Gaudin et al., http://www.jcb.org/cgi/content/full/jcb.201201144/DC1

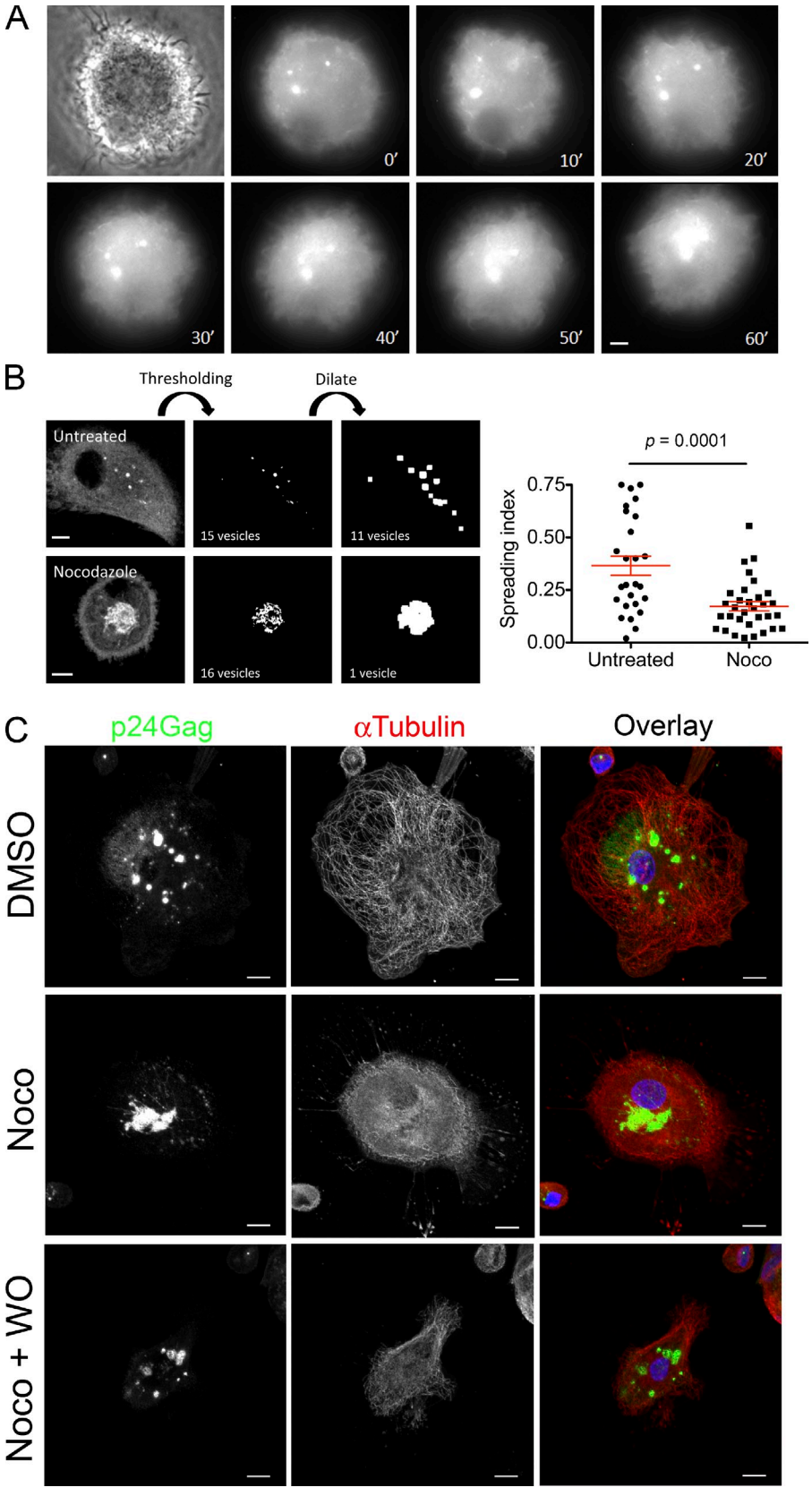


Figure S1. **The distribution of Gag+ compartments depends on the integrity of the microtubule network.** (A) Macrophages infected with HIV Gag-iGFP were imaged every 10 min by epifluorescence before and after exposure to 10 µM nocodazole. Here is shown the transmission light (top first), before treatment (top second), and after treatment (as indicated). VCCs condensed over time. This is a representative experiment out of three. Bar, 5 µm. (B) Schematic representation of the algorithm used to quantify the effect of nocodazole treatment on the spatial distribution of VCC. Macrophages infected with HIV-1 for 7 d, untreated or treated with nocodazole for 1 h at 37°C, were stained for p24 revealed by a secondary antibody coupled to Alexa Fluor 488. Confocal images were acquired, thresholded, and dilated to count Gag+ vesicles using the tools "Dilate" and "Analyze particle" from ImageJ (National Institutes of Health). Examples of images before and after numeric treatments are presented. The spreading index