

AGGREGATION AND NEURODEGENERATION: PARTNERS IN CRIME

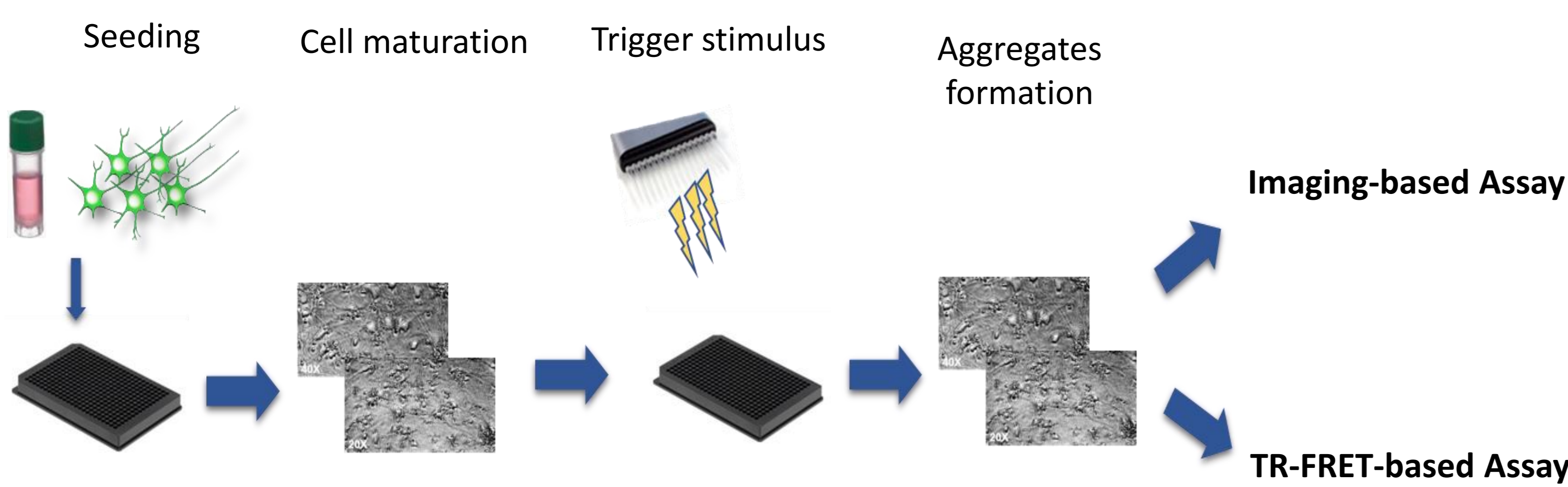
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Summary

Misfolded proteins and protein aggregation are drivers and accelerators for neurodegenerative diseases, including Alzheimer's Disease (AD) and Parkinson's Disease (PD). Interference with these processes holds promise for new treatment opportunities. An essential role is played by iPSC-derived neurons to reproduce the pathways of aggregation in a dish. Apparently Healthy Normal (AHN) iCell® GlutaNeurons (FCDI) and iCell® DopaNeurons (AHN, engineered SNCA A53T, and PD donor-derived LRRK2 G2019S or GBA N370S; FCDI) were cultured in 384-well plate format and seeded with pre-formed fibrils (PFFs) of Tau or α -Synuclein (α -Syn) proteins. The development of pathogenic disease-associated forms of Tau and α -Synuclein was assessed using different readouts including TR-FRET and immuno-staining.

Aggregation assays using iPSC-derived neurons



Tau aggregation in iCell® GlutaNeurons (GNCs)

Figure 1. GNCs showed Tau deposits and increased number of Amytracker spots, by Imaging, and Tau aggregation by TR-FRET (HTRF, Cisbio) after seeding with Tau PFFs.

