

Supplementary material

Table S1. The primer sequences used in this study

Table S2. Baseline demographic and anthropometric characteristics of the study patients

Figure S1-S5.

Table S1. The primer sequences used in this study

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
GAPDH	TCCAAAATCAAGTGGGGCGA	AAATGAGCCCCAGCCTTCTC
P53	CGCTTCGAGATGTTCCGAGA	CTTCAGGTGGCTGGAGTGAG
P16	CCGTGGACCTGGCTGAGGAG	CGGGGATGTCTGAGGGACCTTC
SPHK1	GAGAGTGAGAAGTATCGGCGTCTG	GGGAGGCAGGTGTCTTGGAAC
SPHK2	AACCTCATCCAGACAGAACGACAG	GAGACCGTGACGATGCCATCC
SPNS2	CTGATCCGCCAGAGCACTAAGG	GCCCAGGACCACGACGAAAG
MDSF2B	CATCAACAGGAGCCAGAGGACAG	GCAGGAACCACAGGAAGAAGTAGG
SGPL1	CTTCCCAGGACTACGCAAGATAGA	CAGAAGTCACACATCCACACGAAT
SGPP1	GCCGCTCTACTGCCTGTTCTG	GACCAGCACCCAGATGACCAC

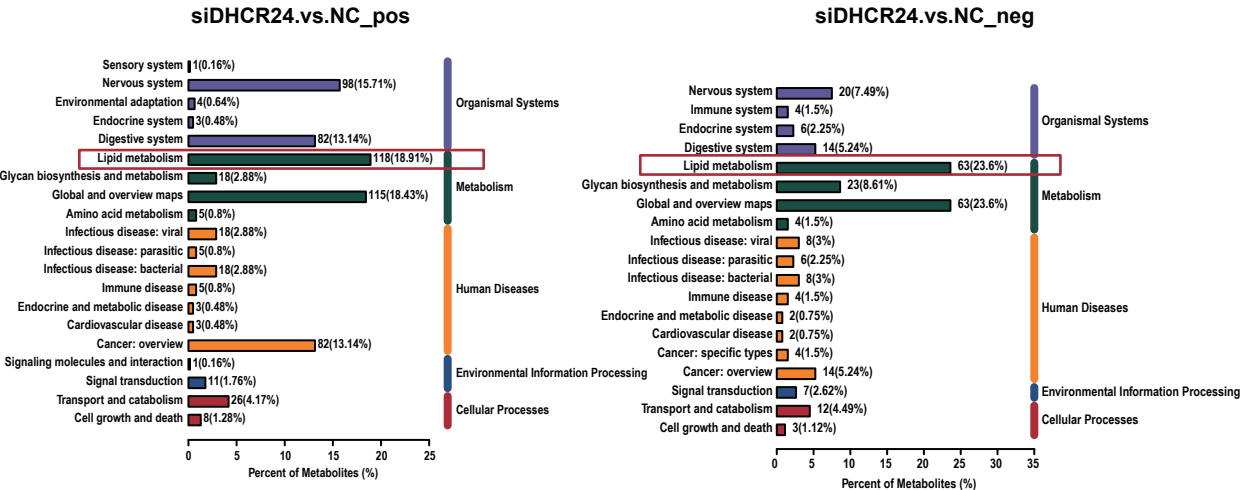
Table S2. Baseline demographic and anthropometric characteristics of the study patients

Patient ID	Sex	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m ²)
P001	Male	25	85.7	171	29.3
P002	Female	43	53.7	164	20
P003	Male	49	77.3	172	26.1
P004	Female	23	45.6	171	15.6
P005	Male	38	65.3	172	22.1
P006	Male	40	72.1	169	25.2
P007	Female	44	52.8	153	22.6
P008	Female	45	50.0	159	19.8
P009	Male	60	66.8	174	22.1
P010	Male	19	56.8	175	18.5
P011	Male	56	62.8	161	24.2
P012	Male	30	51.4	178	16.2
P013	Male	22	84.0	182	25.4
P014	Male	24	68.8	174	22.7
P015	Male	48	72.8	169	25.5
P016	Male	67	71.6	171	24.5
P017	Male	59	67.1	163	25.3
P018	Male	43	85.9	172	29.0
P019	Female	50	58.5	163	22.0
P020	Male	28	80.1	175	26.2

P021	Female	47	56.3	158	22.6
P022	Male	31	65	172	22
P023	Female	32	66.3	153	28.3
P024	Female	53	63.9	165	23.5
P025	Male	40	86.8	183	25.9
P026	Male	35	58.6	166	21.3
P027	Female	37	53.4	158	21.4
P028	Female	42	56.7	158	22.7
P029	Male	61	76.1	178	24
P030	Female	69	52.7	159	20.8
P031	Male	39	84.4	183	25.2

Figure S1

a.



b.

