FLAVIOLA

Targeted delivery of dietary flavanols for optimal human cell function: Effects on cardiovascular health

SFRBM Annual Meeting, Pre-meeting Workshop II

Flavanols in Health and Disease

Flavanols: What Do We Know About Their Mechanisms of Action?

Aalt Bast, Maastricht University

San Diego, 14 November 2012


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(Sub-)cellular targets and mechanisms in cardiovascular cell models

- Characterize cellular antioxidant effects and reactivity of flavanols and metabolites.
- Study the effects of flavanol metabolites on gene expression of specific adhesion and (pro)-inflammatory molecules.
- Identify the flavanol metabolites with most active anti-inflammatory and anti-oxidative activities.
- Determine the role of cell signaling pathways in the cell response to flavanol metabolites.
- Characterize epigenomic effects of flavanols at selected target genes.
- Assess impact of flavanols on mechanisms of vascular repair and regeneration.

Direct and indirect modulation of oxidative processes in human vascular endothelial cells

Evidence as antioxidants?!

Cellular antioxidant effects of flavanols in the cardiovascular system are mediated:

- **Directly**:

  Scavenging of reactive oxygen/nitrogen species (ROS/RNS).

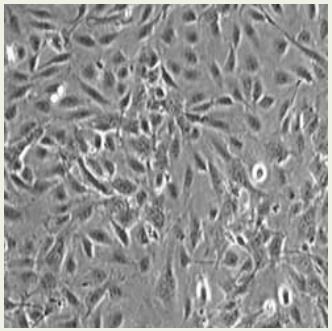
- **Indirectly**:

  Modulation of molecular processes leading to an increased endogenous antioxidant defense or attenuation of ROS generation.

