VU medisch centrum

ESC summerschool Nice 2013

# ENDOTHELIAL INTEGRITY

# The endothelial KLF2-actin balancing act

Prof dr Anton J.G. Horrevoets

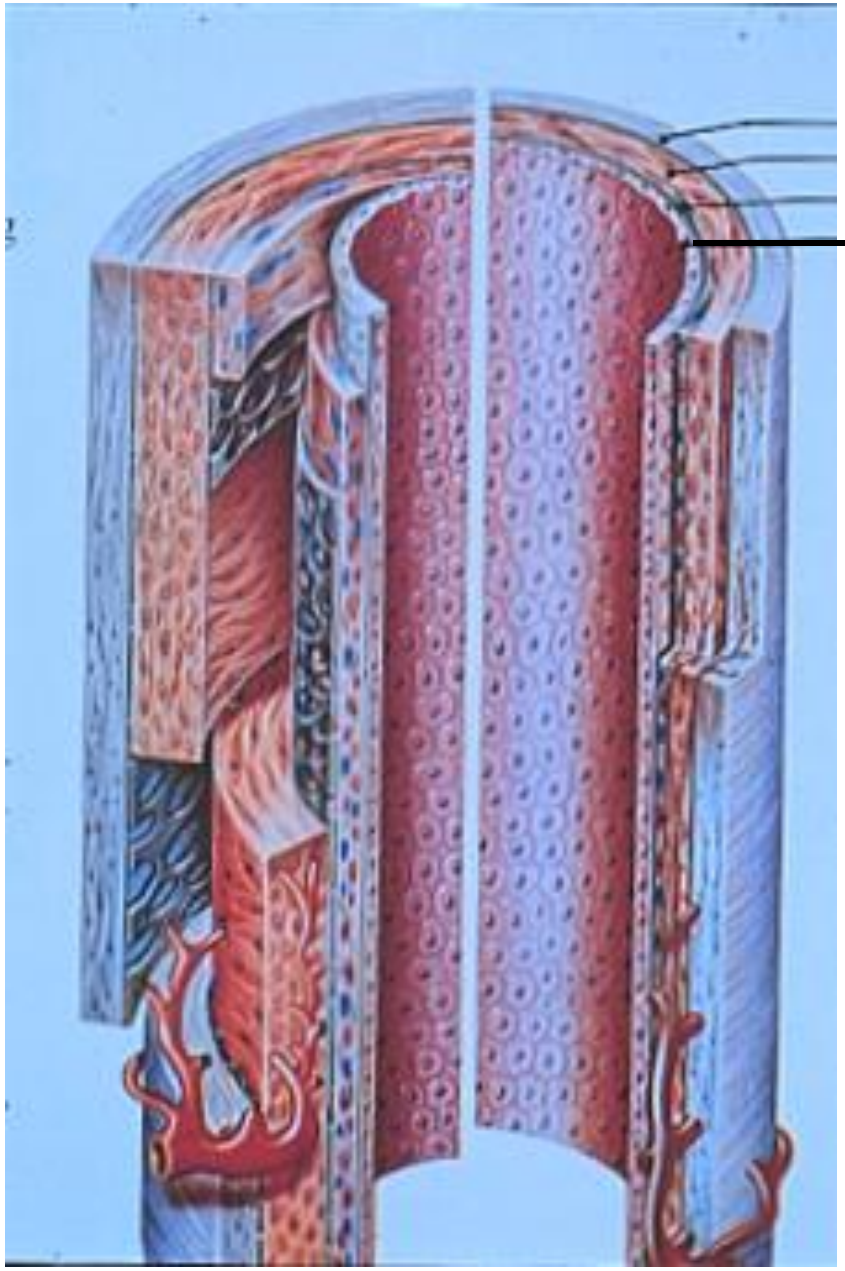
Molecular Cell Biology and Immunology

VU University Medical Center Amsterdam

Institute for Cardiovascular Research

ICaR-VU

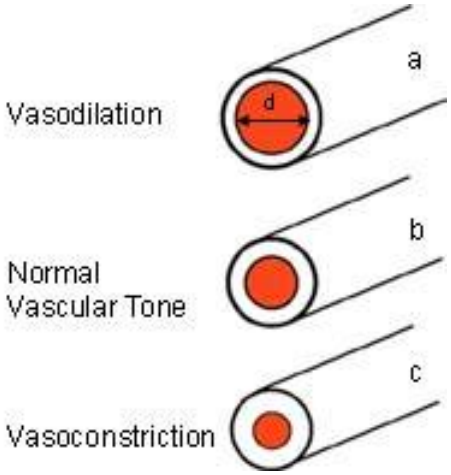
*vrije* **Universiteit** *amsterdam*



## Endothelium as organ

- **720 gram EC, (600 in capillaries)**
- **3000 $m^2$**
- **Total length bloodvessels is over 100,000 km**

| Factor secreted by endothelium | Activities |
| --- | --- |
| prostacyclin | vasodilation, inhibits platelet aggregation |
| nitric oxide | vasodilation, inhibits platelet adhesion and aggregation |
| tissue plasminogen activator (tPA) | regulates fibrinolysis |
| thrombomodulin | anticoagulant activity |
| platelet-activating factor (PAF) | activation of platelets and neutrophils |
| von Willebrand factor (a) | promotes platelet adhesion and activation of blood coagulation |



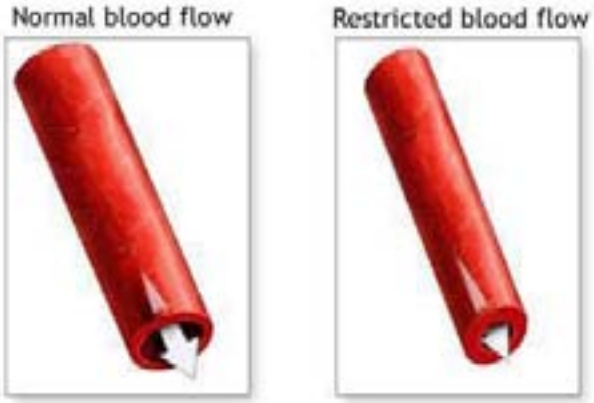

# VASOREGULATION

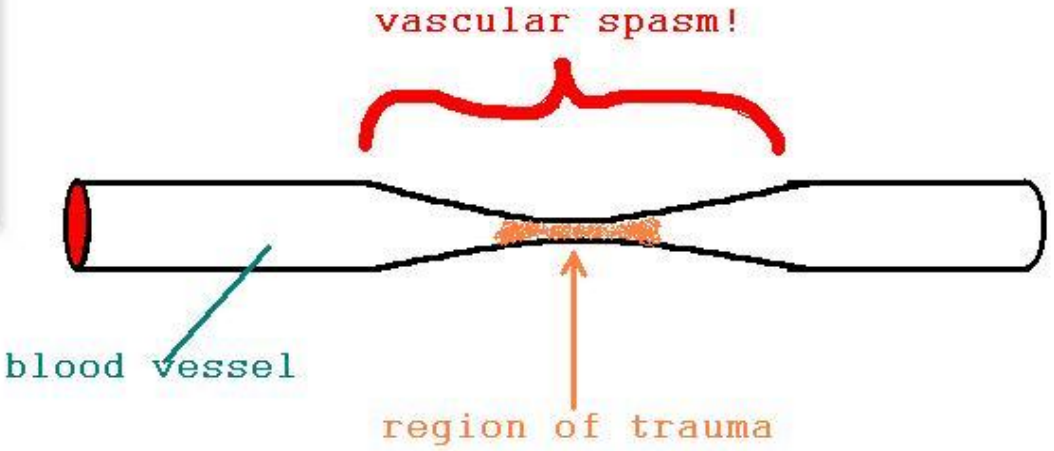
Active Production NO, ET1 and PGI2

Blood pressure

Regulation tissue perfusion

Response to trauma



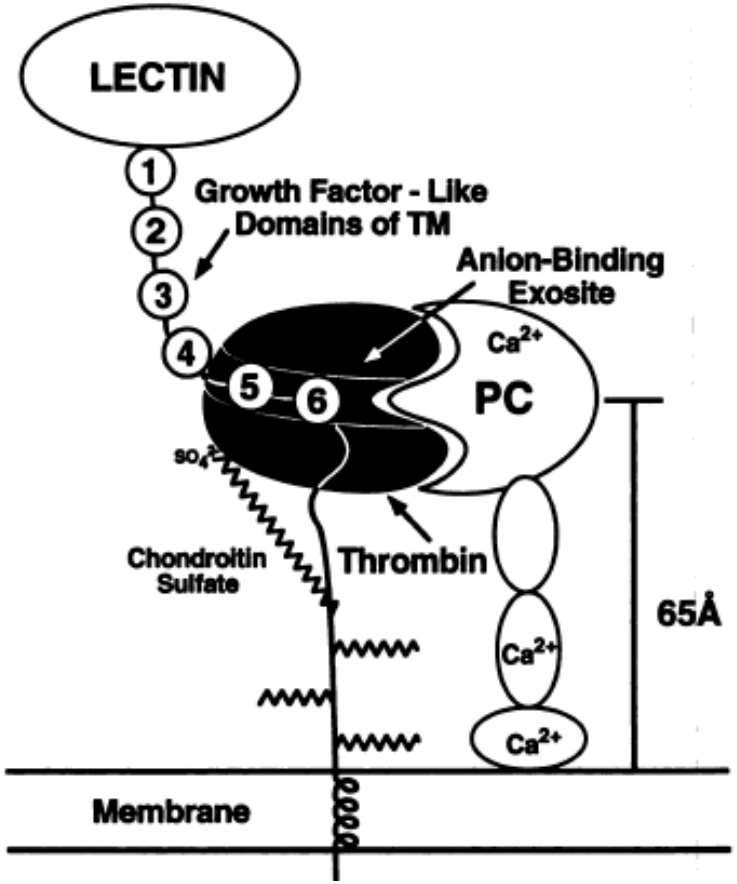



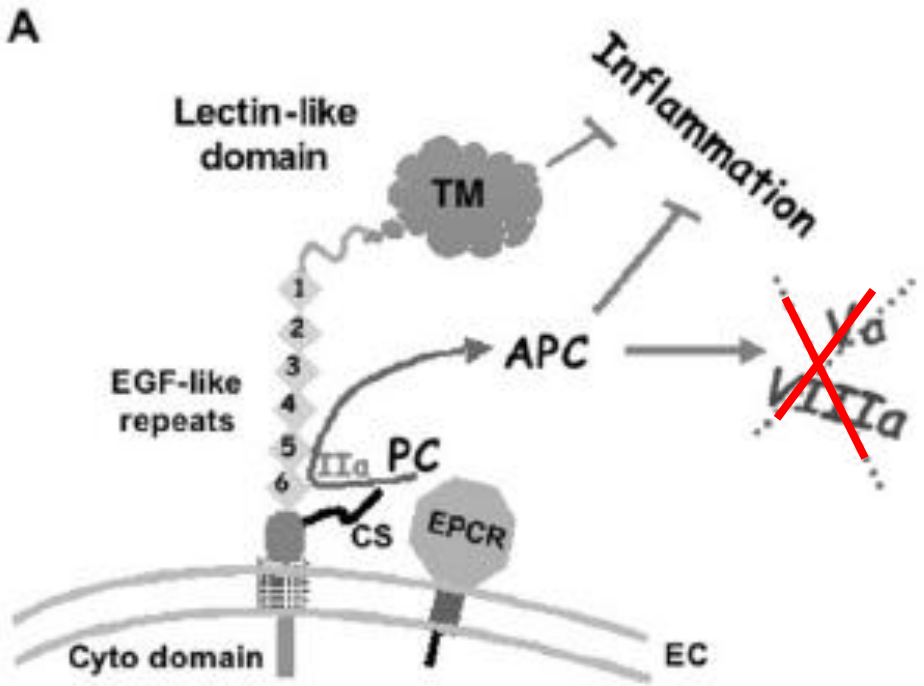

removal endothelium=> vasoconstriction

# Endothelium as anticoagulant:

Prosthesis with and without endothelial cells

# Endothelium as active anti-coagulant:
## thrombomoduline en EPCR



# Activated Endothelium actively recruits monocytes from blood via adhesion molecules

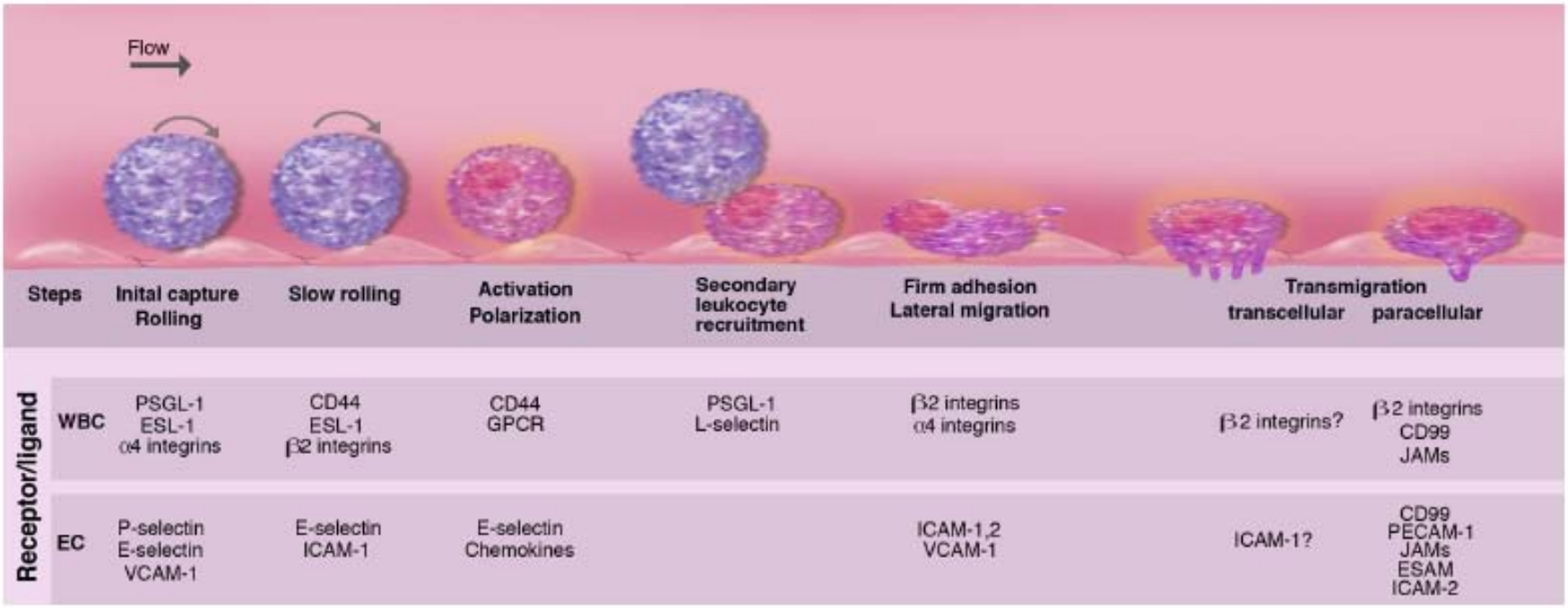
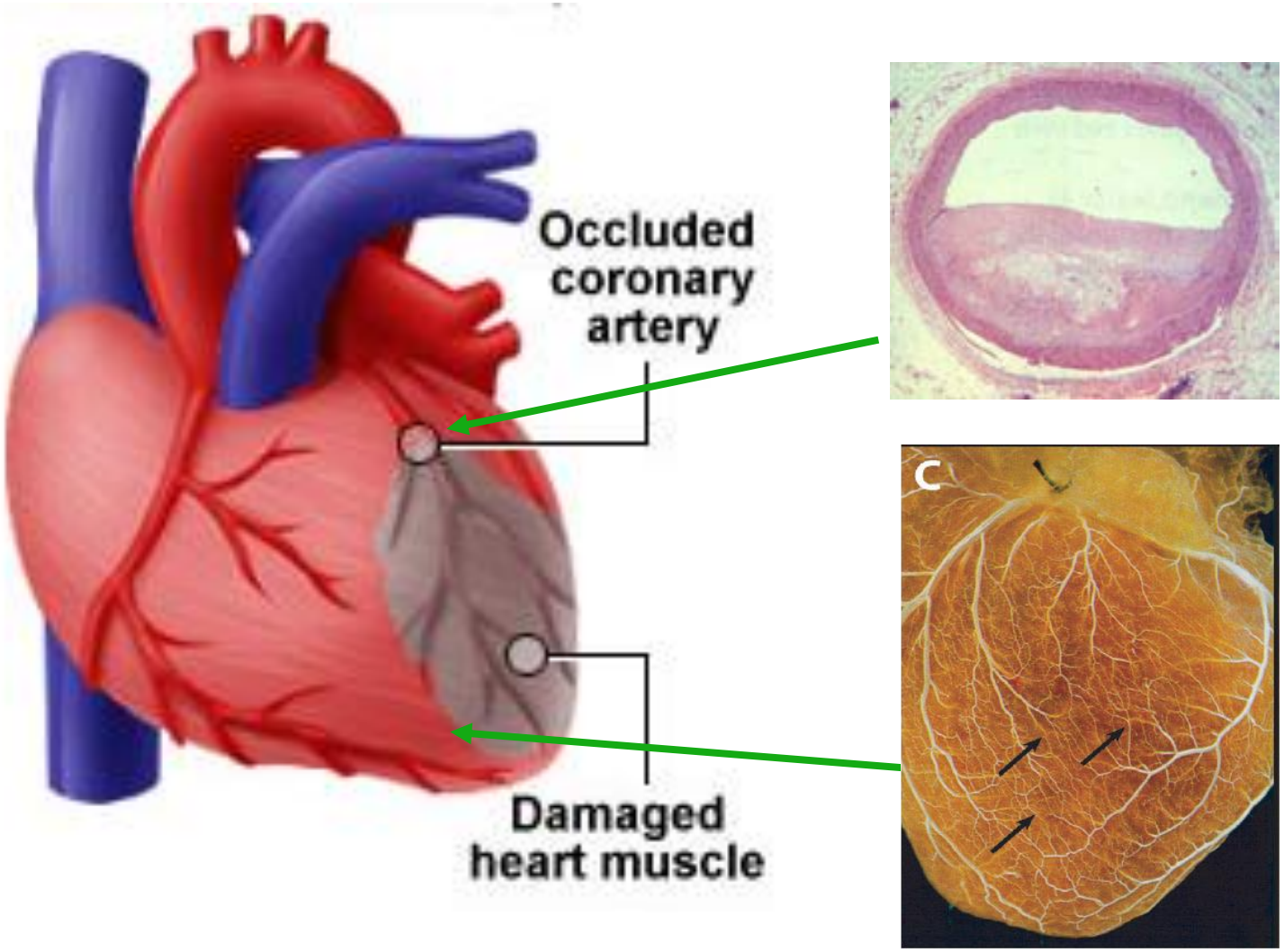


