

Stem Cell Analysis: Advances in Western Blotting Technologies



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CHAPTER 1

Introduction

Stem cell and regenerative medicine offer new therapeutic hope for a range of diseases with limited treatment options—from cancer to neurodegenerative diseases to an array of developmental disorders. This potential is supported by a wealth of basic science research into the biology of stem cells and the signaling pathways that either uphold their pluripotent state or drive cell-type-specific differentiation.

As the stem cell field moves rapidly from bench to bedside, there is a need for improved techniques to study and analyze the complex cellular interactions and dynamic signaling networks that define stem cells. Whether you're performing lineage analysis, tracking cell behaviors such as proliferation, differentiation, migration or cell death, or identifying modulators that control cell behaviors, such as

protein expression levels, changes in cell signaling pathways or epigenetic modifications, you'll need the right tools for your research. Limited sample availability, heterogeneity in cellular behavior, complex collection paradigms and the personalization of treatment strategies require approaches that address research questions faster and comprehensively. From imaging platforms that accommodate traditional Western blotting methods to fully automated Western blotting systems and single-cell protein analysis, ProteinSimple's got your protein analysis of stem cells covered.

At ProteinSimple, we're changing the way researchers analyze proteins. We enable cutting-edge research to uncover the role of proteins in normal biological processes and disease, thereby paving the way for novel approaches to drive the development of protein-based therapeutics. Our innovative product portfolio can help you reveal new insight into proteins, advancing your understanding of protein function. We empower you to make your next discovery by eliminating common protein analysis workflow challenges.

CHAPTER 2

Protein analysis techniques for stem cell research

Protein analysis plays an important role in stem cell research, and for most researchers, Western blotting is a key component of their toolbox. From verifying pluripotency and identifying lineage-specific cell types to identifying key modulators of cell signaling pathways, to evaluating disease models and therapeutic approaches, the right protein analysis techniques can help you achieve success.

Traditional Western Blotting

Western blotting is a widely-used technique to detect and analyze protein expression changes in stem cell sample lysates. This ever-present laboratory technique has seen much innovation in recent years. Gone are the days of multiple film exposures and hours spent in the darkroom developing film; researchers are moving toward digital imaging systems that provide them with a way to rapidly analyze chemiluminescent and fluorescent signals.

FluorChem® Imagers enable fast, high-resolution digital imaging of gels and blots. With a 5-log dynamic range that far outperforms film, FluorChem imagers can detect both faint and bright bands in a single exposure without oversaturation. And you get your data in minutes, with no waste at all.

New to digital imaging technology? Camera resolution and bit depth are commonly considered factors that influence the linear dynamic range and quality of digital images. However, lesser-known factors can also significantly contribute to the real data dynamic range and image quality obtained by a digital imaging system. [Read our technical note](#) to understand key technical specifications of digital imaging systems and get the most out of your imager.



FluorChem R system



WANT TO LEARN HOW FLUORCHEM WORKS?

[Click here to watch a video](#) and see how FluorChem imagers make gel and Western blot imaging a breeze. Simply load your sample, choose your protocol, hit Expose and get perfect, high-quality images.



FROM YOUR PEERS Narendra Sees More in His Immunoblots with the FluorChem Q Imager

"The FluorChem Q gives me clean and publication-quality results. This has helped me enormously to do accurate protein analysis which is very critical for preclinical validation of drugs."

— Narendra Bharathy Elangovan, Ph.D., Postdoctoral Fellow, Children's Cancer Therapy Development Institute

[→ Click here to read his story](#)

Automated Western Blotting

Advances in technology are delivering the reproducibility and reliability that you need to be confident in your research. With automated Western blotting, you can separate and analyze proteins by size or charge with picogram-level sensitivity. Get quantitative results faster, with less hands-on time.

Simple Western® systems reinvent how Western blots are done and automate all assay steps from protein separation, immunoprobings, detection and analysis of data. They also deliver quantitative, reproducible data in hours instead of days. There's no cutting of individual strips, repeated washing steps or incubations. With Simple Western assays, you just pipette your sample and reagents into the wells of an assay plate, set up your run and press Start.

Simple Western assays are automated, capillary-based immunoassays that solve many of the challenges that come with traditional Westerns. There are four Simple Western systems to choose from, all of which generate reproducible data with intra-assay coefficient of variations (CVs) <15%. **Wes™** gives you relative quantitation on up to 25 data points in just three hours with only 30 minutes of hands-on time for setup. If you need higher throughput, **Sally Sue™**, **Peggy Sue™** and **NanoPro 1000** give you 96 data points overnight with only an hour of hands-on time. The Simple Western product line is supported for use in quality control (QC) and good manufacturing practice (GMP) environments. The integrated Compass for Simple Western software (v3.1) has all the 21 CFR Part 11 compliance tools you need to ensure authenticity, integrity, and, when appropriate, confidentiality.



Wes



WANT TO LEARN HOW WES WORKS?

[Click here to watch a video](#) to see how Wes can process up to 25 samples in three hours. This short video shows the step by step process, from sample loading to signal detection.



LEARN MORE Application Note:

[Easy Transfer of Your Traditional Western Blots to Wes](#)

Single-Cell Westerns

Do you need to analyze protein expression at the single-cell level? Milo™ is the world's first Single-Cell Western platform. He measures protein expression in thousands of single cells in a single run so you can profile heterogeneity in your samples. With a wide range of applications, including the profiling of target protein expression heterogeneity and the percent of stem cells that are target positive, to measuring protein isoform heterogeneity, to simplifying phospho-flow signaling studies, to measuring gene editing efficiency and validating single-cell RNA-Seq data, Milo can get you the answers you want at a single stem-cell level.

Want to learn how Milo works?

Milo eliminates the need to fix and permeabilize your stem cell sample so you can save time and hassle. He'll chemically lyse cells before analysis so you can easily access protein epitopes that can be challenging to achieve with intact cells by flow cytometry or fluorescence-activated cell sorting (FACS). Histone modifications, ribosomal proteins, and transcription factors are now just as easy to measure as cytosolic or surface proteins! Just load your stem cell suspension sample, and the scWest chip will capture ~1,000 single-cells. Milo then does a fast, 1-minute SDS-PAGE separation on each single-cell lysate on-chip. Next, just probe with your favorite Western antibodies to measure up to four proteins per cell simultaneously.



Milo



WANT TO LEARN HOW MILO WORKS?

[Click here to watch a short video](#) to see how Milo can automate 1,000 single-cell separations in just 4 hours.



DID YOU KNOW?

Are your phospho-flow or signaling assays challenging to implement? Do some of your phospho-protein targets lack good flow/FACS antibodies? Milo is an open platform that uses conventional Western antibodies so you won't have to struggle with finding high-quality flow-validated antibodies against all of your protein targets again!