corresponds to the number of vesicles counted after dilatation divided by their number before dilatation and is plotted for each cell treatment. Spreading index close to 0 means that Gag vesicles are clustered, whereas a value close to 1 means that they are dispersed. Bar, 5 µm. (C) Confocal micrographs of HIV-1–infected macrophages exposed to DMSO or nocodazole for 1 h (Noco) or Nocodazole for 1 h followed by washes and incubation for 15 min at 37°C (Noco + WO). Cells were fixed and stained with antibodies specific for the indicated proteins (α-tubulin and p24 Gag). Z-projections of image stacks are presented. Upon nocodazole exposure, Gag+ compartments were distributed in the perinuclear area. This effect was reversible, as upon washout of the nocodazole, microtubule growth was accompanied by spreading of the Gag+ compartments. Bar, 5 µm.

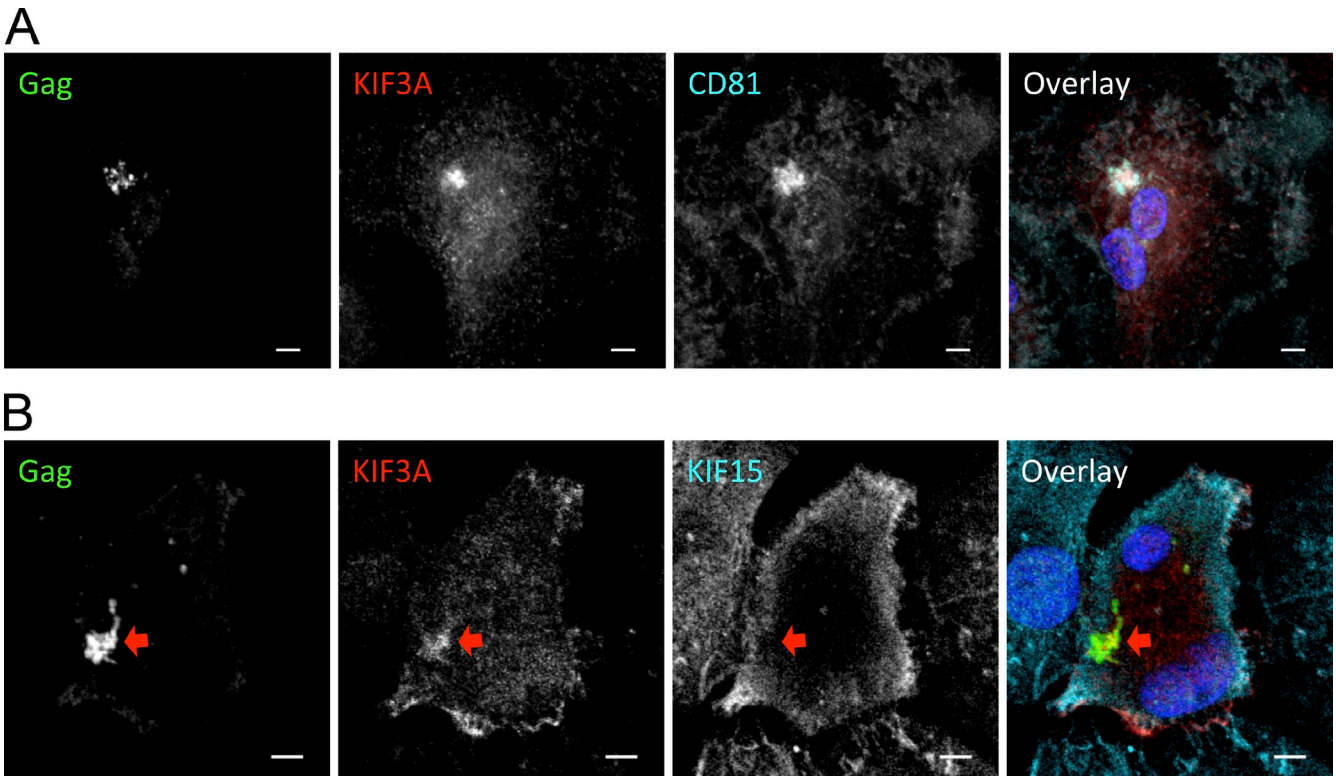


Figure S2. **KIF3A but not KIF15 is associated with the VCCs.** Confocal micrographs of HIV-1–infected macrophages stained for the indicated markers. (A) Z-projections of image stacks are shown. (B) One confocal plane is shown. Bars, 5 µm.

