



Modelling Parkinson's Disease *In Vitro*: Genetic and Chemical Lesions In VTA-Like Spheroids

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Objectives

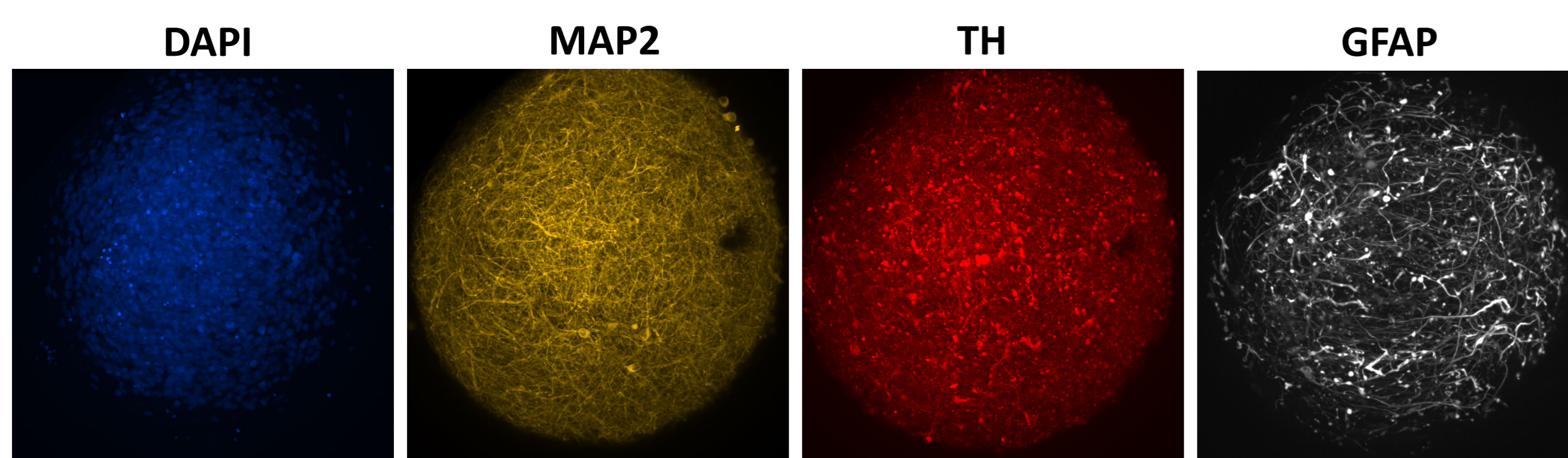
Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons and the accumulation of pathological α -synuclein (α -syn). There is a growing need for advanced *in vitro* systems that can mimic human neurobiology and support translational research. In this study, we utilize 3D spheroids composed of differentiated iPSC-derived neurons, including dopaminergic subtypes, to model the ventral tegmental area (VTA) and investigate PD-related pathology under controlled genetic and chemical stress conditions.

Materials and Methods

A spheroid model was generated by aggregating differentiated iPSC-derived neurons, including the dopaminergic subtype, and astrocytes into 3D structures resembling the VTA. Two lesion types were introduced: a genetic insult via AAV-mediated expression of α -syn carrying the A53T mutation (AAV-A53T), and a chemical challenge using the neurotoxin MPP+. Biomarker responses were evaluated by measuring NF-L and GFAP in the supernatant through immunoassays. Immunocytochemistry was employed to assess dopaminergic neuron integrity and α -syn accumulation. TMRM live-cell assay was used to analyze mitochondrial function.

