

Super-resolution microscopy: nanoscale structure of *amyloid- β plaques* and *synaptic alteration* in Alzheimer's disease

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Chiara Trevisan

SCIENTIFIC REPORTS

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Nanoscale structure of amyloid- β plaques in Alzheimer's disease

Marta Querol-Vilaseca^{1,2}, Martí Colom-Cadena^{1,2}, Jordi Peguerols^{1,2}, Raúl Nuñez-Llaves^{1,2}, Joan Luque-Cabecerans^{1,2}, Laia Muñoz-Llahuna^{1,2}, Jordi Andilla³, Olivia Belbin^{1,2}, Tara L. Spires-Jones⁵, Ellen Gelpi^{4,6}, Jordi Clarimon^{1,2}, Pablo Loza-Alvarez³, Juan Fortea^{1,2} & Alberto Lleó^{1,2}

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Article

Alteration in synaptic nanoscale organization dictates amyloidogenic processing in Alzheimer's disease

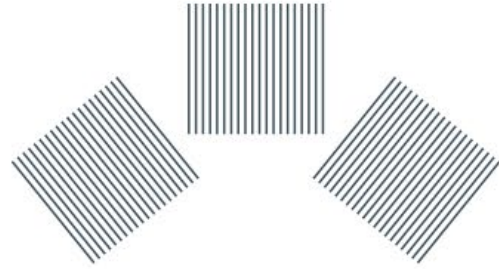
Shekhar Kedia,¹ Pratyush Ramakrishna,² Pallavi Rao Netrakanti,¹ Nivedita Singh,¹ Sangram S. Sisodia,³ Mini Jose,¹ Sathish Kumar,⁴ Anita Mahadevan,⁵ Narendrakumar Ramanan,¹ Suhita Nadkarni,² and Deepak Nair^{1,6,*}

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a SRM techniques

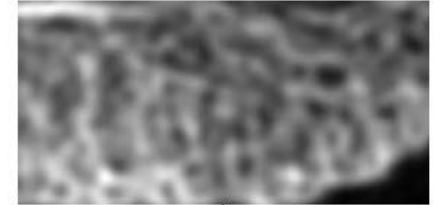
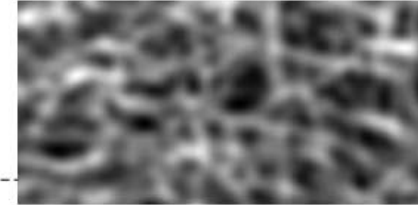
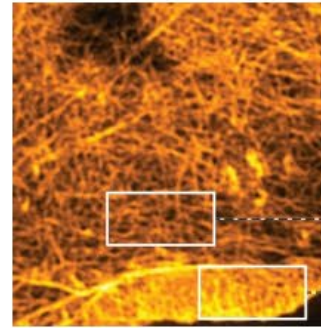
SIM

Increases spatial resolution using a series of regularly spaced illumination patterns



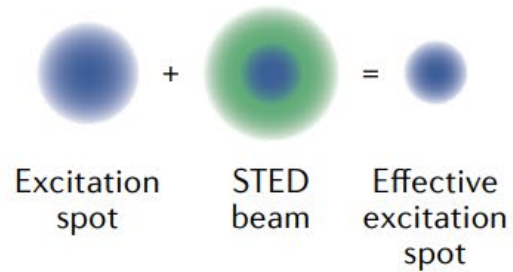
b Actin in COS7 cells

SIM

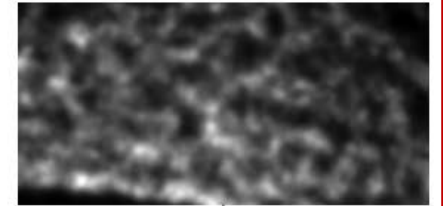
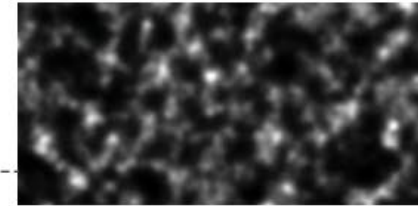
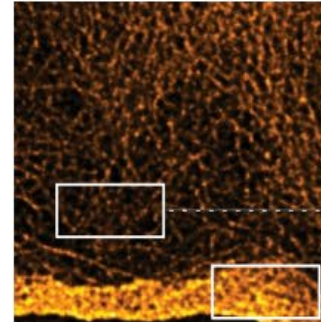


STED microscopy

Reduces excitation spot size using a doughnut-shaped depletion beam



STED microscopy

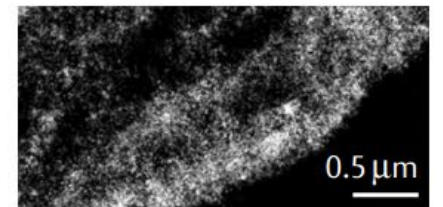
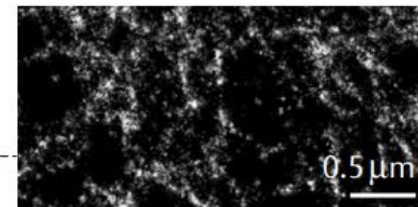
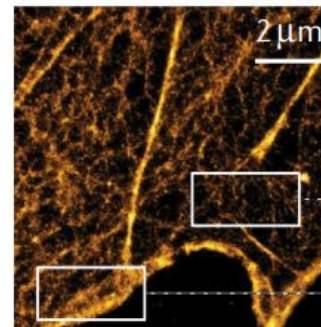


SMLM

Detects single molecules by stochastically activating a sparse set of fluorophores



SMLM



SCIENTIFIC REPORTS

OPEN

Nanoscale structure of amyloid- β plaques in Alzheimer's disease

Marta Querol-Vilaseca^{1,2}, Martí Colom-Cadena^{1,2}, Jordi Pegueroles^{1,2}, Raúl Nuñez-Llaves^{1,2}, Joan Luque-Cabecerans^{1,2}, Laia Muñoz-Llahuna^{1,2}, Jordi Andilla³, Olivia Belbin^{1,2}, Tara L. Spires-Jones⁵, Ellen Gelpi^{4,6}, Jordi Clarimon^{1,2}, Pablo Loza-Alvarez³, Juan Fortea^{1,2} & Alberto Lleó^{1,2}

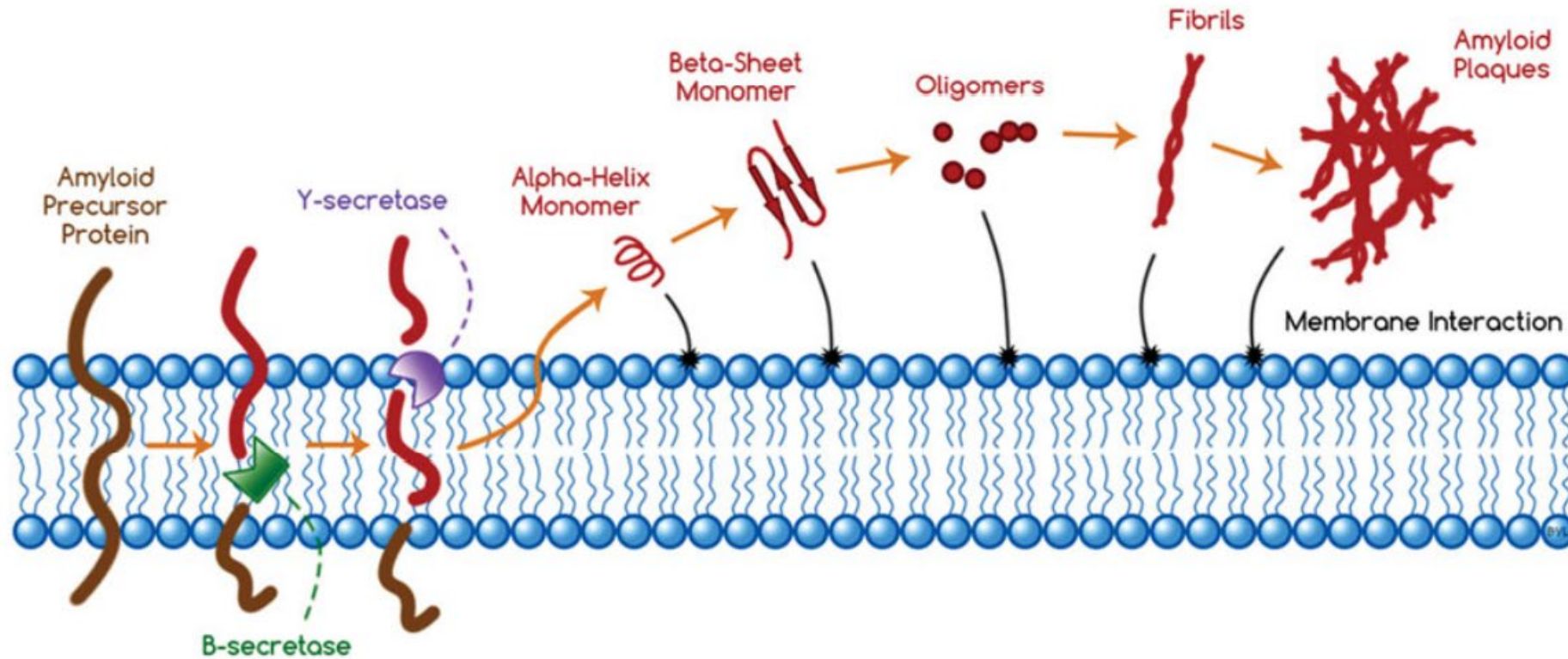
Received: 28 November 2018

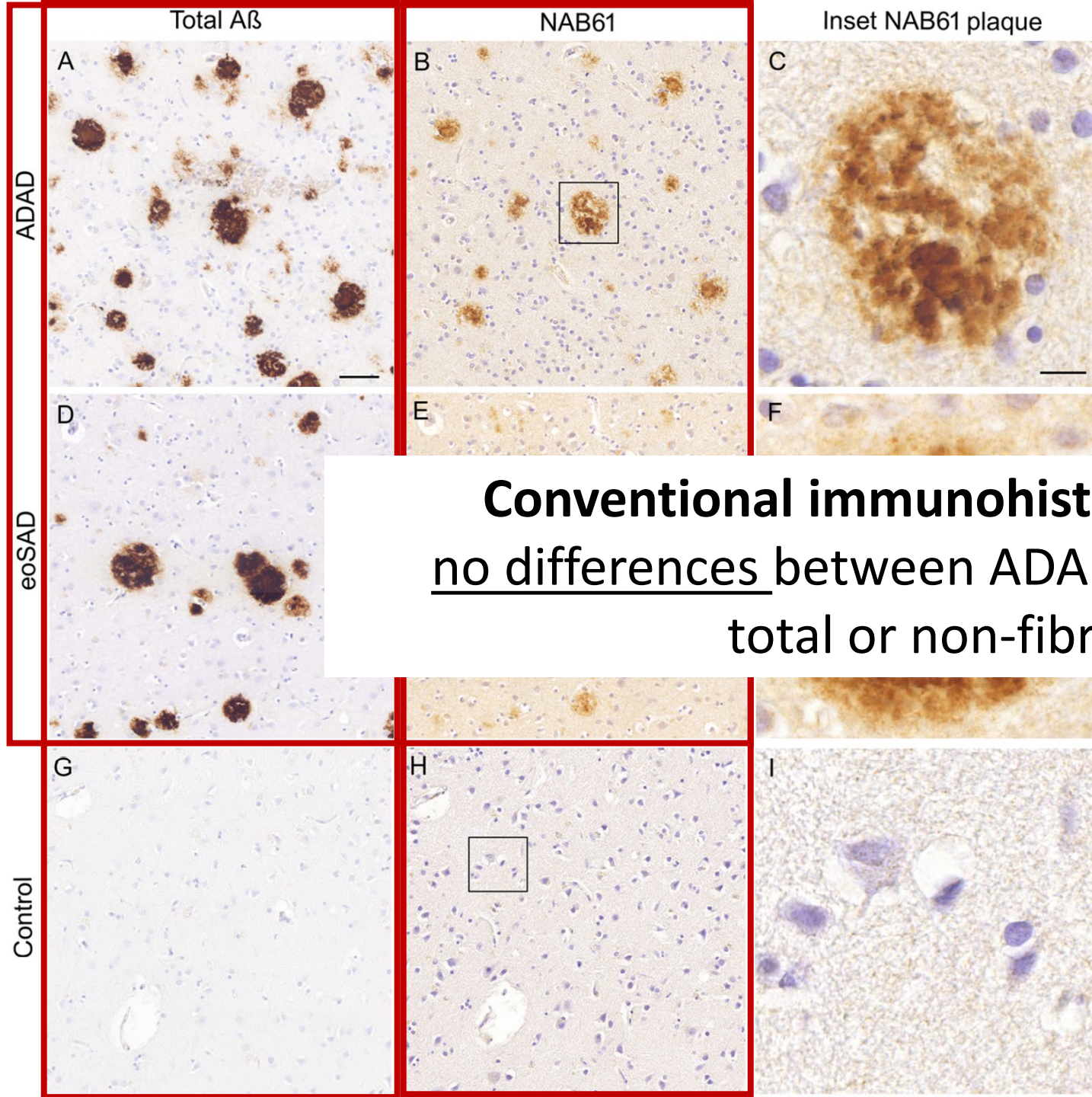
Accepted: 8 March 2019

AIM:

