

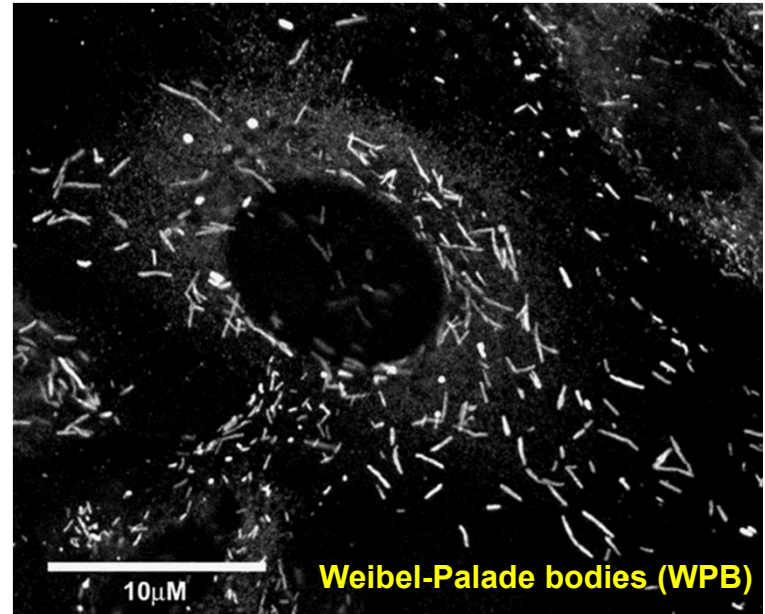
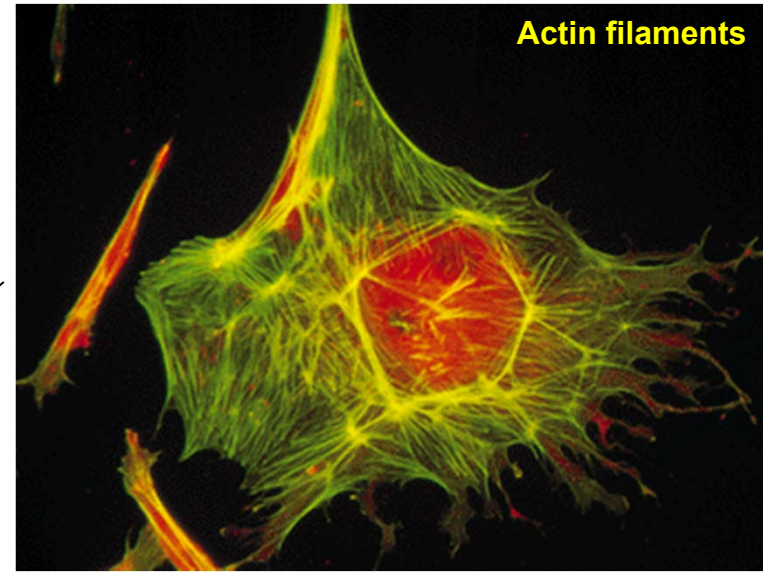
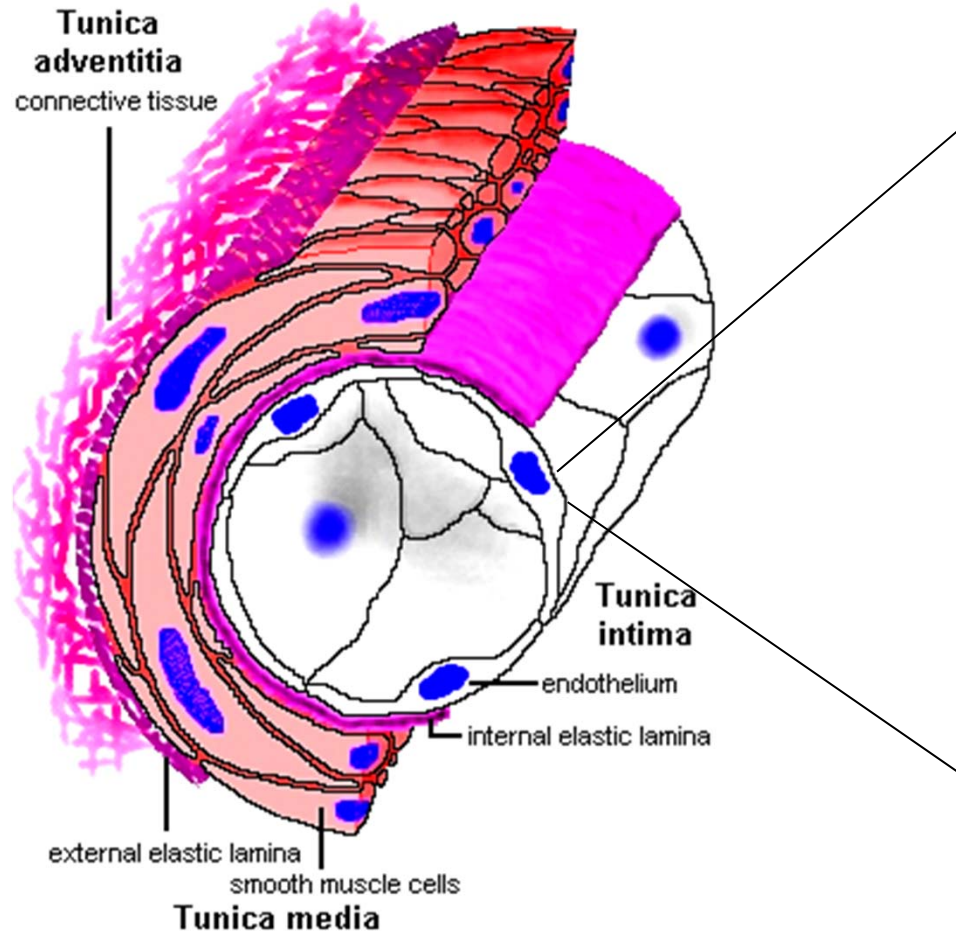


Enhancing & restoring vascular integrity

Young-Guen Kwon

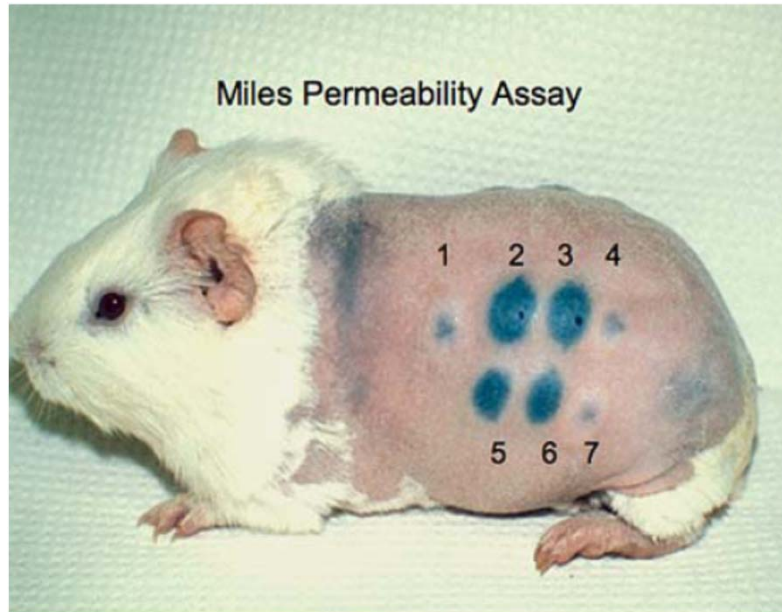
**National Research Laboratory for Vascular Genomics
Yonsei University**

Blood Vessel and Endothelial cells



ECs are critical for both angiogenesis and barrier formation

Vascular leakage (vascular permeability)



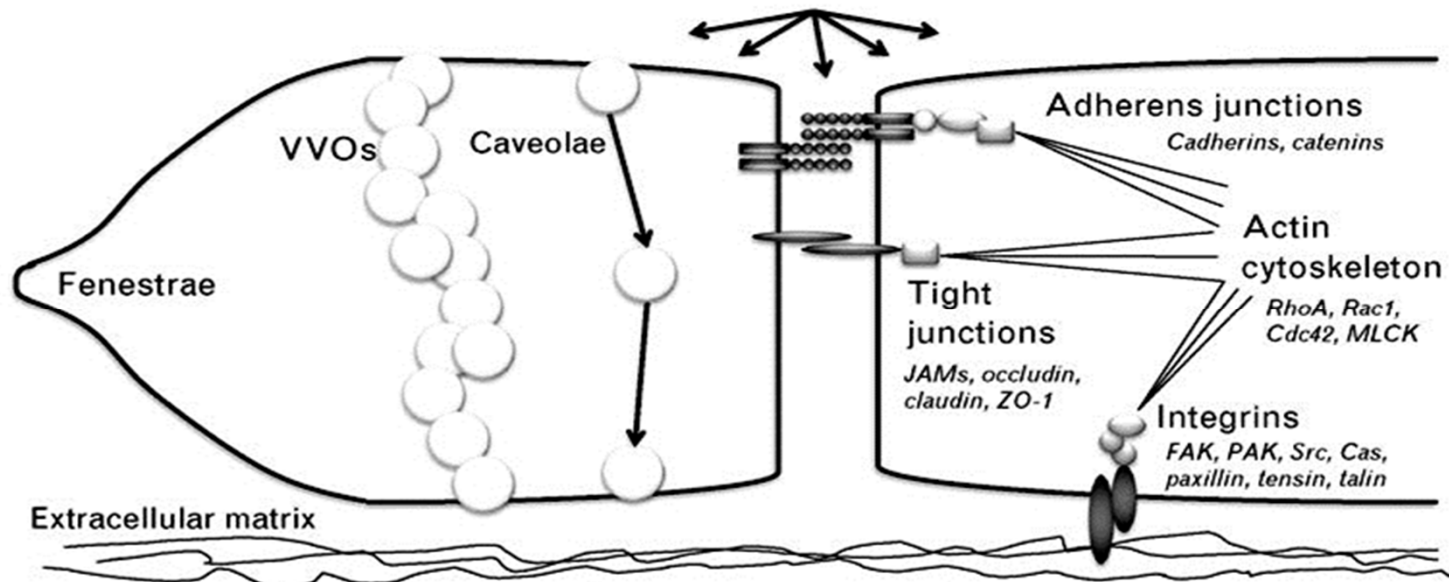
- Vascular permeability (leakiness) commences
 - Transudate gives way to exudate (protein-rich)
 - Increases interstitial osmotic pressure contributing to edema (water and ions)

Five known mechanisms known to cause vascular leakiness

- immediate widening intercellular gaps of venules (not arterioles, capillaries) by histamines, bradykinins, & leukotrienes
- leukocyte-dependent EC injury
- cytokine-mediated EC junction retraction through cytoskeleton reorganization
- direct EC damage by severe injuries
- increased transcytosis via intracellular vesicles

Circulating permeability agents

*Released from platelets, mast cells, monocytes, macrophages,
Endothelial cells, stromal cells, tumor cells*



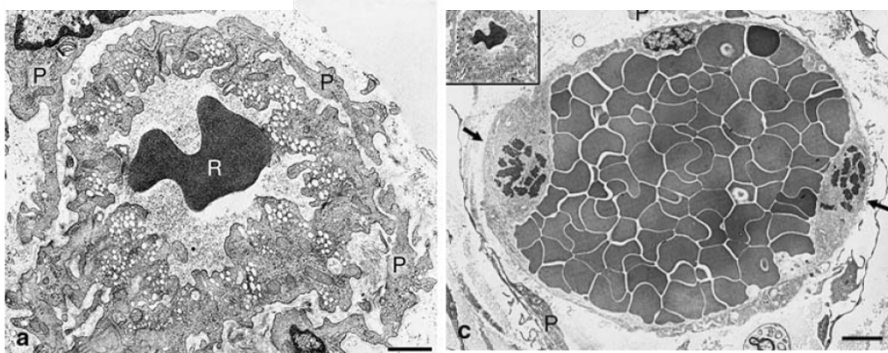
Vascular leak and edema:

*Accumulation of fluid, blood cells, tumor cells in interstitial space
results in tissue damage and prevents recovery*

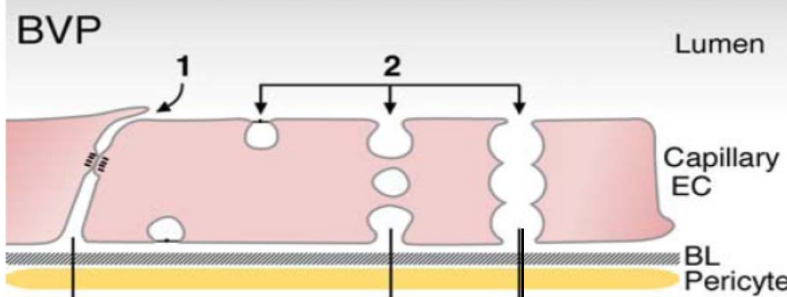
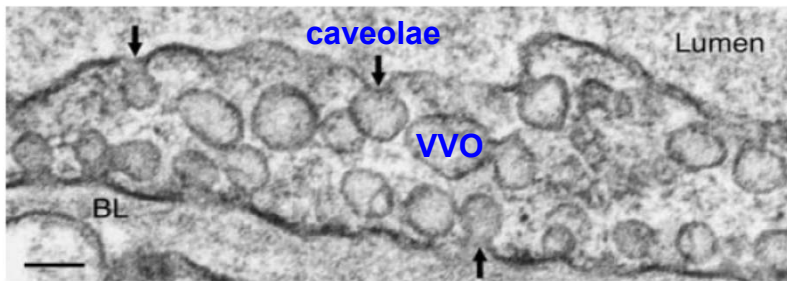
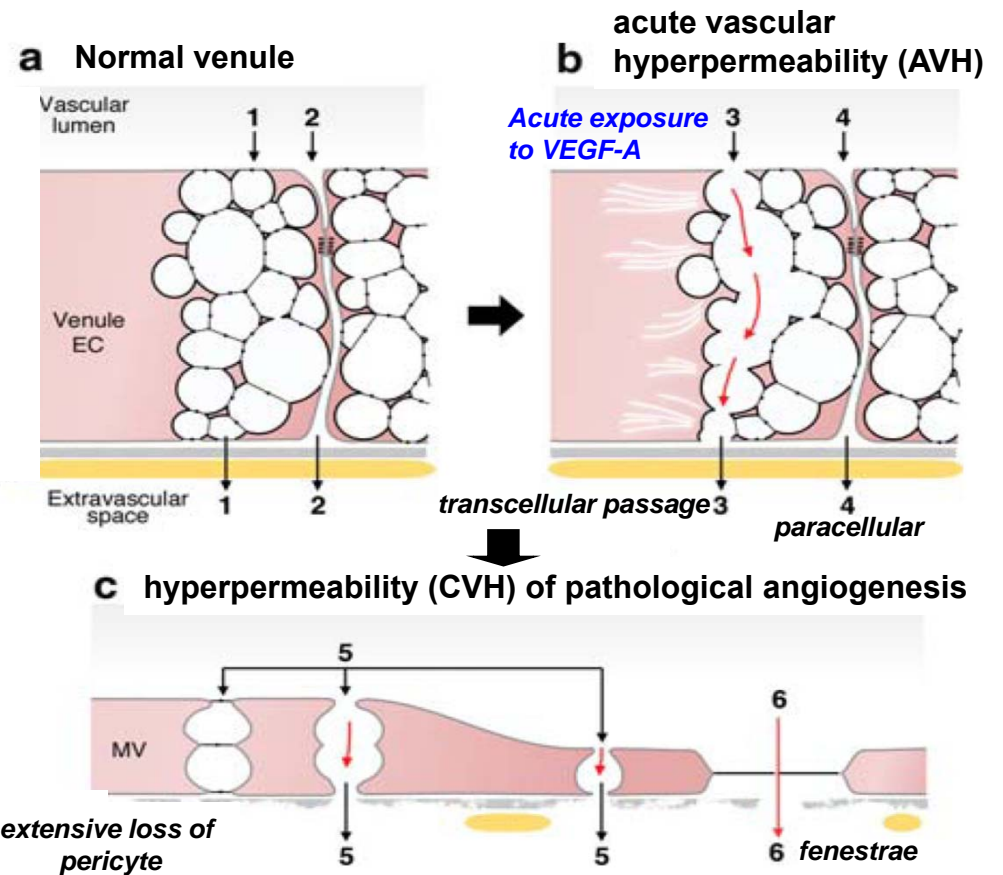
Weis: Curr Opin Hematol, Volume 15(3). 2008.243–249

Normal venule

3 days after VEGF injection

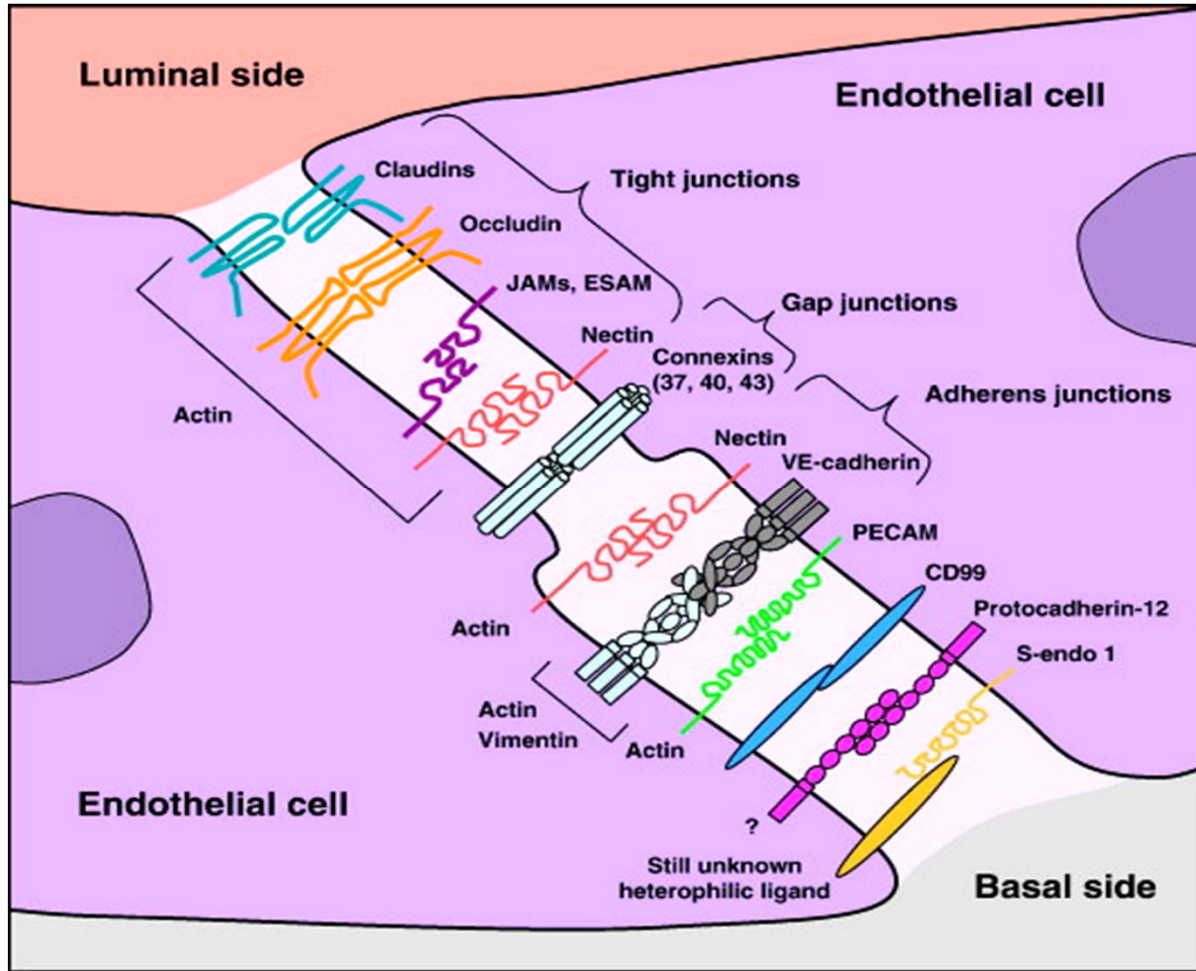


Acute & Chronic Vascular hyperpermeability



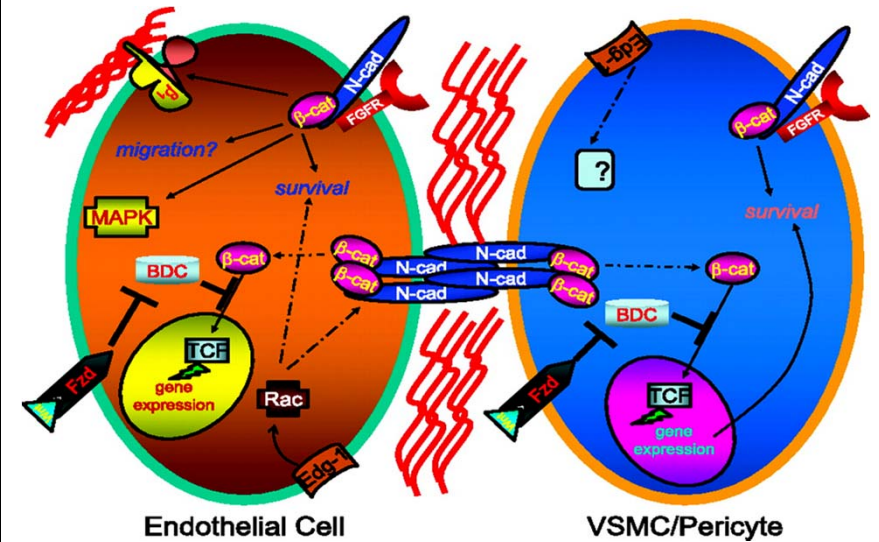
(1) intercellular cleft (2) caveolae (3) vesiculo-vacuolar organelle (VVO)

