

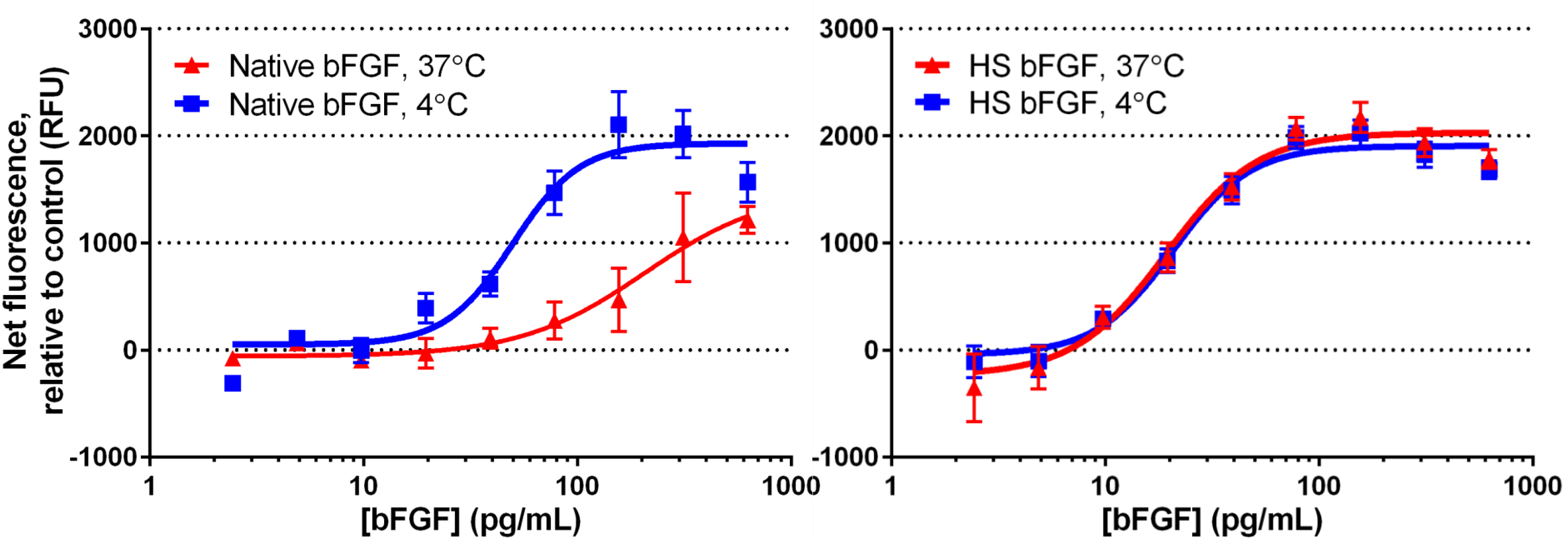
# Novel Engineered Basic Fibroblast Growth Factor Improves Stability and Enables Improved Cell Culture Outcomes

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## Introduction

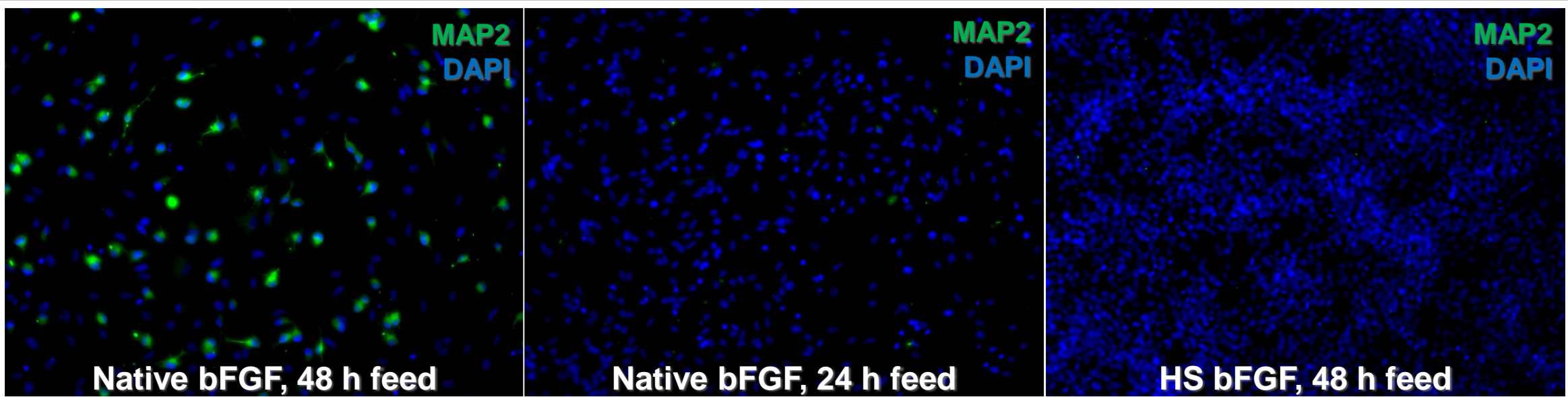
### HS bFGF: Engineered for greater stability