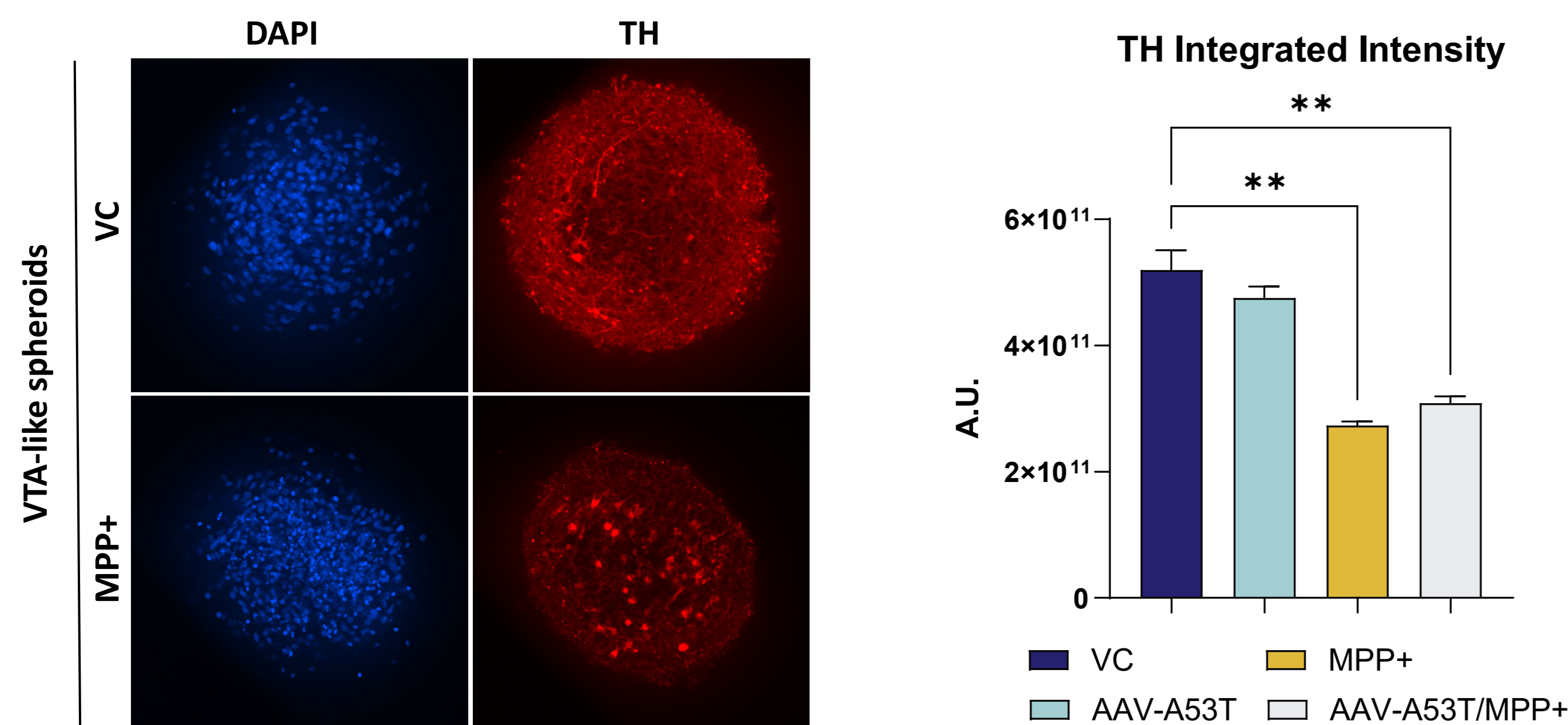
Results

VTA-Like iCell Neurospheres



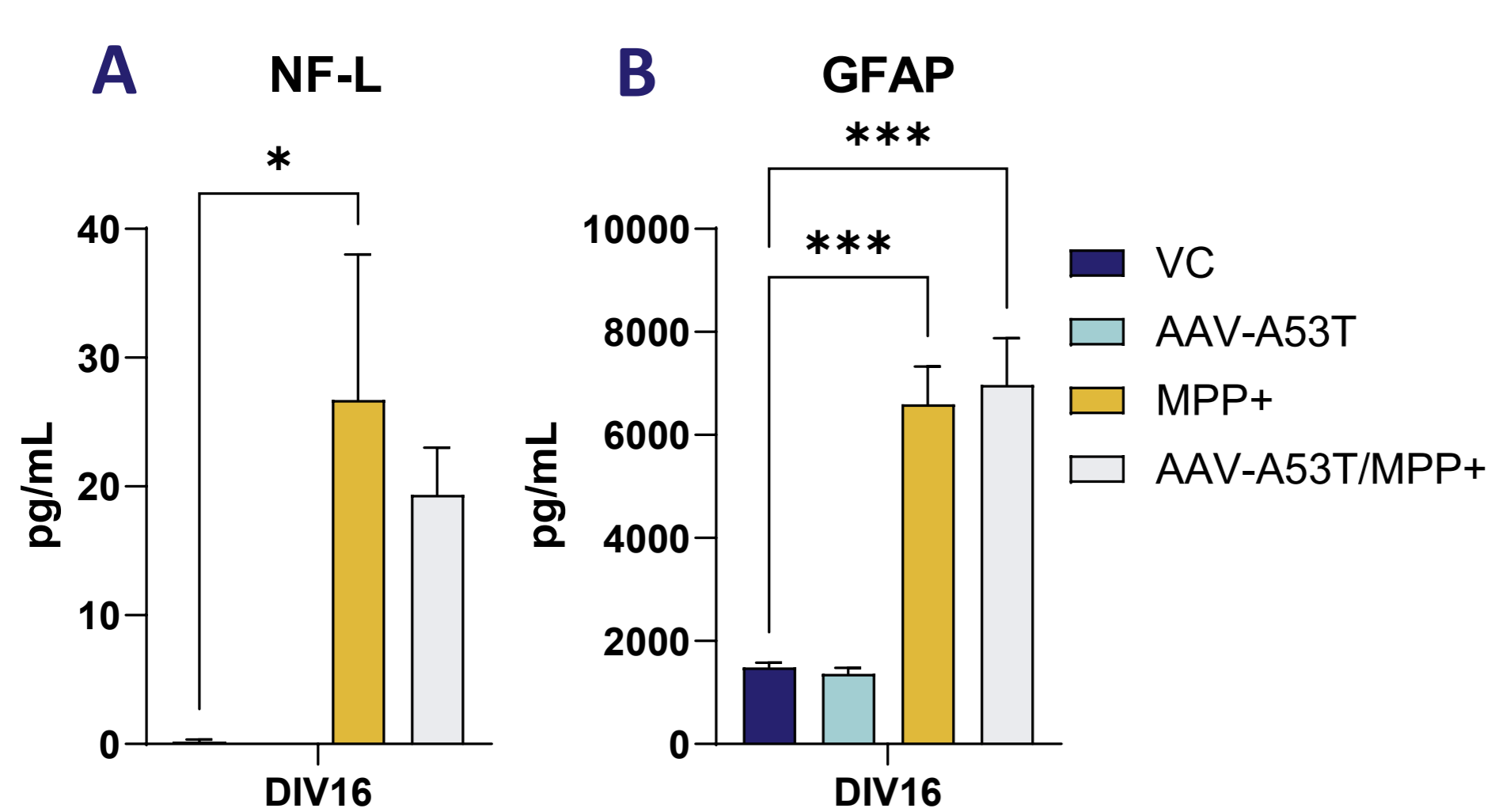
▲ Figure 1: VTA-like iCell NeuroSpheres (FUJIFILM Cellular Dynamics) at DIV21. Immunocytochemistry was performed for MAP2, TH and GFAP markers. Images were acquired with ImageXpress HT.ai confocal microscope (Molecular devices).

