Electronic Supplementary Information

Designing well-defined synthetic matrices using photochemistry for three-dimensional culture and differentiation of induced pluripotent stem cells

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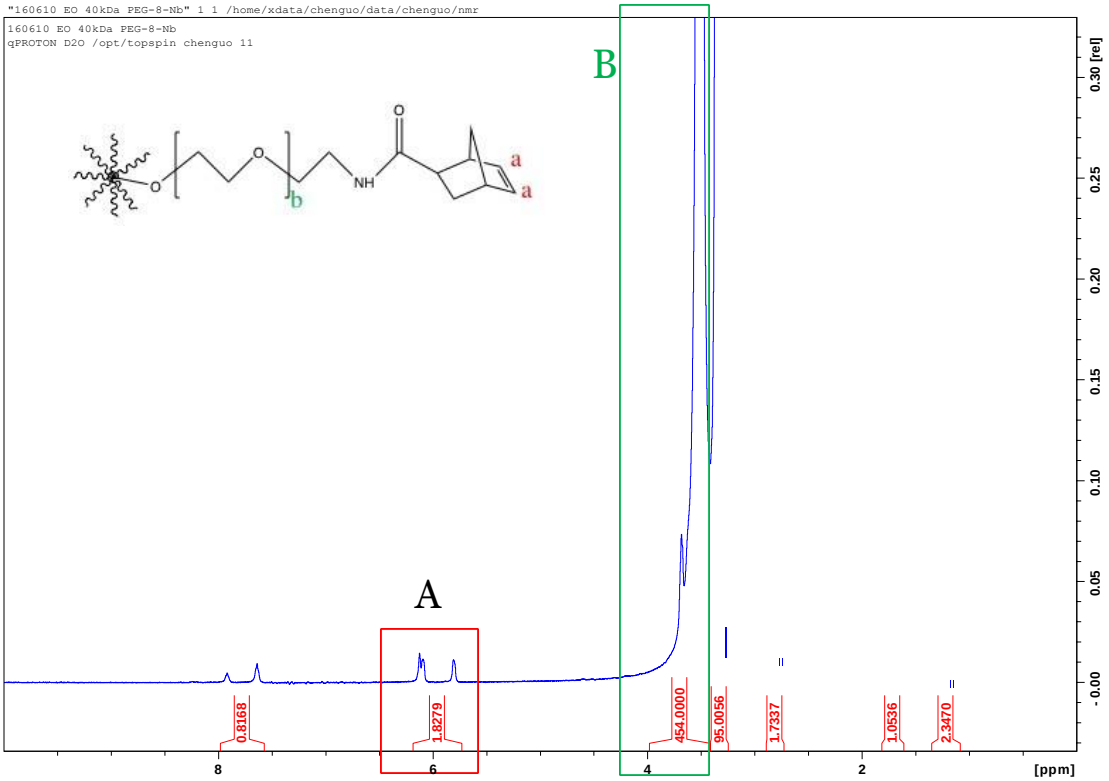
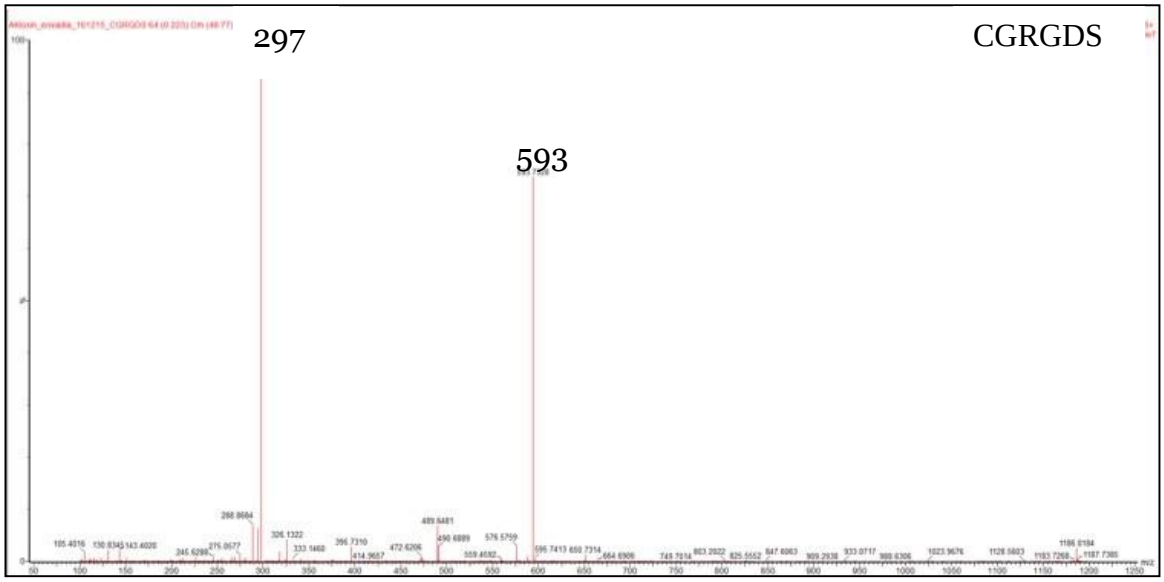
ESI Table 1. Forward and reverse primers for qRT-PCR

