

Additional file

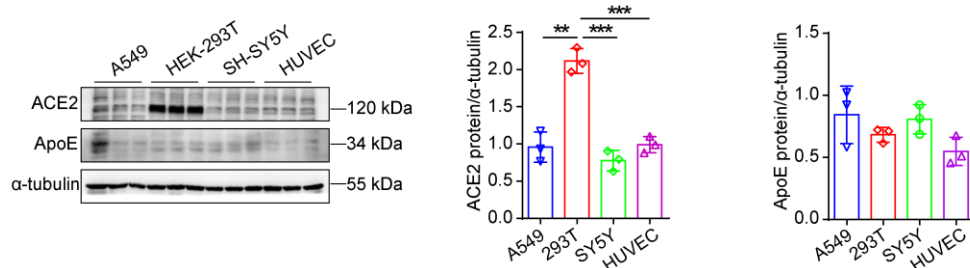


Fig. S1 Expression of ApoE and ACE2 *in vitro*. Representative western blotting analysis of the ApoE and ACE2 protein levels in A549, HEK-293T, SH-SY5Y, and HUVECs; the data are shown as the mean $\pm$ SD of three independent experiments. α -Tubulin was used as a loading control. *P* values were calculated using one-way ANOVA, **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

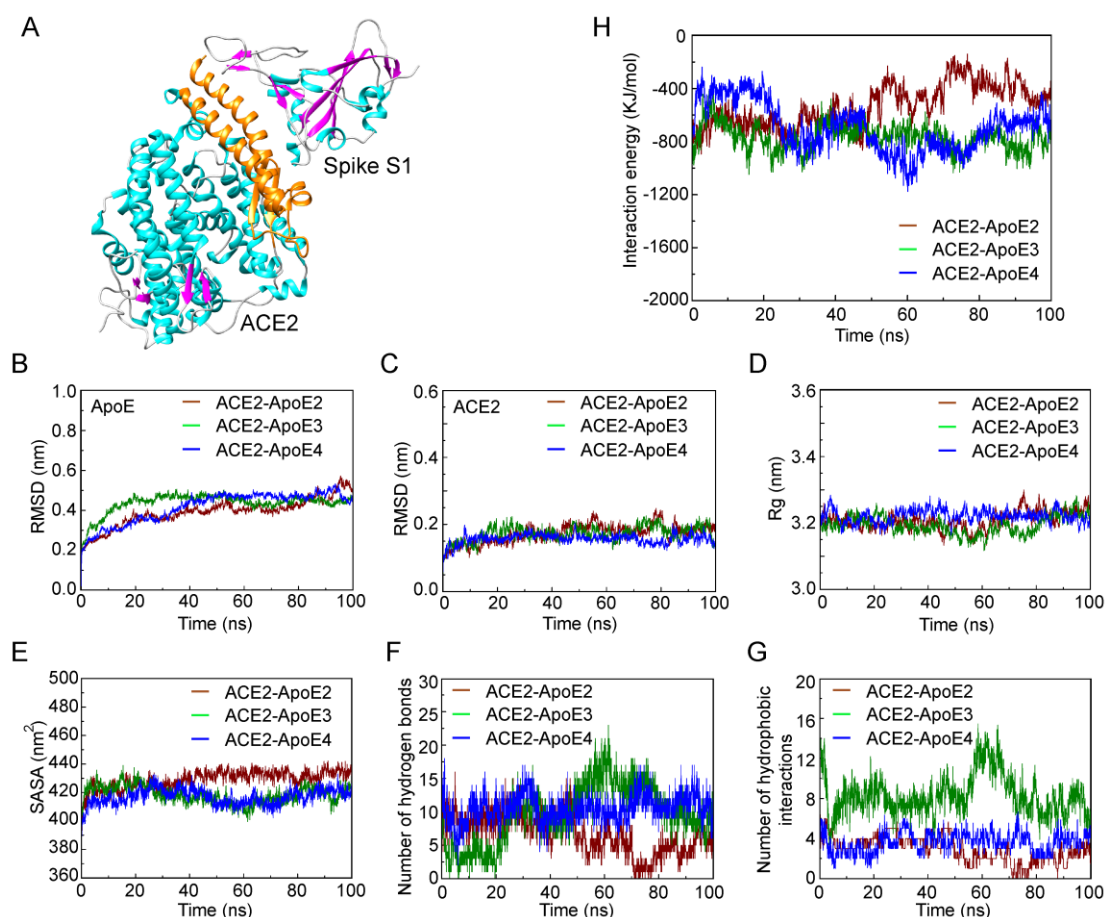


Fig. S2 Molecular docking and simulation analyses of the interaction between ApoE and ACE2. **A** Molecular docking simulation of the SARS-CoV-2 RBD interacting with ACE2 (the orange region of the ACE2 protein represents the region that binds to the spike S1 protein). **B** and **C** Plot of backbone RMSD versus time (ns) for ApoE and ACE2. **D** Plot of Rg versus time (ns) for ApoE-ACE2. **E** Plot of total SASA versus time (ns) for ApoE-ACE2 complexes. **F** Number of hydrogen bonds between ApoE and ACE2. **G** Number of hydrophobic interactions between ApoE and ACE2. **H**

Interaction energy between ACE2 and ApoE. Brown: ApoE2-ACE2, Green: ApoE3-ACE2 and Blue: ApoE4-ACE2.