Adhesive proteins within the interendothelial cleft



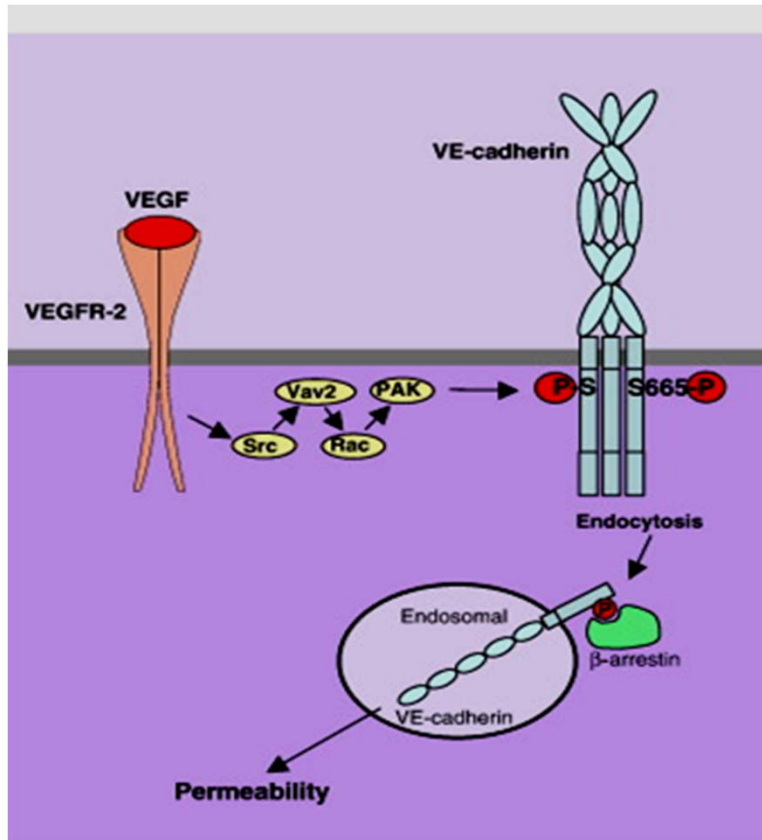
Wallez et al *BBA - Biomembranes* 2008

EC & pericyte adhesion

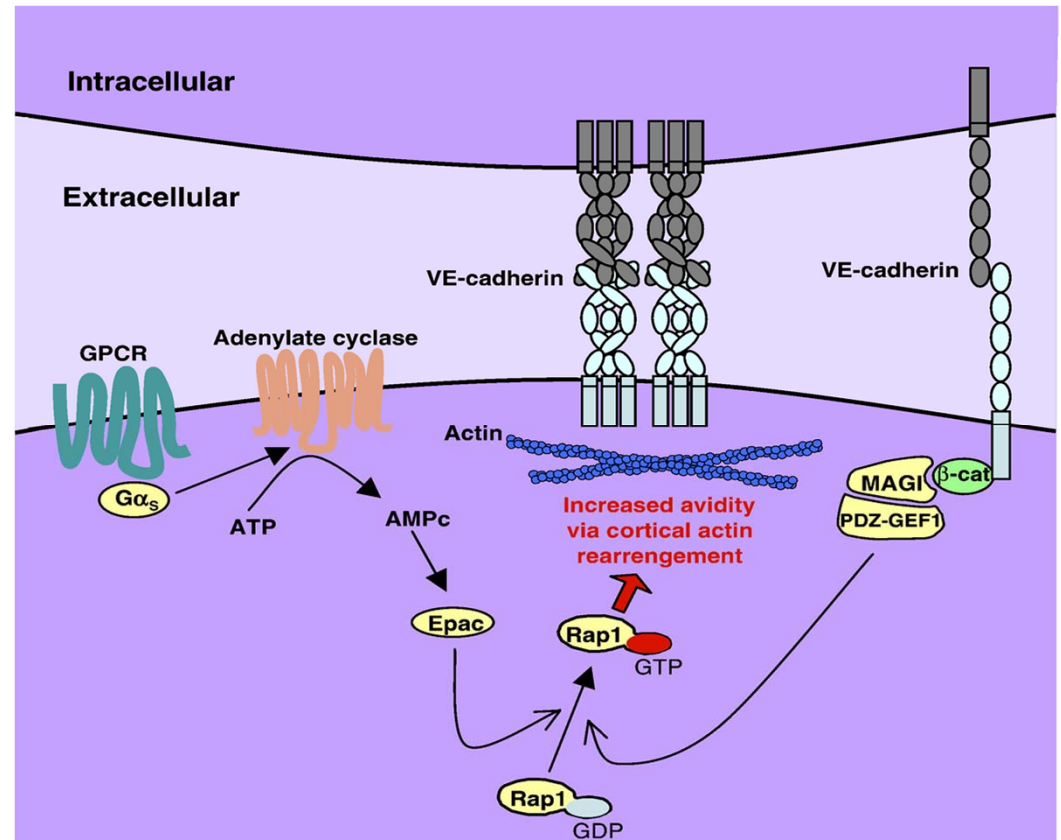


Liebner, S. et al. *ATVB* 2006

Destabilization & stabilization of VE-cadherin junction



VEGF-induced VE-cadherin phosphorylation & internalization via Src kinase



Rap1 activation pathways and stabilization of the endothelial barrier

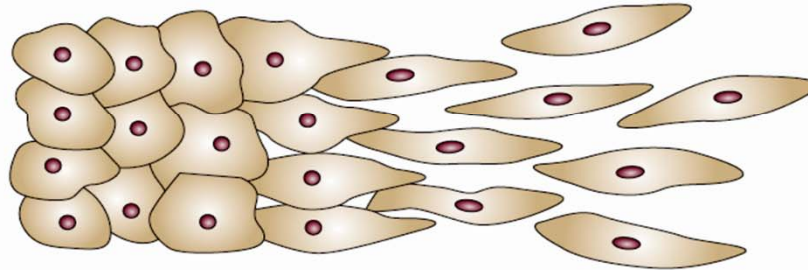
Phenotypes of confluent & sparse cells

Confluent cells

- Epithelioid phenotype
- Contact inhibition of growth and motility
- Rearrangement of actin microfilaments
- Protection from apoptosis
- Apical-basal polarity

Sparse cells

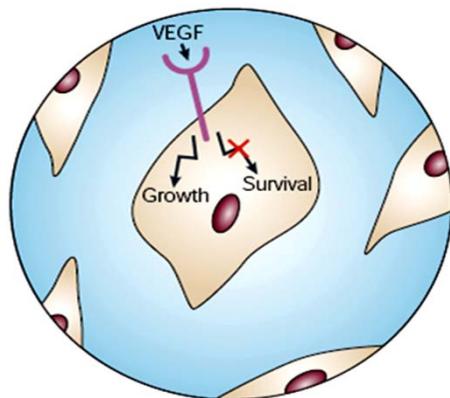
- Fibroblastoid morphology
- Active growth
- Motility



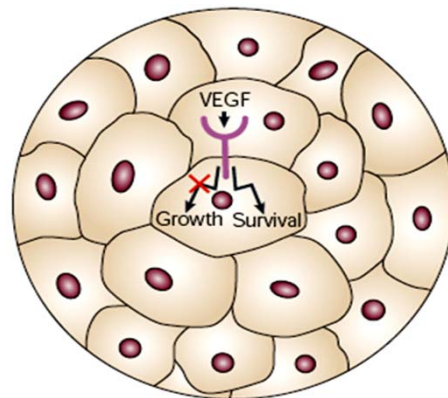
Junctional structures contribute to the 'resting' confluent phenotype by transducing signals within the cells and changing gene expression. Sparse cells, which lack cell-cell junctions, are unable to transduce such signals.

VEGF signaling in confluent and sparse endothelial cells

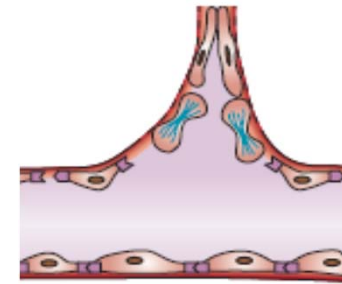
Motility & proliferation



Stabilization

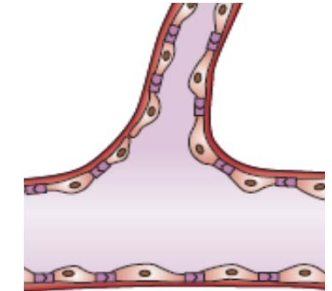


Junction disorganization



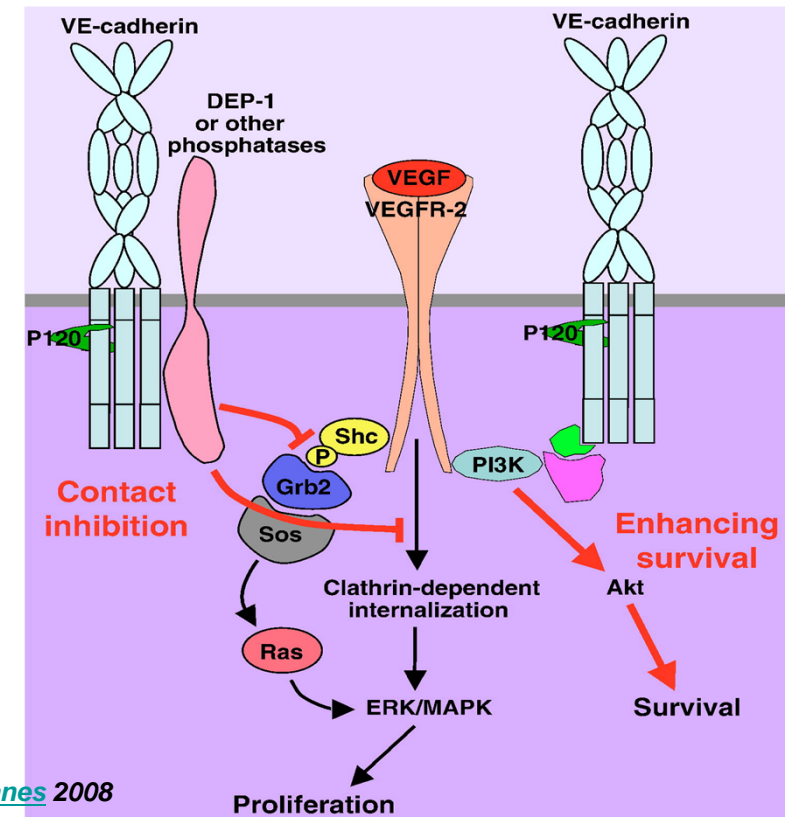
- migrate and proliferate
- increases vascular permeability

Junction maturation

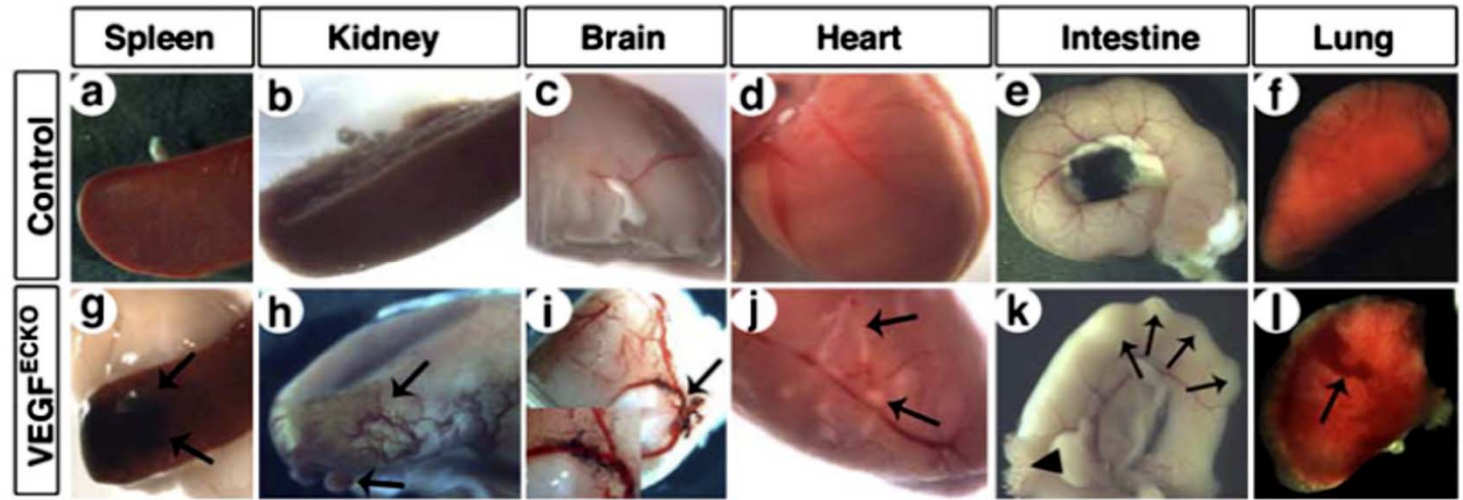
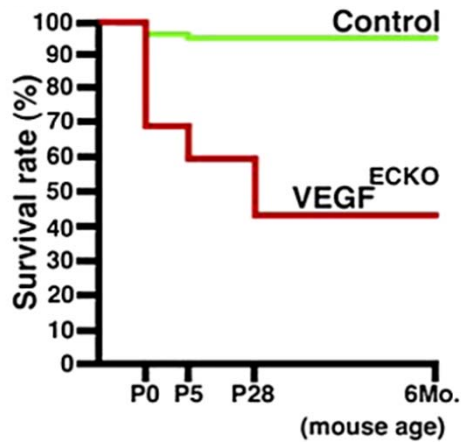


- proliferation & apoptosis are inhibited

VEGFR2 & VE-cadherin

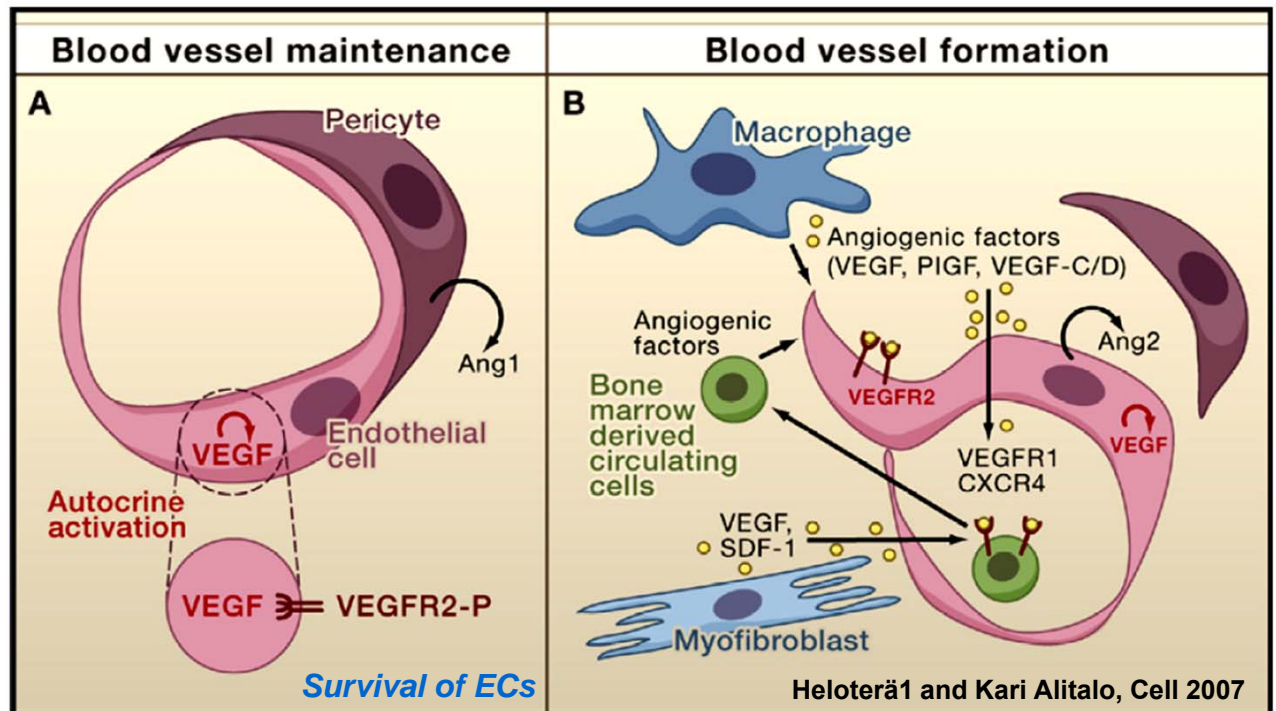
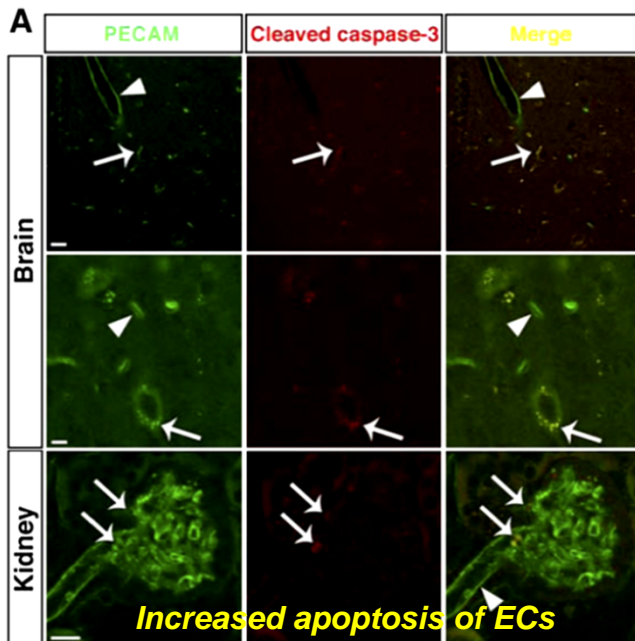


Endogenous VEGF from ECs is crucial for vascular homeostasis

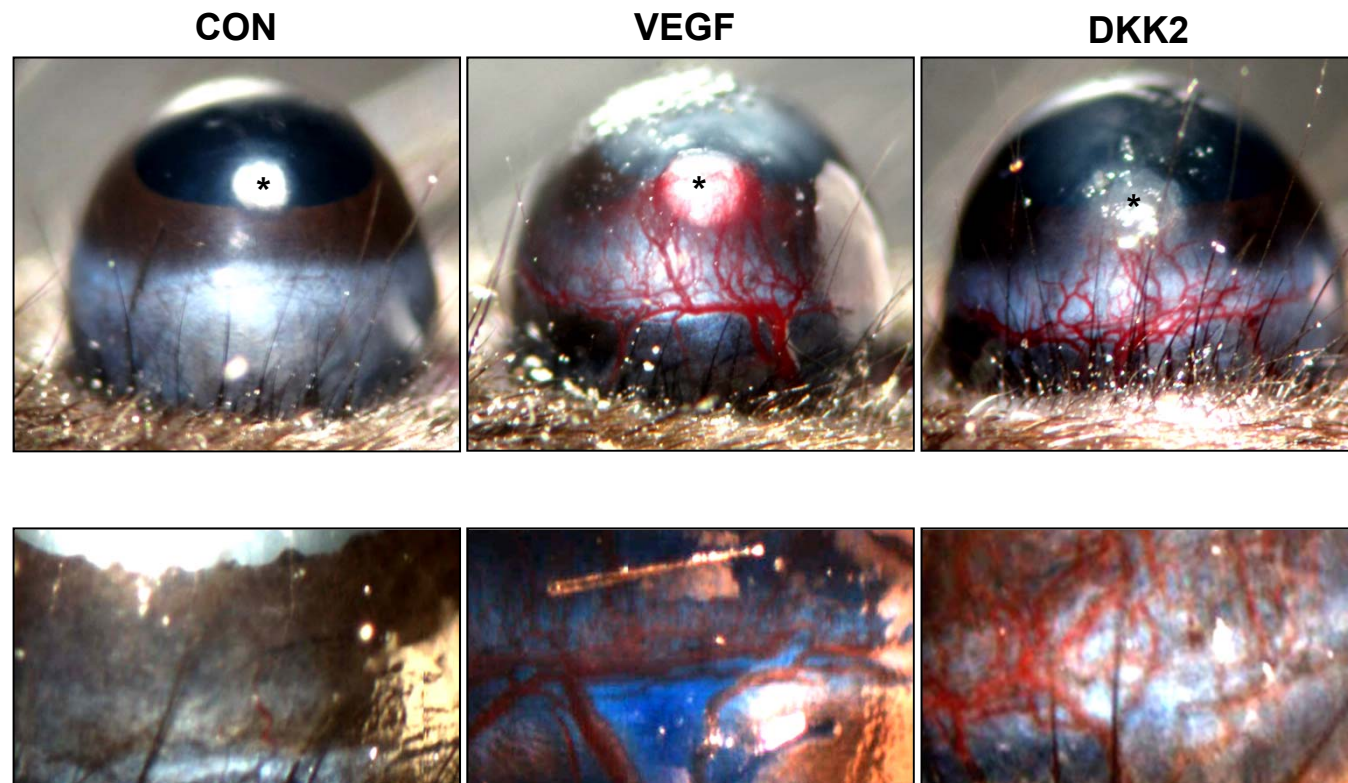


VEGF^{ECKO} organs of older mice show hemorrhage (arrows in g, i, and l), tortuous and dilated vessels (arrows in h), collagen accumulation in the heart indicative of microinfarcts (arrows in j), and intestinal perforations (arrows in k)

Lee et al, Cell 2007

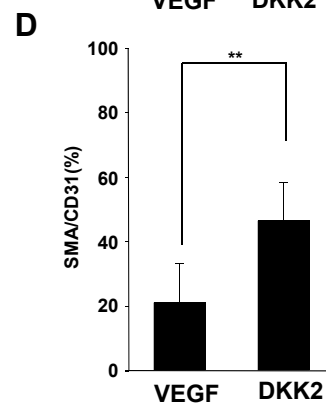
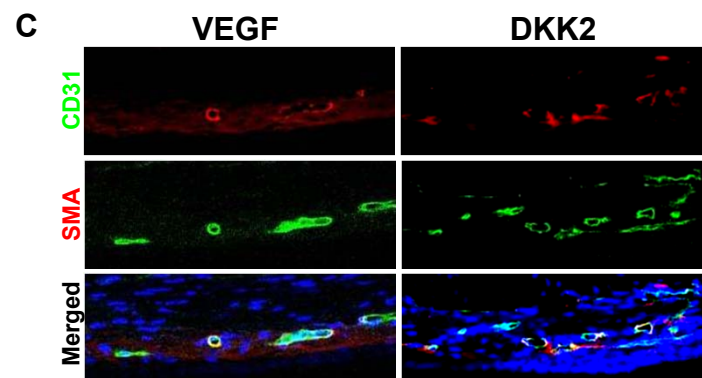
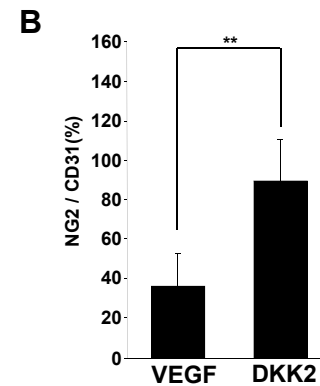
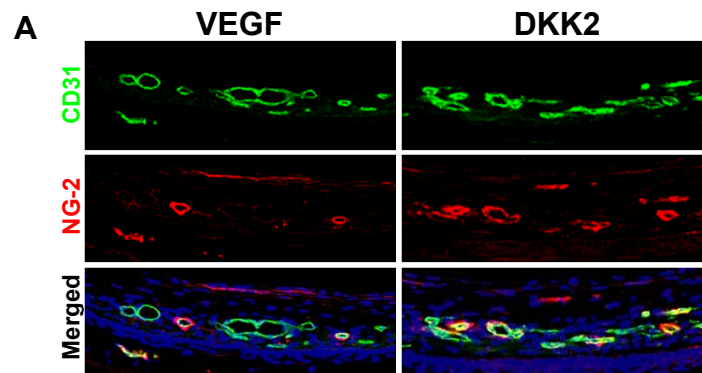


DKK2 recruits normal blood vessels in a mouse cornea micropocket assay



*** Unlikely to VEGF, DKK2-induced blood vessels were well organized and non-leaky

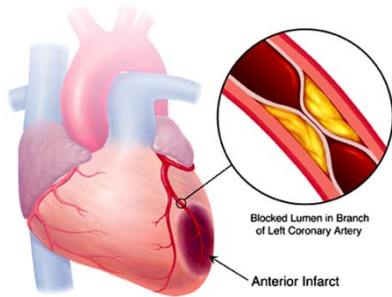
DKK2 induced blood vessels coated with pericytes



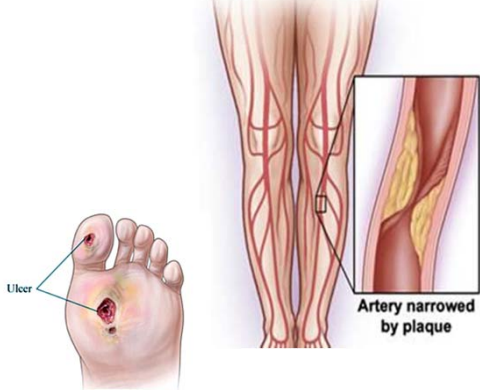
CD31, endothelial cell
NG-2, pericyte
SMA, smooth muscle actin

Vascular abnormalities and diseases

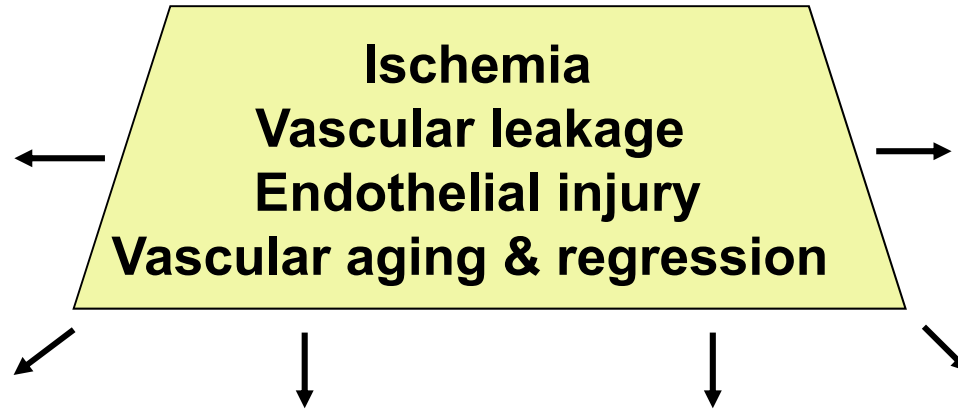
Myocardial Infarction



Peripheral artery diseases



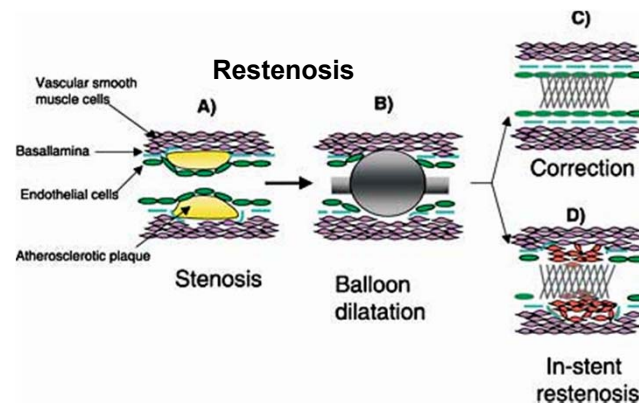
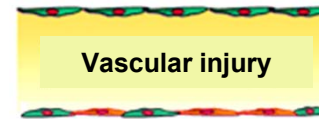
Delay Wound healing
Bone fracture
Baldness
Erectile dysfunction
Preeclampsia etc



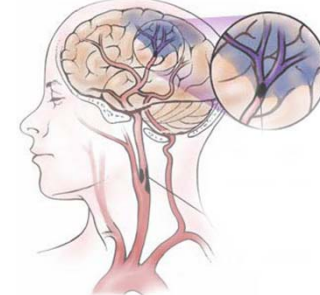
AMD
Diabetic Retinopathy
Macular Edema



Vasculitis



Stroke



Etc.