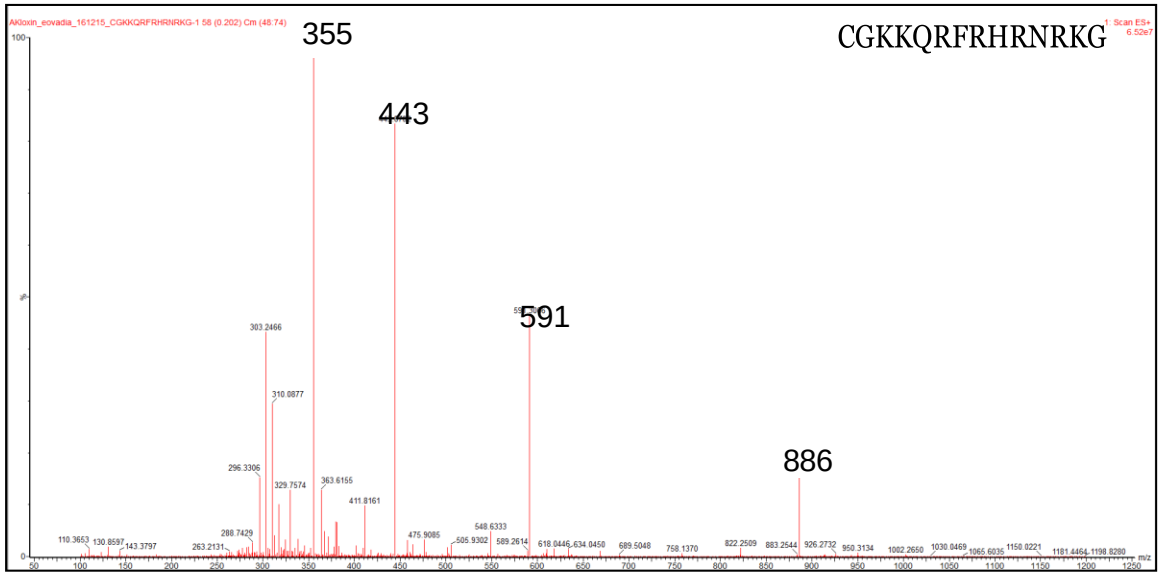
Gene	**Forward primer**	**Reverse primer**
Gapdh	GGCCATCCACAGTCTTCTG	TCATCAGCAATGCCTCCTG
Oct4	CTGAGGGCCAGGCAGGAGCACGAG	CTGTAGGGAGGGCTTCGGGCACTT
Pax6	TCCATCAGTTCCAACGGAGAA	GTGGAATTGGTTGGTAGACAC



