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Native Immunogold Labeling of Cell Surface Proteins and Viral Glycoproteins for Cryo-Electron Microscopy and Cryo-Electron Tomography Applications.

Hong Yi[#], Joshua D. Strauss[#], Zunlong Ke, Eric Alonas, Rebecca S. Dillard, Cheri M. Hampton, Kristen M. Lamb, Jason E. Hammonds, Philip J. Santangelo, Paul W. Spearman and Elizabeth R. Wright*

Robert P. Apkarian Integrated Electron Microscopy Core, Emory University, Atlanta, Georgia, USA (HY, ERW); Division of Pediatric Infectious Diseases, Emory University School of Medicine, Children's Healthcare of Atlanta, Atlanta, GA USA (JDS, RSD, CMH, KML, JEH, PWS, ERW); School of Biology, Georgia Institute of Technology, Atlanta, GA, USA (ZK); and Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University. Atlanta, GA (EA, PJS).

[#]These authors contributed equally.

*To whom correspondence should be addressed.

E-mail: erwrigh@emory.edu; Tel. (+1) 404-727-4665; Fax. (+1) 404-727-9223

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Supplementary Figure and Movie Legends

Supplementary Figure 1. Conventional TEM of immunolabeling controls for tetherin. (A) Native immunolabeled HT1080 cells, transfected with eGFP-tetherin and mCherry-Gag. (B) Capping control of immunolabeled HT1080 cells transfected with eGFP-tetherin and mCherry-Gag. Cells were fixed prior to incubation with antibodies. (C) Tetherin antibody control, immunolabeled HT1080 cells transfected only with mCherry-Gag. Note the absence of tetherin immunolabeling. (A-C) Black arrowheads note 6 nm gold particles. Insets in A, B and C (black boxes) are 2X. Scale bars 200 nm.

Supplementary Figure 2. HRSV F glycoprotein secondary antibody labeling control. Comparison of 2D cryo-TEM images (A and C) and slices (B: 7.37 nm and D: 7.64 nm) through the 3D reconstructions (B and D) of native immunolabeled hRSV F glycoproteins. (A and B) The secondary antibody was a 6 nm gold-conjugated protein G and (C and D) was 6 nm gold-conjugated goat anti-human IgG. Arrowheads indicate immunogold labeling of the hRSV F glycoprotein. Insets are 2X. Gold fiducial markers 10 nm (A and B) or 20 nm (C and D) in diameter were added to the sample and used as image alignment aids during the 3D tomographic reconstruction process. Scale bars 200 nm.

Supplementary Movie 1. Cryo-ET of immunolabeled tetherin and HIV-1 virions attached to an HT1080 microspike. This movie is of a z-slice progression through a tomographic reconstruction of HIV-1 virions tethered to an HT1080 cell extension and corresponds to the data presented in Figure 2. Gold fiducial markers 20 nm in diameter were added to the sample and used as image alignment aids during the 3D tomographic reconstruction process. Scale bar 100 nm.

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Supplementary Movie 2. Cryo-ET of immunolabeled hRSV F glycoproteins at the site of virus assembly on a HeLa cell. This movie is of a z-slice progression through a tomographic reconstruction and the segmentation of the site of hRSV assembly on the surface of a HeLa cell and corresponds to the data presented in Figure 5. The cell membrane is presented in cyan. The filamentous actin network is noted in green. The matrix protein is depicted in blue. The glycoprotein densities are highlighted in magenta. Red tubular densities correspond to the RNP. Gold densities are the 6 nm gold particles conjugated to the secondary antibody. Gold fiducial markers 20 nm in diameter were added to the sample and used as image alignment aids during the 3D tomographic reconstruction process.

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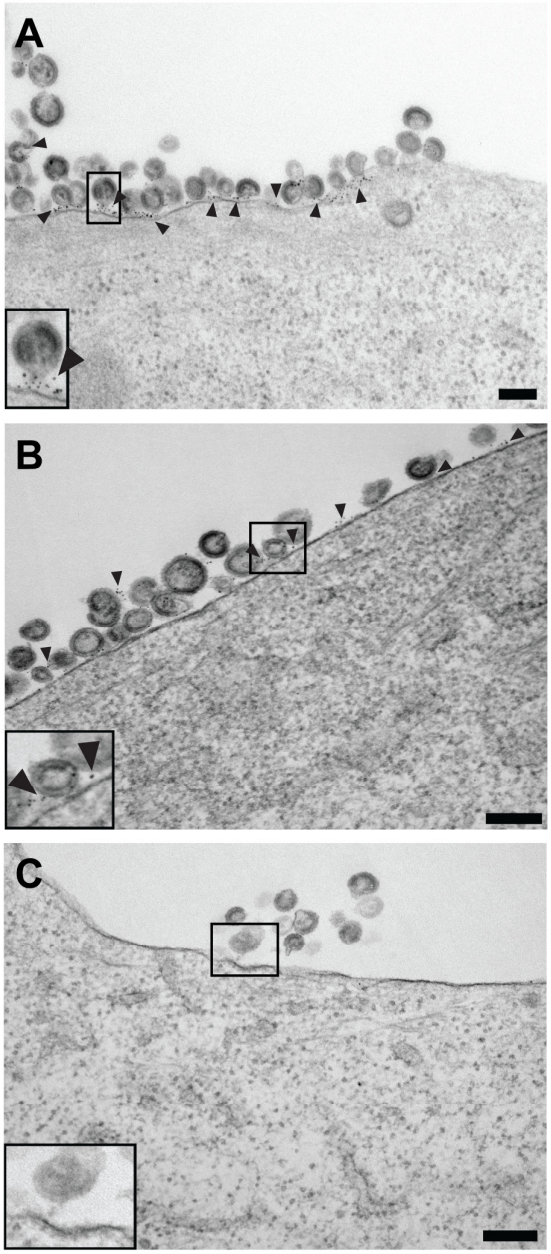

