2019台灣胸腔暨重症加護醫學會
2019 Taiwan Society of Pulmonary and Critical Care Medicine

藉阻斷鈣離子釋放激活鈣離子通道降低大鼠動物呼吸器引起內質網壓力與改善肺損傷

Blocking Calcium Release-activated Calcium Channel Attenuates Ventilator-induced Endoplasmic Reticulum Stress and Lung Injury in Rats

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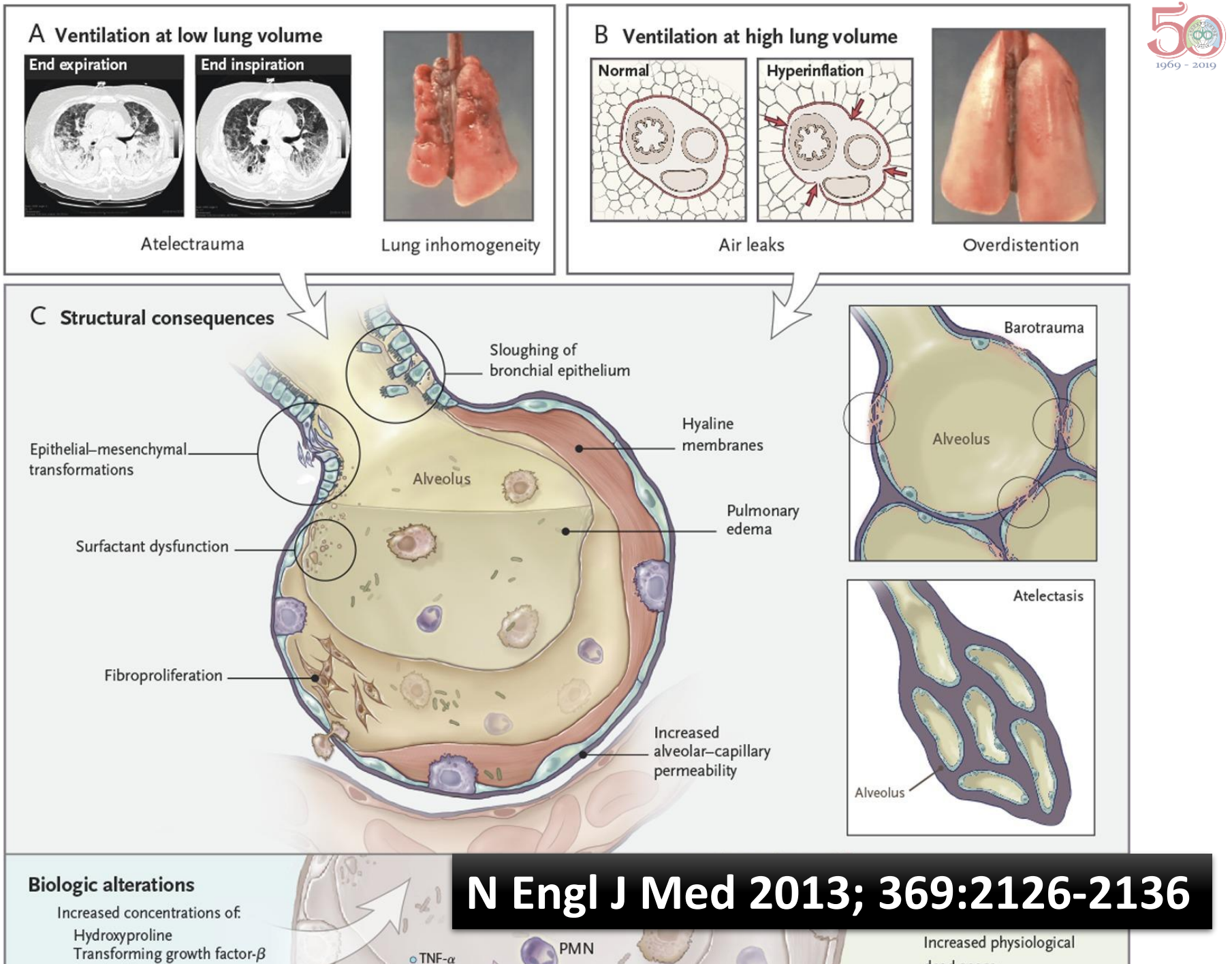
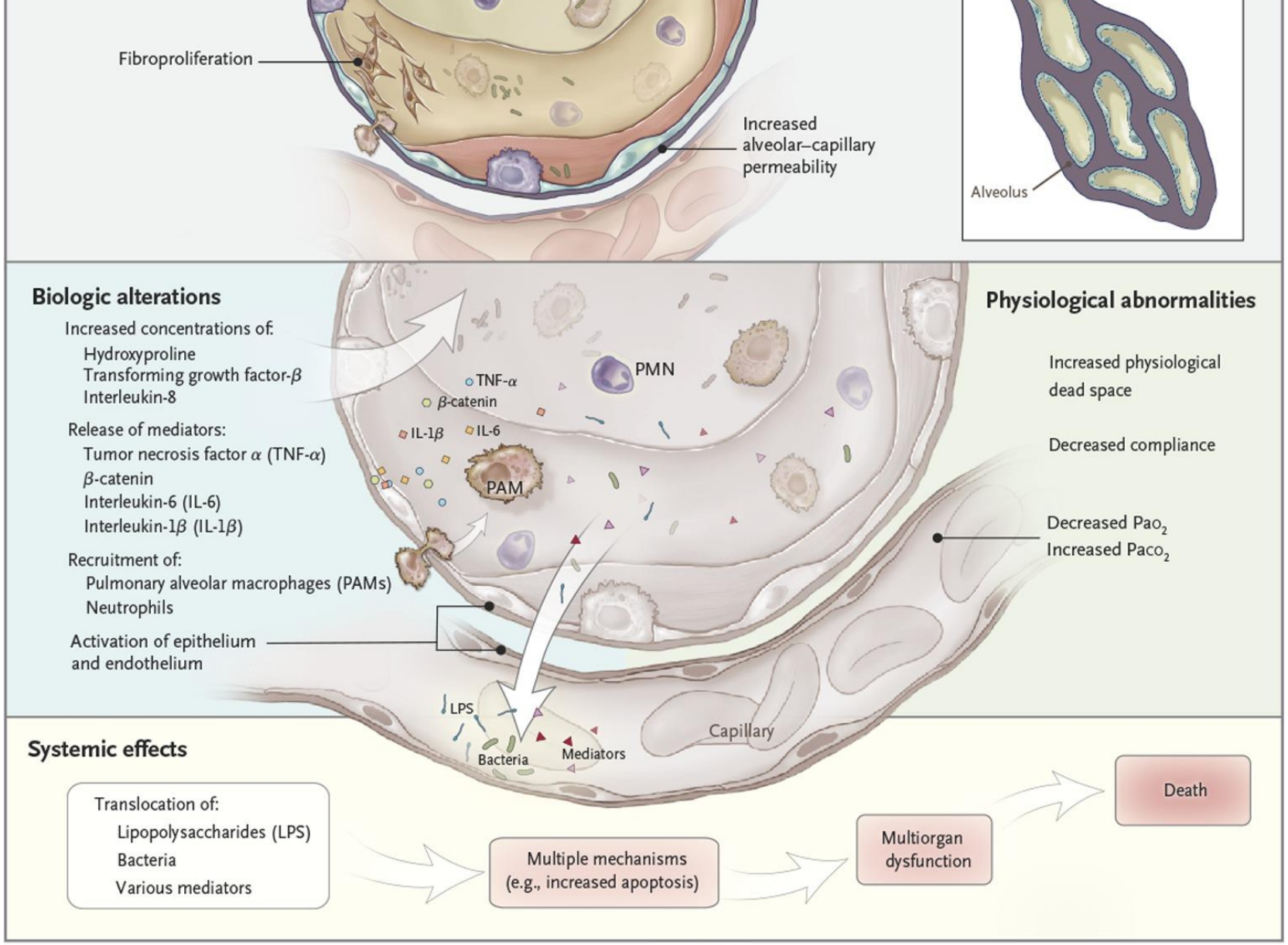


