ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Neural Progenitor Cells Alter Chromatin Organization and Neurotrophin Expression in Response to 3D Matrix Degradability

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Supporting Information

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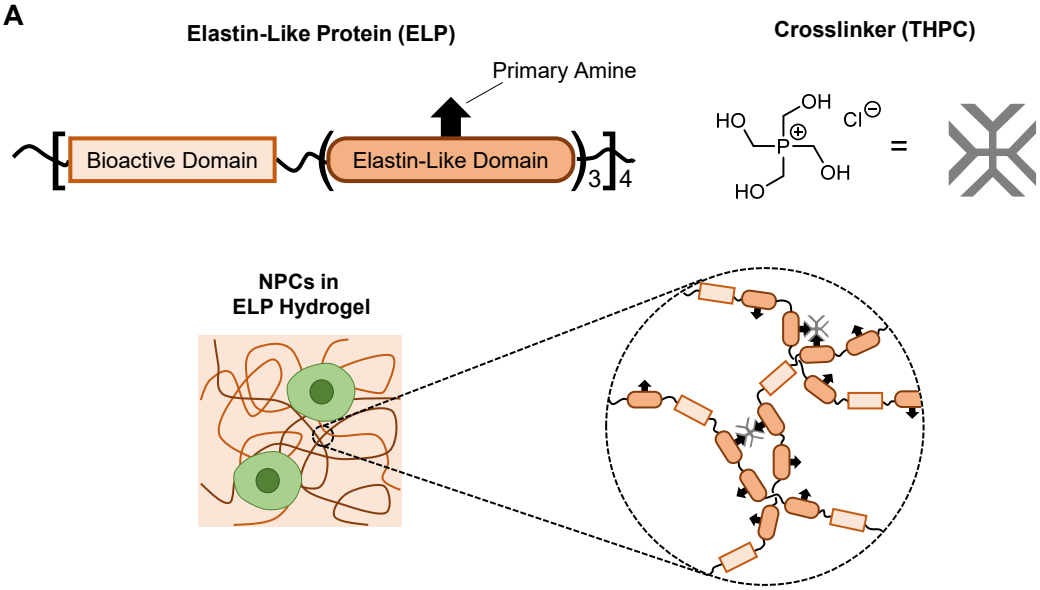
A



B

RGD-ELP (MW ~ 37.9 kDa)

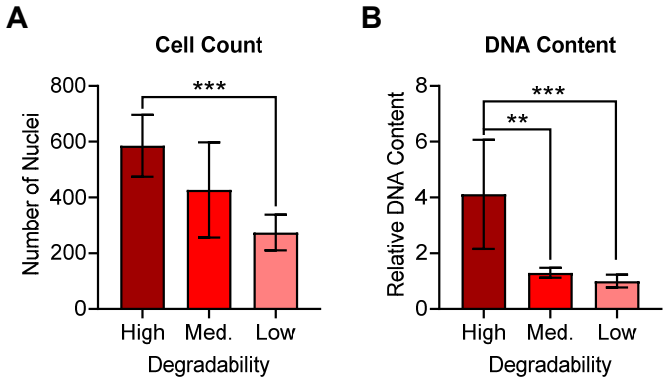
MASMTGGQQMGHHHHHHHDDDDKLQ{LDASTVYAVTG***RGD***SPASSA↑ASA[(VPGIG)$_2$VPGKG(VPGIG)$_2$]$_3$VP}$_4$LE

Tags — Bioactive Domain (RGD – Cell-Adhesive; ↑ – ADAM9 Cleavage site) — Elastin-Like Domain

