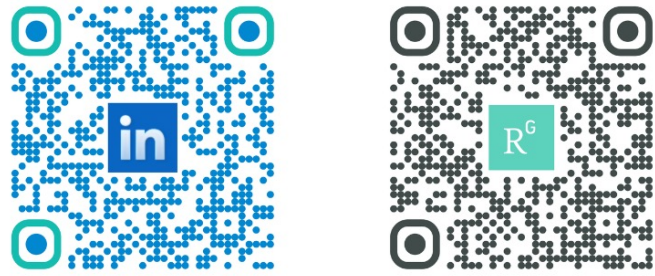


3D bioprinted models of Alzheimer's disease using iPSC-derived neuron and astrocyte co-cultures: Enhancing pathological behaviour.



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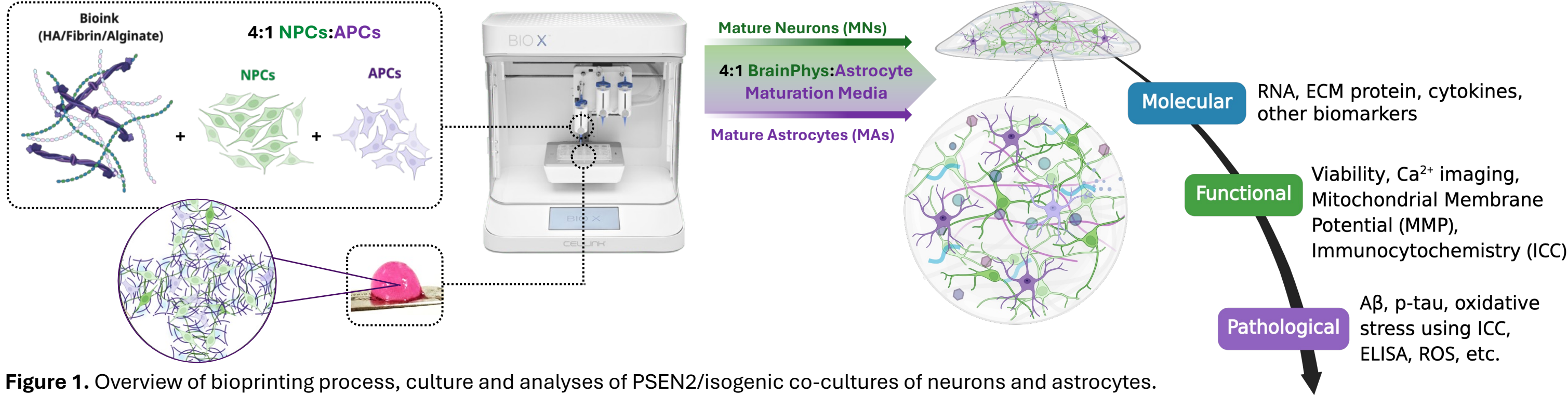
Background

- Three-dimensional (3D) bioprinting can create complex, human-relevant tissue models by precisely depositing multiple cell types within bioinks that mimic the brain's microenvironment. This technique enables a reproducible architecture necessary for mimicking the intrinsic cell behaviour within neurodegenerative disorders such as **Alzheimer's Disease (AD)**.
- Further, human induced pluripotent stem cells (hiPSCs) derived from individuals with AD can generate multiple cell types, enabling the development of multicellular models that better recapitulate AD pathology. For example, incorporating astrocytes into these models captures their contribution to AD pathogenesis, resulting in more representative disease phenotypes¹⁻³.
- This work developed **3D bioprinted neuron-astrocyte co-cultures** from familial AD cell lines (*PSEN2*) and isogenic controls within a recently developed **hyaluronic acid (HA)-based bioink** that mimics the brain **extracellular matrix (ECM)**⁴.
- We assessed whether **3D bioprinted multicellular models could recapitulate key aspects of AD** while supporting multicellular maturation and function, establishing a foundation for future drug screening platforms.

