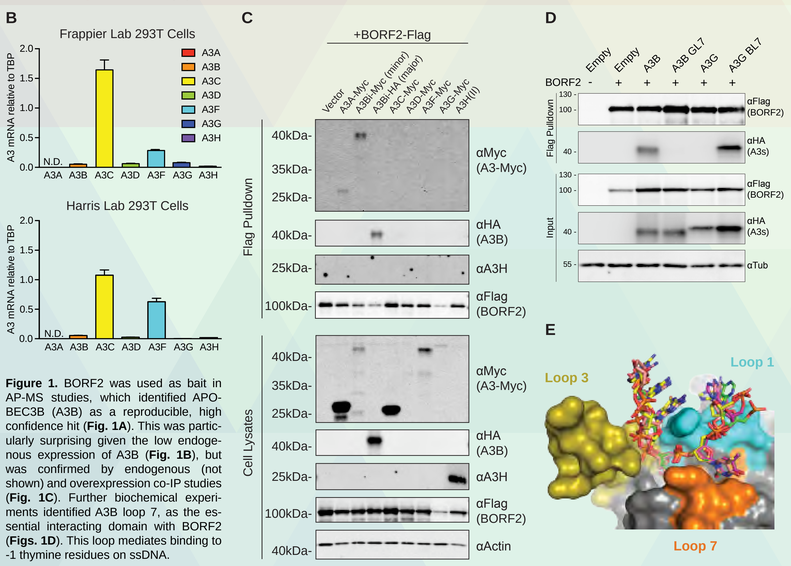


Figure 1. BORF2 was used as bait in AP-MS studies, which identified APOBEC3B (A3B) as a reproducible, high confidence hit (**Fig. 1A**). This was particularly surprising given the low endogenous expression of A3B (**Fig. 1B**), but was confirmed by endogenous (not shown) and overexpression co-IP studies (**Fig. 1C**). Further biochemical experiments identified A3B loop 7, as the essential interacting domain with BORF2 (**Figs. 1D**). This loop mediates binding to -1 thymine residues on ssDNA.

BORF2 inhibits A3B enzymatic activity

Figure 2. In vitro purification and subsequence oligo cleavage assays show that BORF2 directly inhibits A3B.

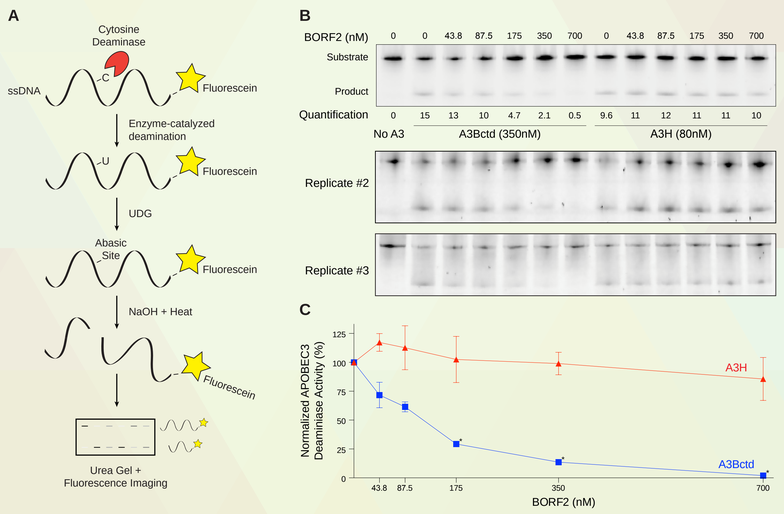


Figure 2. BORF2 and A3s were purified from *E. coli* and protein concentrations were quantified to equimolar concentrations (**Fig. 2C**). A3s were subsequently mixed with 43-mer oligos with TCA sequence context and incubated with increasing amounts of BORF2. Analysis by oligo cleavage assay (**Fig. 2A**) shows that BORF2 inhibits A3B, but not A3H in a dose-dependent, stoichiometric manner (**Fig 2B, C**).

A3B hypermutates EBVΔBORF2

Figure 4. CRISPR/Cas9-mediated deletion of BORF2 leads to increased A3B-mediated hypermutation.

