

Supplement Figures

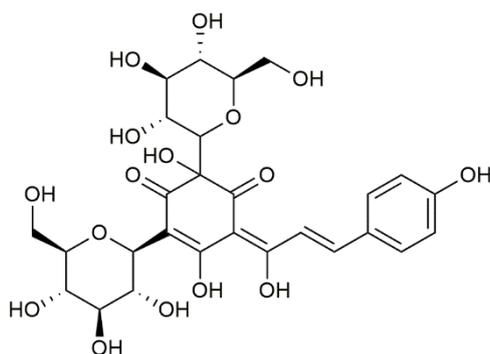


Figure S1. The structure of hydroxysafflor yellow A (HSYA).

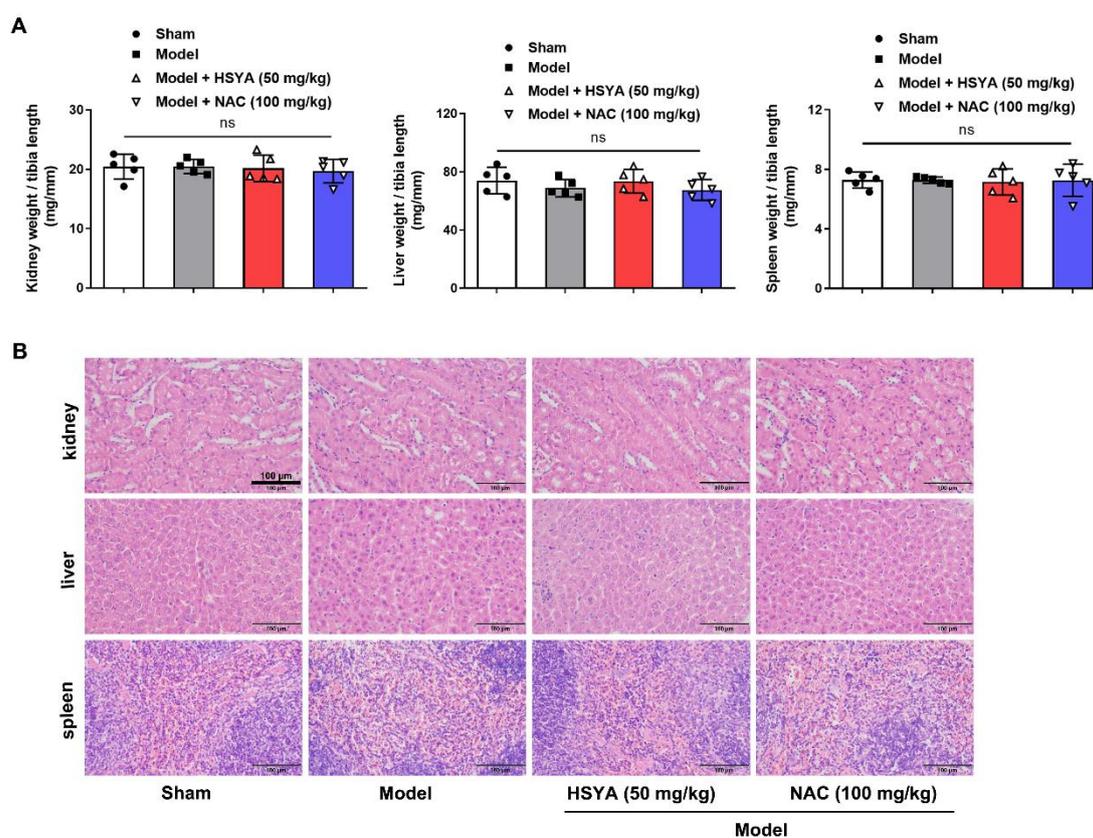


Figure S2. HSYA had no adverse effects on mice. Mice were intraperitoneally injected with HSYA and NAC for 3 days after photothrombotic stroke. **A** The kidney, liver and spleen of the mice were weighted and tibia length was measured. The ratios of kidney, liver and spleen mass to tibia length were calculated. **B** Histopathological examination in the kidney, liver and spleen tissue of the mice (scale bar: 100 μ m). All data were presented as mean \pm SD of five independent experiments. ns: no significant difference.