Meet Your Western Blot Problem Solvers

Whether you're working with traditional Western blot systems, looking to advance your research with an automated Western blot system or trying to analyze your data at the single-cell level, our Western blot squad will take care of you.



Wes



FluorChem



Milo

Chapter 3

Stem cell culture and differentiation: cell type identification

Whatever your model system or specific stem cell research interest, you need to be able to identify and track cell types to verify pluripotency and study lineage differentiation. From traditional Western blotting to single-cell study, protein analysis tools play an important role in cell-type identification. Here's how ProteinSimple technologies are being applied to accomplish such tasks.

To understand how stem cells rebuild an entire tissue, researchers not only have to track their proliferative and self-renewal abilities but also which proteins actively influence this behavior. To support their work, researchers from the Istituto Superiore di Sanità, Rome, Italy used the [FluorChem E](#) to assess the role of β -dystrobrevin, a gene involved in Duchenne muscular dystrophy, as it applies to neural differentiation¹. Their Western blotting experiments and high-quality image acquisition identified β -dystrobrevin protein expression to be downregulated during retinoic acid-induced neuronal differentiation, and that miR-143 controls its expression. This data provides

novel insight into the molecular mechanisms governing neuronal differentiation.

When studying stem cells, with their marked heterogeneity in both proliferative potential and the proteins involved, sometimes more quantitative data than can be achieved with classic Western blotting techniques are required. Looking at this very concept of heterogeneity in human keratinocytes that recapitulate the organization of the epidermis, Roshan et al. uncover not only two modes and populations of keratinocyte proliferation, but, by using *Wes*, they were also able to quantitatively measure the boosting effect of CBX5 on increased colony size and proliferation². These data challenge the idea that stem and progenitor cells exist in a hierarchy within epidermal cultures. Characterizing an observed increased ability of a stem cell population to differentiate requires the analysis of the mechanisms by which the progenitor population develops and expands. Researchers at the Loma Linda University in California recently published an enhanced state of stemness for neonatal human cardiac progenitor cells under low gravity culture conditions induced in spaceflight aboard the International Space Station or on Earth using a 2-D clinostat³. Once returned to Earth, this group observed an increased differentiation ability and

used *Wes* to implicate key intracellular calcium-dependent signaling proteins in progenitor cell population expansion. Their work contributes to our understanding of cardiovascular development and may be a novel approach for increased therapeutic potential following transplantation in the clinic.

A small and distinct population

Stem cells represent a very small population of cells within a tissue, and identifying the proportion that is activated and committed to repair is especially challenging using conventional techniques that require a large sample size and rely on correlation between transcript level and protein. For example, satellite cells are skeletal muscle stem cells that reside on the surface of muscle fibers where they remain in a quiescent state until activated by injury or after exercise. Due to their small population size, detecting and tracking them is difficult. Using the Single-Cell Western platform *Milo*, researchers at Stanford University School of Medicine uncovered that the myogenic differentiation factor, MyoD, is expressed in only a small proportion of muscle stem cells isolated from MyoD^{+/+} mice. **Figure 1** shows that just one of the three cells represented is positive for MyoD, whereas all three contain the loading control β -tubulin. Moreover, when

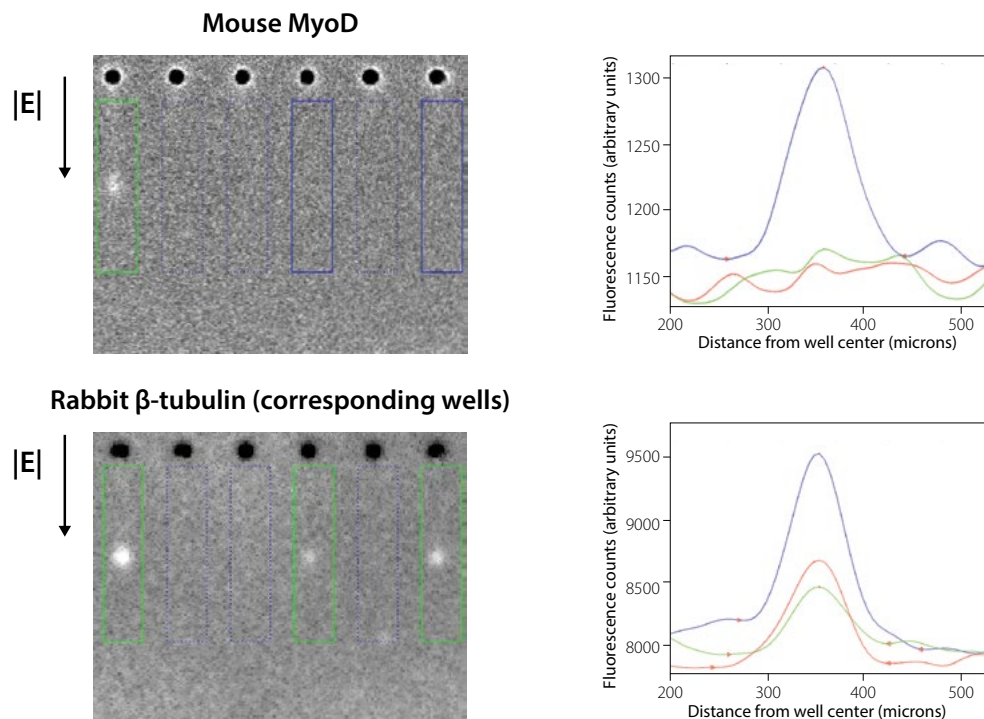


FIGURE 1. MyoD detection in skeletal muscle satellite cells from MyoD^{+/+} mice. Milo automatically detects and quantitates peaks for single-cell protein expression heterogeneity. Just one out of the three cells depicted is positive for MyoD.

