

IGFBP2 IN CELL CULTURE

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. provisional application no. 63/627,359, filed on January 31, 2024, the contents of which are incorporated herein in their entireties by reference thereto.

2. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML Sequence Listing, created on December 12, 2024, is named BRT-009WO_SL.xml and is 361,501 bytes in size.

3. BACKGROUND

[0003] Obtaining an accurate estimate of the number of cells in a cell culture system is important for monitoring cell proliferation and viability, optimizing cell culture conditions, preparing cell-based assays, and processing the cells for downstream use.

[0004] There are a variety of methods to count cultured cells, ranging from counting the cells manually using a microscope, to using an automated cell counter or a flow cytometer. Yet, existing cell counting methods have several shortcomings. Manual cell counting relies on human visualization, which is susceptible to inaccuracies due to subjective differences, selection of an area of the culture dish that is not representative of the entire culture vessel, or improper visualization of a sample associated with cell aggregation, debris, or eyesight issues. Automated cell counting techniques can eliminate subjective errors; however, they may require treatment of cells with a dye or dissociation of cells to generate homogenous cell suspensions, both of which not only disrupt the cells in the culture but also suffer from errors associated with aggregation and debris.

[0005] There is need in the art for a reliable method to estimate the number of cells that can be performed without disrupting cells in a culture system.

4. SUMMARY

[0006] The present disclosure relates to the use of the concentration of secreted IGFBP2 in media of cells in culture as a measure of cell count, cell health, and for normalization of cell product levels. The use of secreted IGFBP2 levels as a surrogate for cell count, cell health, and

for normalization of cell product levels is particularly useful in cultures that involve cells attached on a particular surface, e.g., in roller bottles, tissue culture plates, or cell factories.

[0007] Accordingly, the present disclosure provides methods of quantifying (e.g., measuring the concentration of) IGFBP2 in cell culture media (sometimes referred to herein as “IGFBP2 assays” for convenience), and applications thereof in a number of laboratory, research, and clinical applications.

[0008] Methods of quantifying IGFBP2 levels in media samples are described in Section 6.3 and numbered embodiments 1 to 43, including methods and reagents as described in Section 6.3.1 and numbered embodiments 33 to 35 and timing and frequency of as described in Section 6.3.2 and numbered embodiments 4 to 25. IGFBP2 is expressed as multiple splice variants, which are described in Section 6.2. The methods of the disclosure may measure any single splice variant or combination of (or all) splice variants.

[0009] The IGFBP2 assays of the disclosure can be used to estimate the number of cells and/or to detect an abnormality in a cell culture (e.g., by indicating abnormal proliferation, for example in a cell culture comprising cells undergoing maturation) of a variety of cell types, e.g., as described in Section 6.4 and numbered embodiments 44 to 95. In some embodiments, the cells are pluripotent stem cells (PSCs), e.g., as described in Section 6.4.1, including but not limited to induced pluripotent stem cells (iPSCs), as well as PSC-derived cells, e.g., as described in Section 6.4.2.

[0010] Further applications of the IGFBP2 assays of the disclosure include normalization of cell product levels, e.g., as described in Section 6.5.4 and numbered embodiments 96 to 104 and assessment of cell health, e.g., as described in Section 6.5.5 and numbered embodiments 105 to 142.

5. BRIEF DESCRIPTION OF THE FIGURES

[0011] FIG. 1 is a schematic illustration of exemplary workflows involving IGFBP2 assays as applied to PSCs. The core of the methods (5) entails measuring IGFBP2 levels in the cell culture media (white arrow with solid border), typically (but not necessarily) prior to feeding the cell culture (dotted arrow with solid border). This process can be repeated one or more times (white and dotted arrows with dashed borders). The core (5) of the workflow can be preceded by the optional steps of seeding the PSC cell culture (4), optionally subsequent to generating PSCs (1), thawing preserved PSCs (2), and/or passaging PSCs (3). The core (5) of the workflow can be followed by passaging PSCs (6), differentiating PSCs (7), preserving PSCs (8),

e.g., by cryopreservation, discarding PSCs (9), or any combination thereof. Optional steps in the process are depicted in dashed lines. Although shown as applied to PSCs, the IGFBP2 assays of the disclosure can be used for other cell types, including but not limited to cell types differentiated or undergoing differentiation from PSCs. In some embodiments, the IGFBP2 assays can be applied to cell cultures in which PSCs are being differentiated into dopaminergic (DA) neurons.

[0012] FIGS. 2A and 2B are graphs showing the correlation between concentration of IGFBP2 as measured in culture media and cell density. **FIG. 2A** displays the linear correlation between IGFBP2 expression and iPSC seeding density. **FIG. 2B** displays the concentration of IGFBP2 as measured in culture media and number of PCs.

[0013] FIGS. 3A-3C are graphs showing the correlation between IGFBP2 concentration as measured in culture media and cell counting methods. **FIG. 3A** displays the linearity between PSC seeding density and IGFBP2 levels in three sets of experiments, d1, d2, and d3. Supernatant samples were collected at 24 hours and 48 hours from seeding the culture for the d1 and d2 measurements, respectively, and at 24 hours after a media change and 72 hours from seeding a separate culture for the d3 measurements. **FIG. 3B** displays the correlation between IGFBP2 levels and Incucyte®-based cell count measurements of d1, d2, and d3. **FIG. 3C** shows the IGFBP2 correlation differences between Incucyte®-based and NC200-based cell counting in d3.

[0014] FIGS. 4A-4B show the correlation between IGFBP2 levels and iPSC cell count in four cell factories. One cell factory was harvested each day between D1 and D4 for data collection. **FIG. 4A** is a graph that shows the IGFBP2 levels and cell count for each set. **FIG. 4B** displays the same information in **FIG. 4A** in a tabular format.

[0015] FIGS. 5A-5B show the IL12 levels in individual PSC clones normalized by cell number measured via NC200 or IGFBP2 levels. **FIG. 5A** shows IL12 levels normalized by cell number. **FIG. 5B** shows IL12 levels normalized by IGFBP2 levels in the same set of samples shown in **FIG. 5A**.

[0016] FIGS. 6A-6B show the variation of IGFBP2 levels in different batches of experiments. **FIG. 6A** shows the differences in IGFBP2 levels in distinct datasets. **FIG. 6B** shows the linearity of combined datasets.

[0017] FIGS. 7A-7B show the variation of IGFBP2 levels in PSCs being differentiated to dopaminergic (DA) neurons and correlation between IGFBP2 levels and DA neuron cell yield.

FIG. 7A shows the changes in IGFBP2 levels over time in 11 different batches of DA neurons, 6 of which were differentiated from 2D and 5 of which were differentiated from 3D PSC cultures.

FIG. 7B shows the correlation between IGFBP2 levels on post-differentiation day 12 (D12) and cell number yield on post-differentiation day 16 (D16) in 5 batches of DA neurons that were differentiated from 3D PSC cultures.

[0018] FIGS. 8A-8C show differences in cell counts and IGFBP2 levels associated with culture vessel coating conditions. **FIG. 8A** shows differences in cell counts of cultures that were seeded on cell factories either coated with a substrate overnight at 4 °C (standard coating treatment) or for one hour at room temperature (experimental coating treatment). CF10, 10-layer cell factory; CF1-4, cell factories 1-4 with standard coating treatment; CF5-12, cell factories 5-12 with experimental coating treatment. **FIG. 8B** shows differences in IGFBP2 levels in supernatant of cultures that were seeded on cell factories either coated with a substrate overnight at 4 °C or for one hour at room temperature. **FIG. 8C** shows the linearity of combined datasets.

[0019] FIGS. 9A-9B show the effects of differences in media supplementation on IGFBP2 levels and sphere formation in 3D PSC cultures. **FIG. 9A** shows differences in IGFBP2 levels in PSC cultures, the media of which were supplemented with either Y-27632 or CEPT cocktail. **FIG. 9B** shows differences in sphere counts in the same PSC cultures as in **FIG. 9A**.

[0020] FIGS. 10A-10B show the correlation between IGFBP2 levels and amount of cellular spheres in 3D PSC cultures. **FIG. 10A** shows the correlation between IGFBP2 levels and sphere counts. **FIG. 10B** shows the correlation between IGFBP2 levels and total sphere volume.

[0021] FIGS. 11A-11D show differences in IGFBP2 levels and cell count yields associated with cell seeding density. **FIG. 11A** shows differences in IGFBP2 levels in supernatants of clones seeded at 2.5k, 5k, 10k, and 20k cells/cm² on day 1. **FIG. 11B** shows changes in IGFBP2 levels in supernatants of clones seeded at 2.5k, 5k, 10k, and 20k cells/cm² over time. **FIG. 11C** shows differences in amount of IGFBP2 measured compared to the cell yield from cultures seeded at 5k cells/cm² and harvested on days 3, 4, and 5. **FIG. 11D** shows the relationship between IGFBP2 and differences in cell count yields of clones seeded at 2.5k, 5k, 10k, and 20k cells/cm².

[0022] FIGS. 12A-12H show brightfield images of PSCs seeded at different densities three or four days after seeding. **FIG. 12A** shows cell density of PSCs three days after they were seeded at 2.5k cells/cm². **FIG. 12B** shows cell density of PSCs three days after they were seeded at 5k

cells/cm². **FIG. 12C** shows cell density of PSCs three days after they were seeded at 10k cells/cm². **FIG. 12D** shows cell density of PSCs three days after they were seeded at 20k cells/cm². **FIG. 12E** shows cell density of PSCs four days after they were seeded at 2.5k cells/cm². **FIG. 12F** shows cell density of PSCs four days after they were seeded at 5k cells/cm². **FIG. 12G** shows cell density of PSCs four days after they were seeded at 10k cells/cm². **FIG. 12H** shows cell density of PSCs four days after they were seeded at 20k cells/cm².

[0023] FIGS. 13A-13D show results of FACS analysis of pluripotency markers in PSC clones seeded at 5k cells/cm², assessed 3, 4, and 5 days after seeding. **FIG. 13A** shows the percentage of cells positive for OCT4. **FIG. 13B** shows the percentage of cells positive for NANOG. **FIG. 13C** shows the percentage of cells positive for SOX2. **FIG. 13D** shows the percentage of cells positive for SSEA4.

[0024] FIGS. 14A-14D show the results of a FACS analysis of pluripotency markers in PSC clones seeded at different densities and assessed 4 days after seeding. **FIG. 14A** shows the percentage of cells positive for OCT4. **FIG. 14B** shows the percentage of cells positive for NANOG. **FIG. 14C** shows the percentage of cells positive for SOX2. **FIG. 14D** shows the percentage of cells positive for SSEA4.

[0025] FIGS. 15A-15B show the PSC replating results of PSCs that were initially seeded at 2.5k, 5k, 10k, and 20k cells/cm². **FIG. 15A** shows confluency of replated cells. **FIG. 15B** shows percent recovery rates of replated cells.

[0026] FIGS. 16A-16H show correlations between IGFBP2 levels and various measures. **FIG. 16A** shows the correlation between IGFBP2 levels on day 1 after seeding and seeding density. **FIG. 16B** shows the correlation between IGFBP2 levels on day 4 after seeding and cell number yield on day 4. **FIG. 16C** shows the correlation between IGFBP2 levels on harvest day and percent confluence of cells replated at 200k cells/cm². **FIG. 16D** shows the correlation between IGFBP2 levels on harvest day and percent confluence of cells replated at 100k cells/cm². **FIG. 16E** shows the correlation between IGFBP2 levels on harvest day and percentage of cells positive for OCT4, which includes all data points. **FIG. 16F** shows the correlation between IGFBP2 levels on harvest day and percentage of cells positive for SOX2, which includes all data points. **FIG. 16G** shows the correlation between IGFBP2 levels on harvest day and percentage of cells positive for OCT4, which excludes data from PSCs seeded at 20k cells/cm². **FIG. 16H** shows the correlation between IGFBP2 levels on harvest day and percentage of cells positive for SOX2, which excludes data from PSCs seeded at 20k cells/cm².

[0027] FIGS. 17A-17C show the relationship between IGFBP2 levels and the maturation of cardiomyocytes cultured in different conditions. **FIG. 17A** shows the differences IGFBP2 levels in each culture. **FIG. 17B** and **FIG. 17C** show the results of a FACS analysis of Culture B cardiomyocytes, which were differentiated from PSCs using either an optimal (**FIG. 17B**) or a low (**FIG. 17C**) amount of a culture medium additive.

6. DETAILED DESCRIPTION

[0028] Accurately estimating cell numbers in various culture conditions presents a significant challenge. Traditional methods for cell counting, such as manual counting under a microscope or using automated cell counters, often require cell harvesting, which can be labor-intensive and may disrupt the cell culture environment. These methods are particularly challenging in large-scale cell culture systems, such as cell factories, where visual estimation and/or obtaining a representative cell sample is difficult due to the scale and complexity of the setup. Additionally, in-process monitoring to ensure maintenance of cell health and consistent cell culture (e.g., growth, differentiation, dedifferentiation, or maturation) is crucial for manufacturing of cell therapies, and useful also for research and therapeutic development.

[0029] Current solutions for cell counting and monitoring in cell culture systems have several limitations. Manual counting is prone to human error and variability, while automated systems can be expensive and may not accurately account for cell clumping or non-uniform distribution. Furthermore, these methods often require cell detachment and suspension, which can affect cell viability and alter the culture conditions. In large-scale systems, such as bioreactors or multilayer cell factories, these challenges are increased, making it challenging to obtain accurate and timely cell counts. Moreover, existing methods may not provide real-time data on cell health or the effects of different culture conditions, reducing their usefulness in dynamic and complex cell culture environments.

[0030] The present disclosure addresses these challenges by utilizing IGFBP2 concentration as a novel biomarker for estimating cell numbers and for assessing cell culture status (e.g., detection of an abnormality in a cell culture such as abnormal proliferation in a cell culture comprising cells undergoing maturation) in various culture conditions. By correlating IGFBP2 levels with cell count and/or cell status, the methods of the disclosure provide a non-invasive and efficient way for monitoring cell cultures without the need for cell harvesting. This approach is particularly advantageous in large-scale and visually challenging environments, such as cell factories and 3D cultures, where traditional methods fall short. Additionally, the methods of the disclosure can be applied to provide insights into the effects of different culture conditions, such

as seeding density, substrate coating and media supplements, on cell growth and health, thereby enhancing the ability to optimize and control cell culture processes.

[0031] The methods described herein offer significant advantages over traditional cell counting methods, particularly in terms of cost and time efficiency. By utilizing IGFBP2 concentration as a marker for cell number, these methods provide a rapid in-process assessment of cell culture status without disturbing the culture (e.g., by passaging or harvest), which enables cost-effective decision making (e.g., to stop a culture or add one or more reagents to adjust conditions). The approaches described herein can reduce or eliminate the need for labor-intensive and time-consuming manual cell counting, which often requires cell harvesting and can lead to cell loss or damage. Additionally, the ability to monitor cell status (e.g., health and/or maturation) through IGFBP2 levels allows for early detection of unhealthy or otherwise unsuitable cultures, enabling timely intervention or termination of unsuitable cultures. This proactive management can reduce unnecessary expenditure on media and other resources, as cultures that are unlikely to succeed can be discarded early, preventing further investment in their maintenance. Overall, the methods described herein streamline the cell culture process, offering a more efficient and cost-effective solution compared to conventional cell counting and culture monitoring techniques.

[0032] In certain aspects, the present disclosure provides methods for estimating cell numbers in a pluripotent stem cell culture. The methods typically comprise measuring the concentration of IGFBP2 in the culture supernatant. The concentration of IGFBP2 is indicative of cell count and accordingly in some embodiments the measured concentration can be correlated with cell count. The methods provide efficient means of estimating cell numbers in pluripotent stem cell cultures, reducing the need for disruptive cell harvesting and manual counting, thereby preserving cell viability and culture integrity.

[0033] In certain aspects, the present disclosure provides methods for assessing the health of cultured pluripotent stem cells. The methods typically comprise measuring IGFBP2 concentrations in the culture supernatant. The concentrations of IGFBP2 are indicative of cell health and accordingly in some embodiments the measured concentrations can be compared to a reference indicative of healthy cells. The methods enable real-time assessment of stem cell health by providing a reliable biomarker (IGFBP2) that reflects the physiological state of the cells, allowing for timely interventions to maintain optimal culture conditions. In some embodiments, the IGFBP2 concentration is assessed against a target range, such as 150 ng/ml to 250 ng/ml or 150 ng/ml to 300 ng/ml, to assess cell health.

[0034] In certain aspects, the present disclosure provides methods for normalizing experimental data across multiple batches of pluripotent stem cell cultures. The methods typically comprise using IGFBP2 concentration as a normalization marker to standardize results. Accordingly, in some embodiments, the methods comprise measuring IGFBP2 concentrations across multiple batches of pluripotent stem cell cultures and adjusting other cell culture measurements against the measured IGFBP2 concentrations. The methods allow the use of IGFBP2 concentrations to standardize experimental data across multiple batches, reducing variability and enhancing the reproducibility of results, which is important for consistent and reliable scientific research and applications.

[0035] In certain aspects, the present disclosure provides methods for evaluating the effect of cell culture conditions on pluripotent stem cell growth. The methods typically comprise measuring IGFBP2 concentrations in cultures subjected to different conditions (e.g., coating conditions). Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations under different cell culture conditions and using the measured concentration to identify conditions suitable or optimal for cell growth. The methods can be used to evaluate different cell culture conditions, such as substrate coatings, facilitating the optimization of culture environments for improved cell proliferation and health.

[0036] In certain aspects, the present disclosure provides methods for determining the differentiation efficiency of pluripotent stem cells, e.g., into dopaminergic neurons. The methods typically comprise measuring IGFBP2 concentrations in the culture supernatant during differentiation. IGFBP2 concentrations are associated with the yield of differentiated cells. Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations in differentiating cultures and correlating the concentrations with the success of the differentiation and/or cell yield. The methods provide a quantitative measure of differentiation efficiency, e.g., into dopaminergic neurons, enabling the optimization of differentiation protocols and improving the yield and quality of production of differentiated cell types. In some embodiments, a final measurement target range of IGFBP2 concentration for dopaminergic neuron differentiation of less than 500 ng/ml is used to assess differentiation efficiency.

[0037] In certain aspects, the present disclosure provides methods for determining the maturation efficiency of differentiated cells (e.g., cardiomyocytes), including cells differentiated from pluripotent stem cells. The methods typically comprise measuring IGFBP2 concentrations in the culture supernatant during maturation. IGFBP2 concentrations are associated with the

yield of matured cells. Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations in cultures of cells undergoing maturation and correlating the concentrations with the success of cell maturation and/or yield of mature cells. The methods provide a quantitative measure of maturation efficiency, e.g., of cardiomyocytes, enabling the optimization of maturation protocols and improving the yield and quality of production of mature cell types. In some embodiments, IGFBP2 concentrations are compared to a target range, such as 0 ng/ml to 20 ng/ml when the culture comprises cardiomyocytes, to evaluate maturation efficiency.

[0038] In certain aspects, the present disclosure provides methods for optimizing pluripotent stem cell culture conditions. The methods typically comprise measuring IGFBP2 concentrations in cultures supplemented with different media components. IGFBP2 concentrations are associated with cell growth and health. Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 in cultures supplemented with different media components and identifying media components suitable for cell growth and/or cell health. The methods aid in optimizing culture conditions by correlating IGFBP2 concentrations with cell growth characteristics, allowing for the fine-tuning of media components to enhance cell proliferation and viability.

[0039] In certain aspects, the present disclosure provides methods for estimating cell numbers in three-dimensional pluripotent stem cell cultures. The methods typically comprise measuring IGFBP2 concentrations in the culture supernatant. IGFBP2 concentrations are associated sphere yield and/or volume. Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations in three-dimensional pluripotent cell cultures and correlating the concentrations with sphere formation and/or volume. The methods offer a non-disruptive approach to estimate cell numbers in 3D cultures, and can be used to assess sphere formation and/or volume, overcoming the challenges of traditional counting methods in complex culture systems. In some embodiments, IGFBP2 concentrations are compared to a target range, such as 150 ng/ml to 300 ng/ml, to estimate cell numbers in 3D PSC cultures.

[0040] In certain aspects, the present disclosure provides methods for evaluating the confluence of pluripotent stem cell cultures. The methods typically comprise measuring IGFBP2 concentrations in the culture supernatant. IGFBP2 concentrations are associated with confluence of pluripotent stem cells. Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations in pluripotent stem cell cultures and correlating the concentrations with cell confluence. The methods can be used to evaluate cell

confluence, providing a non-invasive tool to determine optimal cell growth conditions and timing for passaging. In some embodiments, IGFBP2 concentrations are assessed against a target range, such as 150 ng/ml to 250 ng/ml, to evaluate cell confluence.

[0041] In certain aspects, the present disclosure provides methods for estimating cell numbers in visually challenging conditions such as cell factories. The methods typically comprise measuring IGFBP2 concentrations in the culture supernatant. IGFBP2 concentrations are associated with cell numbers. Accordingly, in some embodiments, the methods of the disclosure comprise measuring IGFBP2 concentrations in cell factories and correlating the concentrations with cell count in visually challenging conditions. The methods can be used to estimate cell numbers in cell factories, offering a solution for accurate cell counting in large-scale and visually challenging environments, enhancing production efficiency. In some embodiments, IGFBP2 concentrations are compared to a target range, such as 150 ng/ml to 300 ng/ml for cultures of PSCs, to estimate cell numbers in cell factories.

