Supporting Information

Stress Relaxation and Composition of Hydrazone-Crosslinked Hybrid Biopolymer-Synthetic Hydrogels Determine Spreading and Secretory Properties of MSCs

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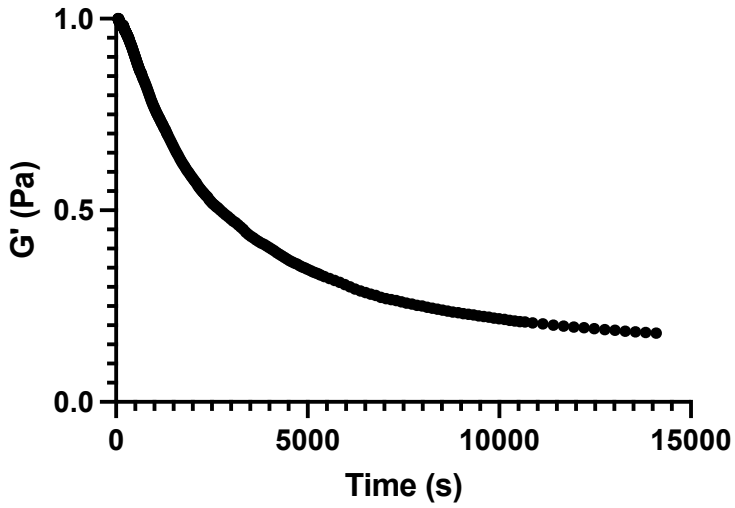

Figure S1. Day 4 Stress Relaxation Curve. The stress relaxation curve for the 88% alkyl hydrazone bonds condition after 4 days, with greater than 80% of the stress relaxed after 4 days. Illuminating how after 4 days the material platform relaxes stress to a similar degree as compared to the in-situ stress relaxation.

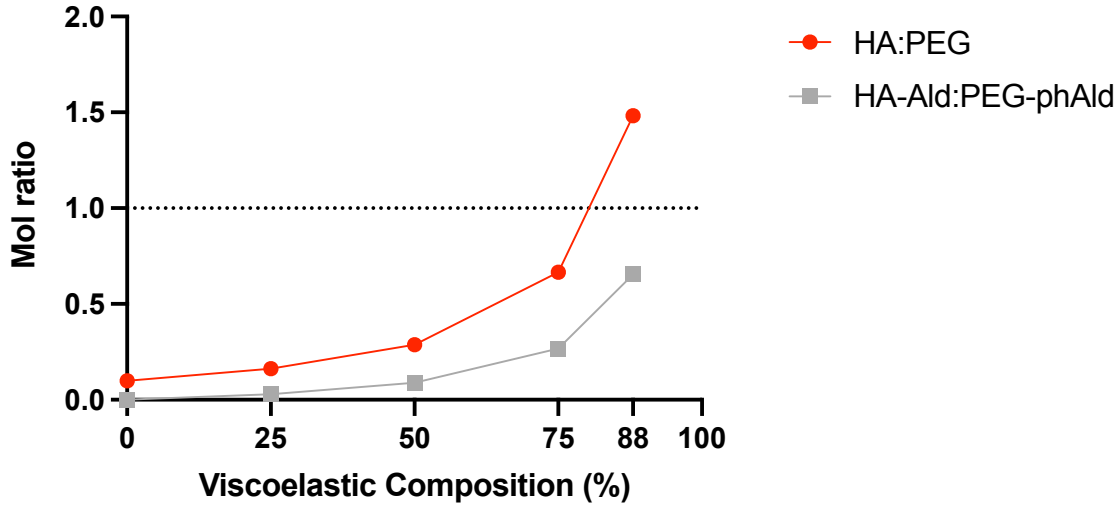

Figure S2. Molar Ratio of HA: PEG. The mol ratios of HA:PEG and HA-Ald: PEG-phAld were calculated for each alkyl hydrazone condition, indicating the 88% alkyl hydrazone condition was the only condition with a HA: PEG > 1. Initially, mass ratios of macromer components were calculated by multiplying the fractional composition of functional groups in the final hydrogel formulations by the molecular weight per functional group in each macromer system (i.e., 5000 Da for 8-arm 40 kDa). Next, these mass ratios were divided by the molecular weight of each macromer to result in the mol ratio of each component.

