Electronic Supplementary Material

Self-assembling peptides cross-linked with Genipin: resilient hydrogels and self-standing electrospun scaffolds for tissue engineering applications

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Experiment

Cell Morphology, Viability and Differentiation Assays

hNSCs were differentiated with basal medium (without growth factors) supplemented with bFGF (10 ng mL^{-1}). After 2h of incubation with basal medium supplemented with bFGF, different genipin concentrations (21mM, 42mM and 64mM) were added to the cells previously seeded on Cultrex or $FAQ(LDLK)_3$ hydrogel and incubated overnight at 37°C. The day after, LIVE/DEAD assay (Thermofisher scientific) was used to evaluate cell viability. Live cells were stained with green-fluorescent calcein-AM, whereas dead cells were identified by red-fluorescent ethidium homodimer-1. Cell Nuclei were stained with Hoechst (1:1000, Invitrogen).

To assess hNSCs morphology and differentiation on electrospun mats made of cross-linked SAPs, hNSCs were differentiated with basal medium (without growth factors) supplemented with bFGF (10 ng mL^{-1}). At 2DIV, bFGF medium was replaced with basal medium supplemented with Leukemia Inhibitory Factor (LIF, 20 ng mL^{-1}; Chemicon) and Brain Derived Neurotrophic Factor (BDNF, 20 ng mL^{-1}; Peprotech). Fresh medium was added every 3 days. After 7DIV, cells were fixed with 4% paraformaldehyde, then samples were washed with DPBS, permeabilized with 0.3% Triton X-100 and blocked with 10% NGS.

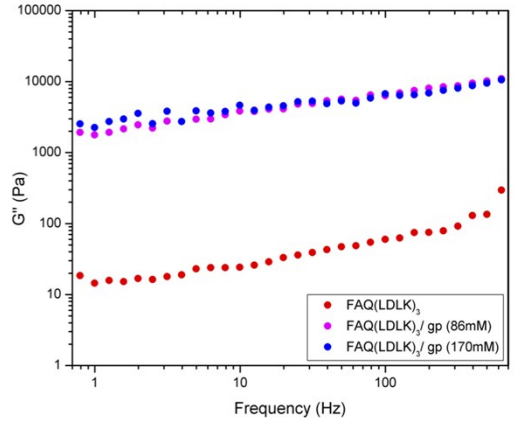
For immunofluorescence tests the following antibodies were used: rabbit anti-GFAP (1:500, DakoCytomation), mouse anti-bIIITubulin (1:500, Covance), mouse anti-GalC (1:200, Chemicon), mouse anti- O4 (1:200, Chemicon), goat anti-mouse Alexa 488 (1:1000, Molecular Probes) and goat anti-rabbit Cy3 (1:1000, Jackson Immunoresearch). Cell nuclei were stained with DAPI (1:500, Molecular probes). Fluorescence images were obtained using Zeiss microscope ApoTome System and processed with ImageJ software. For both viability and differentiation assay three independent experiments were performed.

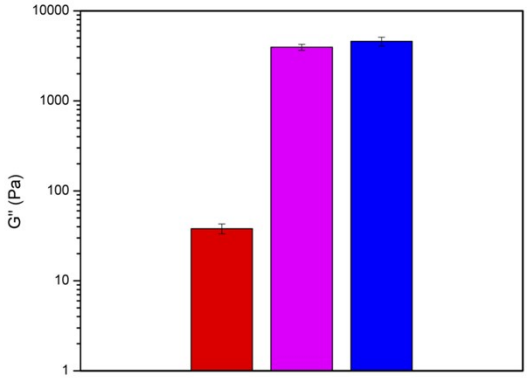
Full name	Abbreviation
Genipin	gp
NH_2-FAQRVPPGGGLDLKLDLKLDLK-$CONH_2$	FAQ$(LDLK)_3$
Peptide cross-linked with genipin	FAQ$(LDLK)_3$/gp
Diffusive cross-linking reaction	DCR
In situ cross-linking reaction	ISCR
In situ partial cross-linking reaction	ISPCR
Peptide cross-linked with genipin using DCR	DCR-FAQ$(LDLK)_3$/gp
Peptide cross-linked with genipin using ISCR	ISCR-FAQ$(LDLK)_3$/gp
Peptide cross-linked with genipin using ISPCR	ISPCR-FAQ$(LDLK)_3$/gp
Elecrospun cross-linked nanofibers	es- FAQ$(LDLK)_3$/gp

