APPENDIX

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

Chemicals/Materials	**Source**
Acetone	Merck, Darmstadt, Germany
Acrylamide	Merck, Darmstadt, Germany
Agarose	Sigma, St. Louis, MO, USA
Anti-CD4 mAb (Leu^{TM}- 3a)	Becton Dickinson, San Jose, CA, USA
Ammonium persulfate	Sigma, St. Louis, MO, USA
Amplicillin	Sigma, St. Louis, MO, USA
Aprotinin	Sigma, St. Louis, MO, USA
Avidin	Sigma, St. Louis, MO, USA
BD IMag™ anti-human CD4 Particles - DM	Becton Dickinson, San Jose, CA, USA
Bisacrylamide	Sigma, St. Louis, MO, USA
10X BM condimed HI	Roche, Mannheim, USA
Bovine serum albumin	Sigma, St. Louis, MO, USA
Bromphenol blue	Merck, Darmstadt, Germany
Chemilumnescent reagent	Pierce, Rockford, IL, USA
Chloramphenicol	Sigma, St. Louis, MO, USA
Chloroquine diphosphate	Sigma, St. Louis, MO, USA
Coomassie brilliant blue R-250	Bio-Rad, Hercules, CA, USA

DEAE-Dextran	Sigma, St. Louis, MO, USA
Developer and replenisher	Kodak, NY, USA
dNTP	Fermentas, MA, USA
DNA ligase	Fermentas, MA, 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
Gentamicin	Russel, London, UK
Glacial acetic acid	Merck, Darmstadt, Germany
Glycerol	Merck, Darmstadt, Germany
50X Hypoxanthine Aminopterin Thymidine (HAT)	Sigma, St. Louis, MO, USA
100X Hypoxanthine Thymidine (HT)	Gibco, Grand Island, NY, USA
Heparin	Lio, Ballerup, Denmark
Hydrochloric acid	Merck, Darmstadt, Germany
Iodoacetamide	Sigma, St. Louis, MO, USA
Isopropyl- β-D-thiogalactopyranoside (IPTG)	Amresco, Solon, OH, USA
Iscove's modified Dulbecco's medium	Gibco, Grand Island, NY, USA

Isopropanol	Merck, Darmstadt, Germany
Isotyping-ELISA kit	Sigma, St. Louis, MO, USA
Kanamycin	Sigma-Aldrich Co.,USA
LB broth base	Gibco, Grand Island, NY, USA
MagnaBind Streptavidin Bead	Pierce, Rockford, IL, USA
2-mercaptoethanol	Merck, Darmstadt, Germany
Methanol	Merck, Darmstadt, Germany
Minimum essential medium	Gibco, Grand Island, NY, USA
MOPS	Amresco, Solon, OH, USA
Nitrocellulose membrane	PALL, East Hill, NY, USA
Nonidet P-40	Pierce, Rockford, IL, USA
Paraformaldehyde	Fluka, Buchs, Switzerland
Potassium chloride	Merck, Darmstadt, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Prestained SDS-PAGE standards	Fermentas, MA, USA
Primer	Invitrogn, Carlsbad, CA, USA
proofStart DNA polymerase	Fermentas, MA, USA
Protein A sepharose	Zymed Laboratories, Inc., CA, USA
QIAGEN Gel Extraction kit	QIAGEN, Hiden, Germany
QIAGEN PCR purification kit	QIAGEN, Hiden, Germany
QIAGEN Plasmid maxi kit	QIAGEN, Hiden, Germany
QIAGEN Plasmid maxi kit	QIAGEN, Hiden, Germany
Rabbit anti-mouse immunoglobulins	Dako, Glostrup, Denmark

Restriction enzymes and 10X reaction buffer	Fermentas, MA, 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	Sigma, St. Louis, MO, USA
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydrogen phosphate	Merck, Darmstadt, Germany
Sodium sulfate	Merck, Darmstadt, Germany
Sulfo-NHS-LC-biotin	Pierce, Rockford, IL, USA
Sreptavidin-HRP	Zymed, South san Francisco, CA
T4 ligase and 10X reaction buffer	Fermentas, MA, USA
Taq DNA polymerase and 10X reaction buffer	Fermentas, MA, USA
TEMED	BioRad Laboratories, Griffin
Tetracycline	Sigma, St. Louis, MO, USA
3,3',5,5;-Tetramethylbenzidine (TMB)	Zymed, South san Francisco, CA
Tris-base	Sigma, St. Louis, MO, USA
Tryptone	Life Technologies, Scotland
Tween 20	Fluka, Buchs, Switzerland
Yeast extract	Life Technologies, Scotland