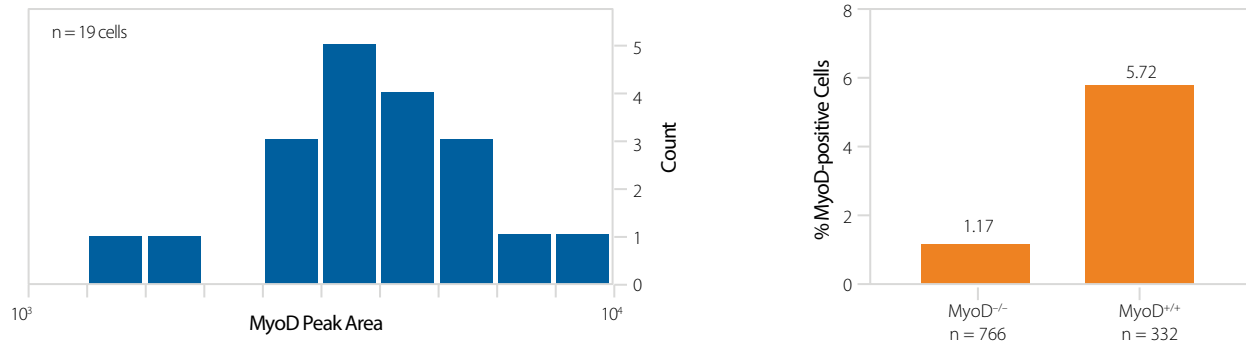


FIGURE 2. Distribution of MyoD signal and percent positive calculation. Data analysis using Scout software uncovered an approximate 1 log variation in MyoD expression and approximately 6% of cells as MyoD⁺, of those freshly-isolated from MyoD^{+/+} mice. Approximately 1% of muscle stem cells from MyoD^{-/-} mice were positive for MyoD.

looking at the overall satellite-cell population, even those cells expressing MyoD had varied expression levels, ranging almost 10-fold. Finally, quantitation studies performed by this research group using Scout software revealed that only 6% of cells analyzed express the marker-of-interest, MyoD (**Figure 2**).

Profiling heterogeneity

R&D Systems tools for [pluripotent stem cell expansion and differentiation](#) are used to create stem cell and differentiated cell populations that are compatible with

Milo for profiling heterogeneity. Following differentiation of induced pluripotent stem cells into neural stem cells using the StemXVivo Neural Progenitor Differentiation Kit (PN SC035), [Milo](#) verified the expression of common neural progenitor differentiation markers in progenitor cells only, namely Pax6 and Oct-3/4. In undifferentiated pluripotent stem cells (**Figure 3**, left), no Pax6 expression is detected, whereas clear upregulation of Pax6 is observed in the differentiated neuronal progenitor population (**Figure 3**, right). Quantitating this variation

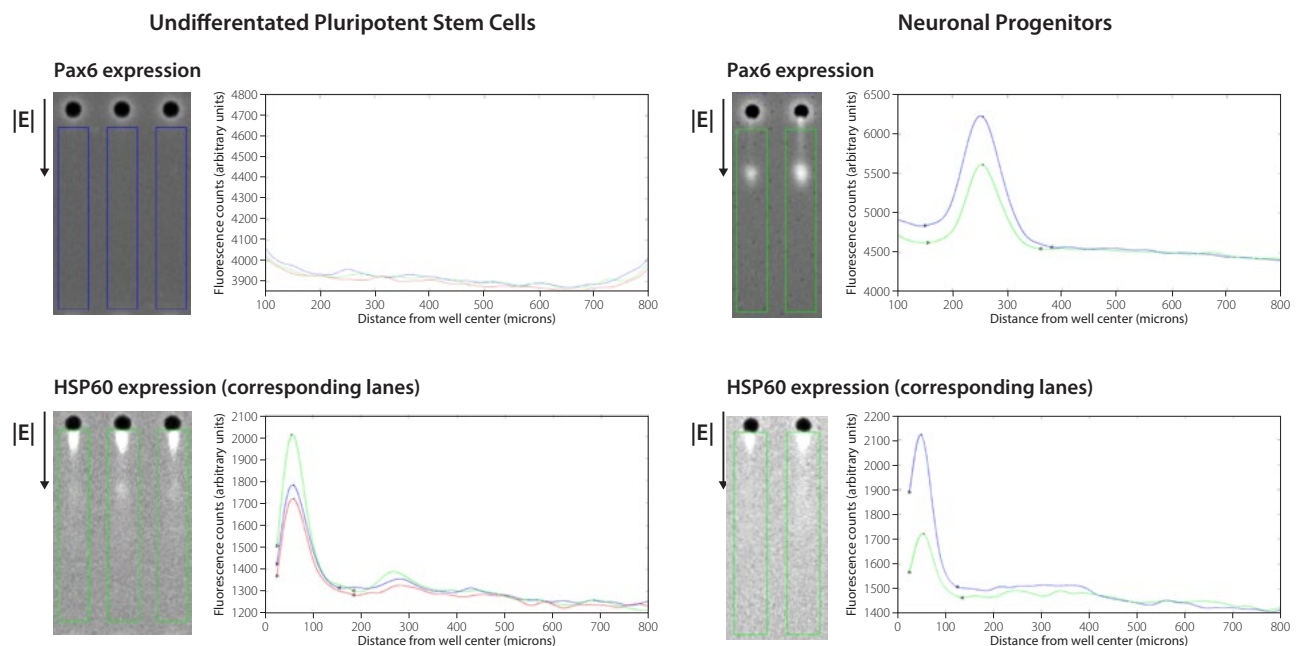


FIGURE 3. Pax6 is not expressed in the undifferentiated pluripotent stem cells analyzed but is upregulated in the neural progenitor population. HSP60, loading control.

between populations using Scout software determined a >100-fold increase in expression of Pax6 in the neuronal progenitor cells (**Figure 4**). In contrast, Oct-3/4 expression was >100-fold higher in the undifferentiated pluripotent population than the neuronal progenitors

analyzed (**Figure 5** and **6**). Milo data allows for the observation of protein heterogeneity on a single-cell level, and the distribution graphs provide for deeper analysis of variance between each cell in a population being analyzed (**Figure 4** and **Figure 6**).

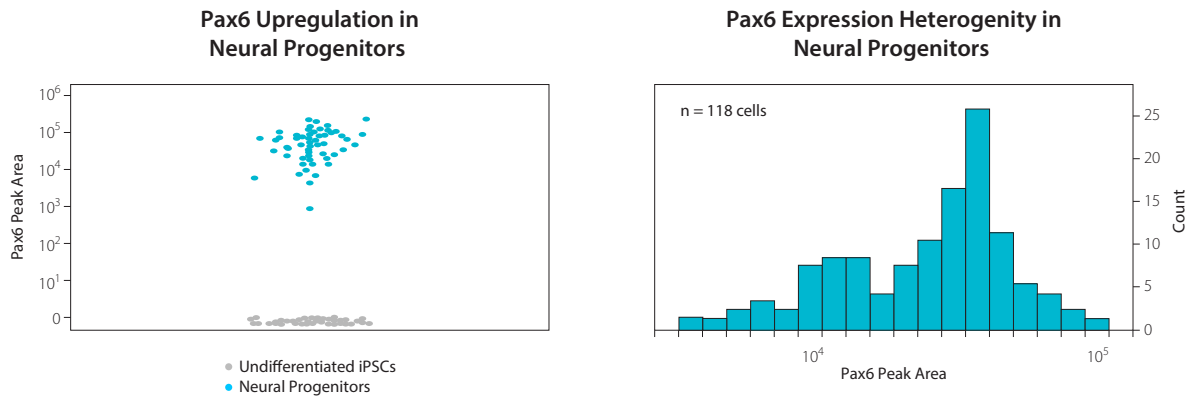


FIGURE 4. Expression heterogeneity and enumeration of Pax6+ in neuronal progenitor cells.

