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Supplementary Materials for

Implantation of hyaluronic acid hydrogel prevents the pain phenotype in a rat model of intervertebral disc injury

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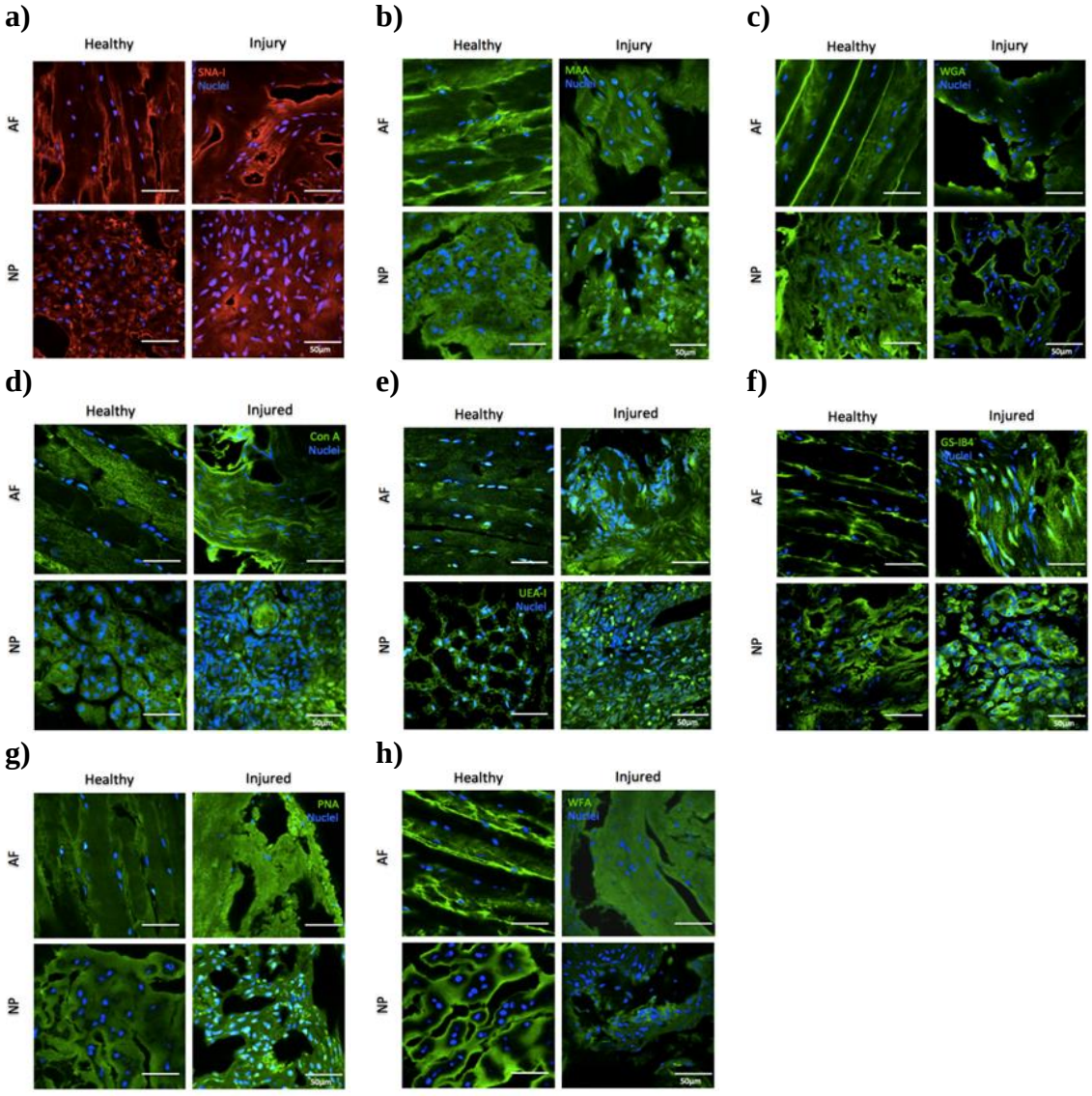


