Supplemental Information

Chronic infusion of ELABELA alleviates vascular remodeling in spontaneously hypertensive rats via anti-inflammatory, anti-oxidative and anti-proliferative effects

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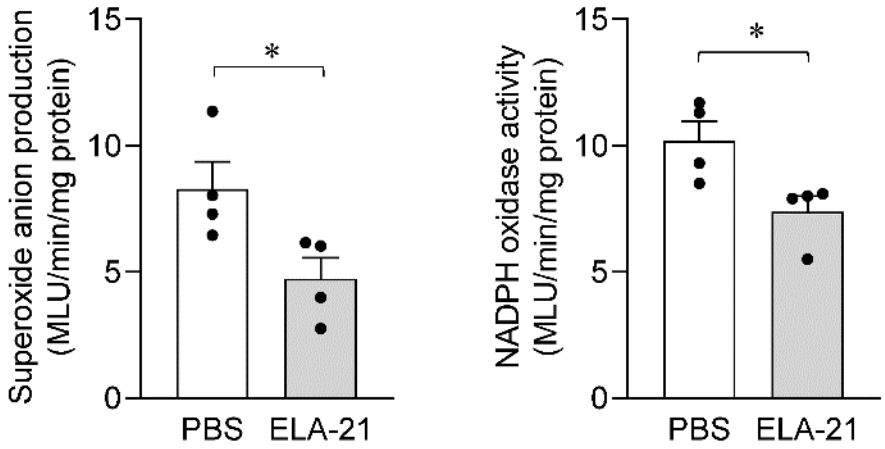
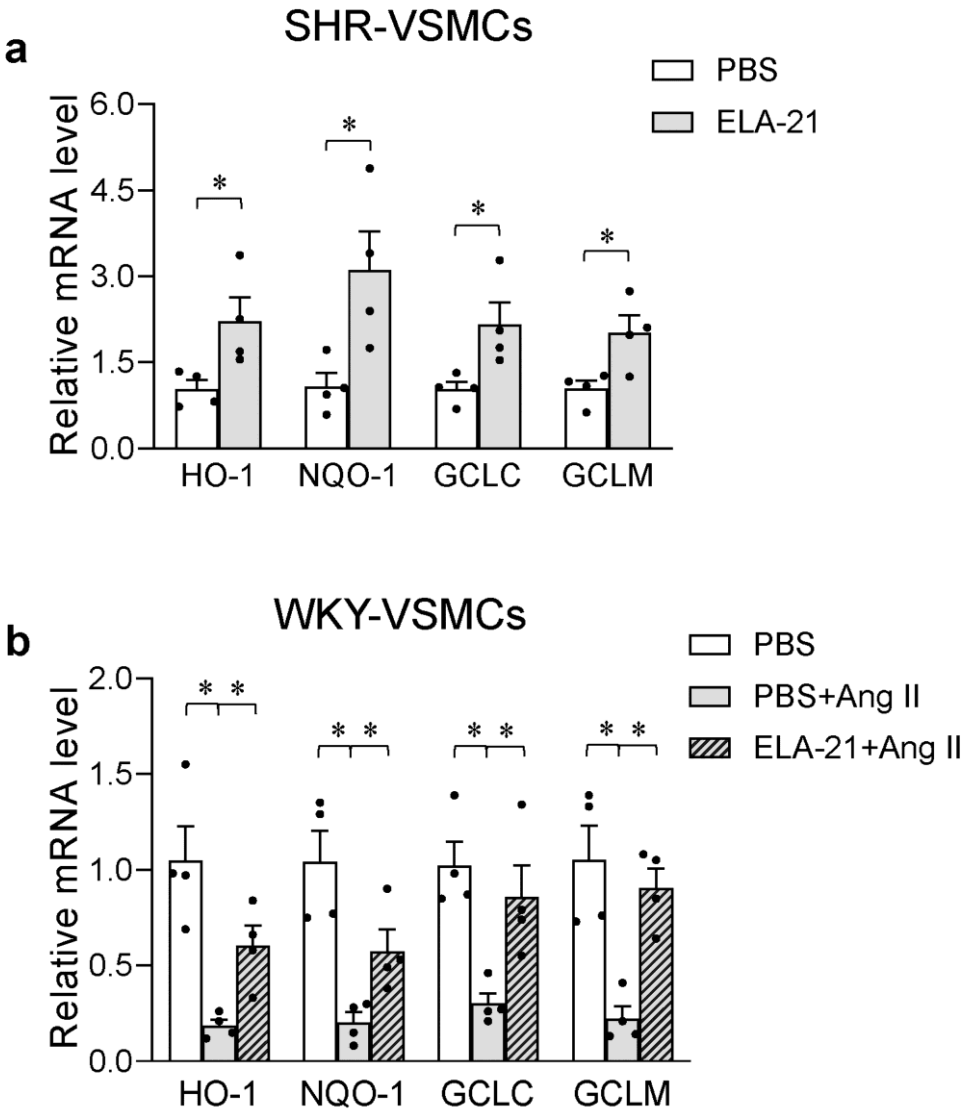
Supplemental Table 1. Primers for semi-quantitative or real-time quantitative PCR analysis.

