Supporting Information

Engineered Phage Matrix Stiffness-Modulating Osteogenic Differentiation

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Table S1. Phage concentrations used for the self-templating assembly and the estimated areal phage density for the observed structures estimated from the phage films.[1]

Self-templated structure	**Initial concentration (mg/mL)**	**Initial concentration (number of phages/mL)**	**Phage density in the resulting film (number of particles/cm^2)**
Nematic stripe pattern	0.1–0.2	3.2–6.4 × 10^{12}	4.9–6.5 × 10^{10}
Cholesteric helical ribbon	0.2–0.5	6.4–16 × 10^{12}	6.2–9.8 × 10^{10}
Nematic orthogonal twist	0.2–1.5	0.64–4.8 × 10^{13}	0.65–4.2 × 10^{11}
Smectic helicoidal nanofilament	4–6	1.3–2.0 × 10^{14}	1.9–2.9 × 10^{12}

Table S2. Primer sequences for pVIII, pIII, and pVII engineering.

Name	Oligonucleotide primer sequence*	Insert Peptide sequence**
p8-fw	5′ ATATAT **CTGCAG** *NK (NNK)$_2$ CGTGGT GAT*	A*XXXRGDXX*DP
RGD	*(NNK)$_2$* GATCCCGCAAAAGCGGCCTTTA ACTC CC 3′	A*DSGRGDTE*DP ***
p8-fw	5′ ATATAT **CTGCAG** *NN GGC CGT GGC GAT TCT*	A*GGRGDSDDY*DP ***
RDD	*GAT GAC GAT* GATCCCGCAAAAGCGGCCTTTAACT CCCTGCAAGCC 3′	
p8-REV	5′ CCT**CTGCAG**CGAAAGACAGCATCGG 3′	
p3-FWD	5′ AAACACT **CGGCCG** AAACTGTTGAAAGT TGTTTAGC 3′	
p3-rev	5′ TATATA **CGGCCG** A *TCCACCGCCGCACG*	
HPQ	*GCGGGCCCTGCGGATGGCACGC* CGAGTGAGAATAGAAAGGAACCACTAAAG GAATTGCG 3′	SHS*ACHPQGPLCGGG*A
P7-Fwd	5′ ATATAT **GGATCC**	
HPQ	ATGGAGTGCNNKCATCCGCAGNNKTGTGTCGCG GATTTCGACACAATTTATCAG 3′	
P7-REV	5′ AAACAC**GGATCC**GTTACTTAGCCGGAACGAGG CGCAGACGGT 3′	ME*CLHPQTC*V

* For primer oligonucleotide sequences, the restriction sites are indicated in **bold** and the insert is *underlined and italicized*.

** For the resulting peptide sequence, the insert is *underlined and italicized*.

*** Constructed using the partial library approach;[2] selected sequence indicated.

Table S3. Phage cloning polymerase chain reaction (PCR) conditions.

PCR ingredients	pVIII PCR conditions	pIII or pVII PCR conditions
~25 ng dsDNA template*		
2.5 µL 10 µM forward primer		
2.5 µL 10 µM reverse primer		
1 µL dNTP (10 mM mixture of A, T, G, and C bases)	98 °C 1 min	98 °C 1 min
1 µL DMSO	/ 98 °C 15 s	/ 98 °C 15 s
10 µL 5X HF Phusion Polymerase buffer	25x < 58 °C** 20 s	25x < 61 °C 20 s
Balance with sterile H_2O to 50 µL	\ 72 °C 3 min 30 s	\ 72 °C 3 min 30 s
1 µL Phusion polymerase Enzyme	72 °C 4 min	72 °C 4 min
	4 °C ∞	4 °C ∞

* ~1 µL; use any template that has a PstI site for pVIII M13 engineering the EagI and the BamHI sites for pIII and pVII M13 engineering

** Primer annealing temperature = Primer Tm (lower of the two primers) − 2

Table S4. Primer sets for mouse osteoblast cell markers.[3]

