

# How plant food bioactives may have benefits for maintaining vascular health in ageing?

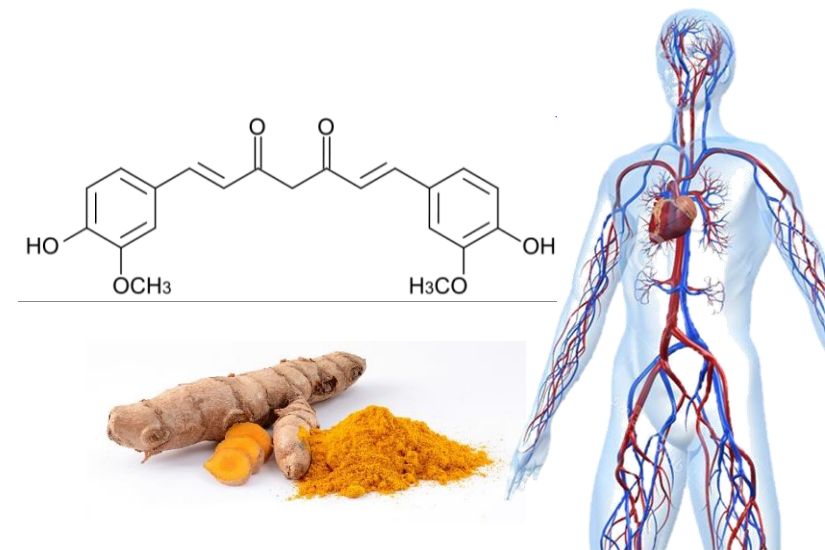
## The case of curcumin

Laurent-Emmanuel Monfoulet

INRA

UMR 1019 - Human Nutrition Unit

Clermont-Ferrand, France

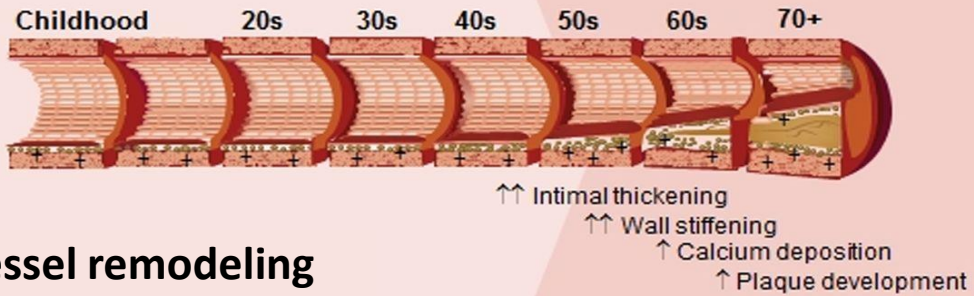
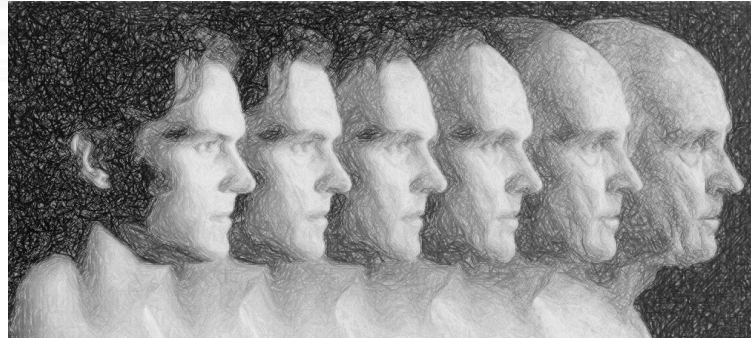


**BORDEAUX VIVA WINTER SCHOOL**  
**XXXIII LIAC MEETING**  
29 nov – 1 dec 2017 Bordeaux (France)



# Vascular Ageing, related diseases & diet

Adapted from Merz et al. Heart 2016



## Vessel remodeling

## Cellular changes

- ↑ Oxidative stress
- ↑ Inflammation
- Impaired autophagy
- ↑ senescence
- ↑ EVs secretion

## Endothelial function

- Shift to dysfunction:
- Pro-adhesive
  - Pro-coagulant
  - ↑ Inflammation
  - Impaired autophagy

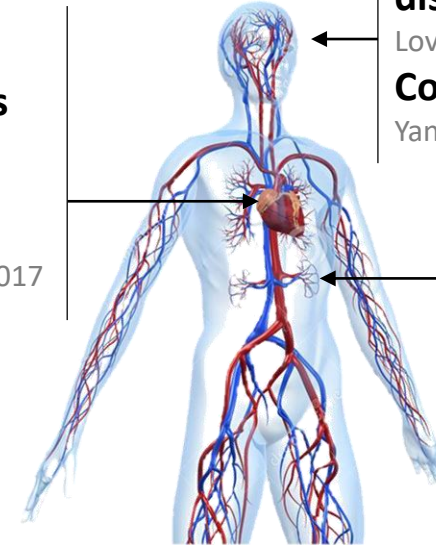
Donato et al. J Mol Cel Card 2015

## Vascular ageing:

### Cardiovascular diseases

Hypertension, PAD, CAD, Atherosclerosis

Costantino et al J Physiol 2016  
 Paneni et al J Am Coll Cardiol 2017



## Microvascular ageing:

### Neurodegenerative diseases

Love et al Acta Neuropathol 2016

### Cognitive decline

Yang et al Ageing Res Rev 2017

### Kidney disease

Kooman et al. Exp Gerontol 2017

And ...

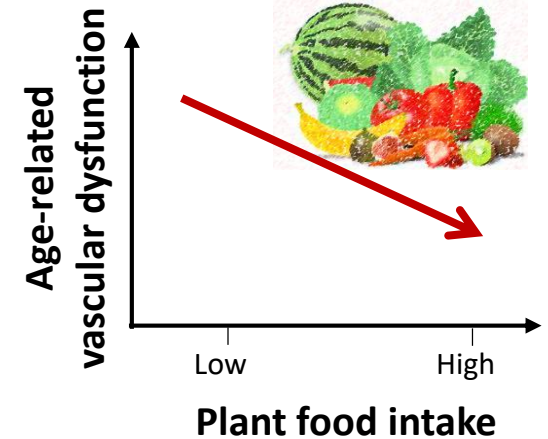


### Epidemiological evidences

Nothlings et al. J nutr 2008  
 Wang et al Br Med J 2014  
 Pistollato et al. Trends Food Sci & Technol 2014  
 Cicero et al. Food Func 2017



### Clinical findings

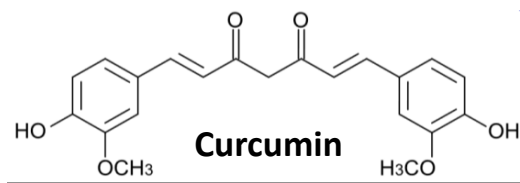


→ Diet represents a crucial factor for the prevention of chronic degenerative diseases

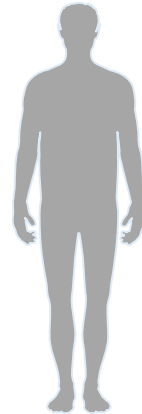


# Curcumin and Health

- Polyphenol abundant in the spice turmeric
- Safe and well tolerated even at high doses (up to 8 g/day)
- Used extensively in Ayurvedic medicine for centuries
- Exhibit in vivo pleiotropic biological activities:



Turmeric (*Curcuma longa*)



**Antioxidant activity**

**Anticancer potential**

**Neuro-protective effect**

**Anti-inflammation activity**

**Anti thrombotic properties**

**Cardioprotection activity**

➔ **Potential interest for prevention and treatment of chronic diseases**

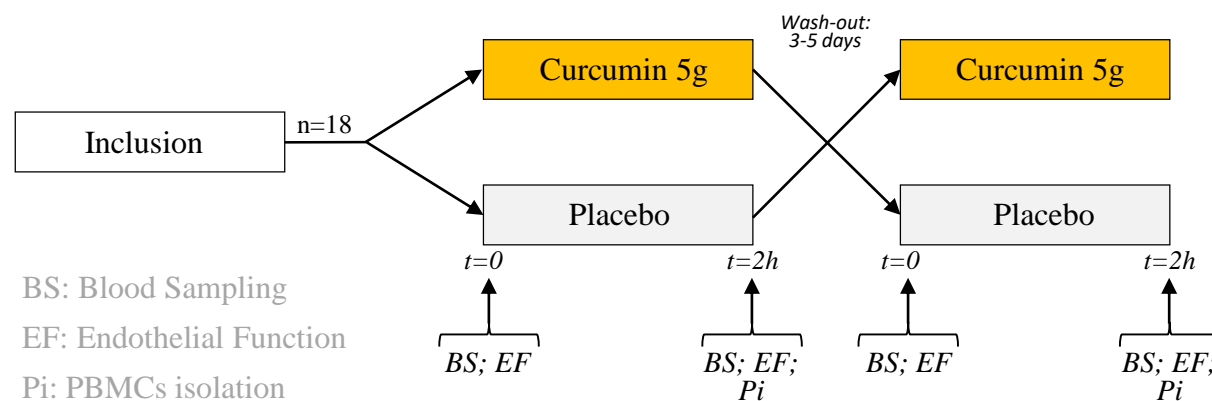
# Vascular effect of an acute intake of curcumin



