

## Supporting Information

### Response to Di-functionalized Hyaluronic Acid with Orthogonal Chemistry Grafting at Independent Modification Sites in rodent models of neural differentiation and spinal cord injury..

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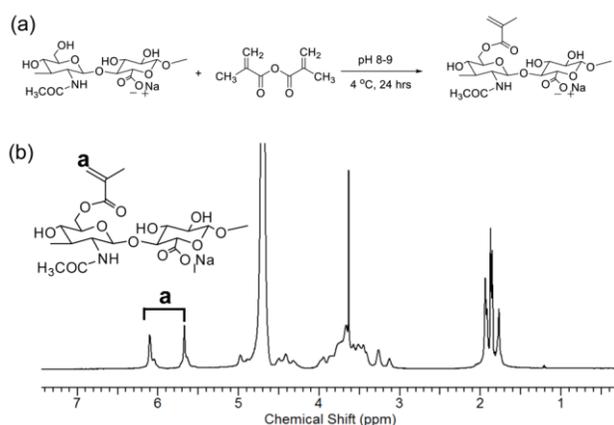
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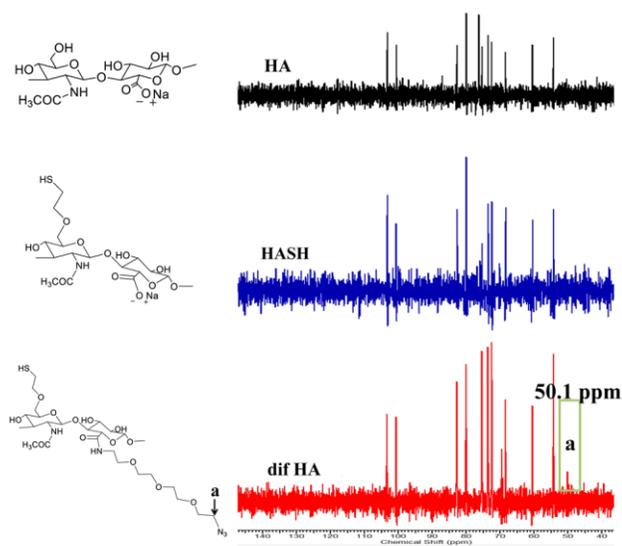
# indicates both authors contributed equally to this work

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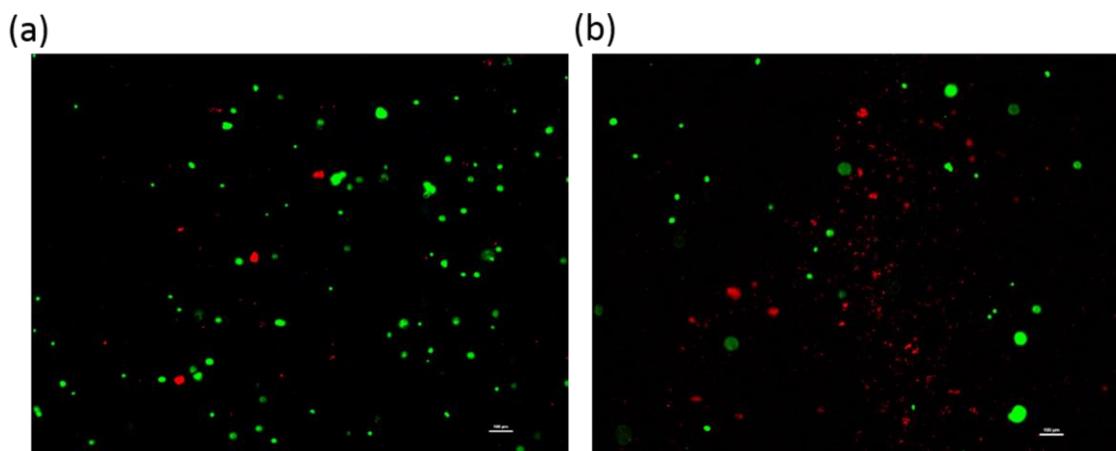
KEYWORDS: hyaluronic acid, thiol, azide, mouse embryonic stem cells, neural differentiation, hydrogel