Table S1. Full name and abbreviation of all cross-linking strategy described in the main manuscript.

a

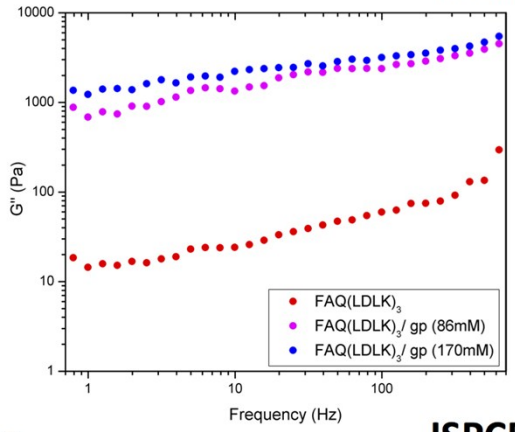
DCR (5% w/v)

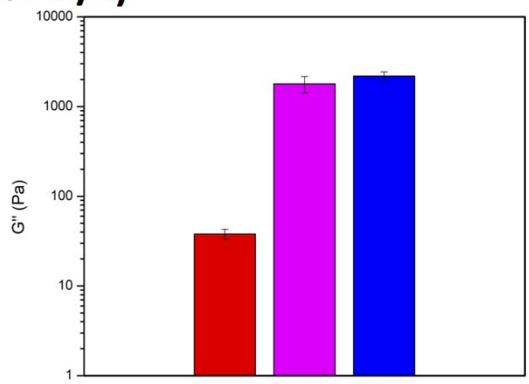




b

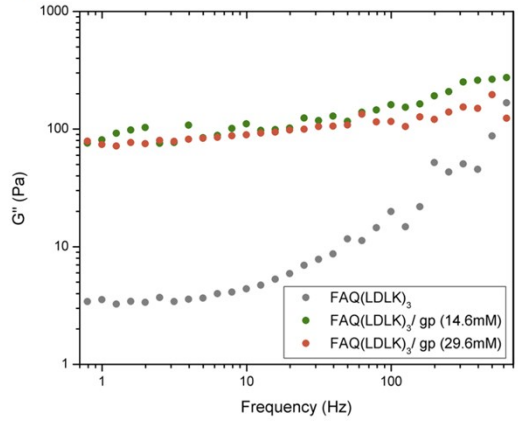
ISCR (5% w/v)





c

ISPCR (1% w/v)



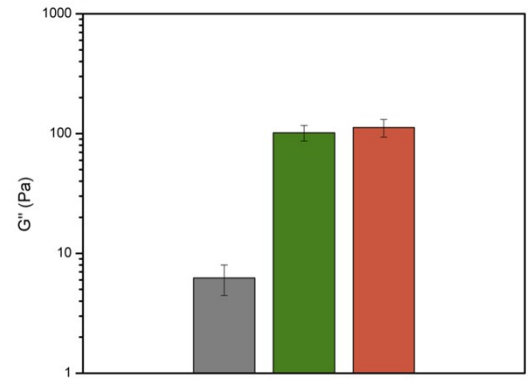


Figure S1. Loss modulus (G") profiles of FAQ(LDLK)$_3$, (a) DCR-, (b) ISCR-, (c) ISPCR-FAQ(LDLK)$_3$/gp peptides, and their average values in the 0.1-1,000 Hz range.

