VU medisch centrum

# Endothelial Integrity:

# KLF2 and the Endothelial Balancing Act

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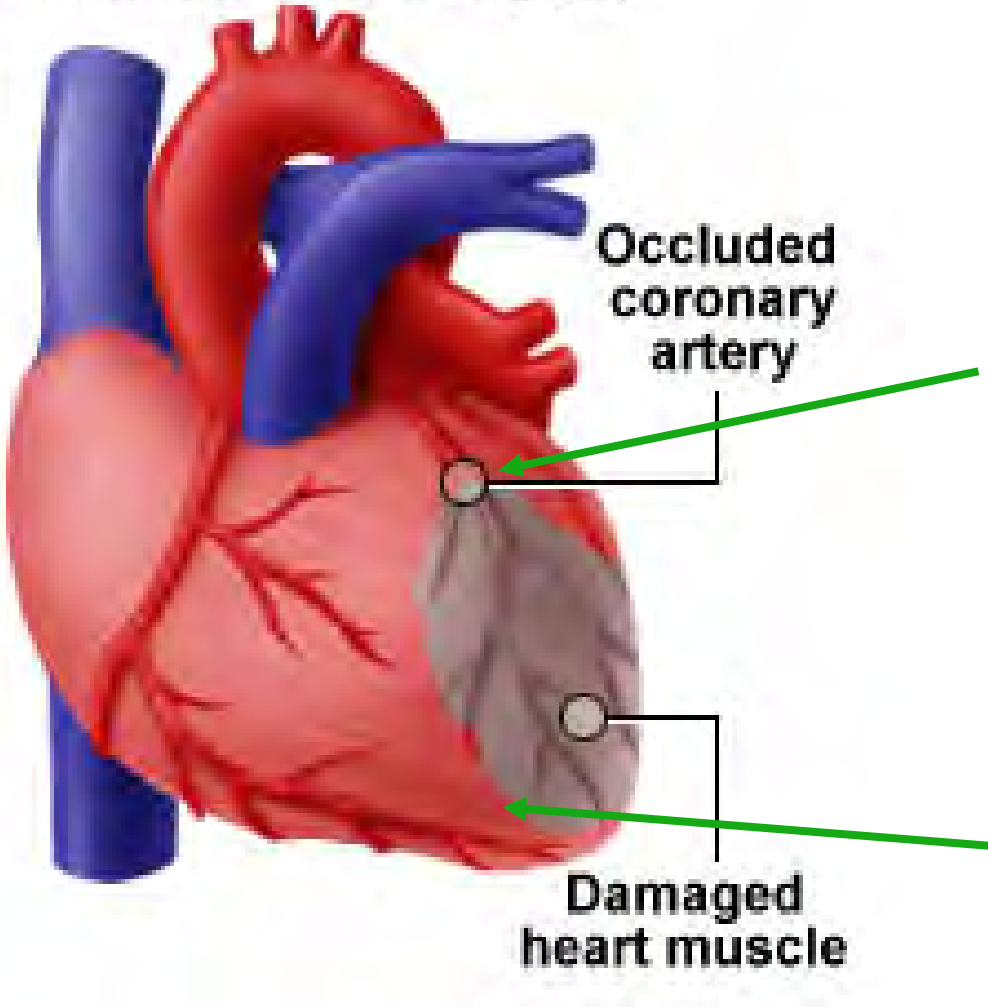
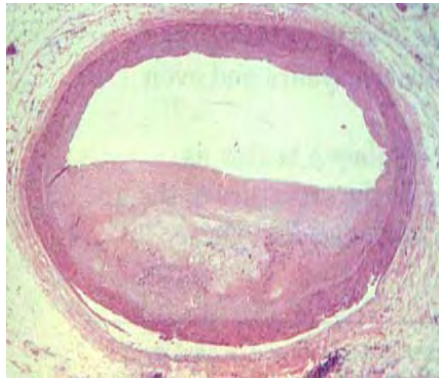
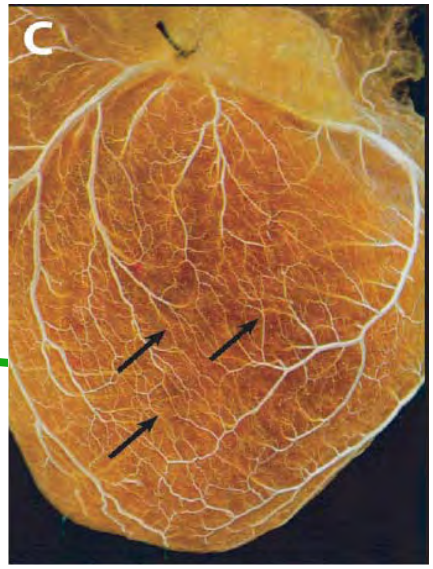
# Shear stress guides vascular remodeling
# Beneficial: outward remodeling, arteriogenesis
# Pathological: atherosclerosis, vascular stenosis

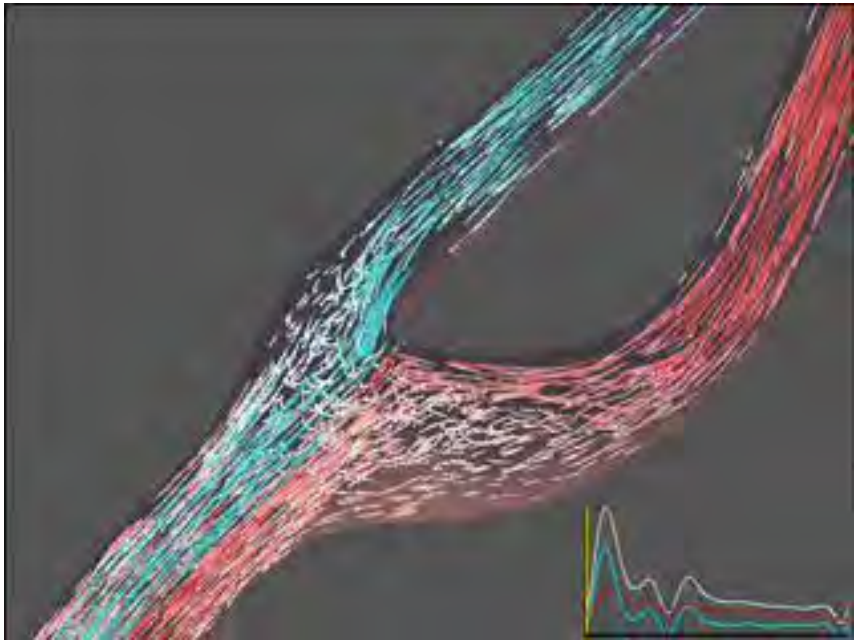


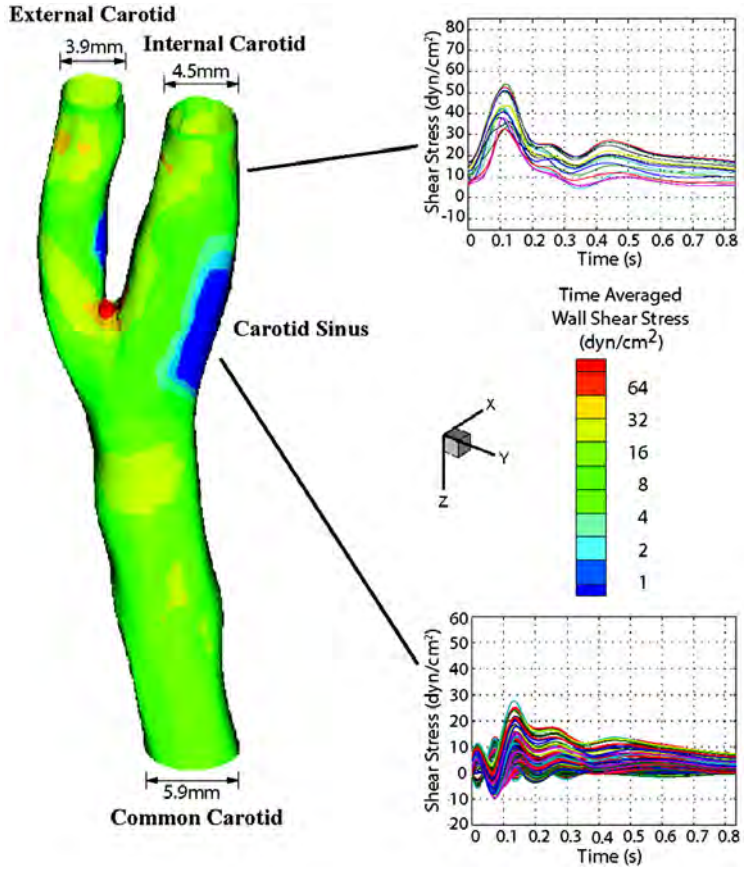


# Focality of lesions correlates with flow patterns: Turbulence result in low shear stress and NFkB activation

**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

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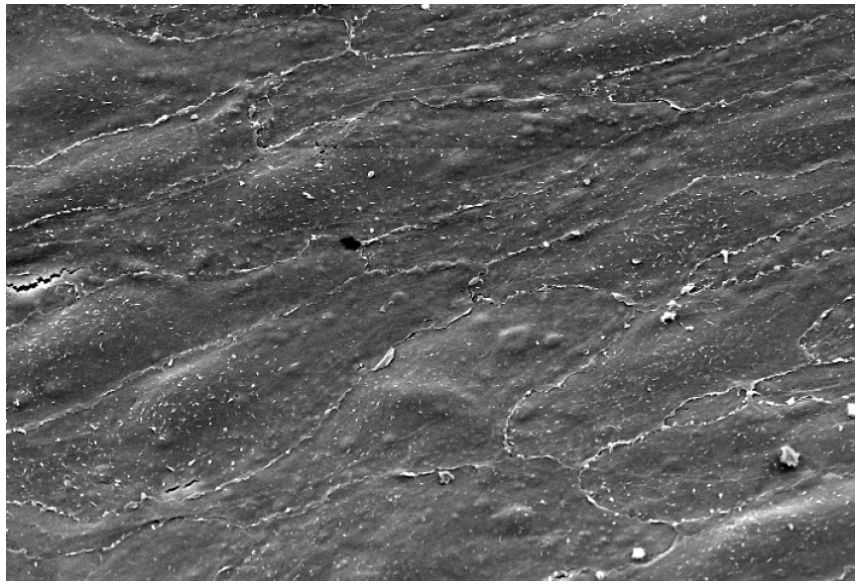
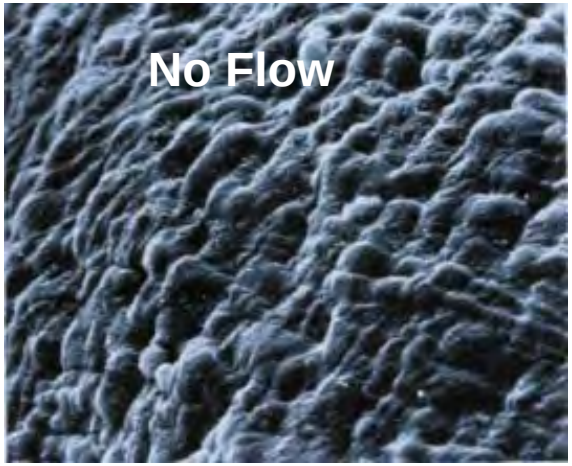
# Endothelium aligns in direction of flow





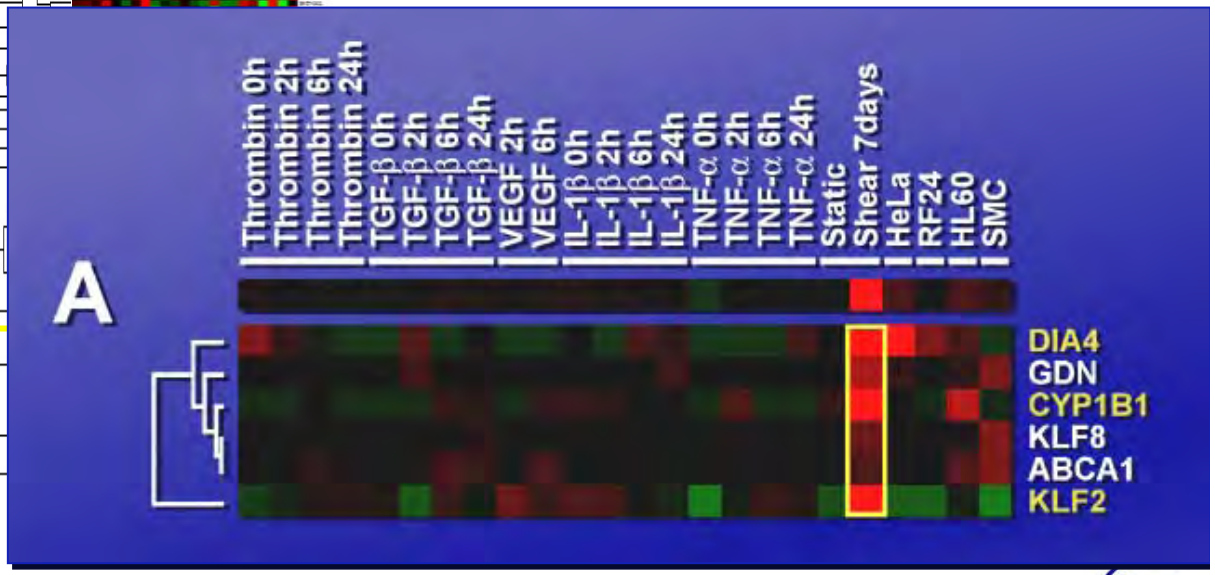
# SOM & hierarchical clustering of genes: digitized cellular phenotypes

