Supporting Information

A Nanoscale Visual Exploration of the Pathogenic Effects of Bacterial Extracellular Vesicles on Host Cells

Minjae Kang[1, ‡], Min Jeong Kim[1, ‡], Dokyung Jeong[1], Hyung-Jun Lim[1], Ga-eun Go[1], Uidon Jeong[1], Eunyoung Moon[2], Hee-Seok Kweon[2], Nae-Gyu Kang[3], Seung Jin Hwang[3], Sung Hun Youn[3], Bo Kyoung Hwang[3], Doory Kim[1, 4, 5, 6, *]

[1]Department of Chemistry, Hanyang University, Seoul 04763, Republic of Korea

[2]Electron Microscopy Research Center, Korea Basic Science Institute, Cheongju 28119, Republic of Korea

[3]R&D Center, LG H&H Co., Ltd., Seoul 07795, Republic of Korea

[4]Research Institute for Convergence of Basic Science, Hanyang University, Seoul 04763, Republic of Korea

[5]Institute of Nano Science and Technology, Hanyang University, Seoul 04763, Republic of Korea

[6]Research Institute for Natural Sciences, Hanyang University, Seoul 04763, Republic of Korea

‡These authors contributed equally.

***Corresponding author: doorykim@hanyang.ac.kr**

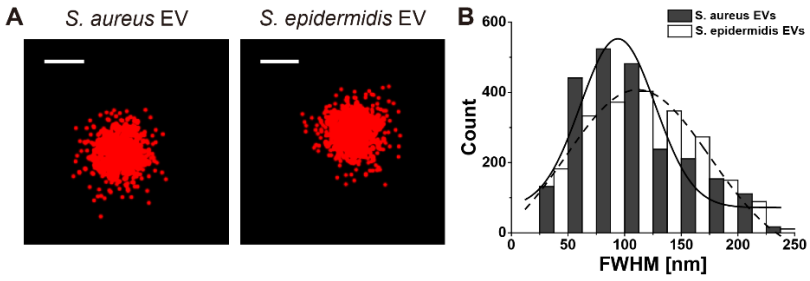


Fig. S1 Representative STORM images of EVs purified from *S. aureus* and *S. epidermidis*. (**A**) Representative STORM images of purified *S. aureus* (left) and *S. epidermidis* (right) EVs labeled with Nile red. Scale bar: 100 nm. (**B**) Size distribution of EVs measured from the STORM images of Nile red-stained purified EV samples. The full width at half maximum (FWHM) of a single-molecule distribution was used as the diameter of each EV particle.

