

## Abstract

Migration of neural progenitors is an important process for the proper development and maintenance of the nervous system. Exposure to neurotoxicants during development can interfere with neural progenitor migration and lead to nervous system defects (for review see Rice and Barone, 2000). In fact, certain toxicants are known to interfere with neural stem cell migration (e.g., Moors, et al., 2009). Recent publications advocate the development of in vitro cell culture systems to identify and prioritize potential human developmental neurotoxicants among >80,000 untested commercial chemicals (Coecke et al., 2007; Gibb, 2008; Lein and Fryer, 2005). To that end, we are developing a high throughput screening (HTS) amenable assay to measure the migration of human embryonic stem cell (hESC) derived neural progenitors (hNP1™; Aruna Biomedical) by using a novel 96-well based cell migration assay platform (Oris™ Cell Migration Assay; Platypus Technologies). Stoppers create central exclusion zones within the wells; cells are plated outside the zones and migrate inward once the stoppers are removed. At the end of the assay period, cells that have migrated into the central zones are stained and detected using fluorescence plate readers and/or visualized by microscopy. Using the Oris™ assay, we demonstrated that cytochalasin D, a disruptor of actin microfilaments, inhibits hNP1™ migration with an IC50 of ~15 nM. We also show that neurotrophic factors, such as basic fibroblast growth factor (bFGF), can accelerate neural progenitor migration as high as two-fold. Thus, this assay can be used to identify factors that inhibit or promote neural progenitor migration. Taken together, our results demonstrate an assay system suitable to help identify novel neurotoxicants among chemicals with unknown toxicological properties.

## Methods

- hNP1™ cells were grown as adherent monolayers using Aruna's defined basal medium plus supplement (AB2 + ANS) with recombinant bFGF (basic fibroblast growth factor) and LIF (leukemia inhibitory factor) included on surfaces coated with Matrigel (1:200).
- hNP1™ were plated at 60,000 cells per well onto Matrigel-coated Oris™ Assay plates in proliferation medium (PRO) for ~16 hrs at 37°C.
- Stoppers were then removed, except in the "no migration" control column (STOPPER), and the plating medium was replaced with test medium (e.g., differentiation medium aka DIF).
- Cells were incubated at 37°C for 72 hrs, stoppers removed from "no migration" control wells and all cells were stained at 37°C for 30-60 minutes with calcein (5 µg/mL) in phenol red-free Neurobasal medium with 1% BSA.
- Plates were read using a Flexstation3 microplate reader (ex 494 nm/ em 517 nm) and then imaged by epifluorescence microscopy.

## References

- Coecke, S, et al. (2007) *Environ. Health Perspect.* **115**: 924-931.
- Gibb S. (2008) *Reprod. Toxicol.* **25**(1): 136-138.
- Lein PJ, Fryer AD. (2005) *Toxicol. Sci.* **83**(1): 166-76.
- Moors M, et al. (2009) *Environ. Health Perspect.* **117**(7): 1131-8.
- Rice D, Barone S, Jr. (2000) *Environ. Health Perspect.* **108** (Suppl 3): 511-533.
- Shin S, et al. (2006) *Stem Cells.* **24**(1): 125-38.
- Young A, et al. (2010) *J Neurosci Res.* **88**(15): 3222-32.
- Zhang JH, et al. (1999) *J Biomol Screen.* **4**(2): 67-73.

