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Supplemental Data

HIV-1 Assembly: Viral Glycoproteins Segregate Quantally to Lipid Rafts that Associate Individually with HIV-1 Capsids and Virions

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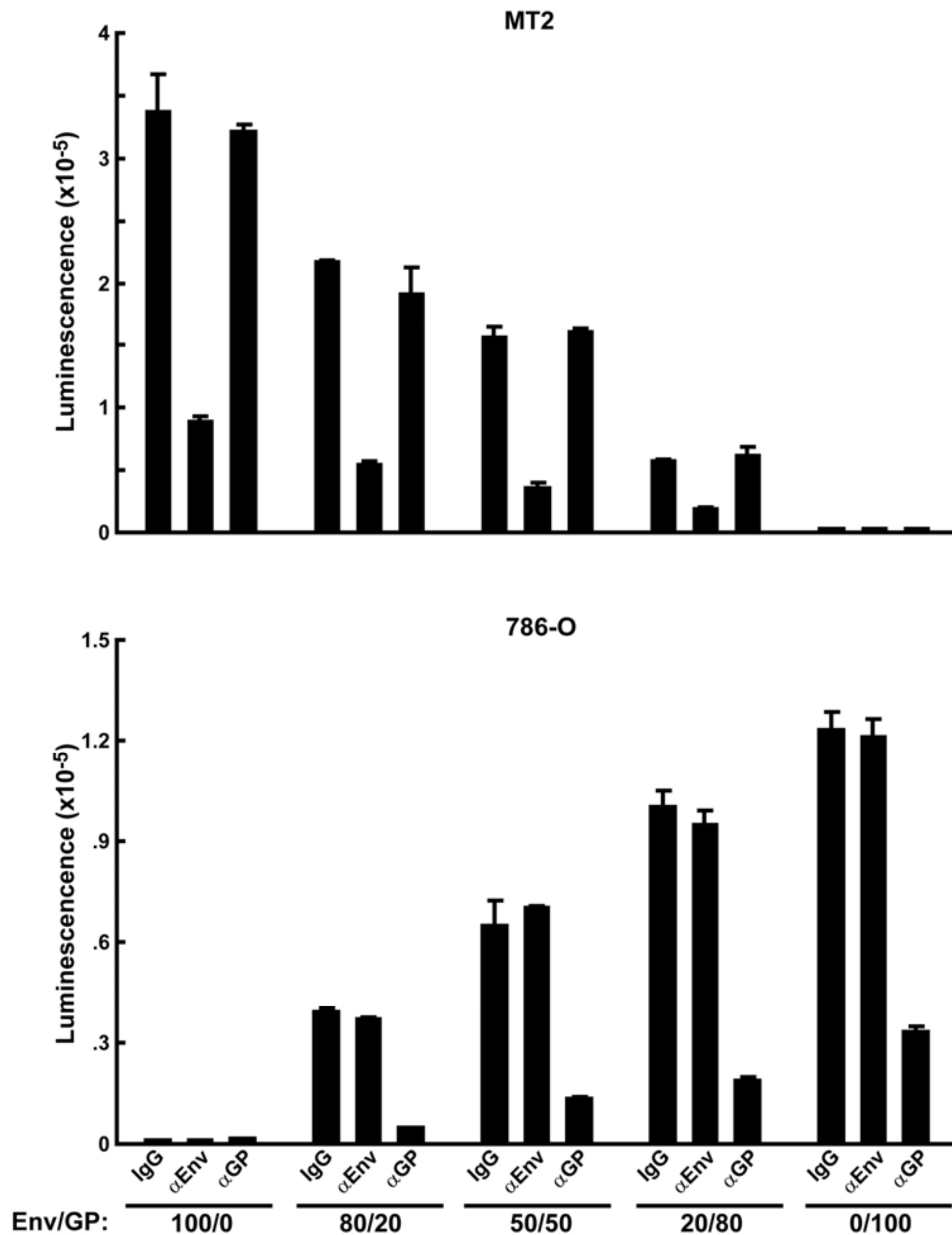