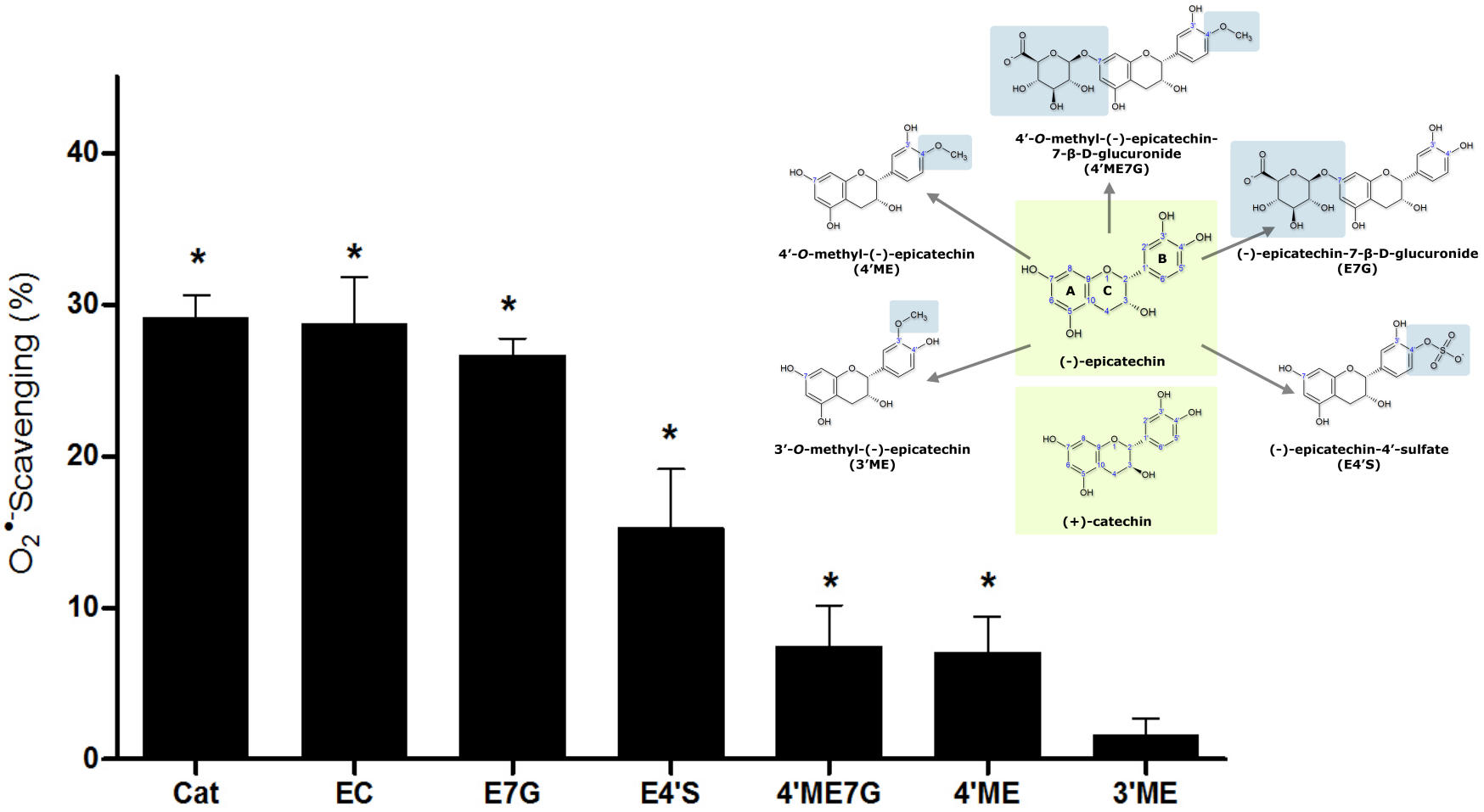
Antioxidant assessment *in vitro*

- In the absence of cells
  - $O_2^{\bullet -}$ scavenging

- In primary human umbilical vein endothelial cells (HUVECs)
  - H_2O_2 induced

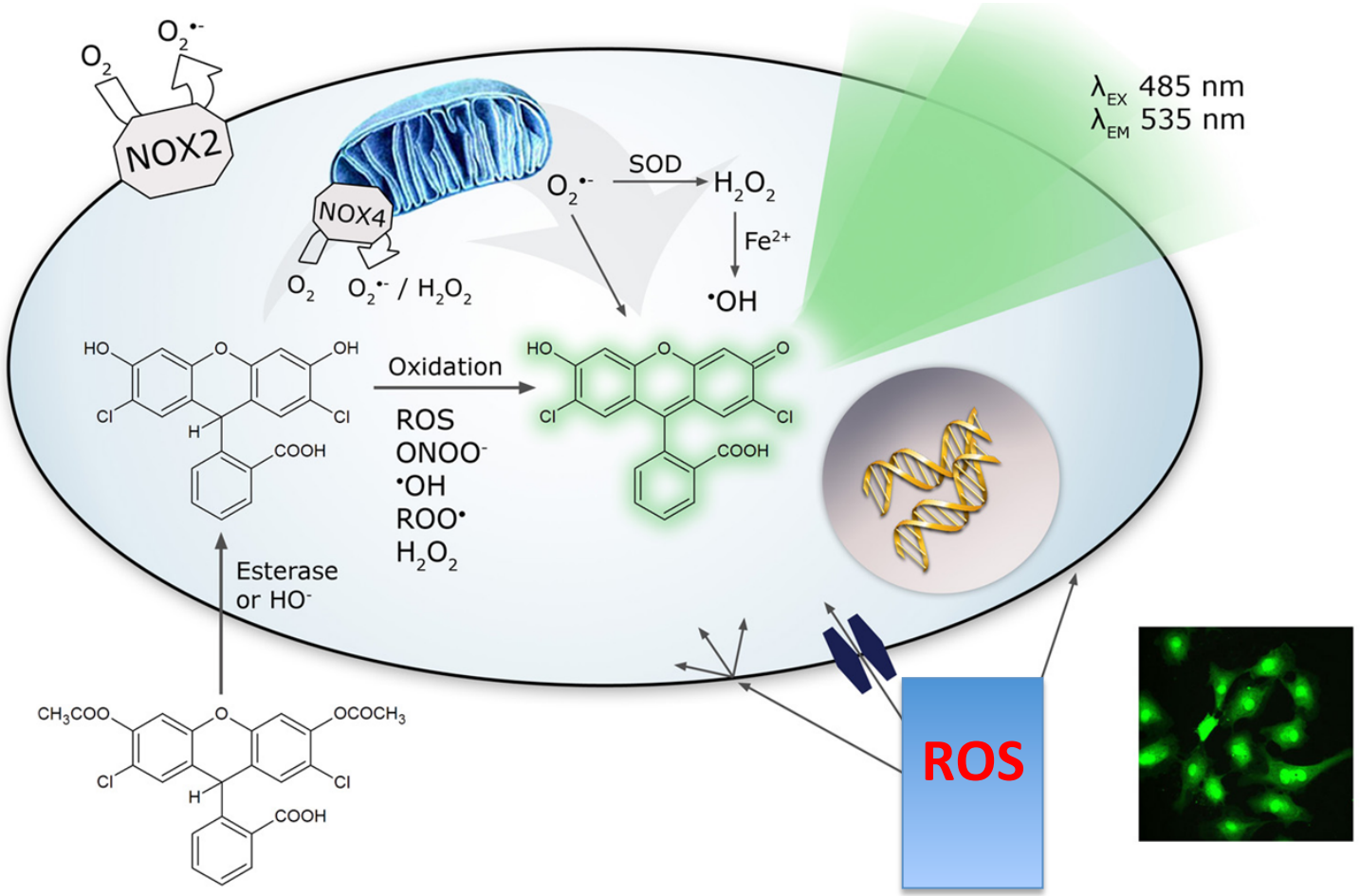


Superoxide scavenging by 10 µM flavanols and metabolites

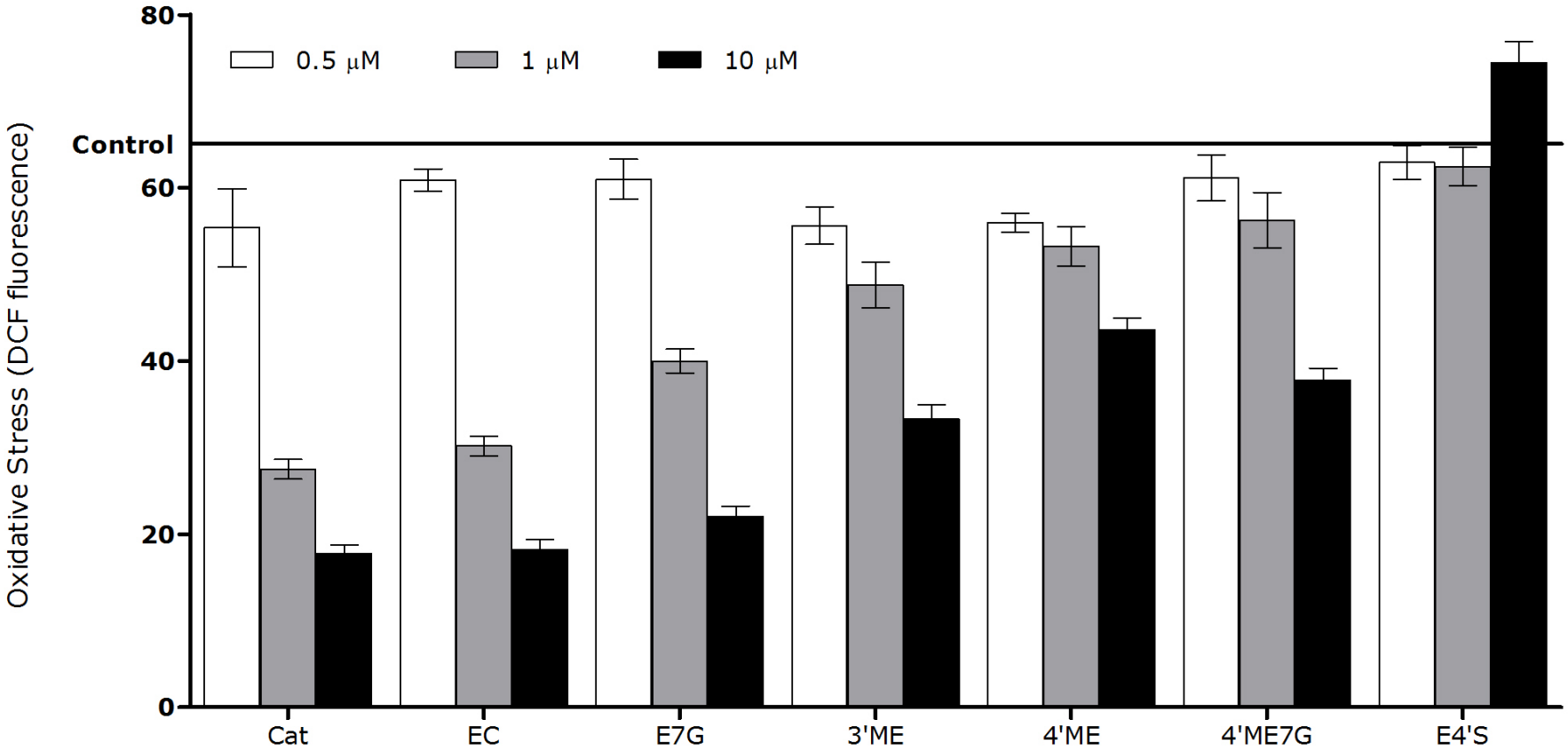


Measurement of intracellular oxidative stress

DCFH assay



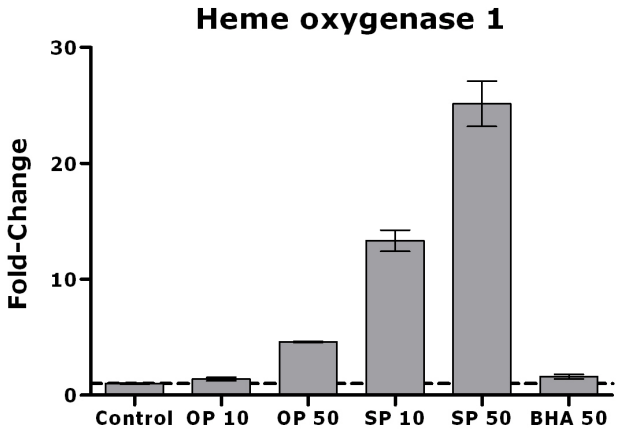
Protection against intracellular DCFH oxidation

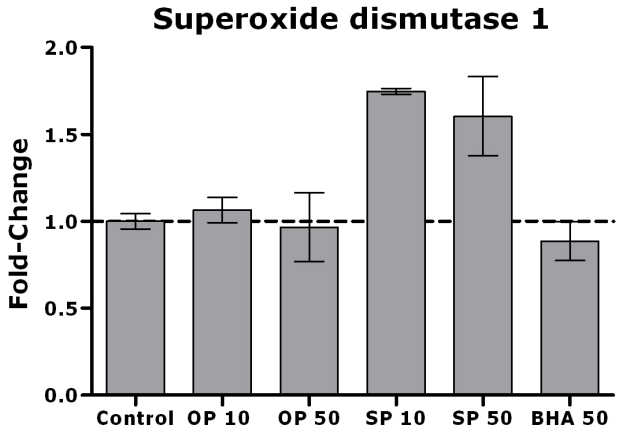


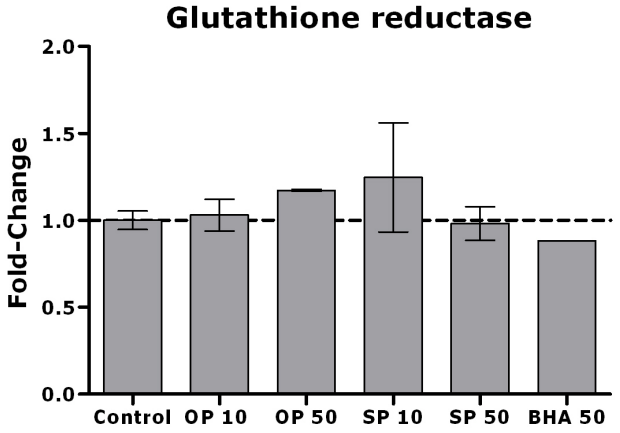
Flavanols and metabolites protect against intracellular DCFH oxidation