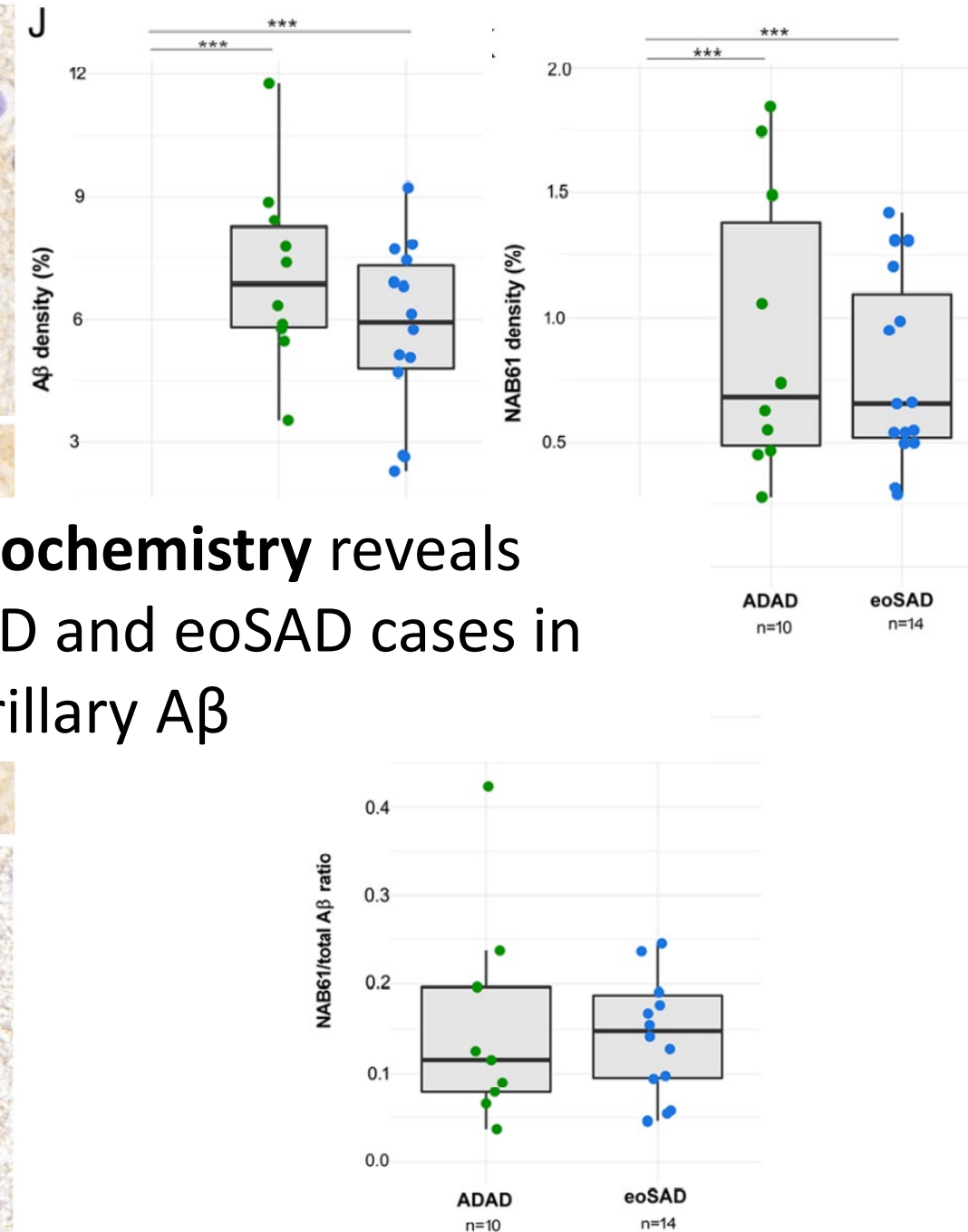
- Propose a new tool to study the nanometric neuropathology of neurodegenerative diseases by combining ultrathin sections used in Array Tomography scanned with super-resolution STED microscopy.
- Investigate the nanoscale architecture of non-fibrillar A β structures in human amyloid plaques.
- Investigated the load and size of these non-fibrillar A β entities in post-mortem human brain tissue in autosomal dominant pattern (ADAD) and early-onset sporadic Alzheimer's disease (eoSAD) patients.

Amyloid origin and the mechanism of the amyloid formation

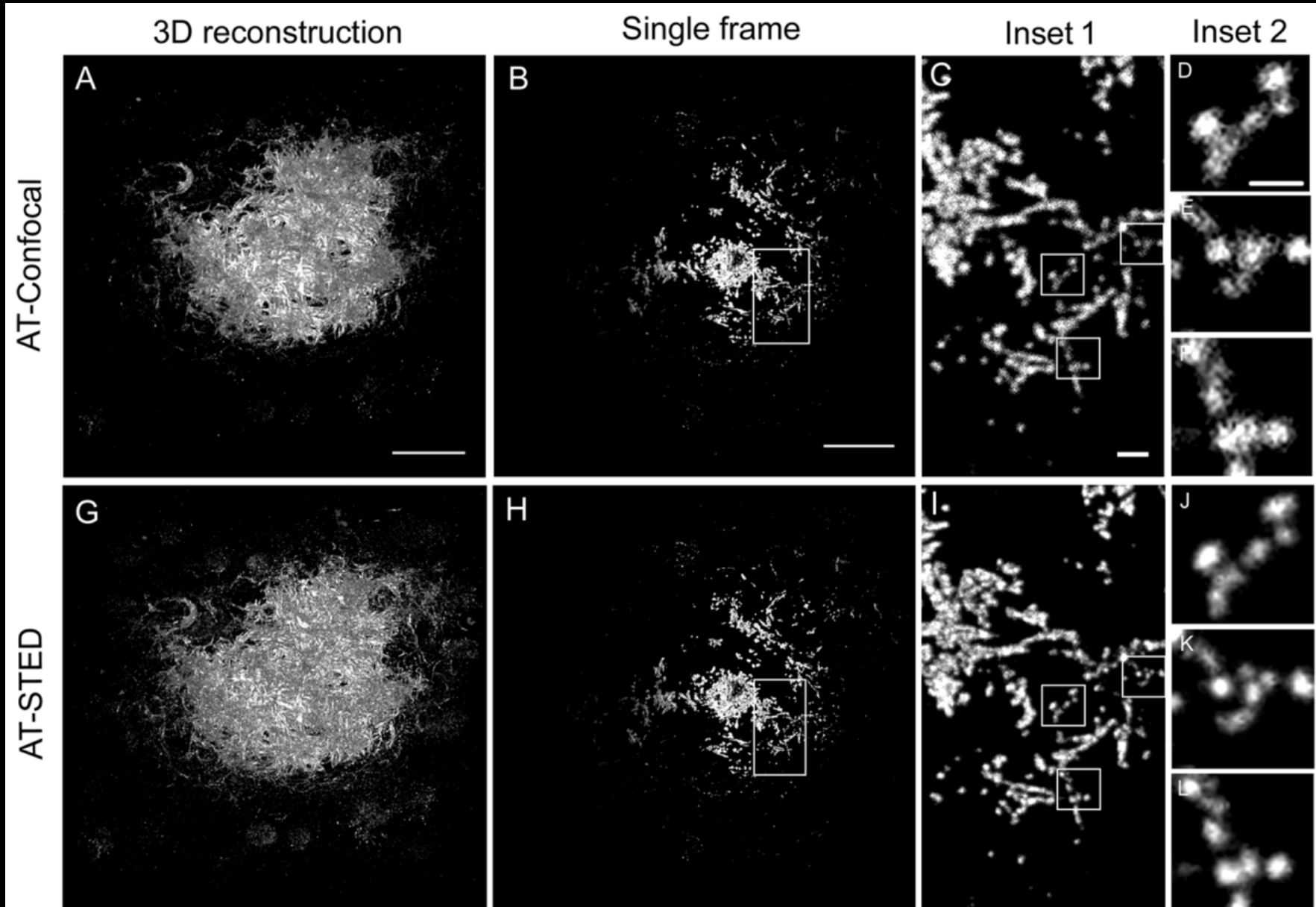




Conventional immunohistochemistry reveals no differences between ADAD and eoSAD cases in total or non-fibrillary Aβ

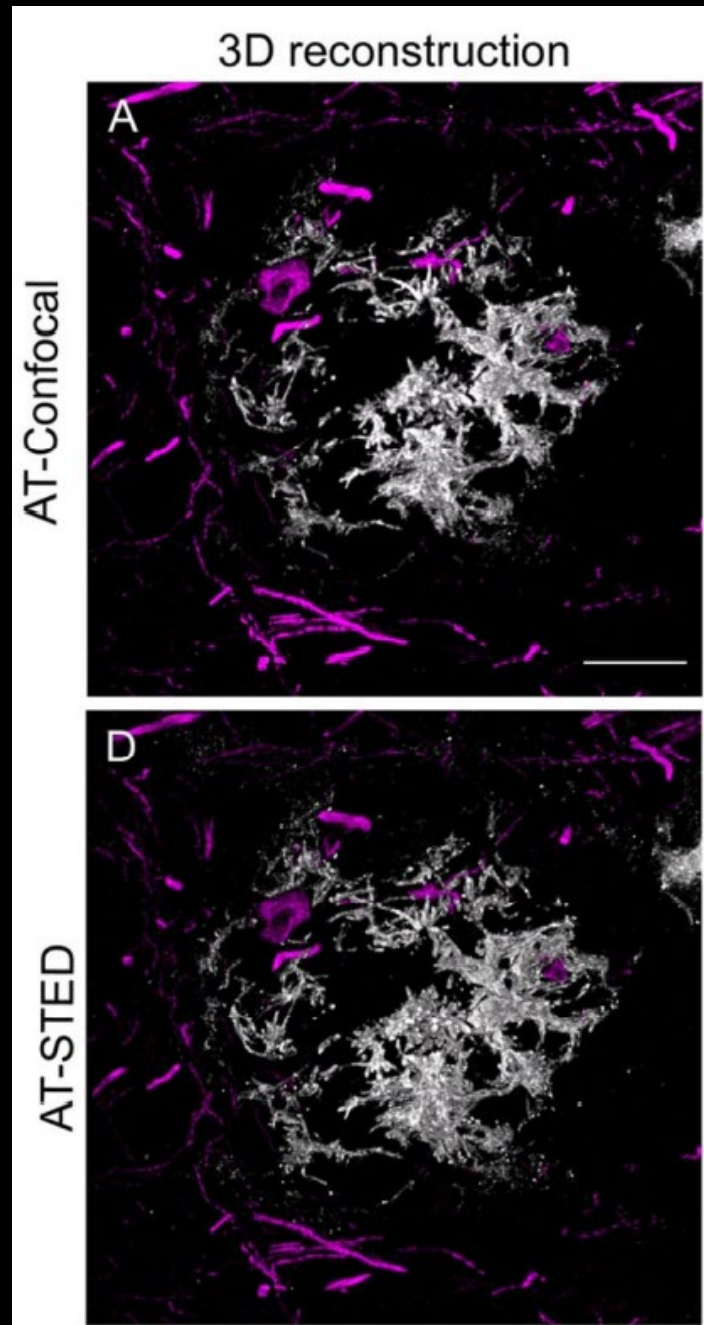


Improving spatial and axial resolution with Array Tomography (AT) and Stimulated Emission Depletion (STED)





The combination of AT and STED allows the identification of smaller non-fibrillar A β structures



Antibodies:

- Anti-Neurofilaments
- Anti-NAB61

A β structure's size:

