Supplementary Figure S1

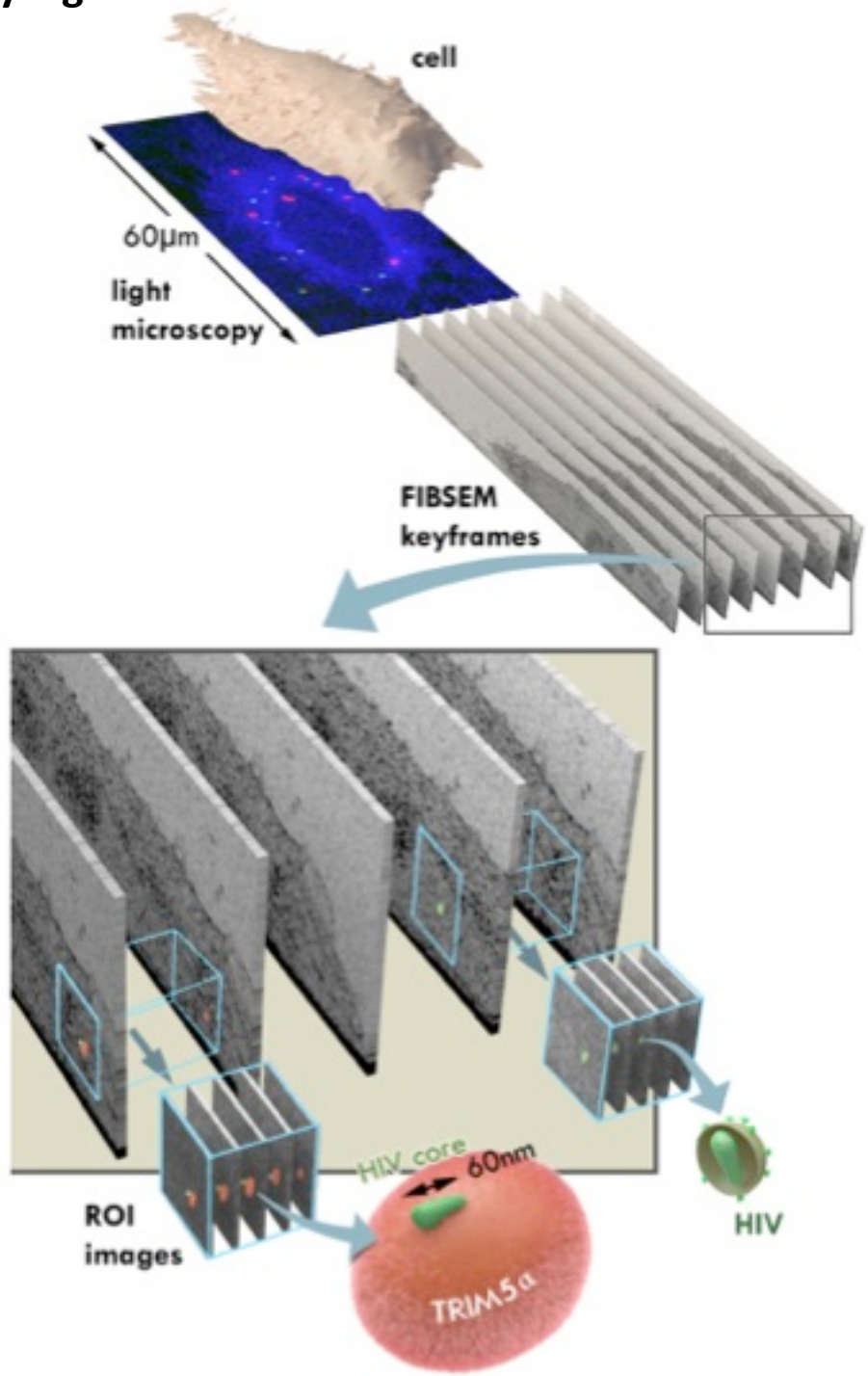

Figure S1. Schematic of targeted high-resolution correlative 3D EM imaging of entire cells and viral cores. Cells with fluorescently labeled targets were imaged by light microscopy, then embedded in resin and imaged in 3D by focused ion beam scanning electron microscopy. "Keyframe" images recorded at lower resolution and sparser intervals capture the whole cell, while "region of interest" (ROI) images target specified locations. ROI images were recorded at high image resolution and fine z spacing, which allowed the accurate reconstruction of endocytosed virions (green) and TRIM bodies (red), as well as the location and visualization of individual HIV cores associated with TRIM5α. This process allows imaging across a 10^9-fold volume span in a single automated overnight run.

