

Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons and the pathological accumulation of α -synuclein (α -syn) aggregates, which form Lewy bodies. To gain deeper insight into the ultrastructural features and cellular effects of α -syn aggregation, we used a midbrain-striatum assembloid model that recapitulates key aspects of PD pathology in a three-dimensional, human-relevant context. To investigate the structural and functional connectivity within the assembloid model, we used large-area correlative light and electron microscopy (CLEM) combined with scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to visualise α -syn aggregates with high spatial precision. CLEM combines the wide-field capabilities of light microscopy with the high-resolution power of electron microscopy, enabling precise analysis of α -syn aggregation patterns, their intracellular localization, and their impact on organelle integrity and neural network connectivity. Our results reveal that α -syn aggregates are frequently associated with organelle damage and impaired neural connectivity, suggesting their direct involvement in the neurodegenerative process. These findings underscore the power of SEM-TEM-based CLEM in elucidating the structural dynamics of PD pathology and highlight its potential for identifying novel therapeutic targets.

Results

Structural analysis of α -syn in SH-SY5Y A53T cells using CLEM technique

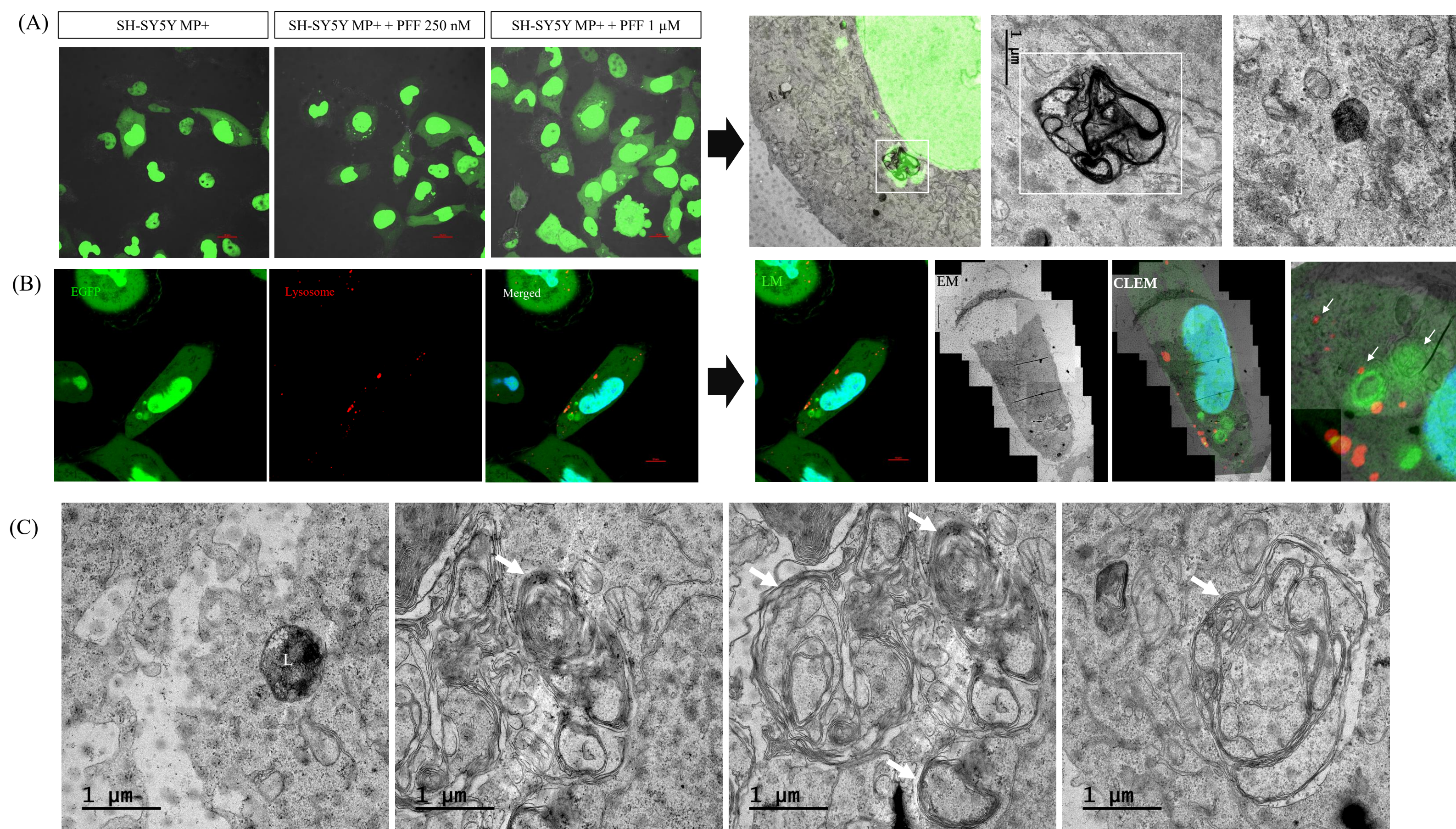


Figure 1. Analysis of α -syn aggregation in SH-SY5Y A53T-EGFP cells after PFF treatment using CLEM. (A-B) Representative CLEM images showing α -syn aggregates in SH-SY5Y cells treated with PFF for 24hr (green : α -syn, red : lysosome, blue : DAPI). (C) High magnification TEM image showing the ultrastructural feature of α -syn aggregates in the cells (L : lysosome, red arrow : α -syn aggregates). The cells and PFFs were provided by Professor Sangmyun Park's lab, Ajou University School of Medicine.

