

Figure S1

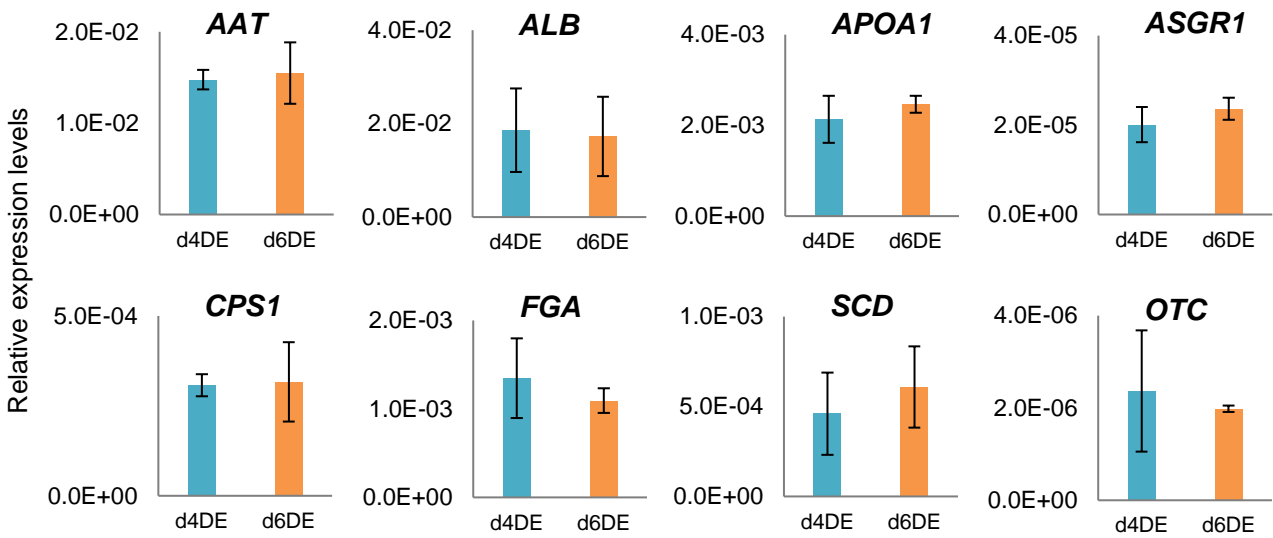


Figure S1. Gene expression analysis in d4DE and d6DE MH, related to Figure 1.

Normalization is performed using the expression level of 18S rRNA.

Data are represented as mean \pm SD. (n = 4 to 6).

Figure S2

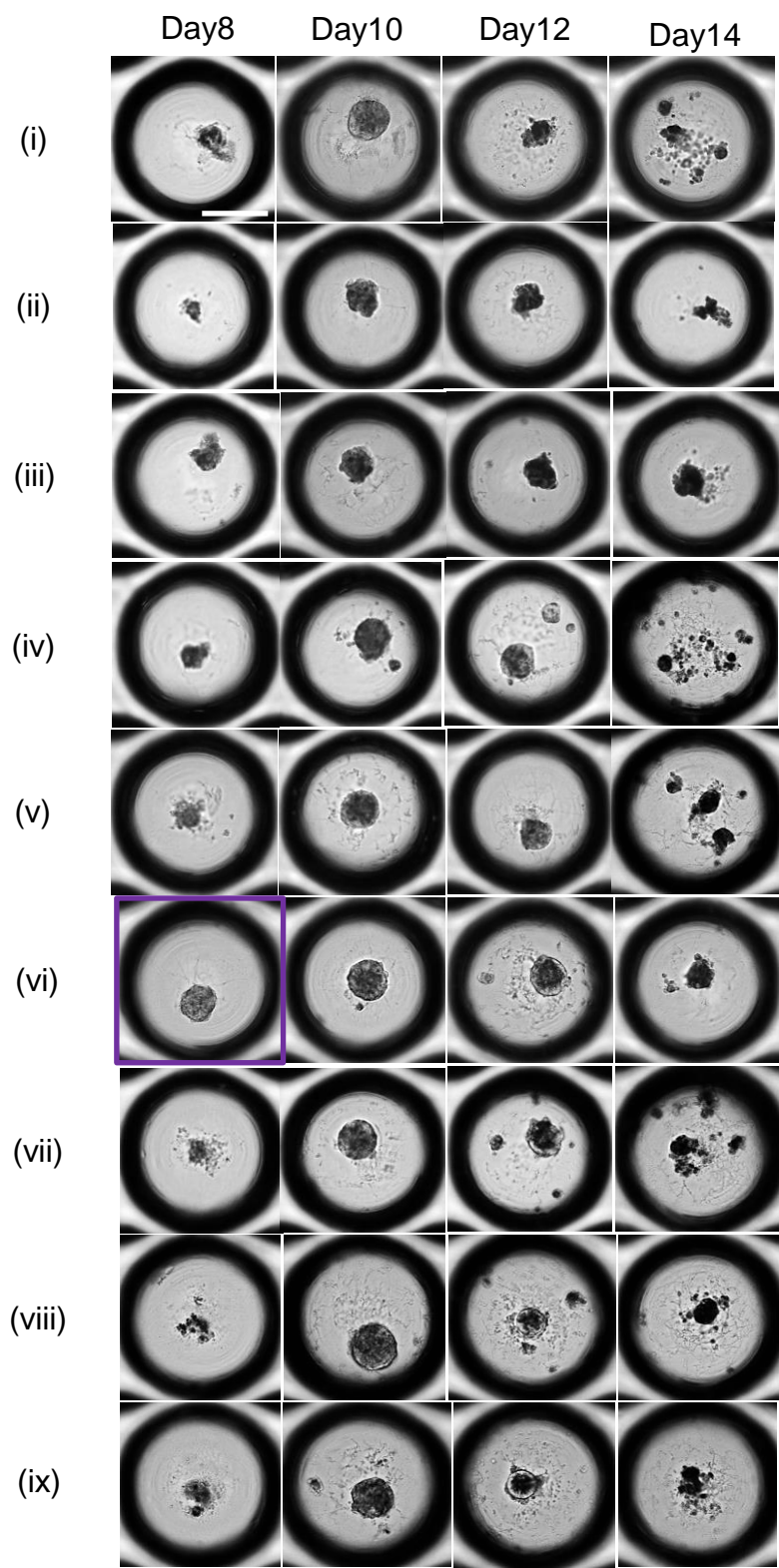


Figure S2. Repeatedly produced HS, related to Figure 2. Morphologies of nine batches of HS produced on days 8, 10, 12 and 14 in the d6DE protocol ((i) to (ix)). HS produced from exceptional d6DE day 8 (Ex-d6DE day 8) cells are outlined in purple (vi). Scale bar, 200 μ m.