Supplementary Figure S2

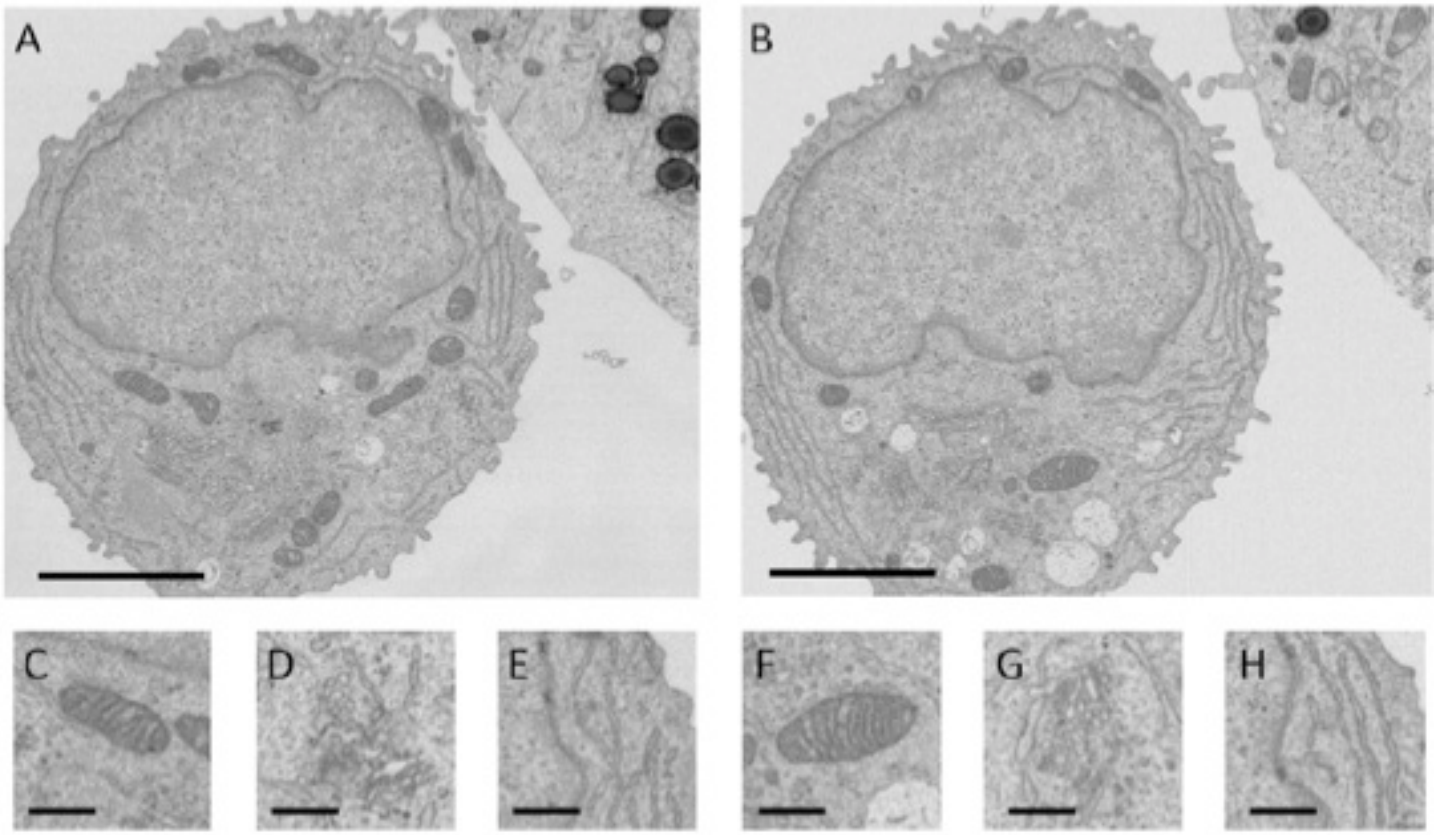

Figure S2. Simultaneous FIB milling and SEM imaging of samples. Resin embedded samples were imaged either by operating both the milling (FIB) and imaging (SEM) beams simultaneously (A) or alternatingly (B). Representative slices and sub-areas from a 3D dataset of embedded dendritic cells acquired at 3nm xy pixel sampling and 50 nm z thickness are shown. Mitochondria (C,F); golgi (D,G) and the endoplasmic reticulum (E,H) are shown. Scale bars 2 μm for whole cells, 400 nm for sub-areas.

