Supplementary Figures

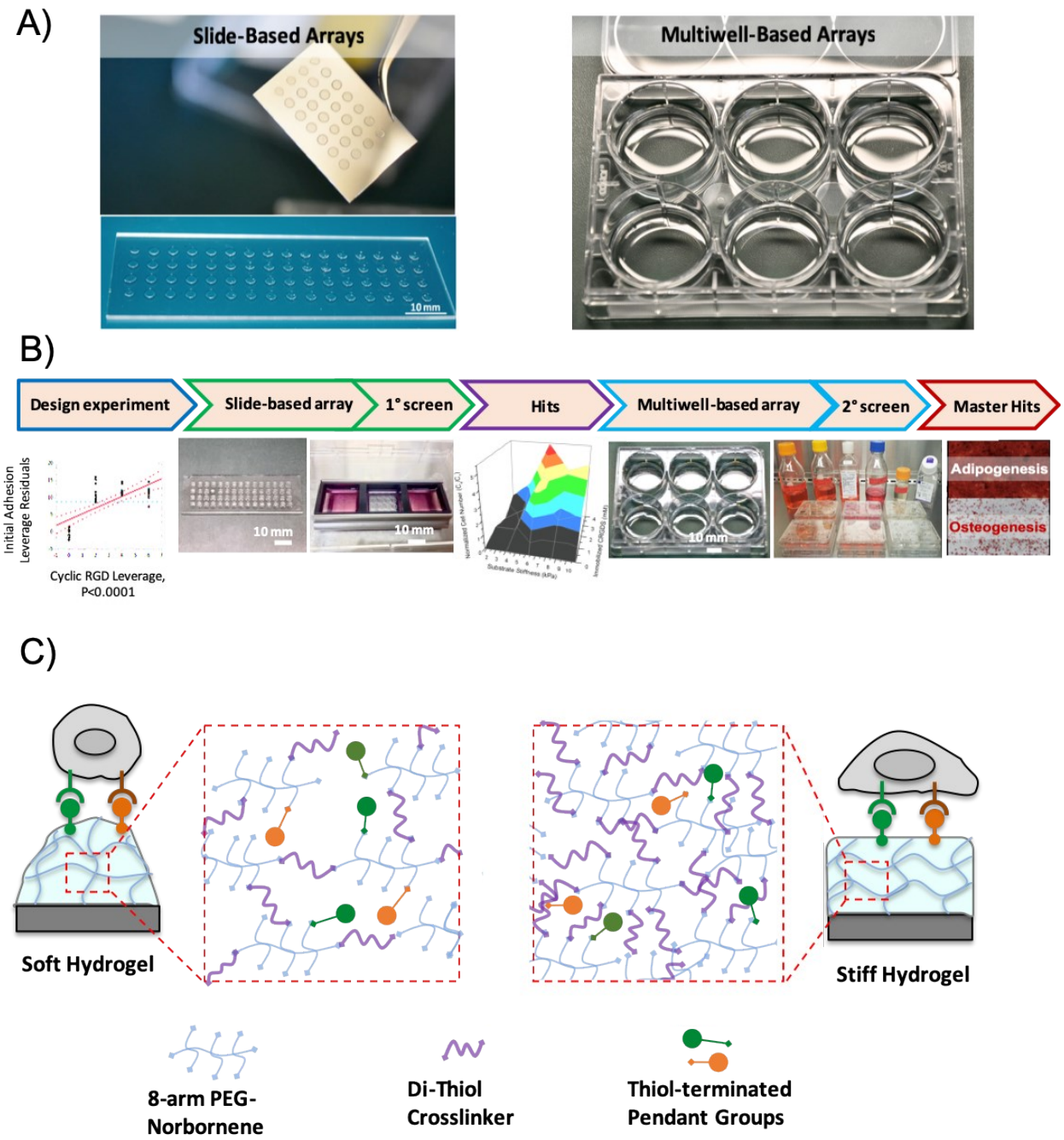
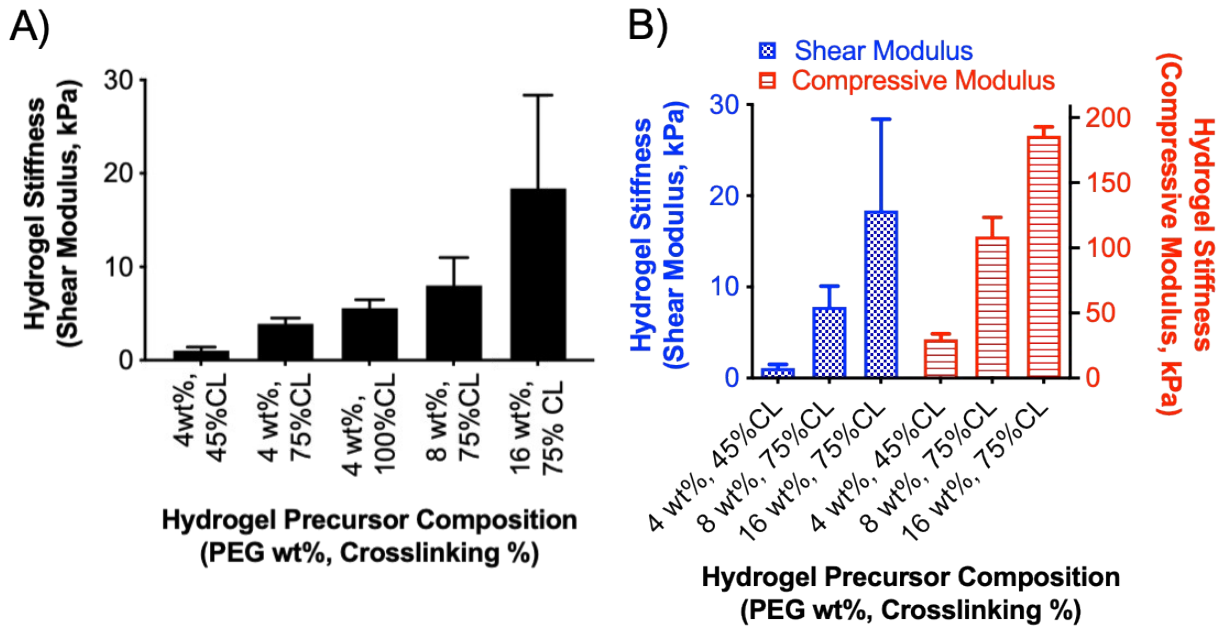