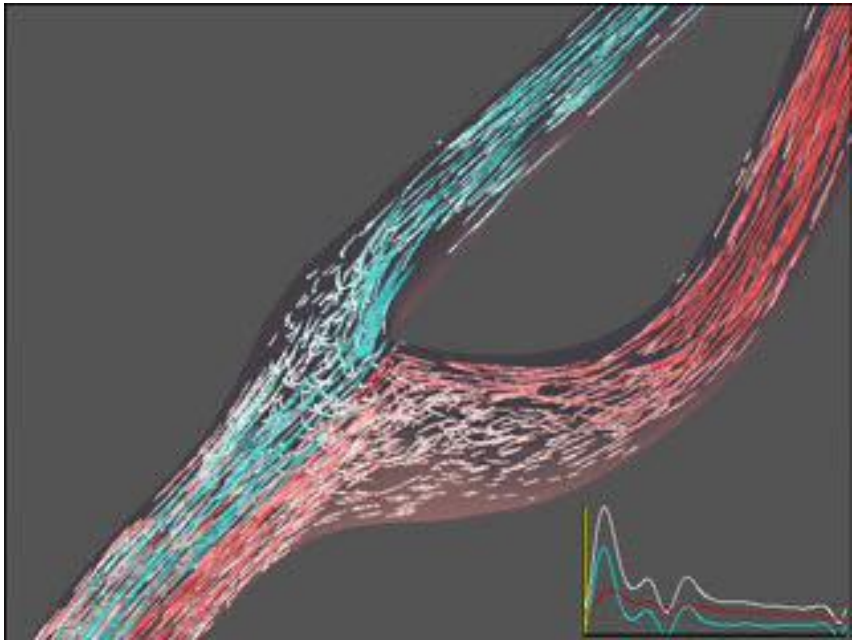

**Figure 5. Multiple sequential steps mediating leukocyte recruitment during inflammation.** Leukocytes are captured and begin to roll on P- and E-selectins and their ligands P-selectin glycoprotein ligand-1 (PSGL-1) and E-selectin ligand-1 (ESL-1). Some leukocytes such as lymphocytes or hematopoietic stem and progenitor cells also roll on α4 integrin and its endothelial receptor vascular cell adhesion molecule-1 (VCAM-1). L-selectin is critical for lymphocyte rolling on HEVs in lymphoid tissues. As inflammation progresses, leukocyte rolling velocity decreases, allowing the integration of activation signals from selectin ligands and G-protein–coupled receptors (GPCRs). These activation signals lead to the polarization of slowly rolling leukocytes and clustering of L-selectin and PSGL-1 to a major pole that allows further leukocyte recruitment through secondary tethers via leukocyte-leukocyte interactions. Leukocyte activation enhances integrin affinity and avidity, leading to firm adhesion on intercellular adhesion molecule-1 (ICAM-1) expressed on endothelial cells. Adherent leukocytes continuously migrate laterally to survey the microvasculature and search for possible sites for transmigration. Leukocytes can transmigrate classically through the junctional (paracellular) pathways via interactions among junctional adhesion molecules (JAMs), CD99 and platelet/endothelial-cell adhesion molecule-1 (PECAM-1), endothelial cell–selective adhesion molecule (ESAM), or alternatively through the endothelial cell (transcellular pathway). Illustration by Marie Dauenheimer.

# Shear stress guides vascular remodeling
# Beneficial: outward remodeling, arteriogenesis
# Pathological: atherosclerosis, vascular stenosis

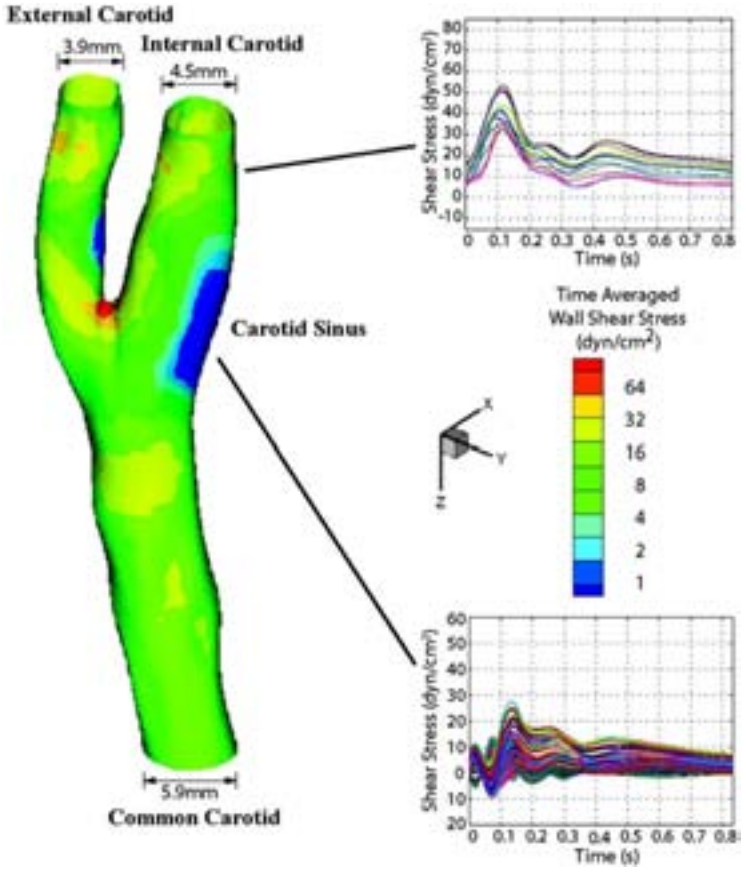


# Focality of lesions correlates with flow patterns: Turbulence result in low shear stress and NFkB activation

**What is protecting the majority of the vasculature???**

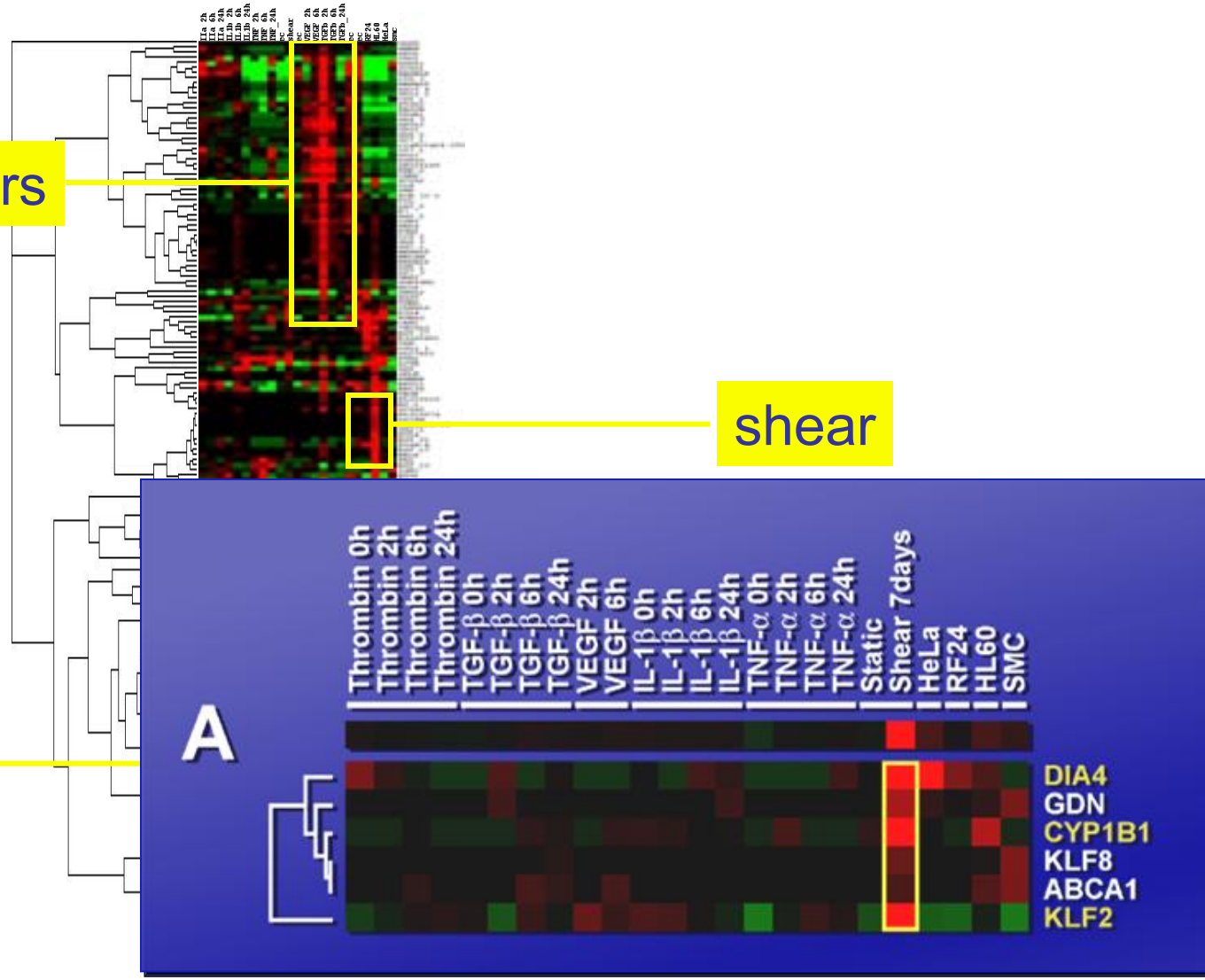


Flow imaging and computing: large artery hemodynamics. Steinman DA Ann Biomed Eng. 2005;33:1704-9.




Dai, G PNAS 2004;101, 14871-14876

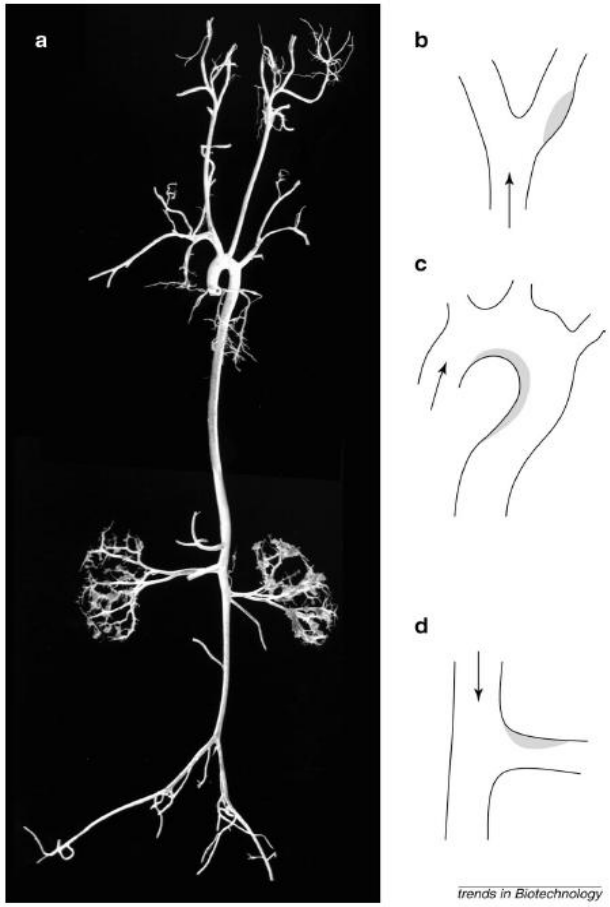
# SOM & hierarchical clustering of genes: digitized cellular phenotypes




