ESSEN BioScience
www.essenbioscience.com

IncuCyte Live-Cell Imaging
Neuroscience Publication Review

Overview

IncuCyte live-cell imaging has been used by researchers around the world to monitor long-term, neuronal cultures and quantify cell behaviors relevant to neuroscience research. Highlighted below are recent literature examples that demonstrate the value of real-time, non-invasive, image-based measurements and show how IncuCyte live-cell imaging has provided true insight into the mechanisms of **neurogenesis** and **neurodegeneration**, enabled phenotypic characterization of genes associated with **neurogenic conditions** and contributed to **neuronal stem cell research**.

IncuCyte measurements of neurite outgrowth, cell migration and proliferation have been used to identify neurotoxic and neuroprotective treatment effects (Tortoriello *et al*., 2014; Woo *et al*., 2014), probe the pathways controlling CNS repair (Oudin *et al*., 2011) and characterize the growth profiles of neuronal stem cells (Gómez-López *et al*. 2011). When used in combination with other end-point imaging devices or biochemical detection methods IncuCyte live-cell kinetic data has been used to explain unexpected temporal events within neuronal cultures (Lodge *et al*. 2010) and resolve time-dependent neuroprotective treatment effects (Lu *et al*. 2013).

For a complete and fully searchable list of all IncuCyte publications visit our online publications library: http://www.essenbioscience.com/resources/publications/

Neurotoxicity and neurogenesis

IncuCyte measurements of neurite outgrowth and neuronal motility have been used to predict neurotoxic and neuroprotective treatment effects (Tortoriello *et al*., 2014; Woo *et al*., 2014) and probe the mechanisms controlling neurogenesis (Oudin *et al*., 2011).

Tortoriello, G. *et al.* **Miswiring the brain: Δ9-tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway**. *EMBO J.* 1–18 (2014).

In a collaborative study researchers at the Karolinska Institutet, Stockholm, Sweden, investigated the neurotoxic effects of THC (Δ^9-tetrahydrocannabinol), the major psychoactive constituent of cannabis, on primary cortical neurons. Children exposed to cannabis in utero have an increased risk of developing neuro-behavioral and cognitive impairments however it is not known whether THC is implicated.

IncuCyte high definition phase contrast images and integrated NeuroTrack image analysis module were used to kinetically quantify the effects of THC on neurite outgrowth in a label free experiment. Pre-treatment reads ensured that control and test wells were phenotypically equivalent prior to THC exposure. THC was shown to impair neurite formation in cortical neurons compared to vehicle controls, a result which supported the authors' conclusion that THC exposure triggers degradation of SCG10, a protein which mediates growth cone development during axonal elongation. SCG10 is the first known molecular target for THC in the developing central nervous system.

Oudin, M. J. *et al.* **Endocannabinoids regulate the migration of subventricular zone-derived neuroblasts in the postnatal brain.** *J. Neurosci.* **31,** 4000–11 (2011).

Oudin *et al*. (Kings College London, UK) used IncuCyte live-cell imaging to examine the role of the endocannabinoid (eCB) signaling in adult neurogenesis. Neural stem (NS) cells in the adult brain proliferate and migrate to populate the olfactory bulb with new neurons. Importantly there is growing evidence that eCB signalling regulates the ability of NS cells to divert away from the olfactory bulb towards damaged areas of the brain where they may act to restore function. IncuCyte migration assays were used to characterize the function of eCB receptors, CB1 and CB2, in primary NS cell migration. Pharmacological inhibition with selective small molecule anatgonists caused substantial reductions in NS cell migration as did inhibition of diacylglycerol lipases, the enzymes that synthesize the major eCB in the brain. In contrast activation of cannabinoid receptors using CB1 or CB2 agonists significantly increased the rate of wound closure, an effect that was reversed when a selective CB1 or CB2 antagonist was co-applied. The results support a novel role for the eCB system in NS cell migration and implicates its importance in regulating adult neurogenesis.

Further reading

Tortoriello, G. *et al.* **Miswiring the brain: Δ9-tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway**. *EMBO J.* 1–18 (2014).

Wiszniak, S. *et al.* **The ubiquitin ligase Nedd4 regulates craniofacial development by promoting cranial neural crest cell survival and stem-cell like properties.** *Dev. Biol.* **383,** 186–200 (2013).

