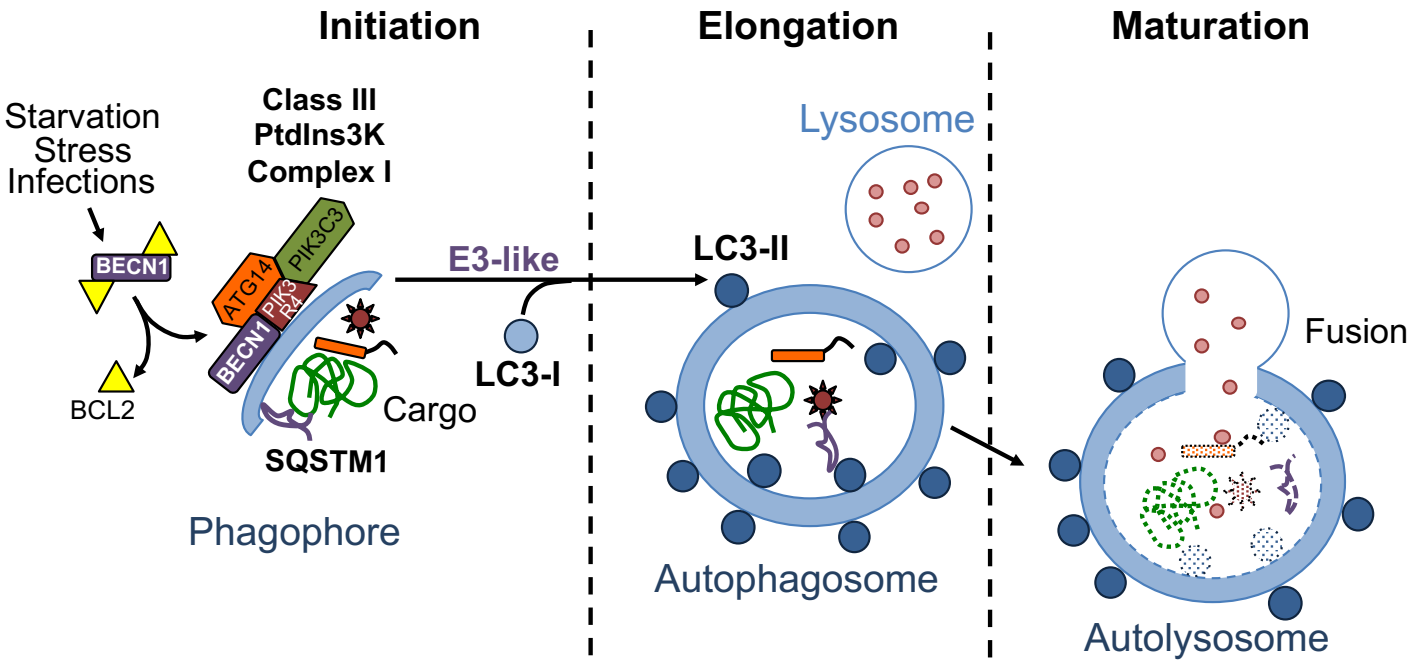

Figure S1. Schematic diagram of the autophagy pathway. Representation of the main steps of autophagy as well as molecules that play a role in the progression of this pathway relevant to this study.