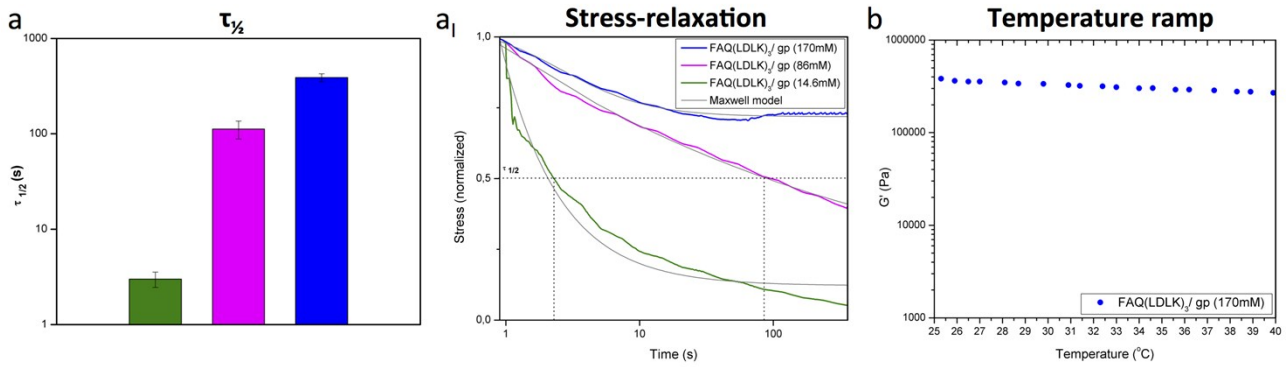


Figure S2. Stress-relaxation tests on DCR- FAQ(LDLK)$_3$/gp. (a) τ½ values of FAQ(LDLK)$_3$/gp 170mM (blue), FAQ(LDLK)$_3$/gp 86mM (magenta), and FAQ(LDLK)$_3$/gp 14.6mM (green). (a_I) Stress-relaxation profiles of FAQ(LDLK)$_3$/gp (170mM), FAQ(LDLK)$_3$/gp (86mM), and FAQ(LDLK)$_3$/gp (14.6mM). Grey lines represent the fitting of stress-relaxation data to two-element Maxwell-Weichert linear viscoelastic model. (b) Temperature ramp of FAQ(LDLK)$_3$/gp (170mM) as a function of G' (T_{rate}= 5°C/min, 1% strain, ω= 1Hz).

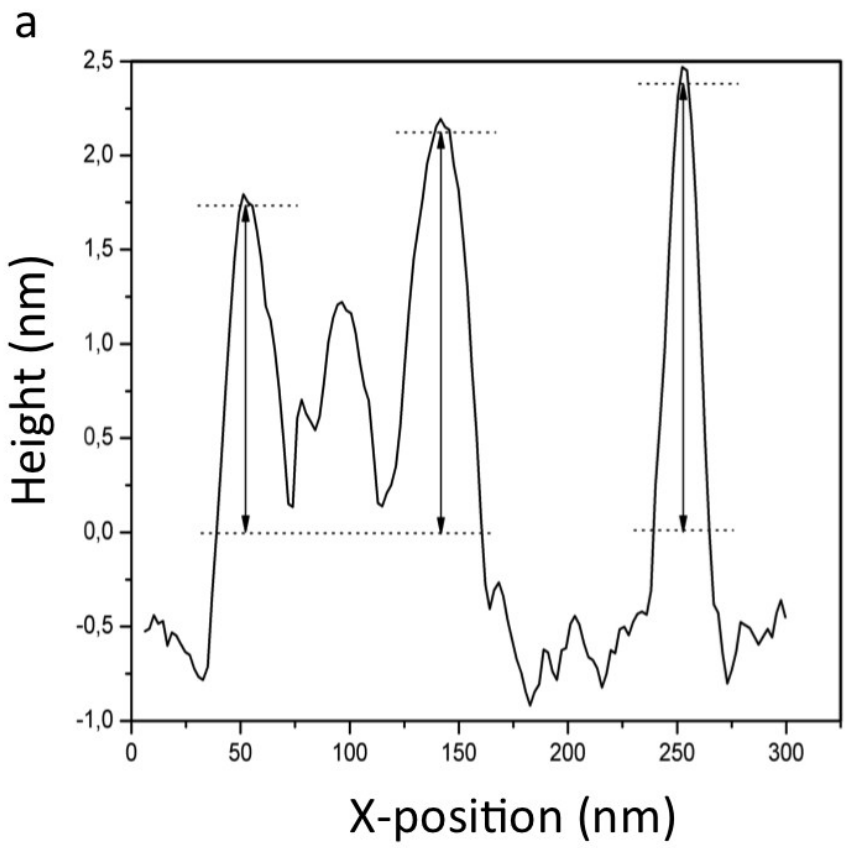


Figure S3. (a) Heights of FAQ(LDLK)$_3$/gp nanofibers obtained from AFM analysis.