Oudin, M. J. *et al.* **Endocannabinoids regulate the migration of subventricular zone-derived neuroblasts in the postnatal brain.** *J. Neurosci.* **31,** 4000–11 (2011).

Wicki-Stordeur, L. E. & Swayne, L. A. **Panx1 regulates neural stem and progenitor cell behaviours associated with cytoskeletal dynamics and interacts with multiple cytoskeletal elements.** *Cell Commun. Signal.* **11,** 62 (2013).

Williams, G. *et al.* **Transcriptional basis for the inhibition of neural stem cell proliferation and migration by the TGFβ-family member GDF11**. *PLoS One* **8,** e78478 (2013).

Parmentier-Batteur, S. *et al.* **Attenuation of scratch-induced reactive astrogliosis by novel EphA4 kinase inhibitors.** *J. Neurochem.* **118,** 1016–31 (2011).

Neurodegeneration

IncuCyte live-cell imaging has enabled detailed inspection of neurodegenerative phenotypes (McLean *et al*. 2014), the development of label-free models that recapitulate protein aggregate mediated neuronal cell death and the measurement of temporal profiles for treatments (Lu *et al*. 2013). These models have been made into easy to perform, high-throughput assay protocols which also enable measurement of neurotoxicity using the Essen BioScience CellPlayer Caspase-3/7 activated fluorescent biosensor (Yao *et al*. 2013).

Lu, B. *et al.* **Identification of NUB1 as a suppressor of mutant Huntington toxicity via enhanced protein clearance.** *Nat. Neurosci.* **16,** 562–70 (2013).

Lu, B. & Palacino, J. **A novel human embryonic stem cell-derived Huntington's disease neuronal model exhibits mutant huntingtin (mHTT) aggregates and soluble mHTT-dependent neurodegeneration.** *FASEB J.* **27,** 1820–9 (2013).

Researchers at Fudan University in Shanghai, China, in collaboration with Novartis, used IncuCyte technology to phenotypically characterize a novel Huntington's disease model. Huntington's disease (HD) is caused by an expansion of the polyglutamine (polyQ) repeats in the mutant Huntington (mhtt) protein. The polyQ length confers a toxic gain of function if it exceeds 36 repeats and the number of repeats is proportional to the severity of HD. Lu *et al*. developed a novel hESC-derived neuronal Huntington's disease model that exhibited both insoluble mutant Huntington (mHTT) protein aggregates and soluble HTT-dependent neurodegeneration. IncuCyte High Definition-phase contrast images and confluence algorithm were used to make detailed assessments of cell morphology and kinetically quantify neurodegenerative phenotypes. IncuCyte time course data revealed that the temporal rescue profile, following siRNA knockdown of mHTT was concentration dependent and enabled calculation of the time taken to achieve a given degree neurodegeneration - a valuable reference for cross study comparison. The authors went on to develop a high-throughput, information-rich IncuCyte screening protocol for genetic modifiers of HD toxicity that was easy to perform and accurately measured the dynamics of neuronal cell death (Yao *et al*.2013)

In a second paper, Lu *et al*. generated neurons from induced pluripotent stem cells (iPSCs) derived from Huntington's disease patients. Using the Essen BioScience CellPlayer Caspase-3/7 reagent they showed that caspase activity, in the absence of BDNF, was reduced to near wild-type level following HTT siRNA knockdown. These results indicate mHTT-dependent neurotoxicity in the absence of BDNF. Furthermore IncuCyte was use to confirm that overexpression of NUB1 (negative regulator of ubiquitin-like protein 1) in diseased neurons protected against caspase-3 mediated apoptosis. These data supported the conclusion that NUB1 is a suppressor of mHTT-induced toxicity may serve as a candidate for novel Huntington's therapeutics.

Further reading

Lu, B. *et al.* **Identification of NUB1 as a suppressor of mutant Huntington toxicity via enhanced protein clearance.** *Nat. Neurosci.* **16,** 562–70 (2013).

Lu, B. & Palacino, J. **A novel human embryonic stem cell-derived Huntington's disease neuronal model exhibits mutant huntingtin (mHTT) aggregates and soluble mHTT-dependent neurodegeneration.** *FASEB J.* **27,** 1820–9 (2013).