- Basic fibroblast growth factor (bFGF) is used in NSC media to maintain multipotency and is known to be present in the tumor microenvironment
- Native bFGF rapidly loses biological activity** when exposed to culture conditions (37°C); we found only ~20% bioactivity after 72 hours
- HS bFGF maintains > 90% homology** to the native protein **and ≥ 80% biological activity**, even after 72 hours of exposure to 37°C



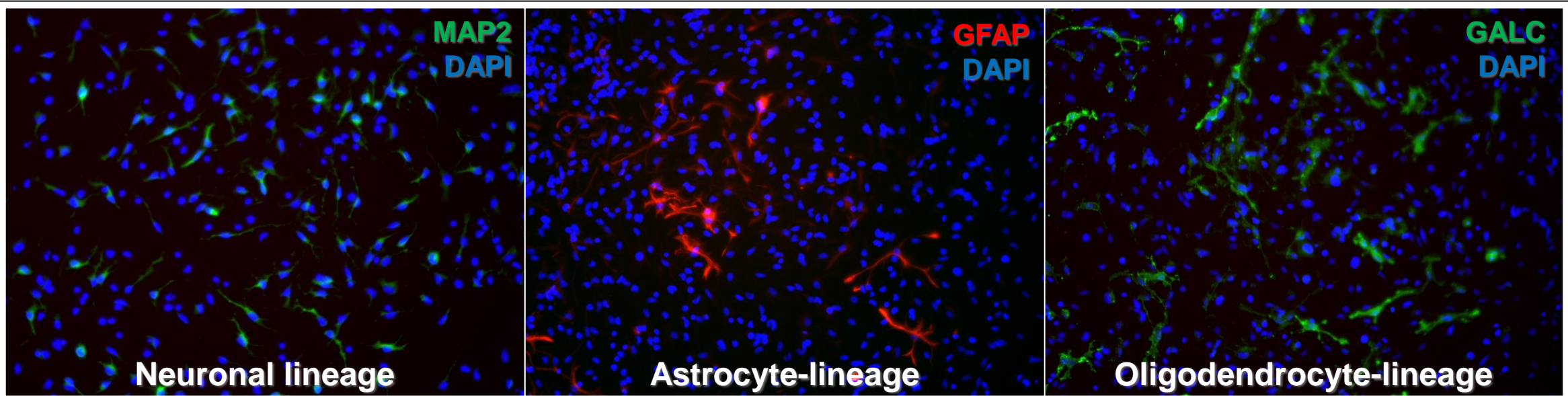
**Figure 1. HS bFGF maintained 95% activity after 72 hours at 37°C.** Dose-response of Balb/3T3 mouse embryonic fibroblast cells to native (top) and HS (bottom) bFGF stored at 4°C or 37°C for 72 hours. Analysis by PrestoBlue® assay after 18 h stimulation. Mean ± SEM.

