

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT IN ENGLISH	v
ABSTRACT IN THAI	viii
TABLE OF CONTENTS	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
ABBREVIATIONS	xxi
CHAPTER I INTRODUCTION	
1.1 Statement of problems	1
1.2 Literature reviews	4
1.2.1 Immune system	4
1.2.1.1 Innate immunity	6
1.2.1.2 Adaptive immunity	7
1.2.1.2.1 Humoral mediated immunity (HMI)	9
1.2.1.2.2 Cell-mediated immunity (CMI)	11
1.3 Lymphocytes	14
1.3.1 T lymphocytes	14
1.3.2 B lymphocytes	16
1.3.3 NK cells	20

1.4 Immunological synapse (IS)	20
1.5 Lipid rafts	22
1.6 Tetraspanins	26
1.7 Leukocyte surface molecules	31
1.7.1 General structure of cell surface proteins	32
1.7.2 Integral membrane proteins	32
1.7.3 Lipid-anchored membrane proteins	33
1.7.4 Peripheral membrane proteins	34
1.8 CD99	36
1.9 Objectives	39
CHAPTER II MATERIALS AND METHODS	
2.2 Production of anti-CD99 monoclonal antibodies	40
2.2.1 Production of CD99 expressing COS7 cells	40
2.2.2 Hybridoma production	41
2.2.3 Indirect immunofluorescence staining of COS7 cells	42
2.2.4 Limiting dilution	42
2.3 Western blotting of cell lysates	43
2.4 Immunoprecipitation and Western immunoblotting of cell surface molecules	44
2.5 Indirect immunofluorescence staining of cell surface molecules	44
2.6 Coimmunoprecipitation of biotinylated cell surface proteins	45
2.7 Coimmunoprecipitation and amino acid sequencing	46

2.8 Production of mAbs against CD99 associated molecules	46
2.8.1 Dot blot assay	47
2.9 Immunomagnetic cell sorting	48
2.10 Plasmid DNA construction and transformation	48
2.10.1 Amplification of gene encoding DDI2, CD99 short form and CD99 long form	48
2.10.2 Plasmid DNA preparation and transformation	49
2.11 Transfection	50
2.12 Transduction and selection of stable clones	51
2.13 Colocalization analysis	51
2.14 Immunological synapse and confocal microscopic analysis	52
2.15 Determination of CD99 in lipid rafts	52
2.16 Proliferation assay	53
2.17 Apoptosis assay	53
2.18 Analysis of protein phosphorylation	54
CHAPTER III RESULTS	
3.1 Production of monoclonal antibodies against CD99 molecules	55
3.1.1 Hybridoma technique for production of anti-CD99 mAbs	55
3.1.2 Western blotting and immunoprecipitation experiments using the generated anti-CD99 mAbs	61
3.2 Characterizations of CD99 molecules	62
3.2.1 Expression of CD99 molecules on various hematopoietic cell lines	62

3.2.2 Biochemical characterization of CD99 molecules on peripheral blood cells and cell lines	62
3.3 Identification of CD99 associated molecules	68
3.3.1 Association of CD99 molecules with various membrane proteins	68
3.3.2 Investigation of the interaction between CD99 and GAPDH	75
3.3.3 Investigation of the interaction between CD99 and DDI2	77
3.3.3.1 Generation of plasmid DNA encoding DDI2 Myc tag fusion protein	77
3.3.3.2 Verification of DDI2-Myc protein expression	78
3.3.3.3 Generation of CD99 expressing and CD99 non-expressing cells	78
3.3.3.4 Verification of the interaction between CD99 and DDI2	82
3.3.4 Production of mAbs to CD99 interacting molecules	84
3.3.4.1 Specificity of the generated mAbs	87
3.3.4.2 Isolation of CD99 associated molecules using the generated mAbs	89
3.3.5 Association of CD99 with MHC class I and MHC class II and tetraspanin CD81	91
3.3.5.1 Association of CD99 with MHC class I and MHC class II	91
3.3.6 Association of CD99-MHC complexes with tetraspanin CD81	94
3.3.7 Colocalization of CD99 with MHC class I, MHC class II and tetraspanin CD81	97
3.4 Investigation of the association of CD99 short and long forms with MHC class I, MHC class II and CD81	98

3.5 Functional study of the CD99 molecules involving T cell activation	104
3.5.1 Recruitment of CD99 into the immunological synapse (IS)	104
3.5.2 Investigation the association between CD99 and lipid raft microdomain	104
3.5.3 Engagement of CD99 molecules inhibits T cell proliferation	105
3.5.4 Engagement of CD99 molecule induces cell apoptosis	105
3.5.5 Analysis of CD99 mediated signaling pathway	110
CHAPTER IV DISCUSSION AND CONCLUSION	114
REFERENCES	124
APPENDIX	134
CURRICULUM VITAE	152