**TGF-β**
**VEGF**
**b-FGF**
**Shear stress**
**NO**
**Thrombin**
**TNF-α**
**IL-1α**

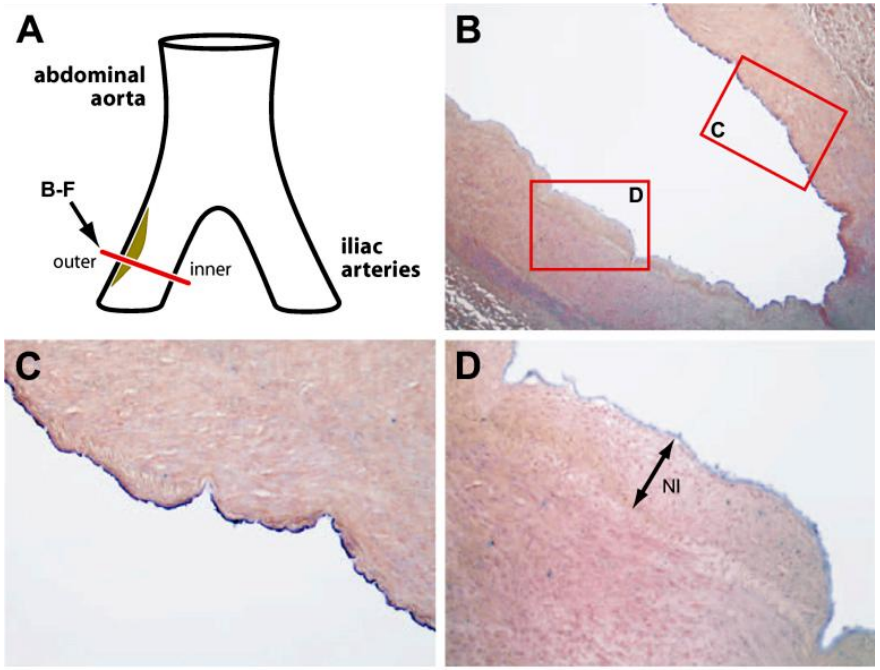
Dekker et al, Blood 2002

# KLF2 localizes to atheroprotected regions




Davies *et al.* (1999) Trends Biotech. **17(9)**, p347

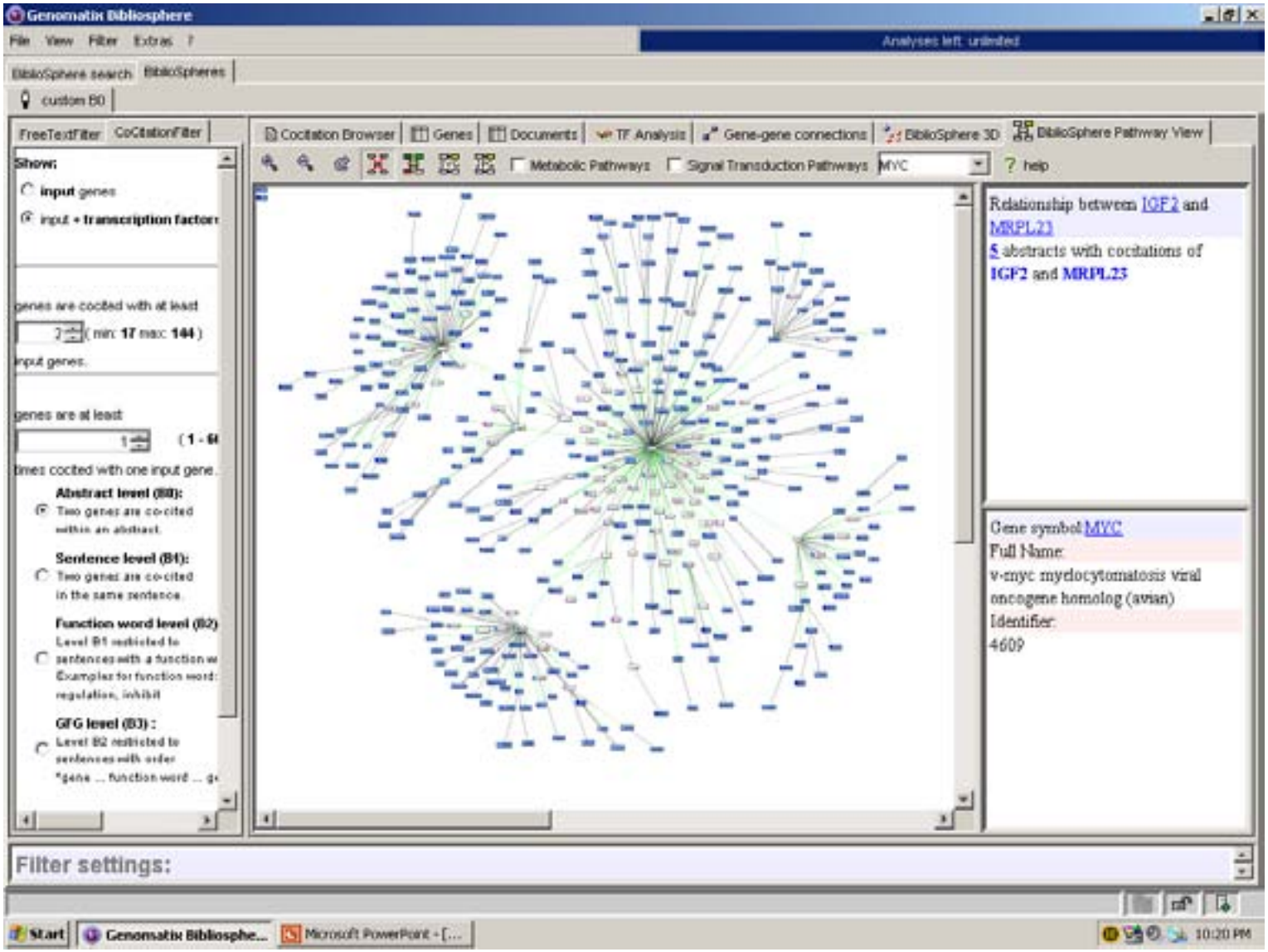
*In situ* hybridisation: KLF2




Dekker *et al.* (2005) Am J Pathol. **167(2)**, p609

# The KLF2-shear stress genetic network

A single transcription factor controls ~ 1000 genes




**Dekker, Boon et al. Blood 2006**

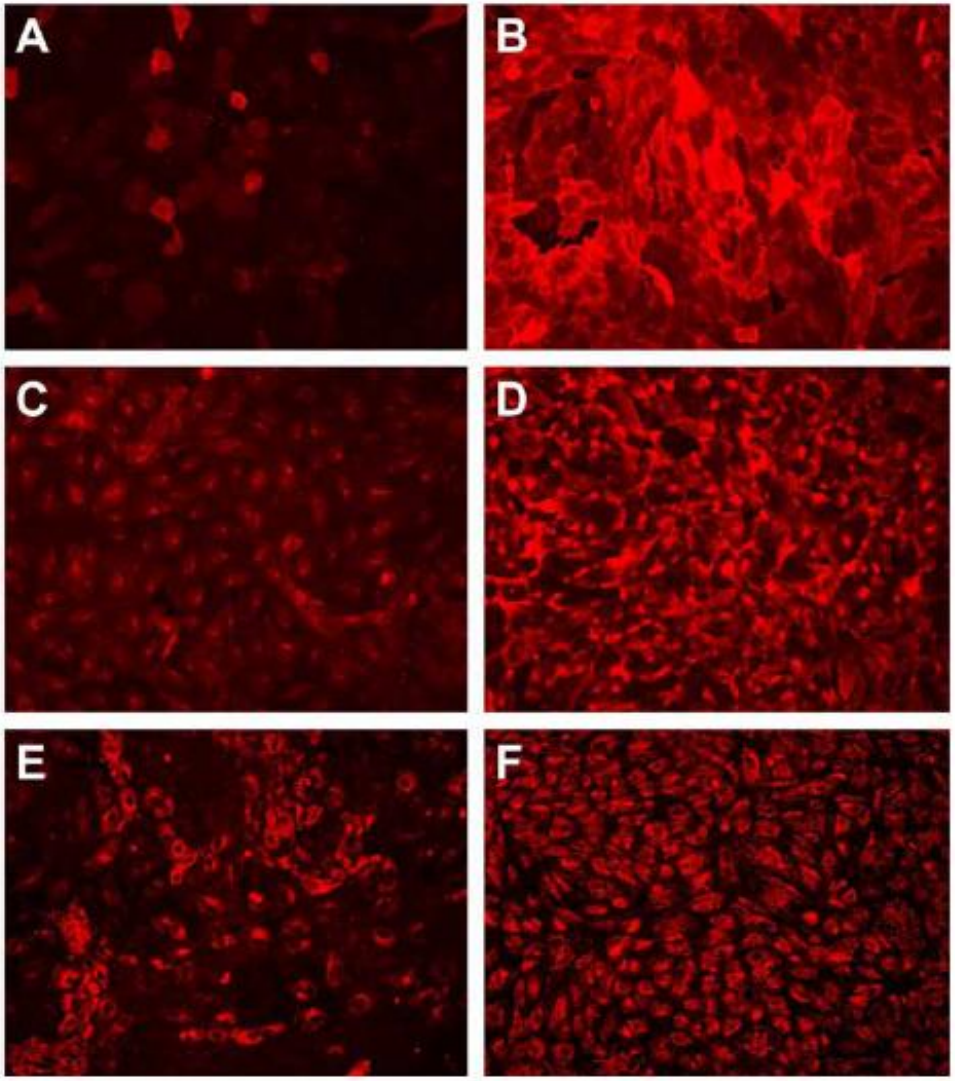
# Augmented KLF2 results in endothelial differentiation
## 1000 genes affected: analysis by PubMed textmining

| Functional category | Genes |
|---|---|
| Haemostasis | THBD↑, VWF↑, SERPINE1↓, RALA↓, PROS1↑, PTGDS↑, TFPI2↓, NOS3↑ |
| Inflammation | CCL2↓, THBD↑, IL8↓ |
| Vascular tone regulation | NOS3↑, ADM↓, ACE↓, EDN↓, PTGDS↑ |
| Cell surface composition (glycocalyx) | HYAL2↑, HPSE↑ |
| Vascular wall matrix composition | ELN↑, FBLN2↑, CTSL↑, TIMP3↑, SERPINE1↓, THBS1↓, COL4A1↓, SEMA3F↑, TNA↓ |

Dekker, Boon et al. Blood 2006

# Augmented KLF2 results in endothelial differentiation

expression of 3 well-known endothelial marker genes



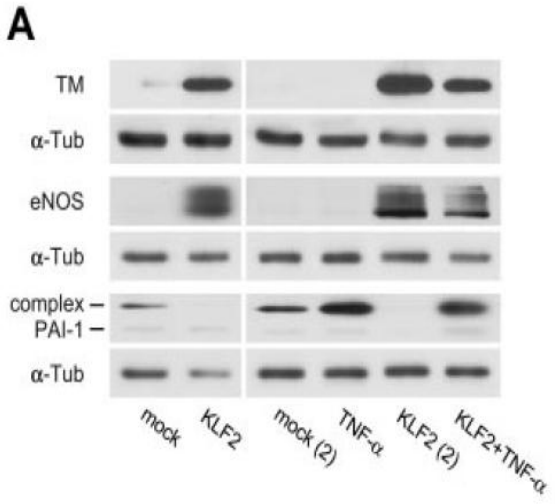

**Thrombomodulin**

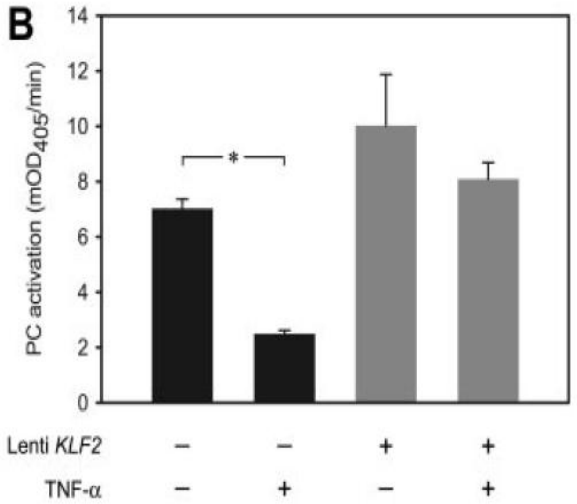
**eNOS**

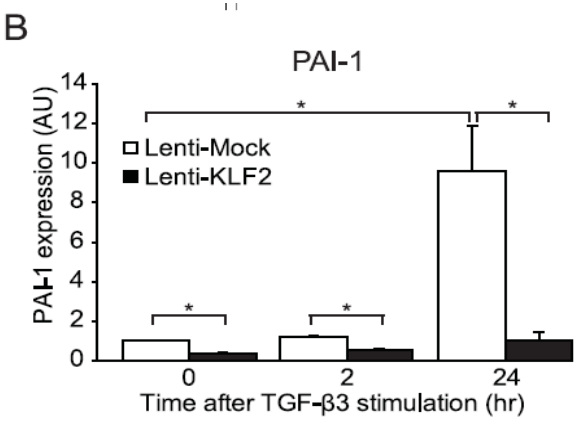
**Von Willebrand Factor**

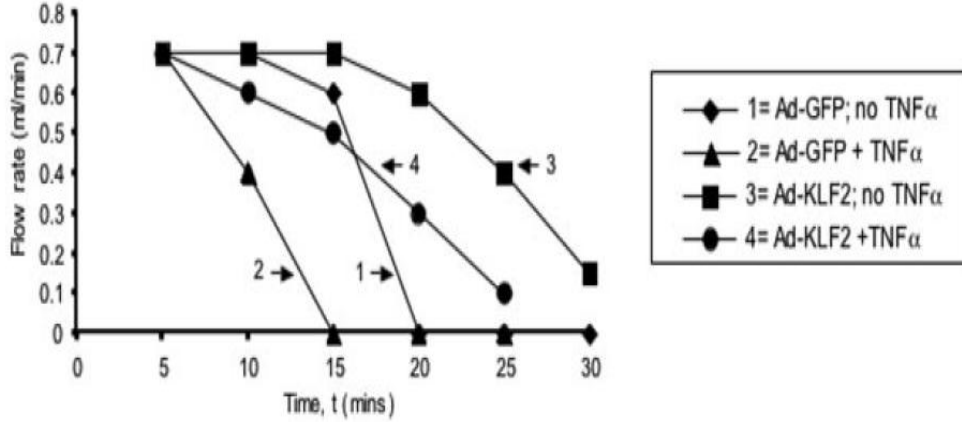
Dekker, Boon et al Blood 2006

# KLF2 inhibits blood clotting by boosting thrombomodullin and repressing PAI-1



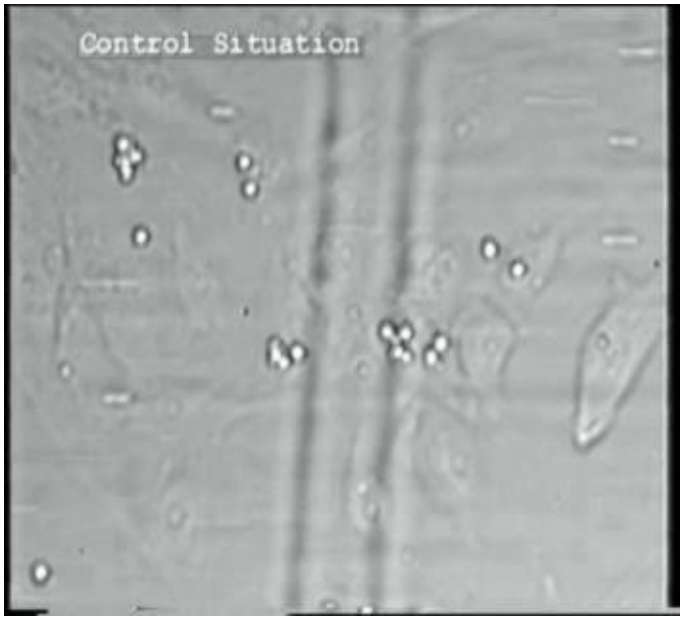



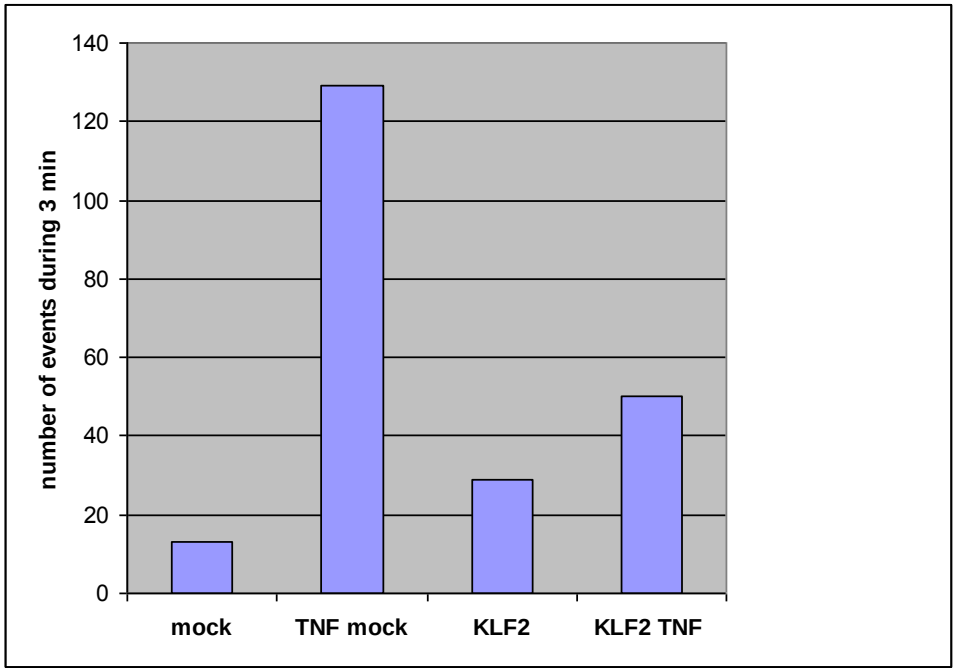




Dekker,Boon Blood 2006
Boon ATVB 2007

Lin Z, et al Circ Res 2005;96:e48

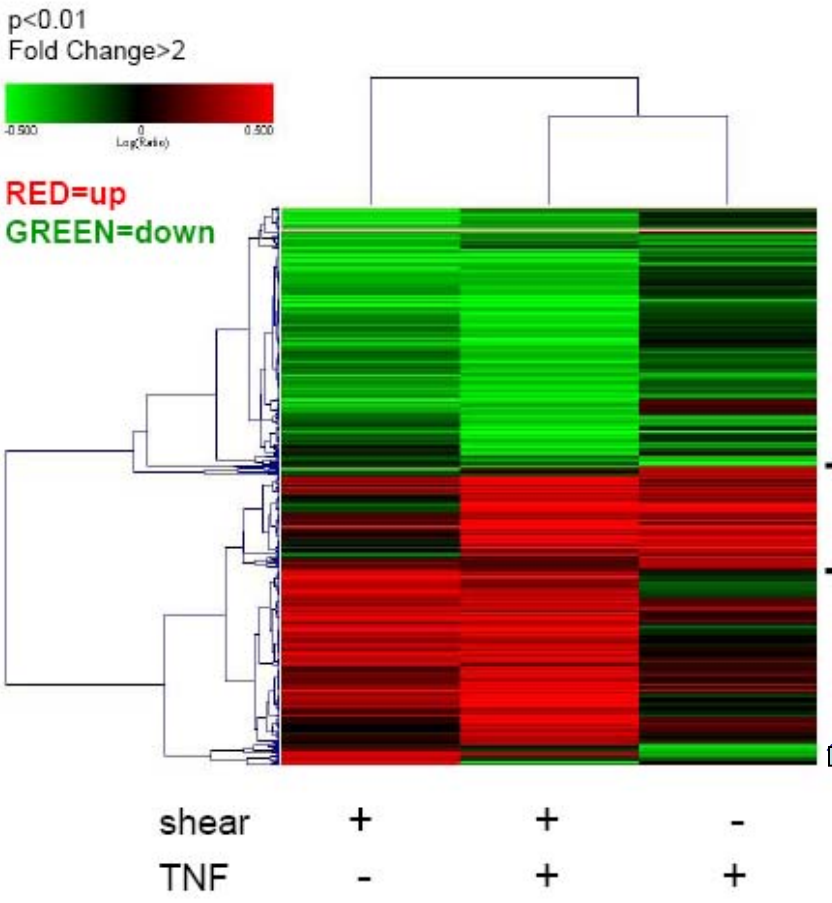
# KLF2 suppresses monocyte adhesion by TNFa



