**Electronic supplementary file**

**Eggshell membrane promotes homeostasis of elastic skin and lung tissue associated with type III collagen and decorin expression and ameliorates pulmonary fibrosis in a bleomycin mouse model**

**Cell and Tissue Research**

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**Online resource 1** Primer sequences (5’-3’)

For amplification in human IMR-90

|  |  |  |
| --- | --- | --- |
| Gene | Forward primer | Reverse primer |
| *GAPDH\*1*  *COL3A1\*2*  *COL1A1\*2*  *DCN\*2*  *MMP2\*3*  *PRRX1\*4* | CGACAGTCAGCCGCATCTTC  GGACCTCCTGGTGCTATAGGT  GGGATTCCCTGGACCTAAAG  GGAGACTTTAAGAACCTGAAGAACC  CCCACTGCCGTTTTCTCGAAT  TGATGCTTTTGTGCGAGAAGA | CGCCCAATACGACCAAATCCG  CGGGTCTACCTGATTCTCCAT  GGAACACCTCGCTCTCCA  CGTTCCAACTTCACCAAAGG  CAAAGGGGTATCCATCGCCAT  AGGGAAGCGTTTTTATTGGCT |
| *AEBP1\*5* | GGTTACGTGATCCCCAACTACG | CTGCCCACTTGTCGGTCTC |
| *LBH\*6* | GCCCCGACTATCTGAGATCG | GCGGTCAAAATCTGACGGGT |
| *TCF21\*7* | TCCTGGCTAACGACAAATACGA | TTTCCCGGCCACCATAAAGG |

\*1Designed by Sigma-Genosys, Japan; \*2 (Ohto-Fujita, et. al., 2011); \*3(Zhao, et al., 2017); \*4(Loh, et al., 2016); \*5 PrimerBank ID 53692188c2 (Spandidos, et al., 2009); \*6(Jiang, et al., 2019); \*7PrimerBank ID 208609971c1 (Spandidos, et al., 2009)

For amplification in mouse lung and skin

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene | Forward primer | | Reverse primer | |
| *GAPDH\*8* | agcttgtcatcaacgggaag | tttgatgttagtggggtctcg | |
| *COL3A1\*8* | tcccctggaatctgtgaatc | | tgagtcgaattggggagaat | |
| *MMP2\*8* | ggagaaggctgtgttcttcg | | aggctggtcagtggcttg | |
| *DCN\*8* | gaggagaagtgaggggagaca | | gattatctcatgtattttcacgacctt | |
| *ELN\*8* | tggagcaggacttggaggt | | cctccagcaccatacttagca | |

\*8 (Ohto-Fujita E, et al., 2019)

Online resource references

Jiang Y, Zhou J, Zou D, Hou D, Zhang H, Zhao J, Li L, Hu J, Zhang Y, Jing Z (2019) Overexpression of Limb-Bud and Heart (LBH) promotes angiogenesis in human glioma via VEGFA-mediated ERK signalling under hypoxia. EBioMedicine 48:36-48

Loh KM, Chen A, Koh PW, Deng TZ, Sinha R, Tsai JM, Barkal AA, Shen KY, Jain R, Morganti RM, Shyh-Chang N, Fernhoff NB, George BM, Wernig G, Salomon REA, Chen Z, Vogel H, Epstein JA, Kundaje A, Talbot WS, Beachy PA, Ang LT, Weissman IL (2016) Mapping the Pairwise Choices Leading from Pluripotency to Human Bone, Heart, and Other Mesoderm Cell Types. Cell 166:451-467

