Presenter Disclosure Information Elements

Esther Lutgens, MD PhD
Immune checkpoint regulators in Atherosclerosis

FINANCIAL DISCLOSURE:

No relevant financial relationship exists

American Heart Association®

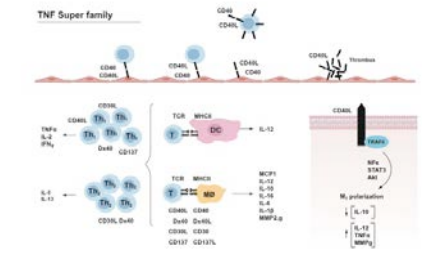
Jeffrey M. Hoeg (1952-1998)

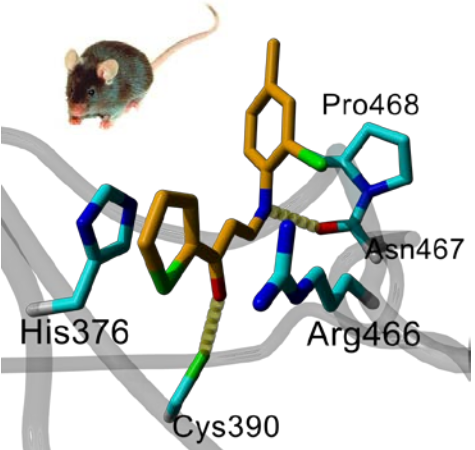
Great Scientist & clinician

Superb mentor

Active member of many societies (ATVB, AAAS, ACC)

American Heart Association.





Immune Checkpoint Regulators in Atherosclerosis

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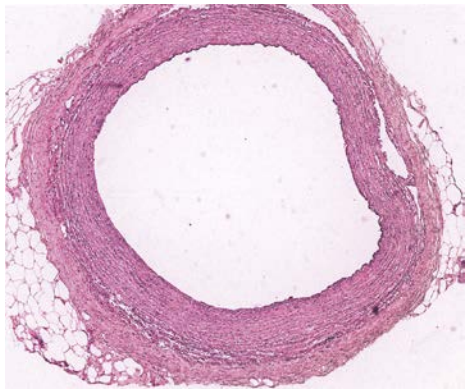
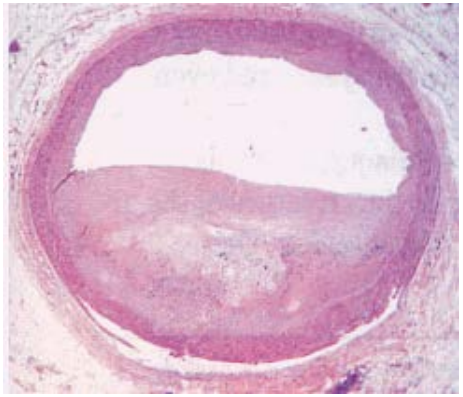
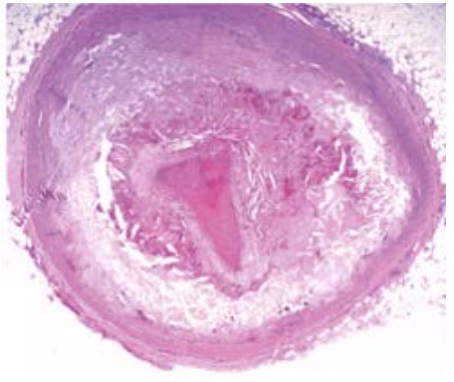
IPEK

Atherosclerosis is a lipid-driven immune disease

Cholesterol- Triglycerides



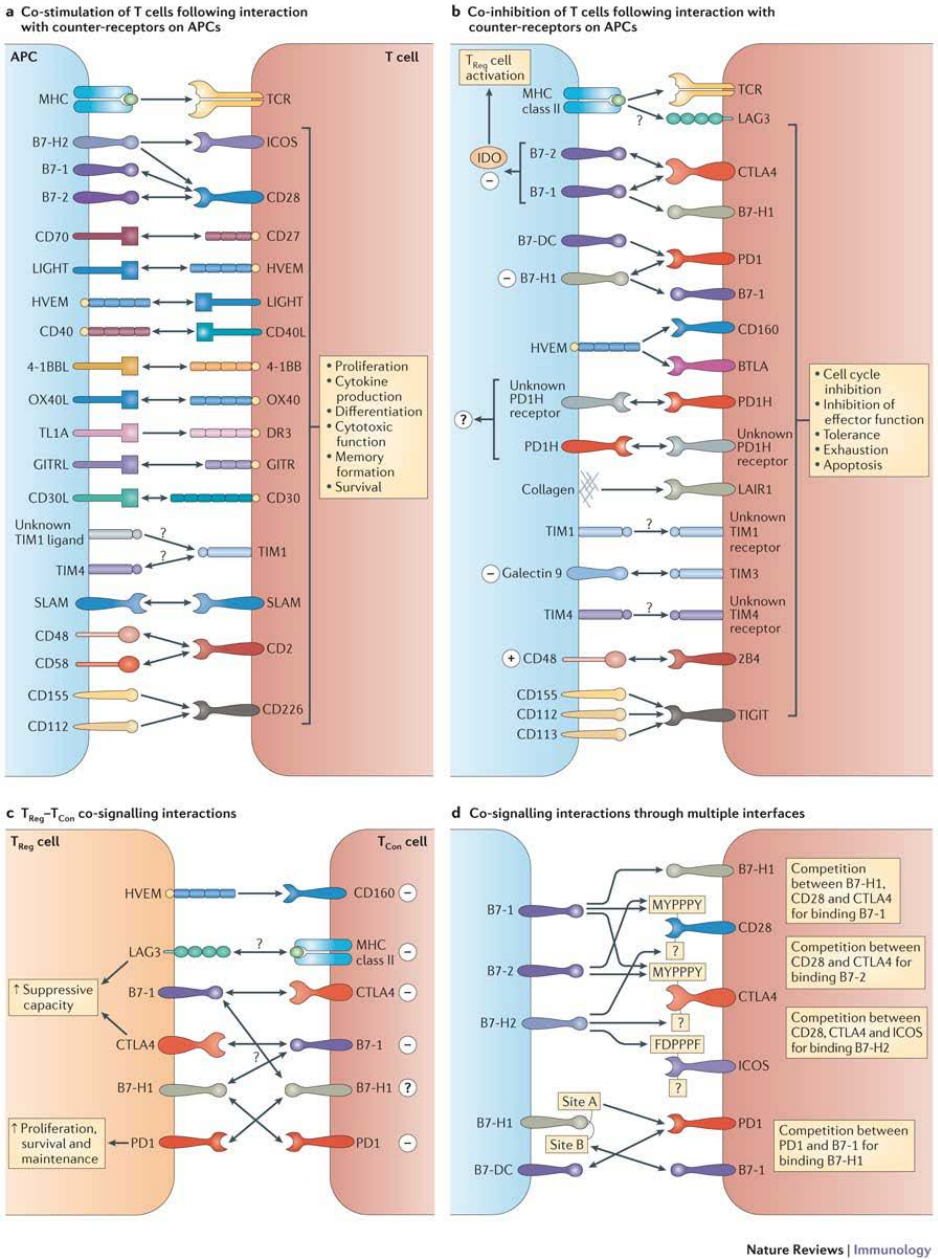


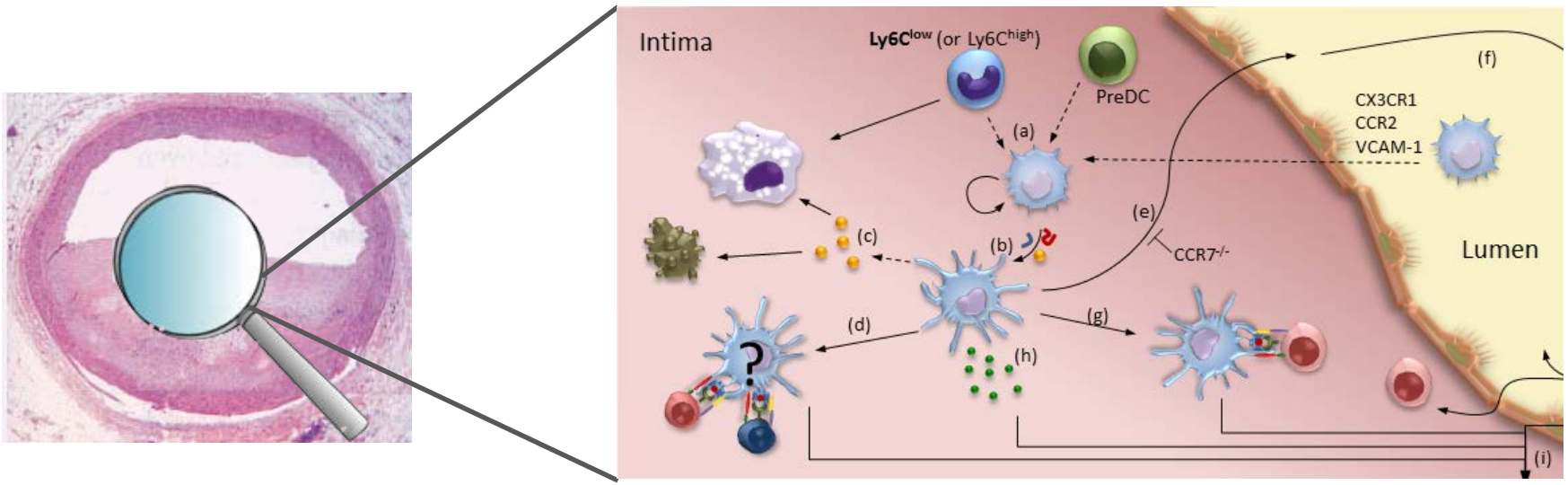


Immune system

IMMUNE CHECKPOINT REGULATORS

Co-stimulatory molecules

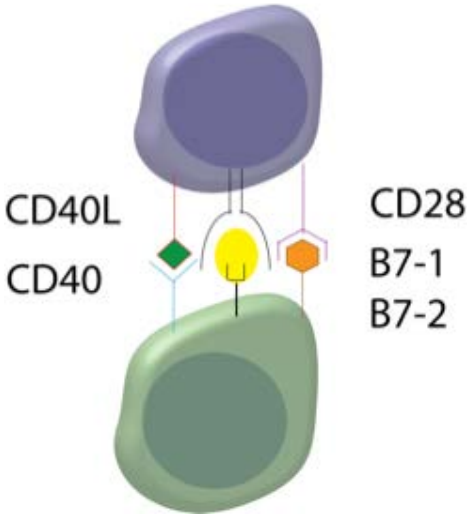




Legein et al, Cell Mol Life Sci, 2013

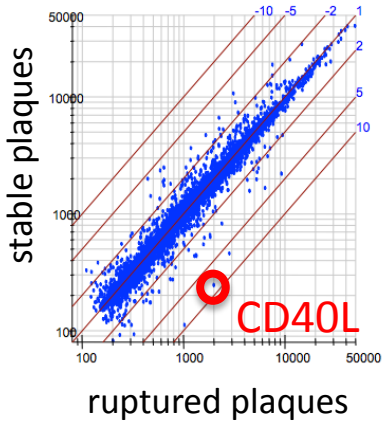
Co-stimulation warrants proper immune reactions

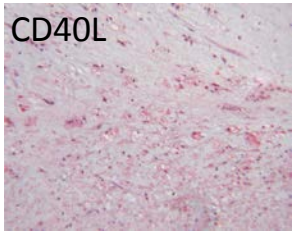
- Signal 2 in T-cell/APC interactions: proliferation and polarisation
- Endothelial cell activation
- Platelet activation

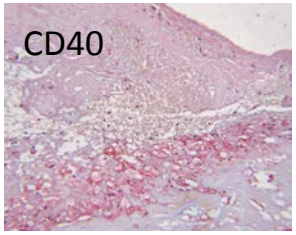


CD40L-CD40 interactions drive atherosclerosis

CD40L



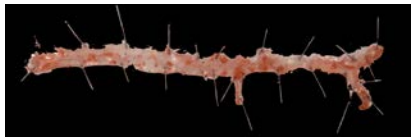


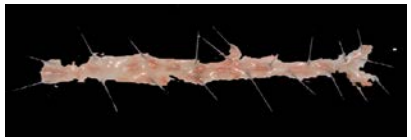


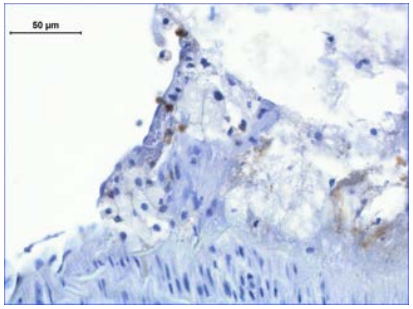
Apoe$^{-/-}$

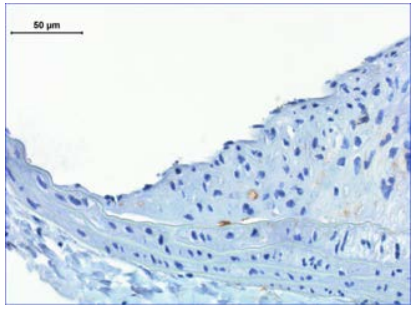
Cd40l$^{-/-}$*Apoe*$^{-/-}$

CD40









Apoe$^{-/-}$

Cd40l$^{-/-}$*Apoe*$^{-/-}$

Lutgens et al, Nat Med, 1999, PNAS 2000, J Exp Med 2010

Inhibition of CD40L or CD40 as therapy for atherosclerosis??

Short term anti-CD40(L) antibody treatment has been tested in phase I/II trials in MS, Crohn's disease, and (hematologic) malignancies

but..

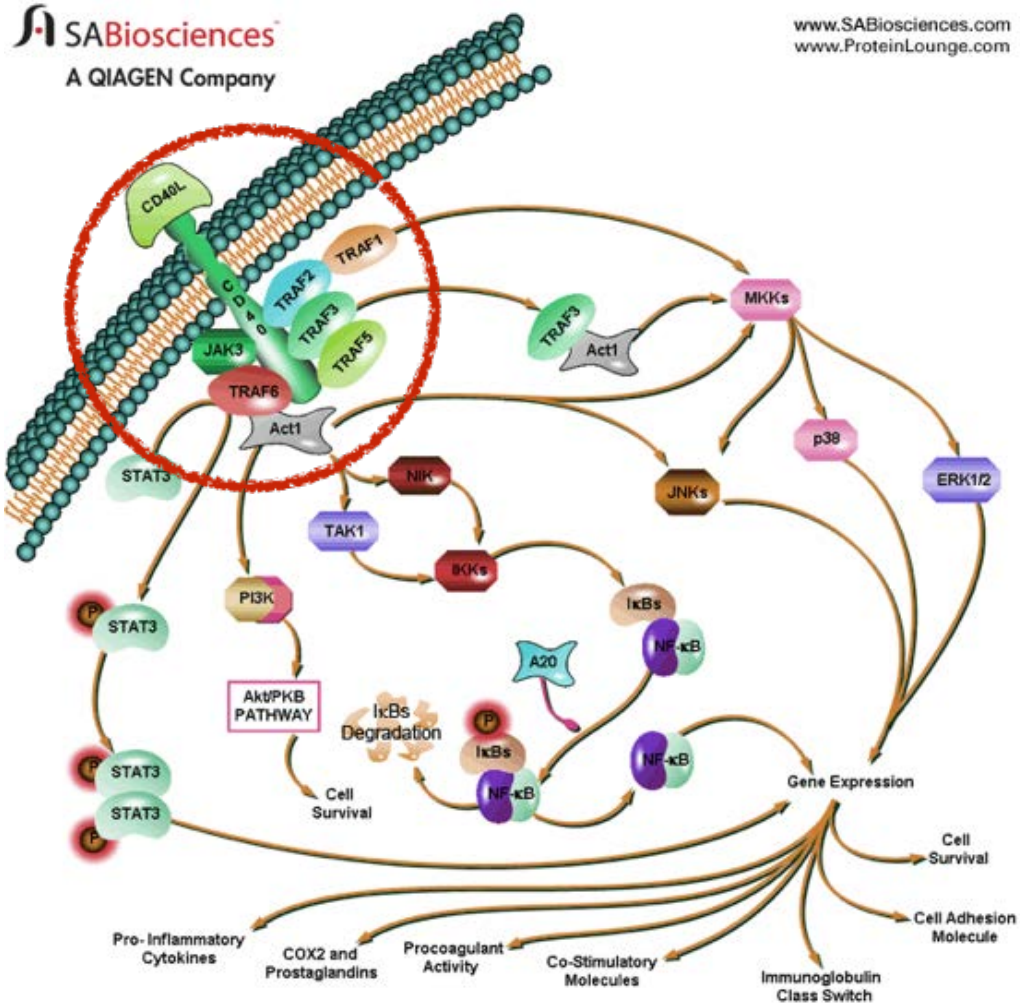
Long-term blockage of CD40-CD40L will result in immune-suppression…

CD40-CD40L as therapeutic target in atherosclerosis: knowledge gaps

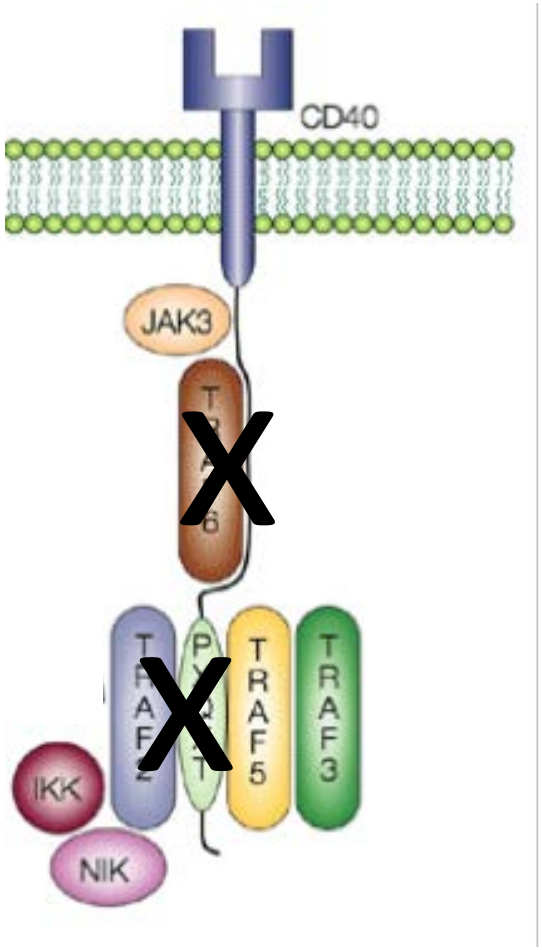
- Which **signaling pathways** are involved?
- Which **cell types** that express CD40(L) are involved in atherosclerosis?
- How does the **co-stimulatory interactome** work in atherosclerosis?