- **Randomized, double-blind, placebo controlled, cross-over study**
- **18 volunteers** (9♀/9♂), aged 50-70 years, healthy without any medication, smokers<sup>1,2</sup>

<sup>1</sup>Impaired endothelial function in chronic smokers (Zeiber et al, Circulation, 1995)

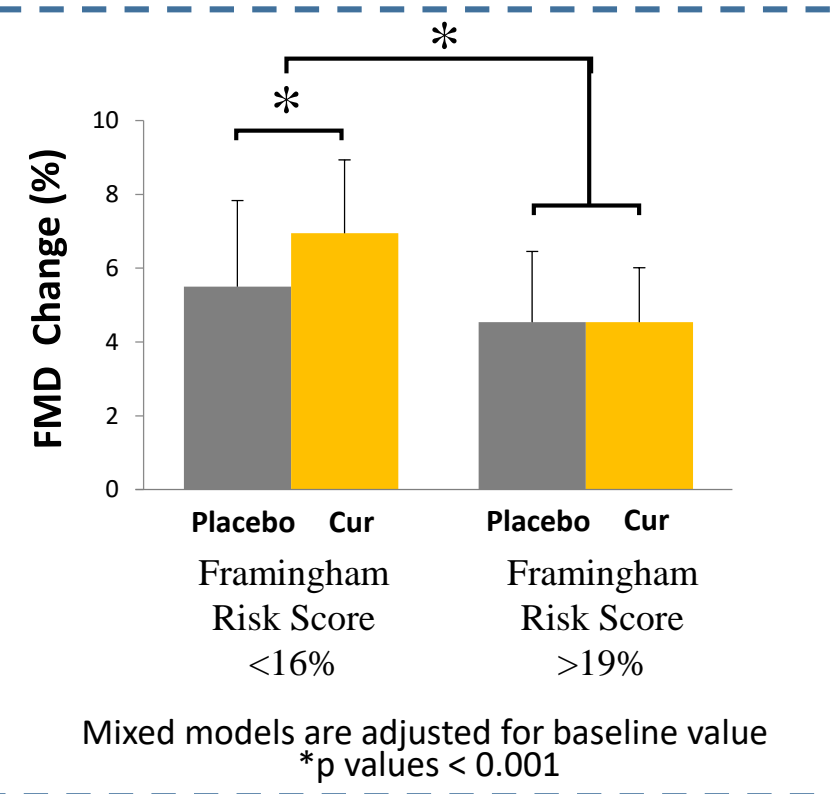
<sup>2</sup>Good model for studying vasoactive properties of food bioactives (Del Bo et al, 2014)



## ➤ Primary Objective :

Evaluate the ability of curcumin to acutely reverse endothelial dysfunction

Population stratification in subgroup according the Framingham Risk Score that estimates the 10-year cardiovascular risk of an individual.



Acute intake of curcumin improves endothelial function (+1.4% on FMD) ONLY in low risk CVD subjects



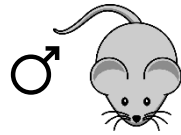
# Curcumin reduces atherosclerosis onset in mice



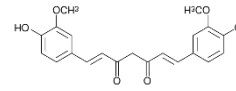
Coban et al.  
MNFR 2012

## Atherosclerotic model:

ApoE<sup>-/-</sup> Mice  
8 week-old



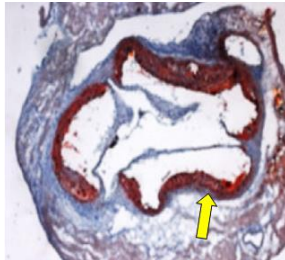
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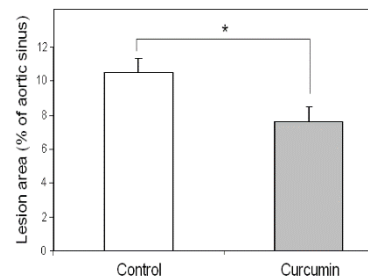
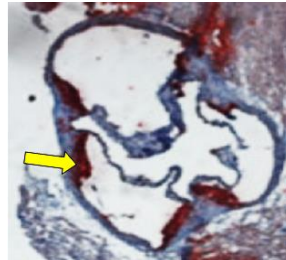
0.2% Curcumin  
for 16 weeks

## Atherosclerotic lesion (aortic sinus)

Control

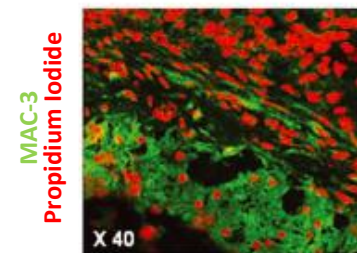


Curcumin

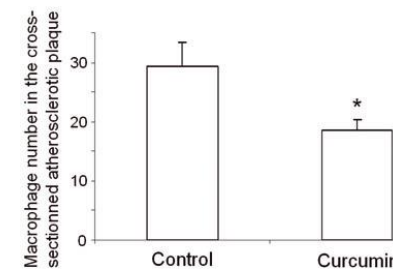
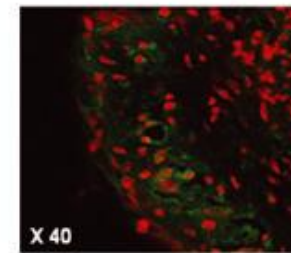


## Macrophages within the lesion

Control



Curcumin



Curcumin intake reduces atherosclerotic lesion development and recruitment of immune cells



### Clinical Trial

Baber-Chamoux et al. *MNFR* 2017  
 Oliver, et al. *J. Nutr. Metab* 2016  
 Usharani et al. *Drugs RD* 2008



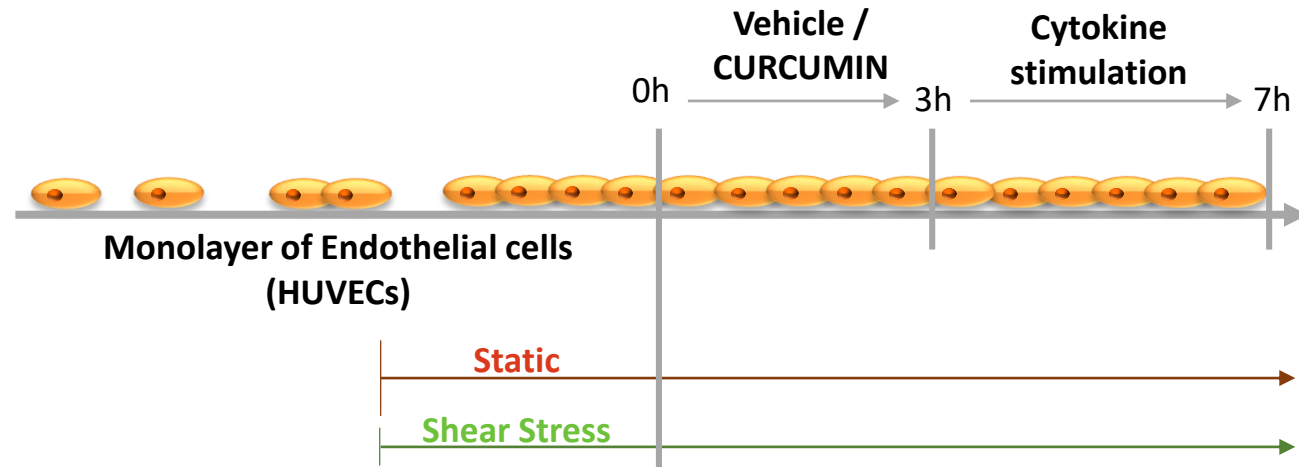
### Preclinical studies

Coban et al. *MNFR* 2012  
 Liu et al. *Inflamm Res* 2014  
 Hao et al. *EBCA med* 2014

