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OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

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Extracellular purines in vascular endothelial barrier preservation

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GRU
GEORGIA REGENTS
UNIVERSITY

VASCULAR
BIOLOGY CENTER

OUTLINE

1. Clinical and physiological importance of lung vascular barrier

2. How to measure vascular permeability *in vitro* and *in vivo*

3. Mechanisms regulating endothelial permeability

4. Extracellular purines and endothelial barrier

5. Mechanisms of purine-induced EC barrier preservation

Lung Vascular Barrier

- Comprise of 3 major components: endothelium, basement membrane and epithelium

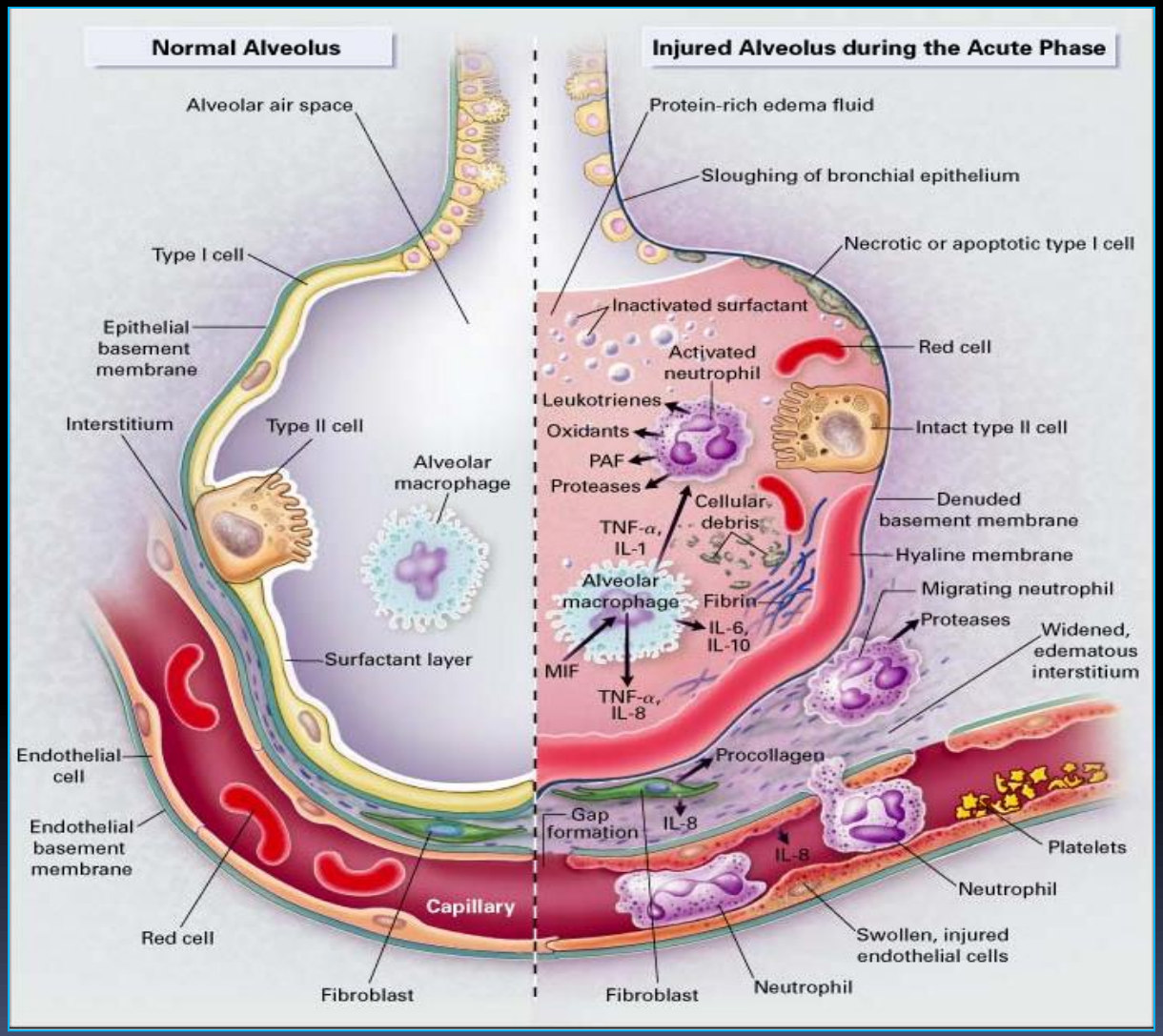
- Regulates exchange of solutes and fluid between blood vessels and alveoli

- Compromise of vascular barrier due to Inflammatory or toxic events results in increased **permeability pulmonary edema** (fluid accumulation) into the lung, which is a cardinal feature of acute lung injury (ALI) and its more severe form acute respiratory distress syndrome (ARDS)

- ALI/ARDS leads to impaired gas exchange and may cause **respiratory failure.**

- There is no standard treatment for **permeability pulmonary edema** only ventilation strategies

The Normal Alveolus (Left-hand side) and the Injured Alveolus in the Acute Phase of ALI (Right-hand side)



Cytokines ⇧

Neutrophils ⇧

Widened Interstitium (leakage of protein rich fluid)

Gap Formation

Alveolar edema fluid flooding

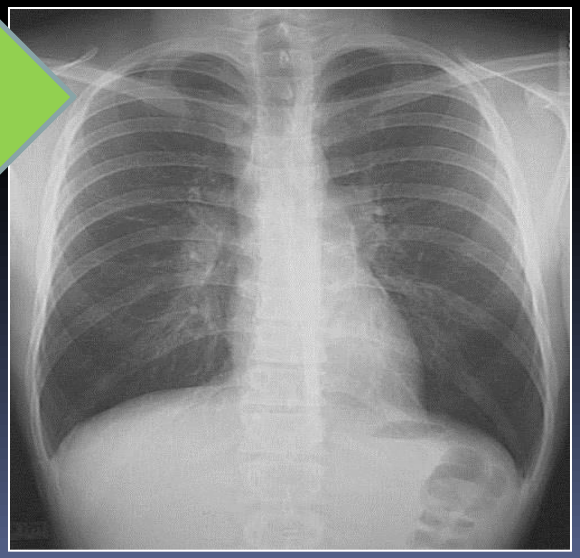
Ware & Matthay, NEJM, 2000

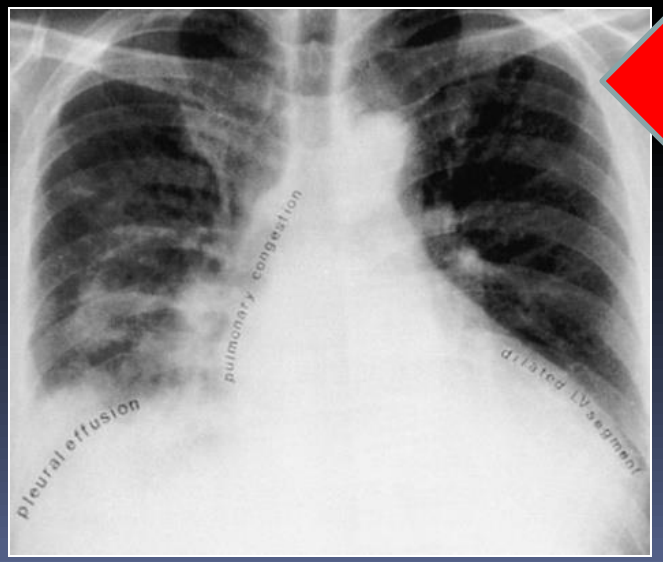
Causes of ALI/ARDS

- Sepsis
- Pneumonia
- Major trauma
- Pulmonary aspiration and near drowning
- Burns
- Inhalation of noxious fumes
- Fat embolism
- Massive blood transfusion
- Amniotic fluid embolism
- Air embolism
- Eclampsia
- Poisoning
- Radiation

Sepsis and pneumonia are the most common, causing about 60% of cases

Chest X-ray healthy lungs





Chest X-ray Injured lungs

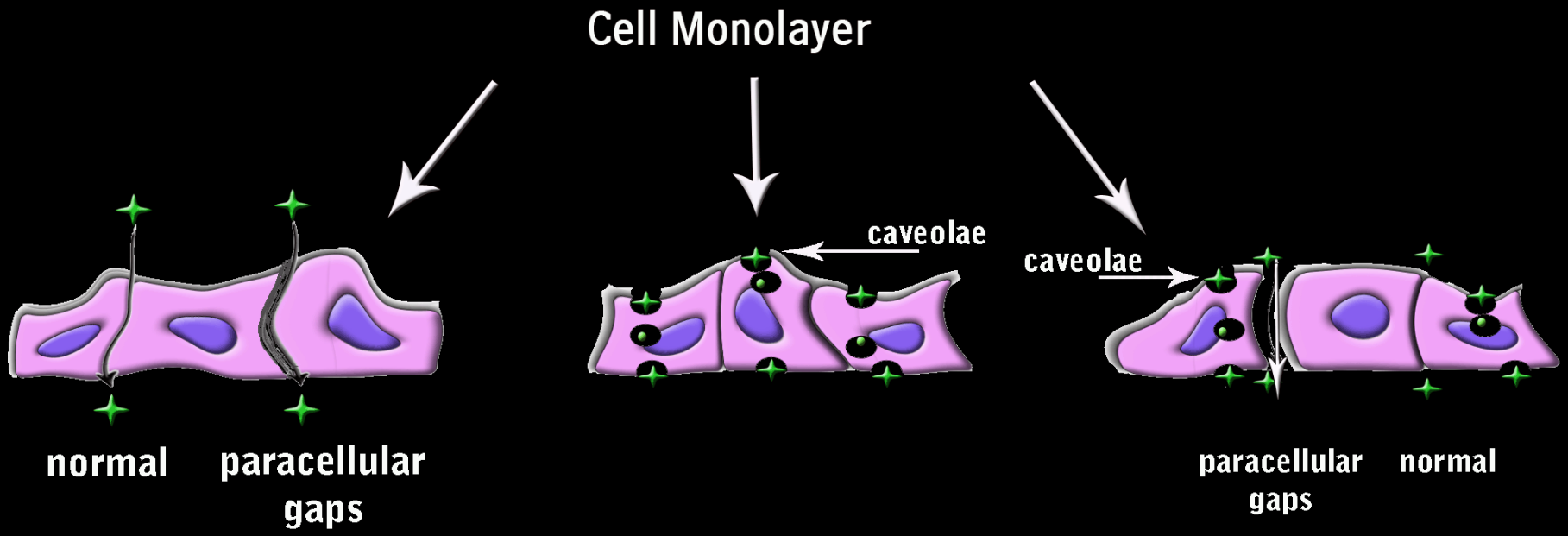
ALI & ARDS - Incidence & Mortality in the US Alone

Table 1. Incidence of Acute Lung Injury and ARDS and Mortality from These Conditions.*

Variable	Acute Lung Injury	ARDS
Cases — no.	1,113	828
Crude incidence — no. per 100,000 person-yr	78.9	58.7
Age-adjusted incidence — no. per 100,000 person-yr†	86.2	64.0
Mortality (95% CI) — %	38.5 (34.9–42.2)	41.1 (36.7–45.4)
Estimated annual cases — no.†	190,600	141,500
Estimated annual deaths — no.†	74,500	59,000
Estimated annual hospital days — no.†	3,622,000	2,746,000
Estimated annual days in ICU — no.†	2,154,000	1,642,000

* ARDS denotes acute respiratory distress syndrome, and CI confidence interval.
† U.S. estimates, age-adjusted to the 2000 Census, are shown.

