Supporting Information

Three-dimensional decellularized tumor extracellular matrices with different stiffness as bioengineered tumor scaffolds

Yonggang Lv[a,b,*], Hongjun Wang[a,b], Gui Li[a,b], Boyuan Zhao[a,b]

[a]Mechanobiology and Regenerative Medicine Laboratory, Bioengineering College, Chongqing University, Chongqing 400044, P. R. China
[b]Key Laboratory of Biorheological Science and Technology (Chongqing University), Ministry of Education, Bioengineering College, Chongqing University, Chongqing 400044, P. R. China

***Corresponding author:**

Dr. Yonggang Lv, Professor

Mechanobiology and Regenerative Medicine Laboratory
Bioengineering College, Chongqing University
174 Shazheng Street, Shapingba District, Chongqing 400044, China
Tel: 86-23-65102507
Fax: 86-23-65102507
E-mail: yglv@cqu.edu.cn
Web: http://yglvbme.com

Supplementary information for the sequence of LOX gene

The sequence of LOX gene:

ATGCGCTTCGCCTGGACCGTGCTCCTGCTCGGGCCTTTGCAGCTCTGCGCGCTAGT
GCACTGCGCCCCTCCCGCCGCCGGCCAACAGCAGCCCCCGCGCGAGCCGCCGGCGGC
TCCGGGCGCCTGGCGCCAGCAGATCCAATGGGAGAACAACGGGCAGGTGTTCAGCTT
GCTGAGCCTGGGCTCACAGTACCAGCCTCAGCGCCGCCGGGACCCGGGCGCCGCCGT
CCCTGGTGCAGCCAACGCCTCCGCCCAGCAGCCCCGCACTCCGATCCTGCTGATCCGC
GACAACCGCACCGCCGCGGCGCGAACGCGGACGGCCGGCTCATCTGGAGTCACCGCT
GGCCGCCCCAGGCCCACCGCCCGTCACTGGTTCCAAGCTGGCTACTCGACATCTAGAG
CCCGCGAAGCTGGCGCCTCGCGCGCGGAGAACCAGACAGCGCCGGGAGAAGTTCCTG
CGCTCAGTAACCTGCGGCCGCCCAGCCGCGTGGACGGCATGGTGGGCGACGACCCTTA
CAACCCCTACAAGTACTCTGACGACAACCCTTATTACAACTACTACGATACTTATGAAA
GGCCCAGACCTGGGGGCAGGTACCGGCCCGGATACGGCACTGGCTACTTCCAGTACGG
TCTCCCAGACCTGGTGGCCGACCCCTACTACATCCAGGCGTCCACGTACGTGCAGAAG
ATGTCCATGTACAACCTGAGATGCGCGGCGGAGGAAAACTGTCTGGCCAGTACAGCAT
ACAGGGCAGATGTCAGAGATTATGATCACAGGGTGCTGCTCAGATTTCCCCAAAGAGT
GAAAAACCAAGGGACATCAGATTTCTTACCCAGCCGACCAAGATATTCCTGGGAATGG
CACAGTTGTCATCAACATTACCACAGTATGGATGAGTTTAGCCACTATGACCTGCTTGAT
GCCAACACCCAGAGGAGAGTGGCTGAAGGCCACAAAGCAAGTTTCTGTCTTGAAGAC
ACATCCTGTGACTATGGCTACCACAGGCGATTTGCATGTACTGCACACACACAGGGATT
GAGTCCTGGCTGTTATGATACCTATGGTGCAGACATAGACTGCCAGTGGATTGATATTAC
AGATGTAAAACCTGGAAACTATATCCTAAAGGTCAGTGTAAACCCCAGCTACCTGGTTC
CTGAATCTGACTATACCAACAATGTTGTGCGCTGTGACATTCGCTACACAGGACATCAT
GCGTATGCCTCAGGCTGCACAATTTCACCGTATTAG.

Supplementary Table

Table S1 PCR primer sequences.

Gene	Primer sequences (5'→3')
LOX	Forward: 5'-GCATACAGGGCAGATGTCAGA-3' Reverse: 5'-TTGGCATCAAGCAGGTCATAG-3'
ABCB1	Forward: 5'-TTGCTGCTTACATTCAGGTTTCA-3' Reverse: 5'-AGCCTATCTCCTGTCGCATTA-3'
ABCC3	Forward: 5'-ATTCCACTCAACGGAGCTGTG-3' Reverse: 5'-GCGCGAGTCCTTCAATTTCAT-3'
ABCG2	Forward: 5'-TGAGCCTACAACTGGCTTAGA-3' Reverse: 5'-CCCTGCTTAGACATCCTTTTCAG-3'
FAK	Forward: 5'-GCTTACCTTGACCCCAACTTG-3' Reverse: 5'-ACGTTCCATACCAGTACCCAG-3'
YAP	Forward: 5'-TAGCCCTGCGTAGCCAGTTA-3' Reverse: 5'-TCATGCTTAGTCCACTGTCTGT-3'
Bcl2	Forward: 5'-GAACTGGGGGAGGATTGTGG-3' Reverse: 5'-CCGGTTCAGGTACTCAGTCA-3'
GAPDH	Forward: 5'-AAATTCCATGGCACCGTCAAGGCT-3' Reverse: 5'-CTCATGGTTCACACCCATGACGAA-3'

