APPENDICES

APPENDIX A

List of the chemicals and materials used in this study

Chemicals	Sources
Acetic acid, glacial	Merck, Darmstadt, Germany
Acetone	Merck, Darmstadt, Germany
Acrylamide	Merck, Darmstadt, Germany
Agar noble	Difco Laboratories, MI, USA
Agarose	FCM Bioproducts, Rockland, ME, USA
Ammonium persulfate	Fluka, Buchs, Switzerland
Amphotericin B	Squibb, NJ, USA
Ampicillin	Sigma, St. Louis, MO, USA
Annexin V-FITC apoptosis detection kit	BD Pharmingen, CA, USA
Aprotinin	Sigma, St. Louis, MO, USA
Avidin-peroxidase	Dako, Glostrup, Denmark
Bisacrylamide	Sigma, St. Louis, MO, USA
Boric acid	Sigma, St. Louis, MO, USA
Bovine serum albumin	Sigma, St. Louis, MO, USA
Bromphenol blue	Merck, Darmstadt, Germany
Chemiluminescent reagent	Pierce, Rockford, IL, USA
Chloroquine diphosphate	Sigma, St. Louis, MO, USA
Citric acid-1-hydrate	Merck, Darmstadt, Germany
Coomassie brillant blue R-250	BioRad Laboratories, Hercules, CA, USA
DEAE-Dextran	Sigma, St. Louis, MO, USA
Dimethyl sulfoxide	Merck, Darmstadt, Germany
Ethylenediaminetetraacetic acid	Fluka, Buchs, Switzerland

Ethyl alcohol	Merck, Darmstadt, Germany
Fetal calf serum	Biochrcom, Leonorenstr, Germany
Ficoll-Hypaque solution	Nycomed pharma AS, Norge, Norway
Fluorescein isothiocyanate	Sigma, St. Louis, MO, USA
Gentamicin	Russel, London, UK
Glycerol	Merck, Darmstadt, Germany
Glycine	Research organic Inc., Cleveland, OH, USA
Heparin	Lio, Ballerup, Denmark
Hydrochloric acid	Merck, Darmstadt, Germany
Iodoacetamide	Sigma, St. Louis, MO, USA
Iscove's modified Dulbecco's medium	Gibco, Grand Island, NY, USA
Isopropanol	Merck, Darmstadt, Germany
LB broth base	Gibco, Grand Island, NY, USA
Methanol	Merck, Darmstadt, Germany
[Methy-3H]Thymidine	Amersham, Buckinhamshire, UK
Minimum essential medium	Gibco, Grand Island, NY, USA
2-mercaptoethanol	Sigma, St. Louis, MO, USA
Nonidet P-40	Sigma, St. Louis, MO, USA
Paraformaldehyde	Fluka, Buchs, Switzerland
Phytoheamagglutinin	Sigma, St. Louis, MO, USA
Phenymethylsulfonyl fluoride	Sigma, St. Louis, MO, USA
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Protein A-Sepharose 4B	Zymed Laboratories, Inc., CA, USA
QIAGEN Plasmid mini kit	QIAGEN, Hiden, Germany
QIAGEN Plasmid midi kit	QIAGEN, Hiden, Germany
Rabbit anti-mouse immunoglobulins	Dako, Glostrup, Denmark
Rat anti-mouse IgM coupled-Sepharose 4B	Zymed Laboratories, Inc., CA, USA
RPMI-1640 medium	Gibco, Grand Island, NY, USA

Sheep anti-mouse immunoglobulins conjugated FITC	Silenus, Melbourne, Australia
Skimmed milk	Difco Laboratories, Detroit MI, USA
Sodium azide	Merck, Darmstadt, Germany
Sodium bicarbonate	Merck, Darmstadt, Germany
Sodium carbonate	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dihydrogen phosphate	Merck, Darmstadt, Germany
Sodium dodecyl sulfate	Sigma, St. Louis, MO, USA
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydrogen phosphate	Merck, Darmstadt, Germany
Sulfo-NHS-LC-biotin	Pierce, Rockford, IL, USA
TEMED	BioRad Laboratories, Griffin, USA
Tetracycline	Sigma, St. Louis, MO, USA
Tris-base	Sigma, St. Louis, MO, USA
Tween 20	Fluka, Buchs, Switzerland

