## Supplemental methods

### Alkaline phosphatase staining

The pluripotency of the encapsulated iPSC colonies was assessed by alkaline phosphatase staining as per manufacturer's instruction (Abcam, Cambridge, MA). Briefly, iPSC colonies encapsulated hydrogels were fixed using fixing solution for 2 min. The colonies were incubated with alkaline phosphatase staining solution for 30 min at room temperature. The phase contrast images of iPSC colonies were taken on EVOS M5000 imaging system (ThermoFisher Scientific, Waltham, MA). The colonies in hematopoietic differentiation media were used as a negative control.

### Immunostaining

On day 3 of hematopoietic differentiation, hydrogels were fixed with 4% paraformaldehyde at room temperature for 30 min. Fixed hydrogels were permeabilized with 0.2% v/v Triton X-100 for 30 min at room temperature. After blocking with 3% BSA for overnight at 4 °C, samples were incubated in NL637- conjugated HAND1 (R&D Systems, Inc., Minneapolis, MN) overnight at 4 °C. DNA was counterstained with Hoechst 33342 (ThermoFisher Scientific). The fluorescence images were taken on EVOS M5000 imaging system (ThermoFisher Scientific).

### Flow cytometry

Cells from hydrogels were collected and suspended in PBS supplemented with 1% FBS. Cells were incubated with flow antibodies (BioLegend, San Diego, CA). For pluripotency testing cells were stained with PE-Tra-1-60 and Alexa Fluor 647-SSEA-4. Following hematopoietic differentiation in low modulus and high modulus hydrogel with varying concentrations of RGD were collected and stained for hemangioblast marker PE-VEGFR2 on day 7 and on day 12 cells were stained for neuronal markers (APC- N-cadherin, FITC-CD24, Pacific Blue-CD56),

adipogenic marker (PE-CD137), osteogenic marker (FITC-CD10) and hematopoietic marker (Brilliant violet 605- CD45). Cells were analyzed using Novocyte 3000 flow cytometer (ACEA Biosciences, San Diego, CA) and NovoExpress software.

**Supplemental figures**

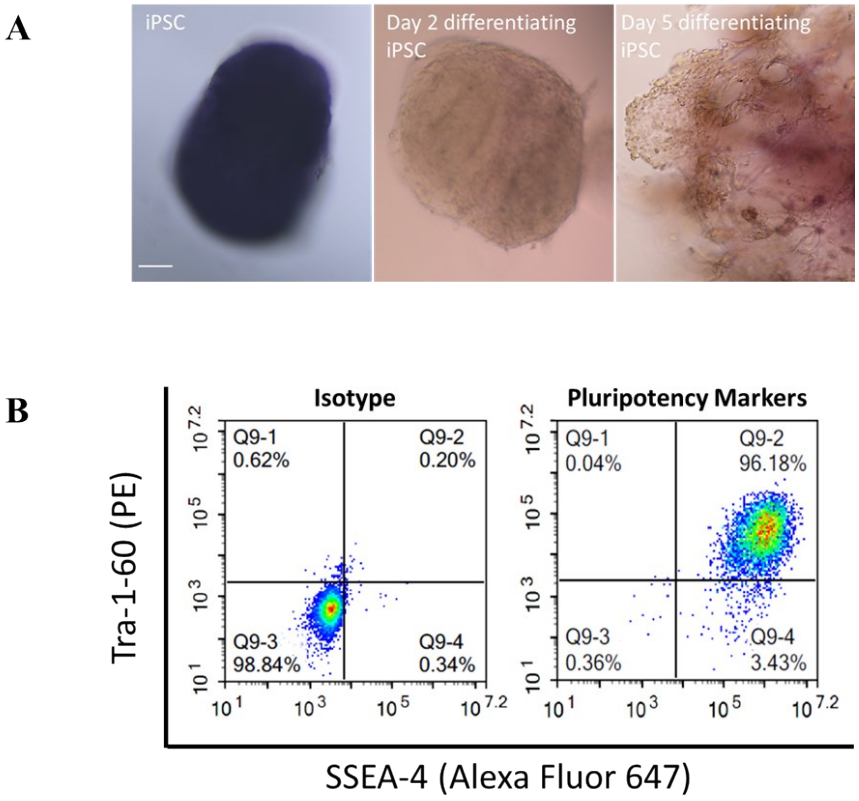


**Fig. S1. A)** Representative optical images of iPSC colonies stained with alkaline phosphatase showing intense purple staining in undifferentiated colonies. **B)** Representative flow plots of pluripotency marker of 3D encapsulated iPSC colonies after 4 days of culture in mTeSR1 medium.

