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Supplementary Materials for

Evolutionarily conserved sequence motif analysis guides development of chemically defined hydrogels for therapeutic vascularization

Jia Jia, Eun Je Jeon, Mei Li, Dylan J. Richards, Soojin Lee, Youngmee Jung, Ryan W. Barrs, Robert Coyle, Xiaoyang Li, James C. Chou, Michael J. Yost, Sharon Gerecht, Seung-Woo Cho*, Ying Mei*

*Corresponding author. Email: seungwoocho@yonsei.ac.kr (S.-W.C.); mei@clemson.edu (Y.M.)

Published 8 July 2020, *Sci. Adv.* **6**, eaaz5894 (2020)
DOI: 10.1126/sciadv.aaz5894

The PDF file includes:

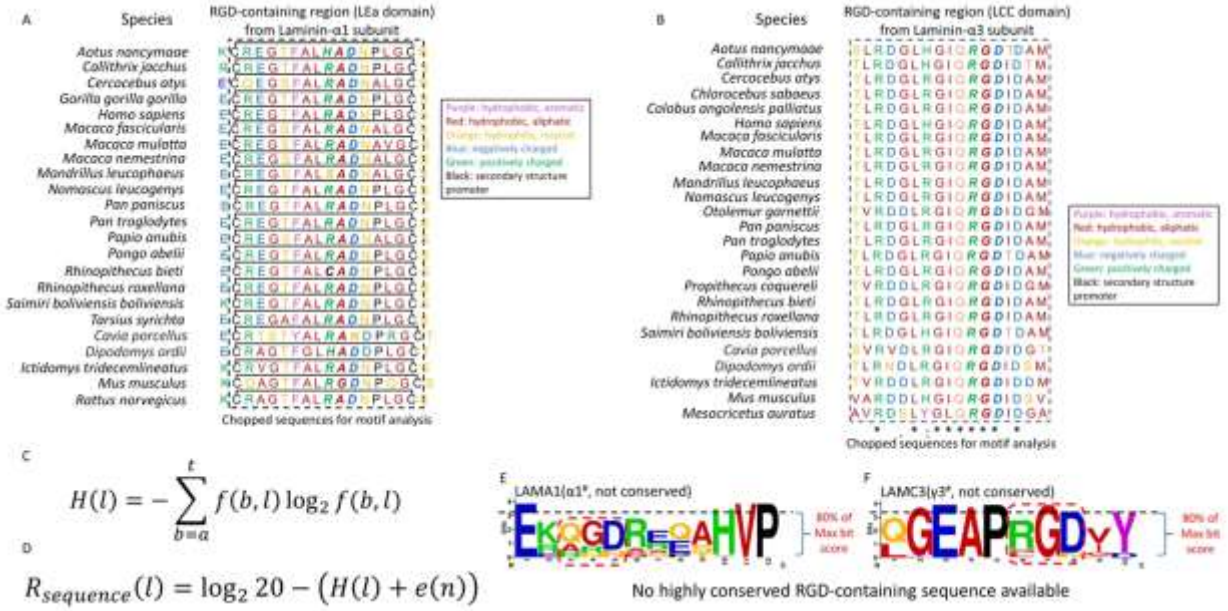
Figs. S1 to S10
Legends for tables S1 to S5

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/28/eaaz5894/DC1)