Key Takeaways

- Bioprinted neuron-astrocyte models could **mimic early pathologies** of Alzheimer's disease
- AD models show **reduced viability**, **dysfunctional mitochondria** and **irregular calcium dynamics**
- AD models have **elevated soluble p-tau** and **Aβ 42/40** compared to isogenic controls after 60 days

Methods



Results

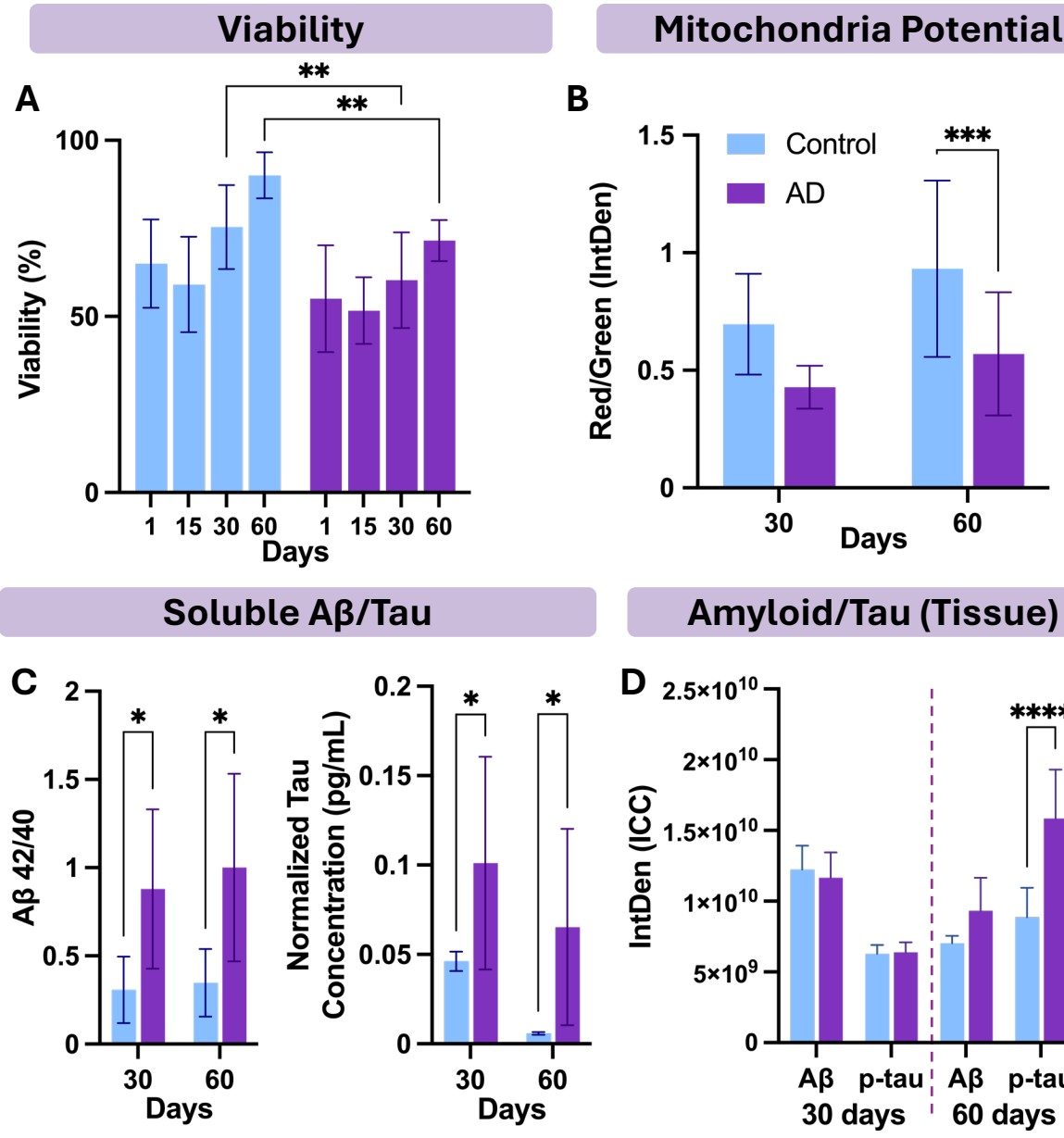


Figure 2. Results of bioprinted co-cultures over 60 days showing (A) Viability, (B) mitochondrial membrane potential, (C) soluble Aβ 42/40, and (D) quantified Aβ and tau within models.

