Identify and Quantify Neural Subtypes with Single-Cell Westerns

INTRODUCTION

The brain and central nervous system (CNS) comprise a complex collection of neurons and non-neuronal cells that work together to coordinate movement, process sensory information, and perform cognitive tasks. CNS cells are all derived from neural stem cells that differentiate into neural progenitor cells before developing into a variety of neural cell subtypes that include neurons, astrocytes, and oligodendrocytes.

Neurons transmit both electrical and chemical signals and are divided into three classes: sensory neurons, motor neurons, and interneurons. Astrocytes are functionally and molecularly diverse glial cells that are thought to contribute to neurotransmitter clearance, maintenance of the blood-brain barrier, and modulation of synaptic transmissions. They also store and release glucose, and promote the myelinating activity of oligodendrocytes, another type of glial cell whose main function is to support and insulate axons in the central nervous system. Dysregulation in these subtypes has been known to play a role in neurodegenerative and neurodevelopmental disorders such as Alzheimer's disease, schizophrenia, and multiple sclerosis.

Individual neural cells, once identified based on cell morphology, are now classified based on the expression of neuronal lineage protein markers in a single cell. However, it is difficult to analyze neurons using flow cytometry, a traditional single-cell analysis tool, due to

the elaborate morphology of neurons which have long axons and convoluted dendrites. This can cause interference in flow cytometry, making it difficult to distinguish signals from individual cell bodies. Immunofluorescence and other single-cell imaging techniques also present challenges if the percentage of target positive cells or the quantification of protein expression in a statistically significant number of single-cells is required.

The Milo™ Single-Cell Western platform allows you to identify and quantify the percentage of neural subtypes in your sample and track differentiation progression based on subtype-specific marker expression analysis. In this application note, we demonstrate how Milo can be used to identify and quantify neural subtypes in a heterogeneous neural sample, and monitor the differentiation of induced pluripotent stem cells (iPSCs) into neurons, astrocytes, and oligodendrocytes using R&D Systems research-grade or GMP differentiation reagents.

MILO MEETS ALL YOUR SINGLE-CELL PROTEIN ANALYSIS NEEDS

Milo measures up to 12 proteins in thousands of single cells in one experiment, so you can quickly and easily identify cell subtypes in your sample. All you have to do is load your neural cell suspension on a scWest chip so that single cells will settle into individual microwells on the chip. Milo then lyses the cells to create single-cell lysates, electrophoretically resolves the proteins in each single-cell lysate by molecular weight, and then captures the proteins in the scWest chip using UV light. The captured proteins can then be detected on the scWest chip by performing an immunoassay using primary antibodies specific for the neural lineage marker of interest and fluorescent secondary antibodies. The chip is imaged using an open format microarray scanner and images are analyzed using Scout™ software for quantitative, automated data analysis.

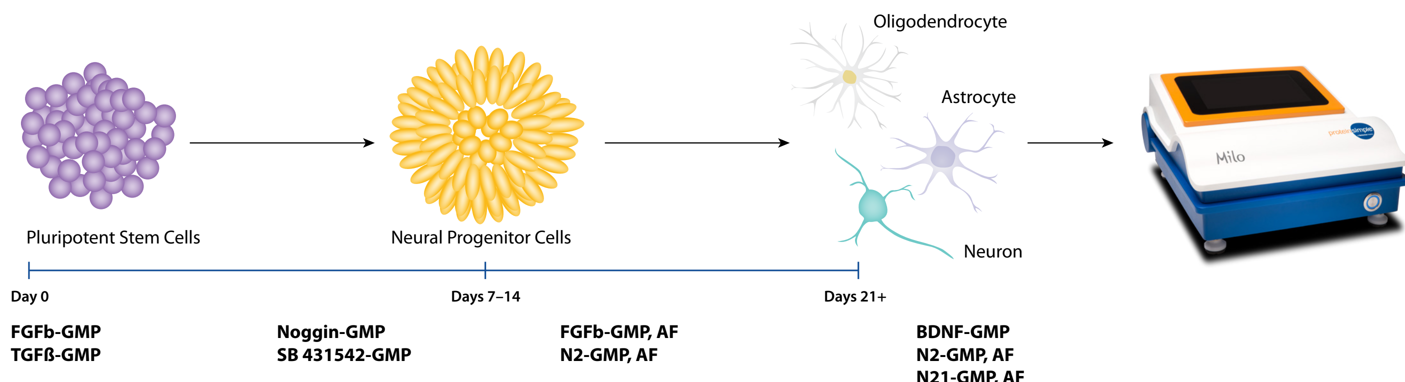


Figure 1. Overview of iPSC differentiation[1] into different neural cell subtypes and identification on Milo.

WORKFLOW OVERVIEW

IBJ6 or IPSK iPSCs were cultured on Cultrex Stem Cell Qualified Reduced Growth Factor (RGF) Basement Membrane Extract (BME) (R&D Systems, 3434-005-02) coated plates and maintained in homemade iPSC media containing research-grade or GMP Recombinant Human FGFb (R&D Systems, 233-FB or 233-GMP, respectively) and research-grade or GMP Recombinant Human TGFβ (R&D Systems, 240-B or 240-GMP, respectively). Media was exchanged daily before they were terminally differentiated into either neuron, astrocyte, or oligodendrocyte cells and then analyzed on Milo (**Figure 1**).

Neural Progenitor Cell Generation

Neural Progenitor Cells (NPCs) were generated from IBJ6 or IPSK iPSCs using a dual SMAD inhibition protocol[1]. Naïve iPSC cells were treated with research-grade or GMP SB 43152 (Tocris, 1614 or TB1614-GMP, respectively) to inhibit TGFβ signaling and research-grade or GMP Recombinant Human Noggin (R&D Systems, 6057-NG or 6057-GMP, respectively) to inhibit BMP signaling for 7 to 9 days. NPCs were either collected for Milo analysis or differentiated into the downstream lineages: neurons, astrocytes, or oligodendrocytes.

