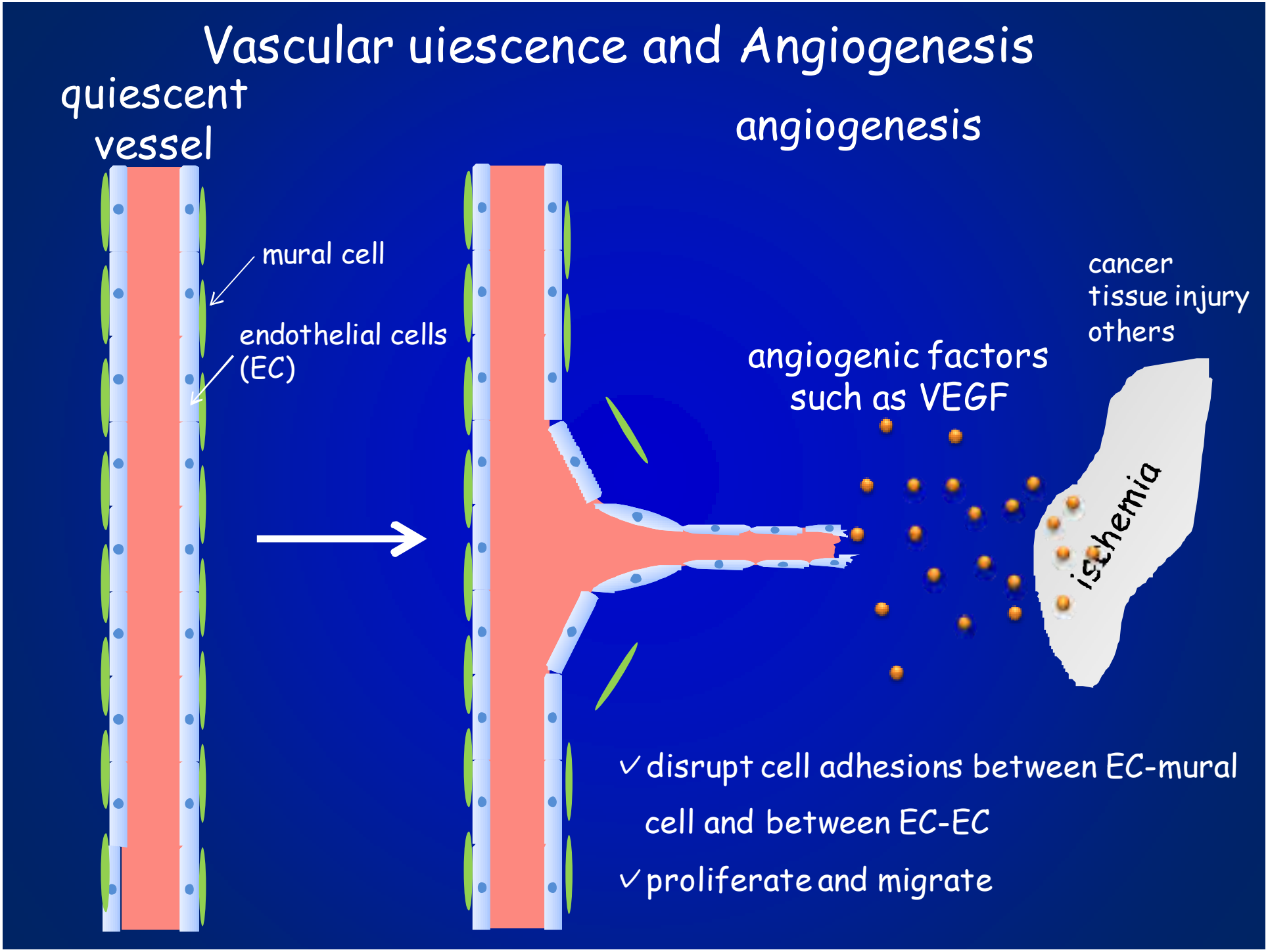
Japan-Mexico Workshop on "Pharmacology" and "Nanobiology"
Feb. 25, 2009; Universidad Nacional Autönoma de Mëxico, Mexico City

Angiostasis and Angiogenesis Regulated by Angiopoietin1-Tie2 Receptor System

Shigetomo Fukuhara

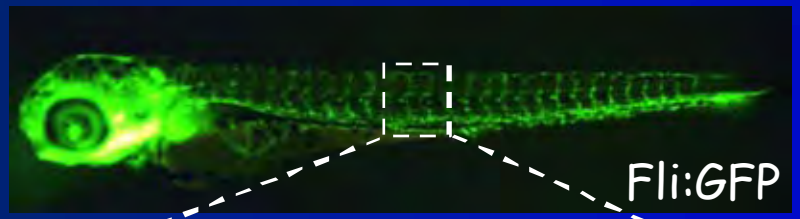
Department of Structural Analysis,
National Cardiovascular Center Research Institute

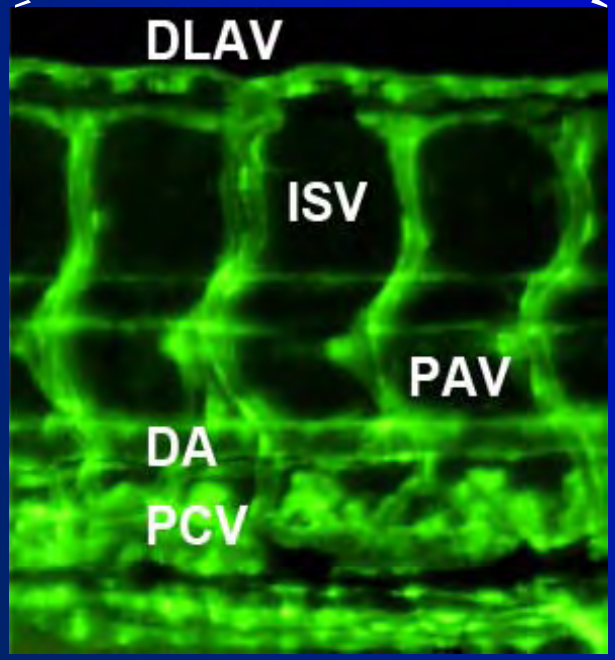
Vascular uiescence and Angiogenesis

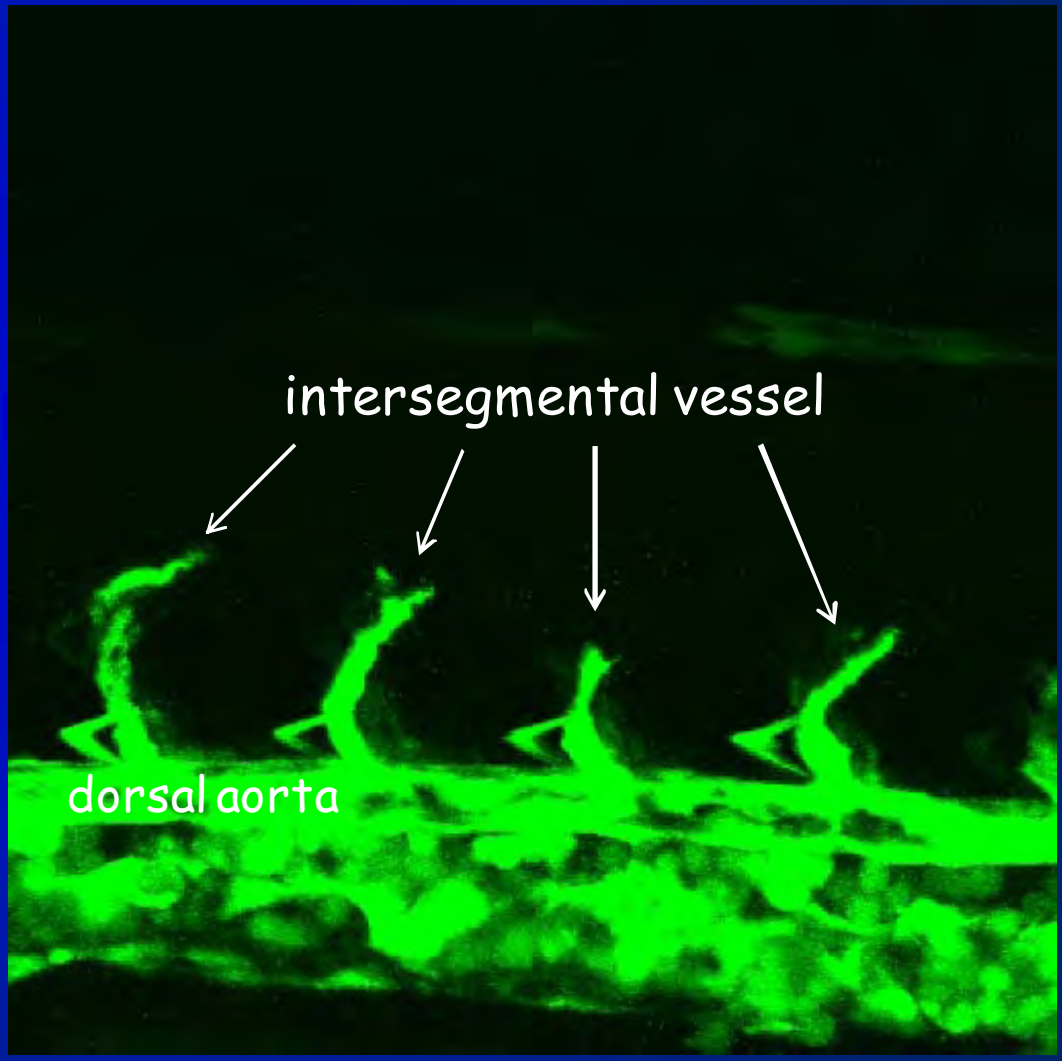


Visualization of in vivo angiogenesis

Transgenic zebrafish embryo expressing GFP in vascular endothelial cells



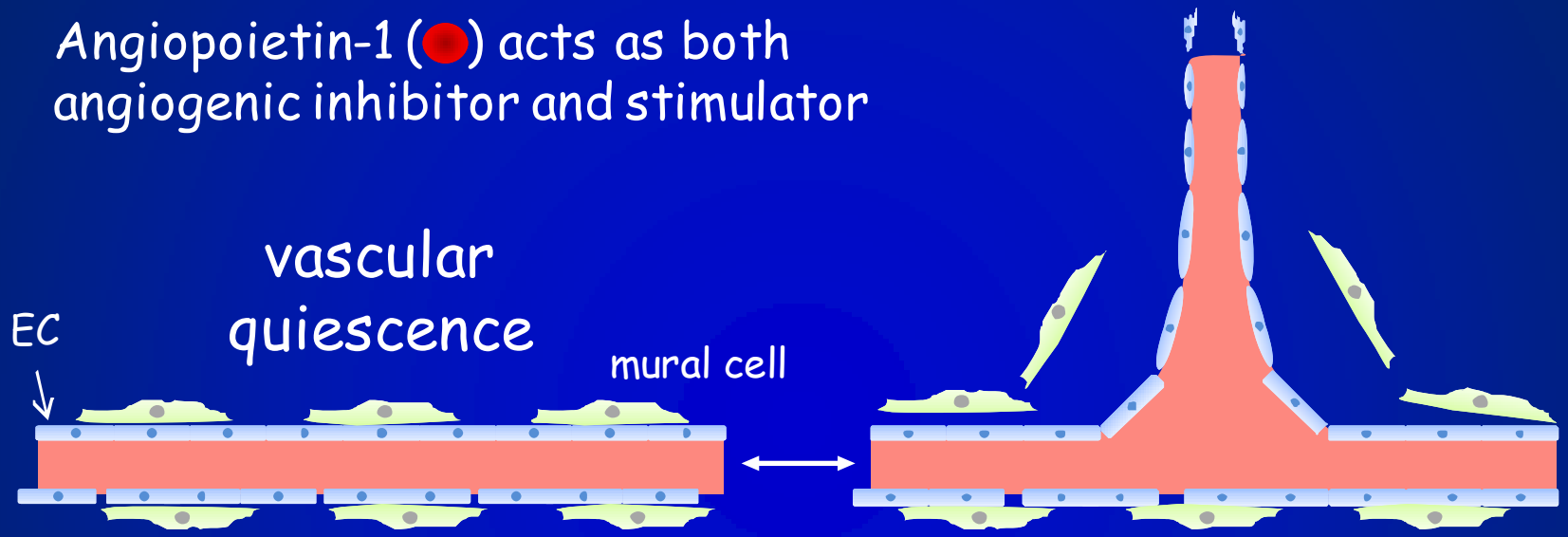




Vascular quiescence vs. Angiogenesis

angiogenesis

Angiopoietin-1 (●) acts as both angiogenic inhibitor and stimulator



Angiogenic inhibitor

Angiogenic stimulator

Angiopoietin-1/Tie2 receptor system

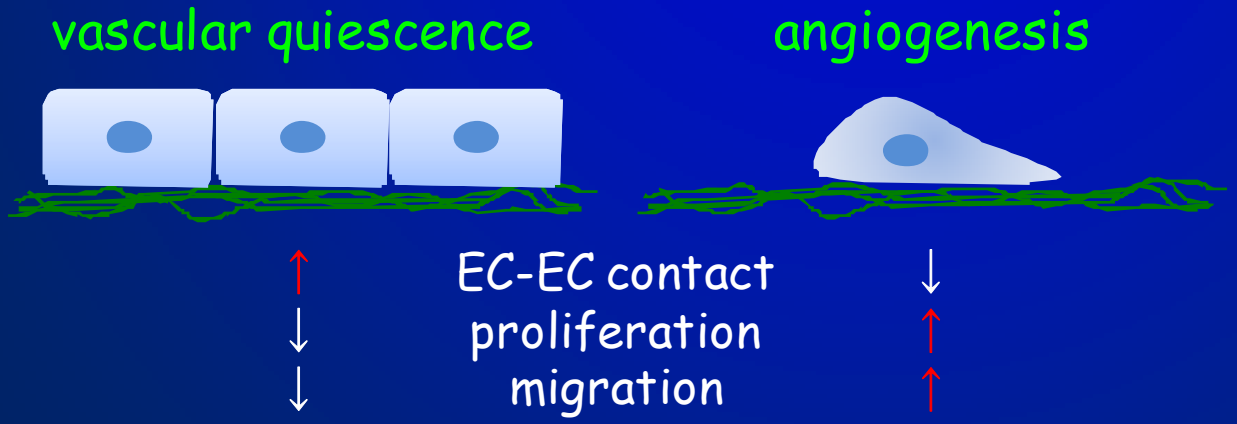
✓ Angiopoietin-1 (Ang1) is a ligand for the receptor tyrosine kinase Tie2, which is expressed on vascular endothelial cells.

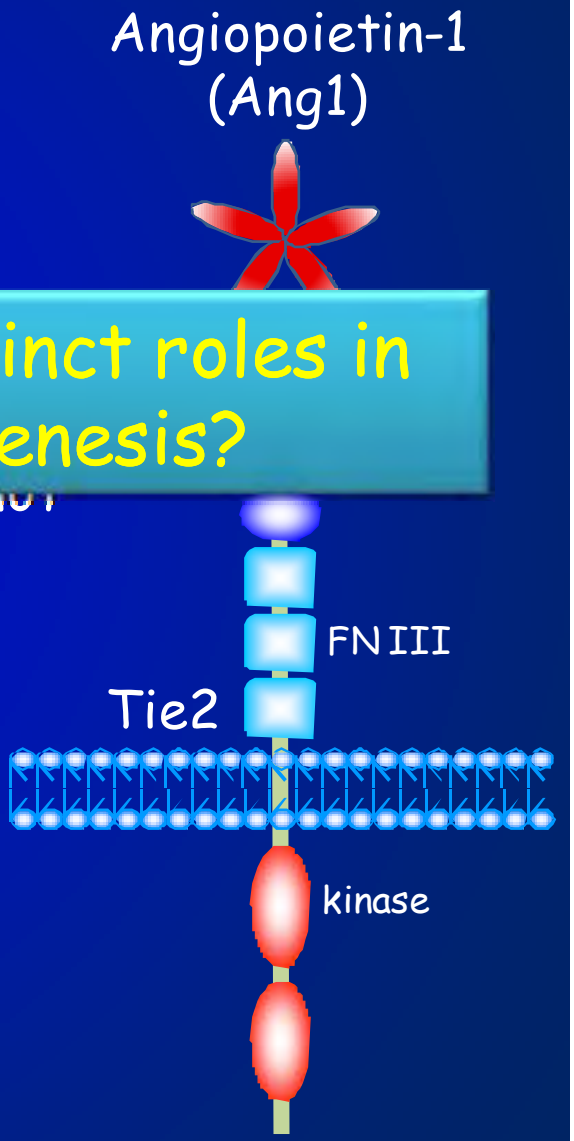
✓ Ang1/Tie2 signal plays a critical role in

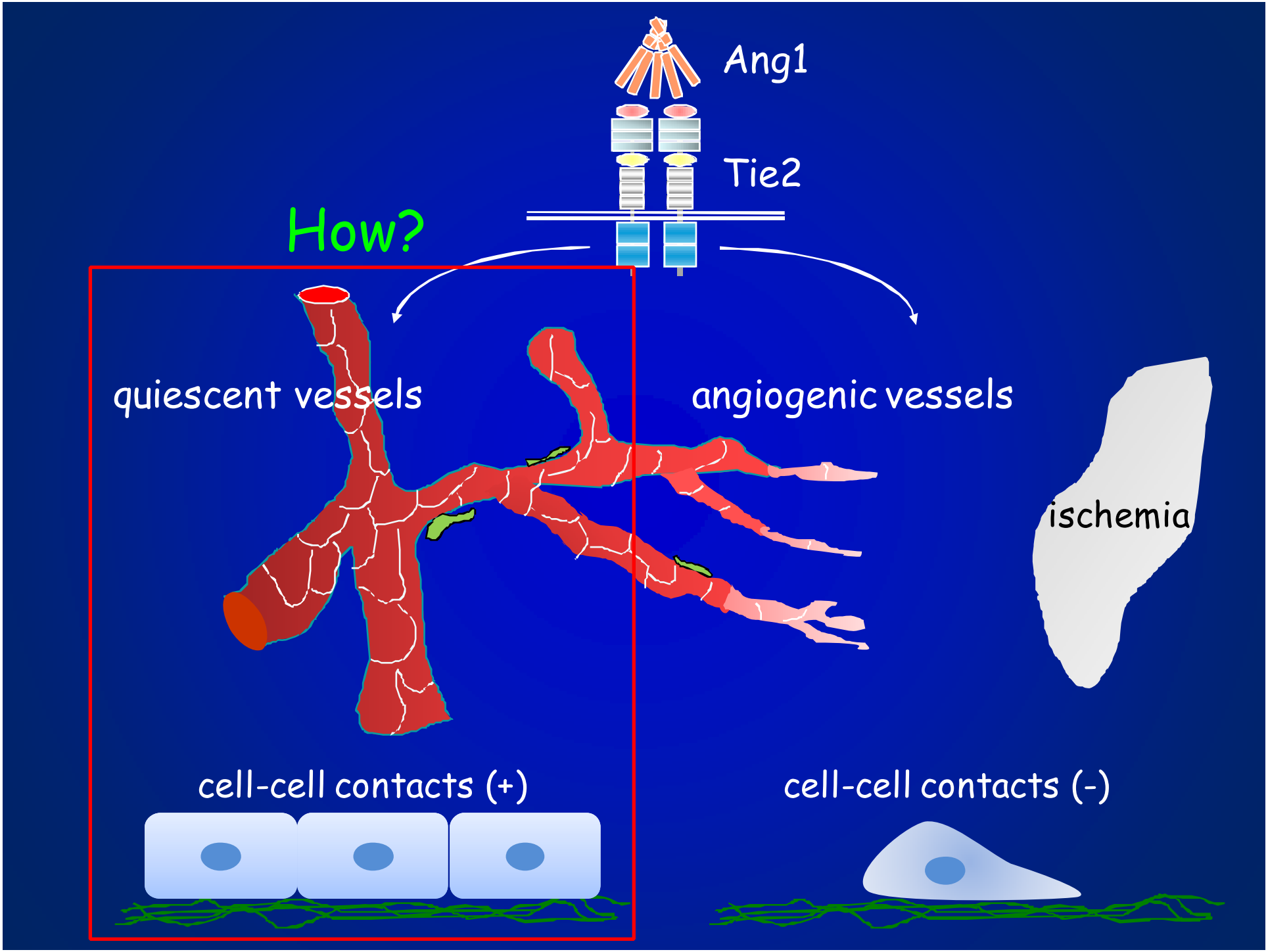
d

How does Ang1/Tie2 signal play distinct roles in both vascular quiescence and angiogenesis?

