Supplementary materials

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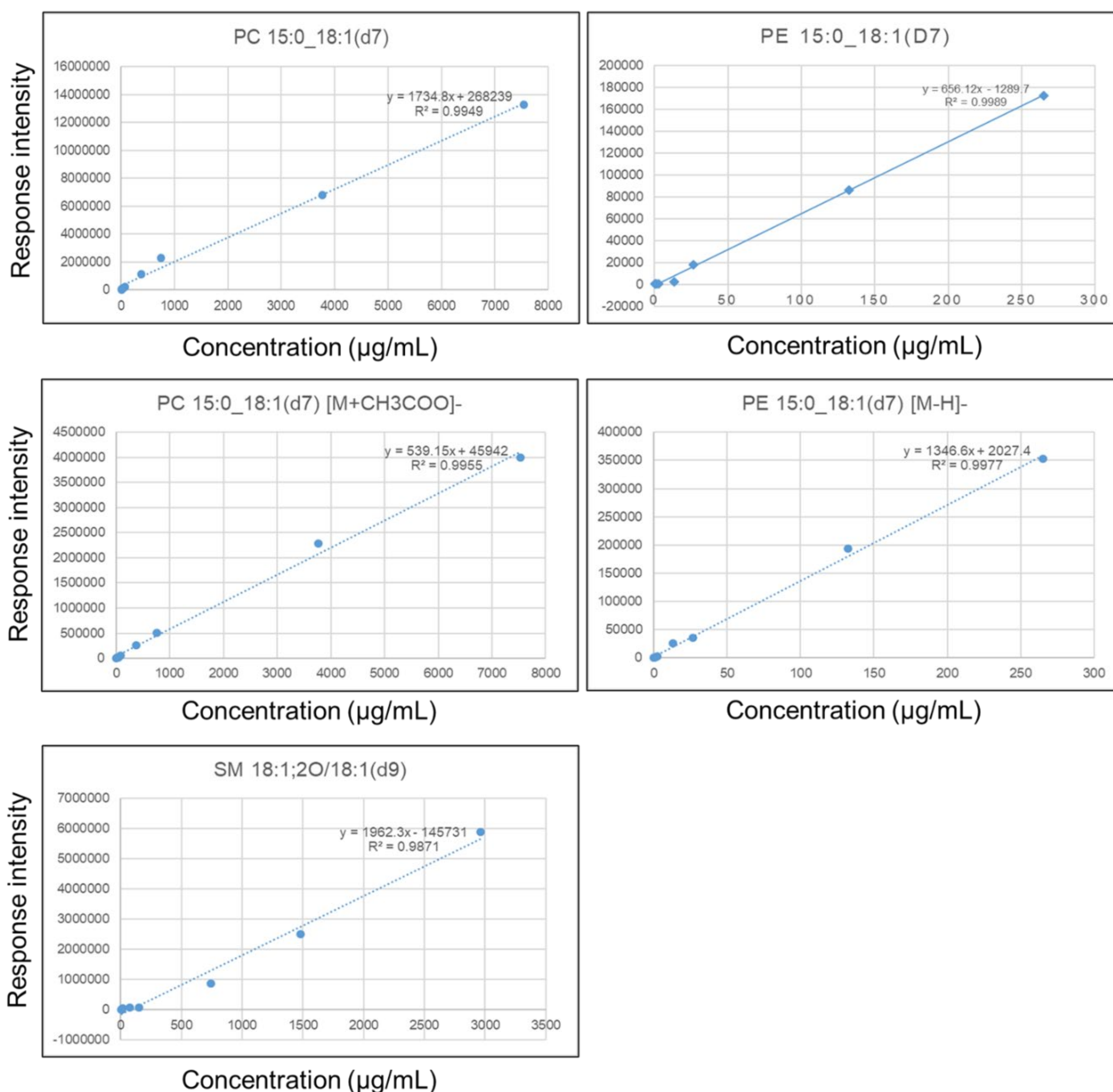

Fig. S1. Linearity plot of internal standards (phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin).

Standards were spiked into pooled QC samples at their respective concentrations to generate response curves. The calibration curves were constructed from eight different gradient concentrations of each standard, and the correlation coefficients were > 0.95. For lipids, for which calibration curves are difficult to apply, signal intensities were used for statistical comparison.