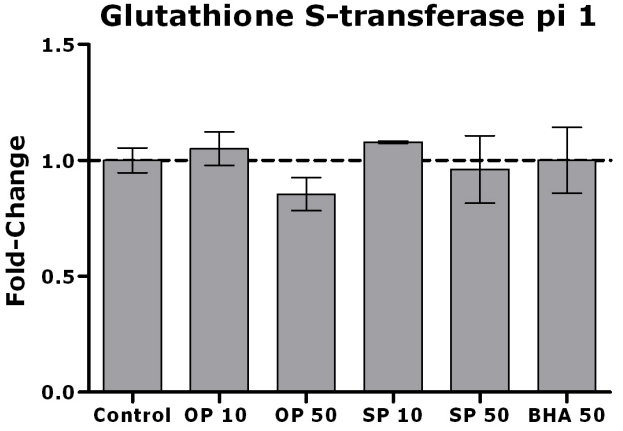
Induction of Nrf2-driven gene expression in HUVECs

OP: Oltipraz, SP: DL-Sulforaphane, BHA: Butylated hydroxyanisole

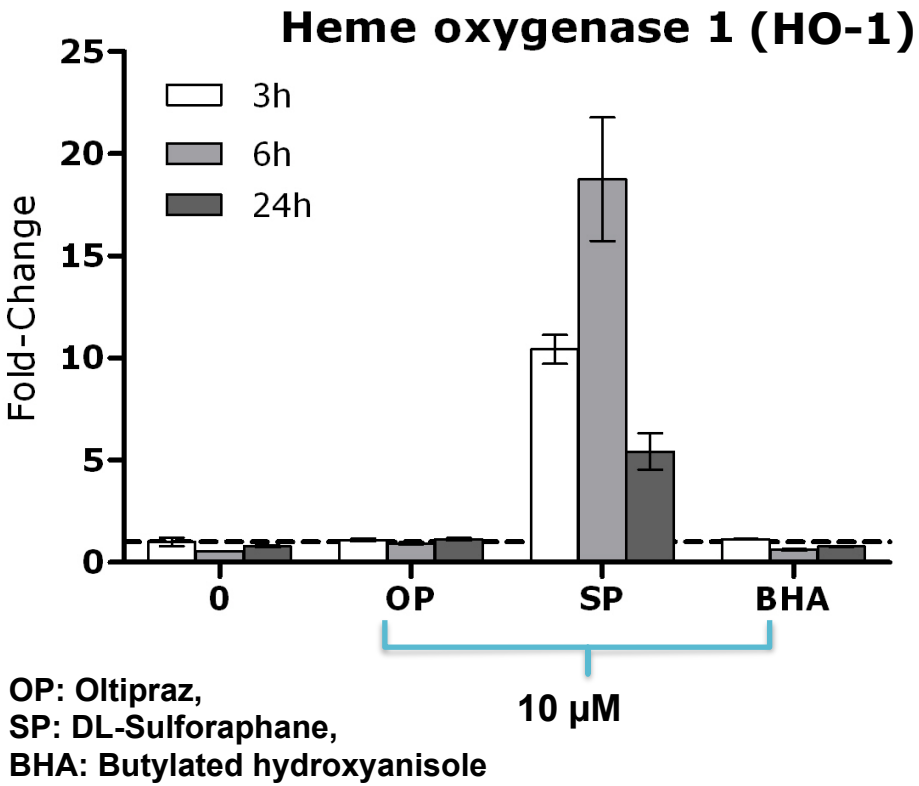




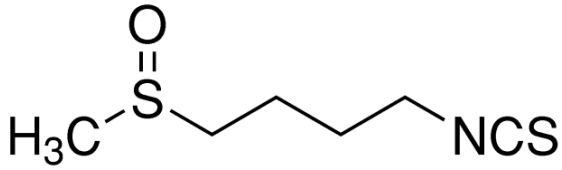




Induction of Nrf2-driven gene expression in HUVECs after different time points

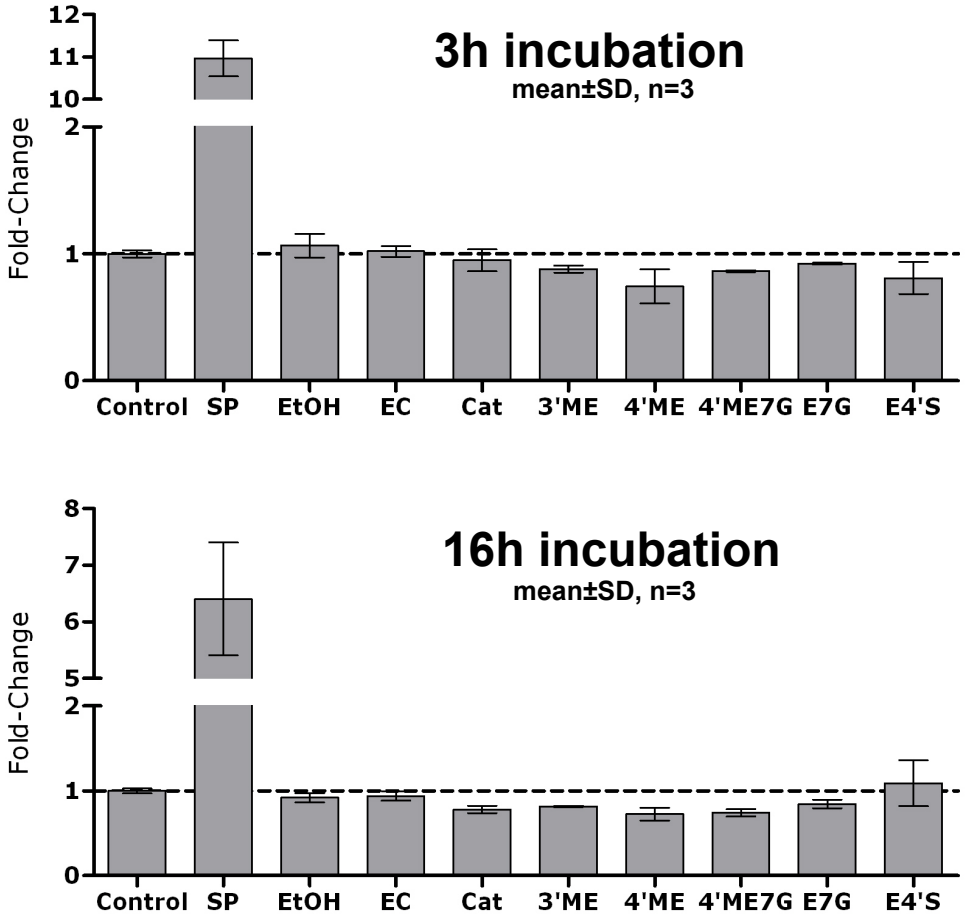


OP: Oltipraz,
SP: DL-Sulforaphane,
BHA: Butylated hydroxyanisole



DL-Sulforaphane

Effects of 10 µM flavanols and metabolites on HO-1 gene expression in HUVECs

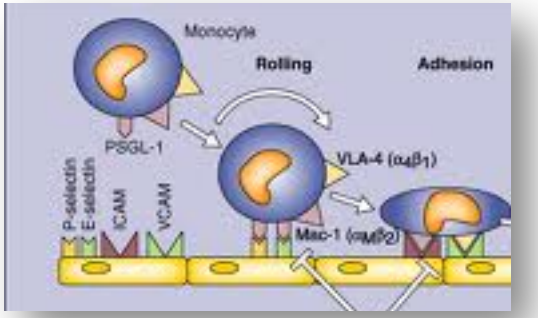


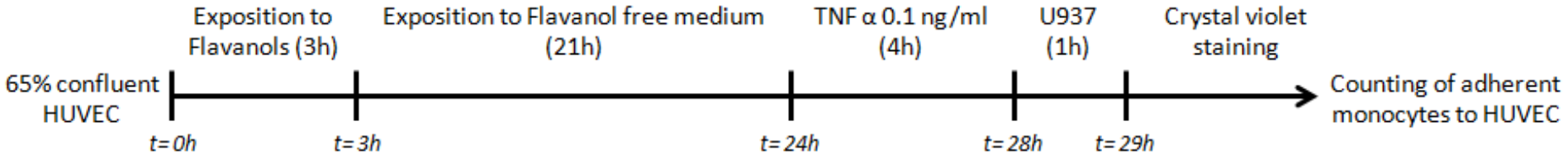
Flavanols and metabolites do not change Nrf2-driven HO-1 gene expression

