



Supplemental Material to:

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**Autophagy plays an important role in the containment of
HIV-1 in nonprogressor-infected patients**

Autophagy 2014; 10(7)

<http://dx.doi.org/10.4161/auto.28678>

www.landesbioscience.com/journals/autophagy/article/28678

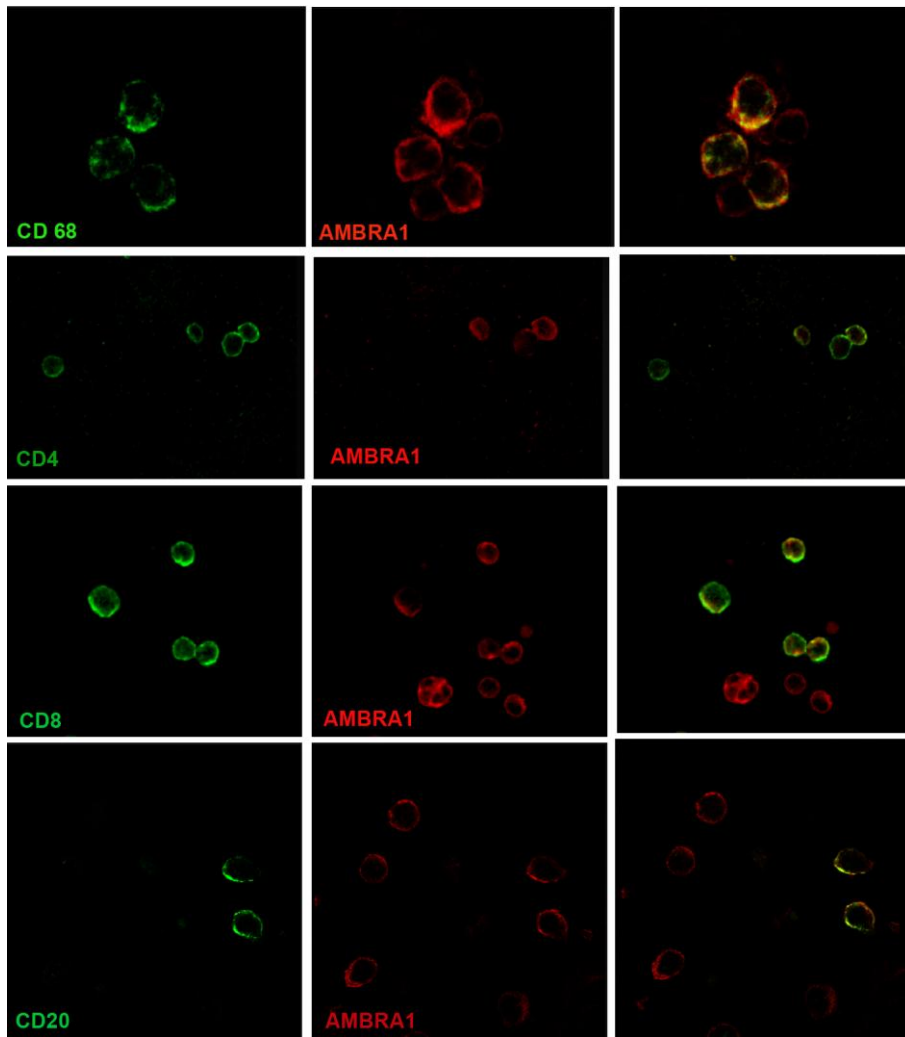


Figure S1. Confocal microscopy cell type immunocharacterization of AMBRA1-positive PBMC. Immunostaining of AMBRA1 (red) and the phenotypic markers CD68, CD4, CD8, MS4A1/CD20 (green) has been performed in PBMC from LTNP. The AMBRA1 positivity was present in all the analyzed subpopulations.