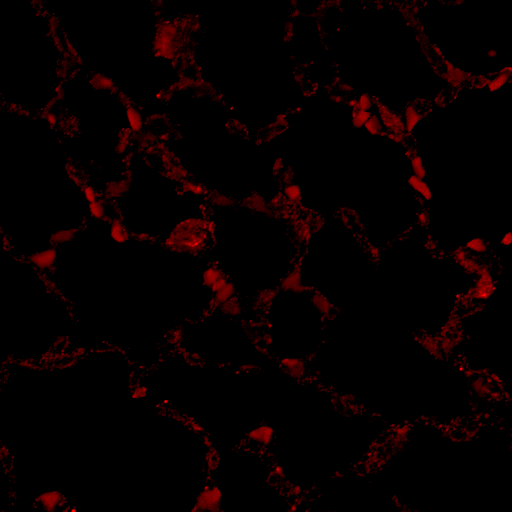
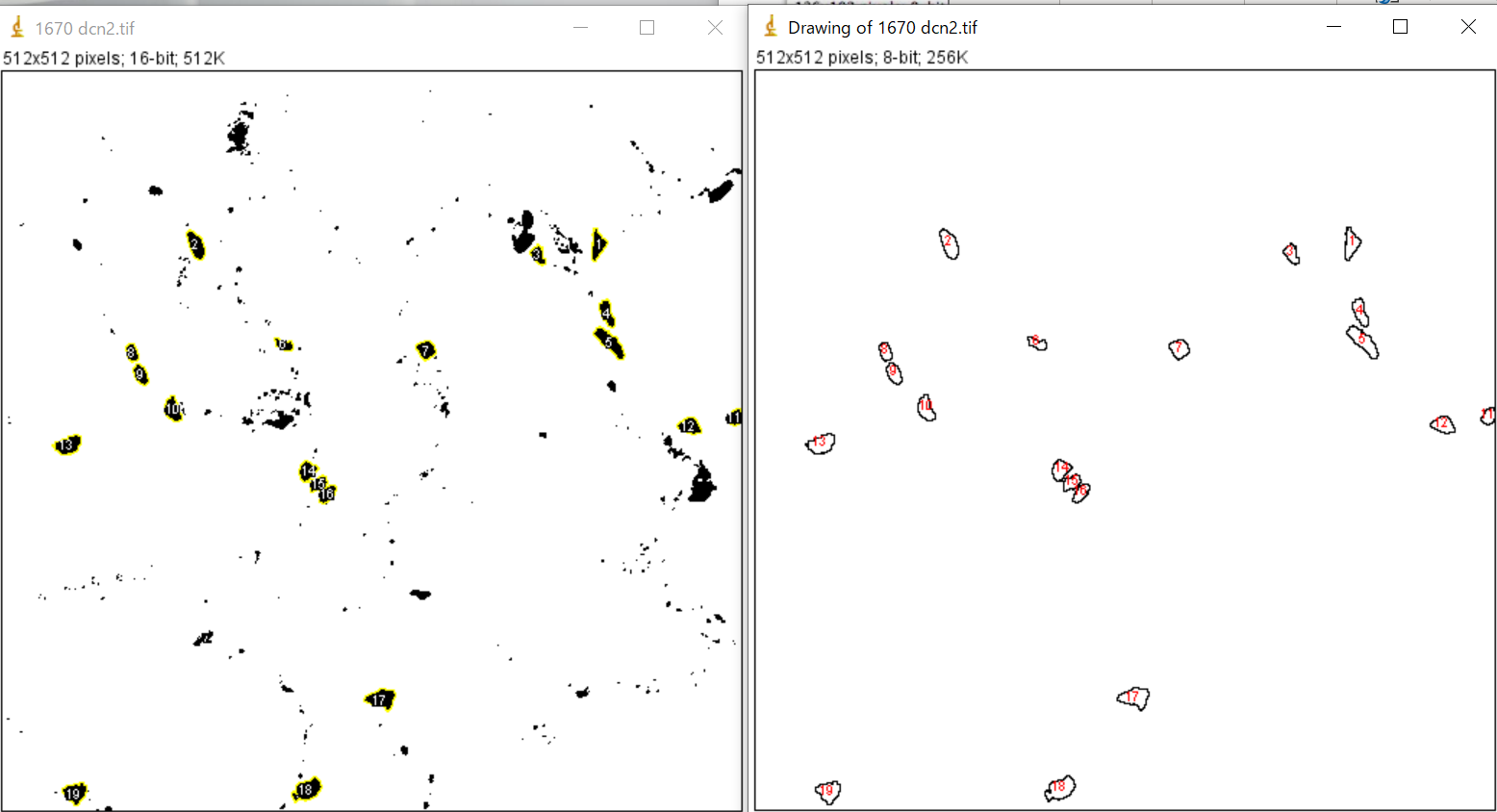
Ohto-Fujita E, Konno T, Shimizu M, Ishihara K, Sugitate T, Miyake J, Yoshimura K, Taniwaki K, Sakurai T, Hasebe Y, Atomi Y (2011) Hydrolyzed eggshell membrane immobilized on phosphorylcholine polymer supplies extracellular matrix environment for human dermal fibroblasts. Cell Tissue Res 345:177-190

