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Supporting Information

Macroporous Chitosan/Alginate Hydrogels Crosslinked with Genipin Accumulate and Retain Glioblastoma Cancer Cells

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Table S1. GNP compositions and CHI/GNP ratios in solutions and gels containing 0.75% w/v CHI.

GNP (%w/v)	0.0125	0.025	0.0375	0.05
CHI / GNP ratio	60	30	20	15

Table S2. GNP compositions and CHI/GNP ratios in solutions and gels containing 0.5% w/v CHI.

GNP (%w/v)	0.025	0.0375	0.05	0.075
CHI / GNP ratio	20	13.33	10	6.67

Table S3. Extracted data from microCT analysis for the PS and PLA domains: volume fractions ϕ , average domain sizes d and specific interfacial area S .

Annealing time (min)	5	10	20	30	45	60
ϕ_{PLA}	41	41	49	48	45	49
ϕ_{PS}	59	59	50	52	55	52
d_{PLA}	12 ± 2	22 ± 6	114 ± 30	187 ± 63	256 ± 77	373 ± 81
d_{PS}	15 ± 6	21 ± 4	105 ± 35	174 ± 49	259 ± 60	333 ± 81
Specific surface PLA (cm^{-1})	1531	752	211	92	70	48

Table S4. Porous gel mechanical properties in compression: effect of genipin crosslinking before VS after crosslinking with $CaCl_2$, ($N = 4$)

	SA 1%	SA 1% CHI 0.5%	SA 1% CHI 0.5% GNP 0.05%	SA 1% CHI 0.5% GNP 0.05% by diffusion
Compression modulus (kPa)	9.4 ± 1.4	5.3 ± 1.6	9.8 ± 3.1	8.2 ± 2.2

Table S5. Bulk and porous hydrogels (180 μm average pore size) compression modulus (kPa) as a function of SA and CHI contents, at 0.025% w/v GNP content.

	SA 0.5% CHI 1%	SA 1% CHI 0.25%	SA 1% CHI 0.5%	SA 1% CHI 0.75%	SA 1% CHI 1%
Bulk gels	11.5 ± 1.0	26.3 ± 9.9	20.5 ± 8.5	24.3 ± 9.4	Not tested
Porous gels	Can't stand	9.9 ± 1.6	12.6 ± 2.4	8.7 ± 2.4	9.8 ± 2.2

Table S6. Compression modulus (kPa) of porous gels (180 μm average pore size) as a function of GNP and CHI compositions.

[GNP] (% w/v)	SA 1% CHI 0.5%	SA 1% CHI 0.75%	SA 1% CHI 1%
0.025	12.5 ± 2.4	8.7 ± 2.4	9.8 ± 2.2
0.0375	6.6 ± 2.5	9.5 ± 3.8	7.3 ± 1.8
0.05	9.8 ± 3.1	10.3 ± 2.6	Not tested
0.1	7.6 ± 2.0	Not tested	Not tested

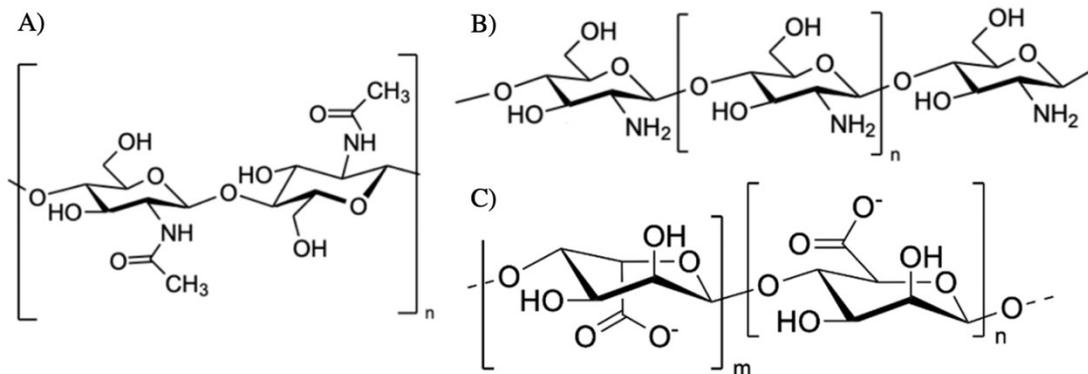


Figure S1. Molecular structure of A) chitin B) chitosan and C) sodium alginate.

