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# Supplementary Materials for

## Biochemical and structural cues of 3D-printed matrix synergistically direct MSC differentiation for functional sweat gland regeneration

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**This PDF file includes:**

**Supplementary Materials**

**Figure legends**

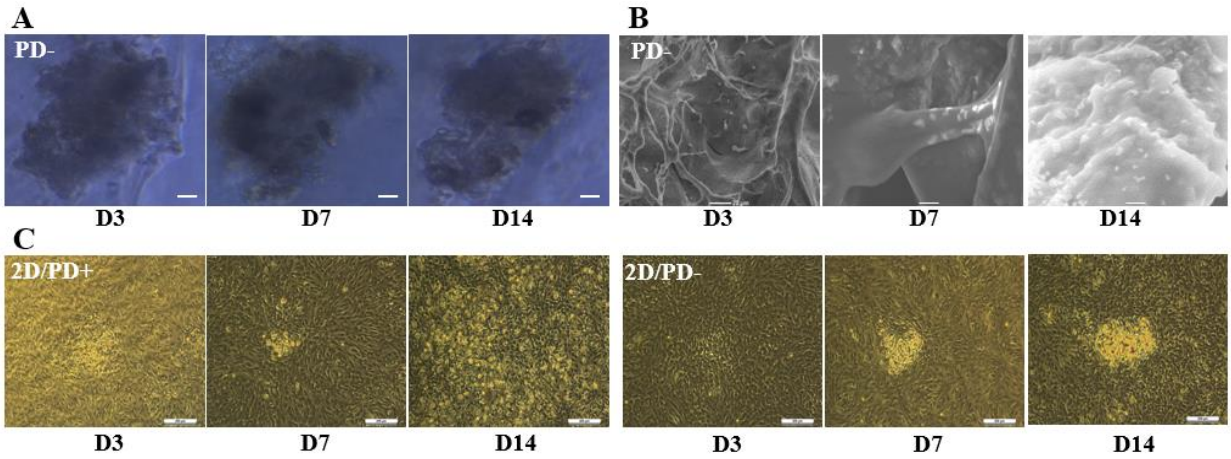


**Fig. S1. Biocompatibility of 3D-bioprinted construct and cellular morphology in 2D monolayer culture.** a Representative microscopy images of cell aggregates at different days (scale bars: 50 μm). b SEM images of 3D structure (scale bars: 20 μm). c Microscopic images of 2D monolayer culture cells with and without application of plantar region dermis (PD). (scale bars: 200 μm).