growth factors

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

cytokines

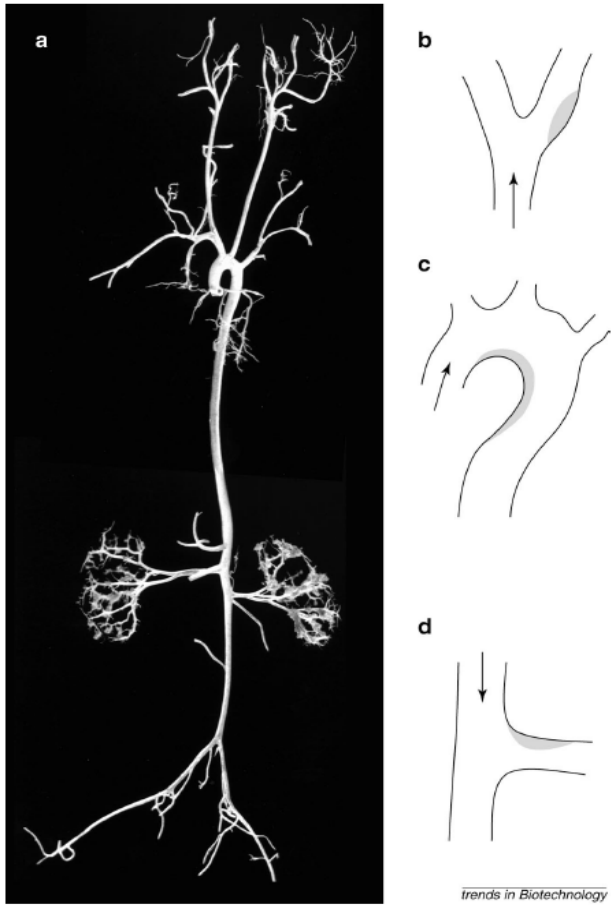
shear





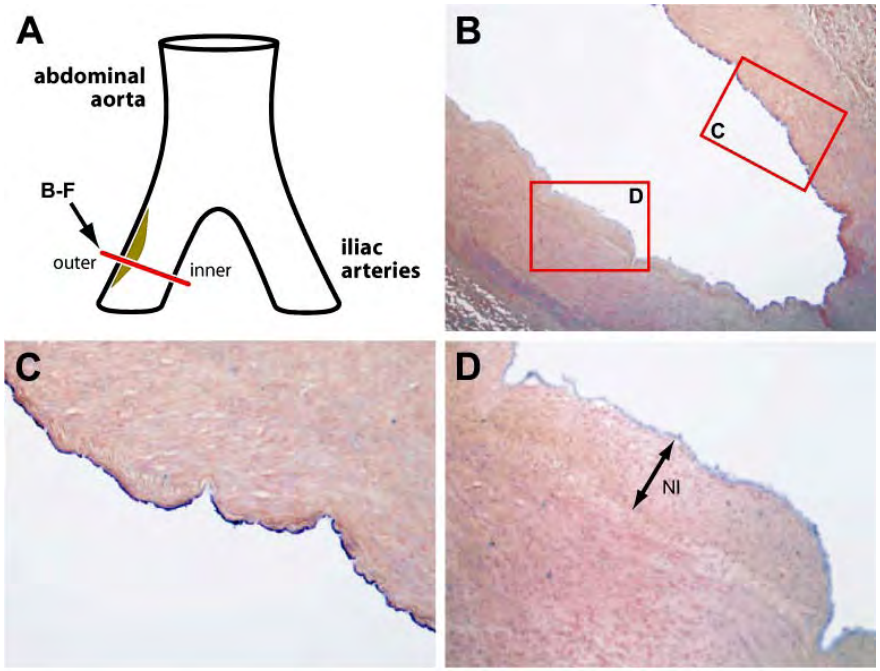

Dekker et al, Blood 2002

# KLF2 localizes to atheroprotected regions




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

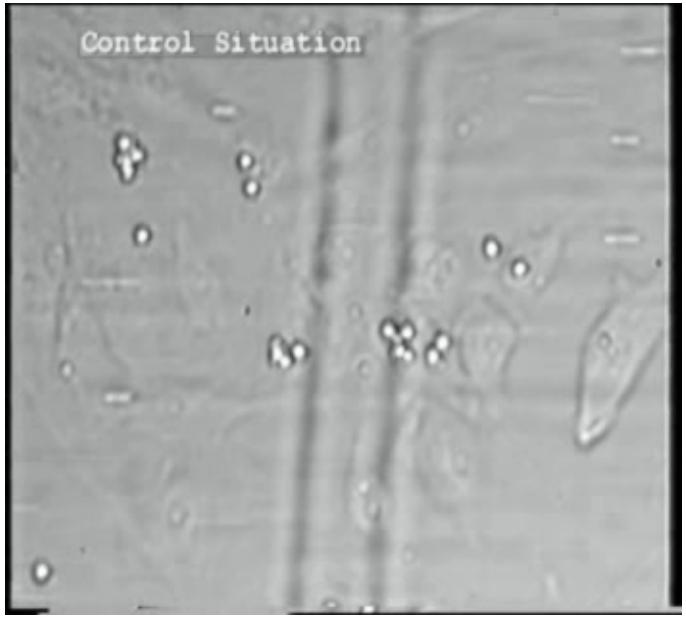
*In situ* hybridisation: KLF2

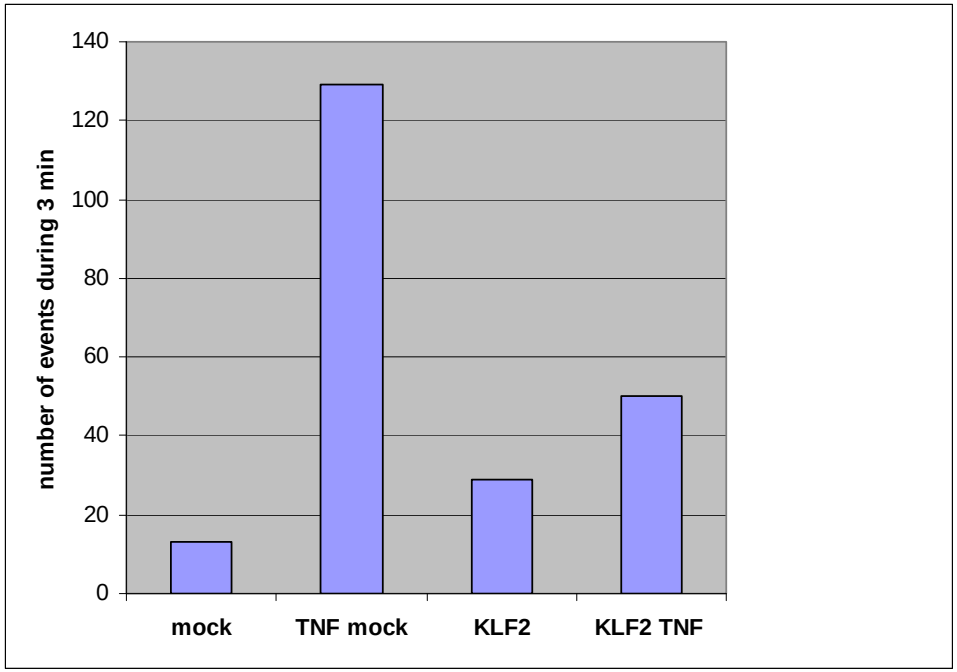



Dekker *et al.* (2005) Am J Pathol. **167(2)**, p609

# Transcription factor KLF2

* directly induces eNOS and Thrombomodulin
* suppresses monocyte adhesion in presence TNFa



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

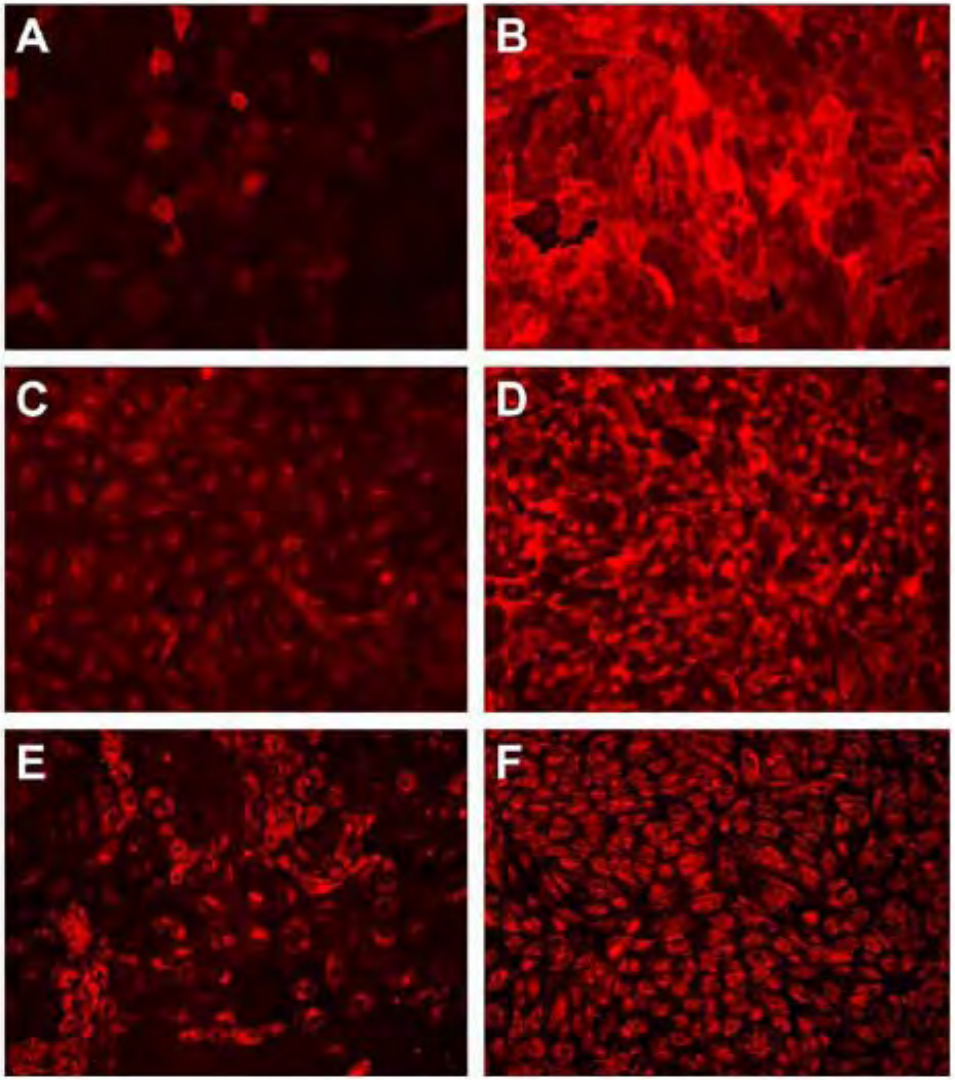
| Functional category | Genes |
|---|---|
| Haemostasis | THBD↑, VWF↑, SERPINE1↓, RALA↓, PROS1↑, PTGDS↑, TFPI2↓, NOS3↑ |
| Inflammation | CCL2↓, THBD↑, IL8↓ |
| Vascular tone regulation | NOS3↑, ADM↓, ACE↓, EDN↓, PTGDS↑ |
| Cell surface composition (glycocalyx) | HYAL2↑, HPSE↑ |
| Vascular wall matrix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

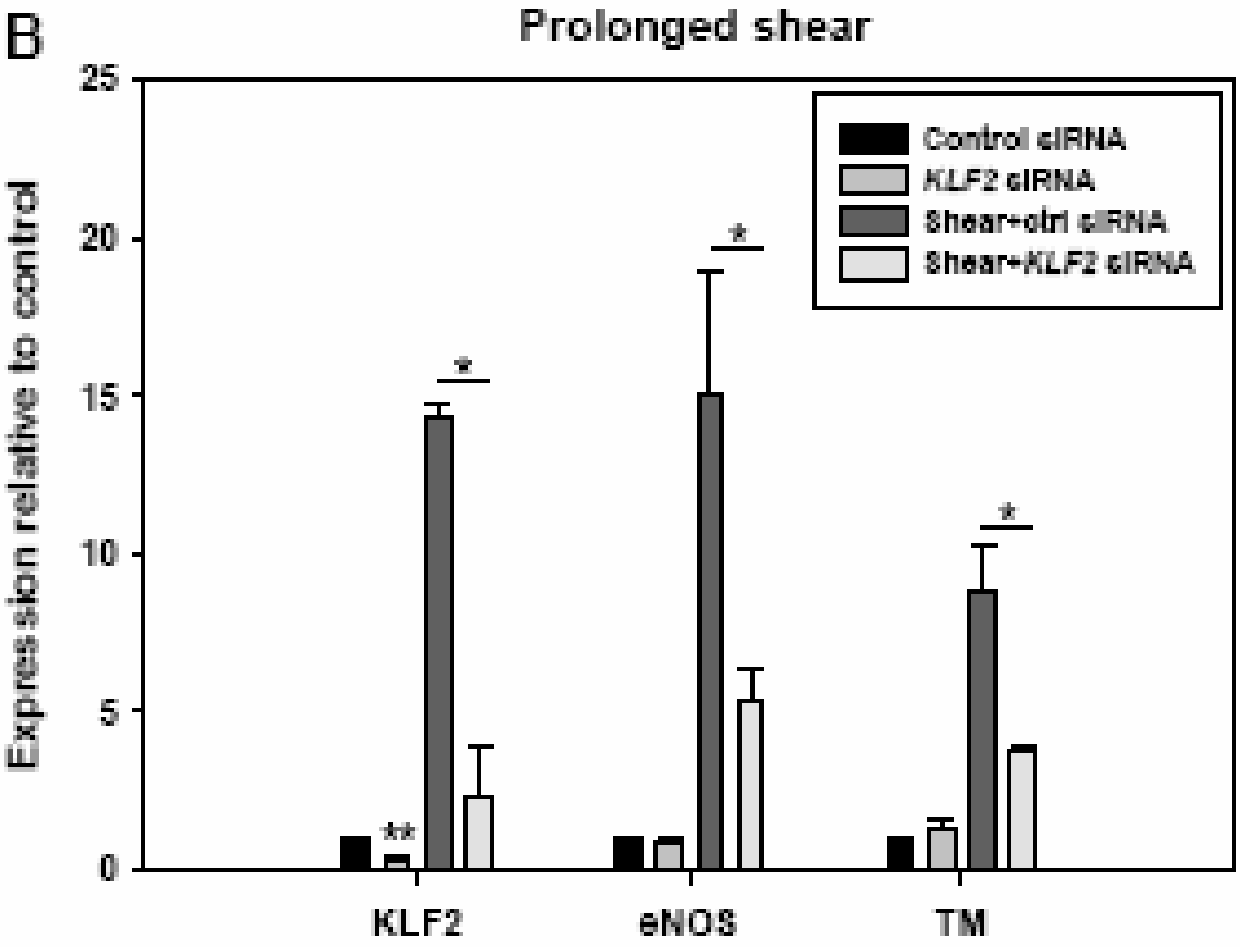
**eNOS**

**Von Willebrand Factor**



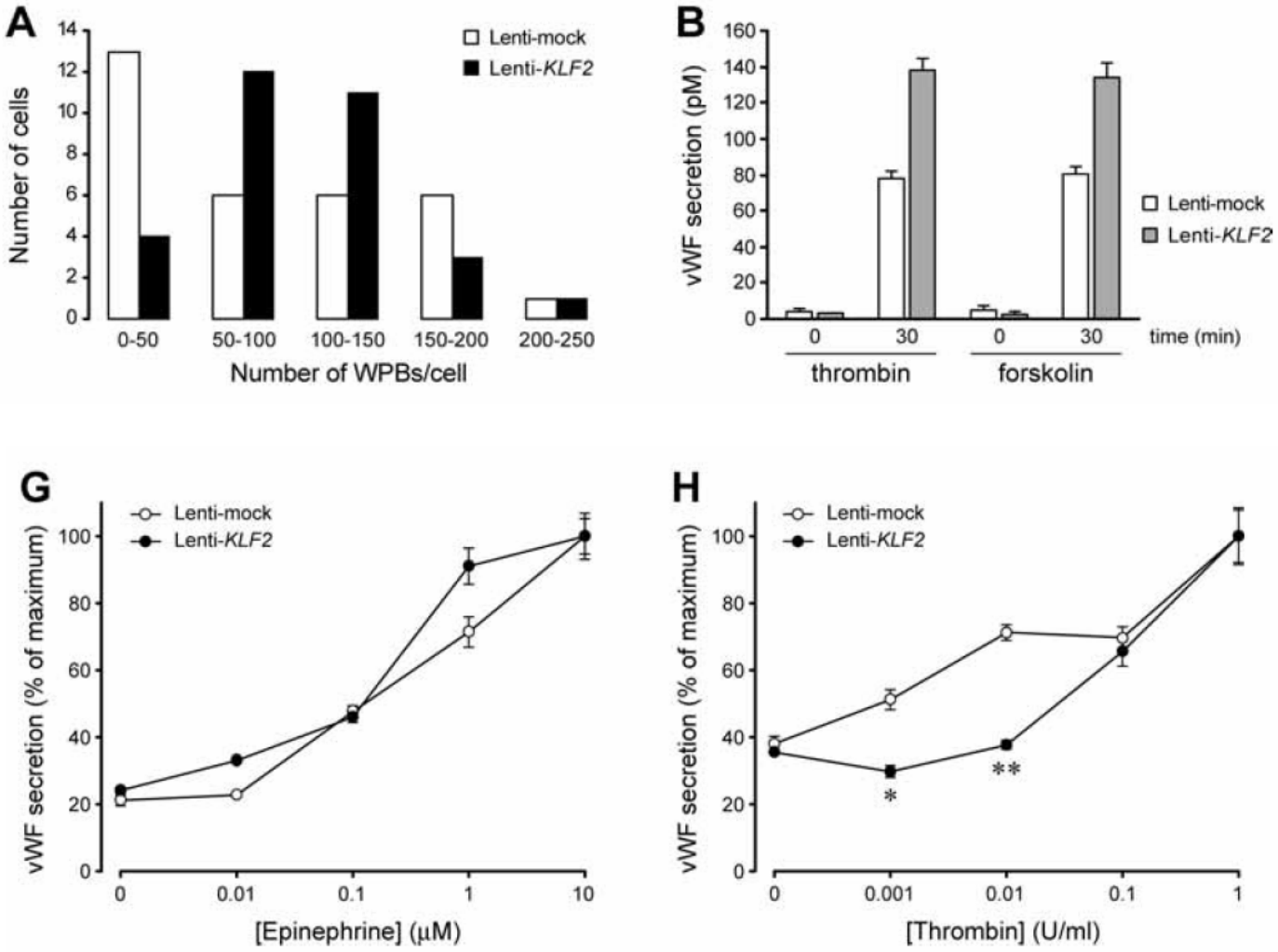
Dekker, Boon et al  Blood 2006

# Shear induction eNOS and TM is KLF2 dependent




Dekker Am J Pathol. 2005
Van Thienen Card Res 2006

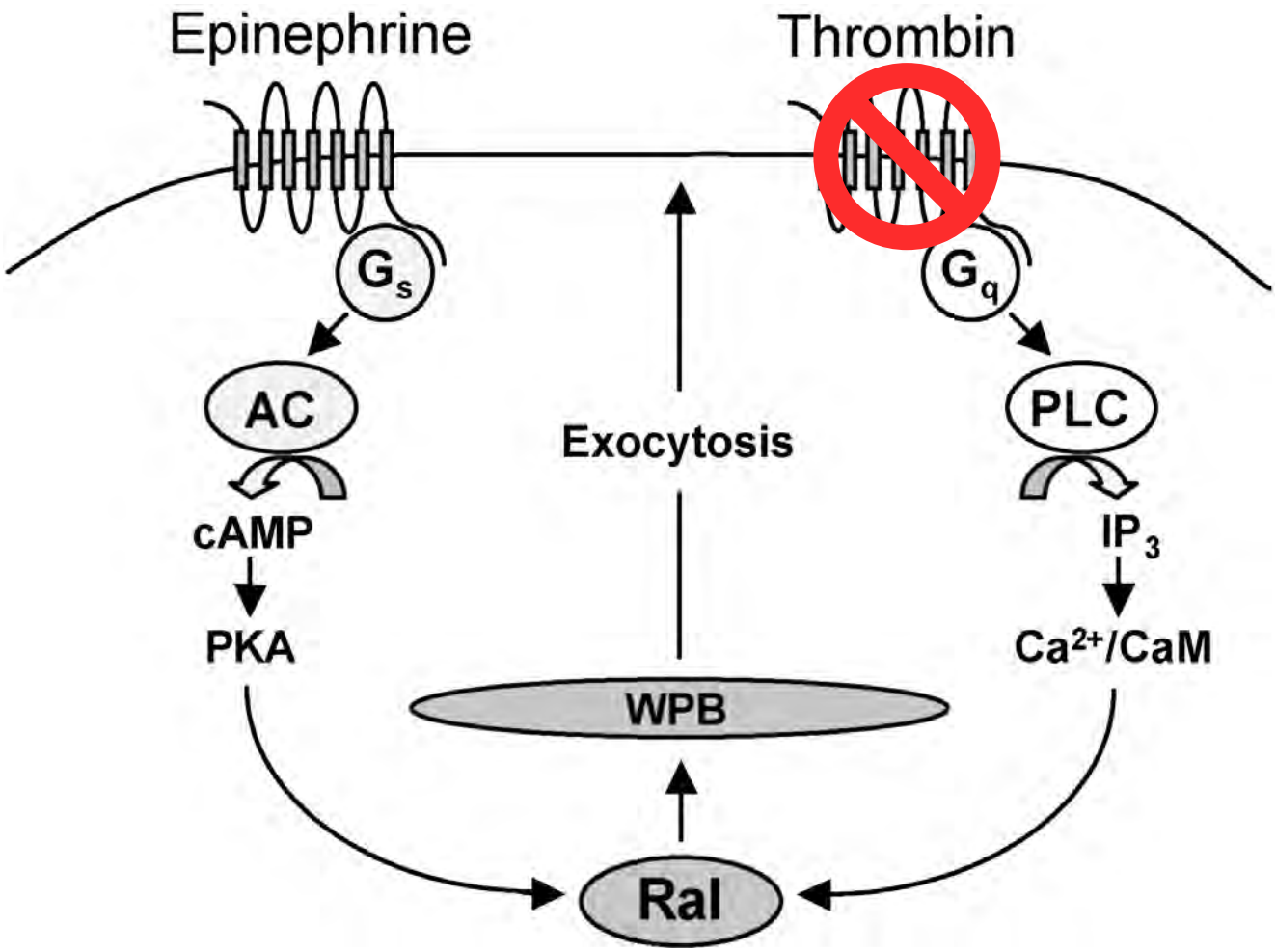
# KLF2 promotes vWF storage and attenuates thrombin-induced vWF release