Figure S1. Screening workflow and platforms. A) Slide- and multiwell-based hydrogel arrays for screening. B) Workflow for enhanced throughput composition screening on hydrogel arrays formed on glass slide and scale up on bulk hydrogels formed in 6-well plate for hMSC expansion and long-term culture. C) Hydrogel networks formed using thiolene chemistry with an 8-arm PEG-norbornene polymer backbone, PEG-dithiol crosslinker, and thiol-terminated peptide pendant groups to promote adhesion. Stiffness is modulated using control of PEG-norbornene and PEG-dithiol crosslinker density in the unpolymerized hydrogel precursor solution.



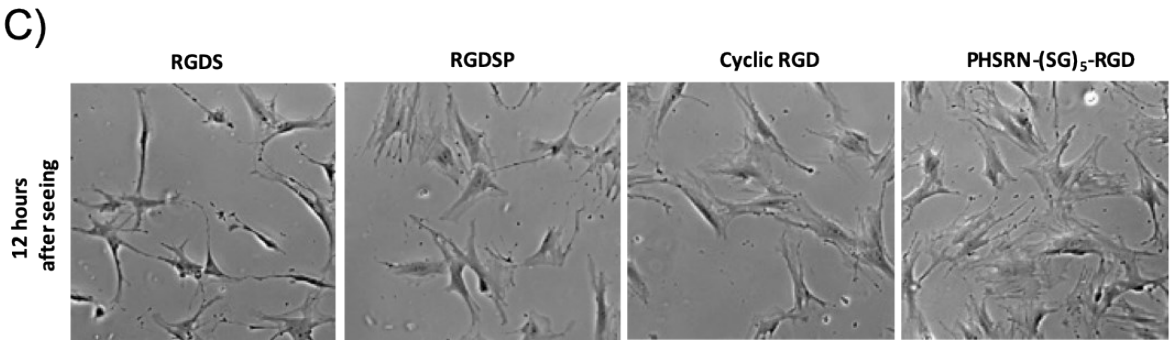


Figure S2. Controllable hydrogel substrate stiffness and adhesivity. A,B) Hydrogel stiffness is tunable by changing the polymer concentration (weight percentage) and crosslinking density (total percentage of norbornene arms crosslinked). C) Adhesivity is controlled by changing the identity and concentration of integrin-binding peptides.