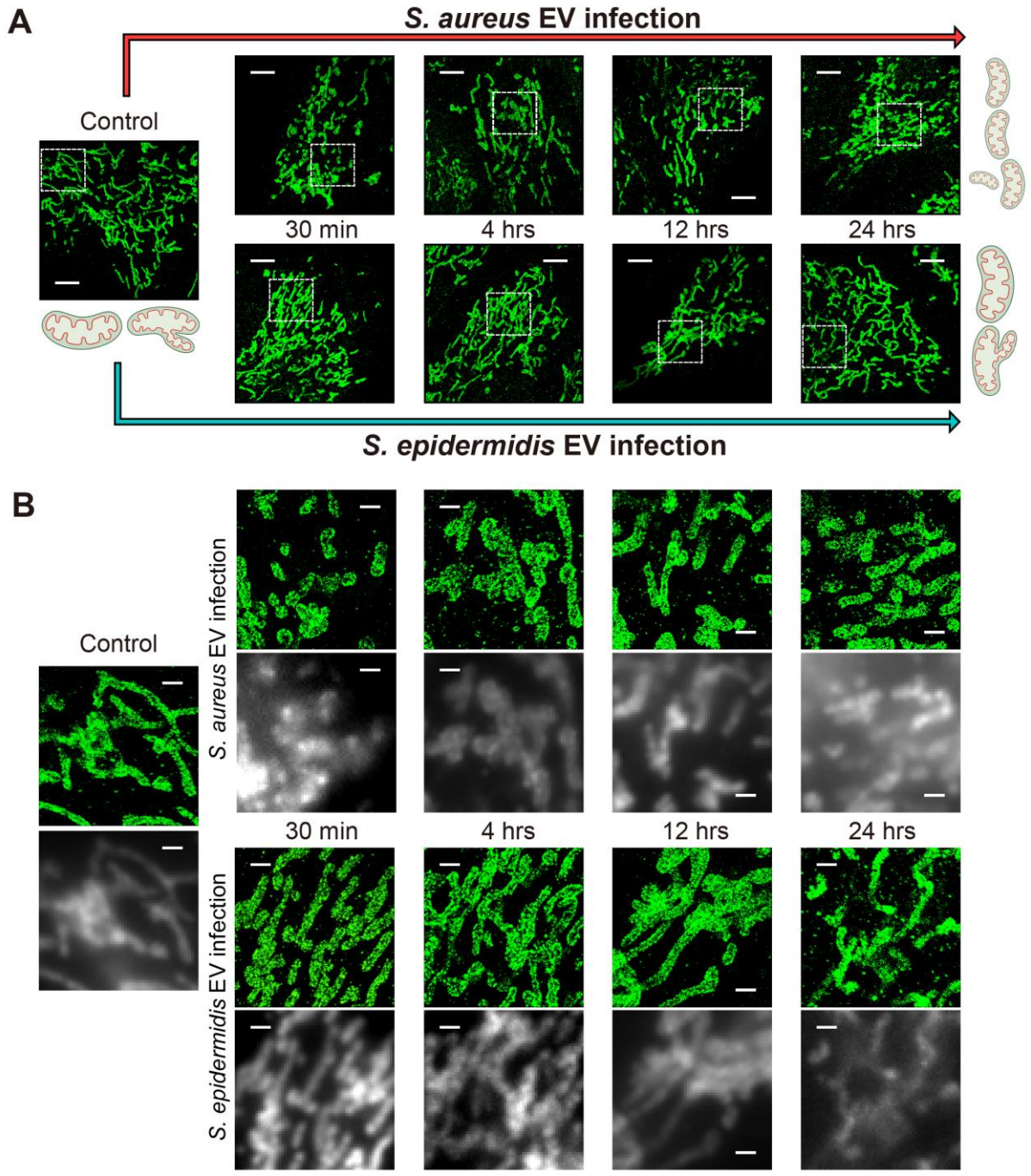


Fig. S2 Mitochondrial change during EV-mediated infection. (**A**) Representative STORM images of mitochondria in epithelial keratinocyte (HaCaT) cells treated with *S. aureus* EVs (top) or *S. epidermidis* EVs (bottom) at different times. Schematic diagrams are shown together. (**B**) Enlargement of the boxed region in (**A**) to compare the diffraction-limited images (bottom) and STORM images (top). Scale bars: 5 μm (top), 1 μm (bottom).

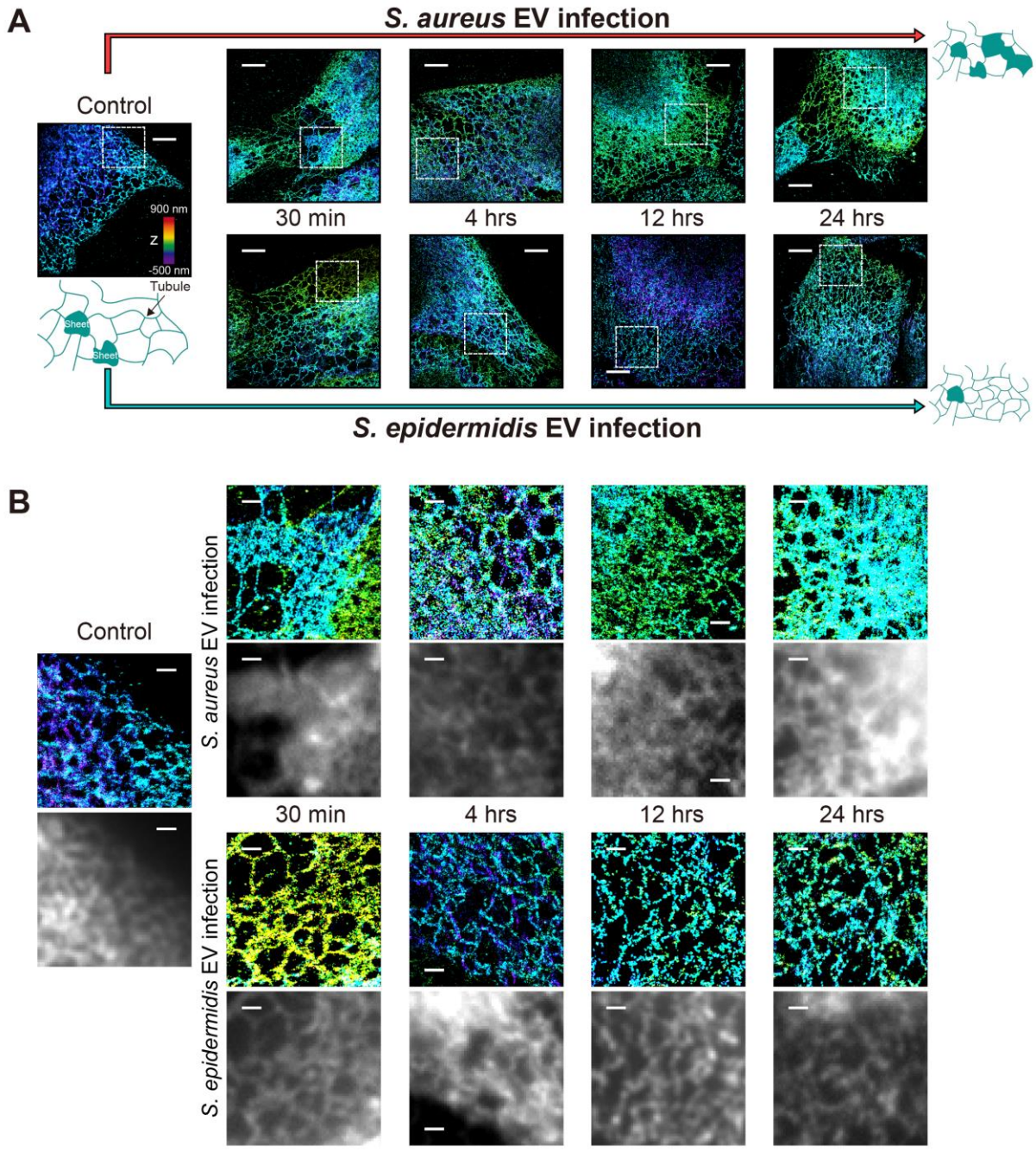


Fig. S3 Transformation of endoplasmic reticulum (ER) between sheet and tubular structures by EV infection. (**A**) Representative STORM images of ER in epithelial keratinocyte (HaCaT) cells treated with *S. aureus* EVs (top) or *S. epidermidis* EVs (bottom) at different times. Schematic diagrams are shown together. (**B**) Enlargement of the boxed region in (**A**) to compare the diffraction-limited images (bottom) and STORM images (top). Scale bars: 5 μm (top), 1 μm (bottom).