## Vascular protective effects of curcumin

# What are the underlying mechanisms of action of curcumin on vascular endothelial cells ?

### In vitro studies Endothelial cells



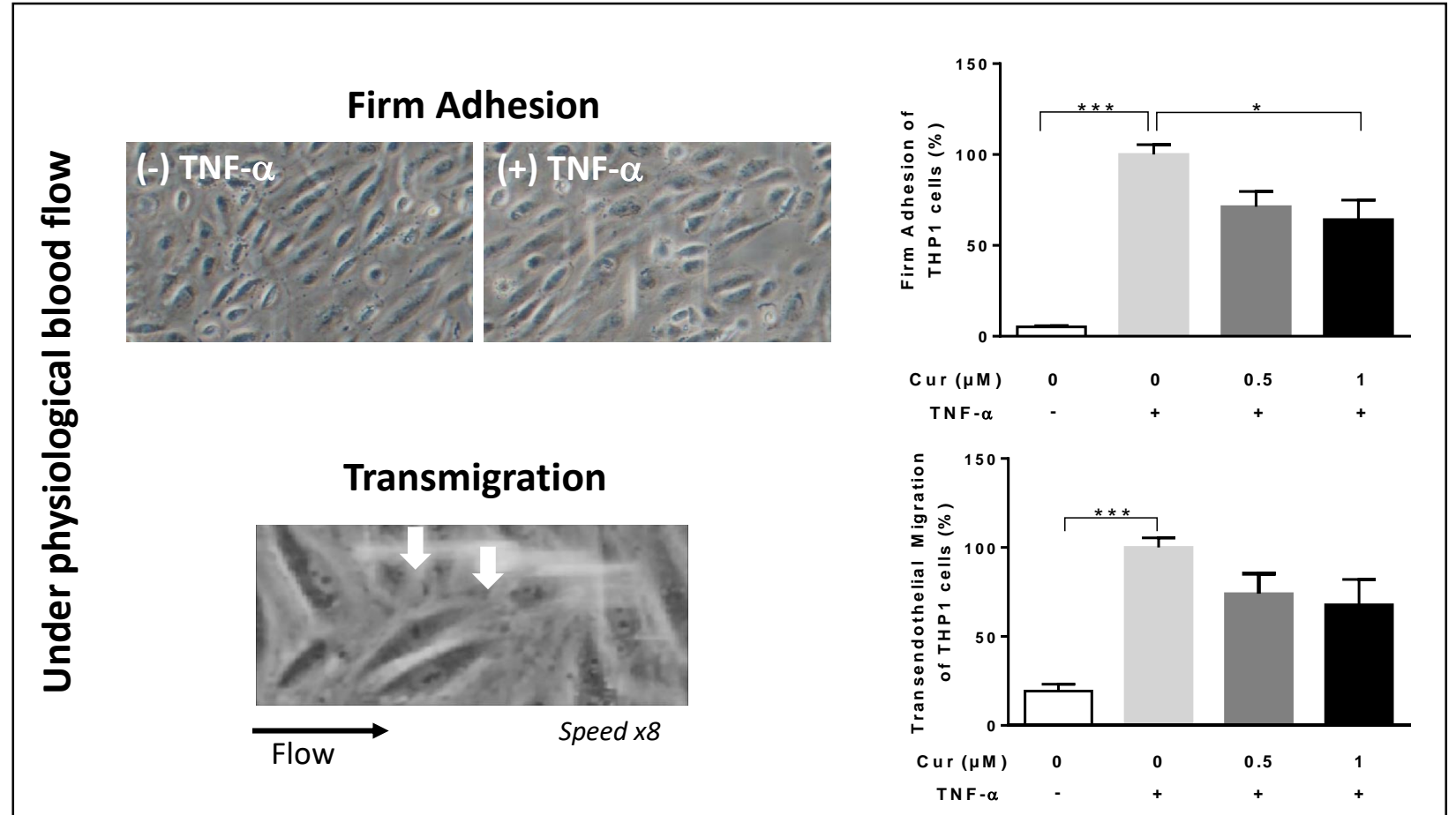
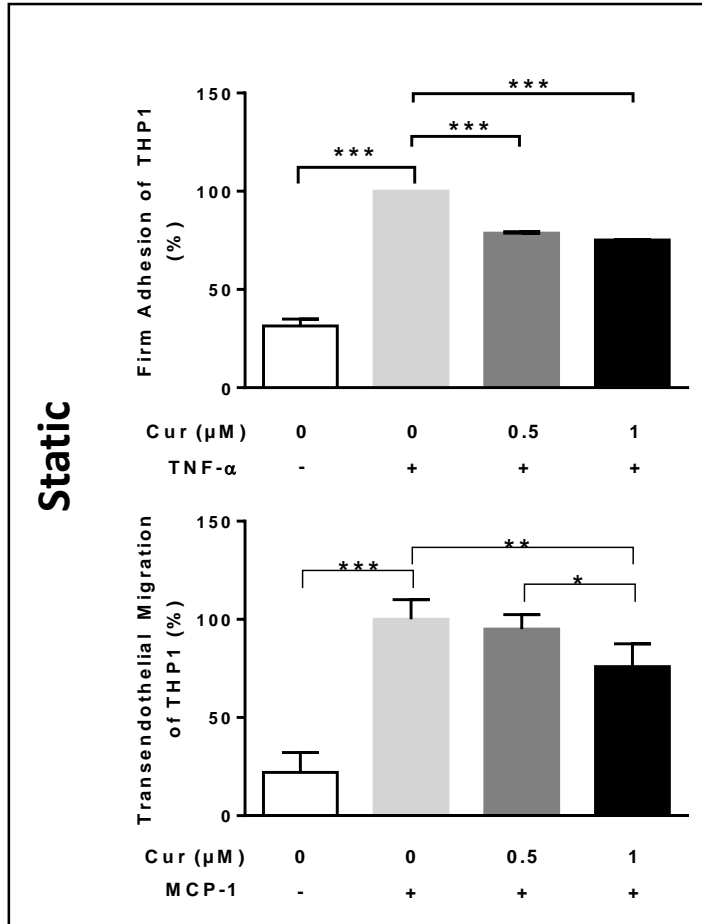
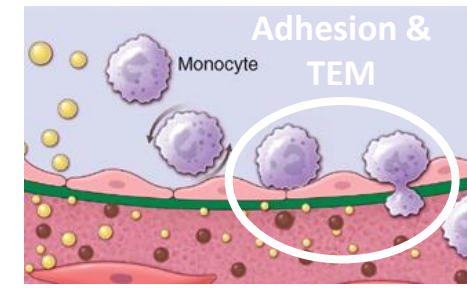
### Endothelial functions:

- Barrier: Permeability assay (a)
- Chemotactism (b)
- Adhesion assay (c)
- TEM assay (d)

### Mechanistic exploration

Monfoulet et al. *FRBM* 2017 Nov;112:109-120. Curcumin modulates vascular endothelial permeability and monocyte TEM by affecting endothelial cells dynamics

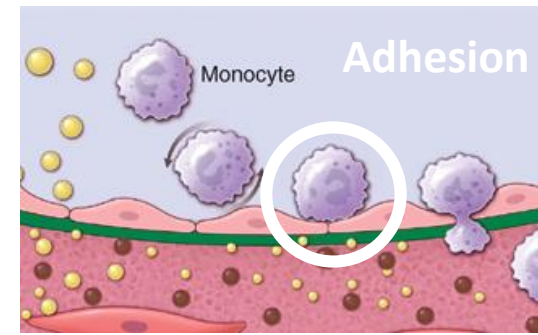
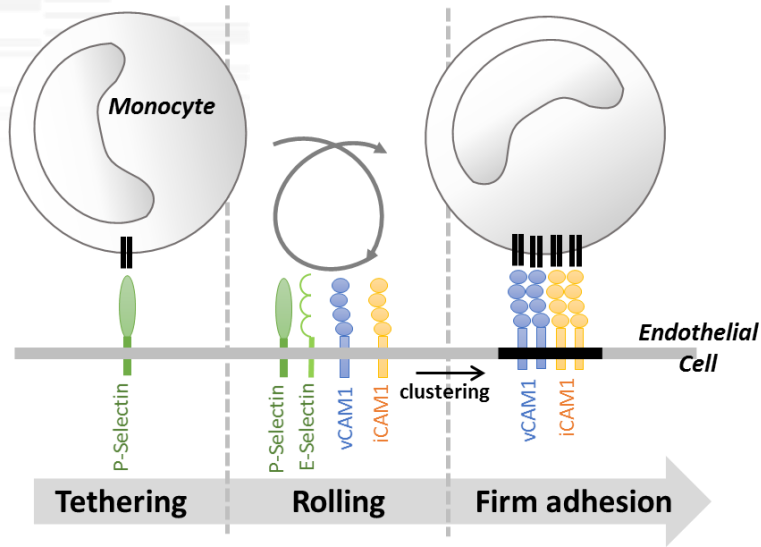
# Effect of curcumin on the adhesive property of endothelial cells



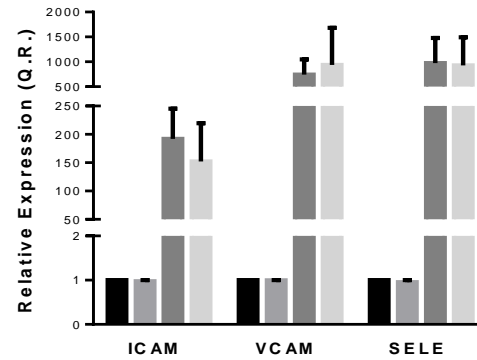
Pre-exposure of endothelial cells to curcumin reduces the adhesion and TEM of monocyte to TNF-activated HUVECs both in static and under vasculo-protective shear-stress



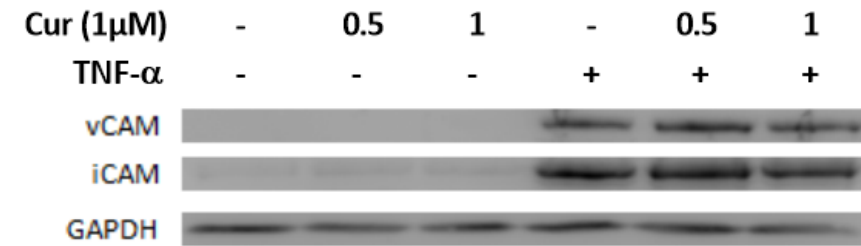
# Underlying mechanism of action?