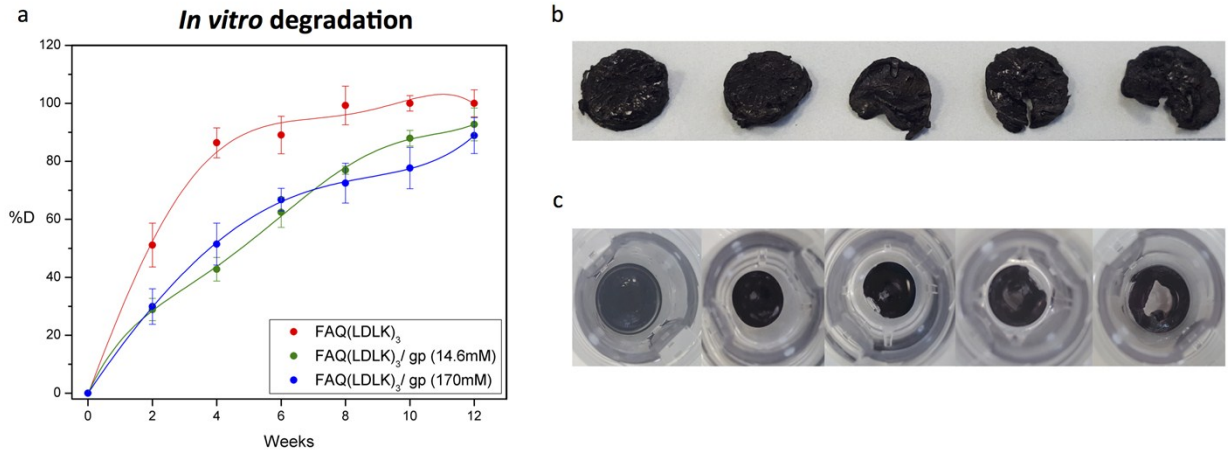


Figure S4. (a) Enzymatic (trypsin) degradation tests of FAQ(LDLK)$_3$ (red), FAQ(LDLK)$_3$/gp 170mM (blue) and FAQ(LDLK)$_3$/gp 14.6mM (green), at pH 7.4 and 37°C. (b) Macroscopic changes of FAQ(LDLK)$_3$/gp 170mM and FAQ(LDLK)$_3$/gp 14.6mM (c) scaffolds in the presence of trypsin observed during the 12 weeks.

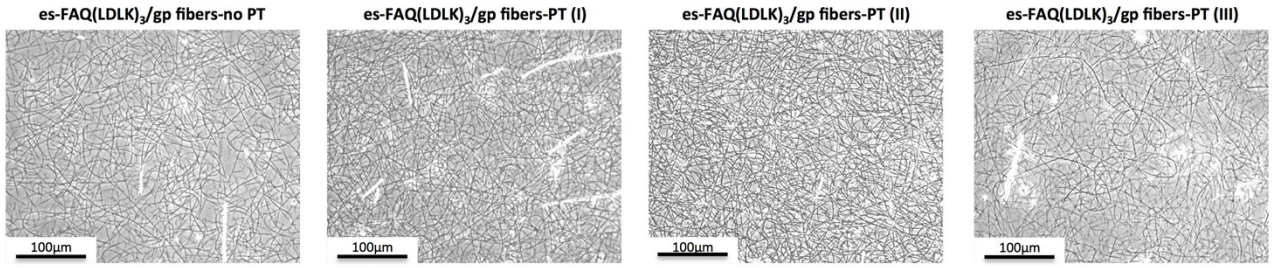


Figure S5. Optical images of es-FAQ(LDLK)$_3$/gp fibers before (no PT) and after different steps of post treatments (PT(I), PT (II), PT (III) see methods for details).