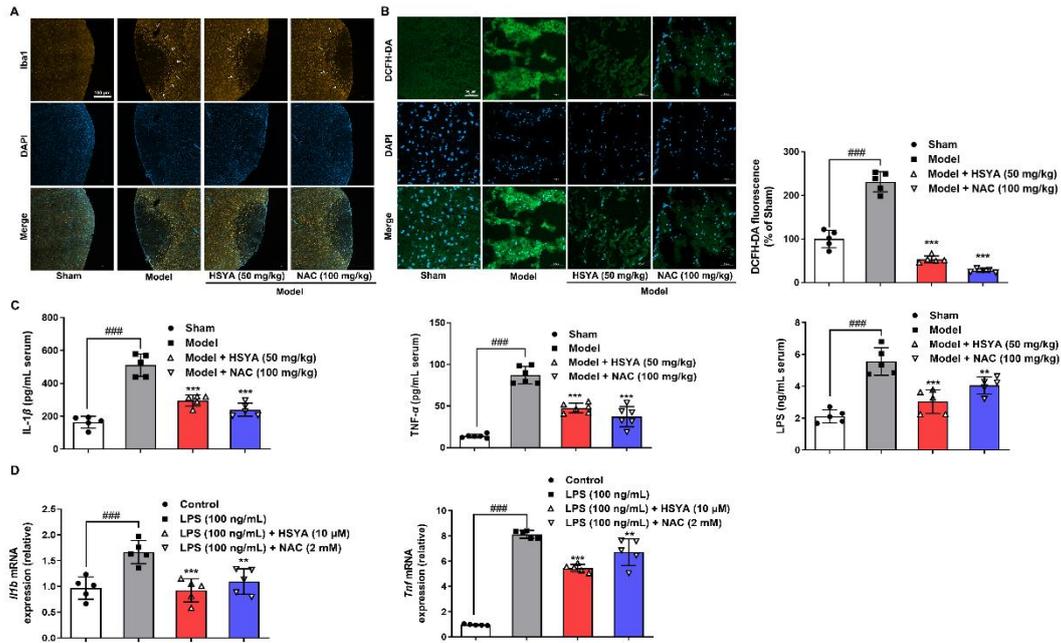


Figure S3. HSYA suppressed inflammatory response. Mice were intraperitoneally injected with HSYA and NAC for 3 days after photothrombotic stroke. **A** Immunofluorescence staining of Iba1 in the peri-infarct zones of brain tissue (scale bar: 100 μm). **B** ROS production was labeled with a DCFH-DA probe in the peri-infarct zones of brain tissue (scale bar: 50 μm). PL: peri-lesion. **C** The levels of IL-1β, TNF-α, and LPS in the serum were measured. **D** Gene expression of *Il1b* and tumor necrosis factor (*Tnf*) in bEnd.3 cells when exposed to LPS. All data were presented as mean ± SD of five independent experiments. ###*P* < 0.001 vs. indicated group, ***P* < 0.01, ****P* < 0.001 vs. LPS group.

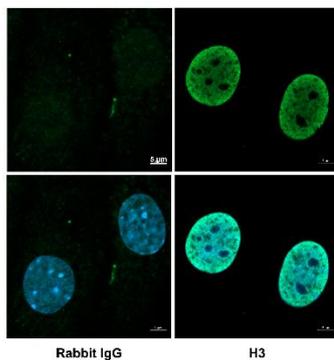


Figure S4. Negative and positive controls for intracellular cytoplasmic proteins and nucleoproteins. The expressions of IgG and histone 3 (H3) in bEnd.3 cells were observed by confocal laser microscopy (scale bar: 5 μm). All data were presented from five independent experiments.

