Experimental Acceptance Criteria

1. hiPSC quality criteria

hiPSC cultures were considered acceptable for subsequent experiments when the following criteria were met: (i) compact colony morphology without substantial spontaneous differentiation; (ii) positive alkaline phosphatase staining; (iii) positive immunofluorescence staining for pluripotency markers OCT4, SOX2, NANOG, TRA-1-60, and TRA-1-81; (iv) a normal karyotype; (v) evidence of trilineage differentiation by embryoid body formation; and (vi) confirmation of the FBN1 genotype by Sanger sequencing.

2. iPSC-derived endothelial cell (iPSC-EC) quality criteria

iPSC-ECs were considered acceptable when they exhibited a cobblestone-like endothelial morphology and positive expression of endothelial markers, including CD31, VE-cadherin, and vWF. Following CD144-based sorting, cell viability was required to be >85%. Cultures were required to be free of bacterial, fungal, and mycoplasma contamination. Cells between passages P4 and P7 were used for experiments.

3. Functional assay acceptance criteria

Functional experiments were performed using at least three independent biological replicates, with technical replicates included where applicable. For qPCR, GAPDH Ct values were required to be within 18–24, and the Ct difference between technical replicates was required to be <0.5. For Western blotting, GAPDH bands were required to be intact and suitable for normalization. For scratch-wound assays, wounds at 0 h were required to be continuous and free of extensive cell detachment. For SA-β-galactosidase assays, baseline positivity in WT-iECs was required to remain low.

Exclusion Criteria

Samples or cultures were excluded when predefined technical or quality-control criteria were not met. hiPSC cultures were excluded in cases of extensive spontaneous differentiation, abnormal karyotype, or substantial loss

of pluripotency-marker expression. iPSC-EC cultures were excluded when endothelial marker expression was lost, microbial contamination was detected, cell viability was <80%, or cells exceeded passage P7.

For molecular assays, samples were excluded when RNA quality was outside the predefined range (A260/A280 ratio of 1.8–2.1), the internal-control Ct value was >26, or the Ct difference between technical replicates was >0.5. Western blot samples were excluded when substantial protein degradation or obvious loading abnormalities compromised quantitative analysis. For functional assays, individual wells were excluded when predefined technical failures occurred, including nonspecific blue staining in SA-β-gal assays, extensive cell detachment during scratch-wound assays, extensive cell death following TNF-α treatment or serum starvation, or confirmed microbial contamination.

Importantly, data were not excluded on the basis of the direction or magnitude of the observed biological effect. Exclusions were based only on predefined technical, sample-quality, or assay-quality criteria.

Quality Control Procedures

1. Cell quality control

Newly established hiPSC lines underwent pluripotency and karyotype characterization before experimental use. Endothelial identity was assessed following CD144-based sorting by evaluating endothelial marker expression. Cell passage numbers were strictly controlled. Following thawing, cell viability and morphology were assessed before cells were used for experiments.

2. Reagent quality control

Active reagents were aliquoted to minimize repeated freeze–thaw cycles. New batches of culture medium were evaluated in small-scale preliminary experiments before use in the main experiments. Appropriate positive and

negative controls were included in immunoblotting and immunofluorescence experiments.