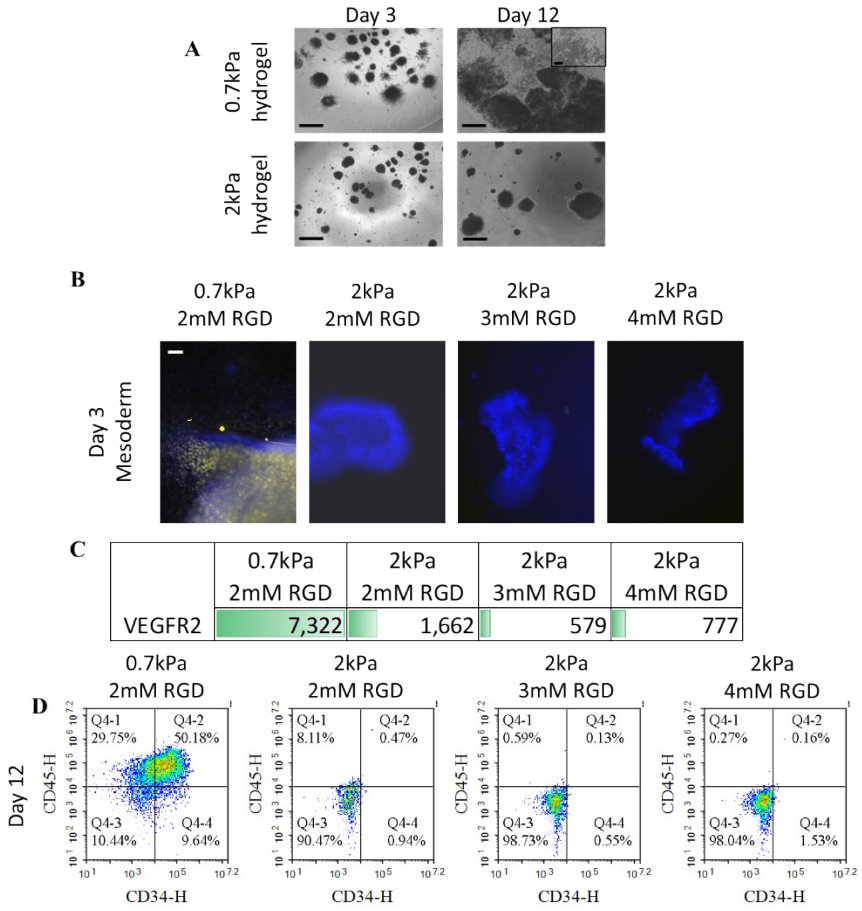


**Fig. S2. A)** Representative optical images of differentiating iPSC in low modulus (0.7kPa) and high modulus gel (2kPa) on day 3 and day 12. Scale bar = 500 µm, scale bar in the insert = 100 µm. **B)** Representative epifluorescence images of mesoderm marker, HAND1 (yellow), in differentiating iPSC in low modulus (0.7kPa) and high modulus gel (2kPa) on day 3. Nuclei counterstained with Hoechst (blue). Scale bar = 100 µm. **C)** Median fluorescence intensity of VEGFR2 normalized to % positive cells **D)** Flow cytometric analysis of hematopoietic markers CD34 and CD45 on day 12 cells collected from low modulus (0.7kPa) and high modulus gel (2kPa)