Neuron Terminal Differentiation

iPSC cells were differentiated into forebrain neurons using a growth factor-withdrawal protocol[2]. Following the aforementioned NPC differentiation protocol using SB 43152 and Noggin, NPCs were passaged onto BME coated tissue culture plates at high density (~ 2.5-4 x10^5 cells/cm^2). NPCs were then transitioned from the NPC differentiation media into media containing research-grade or GMP Recombinant Human FGFb (R&D Systems, 233-FB or 233-GMP, respectively) and research grade or GMP N-2 MAX Media Supplement (100X) (R&D Systems, AR009 or AR016, respectively) and ascorbic acid. After 14-18 days, the cells were re-plated on tissue culture plates coated with Cultrex Mouse Laminin 1, Pathclear (R&D Systems, 3400-010-02) and/or Cultrex BME in media containing N-2 MAX Media Supplement without FGFb. Withdrawal of growth factors in the absence

of other morphogens drives NPC differentiation towards an excitatory cortical neuron fate[2]. The newly differentiated neurons were then transitioned into Neurobasal media containing N-2 MAX Media Supplement (100X) and N21-MAX Media Supplement (50X) (R&D Systems, AR008) and maintained until they were collected on day 24 or 26 for analysis on Milo.

Astrocyte Terminal Differentiation

iPSC cells were differentiated into astrocytes from NPCs using an astrocyte differentiation strategy that exploits the developmental timing of glia cell development[2,3]. Following the dual SMAD inhibition protocol as described above, the cells were passaged and maintained as multipotent neural progenitors for an extended time in culture whereby there is the increased likelihood for glial cell differentiation. The NPC maintenance media was as described above with FGFb and N2-Max supplement. After 28-30 days, the NPCs were passaged and transitioned out of FGFb into neurobasal media supplemented with N21-MAX Media Supplement (50x) and 1% premium select Fetal Bovine Serum (R&D Systems, S11595H). Between day 40 to day 42, the cells were harvested and prepared for analysis on Milo.

Oligodendrocyte Differentiation

Immature oligodendrocytes were generated from iPSC cells by transitioning NPCs to oligodendrocyte progenitor cells (OPCs)[2,3]. This method takes advantage of the delayed development of oligodendrocytes as well as molecular cues involved in oligodendrocyte specification[4]. Briefly, early in NPC generation with the dual SMAD protocol, cultures were treated with increasing levels of retinoic acid and recombinant human SHH (R&D Systems, 8908-SH). The cells were passaged onto Laminin-coated plates and cultured in 2 phases of OPC differentiation conditions. First, the cells were cultured in the presence of SSH and Recombinant Human PDGF-AA (R&D Systems, 221-AA), from day 7 to 13 and then in media, containing research-grade or GMP versions of Recombinant Human NT-3 (R&D Systems, 267-N3 or 267-GMP, respectively) and research-grade or GMP Recombinant Human IGF-1 (R&D Systems, 291-G1 or 291-GMP, respectively), from day 14 to 21. Cells were re-plated on poly-L-lysine/laminin- and/or Cultrex BME-coated plates when cells were close to confluent. Cells were differentiated into oligodendrocytes between day 24-40 using DMEM/F12 media without Vitamin A containing N-2 MAX Media Supplement (100X), N21-MAX Media Supplement (50X), GlutaMax, SHH, NT-3, dCAMP, IGF-1, Noggin, and T3. Cells were maintained in this media until they were collected on day 40 for analysis on Milo.

Sample Prep And Running On Milo

1 mL of a single-cell suspension containing 100,000 cells/mL was loaded onto a standard scWest chip (ProteinSimple, K600) following the normal Single-Cell Western protocol with specific settings as noted. Under non-GMP settings, neurons and NPCs were settled for 4 minutes, astrocytes were settled for 10 minutes, and oligodendrocytes were settled for 7 minutes. Cells were then lysed for 10 seconds. The neurons and NPCs were electrophoresed for 60 seconds and the astrocytes and oligodendrocytes were electrophoresed for 70 seconds. Protein bands were captured in all the scWest chips using 240 seconds of UV exposure.

Under GMP conditions, all cells were settled on the chip for 4 minutes, lysed for 10 seconds, and electrophoresed for 70 seconds. Protein bands were captured in the scWest chips using 240 seconds of UV exposure.

scWest chips were then multiplexed with antibodies specific for the neural lineage markers of interest (**Table 1**). All antibodies were diluted in Milk-free Antibody Diluent (ProteinSimple, 043-524) to the dilutions listed in Table 1. Primary antibodies were all incubated at room temperature for 1 hour, followed by secondary antibody incubation at room temperature for 2 hours. Chips were imaged using a GenePix 4400A (Molecular Devices) microarray scanner and analyzed using Scout software. Chips were probed first with a cocktail of antibodies

against the neural markers (GFAP, Tuj, Nestin and O2 in the non-GMP experiments and Oct4, Tuj, and Pax6 in the GMP experiments) and imaged. Antibody cocktails were then stripped off using the Stripping & Reprobing protocol for Milo chips and each scWest chip was re-probed for a GAPDH internal control.