Increased permeability is associated with a variety of human diseases

Mediators of Increased Endothelial Permeability

Diseases

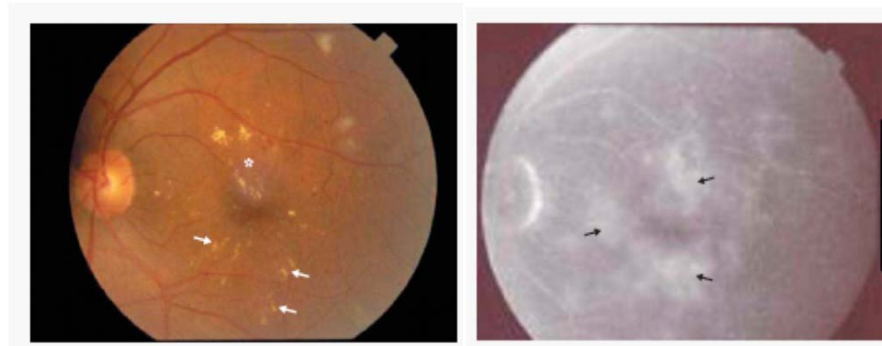
Endothelial Barrier Stabilizing Mediators

Thrombin	diabetic macular edema	stroke	S1P Ang-1
Bradykinin	tumors	acute lung injury	
Histamine	psoriasis	alzheimer disease	
Oxidants	sclerosis	trauma	
VEGF	AMD	nephropathies	
TNF- α	encephalomyelitis	edema	
LPS	inflammatory bowel disease	Rheumatoid arthritis	

- 당뇨병 또는 노인성 황반의 경우, 망막혈관의 누수가 시력을 잃게 되는 직접적인 원인이 됨
- 뇌졸중의 경우, 뇌혈관의 edema가 병증을 악화하는 주원이 됨
- 종양의 경우, 새로 생성되는 혈관이 비상적인 구조로 약물이 종양세포 말단까지 전달되는 것을 막아 항암치료의 효능을 제한하는 원인이 됨
- Restenosis의 경우, stent 삽입 과정에서 혈관내벽의 손상이 재협착증을 유발하는 주원인이 됨
- 이외에도 혈관 누수 및 혈관내피세포의 손상은 다양한 질환의 병증을 악화시키는데 관여함
- 현재까지 혈관의 누수를 차단하거나, 손상된 혈관을 복원하는 약물의 개발은 매우 초기 단계에 있음

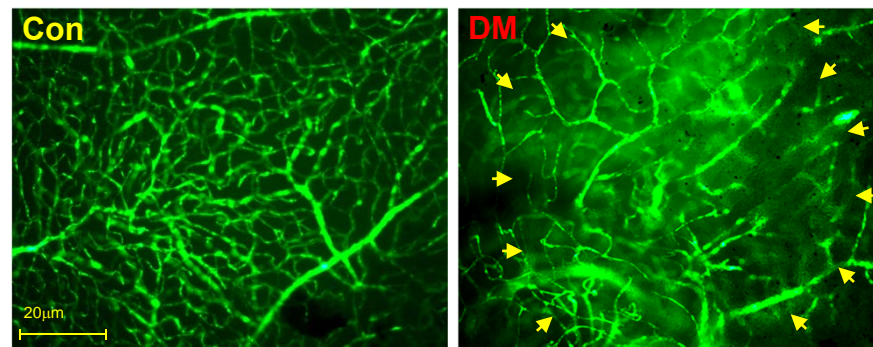
Diabetic Macular Edema

involves **the breakdown of the blood–retinal barrier**, with leakage of plasma from small blood vessels in the macula, the central portion of the retina that is responsible for the major part of visual function.



NEJM
2004

- a ring of hard lipid exudates (white asterisk) superior to the center of the macula
- the leakage of dye (arrows) within the lipid ring and associated with the other lipid clusters



vascular leakage in a streptozocin-induced diabetic mouse model

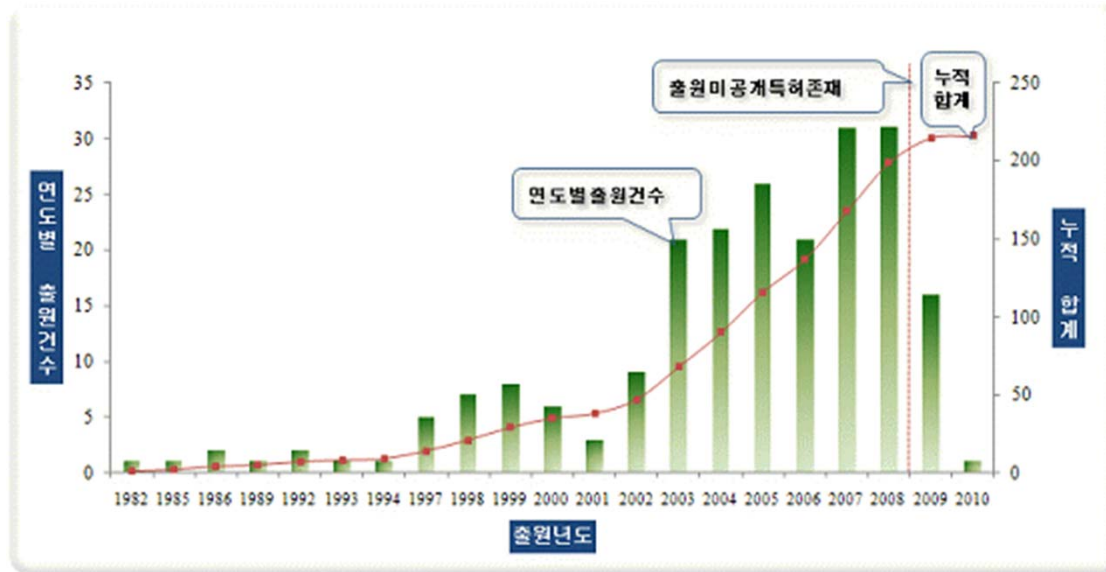
Interventions for diabetic macular edema

황반의 경우, 당뇨 및 고령화로 환자수가 급증하고 있으나, 기존 치료법은 망막혈관의 edema를 막는데는 실패하여 근원적인 치료법으로 자리잡지 못하고 있어서 새로운 개념의 치료법 개발이

- Laser treatment
- Surgical management
- Intravitreal corticosteroids
- NSAID, non-steroidal anti-inflammatory drug
- Intravitreal antiangiogenesis agents

M O'Doherty, I Dooley, M Hickey-Dwyer, Br J Ophthamol 2008

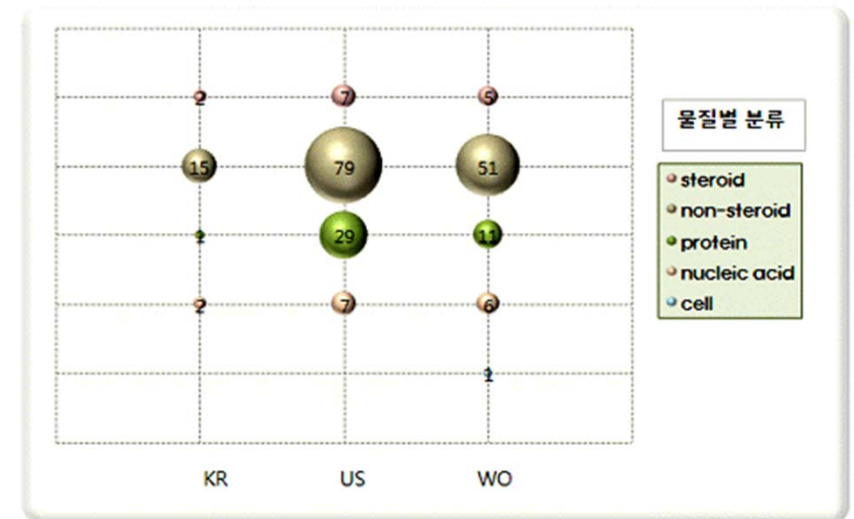
안과혈관질환 분야 연도별 출원 동향



안과혈관질환 특허의 물질별 동향 및 전망

> '혈관누수에 의한 안질환 치료제 개발 기술'은 아직 발명 기전이 명확히 규명되지 않은 분야의 특성 상, 산재된 특허 출원 분포를 보이고 있으며, 이는 신규 약물 타깃을 발굴하기 위한 연구 트렌드를 반영함

> 전세계적으로 비스테로이드 화합물 기술 분야의 특허가 가장 많은 출원이 이루어져 안과혈관 질환 치료제 분야 중, 비스테로이드 화합물에 대한 관심이 집중되고 있는 것으로 파악됨

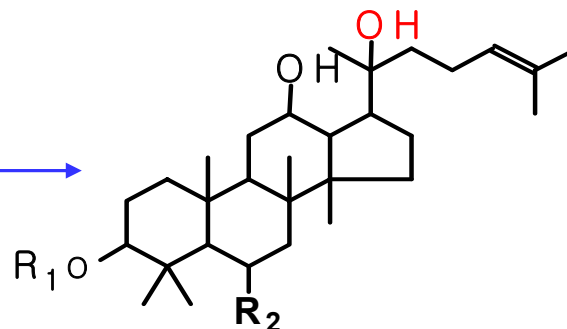
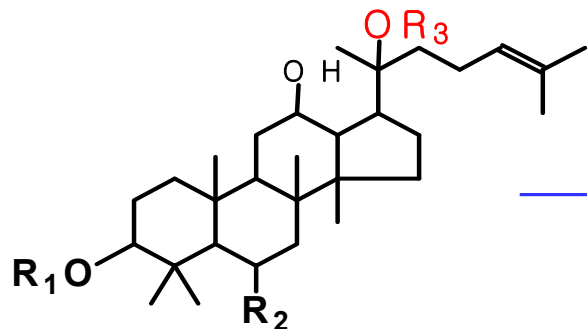


▶안과 질환 치료제 개발 분야는 현재 도입 단계로서, 최근 이에 대한 관심이 급증하면서 2000년대 초반 이후 관련 특허가 급격히 증가

(특허분석자료 인용)

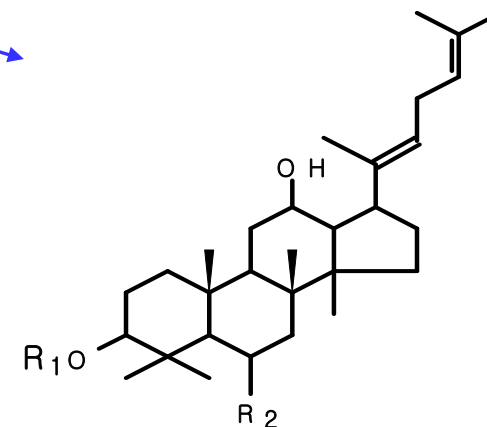
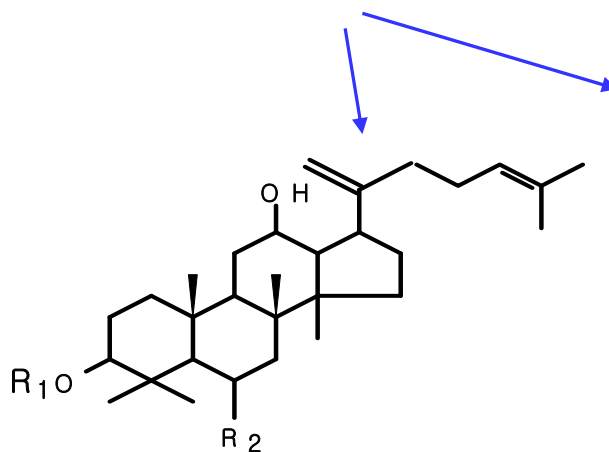
Structural diversity of Ginsenosides

Ginsenoside-a class of steroid-like compounds, triterpene saponins, found exclusively in the plant genus *Panax* (ginseng)



	R_1	R_2
→ Rg_3	glc-glc	H
→ Rh_2	glc	H
→ Rg_2	H	glc-rha
→ Rh_1	H	glc

	R_1	R_2	R_3
ginsenoside			
Rb_1	glc-glc	H	glc-glc
Rb_2	glc-glc	H	glc-ara
Rc	glc-glc	H	glc-ara
Rd	glc-glc	H	glc
Re	H	glc-rha	glc
Rg_1	H	glc	glc



	R_1	R_2
→ Rk_1	glc-glc	H
→ Rk_2	glc	H
→ Rg_6	H	glc-rha
→ Rk_3	H	glc

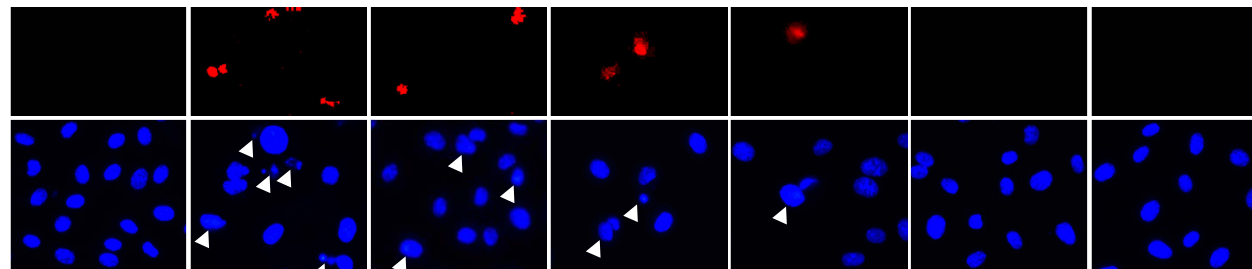
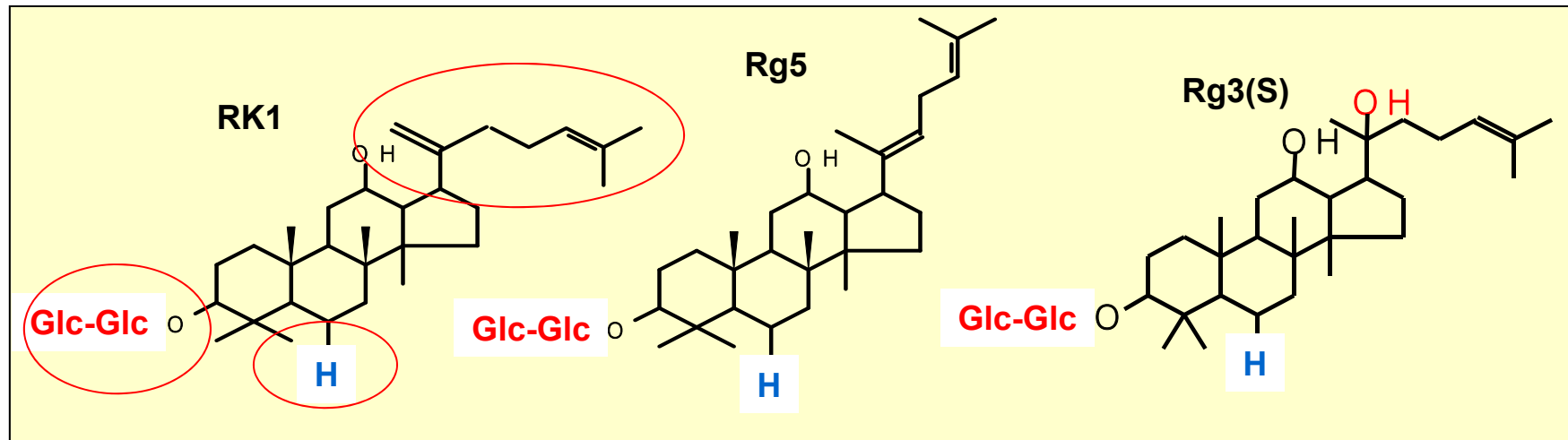
	R_1	R_2
→ Rg_5	glc-glc	H
→ Rh_3	glc	H
→ F_4	H	glc-rha
→ Rh_4	H	glc

From Park JH, SNU

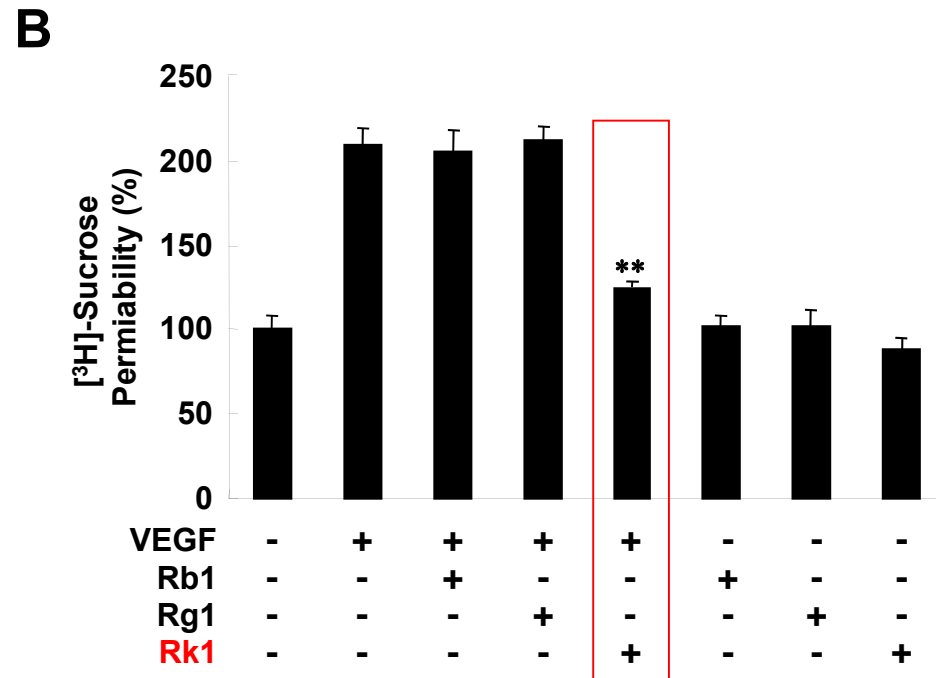
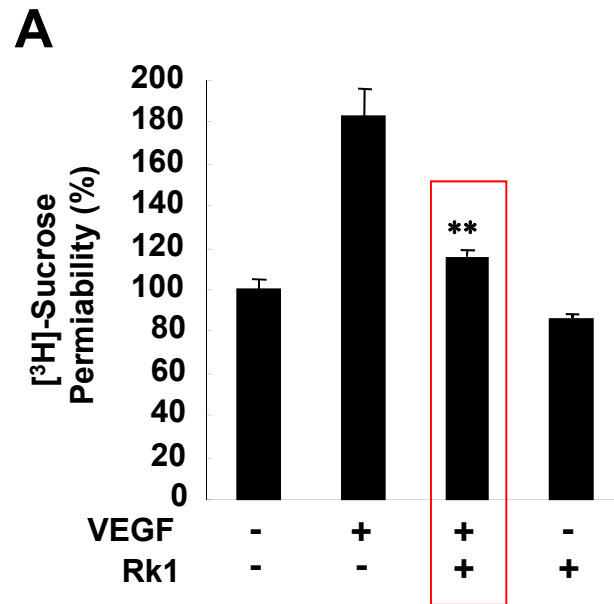
Effects of ginsenosides on retinal endothelial cell survival against serum deprivation-induced apoptosis