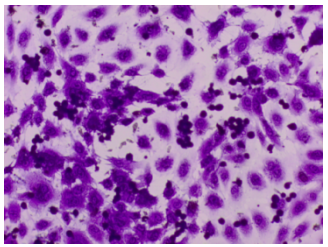
POSTER 200
By Erik Ruijters

Modulation of cellular inflammatory processes: Monocyte adhesion to endothelial cells

Experimental conditions for monocyte adhesion assay

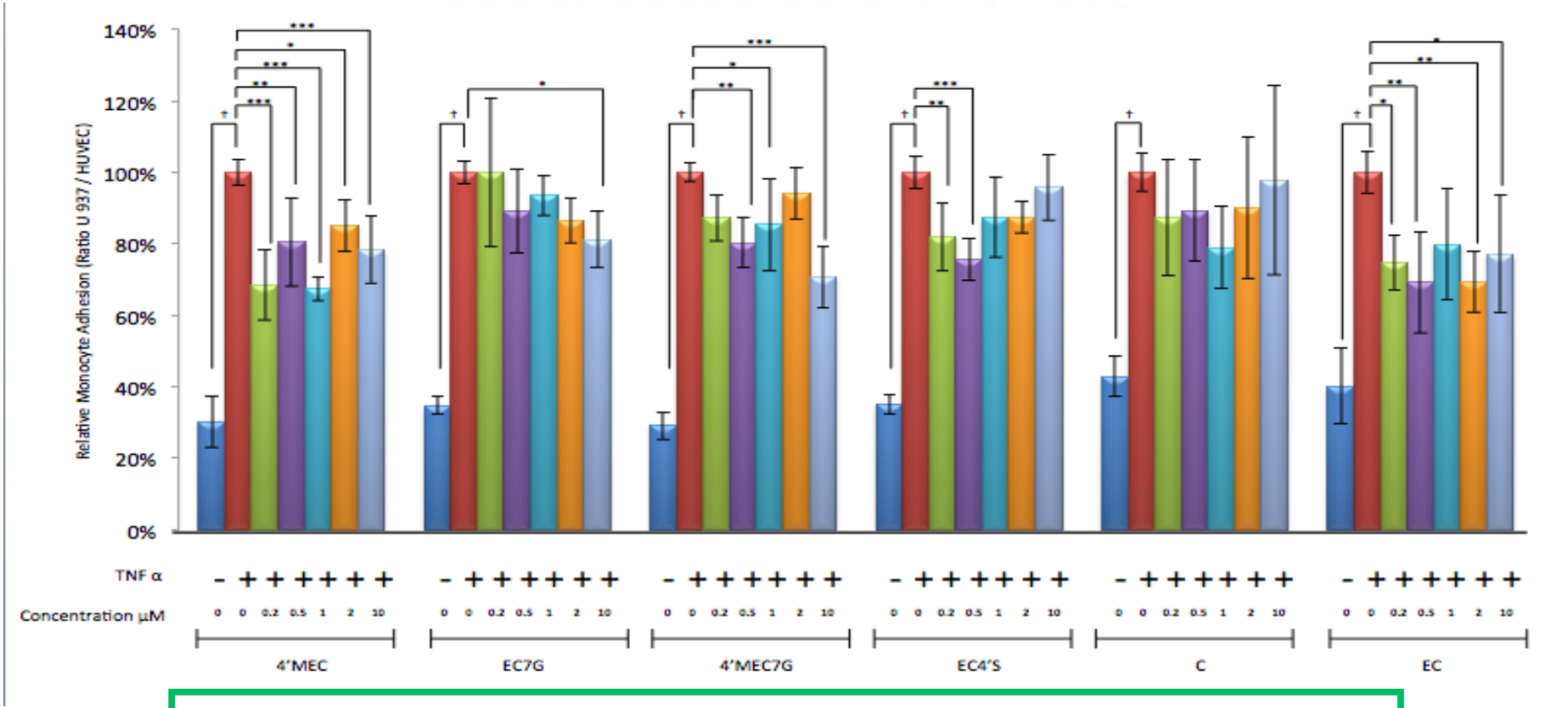






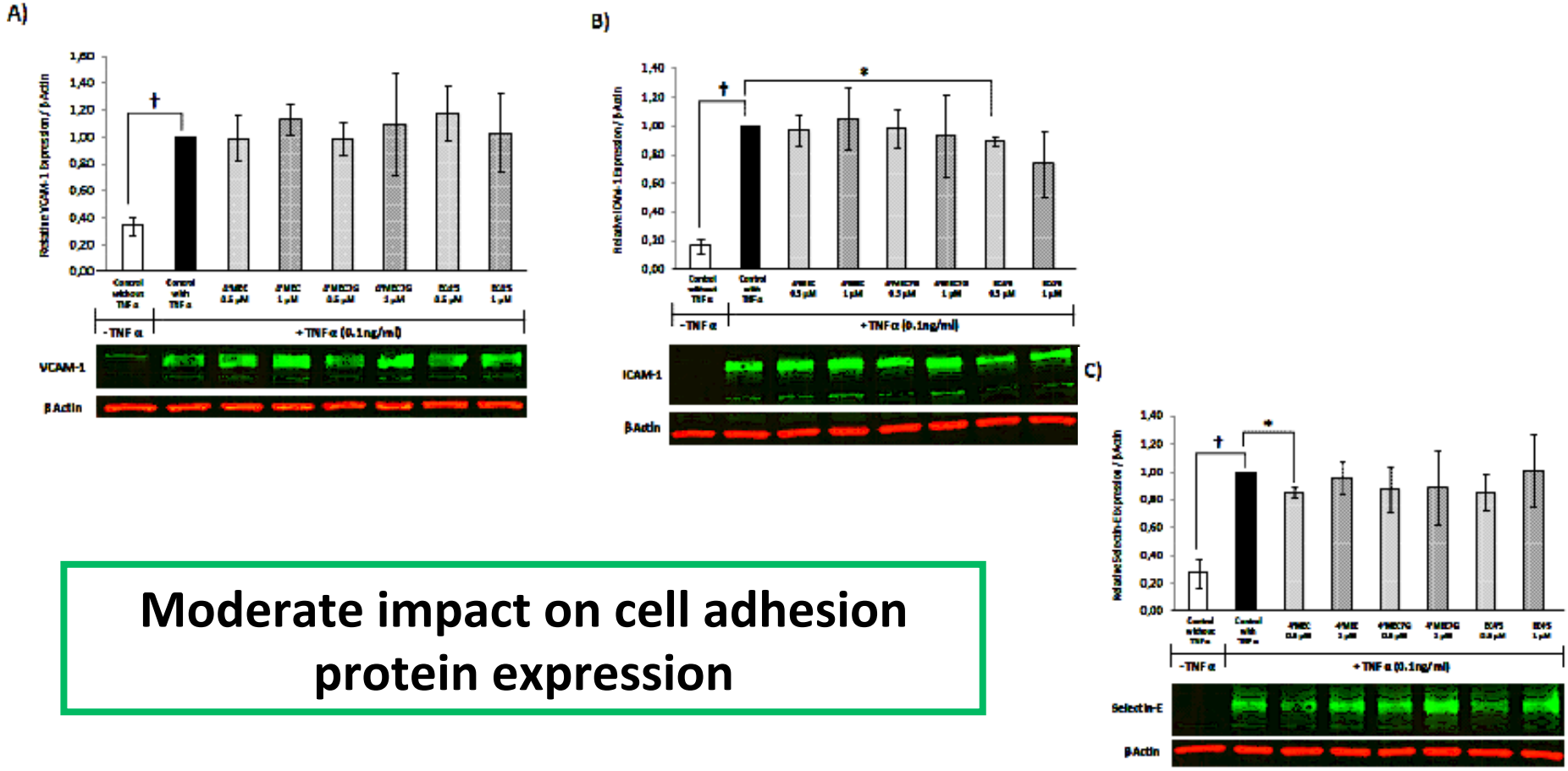
POSTERS 422 and 423
By Sylvain Claude

Inhibition of monocyte (U937 cells) adhesion to TNF-α stimulated HUVECs by flavanols



