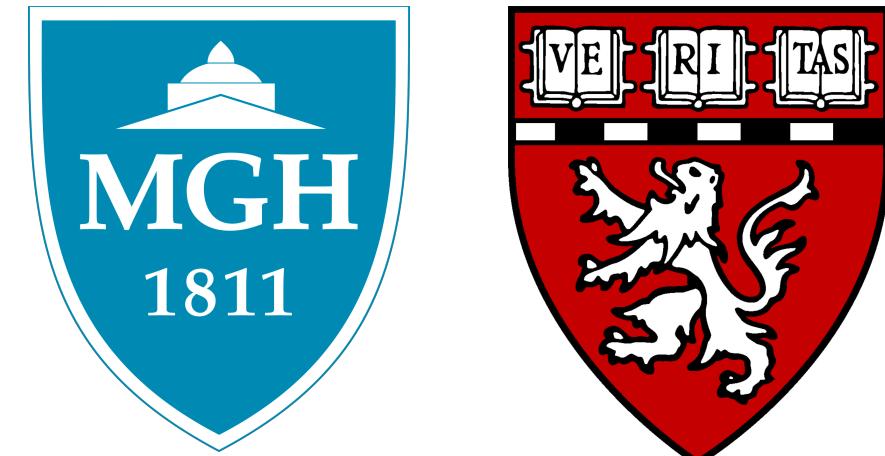


# Visualizing OPA1-Mediated Changes to Inner Mitochondrial Membrane Morphology by Cryo-Electron Tomography



Julie McDonald, Paula Navarro, Yifan Ge, & Luke H. Chao

Department of Molecular Biology, Massachusetts General Hospital; Department of Genetics, Harvard Medical School

## Introduction

Mitochondria are formed by two membranes: the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM). Maintenance of IMM structure is critical in regulating cell metabolism. OPA1, a dynamin family GTPase, is a key player in IMM organization. OPA1 is responsible for both membrane fusion and maintenance of cristae membranes, invaginations of the IMM where respiratory chain complexes reside. Previous studies have shown that disruption of OPA1 expression results in fragmentation of the mitochondrial network, irregular cristae morphology, and cellular susceptibility to apoptosis [1,2].