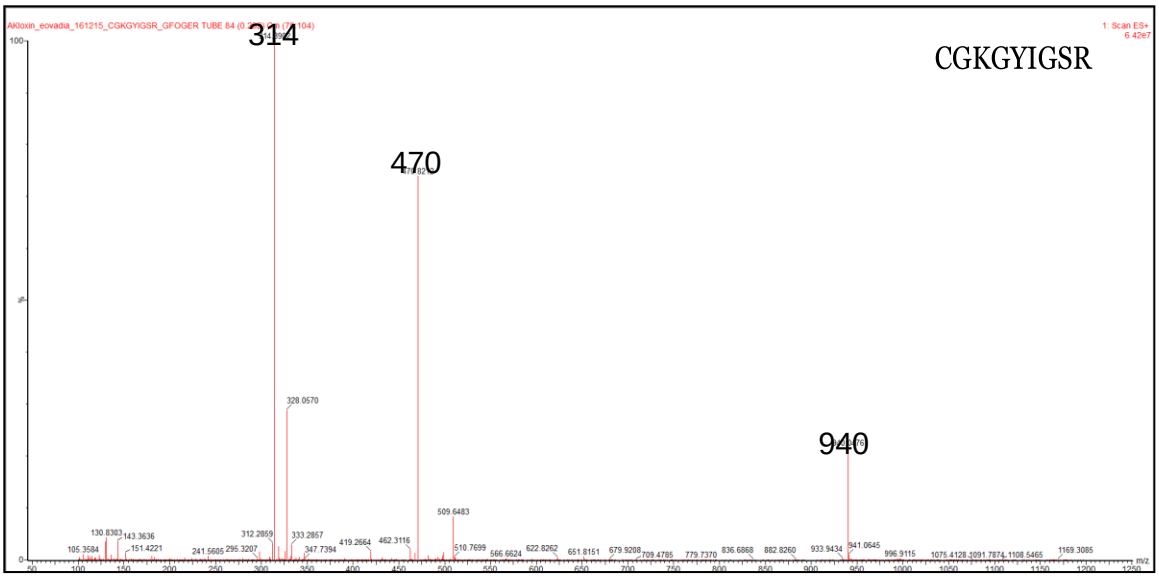
ESI Figure S1. ^{1}H NMR of PEG-8-Nb in DMSO indication 92% functionality. The functionality is based on the number of protons corresponding to norbornene normalized to the PEG backbone. Expected integration for norbornene protons (2H, 6.20 to 5.86 ppm, **A)** The PEG backbone is calibrated to have protons (454 H, 3.65 to 3.40 ppm. **B)** The calculated norbornene functionality is 1.83 divided by 2 equals 92%.



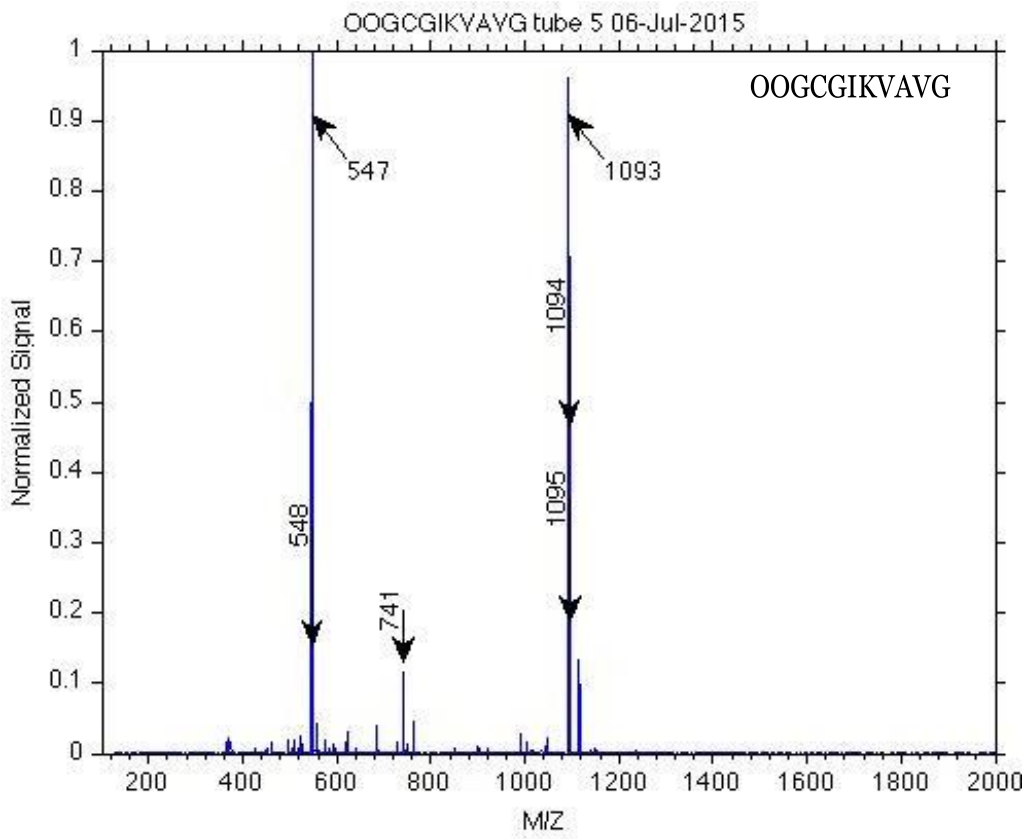
ESI Figure S2. Single Quadruple Detector 2 (SQD2) (Waters; Milford, MA) mass spectrometry of RGDS. Successful synthesis of the bioactive peptide ligand, CGRGDS-amide, was confirmed by SQD2 mass spectrometry. Expected molecular weight of 592.63 g/mol. $[M + H]^+$ = 593 g/mol. $[M + 2H]^+$ = 297 g/mol



