Supporting Information

In Vivo Imaging of Allografted Glial-Restricted Progenitor Cell Survival and Hydrogel Scaffold Biodegradation

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Table S1: Composite make-up of hydrogel formulations.

Hydrogel formulations	Component dilutions (µl) from 20 mg/ml stock (s) in sterile water (w) to prepare 100 µl of hydrogel		
	HA-S (weight fraction=0.4)	**Gel-S (weight fraction=0.4)**	**PEGDA (weight fraction=0.2)**
20 mg/ml (2.0 %)	40 (s)	40 (s)	20 (s)
15 mg/ml (1.5 %)	30 (s)+10 (w)	30 (s)+10 (w)	15 (s)+5 (w)
10 mg/ml (1.0 %)	20 (s)+20 (w)	20 (s)+20 (w)	10 (s)+10 (w)
5 mg/ml (0.5 %)	10 (s)+30 (w)	10 (s)+30 (w)	5 (s)+15 (w)

The mesh sizes of the composite hydrogels were calculated using a Flory-Rehner equation with a swelling (volume fraction) in 1x PBS, HA specific volume= 0.8137, MW of macromer (Mn) =300 kDa, polymer solvent interaction parameter (χ) = 0.473, molar volume of solvent (V_1) =18 mol/cm^3, and a specific volume (v)=0.8137 mL/g. The volume fractions of the gel in relaxed and swollen state ($v_{2,r}$) and ($v_{2,s}$) were calculated first from the swelling weight fractions and the molecular weight between crosslinks (Mc) was estimated as follows:

$$\frac{1}{\mathrm{Mc}} = \frac{2}{\mathrm{Mn}} - \frac{\mathrm{v}/\mathrm{V}_1[\ln(1-\mathrm{v}_{2,s}) + (\mathrm{v}_{2,s}) + \chi(\mathrm{v}_{2,s})]}{\mathrm{v}_{2,r}[(\frac{\mathrm{v}_{2,s}}{\mathrm{v}_{2,r}})^{\frac{1}{3}} - \frac{\mathrm{v}_{2,s}}{2\mathrm{v}_{2,r}}]}$$

The mesh size (ξ, in nm) calculation based on Flory-Rehner equation was adapted from Bryant et al.[1] as:

$$\xi = 0.1743(\mathrm{v}_{2,s})^{\left(-\frac{1}{3}\right)}.(\mathrm{Mc})^{1/2}$$

Table S2: Hydrogel mesh sizes as calculated from swelling data.

Hydrogel formulations	Mesh size (nm)
20 mg/ml (2.0 %)	24.67 ± 3.1
15 mg/ml (1.5 %)	59.11 ± 2.9
10 mg/ml (1.0 %)	81.82 ± 3.8
5 mg/ml (0.5 %)	126.45 ± 4.6

Table S3: Antibodies used for immunohistological characterization.

Antibodies	Company	Cat. #	Source	Dilution
A2B5	Millipore	MAB312	Mouse	1:500
GFAP	Dako	Z0334	Rabbit	1:1000
Ki67	Abcam	ab15580	Rabbit	1:200
NG2	Abcam	ab129051	Rabbit	1:500
Olig1	ThermoFisher	PA5-21613	Rabbit	1:200
Olig2	Millipore	AB9610	Rabbit	1:500
PGDF-B	Abcam	ab23914	Rabbit	1:300
O4	Millipore	MAB345	Mouse	1:200
MBP	Millipore	AB9348	Chicken	1:100
AF-594 anti-Rb	ThermoFisher	A-11037	Goat	1:1000
AF-594 anti-Ch	ThermoFisher	A-11042	Goat	1:1000
AF-647 anti-Ms	ThermoFisher	A-21203	Donkey	1:1000

