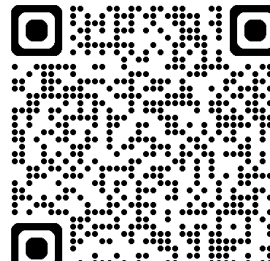


From Patient Cells to Clinical Translation: Development, Manufacturing, and Qualification of an Autologous iPSC-Derived Dopaminergic Cell Therapy for Parkinson's Disease



Alexis N. Corl, Alistair Wilson, Araceli Ramirez, Chase Monckton, Dana Ambruzs, Heidi Olivares, Juan Pintor Cuellar, Matt Goodus, Noelle Ying, Robert Srijemac, Scott Reher, Steve Schreiber, Su-Chun Zhang, Young Lee, Maria-Daniela Cîrnaru

BrainXell Therapeutics, 6370 Nancy Ridge Suite 110 San Diego CA, 92121, USA.



Introduction

Parkinson’s disease (PD) is a common neurodegenerative disorder characterized by the progressive loss of midbrain dopaminergic (mDA) neurons in the substantia nigra [1]. These neurons are essential for dopamine production, having a critical role in motor control, regulating mood and other cognitive functions. Current treatments improve symptoms but do not restore lost neurons or alter disease progression. Restoring the dopaminergic neurons is vital in developing an effective treatment for PD, and the use of induced pluripotent stem cells (iPSCs) provides a promising autologous source of mDA progenitors (mDAPs) for transplantation [2]. As a personalized treatment without complications of immune response, using mDAPs with high purity and differentiation potential from a patient's own reprogrammed iPSCs is an avenue to replenish the lost midbrain dopaminergic neurons. Clinical translation requires a robust and reproducible manufacturing process, GMP-readiness, demonstration of product safety and stability, and consistent quality and functional characteristics across different cell lines, which we address here for BXT-110.

Methods

Our patient-to-product path for an autologous iPSC-derived PD cell therapy product begins with fibroblast cultures from PD patient skin biopsies which are reprogrammed to iPSCs via mRNA technology. Patients are pre-screened to exclude PD mutations. The iPSC lines are banked and tested for genomic integrity, including oncogenic variants, and pluripotency markers OCT4/NANOG co-expression. We generated a total of 28 iPSC lines from normal healthy volunteers and PD patients. To induce mDAPs, we utilize small molecules to achieve dual inhibition of SMAD signaling and drive neurogenesis. Precise regulation of Wnt and Sonic Hedgehog (SHH) signaling promotes appropriate regionalization of midbrain floor plate (FP) progenitors, and FGF8 is used to generate a population of FP-derived ventral dopaminergic progenitors [3]. Conventional 2D adherent culture methods showed high variability and limited scalability. We developed a novel 3D culture system which generates uniform spheroids that respond consistently to differentiation cues. This developmental work was performed across five PD lines (Figs. 1, 2). A 24hr aggregation step was implemented to convert cryopreserved mDAPs – DAP-01 Drug Substance (DS) – into fresh mDAP spheroids – BXT-110 Drug Product (DP). These processes were transferred to GMP manufacturing (Fig. 3) where we quantified cell yield and viability of the DS harvest and, using flow cytometry, we characterized mDAP identity through FOXA2/OTX2 co-expression and impurity through OCT4/NANOG co-expression, indicating the presence of residual iPSCs. BXT-110 DP was manufactured from DAP-01 DS across seven iterations to evaluate the product. Two of these lots were plated as whole spheroids at three timepoints (0, 48, and 72 hours) and differentiated for 14 days to evaluate the differentiation potential *in vitro*. The plates were fixed with paraformaldehyde, and immunocytochemistry (ICC) was used to quantify and characterize the neuronal populations (Fig. 4). To assess the efficacy and its reproducibility, 3D developed BXT-110 from two PD patient lines were transplanted intrastrially into female Rowett nude (RNU) rats with unilateral 6-OHDA lesions of the medial forebrain bundle. Amphetamine-induced rotations were assessed up to 26 weeks post-transplant, after which grafts were analyzed by immunostaining and stereological quantification (Figs. 5, 6). We executed a GLP toxicology study with 92 animals across 5 groups – with an acute timepoint of 29 days and a chronic timepoint of 183 days. Toxicology and biodistribution were evaluated across the vehicle control and high/low doses of BXT-110 DP. Tumorigenicity was evaluated across 100% iPSCs and a high dose BXT-110 DP spiked with 0.1% iPSCs (Fig. 7).

