Ganglioside GA2-mediated caspase-11 activation drives macrophage pyroptosis aggravating intimal hyperplasia after arterial injury

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Supplementary Table

Table S1. Demographic and baseline characteristics of the patients.

Parameter	Total (n=16)	CTL (n=8)	CHD (n=8)	P-value
Age,median(IQR),years	61.0(44.5-69.5)	57.5(44.5-69.5)	64.5(62.0-68.0)	0.4210
Men,n(%)	9(56.2)	4(50)	5(62.5)	>0.9999
SBP,mean±SD,mmHg	130.20±20.27	132.80±24.21	127.6±16.70	0.6298
DBP,mean±SD,mmHg	78.75±13.07	83.50±14.02	74.00±10.86	0.1520
HR,mean±SD,beats/min	75.19±8.57	79.00±9.957	71.38±4.984	0.0732
Laboratory Findings				
WBC,median(IQR),*10^9/L	6.43(5.10-7.83)	6.84(5.28-8.35)	6.01(5.10-6.43)	0.3669
PLT,mean±SD,*10^9/L	180.40±53.11	153.0±44.81	207.8±48.20	0.0338
FBG,mean±SD,mmol/L	5.04±0.59	4.913±0.4486	5.175±0.6541	0.3651
ALT,mean±SD,U/L	27.19±14.54	22.63±13.63	31.75±14.83	0.2208
Cr,mean±SD,μmol/L	85.29±27.34	73.88±25.36	96.71±25.70	0.0952
LDL-C,mean±SD,μmol/L	2.35±0.48	2.231±0.4843	2.468±0.4690	0.3384
Baseline medications, n (%)				
ACEI/ARB	9(56.25)	5(62.50)	4(50.00)	>0.9999
CCB	6(37.50)	3(37.50)	3(37.50)	>0.9999
β-blocker	10(62.50)	3(37.50)	7(87.50)	0.1189
Antiplatelets	3(18.75)	0(0.00)	3(37.50)	0.2000
P2Y12 inhibition	5(31.25)	0(0.00)	5(62.50)	0.0256
Statins	12(75.00)	4(50.00)	8(100.00)	0.0769
Comorbidities, n (%)				
Diabetes	0(0.00)	0(0.00)	0(0.00)	-
Hypertension	7(43.75)	3(37.50)	4(50.00)	>0.9999
Atrial fibrillation	0(0.00)	0(0.00)	0(0.00)	-
Stroke	0(0.00)	0(0.00)	0(0.00)	-

Values are mean ± SD, median (IQR) or n (%). SD: standard deviation; IQR: interquartile range; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; WBC: white blood cell; PLT: platelet; FBG: fast blood glucose; ALT: alanine transaminase; Cr: creatinine; LDL-C: low-density lipoprotein cholesterol; ACEI/ARB: angiotensin-converting enzyme inhibitor or angiotensin receptor blocker; CCB: calcium-channel blocker; β-blocker: β-receptor blocker. P2Y12 inhibition: clopidogrel, prasugrel, ticagrelor.