Large

$>0,015 \mu\text{m}^3$

Medium

$>0,006 < 0,015 \mu\text{m}^3$

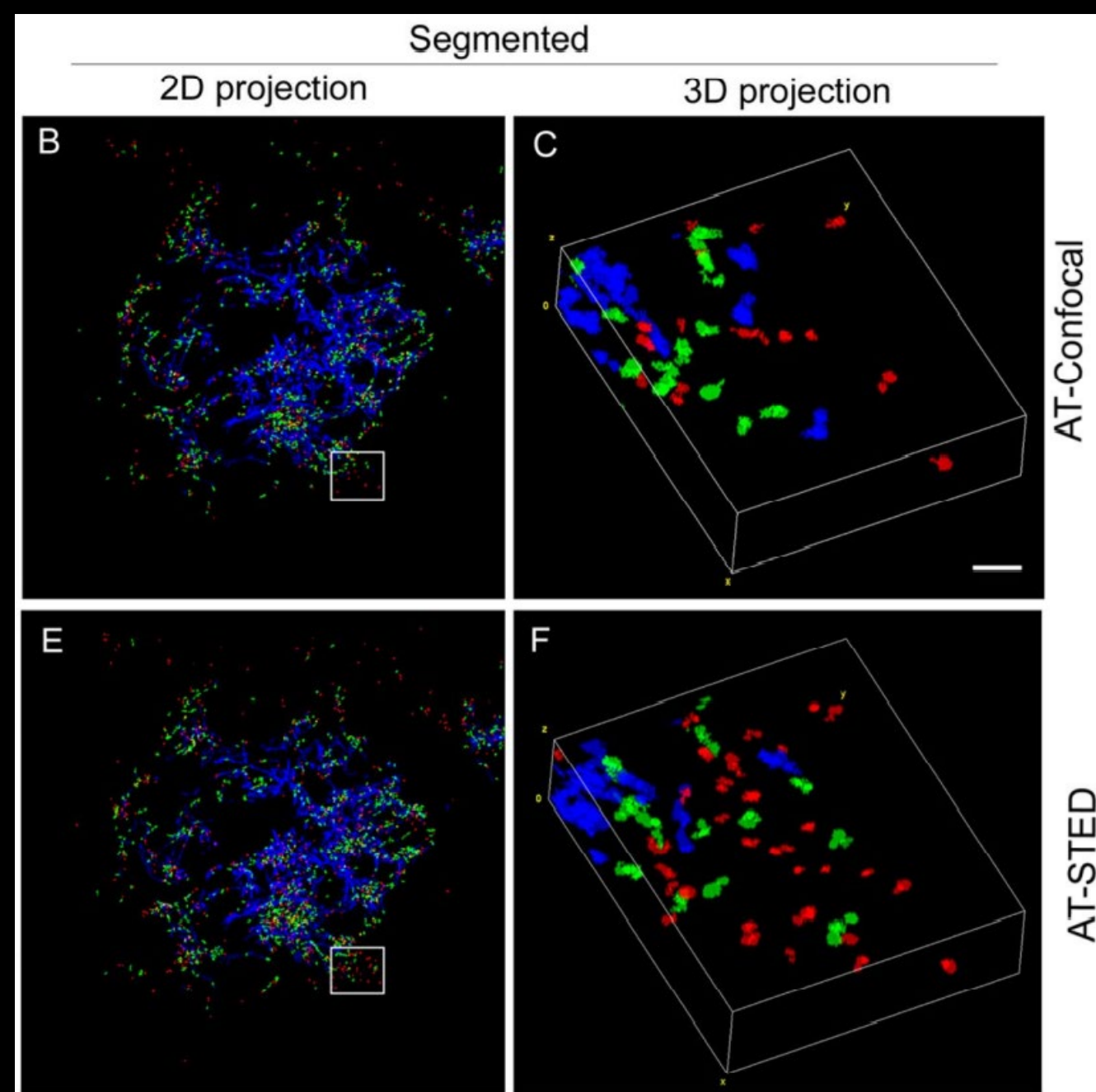
Small

$<0,006 \mu\text{m}^3$

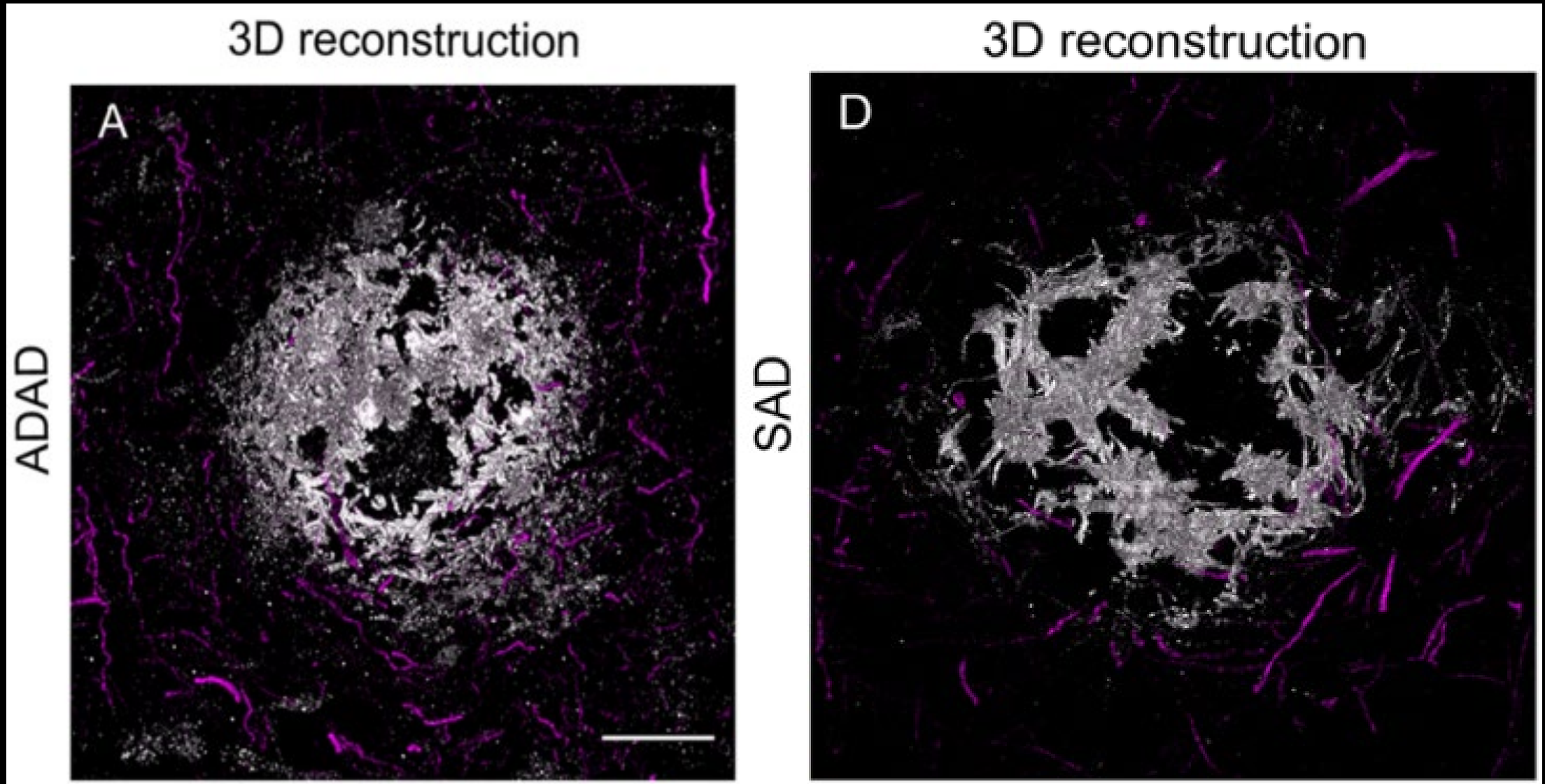
Scale bar:

10 μm

Inset (C-F) 1 μm



AT-STED application: higher levels of non-fibrillar A β structures in ADAD than in SAD



Antibodies: Anti-Neurofilaments , Anti-NAB61

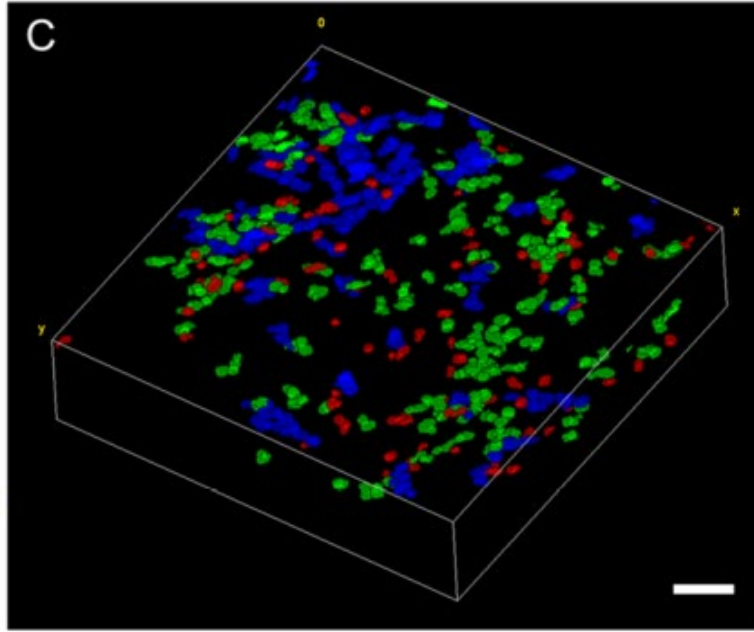
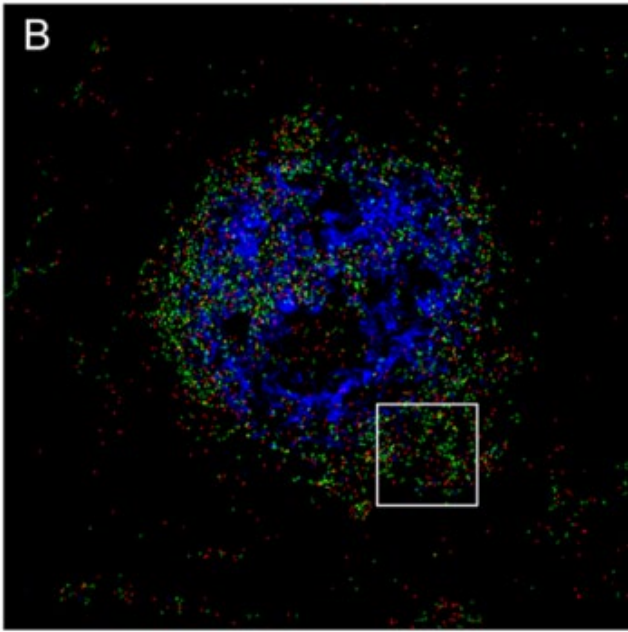
Scale bar: 10 μ m

AT-STED application: higher levels of non-fibrillar A β structures an ADAD than in SAD

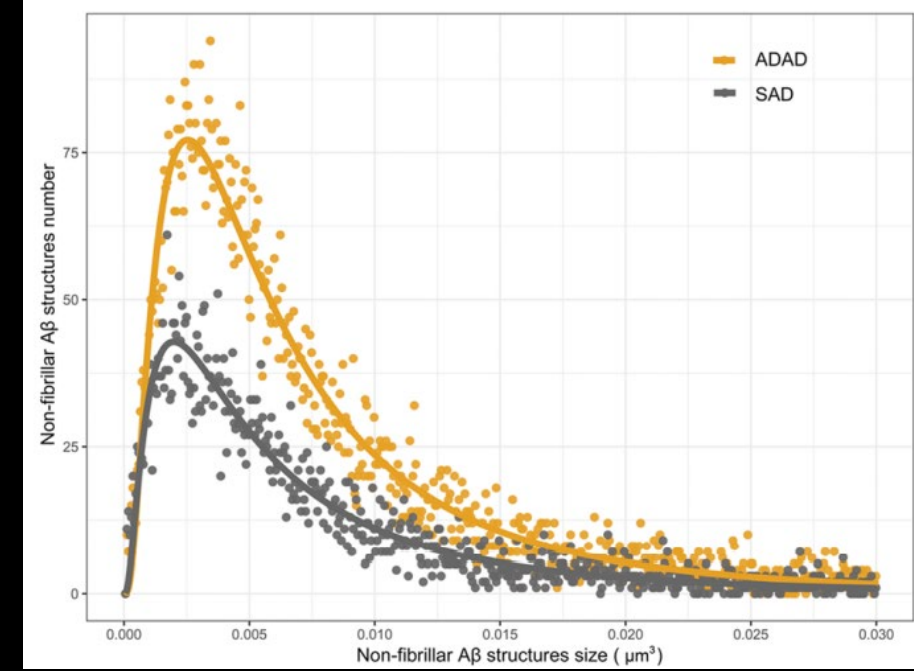
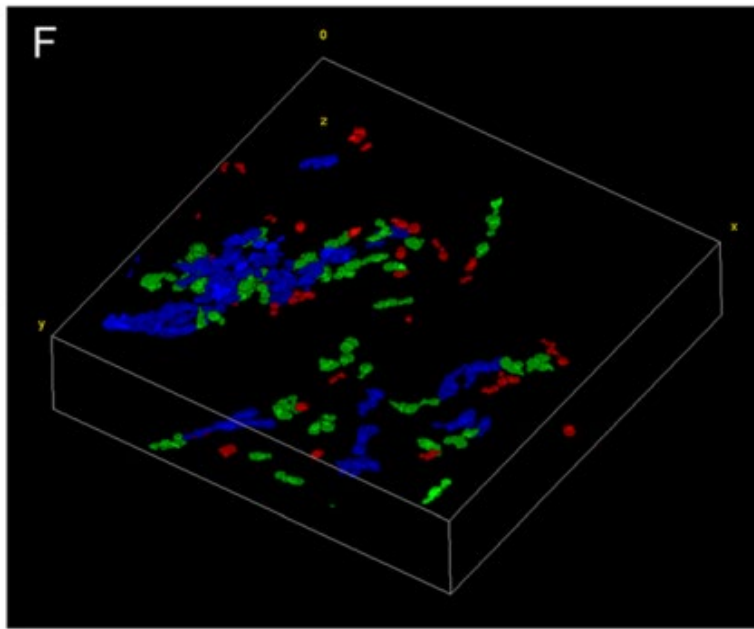
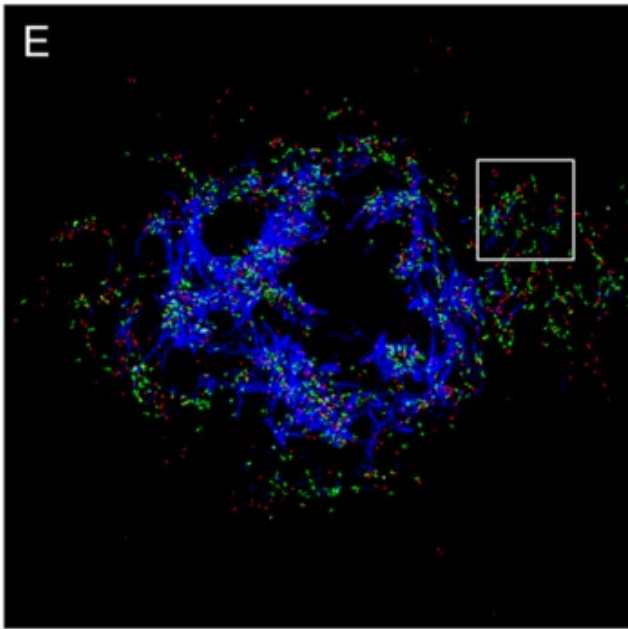
2D projection

3D projection

ADAD



SAD

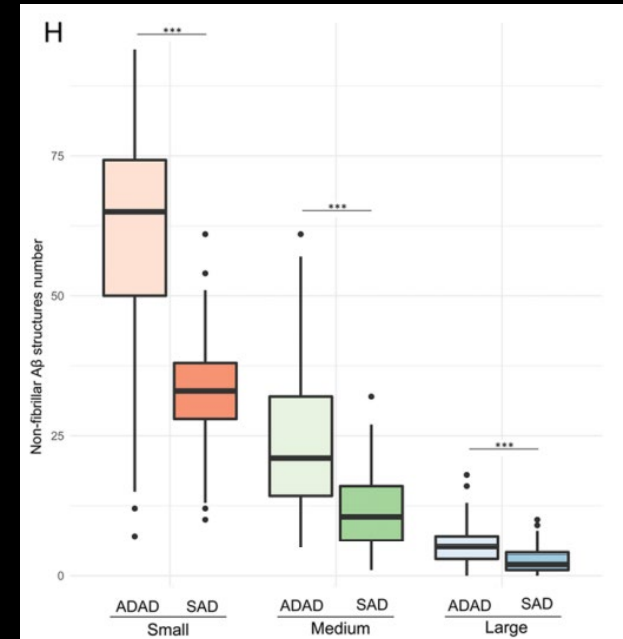


A β structure's size:

Large
 $>0,015 \mu\text{m}^3$

Medium
 $>0,006 < 0,015 \mu\text{m}^3$

Small
 $< 0,006 \mu\text{m}^3$



CONCLUSION

- This study shows that the combination of AT and STED can be successfully applied to investigate non-fibrillar A β structures in AD human brain.
- The obtained nanoscale architecture of human amyloid plaques reveals a dense core with a peripheral halo
- This study provides evidence of higher levels of non-fibrillar A β species in ADAD compared to SAD

This new tool opens an important door for the neuropathology field allowing the characterization of aggregates or structures at a nanometric scale as potential therapeutic targets