Large-area visualization of assembloid using SEM

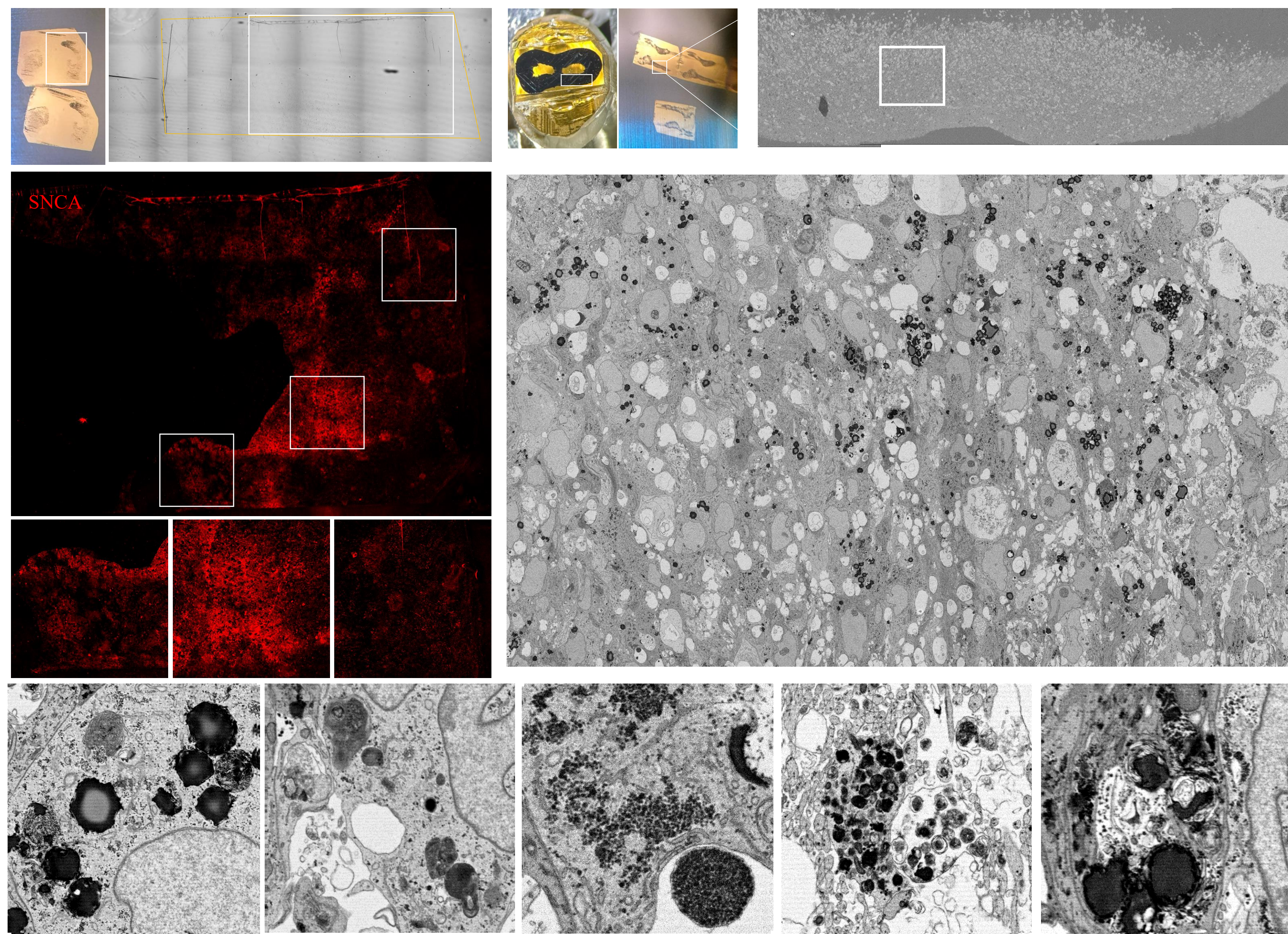


Figure 2. Large area image observation of α -syn accumulation in PFF-injected assembloid using SEM. Assembloid: Sample injected with PFF into the mid-brain. Original SEM image : 39003px X 24291px. 7Gb, imaging time : 6 hr

Large image CLEM of injected with PFF in midbrain and SNCA overexpression assembloids