Vascular permeability pathways



Paracellular Permability

Transcellular Permability

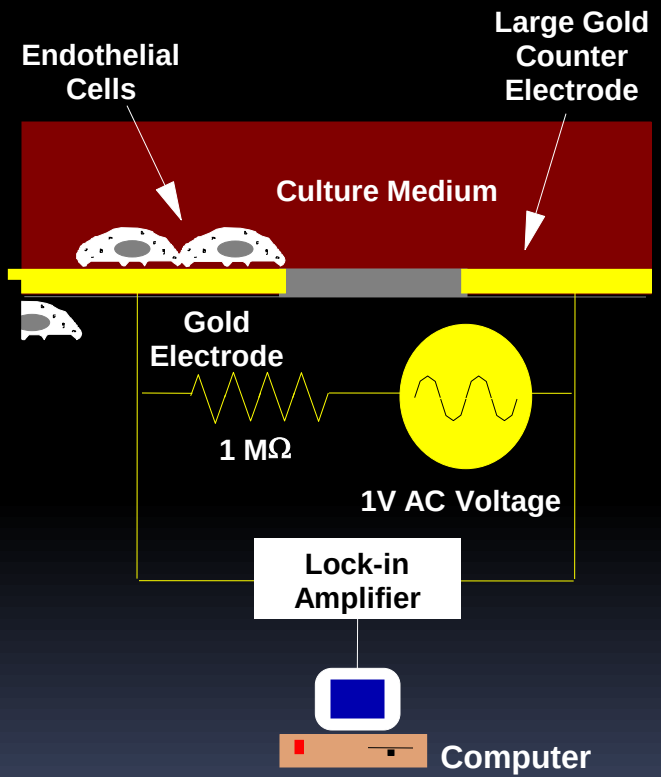
Combination of Para and Transcellular Permability

Permeability across endothelial and epithelial cell monolayers can involve transcellular, paracellular or both pathways. However, the majority of trafficking occurred through paracellular pathway.

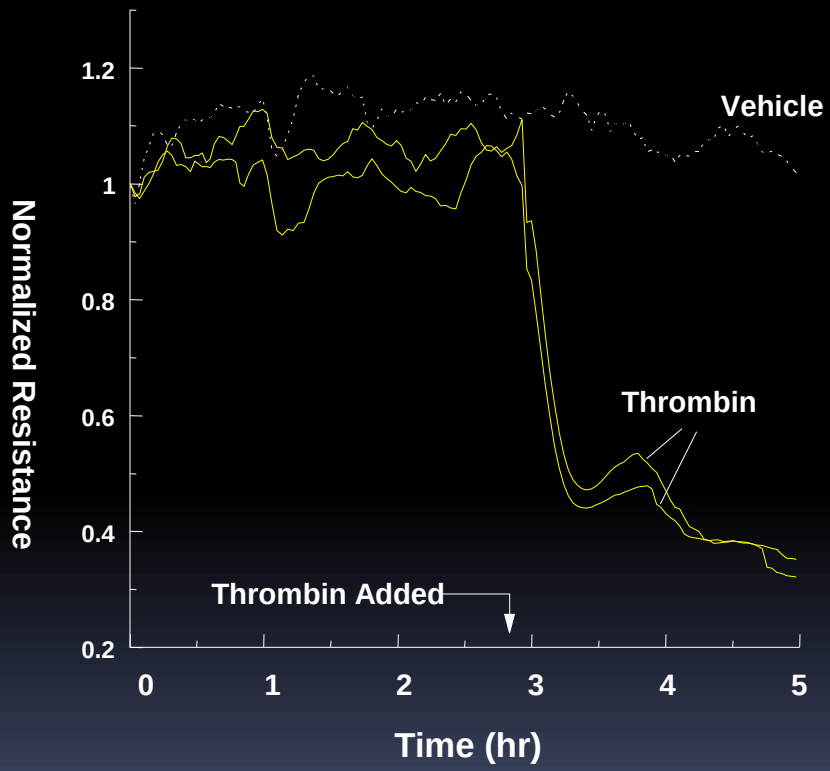
How to measure vascular permeability

Method for assaying endothelial barrier properties *in vitro*

A. Electrical Cell-substrate Resistance Sensor System (ECIS)

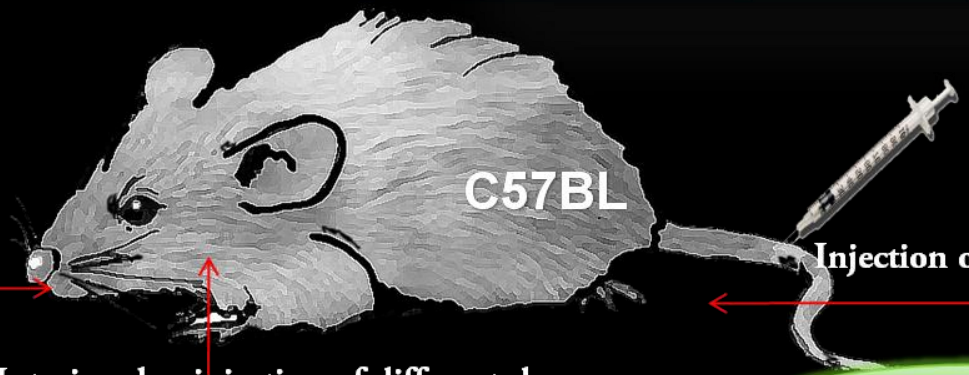


B. Resistance Tracing



Agonist-induced decrease in transendothelial electrical resistance (TER) reflects EC barrier compromise

Schematic of Procedure and Sample Analysis



Intratracheal LPS injection

Injection of EBA – intratail vein

Intrajugular injection of different drugs

Record age

Record body weight

Record weight of lungs

BAL

Protein/ albumin Conc.

BAL Cytokines

Cells

Total cell counts

Differential cell counts

Lung histology

Lung homogenate

Tissue EBA

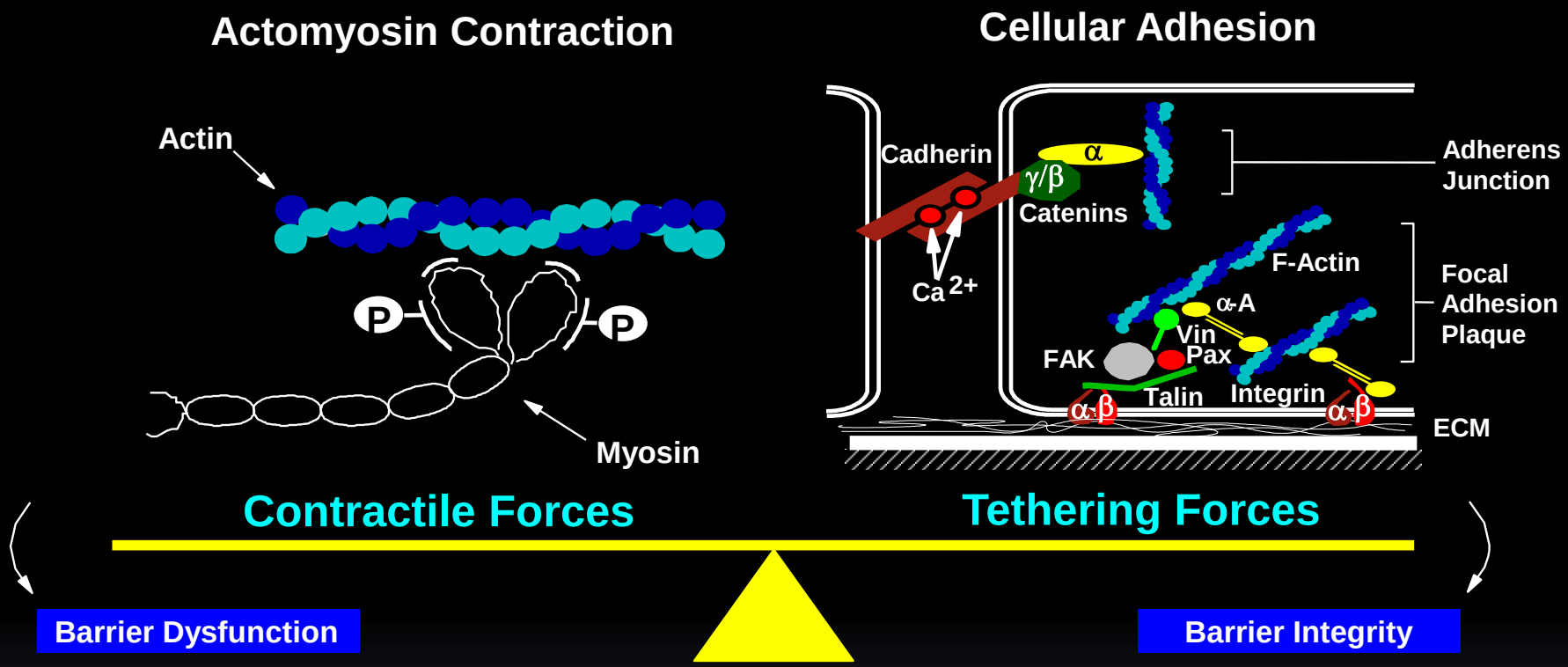
Tissue MPO

Mechanisms regulating vascular permeability

Vascular leakage is primarily caused by an increase permeability of the endothelium