Dopaminergic Neurons Integrity



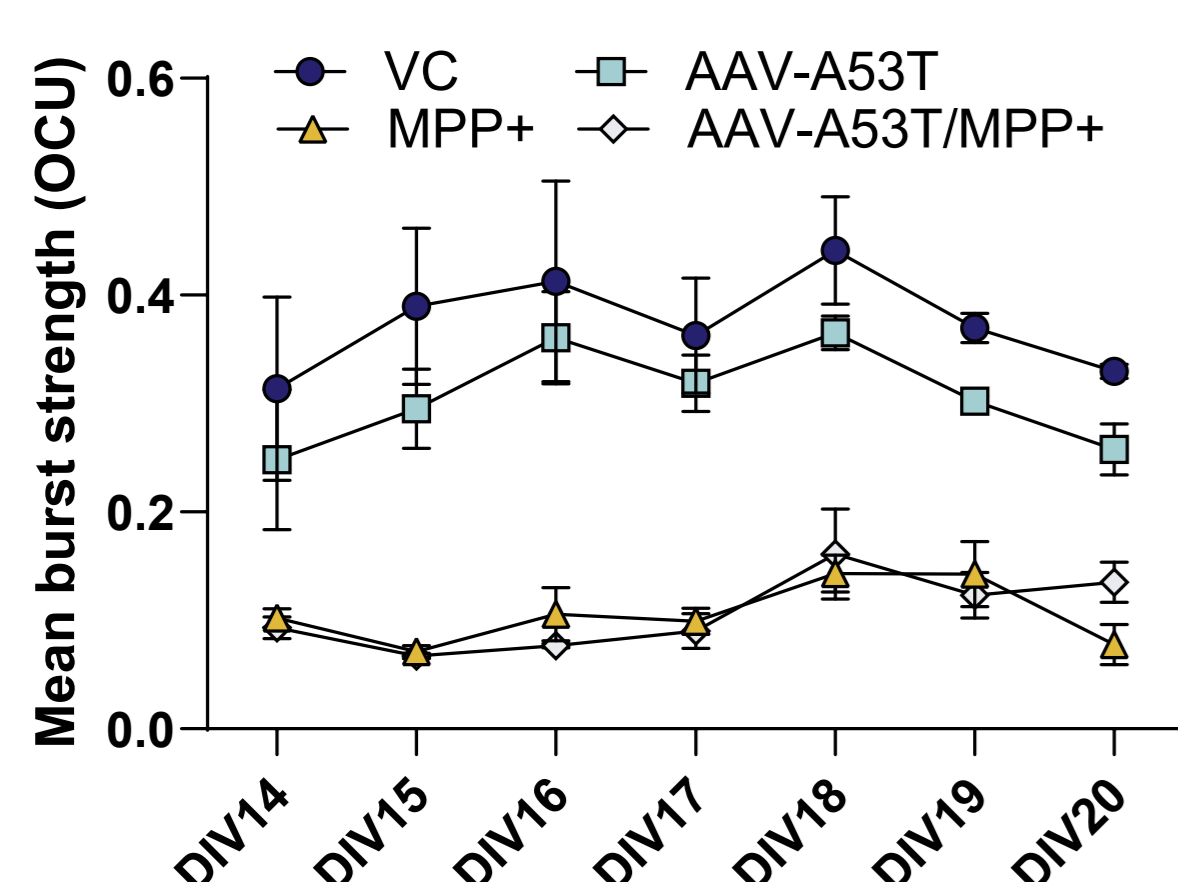
▲ Figure 2: Dopaminergic neurons loss of VTA-like iCell NeuroSpheres (FUJIFILM Cellular Dynamics) after treatment. VTA-like neurospheres were treated with MPP+ for 48 hours. Dopaminergic loss was determined as a reduction of TH intensity assessed with ImageXpress HT.ai confocal microscope (Molecular devices). Mean + SEM; n = 3-5, One-way ANOVA followed by Dunnet's multiple comparisons test. **p < 0.01.

NF-L and GFAP levels



◀ Figure 3: NF-L and GFAP levels in VTA-like iCell NeuroSpheres (FUJIFILM Cellular Dynamics) after treatment. Changes of NF-L (A) and GFAP (B) secretion after treatment with MPP+, AAV-A53T, or both for 48 hours. Analysis was performed by MSD. Mean + SEM; n = 3-5; One-way ANOVA followed by Dunnet's multiple comparisons test. *p < 0.05; ***p < 0.001.

Neuronal Activity - Burst Strength



▲ Figure 4: Neuronal Activity of VTA-like iCell NeuroSpheres after lesions. VTA-like neurospheres were transduced with a genetically encoded calcium indicator (GECI) to indirectly monitor neuronal activity. Imaging was started on DIV14 72h after addition of lesions. Mean ± SEM; n = 3 per group; QR-code videos show DIV17 status. No statistical analysis was performed.