Flavanols and their metabolites can modulate monocyte adhesion

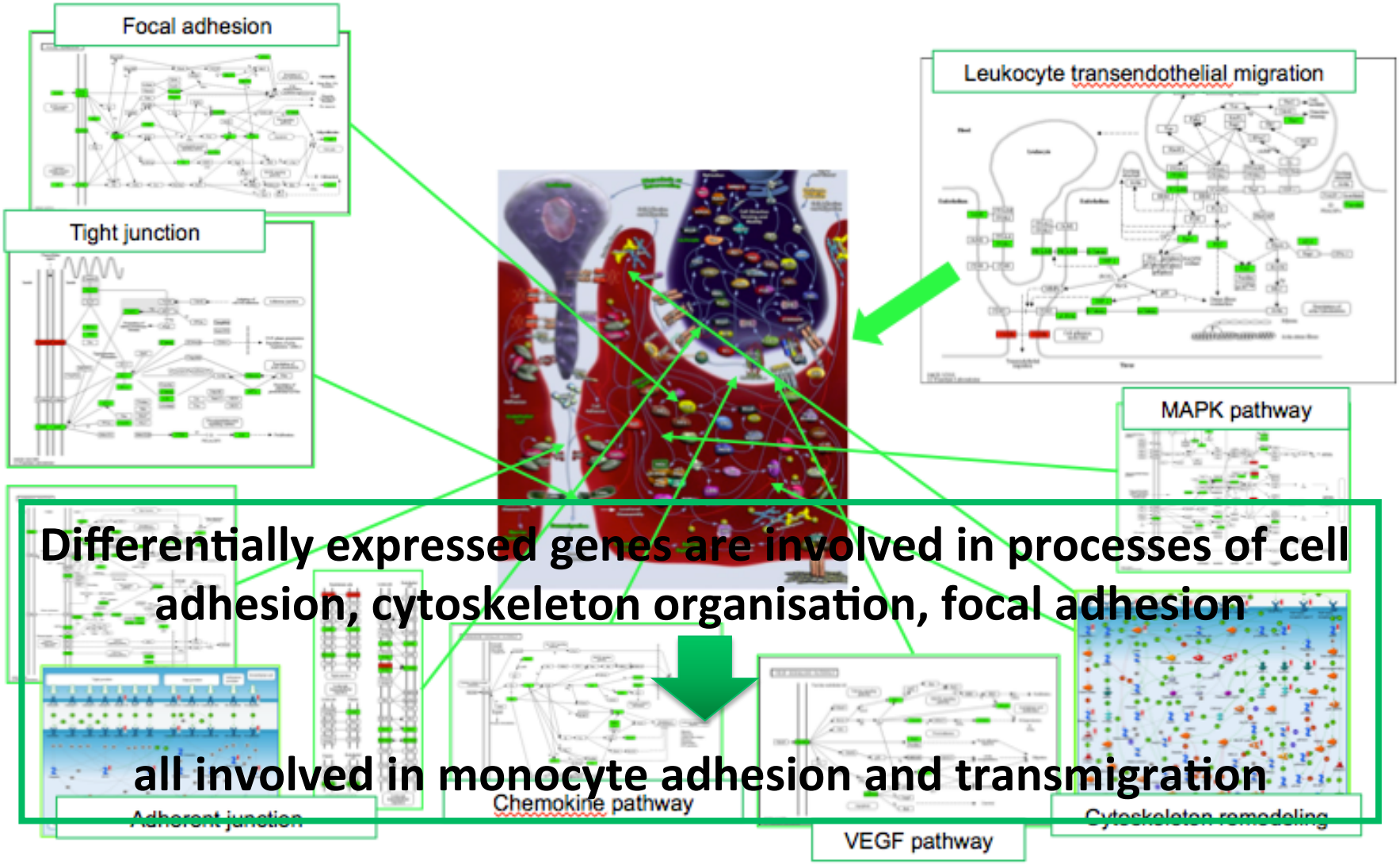
Effect of flavanol metabolites on cell adhesion molecule expression in HUVECs



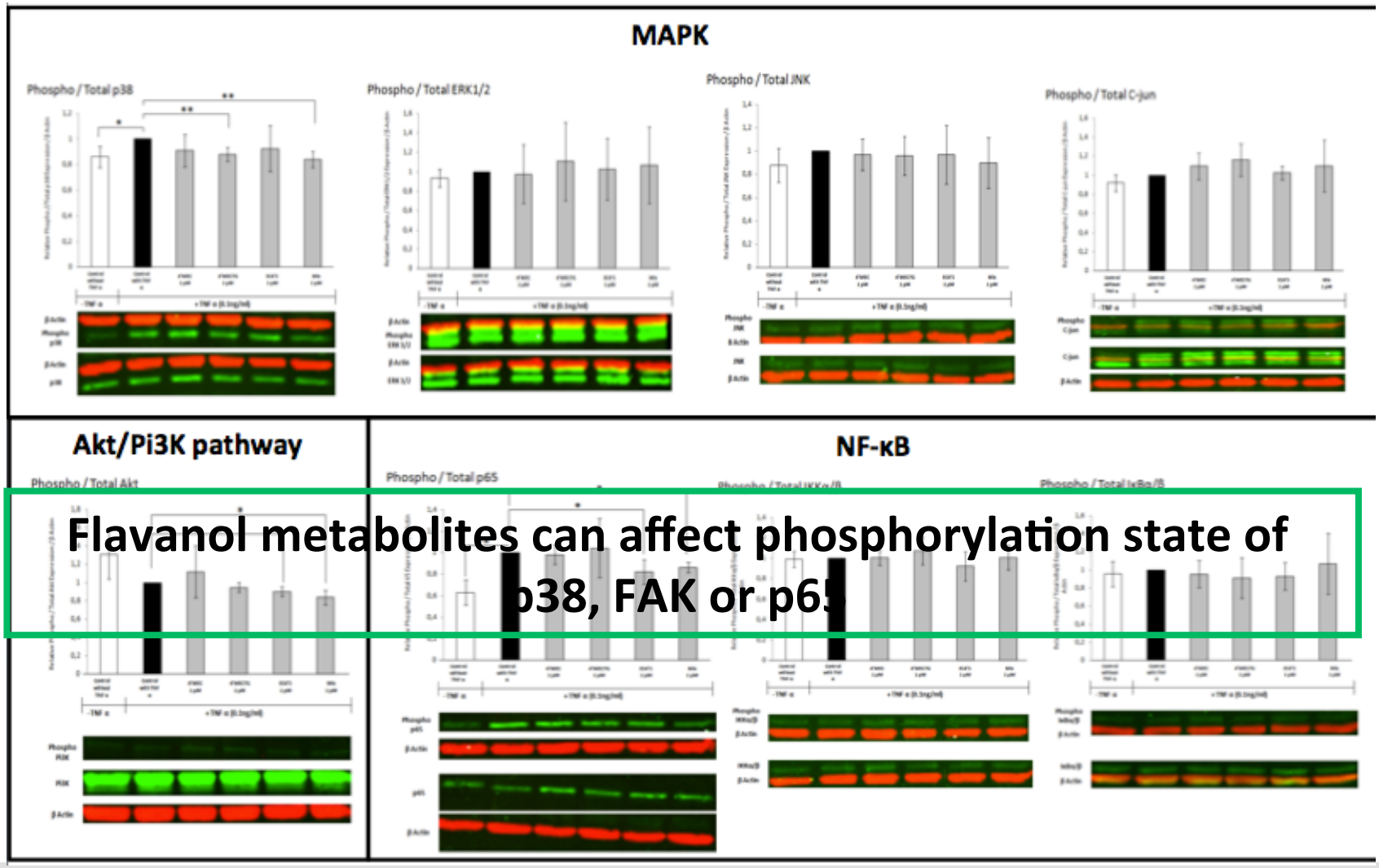
Moderate impact on cell adhesion protein expression