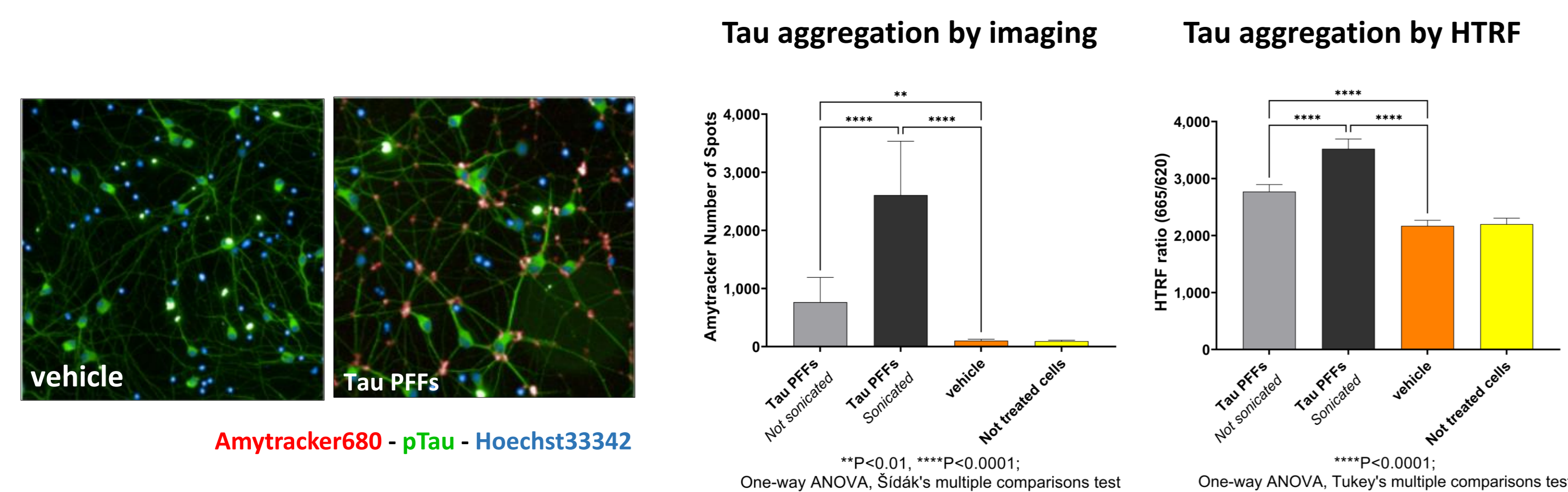
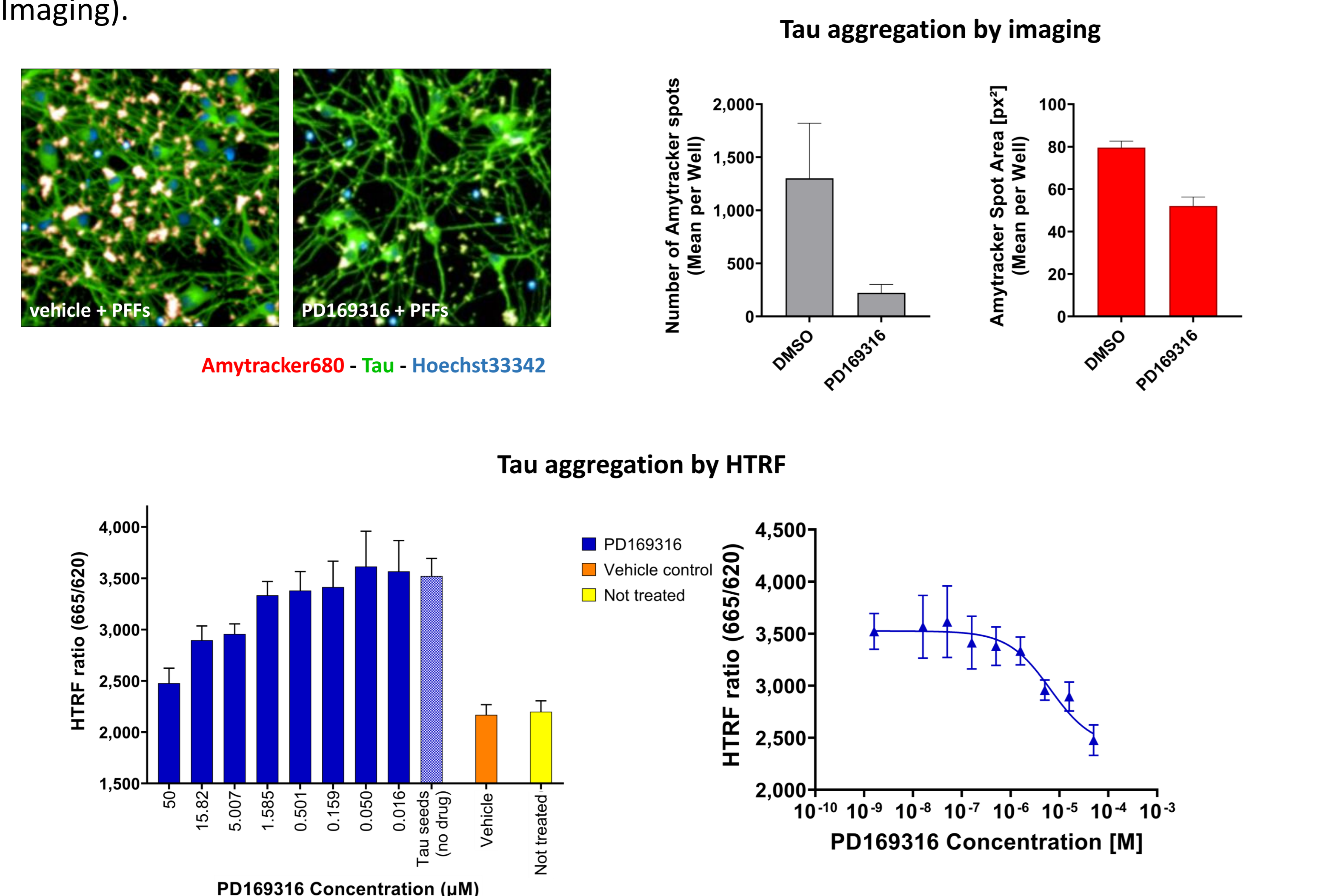