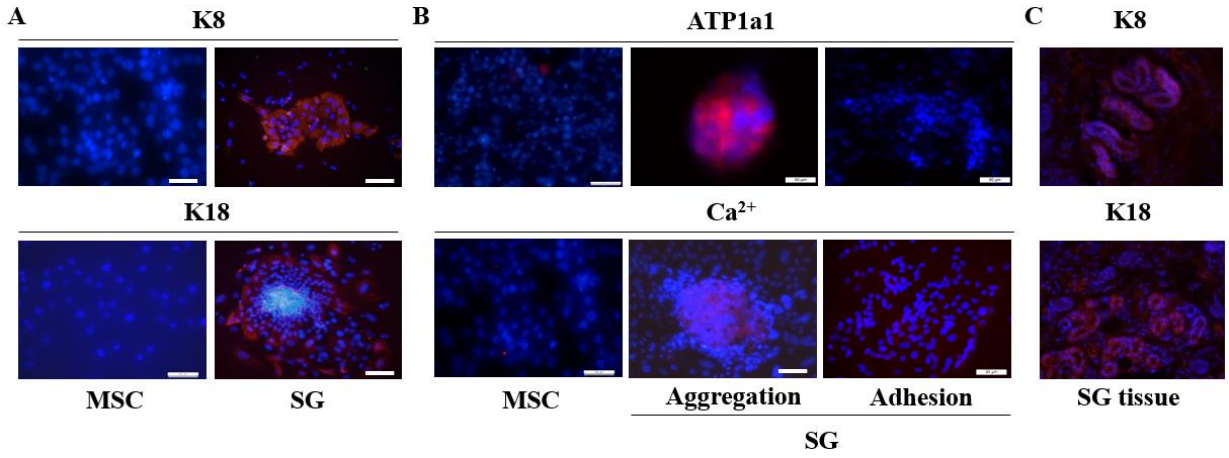


**Fig. S2. Expression of SG-specific and secretion-related markers in MSCs and SG cells in vitro. a** Expression of K8 and K18 in MSCs and SG cells in 2D monolayer culture. (K8, K18: red, DAPI: blue, scale bars: 200 μm) **b** Expression of ATP1a1 and $Ca^{2+}$ in MSCs and SG clusters and monolayer cells. (ATP1a1, $Ca^{2+}$: red, DAPI: blue, scale bars: 50 μm).

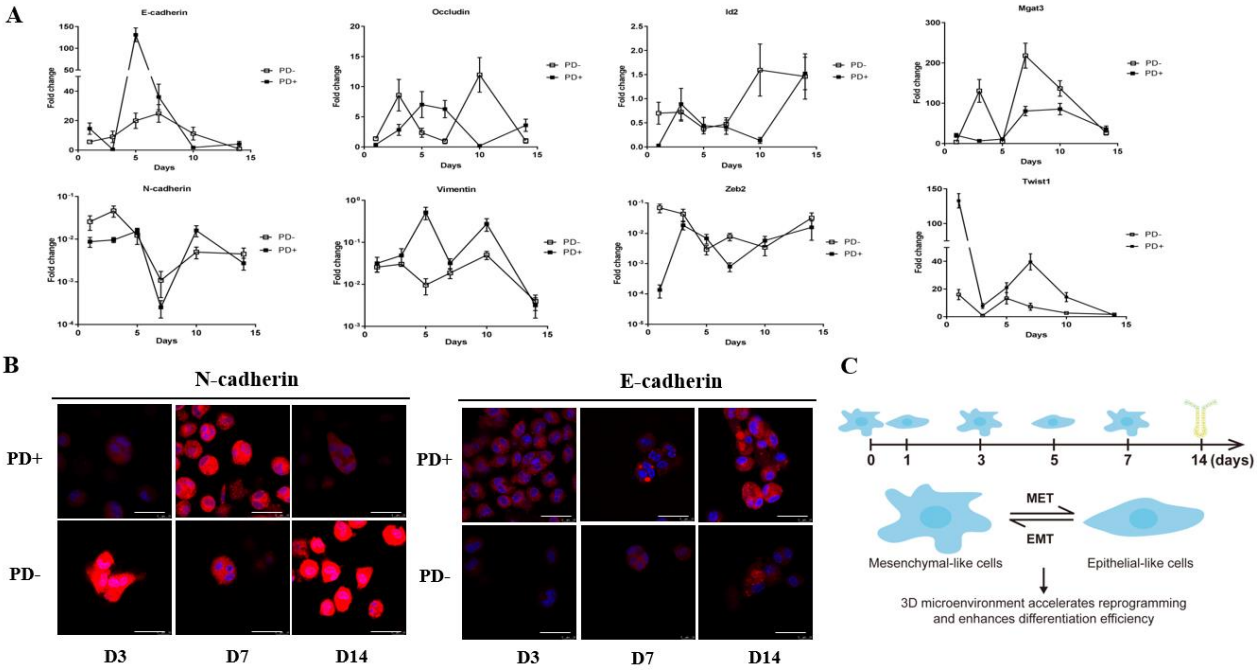


**Fig. S3. Transcriptional and translational expression of epithelial and mesenchymal markers in 3D-bioprinted cells with and without PD. (A)** Transcriptional expression of epithelial markers (E-cadherin, Occludin) and transcription factors of MET (Id2, Mgat3), mesenchymal markers (N-cadherin, Vimentin) and transcription factors of EMT (Twist1, Zeb2) in 3D-bioprinted cells with or without PD at day 1, 3, 5, 7, 10, and 14 culture by qRT-PCR. Data are mean±SEM. **(B)** Comparison of N-cadherin and E-cadherin expression in 3D-bioprinted cells with and without PD (N-cadherin, E-cadherin: red, DAPI: blue, scale bars: 50 μm). (**C)** Schematic illustration of the role of MET-EMT in cell differentiation.

