

SUPPLEMENTARY DATA

Supplementary Materials and Methods

Screening Degradable (SGESPAY↓YTA) : Non-degradable (PEG-dithiol) cross-linker molar ratio (%)

After 14 days in culture, the viability of human neural stem cells (hNSC) within hydrogels cross-linked with different molar ratios (%) of cysteine-flanked matrix metalloproteinase-2 (MMP2)-sensitive peptide (SGESPAY↓YTA) and PEG-dithiol was assessed using a Live/Dead assay, performed according the protocol described in section 2.8. Average occupied area (μm^2) by live (Calcein⁺) cells was assessed in the projected two-dimensional (2D) images through the application of an Otsu thresholding method and determination of the total area occupied by cells in each image.

Cell viability - Immunocytometry

Quantitative analysis of cell viability was conducted by immunocytometry performed in six-pooled hydrogels, after hydrogel dissociation. Briefly, the hydrogels were sequentially incubated with 1.25 mg/mL of collagenase type II (Gibco; 1 h at 37 °C) and StemPro accutase cell dissociation reagent (Life Technologies; 20 min at 37 °C) under stirring (70 rpm). Cells were mechanically dissociated by pipetting, diluted in Glasgow Minimal Essential Medium (GMEM; Life Technologies) supplemented with 10% (v/v) inactivated fetal bovine serum (FBS) and centrifuged. For live/dead staining, cells were then transferred to a round-bottomed 96-well plate and stained with calcein AM (67 nM, 20 min at 37 °C) and propidium iodide (PI; 6 μM , 10 min at 37 °C). Cells were finally washed trice and suspended in FACS buffer (PBS pH 7.4 supplemented with 2% (v/v) FBS) for flow cytometry analysis on BD FACSCanto™ II (BD Biosciences). Unlabeled cells were used to set the fluorescence gates and cells stained with calcein AM and PI only were used to establish the compensation settings. For each flow cytometry analysis, 10,000 events were acquired inside the respective gate.

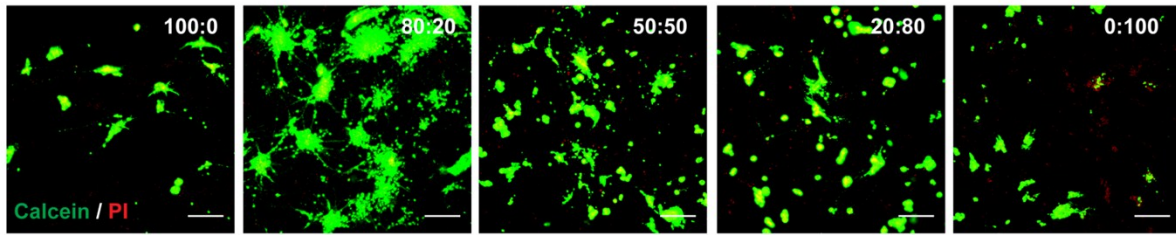
Neurite outgrowth & Phenotypic Analysis - Immunohistochemistry

The effect of laminin site-selective immobilization on hNSC neurite outgrowth and phenotype was assessed after 14 days of cell culture, in samples processed for different intracellular markers. Cell-laden hydrogels were fixed in 3.7% (w/v) paraformaldehyde (PFA) solution diluted 1:1 in culture media (30 min; 37 °C) and permeabilized with 0.2% (v/v) Triton X-100 in PBS (45 min; room temperature (RT)). Samples were then incubated with blocking buffer (5% (v/v) bovine serum albumin (BSA) in PBS) for 1 h at RT, followed by incubation with the primary antibody – mouse anti- β III-tubulin (1:500; Biolegend, 801201); mouse anti-Nestin (1:200; Abcam, AB22035); mouse microtubule-associated protein 2 (MAP2; 1:200; Invitrogen,

13-1500); rabbit anti-Tau (1:100; Sigma-Aldrich, T6402), overnight (ON) at 4 °C. To detect primary antibodies, samples were incubated with Alexa Fluor® 488 conjugated anti-rabbit secondary antibody (1:1000; Life Technologies, A21206) or Alexa Fluor® 594 conjugated anti-mouse secondary antibody (1:1000; Life Technologies, A11020) ON at 4 °C. The nuclei were counterstained with 0.1 µg/mL Hoechst 33342 (Life Technologies, H1399) for 20 min at RT. Samples were observed under confocal laser scanning microscopy (CLSM; TCS SP5II; Leica Microsystems).