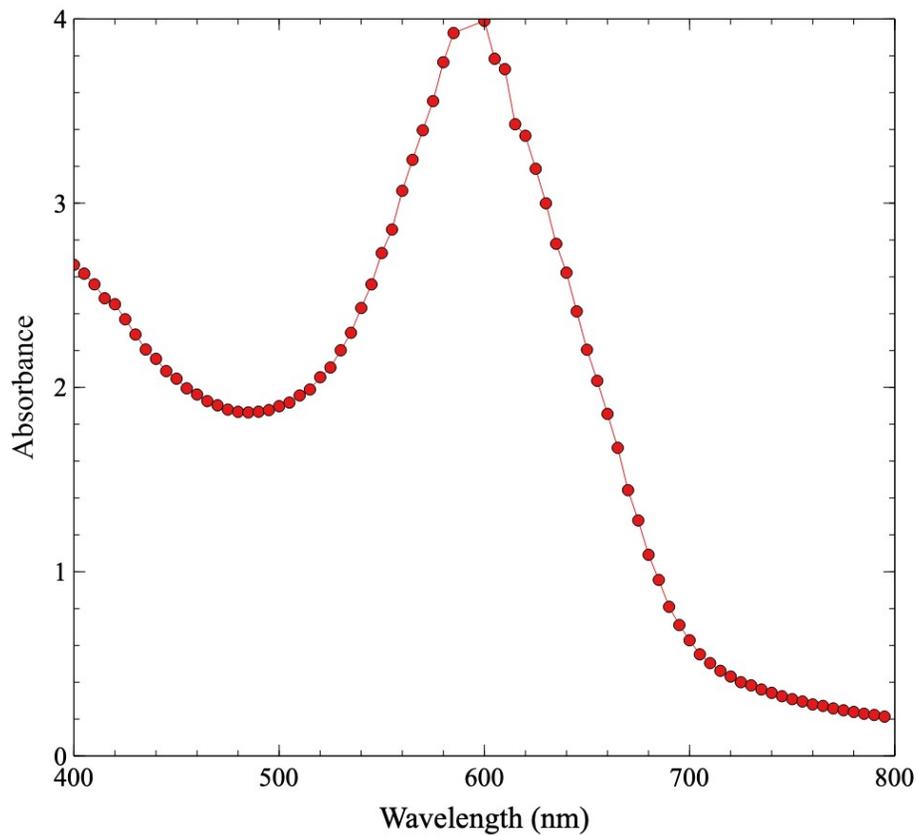


Figure S2. Absorbance master curve of CHI 0.75% GNP 0.05% w/v after 24h of gelation time at 37 °C in a plate well.

