Expanded View Figures

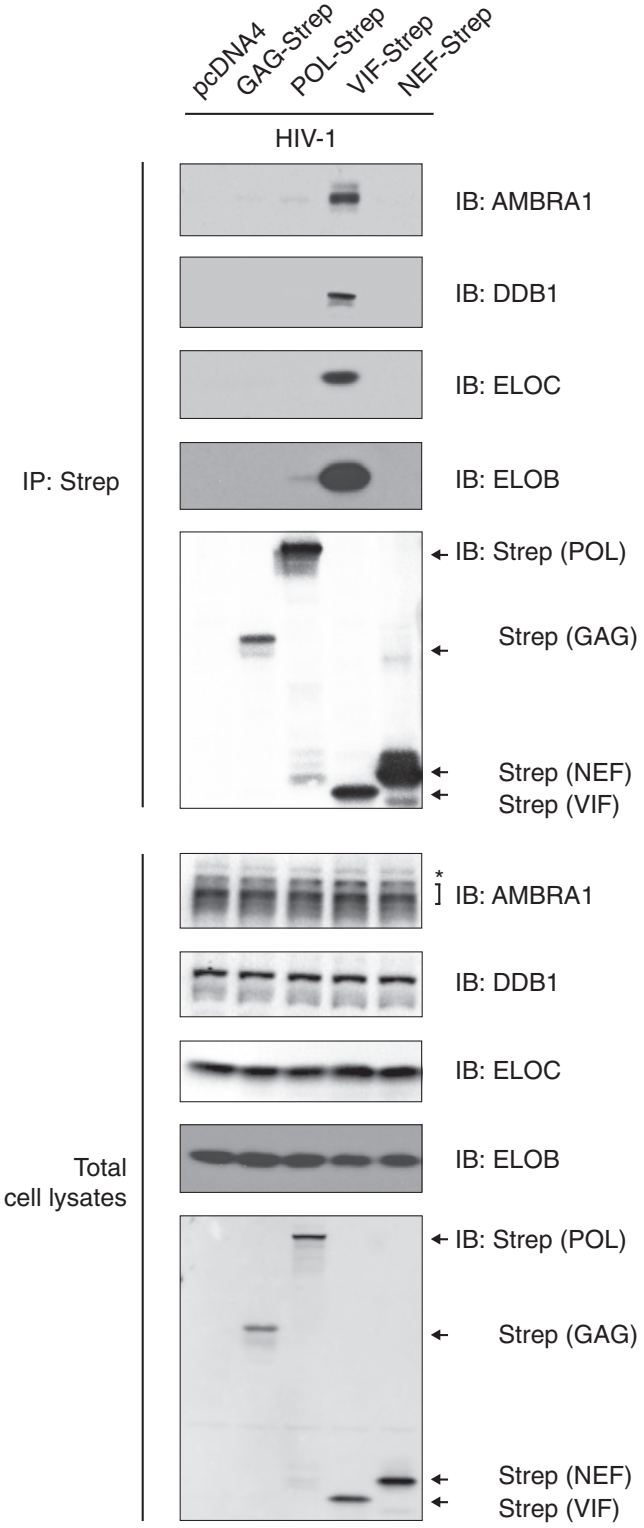


Figure EV1. AMBRA1 specifically binds HIV-1 VIF.

HIV-1 VIF co-purified with AMBRA1, DDB1, and ELOBC. HEK293T cells transfected with empty vector or Strep-tagged HIV-1 constructs were affinity-purified with Strep-Tactin beads and analyzed by immunoblot. Asterisk denotes non-specific bands.

Source data are available online for this figure.