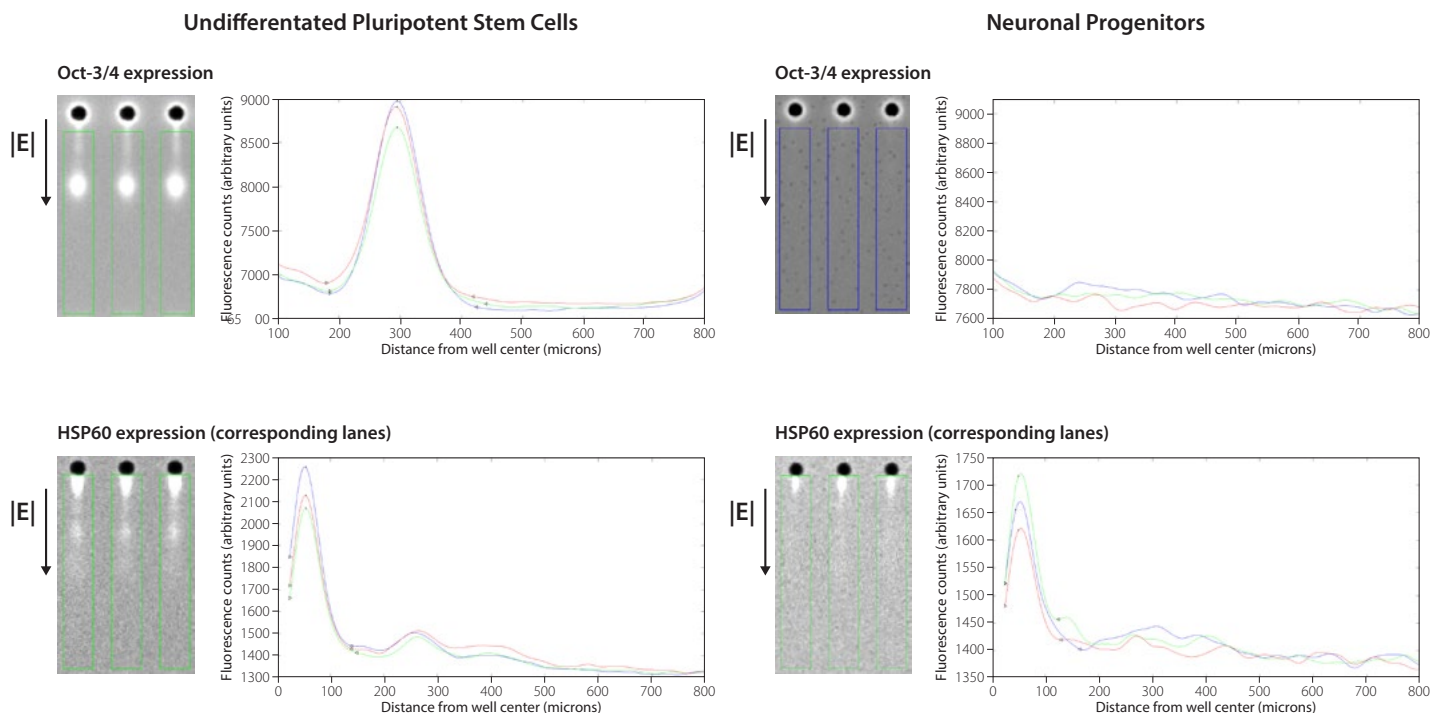


FIGURE 5. Oct-3/4 expression is upregulated in undifferentiated pluripotent stem cells analyzed but is turned off in the neural progenitor population. HSP60, loading control.

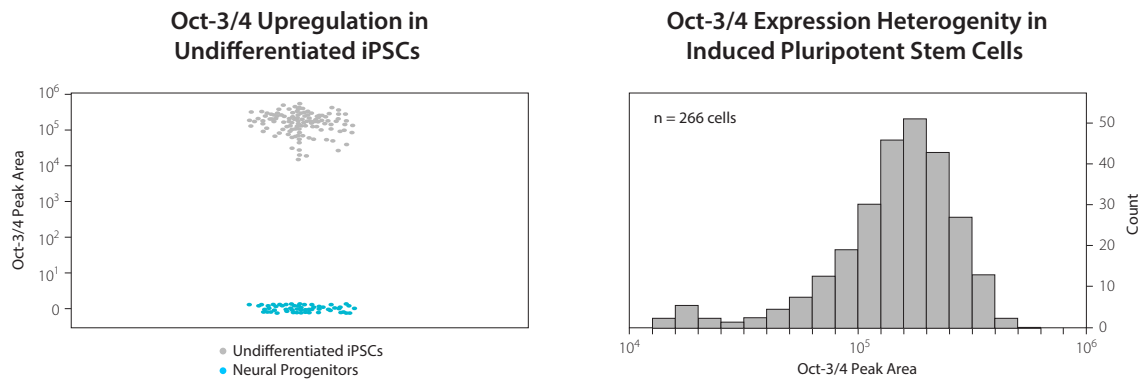


FIGURE 6. Expression heterogeneity and enumeration of Oct-3/4 in neuronal progenitor cells.

The stem cell research field is growing quickly and defining stem cell marker patterns according to the developmental state or degree of differentiation is a key component of most research objectives. The approaches being taken in doing so are wide-spread, making standardization imperative. A consistent and reliable protocol is not possible without a suitable instrument—and for that, ProteinSimple is the answer.

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Chapter 4

Signaling pathway manipulations

Signal transduction pathways are intertwined in a complex network of receptors and intracellular protein components that crosstalk to drive, maintain or halt stem-cell properties and states of existence. They can fluctuate depending on the tissue origin, stage of development or degree of differentiation. Understanding normal tissue regeneration processes and potential therapeutic applications thereof hinges on your

ability to dissect and manipulate the signaling pathways at play. Your efforts are only as good as the protein analysis tools you have in place to do so!

Traditional Western blotting protocols are timely and demanding, so having the right equipment to capture the perfect image at the end of the day is not only gratifying but necessary for making conclusions from your data. We've pulled some examples from the literature to demonstrate how researchers in various stem-cell fields are using **FluorChem systems** to do exactly that.