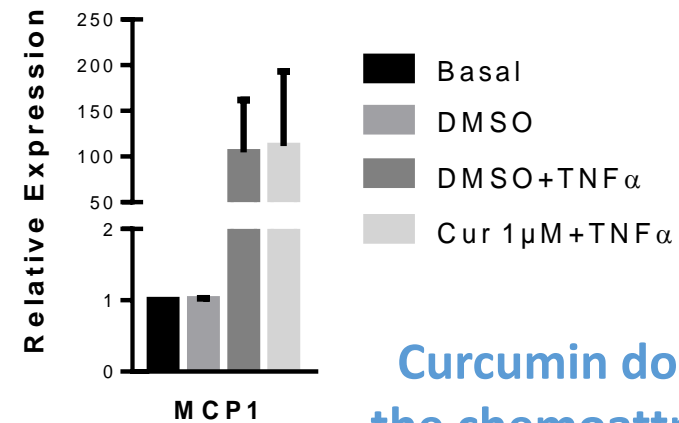
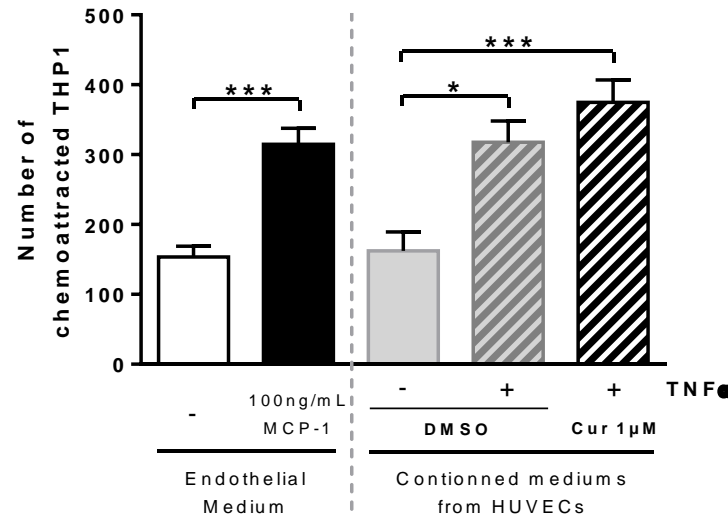
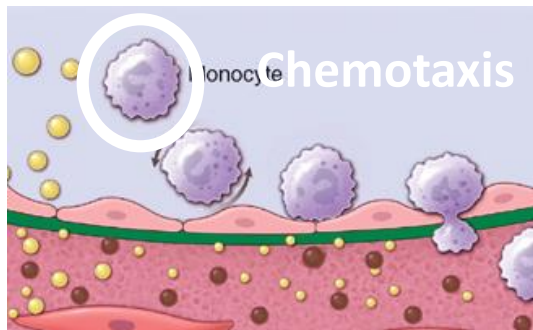
## mRNA level



## Proteic level



Curcumin does not prevent the induced expression of adhesion molecules



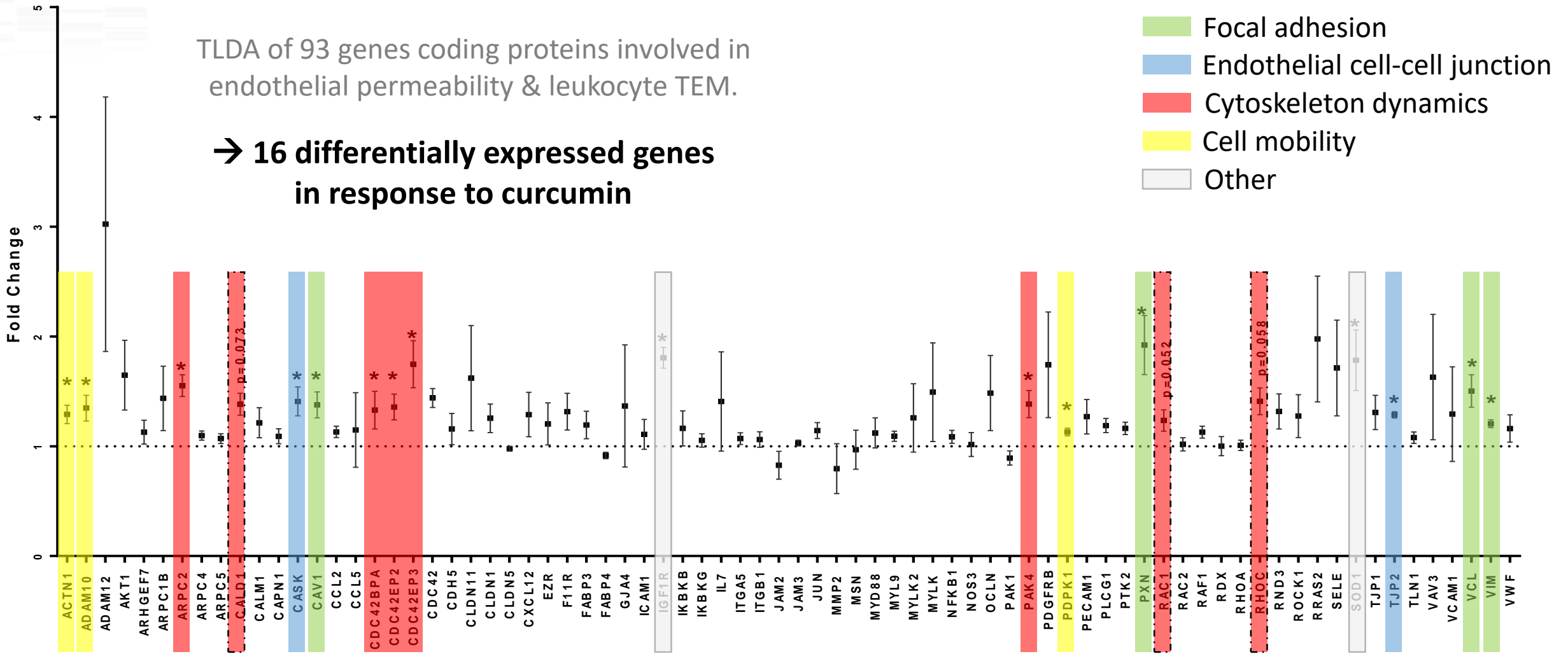
Curcumin does not modulate the chemoattractive potency of activated endothelial cells