Genotype identification

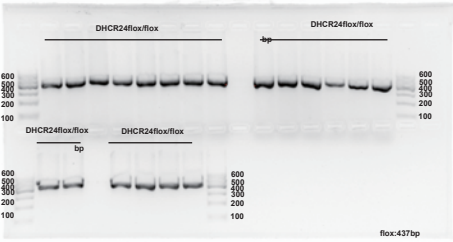
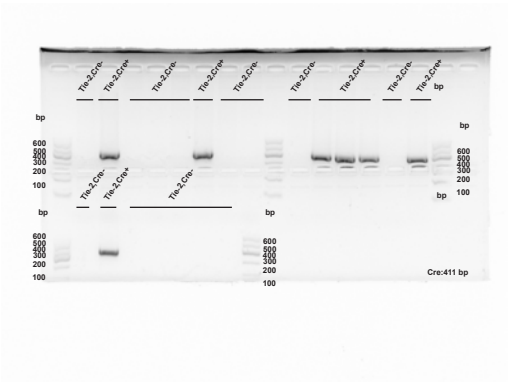


Figure S2

a.

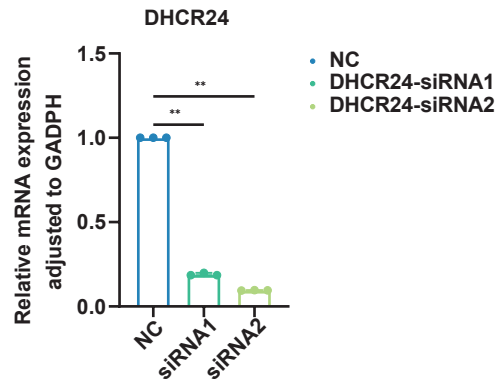


Figure S3

a.