## Acknowledgements

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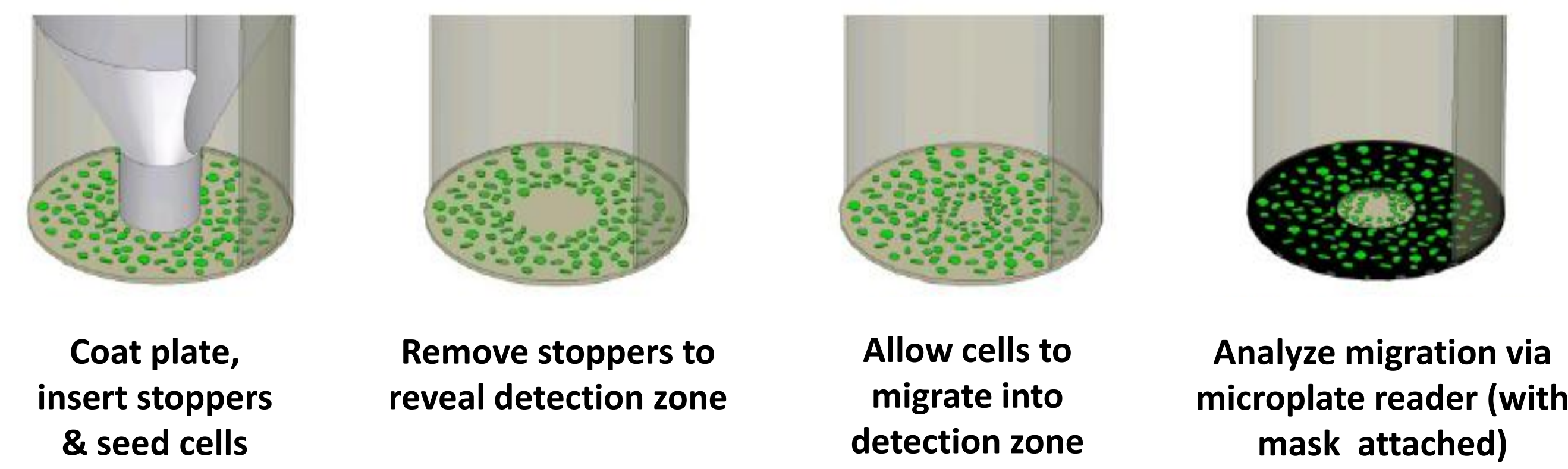


Figure 1. Schematic of the Oris™ Cell Migration Assay

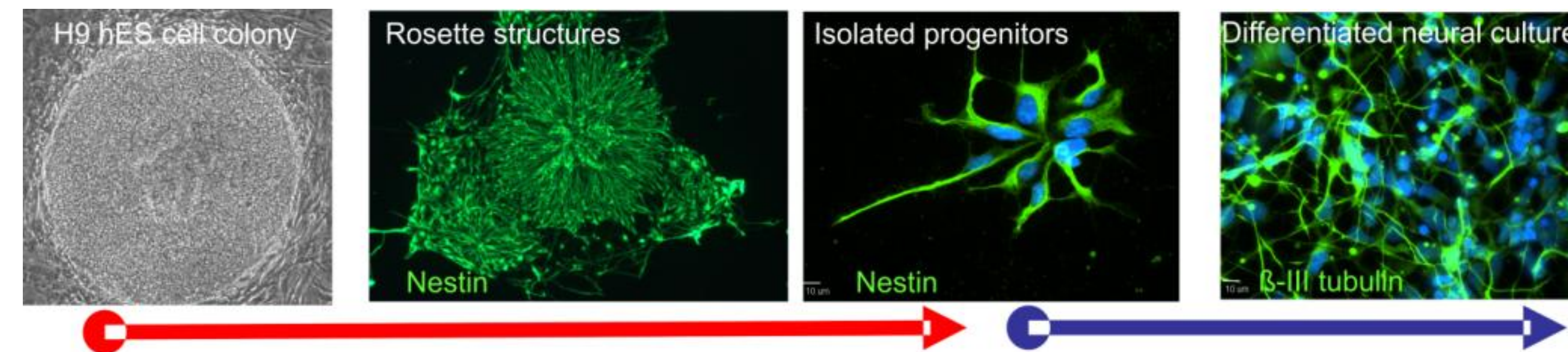


Figure 2. Derivation of hNP1™ neural progenitors. hNP1™ cells were originally derived from the H9 (WA09) human embryonic stem cell line using defined, serum-free conditions (e.g., Shin, et al., 2006), proliferate as adherent monolayers and maintain a stable karyotype for multiple (>10) passages, making them scalable for HTS format (96-, 384-well) assays. hNP1™ express proneural markers (>90% nestin+ and <5% Oct4+) and are capable of differentiation into multiple neuronal phenotypes (e.g., dopaminergic neurons; Young, et al., 2010) upon withdrawal of bFGF.