Figure 2. The treatment with proteasome activator PD169316 reduced Tau aggregation in a concentration/response (c/r) manner (by HTRF), as well as the number of Amytracker spots (by Imaging).



α -Synuclein aggregation

in iCell® DopaNeurons (DNCs) (AHN-SNCA-LRRK2-GBA)

Figure 1. AHN DNCs showed accumulation of total α -Syn aggregates after seeding with α -Syn PFFs. α -Syn aggregation was evaluated measuring α -Syn spots, by Imaging and by TR-FRET (HTRF, Cisbio) in c/r manner (C1: top concentration).

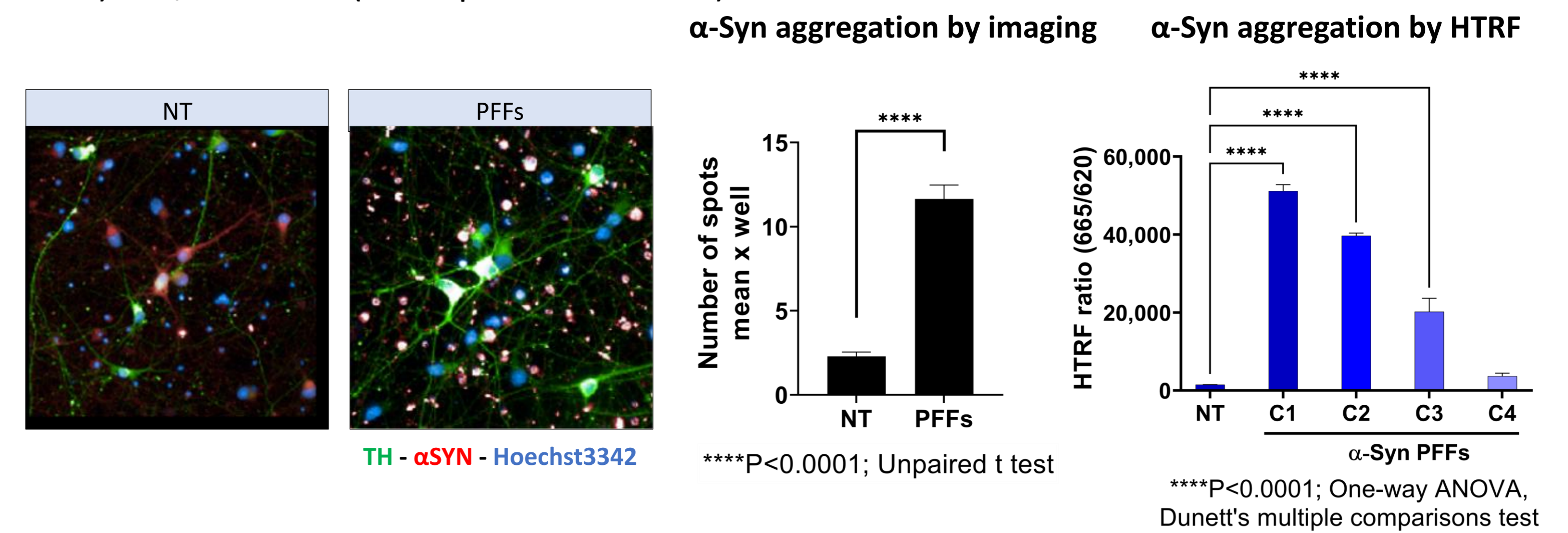


Figure 2. siRNA-mediated knockdown showed abolishment of α -Syn detection in both AHN and SNCA A53T-mutant DNCs.

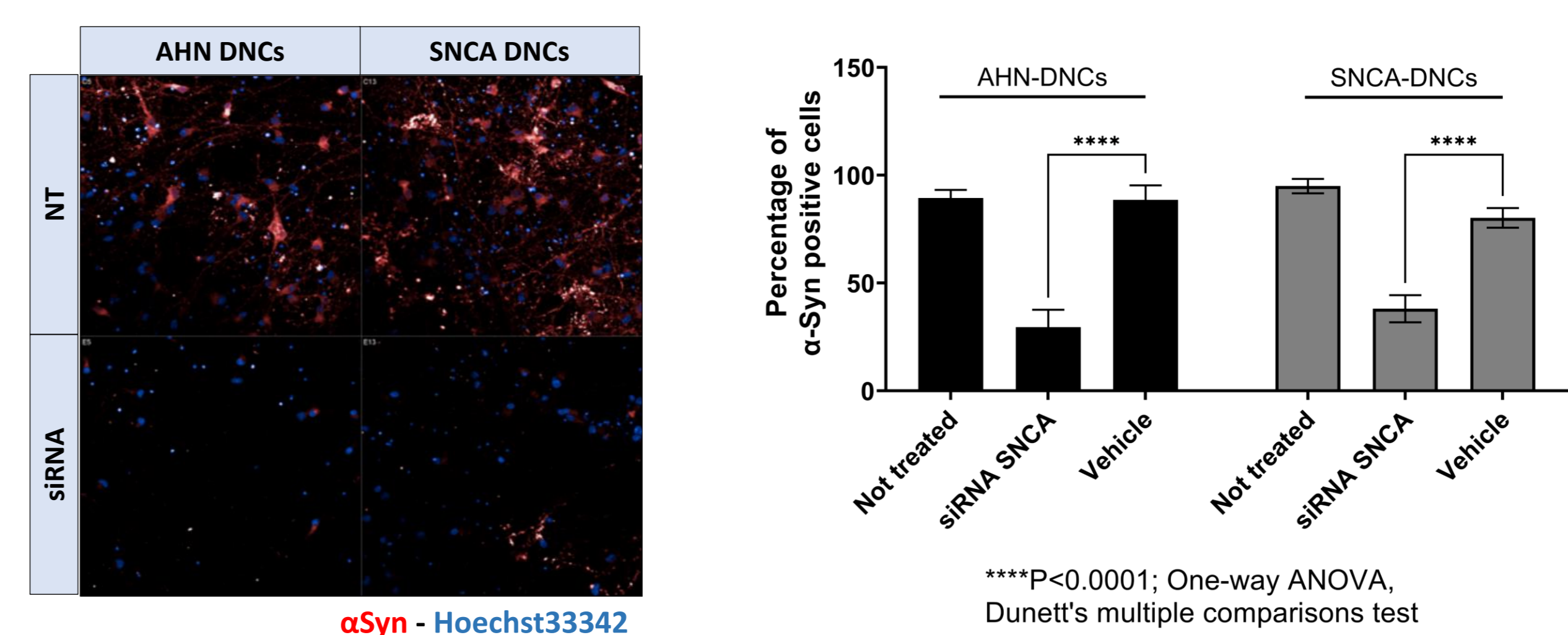


Figure 3. The treatment with proteasome activator PD169316 reduced α -Syn aggregates in a c/r manner, as observed by imaging and TR-FRET readouts.