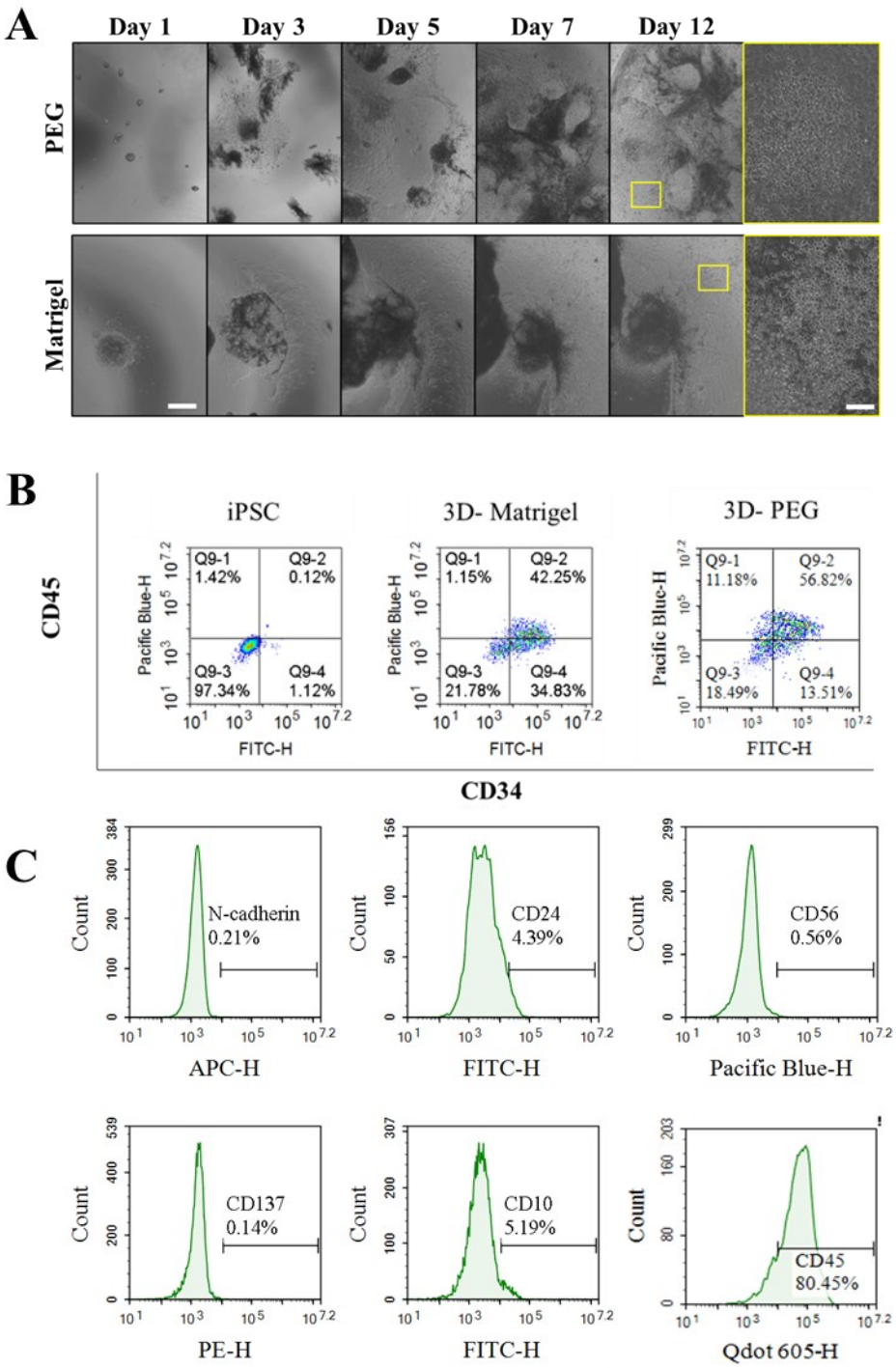
A
Day 1
Day 3
Day 5
Day 7
Day 12
PEG
Matrigel
B
CD45
iPSC
3D- Matrigel
3D- PEG
Q9-1 1.42%
Q9-2 0.12%
Q9-3 97.34%
Q9-4 1.12%
Q9-1 1.15%
Q9-2 42.25%
Q9-3 21.78%
Q9-4 34.83%
Q9-1 11.18%
Q9-2 56.82%
Q9-3 18.49%
Q9-4 13.51%
Pacific Blue-H
FITC-H
CD34
C
Count
N-cadherin 0.21%
APC-H
CD24 4.39%
FITC-H
CD56 0.56%
Pacific Blue-H
CD137 0.14%
PE-H
CD10 5.19%
FITC-H
CD45 80.45%
Qdot 605-H