SIGNALING

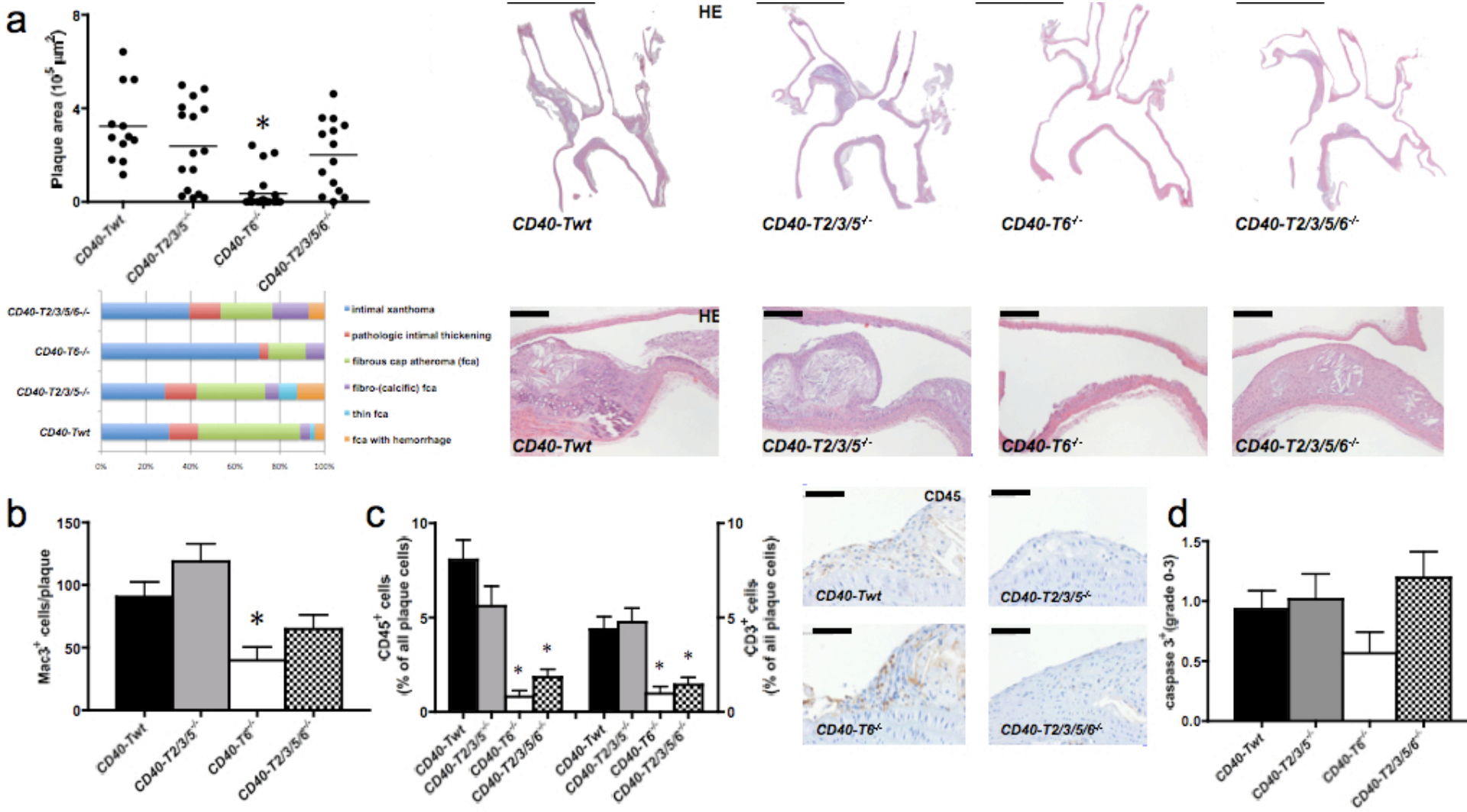
Identification of CD40-downstream pathways in vascular disease



CD40-TRAF interactions: mouse model



CD40-TRAF6 signaling in MHCII+ cells drives atherosclerosis



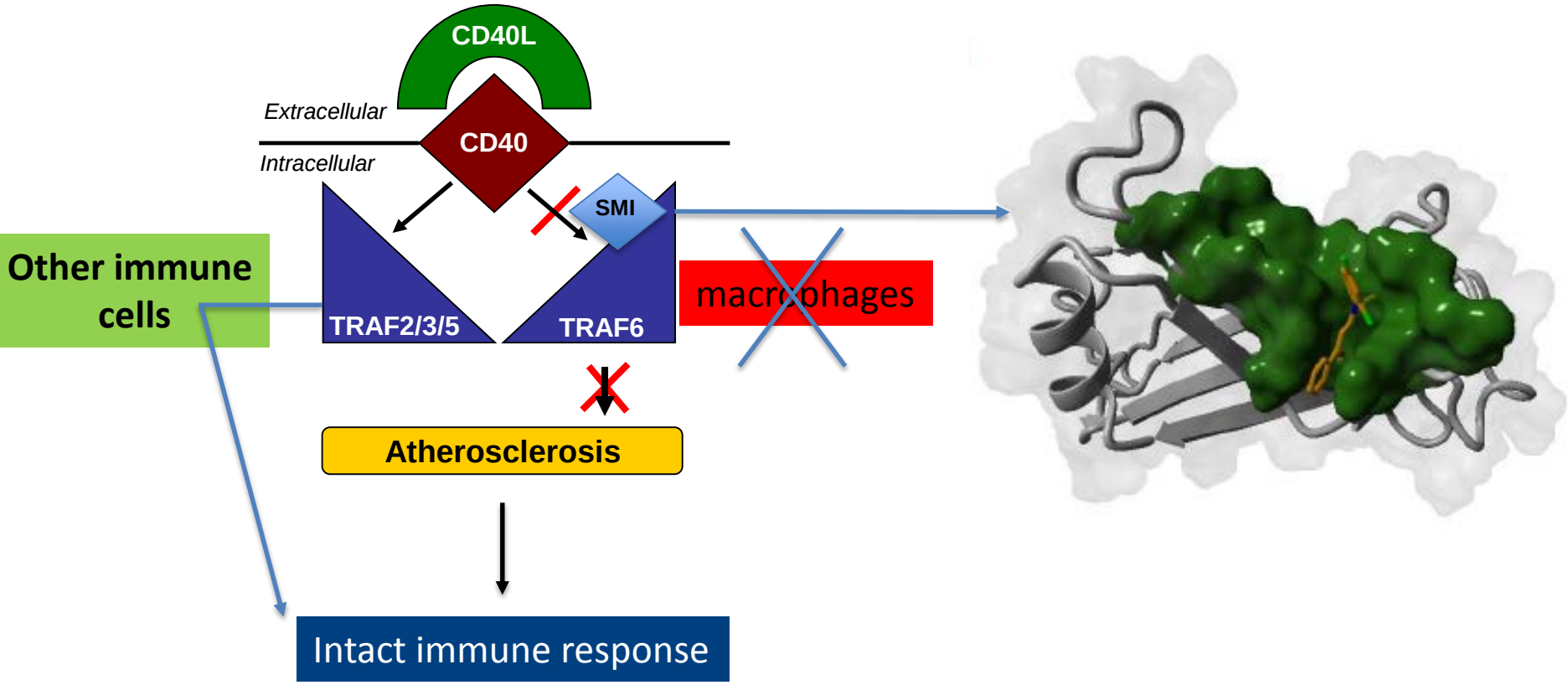
CD40-TRAF interactions in atherosclerosis

MHCII dependent CD40-TRAF6, but not CD40-TRAF2/3/5 signaling inhibits atherosclerosis

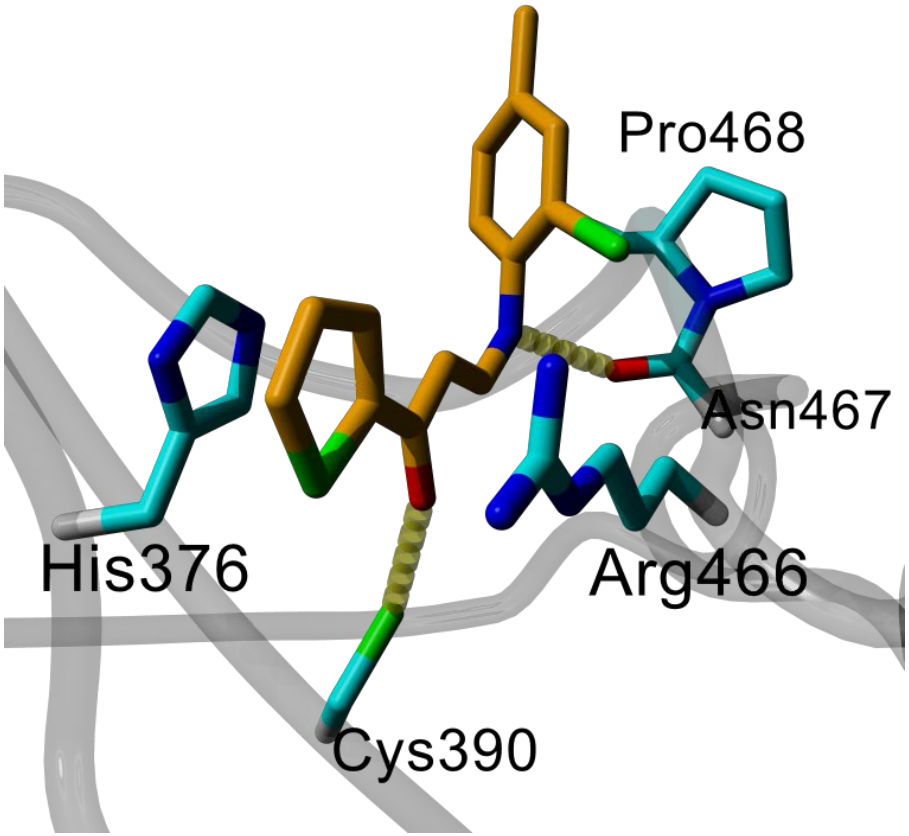
CD40-TRAF6 deficiency omits the Ly6C^{high} monocyte population and polarizes macrophages towards an alternatively activated anti-inflammatory phenotype.

CD40-TRAF6 but not CD40-TRAF2/3/5 interactions drive macrophage activation

CD40-TRAF6: a novel therapeutic target??

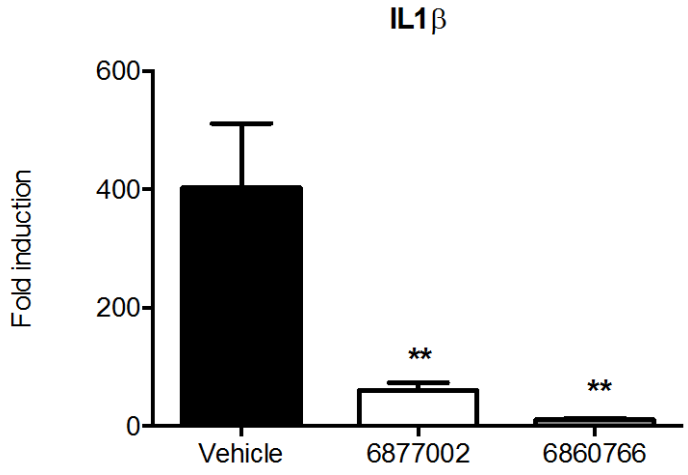


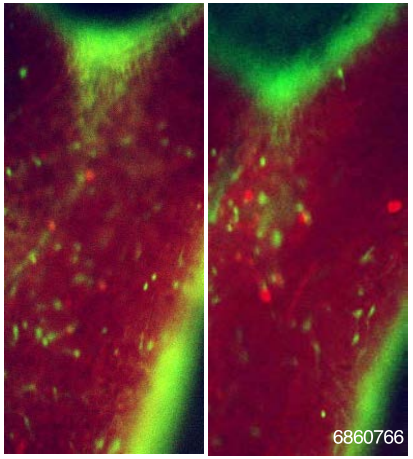
Top-SMIs: TRAF-STOP

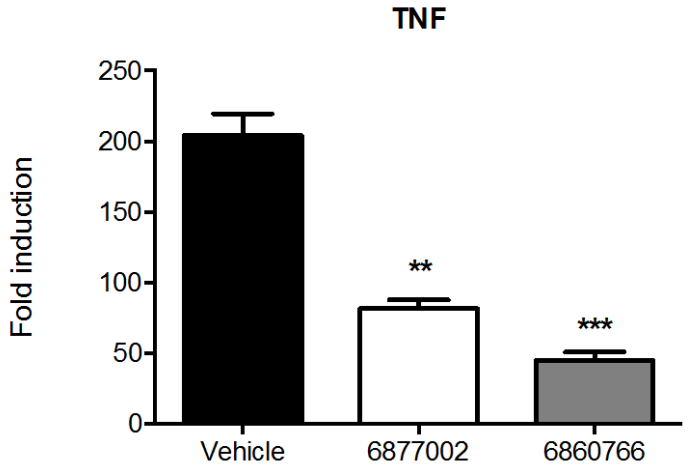


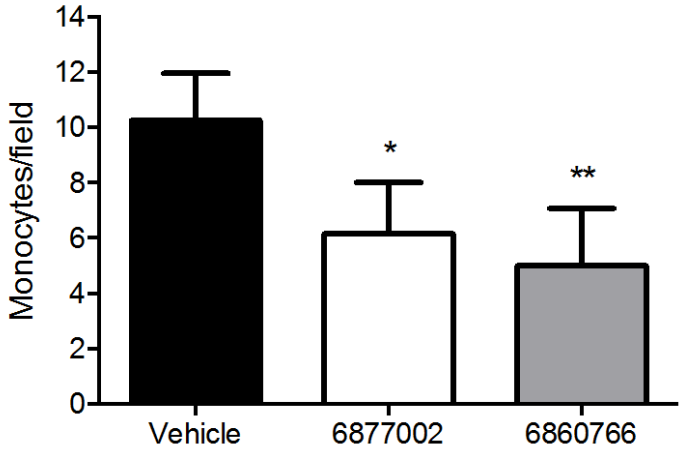
Chatzigeorgiou, Seijkens et al PNAS 2014; Zarzyka et al. J Chem Inf Mod 2015, van den Berg et al. Int J Obes 2015