	TARGET	PRIMARY AB PN	PRIMARY AB CONC/ DILUTION	SECONDARY AB PN	SECONDARY AB DILUTION
Non-GMP Experiments	GFAP	R&D Systems AF2594	100 µg/mL	Thermo A-11015	1:20
	ßIII-tubulin (Tuj)	Sigma T8578	100 µg/mL	Thermo A-31570	1:20
	Nestin	BD Biosciences 611658	1:20	Thermo A-31570	1:20
	O2	Abcam ab136253	1:10	Thermo A-31573	1:20
	GAPDH	Sigma SAB2500450	1:10	Thermo A-11058	1:20
GMP Experiments	Oct4	Cell Signaling 2750	1:10	Thermo A-21206	1:20
	ßIII-tubulin (Tuj)	Sigma T8578	100 µg/mL	Thermo A-21203	1:20
	Pax6	R&D Systems AF8150	100 µg/mL	Thermo A-21448	1:20
	GAPDH	Sigma SAB2500450	1:10	Thermo A-21432	1:20

Table 1. Summary of primary and secondary antibodies, and concentrations used to probe scWest chips. All scWest chips were probed with primary antibody for 1 hour and secondary antibody for 2 hours.

MILO IDENTIFIES CELL SUBTYPES IN A HETEROGENOUS NEURAL SAMPLE

Each differentiated neural sample (iPSC-derived neurons, astrocytes, and oligodendrocytes) and an NPC sample were run on separate scWest chips on Milo and multiplexed for Nestin, Tuj, GFAP, and O2, known neural lineage markers for NPCs, neurons, astrocytes, and oligodendrocytes (**Figure 2A**). The chip was then stripped and re-probed with the internal control GAPDH, increasing the multiplexing capabilities per scWest chip (**Figure 2B**). Scout software was used to calculate the stripping efficiencies for the markers probed in the first probing round to ensure that antibodies were removed during the stripping process. The stripping efficiencies were all above 92%, indicating the stripping efficiency was very good for all four neural lineage markers (**Figure 2C**). This five-protein multiplexed Single-Cell Western assay simultaneously identified the four major neural cell types (NPCs, astrocytes, neurons, and oligodendrocytes) in each of the differentiated, heterogeneous neural samples.

MILO QUANTIFIES DIFFERENTIATION EFFICIENCY IN ENRICHED NEURAL SAMPLES

Differentiated neural cells enriched for neurons, astrocytes, or oligodendrocytes were run on Milo and multiplexed for the four neural lineage markers to evaluate the sample heterogeneity and differentiation efficiency of each sample. One method of classifying neural cell subtypes is to quantify single marker-positive populations, such as cells only expressing a lineage marker like Nestin and the internal control GAPDH (**Figure 3A**). Using such a 1-dimensional (1D) analysis approach, Milo data for the differentiated neuron sample indicated that most of the sample was differentiated, as Nestin was not detected in any cells (**Figure 3A, left**). The sample was also highly enriched for neurons as Tuj was detected in approximately 80% of the population. In comparison, the astrocytes were well-differentiated, as cells expressing Nestin alone were not detected; however, the sample was more heterogeneous compared to the neuron sample, with significant numbers of cells expressing detectable levels of either Tuj or O2 (**Figure 3A, middle**). In this analysis, only ~20% of the cell population could be identified as astrocytes. This may indicate that the astrocyte differentiation protocol requires additional optimization or that the astrocyte population is a mixture of molecularly diverse astrocytes with different protein expression patterns. Re-probing the samples with additional astrocyte markers like S100b+, Glt1, or AldoC could provide insight as to whether further effort should be spent optimizing the astrocyte differentiation protocol. Finally, while the majority of the cells in the oligodendrocyte sample were differentiated into immature oligodendrocytes or into Tuj+ neurons, 35% of the cells still expressed Nestin,