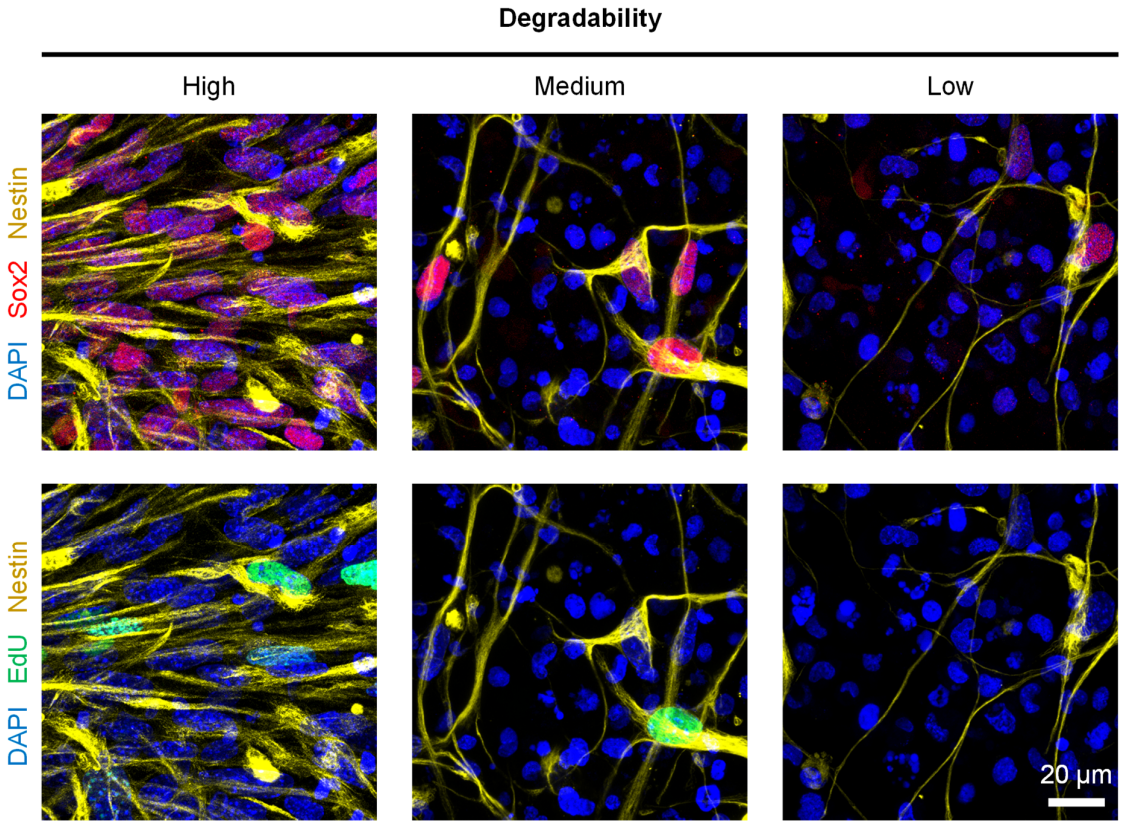
C

Protein Concentration (w/v)	Crosslinker: Protein Reactive Groups	Elastic Modulus [*E*, Pa] (mean±s.d.)	Relative Degradability	Maximum Fraction Degraded (plateau±s.e.)
3%	0.5:1	590±60	High	0.42±0.008
	0.75:1	960±140	Medium	0.27±0.005
	1:1	1450±240	Low	0.19±0.004