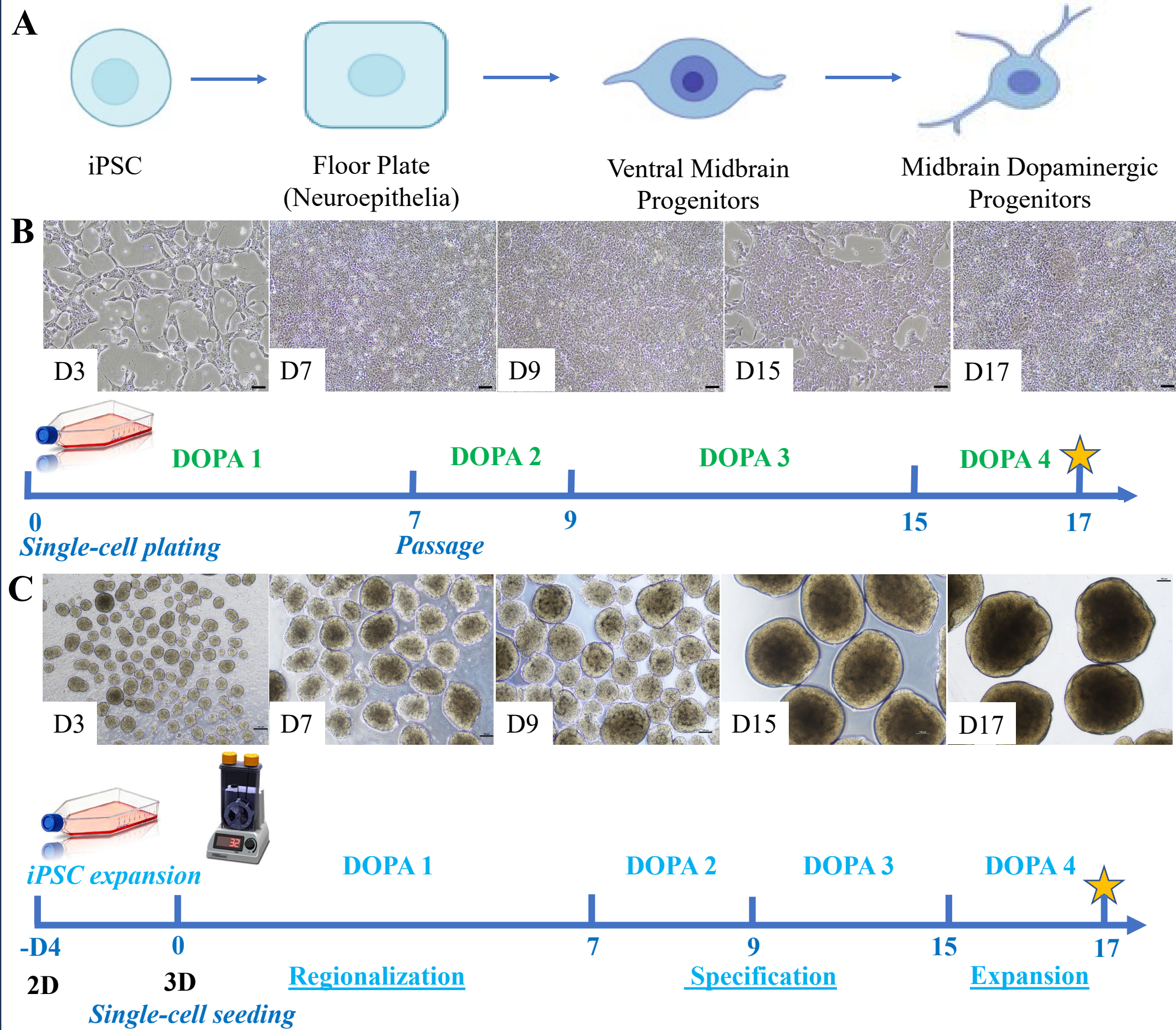


Figure 1. Induction protocol from iPSC to mDAP: **A.** Simplified schematic of the induction pathway. **B.** Format and timeline of 2D induction. Single cell iPSCs are plated into a T25 flask, passed on day 7, and dissociated/cryopreserved on day 17. Images taken before each change in media composition, called DOPA 1-4. Scale bar: 100µm **C.** Format and timeline of 3D suspension induction. Single cell iPSCs are expanded in a T175 flask for 4 days in TeSR AOF medium, harvested at about 70% confluency, and seeded into a 0.5L PBS-mini vertical wheel bioreactor. Spheroids are harvested and dissociated on day 17 and cryopreserved. Images taken before each change in media composition. Scale bar: 100µm

