Supramolecular assembly of multifunctional protein gels via an *N*-glycosylation consensus sequence fusion domain

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Electronic Supplementary Information

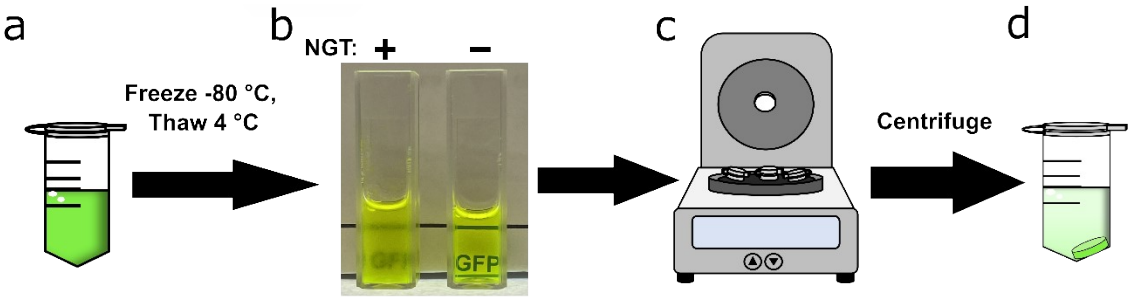

Fig. S1. Schematic of the formation of NGT-fusion protein material. (a) Purified protein is frozen at -80 °C and thawed at 4 °C. (b) The freeze-thaw cycle of NGT-fused proteins causes formation of visible assemblies. 30 μM NGTsfGFP (left) is visibly turbid and obscures the lettering in the background, compared to sfGFP (right), which is optically clear. (c) Assemblies are centrifuged. (d) Assemblies pack into a self-supporting material after centrifugation.

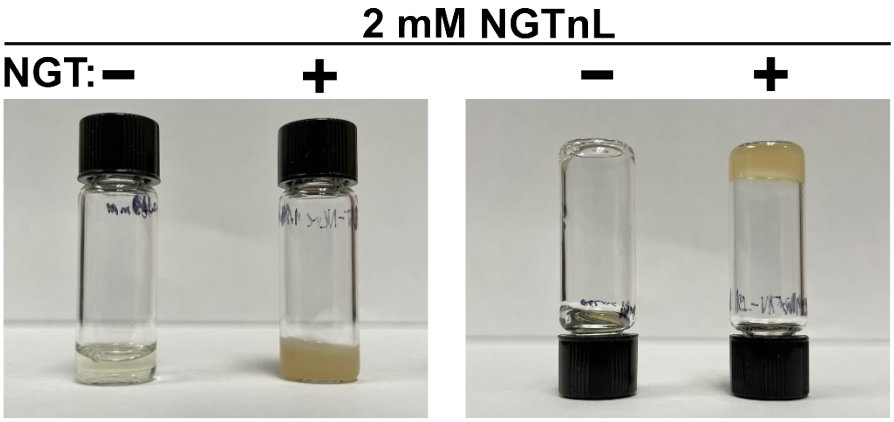

Fig. S2. Vial inversion test of nL (-) and NGTnL (+) at 2 mM after freezing at -80 °C and thawing at 4 °C. nL flowed under these conditions, whereas NGTnL did not.

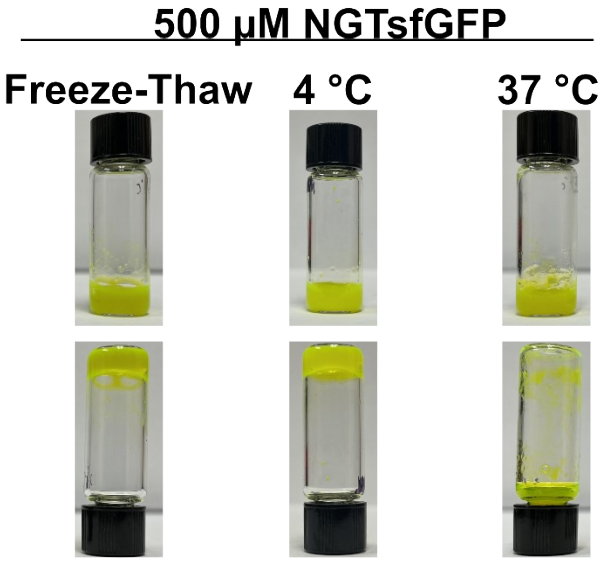

Fig. S3. 500 µM NGTsfGFP processed under different temperature conditions. When frozen at -80 °C and thawed at 4 °C, or kept cool at 4 °C, NGTsfGFP formed a self-supporting material that did not flow due to gravity. When incubated at 37 °C with no exposure to cold temperature, 500 µM NGTsfGFP flowed due to gravity when inverted.