## Primary Rat Neural Stem Cell (NSC) Culture

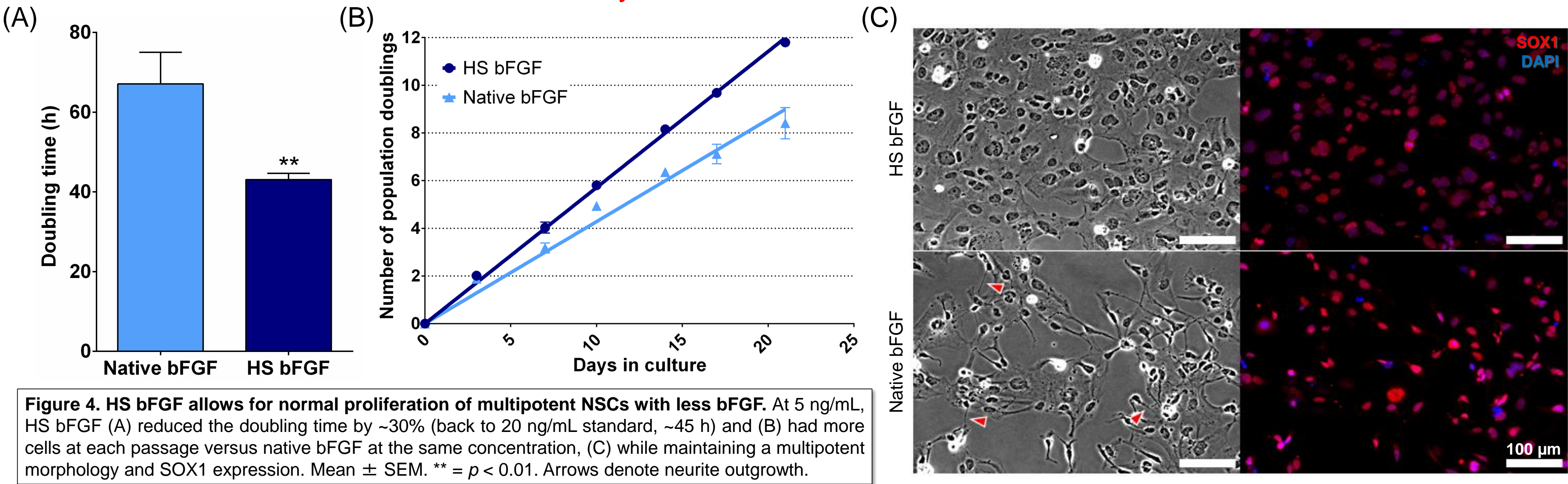


**Figure 2. HS bFGF maintains multipotent NSCs with fewer feeds.** Using 10 ng/mL bFGF, HS bFGF decreased the doubling time and maintained NSC multipotency with feeds every 48 h.

**Figure 3. HS bFGF does not impact NSC differentiation.** Three days after the removal of HS bFGF, the NSCs were stained for differentiation markers and showed trilineage potential.

