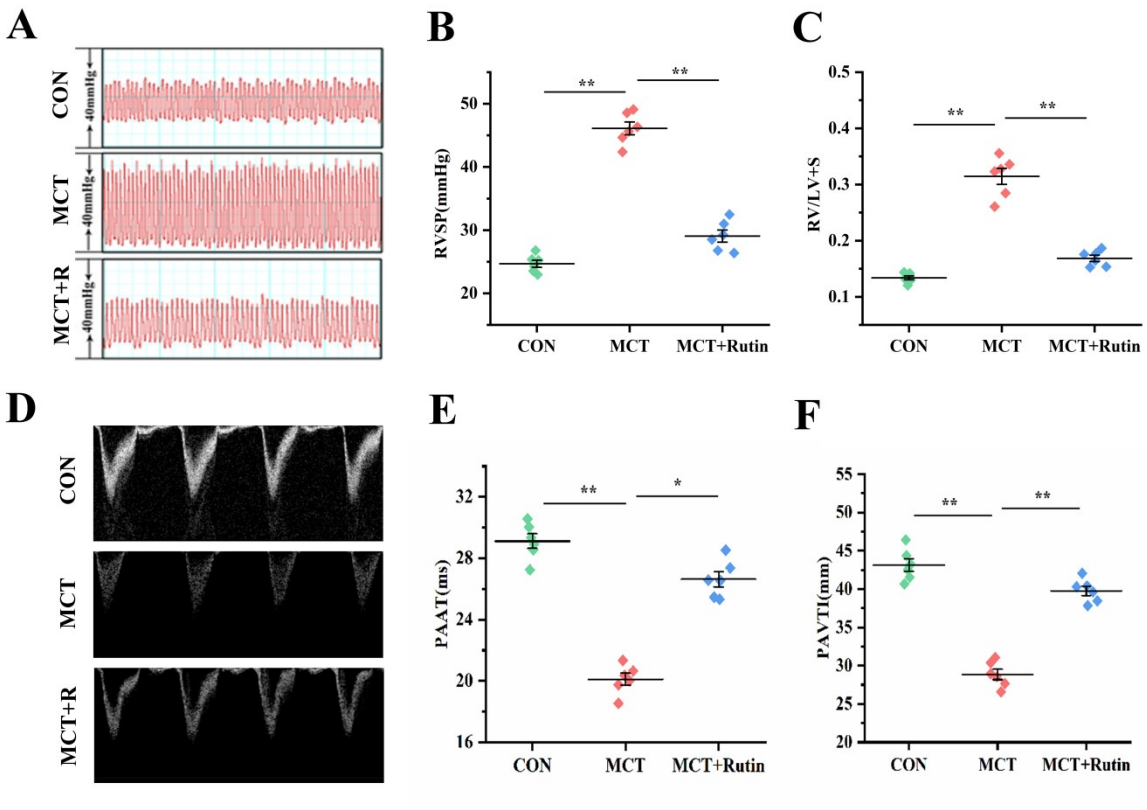
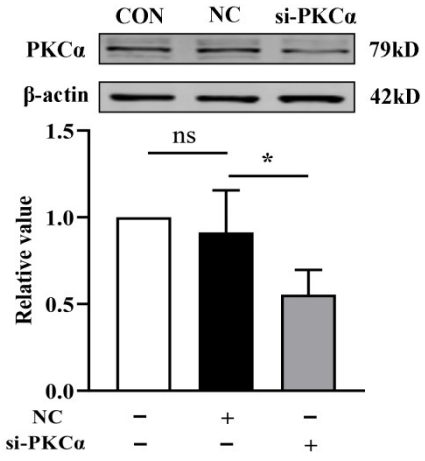
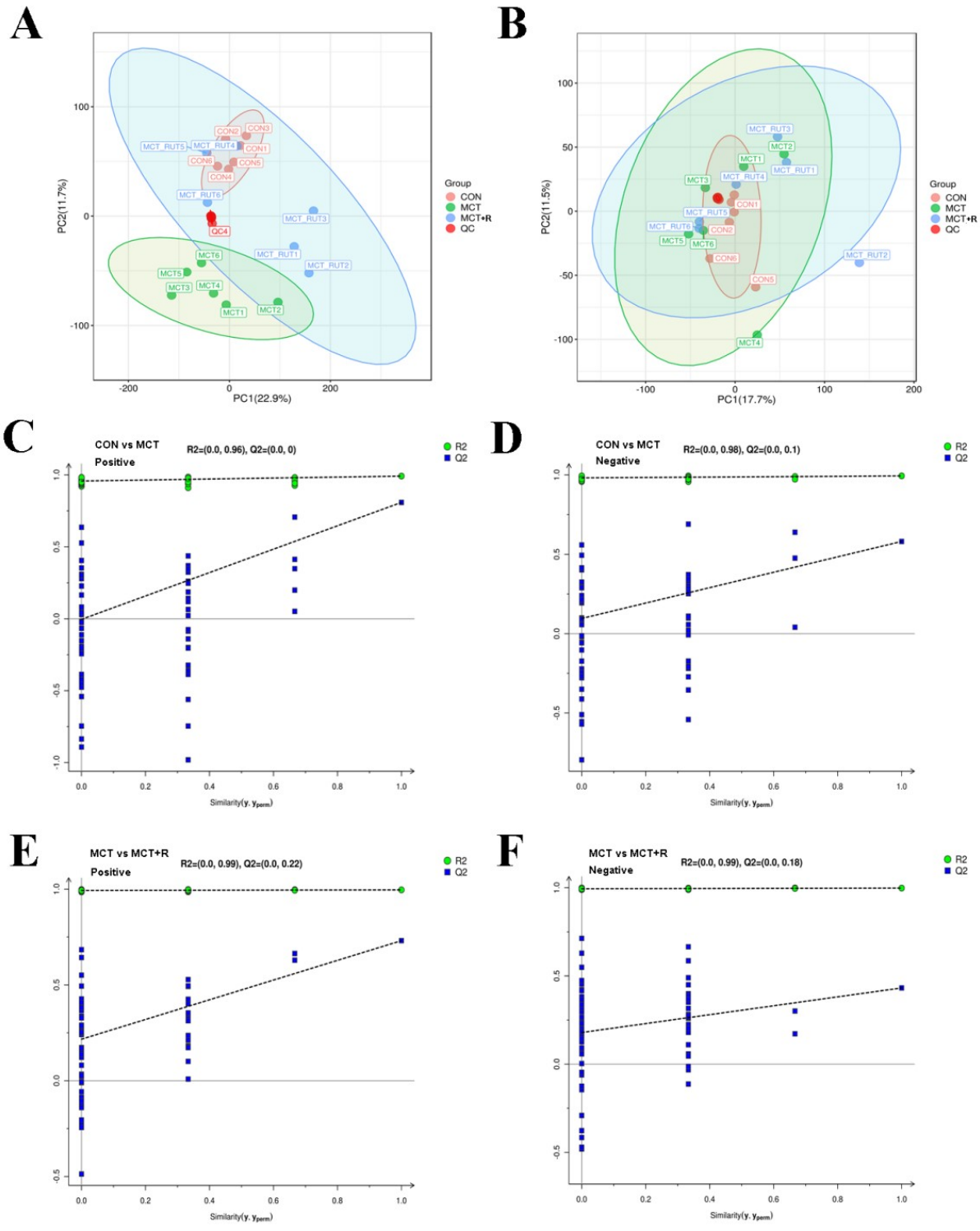