Name		**Sequence 5-3**	**Length (bp)**
Collagen pro-alpha-1 type I chain	COL I-Fw	5′ GGAGAGAGCATGACCGATGGA 3′	102
	COL I-Re	5′ GGTGGACATTAGGCGAGGAA 3′	
Osteopontin	OP-Fw	TGAAAGTGACTGATTCTGGCA	375
	OP-Re	GGACGATTGGAGTGAAAGTGT	
Alkaline phosphatase	ALP-Fw	5′ CCA GCA GGT TTC TCT CTT GG 3′	239
	ALP-Re	5′ CTG GGA GTC TCA TCC TGA GC 3′	
Osteocalcin	OCN-Fw	5′ CTC ACT CTG CTG GCC CTG 3′	257
	OCN-Re	5′ CCG TAG ATG CGT TTG TAG GC 3′	
Dentin matrix protein 1	Dmp I-Fw	5′ CCC AGA GGC ACA GGC AAA TA 3′	211
	Dmp I-Re	5′ TCC TCC CCA CTG TCC TTC TT 3′	
β-Actin	BAT-Fw	GTCCCTCACCCTCCCAAAAG	266
	BAT-Re	GCTGCCTCAACACCTCAACCC	

Table S5. Primer sets for human osteoblast cell markers.

Name		**Sequence 5-3**	**Length (bp)**
Runx2	FW	CCACCACTCACTACCACACC	70
	RE	AAGGGTCCACTCTGGCTTTG	
Alkaline phosphatase (ALP)	FW	CTATCCTGGCTCCGTGCTCC	100
	RE	GCTGGCAGTGGTCAGATGTT	
Osteopontin (OP)	FW	CGAGGTGATAGTGTGGTTTATGG	128
	RE	GCACCATTCAACTCCTCGCTTTC	
Osteocalcin (OCN)	FW	CAAAGGTGCAGCCTTTGTGTC	150
	RE	TCACAGTCCGGATTGAGCTCA	
Collagen pro-alpha-1 type I (COL)	FW	CTGGCAGCCCTGGTGAAA	114
	RE	CACCATCATTTCCACGAGCA	
β-Actin	FW	AGAGCTACGAGCTGCCTGAC	184
	RE	AGCACTGTGTTGGCGTACAG	