Article

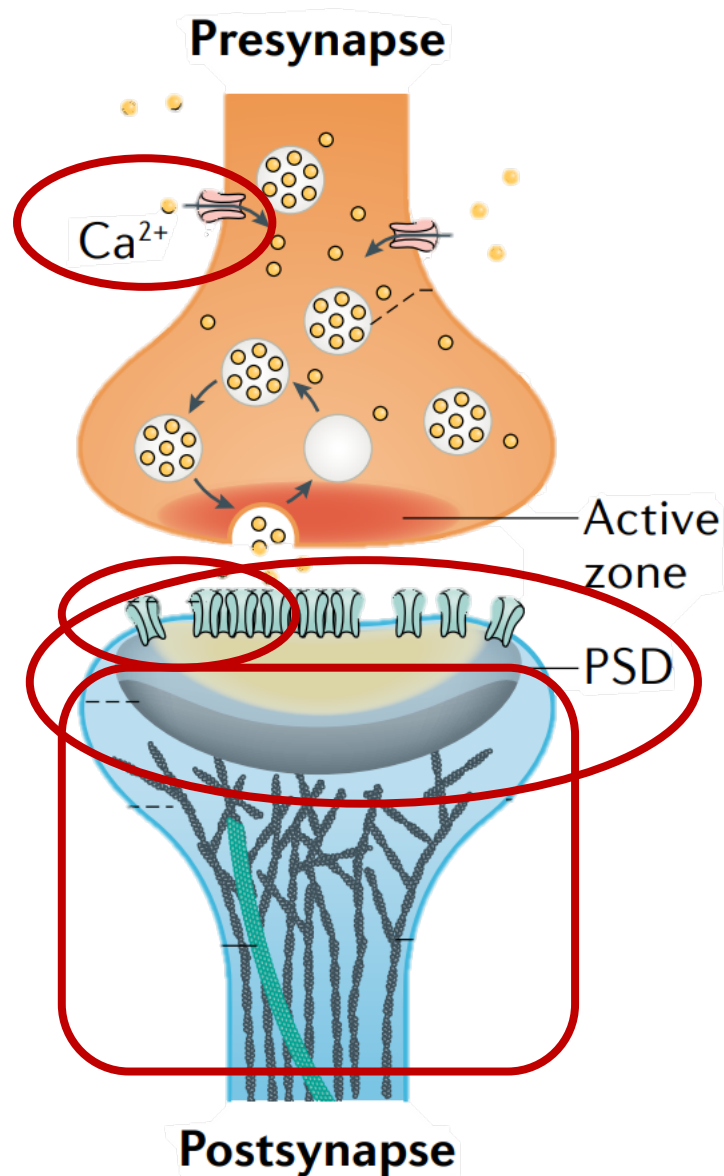
Alteration in synaptic nanoscale organization dictates amyloidogenic processing in Alzheimer's disease

Shekhar Kedia,¹ Pratyush Ramakrishna,² Pallavi Rao Netrakanti,¹ Nivedita Singh,¹ Sangram S. Sisodia,³ Mini Jose,¹ Sathish Kumar,⁴ Anita Mahadevan,⁵ Narendrakumar Ramanan,¹ Suhita Nadkarni,² and Deepak Nair^{1,6,*}

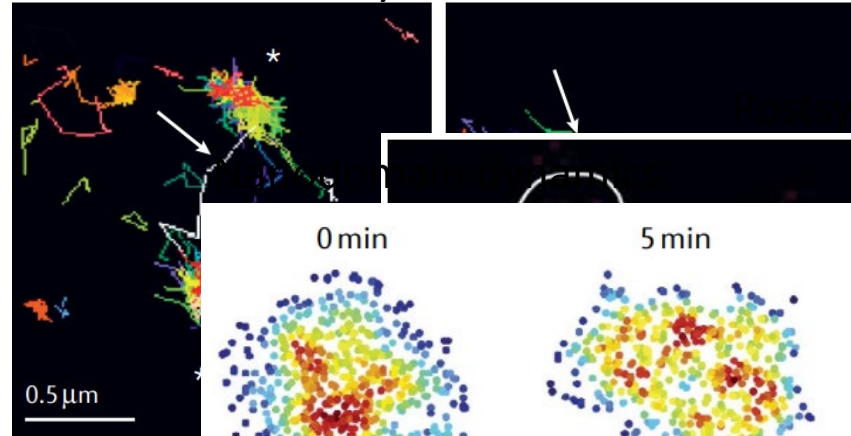
AIM

- Use of **super-resolution imaging** and analysis to reveal the subsynaptic organization of APP, β - and γ -secretases
- Understanding of the association of APP with β -secretases in specialized subsynaptic regions and how this association directly influences the processing of APP through the amyloidogenic pathway.
- Use of transgenic mouse models for AD and postmortem human brain tissues from AD patients, to validate the competency of this molecular model

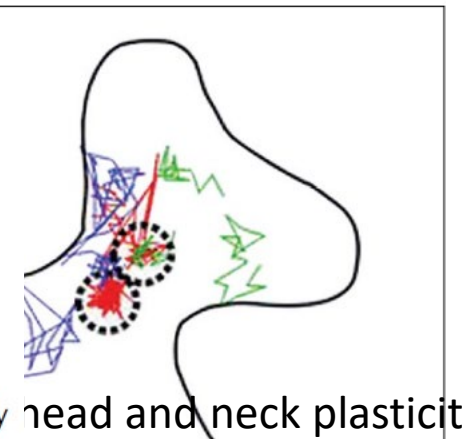
Dynamic of the healthy synapse



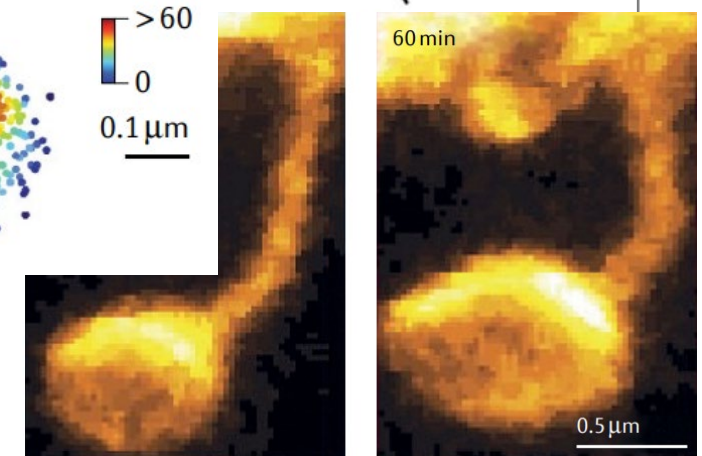
Calcium channel dynamics



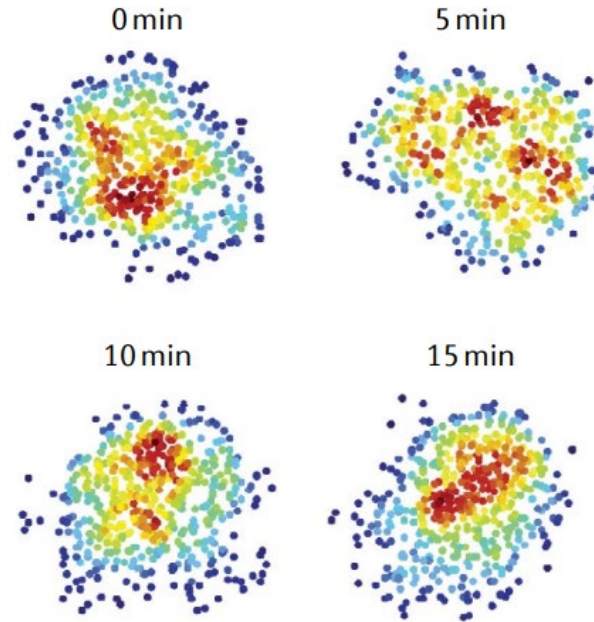
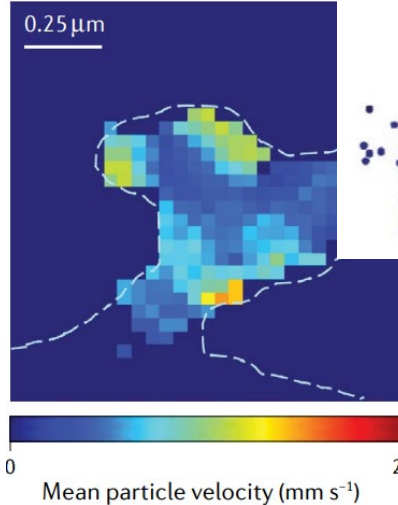
Synaptic receptors dynamics



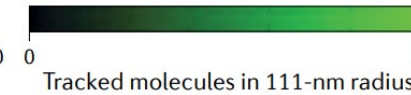
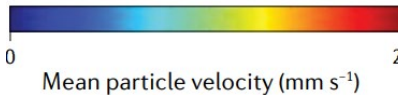
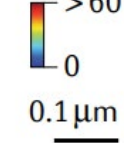
Head and neck plasticity