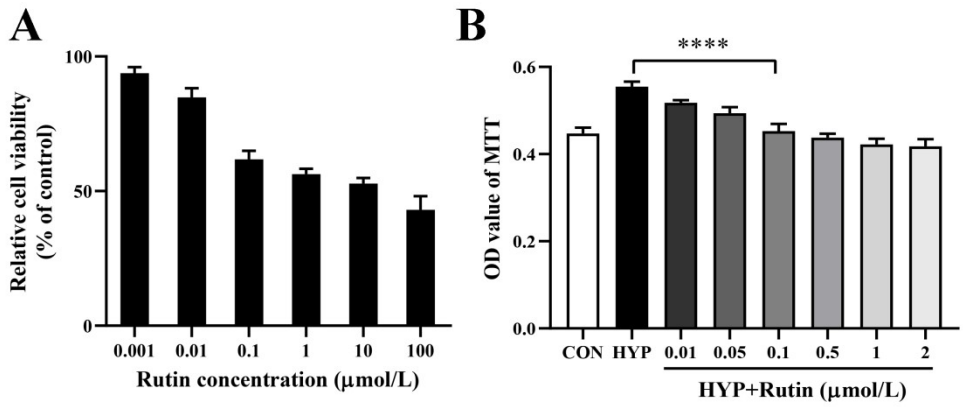
Supplementary Figure 1. Rutin played a preventive role in the progression of PAH. (A-B) The tracings and comparison of RVSP between groups. (C) The statistics of RV/LV + S ratio between groups. Echocardiographic images (D) and bar graph of PAAT (E) and PAVTI (F) in PAH rats.**P<0.01, *P<0.05. CON, control; MCT, monocrotaline; MCT+R, monocrotaline + rutin.



Supplementary Figure 2. The knockdown efficiency of PKCα.



Supplementary Figure 3. Multivariate analyses of mitochondria samples from CON, MCT and MCT+R groups PCA score plots of the three groups in the (A) positive ion mode and (B) negative ion mode. (C-F) Permutation test for the OPLS-DA model. CON, control; MCT, monocrotaline; MCT+R, monocrotaline + rutin; QC, quality control.



Supplementary Figure 4. Determination of concentration of rutin used for PASMCs. (A) MTT assay the IC50 value of the rutin in PASMCs. (B) The numbers of PASMCs after administering various concentrations of rutin. CON, control; HYP, hypoxia. ****P < 0.0001 vs. HYP.