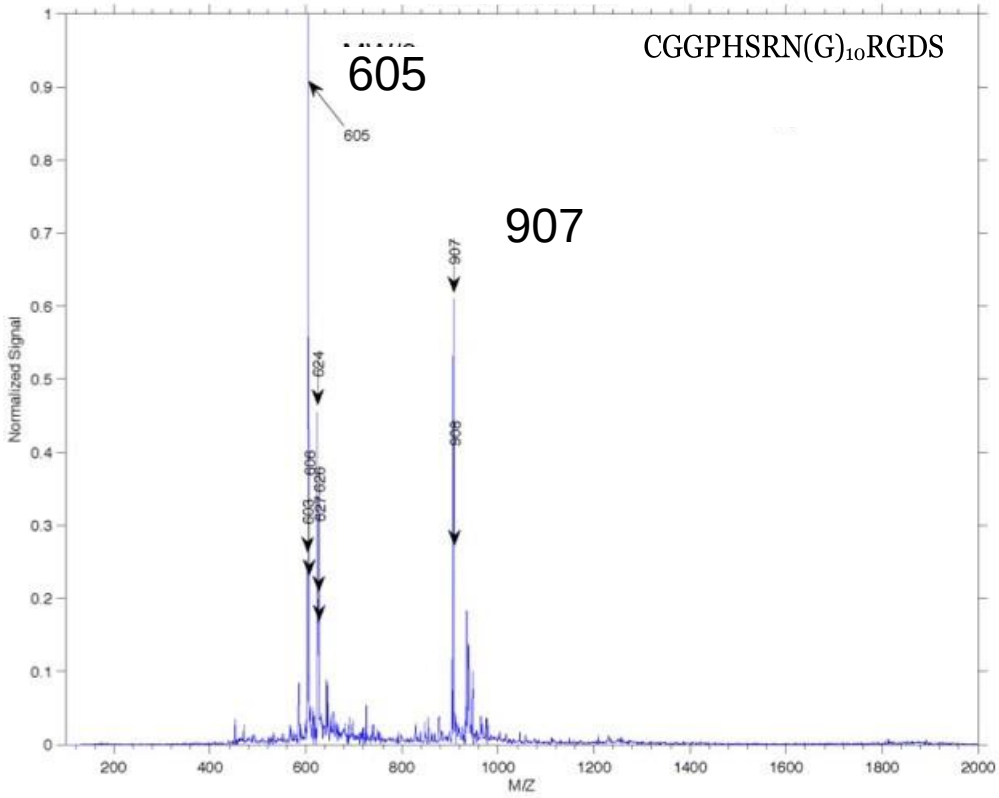
ESI Figure S3. SQD2 mass spectrometry of KKQRFRHRNRKG.
Successful synthesis of the bioactive peptide ligand, CGKKQRFRHRNRKG-amide, was confirmed by SQD2 mass spectrometry. Expected molecular weight of 1770.09 g/mol. $[M + 2H]^+$ = 591 g/mol. $[M + 3H]^+$ = 443 g/mol. $[M + 4H]^+$ = 355 g/mol.



