ADVANCED MATERIALS

Supporting Information

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Stoichiometric Post-Modification of Hydrogel Microparticles Dictates Neural Stem Cell Fate in Microporous Annealed Particle Scaffolds

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Stoichiometric post modification of hydrogel microparticles dictates neural stem cell fate in microporous annealed particle scaffolds

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Supplemental Experimental section/Methods

Generation of Nonporous Scaffolds and Mechanical Testing: Nonporous HA-NB hydrogels were made from the same precursor of HA-NB for making MAP scaffolds. 50 uL of the solution was placed between two sigma coated slides with a 1mm spacer and crosslinked by exposure to 20mW/cm2 of UV light for 2 min. The gels were then allowed to swell overnight in PBS buffer. ALL (RGD (1 mM), IKVAV high (0.3 mM) and YIGSR (0.048 mM)) or YIGSR(0.048mM) only peptides were then added to the gels as well as 0mM, 10mM or 50mM LAP with 2 min of 20mW UV irradiation. After exposure the gels were washed 3x with PBS buffer. A frequency sweep was then performed on each gel at a constant 1% strain, angular frequency of 0.1 to 10 rad at 0.1 rad/s. The raw storage modulus (**Figure S10a**) was plotted as well as the averages (**Figure S10b)** to compare 0 mM + YIGSR group to 10 mM + YIGSR (to see if there was an effect adding LAP and UV exposure) and 10 mM + ALL. There was no significant difference in the storage modulus between groups (**Figure S10b**). We also tested exposure to 50 mM LAP to investigate extreme conditions. We observed an increase in LAP exposure of 50 mM leads in a significant increase in storage modulus (**Figure S10c**). We tested this out with increase LAP without the addition of any crosslinking or peptides and similarly at 50mM to 100mM we began seeing an increase in storage modulus (data not shown). However, this is not observed at the 10mM concentrations we used in the experiment. Therefore, it is likely the increase in moduli is due to unreacted NB groups forming new crosslinking between one another in exposure to excess of free radicals and not in response to the addition of any peptides or left over crosslinker.

