Supplementary material

Paeonol promotes reendothelialization after vascular injury through activating of c-Myc/VEGFR2 signaling pathway

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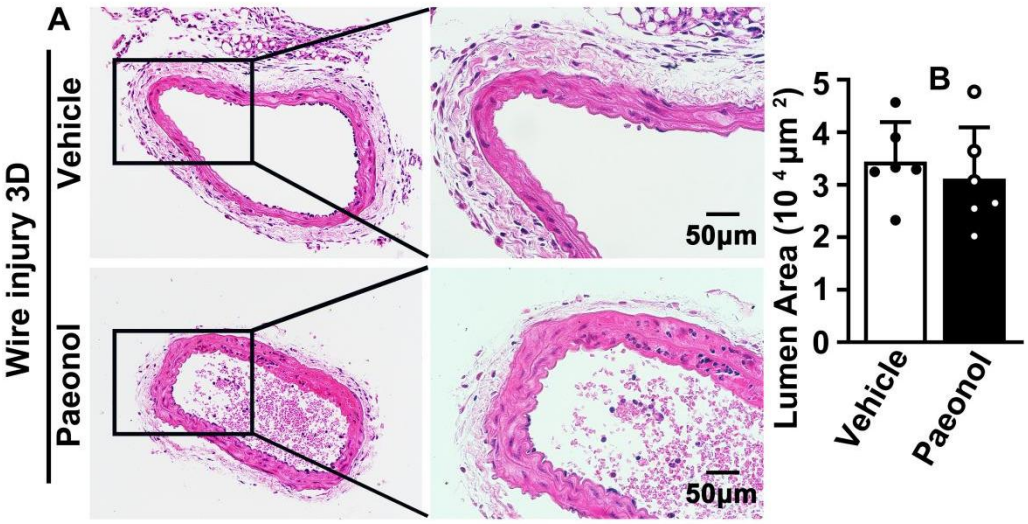
Supplementary Figure 1

List of primer sequences used for Real Time PCR in the study

Gene Name	*Species*	*Sequence*
VEGF121	Human	F:5'-CCCTGATGAGATCGAGTACATCTT-3'
	Human	R:5'-GCCTCGGCTTGTCACATTTT-3'
VEGF165	Human	F:5'-CCCTGATGAGATCGAGTACATCTT-3'
	Human	R:5'-AGCAAGGCCCACAGGGATTT-3'
VEGF189	Human	F:5'-CCCTGATGAGATCGAGTACATCTT-3'
	Human	R:5'-AACGCTCCAGGACTTATACCG-3'
VEGFR1	Human	F:5'-TCTCACACATCGACAAACCAATACA-3'
	Human	R:5'-GGTAGCAGTACAATTGAGGACAAGA-3'
VEGFR2	Human	F:5'-GCAGGGGACAGAGGGACTTG-3'
	Human	R:5'-GAGGCCATCGCTGCACTCA-3'
VEGFR3	Human	F:5'-GACAGCTACAAGTACGAGCATCTG-3'
	Human	R:5'-CGTTCTTGCAGTCGAGCAGAA-3'
c-Myc	Human	F:5'-AGCGACTCTGAGGAGGAACAAG-3'
	Human	R:5'-GTGGCACCTCTTGAGGACCA-3'
Tie2	Human	F:5'-TTGAAGTGGAGAGAAGGTCTG-3'
	Human	R:5'-GTTGACTCTAGCTCGGACCAC-3'
Caspase-3	Human	F:5'-AGTAGATGGTTTGAGCCTGAGC-3'
	Human	R:5'-GTATGGAGAAATGGGCTGTAGG-3'
CD31	Human	F:5'-TTAGCCTGAGGAATTGCTGTGTT-3'
	Human	R:5'-AGCTGTAGGAGGAGAATCCATC-3'
CD148	Human	F:5'-AGTACACACGGCCCAGCAAT-3'
	Human	R:5'-GAGGCGTCATCAAAGTTCTGC-3'
ANGPT1	Human	F:5'-AACATGGGCAATGTGCCTACACTT-3'
	Human	R:5'-CATTCTGCTGTATCTGGGCCATCT-3'
ANGPT2	Human	F:5'-CAGATTTTGGACCAGACCAGTGA-3'
	Human	R:5'-TCAATGATGGAATTTTGCCTTGGA-3'
NRP1	Human	F:5'-CAGAAAAGCCCACGGTCAT-3'
	Human	R:5'-CAGCCAAATTCACAGTTAAAACC-3'
GJC	Human	F:5'-AGCTGTAGGAAGGAGAATCCATC-3'
	Human	R:5'-TGCAAACGCATCATAACAGACA-3'
VWF	Human	F:5'-GTCGAGCTGCACAGTGACATG-3'
	Human	R:5'-GCACCATAAACGTTGACTTCCA-3'
RHOA	Human	F:5'-GAAGAGGCTGGACTCGGATT-3'
	Human	R:5'-AGCAAGCATGTCTTTCCACA-3'
PCNA	Human	F:5'-TCCGCCACCATGTTCGA-3'
	Human	R:5'-TATCCCAGCAGGCCTCGTT-3'
P14arf	Human	F:5'-ATGGTGCGCAGGTTCTTGG-3'
	Human	R:5'-TGCGGGCATGGTTACTGCCTC-3'
P15ink4b	Human	F:5'-CGCAGACCCTGCCACTCT-3'
	Human	R:5'-AGGCATCGCGCACGTC-3'