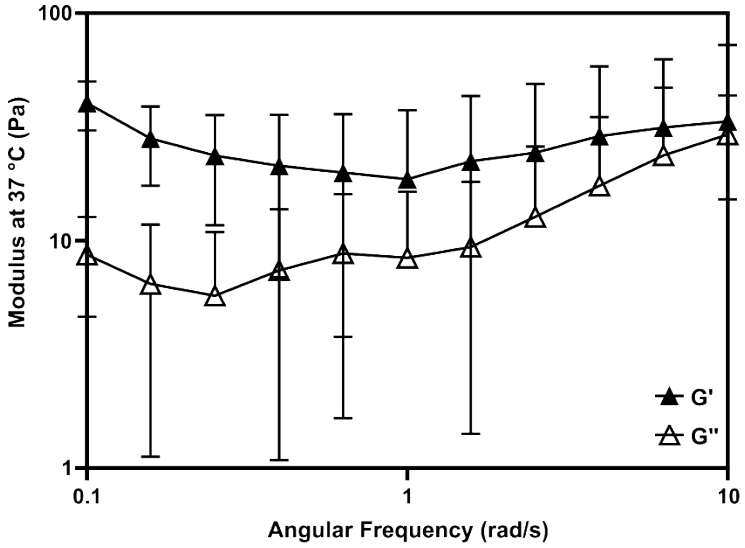

Fig. S4. NGTsfGFP gels formed via refrigeration at 4 °C alone are softer than gels formed using a freeze-thaw cycle. Refrigeration alone gels had an average storage modulus of 40 ± 9.8 Pa at 0.1 rad/s.

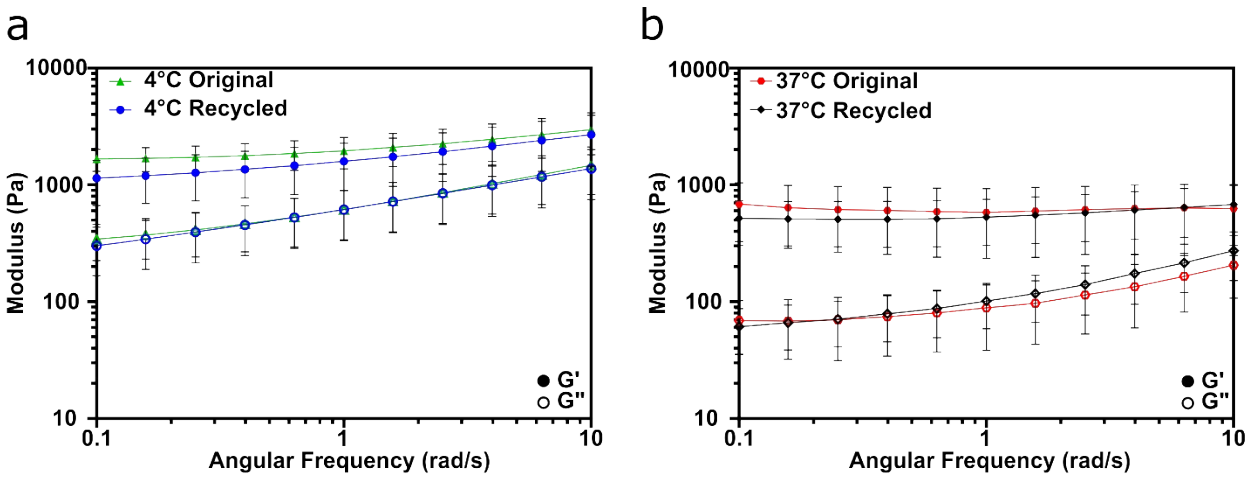

Fig. S5. Frequency sweep from 10 to 0.1 rad/s of 1000 µM NGTsfGFP before and after gel recycling. Storage modulus (G' open circles) and loss modulus (G", open circles) were not significantly different after recycling at 4 °C and 37 °C.