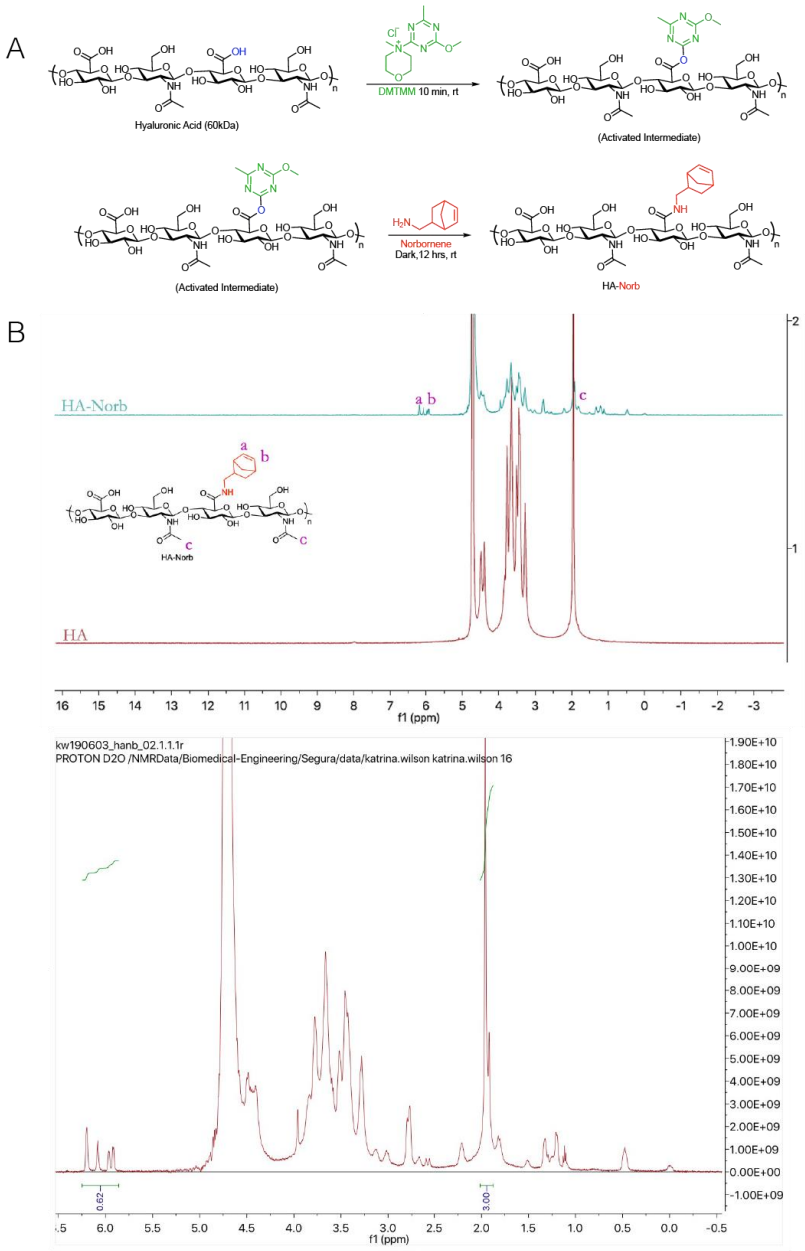

Figure S1. HA modification with NB. A) Schematic of modification. B) H' NMR analysis of modified HA.

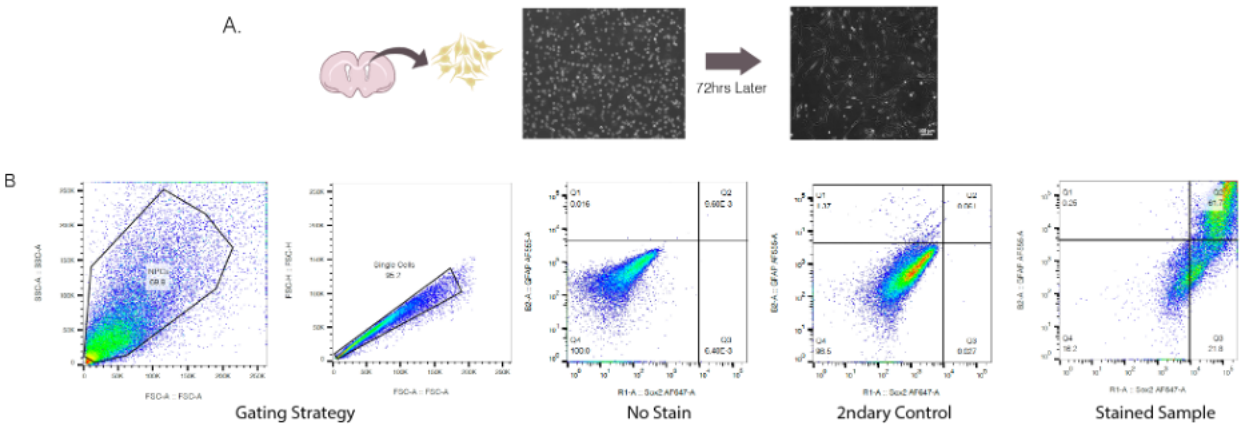

Figure S2. A) Schematic of NPC isolation B) Gating strategy for NPCs. A) NPCs were stained with SOX2 and GFAP.

