SUPPLEMENTARY DATA

Autoantibodies against AT1 Receptor Contribute to Vascular Aging and Endothelial Cell Senescence

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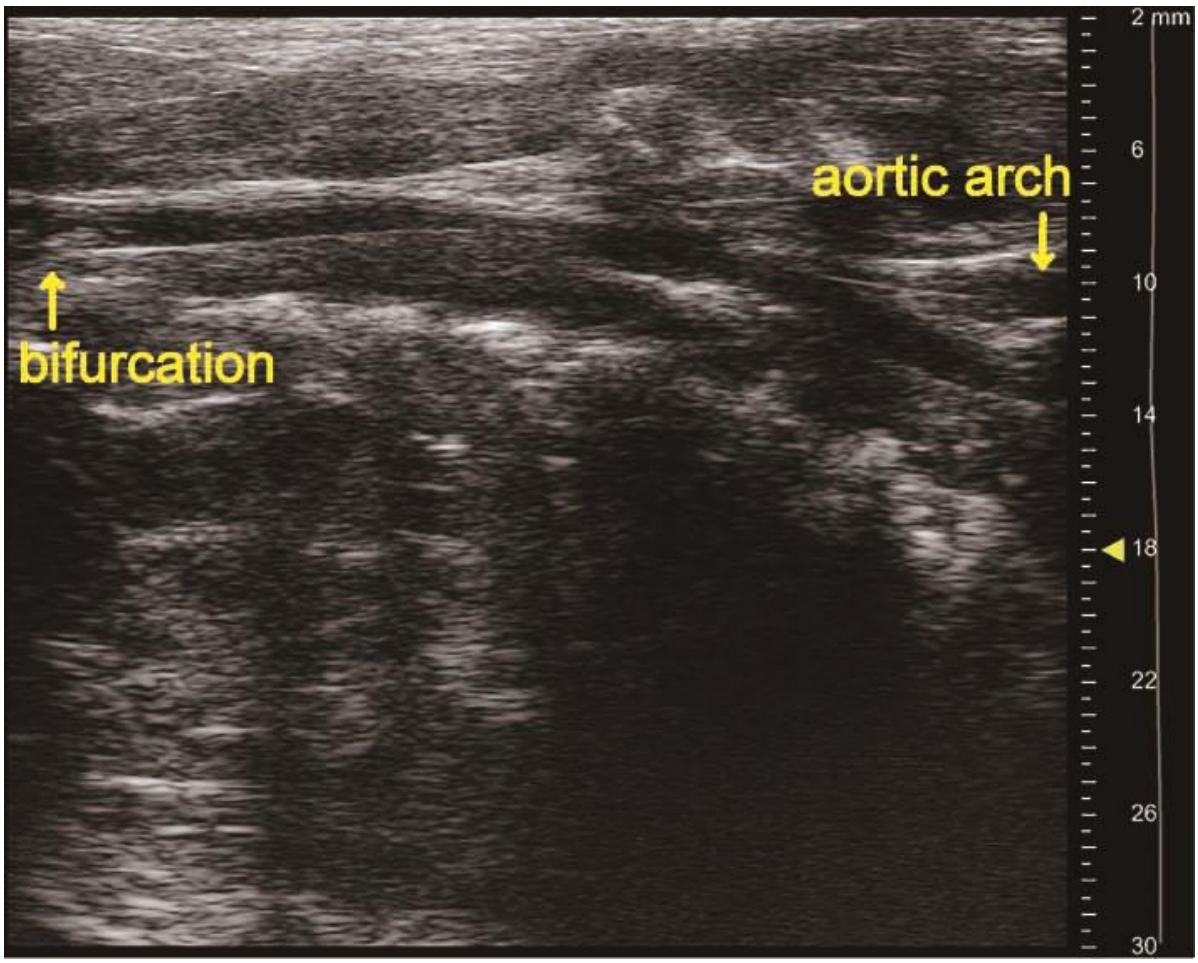
Supplemental Table 1. Primers used in this study.