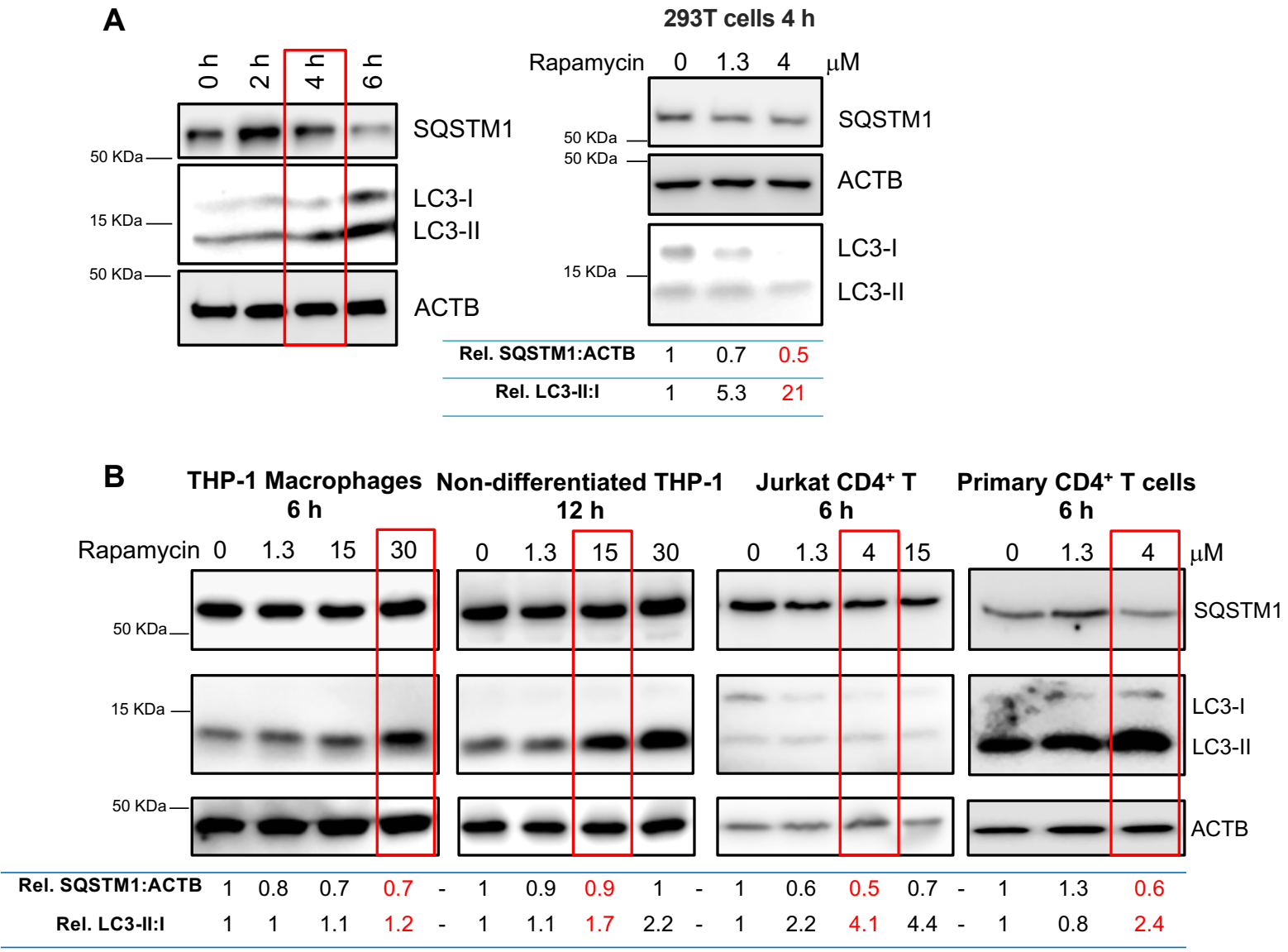

Figure S2. Cell-specific differences in the activation of autophagy in response to rapamycin. (**A, B**) Representative data of HEK293T cells, THP-1-derived macrophages, non-differentiated THP-1 monocytes, Jurkat CD4$^+$ T cells, and primary CD4$^+$ T cells showing their threshold and sensitivity to autophagy activation. Cells were treated with rapamycin (0-30 μM) at different time exposures (0-12 h). Cell lysates were analyzed by western blot for the autophagy markers SQSTM1, LC3, and the housekeeping protein ACTB/β-actin. Densitometric analyses were performed to determine the ratios of LC3-II over LC3-I as well as the SQSTM1:ACTB levels relative to the no-rapamycin treatment. Red boxes indicate optimal conditions for autophagy activation.

