Supplementary Information

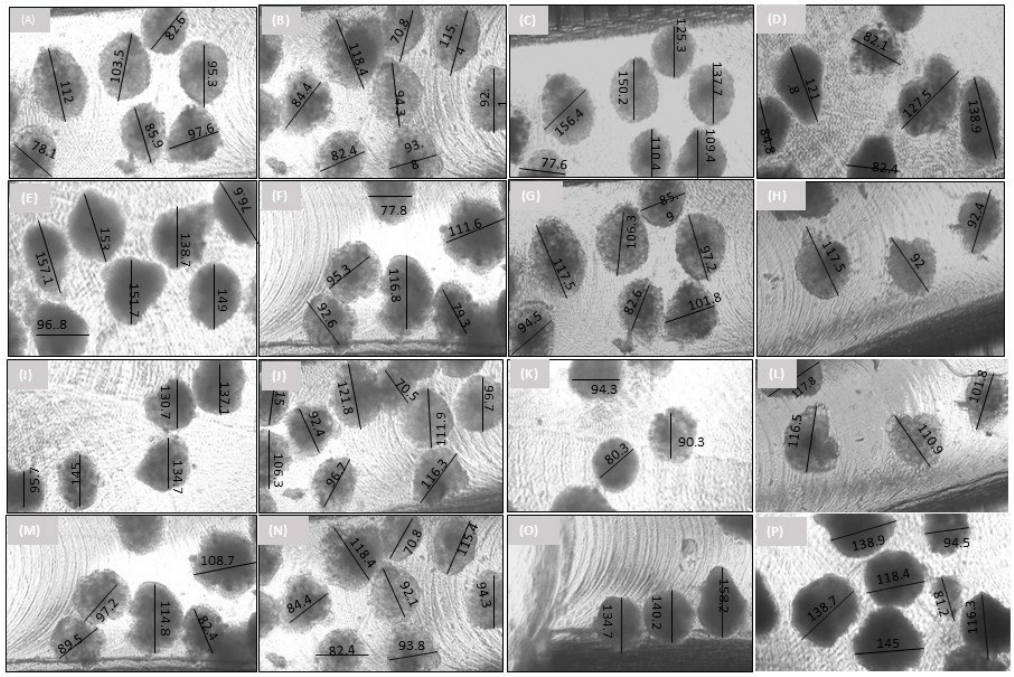


Figure S1. A-P. Neurospheres generated inside microfluidic chip: The size distribution of neurospheres grown in microfluidic chip. Scale bar 10X, 100μm.

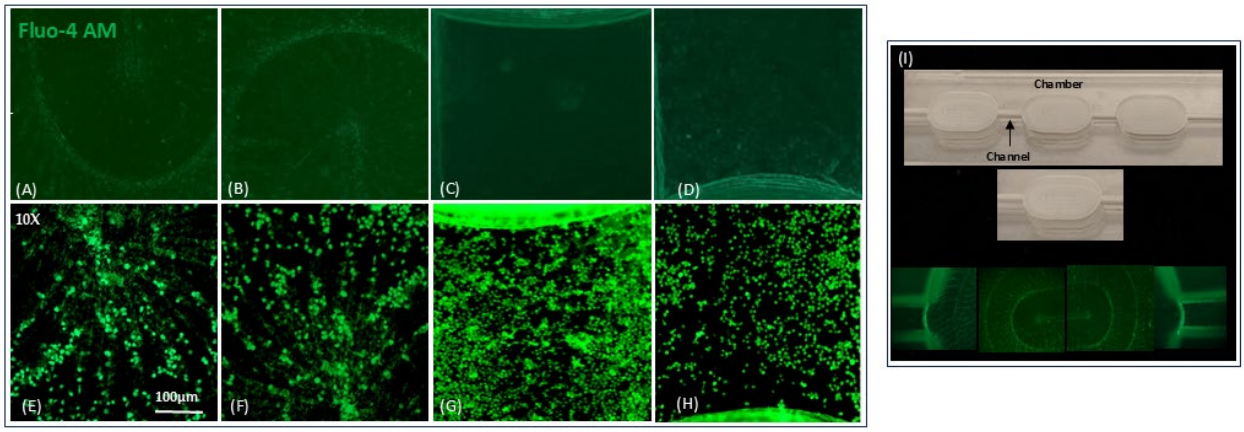


Figure S2. Staining of 2D cells on Microfluidic chip: (A), **(B)**, **(C)**, **(D**) Different area of microfluidic chip without cells stained with Fluo-4 AM for 20 minutes. **(E)**, **(F)**, **(G) and (H**) Different area of microfluidic chip loaded with cells stained with Fluo-4 AM (5μM) for 20 minutes. **(I)** Microfluidic chip where the chamber and channel of the chip are highlighted. Images were captured at 10X magnification, scale bar: 100 μm