	Forward primer (5'-3')	Reverse primer (5'-3')
TNFα	GACAAGCCTGTAGCCCATGT	GGAGGTTGACCTTGGTCTGG
IL-6	TTCGGTCCAGTTGCCTTCTC	CAGCTCTGGCTTGTTCCTCA
MCP-1	GACCATTGTGGCCAAGGAGA	TTGGGTTTGCTTGTCCAGGT
ICAM-1	ACCCCGTTGCCTAAAAAGGA	GCTCCATGGTGATCTCTCCTC
VCAM-1	GGGTCTACCAGCTCCAGAGA	GGCCACCACTCATCTCGATT
PAI-1	GCAACGTGGTTTTCTCACCC	CTCTAGGGGCTTCCTGAGGT



Supplemental Figure 1. Ultrasound imaging of the left common carotid artery in the rat. Longitudinal ultrasound image of the rat left common carotid artery imaged at 15MHz, from the aortic arch to the bifurcation. Transit-time measurements were performed at distal and proximal sites, located 1.5 mm upstream from the bifurcation and 1 mm downstream from the aortic arch, respectively. PWV is calculated using the transit time and the distance between the two sites.

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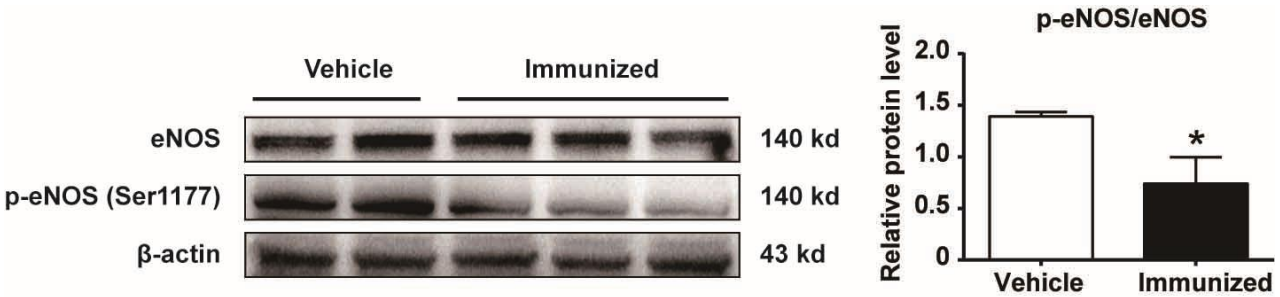
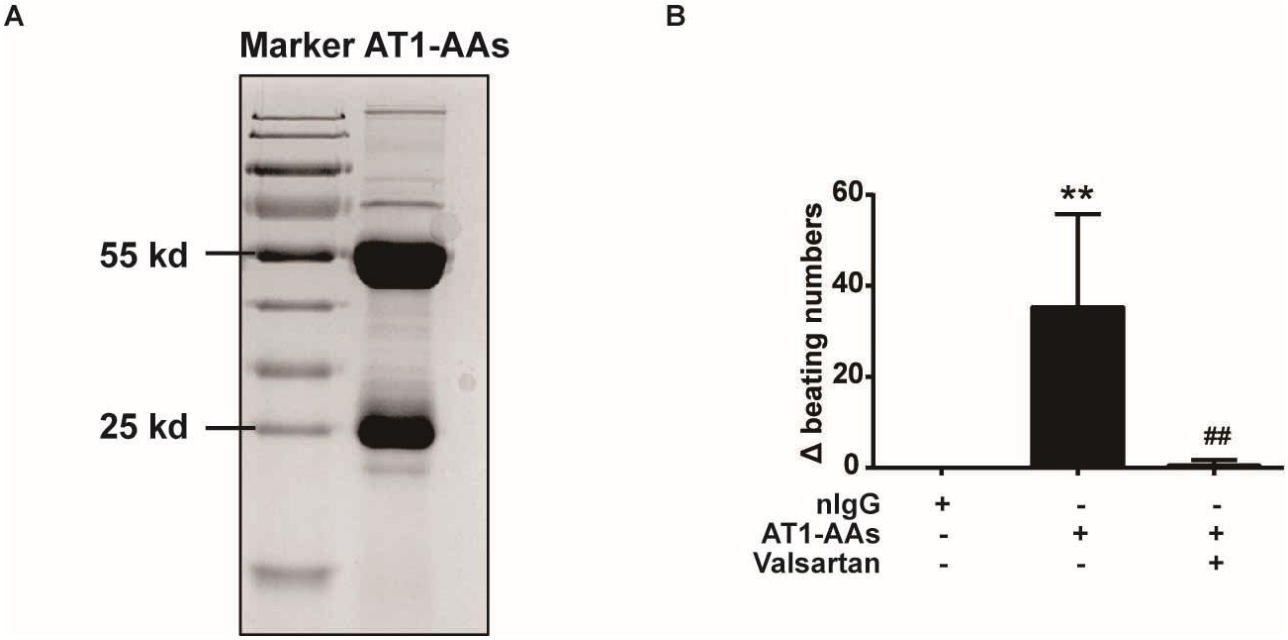


