ACCSCIENCE PUBLISHING

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RESEARCH ARTICLE

Distinct toxicity of microplastics/TBBPA co-exposure to bioprinted liver organoids derived from hiPSCs of healthy and patient donors

Supplementary file

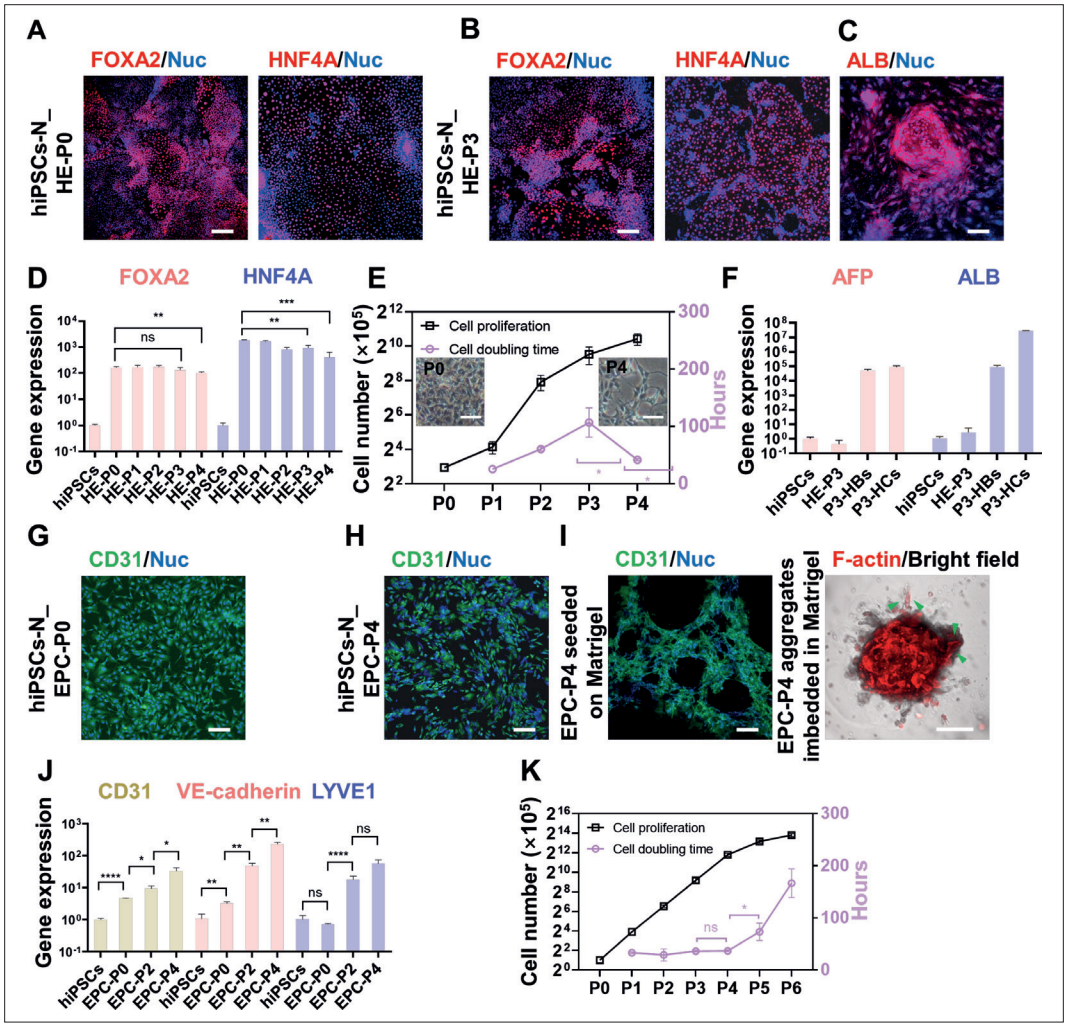

Figure S1. Generation of hepatic endoderm cells (HEs) and endothelial progenitor cells (EPCs) from healthy donor-derived human induced pluripotent stem cells (hiPSCs). (A) Immunofluorescence staining of HE markers (FOXA2 and HNF4A) in HE-P0 and (B) HE-P3. (C) Immunofluorescence staining of ALB in HE-P3-derived hepatocyte-like cells (HCs). (D) qRT-PCR of HE markers from HE-P0 to HE-P4, normalized to *GAPDH* and hiPSCs. (E) The cell proliferation (black curve) and cell number doubling time (pink curve) of HE-P0 to HE-P4. (F) qRT-PCR of hepatocyte markers in HE-P3 and HE-P3-derived hepatoblasts (HBs) and HCs, normalized to *GAPDH* and hiPSCs. (G) Immunofluorescence staining of the endothelial marker CD31 in EPC-P0 and (H) EPC-P4. (I) CD31/DAPI staining of EPC-P4 on Matrigel layer and F-actin/brightfield image of EPC-P4 aggregates embedded in Matrigel hydrogel. Green arrows indicate capillary-like structures. (J) qRT-PCR of endothelial markers in EPC-P0 to EPC-P4, normalized to *GAPDH* and hiPSCs. (K) The cell proliferation (black curve) and cell number doubling time (pink curve) of EPC-P0 to EPC-P6. The data are presented as mean ± SD. Statistical significance was analyzed using the unpaired two-tailed *t*-test, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, 'ns' indicates no significant difference, n=3. Scale bars in (A)–(C) and (G)–(I): 200 μm; scale bar in (E): 100 μm.