Adult stem cells exist in a quiescent state that is reversible in response to injury, upon which those activated to proliferate will either differentiate (opting out of the cell cycle) or self-renew and possibly revert to the quiescent state after regeneration. The underlying mechanisms responsible for the perpetuation of this quiescent state remain to be known and likely involve multiple proteins and pathways. Indeed, in a study looking at adult muscle stem cells, Yue et al. have shed more light on the signaling pathways involved¹. Using the **FluorChem R system**, researchers implicate the phosphatase and tensin homologue (Pten) as a requisite for adult stem cell quiescence and acquire additional images of related pathway proteins such as Akt, FoxO1 and Hes1 on Western blot membranes¹.

Similarly, looking at the role of another adult stem cell population in tissue regeneration, researchers in China investigate the underlying mechanism of mesenchymal stem cell (MSC) migration via adhesion to endothelial cells during an inflammatory response². Using the **FluorChem Q system**, they uncover the importance of the NF κ B, ERK and JNK signaling pathways in overseeing TNF- α induced expression of the vascular cell adhesion molecule 1 (VCAM-1), required for the adhesion of MSCs during tissue repair.

At the cell surface, G-protein coupled receptors (GPCRs) are fundamental for the intracellular signaling events that regulate stem cell survival, growth and ultimately tissue repair. In the case of keratinocytes, two GPCR family members, G α 11 and G α q, are heavily relied upon for cutaneous wound healing—so much so that their deletion results in premature terminal differentiation and defective migration³. To study the effect of over- or under-expression of G α 11 and G α q signaling, Doçi et al. use the **FluorChem E system** to track the chemiluminescent signal of proteins involved in keratinocyte-mediated wound healing with or without applied stimuli³. Their work highlights the incorporation of multiple signaling networks in any given biological process.

Stem cells, in the context of cancer, have some shared features with adult stem cells. The existence of this small subpopulation of cancer stem cells may explain

how and why tumors migrate and recur. Monitoring protein expression and contribution as related to the signaling pathways that drive this population to promote metastasis requires a reliable method of enhanced chemiluminescent signal detection for your Western blotting protocol. The **FluorChem M system** helped researchers at Sun Yat-sen University in China to confidently implicate RSPO2 in a specific and rare subpopulation of colon cancer stem cells that influence metastasis via epithelial-mesenchymal transition⁴.

Influencing stem cell fate with cytokines

Cytokines are often used to strategically direct stem cell differentiation, proliferation, survival and even the activation of other cell types in culture. In addition to being used as a growth matrix for culturing mouse embryonic stem cells (mESCs), **mouse embryonic fibroblasts (MEFs)** also serve as a source of secreted factors required for maintaining pluripotency, can be manipulated as so, and are thus often referred to as feeder cells. The Proteome Profiler™ Antibody Arrays from R&D Systems® and our **FluorChem imagers** enable the quick identification of changes of multiple analytes in your stem cell samples using one simple assay. R&D Systems offers 30 different arrays that cover over 1,000 different analytes for human, mouse and rat. You can also use them for a wide variety of samples like cell and tissue lysates, cell culture supernatants, serum and plasma. The array kits contain all the buffers and reagents needed for chemiluminescence detection; what's more, a simple detection antibody swap lets you do fluorescence detection! Check out the [Proteome Profiler Antibody Arrays web page](#) to learn more about available kits.

Using the Mouse XL Cytokine Antibody Array (R&D Systems, ARY028) to determine the level of selected mouse cytokines, BALB/3T3 MEFs were treated with or without 100 ng/mL of recombinant mouse TNF- α for 24 hours before collecting supernatant for analysis, performed according to the standard protocol outlined in the product insert. Arrays were imaged on the **FluorChem M system** using the chemiluminescence protocol with a series of manual scans. TNF- α induced the expression of CCL2/JE/MCP-1, CCL11/Eotaxin, CCL20/

MIP-3 α , CYCL10/IP-10 and TNF- α itself (**Figure 7**). The induced expression was quantitated to be ~6-fold greater for CCL2/JE/MCP-1 and ~194-fold greater for CCL11/Eotaxin, compared with untreated controls. In the same array, fold increase in expression of CCL20/MIP-3 α and CXCL10/IP-10 was computed to be ~992-fold and ~353-fold, respectively.

Similarly, stem cell-derived human umbilical vein endothelial cells (HUVECs) were cultured with or without 100 ng/mL recombinant human TNF- α for 24 hours before supernatant was collected to run on a Human Cytokine XL Antibody Array (R&D Systems, ARY022B) using fluorophore-conjugated antibodies for detection by fluorescence. Samples were screened for TNF- α induced expression differences in 105 different cytokines. Arrays were imaged using a **FluorChem R system** and the infrared protocol to

capture a series of manual exposures. Quantitative data analysis revealed a ~24-fold increase in GM-CSF expression, ~3-fold increase in GRO α expression, ~2-fold increase in IL-8 expression, ~46-fold increase in RANTES expression and ~43-fold increase in VCAM-1 expression (**Figure 8**).



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Application Note:

Versatile and Simple Imaging of Proteome Profiler Antibody Arrays with FluorChem Imagers
[Click here to read the full application note](#)

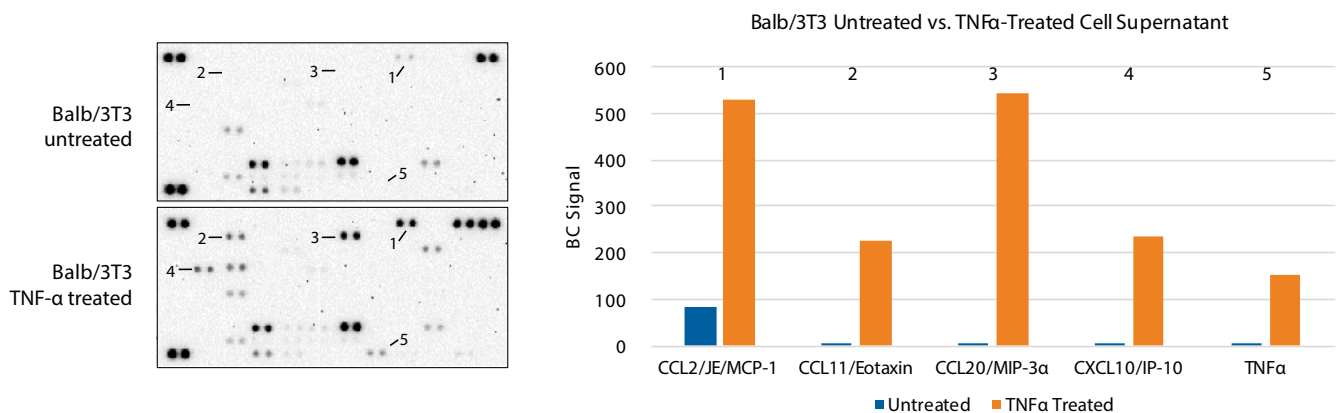


FIGURE 7. Supernatant from Balb/3T3 MEFs treated with, and without 100 ng/mL, recombinant mouse TNF- α were analyzed for changes in cytokine expression. Arrays (left) were imaged for 10 minutes on a FluorChem M system, and AlphaView software was used to quantitate the fold increase in five different cytokines (right).