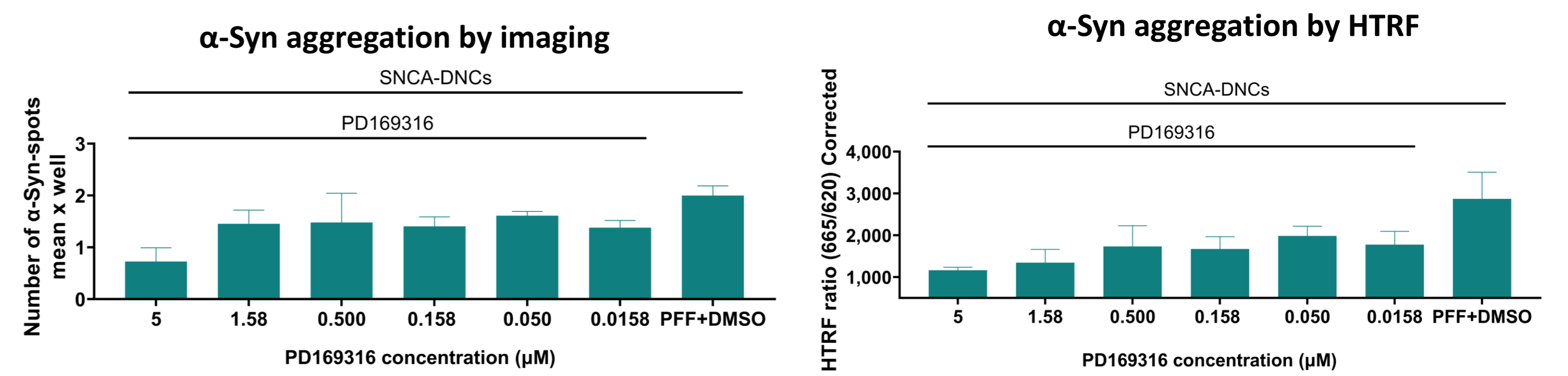


Figure 4. DNCs characterization was performed using tyrosine hydroxylase (TH) staining as marker for dopaminergic neurons in the central nervous system. A similar percentage of TH-positive neurons was observed across cell lines.

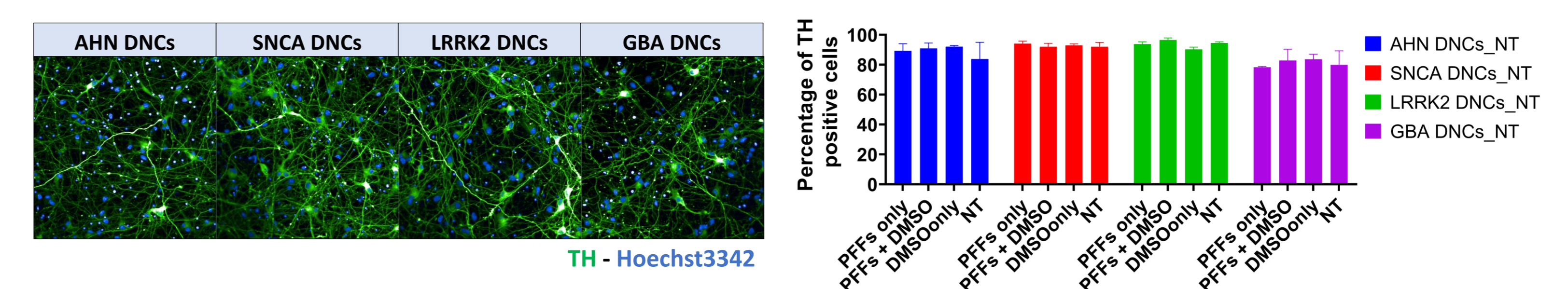


Figure 5. PD-derived DNCs showed higher α -Syn aggregates compared to AHN DNCs when treated with PFFs, as observed by imaging and TR-FRET readouts.