TRAF-STOPs decrease CD40-induced monocyte recruitment and macrophage activation

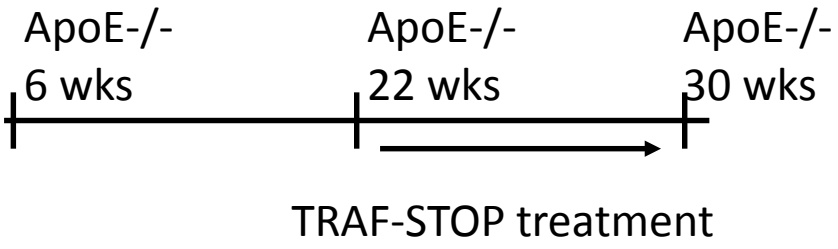


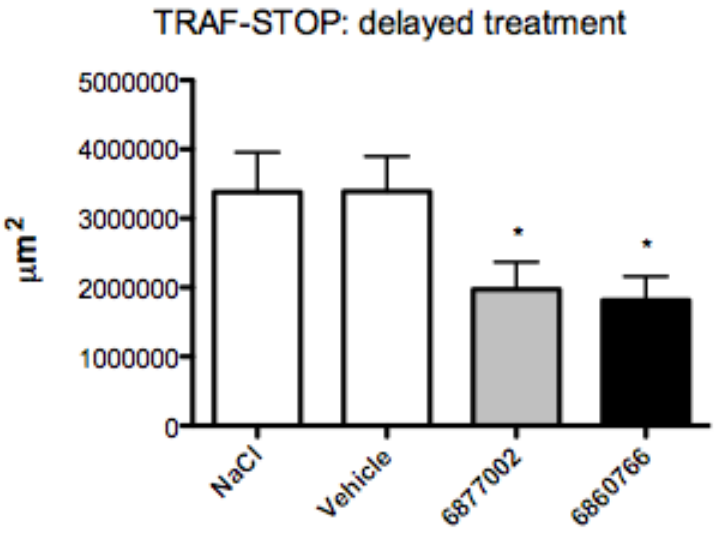


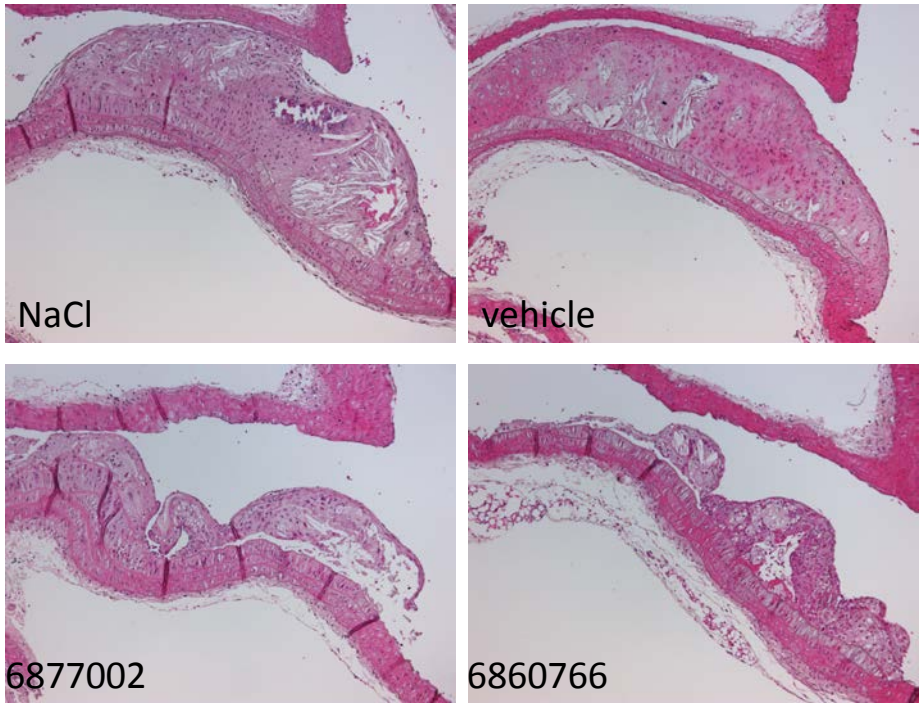




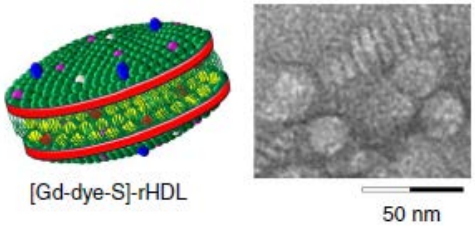
TRAF-STOPs reduce established atherosclerosis







TRAF-STOP rHDL-nanoparticles



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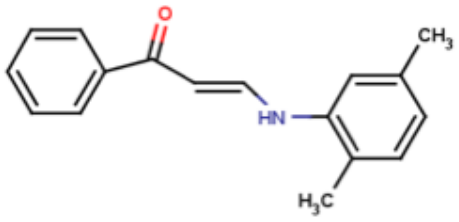
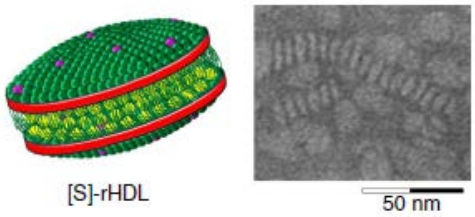
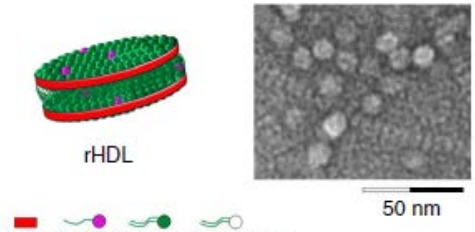
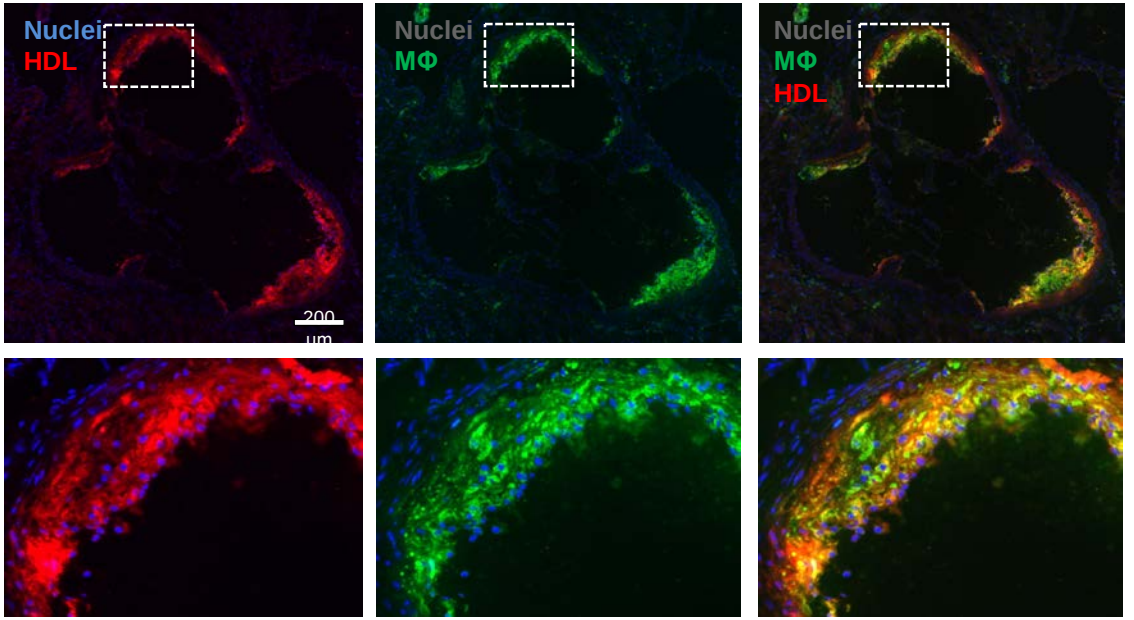


= TRAF-STOP-rHDL

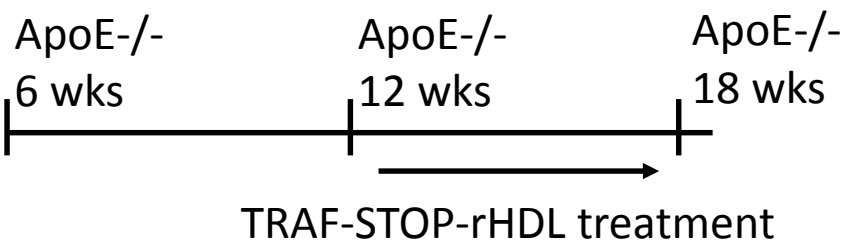


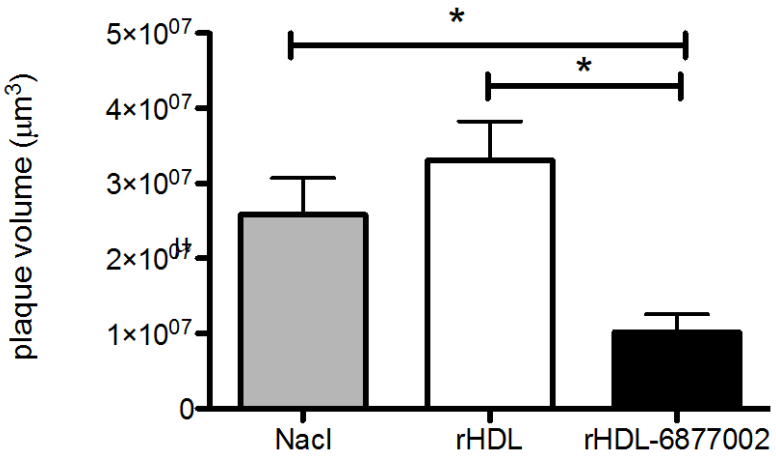


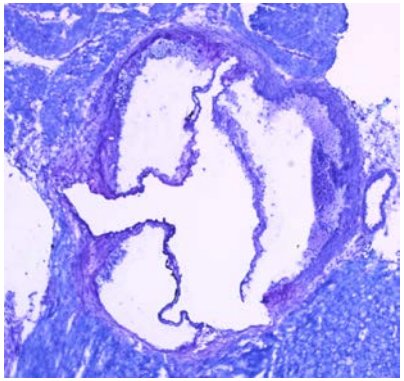


W. Mulder, MSSM & E. Fisher, NYU, NYC

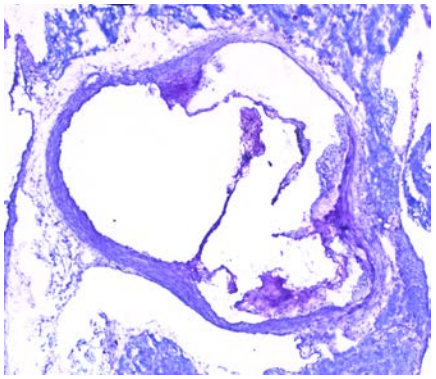
Does TRAF-STOP-HDL treatment reduce atherosclerosis???







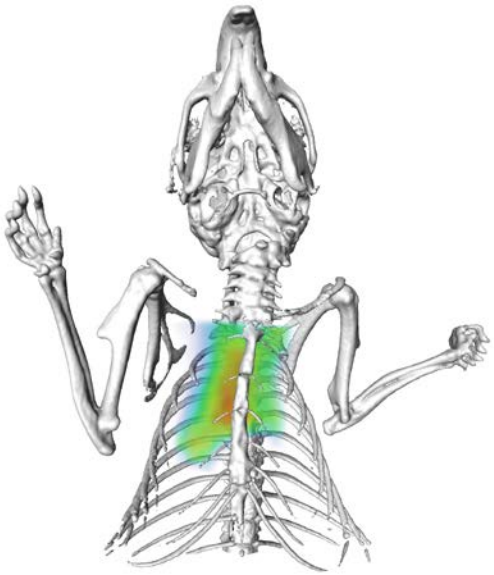
rHDL



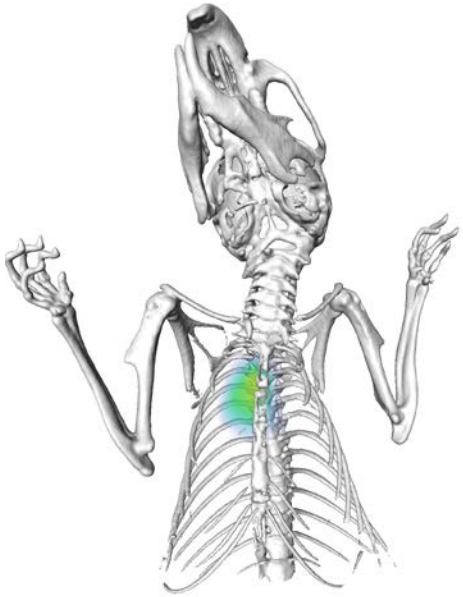
TRAF-STOP-rHDL

Seijkens et al.. In preparation

Does TRAF-STOP-rHDL treatment reduce established atherosclerosis???

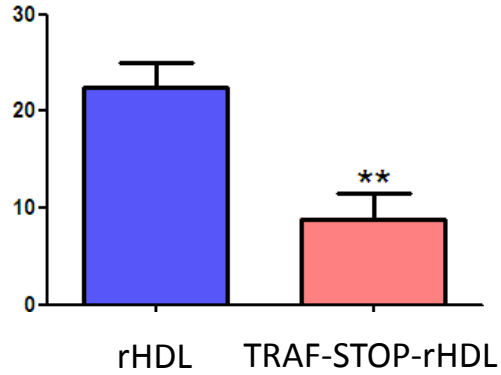


Ctrl-HDL

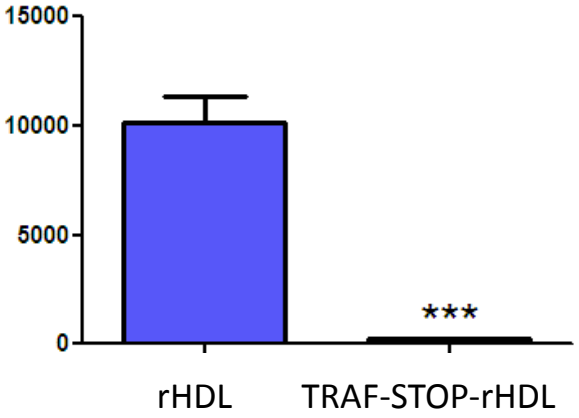


TRAF-STOP-HDL

FMT-CT: Protease activity



Macrophage counts (FACS)



Lameijer, Duivenvoorde, Nahrendorf, Mulder et al.. In preparation

Conclusions

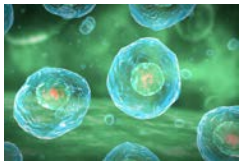
- Macrophage CD40-TRAF6 interactions drive atherosclerosis
- Small molecule mediated inhibition (nanoparticles) of CD40-TRAF6 interactions is a promising therapeutic strategy for the treatment of atherosclerosis
- …but also obesity, EAE, sepsis, peritonitis
- Promising future for TRAF-STOP

CELLS

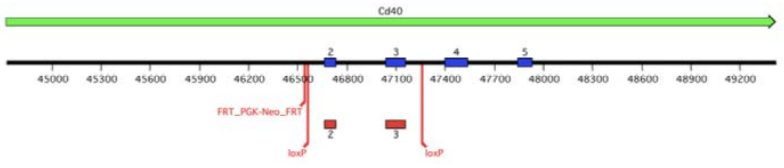
(Plaque) cell type	CD40L	CD40
T-cell	++	+
B-cell	-	++
DC	+	++
macrophage	+-	++
platelet	++	+
VSMC	+	+
Endothelial cell	+	+

Bone marrow transplantation

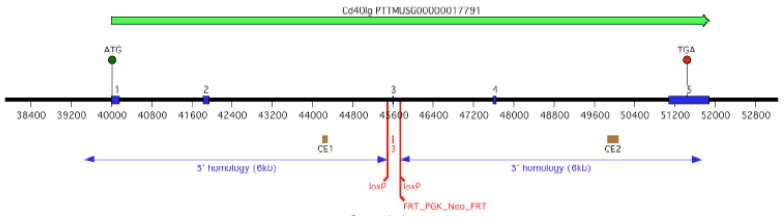
Adoptive transfer



Transgenic mice

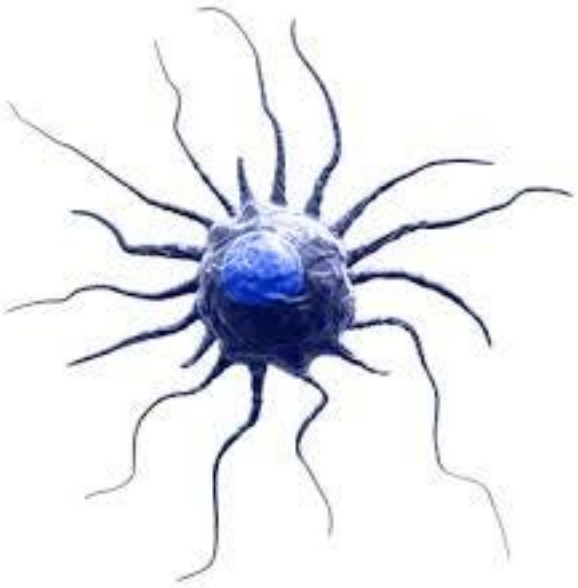


cd40^{flfl}



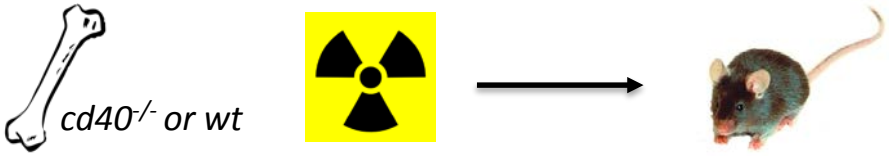
cd40l^{flfl}

Monocytes/macrophages

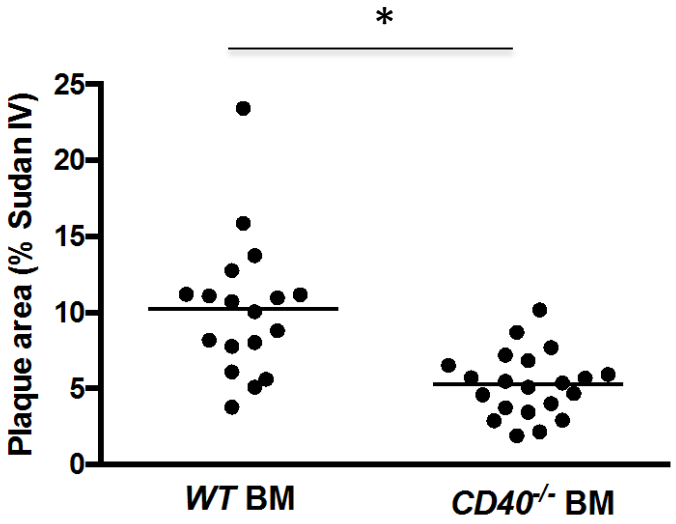


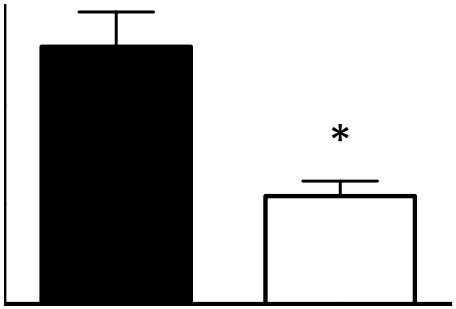
CD40 goes innate….