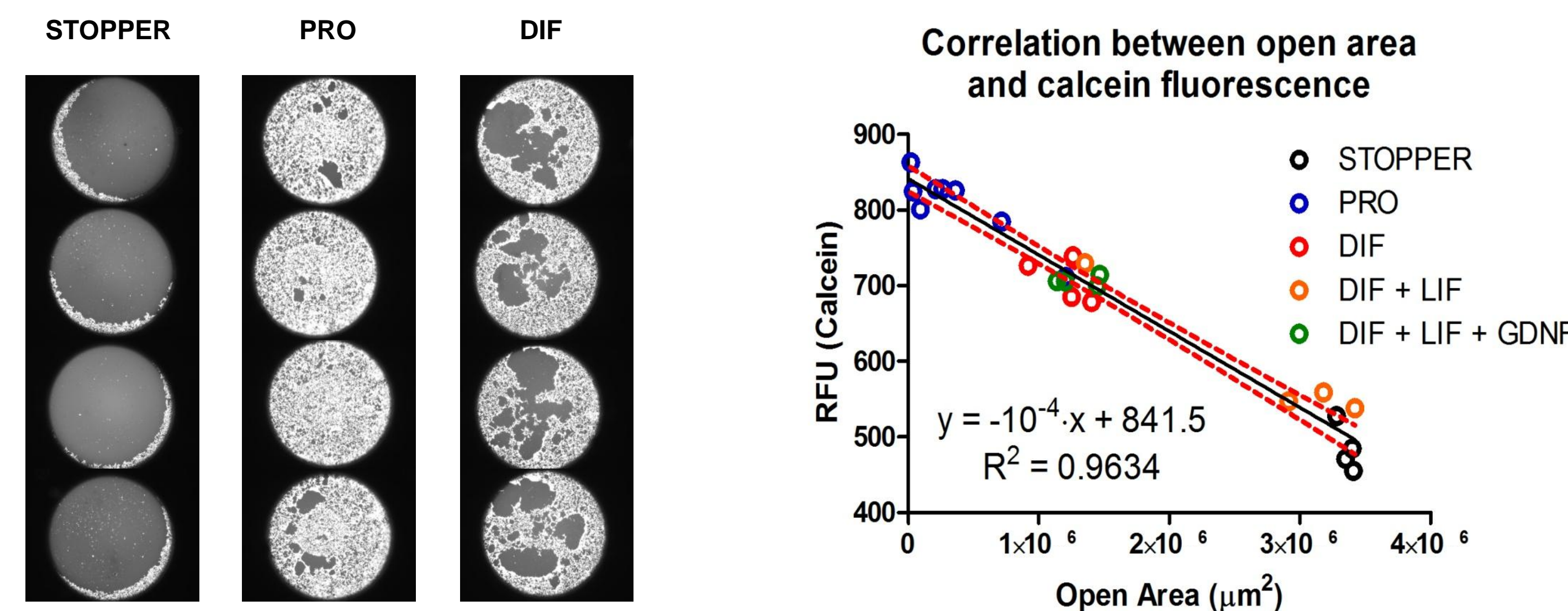


Figure 3. Use of calcein to measure hNP1™ cell migration on a microplate reader. To develop a homogenous HTS-suitable assay, we tested the cytoplasmic dye calcein as a surrogate for time- and computationally-intensive area measurements of detection zone coverage by hNP1™ cells. Cells were incubated for 72 hrs in proliferation (PRO) or differentiation (DIF) media with and without LIF and GDNF. Calcein fluorescence correlated well with area measurements obtained using ImageJ. Subsequent migration experiments were therefore analyzed for fluorescence signal using the microplate reader as a rapid means to capture data.