Structural similarity in active ginsenosides



Rk1 inhibits VEGF-induced retinal endothelial permeability



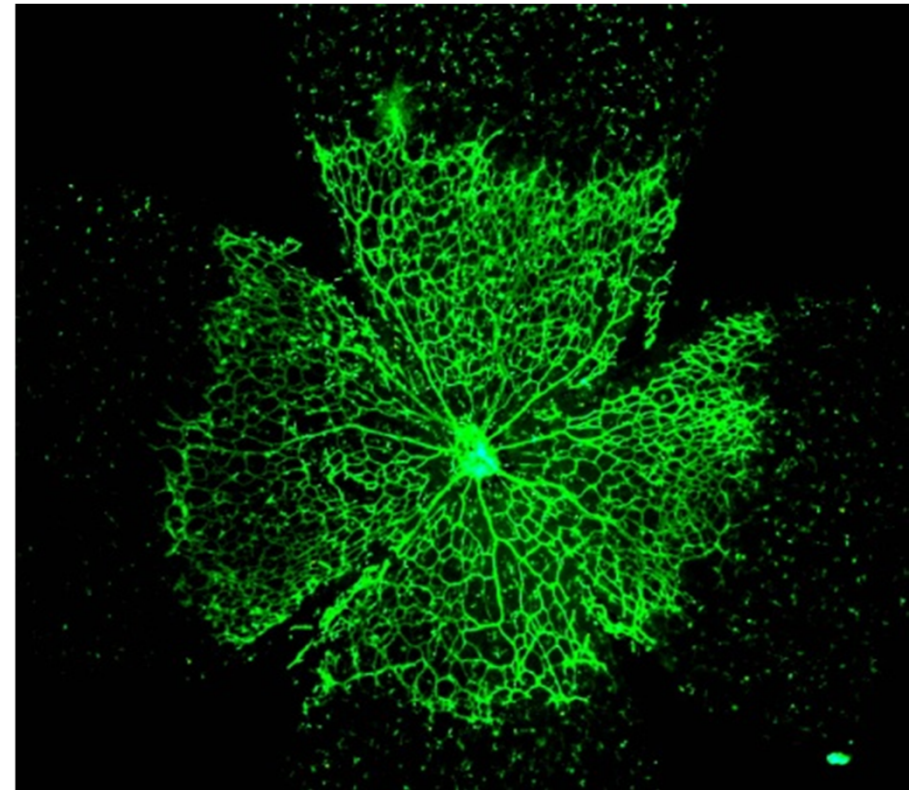
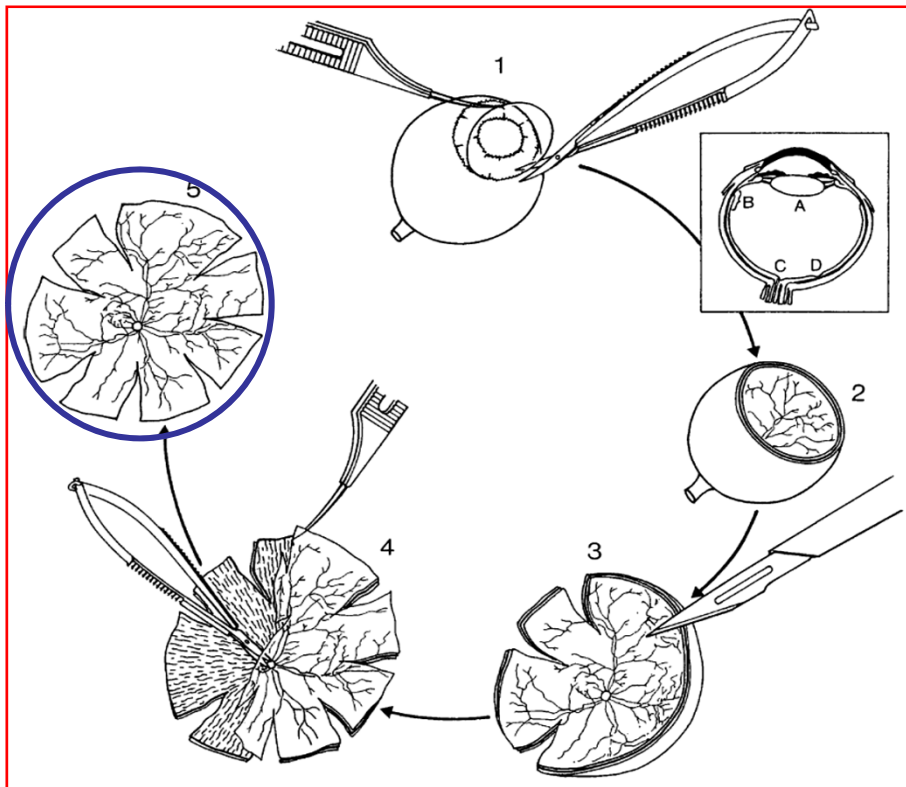
Experimental procedure for measuring retinal vascular permeability

VEGF (w/wt Rk1) injection into the vitreous layer of mouse eye (8 weeks old mice)

→ Cardiac or tail vein injection FITC-dextran

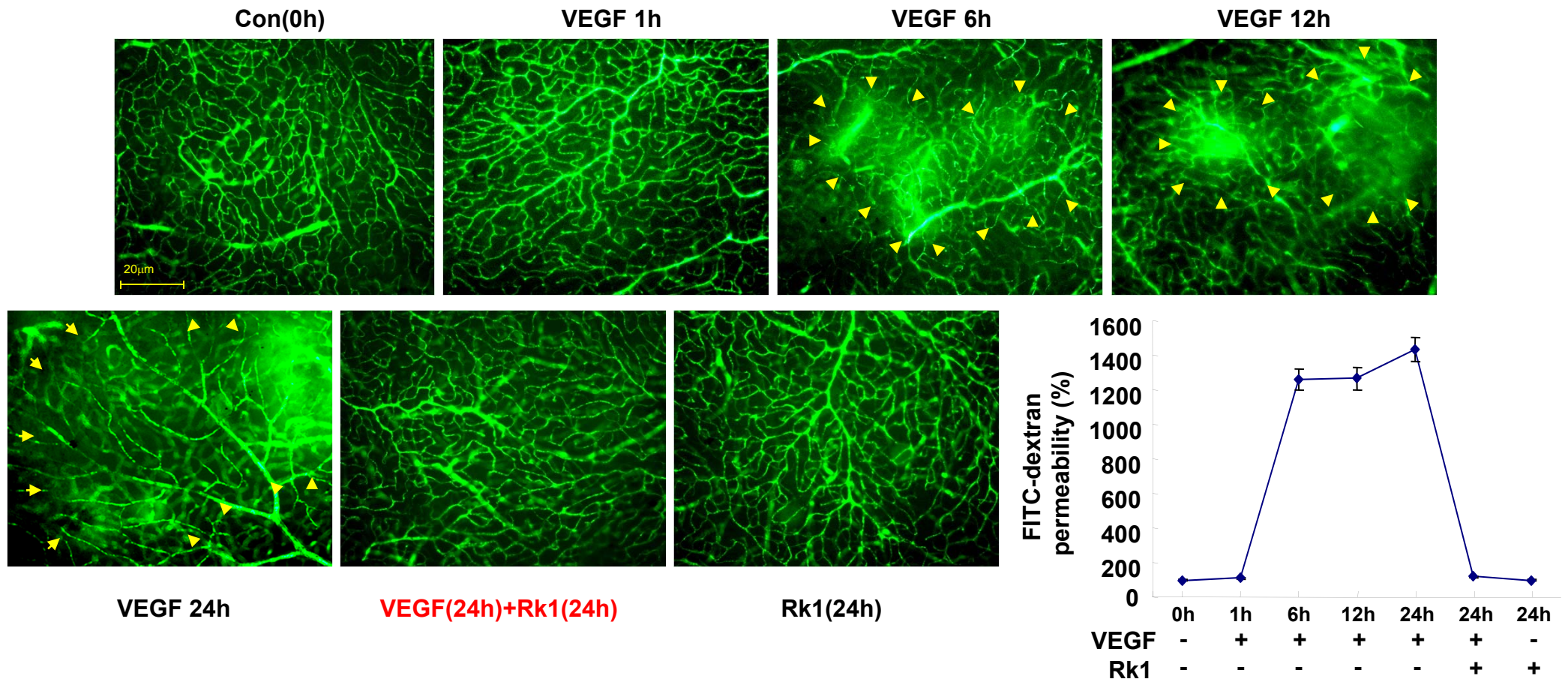
→ Flat mounting of retina

Flat mounting of Retina

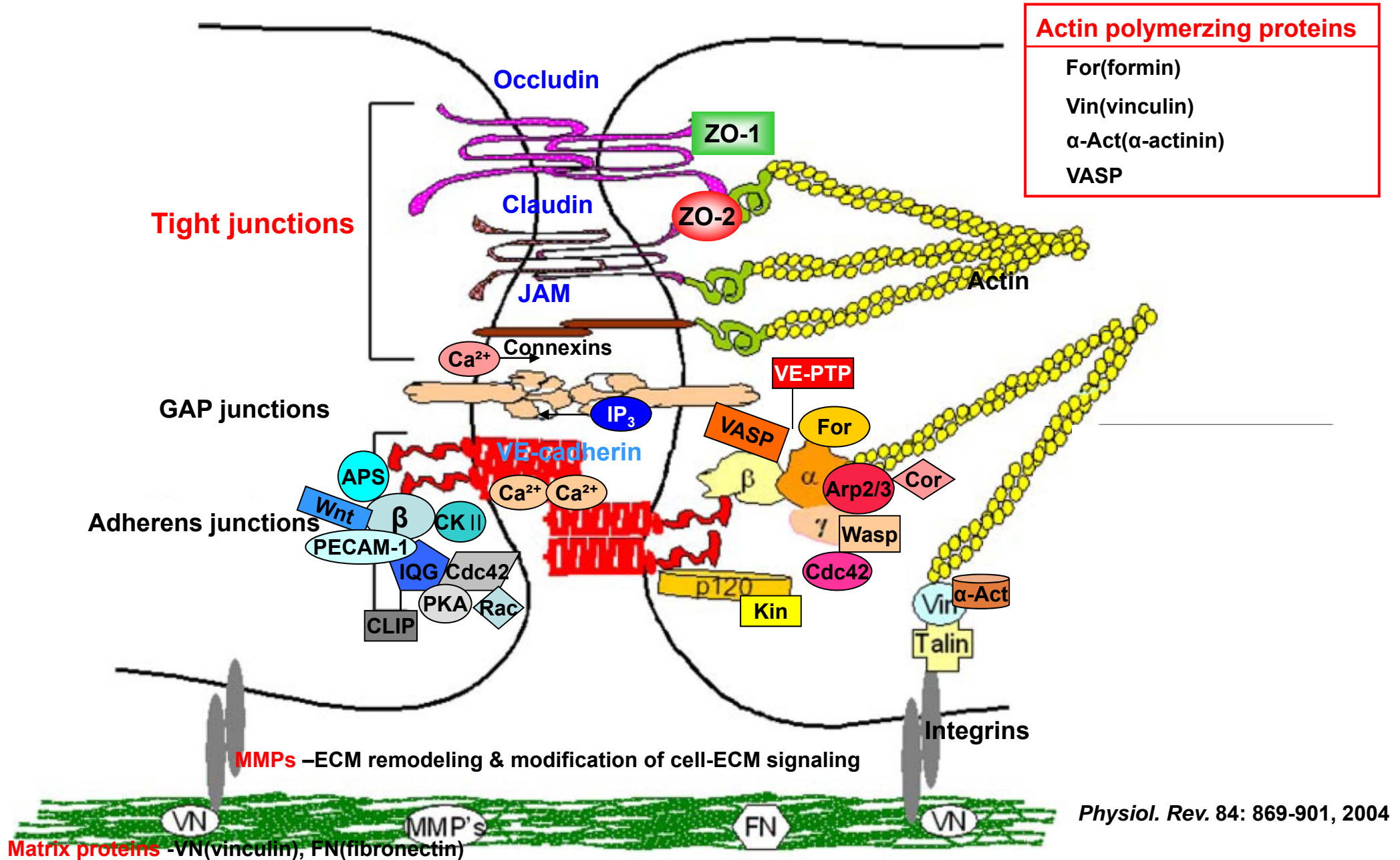


Flat mount staining of retinal vessels at P4

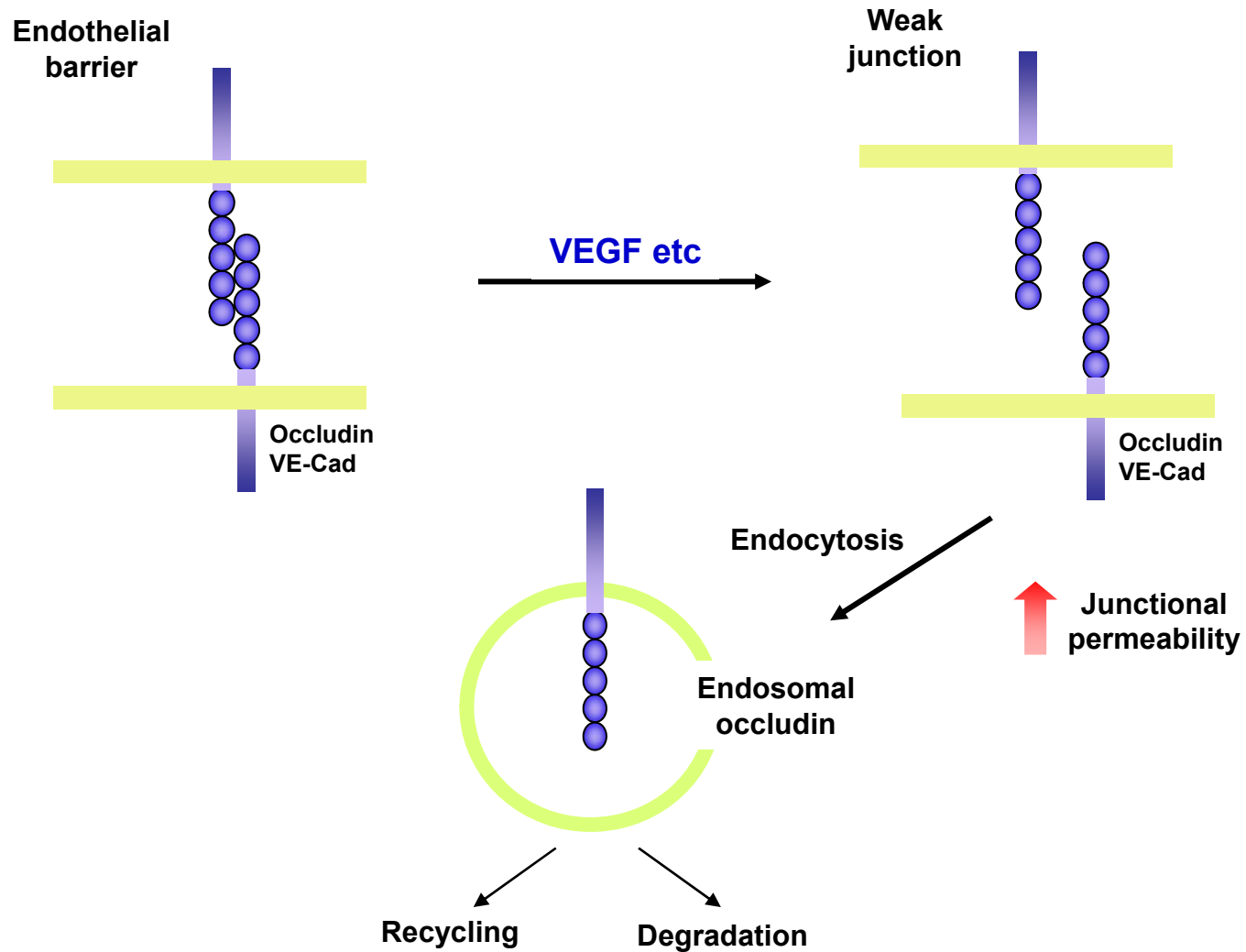
Rk1 inhibits VEGF-induced retinal permeability *in vivo*



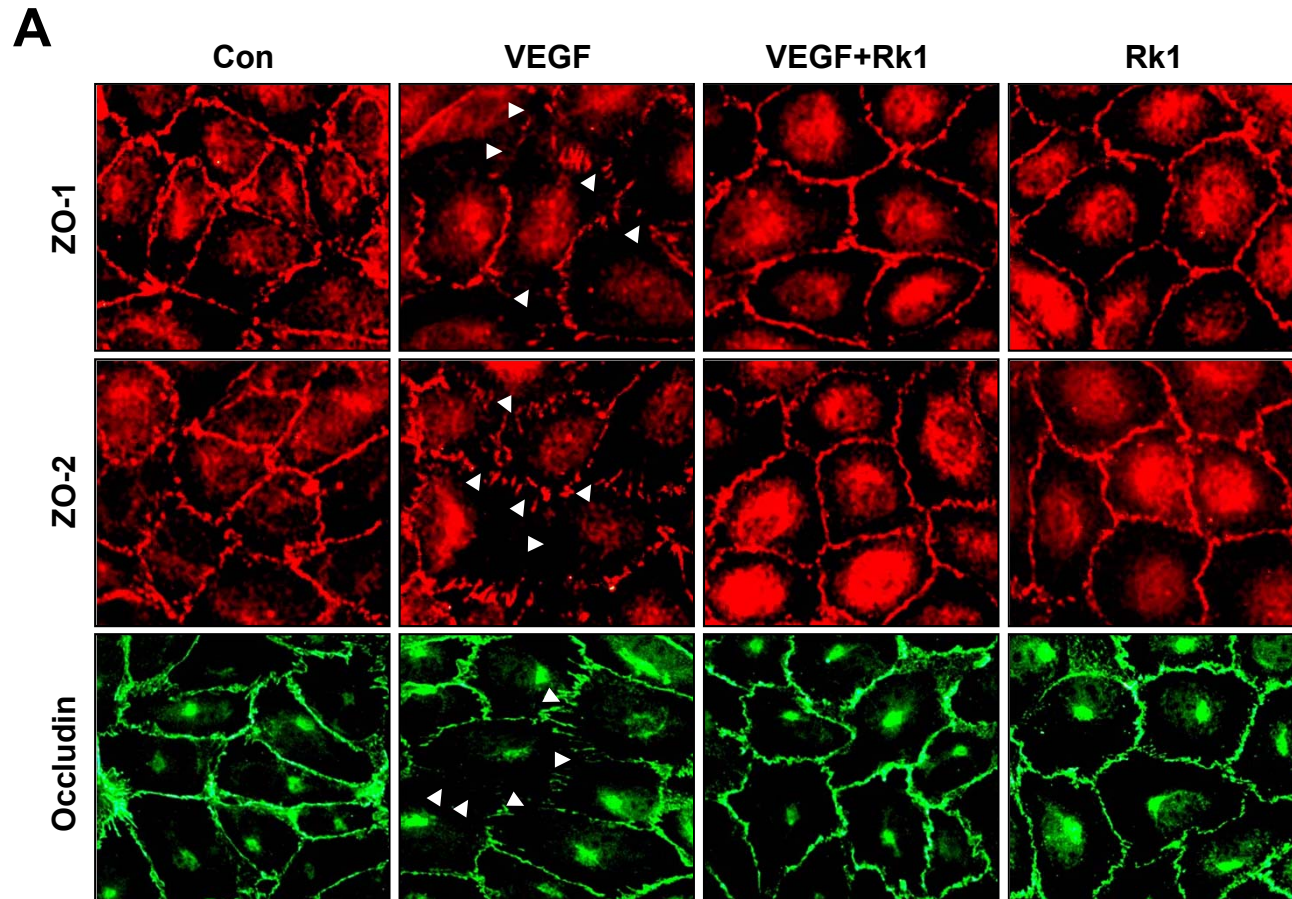
Structural organization of endothelial cell intercellular and matrix interactions



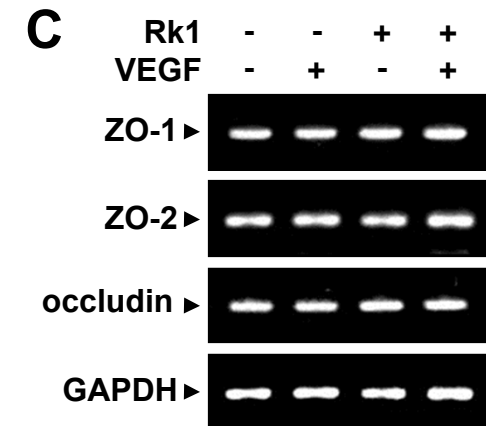
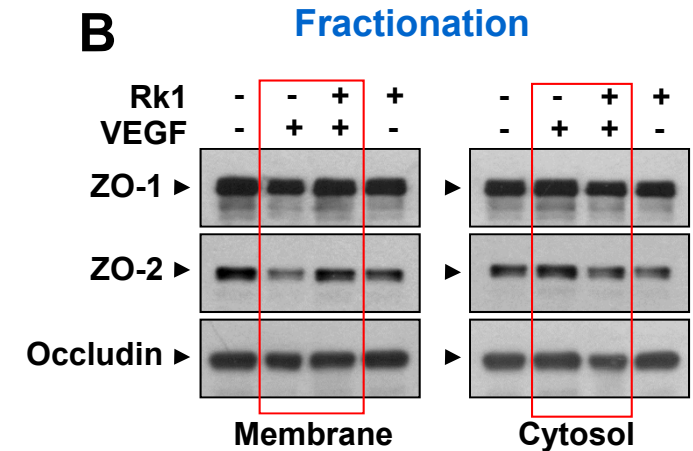
VEGF-stimulated endothelial junction disassembly & permeability



RK1 inhibits VEGF-induced breakdown of blood-retinal barrier



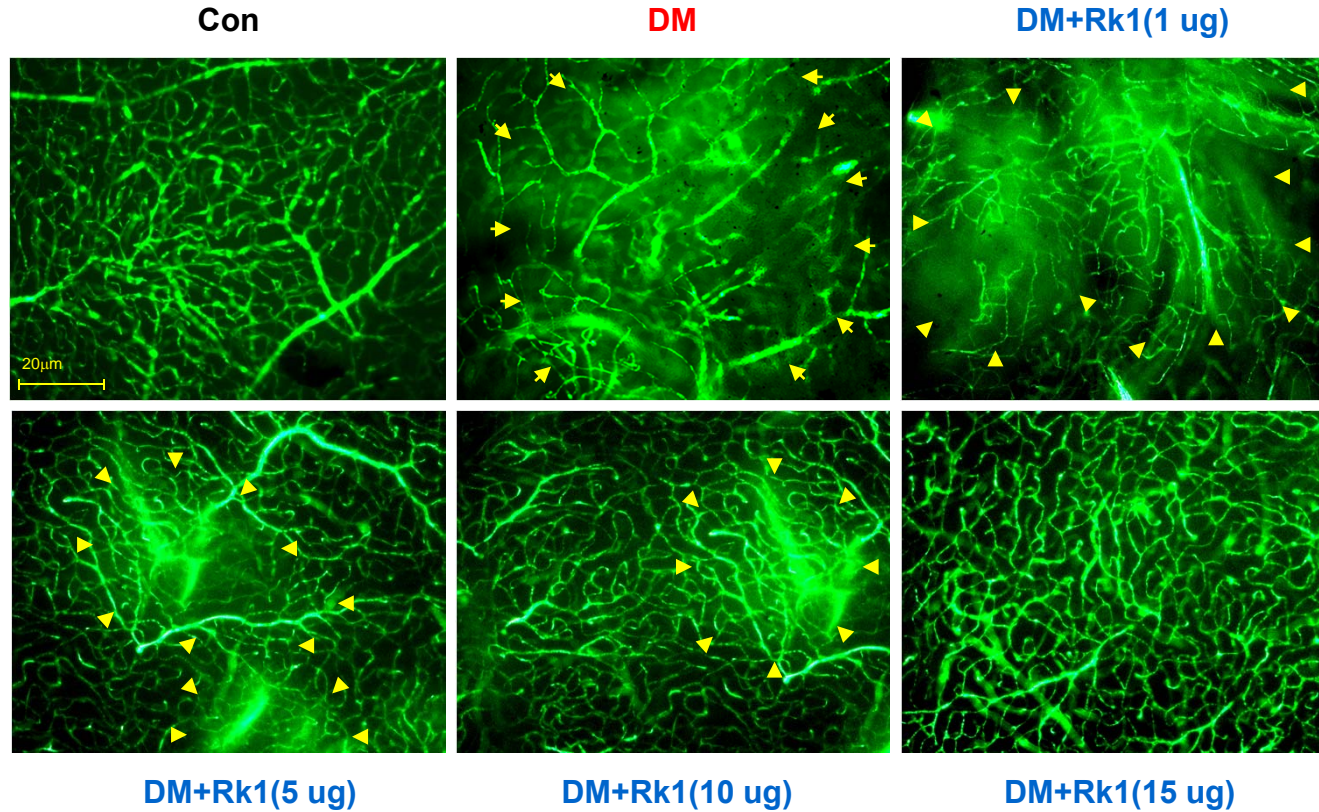
Staining of TJ proteins in retinal endothelial cells