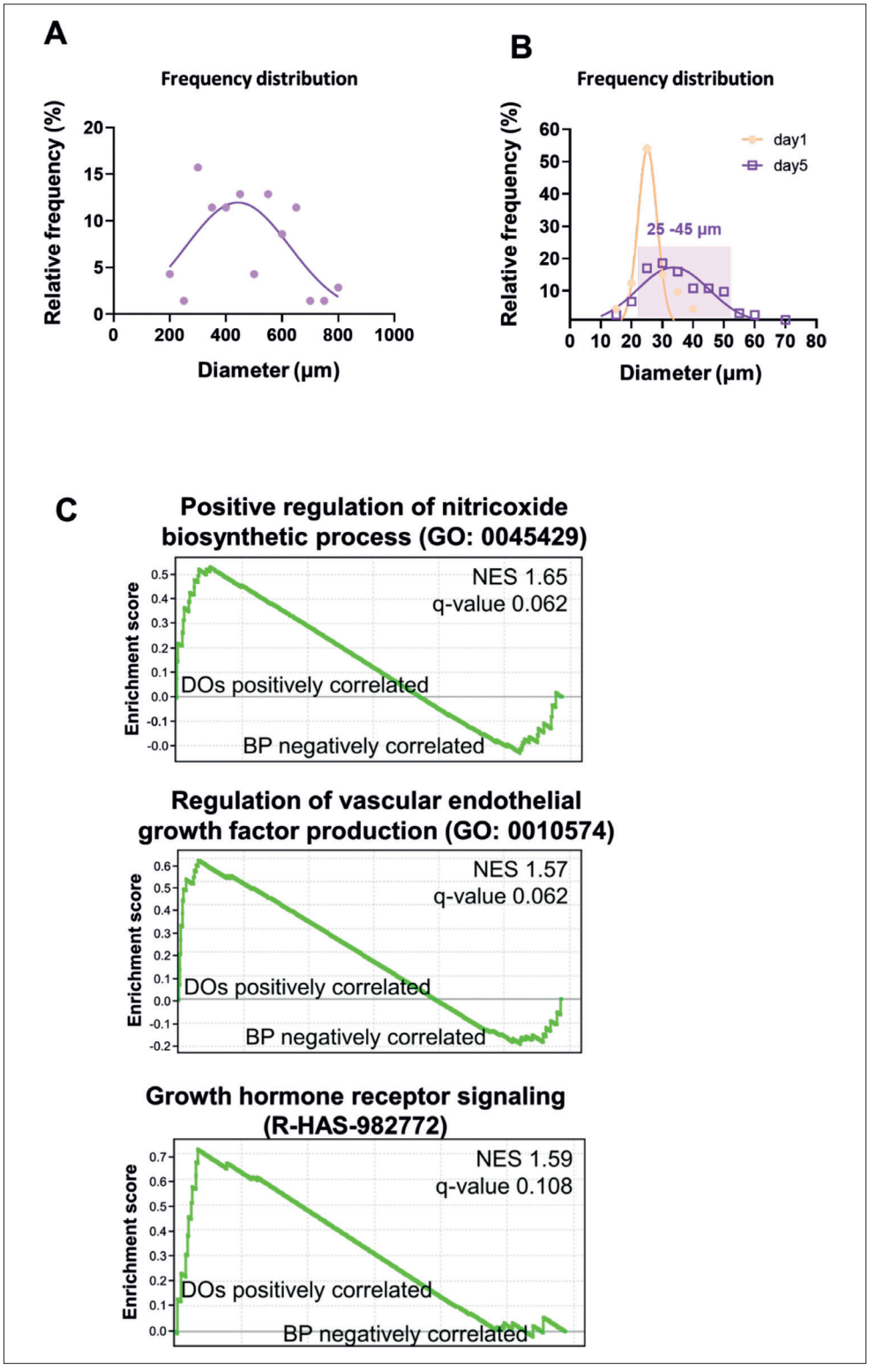

Figure S2. Characterization of microspheres laden with DOs. (A) Frequency distribution of microsphere diameters. (B) Frequency distribution of DO diameters within the microspheres. (C) Representative plots of gene set enrichment analysis (GSEA) comparing DOs and planar cultured cells before printing (BP).