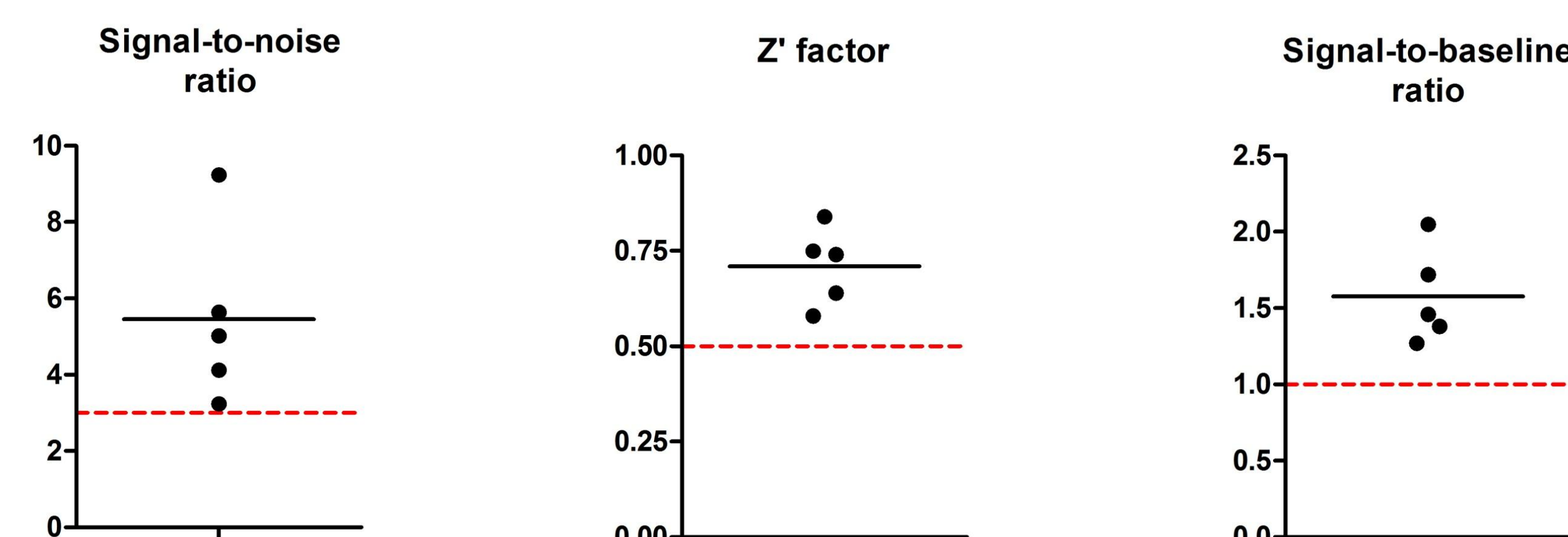


Figure 4. Assay performance measures for the hNP1™ Oris™ Cell Migration Assay. To assess assay performance, we calculated the signal-to-noise ratio, signal-to-baseline ratio, and the Z' factor (Zhang, et al., 1999) for 5 independent experiments using hNP1™ cells migrating in PRO medium. All 3 parameters were in the range considered acceptable for HTS-suitable assays.