[0042] In certain aspects, the present disclosure provides methods for monitoring cell differentiation in pluripotent stem cell cultures. The methods typically comprise measuring IGFBP2 concentrations in the culture supernatant. IGFBP2 concentrations are associated with cell differentiation. Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations in differentiating cultures and correlating the concentrations with the progression of differentiation. The methods can be used to monitor cell differentiation, providing insights into the differentiation process and enabling the optimization of differentiation protocols.

[0043] In certain aspects, the present disclosure provides methods assessing the differentiation of pluripotent stem cells into specific cell lineages. The methods typically comprise measuring IGFBP2 concentrations during the differentiation process. IGFBP2 concentrations are associated with the yield of differentiated cells. Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations in differentiating cultures and correlating the concentrations with the yield of differentiated cells. The methods can be used to assesses differentiation into specific cell lineages, offering a quantitative approach to evaluate and optimize the yield of differentiated cells.

[0044] In certain aspects, the present disclosure provides methods evaluating the differentiation of pluripotent stem cells into dopaminergic neurons. The methods typically comprise measuring IGFBP2 concentrations in the culture supernatant at various stages of differentiation. IGFBP2 concentrations are associated with the yield of dopaminergic neurons. Accordingly, in some

embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations in pluripotent stem cell cultures that are undergoing differentiation into dopaminergic neurons and correlating the concentrations with the efficiency of neuron formation. The method can be used to evaluate the differentiation of stem cells into dopaminergic neurons, providing a tool for optimizing dopaminergic neuron production and improving therapeutic applications. In some embodiments, IGFBP2 concentrations are assessed against a target range, such less than 500 ng/ml, to evaluate differentiation into dopaminergic neurons.

[0045] In certain aspects, the present disclosure provides methods for monitoring cell maturation in differentiated cell cultures (e.g., cultures of cells differentiated from PSCs such as cardiomyocytes). The methods typically comprise measuring IGFBP2 concentrations in the culture supernatant. IGFBP2 concentrations are associated with the degree of cell maturity. Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations in cultures of differentiated cells undergoing maturation and correlating the concentrations with the progression of maturation. The methods can be used to monitor cell maturation, providing insights into the maturation process and enabling the optimization of maturation protocols. In some embodiments, IGFBP2 concentrations are compared to a target range, such as 0 ng/ml to 20 ng/ml for cultures comprising cardiomyocytes, to monitor cell maturation.

[0046] In certain aspects, the present disclosure provides methods for assessing the maturation of differentiated cell cultures (e.g., cultures of cells differentiated from PSCs such as cardiomyocytes). The methods typically comprise measuring IGFBP2 concentrations during the maturation process. IGFBP2 concentrations are associated with the degree of cell maturity. Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations in cell cultures undergoing maturation and correlating the concentrations with the success of the maturation and/or yield of mature cells. The methods can be used to assess maturation, offering a quantitative approach to evaluate and optimize the yield of mature cells.

[0047] In certain aspects, the present disclosure provides methods evaluating the maturation of cardiomyocytes (e.g., cultures of cardiomyocytes differentiated from PSCs). The methods typically comprise measuring IGFBP2 concentrations in the culture supernatant at various stages of cardiomyocyte maturation. IGFBP2 concentrations are associated with the degree of cardiomyocyte maturity. Accordingly, in some embodiments the methods of the disclosure comprise measuring IGFBP2 concentrations in cardiomyocyte cultures undergoing maturation and correlating the concentrations with the success of cardiomyocyte maturation and/or yield of

mature cardiomyocytes. The methods can be used to evaluate the maturation of cardiomyocytes, providing a tool for optimizing cardiomyocyte maturation and improving therapeutic applications. In some embodiments, IGFBP2 concentrations are assessed against a target range, such as 0 ng/ml to 20 ng/ml, to evaluate cardiomyocyte maturation.

[0048] Additional applications for and details on use of IGFBP2 concentrations to assess the status and/or number of cells in culture are set forth in the Sections that follow and specific embodiments 1 to 142.

6.1. Definitions

[0049] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure. In case of conflict, the present specification, including definitions, will control. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, analytical chemistry, synthetic organic chemistry, medicinal and pharmaceutical chemistry, and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Throughout this specification and embodiments, the words "have" and "comprise," or variations such as "has," "having," "comprises," or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. All publications and other references mentioned herein are incorporated by reference in their entirety. Although a number of documents are cited herein, this citation does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[0050] Acceptor Antibody: As used herein, the term "acceptor antibody" refers to a conjugated antibody or a fragment thereof, that is used in HTRF or similar immunological assays. The acceptor antibody is conjugated to a fluorophore with a relatively short fluorescence, or another label. When in close proximity to a donor antibody, the fluorophore of the acceptor antibody transfers energy from the fluorophore of the donor antibody, upon which the fluorophore of the acceptor antibody emits a detectable signal.

[0051] Antibody: As used herein, the term “antibody” refers to a naturally occurring or engineered immunoglobulin molecule or immunologically active fragment thereof, *i.e.*, an antigen-binding portion. The term “antibody” encompasses both monoclonal and polyclonal antibodies.

[0052] Capture Antibody: As used herein, the term “capture antibody” refers to an antibody or a fragment thereof that is immobilized on a solid surface, *e.g.*, the surface of a well such as that of a cell culture plate used for ELISA or similar immunological assays. The capture antibody recognizes a specific biomolecule (*e.g.*, IGFBP2) and binds and retains that biomolecule.

[0053] Cell Count, Cell Counting: As used herein, the term “cell count” and “cell counting” refer to quantification or enumeration of cultured cells. In various embodiments, the cultured cells are adhered to a surface (*e.g.*, the surface of a cell culture vessel, dish, or a well) or suspended in a medium.

[0054] Cell Factory: As used herein the term “cell factory” refers to a large-scale cell culture system which provides a large growth surface in a small area, such as a stack of two or more cell culture chambers sealed together into a single unit, sharing common vent and fill ports to streamline maintenance and processing of the cultured cells.

[0055] Detection Antibody: As used herein, the term “detection antibody” refers to an antibody into which a detection moiety is incorporated, *e.g.*, for use in ELISA or similar immunological assays. The detection antibody can be conjugated to an enzyme, which generates a detectable product upon addition of a substrate, or to a fluorescent or radioactive label that can be detected without the addition of a substrate.

[0056] Donor Antibody: As used herein, the term “donor antibody” refers to a conjugated antibody or a fragment thereof, that is used in HTRF or similar immunological assays. The donor antibody is conjugated to a fluorophore with a relatively long fluorescence. When in close proximity to an acceptor antibody, the fluorophore of the donor antibody transfers energy to the fluorophore of the acceptor antibody, upon which the fluorophore of the acceptor antibody emits a detectable signal.

[0057] Enzyme-Linked Immunosorbent Assay (ELISA): As used herein, the terms “enzyme-linked immunosorbent assay” or “ELISA” refer to an immunological method that is used to quantify a specific protein, peptide, or polypeptide in a complex mixture (*e.g.*, cell culture medium, serum, cell extracts, tissue extracts, saliva, and other bodily fluids). In the context of

the method disclosed herein, ELISA is used to quantify the concentration of IGFBP2 in unfed media samples.

[0058] Encoding: The term “encoding” in relation to a nucleic acid (DNA or RNA) means that the nucleic acid comprises a nucleotide sequence coding for the amino acids of a polypeptide or the nucleotides of an RNA.

[0059] Fluorescence Resonance Energy Transfer (FRET): As used herein, the terms “fluorescence resonance energy transfer,” “Förster resonance energy transfer,” or “FRET” refer to a distance-dependent physical process by which energy is transferred in a nonradiative manner from an excited donor molecular fluorophore to an acceptor fluorophore by means of intermolecular long-range dipole–dipole coupling. Due to its sensitivity to the distance between the donor and the acceptor fluorophores, FRET has been used to investigate colocalization and/or interaction of molecules.

[0060] Homogeneous Time-Resolved Fluorescence (HTRF): As used herein, the terms “homogeneous time-resolved fluorescence”, “HTRF”, “time-resolved fluorescence resonance energy transfer” or “TR-FRET” refer to an immunological method that combines fluorescence resonance energy transfer (FRET) technology with time-resolved measurement of fluorescence. HTRF is used to quantify a specific protein, peptide, or polypeptide in a homogenous format, allowing high-throughput screening. In the context of the methods disclosed herein, HTRF can be used to quantify the concentration of IGFBP2 in unfed media samples.

[0061] Engineered Cell: As used herein, the terms “engineered cell” and “recombinant cell” refer to a cell that has been genetically engineered, *e.g.*, through introduction of a heterologous polypeptide or nucleic acid to express a particular protein or peptide, such as IL12. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. An engineered or recombinant cell may be made by altering a cell’s genome, *e.g.*, by gene editing, and/or by introducing a heterologous nucleic into a cell, *e.g.*, by *e.g.*, by viral delivery or non-viral delivery (*e.g.*, by electroporation, nucleofection, lipofection, or microinjection) of a replicating or non-replicating nucleic acid. A cell can be engineered to ectopically express one or more genes, RNAs, proteins, peptides, or any combination of one or more of the foregoing.

[0062] Final Concentration: As used herein, the term “final concentration” refers to the concentration measured in the final measurement step in an IGFBP2 assay.

[0063] Final Measurement (Step): As used herein, the terms “final measurement” and “final measurement step” refer to the final concentration measurement of an IGFBP2 assay before the culture is manipulated, *e.g.*, differentiated, harvested, preserved, passaged, destroyed, *etc.*

[0064] First Concentration: As used herein, the term “first concentration” refers to the concentration measured in the first measurement step in an IGFBP2 assay.

[0065] First Measurement (Step): As used herein, the terms “first concentration” and “first measurement step” refer to the first concentration measurement of an IGFBP2 assay, *e.g.*, after a cell is seeded or passaged in culture.

[0066] IGFBP2 Assay: As used herein, the term “IGFBP2 assay” refers to an assay that involves measurement of IGFBP2 levels (*e.g.*, IGFBP2 concentration) in cell culture media, *e.g.*, two, three, four or more measurements over multiple days.

[0067] iPSC: As used herein, the terms “induced pluripotent stem cell” and “iPSC” refer to a type of pluripotent stem cell artificially prepared from a non-pluripotent cell, such as an adult somatic cell, partially differentiated cell or terminally differentiated cell, such as a fibroblast, a cell of hematopoietic lineage, a myocyte, a neuron, an epidermal cell, or the like, by introducing or contacting the cell with one or more reprogramming factors. iPSCs can be derived from multiple different cell types, including terminally differentiated cells. iPSCs have an embryonic stem (ES) cell-like morphology, growing as flat colonies with large nucleo-cytoplasmic ratios, defined borders and prominent nuclei. In addition, iPSCs express one or more key pluripotency markers known by one of ordinary skill in the art, including but not limited to Alkaline Phosphatase, SSEA3, SSEA4, SOX2, OCT3/4, NANOG, TRA160, TRA181, TDGF 1, Dnmt3b, FoxO3, GDF3, Cyp26a1, TERT, and zfp42.

[0068] Examples of methods of generating and characterizing iPSCs may be found in, for example, U.S. Patent Publication Nos. US20090047263, US20090068742, US20090191159, US20090227032, US20090246875, and US20090304646 and PCT patent publications WO2013177133 and WO2022204567, the disclosures of each of which are incorporated herein by reference. Generally, to generate iPSCs, somatic cells are provided with reprogramming factors (*e.g.*, OCT4, SOX2, KLF4, MYC, NANOG, Lin28, GLIS1, BCL-XL, *etc.*) known in the art to reprogram the somatic cells to become pluripotent stem cells. In some embodiments, iPSCs are generated by introducing nucleic acids encoding a plurality of Yamanaka factors (*e.g.*, two, three, or all four of OCT4, KLF4, SOX2, and c-MYC) and optionally one or more additional reprogramming factors (*e.g.*, BCL-XL) into somatic cells and culturing the cells under conditions

in which the plurality of Yamanaka factors and optionally one or more reprogramming factors are expressed.

[0069] Maturation: As used herein, “maturation” refers to the cellular development process by which a cell attains its fully developed state. In some embodiments, a cell culture comprising cells undergoing maturation (e.g., cardiomyocytes) will show a decrease in cell proliferation as maturation progresses.

[0070] Normalization Marker: As used herein, a “normalization marker” is a molecule, such as a protein, a peptide, or another biomolecule, that is expressed in a wide variety of cells, including pluripotent cells, such as iPSCs and cells derived thereof, and has utility as an alternative to housekeeping molecules for normalization of measurements in live cells, e.g., due to consistent and/or predictable expression levels. IGFBP2 has utility as a normalization marker for cells in culture, e.g., as described herein for normalization of other measurements across different batches of experiments.

[0071] Nucleic Acid: As used herein, the terms “nucleic acid,” “oligonucleotide” and “nucleic acid” refer to at least two nucleotides covalently linked together. Nucleic acids may be single stranded or double stranded or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods. The depiction of a single strand also defines the sequence of the complementary strand. Thus, reference to a single stranded nucleic acid herein also encompasses the complementary strand of a depicted single strand.

[0072] Polypeptide, Peptide and Protein: As used herein, the terms “polypeptide,” “peptide” and “protein” refer to polymers of any length comprising or consisting of amino acids.

[0073] Pluripotent: As used herein, the term “pluripotent” or “pluripotency” refers to the capacity of a cell to self-renew and to differentiate into cells of any of the three germ layers: endoderm, mesoderm, or ectoderm. “Pluripotent stem cells” or “PSCs” include, for example, embryonic stem cells derived from the inner cell mass of a blastocyst or derived by somatic cell nuclear transfer, and iPSCs derived from non-pluripotent cells.

[0074] Seeding Density: As used herein, the terms “seeding density” or “cell seeding density” refer to the number of cells per unit area that are introduced to a container suitable for cell

growth, such as, but not including, a well (e.g., in a multi-well plate), flask, petri dish, or roller bottle. Typically, the container is sterile upon seeding. The container may be empty or contain a substrate suitable for cell growth.

[0075] Secreted Molecule: As used herein, the term “secreted molecule” refers to a biomolecule produced in and released from a cell. Exemplary types of secreted molecules include, but are not limited to, secreted proteins and peptides, such as growth factors, interleukins, chemokines, interferons, lymphokines, hormones, and neurotransmitters.

[0076] Single Cell Suspension: As used herein, the term “single cell suspension” or equivalents expressions, refers to a mixture of a fluid and a cell, or more typically a plurality of cells, that are separated from each other (e.g., not aggregated), which can be prepared by any available mechanical, biological or chemical means. Single cell suspensions described herein are typically preparations of viable iPSCs or iPSC-derived cells suspended in a physiological solution, such as a basal salt solution, saline, or cell culture media. Several methods exist for dissociating cell clusters to form single cell suspensions from primary tissues, adherent cells in culture, and cell aggregates, including but not limited to, methods that dissociate cells by physical forces (e.g., mechanical dissociation such as cell scraper, trituration through a narrow bore pipette, fine needle aspiration, vortex disaggregation and forced filtration through a fine nylon or stainless steel mesh), enzymatic dissociation methods (e.g., trypsin, collagenase, Accutase™ mediated dissociation), or combinations thereof. Further, methods and culture media conditions capable of supporting growth and viability of single-cell suspensions of iPSCs are useful for expansion, cell sorting, and defined seeding for multi-well plate assays and enable automatization of culture procedures, cell counting, and clonal expansion.

[0077] Spheroid: As used herein, the term “spheroid” refers to a self-assembling cell aggregate or cluster, which is able to form without requiring a scaffolding. Typically, spheroids are spherical in shape and formed in conditions where cell-cell interactions predominate over cell-substrate interactions. A spheroid may be highly organized with a well-defined morphology and mimic different *in vivo* cell interactions, or it may be a simple mass of cells that have clustered or adhered together with minimal organization that relates to the tissue of origin.

[0078] Subsequent Concentration: As used herein, the term “subsequent concentration” refers to any concentration measured a between the first and final measurement steps in an IGFBP2 assay.

[0079] Subsequent Measurement (Step): As used herein, the terms “subsequent measurement” and “subsequent measurement step” refer to any and all concentration measurements between the first and final measurement steps in an IGFBP2 assay.

[0080] Unfed Culture: As used herein, the terms “unfed culture”, “unfed cell culture”, and the like refer to a cell culture whose medium has not been removed, changed, or replenished for a minimum of 12 hours (e.g., for a minimum of 12 hours, for a minimum of 13 hours, for a minimum of 14 hours, for a minimum of 15 hours, for a minimum of 16 hours, for a minimum of 17 hours, for a minimum of 18 hours, for a minimum of 19 hours, for a minimum of 20 hours, for a minimum of 24 hours, for a minimum of 36 hours, or for a minimum of 48 hours). In some embodiments, an unfed culture is one whose medium has not been removed, changed, or replenished for at least 15 hours, for at least 18 hours, or at least 20 hours.

[0081] Unfed Medium: As used herein, the terms “unfed medium,” “unfed media,” “spent medium,” or “spent media” refer to a cell culture medium or media in a culture vessel containing cells, wherein the medium or media have not been removed, changed, or replenished for a minimum of 12 hours (e.g., for a minimum of 12 hours, for a minimum of 13 hours, for a minimum of 14 hours, for a minimum of 15 hours, for a minimum of 16 hours, for a minimum of 17 hours, for a minimum of 18 hours, for a minimum of 19 hours, for a minimum of 20 hours, for a minimum of 24 hours, for a minimum of 36 hours, or for a minimum of 48 hours). In some embodiments, an unfed medium has not been removed, changed, or replenished for at least 15 hours, for at least 18 hours, or at least 20 hours.

[0082] Western Blotting: As used herein, the terms “western blotting” or “immunoblotting” refer to an analytical technique used to detect specific proteins, peptides, or polypeptides in a complex mixture (e.g., cell culture medium, serum, cell extracts, tissue extracts, saliva, and other bodily fluids), wherein proteins, peptides, or polypeptides in the mixture are separated via gel electrophoresis, transferred to a suitable surface (e.g., a membrane) and detected via immunodetection. Western blotting can be used to measure the concentration of IGFBP2 in media samples in the methods of the disclosure.

6.2. IGFBP2

[0083] The present disclosure provides IGFBP2 assays for use in a variety of applications, for example as a measure of cell count or as a normalization marker.

[0084] IGFBP2 is a secreted protein produced in and released from a variety of cells, including but not limited to, pluripotent cells such as iPSCs and cells derived thereof. In the human body,

IGFBP2 can be secreted into the bloodstream, where it binds insulin growth factors IGF-I and IGF-II with high affinity, or it can remain intracellular, interacting with many different ligands. Binding of IGFBP2 to IGFs can prolong the half-life of these growth factors and regulate the interaction of IGFs with their cell surface receptors.

[0085] The IGFBP2 assays of the disclosure can measure concentrations of a wild-type or variant IGFBP2 amino acid sequence. Typically, the IGFBP2 is a mammalian IGFBP2.

[0086] In some embodiments, the mammalian IGFBP2 is a human IGFBP2. The full-length human IGFBP2 is 325 amino acids in length, which corresponds to Uniprot identifier P18065 and has the following amino acid sequence, with the signal peptide sequence underlined:

MLPRVGCPALPLPPPLLPLLLLLLGASGGGGGARA EVLFRCPPTPERLAACGPPPV
APPA AVAAVAGGARMPCAELVREPGCGCCSVCARLEGEACGVYT
PRCGQGLRCYPHPG
SELPLQALVMGE GTCEKRRDAEY GASPEQVADNGDDHSEGLV
ENHVDSTMNMLGGGG
SAGRKPLKSGMKELAVFREKVTEQHRQMKGKHHLGLEEPK
KL RPPPARTPCQQELD
QVLERISTMRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCKMS
LNGQRGECWCVNPN
T GKLIQGAPTIRGDPECHLFYNEQQEARGVHTQRMQ (SEQ ID NO:1)

[0087] Mature human IGFBP2 without the signal peptide has the following amino acid sequence:

AEVLFRCPPCTPERLAACGPPPVAPPA AVAAVAGGARMPCAELVREPGCGCCSVCAR
LEGEACGVYT
PRCGQGLRCYPHPGSELPLQALVMGE GTCEKRRDAEY GASPEQVADN
GDDHSEGLV
ENHVDSTMNMLGGGG
SAGRKPLKSGMKELAVFREKVTEQHRQMKGK
KHHLGLEEPK
KL RPPPARTPCQQELDQVLERISTMRLPDERGPLEHLYSLHIPNCDK
HGLYNLKQCKMS
LNGQRGECWCVNPN
T GKLIQGAPTIRGDPECHLFYNEQQEARGVHTQRMQ (SEQ ID NO:2)

[0088] In some embodiments, the human IGFBP2 is a splice variant of IGFBP2. An IGFBP2 splice variant of 181 amino acids in length corresponding to Uniprot identifier C9JMY1 has the following amino acid sequence:

MPCNNGDDHSEGLV
ENHVDSTMNMLGGGG
SAGRKPLKSGMKELAVFREKVTEQHRQM
GKGGKHHLGLEEPK
KL RPPPARTPCQQELDQVLERISTMRLPDERGPLEHLYSLHIPN
CDKHGLYNLKQCKMS
LNGQRGECWCVNPN
T GKLIQGAPTIRGDPECHLFYNEQQEARGVHTQRMQ (SEQ ID NO:3)

[0089] In some embodiments, the human IGFBP2 splice variant is 98 amino acids in length, which corresponds to Uniprot identifier H7CH0 and has the following amino acid sequence:

KHHLGLEEPK
KL RPPPARQLLAELASGACFVGLLSSLRASPAVCACRLPANRNWTRS
WSGSPPCAFRMSGALWSTSTPCTSTVTSMAC TT SNSARCL (SEQ ID NO:4)

[0090] In some embodiments, the human IGFBP2 splice variant is 78 amino acids in length, which corresponds to Uniprot identifier C9JW52 and has the following amino acid sequence:

MNMLGGGSSAGRKPLKSGMKELAVFREKVTEQHRQMGKGGKHHLGLEEPKKLRPPPA
 RTPCQQELDQVLERISTMRLP (SEQ ID NO:5)

[0091] In some embodiments, the mammalian IGFBP2 is a murine (e.g., mouse) IGFBP2.