Figure S1. Immunodepletion of virion mixtures produced from singly (Env or GP) transfected producer cells. Optiprep band-purified virions prepared from single expression plasmids (Env or GP) were mixed at different ratios as indicated on the X-axis. Immunodepletion was carried out using anti-Env (2F5 and 2G12) or anti-GP (KZ52) as indicated. The infectivity of the immunodepleted supernatants was subsequently tested in MT2 (HIV-1 reporter) and 786-O cells (Ebola GP reporter). The pattern of infectivity observed here was comparable to that presented in Fig. 3A using virions prepared from double expression plasmid (Env/GP) transfection. Error bars indicate standard deviation of the mean derived from three independent transductions.

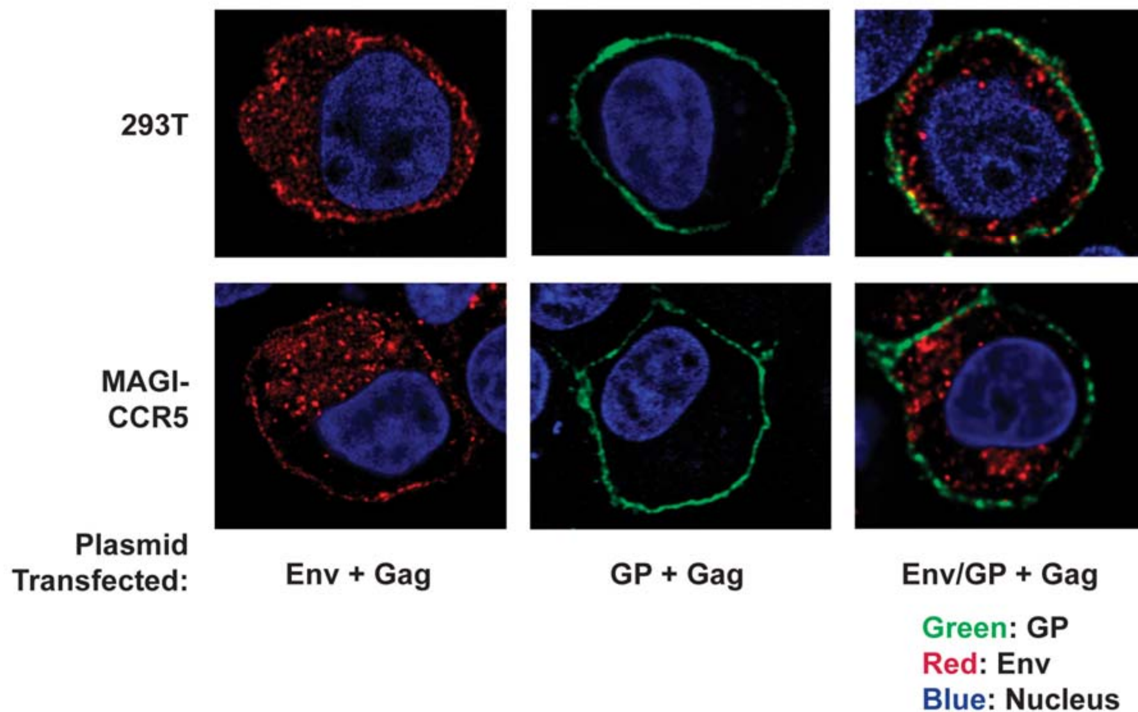


Figure S2. Confocal microscopy of transfected 293T and MAGI-CCR5 cells revealed simlar patterns of envelope glycoprotein expression. 293T and MAGI-CCR5 cells were transiently transfected with Env, GP, or double expression plasmid

(Env/GP) in the presence of packaging vector pCMVΔR8.2 which contains Gag. Immunofluorescent staining was performed 40h later. Single plane images of stained cells were obtained by confocal microscopy.