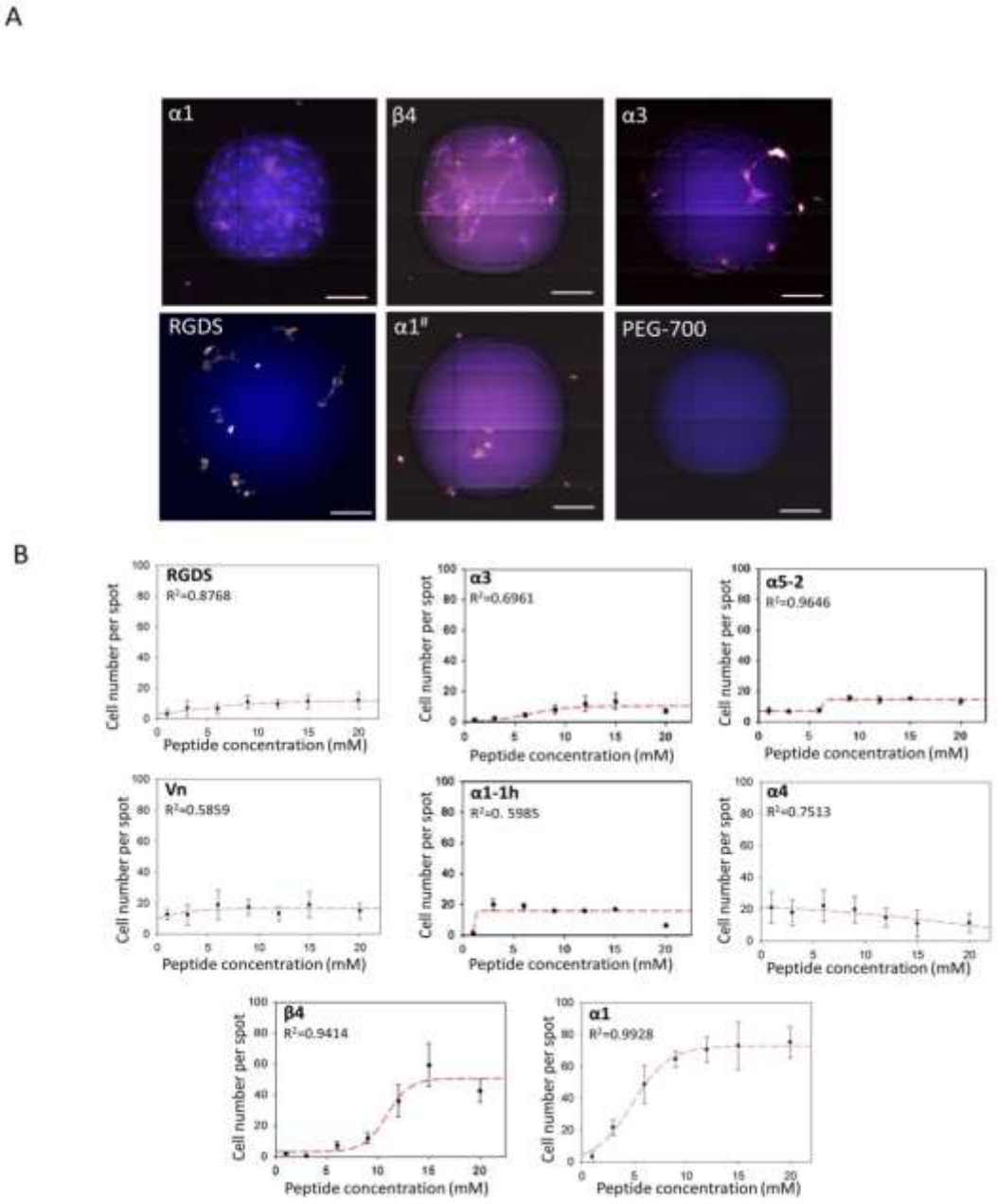
Tables S1 to S5

FIGURE S1



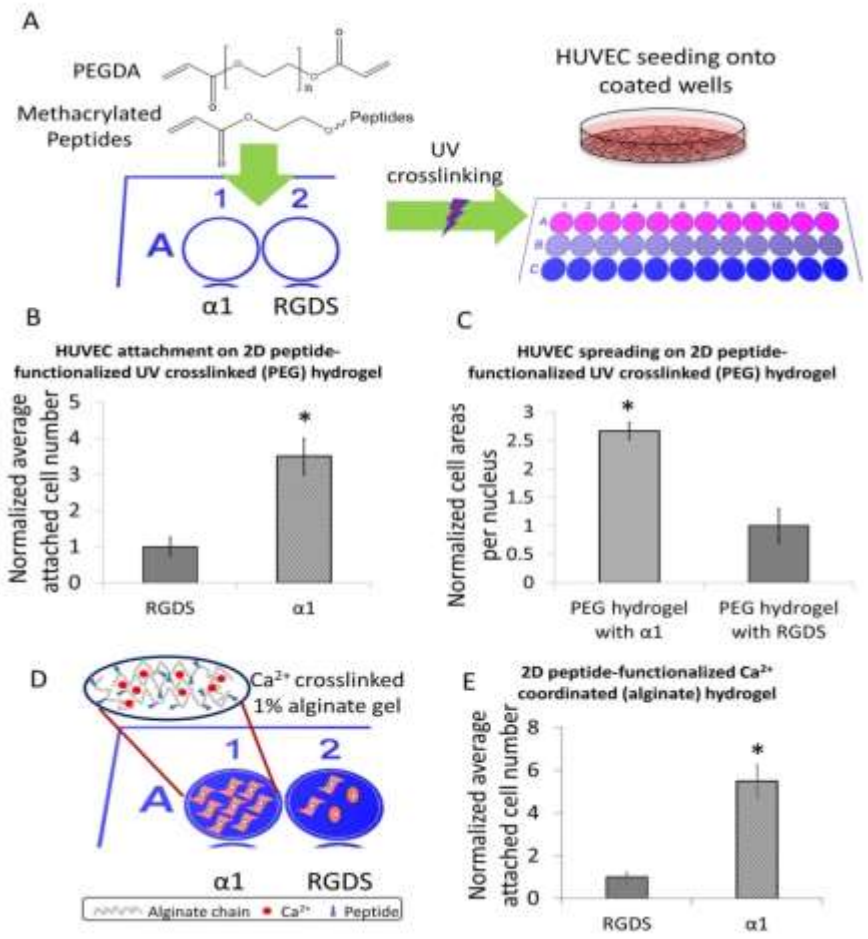
Supplementary Figure 1. The algorithm for the alignment of LM-RGD motifs from the species within Euarchontoglires. A. The chopping strategy of LM-RGD motifs at LEa/LEb/LEc domains. The whole motif between two cysteines has been chopped out for motif analysis. B. The chopping strategy of LM-RGD motifs at non-LEa/LEb/LEc domains (e.g. LCC domains). The motif between two (or more) unconserved AAs (alignment and conservation analyzed by cluster omega) has been chopped out for motif analysis. C. The mathematic definition of the uncertainty measure of a particular position in a series of aligned peptide sequences, where *H(l)* is the uncertainty of at position *l*, *b* is one of the 20 common amino acids and *f(b, l)* is the frequency of amino acid *b* at position *l*. D. The mathematic definition of the total information at a particular position of the aligned peptide sequences, where $R_{sequence}(l)$ is the value of information presented at position *l*, $\log_2 20$ is the maximum uncertainty at any given position and *e(n)* is a correction factor required when the alignment of the given position only contains a few sample sequences ($n \leq 50$) [40]. This mathematic model $R_{sequence}(l)$ represents the importance of at a given position of the given peptide sequences. E, F. The bitmap plot of the non-conserved RGD-containing sequences, ($\alpha 1^{\#}$) from Laminin-α1 (E) and ($\gamma 3^{\#}$) from laminin-γ3 (F), from the species in Euarchontoglires.