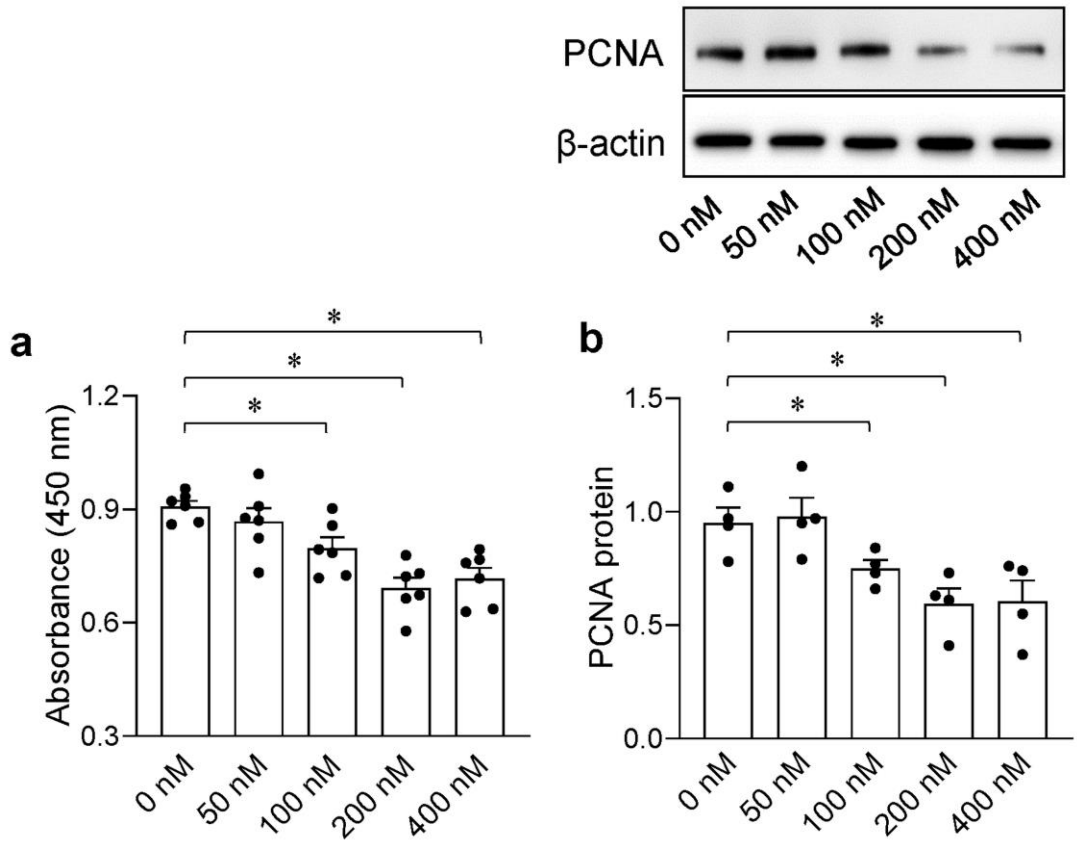
Name	Sequence
ELA	F 5' ATGCGATTCCAGCCCCTTT 3'
	R 5' TCATGGGAAGGGCACTCGAGAAT 3'
TNF - α	F 5' CCAGAACTCCAGGCGGTGTC 3'
	R 5' GGCTACGGGCTTGTCACTCG 3'
iNOS	F 5' GCTCGGGCTGAAGTGGTATGC 3'
	R 5' GAAGTCTCGGACTCCAATCTCGGT 3'
IL-6	F 5' TCCAGCCAGTTGCCTTCTTG 3'
	R 5' AGCCACTCCTTCTGTGACTC 3'
MCP-1	F 5' CTATGCAGGTCTGTCACGCTTC 3'
	R 5' CAGCCGACTCATTGGGATCA 3'
NOX1	F 5' CGGCAGAAGGTCGTGATTA 3'
	R 5' TGGAGCAGAGGTCAGAGT 3'
NOX4	F 5' ACAACTGTTCCTGGCCTGAC 3'
	R 5' CGGGAGGGTGGGTATCTAAA 3'
PCNA	F 5’ TCCGAAGGCTTCGACACATAC 3'
	R 5' GGACATGCTGGTGAGGTTCA 3'
VCAM-1	F 5' GAAGGAAACTGGAGAAGACAATCC 3'
	R 5' TGTACAAGTGGTCCACTTATTTCAATT 3'
ICAM-1	F 5' CACAAGGGCTGTCACTGTTCA 3'
	F 5' CCCTAGTCGGAAGATCGAAAGTC 3'
HO-1	F 5' GAGACGGCTTCAAGCTGGTGATG 3'
	F 5' GTTGAGCAGGAACGCAGTCTTGG 3'
NQO-1	F 5' GGCCATCATTTGGGCAAGTC 3'
	F 5' TCCTTGTGGAACAAAGGCGA 3'
GCLC	F 5' AAACACGCCTTCCTTCCCATTG 3'
	F 5' AGTAAAGTGGCACAG GAGCGAG 3'
GCLM	F 5' GTTCATTGTAGGATCG 3'
	F 5' GGTGCCTATAGCAACAATCT 3'
MMP2	F 5' GCACCGTCGCCCATCA 3'
	F 5' GCACTGCCAACTCTTTGTCTGTT 3'
MMP9	F 5' GGCTTAGATCATTCTTCAGTG 3'
	F 5' GCCTTGGGTCAGGTTTAG 3'
GAPDH	F 5' GAAGGTCGGTGTGAACGGATTTG 3'
	R 5' CATGTAGACCATGTAGTTGAGGTCA 3'