Supplemental Figure 2. AT1-AAs reduced phosphorylation of eNOS in the aorta of AT_1R-EC_{II}-immunized rats. Representative western blot and quantitative graphs of p-eNOS/eNOS levels in the aortas of rats from the immunized and vehicle groups. Data were expressed as mean ± SEM. n=5 for each group, *$p < 0.05$ *vs.* the vehicle group.



Supplemental Figure 3. Purity and biological activity detection of extracted AT1-AA-IgGs from immunized rats. A) IgGs purified from the AT_1R-EC_{II}-immunized rats' sera had high purity, as identified by Coomassie blue staining. **B**) Increases in the beating number of NRCMs treated with nIgGs, AT1-AAs or valsartan plus AT1-AAs. Data in the graphs were from 3 independent experiments and expressed as mean ± SEM. **$p < 0.01$ *vs.* the nIgGs group; ## $p < 0.01$ *vs.* the AT1-AAs group.

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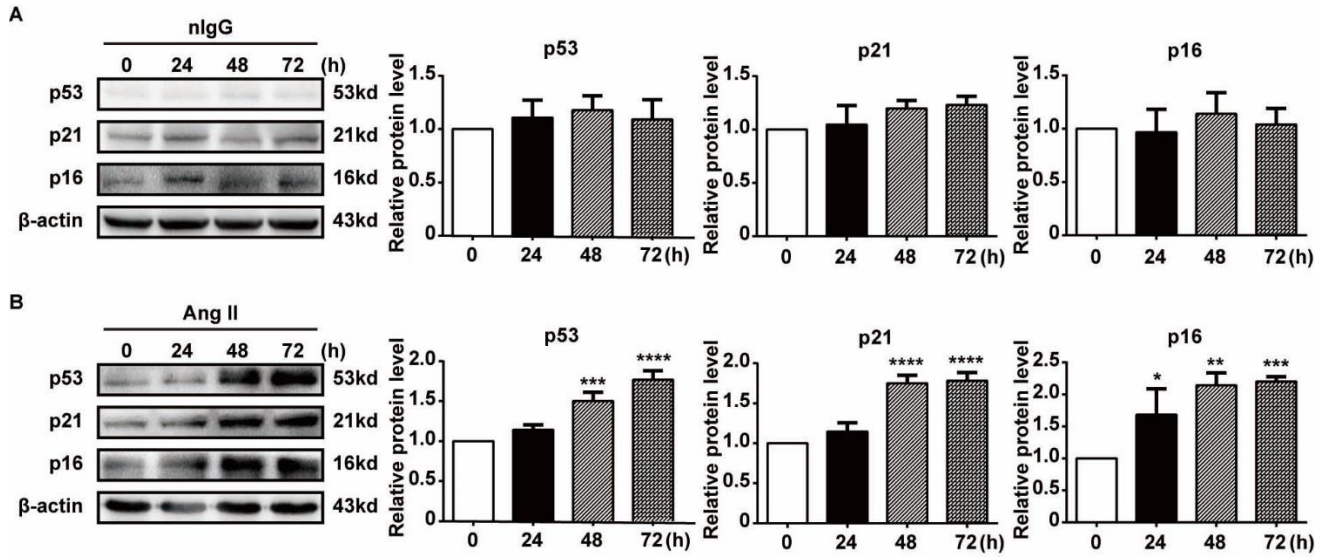
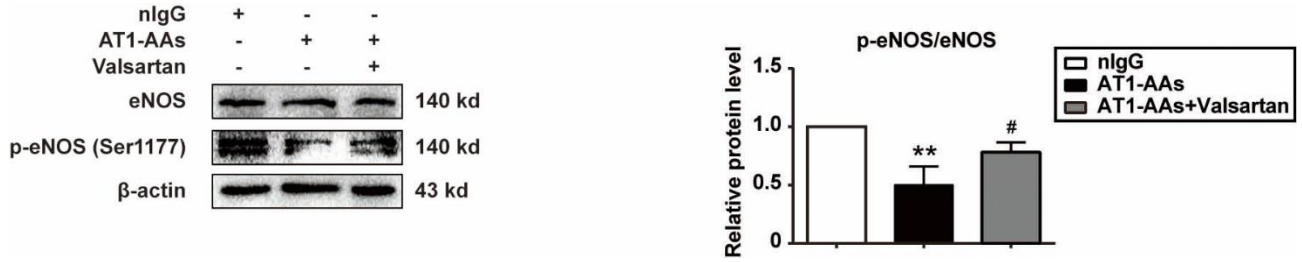