Supplemental Figure 1

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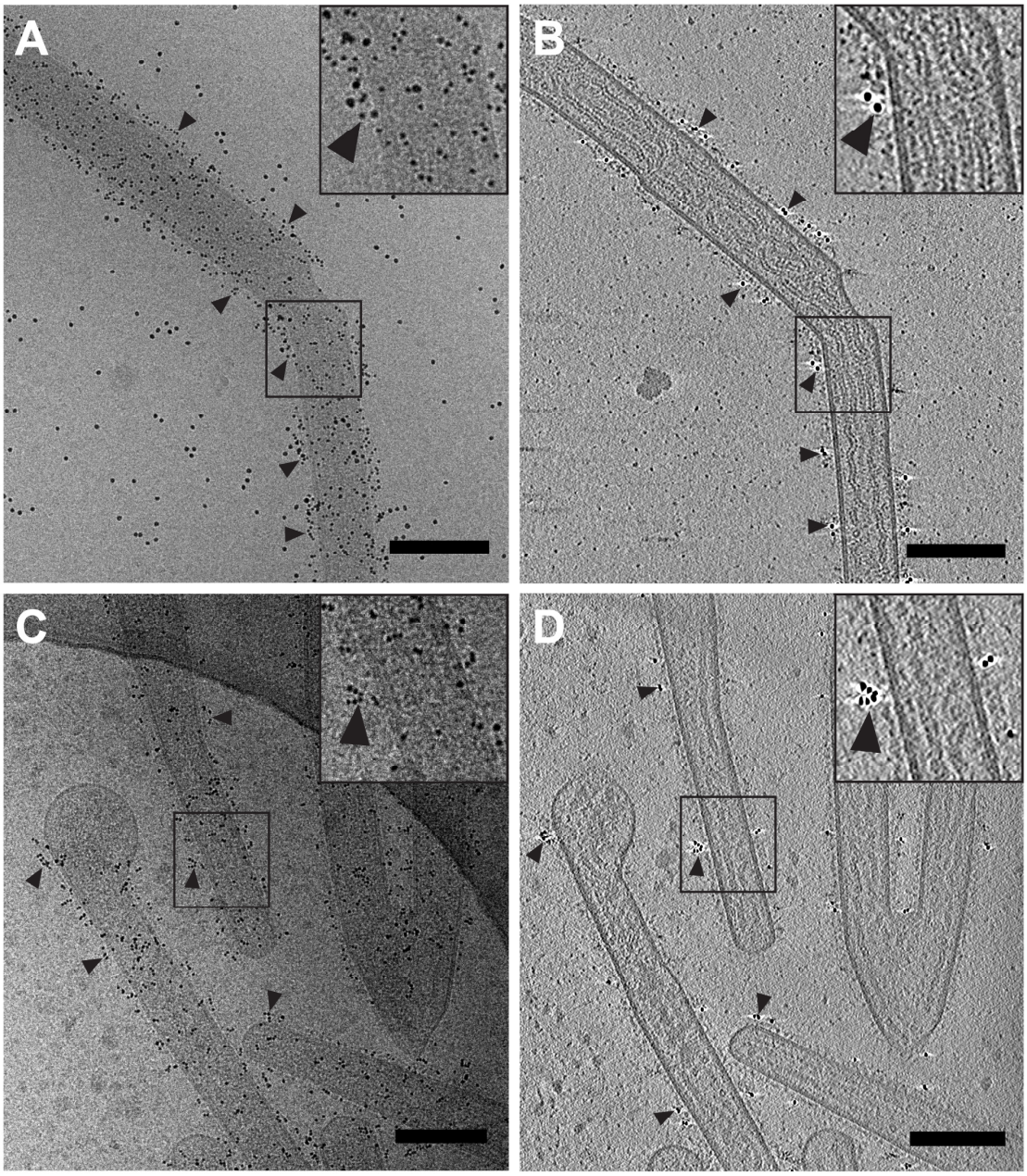

Supplemental Figure 2