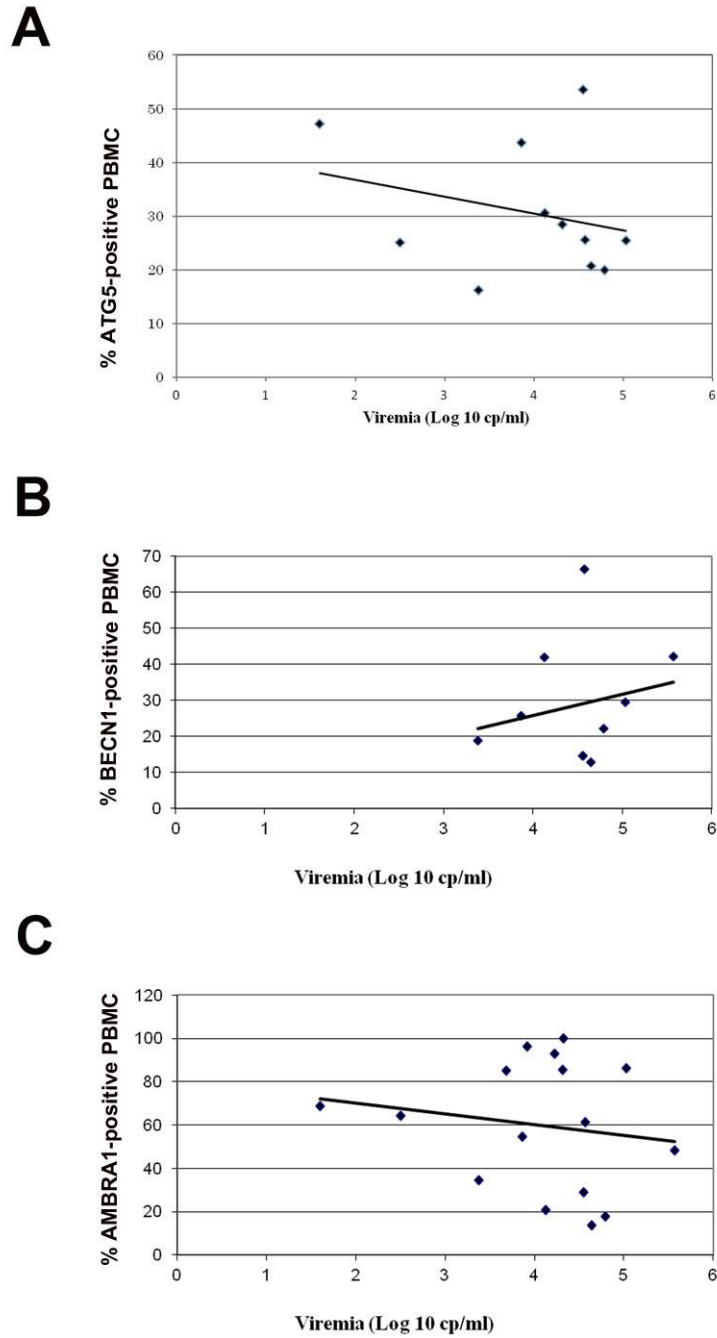


Figure S2. Correlation between autophagic markers expression and plasma viremia in PBMC. We analyzed the relationship existing between the autophagic markers ATG5 (**A**), BECN1 (**B**) and AMBRA1 (**C**) and plasma viremia (Log₁₀cp/ml) in PBMC from HIV-1-infected patients. The data revealed that there is no a linear correlation between these parameters.