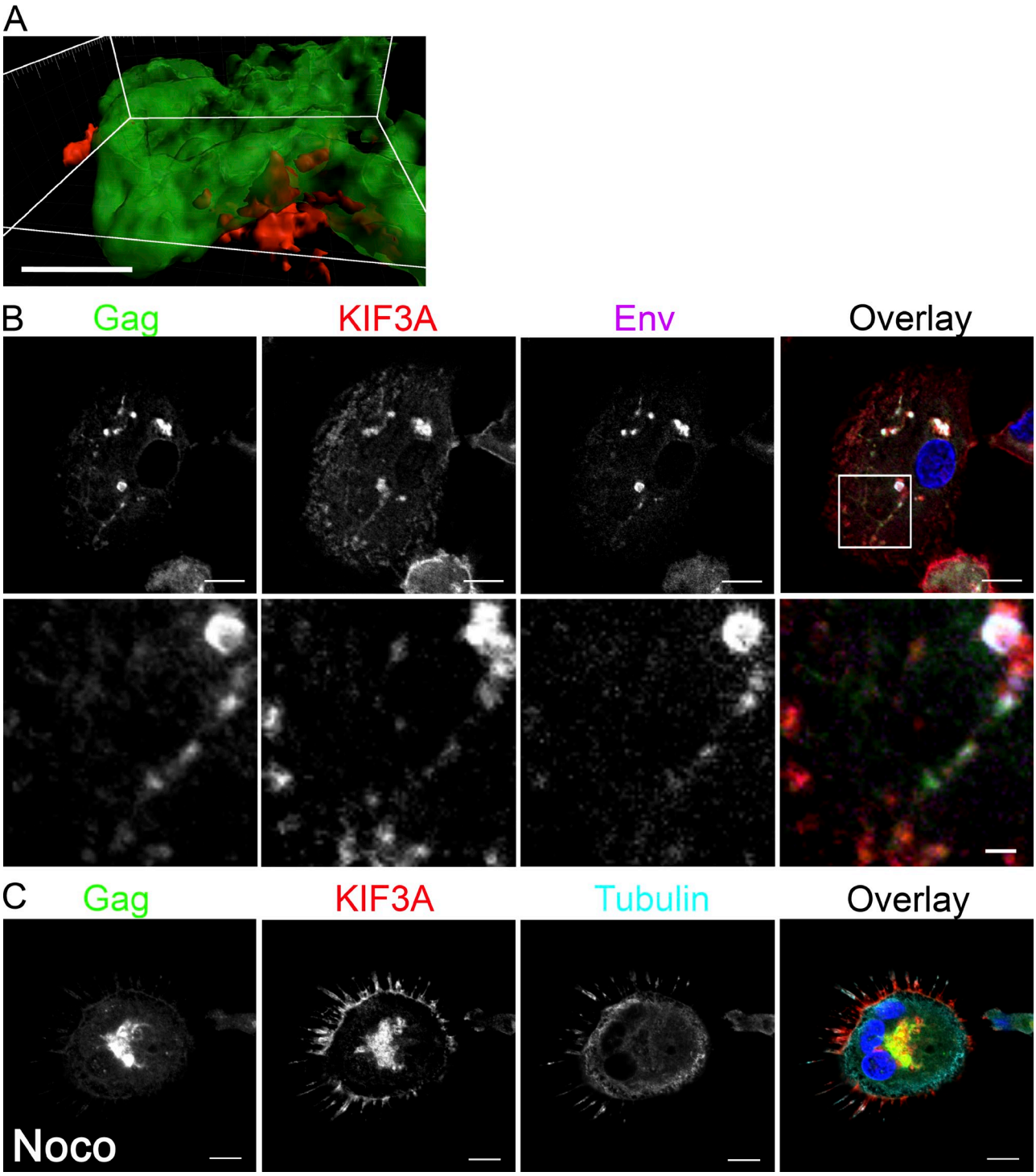


Figure S3. **The kinesin KIF3A is associated with the VCC.** (A) 3D reconstruction of a Gag+ compartment in green with KIF3A distribution in red. This reconstruction was achieved using Imaris software to treat a stack of images acquired by confocal microscopy of HIV-1–infected macrophages stained for Gag (in green) and KIF3A (in red). Bar, 2 µm. (B) Confocal micrographs (one plane) of HIV-1–infected macrophages stained with antibodies specific for the indicated proteins. While Gag and Env stainings overlap extensively, KIF3A staining appears partially codistributed with the viral markers. Bar, 10 µm. The bottom row of images is a magnification of the box shown on the overlay in the top row (bar, 2 µm). (C) Disruption of the microtubule network leads to redistribution of KIF3A in infected macrophages. Confocal