Supplemental Figure 4. The effect of nIgGs and Ang II on HUVECs senescence. A) Representative images and quantitative graphs of p53, p21 and p16^{INK4a} expressions. HUVECs were incubated with nIgGs (1 μM) for indicated times. **B**) Representative images and quantitative graphs of p53, p21 and p16^{INK4a} expressions. HUVECs were incubated with AngII (1 μM) for indicated times. Data in the graphs were from 3 independent experiments and were expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p<0.001, ****p<0.0001 *vs.* the control group.



Supplemental Figure 5. **AT1-AAs-IgGs reduced phosphorylation of eNOS in HUVECs via AT1 receptor.** Representative western blot and quantitative graphs of p-eNOS/eNOS levels in HUVECs treated with nIgGs, AT1-AA or valsartan plus AT1-AA for 72 hrs. Data in the graphs were from 3 independent experiments and were expressed as mean ± SEM. **p < 0.01 *vs.* the nIgGs group; # p < 0.05 *vs.* the AT1-AAs group.

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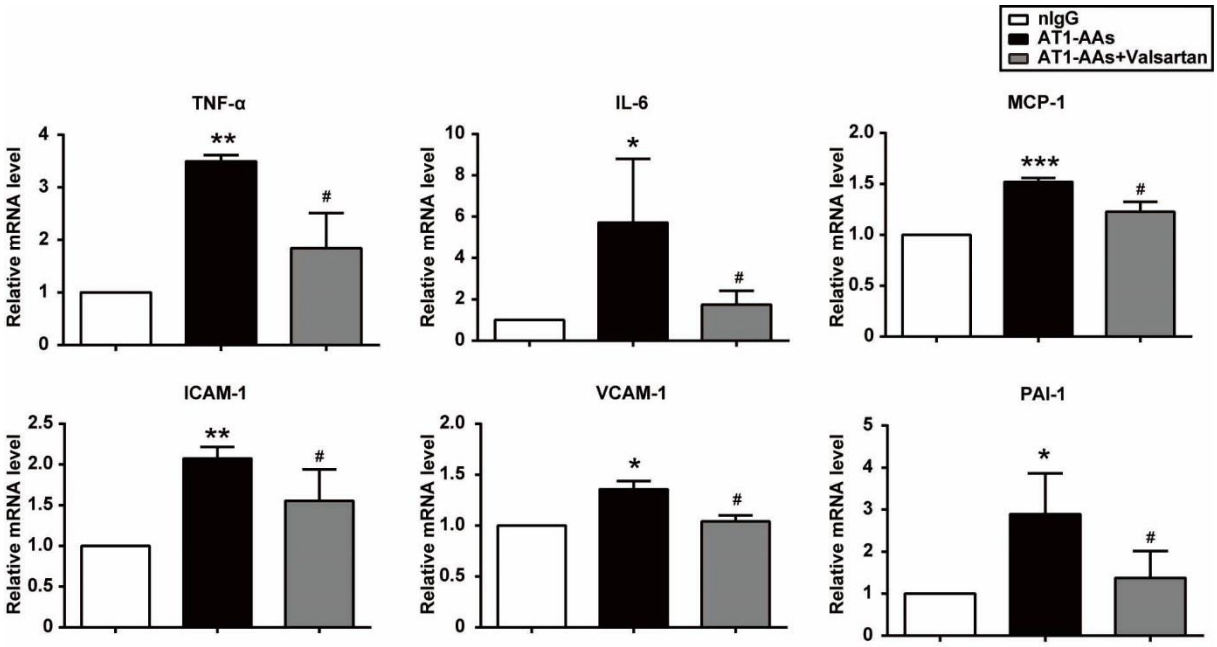
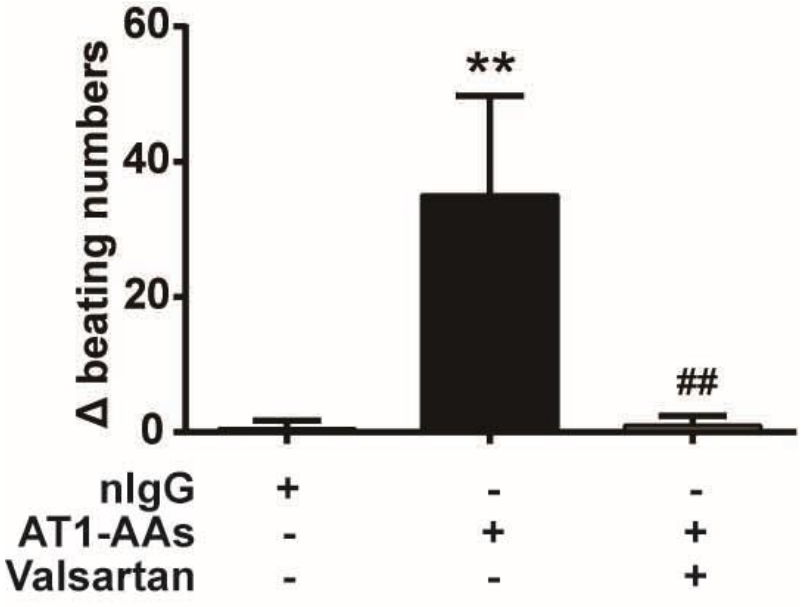


Supplemental Figure 6. AT1-AAs-IgGs increased the levels of inflammatory factors transcription via AT1 receptor. Quantitative graphs of inflammatory factors levels in HUVECs treated with nIgGs, AT1-AA or valsartan plus AT1-AA for 72 hrs. Data in the graphs were from 3 independent experiments and were expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p<0.001$ *vs.* the nIgGs group; # $p < 0.05$ *vs.* the AT1-AAs group.



Supplemental Figure 7. **Biological activity detection of extracted AT1-AA-IgGs from PAD patients.** Increases in the beating number of NRCMs treated with nIgGs, AT1-AAs or valsartan plus AT1-AAs. Data in the graphs were from 3 independent experiments and expressed as mean ± SEM. **$p < 0.01$ *vs.* the nIgGs group; ## $p < 0.01$ *vs.* the AT1-AAs group.

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