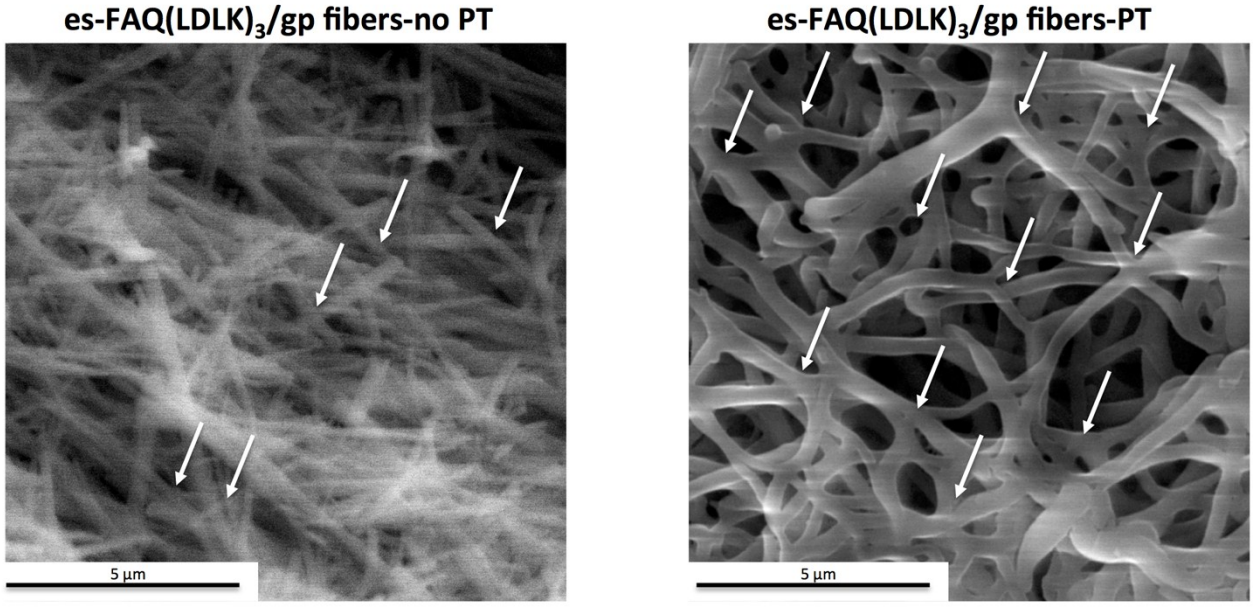


Figure S6. SEM images of es-FAQ(LDLK)$_3$/gp fibers before (no PT) and after post treatment (PT). White arrows point at junctions between adjacent fibers: a significant number of links is observed for es-FAQ(LDLK)$_3$/gp-PT fibers (right) whereas the fibers are not merged in the joints of no-PT es-FAQ(LDLK)$_3$/gp sample (left).