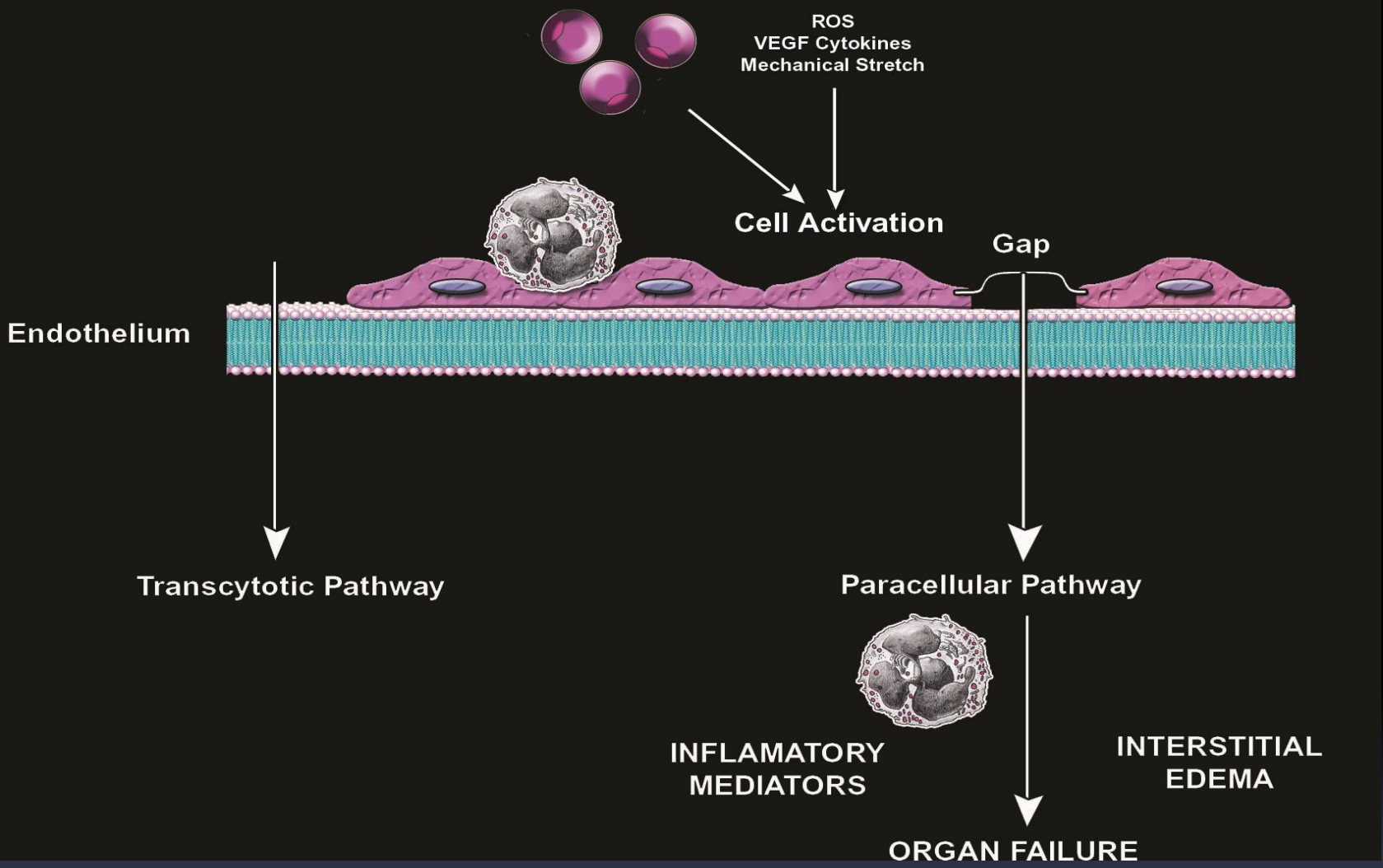
(Michel and Curry, 1999; Renkin, 1985).

Current model for regulation of barrier function in endothelium



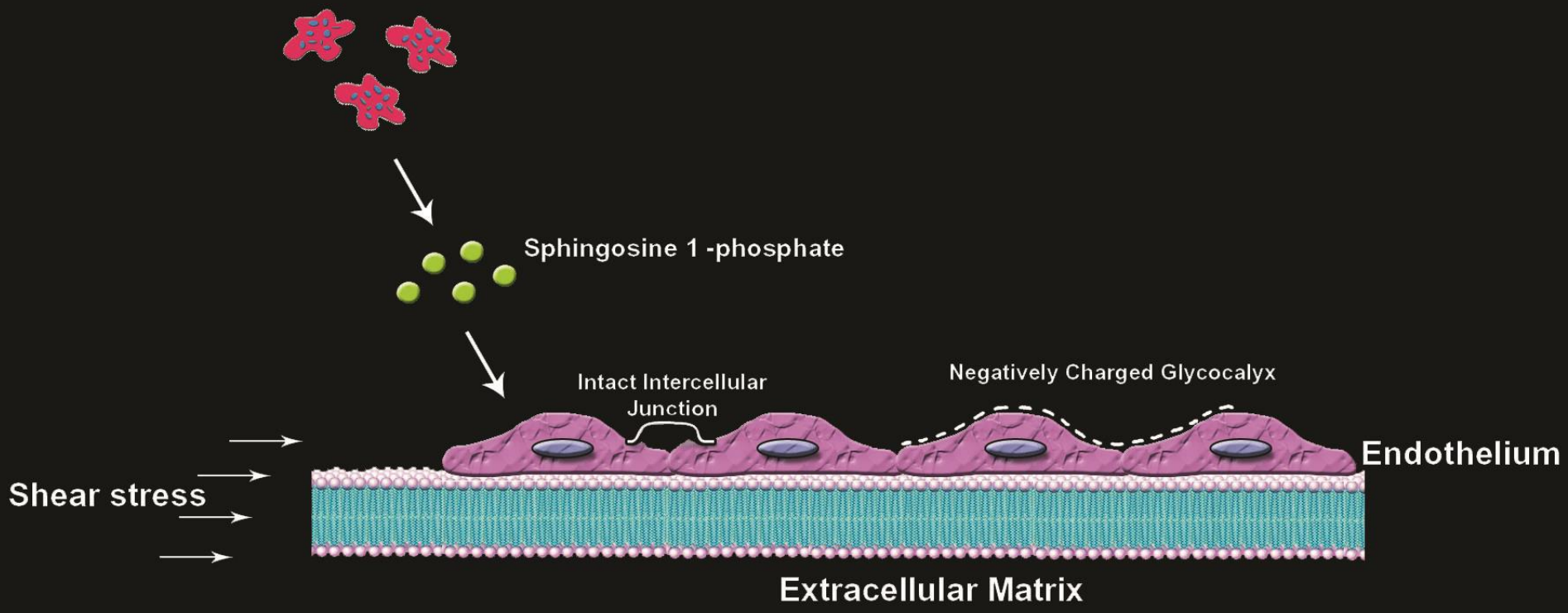
The endothelial cell barrier is regulated by contractile and tethering mechanisms whose effects are critically dependent upon cytoskeletal components.

Edemagenic factors involved in endothelial permeability



Bioactive agonists, growth factors, cytokines and mechanical forces (high shear stress or cyclic stretch), as well as activated leukocytes, serve to activate vascular endothelium. This produces cellular contraction, and increased passage of fluid and cells through intercellular spaces into the interstitial to initiate organ dysfunction.

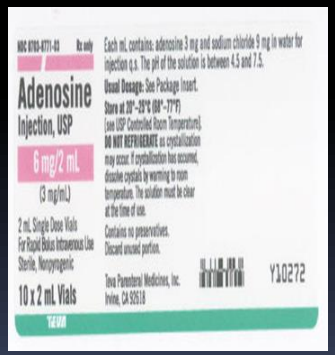
Factors involved in maintaining endothelial integrity/restoration



These include low levels of shear stress, the negatively charged glycocalyx, and barrier protective molecules released by circulating platelets such as sphingosine 1-phosphate

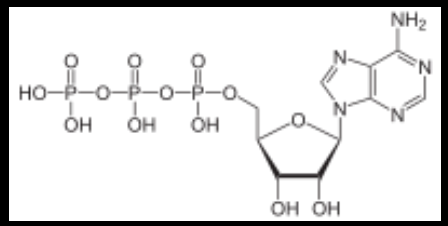
Extracellular purines and endothelial barrier

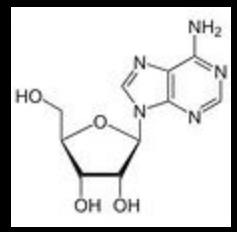
- Extracellular purines such as **ATP**, and its degradation product, **adenosine**, are important vascular mediators
- They are readily present in the surrounding EC micro-environment *in vivo*, and can be released into extracellular fluids under pro-inflammatory conditions from several cell sources including endothelium
- Recently the therapeutic potential of purinergic agonists in the treatment of cardiovascular and pulmonary diseases has been studied
- In the USA, adenosine is clinically used for tachycardia treatment.