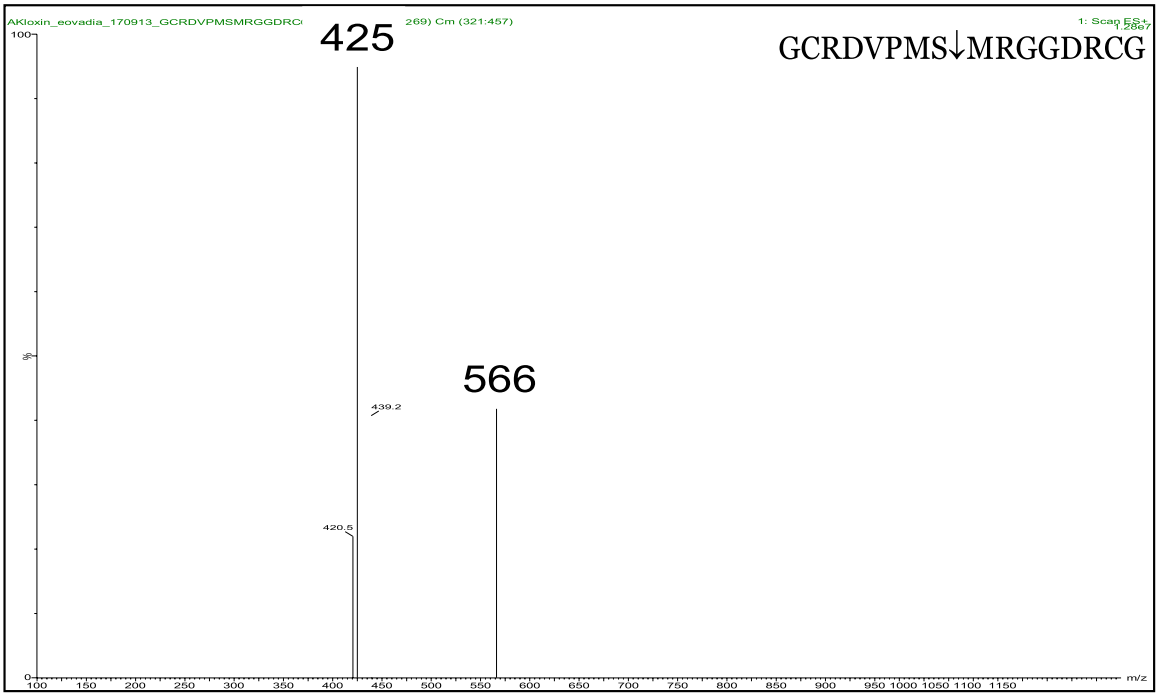
ESI Figure S4. SQD2 mass spectrometry of YIGSR. Successful synthesis of the bioactive peptide ligand, CGKGYIGSR-amide, was confirmed by SQD2 mass spectrometry. Expected molecular weight of 939 g/mol. $[M + H]^+$ = 940 g/mol. $[M + 2H]^+$ = 470 g/mol. $[M + 3H]^+$ = 314 g/mol.



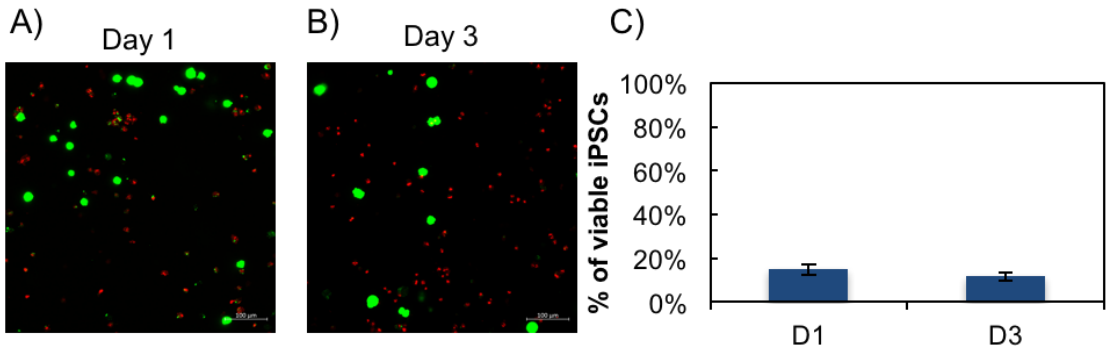
ESI Figure S5. Electrospray ionization mass spectrometry of IKVAV. Successful synthesis of the bioactive peptide ligand, OOGCGIKVAVG-amide, was confirmed by electrospray ionization mass spectrometry on a Shimadzu LCMS 2020. Expected molecular weight of 1093 g/mol. $[M + H]^+$ = 1094 g/mol. $[M + 2H]^+$ = 548 g/mol.