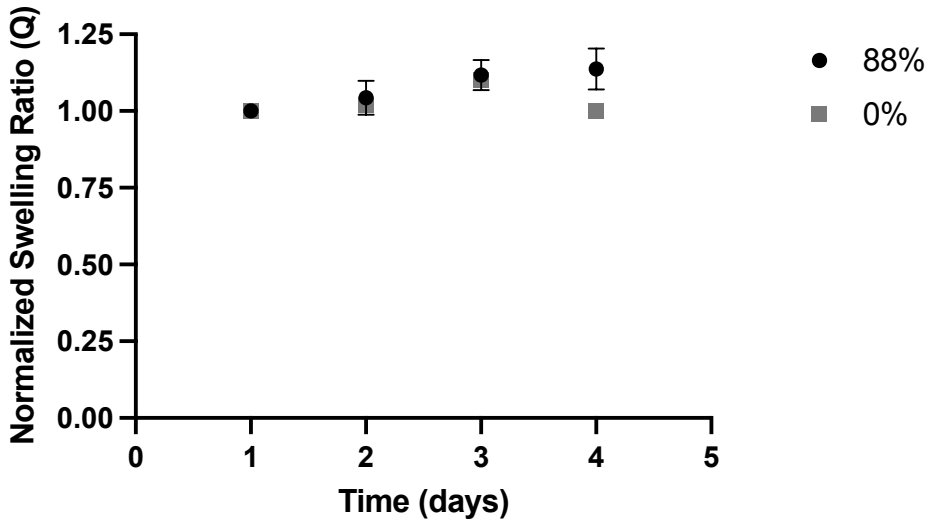

Figure S3. Normalized Swelling Ratio Over 4 Days. The swelling ratios over 4 days, in cell culture media, for the 88% and 0% alkyl hydrazone conditions were analyzed. After 4 days, the swelling ratio had significantly changed from the initial equilibrium swelling ratio achieved after 24 hours ($p<0.05$). However, the swelling ratio had not significantly changed between days 2-4, indicating an equilibrium within the hydrogel.

$$Q = \frac{\text{Swollen Mass}}{\text{Dry Mass}} \tag{S1}$$

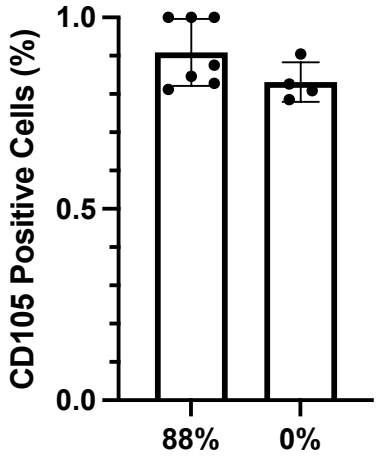

Figure S4. Phenotype of MSCs after 4 Days in Culture. MSCs were stained for CD105, a stem marker, after 4 days in culture, in the 88% and 0% alkyl hydrazone conditions. In the 88% alkyl hydrazone condition showed greater than 90% of the MSCs expressed the integrin for CD105, including the small cluster formations, and greater than 80% expressed the integrin for CD105 in the 0% alkyl hydrazone condition. This indicates that after 4 days in culture the MSCs remain multipotent.