Supplementary Figure S3

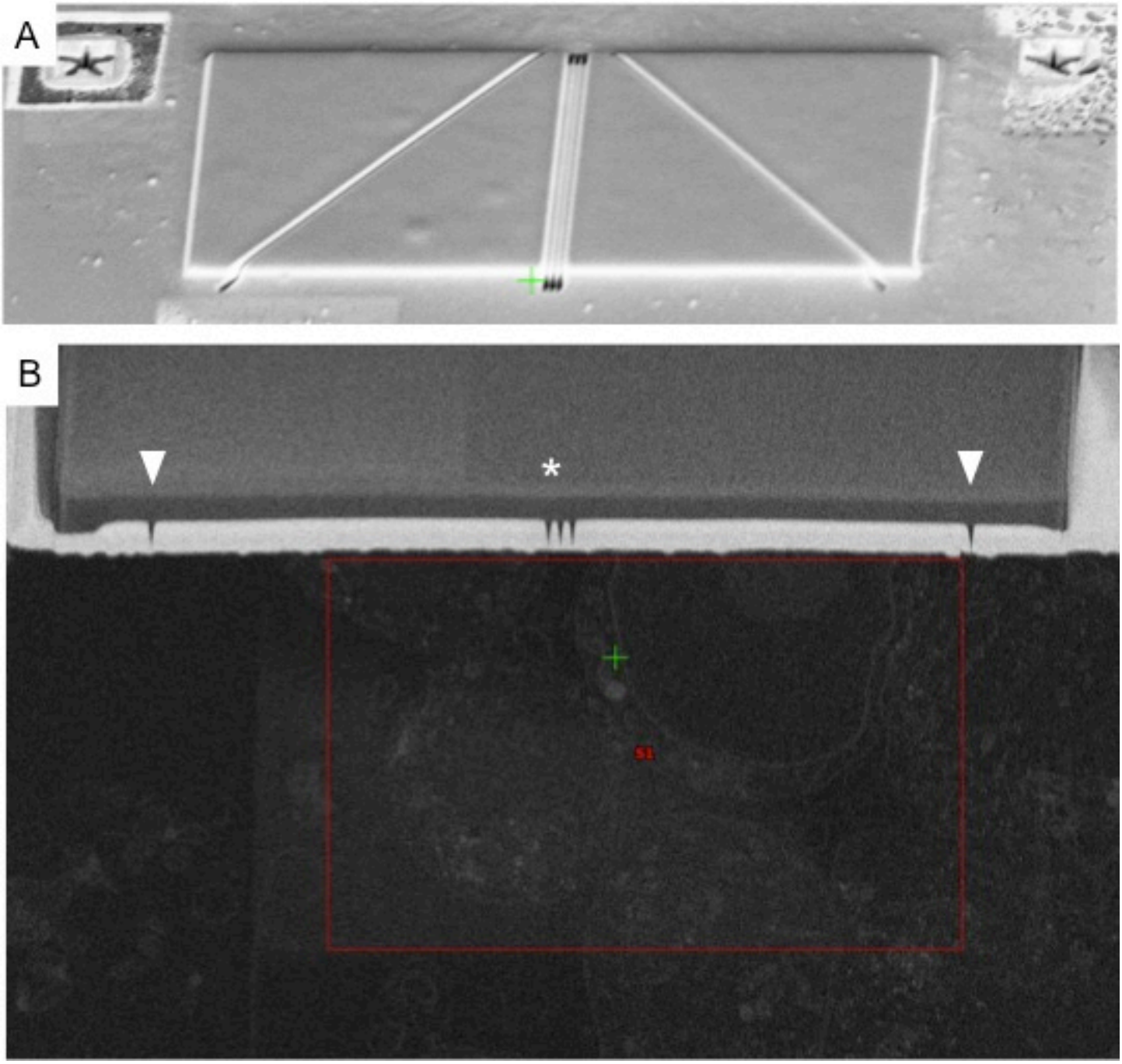

Fig S3. Fine control of drift correction and focus/stigmation. (A) Protective coating of platinum, into which a chevron-shaped pattern has been etched, on the resin surface atop a region of interest. A carbon coat was then applied on top of the platinum coat (B) EsB keyframe image of a cross-section through the protective coating; the contrast between the layers is due to m/z differences between C and Pt. The chevron notches (arrowheads) are used as fiducials to monitor slice thickness and correct for drift in z, while the triplet of straight notches in the middle (*)are used to maintain focus and stigmation, as well as correct for drift in x and y. The red box indicates the ROI for high resolution imaging.