Spine actin dynamics

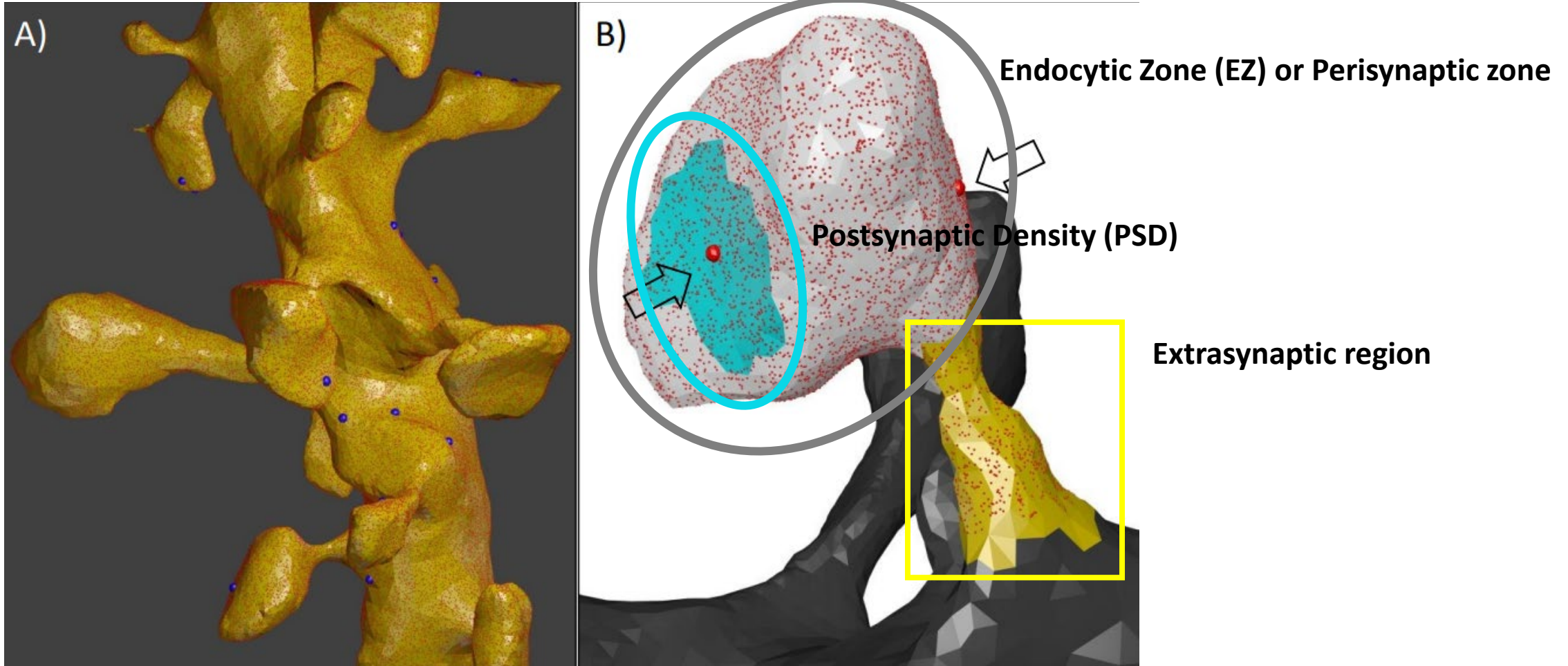


Local density



Insight into nanodomains

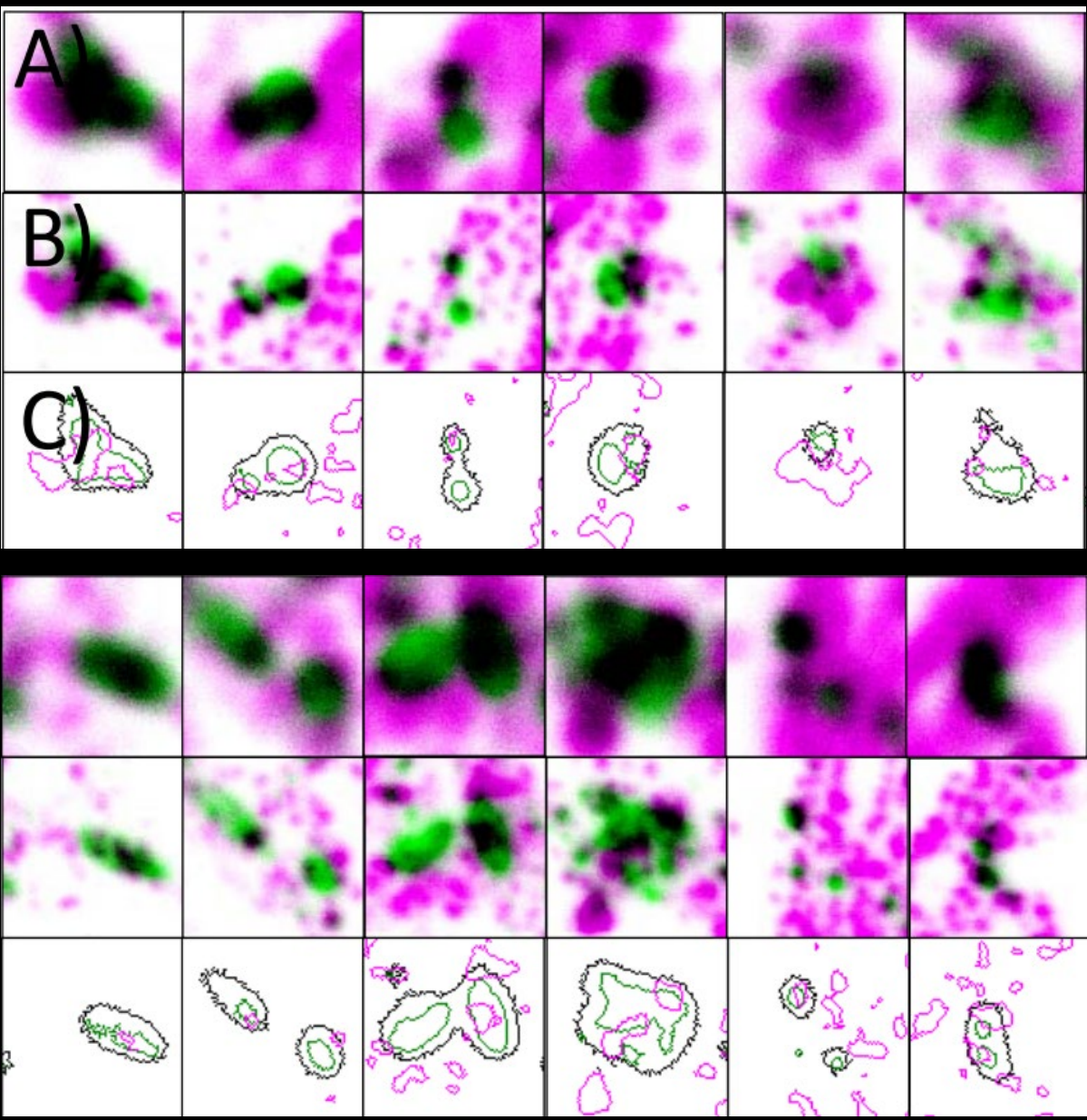
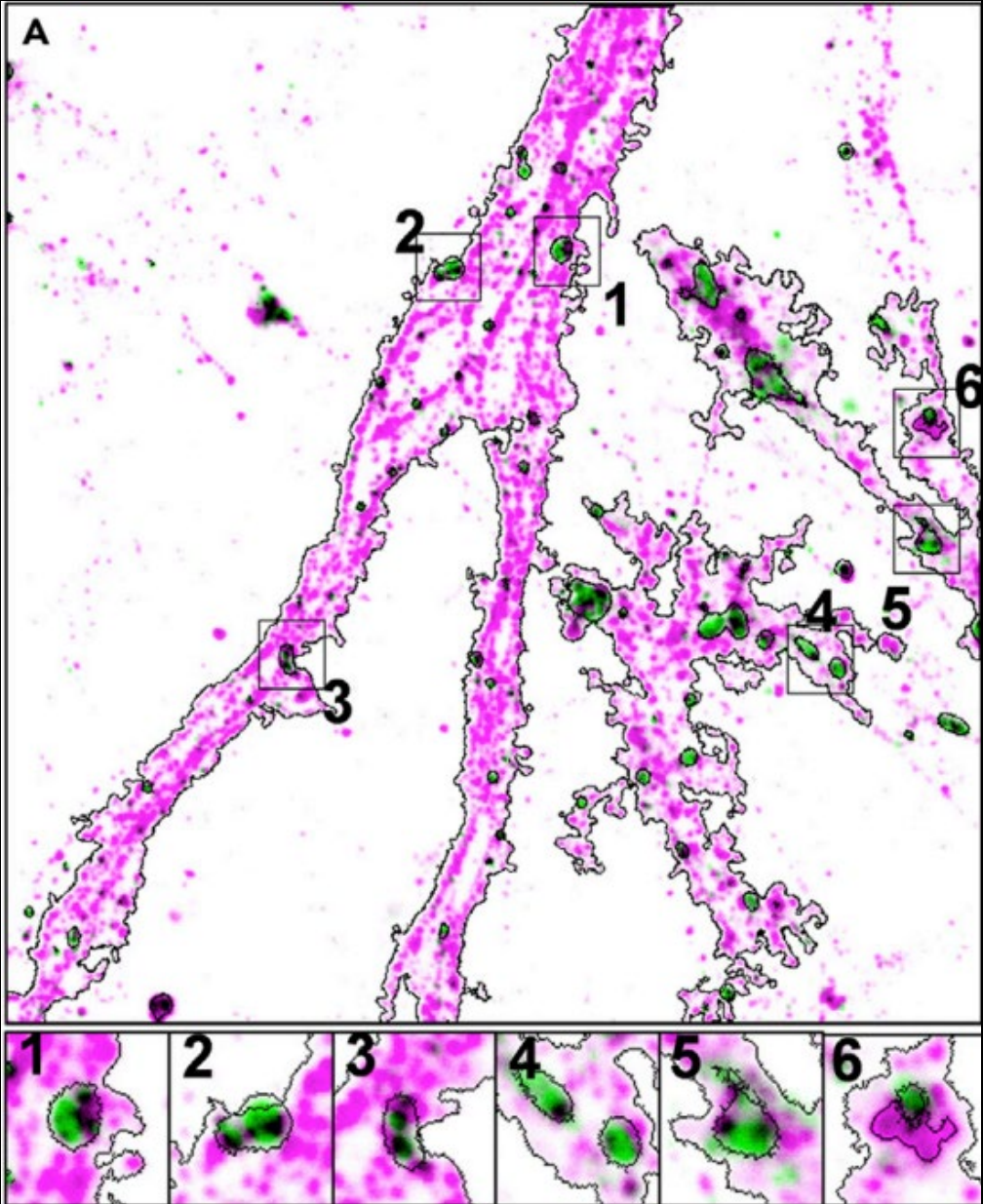
Reconstruction of nanoscale distribution of APP on the dendritic shaft of pyramidal neuron



Nanoorganization of
amyloidogenic proteolytic machinery in the
functional domains of an excitatory synapse

Nanoscale distribution of β -secretase in the functional zones of excitatory postsynapse using STED microscopy

Postsynaptic Density / β -Secretase



Confocal

STED

Automatically
detected
regions

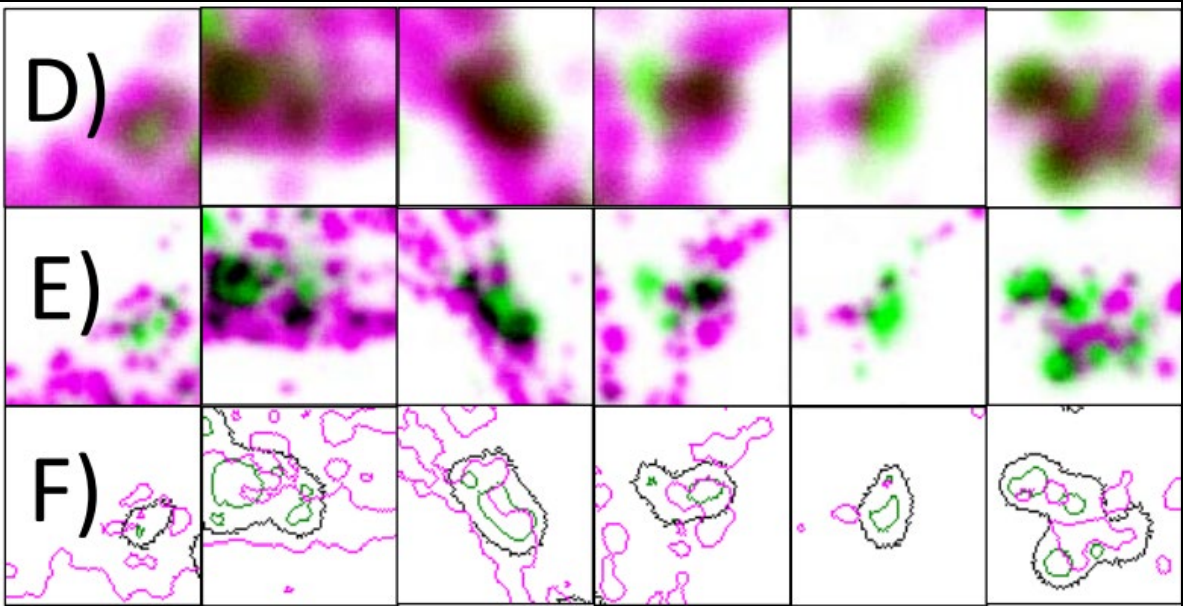
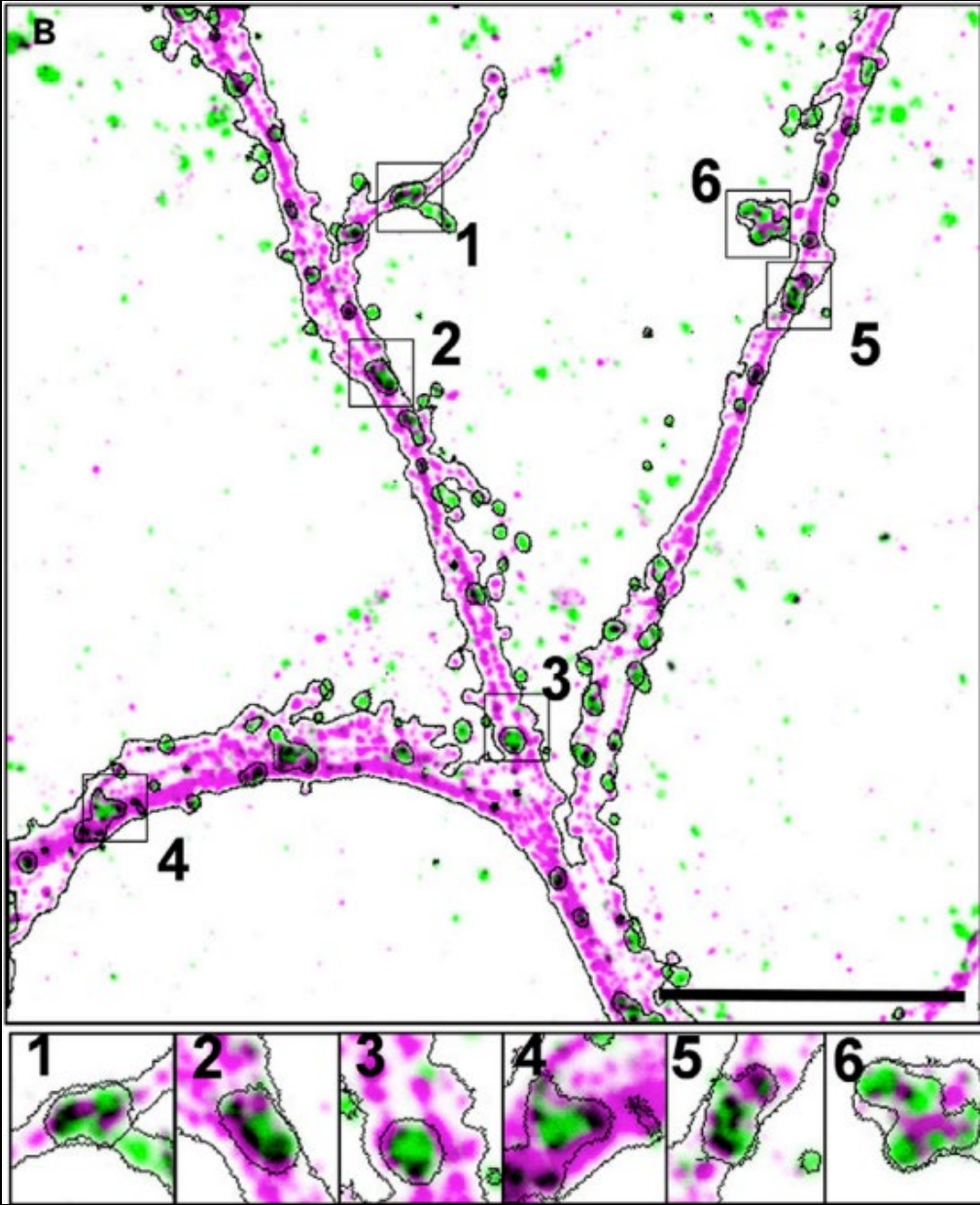
Confocal

STED

Automatically
detected
regions

Nanoscale distribution of β -secretase in the functional zones of excitatory postsynapse using STED microscopy