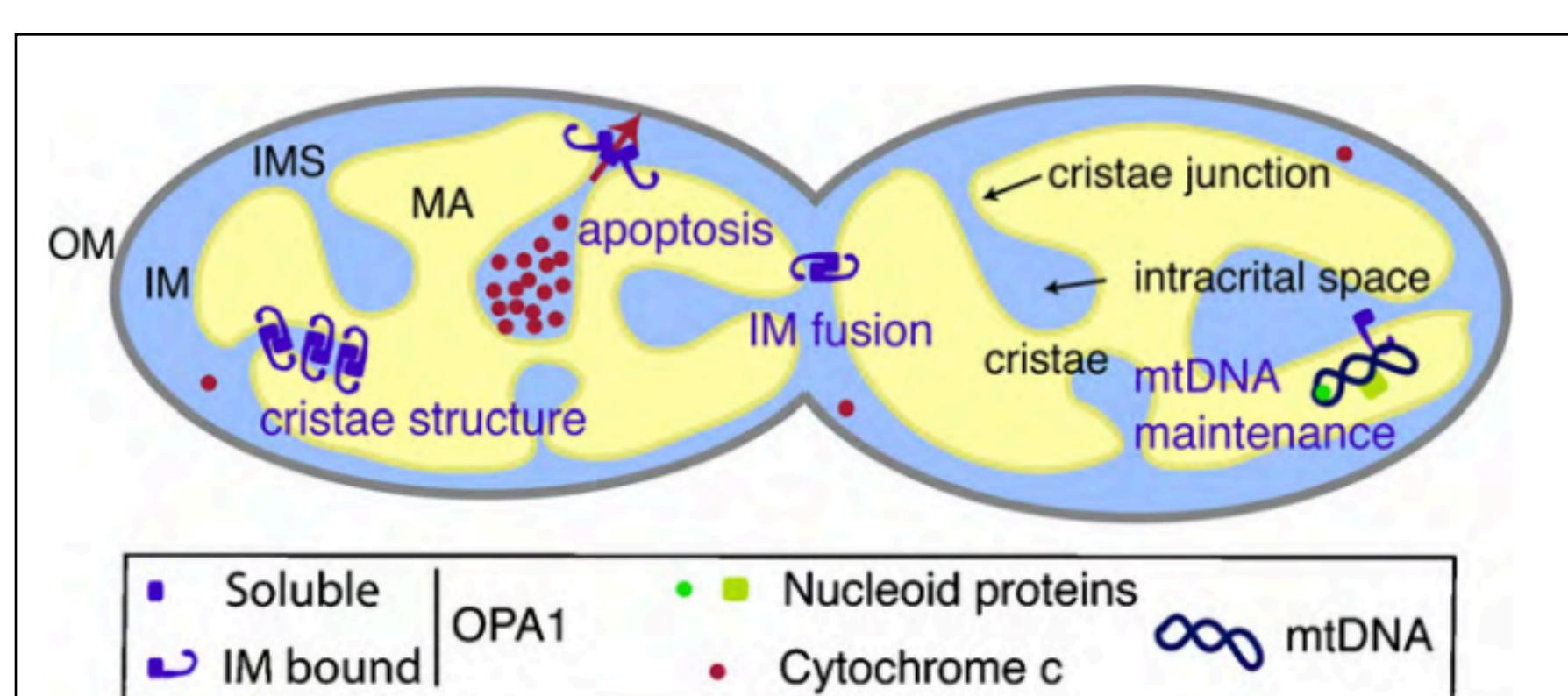


Fig. 1. OPA1 functions in mitochondria.

Transmembrane and soluble forms of OPA1 work together to complete mitochondrial fusion, secure cristae junctions, and maintain mitochondrial DNA. From [1].

We apply *in situ* cryo-electron tomography (cryo-ET) and sub-tomogram averaging to study the shape of the IMM under different protein states. This allows us to probe the effects of varying expression levels of OPA1 on membrane structure.

## Experimental Approach

1. Culture/deposit cells under different protein expression states on coated gold grids.
2. Plunge freeze grids
3. Generate cell lamellae using cryo-focused ion beam (cryo-FIB) milling
4. Visualize native mitochondria by cryo-ET