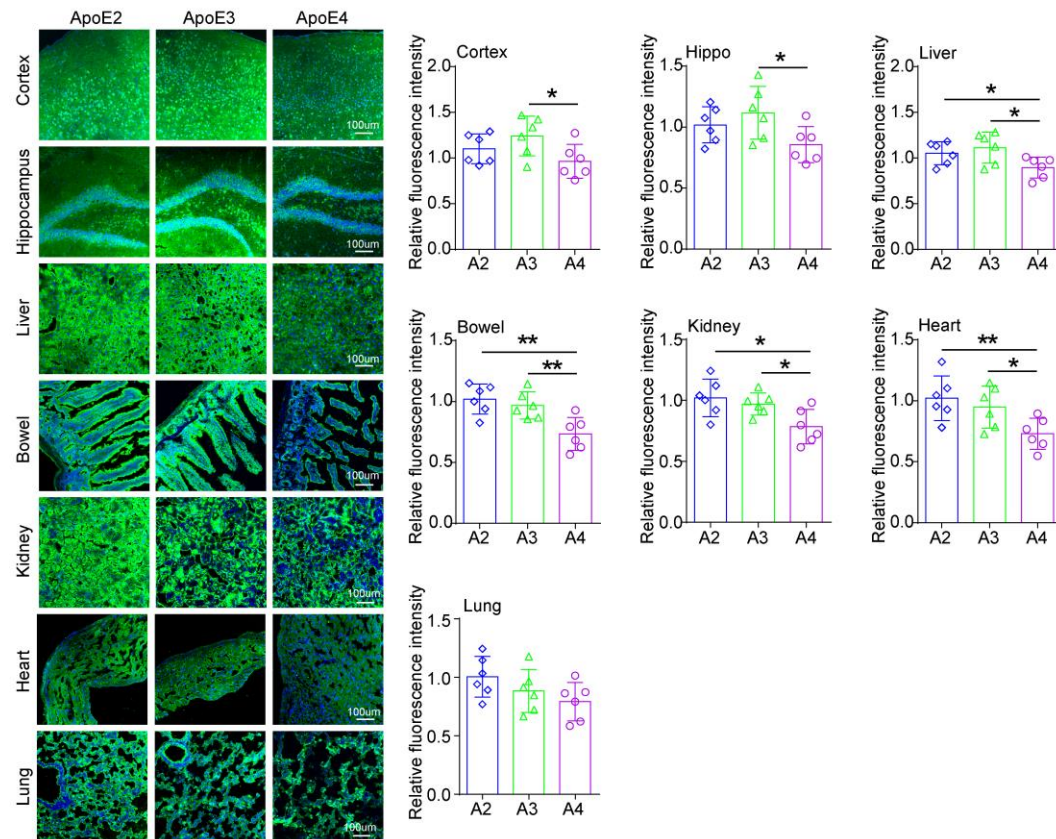


Fig. S3 ApoE4 downregulates ACE2 protein expression *in vivo*. ACE2 protein levels in the cortex, hippocampus, liver, bowel, spleen, kidney, heart and lung of ApoE2-TR, ApoE3-TR, and ApoE4-TR mice were assessed by immunofluorescence staining. The results were normalized to the expression of a-tubulin. n = 6 mice per group. The sections were stained with an anti-ACE2 (green) antibody and counterstained with DAPI (blue). The data are expressed as the mean $\pm$ SD. Statistical differences were evaluated by one-way ANOVA. Scale bars, 100 μ m. * p < 0.05; ** p < 0.01; *** p < 0.001.

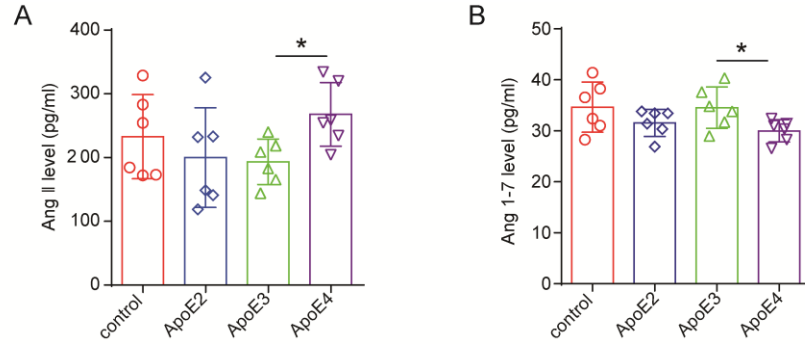


Fig. S4 ApoE4 regulates Ang II and Ang 1-7 protein expression in vitro. Expression of the Ang II **A** and Ang 1-7 **B** proteins in HEK-293T cells as shown by ELISA after transfection with 1 μ g/ml Flag, ApoE2-Flag, ApoE3-Flag or ApoE4-Flag plasmids for 48 h. The data are expressed as the mean $\pm$ SD. One-way ANOVA tests were used. * p <0.05.

Table S1. Characteristics of the included studies.

Study (author, reference)	Ethnity	Gender (M/F)		Genotypes						Phenotype
				ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	
Al-Jaf, 2021 (29)	Iraq	48/57	control	0	14	0	92	8	0	risk
			case	0	10	3	77	15	0	