Lazzeroni, G. *et al.* **A phenotypic screening assay for modulators of huntingtin-induced transcriptional dysregulation.** *J. Biomol. Screen.* **18,** 984–96 (2013).

Yao, Y. *et al.* **High-throughput high-content detection of genetic modifiers of neurodegeneration in human stem cell derived neurons.** *Protoc. Exch.* (2013).

McLean, J. R. *et al.* **ALS-associated peripherin spliced transcripts form distinct protein inclusions that are neuroprotective against oxidative stress.** *Exp. Neurol.* (2014).

Neurogenetic conditions: Characterization of gene overexpression and disruption

Real-time measurements of neurite dynamics, without the need for fluorescent labels, have been used to investigate the phenotypic consequences of genetic abnormalities associated with neurogenetic conditions such as Down and 2q23.1 micro deletion syndromes (Soppa *et al*. 2014; Camarena *et al*. 2014).

Soppa, U. *et al.* **The Down syndrome-related protein kinase DYRK1A phosphorylates p27(Kip1) and Cyclin D1 and induces cell cycle exit and neuronal differentiation.** *Cell Cycle* **13,** 2084–100 (2014).

Researchers at Aachen University, Germany used IncuCyte confluence and fluorescence measurements in combination with the IncuCyte NeuroTrack analysis software to investigate the effects of DYRK1A overexpression (a candidate gene for Down syndrome) in a human neuronal cell model. High definition IncuCyte phase contrast images revealed that DYRK1A overexpression triggered proliferation arrest without inducing cell death - a conclusion that could not be drawn from parallel impedance studies and which was further confirmed by IncuCyte GFP nuclear counts. In contrast overexpression of a kinase-deficient point mutant (DYRK1A-KR) had no effect on proliferation indicating that growth inhibition was dependent on DYRK1A kinase activity. Automated analysis of neurite outgrowth using IncuCyte NeuroTrack analysis software confirmed that long-term overexpression of DYRK1A significantly increased total neurite length per cell compared to DYRK1A-KR overexpressing cells. The results support a novel mechanism by which overexpression of DYRK1A may promote premature neuronal differentiation and thus contribute to altered brain development in Down syndrome

Camarena, V. *et al.* **Disruption of Mbd5 in mice causes neuronal functional deficits and neurobehavioral abnormalities consistent with 2q23.1 microdeletion syndrome.** *EMBO Mol. Med.* 1–13 (2014).

2q23.1 microdeletion syndrome is caused by disruption of the MBD5 gene and characterized by learning disability, behavioral difficulties and craniofacial abnormalities. The IncuCyte NeuroTrack module was used by the University of Miami, USA in collaboration with Kumamoto University, Japan to investigate a novel murine model carrying an insertional mutation in Mbd5. Cortical neuronal cultures isolated from heterozygous mutant mice showed impaired neurite outgrowth and branching. The authors conclude that the findings suggest a role for MBD5 in neuronal processes and support a causal role of MBD5 in 2q23.1 microdeletion syndrome.

Neuronal stem cells: Characterization, differentiation and use in screening

IncuCyte live-cell imaging has been used by researchers to characterize neuronal stem cell proliferation (Sun *et al*. 2011), investigate genetic factors controlling self-renewal (Gómez-López *et al*. 2011) and identify treatments that induce neuronal differentiation (Lodge *et al*. 2010). Integrated IncuCyte NeuroTrack software has been used to quantify neuronal differentiation in real time (Efthymiou *et al*. 2014) and IncuCyte image based screens, employing NSC, have identified new leads in the hunt for therapies for brain tumours (Danovi *et al*. 2013).

Lodge, A. P. *et al.* **Performance of mouse neural stem cells as a screening reagent: characterization of PAC1 activity in medium-throughput functional assays.** *J. Biomol. Screen.* **15,** 159–68 (2010).