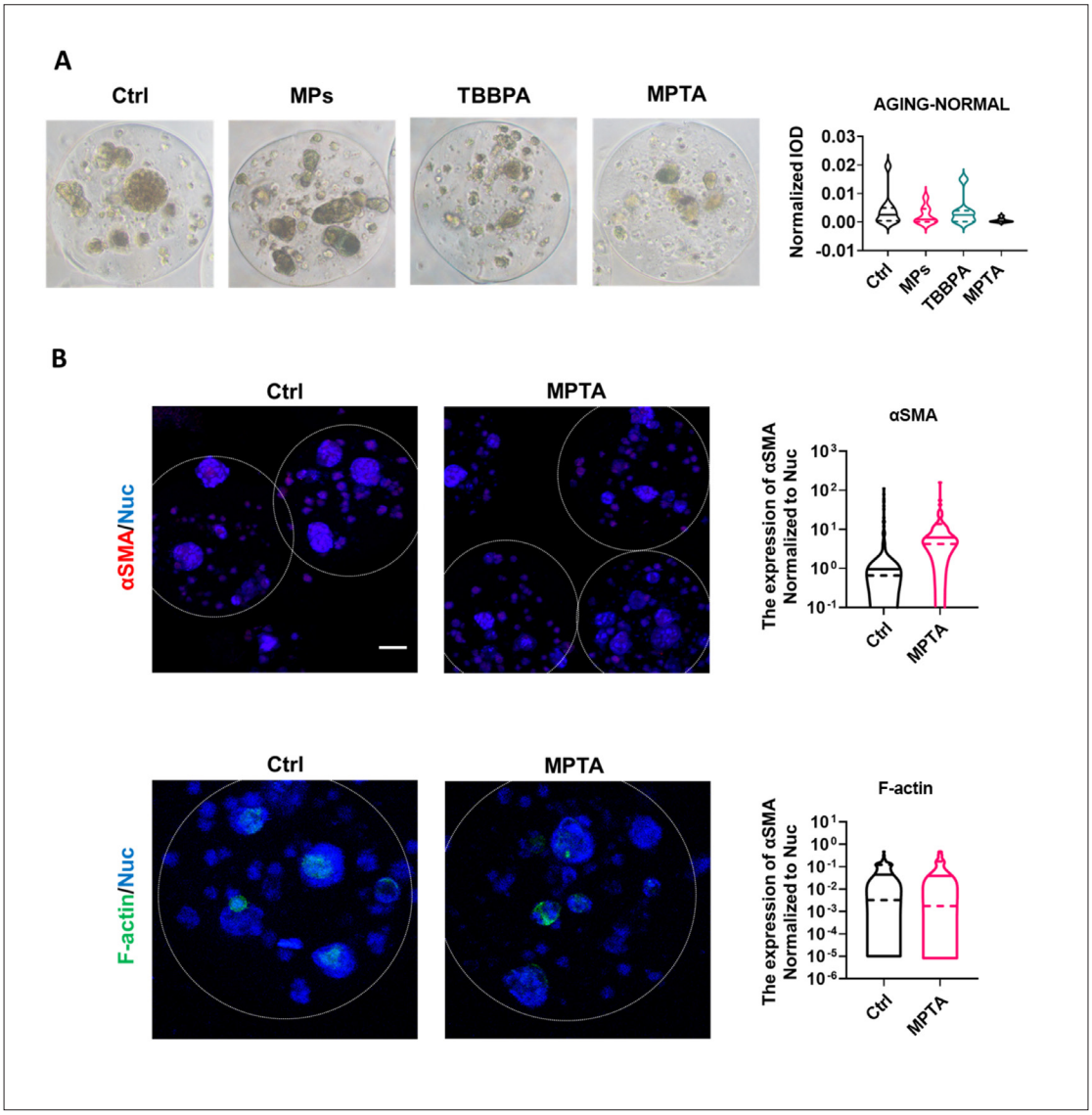

Figure S3. The effects of PS-MPs and TBBPA on N_Dos. (A) Staining of β-galactosidase (SA-β-Gal). The right panel shows the quantification based on the Integrated Optical Density (IOD) values of the images, *n*>3. (B) *In situ* staining of F-actin, COL1A1, and αSMA (the maximal projection), with treatments of vehicle and MPTA. Nucleus (Nuc) were stained by DAPI. Scale bar: 100 μm. The right panels show the quantification based on the staining images. The data are presented as mean ± SD. Statistical significance was analyzed using the unpaired two-tailed *t*-test; 'ns' indicates no significant difference.

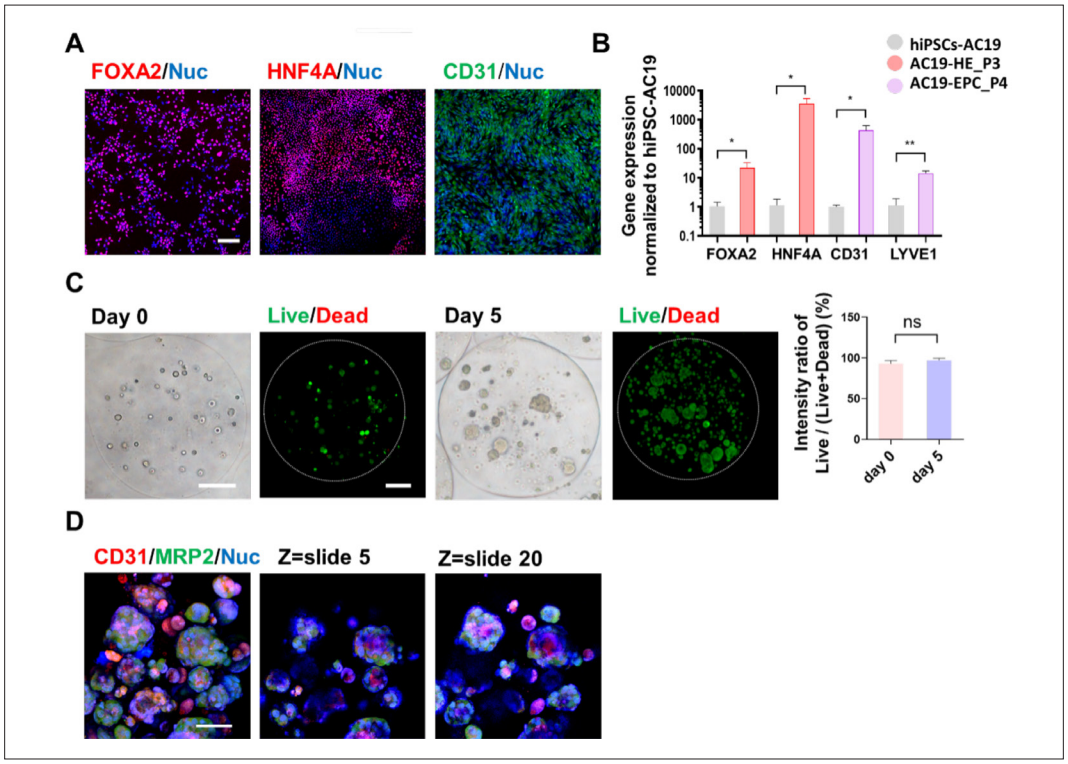

Figure S4. Generation of AC19_DOs derived from ALD patient-derived hiPSCs. (A) Immunostaining of HE markers (FOXA2 and HNF4A) in hiPSCs-AC19_HE-P3 and endothelial marker (CD31) in hiPSCs-AC19_EPC-P4. Nucleus (Nuc) were stained by DAPI. Scale bar: 200 μm. (B) qPCR of the markers of HEs and endothelial cells in hiPSCs-AC19, hiPSCs-AC19_HE-P3, and hiPSCs-AC19_EPC-P4, normalized to *GAPDH* and hiPSCs. (C) Optical images and maximal projection of Live/Dead fluorescent images of postprinted cell-laden microspheres at day 0 and day 5. Scale bar: 100 μm. (D) Maximal projection of CD31/MARP2/DAPI staining in AC19_DOs harvested from microspheres. The right two panels are slides 5 and 20. Scale bar: 50 μm.