# Functional transcriptomics-promoter analysis:

the KLF2 transcriptional network consists of 4 clusters, 3 transcription factors



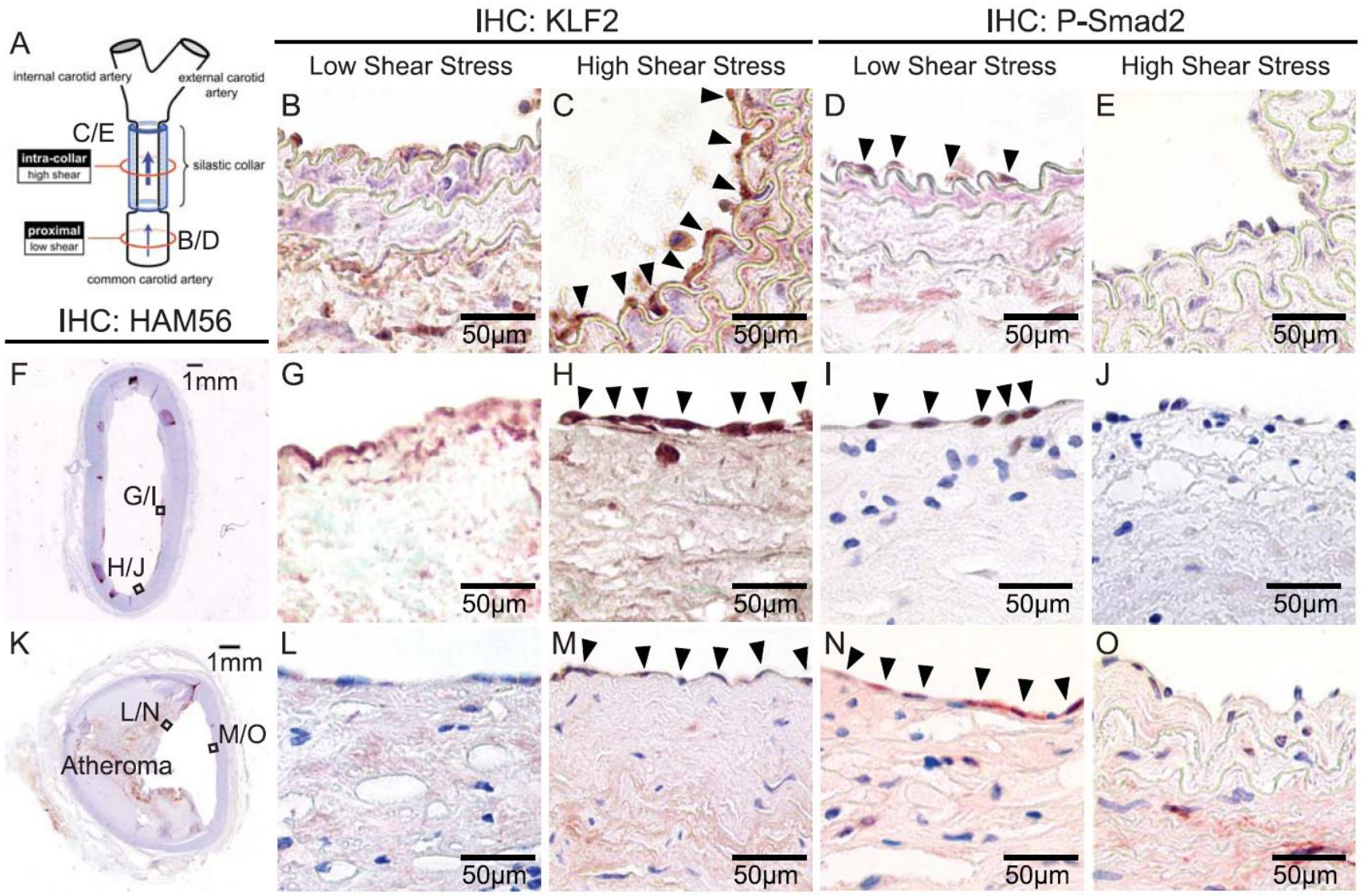

| Group | Description | Over-represented TFBS in 500bp upstream region |
|---|---|---|
| A | Shear inhibited | ATF / SMAD / SOX |
| B | TNF induced | NF-kB / AP-1 / ATF / NRF2 / ETS / SOX |
| C | Shear induced | AP-1 / NRF2 / XR / PR |

**KLF2, eNOS, TM (direct targets)**

Fledderus. Blood 2007

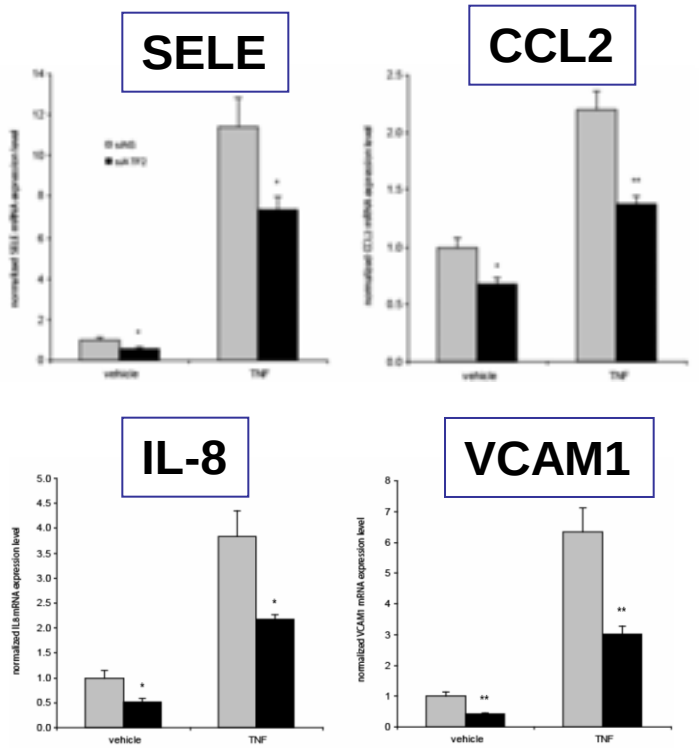
# KLF2 and TGFbeta signaling are mutually exclusive in vivo

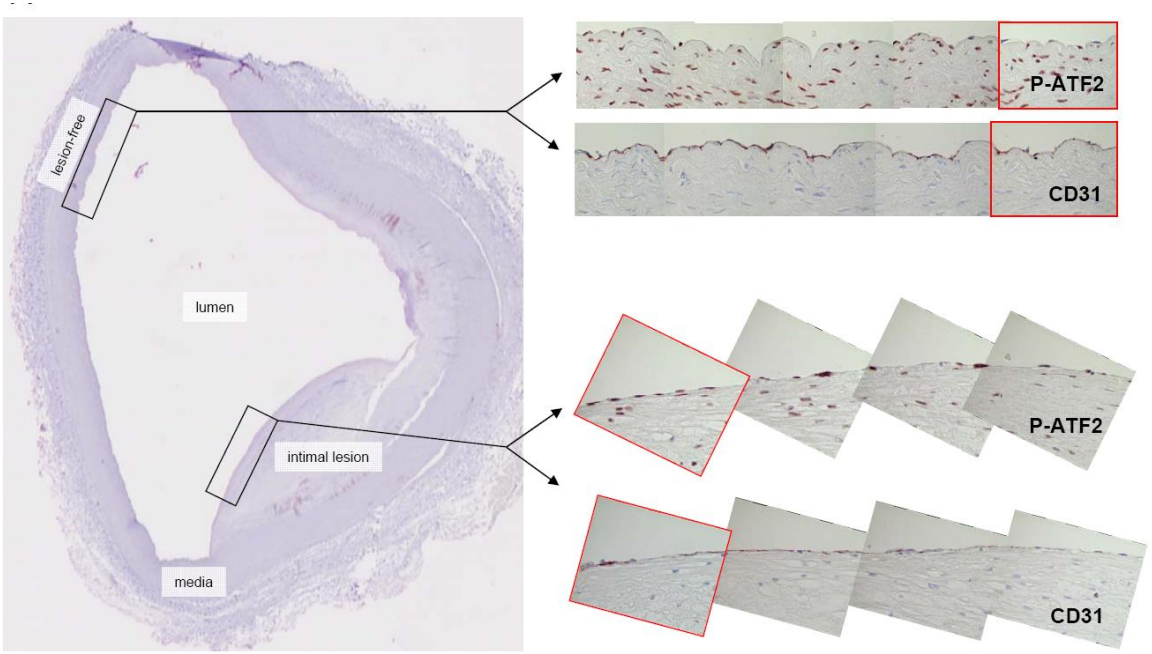
## detection by phosphospecific antibody: activated proteome




Boon et al., ATVB 2007

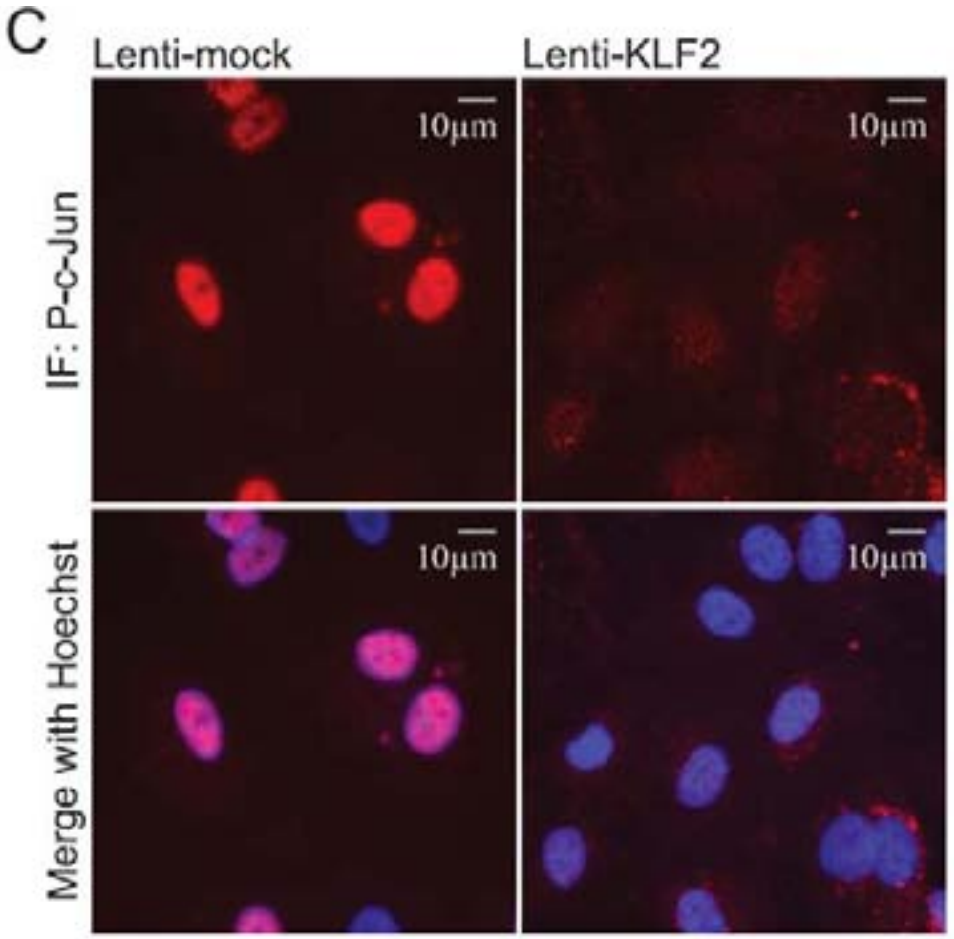
# si-ATF2 suppresses basal and inducible levels inflammatory genes
# Active phospho-ATF2 only in human atherosclerotic lesion EC