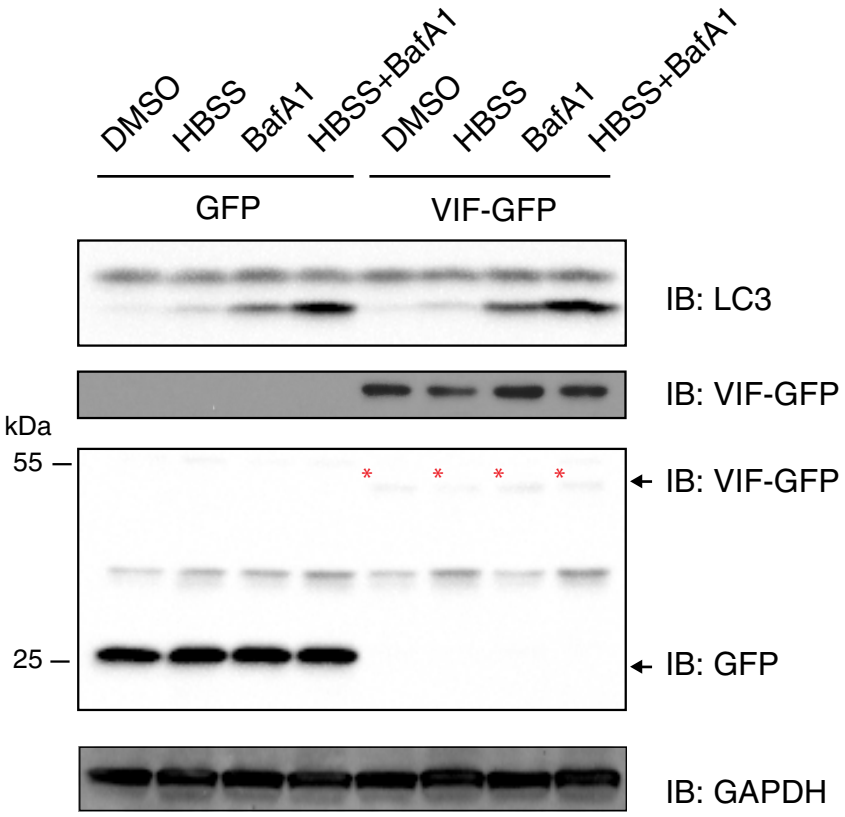
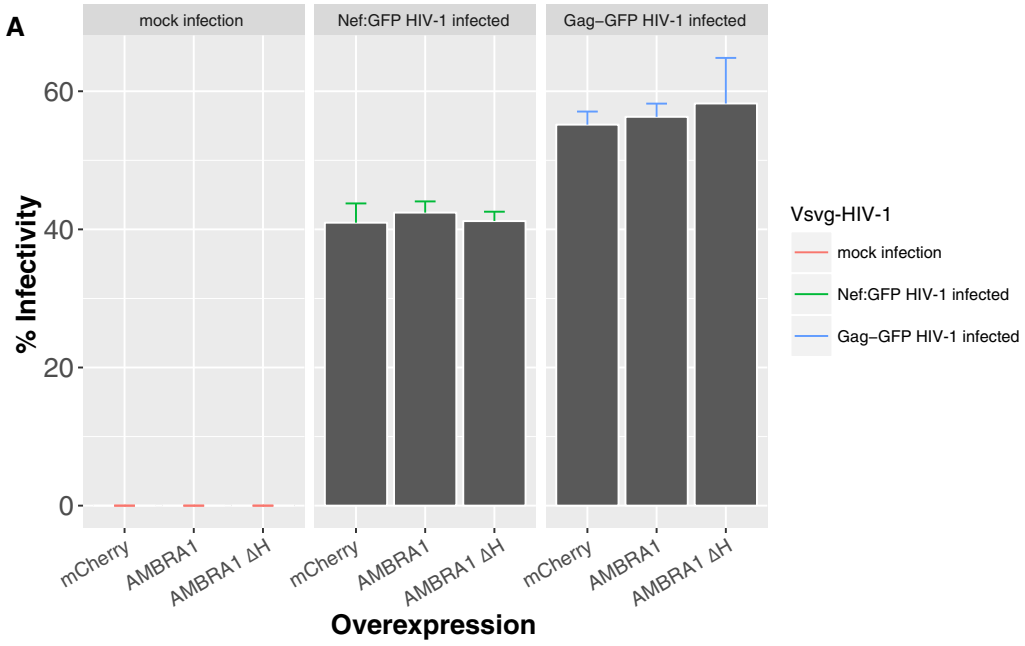
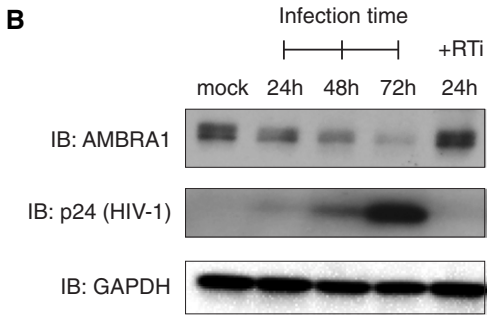