Results

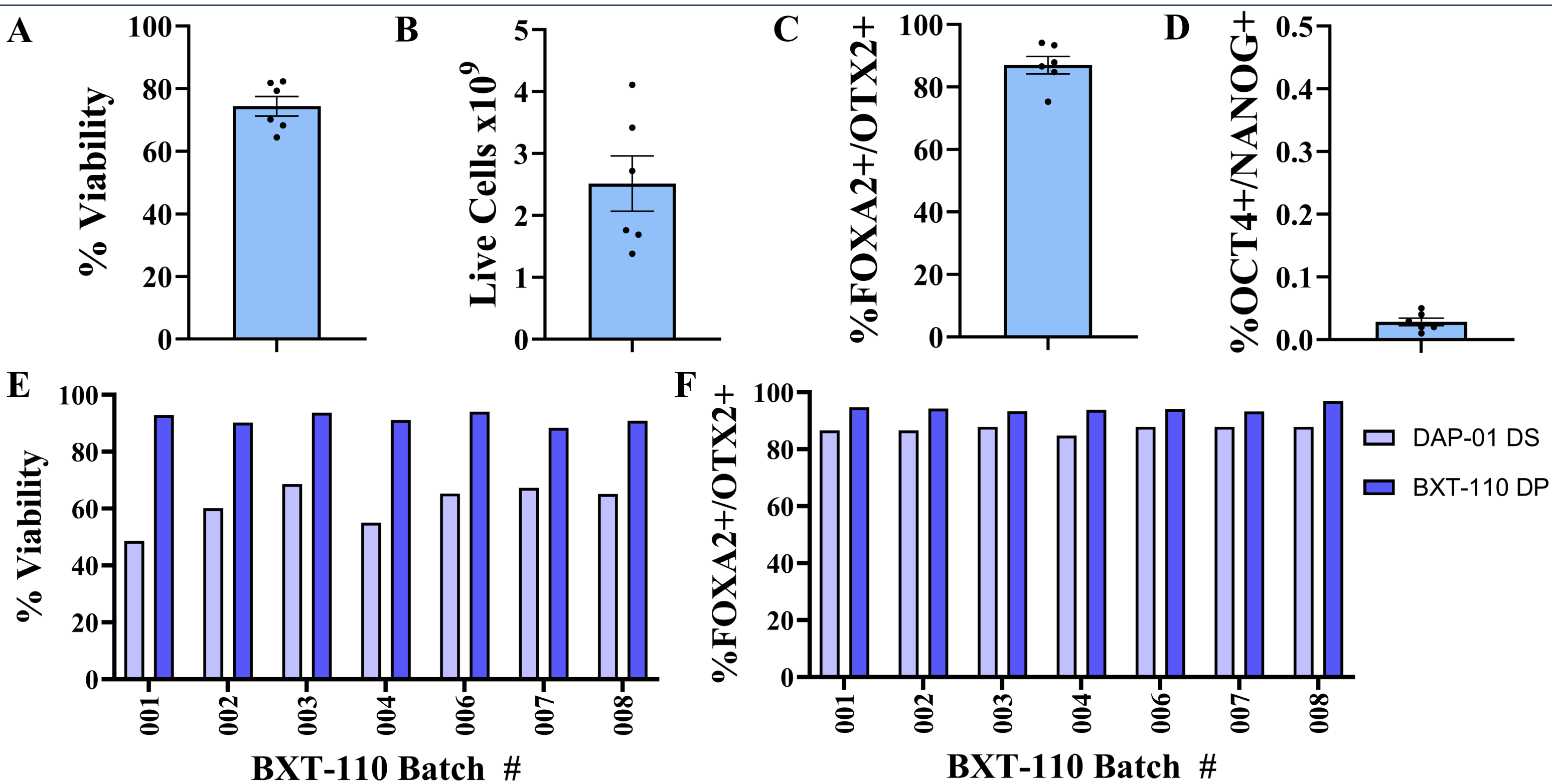
