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Supplemental information

A scalable and tunable thermoreversible polymer for 3D human pluripotent stem cell biomanufacturing

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Supplement Figures

A

Observation	Semi-Toxic	Toxic			Non-Toxic	
Independent Lot	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
LCST	21°C	17°C	16°C	14°C	21°C	20°C

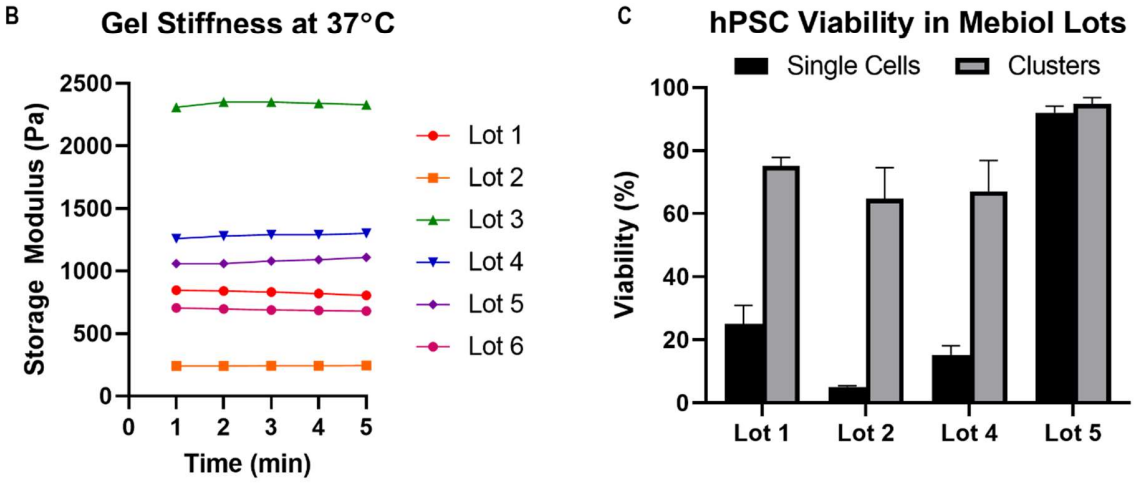

Figure S1. Lot-to-lot variability of Mebiol PEG-PNIPAAm hydrogels, Related to Figure 1 and 2. A) Mebiol lot designation, observed toxicity, and resulting LCST, as determined via rheometry. B) Gel stiffness maintained over time at 37°C for various Mebiol lots. C) Flow cytometry live/dead assay of hPSCs cultured in various Mebiol lots as either single cells or clusters (~5 cells) by day 4. n=3, error bars = std. dev.

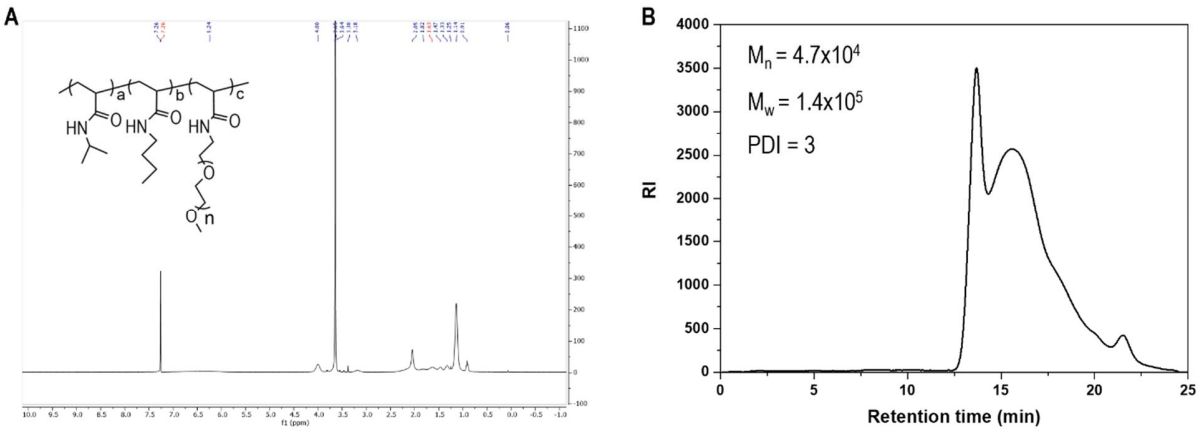

Figure S2. Thermoreversible graft copolymer material characterization, Related to Figure 1. A) H-NMR analysis of final polymer structure. B) GPC distribution of polymer retention time and resulting molecular weights, calibrated using PEO standards (Fluka).