Figure EV2. HIV-1 VIF expression in HEK293 cells does not affect autophagy responses.

HEK293 cells stably expressing tet-on GFP or VIF-GFP were treated overnight with 1 μg/ml of doxycycline (dox) for protein expression, followed by the addition of DMSO, 100 nM Bafilomycin A1 (BafA1), or starvation media (HBSS supplemented with DMSO or BafA1) for 4 h before lysis and analysis by immunoblot. Red asterisks denote VIF-GFP.

Source data are available online for this figure.





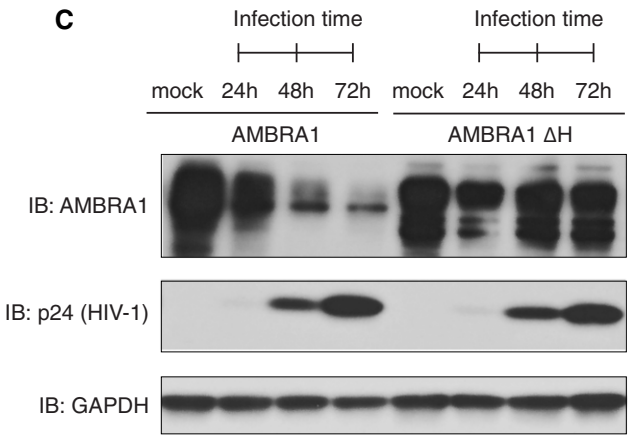


Figure EV3. AMBRA1 overexpression does not affect HIV-1 infection due to its downregulation by the virus.

A SupT11 cells stably expressing APOBEC3G (SupT11-APOBEC3G) cells overexpressing mCherry, AMBRA1, or the ΔH mutant were infected with Vsvg-pseudotyped reporter HIV-1 *Nef:GFP* or Gag-GFP viruses, followed by flow cytometry analysis. Each data point represents mean ± SEM, n = 3.

B SupT11-APOBEC3G cells were infected with Vsvg-pseudotyped HIV-1 at a MOI of 2. The infected cells were harvested at the indicated time points and were analyzed by immunoblot.

C Similar to B, SupT11-APOBEC3G cells overexpressing AMBRA1 WT or ΔH were infected with Vsvg-HIV-1 at a MOI of 1.

Source data are available online for this figure.