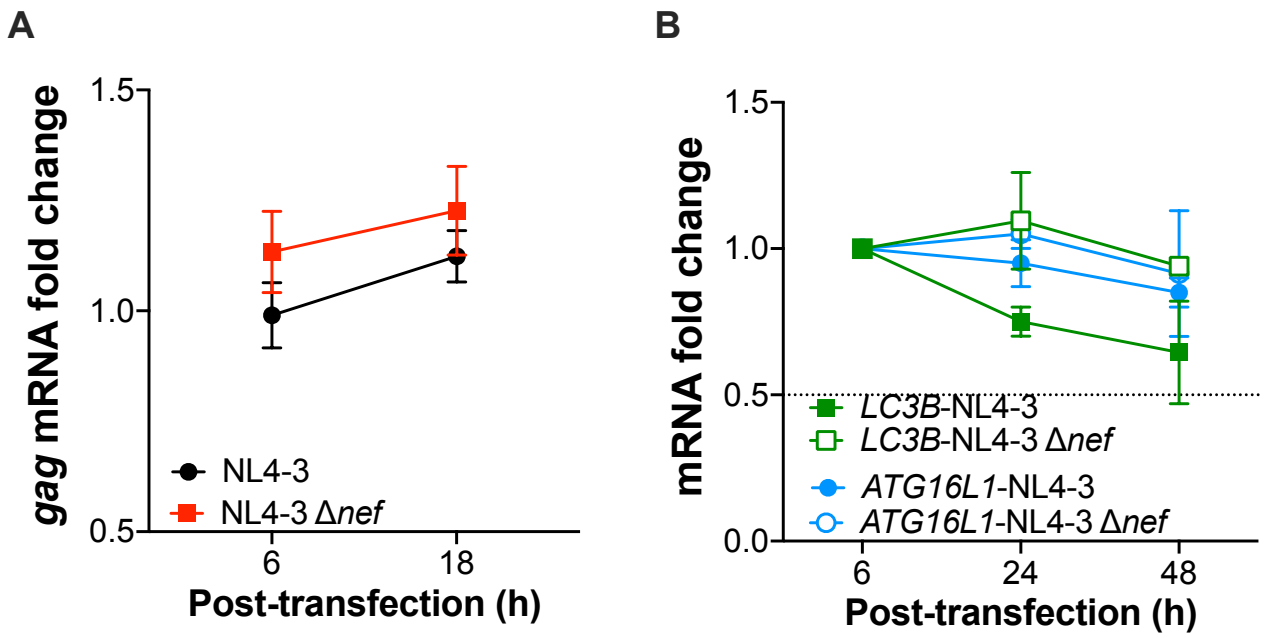

Figure S3. Autophagy effects on *gag* and *LC3B* mRNAs. (**A**) HEK293T cells were co-transfected with the proviral constructs of HIV-1 NL4-3 and HIV-1 NL4-3 Δ*nef*. 6 and 18 h post-transfection, cells were lysed, total RNA was extracted, converted to cDNA by reverse transcription, and analyzed for the mRNA levels of *gag* relative to the first time point of HIV-1 NL4-3, after normalization to *GAPDH*. (**B**) HEK293T cells were co-transfected with HIV-1 NL4-3 or HIV-1 NL4-3 Δ*nef* proviral DNA. Next, the mRNA levels of *LC3B* and *ATG16L1* were assessed at the selected time points by RT-qPCR and expressed as fold change relative to the first data point after normalization to *GAPDH*. The dashed line represents cutoff for biologically relevant differences. Data represent the mean and SEM from 3 independent biological replicates.

