SUPPLEMENT MATERIAL
Supplemental Methods:

1. Study population

Serum samples of preeclampsia patients and healthy individuals were collected from Taiyuan Central Hospital, Shanxi, China. 21 patients and 21 normotensive pregnant women were informed of the study’s purpose and protocol and asked to participate. Based on the criteria set by the International Society for the Study of Hypertension in Pregnancy (ISSHP)\(^1\), Preeclampsia is characterized by the new onset of hypertension (>140 mmHg systolic or >90 mmHg diastolic) after 20 weeks gestation with one or more of the following new-onset conditions: 1) Proteinuria, 2) maternal organ dysfunction, and 3) uteroplacental dysfunction. Women with normal pregnancy, defined as normal blood pressure during pregnancy and normal full-term delivery, were recruited as controls. Blood pressure was measured by traditional mercury column sphygmomanometer with standard a cuff with 12-13×35 cm, which was used by qualified nurses. Before the test, the subjects were asked to rest for 5-10 min, and they assumed a supine or sitting position. Their upper arm was bare, straight, and mildly abducted, and the elbow was at the same level as the heart. We took two readings at least two minutes apart and averaged the results. The blood pressure value of each patient was taken at least three consecutive days and averaged again. All participants were excluded from the study if they had complications of pre-existing hypertension, autoimmune disease, diabetes, endocrine dysfunction, cancer, chronic wasting, renal injury, or any implants. Blood samples for the study were obtained shortly after diagnosis and allowed to clot before centrifugation at 3000 rpm for 15 min, and then stored at -80°C.

The research conformed to the declaration of Helsinki. All participants were anonymized. The study was approved by the local research ethics committee.
(Taiyuan Central Hospital and the School of Basic Medical Sciences, Capital Medical University, Beijing, China) and written informed consent was obtained from all participants before the study commenced.

2. Enzyme-linked immunosorbent assay (ELISA)

The AT1-AA titers in sera of pregnant women or rats were detected by modified ELISA as previously reported\(^2\). Briefly, the peptide corresponding to the sequence of the human AT\(_1\)-R-ECII (165–191, I-H-R-N-V-F-I-I-N-T-N-I-T-V-C-A-F-H-Y-E-SQ-N-S-T-L) was synthesized as antigen by GL Bio-chem Ltd (Shanghai, China). 96-well microtiter plates were coated with 10 \(\mu\)g/mL human AT\(_1\)-R-ECII peptide, which was dissolved in \(\text{Na}_2\text{CO}_3\) solution (0.1 mol/L, pH 11.0) and incubated overnight at 4\(^\circ\)C. After washing with 0.1\% (v/v) Tween 20 in phosphate-buffered saline (PBS-T) three times, the plates were saturated with 5\% (w/v) defatted milk in PBS-T at 37\(^\circ\)C for 1 h. After three washings, 50 \(\mu\)L serum sample dilutions (pregnant women: 1: 10; rat: 1:100) in 5\% (w/v) defatted milk were added to the plates and incubated at 37\(^\circ\)C for 1 h. After three washings, peroxidase-conjugated goat anti-human IgG antibodies or goat anti-rat IgG antibodies (1:500 dilutions in 5\% (w/v) defatted milk, Zhongshan, China) were added for 1 h at 37\(^\circ\)C. Finally, 2,2-azino-di(3-ethylbenzothiazoline) sulfonic acid (ABTS)-\(\text{H}_2\text{O}_2\) (Roche, Switzerland) substrate buffer was added and reacted in the dark at 37\(^\circ\)C for 30 min. The optical densities (OD) were measured at 405 nm in an ELISA reader (Spectra Max Plus; Molecular Devices, Sunnyvale, CA, US). Results were also judged by the P/N value [(specimen OD - blank control OD)/(negative control OD - blank control OD)] of each sample. Negative control samples were prepared as described before (Liu et al., 1999). The positivity of the serum sample to AT1-AA was defined as P/N >2.1.

3. Preparation of AT1-AA from AT1-AA-positive IgG

The AT1-AA was purified from AT1-AA-positive IgG using the peptide corresponding
to second extracellular loop of human AT\(_1\)R (AT\(_1\)-R-ECII), linked to CNBr-activated Sepharose 4B according to the manufacturer’s instructions. Briefly, the AT\(_1\)-R-ECII was dissolved in coupling buffer (0.1 mol/L NaHCO\(_3\), 0.5 mol/L NaCl, pH 8.3), and it was added to the prepared CNBr-activated Sepharose 4B medium suspension in a stoppered vessel. Then the mixture was rotated end-over-end for overnight at 4°C. After washing away excess ligand with at least 5 medium (gel) volumes of coupling buffer, the medium was transferred to 0.1 mol/L Tris-HCl (pH 8.0) and stood for 2 h to block any remaining active groups.

4. Immunofluorescence (IF) microscopy

The IF staining was performed to identify primary VSMCs. Cells were grown on chamber slides and then were washed twice with ice-cold PBS and fixed with cold methanol for 10 min. After blocking with 5% BSA in PBS at room temperature for 30 min, the cells were incubated overnight at 4°C with an antibody against α-SMA (Abcam; 1:1000) or with calponin (Abcam; 1:500). Then the cells were washed with PBS and incubated them with Alexa Fluor 488 anti-mouse IgG (Thermo Fisher) or Alexa Fluor 568 anti-rabbit IgG (Abcam) at 37°C for 30 min. After rinsing 3 times with PBS, we mounted coverslips with ProLong gold anti-fade reagent with DAPI (Thermo Fisher) and observed the cells under an Imager A2 fluorescence microscope (Zeiss).
# Table S1: Clinical profiles of patients and healthy controls (normal-pregnancy group).

| Clinical profiles                      | Preeclampsia (n=21) | Normal pregnancy (n=21) | P value |
|----------------------------------------|---------------------|-------------------------|---------|
| Maternal age (years)                   | 29 (24–35)          | 28 (26–34)              | NS      |
| Gestational age at sampling (weeks)    | 38±1.2              | 39±1.1                  | NS      |
| Ethnic background                      | Han                 | Han                     | --      |
| Systolic blood pressure (mmHg)         | 162±9.7             | 122.8±11.2              | <0.001  |
| Diastolic blood pressure (mmHg)        | 96.7±10.5           | 78.4±7.3                | <0.001  |
| Proteinuria¹                           | (+~+++              | (−)                    | <0.001  |

Values are expressed as mean ± SD.