FIGURE S2



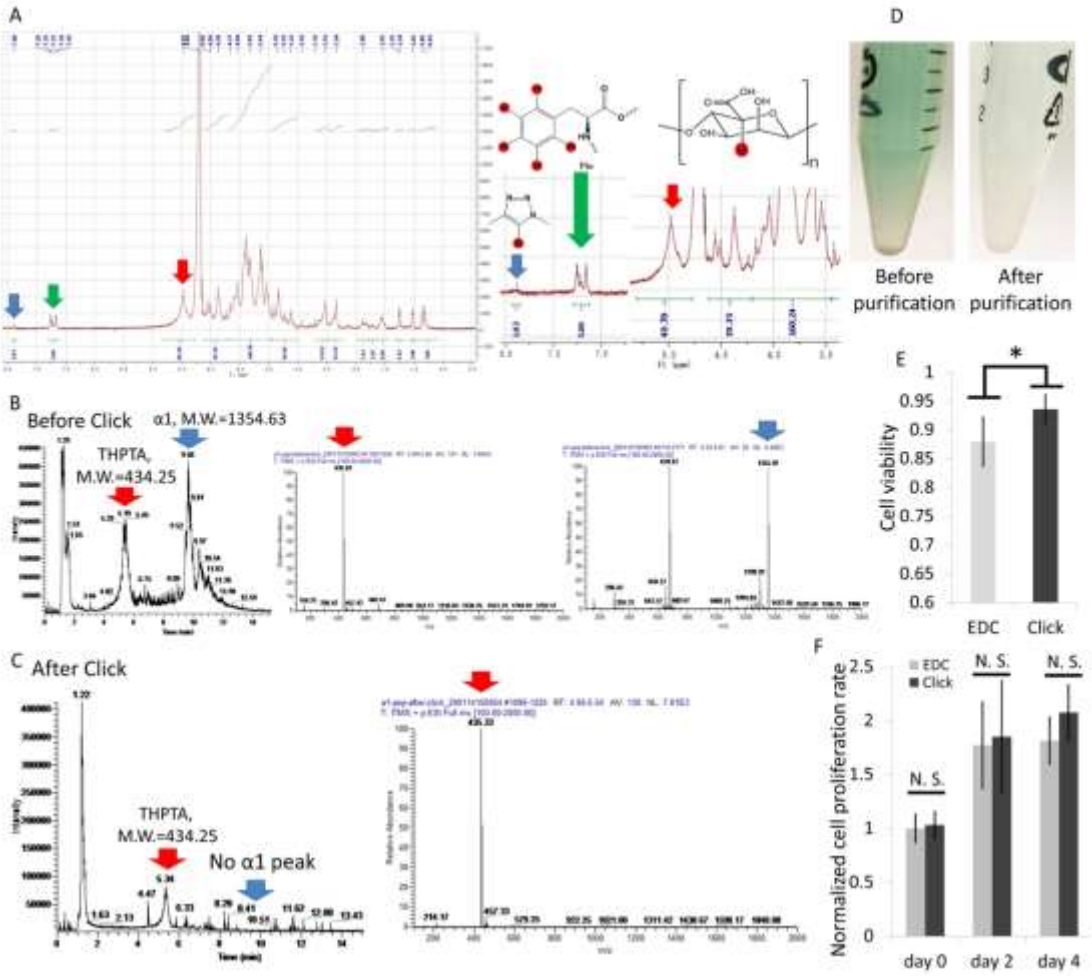
Supplementary Figure 2. HUVEC adhesion on peptide-functionalized hydrogel microarrays. A. The representative images of HUVEC attachment on high (α1), medium (β4) and minimal ($\alpha1^{\#}$) cell-adhesive hydrogels surfaces (RGDS surface included as positive controls; PEG-700, no peptide modified surface, included as negative controls). B. The plots of attached HUVECs on each spots versus peptide concentration conjugated on hydrogels for all LM-RGDs, $n \geq 10$ for each group. Most of the LM-RGDs showed the sigmoidal relationship. All plots represent mean $\pm$ SEM.