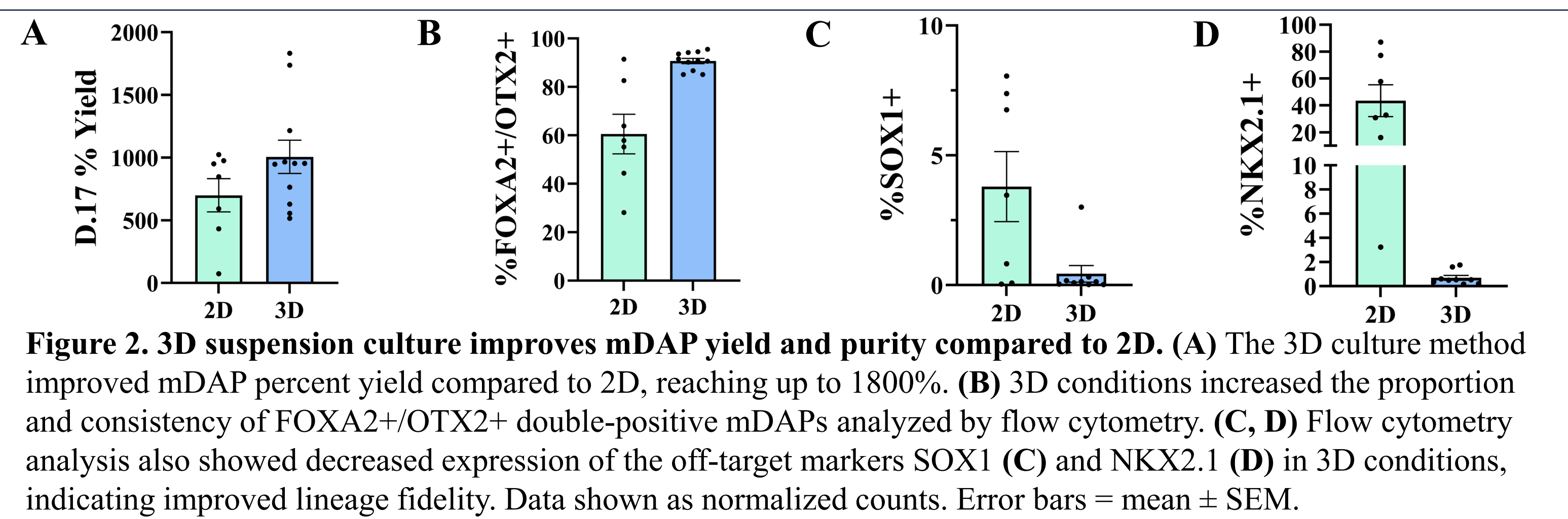