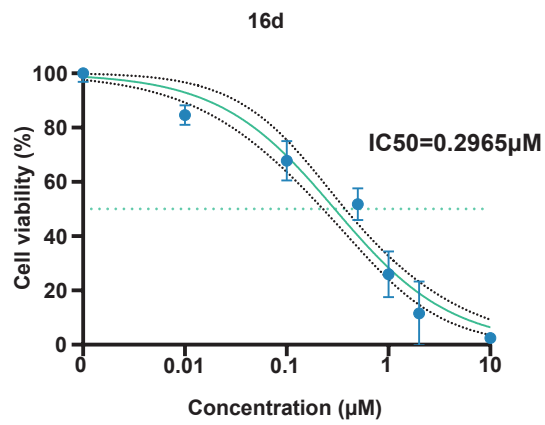


Figure S4

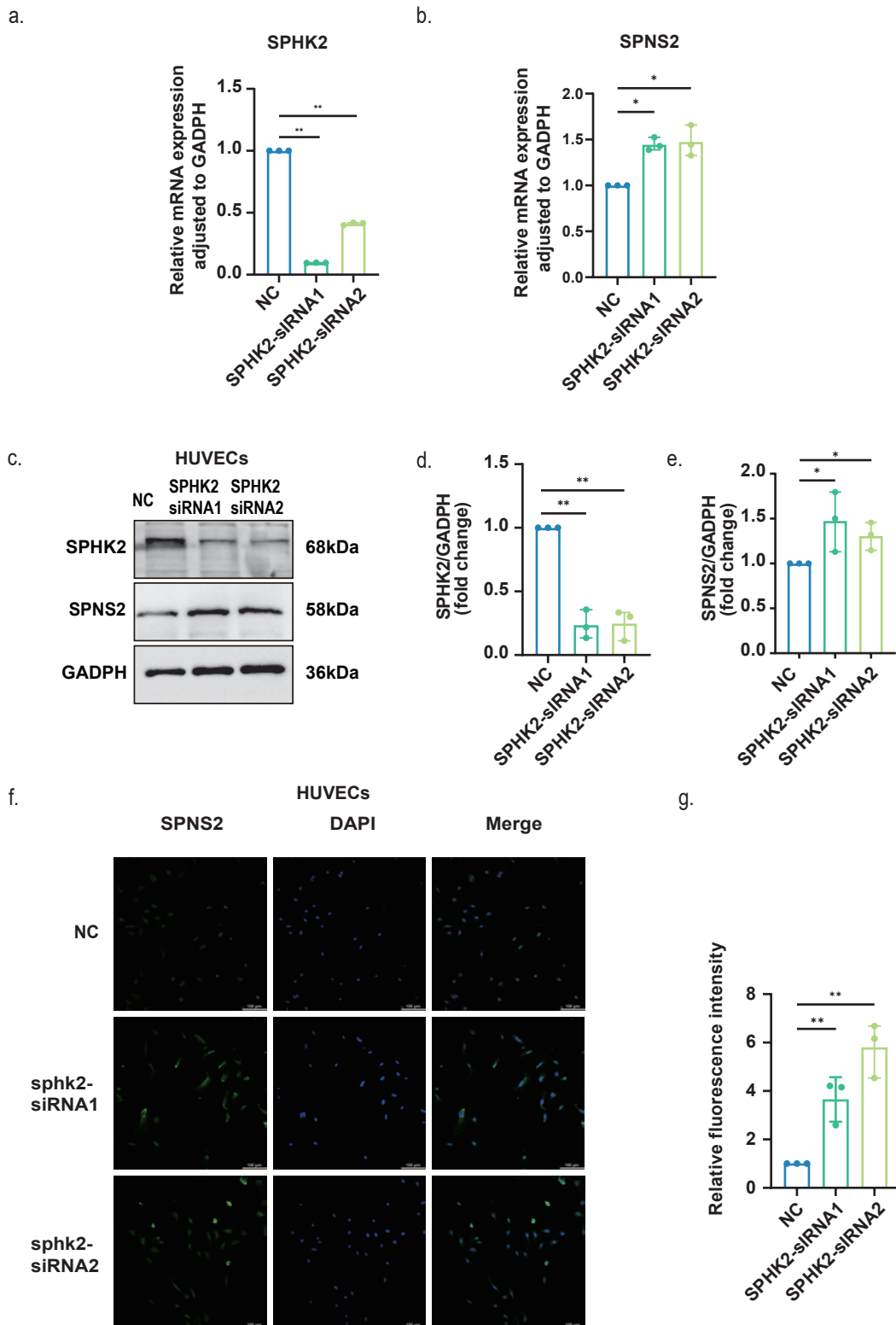


Figure S5

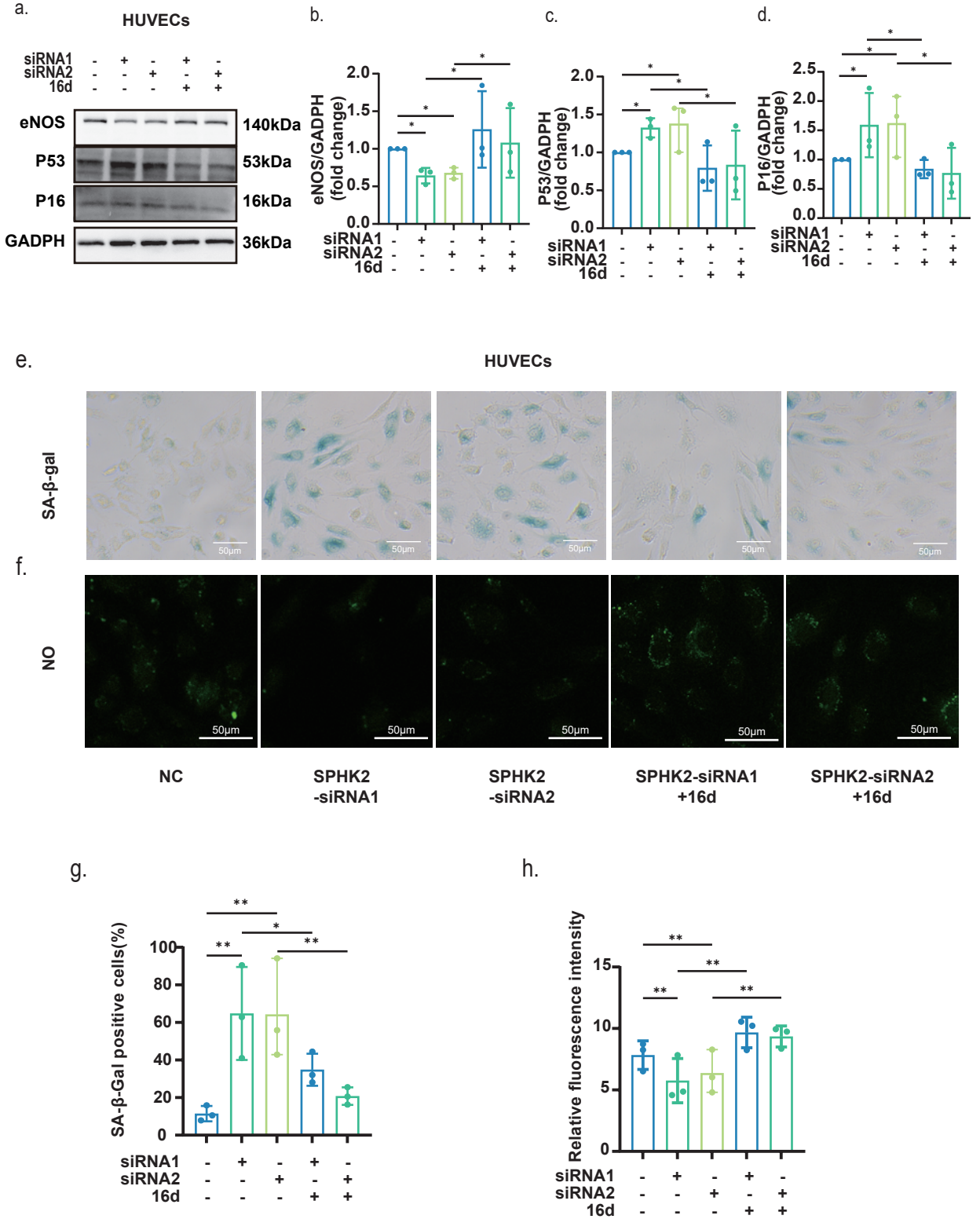


Table S1. Primer sequences used for quantitative real-time PCR (qRT-PCR) in this study.

Table S2. Baseline demographic and clinical characteristics of the 31 enrolled human subjects.

Fig S1 Lipid clustering heatmaps and genotyping validation

- (a) KEGG pathway classification analysis of differentially abundant metabolites in DHCR24 knockdown (si-DHCR24) and negative control (NC) HUVECs detected by liquid chromatography–mass spectrometry (LC-MS).
- (b) Genotyping of Tie2-Cre endothelial-specific DHCR24 conditional knockout mice.

Fig S2 Validation of DHCR24 knockdown efficiency in HUVECs

- (a) Relative mRNA expression of DHCR24 in negative control (NC) and DHCR24-knockdown (siRNA1, siRNA2) HUVECs, as determined by quantitative real-time PCR (qRT-PCR). GAPDH was used as an internal reference. Statistical significance was determined using Student's t test. $n = 3$. The values are presented as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$.

Fig S3 Dose-response curve of 16d on HUVEC viability and calculated IC_{50} .

- (a) HUVECs were treated with increasing concentrations of 16d (0, 0.01, 0.1, 1, 2, 10 μ M) for 24 h. Cell viability was determined by the CCK-8 assay, and data are presented as mean $\pm$ SEM ($n = 3$). The half-maximal inhibitory concentration (IC_{50}) of 16d was calculated using a four-parameter logistic regression model, with the 95% confidence interval shown as the dotted band around the fitted curve. The green dotted line indicates the 50% cell viability threshold.