Figure S3

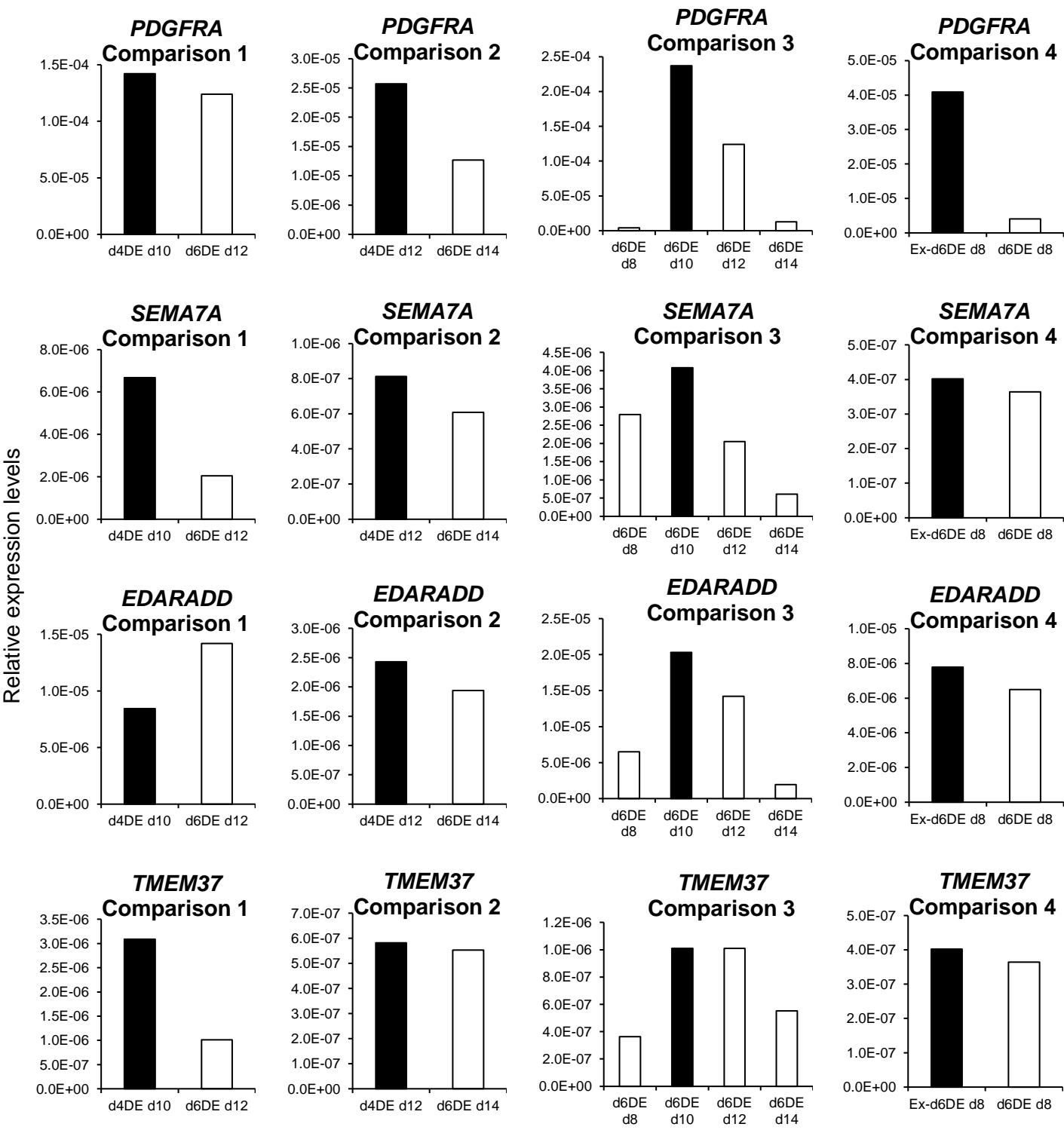


Figure S3. Verification of microarray analysis by qRT-PCR, related to Figure 2.
The black and white bars represent the cells with better and worse HS-forming ability, respectively. Normalization is performed using the expression level of 18S rRNA.