Supplementary Figure S4

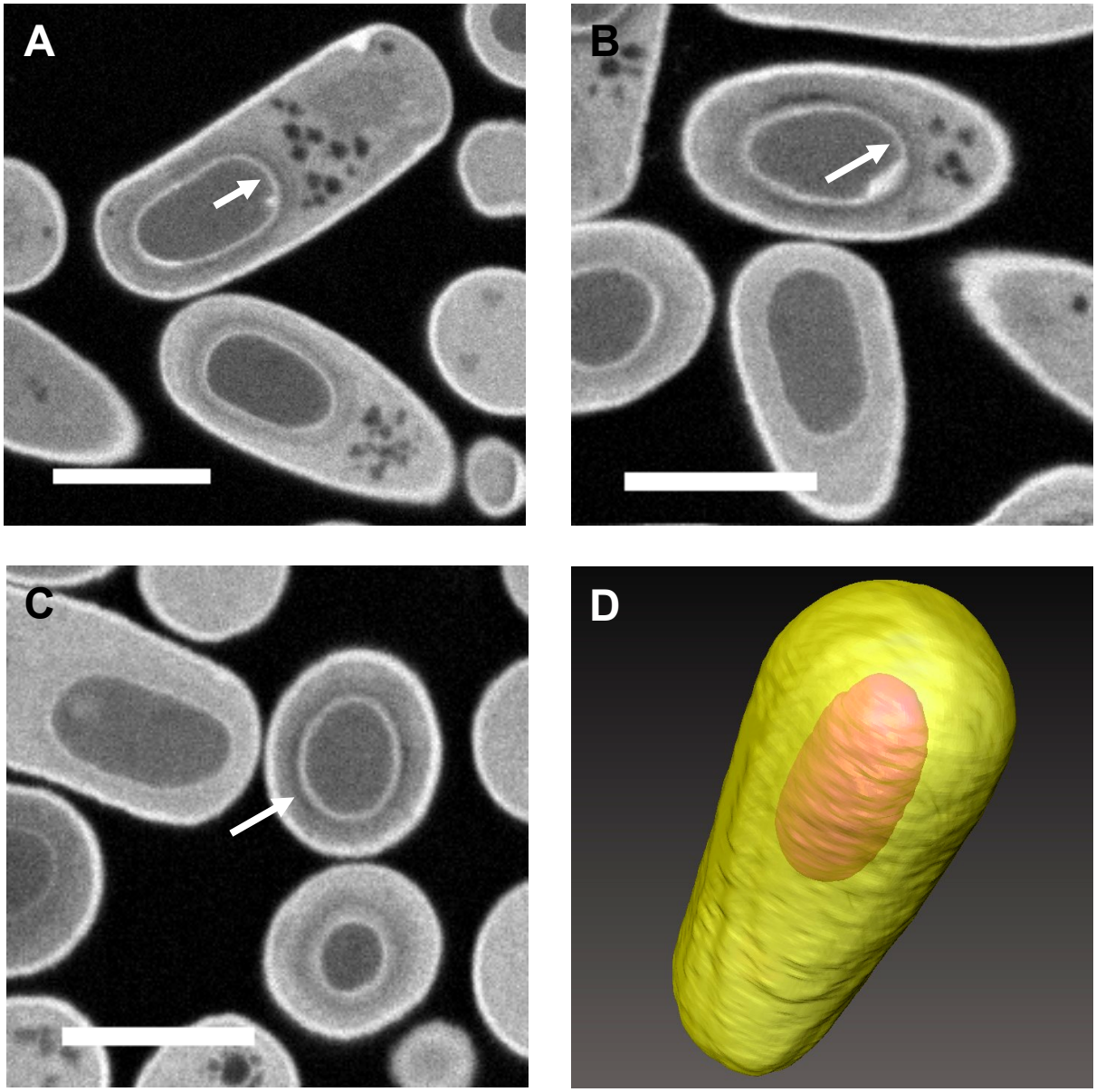

Figure S4. 3D imaging of B subtilis at 3 x 3 x 3 nm pixel sampling by FIB-SEM. Stained and embedded bacterial cells were imaged in 3D, and the dataset was aligned and binned by 4. Fine features such as the spore coats (20-100 nm) surrounding the bacterial forespore (white arrows) could be discerned in all 3 axes, (A) xy, which is the "imaging plane", (B) yz, and (C) xz. (D) Simple segmentation of the bacterial cell; highlighted are the membrane (gold) and forespore (salmon). Scale bar 1 µm.