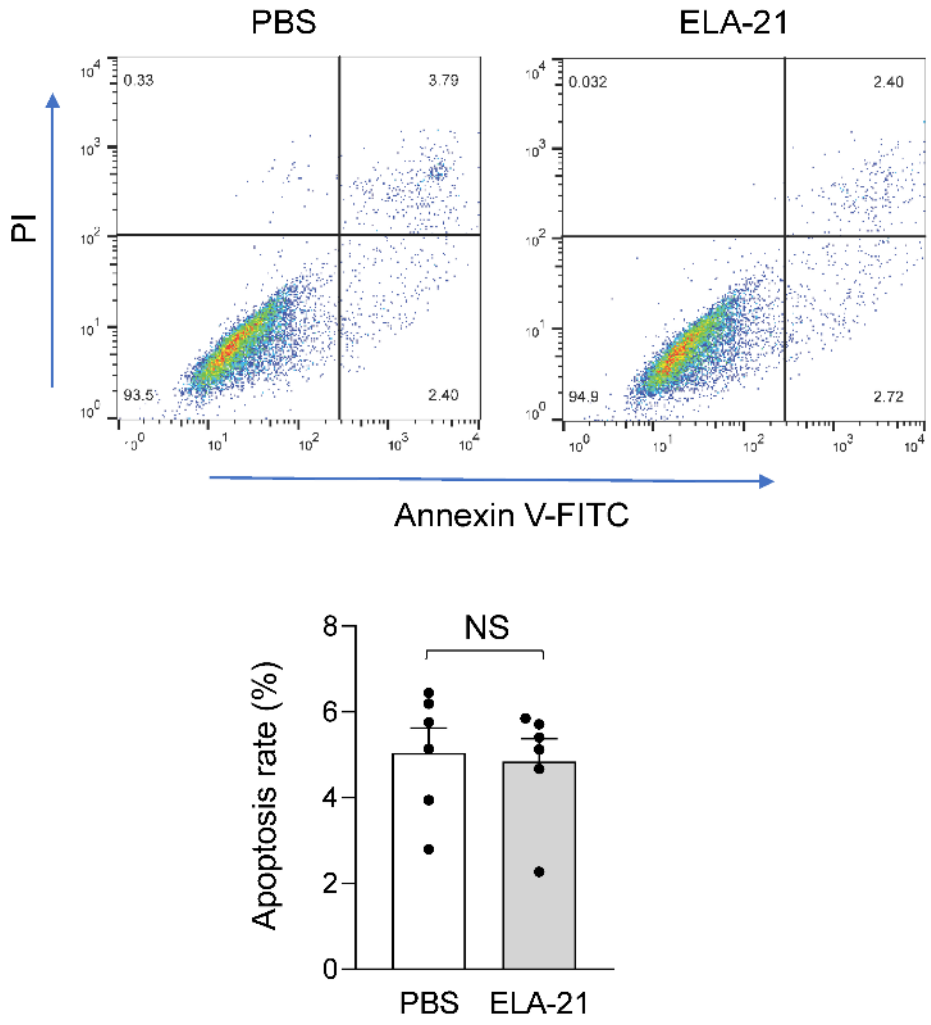
Supplementary Figure 1. ELA-21 reduced superoxide anion production and NADPH oxidase activity in SHR-VSMCs. Superoxide anion levels and NADPH oxidase activity were measured with lucigenin-derived chemiluminescence method. The measurements were made 24 h after ELA-21 treatment. n=4 per group. Values are mean±SE. *P < 0.05.