✓ In addition, Ang1/Tie2 signal regulates not only vascular quiescence, but also physiological and pathological angiogenesis.





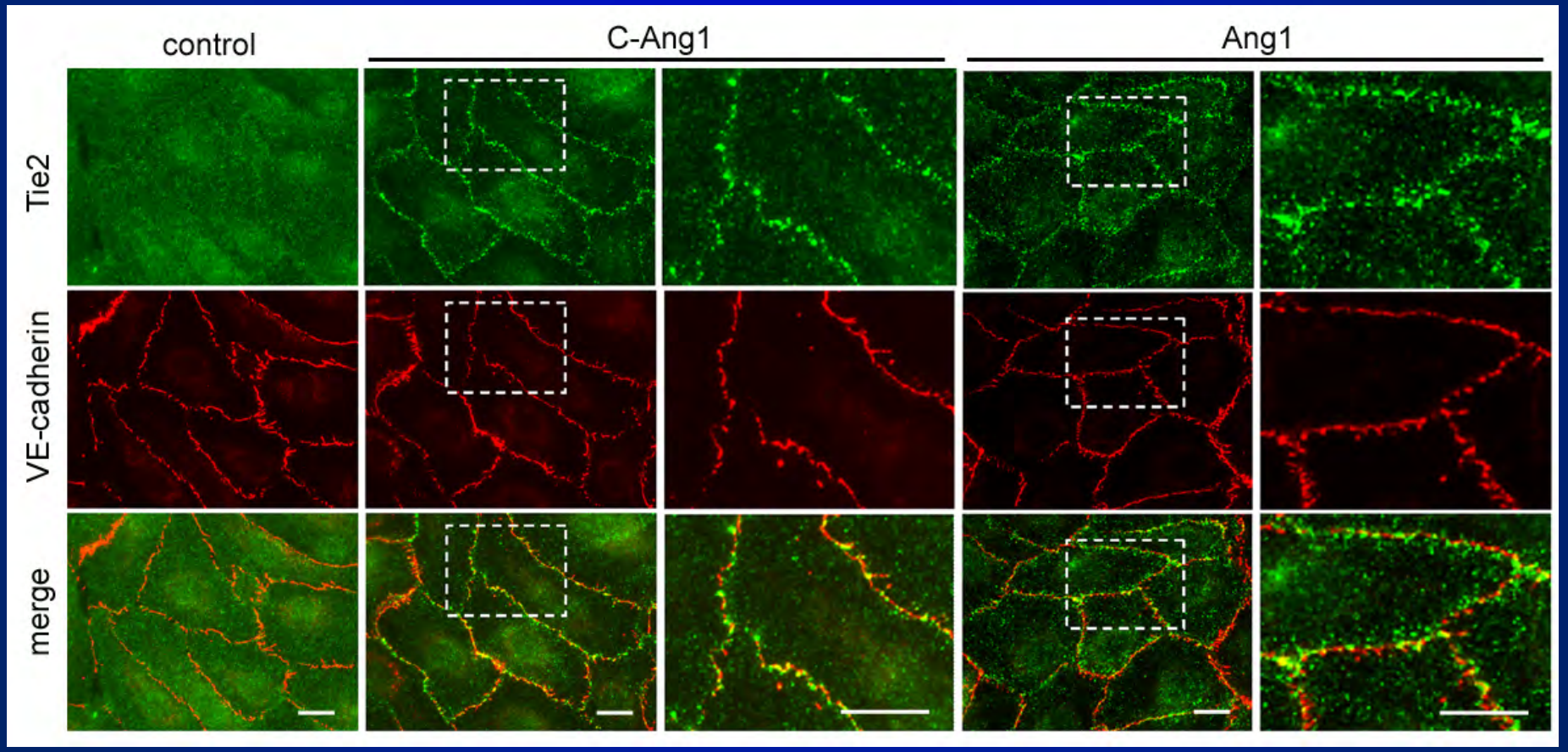

Ang1
Tie2
How?
quiescent vessels
angiogenic vessels
ischemia
cell-cell contacts (+)
cell-cell contacts (-)

Ang1 induces recruitment of Tie2 at cell-cell contacts

COMP-Ang1

Ang1

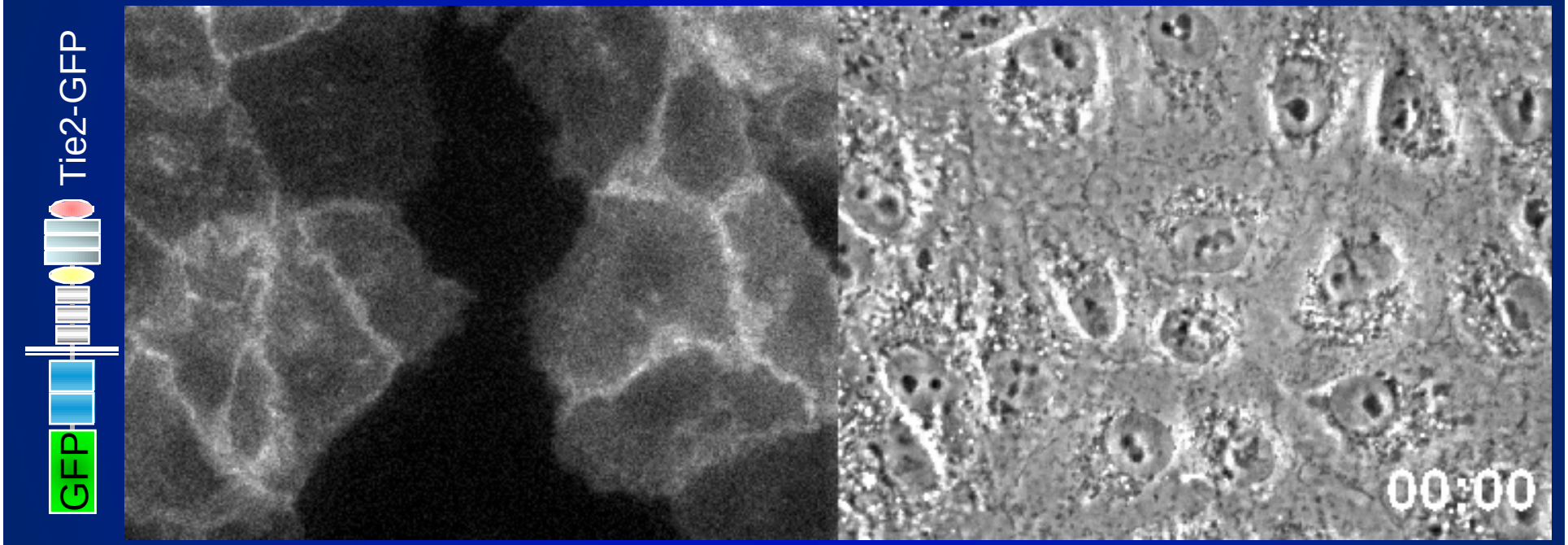
HUVEC



COMP (Cartilage oligomeric matrix protein)

Ang1 induces recruitment of Tie2 at cell-cell contacts

CHO cells expressing Tie2-GFP were stimulated with COMP-Ang1

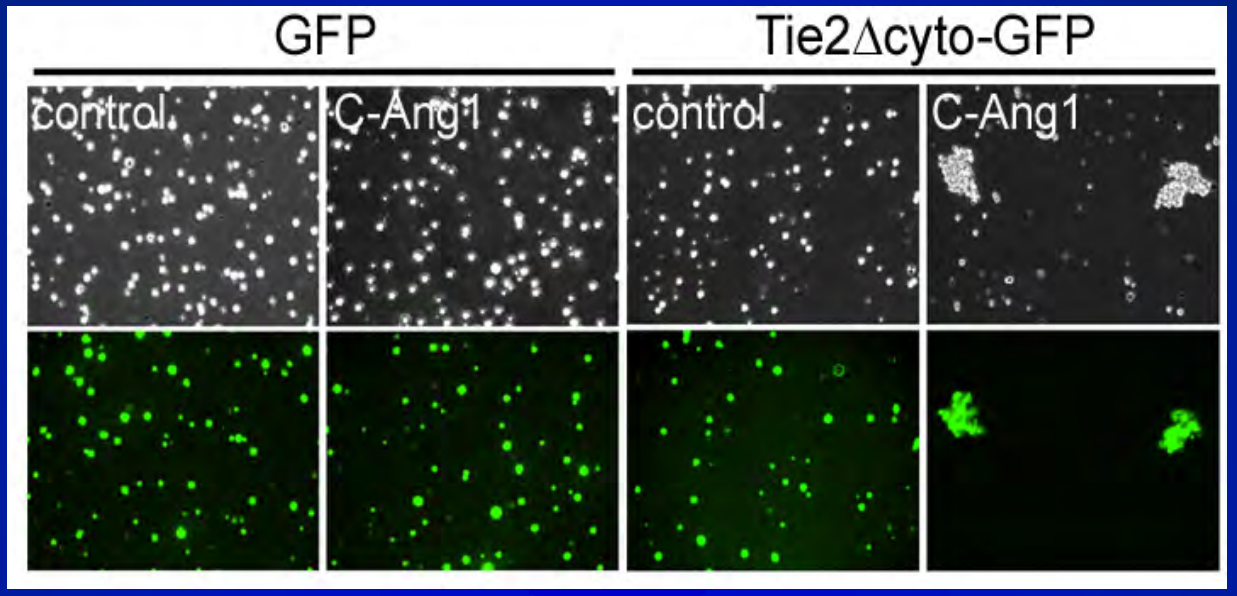


Accumulation of Tie2 at cell-cell contacts requires Tie2 expression in adjacent cells.

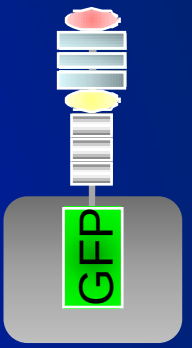
Ang1 may induce *trans*-association of Tie2 at cell-cell contacts

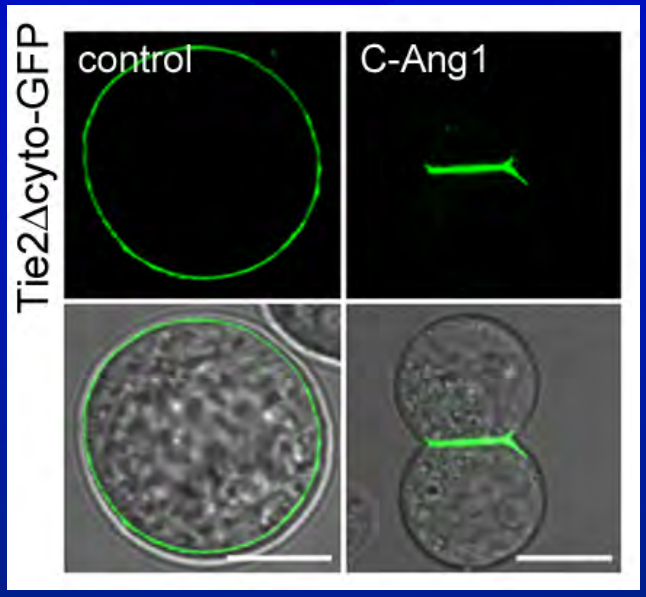
Ang1 induces aggregation of 293F cells expressing Tie2Δcyto-GFP in suspension.

Cell expressing GFP

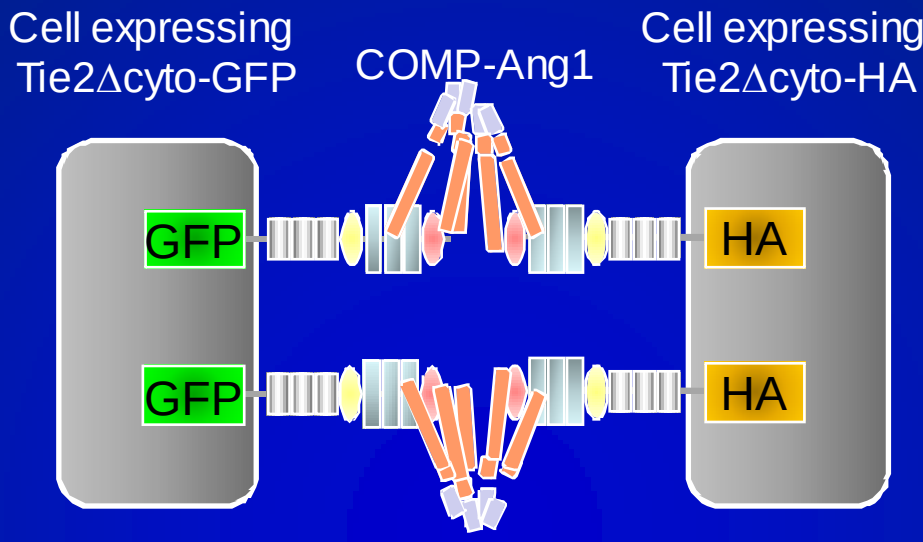


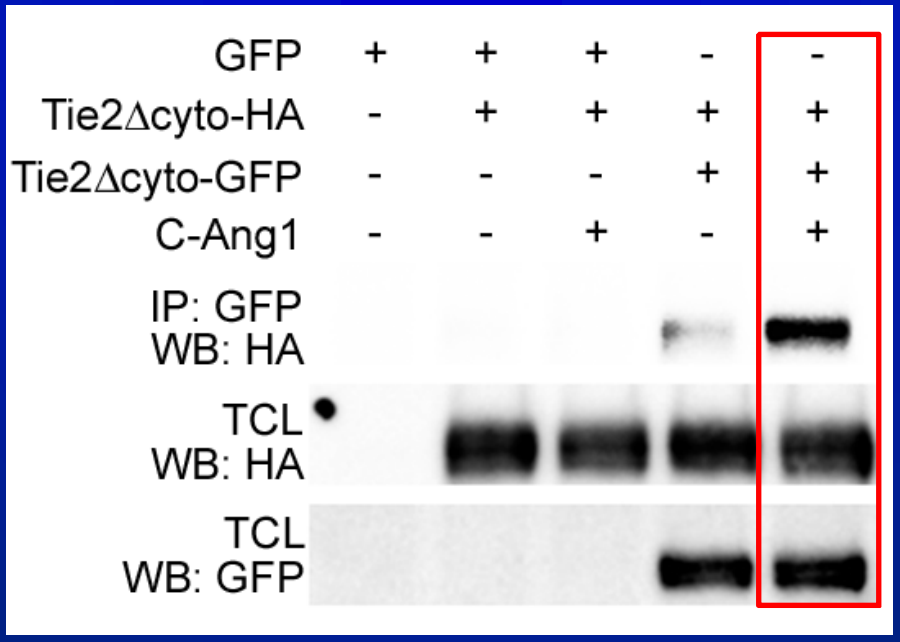
Cell expressing Tie2Δcyto-GFP





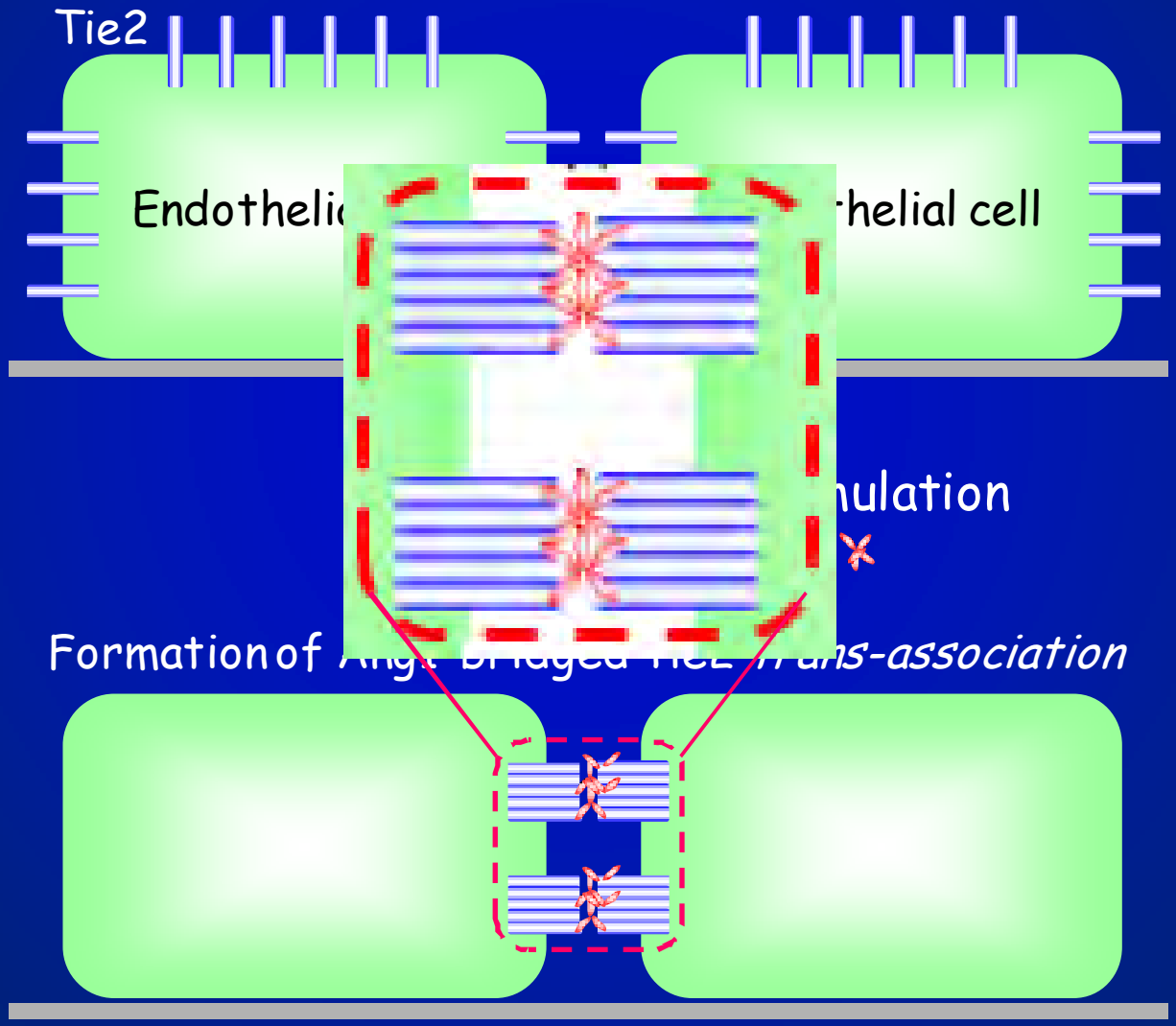
Ang1 induces *trans*-association of Tie2 at cell-cell contacts