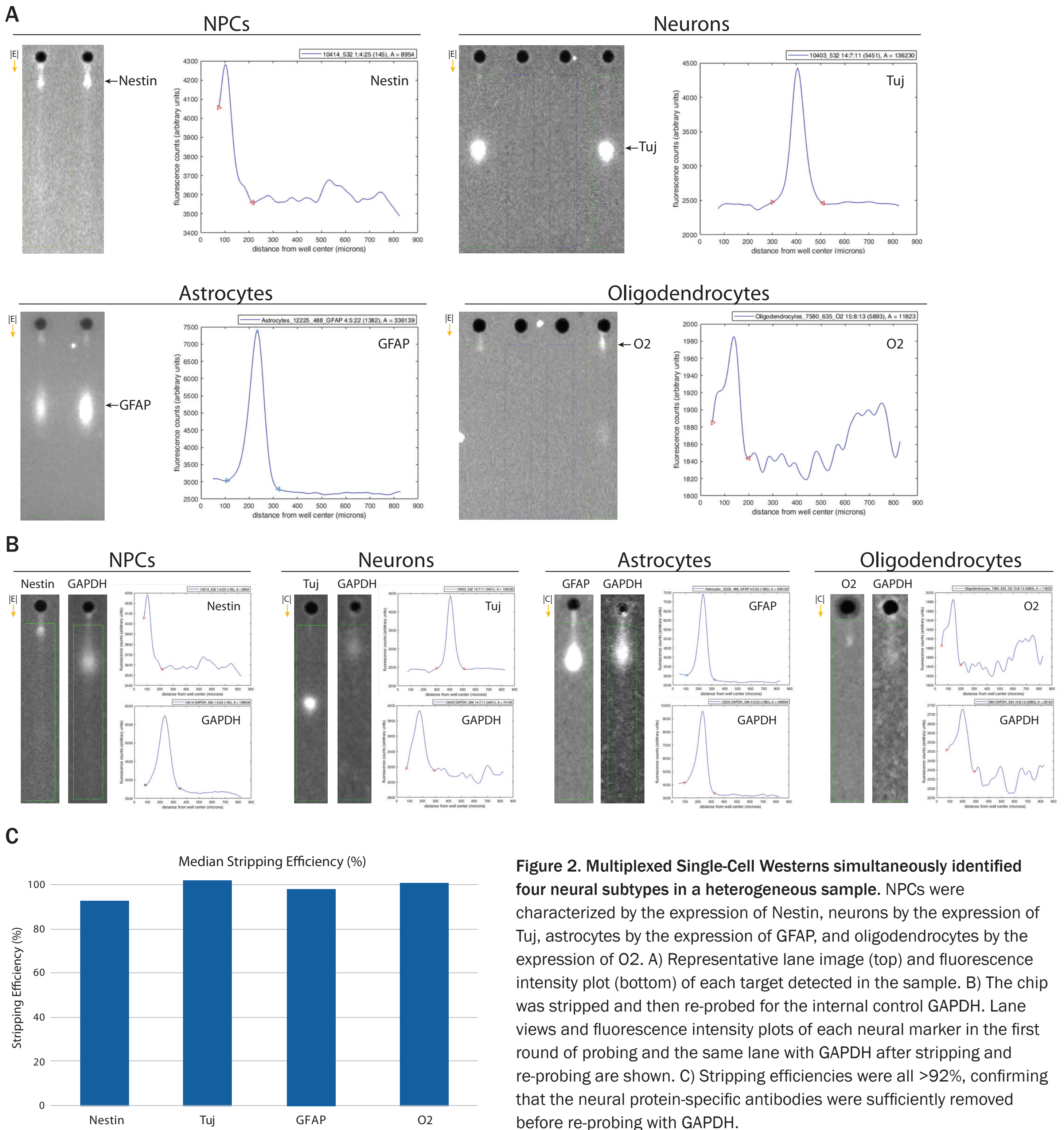


Figure 2. Multiplexed Single-Cell Westerns simultaneously identified four neural subtypes in a heterogeneous sample. NPCs were characterized by the expression of Nestin, neurons by the expression of Tuj, astrocytes by the expression of GFAP, and oligodendrocytes by the expression of O2. A) Representative lane image (top) and fluorescence intensity plot (bottom) of each target detected in the sample. B) The chip was stripped and then re-probed for the internal control GAPDH. Lane views and fluorescence intensity plots of each neural marker in the first round of probing and the same lane with GAPDH after stripping and re-probing are shown. C) Stripping efficiencies were all >92%, confirming that the neural protein-specific antibodies were sufficiently removed before re-probing with GAPDH.

suggesting that these conditions may result in an incomplete differentiation with many cells still in an immature progenitor phase. Indeed, previous work has demonstrated that Nestin is still abundantly expressed in oligodendrocyte progenitors, but decreases as oligodendrocytes become fully functional[5]. The remaining sample was a mix of neurons and immature oligodendrocytes, as approximately 25% of the cells in the sample expressed Tuj only and approximately 10% of the cells expressed O2 only (**Figure 3A, right**). Further optimization and extended time-points would be required to determine if these Nestin+ progenitors and O2+ cells would differentiate further into fully mature oligodendrocytes.

Viewing the data in 1D as shown in **Figure 3A** gives one view into which cell subtypes are present in each differentiated sample, informing optimization of your differentiation protocol or enabling further detailed study of these neural subtypes. However, it is not always sufficient when analyzing heterogeneous samples and can result in situations where the sum of the % of the population expressing each marker is greater than 100%, as we observed for the astrocytes. Multidimensional analysis approaches can add granularity to single-cell data that will help identify different cell types in a mixed population. One possible multidimensional analysis is a principal component analysis (PCA), which can display multi-dimensional data in a lower-dimensional picture to visualize relatedness between populations. Using a PCA, we combined the Single-Cell Western data for neuron, astrocyte, and oligodendrocyte samples *in silico* and observed that the astrocyte sample contains a population of neuron-like cells and a separate population of GFAP expressing astrocytes that are uncorrelated with the neuron-like population (**Figure 3B**). A closer look at the data indicate that the astrocyte population that overlaps with the neuron population on the PCA plot are not just Tuj+ cells but also include some Tuj+/Nestin+ cells as well. The observations using this multidimensional analysis approach is biologically consistent with our knowledge of how neural cells differentiate, as neurons emerge first, followed by astrocytes, and then oligodendrocytes. Thus, the use of a multidimensional analysis approach with Milo is another powerful tool that can be used to assess differentiation quality.