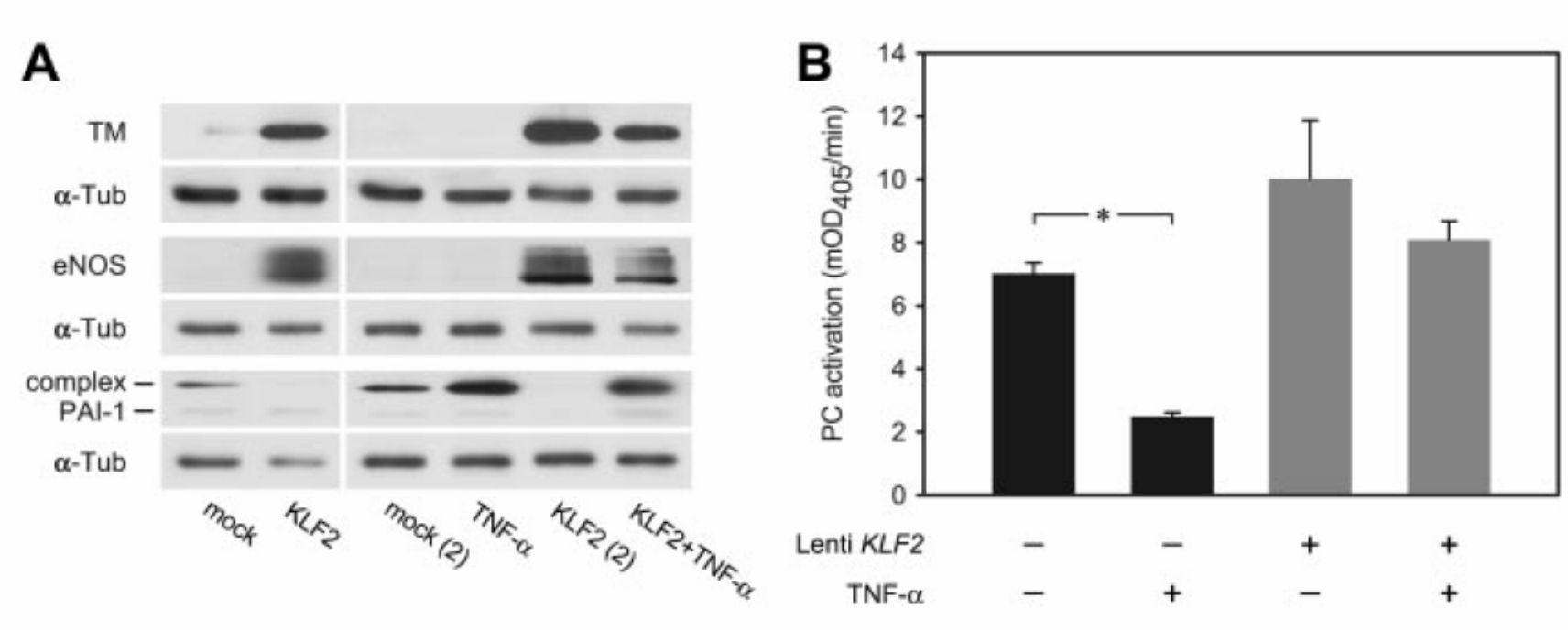

Dekker, Boon et al Blood 2006

# Mechanism of **exocytosis** of vWF containing Weibel-Palade bodies



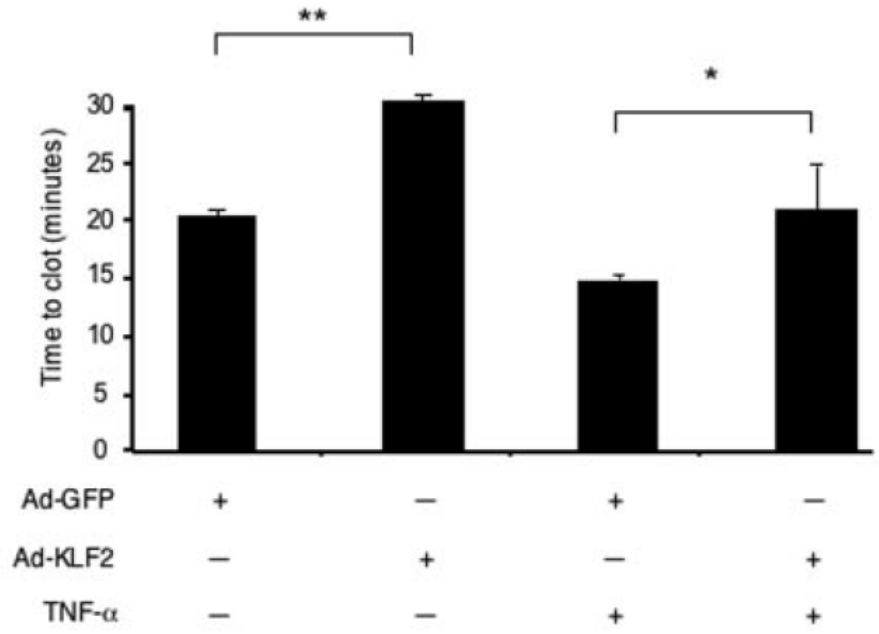

Rondaij *et al.* (2004)

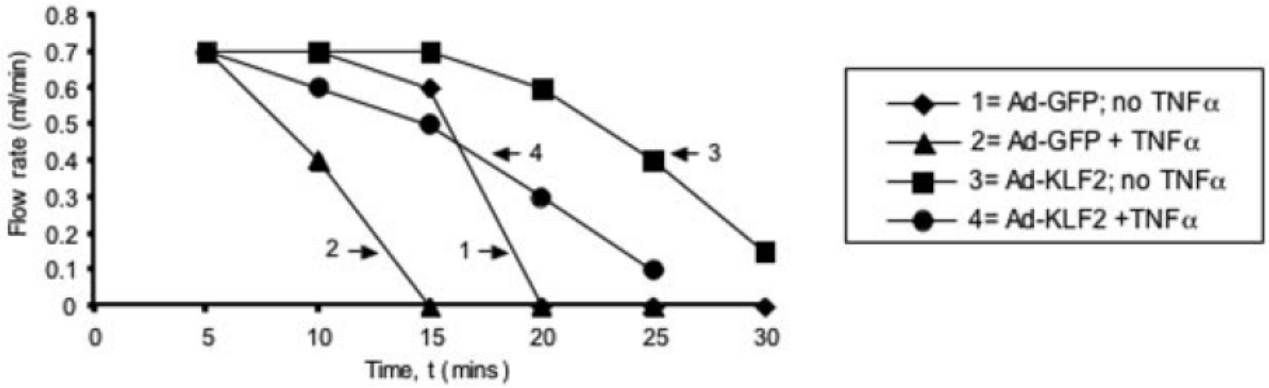
# KLF2 neutralizes effects TNFa on anti-coagulation




Dekker,Boon Blood 2006

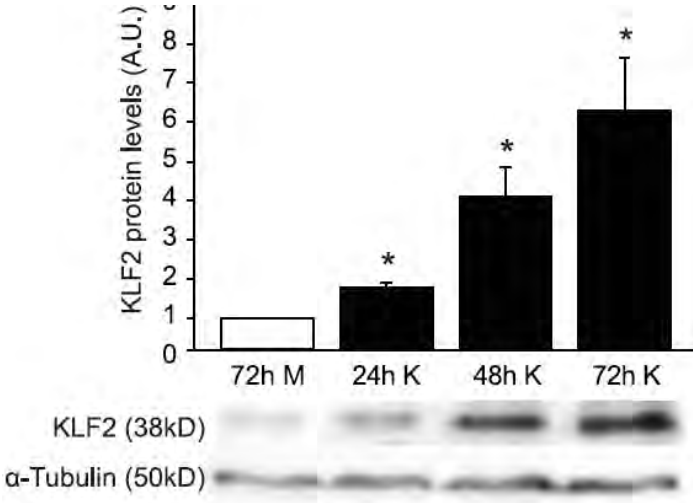
# Endothelial KLF2 attenuates blood clotting under flow