# Underlying mechanism of action?

## Genomic effect of curcumin related to TEM process

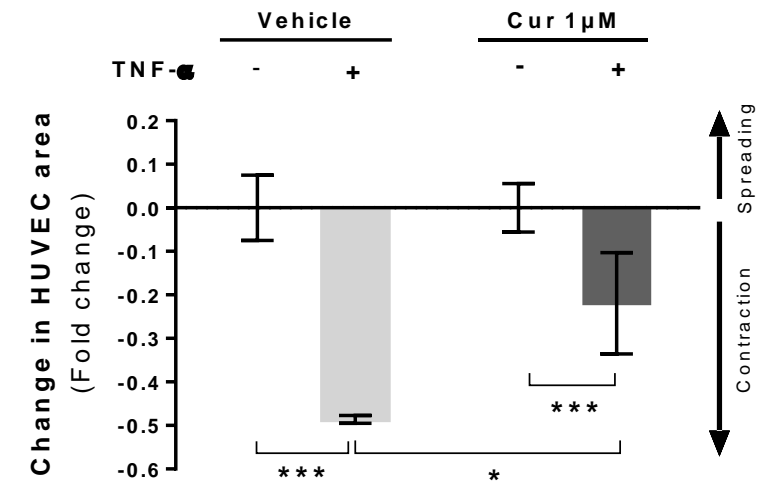
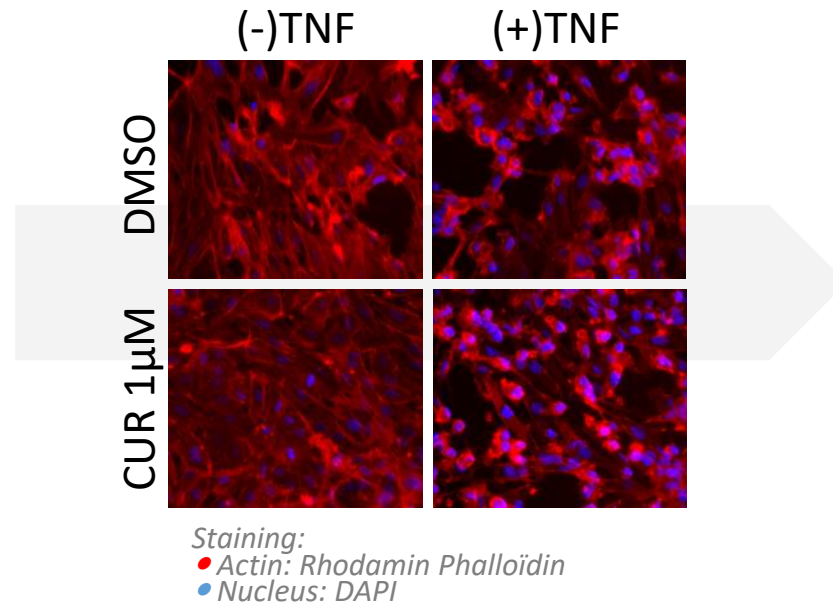
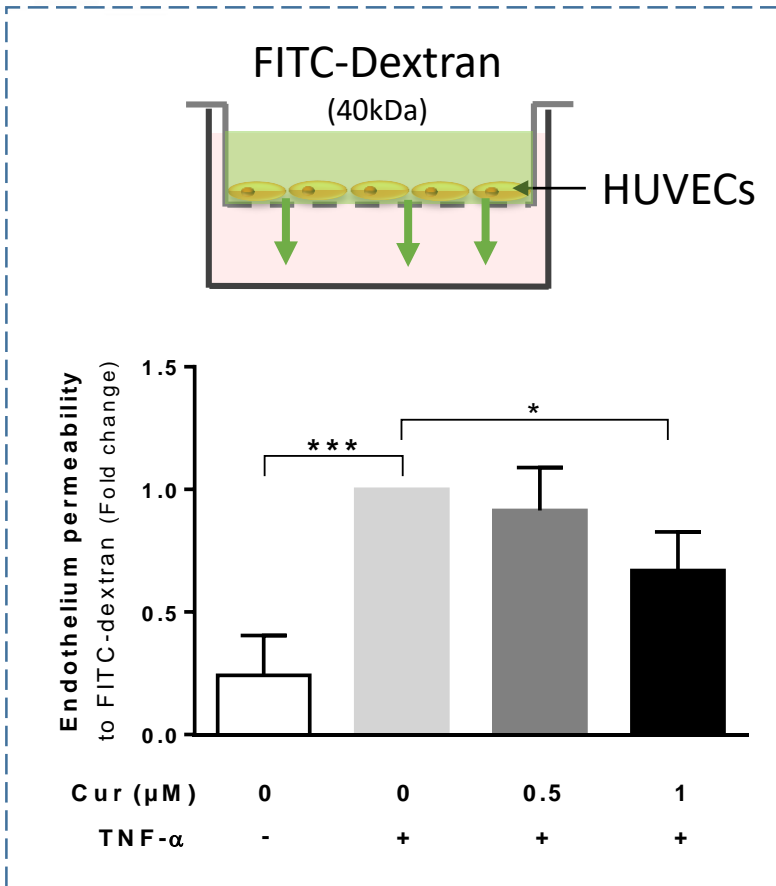
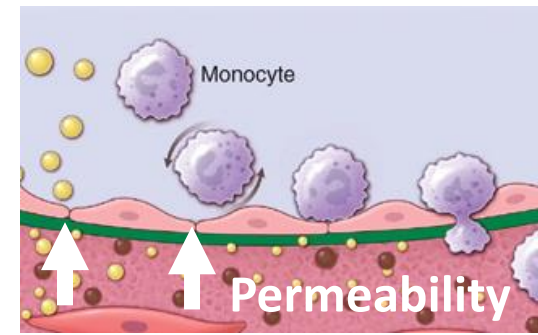
TLDA of 93 genes coding proteins involved in endothelial permeability & leukocyte TEM.

→ 16 differentially expressed genes in response to curcumin



Curcumin could maintain endothelium integrity by reinforcing cell-cell and cell-matrix interactions, and by stabilizing endothelial cell cytoskeleton through nutrigenomic effect

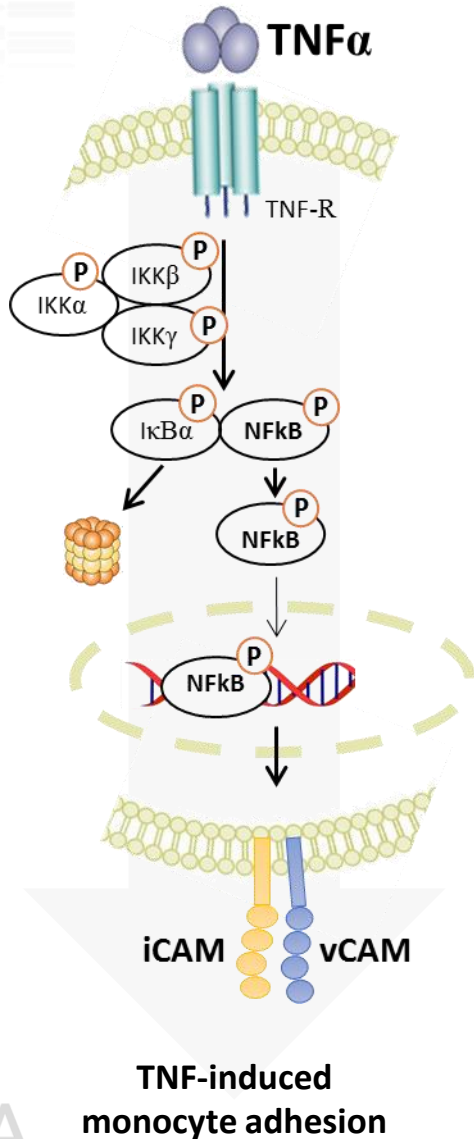
# Effect of curcumin on endothelial permeability



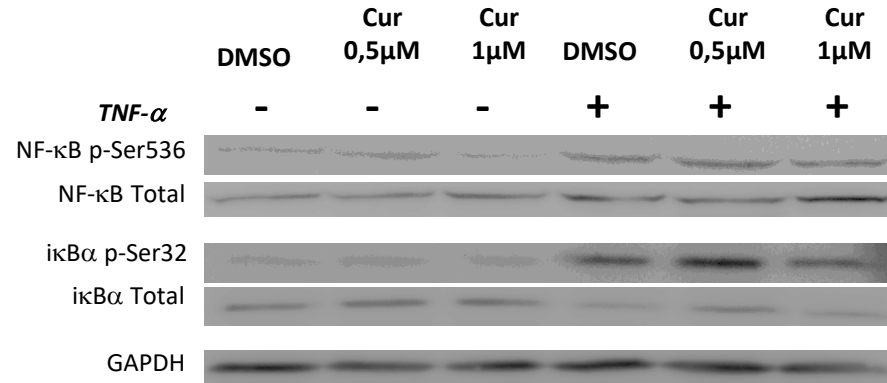
Pre-exposure of Endothelial Cells to curcumin inhibits permeability changes, probably acting on cytoskeleton reorganisation

# Underlying mechanism of action?

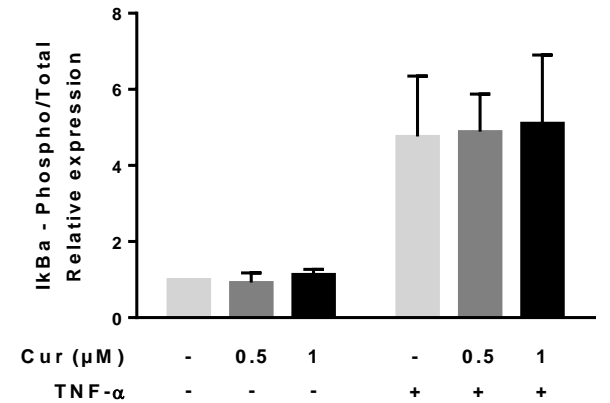
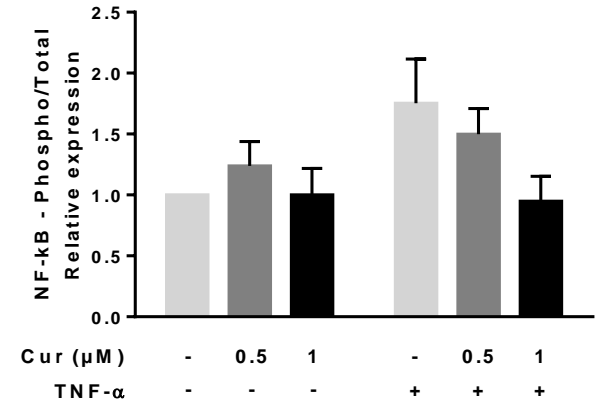
## Cell signalling