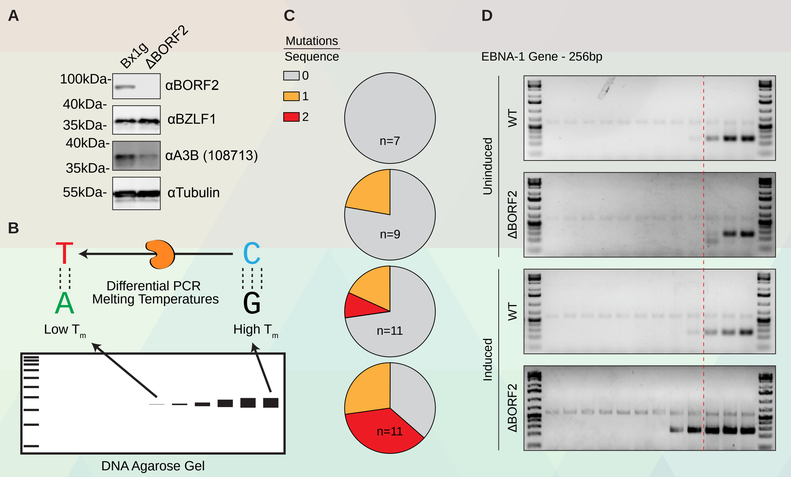


Figure 4. Guide RNAs were generated to target BORF2 at +122bp in AGS cells harboring EBV-GFP (AGS Bx1g), which resulted in deletion of BORF2 (**Fig. 4A**). 3D-PCR was used as to asses A3-mediated hypermutation (**Fig. 4B**). Representative data from the EBNA-1 gene shows that EBVΔBORF2 generates PCR amplicons at lower melting temperatures, suggesting increased hypermutation compared to wild-type controls (**Fig. 4D**). Sanger sequencing on these fragments show increased C-to-T mutations in EBVΔBORF2 (**Fig. 4C**).

BORF2 alters A3B localization

Figure 3. BORF2 stabilizes A3B and translocates A3B from the nucleus to perinuclear punctate structures.

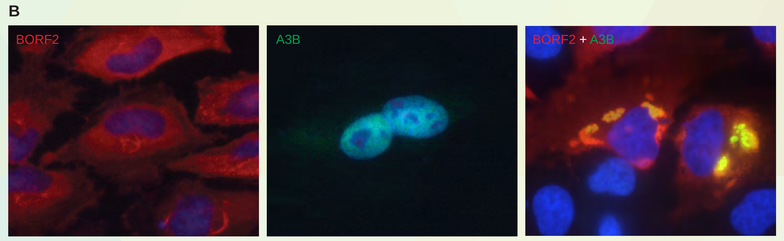
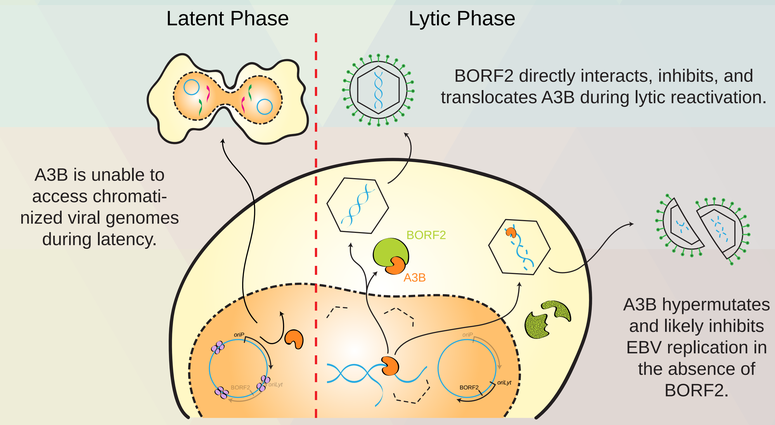


Figure 3. In contrast to canonical mechanisms of A3 counteraction by proteosomal degradation (*e.g.*, HIV Vif), EBV BORF2 stabilizes A3B in cells (**Fig. 3A**). Visualization by immunofluorescence microscopy shows translocation of A3B from the nucleus to the perinuclear punctate structures (**Fig. 3B**). These results were confirmed by conventional TEM and are structurally similar to endolysosomes (**Fig. 3C**).

Working model



Acknowledgements

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