LIST OF TABLES

Table	Page
1.1 Size and genomics of human tetraspanins	30
3.1 Reactivity of the generated mAbs with CD99 transfected and un-transfected COS cells	57
3.2 Summary of generated mAbs against CD99 associated molecules	85

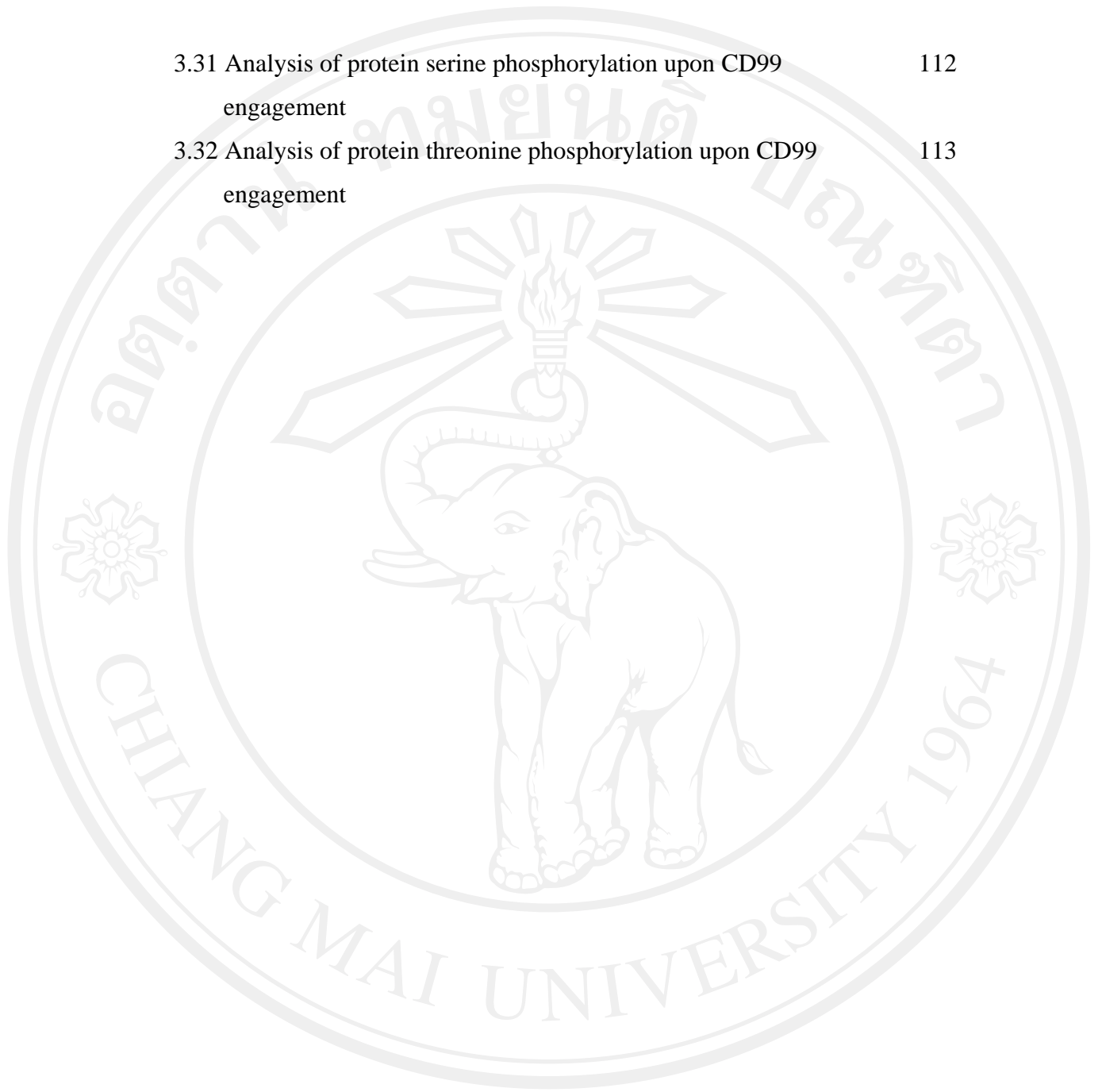
LIST OF FIGURES

Figure	Page
1.1 Innate and adaptive immunity	5
1.2 Types of adaptive immunity	8
1.3 The functions of antibodies in elimination of the invaders	10
1.4 The initiation and effector phases of cell-mediated immunity	13
1.5 TCR activation signaling pathway	18
1.6 Model for activation of BCRs signaling	19
1.7 Architecture of the conventional immunological synapse	24
1.8 The central event of T cell activation occurs in lipid rafts of the conjugation of T cell and APC	25
1.9 Structure of a typical tetraspanin	28
1.10 Dual role for CD81 in the antigen-presenting cell–T-cell immune synapse	29
1.11 Diagram of integration of proteins associate with the lipid bilayer	35
3.1 Immunofluorescence analysis of reactivity of mAb MT99/1 and MT99/2 with CD99-DNA transfected COS cells	58
3.2 Immunofluorescence analysis of the reactivity of mAbs MT99/1, MT99/2 and MT99/3 with peripheral blood leukocytes	59