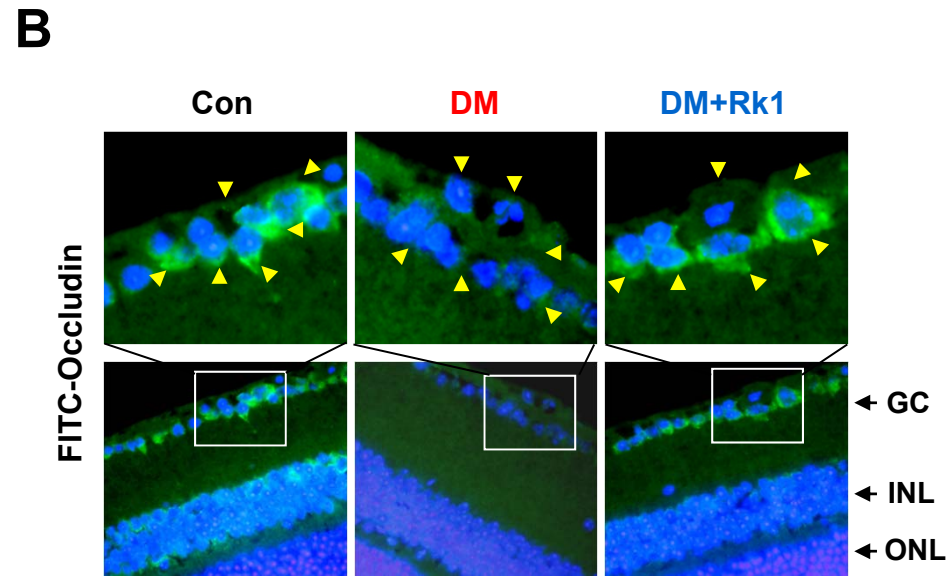
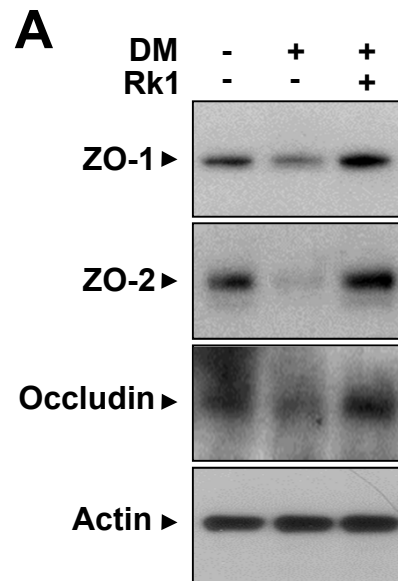
No effects on mRNA expression of TJ proteins

RK1 inhibits retinal vascular leakage in a streptozozan-induced diabetic mouse model

Streptozozan-induced DM → RK1 treatment for 24 h → Tail vein injection of FITC-dextran → Flat mounting for 10 min

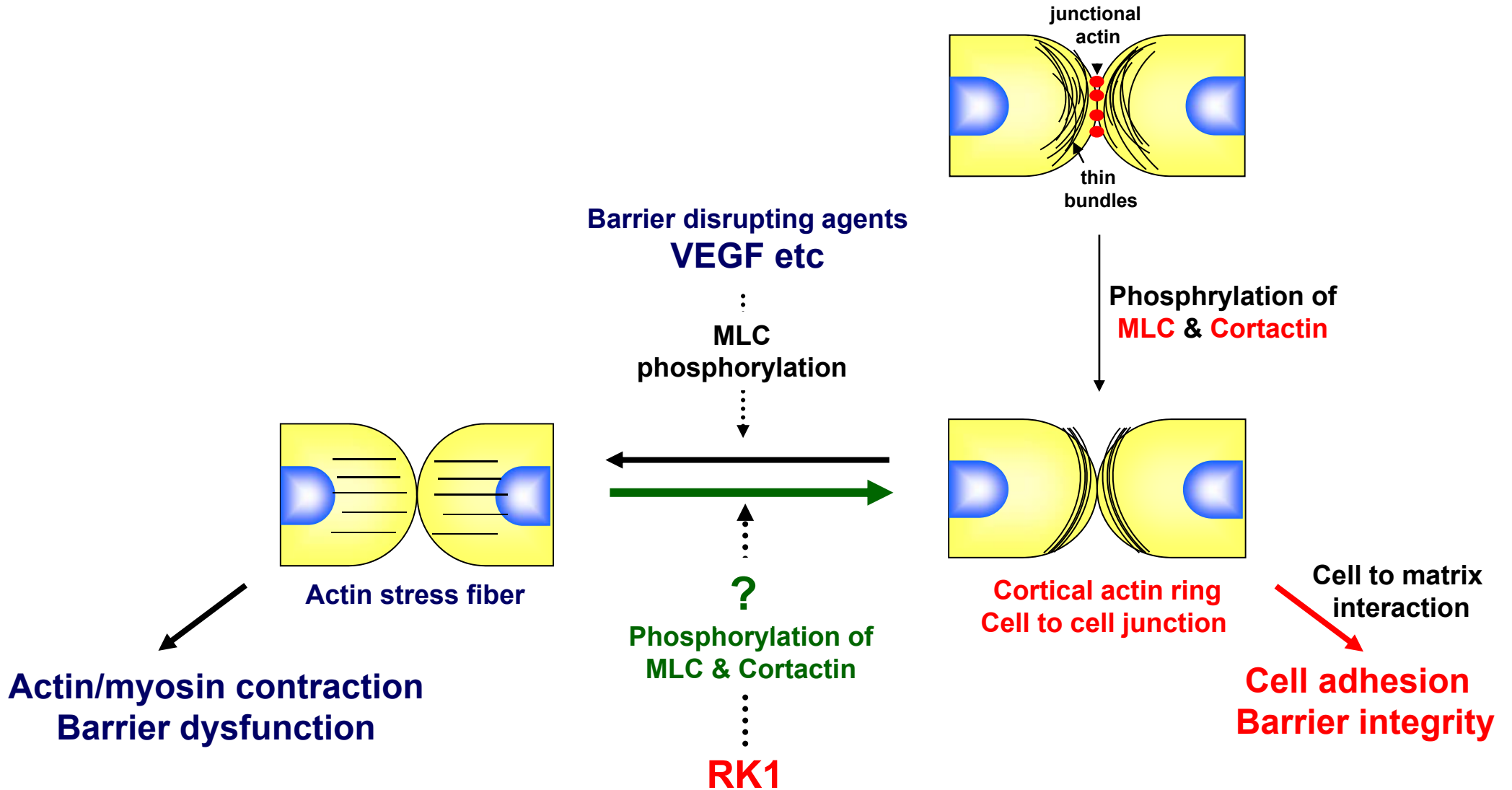


Rk1 restored presence of TJ proteins at cell to cell contacts in the retinal blood vessels of diabetic mouse



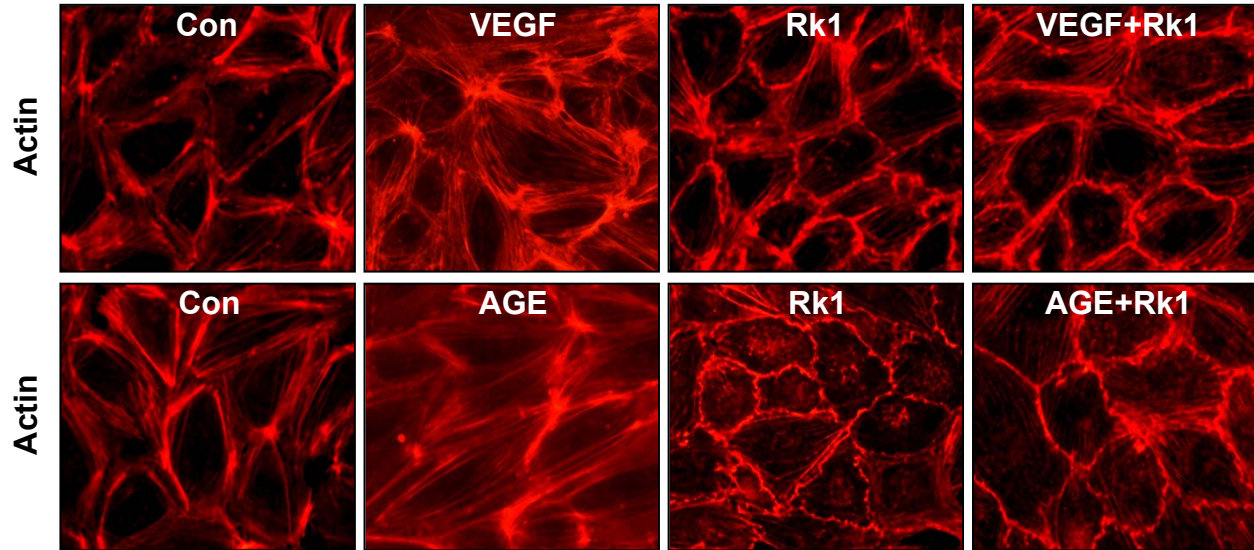
TJ proteins at the mouse retina

Actin structure & endothelial integrity

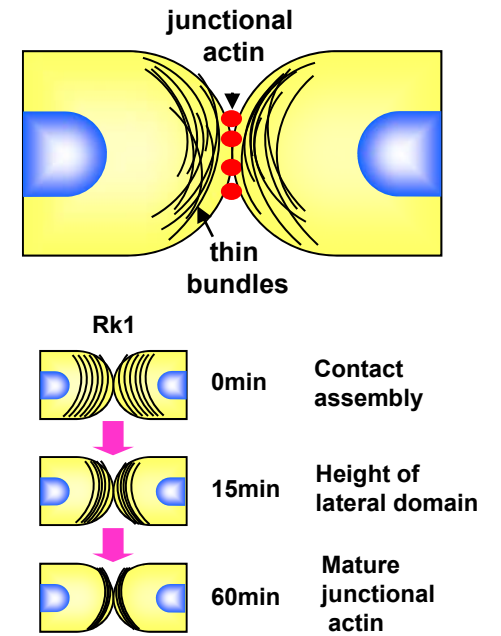


Rk1 blocks stress fiber formation induced by VEGF or AGE in retinal endothelial cells

A

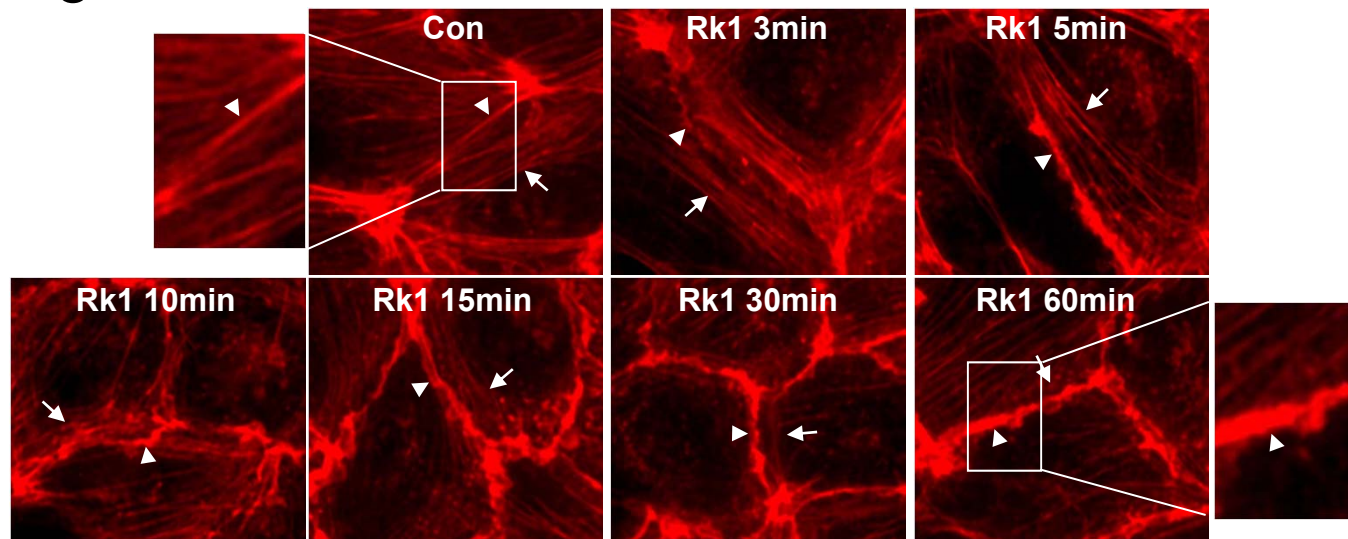


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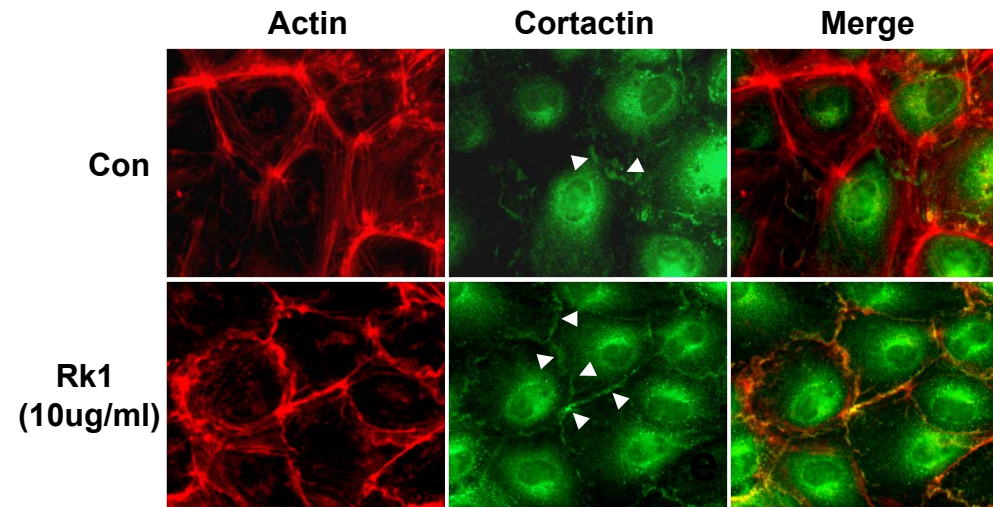


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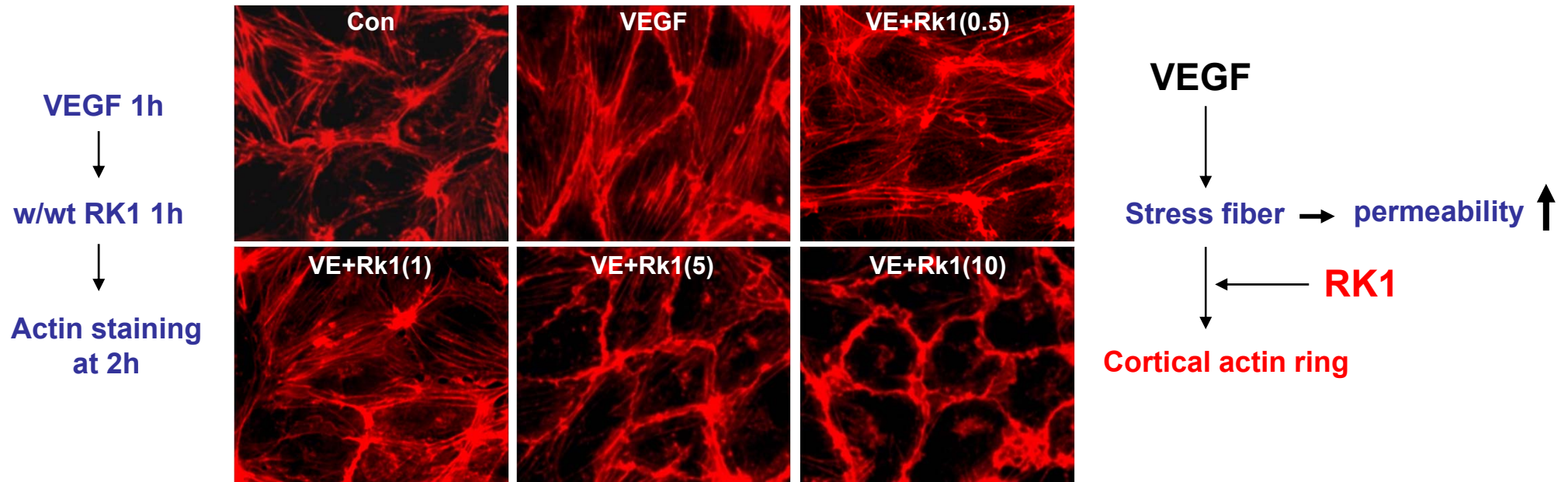
Rk1 induces cortical actin ring formation



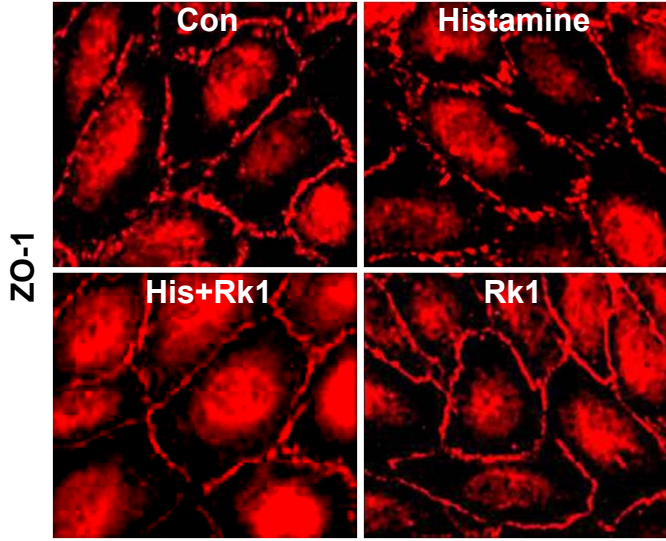
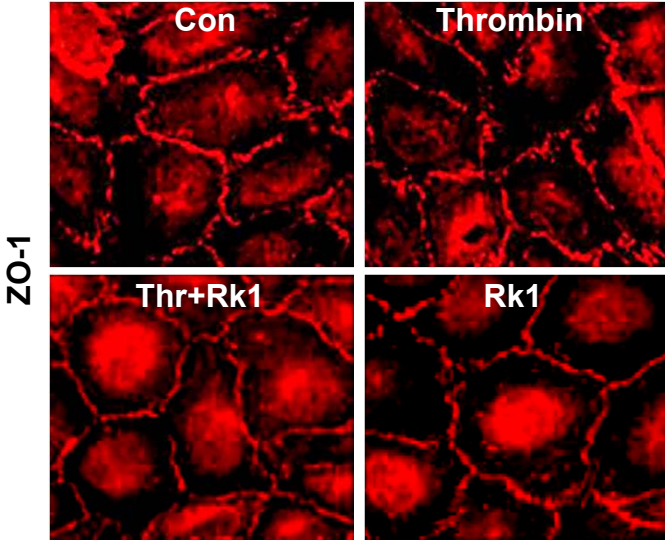
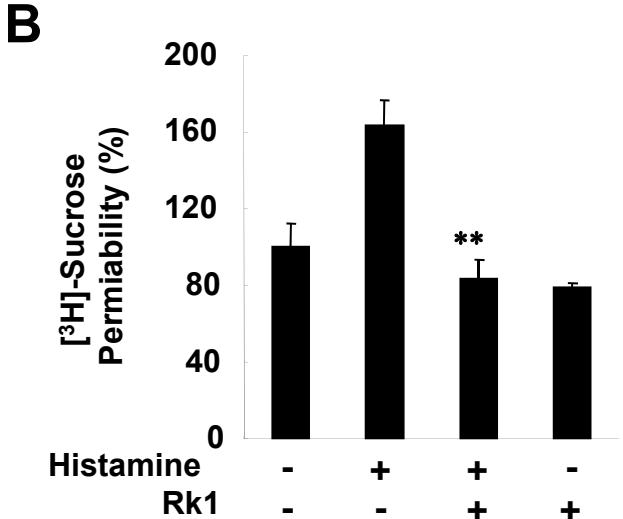
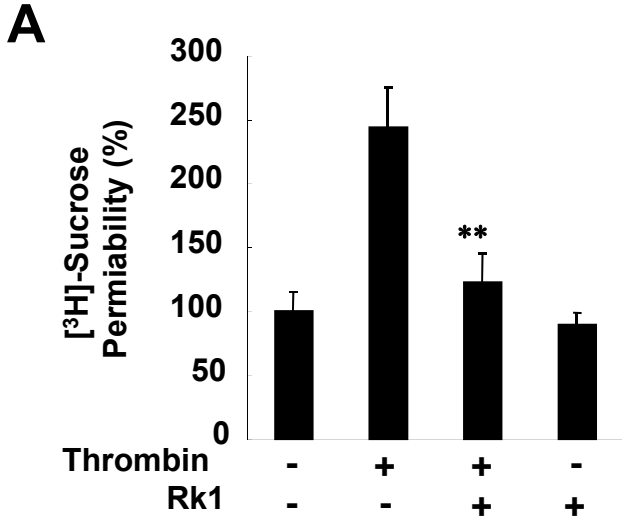
Rk1 induces cortactin localization at boundaries between cells



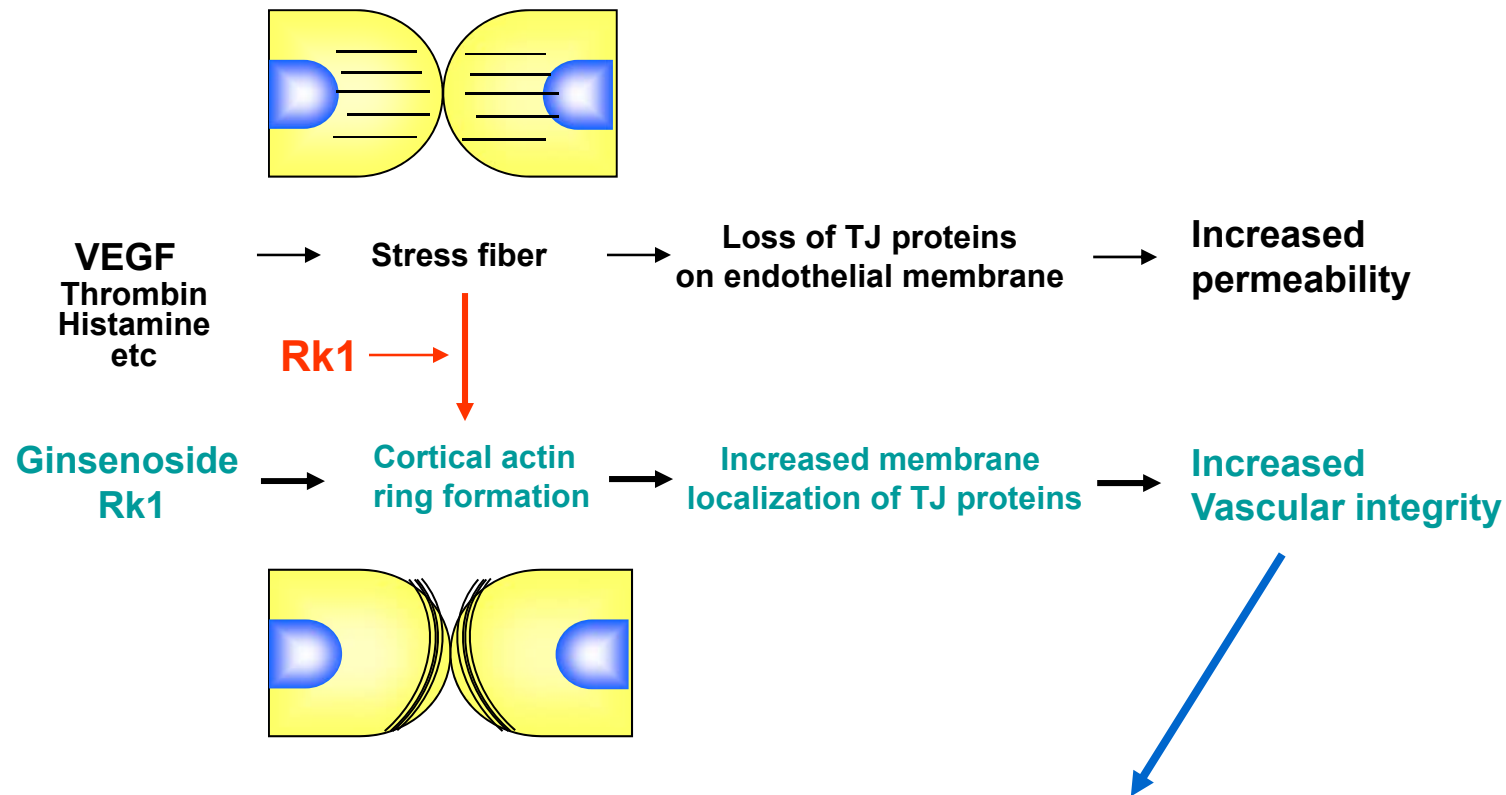
RK1 switches VEGF-induced stress fibers into the cortical actin ring structure



