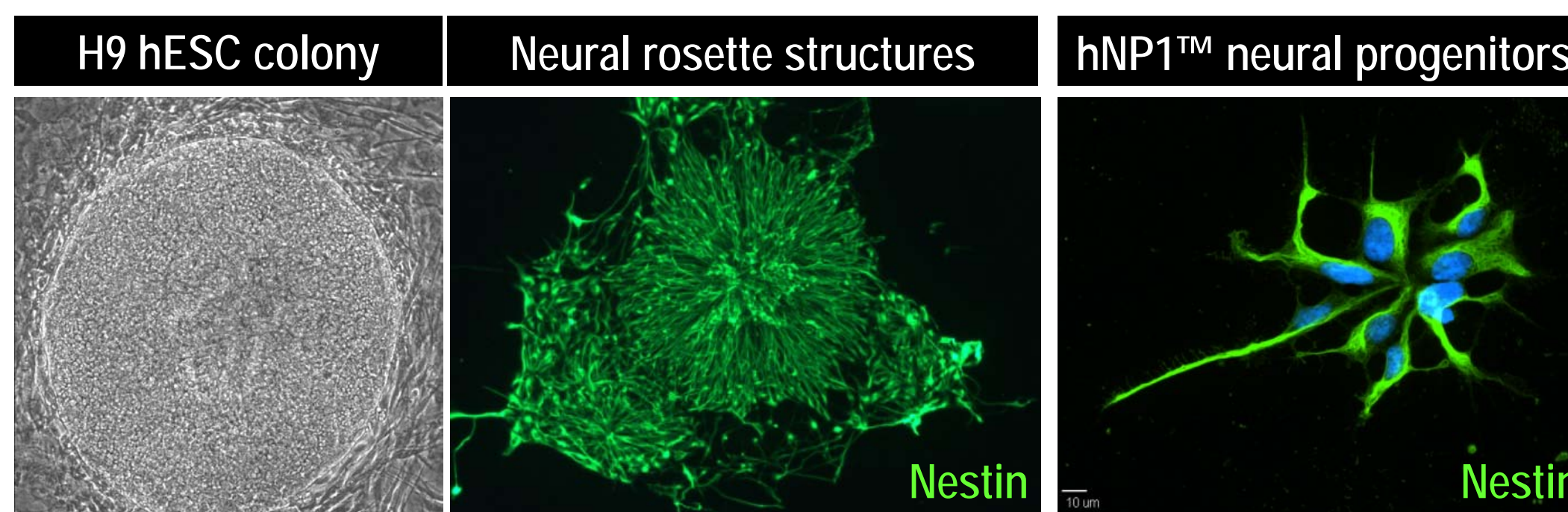


Abstract

- Neural progenitor migration is an important process for the proper development and maintenance of the nervous system. Derived from proliferative zones within the brain, neural progenitors migrate to specific destinations guided by various extracellular cues, such as growth factors.
- Exposure to neurotoxicants during development can interfere with neural progenitor migration and lead to nervous system defects (for review see Rice and Barone, 2000). 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 identify modulators of neural progenitor cell migration using human embryonic stem cell (hESC) derived neural progenitors (hNP1™; Aruna Biomedical) and a novel 96-well based cell migration assay platform (Oris™ Cell Migration Assay; Platypus Technologies).
- Our results show that this assay can be used to identify both inhibitors and stimulators of neural progenitor migration. The combination of this novel cell migration assay and neural stem cells provides a powerful tool for understanding proper nervous system development, identifying neurotoxicants, and developing therapies for migration defects in neurological syndromes.

hNP1™ Neural Progenitors



Marker	%positive
Nestin	>90
Musashi1	>90
SOX2	>90
Oct4	<5

Figure 1. Characteristics 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- or 384-well) assays. hNP1™ express proneural markers (nestin, green; see also table) and are capable of differentiation into multiple neuronal phenotypes.