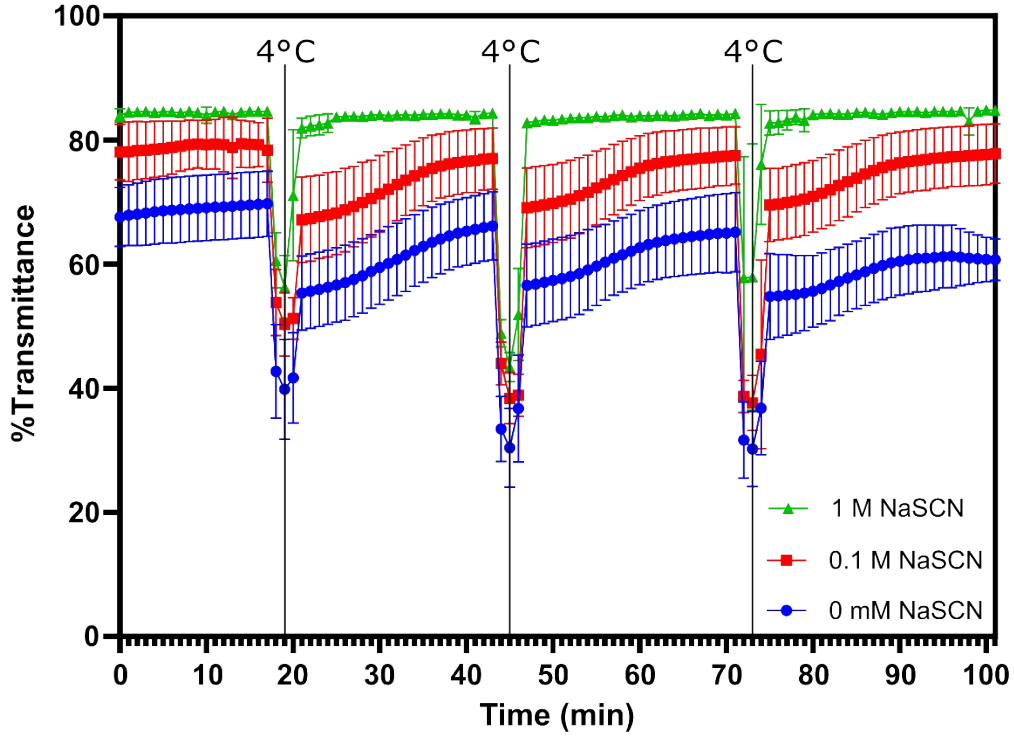

Fig. S6. Transmittance at λ = 600 nm of 100 µM NGTsfGFP from 4 °C to 55 °C over multiple cycles in the presence of 0 M, 0.1 M, or 1 M NaSCN. Vertical lines denote samples cooled to 4 °C to restart the cycle. Trends observed in Fig. 3d persist over these heating and cooling cycles.

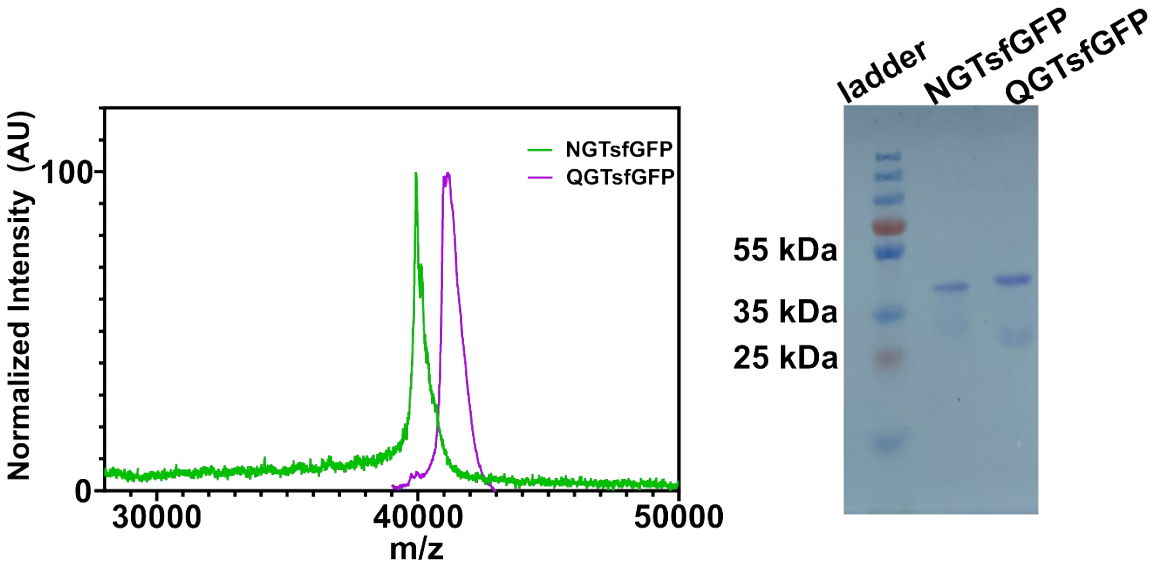

Fig. S7. MALDI-TOF MS spectra and SDS-PAGE analysis of QGTsfGFP. The mass spectra and SDS-PAGE agree with the QGTsfGFP expected molecular weight of 41.1 kDa.