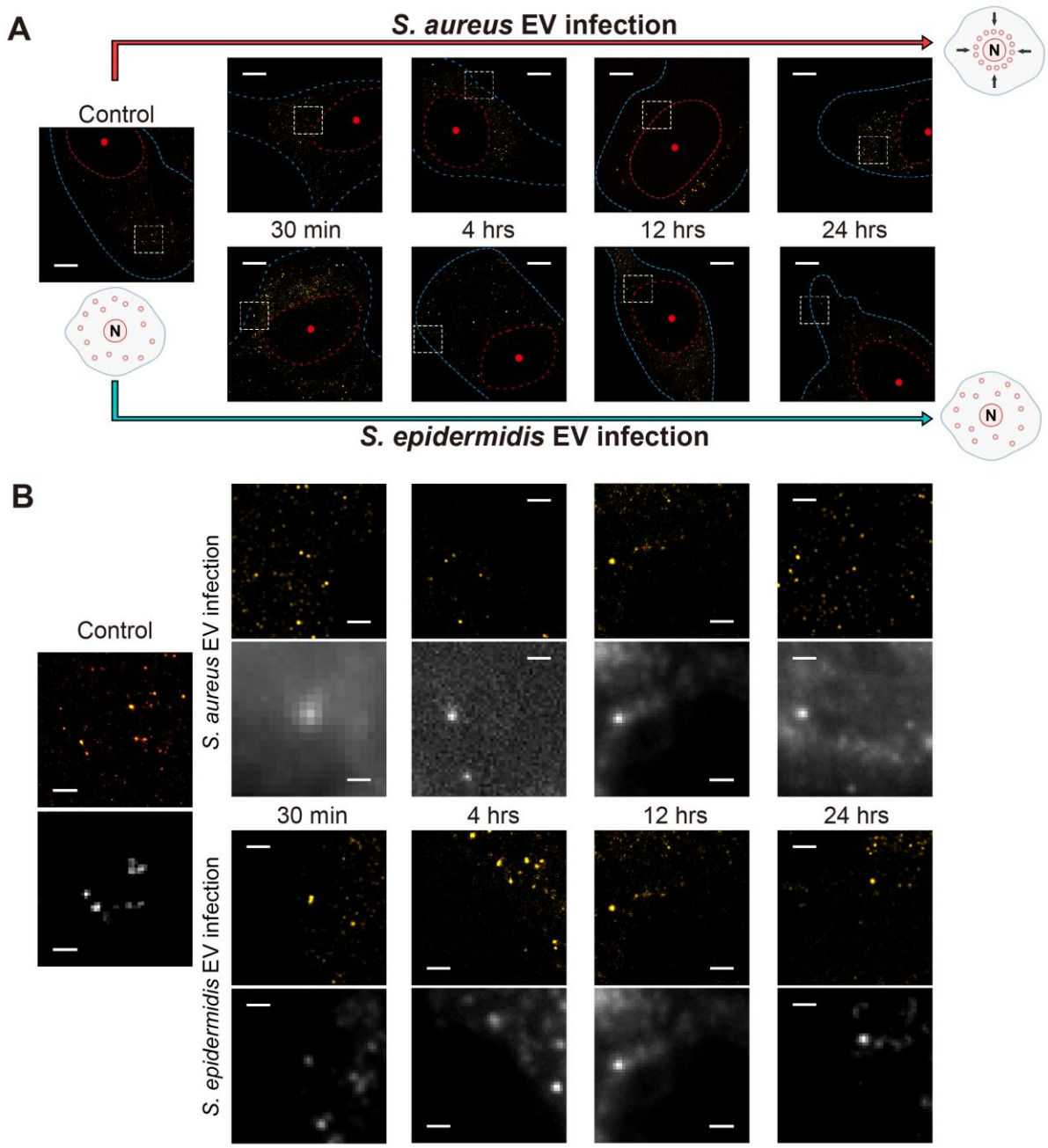


Fig. S4 Lysosome distribution change during EV-mediated infection. (**A)** Representative STORM images of lysosomes in epithelial keratinocyte (HaCaT) cells treated with *S. aureus* EVs (top) or *S. epidermidis* EVs (bottom) at different times. Schematic diagrams are shown together. Dashed lines: cell membrane boundary (blue) and nucleus boundary (red). (**B**) Enlargement of the boxed region in (**A**) to compare the diffraction-limited images (bottom) and STORM images (top). Scale bars: 5 μm (top), 1 μm (bottom).