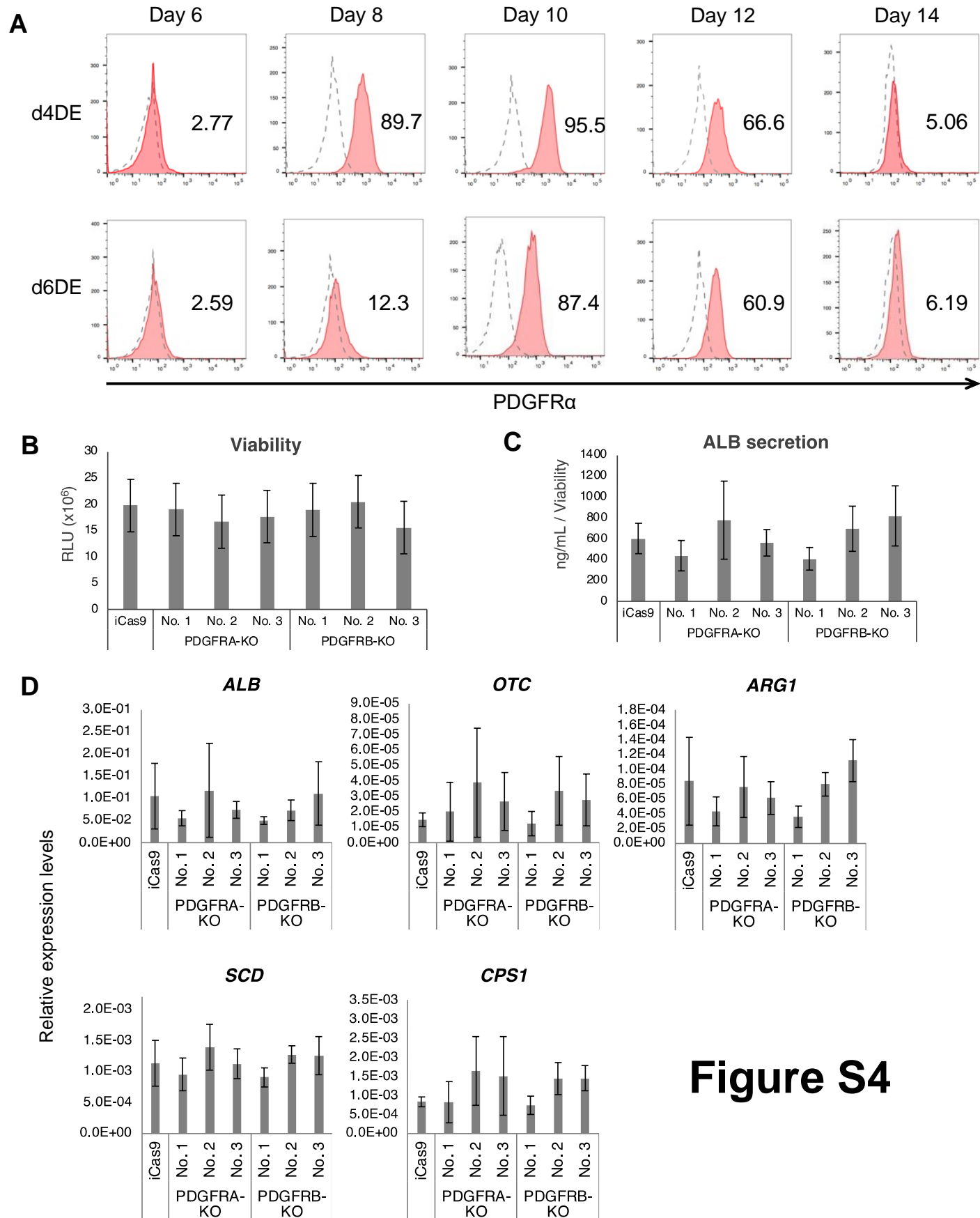


Figure S4

Figure S4. Functional analyses of PDGFRs in differentiating cells and HS, related to Figure 3.

(A) Flow cytometric analysis of PDGFRα. The dotted lines are the isotype control, the red lines are the sample stained with PDGFRα Alexa Fluor 647-conjugated antibody, and the numbers represent the positive rate (%).

(B) Cell viability within HS by measuring intracellular ATP levels in iCas9, PDGFR-KO, and PDGFRB-KO cell lines. The bars represent mean ± SD. (n = 3). RLU: relative luminescent unit.

(C) Albumin secretion of each HS on day 11 after HS production in iCas9, PDGFR-KO, and PDGFRB-KO cell lines. The bars represent mean ± SD. (n = 3).

(D) Expression levels of hepatocyte marker genes in iCas9, PDGFR-KO, and PDGFRB-KO cell lines.

Normalization is performed using the expression level of 18S rRNA. The bars represent mean ± SD. (n = 4).

Figure S5

A

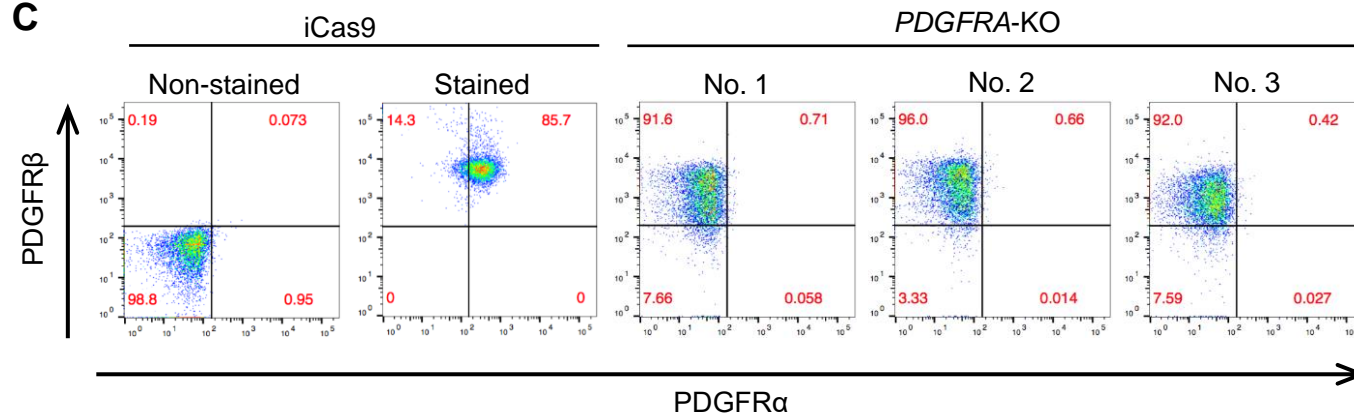
iCas9: GGACTTCCCATCCGGCGTTCCTGGTCTTAGGCTGT
PDGFRA-KO No. 1: GGACTTCCCATCTTAAGACCGGCGTTCCTGGTCTTAGGCTGT 7 bases insertion
PDGFRA-KO No. 2: GGACTTCCCATC-----TTAGGCTGT 14 bases deletion
PDGFRA-KO No. 3: GGACTTCCCATC-----CTTAGGCTGT 13 bases deletion

B

iCas9: CCTTCGTTCTGACCTGCTCGGGTTCAGCTCCGGTGGTGTGGGAACGGATGTCCCAGGAGCCCCACAGGAAATGGC
CAAGGCCAGGATGGCACCTTCTCCAGCGTGCTCACACTGACCAACCTCACTGGGCTAGACACGGGAGAATACTTT
TGCACCCACAATGACTCCCGTGGACTGGAGACCGATGAGCGGAAACGGCTCTAC

PDGFRB-KO No. 1: CCTTCG---(183 bases deletion)---AGCGGAAACGGCTCTAC
PDGFRB-KO No. 2: CCTTCG---(183 bases deletion)---AGCGGAAACGGCTCTAC
PDGFRB-KO No. 3: CCTTCG---(183 bases deletion)---AGCGGAAACGGCTCTAC