**Fig. S3. A)** The morphological characterization of hematopoietic differentiation at indicated timepoints in 3D PEG and Matrigel. **B)** Flow cytometric analysis of hematopoietic markers CD34 and CD45 on 3D generated HSPC on day 12. **C)** Representative flow plots for neuronal differentiation markers (N-cadherin, CD24, CD56), adipogenic differentiation marker (CD137), osteogenic differentiation marker (CD10) and hematopoietic differentiation marker (CD45).

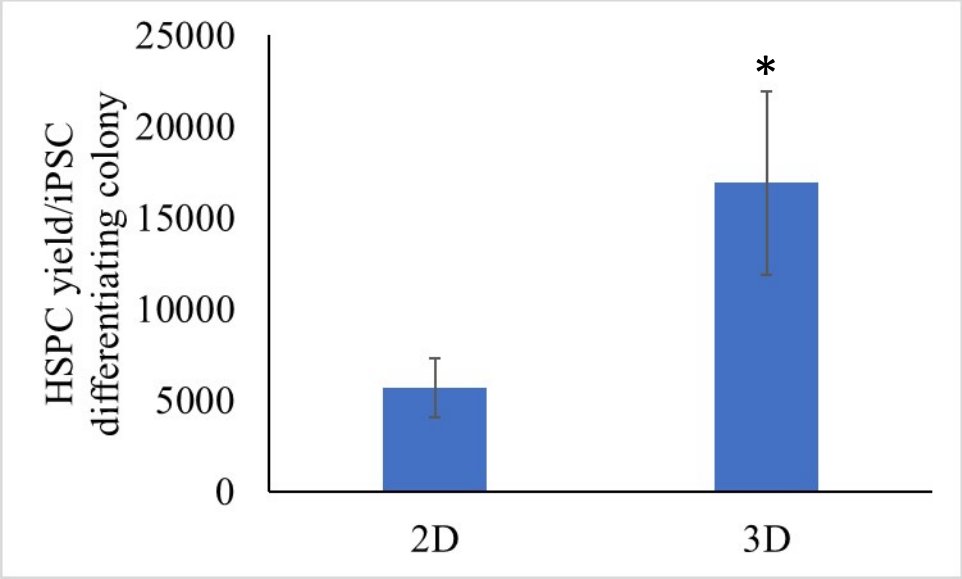


**Fig. S4.** Yield of hematopoietic cells generated per differentiating iPSC colony from 2D culture and 3D PEG hydrogel. Average data from 3 independent experiments is plotted. Error bars indicate standard deviation. *$p<0.05$.