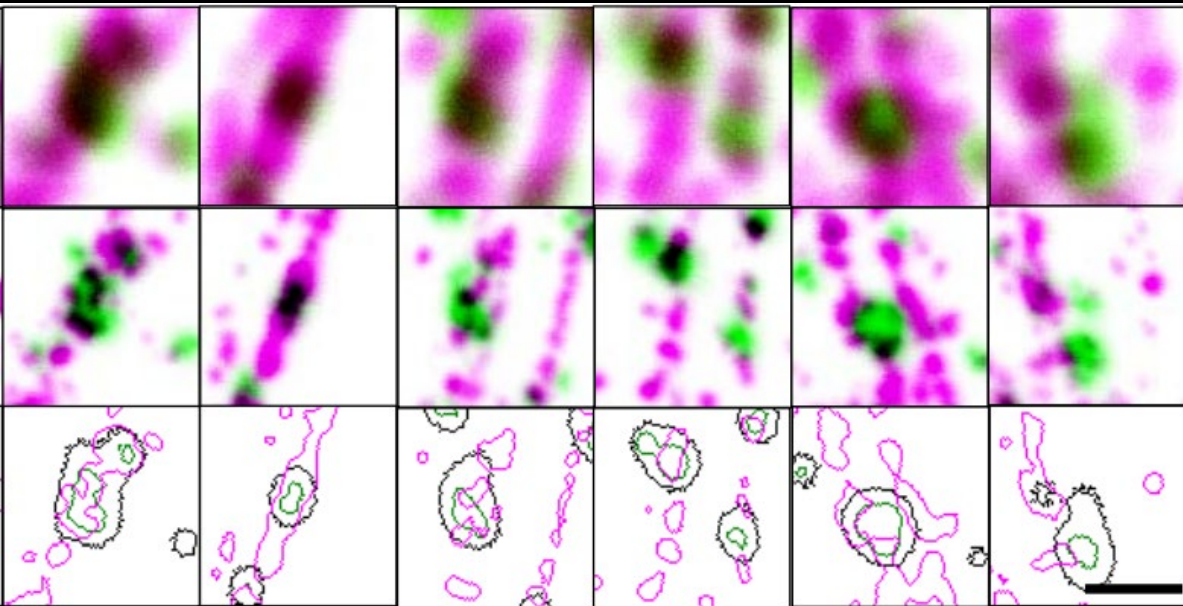
Endocytic Zone / β -Secretase



Confocal

STED

Automatically
detected
regions



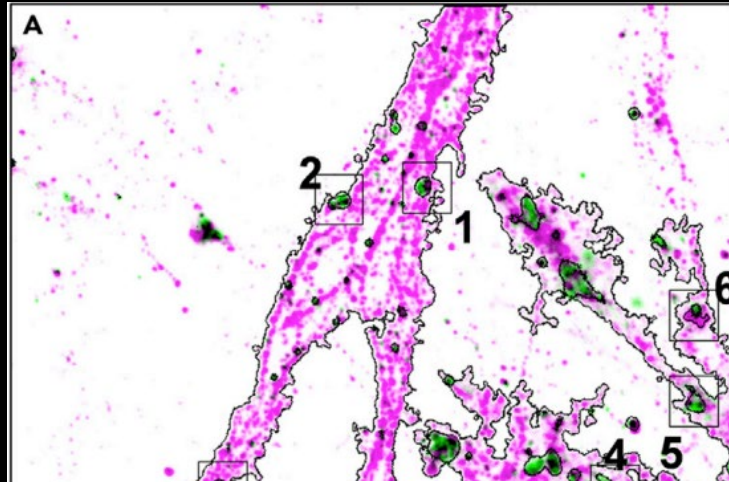
Confocal

STED

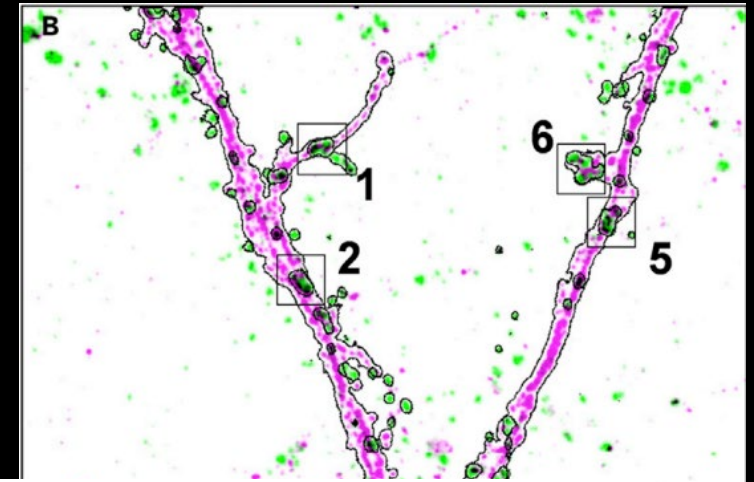
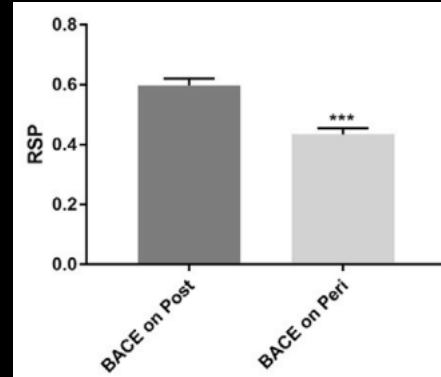
Automatically
detected
regions

Postsynaptic Density / β -Secretase

Endocytic Zone / β -Secretase

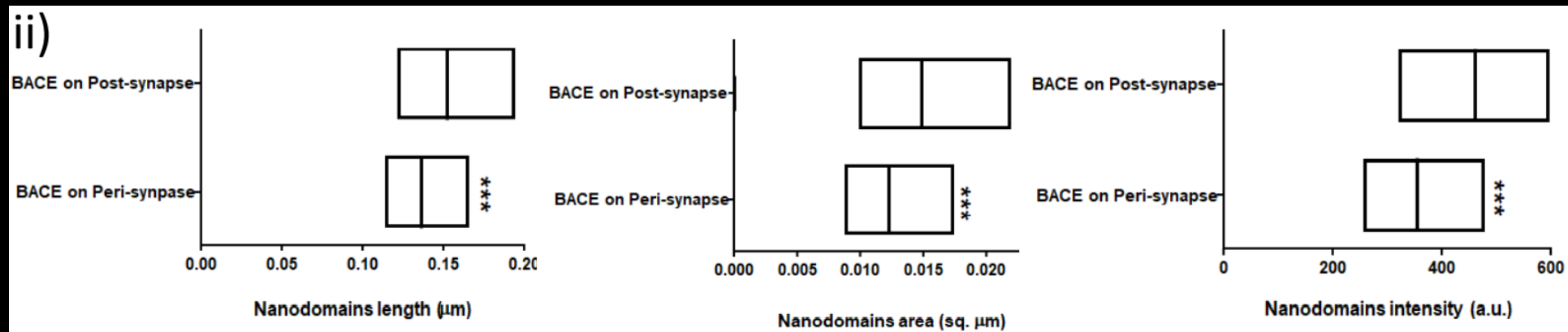


Colocalization



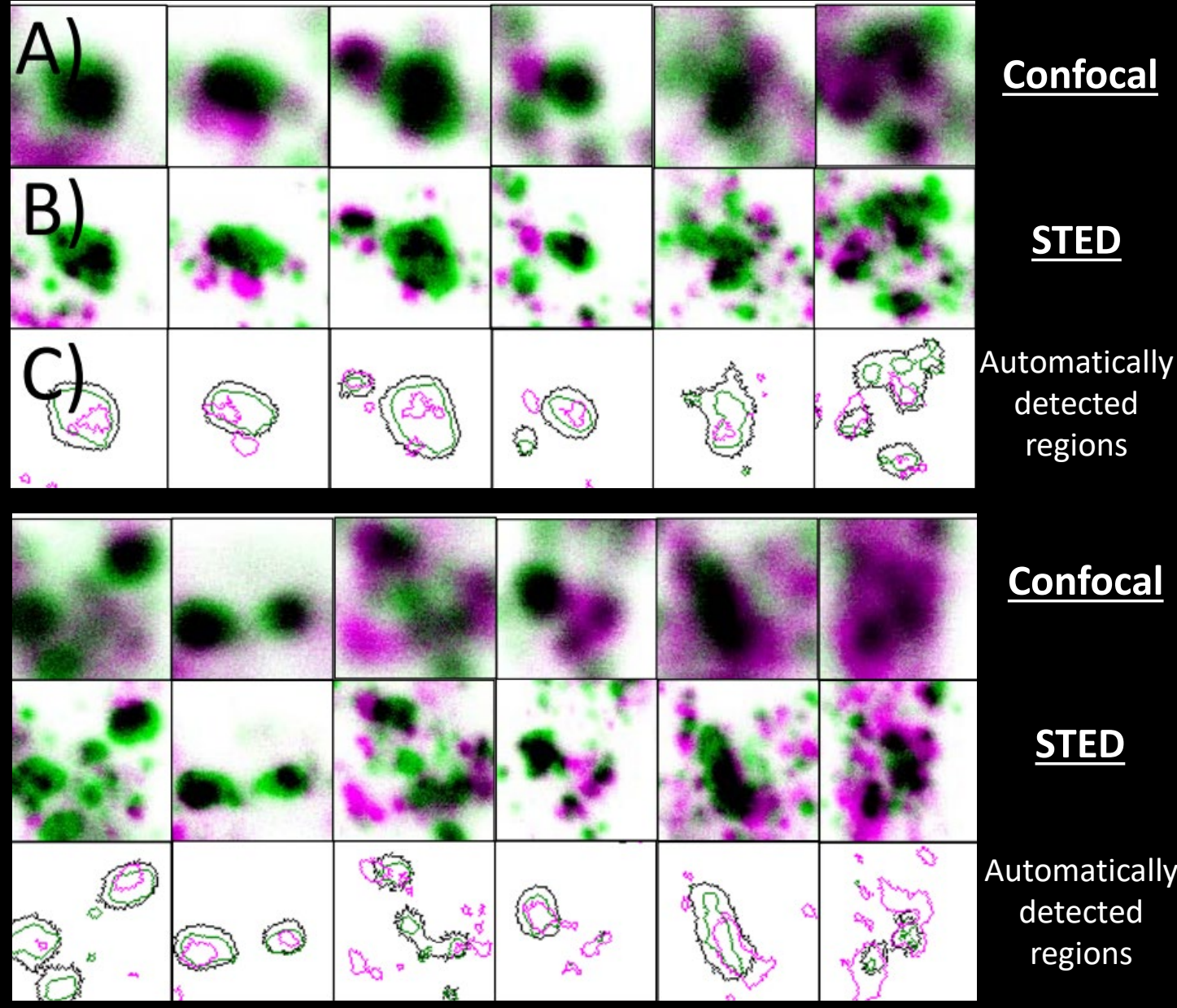
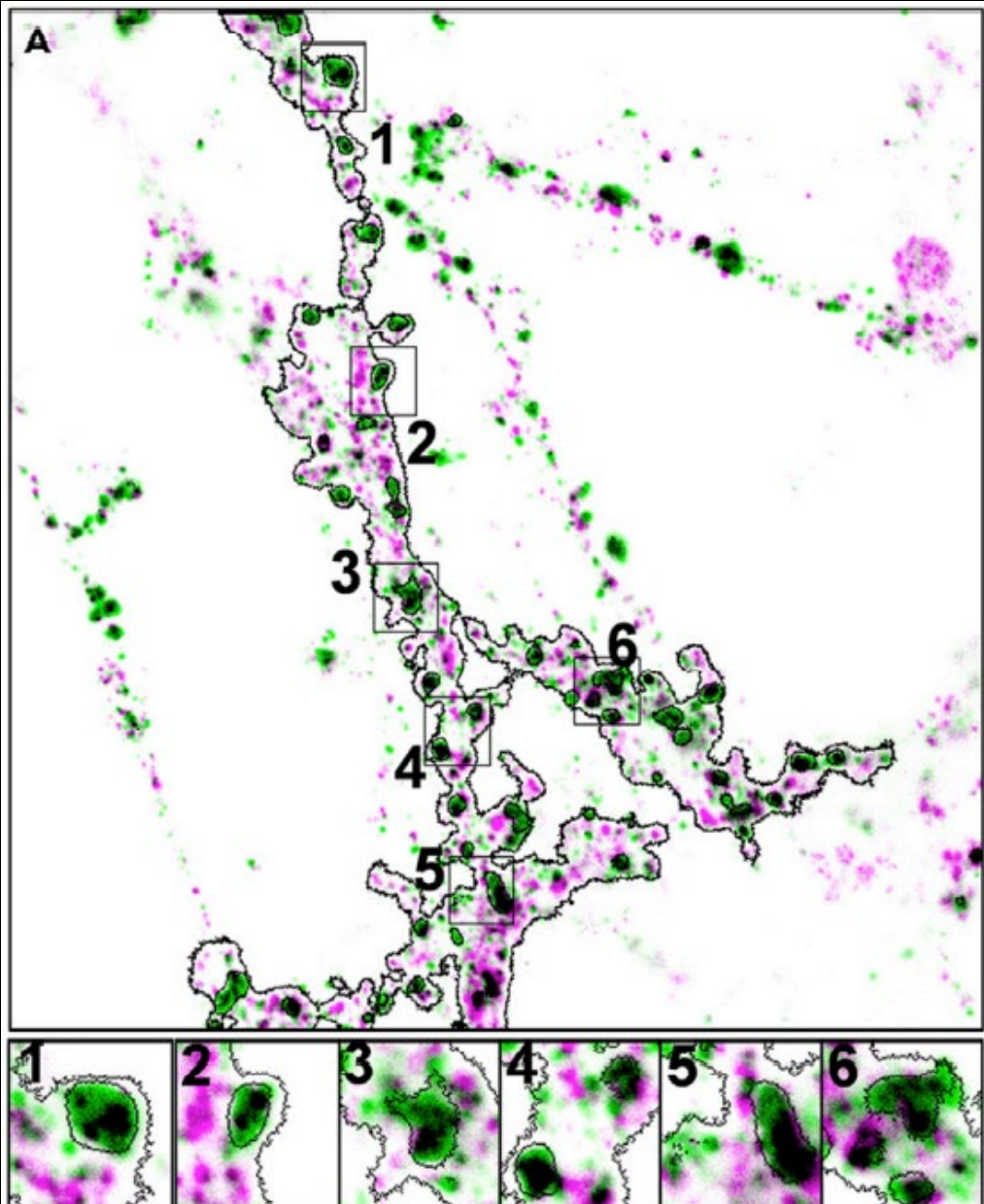
Conclusion: - The length, area, and intensity were significantly higher for nanodomain β / PSD in comparison to nanodomain β / EZ.