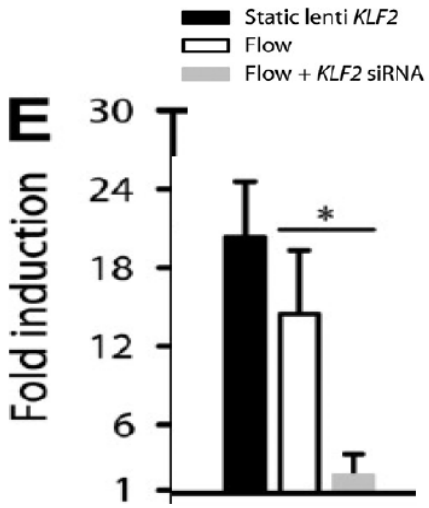


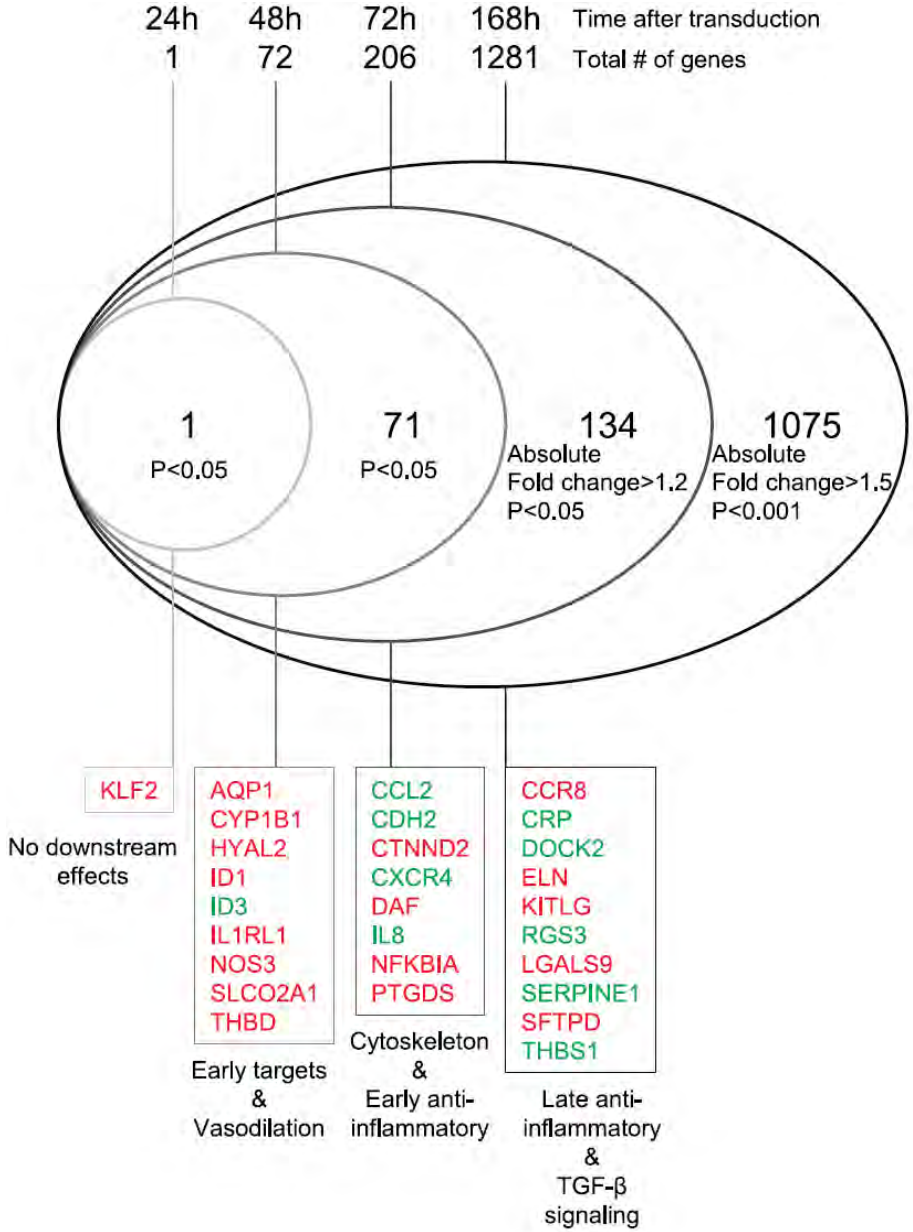




Lin Z, et al Circ Res 2005;96:e48

# Effects of KLF2

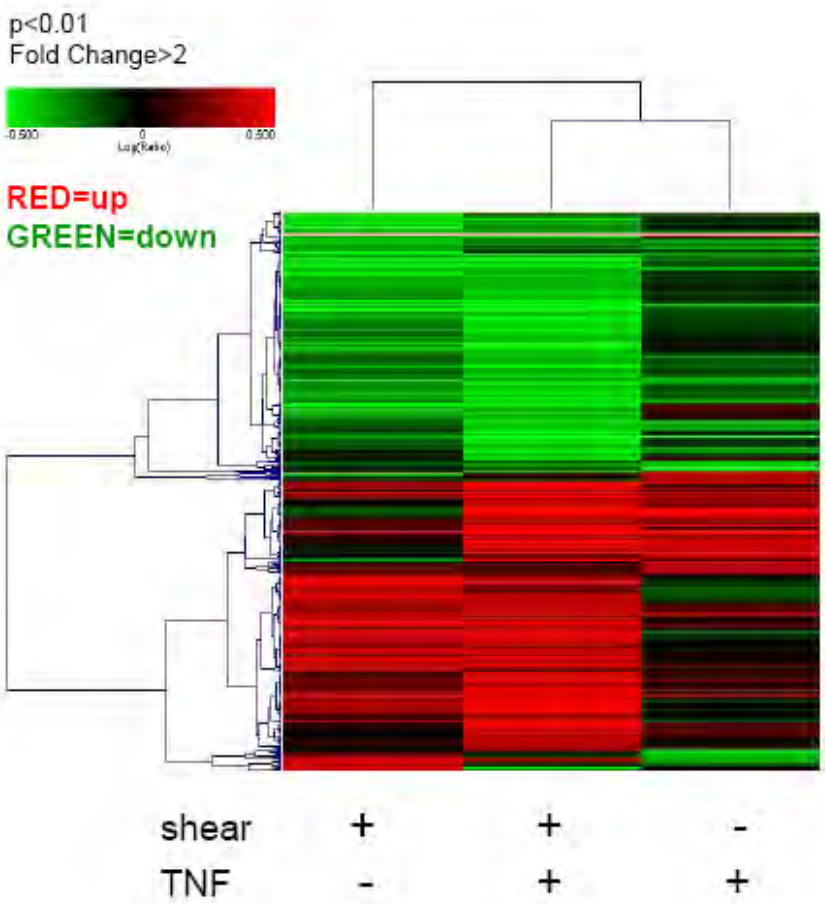


# Functional transcriptomics-promoter analysis

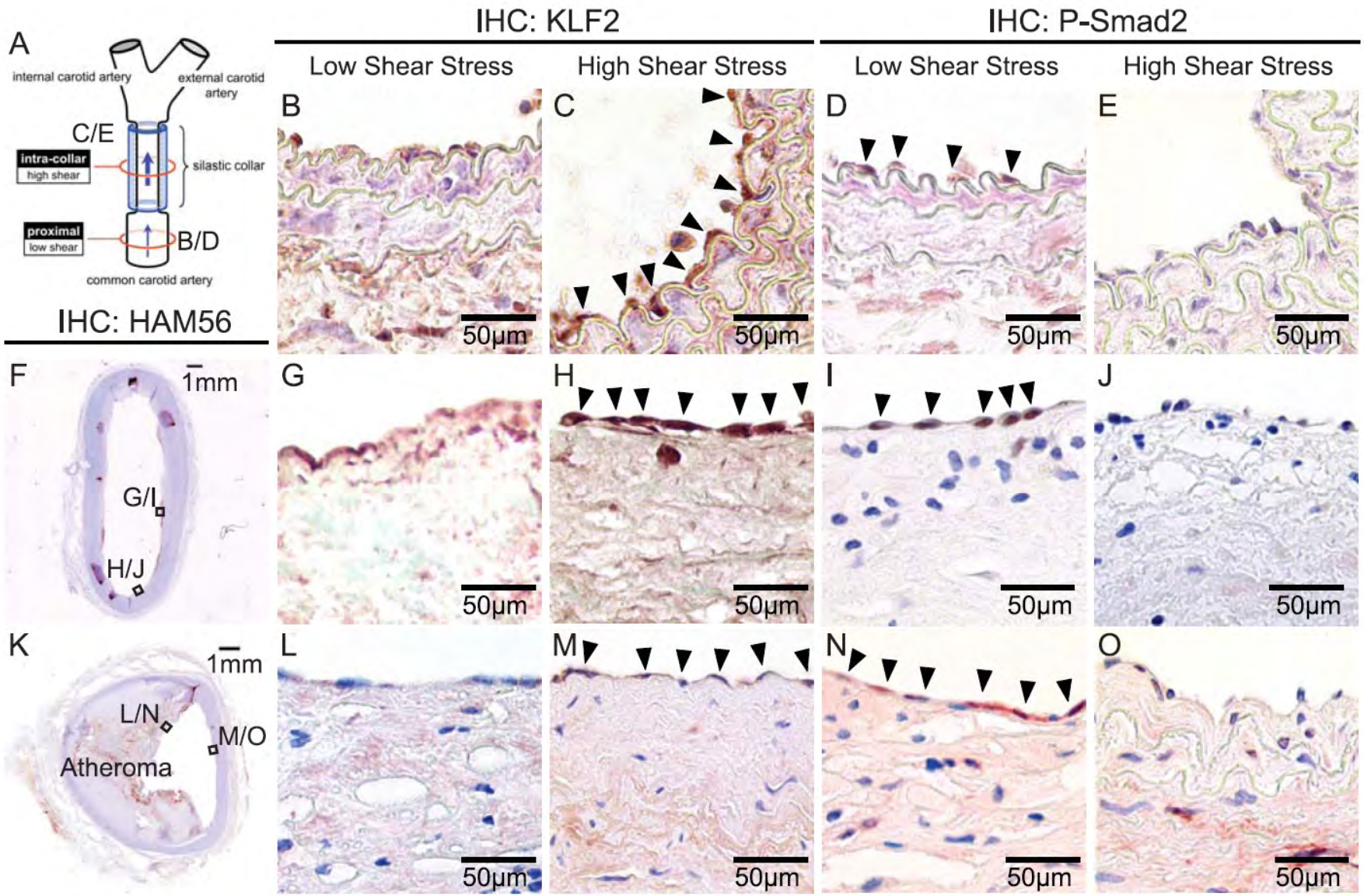
deciphering the KLF2 transcriptional network




| 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 |

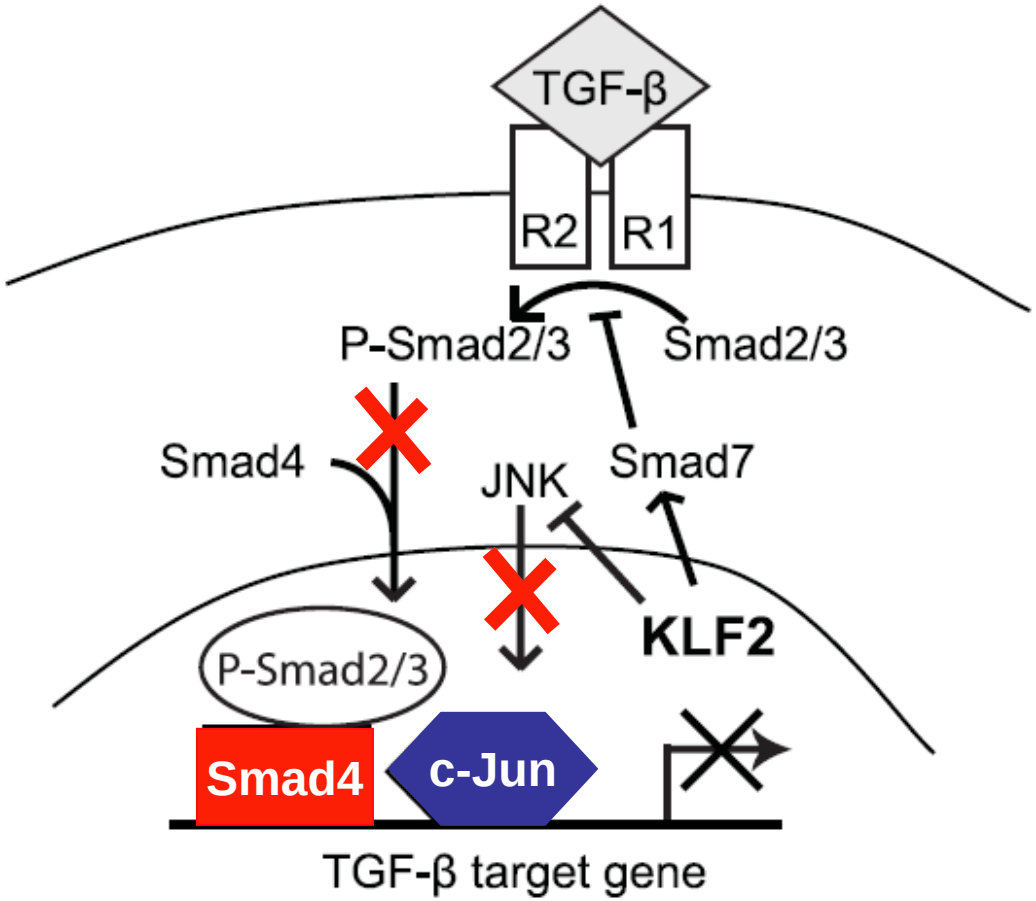
# KLF2 and TGFbeta signaling are mutually exclusive in vivo

## detection by phosphospecific antibody: activated proteome



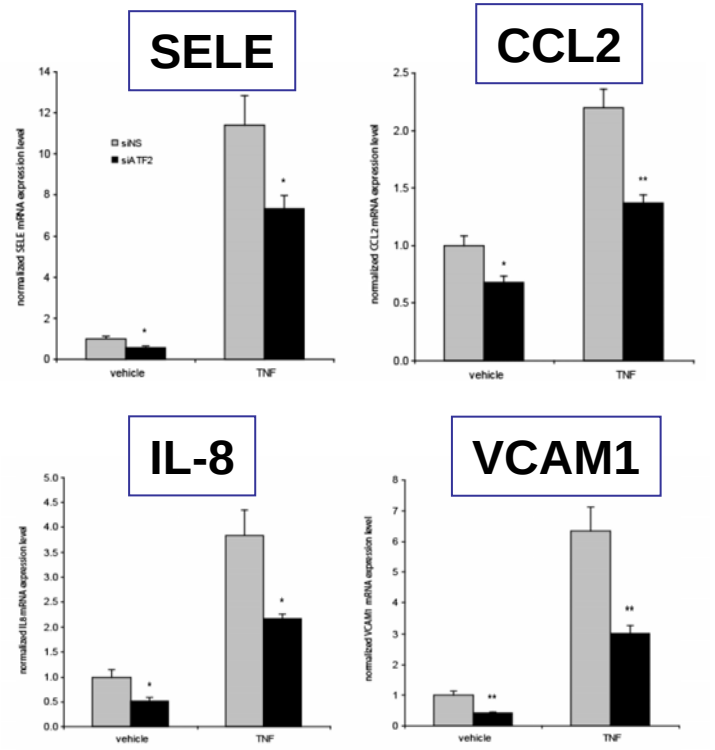

Boon et al., ATVB 2007

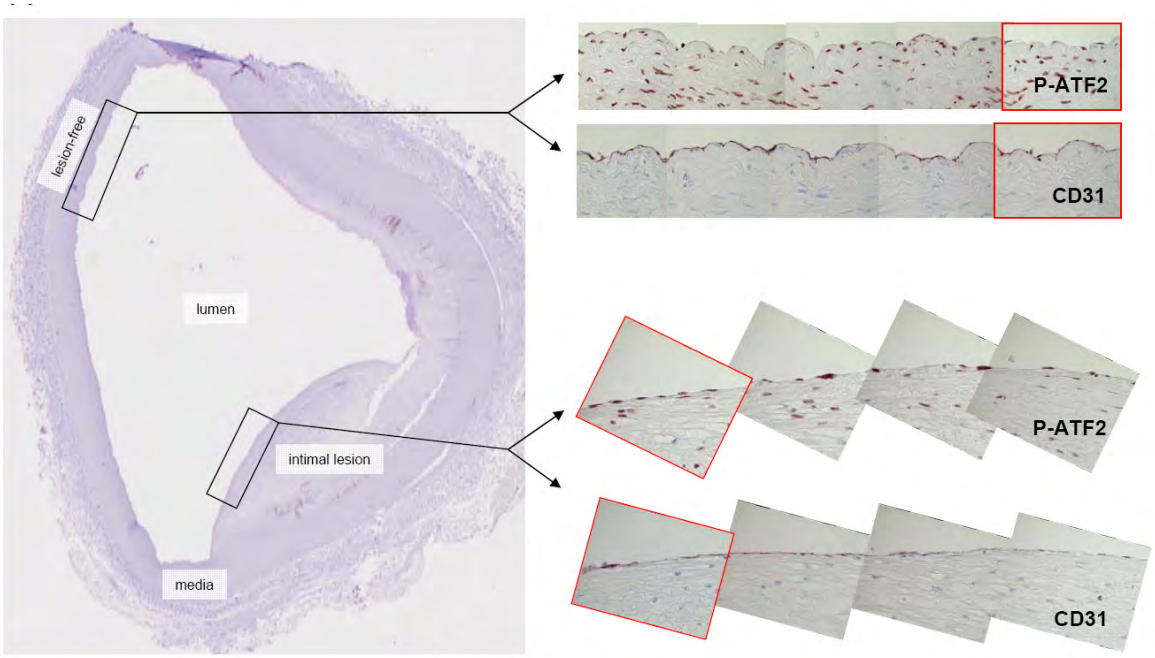
# KLF2 supresses TGFbeta signalling




Boon ATVB 2007

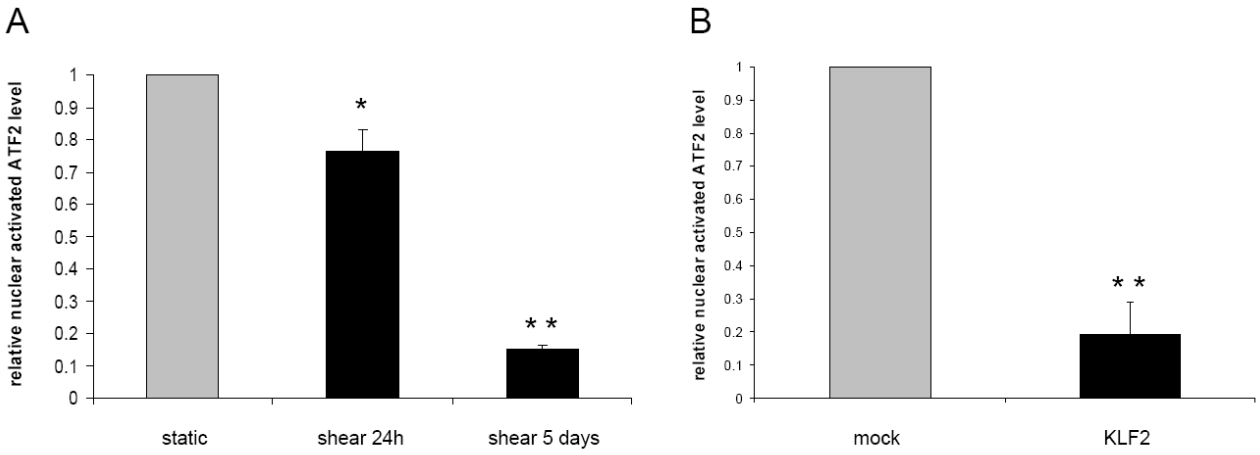
# si-ATF2 suppresses basal and inducible levels inflammatory genes
# Active phospho-ATF2 only in human atherosclerotic lesion EC