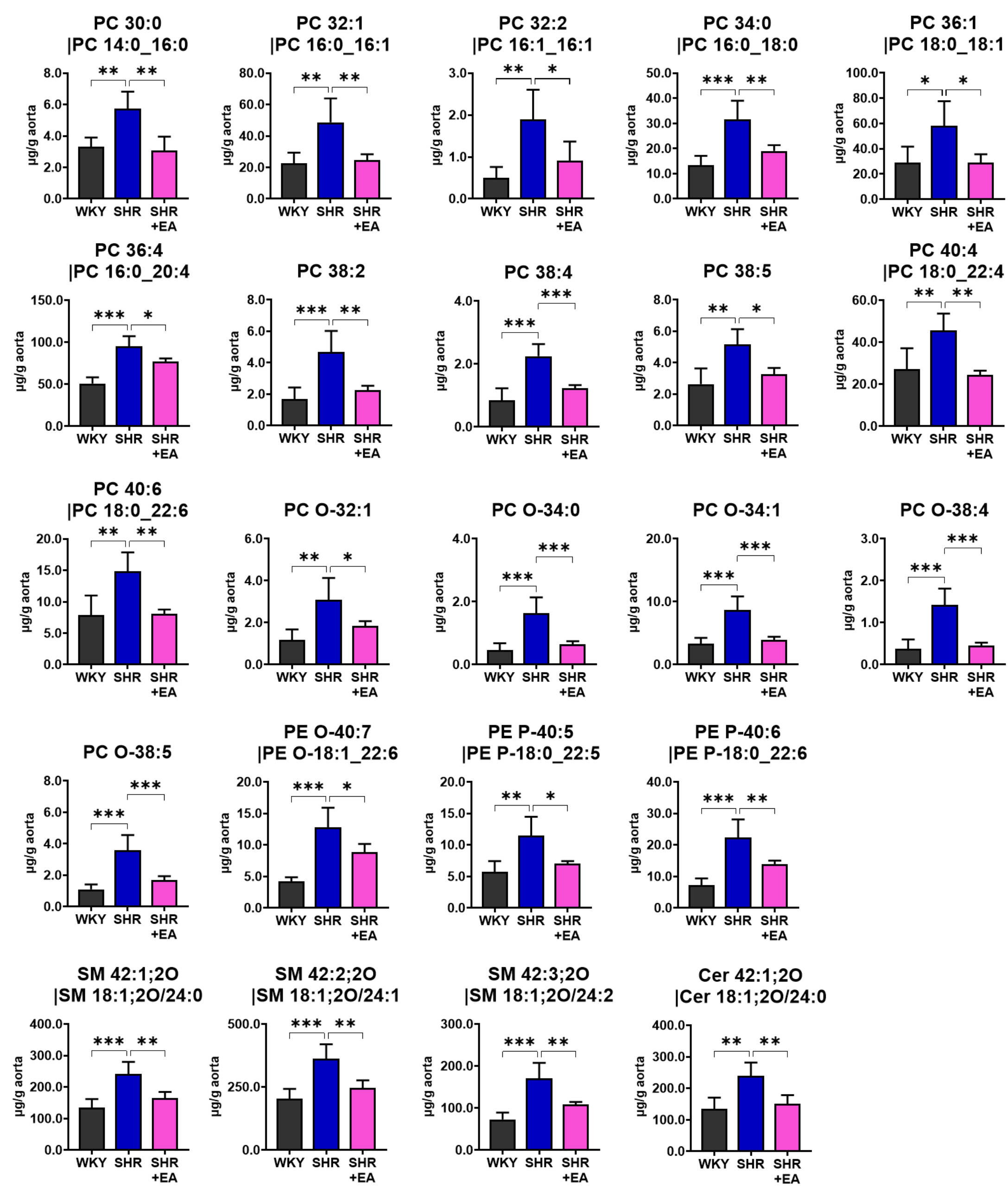

Fig. S2. **Semi-quantification results of significantly altered lipids by the EA treatment in SHR.**