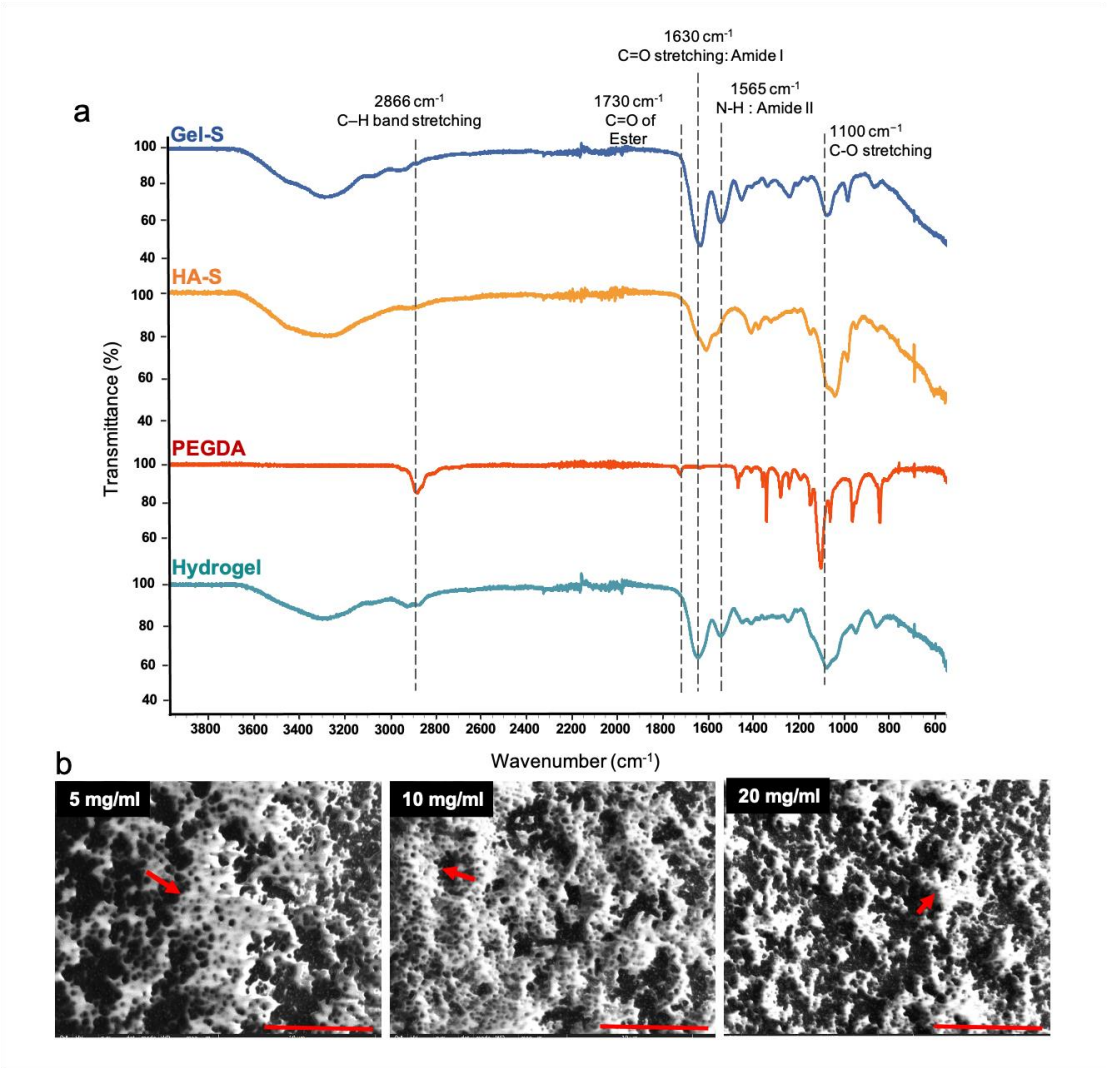

Figure S1: (a) FTIR spectra of hydrogel precursors and crosslinked hydrogel. (b) SEM micrographs of crosslinked 5, 10 and 20 mg/ml hydrogels. Red arrows point to honeycomb-like porous regions. Scale bar = 10 µm.