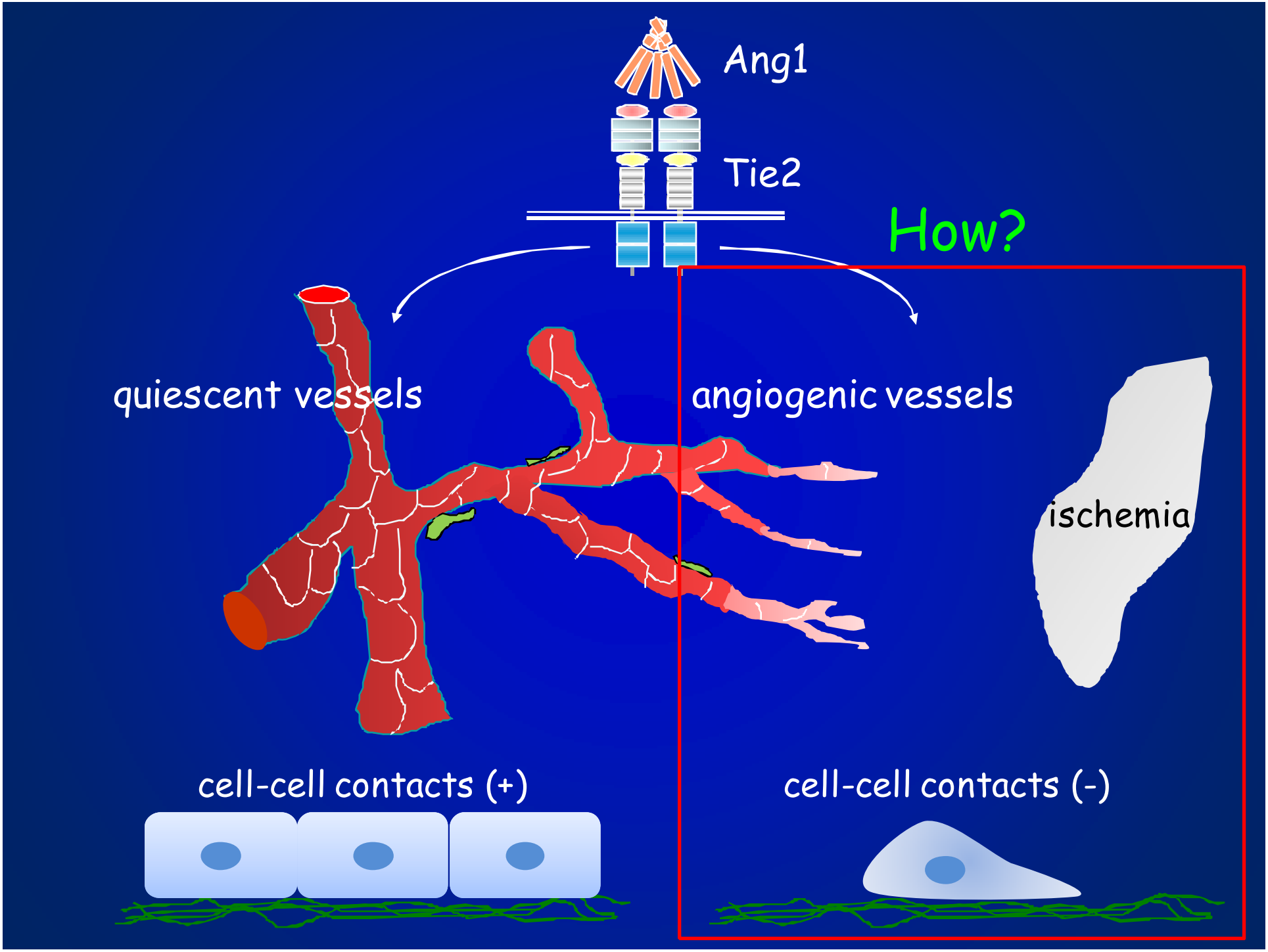




Conclusion Part1: in the presence of cell-cell contacts

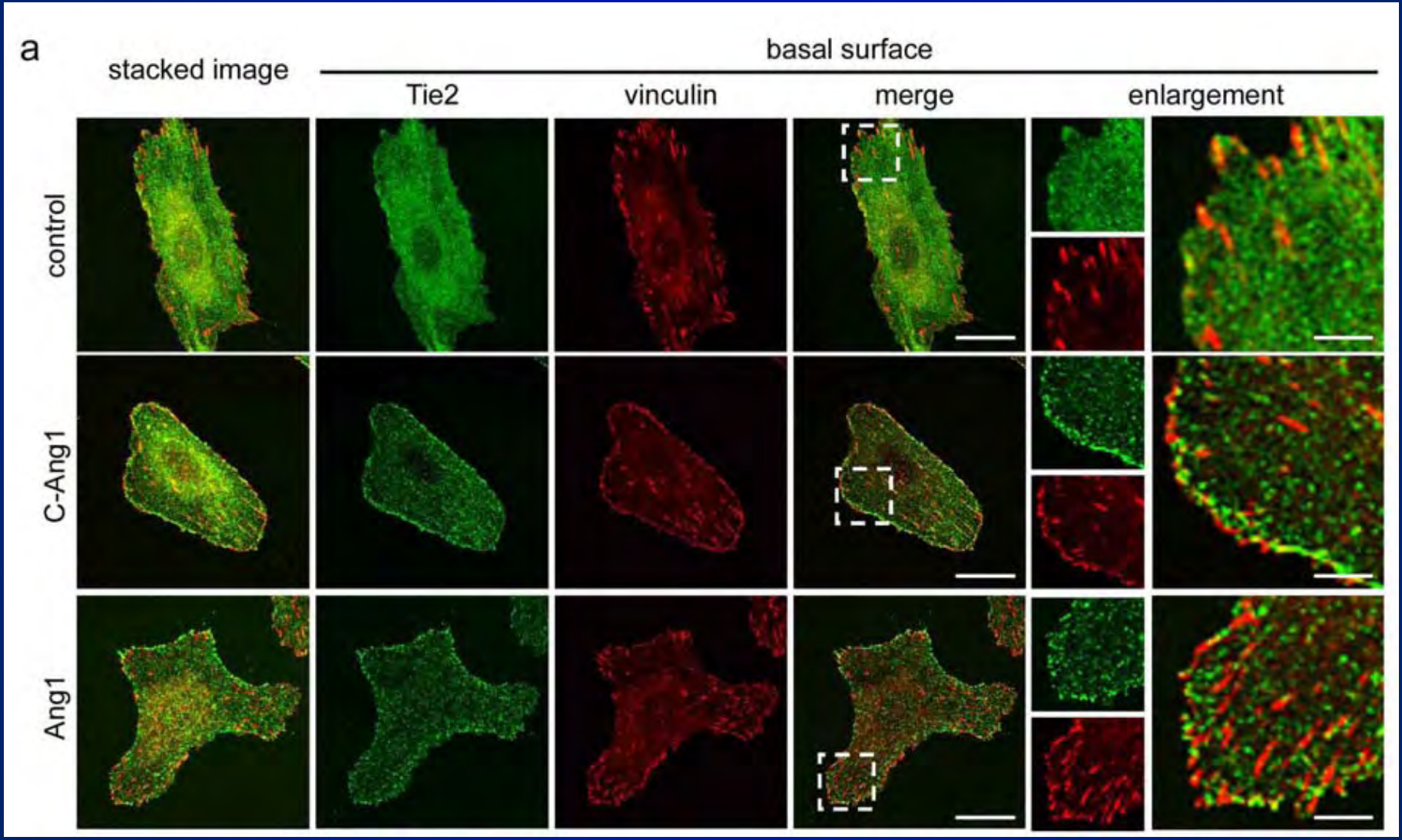
Tie2 is broadly expressed on plasma membrane in the absence of Ang1




Ang1
Tie2
How?
quiescent vessels
angiogenic vessels
ischemia
cell-cell contacts (+)
cell-cell contacts (-)

Ang1 induces accumulation of Tie2 at cell-substratum contacts, which are different from focal adhesions.

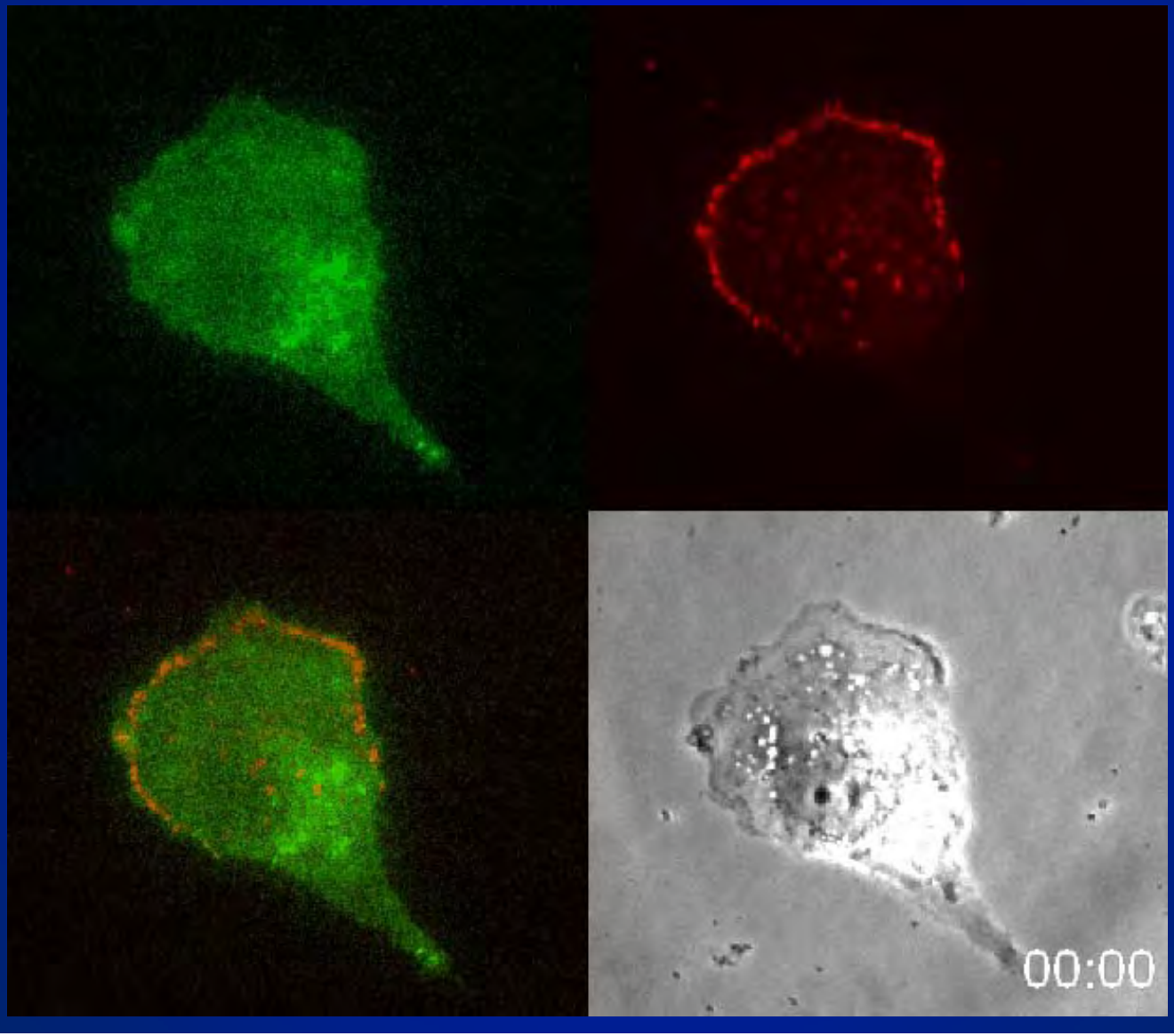
Sparse HUVECs were stimulated with COMP-Ang1 and Ang1.



Extracellular domain is sufficient for accumulation of Tie2 at cell-substratum interface

Sparse HUVEC expressing Tie2Δcyto-GFP and RFP-Crk (focal adhesion marker) were stimulated with COMP-Ang1

Tie2Δcyto -GFP

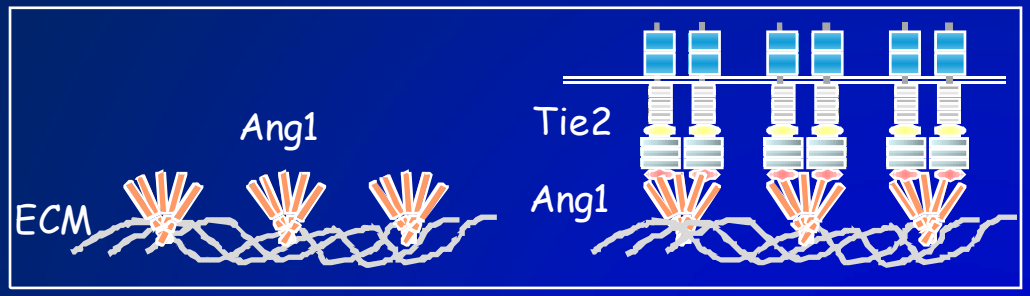


RFP-Crk (focal adhesion marker)

merge

phase contrast

Tie2 is anchored by ECM-bound Ang1 to cell-substratum contacts

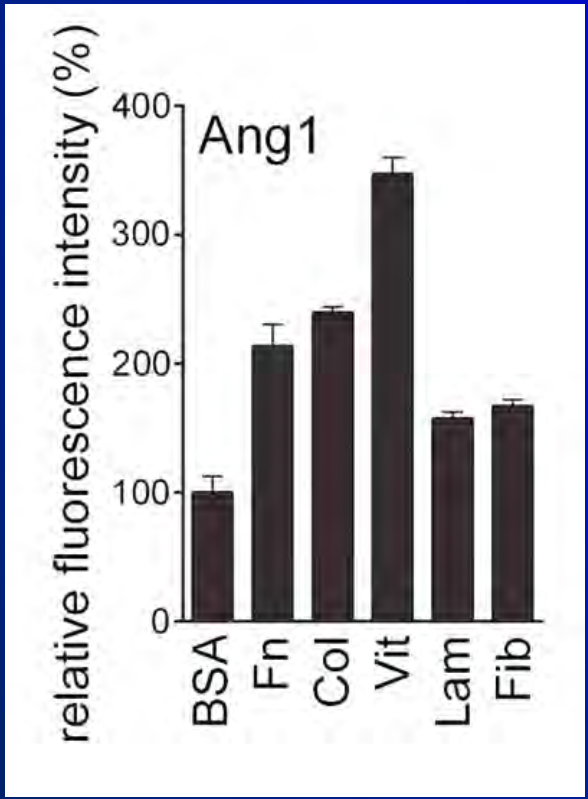


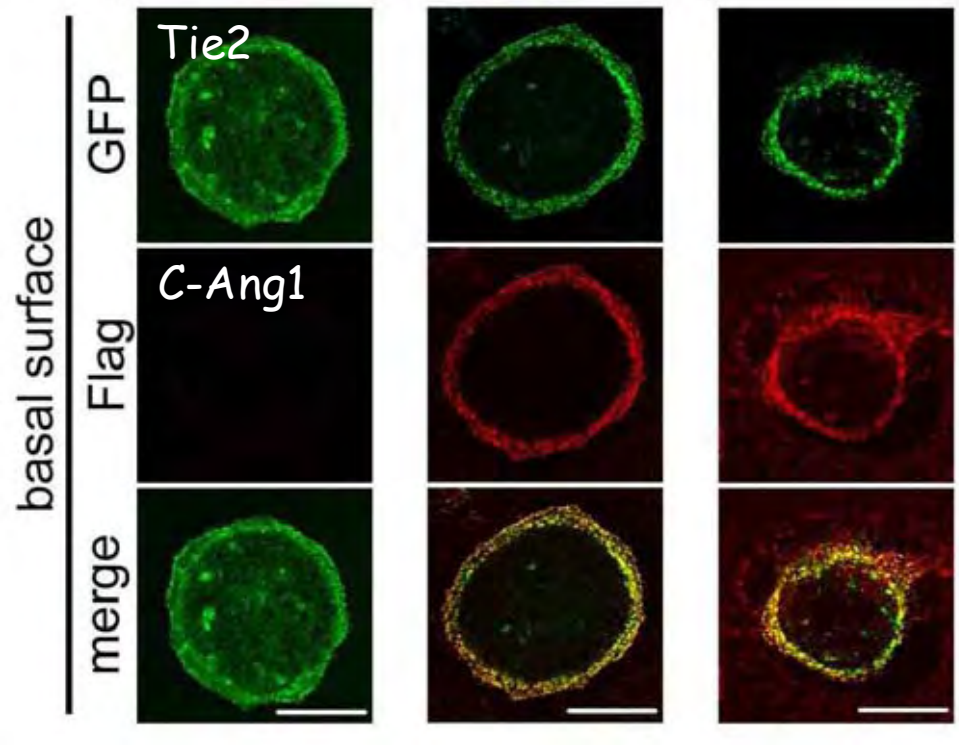
Tie2-GFP-expressing CHO cells plated on collagen-coated

COMP-Ang1 stimulation (min)