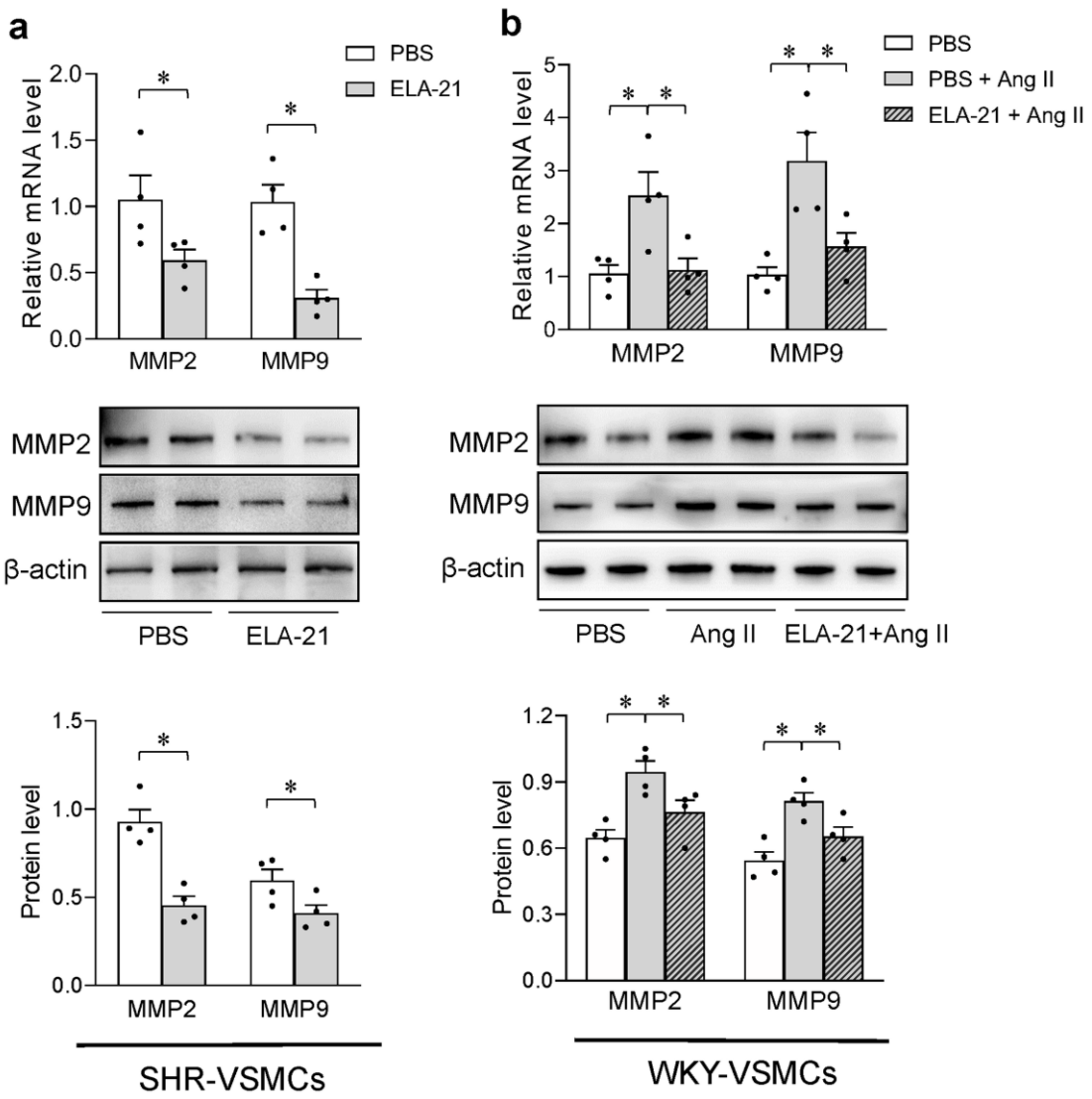
Supplementary Figure 2. ELA-21 enhanced the expression of Nrf2 downstream genes (HO-1, NQO-1, GCLC and GCLM) in SHR-VSMCs and Ang II-stimulated WKY-VSMCs. For SHR-VSMCs, the cells were treated with ELA-21 (200 nM) for 24 h. For WKY-VSMCs, the cells were treated with ELA-21 (200 nM) for 2 h followed by Ang II (100 nM) for 24 h. n=4 per group. Values are mean±SE. *P < 0.05.



