

Supplementary Materials for

**NR4A1 Deficiency promotes Carotid Plaque Vulnerability by
activating Integrated Stress Response via targeting Bcat1**

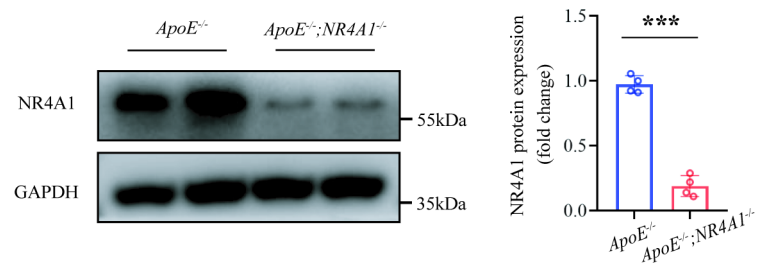


Figure S1. NR4A1 is ablated in macrophages in *ApoE*^{-/-};NR4A1^{-/-} mice.

BMDMs were isolated from *ApoE*^{-/-} and *ApoE*^{-/-};NR4A1^{-/-} mice. The protein level of NR4A1 was determined by western blot. p values were determined using student's t-test. For all panels, *p < 0.05; **p < 0.01; ***p < 0.001.

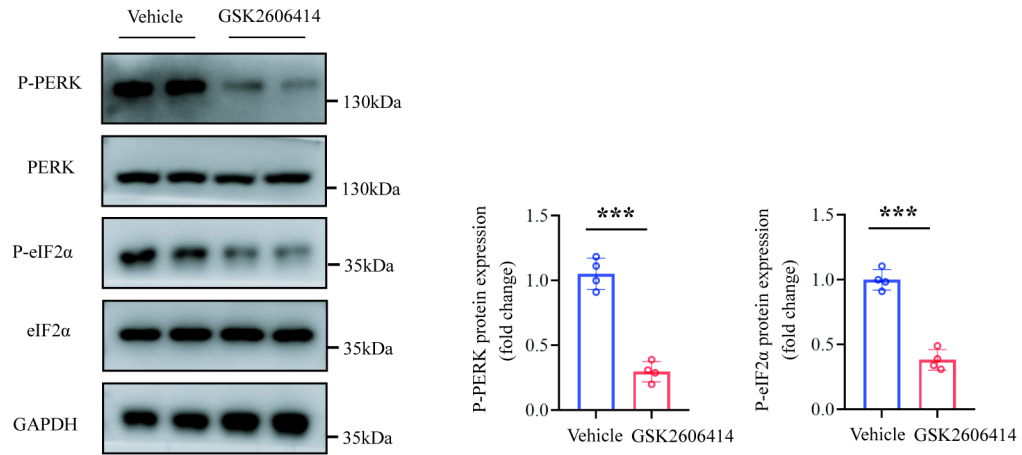


Figure S2. GSK2606414 significantly suppresses the activation of PERK and eIF2α in macrophage.

BMDMs were treated with vehicle or GSK2606414 (2 μmol/L) for 12 hours. The protein levels of PERK, P-PERK, eIF2α, and P-eIF2α were determined by western blot. p values were determined using student's t-test. For all panels, *p < 0.05; **p < 0.01; ***p < 0.001.

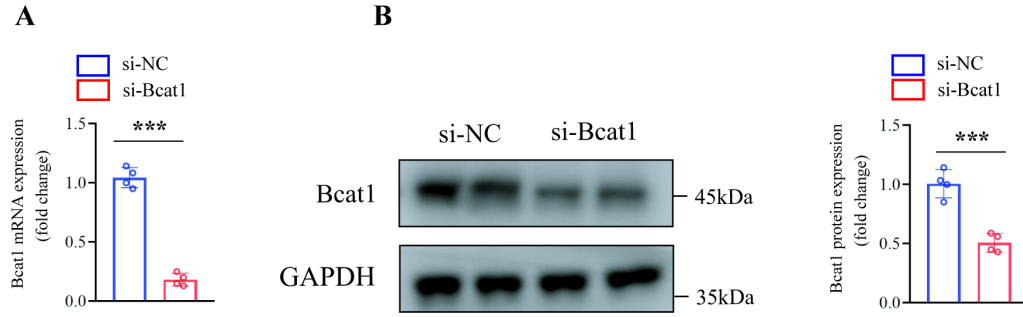


Figure S3. The knockdown efficiency of Bcat1 is verified.