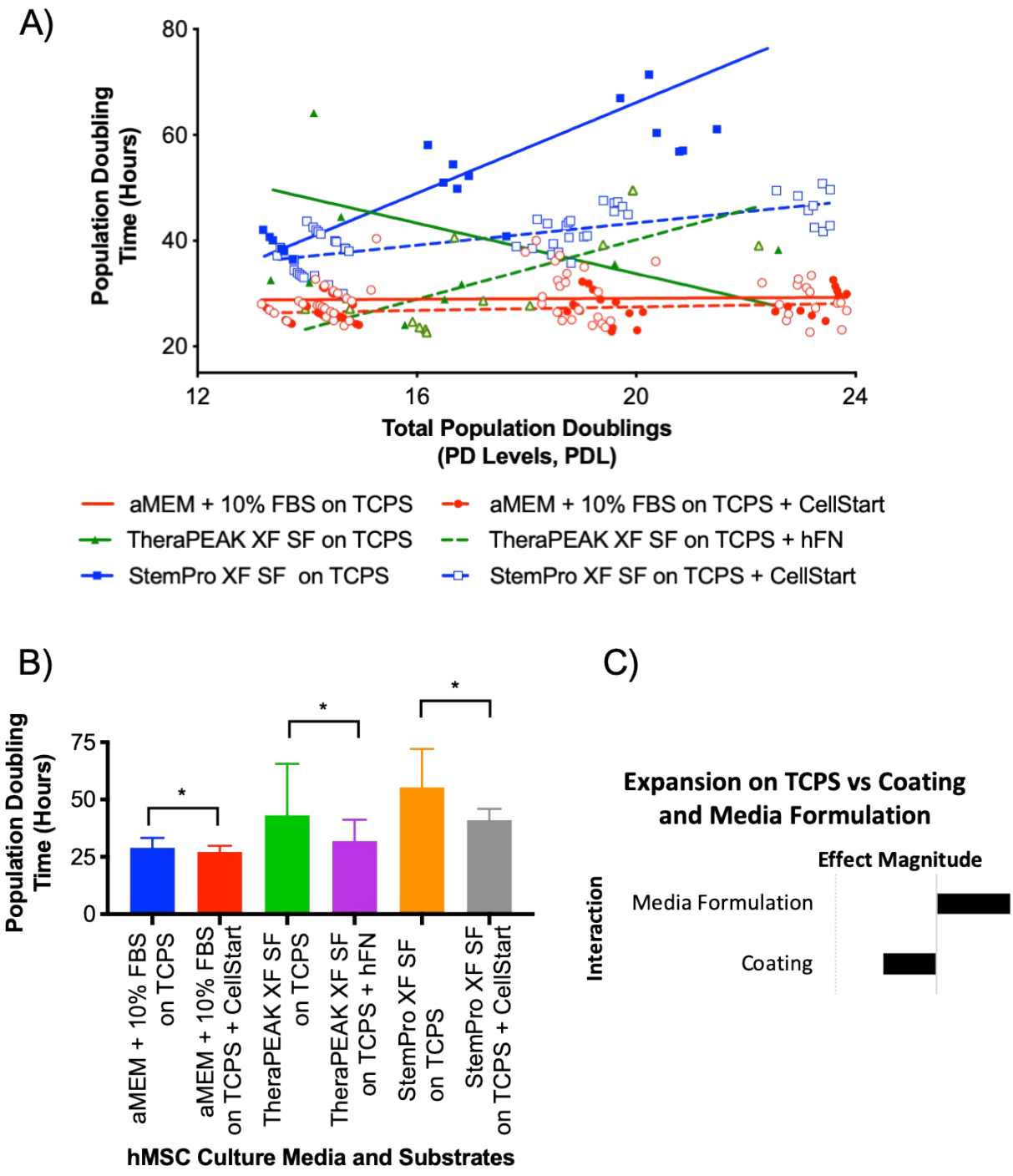


Figure S3. hMSC growth and maintenance in different xeno-free (XF) and serum-free (XF) media and protein-coated tissue-culture polystyrene (TCPS) substrates. A,B) Media and substrate properties independently and combinatorially affect age-dependent hMSC reduction in proliferative capacity. C) Multivariate analysis of previous quality control data reveals the importance of both media formulation and substrate adhesivity on hMSC adhesion.

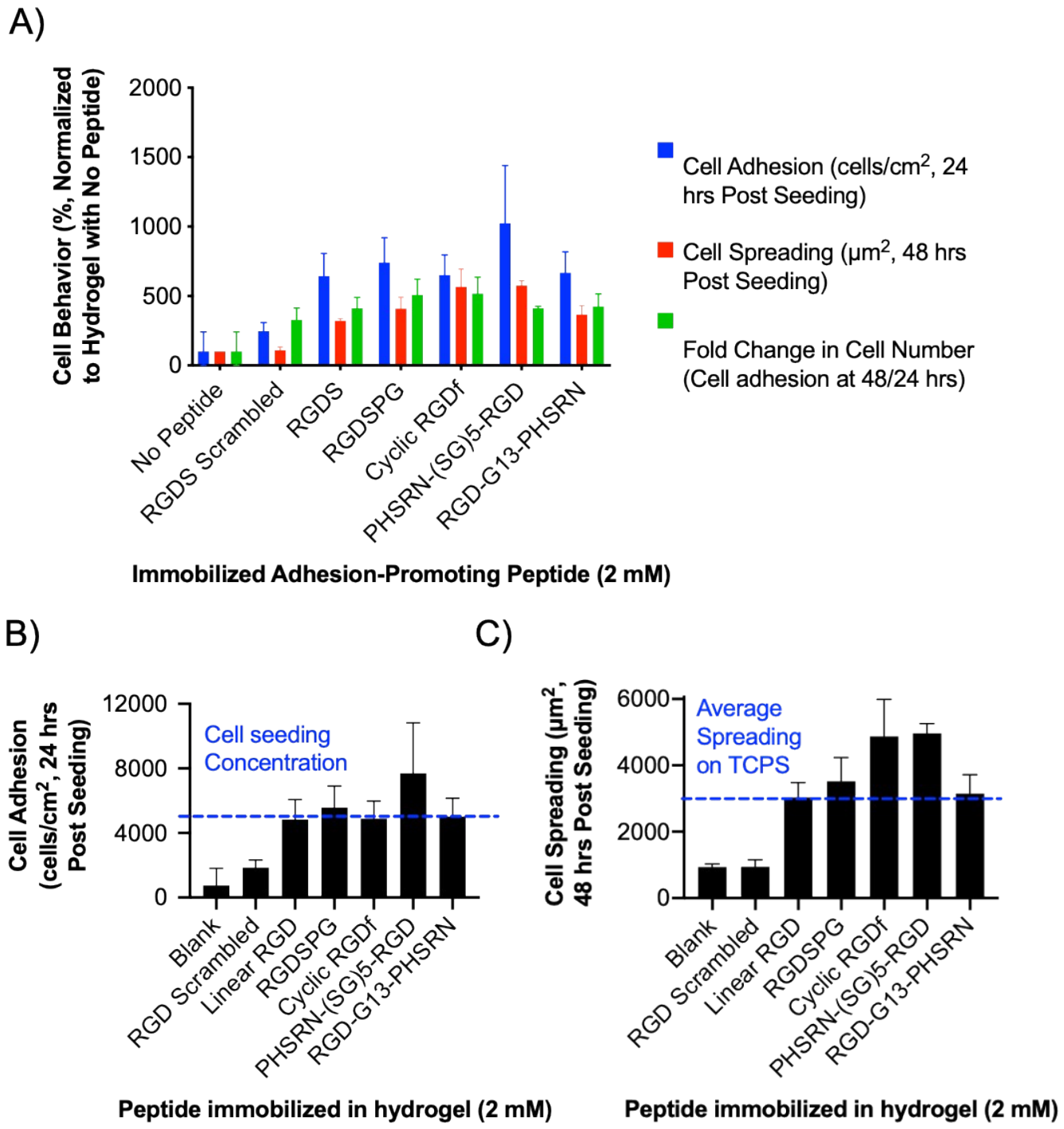
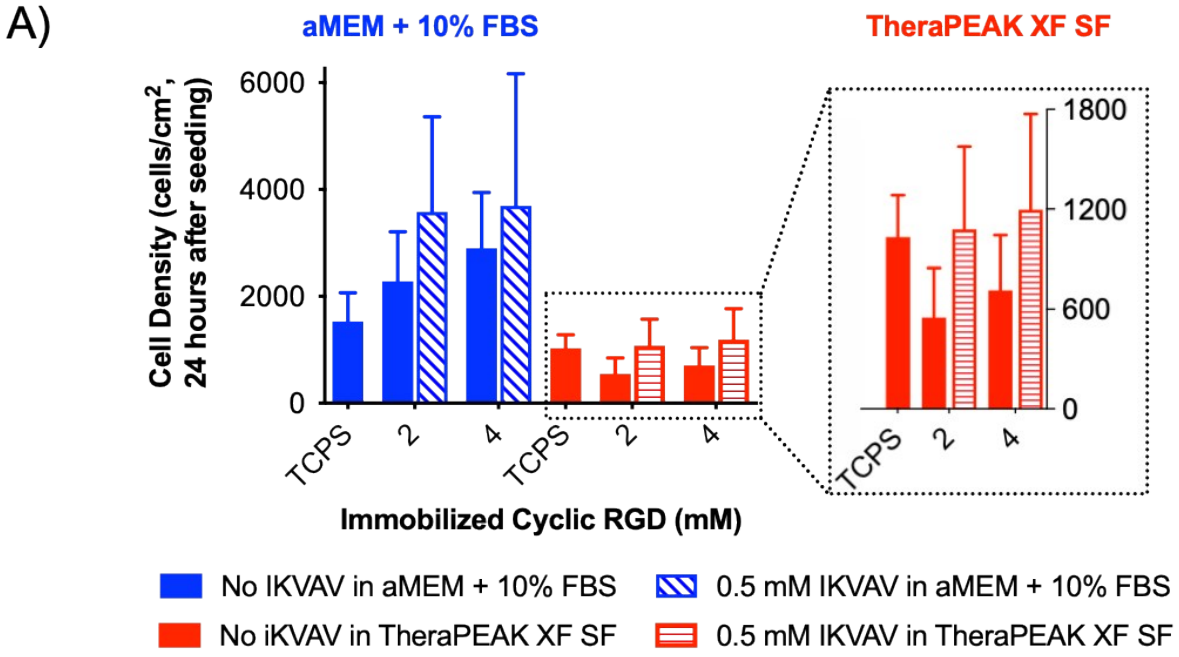
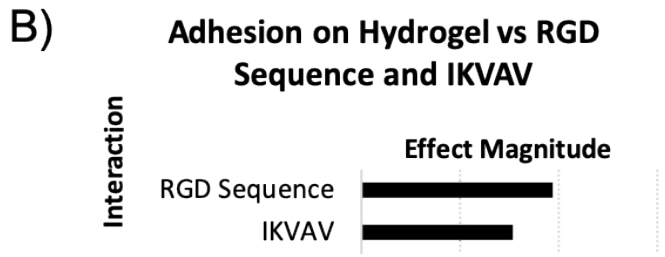


