

## Acknowledgements

si un dia me faltas no sere nada  
y al mismo tiempo lo sere todo



Dear friends, colleagues and family,

when I started this PhD project I knew more or less what I was getting myself into, since you already showed me: Hard work, long days, lots of disappointments and a booklet that seems, and actually still is, a never-ending story. However, we already had a pretty well working method to put these things in perspective. All it took were almost weekly dinners with delicious homemade tapas, soups, paellas, meatballs and some vegetarian stuff. Occasionally some Surinamese, Swedish or “Limburgian” food was allowed as well. And, very much-missed...desserts, in cake form and any other form! Maybe I should mention that for me, this whole dinner-ritual started with a chocolate-brick, or that since you all left Amsterdam I lost almost 10 kilos, but then again...

“In der Beschränkung zeigt sich der Meister”, right Victor? You have taught me well. I have always enjoyed working with you. Thank you for all your time and good advise. I will try to use it wisely.

I could not have managed to finish this book without the support and friendship of all the people that were regular guests at that dinner table, or who were at least regularly invited.... Even though we have sometimes not seen or even spoken to each other for a long time, to all of you I want to say this: I hope to see you after the defense, dinner will be served!

Geerten, during these years we have said almost everything there is to say. And yes, “alles is veel omvattend”. Sometimes we bit our tongs, and sometimes we did not. The latter usually resulted in me struggling to apologize, while you sat behind your desk with an enormous grin on your face.... you have truly given punishment a new dimension! Yet, passionately and patiently, you have mainly inspired me, both professionally and personally. Thank you for everything! I wish you all the best, and I hope you let me know when more “seafood” is required.

All other colleagues and friends from physiology, oncology, pathology, photography and the technical services: without your good fellowship, friendly resignation and your commitment to foolery, I would not have been able to enjoy going to work every morning. Thanks a lot! See you in Cobra!

*Cora*



**Curriculum Vitae**

Cora Maria Leo Beckers was born in Heerlen November 2nd 1978. After graduating high school in 1998 she started her study Medical Biology at the VU University in Amsterdam. During her first research training at the department of Experimental Oncology of the VU University medical center she assessed the effect of Doxorubicin on HIF-1 $\alpha$  protein and VEGF mRNA levels in normoxic and hypoxic human ovarian cancer cell lines under supervision of dr. M.C.A. Duyndam. She decided to stay in Amsterdam and continued her research training at the department of Tumor Immunology at the Dutch Cancer Institute (NKI). Under supervision of dr. R.M. Luiten she worked on a project to isolate CD4<sup>+</sup> T cells reactive with MART-1 or NYESO-1 antigens for adoptive transfer therapy of human melanoma. After writing her small thesis on  $\beta$ -cell transplantation at the department of Immunohematology and Blood Transfusion of the Leiden University medical center under supervision of dr. B.O. Roep, she moved to Málaga, Spain. Here, she did an additional internship at the Immunology department and Human Pancreatic Islet Laboratory of dr. A. Alonso Ortiz, which she combined with a study Spanish at the Málaga University. In February 2004 she started her PhD project financed by the Institute for Cardiovascular Research of the VU University medical center. With great pleasure she worked at the department for Physiology, under supervision of prof.dr. V.W.M. van Hinsbergh and dr. G.P. van Nieuw Amerongen. This resulted in the here presented thesis entitled: "Spatio-temporal signaling controlling endothelial permeability." Recently, she transferred to the Cardiovascular Research Institute Maastricht. In the Experimental Vascular Pathology group of prof.dr. E.A.L. Biessen she is currently studying the basic mechanisms in atherogenesis.