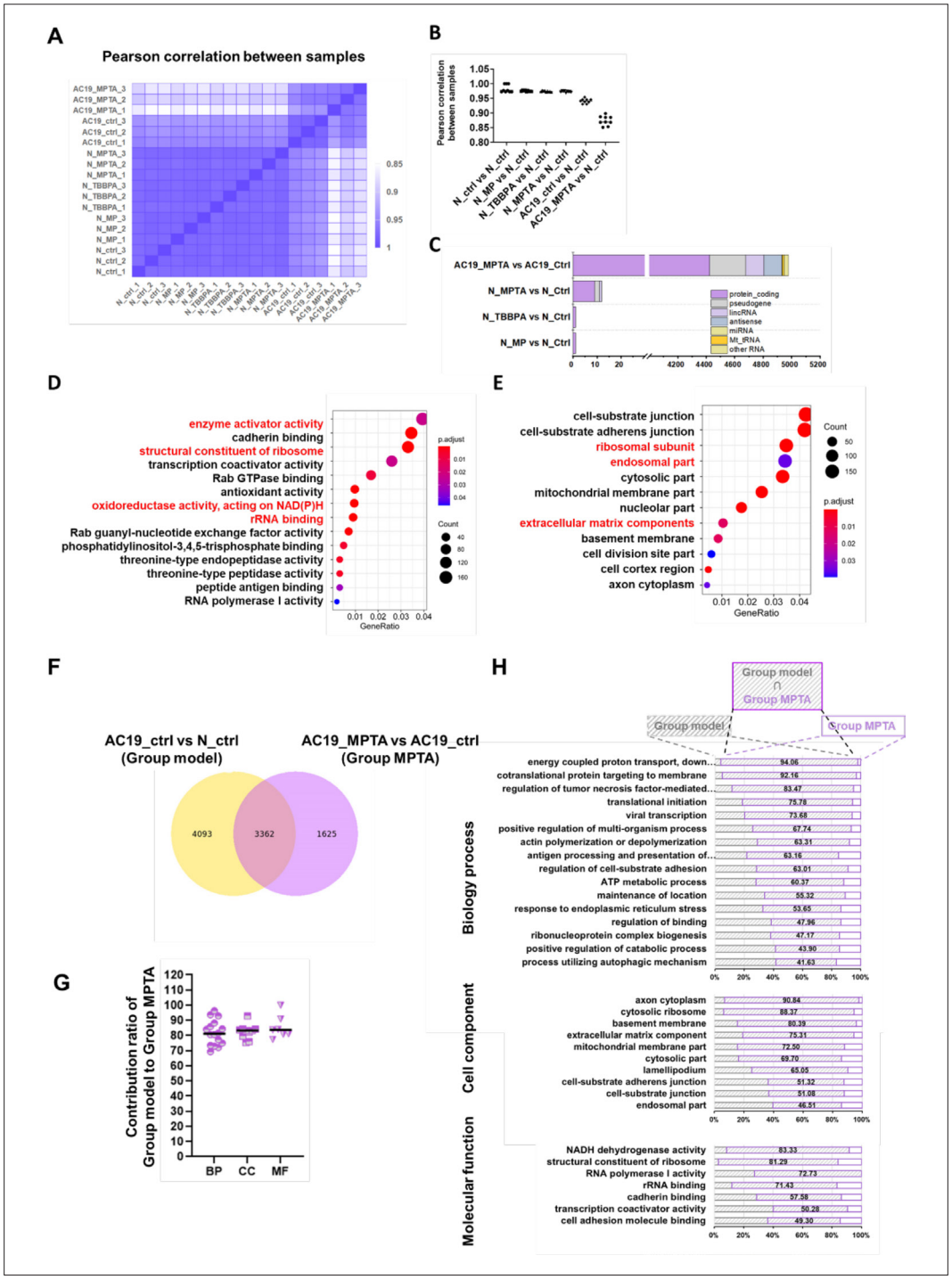

Figure S5. Transcriptomic analysis of hepatotoxicity of MPTA in N_DOs and AC19_DOs. (A) Heatmap showing the Pearson correlation between samples of transcriptome treated with vehicle (Ctrl), PS-MPs, TBBPA, and MPTA. (B) Plot of the Pearson correlation coefficients. (C) The number of DEGs compared between different treatments. (D) Bubble chart depicting GO enrichment in molecular functions. (E) Bubble chart describing GO enrichment in cellular components. (F) Venn diagram displaying AC19_ctrl *vs.* N_ctrl and AC19_MPTA *vs.* AC19_ctrl. (G) Plot illustrating the contribution ratio of ALD genetic inheritance to the hepatotoxicity of MPTA. Each dot represents the ratio of the number of shared genes in each GO term to the number of DEGs affected by MPTA (Group MPTA). (H) Analysis of ALD genetic inheritance contribution to hepatotoxicity of MPTA. The bars with diagonal lines represent the number of genes enriched in GO terms for the Group model (AC19_ctrl *vs.* N_ctrl), while the pink bars represent the number of genes enriched in GO terms for the group MPTA (AC19_MPTA *vs.* AC19_ctrl). The overlapping regions between the diagonal and pink bars represent the number of overlapping genes.

Table S1. qRT-PCR primer list

Gene	Forward sequence (5' to 3')	Reverse sequence (5' to 3')