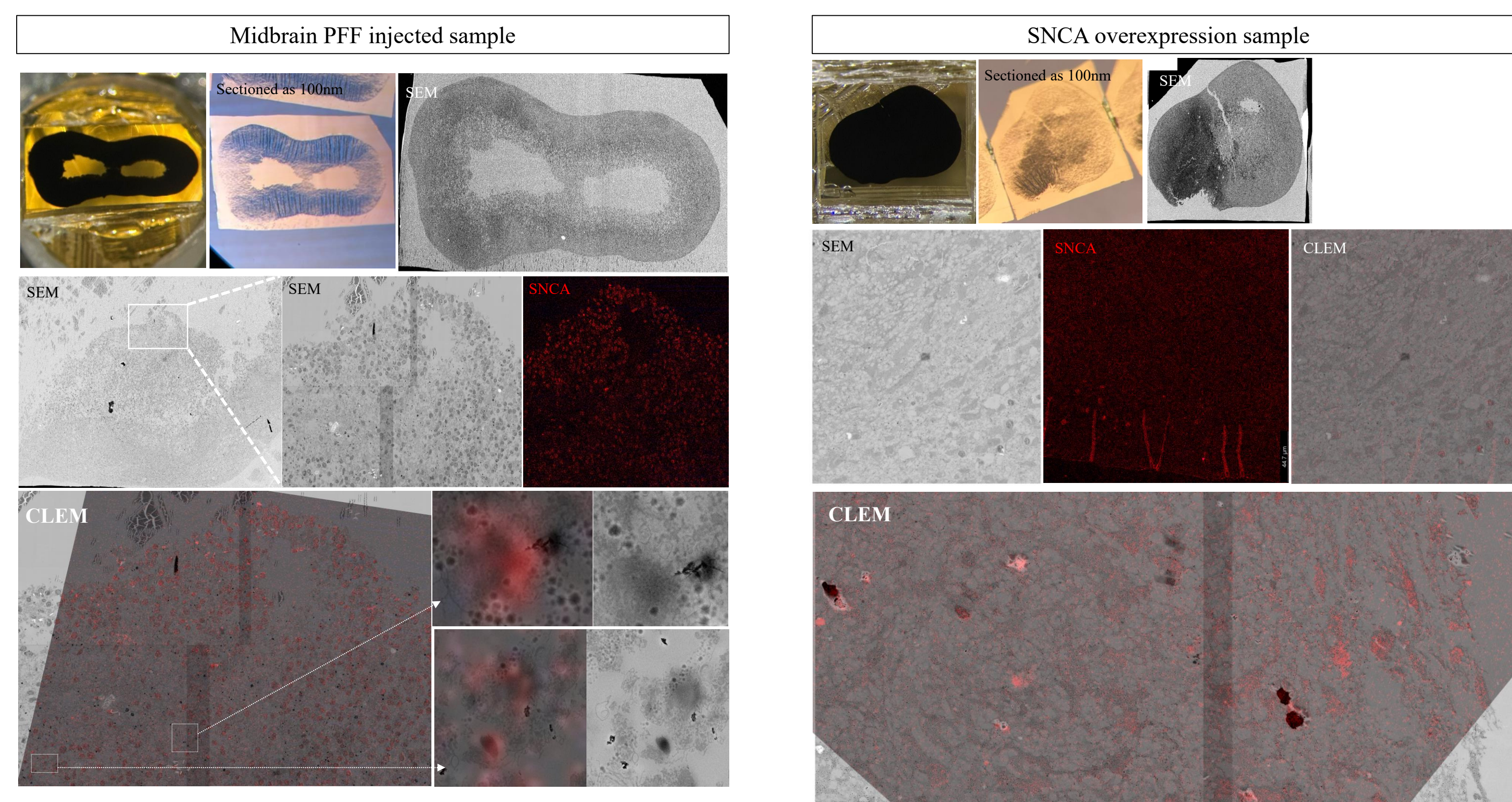
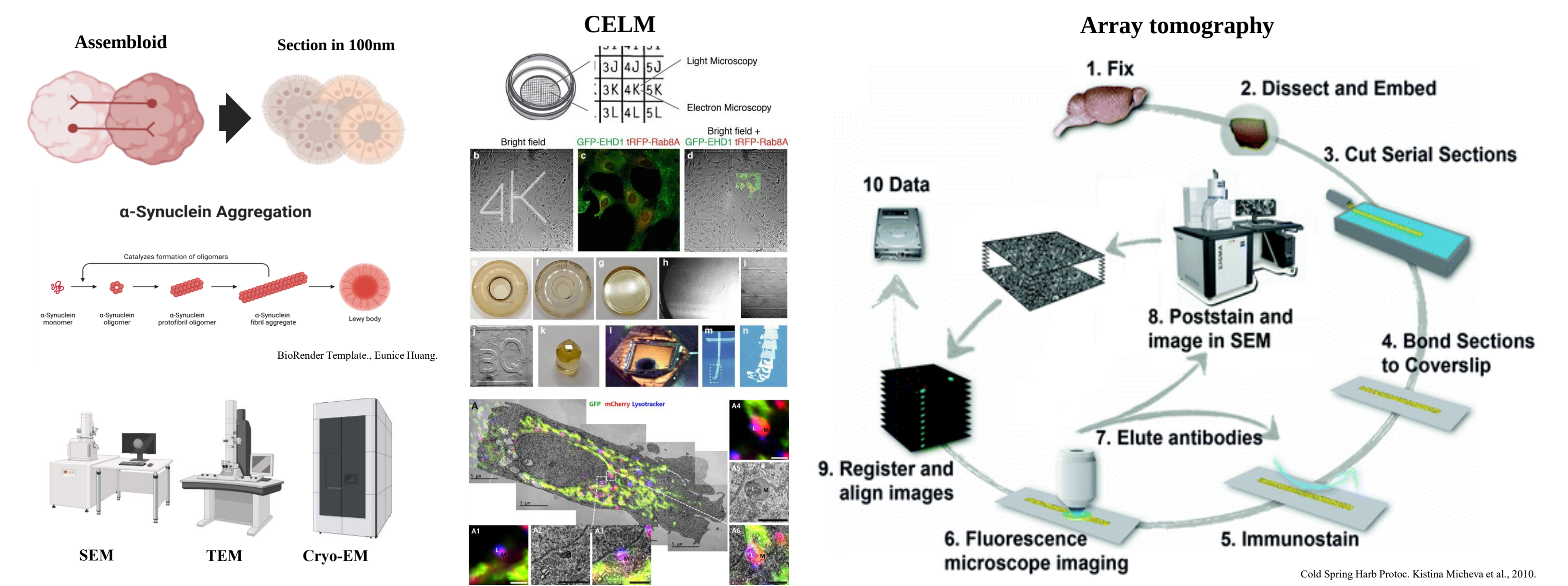


Figure 3. Comparison of α -syn aggregation in midbrain injected with PFF and SNCA OE assembloid using large-area CLEM.

Material and Method



- **SEM and TEM** : Uses an electronic source and an electronic aperture; uses a series of electromagnetic and electrostatic lenses that control the shape and trajectory of the electron beam. However, The main difference between SEM and TEM is that SEM produces images by detecting reflected or knocked-out electrons, whereas TEM uses transmitted electrons (electrons that pass through the sample) to produce images. As a result, TEM provides important information about the internal structure of a sample, such as crystal structure, morphology, and stress state information, while SEM provides information about the surface and composition of a sample.
- **Array tomography** : used to obtain high-resolution, 3D reconstructions of biological samples by imaging ultrathin serial sections. This technique combines imaging modalities like fluorescence and electron microscopy for comprehensive molecular and structural analysis.
- **Cryo-electron microscopy** : Uses an ion beam to create thin lamellae, which are then rapidly frozen at cryogenic temperatures below minus 180 degrees Celsius to preserve the original structure and rotated for more accurate three-dimensional imaging.
- **CLEM** combines the two powerful techniques of light microscopy and electron microscopy to obtain detailed information about intracellular organelles from fluorescently labelled live or fixed cells.