P< 0.001 vs. normal pregnancy

1: Mann-Whitney Test compared between preeclampsia and normal pregnancy group
Figure S1: AT1-AA levels were significantly greater in preeclamptic patients. A: The P/N value of pregnant women were detected by modified ELISA, the dotted line represents the value of 2.1 (***P < 0.001 vs. normal pregnancy). B: The positive rate of AT1-AA both normal pregnancy and preeclampsia, Chi-square Test was used to compare preeclampsia to normal pregnancy group (***P < 0.001 vs. normal pregnancy).
Figure S2: The AT1-AA and AT1-AA-positive IgG were compared. A: The combining capacity with AT1-R-ECII was detected by ELISA, the numerals 1, 2, 3, 4, and 5 represent AT1-AA-positive IgG, AT1-AA, negative control, positive control, and blank control, respectively. B: The purity was analyzed using SDS-PAGE, the characters M, 1, and 2 represent marker, AT1-AA-positive IgG, and AT1-AA, respectively. C: The biological activity was compared by intracellular Ca^{2+} detection (n=3).
Figure S3: AT1-AA-positive IgG had high capacity to combine with human AT₁R-ECII. AT1-AA-positive and negative IgG were diluted with a serial concentration and detected the amount of binding with AT₁R-ECII by ELISA. The OD value (405 nm) was positively correlated with the degree of binding. By fitting the curve and selecting a 95% confidence interval, two dashed lines divided the image into four intervals. Horizontal dashed line: confidence limit of background scatter. Vertical dashed line: specificity cut-off.
Figure S4: Intact and damaged endothelia of vascular rings were identified. A: Acetylcholine exerted concentration-dependent relaxation effects against norepinephrine-induced contractions of arterial rings with intact endothelia. B: Sodium nitroprusside (SNP) exerted relaxation effects against norepinephrine-induced contractions of vascular rings with their endothelia removed, whereas acetylcholine did not show concentration-dependent relaxation effects. (NE: norepinephrine; Ach: acetylcholine; SNP: sodium nitroprusside).
Figure S5: VSMCs were identified. A: VSMCs successfully developed from the aortic tissue of rats. B: The cultured cells were immunofluorescence stained for markers of VSMCs (α-SMA and calponin).
Figure S6: The endocytic inhibitor Dynasore abolished Ang II-induced AT$_1$R internalization. Representative images and line chart of membrane AT$_1$R trafficking by TIRF are shown. The images were acquired at 1 frame/second for 33 min. The ligand was added at the 3-minute mark. Data show that administration of Ang II did not induce AT$_1$R internalization after 30 min of pre-incubation with Dynasore.
Figure S7: Ang II induced persistent AT₁R activation and sustained vasoconstriction after endocytosis inhibition. We pre-incubated an endocytosis inhibitor, Dynasore, with a vascular ring or VSMCs before administering Ang II. A: With Dynasore pre-incubation, Ang II prolonged the duration of PKC and ERK1/2 hyperphosphorylation (n=6; *P < 0.05 vs. 0 min). B: With Dynasore pre-incubation, Ang II prolonged [Ca²⁺] elevation, (n=5; *P < 0.05; **P < 0.01 vs. baseline; #P < 0.05 vs. Ang II group). C: With Dynasore pre-incubation, Ang II induced prolonged vasoconstriction (n=6, **P < 0.01 vs. Ang II group, ns: not significant). The white arrows indicate the VSMCs of [Ca²⁺] elevation; the black arrows indicate the time point of adding ligand.
Supplementary Video 1: The [Ca\(^{2+}\)]\(_i\) increased in response to stimulation with Ang II (1\(\mu\)mol/L)

Supplementary Video 2: The [Ca\(^{2+}\)]\(_i\) increased in response to stimulation with AT1-AA (1\(\mu\)mol/L)

Supplementary Video 3: The internalization of AT\(_1\)R in transiently transfected cells by RFP-fused AT\(_1\)R upon Ang II (1\(\mu\)mol/L) stimulation. The red puncta represent the RFP-fused AT\(_1\)R on the membrane.

Supplementary Video 4: The internalization of AT\(_1\)R in transiently transfected cells by RFP-fused AT\(_1\)R upon AT1-AA (1\(\mu\)mol/L) stimulation.

Supplemental References
1. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, Zeeman GG, Brown MA. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. PREGNANCY HYPERTENS. 2014;4:97-104.
2. Zhang S, Zheng R, Yang L, Zhang X, Zuo L, Yang X, Bai K, Song L, Tian J, Yang J, Liu H. Angiotensin type 1 receptor autoantibody from preeclamptic patients induces human fetoplacental vasoconstriction. J CELL PHYSIOL. 2013;228:142-148.
3. Yang X, Wang F, Chang H, Zhang S, Yang L, Wang X, Cheng X, Zhang M, Ma XL, Liu H. Autoantibody against AT1 receptor from preeclamptic patients induces vasoconstriction through angiotensin receptor activation. J HYPERTENS. 2008;26:1629-1635.