- β -Secretase associated closely with Postsynaptic Density



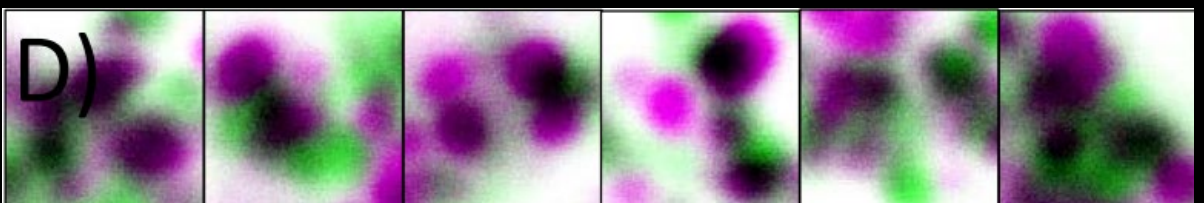
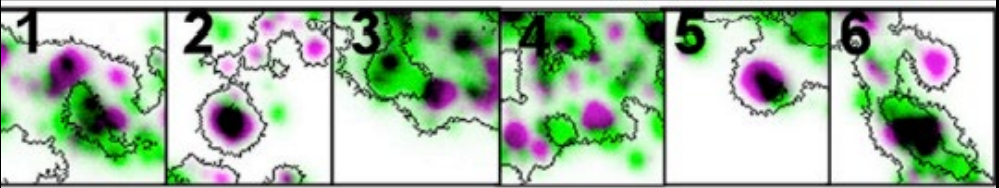
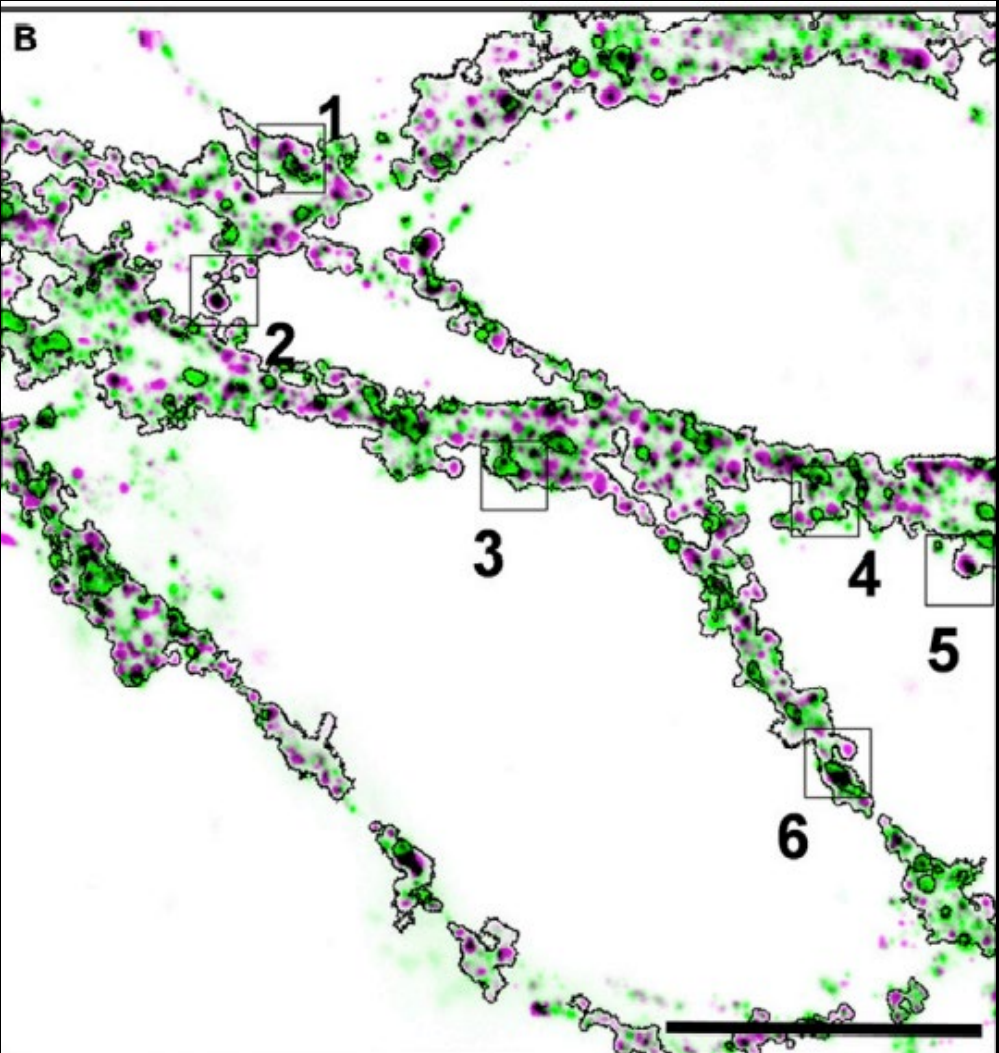
Nanoscale distribution of γ -secretase in the functional zones of excitatory postsynapse using STED microscopy

Postsynaptic Density / γ -Secretase

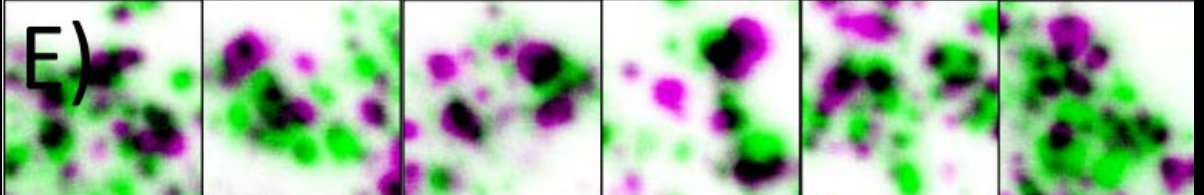


Nanoscale distribution of γ -secretase in the functional zones of excitatory postsynapse using STED microscopy

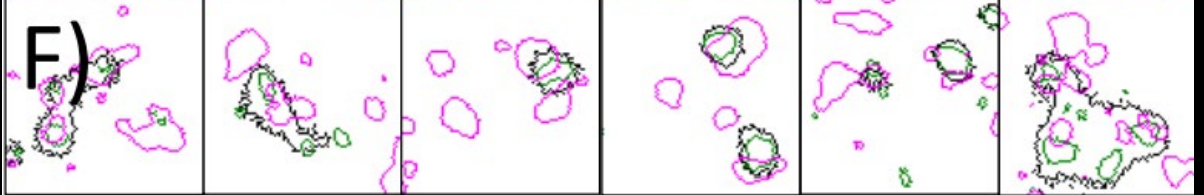
Endocytic Zone / γ -Secretase



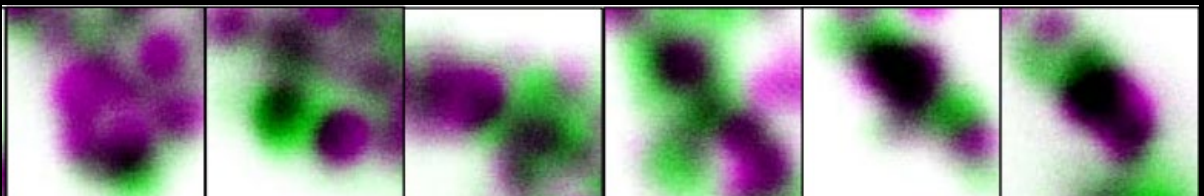
Confocal



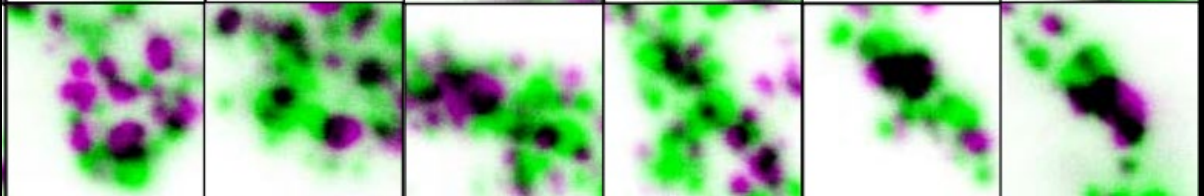
STED



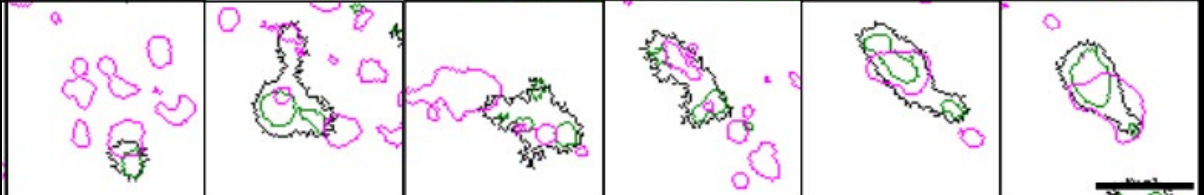
Automatically
detected
regions



Confocal



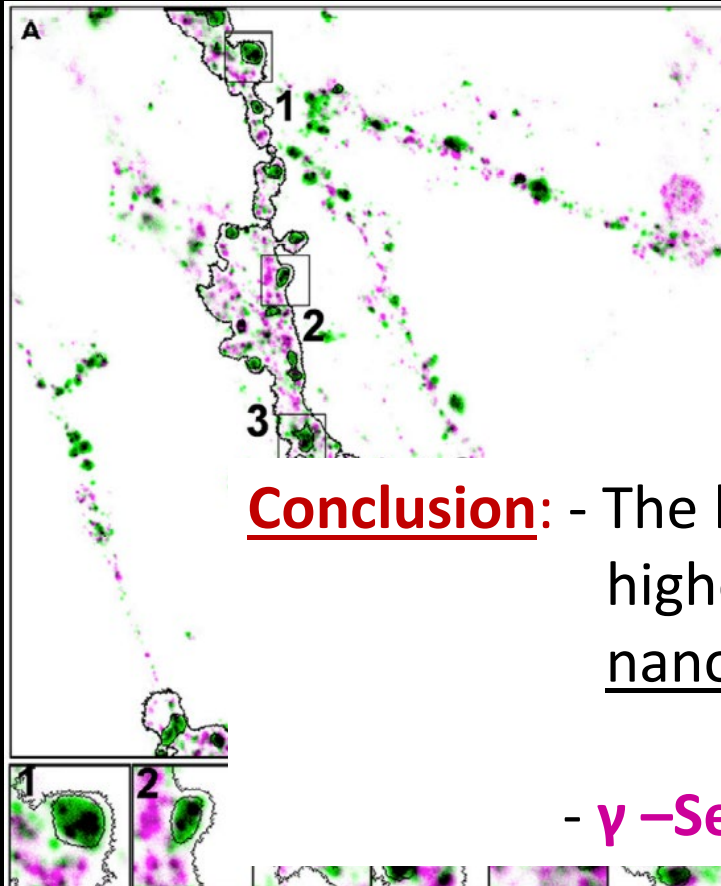
STED



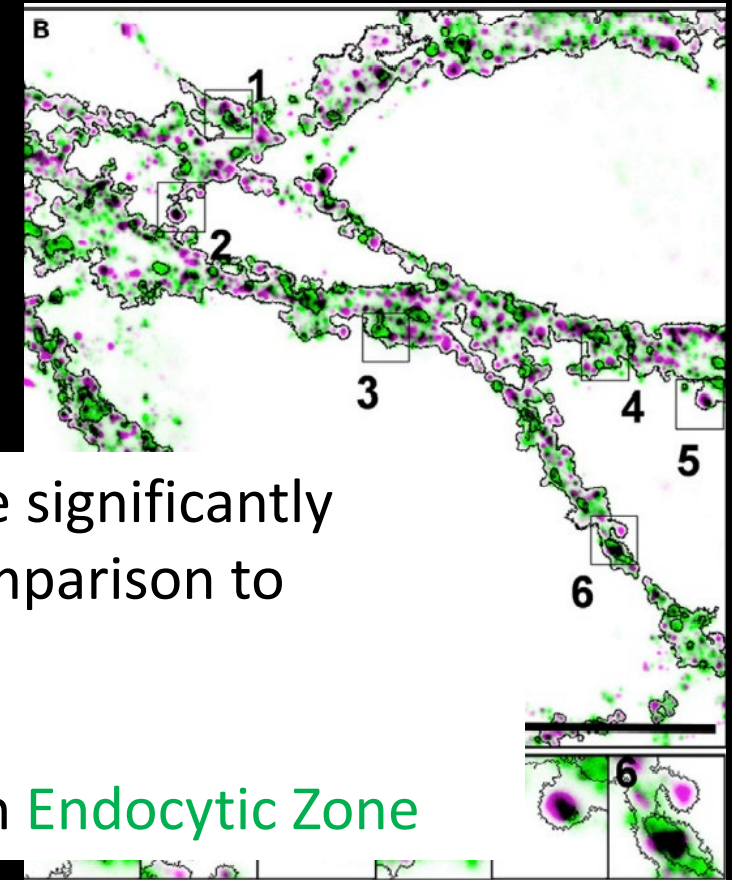
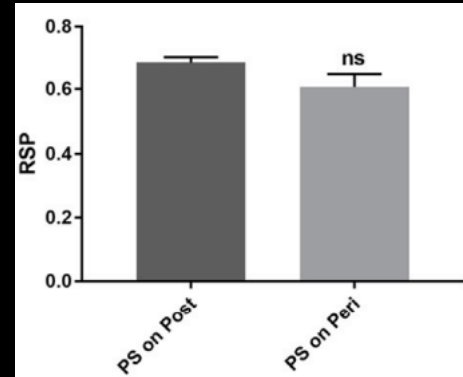
Automatically
detected
regions

Postsynaptic Density / γ -Secretase

Endocytic Zone / γ -Secretase

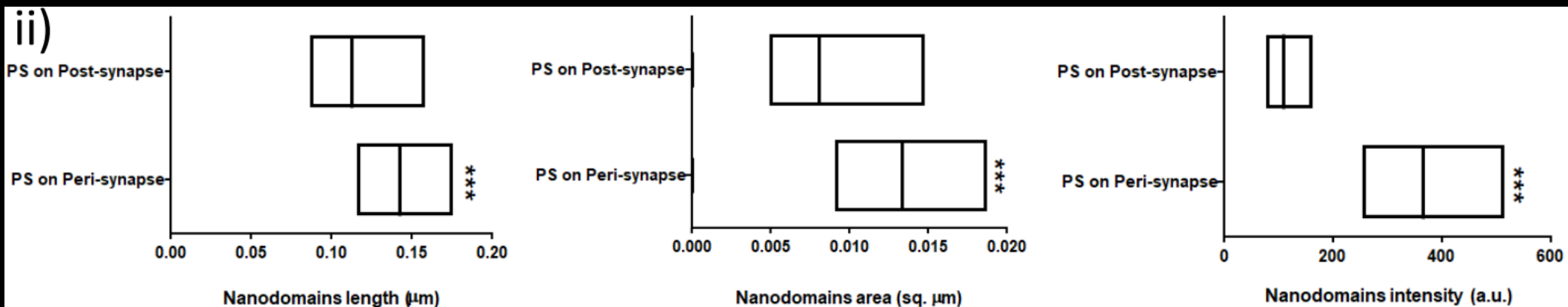


Colocalization



Conclusion: - The length, area, and intensity were significantly higher for nanodomain γ /EZ in comparison to nanodomain γ /PSD.