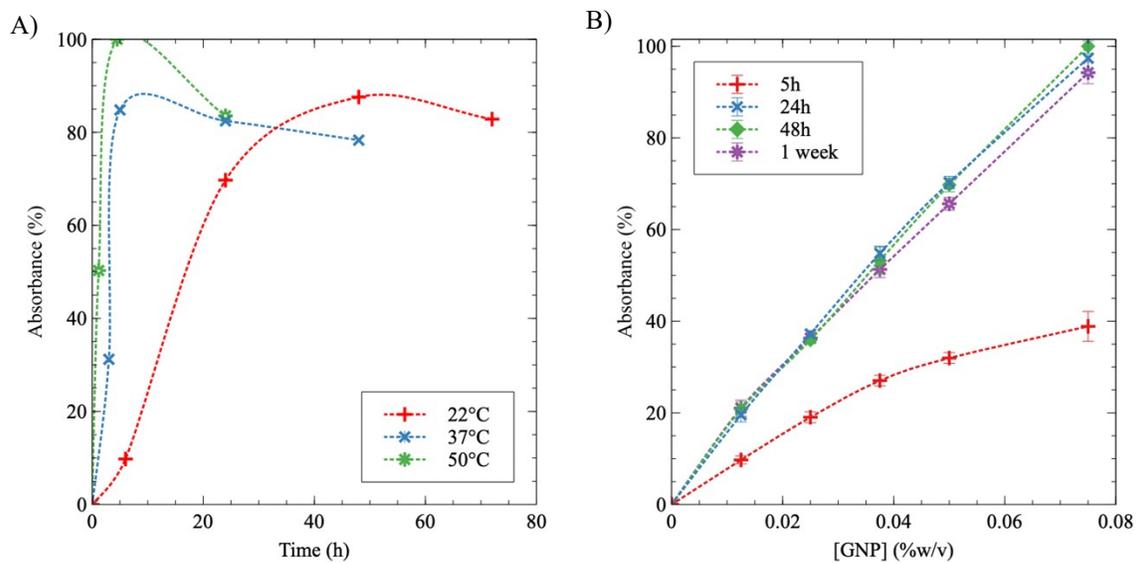


Figure S3. Chitosan (CHI) crosslinking kinetics using genipin (GNP) as monitored by UV-Vis spectroscopy. A) Absorbance as a function of time t and temperature T , at $[\text{CHI}] = 0.5\% \text{ w/v}$ and $[\text{GNP}] = 0.025\% \text{ w/v}$; B) Absorbance as a function of time t and GNP composition, at $[\text{CHI}] = 0.5\% \text{ w/v}$ and 37°C .

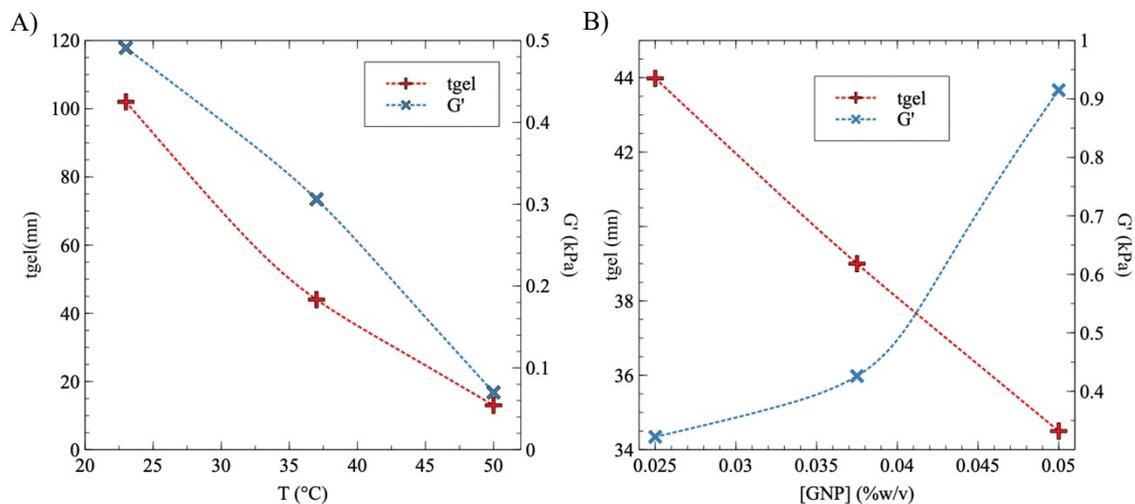


Figure S4. A) Gelation time (t_{gel}), and storage modulus at equilibrium (G') as a function of temperature T , for SA 1% CHI 0.5% GNP 0.05% w/v solutions; B) Gelation time (t_{gel}) and storage modulus at equilibrium (G') as a function of GNP concentration for SA 1% CHI 0.5% w/v at 37 °C. The decrease in G' observed in (A) is due to syneresis.