FIGURE S3



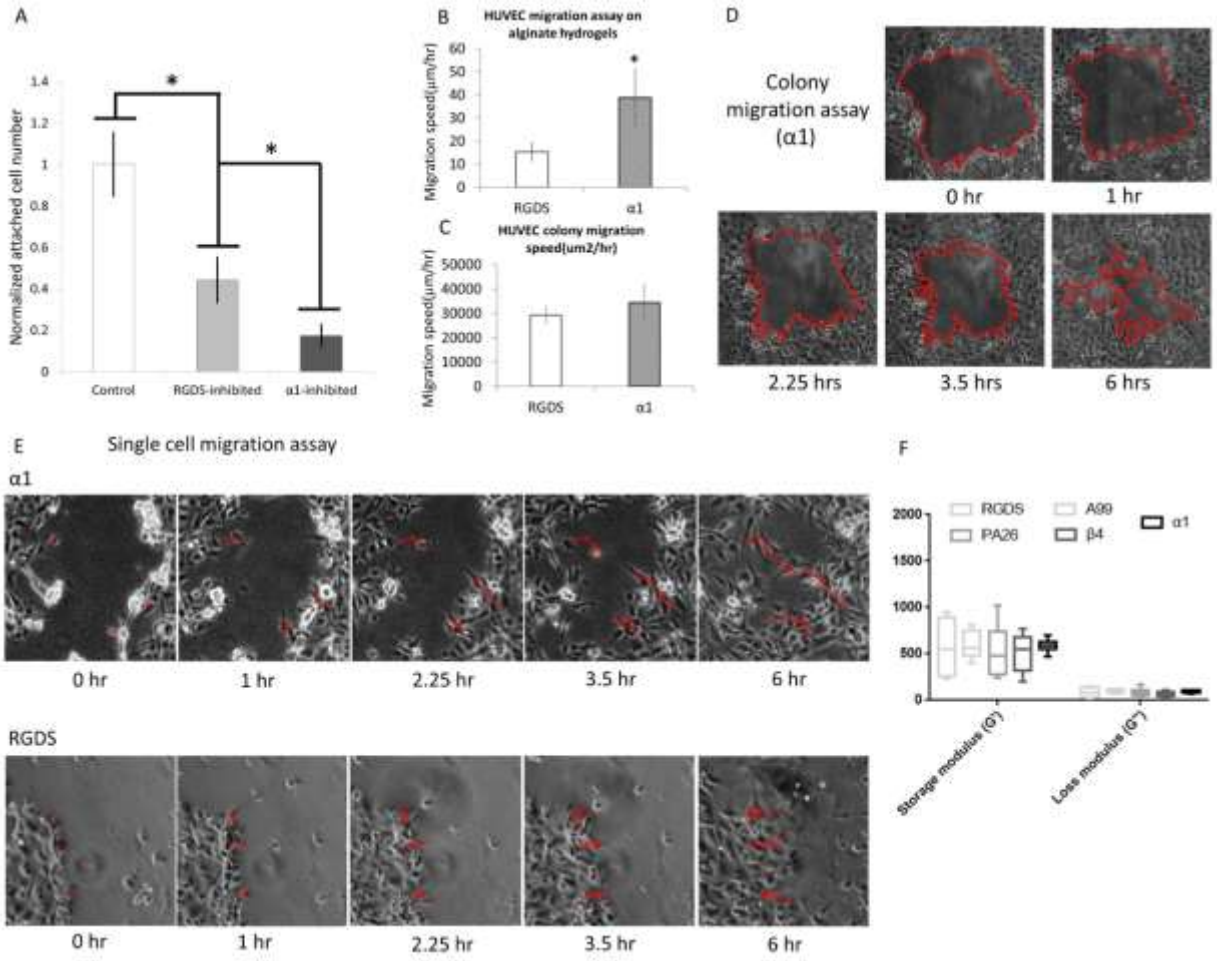
Supplementary Figure 3. HUVEC attachment and spreading on α1-modified hydrogels. A. The scheme of the experimental design of the peptides modified chemically crosslinked PEG hydrogel substrates (2D) for HUVEC attachment. B. The quantification of attached HUVECs on different peptides modified PEG hydrogel surface, n≥11 for each group. * indicates the significant difference of the attached HUVEC number on α1-modified hydrogel (2D) versus the cell number on RGDS modified hydrogel (2D), $P<0.05$. C. The quantification of the cell areas of attached HUVECs on different peptides modified PEG hydrogel surface. * indicates the significant difference of the cell area on α1-modified hydrogel (2D) versus the cell area on RGDS modified hydrogel (2D), $P<0.05$. D. The scheme of the experiment of the peptides modified physically crosslinked alginate hydrogel substrates (2D) for HUVEC attachment. E. The quantification of number of attached HUVECs on different peptides modified alginate hydrogel surface, n=10 for each group. * indicates the significant difference of the attached HUVEC number on α1-modified hydrogel (2D) versus the cell area on RGDS modified hydrogel (2D), $P<0.05$. All plots represent mean $\pm$ S.D.

FIGURE S4



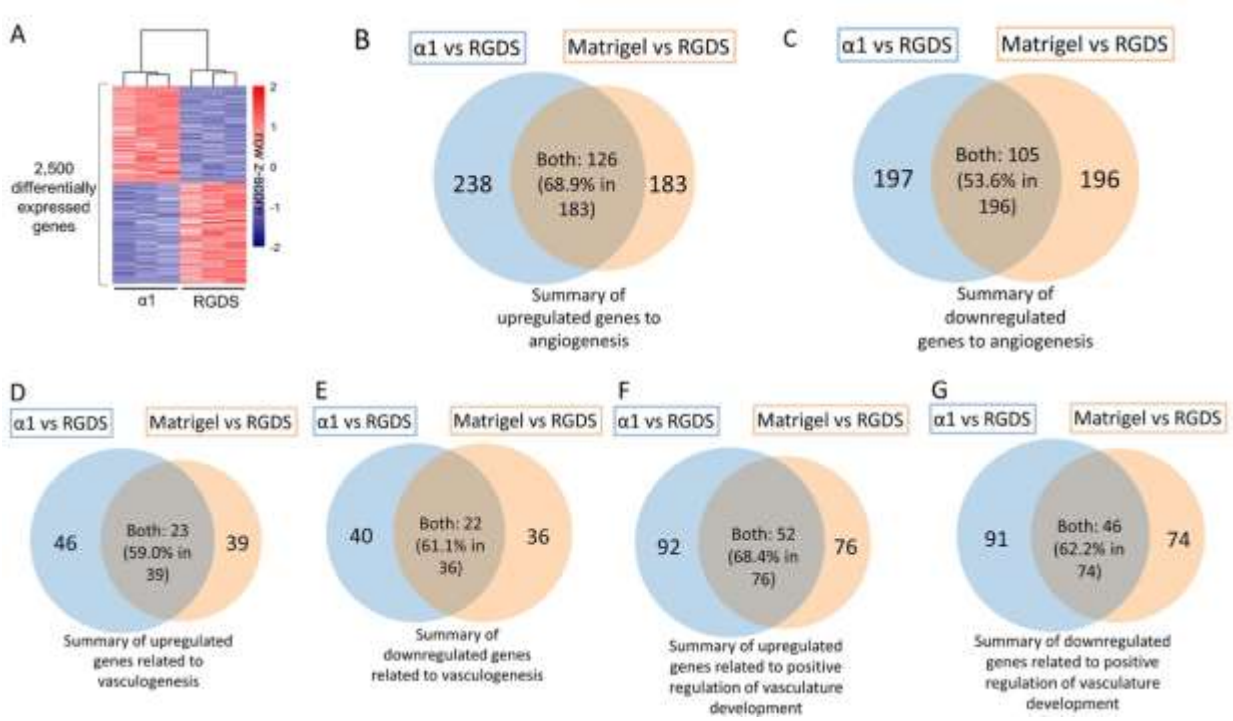
Supplementary Figure 4. Validation of peptide conjugation efficiency to alginate and the cytocompatibility of peptide-conjugated alginate through CuAAC. A. NMR spectrum of α1 functionalized alginate. Green, red and blue arrow indicates the protons on the benzene ring from phenylalanine (Phe, F) in α1 peptide, the proton on alginate backbone and the proton on triazole formed through click chemistry, respectively. B, C. LC and MS spectrum of α1 and CuAAC catalytic complex before and after Click conjugation. Red and blue arrow indicates the corresponding peaks of THPTA (M.W.=434.25, copper coordinating compound) and α1 peptide (M.W.=1354.63), respectively. D. The pictures of the alginate solution before and after purification. The colorless solution after purification indicates the removal of copper. E. Viability test of HUVECs on α1-functionalized hydrogels after 4 day culture. HUVEC viability on α1-conjugated hydrogel through EDC chemistry was used as control, $n \geq 10$. * indicates the significant difference, $P<0.05$. F. The proliferation rate of HUVECs on α1-functionalized hydrogels at day 0, 2 and 4. HUVEC proliferation rate on α1-conjugated hydrogel through EDC chemistry was used as control. No significant difference between the groups on each day. All plots represent mean ± S.D.