Supplementary Figures

A



B

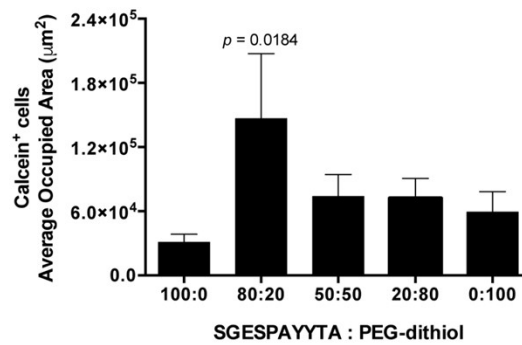


Figure S1. Optimization of MMP2-sensitive peptide (SGESPAY↓YTA): PEG-dithiol molar ratio (%) in affinity-bound laminin PEG-4MAL hydrogels (50 μM NtA; 20 μg/mL rhLn-521 (Biolamina)). **A)** Representative 2D projections of CLSM three-dimensional (3D) stack images of cell-laden affinity-bound laminin PEG-4MAL hydrogels at day 14 of cell culture covering a thickness of 200 μm, showing the distribution of live (green) and/or dead (red) cells. Scale Bar = 200 μm. **B)** Calcein⁺ cells average occupied area (μm²) after 14 days of culture, as determined by quantitative image analysis of 2D projections of CLSM 3D stack images of cell-laden hydrogels incubated with calcein AM (viable cells) and PI (dead cells). Data represent mean ± standard error of the mean (SEM) of 6-9 images from two independent experiments; one-way ANOVA followed by Dunnett's test (vs. 100:0)

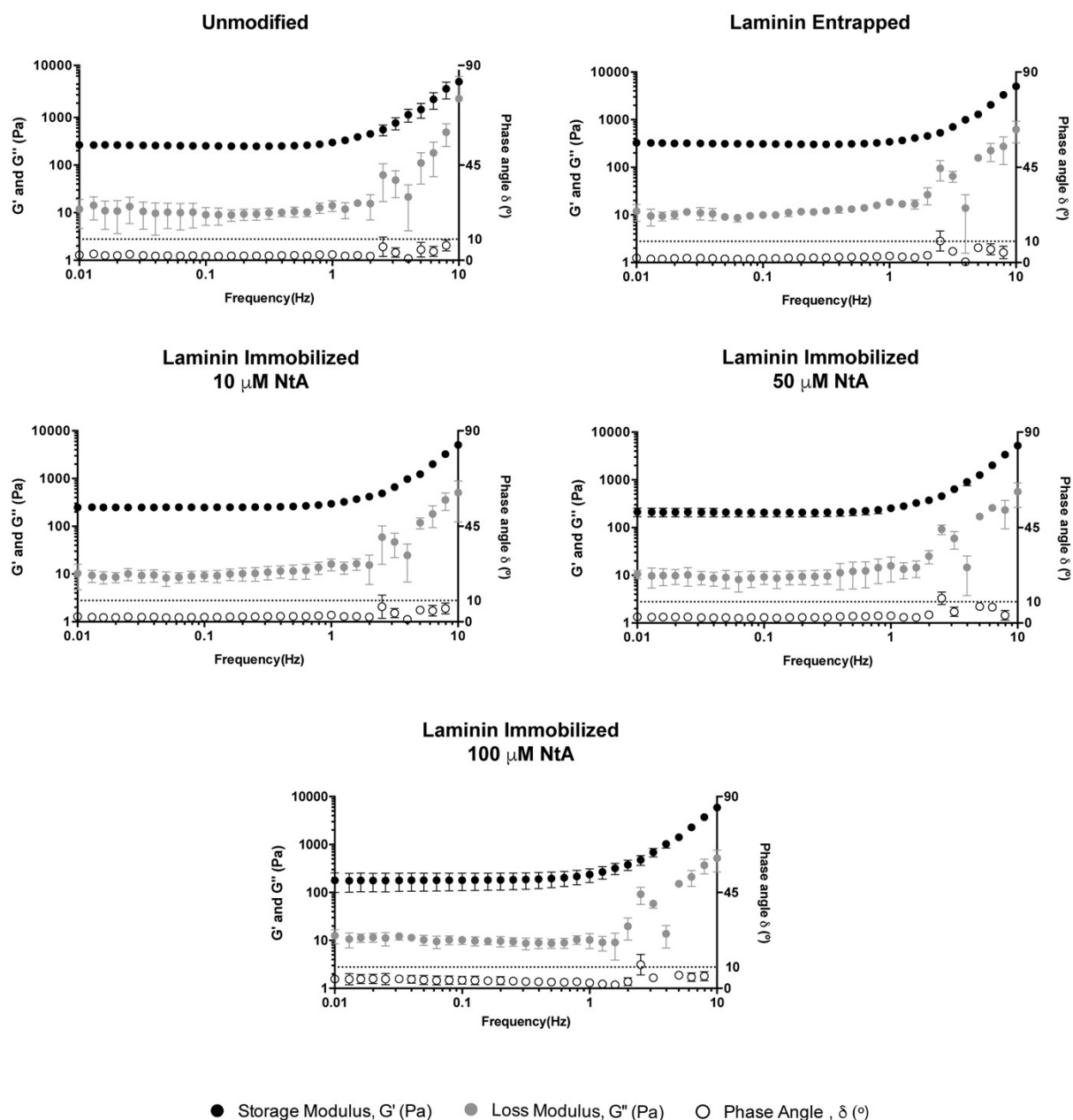


Figure S2. Frequency sweep analysis of unmodified, physically entrapped laminin and affinity-bound laminin (10, 50 and 100 μ M NtA) hydrogels after stabilization in PBS (pH 7.4) for 24 h. The analysis was performed within a frequency range of 0.01 – 10 Hz, at a constant strain of 1% and 37 °C. Data represent mean \pm standard deviation (SD); n = 6.