**List of publications**

- (1) RG Naik, CML Beckers, R Wentwoord, A Frenken, G Duinkerken, B Brooks-Worrell, NC Schloot, JP Palmer and BA Roep (2004). Precursor frequencies of T-cells reactive to insulin in recent onset of type I diabetes mellitus. ***Journal of Autoimmunity***, 23: 55-61.
- (2) P da Costa Martins, JJ García Vallejo, JV van Thienen, M Fernandez-Borja, JM van Gils, CML Beckers, AJ Horrevoets, PL Hordijk, JJ Zwaginga (2007). P-Selectin glycoprotein ligand-1 is expressed on endothelial cells and mediates monocyte adhesion to activated endothelium. ***Arteriosclerosis, Thrombosis and Vascular Biology***, 27(5):1023-9.
- (3) GP van Nieuw Amerongen, CML Beckers, ID Achekar, S Zeeman, RJP Musters, VWM van Hinsbergh (2007). Involvement of Rho kinase in endothelial barrier maintenance. ***Arteriosclerosis, Thrombosis and Vascular Biology***, 27;2332-2339.
- (4) JJ García Vallejo, E van Liempt, P da Costa Martins, CML Beckers, B van het Hof, SI Gringhuis, JJ Zwaging, W van Dijk, TBH Geijtenbeek, Y van Kooyk, I van Die (2008). DC-SIGN mediates adhesion and rolling of dendritic cells on primary human umbilical vein endothelial cells through Lewis<sup>Y</sup> antigen expressed on ICAM-2. ***Molecular immunology***, 45:2359-2369.
- (5) CML Beckers, JJ García Vallejo, VWM van Hinsbergh and GP van Nieuw Amerongen (2008). Nuclear targeting of  $\beta$ -catenin and p120ctn during thrombin induced endothelial barrier dysfunction. ***Cardiovascular Research***, 79;679-688.
- (6) R Vlasblom, A Muller, CML Beckers, GP van Nieuw Amerongen, M Zuidwijk, C van Hardeveld, W Paulus, W Simonides (2009). RhoA-ROCK signaling is involved in contraction-mediated inhibition of SERCA2a expression in cardiomyocytes. ***European Journal of Physiology***, 458:785-793.
- (7) CML Beckers, VWM van Hinsbergh and GP van Nieuw Amerongen (2009). Driving Rho GTPase activity in endothelial cells regulates barrier integrity. ***Thrombosis and Haemostasis***, in press.



**Abbreviations**

AC	adenyl cyclase
AJ	adherens junction
Ang	angiopoietin
APC	adenomatosis poliposis coli
ARDS	acute respiratory distress syndrome
Ca <sup>2+</sup>	calcium
CaM	calmodulin
cAMP	cyclic adenosine monophosphate
CCM	cerebral cavernous malformations proteins
CFP	cyan fluorescent protein
CK	casein kinase
DAG	diacylglycerol
DH	Dbl homology
EBA	Evans blue conjugated albumin
EC	endothelial cell
ECIS	electrical cell substrate impedance sensing
eNOS	endothelial nitric oxide synthase
ERM	ezrinn, radixin and moesin
FAK	focal adhesion kinase
Fasudil	Rho kinase inhibitor
FRET	flourescent resonance energy transfer
FRETc	calculated FRET
FRETc/A	calculated FRET corrected for acceptor amount
GAP	GTPase activating protein
GDI	guanine nucleotide dissociation inhibitor
GEF	guanine nucleotide exchange factor
G-LISA	Rho GTPase activation assay
GPCR	G-protein coupled receptor
GSK-3 $\beta$	glycogen synthase kinase 3 $\beta$
H1152	Rho kinase inhibitor
HEG	heart of glass
HRP	horseradish peroxidase
HSP	heat shock protein
HUVEC	human umbilical vein endothelial cell
IL	interleukin
IP3	inositol triphosphate
IQGAP1	IQ motif containing GTPase-activating protein 1

## Abbreviations

KRIT1	also known as CCM1 (see CCM)
LARG	leukemia-associated RhoGEF
LiCl	lithiumchloride
LPA	lysophosphatidic acid
MAGI-1	membrane-associated guanylate kinase with inverted domain structure-1
MAPK	mitogen-activated protein kinase
MBS	myosin binding subunit
MG132	proteasome inhibitor
MLC	myosin light chain
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase
MMP	matrix metalloproteinase
MP	myosin phosphatase
MT	microtubule
MYPT1	myosin phosphatase targeting subunit
p120ctn	p120catenin
PAR	protease-activated receptor
PH	plekstrin homology
PI3K	phosphatidylinositol 3-kinase
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PRK or PKN	protein kinase C-related kinase
PTK	protein tyrosine kinase
Raichu	Ras and interacting protein chimeric unit
RBD	Rho binding domain
ROCK-I	Rho kinase isoform 1
ROCK-II	Rho kinase isoform 2
ROS	reactive oxygen species
RTK	receptor tyrosine kinase
S1P	shingosine-1-phosphate
SAPK	stress-activated protein kinase
SF	stress fiber
SH	Src homology
siRNA	short interfering RNA
Slx-2119	ROCK-II specific inhibitor
Slx-3242	Rho kinase inhibitor
Syx	synectin-binding RhoA exchange factor