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APPENDIX

Appendix A: List of the chemicals and materials used in this study

Chemicals/Materials	Source
Acetone	Merck, Darmstadt, Germany
Acrylamide	Merck, Darmstadt, Germany
Alexa Fluor 488-labeled goat anti-mouse IgG antibodies	Invitrogen, Carlsbad, CA, USA
Alexa Fluor 568-labeled goat anti-mouse IgM antibodies	Invitrogen, Carlsbad, CA, USA
Ammonium persulfate	Sigma, St. Louis, MO, USA
Amplicillin	Sigma, St. Louis, MO, USA
Anti-FITC MicroBeads	Miltenyi Biotec, Bergisch Gladbach, Germany
Aprotinin	Sigma, St. Louis, MO, USA
Bisacrylamide	Sigma, St. Louis, MO, USA
Bovine serum albumin	Sigma, St. Louis, MO, USA
Brij-58	Pierce, Rockford, IL, USA
Brij-98	Sigma, St. Louis, MO, USA
Carboxyfluorescein diacetate succinimidyl ester (CFSE)	Sigma, St. Louis, MO, USA
CellTrace™ Far Red DDAO-SE	Invitrogen, Carlsbad, CA, USA
Chemilumnescent reagent	Pierce, Rockford, IL, USA
Coomassie brilliant blue R-250	Bio-Rad, Hercules, CA, USA
Coomassie brilliant blue G-250	Bio-Rad, Hercules, CA, USA

Chemicals/Materials	**Source**
Developer and replenisher	Kodak, NY, USA
Dimethyl sulfoxide	Sigma, St. Louis, MO, USA
Ethylenediaminetetraacetic acid	Fluka, Buchs, Switzerland
Ethyl alcohol	Merck, Darmstadt, Germany
Fetal calf serum	Gibco, Grand Island, NY, USA
Ficoll-Hypaque solution	Sigma, St. Louis, MO, USA
FITC-conjugated sheep $F(ab')_2$ anti-mouse Igs	Silenus, Boronia, Victoria, Australia
FITC-conjugated annexin V	Becton Dickinson, San Jose, CA, USA
Gentamicin	Russel, London, UK
Heparin	Lio, Ballerup, Denmark
Hoechst 33258 dye	Invitrogen, Carlsbad, CA, USA
HRP-conjugated rabbit anti-mouse Igs antibodies	Dako, Glostrup, Denmark
HRP-conjugated streptavidin	Dako, Glostrup, Denmark
HRP-conjugated goat anti-mouse Igs light chain specific antibodies	Jackson ImmunoResearch Laboratories, PA, USA
HRP-conjugated anti-phosphoserine antibodies	Merck, Darmstadt, Germany
HRP-conjugated anti-phosphothreonine antibodies	Merck, Darmstadt, Germany
Iodoacetamide	Sigma, St. Louis, MO, USA
Iscove's modified Dulbecco's medium	Gibco, Grand Island, NY, USA
Isopropanol	Merck, Darmstadt, Germany
Isotyping-ELISA kit	Sigma, St. Louis, MO, USA

Chemicals/Materials	Source
Laurylmaltoside (n-dodecyl-β-D-maltoside	Calbiochem/Merck, Darmstadt, Germany
Lipofectamine	Invitrogen, Carlsbad, CA, USA
2-mercaptoethanol	Merck, Darmstadt, Germany
Methanol	Merck, Darmstadt, Germany
Nitrocellulose membrane	PALL, East Hill, NY, USA
Nonidet P-40	Pierce, Rockford, IL, USA
Paraformaldehyde	Fluka, Buchs, Switzerland
Polybrene	Sigma, St. Louis, MO, USA
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Prestained SDS-PAGE standards	Fermentas, MA, USA
Sepharose 4B column	Sigma, St. Louis, MO, USA
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	Merck, Darmstadt, Germany
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydrogen phosphate	Merck, Darmstadt, Germany