3.3 Reactivity of mAbs MT99/1, MT99/2 and MT99/3 with red blood cells	60
3.4 Western blotting analysis of cell surface antigen recognized by mAbs MT99/1, MT99/2 and MT99/3	64
3.5 Immunoprecipitation analysis of cell surface antigen recognized by mAbs MT99/1, MT99/2 and MT99/3	65
3.6 Distribution of the CD99 molecule on various hematopoietic cell lines	66
3.7 Expression of CD99 isoforms on peripheral blood cells (A) and hematopoietic cell lines (B)	67
3.8 Coimmunoprecipitation of CD99 with other membrane proteins.	70
3.9 Coimmunoprecipitation of CD99 with other membrane proteins using cell lysates of various cell types	71
3.10 Coimmunoprecipitation of CD99 with other membrane proteins using raft-disrupting detergent 1% laurylmaltoside and mild detergent 1% Brij-58	72
3.11 Silver stained gel of CD99 immunoprecipitates	74
3.12 Investigation of the interaction between CD99 and GAPDH	76
3.13 Agarose gel electrophoresis of amplified PCR product and restriction fragment analysis of pMSCV-DDI2Myc	79
3.14 Western blot analysis of pMSCV-DDI2Myc transfected HEK293	80
3.15 Immunofluorescence analysis of CD99 expression on Ramos wild type and CD99 sorted cells	81

3.16 Verification of the interaction between CD99 and DDI2 protein	83
3.17 Reactivity of the generated mAbs with the CD99 precipitated proteins	86
3.18 Verification of the interaction between the proteins recognized by the generated mAb and CD99 molecules	88
3.19 Silver stained gel of immunoprecipitated protein using the generated mAbs against CD99 associated molecules	90
3.20 Interaction between CD99 and MHC class I and MHC class II	93
3.21 Association of CD99-MHC class II complexes with CD81	96
3.22 Colocalization of CD99 and MHC class I, MHC class II and CD81	100
3.23 Verification of pMSCV-CD99SF and pMSCV-CD99LF	101
3.24 Flow cytometric analysis of CD99 expression of Ramos expressing CD99 short and long forms	102
3.25 Interaction between CD99 isoforms with MHC class I, MHC class II and CD81	103
3.26 CD99 is recruited into the IS	106
3.27 The association between CD99 and lipid raft microdomain	107
3.28 Engagement of CD99 inhibits T cell proliferation	108
3.29 mAbs MT99/1 and MT99/3 induced cell apoptosis of Jurkat cells	109
3.30 Analysis of protein tyrosine phosphorylation upon CD99 engagement	111

3.31 Analysis of protein serine phosphorylation upon CD99 engagement	112
3.32 Analysis of protein threonine phosphorylation upon CD99 engagement	113