C



D

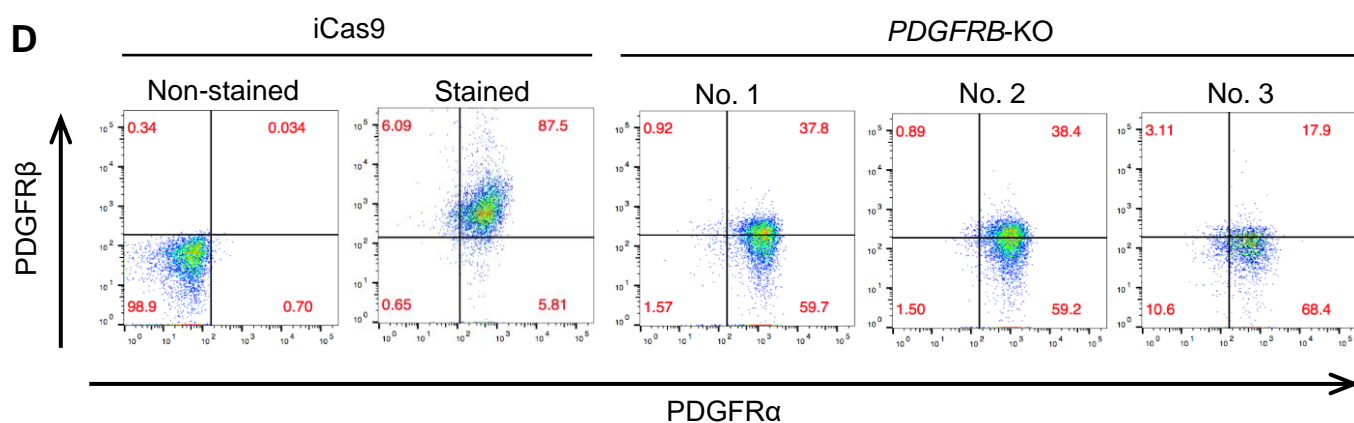


Figure S5. Validation of *PDGFRA*-KO and *PDGFRB*-KO cell lines, related to Figure 3.
(A) Sequence analysis of *PDGFRA*-KO cell lines. The yellow bases represent a target sequence, the underlined bases are protospacer adjacent motif in CRISPR/Cas9 system.
(B) Sequence analysis of *PDGFRB*-KO cell lines. The cyan and magenta bases represent target sequences, the underlined bases are protospacer adjacent motifs in CRISPR/Cas9 system.
(C) (D) Flow cytometric analysis of PDGFRα and PDGFRβ in *PDGFRA*-KO and *PDGFRB*-KO cell lines. Each cell line was differentiated into MC using PDGF-BB (C) or PDGF-AA (D) and then analyzed. The numbers represent the population rates (%).

Figure S6

A

PDGFRB-KO No. 1 :	GGACTT	<u>CCCATCCGGCGTTCCTGGTCTTA</u>	GGCTGT			
PDGFRA/B-KO No. 1:	GGACTT	<u>CCCATC</u>	-----	<u>GGTCTTA</u> GGCTGT	10 bases deletion	
PDGFRA/B-KO No. 2:	GGACTT	<u>CCCATC</u>	-----	<u>CTTA</u> GGCTGT	13 bases deletion	
PDGFRA/B-KO No. 3:	GGACTT	<u>CCCATC</u>	-----	-----	GGCTGT	17 bases deletion
PDGFRA/B-KO No. 4:	GGACTT	<u>CCCATC</u>	-----	<u>GTCTTA</u>	GGCTGT	11 bases deletion

B

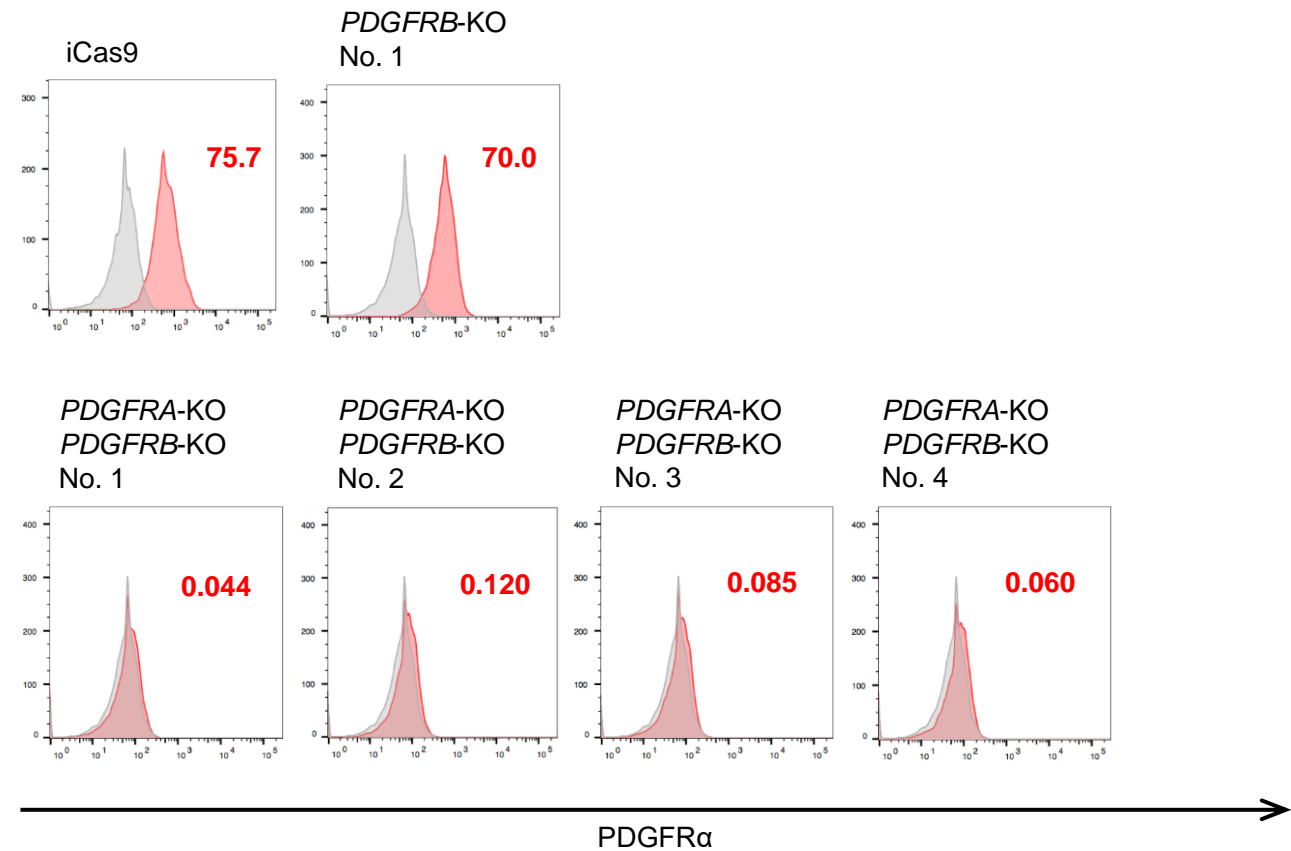


Figure S6. Validation of *PDGFRA/B* double KO cell lines, related to Figure 4.
(A) Sequence analysis of *PDGFRA* gene locus in *PDGFRA/B* double KO cell lines. *PDGFRA/B* double KO cell lines originate from *PDGFRB*-KO No. 1. The yellow bases represent a target sequence, the underlined bases are protospacer adjacent motif in CRISPR/Cas9 system.
(B) Flow cytometric analysis of PDGFRα in *PDGFRA/B* double KO cell lines. Each cell line was differentiated into HE by the d6DE protocol and used for the analysis. The grey lines are the isotype control, the red lines are the sample stained with PDGFRα Alexa Fluor 647-conjugated antibody, and the numbers represent the positive rate (%).