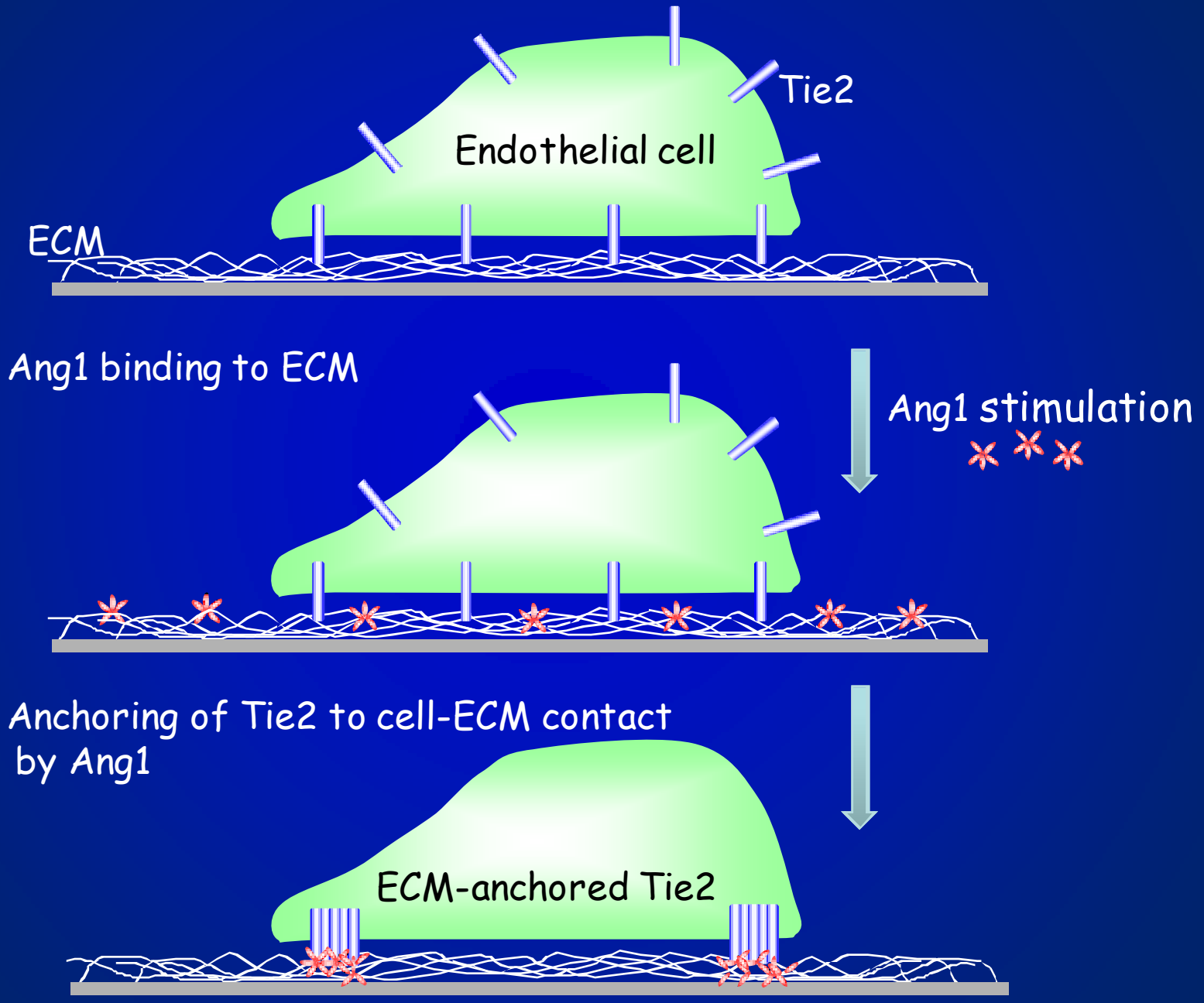
0 30 60

ECM binding assay





Conclusion Part2: in the absence of cell-cell contacts



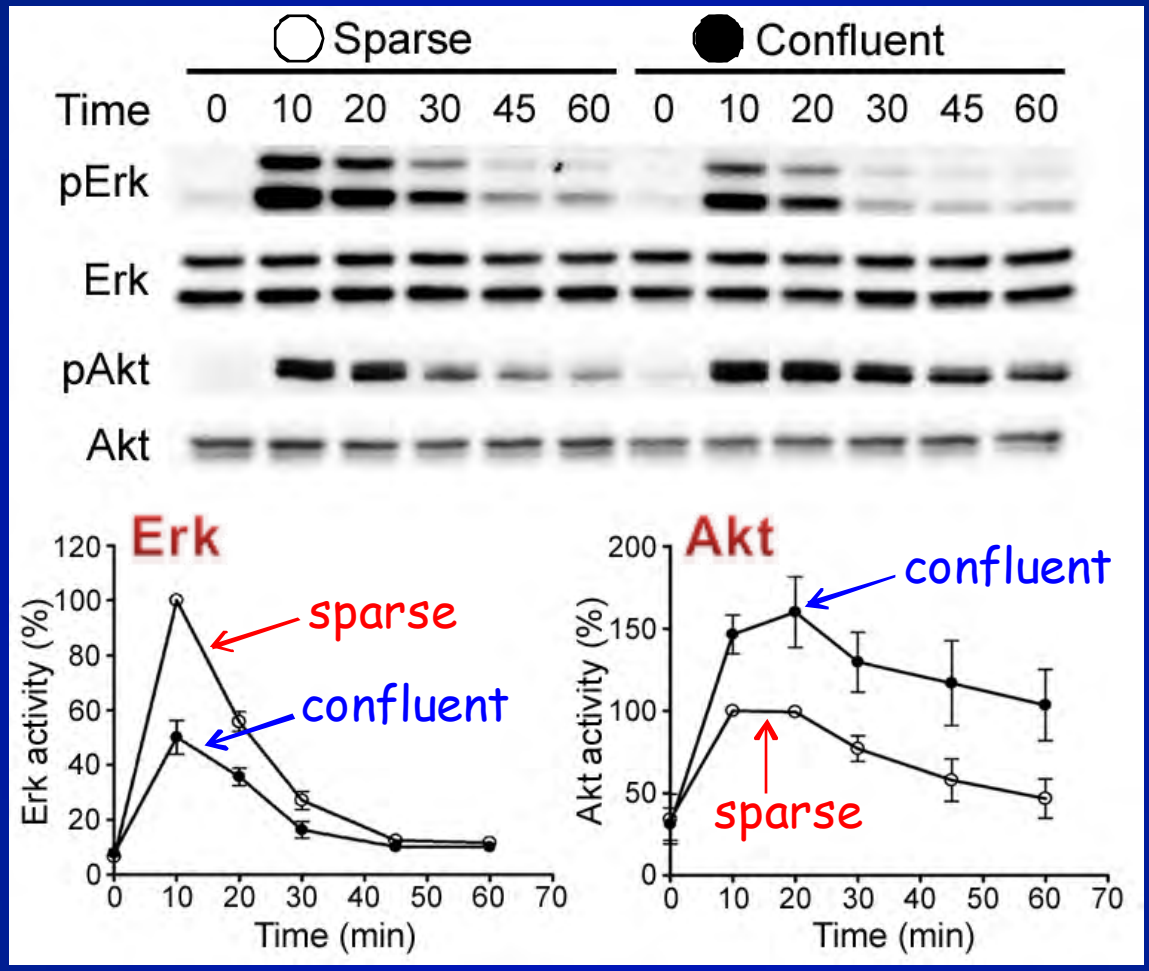
Biological significance of ECM-anchored Tie2 and trans-associated Tie2

Sparse and confluent HUVECs were stimulated with Ang1.

Erk
↓
migration
proliferation
↓
angiogenesis

Akt
↓
cell survival
↓
vascular quiescence

Preferential activation of Erk in sparse endothelial cells and that of Akt in confluent cells upon Ang1 stimulation



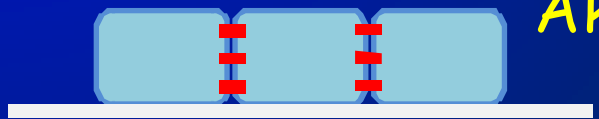
sparse

Erk>Akt

ECM-anchored Tie2

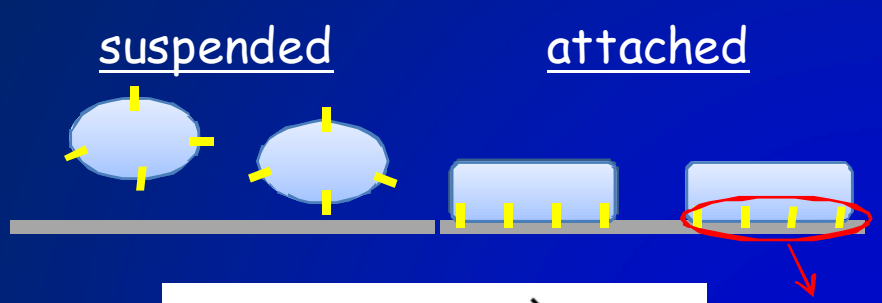
confluent

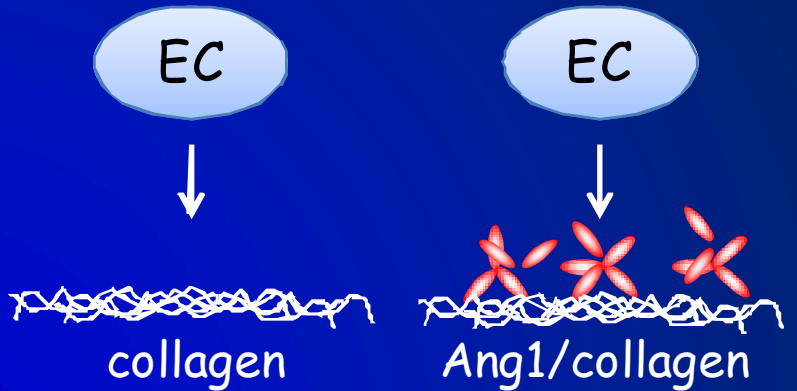
Akt>Erk

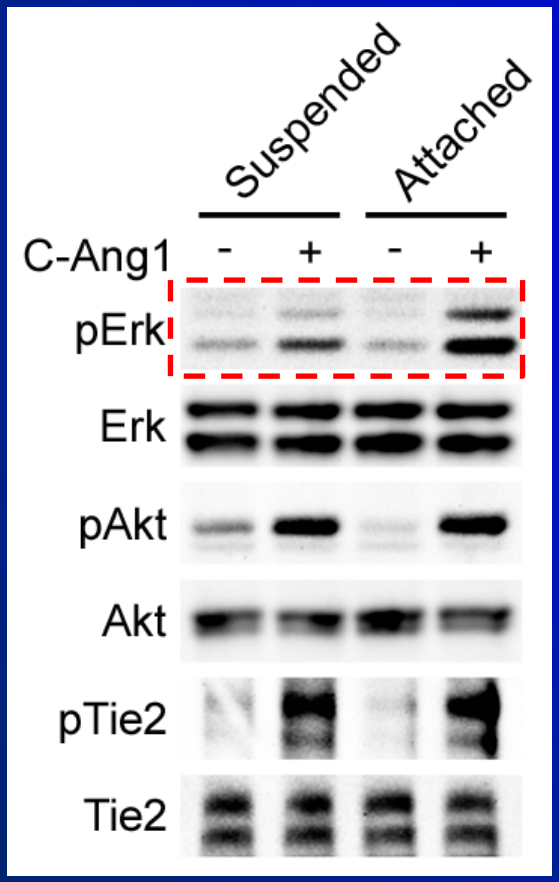


trans-associated Tie2

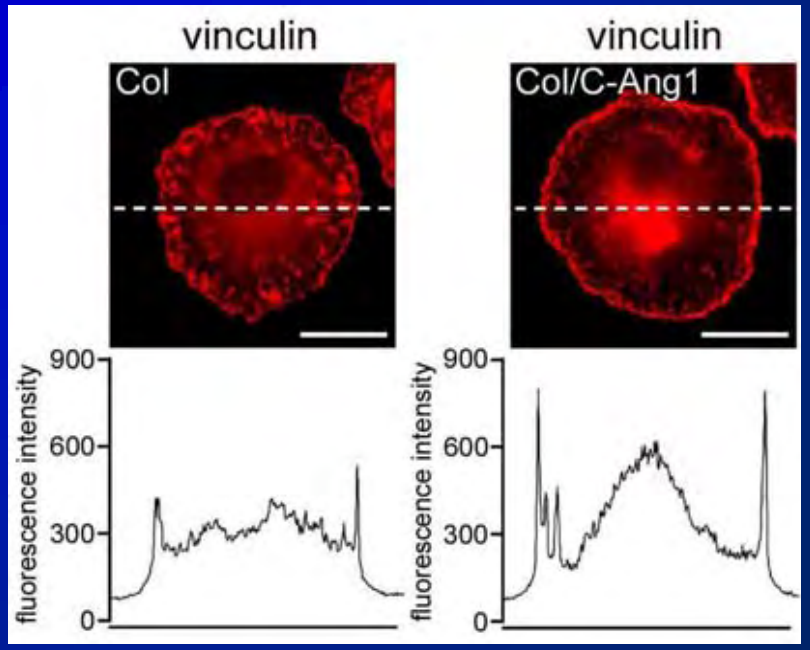
Anchoring of Tie2 to ECM facilitates Erk pathway and induces focal complex assembly



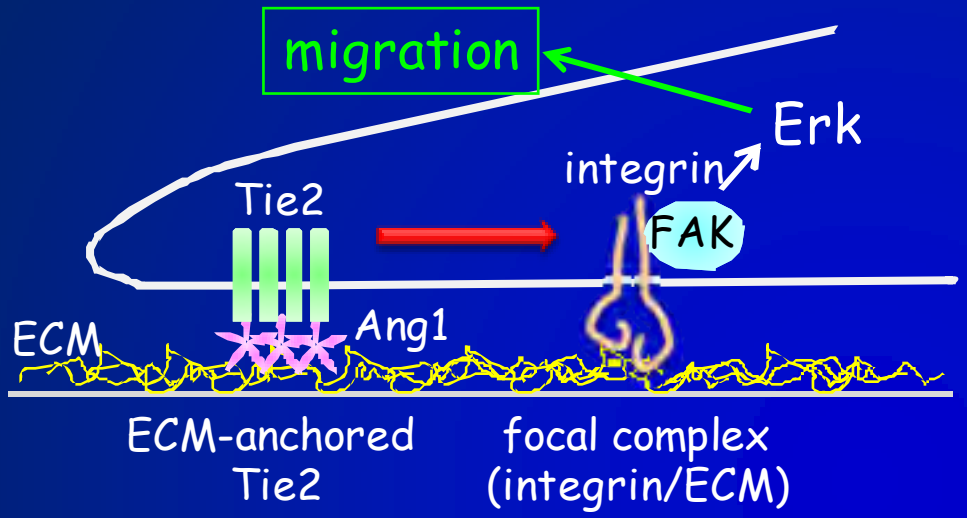




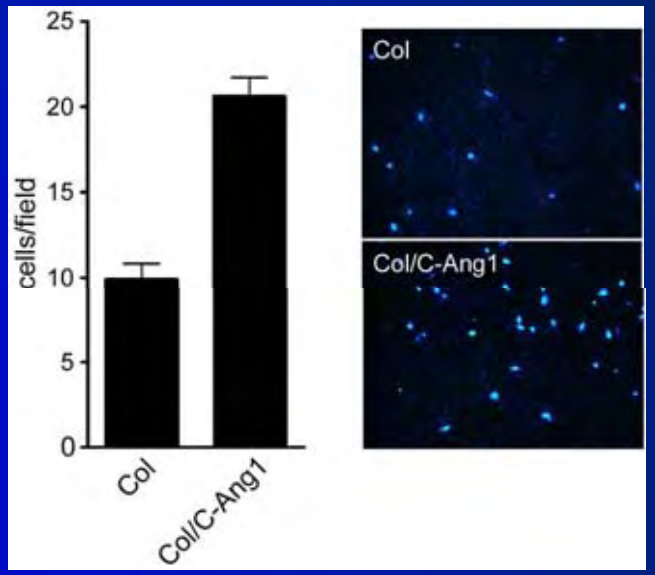
vinculin staining



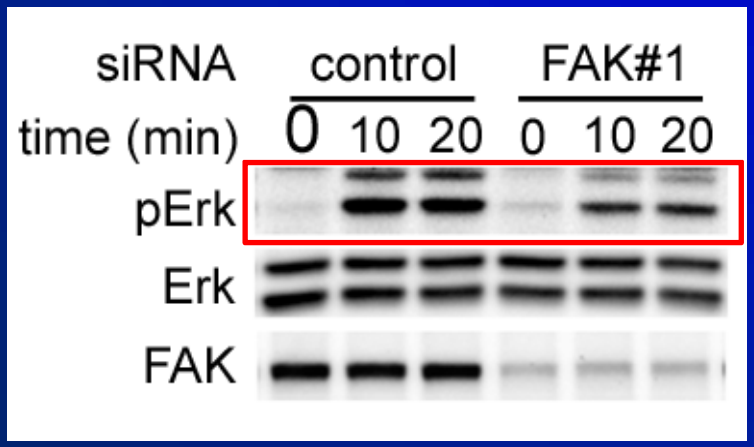
ECM-anchored Tie2 activates Erk pathway partly via FAK, leading to the enhanced endothelial cell migration