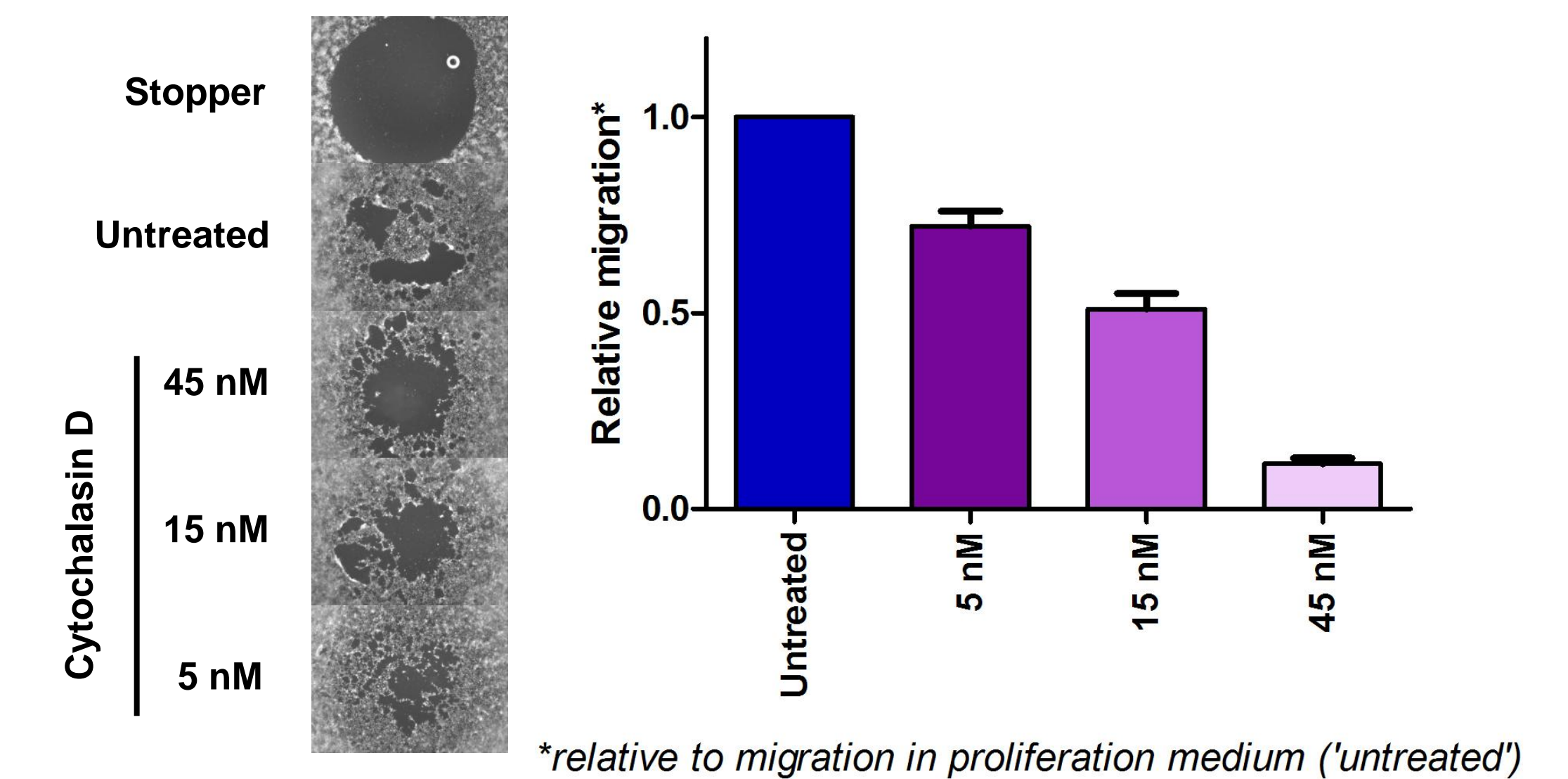


Figure 5. Inhibition of hNP1™ cell migration by Cytochalasin D. Cytochalasin D inhibited hNP1™ migration in a concentration dependent manner with an IC<sub>50</sub> of ~15 nM (72 hr treatment; the mean of 2 independent experiments shown here), indicating that inhibitors of migration can be readily detected.