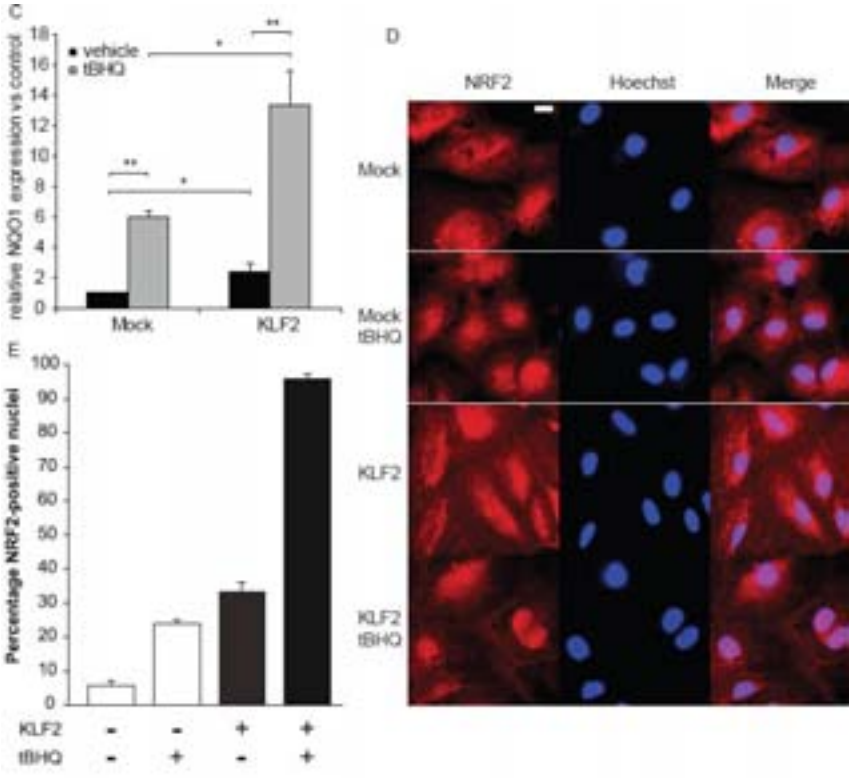

Fledderus, Blood 2007

# KLF2 leads to nuclear exclusion of proinflammatory ATF2 and c-Jun



# KLF2 augments nuclear localization of anti-oxidant NRF2

## This results in enhanced expression of multiple anti-oxidant enzymes



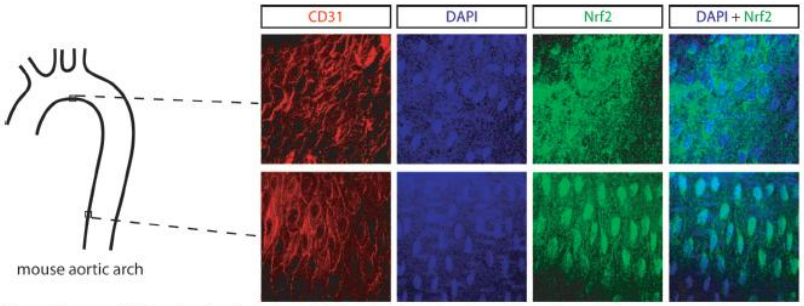



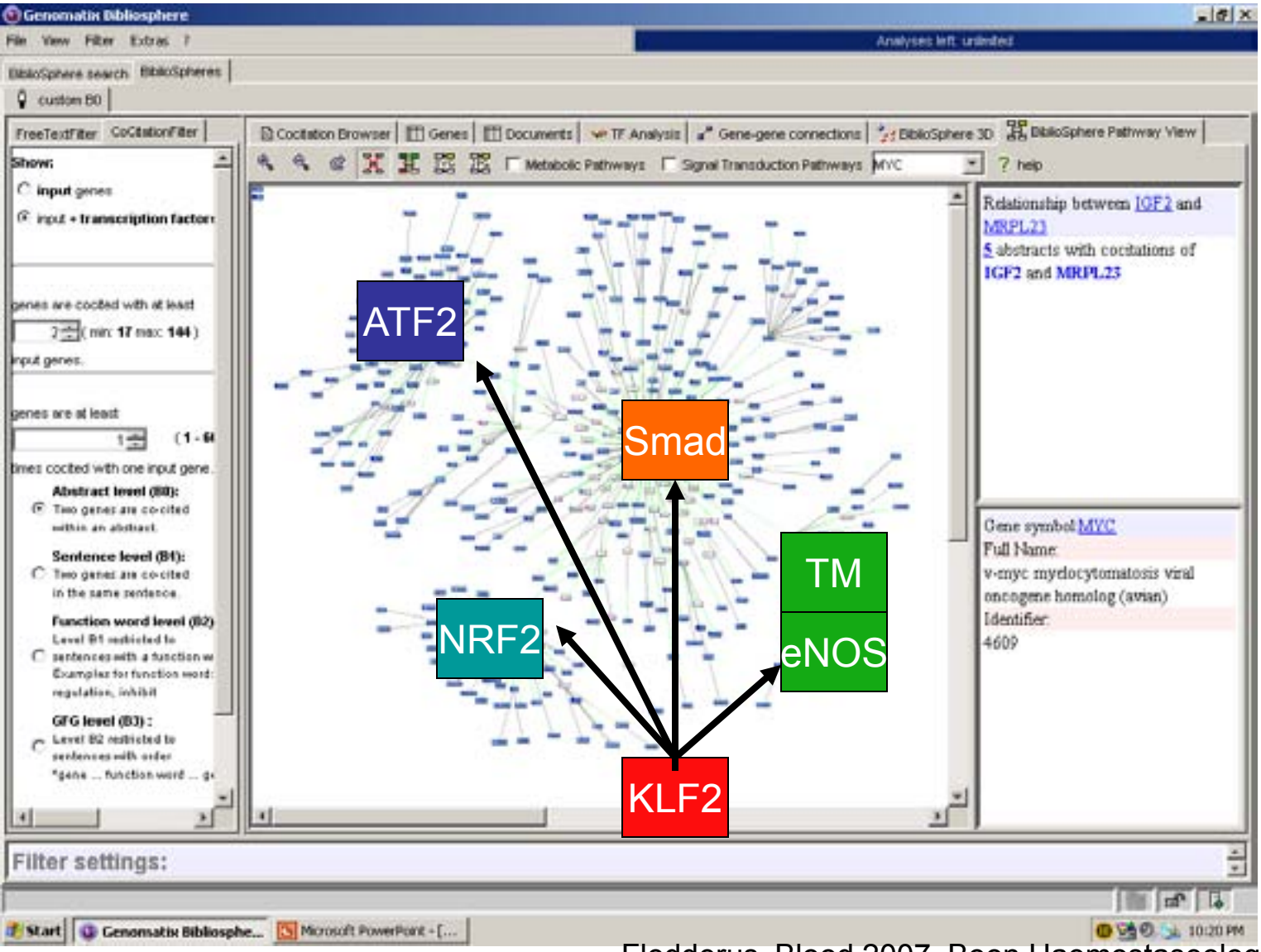

**Figure 6.** Increased Nrf2 nuclear localization in endothelium in atherosclerosis-resistant regions of the mouse aorta. The aortic

Dai et al. Circ Res. 2007;101:723-733.

Fledderus, Boon et al, ATVB 2008;28:1339-46.

# The KLF2-shear stress genetic network

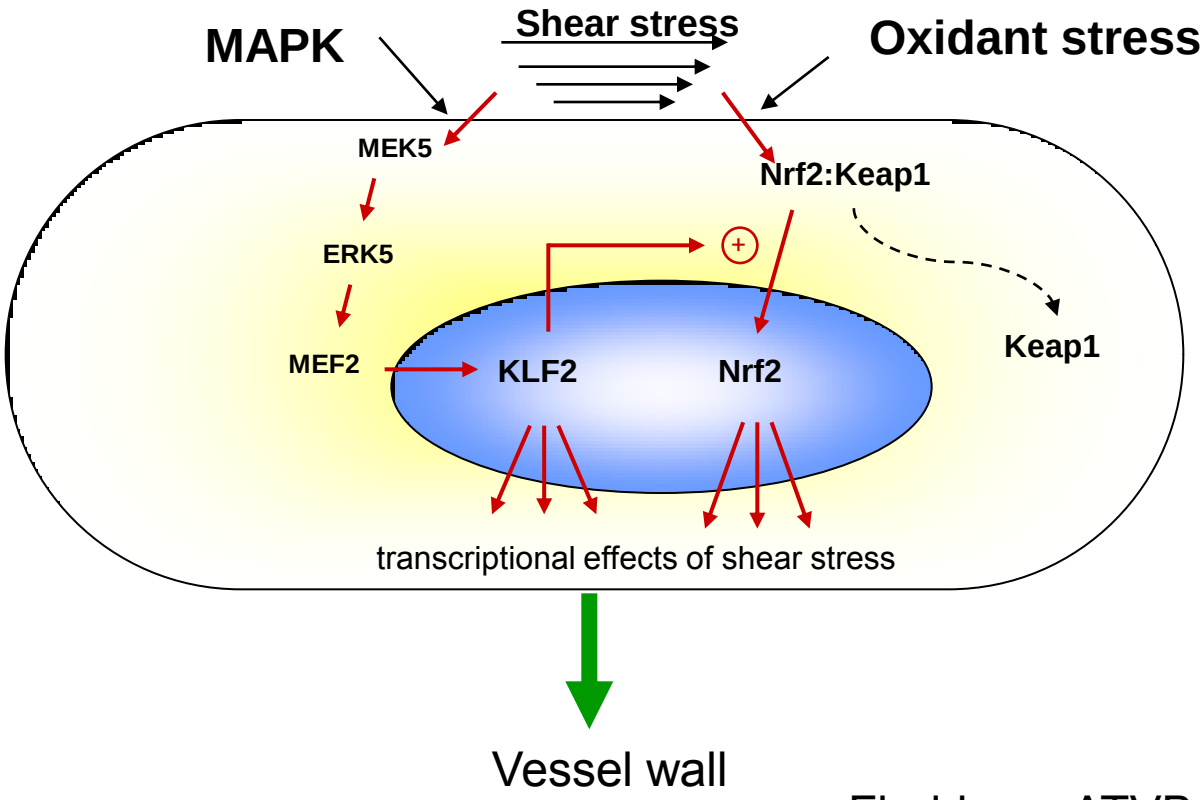
## How a single transcription factor controls ~ 1000 genes




Fledderus, Blood 2007, Boon Haemostaseologie 2010

# The KLF2-NRF2 axis in the shear stress genetic network

2 transcription factors control ~ 70% of shear controlled gene modules




Fledderus ATVB 2009
Boon, Haemostasiologie 2010

# KLF2-induced miRNAs communicate with the underlying vessel wall

nature
cell biology

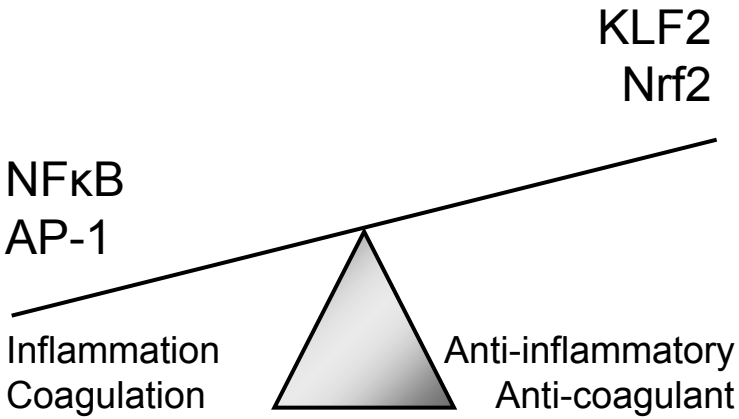
---

## Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs

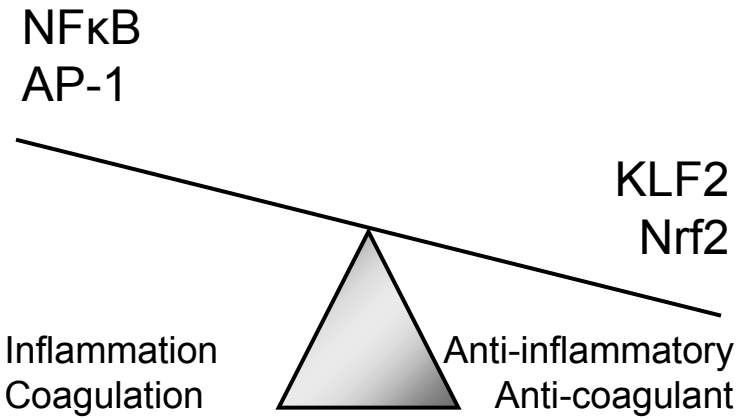
Eduard Hergenreider[1], Susanne Heydt[1], Karine Tréguer[1], Thomas Boettger[2], Anton J. G. Horrevoets[3], Andreas M. Zeiher[4], Margot P. Scheffer[5], Achilleas S. Frangakis[5], Xiaoke Yin[6], Manuel Mayr[6], Thomas Braun[2], Carmen Urbich[1], Reinier A. Boon[1,7] and Stefanie Dimmeler[1,7,8]

# How can we tip this balance?

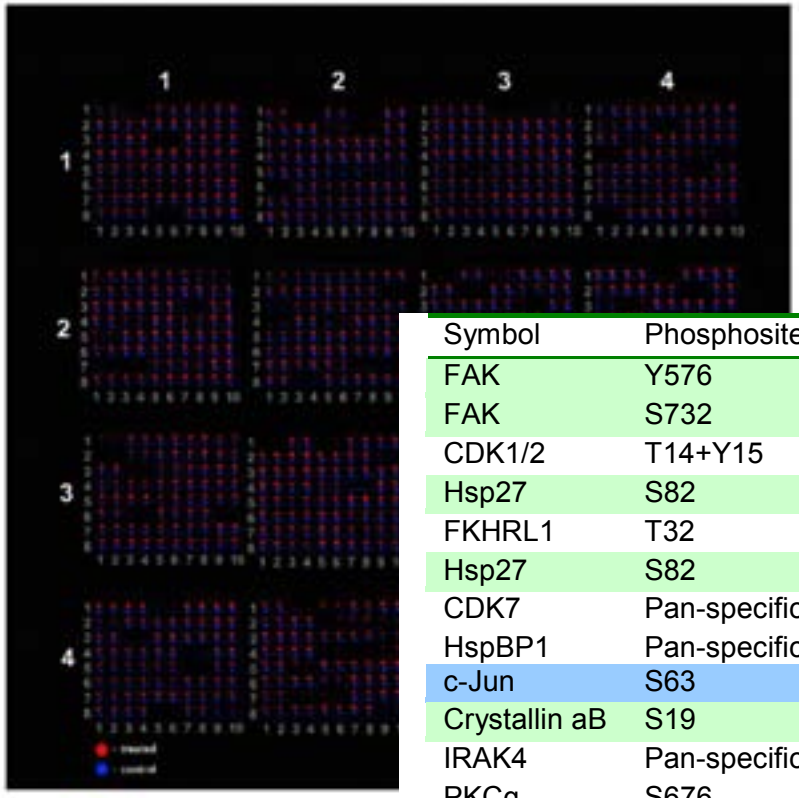
**Onset or Oscillatory Shear stress**




**Prolonged laminar Shear stress**



# Kinome analysis: Actin rather than MAPK

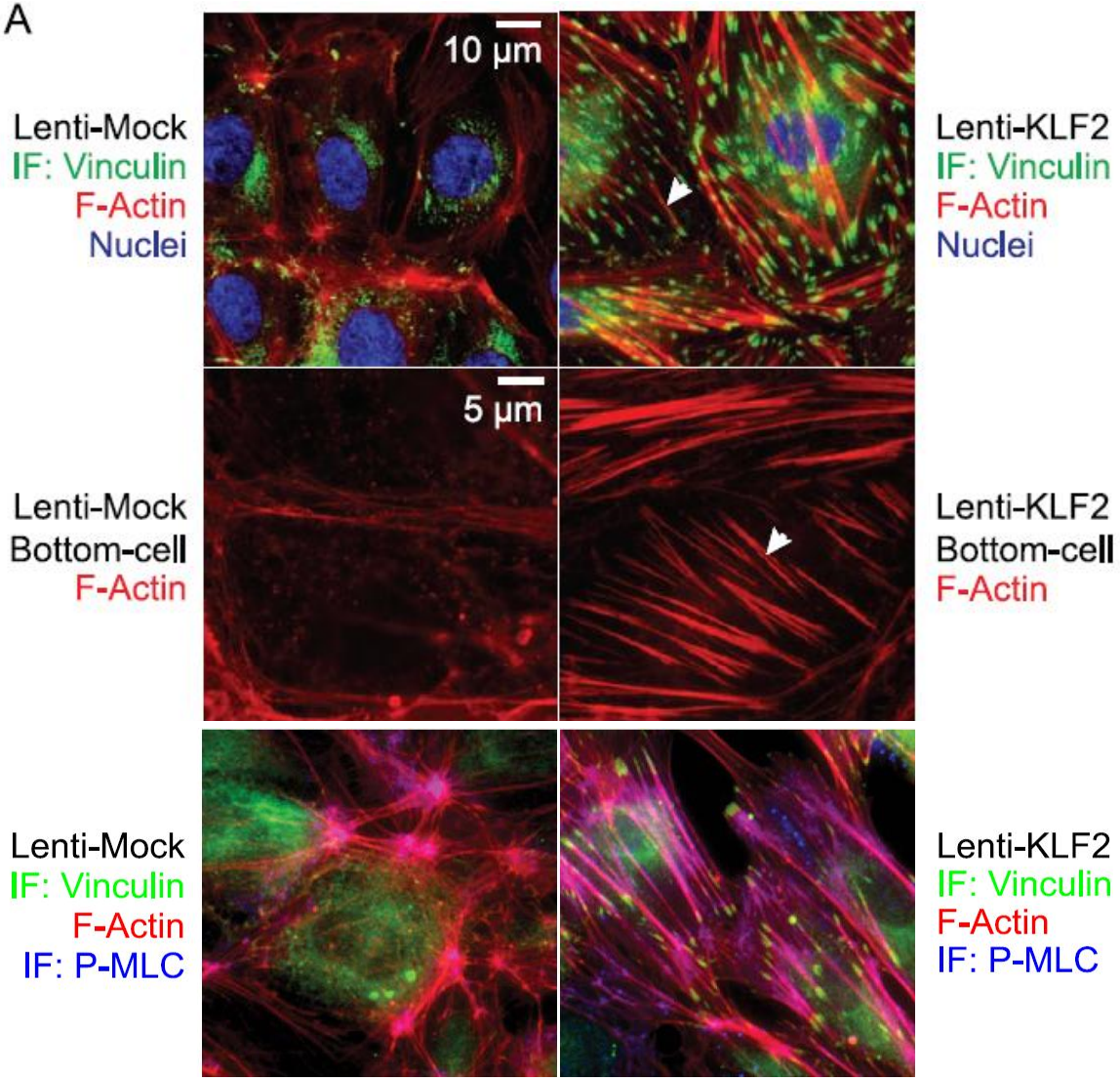



| Symbol | Phosphosite | Bayes.p | Fold |
|---|---|---|---|
| FAK | Y576 | 0.0005 | -3.02 |
| FAK | S732 | 0.0008 | -2.81 |
| CDK1/2 | T14+Y15 | 0.0091 | -1.67 |
| Hsp27 | S82 | 0.0150 | -1.93 |
| FKHRL1 | T32 | 0.0155 | -3.65 |
| Hsp27 | S82 | 0.0169 | -2.41 |
| CDK7 | Pan-specific | 0.0203 | -1.45 |
| HspBP1 | Pan-specific | 0.0217 | -1.49 |
| c-Jun | S63 | 0.0324 | -3.54 |
| Crystallin aB | S19 | 0.0351 | -1.57 |
| IRAK4 | Pan-specific | 0.0426 | -4.10 |
| PKCq | S676 | 0.0472 | -1.39 |
| Ksr1 | Pan-specific | 0.0489 | -1.68 |
| Rb | S780 | 0.0498 | -1.62 |
| PP6C | Pan-specific | 0.0586 | -1.34 |
| Tau | S518 | 0.0595 | -1.28 |
| EGFR | Y1148 | 0.0602 | -1.94 |
| ERK5 | T218+Y220 | 0.0618 | -1.44 |

| Symbol | Phosphosite | Bayes.p | Fold |
|---|---|---|---|
| PKCg | T514 | 0.0036 | 2.24 |
| Abl | Y412 | 0.0048 | 1.62 |
| RSK1/2 | S363/S369 | 0.0117 | 3.16 |
| Src | Pan-specific | 0.0179 | 1.61 |
| HO2 | Pan-specific | 0.0267 | 2.04 |
| ATF2 | T51+T53 | 0.0273 | 1.44 |
| p38a | Pan-specific | 0.0336 | 1.48 |
| PKCb2 | T641 | 0.0380 | 2.42 |
| STAT5A | Y694 | 0.0418 | 3.41 |
| S6Ka | T389 | 0.0462 | 2.53 |
| Bad | S75 | 0.0506 | 1.42 |
| EGFR | Pan-specific | 0.0570 | 1.54 |
| PKCg | Pan-specific | 0.0575 | 2.04 |
| FAK | Y397 | 0.0667 | 1.81 |
| Erk4 | Pan-specific | 0.0668 | 1.33 |
| Kit | Y730 | 0.0690 | 1.20 |