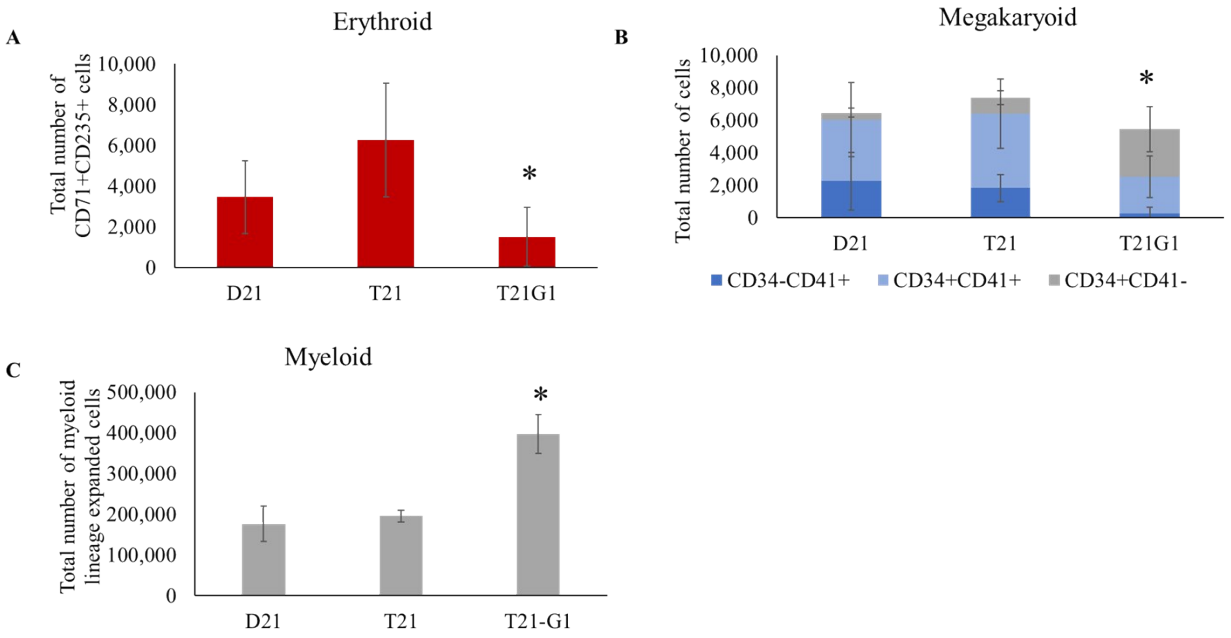


**Fig. S5. A)** Total number of erythroid cells (CD71+CD235+) on day12. **B)** Total number of megakaryoid subpopulation (CD34-CD41+, CD34+CD41+) and CD34+ CD41- cells on day12. **C)** Total number of myeloid cells collected after expansion in myeloid specific media on day 7. Average data from 3-5 independent experiments is plotted. Error bars indicate standard deviation. *p<0.05.

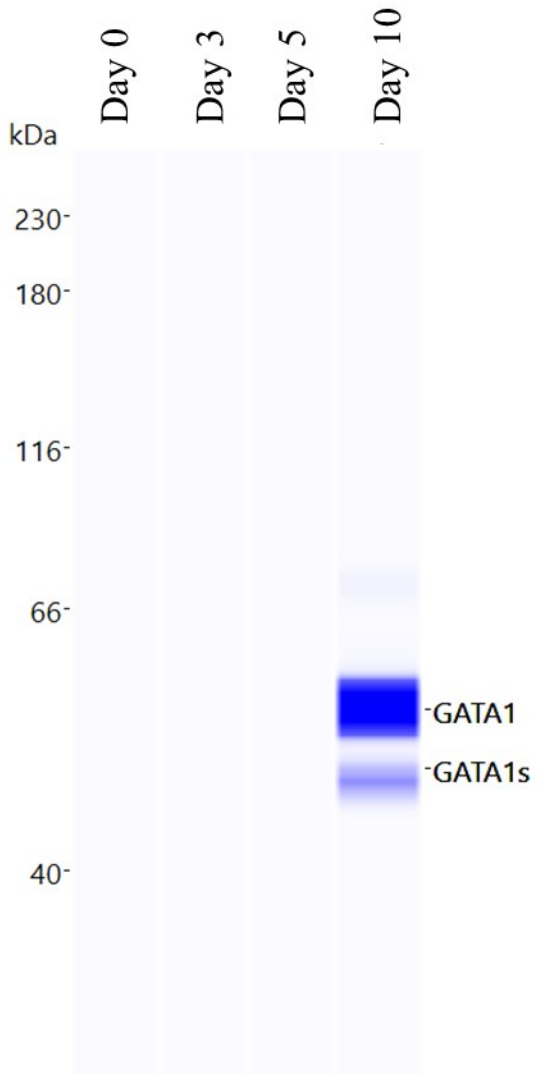


**Fig. S6.** Immunoblots using the automated Western blotting system Wes in band view to detect the presence of GATA1 protein expression.