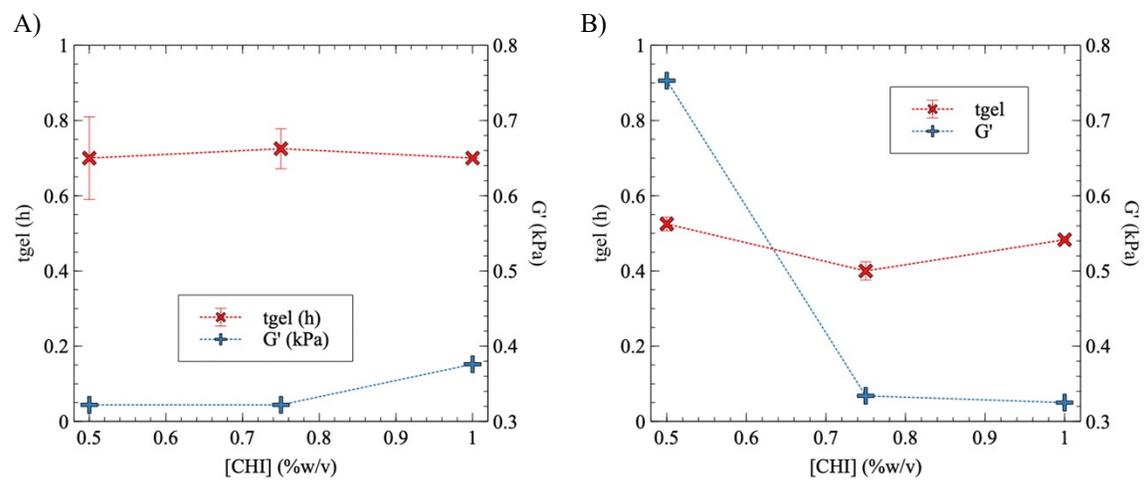


Figure S5. Influence of CHI composition on t_{gel} and final G' values, at constant SA (1% w/v) content, and at GNP compositions of (A) 0.025% and (B) 0.05% w/v. The decrease in G' in (B) is due to syneresis.

