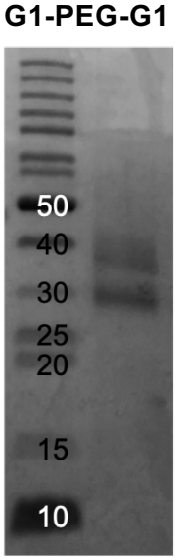
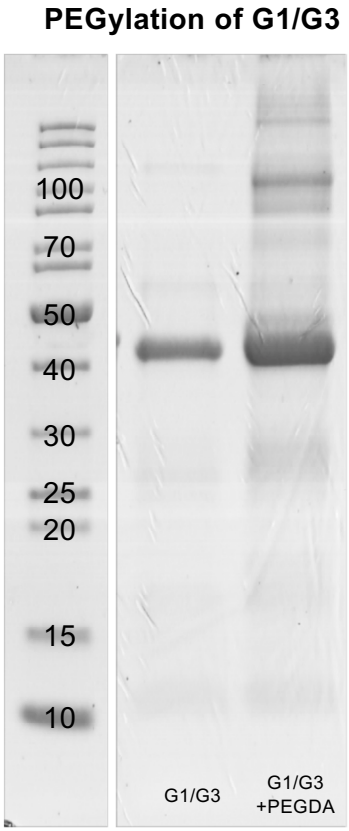
Electronic Supplementary Material (ESI) for Biomaterials Science.

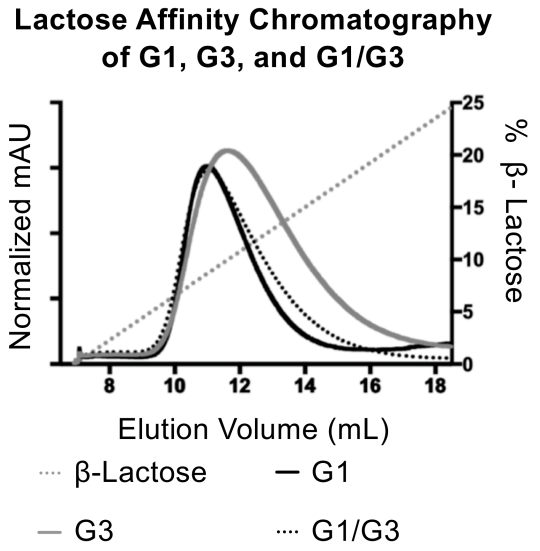
Supplemental Information



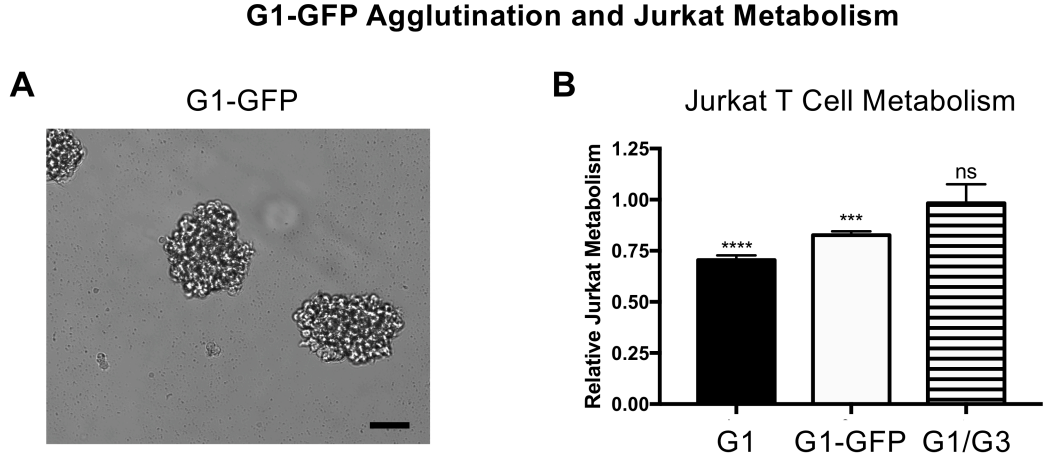
Supplemental Figure 1: Synthesis of G1-PEG-G1.
To synthesize a stable homodimeric G1 variant ("G1-PEG-G1") we used an previously reported protocol.[1] In particular, 30 μM of a G1 variant having one cysteine residue on its surface ("C2S/C16S/C88S G1") was stirred with 25-fold excess of poly(ethylene glycol) diacrylate (M_n= 2000 Da) overnight protected from light at room temperature. The resulting product was characterized with SDS-PAGE.