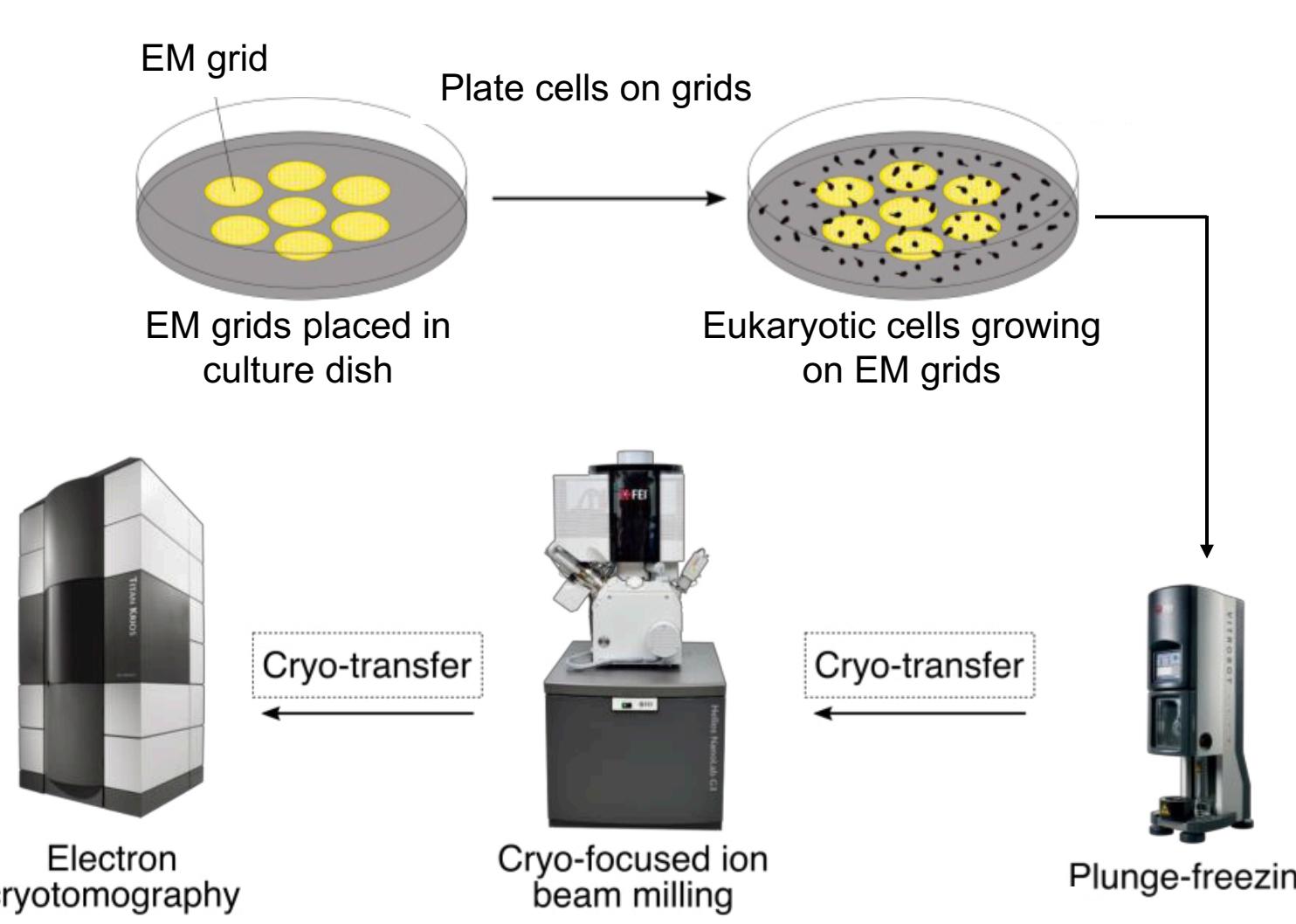


Fig. 2. Cryo-FIB / Cryo-ET pipeline for imaging eukaryotic cells. Adapted from [3].

## Cryo-Focused Ion Beam Milling

During cryo-focused ion beam (cryo-FIB) milling, a gallium ion beam is used to mill excess layers of sample in a vitrified target, leaving 100-300 nm-thick lamella that can be subsequently imaged by cryo-EM. Cryo-FIB milling overcomes the sample thickness limit, enabling cryo-ET on eukaryotic cell cytoplasm and tissue [4].