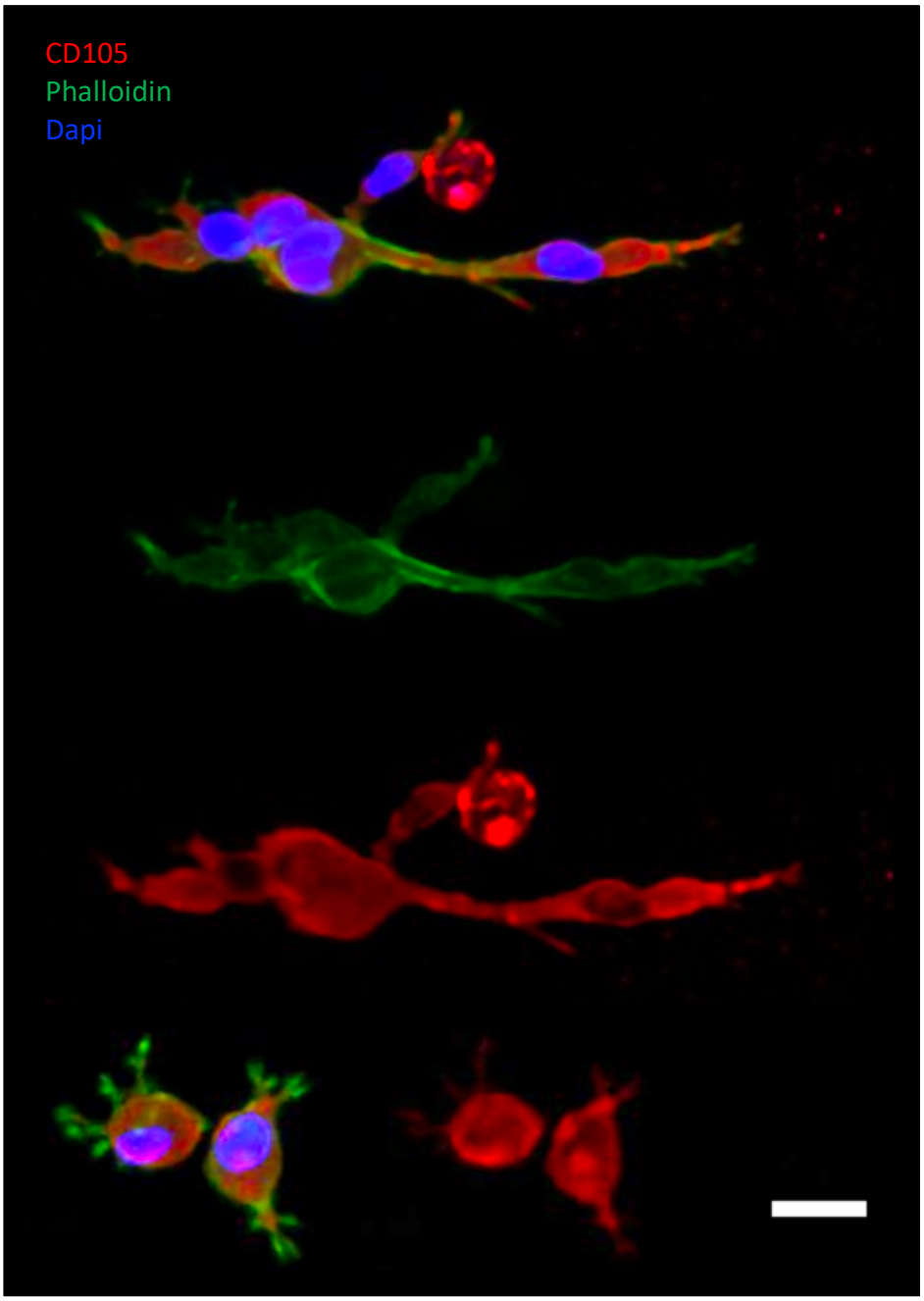

Figure S5. MSCs Maintain Multipotency. CD105 integrin expression was identified in both single cells and cell clusters in the 88% alkyl hydrazone condition indicating MSC multipotency after 4 days in culture. Scale bar = 20 μm.

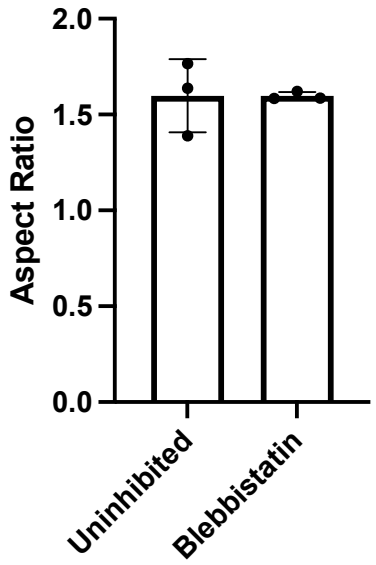

Figure S6. MSCs Retain Spread Morphology in Presence of Blebbistatin. The 88% alkyl hydrazone condition was utilized to investigate the influence of actomyosin contractility, and it was determined that the spreading of the MSCs was not dependent upon Myosin II.