Recent data implicate the involvement of extracellular purines in EC barrier enhancement/protection

Extracellular purine-induced signaling in endothelium





Blood

ectonucleotIdases

ATP → ADP → AMP → Adenosine

P2Y2 P2Y13 A2A A2B

Apical

TJ TJ

P2Y1 P2Y11 A2A A2B

Endothelial cell

Basolateral

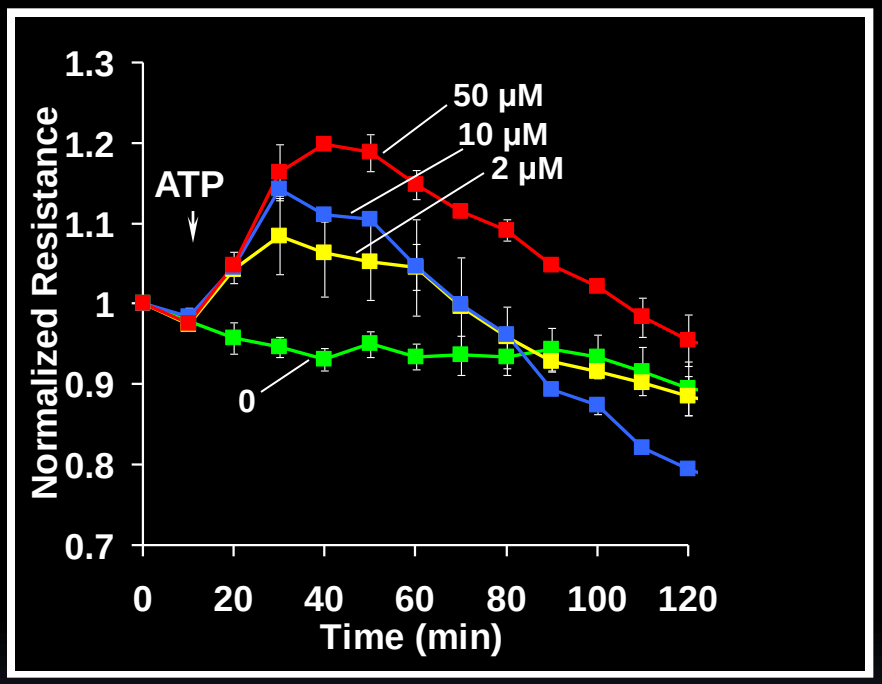
P2Y13

ATP → ADP → AMP → Adenosine

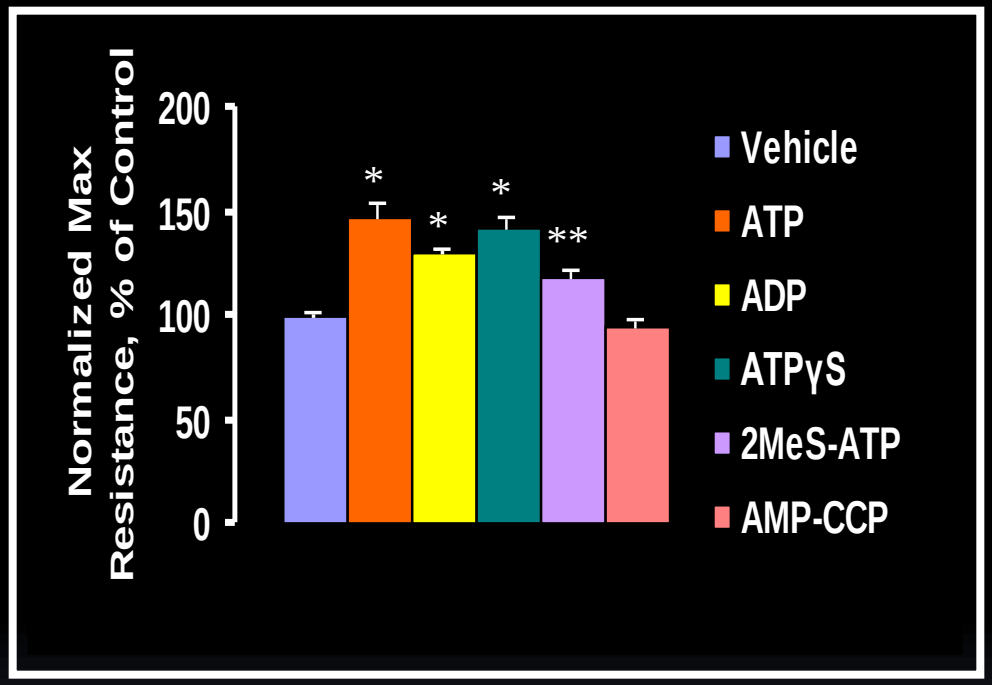
ectonucleotIdases

Basement membrane and alveolar epithelium

Effect of purinergic stimulation on EC permeability

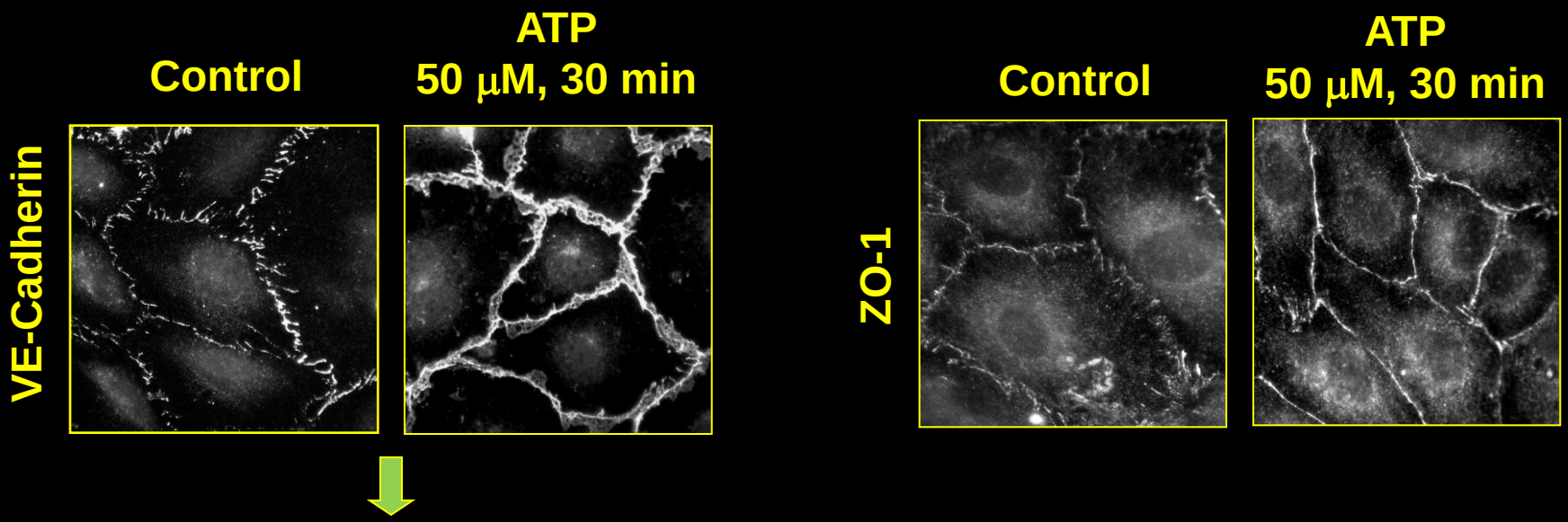


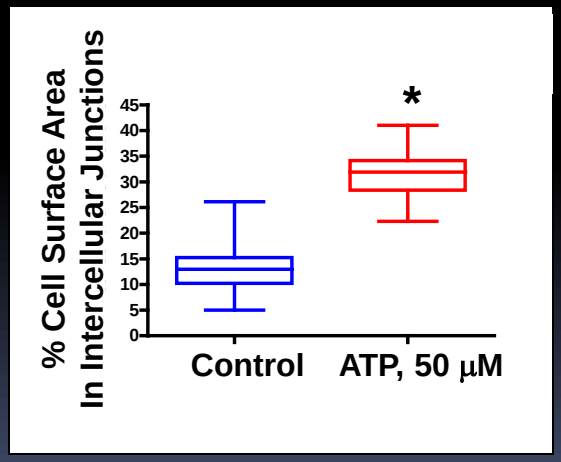
Dose-dependent effect of ATP on TER



Agonists of P2 receptors (50 μM each, 30 min) increase TER

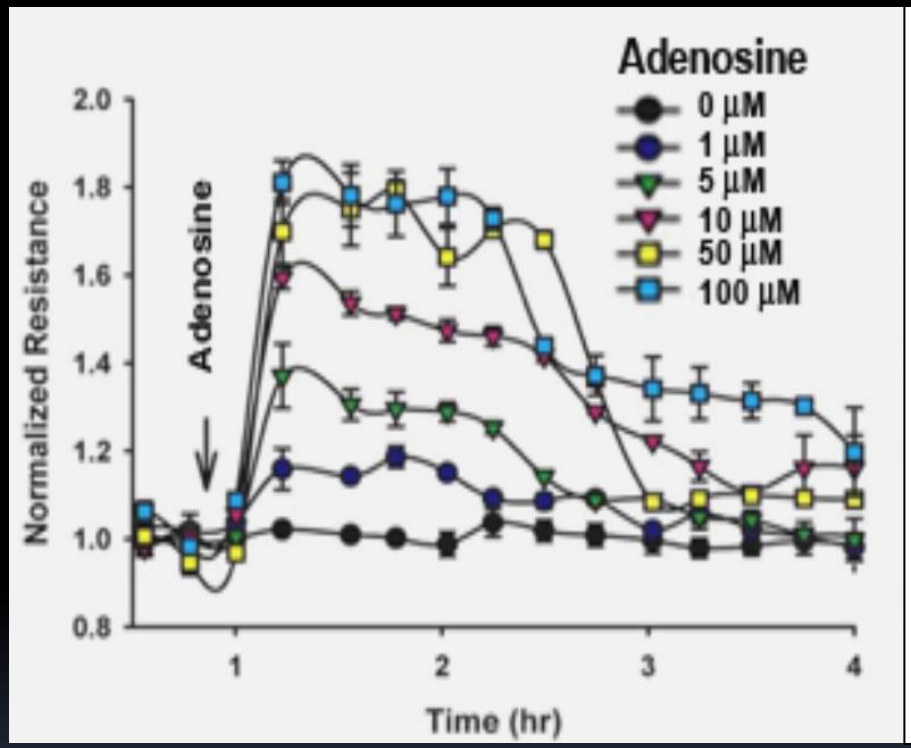
Effect of ATP on cell-cell junctions