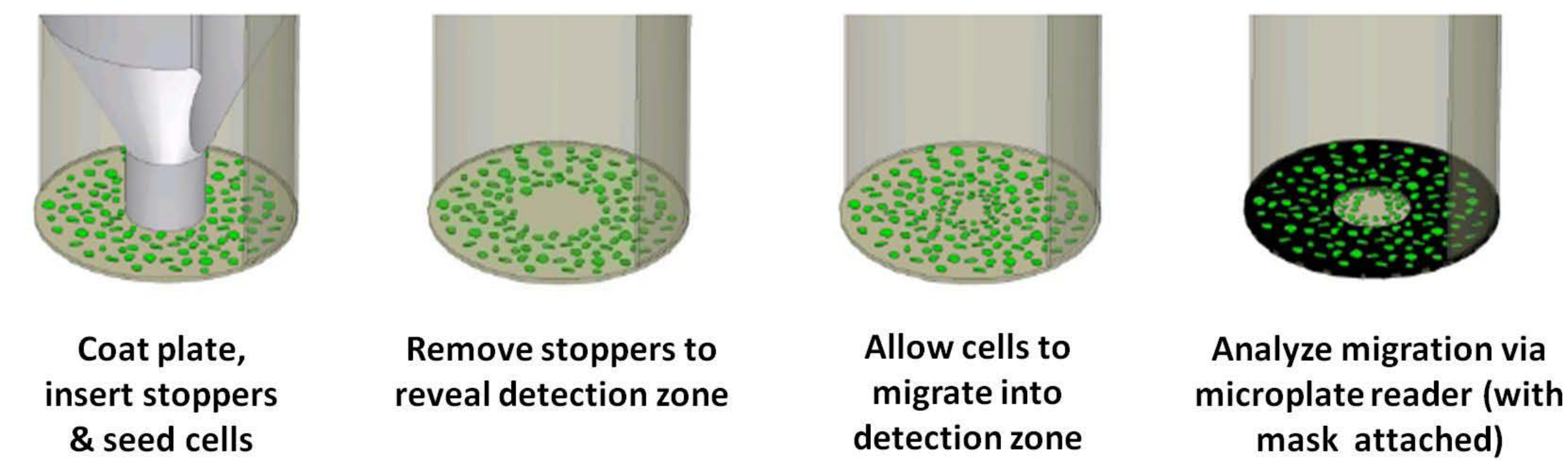


Figure 2. Design of the Oris™ Cell Migration Assay using hNP1™ Neural Progenitors

hNP1™ were plated at 60,000 cells per well onto Matrigel in basal medium with LIF and bFGF for ~16 hrs at 37°C. Stoppers were then removed, except in the "no migration" control column. The plating medium was replaced with test medium and cells were then incubated at 37°C for 72 hrs to allow for migration. At the end of the migration period, stoppers were removed from "no migration" control wells, all cells were stained at 37°C for 30-60 minutes with calcein-AM (5 µg/mL), and plates were read using a Flexstation3 microplate reader (ex 494 nm/ em 517 nm) followed by imaging with epifluorescence microscopy.

