

Age-preserving 3D iN constructs reveal amyloid-independent, intrinsic neuronal vulnerability in sporadic Alzheimer's disease

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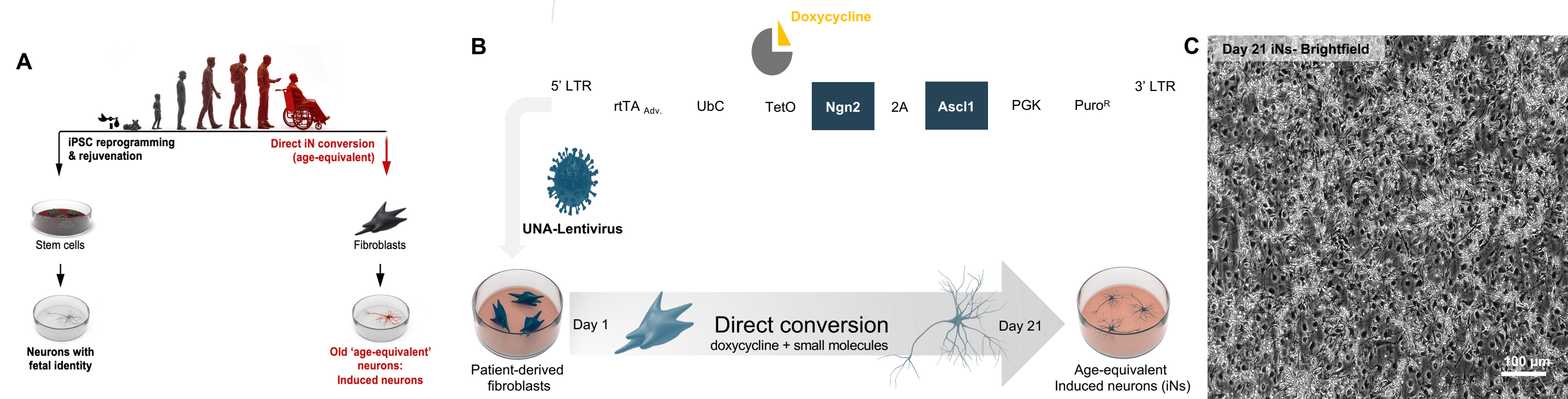
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I. Directly converted induced neurons (iNs) preserve donor aging signatures

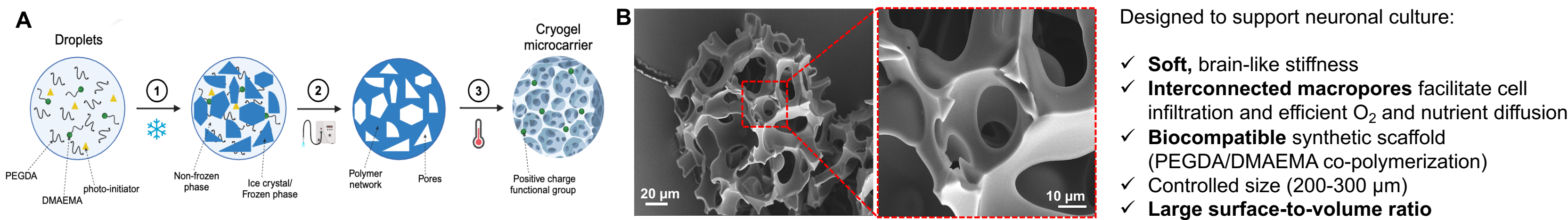
Aging is the greatest risk factor for Alzheimer's disease (AD), yet many in vitro models fail to capture age-associated neuronal features. Unlike induced pluripotent stem cell (iPSC)-derived neurons, directly converted induced neurons (iNs) **retain donor age-associated epigenetic and transcriptional signatures**, providing a physiologically relevant platform to study age-dependent AD mechanisms.



Directly converted iNs retain donor-specific aging signatures, providing an age-relevant human neuronal model for Alzheimer's disease