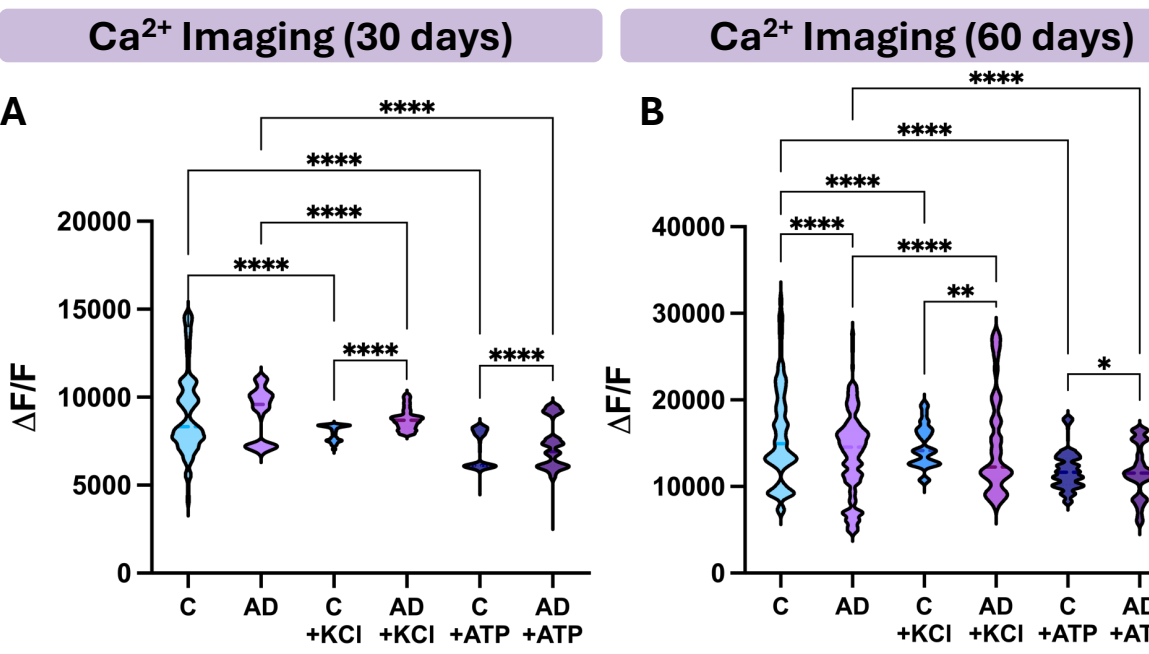


Figure 3. Change in fluorescence (ΔF/F) of live cell calcium (Ca²⁺) imaging after 30 (A) and 60 days (B) for isogenic controls (C) and AD co-culture models. Results show spontaneous calcium dynamics, and activity induced with KCl or ATP.

- AD co-cultures have decreased cell viability overall compared to controls, which worsens over longer culture
- AD models have decreased mitochondrial membrane potential, indicating less healthy and stressed cultures

- AD co-cultures have higher levels of soluble Aβ 42/40 and tau after 30 and 60 days.
- Over time, AD co-cultures show increasing levels of Aβ and significantly higher levels of phosphorylated tau (p-tau) in the models after 60 days.

- Overall, both models have higher calcium activity after 60 days (ΔF/F values).
- AD models have exaggerated calcium responses following KCl depolarization, similar to AD patients⁵.
- AD co-cultures had less synchronous networks, consistent with other analyses (ICC).

