Supplemental Methods and Data

Methods

Antibodies Used for IHC

Stain	Primary Antibody	Vendor, Catalog #	Dilution	Secondary Antibody	Vendor, Catalog #	Dilution
SDF-1/CXCL12	Rabbit Polyclonal anti-SDF-1	Abcam, Ab25117	1:500	Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 555	ThermoFisher Scientific, A-21429	1:750
Doublecortin	Rabbit polyclonal to Doublecortin	Abcam, ab18723	1:2000	Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 555	ThermoFisher Scientific, A-21429	1:500
Nestin	Chicken polyclonal to Nestin	Novus Biological, NB100-1604	1:400	Goat anti-Chicken IgY (H+L) Secondary Antibody, Alexa Fluor 555	ThermoFisher Scientific, A-21437	1:500
GFAP	Goat polyclonal to GFAP	Abcam, ab53554	1:1000	Donkey anti-Goat IgG (H+L) Secondary Antibody, Alexa Fluor 555	ThermoFisher Scientific, A-21432	1:500
Iba-1	Rabbit polyclonal to Iba1	Wako Pure Chemical Industries Ltd, 019-19741	1:250	Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 555	ThermoFisher Scientific, A-21429	1:750

NPSC Harvest and Culture

Murine fetal derived neural progenitor/stem cells (NPSCs) were isolated from the medial and lateral germinal eminences of E14.5 C57BL/6 mice based on previously published protocols and in accordance with approval by the Institutional Animal Care and Use Committee at Arizona State University [25]. The germinal eminences were harvested, mechanically disassociated and cultured in NPSC medium (Dulbecco's modified eagle medium (DMEM:F12) with 2.4mg/mL sodium bicarbonate ($NaHCO_3$), 6 mg/mL glucose, 5mM HEPES, 62.9 ng/mL progesterone, 9.6 μg/mL putrescine, 1.83 μg/mL heparin, 1X B27 growth supplement, 20 ng/mL epidermal growth factor (EGF), 5 ng/mL bFGF, 5 μg/mL insulin, 5 μg/mL transferrin, and 5 ng/mL sodium selenite). NPSCs were cultured as non-adherent neurospheres and used for experimentation between passages 3-6.

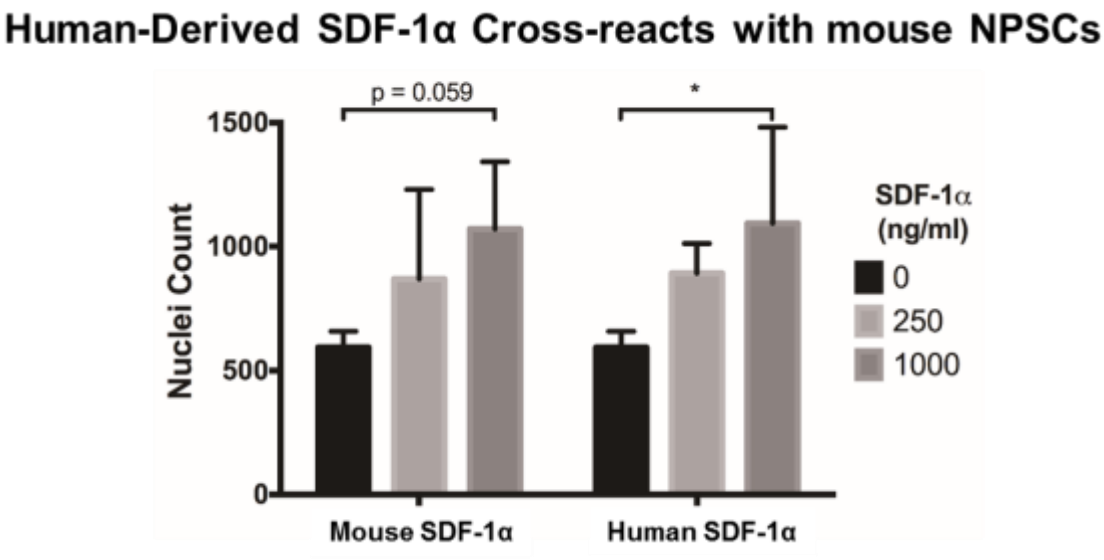
Chemotactic NPSC Migration Assay

NPSC chemotaxis in a modified-Boyden chamber assay was used to validate AFSDF-1 bioactivity on mouse-derived NPSCs as previously described [26]. In short, disassociated NPSCs were plated (70,000 cells/cm^2) on laminin-coated transwell inserts with 8μm pore diameter (Millipore, Temecula, CA). Growth factor-free NPSC medium (no EGF or bFGF) with 0, 250, or 1000ng/mL SDF-1α was added in the bottom chamber. NPSCs were then allowed to undergo chemotaxis for 24hrs in an incubator (37^0C and 5% CO_2). Subsequently, cells on the top side of the transwell membrane were removed using a cotton swab whereas migrated cells that reached the bottom were fixed, underwent a DAPI nuclear stain and imaged. NPSC nuclei have diameters of approximately 20μm [27]. After intensity thresholding, nuclei count was quantified using a particle count

algorithm in ImageJ where stained nuclei 10-30μm in diameter were counted as individual cells. Nuclei count was determined by imaging and quantifying whole transwell membranes.

Supplemental Data

The N-terminus of SDF-1 is critical for binding and activation of CXCR4 [32]. Thus, site-specific modifications for fluorescent tagging at the C-terminus is predicted to better maintain SDF-1 bioactivity [33]–[36]. The only commercially available fluorescently-tagged SDF-1 was human-derived recombinant SDF-1 with AlexaFluor-647 conjugated to the C-terminus (AFSDF-1) from Almac Chemokines (Craigavon, UK). Previous studies have noted cross-reactivity between diverse species, yet we verified that mouse NPSCs respond to human AFSDF1. A modified-Boyden chamber migration assay was used with 8μm pore width membranes. NPSCs were plated on the topside of membrane and the nuclei of cells that migrate to reach the bottom in response to SDF-1α were counted after a 24hr incubation period. Since the SDF-1 gene is over 90% homologous between mouse to human species, both types of SDF-1α elicited a similar pattern of migratory behavior from mouse-derived NPSCs (Supplemental Figure 1) [37]. Three different SDF-1α concentrations (0, 250 & 1000ng/ml) were evaluated in the bottom chamber of the Boyden assay. Nuclei count increased in a SDF-1α dose-dependent manner although only the human SDF-1α at 1000ng/ml was able to garner a statistically significant change in nuclei count. Regardless, similar patterns in NPSC response validated the bioactivity of human-derived SDF-1α on mouse NPSCs.



Supplemental Figure 1: Boyden chamber migration assay verified bioactivity of human-derived SDF-1α on mouse NPSCs. Human SDF-1α was compared to mouse SDF-1α at 0, 250 and 1000ng/ml. Only the human SDF-1α at 1000ng/ml was statistically different compared to control. However, both human and mouse SDF-1α exhibited similar trends in eliciting a migratory response from mouse NPSCs. (* represents $p < 0.05$)