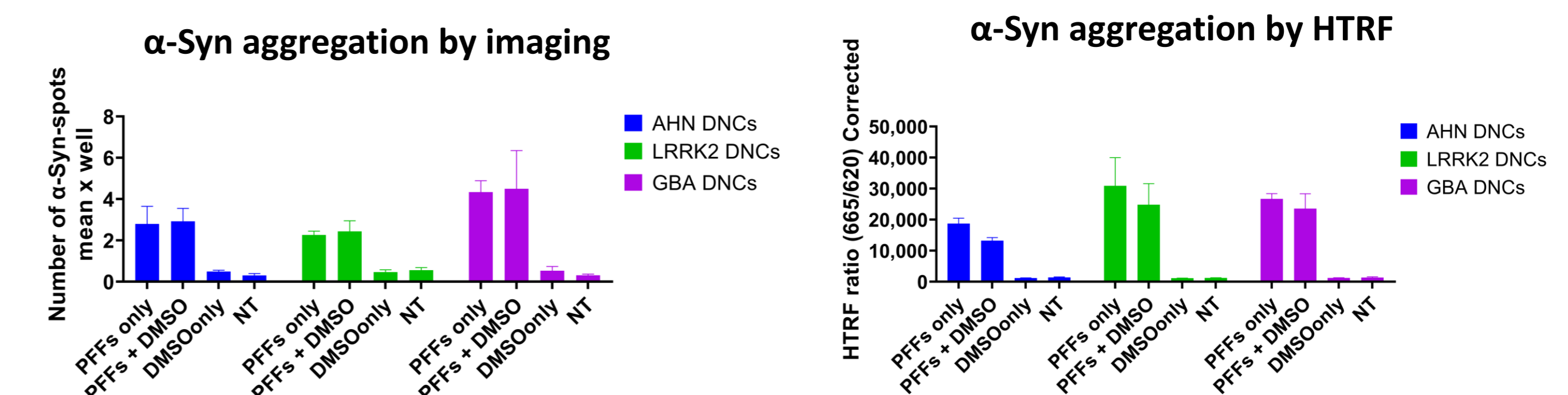
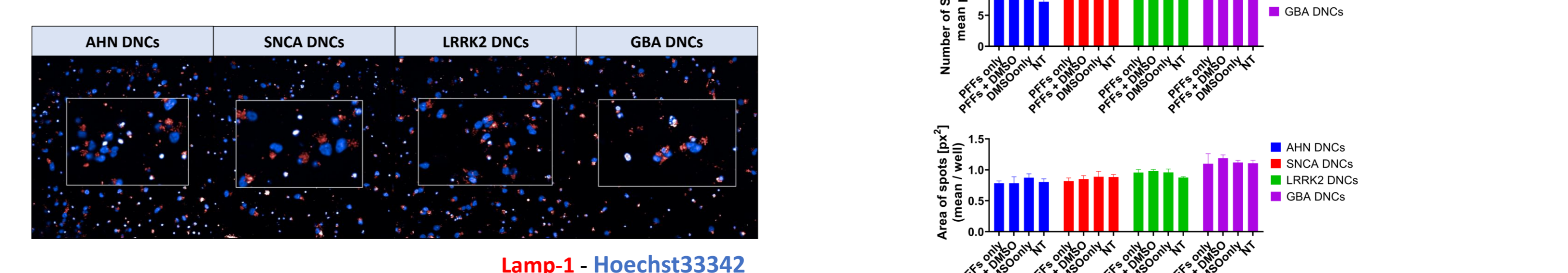


Figure 6. Lamp-1 staining was used to measure autophagy in DNCs. GBA-mutant DNCs show increased area of Lamp-1 puncta (by Imaging).



Conclusions

We provide a physiologically relevant assay platform to model PD and AD for drug discovery, multiplexable with several readouts, and suitable for compound profiling and screening campaigns.