Kuo, 2020 (8)	UK	14997/ 176951	control	-	-	-	223056	90285	8985	risk
			case	-	-	-	401	184	37	
Lord, 2022 (30)	Farmanf ar-mayan	122/79	control	2	7	2	83	7	0	risk
			case	1	10	1	61	13	14	
Kuo, 2020 (9)	England	142909/ 174667	control	-	-	-	219084	88561	8708	risk
			case	-	-	-	663	321	59	
Kurki, 2021 (11)	Finns	1021/ 1590	control	APOE4+ (APOE4/E4 + APOE4/E3 + APOE4/E2) = 832						risk
			Case in ICU	APOE4+ (APOE4/E4 + APOE4/E3 + APOE4/E2) = 24						
Hubacek, 2021 (31)	Czech	185/223	control	APOE4+ (APOE4/E4 + APOE4/E3 + APOE4/E2) = 475						risk
			case	APOE4+ (APOE4/E4 + APOE4/E3 + APOE4/E2) = 88						
Hubacek, 2021 (31)	Czech	185/223	asymptomatic	APOE4+ (APOE4/E4 + APOE4/E3 + APOE4/E2) = 29						severity
			symptomatic	APOE4+ (APOE4/E4 + APOE4/E3 + APOE4/E2) = 88						
Kuo, 2020 (9)	England	142909/ 174667	case	-	-	-	219084	88561	8708	severity
			case with dead	-	-	-	79	42	13	
del Ser, 2021 (32)	Spain	323/590	asymptomatic	APOE4+ (APOE4/E4 + APOE4/E3 + APOE4/E2) = 140						severity
			symptomatic	APOE4+ (APOE4/E4 + APOE4/E3 + APOE4/E2) = 20						
Kuo, 2022 (8)	England	3595/ 3830	case	-	-	-	941	358	64	severity
			Case with delirium	-	-	-	52	43	13	
Tavares-Júnior, 2022 (34)	Brazil	52/89	control	0	2	0	37	8	1	severity
			Case with cognitive impairment	0	2	0	16	6	1	