Chemicals/Materials	**Source**
Sulfo-NHS-LC-biotin	Pierce, Rockford, IL, USA
Sreptavidin-HRP	Zymed, South san Francisco, CA
Staphylococcal enterotoxin B	Sigma, St. Louis, MO, USA
TEMED	BioRad Laboratories, Griffin
Tris-base	Sigma, St. Louis, MO, USA
TRITC-phalloidin	Sigma, St. Louis, MO, USA
Tween 20	Fluka, Buchs, Switzerland

Appendix B: List of antibodies used in this study

Monoclonal antibodies	Isotype	Recognized antigen
MT99/1[a]	IgM	CD99
MT99/2[a]	IgM	CD99
MT99/3[a]	IgG2a	CD99
MEM-55[b]	IgG1	CD45
M38[b]	IgG1	CD81
MEM-111[b]	IgG2a	CD54
MT4[a]	IgM	CD4
OKT3[a]	IgG1	CD3ε
M6-1D4[a]	IgM	CD147
M6-1E9[a]	IgG2a	CD147
MEM-136[b]	IgG1	MHC class II
HC10[b]	IgG1	MHC class I
NAP-07[b]	IgG1	NTAL
LCK-01[b]	IgG1	Lck
SKAP55[b]	IgG2a	SKAP-55
TRAP02[b]	IgG2a	Transmembrane adapter protein
4G10[b]	IgG1	Tyrosine phosphorylated protein

Monoclonal antibodies	Isotype	Recognized antigen
PB-1[a]	IgG1	Hemoglobin Bart's
13M[a]	IgG2a	Bacteriophage protein
4G2[c]	IgG2a	Dengue viral protein

[a] Produced in our laboratory, Division of Clinical Immunology, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand

[b] Kindly provided by Prof.Vaclav Horejsi, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

[c] Obtained from Dr. Prida Malasit, Division of Medical Molecular Biology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Appendix C: List of cell lines used in the this study

The description of cells lines in this study

Cell line	Cell type	Origin	References
COS7 cells	Kidney cell line	Kidney cell	Gluzman, 1981
Ramos cells	B cell line	Burkitt's lymphoma	Benjamin *et al.*, 1982
Raji cells	B cell line	Burkitt's lymphoma	Pulvertaft, 1964
Jurkat cells	T cell line	Acute lymphoblastic leukemia	Schneider *et al.*, 1997
SUP-T1 cells	T cell line	Acute lymphoblastic leukemia	Smith *et al.*, 1984
U937 cells	Monocyte cell line	Histiocytic lymphoma	Sundstrom and Nilsson, 1976
THP-1 cells	Monocyte cell line	Monocytic leukaemia	Tsuchiya *et al.*, 1980
HEK293 cells	Kidney cell line	human embryonic kidney cells	Graham *et al.*, 1977
Phoenix-Ampho cells	Retroviral producer cell line	HEK293T cells	Fujita *et al.*, 1992

Appendix D: Reagent and buffer preparation

1. Reagents for cell culture

1.1 Incomplete IMDM medium

IMDM powder	1	pack
$NaHCO_3$	3.024	g
Gentamycin (40 mg/ml)	1	ml
Dissolved in distilled water and adjust volume to	1000	ml
Filtrated through 0.2 µm Millipore membrane filter		
Added Fungizone (5 mg/ml)	500	µl
Mixed and stored at 4 ^{o}C		

1.2 Complete IMDM medium

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

1.3 Incomplete RPMI 1640 medium

RPMI 1640 powder	1	pack
$NaHCO_3$	2	g
Streptomycin (0.2 g/ml)	500	µl
Penicillin ($1x10^6$ U/ml)	100	µl

Dissolved in distilled water and adjusted volume to 1000 ml and pH to 7.2

Filtered through 0.2 µm millipore membrane filter

then added Fungizone (5 mg/ml)	500	µl
and stored at 4°C		

1.4 Complete RPMI1640 medium

RPMI 1640 medium	90	ml
Fetal bovine serum (FBS)	10	ml

1.5 Incomplete MEM medium

MEM powder	1	pack
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		
Filtrated with 0.2 µm Millipore filter		
Sterile fungizone (2.5 mg/ml) 500 µl		
Checked sterility before used		

