

High-Resolution Analysis of Organelle Structures and Contacts through Correlative Light and Electron Microscopy

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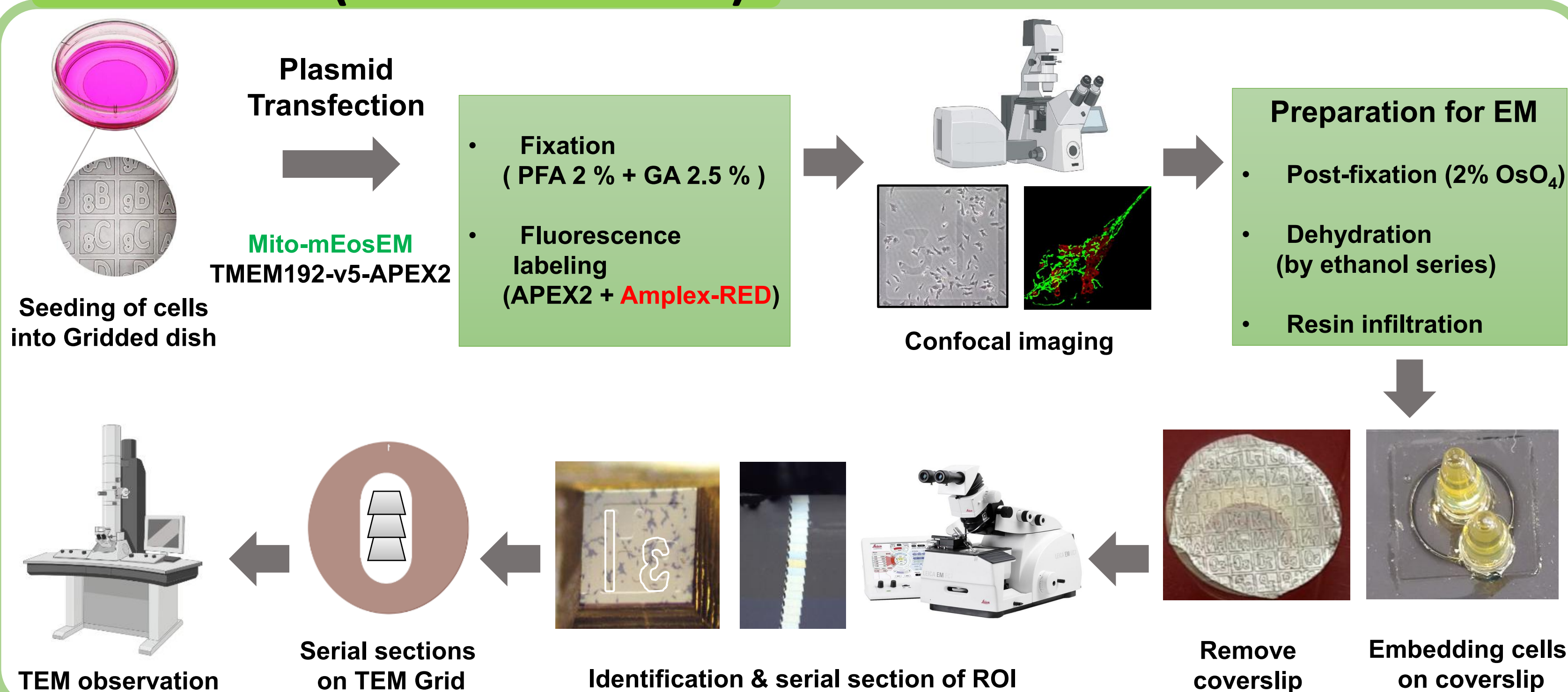
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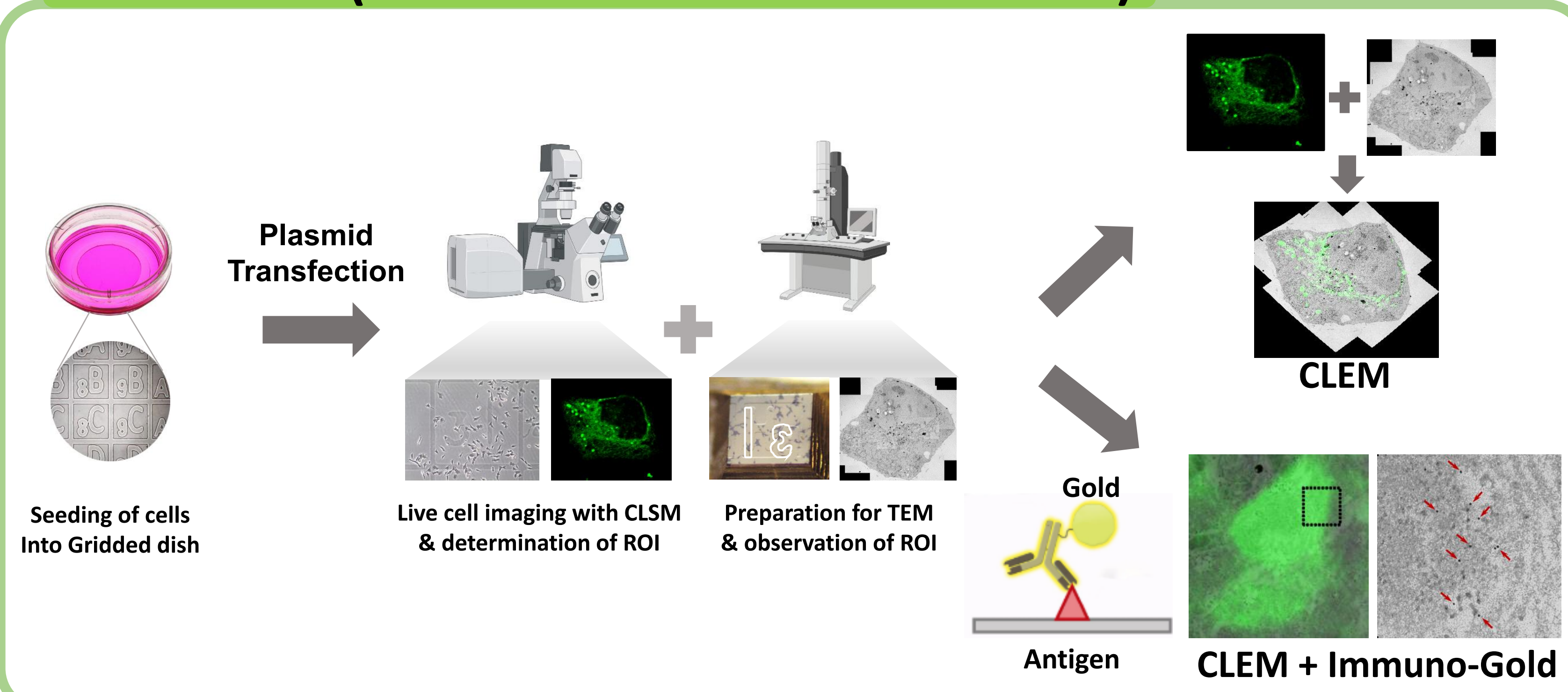
Abstract