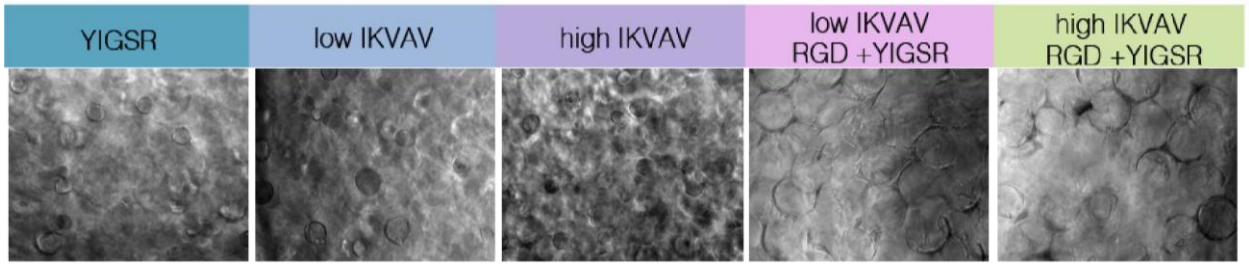

Figure S3. Control D7. Peptides were made without thiol or tetrazine handles. Only RGD was left with an available thiol for crosslinking. We observe no spreading in conditions without RGD
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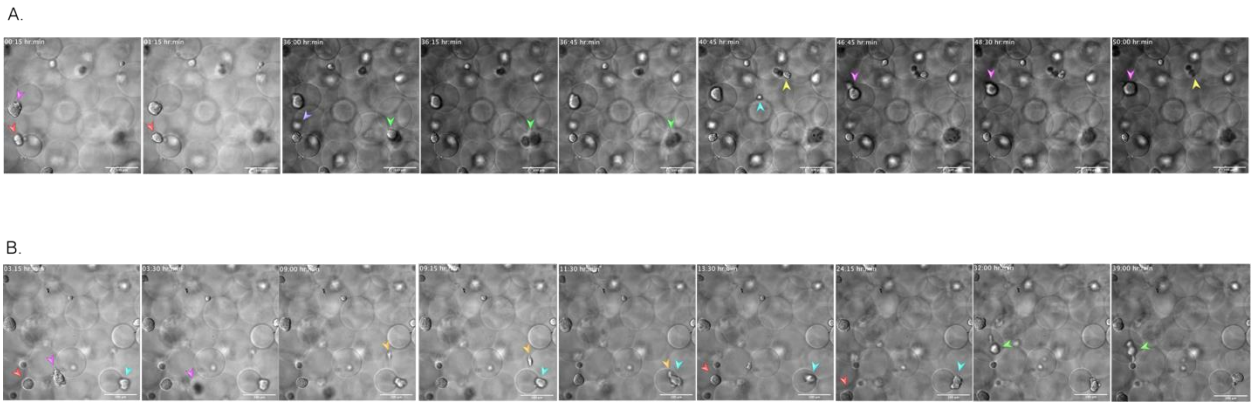

Figure S4. Neurosphere migration within YIGSR scaffold over 2 days. A) Images taken from supplemental video 5. B) Images taken from supplemental video 6. Arrows indicate neurosphere that will either move in the xy or z direction or fuse with another neurosphere.

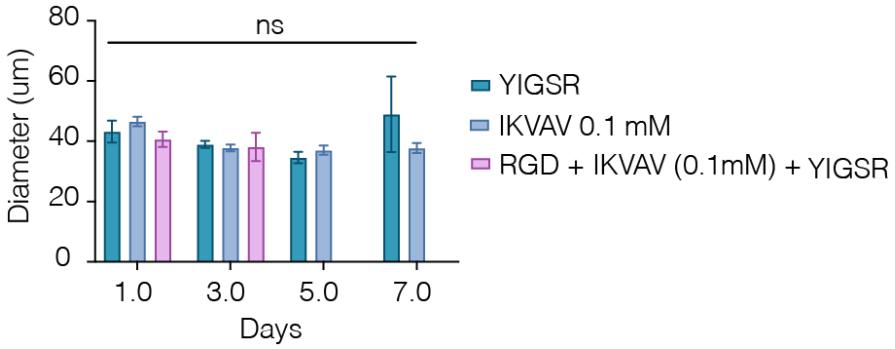

Figure S5. Neurosphere diameter measurements in all conditions over 7 days.