Rk1 inhibits thrombin or histamin-induced retinal endothelial permeability



Proposed action mechanism of RK1 in the vasculature

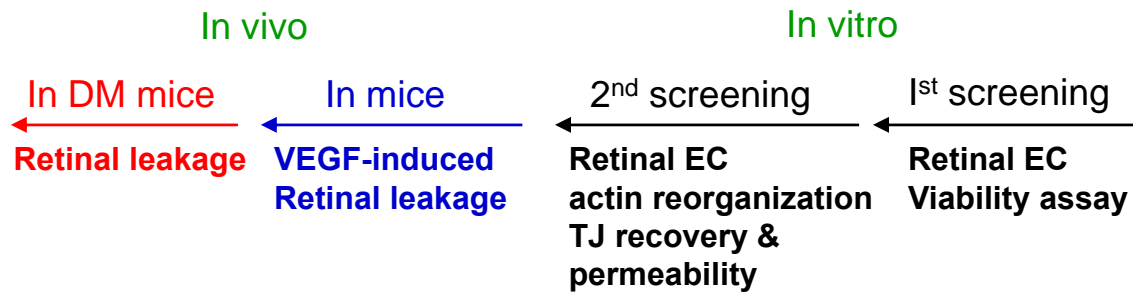
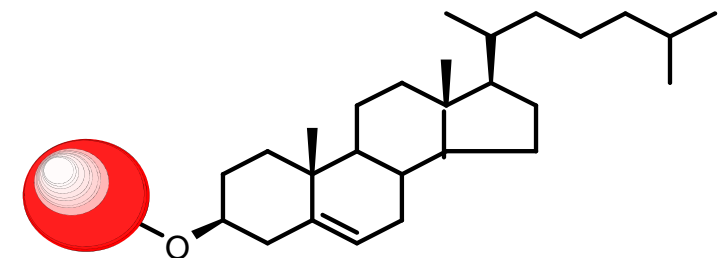
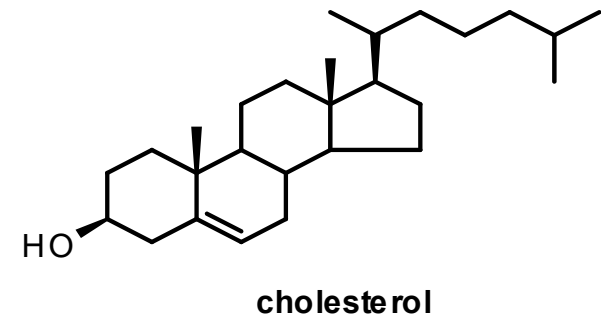
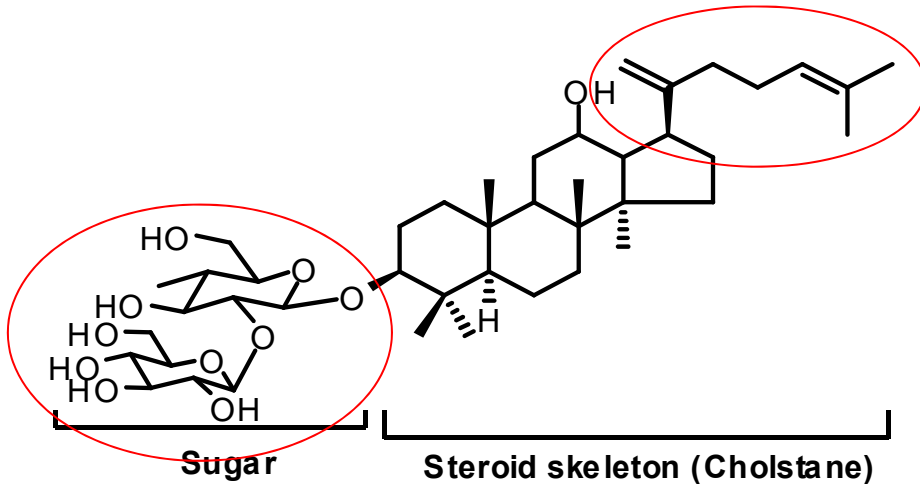


Restores vascular integrity & blocks vascular leakage

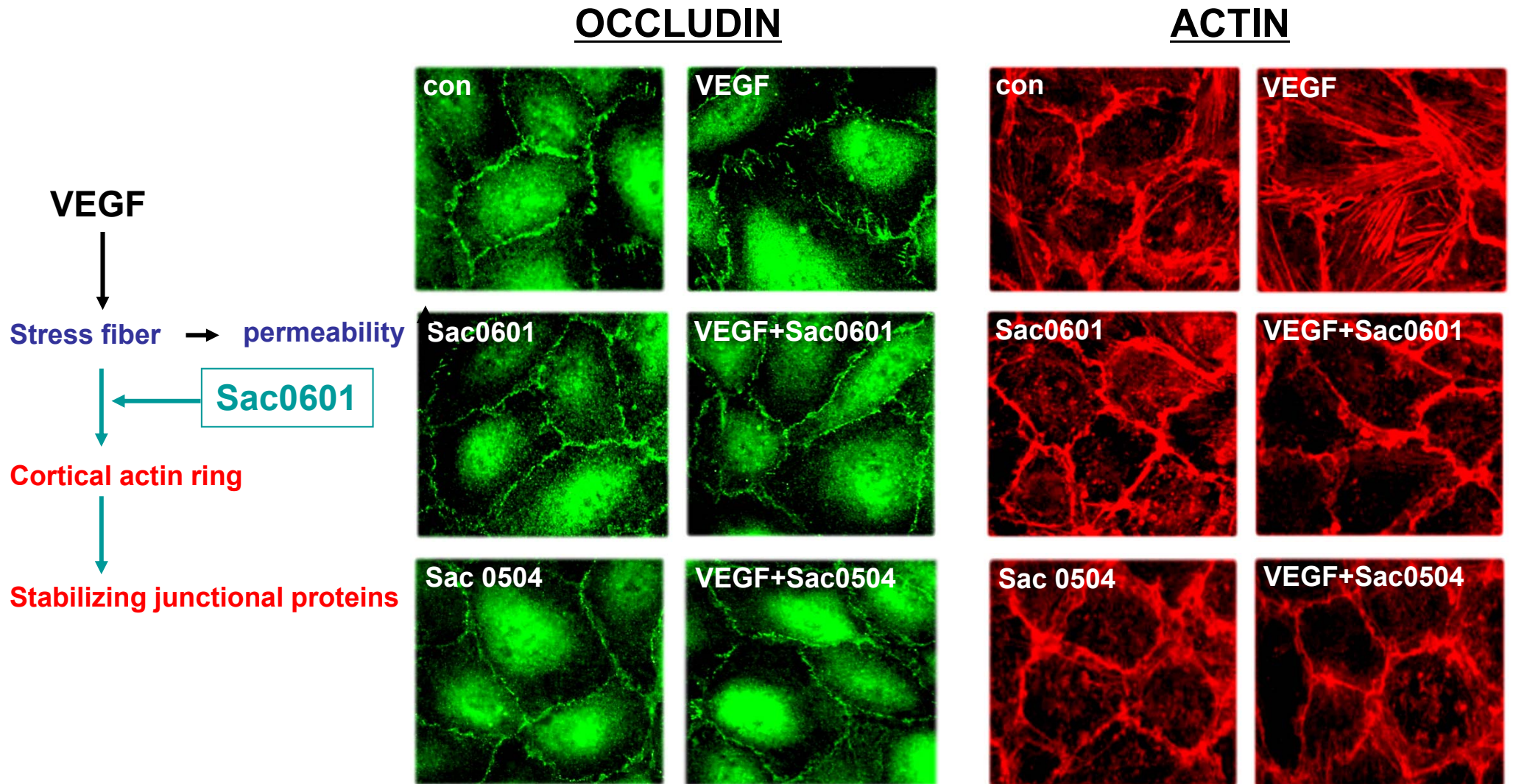
Novel pharmaceutical core for developing anti-permeability agent

Design of Synthetic Analogues

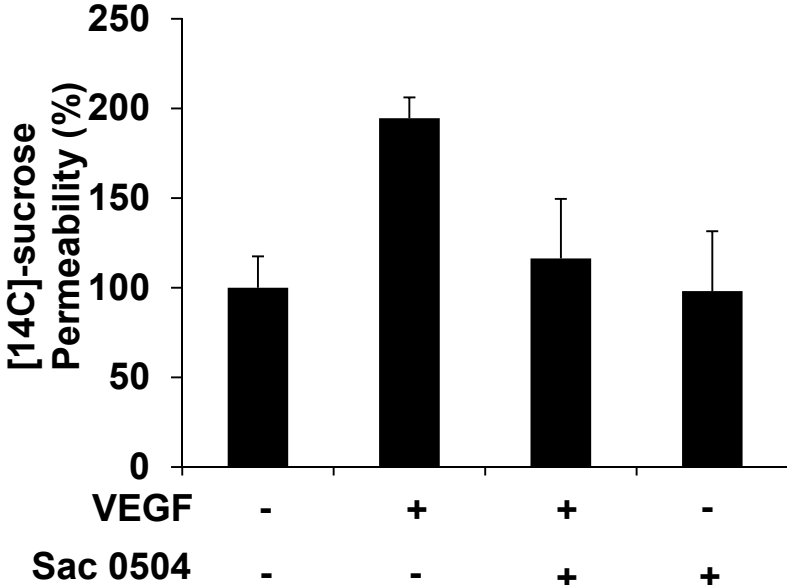
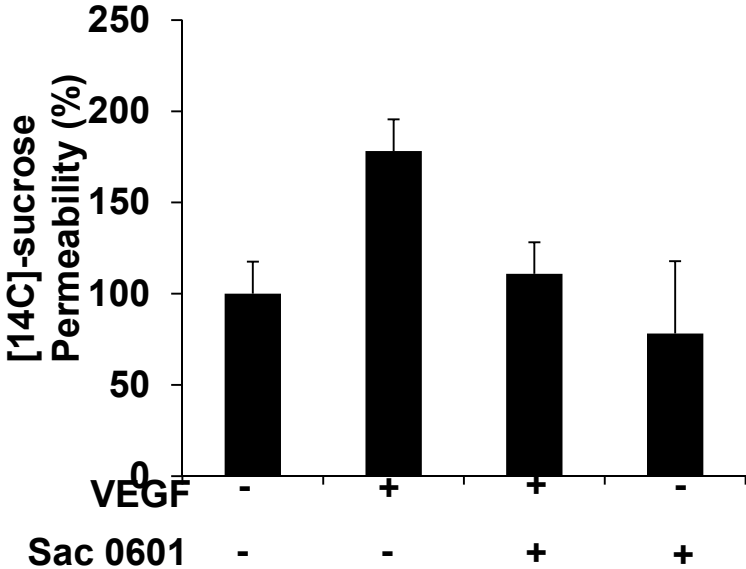
> Common structural features in active ginsenosides



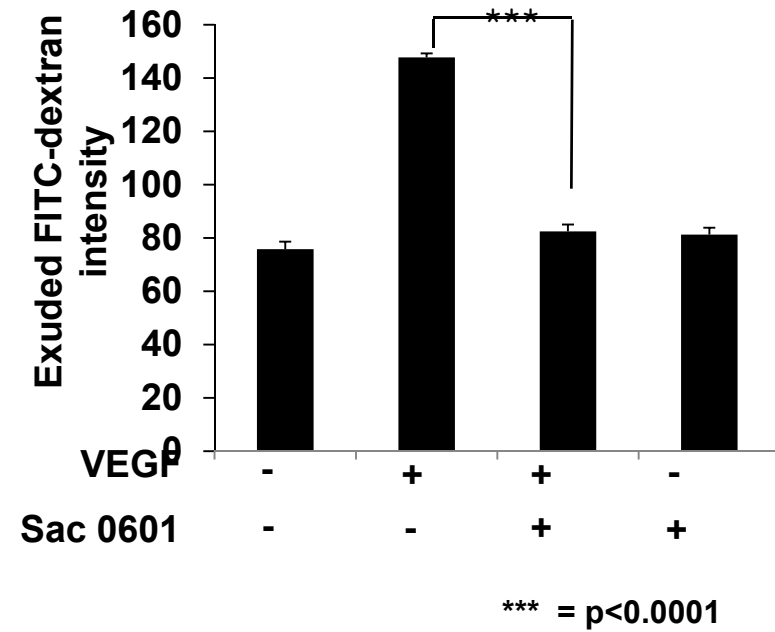
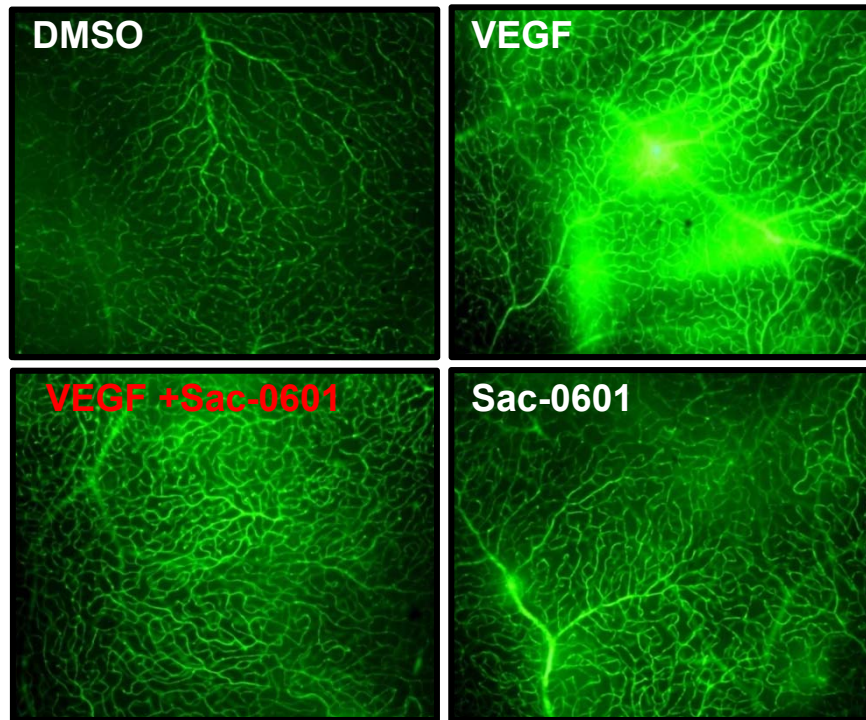
Sac-0601 and Sac-0504 reverse the VEGF-induced actin cytoskeleton and tight junction rearrangement



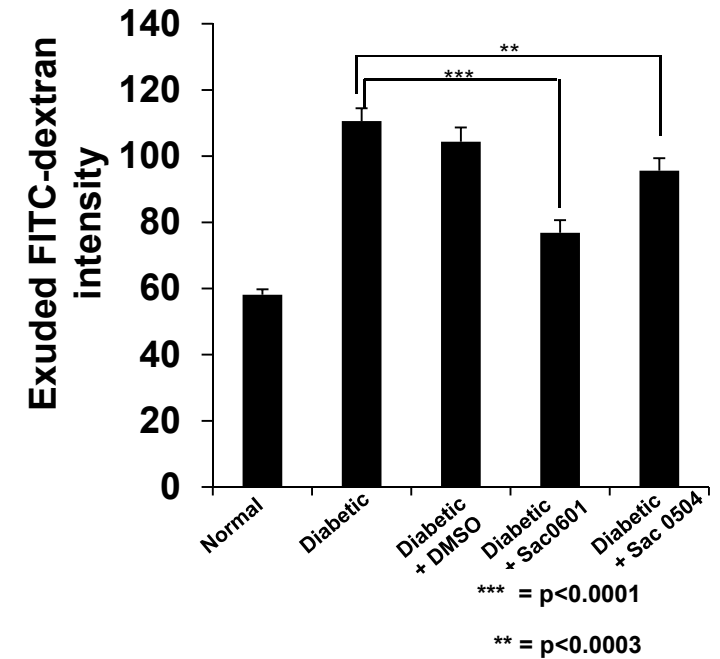
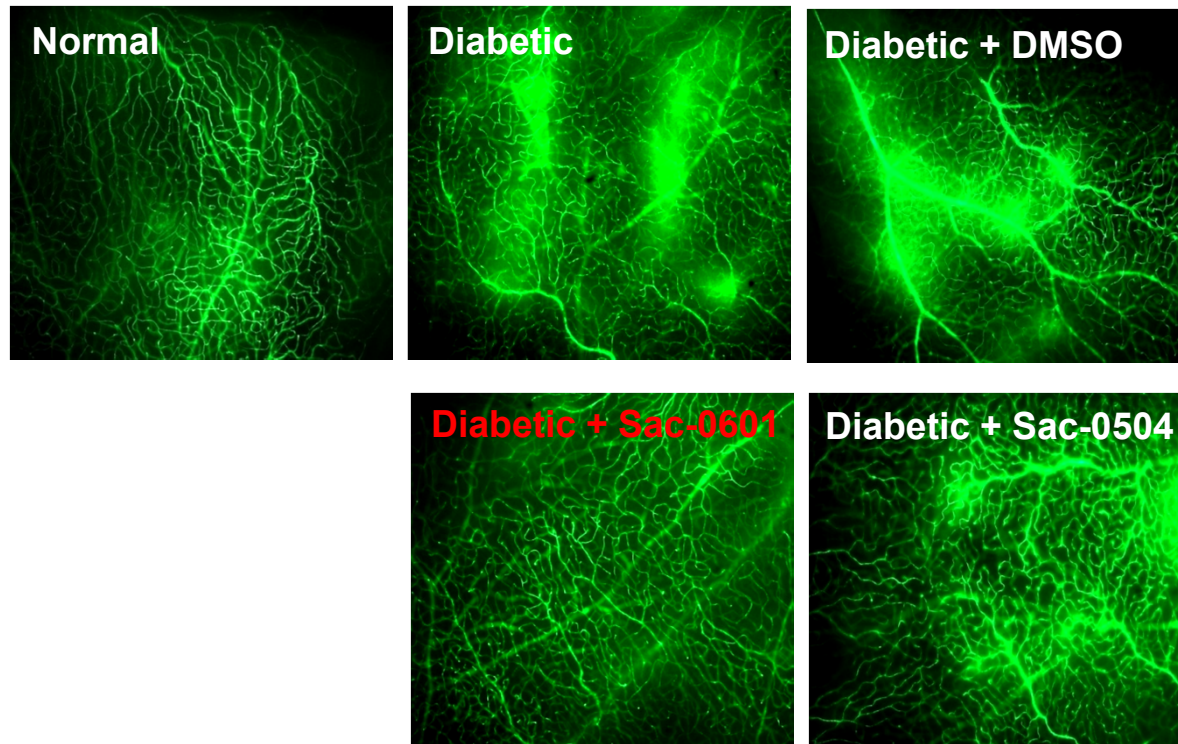
Sac-0601 and Sac-0504 inhibit VEGF-induced retinal endothelial permeability *in vitro*



Sac-0601 Inhibits VEGF induced-vascular leakages of retina in a mouse model

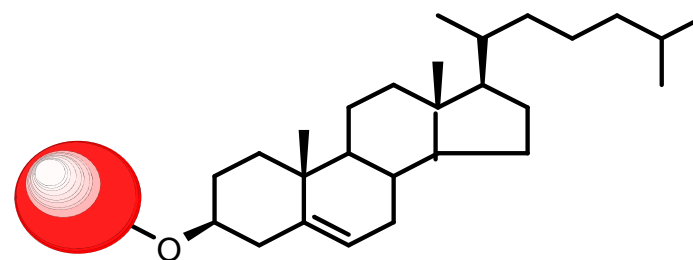
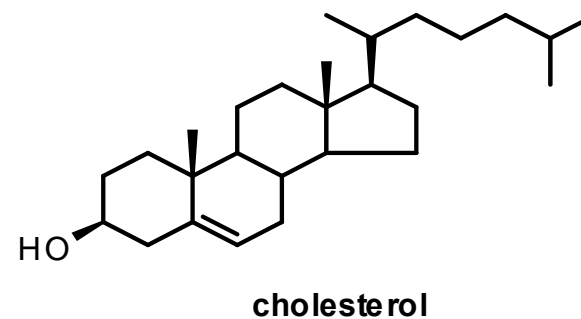
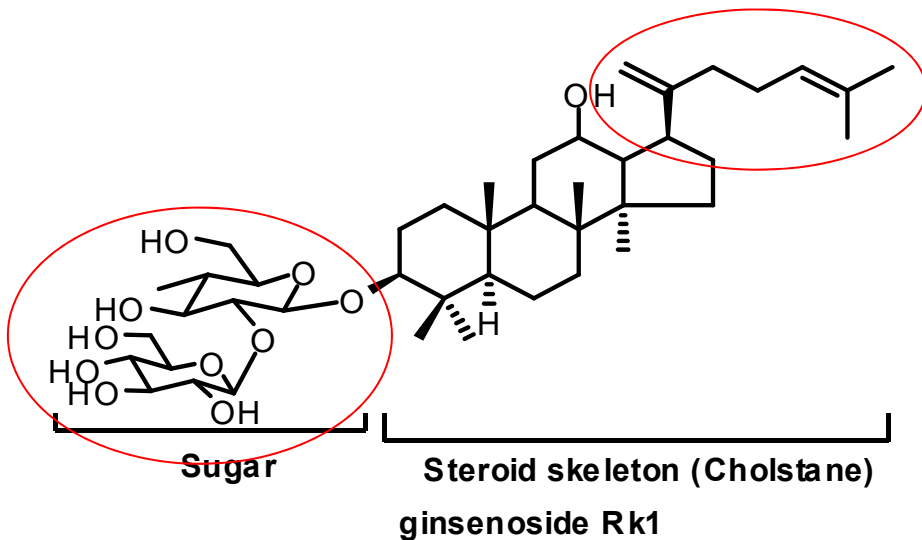