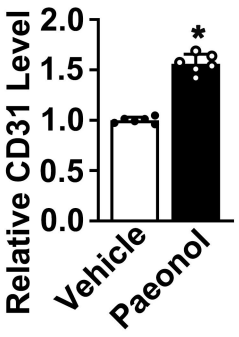
Gene Name	*Species*	*Sequence*
P16ink4a	Human	F:5'-CCCAACGCACCGAATAGTTA-3'
	Human	R:5'-ACCAGCGTGTCCAGGAAG-3'
P18ink4c	Human	F:5'-CATCATGCAGCCTGGTTAGG-3'
	Human	R:5'-GCTGGCCGTGTGCTTCAC-3'
P19arf	Human	F:5'-CCCTCGTGCTGAGCTACTGA-3'
	Human	R:5'-ACCACCAGCGTGTCCAGGAA-3'
P27kip1	Human	F:5'-TGGAGAAGCACTGCAGAGAC-3'
	Human	R:5'-GCGTGTCCTCAGAGTTAGCC-3'
CCND1	Human	F:5'-GTGCTGCGAAGTGGAAACC-3'
	Human	R:5'-ATCCAGGTGGCGACGATCT-3'
MMP9	Human	F:5'-CCTGGGCAGATTCCAAACCT-3'
	Human	R:5'-GCAAGTCTTCCGAGTAGTTTTGGAT-3'
VE-Cadherin	Human	F:5'-GAGCCGCCGCCGCAGGAAG-3'
	Human	R:5'-CGTGAGCATCCAGCAGTGGTAGC-3'
Collagen1	Human	F:5'-ATGCCTGGTGAACGTGGT-3'
	Human	R:5'-AGGAGAGCCATCAGCACCT-3'
Fibronectin	Human	F:5'-CCGCCGAATGTAGGACAAGA-3'
	Human	R:5'-TGCCAACAGGATGACATGAAA-3'
β-actin	Human	F:5'-CGAGGCCCAGAGCAAGAGAG-3'
	Human	R:5'-CTCGTAGATGGGCACAGTGTG-3'