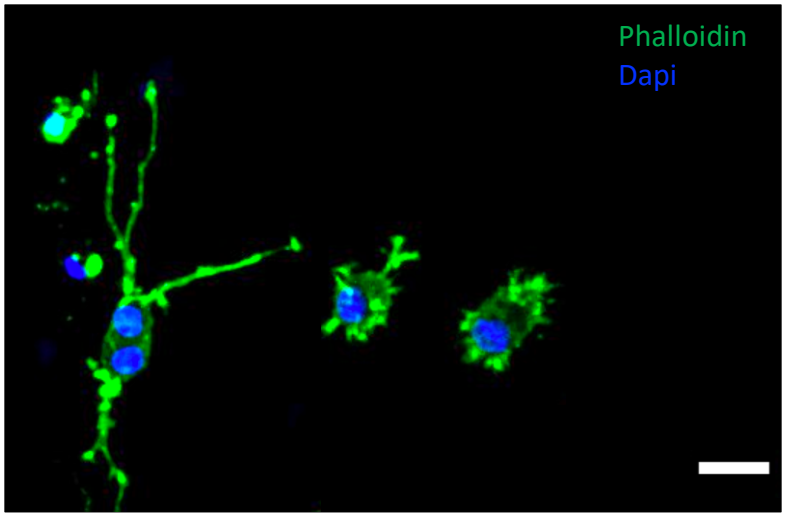

Figure S7. Visualization of MSC Spreading in the Blebbistatin Inhibited Condition. In the 88% alkyl hydrazone condition, with Blebbistatin inhibition, there was a distinct change in MSC spreading shape, as the filipodia were seen to be thinner spindle-like structures, with the cell body remaining relatively rounded, and nearby cells no longer forming clusters, scale bar = 20 μm.

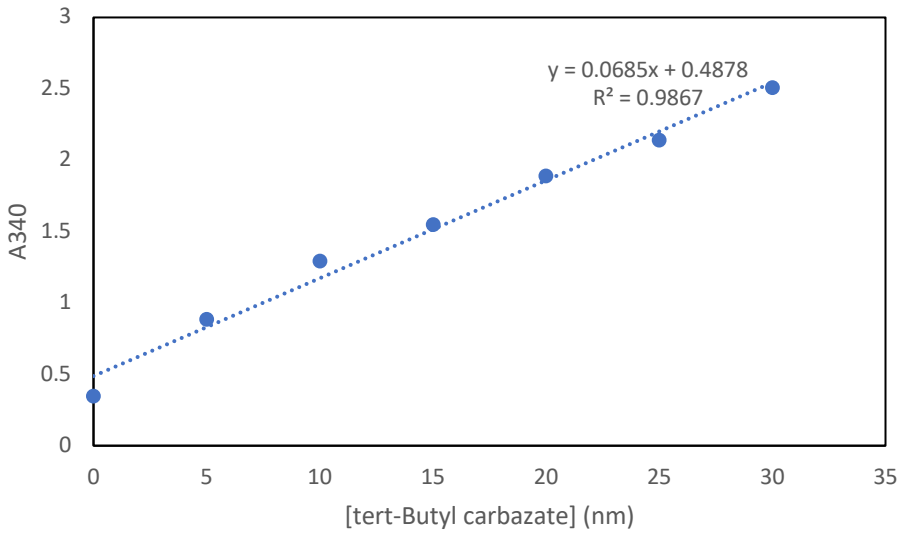

Figure S8. Characterization of HA-Ald. The HA-Ald modification was quantified via a TNBS assay, where tert-Butyl carbazate was reacted with the HA-Ald and quantified with TNBS. HA-Ald was found to have ~37% modification of the disaccharide repeat units.

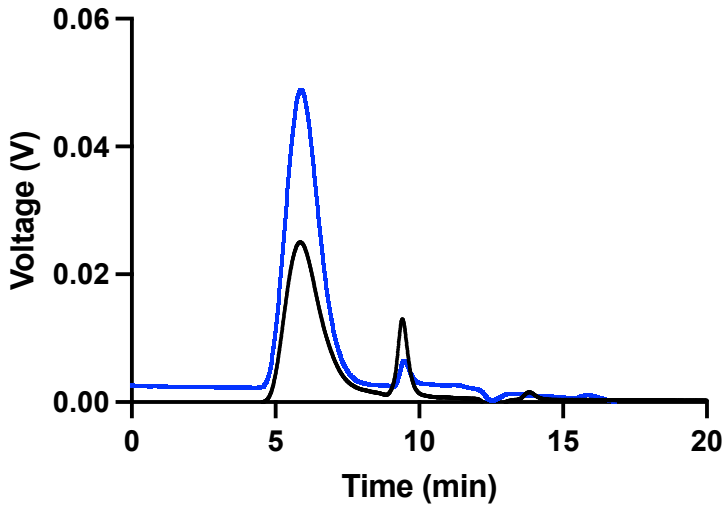

Figure S9. GPC Characterization of HA-Ald and HA-Hyd. GPC traces of HA-Ald (black) and HA-Hyd (blue), determined to be ~96 kDa and ~85 kDa, respectively.