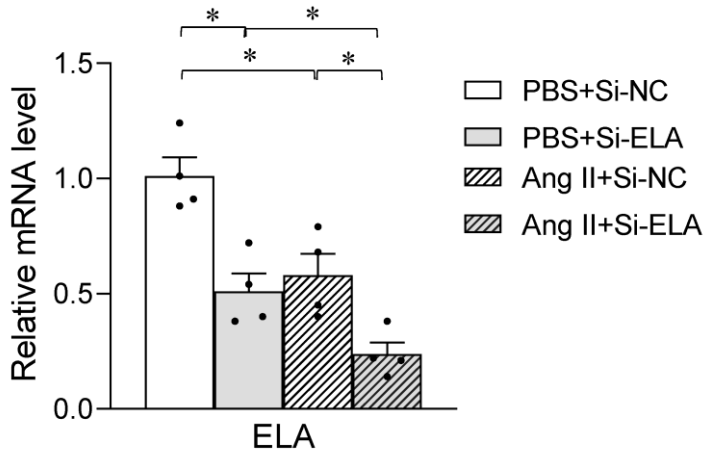
Supplementary Figure 3. Dose effect of ELA-21 on VSMC proliferation of SHR. The cells were treated with PBS or ELA-21 (50, 100, 200 and 400 nM) for 24 h. The VSMC proliferation was evaluated with CCK-8 kits (a) and PCNA expression (b). n=6 in a and n=4 in b. Values are mean±SE. *P < 0.05.



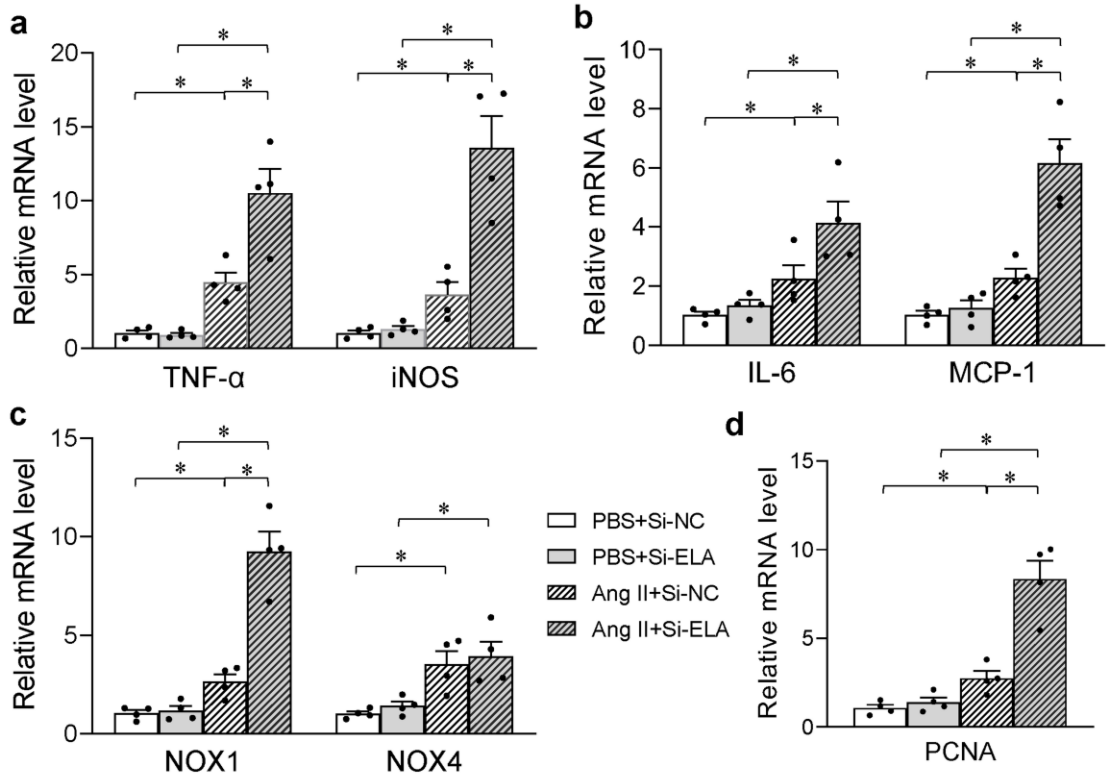
Supplementary Figure 4. Effects of ELA-21 on apoptosis in SHR-VSMCs. Annexin -V/PI staining and flow cytometry was used to determine the level of apoptosis. Cells in upper and lower right quadrants were classed as apoptotic and the results were analyzed quantitatively. The measurements were made 24 h after ELA-21 (200 nM) treatment. n=6 per group. Values are mean±SE. NS, no significant difference.