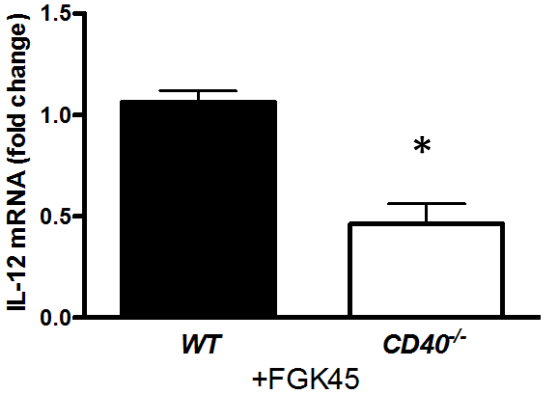
CD40 and macrophages

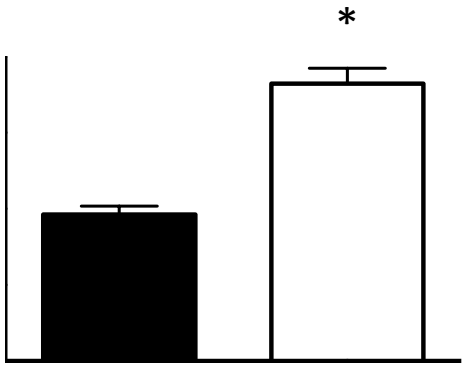


Ldlr$^{-/-}$ *cd40*$^{-/-}$ or *wt Ldlr*$^{-/-}$ chimeras

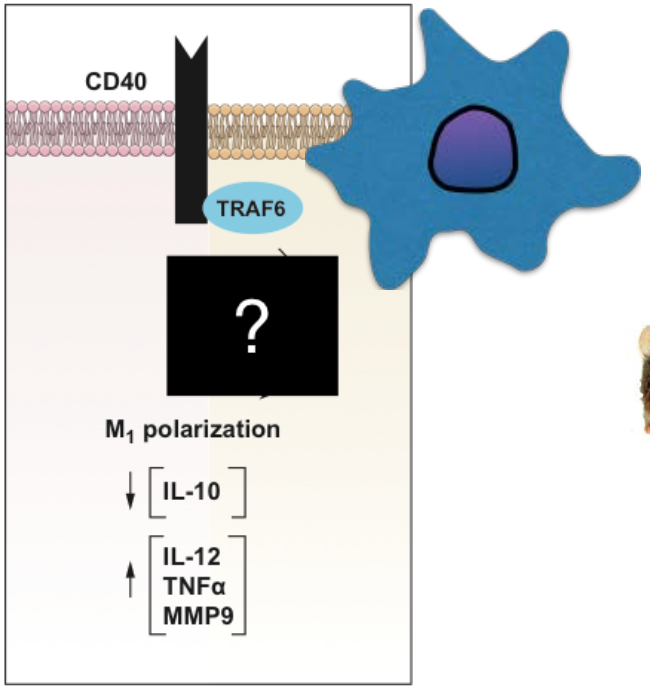


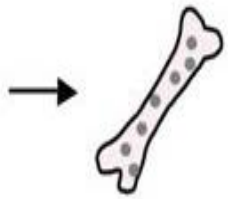






Lutgens et al, J Exp Med 2010 & unpublished





Isolation and culture of BMDMs

macrophages

-

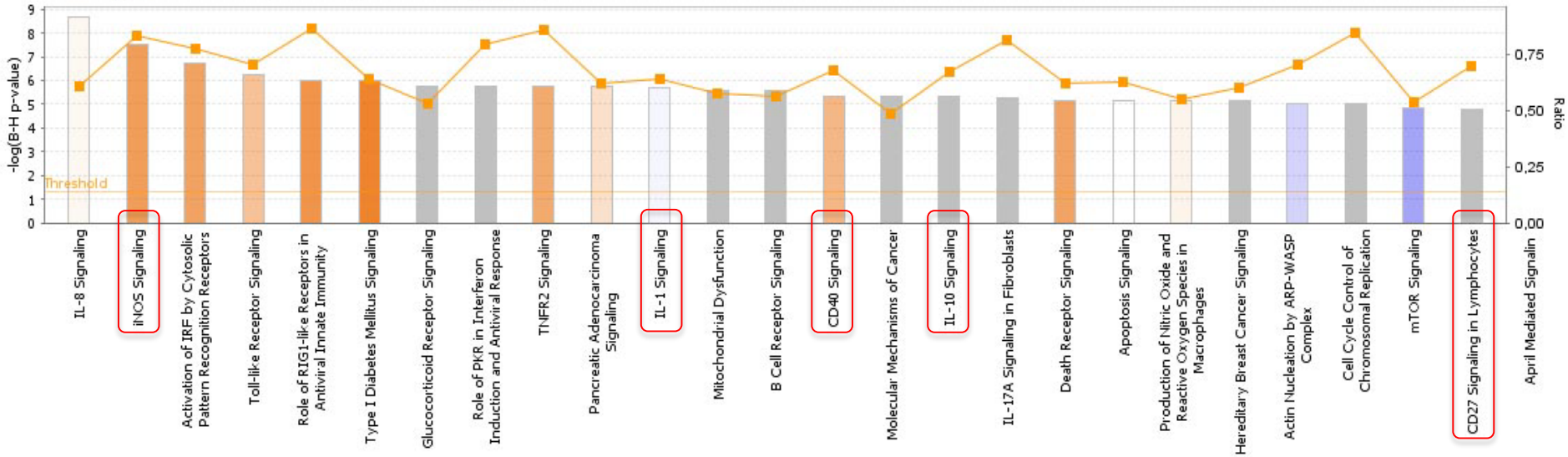
FGK
(CD40 activation)

foam cells

oxLDL

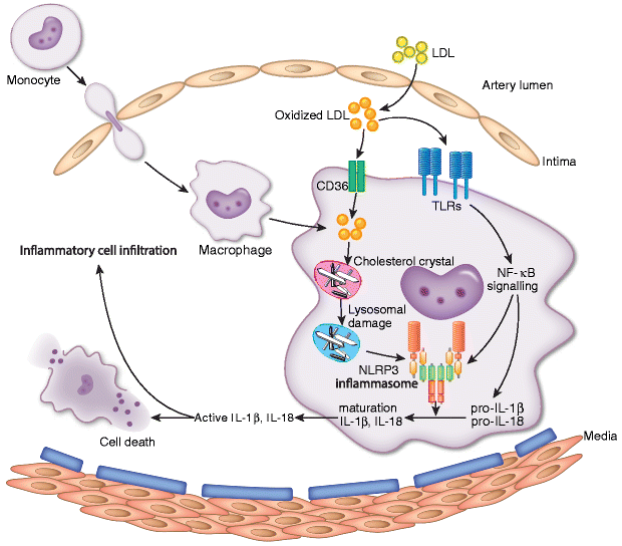
oxLDL +FGK
(CD40 activation)

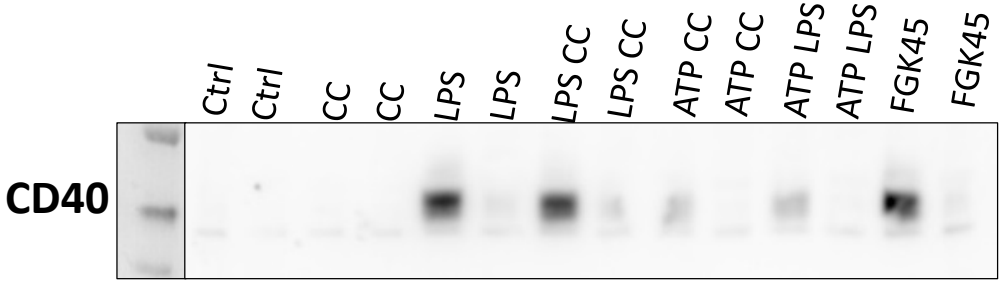
FGK-stimulated vs control **foam cells**

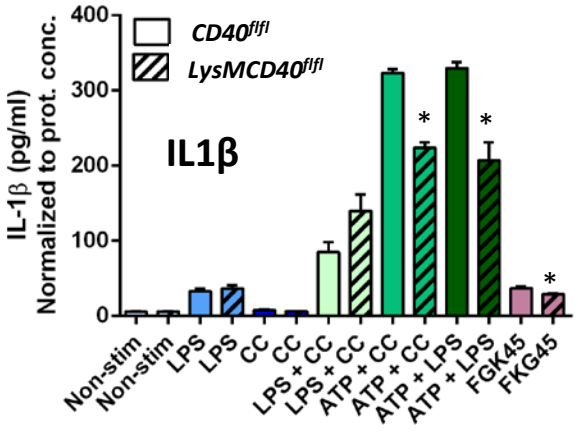


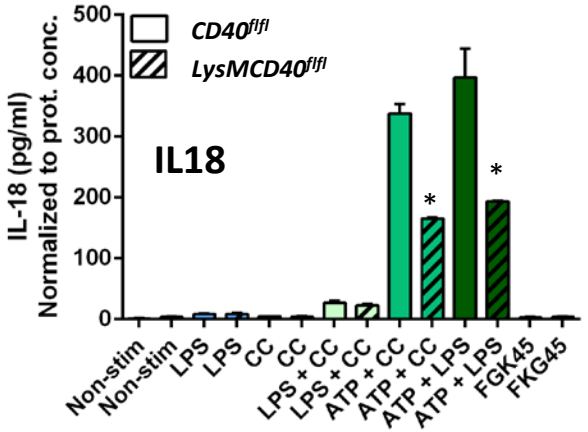
Illumina mouse WG-6 arrays

Macrophage CD40 activates the inflammasome

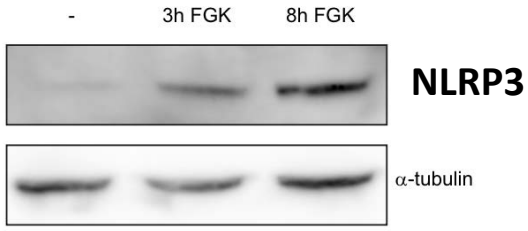








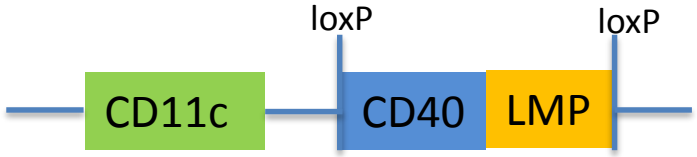
!	Macrophages! !		Foam cells! !	
Gene!	Fold change!	Adj. p-value!	Fold change!	Adj. p-value!
NLRP3!	2.6 1.9!	$9.3x10^{-7}$ $6.0x10^{-5}$!	3.6 3.4!	$4.0x10^{-8}$ $4.8x10^{-8}$!
ASC (Pycard)!	1.2 0.9!	0.26 0.72!	0.6 0.6!	$1.8x10^{-4}$ 0.003!
Caspase-1!	2.5!	$8.4x10^{-9}$!	2.0!	$1.9x10^{-7}$!
IL1b!	549!	$4.2x10^{-10}$!	416 !	$6.5x10^{-10}$!



Shami, van Tiel, unpublished

Dendritic cells

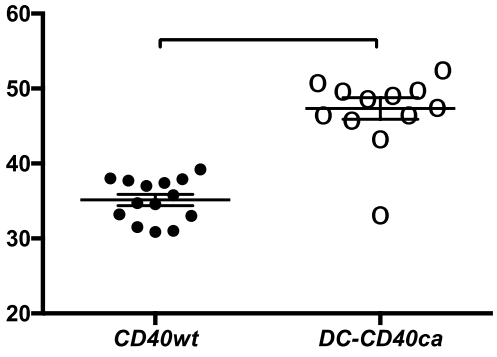
CD40: dendritic cells

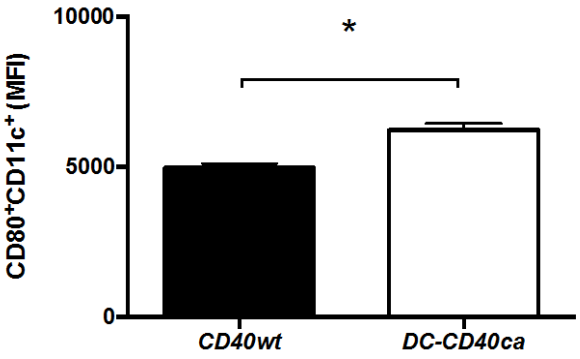


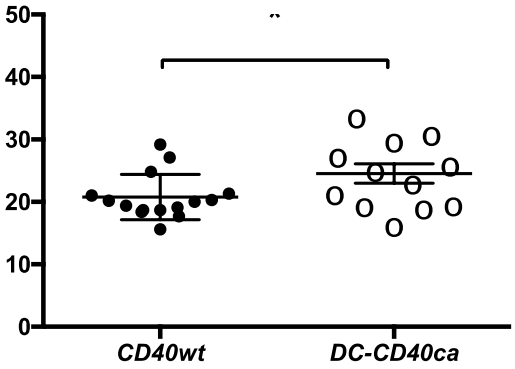
Constutively active CD40 signaling in DCs

DC-CD40ca
wt

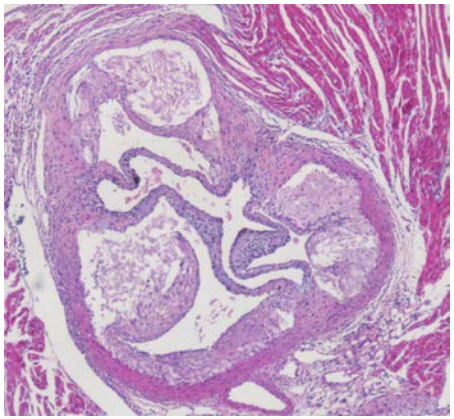
DC-CD40ca-ldlr-/- chimeras
Wt-ldlr-/- chimeras

