APPENDIX A

(List of chemical and material used in the study)

Name of chemicals	Company
Acrylamide	Sigma-Aldrich, USA
Akt antibody	Cell signaling, USA
Ammonium persulfate	Applichem, Germany
Aprotinin	Sigma-Aldrich, USA
Bovine serum albumin	Sigma-Aldrich, USA
C-JUN antibody	Cell signaling, USA
Coomassie plus™ protein assay reagent	Thermo scientific, USA
COX-2 antibody	Cell signaling, USA
Dimethyl sulfoxide	Merck, Germany
Dulbecco's Modified Eagle Medium	GIBCO, USA
EDTA	GIBCO, USA
ERK1/2 antibody	Cell signaling, USA
Fetal bovine serum	Hyclone,USA
Glycine	Vivantis Inc, USA
Griess buffer	Sigma-Aldrich, USA
HEPES	Sigma-Aldrich, USA
HRP-linked anti mouse IgG	Cell signaling, USA
HRP-linked anti rabbit IgG	Cell signaling, USA
iNOS antibody	Merck Millipore, USA
JNK antibody	Cell signaling, USA
Leupeptin	Sigma-Aldrich, USA
Lipopolysaccharide	Sigma-Aldrich, USA
LY294002	Cell signaling, USA
Methanol	Sigma-Aldrich, USA

Mercaptoethanol	Sigma-Aldrich, USA
Mouse IL-6 enzyme-linked immunosorbent assay kits	Biolegend, USA
Mouse TNF-α enzyme-linked immunosorbent assay kits	Biolegend, USA
MTT dye	USB Corporation, USA
NF-κB p65 antibody	Cell signaling, USA
p38 antibody	Cell signaling, USA
PAPR antibody	Santa Cruz, USA
PD98059	Cell signaling, USA
Penicillin-streptomycin	GIBCO, USA
PGE_2 Immunoassay ELISA kit	Abcam, USA
Pierce® RIPA buffer	Thermo scientific, USA
Pierce® ECL plus western blotting substrate	Thermo scientific, USA
Phospho p38 antibody	Cell signaling, USA
Phospho Akt antibody	Cell signaling, USA
Phospho C-JUN antibody	Cell signaling, USA
Phospho ERK1/2 antibody	Cell signaling, USA
Phospho JNK antibody	Cell signaling, USA
Phospho NF-κB p65 antibody	Cell signaling, USA
Potassium chloride	VWR chemical, Belgium
Potassium phosphate	VWR chemical, Belgium
SB202190	Invivogen, USA
Skim milk	Sigma-Aldrich, USA
Sodium hydrogen carbonate	VWR chemical, Belgium
Sodium hydrogen phosphate anhydrous	VWR chemical, Belgium
Sodium chloride	Vivantis Inc, USA
Sodium dodecyl sulfate	Vivantis Inc, USA
Sodium nitrite	Sigma-Aldrich, USA
SP600125	Invivogen, USA
Restore™ western blot stripping buffer	Thermo scientific, USA
TEMED	Applichem, Germany
Tris	Vivantis Inc, USA

Triton-X100	Sigma-Aldrich, USA
Tween-20	Sigma-Aldrich, USA

APPENDIX B

(List of instruments used in the study)

Name of instruments	Company
6 or 12 or 96-well plates	Thermo scientific, USA
Automatic pipette	Labnet, USA
Carbon dioxide incubator	Thermo scientific, USA
Deionized water machine	Thermo scientific, USA
Distilled water machine	Thermo scientific, USA
Electrophoresis system	Bio-Rad, USA
Filter paper	GE Healthcare, UK
Freezer (-80 ^{o}C)	Thermo scientific, USA
Freezer (-20 ^{o}C)	Sanyo, Japan
High performance chemiluminescence film	GE Healthcare, UK
High-pressure steam sterilizer	Tomy Seiko, Japan
High speed refrigerated micro centrifuge	Tomy Seiko, Japan
Hot air oven	Heraeus, Germany
Light microscope	Olympia, Japan
Laminar flow biological cabinet	NUAIRE, USA
Magnetic stirrer	Thermolyne
Microcentrifuge tube	Thermo scientific, USA
Micro-plate spectrophotometer	Bio-Tek Instrument
Nitrocellulose membrane	GE Healthcare, UK
pH meter	EzDo, UK
Power supply	EC Appraratus Corporation
Sterile Vacuum Bottle-Top Filters	Merck, Germany
Trans-blot® electrophoretic transfer cell	Bio-Rad, USA
Vortex	Thermo scientific, USA

APPENDIX C

(Preparation of some reagents and buffers)

Cell culture

1. Incomplete DMEM medium

DMEM	1	package (13.5 g)
HEPES	2.603	g
$NaHCO_3$	3.7	g
Penicillin/streptomycin	10	mL
Deionize water	800	mL