Understanding the structural basis of organelle interactions is essential for elucidating cellular functions and maintaining homeostasis. Correlative light and electron microscopy (CLEM) combines the molecular specificity of fluorescence microscopy with the ultrastructural resolution of electron microscopy. This enables precise visualization of subcellular architecture and dynamic organelle contacts. In this study, we used a CLEM approach to examine the spatial organization and interactions of mitochondria, the endoplasmic reticulum (ER) and lysosomes in normal and stressed conditions. Fluorescent labelling enabled the identification of organelles, which were then examined using high-resolution EM imaging for structural analysis. Using this workflow, we visualized mitochondrial-lysosomal contacts and ER membrane remodeling, including tubular ER structures induced by ER stress. Our results demonstrate the effectiveness of CLEM in capturing fine morphological details and contact sites between organelles, offering valuable insights into the cellular response to stress. This imaging strategy offers a powerful platform for studying organelle interactions with high spatial accuracy, bridging molecular dynamics and structural context.

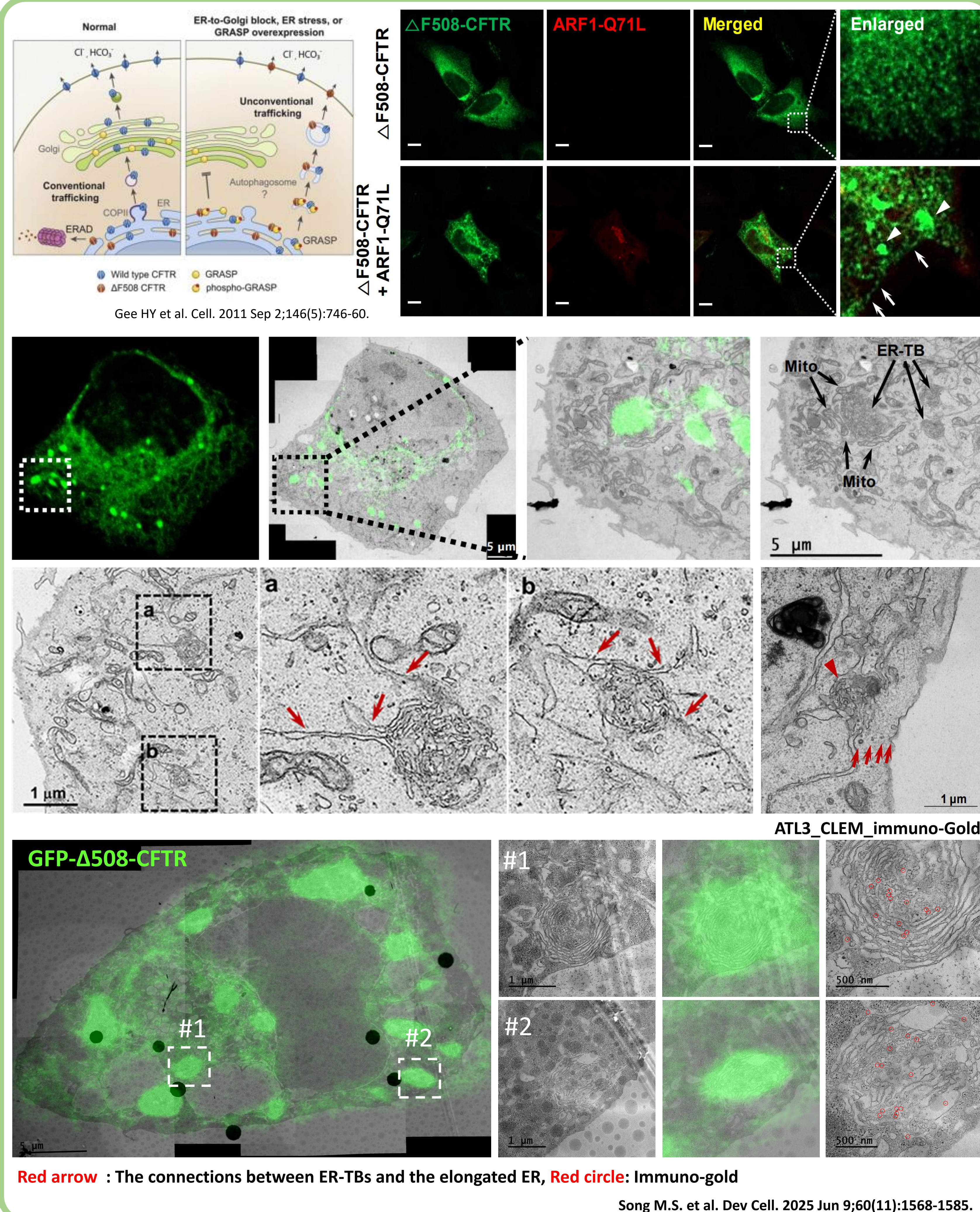
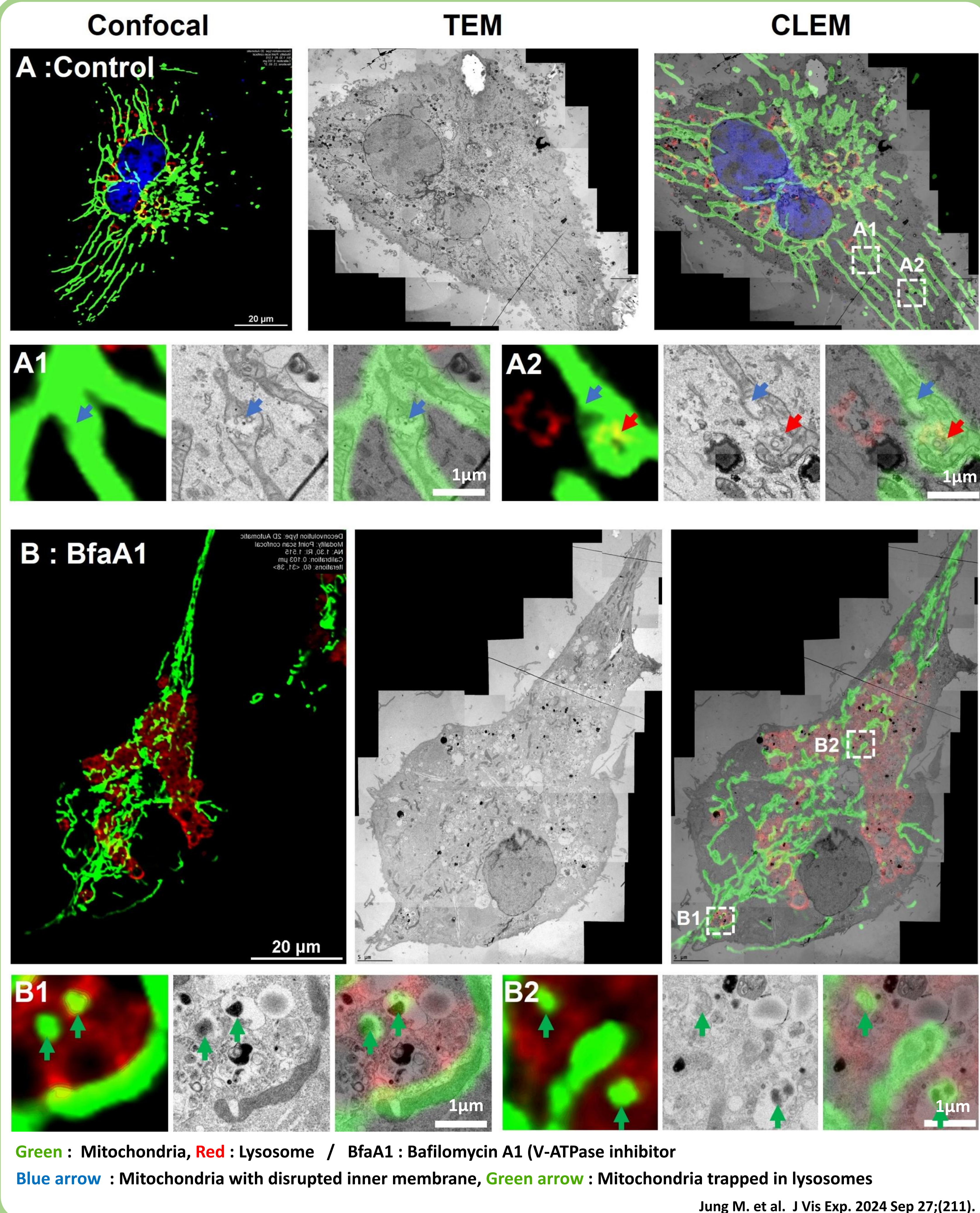
Methods I (Fixed cell CLEM)



Methods II (Live cell CLEM + Immuno-Gold)



Results



Discussion

- Combining Mito-mEosEM and TMEM192-V5-APEX2 (with an Amplex-Red probe) enables the detection of fluorescent signals from cells that have been **fixed using strong fixatives**.
- The use of dual-color CLEM enabled the **precise distinction of mitochondria** that had become **trapped within lysosomes**.
- We have identified a tubular ER structure (ER tubular body [ER-TB]) using correlative light-electron microscopy (CLEM) analyses.
- The combination of **live cell imaging, CLEM, and immunogold labeling** has enabled highly accurate, high-resolution localization of specific proteins in the same sample.