micrographs of HIV-1–infected macrophages exposed to 10 µM nocodazole for 1 h. Cells were fixed and stained with antibodies specific for the indicated proteins. A z-projection of an image stack is presented. Bar, 10 µm. Experiments presented are representative of at least two independent experiments.

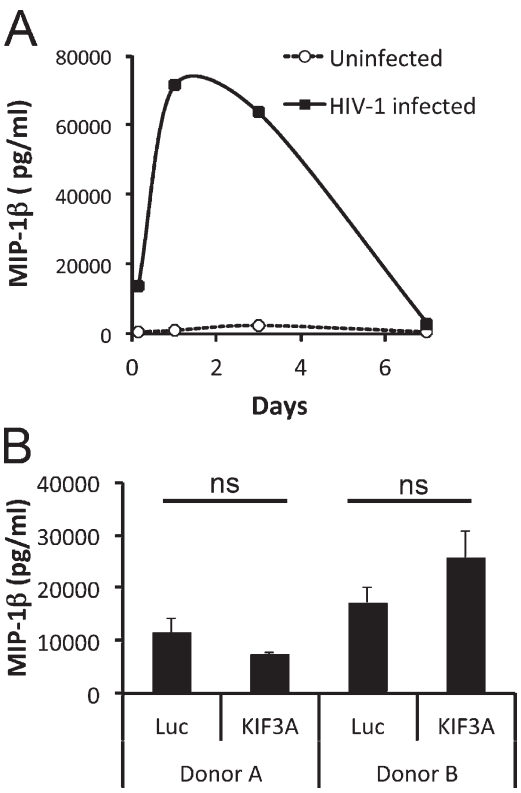


Figure S4. **KIF3A depletion does not affect MIP-1β secretion by HIV-infected macrophages.** (A) Macrophages were uninfected or infected with HIV NLAD8 (VSV-G). MIP-1β released in the supernatant was measured at 4 h and 1, 3, and 7 d after infection by Cytometric Bead Array (BD). (B) Macrophages from two healthy donors were transfected with siRNA and infected 2 d later. MIP-1β released in the supernatant was measured 24 h after infection. Experiments presented have been repeated twice in triplicate.