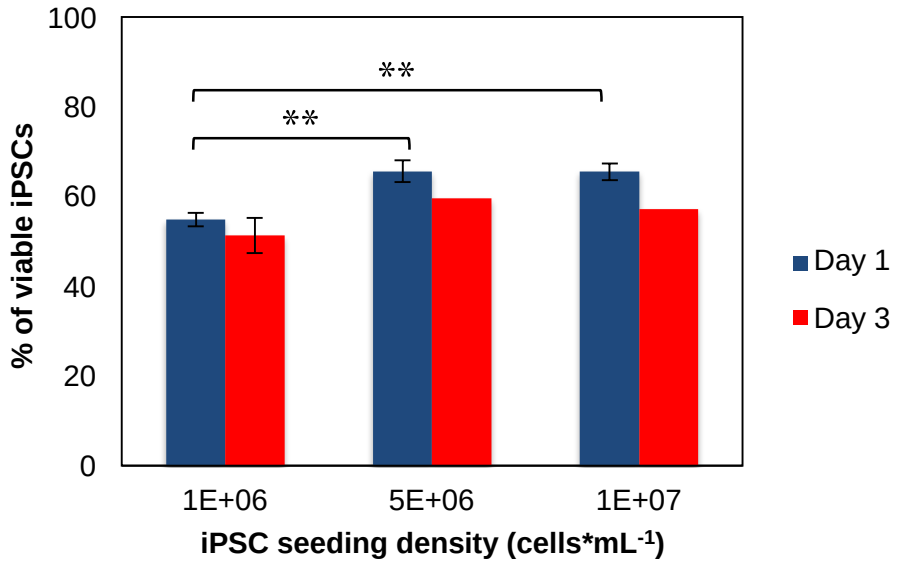
ESI Figure S6: Electrospray ionization mass spectrometry of PHSRN$(G)_{10}$RGDS. Successful synthesis of the bioactive peptide ligand, CGGPHSRN$(G)_{10}$RGDS-amide, was confirmed by electrospray ionization mass spectrometry on a Shimadzu LCMS 2020. Expected molecular weight of 1811 g/mol. $[M + H]^+$ = 907 g/mol. $[M + 2H]^+$ = 605 g/mol.



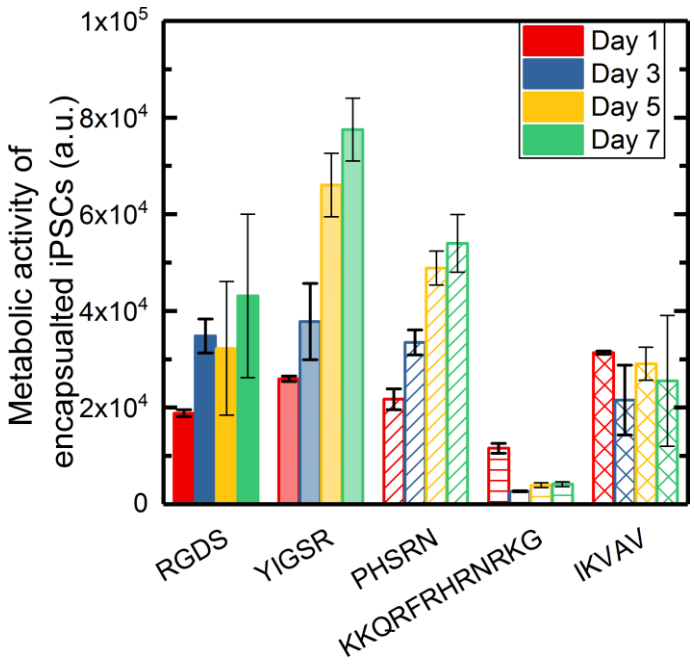
ESI Figure S7. SQD2 mass spectrometry of VPMS↓MRGG. Successful synthesis of the peptide crosslinker, CGRDVPMS↓MRGGDRCG-amide, was confirmed by SQD2 mass spectrometry. Expected molecular weight of 996 g/mol. $[M + 3H]^+$ = 566 g/mol. $[M + 4H]^+$ = 425 g/mol.