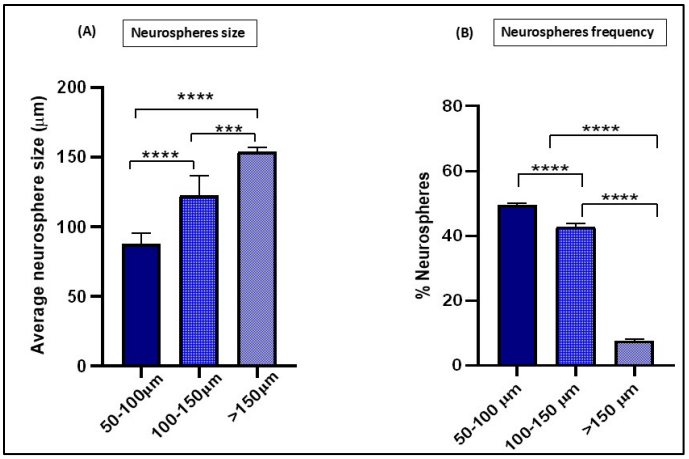


Figure S3. Size of primary 3D neurospheres generated from the microfluidic chip. (A) Quantitative analysis of the average size of neurospheres. Bar graph representing the statistical comparison of the average size of neurospheres using One-way ANOVA followed by Tukey's multiple comparisons test revealed significant differences among all groups: 50–100 μm vs. 100–150 μm: ****p = 0.0001, 50–100 μm vs. >150 μm: ****p = 0.0001, 100–150 μm vs. >150 μm: ***p = 0.0002, (N = 3 experiments, M=2 replicates). **(B)** Percentage distribution of neurospheres by size 50-100 μm, 100-150 μm, and >150 μm. One-way ANOVA followed by Tukey's multiple comparisons test revealed significant differences: 50–100 μm vs. 100–150 μm (****p = 0.0001), 50–100 μm vs. >150 μm (****p = 0.0001), 100–150 μm vs. >150 μm (****p = 0.0001). Data represent mean ± SEM . Asterisks signify **** p< 0.0001, *** p< 0.001, * p< 0.01.