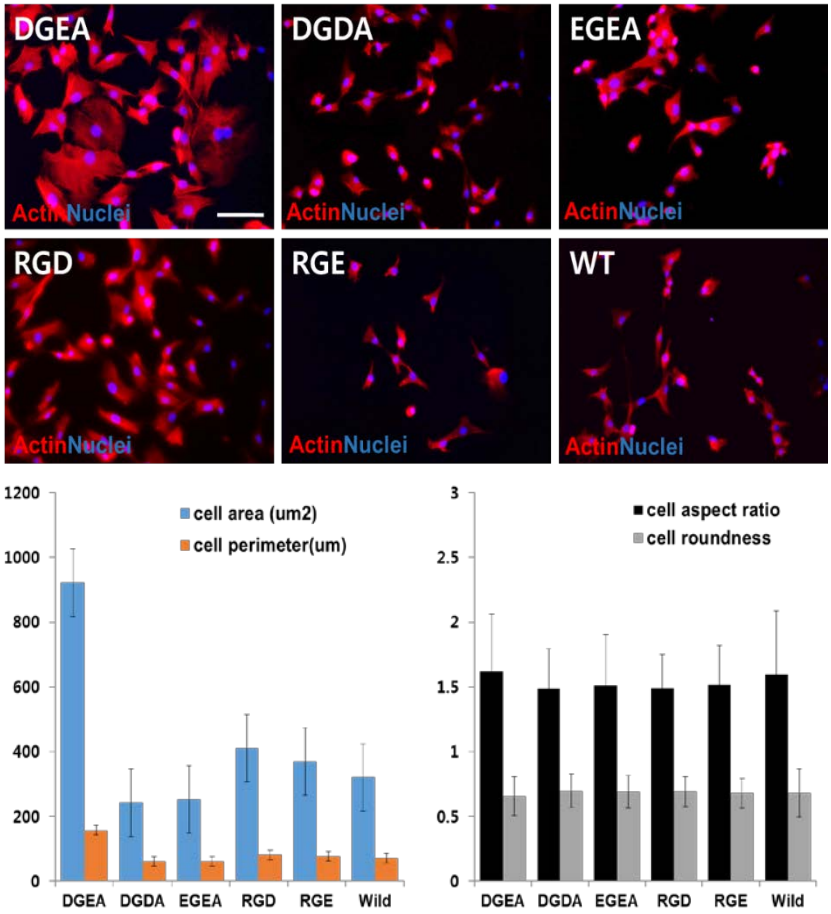

Figure S1. Morphological anaylsis of MC3T3 preosteoblast cells on different phages (DGEA, DGDA, EGEA, RGD, RGE-diplaying or wild-type phages). Fluorescent microscopy image of preosteoblast on different type of phage matrices (Up, Actin was stained with phalloidin (red) and nucleus was stained with DAPI (blue). Scale bar = 100μm). The bar graph shows the quantification of area, perimeter, AR, and roundness of cells on each phage (Bottom, Two to three fields of views were taken to anlayze each images. cell *n*= 5 per one filed of view).

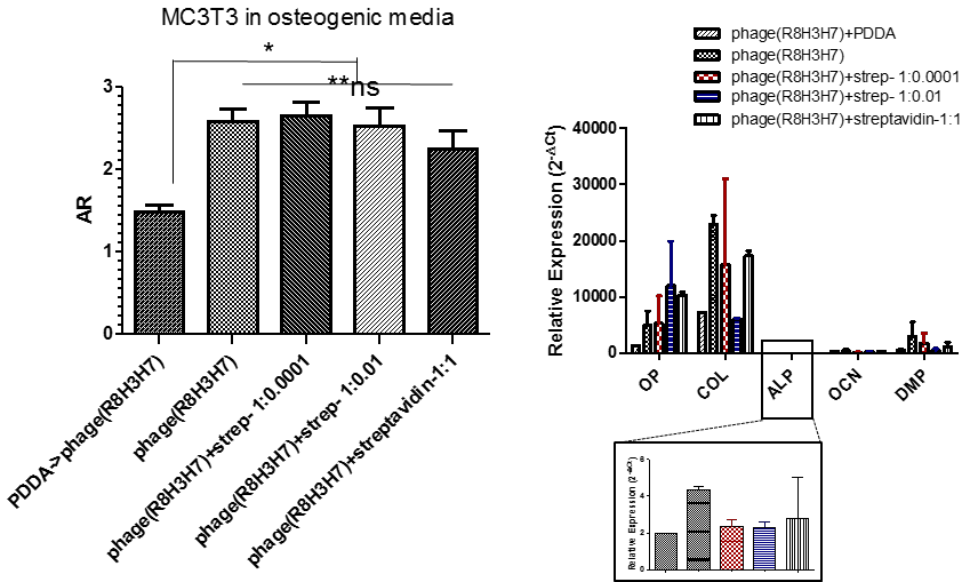

Figure S2. Morphology of preosteoblasts on top of different phage matrixs (left) and quantitative osteogenic differentiation marker expression of the cells on the different stiffness ranged phage matrix (right) in osteogenic differentiation media. With ostegenic induction media, there was no significant difference in AR (left) and osteogenic marker expression (right) of MC3T3s on the different stiffness ranged phage matrix (phage only, phage+strep-1:0.0001, phage+strep-1:0.01 and phage+strep-1:1, Most of them have about 2.5 of ARs at 24h culture; **ns $p>0.05$, ANOVA) whereas ARs of MC3T3 on phage matrix with low stiffenss (3-6kPa, phage+PDDA) are around 1.5 (*$p<0.05$ *vs* phage(R8H3H7) phage matrix, *t*-test). Two to three fields of views were taken to anlayze each images. cell $n= 10$ per one filed of view.