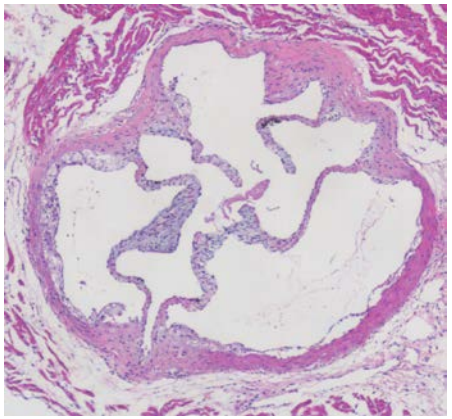




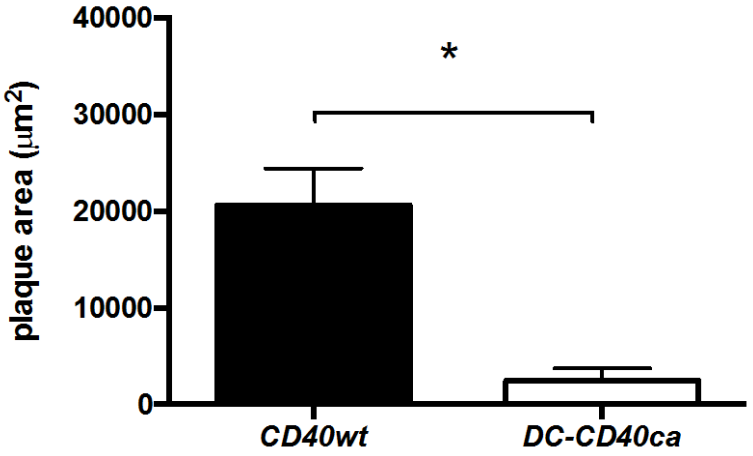


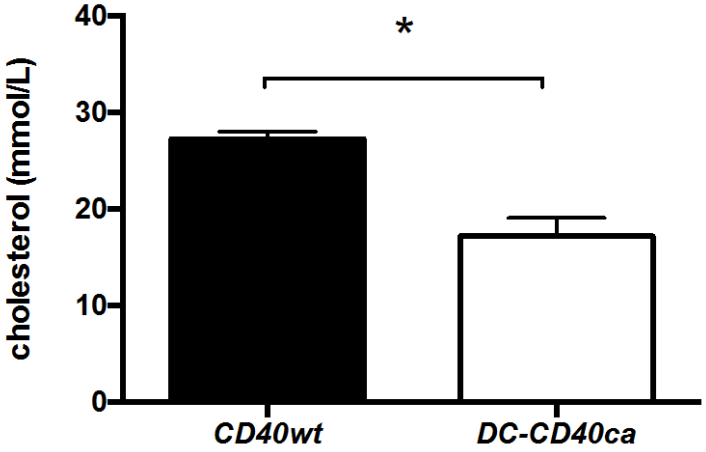
Constitutive activation of DC CD40

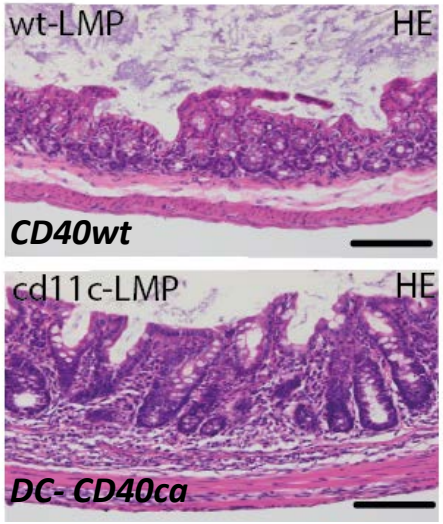


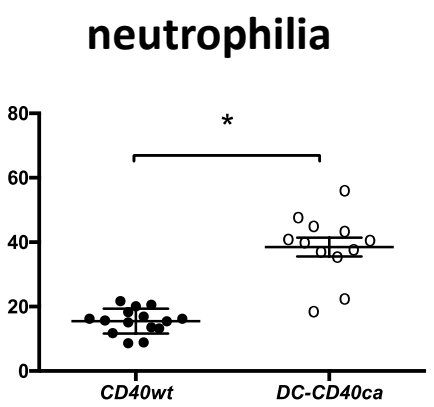


CD40wt ***DC- CD40ca***



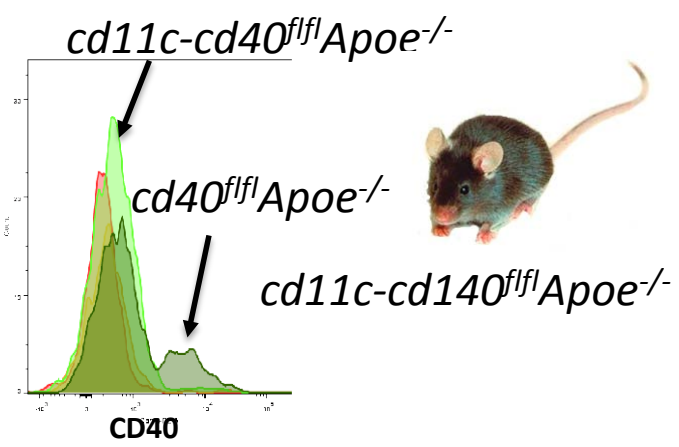


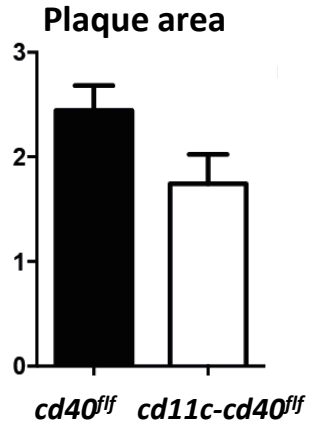


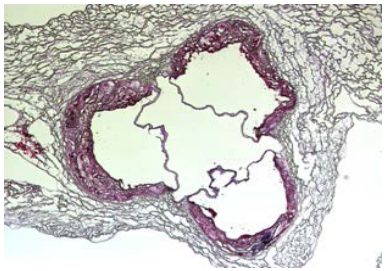


Colitis!!!

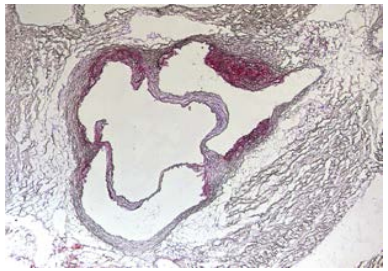
Deficiency of CD40 on DCs



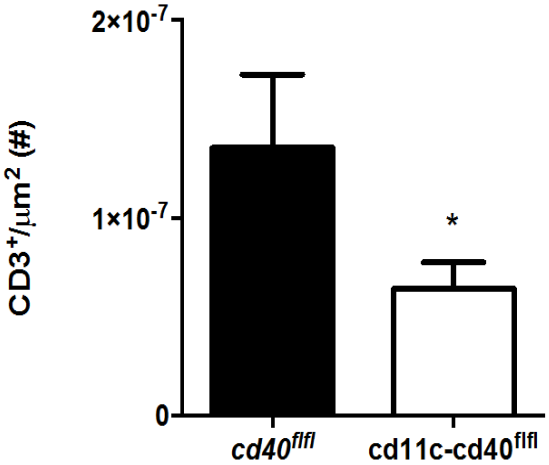


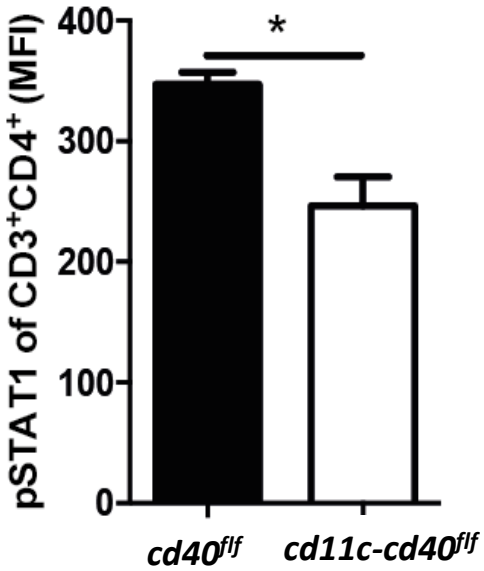


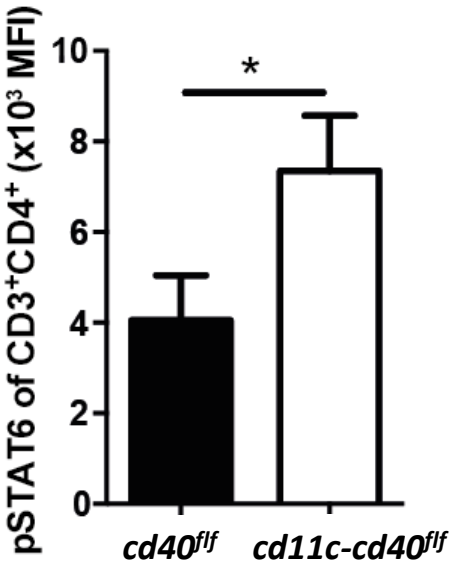
cd40flflApoe-/-



cd11c-cd140flflApoe-/-

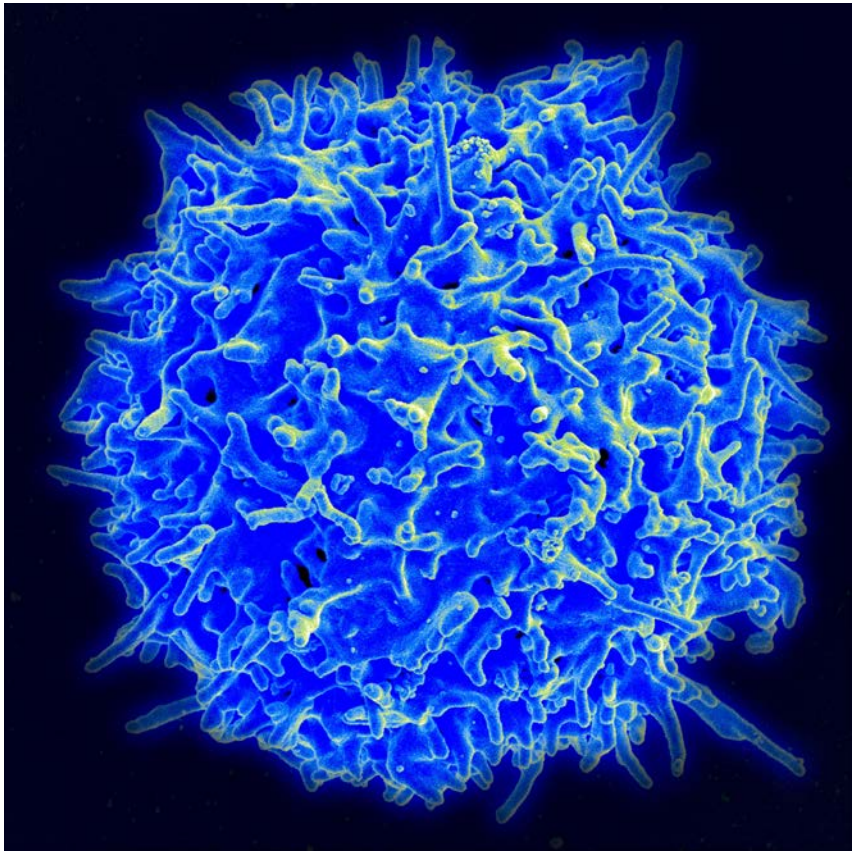






Shift from Th1 to Th2

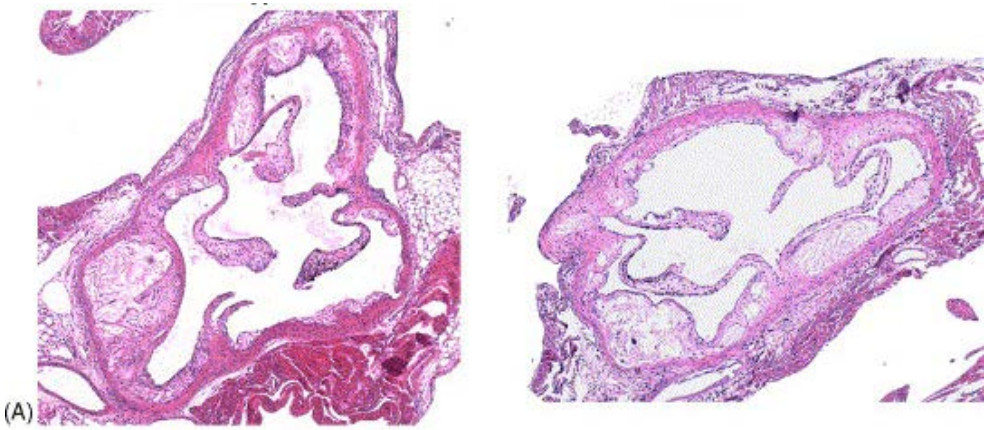
CD40L: T cell



CD40L^{+} T cells: of importance in CVD?

cd40l$^{-/-}$
wt

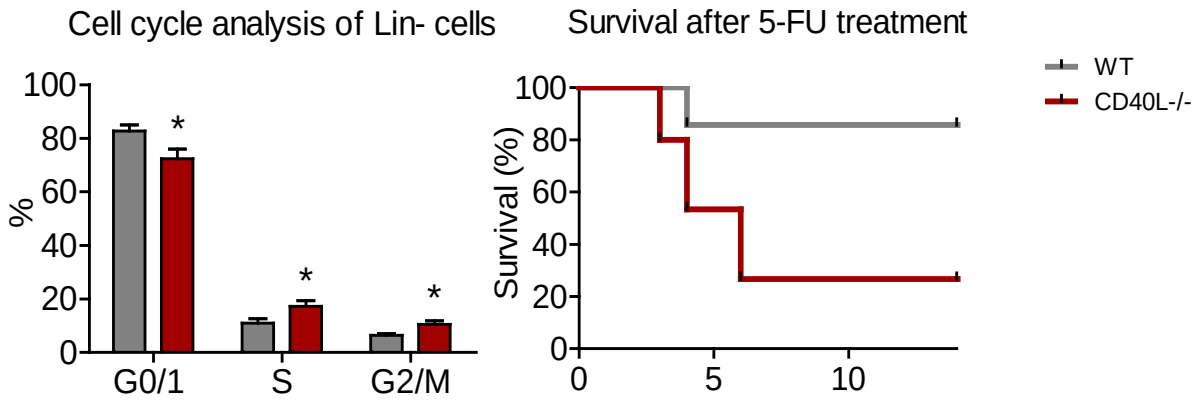
cd40l$^{-/-}$ *ldlr*$^{-/-}$ chimeras
wt ldlr$^{-/-}$ chimeras

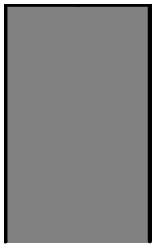


No effect of hematopoietic CD40L on atherosclerosis !?!?!?

Smook, Atherosclerosis 2005, Bavendieck, 2005

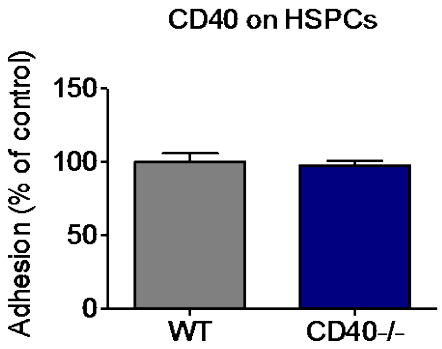
CD40L mediates homing of HSPCs!!

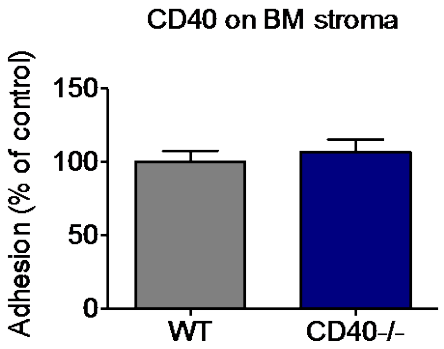




Seijkens, submitted

Independent of CD40!!!