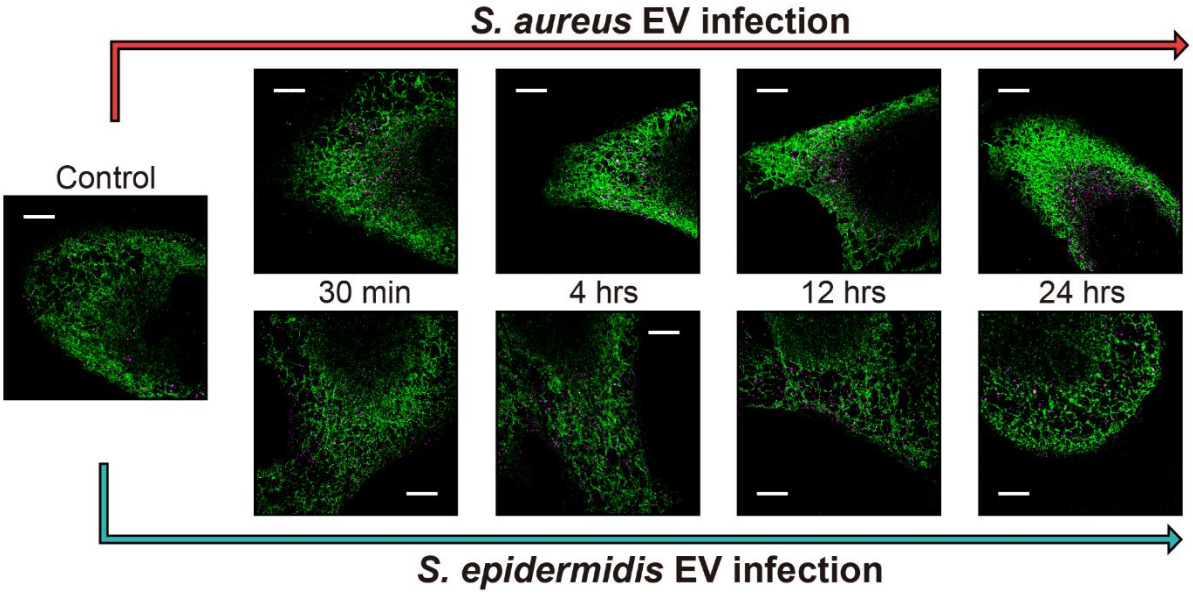


Fig. S5 Representative two-color STORM images of ER (green) and lysosomes (magenta) in epithelial keratinocyte (HaCaT) cells treated with *S. aureus* EVs (top) or *S. epidermidis* EVs (bottom) at different times. Scale bar: 5 μm.