APPENDIX B

List of antibodies used in this study

Antibody	Isotype	Recognized antigen
M6-2F9	IgM	CD147
M6-2B1	IgM	CD147
M6-1F3	IgM	CD147
M6-1D4	IgM	CD147
M6-1B9	IgG3	CD147
M6-1E9	IgG2a	CD147
MT8	IgM	CD8
MT99/3	IgM	CD99
BF1/4G2	IgG2a	Dengue virus
CA-1H4	IgM	Un-defined
FE-1H10	IgM	Un-defined
OKT3	IgG1	CD3
MT54	IgG2a	CD54
UPC-10	IgG2a	β-2-6-linked fructosan

APPENDIX C

List of cell lines used in this study

Cell line Destination	Cell type	Origin / Source	Reference
COS7 cells	Kidney cell line	Kidney cell / African green monkey	Gluzman, 1981
U937	Myeloid cell line	Histiocytic lymphoma / Human	Sundstrom and Nilsson, 1976
Sup-T1	T cell line	Acute Lymphoblastic leukemia / Human	Smith *et al.*, 1984
KG1a	Myeloid cell line	Bone marrow acute myelogenous leukemia / Human	Koeffler *et al.*, 1980

APPENDIX D

List of instruments used in this study

Instrument-model	Source
β counter (liquid scintillation counter)	Wallac, Finland
Analytical balance	Mettler Toledo, Switzerland
Electrophoresis & electrotransfer unit	Amersham, USA
Flow cytometer-FACSCalibur	Beckton Dickinson, USA
Fluorescent microscope	Olympus, USA
Heat sealer	Wallac, Finland
High-speed micro refrigerated centrifuge	Tommy, USA
Inverted microscope	Olympus, USA
Laminar flow	Nuaire, Inc., USA
Light microscope	Olympus, USA
Microcentrifuge	Sorvall, Germany
Microdialyzer system	Pierce, USA
pH meter	Pierce, USA
Refrigerated centrifuge	Sorvall, Germany
Refrigerator (-20°C)	Sanyo, Thailand
Refrigerator (-70°C)	Foma Sciencific, USA
Spectrophotometer UV1201	Shimadzu Co., Japan
Ultracentrifuge	Beckman, USA
Water bath	Thermoline, Australia

APPENDIX E

Reagents and buffers preparation

1. Reagents for mouse immunoglobulins purification.

1.1 20 mM Sodium phosphate (pH 7.0)

1M Na_2HPO_4	5.8 ml
1M NaH_2PO_4	4.2 ml
Distilled water	400 ml

Adjust pH to 7.0 by HCl or NaOH

Adjust final volume to 500 ml

Filtered with 0.2μ Millipore filter, stored at 4°C

1.2 Elution buffer for anti-mouse IgM coated Sepharose column (0.2 M Glycine pH 2.8)

Glycine	1.5014 g
Distilled water	90 ml

Adjust pH to 2.8 by 1N NaOH

Adjust final volume to 100 ml

Filtered with 0.2μ Millipore filter, stored at 4°C

1.3 Elution buffer for Protein A coated Sepharose column (0.1 M citric acid pH 3.0)

Citric acid-1-hydrate	2.1 g
Distilled water	70 ml

Adjust pH to 3.0 by 5N NaOH

Adjust final volume to 100 ml

Filter with 0.2 μ Millipore filter, stored at 4°C

1.4 Neutralizing buffer (2M Tris-HCl pH 8.0)

Tris-base	24.22 g
Distilled water	60 ml
Adjust pH to pH 8.0 by concentrate HCl	
Adjusted final volume to 100 ml, stored at room temperature	

1.5 Storage buffer (0.05% NaN_3-PBS pH 7.4)

$Na_2HPO_4.12H_2O$	1.15 g
KH_2PO_4	0.1g
NaCl	0.877g
10% NaN_3 in PBS	500 µl
Adjust pH to pH 7.4 by concentrate 5N NaOH	
Adjusted final volume to 100 ml, stored at 4°C	

2. Reagents for human blood cell and cell lines culture

2.1 Incomplete RPMI-1640 medium

RPMI-1640 powder	10.4 gm (1 package)
Distilled water	900 ml
$NaHCO_3$	2 g
Stirred until dissolved	
Gentamycin (40 mg/ml)	1 ml
Adjusted final volume to 1000 ml with distilled water	
Filtered with 0.2µ Millipore filter	
Sterile fungizone (2.5 mg/ml)	500 µl
Checked sterility before used	