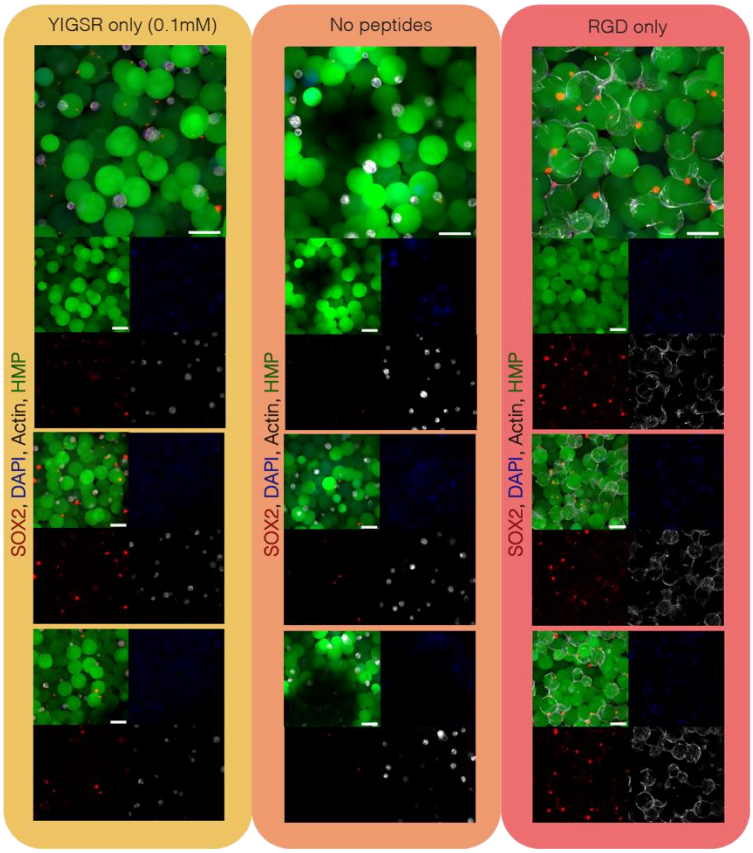

Figure S6. Confocal images of *in vitro* D7 high [YIGSR] only, no peptide, and RGD only conditions. Same batch of cells, technical replicates. Scale bar 100 μm.