ALB	GATGAGATGCCTGCTGACTTGC	CACGACAGAGTAATCAGGATGCC
CYP3A4	CCGAGTGGATTTCCTTCAGCTG	TGCTCGTGGTTTCATAGCCAGC
AFP	GCAGAGGAGATGTGCTGGATTG	CGTGGTCAGTTTGCAGCATTCTG
CD31	AAGTGGAGTCCAGCCGCATATC	ATGGAGCAGGACAGGTTCAGTC
CD34	CCTCAGTGTCTACTGCTGGTCT	GGAATAGCTCTGGTGGCTTGCA
LYVE1	GCCGACAGTTTGCAGCCTATTG	CCGAGTAGGTACTGTCACTGAC
STAB2	ACTGGCTCCTTACCAAACCTGC	GAGCAAACACTGTGTAGGCATCG
ALCAM	TCCAGAACACGATGAGGCAGAC	GTAGACGACACCAGCAACAAGG
PDGFRB	TGCAGACATCGAGTCCTCCAAC	GCTTAGCACTGGAGACTCGTTG
COL1A1	GATTCCCTGGACCTAAAGGTGC	AGCCTCTCCATCTTTGCCAGCA
APOA1	GTGGATGTGCTCAAAGACAGCG	GCTTGCTGAAGGTGGAGGTCAC
SREBF1	ACTTCTGGAGGCATCGCAAGCA	AGGTTCCAGAGGAGGCTACAAG
SREBF2	CTCCATTGACTCTGAGCCAGGA	GAATCCGTGAGCGGTCTACCAT
CYP2E1	GAGCACCATCAATCTCTGGACC	CACGGTGATACCGTCCATTGTG
SIRT1	TAGACACGCTGGAACAGGTTGC	CTCCTCGTACAGCTTCACAGTC
PPARA	TCGGCGAGGATAGTTCTGGAAG	GACCACAGGATAAGTCACCGAG
FXR2	TGAGGGTTCGAGTGGAAGGTGA	GGTGATACTCCAGCAAAGCCTG
THRB	GGTTGACTTGGAAGCCTTCAGC	GGATGATCTGGTCTTCACATGGC
LOXL2	TGACTGCAAGCACACGGAGGAT	TCCGAATGTCCTCCACCTGGAT
TIMP1	GGAGAGTGTCTGCGGATACTTC	GCAGGTAGTGATGTGCAAGAGTC
MMP3	CACTCACAGACCTGACTCGGTT	AAGCAGGATCACAGTTGGCTGG
MMP1	ATGAAGCAGCCCAGATGTGGAG	TGGTCCACATCTGCTCTTGGCA
FOXA2	GGAACACCACTACGCCTTCAAC	AGTGCATCACCTGTTCGTAGGC
HNF4A	GGTGTCCATACGCATCCTTGAC	AGCCGCTTGATCTTCCCTGGAT
VE-cadherin	GAAGCCTCTGATTGGCACAGTG	TTTTGTGACTCGGAAGAACTGGC