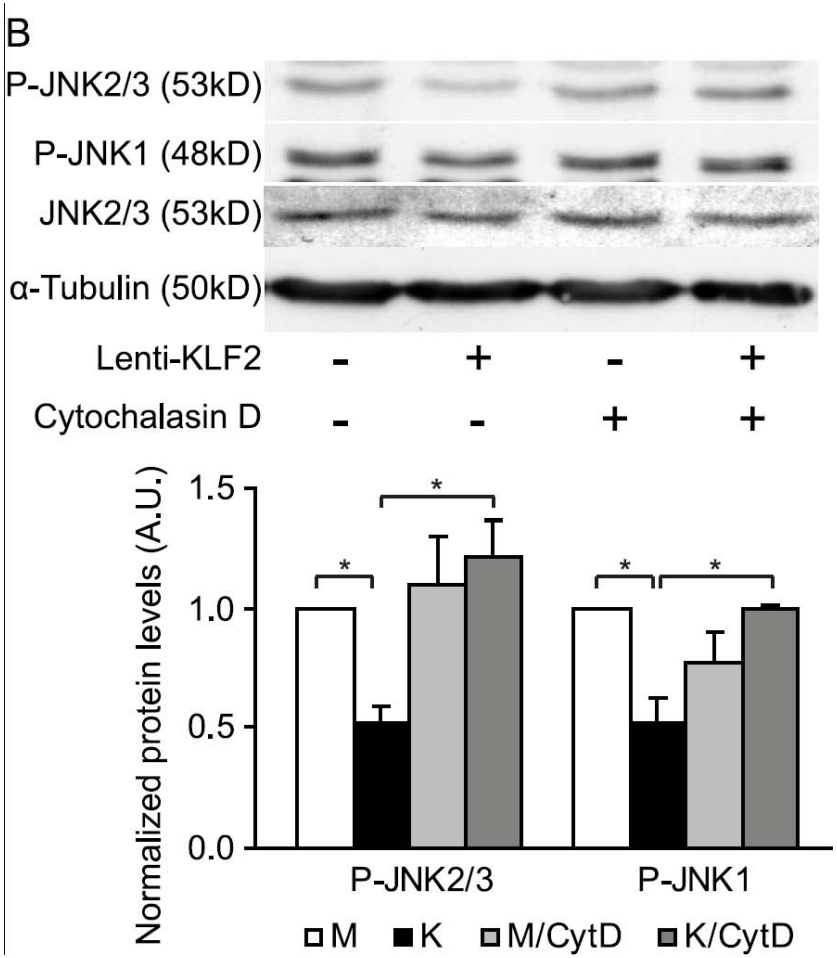
Boon, Blood 2010

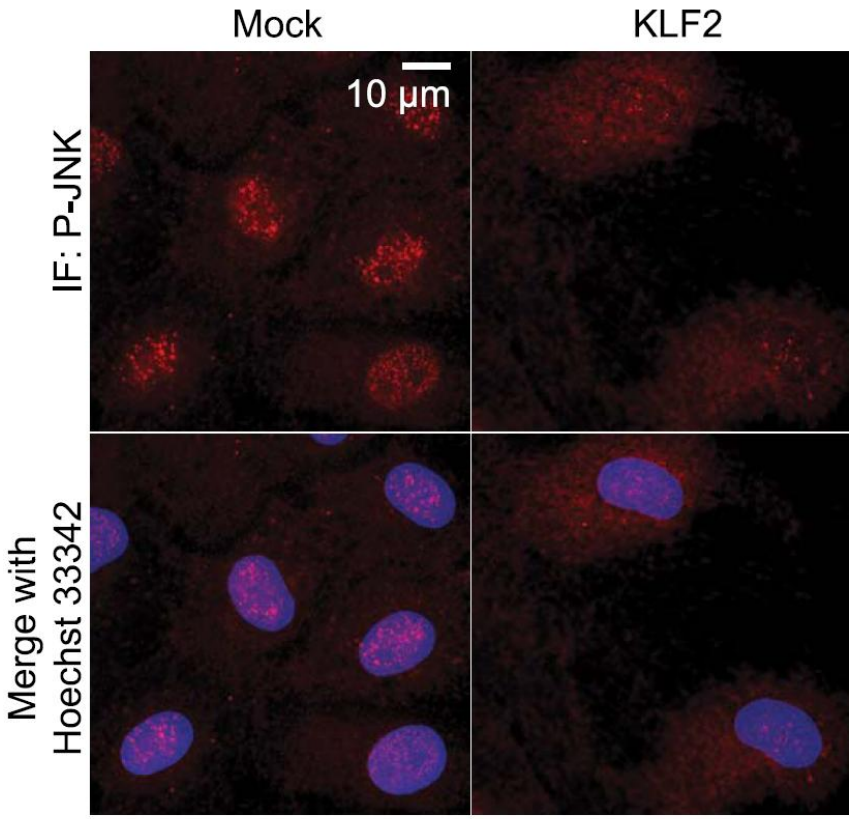
# KLF2 actin fibers localize on the basal side of endothelium and contain phosphoMyosin


Boon et al, Blood 2010

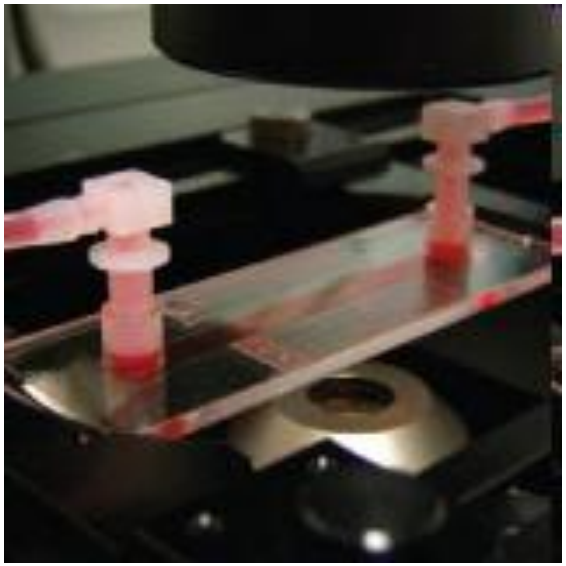
# Actin-dependent exclusion JNK,c-JUN and ATF2 from nucleus leads to anti-inflammatory effects

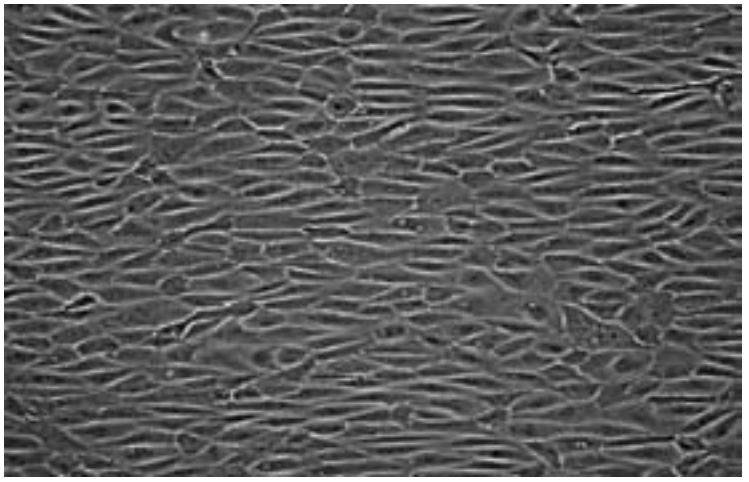


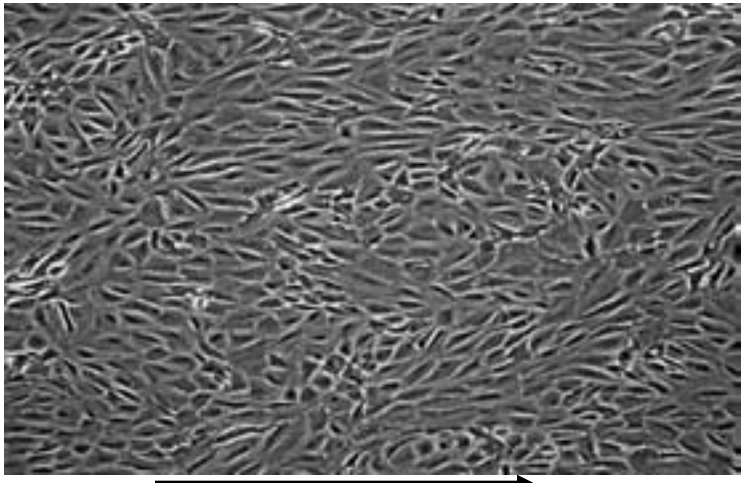

Boon et al Blood 2010

# KLF2 directs the alignment of EC with flow





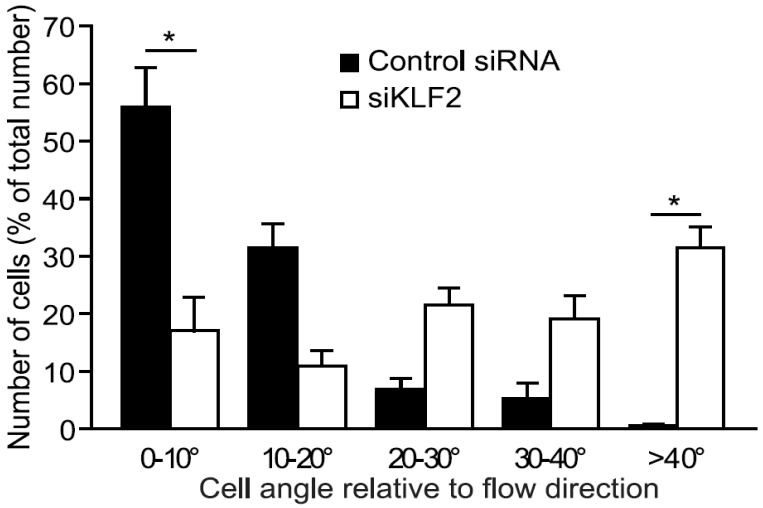
Control shRNA



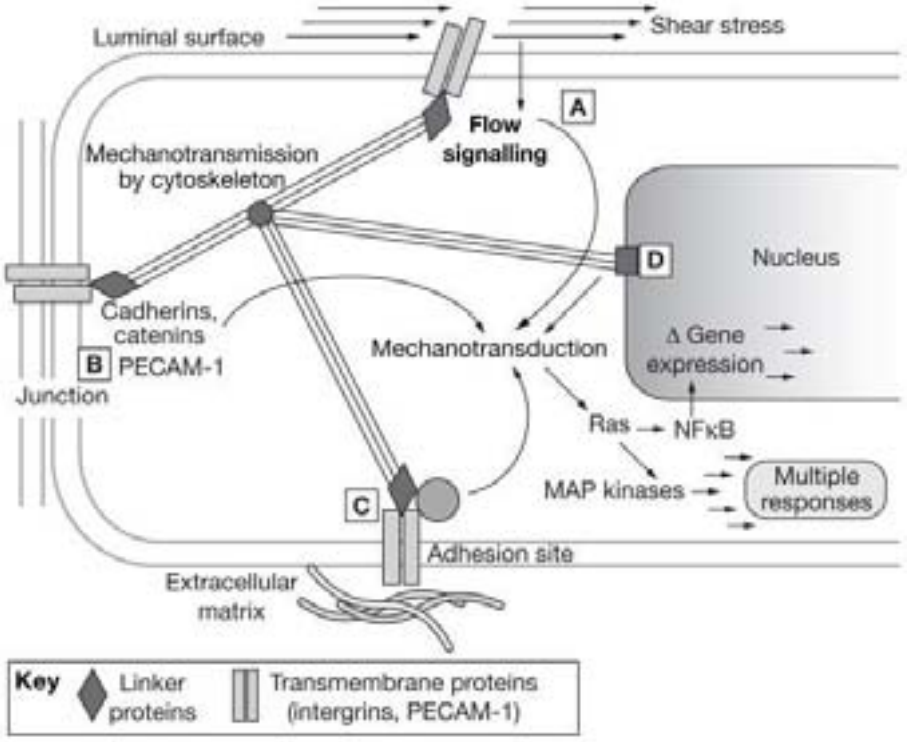
KLF2 shRNA

Flow

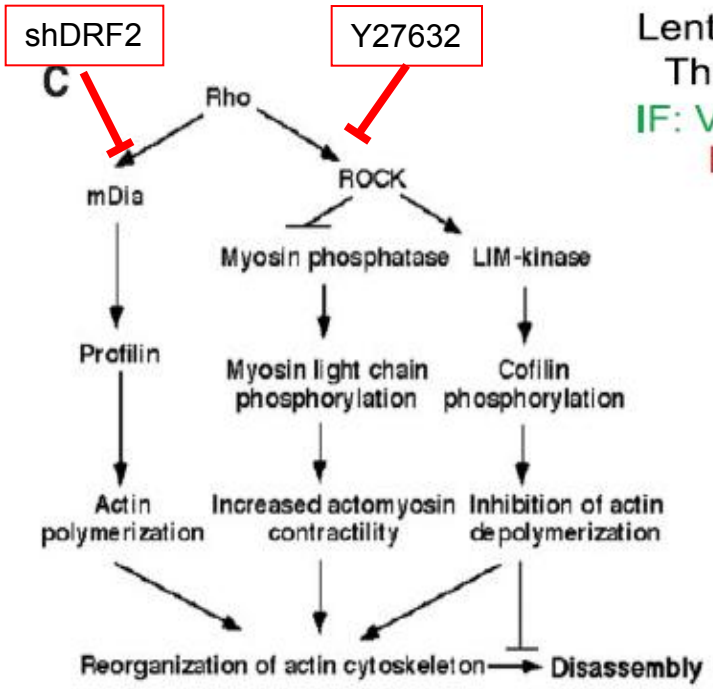
A

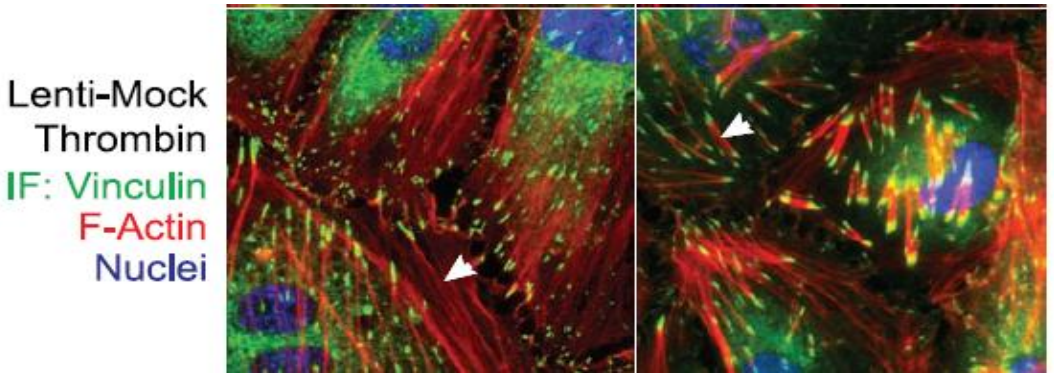


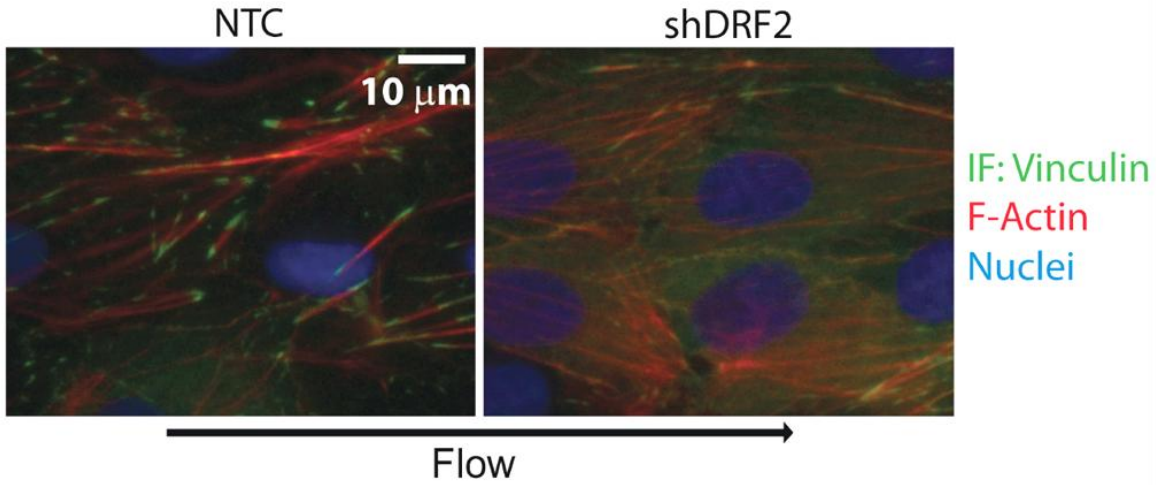
# Mechanosensor or inhibitor?