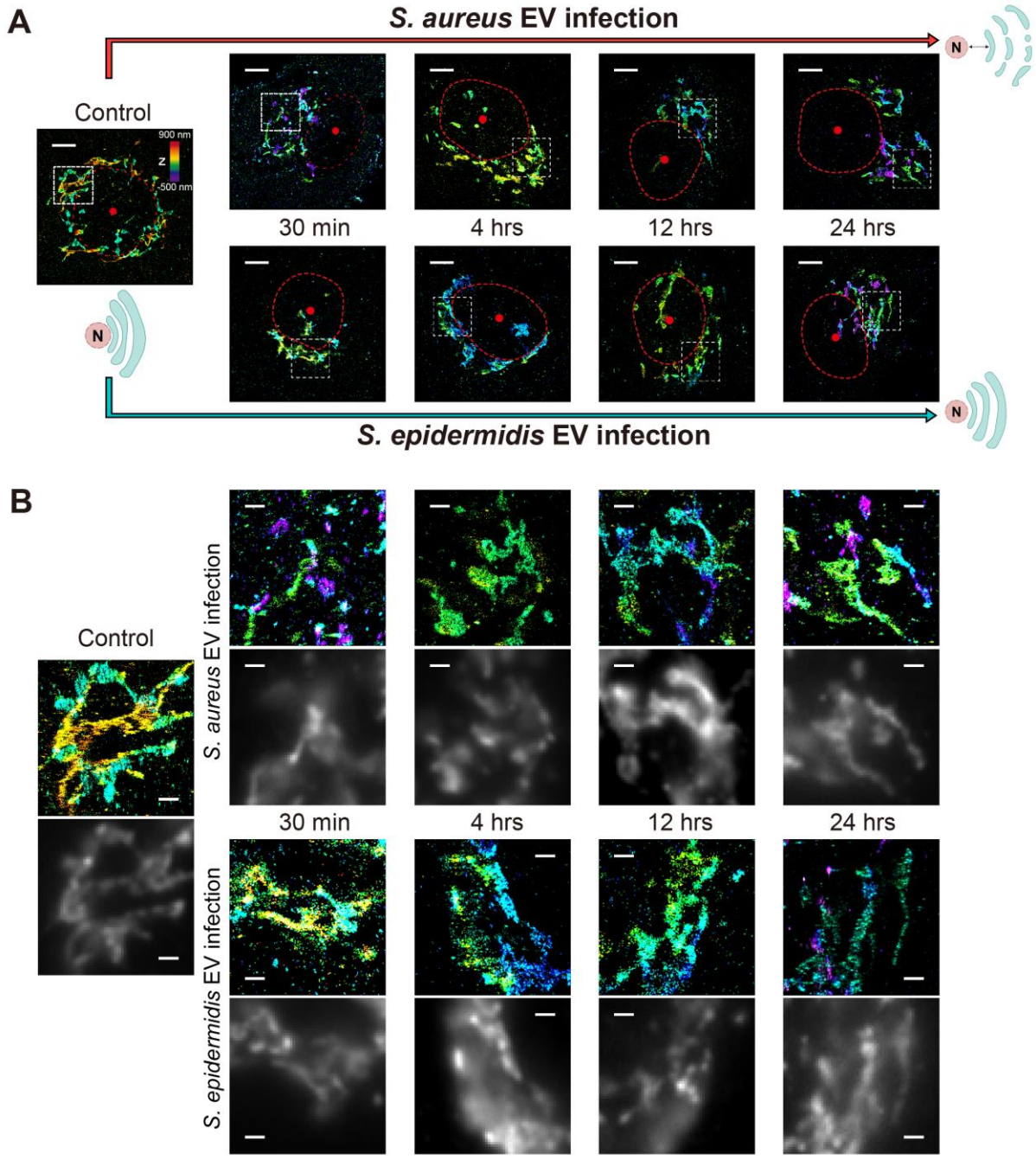


Fig. S6 Golgi dispersion and fragmentation by *S. aureus* EV infection. (**A**) Representative STORM images of Golgi in epithelial keratinocyte (HaCaT) cells treated with *S. aureus* EVs (top) or *S. epidermidis* EVs (bottom) at different times. Schematic diagrams are shown together. (**B**) Enlargement of the boxed region in (**A**) to compare the diffraction-limited images (bottom) and STORM images (top). Scale bars: 5 μm (top), 1 μm (bottom).

