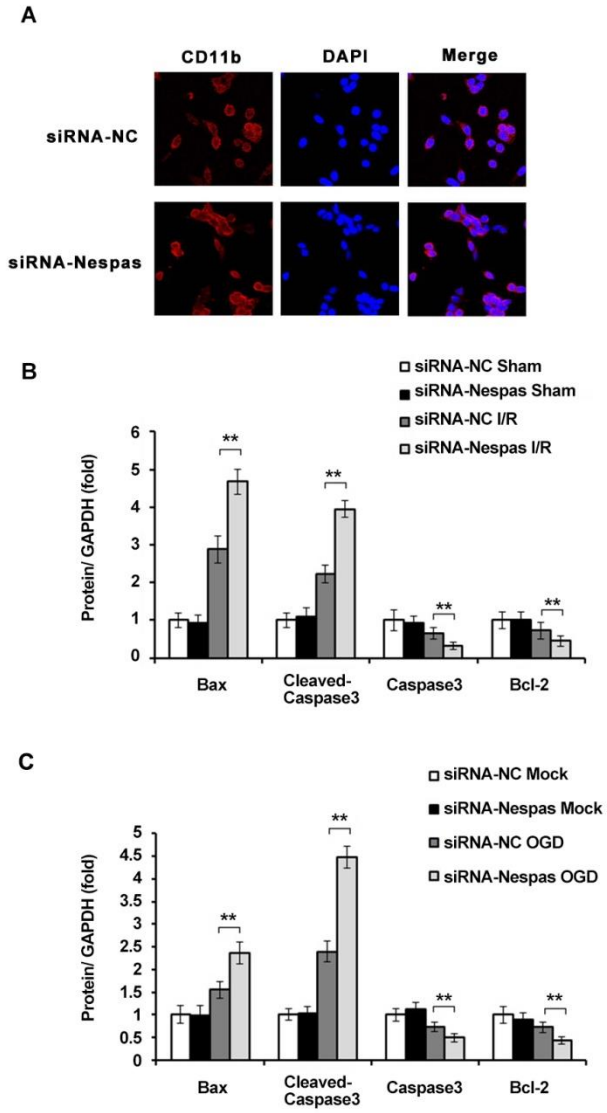
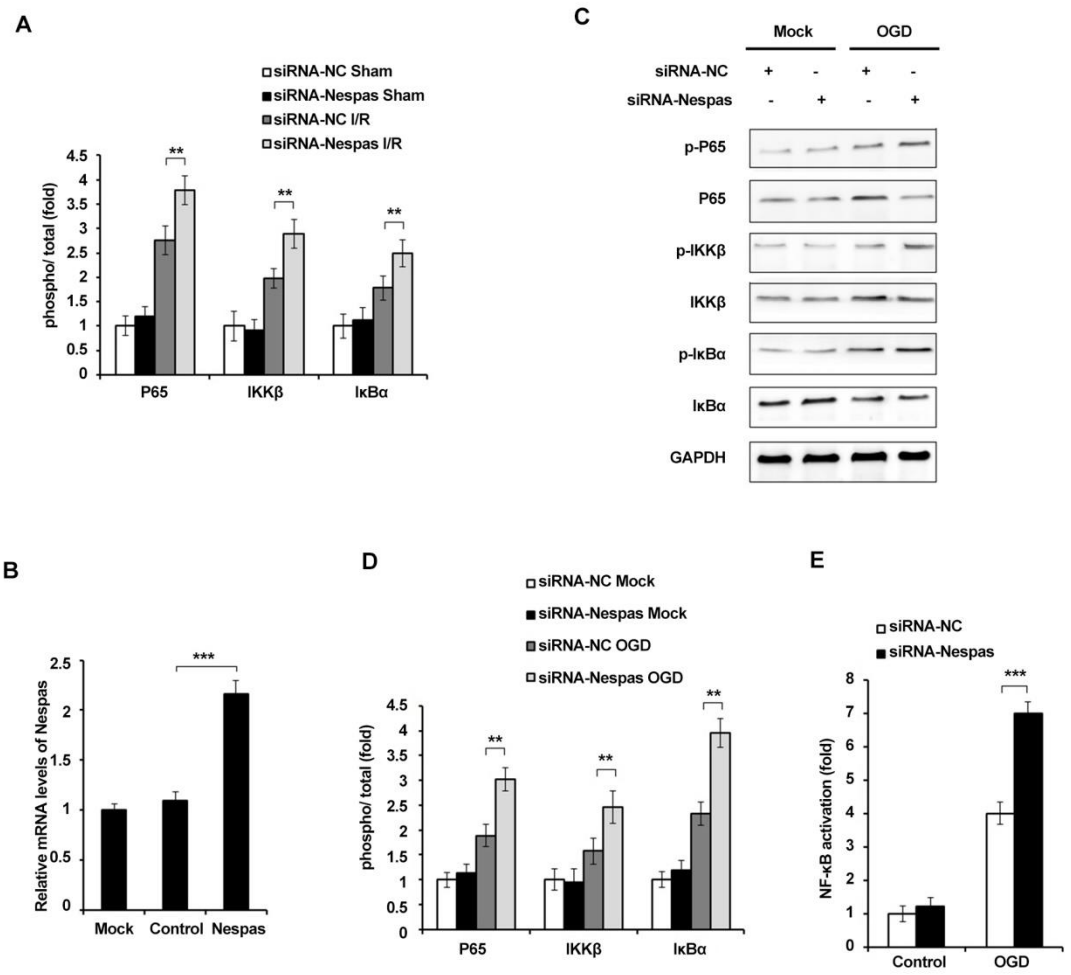
SUPPLEMENTAL MATERIAL

Table I. List of primers used in the study.