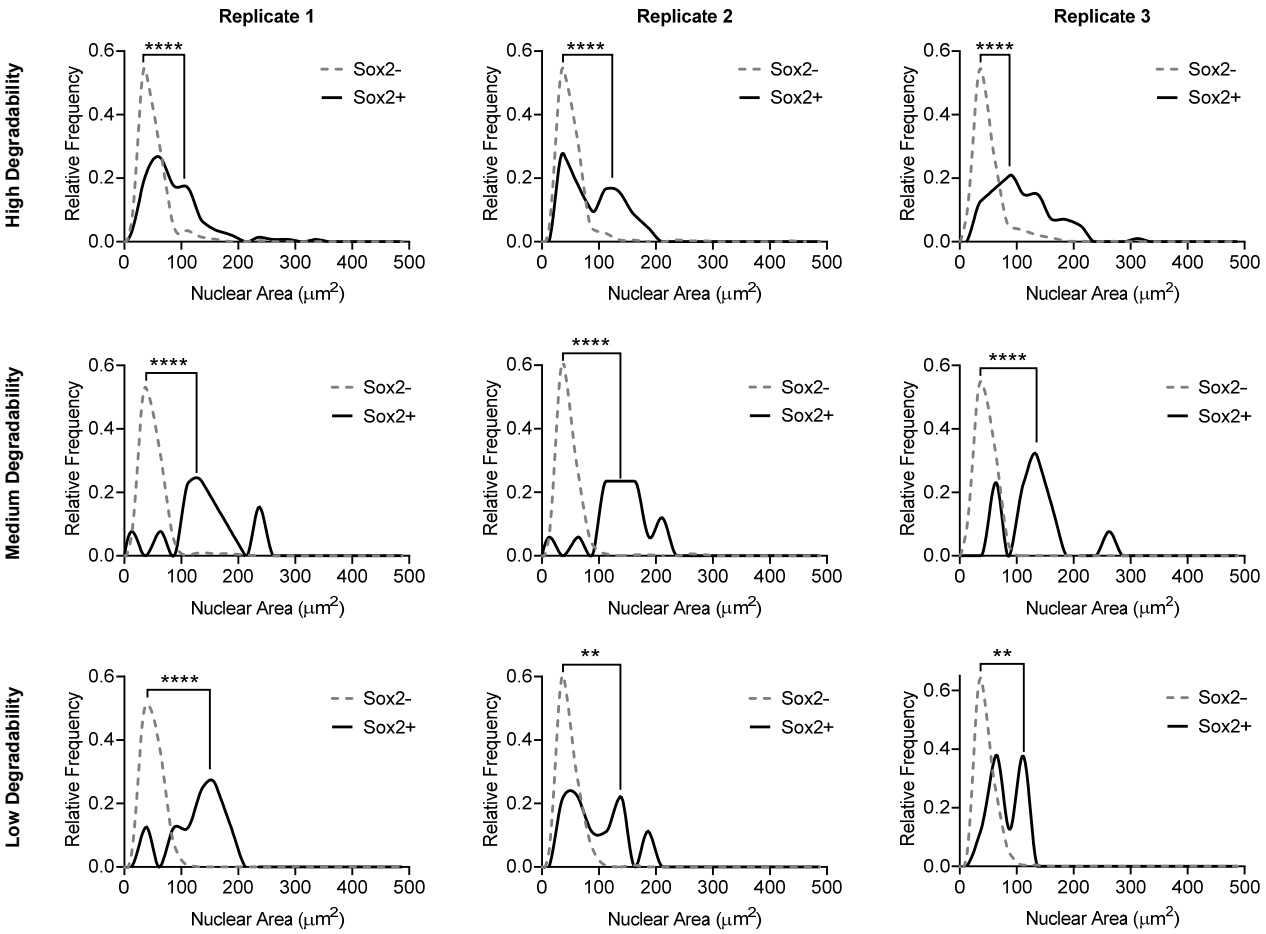
Supporting Figure S1. Elastin-like protein (ELP) hydrogels with tunable degradability for 3D NPC culture. (A) Schematic depicting modular structure of ELPs, the tetrafunctional amine reactive crosslinker THPC, and NPCs embedded within an ELP hydrogel crosslinked by THPC. **(B)** Amino acid sequence of the recombinant ELP used in this study. **(C)** Characterization of ELP hydrogel elastic moduli and degradability for 3% (w/v) hydrogels with varying crosslinker concentration. n = 4 independent gels for elastic moduli measurements and 3 independent gels for degradability measurements.