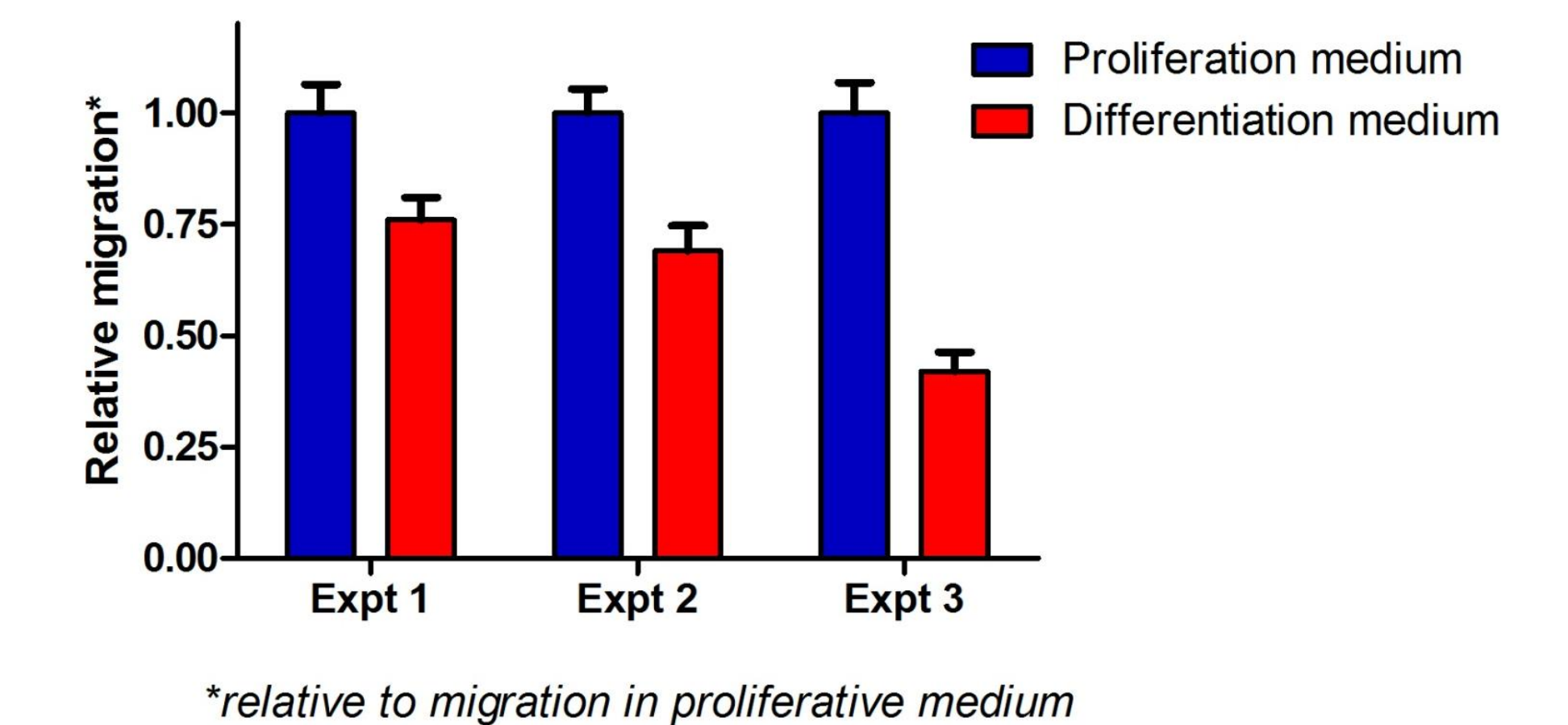


Figure 6. Differences in hNP1™ cell migration under various culture conditions. hNP1™ cells showed reduced migration in differentiation medium (basal medium alone) than in proliferation medium (basal medium plus bFGF and LIF), suggesting that either bFGF, LIF or both have a chemokinetic effect on neural progenitors.