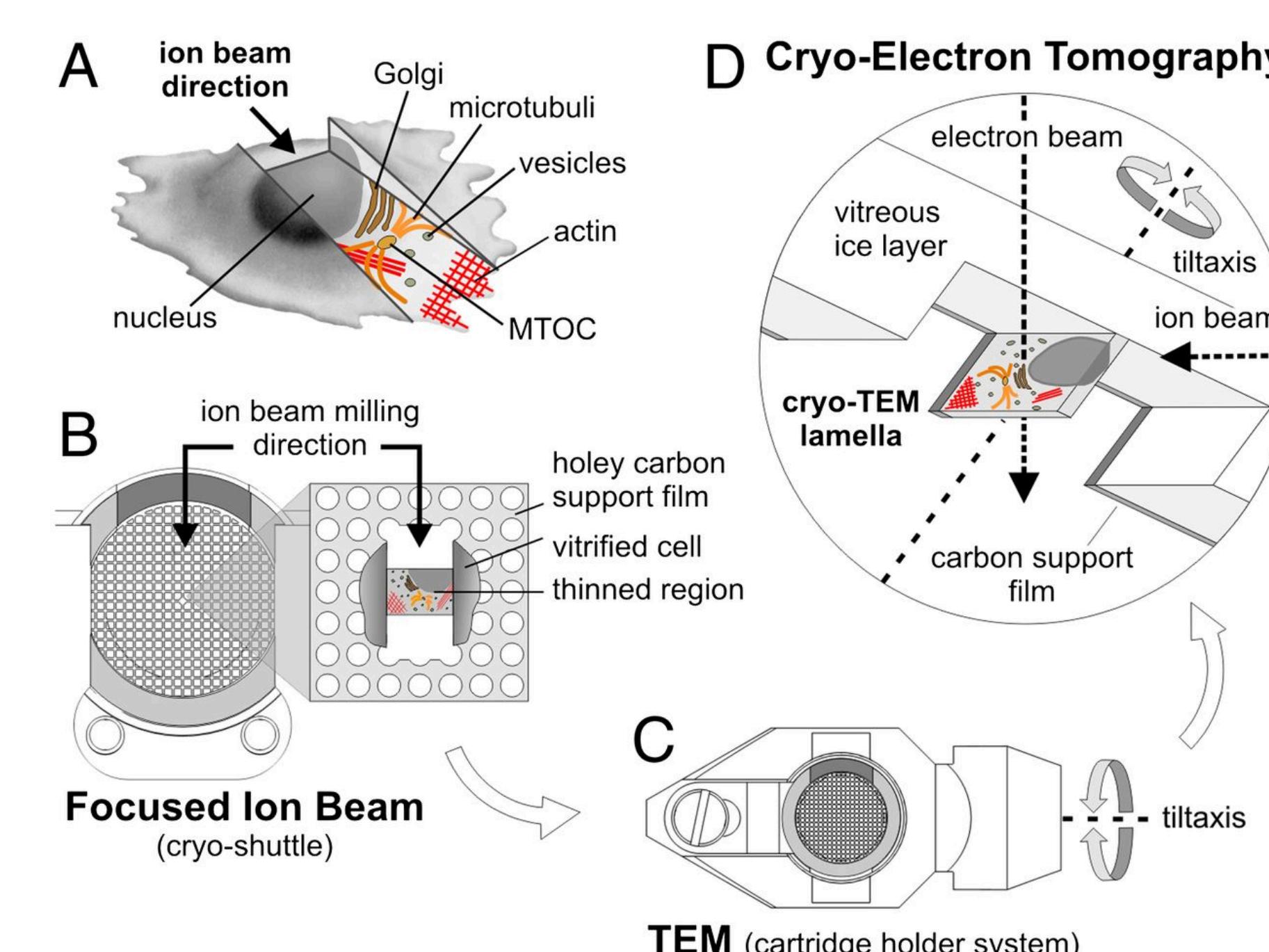


Fig. 3. *In situ* lamella preparation method.

(A) Cartoon illustrating the thinning of an adherent eukaryotic cell by FIB milling. (B) Transmission electron microscope (TEM) lamella preparation on an EM grid. (C) The grid, containing the *in situ* lamella, is transferred into a TEM holder with respect to the required tilt axis geometry. (D) TEM imaging of the lamella. From [4].

## Cryo-Electron Tomography

Cryo-ET allows for 3D visualization of vitrified biological samples, such as protein complexes and cell organelles, in near-native environments at nanometer scale. Inside a TEM microscope, the specimen is rotated around a single axis perpendicular to the optical axis of the electron beam. Images are collected each rotation angle, resulting in a tilt series. The tilt series are then computationally aligned and reconstructed into 3D tomograms.