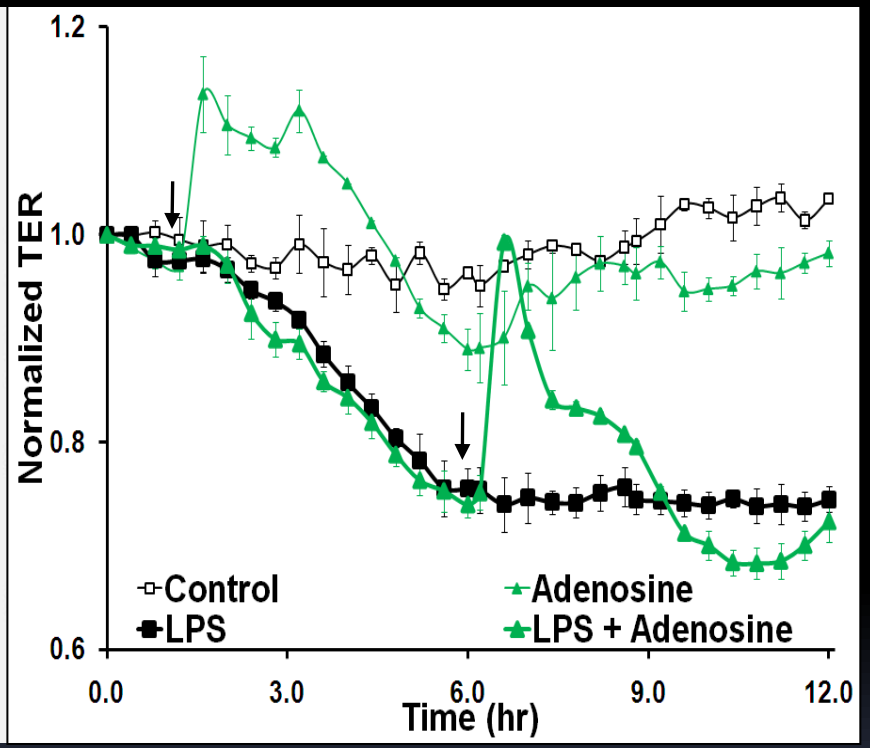




Quantification of the surface area of the cell-cell interface. The percentage of total cell surface area occupied by VE-cadherin-labeled cell-cell junctions was calculated for 20 cells in each group (* $p < 0.001$ compared to control).

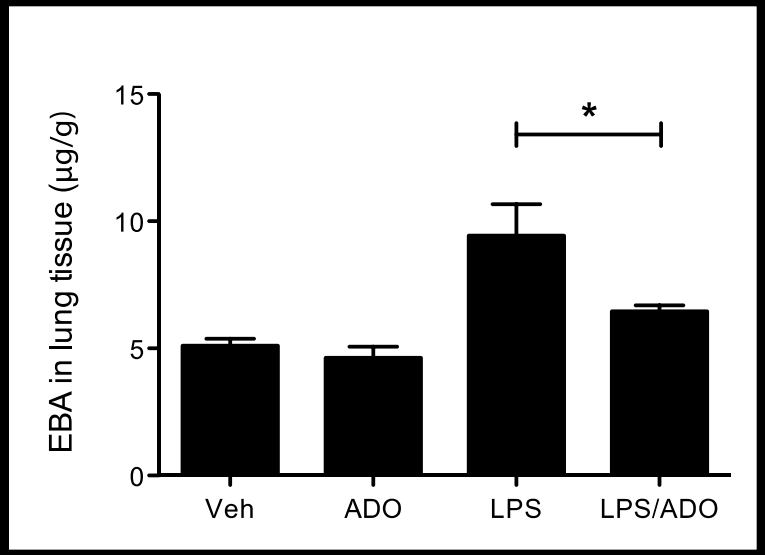
Adenosine enhances and restores EC barrier *in vitro*



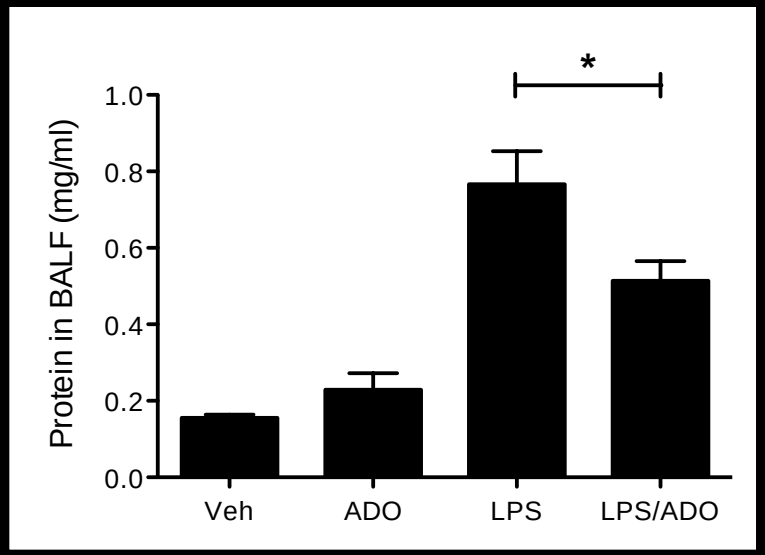


Effect of adenosine post-treatment on vascular permeability and inflammation in murine model of LPS-induced ALI

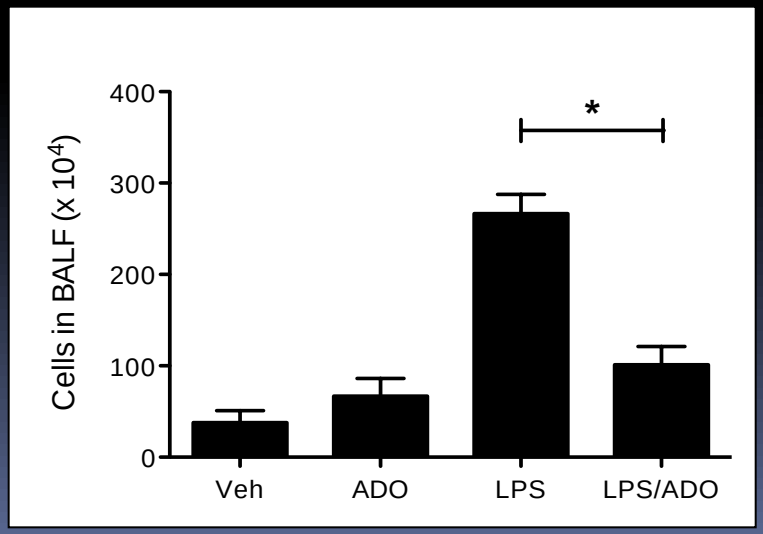
A



B

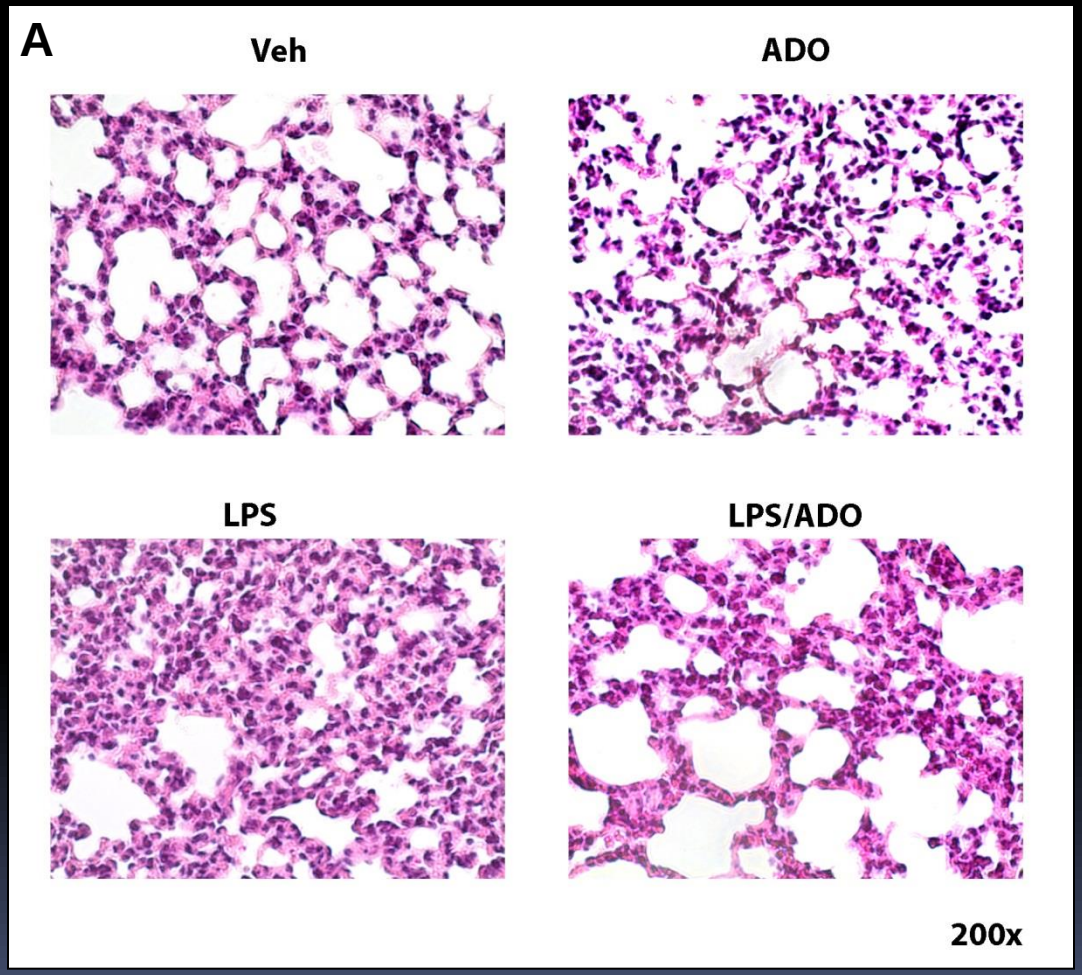


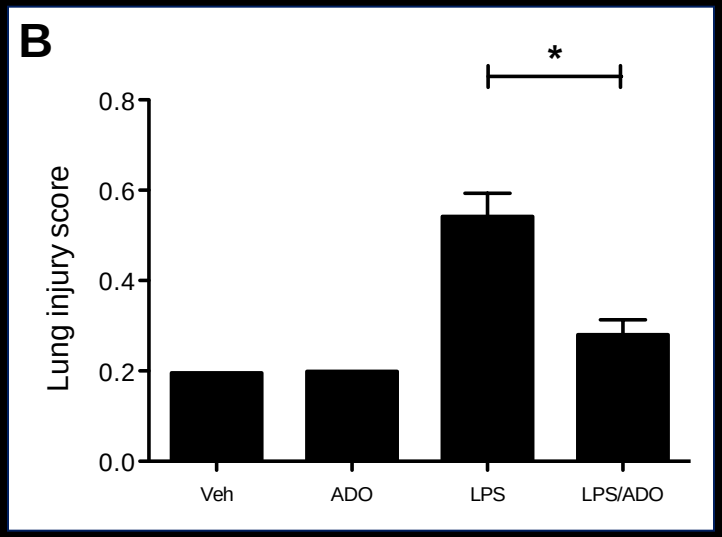
C



Adenosine (i.v., 100 µM in blood, added 3 hr after LPS) significantly attenuates LPS (i.t., 0.9 mg/kg)-induced vascular leak and inflammation in mice.