FIGURE S5



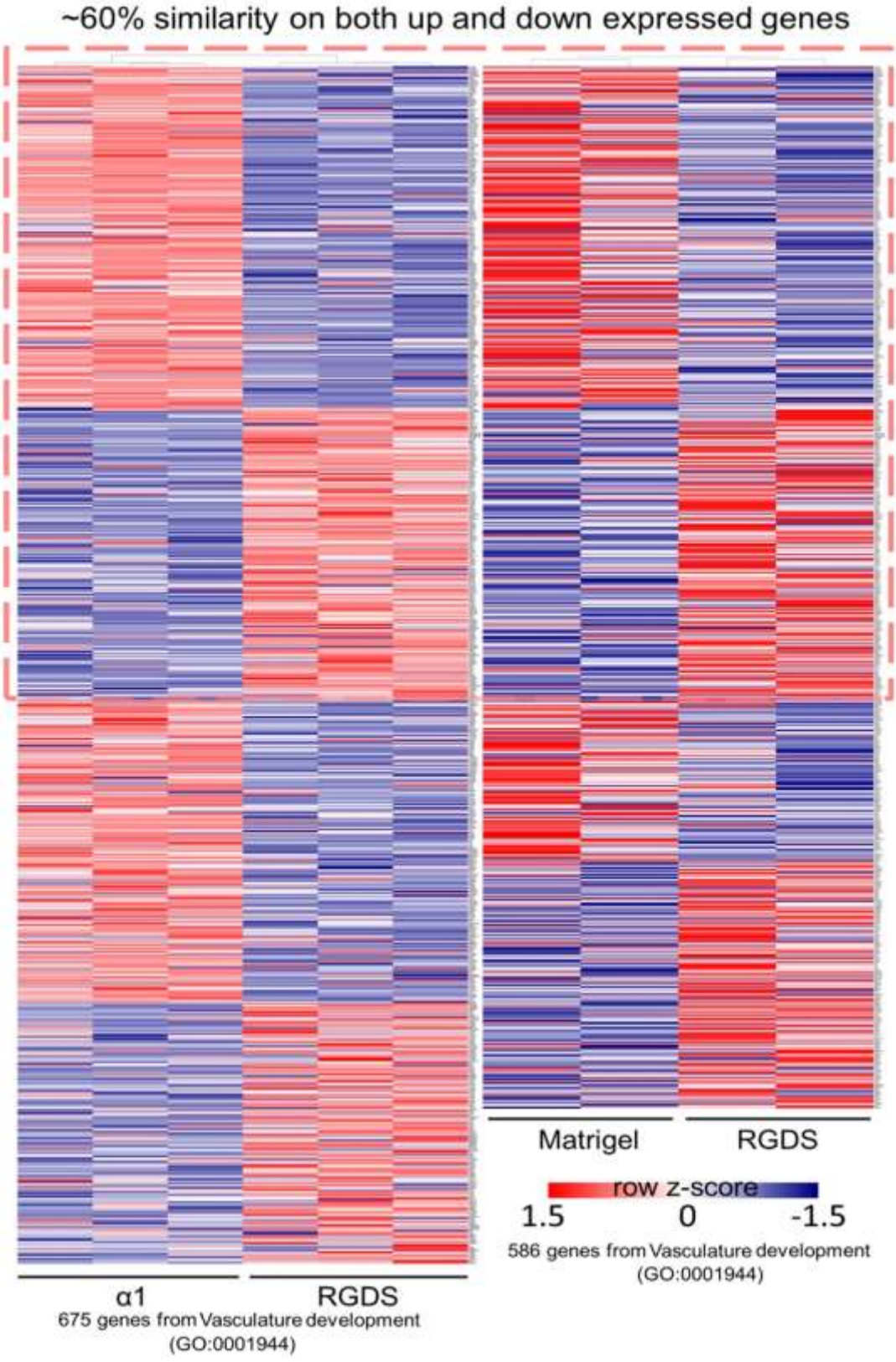
Supplementary Figure 5. In vitro analysis of HUVEC behaviors on α1-functionalized hydrogels. A. The inhibitory effects of soluble α1 and RGDS peptides on the HUVEC attachment to α1-functionalized hydrogels. (n≥11. * indicates significant difference, P<0.05. All plots represent mean + SD). B. Quantification of HUVEC migration in different peptides modified alginate hydrogels, n≥6 for each group. * indicates significant faster migration speed on α1-modified alginate hydrogels than on RGDS modified hydrogels, *P*<0.05. C. The quantification of the HUVEC colony migration speed within first 6 hours after the scratch on the peptides modified alginate hydrogels, n=6 for each group. D. The representative images of scratch based HUVEC colony migration assay at 0, 1, 2.25, 3.5 and 6 hours after scratch. Red loop indicates the unclosed areas measured for the calculation of migration speed. E. The representative images of scratch based single HUVEC migration assay at 0, 1, 2.25, 3.5 and 6 hours after scratch. The migration path of each cell has been marked in red. F. Mechanical properties of RGDS/α1/β4-modified 2% (w/w) alginate hydrogels, n=12 for each group. All plots represent mean ± S.D.