Effects on cellular gene expression

Pathway analyses of differentially expressed genes

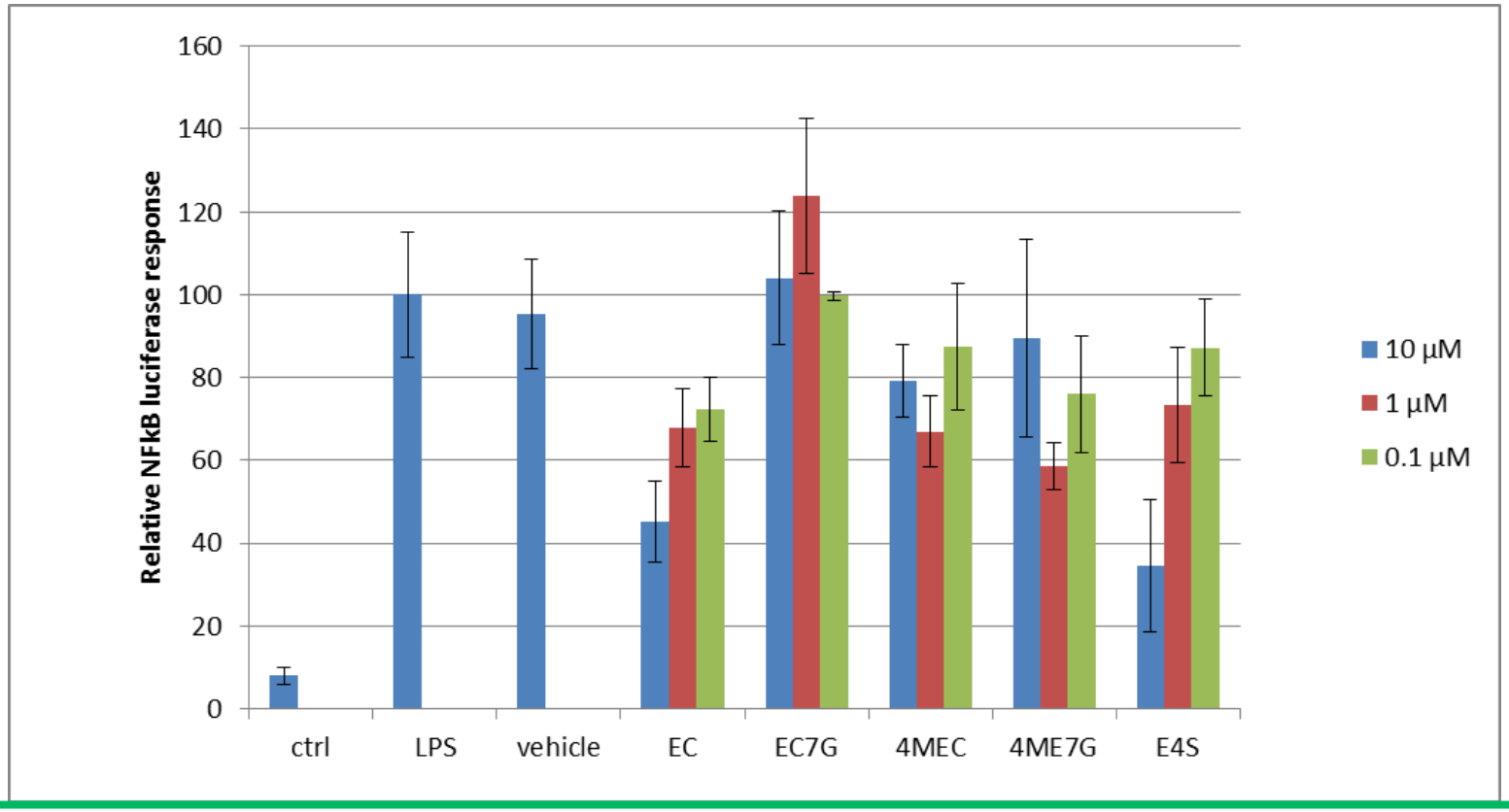


Signaling pathway involved in the cellular response to flavanol metabolites



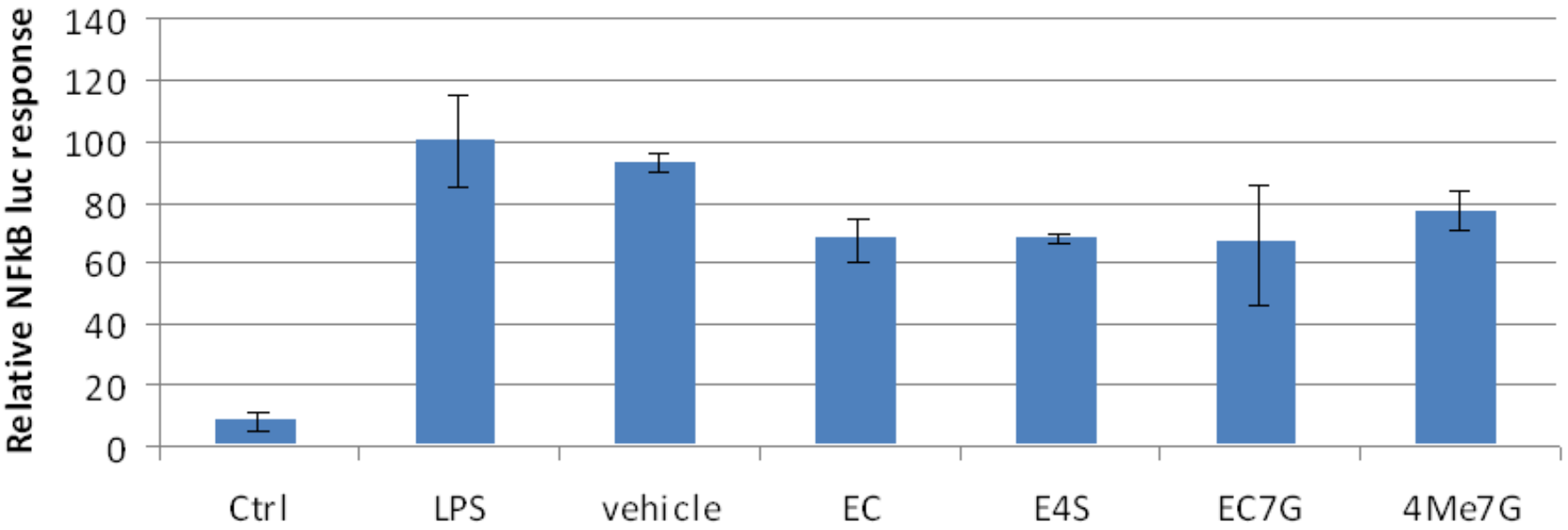
Flavanol metabolites can affect phosphorylation state of p38, FAK or p65

Effects of flavanol metabolites on NF-κB response in differentiated human monocytes



Epicatechin and its sulfate metabolite significantly inhibit NF-κB-driven gene expression in low µM concentrations

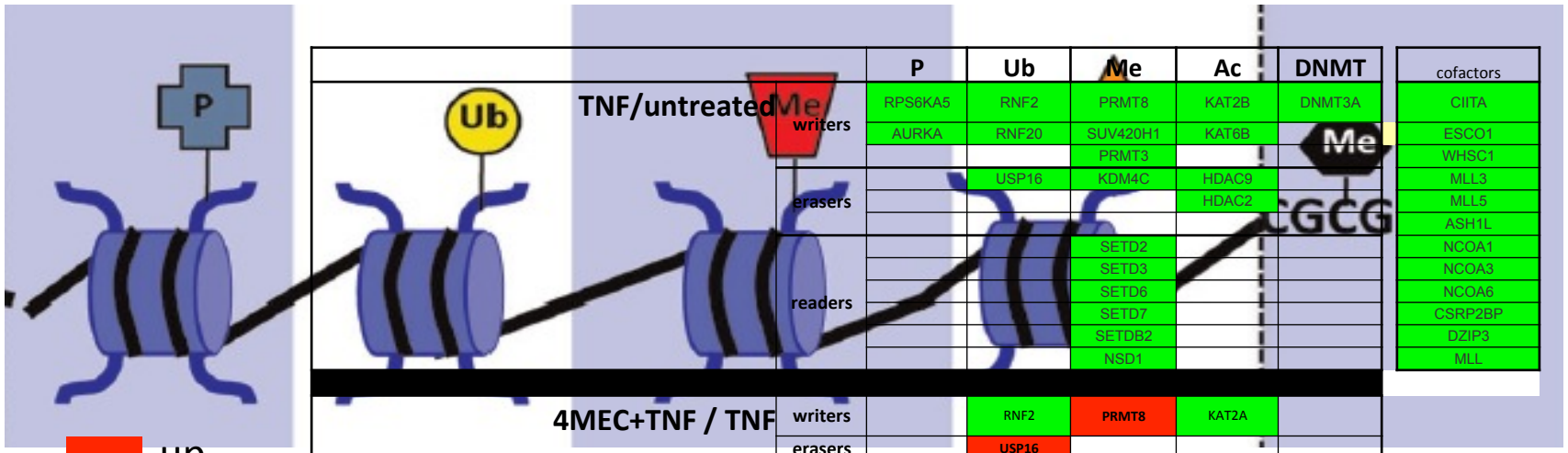
Effects of flavanol metabolites on monocyte differentiation and subsequent NF-kB response