Supplementary figure 2



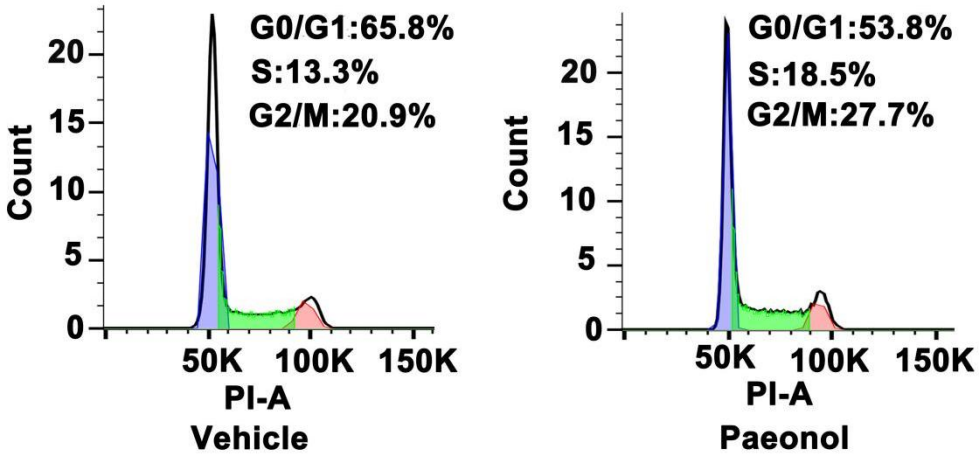
Supplementary Figure 2. (A) Representative images of H&E staining of left carotid artery catheter guide wires injury after Paeonol treatment for 3 days. Whole images exhibited in left and high resolution of corresponding areas exhibited in right. (Scale bar: 50 μm). (B) Quantification of lumen area (n=6). Data presented as mean ± SEM.

Supplementary figure 3



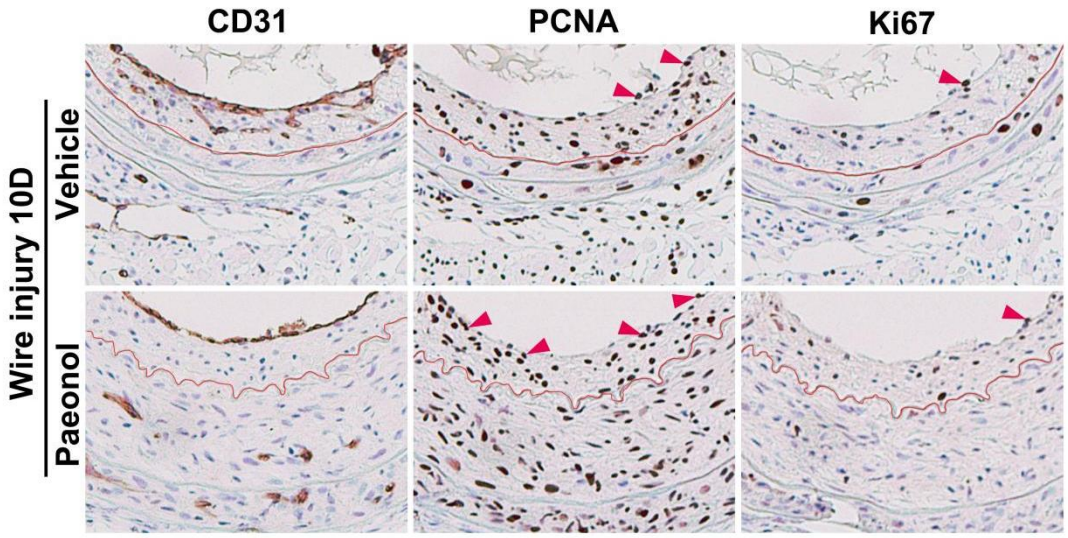
Supplementary Figure 3. The quantification of relative CD31expression on slides from carotid artery after Paeonol treatment for 7 days (n=6). Data presented as mean ± SEM, *P < 0.05 was considered significant.