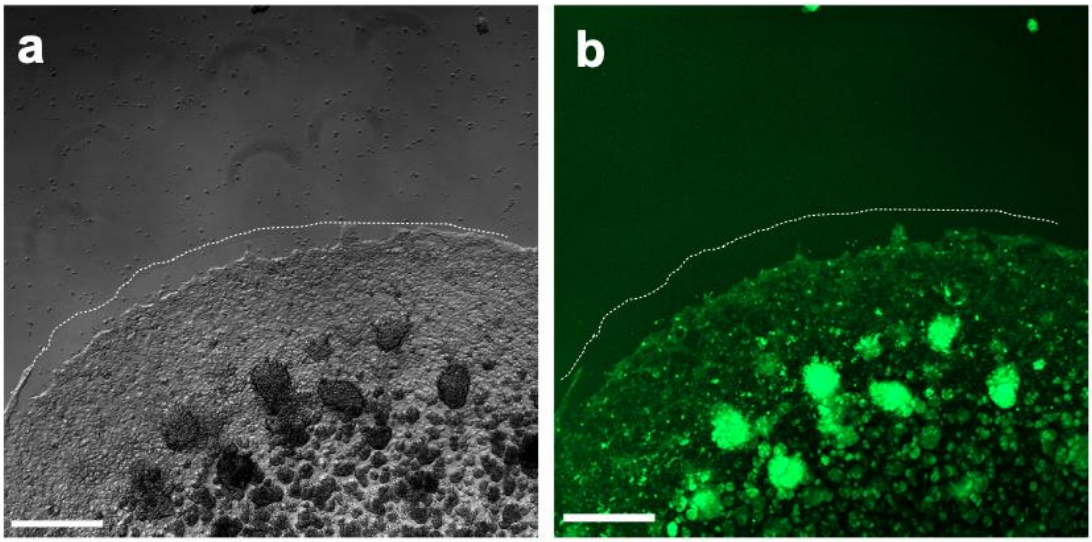

Figure S2: Morphology of scaffolded mGRPs in 10 mg/ml composite gels at day 14. (a) Phase contrast, (b) GFP fluorescence. Dotted line indicates hydrogel boundary. Scale bar=200 μm

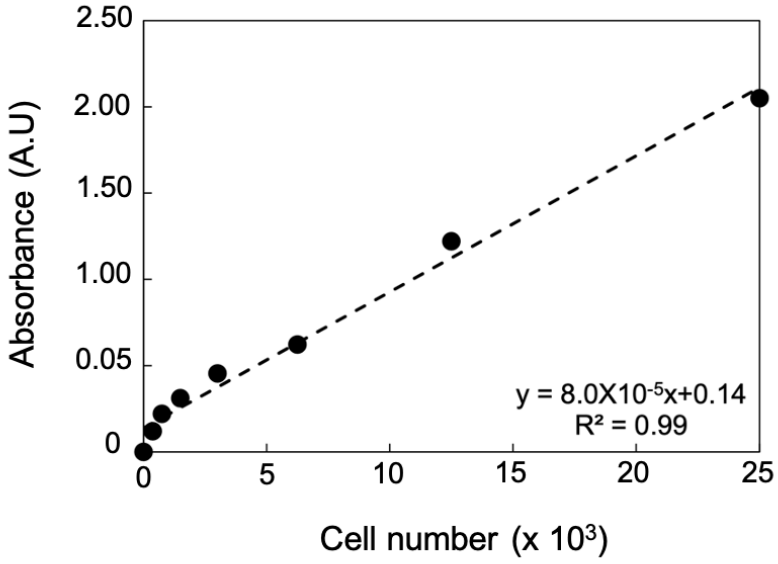

Figure S3: Calibration curve of absorbance vs. mGRP cell number for the CCK-8 proliferation assay.