Figure S4. First screen of hydrogels containing various RGD-containing adhesion-promoting peptides and their effects on hMSC A,B) adhesion, A,C) spreading, and A) expansion.





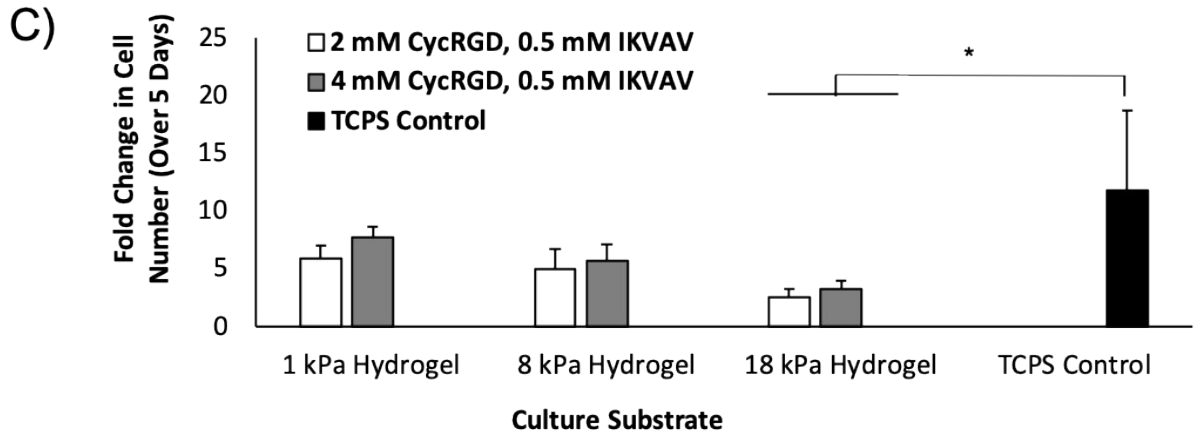


Figure S5. Second screen of the effects of hydrogels with immobilized A) Cyclic RGDf and IKVAV on hMSC adhesion in SC and SF media. B) MVA of RGD and IKVAV and their effects on hMSC adhesion. A,C) The combinatorial effects of adding IKVAV to Cyclic RGDf-containing hydrogels on stable hMSC adhesion and long-term expansion in SF culture.