Mouse IGFBP2 has the Uniprot identifier P47877 and has the following amino acid sequence, with the signal sequence underlined:

MLPRLGGPALPLLLPSLLLLLLLGAGGCGPGVRAEVLFRCPPTPERLAACGPPPPDA
 PCAELVREPGCGCCSVCARQEGEACGVYIPRCAQTLRCYPNPGSELPLKALVTGAGT
 CEKRRVGTTPQQVADSDDDHSEGGLVENHVDGTMMNMLGGGSSAGRKPLKSGMKELAV
 FREKVNEQHRQMGKGAHLSLEEPKKLRPPPARTPCQQELDQVLERISTMRLPDDRG
 PLEHLYSLHIPNCDKHGRYNLKQCKMSLNGQRGECWCVPNTGKPIQGAPTIRGDPE
 CHLFYNEQQETGGAHAQSVQ (SEQ ID NO:6)

[0092] Mature mouse IGFBP2 without the signal peptide has the following amino acid sequence:

EVLFRCPPTPERLAACGPPPDAPCAELVREPGCGCCSVCARQEGEACGVYIPRCAQ
 TLRCYPNPGSELPLKALVTGAGTCEKRRVGTTPQQVADSDDDHSEGGLVENHVDGTMM
 NMLGGGSSAGRKPLKSGMKELAVFREKVNEQHRQMGKGAHLSLEEPKKLRPPPART
 PCQQELDQVLERISTMRLPDDRGPLEHLYSLHIPNCDKHGRYNLKQCKMSLNGQRGE
 CWCVPNTGKPIQGAPTIRGDPECHLFYNEQQETGGAHAQSVQ (SEQ ID NO:7)

[0093] In some embodiments, the mammalian IGFBP2 is a monkey (e.g., *C. saebaeus*) IGFBP2.

Monkey IGFBP2 has the Uniprot identifier A0A0D9R9L4 and has the following amino acid sequence, with the signal sequence underlined:

MLPRVGC PALPLPPPPLPLLLLLLLLGASGGGGGAHAEVLFRCPPTPERLAACGPP
 VAPPAVA AVAGGARMPCAE LVREPGCGCCSVCARLEGEACGVYTPRCGQGLRCYPH
 PGSELPLQALVMGEGTCEKRRDAEYGASPEQVADNGDDHSEGGLVENHVDSTMMNLG
 GGSAGRKPLKSGMKELAVFREKVTEQHRQMGKGGKHHLGLEEPKKLRPPPARTPCQ
 QELDQVLERISTMRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWC
 VNPNTGKLIQGAPTIRGDPECHLFYNEQQEARGVHTQRMQ (SEQ ID NO:8)

[0094] Mature monkey IGFBP2 without the signal peptide has the following amino acid sequence:

EVLFRCPPTPERLAACGPPPVAPPAVA AVAGGARMPCAE LVREPGCGCCSVCARL
 EGEACGVYTPRCGQGLRCYPH PGSELPLQALVMGEGTCEKRRDAEYGASPEQVADNG
 DDHSEGGLVENHVDSTMMNLGGGSSAGRKPLKSGMKELAVFREKVTEQHRQMGKGGK
 HHLGLEEPKKLRPPPARTPCQQELDQVLERISTMRLPDERGPLEHLYSLHIPNCDKH
 GLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFYNEQQEARGVHT
 QRMQ (SEQ ID NO:9)

[0095] In some embodiments, the mammalian IGFBP2 is a hamster (e.g. Chinese hamster)

IGFBP2. Hamster IGFBP2 has the Uniprot identifier A0A8C2MM64 and has the following amino acid sequence, with the signal sequence underlined:

MLPRLGGPALSLLLPSLLLLLLLGAGGCGPGVRAEVLFRCPPTPERLAACGPPPDG
 PCAELVREPGCGCCSVCARLEGEACGVYTPRCAQTLRCYPNPGSELPLKALVSGAGT
 CEKSRVGATPQQVADNGDDHSEGGGLVENHVDGTMNMLGGGSSAGRKPLKSGMKELAV
 FREKVSEQHRQMGKGKHHLSLEEPKKLRPPPARTPCQQELDQVLERISTMRLPDDR
 GPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNNTGKLIQGAPTIRGDP
 ECHLFYNEQQETGGAHAQRVQ (SEQ ID NO:10)

[0096] Mature hamster IGFBP2 without the signal peptide has the following amino acid sequence:

EVLFRCPPTPERLAACGPPPDGPCAELVREPGCGCCSVCARLEGEACGVYTPRCAQ
 TLRCYPNPGSELPLKALVSGAGTCEKSRVGATPQQVADNGDDHSEGGGLVENHVDGTM
 NMLGGGSSAGRKPLKSGMKELAVFREKVSEQHRQMGKGKHHLSLEEPKKLRPPPAR
 TPCQQELDQVLERISTMRLPDDRGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRG
 ECWCVNPNNTGKLIQGAPTIRGDPECHLFYNEQQETGGAHAQRVQ (SEQ ID
 NO:11)

6.3. IGFBP2 Assays

[0097] The present disclosure provides IGFBP2 assays, typically conducted under conditions in which the IGFBP2 concentration is a reflection of cell count or cell health in the culture. The IGFBP2 assays may entail repeat measurements of IGFBP2 concentrations over the life of a cell culture. In general, IGFBP2 concentration is measured by removing an appropriate volume of a cell culture medium from the cell culture vessel with live cells and using a suitable assay method to measure IGFBP2 concentration in the cell culture medium. Suitable assay methods are further described in Section 6.3.1.

[0098] In some embodiments, an IGFBP2 assay comprises:

- (a) a first measurement step, comprising measuring a first concentration of IGFBP2 in the medium of a cell culture;
- (b) optionally, a subsequent measurement step, comprising measuring at least one subsequent concentration of IGFBP2 in the medium of the cell culture; and
- (c) optionally, a final measurement step, comprising measuring a final concentration of IGFBP2 in the medium of the cell culture.

[0099] Typically, the medium of the cell culture used for measuring the concentration of IGFBP2 is an unfed medium. The timing of the measurements relative to feeding of the cell culture is further described in Section 6.3.2.

[0100] In some embodiments, the first measurement step is followed by one or more subsequent measurement steps and/or a final measurement step. The frequency and timing of the subsequent and/or final measurement steps are further described in Section 6.3.2.

6.3.1. Assay Methods

[0101] The IGFBP2 concentration can be measured in a cell culture medium by removing an appropriate volume of the cell culture medium from a culture vessel (e.g., a culture dish) containing cells and measuring IGFBP2 concentration in the sampled cell culture medium using a suitable assay method, e.g., an immunological assay method. A cell culture medium may contain live cells, dead cells, or live and dead cells.

[0102] Anti-IGFBP2 antibodies that can be used in an immunological assay to determine the concentration of IGFBP2 in cell culture media can be purchased commercially or can be readily generated by a practitioner using ordinary procedures known in the art.

[0103] Nonlimiting examples of anti-IGFBP2 antibodies that recognize and bind to IGFBP2 that can be used in immunological assays of the disclosure are set forth in Table 1 below.

Table 1: Anti-IGFBP2 Antibodies	
Description	Reference and/or Catalog #
IGFBP2 neutralizing antibody M14	VH: SEQ ID NO:9 of U.S. Patent No. 10,301,684 VL: SEQ ID NO:10 of U.S. Patent No. 10,301,684
Polyclonal anti-IGFBP2 antibody	Abcam #AB227881
Monoclonal recombinant Anti-IGFBP2 antibody	Abcam #AB188200
Polyclonal anti-IGFBP2 antibody	R&D Systems #AF674
Polyclonal anti-IGFBP2 antibody	ThermoFisher Scientific #PA5-79449
Monoclonal anti-IGFBP2 antibody	ThermoFisher Scientific #MA5-15400
Monoclonal recombinant Anti-IGFBP2 antibody	ThermoFisher Scientific #MA5-41248

[0104] Nonlimiting examples of immunological assays that can be used to determine the concentration of IGFBP2 in cell culture media include enzyme-linked immunosorbent assay (ELISA) and homogeneous time-resolved fluorescence (HTRF), which are further described in Section 6.3.1.1 and Section 6.3.1.2, respectively.

[0105] In some embodiments, all IGFBP2 concentration measurements in media of the same culture utilize the same method. In other embodiments, different IGFBP2 concentration measurements in media of the same culture utilize two or more methods.

[0106] In some embodiments, the first measurement step utilizes ELISA. In other embodiments, the first measurement step utilizes HTRF. In other embodiments, the first measurement step utilizes Western blotting.

[0107] In some embodiments, one or more (or all) subsequent measurement steps utilize ELISA. In other embodiments, one or more (or all) subsequent measurement steps utilizes HTRF. In some embodiments, one or more (or all) subsequent measurement steps utilize Western blotting.

[0108] In some embodiments, the final measurement step utilizes ELISA. In other embodiments, the final measurement step utilizes HTRF. In other embodiments, the final measurement step utilizes Western blotting.

[0109] In some embodiments, the first measurement step, all subsequent measurement steps and the final measurement step in an IGFBP2 assay utilize ELISA.

[0110] In some embodiments, the first measurement step, all subsequent measurement steps and the final measurement step in an IGFBP2 assay utilize HTRF.

[0111] In some embodiments, the first measurement step, all subsequent measurement steps and the final measurement step in an IGFBP2 assay utilize Western blotting.

6.3.1.1. ELISA

[0112] ELISA is an immunological assay commonly used to measure antibodies, antigens, proteins, and glycoproteins in biological samples and can be used to determine the concentration of IGFBP2 in cell culture media samples.

[0113] ELISA-based measurement of IGFBP2 concentration in cell culture media involves four steps. In the first step, protein standards and cell culture media samples are added into appropriate wells of a plate (e.g. a 96-well plate) coated with a capture antibody followed by incubation of the plate at room temperature for a suitable incubation duration to allow the IGFBP2 in the media samples to bind to the capture antibody (first incubation step). In the second step, the wells are washed, an appropriate conjugated antibody is added to each well, and plates are incubated at room temperature for a suitable incubation duration to allow the conjugated antibody to bind to the IGFBP2 immobilized by the capture antibody (second

incubation step). In the third step, the unbound conjugated antibody is washed off, a substrate solution is added to the wells and incubated in the dark to allow color development for a suitable incubation duration (third incubation step). In the last step, color development is stopped with the addition of a stop solution and the color intensity in each well is measured at an appropriate wavelength (e.g., 450 nm) (measurement step), wherein the color intensity in a well is proportional to the amount of IGFBP2 in that well. A representation of an ELISA calibration curve is performed using known concentrations of standards and the concentration of IGFBP2 in the media samples can be determined by comparing the signal observed therein with the signal observed in the calibration.

[0114] In some embodiments ELISA is performed using an automated system. Automated ELISA systems useful in the disclosed IGFBP2 assay methods include, for example, microfluidics-based immunoassay systems such as the ELLA™ Automated Immunoassay System (R&D Systems) and the Agility® ELISA system (Dynex Technologies), as well as systems described in patent publications EP3311163B1 and WO2013010178A1 (each incorporated herein by reference in its entirety).

[0115] In some embodiments, the incubation duration in the first incubation step is one to two hours.

[0116] In some embodiments, the incubation duration in the first incubation step is two hours.

[0117] In some embodiments, the incubation duration in the second incubation step is one to two hours.

[0118] In some embodiments, the incubation duration in the second incubation step is one hour.

[0119] In some embodiments, the incubation duration in the third incubation step is quarter of an hour to one hour.

[0120] In some embodiments, the incubation duration in the third incubation step is half an hour.

[0121] In some embodiments, ELISA is performed to determine a first concentration of IGFBP2 in a medium of a cell culture.

[0122] In some embodiments, ELISA is performed to determine one or more subsequent concentrations of IGFBP2 in cell culture media. In some embodiments, ELISA is performed to determine only one subsequent concentration of IGFBP2 in a medium of a cell culture. In other

embodiments, ELISA is performed to determine a plurality of subsequent concentrations of IGFBP2 in cell culture media.

[0123] In some embodiments, ELISA is performed to determine a final concentration of IGFBP2 in a medium of the cell culture.

[0124] In some embodiments, ELISA is performed to determine a first concentration of IGFBP2, one or more subsequent concentrations of IGFBP2 and a final concentration of IGFBP2.

6.3.1.2. Homogeneous Time-Resolved Fluorescence (HTRF)

[0125] Homogeneous time-resolved fluorescence (HTRF) is an immunological assay that combines time-resolved fluorescence (TRF) detection with fluorescence resonance energy transfer (FRET), eliminating short-lived background fluorescence. HTRF cell-based assays are described in U.S. Patent. No. 5,527,684, the disclosure of which is incorporated by reference herein.

[0126] HTRF-based measurement of IGFBP2 concentration in cell culture media involves addition of standards or cell culture media samples into reservoirs, e.g., the wells of a suitable 96-well plate, and incubation of the standards and samples with two anti-IGFBP2 antibodies provided at a suitable weight ratio (e.g., at a 1:1, 1:2, 2:5, 1:4, 1:5, 1:8, 1:10, 1:16, 1:20, 1:40, 5:4, 5:2, 2:1, 4:1, or 5:1 weight ratio) for an appropriate duration (dual antibody incubation step), wherein the first antibody is coupled to a donor (donor antibody) and the second antibody is coupled to an acceptor (acceptor antibody). In some embodiments, the two anti-IGFBP2 antibodies are in a range of 1:2 to 2:1, optionally at a 1:1, weight ratio. Following the completion of the dual antibody incubation step, the light emission from each well is detected on an HTRF-compatible reader, e.g., a microplate reader, wherein the emission by the acceptor antibody is proportional to the level of interaction. A representation of an HTRF calibration curve is performed using known concentrations of IGFBP2 standards and the concentration of IGFBP2 in the media samples can be determined by comparing the signal observed therein with the signal observed in the calibration.

[0127] In some embodiments, the concentrations of a donor antibody and an acceptor antibody are provided at a 1:20 weight ratio.

[0128] In some other embodiments, the concentrations of a donor antibody and an acceptor antibody are provided at a 1:40 weight ratio.

[0129] In some embodiments, the incubation duration of the dual antibody incubation step is two to six hours (e.g., 2-6 hours, 2-5 hours, 2-4 hours, 2-3 hours, 3-4 hours, 3-5 hours, 3-6 hours, 4-5 hours, 4-6 hours, or 5-6 hours).

[0130] In various embodiments, the incubation duration of the dual antibody incubation step is three to five hours.

[0131] In some embodiments, the incubation duration of the dual antibody incubation step is three hours.

[0132] In some embodiments, the incubation duration of the dual antibody incubation step is four hours.

[0133] In some embodiments, the incubation duration of the dual antibody incubation step is five hours.

[0134] In some embodiments, HTRF is performed to determine a first concentration of IGFBP2 in a medium of a cell culture.

[0135] In some embodiments, HTRF is performed to determine one or more subsequent concentrations of IGFBP2 in cell culture media. In some embodiments, HTRF is performed to determine only one subsequent concentration of IGFBP2 in a medium of a cell culture. In other embodiments, HTRF is performed to determine a plurality of subsequent concentrations of IGFBP2 in cell culture media.

[0136] In some embodiments, HTRF is performed to determine a final concentration of IGFBP2 in a medium of the cell culture.

[0137] In some embodiments, HTRF is performed to determine a first concentration of IGFBP2, one or more subsequent concentrations of IGFBP2 and a final concentration of IGFBP2.

6.3.1.3. Western Blotting

[0138] Western blotting or immunoblotting is an analytical technique used to detect specific proteins in a given sample of a cell lysate, cell or tissue homogenate, or other protein containing samples, wherein proteins in the sample are separated via gel electrophoresis under non-denaturing (native) or denaturing conditions, transferred from the gel (e.g. a polyacrylamide gel) onto a suitable surface (e.g., a nitrocellulose membrane or a PVDF membrane) and detected via antibody detection methods and quantified using suitable image analysis software (e.g. ImageJ software). Western blot methods are described in U.S. Patent. No. 8,592,141 and PCT Publication No. 2012/057689 A1, the disclosures of which are incorporated by reference herein.

[0139] In some embodiments, proteins in a cell lysate are separated under denaturing conditions.

[0140] In other embodiments, proteins in a cell lysate are separated under non-denaturing conditions.

[0141] In some embodiments, proteins are transferred from the gel onto a nitrocellulose membrane.

[0142] In some embodiments, proteins are transferred from the gel onto a PVDF membrane.

[0143] In some embodiments, the protein-containing membrane is incubated with an unlabeled antibody (primary antibody) against IGFBP2 and then incubated with a labeled antibody (secondary antibody) against the primary antibody.

[0144] In some embodiments, the secondary antibody has a fluorescent label or tag.

[0145] In some embodiments, the secondary antibody has a chemiluminescent label or tag.

[0146] In some embodiments, the secondary antibody has a radioactive label or tag.

[0147] In some embodiments, Western blotting is used to quantify only IGFBP2 concentration in a sample.

[0148] In some embodiments, Western blotting is performed to determine a first concentration of IGFBP2 in a medium of a cell culture.

[0149] In some embodiments, Western blotting is performed to determine one or more subsequent concentrations of IGFBP2 in cell culture media. In some embodiments, Western blotting is performed to determine only one subsequent concentration of IGFBP2 in a medium of a cell culture. In other embodiments, Western blotting is performed to determine a plurality of subsequent concentrations of IGFBP2 in cell culture media.

[0150] In some embodiments, Western blotting is performed to determine a final concentration of IGFBP2 in a medium of the cell culture.

[0151] In some embodiments, Western blotting is performed to determine a first concentration of IGFBP2, one or more subsequent concentrations of IGFBP2 and a final concentration of IGFBP2.

6.3.2. Assay Timing

[0152] In some embodiments, the measurement of IGFBP2 is performed on a sample of unfed medium sampled after at least 12 hours (e.g., at least 12 hours, at least 13 hours, at least 14

hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, or at least 24 hours) have elapsed from the prior feeding.

[0153] In some embodiments, IGFBP2 measurement is performed on a sample of unfed medium sampled after 12 hours have elapsed from the prior feeding, and optionally after up to 30 hours or up to 36 hours have elapsed from the prior feeding.

[0154] In some embodiments, IGFBP2 measurement is performed on a sample of unfed medium sampled after 16 hours have elapsed from the prior feeding, and optionally after up to 30 hours or up to 36 hours have elapsed from the prior feeding.

[0155] In some embodiments, IGFBP2 measurement is performed on a sample of unfed medium sampled after 18 hours have elapsed from the prior feeding, and optionally after up to 30 hours or up to 36 hours have elapsed from the prior feeding.

[0156] In some embodiments, IGFBP2 measurement is performed on a sample of unfed medium sampled after 20 hours have elapsed from the prior feeding, and optionally after up to 30 hours or up to 36 hours have elapsed from the prior feeding.

[0157] In some embodiments, IGFBP2 measurement is performed on a sample of unfed medium sampled after 22 hours have elapsed from the prior feeding, and optionally after up to 30 hours or up to 36 hours have elapsed from the prior feeding.

[0158] In some embodiments, IGFBP2 measurement is performed on a sample of unfed medium sampled after 24 hours have elapsed from the prior feeding, and optionally after up to 30 hours or up to 36 hours have elapsed from the prior feeding.

[0159] In various embodiments, the unfed medium is sampled 12-36 hours following the prior feeding, 15-30 hours following the prior feeding, 18-24 hours following the prior feeding, or 20-24 hours following the prior feeding. In some embodiments, the unfed medium is sampled 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 25 hours, 26 hours, 27 hours, 28 hours, 29 hours, 30 hours, or longer following the prior feeding.

[0160] In some embodiments, the first measurement is performed by measuring the IGFBP2 concentration in an unfed medium sampled when at least 12 hours have elapsed from the prior feeding. In various embodiments, the first measurement is performed by measuring the IGFBP2 concentration in an unfed medium that is sampled 12-36 hours following the prior feeding, 15-30

hours following the prior feeding, 18-24 hours following the prior feeding, or 20-24 hours following the prior feeding. In some embodiments, the first measurement is performed by measuring the IGFBP2 concentration in an unfed medium that is sampled 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 25 hours, 26 hours, 27 hours, 28 hours, 29 hours, 30 hours, or longer following the prior feeding.

[0161] In some embodiments, one or more (or all) subsequent measurements are performed measuring the IGFBP2 concentration in an unfed medium sampled when at least 12 hours have elapsed from the prior feeding. In various embodiments, one or more (or all) subsequent measurements are performed by measuring the IGFBP2 concentration in an unfed medium that is sampled 12-36 hours following the prior feeding, 15-30 hours following the prior feeding, 18-24 hours following the prior feeding, or 20-24 hours following the prior feeding. In some embodiments, the one or more (or all) subsequent measurements are performed by measuring the IGFBP2 concentration in an unfed medium that is sampled 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 25 hours, 26 hours, 27 hours, 28 hours, 29 hours, 30 hours, or longer following the prior feeding.