II. Engineering a soft cryogel microcarrier platform for 3D neuronal culture

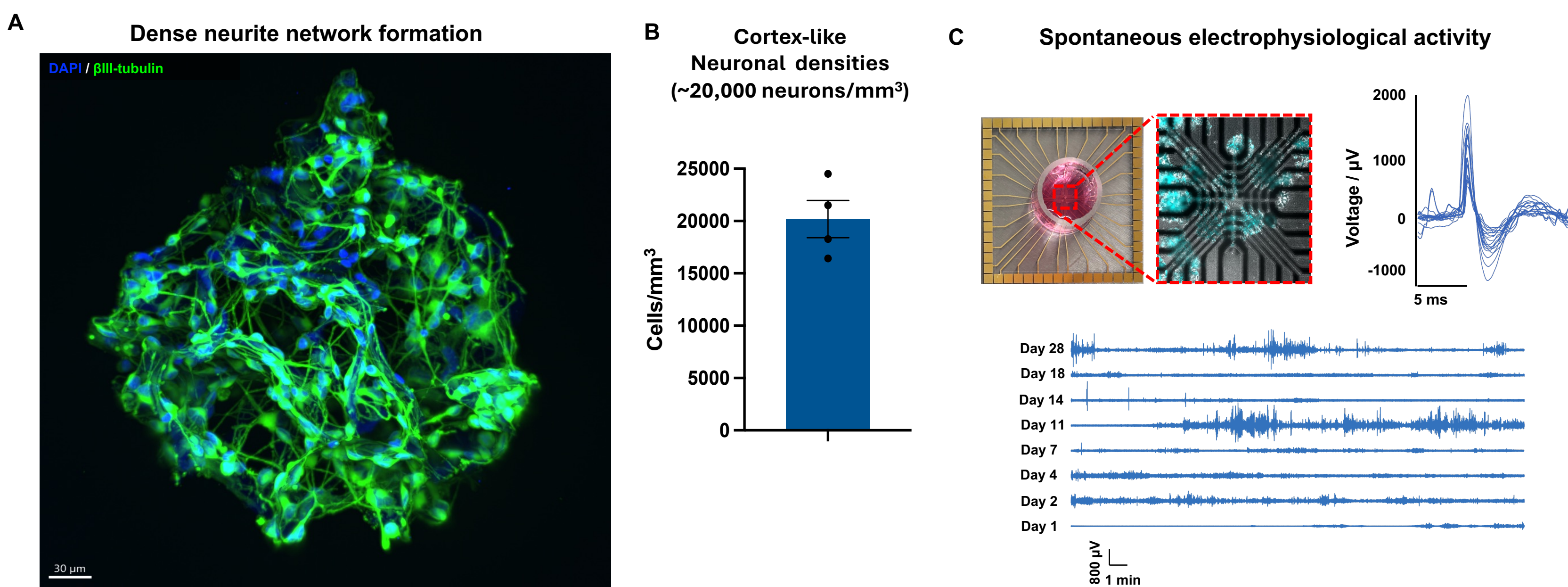
Conventional iN cultures are primarily maintained in two-dimensional (2D) environments, which fail to recapitulate the structural and extracellular complexity of the human brain. To better mimic the native neuronal microenvironment, we developed a **three-dimensional (3D) cryogel microcarrier platform (3D iN constructs)** that supports long-term culture and neuronal organization.



Soft cryogel microcarriers provide a brain-like microenvironment that supports 3D neuronal culture

III. 3D iN constructs form dense, functional neuronal networks

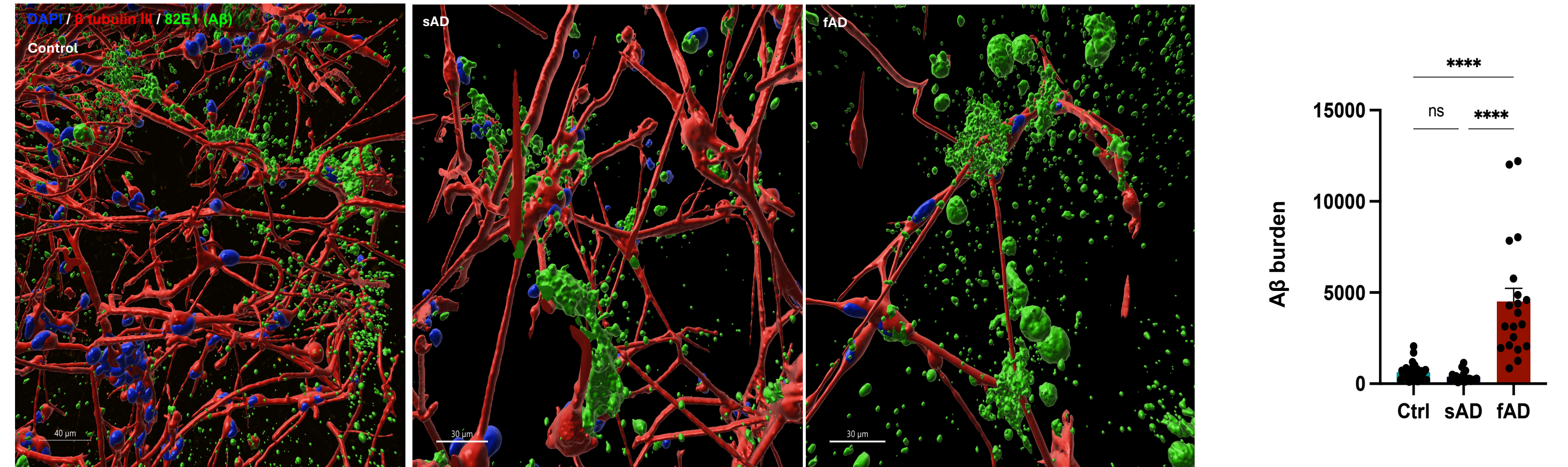
The 3D iN constructs established dense neuronal networks that achieved **cortex-like neuronal densities** and exhibited **spontaneous electrical activity**.