Figure S7

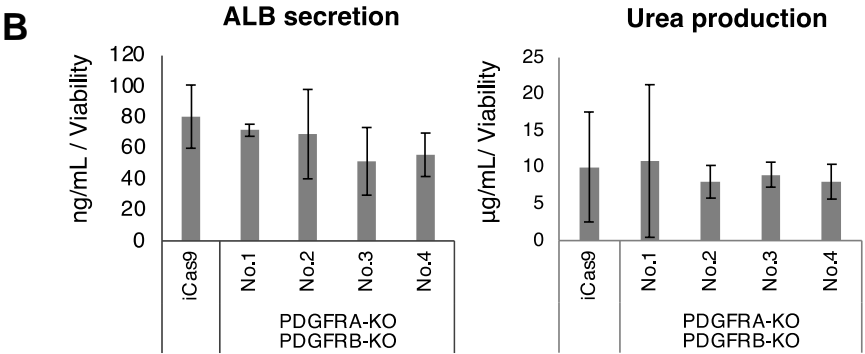
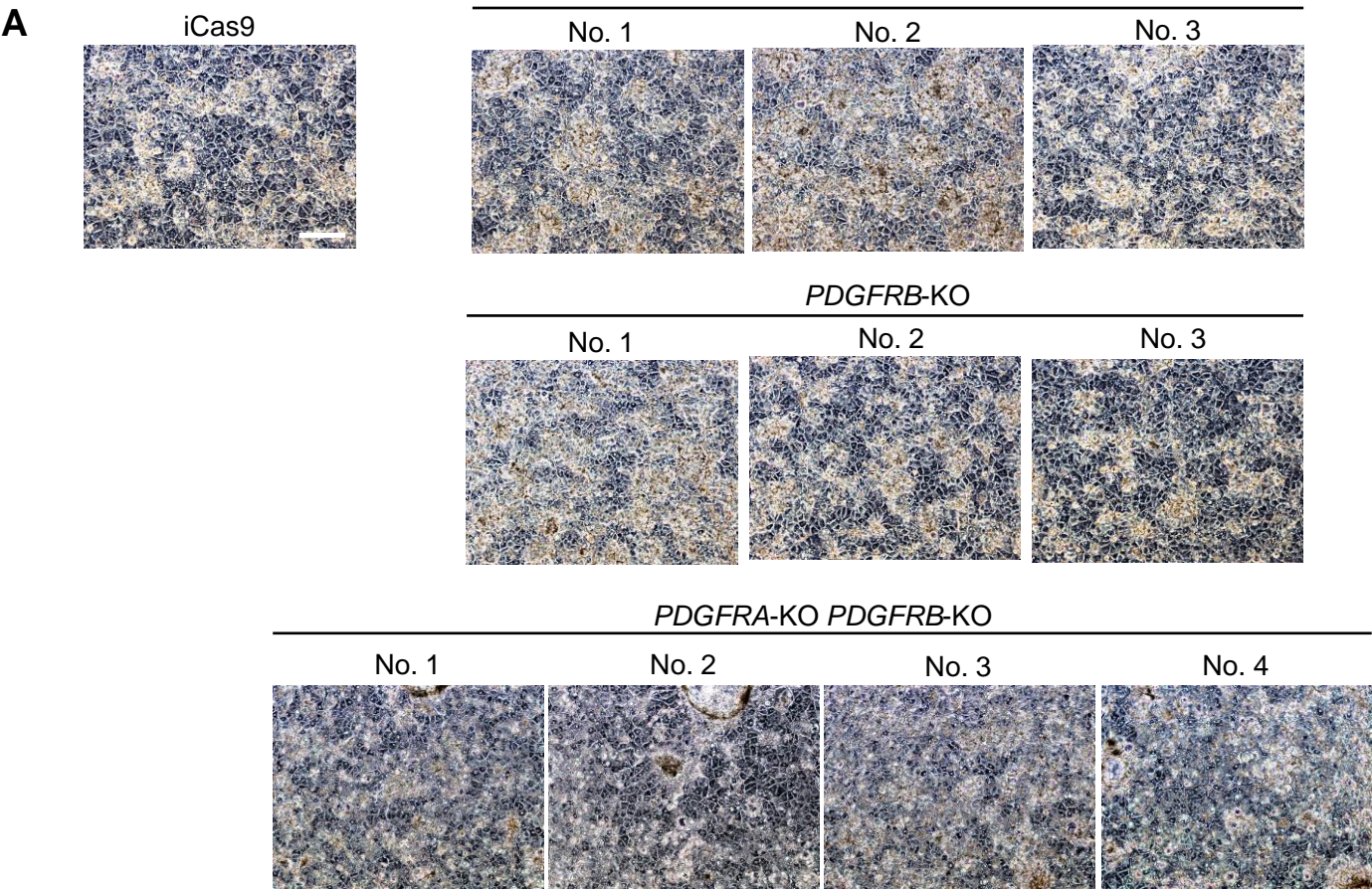
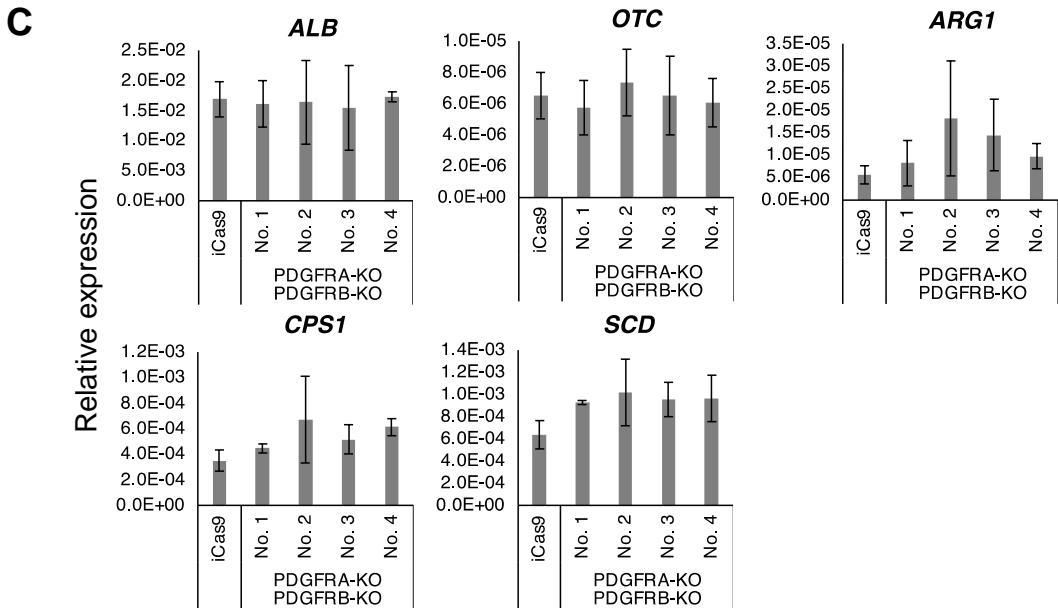


Figure S7. The phenotype analyses of *PDGFRA/B* double KO cell lines in 2D-cultured MH, related to Figure 4. (A) Morphologies of 2D-cultured MH in iCas9, *PDGFRA*-KO, *PDGFRB*-KO, *PDGFRA/B* double KO cell lines. Scale bar, 200 μm. (B) Albumin secretion and urea production per cell viability of MH in each cell line. The bars represent mean ± SD. (n = 3).