FIGURE S6



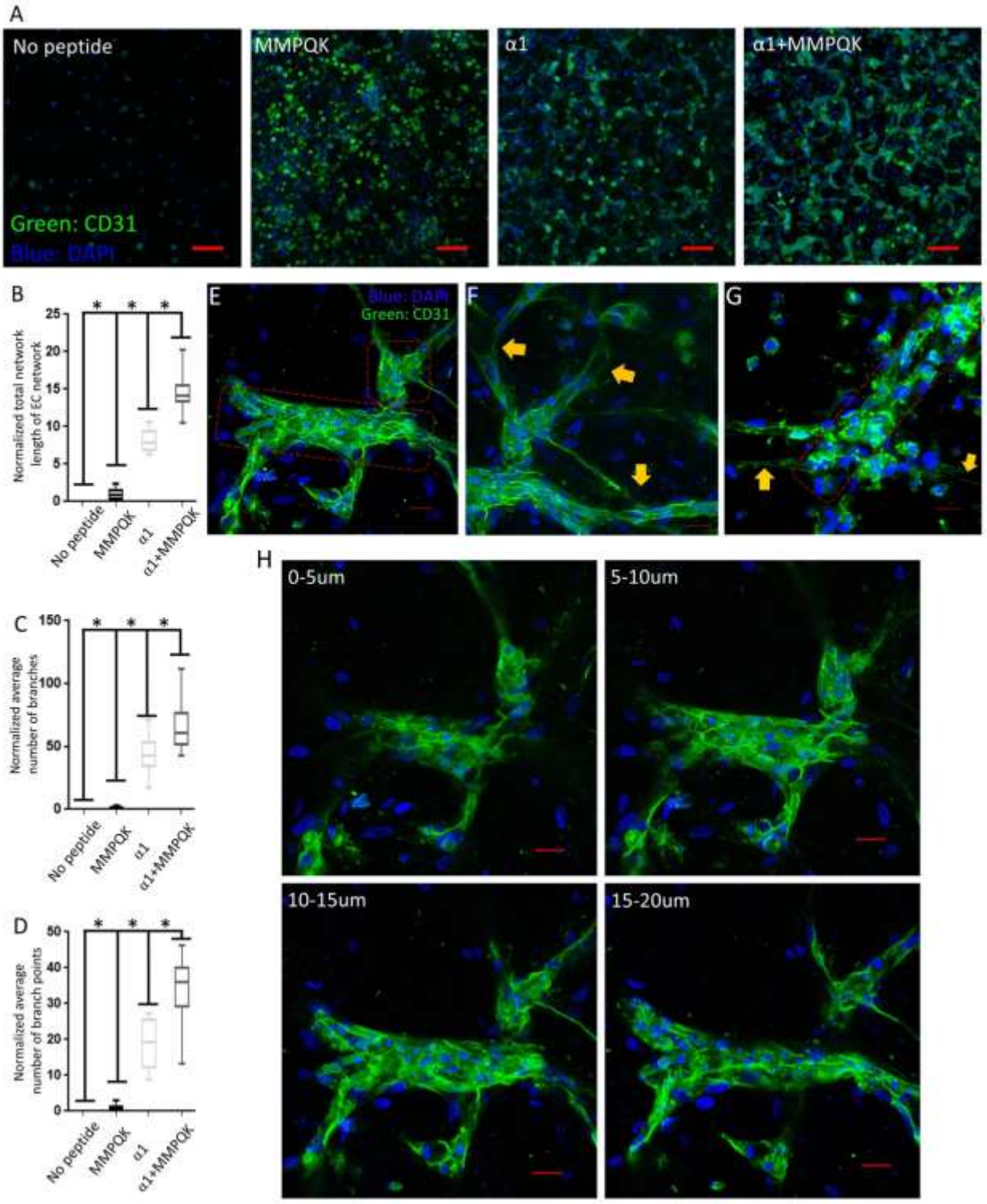
Supplementary Figure 6. Transcriptomic analyses of HUVEC cultured in α1-/RGDS-modified hydrogels. A. The heatmap of the total 2500 differential expressed genes (fold change >1.5 or <-1.5, adjusted p-value<0.05) from the transcriptomic assays of HUVECs cultured in α1-/RGDS-modified alginate hydrogels, ordered by fold change. B, C. The Venn diagram of upregulated (B) and downregulated (C) DE genes from transcriptomic assay of α1/RGDS and Matrigel/RGDS in the "angiogenesis" GO-BP term (GO:0001525). D, E. The Venn diagram of upregulated (D) and downregulated (E) DE genes from transcriptomic assay of α1/RGDS and Matrigel/RGDS in the "vasculogenesis" GO-BP term (GO:0001570). F, G. The Venn diagram of upregulated (F) and downregulated (G) DE genes from transcriptomic assay of α1/RGDS and Matrigel/RGDS in the "positive regulation of vasculature development" GO-BP term (GO:1904018).

FIGURE S7


~60% similarity on both up and down expressed genes
α1
RGDS
675 genes from Vasculature development
(GO:0001944)
Matrigel
RGDS
row z-score
1.5
0
-1.5
586 genes from Vasculature development
(GO:0001944)

Supplementary Figure 7. The transcriptomic comparison of the HUVECs cultured in α1/RGDS modified hydrogels versus those in Matrigel/RGDS-modified PEG hydrogel (GSE93511) from literature. The heatmap of 675 genes from GO-BP "vasculature development" term (GO:0001944) gene list of HUVECs cultured in α1/RGDS modified hydrogels and its comparison with the 586 genes from GO-BP "vasculature development" term of HUVECs cultured in Matrigel/RGDS-modified PEG hydrogel (GSE93511) from literature. The dashed red box summarized the shared "vasculature development" genes between α1/RGDS modified hydrogels and Matrigel/RGDS-modified PEG hydrogel, which revealed ~60% similar gene expression profiles.