Video of a VTA-like spheroid treated with vehicle control ▶

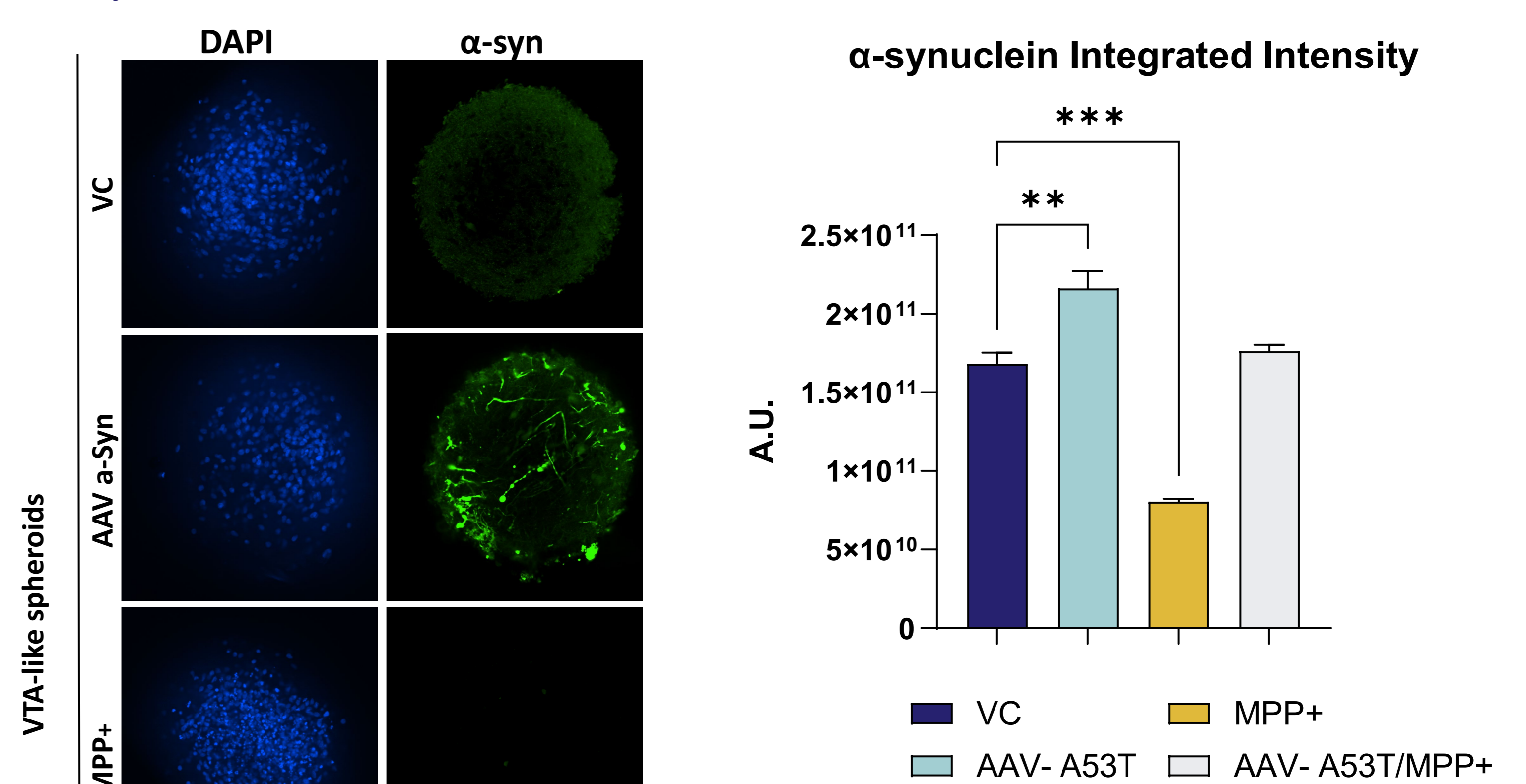
◀ Video of a VTA-like spheroid treated with mitochondria targeting neurotoxin MPP+

Video of a VTA-like spheroid transduced with an AAV overexpressing a-syn carrying A53T mutation ▶

◀ Video of a VTA-like spheroid transduced with an AAV overexpressing a-syn carrying A53T mutation and MPP+