N Engl J Med 2013; 369:2126-2136

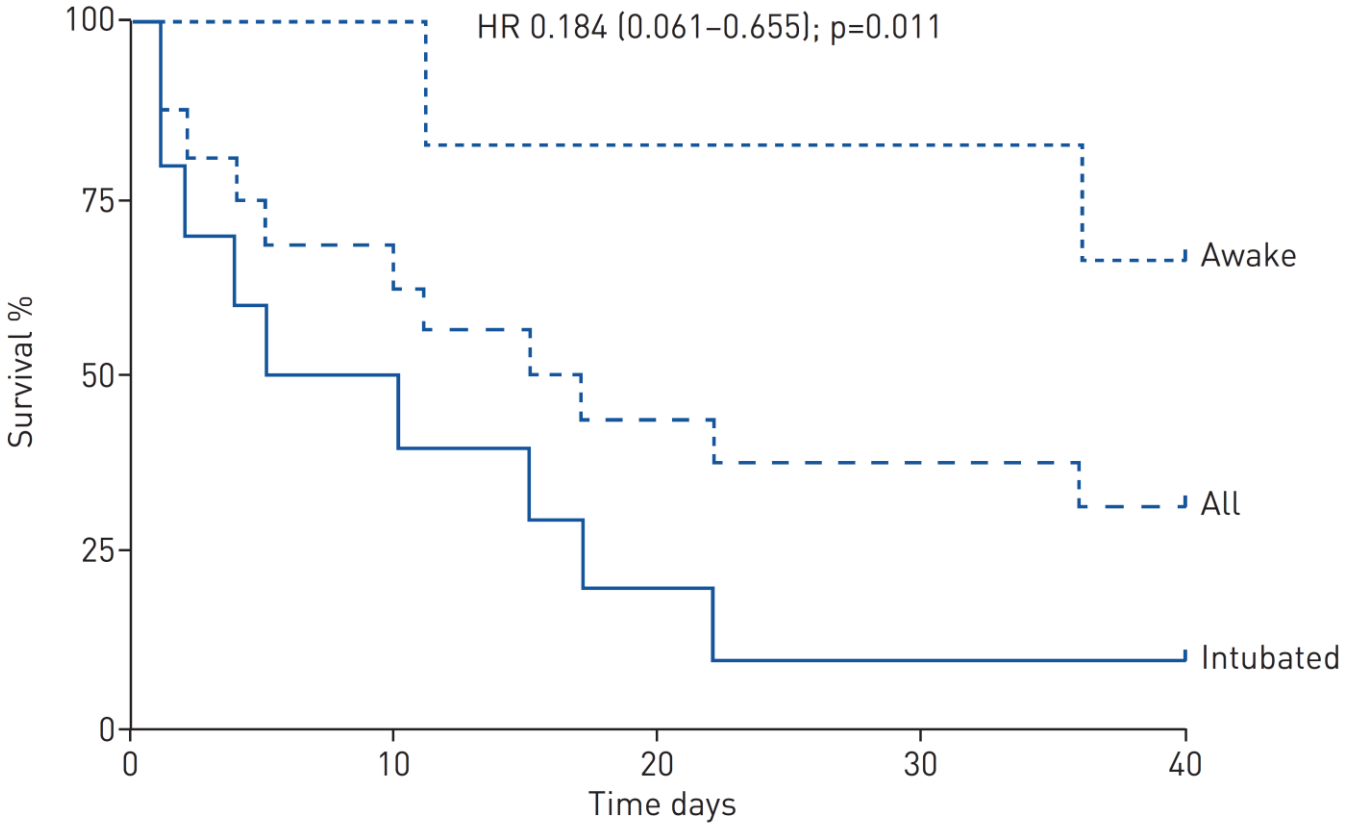


N Engl J Med 2013; 369:2126-2136

ECMO in a non-intubated patient with ARDS (Awake ECMO)

European Respiratory Journal 2012 40: 1296-1298

Awake ECMO for ARDS due to Pneumocystis pneumonia



Eur Respir J 2019; 54: 1900410

Introduction

Acute respiratory distress syndrome (ARDS)
Unfortunately, there is no specific therapy for ALI/ARDS.