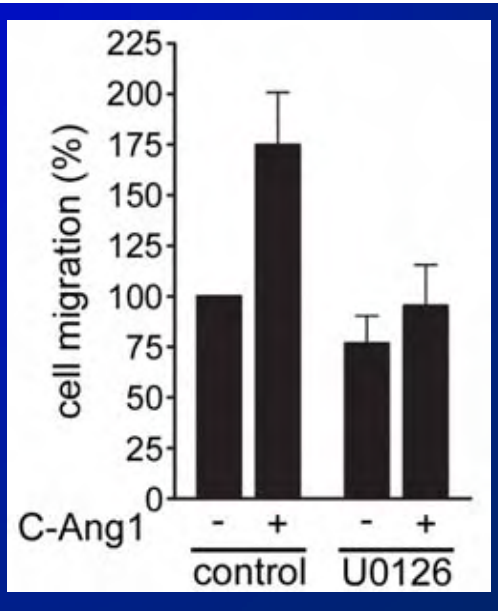


Endothelial cell migration

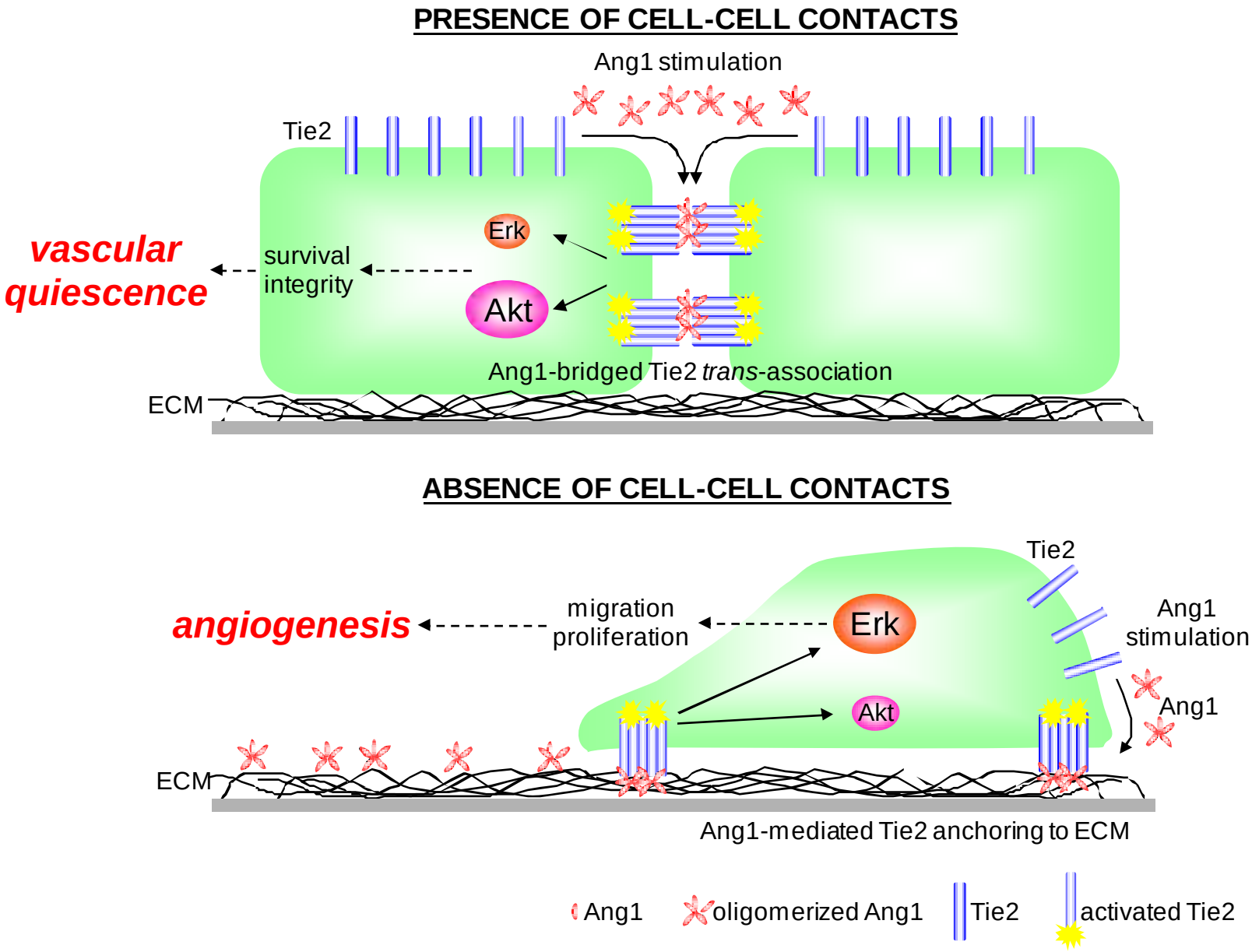


Knock-down of FAK



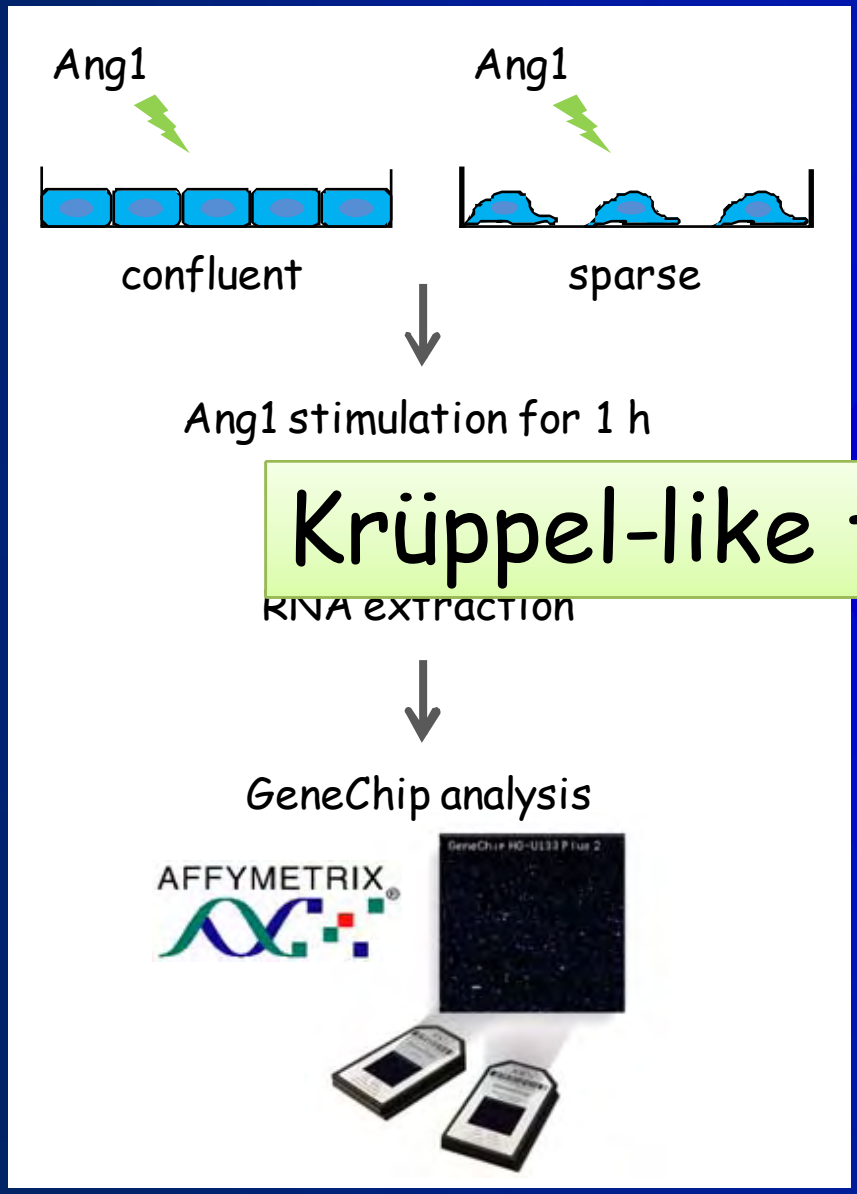


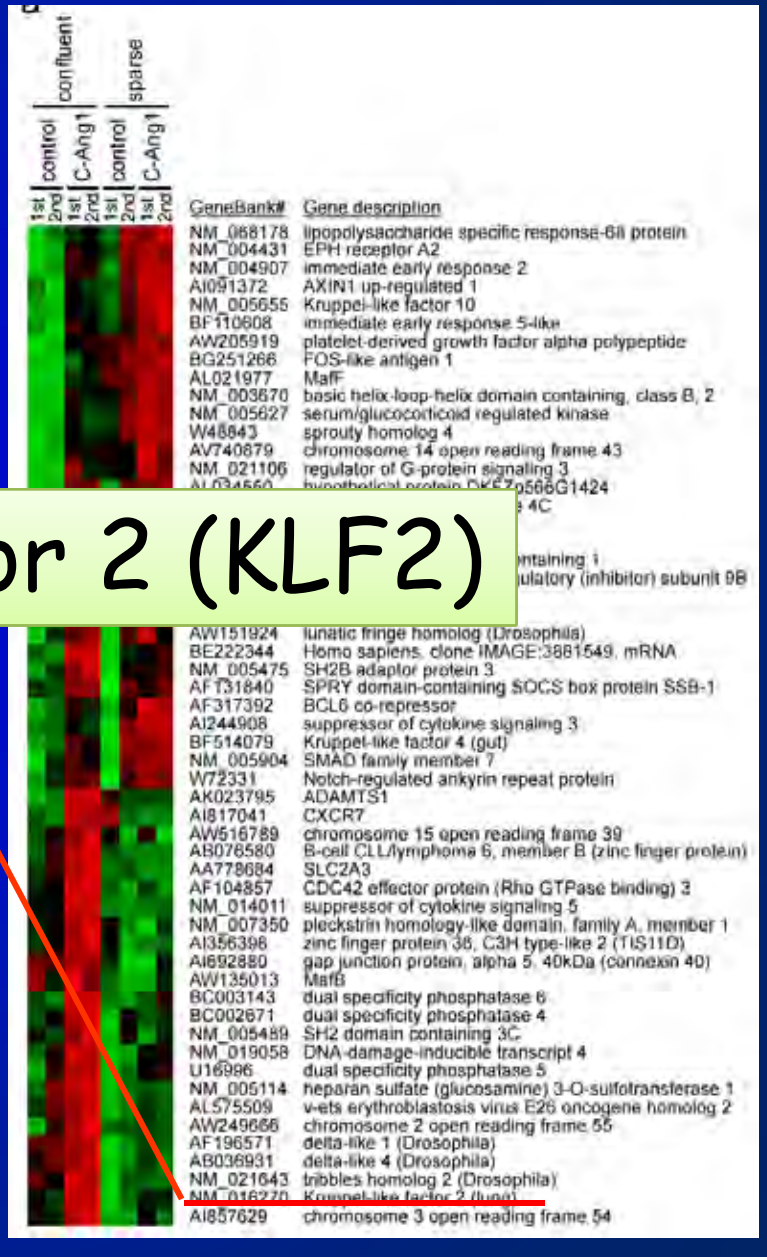
Conclusion Part3



Nat. Cell Biol. 10: 513-526, 2008

Distinct sets of genes are regulated by *trans*-associated Tie2 and ECM-anchored Tie2





Krüppel-like factor 2 (KLF2)

Role of KLF2 in blood vessels

- ✓ KLF2 is a zinc finger family of transcription factor.
- ✓ KLF2 expression is induced by laminar shear stress in endothelial cells, but not induced in the region of disturbed blood flow.
- ✓ KLF2 has anti-inflammatory, anti-thrombotic and anti-angiogenic effects.

Summary of KLF2 target genes

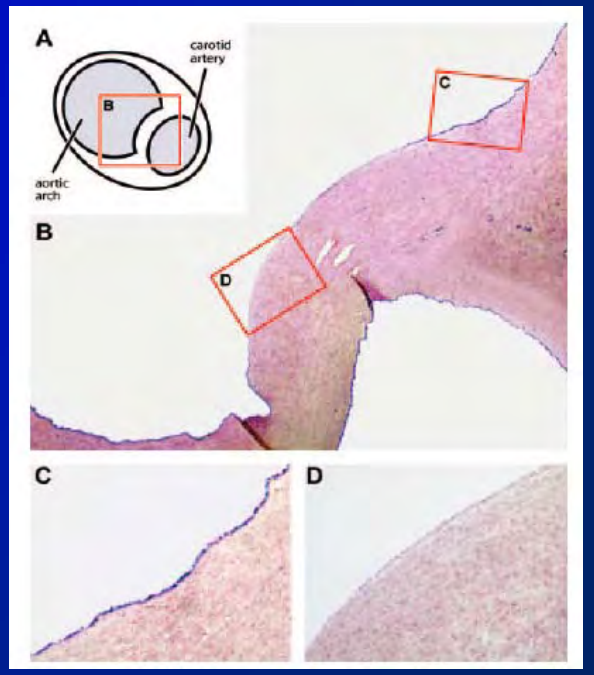
anti-inflammatory: VCAM-1 ↓ , E-selectin ↓ , eNOS ↑

anti-thrombotic: PAI-1 ↓ , TF ↓ , TM ↑ , eNOS ↑ , tPA ↑

vasodilatory: ET-1 ↓ , CNP ↑ , eNOS ↑ , ASS ↑

anti-angiogenic: VEGFR2 ↓ , SEMA3F ↑

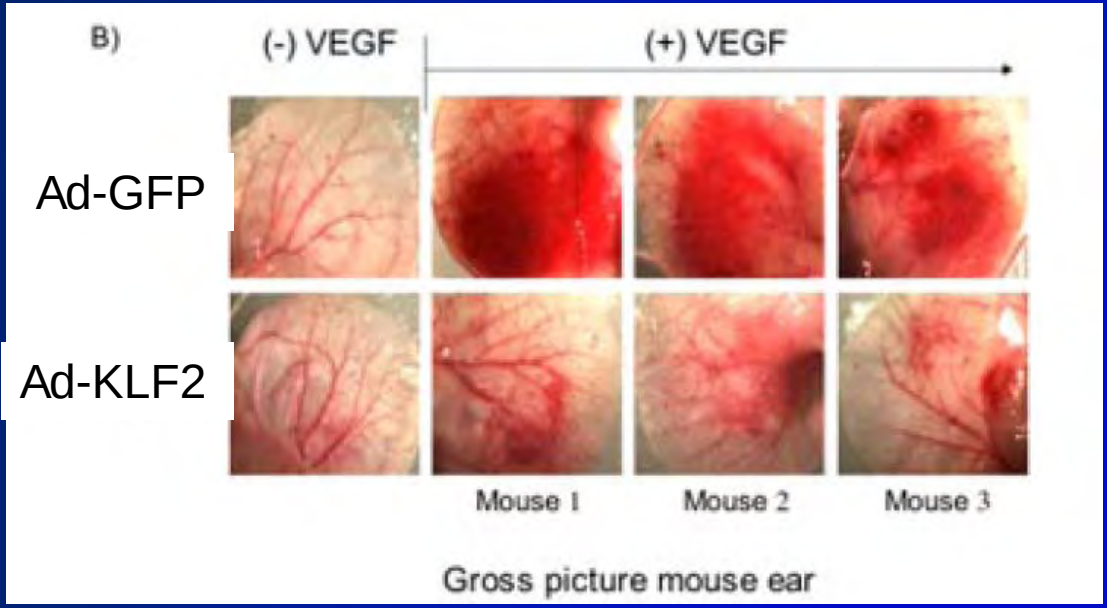
Role of KLF2 in vascular quiescence!



Dekker et al. *Am. J. Pathol.*, 2005

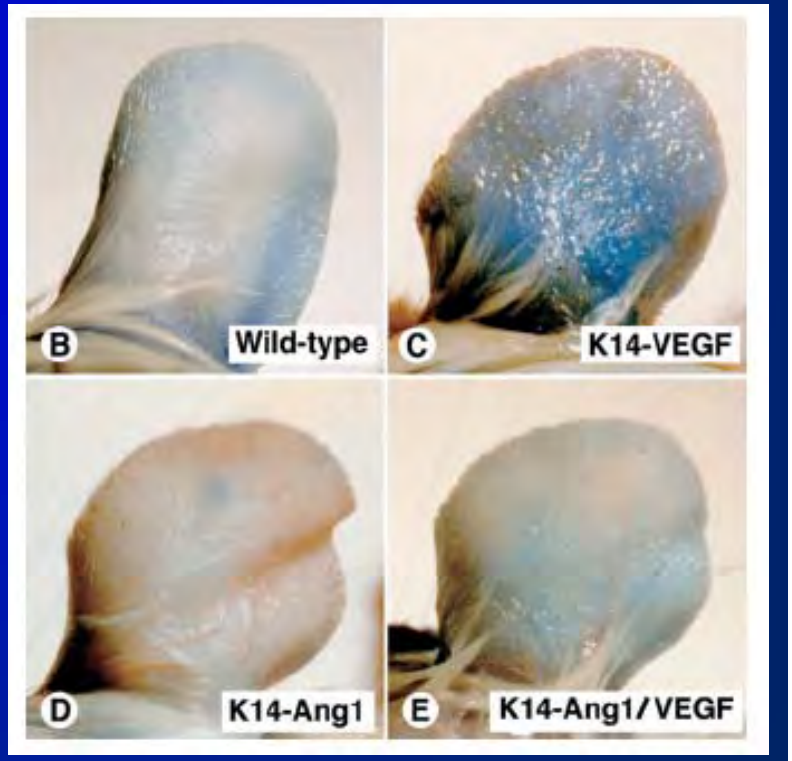
Inhibitory effects of Ang1 and KLF2 on VEGF-induced vascular leakage

KLF2 inhibits VEGF-induced vascular leakage



Bhattacharya et al. *J. Biol. Chem.*, 2005

Ang1 inhibits VEGF-induced vascular leakage.



Thurston et al. *Science*, 1999

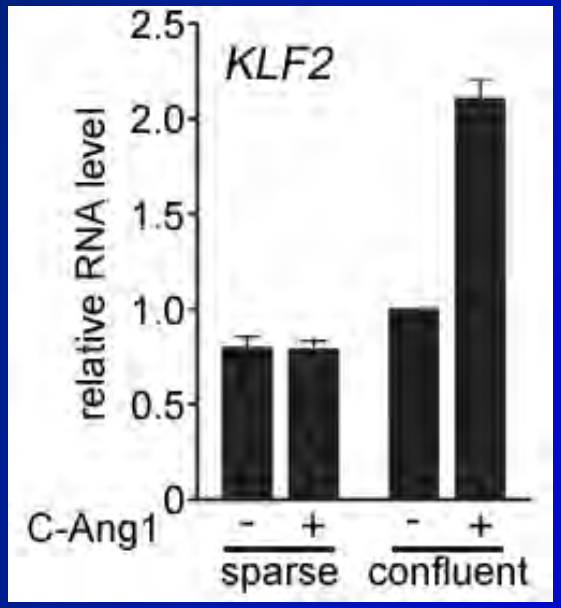
Hypothesis

Ang1/Tie2 signal may induce vascular quiescence through KLF2 expression.

