# Supporting Information

# N-Cadherin Adhesive Ligation Regulates Mechanosensitive Neural Stem Cell Lineage Commitment in 3D Matrices

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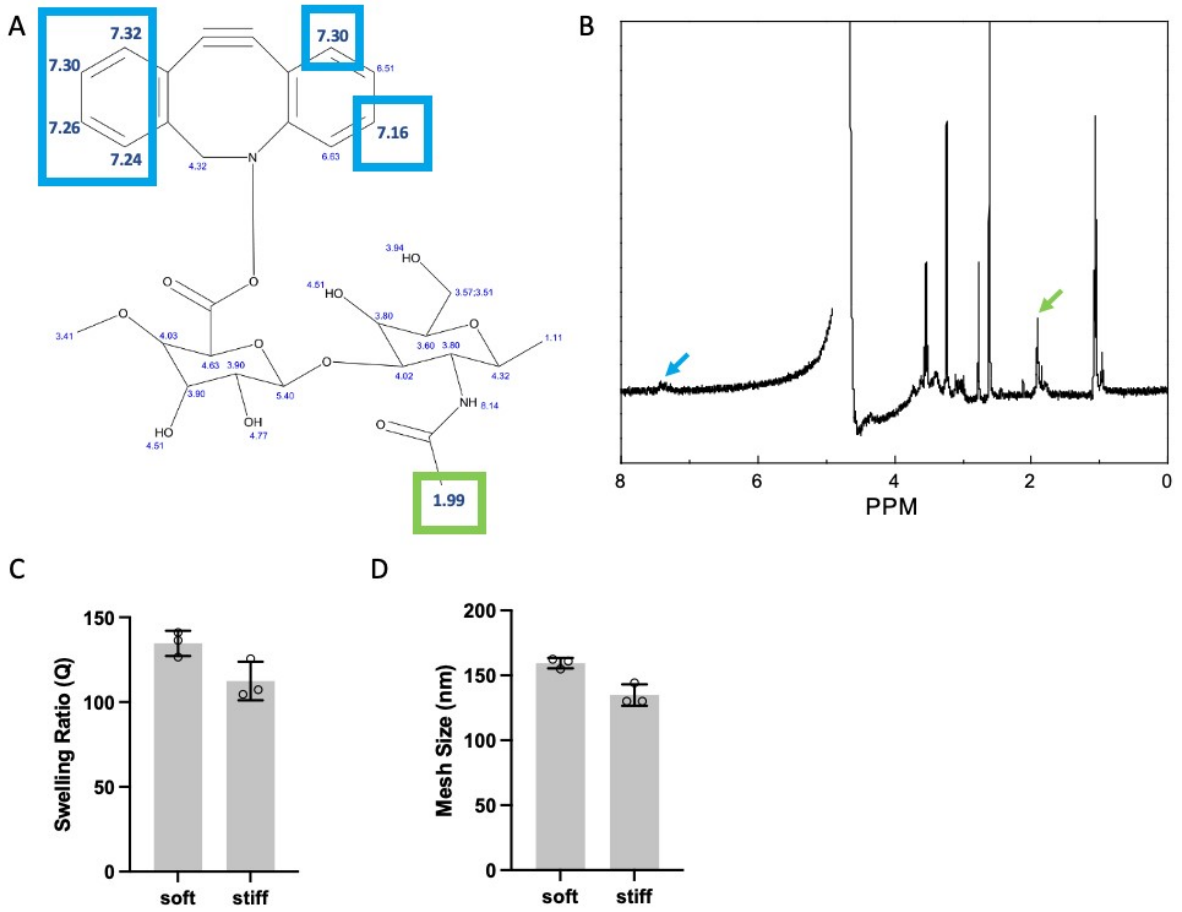


**Figure S1. A**, Chemical structure of DBCO-functionalized HA (HA-DBCO) with corresponding ppm values for each hydrogen in the structure. **B**, $^{1}$H Nuclear magnetic resonance (NMR) spectroscopy of the HA-DBCO. Blue and green square boxes & arrows indicate proton peaks present only in DBCO and HA chains, respectively. **C**, Swelling ratio and **D**, mesh size of soft (90 Pa) and stiff (600 Pa) hydrogels after swelling in DMEM overnight. n=3 technical replicates.