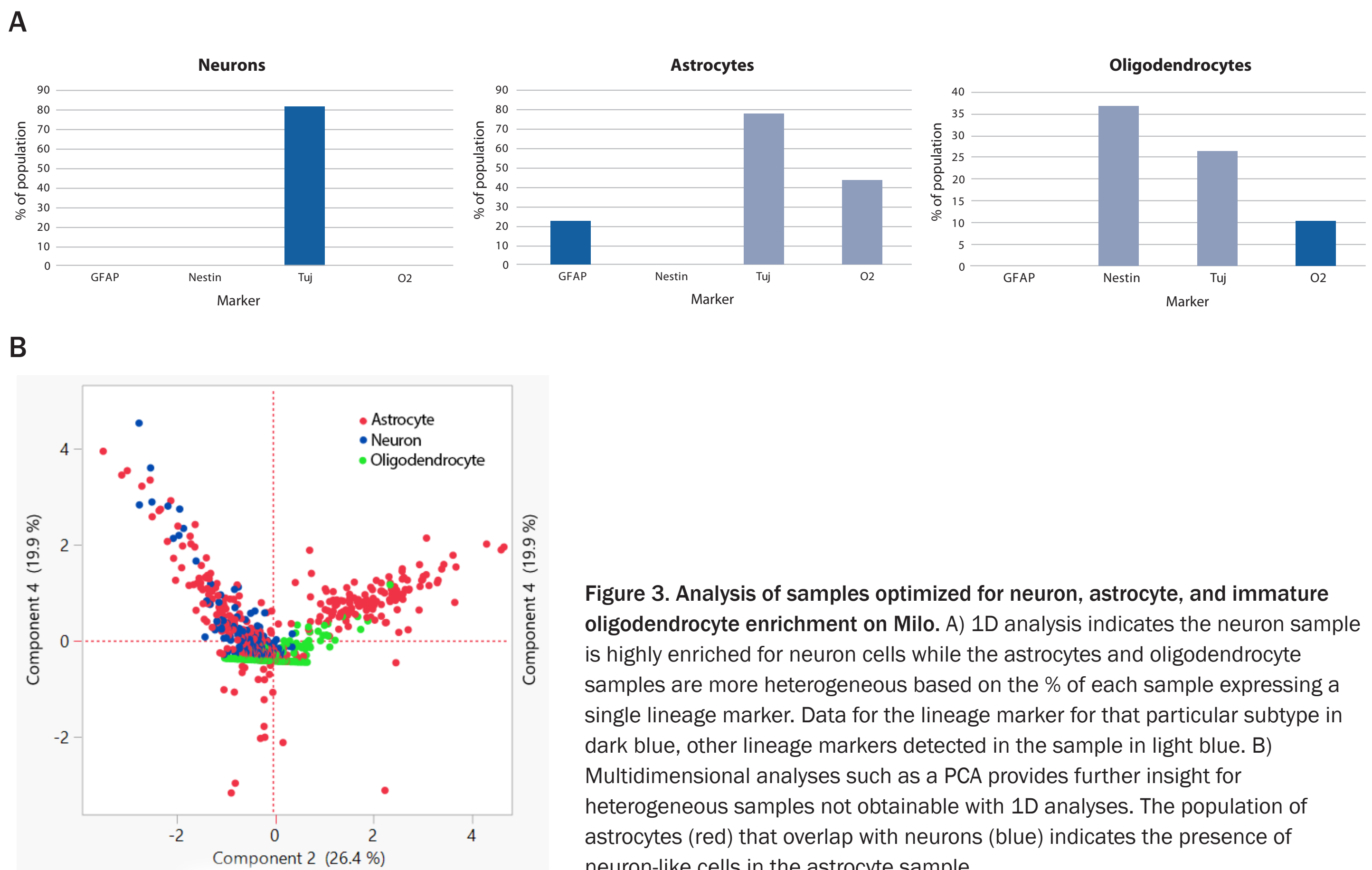


Figure 3. Analysis of samples optimized for neuron, astrocyte, and immature oligodendrocyte enrichment on Milo. A) 1D analysis indicates the neuron sample is highly enriched for neuron cells while the astrocytes and oligodendrocyte samples are more heterogeneous based on the % of each sample expressing a single lineage marker. Data for the lineage marker for that particular subtype in dark blue, other lineage markers detected in the sample in light blue. B) Multidimensional analyses such as a PCA provides further insight for heterogeneous samples not obtainable with 1D analyses. The population of astrocytes (red) that overlap with neurons (blue) indicates the presence of neuron-like cells in the astrocyte sample.

MILO PROVIDES MORE QUANTITATIVE DATA COMPARED TO IMMUNOFLUORESCENCE

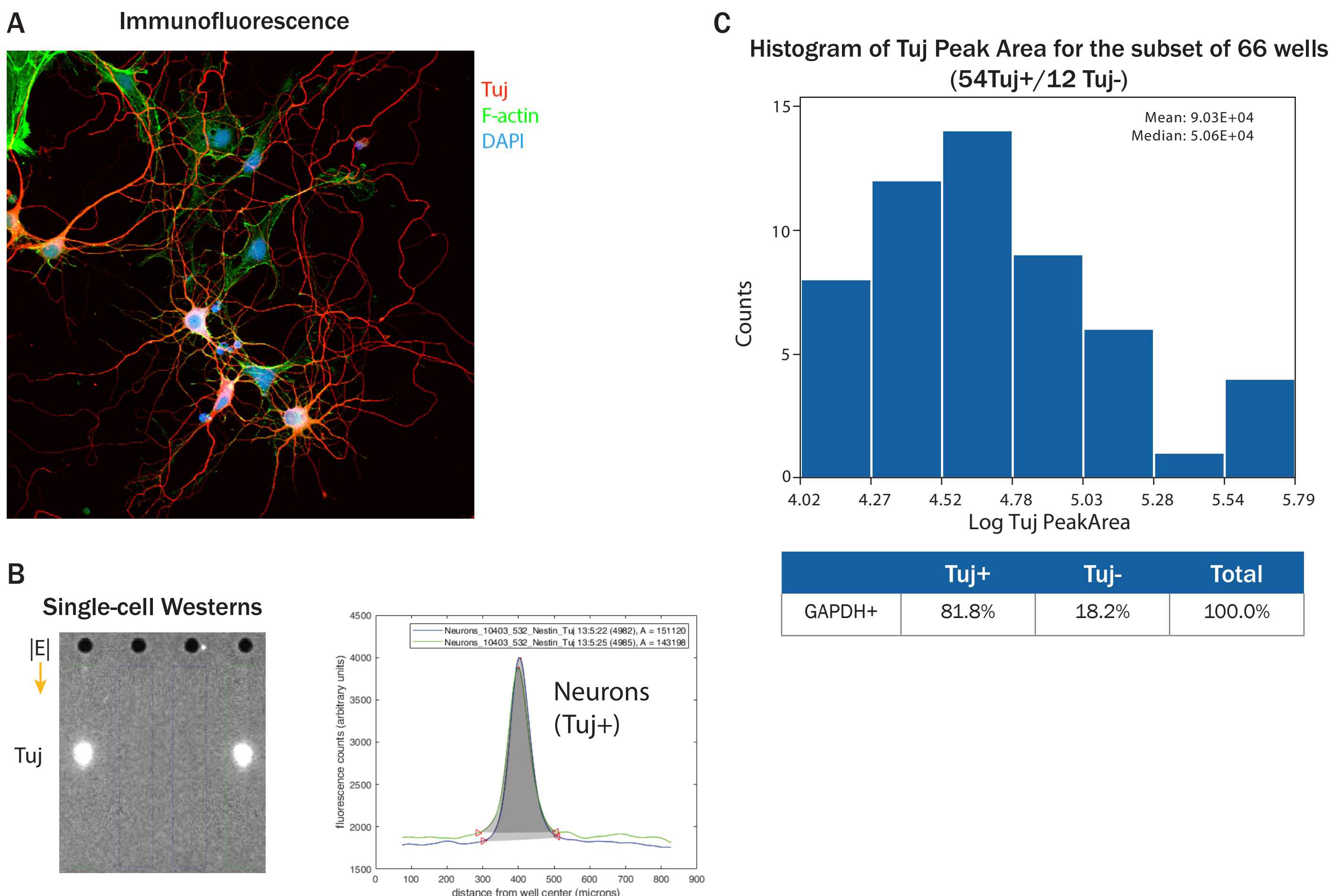
Immunofluorescence (IF) is a well-established tool used to characterize protein expression in neural samples. To compare IF and Single-Cell Western approaches, the differentiated neuron sample was analyzed using Milo and IF. For IF, the neurons were stained for Tuj and F-actin and cells counterstained with DAPI (**Figure 4A**). While it was possible to detect the expression of multiple proteins to identify a single cell using immunofluorescence, overlapping signal, poor resolution of individual cells, and low statistical power due to a limited field of view for IF made it difficult to quantify how enriched the sample was for neuronal cells and arrive at an accurate measurement of the number of neurons in the sample. In contrast, fluorescence intensity plots of each single-