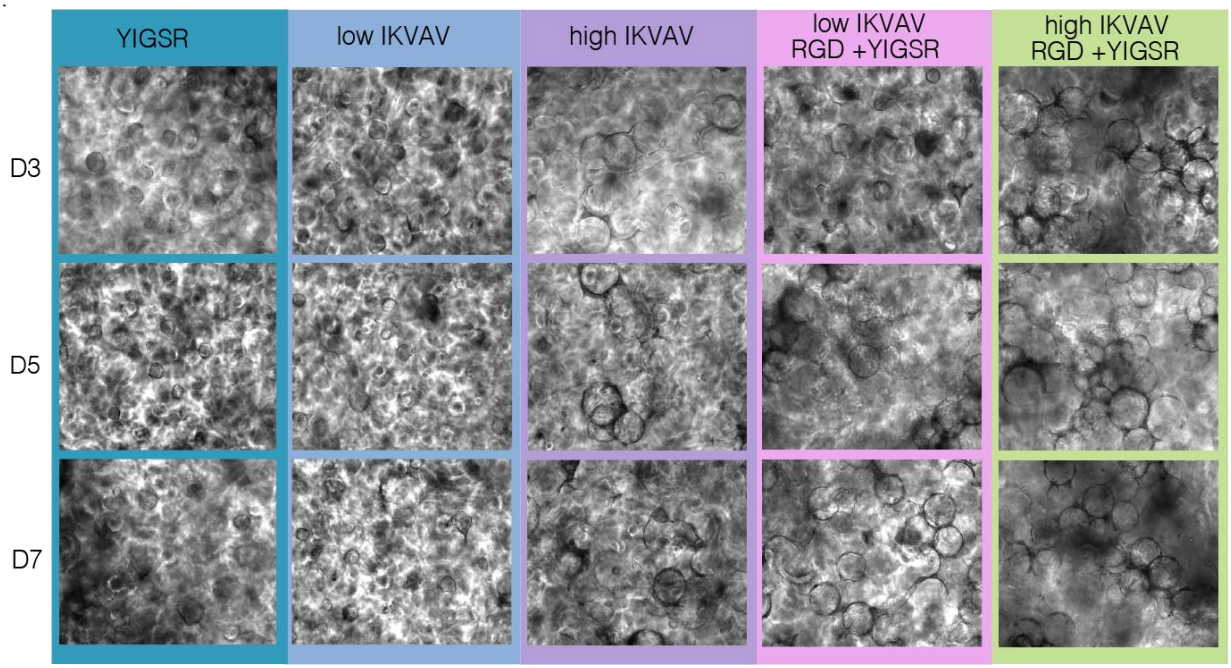

Figure S7. Phase images of all groups from D3 to D7.

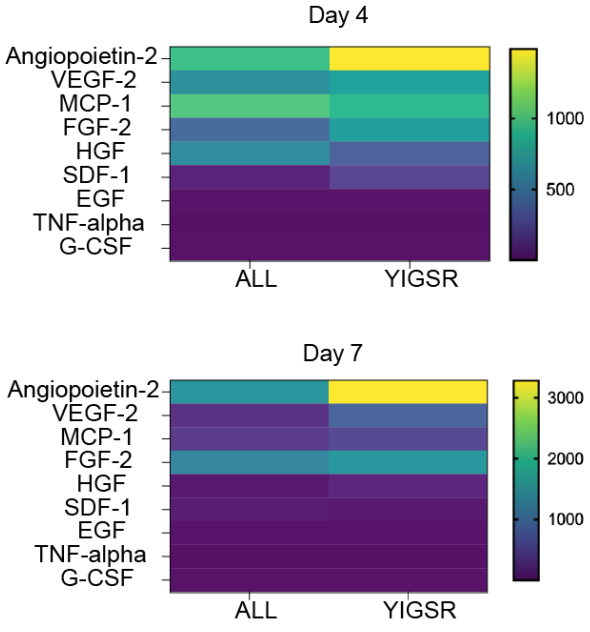

Figure S8. Heat map of luminex multiplex data. No significant difference.