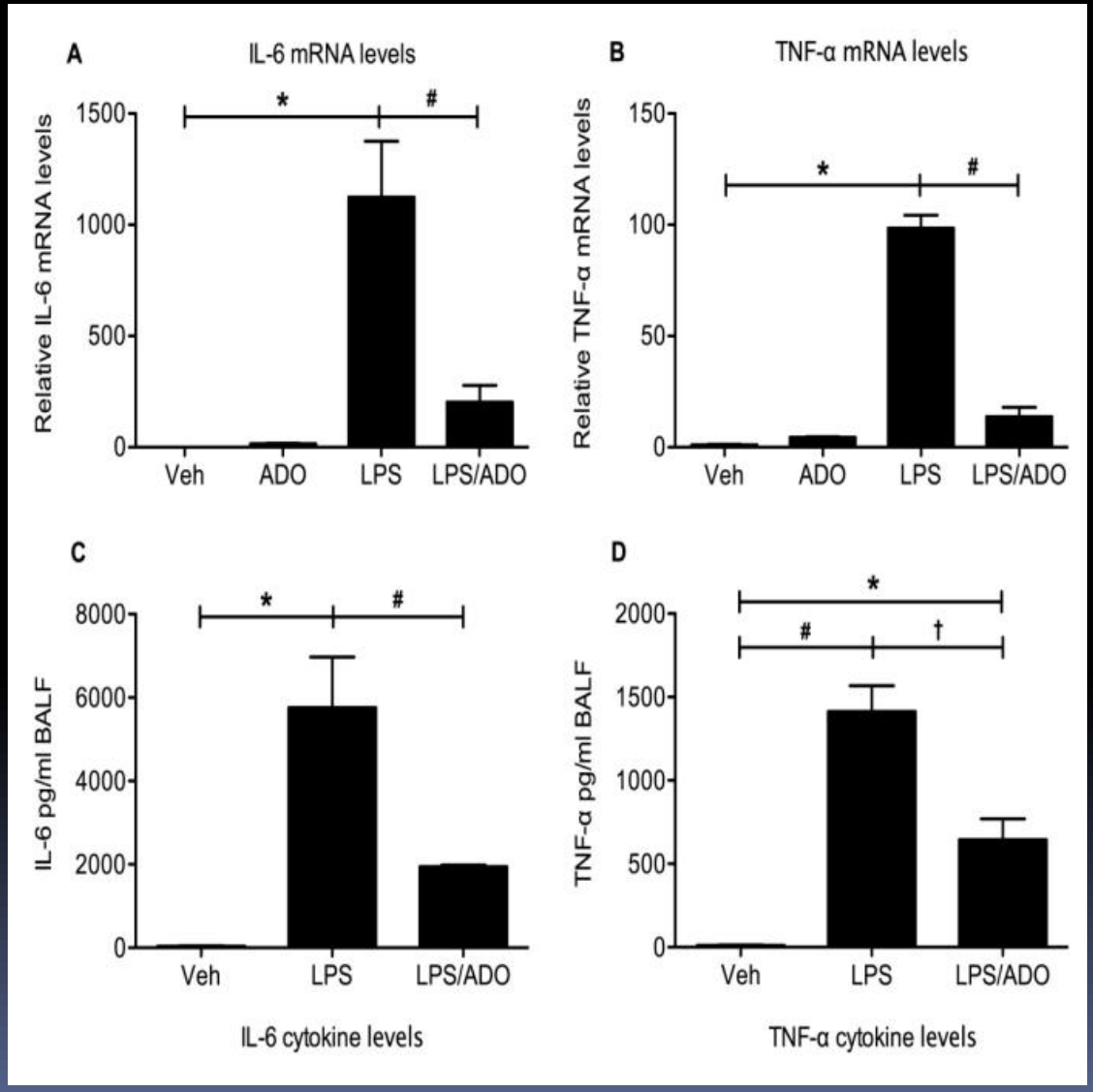
Histological assessment of the effect of adenosine on LPS-induced lung inflammation and injury.





H&E staining (A) and lung injury score (B) demonstrate prominent lung inflammation in mice exposed to i.t. LPS compare to vehicle. Treatment with adenosine attenuates LPS-induced response.

Adenosine attenuates LPS-induced pro-inflammatory cytokine production in murine model of ALI.



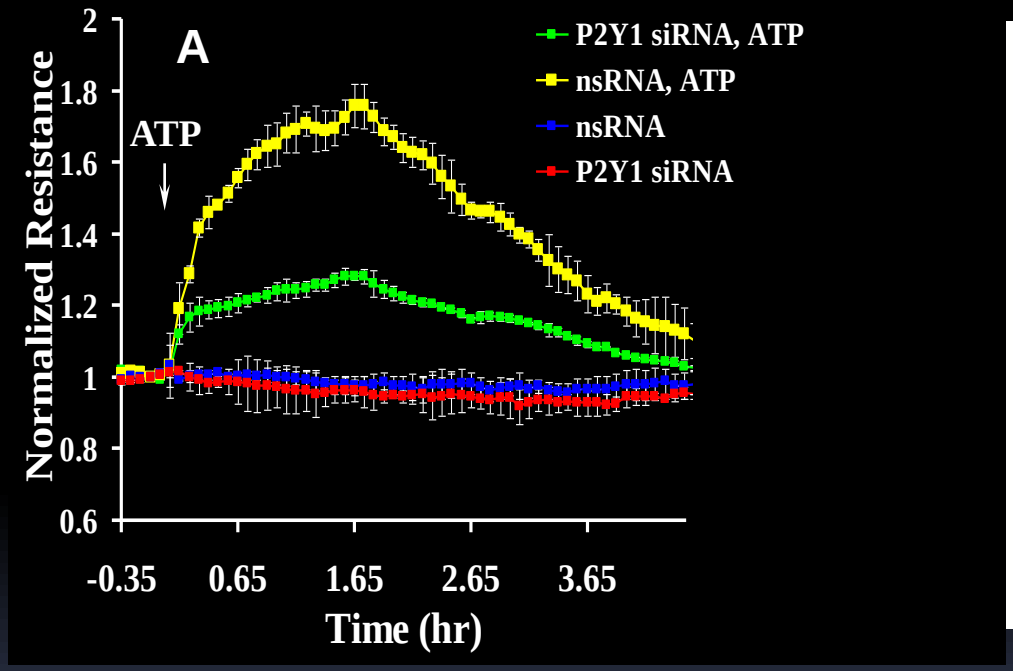
Summary (1):

1. Extracellular purines, ATP and adenosine, enhances and restores endothelial barrier *in vitro*

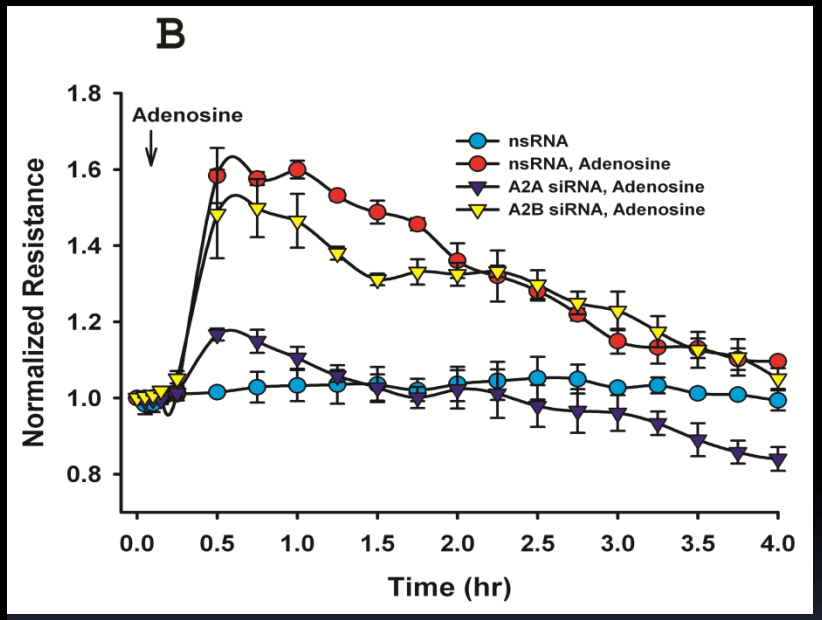
2. Extracellular purines protect lung vascular barrier and reduce inflammation in murine model of LPS-induced lung injury

Mechanisms of purine-induced EC barrier preservation

Effect of purinergic receptors depletion on EC barrier enhancement induced by extracellular purines