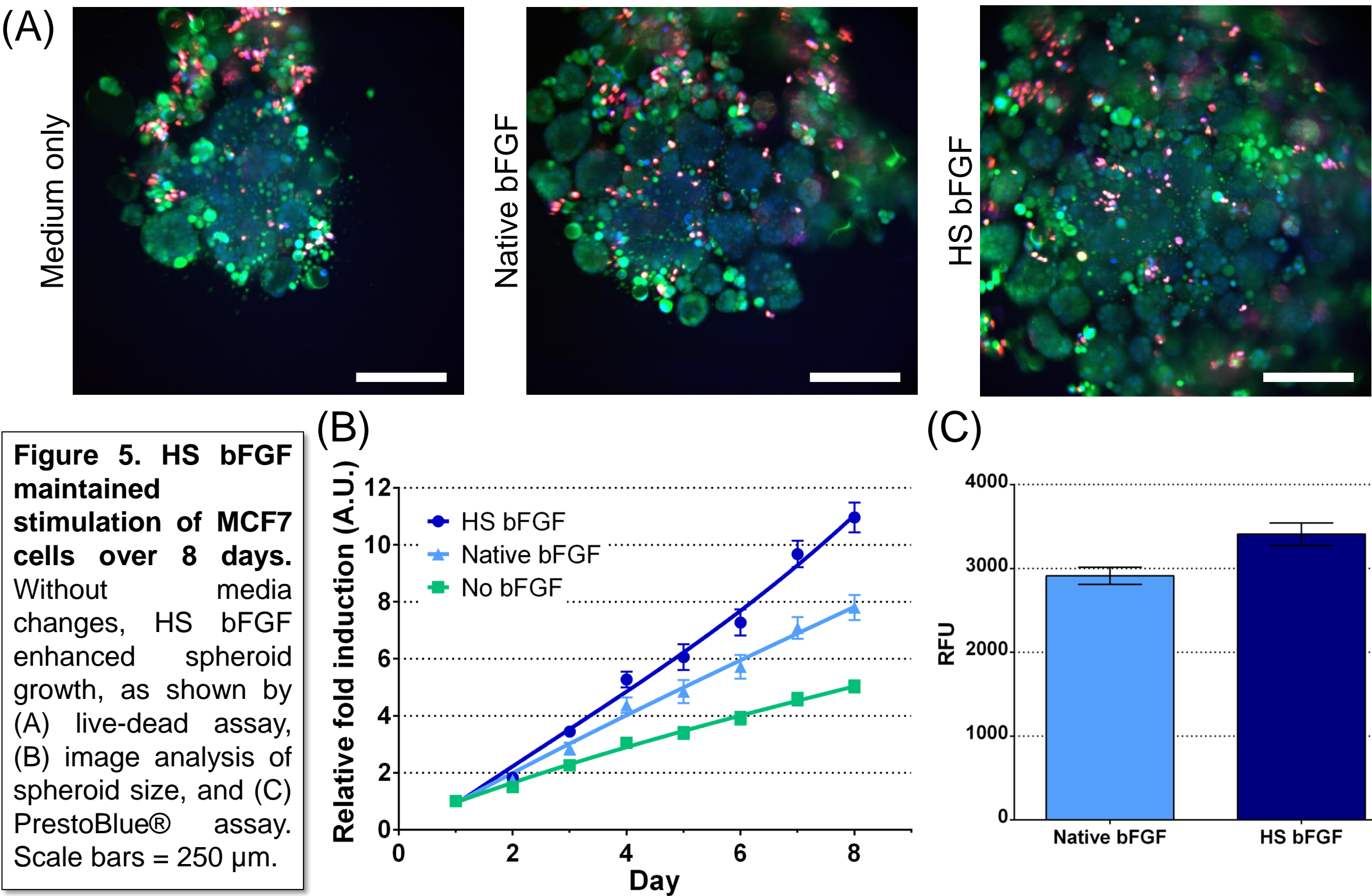


## Human Embryonic Stem Cell-Derived NSC Culture



**Figure 4. HS bFGF allows for normal proliferation of multipotent NSCs with less bFGF.** At 5 ng/mL, HS bFGF (A) reduced the doubling time by ~30% (back to 20 ng/mL standard, ~45 h) and (B) had more cells at each passage versus native bFGF at the same concentration, (C) while maintaining a multipotent morphology and SOX1 expression. Mean ± SEM. \*\* =  $p < 0.01$ . Arrows denote neurite outgrowth.

## Human Breast Cancer Spheroid Culture



**Figure 5. HS bFGF maintained stimulation of MCF7 cells over 8 days.** Without media changes, HS bFGF enhanced spheroid growth, as shown by (A) live-dead assay, (B) image analysis of spheroid size, and (C) PrestoBlue® assay. Scale bars = 250 μm.

## Conclusions

- In primary rat NSCs, using **HS bFGF allows for a more user-friendly workflow** while maintaining multipotency
- In human ESC-derived NSCs, HS bFGF can maintain multipotency and standard doubling times with **reduced bFGF concentrations**
- After expansion, **HS bFGF can be removed just as easily as native bFGF** to allow for downstream differentiation into neurons and glial cells
- HS bFGF can be used for spheroid culture**, or other systems where media changes are undesirable

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