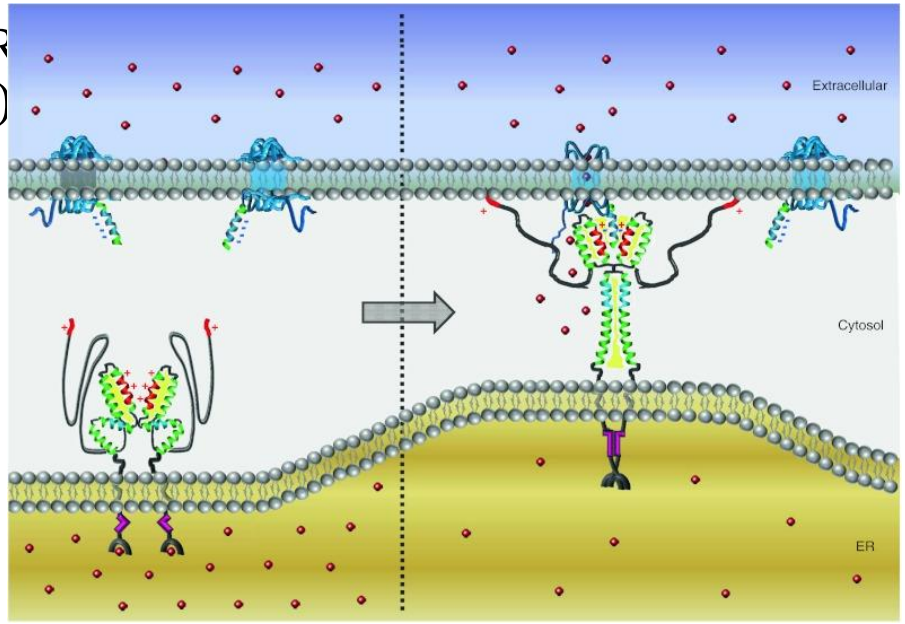
Hypothesis

Calcium release-activated Calcium Channel participate in the pathogenesis of ventilator-induced lung injury.

Calcium release-activated Calcium (CRAC) Channel

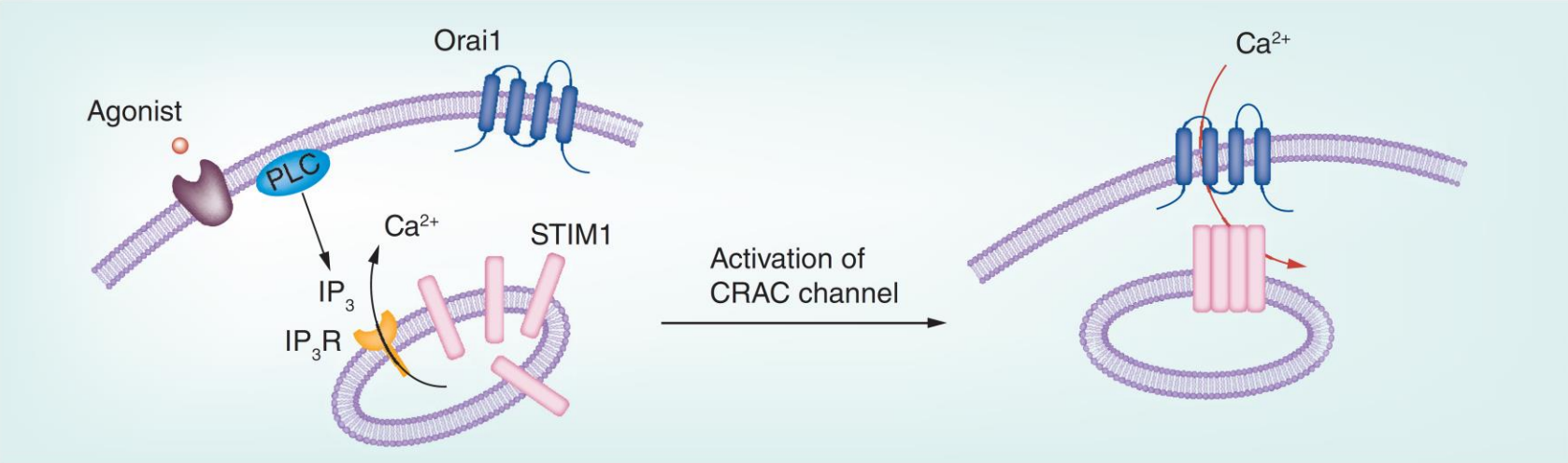
- **Store-operated calcium (Ca(2+)) entry (SOCE)** : molecules located on the endo/sarcoplasmic reticulum (ER/SR respond to decreased luminal Ca(2+) levels by signaling Ca(2+) release activated Ca(2+) channels (CRAC) channels to open on the plasma membrane (PM).
- ER: **stromal interaction molecules (STIMs)**: functioning as the ER/SR luminal Ca(2+) sensors