Epicatechin and its metabolites persistently inhibit NF-κB-driven gene expression when added during monocyte differentiation prior to the inflammatory stimulus

Modulation of subcellular epigenetic processes

Effects of TNF-α, flavanols and metabolites on gene expression of chromatin modifier in HUVECs



		P	Ub	Me	Ac	DNMT	cofactors
TNF/untreated	writers	RPS6KA5	RNF2	PRMT8	KAT2B	DNMT3A	CIITA
		AURKA	RNF20	SUV420H1	KAT6B		ESCO1
				PRMT3			WHSC1
	erasers		USP16	KDM4C	HDAC9		MLL3
					HDAC2		MLL5
							ASH1L
	readers			SETD2			NCOA1
				SETD3			NCOA3
				SETD6			NCOA6
				SETD7			CSRP2BP
				SETDB2			DZIP3
				NSD1			MLL
4MEC+TNF / TNF	writers		RNF2	PRMT8	KAT2A		
	erasers		USP16				
	readers						

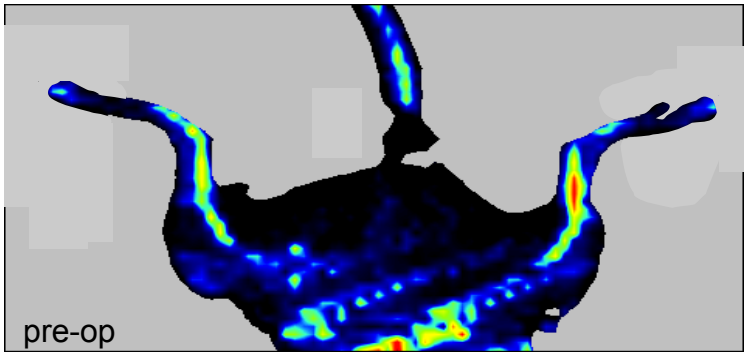
		P	Ub	Me	Ac	DNMT	cofactors
4MEC7G+TNF / TNF	writers			PRMT8			NCOA1
	erasers		USP16		HDAC2		MLL
	readers						ESCO1
							MLL3
							CIITA
EC4S+TNF / TNF	writers			PRMT8			SMARCA4
	erasers		USP16				
	readers						

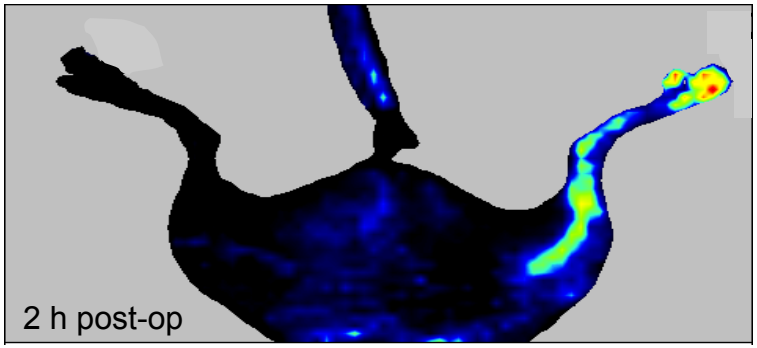
		P	Ub	Me	Ac	DNMT	cofactors
4MEC+4MEC7G+EC4S+TNF/TNF	writers			PRMT8			NCOA1
	erasers		USP16		HDAC2		CIITA
	readers						

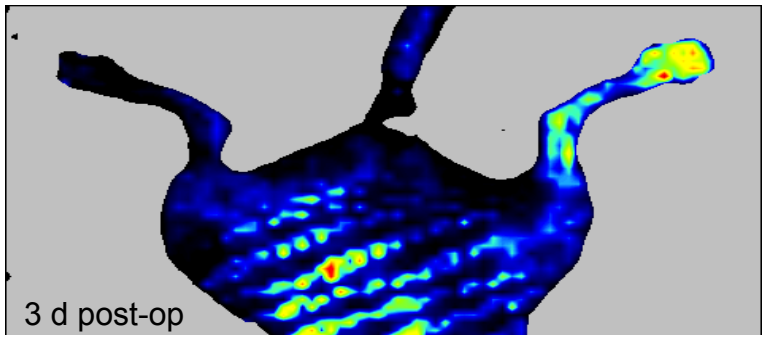
Flavanols and metabolites counteract TNF-α induced changes in gene expression of chromatin modifiers

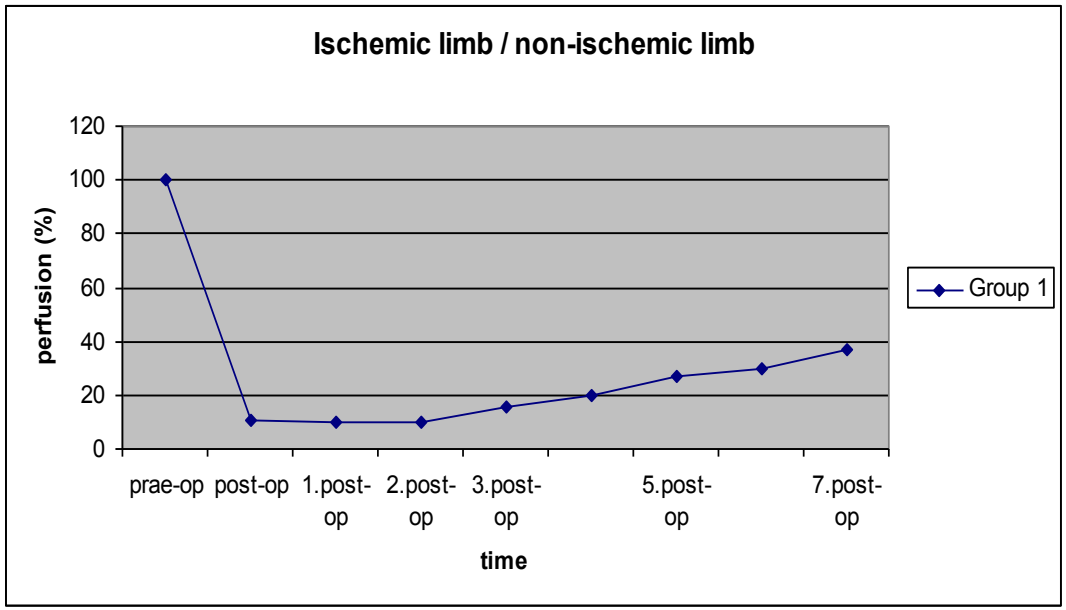
Modulation of ischemia injury *in vivo*

Hindlimb ischemia mice model



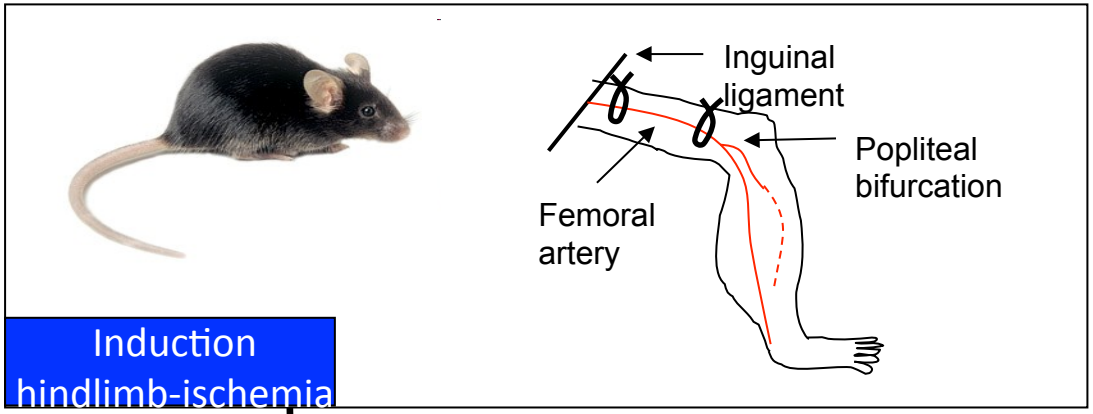






Analysis of the perfusion of the ischemic hindlimb in relation to the non-ischemic hindlimb through laser doppler imaging