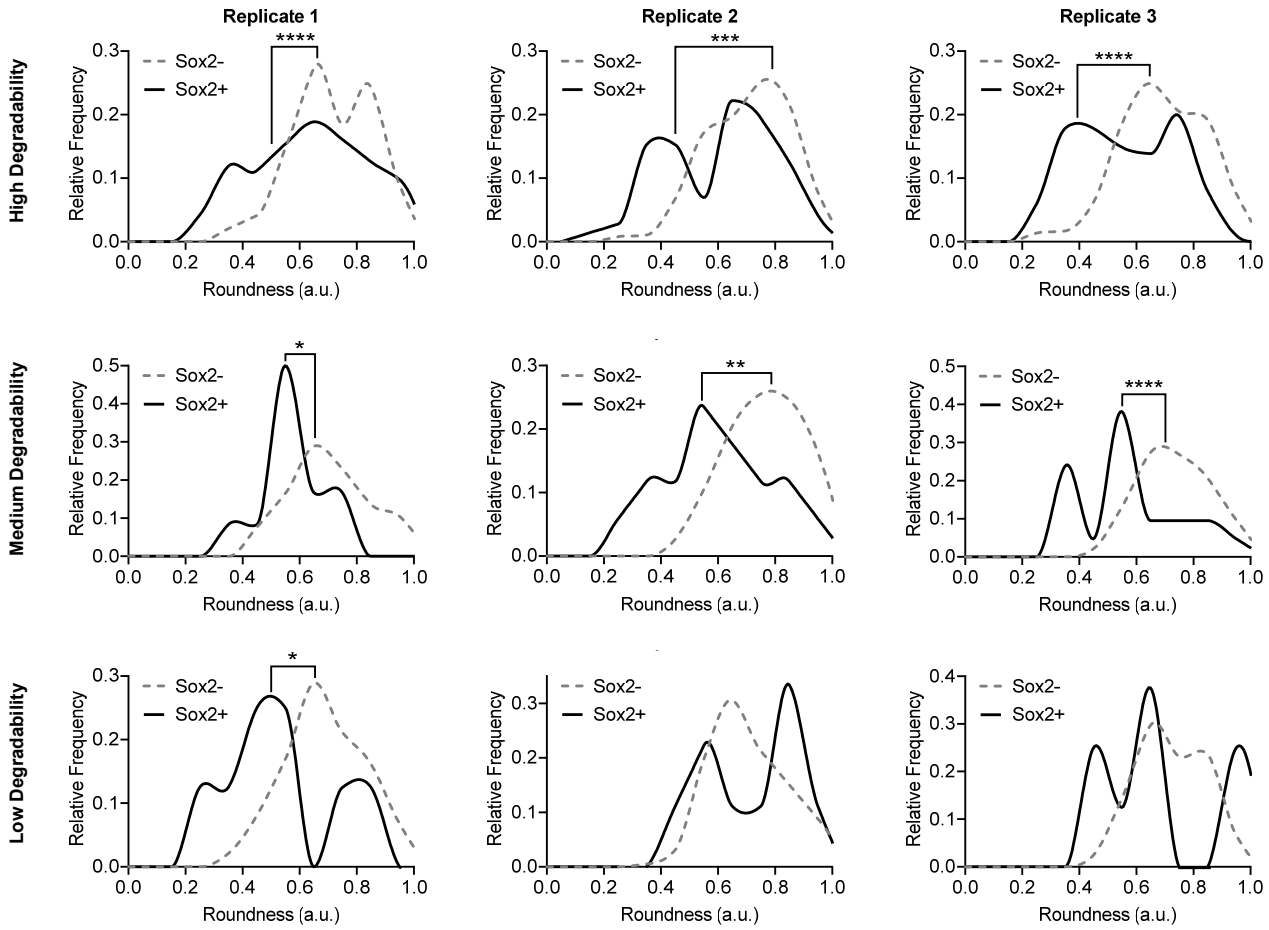
Supporting Figure S2. NPC proliferation decreases with decreasing hydrogel degradability. **(A)** The number of nuclei counted per sample is significantly lower in low degradability gels than in high degradability gels after 7 days in culture. **(B)** Measuring proliferation by quantifying DNA content after 7 days in culture reveals a similar trend, with decreased DNA content in less degradable gels. **p<0.01, ***p<0.001, one-way ANOVA with Bonferroni post-hoc test. In A, n = 8 independent gels per condition. In B, n = 6 independent gels per condition.



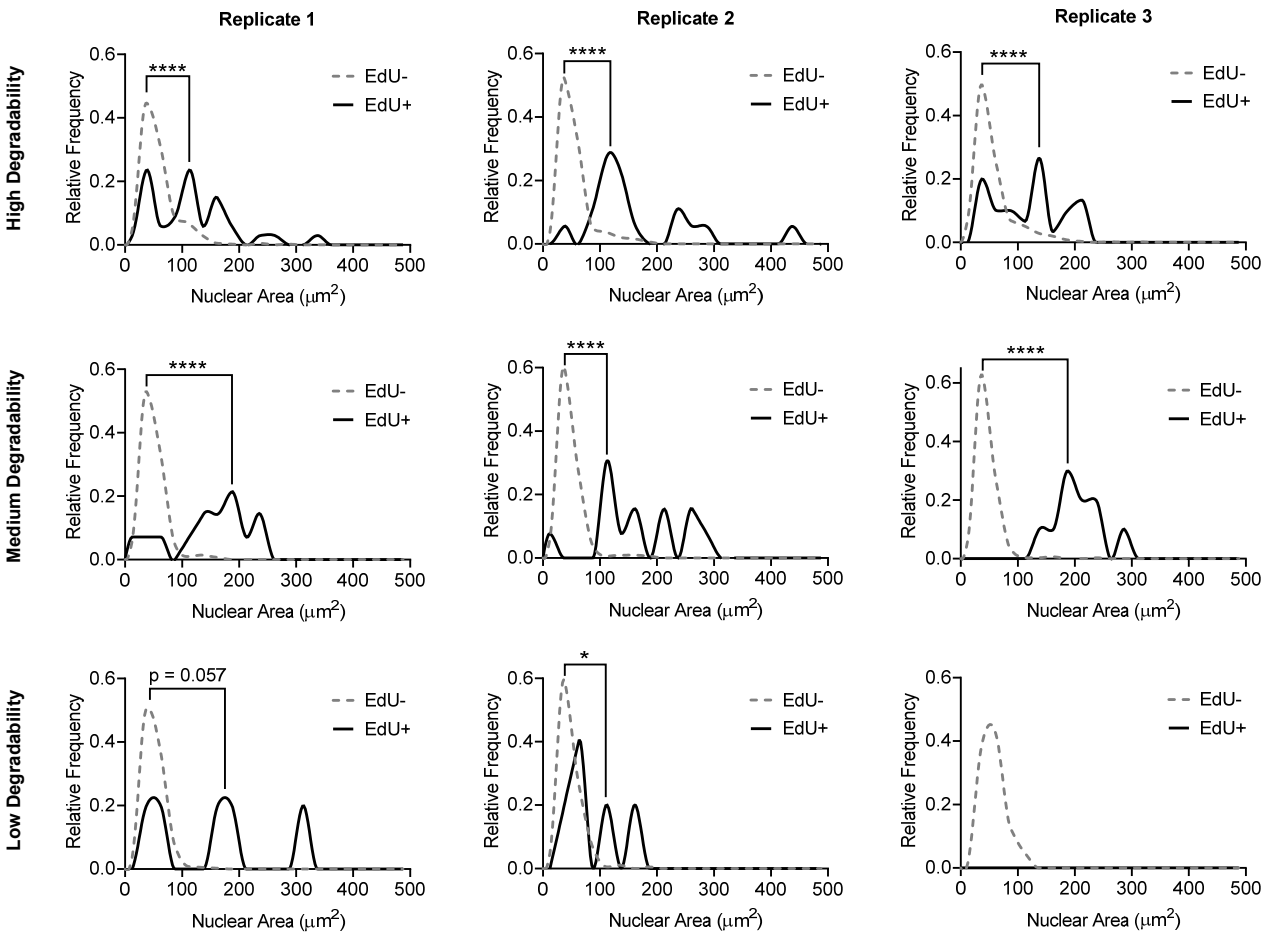
Supporting Figure S3. Immunofluorescence characterization of NPCs cultured in ELP hydrogels with high, medium, or low degradability. The fraction of cells staining positive for the stemness markers nestin and Sox2 and for incorporation of the proliferation indicator EdU decrease with decreasing hydrogel degradability. Cells that stain positive for Sox2 expression and EdU incorporation co-stain for nestin. Blue: DAPI (DNA); Green: EdU; Red: Sox2; Yellow: nestin.