Table S1. Operational conditions and parameters of the UHPLC-Q-TOF MS system

LC conditions			
Instrument system	1290 Infinity II UHPLC system (Agilent)		
Column	ACQUITY CSH C18 Column, 2.1×100 mm, 1.7 μm (Waters)		
Column oven temperature	35 ℃		
Injection volume	1 μL		
Flow rate	0.25 mL/min		
Sequential gradient mobile phase system	Time (min)	A (%)	B (%)
	0.0	60	40
	5.0	35	65
	12.0	30	70
	15.0	1	99
	17.0	1	99
	17.1	60	40
	20.0	60	40
MS conditions			
Sheath gas temperature and flow	350 ℃ and 11 L/min		
Gas temperature and drying gas flow	325 ℃ and 8 L/min		
Fragmentor and skimmer voltage	125 and 65 V		
Capillary and nozzle voltage	3500 and 1000 V		

Table S2. PCR primer sequences

Gene	Primer sequence
Cers2	sense: 5´-CCG ATT ACC TGC TGG AGT CAG-3´
	anti-sense: 5´-GGC GAA GAC GAT GAA GAT GTT G-3´
Gnpat	sense: 5´- CGC ATAGGA GCC ATT CGG TT-3´
	anti-sense: 5´-AGT GGT GGA CTC CTT CGG CT-3´;
Actb	sense: 5´-ATT GGC AAT GAG CGG TTC C-3´
	anti-sense: 5´-GGT AGT TTC GTG GAT GCC ACA-3´

Method S1. Immunostaining for hypertension-related neuronal signal analysis

For immunohistochemistry, the brain tissues were washed in PBS containing 0.1% Triton X-100 (PBST) and endogenous peroxidase inactivated by 3% H_2O_2 for 20 min. The tissue samples were then incubated with 1% bovine serum albumin (BSA, BOVOGEN, Williams Ave, Keilor East VIC, Australia) at room temperature (RT) for 1 h and reacted at 4 °C overnight with the primary antibodies as follows: c-Fos (1:250; Abcam, Cambridge, MA, USA) and angiotensin-converting enzyme (ACE) (1:100, Santacruz, Dallas, TX, USA). After washing, the samples were incubated with biotinylated secondary antibody (anti-mouse IgG (H+L), 1:500 for c-Fos and ACE, Vector Laboratories, Burlingame, CA, USA) at RT for 1 h. The tissues were incubated with ABC reagent (Vector Laboratories, Burlingame, CA, USA) and then reacted with a DAB kit (Vector Laboratories, Burlingame, CA, USA). Dehydration was then performed in a gradient of 70-100% ethyl alcohol and 100% xylene. The stained brain sections were mounted using Permount solution (Fisher chemical, Hampton, NH, USA) and then observed under microscope (Nikon, Minato, Japan). The number of stained cells in each brain region was counted within a square of 300 × 300 µm, and the mean values of the left and right regions were calculated.

For immunofluorescence, brain tissues were washed with PBST and then incubated with 1% BSA at RT for 1 h. The brain tissues were reacted with primary antibodies against AT1 (1:50, Enzo Life sciences, Farmingdale, NY, USA) and AT2 (1:50, Enzo Life sciences, Farmingdale, NY, USA) at 4 °C overnight, incubated with goat anti-rabbit IgG secondary antibodies (1:500 for AT1 and AT2; Alexa Fluor 488; Invitrogen, Waltham, Massachusetts, USA) at RT for 2 h, and then mounted using VECTASHIELD mounting medium stained with 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA). The brain tissues were observed using a fluorescence microscope (Nikon, Minato, Japan).

Method S2. Sample preparation for lipid profiling

Ten milligrams of aortic vessels were transferred to a 2 mL Precellys lysing tube containing zirconium oxide beads and homogenized twice at 5000 rpm for 20 s with 700 μL of a cold methanol-water mixture (1:1, v/v) using a tissue grinder (Precellys 24, Bertin Technologies, Montigny-le-Bretonneux, France), and 700 μL of cold chloroform. After vortexing for 1 min, the mixture was rested for 15 min at 4 °C, followed by centrifugation for 20 min (13,200 rpm, 4 °C). The bottom layer was separated, transferred to a 1.5 mL tube, and evaporated using a speed vacuum concentrator (Hanil Science Medical, Gwangju, Republic of Korea). The resulting lipid pellet was reconstituted with 200 μL of an isopropanol-acetonitrile mixture (4:1, v/v).