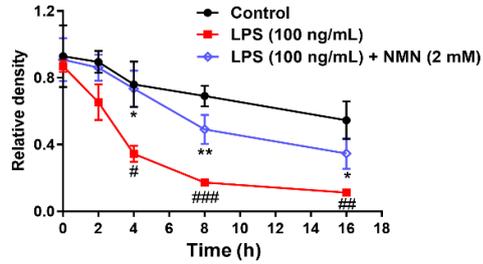
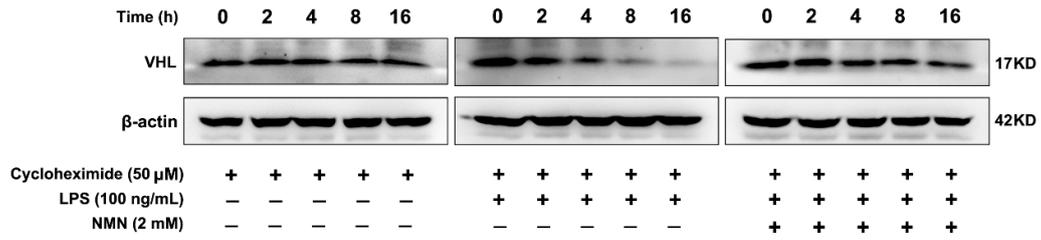


Figure S5. HSYA prevented VHL degradation. When protein synthesis was inhibited by cycloheximide in bEnd.3 cells, VHL protein degradation from 2 h to 16 h was determined by western blot. All data were presented as mean \pm SD of five independent experiments. # $P < 0.05$, ## $p < 0.01$, ### $P < 0.001$ vs. Control group, * $P < 0.05$, ** $P < 0.01$ vs. LPS group.