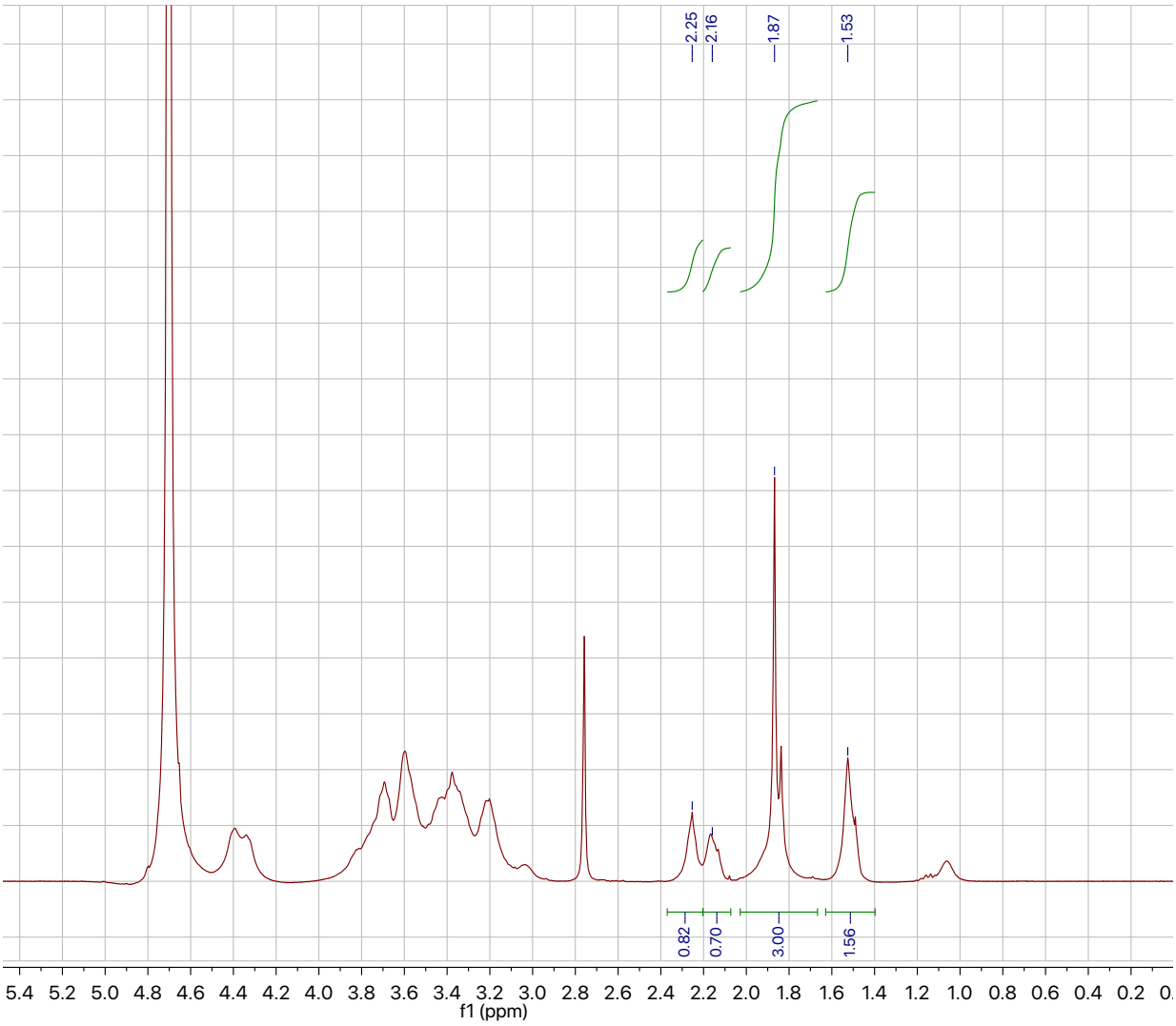

Figure S10. ^{1}H NMR characterization of HA-Hyd. The HA-Hyd modification was determined using ^{1}H NMR (400 MHZ, deuterium oxide) and integrating the butyl linker (8H, 2.1-2.4 ppm, 1.4-1.6 ppm) relative to the methyl group of HA (3H, 1.7-2.0 ppm). Functionalization was found to be ~39% of disaccharide repeat units.