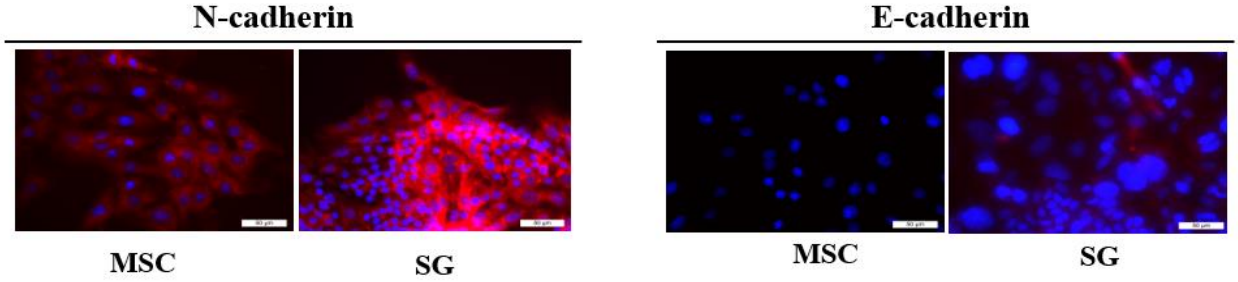


**Fig. S4. Expression of N- and E-cadherin in MSCs and SG cells in 2D monolayer culture.** (N-cadherin, E-cadherin: red, DAPI: blue, scale bars: 50 μm).

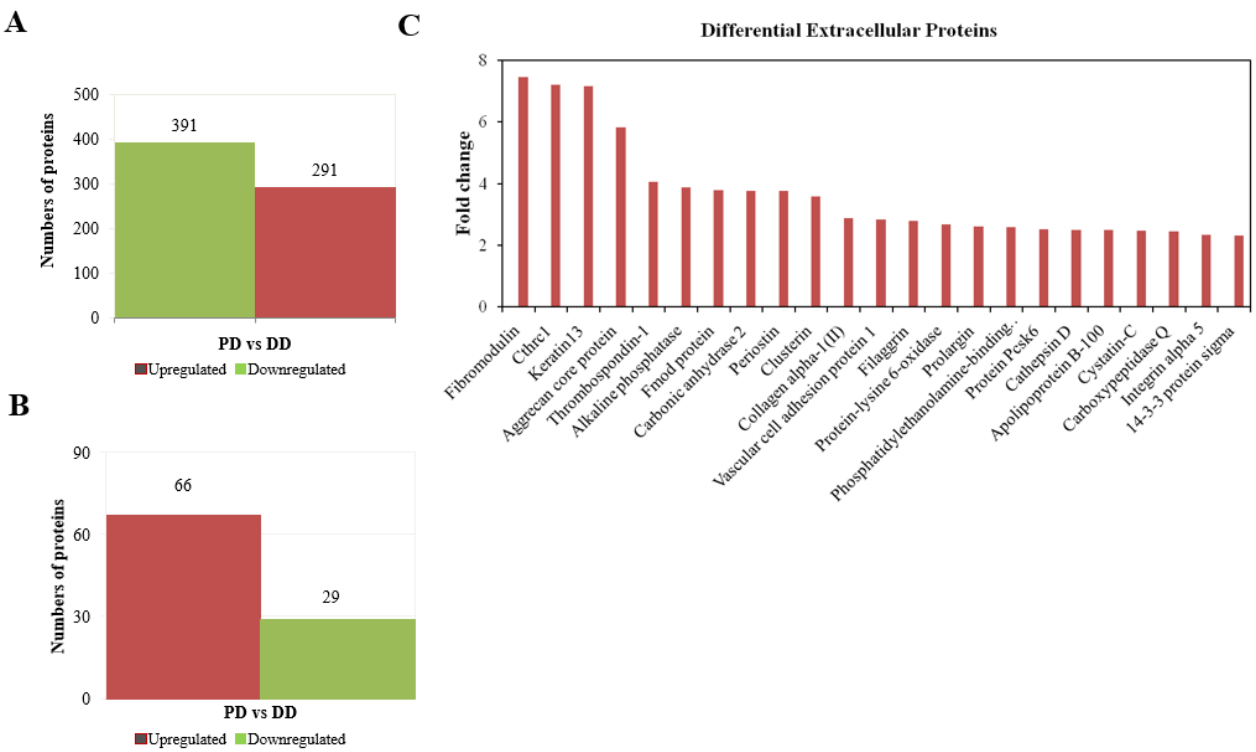


**Fig. S5. Proteomic microarray assay of differential gene expression between PD and DD ECM in postnatal mice. a** Comparison of proteins down- and upregulated between PD and DD ECM in postnatal mice. **b** Number of proteins in PD and DD ECM in postnatal mice. **c** Proteins with significant differential expression between PD and DD ECM in postnatal mice.

