SUPPLEMENTAL INFORMATION:

Oxidized high-density lipoprotein induces macrophage apoptosis via toll-like receptor 4-dependent CHOP pathway

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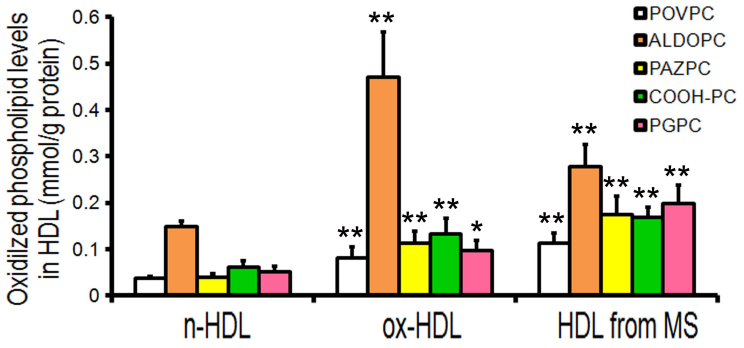
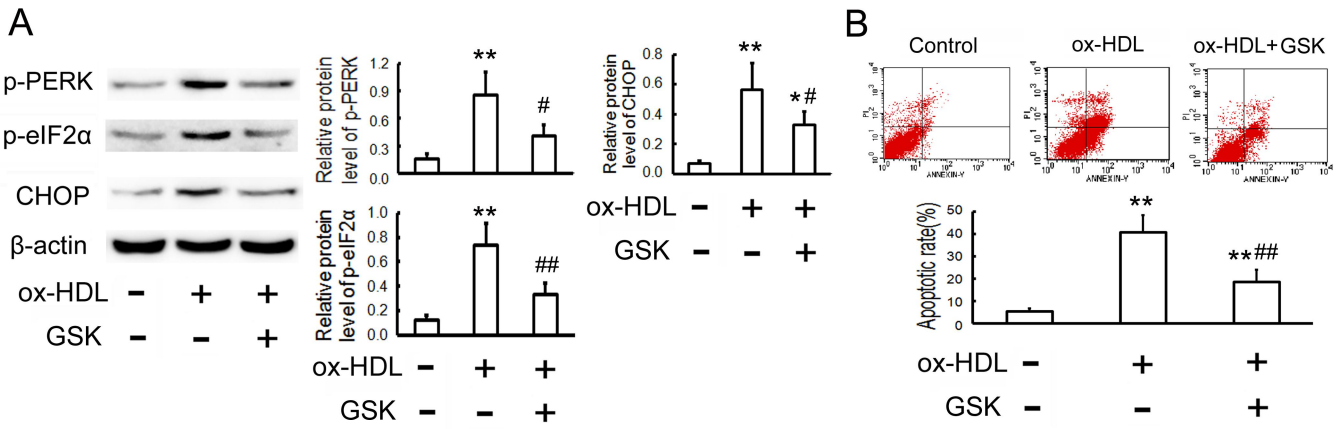
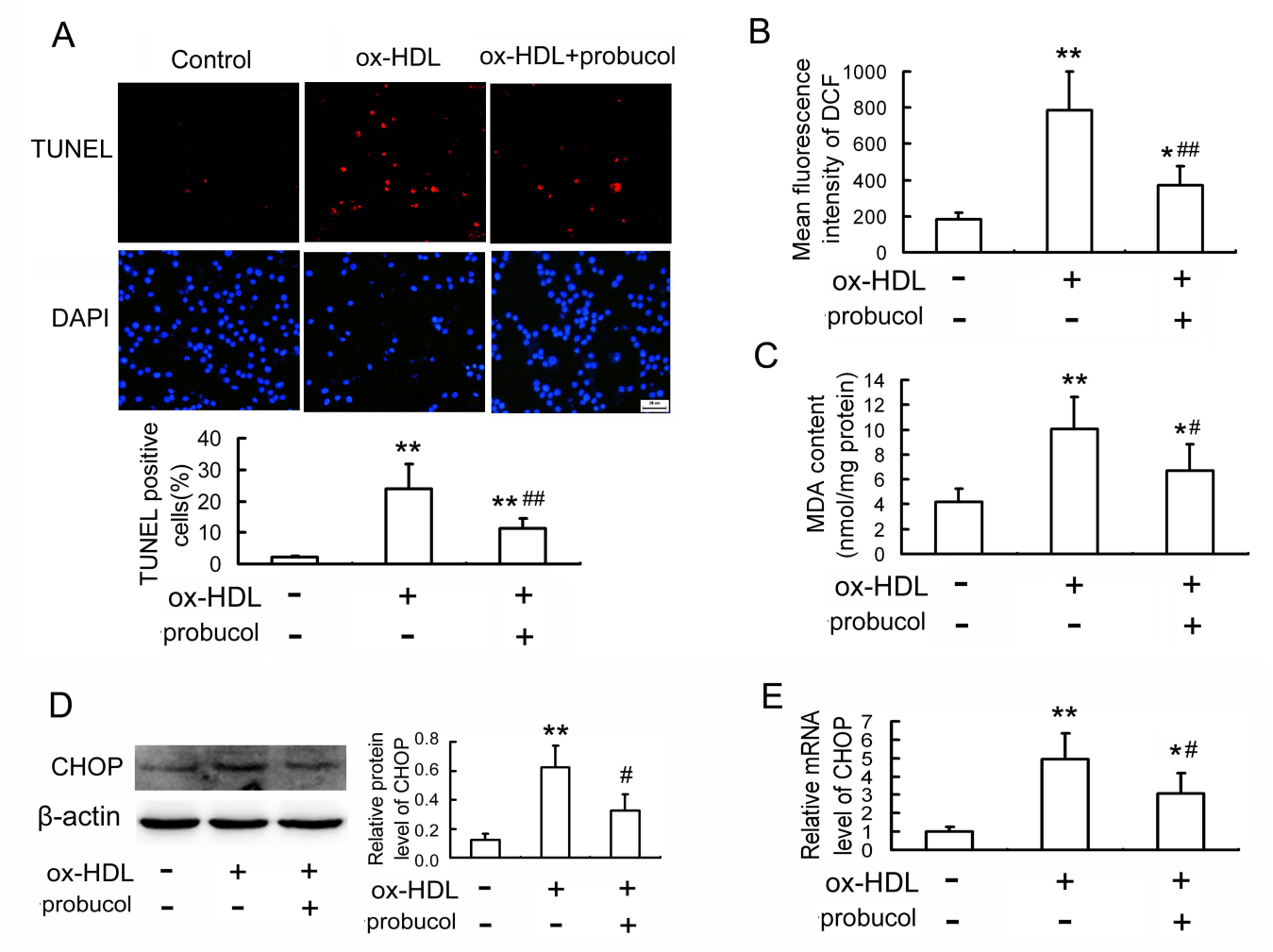