Sac-0601 Inhibits the vascular leakages of retina in a diabetic mouse model

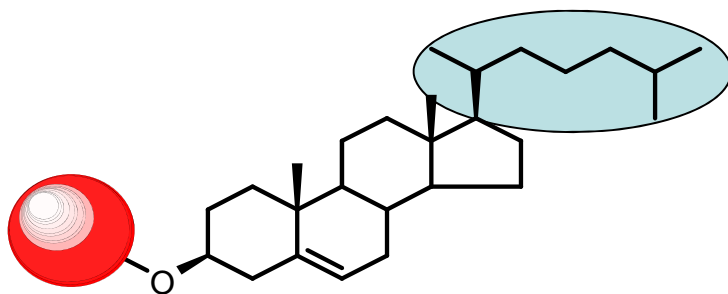


Design of Synthetic Analogues

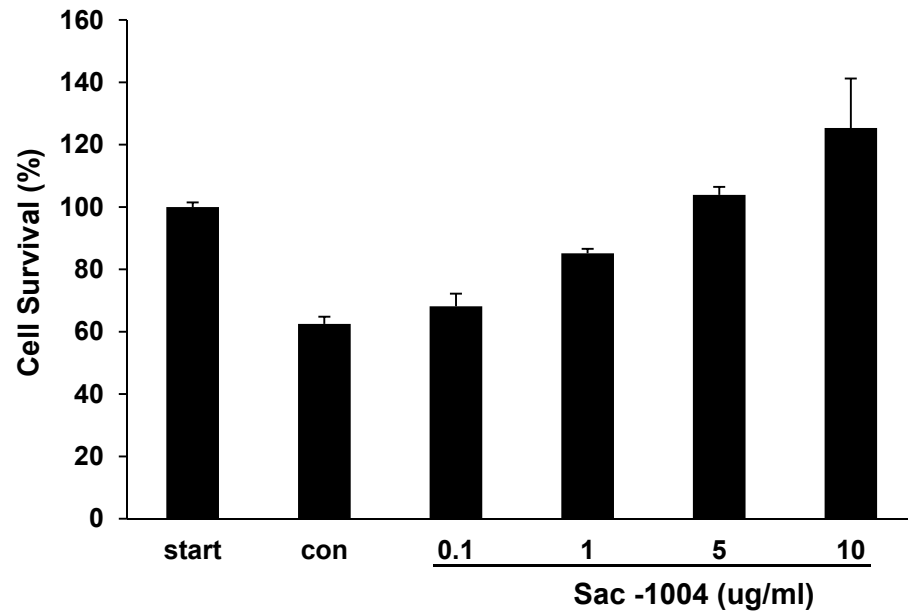
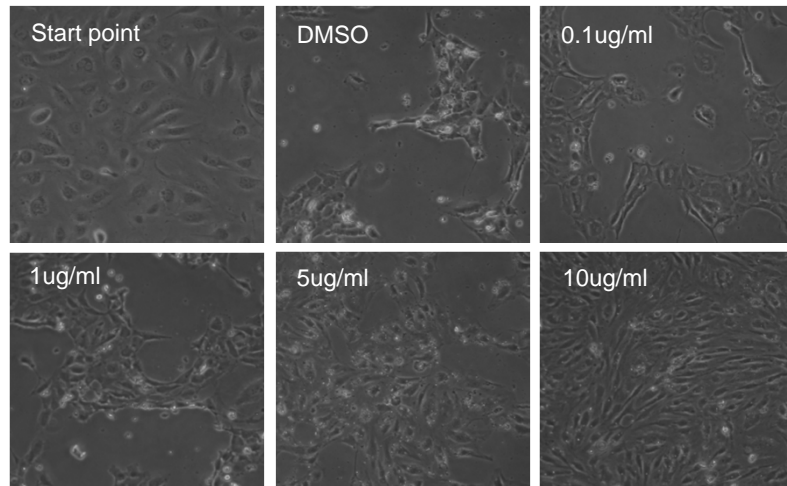
> Common structural features in active ginsenosides



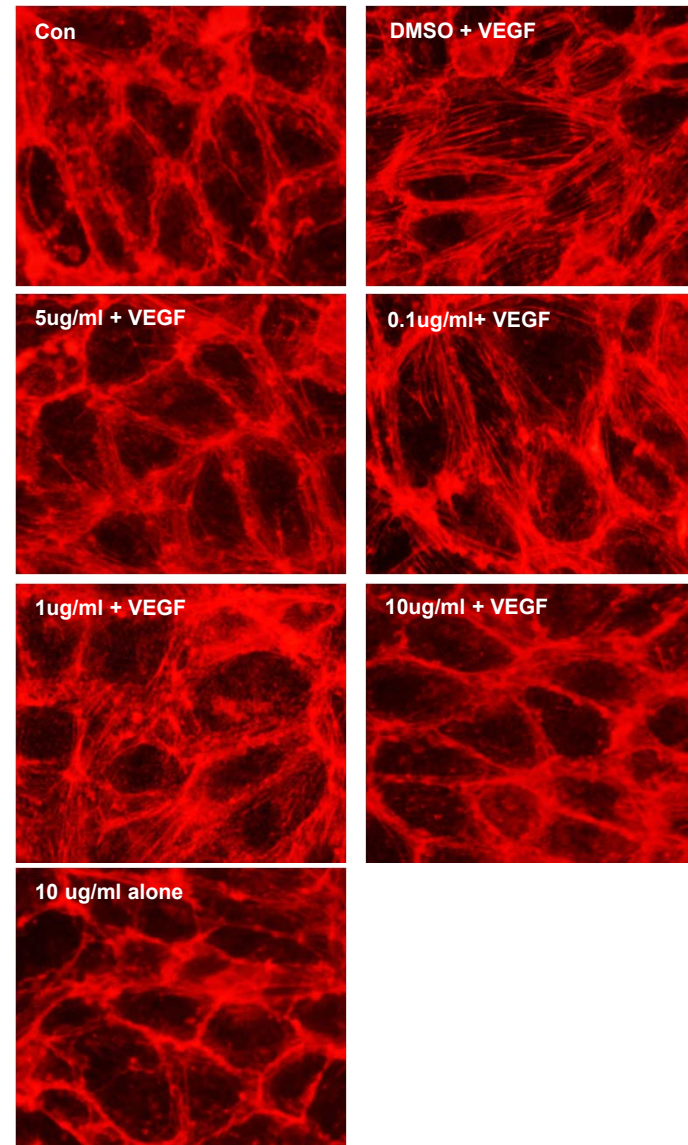
Introduction of pseudo sugar moiety to alcohol



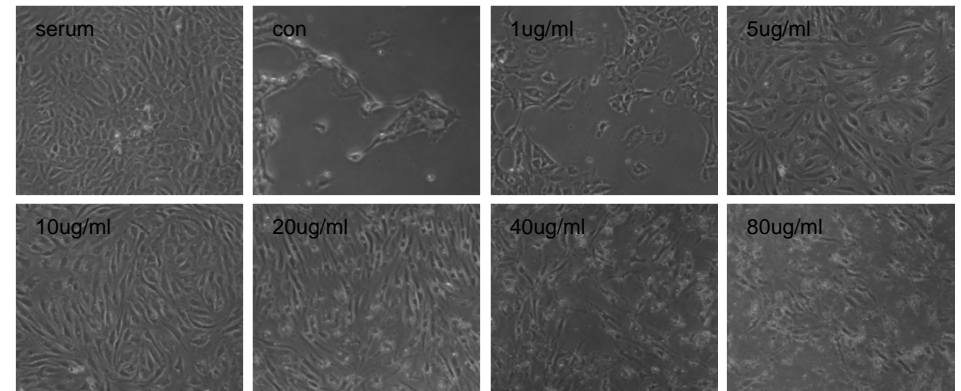
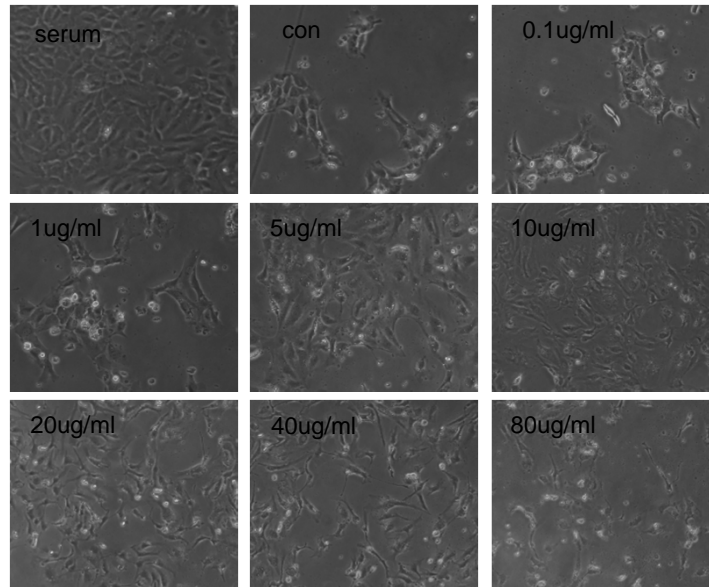
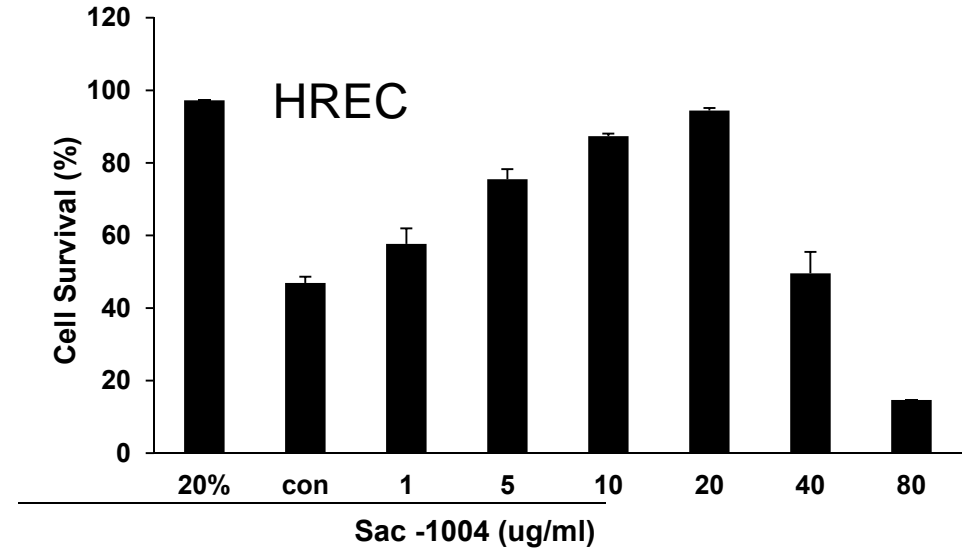
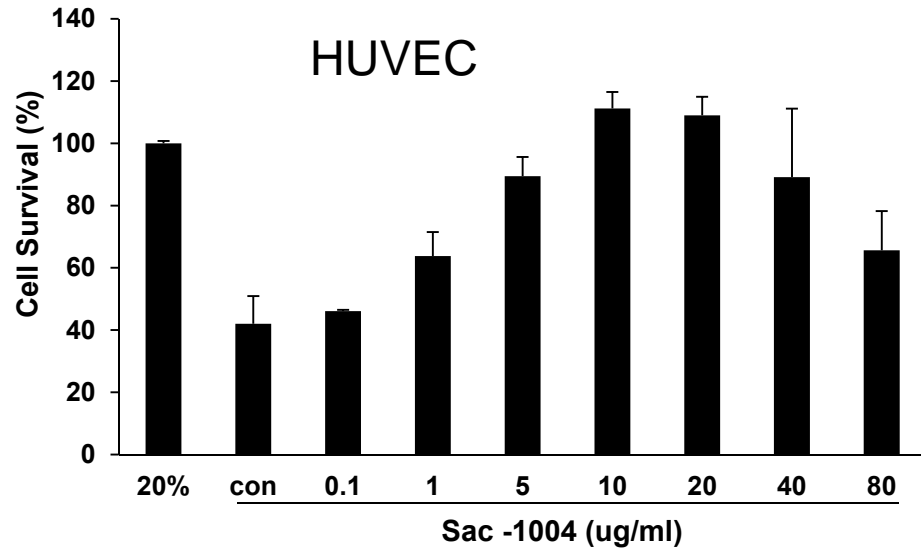
Sac-1004 protects HRECs from serum deprivation-induced cell death in concentration dependent manner.



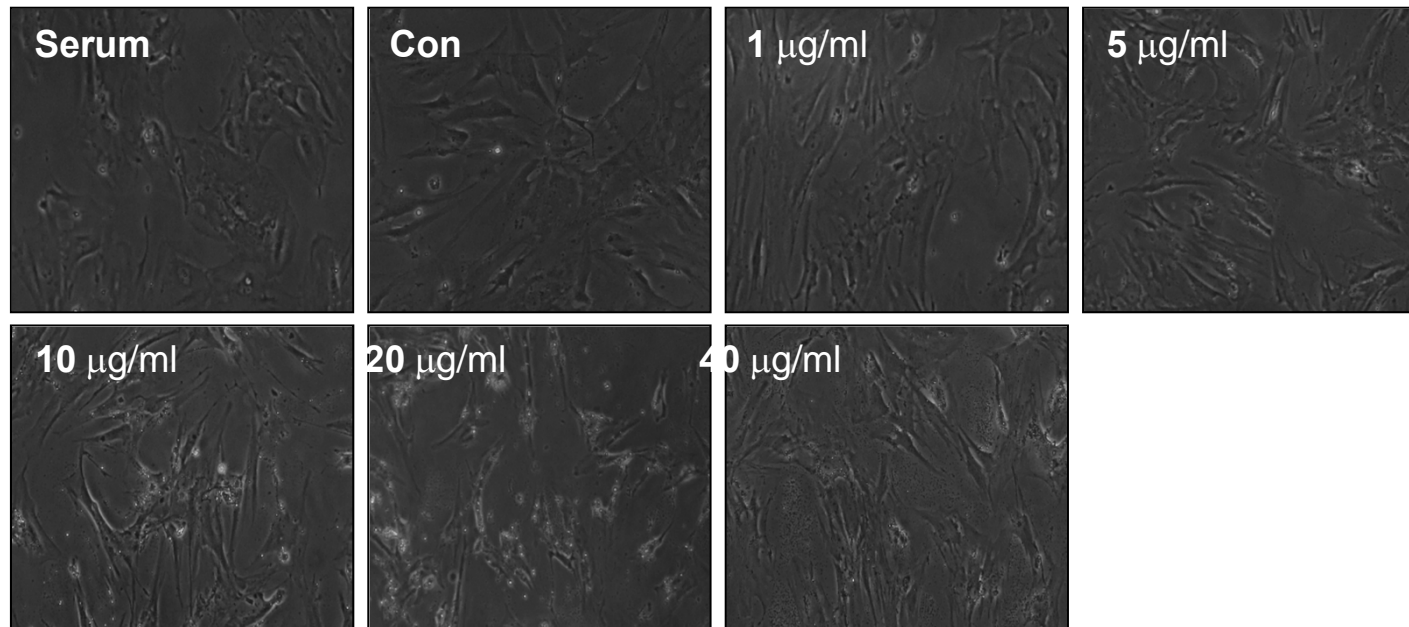
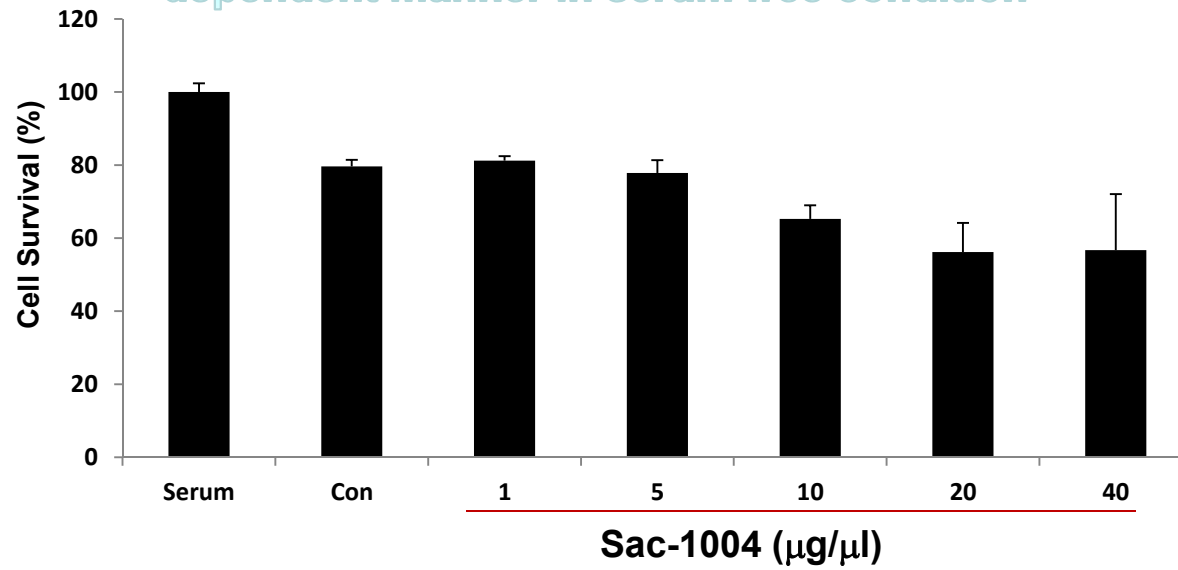
Sac-1004 blocks the actin stress fiber formation induced by VEGF and forms cortical actin ring



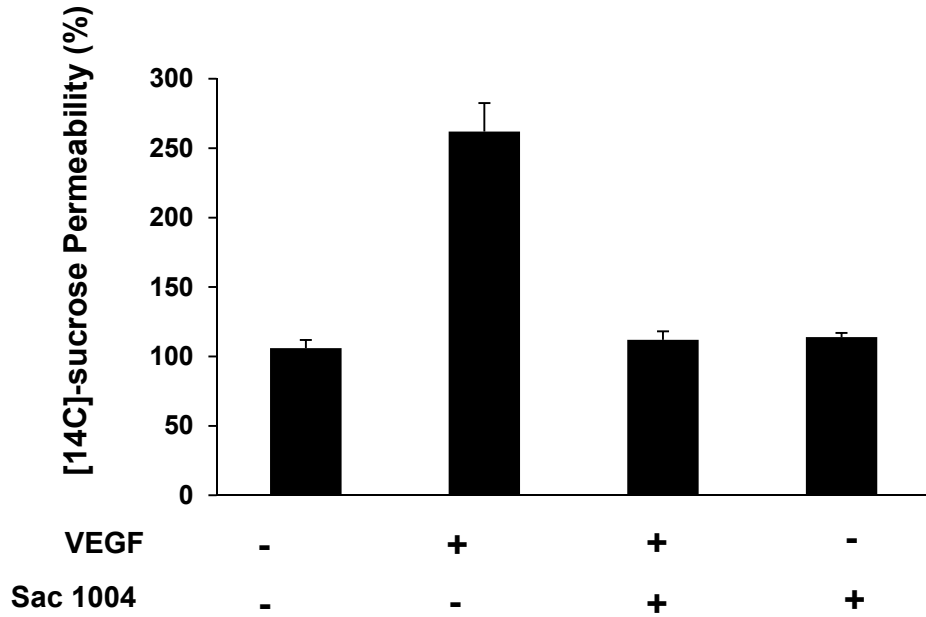
Sac-1004 protects ECs from serum deprivation-induced cell death in concentration dependent manner.



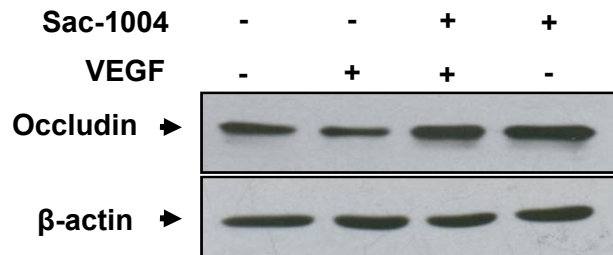
The effect of Sac 1004 drug in Smooth Muscle cells in concentration dependent manner in serum free condition



Sac-1004 inhibits VEGF-induced retinal endothelial permeability.

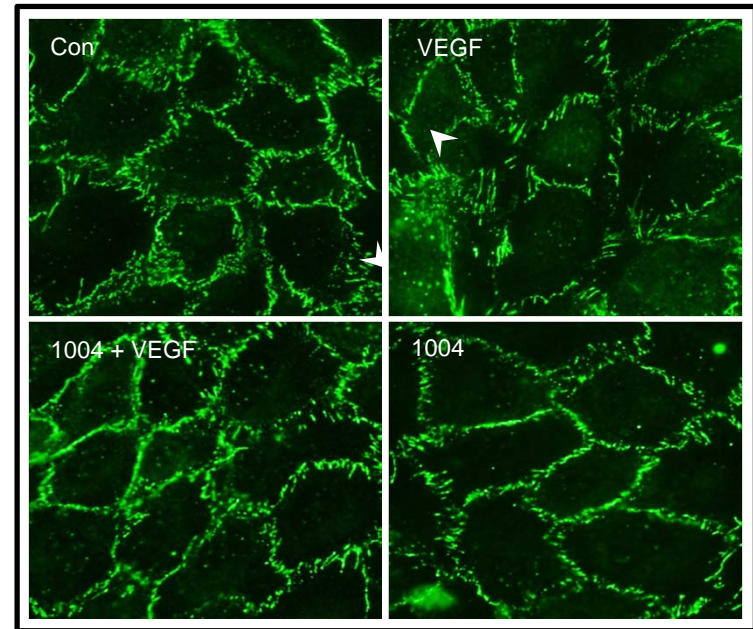


Sac-1004 restores the tight junction protein, occludin by preventing the action of VEGF