Figure 3. Reproducible GMP manufacturing of DAP-01 Drug Substance and BXT-110 Drug Product. **(A–D)** Characterization of six GMP DAP-01 DS manufacturing lots derived from four iPSC banks. **(A)** Percentage of viable cells at harvest **(B)** Total live cell yield (x10⁹) at harvest. **(C)** Consistent co-expression of FOXA2+/OTX2+ midbrain dopaminergic progenitor markers averaging above 80%. **(D)** Minimal expression of the OCT4+/NANOG+ pluripotency markers (≤ 0.05%), indicating highly enriched mDAPs with negligible residual undifferentiated iPSCs. **(E, F)** Comparison of DAP-01 DS (light blue) and the respective BXT-110 DP (dark blue) across seven DP manufacturing runs generated from four different DS lots. **(E)** Live cell viability of BXT-110 at release showed consistently higher viability than the starting DAP-01 DS at thaw, with over 90% viability in all runs. **(F)** FOXA2+/OTX2+ expression was maintained and enriched in BXT-110 DP, with over 90% FOXA2+/OTX2+ cells in all runs. Error bars = mean ± SEM.

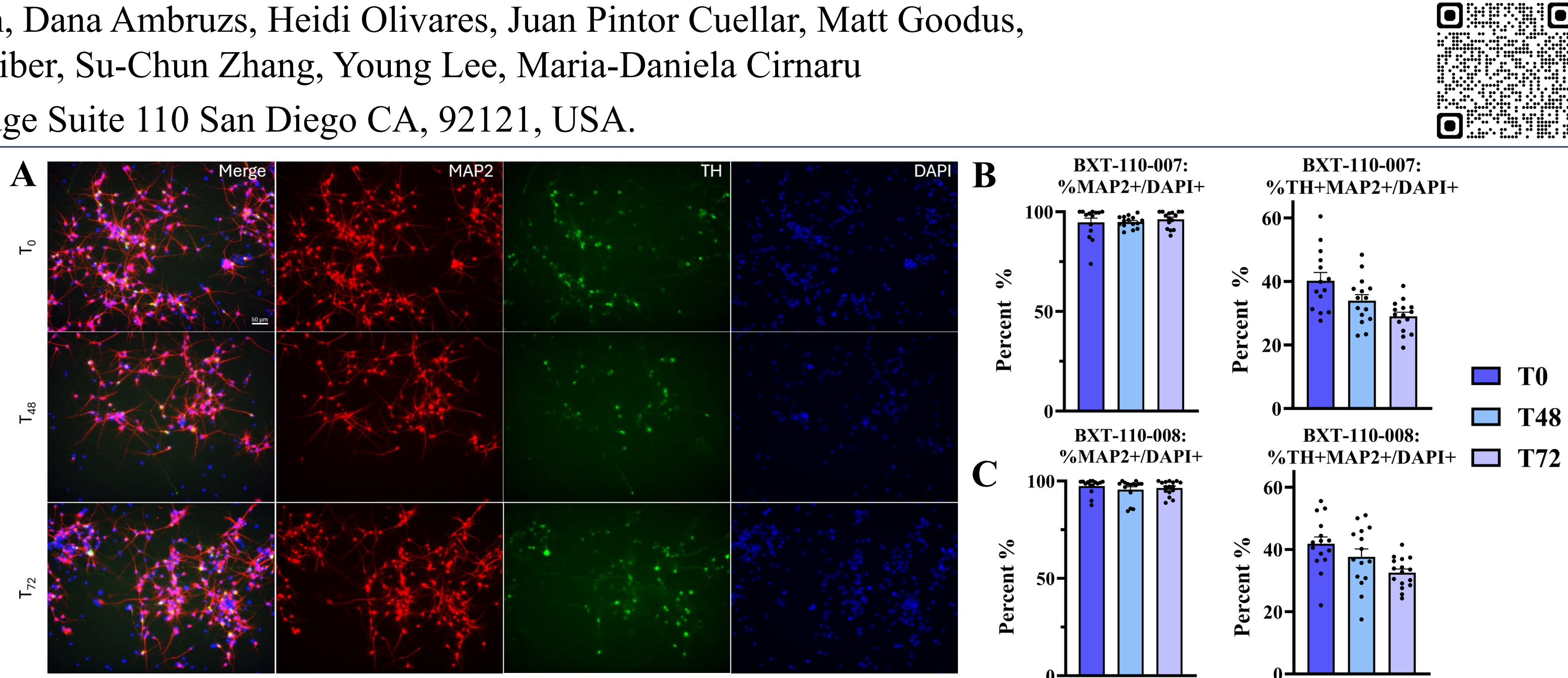


Figure 4: Dopaminergic Neuronal Differentiation of BXT-110 at T0, T48, and T72. **(A)** Representative fluorescent images of BXT-110 neurons after 14 days of differentiation, immunolabeled for the neuronal marker MAP2 (microtubule-associated protein 2), dopaminergic marker TH (tyrosine hydroxylase), and DAPI nuclear counterstain, acquired with Keyence automated microscope for quantitative assessment. Scale bar 50 µm. **(B, C)** Quantification of MAP2 and TH show the maintained neuronal identity and dopaminergic potential across time points for both lots.