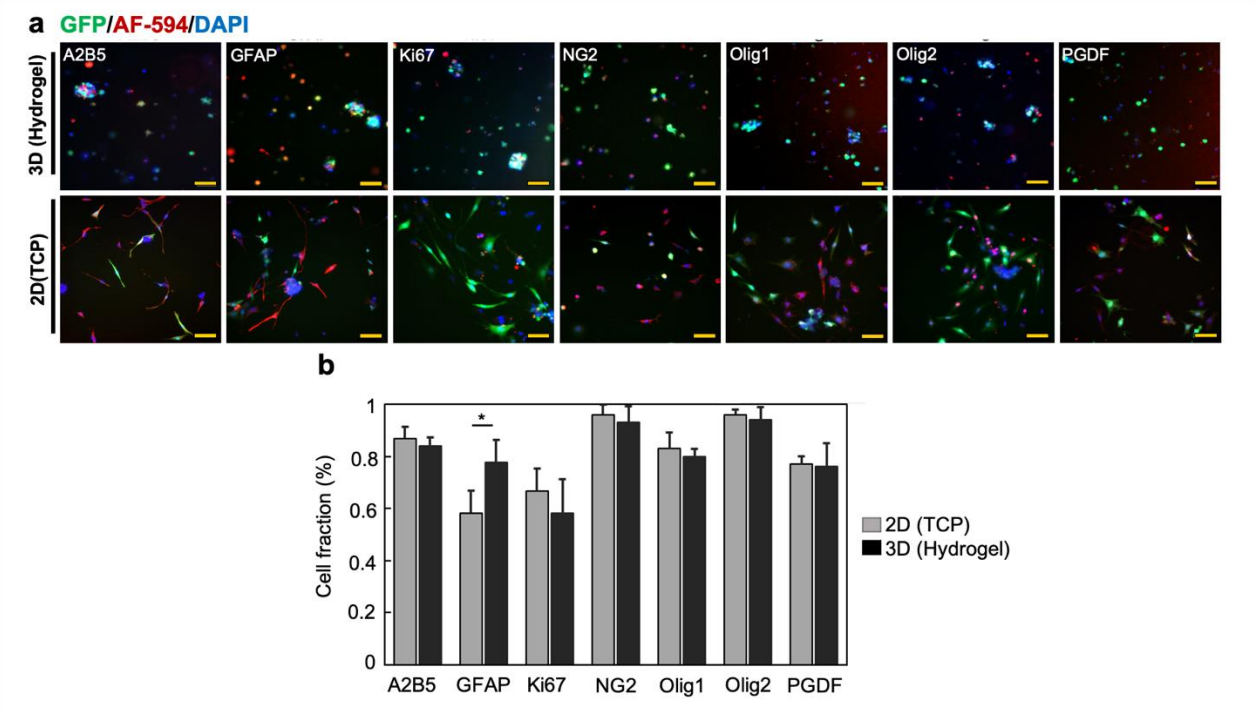

Figure S4: (a) Immunocytochemistry of GFP$^+$ mGRPs (day 4, green) grown either as 3D cultures (10 mg/ml hydrogel) or as plate surface 2D cultures showing expression of GRP phenotype markers (red). Nuclei are counterstained with DAPI. TCP=tissue culture plate. Scalebar = 100 µm. (b) Quantification of cells expressing phenotypic markers. *p<0.05 (n=3).