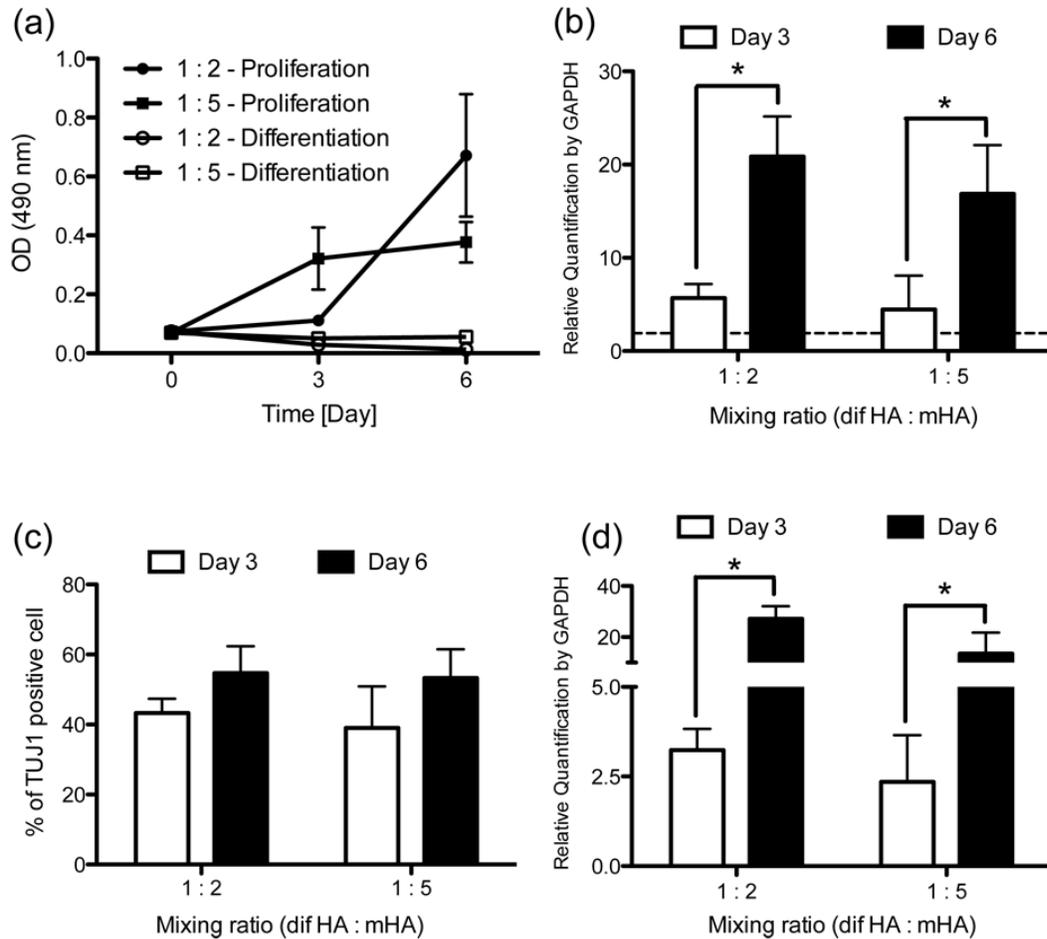
**Figure S1.** Methacrylated hyaluronic acid (mHA) modification and characterization (a) Scheme of hyaluronic acid methacrylation and (b) <sup>1</sup>H-NMR of mHA. Peaks at 5.6 and 6.1 ppm on <sup>1</sup>H-NMR spectrum indicate the presence of a double bond on the hyaluronic acid backbone.