Inflammatory stimuli: ↑ CD40L expression

CD40

Non-classical receptor

Activation of mature immune cells

Adhesion-mediated quiescence of HSPC

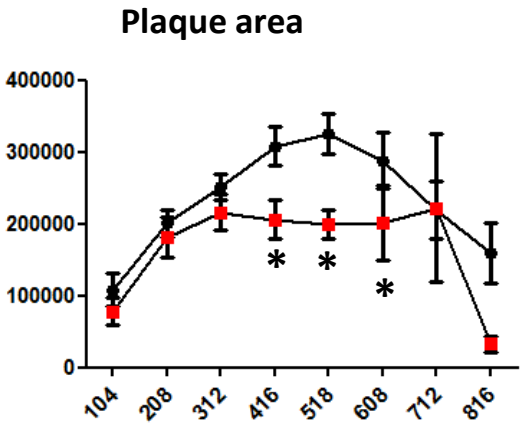
Immune activation and inflammatory disease

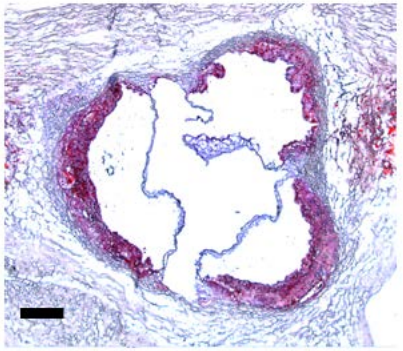
Securing long term hematopoiesis

Seijkens, submitted

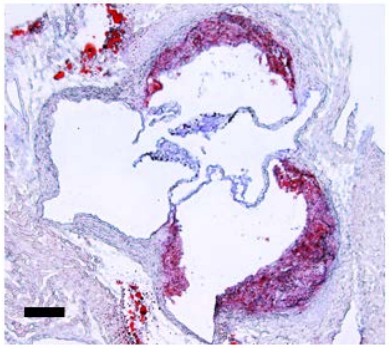
T cell CD40L drives atherosclerosis

cd4cre⁺cd40l^{flfl}ApoE$^{-/-}$
cd40l^{flfl}ApoE$^{-/-}$

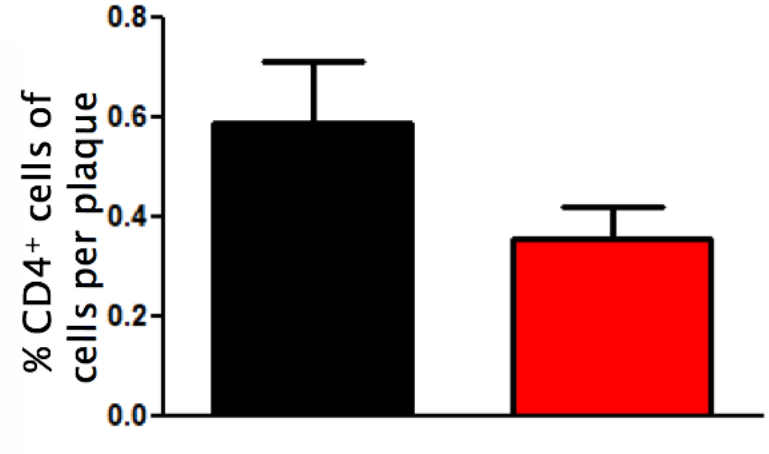


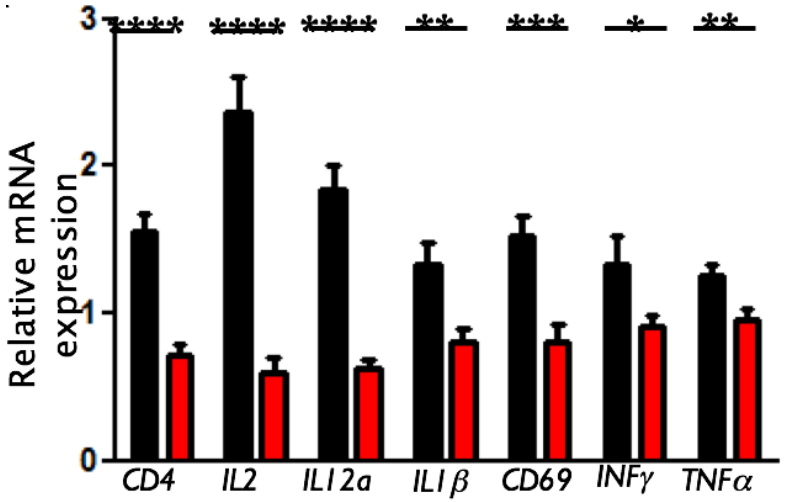


cd40l^{flfl}ApoE$^{-/-}$



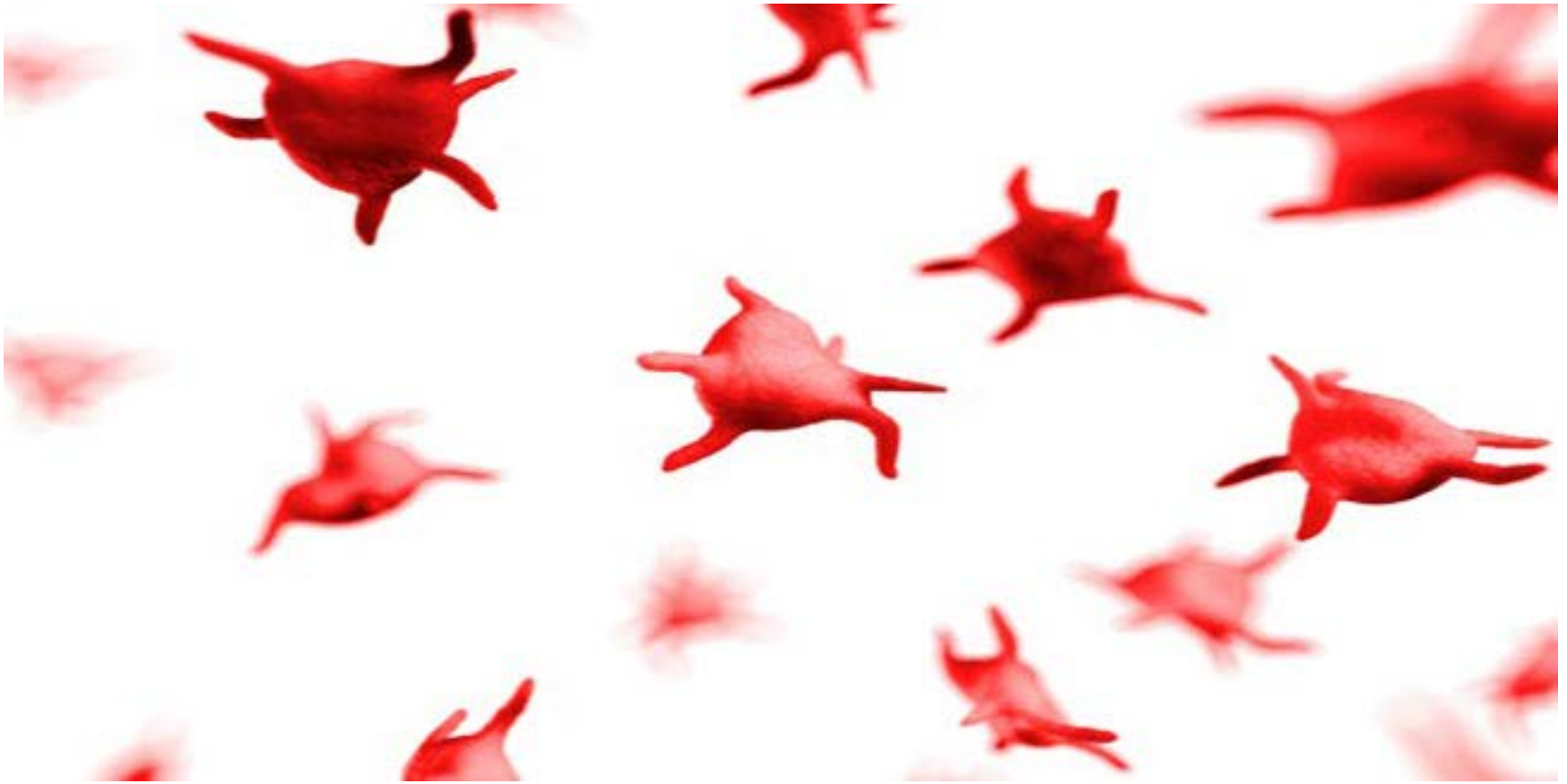
cd4cre⁺cd40l^{flfl}ApoE$^{-/-}$



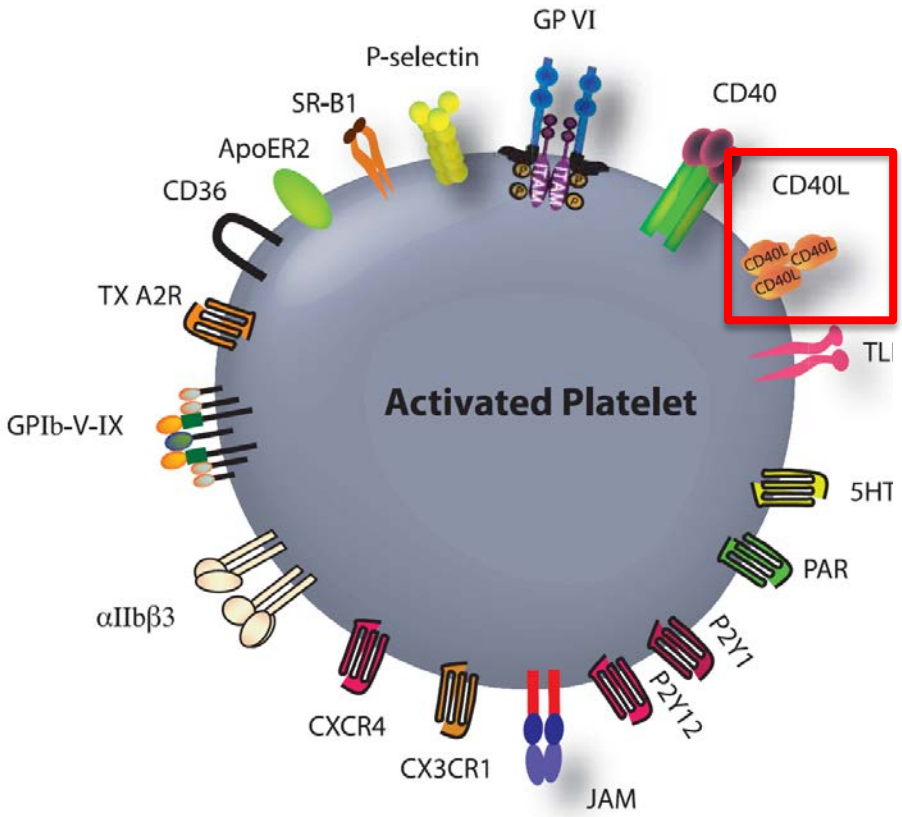


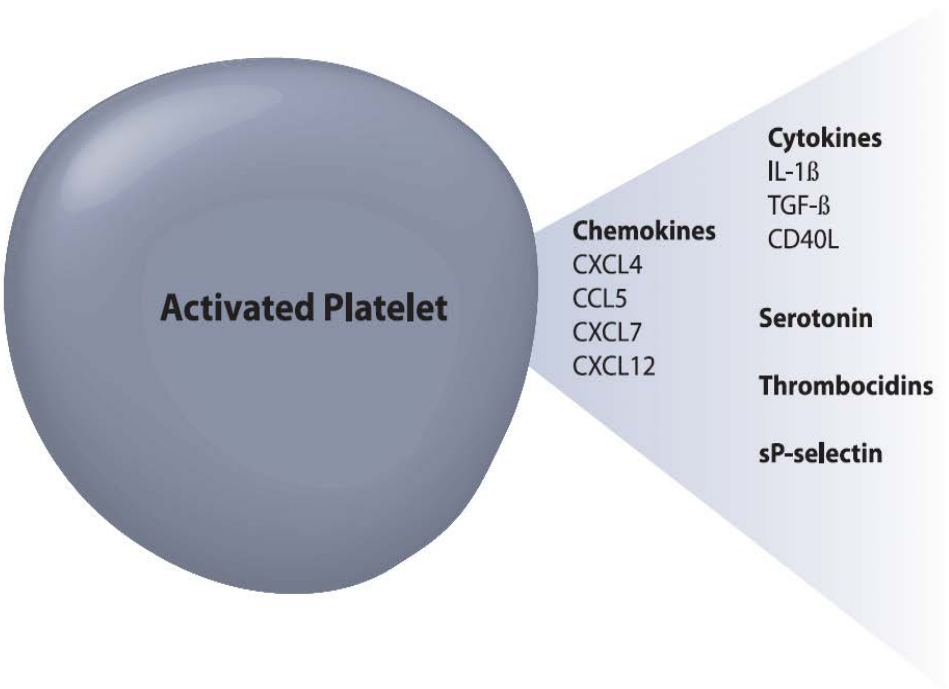
Bürger, Gerdes, unpublished

CD40L & CD40: platelets



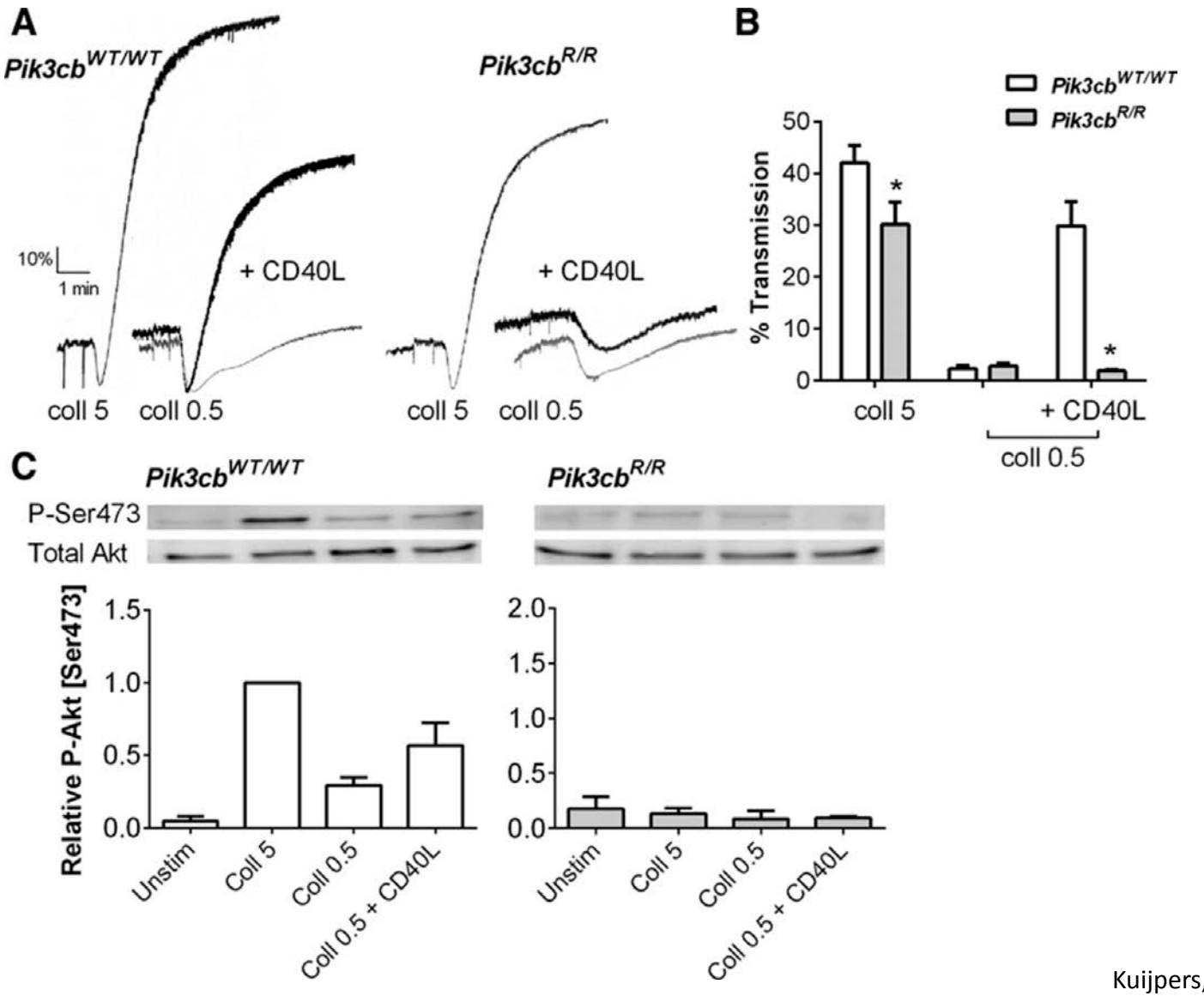
Platelets participate in inflammation by expression of cell surface molecules and secretion of soluble mediators