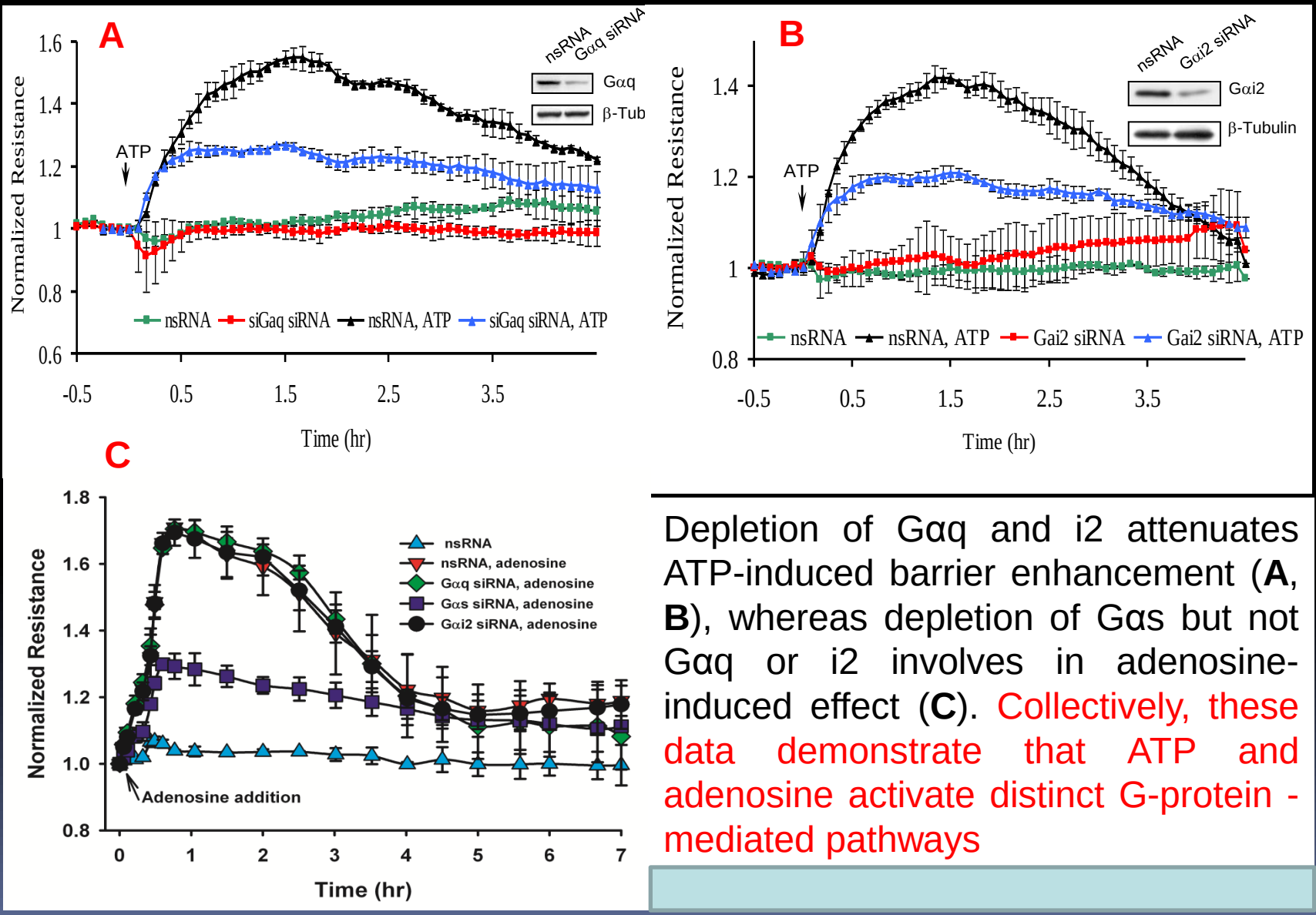


P2Y1 is involved in EC barrier regulation



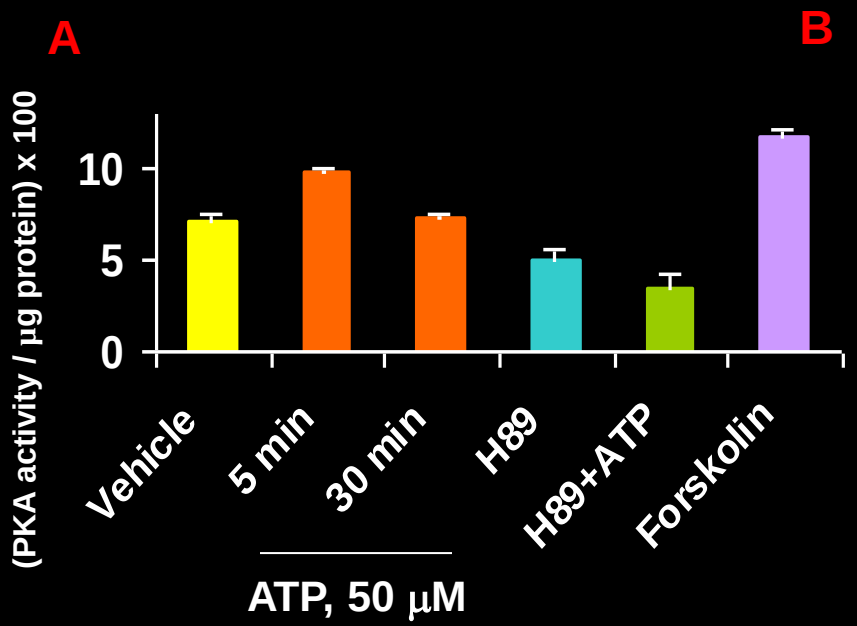
A2A, but not A2B receptor is involved in EC barrier regulation

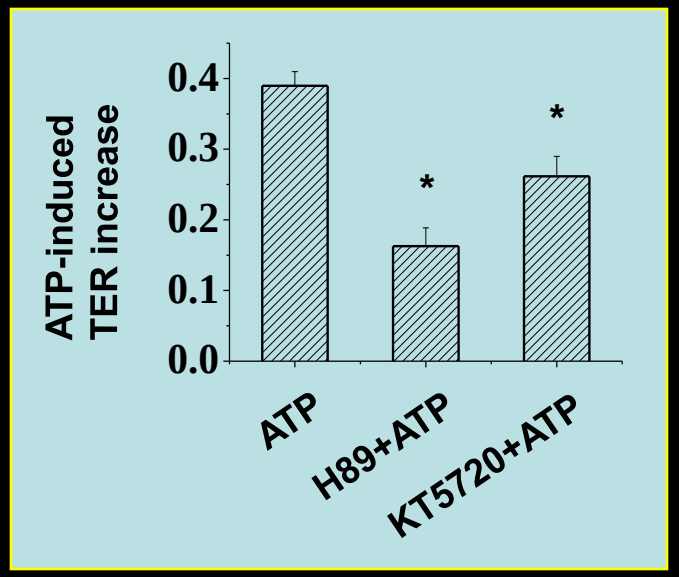
Extracellular purines enhance endothelial barrier via G protein-coupled mechanism

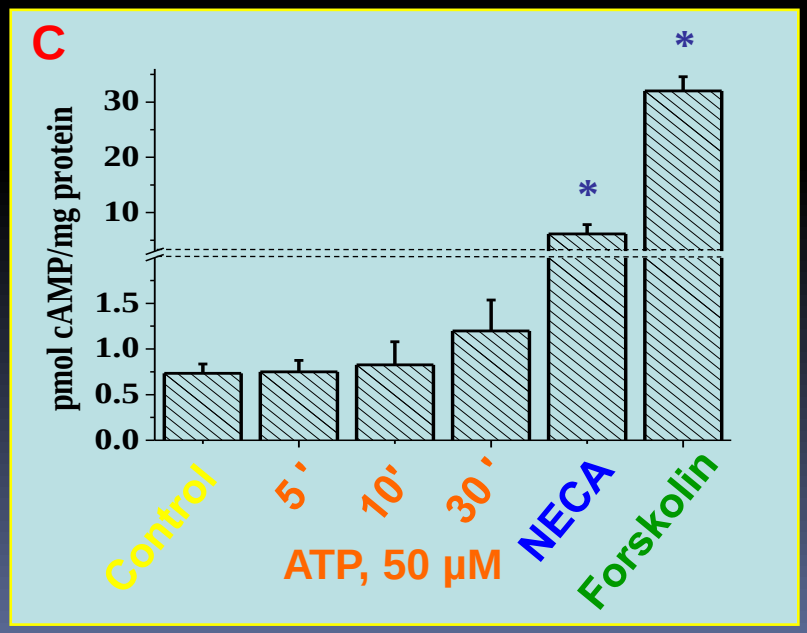


Depletion of Gαq and i2 attenuates ATP-induced barrier enhancement (**A**, **B**), whereas depletion of Gαs but not Gαq or i2 involves in adenosine-induced effect (**C**). Collectively, these data demonstrate that ATP and adenosine activate distinct G-protein - mediated pathways