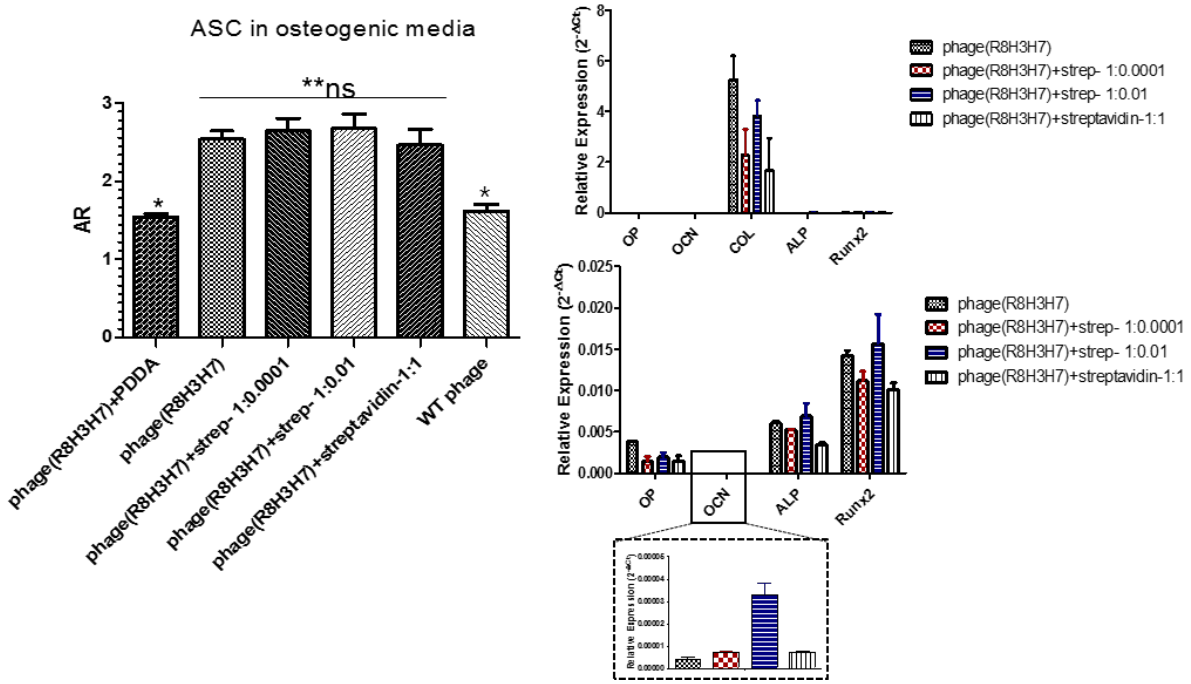

Figure S3. Morphology of human adipose-derives stem cells (ASCs) in osteogenic differentiation media on top of different phage matrixs (left) and quantitative osteogenic differentiation marker expression of the cells on the different stiffness ranged phage matrix (right). With ostegenic induction media, there was no significant difference in AR (left) and osteogenic marker expression (right) of ASCs on the different stiffness ranged phage matrix (phage only, phage+strep-1:0.0001, phage+strep-1:0.01 and phage+strep-1:1, ARs are ~ 2.5 at 24h culture; **ns p>0.05, ANOVA) whereas ARs of ASCs on phage matrix with low stiffenss (3-6kPa, phage+PDDA) or phage matix without RGD motif (20-40kPa, WT phage matrix) are around 1.5 (*p<0.05 *vs* phage(R8H3H7) phage matrix, *t*-test). Two to three fields of views were taken to anlayze each images. cell n= 10 per one filed of view.