cell separation on Milo can be extracted from the lane images. Scout software automatically calculates Tuj abundance in each single cell by determining the area under the curve for each neuron cell analyzed (**Figure 4B**). An expression histogram and an enumeration table of Tuj expression confirmed that the neuron samples were highly enriched, as 81.8% of the cells analyzed using the scWest chip expressed Tuj (**Figure 4C**). Further, the heterogeneity of Tuj expression across the single-cells analyzed can be quantified and was measured to be almost 100-fold based on peak area.

MILO AIDS THE TRANSITION TO GMP-DIFFERENTIATED SAMPLES

Milo can be a critical asset as scientists and companies take research-designed differentiation protocols and transition them into scalable, reproducible, and robust processes for cell therapy manufacturing. During this transition, scientists usually progress from research-grade to GMP-grade reagents. Milo can analyze the differentiation efficiency during scale-up and during the switch to GMP-grade reagents to inform on how to modify individual protocols to preserve or improve differentiation efficiencies.

With this in mind, we ran iPSCs, NPCs, and neurons differentiated with GMP-reagents on Milo. Each chip was multiplexed for the iPSC marker Oct4, the NPC marker Pax6, and the neuron cell marker Tuj to evaluate the differentiation efficiency (**Figure 5A**). Chips were then stripped and re-probed for the loading control GAPDH. 1D scatter plots of marker expression across the three samples show that the majority of the iPSC cells (defined as GAPDH+) expressed Oct4, indicating that the starting iPSC sample was undifferentiated before the cells



	Tuj+	Tuj-	Total
GAPDH+	81.8%	18.2%	100.0%

Figure 4. Single-Cell Western provides quantitation not possible with immunofluorescence. A) The neuron sample was stained with Tuj (red, R&D Systems MAB1195), F-actin labeled with Alexa Fluor™ 488 Phalloidin (green, Life Technologies), and DAPI (blue). Immunofluorescence identified neurons by the expression of Tuj, but could not quantify target abundance. B) The neuron sample analyzed with Milo. Tuj was detected in neuron cells and the target abundance determined by calculating the area under the curve (grey). C) An expression histogram and enumeration table of Tuj expression in the neuron samples confirmed that the sample was highly enriched for Tuj+/GAPDH+ neuron cells.

underwent differentiation. In contrast, none of the cells in the NPC and differentiated neuron samples had detectable levels of Oct4, suggesting they were successfully differentiated (**Figure 5B, left**). Pax6 was not detectable in iPSCs but elevated in increasing percentages of cells in the NPC and neuron samples showing the progression of those cell populations through the differentiation process. The percentage of Tuj+ cells increases dramatically in the differentiated neuron sample compared to the iPSC and NPC samples. A subset of cells in the iPSC and NPC samples were also measured to be Tuj+, where Tuj expression levels in this subpopulation were quantified to be similar in the iPSC and NPC samples and increased in the differentiated neuron sample, confirming the observation made by Kuang *et al.*[6] Together, these results indicate that these cells successfully differentiated into neurons (**Figure 5B**).