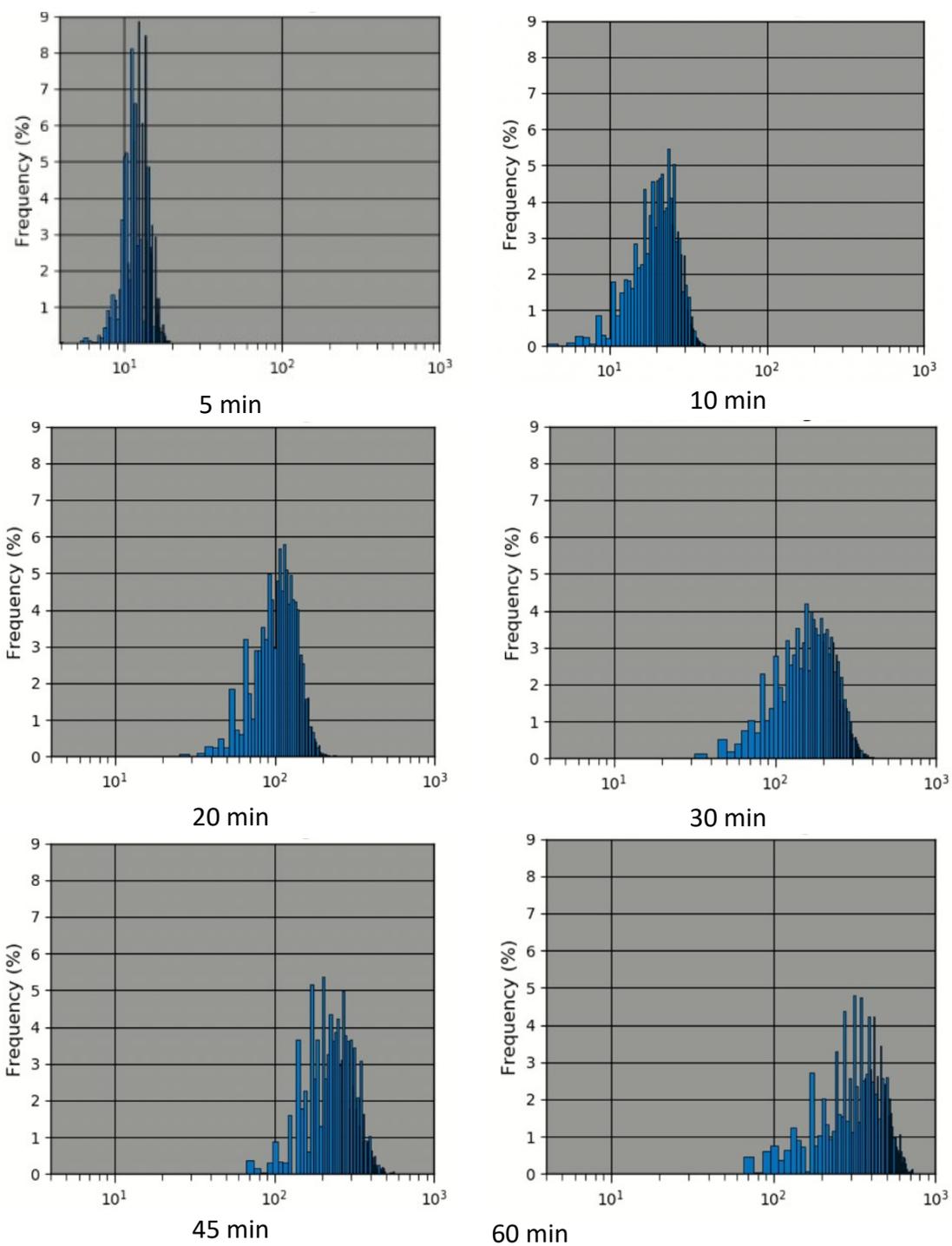


Figure S6. Pore size distributions for different annealing times. The x -axis is in μm .

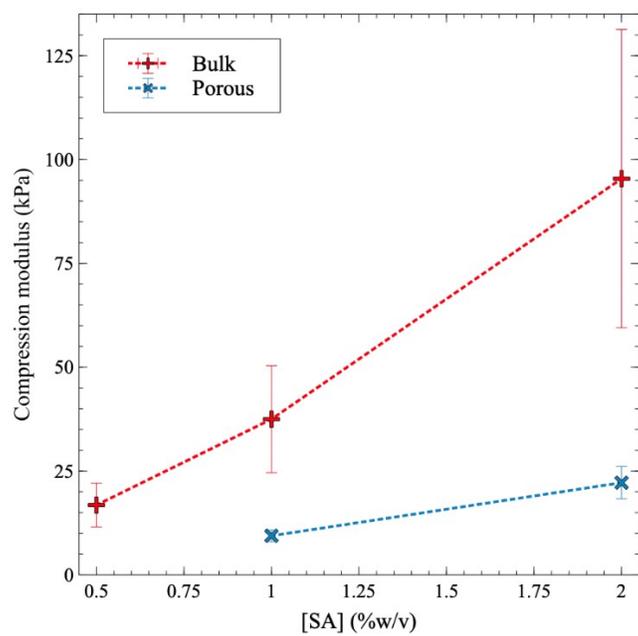


Figure S7. Compression modulus of sodium alginate (SA) bulk and porous (pore size = 180 μm) gels as a function SA composition.



Figure S8. SA 1% CHI 0.75% GNP 0.025% w/v (left) and SA1% CHI0.5% GNP0.025% w/v (right) after (A) 1 week in PBS and (B) 2 weeks in DMEM medium.

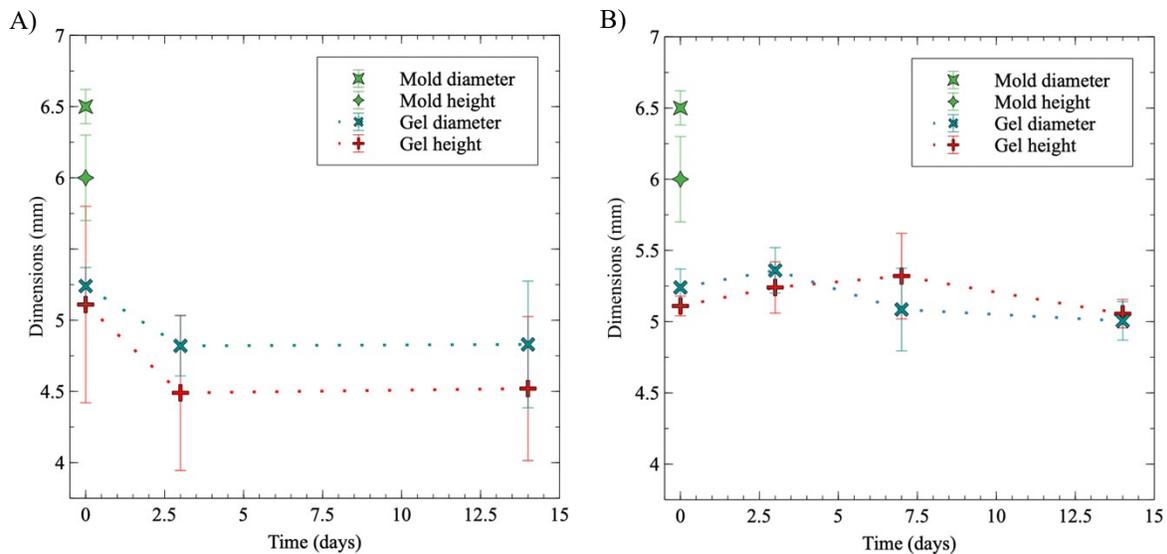


Figure S9. Evolution of porous gels dimensions (1% w/v SA, 0.75% w/v CHI, 0.025% w/v GNP, average pore size = 180 μm) in a) PBS and b) DMEM medium, as a function of time, and compared to the initial PLA molds dimensions.