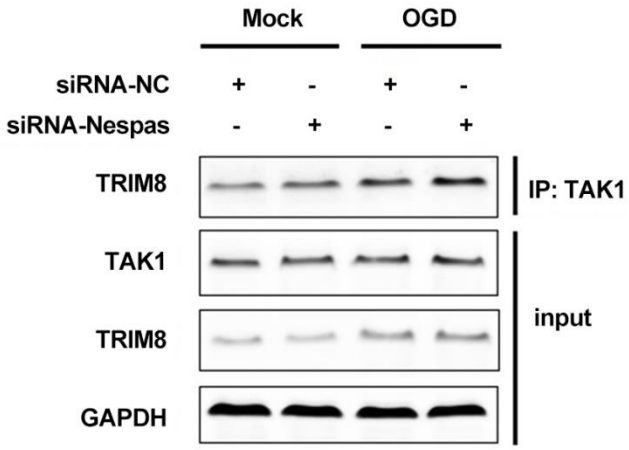
Number	Gene		Sequence (5'-3')
1	Nespas	F	AGA TGC TGA ACC CTG CAC AA
		R	TGG CAG TTG TTT TGT GGT GC
2	TNF-α	F	CTA AGA GGG AGA GAA GCA
		R	AGA GGC TGA GGA ACA AGC
3	IL-1β	F	AGC TAC GAA TCT CCG ACC
		R	TGG CCA CAA CAA CTG ACG
4	IL-6	F	CCT TCG GTC CAG TTG CCT TCT
		R	CCA GTG CCT CTT TGC TGC TTT
5	MCP-1	F	TAC ATG GGA GAC TCT GGG GG
		R	TTC TGG TGA AGA CTG CAG GG
6	VCAM-1	F	TCA GGA AAT GCC ACC CTC AC
		R	TGG CAA ACA TTA GGT GTA CAG T
7	ICAM-1	F	TGG CGG GAA AGT TCC TGT TT
		R	AGT CTG CTG AGA CCC CTC TT



Supplemental Figure I. A. Immunofluorescence labeling of CD11b in microglial cells of MCAO mice (200×). B, Quantification of the protein level of Bax, cleaved-caspase3, caspase3, Bcl-2 expression of Figure 3B. C, Quantification of the protein level of Bax, cleaved-caspase3, caspase3, Bcl-2 expression of Figure 3D. Data are the representative of 3 independent experiments with similar results (mean± SD). **P < 0.01.



Supplemental Figure II. A, Quantification of phosphorylation level of P65, IKKβ, IκBα of Figure 4B. **B**, Efficiency of Nespas overexpression in BV2 cells. **C** and **D**, Western blot analysis (C) and quantification (D) of phosphorylation level of P65, IKKβ, IκBα in BV2 microglial cells after OGD treatment for 24h. **E**, Dual-Luciferase reporter gene assay was performed to examine the activation of NF-κB in Nespas silenced BV2 cell after OGD treatment for 24h. Data are the representative of 3 independent experiments with similar results (mean± SD). **P < 0.01, ***P < 0.001.



Supplemental Figure III. Immunoprecipitation assay was used to detect the interaction between TRIM8 and TAK1 in Nespas silenced BV2 cells after OGD treatment for 24h.

*** Preclinical Checklist**

Preclinical Checklist: Prevention of bias is important for experimental cardiovascular research. ***This short checklist must be completed, and the answers should be clearly presented in the manuscript.*** *The checklist will be used by reviewers and editors and it will be published. See "Reporting Standard for Preclinical Studies of Stroke Therapy" and "Good Laboratory Practice: Preventing Introduction of Bias at the Bench" for more information.*

This study invovles animal models:
Yes

Experimental groups and study timeline

The experimental group(s) have been clearly defined in the article, including number of animals in each experimental arm of the study:	Yes
An account of the control group is provided, and number of animals in the control group has been reported. If no controls were used, the rationale has been stated:	Yes
An overall study timeline is provided:	Yes

Inclusion and exclusion criteria

A priori inclusion and exclusion criteria for tested animals were defined and have been reported in the article:	Yes

Randomization

Animals were randomly assigned to the experimental groups. If the work being submitted does not contain multiple experimental groups, or if random assignment was not used, adequate explanations have been provided:	Yes
Type and methods of randomization have been described:	Yes
Methods used for allocation concealment have been reported:	Yes

Blinding

Blinding procedures have been described with regard to masking of group/treatment assignment from the experimenter. The rationale for nonblinding of the experimenter has been provided, if such was not feasible:	Yes
Blinding procedures have been described with regard to masking of group assignment during outcome assessment:	Yes

Sample size and power calculations

Formal sample size and power calculations were conducted based on a priori determined outcome(s) and treatment effect, and the data have been reported. A formal size assessment was not conducted and a rationale has been provided:	Yes

Data reporting and statistical methods

Number of animals in each group: randomized, tested, lost to follow-up, or died have been reported. If the experimentation involves repeated measurements, the number of animals assessed at each time point is provided, for all experimental groups:	Yes
Baseline data on assessed outcome(s) for all experimental groups have been reported:	Yes
Details on important adverse events and death of animals during the course of experimentation have been provided, for all experimental arms:	Yes
Statistical methods used have been reported:	Yes
Numeric data on outcomes have been provided in text, or in a tabular format with the main article or as supplementary tables, in addition to the figures:	Yes

Experimental details, ethics, and funding statements

Details on experimentation including stroke model, formulation and dosage of therapeutic agent, site and route of administration, use of anesthesia and analgesia, temperature control during experimentation, and postprocedural monitoring have been described:	Yes
Different sex animals have been used. If not, the reason/justification is provided:	Yes

Statements on approval by ethics boards and ethical conduct of studies have been provided:	Yes
Statements on funding and conflicts of interests have been provided:	Yes

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