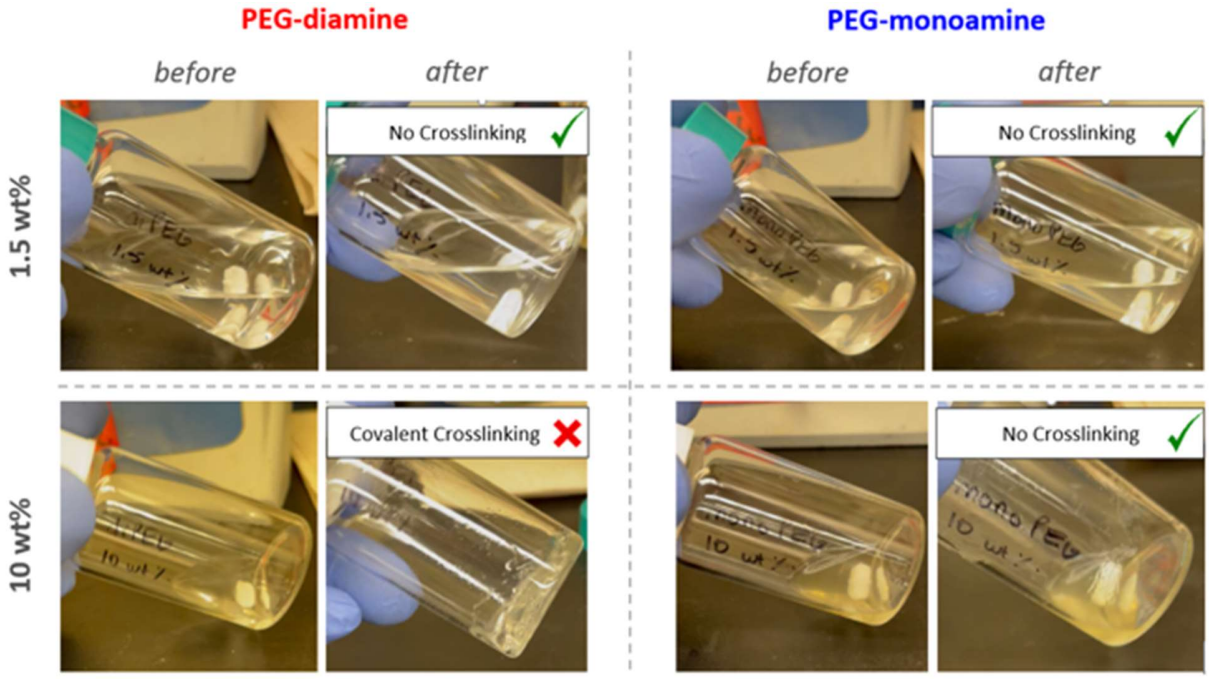

Figure S3. R**ole of mono vs diamino-PEG in polymer synthesis, Related to Figure 1 and 2**. Representative images of final polymer product in chloroform before and after reaction part 2.