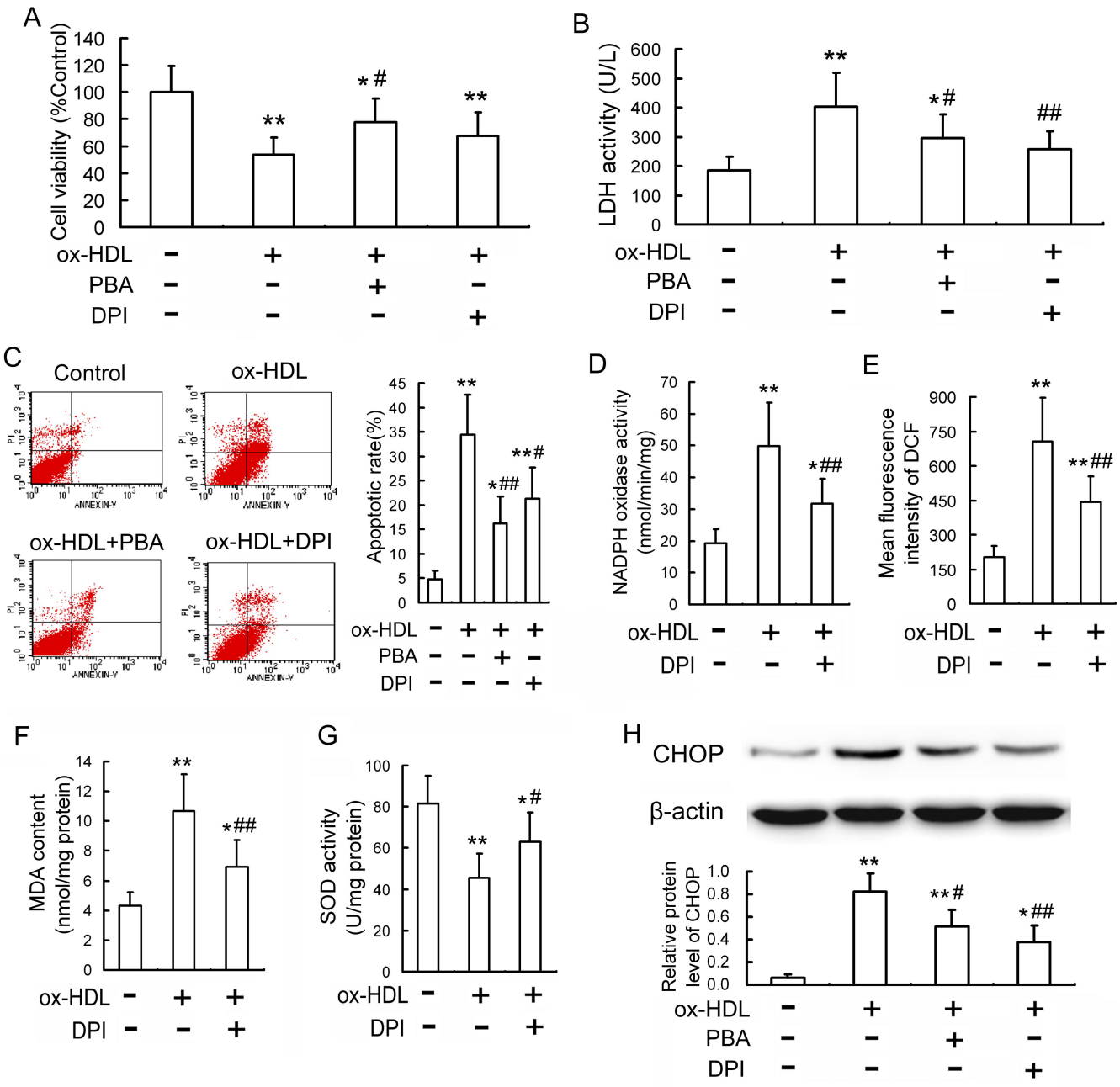
Supplemental Figure S1: Liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis of oxidized phosphatidylcholines in n-HDL, ox-HDL and HDL from metabolic syndrome (MS) patients. Data are expressed as the mean ± SD of six independent experiments. **P*<0.05, ***P*<0.01 versus n-HDL. POVPC, 1-palmitoyl-2-(5'-oxo-valeroyl)-*sn*-glycero-3-phosphocholine; ALDOPC, 1-palmitoyl-2-(9'-oxo-nonanoyl)-*sn*-glycero-3-phosphocholine; PAZPC, 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine; COOH-PC, 1-hexadecyl-2-azelaoyl-*sn*-glycero-3-phosphocholine; PGPC, 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine.



