Supplemental methods

Animals

Male C57BL/6J WT mice, aged 8 weeks, were purchased from CLEA Japan (Osaka, Japan) and used as control. cPLA$\alpha^{-/-}$ were generated as previously reported$^{15}$. All the experiments used mice aged 8 weeks unless otherwise indicated. As homozygous null females have a parturition defect, breeding was carried out using heterozygote pairs. The genotyping was determined by polymerase chain reaction (PCR) as described in supplemental data. The experimental protocols were approved by the Institutional Animal Care and Use Committee, Faculty of Medicine, Tottori University.

Bone marrow transplantation (BMT) experiments

To investigate whether cPLA$_2$$\alpha$ in blood cells participates in BP elevation induced by L-NAME treatment, we carried out BMT between the WT and cPLA$_2$$\alpha^{-/-}$. Bone marrow cells from the donor mice were obtained by flushing
femurs and tibias with phosphate-buffered saline (PBS). After lethal whole body irradiation (9Gy), the recipient WT or cPLA₂α⁻/⁻ were injected intravenously with $1 \times 10^7$ bone marrow cells from WT or cPLA₂α⁻/⁻. Four weeks after BM implantation, the BMT-mice were used in subsequent experiments. Confirmation of the completion of BMT four weeks after transplantation was performed by PCR (details were mentioned in supplemental data).

**Western blot analysis**

Protein was extracted from the thoracic aorta, peripheral blood mononuclear cells (PB-MNCs), kidney and heart using extraction buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40 (Sigma Aldrich Inc, MO, USA), 0.5% sodium deoxycholate, 2mM sodium fluoride, 2mM ethylenediaminetetraacetic acid (EDTA), 0.1% sodium dodecyl sulfate (SDS), and a protease inhibitor cocktail tablet (Roche Diagnostics, IN, USA). Mono-nuclear cells were separated from blood by gradient separation on Ficoll-Paque Plus (GE Healthcare Bio-Sciences, Buckinghamshire, UK) prior to protein extraction, as reported previously¹. In some experiments, the
aortic endothelium was removed by gently rubbing the inner thoracic aortas with a 22 gauge needle. Protein was extracted by the same method.

The protein was separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to a nitrocellulose membrane (Millipore, MA, USA).

The antibodies against cPLA$_2$ (Santa Cruz Biotechnology, CA, USA), phospho-cPLA$_2$ (Cell Signaling Technology Inc, MA, USA), alpha-tubulin (Abcam, Cambridge, UK) and inducible PLA$_2$ (Sigma Aldrich Inc.) were used as primary antibodies, while horseradish peroxide conjugate (BioRad, CA, USA) was used as the secondary antibody. The immunoblots were visualized using the ECL Reagent (GE Healthcare Bio-Sciences, Buckinghamshire, UK). The signals were then scanned and the intensities quantified using ImageJ software (National Institutes of Health), with signal intensities being adjusted against those recorded for alpha-tubulin.

**Plasma renin activity assay**

Plasma samples were collected after four weeks L-NAME treatment. Plasma renin activity was quantified by commercially available kit for mice (AnaSpec, Inc. CA, USA).
**PLA₂ activity**

Before and after four weeks L-NAME treatment, thoracic aortas were rapidly extracted and placed in an ice-cold PBS. The fat and adventitia were removed and homogenized in 50mM Hepes containing 1mM EDTA. The supernatant was corrected and PLA₂ activity was quantified by commercially available cPLA₂ Assay Kit (Cayman Chemical Co, MI, USA) according to the manufacturer’s instructions.

**Angiotensin II induced hypertension**

Mice were infused with angiotensin II (1μg/kg per minute) subcutaneously for 28 days using ALZET osmotic minipumps (Model 2004, Durect Corp, Cupertino, CA).
Supplemental figure legends

Supplemental Fig I

Effect of 4-weeks L-NAME treatment on plasma eicosanoid levels.

Plasma concentrations of TXB\(_2\) (A), 6-keto-PGF\(_1\alpha\) (B), LTB\(_4\) (C) and cysLTs (D) in cPLA\(_2\alpha/-\) were significantly lower than WT irrespective of L-NAME treatment. L-NAME administration caused no significant changes in plasma eicosanoid levels in WT and cPLA\(_2\alpha/-\). n=11 per group. *p<0.05 versus the untreated WT.

Supplemental Fig II

Effect of cPLA\(_2\alpha\) gene deficiency in blood cells on plasma eicosanoids level.

Four weeks after BMT procedure, plasma levels of TXB\(_2\) (A), LTB\(_4\) (C) and cysLTs (D) but not 6-keto-PGF\(_1\alpha\) (B) of cPLA\(_2\alpha/-\)→WT or cPLA\(_2\alpha/-\)→cPLA\(_2\alpha/-\) were significantly lower than WT→WT or WT→cPLA\(_2\alpha/-\), respectively. Plasma 6-keto-PGF\(_1\alpha\) levels of WT→cPLA\(_2\alpha/-\) or cPLA\(_2\alpha/-\)→cPLA\(_2\alpha/-\) were significantly lower than WT→WT or cPLA\(_2\alpha/-\)→WT, respectively (B). n=6 per group. *p<0.05. NS: not significant.
Supplemental Fig III

Effect of L-NAME treatment on protein expression and phosphorylation of cPLA$_2$$\alpha$ in kidney (A) and heart (B) of WT. No significant change of cPLA$_2$$\alpha$ expression or phosphorylation was observed. n=6 per group.

Supplemental Fig IV

Hematoxylin-eosin staining (A)-(C) and Masson-trichrome staining (D)-(F) of arteries in heart of untreated WT (A)(D), 4 weeks of L-NAME treatment in WT (B)(E) and 4 weeks of L-NAME treatment in cPLA$_2$$\alpha$-/- (C)(F). No inflammation or vascular hypertrophy was observed.

Supplemental table

BP changes following four weeks angiotensin II treatment in WT and cPLA$_2$$\alpha$-/-. There was no significant difference in SBP and DBP between WT and cPLA$_2$$\alpha$-/-.
Reference

Supplemental figure III

A

- cPLA2α
- p-cPLA2α
- α-tubulin

L-NAME - +

B

- cPLA2α
- p-cPLA2α
- α-tubulin

L-NAME - +

not detected

Supplemental figure IV

A

B

C

D

E

F

100 μm

100 μm

100 μm

100 μm

Supplemental table

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<th>diastolic blood pressure (mmHg)</th>
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