A

Observation	Non-Toxic					
Independent GCP Batch	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
LCST	27°C	27°C	28°C	27°C	25°C	26°C

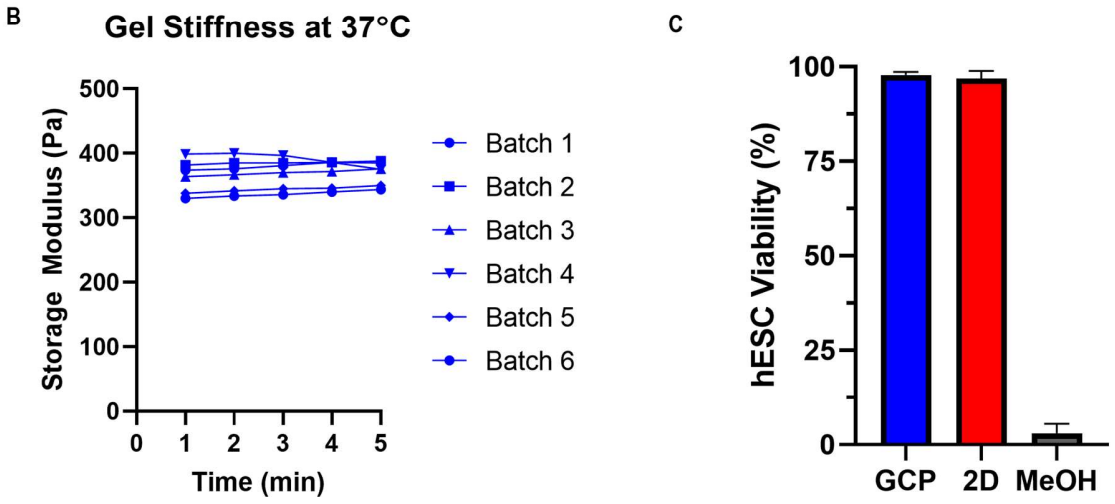

Figure S4. **Lot-to-lot variability of the thermoreversible graft copolymer, Related to Figure 2**. A) Graft copolymer (GCP) batch designation, observed toxicity, and resulting LCST, as determined via rheometry. B) Gel stiffness maintained over time at 37°C for various GCP batches. C) Representative flow cytometry live/dead assay of hPSCs cultured in the thermoreversible GCP batch 4, in 2D Matrigel (2D), and the negative methanol control (MeOH). n=3, error bars = std. dev.