3D iN constructs establish dense, functional neuronal networks with cortex-like densities for modeling age-related neurodegeneration

IV. 3D iN constructs support amyloid deposition

To determine whether 3D iN constructs develop AD-relevant pathology, we assessed **β-amyloid (Aβ) deposition** across healthy controls, sporadic AD (sAD), and familial AD (fAD) cultures.

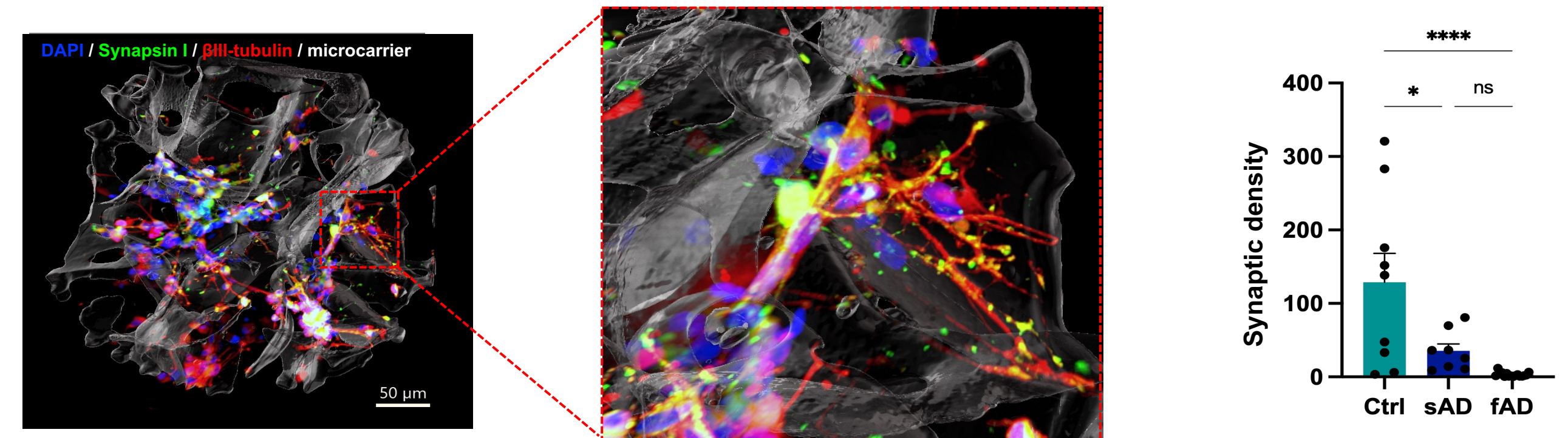


3D iN constructs support Aβ deposition, with the greatest amyloid burden observed in familial AD cultures. In contrast, control and sporadic AD cultures exhibit comparable Aβ burden.

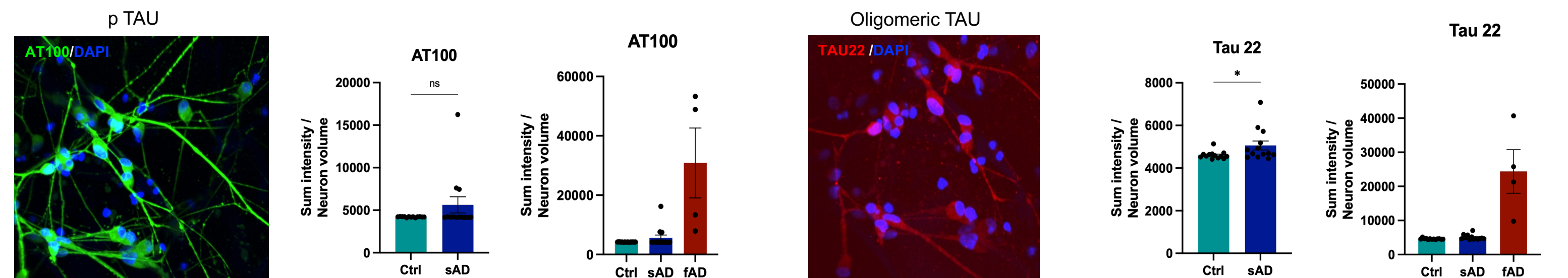
V. Intrinsic neuronal vulnerability emerges in sporadic AD despite comparable amyloid burden