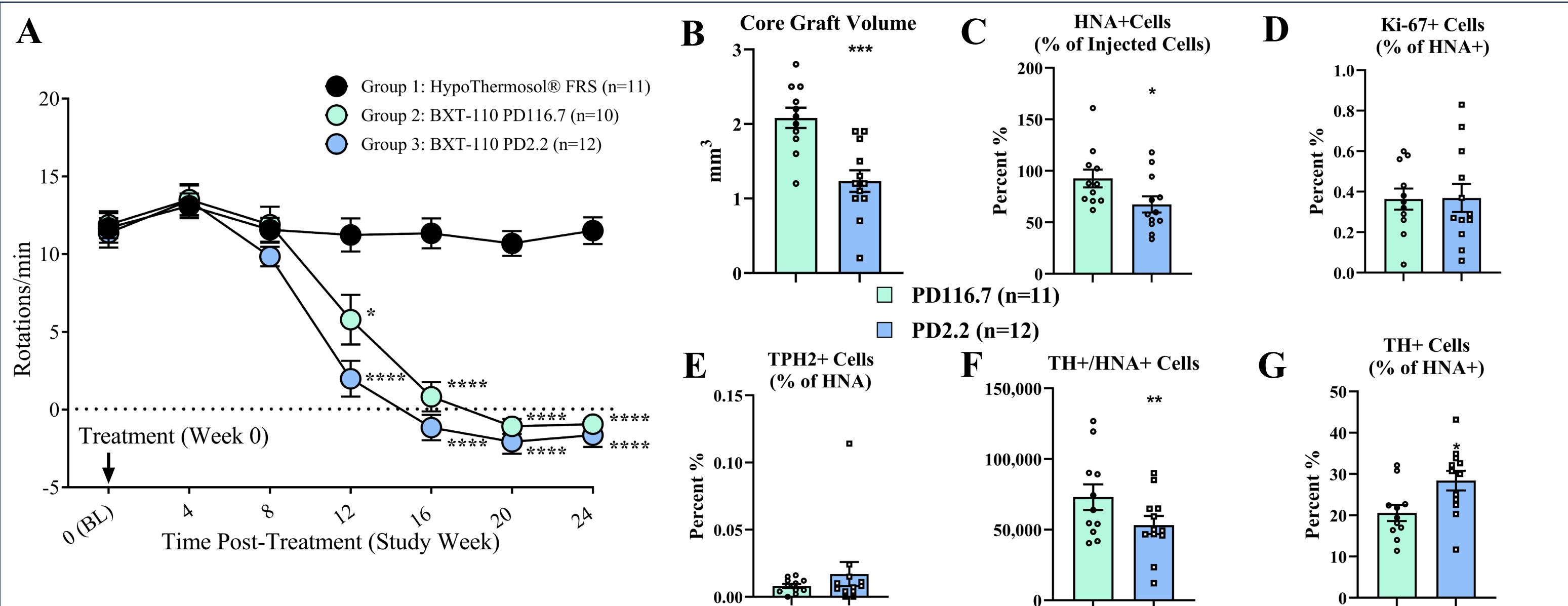


Figure 5. Reproducible efficacy, graft survival and dopaminergic differentiation of BXT-110 derived from two PD patient lines at 26 weeks post transplantation. **(A)** Intrastriatal brain administration of BXT-110 Test Article (viability higher than 90% at transplantation) robustly reverts the amphetamine-induced rotational asymmetry as early as 12 weeks, with sustained efficacy up to 26 weeks post-transplant, compared to Vehicle Control Article in female 6-OHDA-lesioned RNU rats. Data are graphed as net ipsiversive rotations during the 0–90 min testing period beginning 10–27 minutes following amphetamine administration. Statistical significance represents *p<0.05, ****p<0.0001 BXT-110 Drug Product Test Article vs. HypoThermosol® FRS Vehicle Control Article at each time point (two-way repeated measures ANOVA with Tukey’s multiple comparisons post hoc test). BL, baseline. **(B)** Individual and group mean core HNA+ graft volume (mm³) **(C)** Percentage of HNA+ cells that survived the transplant within the striatum for each treatment group (***p<0.001 unpaired t-test), indicating over 60% cell survival in both PD patient donor lines analyzed. **(D)** Quantification of Ki67+/HNA+ cells and **(E)** TPH2+/HNA+ cells in BXT-110 Test Article grafts from PD116.7 and PD2.2, showing minimal expression of these off-target markers. **(F)** Stereological quantification of TH+/HNA+ cells identified in the transplanted hemisphere in BXT-110 Test Article grafts from both PD lines (**p<0.01 unpaired t-test). **(G)** Stereological quantification of TH+/HNA+ cells expressed as percentage of total HNA+ cells showed that BXT-110 from both cell lines generated more than 20% TH+ cells in vivo at 26 weeks post-transplant. Error bars = mean ± SEM throughout. SEM, standard error of the mean.