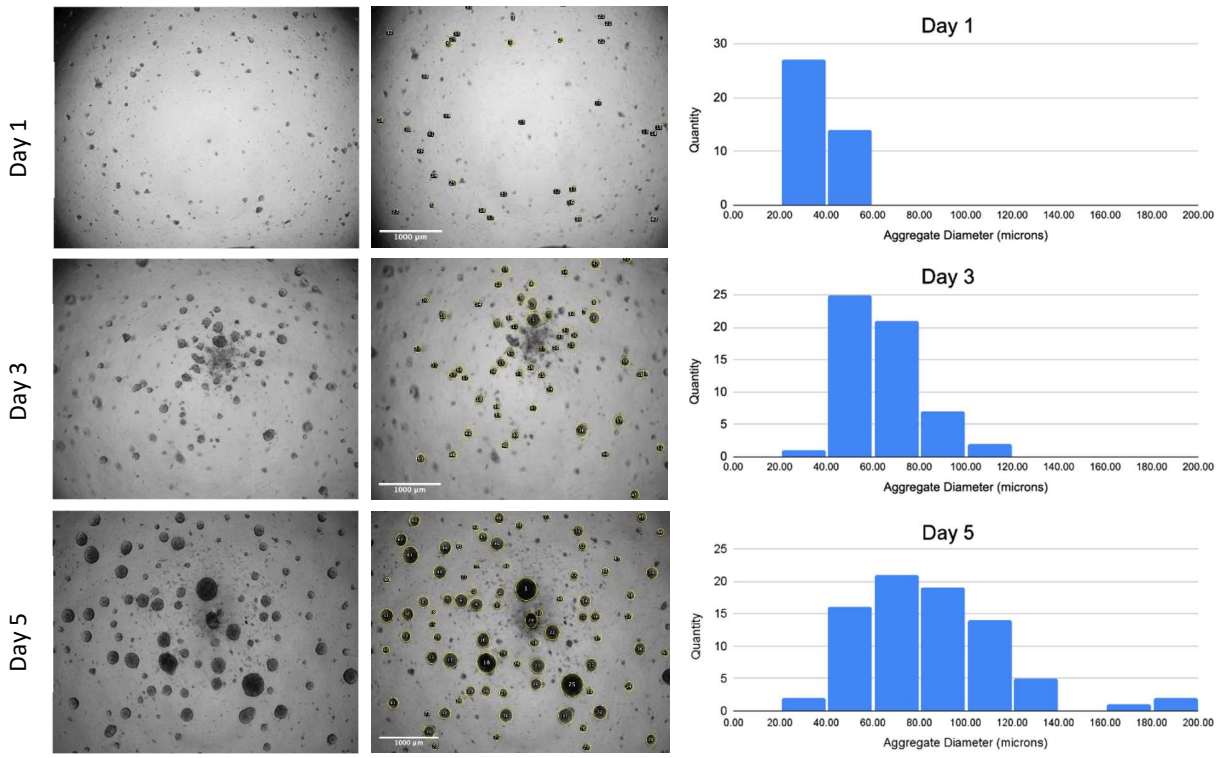

Figure S5. hPSC aggregate size distribution over one passage in the thermoreversible hydrogel, Related to Figure 3. Representative images of hPSC aggregate size (left) and aggregates size distribution (right) in the thermoreversible polymer after 1, 3, or 5 days in mTeSR medium (Figure 3A protocol). Aggregate size distribution calculated using ImageJ from bright field images. Scale bar = 1000 μm.