2.2 Complete RPMI-1640 medium

Incomplete RPMI-1640 medium	90 ml
Fetal calf serum	10 ml
Checked sterility before used	

2.3 Incomplete IMDM medium

IMDM powder	17.7 g (1 package)

Distilled water	900 ml
$NaHCO_3$	3.024 g
Stirred until dissolved	
Gentamycin (40 mg/ml)	1 ml
Adjusted final volume to 1000 ml with distilled water	
Filtered with 0.2μ Millipore filter	
Sterile fungizone (2.5 mg/ml)	500 μl
Checked sterility before used	

2.4 Complete IMDM medium

Incomplete IMDM medium	90 ml
Fetal calf serum	10 ml
Checked sterility before used	

3. Reagents for direct and indirect immunofluorescence staining

3.1 Phosphate buffered saline (PBS)

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.15 g
KH_2PO_4	0.2 g
Distilled water	900 ml
Adjusted pH to 7.2	

3.2 1%BSA-0.02%NaN_3 in PBS

Bovine serum albumin fraction V	10 g
PBS (pH 7.2)	1000 ml
Mixed well until BSA completely dissolved	
Added 10% (w/v) NaN_3 to final concentration 0.02%, mixed well	
Stored at 4°C	

3.3 1%Paraformaldehyde

Paraformaldehyde	1 g
PBS pH 7.2	100 ml

Heat at 56°C until dissolved

Adjust pH to 7.4 by 0.1M NaOH or 0.1M HCl

Filtered with 0.2 μ Millipore filter, stored at 4°C

4. Reagents for DEAE-Dextran transfection

4.1 Incomplete MEM medium

MEM powder	9.6 g (1 package)
Distilled water	900 ml
$NaHCO_3$	2.2 g
Stirred until dissolved	
Gentamycin (40 mg/ml)	1 ml
Adjusted final volume to 1000 ml with distilled water	
Filtered with 0.2μ Millipore filter	
Sterile fungizone (2.5 mg/ml)	500 μl
Checked sterility before used	

4.2 Complete MEM medium

Incomplete MEM medium	90 ml
Fetal calf serum	10 ml
Checked sterility before used	

4.3 0.5 mM EDTA-PBS

PBS pH 7.2	100 ml
0.5 M EDTA pH 8.0	100 μl
Filtered with 0.2 μ Millipore filter, stored at room temperature	

4.4 DEAE-Dextran stock solution (10 mg/ml)

DEAE-Dextran (M.W. 500,000)	0.1 g
PBS pH 7.2	10 ml
Filter with 0.2 μ Millipore filter	
Aliquot to vials and stored at -20°C	

4.5 Chloroquine diphosphate stock solution (10 mM)

Chloroquine diphosphate	0.103 g

PBS pH 7.2	20 ml

Filter with 0.2 μ Millipore filter

Aliquot to vials and stored at -20°C

4.6 10% DMSO-PBS

Dimethyl sulfoxide	10 ml
PBS pH 7.2	90 ml

Filtered with 0.2 μ Millipore filter, stored at room temperature

5. Reagent for SDS-PAGE

5.1 4X Separating gel buffer (1.5M Tris HCl pH 8.8)

Tris base	18.15 g
Deionized distilled water	80 ml

Adjusted pH to 8.8 by concentrate HCl

Adjusted final volume to 100 ml

Stored at room temperature

5.2 4X Stacking gel buffer (0.5M Tris HCl pH 6.8)

Tris base	6.0 g
Deionized distilled water	80 ml

Adjusted pH to 6.8 by concentrate HCl

Adjusted final volume to 100 ml

Stored at room temperature

5.3 2x non-reducing buffer

0.5M Tris HCl pH 6.8	2.5 ml
87% glycerol	2.3 ml
Sodium dodecyl sulfate	0.4 g
Distilled water	5.16 ml
1% Bromphenol blue	40 μl

Mixed well, aliquot and stored at -20°C

5.4 2x reducing buffer

0.5M Tris HCl pH 6.8	2.5 ml

87% glycerol	2.3 ml
Sodium dodecyl sulfate	0.4 g
Distilled water	2.2 ml
2-ME	1 ml
1% Bromphenol blue	40 µl

Mixed well, aliquot and stored at -20°C

5.5 Running buffer

Tris base	3.028 g
Glycine	14.413 g
Sodium dodesyl sulfate	1.0 g
Distilled water	1000 ml