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Spandidos A, Wang X, Wang H, Seed B (2009) PrimerBank: a resource of human and mouse PCR primer pairs for gene expression detection and quantification. Nucleic Acids Research 38:D792-D799

Zhao S, Zhao Y, Guo J, Fei C, Zheng Q, Li X, Chang C (2017) Downregulation of MMP1 in MDS-derived mesenchymal stromal cells reduces the capacity to restrict MDS cell proliferation. Sci Rep 7:43849

**Online resource 2** Quantification of the number of alveolar stromal decorin-positive cells by Image J.



20 mm

**a**

**b**

The number of decorin-positive cells in the alveolar interstitium was measured using Image J. Lung paraffin sections immunostained with anti-decorin-Alexa546 were observed with a confocal microscope, and the acquired images (a) were converted to 16 bit by Image J. After binarization, the size was set to 70-350 pixel2 (4.5-22.6 mm2) and the circularity was set to (0.5-1.0), and the number of extracted objects was counted (b).

**Online resource 3** Large view of Figure 5 (picrosirius red staining images).

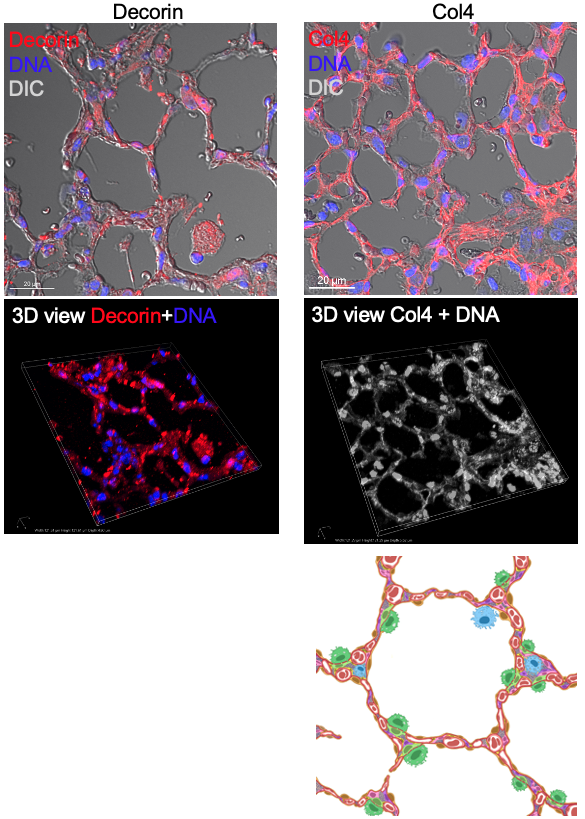
Bar is 10 mm.

ぼやけた背景

低い精度で自動的に生成された説明

**Online resource 4.** Alveolar staining of decorin (a, c) and Col4 (basement membrane) (b, d).

(e) Illustration of cells and basement membrane in alveolar area.