Supplemental Figure S2: GSK2606414 inhibits CHOP-mediated macrophage apoptosis induced by ox-HDL. RAW264.7 cells were pretreated with or without 40 nmol/L GSK2606414 (PERK inhibitor) for 1 h, and then stimulated with ox-HDL (100 mg/L) for 24 h. The protein levels of ER stress markers and apoptosis were determined by Western blot (A) and flow cytometry (B), respectively. Data are expressed as the mean ± SD of at least three independent experiments. **P*<0.05, ***P*<0.01 versus control group; #*P*<0.05, ##*P*<0.01 versus ox-HDL group.

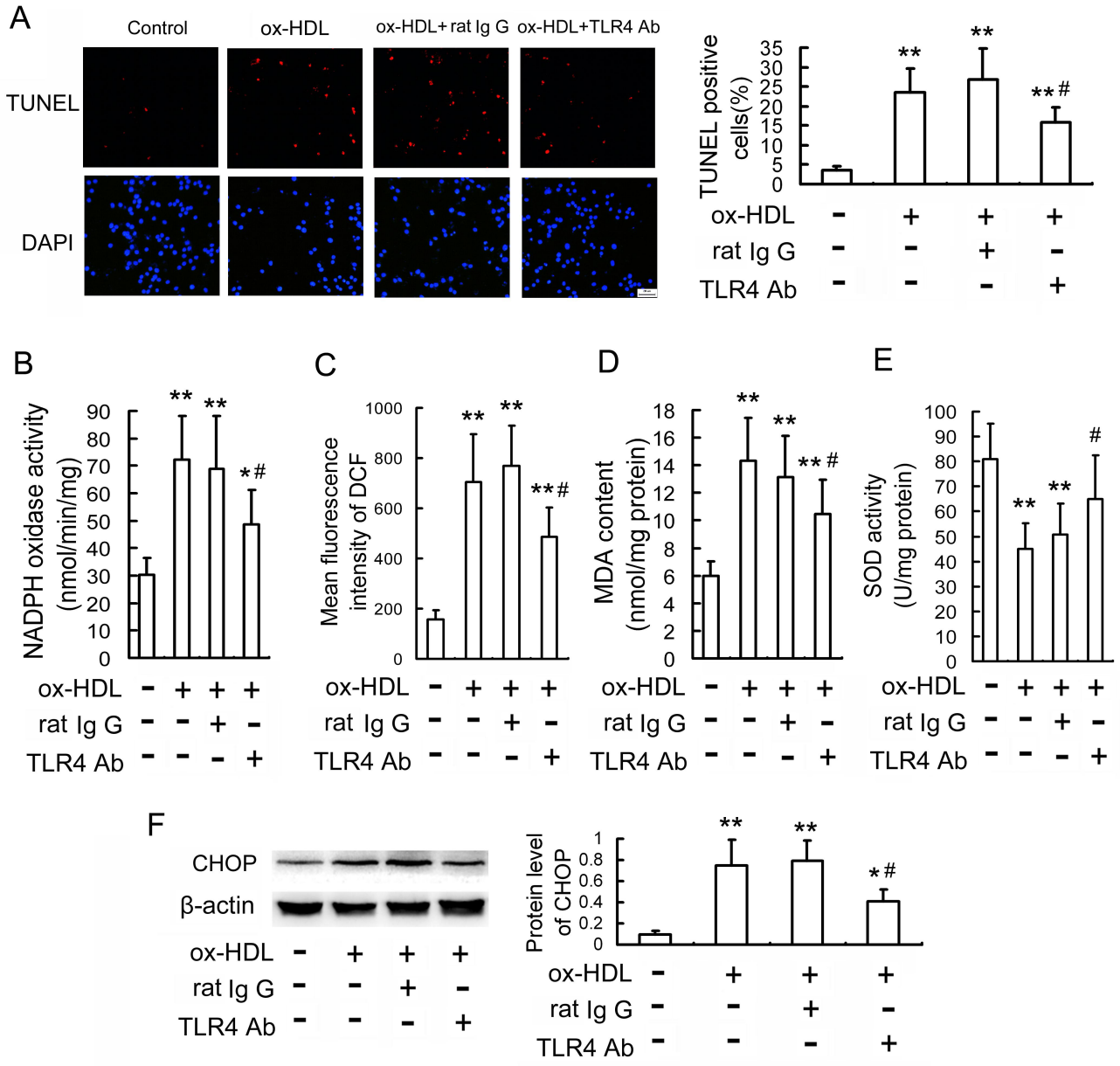


Supplemental Figure S3: Probucol inhibits CHOP-mediated macrophage apoptosis induced by ox-HDL. RAW264.7 cells were pretreated with or without 50 μmol/L probucol (a ROS scavenger) for 1 h and then stimulated with ox-HDL (100 mg/L) for 24 h. (A) Cell apoptosis was measured by TUNEL assay. Scale bar =20 μm. (B) Intracellular ROS levels were measured by DCF analysis using a flow cytometer. (C) MDA content was determined using commercial kits. (D and E) The protein and mRNA levels of CHOP were determined by Western blot and quantitative real-time PCR, respectively. Data are expressed as the mean ± SD of at least three independent experiments. **P*<0.05, ***P*<0.01 versus control group; #*P*<0.05, ##*P*<0.01 versus ox-HDL treatment.