Mixed well, prepare before use

5.6 Blotting buffer

Tris-base	1.515 g
Glycine	7.205 g
Sodium dodesyl sulfate	0.5 g
Distilled water	350 ml

Mixed well

Methanol	100 ml

Adjust final volume to 500 ml

Filtrated with 0.2µ filter, stored at room temperature

5.7 Slab gel

	12.5% separating gel	4% stacking gel
Distilled water	3.2 ml	1.5 ml
Monomer	4.2 ml	332.5 µl
4X Separating gel buffer	2.5 ml	-
4X Stacking gel buffer	-	625 µl
10% SDS (in distilled water)	100 µl	25 µl
10% APS (in distilled water)	50 µl	12.5 µl
TEMED	10 µl	5 µl

5.8 Staining solution

Coomassie brilliant blue R-250	0.125 g
Methanol	200 ml
Acetic acid	35 ml

Adjusted final volume with distilled water to 500 ml

Stored at room temperature

5.9 Destaining solution I

Methanol	400 ml
Acetic acid	70 ml

Adjusted final volume with distilled water to 1000 ml

5.10 Destaining solution II

Methanol	50 ml
Acetic acid	70 ml

Adjusted final volume with distilled water to 1000 ml

6. Reagents for immunoprecipitation

6.1 Tris lysis buffer pH 8.2 (100mM NaCl, 50mM Tris-base, 2 mM EDTA, 0.02% NaN_3)

Tris base	3.03 g
NaCl	2.922 g
EDTA (M.W. 292.25)	0.292 g
NaN_3	0.1 g
Distilled water	200 ml

Adjust pH to 8.2 by 0.1M NaOH

Adjusted final volume to 500 ml, stored at room temperature

6.2 Lysis buffer

Phenylmethylsulfonyl fluoride (PMSF)	100 µl
(100 mM in acetone)	
Iodoacetamide (0.5M in distilled water)	100 µl
Aprotinin (1 mg/ml in PBS)	100 µl

10% NP40 (in Tris lysis buffer)	1 ml
Tris-lysis buffer pH 8.2	8.7 ml

Mixed well, aliquot to vial and stored at -20°C

6.3 5 mM Biotin in PBS

Sulfo-NHS-LC-biotin	0.00278 g
PBS pH 7.2	1 ml

Freshiy prepared before use

6.4 1 mM Glycine in PBS

Glycine	0.0375 g
PBS pH 7.2	500 ml

Stored at 4°C

6.5 Coating buffer (0.1M Carbonate-bicarbonate buffer pH 9.6)

Na_2CO_3	1.06 g
$NaHCO_3$	1.26 g
Distilled water	200 ml

Adjust pH to 9.6 by 10% acetic acid

Adjusted final volume to 250 ml, stored at room temperature

6.6 Blocking buffer (2.5% BSA-PBS pH 7.2)

Bovine serum albumin fraction V	1.25 g
PBS (pH 7.2)	50 ml

Stored at 4°C

6.7 5% skimmed milk in PBS

Skimmed milk	5 g
PBS (pH 7.2)	100 ml

Mixed well, prepared before use

7. Reagents for bacterial culture

7.1 LB broth

LB broth base	20 g
Distilled water	1000 ml

Sterilized in Autoclave at 121°C 15 minutes

Stored at 4°C

Checked sterility before used

7.2 LB broth contain ampicillin and tetracycline

LB broth	100 ml
Ampicillin (50 mg/ml)	30 µl
Tetracycline (30 mg/ml)	33.6 µl

Checked sterility before used

7.3 LB agar contain ampicillin and tetracycline

LB broth base	10 g
Agar noble	7.5 g
Distilled water	500 ml

Sterilized with Autoclave at 121°C 15 minutes

Ampicillin (50 mg/ml)	150 µl
Tetracycline (30 mg/ml)	168 µl

Pour plate 25 ml/plate, stored at 4°C

Checked sterility before used

8. Reagents for production of plasmid DNA

8.1 TE buffer

Tris-base	0.121 g
EDTA	0.307 g
Distilled water	80 ml

Adjusted pH to 7.0 by 1N HCl

Adjusted final volume to 100 ml, stored at room temperature

8.2 TBE buffer

Tris-base	27 g
Boric acid	13.75 g
0.5 M EDTA (pH 8.0)	8 ml

Adjusted final volume to 500 ml, stored at room temperature

8.3 1% Agarose gel

Agarose gel	1 g
TBE buffer	100 ml

Heated until dissolved

CIRRICULUM VITAE

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