**c**

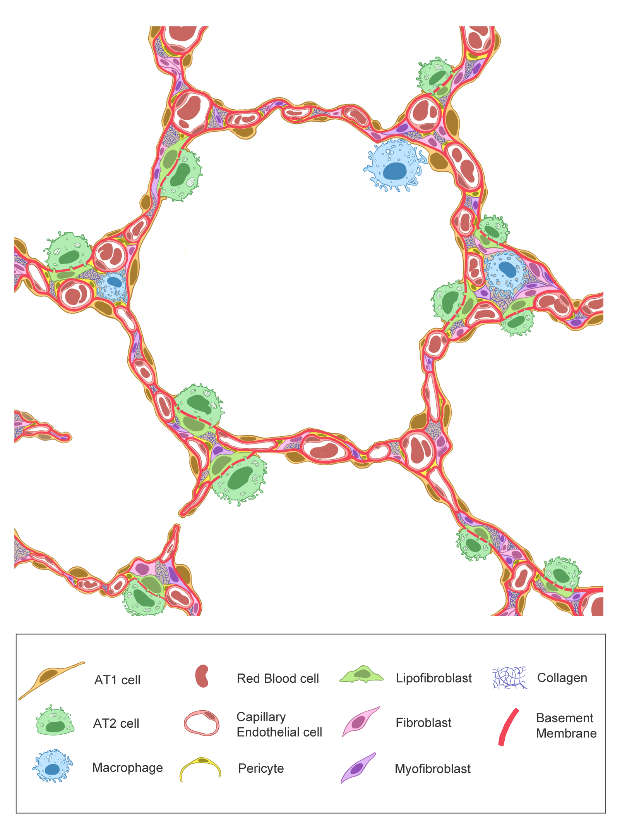
**b**

**a**

**d**

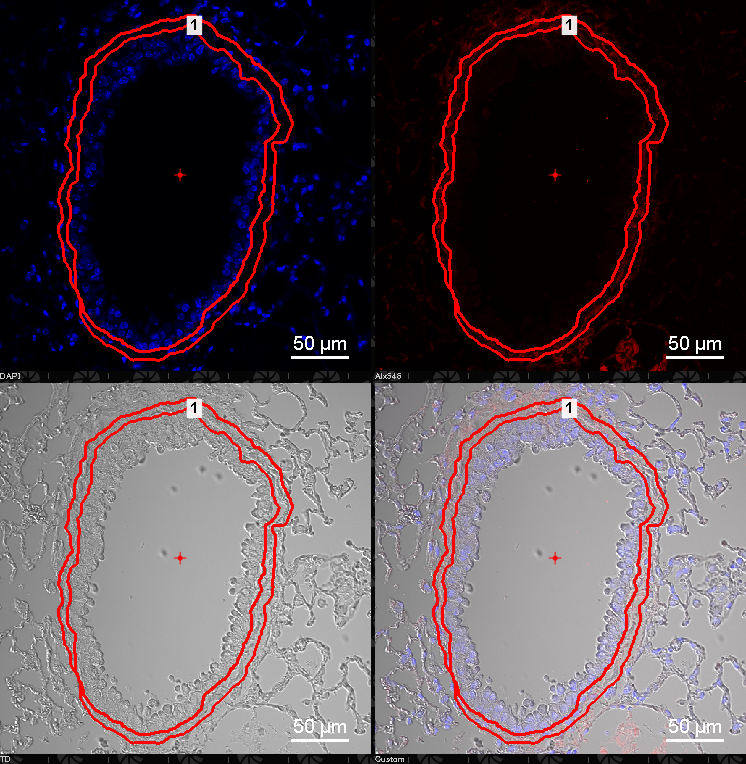
**Basement**

**membrane**



**e**

**Online resource 5** Quantification of fluorescence intensity of type III collagen-stained area around bronchi in lung tissue sections



Interstitial fluorescence of type III collagen-Alexa546 around bronchioles (size: about 200 µm in diameter/ µm2) was obtained from two bronchioles per individual using a Nikon A1 RMP equipped with NIS-Elements software