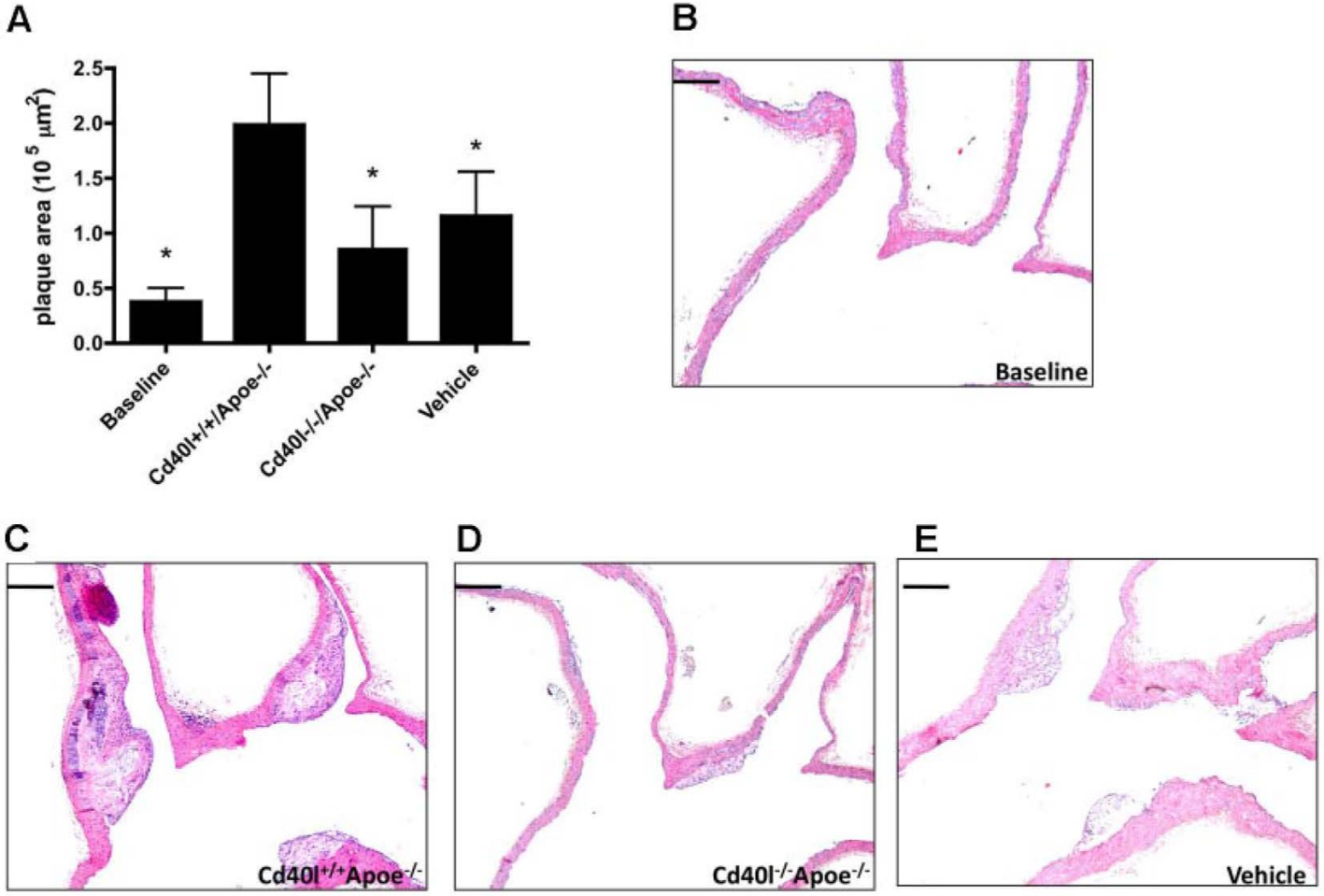
Lievens, von Hundelshausen, Thomb. Hemost., 2011

Platelet CD40L mediates platelet aggregation via PI3kβ



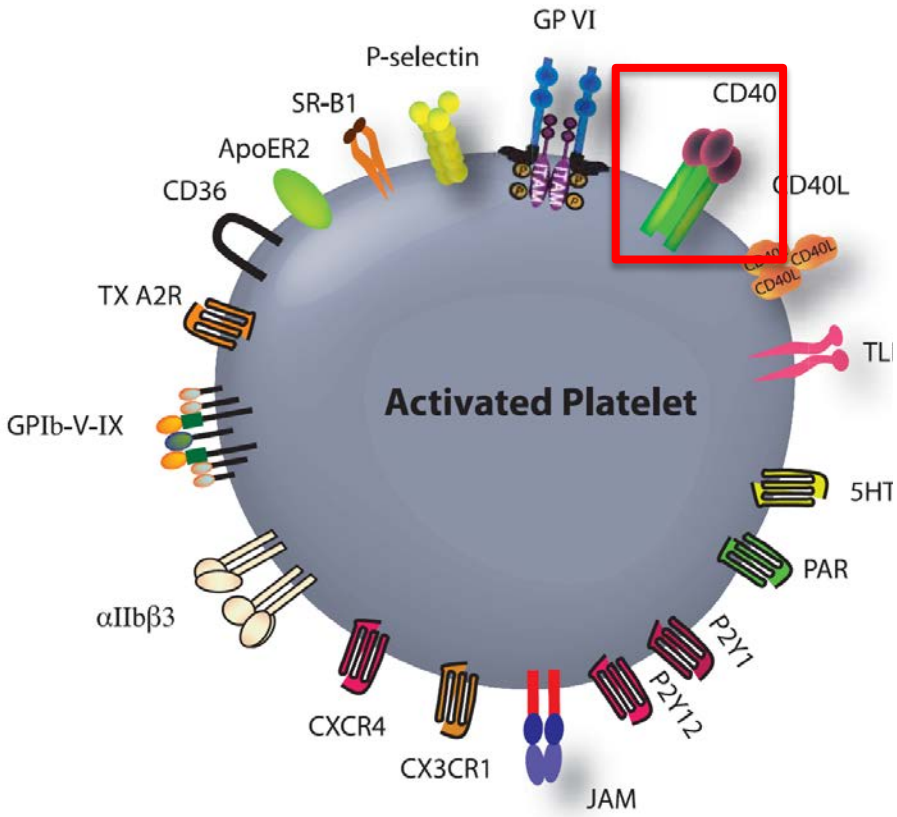
Kuijpers, ATVB 2015

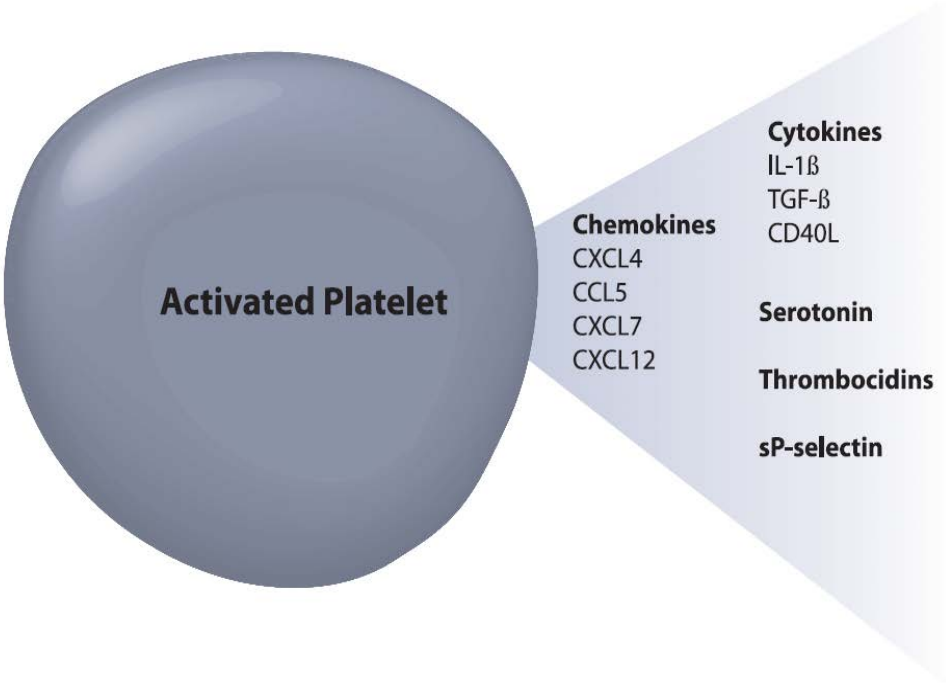
Platelet CD40L contributes to the progression of established plaques



Lievens et al., Blood, 2010

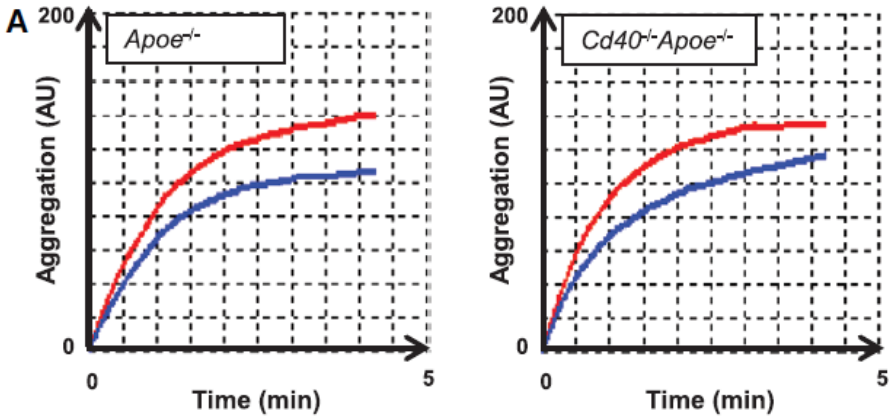
Platelets participate in inflammation by expression of cell surface molecules and secretion of soluble mediators

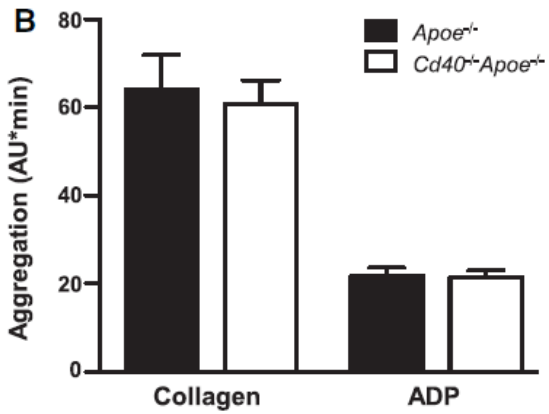


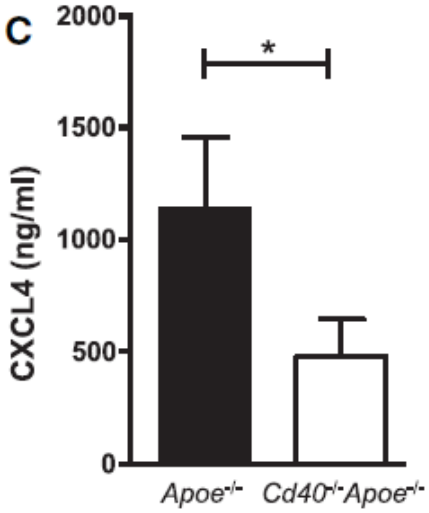


Lievens, von Hundelshausen, Thomb. Hemost., 2011

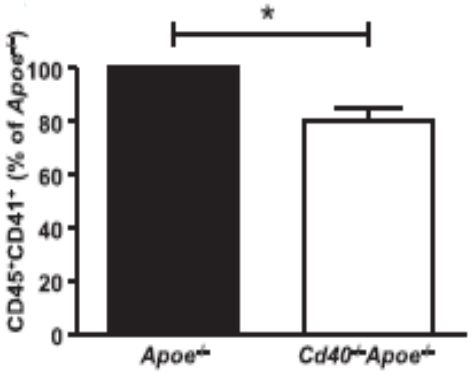
CD40-deficient platelets exhibit normal platelet activation secrete less PF4/CXCL4, and impair PLA formation