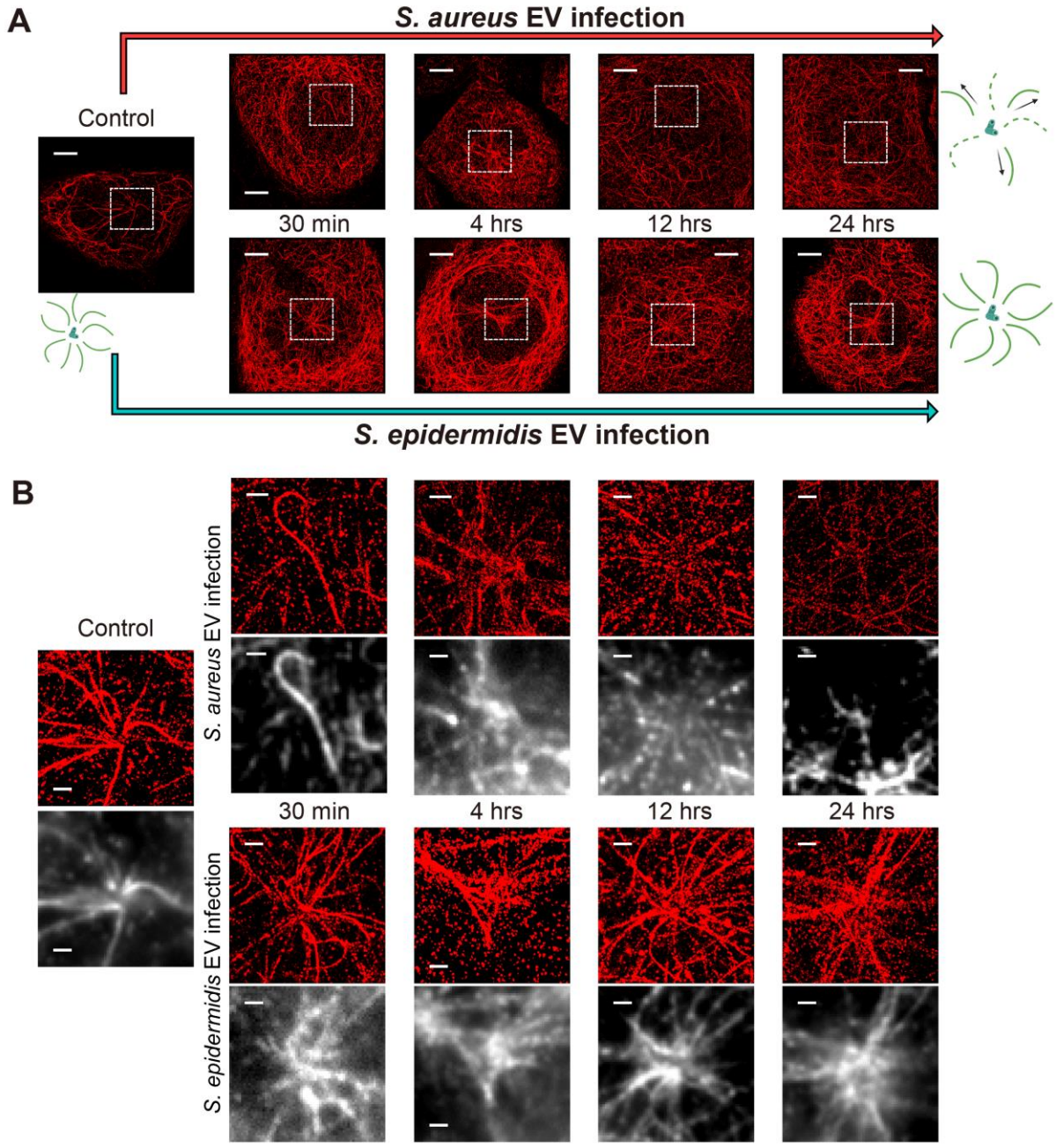


Fig. S7 Depolymerized microtubule organizing center (MTOC) by *S. aureus* EV infection. (**A**) Representative STORM images of acetylated tubulin to reveal the MTOC in epithelial keratinocyte (HaCaT) cells treated with *S. aureus* EVs (top) or *S. epidermidis* EVs (bottom) at different times. Schematic diagrams are shown together. (**B**) Enlargement of the boxed region in (**A**) to compare the diffraction-limited images (bottom) and STORM images (top). Scale bars: 5 μm (top), 1 μm (bottom).

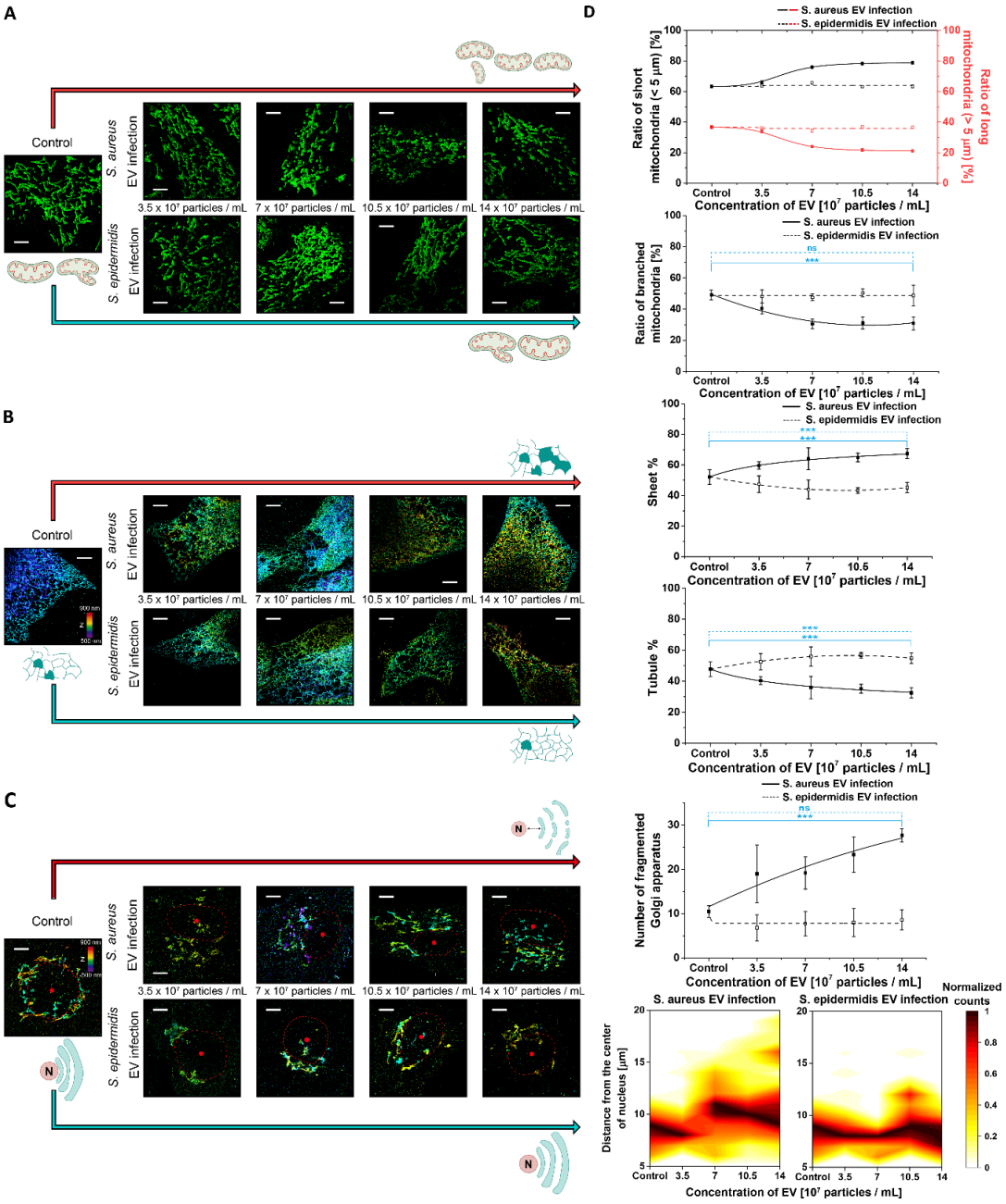
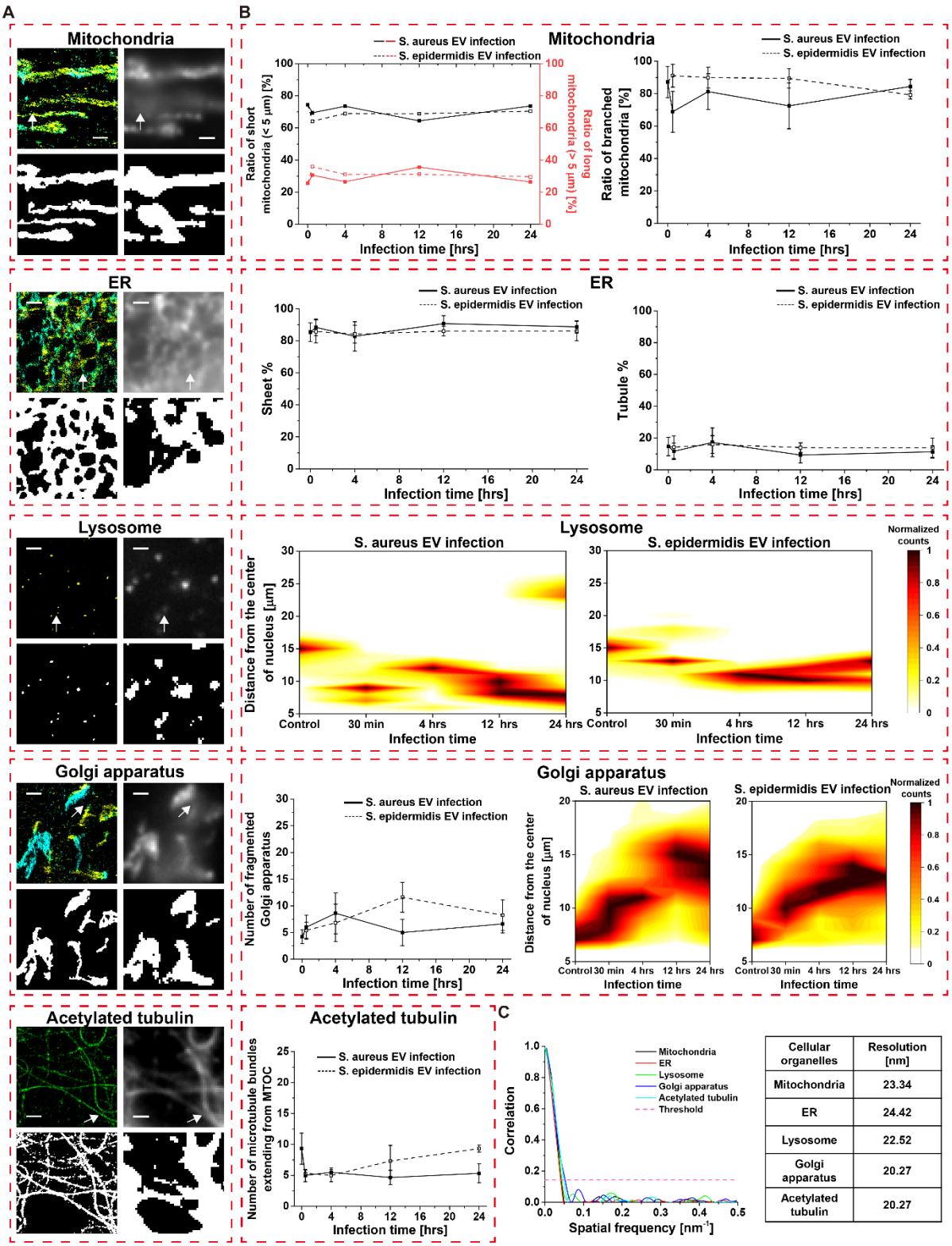