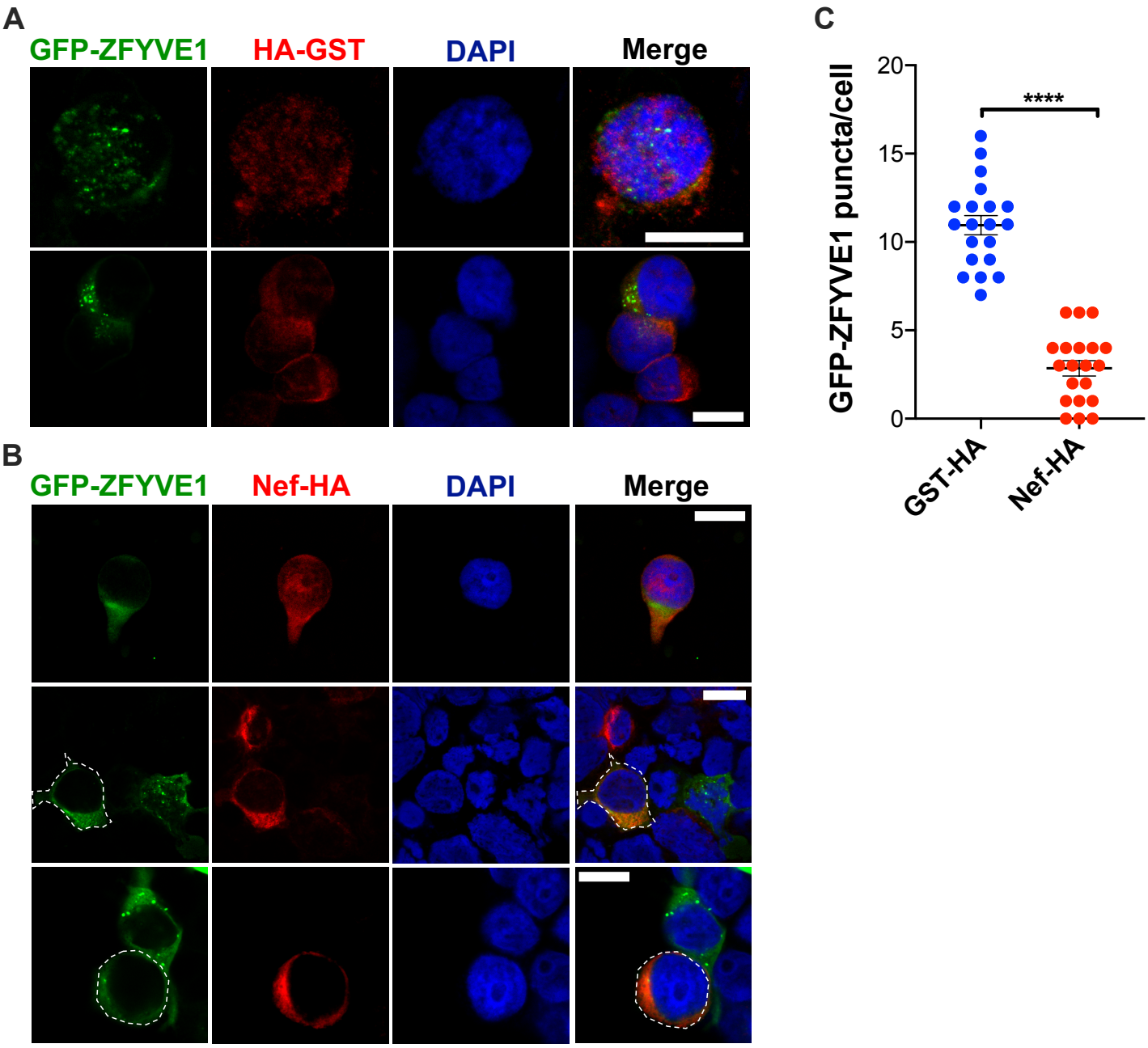

Figure S4. Nef reduces ZFYVE1 puncta formation. (**A, B**) HEK293T cells were co-transfected with GFP-*ZFYVE1* and either *GST*-HA (A) or NL4-3 *nef*-HA (B) and treated with 4 μM of rapamycin for 4 h. 48 h later, cells were stained for GFP (green), HA (DyLight-550; red) and the nuclei (DAPI; blue). (**C**) Data correspond to the mean and SEM of GFP-ZFYVE1 puncta present in 20 randomly selected cells expressing GST-HA or NL4-3 Nef-HA. ****: $p \leq 0.0001$. White scale bar: 10 μm. Cells surrounded by white borders are Nef$^+$.