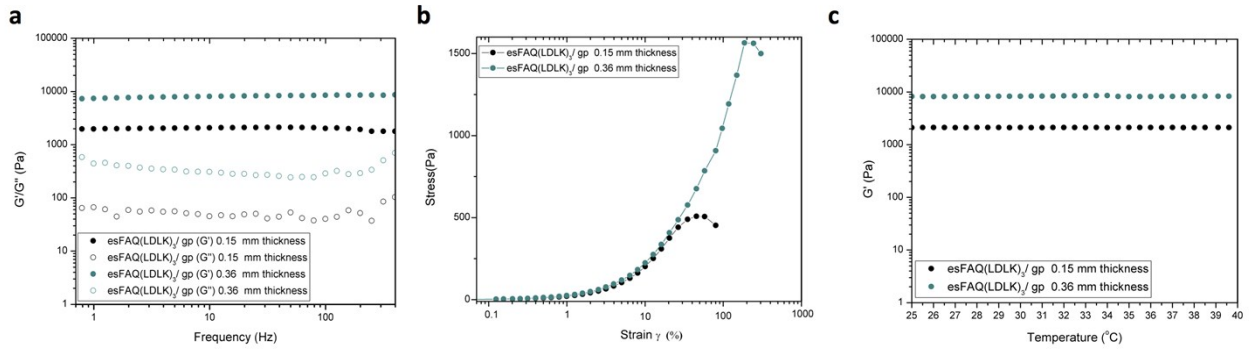


Figure S7. Rheological characterization of es-FAQ(LDLK)$_3$/gp nanofibrous mats with thickness of 0.185 mm (black dots) and 0.36 mm (cyan dots). (a) Trends of G' and G" by frequency sweep tests (0.1-1,000 Hz). (b) In stress-failure tests, the es-FAQ(LDLK)$_3$/gp with thickness of 0.36 mm showed a substantial failure stress increase compared with es-FAQ(LDLK)$_3$/gp with thickness of 0.185 mm, probably thanks to the greater number of covalent interactions due to the increased mat thickness. c) Temperature ramp of G' in the 25-40°C range (T_{rate}= 5°C/min, 1% strain, ω= 1Hz).

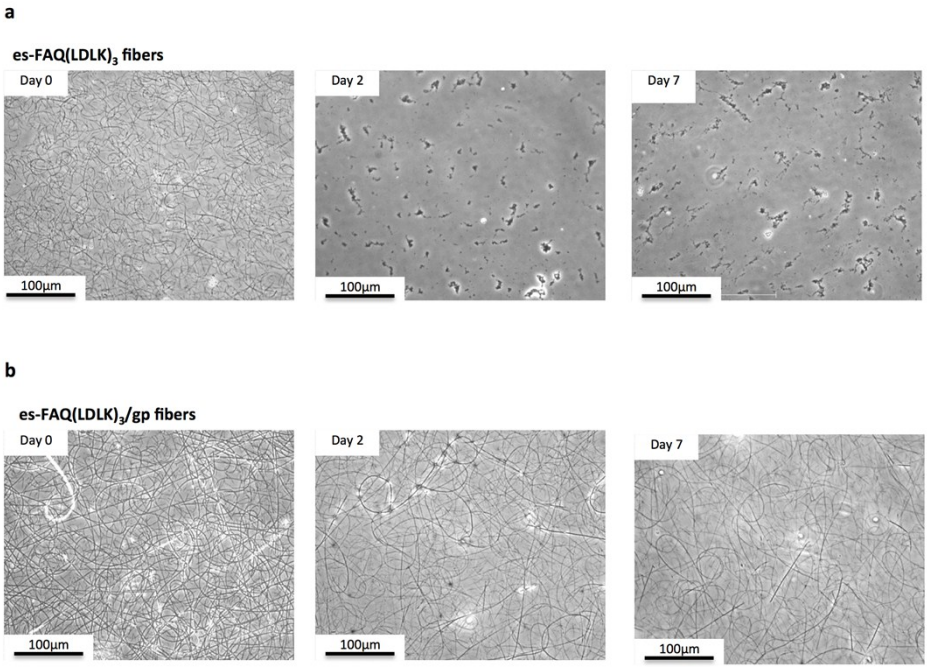


Figure S8. (a) Optical images of uncross-linked es-FAQ(LDLK)$_3$ and (b) cross-linked es-FAQ(LDLK)$_3$/gp fibers at dry conditions (Day 0) and upon exposure to PBS at 37°C (Day 2-7).

Contrarily to uncross-linked es-FAQ$(LDLK)_3$, electrospun fibers of FAQ$(LDLK)_3$/gp are stable in wet state over time, allowing for their long-term use.