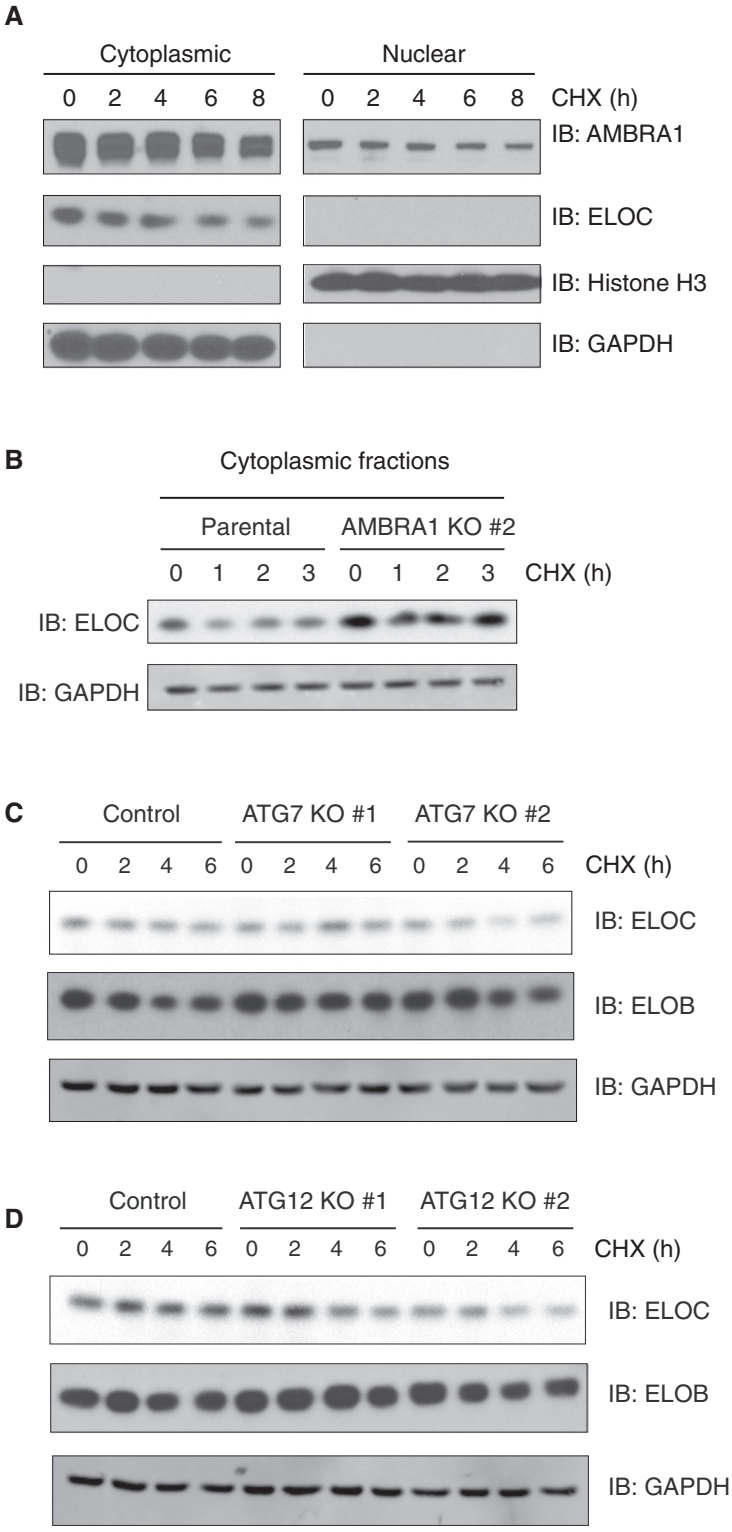


Figure EV4. Depletion of AMBRA1, not ATG proteins, promotes ELOC accumulation.

A HEK293T cells were treated with CHX at different time points and followed by cytoplasmic/nuclear fractionation. The fractionated samples were analyzed by immunoblot.

B Parental HEK293T and AMBRA1 KO #2 cells were treated with CHX at different time points and followed by cytoplasmic enrichment. The fractionated samples were analyzed by immunoblot.

C, D ELOC and ELOB stability was monitored by CHX chase assay in NT or AMBRA1 shRNA-expressing HEK293T cells (B), or in scramble control, ATG7-, or ATG12-knockout HEK293T cells (C, D). Cells were treated with 100 μg/ml CHX at different time points. Lysates were analyzed by immunoblot.

Source data are available online for this figure.

