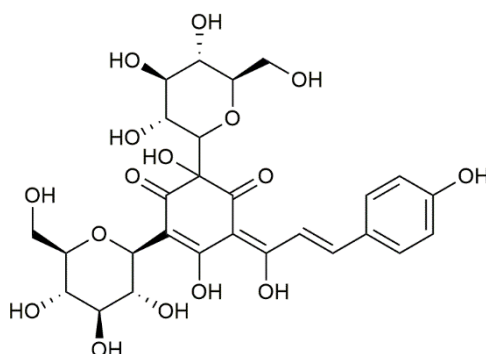
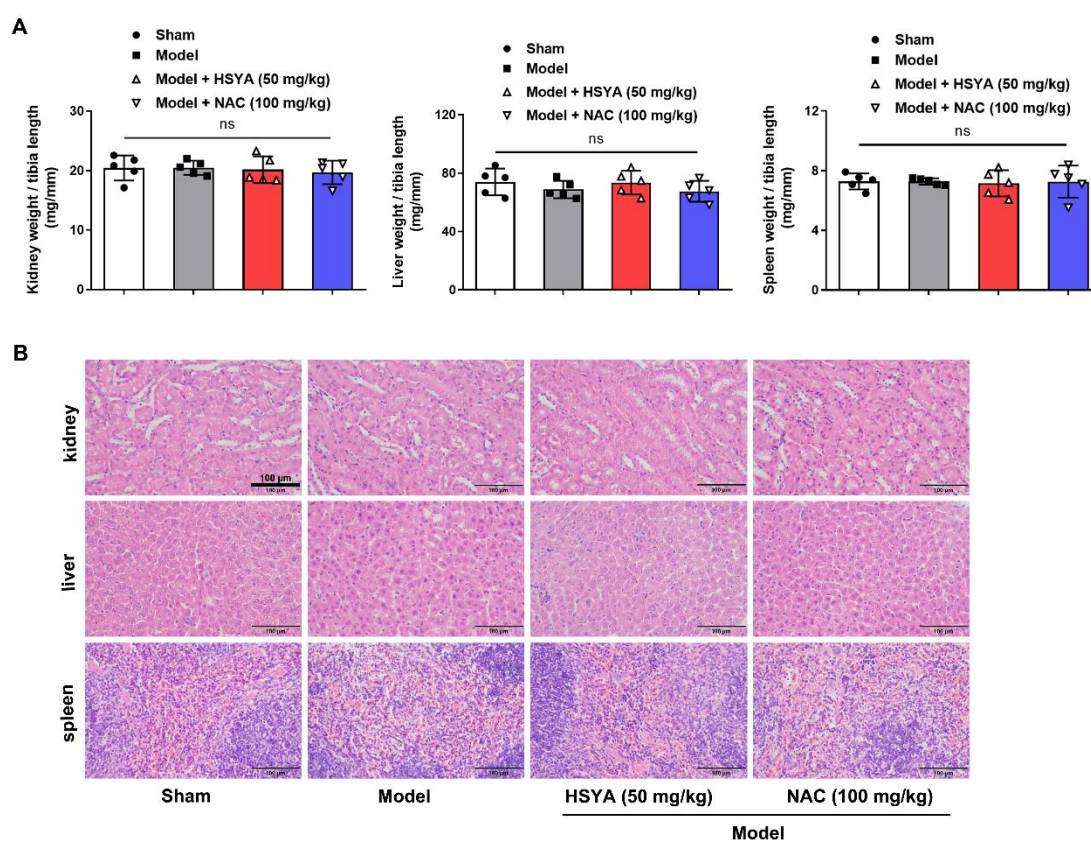


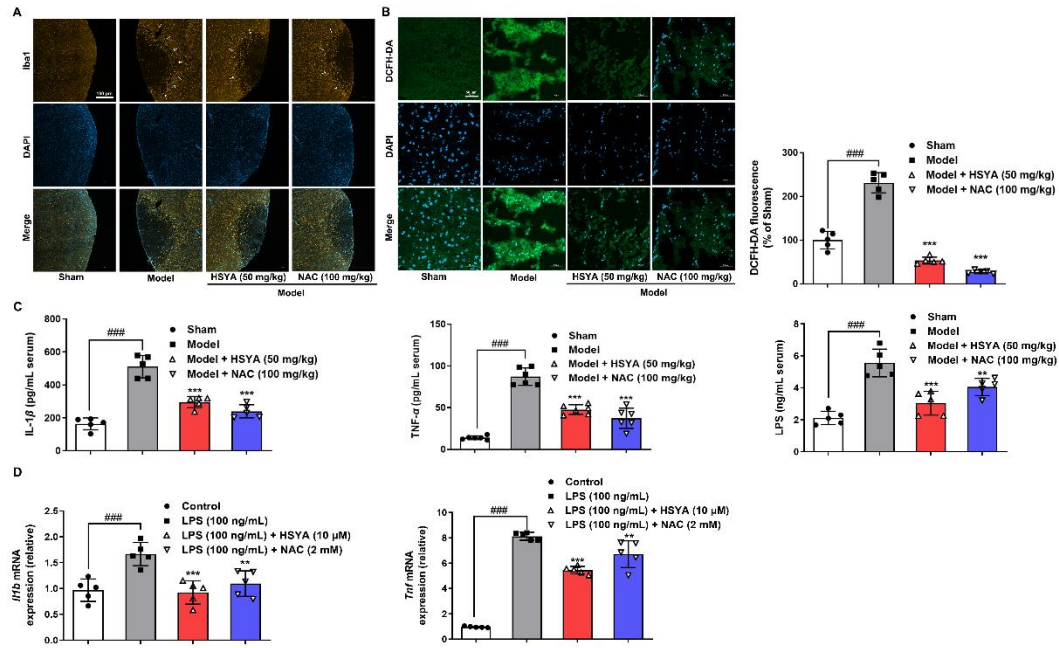
## Supplement Figures



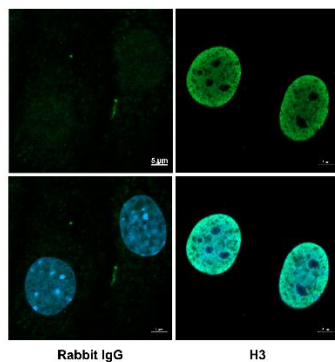
**Figure S1.** The structure of hydroxysafflor yellow A (HSYA).



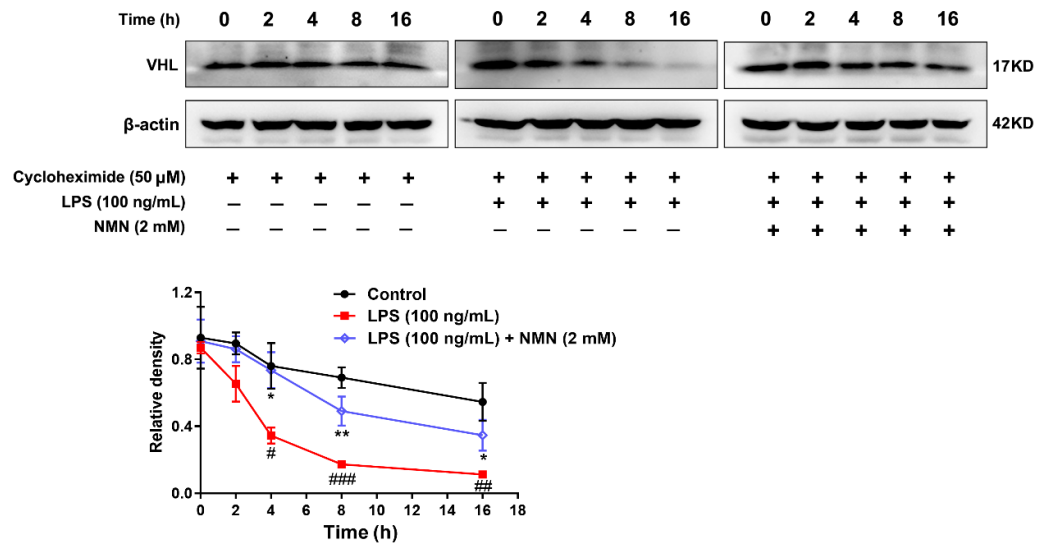
**Figure S2. HSYA had no adverse effects on mice.** Mice were intraperitoneally injected with HSYA and NAC for 3 days after photothrombotic stroke. **A** The kidney, liver and spleen of the mice were