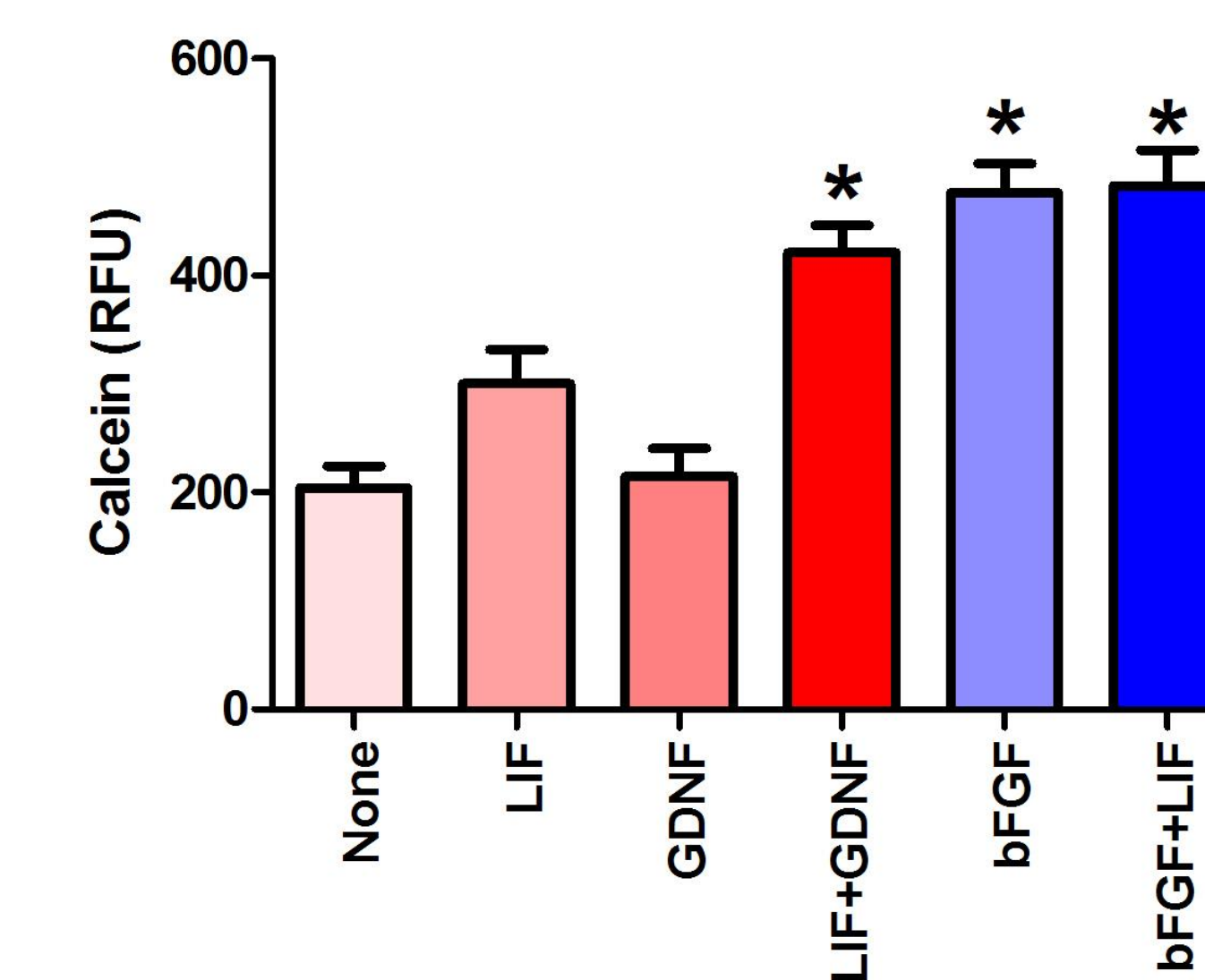


Figure 7. Effect of growth factor combinations on hNP1™ cell migration. Preliminary results indicate that bFGF alone is primarily responsible for driving migratory behavior under proliferative conditions (\*, P < 0.05 when compared to no growth factor control). In contrast, the combination of LIF and GDNF, which differentiates hNP1™ into dopaminergic neurons (Young, et al., 2010), has a synergistic chemokinetic effect (\*, P < 0.05).

## Conclusions

- The hNP1™ Oris™ Cell Migration Assay can quantitatively detect both stimulators and inhibitors of cell migration.
- This assay has the potential for adaptation as a homogenous HTS-suitable cell-based assay.
- Preliminary results suggest that bFGF alone has a potent chemokinetic effect while LIF and GDNF act synergistically to drive migratory behavior during dopaminergic differentiation.