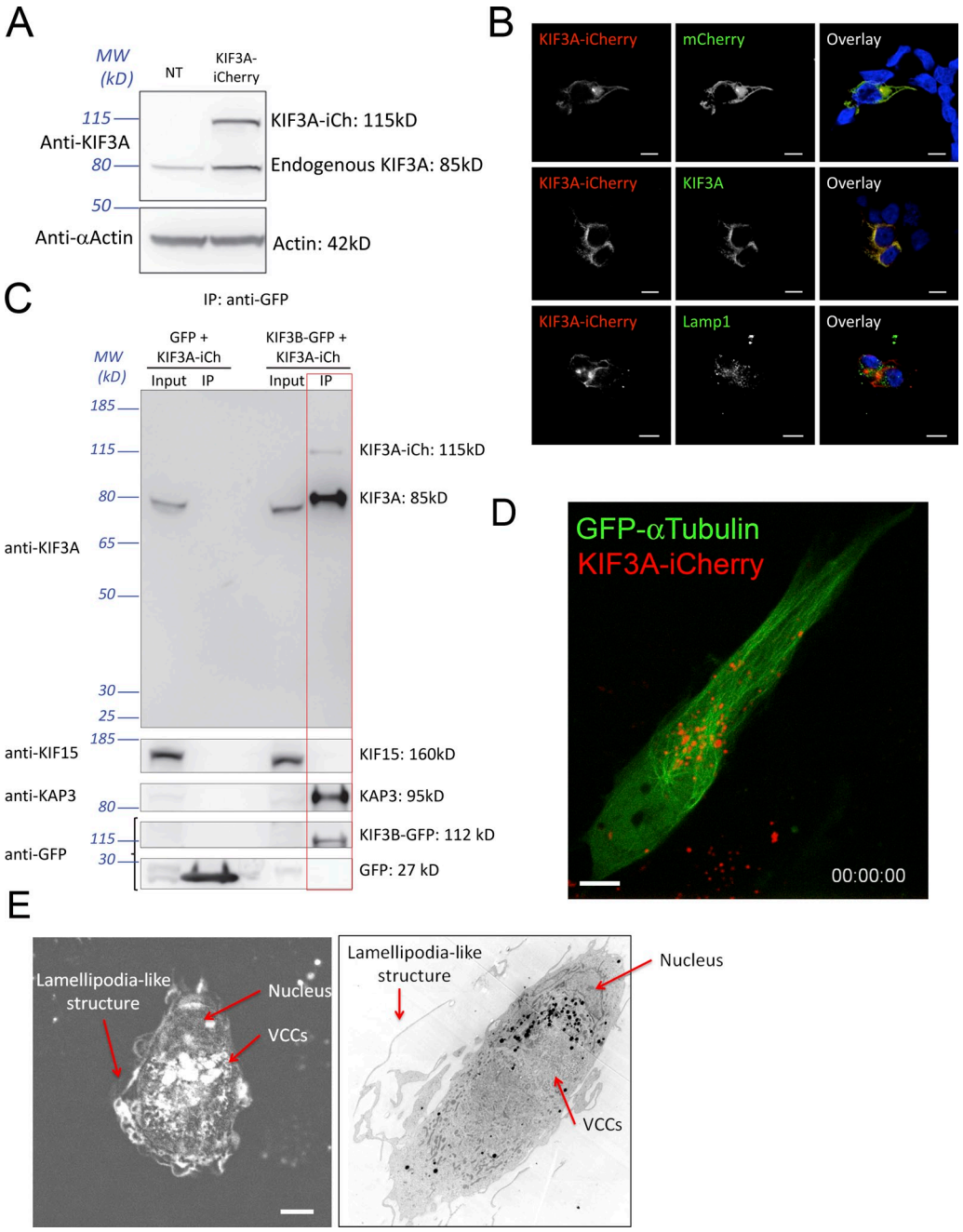
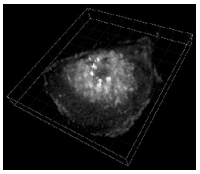
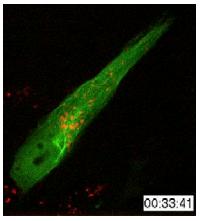


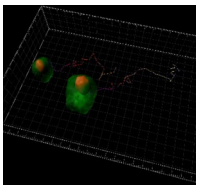
Figure S5. **Characterization of the KIF3A-iCherry construct.** (A) 293T cells were transfected or not with KIF3A-iCh, and analyzed by immunoblot revealed by antibodies specific for KIF3A or actin. (B) Confocal micrographs (one plane) of 293T cells expressing KIF3A-iCh and stained for the indicated proteins. (C) KIF3A-iCh specifically associates with KIF3B. HeLa cells were cotransfected with pExp-DH1-KIF3A-iCh and pEGFP-C1-KIF3B (kindly given by T. Akiyama [University of Tokyo, Tokyo, Japan] via S. Linder [Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany]; Haraguchi et al., 2006; Wiesner et al., 2010) or with pExp-DH1-KIF3A-iCh and pEGFP. 2 d after transfection, cells were harvested and lysed in 0.5% PBS-NP40 for 30 min on ice. GFP immunoprecipitations were performed using GFP-TRAP beads (ChromoTek) according to the manufacturer's instructions, and analyzed by immunoblotting using rabbit polyclonal antibodies anti-KIF3A (ab11259; Abcam), mouse anti-KIF15 (ab57521; Abcam), rabbit anti-KAP3 (ab109026; Abcam), rabbit anti-GFP (Takara Bio Inc.), and appropriate secondary antibodies coupled to HRP (Jackson ImmunoResearch Laboratories). IP, immunoprecipitation with GFP-TRAP beads; Input, total cell lysates loaded after total protein normalization. Immunoprecipitation of KIF3B-GFP brings down endogenous KIF3A, KIF3A-iCherry, and KAP3 as expected, but not KIF15. On the GFP immunoblot, the presence in the input lanes of a band slightly higher than the GFP, at around 28 kD, corresponds to a band developed by the anti-KIF15 antibody. (D) Confocal spinning disk micrograph (one plane) of RPE1 cells coexpressing KIF3A-iCh and GFP-tubulin. KIF3A is aligned on microtubules and moves along them (see Video 2). Bar, 5 µm. (E) Correlation between photonic and electron microscopy from an HIV Gag-iGFP– and KIF3A-iCherry–infected primary macrophage. (Left) Luminosity and contrast from the KIF3A-iCh fluorescent micrograph of Fig. 6 B have been enhanced to better appreciate the position of the VCCs below the nucleus, as well as a large a lamellipodia-like structure on the left. Bar, 5 µm. Electron micrograph of the very same cell oriented in a similar way is presented on the right.