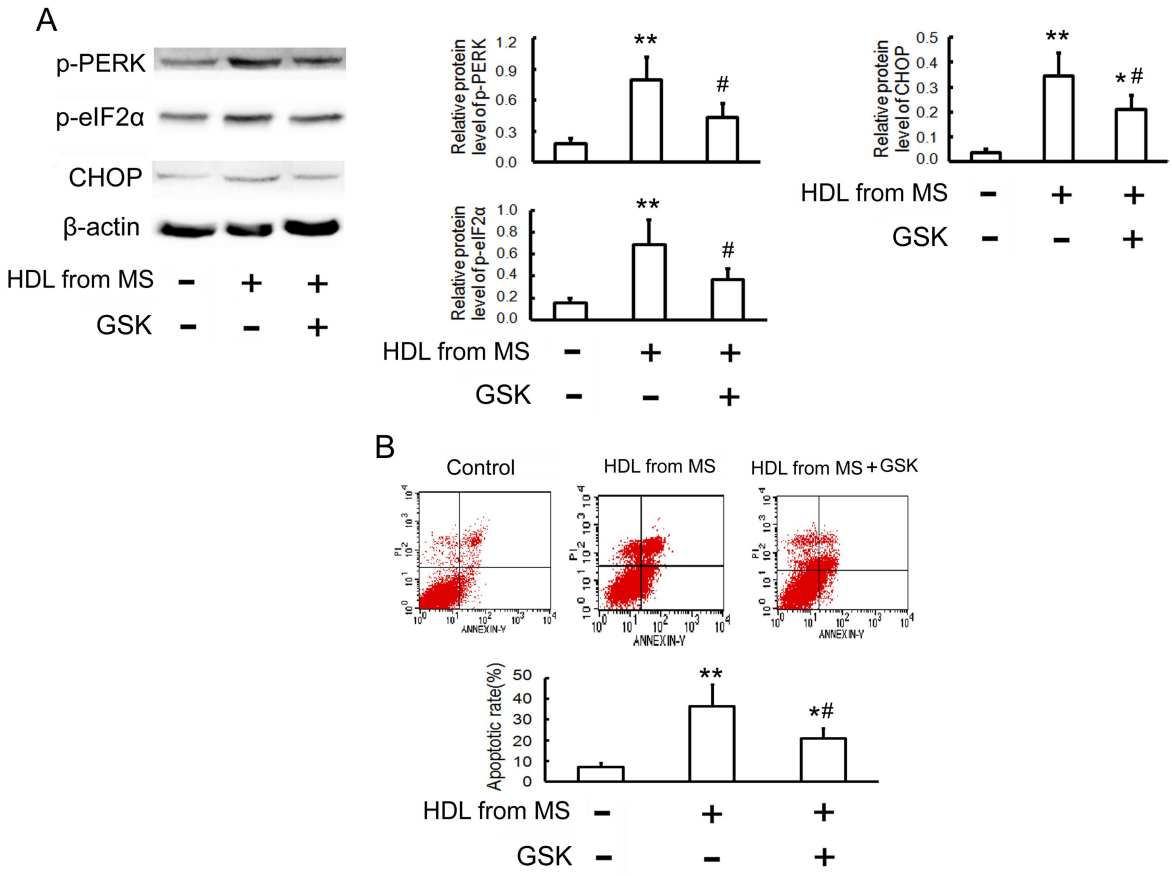


Supplemental Figure S4: Ox-HDL induces oxidative stress and CHOP-mediated apoptosis in mouse peritoneal macrophages. Peritoneal macrophages from C57BL/6J mice were harvested with PBS 3 days after intraperitoneal injection with 1 ml of 4% thioglycollate and maintained in DMEM with 10% FBS. Cells were pretreated with or without PBA (5 mmol/L) or DPI (5 μmol/L) for 1 h, and then stimulated with ox-HDL (100 mg/L) for 24 h. Cell viability (A) and LDH activity in media (B) were determined by MTT assay and a kit, respectively. (C) Cell apoptosis was detected using flow