Supplementary Figures

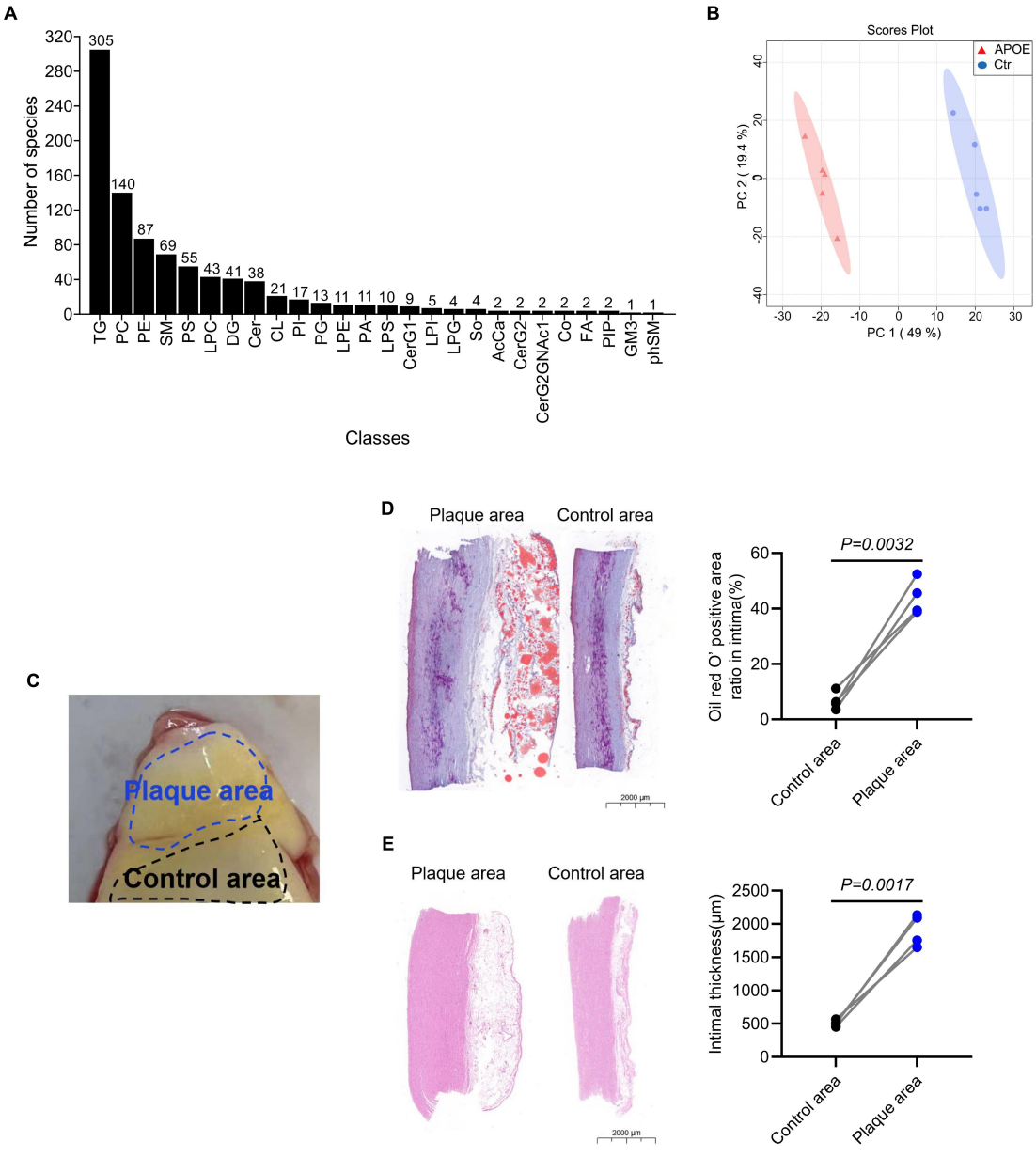

Figure S1. (A) Total lipidomic classes and species were identifed in mouse aortae. **(B)** Scores scatter plot corresponding to a principal component analysis of the Lipidomics concentration in the aortae of mice (n = 5). Wild type (WT) mice fed a normal laboratory diet for 16 weeks served as control (Ctr) group. APOE$^{-/-}$ mice fed high-fat high-cholesterol diet for 16 weeks served as APOE group. **(C)** Representative image of atherosclerotic lesions in the human thoracic aorta. **(D)** Oil-red O staining in plaque and non-plaque areas in human arteries (N=4 patients). Scale bars: 2 mm. **(E)** Intimal thickness by hematoxylin and eosin-staining (H&E) in plaque and non-plaque areas in human arteries (N=4 patients). Scale

bars: 2 mm. **D** and **E** was tested using a two-tailed paired *t*-test.

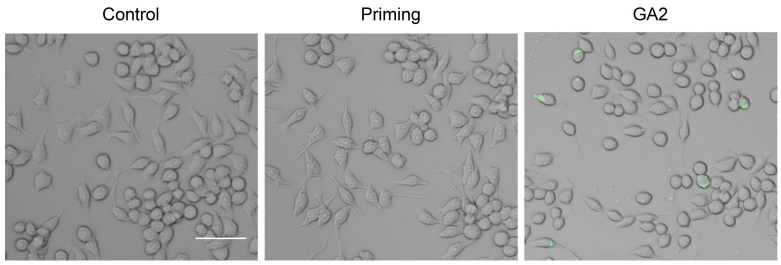

Figure S2. The endocytosis of ganglioside GA2 in RAW264.7 cells.