[0162] In some embodiments, the measurement of IGFBP2 is performed measuring the IGFBP2 concentration in an unfed medium, wherein the first measurement step, one or more subsequent measurement steps, if performed, and/or the final measurement step, if performed, is carried out on unfed medium sampled after at least 12 hours have elapsed from the prior feeding. In various embodiments, in each case the unfed medium is sampled 12-36 hours following the prior feeding, 15-30 hours following the prior feeding, 18-24 hours following the prior feeding, or 20-24 hours following the prior feeding. In some embodiments, the first measurement step, one or more subsequent measurement steps, if performed, and/or the final measurement step, if performed, is carried out on unfed medium sampled 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 25 hours, 26 hours, 27 hours, 28 hours, 29 hours, 30 hours, or longer following the prior feeding.

[0163] In some embodiments, the first concentration measurement of IGFBP2 is followed by at least one subsequent concentration measurement of IGFBP2, wherein each subsequent concentration measurement is repeated when the cell culture is unfed, using a new sample of unfed medium.

[0164] In some embodiments, the subsequent measurement step comprises measuring a plurality of subsequent concentrations of IGFBP2 in the medium of the cell culture, wherein measurement of subsequent concentrations is repeated once daily, twice daily, or every other day. In some embodiments, the first of the subsequent measurement steps is performed on a cell culture medium sampled one day to one week (e.g., one day, two days, three days, four days, five days, six days or seven days) after sampling the cell culture medium for the first measurement step.

[0165] In some embodiments, the subsequent measurement step comprises measuring a plurality of subsequent concentrations of IGFBP2 in the medium of the cell culture, wherein measurement of subsequent concentrations is repeated once daily for at least 3 days, at least 4 days, or at least 5 days. In some embodiments, the first of the subsequent measurement steps is performed on a cell culture medium sampled one day to one week (e.g., one day, two days, three days, four days, five days, six days or seven days) after sampling the cell culture medium for the first measurement step.

[0166] In some embodiments, the subsequent measurement step comprises measuring a plurality of subsequent concentrations of IGFBP2 in the medium of the cell culture, wherein measurement of subsequent concentrations is repeated twice daily for at least 3 days, at least 4 days, or at least 5 days. In some embodiments, the first of the subsequent measurement steps is performed on a cell culture medium sampled one day to one week (e.g., one day, two days, three days, four days, five days, six days or seven days) after sampling the cell culture medium for the first measurement step.

[0167] In other embodiments, the subsequent measurement step comprises measuring a plurality of subsequent concentrations of IGFBP2 in the medium of the cell culture, wherein measurement of subsequent concentrations is repeated every other day for a period of at least 6 days, at least 8 days, at least 10 days, at least 12 days, at least two weeks, or at least three weeks. In some embodiments, the first of the subsequent measurement steps is performed on a cell culture medium sampled one day to one week (e.g., one day, two days, three days, four days, five days, six days or seven days) after sampling the cell culture medium for the first measurement step.

[0168] In some embodiments, the subsequent measurement step comprises measuring a plurality of subsequent concentrations of IGFBP2 in the medium of the cell culture, wherein measurement of subsequent concentrations is repeated every other day for a period of up to four weeks. In some embodiments, the first of the subsequent measurement steps is performed

on a cell culture medium sampled one day to one week (e.g., one day, two days, three days, four days, five days, six days or seven days) after sampling the cell culture medium for the first measurement step.

[0169] The IGFBP2 assays are typically performed on an unfed medium. Upon removal of the unfed cell culture medium sample for IGFBP2 concentration measurement, the cell culture can be fed and placed into the incubator until the next measurement. In some embodiments, the cell culture is fed daily.

6.4. Cell Cultures

[0170] The methods of the disclosure for measuring the concentration of IGFBP2 in a sample of a cell culture medium can be used with any suitable type of cells that are (i) in culture, and (ii) capable of expressing IGFBP2. The cultured cells used for measuring the concentration of IGFBP2 may be from established cell lines or they may be primary cells.

[0171] In some embodiments, cultured cells are cell lines. In some embodiments, cell lines are mammalian cell lines. Nonlimiting examples of mammalian cell lines include Human Embryonic Kidney (HEK) cells (e.g., HEK293 cells), Chinese hamster ovary cells (CHO cells), HeLa cells, pluripotent stem (PSC) cell (e.g. iPSC) lines, and COS-7 cells.

[0172] In some embodiments, cultured cells are primary cells, where “primary cells”, “primary cell lines”, and “primary cultures” are used interchangeably herein to refer to cells and cells cultures that have been derived from a subject and allowed to grow *in vitro* for a limited number of passages, e.g., splittings, of the culture. For example, primary cultures include cultures that may have been passaged 0 times, 1 time, 2 times, 4 times, 5 times, 10 times, or 15 times, but not enough times go through the crisis stage. Primary cell lines can be maintained for fewer than 10 passages *in vitro*.

[0173] Any type of cells can be used (e.g., a stem cell, e.g., a human embryonic stem cell (hESC), a pluripotent stem cell (PSC) an induced pluripotent stem cell (iPSC), a germ cell; a somatic cell, e.g., a fibroblast, a hematopoietic cell, a neuron, a glial cell, an oligodendrocyte, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell, a myeloid cell or a myeloid progenitor cell, e.g., a primitive myeloid progenitor cell, a microglial cell or a microglial progenitor cell, a T cell, e.g., a CD4+ T cell, such as a Treg).

[0174] In some embodiments, the mammalian cultured cells are cells of human origin.

[0175] In some embodiments, host cells are pluripotent stem cells (PSCs), e.g., as described in Section 6.4.1.

[0176] If the cultured cells are primary cells, such cells may be harvested from an individual by any suitable method. For example, leukocytes may be suitably harvested by apheresis, leukocytapheresis, density gradient separation, etc., while cells from tissues such as skin, muscle, bone marrow, spleen, liver, pancreas, lung, intestine, stomach, etc. are most suitably harvested by biopsy. An appropriate solution may be used for dispersion or suspension of the harvested cells. The harvested cells may be used immediately, or they may be stored, frozen (cryopreserved), for long periods of time, being thawed and capable of being reused. In such cases, the cells will generally be frozen in 10% dimethyl sulfoxide (DMSO), 50% serum, 40% buffered medium, or some other such solution as is commonly used in the art to preserve cells at such freezing temperatures and thawed in a manner as commonly known in the art for thawing frozen cultured cells.

[0177] Forming a cell culture comprises seeding a culture with a population of cells. In some embodiments, the population of cells is seeded to a density of 5,000 cells/cm² to 20,000 cells/cm².

[0178] Any suitable cell culture medium can be used to maintain and passage the cultured cells, and for IGFBP2 measurements. Some nonlimiting examples of culture media include Essential 8 (E8), MEM, DMEM, DMEM F12, RPMI 1640, SFM, HPLM, IMDM, Gibco Media 199, Ham's F-12, Ham's F10, Medium 106. The media can also contain supplements, such as FBS, ROCK inhibitors, CEPT cocktail, antibiotics, or additional nutrients.

[0179] In some embodiments, the culture medium is E8 medium. In some embodiments, the E8 medium is further supplemented with the ROCK inhibitor Y-27632. In other embodiments, the E8 medium is further supplemented with CEPT cocktail.

[0180] The cells can be cultured in two dimensional (2D) cultures or three dimensional (3D) cultures. 3D cultures allow cells to grow and interact in all three dimensions.

[0181] In some embodiments, the cells are cultured in 2D cultures. 2D cultures typically involve growing cells as a monolayer on a flat surface.

[0182] In other embodiments, the cells are cultured in 3D cultures. 3D cultures typically involve growing cells in a manner that allows the cells to grow and interact in all three dimensions, for example by culturing them on a non-adherent surface and/or a bioreactor.

[0183] In some embodiments, the 3D culture can utilize a scaffold that provides support and guidance for cell growth. In some embodiments, the scaffolds are hydrogel-based scaffolds. In other embodiments, the 3D culture is performed under conditions where the cells themselves form clusters of spheroids. Clusters or spheroids can be generated when cells are cultured on low-adhesion culture plates or micropatterned surfaces that induce cell clumping and extracellular matrix production. Thus, in some embodiments, the term “3D culture” as used herein encompasses 2D culture methods on low adherence materials, resulting in formation of cell clumps (aggregates), including spheroids, in suspension.

[0184] The cells in the culture can be suspended in media or adherent to the surface of a suitable cell culture vessel. Suitable cell culture vessels and culturing systems include but are not limited to, cell culture dishes, flasks, plates, tubes, slides, cell factories and bioreactors.

[0185] In some embodiments, the cell culture is an adherent culture.

[0186] In other embodiments, the cell culture is a cell culture suspension.

[0187] In some embodiments, the cell culture is in a bioreactor.

[0188] In some embodiments, the cell culture is in a roller bottle.

6.4.1. Pluripotent Stem Cells (PSCs)

[0189] Stem cells are the starting point for the potential generation of large numbers of a specific cell type that can be delivered for regenerative medicine in patients with many different diseases.

[0190] In some embodiments, methods of the disclosure are used for measuring the concentration of IGFBP2 in a medium sample of cultured stem cells.

[0191] In some embodiments, the stem cells are pluripotent stem cells (PSCs), such as induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs).

[0192] In some embodiments, the cells are iPSCs.

[0193] In some embodiments, the iPSCs are cultured by seeding preserved (*e.g.* cryopreserved) iPSCs onto a suitable cell culture dish.

[0194] In other embodiments, iPSCs are produced by a process comprising: (a) dedifferentiating adult cells; (b) introducing one or more nucleic acids encoding Yamanaka factors into the adult cells; and (c) culturing the adult cells under conditions in which the Yamanaka factors are expressed. Yamanaka factors are the original factors discovered to be

capable of reprogramming an adult mammalian cell into an iPSC and include OCT4, KLF4, SOX2, and c-MYC. In some embodiments, the nucleic acids encode at least two, at least three or all four of OCT4, KLF4, SOX2, and c-MYC. The nucleic acids introduced to the adult cells can include additional reprogramming factors (e.g., one or more of NANOG, Lin28, GLIS1, and BCL-XL).

[0195] In some embodiments, methods of the disclosure are used to determine the IGFBP2 concentration in a PSC cell culture before differentiation of PSCs into other cells.

[0196] In some embodiments, PSCs from the PSC cell culture are differentiated if the final concentration of IGFBP2 in the cell culture medium is above a threshold amount. The term “threshold amount” is used interchangeably herein with the term “threshold level.”

[0197] In some embodiments, PSCs from the PSC cell culture are differentiated if the final concentration of IGFBP2 in the cell culture medium is within a target range.

[0198] In some embodiments, the target range is 50 ng/ml to 200 ng/ml.

[0199] In some embodiments, PSCs from the PSC cell culture are differentiated into dopaminergic neurons.

6.4.2. PSC-Derived Cells

[0200] The methods of the disclosure can be used for measuring the concentration of IGFBP2 in a sample of a cell culture medium of a culture comprising PSC-derived cells or cells undergoing differentiation from PSCs into another cell type.

[0201] In some embodiments, the concentration of IGFBP2 is measured one or more times during the first week or first month following initiating differentiation of the PSCs into different cell types, e.g., dopaminergic (DA) neurons. In some embodiments, the concentration of IGFBP2 in a culture is measured at least once, at least twice or at least three times during the first three days, first four days or first five days following initiating differentiation of PSCs into another cell type.

[0202] In some embodiments, the cells are undergoing differentiation from PSCs (or have been differentiated from PSCs) into cells of the endoderm (e.g., lung, thyroid, or pancreatic cells, or progenitors thereof), ectoderm (e.g., skin, neuronal, or pigment cells, or progenitors thereof) and mesoderm (e.g., cardiac cells, skeletal muscle cells, red blood cells, smooth muscle cells, or progenitors or precursors thereof) lineages.

[0203] In some embodiments, the cells are undergoing differentiation from PSCs (or have been differentiated from PSCs) into neural lineage cells, for example neural crest cells, astrocytes, dopaminergic neuron progenitor cells, dopaminergic (DA) neurons, midbrain dopaminergic neuron progenitor cells, midbrain dopaminergic neurons, an authentic midbrain dopamine (DA) neuron, a dopaminergic neuron precursor cell, a floor plate midbrain progenitor cell, or a floor plate midbrain DA neuron.

[0204] In some embodiments, the cells are undergoing differentiation from PSCs (or have been differentiated from PSCs) into dopaminergic (DA) neurons. PSCs can be differentiated into DA neurons using a differentiation protocol, such as a protocol adapted from Piao *et al.*, 2021, Cell Stem Cell 28(2):217-229.e7, PCT Publication Nos. WO 2016/196661 A1, WO 2010/096496 A2, WO 2013/067362 A1, WO 2021/042027 A1, WO 2021/203009 A1, and/or US Patent No. 10,711,243, the contents of which are hereby incorporated by reference in their entireties.

[0205] In some embodiments, the cells are undergoing differentiation from PSCs (or have been differentiated from PSCs) into oligodendrocyte progenitor cells or oligodendrocytes.

[0206] In some embodiments, the cells are undergoing differentiation from PSCs (or have been differentiated from PSCs) into cells of the ocular system, such as photoreceptor cells, photoreceptor precursor cells, retinal pigmented epithelium cells, neural retinal cells, or neural retinal progenitor cells.

[0207] In some embodiments, the cells are undergoing differentiation from PSCs (or have been differentiated from PSCs) into microglial cells or microglial progenitor cells.

[0208] In some embodiments, the cells are undergoing differentiation from PSCs (or have been differentiated from PSCs) into human immune cells such as macrophages, T cells, myeloid cells, dendritic cells, or T cells expressing a chimeric antigen receptor (CAR) or recombinant TCR.

[0209] In some embodiments, the cells are undergoing differentiation from PSCs (or have been differentiated from PSCs) into enteric progenitor cells or enteric cells.

[0210] In some embodiments, the cells are undergoing differentiation from PSCs (or have been differentiated from PSCs) into cells of the human metabolic system such as hepatocytes, cholangiocytes, or pancreatic beta cells. In some embodiments, the cells are undergoing differentiation from PSCs (or have been differentiated from PSCs) into cardiomyocyte (CM) cells. In some embodiments, the cells are cardiomyocytes being cultured. In some embodiments, the cells are cardiomyocytes undergoing maturation. In some embodiments, the

concentration of IGFBP2 is measured one or more times to assess the maturation of cells differentiated from PSCs (e.g., cardiomyocytes).

6.5. IGFBP2 Assay Applications

[0211] IGFBP2 assays of the disclosure allow determination of IGFBP2 concentration in cell culture media. Measurement of IGFBP2 concentration in a culture medium sample via the methods of the disclosure has multiple applications.

[0212] IGFBP2 assays of the disclosure can be used to estimate the number of cells in a cell culture, as described in Section 6.5.2. For example, the cell culture can be a cell culture comprising PSCs, cells differentiated from PSCs, or cells being dedifferentiated (e.g., to make PSCs). In some embodiments, the cell culture comprises PSCs. In some embodiments, the cell culture comprises cells differentiated from PSCs. In some embodiments, the cell culture comprises cells undergoing dedifferentiation to PSCs. In some embodiments, the cell culture comprises cells being cultured for cell maintenance (e.g., without a change from one cell type to another).

[0213] IGFBP2 assays of the disclosure can also be used to identify cell lines suitable for production of a secreted polypeptide, as described in Section 6.5.3.

[0214] Furthermore, IGFBP2 assays of the disclosure can be used as normalization markers as an alternative to housekeeping molecules for normalization of measurements in live cells, as described in Section 6.5.4.

[0215] IGFBP2 assays of the disclosure can also be used to assess the health of the cells in a cell culture, as described in Section 6.5.5.

[0216] Another application of IGFBP2 assays of the disclosure is identification and selection of improved cell culture parameters and/or optimization of cell culturing methods, as described in Section 6.5.6.

[0217] Yet another application of IGFBP2 assays of the disclosure is assessment of a cell culture comprising cells undergoing maturation, as described in Section 6.5.7.

6.5.1. Cell Line Evaluation

[0218] The methods of the disclosure can be used to evaluate a population of cells (e.g., a sample or aliquot of cells from a cell line). In some embodiments, the methods comprise evaluation of cell population, such as a sample or aliquot of cells from a cell line, to determine a target IGFBP2 concentration range. Establishing a target IGFBP2 concentration range may be

used as a benchmark for future culturing of samples or aliquots of cells from the cell line, and can be useful for applications such as, for example, cell number estimation (e.g., as described in Section 6.5.2), identification of cells as suitable for production of a secreted polypeptide (e.g., as described in Section 6.5.3), normalizing outputs from different batches of experiments (e.g., as described in Section 6.5.4), assessment of cell health (e.g., as described in Section 6.5.5), and testing cell culture parameters (e.g., as described in Section 6.5.6).

[0219] Establishment of a target range of IGFBP2 concentration may comprise culturing cells and evaluating IGFBP2 at different points in the culture, e.g., before feeding the cells and/or after recovery from a splitting. The IGFBP2 measurements can be compared to analogous IGFBP2 measurements from another cell line (e.g., another cell line of the same type or for historical data for the same cell line) and/or alongside one or more known markers of cell health, viability, and/or (for PSCs) differentiation potential. Such markers may include cell count, cell density, cell confluency, cell metabolism, ATP production, and protease activity, as well as various recognized secreted markers or cell health and/or death, such as lactate dehydrogenase (LDH) and secreted DNA. A target IGFBP2 concentration range can be determined based on correlation with one or more of such known IGFBP2 benchmarks and/or one or more known markers of cell health, viability, and/or differentiation potential.

[0220] For example, in some embodiments, cells (e.g., PSCs such as those described in Section 6.4.1) are cultured and IGFBP2 concentration measured as described in Section 6.3. One or more markers of cell health and/or viability are measured. IGFBP2 concentration is correlated with the markers of cell health and/or viability to determine a target range of IGFBP2. Such a target range may then be used for various applications, such as those described herein, using cells of the same type.

[0221] Exemplary target ranges for different cell types and cell culture vessels are listed below:

Cell Type	Vessel	Day 1 after seeding	Final Measurement
PSCs	T-flask	5-15 ng/mL	150-250 ng/mL
PSCs	Cell factory	5-15 ng/mL	150-250 ng/mL
PSCs	Bio-Reactor	20-60 ng/mL	150-300 ng/mL
Cardiomyocytes (Maturing)	Roller bottle	20-40 ng/mL	< 20 ng/mL
DA cells (Day 11)	T-flask	250-500 ng/mL	< 500 ng/mL

[0222] Additional target ranges can be determined for a given cell type and culture conditions (e.g., vessel type) as described herein.

6.5.2. Cell Number Estimation

[0223] The methods of the disclosure can be used to estimate the number of cells in a culture, wherein the concentration of IGFBP2 in the medium is indicative of the number of cells in the cell culture. A measurement of cell number can be useful to bring consistency in cell storage, e.g., to consistently store a certain number of cells per vial.

[0224] In some embodiments, one or more (or all) of the first measurement step, one or more subsequent measurement steps, if performed, and/or the final measurement step, if performed, is performed such that the measurement is indicative of the number of cells in the cell culture.

[0225] Estimating the number of cells in a cell culture using an IGFBP2 assay can be used for, but is not limited to, monitoring cell proliferation, testing (e.g., optimizing) cell culture conditions (e.g., by varying cell culture parameters and assessing the effect on IGFBP2 production), preparing cell-based assays, and processing the cells for downstream use. The number of cells in the cell culture can be estimated based on the concentration of IGFBP2 in the cell culture.

[0226] Upon estimation of the number of cells in culture with a final measurement step, the cells can be processed further, wherein further processing of the cell culture comprises:

- (a) passaging cells in the cell culture (or the entire cell culture);
- (b) preserving cells from the cell culture (or the entire cell culture);
- (c) destroying cells in the cell culture (or the entire cell culture);
- (d) if the cell culture comprises PSCs, differentiating PSCs in the PSC cell culture (or the entire PSC cell culture); or
- (e) any combination of one, two, three, or all four of (a)-(d).

[0227] In some embodiments, the cell culture is passaged if the final concentration measurement of IGFBP2 is above a threshold level.

[0228] In some embodiments, the cell culture is passaged if the final concentration measurement of IGFBP2 is within a target range. In some embodiments, the target range is based on a historical control, e.g., a previously determined range for the cell type or cell line, e.g., as described in Section 6.5.1. In some embodiments, e.g., where the cells are PSCs, the target range is 50 ng/ml to 200 ng/ml. In some embodiments, e.g., where the cells are PSCs,

the target range is 150 ng/ml to 250 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 300 ng/ml.

[0229] In some embodiments, the cell culture is preserved if the final concentration measurement of IGFBP2 is above a threshold level. In some embodiments, e.g., where the cells are PSCs, the threshold level is 150 ng/ml.

[0230] In some embodiments, the cell culture is preserved if the final concentration measurement of IGFBP2 is within a target range. In some embodiments, the target range is based on a historical control, e.g., a previously determined range for the cell type or cell line, e.g., as described in Section 6.5.1 In some embodiments, e.g., where the cells are PSCs, the target range is 50 ng/ml to 200 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 250 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 300 ng/ml.

[0231] In some embodiments, the cell culture is discontinued (e.g., by destroying the cell culture or discarding the cell culture) if the final concentration measurement of IGFBP2 is below a threshold level. In some embodiments, e.g., where the cells are PSCs, the threshold level is 150 ng/ml.

[0232] In some embodiments, the cell culture is destroyed if the final concentration measurement of IGFBP2 is below a threshold level. In some embodiments, e.g., where the cells are PSCs, the threshold level is 150 ng/ml.