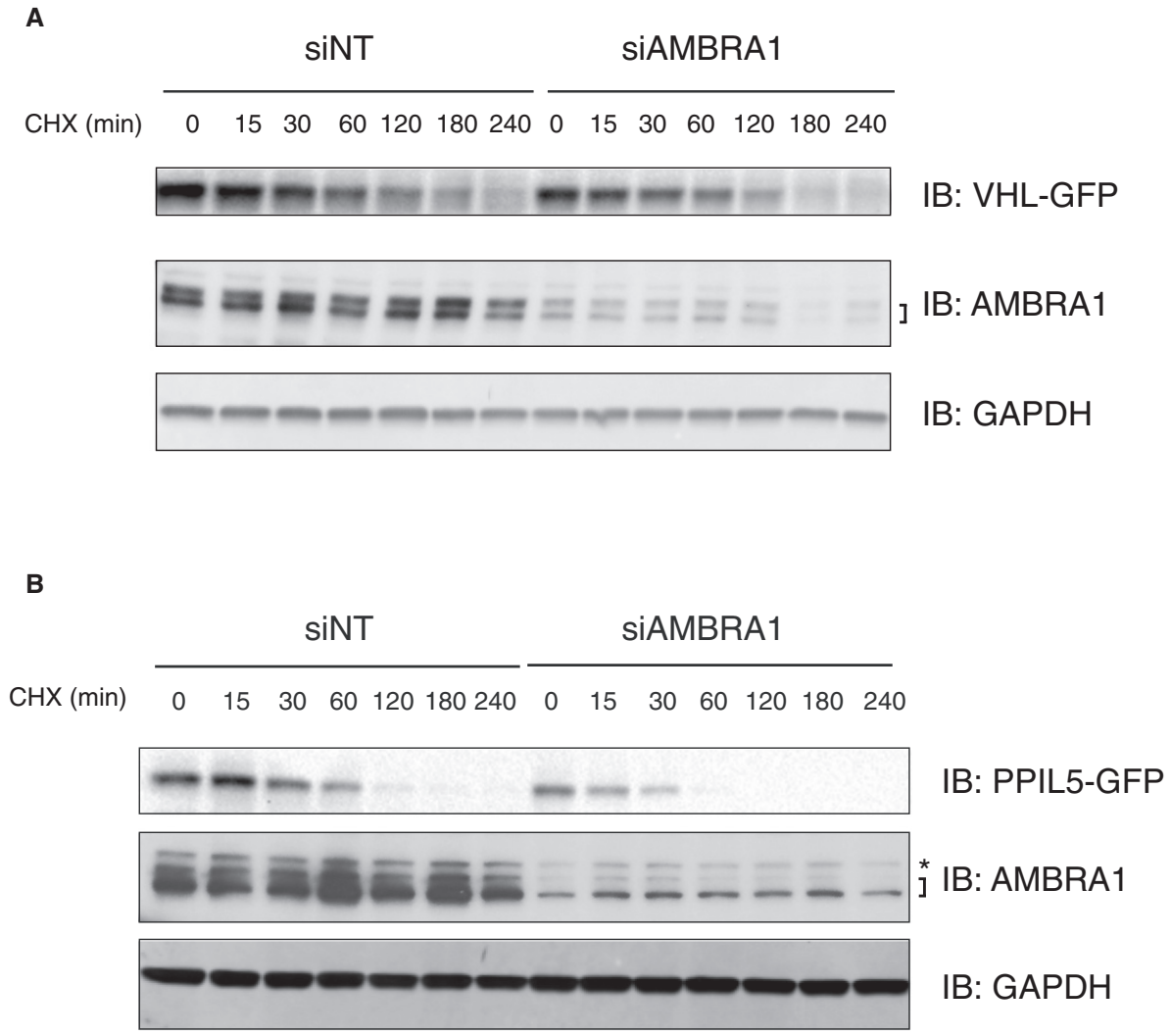


Figure EV5. AMBRA1 regulates CRL2-mediated autocatalytic degradation of VHL and PPIL5.

A, B HEK293 cells stably expressing tet-on VHL-GFP (A) or PPIL5-GFP (B) were transfected with 10 nM NT or AMBRA1 siRNA for 72–96 h. Protein expression was induced overnight with 1 μg/ml of dox, followed by the addition of 100 μg/ml CHX at different time points. Asterisk denotes non-specific bands.

Source data are available online for this figure.