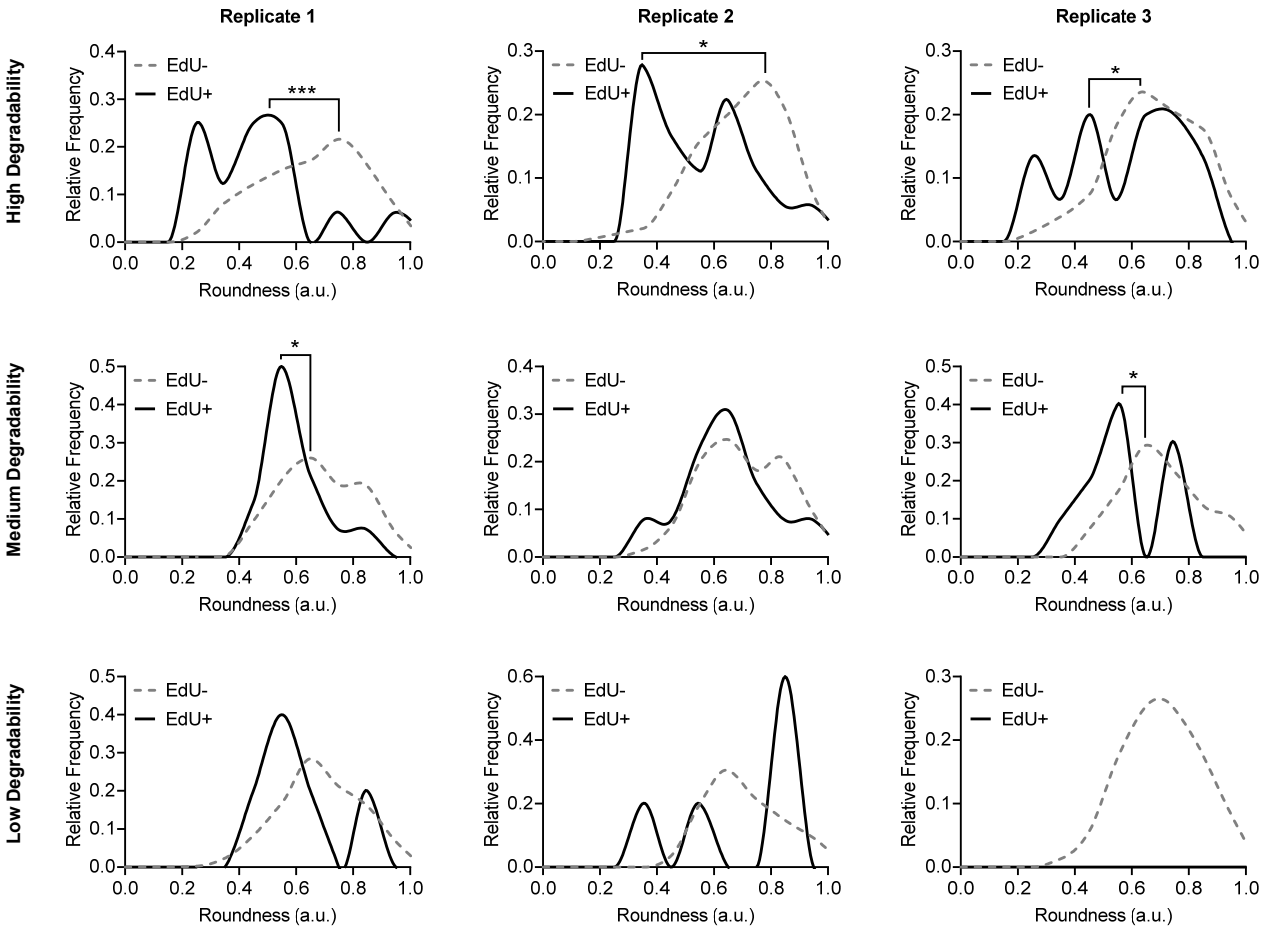
Supporting Figure S4. Nuclear size correlates with Sox2 expression. Histograms of nuclear area for Sox2+ and Sox2− nuclei from 3 representative independent samples for high, medium, and low degradability gels. **p<0.01, ****p<0.0001, Kolmogorov-Smirnov test.