A and B, BMDMs were transfected with control siRNA (si-NC) and Bcat1 siRNA (si-Bcat1) for 48 hours. Bcat1 mRNA and protein levels were determined by real-time quantitative polymerase chain reaction (RT-qPCR, A) and western blot (B). p values were determined using student's t-test. For all panels, *p < 0.05; **p < 0.01; ***p < 0.001.

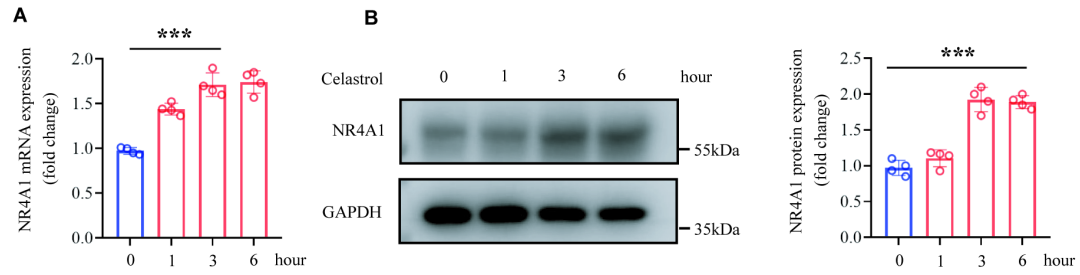


Figure S4. Celastrol induces a time-dependent increase of NR4A1 expression in macrophages.

A and B, BMDMs were treated vehicle or celastrol (0.3 μ M) for the indicated time periods (0, 1, 3, and 6 hour). NR4A1 mRNA and protein levels were determined by RT-qPCR (A) and western blot (B). p values were determined using student's t-test. For all panels, *p < 0.05; **p < 0.01; ***p < 0.001.

Table S1. The primer sequences for qPCR in this study.

Gene	Primer	Sequence (5'-3')	Application
Gapdh	Forward	AGGTCGGTGTGAACGGATTTG	RT-qPCR
	Reverse	TGTAGACCATGTAGTTGAGGTCA	RT-qPCR
NR4A1	Forward	TGTCCGCTCTGGTCCTCATCAC	RT-qPCR
	Reverse	TCTCCTGCCACGGTAGCCATG	RT-qPCR
Bcat1	Forward	GAGCCAGAGGTCGGA CTGTA	RT-qPCR
	Reverse	CTCTTTCTCCAGCAAACGGC	RT-qPCR
TNF- α	Forward	TCCCAGGTTCTCTTCAAGGGA	RT-qPCR
	Reverse	GGTGAGGAGCACGTAGTCGG	RT-qPCR
MCP-1	Forward	ACGCCCCACTCACCTGCTG	RT-qPCR
	Reverse	CCTGCTGCTGGTGATCCTCTTG	RT-qPCR
IL-6	Forward	GCCTTCTTGGGACTGATGCT	RT-qPCR
	Reverse	GGTCTGTTGGGAGTGGTATCC	RT-qPCR
IL-1 β	Forward	GATGGCTGCACTATTCCTAATGCC	RT-qPCR
	Reverse	ATGGCTCTGAGAGACCTGACTTG	RT-qPCR

Table S2. Si-RNA sequences.

Gene		Sequence (5'-3')
Bcat1	Forward	GCUUCUACAGAU CGACCAA
	Reverse	UUGGUCGAUCUGUAGAAGC