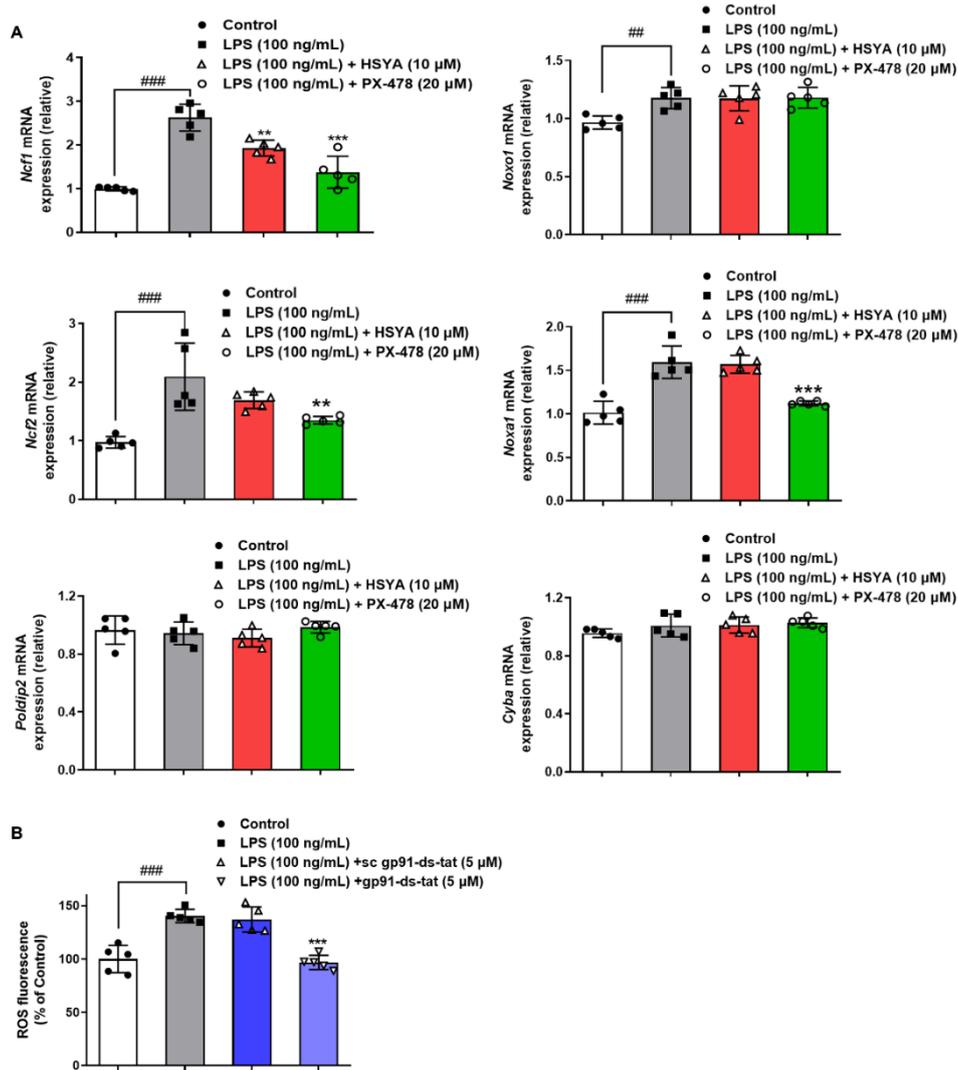


Figure S6. The expression of NOXs in bEnd.3 cells. A Gene levels of *Ncf1*, *Nox1*, *Nox2*, *Poldip2* and *Cyba* in LPS-induced bEnd.3 cells. **B** bEnd.3 cells were treated with sc gp91-ds-tat or gp91-ds-tat, and the intracellular ROS content was detected by fluorescein microplate analyzer. All data were presented as mean \pm SD of five independent experiments. ## $P < 0.01$, ### $P < 0.001$ vs. Control group, ** $P < 0.01$, *** $P < 0.001$ vs. LPS group.

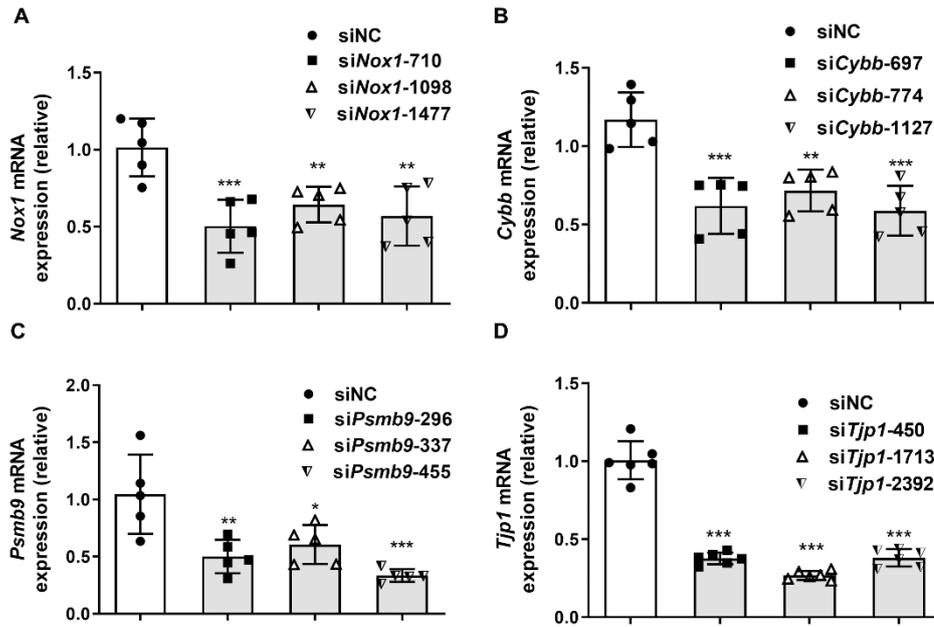


Figure S7. Small interfering RNA (siRNA) screening. bEnd.3 cells were transfected with si*Nox1*, si*Cybb*, si*Psm9*, si*Tjp1* or siNC using lipofectamine 3000 transfection reagent for 48 h. **A-D** the gene levels of *Nox1*, *Cybb*, *Psm9* and *Tjp1* were detected by qRT-PCR. All data were presented as mean \pm SD of five or six independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. siNC treatment.