cytometry and the total apoptotic cells were represented by the right side of the panel (Annexin V staining alone or together

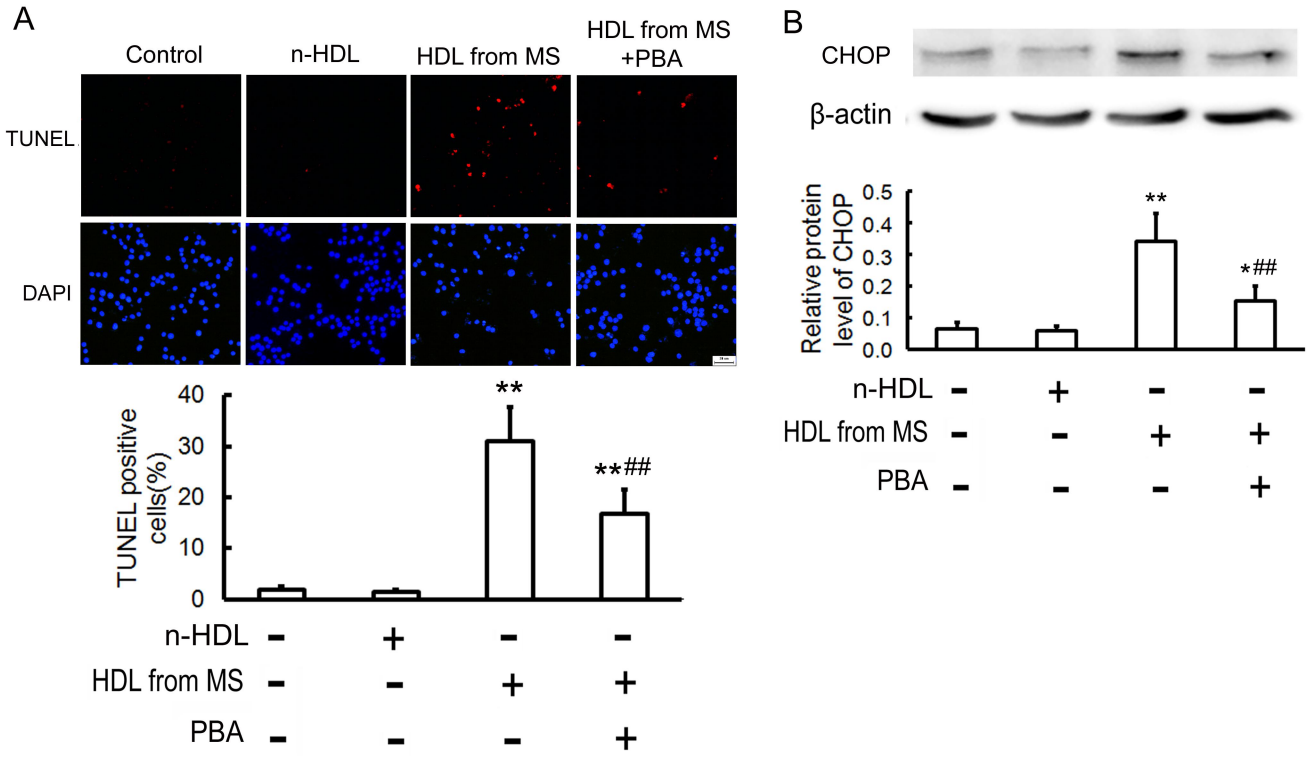
with PI). (D) NADPH oxidase activity was determined by cytochrome C chromometry. (E) Intracellular ROS levels were measured by DCF analysis using a flow cytometer. (F and G) MDA content and SOD activity were determined using commercial kits. (H) Western blot analysis of CHOP. Data are expressed as the mean ± SD of at least three independent experiments. **P*<0.05, ***P*<0.01 versus control group; #*P*<0.05, ##*P*<0.01 versus ox-HDL treatment.