(C) Expression levels of hepatocyte marker genes of MH in each cell line. Normalization is performed using the expression level of 18S rRNA. The bars represent mean ± SD. (n = 3).

Figure S8

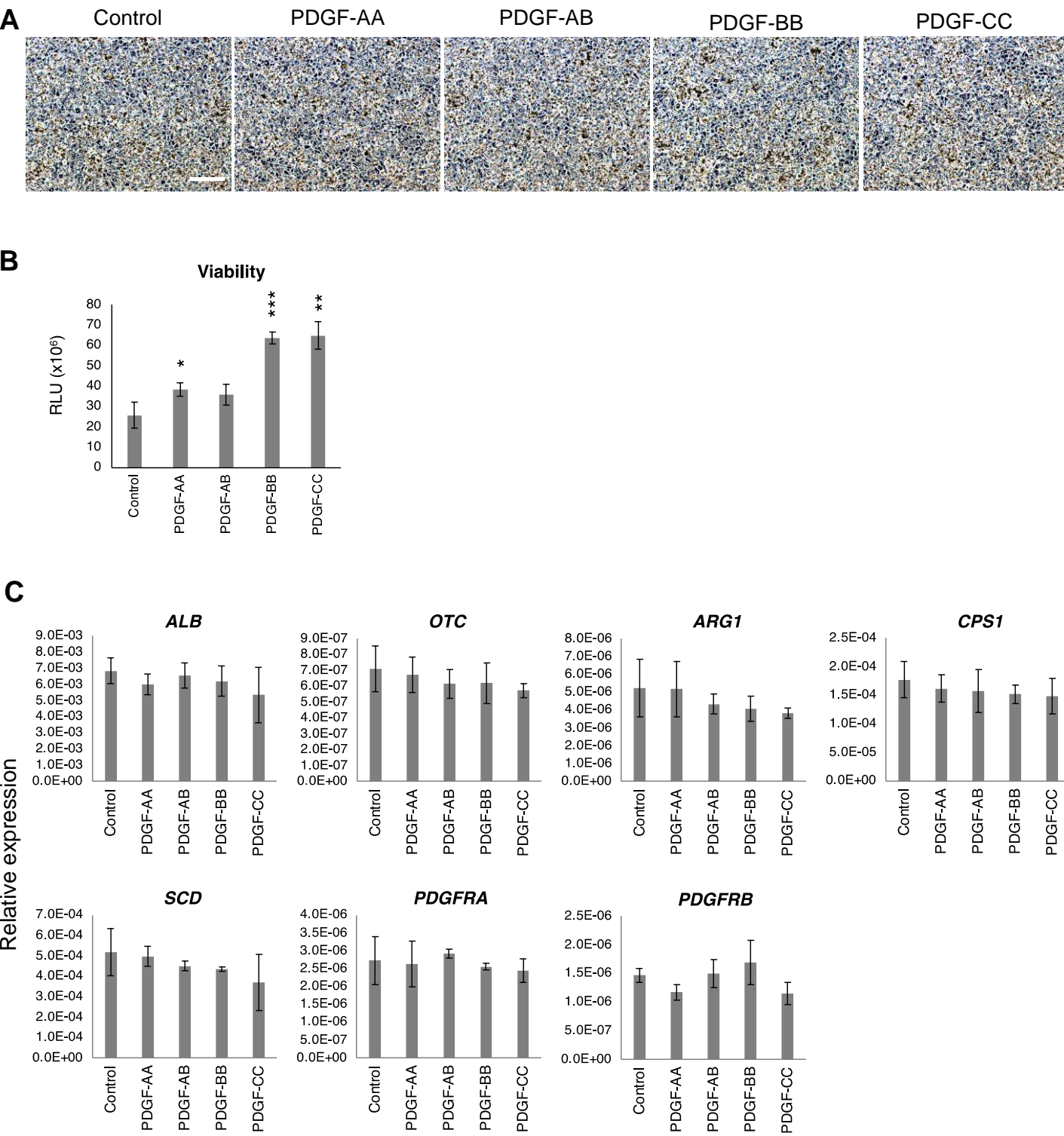


Figure S8. Data of each PDGF-treated 2D-cultured MH, related to Figure 5.
(A) Morphologies of mock control and each PDGF-treated MH. Scale bar, 200 μ m.
(B) Cell viability of mock control and each PDGF-treated MH. The bars represent mean \pm SD. (n = 3; *p < 0.05; **p < 0.01; ***p < 0.001).
(C) Expression levels of hepatocyte marker and PDGFR genes of mock control and each PDGF-treated MH. Normalization is performed using the expression level of 18S rRNA. The bars represent mean \pm SD. (n = 3).