[0233] In some embodiments, the cell culture is discontinued (e.g., by destroying the cell culture or discarding the cell culture) if the final concentration measurement of IGFBP2 is outside a target range. In some embodiments, the cell culture is destroyed if the final concentration measurement of IGFBP2 is outside a target range. In some embodiments, the target range is based on a historical control, e.g., a previously determined range for the cell type or cell line, e.g., as described in Section 6.5.1 In some embodiments, e.g., where the cells are PSCs, the target range is 50 ng/ml to 200 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 250 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 300 ng/ml.

[0234] In some embodiments, PSCs from the PSC cell culture are differentiated if the final concentration of IGFBP2 in the cell culture medium is above a threshold amount. In some embodiments, e.g., where the cells are PSCs, the threshold amount is 150 ng/ml.

[0235] In some embodiments, PSCs from the PSC cell culture are differentiated if the final concentration of IGFBP2 in the cell culture medium is within a target range. In some embodiments, the target range is based on a historical control, e.g., a previously determined range for the cell type or cell line, e.g., as described in Section 6.5.1 In some embodiments, e.g., where the cells are PSCs, the target range is 50 ng/ml to 200 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 250 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 300 ng/ml.

6.5.3. Secreted Molecule-Producing Cells

[0236] Cultured cells can be used to produce secreted molecules, e.g., secreted peptides or polypeptides. IGFBP2 assays can be used to identify cell lines suitable for production of a secreted polypeptide performing an IGFBP2 assay in a cell culture of a cell line and concurrently measuring the concentration of the secreted polypeptide in the same cell culture.

[0237] In some embodiments, identifying a cell line suitable for production of a secreted polypeptide comprises: (i) performing an IGFBP2 assay as described in Section 6.3; and (ii) concurrently with measuring IGFBP2, measuring concentration of the secreted polypeptide in the cell culture, wherein the concentration of the secreted polypeptide is measured via an immunoassay. As used herein, reference to “concurrently measuring concentration of the secreted polypeptide” performing a measurement of the concentration of secreted polypeptide in the same sample of cell culture medium on which the IGFBP2 assay is performed, or on a separate sample obtained within an hour (optionally within half an hour, quarter of an hour, or 5 minutes) of the cell culture medium on which the IGFBP2 assay is performed.

[0238] In some embodiments, a concentration of secreted polypeptide that is above a threshold amount relative to the amount of IGFBP2 is indicative that the cell line is suitable for production of the secreted polypeptide, whereas a concentration of secreted polypeptide that is below a threshold amount relative to the amount of IGFBP2 is indicative that the cell line is unsuitable for production of the secreted polypeptide.

[0239] The secreted polypeptide can be endogenously or recombinantly expressed in the cell line.

[0240] In some embodiments, the secreted polypeptide is endogenously expressed in the cell line.

[0241] In other embodiments, the secreted polypeptide is recombinantly expressed in the cell line.

[0242] Concurrently measuring concentration of IGFBP2 and the secreted polypeptide in the cell culture can be performed with any type of secreted protein, peptide, and polypeptide, including but not limited to as growth factors, interleukins, chemokines, interferons, lymphokines, hormones, and neurotransmitters.

[0243] Nonlimiting examples of secreted proteins, peptides, and polypeptides include interleukins (e.g., interleukin 12 (IL-12), IL-1 α , IL-1 β , IL-1R4 (ST2), IL-2, IL-2R, IL-3, IL-3R α , IL-5, IL-6, IL-6R, IL-7, IL-8, IL-8 RB, IL-11, IL-12p40, IL-12p70, IL-13, IL-13 R1, IL-13R2, IL-15, IL-15R α , IL-16, IL-17, IL-17C, IL-17E, IL-17F, IL-17R, IL-18, IL-18BP α , IL-18 R α , IL-20, IL-23, IL-27, IL-28, IL-31, and IL-33), growth factors (e.g., angiogenin, angiopoietin-1, angiopoietin-2, bNGF, VEGF-A, VEGF-C, VEGF-D, VEGF-R1, VEGF-R2, VEGF-R3, G-CSF, GM-CSF, P1GF, P1GF-2, BDNF, BMP4, EGF, FGF-1, FGF-2, FGF-7, FGF-21, Follistatin, GDF-15, HB-EGF, HGF, LAP, NT-3, NT-4, TGF- α , TGF- β , and TGF- β 3), cytokines and chemokines (e.g., GCP-2, GM-CSF, PDGF-AA, PDGF-BB, PDGF-AB, SDF-1, 6CKine, BLC, BRAK, CD186, ENA-78, Eotaxin-1, Eotaxin-2, Eotaxin-3, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1 gamma, MIP-1 α , MIP-1 β , MIP-1 δ , MIP-3 α , MIP-3 β , MPIF-1, PARC, PAI-1, IP-10, I-TAC, LIF, LIX, M-CSF, MIF, MIG, GDF-15, GM-CSF, GRO, HCC-4, PF4, RANTES, Resistin, SCF, SCYB16, TARC, TSLP, TNF- α , TRAIL-R4, Activin A, Amphiregulin, Axl, and I-309, RANTES and CXCR4), interferons (e.g., IFN- α , IFN- β , and IFN- γ), differentiation markers (e.g., TFF3, PTN, and LGI1) and other secreted proteins, peptides, and polypeptides (e.g., cathepsin S, Galectin-7, Tie2, EpCAM, LRP6, TACI, TNF-R1, TREM-1, Galectin-7, Gash, IGFBP-1, IGFBP-3, IGFBP-6, MADCam-1, NGF R, NrCAM, TRAIL-R4, ADAMTS1, MMP-9, pro-MMP9, RANK, and RANKL).

[0244] In some embodiments, the secreted polypeptide is interleukin-12 (IL-12). Human IL-12 is a dimer, composed of a p40 subunit and a p35 subunit. Full-length human p40 has the following amino acid sequence, corresponding to Uniprot identifier P43432, shown below with the signal sequence underlined:

MCHQQLVISWFSFLVFLASPLVAIWELKKDVYVVVELDWYPDAPGEMVVLTC
 DTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHS
 LLLLHKKEDGIWSTDILKDQKEPKNKTFRLRCEAKNYSGRFTCWWLTTIST
 DLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVEQEDSACP
 AAEEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSR
 QVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVIC
 RKNASISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO:14)

[0245] Full-length human p35 has the following amino acid sequence, corresponding to Uniprot identifier P29459, shown below with the signal sequence underlined:

MCPARSLLLVATLVLLDHLSLARNLPVATPDPGMFPC LHHSQNLLRAVSN
MLQKARQTLEFYPC TSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSR
ETSFITNGSCLASRKTSFMMALCLSS IYEDLKMYQVEFKTMNAKLLMDPK
RQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLH
AFRIRAVTIDRVMSYLNAS (SEQ ID NO:15)

[0246] In some embodiments, IL-12 and IGFBP2 concentrations are measured in a DA neuron cell culture. IL-12 concentration can be measured using the methods described in Section 6.3 for IGFBP2, for example using an anti-IL12 antibody as described in U.S. Patent. No. 11,197,913, the disclosure of which is incorporated by reference herein.

[0247] In some embodiments, the secreted polypeptide is trefoil factor 3 (TFF3). The full length human TFF3 is 80 amino acids in length, has the following amino acid sequence, corresponding to Uniprot identifier Q07654, shown below with the signal sequence underlined:

MAARALCMLGLVLALLSSSSAEEYVGLSANQCAVPAKDRVDCGYPHVTPK
ECNNRGCCFDSRIPGVWCFKPLQEAECTF (SEQ ID NO:12)

[0248] In some embodiments, TFF3 and IGFBP2 concentrations are measured in a DA neuron cell culture. TFF3 concentration can be measured using the methods described in Section 6.3 for IGFBP2, for example using an anti-TFF3 antibody as described in PCT Publication No. WO 2005/013802 A2 and U.S. Patent No. 10,551,392, the disclosures of which are incorporated by reference herein.

[0249] In some embodiments, the secreted polypeptide is pleiotrophin (PTN). The full length human PTN is 168 amino acids in length, has the following amino acid sequence, corresponding to Uniprot identifier P21246, shown below with the signal sequence underlined:

MQAQQYQQQRRKFAAAFLAFIFILAAVDTAEAGKKEKPEKKVKKSDCGEW
QWSVCVPTSGDCGLGTREGTRTGAECKQTMKTQRCKIPCNWKKQFGAECK
YQFQAWGECDLNTALKTRTGSLKRALHNAECQKTVTISKPCGKLTKPKPQ
AESK KKKKEGKKQEKMLD (SEQ ID NO:13)

[0250] In some embodiments, PTN and IGFBP2 concentrations are measured in a DA neuron cell culture. PTN concentration can be measured using the methods described in Section 6.3 for IGFBP2, for example using an anti-PTN antibody as described in U.S. Publication. No.

2014/0294845 A1 and U.S. Patent No. 7,608,264, the disclosures of which are incorporated by reference herein.

[0251] In some embodiments, the secreted polypeptide is leucine rich glioma inactivated 1 (LGI1). The full length human LGI1 is 557 amino acids in length, has the following amino acid sequence corresponding to Uniprot identifier O95970, shown below with the signal sequence underlined:

MESERSKRMGNACIPLKRIAYFLCLLSALLLTEGKKPAKPKCPAVCTCTKD
 NALCENARSIPRTVPPDVISLSFVRSGFTEISEGSFLFTPSLQLLLFTSNS
 FDVISDDAFI GLPHLEYLFIENNNIKSISRHTFRGLKSLIHLSLANNNLQT
 LPKDIFKGLDSL TNVDLRGNSFNCDCKLKWLV EWLGH TNATVEDIYCEGPP
 EYKKRKINSLSKDFDCIITEFAKSQDL PYQSLSIDTF SYLNDEYV VIAQP
 FTGKCI FLEWDHVEKTF RNYDNITGTSTV VCKPIVIETQLYVIVAQLFGGS
 HIYKRDSFANKFIKI QDIEILKIRKPNDIETFKIENN WYFVVADSSKAGFT
 TIYKWNNGFGYSHQSLHAWYRDTDVEYLEIVRTPQT LRTPHLILSSSSQRP
 VIYQWNKATQLFTNQTDIPN MEDVYAVKHFSVKG DVYICLTRFIGDSKVMK
 WGGSSFQDIQRMP SRGSMVFQPLQINNYQYAILGSDYSFTQVYNWDAEKAK
 FVKFQELNVQAPRSFTHVSINKRNFLFASSFKGNTQIYKHVIVDL SA
 (SEQ ID NO:16)

[0252] In some embodiments, LGI1 and IGFBP2 concentrations are measured in a DA neuron cell culture. LGI1 concentration can be measured using the methods described in Section 4.3 for IGFBP2, for example using an anti-LGI antibody as described in U.S. Patent No. 9,250,250, the disclosure of which is incorporated by reference herein.

6.5.4. Normalizing Outputs from Different Batches of Experiments

[0253] Cell behavior can vary between different batches of experiments. Therefore, the outputs of different batches of cell culture experiments cannot be compared to one another or analyzed together unless these outputs are normalized by a control output, such as the expression of a housekeeping molecule. Assessment of housekeeping molecules necessitates manipulation of cells, such as preparation of cell extracts, and cannot be done easily in live cultures without disrupting the cells. Therefore, using IGFBP2 as a normalization marker in different batches of cell culture experiments can be used as an alternative to housekeeping molecules for normalization of measurements in live cells.

[0254] Methods of controlling for cell behavior in a cell culture experiment can comprise: (i) performing a cell culture study; and (ii) performing an IGFBP2 assay as described in Section

6.3, wherein the cell culture is deemed as behaving normally if the IGFBP concentration in the cell culture is in a target range.

[0255] In some embodiments, the target range is based on a historical control, e.g., a previously determined range for the cell type or cell line, e.g., as described in Section 6.5.1. In some embodiments, e.g., where the cells are PSCs, the target range is 50 ng/ml to 200 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 250 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 300 ng/ml. In some embodiments, e.g., where the cells are cardiomyocytes, the target range is 0 ng/ml to 20 ng/ml. In some embodiments, e.g., where the cells are DA neurons, the target range is 0 ng/ml to 20 ng/ml.

[0256] The methods of controlling for cell behavior can be used for cell culture experiments comprising the same cell line under different conditions. Nonlimiting examples of cell culture experiments comprising the same cell line under different conditions include experiments with the same cell line that are performed at different times; experiments with the same cell line performed in parallel but in a different type or size of culture vessel; experiments with the same cell line performed in parallel but with a new batch of medium and/or supplement; and experiments with the same cell line performed by different experimenters.

[0257] The methods of controlling for cell behavior can also be used for cell culture experiments comprising culturing different cell lines under the same conditions. The different cell lines in such experiments can comprise one or more test cell lines and one or more control cell lines, wherein the one or more control cell lines can comprise one or more positive control cell lines and one or more negative control cell lines.

[0258] In some embodiments, the cell culture experiment comprises culturing the same cell line under different conditions.

[0259] In some embodiments, the cell culture experiment comprises culturing different cell lines under the same conditions, wherein different cell lines comprise one or more test cell lines and one or more control cell lines.

[0260] In some embodiments, one or more control cell lines comprise a positive control cell line and/or a negative control cell line.

[0261] In some embodiments one or more control cell lines comprise a positive control cell line.

[0262] In some embodiments one or more control cell lines comprise a negative control cell line.

[0263] In some embodiments one or more control cell lines comprise both a positive control cell line and a negative control cell line.

[0264] In some embodiments, culturing the different cell lines is performed concurrently.

6.5.5. Assessment of Cell Health

[0265] IGFBP2 assays of the disclosure can be used for assessing the health of cultured cells in a test cell line, wherein the test cell line is deemed healthy if the IGFBP2 concentration is in a target range.

[0266] Methods for the assessment of the health of a test cell line can comprise: (i) seeding the test cell line into cell culture at a low density; and (ii) performing an IGFBP2 assay as described in Section 6.3, wherein the IGFBP2 target range can be determined by: (a) seeding in parallel one or more control cell lines into cell culture at a low density; and (b) performing an IGFBP2 assay as described in Section 6.3.

[0267] In some embodiments, assessing the health of a test cell line comprises: (i) seeding the test cell line into cell culture at a low density; (ii) performing an IGFBP2 assay as described in Section 6.3; and (iii) comparing the IGFBP2 concentration to a target range.

[0268] In some embodiments, the IGFBP2 target range is determined by: (i) seeding in parallel one or more control cell lines into cell culture at a low density; and (ii) performing an IGFBP2 assay as described in Section 6.3.

[0269] In some embodiments, assessing the health of a test cell line comprises: (i) seeding the test cell line into cell culture at a low density; (ii) performing an IGFBP2 assay as described in Section 6.3, and determining the IGFBP2 target range by (a) seeding in parallel one or more control cell lines into cell culture at a low density; and (b) performing an IGFBP2 assay as described in Section 6.3, wherein the seeding density of the test cell line and the seeding density of the control cell line are the same.

[0270] In some embodiments, the control cell lines comprise a positive cell line known to be healthy, wherein the test cell line is deemed healthy if the IGFBP2 concentration in the test cell culture is at least 75% of the IGFBP2 concentration of the positive control cell culture.

[0271] In some embodiments, the control cell lines comprise a negative control cell line known to be unhealthy, wherein the test cell line is deemed healthy if the IGFBP2 concentration in the test cell culture exceeds the IGFBP2 concentration of the negative control cell culture.

[0272] In some embodiments, the control cell lines comprise a negative control cell line known to be unhealthy, and a positive control cell line known to be healthy, wherein the test cell line is deemed healthy if the IGFBP2 concentration in the test cell culture (i) exceeds the IGFBP2 concentration of the negative control cell culture and (ii) is at least 75% of the IGFBP2 concentration of the positive control cell culture.

[0273] In some embodiments, the target range is based on a historical control, e.g., a previously determined range for the cell type or cell line, e.g., as described in Section 6.5.1 In some embodiments, e.g., where the cells are PSCs, the target range is 50 ng/ml to 200 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 250 ng/ml. In some embodiments, e.g., where the cells are PSCs, the target range is 150 ng/ml to 300 ng/ml. In some embodiments, e.g., where the cells are cardiomyocytes, the target range is 0 ng/ml to 20 ng/ml. In some embodiments, e.g., where the cells are DA neurons, the target range is 0 ng/ml to 20 ng/ml.

6.5.6. Testing Cell Culture Parameters

[0274] IGFBP2 assays of the disclosure can be used for testing cell culture workflow and parameters. Without wishing to be bound by theory, the concentration of IGFBP2 in medium of a cell culture is understood, as described herein, is indicative of the health of a cell culture, and thus, alterations in IGFBP2 concentrations in response to alterations of cell culture parameters is believed to be indicative of whether a cell culture parameter (e.g., cell culture medium, seeding density, medium, media supplements, culture time, splitting density, etc.) or change therein has a positive impact on growth and/or viability of cells.

[0275] Methods for the testing cell culture parameters can comprise (i) culturing a cell line under a first set of parameters and performing an IGFBP2 assay as described in Section 6.3 and (ii) comparing the IGFBP2 measurements with IGFBP2 measurements from culturing the cell line under a second set of parameters that differs from the first set of parameters by at least one parameter (e.g., a single parameter) and performing an IGFBP2 assay as described in Section 6.3 under the second set of parameters. Culturing a cell line under a first set of parameters under a second set of parameters may be performed in parallel (e.g., simultaneously) or in series (e.g., successively).

[0276] Thus, in some embodiments, methods for the testing cell culture parameters can comprise (i) culturing a cell line under a first set of parameters and performing an IGFBP2 assay as described in Section 6.3, (ii) culturing the cell line under a second set of parameters that differs from the first set of parameters by at least one parameter (e.g., a single parameter) and performing an IGFBP2 assay as described in Section 6.3, and (iii) comparing the IGFBP2 measurements from both sets of parameters, e.g., to determine whether the differing parameters contributed to cell culture health.

[0277] In some embodiments, culturing in series may have been previously cultured and tested to determine a target IGFBP2 concentration range (e.g., as described in Section 6.5.1).

[0278] In some embodiments, the methods for testing cell culture parameters comprise evaluating use of a second culture medium that is different from a first culture medium by (i) culturing a cell line using a first medium; (ii) culturing (in parallel or at separate times) the same cell line using a second, different medium; and (b) performing an IGFBP2 assay as described in Section 6.3 for both cell cultures.

[0279] Various cell culture media are recognized in the art and may be evaluated using the disclosed methods. Non-limiting examples of media that can be evaluated include Essential 8 (E8), MEM, DMEM, DMEM F12, RPMI 1640, SFM, HPLM, IMDM, Gibco Media 199, Ham's F-12, Ham's F10, and Medium 106.

[0280] In some embodiments, the methods for testing cell culture parameters comprise evaluating use of a particular cell culture media supplement by (i) culturing a cell line in the absence of the supplement; (ii) culturing (in parallel or at separate times) the same cell line in the presence of the supplement; and (b) performing an IGFBP2 assay as described in Section 6.3 for both cell cultures.

[0281] Various cell culture supplements are recognized in the art and may be evaluated using the disclosed methods. Non-limiting examples of cell culture media supplements that can be evaluated are FBS, ROCK inhibitors (e.g., Y-27632), CEPT cocktail, antibiotics, and nutrients.

[0282] In some embodiments, the methods for testing cell culture parameters comprise evaluating use of a particular cell culture vessel (e.g., cell culture dishes, multilayer cell factories, etc.) by (i) culturing a cell line in a first cell culture vessel; (ii) culturing (in parallel or at separate times) the same cell line in a second, different cell culture vessel; and (b) performing an IGFBP2 assay as described in Section 6.3 for both cell cultures. In some embodiments, the methods for testing cell culture parameters comprise evaluating use of a particular set of cell

culture vessel conditions (e.g., substrate coating temperature and/or duration) by (i) culturing a cell line in a first set of conditions (e.g., a vessel coated with a coating substrate at a first temperature and/or for a first duration); (ii) culturing (in parallel or at separate times) the same cell line in a second, different set of conditions (e.g., a vessel coated with a coating substrate at a second temperature and/or for a second duration); and (b) performing an IGFBP2 assay as described in Section 6.3 for both cell cultures.

[0283] In some embodiments, the type of coating substrate is evaluated. Certain non-limiting examples of culture vessel coating substrates are phospholipids, streptavidin, antibodies, collagen, cadherin, laminin, vitronectin, Poly-d-lysine (PDL), nickel chelate, protein A, WGA (wheat germ agglutinin) and hydrogels.

[0284] In some embodiments, the duration and/or the temperature of coating is evaluated.

[0285] In some embodiments, the culture vessel is coated with a coating substrate for 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 1 day, 2 days, 3 days, or 4 days or longer.

[0286] In some embodiments, the culture vessel is coated with a coating substrate for one hour. In some other embodiments, the culture vessel is coated with a coating substrate overnight (e.g., between 16-20 hours).

[0287] In some embodiments, the culture vessel is coated with a coating substrate at room temperature (e.g., between 20 °C and 25 °C). In some other embodiments, the culture vessel is coated with a coating substrate at 4°C or about 4°C (e.g., between 3°C and 5°C).

[0288] In some embodiments, the methods for testing cell culture parameters comprise evaluating the seeding density and/or maturation of cells to confluency by (i) culturing a cell line at a first density; (ii) culturing (in parallel or at separate times) the same cell line at a second, different density (e.g., a higher density or a lower density); and (b) performing an IGFBP2 assay as described in Section 6.3 for both cell cultures.