ATP –induced EC barrier enhancement involved PKA activation

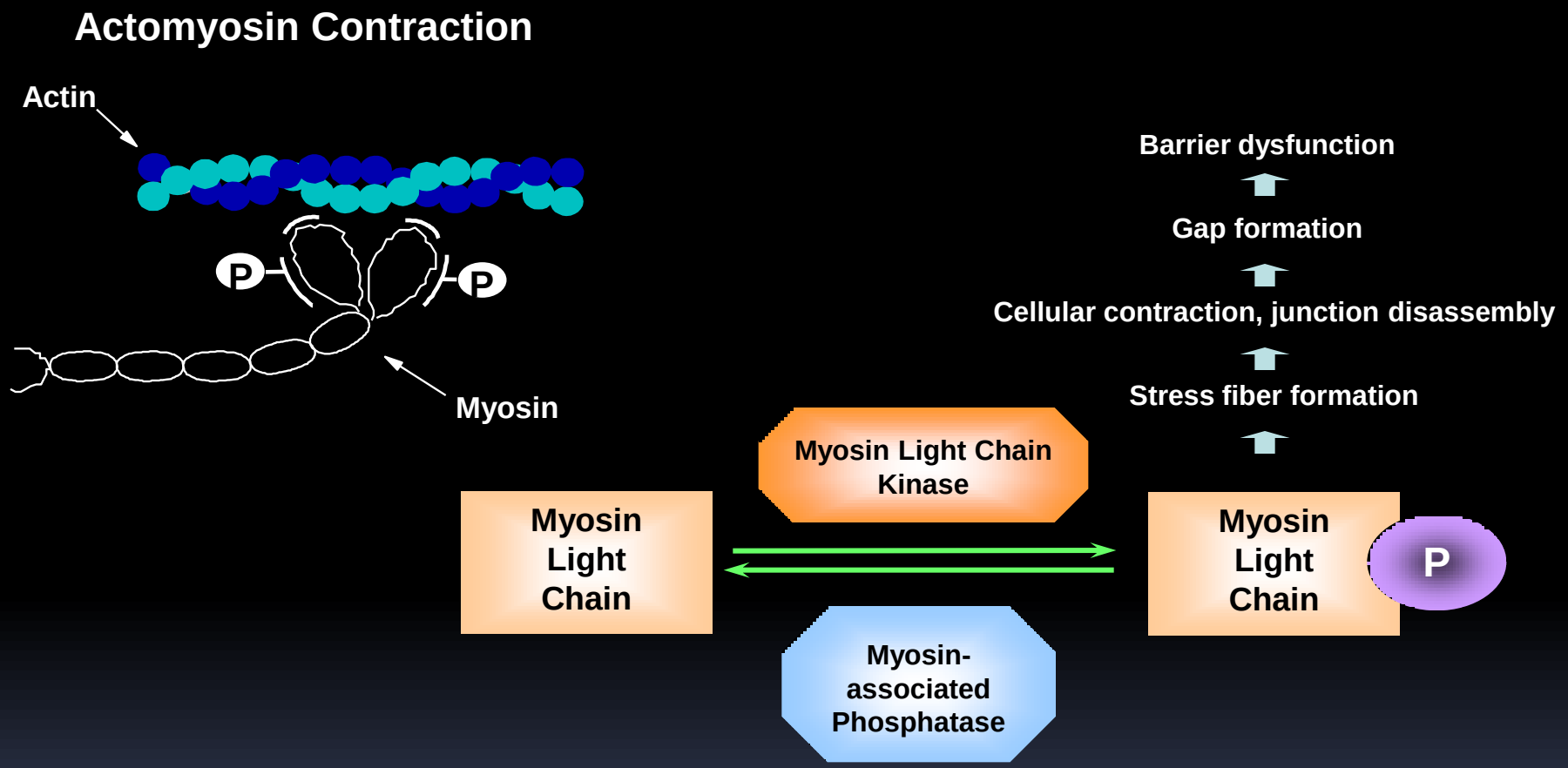






A. ATP increases PKA activity. **B.** Inhibition of PKA attenuates EC ATP-induced EC barrier enhancement **C.** ATP does not increases cAMP in EC. In contrast, adenosine agonist, NECA significantly increases cAMP suggesting distinct signaling involved in ATP and adenosine-induced PKA activation

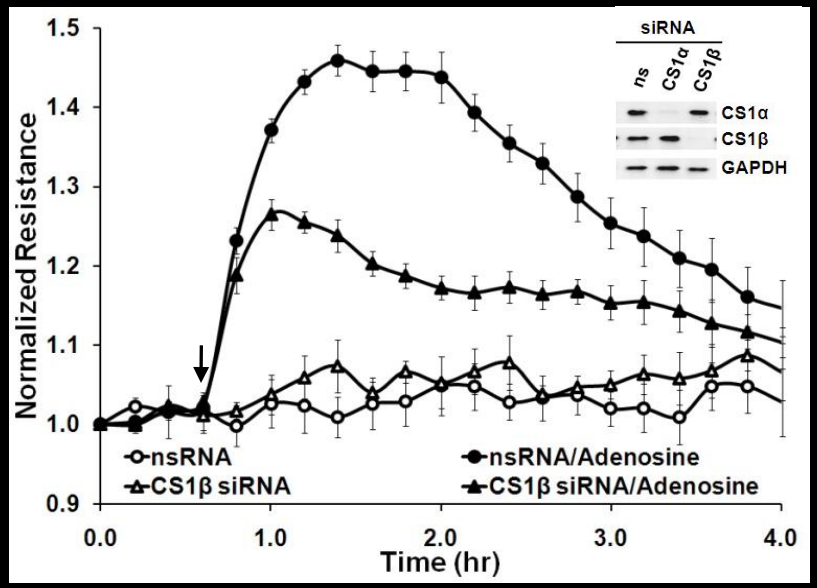
Role of MLC phosphorylation in the regulation of EC barrier



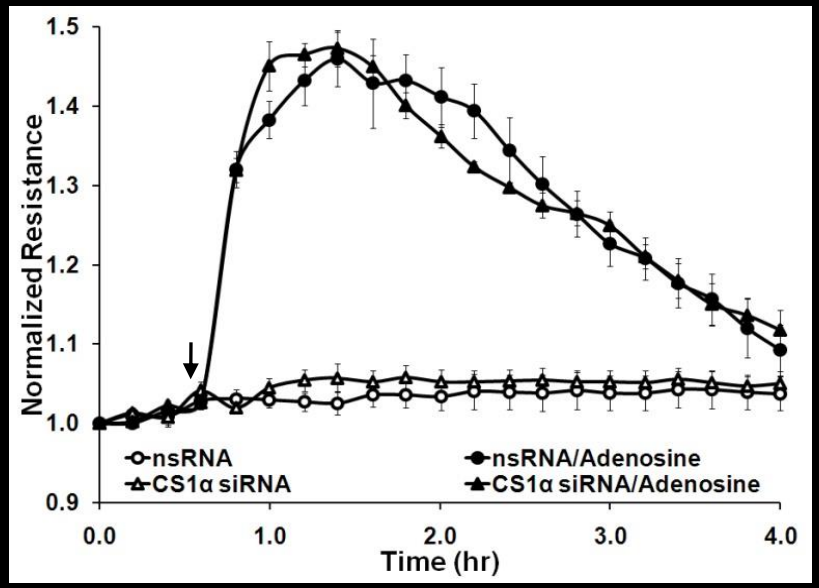
Myosin-associated phosphatase (MLCP) by dephosphorylating MLC may be involved in EC barrier preservation

Effect of MLCP depletion on adenosine-induced EC barrier enhancement

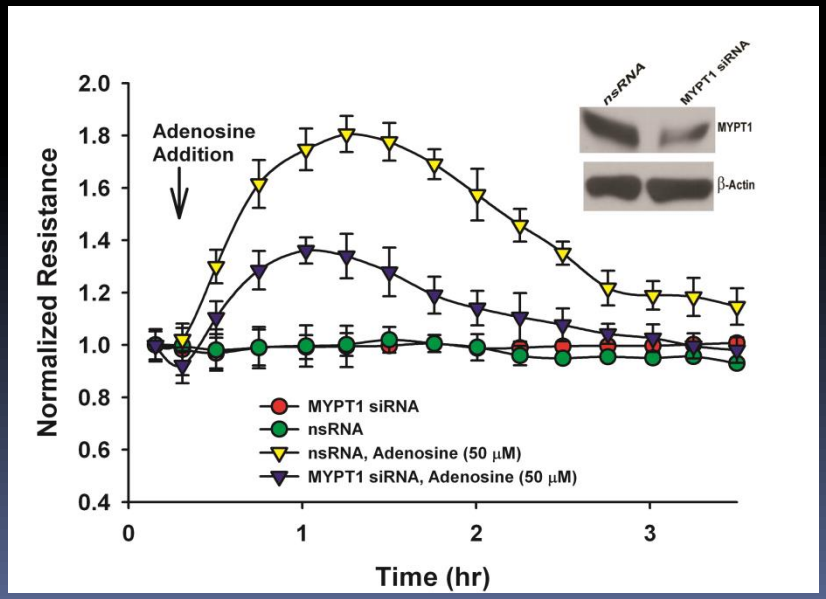
A



B



C



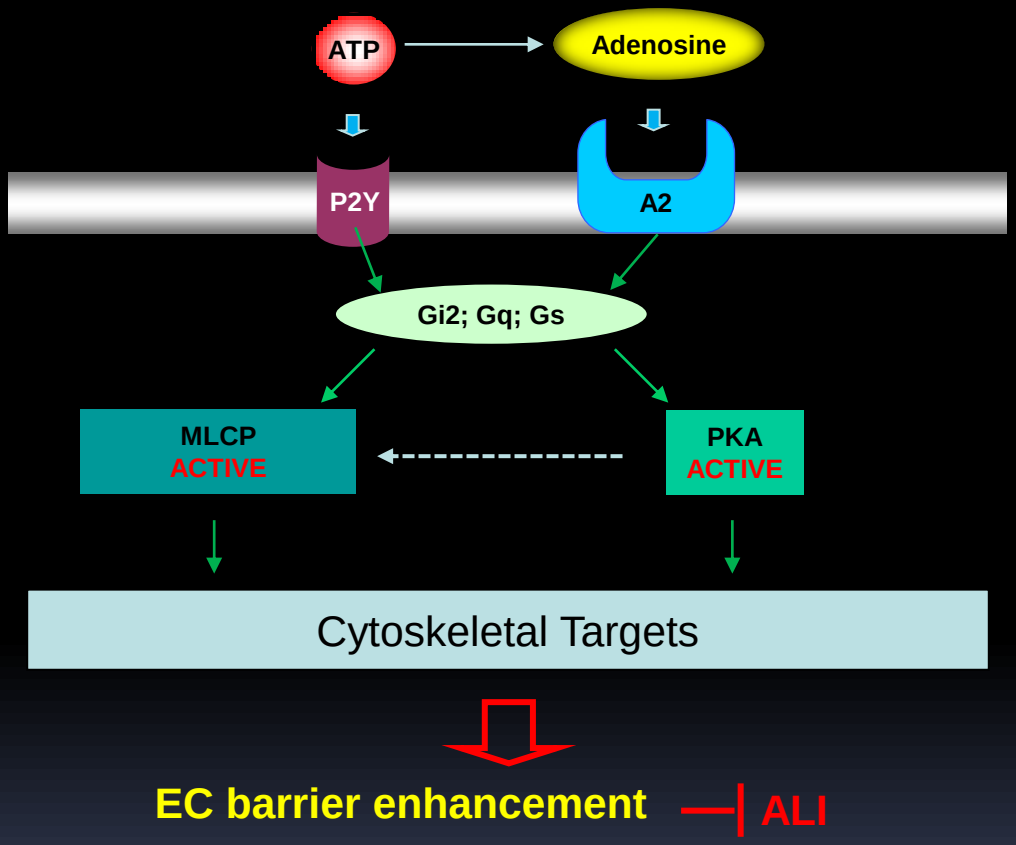
A, B. Depletion of catalytic MLCP subunit (CS1β), but not CS1α-control attenuates adenosine-induced EC barrier enhancement. Depletion of MLCP regulatory subunit (MYPT 1) demonstrates the same effect (**C**)

Summary (2):

1. ATP and adenosine enhances EC barrier by activation of different signaling

2. Purine-induced EC barrier enhancement involves activation of protein kinase A and myosin phosphatase

CONCLUSION



Increase of extracellular purines

Receptors activation

Trimeric G-proteins engagement
Small G-proteins activation

Activation of regulatory enzymes

Changes in phosphorylation
subcellular localization
of cytoskeletal targets

Lung injury prevention

Dr. Verin's lab

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David Fulton, PhD

Rudolph Lucas, PhD

National Heart Lung and Blood Institute

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