# KLF2 and shear induced actin fibers are ROCK independent, DRF2 dependent



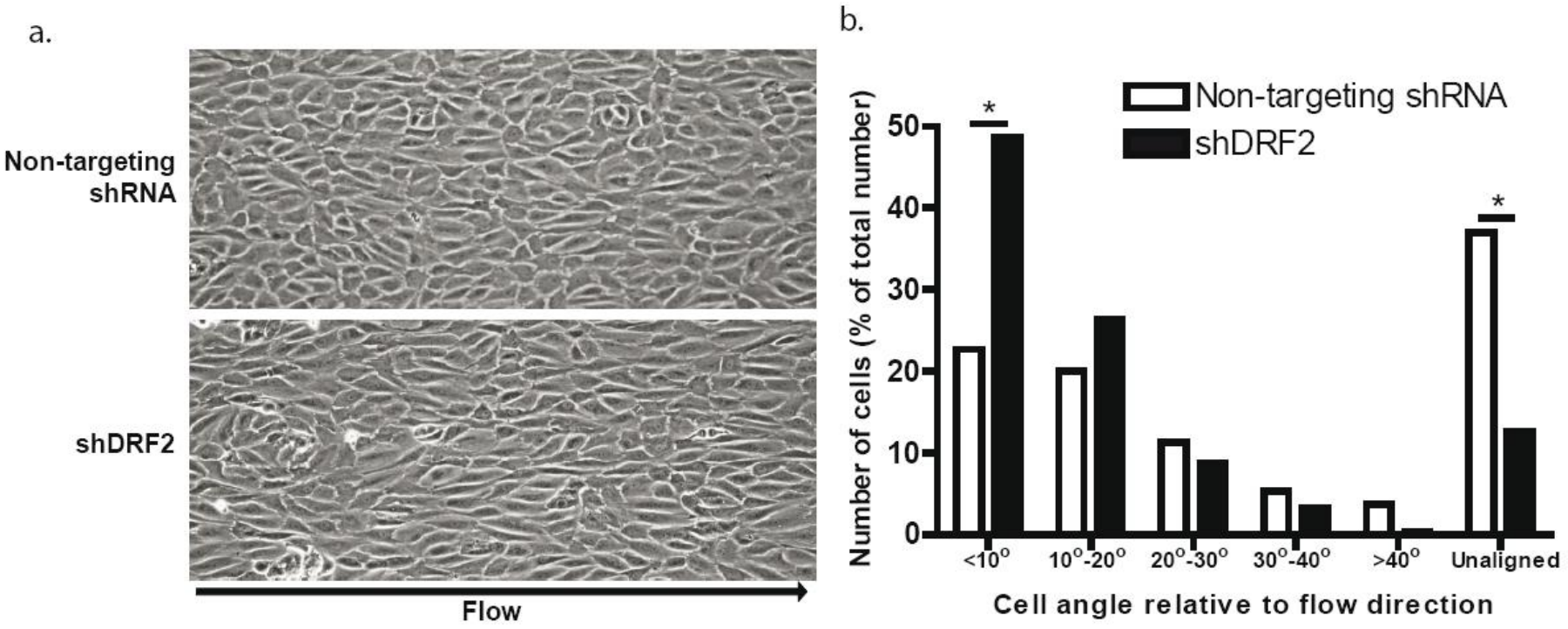



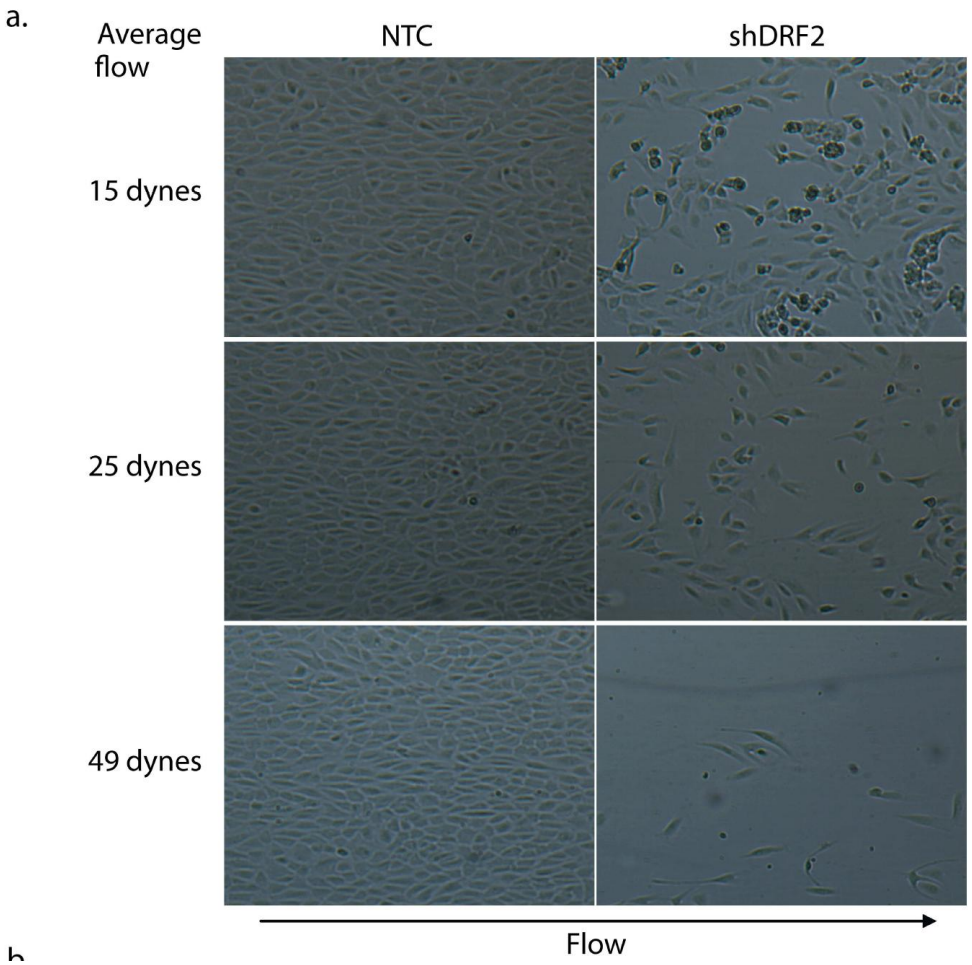

Diaphenous-Related Formin-2 (mDia)