[0289] Testing methods may further include determining whether there is a difference in IGFBP2 concentrations between the first and second set of parameters. Where there is no such difference, the second set of parameters may be deemed not to improve health of the cell line. Where such a difference does exist, the second set of parameters may be deemed to improve the health of the cell line if the IGFBP2 concentration measured from the first set of parameters is below a target range while the second set of parameters is within a target range. That is, IGFBP2 concentration may be used to identify whether a particular cell culture parameter, or set

of parameters, contributes to the health of a cell line by identifying a set of parameters that brings IGFBP2 concentration into a desired, target range (e.g., a pre-determined target range based on previous measurements of cells known to be healthy or as having desired parameters such as differentiation).

6.5.7. Assessment of Cell Maturation

[0290] The methods of the disclosure can be used to assess the maturation of differentiated cells in a culture, wherein the concentration of IGFBP2 in the medium is indicative of the maturation state of the cells in the cell culture.

[0291] In some embodiments, one or more (or all) of the first measurement step, one or more subsequent measurement steps, if performed, and/or the final measurement step, if performed, is performed such that the measurement is indicative of the maturation of cells in the cell culture.

[0292] Upon assessment of the maturation of cells in culture with a final measurement step, the cells can be processed further, wherein further processing of the cell culture comprises:

- (a) passaging cells in the cell culture (or the entire cell culture);
- (b) preserving cells from the cell culture (or the entire cell culture);
- (c) destroying cells in the cell culture (or the entire cell culture);
- (e) any combination of one, two, or all three of (a)-(c).

[0293] In some embodiments, the cell culture is destroyed if the final concentration of IGFBP2 in the cell culture medium is above a threshold amount and/or outside a target range. In some embodiments, the target range is based on a historical control, e.g., a previously determined range for the cell type or cell line, e.g., as described in Section 6.5.1. In some embodiments, e.g., where the cells are cardiomyocytes, the threshold amount is 20 ng/ml. In some embodiments, e.g., where the cells are cardiomyocytes, the target range is 0 ng/ml to 20 ng/ml.

[0294] In some embodiments, the cell culture is preserved if the final concentration measurement of IGFBP2 is below a threshold level and/or within a target range. In some embodiments, the target range is based on a historical control, e.g., a previously determined range for the cell type or cell line, e.g., as described in Section 6.5.1. In some embodiments, e.g., where the cells are cardiomyocytes, the threshold amount is 20 ng/ml. In some embodiments, e.g., where the cells are cardiomyocytes, the target range is 0 ng/ml to 20 ng/ml.

7. SPECIFIC EMBODIMENTS

[0295] The present disclosure is exemplified by the specific embodiments below.

1. A method comprising:
 - (a) measuring a first concentration of insulin like growth factor binding protein 2 (IGFBP2) in the medium of a cell culture ("first measurement step");
 - (b) optionally, measuring at least one subsequent concentration of IGFBP2 in the medium of the cell culture (each a "subsequent measurement step"); and
 - (c) optionally, measuring a final concentration of IGFBP2 levels in the medium of the cell culture ("final measurement step").
2. The method of embodiment 1, which comprises performing step (b).
3. The method of embodiment 2, wherein step (b) comprises measuring a plurality of subsequent concentrations of IGFBP2 in the medium of the cell culture.
4. The method of embodiment 3, wherein step (b) is repeated once or twice daily for a period of at least three days.
5. The method of embodiment 3, wherein step (b) is repeated once or twice daily for a period of at least four days.
6. The method of embodiment 3, wherein step (b) is repeated once or twice daily for a period of at least five days.
7. The method of any one of embodiments 4 to 6, wherein step (b) is repeated daily until one or two days before the cell culture is passaged.
8. The method of any one of embodiments 3 to 7, wherein step (b) is repeated every other day for a period of at least 6 days.
9. The method of any one of embodiments 3 to 7, wherein step (b) is repeated every other day for a period of at least 8 days.
10. The method of any one of embodiments 3 to 7, wherein step (b) is repeated every other day for a period of at least 10 days.

11. The method of any one of embodiments 3 to 7, wherein step (b) is repeated every other day for a period of at least two weeks.
12. The method of any one of embodiments 3 to 7, wherein step (b) is repeated every other day for a period of at least three weeks.
13. The method of any one of embodiments 8 to 12, wherein step (b) is repeated every other day for a period of up to four weeks.
14. The method of any one of embodiments 1 to 13, wherein each subsequent measurement step is performed when the cell culture is unfed.
15. The method of any one of embodiments 1 to 14, wherein the concentration of IGFBP2 in the medium is indicative of the number of cells in the cell culture.
16. The method of any one of embodiments 1 to 15, which further comprises feeding the cell culture after one or more subsequent measurement steps.
17. The method of embodiment 16, which further comprises feeding the cell culture after the majority of the subsequent measurement steps.
18. The method of embodiment 16 or embodiment 17, wherein the cell culture is fed daily.
19. The method of any one of embodiments 1 to 18, which further comprises performing step (c).
20. The method of embodiment 19, wherein the final measurement step is performed when the cell culture is unfed.
21. The method of any one of embodiments 1 to 20, wherein one or more (or all) of the first measurement step, one or more subsequent measurement steps (if performed), and/or the final measurement step (if performed) is performed such that the measurement is indicative of the number of cells in the cell culture.
22. The method of any one of embodiments 1 to 21, wherein one or more (or all) of the first measurement step, one or more subsequent measurement steps (if performed), and/or

the final measurement step (if performed) is performed such that the measurement is indicative of the maturation of cells in the cell culture.

23. The method of any one of embodiments 1 to 22, wherein one or more (or all) of the first measurement step, one or more subsequent measurement steps (if performed), and/or the final measurement step (if performed) is performed when at least 12 hours have elapsed from the prior feeding.

24. The method of any one of embodiments 1 to 22, wherein each subsequent measurement step is performed when at least 15 hours have elapsed from the prior feeding.

25. The method of any one of embodiments 1 to 22, wherein each subsequent measurement step is performed when at least 18 hours have elapsed from the prior feeding.

26. The method of any one of embodiments 1 to 25, which comprises performing step (c) and further comprises performing step (d), wherein step (d) comprises any combination of one, two, three or all four of:

- (i) destroying cells in the cell culture (or the entire cell culture);
- (ii) passaging cells in the cell culture (or the entire cell culture);
- (iii) preserving cells from the cell culture (or the entire cell culture); and
- (iv) if the cell culture comprises pluripotent stem cells (PSCs), differentiating PSCs in the PSC cell culture (or the entire PSC cell culture).

27. The method of embodiment 26, wherein step (d) comprises destroying the cell culture if the final concentration of IGFBP2 in the cell culture medium is (a) below a threshold amount and/or (b) outside a target range.

28. The method of embodiment 26, wherein step (d) comprises passaging cells in the cell culture (or the entire cell culture) if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) within a target range.

29. The method of embodiment 26 or embodiment 28, wherein step (d) comprises preserving cells from the cell culture (or the entire cell culture) if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) within a target range.

30. The method of embodiment 26, wherein step (d) comprises destroying the cell culture if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) outside a target range.

31. The method of embodiment 26, wherein step (d) comprises preserving cells from the cell culture (or the entire cell culture) if the final concentration of IGFBP2 in the cell culture medium is (a) below a threshold amount and/or (b) within a target range.

32. The method of any one of embodiments 27 to 31, wherein the target range is 50 ng/ml to 200 ng/ml.

33. The method of any one of embodiments 1 to 32, wherein the measurement steps are performed via immunoassay, optionally wherein the immunoassay is an enzyme-linked immunosorbent assay (ELISA), a fluorescence resonance energy transfer (FRET) assay, or a Western blot.

34. The method of embodiment 33, wherein the immunoassay is an enzyme-linked immunosorbent assay (ELISA).

35. The method of embodiment 33, wherein the immunoassay is a fluorescence resonance energy transfer (FRET) assay.

36. The method of any one of embodiments 1 to 35, wherein the cell culture is a two-dimensional (2D) cell culture.

37. The method of any one of embodiments 1 to 35, wherein the cell culture is a three-dimensional (3D) cell culture.

38. The method of any one of embodiments 1 to 35, wherein the cell culture is an adherent culture.

39. The method of any one of embodiments 1 to 35, wherein the cell culture is a suspension.

40. The method of embodiment 39, wherein the culture is in a bioreactor.

41. The method of any one of embodiments 1 to 40, which further comprises, prior to step (a), forming the cell culture.

42. The method of embodiment 41, wherein forming the cell culture comprising seeding a culture with a population of cells.

43. The method of embodiment 42, wherein the population is seeded to a density of 5,000 cells/cm² to 20,000 cells/cm².

44. The method of any one of embodiments 1 to 43, wherein the cell culture comprises PSCs and/or cells undergoing differentiation from PSCs into another cell type.

45. The method of embodiment 43 or embodiment 44, wherein the cell culture is seeded with a population of preserved (e.g., cryopreserved) PSCs.

46. The method of embodiment 44 or embodiment 45, wherein the PSCs are iPSCs.

47. The method of embodiment 46, wherein the iPSCs are the product of a process comprising:

- (a) dedifferentiating adult cells;
- (b) introducing one or more nucleic acids encoding Yamanaka factors into the adult cells; and
- (c) culturing the adult cells under conditions in which the Yamanaka factors are expressed,

thereby generating the population of iPSCs.

48. The method of any one of embodiments 44 to 47, wherein step (d), if present, comprises differentiating PSCs from the PSC cell culture if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) within a target range.

49. The method of embodiment 48, wherein the target range is 150 ng/ml to 300 ng/ml.

50. The method of embodiment 48, wherein the target range is 150 ng/ml to 250 ng/ml.

51. The method of embodiment 48, wherein the target range is 50 ng/ml to 200 ng/ml.

52. The method of any one of embodiments 44 to 51, further comprising, prior to differentiating the PSCs from the PSC cell culture, measuring an additional concentration of IGFBP2 levels in the medium of the cell culture.

53. The method of any one of embodiments 44 to 52, further comprising, while differentiating the PSCs from the PSC cell culture, measuring an additional concentration of IGFBP2 levels in the medium of the cell culture.

54. The method of any one of embodiments 44 to 53, further comprising, after differentiating the PSCs from the PSC cell culture, measuring an additional concentration of IGFBP2 levels in the medium of the cell culture.

55. The method of any one of embodiments 48 to 54, wherein the PSCs from the PSC cell culture are differentiated into dopaminergic neurons.

56. The method any one of embodiments 48 to 55, which further comprises repeating the method of any one of embodiments 1 to 43 during and/or following differentiation.

57. The method of any one of embodiments 48 to 56, wherein the cell culture comprises PSCs undergoing differentiation into dopaminergic neurons.

58. The method of any one of embodiments 1 to 57, wherein the cell culture comprises dopaminergic neurons.

59. The method of embodiment 58, wherein step (c) is performed at least 5 days after seeding of the dopaminergic neurons or precursors thereof (e.g., PSCs).

60. The method of embodiment 58, wherein step (c) is performed at least 6 days after seeding of the dopaminergic neurons or precursors thereof (e.g., PSCs).

61. The method of embodiment 58, wherein step (c) is performed at least 7 days after seeding of the dopaminergic neurons or precursors thereof (e.g., PSCs).

62. The method of embodiment 58, wherein step (c) is performed at least 8 days after seeding of the dopaminergic neurons or precursors thereof (e.g., PSCs).

63. The method of embodiment 58, wherein step (c) is performed between 9 and 12 days after seeding of the dopaminergic neurons or precursors thereof (e.g., PSCs).

64. The method of embodiment 58, wherein step (c) is performed between 10 and 12 days after seeding of the dopaminergic neurons or precursors thereof (e.g., PSCs).

65. The method of embodiment 58, wherein step (c) is performed between 9 and 11 days after seeding of the dopaminergic neurons or precursors thereof (e.g., PSCs).

66. The method of embodiment 58, wherein step (c) is performed between 9 and 10 days after seeding of the dopaminergic neurons or precursors thereof (e.g., PSCs).

67. The method of embodiment 58, wherein step (c) is performed between 11 and 12 days after seeding of the dopaminergic neurons or precursors thereof (e.g., PSCs).

68. The method of embodiment 58, further comprising harvesting the dopaminergic neurons if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) within a target range.

69. The method of embodiment 68, further comprising formulating the harvested dopaminergic neurons in a composition.

70. The method of embodiment 69, wherein the composition is a pharmaceutical composition for use in therapy.

71. The method of embodiment 58, further comprising maintaining the dopaminergic neurons in culture if the final concentration of IGFBP2 in the cell culture medium is (a) below a threshold amount and/or (b) outside a target range.

72. The method of embodiment 71, further comprising measuring an additional concentration of IGFBP2 levels in the medium of the dopaminergic neuron culture.

73. The method of embodiment 72, wherein the additional concentration is measured at least 24 hours after the final concentration.

74. The method of embodiment 72, wherein the additional concentration is measured at least 2 days after the final concentration.

75. The method of embodiment 72, wherein the additional concentration is measured at least 3 days after the final concentration.

76. The method of any one of embodiments 72 to 75, further comprising discontinuing the cell culture (e.g., destroying or discarding the cell culture) if the additional concentration of IGFBP2 in the cell culture medium is (a) below a threshold amount and/or (b) outside a target range.

77. The method of any one of embodiments 72 to 75, further comprising destroying the dopaminergic neurons if the additional concentration of IGFBP2 in the cell culture medium is (a) below a threshold amount and/or (b) outside a target range.

78. The method of embodiment 58, further comprising discontinuing the cell culture (e.g., destroying or discarding the cell culture) if the final concentration of IGFBP2 in the cell culture medium is (a) below a threshold amount and/or (b) outside a target range.

79. The method of embodiment 58, further comprising destroying the dopaminergic neurons if the final concentration of IGFBP2 in the cell culture medium is (a) below a threshold amount and/or (b) outside a target range.

80. The method of any one of embodiments 1 to 43, wherein the cell culture comprises cells undergoing maturation.

81. The method of embodiment 80, wherein the cell culture comprises cells cardiomyocytes undergoing maturation.

82. The method of embodiment 80 or embodiment 81, further comprising discontinuing the cell culture (e.g., destroying or discarding the cell culture) if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) outside a target range.

83. The method of embodiment 80 or embodiment 81, wherein step (d), if present, comprises destroying the cell culture if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) outside a target range.

84. The method of embodiment 80 or embodiment 81, wherein step (d), if present comprises preserving cells from the cell culture (or the entire cell culture) if the final concentration of IGFBP2 in the cell culture medium is (a) below a threshold amount and/or (b) within a target range.

85. The method of any one of embodiments 82 to 84, wherein the threshold amount is 20 ng/ml.

86. The method of any one of embodiments 82 to 85, wherein the target range 0 ng/ml to 20 ng/ml.

87. A method of identifying a cell line suitable for production of a secreted polypeptide comprising:

- (a) measuring IGFBP2 concentration in a cell culture of the cell line according to the method of any one of embodiments 1 to 43; and
- (b) concurrently with measuring IGFBP2, measuring concentration of the secreted polypeptide in the cell culture.

88. The method of embodiment 87, wherein concurrently measuring concentration of IGFBP2 and the secreted polypeptide in the cell culture comprises performing a measurement of the concentration of secreted polypeptide in the same sample of the cell culture supernatant.

89. The method of embodiment 87, wherein concurrently measuring concentration of IGFBP2 and the secreted polypeptide in the cell culture comprises performing a measurement of the concentration of secreted polypeptide in a separate sample of the cell culture supernatant obtained within an hour (optionally within half an hour, quarter of an hour, or 5 minutes) of the cell culture supernatant in which IGFBP2 is measured.

90. The method of any one of embodiments 87 to 89, wherein the concentration of the secreted polypeptide is measured via an immunoassay.

91. The method of any one of embodiments 87 to 90, wherein the secreted polypeptide is interleukin-12, Tff3, PTN, LGI1, or any combination of two or more of the foregoing.

92. The method of any one of embodiments 87 to 91, wherein a concentration of secreted polypeptide that is above a threshold amount relative to the amount of IGFBP2 is indicative that the cell line is suitable for production of the secreted polypeptide.

93. The method of any one of embodiments 87 to 92, wherein the secreted polypeptide is recombinantly expressed in the cell line.

94. The method of any one of embodiments 87 to 92, wherein the secreted polypeptide is endogenously expressed in the cell line.
95. The method of any one of embodiments 87 to 94, wherein the cell line is a therapeutic cell line.
96. A method of controlling for cell behavior in a cell culture experiment:
- (a) performing a cell culture study; and
 - (b) measuring IGFBP2 concentration as a positive control in the cell culture study according to the method of any one of embodiments 1 to 43.
97. The method of embodiment 96, wherein the cell culture is behaving normally if the IGFBP concentration is in a target range.
98. The method of embodiment 97, wherein the target range is 50 ng/ml to 200 ng/ml.
99. The method of any one of embodiments 96 to 98, wherein the cell culture experiment comprises culturing different cell lines under the same conditions.
100. The method of embodiment 99, wherein the different cell lines comprise one or more test cell lines and one or more control cell lines.
101. The method of embodiment 100, wherein the one or more control cell lines comprise a positive control cell line and/or a negative control cell line.
102. The method of any one of embodiments 98 to 101, wherein the cell culture experiment comprises culturing the same cell line under different conditions.
103. The method of any one of embodiments 99 to 102, wherein culturing the different cell lines is performed concurrently.
104. The method of any one of embodiments 99 to 103, wherein IGFBP2 concentration is measured according to the method of any one of embodiments 1 to 43 for each cell culture in the experiment.
105. A method of assaying the health of a test cell line, comprising:

- (a) seeding the test cell line into cell culture at a low density; and
- (b) measuring IGFBP2 concentration in the test cell culture according to the method of any one of embodiments 1 to 43.

106. The method of embodiment 105, wherein the cell line is deemed healthy if the IGFBP2 concentration is in a target range.

107. The method of embodiment 105, wherein the cell line is deemed overconfluent if the IGFBP2 concentration is below a target range.

108. The method of embodiment 106 or 107, wherein the target range is determined by:

- (a) seeding one or more control cell lines into cell culture at the low density; and
- (b) measuring IGFBP2 concentration in the control cell culture(s) according to the method of any one of embodiments 1 to 43.

109. The method of embodiment 108, wherein the one or more control cell lines are seeded in parallel with the test cell line.

110. The method of embodiment 108, wherein the one or more control cell lines are seeded prior to the test cell line.

111. The method of any one of embodiments 108 to 110, further comprising measuring IGFBP2 concentration in the control cell culture(s) according to the method of any one of embodiments 1 to 43.

112. The method of any one of embodiments 108 to 111, wherein the control cell lines comprise a positive control cell line known to be healthy.

113. The method of embodiment 112, wherein the test cell line is deemed healthy if the IGFBP2 concentration in the test cell culture is at least 75% of the IGFBP2 concentration of the positive control cell culture.

114. The method of any one of embodiments 108 to 113, wherein the control cell lines comprise a negative control cell line known to be unhealthy.

115. The method of embodiment 114, wherein the test cell line is deemed healthy if the IGFBP2 concentration in the test cell culture exceeds the IGFBP2 concentration of the negative control cell culture.
116. The method of any one of embodiments 106 to 115, wherein the target range is 50 ng/ml to 200 ng/ml.
117. The method of any one of embodiments 105 to 116, wherein the test cell line is a PSC.
118. The method of embodiment 117, wherein the PSCs are preserved (e.g., cryopreserved).
119. The method of embodiment 117 or embodiment 118, wherein the PSCs are iPSCs.
120. The method of embodiment 105, which comprises measuring a subsequent concentration of IGFBP2 and measuring a final concentration of IGFBP2 levels, wherein the cell line is deemed overconfluent if the final concentration of IGFBP2 levels is lower than the subsequent concentration of IGFBP2 levels.
121. The method of any one of embodiments 105 to 120, further comprising passaging cells in the cell culture (or the entire cell culture).
122. The method of any one of embodiments 105 to 120, further comprising discontinuing the cell culture (e.g., by discarding or destroying the culture).
123. The method of any one of embodiments 105 to 120, further comprising preserving cells in the cell culture (or the entire cell culture).
124. The method of any one of embodiments 105 to 120, further comprising destroying cells in the cell culture (or the entire cell culture).
125. A method of testing cell culture parameters, e.g., for optimization of cell culture conditions, comprising:
- (a) culturing a cell line under a first set of parameters;

- (b) culturing the cell line under a second set of parameters that differs from the first set of parameters by at least one parameter; and
- (c) measuring IGFBP2 concentrations in (a) and (b) according to the method of any one of embodiments 1 to 43.

126. The method of embodiment 125, wherein the second set of parameters differs from the first set of parameters by only one parameter.

127. The method of embodiment 125 or 126, which further comprises determining if the IGFBP2 concentrations measured in (a) and (b) are different.

128. The method of any one of embodiments 125 to 127, wherein the IGFBP2 concentration measured in (a) is below a target range.

129. The method of embodiment 128, wherein the one parameter is deemed to improve health of the cell line if the IGFBP2 concentration in (b) is within a target range.

130. The method of embodiment 128, wherein the one parameter is deemed not to improve health of the cell line if the IGFBP2 concentration in (b) is below a target range.

131. The method of any one of embodiments 125 to 130, wherein steps (a) and (b) are performed in parallel.

132. The method of any one of embodiments 125 to 130, wherein steps (a) and (b) are performed at separate times.

133. The method of embodiment 132, wherein step (a) is performed prior to step (b).

134. The method of any one of embodiments 125 to 133, wherein one the parameter is a culture medium.

135. The method of any one of embodiments 125 to 133, wherein one the parameter is a cell culture media supplement.

136. The method of any one of embodiments 125 to 133, wherein one the parameter is a cell culture vessel coating parameter.