The endocytosis of ganglioside GA2 (GA2) by inflammatory macrophages (n = 3). Green indicates the GA2. Priming cells were treated with 4 μg/mL fluorescent probe labeled-GA2 for 8 h. Priming or non-priming cells stimulated with equimolar DMSO were set as a priming group or a control group. Scale bars: 50 μm

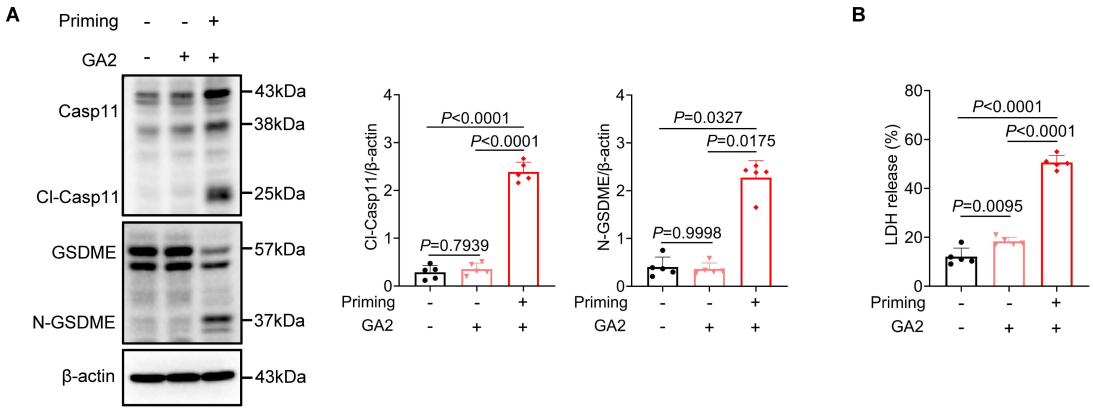

Figure S3. GA2 triggers pyroptosis in the priming mouse macrophages.

(A) Representative immunoblots of Casp11, Cl-Casp11, GSDME and N-GSDME in RAW264.7 cells and analysis results (n = 5). Priming or non-priming cells were transfected with 6 μg GA2 for 20 h. Non-priming cells transfected with equimolar DMSO were set as a control group. **(B)** LDH release of RAW264.7 cells (n = 5). **A** (Cl-Casp11) and **B** were tested using a One-way ANOVA test; **A** (N-GSDME) was tested using a Kruskal Wallis test.

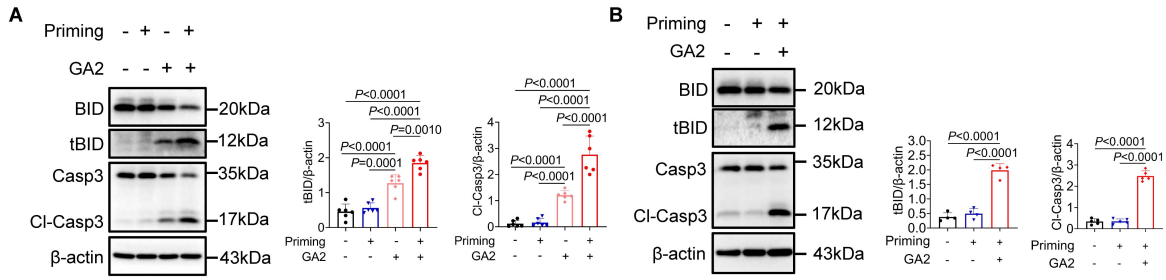

Figure S4. GA2 induces Casp3 and BID activation in macrophages.

(A) Representative immunoblots of BID, tBID, Casp3 and Cl-Casp3 in HMDM cell lysates and analysis results (n = 6). **(B)** Representative immunoblots of BID, tBID, Casp3 and Cl-Casp3 in RAW264.7 cells and analysis results (n = 5). **A** (tBID) and **B** (Cl-Casp3) were tested using a One-way ANOVA test; **A** (Cl-Casp3) and **B** (tBID) were tested using a Welch's ANOVA test.