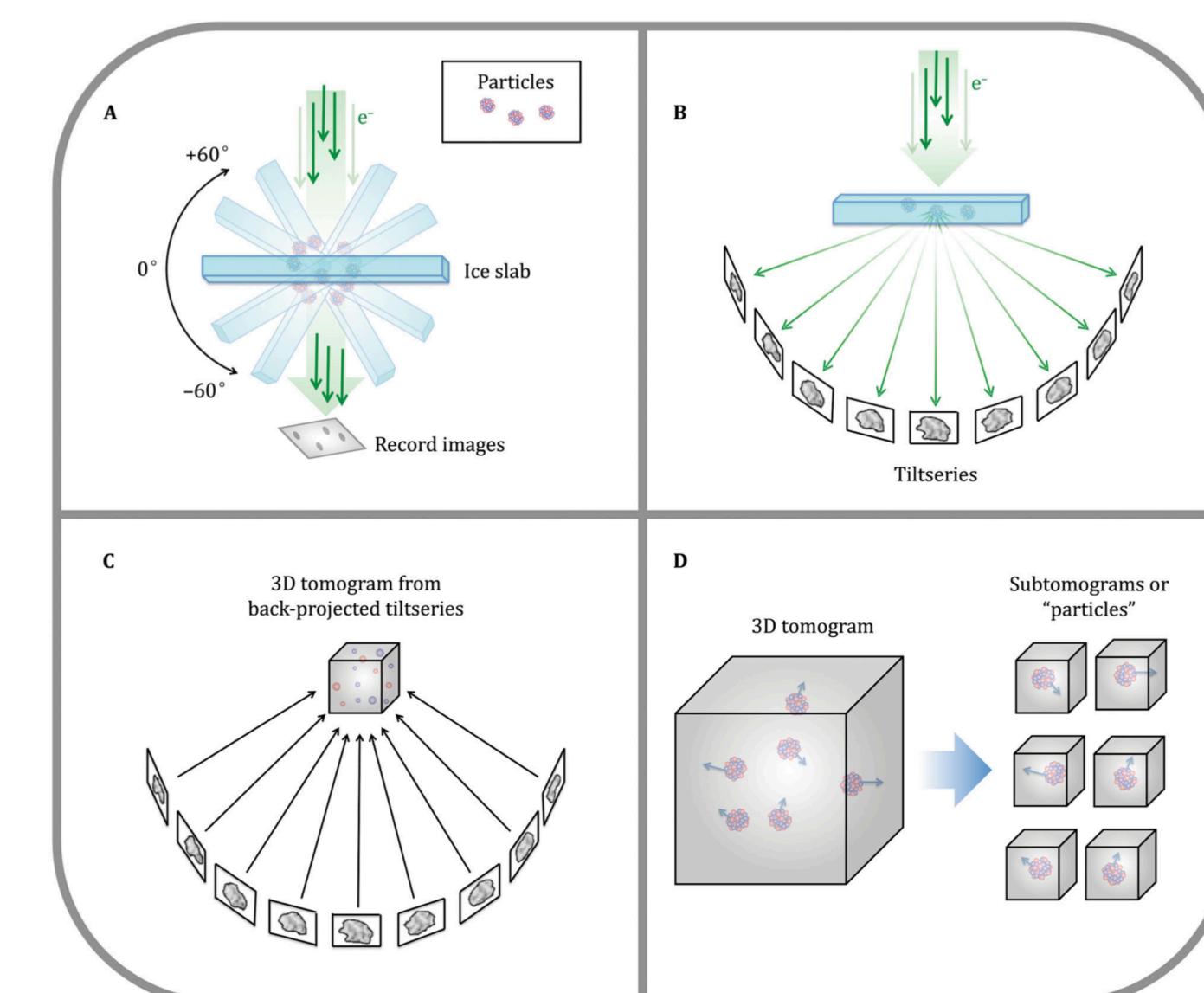


Fig. 4. Cryo-ET schematic.

In cryo-ET, the sample is tilted over a wide range of angles and images are recorded at each tilt angle. These images are combined to create a tilt series, which can be computationally aligned and reconstructed to create a three-dimensional tomogram. From [5].

## Results

Here, we apply cryo-FIB on OPA1 overexpression HEK293 cells to obtain thin lamellae of their cytoplasm.