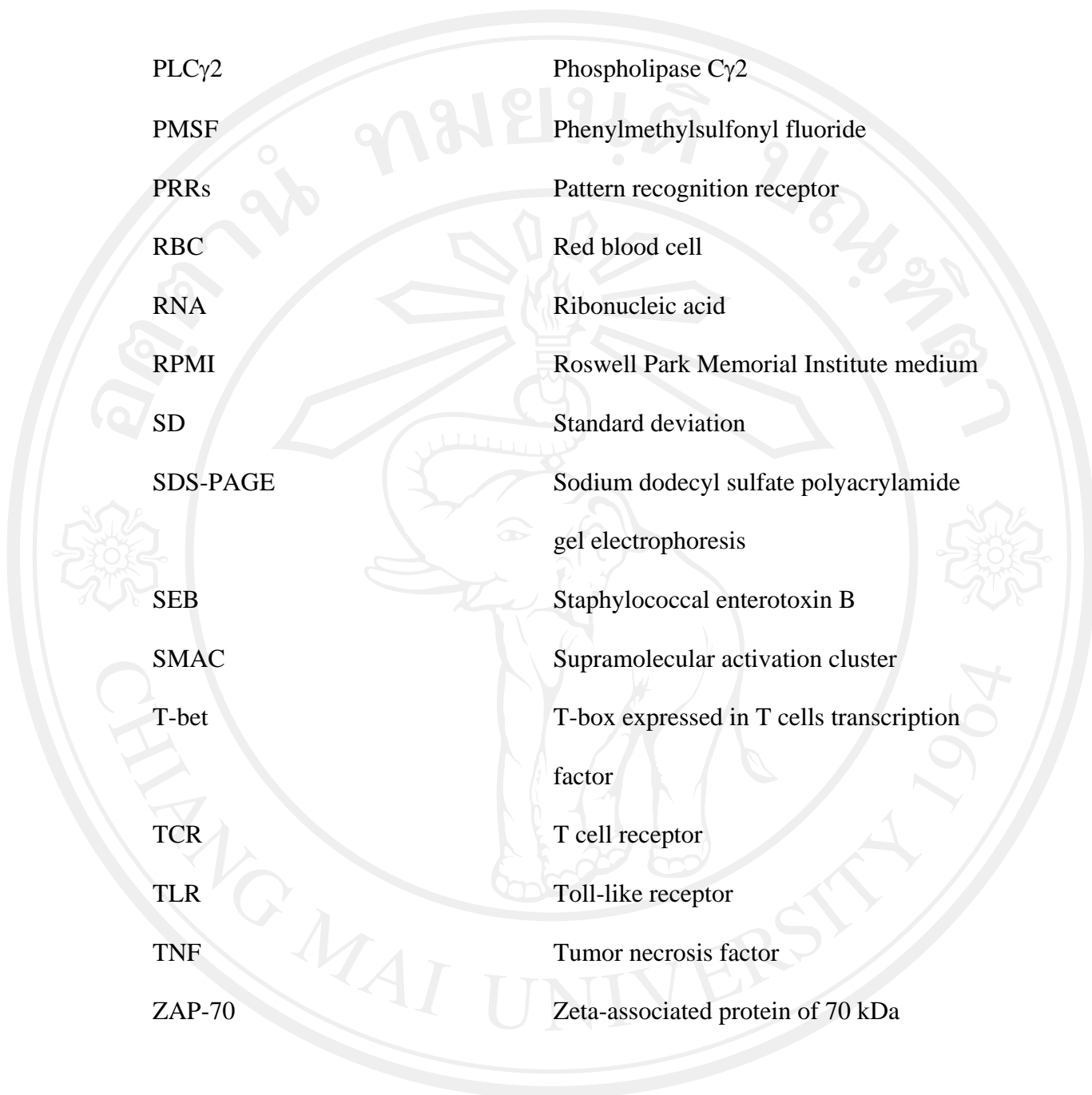
ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

ABBREVIATIONS

%	Percentage
α	Alpha
β	Beta
$^{\circ}\text{C}$	Degree Celsius
μCi	Microcurie
μg	Microgram
μl	Microlitre
μM	Micromolar
APC	Antigen-presenting cell
APS	Ammonium persulfate
BCR	B cell receptor
BSA	Bovine serum albumin
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CFSE	Carboxyfluorescein diacetate, succinimidyl ester
CMI	Cell mediated immunity
CTL	Cytotoxic T lymphocyte
DEAE-Dextran	Diethylaminoethyl-Dextran
DMEM	Dulbecco's modified Eagle's medium
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid

FAK	Focal adhesion kinase
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
g	Gram
HIV	Human immunodeficiency virus
HLDA	Human leukocyte differentiation antigens workshops
HMI	Humoral mediated immunity
HRP	Horseradish peroxidase
IFN	Interferon
Ig	Immunoglobulin
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Igs	Immunoglobulins
IL	Interleukin
IS	Immunological synapse
ITAM	Immunoreceptor tyrosinebased activation motif
kDa	Kilodalton
L	Liter
LAT	Linker for activation of T cells
LB	Luria Bertani

LM	laurylmaltoside or n-dodecyl-b-D-maltoside
LPS	Lipopolysaccharide
M	Molarity
mAb	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
MEM	Minimum essential medium
mg	Milligram
MHC	Major histocompatibility complex
ml	Milliliter
NaCl	Sodium chloride
NaHCO ₃	Sodium bicarbonate
ng	Nanogram
NK cells	Natural killer cells
PAMPs	Pathogen-associated molecular patterns
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PE	Phycoerythrin
PEG	Polyethylene glycol
pH	Power of hydrogen
PHA	Phytohaemagglutinin
PI3K	Phosphoinositide 3-kinase
PKC	Protein kinase C



PLC γ 2	Phospholipase C γ 2
PMSF	Phenylmethylsulfonyl fluoride
PRRs	Pattern recognition receptor
RBC	Red blood cell
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute medium
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEB	Staphylococcal enterotoxin B
SMAC	Supramolecular activation cluster
T-bet	T-box expressed in T cells transcription factor
TCR	T cell receptor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
ZAP-70	Zeta-associated protein of 70 kDa