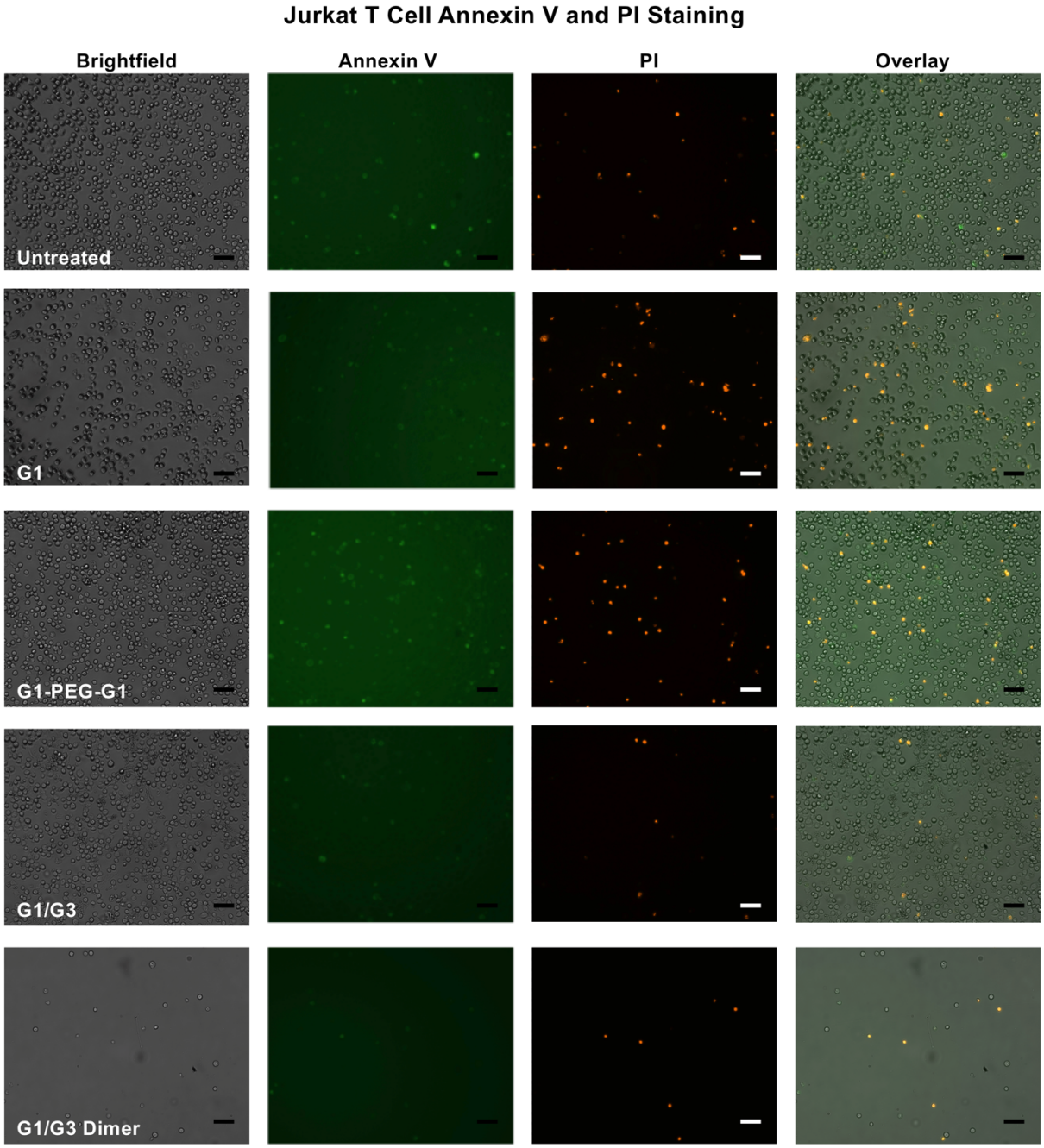
Supplemental Figure 2: PEGylation of G1/G3.
A G1/G3 variant with one surface cysteine ("(C2S/C16S/C88S)G1/G3") was expressed and purified using similar methods as reported for G1/G3. We attempted to form a G1/G3 dimer via PEGylation ("G1/G3-PEG-G1/G3") by adapting the previously reported protocol for synthesis of G1-PEG-G1.[1] The resulting product was characterized with SDS-PAGE. The product of the G1/G3 + PEGDA reaction migrated to a similar distance as G1/G3 in the absence of PEGDA demonstrating that G1/G3-PEG-G1/G3 was not formed.



Supplemental Figure 3: **Lactose affinity chromatography of G1, G3, and G1/G3.** Proteins were eluted with a linear gradient of β-lactose in PBS. Proteins were detected via absorbance at 280 nm. The right shifted elution volume for G3 indicated it had higher affinity for immobilized lactose than G1 or G1/G3.



Supplemental Figure 4: G1-GFP Bioactivity on Jurkat T cells.
(A) Brightfield micrographs of Jurkat T cells after 18 h treatment with 5 μM G1-GFP. (B) Normalized metabolic activity of Jurkat T cells incubated with G1, G1/G3, and G1-GFP. *** $p < 0.001$ and **** $p < 0.0001$. Scale bar is 50 μM.



Supplemental Figure 5: Annexin V and PI staining of protein treated Jurkat T Cells.
Phosphatidylserine exposure was determined via Annexin V-FITC staining and membrane permeability was determined via propidium iodide (PI) after Jurkat T cells were treated with 5 μM protein for 4 hours. Scale bars are 50 μM

Reference

1. M. Fettis and G. Hudalla, *Bioconjugate Chemistry*, 2018, **29**, 2489-2496.