Figure S9

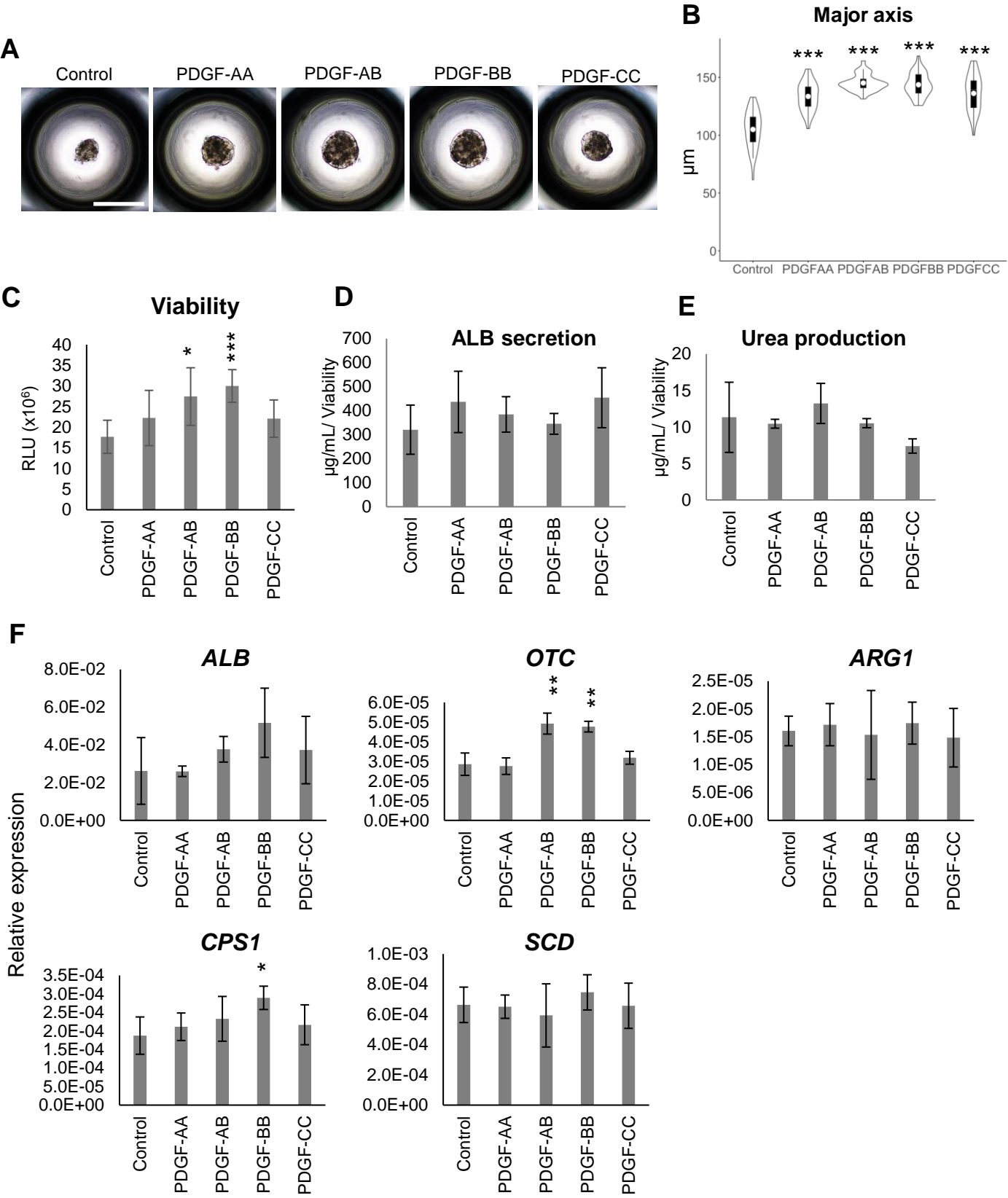


Figure S9. Evaluation of PDGFs effect on HS produced from d6DE day10 cells, related to Figure 5.

(A) Morphologies of mock control and each PDGF-treated HS on day 11 after production. Scale bar, 200 μ m.

(B) Violin plot of major axis length of mock control and each PDGF-treated HS on day 11. (n = 30 to 33; ***p < 0.001)

(C) Cell viability within HS by measuring intracellular ATP levels in each HS. The bars represent mean \pm SD. (n = 6; *p < 0.05; ***p < 0.001). RLU: relative luminescent unit.

(D) Albumin secretion per cell viability in d each HS. The bars represent mean \pm SD. (n = 3; *p < 0.05; **p < 0.01).

(E) Urea production per cell viability in each HS. The bars represent mean \pm SD. (n = 3).

(F) Expression levels of hepatocyte marker genes in each HS. Normalization is performed using the expression level of 18S rRNA. The bars represent mean \pm SD. (n = 3; *p < 0.05; **p < 0.01).