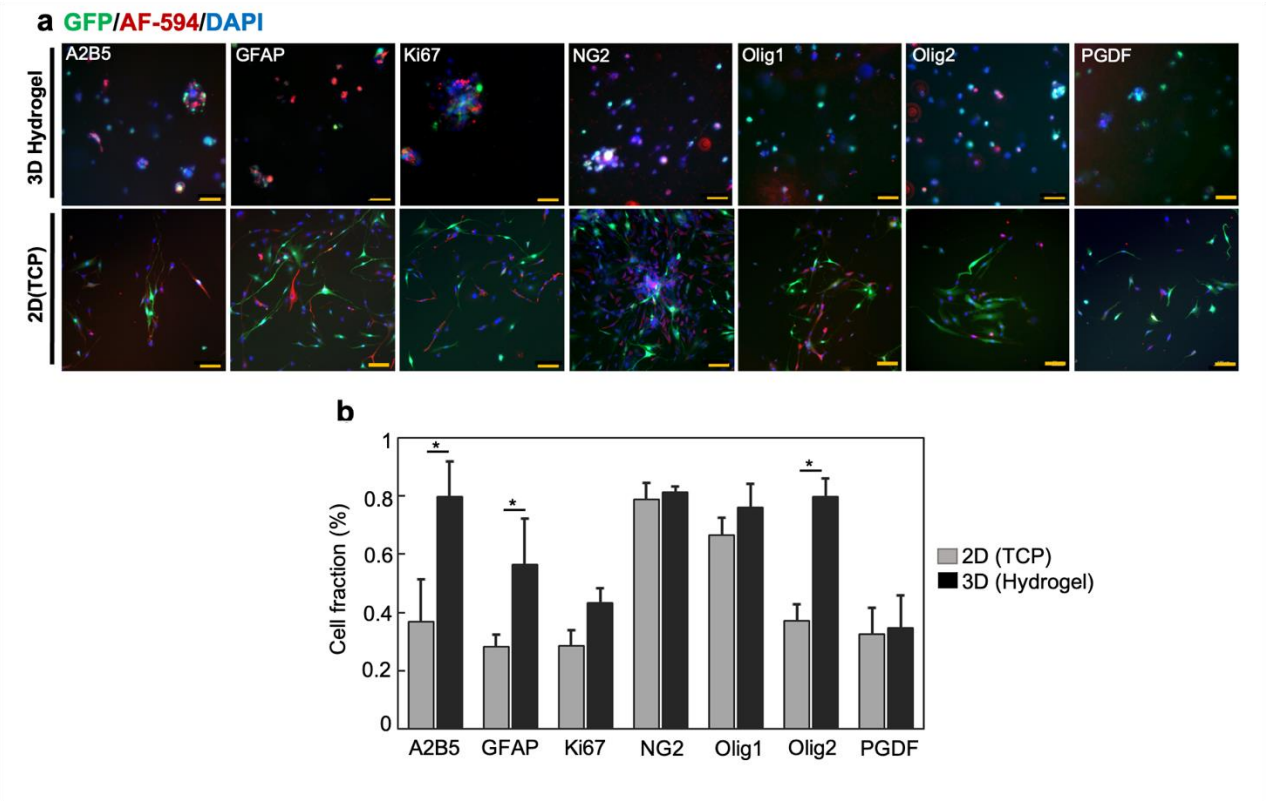

Figure S5: (a) Immunocytochemistry of GFP$^+$ mGRPs (day 16, green) grown either as 3D cultures (10 mg/ml hydrogel) or as plate surface 2D cultures showing the expression of GRP phenotype markers (red) in both single cells and 3D cell cultures. Nuclei are counterstained with DAPI (blue). TCP=tissue culture plate. Scale bars=100 µm. (b) Quantification of cells expressing phenotypic markers. *$p<0.05$ (n=3).