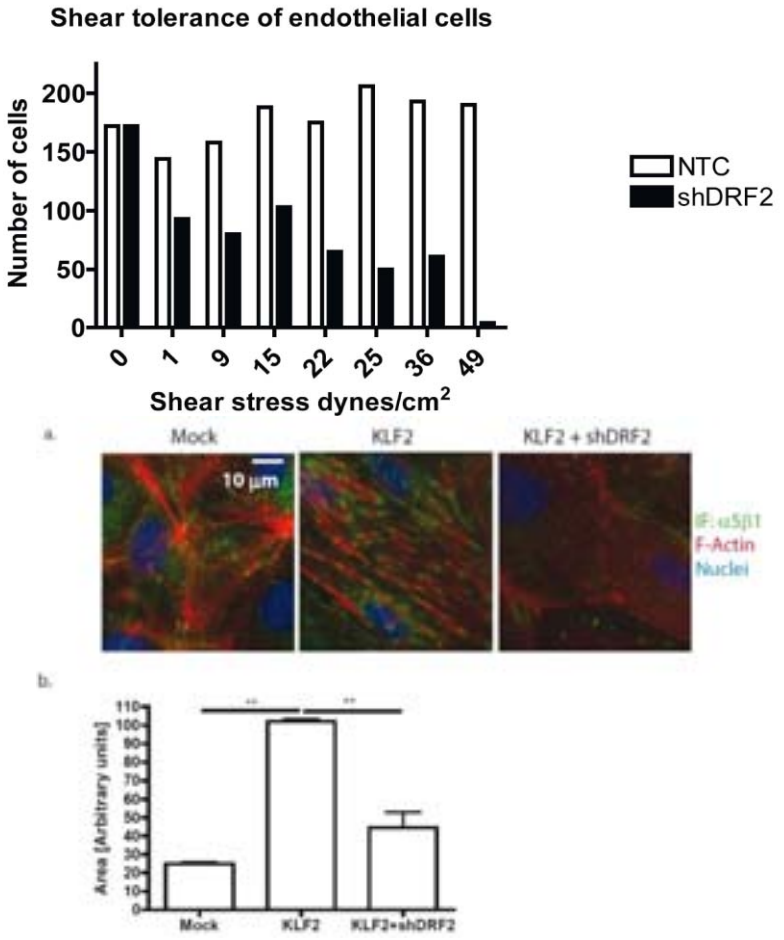
Thomas Leyen, 2012

# Better cell alignment without shear fibers



# DRF2-dependent shear fibers prevent flow-erosion of EC by inducing focal adhesion plaques

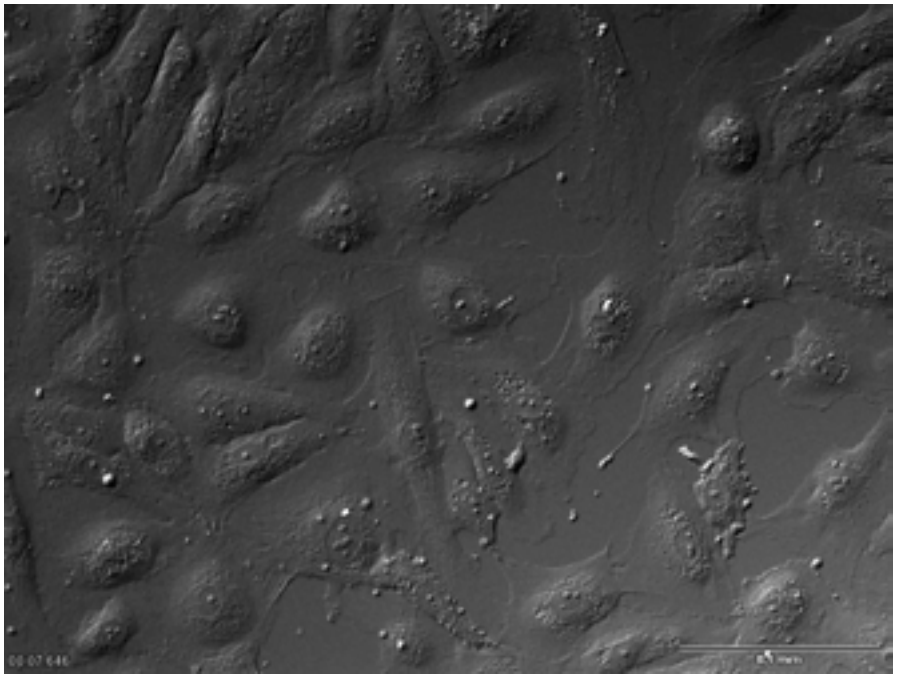


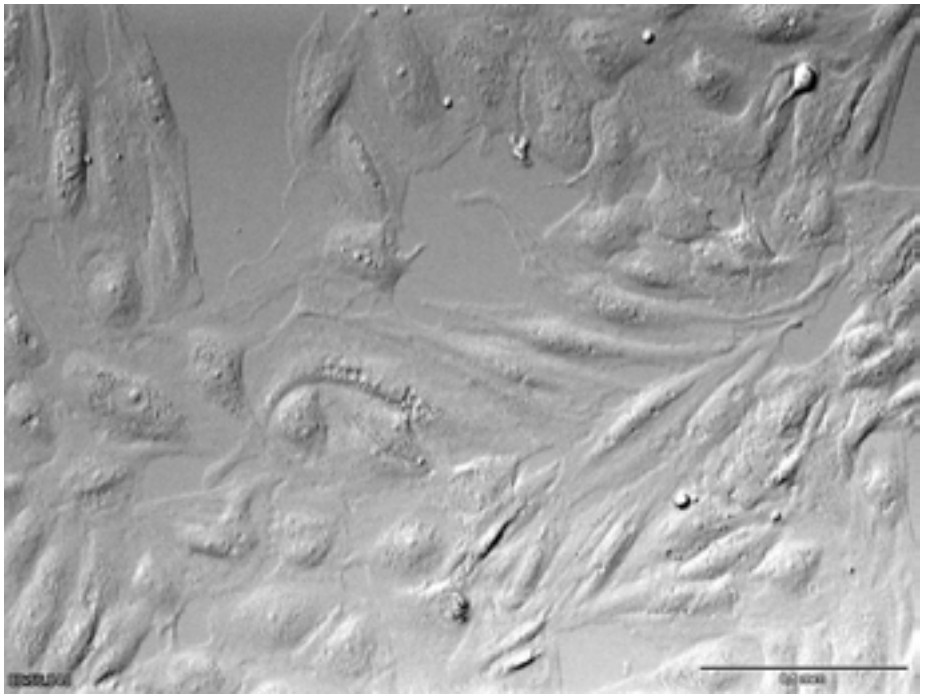

Thomas Leyen 2012

# Firm attachment to extracellular matrix: reduced motility by KLF2 actin rearrangements

**EC mock**

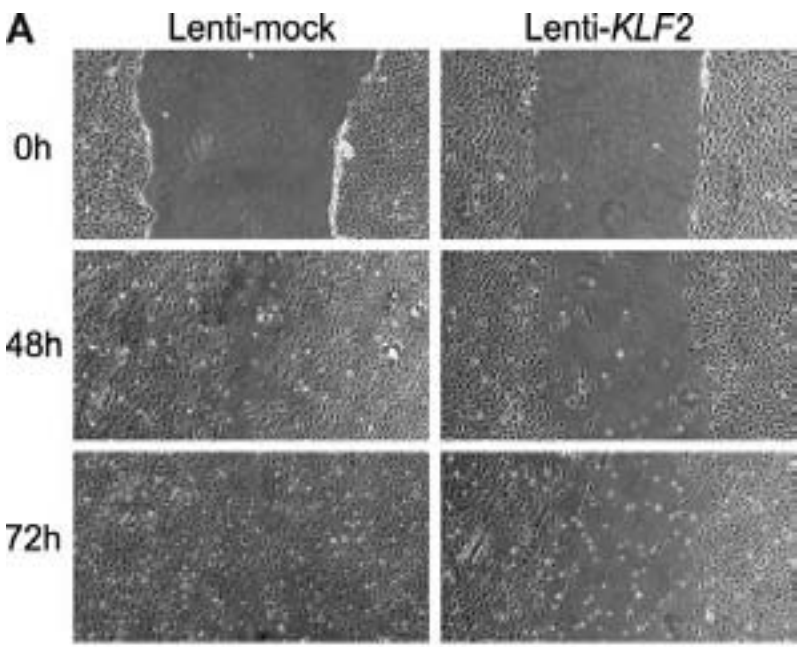


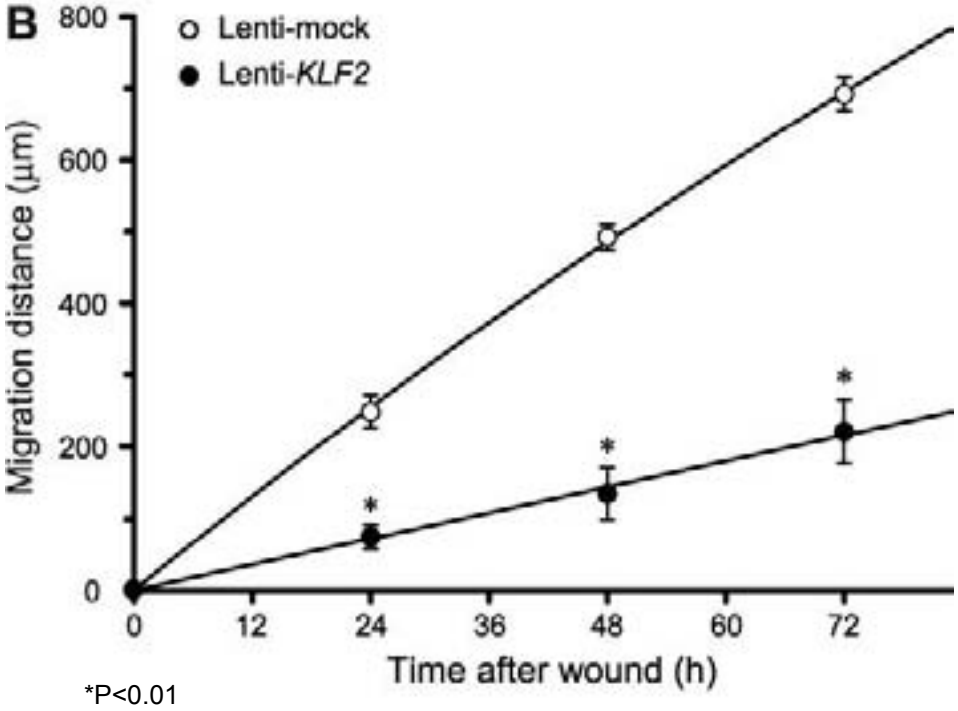
**EC KLF2**



Thomas Leyen, 2012

# KLF2 attenuates endothelial cell migration: anti angiogenic

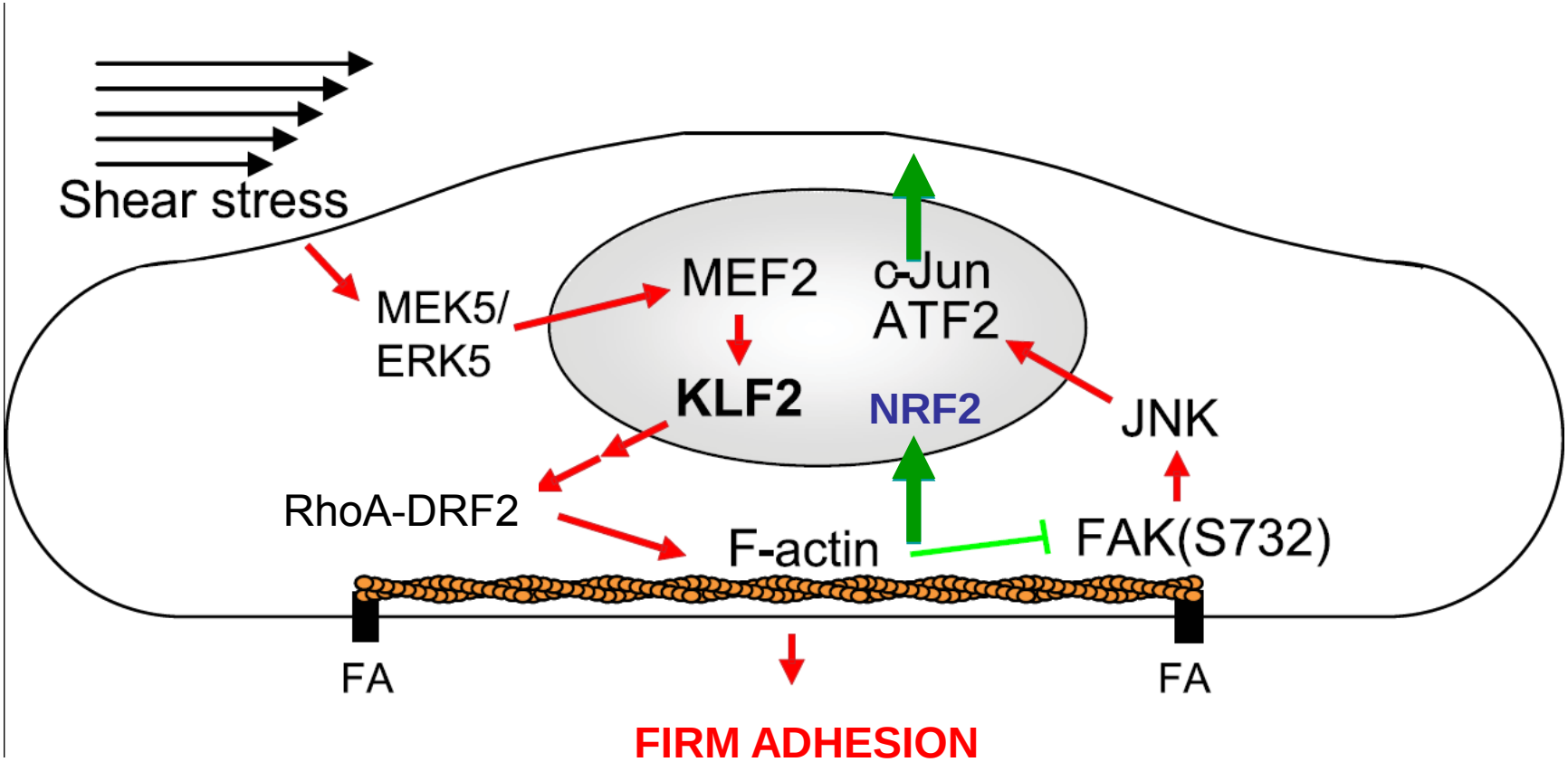



*P<0.01

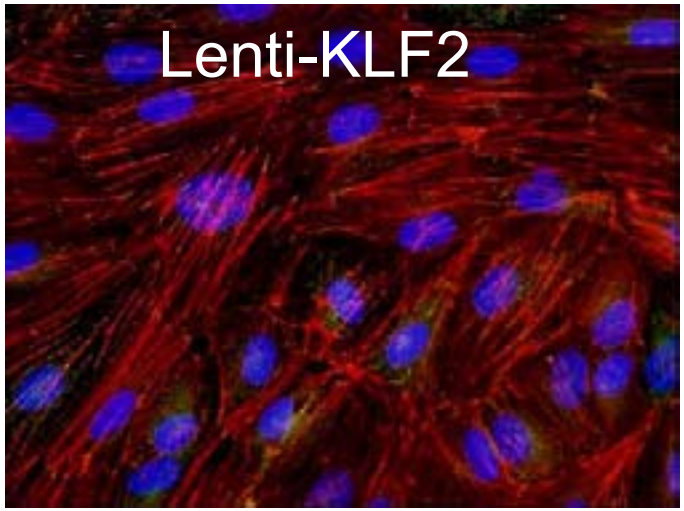
Dekker, Boon Blood 2006

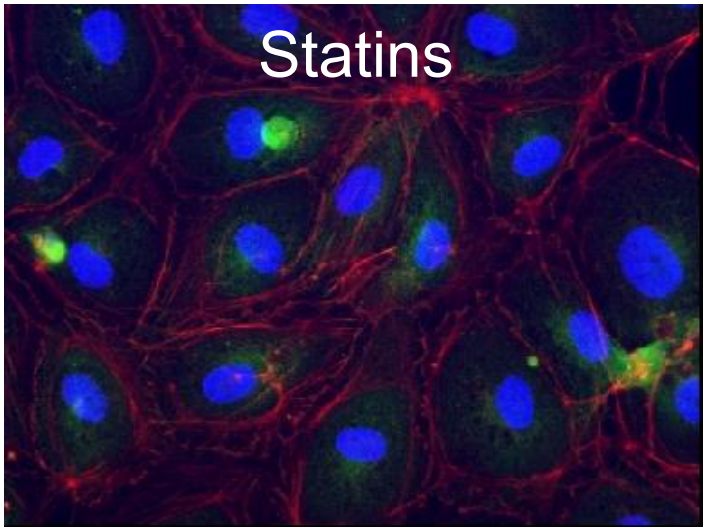
# Cell resistance to flow is directly coupled to anti-inflammatory effect through actin cytoskeleton

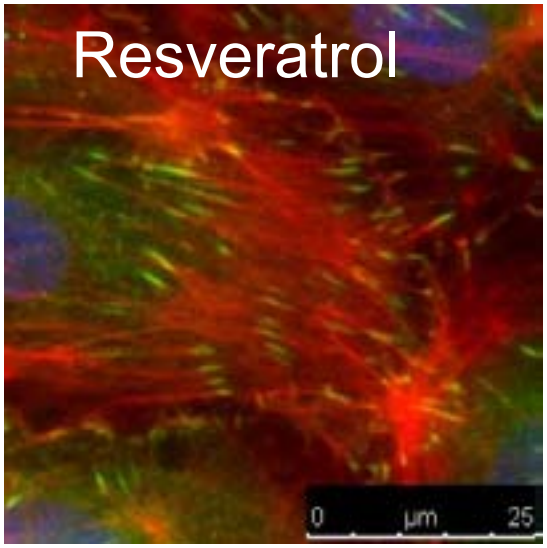


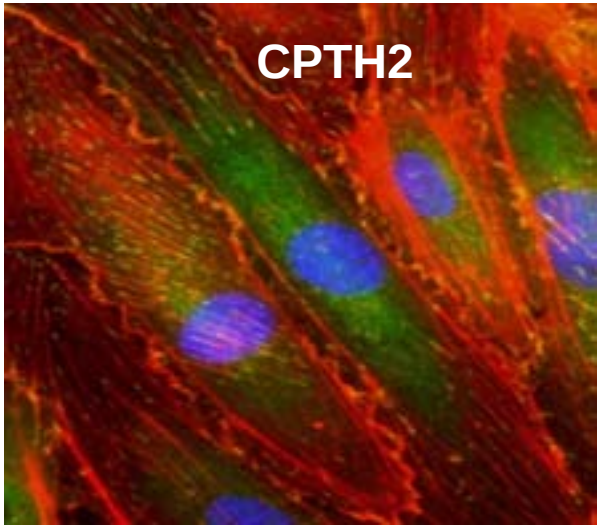

Fledderus Blood 2007, ATVB 2008; Boon. Blood 2010, Leyen 2012

# Is shear stress a drugable target for small molecules ?



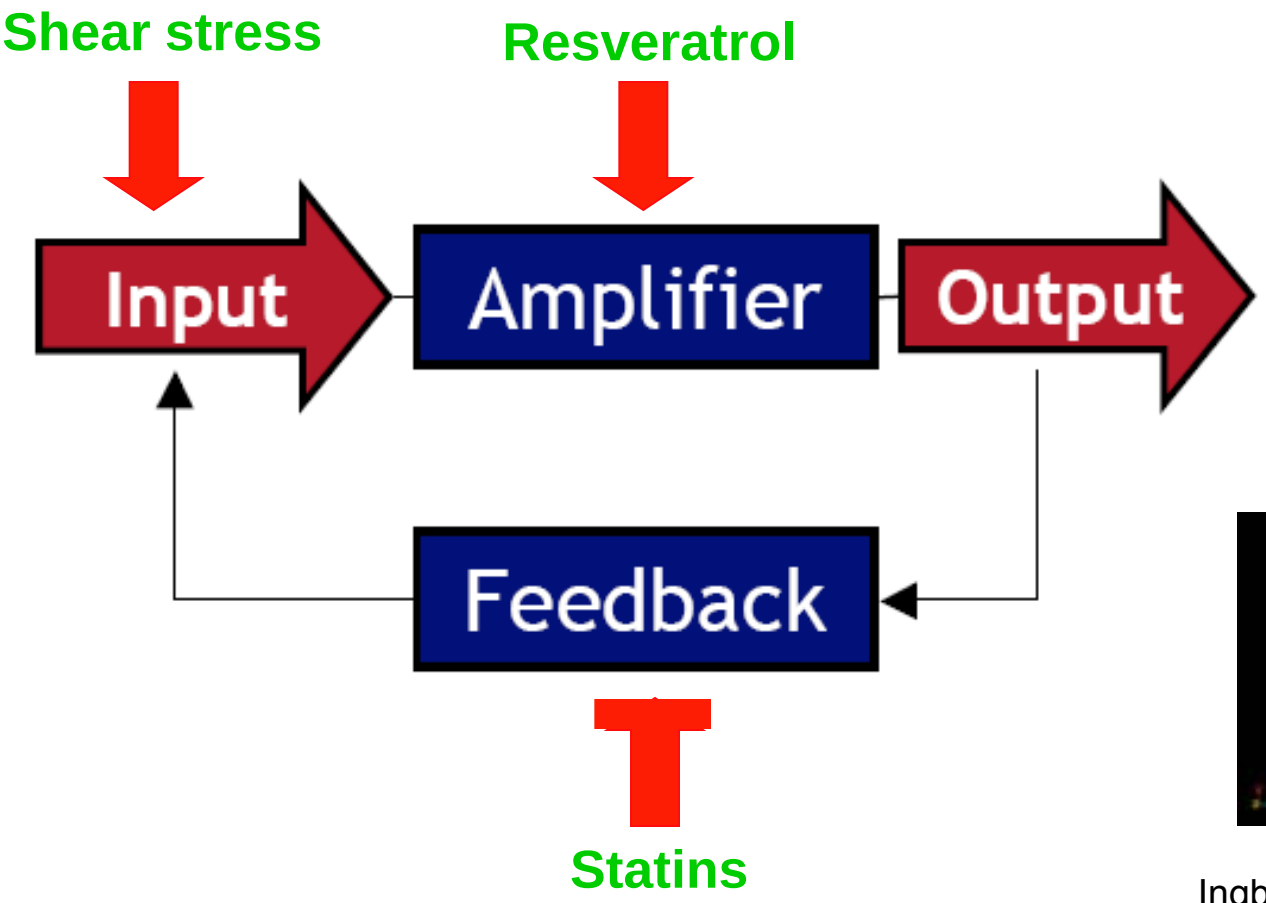



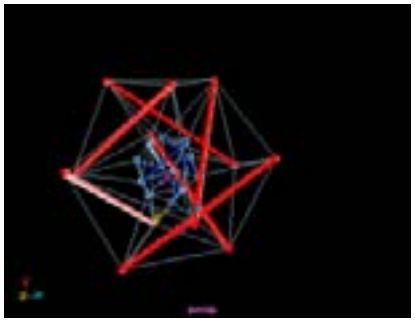



# A Negative Feedback Amplifier:

## A non-linear response module




KLF2



Ingber Tensegrity website

# Simplistic view of an endothelial cell

# The protective endothelial shear response

Flow-induced KLF2 is a central mediator of quiescent endothelial phenotype

Protective effects are multiscale: biomechanics, gene expression, kinome, protein localization,cytoskeleton, miRNA

Anti-inflammatory, anti-coagulant, anti-oxydant, anti-angiogenic effects are driven by actin rearrangements and protein relocation

The KLF2/shear modules can be modified by small molecules, based on computational modelling

# Acknowledgements


## VUmc Amsterdam
Thomas Leyen
Reinier Boon
Rob Dekker
Joost Fledderus
Ruud Fontijn
Jakub Rohlena
Oscar Volger
Cansu Yildirim
Tineke van der Pouw Kraan
Geerten van Nieuw Amerongen
Niels van Royen
Victor van Hinsbergh

## AMC Amsterdam
Perry Moerland
Ed van Bavel
Stephan Schirmer
Anja van der Laan
Jan Piek

## Univ Maastricht-Pathology
Mat Daemen
Erik Biessen

## Univ Leiden-LACDR
Johan Kuiper
Theo van Berkel

## Univ Leiden-MCB-II
Lia Pardali
Peter ten Dijke

## Sanquin Research Amsterdam
Jaap van Buul
Peter Hordijk

## Goethe University Frankfurt
Reinier Boon
Stefanie Dimmeler

EUROPEAN VASCULAR GENOMICS NETWORK

Nederlandse Hartstichting
Molecular Cardiology Program

NWO
Genomics

IOP
Genomics

Institute for Cardiovascular Research

ICaR-VU
vrije Universiteit amsterdam