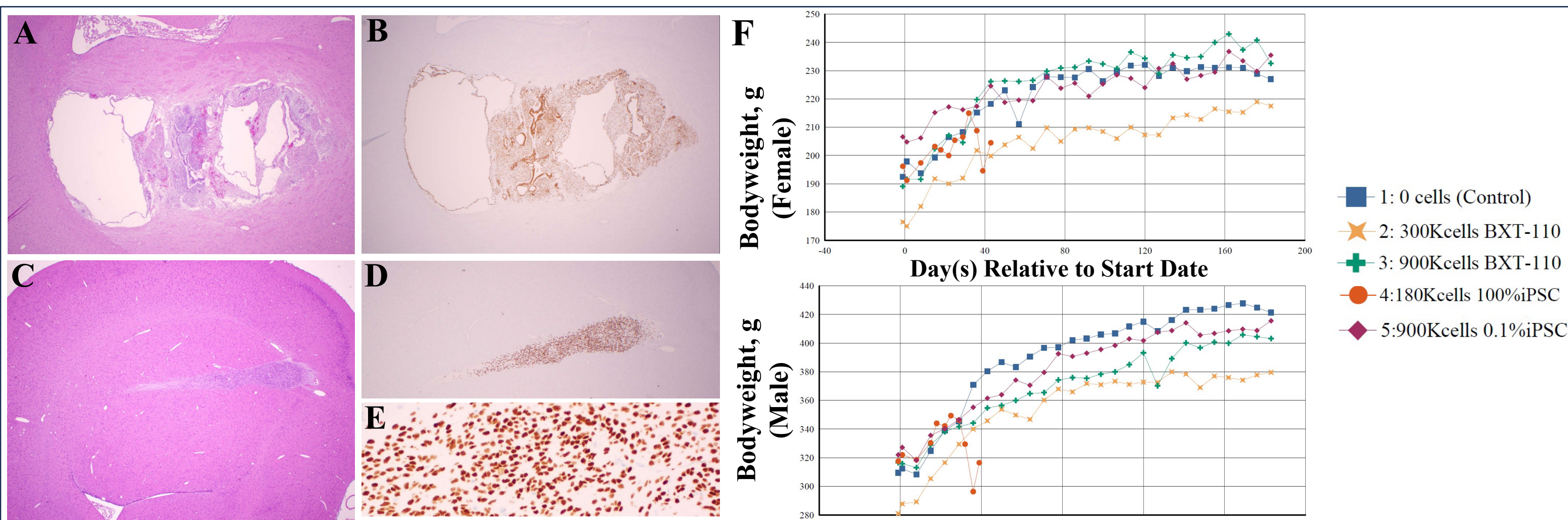
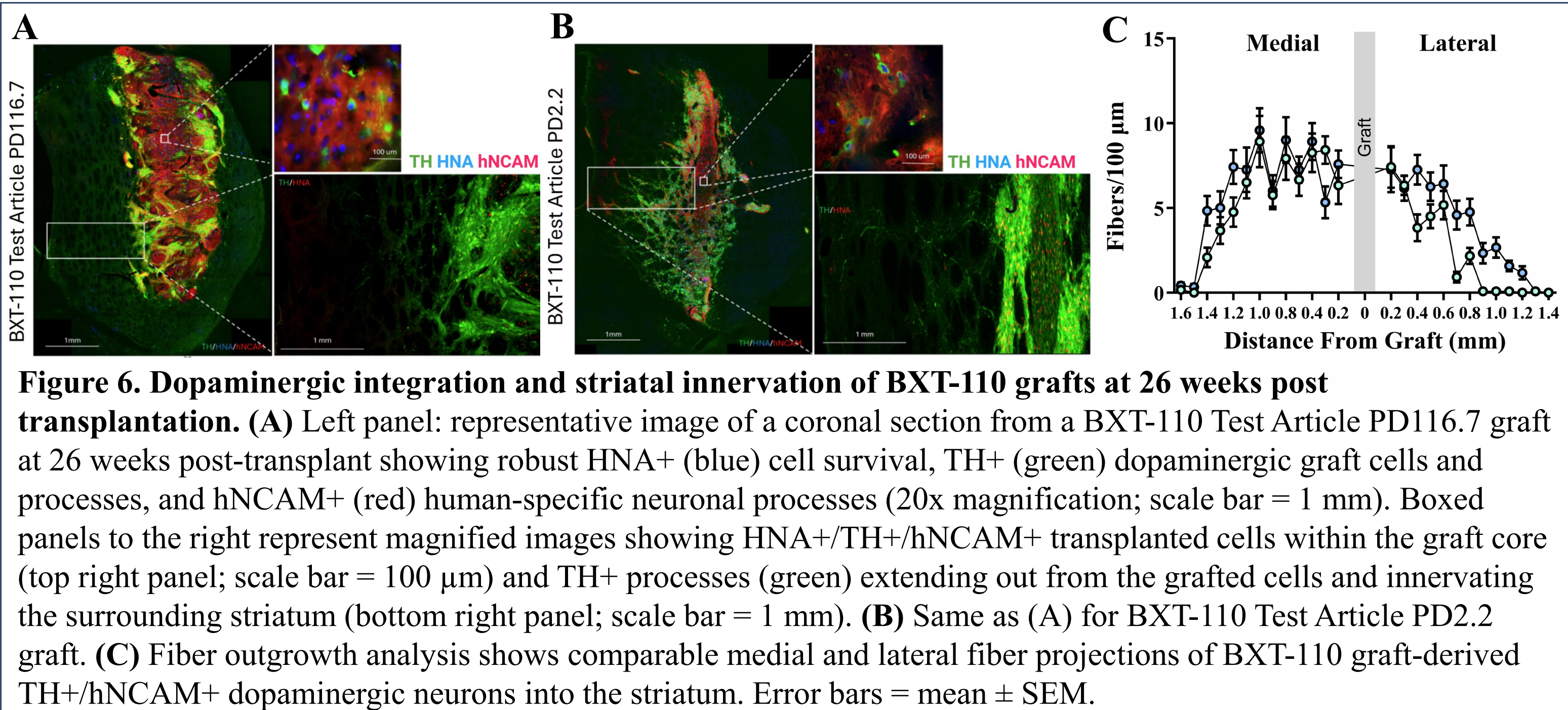