Researchers at GlaxoSmithKline characterized the morphology and long-term growth/differentiation profiles of mouse neural stem cells (mNSC) for use as a physiologically relevant screening reagent. IncuCyte confluence measurements were used to examine the effects of growth factor conditions and the function of a natively expressed neural peptide receptor PAC1 (pituitary adenylate cyclase activating peptide receptor 1) on proliferation. Surprisingly stimulation with high concentrations of the PAC1 ligand PACAP-38 markedly reduced mNSC confluence after 70h in culture. This observation helped to resolve a conflict between two parallel end-point methodologies for measuring cell proliferation. In the presence of comparable concentrations of PACAP-38 a fluorescence microscopy fix-and-stain assay recorded a sharp decline in mNSC cell numbers whereas estimates of cell number from ATP measurements had remained high. The ability to review IncuCyte time lapse images revealed that the PACAP-38 induced decrease in confluence was caused by a pronounced change in cell phenotype consistent with neuronal differentiation. Taken together, the authors concluded that high concentrations of PACAP-38 induced mNSC differentiation and that this process caused increased metabolic activity in the cells that do not undergo apoptosis.

Efthymiou, A. *et al.* **Functional screening assays with neurons generated from pluripotent stem cell-derived neural stem cells.** *J. Biomol. Screen.* **19,** 32–43 (2014).

Efthymiou and colleagues at NIH, Bethesda, USA, describe a protocol to culture pure populations of neurons derived from human pluripotent stem cells (PSCs) capable of generating patient derived

neurons in quantities suitable for high-throughput, phenotypic screens for neurodegenerative diseases. The IncuCyte NeuroTrack image analysis module was used to monitor neurite length as a marker of neuronal maturation in human PSC derived neurons. The authors note that the ability to track real-time phenotypic changes in neurons is critical for the successful interrogation of diseases that affect synaptic development and axonal outgrowth and that these data pave the way for high-throughput screens on neurons derived from patients with neurodegenerative disorders.

Danovi, D. *et al.* **A high-content small molecule screen identifies sensitivity of glioblastoma stem cells to inhibition of polo-like kinase 1.** *PLoS One* **8,** e77053 (2013).

University College London screened chemical libraries against normal (NS) and glioblastoma-derived neural stem (GNS) cells in the hunt for new therapies for Glioblastoma Multiforme (GM). GM is the most common and aggressive malignant primary brain tumor in humans with no known therapeutic cure. IncuCyte was used to identify small molecules which selectively elicit cytotoxic or cytostatic effects on GNS cells without perturbing NS cell proliferation. 160 kinase inhibitors were tested in 96-well format against three patient derived GNS cell lines. Effects on cell proliferation were quantified by segmenting and analyzing IncuCyte high definition phase contrast images using an open source software package (Cell-Profiler). Based on morphological parameters, the number of mitotic cells at each time point was quantified. The screen identified JNJ-10198409 (J101) as a compound that induced mitotic arrest in GNS but not NS cells. Subsequent proteomic analysis suggested that J101 may inhibit polo-like kinase 1 (PLK-1) an early effector of G2/M transition. Further studies using known potent and specific Plk1 inhibitors phenocopied J101 and were selective against GNS cells. Plk1 inhibitors may provide a promising starting point for the development of novel glioblastoma therapies.

Further reading

Lodge, A. P., Langmead, C. J., Daniel, G., Anderson, G. W. & Werry, T. D. Performance of mouse neural stem cells as a screening reagent: characterization of PAC1 activity in medium-throughput functional assays. *J. Biomol. Screen.* **15,** 159–68 (2010).

Gómez-López, S. *et al.* Sox2 and Pax6 maintain the proliferative and developmental potential of gliogenic neural stem cells In vitro. *Glia* **59,** 1588–99 (2011).

Efthymiou, A. *et al.* Functional screening assays with neurons generated from pluripotent stem cell-derived neural stem cells. *J. Biomol. Screen.* **19,** 32–43 (2014).

Sun, Y., Hu, J., Zhou, L., Pollard, S. M. & Smith, A. Interplay between FGF2 and BMP controls the self-renewal, dormancy and differentiation of rat neural stem cells. *J. Cell Sci.* **124,** 1867–77 (2011).

Danovi, D. *et al.* A high-content small molecule screen identifies sensitivity of glioblastoma stem cells to inhibition of polo-like kinase 1. *PLoS One* **8,** e77053 (2013).

Tailor, J. *et al.* Stem cells expanded from the human embryonic hindbrain stably retain regional specification and high neurogenic potency. *J. Neurosci.* **33,** 12407–22 (2013).