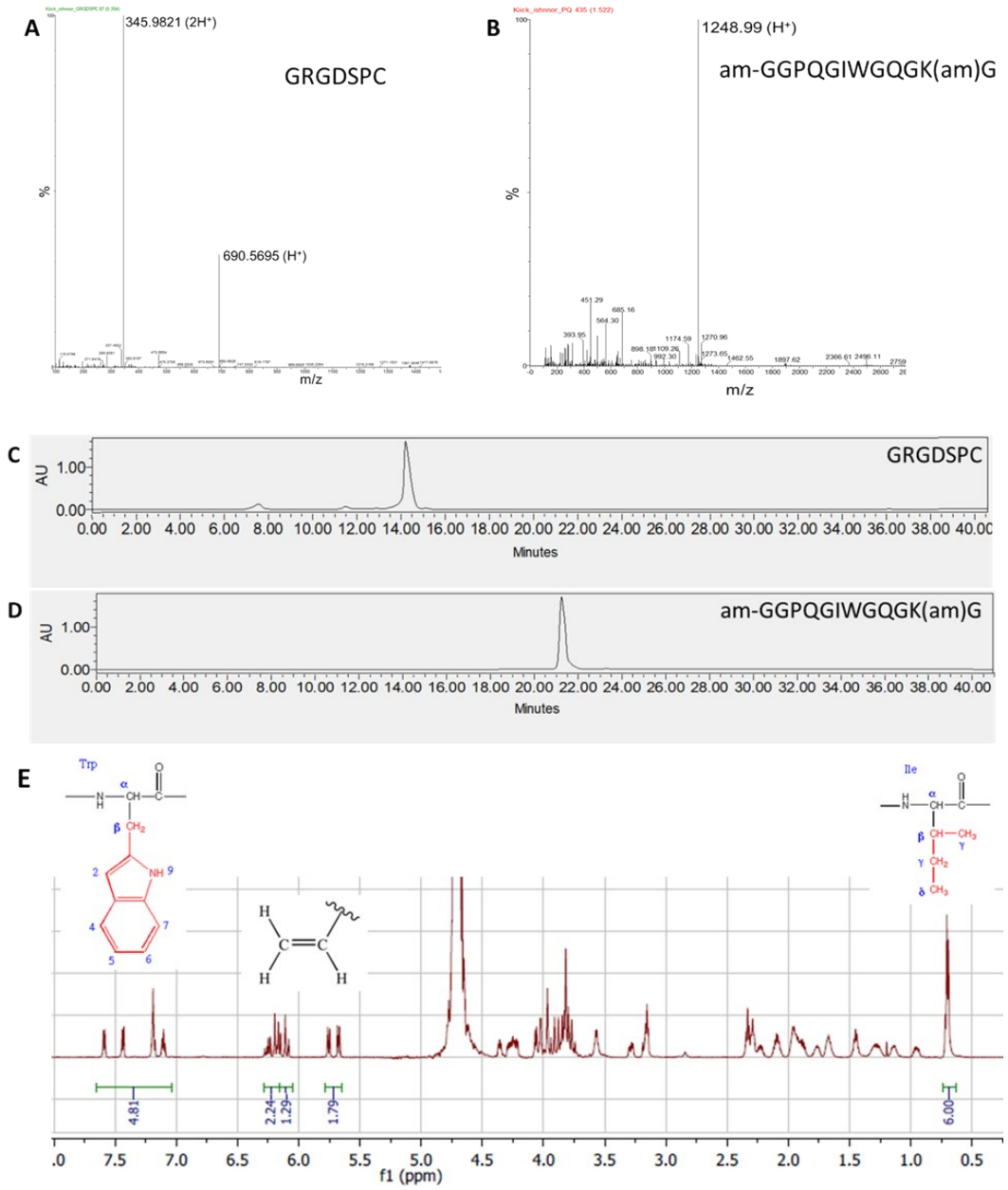


**Fig. S7.** Characterization of synthetic peptides using ESI-MS (positive ion mode). **A)** GRGDSPC (690 g/mol) and **B)** PQ-2-Am (1249 g/mol). Purity of peptide assessed by analytical HPLC **C)** 92% for RGD peptide and **D)** 97% for PQ-2-Am peptide. **E)** Average number of acrylamide group on PQ-2-Am was determined to be ~1.8 via $^{1}$H NMR