Figure 7. GLP-toxicology study to evaluate the potential toxicity, tumorigenicity, and biodistribution of BXT-110 DP. Our product showed no safety concern – including macroscopic, organ weight or microscopic findings – an isolated biodistribution, and consistently low proliferative profiles. Changes related to the injection procedure were minimal to mild and showed no differences between the groups. Animals receiving iPSCs had severe tumor cell infiltration. **(A)** Group 4, 100% iPSC positive control, sacrificed on D29: H&E slide of the brain and **(B)** BXT-110 cells in the brain stained positive by immunohistochemistry (IHC) for Human Nuclear Antigen (HuNu) **(C)** Group 3, High Dose BXT-110 DP – 900K cells, sacrificed on D29: H&E slide of the brain, **(D)** HuNu labelling, and **(E)** magnification of the HuNu staining, identifying the nuclei of human derived cells. **(F)** Body weight curves of all animals in the study. Females are plotted on the top, males are plotted on the bottom. The decline of Group 4 aligns with observations that led to early termination at D43. Error bars = mean ± SEM.

Conclusion

Our novel 3D suspension-based induction improves yield, identity, and lineage fidelity. The 24h formulation of the BXT-110 DP increases cell viability and purity prior to transplantation as well as cell survival and innervation *in vivo*. The combination of reproducible autologous manufacturing, controlled *in vivo* differentiation, durable functional efficacy and a favorable nonclinical safety profile illustrate BXT-110 readiness to be tested in the clinic as a promising next-generation, regenerative, cell-based therapy for PD.

References:

1. Surmeier DJ, Obeso JA, Halliday GM. (2017). Nat. Rev. Neurosci., 18(2), 101–113.
2. Song B, et al. (2018). J.Clin. Invest., 128(4), 1028–1042.
3. Xi J, Liu Y, Liu H, Chen H, Emborg ME, Zhang S-C. (2012). Stem Cells, 30(8), 1655–1663.



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