fig. S1. Confocal microphotographs of glycan expressions in IVD at post-injury day 29. **(a–c)** Detection of sialylation. SNA-I lectin binds α-(2,6)-linked sialic acid, MAA binds α-(2,3) linked sialic acid, and WGA binds GlcNAc and sialic acid in nucleus pulposus (NP) tissue in injured and uninjured rats. **(d)** Binding of Con A lectin (indicating mannose) was determined in annulus fibrosus (AF) and NP tissues in injured and uninjured rats. **(e)** Binding of UEA-I (which binds to α-(1,2)-linked fucose) was observed in AF and NP tissues. **(f,g)** PNA binds to non-sialylated Gal-β-(1,3)-GalNAc, and GS-I-B4 binds to terminal α-galactose was expressed in AF and NP tissues. **(h)** WFA binds to either α-linked or β-linked terminal GalNAc, including that in chondroitin sulfate (n = 3).

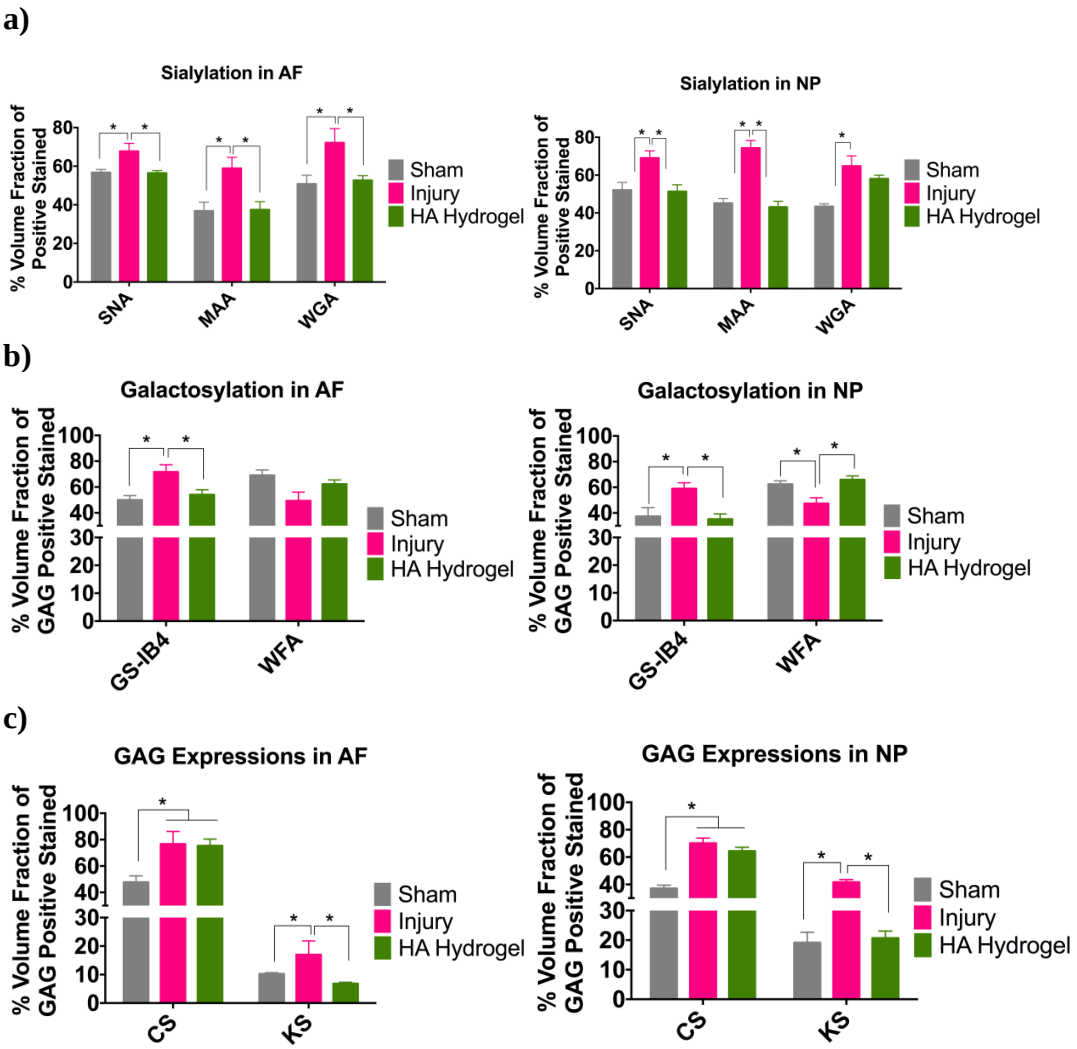
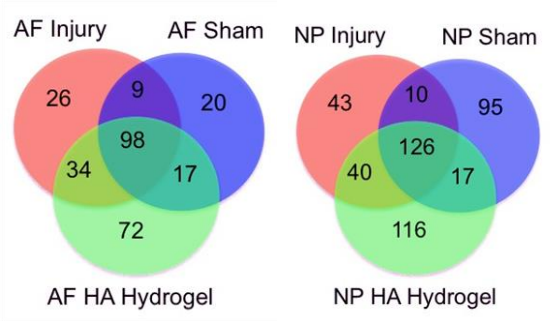