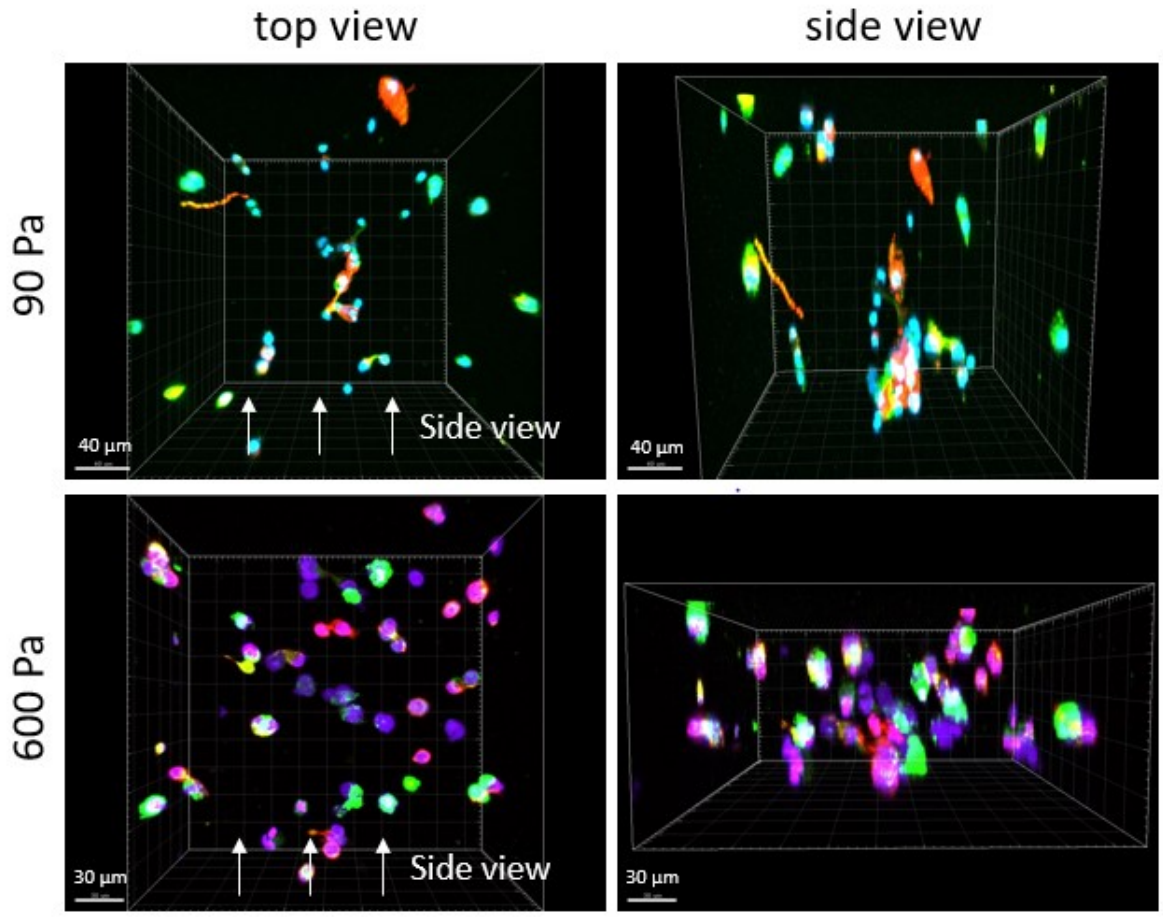


**Figure S2.** Immunofluorescence images (top view and side view) of hNSCs differentiated in 90-Pa and 600-Pa 3D HA hydrogels. The cells were stained for nuclei (DAPI, blue), neuronal (β-tubulin III, green) and astrocyte (GFAP, red) lineages. Scale bar is 30 $\mu$m and 40 $\mu$m for 90-Pa and 600-Pa gels, respectively.