Supplementary Figures

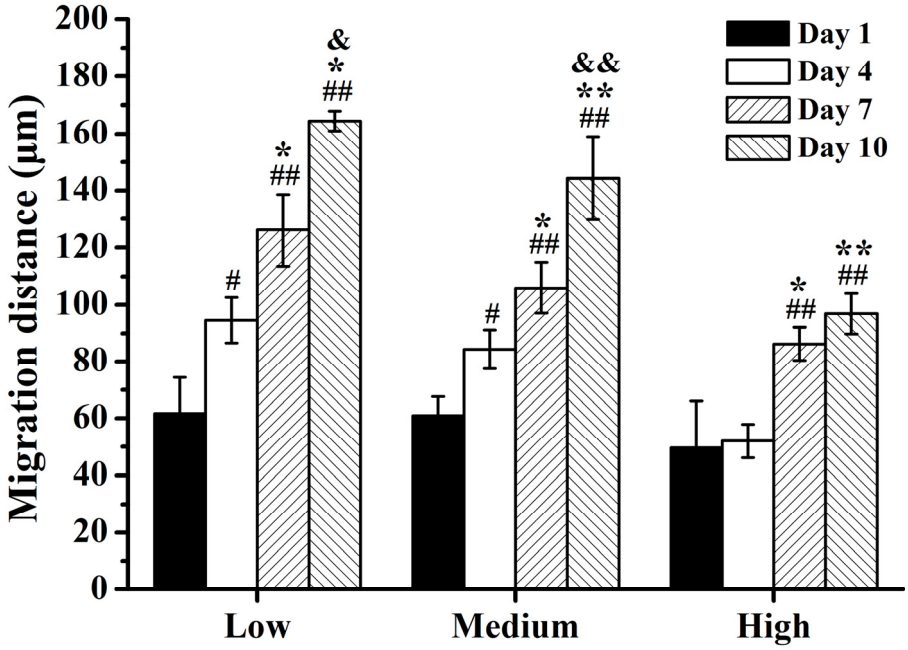

Fig. S1. Statistics of cells maximum migration distance in the scaffold. # $P < 0.05$ and ## $P < 0.01$ represent significant and extremely significant difference compared to that of Day 1; * $P < 0.05$ and ** $P < 0.01$ represent significant and extremely significant difference compared to that of Day 4; & $P < 0.05$ and && $P < 0.01$ represent significant and extremely significant difference compared to that of Day 7. n = 3.