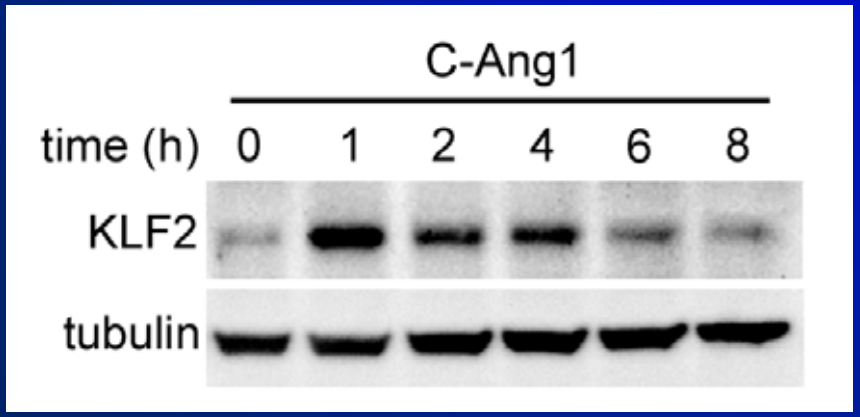
- ✓ Intracellular signaling pathway involved in Ang1-induced KLF2 expression
- ✓ Biological consequence of Ang1-induced KLF2 expression

Trans-associated Tie2 induces KLF2 expression

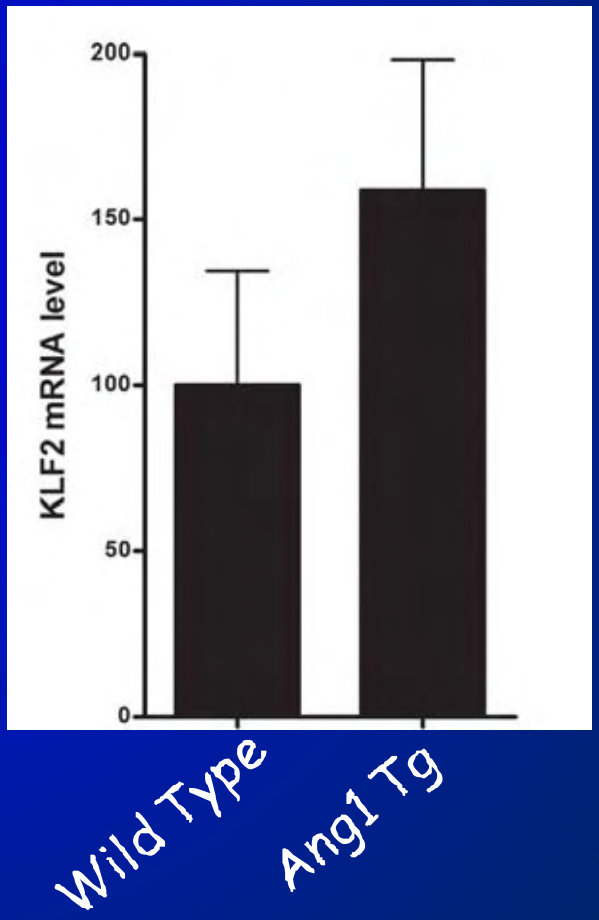
Real-time PCR



Western blot analysis

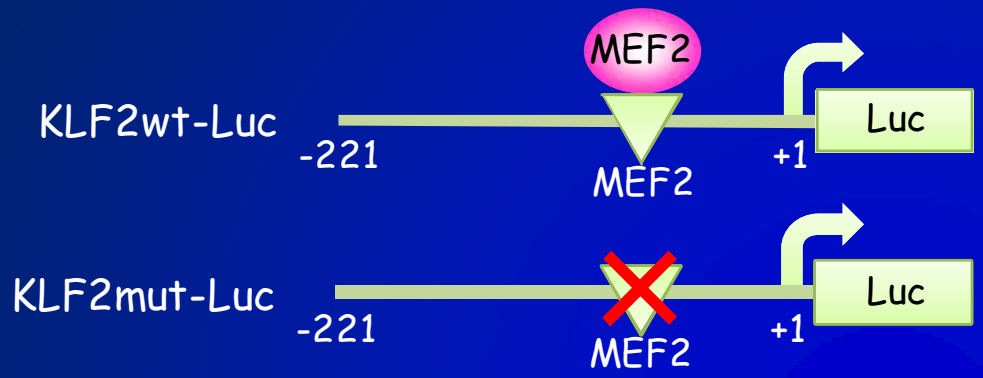


KLF2 expression in endothelial cells from Ang1 transgenic mice

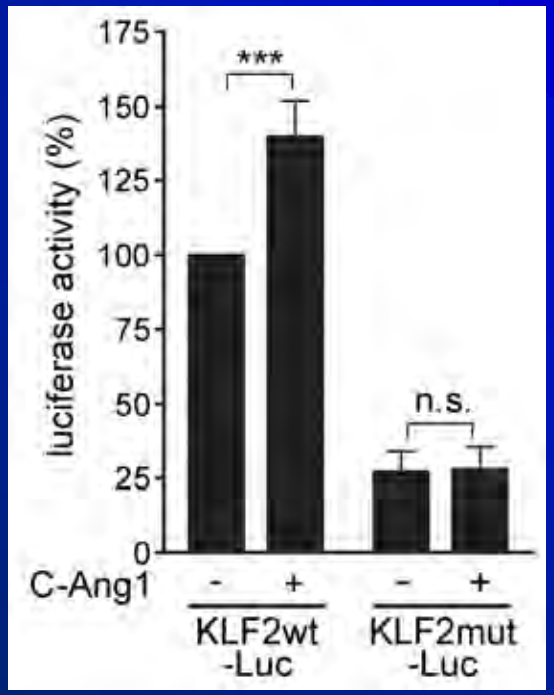


Trans-associated Tie2 induces KLF2 expression through MEF2

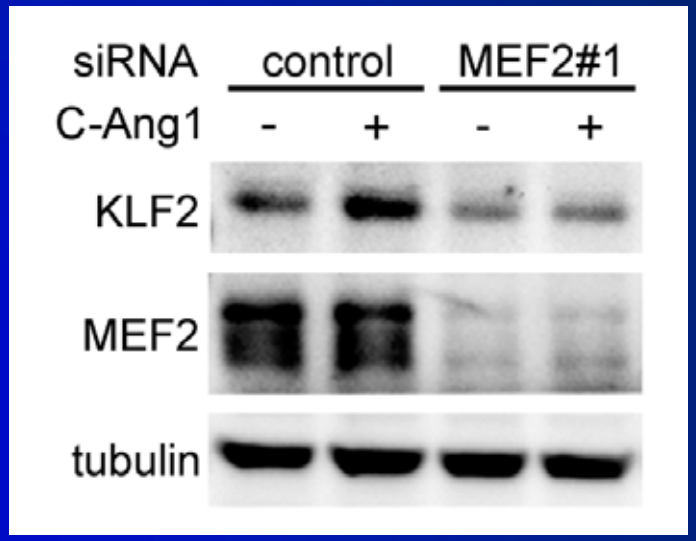
KLF2 promoter/luc construct



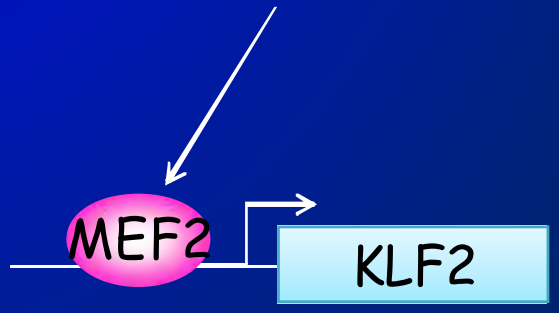
MEF2: myocyte enhancer factor 2



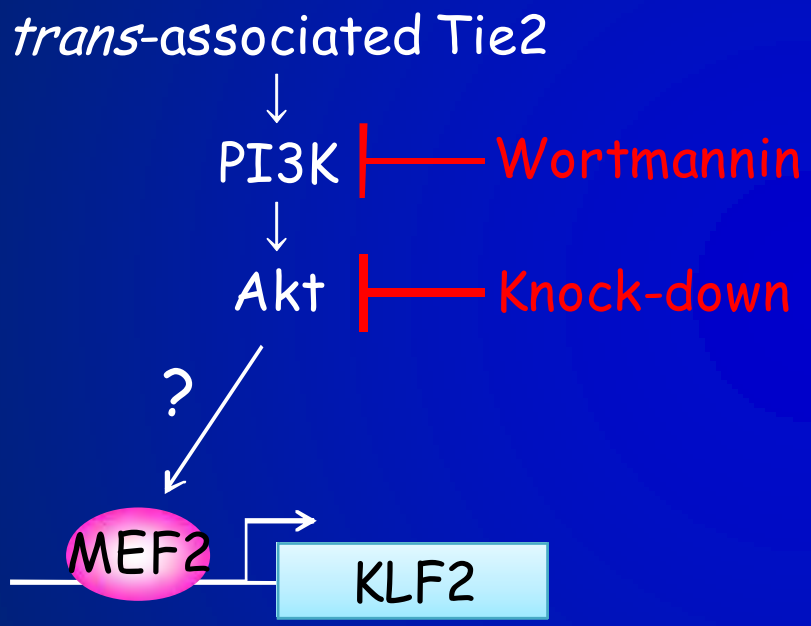
Knock-down of MEF2



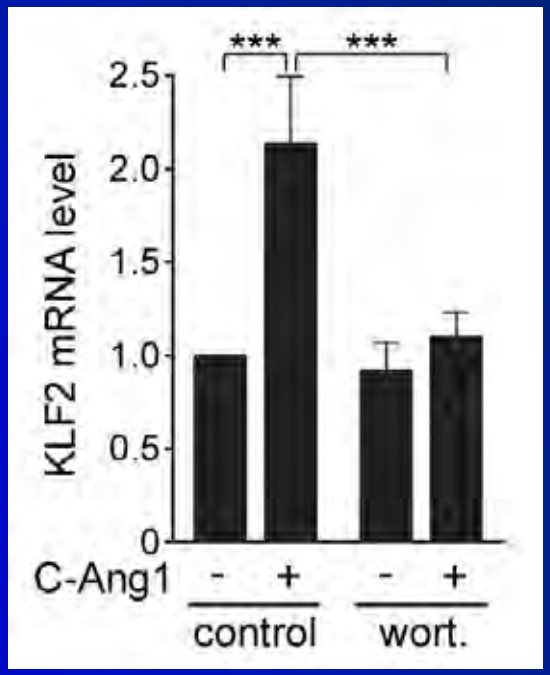
Ang1-induced *trans*-associated Tie2



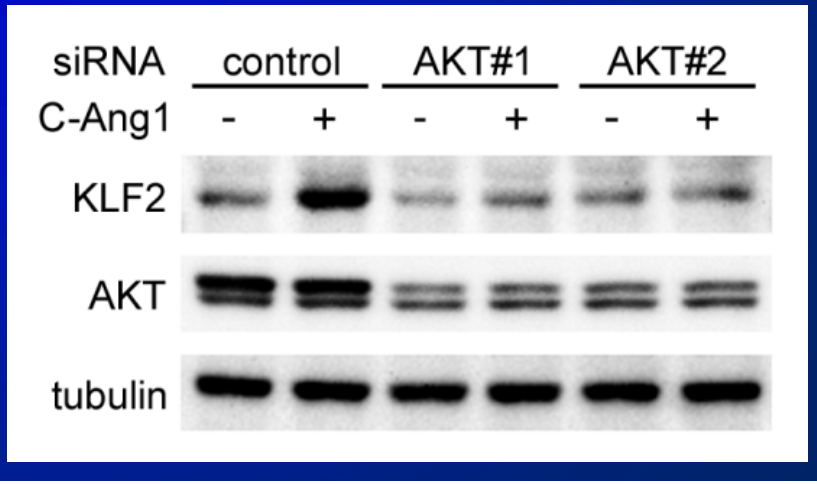
A PI3K/Akt pathway is involved in Ang1-induced KLF2 expression



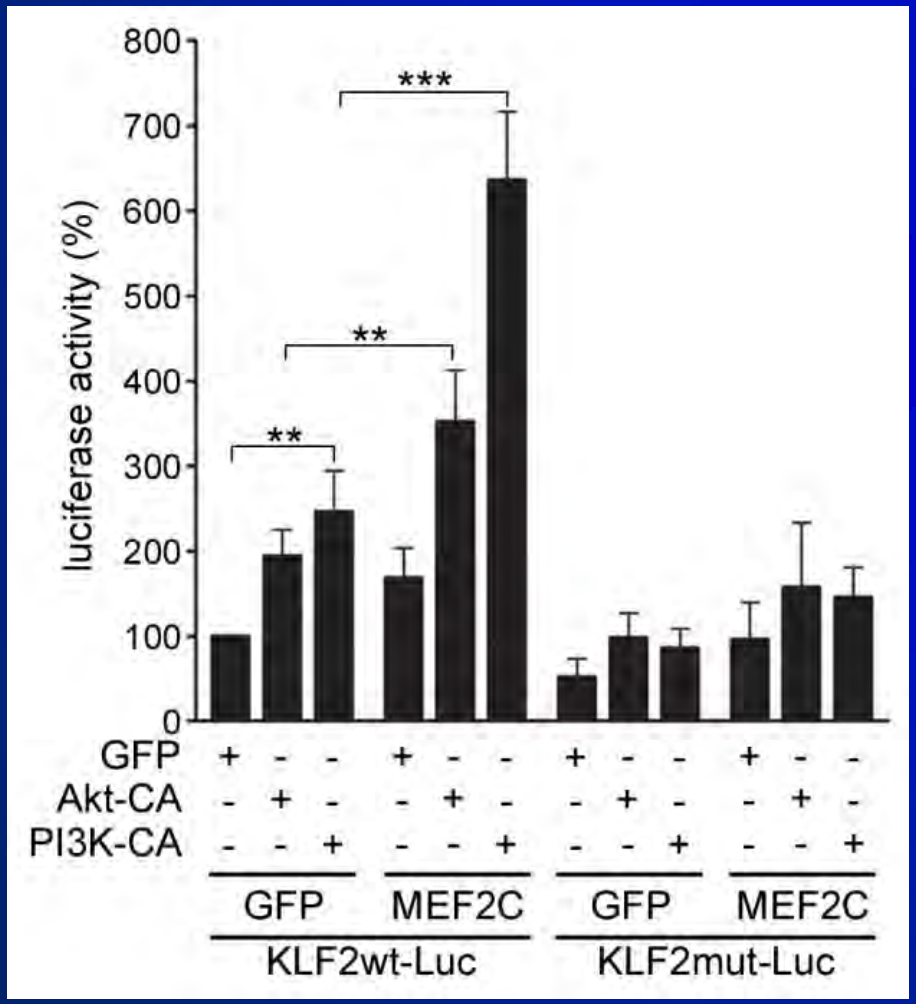
PI3K inhibitor

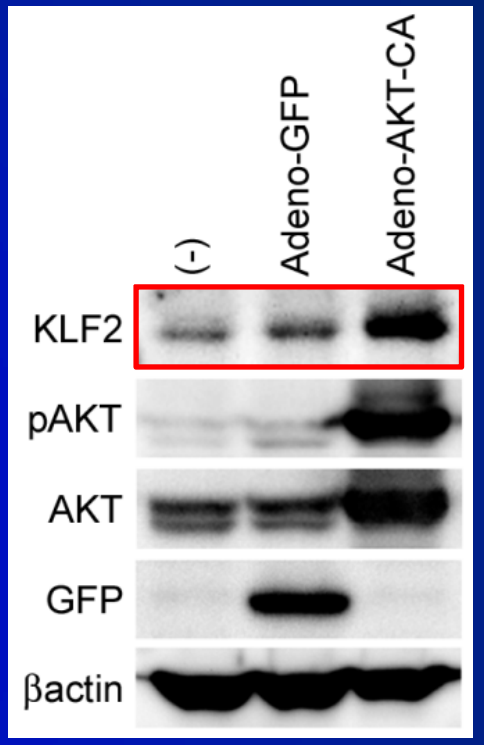


Knock-down of Akt



A PI3K/Akt/MEF2 signaling pathway induces KLF2 expression

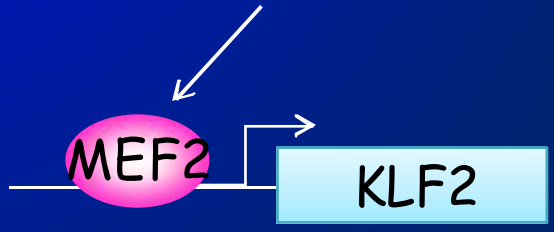




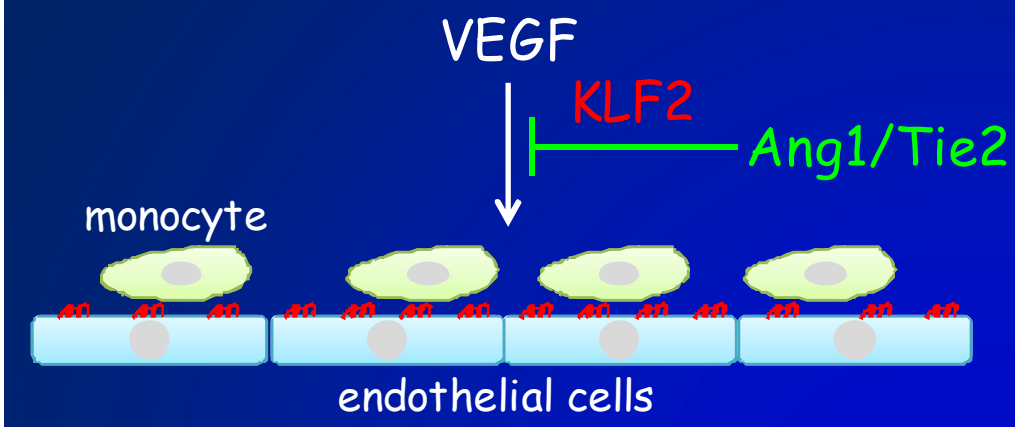
trans-associated Tie2

↓

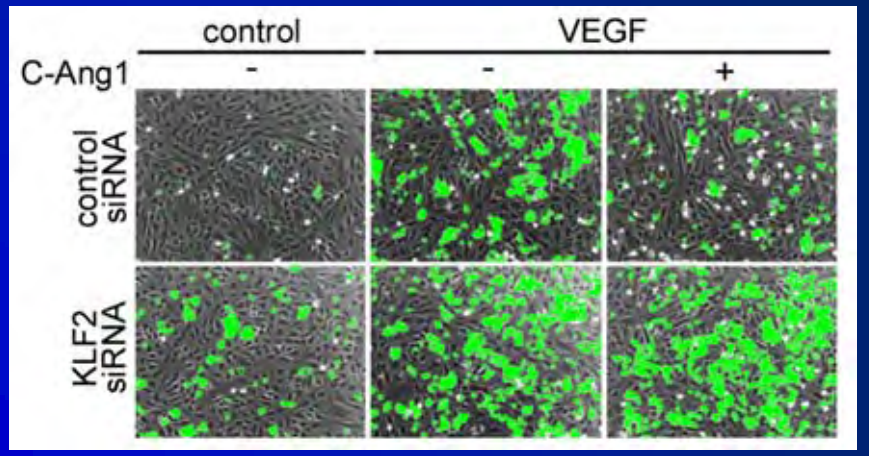
PI3K/Akt



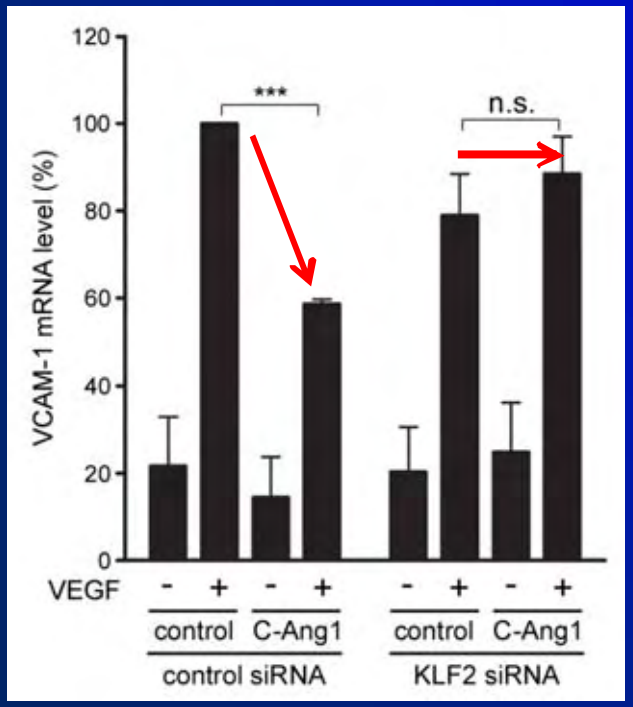
Ang1 inhibits VEGF-induced inflammation through KLF2