fig. S2. Glycosignatures of IVD post-implantation of HA hydrogel at day 29. (a) SNA-I and MAA binding to α-(2,6)-linked sialic acid and α-(2,3) sialylated galactose respectively, was significantly higher in the untreated injury group than in the sham control or the HA-hydrogel-treated injury groups, in annulus fibrosus (AF) and nucleus pulposus (NP) tissues. Binding of WGA to N-acetyl-D-glucosamine or sialic acid was not significantly affected by implantation of the HA-hydrogel. (b) GS-I-B4 binding to α-galactose was significantly higher in the untreated injury group compared to the sham control or HA-hydrogel-treated injury groups, in AF and NP tissues. WFA binding to terminal GalNAc motifs was significantly lower in the untreated injury group than in the sham control or HA-hydrogel-treated injury groups, in NP tissues. (c) Chondroitin sulfate expression was significantly higher in the untreated injury and HA-hydrogel-treated groups than in the sham control group, in AF and NP tissues. Keratan sulfate expression was significantly higher in the untreated injury group compared to the sham control group in NP tissues, or the HA-hydrogel-treated injury groups, in AF and NP tissues. *Significant differences between groups, by one-way ANOVA (n = 4, p <0.05). Area fraction data were normalized to the total area and are presented as volume fraction and represented as the mean ± standard error of the mean.

a)



b)

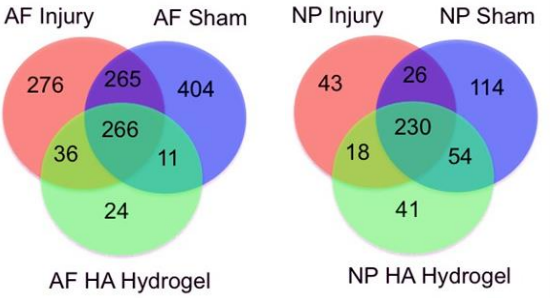


fig. S3. Number of extracted proteins in the disc. Venn diagrams showing the numbers of proteins extracted with **(a)** proteinase K digestion (of ECM) and **(b)** trypsin digestion of cells and extracellular matrix, in annulus fibrosus (AF) and nucleus pulposus (NP) tissues of the sham control, intervertebral disc injury and HA-hydrogel-treated injury groups.