Fig S4 Knockdown of SPHK2 upregulates SPNS2 expression in HUVECs

(a, b) Relative mRNA levels of SPHK2 and SPNS2 in HUVECs transfected with SPHK2 siRNA1 were determined by RT-qPCR. (c-e) Protein expression of SPHK2 and SPNS2 in HUVECs after SPHK2 knockdown was detected by Western blotting. GAPDH served as a loading control. Quantitative analysis of band intensity is shown on the right. (f, g) Immunofluorescence staining of SPNS2 (green) in control (NC) and SPHK2-knockdown HUVECs. Nuclei were counterstained with DAPI (blue). Relative fluorescence intensity was quantified and shown on the right. The values are presented as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$.

Fig S5 Inhibition of SPNS2 rescues endothelial senescence induced by SPHK2 knockdown in HUVECs

(a-d) Western blot analysis of P53, P16, and eNOS protein levels in HUVECs transfected with SPHK2 siRNA or treated with 16d. GAPDH was used as the loading control. Quantitative analysis of relative protein expression is shown on the right. (e) SA- β -gal staining analysis showing senescent cell distribution under different experimental conditions. (f) Fluorescence imaging of nitric oxide (NO) detection using DAF-FM DA staining (green fluorescence), showing spatial NO distribution patterns. (g, h) Quantitative evaluation of SA- β -gal-positive cell ratio (G) and NO fluorescence intensity (H) across different treatment groups. All experiments were performed in triplicate. Data are presented as mean $\pm$ standard deviation (SD). Statistical significance was determined using Student's t-test or one-way ANOVA where appropriate. * $p < 0.05$, ** $p < 0.01$.