Table S2. Newcastle-Ottawa Scale to assess quality of the involved studies.

Study (first author, year, reference)	Selection			Comparability			outcome		Total score
	Adequate	Representa-	Selection	Definition	Control of	Ascertainment	Same method of	Non-Response	
	case	tiveness of	of control	of control	important	of exposure	ascertainment for	rate	
	definition	the cases			confusion factors		cases and controls		
Al-Jaf, 2021 (29)	☆	☆	☆	☆	–	☆	☆	☆	7
Kuo, 2020 (8)	☆	☆	☆	☆	☆	☆	☆	☆	8
Lord, 2022 (30)	☆	☆	☆	☆	☆	☆	☆	☆	8
Hubacek, 2021 (31)	☆	☆	☆	☆	–	☆	☆	☆	7
Kuo 2020 (9)	☆	☆	☆	☆	☆	☆	☆	☆	8
del Ser 2021 (32)	☆	☆	☆	☆	☆	☆	☆	☆	8
Kuo 2022 (33)	☆	☆	☆	☆	☆	☆	☆	☆	8
Kurki 2021 (11)	☆	☆	☆	☆	☆	☆	☆	☆	8
Tavares-Júnior 2022 (34)	☆	☆	☆	☆	–	☆	☆	☆	7

☆ Represents one point.

Table S3. Kinetic parameters of ApoE and ACE2 calculated by BLI.

Sample	Loading Sample	Concentration (nM)	Response	KD (M)	Kon (1 Ms)	Kdis (1/s)	Full R ²
ApoE2	ACE2	5882	0.0914	6.73E-07	3.42E+03	2.30E-03	0.93
		2941	0.0503				
		1471	0.0353				
		735.3	0.0242				
ApoE3	ACE2	5882	0.0896	7.08E-07	6.42E+03	4.54E-03	0.92
		2941	0.0614				
		1471	0.0436				
		735.3	0.0268				
ApoE4	ACE2	5882	0.1058	1.07E-06	2.85E+03	3.05E-03	0.93
		2941	0.064				
		1471	0.0421				
		735.3	0.0303				

Table S4. Kinetic parameters of ApoE and SARS-CoV-2 (RBD) calculated by BLI.

Sample	Loading Sample	Concentration (nM)	Response	KD (M)	Kon (1 Ms)	Kdis (1/s)	Full R ²
ApoE2	SARS-Cov-2	5882	0.1713	1.71E-06	1.00E+04	1.72E-02	0.93
		2941	0.1229				
		1471	0.0905				
		735.3	0.0637				
		367.6	0.0606				
ApoE3	SARS-Cov-2	5882	0.1679	5.89E-07	1.22E+04	7.18E-03	0.91
		2941	0.1183				
		1471	0.0884				
		735.3	0.0569				
		367.6	0.0562				
ApoE4	SARS-Cov-2	5882	0.1476	4.88E-07	12530	0.00611	0.89
		2941	0.1074				
		1471	0.0779				
		735.3	0.0583				
		367.6	0.0539				