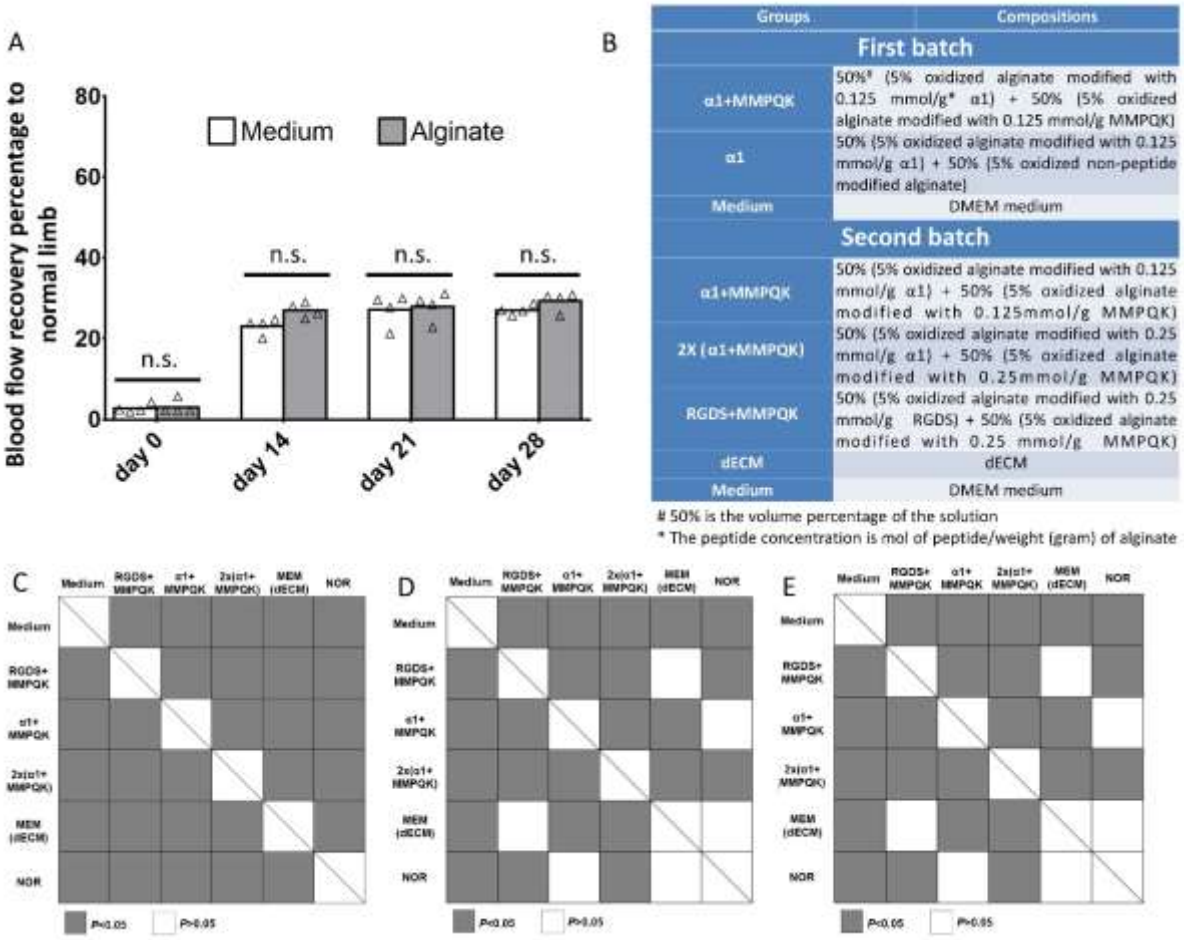
FIGURE S8



Supplementary Figure 8. The vasculogenesis assay of no peptide, MMPQK only, α1 only, α1+MMPQK-modified hydrogels revealed the formulation of α1+MMPQK promoted the