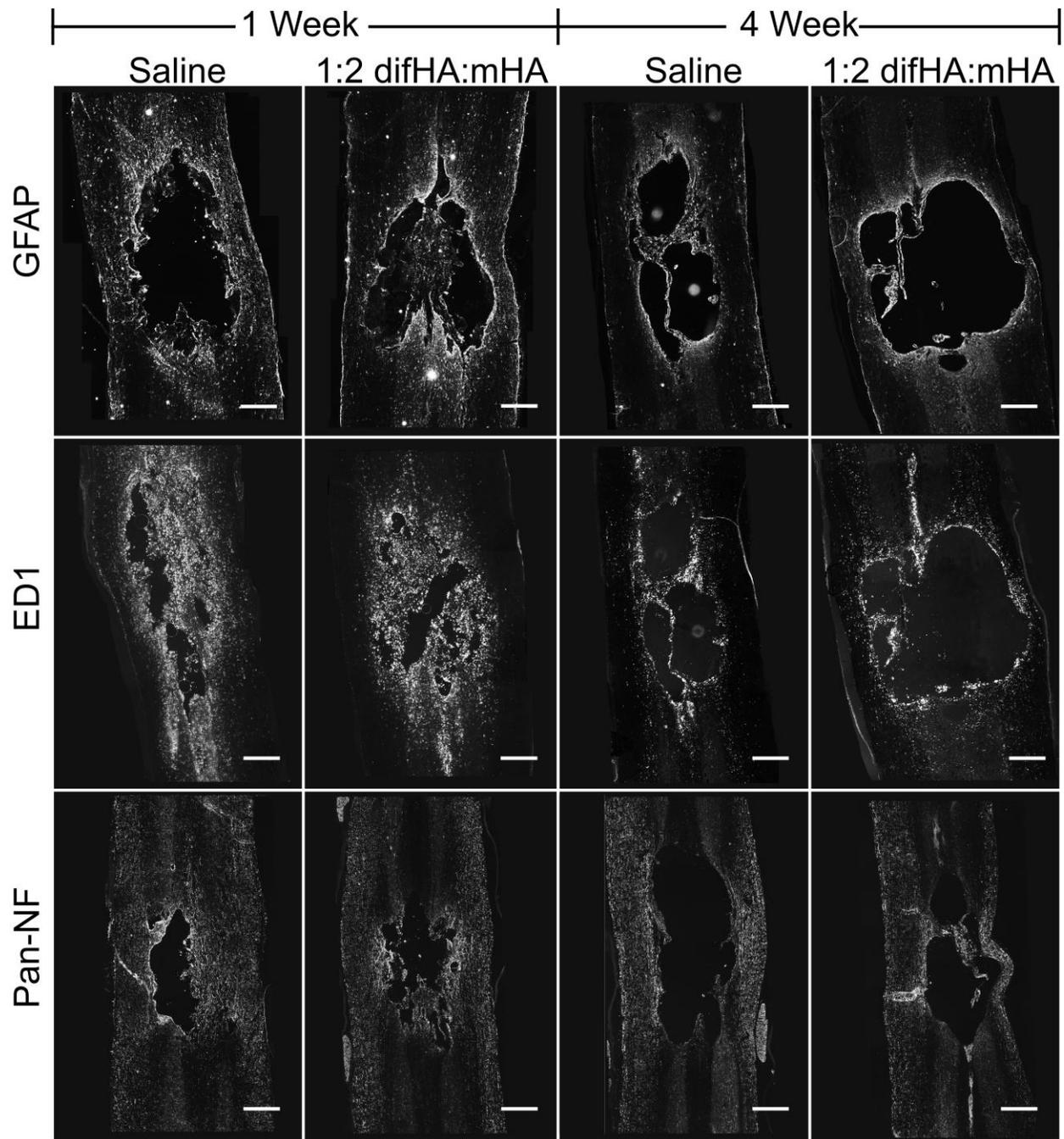
**Figure S2.** Characterization of azide group on hyaluronic acid (HA), HA-sulphydryl (HASH), and di-functional HA (dif HA) by  $^{13}\text{C}$ -NMR. The azide peak was presented at 50.1 ppm on the  $^{13}\text{C}$ -NMR on only the dif HA group.



**Figure S3.** Viability staining (live=green and dead= red) of mouse embryonic stem cells (mES) after 24hr of encapsulation in 1:2 (a) and 1:5 (b) di-functional hyaluronic acid (dif HA)/methacrylate hyaluronic acid (mHA) hydrogels. Scale bar=100  $\mu\text{m}$ .



**Figure S4.** Biological properties of di-functional hyaluronic acid (dif HA)/methacrylate hyaluronic acid (mHA) hydrogels composed of various mixing ratios. (a) Proliferation curves of HA hydrogels by MTS assay, (b) PAX6, early stage marker for neural differentiation, gene expression of mouse embryonic stem cells (mES) in dif HA/mHA hydrogel at Day 3 and 6 of neural differentiation (n=3). (c) Percentage of cells staining positive for  $\beta$ -tubulin III, an early stage marker for neural differentiation, in dif HA/mHA hydrogels at Day 3 and 6 of neural differentiation (n=5), and (d)  $\beta$ -tubulin III gene expression of mES in dif HA/mHA hydrogels at Day 3 and 6 of neural differentiation (n=3). \* indicates a  $p < 0.05$ .



**Figure S5.** Representative images of GFAP, ED1 and pan-axonal neurofilament (PanNF) staining of the lesion area 1 and 4 weeks after injection into a spinal cord, which corresponds to 3 and 6 weeks after the initial contusion injury. Scale bar= 500 $\mu$ m.