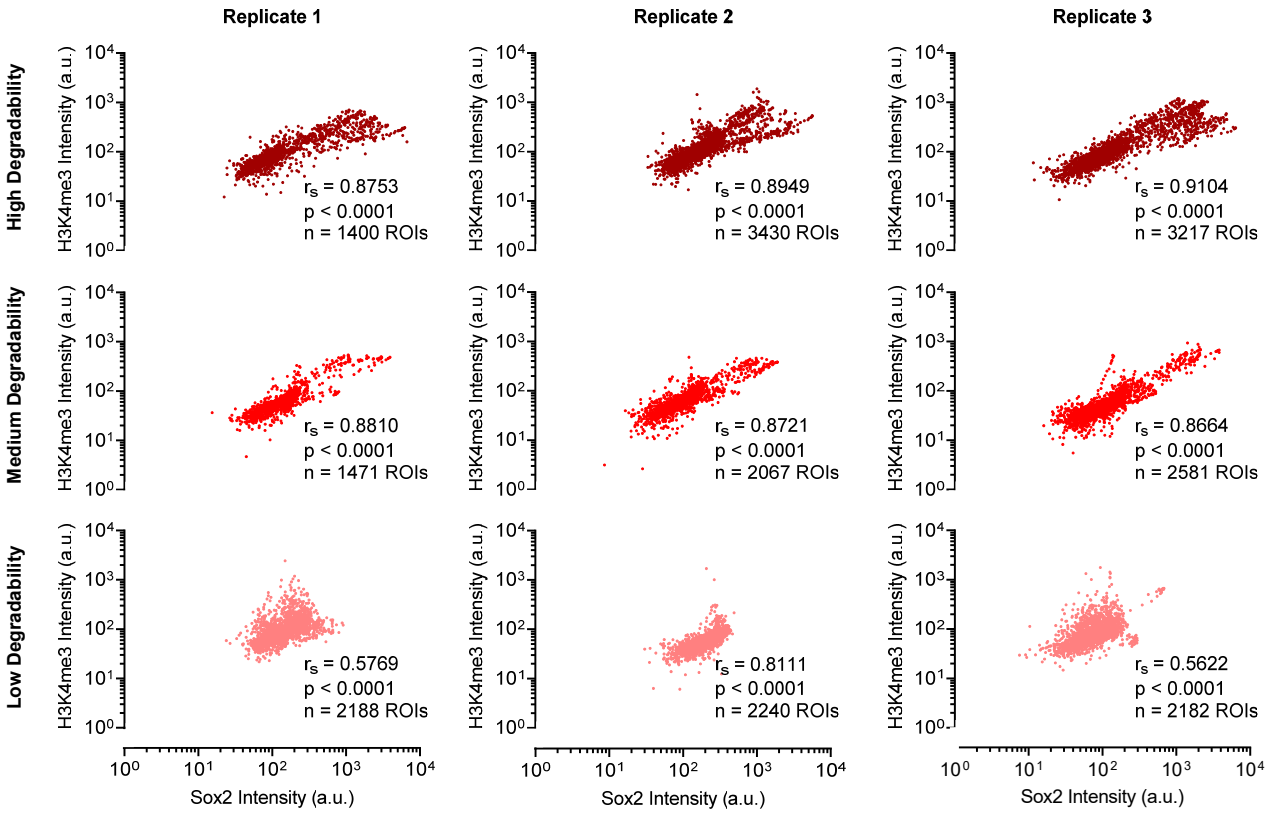
Supporting Figure S5. Nuclear roundness correlates with Sox2 expression. Histograms of nuclear roundness for Sox2+ and Sox2− nuclei from 3 representative independent samples for high, medium, and low degradability gels. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, Kolmogorov-Smirnov test.