Supplementary figure 4



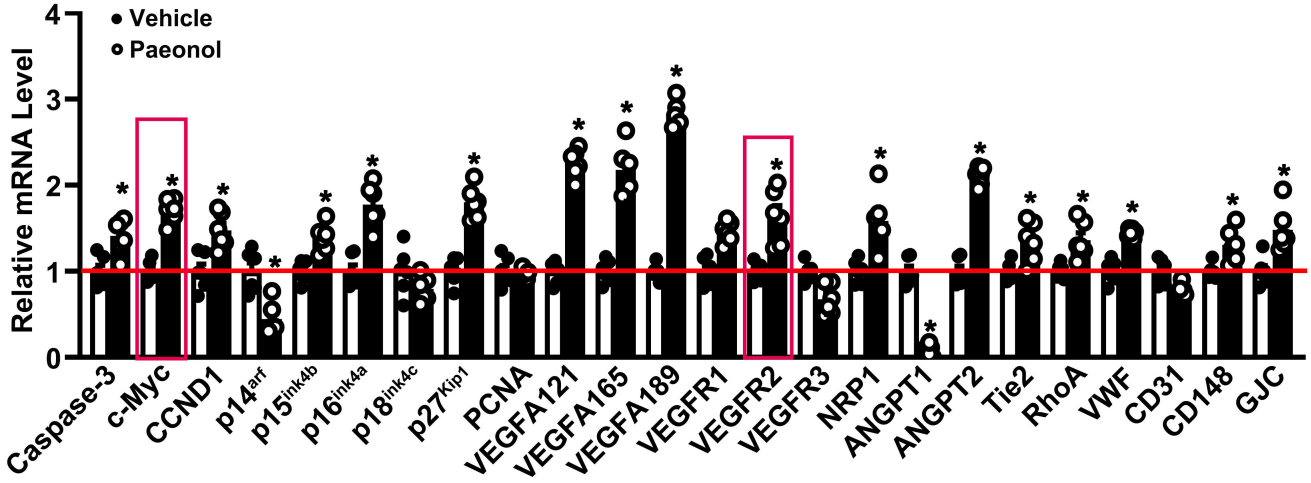
Supplementary Figure 4. Cell cycle of HUVEC-C was evaluated by flow cytometry analysis after Paeonol treatment following PI staining.

Supplementary figure 5



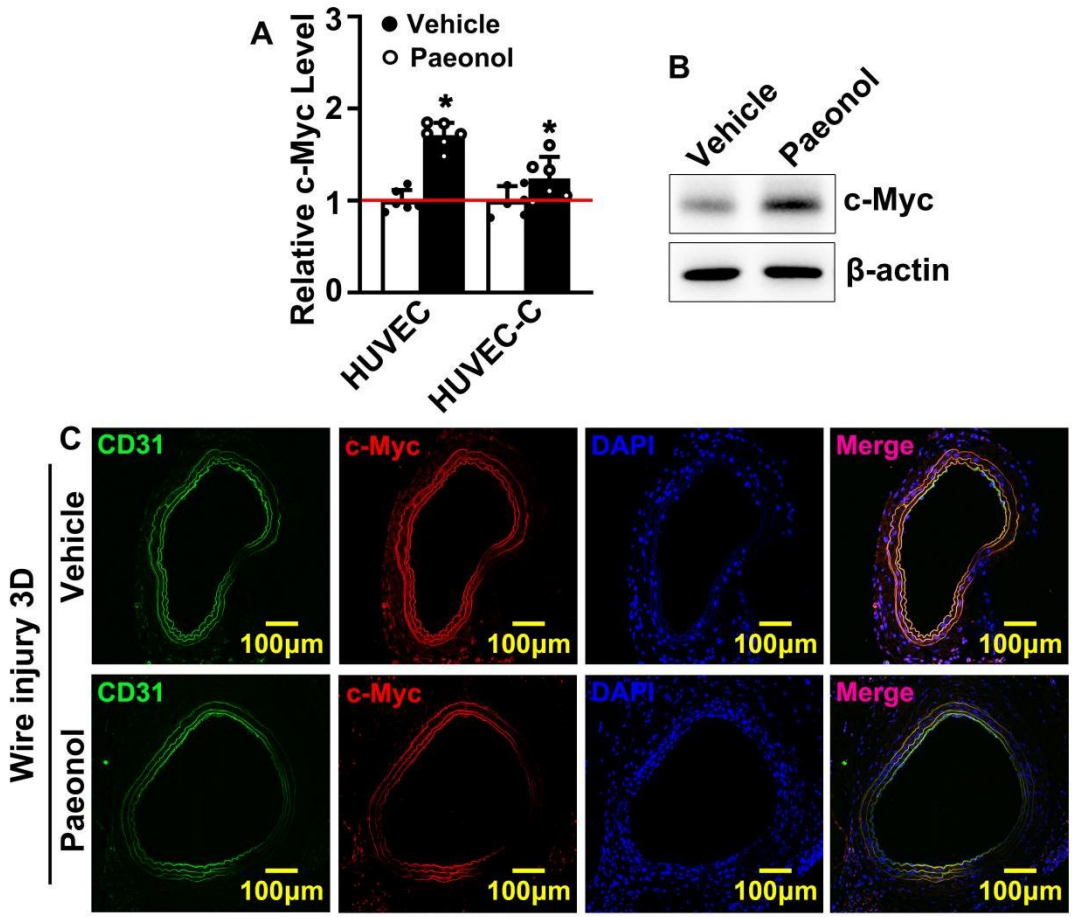
Supplementary Figure 5. IHC staining against CD31, PCNA and Ki67 antibodies on slides from left carotid arteries after Paeonol treatment for 10 days (Scale bar: 50 μm).