### Western-blot Analysis

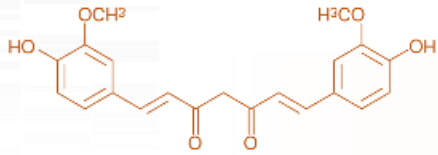


### Phosphorylation rate



Curcumin inhibits the activation of NFκB pathway, independently of IκBα

# To summarize



Curcumin

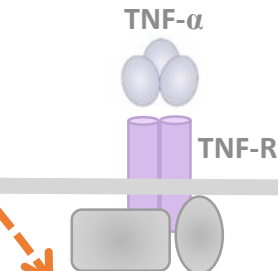
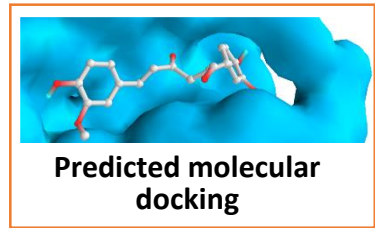
1. Tethering

3. Firm adhesion

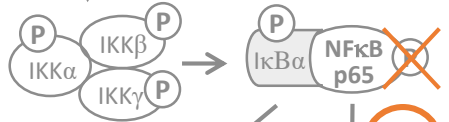
4. Transendothelial migration

2. Rolling

Blood flow



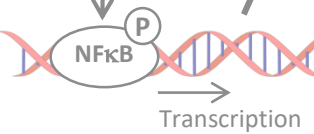
PDK1-TAK1  
AKT1-AKT2



Proteasome



TNF- $\alpha$ -induced mRNA



clustering

PAK4  
Cytoskeleton remodelling

Rac1  
CDC42

Endothelial permeability

Endothelial junction

Focal Adhesion

Extracellular Matrix

mRNAs modulated by CURCUMIN

CDC42EP3	ADAM10	TJP2
CDC42BPA	PLCG1	VIM
CDC42EP2	IGF1R	VCL
ARPC2	CAV1	PXN
CASK	SOD1	
PAK4		

Putative modulated miRNAs

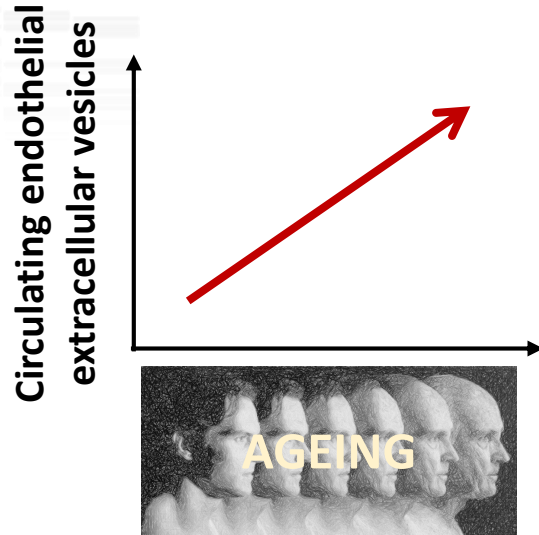
miR-137	miR181a	miR181b
miR-142-5p	miR18a	miR27a
miR-142-3p	miR150	miR155
miR-335-5p	miR101	miR20a
miR-423-5p	miR126	...

Endothelial cell

Unpublished results  
Submitted for publication  
to FRBM

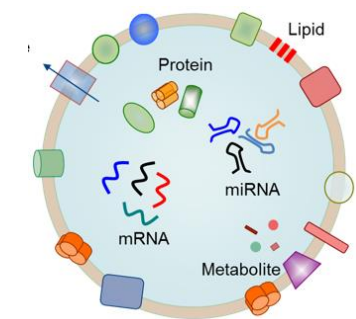
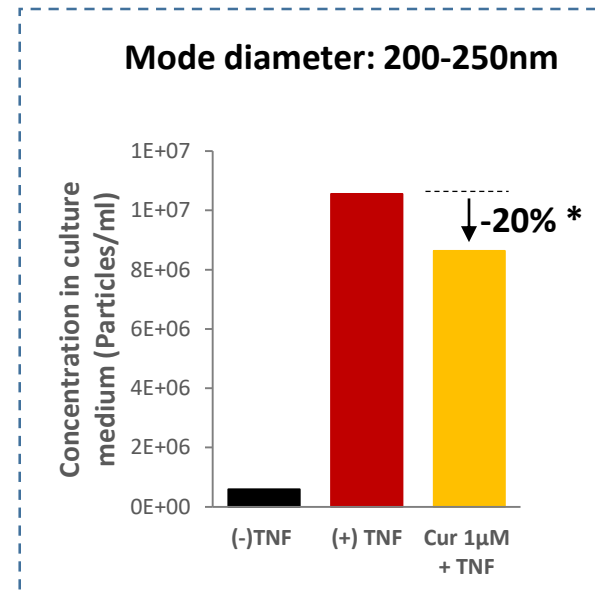
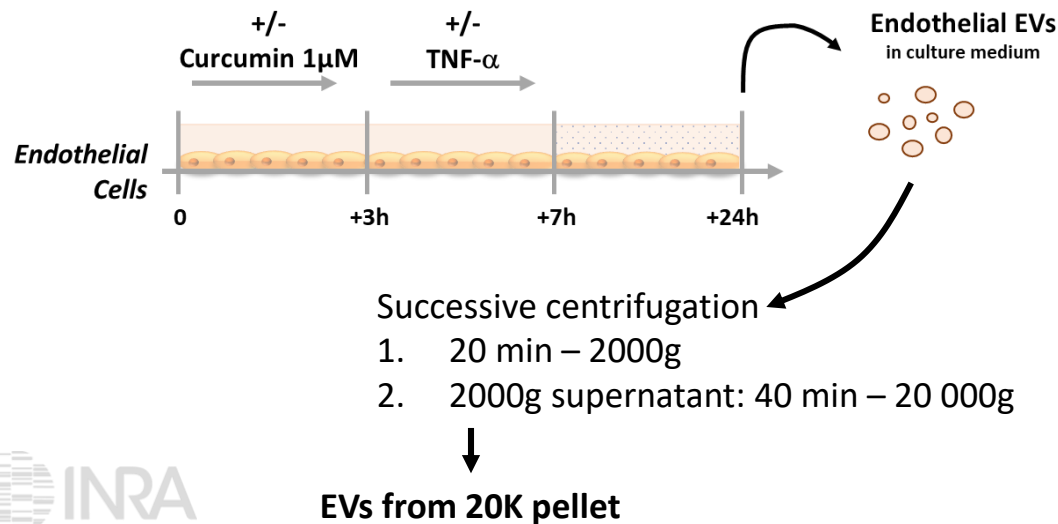


# A new mechanism of action of curcumin (Preliminary results)



- EVs are shed membrane particles of less than 1µm in diameter emerging as a hot topic in biology in view of their ability to sustain a cell-to-cell communication in **physiology and pathology**. (Yanez-Mo et al, J Extracell Vesicles 2015)
- **↑ Endo EVs in patients with Cardiometabolic Diseases** (CAD, Metabolic Syndrome, Diabetes, hypertension...)
- **Diet may modify EVs secretion and their bioactivity** (Vafeiadou 2012, Andres 2012, Horn 2014, Heinrich 2015, Ousmaal 2016)

➔ **What about the effect of curcumin on endothelial EVs?**

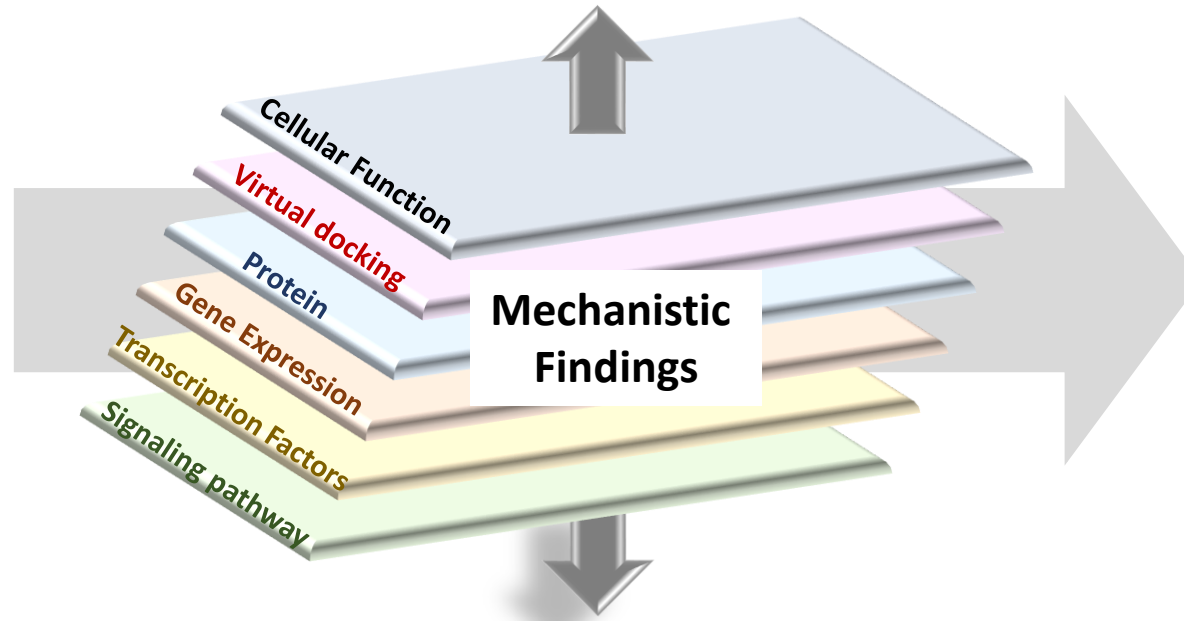
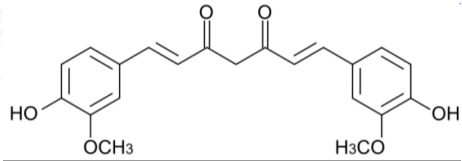