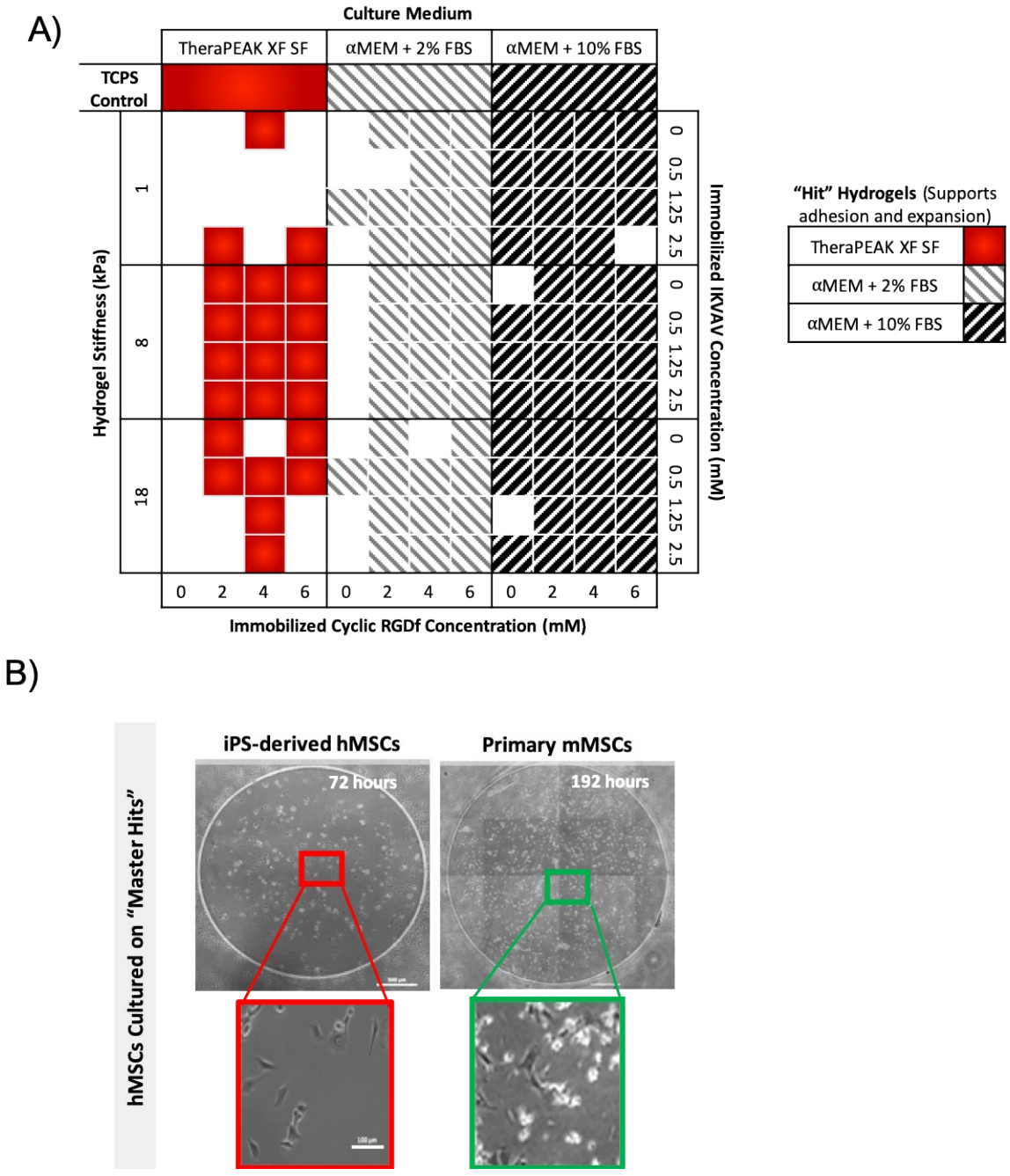


Figure S6. Screening for A) hydrogel substrates that support hMSC adhesion and expansion in TheraPEAK XF SF, αMEM + 2% FBS, and αMEM + 10% FBS media ("hits") and B) "master hits" that support media- and cell source-agnostic hMSC culture.

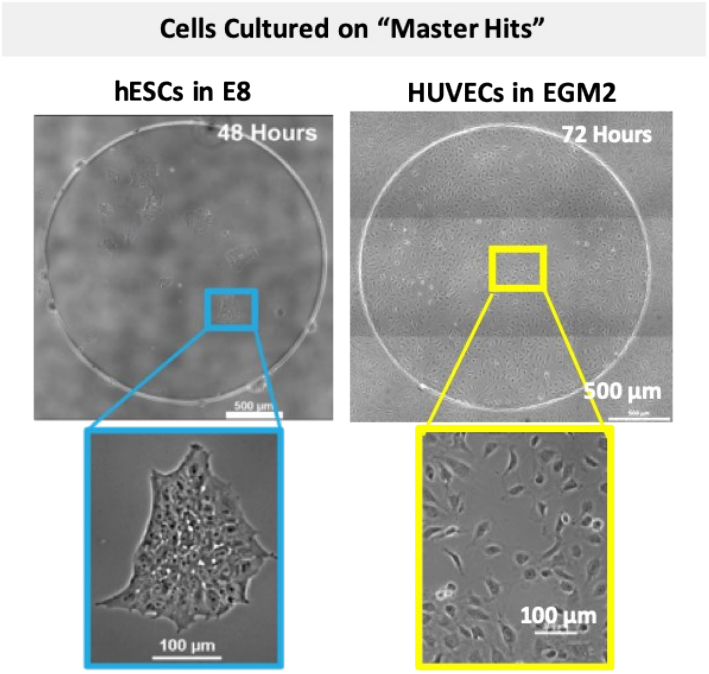


Figure S7. Screening workflow for use in identifying substrates for serum-free hESC and -HUVEC attachment and proliferation