137. The method of embodiment 136, wherein the cell culture vessel coating parameter is a type of coating substrate.

138. The method of embodiment 136, wherein the cell culture vessel coating parameter is a duration of coating of a cell culture vessel with a substrate.

139. The method of embodiment 136, wherein the cell culture vessel coating parameter is a temperature of coating of a cell culture vessel with a substrate.

140. The method of any one of embodiments 125 to 133, wherein the cell culture parameter is seeding density.

141. The method of any one of embodiments 1 to 140, wherein the cell culture is a mammalian cell culture.

142. The method of embodiment 141, wherein the mammalian cell culture is a human cell culture.

8. EXAMPLES

8.1. Materials and Methods

8.1.1. Pluripotent Stem Cell (PSC) Culture and Maintenance

[0296] Before culturing PSCs, culture vessels were coated with a substrate for a minimum of two hours at room temperature or overnight at 4° C. Cells suspended in Essential 8 (E8) medium supplemented with Y-27632 or CEPT cocktail were plated at preferred seeding density (cells/cm²), ensuring even distribution of cells on the culturing surface. Culture vessels were incubated overnight at 37° C in an incubator with 5% CO₂. The next day and daily after until the cells reach 70 to 80% confluency, culture vessels were removed from the incubator and cells were observed under a microscope. Media in each vessel compartment was aspirated and replaced with fresh E8 media without Y-27632, and culture vessels were placed back into the incubator until passaging or preparation of a single cell suspension using standard methods using Trypsin-EDTA or Accutase™.

8.1.2. PSC Passaging Protocol

[0297] Cell culture vessels were removed from the incubator and media was aspirated from each compartment. Cells were washed with PBS-/- and incubated with 0.5 mM EDTA in PBS for 5 to 7 minutes at room temperature. The culturing surface of the vessel was flushed with E8

twice the volume of the EDTA-containing PBS. Pipetting up and down gently to preserve cell clumps, the culturing surface was washed no more than two times to detach any loosely attached cell colonies, and the cell suspension was transferred to a tube. The culturing surface of the vessel was washed once more with an additional volume of E8 and the resulting cell suspension was added to the tube. The cell suspension in the tube was pipetted up and down gently to ensure even distribution of cells. An appropriate volume of cell suspension was transferred into a new centrifuge tube for a 1:5 split, and the tubes were centrifuged at 300g for 3 minutes. After the removal of supernatant, cell pellets were loosened by flicking and/or tapping the tube and resuspended in an appropriate volume of E8 with Y-27632 for the preferred seeding density. The resuspended cells were seeded on substrate-coated vessels, and after ensuring even distribution of cells, culturing vessels were placed in an incubator set to 37° C and 5% CO₂.

[0298] For Accutase™ protocols, cells were washed with PBS/- and incubated with Accutase™ instead of 0.5 mM EDTA in PBS for 5 to 7 minutes at room temperature. The culturing surface of the vessel was flushed with E8 twice the volume of the EDTA-containing PBS. Pipetting up and down gently to preserve cell clumps, the culturing surface was washed no more than two times to detach any loosely attached cell colonies, and the cell suspension was transferred to a tube. The culturing surface of the vessel was washed once more with an additional volume of E8, and the resulting cell suspension was added to the tube. The cell suspension in the tube was pipetted up and down gently to ensure even distribution of cells. Cells were counted before proceeding to the next protocol.

8.1.3. Cell Counting Protocols

8.1.3.1. Incucyte®-Based Cell Counting

[0299] Incucyte® Nuclight Rapid NIR dye was brought to room temperature and diluted to 1:750 in complete media. Spent media from culture vessels were aspirated and cells in each compartment were washed with an equal volume of 1 x HBSS. After the addition of the equal volume of dye-containing medium, culture vessels were placed into the Incucyte® and allowed to equilibrate for 30 minutes. A standard scan was conducted with both Phase and NIR options selected. Cell numbers were calculated by setting up an analysis scheme that counts NIR objects.

8.1.3.2. NC200-Based Cell Counting

[0300] A minimum of 200 µL of a homogenous single cell suspension was sampled for measurements. The tip of a Via-1 cassette was inserted into the sample and the piston was

depressed to take up sample, upon which the cassette was inserted into the NC-200 (Chemometec, Denmark). From the main screen, the "Viability and Cell Count Assay" was selected. Data pertaining to the percent viability, total cell count per mL, counts of viable and non-viable cells, and an approximation of the cell diameter (in μm) was collected together with information about cell aggregation.

8.1.3.3. Cellometer K2-Based Cell Counting

[0301] 20 μL of a homogenous single cell suspension was mixed with 20 μL of AOPI solution. 20 μL of the mixture was transferred into a disposable cell counting chamber and place the chamber into the Cellometer K2 (Nexcelom, MA, USA). In the setup screen, the assay "hES cells AOPI" and the cells "hES cells AO" were selected. When necessary, focus was adjusted in preview mode. Data pertaining to percent viability, total cell count per mL, and counts of viable and non-viable cells were collected.

8.1.4. Enzyme-Linked Immunosorbent Assay (ELISA)

[0302] IGFBP2 measurements via enzyme-linked immunosorbent assay (ELISA) was carried out using R&D Systems Quantakine™ ELISA (R&D Systems Catalog# DGB200; MN, USA), following the manufacturer's protocol. Briefly, all reagents and samples were brought to room temperature before use. ELISA wash buffer was prepared by diluting 20 mL of Wash Buffer Concentrate with 480 mL of deionized or distilled water (ddH_2O). An IGFBP2 standard curve was prepared by reconstituting the standard with ddH_2O using the volume listed on the vial label, to yield a stock solution with a concentration of 200 ng/mL. After the standard stock has equilibrated at room temperature for 15 minutes, standard dilutions were generated, with the highest standard having a 20 mg/mL concentration, generated by diluting 100 μL of stock solution with 900 μL of Calibrator Diluent RD5-20, upon which the rest of the standards were prepared for an eight-point standard curve by making 2-fold serial dilutions. After 100 μL of Assay Diluent was added to each well of a plate precoated with the capture antibody, 50 μL of Standard, control, or sample was added to each well as designated. The plate was covered with a plate sealer and incubated at room temperature for 2 hours. Each well was aspirated and washed, repeating the process for a total of 4 washes, and 200 μL of Conjugate was added to each well. The plates were sealed and incubated at room temperature for 1 hour, each well was aspirated and washed 4 times. Next, 200 μL Substrate Solution was added to each well and the plates were incubated at room temperature for 30 minutes, protected from light. The reaction was stopped by adding 50 μL of Stop Solution to each well. The plates were placed into the reader and data was collected within 30 min at 450 nm, with the wavelength correction set to

540 nm or 570 nm. All samples, standards, and controls were assayed in duplicate. A standard curve was generated to interpolate sample concentrations from absorbance values.

8.1.5. Automated Immunoassay

[0303] IGFBP2 measurements with an automated ELISA system were performed using ELLA™ Automated Immunoassay System (ProteinSimple Catalog #600-100), following the manufacturer's protocol. Briefly, the IGFBP2 cartridge barcode on the outside of the vacuum bag was scanned and the cartridge was removed from bag. 1 mL Wash Buffer was pipetted into appropriate trough and samples were diluted 1:10 with sample diluent included. 50 µL of each diluted sample was pipetted into appropriate well. The protective lining was peeled off from the bottom of the cartridge and the cartridge was loaded into the ELLA system to perform the measurement.

8.1.6. Homogeneous Time-Resolved Fluorescence (HTRF)

[0304] Cell culture medium or a diluent solution of PBS+0.1% BSA was used for dilutions. Briefly, a reconstituted stock solution was used to prepare a 12-point standard curve (std 0 – std11) by making 2-fold serial dilutions with the highest standard concentration, std 11, being 1000ng/mL, whereas std 0 was a negative control with no protein. 16µL of standard was added into each standard well as designated. The acceptor (D2) antibody was diluted to 5µg/mL and the donor (europium cryptate) antibody was diluted to 0.25µg/mL in assay diluent, and the two antibody dilutions were combined at a 1:1 ratio. 4µL of the combined antibody mixture was added to each well, the plate was sealed and incubated for 4 hours at 4°C. Following incubation, the plate sealer was removed, and time resolved fluorescence was read on a CLARIOstar (BMG Labtech, Germany) or a similar HTRF-compatible plate reader. All samples and standards were assayed in triplicate. The ratio of the acceptor and donor emission signals for each individual well was calculated using the formula: Ratio = (Signal 665nm) / (Signal 620nm). A 4-Parameter Logistic curve was fitted to the standard ratios to interpolate sample concentration.

8.1.7. Differentiation of PSCs into DA Neurons

[0305] PSCs were differentiated into DA neurons for 16 days using a differentiation protocol adapted from Piao et al., 2021, Cell Stem Cell 28(2):217-229.e7, PCT Publication Nos. WO 2016,196661 A1, WO 2010/096496 A2, WO 2013/067362 A1, WO 2021/042027 A1, WO 2021/203009 A1, and US Patent No. 10,711,243, which are hereby incorporated by reference in their entireties.

8.1.8. 3D Expansion of PSCs

[0306] To generate 3D expanded PSCs, a PSC population was plated on Day 0 in Stemscale media with L-glutamine and medium comprising either 1x CEPT cocktail or 10 μ M of Y-27632 and placed in an incubator set to 37° C and 5% CO₂. Media was replaced daily with Stemscale media with L-Glutamine. Cells were dissociated on Day 4 using standard methods utilizing EDTA, resuspended in medium, and replated. Media was replaced daily with Stemscale media with L-Glutamine. At Day 8, cells were dissociated using standard methods utilizing Accutase™ and resuspended in medium, thereby preparing the starting PSCs for 3D culture. The starting PSCs were seeded into suitable bioreactors (Eppendorf). Cells in bioreactors were continuously perfused with alternating tangential flow (ATF) perfusion to facilitate media exchange and generate smaller aggregates.

8.2. Example 1: IGFBP2 Expression Correlates Closely With Seeding Density and Cell Count

[0307] To determine whether there is a correlation between cell seeding density and IGFBP2 concentration in supernatant, supernatant collected from iPSC cultures seeded at various concentrations was evaluated for IGFBP2 concentration. iPSCs were harvested using Accutase™ and cell count was determined using the NC200-based method as described in Section 8.1.3.2. Cells were seeded at 25k, 50k, 100k, and 200k cells/cm² and placed into the incubator as described in Section 8.1.1. Supernatant was collected 24 hours after seeding, the media was replenished in each compartment, the cells were returned to the incubator, and the supernatant was collected again after another 24 hours. Both sets of supernatants were used to determine IGFBP2 concentration by ELISA using the method described in Section 8.1.4. The results of this assessment revealed a strong linear correlation between the seeding cell density of iPSCs and IGFBP2 concentration after 24 and 48 hours (FIG. 2A).

[0308] In the next assessment, the relationship between cell counts and IGFBP2 concentration in supernatant was evaluated. Five different iPSC clones that were engineered to express IL12 were harvested using Accutase™ and counted using the NC200 as described in Section 8.1.3.2. The supernatants were tested for IGFBP2 using the HTRF as described in section 8.1.6. The results of this assessment revealed a linear correlation between the harvested cell count and IGFBP2 concentration with an $R^2 = 0.9591$ (FIG. 2B).

[0309] Taken together, the results of these assessments provided evidence for a correlation between IGFBP2 and cell seeding density and between IGFBP2 and cell count, suggesting IGFBP2 can indicate numbers of cells in a cell culture setup.

8.3. Example 2: Comparison of IGFBP2 Expression to Cell Counting Protocols

[0310] In this assessment, IGFBP2 expression was compared to Incucyte® and NC200-based cell counting methods. Briefly, iPSCs were first counted using the NC200 as described in Section 8.1.3.2. upon which they were seeded at 13k, 25k, 50k, and 100k cells/cm². Over the next three days, supernatant was collected from the cells, and media was refreshed as described in Section 8.1.1. Each day, cells were counted using the Incucyte® method as described in Section 8.1.3.1. On the third day, cells were harvested with Accutase™ and counted using the NC200-based method as described in Section 8.1.3.2.

[0311] The IGFBP2 concentration in supernatant samples for each seeding density displayed a linear correlation, albeit each set of experiment displayed a distinct slope (FIG. 3A). When IGFBP2 concentration was compared to Incucyte®-based cell counts, the relationship was non-linear (FIG. 3B). Since cell numbers in d3 supernatant samples was evaluated with both Incucyte® and NC-200-based methods, the IGFBP2 concentration at d3 was compared to both of these cell counting methods. The results of this evaluation showed that there was better linearity between NC200-based cell counting and IGFBP2 concentration, whereas the Incucyte®-based method appeared to underestimate cell numbers (FIG. 3C), which is potentially due to the inability of the Incucyte®-based method to correctly estimate cell counts when cells are clumped or aggregated.

8.4. Example 3: IGFBP2 Expression and Cell Number Estimation in Cell Factories

[0312] Cell number estimations in cell factories can be challenging if the cells are to be counted using visual methods, such as when cell numbers need to be estimated in culture plates without harvesting cells into a cell suspension. Therefore, the utility of IGFBP2 concentration for cell number estimation was evaluated using cell factories. Briefly, iPSCs growing in culture were passaged using EDTA into four 1-layer cell factories at approximately 4 million cells/layer. 24 hours later, supernatant from one cell factory was collected. Cells from that cell factory were harvested using Accutase™ and counted using the Cellometer K2 as described in Section 8.1.3.3. The remaining three cell factories received a media change. This process was repeated 3 more times on day 2, day 3, and day 4. Supernatants were tested for IGFBP2 as described in Section 8.1.4.

[0313] The results of this evaluation showed that the IGFBP2 increase over time correlated well with increases in NC200-based cell counts over time (FIGS. 4A and 4B), suggesting that

IGFBP2-based cell number estimation may be suitable to estimate cell numbers in visually challenging conditions, such as cell factories.

8.5. Example 4: IGFBP2 Expression and Cell Number Estimation in Clones Producing Secreted Molecules

[0314] The suitability of IGFBP2-based cell number estimation for cells producing secreted molecules was evaluated. Briefly, iPSCs engineered to express the secreted molecule IL12 were passaged, and supernatants from 18 different iPSC clones were collected using Accutase™ 24 hours after a media change. Cells were counted using the NC200-based method as described in Section 8.1.3.2 and IL12 concentration in supernatant samples were determined using a commercially available HTRF kit from Cisbio (CT, USA). IGFBP2 concentration was determined using the protocol described in Section 8.1.6.

[0315] Figure 5A shows the IL12 concentration normalized to NC200-based cell count. Figure 5B shows the IL12 concentration normalized to IGFBP2 concentration. The high level of similarity of the two graphs suggests IGFBP2 expression can be used to estimate cell numbers in clones producing secreted molecules, such as IL12.

8.6. Example 5: IGFBP2 as a Normalization Marker for Individual Batches of Experiments

[0316] Briefly, iPSCs engineered to express heterologous polypeptides were passaged and 24 hours after experiencing a media change, supernatants were collected from 43 clones across 5 independent experiments. Cells were harvested using Accutase™ and counted using the NC200 as described in Section 8.1.3.2. Supernatants were tested for IGFBP2 using the ELISA described in Section 8.1.4. Figure 6A shows the correlation of IGFBP2 concentration by experiment, whereas Figure 6B shows the combined data for all experiments, which displayed linearity with an R^2 value of 0.85, suggesting IGFBP2 concentration can be used as normalization marker across batches of experiments.

8.7. Example 6: IGFBP2 Expression to Assess Cell Health

[0317] To evaluate whether IGFBP2 concentration measurements can be used to evaluate the health of cultured cells, IGFBP2 levels can be assessed in healthy and unhealthy iPSCs. In an exemplary assessment, iPSCs can be cultured as described in Section 8.1.1. Next, iPSC culture vessels can be assigned into a healthy control group or treatment groups that render the cells unhealthy. For instance, different types of treatments (e.g., mechanical stress, chemical manipulation, non-optimal culturing incubator conditions, etc.) or different levels of a single type

of treatment (e.g., different concentrations of one chemical or varying application durations of a chemical manipulation for the same chemical, which lead to different levels of change in cell health) can be evaluated by collecting supernatant samples from each treatment condition and comparing the absolute IGFBP2 concentration of each treatment condition measured with a suitable protocol such as those described in Sections 8.1.4 and 8.1.6, against the IGFBP2 concentration in supernatant samples of the untreated healthy control group.

8.8. Example 7: IGFBP2 Expression in DA Neurons Differentiated from PSCs

[0318] The utility of IGFBP2 concentration measurements was evaluated in DA neurons differentiated from PSCs. Briefly, PSCs were maintained as described in Section 8.1.1 and cells were differentiated into DA neurons as described in Section 8.1.7. Supernatants of 11 different batches of cells were collected on post-differentiation days 12, 13, 14, 15, and 16 (D12-16). IGFBP2 measurement was carried out as described in Section 8.1.5, cells were harvested using Accutase™ and cell count yield on D16 was calculated as described in Section 8.1.3.2.

[0319] Changes in IGFBP2 levels of individual batches of DA neurons over time are shown in FIG. 7A. The correlation between IGFBP2 levels in DA neurons differentiated from 3D PSC cultures on post-differentiation day 12 (D12) and cell count yield on day 16 (D16) are shown in FIG. 7B, which displayed linearity with an R^2 value of 0.78, suggesting IGFBP2 concentration may be suitable to estimate cell numbers of cells that are in the process of differentiating from PSCs into DA neurons or that are differentiated into DA neurons.

8.9. Example 8: Effect of Coating Conditions on IGFBP2 Expression and Cell Count in Cell Factories

[0320] IGFBP2 concentration for cell number estimation was evaluated using cell factories subjected to different coating conditions. Briefly, iPSCs growing in culture were passaged using EDTA into 12 individual 10-layer cell factories into vessels that were either coated with a substrate overnight at 4 °C (Cell factories 1-4) or for one hour at room temperature (Cell factories 5-12). Four days later, supernatant from each cell factory was collected, cells were harvested using Accutase™ and counted as described in Section 8.1.3.2. Supernatants were tested for IGFBP2 as described in Section 8.1.5.

[0321] Cell counts were lower in cell factories grown in vessels that were coated with a substrate for only one hour relative to those grown in vessels that were coated overnight (FIG. 8A). Similarly, IGFBP2 level was lower in supernatants collected from cell factories grown in vessels that were coated with a substrate for only one hour relative to those grown in vessels

that were coated overnight (FIG. 8B). IGFBP2 levels correlated well with cell counts (FIG 8C). These results suggest that IGFBP2 reflects culture condition-associated differences in cell counts in multilayer cell factories and may be suitable to estimate cell numbers in visually challenging cell factories.

8.10. Example 9: Effect of Different Supplements on IGFBP2 Expression and Sphere Formation in 3D PSC Cultures.

[0322] To determine the effect of different supplements on IGFBP2 levels and formation of spheres, eight 3D PSC cultures, four of which utilized media supplemented with CEPT cocktail and the remaining four of which utilized media supplemented with Y-27632, were generated and maintained as described in Section 8.1.8. Supernatant was collected and the number of spheres and total sphere volume in each culture was assessed using the FlowCam, wherein the samples run through a flow cell and images of the supernatant are captured rapidly using a 4X objective lens. The objects detected in the images are analyzed by FlowCam software, generating reports over 40 morphological parameters, including diameter, for each object. Total sphere volume was estimated using the diameter of all objects larger than 50 μ m. Supernatants were also tested for IGFBP2 as described in Section 8.1.5.

[0323] IGFBP2 levels in 3D PSC cultures in media supplemented with Y-27632 were lower than those in media supplemented with CEPT cocktail (FIG. 9A). Similarly, sphere counts in 3D PSC cultures in media supplemented with Y-27632 were lower than those in media supplemented with CEPT cocktail (FIG. 9B). Overall, IGFBP2 level correlated with sphere count (FIG. 10A) and total sphere volume (FIG. 10B). These results suggest that IGFBP2-based cell number estimation may be suitable to estimate sphere numbers and cell numbers in visually challenging 3D cell cultures.

8.11. Example 10: Effect of Seeding Density on PSC Cell Count, IGFBP2 Levels, Pluripotency Markers, and Confluency after Replating

[0324] To further assess the relationship between cell seeding density, IGFBP2 concentration in supernatant, total cell counts, and cell density after replating, PSC cultures seeded at various concentrations were evaluated. Briefly, PSC cells from a single clone were passaged 1:10, and passaged again to generate a total of six clones, one clone seeded at 2.5k cells/cm² (clone 1), three clones seeded at 5k cells/cm² (clones 2, 3, and 4) one clone seeded at 10k cells/cm² (clone 5) and one clone seeded at 20k cells/cm² (clone 6) and placed into an incubator as described in Section 8.1.1. Supernatant was collected once per day for four days from all clones. After collection of supernatant, media was replenished, and the cells were returned to

the incubator. Supernatants were used to determine IGFBP2 concentration using the method described in Section 8.1.5. Cells were collected from clones 1, 2, 5, and 6 at 3 days after seeding, from clone 3 at 4 days after seeding, and from clone 4 at 5 days after seeding as described in Section 8.1.2. Cell count was assessed as described in Section 8.1.3.2.

Pluripotency markers (NANOG, OCT4, SOX2, SSEA4) were assessed with FACS. Cells from each clone were replated at 50k, 100k, 200k, and 400k cells/cm² and cell confluency and % recovery was evaluated with Incucyte Live Cell Imager 3 and 4 days after replating.