weighted and tibia length was measured. The ratios of kidney, liver and spleen mass to tibia length were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



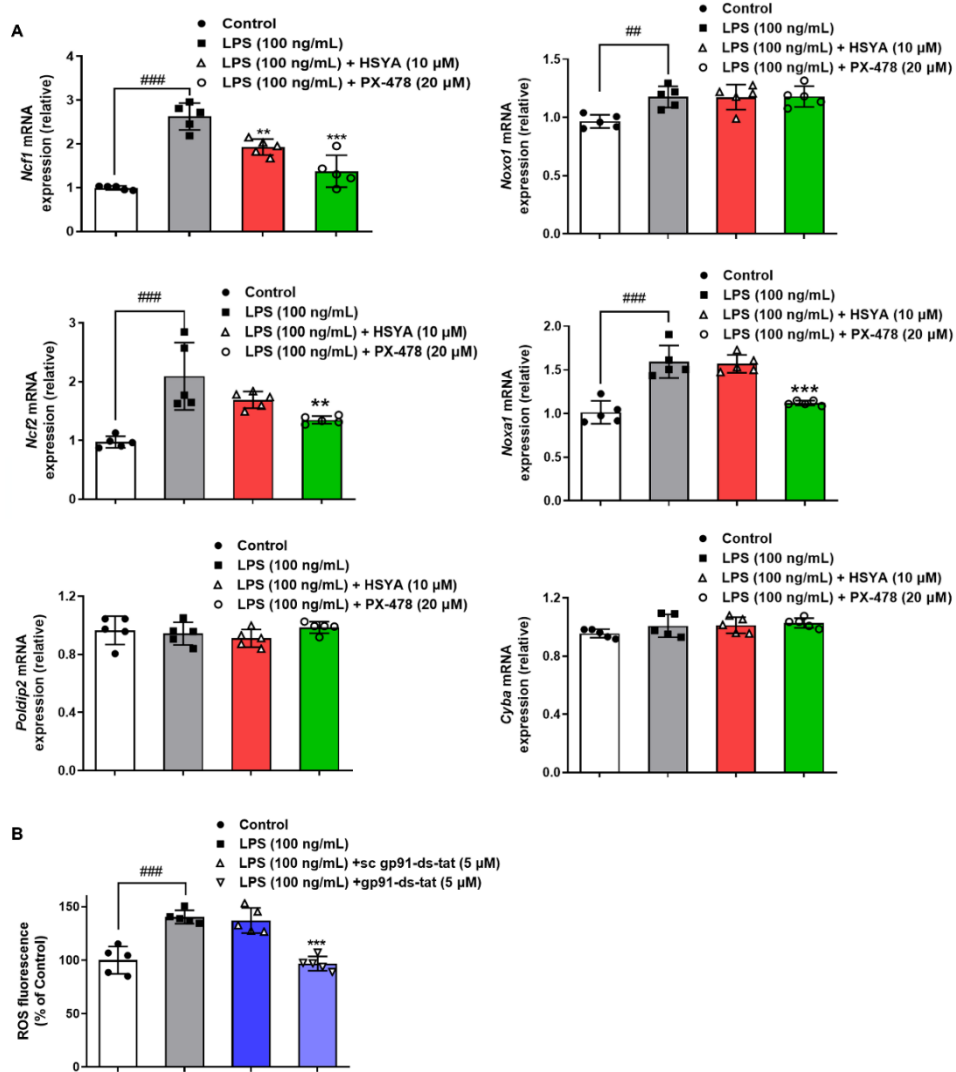
**Figure S3. HSYA suppressed inflammatory response.** Mice were intraperitoneally injected with HSYA and NAC for 3 days after photothrombotic stroke. **A** Immunofluorescence staining of Iba1 in the peri-infarct zones of brain tissue (scale bar: 100  $\mu$ m). **B** ROS production was labeled with a DCFH-DA probe in the peri-infarct zones of brain tissue (scale bar: 50  $\mu$ m). PL: peri-lesion. **C** The levels of IL-1 $\beta$ , TNF- $\alpha$ , and LPS in the serum were measured. **D** Gene expression of *Ilf1* and tumor necrosis factor (*Tnf*) in bEnd.3 cells when exposed to LPS. All data were presented as mean  $\pm$  SD of five independent experiments. <sup>###</sup> $P$  < 0.001 vs. indicated group, <sup>\*\*</sup> $P$  < 0.01, <sup>\*\*\*</sup> $P$  < 0.001 vs. LPS group.