Oct4, Pax6, and Tuj were also detected in the iPSC, NPC, and neuron cells using immunofluorescence (**Figure 5C**). The protein expression patterns observed in each sample using immunofluorescence was

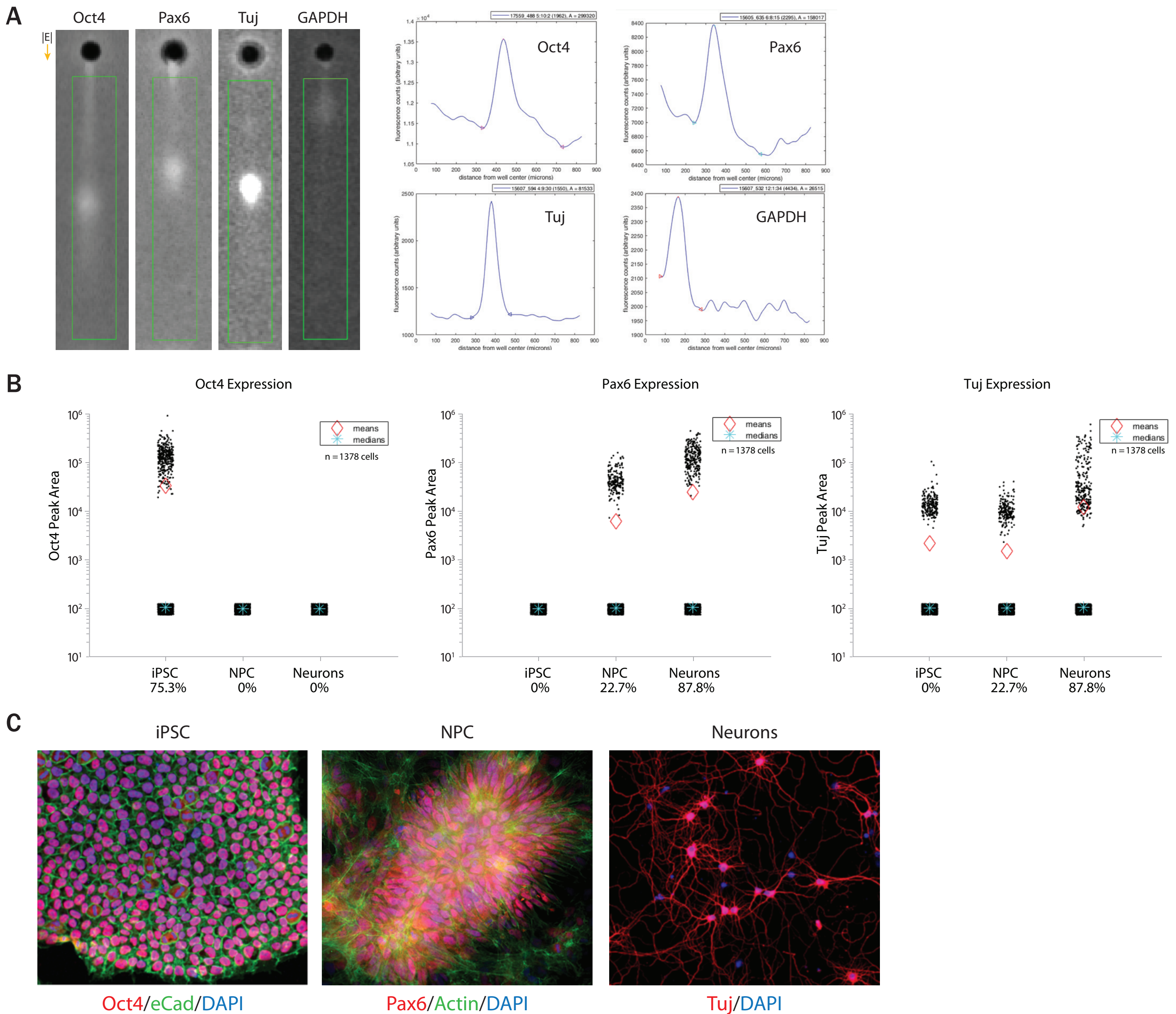
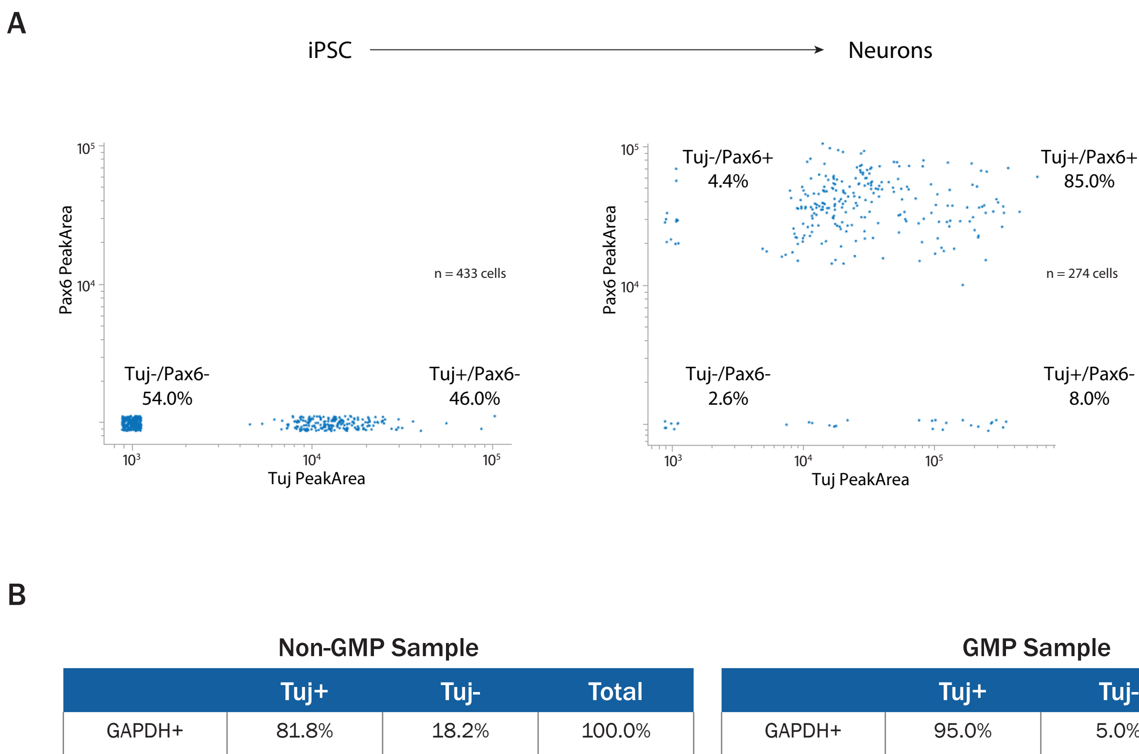