[0325] On day 1, the levels of IGFBP2 reflected the initial seeding density in each condition (FIG. 11A). IGFBP2 levels increased in supernatants of all clones over the course of four days, with the highest level of IGFBP2 associated with clone 6 (20k cells/cm²) and the lowest level with clone 1 (2.5k cells/cm²) for days 1-3 (FIG. 11B). On day 4, supernatant IGFBP2 levels were comparable between clone 5 (10k cells/cm²) and clone 6 (20k cells/cm²) (FIG. 11B).

[0326] Cell count assessments of clone 2 on day 3, clone 3 on day 4, and clone 4 on day 5, all of which seeded with 5k cells/cm², showed a steady increase in number of cells each day (FIG. 11C). The comparison of number of cells for each seeding density condition on day 4 showed an increase in cell numbers in clones that reflected their seeding density for clones with lower seeding densities, which was not seen with overconfluent cells (FIG. 11D).

[0327] Confluence of cells were assessed on days 3 and 4. On day 3, cells seeded at 2.5k cells/cm² and 5k cells/cm² were underconfluent (FIGS. 12A and 12B, respectively), cells seeded at 10k cells/cm² displayed confluency within the optimal range (FIG. 12C) and cells seeded at 20k cells/cm² were overconfluent (FIG. 12D). On day 4, cells seeded at 2.5k cells/cm² were underconfluent (FIG. 12E), cells seeded at 5k cells/cm² displayed confluency within the optimal range (FIG. 12F) and cells seeded at 10k cells/cm² and 20k cells/cm² were overconfluent (FIG. 12G and 12H).

[0328] The expression of pluripotency markers was also affected by seeding density and confluence of clones at the time of assessments. The percentage of cells expressing pluripotency markers in each condition are presented in Table 2 below and plotted in FIGS. 13A-13D and 14A-14D.

Table 2						
Sample	Concentration	Day of Assessment	NANOG+	OCT4+	SOX2+	SSEA4+
Clone 1	2.5k cells/cm ²	4	97.1	92.9	93.1	92.6
Clone 2	5k cells/cm ²	3	98.1	93.7	92.1	94.1
Clone 3	5k cells/cm ²	4	96.7	91.6	91.7	90.1

Clone 4	5k cells/cm ²	5	93.7	86.3	89	88.9
Clone 5	10k cells/cm ²	4	95.9	89.9	91.2	88.7
Clone 6	20k cells/cm ²	4	92.4	82.6	80	87.6
Unstained	-	-	0.1	0.1	0.1	0.8

[0329] Briefly, increased confluence among the clones with the 5k cells/cm² seeding density resulted in a decrease in percentage of cells positive for pluripotency markers (Table 2 and FIGS. 13A-13D). Similarly, increased confluence among all clones assessed on day 4 resulted in a decrease in percentage of cells positive for pluripotency markers (Table 2 and FIGS. 14A-14D). Results of the replating assessment also were associated with the confluency of cells, wherein overconfluent clones performed worse than other clones in terms of confluence after replating (FIG. 15A) and percent recovery (FIG. 15B).

[0330] Taken together, the results of these assessments provided evidence for a correlation between IGFBP2 levels and cell seeding density (FIG. 16A) and between IGFBP2 levels and cell count on day 4 (FIG. 16B). There was also a correlation between IGFBP2 levels percent confluence (FIGS. 16C and 16D). The correlation between IGFBP2 levels and pluripotency markers OCT4 and SOX2 was affected by confluency (FIGS. 16E and 16F, respectively), as reanalysis without the 20k cells/cm² data resulted in stronger correlations for both markers (FIGS. 16G and 16H). These results suggest that IGFBP2 levels can indicate numbers of cells in cultures of non-overconfluent healthy cells.

8.12. Example 11: IGFBP2 Expression in Cardiomyocytes Differentiated from PSCs

[0331] The utility of IGFBP2 concentration measurements was evaluated in cardiomyocytes differentiated from PSCs. Supernatants of five different cardiomyocyte cultures were collected every other day on maturation days 0 to 34. IGFBP2 measurement was carried out as described in Section 8.1.5.

[0332] Changes in IGFBP2 levels of each individual cardiomyocyte culture over time are shown in FIG. 17A. Culture A, Culture B, and Culture C differed in culture conditions; for instance, Culture A was seeded at a high seeding density. Culture B (low) and Culture B (high) differed in the amount of a culture medium additive. The pattern of IGFBP2 increase for Culture B (low) cells indicated suboptimal maturation of these cells, as a comparative FACS analysis of Culture B cells that had optimal levels of the culture medium additive (FIG. 17B) and Culture B (low) cells that had low levels of the culture medium additive (FIG. 17C) showed higher levels of impurities for the latter (FIGS. 17B-17C and Table 3).

Table 3	
Sample	% Impurity as measured by FACS
Culture A	0.24
Culture C	0.64
Culture B	0.09
Culture B (low)	15.1
Culture B (high)	0.31

9. SEQUENCE LISTING

[0333] Exemplary sequences of the present disclosure are provided in Table 4 below.

Table 4		
SEQ ID NO	Description	Sequence
1	Human IGFBP2 aa sequence - full length, signal peptide sequence underlined (UniProt # P18065)	MLPRVGC PALPLPPP LLLPLLLLLLGASGGGGGARA EVLFRCP PCTPE RLAACGPPP VAPPA AAVAGGARMPCAELVREPGCGCCSVCARLEGEACGVYT PRCGQGLRCYPHPGSELPLQALVMGEGTCEKRRDAEYGASPEQVADNGDDHSEGLVENHVDSTMNMLGGGGSAGRKPLKSGMKELAVFR EKVTEQHRQMGKGKHHLGLEEPKKLRPPP PARTPCQQELDQVLERIST MRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNP NTGKLIQGAPTIRGDPECHLFYNEQQEARGVHTQRMQ
2	Human IFGBP2 aa sequence - mature	AEVLFRCP PCTPERLAACGPPP VAPPA AAVAGGARMPCAELVREPGCGCCSVCARLEGEACGVYT PRCGQGLRCYPHPGSELPLQALVMGEGTC EKRRDAEYGASPEQVADNGDDHSEGLVENHVDSTMNMLGGGGSAGRKPLKSGMKELAVFREKVTEQHRQMGKGKHHLGLEEPKKLRPPP PARTPCQQELDQVLERIST MRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLFYNEQQEARGVHTQRMQ
3	Human IFGBP2 aa sequence – splice variant 1 (UniProt # C9JMY1)	MPCNNGDDHSEGLVENHVDSTMNMLGGGGSAGRKPLKSGMKELAVFR EKVTEQHRQMGKGKHHLGLEEPKKLRPPP PARTPCQQELDQVLERIST MRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNP NTGKLIQGAPTIRGDPECHLFYNEQQEARGVHTQRMQ
4	Human IFGBP2 aa sequence – splice variant 2	KHHLGLEEPKKLRPPP PARQLLAELASGACFVGLLSSLRASPAVCACRL PANRNWTRSWSGSPCAFRMSGALWSTSTPCTSP T V T S M A C T T S N S A R C L

Table 4

SEQ ID NO	Description	Sequence
	(UniProt # H7CH0)	
5	Human IFGBP2 aa sequence – splice variant 2 (UniProt # C9JW52)	MNMLGGGGSAGRKPLKSGMKELAVFREKVTEQHRQMKGKGGKHHLGLLEE PKKLRPPPARTPCQQELDQVLERISTMRLP
6	Mouse IGFBP2 aa sequence - full length, signal peptide sequence underlined (UniProt # P47877)	<u>MLPRLGGPALPLLLPSLLLLLLLGAGGCGPGVRAEVLFRCPPTPERL</u> <u>AACGPPPDAPCAELVREPGCGCCSVCARQEGEACGVYIPRCAQTLRCY</u> <u>PNPGSELPLKALVTGAGTCEKRRVGTTPQQVADSDDDHSEGGLVENHV</u> <u>DGTMNMLGGGSSAGRKPLKSGMKELAVFREKVNEQHRQMKGAKHLSL</u> <u>EEPKKLRPPPARTPCQQELDQVLERISTMRLPDDRGPLEHLYSLHIPN</u> <u>CDKHGRYNLKQCKMSLNGQRGECWCVPNPNTGKPIQGAPTIRGDPECHL</u> <u>FYNEQQETGGAHAQSVQ</u>
7	Mouse IFGBP2 aa sequence - mature	EVLFRCPPTPERLAACGPPPDAPCAELVREPGCGCCSVCARQEGEAC GVYIPRCAQTLRCYPNPGSELPLKALVTGAGTCEKRRVGTTPQQVADS DDDHSEGGLVENHVDGTMNMLGGGSSAGRKPLKSGMKELAVFREKVNE QHRQMKGAKHLSLEEPKKLRPPPARTPCQQELDQVLERISTMRLPDD RGPLEHLYSLHIPNCDKHGRYNLKQCKMSLNGQRGECWCVPNPNTGKPI QGAPTIRGDPECHLFYNEQQETGGAHAQSVQ
8	Monkey IGFBP2 aa sequence - full length, signal peptide sequence underlined (UniProt # A0A0D9R9L4)	<u>MLPRVGC PALPLPPPPLPLLLLLLLLGASGGGGGAHA</u> <u>EVLFRCPPTPE</u> <u>RLAACGPPPVAPPAVA</u> <u>AVAGGARMPCAE</u> <u>LVREPGCGCCSVCARLEGE</u> <u>ACGVYTPRCGQGLRCYPHPGSELPLQALVMGEGTCEKRRDAEYGASPE</u> <u>QVADNGDDHSEGGLVENHVDSTMNMLGGGSSAGRKPLKSGMKELAVFR</u> <u>EKVTEQHRQMKGKGGKHHLGLLEEPKKLRPPPARTPCQQELDQVLERIST</u> <u>MRLPDERGP</u> <u>LEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVPN</u> <u>NTGKLIQGAPTIRGDPECHLFYNEQQEARGVHTQRMQ</u>
9	Monkey IFGBP2 aa sequence - mature	EVLFRCPPTPERLAACGPPPVAPPAVA <u>AVAGGARMPCAE</u> <u>LVREPGC</u> <u>GCCSVCARLEGEACGVYTPRCGQGLRCYPHPGSELPLQALVMGEGTCE</u> <u>KRRDAEYGASPEQVADNGDDHSEGGLVENHVDSTMNMLGGGSSAGRK</u> <u>PLKSGMKELAVFREKVTEQHRQMKGKGGKHHLGLLEEPKKLRPPPARTPCQ</u> <u>QELDQVLERISTMRLPDERGP</u> <u>LEHLYSLHIPNCDKHGLYNLKQCKMSL</u> <u>NGQRGECWCVPNPNTGKLIQGAPTIRGDPECHLFYNEQQEARGVHTQRM</u> <u>Q</u>
10	Hamster IGFBP2 aa sequence - full length, signal peptide	<u>MLPRLGGPALSLLLPSLLLLLLLGAGGCGPGVRAEVLFRCPPTPERL</u> <u>AACGPPPDGCAELVREPGCGCCSVCARLEGEACGVYTPRCAQTLRCY</u> <u>PNPGSELPLKALVSGAGTCEKSRVGATPQQVADNGDDHSEGGLVENHV</u> <u>DGTMNMLGGGSSAGRKPLKSGMKELAVFREKVSEQHRQMKGKGGKHHLS</u> <u>LEEPKKLRPPPARTPCQQELDQVLERISTMRLPDDRGP</u> <u>LEHLYSLHIP</u>

Table 4

SEQ ID NO	Description	Sequence
	sequence underlined (UniProt # A0A8C2MM64)	NCDKHGLYNLKQCKMSLNGQRGECWCVPNPNTGKLIQGAPTIRGDPECHLFYNEQQETGGAHAQRVQ
11	Hamster IFGBP2 aa sequence - mature	EVLFRCPPTPERLAACGPPPDGPCAELVREP GCGCCSVCARLEGEACGVYTPRCAQTLRCYPNPGSELPLKALVSGAGTCEKSRVGATPQQVADNGDDHSEGLLVENHVDGTMNMLGGGSSAGRKPLKSGMKELAVFREKVSEQHRQMGKGGKHHLSLEEPKKLRPPPARTPCQQELDQVLERISTMRLPDRGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVPNPNTGKLIQGAPTIRGDPECHLFYNEQQETGGAHAQRVQ
12	Human TFF3 aa sequence (UniProt # Q07654)	MAARALCMLGLVLALLSSSSAE EYVGLSANQCAVPAKDRVDCGYPHVTPKECNRRGCCFDSRIPGVFWCFKPLQEAECTF
13	Human PTN aa sequence (UniProt # P21246)	MQAQQYQQRRKFAAAFLAFIFILAAVDTAEAGKKEKPEKKVKKSDCGEWQWSVCVPTSGDCGLGTREGTRTGAECKQTMKTQRCKIPCNWKKQFGAECKYQFQAWGECDLNTALKTRTGS LKRALHNAECQKTVTISKPCGKLT KPKPQAESKKKKKEGKKQEKMLD
14	Human IL-12 p40 subunit aa sequence (UniProt # P43432)	MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTL DQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTS SFFIRDI IKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVIC RKNASISVRAQDRYYSSSWSEWASVPCS
15	Human IL-12 p35 subunit aa sequence (UniProt # P29459)	MCPARSLLLVATLVLLDHL SLARNLPVATPDPGMF PCLHHSQNLLRAVSNMLQKARQTLEFY PCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS
16	Human LGI1 aa sequence (UniProt # O95970)	MESERSKRMGNACIPLKRIAYFLCLLSALLL TEGKKPAKPKCPAVCTCTKDNALCENARSIPRTVPDPVISLSFVRS GFTEISEGSFLFTPSLQLLLFTSNSFDVISDDAFIGLPHLEYLFIENNNIKSISRHTFRGLKSLIHL SLANNNLQTLPKDIFKGLDSL TNVDLRGNSFNCDCKLKWLV EWLGHNTNATVEDIYCEGPPEYKKRKINSLSSKDFDCIITEFAKSQDLPYQSLSIDTFSYLNDEYVVI AQPFTGKCIFLEWDHVEKTFRNYDNITGTSTV VCKPVIETQLYVIVAQLFGGSHIYKRDSFANKFIKIQDIEILKIRKPN DIE TFKIENNWYFV VADSSKAGFTTIYKWNNGNFYSHQSLHAWYRDTDVEYLEIVRTPQTLRTPHLILSSSSQRPVIYQWNKATQLFTNQTDIPNMEDVYAVKHFSVKGDVYICLTRFIGDSKVMKWGGSSFQDIQRMP SRGSMVFQPLQINNYQYAILGSDYSFTQVYNWDAEKAKFVKFQELNVQAPRSFTHV SINKRNFLFASSFKGNTQIYKHVIVDLSA

10. CITATION OF REFERENCES

[0334] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes. In the event that there is an inconsistency between the teachings of one or more of the references incorporated herein and the present disclosure, the teachings of the present specification are intended.

WHAT IS CLAIMED IS:

1. A method comprising:
 - (a) measuring a first concentration of insulin like growth factor binding protein 2 (IGFBP2) in the medium of a cell culture ("first measurement step");
 - (b) optionally, measuring at least one subsequent concentration of IGFBP2 in the medium of the cell culture (each a "subsequent measurement step"); and
 - (c) optionally, measuring a final concentration of IGFBP2 levels in the medium of the cell culture ("final measurement step").
2. The method of claim 1, which comprises performing step (b).
3. The method of claim 2, wherein step (b) comprises measuring a plurality of subsequent concentrations of IGFBP2 in the medium of the cell culture.
4. The method of claim 3, wherein step (b) is repeated once or twice daily for a period of at least three days.
5. The method of claim 4, wherein step (b) is repeated daily until one or two days before the cell culture is passaged.
6. The method of any one of claims 3 to 5, wherein step (b) is repeated every other day for a period of at least 6 days.
7. The method of any one of claims 1 to 6, wherein each subsequent measurement step is performed when the cell culture is unfed.
8. The method of any one of claims 1 to 7, wherein the concentration of IGFBP2 in the medium is indicative of the number of cells in the cell culture.
9. The method of any one of claims 1 to 7, wherein the concentration of IGFBP2 in the medium is indicative of the maturation of cells in the cell culture.
10. The method of any one of claims 1 to 9, which further comprises feeding the cell culture after one or more subsequent measurement steps.
11. The method of claim 10, wherein the cell culture is fed daily.

12. The method of any one of claims 1 to 11, which further comprises performing step (c).

13. The method of claim 12, wherein the final measurement step is performed when the cell culture is unfed.

14. The method of any one of claims 1 to 13, wherein one or more (or all) of the first measurement step, one or more subsequent measurement steps (if performed), and/or the final measurement step (if performed) is performed such that the measurement is indicative of the number of cells in the cell culture.

15. The method of any one of claims 1 to 14, wherein one or more (or all) of the first measurement step, one or more subsequent measurement steps (if performed), and/or the final measurement step (if performed) is performed when at least 12 hours have elapsed from the prior feeding.

16. The method of any one of claims 1 to 15, which comprises performing step (c) and further comprises performing step (d), wherein step (d) comprises any combination of one, two, three or all four of:

- (i) destroying cells in the cell culture (or the entire cell culture);
- (ii) passaging cells in the cell culture (or the entire cell culture);
- (iii) preserving cells from the cell culture (or the entire cell culture); and
- (iv) if the cell culture comprises pluripotent stem cells (PSCs), differentiating PSCs in the PSC cell culture (or the entire PSC cell culture).

17. The method of claim 16, wherein step (d) comprises destroying the cell culture if the final concentration of IGFBP2 in the cell culture medium is (a) below a threshold amount and/or (b) outside a target range.

18. The method of claim 16, wherein step (d) comprises passaging cells in the cell culture (or the entire cell culture) if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) within a target range.

19. The method of claim 16 or claim 18, wherein step (d) comprises preserving cells from the cell culture (or the entire cell culture) if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) within a target range.

20. The method of claim 16, wherein step (d) comprises destroying the cell culture if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) outside a target range.
21. The method of claim 16, wherein step (d) comprises preserving cells from the cell culture (or the entire cell culture) if the final concentration of IGFBP2 in the cell culture medium is (a) below a threshold amount and/or (b) within a target range.
22. The method of any one of claims 17 to 19, wherein the target range is 50 ng/ml to 200 ng/ml.
23. The method of any one of claims 1 to 22, wherein the measurement steps are performed via immunoassay, optionally wherein the immunoassay is an enzyme-linked immunosorbent assay (ELISA), a fluorescence resonance energy transfer (FRET) assay, or a Western blot.
24. The method of any one of claims 1 to 23, wherein the cell culture is an adherent culture.
25. The method of any one of claims 1 to 23, wherein the cell culture is a suspension.
26. The method of any one of claims 1 to 23, wherein the cell culture is a three-dimensional (3D) cell culture.
27. The method of any one of claims 1 to 26, which further comprises, prior to step (a), forming the cell culture.
28. The method of any one of claims 1 to 27, wherein the cell culture comprises PSCs and/or cells undergoing differentiation from PSCs into another cell type.
29. The method of claim 28, wherein the cell culture is seeded with a population of preserved (e.g., cryopreserved) PSCs.
30. The method of any one of claims 27 to 29, wherein the PSCs are iPSCs.
31. The method of claim 30, wherein the iPSCs is the product of a process comprising:
- (a) dedifferentiating adult cells;

- (b) introducing one or more nucleic acids encoding Yamanaka factors into the adult cells; and
- (c) culturing the adult cells under conditions in which the Yamanaka factors are expressed,

thereby generating the population of iPSCs.

32. The method of any one of claims 28 to 31, wherein step (d), if present, comprises differentiating PSCs from the PSC cell culture if the final concentration of IGFBP2 in the cell culture medium is (a) above a threshold amount and/or (b) within a target range.

33. The method of claim 32, wherein the target range is 50 ng/ml to 200 ng/ml.

34. The method of any one of claims 28 to 33, further comprising, prior to, during, or after differentiating the PSCs from the PSC cell culture, measuring an additional concentration of IGFBP2 levels in the medium of the cell culture.

35. The method of any one of claims 32 to 34, wherein the PSCs from the PSC cell culture are differentiated into dopaminergic neurons.

36. The method any one of claims 32 to 35, which further comprises repeating the method of any one of claims 1 to 27 during and/or following differentiation.

37. The method of any one of claims 1 to 36, wherein the cell culture comprises dopaminergic neurons.

38. The method of any one of claims 1 to 27, wherein the cell culture comprises cells undergoing maturation (e.g., cardiomyocytes).

39. A method of identifying a cell line suitable for production of a secreted polypeptide comprising:

- (a) measuring IGFBP2 concentration in a cell culture of the cell line according to the method of any one of claims 1 to 27;
- (b) concurrently with measuring IGFBP2, measuring concentration of the secreted polypeptide in the cell culture.

40. A method of controlling for cell behavior in a cell culture experiment:

- (a) performing a cell culture study; and

- (b) measuring IGFBP2 concentration as a positive control in the cell culture study according to the method of any one of claims 1 to 27.
- 41. A method of assaying the health of a test cell line, comprising:
 - (a) seeding the test cell line into cell culture at a low density; and
 - (b) measuring IGFBP2 concentration in the test cell culture according to the method of any one of claims 1 to 27.
- 42. A method of testing cell culture parameters, comprising:
 - (a) culturing a cell line under a first set of parameters;
 - (b) culturing the cell line under a second set of parameters that differs from the first set of parameters by at least one parameter; and
 - (c) measuring IGFBP2 concentrations in (a) and (b) according to the method of any one of claims 1 to 27.