Supplemental Figure S5: Anti-TLR4 antibody inhibits ox-HDL-induced oxidative stress and CHOP-mediated macrophage apoptosis. RAW264.7 cells were preincubated with 2 mg/L of anti-TLR4 antibody (TLR4 Ab) or rat isotype IgG for 30 min and then treated with ox-HDL (100 mg/L) for 24 h. (A) Cell apoptosis was measured by TUNEL assay. Scale bar =20 μm. (B) NADPH oxidase activity was determined by cytochrome C chromometry. (C) Intracellular ROS levels were measured by DCF analysis using a flow cytometer. (D and E) MDA content and SOD activity were determined using commercial kits. (F) CHOP protein level was determined by Western blot. Data are expressed as the mean ± SD of at least three independent experiments. **P*<0.05, ***P*<0.01 versus control group; #*P*<0.05 versus ox-HDL treatment.



Supplemental Figure S6: GSK2606414 inhibits CHOP-mediated macrophage apoptosis induced by HDL from MS patients. RAW264.7 cells were pretreated with or without 40 nmol/L GSK2606414 (PERK inhibitor) for 1 h, and then stimulated with HDL from MS patients (100 mg/L) for 24 h. The protein levels of ER stress markers and apoptosis were determined by Western blot (A) and flow cytometry (B), respectively. Data are expressed as the mean ± SD of at least three independent experiments. **P*<0.05, ***P*<0.01 versus control group; #*P*<0.05 versus HDL from MS patients treatment.



Supplemental Figure S7: HDL from MS patients induces macrophage apoptosis and CHOP upregulation. HDL samples were isolated from individual fasting plasma of six MS patients and six healthy subjects, respectively. RAW264.7 cells were pretreated with or without PBA (5 mmol/L) for 1 h, followed by stimulation with HDL from MS patients (100 mg/L) or HDL from healthy subjects (n-HDL, 100 mg/L) for 24 h. (A) Cell apoptosis was measured by TUNEL assay. Scale bar =20 μm. (B) CHOP protein level was analyzed by Western blot. Data are expressed as the mean ± SD of six independent experiments. *P<0.05, **P<0.01 versus control group; ##P<0.01 versus treatment with HDL from MS patients.

Supplemental Table S1: Clinical and biochemical characteristics of metabolic syndrome patients and healthy controls.

	Metabolic syndrome	Healthy controls	p
Age, years	49.5 ± 9.8	43.8 ± 8.6	0.02
Waist circumference, cm	101.3 ± 13.6	76.6 ± 7.2	<0.01
Body mass index, kg/m^2	28.5 ± 4.0	21.6 ± 2.8	<0.01
Systolic blood pressure, mmHg	145.6 ± 21.7	128.5 ± 12.8	<0.01
Diastolic blood pressure, mmHg	86.2 ± 11.8	81.6 ± 9.1	0.096
Glucose, mmol/L	7.08 ± 2.06	5.12 ± 0.57	<0.01
Triglycerides, mmol/L	1.93 ± 0.65	0.96 ± 0.23	<0.01
HDL-cholesterol, mmol/L	1.24 ± 0.32	1.49 ± 0.35	<0.01
LDL-cholesterol, mmol/L	2.80 ± 0.82	2.48 ± 0.56	0.076

Data are expressed as the mean ± SD. Differences between metabolic syndrome group and control group were tested using the Student's t test.