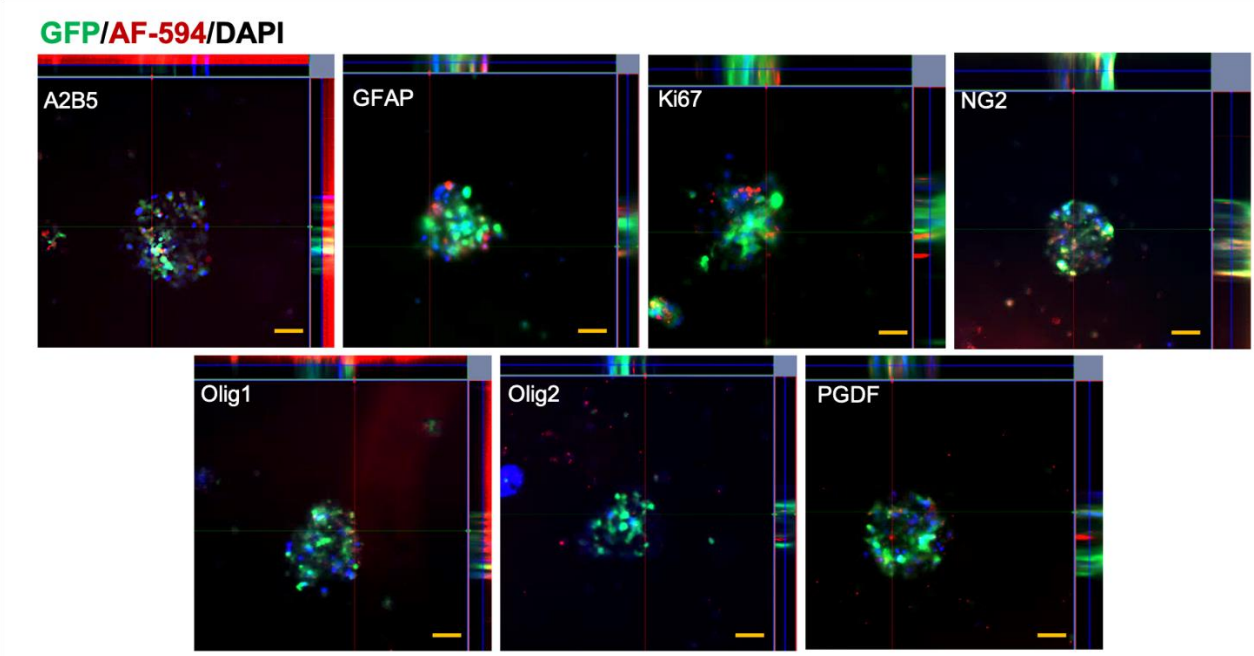

Figure S6: Immunocytochemistry of mGRP spheres (day 16, green) grown as 3D cultures (10 mg/ml hydrogel) showing the expression of GRP phenotype markers (red). Nuclei are counterstained with DAPI (blue). Scale bar=50 µm.