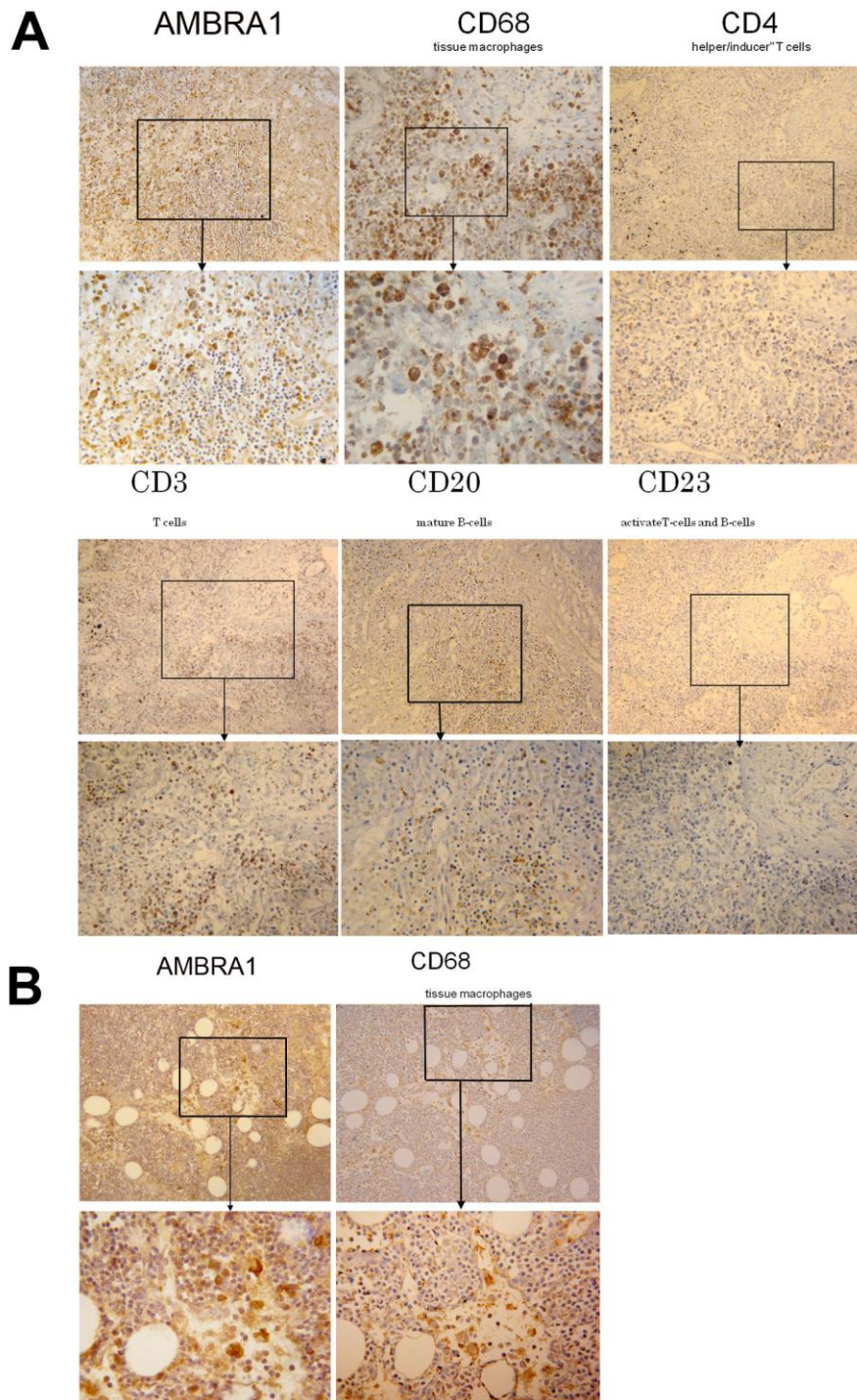


Figure S3. Cell type immunocharacterization of AMBRA1-positive lymph node cells. **(A)** Immunostaining of AMBRA1 and the phenotypic markers CD68, CD4, CD3, MS4A1/CD20, and FCER2/CD23 in consecutive sections of HIV-infected lymph nodes. The AMBRA1 positive cells showed preferentially colocalization with CD68 (tissue macrophages marker) positive cells **(B)** Axillary lymph node consecutive sections from HD showing few AMBRA1-positive cells that colocalize with CD68. Original magnifications: 20X (insets 40X, 63X).