Supplementary figure 6



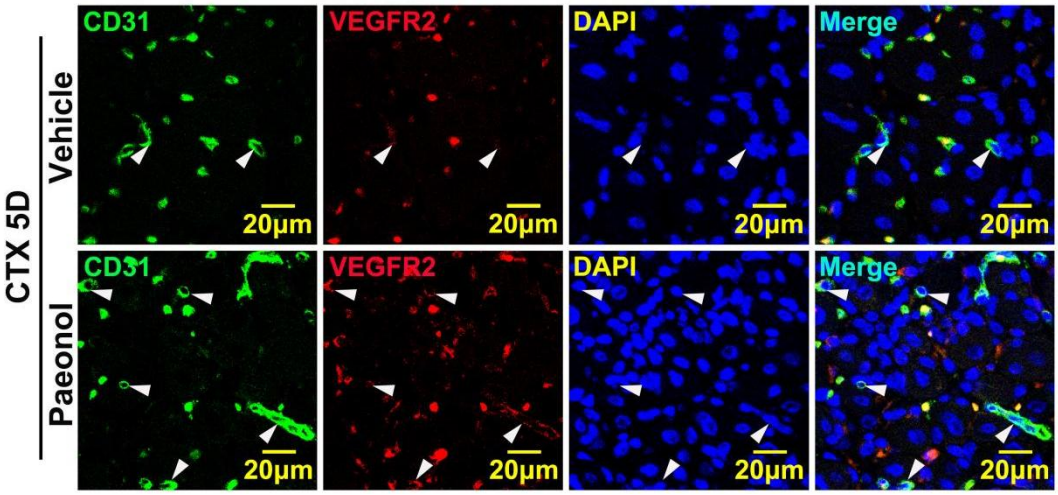
Supplementary Figure 6. HUVECs were treated with Paeonol, and Real Time PCR performed to determine angiogenesis related genes (n=6, unpaired *t* test). Quantitative data presented as mean ± SEM, *P < 0.05 was considered significant.

Supplementary figure 7



Supplementary Figure 7. (A) The mRNA level of c-Myc in HUVECs and HUVEC-Cs was analyzed by Real Time PCR after Paeonol treatment (n=6, unpaired *t*-test). (B) Western blot analysis of c-Myc protein expression in HUVEC-Cs after Paeonol treatment for 48 hours. (C) IF staining against c-Myc antibody performed on carotid artery after Paeonol treatment for 3 days (Scale bar: 100 μm). Quantitative data presented as mean ± SEM, *P < 0.05 was considered significant.