3. Molecular assay quality control

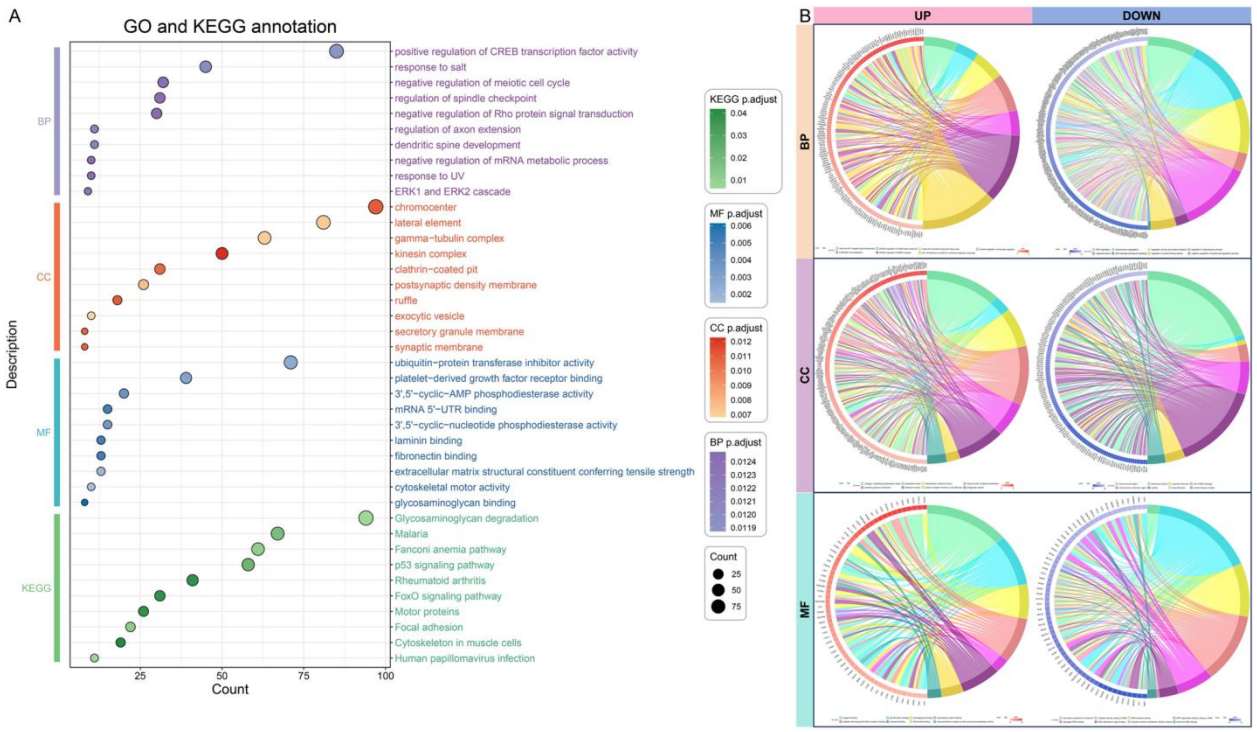
RNA quality was assessed based on A260/A280 and A260/A230 ratios, with predefined acceptance criteria of 1.8–2.1 and >1.8, respectively. No-template controls (NTCs) were included in qPCR assays. For Western blotting, molecular-weight markers and GAPDH normalization were used, and band intensities were quantified using ImageJ. For RNA-seq experiments, samples with an RNA integrity number (RIN) ⩾7 were considered acceptable for sequencing.

4. Functional assay quality control

Functional experiments included at least three independent biological replicates. TNF-α stimulation experiments included unstimulated controls. SA-β-gal staining solutions were freshly prepared, and samples were processed simultaneously when possible to minimize batch-to-batch variation. For image-based assays, at least five randomly selected, non-overlapping fields were analyzed per group, and quantitative image analysis was performed using ImageJ.