Figure 5. Milo easily tracks neural differentiation. A) iPSCs were GMP-differentiated and the iPSCs, NPCs, and neuron samples were run on separate scWest chips and multiplexed for Oct4, Pax6, and ßIII-tubulin (Tuj). Chips were then stripped and re-probed for the loading control GAPDH. Representative lanes demonstrate protein expression in the lane image (left) and fluorescence intensity plot (right). B) 1D scatter plots of marker expression across the three scWest chips indicate iPSC samples were pure before differentiation and that they progressed from iPSC to neuron cells as expected based on expression of Pax6 and Tuj. C) Immunofluorescence micrographs of the three cell types and their respective markers.

consistent with that reported by Milo; however, for the reasons described above, protein expression and the percentage of cells in each sample expressing each neural marker couldn't easily be quantified using IF.

The GMP-reagents yielded high-quality differentiation from iPSCs to neurons (**Figure 6A**). 2D scatter plots showing subpopulations of cells expressing Tuj and/or Pax6 demonstrate the differentiation efficiency. Pax6 was undetectable in the iPSCs while 46% of the cells expressed Tuj. After differentiation, 85% of the cells were Tuj+/Pax6+. Milo data also indicate that the differentiation protocols performed with both non-GMP and GMP-reagents resulted in robust neuronal differentiation, confirming a seamless transition between research-use kits to GMP-reagents as minimal protocol modification will be required to maintain differentiation efficiencies (**Figure 6B**). The differences between the percentage of Tuj+/GAPDH+ cells in the non-GMP (81.8%) and GMP-reagents (95%) can be attributed to differences in harvest day, as the non-GMP differentiated neuron were harvested after 24 days and the GMP differentiated neurons were harvested after 26 days. This suggests harvesting cells after 26 days will result in a more pure neuron population.



Non-GMP Sample

	Tuj+	Tuj-	Total
GAPDH+	81.8%	18.2%	100.0%

GMP Sample

	Tuj+	Tuj-	Total
GAPDH+	95.0%	5.0%	100.0%

Figure 6. GMP-reagents yielded high-quality differentiation from iPSCs to neurons. A) Pax6 was undetectable in iPSCs while 46% of the cells expressed Tuj. 85% of the neuron cells were Tuj+/Pax6+. B) A comparison of non-GMP and GMP reagents indicates the transition between differentiation kits and GMP-reagents is seamless. Minor differences in the percentages in Tuj+/GAPDH+ cells in the non-GMP and GMP sample can be attributed to different harvest days.

CONCLUSION

The intricacies of the brain and the numerous CNS cell types and networks that underpin its function require powerful high-resolution single-cell tools to understand this complexity. This cellular diversity makes population-level analyses inadequate and drives a critical need for tools that can identify and quantify neural cell subtypes to better understand the neural processes in health and disease. The Milo Single-Cell Western platform does just this by analyzing the neural cells that flow cytometry cannot and providing the quantitation that immunofluorescence does not. This makes Milo the perfect fit for your neuroscience studies as it offers a way to quantify and study neural cell types that can be challenging to do with conventional tools.

Milo is also a powerful asset that can be used to evaluate differentiation efficiency and help evaluate quality in scale-up processes as scientists and companies working with differentiated samples transition from research to production. Milo can confirm that your differentiation protocols have yielded high-quality differentiations for your cell subtype of interest. This includes the detection of residual iPSCs, which could be tumorigenic if included in a cell therapy product. R&D Systems' research-grade and GMP reagents all resulted in robust differentiation, offering a seamless transition as your project progresses. Additionally, analyzing the data using multidimensional analysis tools like PCA provides further insight into complex expression patterns. Thus, no matter where you are with your project, you'll have confidence that your protocols are fully optimized and that your samples are pure and enriched.

REFERENCES

1. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. SM Chambers, CA Fasano, EP Papapetrou, M Tomishima, M Sadelain, and L Struder, *Nat Biotechnol*, 2009; 3:275-280.

2. Efficient generation of region-specific forebrain neurons from human pluripotent stem cells under highly defined condition. F Yuan, KH Fang, SY Cao, ZY Qu, R Krencik, M Xu, A Bhattacharyya, YW Su, DY Zhu, and Y Liu, *Sci Rep*, 2015, 5:18550

3. Neural subtype specification from human pluripotent stem cells. Y Tao and SC Zhang, *Cell Stem Cell*, 2016, 19(5): 573-586

4. Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. S Wang, J Bates, X Li, S Schanz, D Chandler-Militello, C Levine, N Maherali, L Studer, K Hochedinger, M Windrem, and SA Goldman, Cell Stem Cell, 2013, 12(2): 252-264

5. Developmental and growth factor-induced regulation of nestin in oligodendrocyte lineage cells. V Gallo and RC Armstrong, *J Neurosci*, 1995, 15(1): 394-406.

6. Evaluation of commonly used ectoderm markers in iPSC trilineage differentiation. YL Kuang, A Munoz, G Nalula, KE Santostefano, V Sanghez, G Sanchez, N Terada, AN Mattis, M Iacovino, C Iribarren, RM Krauss, and MW Medina, *Stem Cell Res*, 2019; 37: 101434.

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