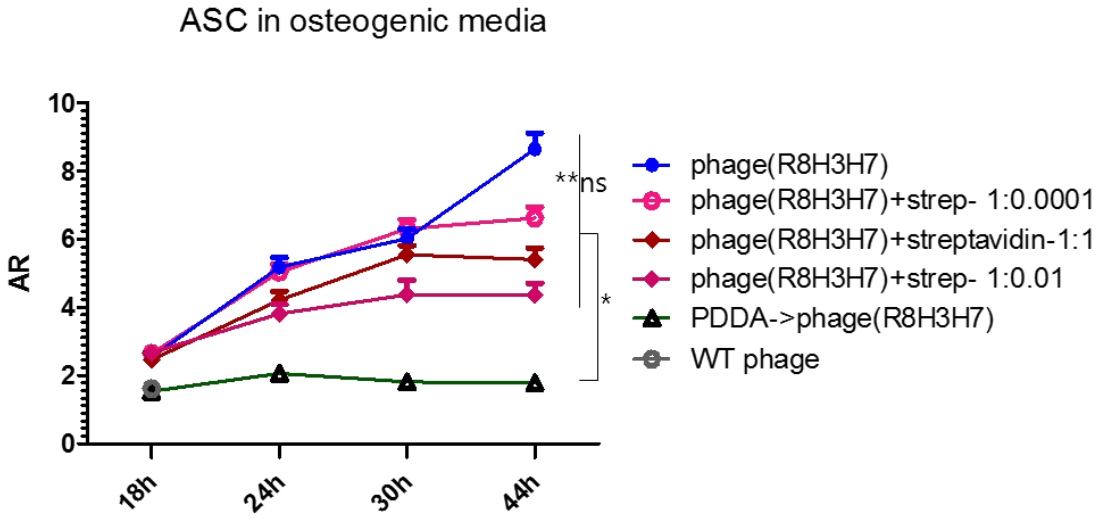

Figure S4. AR changes of ASCs on the different stiffness ranged phage matrices with osteogenic differentation media according to the culture time. There was no significant difference in AR chanages of ASCs on the different stiffness ranged phage matrix (phage only, phage+strep-1:0.0001, phage+strep-1:0.01 and phage+strep-1:1; **ns p>0.05, ANOVA) whereas that of ASCs on phage matrix with low stiffenss (3-6kPa, phage+PDDA) are kept as around 1.5 (*p<0.05 *vs* phage(R8H3H7) matrix with stiffness ranges of higher than 20 kPa, *t*-test). Two to three fields of views were taken to anlayze each images. cell n= 10 per one filed of view.

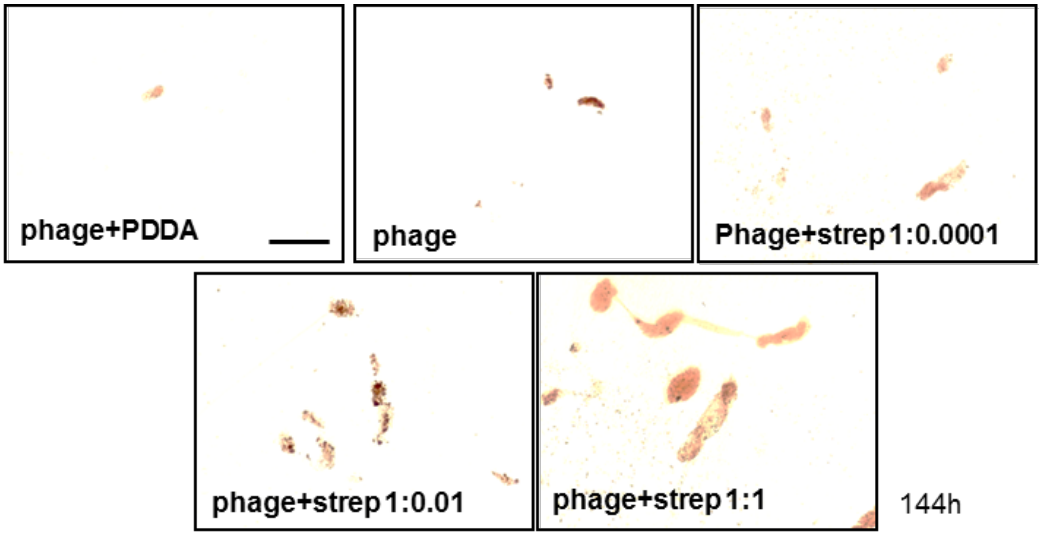

Figure S5. Alizarin Red staining of ASCs on the different stiffness ranged phage matrices with osteogenic differentation media for 144h. Correlation between mineralization and phage matrix stiffness is apparently oberved with Alizarin Red staining of ASCs on the different stiffness ranged phage matrix even at 144h culture (at 6 day, phage+strep 1:1 > phage+strep1:001 > phage+strep 1:0.0001 > phage only > phage+PDDA, Scale bar = 20 μm).

References

(1) Chung, W.-J.; Oh, J.-W.; Kwak, K.; Lee, B. Y.; Meyer, J.; Wang, E.; Hexemer, A.; Lee, S.-W. Biomimetic self-templating supramolecular structures. *Nature* **2011,** *478* (7369), 364-368, DOI: 10.1038/nature10513.

(2) Merzlyak, A.; Indrakanti, S.; Lee, S. W. Genetically engineered nanofiber-like viruses for tissue regenerating materials. *Nano Lett* **2009,** *9* (2), 846-52, DOI: 10.1021/nl8036728 10.1021/nl8036728 [pii].

(3) Yoo, S. Y.; Kobayashi, M.; Lee, P. P.; Lee, S. W. Early osteogenic differentiation of mouse preosteoblasts induced by collagen-derived DGEA-peptide on nanofibrous phage tissue matrices. *Biomacromolecules* **2011,** *12* (4), 987-996, DOI: 10.1021/bm1013475.