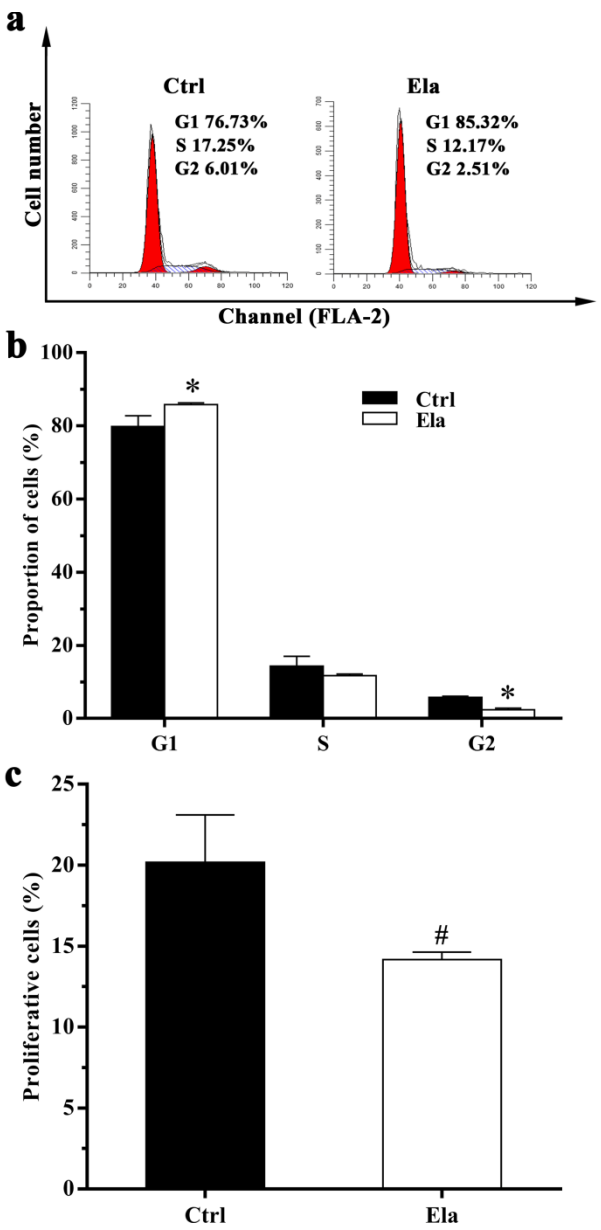

Fig. S2. The cell cycle of MDA-MB-231 cells cultured in the DECM scaffold with high stiffness before and after treated with 2μM elacridar. (a) The typical graph of cell cycle. (b) Statistical data of cell cycle (n = 3). * $P < 0.05$ represents statistically significant difference compared with the group without elacridar treatment in the same cell cycle. (c) Statistical data of cell proliferation (n = 3). # $P < 0.05$ represents statistically significant difference compared with the group without elacridar treatment. Statistical significance was determined by ANOVA followed by Tukey's post hoc test. Ctrl: group without elacridar treatment. Ela: group treated with 2 μM elacridar.

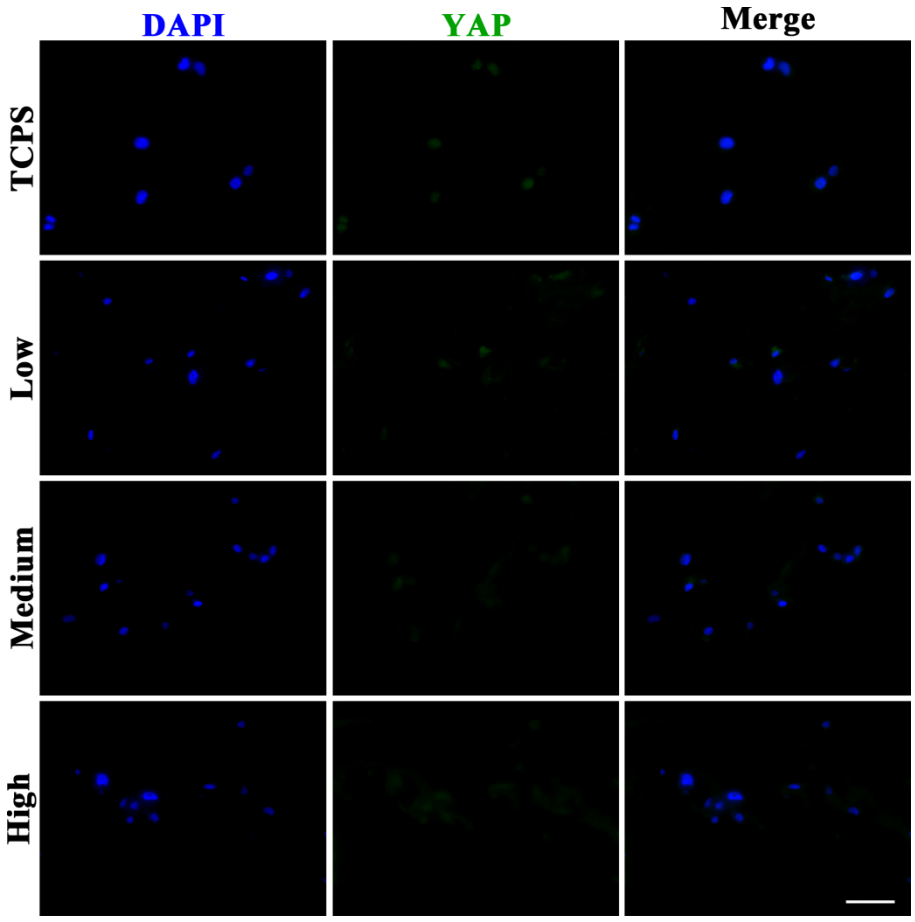

Fig. S3. Immunofluorescence detection of YAP expression for repopulation cultured in DECM scaffolds with different stiffness for 10 days. Scale bar: 50 μm. Low: low stiffness group; Medium: medium stiffness group; High: high stiffness group. Samples were fixed with 4% (v/v) paraformaldehyde for 20 min at room temperature, and then permeabilized with 0.25% (v/v) TritonX-100 in PBS for 15 min. After being blocked with normal goat serum (Boster, China) for 20 min at room temperature, the samples were incubated with mouse YAP antibody (1:100, Santa Cruz, USA) at 4°C overnight. Finally, the samples were incubated with DAPI solution (Solarbio, China) for 10 min and captured by an inverted fluorescence microscope (Olympus IX71, Japan).