Results

Structural analysis of α -syn aggregates and Lewy body-like structures using TEM and SEM

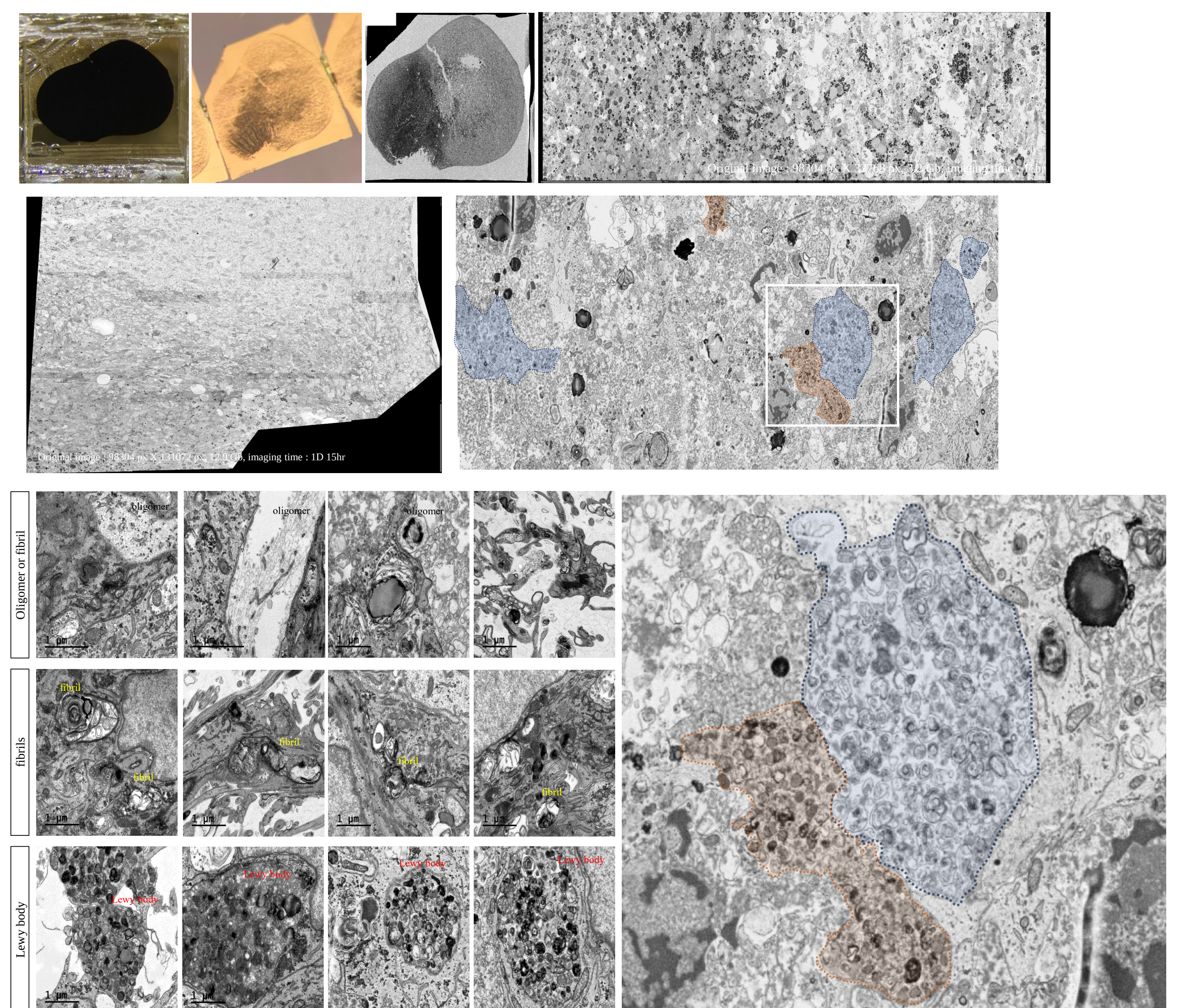


Figure 4. Comparison structural changes of α -syn aggregates and Lewy body-like structure in the SNCA OE assembloid using TEM and SEM.