Protein and miRNA content analysis are ongoing

**Pre-exposure of Endothelial Cells to curcumin reduces EVs secretion and probably affects proteins and miRNA conveyed.**

# Conclusion

## CURCUMIN



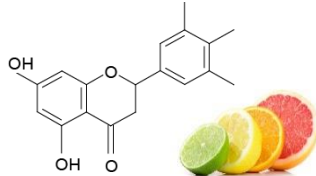
An improved vascular integrity and health



## Results from our group showing that other polyphenols exert cardioprotective effects

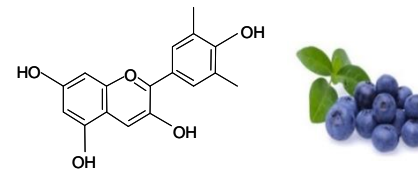
Reviews: Habauzit et al. *Ther Adv Chronic Dis* 2012; Milenkovic et al *PlosOne* 2012 ; Krga et al. *Food Func* 2016

### Flavanones



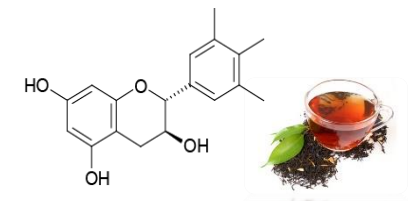
Morand et al *AJCN* 2011  
Milenkovic et al. *PlosOne* 2011  
Chanet et al. *JNB* 2012  
Chanet et al *BJN* 2013  
Habauzit et al *AJCN* 2015

### Anthocyanins



Mauray et al. *JAFC* 2009  
Krga et al. *ABB* 2016

### Flavanols - Catechins



Auclair et al. *Atherosclerosis* 2009  
Claude et al. *MNFR* 2014

# Acknowledgments



## NutriVasc TEAM

S.Mercier  
M-A Verny  
D.Coban  
D.Bayle  
C.Morand  
D.Milenkovic  
N.Baber-Chamoux



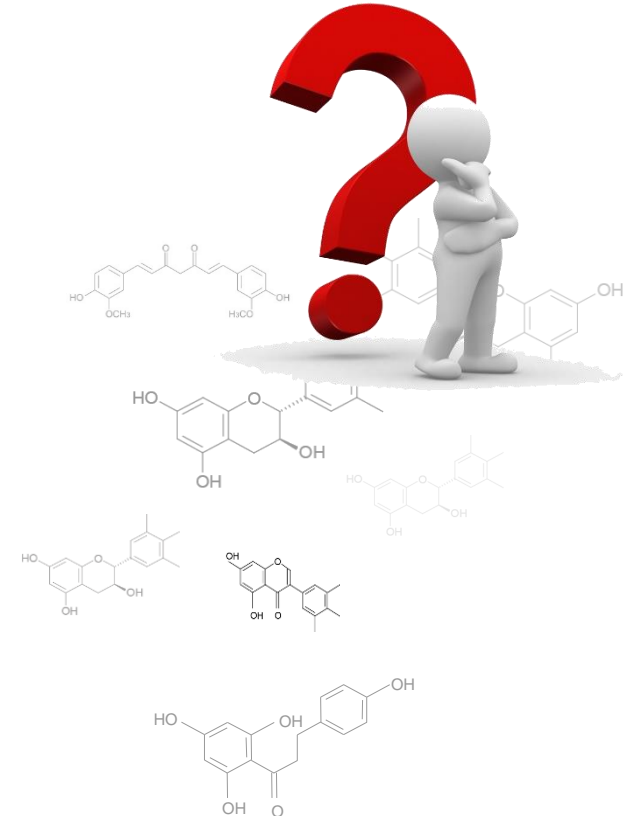
**Centre Imagerie Cellulaire Santé**  
→ Flow Cytometry analysis



**Radu Tamaian (Romania)**  
→ Docking Analysis



**Camille Roesch**  
→ TRPS analysis of endothelial EVs

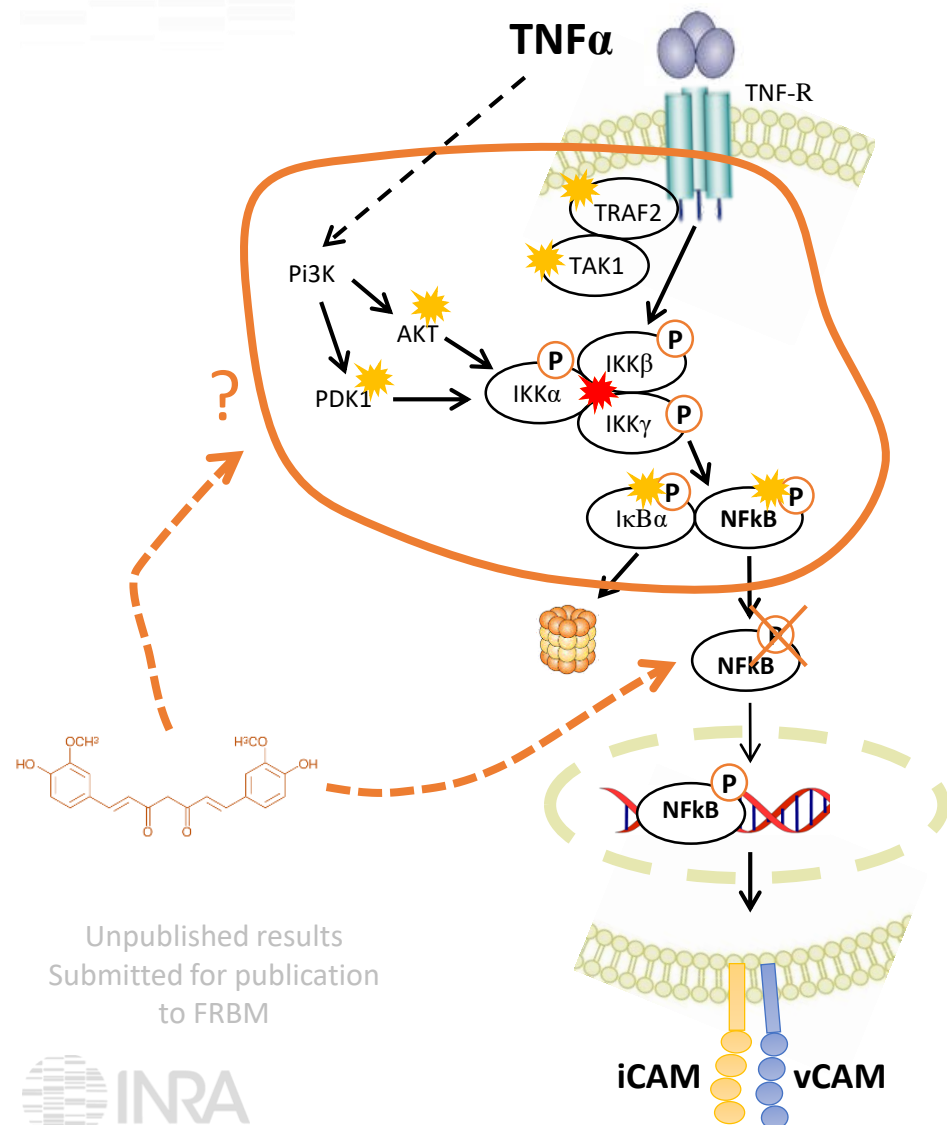


# Underlying mechanisms of action (5)

## Molecular docking to predict/identify putative targets of curcumin

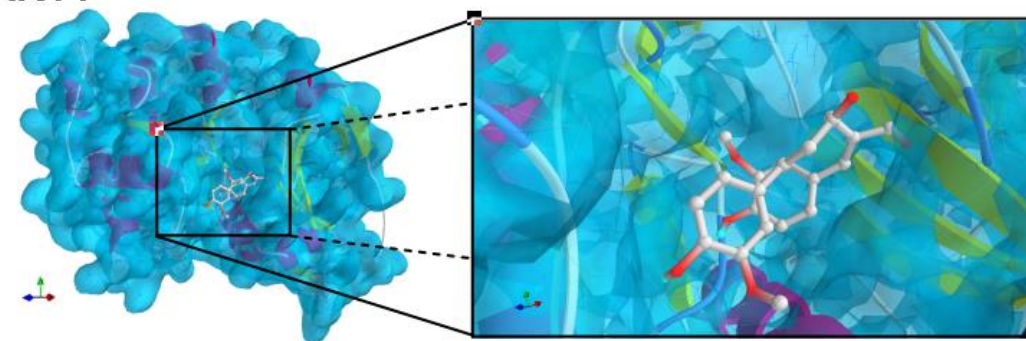
Radu Tamaian  
(Romania)