About the author:

Essen BioScience Applications Specialist, Tim O'Callaghan, reviews the IncuCyte neuroscience literature to reveal how IncuCyte live cell imaging has been used by researchers around the world to drive neuroscience discovery. Exploring broad themes from a wide range of manuscripts, Tim examines how researchers have used IncuCyte to make kinetic measurements of neurotoxicity, probe the pathways governing neurogenesis, and investigate the role of candidate genes in neurogenetic conditions.

Neuroscience Publications List – By Topic

Neurotoxicity and neurogenesis

1. Tortoriello, G. *et al.* **Miswiring the brain: Δ9-tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway.** *EMBO J.* 1–18 (2014). doi:10.1002/embj.201386035
2. Wiszniak, S. *et al.* **The ubiquitin ligase Nedd4 regulates craniofacial development by promoting cranial neural crest cell survival and stem-cell like properties**. *Dev. Biol.* **383,** 186–200 (2013).
3. Wicki-Stordeur, L. E. & Swayne, L. A. **Panx1 regulates neural stem and progenitor cell behaviours associated with cytoskeletal dynamics and interacts with multiple cytoskeletal elements**. *Cell Commun. Signal.* **11,** 62 (2013).
4. Nam, S. T. *et al.* **Insect peptide CopA3-induced protein degradation of p27Kip1 stimulates proliferation and protects neuronal cells from apoptosis.** *Biochem. Biophys. Res. Commun.* **437,** 35–40 (2013).
5. Oudin, M. J. *et al.* **Endocannabinoids regulate the migration of subventricular zone-derived neuroblasts in the postnatal brain.** *J. Neurosci.* **31,** 4000–11 (2011).
6. Parmentier-Batteur, S. *et al.* **Attenuation of scratch-induced reactive astrogliosis by novel EphA4 kinase inhibitors.** *J. Neurochem.* **118,** 1016–31 (2011).
7. Goncalves, M. B. *et al.* **The COX-2 inhibitors, meloxicam and nimesulide, suppress neurogenesis in the adult mouse brain.** *Br. J. Pharmacol.* **159,** 1118–25 (2010).
8. Niego, B. *et al.* **Thrombin-induced activation of astrocytes in mixed rat hippocampal cultures is inhibited by soluble thrombomodulin.** *Brain Res.* **1381,** 38–51 (2011).
9. Wan Woo, K. *et al.* **Phenolic derivatives from the rhizomes of Dioscorea nipponica and their anti-neuroinflammatory and neuroprotective activities**. *J. Ethnopharmacol.* 1–7 (2014). doi:10.1016/j.jep.2014.06.043
10. Sidrauski, C. et al. Pharmacological brake-release of mRNA translation enhances cognitive memory. Elife 2, e00498 (2013).

Neuro-degeneration

1. McLean, J. R. et al**. ALS-associated peripherin spliced transcripts form distinct protein inclusions that are neuroprotective against oxidative stress.** Exp. Neurol. (2014). doi:10.1016/j.expneurol.2014.05.024

2. Lu, B. & Palacino, J. **A novel human embryonic stem cell-derived Huntington's disease neuronal model exhibits mutant huntingtin (mHTT) aggregates and soluble mHTT-dependent neurodegeneration**. *FASEB J.* **27,** 1820–9 (2013).

3. Lu, B. *et al.* **Identification of NUB1 as a suppressor of mutant Huntington toxicity via enhanced protein clearance.** *Nat. Neurosci.* **16,** 562–70 (2013).

4. Lazzeroni, G. *et al.* **A phenotypic screening assay for modulators of huntingtin-induced transcriptional dysregulation.** *J. Biomol. Screen.* **18,** 984–96 (2013).

5. Yao, Y. *et al.* **High-throughput high-content detection of genetic modifiers of neurodegeneration in human stem cell derived neurons.** *Protoc. Exch.* (2013). doi:10.1038/protex.2013.085

Neuro-genetic conditions

1. Soppa, U. *et al.* **The Down syndrome-related protein kinase DYRK1A phosphorylates p27(Kip1) and Cyclin D1 and induces cell cycle exit and neuronal differentiation.** *Cell Cycle* **13,** 2084–100 (2014).