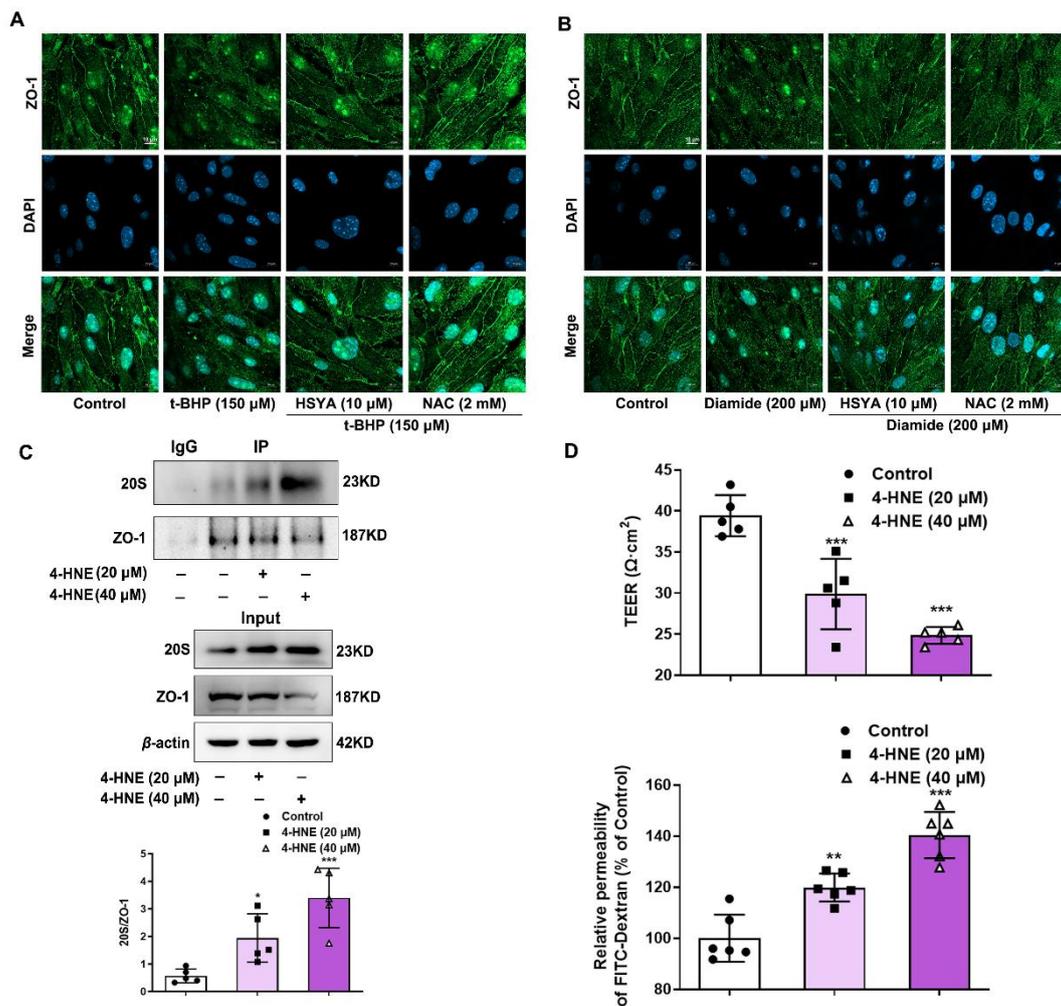


Figure S8. HSYA preserved ZO-1 protein stability. A-B Immunofluorescence observed ZO-1 protein expression in the presence of t-BHP or diamide (scale bar: 10 μm). C the binding of 20S to ZO-1 was determined by immunoprecipitation. D the TEER values and the absorbance of FITC-dextran across the bEnd.3 monolayer cells. All data were presented as mean \pm SD of five independent experiments. $**P < 0.01$, $***P < 0.001$ vs. Control group.