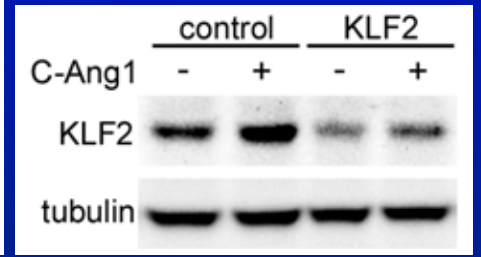
monocyte-EC adhesion

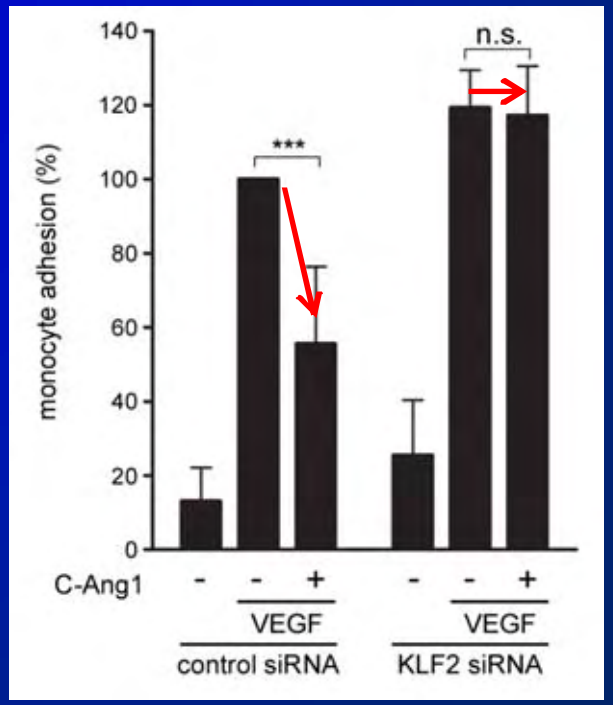


VCAM1 expression

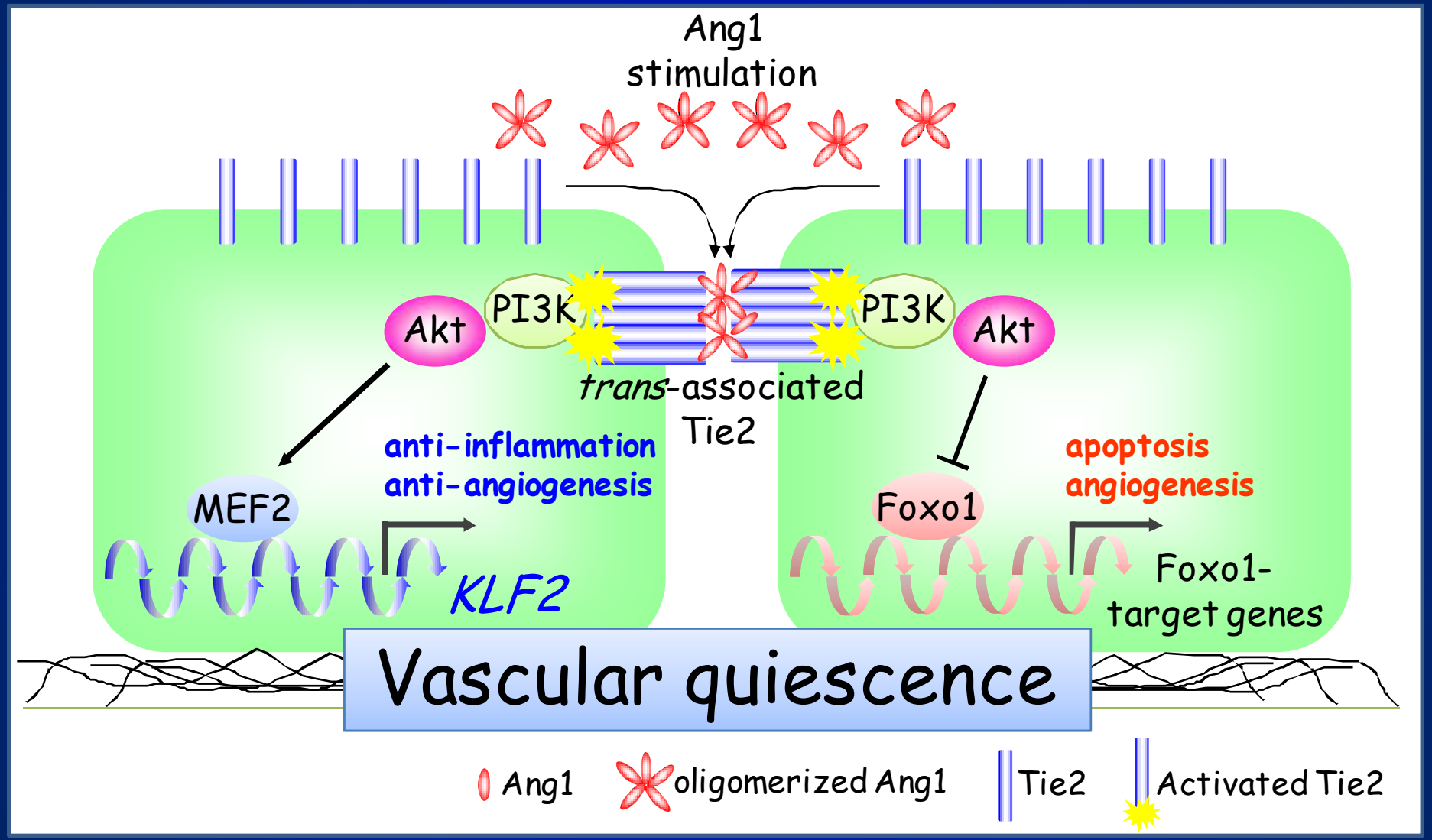


Knock-down of KLF2



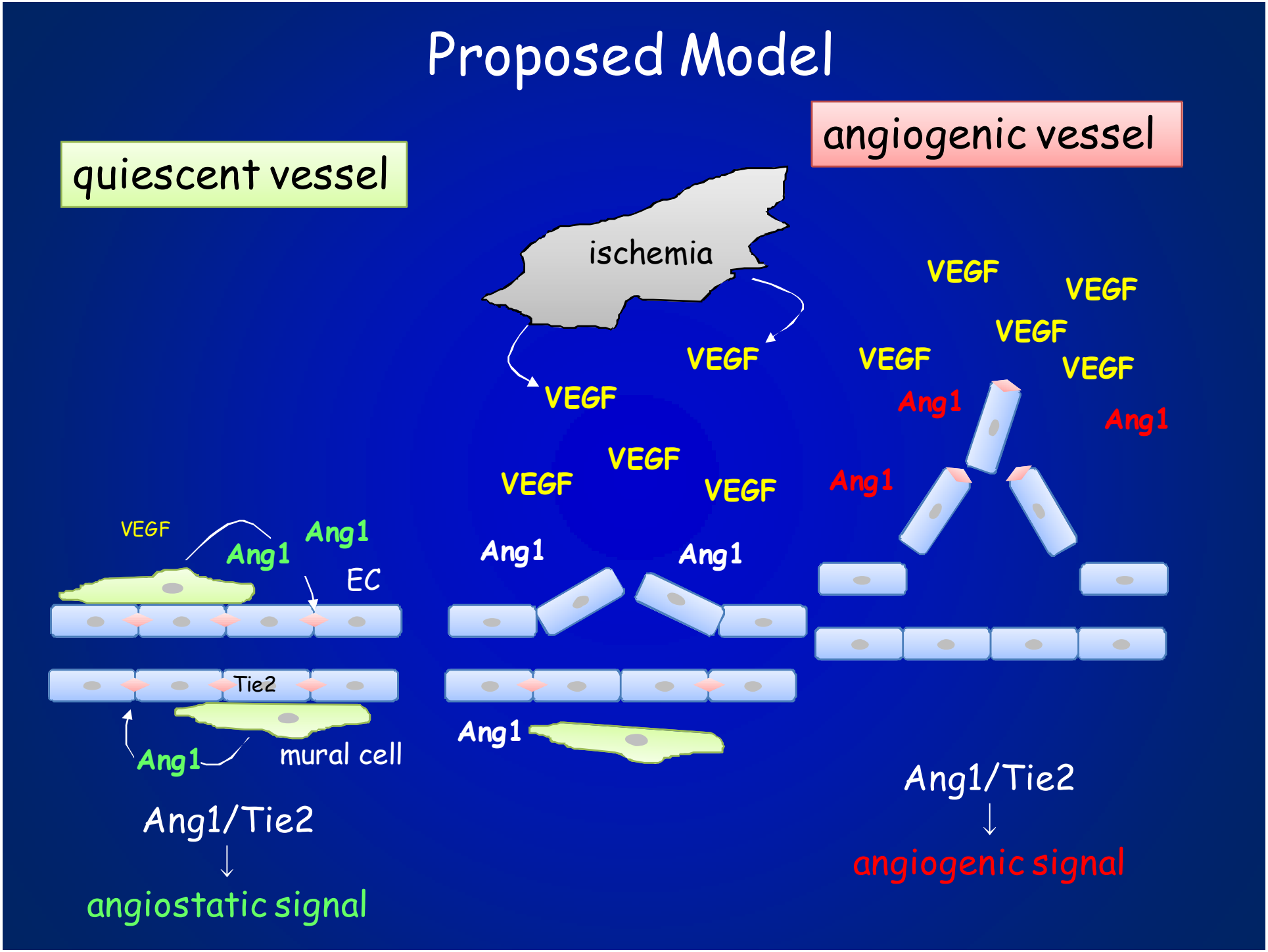


Vascular quiescence regulated by Ang1/Tie2 signal



J. Biol. Chem., in press

Proposed Model

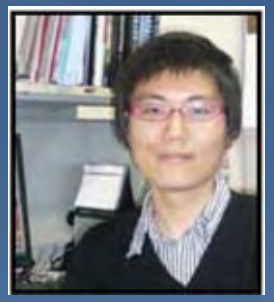


Acknowledgments

NCVC Research Institute

Naoki Mochizuki

Keisuke Sako



Kazuomi Noda

Korea Advanced Institute for Science and Technology

Gou Young Koh

LSBM, Tokyo University

Takashi Minami

Tatsuhiko Kodama

Takao Hamakubo

Keio University

Toshio Suda

The University of Osaka

Nobuyuki Takakura

National Institute of Dental and Craniofacial Research, NIH

J. Silvio Gutkind

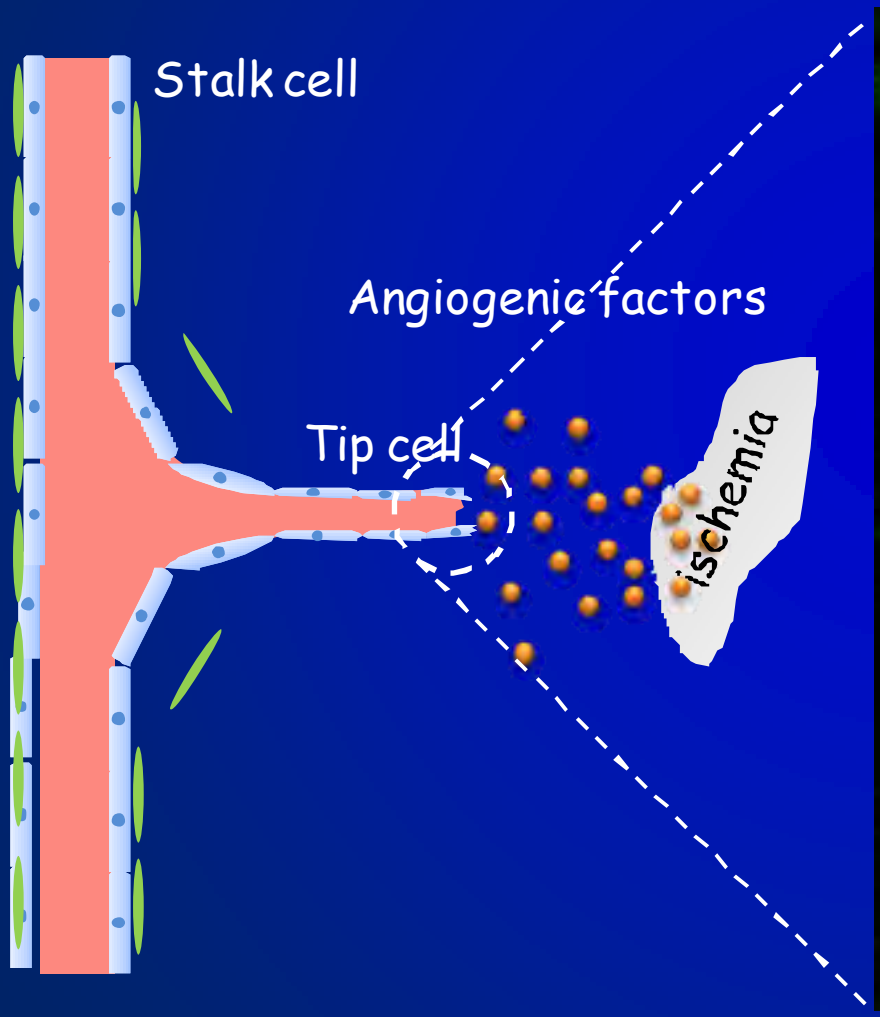
Tokyo Medical and Dental University

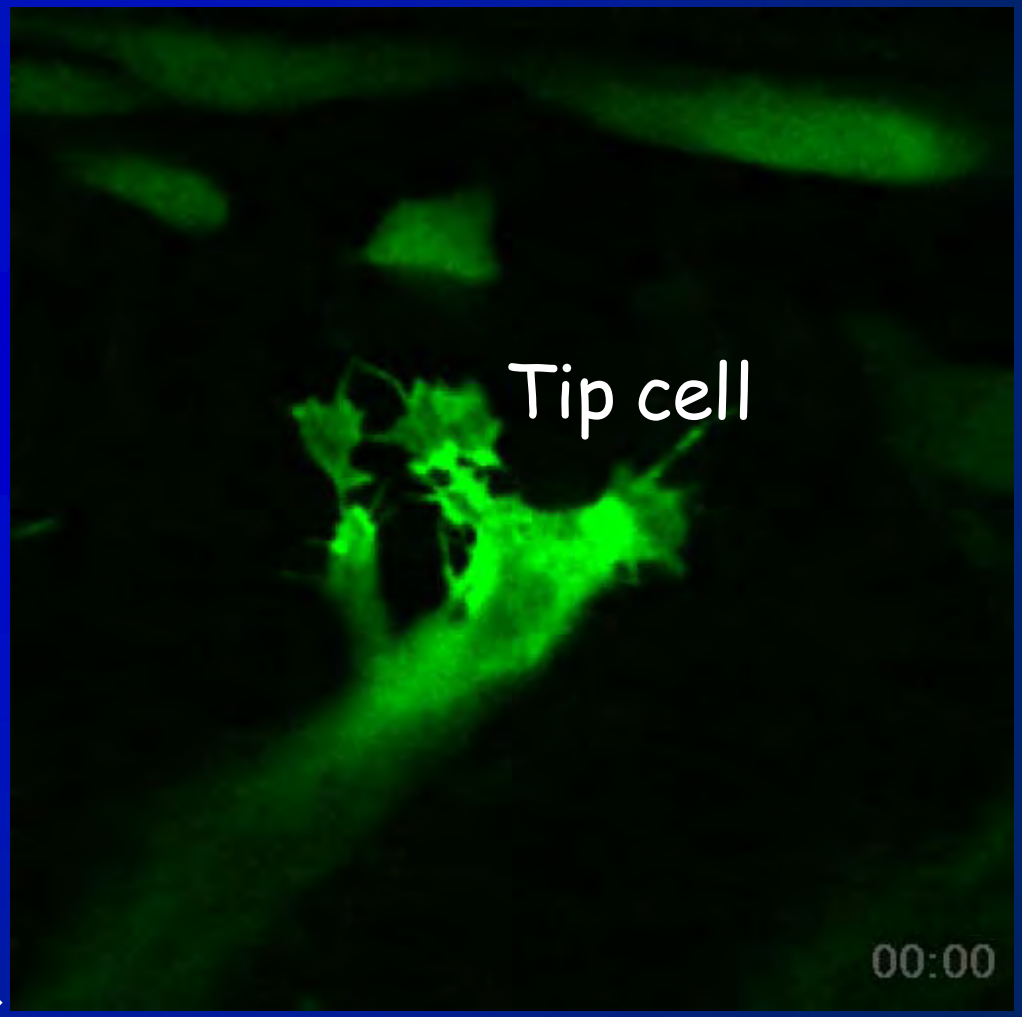
Masabumi Shibuya

University of Tsukuba

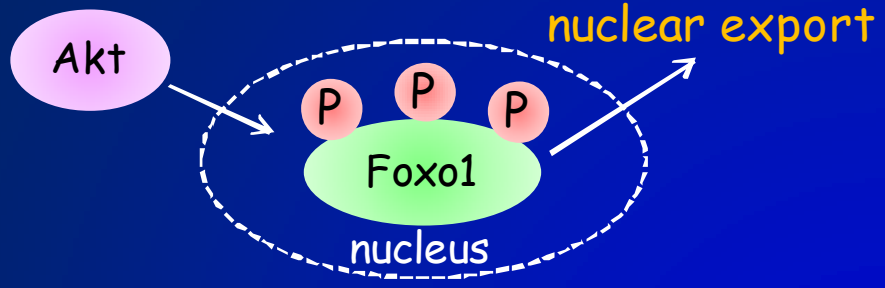
Akiyoshi Fukamizu

Filopodia extensions are actively produced by endothelial tip cells in response to angiogenic factors





Akt-Foxo1 pathway is preferentially induced by *trans*-associated Tie2 at cell-cell contacts

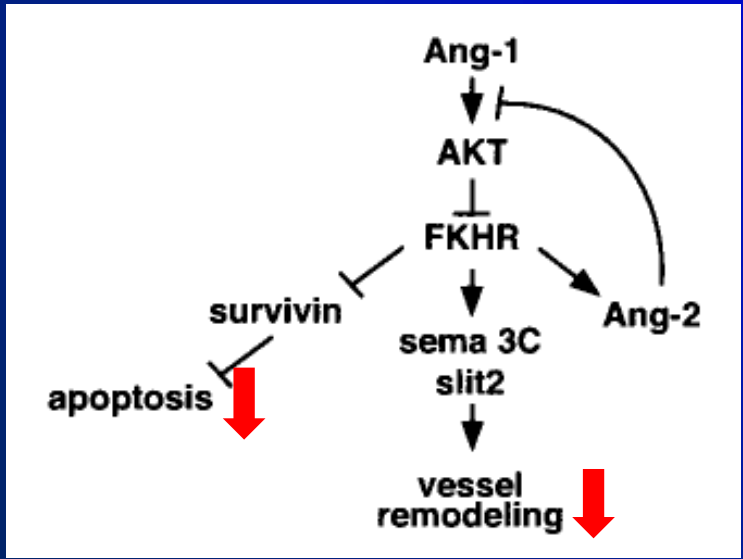


GENES & DEVELOPMENT 18:1060–1071 © 2004

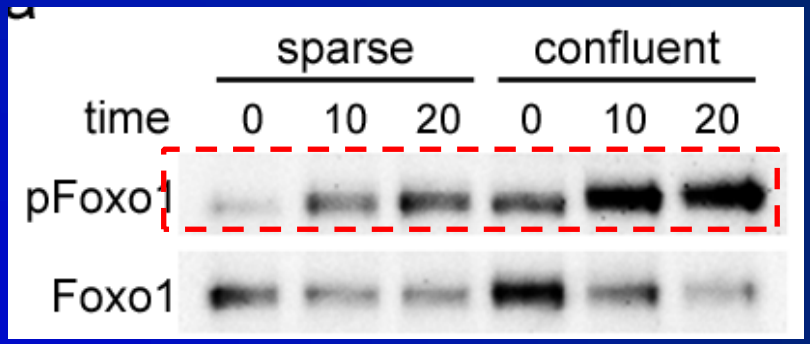
Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1)