2. Camarena, V. *et al.* **Disruption of Mbd5 in mice causes neuronal functional deficits and neurobehavioral abnormalities consistent with 2q23.1 microdeletion syndrome.** *EMBO Mol. Med.* 1–13 (2014). doi:10.15252/emmm.201404044

Neuronal stem cells

1. Efthymiou, A. *et al.* **Functional screening assays with neurons generated from pluripotent stem cell-derived neural stem cells.** *J. Biomol. Screen.* **19,** 32–43 (2014).

2. Ninomiya, E. *et al.* **Glucocorticoids promote neural progenitor cell proliferation derived from human induced pluripotent stem cells.** *Springerplus* **3,** 527 (2014).

3. Danovi, D. *et al.* **A high-content small molecule screen identifies sensitivity of glioblastoma stem cells to inhibition of polo-like kinase 1**. *PLoS One* **8,** e77053 (2013).

4. Tailor, J. *et al.* **Stem cells expanded from the human embryonic hindbrain stably retain regional specification and high neurogenic potency.** *J. Neurosci.* **33,** 12407–22 (2013).

5. Williams, G. *et al.* **Transcriptional basis for the inhibition of neural stem cell proliferation and migration by the TGFβ-family member GDF11**. *PLoS One* **8,** e78478 (2013).

6. Mclaren, D. *et al.* **Automated large-scale culture and medium-throughput chemical screen for modulators of proliferation and viability of human induced pluripotent stem cell-derived neuroepithelial-like stem cells**. *J. Biomol. Screen.* **18,** 258–68 (2013).

7. Falk, A. *et al.* **Capture of neuroepithelial-like stem cells from pluripotent stem cells provides a versatile system for in vitro production of human neurons**. *PLoS One* **7,** e29597 (2012).

8. Sun, Y. *et al*. **Interplay between FGF2 and BMP controls the self-renewal, dormancy and differentiation of rat neural stem cells**. *J. Cell Sci.* **124,** 1867–77 (2011).

9. Gómez-López, S. *et al.* **Sox2 and Pax6 maintain the proliferative and developmental potential of gliogenic neural stem cells In vitro.** *Glia* **59,** 1588–99 (2011).

10. Lodge, A. P. *et al.*. **Performance of mouse neural stem cells as a screening reagent: characterization of PAC1 activity in medium-throughput functional assays.** *J. Biomol. Screen.* **15,** 159–68 (2010).

11. Sun, Y. *et al.* **Long-term tripotent differentiation capacity of human neural stem (NS) cells in adherent culture.** *Mol. Cell. Neurosci.* **38,** 245–58 (2008).

Neuro-oncology

1. Mulcahy Levy, J. M. *et al.* **Autophagy Inhibition Improves Chemosensitivity in BRAFV600E Brain Tumors.** *Cancer Discov.* (2014). doi:10.1158/2159-8290.CD-14-0049

2. Balvers, R. K. *et al.* **Serum-free culture success of glial tumors is related to specific molecular profiles and expression of extracellular matrix-associated gene modules.** *Neuro. Oncol.* **15,** 1684–95 (2013).

3. Presneau, N. *et al.* **MicroRNA profiling of peripheral nerve sheath tumours identifies miR-29c as a tumour suppressor gene involved in tumour progression.** *Br. J. Cancer* **108,** 964–72 (2013).

4. Liu, Z. *et al.* **CASZ1, a candidate tumor-suppressor gene, suppresses neuroblastoma tumor growth through reprogramming gene expression.** *Cell Death Differ.* **18,** 1174–83 (2011).

5. Ward, R. J. *et al.* **Multipotent CD15+ cancer stem cells in patched-1-deficient mouse medulloblastoma**. *Cancer Res.* **69,** 4682–90 (2009).

6. Pollard, S. M. *et al.* **Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens**. *Cell Stem Cell* **4,** 568–80 (2009).

7. Camand, E., Peglion, F., Osmani, N., Sanson, M. & Etienne-Manneville, S. **N-cadherin expression level modulates integrin-mediated polarity and strongly impacts on the speed and directionality of glial cell migration.** *J. Cell Sci.* **125,** 844–57 (2012).

8. Danovi, D. *et al.* **High content screening of defined chemical libraries using normal and glioma-derived neural stem cell lines.** *Methods Enzymol.* **506,** 311–29 (2012).

9. Danovi, D. *et al.* **Imaging-based chemical screens using normal and glioma-derived neural stem cells.** *Biochem. Soc. Trans.* **38,** 1067–71 (2010).