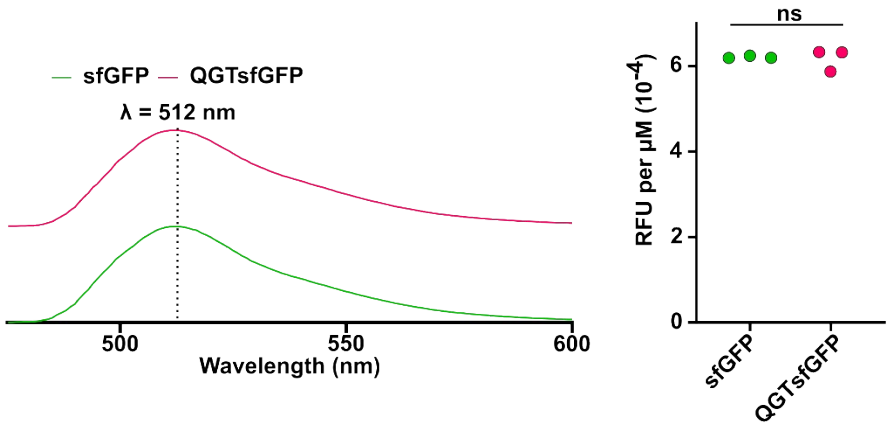

Fig. S8. Offset emission spectra and relative fluorescent units of sfGFP and QGTsfGFP in the presence of λ = 465 nm wavelength light, n=3 technical replicates, significance determined by Student's t-test (pairwise comparison). No shift in fluorescence emission or change in RFU was detected between these proteins.

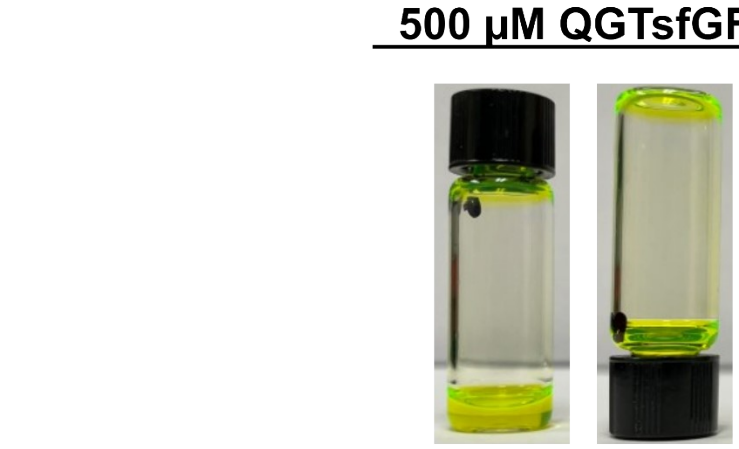

Fig. S9. 500 µM QGTsfGFP after being frozen at -80 °C and thawed at 4 °C. Vial inversion demonstrated that QGTsfGFP under these conditions flowed due to gravity and did not form a gel.