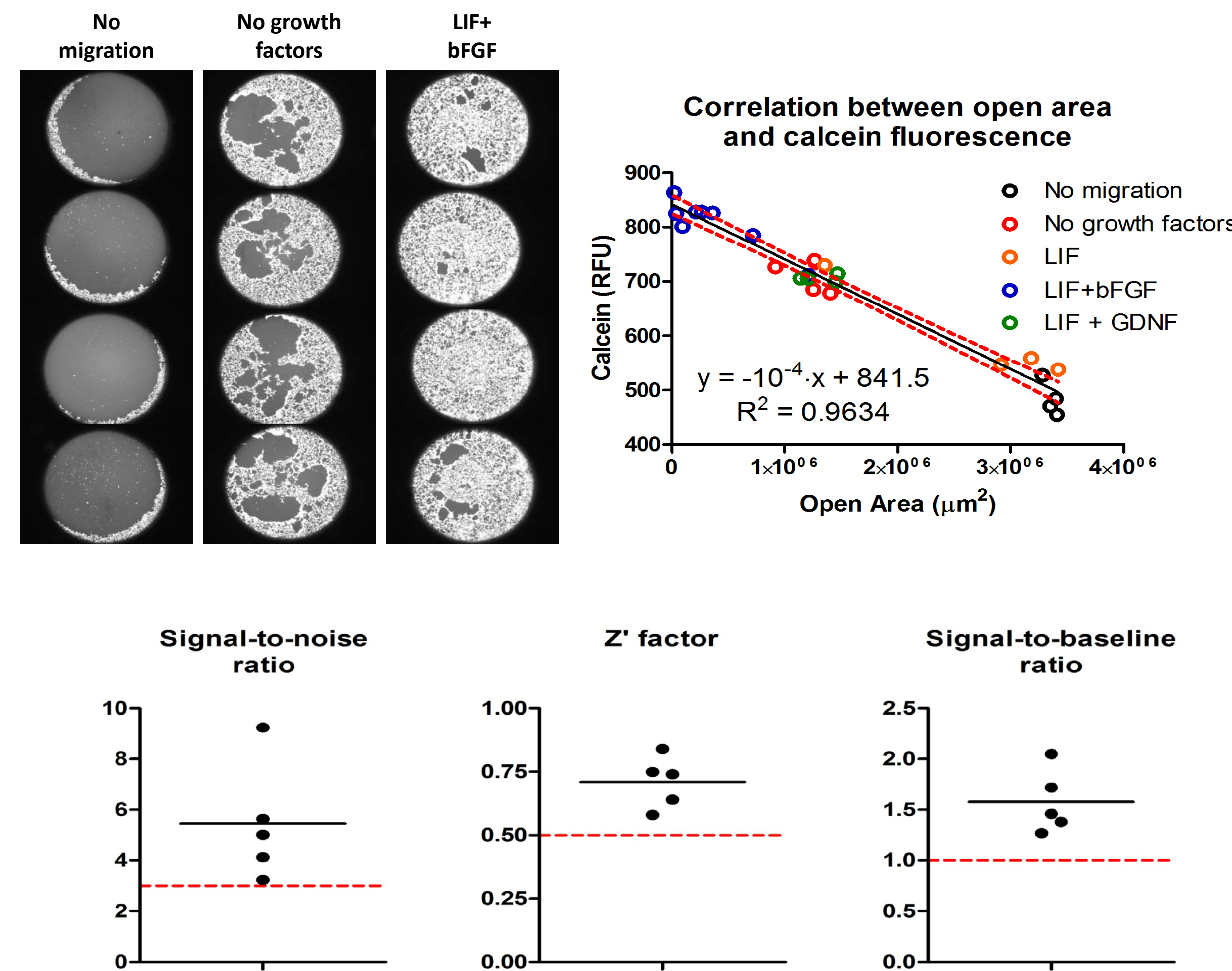


Figure 3. Validation and Performance Measures of hNP1™ Oris™ Cell Migration Assay

To develop a homogenous HTS-amenable 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 basal medium with and without LIF, bFGF and/or GDNF. Calcein fluorescence correlated well with area measurements obtained using ImageJ. 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.