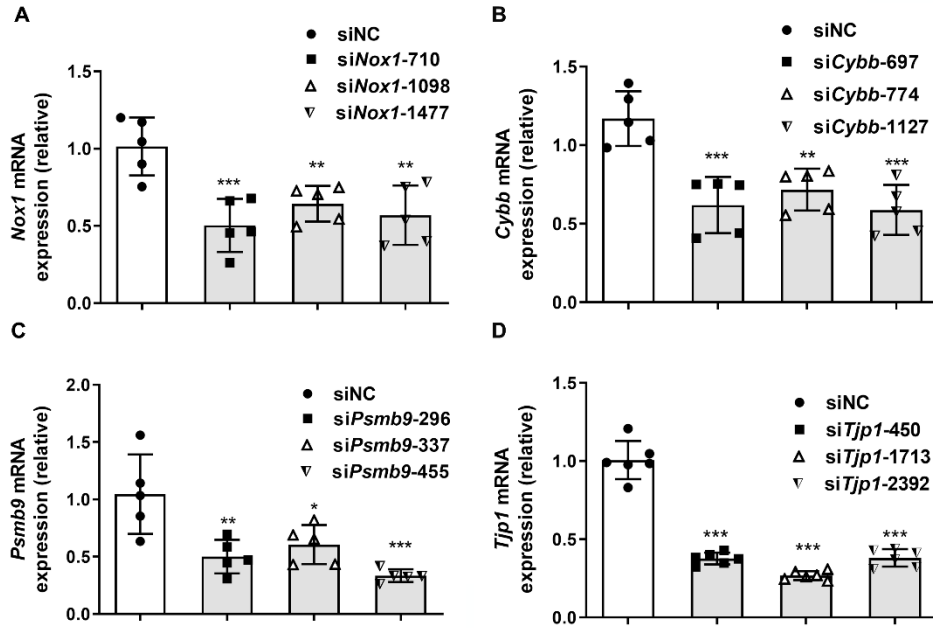
**Figure S4. Negative and positive controls for intracellular cytoplasmic proteins and nucleoproteins.** The expressions of IgG and histone 3 (H3) in bEnd.3 cells were observed by confocal laser microscopy (scale bar: 5  $\mu$ m). All data were presented from five independent experiments.