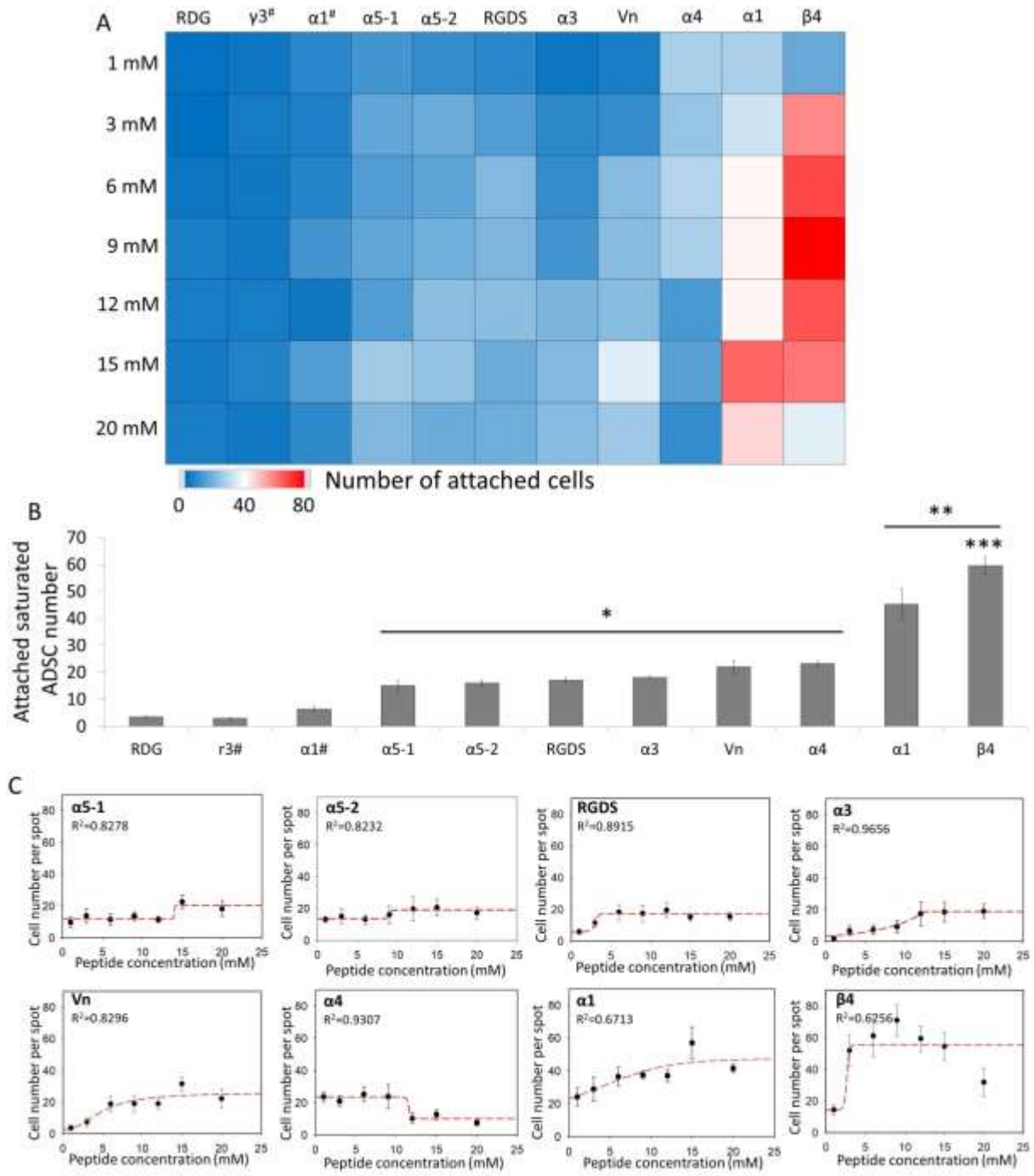
highest vasculogenic network formation. A. The representative images of HUVEC vascular network in no peptide, MMPQK only, α1 only, α1+MMPQK-modified hydrogels (Scale bars=100 μm). Green: CD31; blue: DAPI. Sprouting HUVEC clusters were found with elongated HUVECs in α1 only and α1+MMPQK-modified hydrogels. B, C, D. The quantification of total network length, branches and branch points of EC network in 3D hydrogel culture (no peptide, MMPQK only, α1 only, α1+MMPQK-modified hydrogels), n≥11 for each group. * indicates significant difference, $P<0.05$. E. Zoomed-in image of the EC "clusters" in the α1-modified hydrogels (E, F) and Matrigel (G) from Figure 4. Red box highlighted the EC clusters with established connection to the surrounding EC network (Scale bar=25 μm). Orange arrows highlighted the elongated ECs from EC "clusters" connected with the surrounding ECs. H. The image of each of 5 μm thickness layer of the EC cluster viewed from top to bottom (total thickness = 20 μm). Scale bar = 25μm. All plots represent mean ± S.D.

FIGURE S9



Supplementary Figure 9. The in vivo testing of α1+MMP-QK peptide-modified alginates. A. The experiment of blank alginate (non-peptide conjugated) effects in the murine hindlimb ischemia experiments, n=4 for each group. No significant difference was observed between media injected group and blank alginate (non-peptide conjugated) injected group at day 0, 14, 21 and 28. B. The table of all the treatment groups from first and second batches used in the murine hindlimb ischemia experiments. C, D, E. The heatmap of the significance analysis (t-test) in fibrotic area (%) (C), α-SMA+ arteries (D) and vWF+ capillaries (E) from the groups of the second batch at day 28.

FIGURE S10



Supplementary Figure 10. hADSC adhesion on peptide-functionalized hydrogel microarray. A, B. Heatmap of hADSC attachment and quantification of saturated attachment (from sigmoidal plots of peptide concentration versus attached cell number) on the LM-RGD derived hydrogel microarray, n≥10 for each group. * indicates significantly more hADSC attachment than on

hydrogels modified with RDG, $\alpha 1^{\#}$, $\gamma 3^{\#}$, $P<0.05$. ** indicates significantly more hADSC attachment than remaining groups, $P<0.05$. *** indicates significantly more hADSC attachment than on α1 modified hydrogels, $P<0.05$. C. The plots of attached hADSCs on each spot versus peptide concentration conjugated on hydrogels for all LM-RGDs. Most of the LM-RGDs showed the sigmoidal relationship. All plots represent mean $\pm$ SEM.

SUPPLEMENTARY TABLES

Supplementary Table 1. The list of proteins used for the bioinformatics screening to identify the evolutionarily conserved sequences.

Supplementary Table 2. The list of all DE genes (at least 1.5 fold change in positive or negative direction and q-value <0.05) from RNA sequencing of HUVECs in α1- versus RGDS-functionalized hydrogels.

Supplementary Table 3. The tables of all (max. 350 terms) significant ($p<0.05$) gene ontology (GO) terms of differentially expressed (DE) genes (fold change >1.5 or <-1.5, adjusted p-value<0.05) from HUVECs in α1- versus RGDS-functionalized hydrogels for the visualization of the cellular component (CC), molecular function (MF), and biological process (BP).

Supplementary Table 4. The tables of all the "vasculature development (GO:0001944)", "angiogenesis (GO:0001525)", "vasculogenesis (GO:0001570)" and "positive regulation of vasculature development (GO:1904018)" related genes from RNA sequencing of HUVECs in α1- versus RGDS-functionalized hydrogels and HUVECs in Matrigel- versus RGDS-functionalized PEG hydrogels. Upregulated/downregulated genes are categorized separately and the Venn diagrams have been generated based on this table in Figure 4E & 4F, Supplementary Figure 8B-G.

Supplementary Table 5. The groups and formulations used for vasculogenesis assay.