Despite Aβ deposition comparable to aged controls, sporadic AD neurons exhibited **reduced synaptic density** and a trend toward increased **tau pathology**, revealing an **intrinsic neuronal vulnerability** phenotype independent of amyloid burden.

1. Synaptic vulnerability



2. TAU pathology



sAD neurons exhibit intrinsic neuronal vulnerability through synaptic loss and increased tau pathology despite comparable amyloid burden

Conclusions:

- Age-preserving 3D iN constructs provide a physiologically relevant platform for modeling Alzheimer's disease.
- The model recapitulates key AD pathologies, including Aβ deposition, tau pathology, and synaptic degeneration.
- Sporadic AD neurons exhibit intrinsic neuronal vulnerability despite comparable amyloid burden, suggesting mechanisms beyond Aβ accumulation.

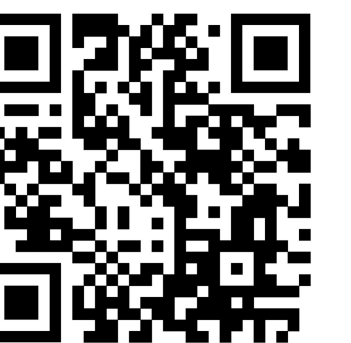
References:

Mertens J, Herdy JR, Traxler L, et al. Age-dependent instability of mature neuronal fate in induced neurons from Alzheimer's patients. *Cell Stem Cell* 2021;28(9):1533-1548.e6, doi:10.1016/j.stem.2021.04.004

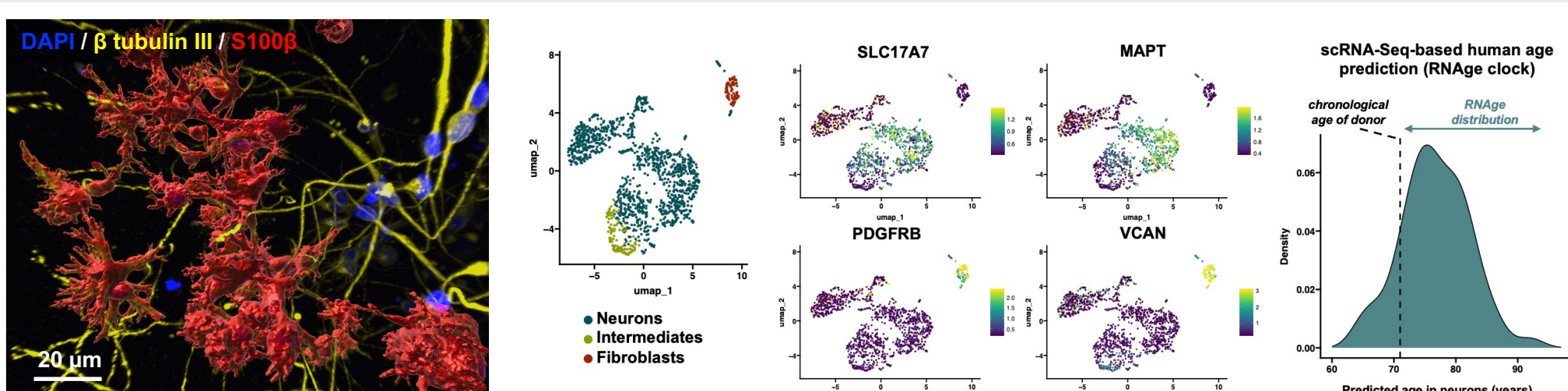
Newland B, Ehret F, Hoppe F, et al. Macroporous heparin-based microcarriers allow long-term 3D culture and differentiation of neural precursor cells. *Biomaterials* 2020/02/01;230(doi:10.1016/j.biomaterials.2019.119540

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Platform applications:



- Astrocyte-iN co-cultures
- Single-cell RNA sequencing
- ECM remodeling
- Proteomic profiling