Fig. S8 Representative STORM images of (A) mitochondria, (B) ER, and (C) Golgi in epithelial keratinocyte (HaCaT) cells treated with *S. aureus* EVs (top) or *S. epidermidis* EVs (bottom) at different concentration of EVs after 30 min of EV infection. (D) Quantitative analysis of the ultrastructural changes of mitochondria in terms of the ratio of long (with > 5 μm longest diameter) and short mitochondria (with < 5 μm longest diameter) and ratio of branched mitochondria from STORM images (n= 10, mean±SD), ER in terms of the percentage of sheet-like and tubular structures (n = 10, mean±SD), Golgi apparatus in terms of the number of Golgi fragments and the radial distribution from the center of the nucleus. (n = 10, mean±SD) (ns: $p > 0.05$, ***$p < 0.001$) Scale bars: 5 μm



Cellular organelles	Resolution [nm]
Mitochondria	23.34
ER	24.42
Lysosome	22.52
Golgi apparatus	20.27
Acetylated tubulin	20.27

Fig. S9 Super-resolution imaging capability of STORM in this study. (A) Ultrastructures of various organelles revealed by STORM imaging compared with diffraction-limited fluorescence microscopy. The bottom row shows the identified boundaries of each organelle segment, indicating errors in detecting the fine structures of the

organelles due to optical blurring. Arrows: filtered background signal. Scale bar: 1 μm. (B) Quantitative analysis of the structural changes of organelles from the diffraction-limited fluorescence images corresponding to the STORM images analyzed in Figs. 1, 3, and 4. (C) Fourier ring correlation (FRC) analysis of STORM images for each organelle.