5. Statistical quality control

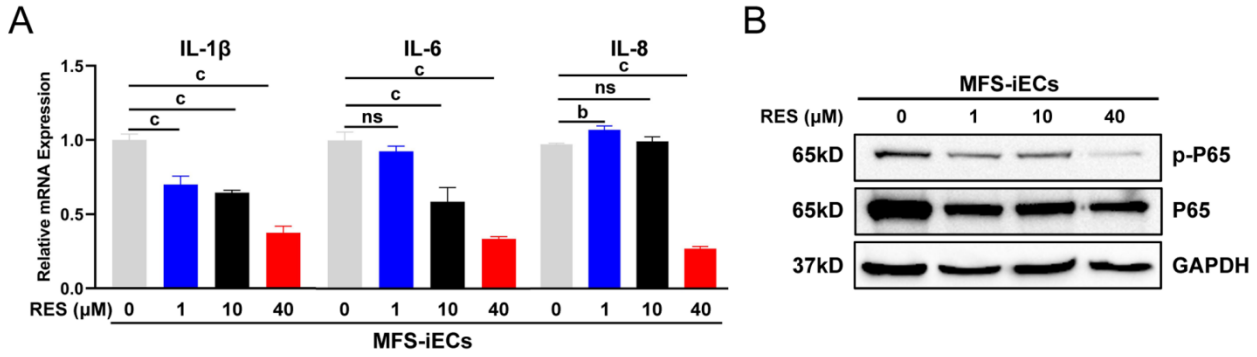
Statistical analyses were performed using GraphPad Prism 8. Data were presented as mean ± SD unless otherwise indicated. For comparisons between two independent groups, an unpaired two-tailed Student's *t*-test was used when the assumptions of the test were satisfied. For comparisons among more than two groups, one-way analysis of variance (ANOVA) was used, followed by an appropriate multiple-comparison procedure as specified in the corresponding figure legend. Statistical significance was defined as $P < 0.05$. Exact P values are reported in the main text and/or figure legends where applicable.



Supplementary Figure 1 GO and KEGG Annotation in MFS-iECs.

(A) GO and KEGG annotation diagrams illustrating results in MFS-iECs.

(B) GO enrichment analysis for Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) in MFS-iECs.



Supplementary Figure 2 The Inflammation-Related Phenotypes of MFS-iECs Can Be Ameliorated by Resveratrol.

(A) RT-qPCR analysis of the expression changes of relevant inflammatory factors after treating MFS-iECs with various concentrations of resveratrol or leaving them untreated for 24 hours.

(B) Western blot analysis of phosphorylated p65 following treatment of MFS-iECs with various concentrations of resveratrol or remaining untreated for 24 hours.

Data are presented as mean ± SD (n = 3 independent differentiation batches). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$. Statistical significance was determined *via* one-way ANOVA for panel A.

Supplementary Table 1. Patients information

Variable	Control	MFS-1	MFS-2
Sex	Male	Male	Male
Age, years	75	28	56
Clinical diagnosis	Severe mitral regurgitation and heart failure	Aortic root dilation and sinus of Valsalva aneurysm	Aortic sinus and ascending aortic dilation; moderate-to-severe aortic regurgitation
MFS diagnosis	No	Yes	Yes
FBN1 variant	Not identified	c.C3442G,p.P1148A	c.C4567T,p.R1523X
Exon	/	28	37
hiPSC line	WT-iPSC	MFS-iPSC-1	MFS-iPSC-2
Independent clones	3	3	3
EC differentiations	3-5	3-5	3-5
EC passage used	4-7	4-7	4-7

Supplementary Table 2. Antibodies Used

Antibodies	Source	Identifier
Anti-CD31	R&D	Cat#AF806
Anti-vWF	Boster	Cat#PB0273
Anti-VE-CAD	CST	Cat#2158s
Anti-P53	HuaBio	Cat#EM20603
Anti-P21	ABclonal	Cat#A19094
Anti- Phospho-NF-κB p65	CST	Cat#3033S
Anti-NF-κB p65	HuaBio	Cat#ET1603-12
Anti-Ki67	BD Biosciences	Cat#550609
Anti-OCT4	STEMGENT	Cat# 090023
Anti-NANOG	STEMGENT	Cat#0906020
Anti-SOX2	STEMGENT	Cat#0906024
Anti-TRA-1-60	STEMGENT	Cat# 09-06010
Anti-TRA-1-81	STEMGENT	Cat# 09- 0011
Anti-α-SMA	Sigma	Cat# A5228
Anti-α-fetoprotein	Abcam	Cat# ab169552
Anti-TUJ 1	Covance	Cat# MMS-435P
Anti-eNOS	ABclonal	Cat#A1548
Goat Anti-Rabbit IgG(H+L), Mouse/Human ads-HRP	SouthernBiotech	Cat#SBA-4050-05
Goat Anti-Mouse Ig, Human ads-HRP	SouthernBiotech	Cat#1010-05
AF488 goat anti-rabbit IgG	Invitrogen	Cat# A11008
AF488 goat anti-mouse IgG	Invitrogen	Cat# A11001
AF594 goat anti-rabbit IgG	Invitrogen	Cat# A11012
AF594 goat anti-Mouse IgG	Invitrogen	Cat# A21044