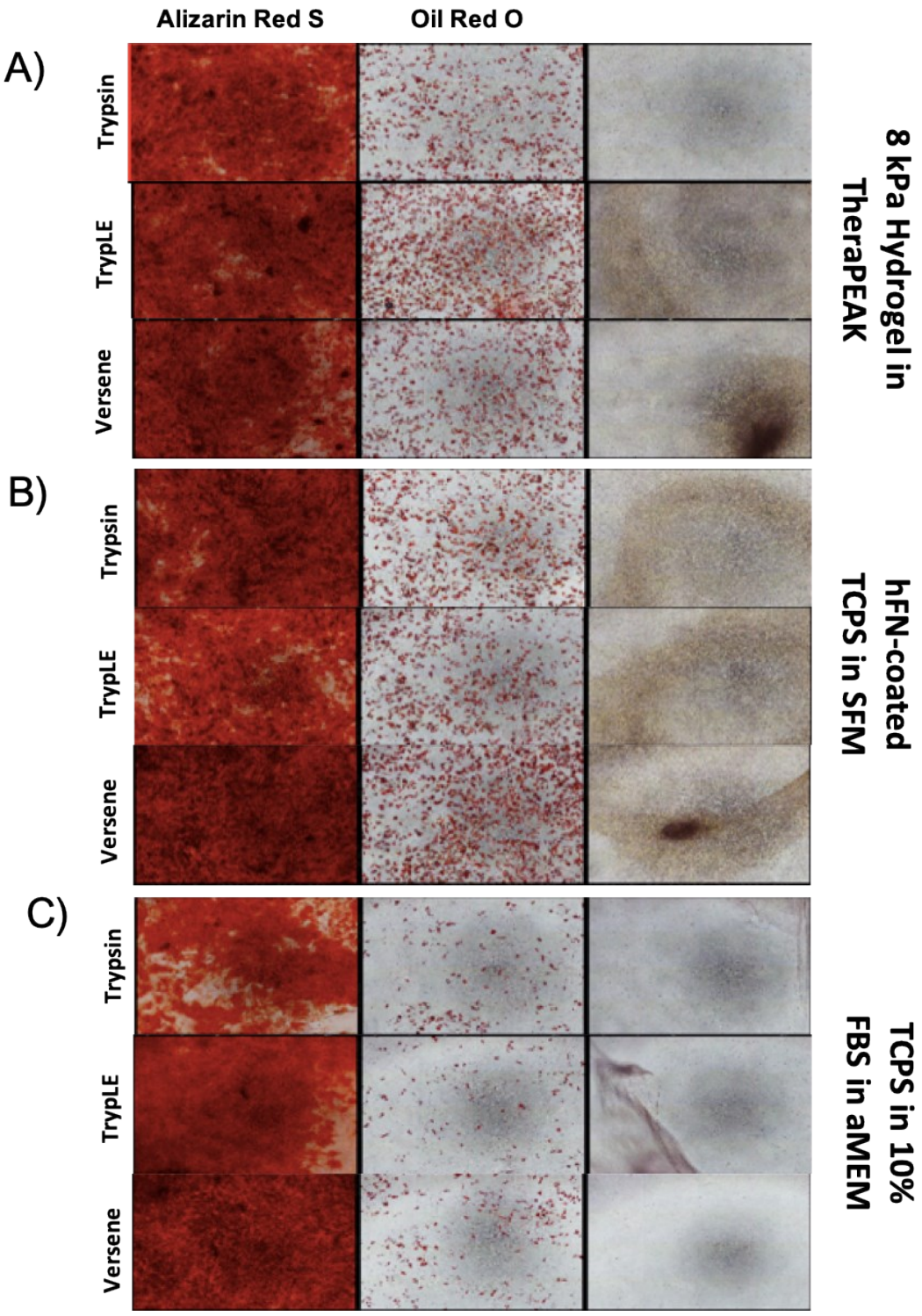


Figure S8. hMSC multipotency analysis after 8 days of culture on A) 8 kPa hydrogel in SFM, B) hFN-coated TCPS in SFM, or C) TCPS in 10% FBS in a αMEM and dissociated with trypsin, trypLE, or versene during harvest. All differentiation experiments conducted on collagen-coated TCPS. Osteogenic differentiation and no differentiation control (culture in αMEM + 2% FBS) assessed with Alizarin Red S staining and adipogenic differentiation staining assessed with Oil Red O staining after 28 days of culture in differentiation media.

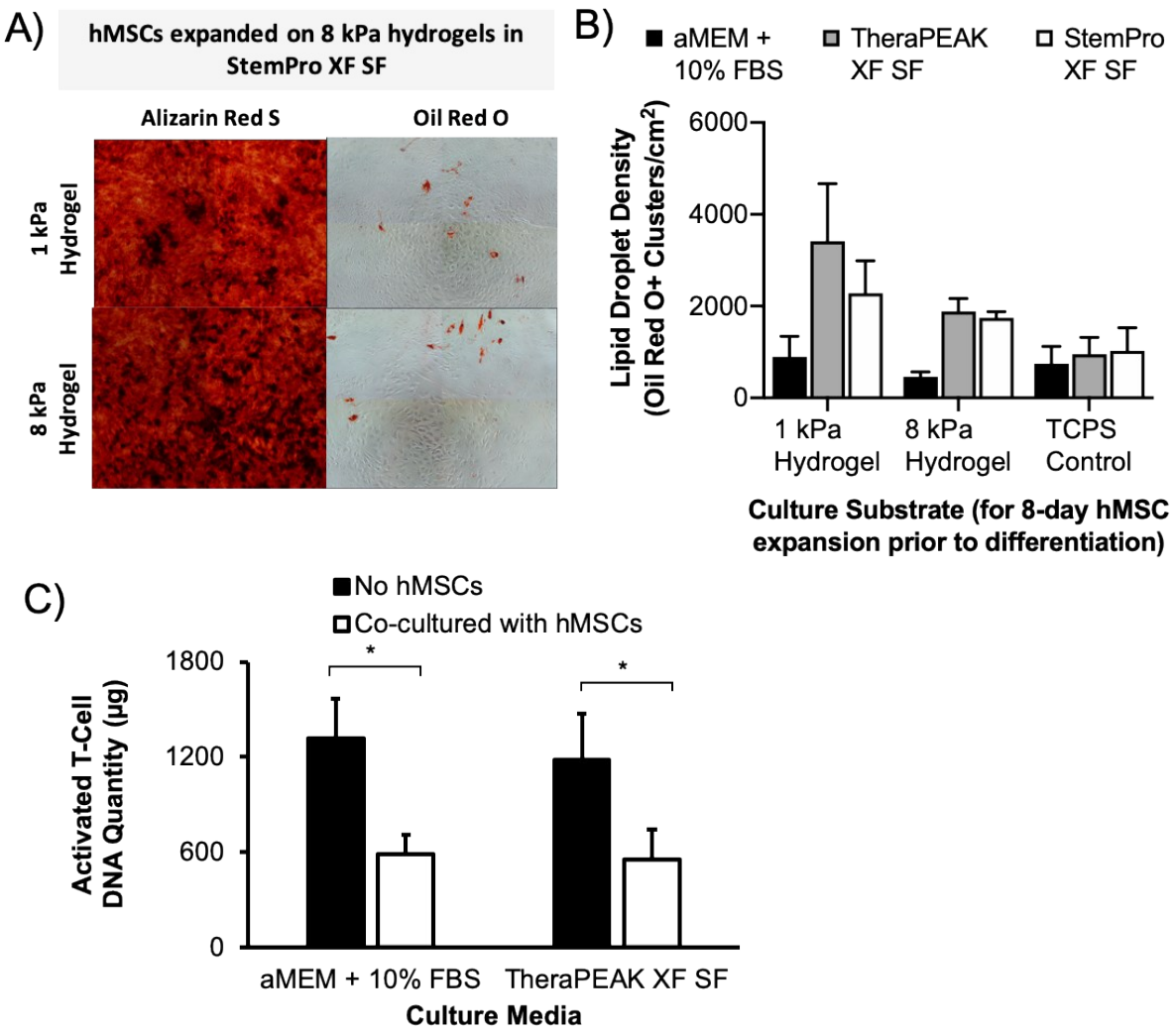


Figure S9. Confirmation of functional hMSCs after 8-days of expansion on hydrogels in SC and SF media. A,B) Directed differentiation and C) immunomodulatory activity by controlling T-cell proliferation.