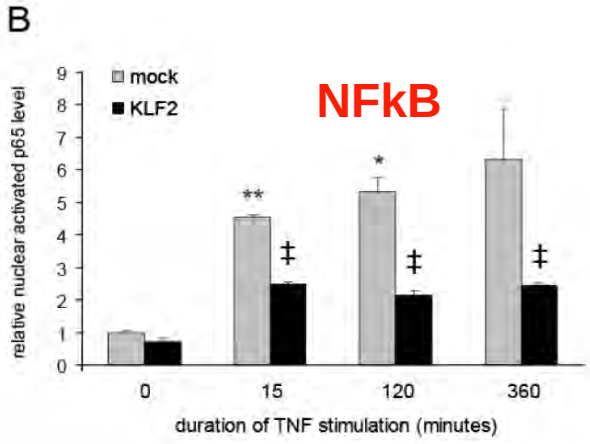


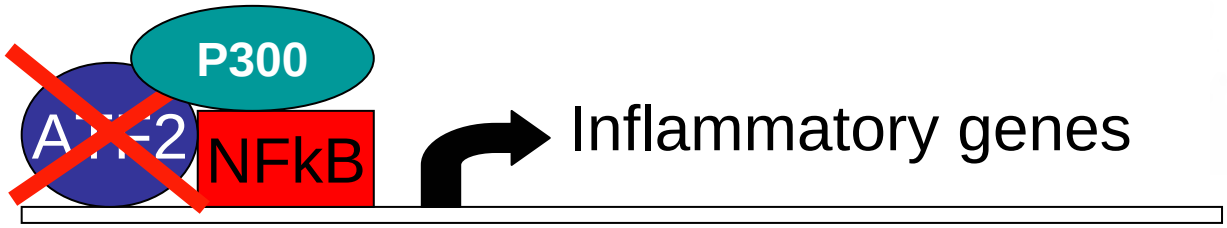



# Shear and KLF2 provoke a similar suppression of ATF2:

# this desensitizes NFkB response



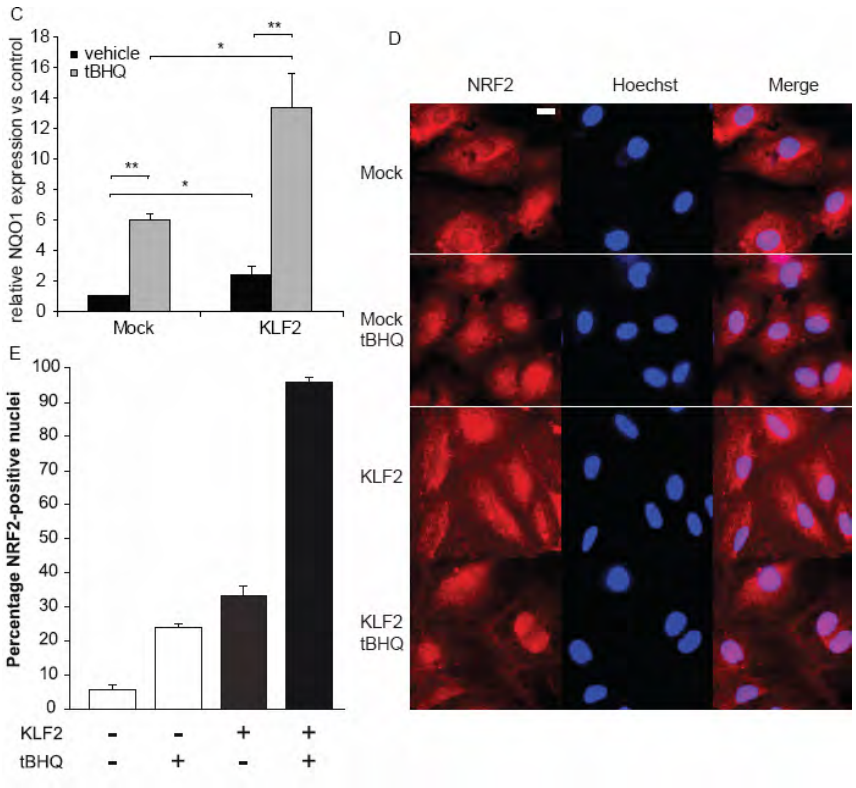

MCP-1, VCAM1, IL-8, PAI-1



Fledderus Blood 2008

# KLF2 augments nuclear localization of anti-oxidant NRF2

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




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

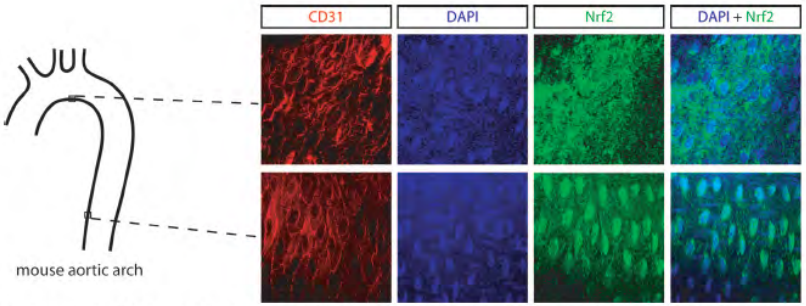
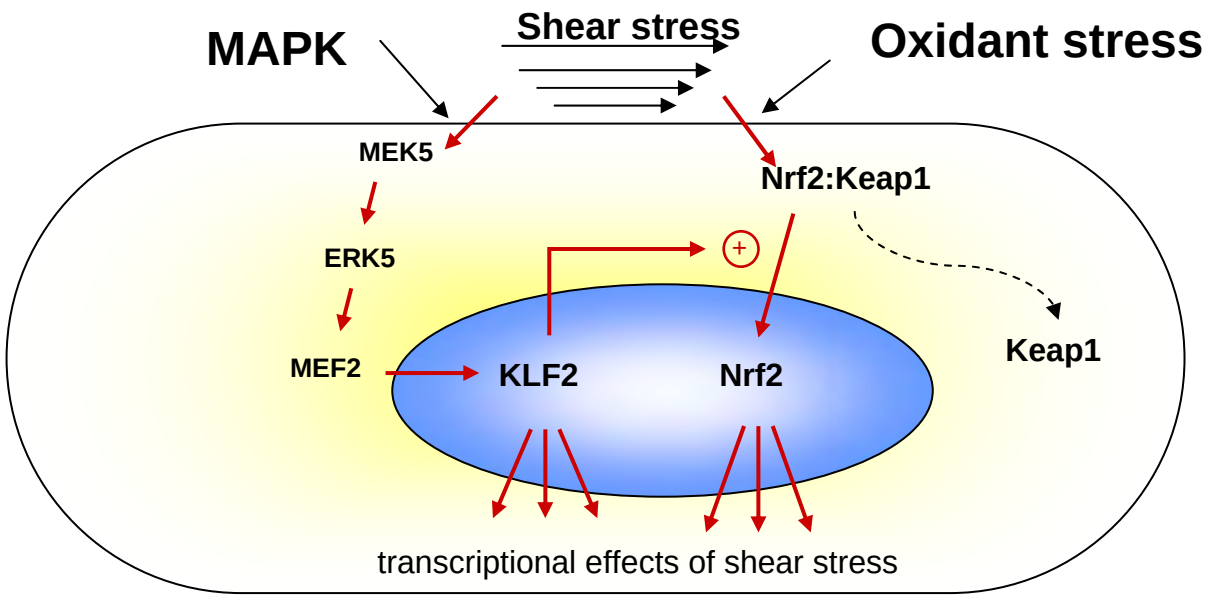



**Figure 6.** Increased Nrf2 nuclear localization in endothelium in atherosclerosis-resistant regions of the mouse aorta. The aortic

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

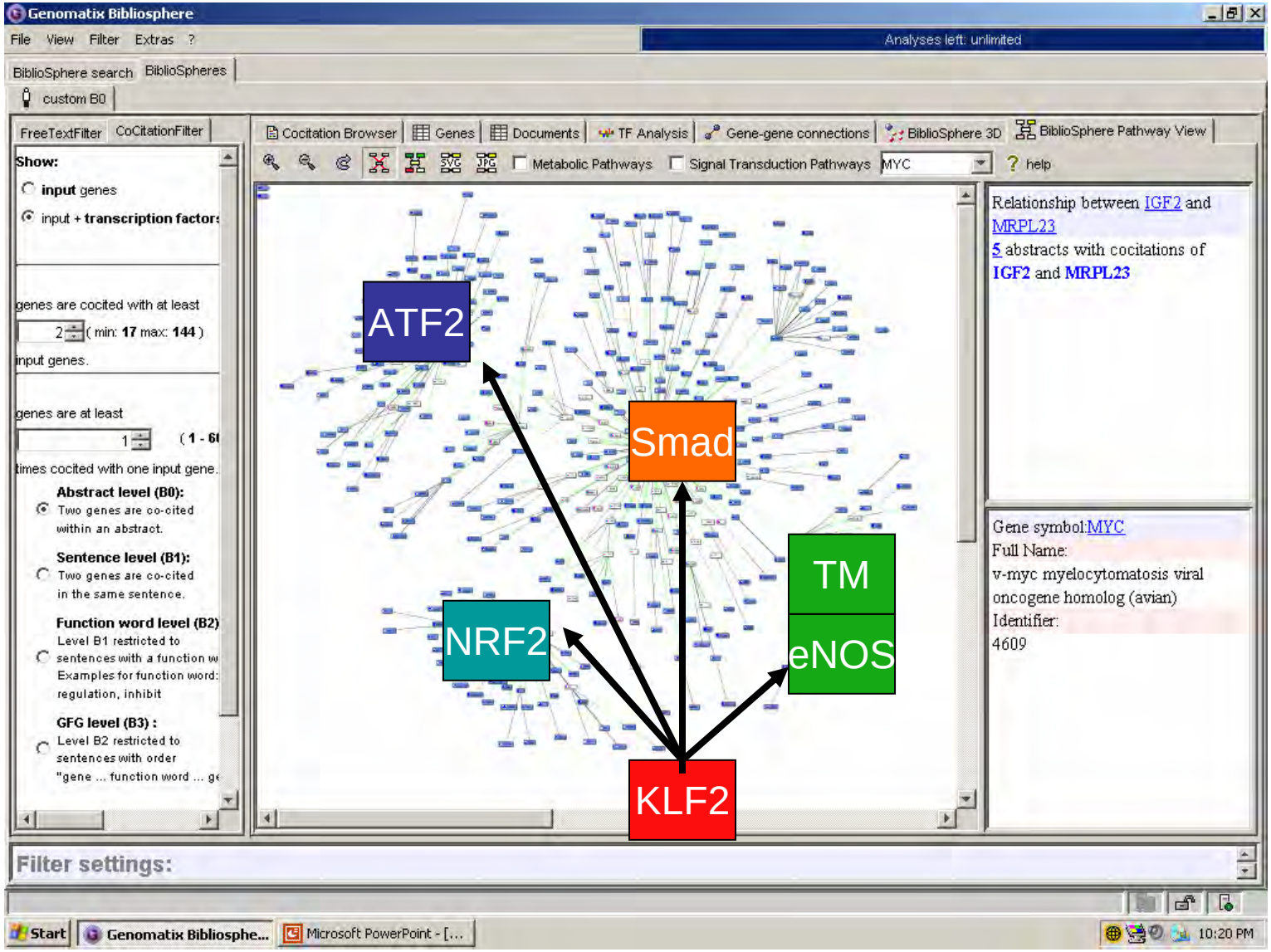
# The KLF2-NRF2 axis in the shear stress genetic network

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



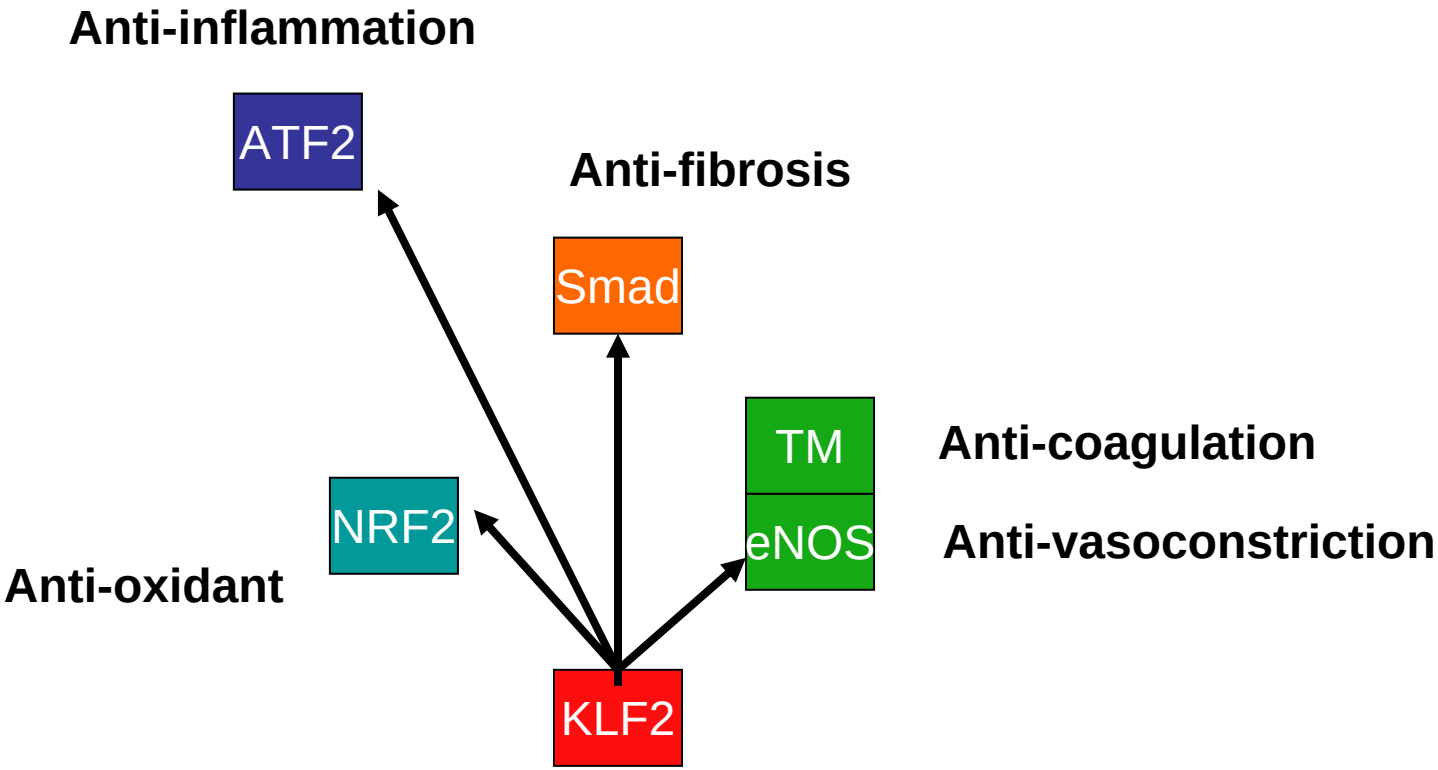
# The KLF2-shear stress genetic network

## How a single transcription factor controls ~ 1000 genes



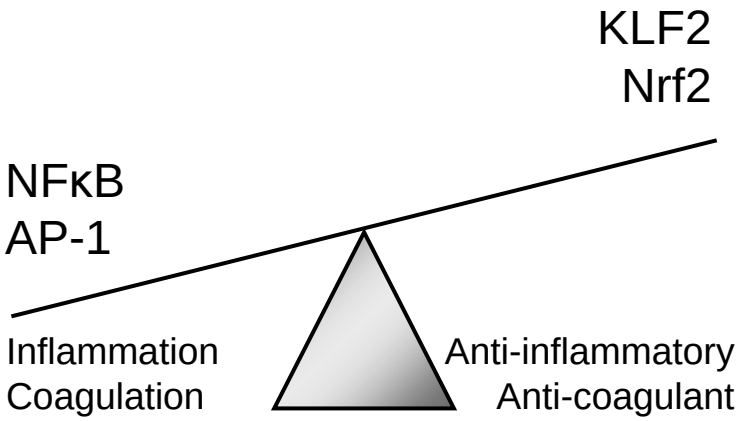
# The KLF2-shear stress genetic network

How a single transcription factor controls ~ 1000 genes

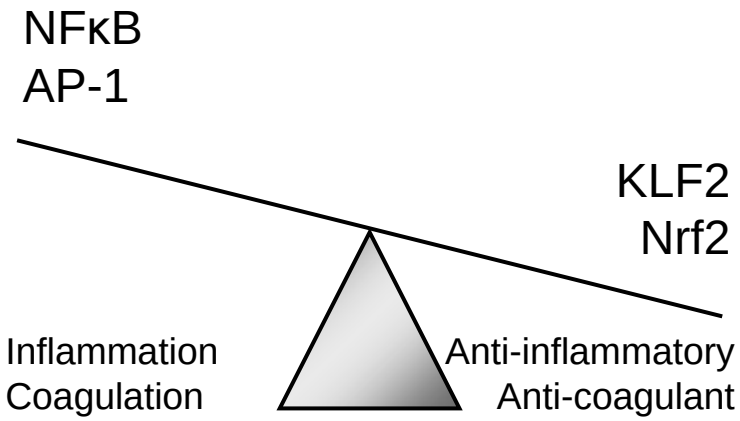


# How can we tip this balance?

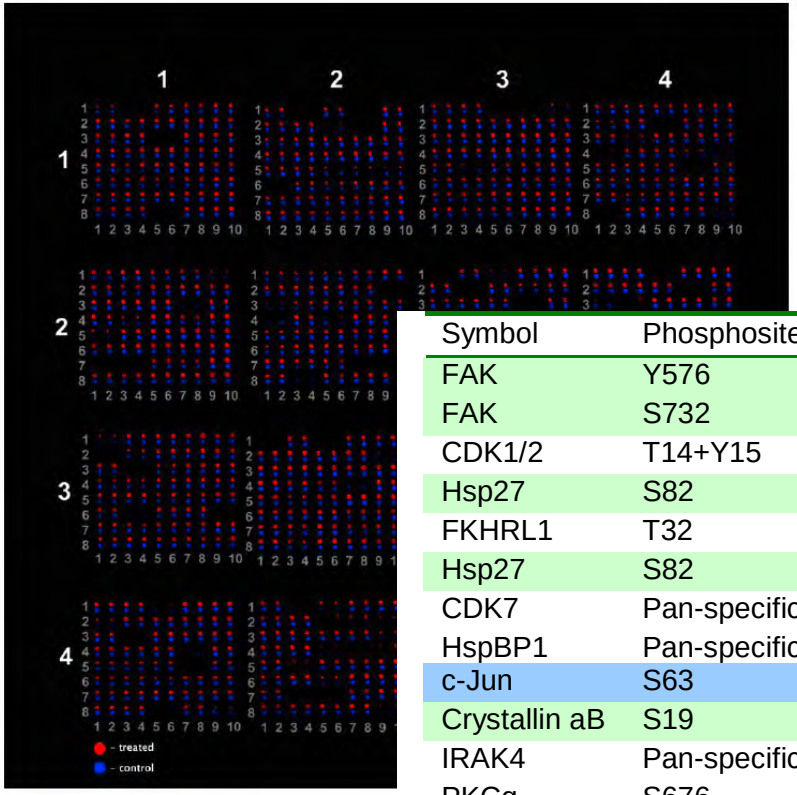
**Onset or Oscillatory Shear stress**




**Prolonged laminar Shear stress**



# Kinome analysis: Actin rather than MAPK

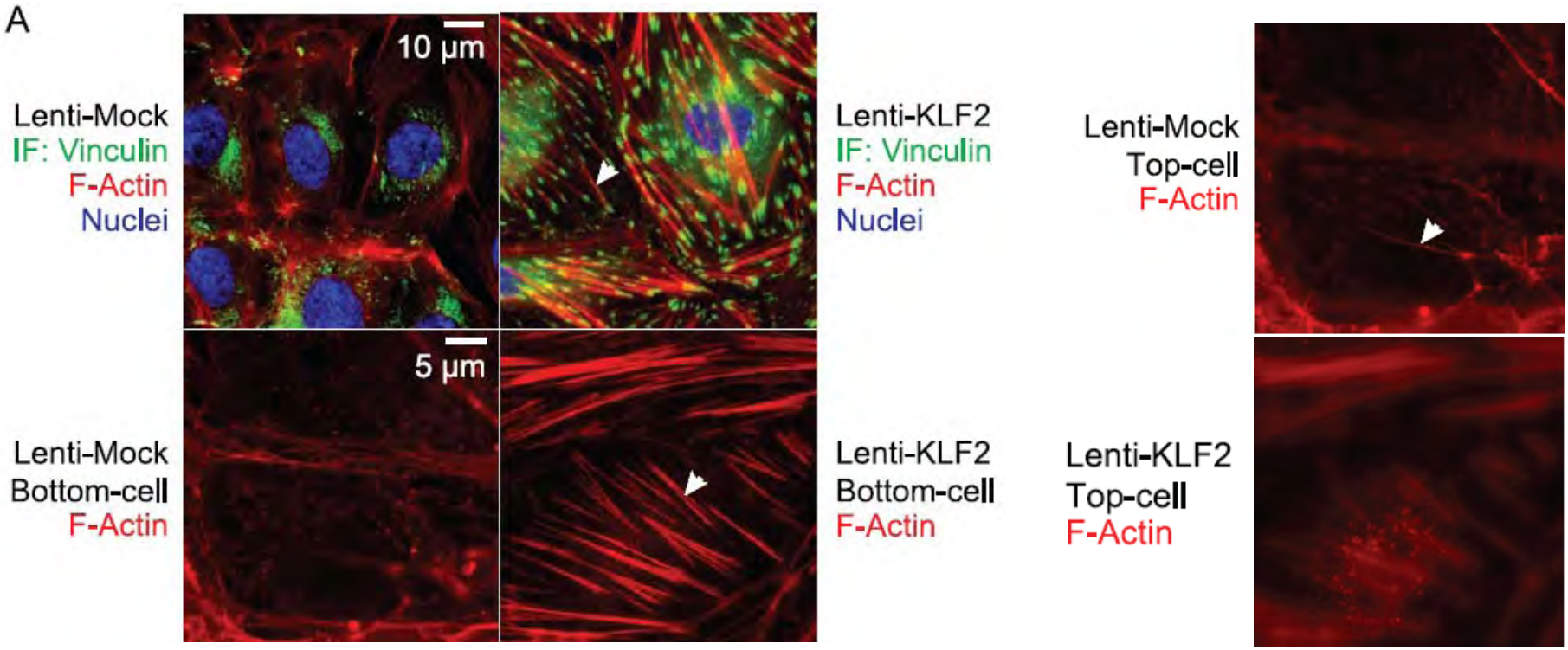


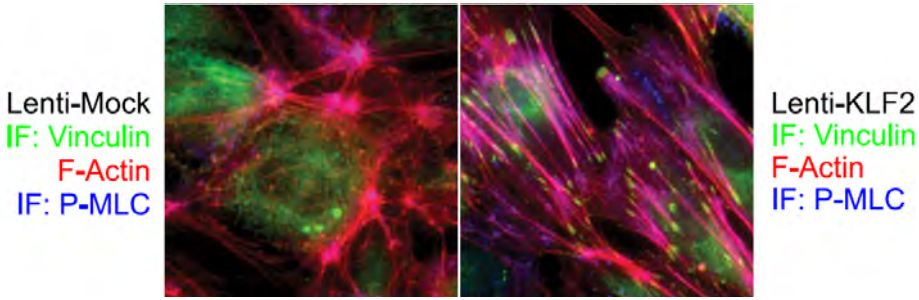

| Symbol | Phosphosite | Bayes.p | Fold |
|---|---|---|---|
| FAK | Y576 | 0.0005 | -3.02 |
| FAK | S732 | 0.0008 | -2.81 |
| CDK1/2 | T14+Y15 | 0.0091 | -1.67 |
| Hsp27 | S82 | 0.0150 | -1.93 |
| FKHRL1 | T32 | 0.0155 | -3.65 |
| Hsp27 | S82 | 0.0169 | -2.41 |
| CDK7 | Pan-specific | 0.0203 | -1.45 |
| 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 |