Experimental Design and Results

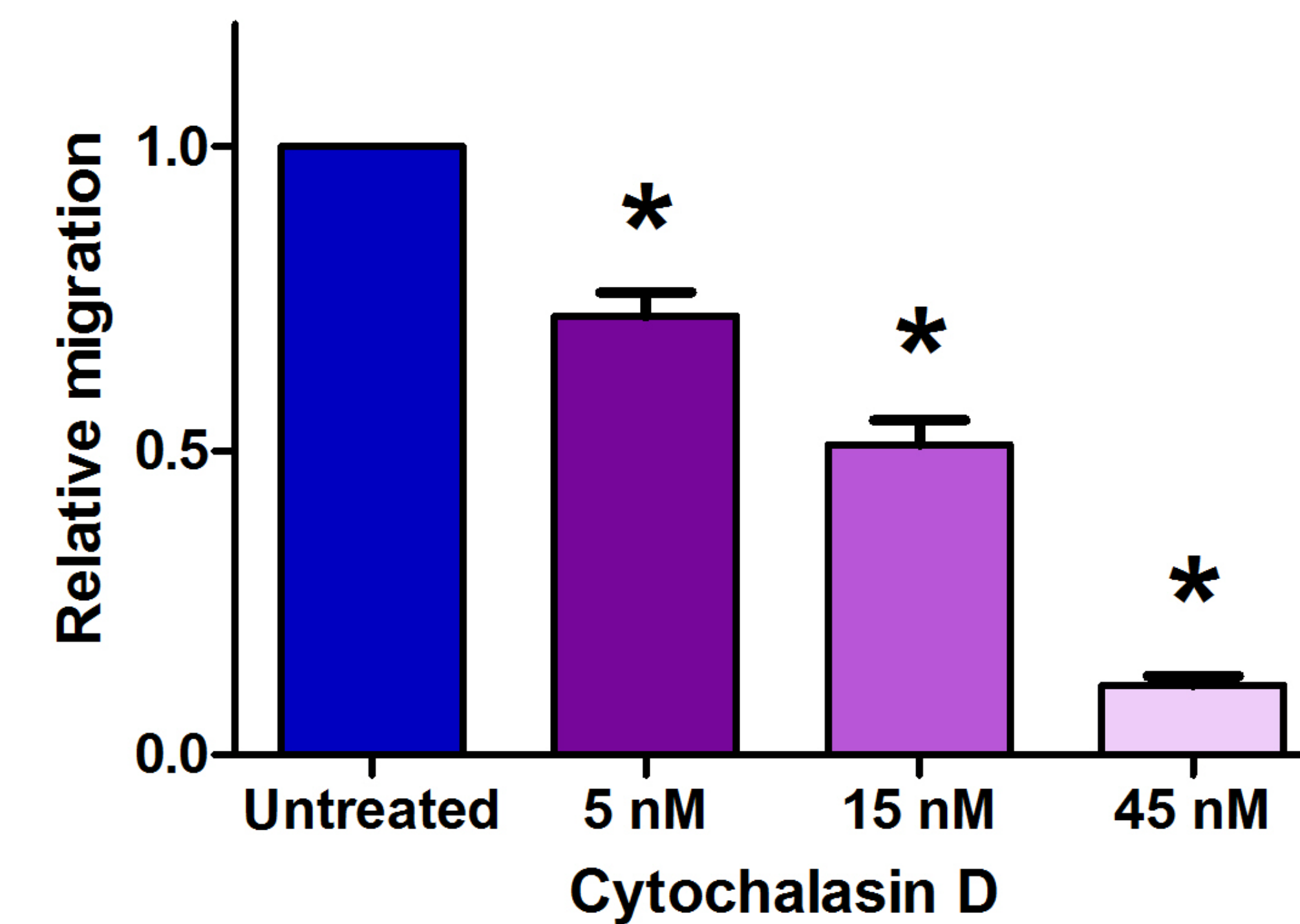


Figure 4. Inhibition of hNP1™ cell migration by cytochalasin D

Cytochalasin D inhibited hNP1™ migration in a concentration dependent manner with an IC₅₀ of ~15 nM, indicating that inhibitors of migration can be readily detected (*, P < 0.05 compared to untreated).