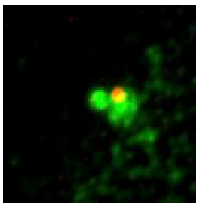
Video 1. **Animation of a 3D reconstruction of an HIV-1–infected macrophage fixed and stained for Gag (gray levels) presented in Fig. 1 B.** This movie allows for appreciating the general shape of the macrophages, which look like a sunny-side up egg. Gag is present throughout the cytosol as diffuse staining and also concentrated in large compartments located in the central thick part of the cell. Images were acquired using a confocal microscope (A1R; Nikon) with a 60x objective. Z-step was 0.5 µm. Related to Fig. 1 B.



Video 2. **Dynamic of KIF3A-iCh and GFP-Tubulin in live RPE1 cells.** RPE1 cells were transfected with KIF3A-iCh and GFP-tubulin constructs. 2 d later they were imaged by time-lapse spinning disc microscopy. In the video presented, one plane was acquired every 10 s for 1 h. Spots of KIF3A-iCh (red) of various sizes can be seen moving along microtubules (green). The mean velocity of these movements was estimated from other videos acquired at higher frequency (1 image/s). Using the particle-tracking option from the Imaris x64 7.0 software, the mean speed of KIF3A-iCh+ structures was 0.59 µm/s. Related to Figs. 6 and S5.



Video 3. **Dynamics of KIF3A-iCherry and HIV-1 Gag-iGFP in primary macrophages.** Time-lapse imaging by spinning disc confocal microscopy of macrophages coinfected with HIV-1 Gag-iGFP ΔEnv (green) and KIF3A-iCherry lentiviral vector (red) and cultured for 5 d. Images were acquired every 5 min for 4 h. The video represents a 5D reconstruction performed with the Imaris x64 7.0 software. Related to Fig. 7.



Video 4. **Dynamics of KIF3A-iCherry and HIV-1 Gag-iGFP in primary macrophages.** Time-lapse imaging by spinning disc confocal microscopy of macrophages coinfected with HIV-1 Gag-iGFP ΔEnv (green) and KIF3A-iCherry lentiviral vector (red) and cultured for 5 d. Images were acquired every 5 min for 4 h. The video was obtained by performing a z-projection of the images acquired. Related to Fig. 7.

Table S1. **Name and target sequences of the siRNA used**

Name	Target sequence
KIF3A#1	GGUUCAGAAAGACAGGCAA
KIF3A#2	GACCUGAUGUGGGAGUUUA
Luc	CGUACGCGGAAUACUUCGA
Tsg101	CCUCCAGUCUUCUCUCGUC

TT DNA modifications have been added at the 3′ end of all siRNA sequences, which were purchased from Eurogentec.

Table S2. **Name and TRC number for shRNAs purchased from MISSION (Sigma-Aldrich)**

Name	Identification
Scramble	Non-target shRNA catalogue n. SHC002
KIF3A#1	TRCN0000116812
KIF3A#2	TRCN0000116814
Tsg101	TRCN0000007564

References

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Wiesner, C., J. Faix, M. Himmel, F. Bentzien, and S. Linder. 2010. KIF5B and KIF3A/KIF3B kinesins drive MT1-MMP surface exposure, CD44 shedding, and extracellular matrix degradation in primary macrophages. *Blood*. 116:1559–1569. http://dx.doi.org/10.1182/blood-2009-12-257089