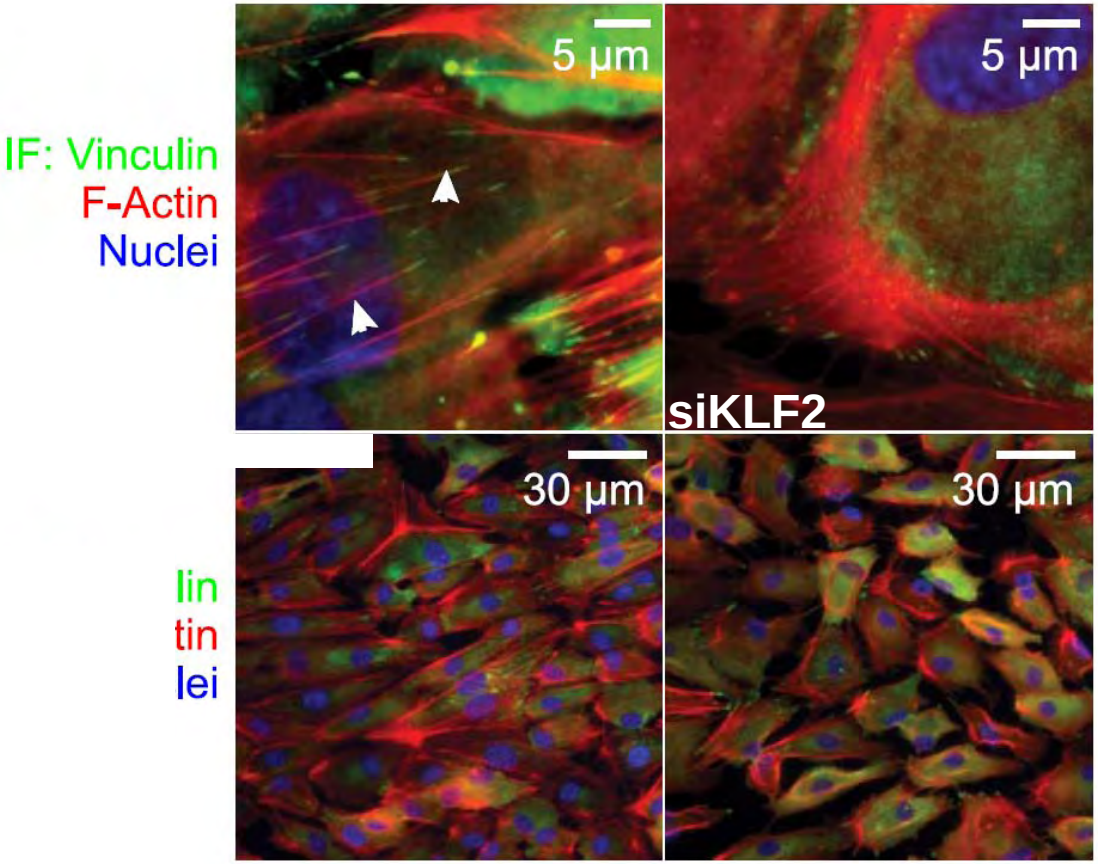
Boon, Blood 2010

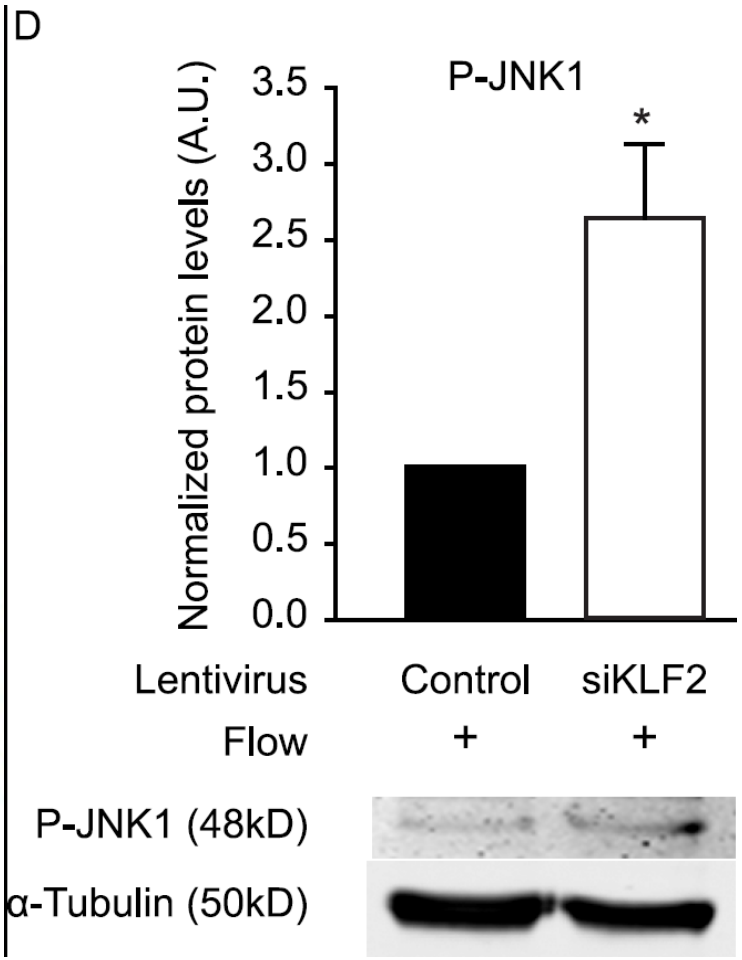
# KLF2 fibers localize on the basal side of endothelium and contain phosphoMyosin



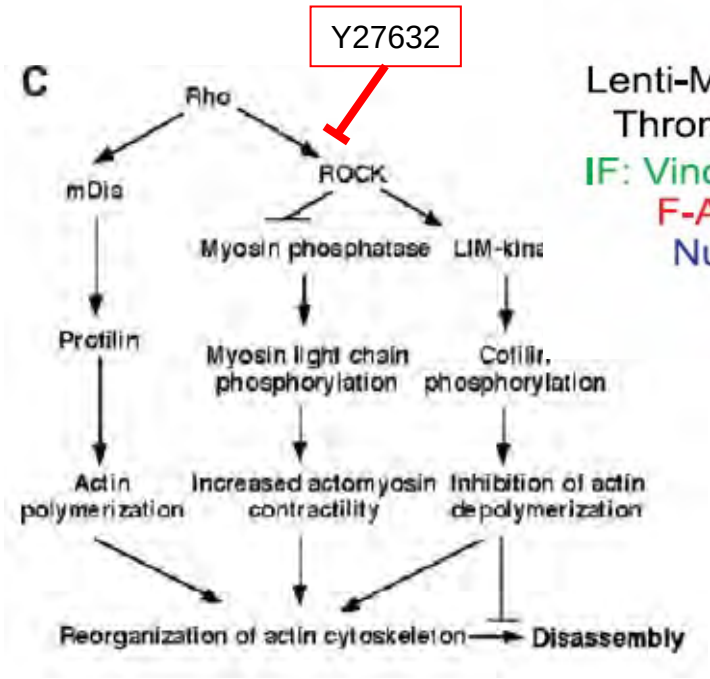



# KLF2 essential for shear fiber induction and JNK suppression

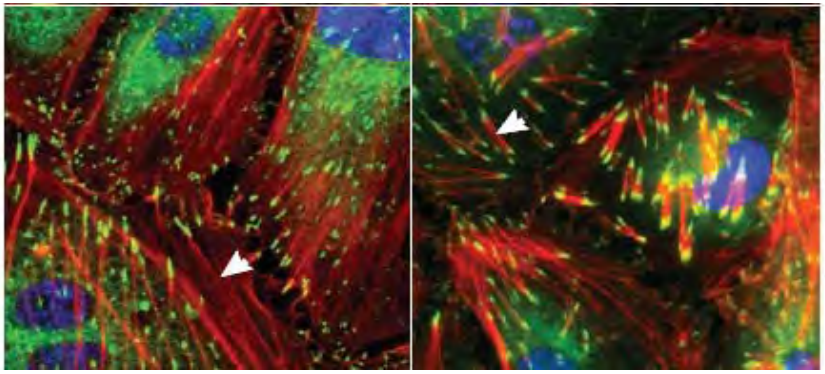


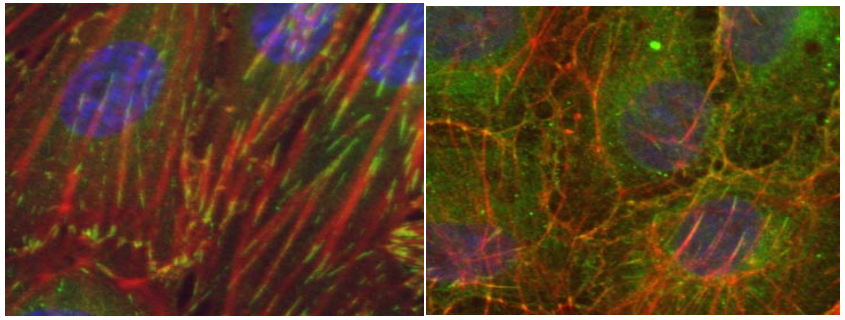
# KLF2 induced shear fibers are ROCK independent, mDIA dependent




Lenti-Mock
Thrombin
IF: Vinculin
F-Actin
Nuclei



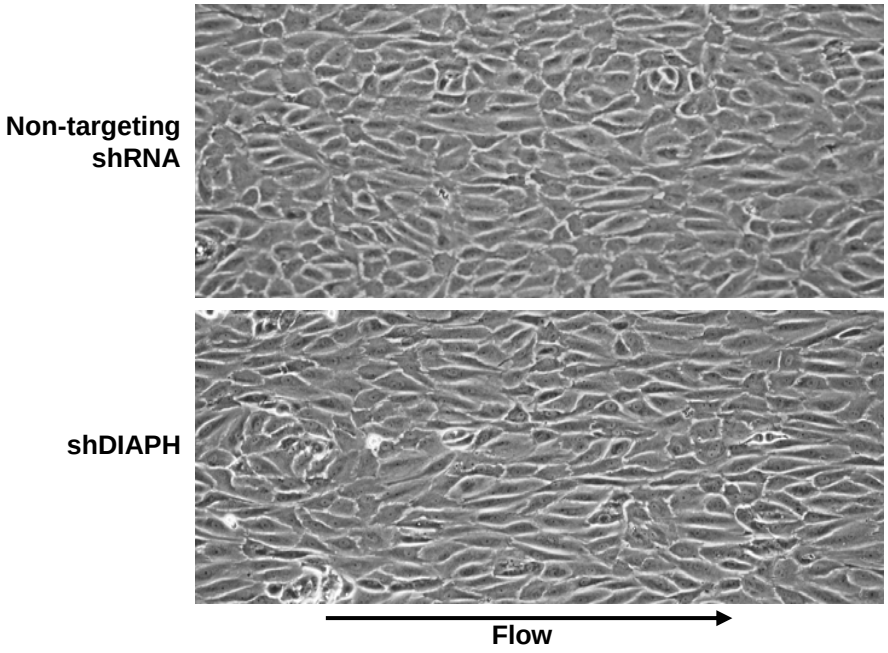
Lenti-KLF2
Y27632
IF: Vinculin
F-Actin
Nuclei

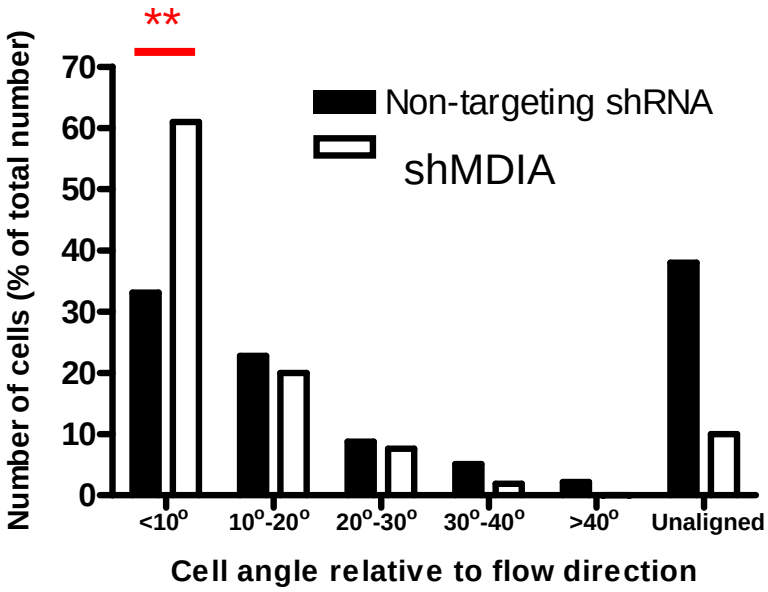


**Lenti-KLF2**
**shMDIA**
**Vinculin**
**F-Actin**
**Nuclei**

Thomas Leyen, 2010

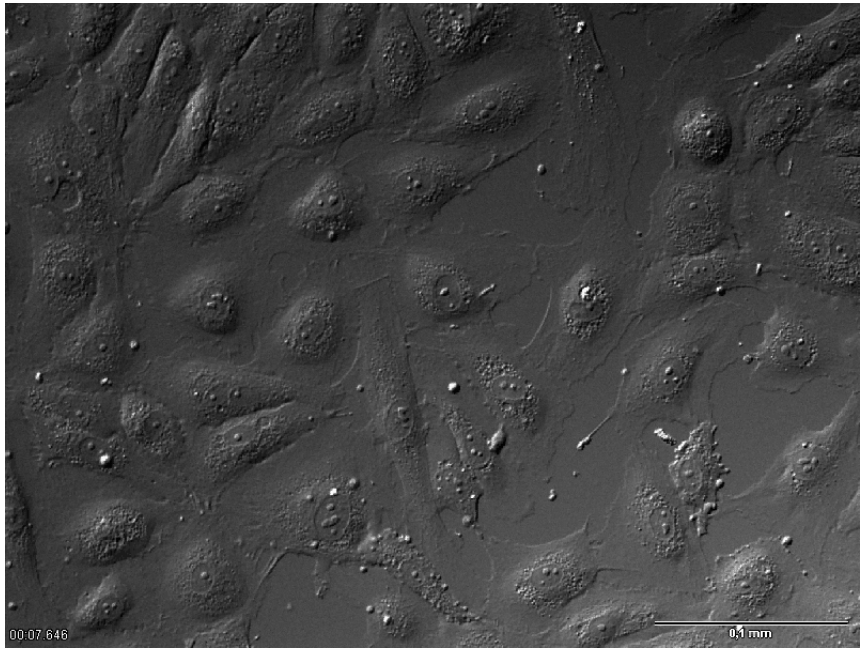
# Absence of shear fibers leads to improved endothelial cell alignment to flow