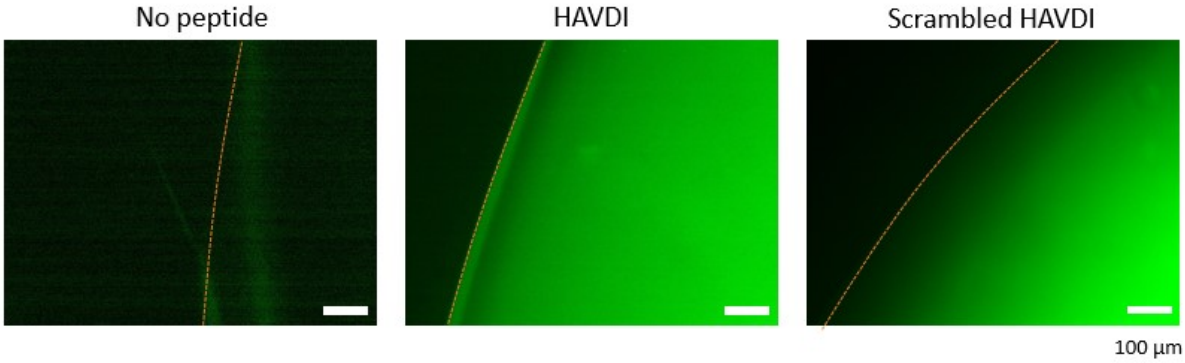


**Figure S3.** Fluorescence tagging of peptides (HAVDI and scrambled HAVDI) to visualize the peptide incorporation and localization in HA-DBCO gels.

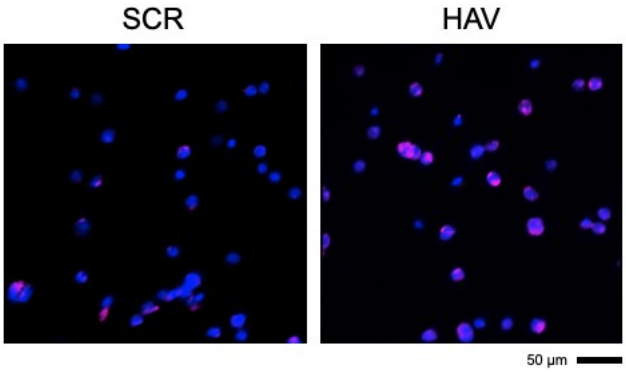


**Figure S4.** Immunofluorescent staining of N-cadherin in hNSCs encapsulated with SCR or HAV gels (200 Pa) after 24 hr. Scale bar, 50 μm.