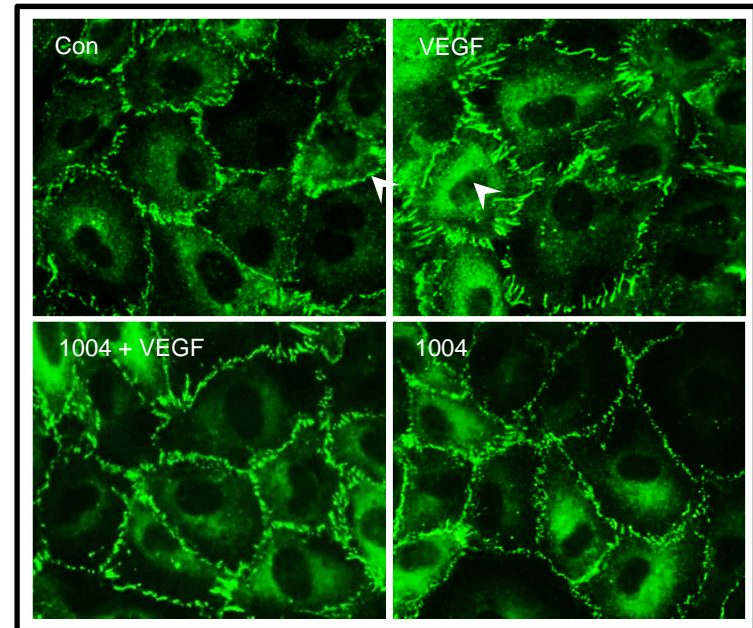


Sac-1004 displays the linear pattern of the tight junction proteins

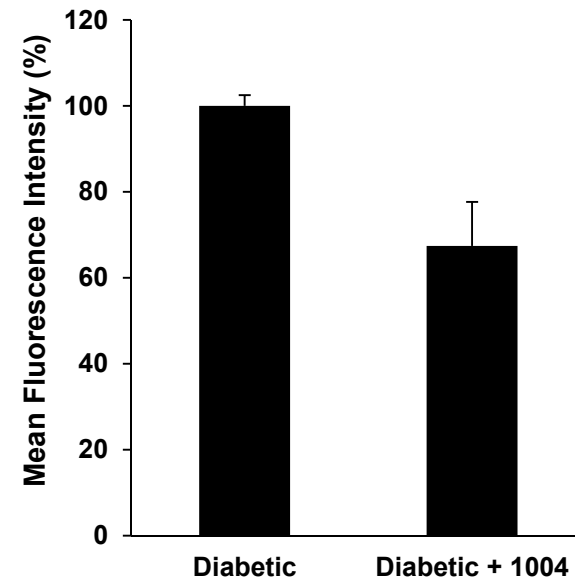
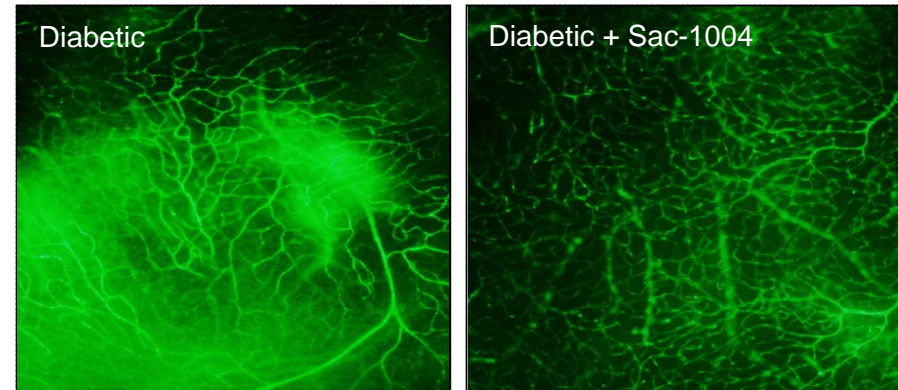
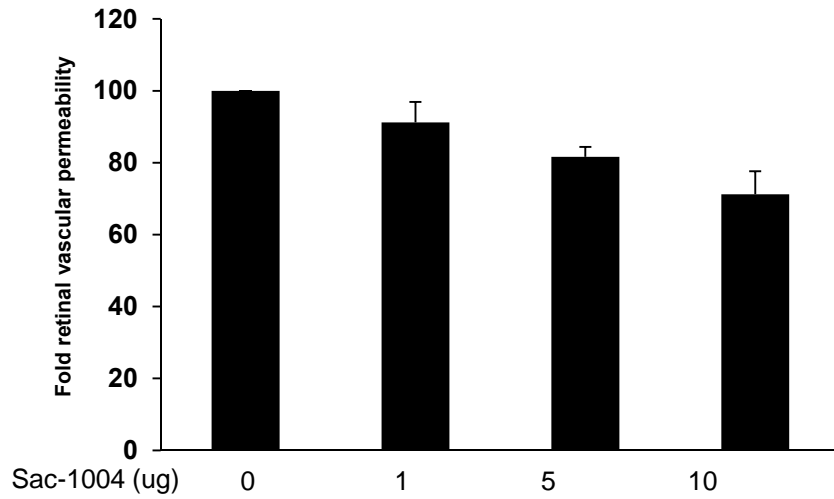
ZO-1



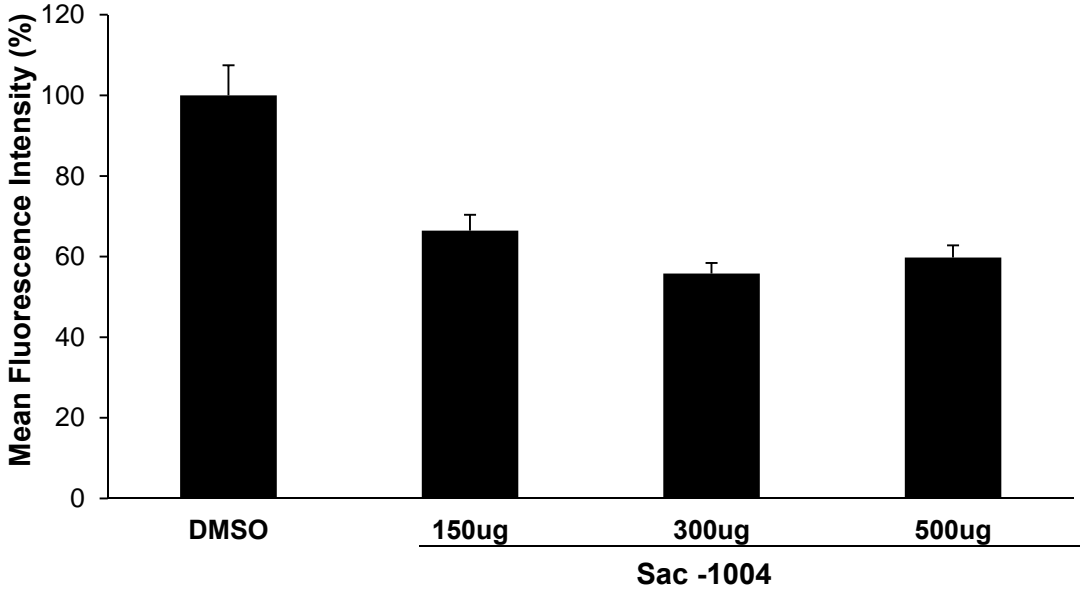
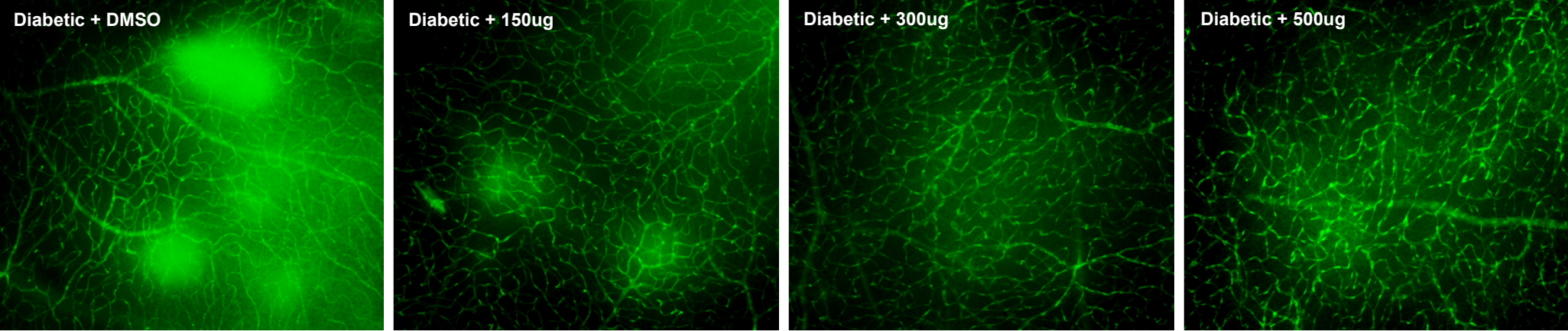
ZO-2



Intravitreal injection of Sac-1004 Inhibits the vascular leakages of retina in a diabetic mouse model

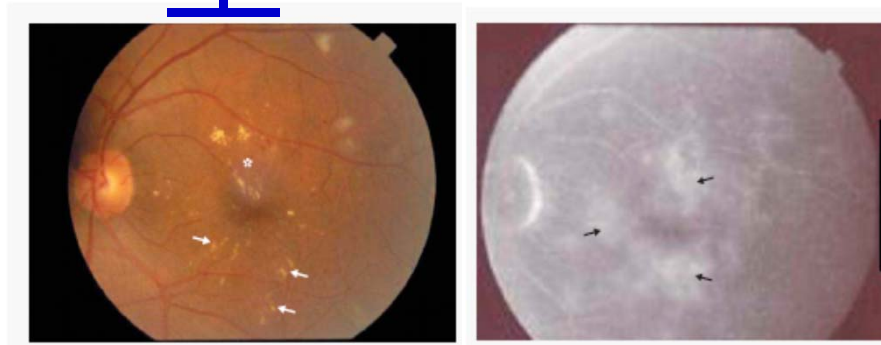


Intra venous injection of Sac-1004 Inhibits the vascular leakages of retina in a diabetic mouse model



New means for vascular leakage-related diseases

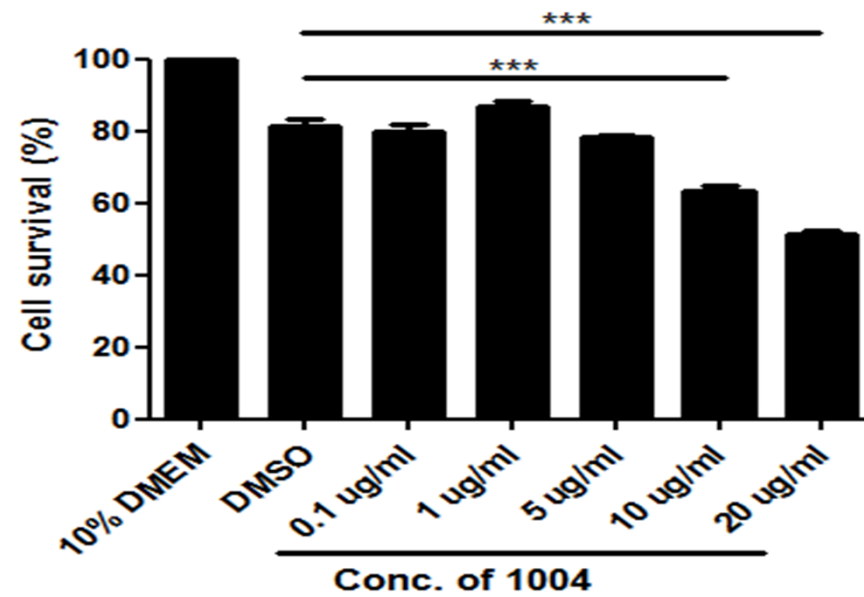
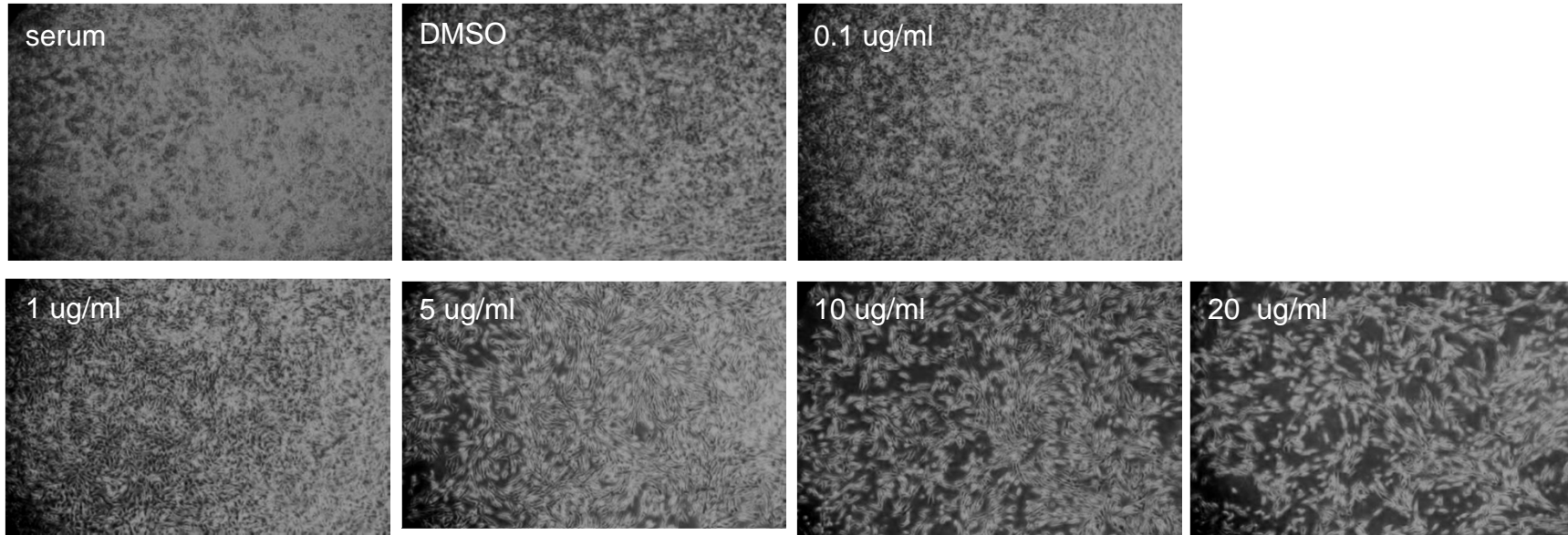
Anti-VEGF therapy



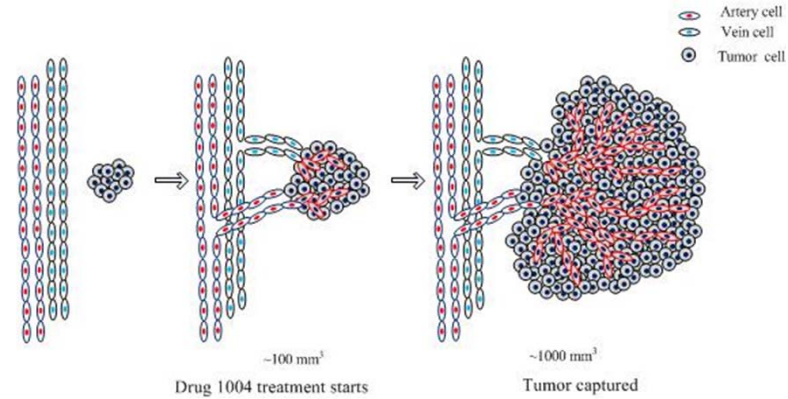
Integrity promoting therapy (Sac-1004 analogs)

- Increase water solubility
- Identify a cognate receptor
- Preclinical & clinical evaluation

The effect of Sac 1004 drug in B16F10 cells in concentration dependent manner in serum free condition

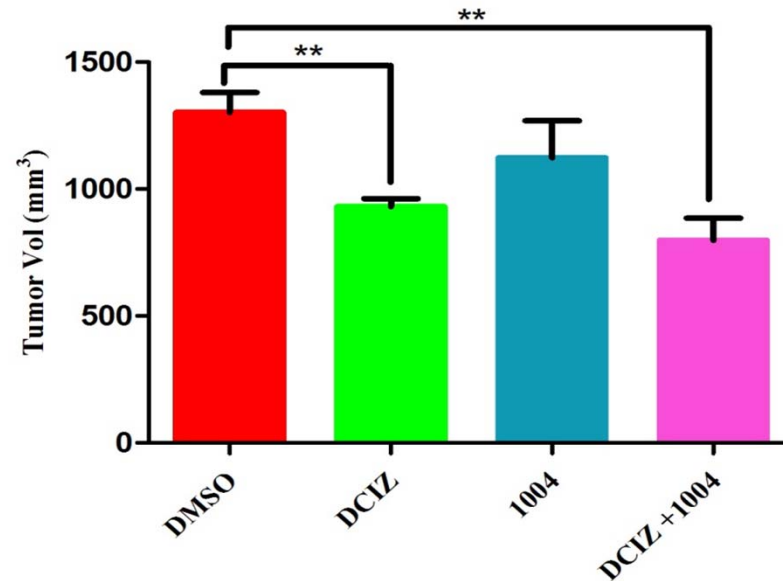


Sac-1004의 암혈관 구조개선 및 항암제 병행치료 효과



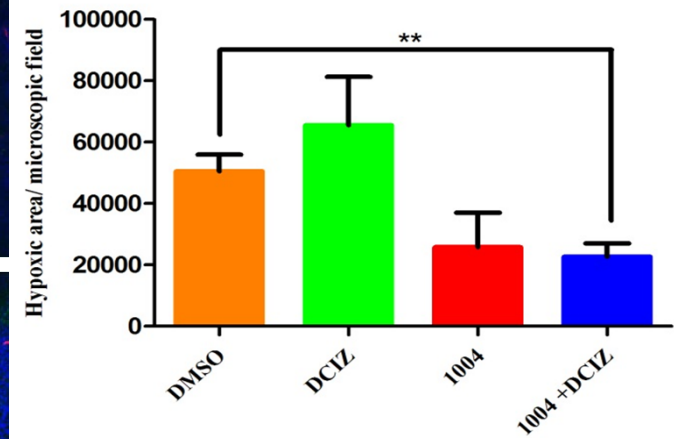
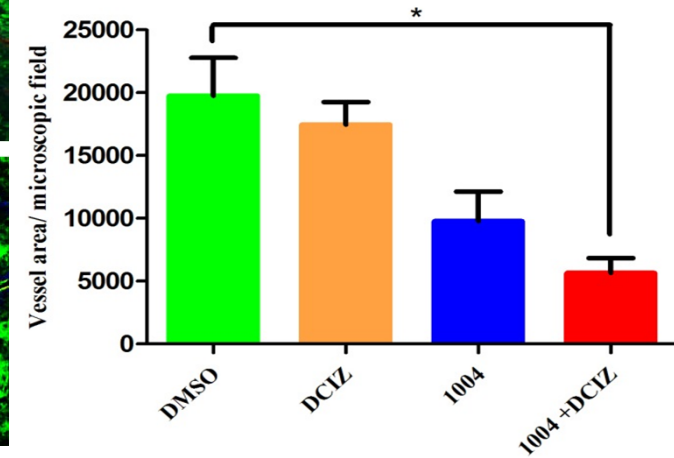
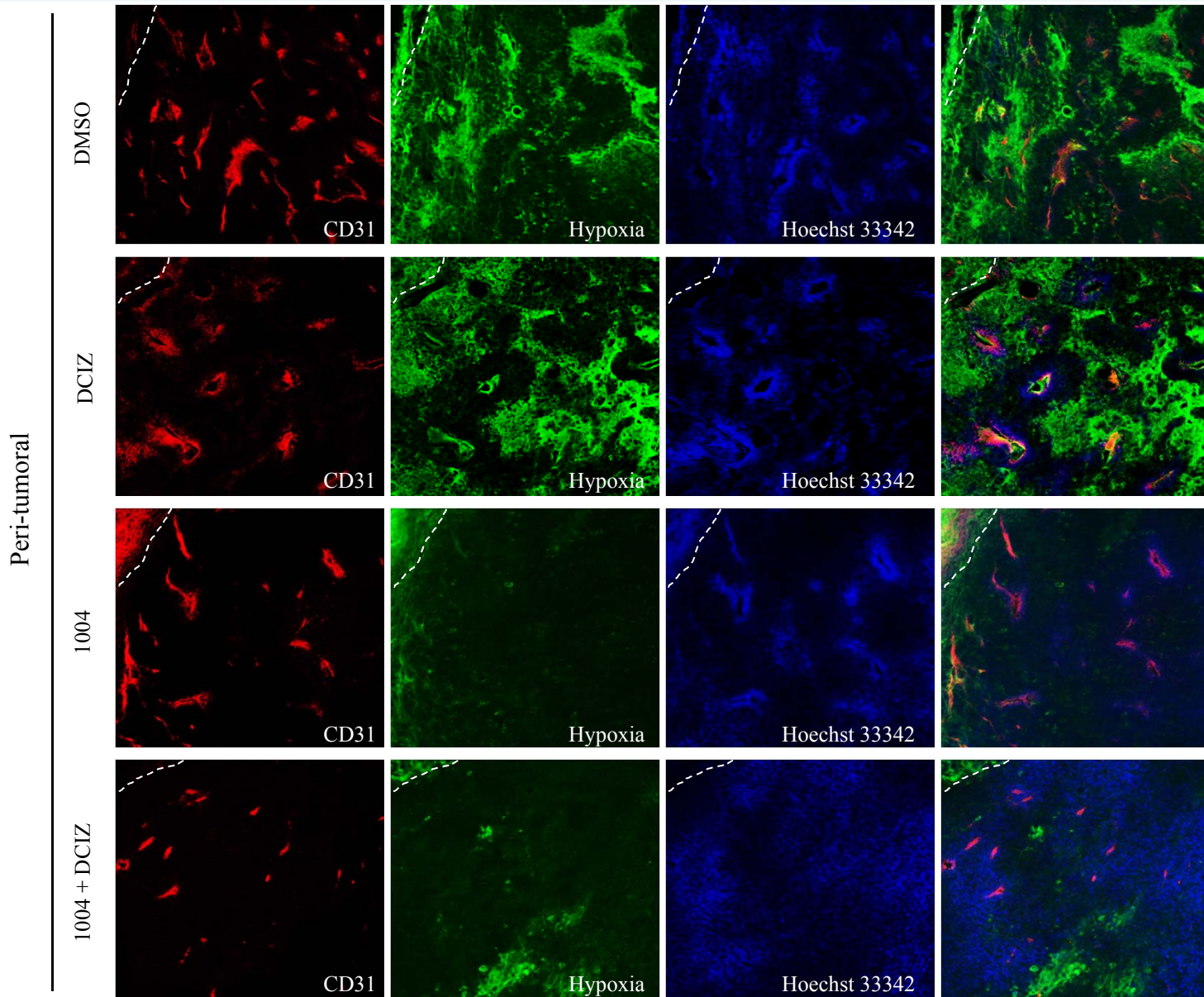
Combination Drug Treatment Schedule (i.v)

Day	1	2	3	4	5	6	7
	1004	1004	1004 + DCIZ	1004	1004	1004 + DCIZ	1004 + DCIZ



1004 = 50 mg/kg
DCIZ = 20 mg/kg

EFFECT OF 1004 AND DACARBAZINE COMBINATION ON VESSEL DENSITY AND HYPOXIA IN B16F10 MELANOMA



50 μ m

Sac-1004 암혈관 구조개선 및 항암제 병행치료 효과

- **Sac-1004**의 **IV** 투여는 암혈관의 누수를 억제
- **Sac-1004**는 암혈관 내부 **hypoxia**를 현격히 개선
- 항암제와 병행치료시 암세포의 **necrosis**를 현격히 증가 시킴
- 이상으로 볼 때, **Sac-1004**는 암조직내 혈관구조를 개선하여 암조직 내부로 **perfusion**을 증가시켜, 항암제의 전달을 용이하게 하고, 암세포의 **aggressiveness**를 감소시켜 암세포의 성장과 전이를 차단할 가능성 큼

Therapeutic implication of vascular integrity promoting agents In human diseases

1. **Macular edema** 등 안과 질환
2. **Stroke**
3. 항암제 병행치료제
4. 혈관재협착 방지용 **Stent coating**제
5. 혈관누수가 관련된 기타 질환

Acknowledgements

Vascular Genomics Lab

(www.bio1004.org)

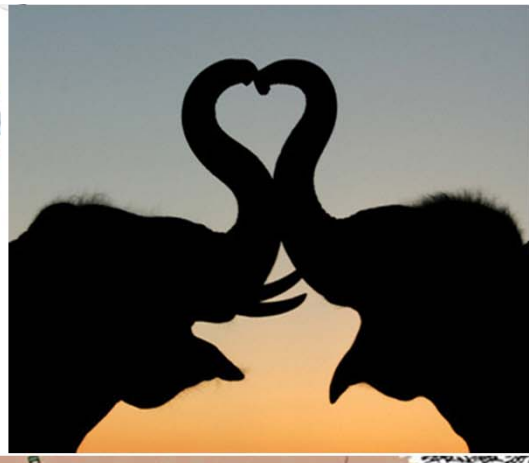
Yong-Sun Maeng
Sony Maharjan
Vijayendra Agrawal
Hong-Ryul Park
Hyun-Jung Choi
Shin-Jung Lee
Bo-Jung Pyun
Seong-Sik Rho
Ji-Hye Kim
Yeon-Suk Choi
Ji-Hye Jung
Hyo-Jin Park
Jung-Yeon Choi
Mi-Sun Cho
Jeong-Ae Park

SNU

Kyu-Won Kim
Young-Ger Sur
Jeong-Il Park

Kangwon Natl Univ.

Young-Myoung Kim



Thank You 