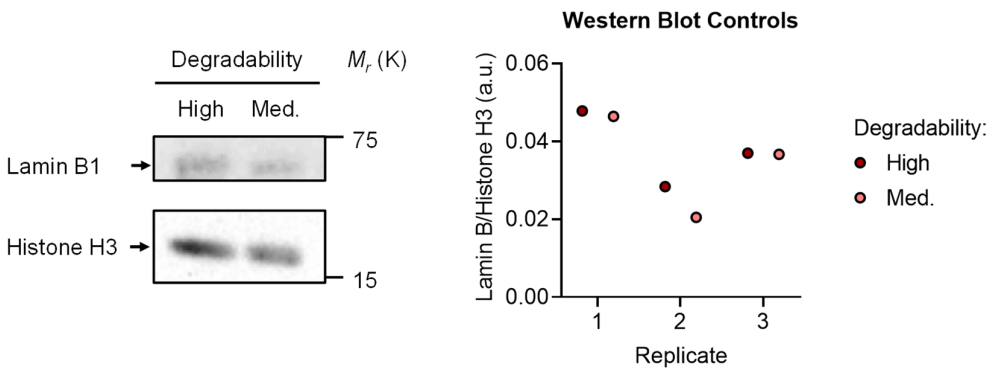
Supporting Figure S6. Nuclear size correlates with EdU incorporation. Histograms of nuclear area for EdU+ and EdU− nuclei from 3 representative independent samples for high, medium, and low degradability gels. Only 2 low degradability samples contained EdU positive nuclei, so the third replicate only shows a histogram for the EdU− nuclei. *p<0.05, ****p<0.0001, Kolmogorov-Smirnov test.



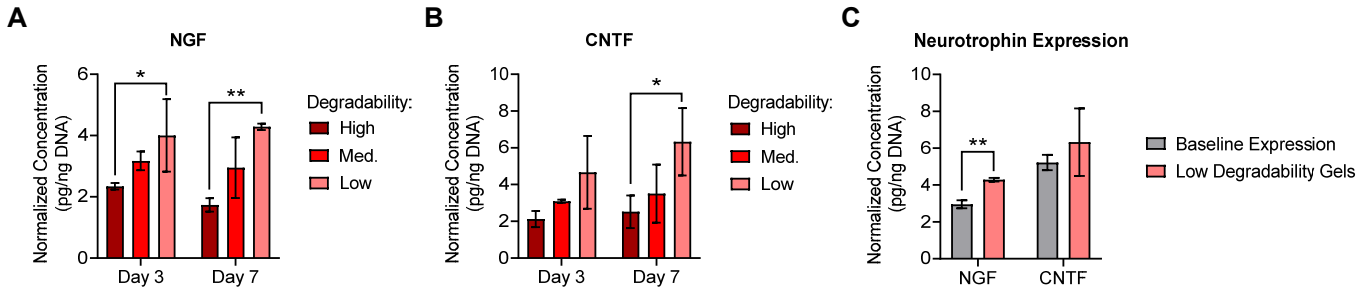
Supporting Figure S7. Nuclear roundness correlates with EdU incorporation. Histograms of nuclear roundness for EdU+ and EdU− nuclei from 3 representative independent samples for high, medium, and low degradability gels. Only 2 low degradability samples contained EdU positive nuclei, so the third replicate only shows a histogram for the EdU− nuclei. *p<0.05, ***p<0.001, Kolmogorov-Smirnov test.