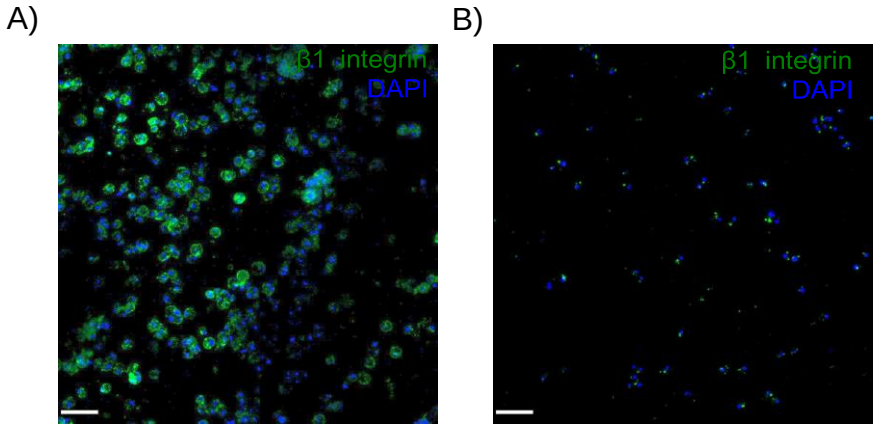
ESI Figure S8. Viability of iPSCs cultured in PEG-only hydrogels. iPSCs were encapsulated and cultured in 3 wt% PEG-only hydrogels as a 'blank slate' control (no integrin-binding peptide or ROCK inhibitor) and cultured for 3 days. Viability of iPSCs was determined by a Live/Dead cytotoxicity assay for iPSCs cultured in hydrogels at **A)** day 1 and **B)** day 3, where live cells were stained green and dead cells red. **C)** iPSC viability was observed to be low both initially and over time (15 ± 2% at day 1 (D1) and 12 ± 2% at day 3 (D3)). iPSC viability in this 'blank slate' control was statistically lower at both day 1 and day 3 in comparison to iPSCs in 3D culture with ROCK inhibitor alone (p-value < 0.01) and at day 1 in comparison to iPSCs in 3D culture in hydrogels presenting RGDS alone (p-value < 0.01).



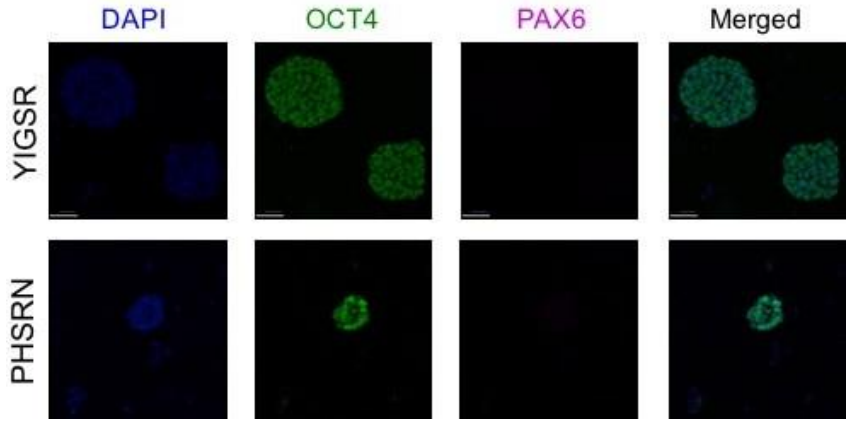
ESI Figure S9. Viability of encapsulated iPSCs at varying seeding densities. To test the effect of initial iPSC seeding density for encapsulation on cell viability, iPSCs were encapsulated at seeding densities of 10^6, 5×10^6, and 10^7 cells per mL in RGDS presenting hydrogels and cultured in mTeSR1 with ROCK inhibitor Y-27632. A Live/Dead cytotoxicity assay was used to assess the percentage of live and dead cells at days 1 and 3. Viability at day 1 was statistically lower (** $p < 0.01$) for the lowest seeding density of 10^6 cells per mL, with 55 ± 2% viable cells, relative to both seeding densities of 5×10^6 and 10^7 cells per mL. Seeding densities of 5×10^6 and 10^7 iPSCs per mL resulted in the similar viability at day 1 (66 ± 2%). After 3 days in culture there was no statistical difference in iPSC viability amongst the different seeding densities: encapsulated iPSC viabilities of 51 ± 3%, 60 ± 5%, and 57 ± 6% were measured at seeding densities of 10^6, $5x10^6$, and 10^7 cells per mL, respectively. A seeding density of $5x10^6$ iPSCs per mL was chosen for all future experiments, as this seeding density provides a good compromise between cell-cell contact and cell-matrix interactions.