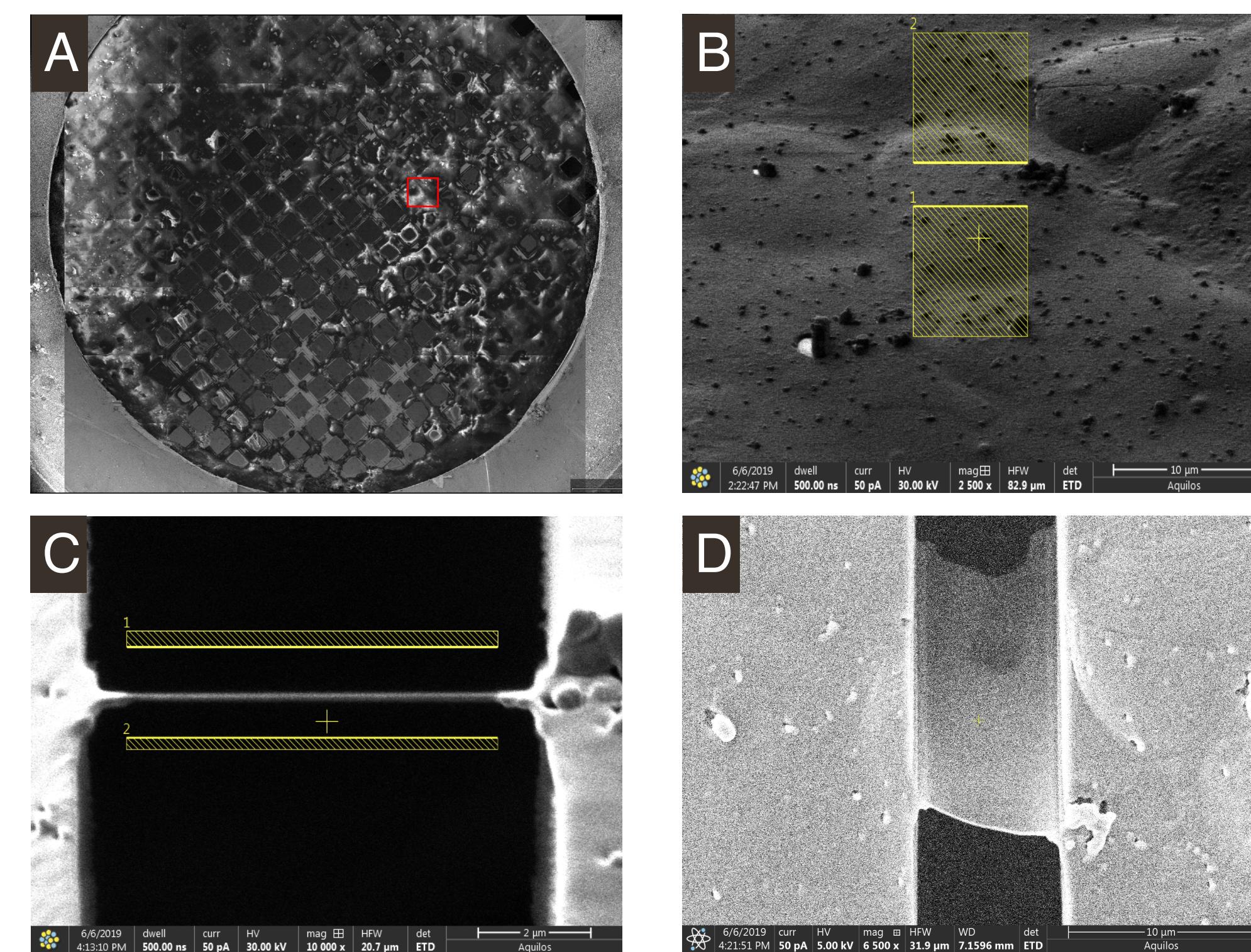


Fig. 5. Cryo-FIB milling of HEK293 cells.

(A) Scanning electron microscope (SEM) overview image of HEK293 cells on EM grid. Red box indicates lamella milling site. (B) Cell indicated in (A) imaged with the FIB. Yellow boxes show the milling pattern where the beam will concentrate. (C) Milled lamella imaged with the FIB. Final thickness = 140 nm. (D) Top-down view of the same lamella imaged with the SEM.