Table S1. Primer sequences for qRT-PCR

Primers	Sequences	Gene accession number
<i>Sod1</i>	Forward 5'- GACTGCTGGAAAGGACGGTGTG -3'	AK020624.1
	Reverse 5'- ACGGCCAATGATGGAATGCTCTC -3'	
<i>Cat</i>	Forward 5'- CATAGCCAGAAGAGAAACCCACAGAC -3'	AK075853.1
	Reverse 5'- AATCCCTCGGTCCTGAACAAGAAAG -3'	
<i>Ncf1</i>	Forward 5'- TGGTGGGTGGTCAGGAAAGGG -3'	AK149668.1
	Reverse 5'- ATGCTCTGTGCGTTGCGGATG -3'	
<i>Cybb</i>	Forward 5'- CGAAGACAACCTGGACAGGAACCTC -3'	AK053920.1
	Reverse 5'- AAATCCCGACTCTGGCATTACACAC -3'	
<i>Tjp1</i>	Forward 5'- GACCTTGAGCAGCCGTCATACAG -3'	AK144506.1
	Reverse 5'- CGTAGGCGATGGTCATAGTTCCG -3'	
<i>Ocln</i>	Forward 5'- TGGCTATGGAGGCGGCTATGG -3'	AK019880.1
	Reverse 5'- AAGGAAGCGATGAAGCAGAAGGC -3'	
<i>Cldn 5</i>	Forward 5'- TGGTGCTGTGTCTGGTAGGATGG -3'	AK077282.1
	Reverse 5'- GTCACGATGTTGTGGTCCAGGAAG -3'	
<i>Il1b</i>	Forward 5'- TCGCAGCAGCACATCAACAAGAG -3'	AK225002.1
	Reverse 5'- AGGTCCACGGGAAAGACACAGG -3'	
<i>Tnf</i>	Forward 5'- ATGTCTCAGCCTCTTCTCATTG -3'	AK153319.1
	Reverse 5'- GCTTGTCCTCGAATTTTGAGA -3'	
<i>Psmb9</i>	Forward 5'- GCGGGAACAGCAGTGGTGAAC -3'	AK008429.1
	Reverse 5'- CTTGGGCATCAGCAGCGGAAC -3'	
<i>Nox1</i>	Forward 5'- CCTGATTCCTGTGTGTCGAAA -3'	AK136432.1
	Reverse 5'- TTGGCTTCTTCTGTAGCGTTC -3'	
<i>Nox4</i>	Forward 5'- CCTTTTACCTATGTGCCGGAC -3'	AK050371.1
	Reverse 5'- CATGTGATGTGTAGAGTCTTGCT -3'	
<i>Noxol</i>	Forward 5'- CTTGGTGCAAATGGACCGACT -3'	AF399754.1
	Reverse 5'- CCAGCTCCTCCGCACAAAT -3'	
<i>Noxa1</i>	Forward 5'- CTCTCTAGGGGATCAGATACGG -3'	AK087263.1
	Reverse 5'- GTCCTGCTTGGTCAAATGCC -3'	
<i>Ncf2</i>	Forward 5'- GGAGAAGTACGACCTTGCTATCA -3'	AK036379.1
	Reverse 5'- ACAGGCAAACAGCTTGAAGT -3'	
<i>Poldip2</i>	Forward 5'- TTTGAGGTGCCAAAACAAAATGG -3'	AK003702.1

	Reverse	5'- CCCTCGGTAGCCAAAAACAC -3'	
<i>Cyba</i>	Forward	5'- CTA CTGCTGGACGTTTCACAC -3'	AK018713.1
	Reverse	5'- GGTGGACCCCTTTTCTCTT -3'	
<i>Actb</i>	Forward	5'- CTACCTCATGAAGATCCTGACC -3'	AK147787.1
	Reverse	5'- CACAGCTTCTCTTTGATGTCAC -3'	

Table S2. Primer sequences for ChIP-qPCR

Primers		Sequences	Gene accession number
<i>Ncf1</i> promoter (site 1)	Forward	5'- TCCTCCTCCACTGCTGGAAT -3'	NC_000072
	Reverse	5'- CAGGAGGTCTCTGGGACTCA -3'	
<i>Ncf1</i> promoter (site 2)	Forward	5'- ATCAGTCCCTGCTTTCTCCG -3'	
	Reverse	5'- AGATAGCCTGGCCTACGTGA -3'	
<i>Cybb</i> promoter (site 1)	Forward	5'- GCTGTCAGCATGGGTCTCTT -3'	NC_000086
	Reverse	5'- ACAGTTGTTGCCCTGGAGTC -3'	
<i>Cybb</i> promoter (site 2)	Forward	5'- AATGTGGGGCCTGAGGAATG -3'	
	Reverse	5'- TATCTTCCGCACCTCCAGC -3'	