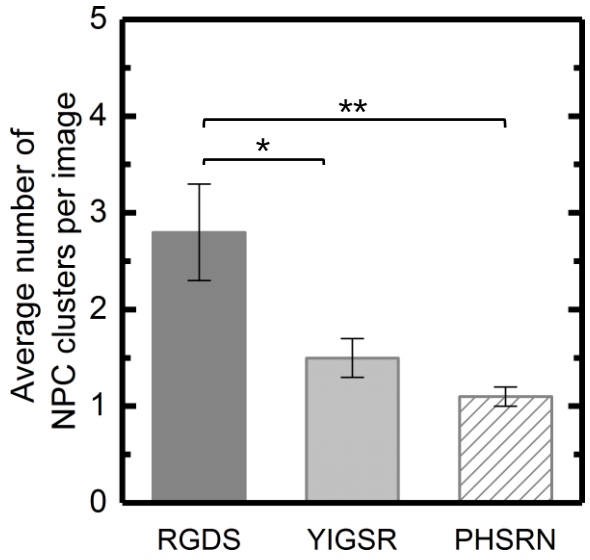
ESI Figure S10. Metabolic activity of iPSCs in cultured in 3D peptide-containing PEG hydrogels. Metabolic activity was measured over 1 week (alamarBlue assay, Ex/Em ~ 560/590 nm). Over 7 days, iPSCs cultured in the YIGSR or PHSRNG_{10}RGDS (labeled as PHSRN) gels have increased metabolic activity, indicating the iPSCs are surviving and growing in 3D culture. Of note, by day 7, iPSCs cultured in YIGSR-presenting hydrogels have the highest metabolic activity. Cells in the RGDS-presenting hydrogel exhibited an initial increase in metabolic activity from day 1 to day 3 and then a plateau in activity by day 7. The iPSCs in the IKVAV-presenting hydrogel had the highest metabolic activity at day 1 and that remained constant over 1 week; in the context of the live/dead data for this composition and comparison of metabolic activity increases observed in other compositions, these data may indicate limited cell proliferation or a balance between proliferation and cell death. Cells in hydrogels presenting KKQRFRHRNRKG had the lowest and minimal metabolic activity over the course of 1 week, indicating cell death in this composition and consistent with observations made with the live/dead assay.



ESI Figure S11. Confirmation of β1 blocking. iPSCs in 3D culture for 3 days in YIGSR-presenting hydrogels were immunonstained for β1 integrin in conditions cultured either **A)** without β1-blocking antibody or **B)** with β1-blocking antibody. At day 3, samples were fixed and immunostained with a β1 antibody (green, AF488) and DAPI (blue). Without the β1-blocking antibody in culture, consistent β1 integrin expression was observed on iPSC in 3D culture. Importantly, with the β1-blocking antibody in culture, little β1 integrin was observed as available for binding on iPSCs in 3D culture. (representative confocal z-stack projections shown; scale bar, 200 μm.)



ESI Figure S12. iPSC expression of pluripotency (OCT4) and NPC (PAX6) markers in PEG-peptide hydrogels. iPSCs were cultured in growth medium (mTeSR1 with ROCK inhibitor) for 6 days in hydrogels presenting YIGSR and PHSRNG_{10}RGDS (labeled as PHSRN in figure panel). iPSCs cultured in these hydrogels exhibited similar OCT4 expression to iPSCs cultured in RGDS-presenting PEG hydrogels (**Figure 6a**). No expression of the differentiation marker PAX6 was detectable. Overall, these data support that iPSCs retain pluripotency over 6 days of culture in PEG-peptide hydrogels when culture in growth medium.



ESI Figure S13. Average number of NPC clusters per image after differentiation. Multiple NPC clusters were observed in each hydrogel composition with confocal microscopy. To quantify this, the average number of cell clusters per image was analyzed and ranged from the most clusters to the least in RGDS, YIGSR, or PHSRN peptide-presenting hydrogels. These correlate to values of 2.8 ± 0.5, 1.5 ± 0.2, 1.1 ± 0.1 number of clusters per image, respectively. The number of NPC clusters cultured in RGDS was statistically different from the YIGSR and $PHSRNG_{10}RGDS$ (labeled as PHSRN) conditions at p-values of 0.029 and 0.007, respectively. There is no statistically difference between the number of clusters per image between YIGSR and $PHSRNG_{10}RGDS$ conditions. (n = 3 replicates per condition; statistical differences determined by Student's t-test; *p-value < 0.05, ** p-value < 0.01.)