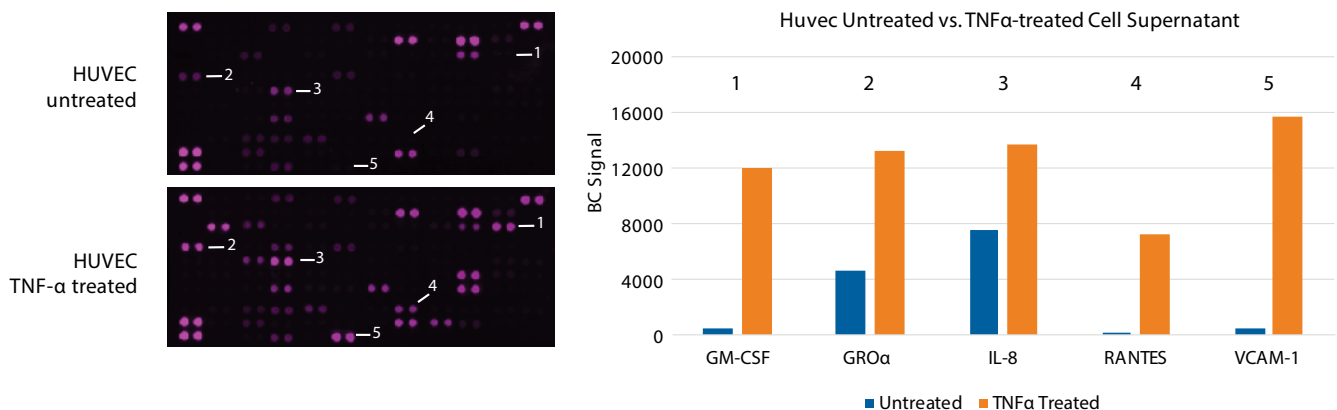


FIGURE 8. Supernatant from HUVECs treated with and without TNF- α were screened for changes in cytokine expression. Arrays (left) were imaged for 10 minutes on a FluorChem R system, and AlphaView software was used to quantitate the fold change in cytokine expression (right).

Multiple pathways, multiple proteins

With so little sample yet so many potential players in any given pathway, obtaining more data points from one sample and in less time would mean you're well ahead of the curve. Multiplexing can give you this flexibility. Our [Simple Western](#) and [Single-Cell Western](#) platforms can multiplex, so you get the most data out of each sample.

Single-Cell Westerns on [Milo](#) multiplex to measure approximately four proteins per single cell via spectral multiplexing and size-based multiplexing. To spectrally

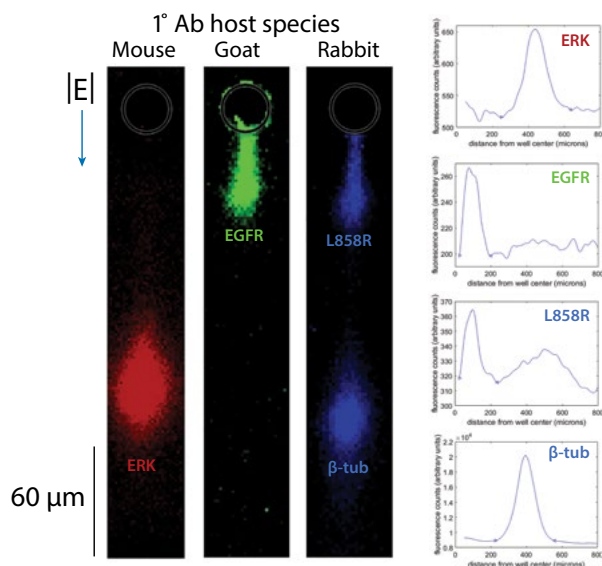


FIGURE 9. Multiplexing with Single-Cell Western. Data for four proteins detected in a single cell. Two different spectral channels were used to detect anti-mouse ERK and anti-goat EGFR, while a third was used to detect β -tubulin and L858R with two anti-rabbit antibodies which were resolved based on their different molecular weights.

multiplex, first probe your targets of interest with primary antibodies raised in different host species—mouse and rabbit for example. Next, probe with host-specific secondary antibodies tagged with different fluorophores—for example, donkey anti-mouse Cy3 and donkey anti-rabbit Cy5. For size-based multiplexing, if there's more than a 30% molecular weight difference between your proteins of interest, you can probe for them with primary antibodies raised in the same host species and image them in the same spectral channel. These targets can be resolved in the SDS-PAGE separation on the scWest chip and differentiated based on molecular weight. scWest chips can also be stripped and re-probed up to nine times for additional multiplexing. **Figure 9** shows Single-Cell Western data for four proteins detected in a single cell. Three different spectral channels were used to detect three protein targets, while β -tubulin and L858R were resolved based on their different molecular weights.

Single-cell resolution is especially important for understanding the interactions between different proteins and signaling events that make up a bulk, heterogeneous sample of stem cells. Milo delivers greater insight into the heterogeneity that underpins these complex samples. With Milo, you can uncover distinct cell subpopulations based on single- or co-expression of different protein targets, detect discrete cell-signaling states driven by multiple phosphorylated proteins in an individual cell, and all the while simultaneously quantify both phosphorylated and total protein expression across stimulated and unstimulated stem cell populations. An example of this is shown in **Figure 10**.