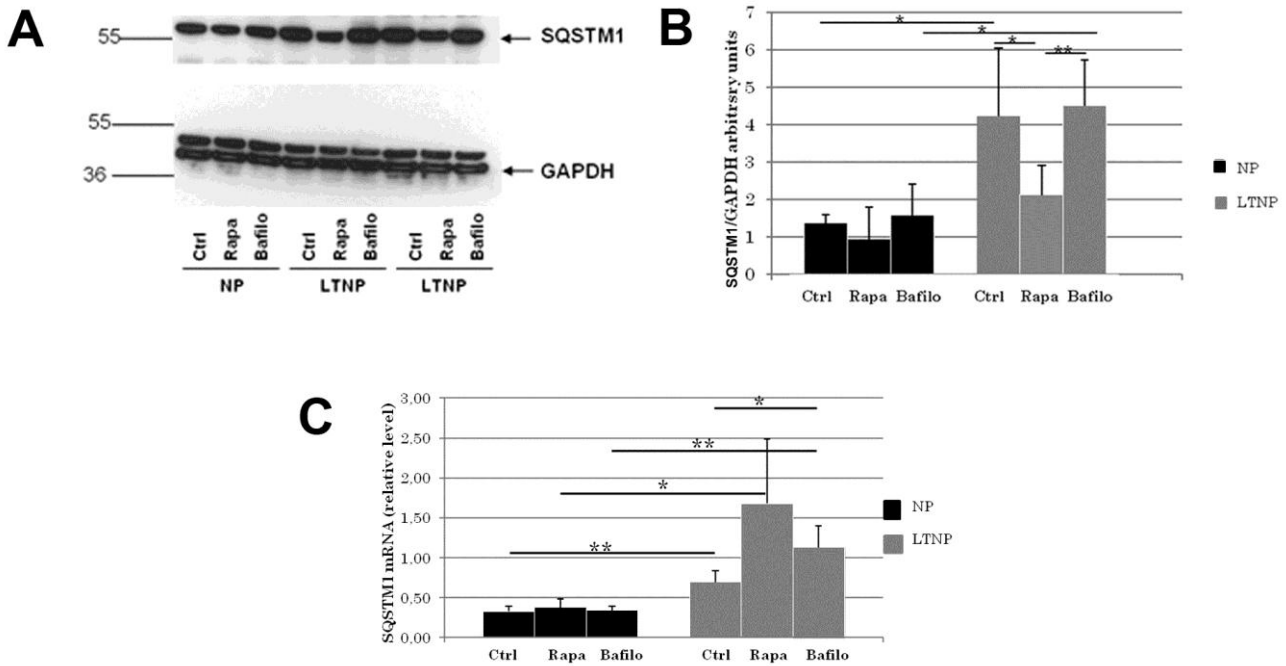


Figure S4. Pharmacological modulation of autophagy. Analysis of SQSTM1. The chemical compounds rapamycin and bafilomycin A₁, were used to try to modulate autophagy in PBMC by *in-vitro* treatment. (A,B) WB analysis of SQSTM1 protein. (C) Quantification of *SQSTM1* mRNA relative level.

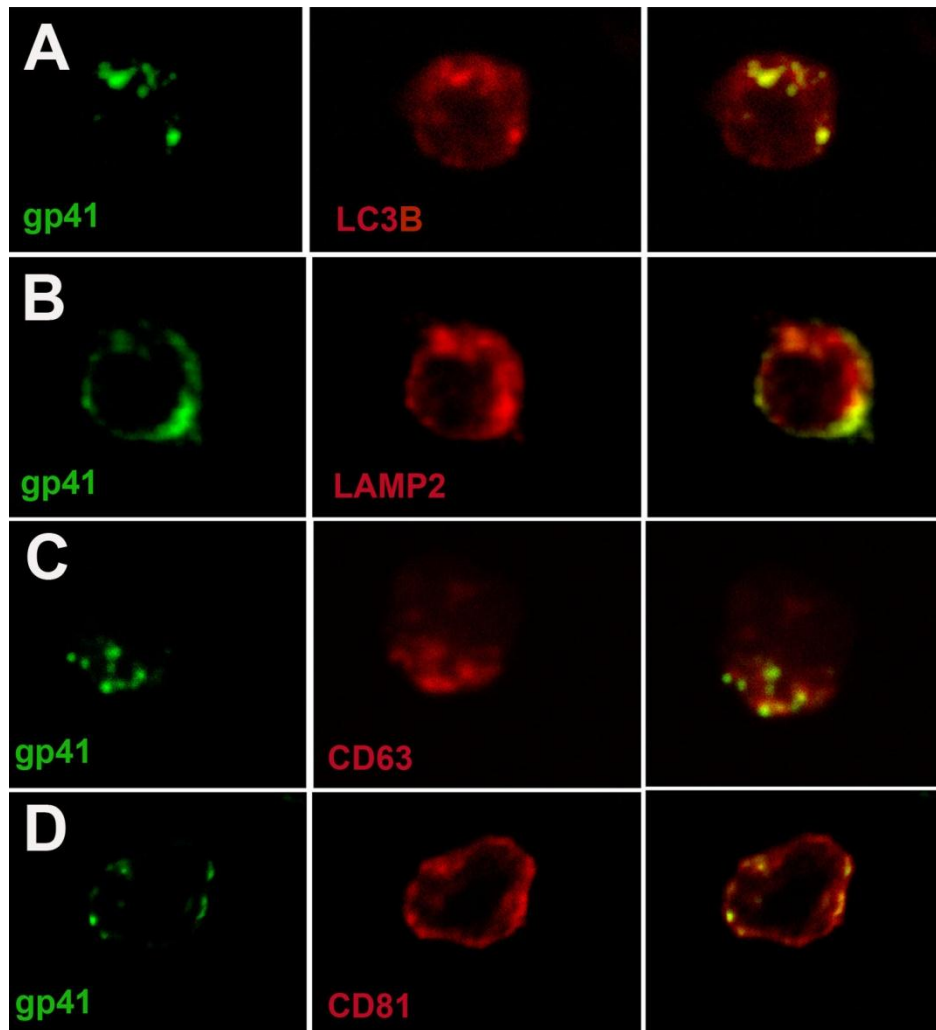


Figure S5. Confocal immunolocalization of the HIV-1 protein gp41 inside cellular compartments. (A) Confocal microscopy immunolocalization of the HIV-1 protein gp41 (green) and the AV membrane protein LC3B (red) in PBMC from LTNP. (B) Immunolocalization of the HIV-1 protein gp41 (green) and the lysosomal membrane protein LAMP2 (red) in PBMC from LTNP. (C) Confocal microscopy immunolocalization of the HIV-1 protein gp41 (green) and the tetraspanins CD63 (red). (D) Immunolocalization of the HIV-1 protein gp41 (green) and CD81 (red).