1.6 Complete MEM medium

Incompleat MEM medium	90	ml
Fetal bovine serum (FBS)	10	ml

1.7 Incomplete DMEM medium

DMEM powder	1	pack
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

Filtrated with 0.2 μm Millipore filter

Sterile fungizone (2.5 mg/ml) 500 μl

Checked sterility before used

1.8 Complete DMEM medium

Incompleat DMEM medium	90	ml
Fetal bovine serum (FBS)	10	ml

1.9 Freezing medium (10%DMSO in 25%FCS-IMDM)

Incomplete IMDM	65	ml
Fetal calf serum	25	ml
DMSO (Hybrimax)	10	ml

Mixed well and stored at 4°C

1.10 0.6% 2-mercaptoethanol (2-ME)

Incomplete IMDM	5	ml
2-mercaptoethanol	30	μl

Filtrated through 0.2 μm Millipore membrane filter

Aliquot 50 μl/tube, stored at -20°C

1.11 1xHAT medium

Incomplete IMDM	78	ml
Heat inactivated FCS	10	ml
BM condimed HI	10	ml
0.6% 2-ME	30	μl
50X HAT	2	ml

Stored at 4°C

1.12 1XHT medium

Incomplete IMDM	119	ml
Heat inactivated FCS	15	ml
BM condimed HI	15	ml
0.6% 2-ME	30	µl
100X HT	1	ml

Stored at 4°C

2. Reagents for Immunoprecipitation

2.1 Tris lysis buffer pH 8.2 (100mM NaCl, 50mM Tris-base, 2 mM EDTA, 0.02% NaN3)

Tris base	3.03	g
NaCl	2.922	g
NaF	1.05	g
EDTA	0.292	g
NaN_3	0.1	g
Distilled water	200	ml

Adjusted pH to 8.2 by 0.1M NaOH

Adjusted final volume to 500 ml, stored at room temperature

2.2 Lysis buffer

Phenylmethylsulfonyl fluoride (PMSF) (100 mM in acetone)	100	µl
Iodoacetamide (0.5M in distilled water)	100	µl
Aprotinin (1 mg/ml in PBS)	100	µl
10% detergent solubilization (in Tris lysis buffer)	1	ml

Tris-lysis buffer pH 8.2	8.7	ml
Pepstatin A	10	μl

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

2.3 1mM Glycine in PBS

Glycine	0.0375	g
PBS pH 7.2	500	ml

Stored at 4°C

2.4 5mM Biotin in PBS

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

Freshly prepared before used

3. Reagents for SDS-PAGE

3.1 4X Separating gel buffer (1.5M Tris HCl pH 8.8)

Tris base	18.15	g
Distilled water	80	ml

Adjusted pH to 8.8 by concentrate HCl

Adjusted final volume to 100 ml

Filtrated 0.2 μm Millipore membrane filter

Stored at 4°C

3.2 4X Stacking gel buffer (0.5M Tris HCl pH 6.8)

Tris base	6.0	g
Distilled water	80	ml

Adjusted pH to 6.8 by concentrate HCl

Adjusted final volume to 100 ml

Filtrated 0.2 μm Millipore membrane filter

Stored at 4°C

3.3 2x non-reducing buffer

0.5 M 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

3.4 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	4.16	ml
2-ME	1	ml
1% Bromphenol blue	40	μl

Mixed well, aliquot and stored at -20°C

3.5 1X Running buffer

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

Mixed well, prepared before used

3.6 30% Monomer (30.8% acrylamide, 2.7% bis-acrylamide)