Supplementary Figure S5

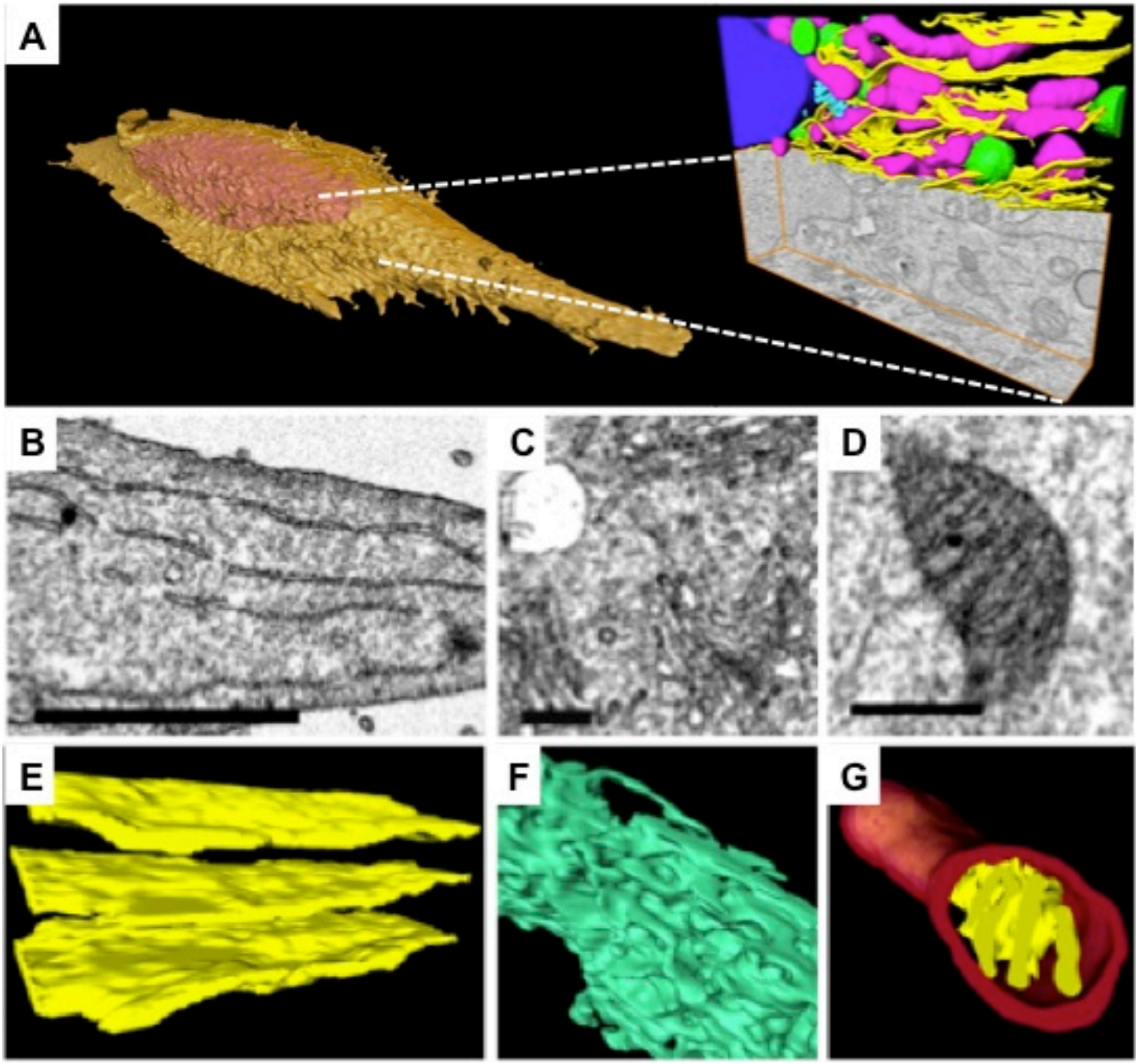

Figure S5. 3D cellular imaging by FIB-SEM and segmentation (A) Automated segmentation of cytoplasm (brown) and nucleus (salmon) of a HeLa cell. Images from the 3D volume and segmentation of a perinuclear area (nucleus, blue; ER, yellow; golgi, teal; mitochondria, pink; vesicles, geen. Slices through, and segmentation of ER (B,E), golgi (C,F), mitochondria (D,G). Scale bar 2µm (B), 400 nm (C,D).