10. Galavotti, S. *et al.* **The autophagy-associated factors DRAM1 and p62 regulate cell migration and invasion in glioblastoma stem cells**. *Oncogene* **32,** 699–712 (2013).

Neuroscience Publications List – By First Author

1. Balvers, R. K. *et al.* **Serum-free culture success of glial tumors is related to specific molecular profiles and expression of extracellular matrix-associated gene modules.** *Neuro. Oncol.* **15,** 1684–95 (2013).

2. Camand, E., Peglion, F., Osmani, N., Sanson, M. & Etienne-Manneville, S. **N-cadherin expression level modulates integrin-mediated polarity and strongly impacts on the speed and directionality of glial cell migration.** *J. Cell Sci.* **125,** 844–57 (2012).

3. Camarena, V. *et al.* **Disruption of Mbd5 in mice causes neuronal functional deficits and neurobehavioral abnormalities consistent with 2q23.1 microdeletion syndrome.** *EMBO Mol. Med.* 1–13 (2014). doi:10.15252/emmm.201404044

4. Danovi, D. *et al.* **Imaging-based chemical screens using normal and glioma-derived neural stem cells.** *Biochem. Soc. Trans.* **38,** 1067–71 (2010).

5. Danovi, D. *et al.* **High content screening of defined chemical libraries using normal and glioma-derived neural stem cell lines.** *Methods Enzymol.* **506,** 311–29 (2012).

6. Danovi, D. *et al.* **A high-content small molecule screen identifies sensitivity of glioblastoma stem cells to inhibition of polo-like kinase 1.** *PLoS One* **8,** e77053 (2013).

7. Efthymiou, A. *et al.* **Functional screening assays with neurons generated from pluripotent stem cell-derived neural stem cells.** *J. Biomol. Screen.* **19,** 32–43 (2014).

8. Falk, A. *et al.* **Capture of neuroepithelial-like stem cells from pluripotent stem cells provides a versatile system for in vitro production of human neurons**. *PLoS One* **7,** e29597 (2012).

9. Galavotti, S. *et al.* **The autophagy-associated factors DRAM1 and p62 regulate cell migration and invasion in glioblastoma stem cells**. *Oncogene* **32,** 699–712 (2013).

10. Gómez-López, S. *et al.* **Sox2 and Pax6 maintain the proliferative and developmental potential of gliogenic neural stem cells In vitro.** *Glia* **59,** 1588–99 (2011).

11. Goncalves, M. B. *et al.* **The COX-2 inhibitors, meloxicam and nimesulide, suppress neurogenesis in the adult mouse brain.** *Br. J. Pharmacol.* **159,** 1118–25 (2010).

12. Lazzeroni, G. *et al.* **A phenotypic screening assay for modulators of huntingtin-induced transcriptional dysregulation.** *J. Biomol. Screen.* **18,** 984–96 (2013).

13. Liu, Z. *et al.* **CASZ1, a candidate tumor-suppressor gene, suppresses neuroblastoma tumor growth through reprogramming gene expression.** *Cell Death Differ.* **18,** 1174–83 (2011).

14. Lodge, A. P. *et al.* **Performance of mouse neural stem cells as a screening reagent: characterization of PAC1 activity in medium-throughput functional assays.** *J. Biomol. Screen.* **15,** 159–68 (2010).

15. Lu, B. *et al.* **Identification of NUB1 as a suppressor of mutant Huntington toxicity via enhanced protein clearance.** *Nat. Neurosci.* **16,** 562–70 (2013).

16. Lu, B. & Palacino, J. **A novel human embryonic stem cell-derived Huntington's disease neuronal model exhibits mutant huntingtin (mHTT) aggregates and soluble mHTT-dependent neurodegeneration.** *FASEB J.* **27,** 1820–9 (2013).

17. Mclaren, D. *et al.* **Automated large-scale culture and medium-throughput chemical screen for modulators of proliferation and viability of human induced pluripotent stem cell-derived neuroepithelial-like stem cells**. *J. Biomol. Screen.* **18,** 258–68 (2013).