Appendix B: List of antibodies used in this study

Monoclonal antibodies	Isotype	Recognized antigen
MT4	IgM	CD4
Leu3a	IgG1	CD4
VIT4	IgG2a	CD4
MT310	IgG1	CD4
L200	IgG1	CD4
MT8	IgM	CD8
MT99/3	IgG2a	CD99
M6-1E9	IgG2a	CD147
M6-1B9	IgG3	CD147
OKT-3	IgG1	CD3

Appendix C: List of cell lines and microorganism used in this study

1. Cell lines

Cell line **Destination**	**Cell type**	**Origin / Source**	**Reference**
COS7 cells	Kidney cell line	Kidney cell / African green monkey	Gluzman, 1981

2. Microorganism

- XL1-Blue (Stratagene, USA))

Genotype: *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*qZΔ*M15* Tn*10* (Tetr)]

- *Escherichi coli* Origami B (Novagen, Madison, WI)

Genotype: F– *ompT hsdS*B(rB– mB–) *gal dcm lacY1 gor522*::Tn*10* (TcR) *trxB*::kan

- *Escherichi coli* MC1061/P3

Genotype: F- *hsd*R (rk-, mk+) *ara*D139 Δ(*ara*ABC-*leu*)7679 *gal*U *gal*K Δ*lac*X74 *rps*L *thi mcr*B {P3: KanR AmpR (am) TetR (am)}

Appendix D: List of instruments used in this study

Instrument-Model	**Source**
2-20 µl Autopipette	Bio-rad, USA
20-200 µl Autopipette	Bio-rad, USA
100-1000 µl Autopipette	Bio-rad, USA
40-350 µl multichanel autopipette	Socorex, Switzerland
Analytical balance	Mettler Toledo, Canada
Autoclave	Huxey, Taiwan
CO_2 incubator	Thermo electron corporation, USA
Electrophoresis and Electrotransfer unit	Amersham,,USA
Flow cytometer-FACSCalibur	Beckton Dickinson, USA
Fluorescent microscope	Olympus, Japan
High speed micro refrigerated centrifuge	Tomy, Japan
Inverted microscope	Olympus, Japan
Laminar flow	Nuaire, USA
Light microscope	Olympus, Japan
Microcentrifuge	Sorvall, Germany
Microplate reader	Sunrise tecan, Austria
PCR Mastercycler personal	Eppendrof, USA
pH meter	Precisa, Switzerland
Refrigerated centrifuge	Sorvall, Germany
Refrigerat(-20°C)	Sanyo, Thailand
Rotator	Technomara, Switzerland

Semi-dry blotting	Amershame Biosciences, Sweden
Spectrophotometer UV-1201	Shimadzu, Japan

Appendix E: Reagents and buffers preparation

1. Reagents for immunoprecipitation

1.1 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

Adjusted pH to 8.2 by 0.1M NaOH

Adjusted final volume to 500 ml, stored at room temperature

1.2 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% 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

1.3 5 mM Biotin in PBS

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

Freshly prepared before use

1.4 1 mM Glycine in PBS

Glycine	0.0375 g
PBS pH 7.2	500 ml

Stored at 4°C

2. Reagent for SDS-PAGE

2.1 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 4°C

2.2 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 4°C

2.3 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

2.4 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

2.5 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

2.6 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

Adjusted final volume to 500 ml

Filtrated with 0.2 μm filter, stored at room temperature

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

Acrylamide	60	gm
Bis-acrylamide	1.6	gm
ddH_2O	200	ml

Mix thoroughly and filtrated through 0.2 μm Millipore membrane filter, kept in dark at 4°C