Adjust pH to 7.4 then topped up volume with deionized water to 1,000 mL, sterile by Sterile vacuum bottle-top filters (membrane pore size 0.2 µM) and stored at 4 ^{o}C

2. Completed DMEM with 10% FBS medium

Incomplete DMEM medium	225	mL
Fetal bovine serum	25	mL

Stored at 4 ^{o}C

3. Freezing solution

Incomplete DMEM medium	15	mL
Fetal bovine serum	4	mL
Dimethyl sulfoxide	1	mL

Stored at 4 ^{o}C

Measurement of cell viability

1. Phosphate buffer saline (PBS) pH 7.4

NaCl	8	g
KCl	0.2	g
Na_2HPO_4	1.44	g
KH_2PO_4	0.24	g
Deionize water	800	mL

Adjust pH to 7.4 then topped up volume with deionized water to 1,000 mL and sterile by High-pressure steam sterilizer. Then, stored at 4 ^oC

2. MTT stock dye solution

MTT dye	1	g
PBS (pH 7.4)	200	mL

Filtrate with membrane filter pore size 0.22 µM, collected in dark container and stored at 4 ^oC

Western blot analysis

1. Whole cells lysis buffer (prepare before used)

RIPA buffer	1	mL
PMSF	5	µL
Aprotinin	10	µL
Leupeptin	2	µL

2. Hypotonic buffer

10 mM HEPES, pH 7.9	0.595	g
10 mM KCL	0.186	g
0.1 mM EDTA	0.009	g
0.1 mM EGTA	0.009	g
1 mM NaF	0.010	g

Add Deionize water to 250 mL and sterilize by autoclaving.

3. Extraction buffer

20 mM HEPES, pH 7.9	0.477	g
400 mM NaCl	2.338	g
1 mM EDTA	0.037	g
1 mM EGTA	0.038	g
1 mM NaF	0.004	g

Add Deionize water to 100 ml and sterilize by autoclaving.

4. Cytoplasmic fraction lysis buffer (prepare before used)

Hypotonic buffer	1	mL
1 mM DTT	10	µL
0.5 mM PMSF	2.5	µL
1 mM Na_3VO_4	5	µL
Aprotinin	2	µL
Leupeptin (5 mg/mL)	2	µL

5. Nuclear fraction lysis buffer (prepare before used)

Extraction buffer	1	mL
1 mM DTT	10	µL
1 mM Na_3VO_4	10	µL

6. 10% NP-40

NP-40	1	mL

Add DI water to 10 mL mix gently by inverting.

7. PBST buffer

NaCl	8.18	g
KCl	0.201	g
Na_2HPO_4	1.42	g
KH_2PO_4	0.244	g
Deionize water	800	mL
Tween-20	3	mL

Adjust pH to 7.4 then topped up volume with deionized water to 1,000 mL and stored at 4 ^oC

8. Running buffer

Tris	3.04	g
Glycine	14.44	g
SDS	1	g
Deionize water	1,000	mL

9. Transfer buffer

Tris	3.03	g
Glycine	14.4	g
Deionize water	500	mL
Methanol	200	mL

10. 5% Blocking solution

Skim milk	5	g
PBST buffer	100	mL

11. Stock solution A : Separating gel buffer 1.5 mM Tris HCl, pH 8.8

Tris	18.18	g
Deionize water	80	mL

Adjust pH to 7.4 then topped up volume with deionized water to 100 mL, filtrate with membrane filter pore size 0.22 µM, collected in dark container and stored at 4 ^oC

12. Stock solution C : 30% w/v Acrylamide

Acrylamide	29.2	g
Bis-acrylamide	0.8	g
Deionize water	80	mL

Topped up volume with deionized water to 100 mL, filtrate with membrane filter pore size 0.22 µM, collected in dark container and stored at 4 ºC

13. Stock solution D : separating gel buffer 1.5 mM Tris HCl, pH 6.8

Tris	6.05	g
Deionize water	80	mL

Adjust pH to 7.4 then topped up volume with deionized water to 100 mL, filtrate with membrane filter pore size 0.22 µM, collected in dark container and stored at 4 ºC

14. 10 % w/v APS

Ammonium persulfate	1	g
Deionize water	10	mL

Stored at -20 ºC

15. 10% w/v SDS

Sodium dodecyl sulfate	10	g
Deionize water	100	mL

16. 10% Separating gel SDS-PAGE

Stock solution C	3.33	mL
Stock solution A	2.5	mL
Deionize water	3.5	mL
10% SDS	100	µL
10% APS	100	µL
TEMED	5	µL

17. 3.9% Acrylamide stacking gel SDS-PAGE

Stock solution D	1.25	mL
Deionize water	3.05	mL
Stock solution C	650	µL
10% SDS	50	µL
10% APS	50	µL
TEMED	10	µL

CURRICULUM VITAE

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