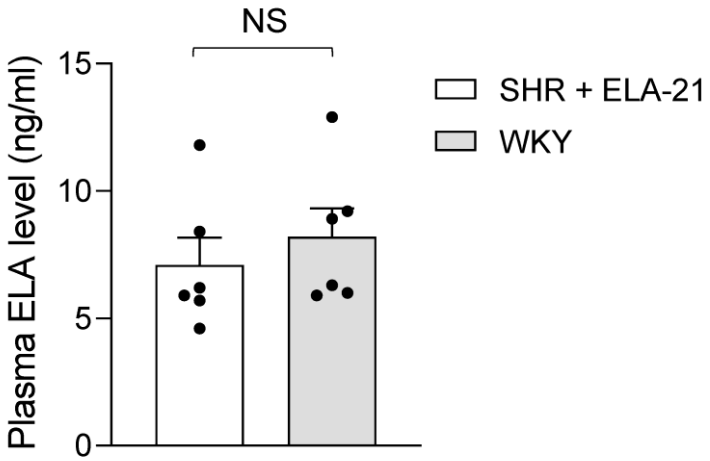
Supplementary Figure 5. ELA-21 suppressed expression of MMP2 and MMP9 in SHR-VSMCs and Ang II-stimulated WKY-VSMCs. For SHR-VSMCs, the cells were treated with ELA-21 (200 nM) for 24 h. For WKY-VSMCs, the cells were treated with ELA-21 (200 nM) for 2 h followed by Ang II (100 nM) for 24 h. n=4 per group. Values are mean±SE. *P < 0.05.



Supplementary Figure 6. ELA knockdown by siRNA reduced ELA expression in VSMCs of WKY. WKY-VSMCs were treated with ELA siRNA (Si-ELA, 50 nM) or negative control siRNA (Si-NC, 50 nM) using lipofectamine 3000 reagent for 48 h followed by Ang II (100 nM) treatment for 24 h. n=4 per group. Values are mean±SE. *P < 0.05.



Supplementary Figure 7. ELA knockdown by siRNA deteriorated Ang II-induced inflammation, oxidative stress and VSMC proliferation of WKY. (a, b) inflammatory gene expression; (c) NOX1 and NOX4 gene expression; (d) PCNA gene expression. WKY-VSMCs were treated with ELA siRNA (Si-ELA, 50 nM) or negative control siRNA (Si-NC, 50 nM) using lipofectamine 3000 reagent for 48 h followed by Ang II (100 nM) treatment for 24 h. n=4 per group. Values are mean±SE. *P < 0.05.



Supplementary Figure 8. Comparison of the ELA level between ELA-21-infused SHR and WKY in plasma. ELA-21 was infused by osmotic minipumps for 4 weeks in SHR. ELA level in plasma was determined by ELISA. Values are mean ± SE. NS, no significant difference. n=6.