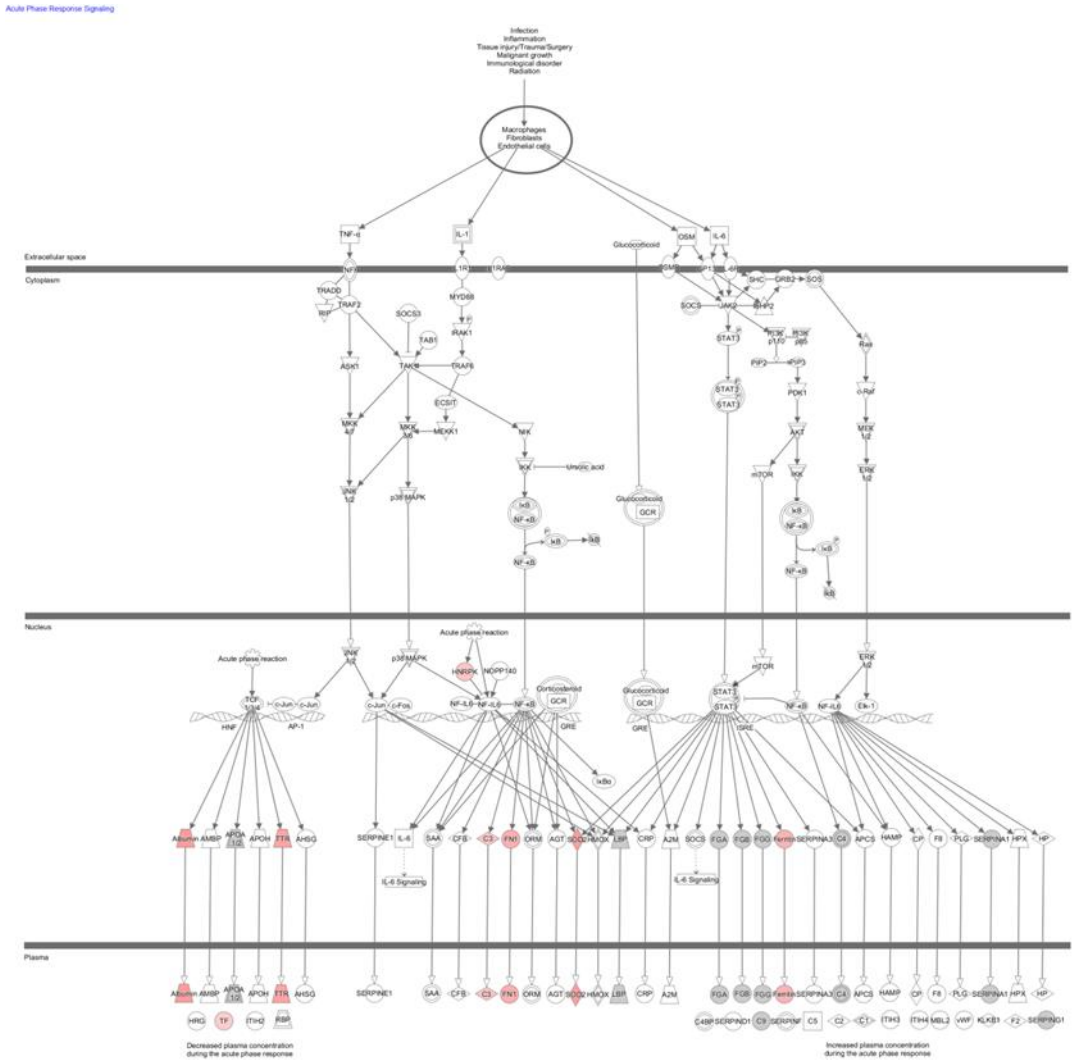


fig. S4. IPA revealed canonical "acute-phase signaling" in AF and NP tissues from the data set of differentially expressed proteins in our experimental groups. Red symbols indicated activation of acute phase proteins from injured tissue that were presented in the signaling pathways associated with inflammation, pain and dysregulation of matrix.