Effects of epicatechin on angiogenesis in the hindlimb ischemia mice model



Induction hindlimb-ischemia

Vehicle or (-)-epicatechin feeding

Time (d)

-5 -1 0 2h 1 2 3 4 5 6 7 8 9 10 11 12 13 14

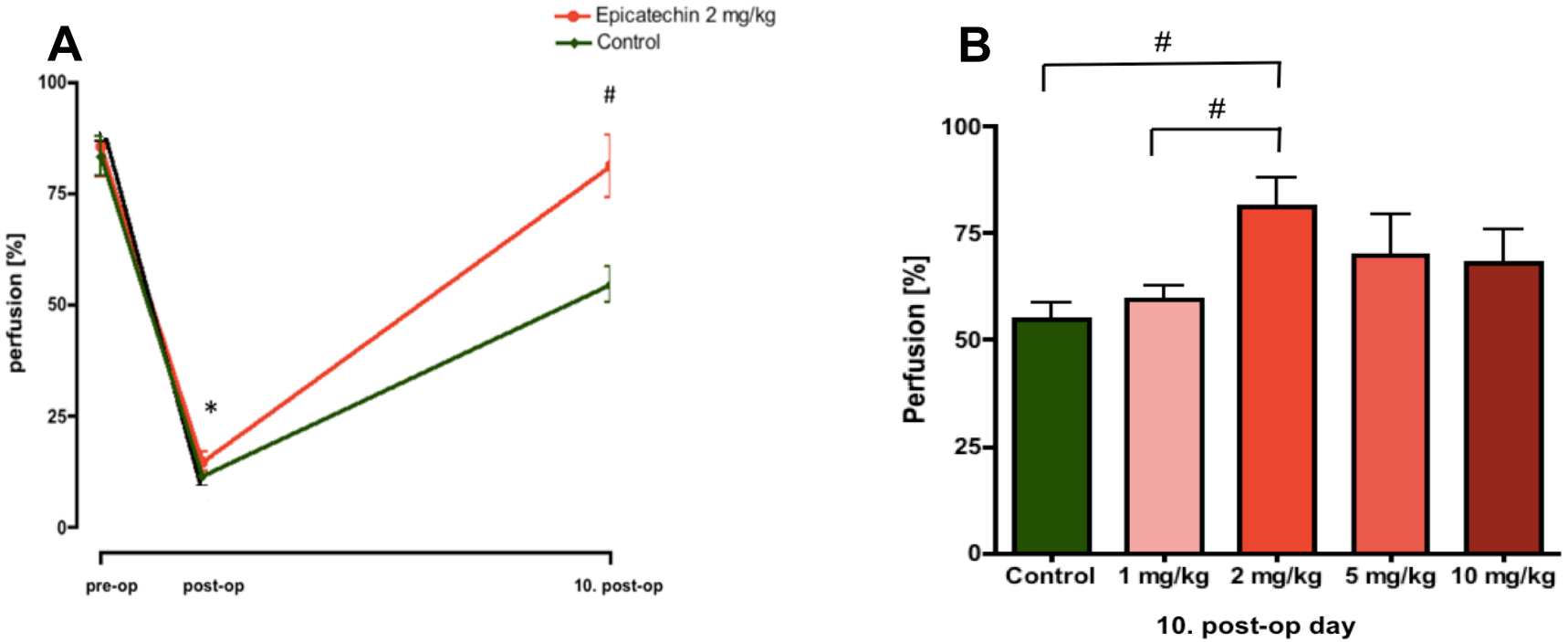
Laser Doppler Imaging (hindlimb perfusion)

Groups:

1) Control
2) 1 mg/kg BW
3) 2 mg/kg BW
4) 5 mg/kg BW
5) 10 mg/kg BW

Effects of epicatechin on hindlimb perfusion of mice after ischemia

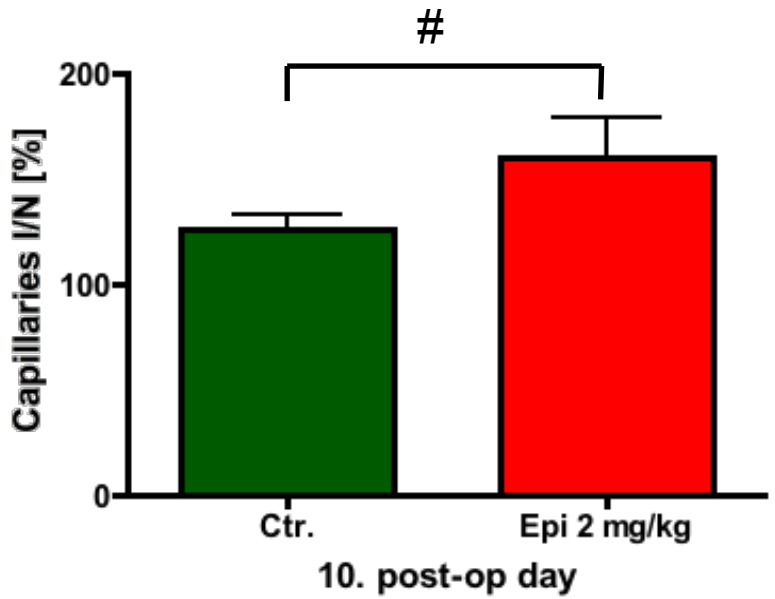
Laser Doppler Perfusion Imaging

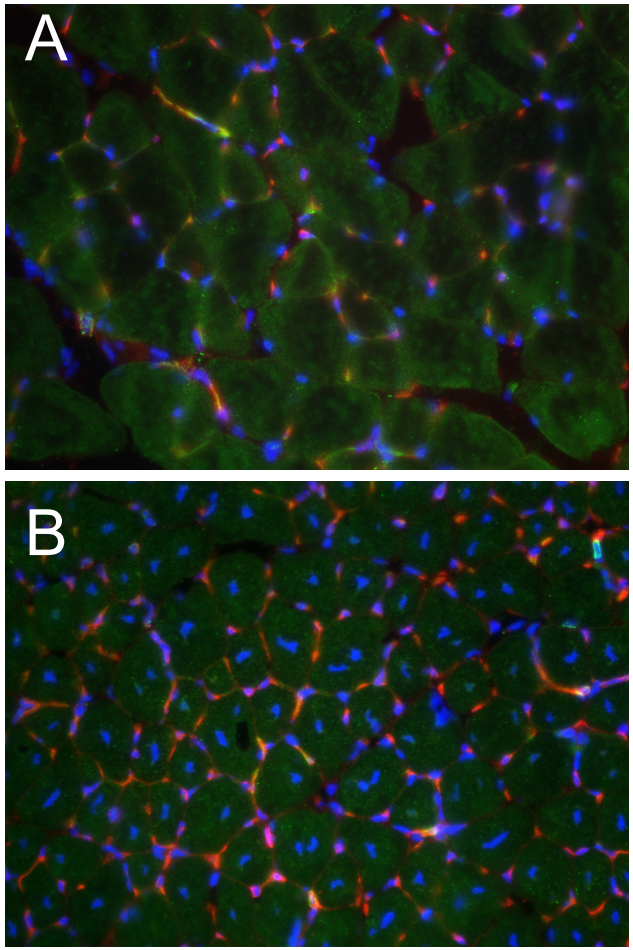


Epicatechin increases blood flow after ischemia

Effects of epicatechin on angiogenesis of mice after ischemia

Morphometric analysis





Epicatechin augments capillary formation after ischemia

Summary

Flavanols and flavanol metabolites...

- Protect HUVECs intracellularly against oxidative damage without modulating Nrf-2 mediated gene expression.