Supplementary figure 8



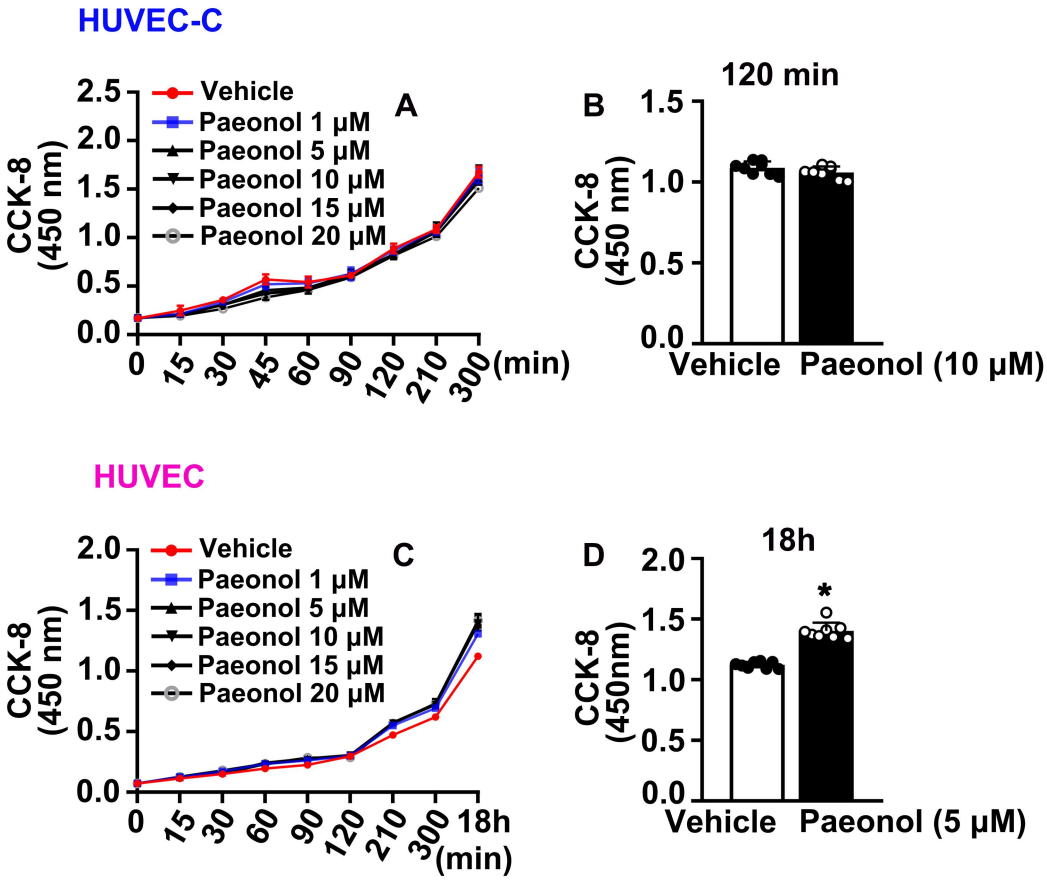
Supplementary Figure 8. IF staining against CD31 and VEGFR2 antibodies in skeletal muscle which damaged by cardiotoxin injection following Paeonol treatment for 5 days (Scale bar: 20 μm).

Supplementary figure 9

Complex	Interface area (Å2)	Solvation free energe (kcal/mol)
Complex 1	1049.5	-12.6
Complex 2	1001.1	-12.2
Complex 3	1123.4	-11.6
Complex 4	1000.4	-12.2
Complex 5	1069.4	-12.4

Supplementary Figure 9. Interface area and solvation free energy of complexes obtained from c-Myc and VEGFR2 protein docking.

Supplementary figure 10



Supplementary Figure 10. (A, B) HUVEC-C treated with different doses of Paeonol and cell viability determined by CCK8 assay. (C, D) HUVEC treated with different doses of Paeonol and cell viability determined by CCK8 assay. Quantitative data presented as mean ± SEM, *P < 0.05 was considered significant.