Supplementary Figure S6

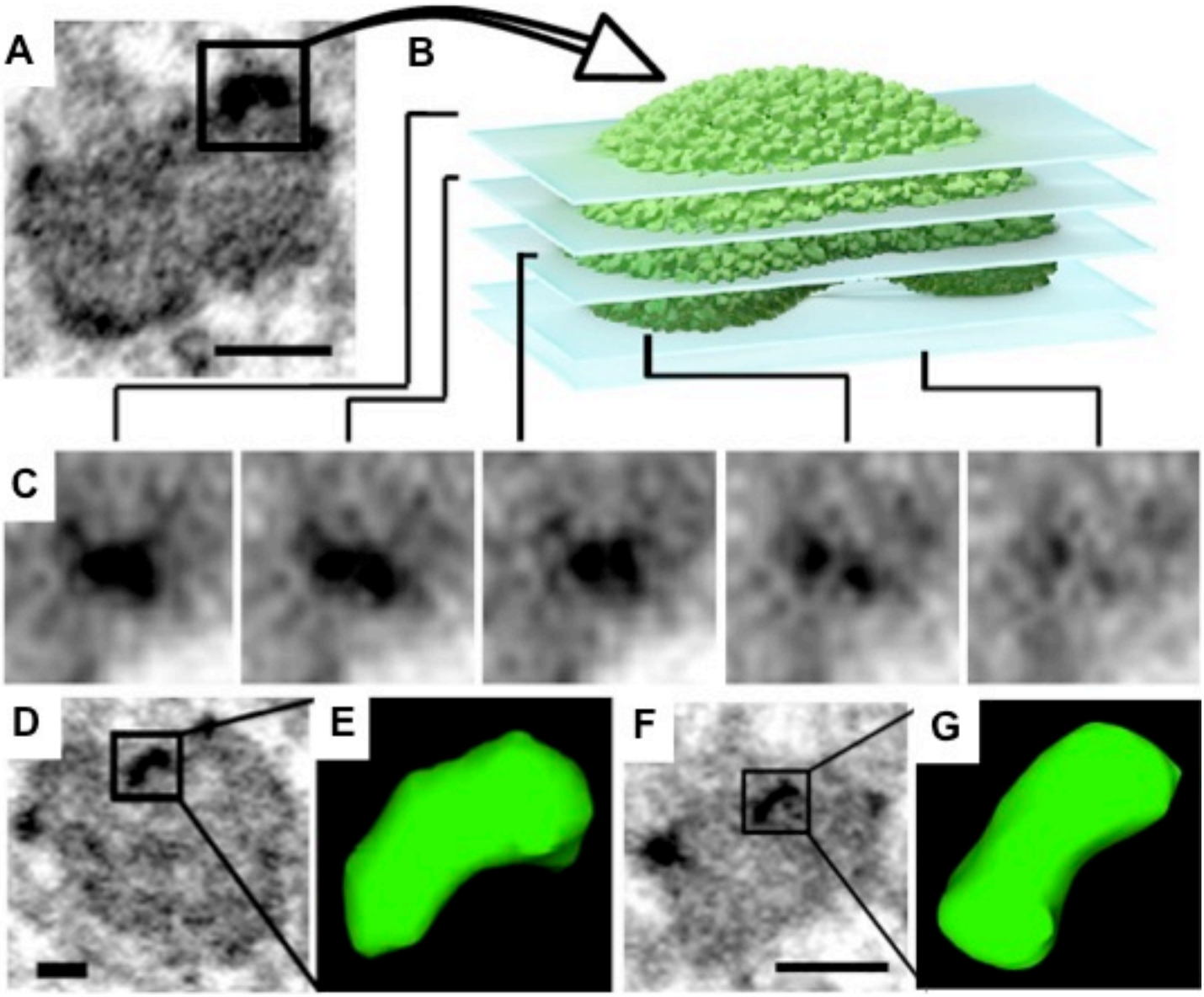

Figure S6. Images of HIV cores associated with TRIM bodies. (A) oblique slice through TRIM associated HIV core (B) schematic of bent or curved core with a molecular rendering of capsid hexamers, with "slices" through the density (C) Image slices through 3D data showing single area of density splitting into two, suggestive of curvature. (D-G) Images and segmentation of other examples of similar intracellular TRIM body-associated HIV cores. Scale bars 200 nm for A,F, 100 nm for D.

Supplementary Movies

Movie M1. Keyframe image stack of an entire HeLa cell (~65 μm wide), imaged at 12 nm pixel sampling in xy, and 120 nm z spacing

Movie M2. ROI image stack of the leading edge of the same HeLa cell; 250 slices are shown, imaged at 4 nm pixel sampling in xy and 12 nm z slice thickness. A TRIM body appears in the frames between 11 and 17 seconds; it is the <1 μm sized moiety with even grey contrast located in the thin area of the cytoplasm between the nuclear membrane and the bottom of the cell.