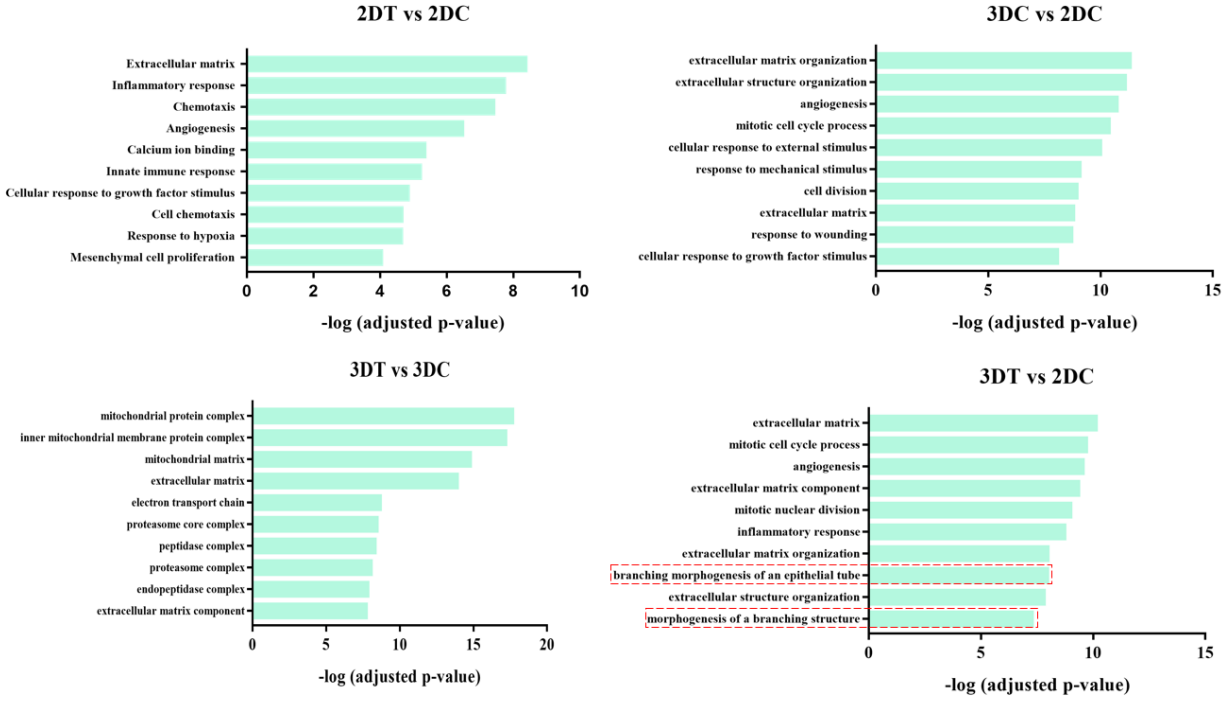


**Fig. S6. GO term analysis of differentially expressed pathways.**

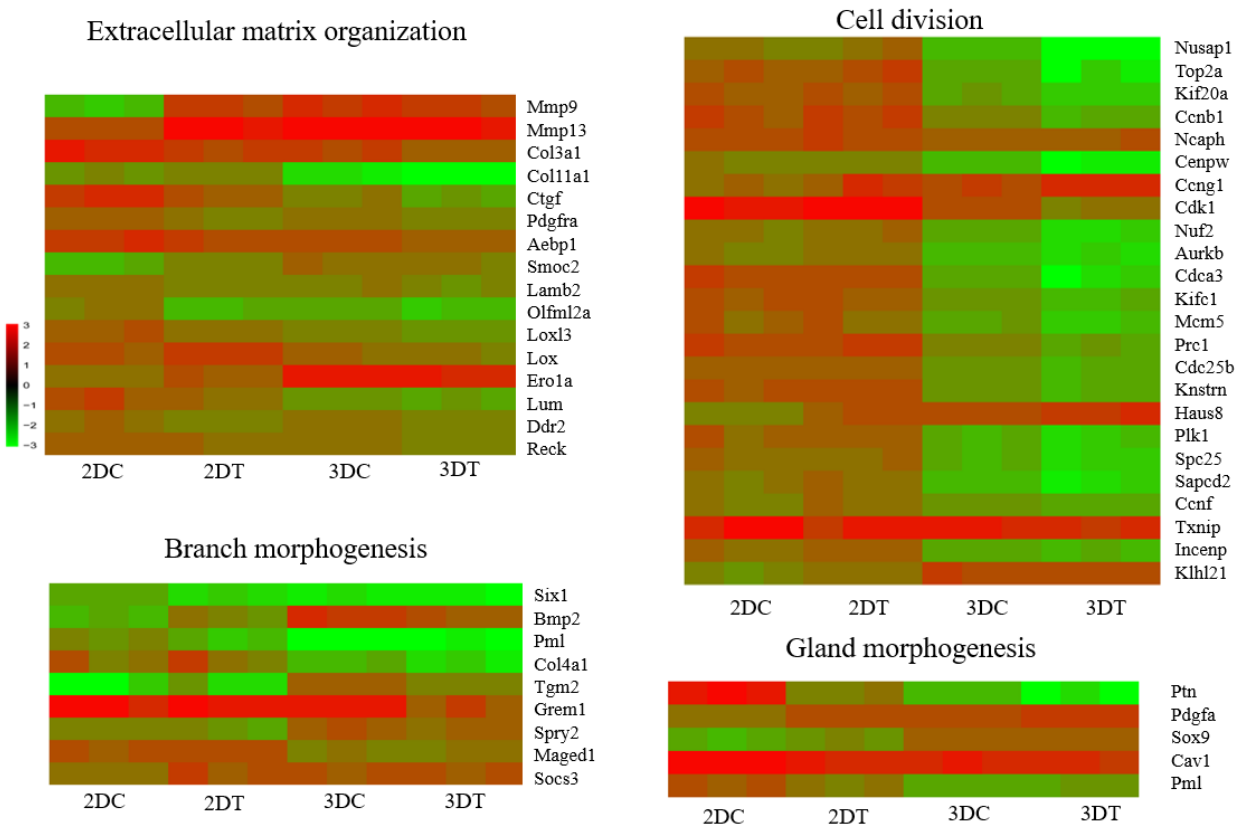


**Fig. S7. Heat maps illustrating differential expression of genes implicated in ECM organization, cell division, and gland and branch morphogenesis.**