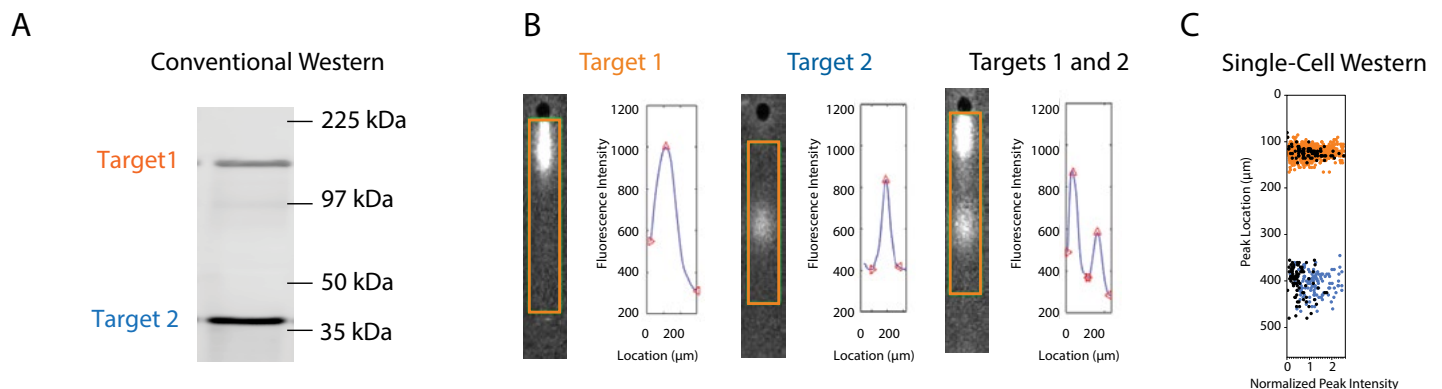


FIGURE 10. Milo Single-Cell Westerns reveal three subpopulations of cells not visible on a conventional Western. (A) Traditional Western blot shows two distinct proteins (Target 1 and Target 2) within the bulk population. (B) Single-Cell Western analysis identifies three cell subpopulations within the overall population, one expressing only Target 1, one expressing only Target 2, and one expressing both Target 1 and Target 2. Representative single-cell separation images from each subpopulation (B) and fluorescent intensity plots (C) generated by Scout software show the fluorescence intensity measured along the separation lane. The red diamonds indicate the start, middle, and end points of the peak automatically detected by Scout (C).



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Application Note:

Reveal Cell Subtypes, Protein Isoforms, and Phospho-Protein Heterogeneity with Milo.

[Click here to read the full application note](#)

Epigenetic and genetic modifications

Histones are critical protein regulators of gene expression. The dysregulation of histone modifications has pleiotropic signaling effects that are associated with a number of diseases. Therefore, manipulating this activity is an attractive approach to generating specific cell types for transplantation. The detection and quantitation of histones, however, can be challenging using traditional proteomic methods.

New measurement capabilities for histones could be key to unlocking discoveries in the field of stem cells and epigenetics, and how they relate to cancer and aging, for

example. Because Milo lyses the cells before analysis and the workflow is flexible to allow for sample pre-treatments and digestions, highly complexed proteins can be measured in single cells to reveal expression heterogeneity of histone modifications. In **Figure 11** we demonstrate the ability of Milo to measure heterogeneity in H3K27me3 and total histone H3 expression in single HeLa cells treated with the on-chip chromatin digestion protocol.

In a recent *Frontiers in Molecular Neuroscience* publication, Bailey et al. from Virginia Tech measured histone acetylation in tissue isolated from the prefrontal cortex of Sprague Dawley rats in a model of blast-induced neurotrauma⁵. Herein, the Simple Western Size Assay on *Wes* helps researchers to differentiate between no change in total histone protein expression level and decreased levels of acetylation of histone H2b, H3 and H4 following injury. In another publication, McConnell et al. from Université Paris Diderot, used Simple Western Charge Assays on *NanoPro 1000* to detect acetylated and nonacetylated isoforms of promyelocytic leukemia zinc finger protein (PLZF), a pivotal stem cell regulator, in cell extracts⁶. Their work measured changes in levels of PLZF acetylated isoforms in the presence of HDAC3 and SIRT1.5, controlling factors not previously well understood but

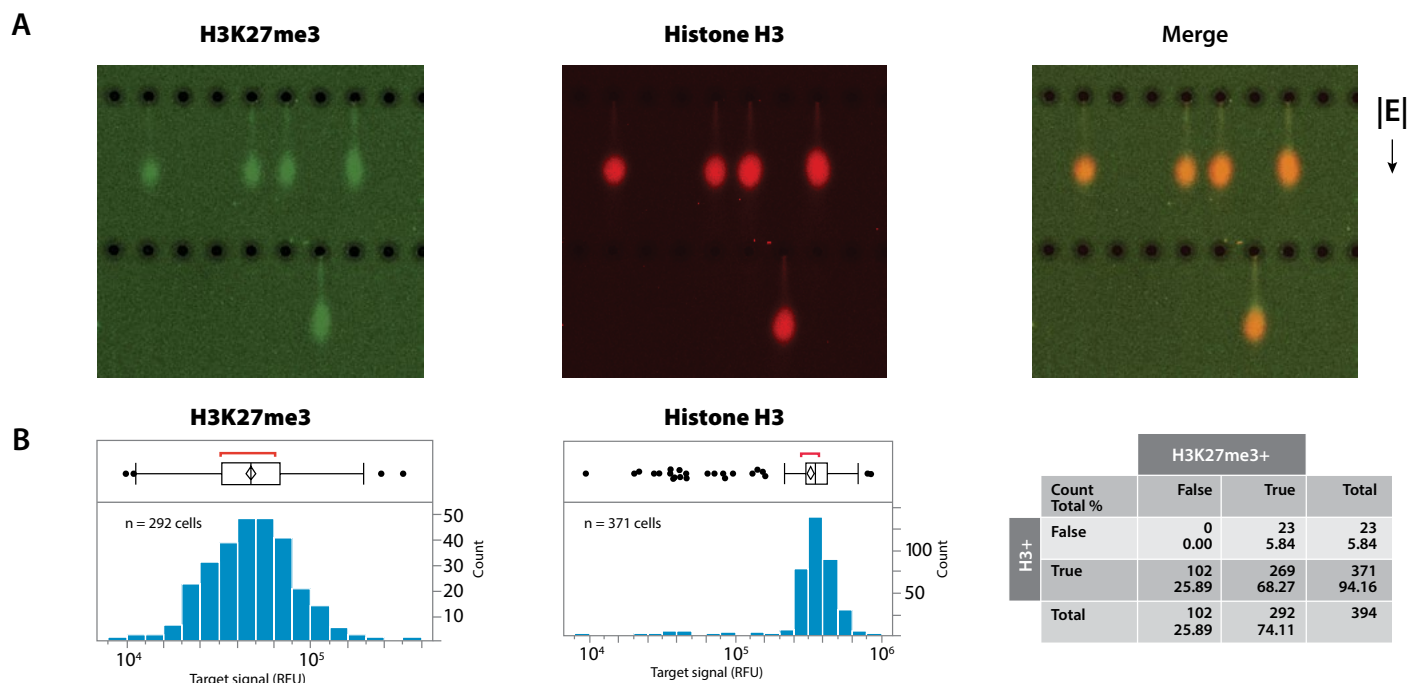


FIGURE 11. H3K27me3 signal co-localizes with total histone H3. Single-Cell Western detection of H3K27me3 (green) was validated by comigration with total histone H3 (red) (A). H3K27me3 and histone H3 signal distributions (RFU) and co-detection analysis (B). H3K27me3 was detected in ~68% of cells identified by histone H3 expression.

whose interaction is essential in keeping PLZF balance for stem cell differentiation.