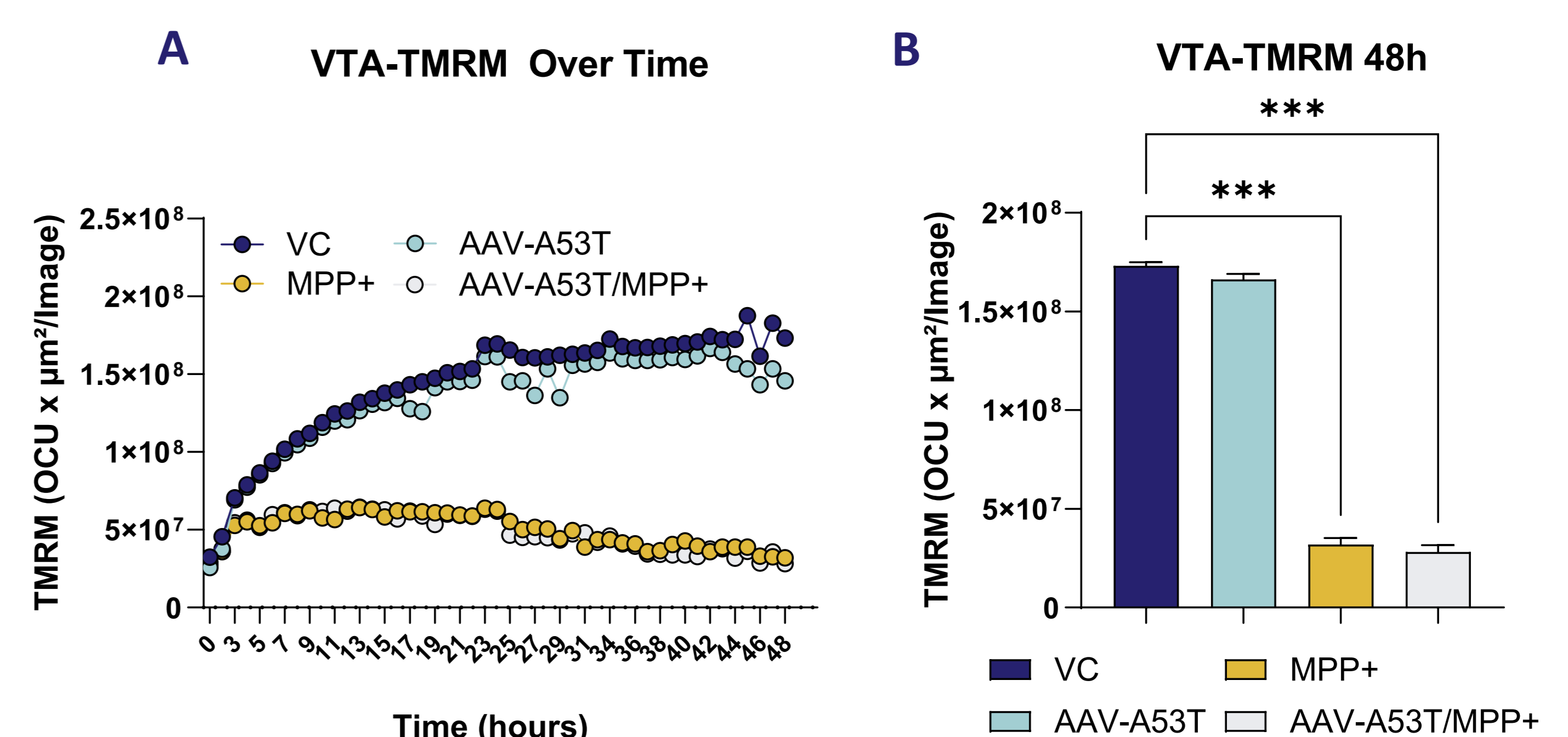
Results

α -synuclein Accumulation



◀ ▲ Figure 5: α -synuclein levels in VTA-like iCell NeuroSpheres (FUJIFILM Cellular Dynamics) after treatment. VTA-like neurospheres were treated with MPP+, AAV-A53T, or both for 48 hours. α -syn accumulation was determined as an increase of intensity assessed with ImageXpress HT.ai confocal microscope (Molecular devices). Mean + SEM; n = 3-5; One-way ANOVA followed by Dunnet's multiple comparisons test. **p < 0.01, ***p < 0.001.

Mitochondrial Activity



▲ Figure 6: Mitochondrial activity in VTA-like iCell NeuroSpheres (FUJIFILM Cellular Dynamics) was evaluated by TMRM assay using Incucyte®. VTA-like neurospheres were treated with MPP+, AAV-A53T, or both for 48 hours. A: Total TMRM intensity over time. B: Total TMRM intensity at 48 h. Mean + SEM; n = 5. One-way ANOVA followed by Dunnet's multiple comparison test. ***p < 0.001. OCU: Total orange object intensity.

Conclusion

Spheroid models including dopaminergic neurons provide a robust and scalable approach for studying Parkinson's disease mechanisms, offering insights into neurodegenerative processes and potential therapeutic targets.

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For more information about the models please visit: www.scantox.com
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