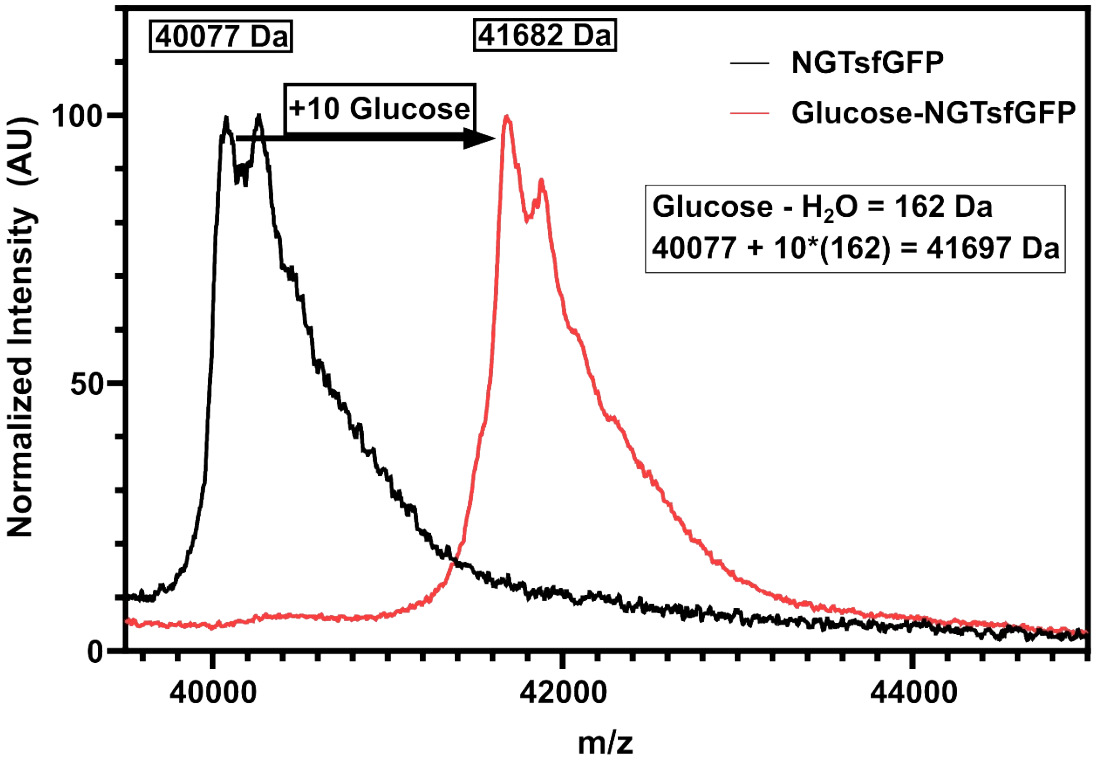

Fig. S10. MALDI-TOF MS spectra of NGTsfGFP (black trace) and glucose modified NGTsfGFP (blue trace). The difference between the intact mass peaks is the exact mass of ten covalently linked glucose molecules.

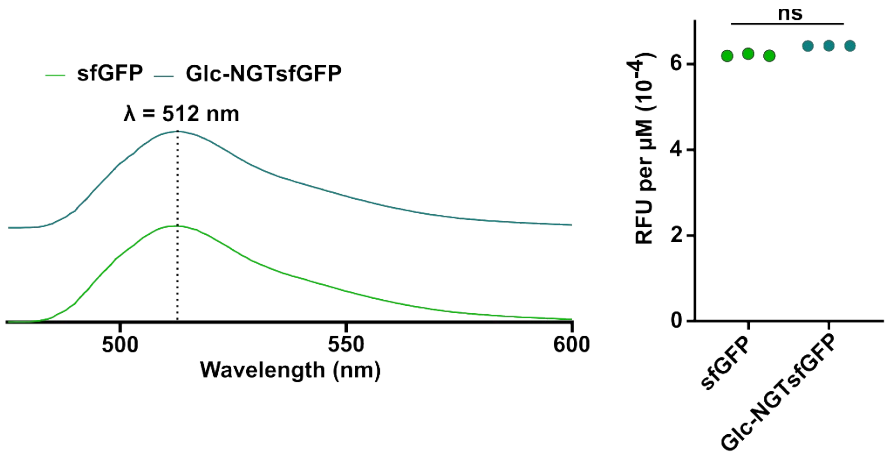

Fig. S11. Offset emission spectra and relative fluorescent units of sfGFP and Glc-NGTsfGFP in the presence of λ = 465 nm wavelength light, n=3 technical replicates, significance determined by Student's t-test (pairwise comparison). No shift in fluorescence emission or change in RFU was detected between these proteins.