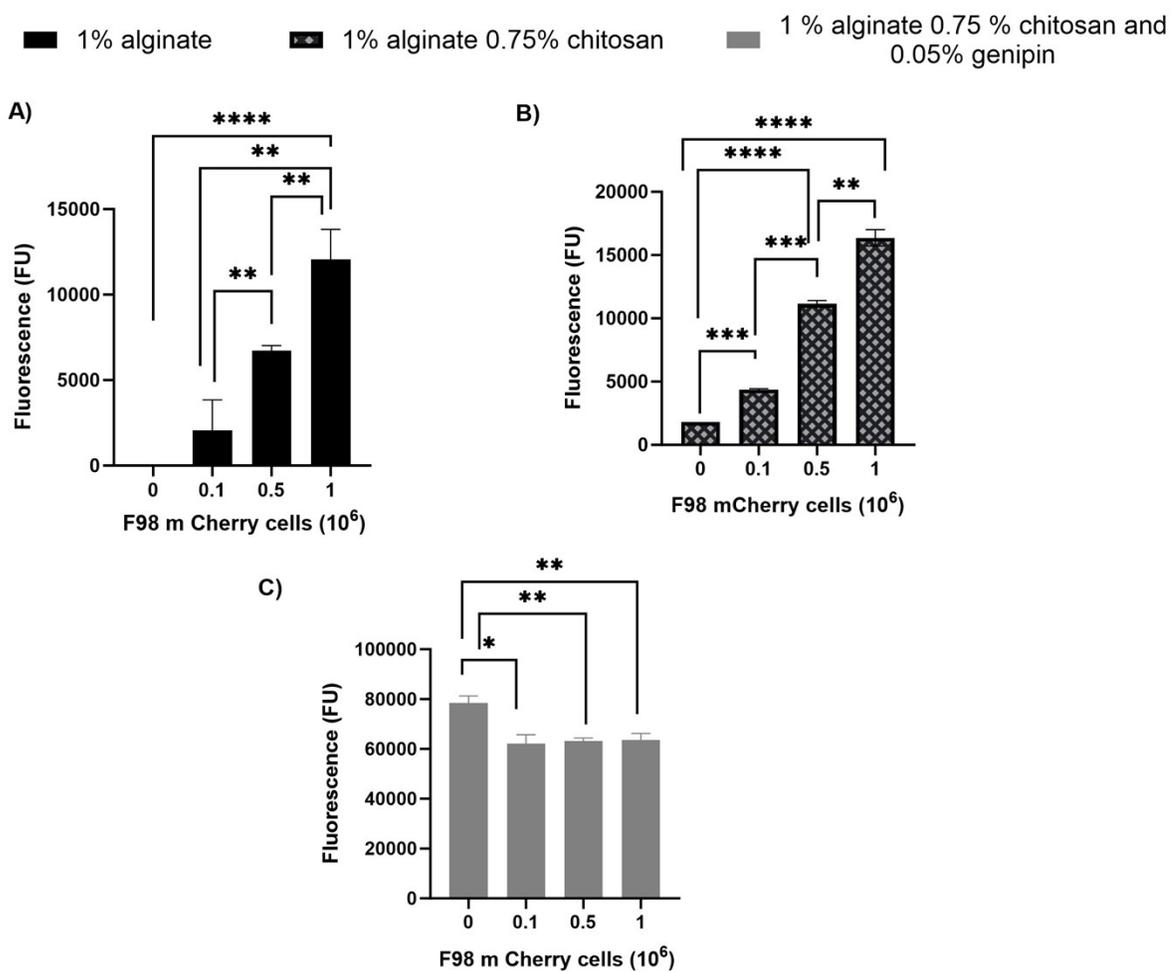


Figure S10. F98 mCherry cells fluorescence intensity in SA 1% hydrogels (A), SA 1% CHI 0.75% hydrogels (B), and SA 1% CHI 0.75% GNP 0.05% w/v hydrogels (C).