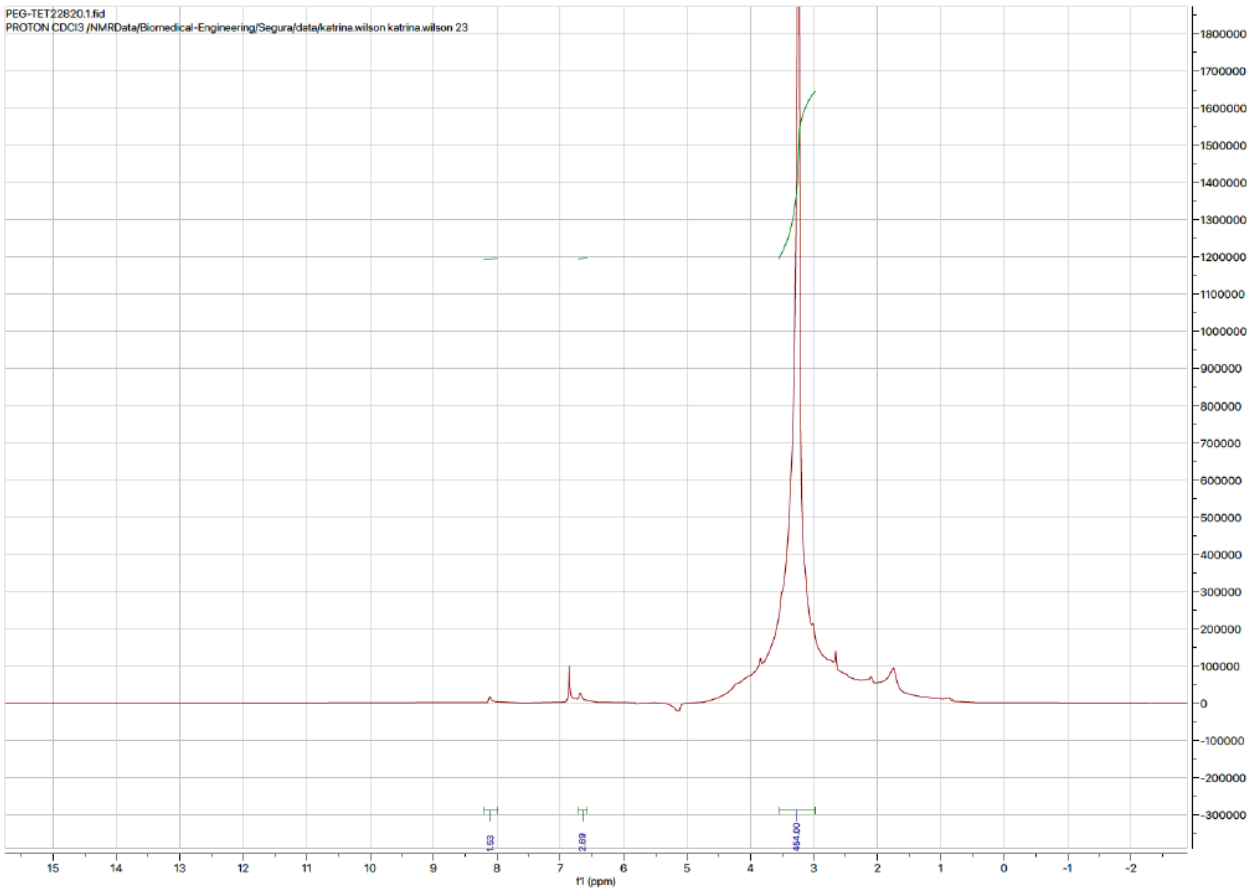

Figure S9. H'NMR of PEG-Tet

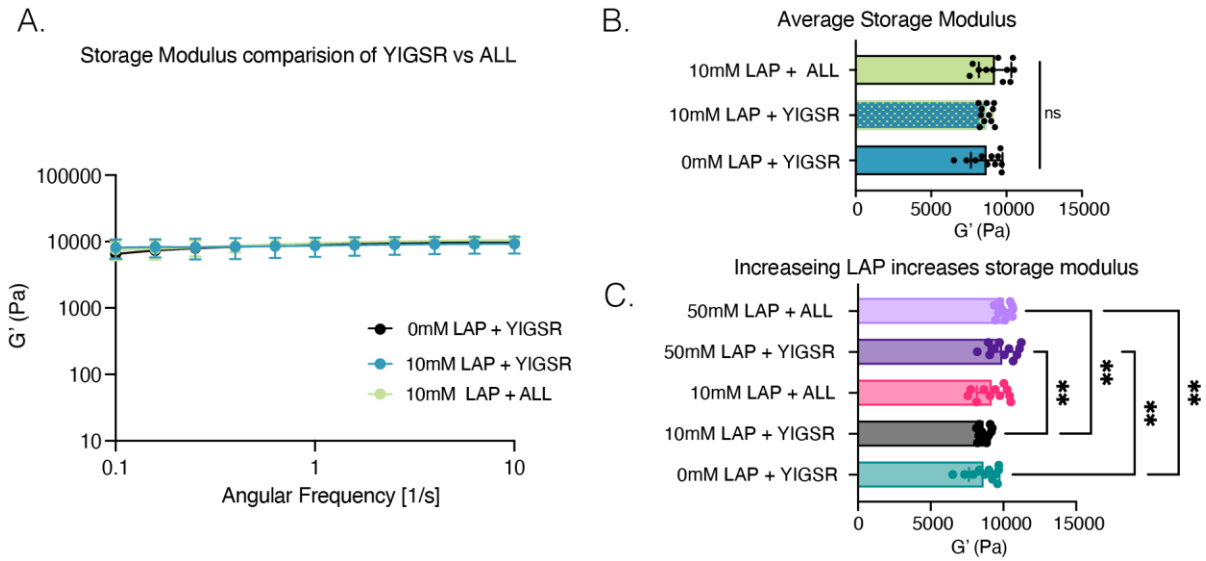

Figure S10. Rheological data of Nonporous HANB at different concentrations of LAP