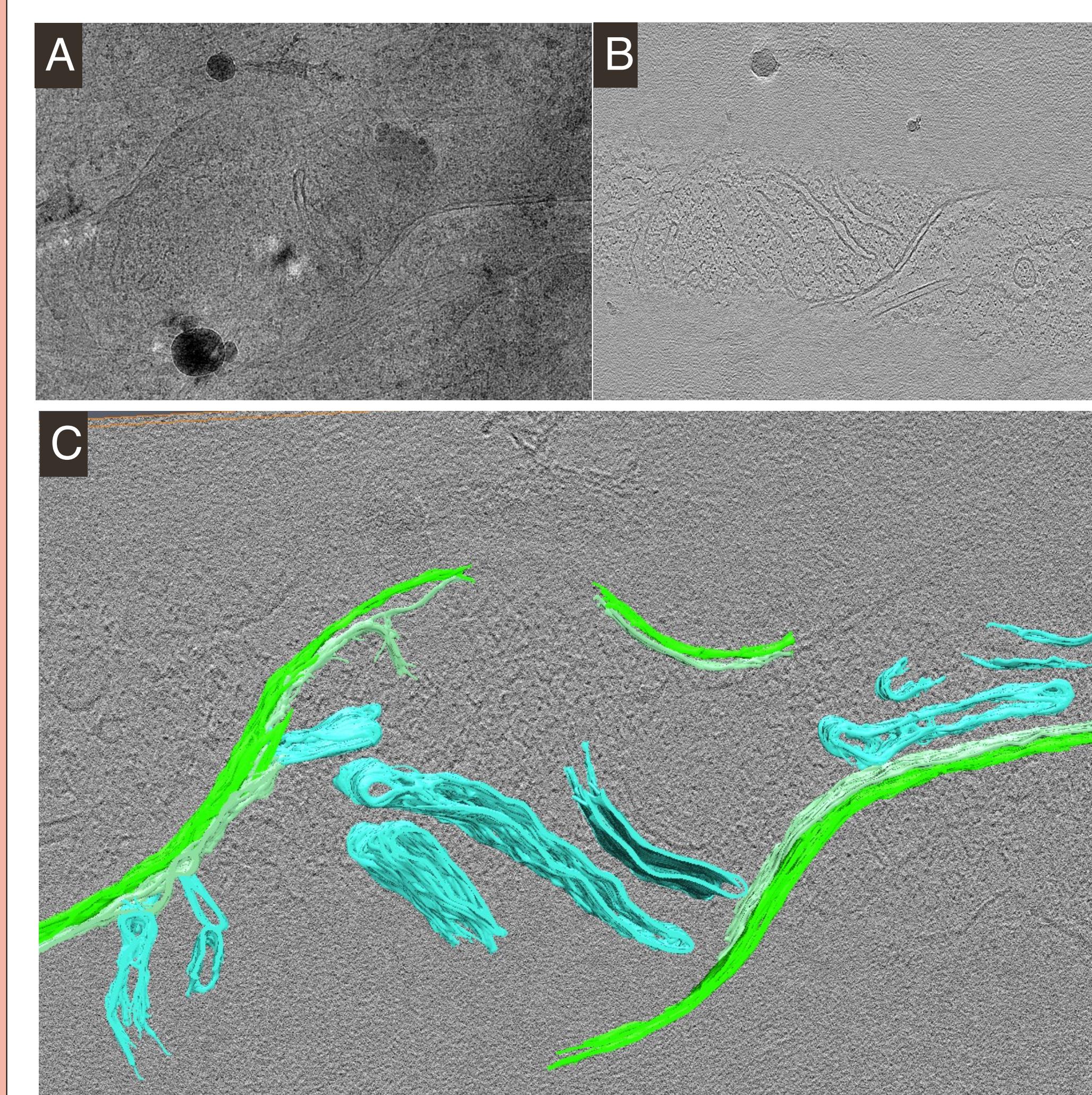


Fig. 6. Tomographic reconstruction of mitochondria from COS-7 cells overexpressing OPA1.

(A) Aligned tilt series, screenshot taken at zero tilt  
(B) Reconstructed tomogram  
(C) Segmented tomogram illustrated with Thermo Scientific Amira. OMM is displayed in green and IMM is displayed in blue.