Supplementary Table 3. Primers Used

Primer name	sequence (5' to 3')
hOCT4-F	AGTGCCCGAAACCCACACTG
hOCT4-R	ACCACACTCGGACCACATCCT
hSOX2-F	CTCGTGCAGTTCTACTCGTCG
hSOX2-R	AGCTCTCGGTCAGGTCCTTT
hNANOG-F	TGAACCTCAGCTACAAACAG
hNANOG-R	TGGTGGTAGGAAGAGTAAAG
hCD31-F	AACAGTGTTGACATGAAGAGCC
hCD31-R	TGTAAAACAGCACGTCATCCTT
hVE-Cad-F	TTGGAACCAGATGCACATTGAT
hVE-Cad-R	TCTTGCGACTCACGCTTGAC
hvWF-F	CCGATGCAGCCTTTTCGGA
hvWF-R	TCCCCAAGATACACGGAGAGG
hPCNA-F	TCAGGTACCTCAGTGCAAAAG
hPCNA-R	TGCAAGTGGAGAACTTGGAA
hMKi67-F	TGACTTCCTTCCATTCTGAAGAC
hMKi67-R	TGGGTCTGTTATTGATGAGCC
hICAM1-F	ATGCCCAGACATCTGTGTCC
hICAM1-R	GGGGTCTCTATGCCCAACAA
heNOS-F	TGATGGCGAAGCGAGTGAAG
heNOS-R	ACTCATCCATACACAGGACCC
hTFAM-F	CCGAGGTGGTTTTCATCTGT
hTFAM-R	GCATCTGGGTTCTGAGCTTT
hUCP2-F	CCCCGAAGCCTCTACAATGG
hUCP2-R	CTGAGCTTGGAATCGGACCTT
hPPARA-F	CTATCATTTGCTGTGGAGATCG
hPPARA-R	AAGATATCGTCCGGGTGGTT
hPGC-1β-F	GATGCCAGCGACTTTGACTC
hPGC-1β-F	ACCCACGTCATCTTCAGGGA

hIL-1β-F	ATTACAGTGGCAATGAGGAT
hIL-1β-R	TAGTGGTGGTCGGAGATT
hP53-F	GCGTGTGGAGTATTTGGATGAC
hP53-R	ATGTAGTTGTAGTGGATGGTGGTA
hP21-F	AGTCAGTTCCTTGTGGAGCC
hP21-R	GAAGGTAGAGCTTGGGCAGG
hIL-6-F	ACTCACCTCTTCAGAACGAATTG
hIL-6-R	CCATCTTTGGAAGGTTCAGGTTG
hIL-8-F	ACCGGAAGGAACCATCTCAC
hIL-8-R	GGCAAAACTGCACCTTCACAC
hIL-10-F	TCAAGGCGCATGTGAACTCC
hIL-10-R	GATGTCAAACTCACTCATGGCT
hMMP2-F	TACAGGATCATTGGCTACACACC
hMMP2-R	GGTCACATCGCTCCAGACT
hMMP3-F	AGTCTTCCAATCCTACTGTTGCT
hMMP3-R	TCCCCGTCACCTCCAATCC
hMMP12-F	ACACCTGACATGAACCGTGAG
hMMP12-R	ATGCCTGTGTTAATCTTGCTGA
hGAPDH-F	AAGGTGAAGGTCGGAGTCAAC
hGAPDH-R	GGGGTCATTGATGGCAACAATA