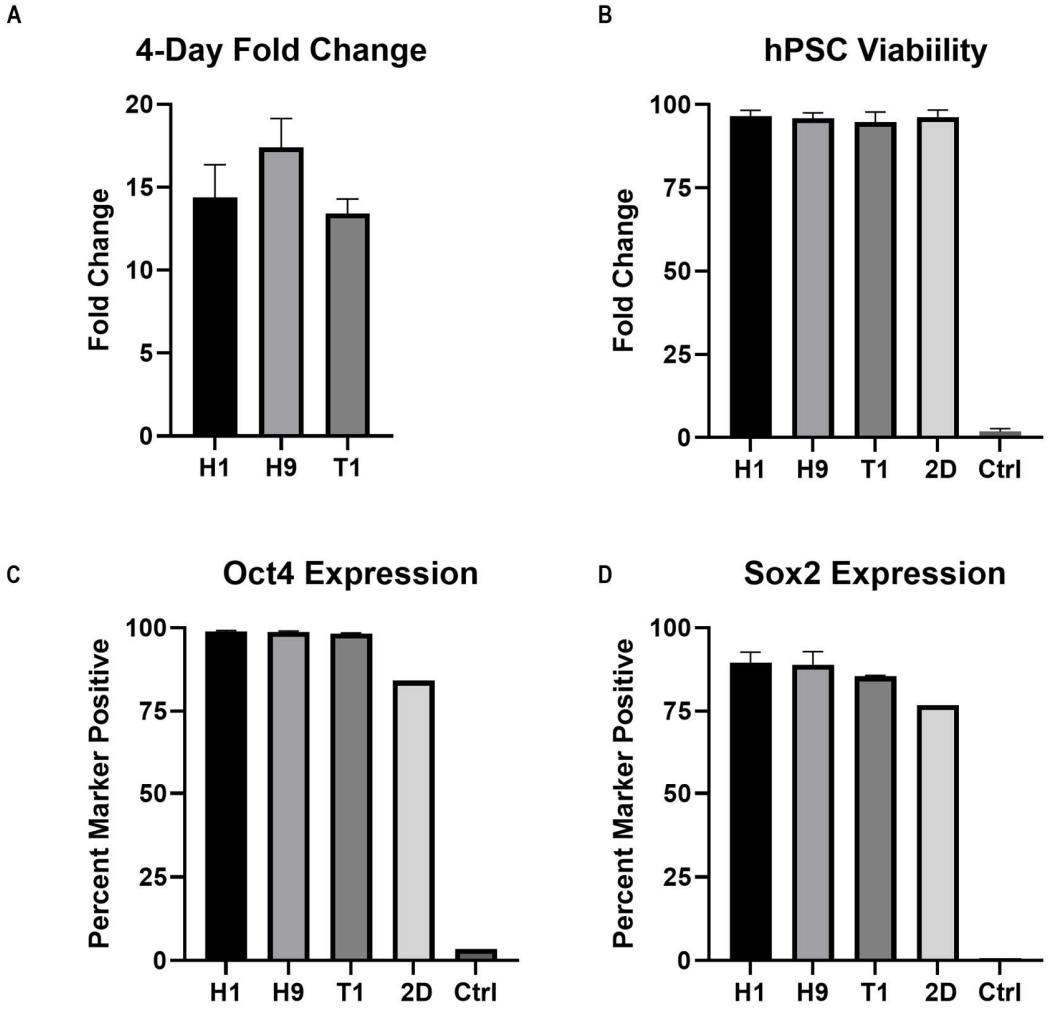

Figure S6: **Comparison of hPSC expansion and performance between H9 and H1 hESCs (WiCell) and T1 iPSCs (TMOi001-A, ThermoFisher) in the thermoreversible hydrogel, Related to Figure 3**. A) Comparison of 4-day fold change between the hPSCs seeded in the thermoreversible polymer and expanded in mTeSR (Figure 3A). Fold change calculated via comparing cell counts before and after expansion. B) comparison of hPSC viability after 4-days of expansion in the thermoreversible polymer, calculated via TO-PRO™3 Ready Flow™ Reagent after singularization. Ctrl represents methanol treated hPSCs. C) Comparison of Oct4+ hPSC after 4-days of expansion in the thermoreversible hydrogel, calculated via flow cytometry. Ctrl represents primary only stained samples. D) Comparison of Sox2+ hPSC after 4-days of expansion in the thermoreversible hydrogel, calculated via flow cytometry. Ctrl represents primary only stained samples. Error bars = std. dev. n=3.

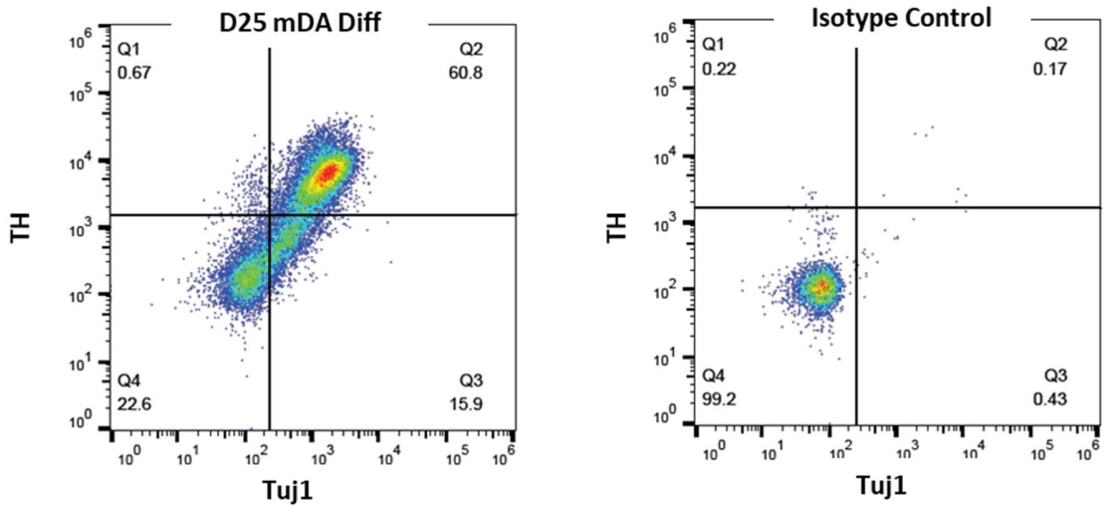

Figure S7. Representative flow cytometry dot-plots of neuronal markers at day 25 of dopaminergic differentiation from hPSCs in the 3D thermoreversible hydrogel, Related to Figure 4. Tyrosine hydroxylase (TH) and neuronal marker Tuj1 expression demonstrates a TH+/Tuj1+ and TH-/Tuj1+ positive population. Marker gates are drawn on the isotype control.