Supplementary Information

Scanning electron-assisted dielectric microscopy reveals autophagosome formation by LC3 and ATG12 in cultured mammalian cells

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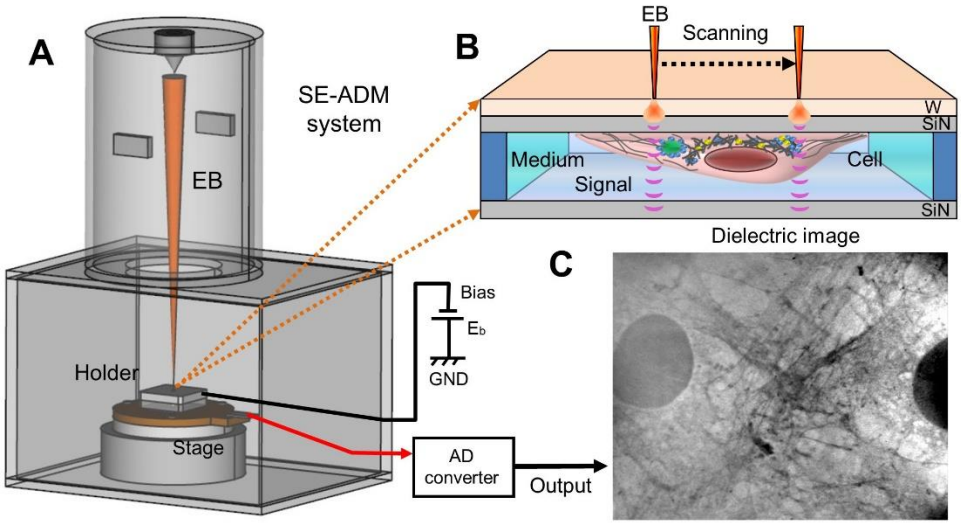
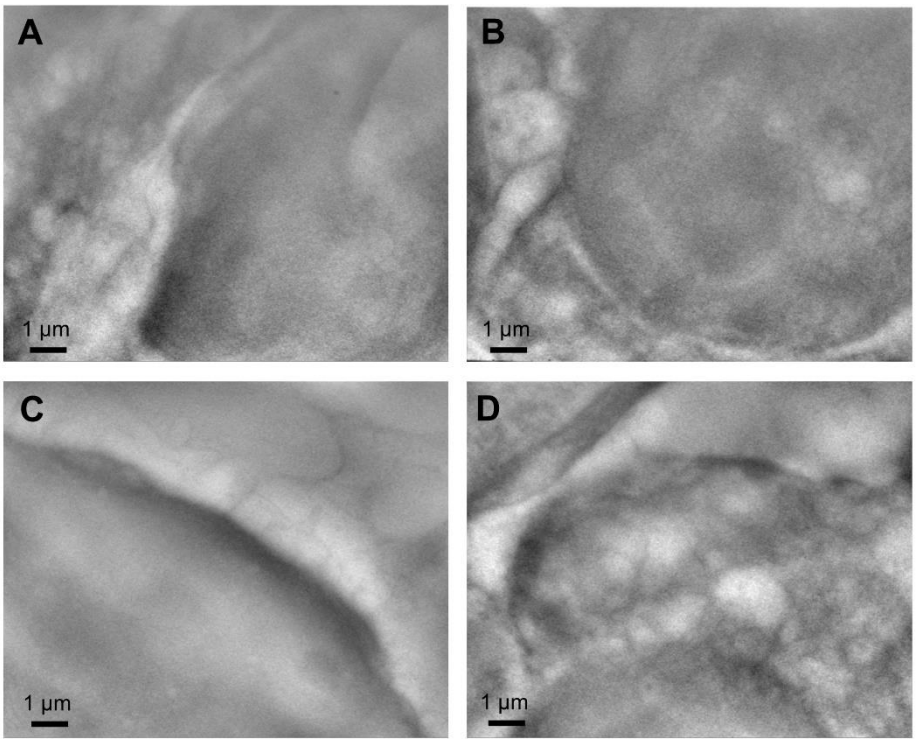