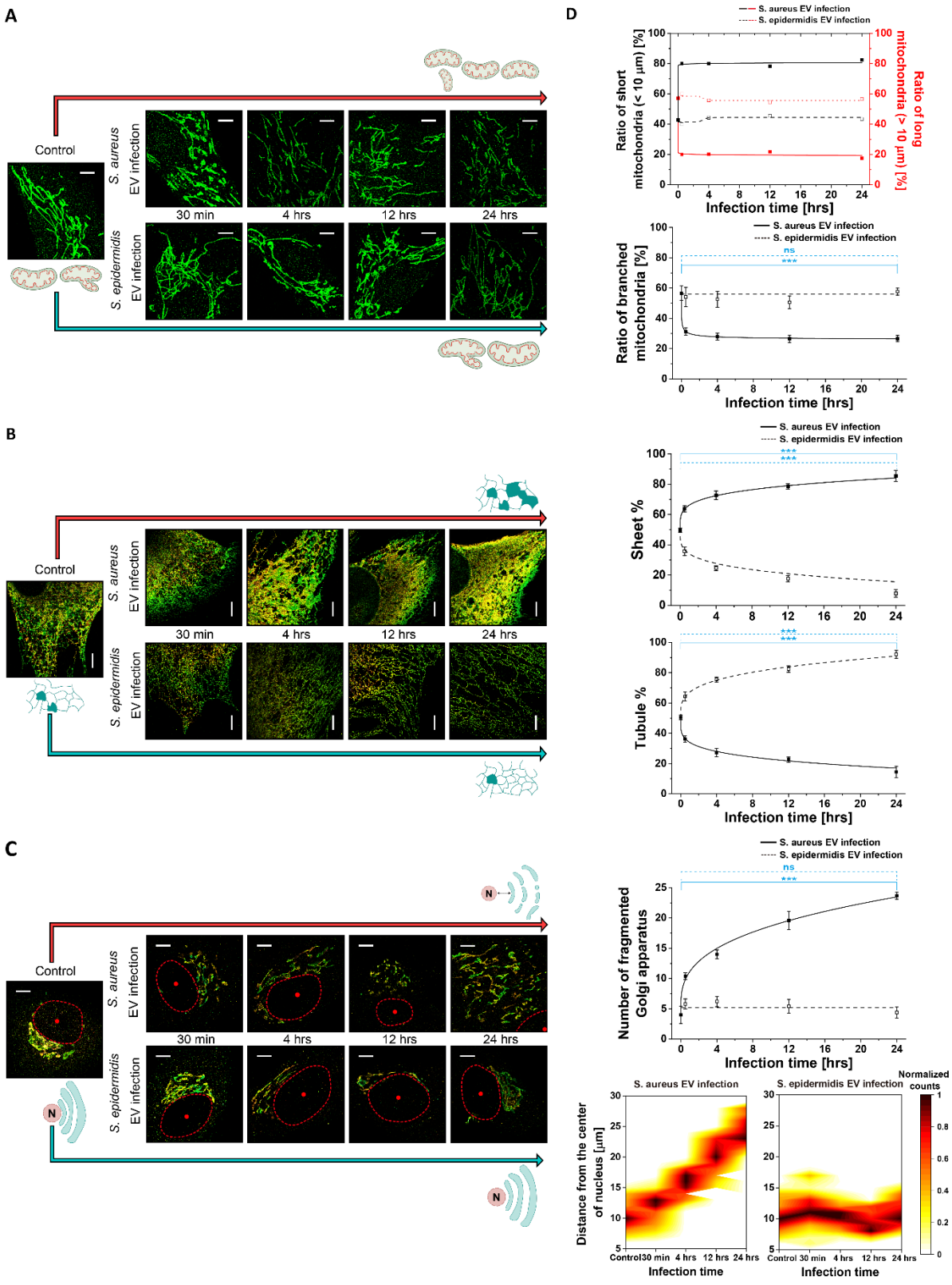


Fig. S10 Representative STORM images of (**A**) mitochondria, (**B**) ER, and (**C**) Golgi apparatus in human dermal fibroblasts (HDF) cells treated with *S. aureus* EVs (top) or *S. epidermidis* EVs (bottom) at different times. (**D**) Quantitative analysis of the STORM images of each organelle. (the ratio of long (with > 10 μm longest diameter)

and short mitochondria (with < 10 μm longest diameter) and the ratio of branched mitochondria; percentage of ER tubular and sheet-like structures; the number of Golgi fragments and radial distribution from the center of the nucleus). (n= 10, mean±SD) (ns: $p > 0.05$, ***$p < 0.001$) Scale bar: 5 μm.

Supplementary Movie 1. The 3D reconstructed HVEM movie of a representative mitochondrion in epithelial keratinocyte (HaCaT) cells as a control sample.

Supplementary Movie 2. 3D reconstructed HVEM movie of a representative mitochondrion in epithelial keratinocyte (HaCaT) cells treated with *S. aureus* EVs.

Supplementary Movie 3. 3D reconstructed HVEM movie of a representative mitochondrion in epithelial keratinocyte (HaCaT) cells treated with *S. epidermidis* EVs.

Supplementary Movie 4. The 3D reconstructed HVEM movie of a representative Golgi in epithelial keratinocyte (HaCaT) cells as a control.

Supplementary Movie 5. 3D reconstructed HVEM movie of a representative Golgi in epithelial keratinocyte (HaCaT) cells treated with *S. aureus* EVs.

Supplementary Movie 6. 3D reconstructed HVEM movie of a representative Golgi in epithelial keratinocyte (HaCaT) cells treated with *S. epidermidis* EVs.