Supplementary Table 1. LC parameters for detection of mitochondria samples

UHPLC	Parameters
Column	ACQUITY UPLC ® HSS T3 (150 × 2.1 mm, 1.8 μm)
Column temperature	40 °C
Flow rate	0.25 mL/min
Injection volume	2 μL
Mobile phases	A: 0.1% formic acid in water (v/v)
	B: 0.1% formic acid in acetonitrile (v/v)
	C: ammonium formate (5 mM)
	D: acetonitrile
Gradient profile	(time (min), A/C(%)): (0, 98), (1,98), (9,50), (12, 2), (13.5, 2), (14, 98), (20, 98)

Supplementary Table 2. MS/MS parameters for detection of mitochondria samples

MS/MS	Parameters
Sheath gas pressure	30 arb
Aux gas flow	10 arb
Spray voltage	3.50 kV and -2.50 kV for ESI(+) and ESI(-)
Capillary temperature	325 °C
MS1 range	m/z 100-1000
MS1 resolving power	60000 FWHM
Number of data dependant scans per cycle	4
MS/MS resolving power	15000 FWHM
Normalized collision energy	30%
Dynamic exclusion time, automatic.	Automatic

Supplementary Table 3. KEGG pathway enrichment analysis of CON vs. MCT group.

Pathways	Hits	P value
Glutathione metabolism	2	0.011032
Alanine, aspartate and glutamate metabolism	2	0.011032
Arginine and proline metabolism	2	0.019889
Aminoacyl-tRNA biosynthesis	2	0.030935
Nitrogen metabolism	1	0.035314
D-Glutamine and D-glutamate metabolism	1	0.035314
Ascorbate and aldarate metabolism	1	0.058236
Arginine biosynthesis	1	0.080673
Butanoate metabolism	1	0.086207
Histidine metabolism	1	0.091712

Supplementary Table 4. KEGG pathway enrichment analysis of MCT vs. MCT+R group.

Pathways	Hits	P value
Glutathione metabolism	2	0.011032
Alanine, aspartate and glutamate metabolism	2	0.011032
Arginine and proline metabolism	2	0.019889
Aminoacyl-tRNA biosynthesis	2	0.030935
Nitrogen metabolism	1	0.035314+
D-Glutamine and D-glutamate metabolism	1	0.035314
Ascorbate and aldarate metabolism	1	0.058236
Arginine biosynthesis	1	0.080673
Butanoate metabolism	1	0.086207
Histidine metabolism	1	0.091712

Supplementary Table 5. The top 10 of GO enrichment analysis of common targets between rutin and glutamate metabolism.

GO term	Subgroup	Enrichment score
protein phosphorylation	Biological Processes	32.71
positive regulation of cell migration	Biological Processes	25.23
regulation of kinase activity	Biological Processes	20.56
cellular response to nitrogen compound	Biological Processes	20.56
regulation of ion transport	Biological Processes	20.56
regulation of proteolysis	Biological Processes	20.56
regulation of cellular catabolic process	Biological Processes	19.63
regulation of system process	Biological Processes	18.69
positive regulation of protein localization	Biological Processes	17.76
cell activation	Biological Processes	17.76
perinuclear region of cytoplasm	Cellular Components	15.89
side of membrane	Cellular Components	14.95
postsynapse	Cellular Components	13.08
axon	Cellular Components	12.15
membrane raft	Cellular Components	11.21
centrosome	Cellular Components	9.35
basal plasma membrane	Cellular Components	8.41
transferring phosphorus-containing groups	Cellular Components	8.41
cell projection membrane	Cellular Components	8.41
vesicle lumen	Cellular Components	7.48
protein kinase activity	Molecular Functions	29.91
kinase binding	Molecular Functions	23.36
oxidoreductase activity	Molecular Functions	18.69
protein homodimerization activity	Molecular Functions	15.89
protein domain specific binding	Molecular Functions	14.02
hydrolase activity, acting on ester bonds	Molecular Functions	12.15
phosphatase binding	Molecular Functions	9.35
carbonate dehydratase activity	Molecular Functions	8.41
calcium-dependent protein kinase C activity	Molecular Functions	7.48
serine hydrolase activity	Molecular Functions	7.48

Supplementary Table 6. The top 20 of KEGG pathway enrichment analysis of common targets between rutin and glutamate metabolism.

Pathways	enrichment	Count
Pathways in cancer	21.5	23
PI3K-Akt signaling pathway	15.89	17
Pathways of neurodegeneration - multiple diseases	11.21	12
AGE-RAGE signaling pathway in diabetic complications	10.28	11
Lipid and atherosclerosis	10.28	11
Inflammatory mediator regulation of TRP channels	9.35	10
Chemical carcinogenesis - reactive oxygen species	9.35	10
Human papillomavirus infection	9.35	10
Nitrogen metabolism	8.41	9
cGMP-PKG signaling pathway	7.48	8
Human T-cell leukemia virus 1 infection	7.48	8
Platelet activation	6.54	7
Ovarian steroidogenesis	5.61	6
Adherens junction	5.61	6
Arachidonic acid metabolism	4.67	5
Alcoholic liver disease	4.67	5
Adrenergic signaling in cardiomyocytes	4.67	5
Antifolate resistance	3.74	4
Acute myeloid leukemia	3.74	4
Folate biosynthesis	2.8	3