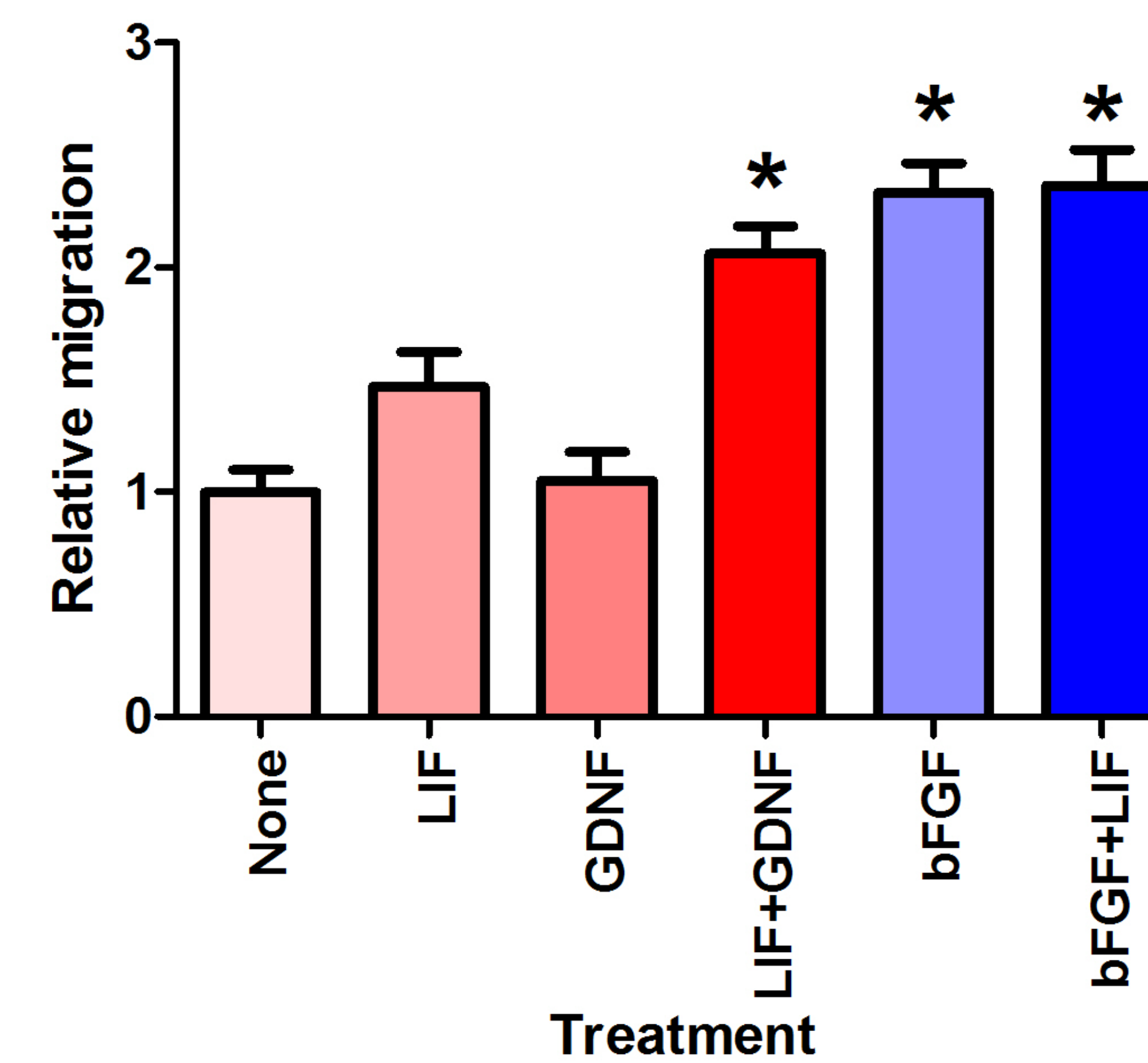


Figure 5. Stimulation of hNP1™ cell migration by growth factors

bFGF alone stimulates hNP1™ migration (*, P < 0.05 when compared to no growth factor control). In contrast, the combination of LIF and GDNF has a synergistic chemokinetic effect (*, P < 0.05).

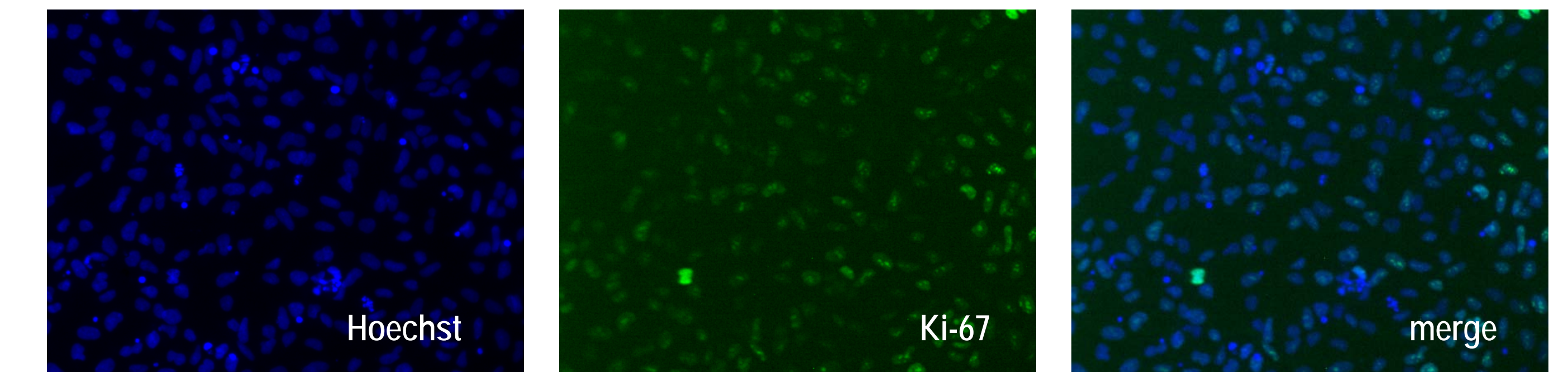


Figure 6. hNP1™ proliferation within the detection zone. Preliminary results indicate that ~60% of hNP1™ cells in the presence of LIF and bFGF within the detection zone are positive for Ki-67, a marker for proliferation. This suggests the potential for using the assay to measure neural progenitor proliferation.

Conclusions

The hNP1™ Oris™ Cell Migration Assay:

- Can quantitatively detect both stimulators and inhibitors of neural cell migration,
- Can be adapted into a homogenous HTS-amenable cell-based assay, and
- May be able to measure neural progenitor proliferation.

References

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