Finally, endless possibilities exist for pathway manipulation and therapeutic strategy in genomic and regenerative medicine when CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and stem cells are combined. Confirming your edited transcript translates to the desired protein product and function in vitro and in vivo is critical for progressing to the clinic, and you can't get quantitative and meaningful results using conventional Western blotting techniques! For instance, Harvard researchers striving to restore dystrophin expression in Duchenne muscular dystrophy (DMD) employed CRISPR technology to create active complexes that could be delivered locally and systemically in both neonatal and adult mice⁷. Targeted cells include terminally differentiated skeletal muscle fibers, cardiomyocytes and satellite cells, where the modulated gene function was quantitatively analyzed and confirmed on a protein level using *Wes* to detect changes in dystrophin protein expression in the indicated muscles of mice receiving the systemic CRISPR therapy⁷.

Simple Western technology is a solution that will have you running quantitative, multiplexed assays quickly and efficiently in no time. See our [how-to guide](#) to learn more, plus tips on implementing the workflow in your lab!

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Chapter 5

Disease models and therapeutic strategies

Developing pre-clinical stem cell-based therapies and disease models is interdisciplinary. Cells are often modified or induced ex vivo to correct, replace, or regenerate cells, tissues, or organs in vivo. Whatever your disease model system or therapeutic approach, protein expression evaluation and quantitation are integral components of making sure your model or strategy works as proposed. However, doing so using traditional protein analysis techniques is both laborious and inaccurate. Here's how scientists are using ProteinSimple technologies to advance their research confidently.

Currently, no effective treatment for peripheral nerve injury (PNI) exists. Using an in vivo nerve transection surgery model, researchers in China implicate conserved dopamine neurotrophic factor (CDNF), that's been transduced into mesenchymal stem cells (MSCs) for delivery, in the augmentation of axonal and Schwann cell regeneration. To measure CDFN transduction in vitro, and transfer efficiency in vivo, the group used the [FluorChem E system](#) to draw relative density conclusions from Western blot imaging studies¹. Taken together, the data provide evidence for CDFN gene therapy to be significantly neuroprotective in a PNI disease setting. Similarly, the search for effective therapies for Parkinson's disease (PD) continues to come up short. Zhao et al. at the University of North Carolina at Chapel Hill previously published a strategy that promotes regeneration of dopaminergic neurons, which may overcome the limitations of tried therapeutics. The group

used autologous macrophages that were transfected ex vivo with glial cell-line derived neurotrophic factor (GDNF) followed by return delivery to the inflamed brain of mice². To validate the expression of GDNF protein in transfected macrophages, and the subsequent exosomes they secrete, researchers utilized the **FluorChem E system**, which allowed them to proceed to their in vivo disease model confidently².

Outside of neuroscience, MSCs are commonly investigated for their potential to repair the injured myocardium. But for their use as a cellular therapy to be successful, much work has focused on enhancing their viability in the harsh conditions that make up the microenvironment of an ischemic heart. Tian et al. uncovered a protective effect of globular adiponectin (gAPN) for MSC survival in an in vitro model mimicking the ischemic conditions via hypoxia and serum starvation³. Using a **FluorChem M system** this group further teased out that the anti-apoptotic function of gAPN is assisted by various proteins in the AMPK signaling pathway. The phenotypic assays used to detect morphological changes and consequential apoptosis allowed researchers to make general conclusions about MSC survival, but it was the protein expression analysis and pathway delineation, resolved by the FluorChem M, that generated data specific to implicating gAPN as a novel survival factor, of which the strategic efficacy can now be tested in vivo.

Simple Western technology is actively helping researchers undertake the complex process of decoding human

neurodevelopment to build reliable models of disease. Recently used by Keio University School of Medicine in Japan to address the dysfunction of the chromatin-remodeling factor, CHD7, in human neuroectodermal development, Chai et al. used induced pluripotent stem cell-derived neuroepithelial (iPSC-NE) cells, primary cells derived from CHARGE patients and a brain organoid model derived from iPSCs to establish CHD7 as a key player in NE identity and central nervous system (CNS) lineage development⁴. In this work, Simple Western provided insight into the causes of several known abnormalities characteristic of CHARGE syndrome by profiling protein expression and thereby confirming that human iPSCs can be used when designing models for the understanding of neurodevelopmental disorders.

Alterations in neurogenesis may be related to various early life stressors. To study the proposed link between Toll-like receptor 9 (TLR-9) and microglial activation in situations of early life stress as proponents of changes in neurogenesis that result in neuropsychiatric disease, researchers at the Medical College of Wisconsin developed a mouse model of adverse early life environment (AELE) and measured hippocampal cell numbers of microglia, neural stem cell and neuron and hippocampal TLR-9 expression⁵. Using **Wes** to analyze TLR-9 protein expression in mouse-derived hippocampi, they uncovered a process by which AELE induces immune responses that may damage offspring hippocampal neurogenesis⁴.



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A myriad of reasons for a quantitative approach

The basis of obesity, a surplus of adipose tissue, is multifactorial. As the master regulator of adipogenesis, proliferator-activated receptor gamma (PPARG) activation by ligand/chemical binding is thought to drive adipogenesis and adipocyte hyperplasia during gestation, and thereby predisposition to childhood obesity. To uncover factors contributing to the developmental origins of obesity, researchers at The Hamner Institutes for Health Sciences used human adipose-derived stem cells (hASC) isolated from adipose tissue as the first human primary-cell model of adipogenesis to screen a set of 60 chemical compounds that were either PPARG active or inactive. Those showing activity were evaluated for their effect on key events in PPARG-dependent adipogenesis, including gene transcription and quantitative protein expression analysis of phenotypic markers using *Wes*⁶. This study draws awareness to the contributing power of PPARG-activating chemicals in obesity and the “obesogen hypothesis.”

Likewise, colorectal cancer progression can be attributed to a multitude of factors, for which a disease model that physiologically recapitulates the tissue structure and organization is imperative to understanding the contributing power of each. To quantitatively measure the cooperation of oncogenic Wnt- β -catenin signaling and PI3K activity, researchers in Germany utilized *Wes* for protein expression and phosphorylation analysis of organoid samples of mouse small intestine⁷. They used a combination of pharmaceutical inhibitors, phenotypic assays and phosphoprotein profiling to get to the bottom of cell survival and motility. With *Wes*, this group could confidently associate cell signaling networks in intestinal organoids to cancer-related phenotypes.

Therapeutic approaches and pre-clinical disease models that are stem cell-derived are complex and call for a comprehensive approach to ensure physiological relevance and biological safety. Investigating, characterizing and validating protein expression is indispensable to moving forward with your design. Don't worry, ProteinSimple has the tools you'll need to streamline your protein characterization workflows.

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