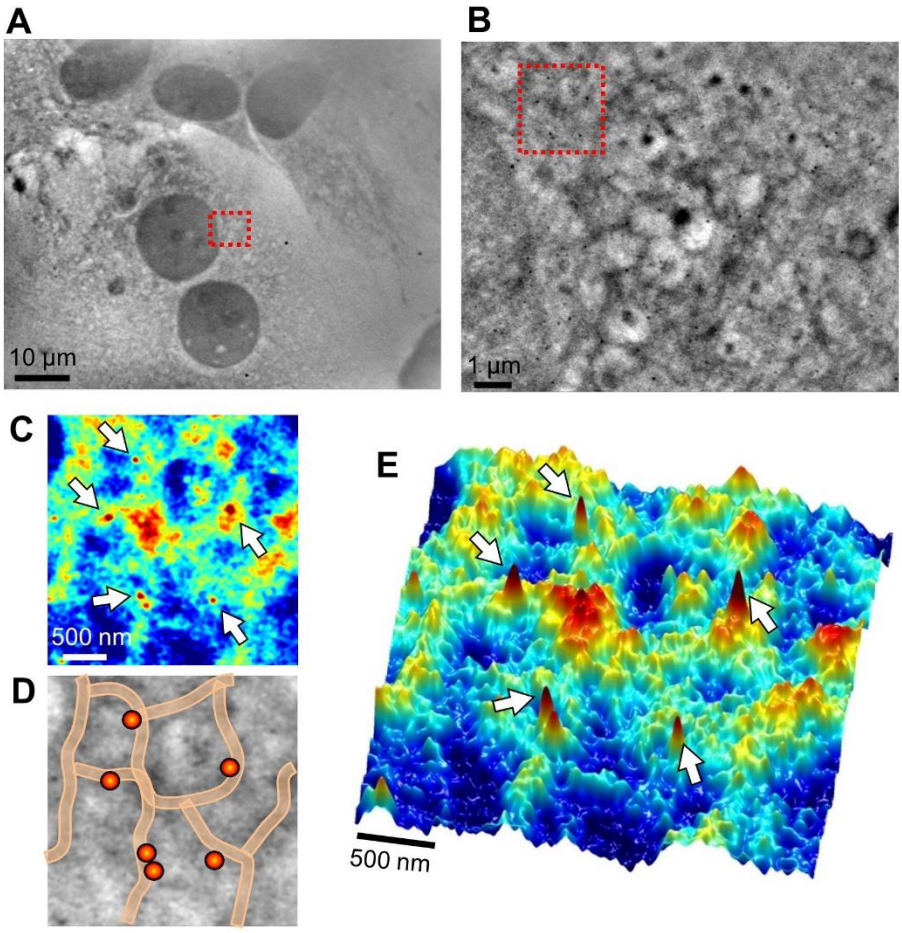
Supplementary Figure S1

Overview of autophagosome observation in cells using the SE-ADM system. (**A**) A schematic illustration of the SE-ADM system based on FE-SEM with a liquid sample holder. The sample holder containing the cultured cells is mounted on the stage attached to the pre-amplifier. This whole apparatus is introduced to the SEM chamber. (**B**) A schematic illustration of the liquid holder containing the cells and 60 nm colloidal gold particles bound to LC3 or Atg12. The W-coated SiN film is scanned with the electron beam. The measurement terminal under the holder detects the electrical signal through the cells in the liquid medium. (**C**) A typical SE-ADM image of the cells in the liquid holder.



Supplementary Figure S2

Dielectric images of 4T1E/M3 cells under normal condition stained with anti-LC3 antibody conjugated to 60 nm colloidal gold particles. (**A**–**D**) The high-magnification images of 4T1E/M3 cells stained with 60 nm colloidal gold particles after paraformaldehyde fixation and permeabilisation (10,000× magnification, 10–13 kV EB). Without the treatment of starvation, gold particles are not seen in these SE-ADM images. Scale bars: 1 µm in (A–D).



Supplementary Figure S3

SE-ADM images of REF cells stained with anti-Atg12 antibody conjugated to 60 nm colloidal gold particles. (**A**) Another low-magnification dielectric image of REF cells stained with anti-Atg12 antibody conjugated to 60 nm colloidal gold particles after paraformaldehyde fixation and permeabilisation (1,000× magnification, 6-kV EB). (**B**) A high-magnification image (10,000×) of the red framed area in (A), showing Atg12 conjugated to 60 nm colloidal gold particles. (**C**) An enlarged pseudo-colour map of the red framed area in (B). Atg12 is localised along the meshwork structures. (**D**) A schematic diagram of meshwork structures and colloidal gold particles superimposed on a dielectric image of (C). (**E**) A 3D colour map of (D). Scale bars: 10 µm in (A), 1 µm in (B), 500 nm in (C, E).