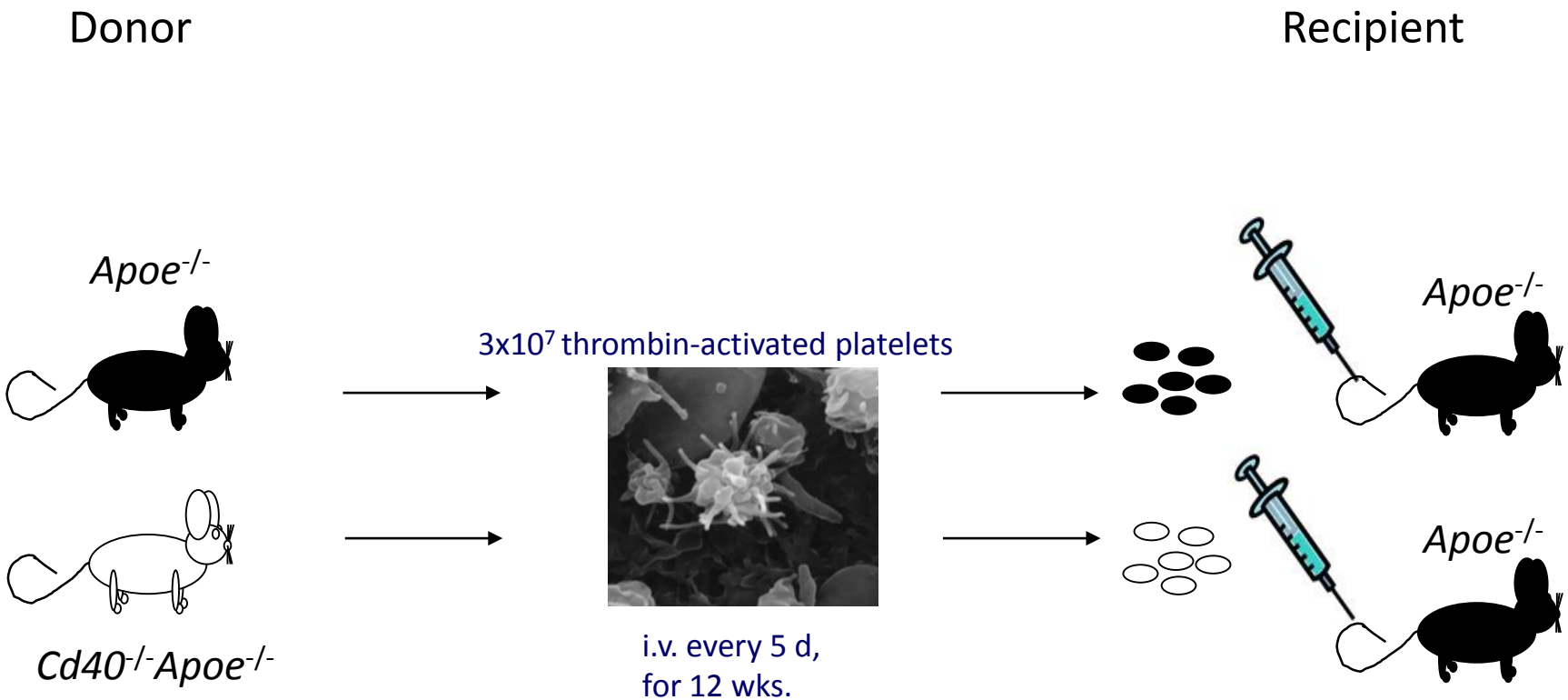


Platelet-leukocyte aggregates



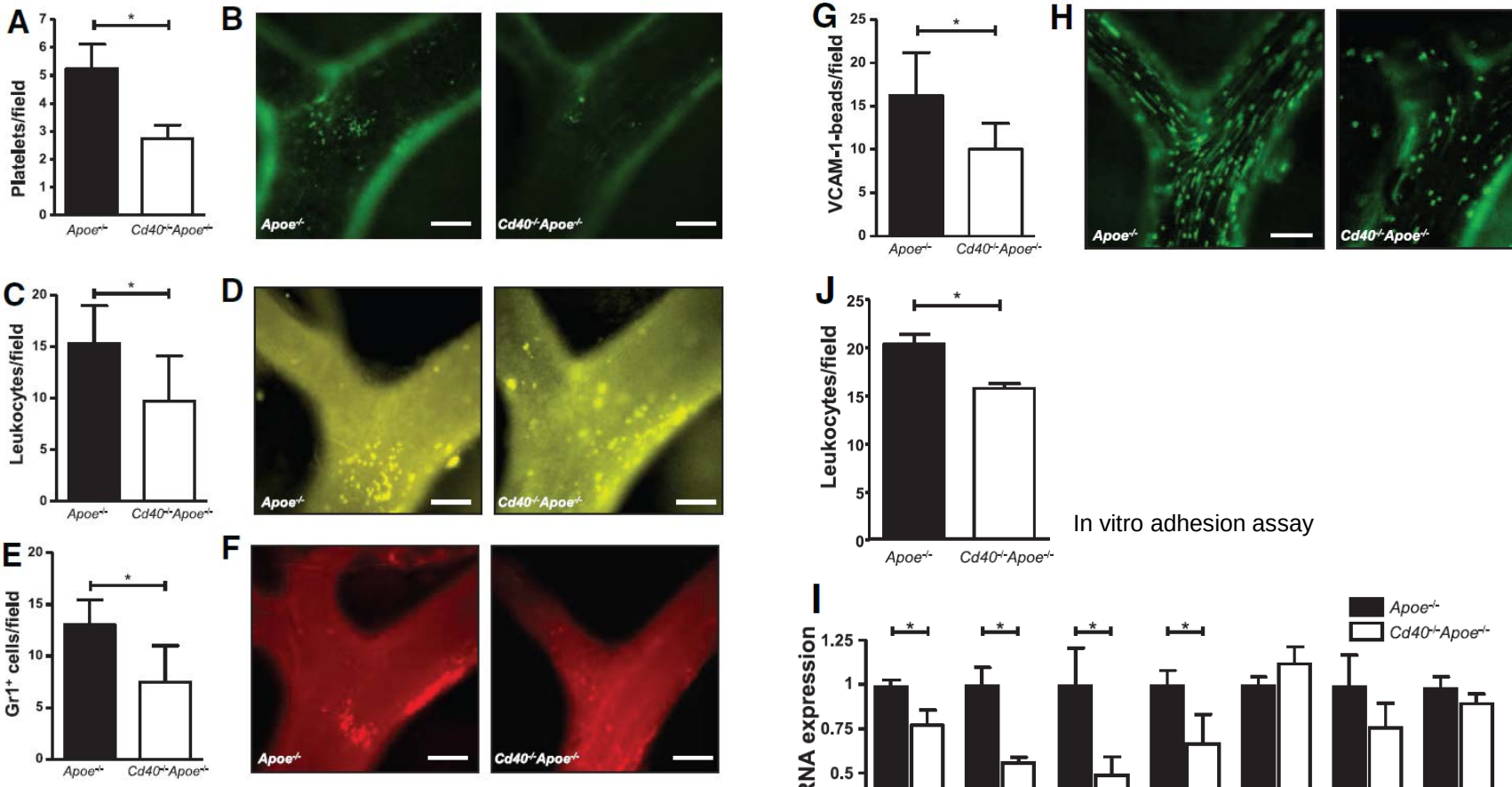
Gerdes et al., ATVB, 2016

Platelet transfusion model



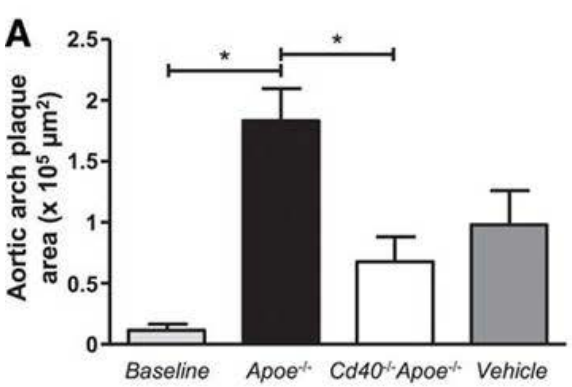
Gerdes et al., ATVB, 2016

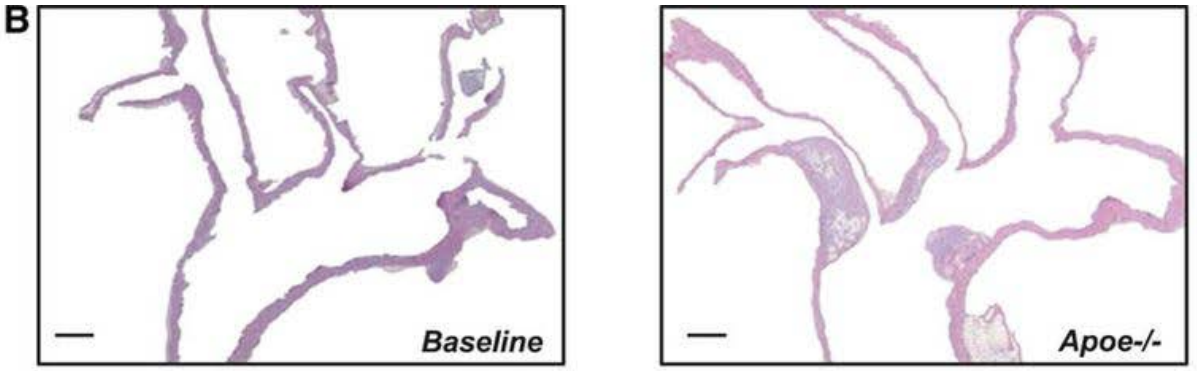
Platelet CD40 deficiency reduces endothelial activation and neutrophil recruitment

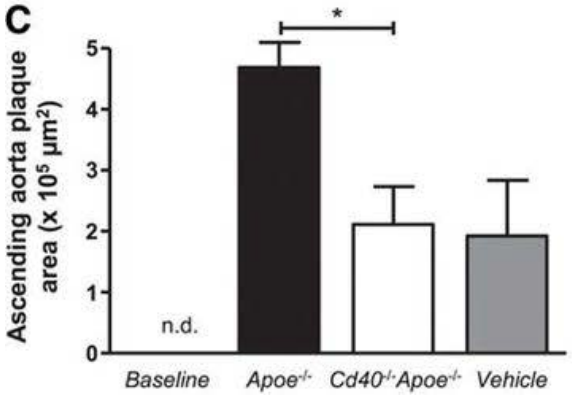


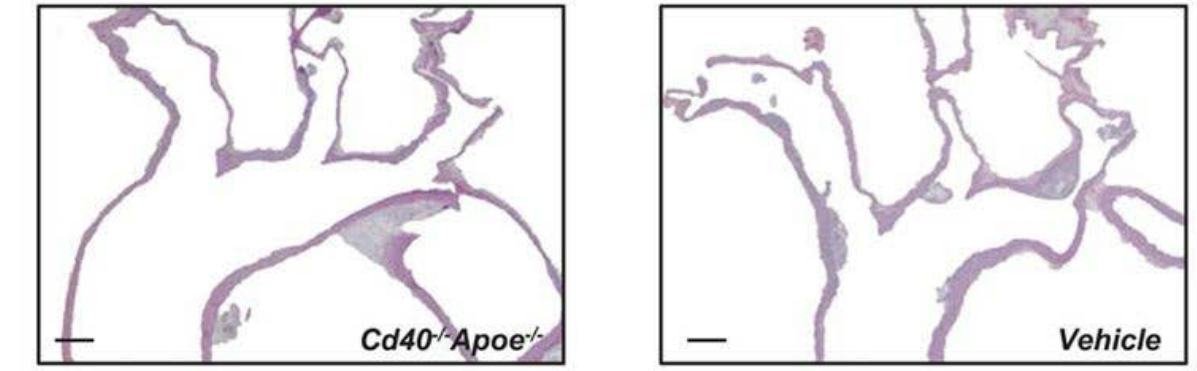
Gerdes et al., ATVB, 2016

Platelet CD40 contributes to atherosclerosis

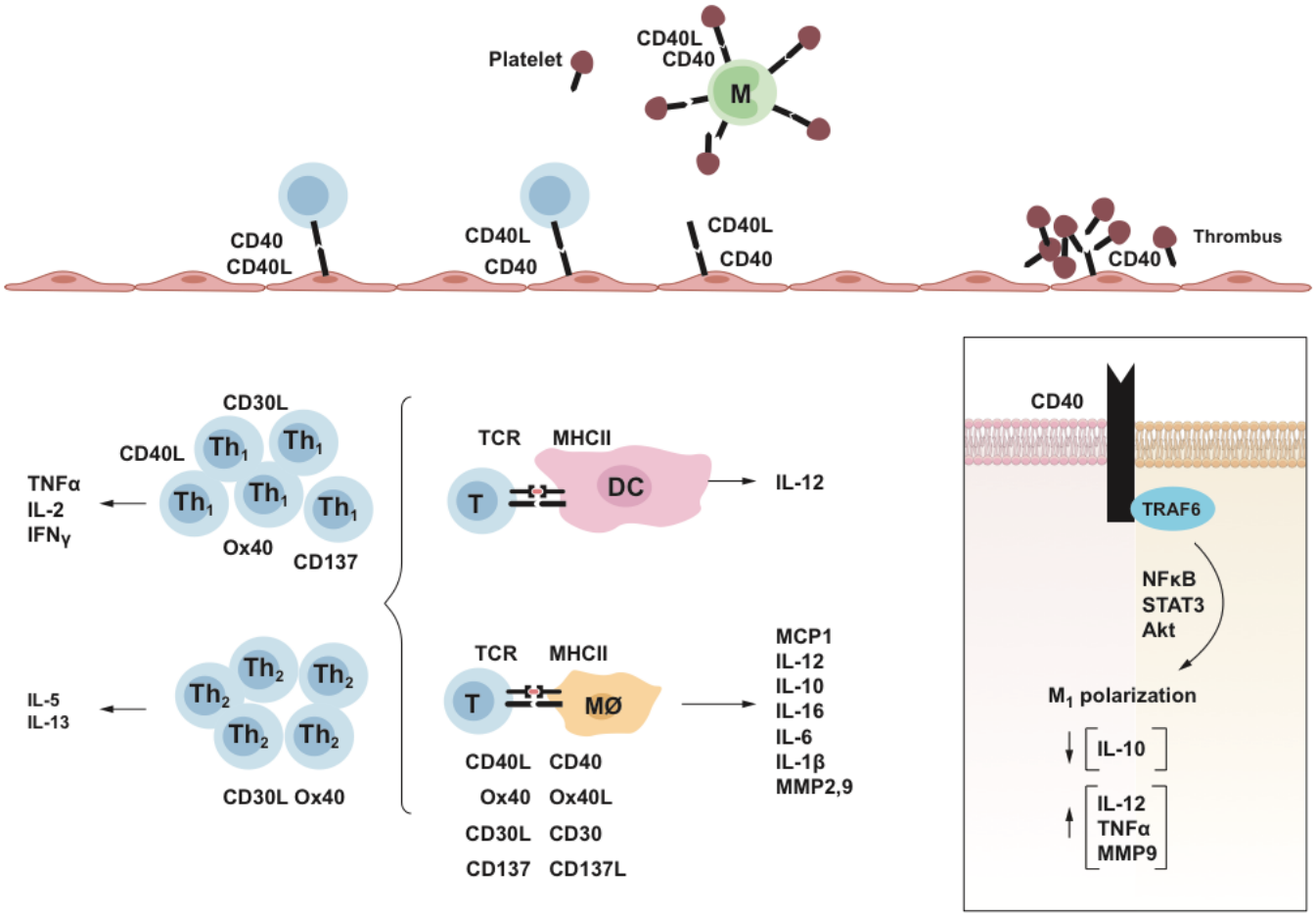






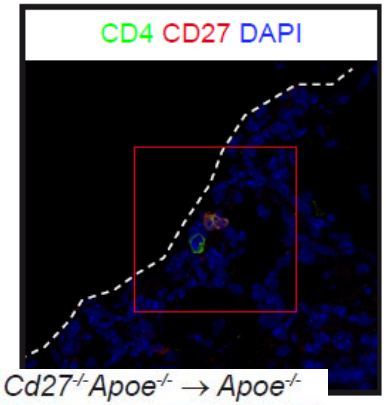


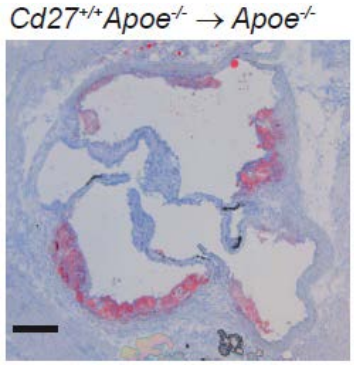
Gerdes, ATVB 2016

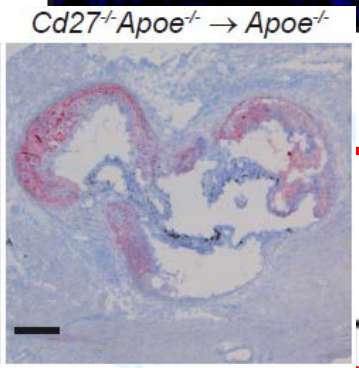


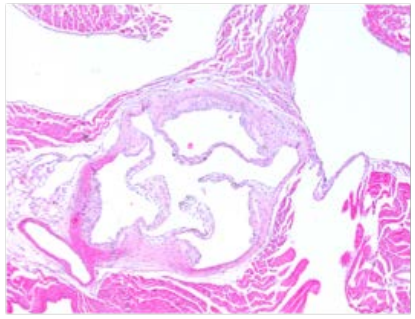
Bäck, Weber, Lutgens, JIM, 2016

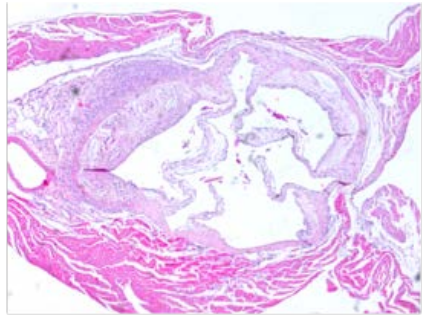
INTERACTOME OF IMMUNE CHECKPOINT INHIBITORS





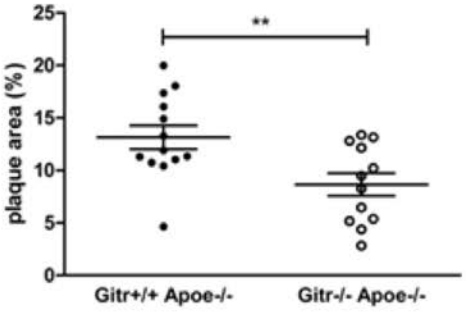


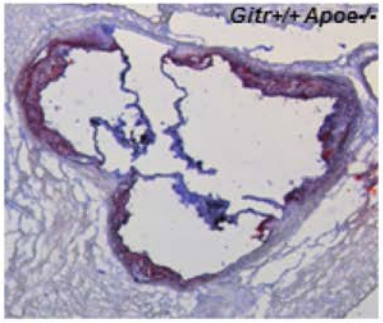


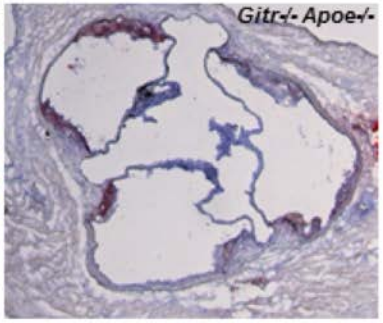


Apoe-/-

Cbl-b/Apoe-/-





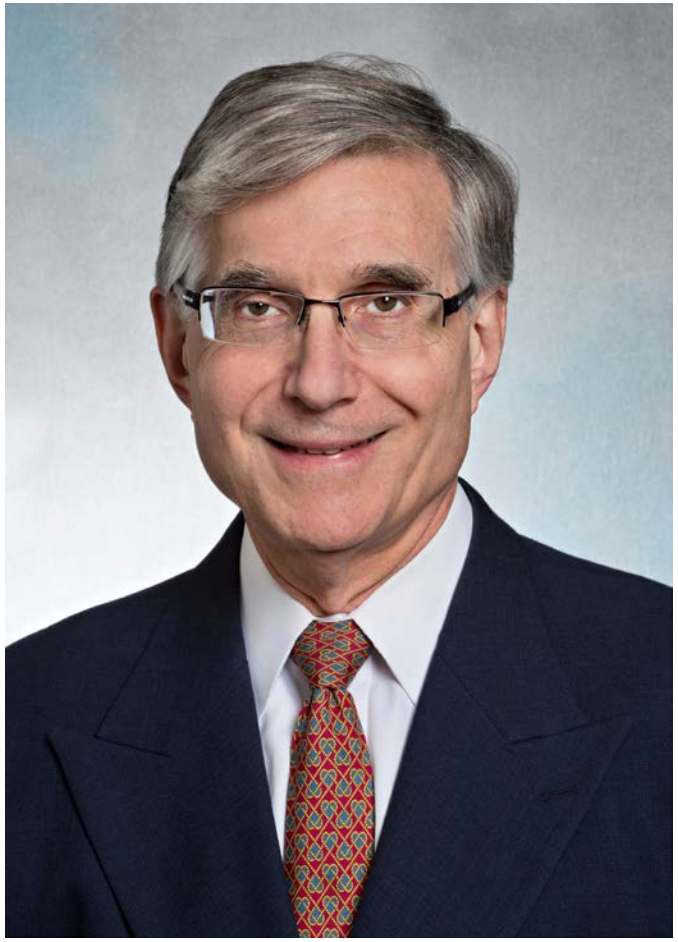



TSA CHECKPOINT

My “Jeffrey Hoegs”

Prof. Mat Daemen

Prof. Christian Weber



Prof. Peter Libby

Prof. Michael Simons

My Scientific BFFs

Prof. Menno de Winther

Dr. Norbert Gerdes

The Amsterdam Laboratory

The Munich Laboratory







My family

Acknowledgements

Academic Medical Center, AMC Amsterdam
Tom Seijkens
Svenja Meiler
Esther Smeets
Pascal Kusters
Claudia van Tiel
Patrick Burger
Linda Beckers
Myrthe den Toom
Suzanne Aarts
Susan van den Berg
Annelie Shami
Matthijs Janssen
Marion Gijbels
Menno de Winther
Noam Zelcer

IPEK, LMU, Munich
Norbert Gerdes
Holger Winkels
Christina Bürger
Charlotte Spitz
Maiwand Ahmadsei
Sigrid Reim

Oliver Soehnlein
Christian Weber

Icahn School of Medicine, Mount Sinai, NY, USA
Jun Tan
Francois Fay
Willem Mulder

Sanquin, Amsterdam
Jaap van Buul
Peter Hordijk
Martijn Nolte

Bioceros BV, Utrecht
Louis Boon

Radboud UMC, Nijmegen
Gert Vriend
Sander Nabuurs

NKI, Amsterdam
Jannie Borst

VU, Amsterdam
Christien Dijkstra
Gijs Kooij

CARIM, University of Maastrich
Barbara Zarzycka
Gerry Nicholaes

European Research Council
Established by the European Commission

Netherlands Organisation for Scientific Research

funded by the dutch heart foundation
Hartstichting

Deutsche Forschungsgemeinschaft
DFG

Alexander von Humboldt
Stiftung/Foundation