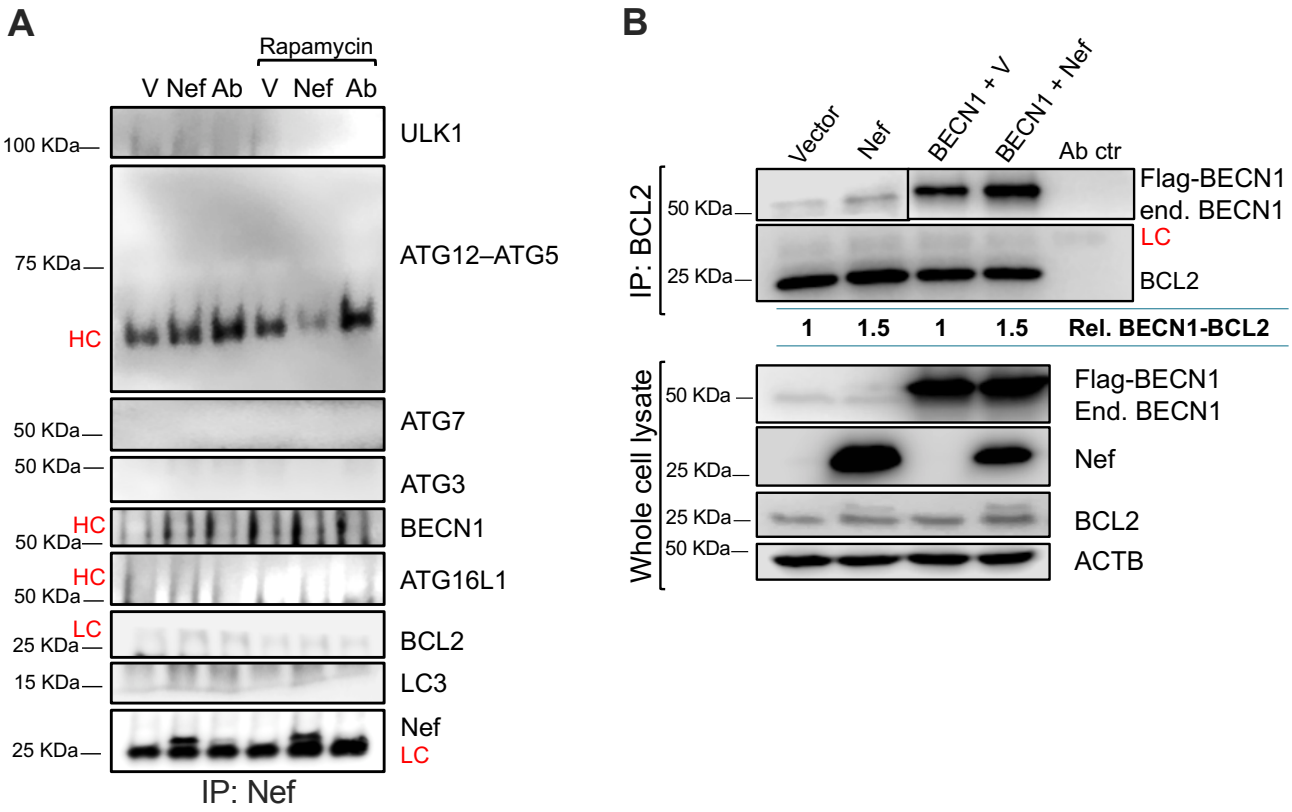

Figure S5. Nef arrests autophagy initiation by enhancing the association between BECN1 and BCL2. (**A**) HEK293T cells were transfected with NL4-3 *nef* or an empty vector. 48 h later, cells were exposed to rapamycin (4 μM) for 4 h. The cell lysates were subjected to immunoprecipitation for Nef, and the pulldown fraction was evaluated by western blot for ULK1, ATG12–ATG5, ATG7, ATG3, BECN1, ATG16L1, BCL2, LC3 and Nef. (**B**) HEK293T cells were co-transfected with *BECN1*-Flag and NL4-3 *nef* or an empty vector. 48 h later, the cell lysates were subjected to immunoprecipitation for BCL2. The pulldown fraction was then examined for the presence of BECN1 and BCL2. The cell lysates were also analyzed by western blot to assess the cellular levels of BECN1, Nef, BCL2, and ACTB. Densitometric analyses were used to determine the relative levels of interaction between BECN1 and BCL2. V: vector; HC: heavy chain; LC: light chain. Ab ctr: antibody control.