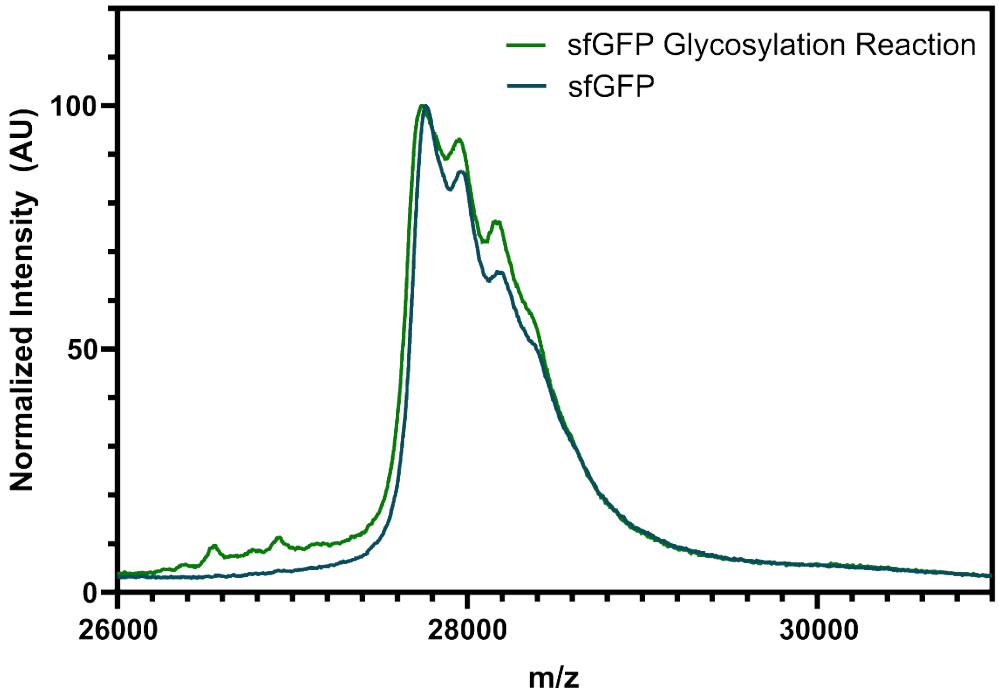

Fig. S12. MALDI-TOF MS spectra of unmodified sfGFP (blue trace) and sfGFP that was incubated with ApNGT and UDP-glucose (green trace). No detectable difference in intact mass peaks demonstrates that glucose is not conjugated to sfGFP under these conditions.

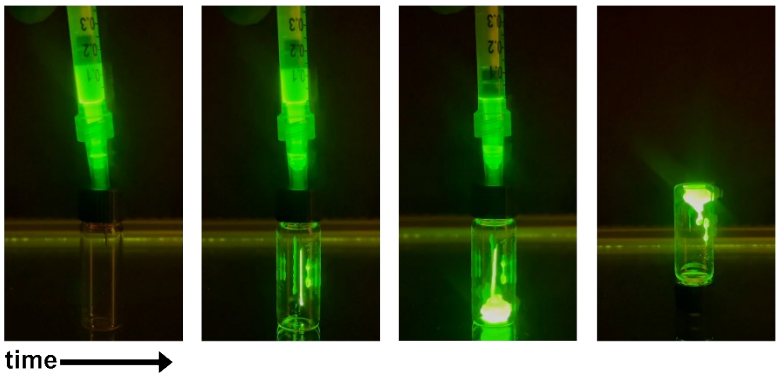

Fig. S13. Still images of 1 mM NGTsfGFP before and after extrusion from a 25-gauge syringe. The last still shows that directly after extrusion the NGTsfGFP gel does not flow when the vial is inverted.

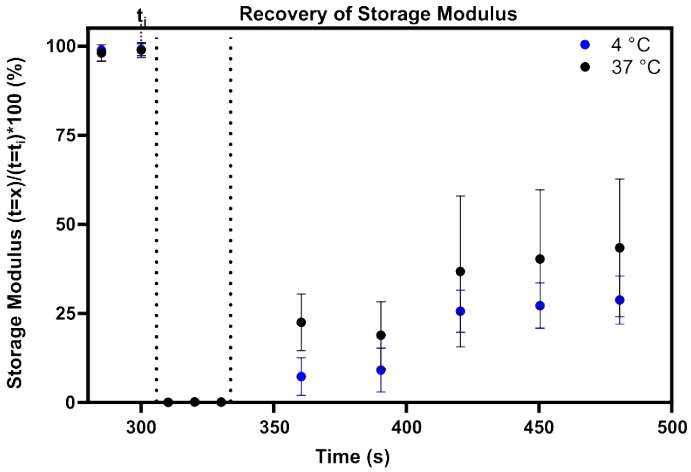

Fig. S14. Recovery of the storage modulus of 1 mM NGTsfGFP after high strain disruption at 4 °C and 37 °C, normalized to t = t_i = 300 s. Storage modulus measured at 0.3% strain before and after dashed lines, and 1000% strain in-between dashed lines. Recovery of the storage modulus was not noticeably impacted by temperature of the gel.