Structural analysis of Lewy body-like structure using Cryo-tomography

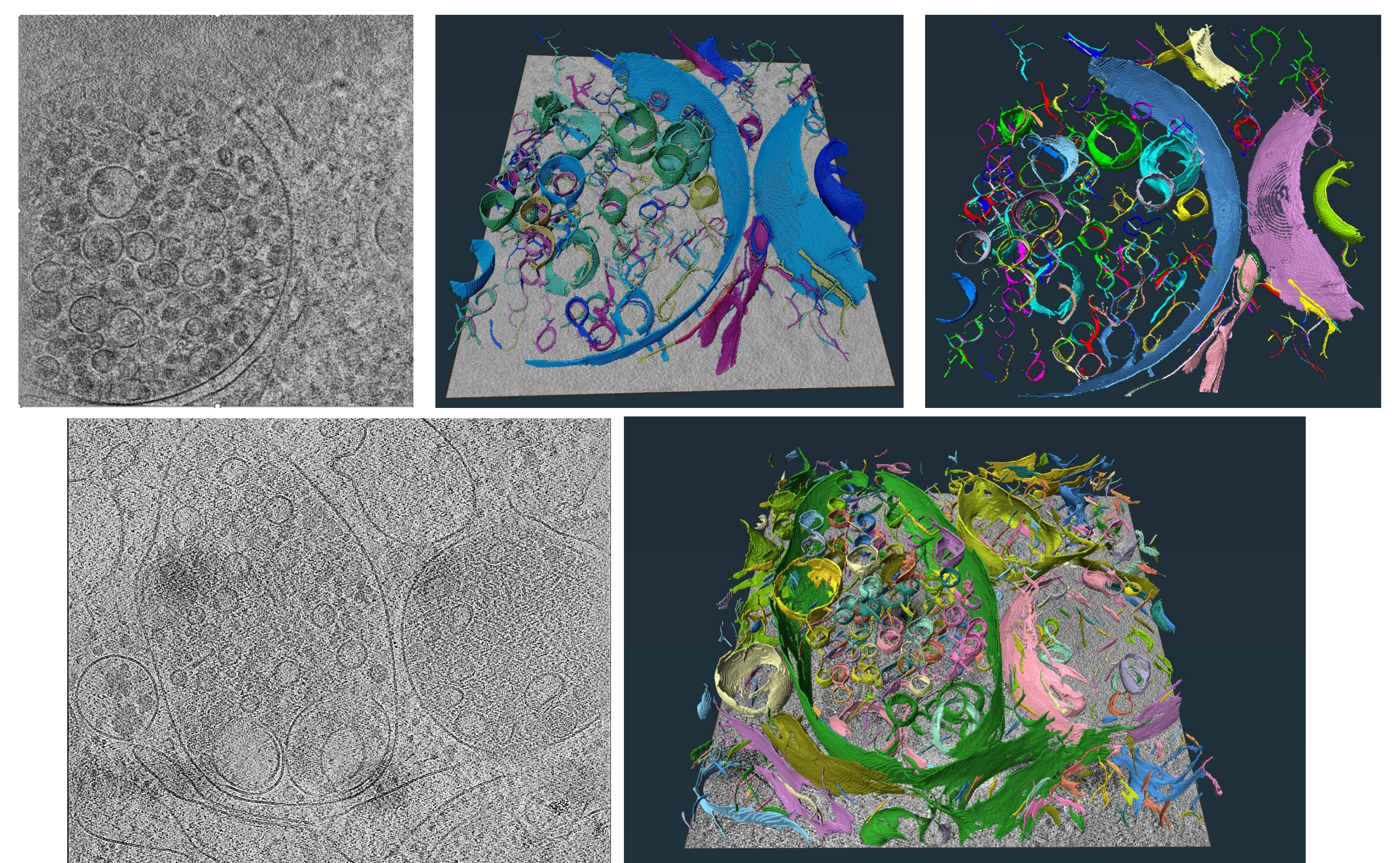


Figure 5. Characterization of Lewy body-like structure in A53T α -syn-EGFP SH-SY5Y cells using Cryo-electron tomography.

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