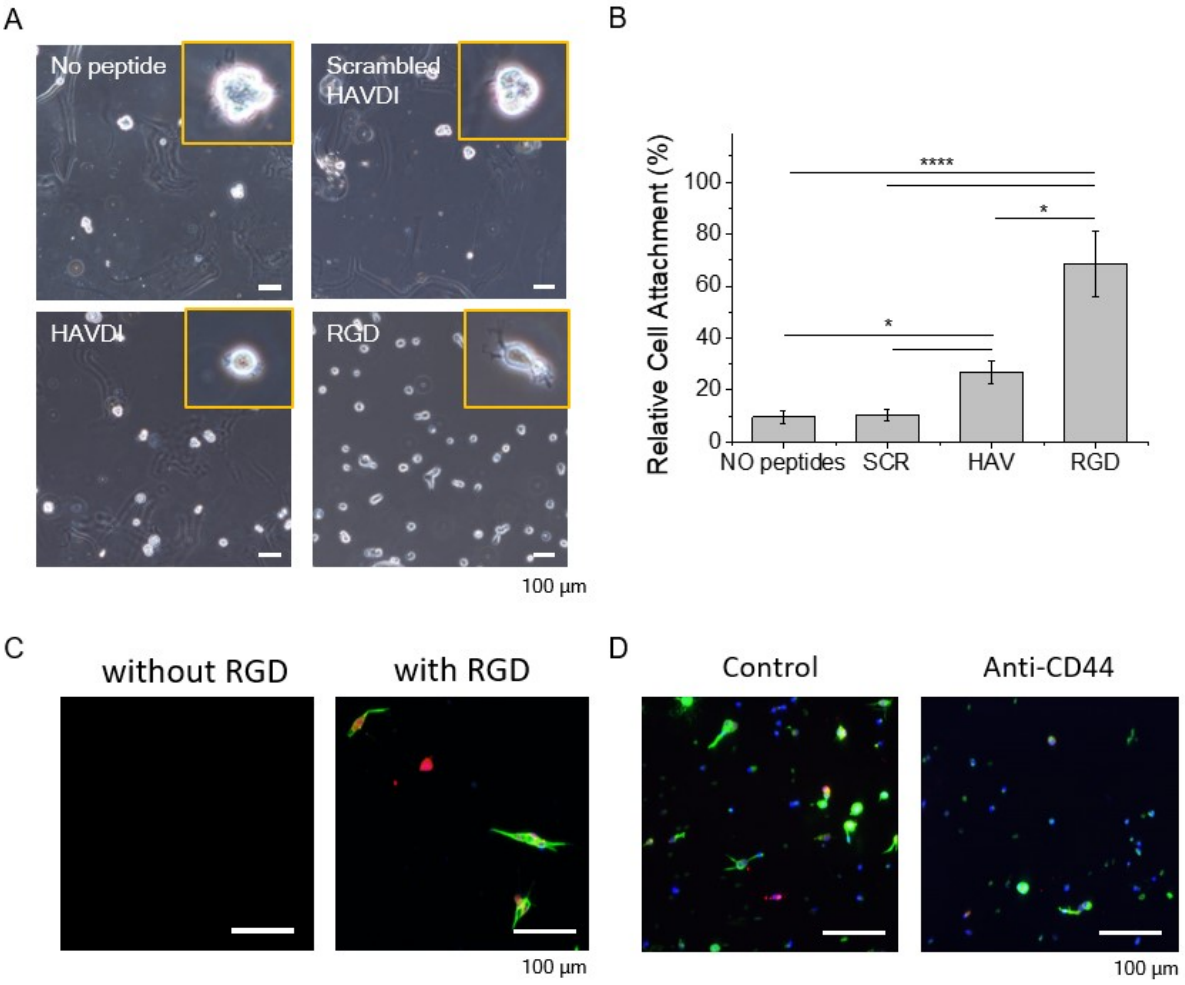


**Figure S5. A,** Bright field images and **B,** cell number quantification of hNSCs attached on each peptide (1 mM)-modified 3D gels: non-functionalized (no peptide), scrambled HAVDI, HAVDI, and RGD. The stiffness of each gel was maintained as 200 Pa, and the cells were washed with DMEM/F-12 for two times after 24 hr of adhesion. n=5 technical replicates with n=3 biological replicates. Scale bar, 100 μm. **C**, Live/Dead assay for the cells treated with anti-CD44 (CD44-blocked) on the 2D HA gels with and without RGD peptide. Live cells were stained with Calcein-AM (green) and dead cells were stained with EthD-1 (red). Scale bar, 100 $\mu$m. **D**, Immunofluorescence

images of control and CD44-blocked NSCs in 3D HA gels without RGD peptide. Stains are β-tubulin III (green), GFAP (red), and DAPI (blue). Scale bar, 100 $\mu$m.

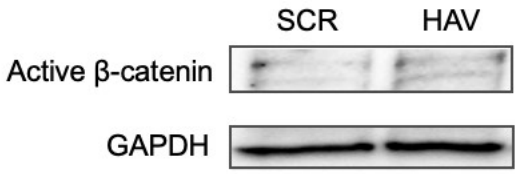


**Figure S6.** Western blotting for active β-catenin in hNSCs encapsulated with SCR or HAV gels (200 Pa) for 3 days.