Christopher Daly,[1] Vivian Wong, Elena Burova, Yi Wei, Stephanie Zabski, Jennifer Griffiths, Ka-Man Lai, Hsin Chieh Lin, Ella Ioffe, George D. Yancopoulos, and John S. Rudge

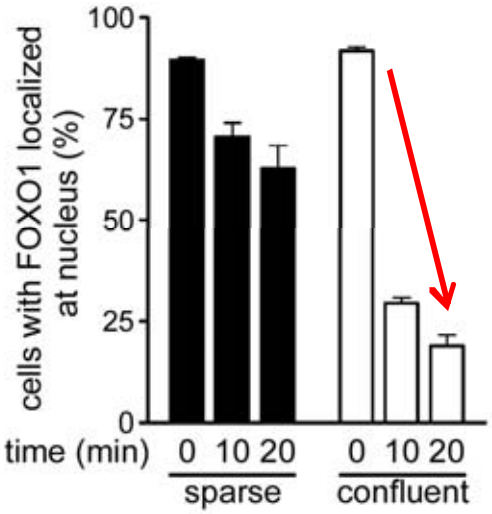
Regeneron Pharmaceuticals, Inc., Tarrytown, New York 10591, USA

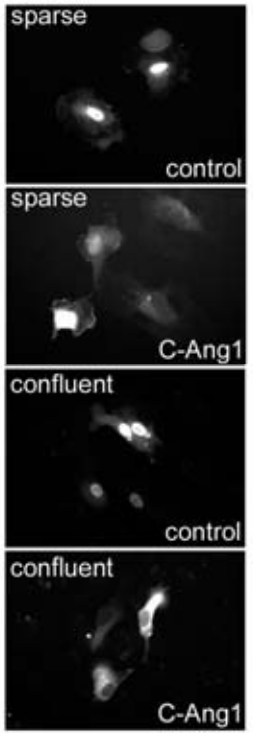


Foxo1 phosphorylation by COMP-Ang1

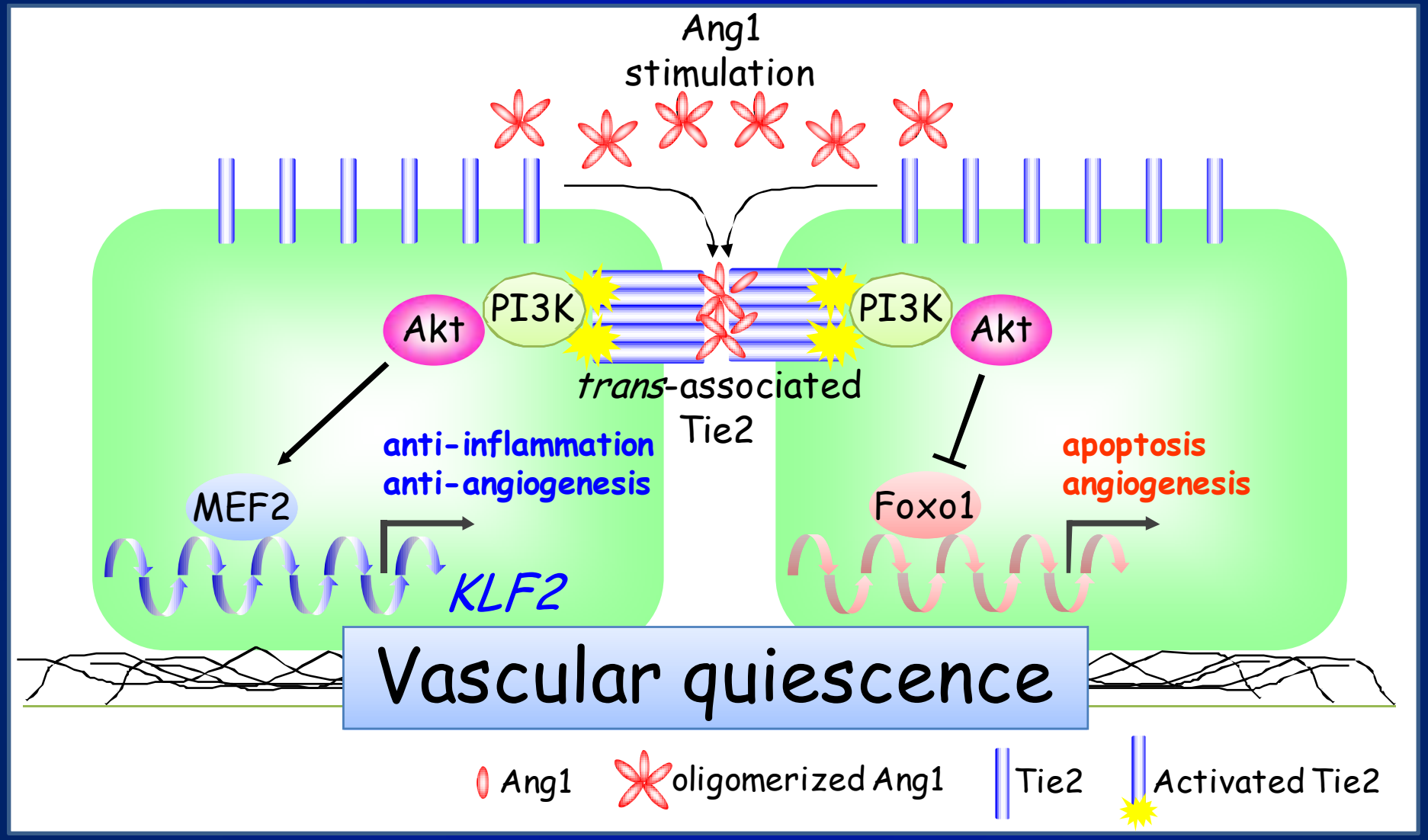


Nuclear export





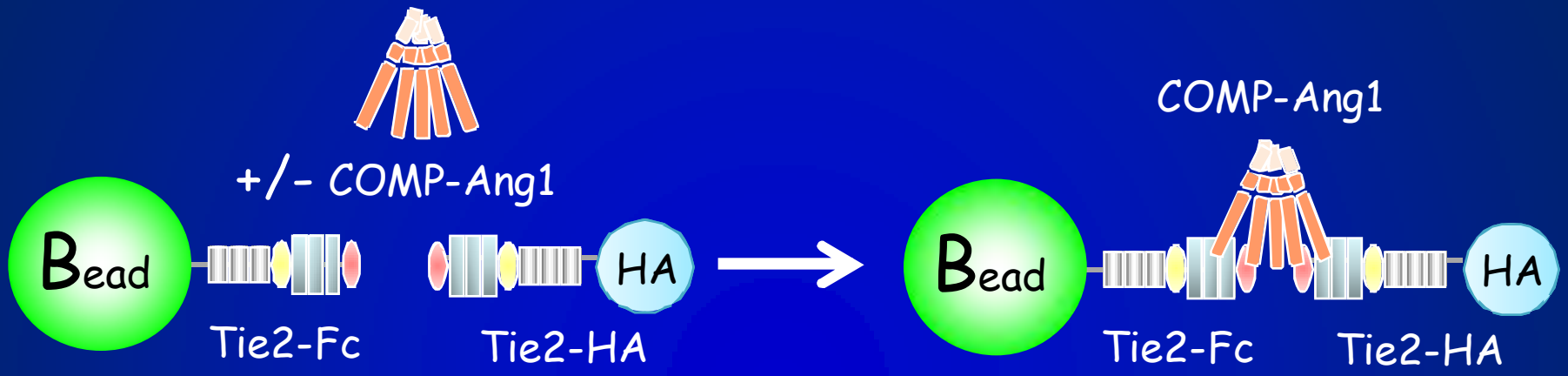
Vascular quiescence regulated by Ang1/Tie2 signal

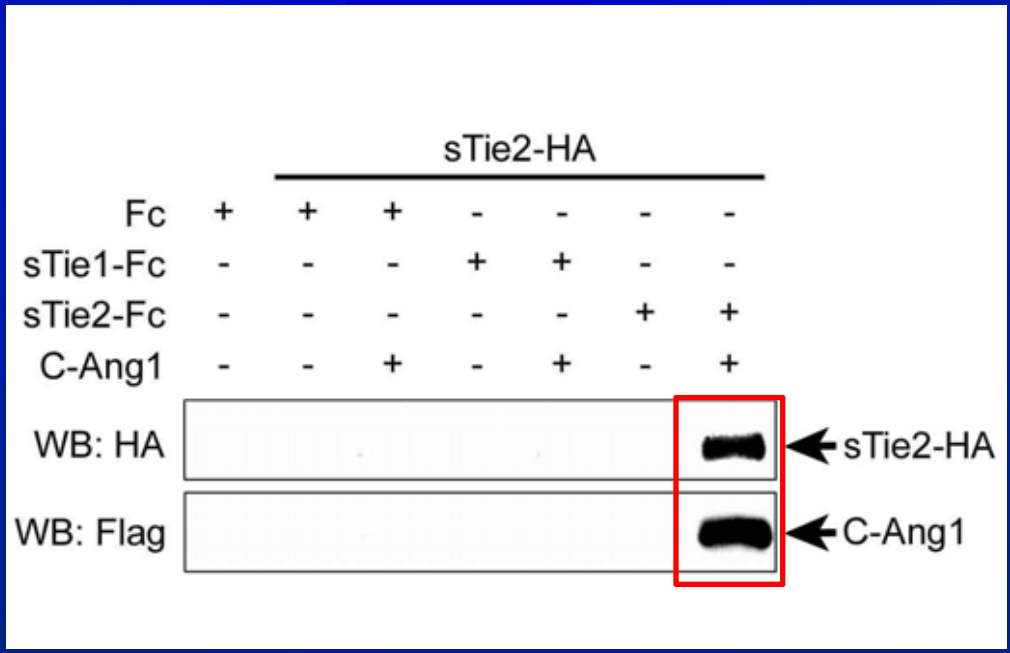


J. Biol. Chem., in press

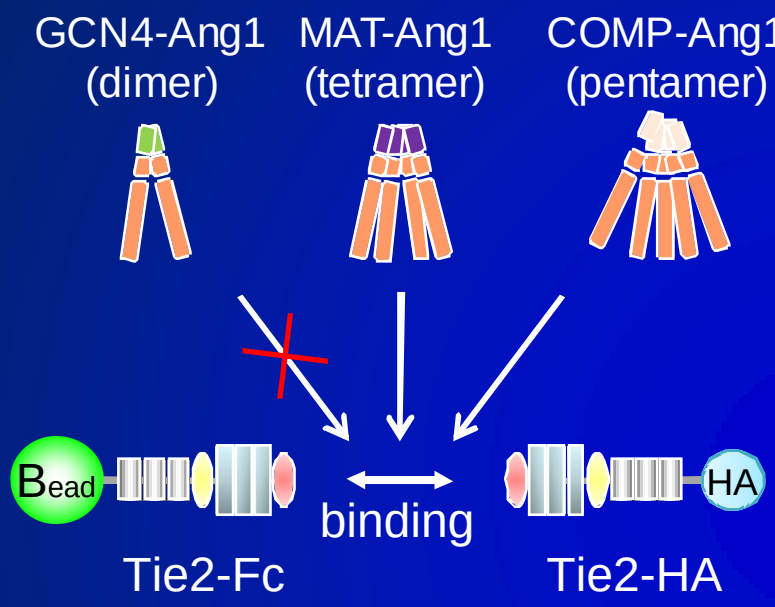
Ang1 induces *trans*-association of Tie2 in vitro

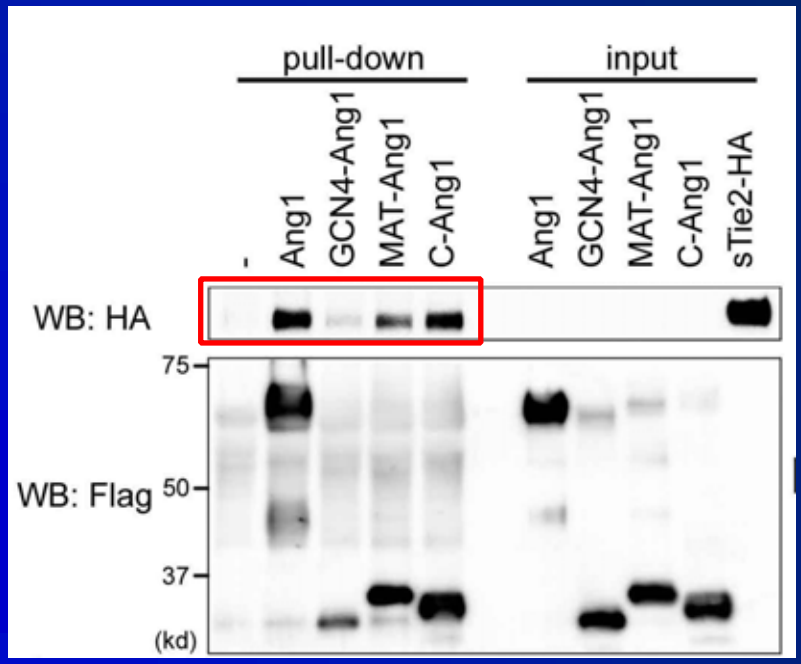
in vitro Tie2 *trans*-association assay



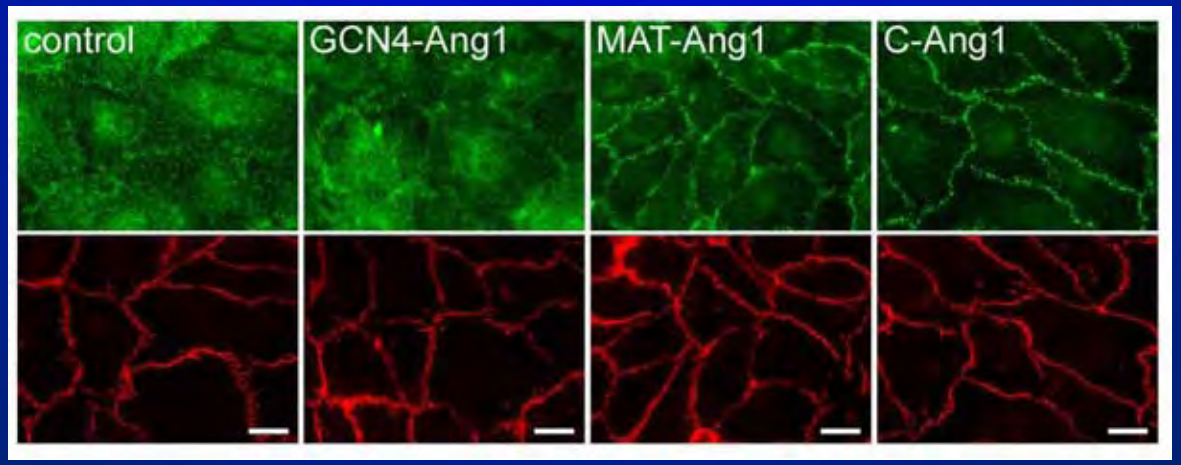


Multimerization of Ang1 is required for *trans*-association of Tie2 at cell-cell contacts





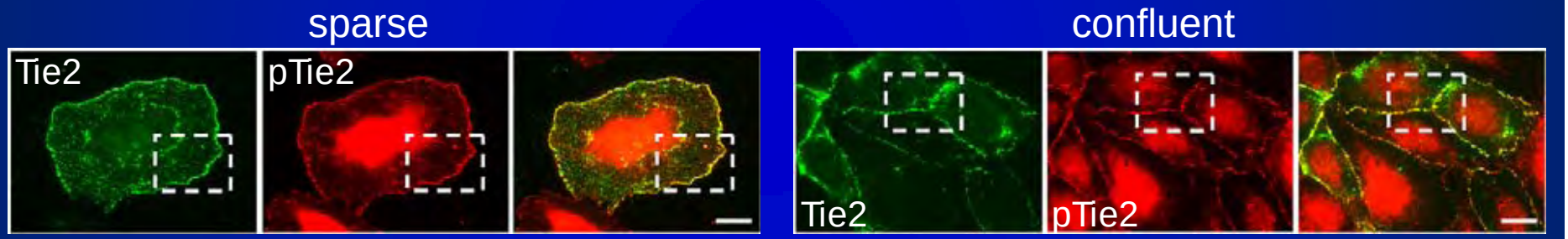
Localization of Tie2 at cell-cell contacts by Ang1 mutants

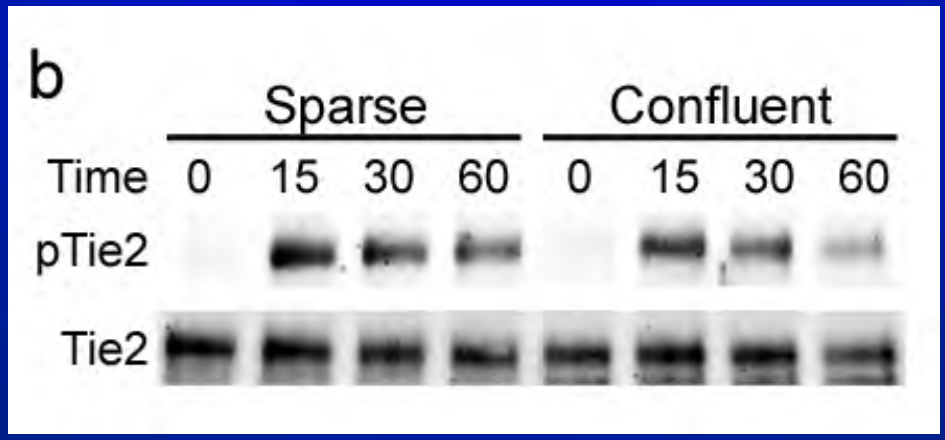


Biological significance of ECM-anchored Tie2 and trans-associated Tie2

Sparse and confluent HUVECs were stimulated with COMP-Ang1.

Tie2 activation





Endothelial cell-cell contacts do not affect Tie2 activation by Ang1.