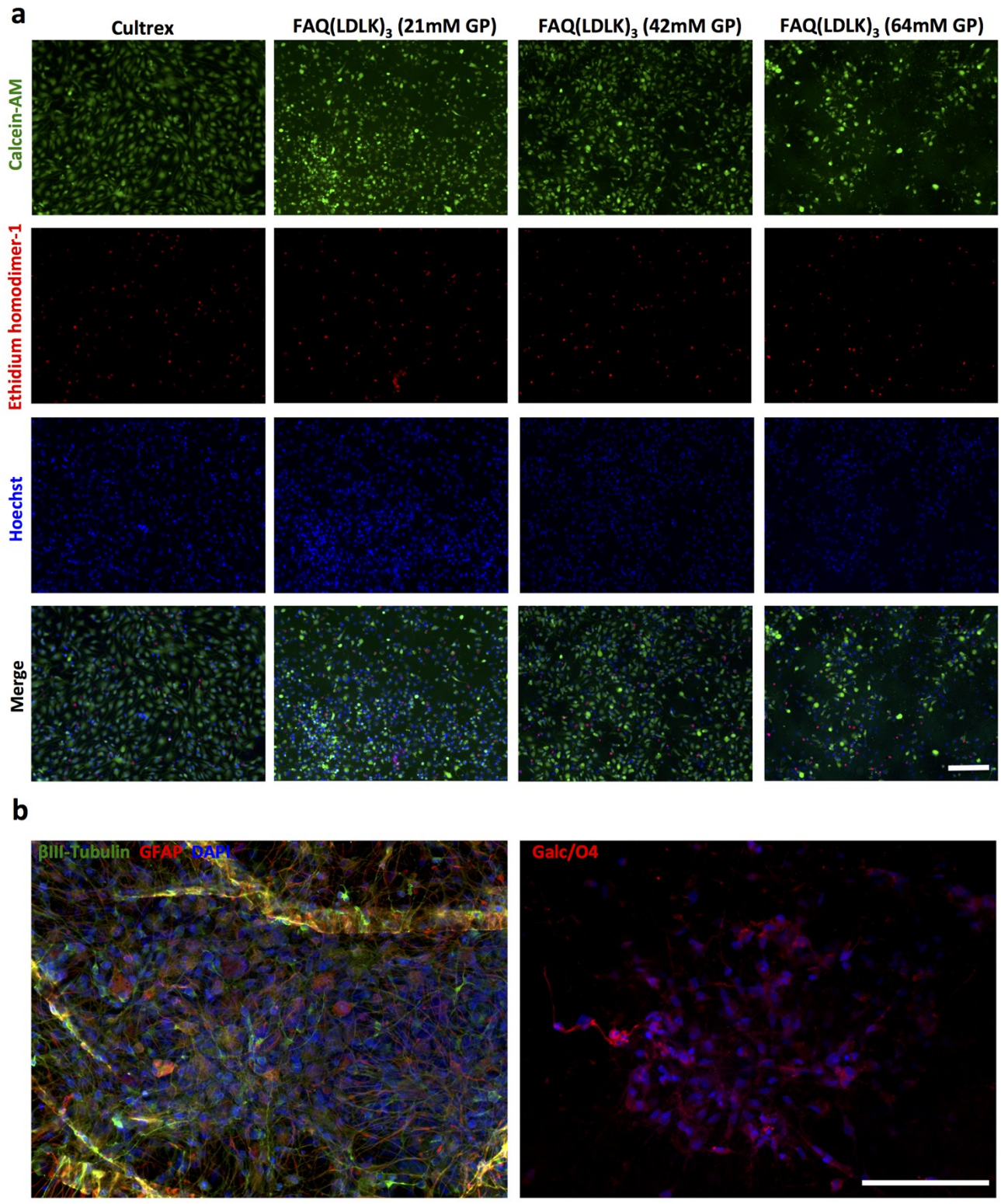


Figure S9. (a) Representative images of LIVE/DEAD assay on hNSC cells. Fluorescence staining of viable cells (green) and dead cells (red); Hoechst staining (blue) to highlight the nuclei. (b) Fluorescent micrographs of hNSC differentiated progeny on cross-linked electrospun FAQ(LDLK)$_3$/gp nanofibrous mats at 7DIV. Cells were stained for βIII-Tubulin

maker (neurons) in green, GFAP (astrocytes) in red and Galc/O4 (oligodendrocytes) in red. Cell nuclei were stained with DAPI. Scale bar in all images are 100 μm.