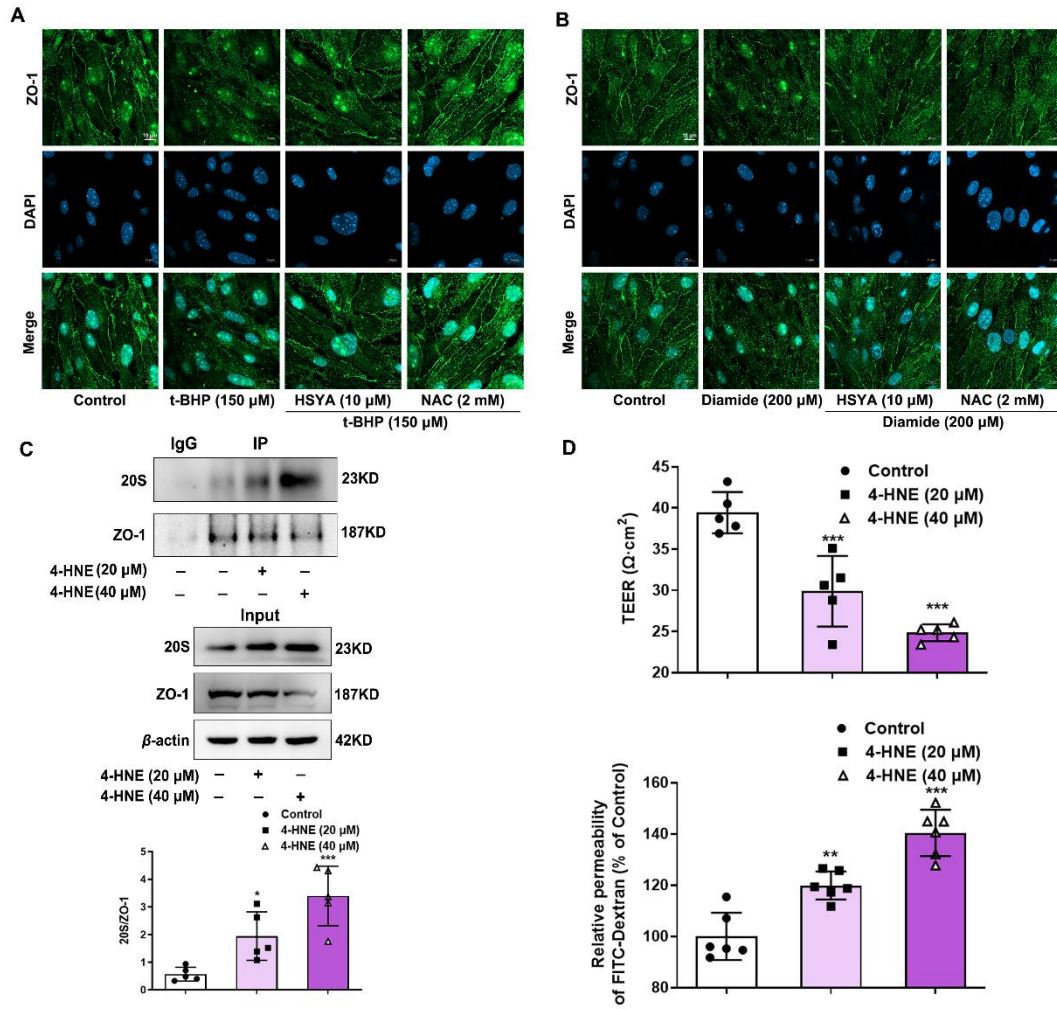
**Figure S5. HSYA prevented VHL degradation.** When protein synthesis was inhibited by cycloheximide in bEnd.3 cells, VHL protein degradation from 2 h to 16 h was determined by western blot. All data were presented as mean  $\pm$  SD of five independent experiments. #  $P < 0.05$ , ##  $p < 0.01$ , ###  $P < 0.001$  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 siNox1, siCybb, siPsm9, siTjp1 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  $\mu$ m). 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'- AATCCCTCGGTCACTGAACAAGAAAG -3'	
<i>Ncf1</i>	Forward	5'- TGGTGGGTGGTCAGGAAAGGG -3'	AK149668.1
	Reverse	5'- ATGCTCTGTGCGTTGCGGATG -3'	
<i>Cybb</i>	Forward	5'- CGAAGACAACTGGACAGGAACCTC -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'- ATGTCTCAGCCTCTTCTCATTC -3'	AK153319.1
	Reverse	5'- GCTTGTCACCTCGAATTTTGAGA -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>Noxo1</i>	Forward	5'- CTTGGTGCAAATGGACCGACT -3'	AF399754.1
	Reverse	5'- CCAGCTCCTCCGCACAAAT -3'	
<i>Noxa1</i>	Forward	5'- CTCTCTAGGGGATCAGATACGG -3'	AK087263.1
	Reverse	5'- GTCAGTCTTGGTCAAATGCC -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'- CTACTGCTGGACGTTTCACAC -3'	AK018713.1
	Reverse	5'- GGTGGACCCCTTTTTCCTCTT -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'- TATCTTTCCGCACCTCCAGC -3'	