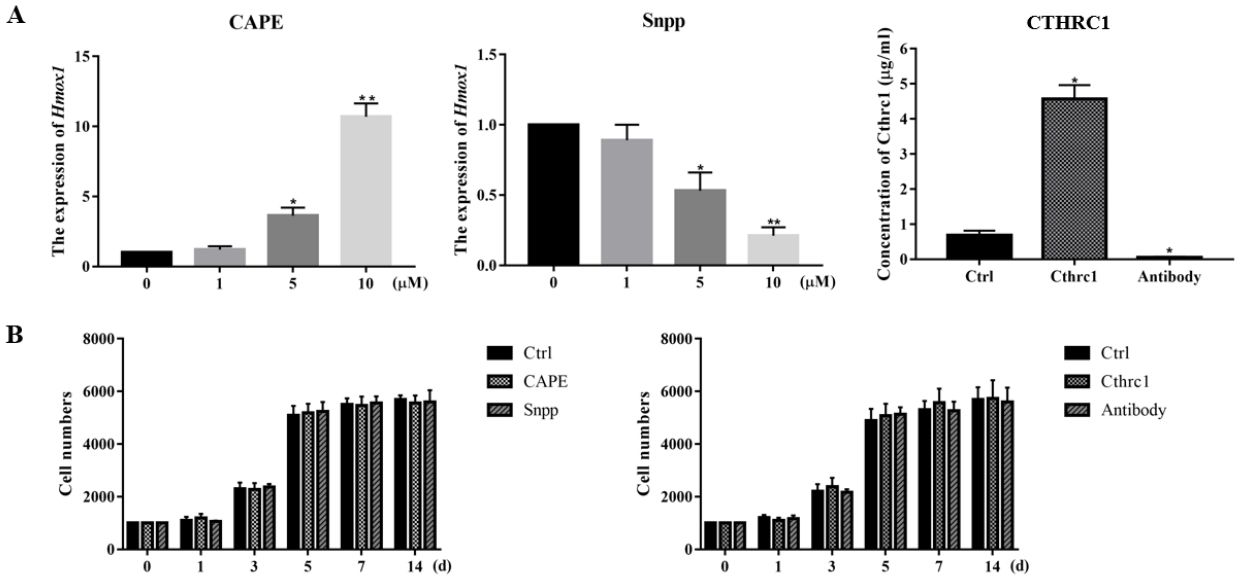


**Fig. S8. The expression of *Hmox1* and the concentration of CTHRC1 on treatment and the related effects on cell proliferation.** Effects of CAPE and Snpp on the expression of *Hmox1* and effects of recombinant CTHRC1 and CTHRC1 antibody on the concentration of CTHRC1 (A) and the related effects on cell proliferation (B) **p*<0.05.

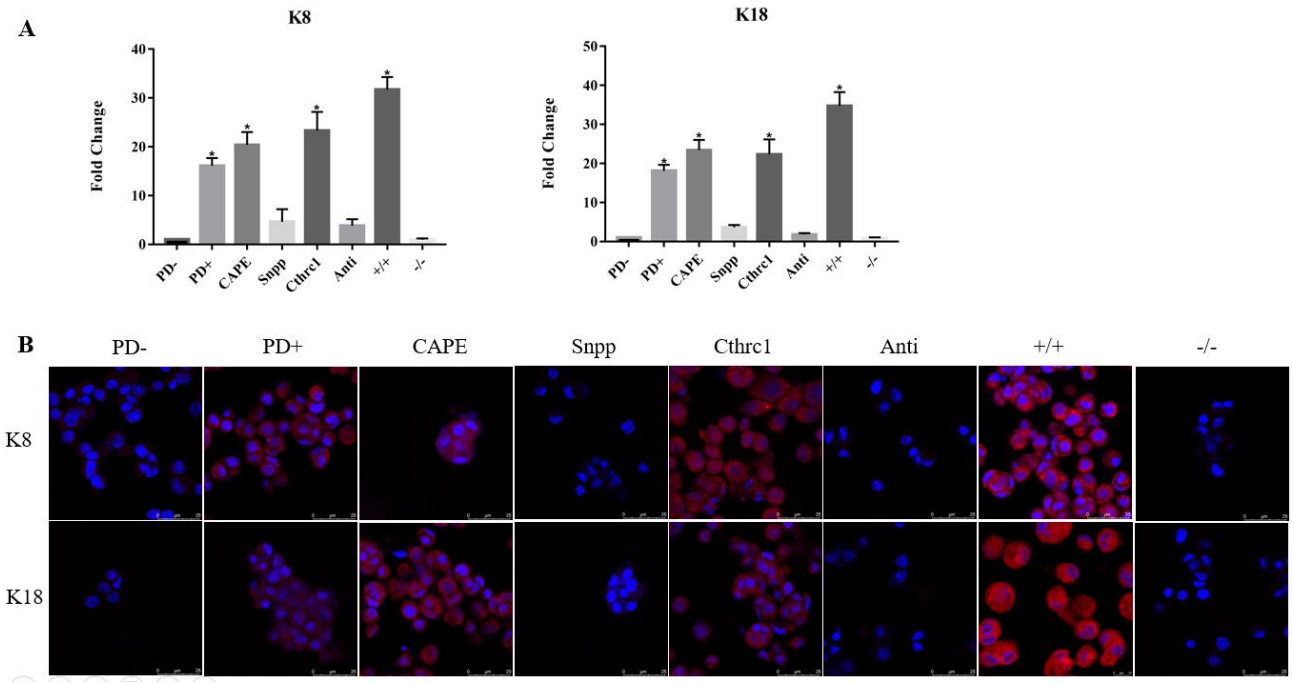


**Fig. S9. The expression of K8 and K18 with *Hmox1* and CTHRC1 regulation.** Expression of K8 and K18 at transcriptional (A) and translational (B) level with *Hmox1* and CTHRC1 regulation (K8, K18: red, DAPI: blue, scale bars: 200 μm) *$p$<0.05.