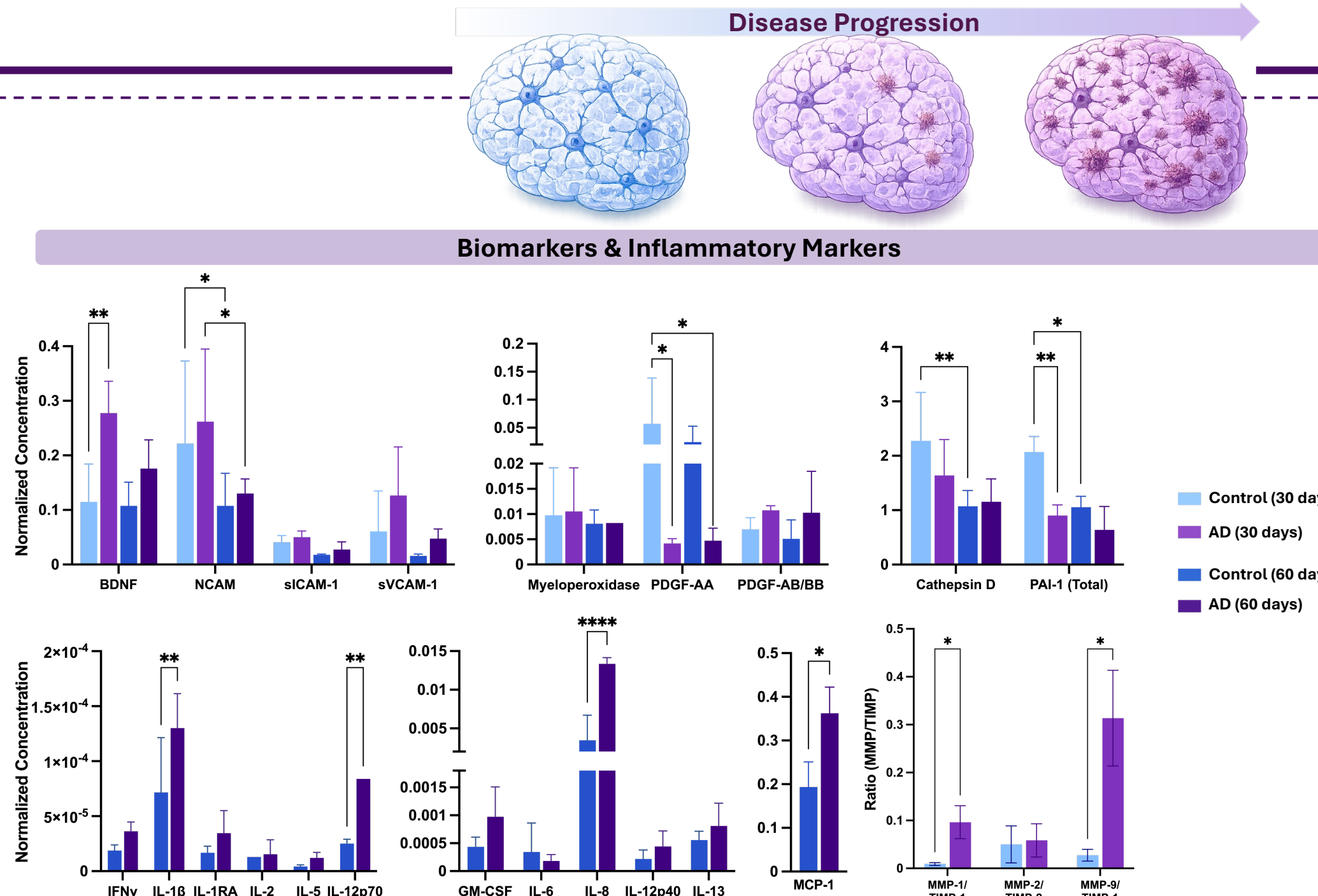


Figure 4. Normalized concentration (pg/mL) of biomarkers and inflammatory markers over 60 days.

- AD models show increased levels of certain inflammatory markers (IL-1β, IL-12p70, IL-8, and MCP-1) after 60 days and matrix metalloproteinase ratios (MMP-1/TIMP-1), especially MMP-9/TIMP-1 after 30 days, with similar trends to AD patient plasma⁶.
- Lower levels of PAI-1 are also similar to plasma levels in early AD pathology⁷.