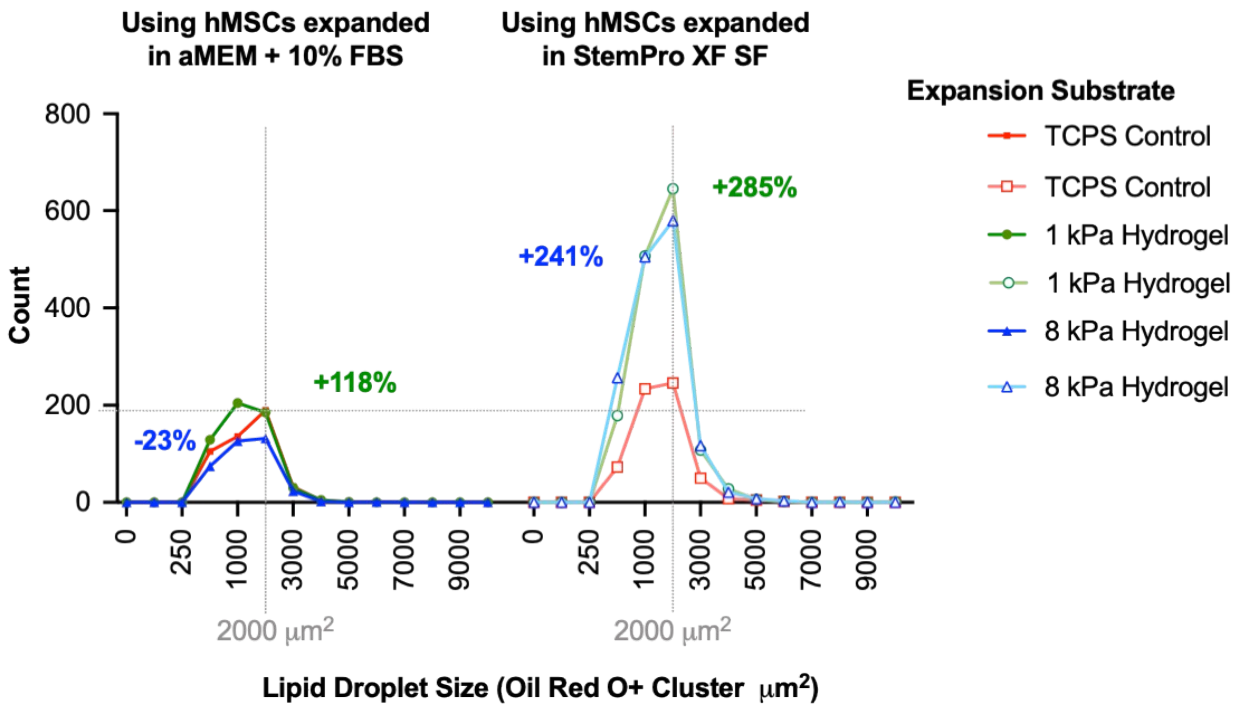


Figure S10. Directed hMSC adipogenic differentiation and Oil Red O+ quantification of hMSCs expanded for 8 days in αMEM + 10% FBS or StemPro XF SF media on TCPS controls or hydrogels of varying stiffness. Note TCPS control for αMEM + 10% FBS is uncoated TCPS and for StemPro XF SF is CellStart-coated TCPS.