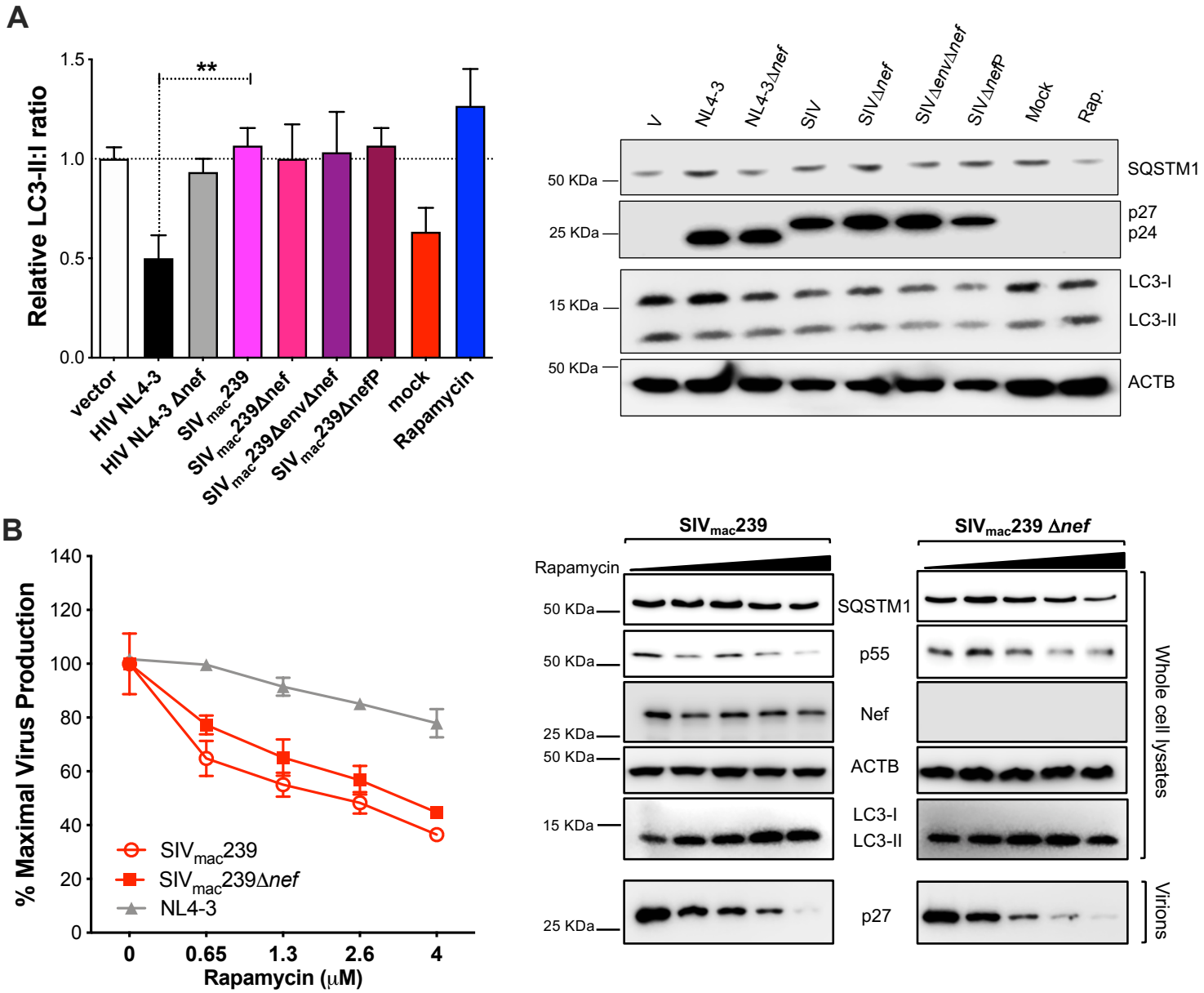

Figure S6. SIV$_{mac}$239 is sensitive to autophagy restriction. (**A**) Left panel: HEK293T cells were transfected with the full-length proviral DNA of SIV$_{mac}$239 or mutants of this molecular clone, such as Δ*nef*, Δ*env*Δ*nef* and Δ*nefP*. As controls, the proviral DNA of full-length HIV-1 NL4-3, NL4-3 Δ*nef*, and an empty retroviral vector were included. 48 h later, the ratio of LC3-II:I relative to the vector control was calculated for each sample. Right panel: western blot showing the levels of SQSTM1, capsid p27 and p24, LC3, and ACTB. (**B**) Left panel: HEK293T cells were transfected with the proviral DNA of SIV$_{mac}$239, SIV$_{mac}$239 Δ*nef* and HIV-1 NL4-3, as a control. Cells were then exposed to rapamycin at the selected concentrations for 12 h and the percentage of maximal virus production was measured by the amounts of SIV p27 or HIV p24 accumulated in the culture supernatant, as described previously. Data correspond to the mean and SEM of 3 independent experiments. Right panel: Representative western blot for SQSTM1, Gag p55, Nef, ACTB , and LC3. Capsid p27 from pelleted virions is also provided. V: vector. **: $p \leq 0.01$.