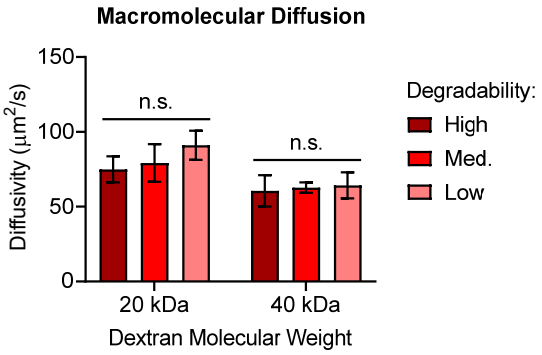
Supporting Figure S8. Sox2 and H3K4me3 intensity are positively correlated. All replicates in all three hydrogel degradability conditions exhibit positive correlations between Sox2 and H3K4me3 intensity (Spearman correlation). The correlation in high and medium degradability gels is stronger than in low degradability gels due to the higher number of strongly Sox2+/H3K4me3+ cells in the high and medium degradability gels.



Supporting Figure S9. Lamin B1 co-varies with Histone H3 as nuclear loading controls. To validate that Lamin B1 level serve as an appropriately invariant control for comparing Lamin A/C expression levels, Lamin B1 expression was compared to Histone H3 expression as another constitutively expressed nuclear-localized protein to serve as loading control. Lamin B1 intensity tracks well with Histone H3 intensity, validating that Lamin B1 is also a valid control for these experiments.



Supporting Figure S10. Pairwise comparisons for NGF and CNTF expression in NPCs cultured in hydrogels with tunable degradability. **(A,B)** Normalized NGF and CNTF concentrations increase with decreasing hydrogel degradability. **(C)** NGF concentration after 7 d in culture in low degradability gels is higher than in freshly encapsulated NPCs. Data are presented as mean ± s.d. n = 3 independent gels. In A and B, *p<0.05, **p<0.01, one-way ANOVA with Bonferroni post-hoc test. In C, **p<0.01, two-tailed Student's *t*-test with Holm-Sidak multiple comparisons correction.



Supporting Figure S11. Macromolecular diffusivity does not vary with hydrogel degradability. Diffusion coefficients determined from FRAP analysis of fluorescently-labeled dextrans with molecular weights similar to the neurotrophic factors studied. Data are presented as mean ± s.d. n = 3-4 independent gels. n.s. = not significant, two-way ANOVA with Bonferroni post-hoc test.

Supporting Table S1. Primary antibodies.
ICC = Immunocytochemistry. WB = Western blot.

Target	Host Species	Supplier	Catalog Number	Dilution
Nestin	Mouse	BD Pharmingen	556309	ICC: 1:400
Sox2	Rabbit	Cell Signaling Technology	23064S	ICC: 1:400
H3K4me3	Mouse	Abcam	ab185637	ICC: 1:200
H3K9me3	Rabbit	Abcam	ab8898	ICC: 1:500
Lamin A/C	Mouse	Cell Signaling Technology	4777S	WB: 1:2000
Lamin B1	Rabbit	Cell Signaling Technology	12586S	WB: 1:1000
Histone H3	Rabbit	Cell Signaling Technology	4499S	WB: 1:1000

Supporting Table S2. Primers used in qRT-PCR.

Target	Primers
Nestin	Fwd: CCCTGAAGTCGAGGAGCTG Rev: CTGCTGCACCTCTAAGCGA
Sox2	Fwd: GCGGAGTGGAAACTTTTGTCC Rev: CGGGAAGCGTGTACTTATCCTT
p16	Fwd: CGCAGGTTCTTGGTCACTGT Rev: TGTTCACGAAAGCCAGAGCG
p21	Fwd: CCTGGTGATGTCCGACCTG Rev: CCATGAGCGCATCGCAATC
p53	Fwd: GCGTAAACGCTTCGAGATGTT Rev: TTTTTATGGCGGGAAGTAGACTG
p19	Fwd: CTGAACCGCTTTGGCAAGAC Rev: GCCCTCTCTTATCGCCAGAT
NGF	Fwd: CCAGTGAAATTAGGCTCCCTG Rev: CCTTGGCAAAACCTTTATTGGG
GDNF	Fwd: TCCAACTGGGGGTCTACGG Rev: GCCACGACATCCCATAACTTCAT
NT-3	Fwd: GGAGTTTGCCGGAAGACTCTC Rev: GGGTGCTCTGGTAATTTTCCTTA
NT-4/5	Fwd: TGAGCTGGCAGTATGCGAC Rev: CAGCGCGTCTCGAAGAAGT
BDNF	Fwd: TCATACTTCGGTTGCATGAAGG Rev: AGACCTCTCGAACCTGCCC
CNTF	Fwd: TCTGTAGCCGCTCTATCTGG Rev: GGTACACCATCCACTGAGTCAA