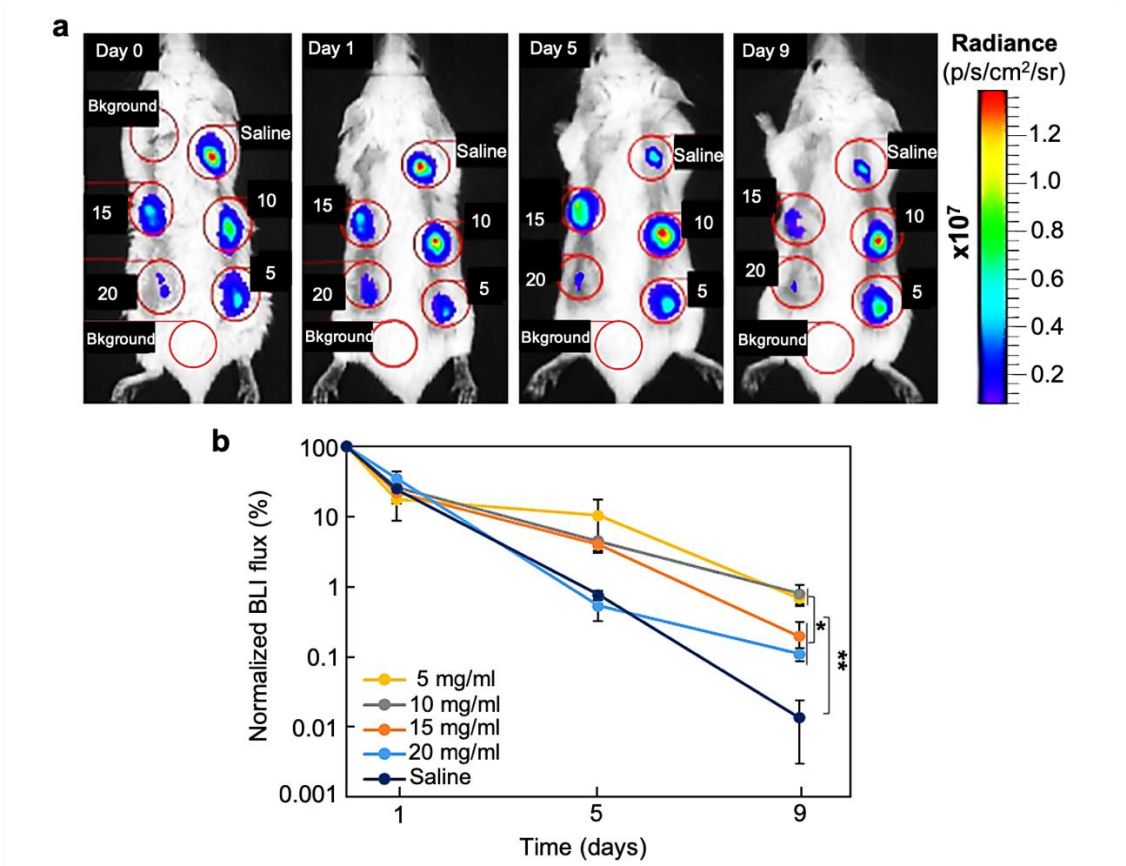

Figure S7: S.c. transplanted mGRP survival: (a) Representative in vivo BL images and (b) signal quantification of allogeneic mGRPs scaffolded in various hydrogel formulations or saline transplanted s.c. in immunocompetent Balb/C mice (n=4). Values represent different composite hydrogel densities expressed as mg/ml. Signal was normalized to the day of transplantation (day 0). *p<0.05 between scaffolded cell groups and **p<0.01 between scaffolded cell groups and saline group at day 9.

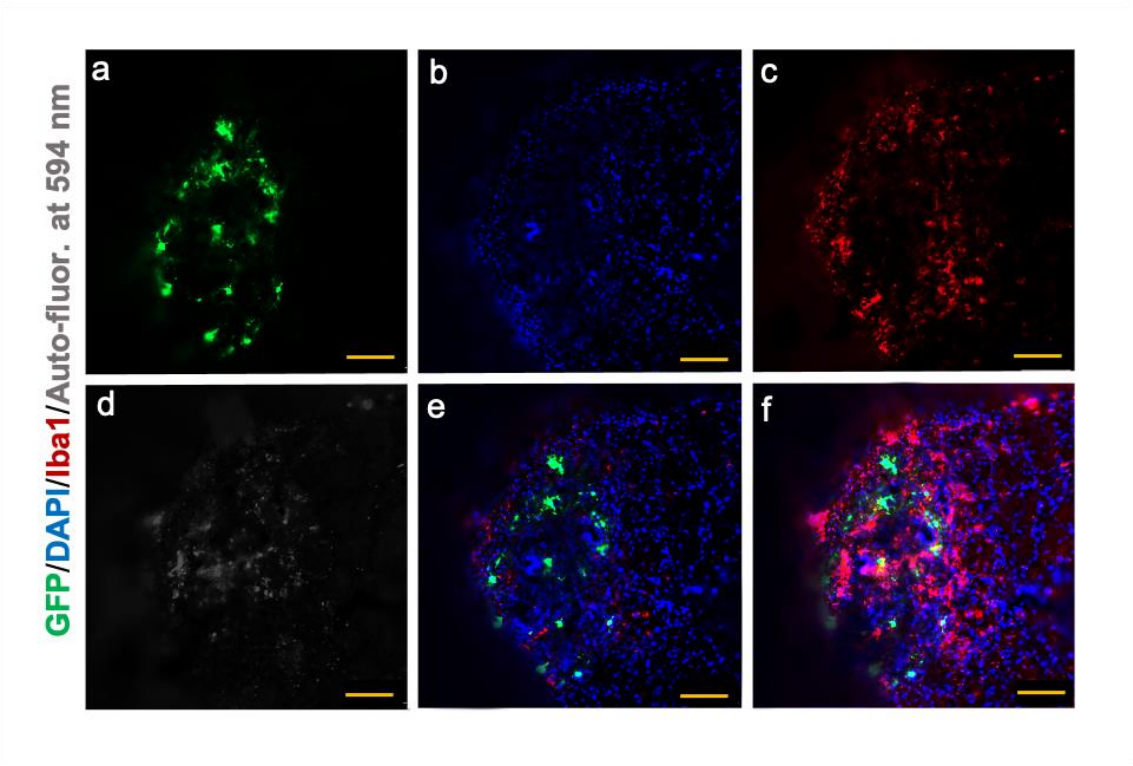

Figure S8: Immunohistology of (a) GFP^+ mGRP graft (green) stained with (b) DAPI (nuclear counterstain, blue) and (c) anti-Iba-1 (red) showing (d) autofluorescence from secondary antibody at 594 nm and (e, f) merged images without (e) and with (f) 594 nm autofluorescence. Scale bar=100 µm.

Reference

1) Bryant, S. J.; Anseth, K. S. Hydrogel Properties Influence ECM Production by Chondrocytes Photoencapsulated in Poly(ethyleneGlycol) Hydrogels. J. Biomed. Mater. Res. **2002**, 59, 63−72