- γ -Secretase associated closely with Endocytic Zone



Nanoscale association of β/γ -secretase with APP in the neuronal processes, using STED microscopy

β -Secretase / APP

γ -Secretase / APP

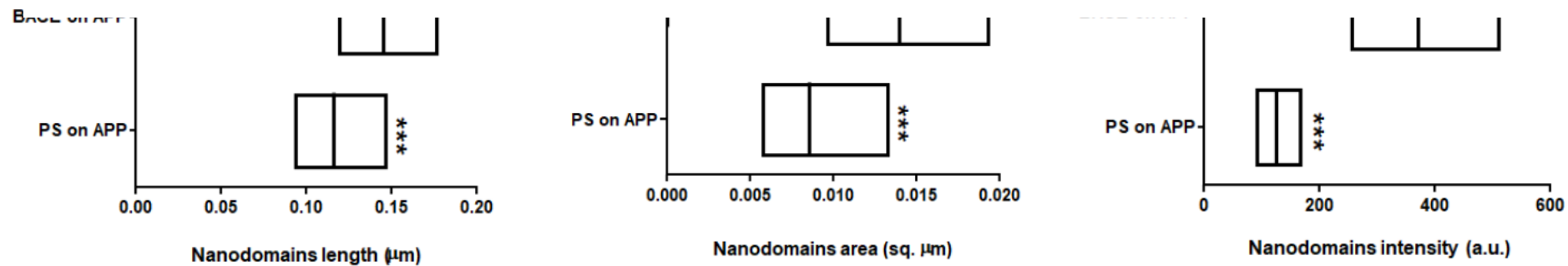
Confocal

Conclusion: - Discrete association of APP with β -Secretase in contrast to γ -Secretase nanodomain

- High variability in the intensity of nanodomain β /APP compared to nanodomain γ /APP

The concentration of β -Secretase associated with the nanodomains is critical for the amyloidogenic processing of APP

ii



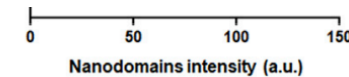
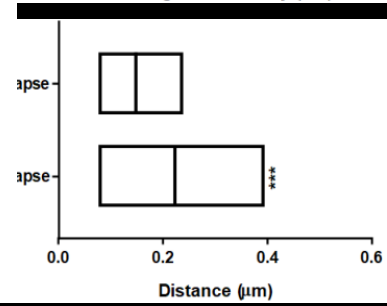
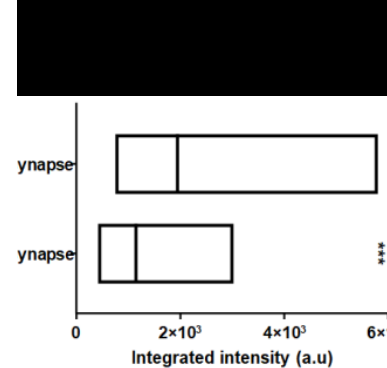
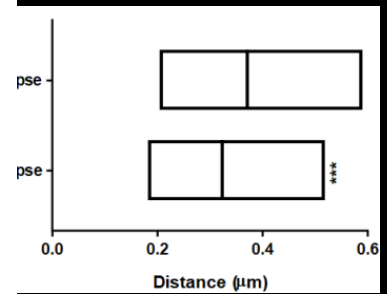
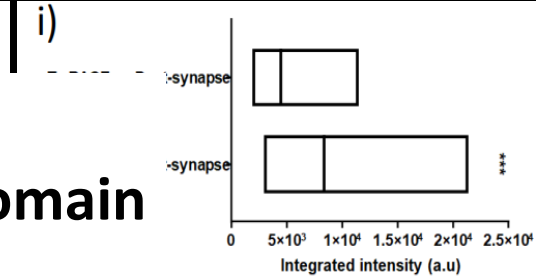
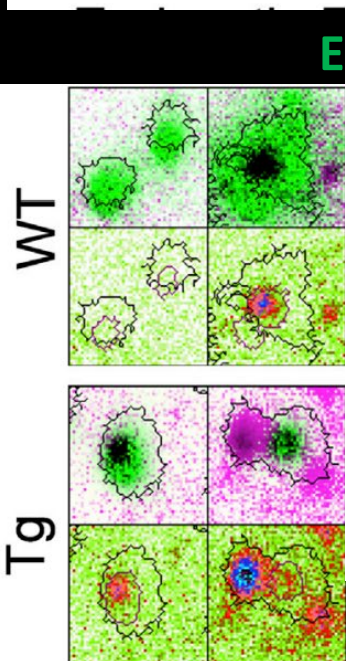
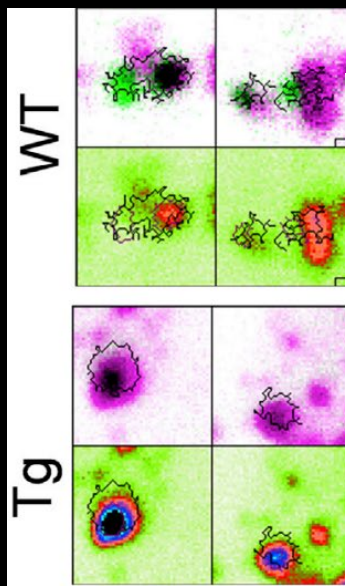
Nanoscale alteration of molecular fingerprints of
amyloidogenic machinery in
multiple models of AD

Postsynaptic Density / β -Secretase

Conclusion:

- The length and intensity of **nanodomain β /PSD** and **nanodomain β /EZ** were significantly altered between WT and Tg mice
- The cumulative β -secretase levels were decreased in PSD, while it increased in EZ in Tg mice
- The proximity of β -secretase to PSD and EZ were altered antagonistically. The distance of β -secretase to PSD increased, while a significant decrease was observed for EZ in Tg mice

Taken together, the β -secretase levels in Tg mice were augmented significantly in EZ, both inside and outside of nanodomains, along with a decrease in the length of nanodomains.

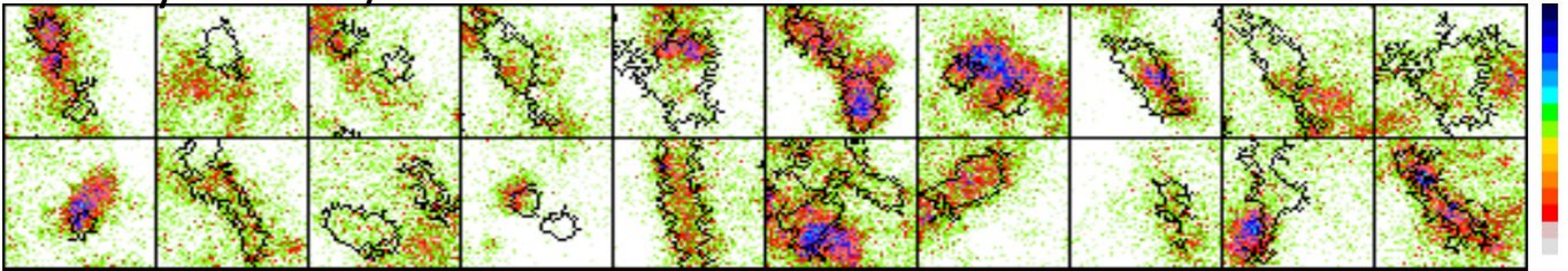


Alteration of nanoscale molecular determinants involved in β -amyloidogenic machinery in human brain slices using STED

Endocytic Zone / APP

Control

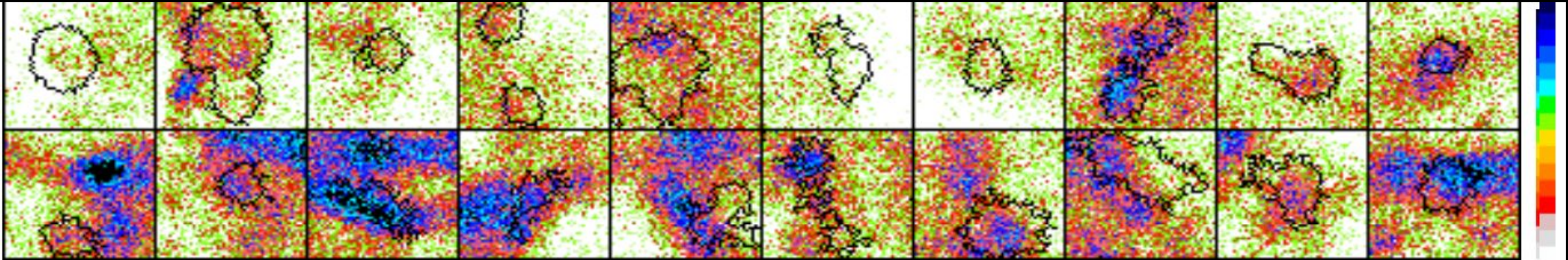
AD



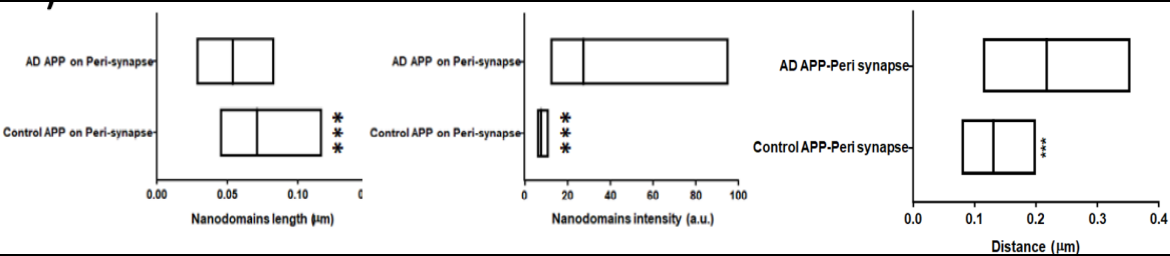
Endocytic Zone / β -Secretase

Control

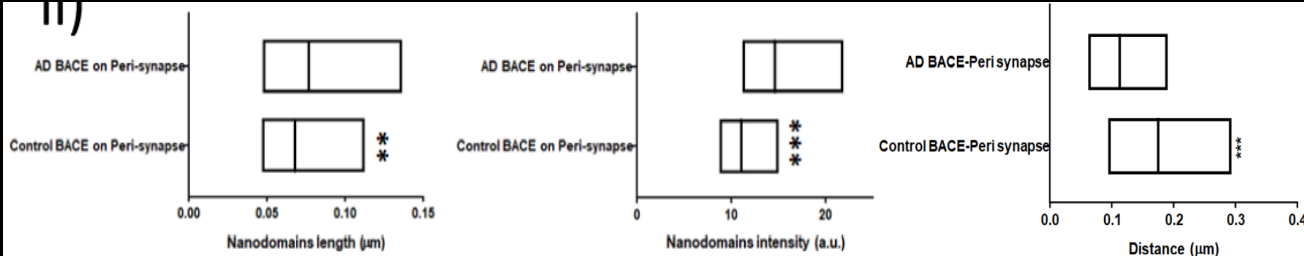
AD



Endocytic Zone / APP



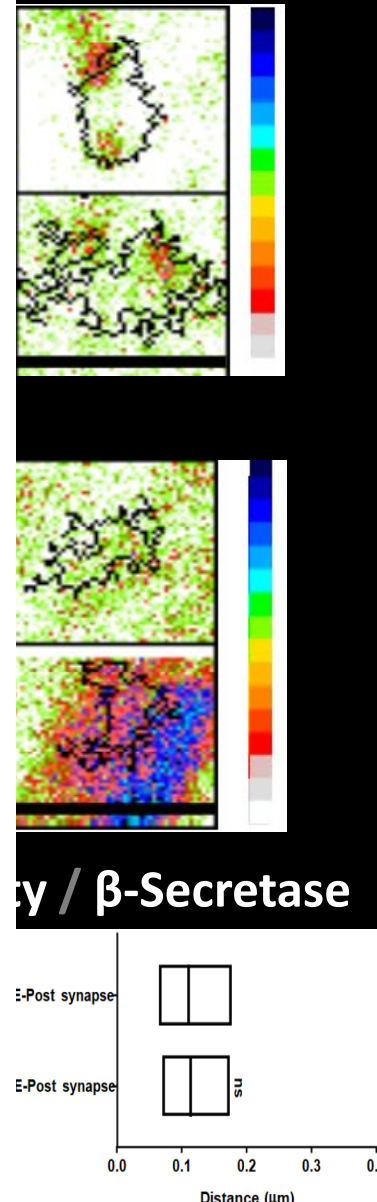
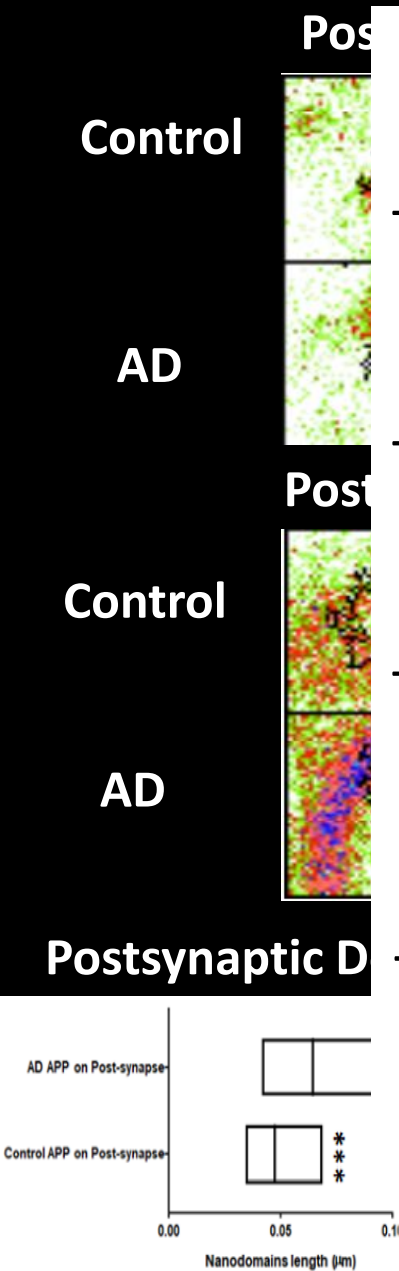
Endocytic Zone / β -Secretase



Conclusion:

- The cumulative APP and β -Secretase levels were found to be increased in EZ in AD, compared to PSD
- APP levels were augmented in PSD while β -Secretase levels decreased in PSD in AD
- The distance of APP and β -Secretase did not differ significantly in the PSD, while at EZ it was reduced for β -Secretase and augmented for APP

These observations in humans reinforce the observations in Tg mice, **confirming higher β -Secretase levels at EZ leading to an increased load of β -Secretase per endocytic event**



CONCLUSION

- The different models used to evaluate the nanoscale organization of β -amyloidogenic machinery converge to provide evidence that the compositionality of this machinery is altered at synapses, and is a critical determinant in deciding the shift in equilibrium toward β -amyloidogenic pathway.
- The authors show an increase in the content of β -secretase and APP in nanodomains. This supports our hypothesis that the availability of β -secretase and APP in nanodomains of subsynaptic compartments can be a limiting or a contributing factor for β -amyloidogenic processing of APP.
- In this study was described a holistic approach for the systematic investigation of AD as a synaptopathy. This approach uncovers a fundamental nanomachinery, where alteration in real-time molecular interactions in the scale of milliseconds to minutes can contribute toward long-term deficits such as those seen in AD.

Thanks for your attention