Acrylamide	60	g
Bis-acrylamide	1.6	g
ddH_2O	200	ml

Mixed thoroughly and filtrated through 0.2 μm

Millipore membrane filter, kept in dark at 4°C

3.7 Slab gel

	Separating gel						Stacking gel	
	12.5%		**10%**		**7.5%**		**4%**	
Distilled water	3.2	ml	4	ml	4.85	ml	1.5	ml
30% Monomer	4.2	ml	3.3	ml	2.5	ml	332.5	μl
4X Separating gel buffer	2.5	ml	2.5	ml	2.5	ml	-	
4X Stacking gel buffer	-		-		-		625	μl
10% SDS (in distilled water)	100	μl	100	μl	100	μl	25	μl
10% APS (in distilled water)	50	μl	50	μl	50	μl	12.5	μl
TEMED	10	μl	10	μl	10	μl	5	μl

3.8 10% APS

Ammonium persulfate	0.1	g
Distilled water	1	ml

Mixed well, aliquot and stored at -20°C

3.9 10% SDS

Sodium dodecyl sulfate	10	g
Distilled water	100	ml

Mixed well, aliquot and stored at -20°C

3.10 1X Blotting buffer

Tris-base	1.515	g
Glycine	7.205	g
Sodium dodecyl sulfate	0.5	g
Distilled water	350	ml
Mixed well		
Methanol	100	ml
Adjusted final volume to	500	ml

Filtrated with 0.2 μm filter, stored at room temperature

4. Reagents for indirect immunofluorescence staining

4.1 Phosphate buffer 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 by 5N NaOH

Adjusted volume to 1000 ml, stored at room temperature

4.2 1 % BSA-0.02%NaN_3 in PBS

Bovine serum albumin fraction V	10	g
PBS pH 7.2	1000	ml
10% NaN_3 in PBS	2000	μl

Mixed well until BSA completely dissolved, stored at 4°C

4.3 1% Para-formaldehyde in PBS

Para-formaldehyde	5	g
PBS pH 7.2	500	ml

Heat at 56°C until dissolved

Filtrated with 0.2 μm millipore filter, stored at 4°C

5. Reagents for IgG purification

5.1 Binding buffer

1 M Na_2HPO_4	11.6	ml
1 M NaH_2PO_4	8.4	ml
ddH_2O	800	ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 1000 ml. with distilled water

Mixed thoroughly and filtrated through 0. 2 μm Millipore membrane filter

Kept at 4 °C, degas for 30 min before used

5.2 Eluting buffer (Tris-Glysine pH 2.7)

Tris-base	1.515	g
Glycine	7.205	g
Distilled water	900	ml

Adjusted the pH to 2.7 with 5 N HCl

Adjusted the volume to 1000 ml. with distilled water

Mixed thoroughly and filtrated through 0. 2 μm Millipore membrane filter

Kept at 4 °C, degas for 30 min before used

6. Reagents for IgM purification

6.1 Binding buffer (20 mM sodium phosphate, 0.8 M $(NH_4)_2SO_4$, pH 7.5)

1 M Na_2HPO_4	5.8	ml
1 M NaH_2PO_4	4.2	ml
$(NH_4)_2SO_4$	52.856	g
ddH_2O	400	ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 500 ml with distilled water

Mixed thoroughly and filtrated through 0.2 μm Millipore membrane filter

Kept at 4 ºC, degas for 30 min before used

6.2 Eluting buffer (20 mM sodium phosphate pH 7.5)

1 M Na_2HPO_4	11.6	ml
1 M NaH_2PO_4	8.4	ml
ddH_2O	800	ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 1000 ml. with distilled water

Mixed thoroughly and filtrated through 0. 2 μm Millipore membrane filter

Kept at 4 ºC, degas for 30 min before used

6.3 Regeneration buffer

1 M Na_2HPO_4	5.8	ml
1 M NaH_2PO_4	4.2	ml
Isopropanol	150	ml
ddH_2O	200	ml

Adjusted the pH to 7.5 with 5 N NaOH

Adjusted the volume to 500 ml. with distilled water

Mixed thoroughly and filtrated through 0.2 μm Millipore membrane filter

Kept at 4 °C, degas for 30 min before used

CURRICULUM VITAE

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Oral presentation

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