**Table S1. Primers for qRT-PCR of all the genes.**

| Primers | Sequence(5'to3') |
|---|---|
| K8-F | GGAGGACTGAGTTCATCCTACGGG |
| K8-R | GGGTTTCAATCTTCTTCACGACCA |
| K18-F | CCGTCTTGCCGCTGATGACTTT |
| K18-R | TCCGCCATGATCTTGCTGAGGT |
| ATP1a1-F | GACGCCTTTCAGAATGCCTACC |
| ATP1a1-R | TGTGACCATGATGACCTTAATCCC |
| Fxyd2-F | ATGGACAGGTGGTACTTGGGTG |
| Fxyd2-R | CATTGACCTGCCTATGCTTCTTACT |
| Aqp5-F | GCCTTATCCATTGGCTTGTCTGTC |
| Aqp5-R | CCCAGTCCTCCTCCGGCTCATA |
| E-cadherin-F | CCATCGCCTACACCATCCTCAG |
| E-cadherin-R | TACGGGCACCGACCTCATTCTC |
| Occludin-F | GCTTGGGACAGAGCCTATGGAA |
| Occludin-R | CCCGATCTAATGACGCTGGTAA |
| Mgat3-F | CCGACTTGTTACGGACTCCACTCTA |
| Mgat3-R | TCAGCCACCTTGGTCTTCTCCTCT |
| Id2-F | TCCGGTGAGGTCCGTTAGGAAA |
| Id2-R | GGTGCAGGCTGACGATAGTGGG |
| Vimentin-F | TGACCGCTTCGCCAACTACATC |
| Vimentin-R | GCTTCCTCCCTCTGGAGCATCTC |
| N-cadherin-F | CCTCATGTTCGTGGTATGGATG |
| N-cadherin-R | TATTGTGGCTCAGCGTGGATAG |
| Zeb2-F | GTTCGGCATGAACCCATTTAGT |
| Zeb2-R | ATCCTTGTTTCCGCTGGTATTT |
| Twist1-F | ACCCAGTCGCTGAACGAGGCATT |
| Twist1-R | TCCATCCTCCAGACGGAGAAGGC |
| GAPDH-F | CTCTGGAAAGCTGTGGCGTGAT |
| GAPDH-R | GGAGACAACCTGGTCCTCAGTGTAG |
| IL-10-F | GCTCTTACTGACTGGCATGAG |
| IL-10-R | CGCAGCTCTAGGAGCATGTG |
| Mmp9-F | CTGGACAGCCAGACACTAAAG |
| Mmp9-R | CTCGCGGCAAGTCTTCAGAG |
| Ptges-F | GGATGCGCTGAAACGTGGA |
| Ptges-R | CAGGAATGAGTACACGAAGCC |
| Vsig-F | TGCAGGTCAGGTCAGTATGGT |
| Vsig-R | CTCGGTGGTGGTATAGAGGCA |
| Vwa1-F | GGGGGACCTGTTGTTCCTG |
| Vwa1-R | CATCCTGTATAGCCTGGCCT |
| Hmox1-F | AAGCCGAGAATGCTGAGTTCA |
| Hmox1-R | GCCGTGTAGATATGGTACAAGGA |

| | |
|---|---|
| **Bmp2-F** | **GGGACCCGCTGTCTTCTAGT** |
| **Bmp2-R** | **TCAACTCAAATTCGCTGAGGAC** |
| **Sox9-F** | **GAGCCGGATCTGAAGAGGGA** |
| **Sox9-R** | **GCTTGACGTGTGGCTTGTTC** |
| **Tgm2-F** | **GACAATGTGGAGGAGGGATCT** |
| **Tgm2-R** | **CTCTAGGCTGAGACGGTACAG** |