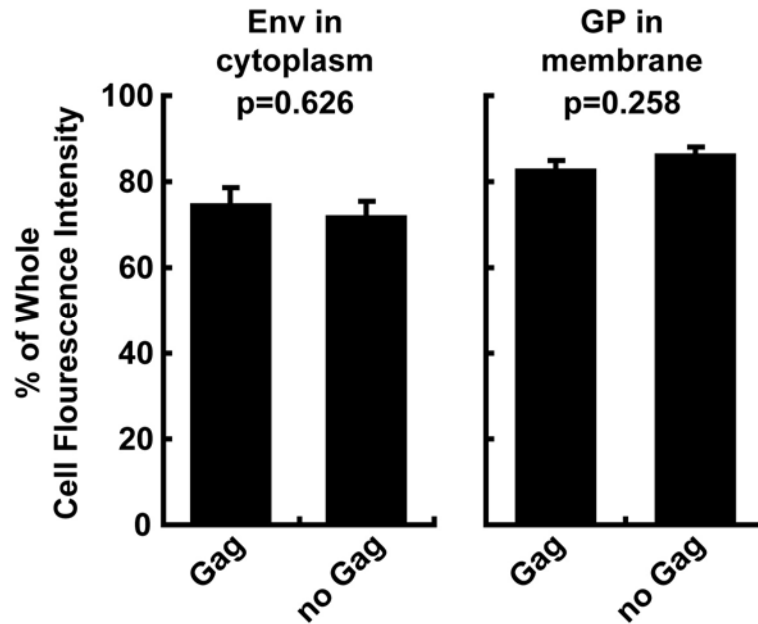
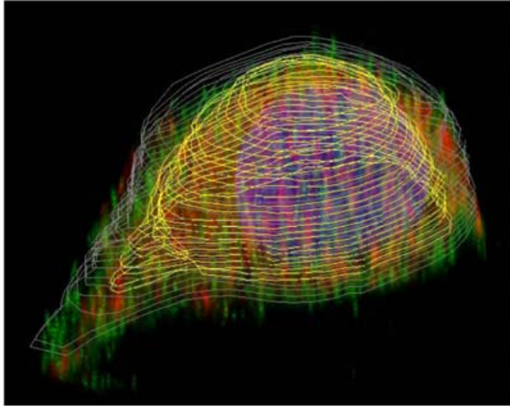


Figure S3. Distribution of Env in the cytoplasm and GP in the plasma membrane independent of Gag expression. The presence of Gag did not alter the subcellular distribution of viral spike proteins. MAGI-CCR5 cells were transfected with the indicated viral spike proteins with or without Gag. Forty hours later, transfected cells were immunostained, and confocal imaging was performed. Data from sequential Z-sections of 6 individual cells per group were collected to generate 3-D images. Signals of the fluorescence intensity of Env or GP in plasma membrane and cytoplasm were then partitioned using the contour surface modeling function of the Imaris software. Error bars indicate standard deviation of the mean for measurements from six individual cells.



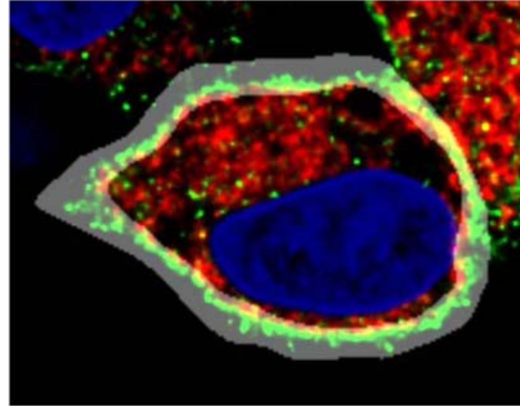


Figure S4. Images showing construction of two separate 3D regions for evaluation of cytoplasmic and cell membrane co-localization. (A) The image shows two contour surfaces, one in yellow and the second in grey, depicting the borders encompassed by the membrane of the cell. The mask generated from these two contour surfaces was then used to define the region utilized for calculation of specific membrane and cytoplasm co-localization statistics. (B) This image depicts a representative middle slice of the whole 3D cell volume. The white transparent region is the constructed mask used for calculation of membrane co-localization. Gag signals were removed for clarity of presentation.