## Troubleshooting

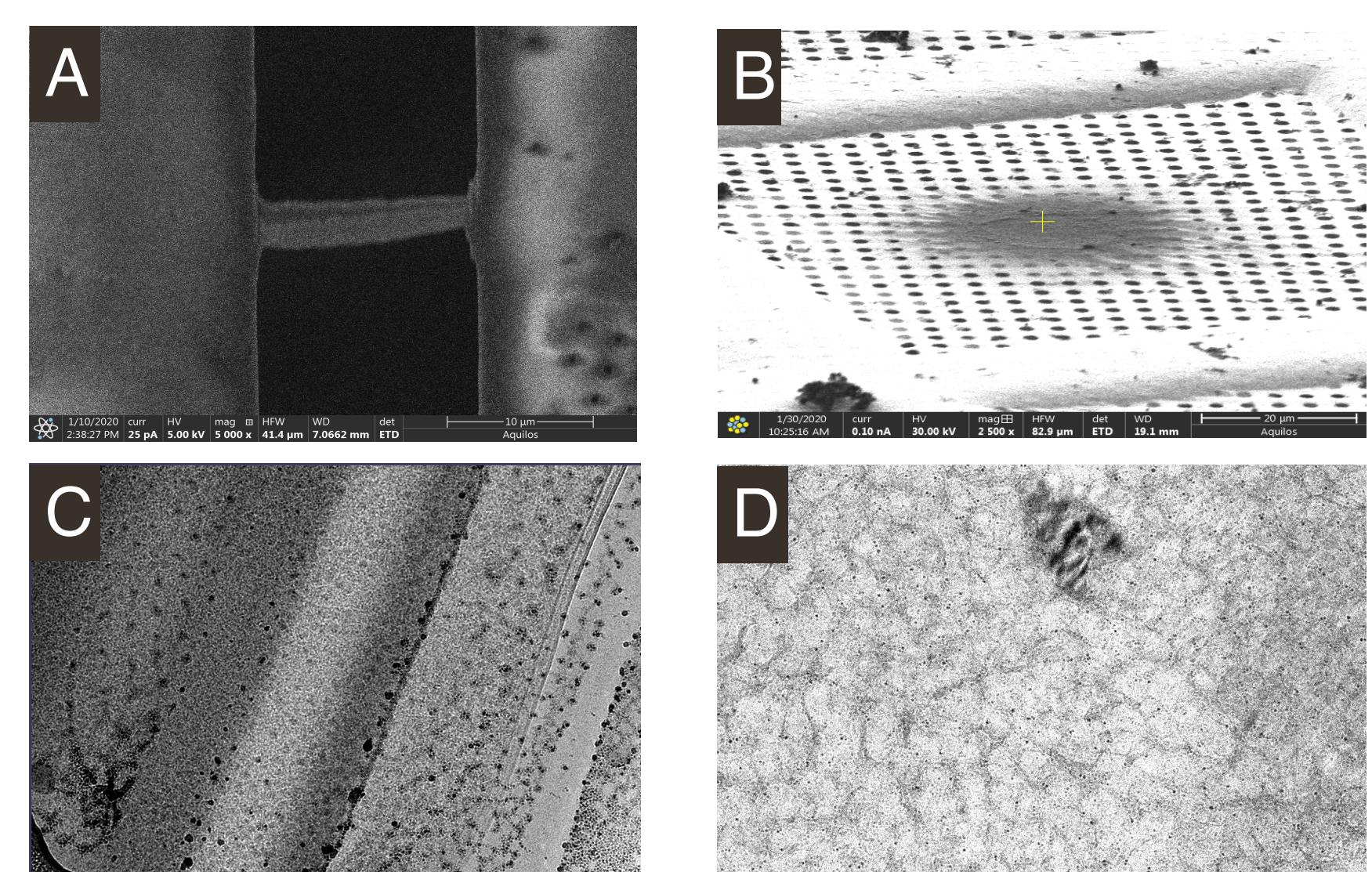


Fig. 7. Cryo-FIB and cryo-ET troubleshooting. (A) Shortened cell lamella after FIB milling (B) Cellular residue from poor freezing conditions (C) Platinum and ice contamination of lamella viewed in TEM (D) Devitrification of lamella viewed in TEM.

## Conclusions

Currently, cryo-ET is the only technique that allows visualization of intact cell ultrastructure in 3D at nanometer resolution and in near-native contexts. We are able to reproducibly freeze and mill mammalian cells to image mitochondria using cryo-ET. We expect that these studies will help to understand the molecular basis of mitochondrial ultrastructure, coupling structure to function. Importantly, knowledge on the basic determinants for the functional organization of mitochondria will answer fundamental questions on mitochondrial biogenesis and its disease-related phenotypes.

## Key References

1. Landes, et al. *J. Biol. Chem.* 2003, 278(10), 7743–46.
2. Frezza, C., et al. *Cell.* 2006, 126(1), 177-189.
3. Böck et al. *Science.* 2017 Vol. 357, Issue 6352, pp. 713-717
4. Rigot et.al. *PNAS.* 2012. 109 (12) 4449-4454.
5. Galaz-Montoya and Ludtke. *Biophys Rep.* 2017;3(1):17-35

## Acknowledgements

The authors would like to thank:

- The MGH Department of Molecular Biology and the Hood Foundation for financial support.
- The Harvard Cryo-Electron Microscopy Center for Structural Biology, the Cryo-Electron Microscopy Center at MIT.nano, and the Cryo-EM Facility at UMASS Worcester for facility support.