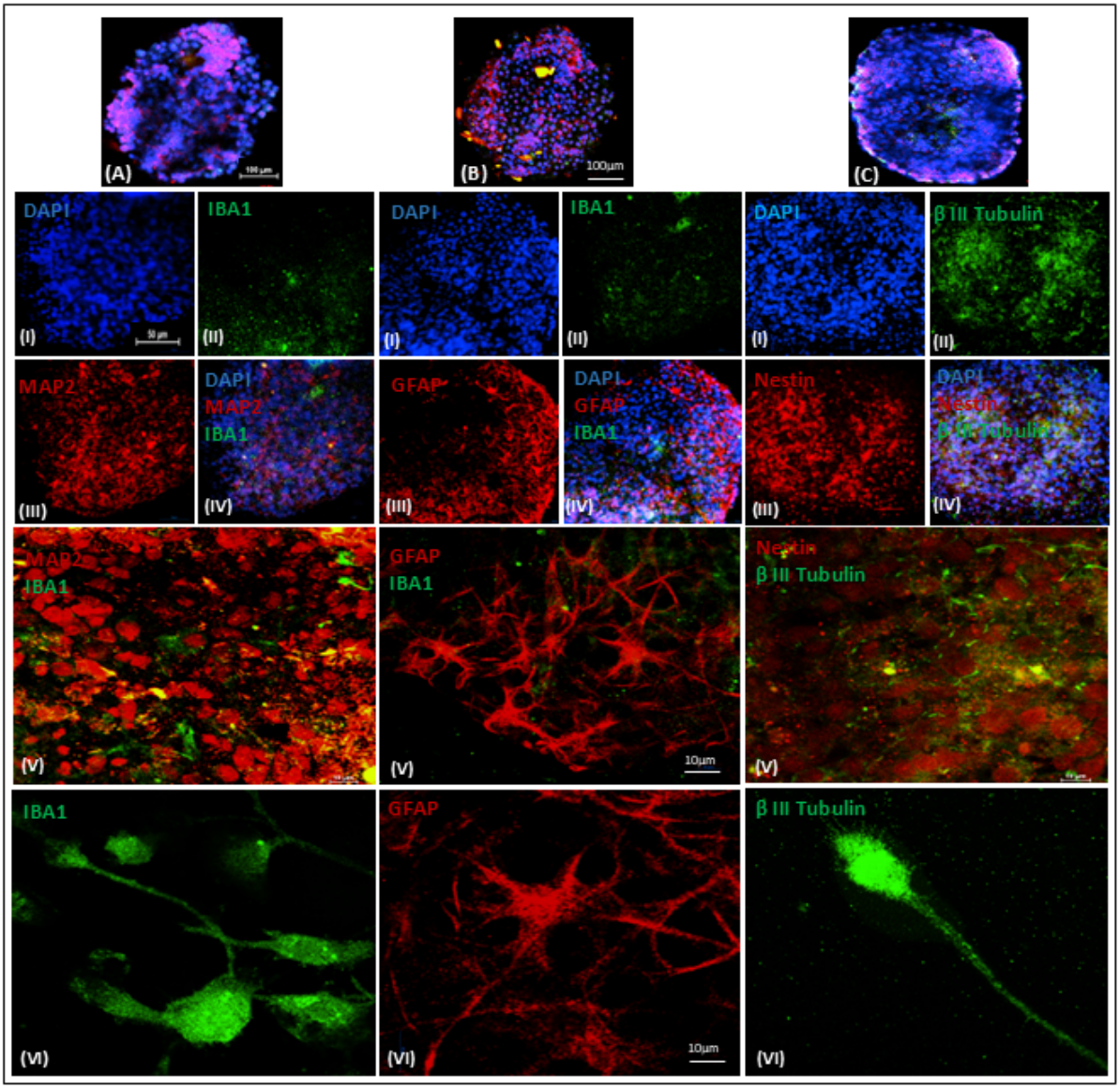


Figure S4. Immunocytochemical characterization of neurospheres generated from the microfluidic chip stained with lineage-specific markers: (A), (B), (C) Confocal images are from three independent neurospheres generated from the microfluidic chip (top-most, scale bar: 100 µm), images were captured at 20X magnification. Neurospheres were positive for neural stem/progenitor markers Nestin **(C)**, the neuronal marker β III Tubulin **(C)**, astroglial marker GFAP **(B)**, microglia marker IBA1 **(A, B)**, neuronal dendrites MAP2 **(A)**, DAPI (nuclei) in blue. The top panel **(I)** to **(IV)** are images of neurospheres with magnification 60X (top, scale bar: 50 µm). The middle panel **(V)** are magnified image of neurospheres captured at 60X magnification (middle, scale bar: 10 µm). The bottom panel **(VI)** is highly magnified image of neurospheres captured at 60X magnification (bottom, scale bar: 10 µm). The generated primary neurospheres comprise a mixed cell population characterized by immunolabelling.

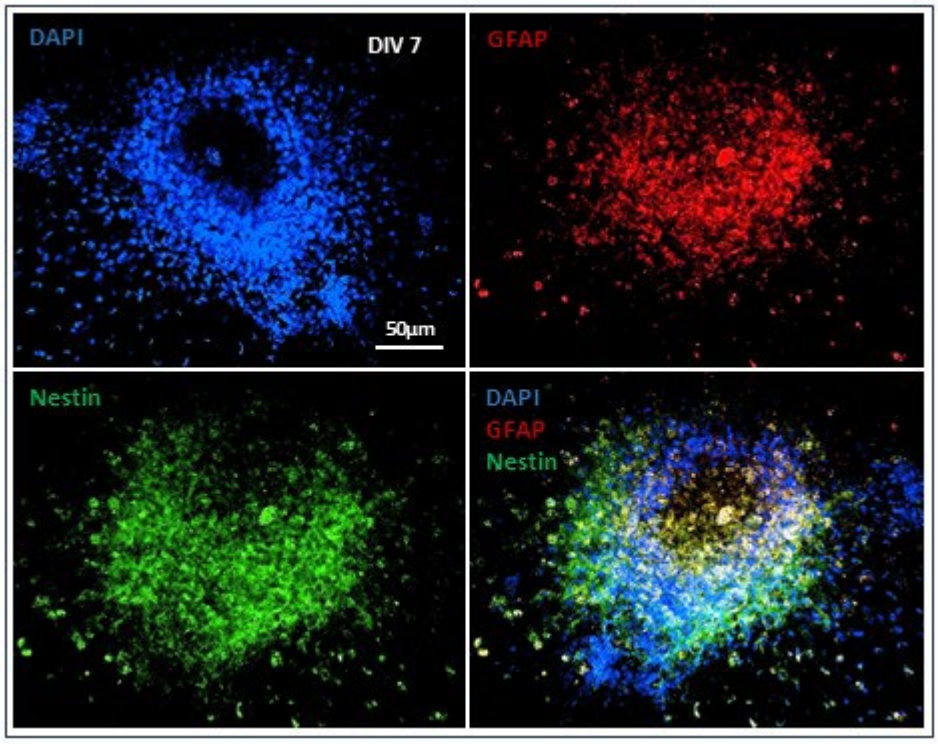


Figure S5. Immunocytochemical characterization of neurospheres differentiated on the microfluidic chip: (A), **(B)**, **(C)**, Fluorescence microscopy images of neurospheres differentiated on the microfluidic chip with DAPI (nuclei) in blue, GFAP (red) and Nestin (green) respectively. **(D)** The overall staining with both GFAP, Nestin along with DAPI, indicating cellular subtype composition of cultures. It reveals the presence of both differentiated astrocytes and residual progenitor populations within the neurospheres-derived culture. Images were captured at 20X magnification, scale bar: 50 µm.