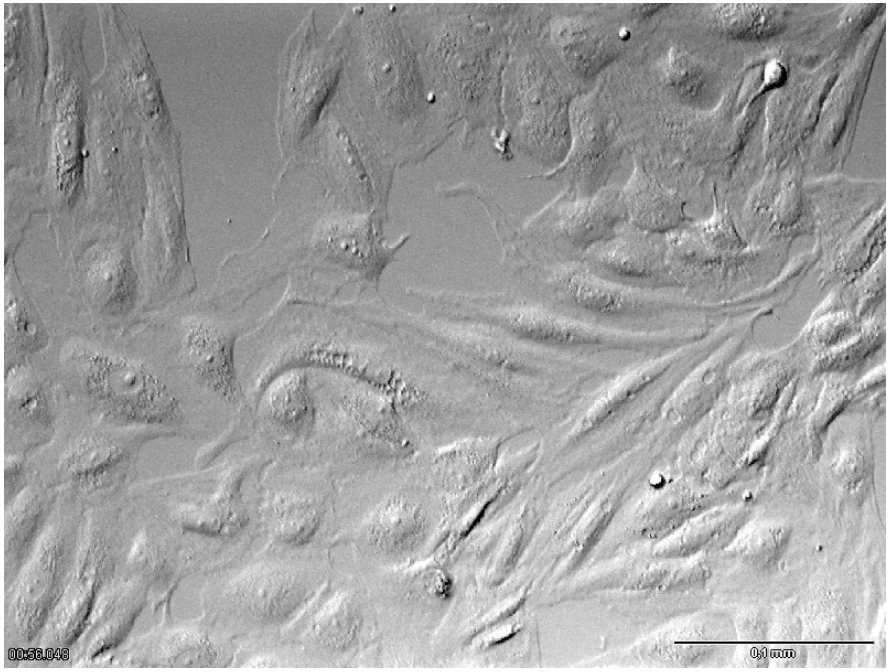



# Shear fibers are important for firm attachment to extracellular matrix

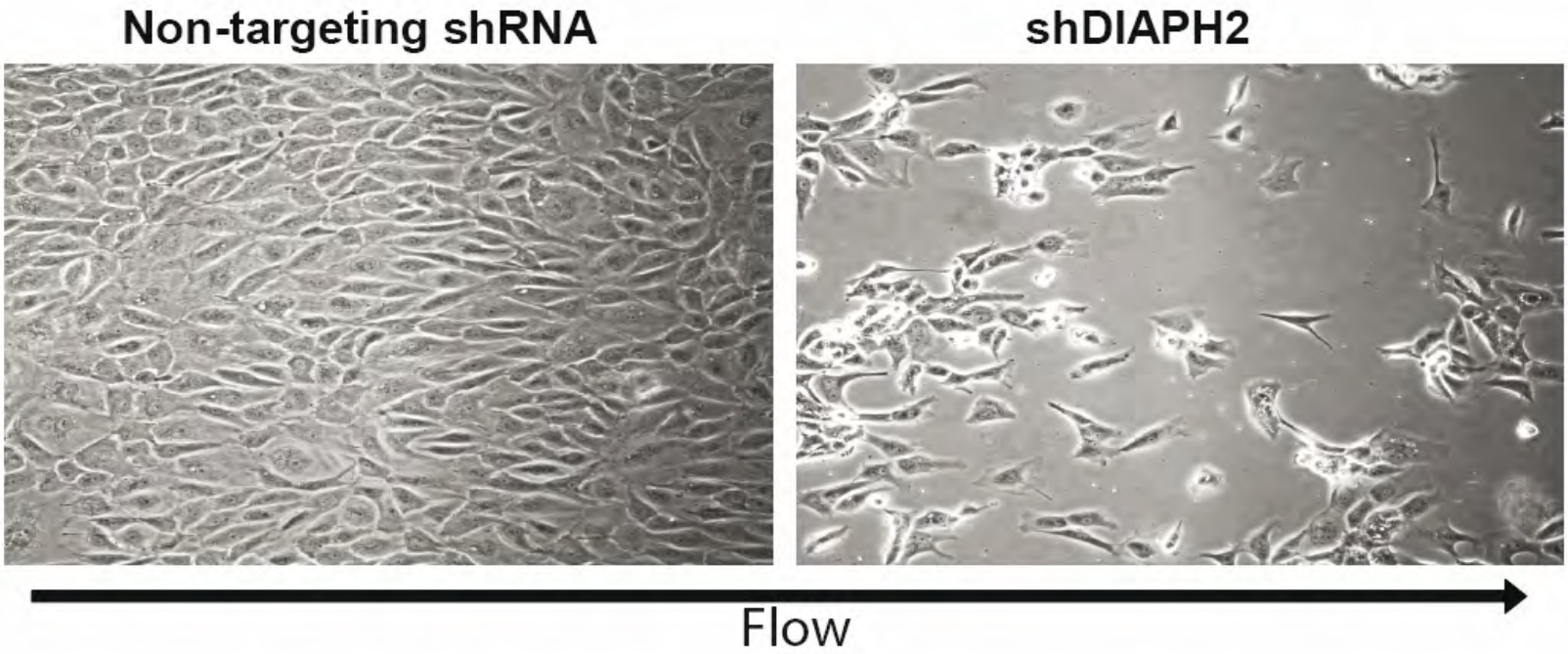
**EC mock**



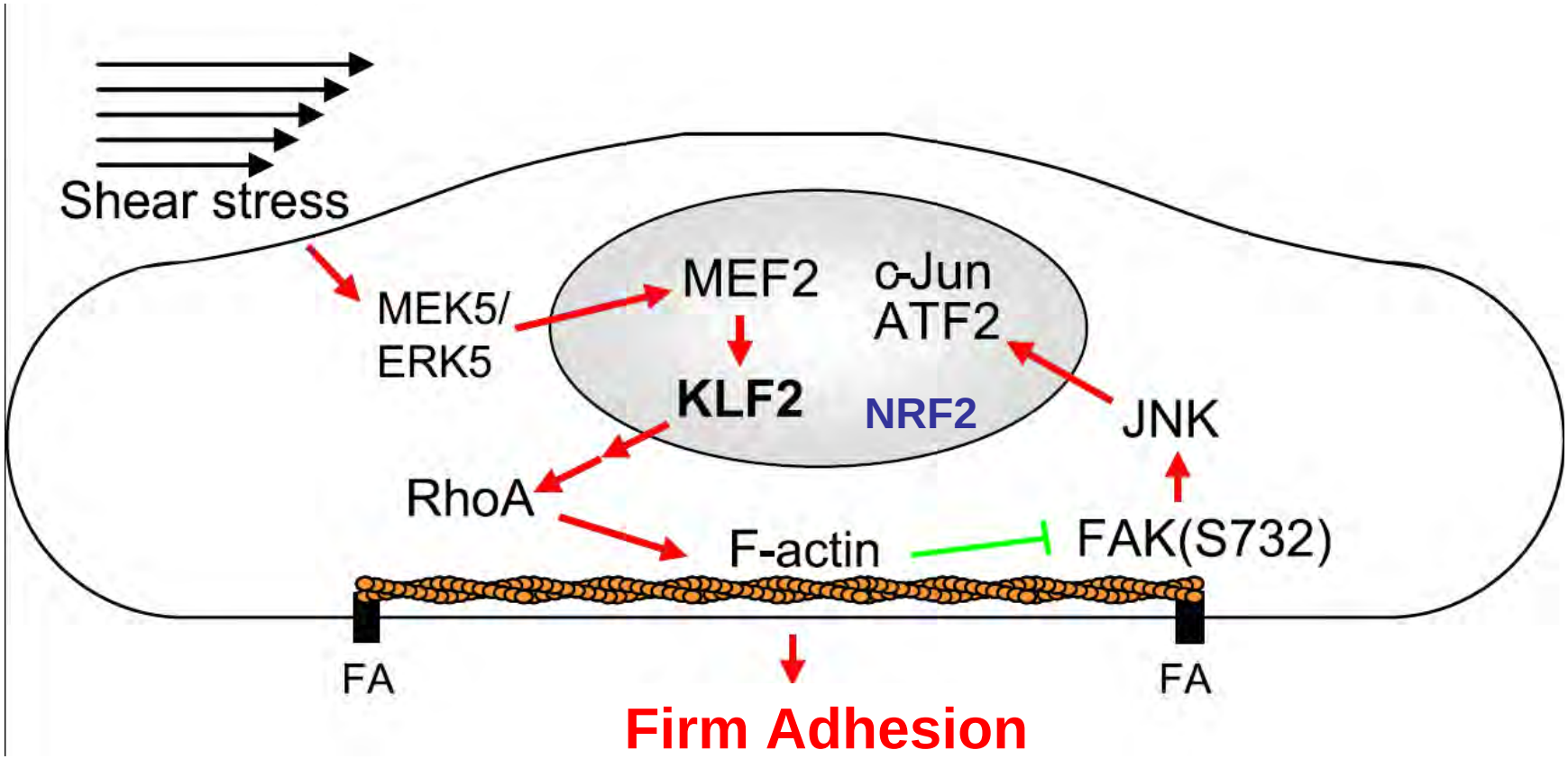

**EC KLF2**



# Shear fibers needed for firm adhesion under flow



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




Boon, Blood 2010

# Shear Stress in a pill ?!

ELSEVIER

Cardiovascular Research 72 (2006) 193 – 195

Cardiovascular Research

www.elsevier.com/locate/cardiores

Editorial

## Sports or statins for atheroprotection? New insight from Kruppel-like factor 2

Thomas Thum [a,b,*], Johann Bauersachs [a]

[a] *Universität Würzburg, Universitätsklinikum, Medizinische Klinik und Poliklinik I, Kardiologie, Würzburg, Germany*
[b] *Universität Würzburg, Interdisciplinary Center for Clinical Research (IZKF), Junior Research Group "Cardiac Wounding/Healing", Würzburg, Germany*

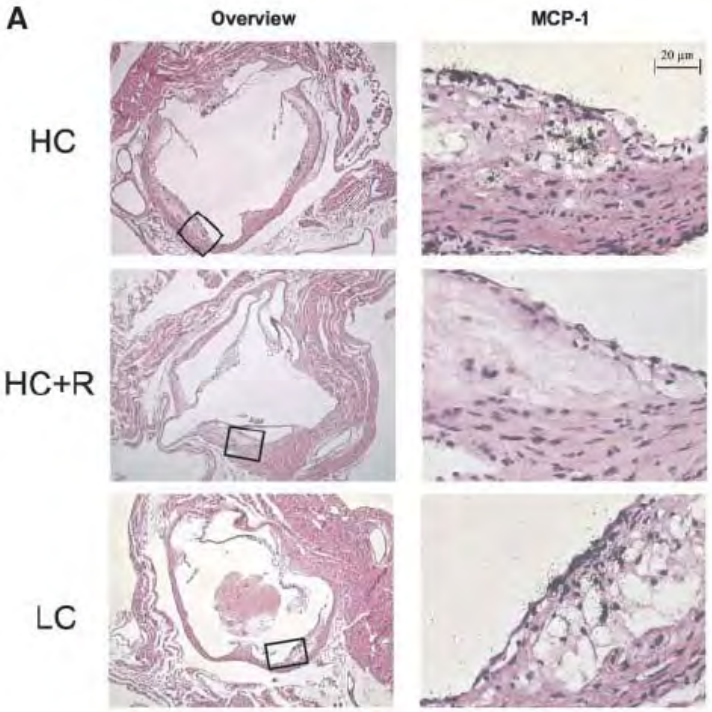
Received 24 July 2006; accepted 24 August 2006
Available online 30 August 2006

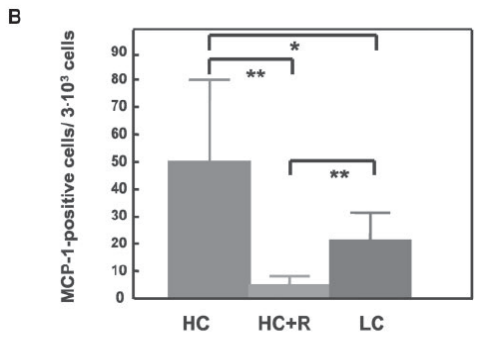
# Rosuvastatin Reduces Atherosclerosis Development Beyond and Independent of Its Plasma Cholesterol–Lowering Effect in APOE*3-Leiden Transgenic Mice

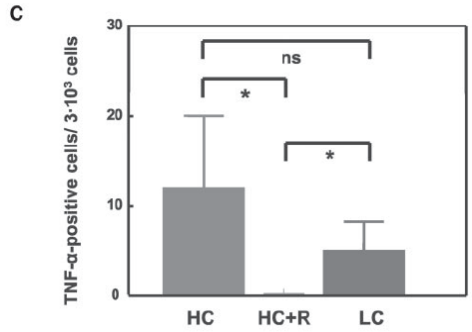
## Evidence for Antiinflammatory Effects of Rosuvastatin

Robert Kleemann, PhD; Hans M.G. Princen, PhD; Jef J. Emeis, PhD; J. Wouter Jukema, MD, PhD; Ruud D. Fontijn, MSc; Anton J.G. Horrevoets, PhD; Teake Kooistra, PhD; Louis M. Havekes, PhD

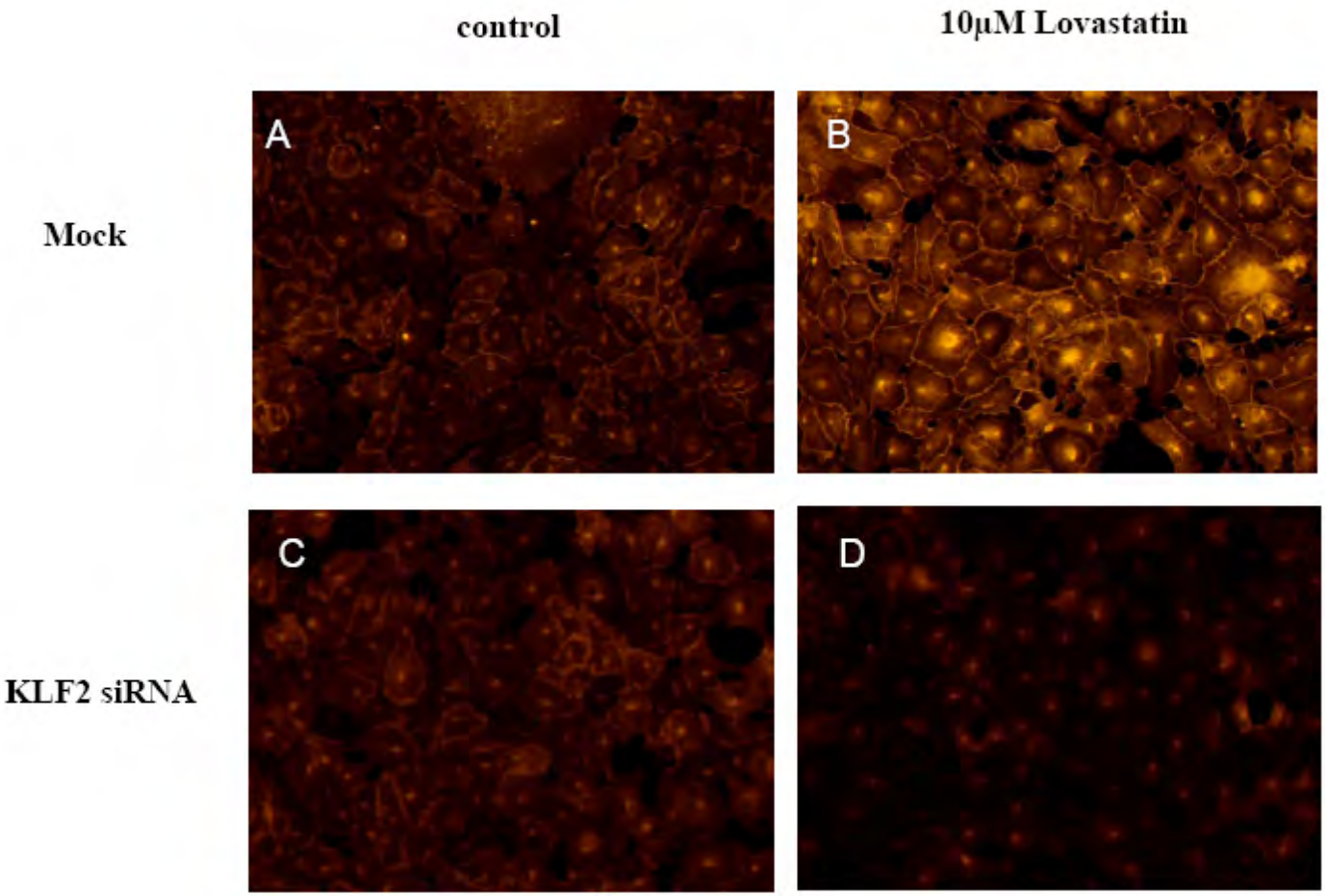
(*Circulation*. 2003;108:1368-1374.)