Figure S10

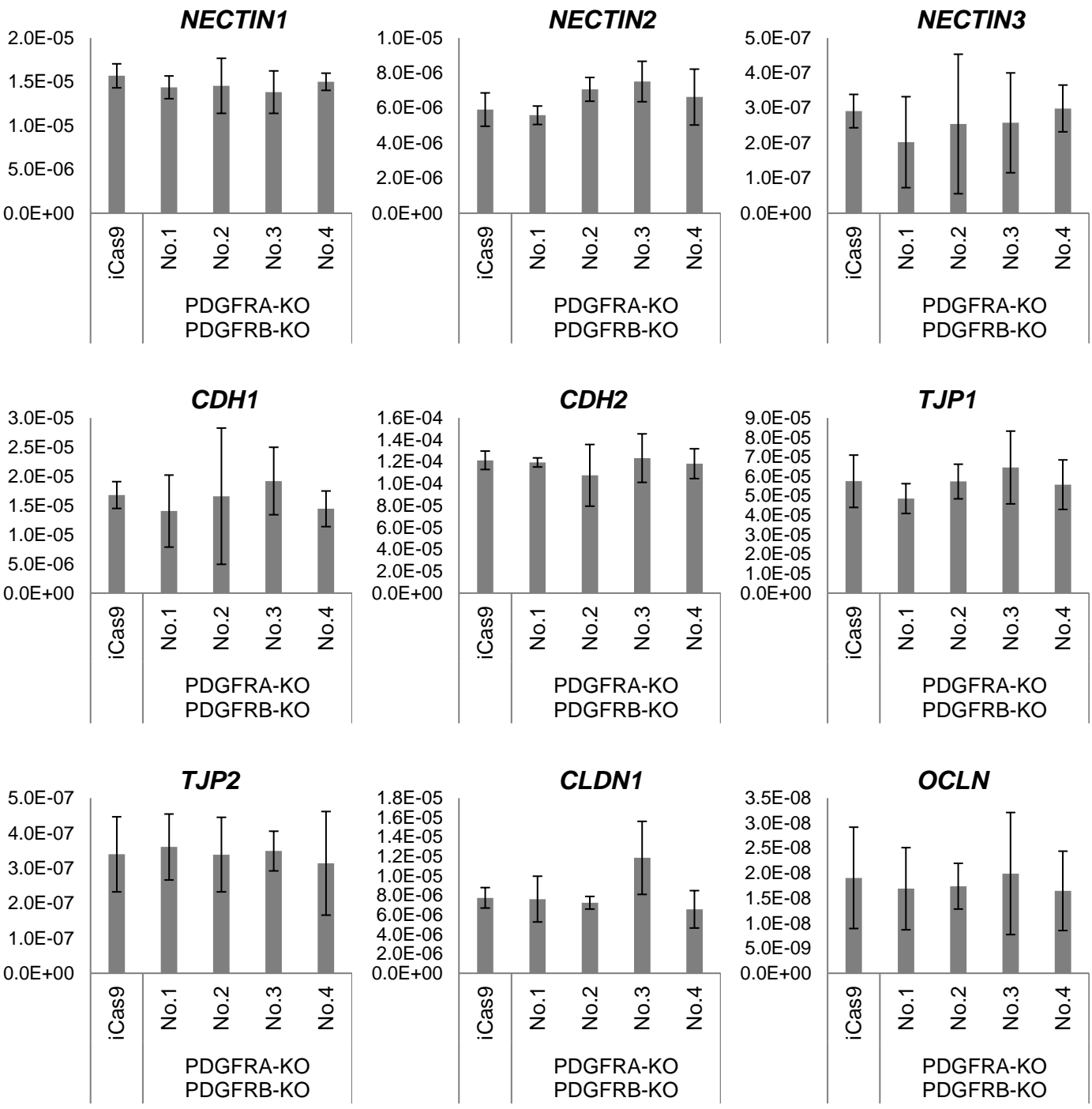


Figure S10. Expression levels of major cell adhesion molecules on d6DE day 10 cells, related to Figure 6. Normalization is performed using the expression level of 18S rRNA. The bars represent mean \pm SD. (n = 3).

Table S1: Genes encoding cell membrane protein screened by Comparison 1 to 4

Gene symbol	Comparison 1 FC	Comparison 2 FC	Comparison 3 FC	Comparison 4 FC
<i>PDGFRA</i>	6.95	4.61	3019.15	1757.08
<i>SEMA7A</i>	33.95	5.09	18.83	1.55
<i>EDARADD</i>	7.48	36.41	3.32	4.25
<i>TMEM37</i>	10.67	4.22	27.16	11.25
<i>SLC39A4</i>	9.54	4.91	10.46	14.86
<i>SLC7A11</i>	4.14	12.89	2.91	2.39

FC: fold change.

Table S2: Antibodies used in this study.

Antibody	Vender	Catalog No.
Anti-human FOXA2	Millipore	07-633
Anti-human SOX17	R&D SYSTEMS	AF1924
Anti-human NECTIN3	Millipore	MABT63
Anti-human NECL5	Millipore	MABF2245
Anti-human E-cadherin	R&D SYSTEMS	MAB1838
Anti-mouse HNF4A	Thermo Fisher Scientific	MA1-199
Anti-mouse PDGFR α	R&D SYSTEMS	AF1062
Anti-mouse NECL5	R&D SYSTEMS	MAB6909
Anti-mouse NECTIN3	abcam	ab63931

Table S3: Primers and probes used for qRT-PCR in this study.

Gene	Forward	Reverse	Probe No.
<i>CER1</i>	GCCATGAAGTACATTGGGAGA	CACAGCCTTCGTGGGTTATAG	41
<i>CXCR4</i>	ATTGGGATCAGCATCGACTC	CAAACCTCACACCCTTGCTTG	79
<i>TBX3</i>	GCAGCTTTCAACTGCTTCG	ACCCTCGCTGGGACATAAAT	19
<i>HNF4A</i>	TCAGACCCTGAGCCACCT	AGCAACGGACAGATGTGTGA	27
<i>AAT</i>	AATGGGGCTGACCTCTCC	GTCAGCACAGCCTTATGCAC	82
<i>ALB</i>	AATGTTGCCAAGCTGCTGA	CTTCCCTTCATCCCGAAGTT	27
<i>APOA1</i>	CCTTGGGAAAACAGCTAAACC	CCAGAACTCCTGGGTCACA	39
<i>ASGR1</i>	TGACCACCATCAGCTCAGAA	ACAGACAACCACAAGCAGCA	8
<i>ARG1</i>	CAAGGTGGCAGAAAGTCAAGA	GCTTCCAATTGCCAAACTGT	64
<i>CPS1</i>	ATTCCTGCACAAAGTGGACCT	TGACTCGGAAGCTGCTCAC	8
<i>FGA</i>	GGAAATTTTGAGAGGCGATTT	CCTCTGACACTCGGTTGTAGG	30
<i>SCD</i>	CCTAGAAGCTGAGAAACTGGTGA	ACATCATCAGCAAGCCAGGT	82
<i>OTC</i>	CAGCGAAATTCGGAATGC	CTAGCATCCGGCTCATAACC	27
<i>PDGFRA</i>	CCACCTGAGTGAGATTGTGG	TCTTCAGGAAGTCCAGGTGAA	27
<i>PDGFRB</i>	CATCTGCAAAACCACCATTG	GAGACGTTGATGGATGACACC	10
<i>SEMA7A</i>	CCTTTCATGTGCTTTACCTAACTACA	GATGTTGAAGGCGAAGCTGT	15
<i>EDARADD</i>	ACTGCCACGAAATTCAGATA	TGGAAGAGGATCTCCAGTGC	7
<i>TMEM37</i>	CCCAGGGTTGTTAAGAATGG	GAAAGTCCTAGGCACTGATTGG	11
<i>PDGFA</i>	GATGAGGACCTTGGCTTGC	CCAGCCTCTCGATCACCTC	68
<i>PDGFB</i>	CTGGCATGCAAGTGTGAGAC	CGAATGGTCACCCGAGTTT	68
<i>PDGFC</i>	CAGCAACAAGGAACAGAACG	TGGGCTGTGAATACTTCCATT	73
<i>PDGFD</i>	ACCATGACCGGAAGTCAAAA	CTGGGAGTGCAACTGTAACG	85
<i>CDH1</i>	AGGGGTCTGTCATGGAAGGT	GCGGCATTGTAGGTGTTCA	5
<i>CDH2</i>	GGTGGAGGAGAAGAAGACCAG	GGCATCAGGCTCCACAGT	66
<i>NECTIN1</i>	GACTCGCTCTCGGCTTGA	CGTTCACCTGGACCACCT	69
<i>NECTIN2</i>	GAGGACGAGGGCAACTACAC	TTGGGCTTGGCTATGACTCT	60
<i>NECTIN3</i>	TGACCAAAAAGTCATCTACATTTC	TTGAGGGATGCCACTGAAT	41
<i>TJP1</i>	CCAGCTGGTATGGGTTTCC	TCTACTGTCCGTGCTATACATTGAGT	1
<i>TJP2</i>	GAGGCGCCTACACTGACAAT	TTCTGGGCAATTTGATCTC	42
<i>CLDN1</i>	TTGACTCCTTGCTGAATCTGAG	GGCCACAAAGATTGCTATCAC	79
<i>OCLN</i>	GTCATCCAGGCCTCTTGAAA	AATGGCAATGGCAATTCATC	10