**ON.ERGHY**  
RESEARCH ON FUEL CELLS & HYDROGEN



Target	Binding affinity (Kcal/mol)	Number of H-Bonds	Location of H-bonds
TRAF2	-7.6	2	Tyr350 (coil) and Thr405 (coil) of the MATH domain
	-5.8	1	Gly127 (coil) of the Zinc finger (TRAF-type 1)
PDK1	-6.5	1	Tyr126 (alpha helix) of the active site
AKT1	-5.8	1	Lys8 of PH domain (beta strand)
AKT2	-7.3	0	N/A
AKT3	-6.3	0	N/A
AKT3	-7.1	3	Glu96 of PH domain (chain D - alpha helix), Lys63 (chain H - beta strand) of PH domain and Glu114 (chain H - coil)
TAK1	-7.8	1	Lys227 (coil) of the protein kinase domain, blocking the access at the ATP binding site and interaction with MAPK8IP1
IKK α	-7.6	1	Gln52 (chain C - alpha helix) of NEMO-binding region
IKK β	-7.6	1	Arg427 (alpha helix) - non specific binding
IKK γ	-5.7	1	Ser740 (3/10 helix) of NEMO-binding region
	-5.4	0	N/A
I <sub>κ</sub> B <sub>α</sub>	-5.6	3	Thr179 (two H-bods - coil), Gln212 (coil)
I <sub>κ</sub> B <sub>β</sub>	No experimental structures found		
NFκB p65	-6.2	1	Asn137 (coil) of the RHD (Rel-like) domain
Rel B	No experimental structures found		

### AKT1



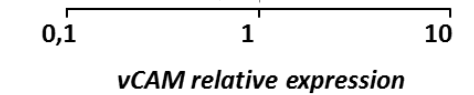
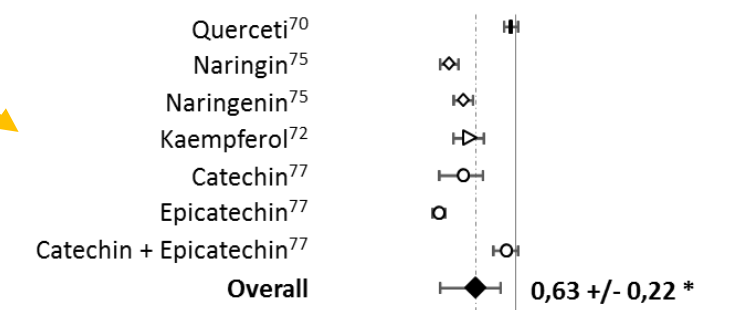
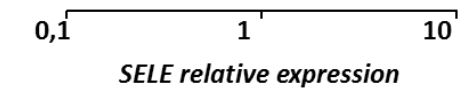
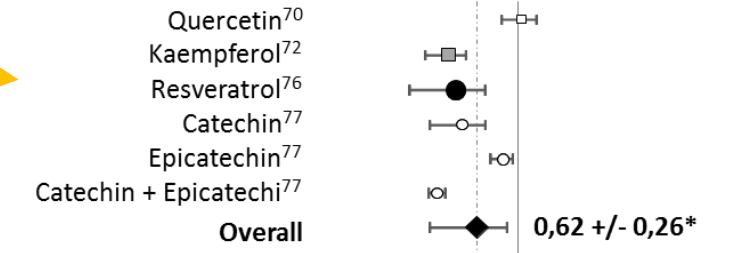
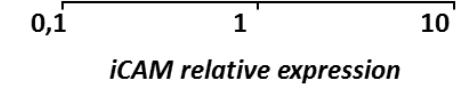
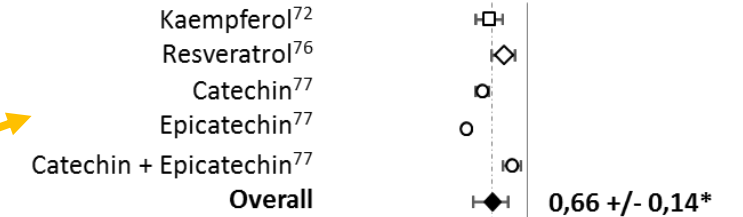
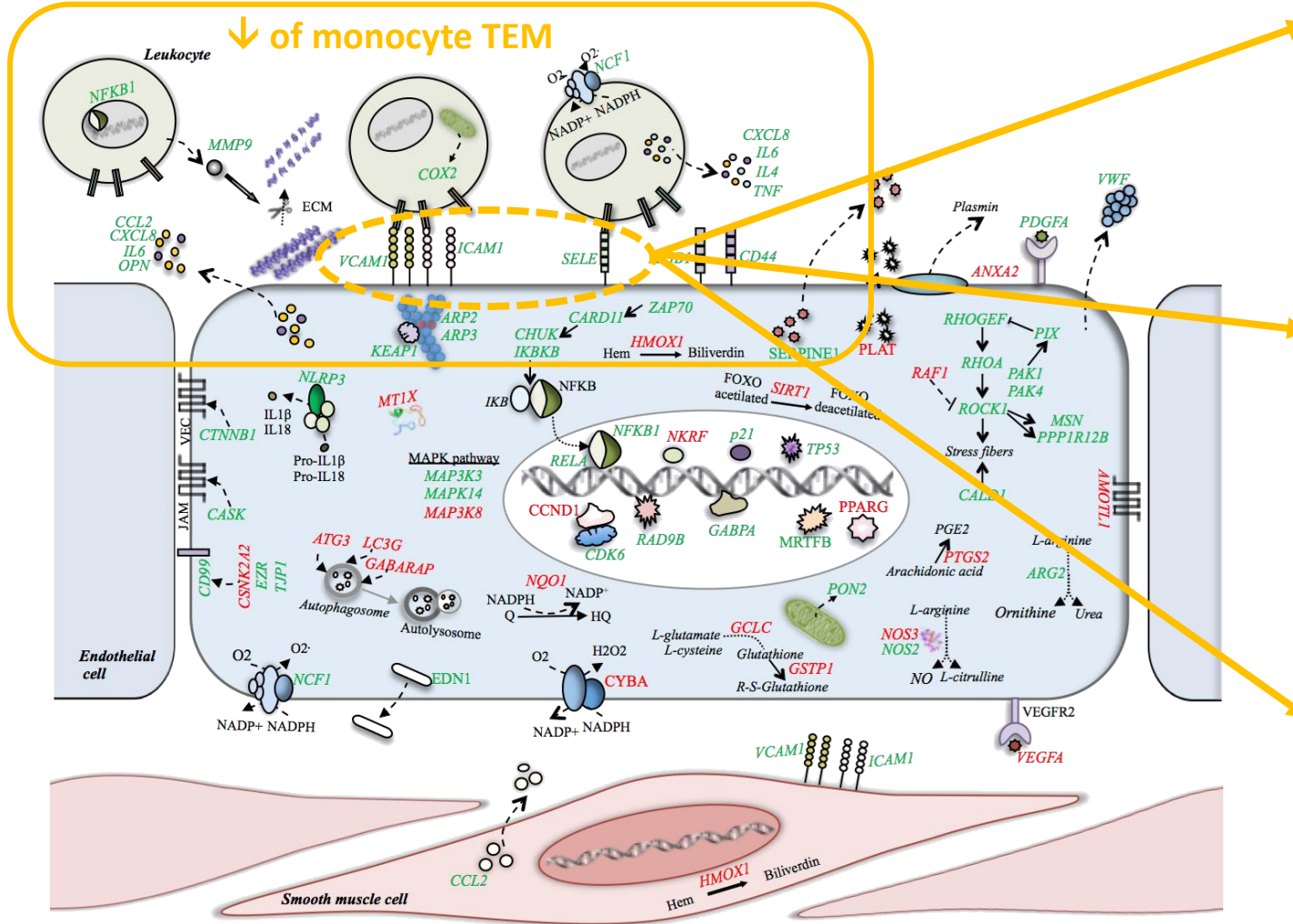


# Gene expression in response to (fruit) polyphenols

*Aortic expression*

*In vitro evidence*

↓ of monocyte TEM



Krga et al.  
 Food & Function  
 2016

Regulation ↑ ↓

Positive roles of polyphenols in preventing vascular dysfunction associated with the development of CVD



# Acknowledgments



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