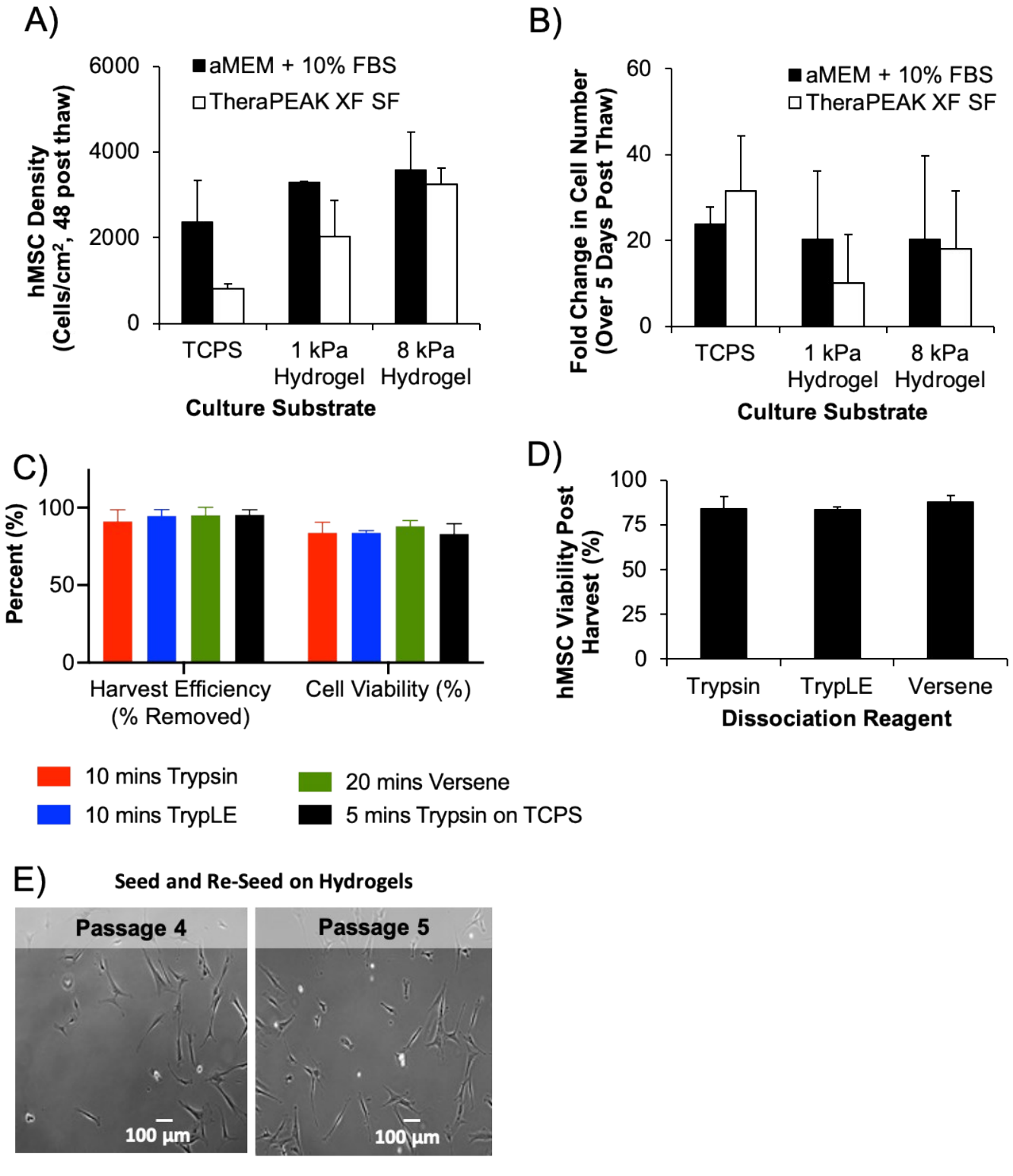


Figure S11. Integration of "hit" hydrogel substrates into standard hMSC culture workflow. A) hMSC adhesion and B) expansion following thaw directly onto hydrogels. hMSC C,D) viability following harvest with varying enzymatic and non-enzymatic dissociation reagents and E) after re-seeding onto new hydrogel substrates for continued expansion.

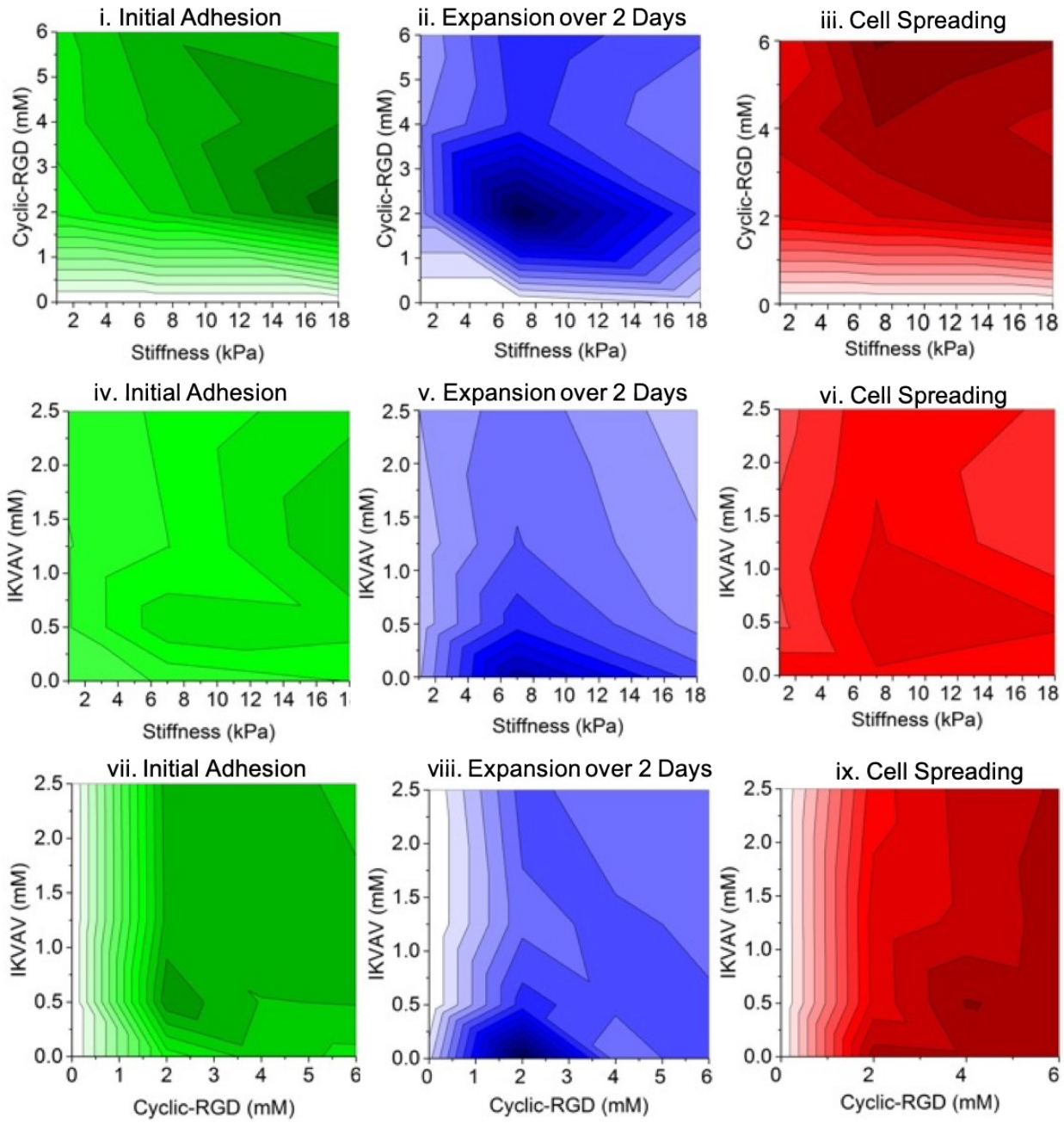


Figure S12. Heat map of hMSC i, iv, vii) adhesion, ii, v, viii) expansion and ii, vi, ix) spreading in TheraPEAK chemically-defined XF SF medium. Increasing color intensity indicates increasing adhesion, expansion, or spreading.

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