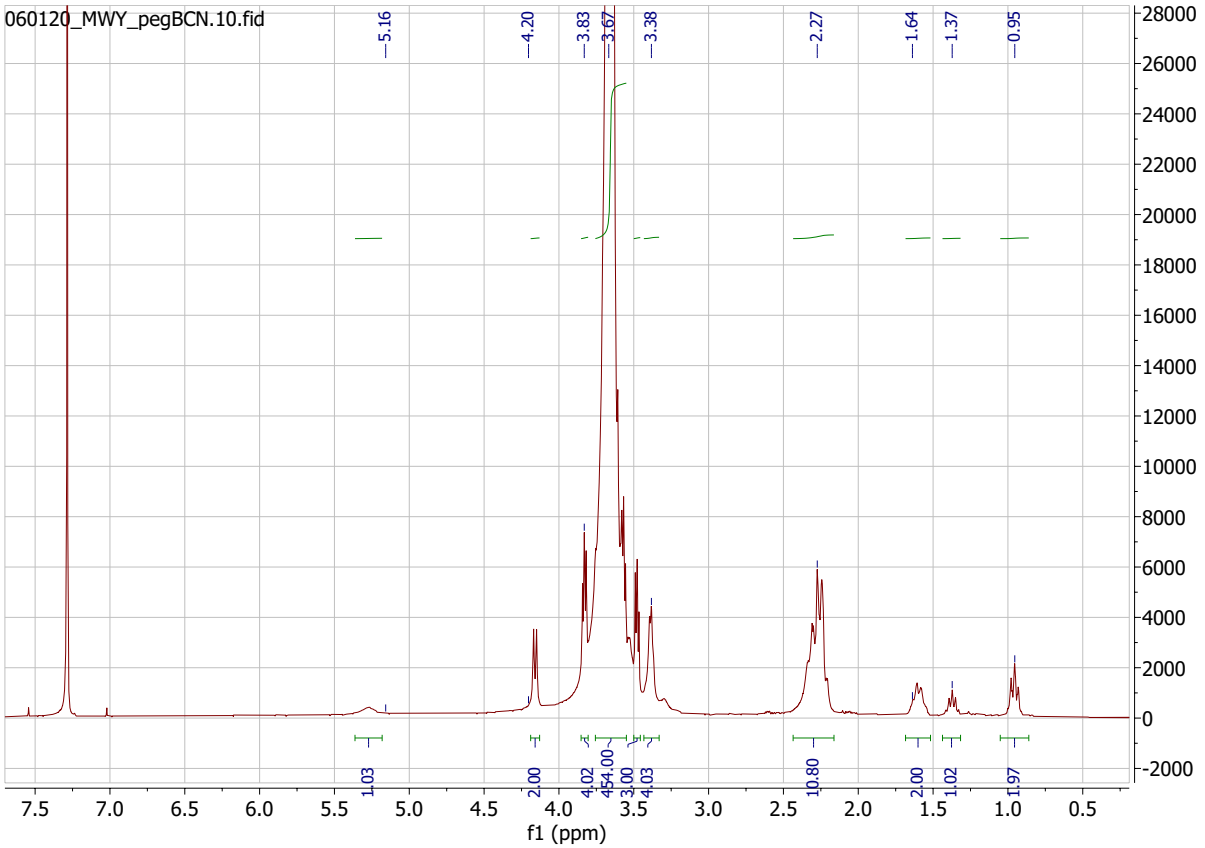

Figure S11. ^{1}H NMR characterization of Peg-BCN. The end group functionality of Peg-BCN was determined by using ^{1}H NMR (400 MHZ, $CDCl_3$) and comparing the integrated values of characteristic BCN peaks 5.16 ppm (1H), 1.64 ppm (2H), 1.37 ppm (1H), and 0.95 ppm (2H) to those of the PEG backbone 3.67 ppm (454H per Peg arm). Functionalization was found to >95%.