18. McLean, J. R. *et al.* **ALS-associated peripherin spliced transcripts form distinct protein inclusions that are neuroprotective against oxidative stress.** *Exp. Neurol.* (2014). doi:10.1016/j.expneurol.2014.05.024

19. Mulcahy Levy, J. M. *et al.* **Autophagy Inhibition Improves Chemosensitivity in BRAFV600E Brain Tumors.** *Cancer Discov.* (2014). doi:10.1158/2159-8290.CD-14-0049

20. Nam, S. T. *et al.* **Insect peptide CopA3-induced protein degradation of p27Kip1 stimulates proliferation and protects neuronal cells from apoptosis.** *Biochem. Biophys. Res. Commun.* **437,** 35–40 (2013).

21. Niego, B. *et al.* **Thrombin-induced activation of astrocytes in mixed rat hippocampal cultures is inhibited by soluble thrombomodulin.** *Brain Res.* **1381,** 38–51 (2011).

22. Ninomiya, E. *et al.* **Glucocorticoids promote neural progenitor cell proliferation derived from human induced pluripotent stem cells.** *Springerplus* **3,** 527 (2014).

23. Oudin, M. J. *et al.* **Endocannabinoids regulate the migration of subventricular zone-derived neuroblasts in the postnatal brain.** *J. Neurosci.* **31,** 4000–11 (2011).

24. Parmentier-Batteur, S. *et al.* **Attenuation of scratch-induced reactive astrogliosis by novel EphA4 kinase inhibitors.** *J. Neurochem.* **118,** 1016–31 (2011).

25. Pollard, S. M. *et al.* **Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens**. *Cell Stem Cell* **4,** 568–80 (2009).

26. Presneau, N. *et al.* **MicroRNA profiling of peripheral nerve sheath tumours identifies miR-29c as a tumour suppressor gene involved in tumour progression.** *Br. J. Cancer* **108,** 964–72 (2013).

27. Sidrauski, C. *et al.* **Pharmacological brake-release of mRNA translation enhances cognitive memory.** *Elife* **2,** e00498 (2013).

28. Soppa, U. *et al.* **The Down syndrome-related protein kinase DYRK1A phosphorylates p27(Kip1) and Cyclin D1 and induces cell cycle exit and neuronal differentiation.** *Cell Cycle* **13,** 2084–100 (2014).

29. Sun, Y. *et al.* **Interplay between FGF2 and BMP controls the self-renewal, dormancy and differentiation of rat neural stem cells.** *J. Cell Sci.* **124,** 1867–77 (2011).

30. Sun, Y. *et al.* **Long-term tripotent differentiation capacity of human neural stem (NS) cells in adherent culture.** *Mol. Cell. Neurosci.* **38,** 245–58 (2008).

31. Tailor, J. *et al.* **Stem cells expanded from the human embryonic hindbrain stably retain regional specification and high neurogenic potency**. *J. Neurosci.* **33,** 12407–22 (2013).

32. Tortoriello, G. *et al.* **Miswiring the brain: Δ9-tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway**. *EMBO J.* 1–18 (2014). doi:10.1002/embj.201386035

33. Wan Woo, K. *et al.* **Phenolic derivatives from the rhizomes of Dioscorea nipponica and their anti-neuroinflammatory and neuroprotective activities**. *J. Ethnopharmacol.* 1–7 (2014). doi:10.1016/j.jep.2014.06.043

34. Ward, R. J. *et al.* **Multipotent CD15+ cancer stem cells in patched-1-deficient mouse medulloblastoma**. *Cancer Res.* **69,** 4682–90 (2009).

35. Wicki-Stordeur, L. E. & Swayne, L. A. **Panx1 regulates neural stem and progenitor cell behaviours associated with cytoskeletal dynamics and interacts with multiple cytoskeletal elements.** *Cell Commun. Signal.* **11,** 62 (2013).

36. Williams, G. *et al.* **Transcriptional basis for the inhibition of neural stem cell proliferation and migration by the TGFβ-family member GDF11**. *PLoS One* **8,** e78478 (2013).

37. Wiszniak, S. *et al.* **The ubiquitin ligase Nedd4 regulates craniofacial development by promoting cranial neural crest cell survival and stem-cell like properties.** *Dev. Biol.* **383,** 186–200 (2013).

38. Yao, Y. *et al.* **High-throughput high-content detection of genetic modifiers of neurodegeneration in human stem cell derived neurons.** *Protoc. Exch.* (2013). doi:10.1038/protex.2013.085