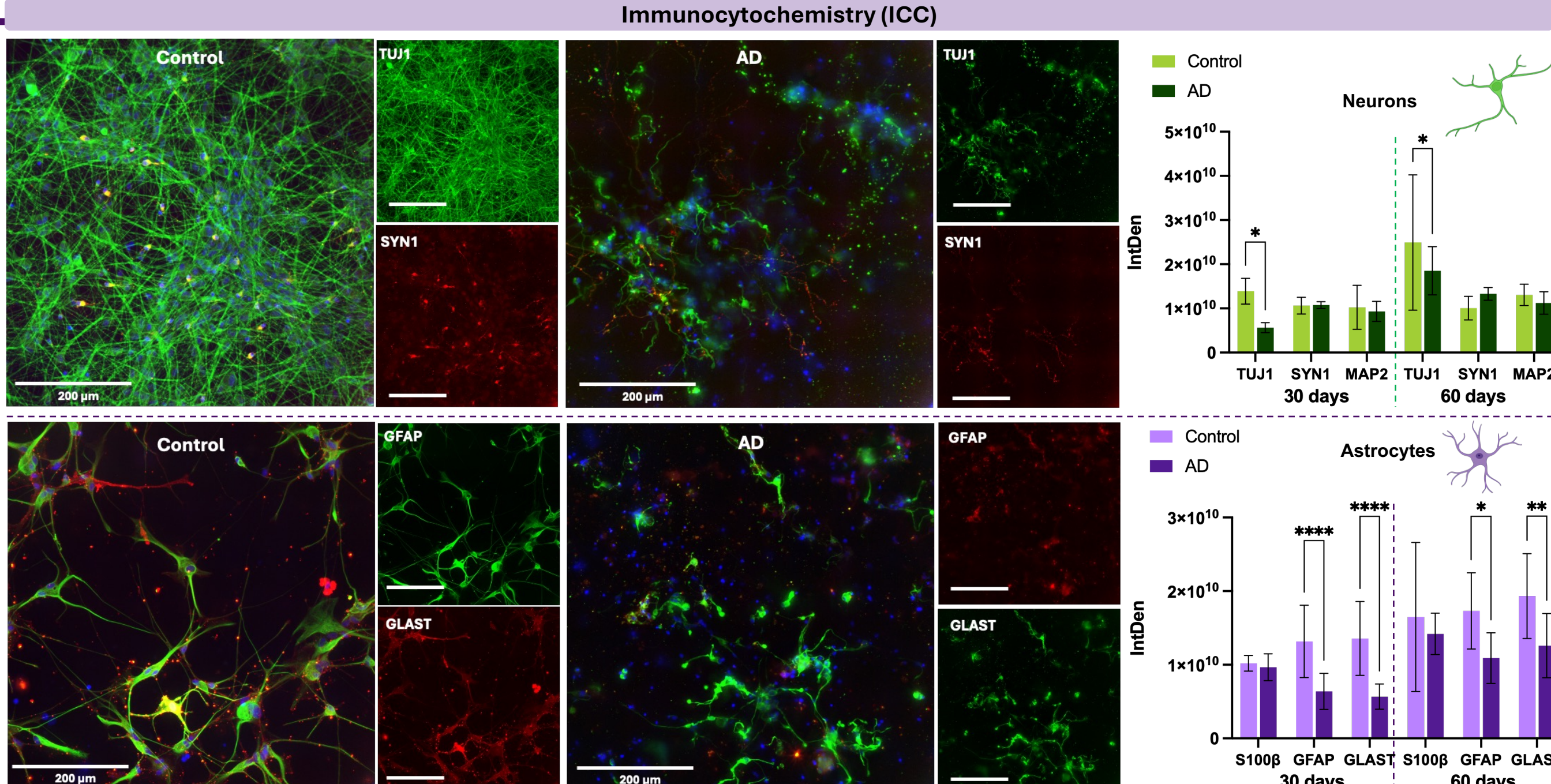


Figure 5. ICC of neural (A) and astrocytes (B) markers for control and AD models after 60 days. Right panel is quantified ICC data at 30 and 60 days.

- AD co-cultures have decreased mature neural network formation and synaptic density, with significantly lower TUJ1.
- Isogenic controls have more homogeneous structural astrocyte formation (GFAP⁺) and functional networks (GLAST⁺).
- AD models have localized GLAST, and lower GFAP/GLAST consistently over time.

Conclusions

- Astrocyte inclusion within 3D bioprinted models recapitulates key functional and structural roles that promote AD phenotypes *in vitro*.
- Relevant AD pathologies were observed in the co-cultured models, including decreased cell viability, neurite network formation (TUJ1⁺), and functional network formation (lower GLAST, SYN1 distribution).
- Elevated levels of Aβ42/40 and tau support the formation of early AD markers *in vitro*
- These models can serve as a foundational basis for studying disease model progression and viable tools for evaluating potential therapies with pathological human relevance for AD.