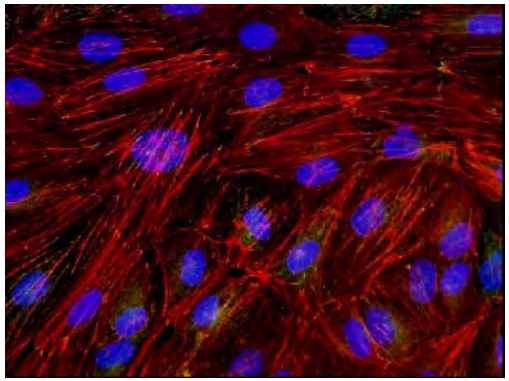
# Statins induce eNOS and thrombomodulin through KLF2
## (involves MAPK ERK5 and Rho)



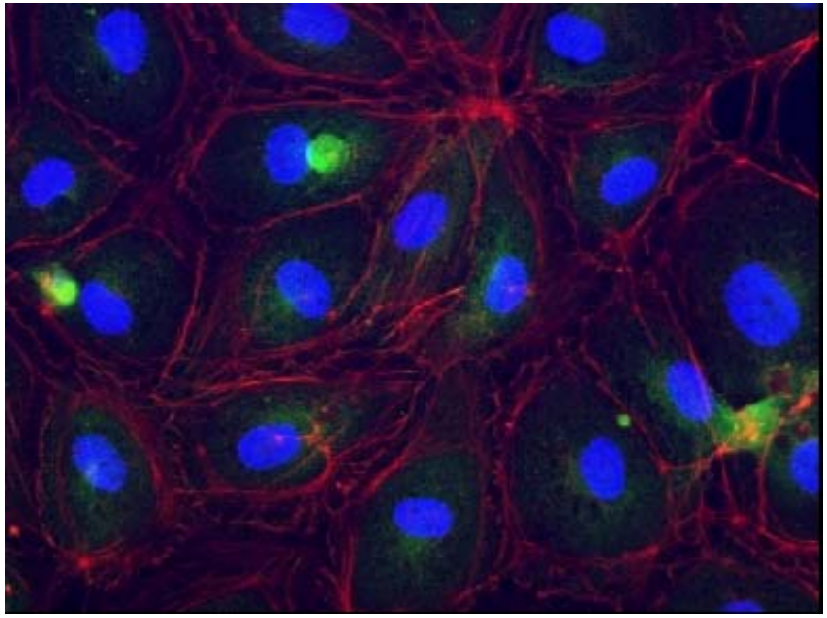

Van Thienen, Cardiovasc. Res 2006

# Is shear stress a drugable target?

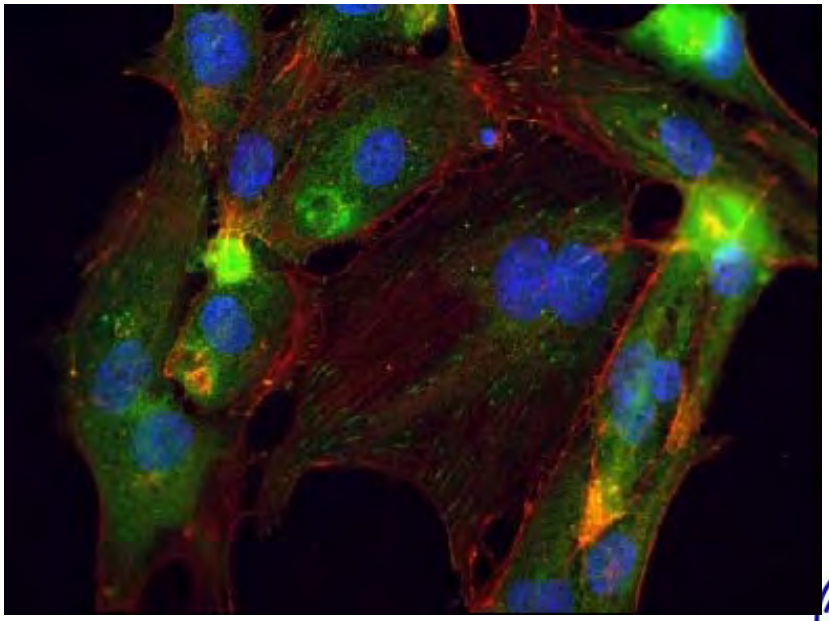
Lenti-KLF2



Statins



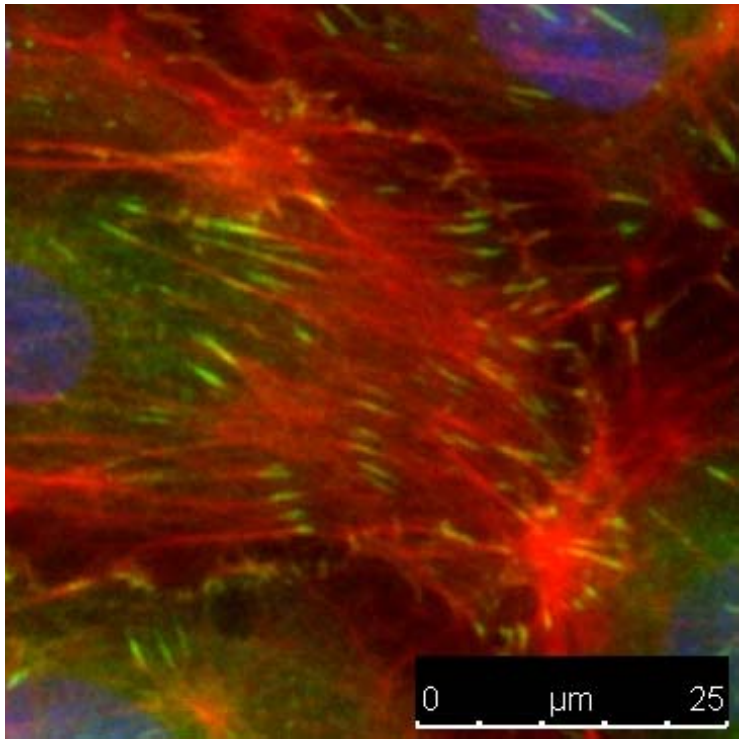
Resveratrol



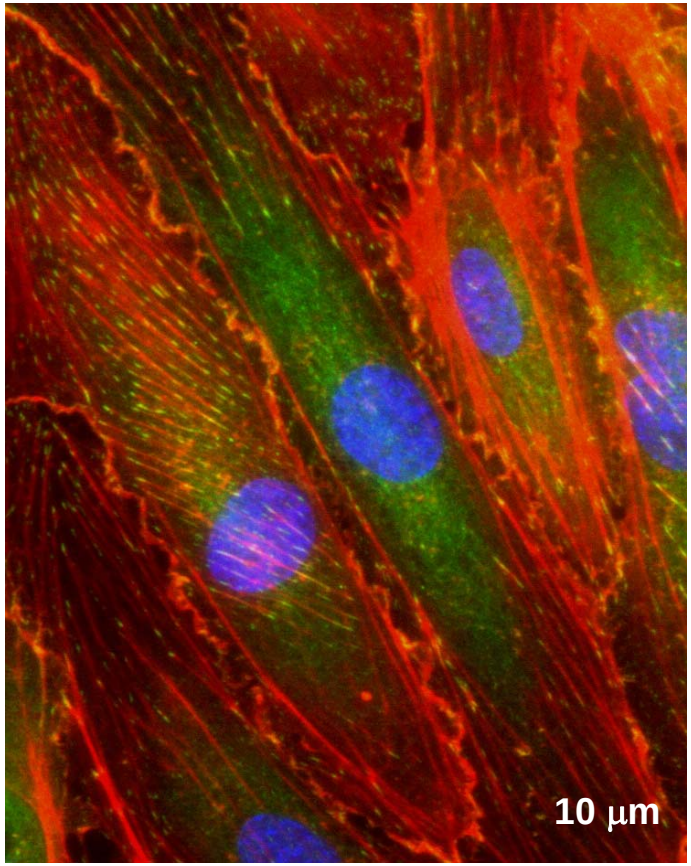
# Resveratrol activates SIRT1, CPTH2 inhibits GCN5
# Both induce KLF2 and shear fibers, MEF2 dependent

**72h CPTH2 0,2 mM**

**50 μM**
**Resveratrol 72h**

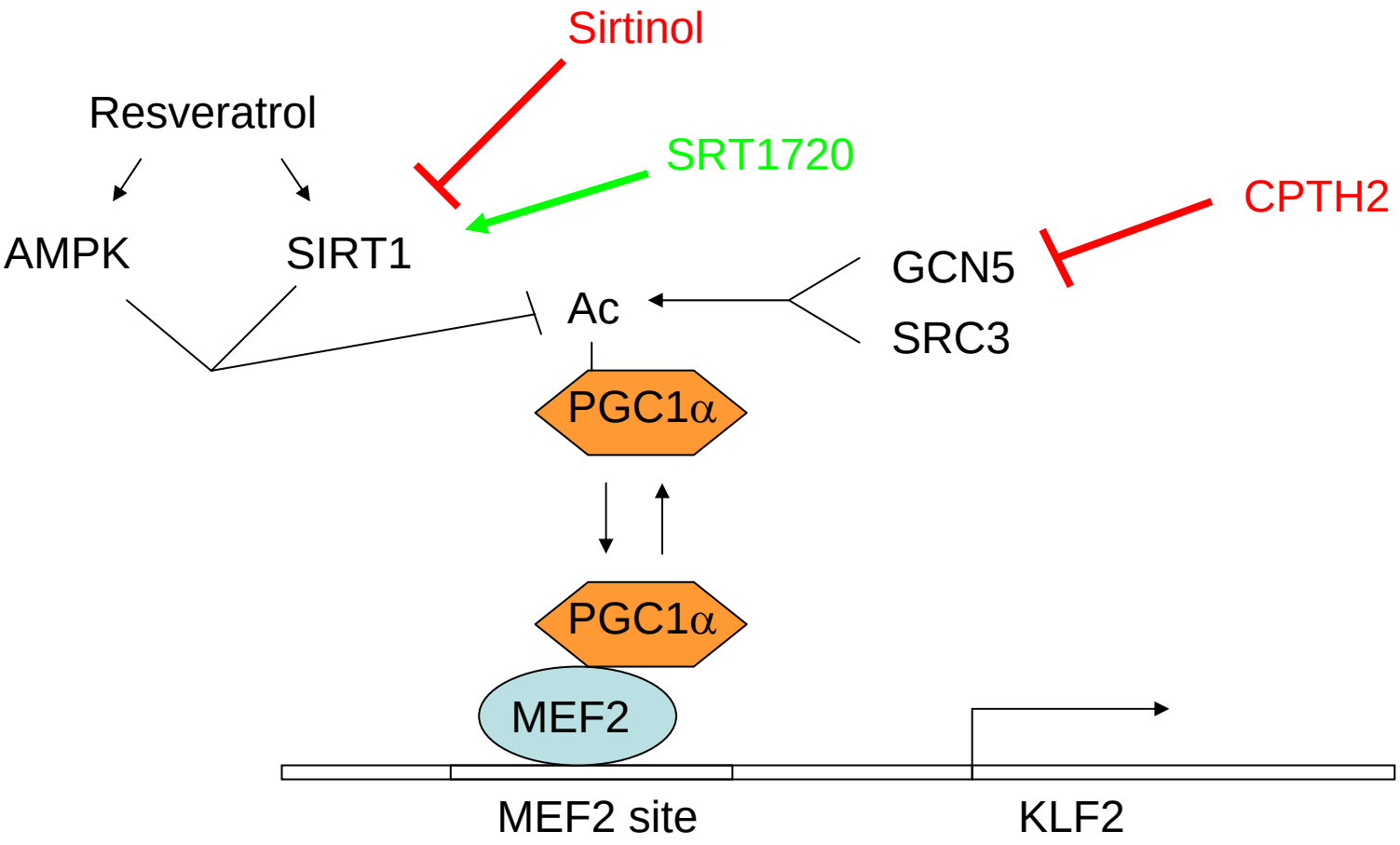



IF: Vinculin
F-Actin
Nuclei

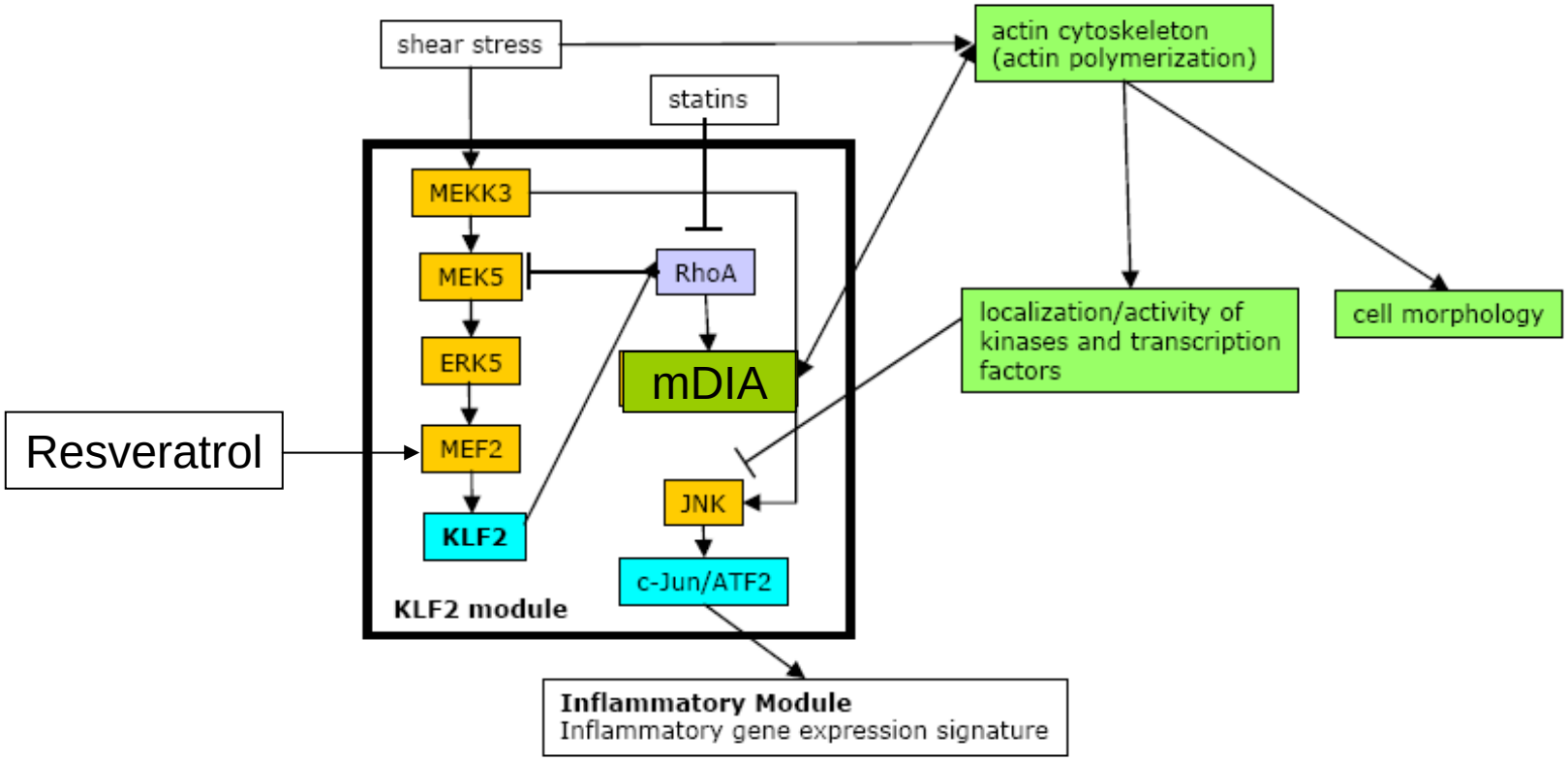


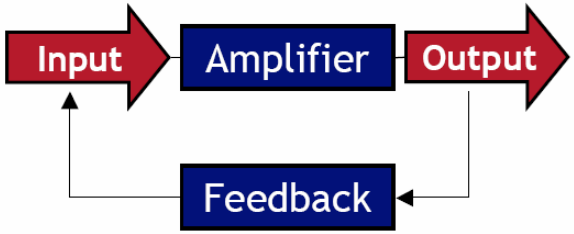

Thomas Leyen 2010

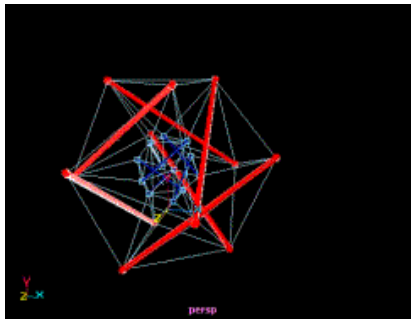
# Possible pathway for small molecule-mediated KLF2 expression involve PGC1a



# An integrated KLF2 module for vascular protection





Ingber Tensegrity website

# Endothelial Balancing Act: Actin

Ingber, D. E. J Cell Sci 2003;116:1157-1173

# The protective endothelial shear response

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

-induction of eNOS and thrombomodulin
-promotes activity of anti-oxydant transcription factor NRF2
-focal adhesion capped shear fibers
    -results in firm adhesion of EC to substratum
    - less alignment (flow resistance)
    - suppresses SMAD, c-Jun and ATF2 activity / antiinflammatory

## The KLF2/shear modules can be modified by small molecules

-Resveratrol induces KLF2 and shear fibers,mediated through SIRT1 and MEF2
-CPTH2 induces shear fibers indicating a role for PGC1a acetylation

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