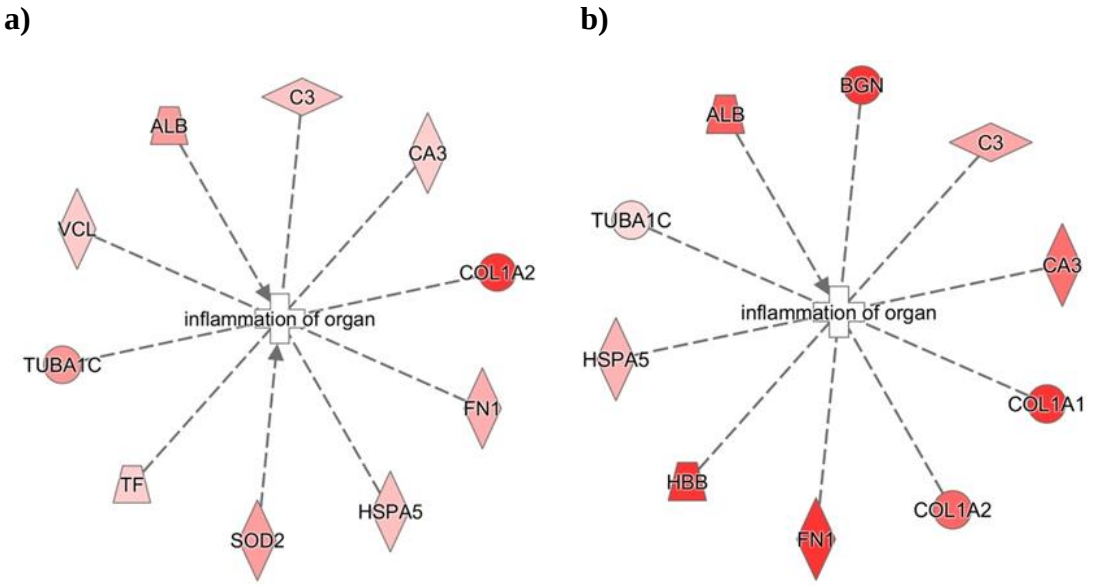


fig. S5. Inflammation network in the injured disc analyzed by IPA. Inflammation networks in the injured **(a)** annulus fibrosus (AF) and **(b)** nucleus pulposus (NP) tissues, determined by Ingenuity Pathway Analysis (IPA) of the dataset of differentially expressed proteins in our experimental groups.

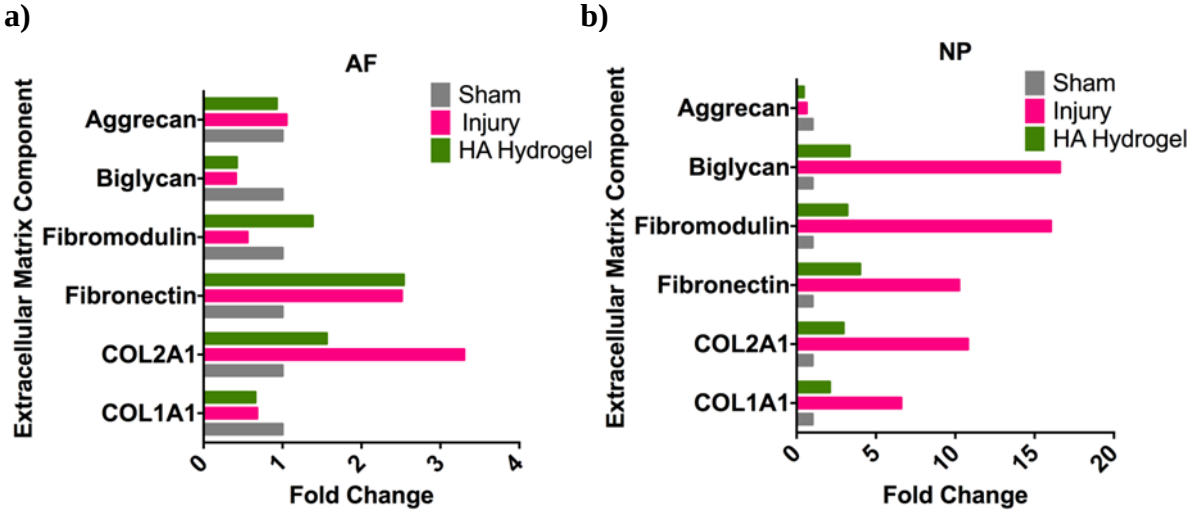


fig. S6. Dysregulation of ECM proteins in the disc. Dysregulation of ECM proteins in sham control, intervertebral disc injury and HA-hydrogel-treated injury groups in **(a)** annulus fibrosus (AF) and **(b)** nucleus pulposus (NP) tissues on post-operation day 29. Abbreviations: COL1A1, α1 type I collagen; COL2A1, α1 type II collagen.