2.8 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

2.9 10% SDS

Sodium dodecyl sulfate	10	g
Distilled water	100	ml

Mix well, aliquot and store at -20°C

2.10 10% APS

Ammonium persulfate	0.1	g
Distilled water	1	ml

Mix well, aliquot and store at -20°C

3. Reagent for gel electrophoresis

3.1 10x TAE buffer

Tris-base	24.20	g
Glacial acetic acid	5.71	ml
0.5 M EDTA (pH 8.0)	10	ml

Adjusted final volume to 500 ml, stored at room temperature

3.2 1% agarose gel

Agarose gel	1	g
TBE buffer	100	ml

Heated until dissolved

3.3 Ethidium bromide working solution(10 mg/ml)

Ethidium bromide	1	g
Distilled water	100	ml

Kept in the dark bottle and stored at room temperature

4. Regent for bacterial culture

4.1 LB broth

Sterilized with Autoclave at 121°C 15 minutes

Stored at 4°C

Checked sterility before used

4.2 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

4.3 Super Broth

Tryptone	30	g
Yeast extracts	20	g
Morpholinepropanesulphonic acid(MOPS)	10	g

Distilled water 1000 ml

Sterilized with Autoclave at 121°C 15 minutes and stored at 4°C

5. Reagent for plasmid mini-preparation

5.1 3 M Sodium acetate pH 7.0

Sodium acetate

Distilled water	100	ml

Adjusted pH to 7.0 by HCl/NaOH

5.2 Potassium Acetate

Potassium Acetate	29.4	g
Glacial acetic acid	11.5	ml

Adjusted final volume to 100 ml with distilled water

5.3 10 M NaOH

NaOH	200	g
Distilled water	500	ml

5.4 7.5 M NH4Acetate

NH4Acetate	57.8	g
Distilled water	100	ml

5.5 1 M glucose buffer

D-glucose	18.2	g
Distilled water	100	ml

Sterilized with Autoclave at 121°C 15 minutes and stored at 4°C

5.6 0.5 M EDTA pH 8.0

EDTA	32.22	g
Distilled water	150	ml

Adjusted pH to 8.0 by HCl/NaOH

Adjusted final volume to 200 with distilled water

5.7 10X glucomix

1 M glucose buffer	50	ml
0.5 M EDTA pH 8.0	20	ml
1 M Tris pH 8.0	25	ml
Distilled water	5	ml

Sterilized with Autoclave at 121°C 15 minutes and stored at 4°C

5.8 1X glucomux-lysozyme solution

10X glucomix	300	ml
Lysozyme(50 mg/ml in DW)	300	µl
Distilled water	2.4	ml

Freshly prepared before used

6. Reagents for indirect immunofluorescence staining

6.1 Phosphate buffer saline (PBS)

NaCl	8	g
KCl	0.2	g
Na_2HPO_4	1.15	g
KH_2HPO_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

6.2 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	200	µl

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

6.3 1%Paraformaldehyde in PBS

Paraformaldehyde	5	g
PBS pH 7.2	500	ml

Heat at 56°C until dissolved

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

7. Reagents for ELISA

7.1 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

Mixed and adjusted pH to 9.6 with concentrated HCl

Adjusted final volume to 250 ml with distilled water, store at 4°C

7.2 0.05% Tween-PBS

PBS pH 7.2	500	ml
Tween 20	250	μl

Mixed and stored at room temperature

7.3 Blocking buffer (2% BSA-PBS)

Bovine serum albumin	2	g
PBS pH 7.2	100	ml

Freshly prepared before used

7.4 Stop reaction solution (1N HCL)

Concentrate HCl	8.3	ml
Distilled water	91.7	ml

Slowly dropwise HCl to distilled water, store at room temperature

8. Reagents for DEAE-Dextran transfection

8.1 0.5 mM EDTA-PBS

PBS pH 7.2	100	ml
0.5 M EDTA pH 8.0	100	µl

Filtrated with 0.2 µm Millipore filter, stored at room temperature

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

DEAE-Dextran (M.W. 500,000)	0.1	g
PBS pH 7.2	10	ml

Filtrated with 0.2 µm Millipore filter

Aliquot to vials and stored at -20oC

8.3 Chloroquine diphosphate stock solution (10 mM)

Chloroquine diphosphate	0.103	g
PBS pH 7.2	20	ml

Filtrated with 0.2 µm Millipore filter

Aliquot to vials and stored at -20°C

8.4 10% DMSO-PBS

Dimethyl sulfoxide	10	ml
PBS pH 7.2	90	ml

Filtrated with 0.2 µm Millipore filter, stored at room temperature

9. Reagents for human blood cell and cell lines culture

9.1 Incomplete IMDM medium

IMDM powder	1	pack
$NaHCO_3$	3.024	g
Gentamycin (40 mg/ml)	1	ml

Dissolved in ddH_20 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°C		

9.2 Complete IMDM medium

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

9.3 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		

9.4 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		

9.5 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

9.6 Incomplete MEM medium

MEM powder	1	pack
Deionized 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

9.7 Hypotonic solution (0.083% NH_4Cl) for RBC lysing

NH_4Cl	0.829	g
$KHCO_3$	0.1	g
EDTA	0.0037	g
Deionized distilled water	90	ml

Adjusted pH to 7.2 with 1N HCl

Adjusted volume to 100 ml

Filtrated 0.4 µm Millipore membrane filter

Stored at 4°C

9.8 Complete MEM medium

Incomplete MEM medium	90	ml
Heat inactivated fetal calf serum	10	ml

Checked sterility before used

CURRICULUM VITAE

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Publication :

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Poster Presentation:

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