Plasma membrane: CRAC channels: Orai proteins

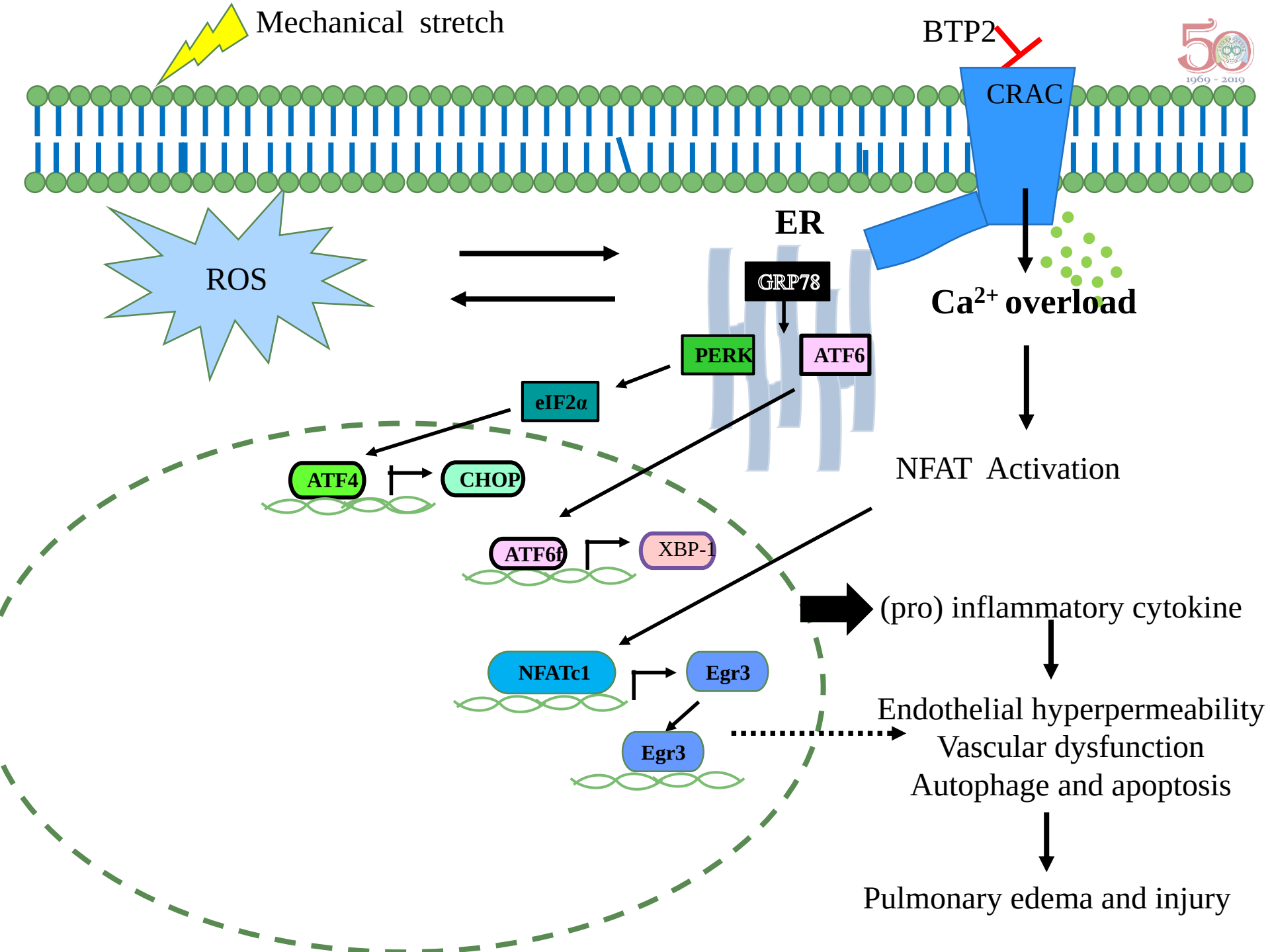


Cell Mol Life Sci. 2012; 69: 4163–4176.

Activation of release-activated Ca2+ channel



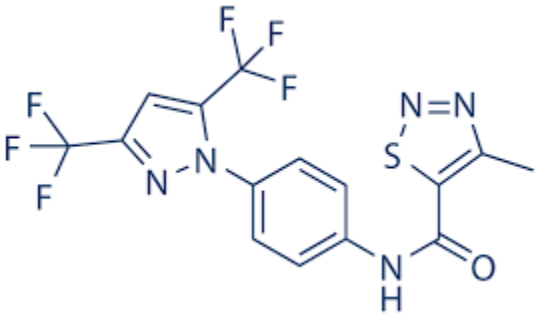
Future Med. Chem. (2016) 8(7), 817–832

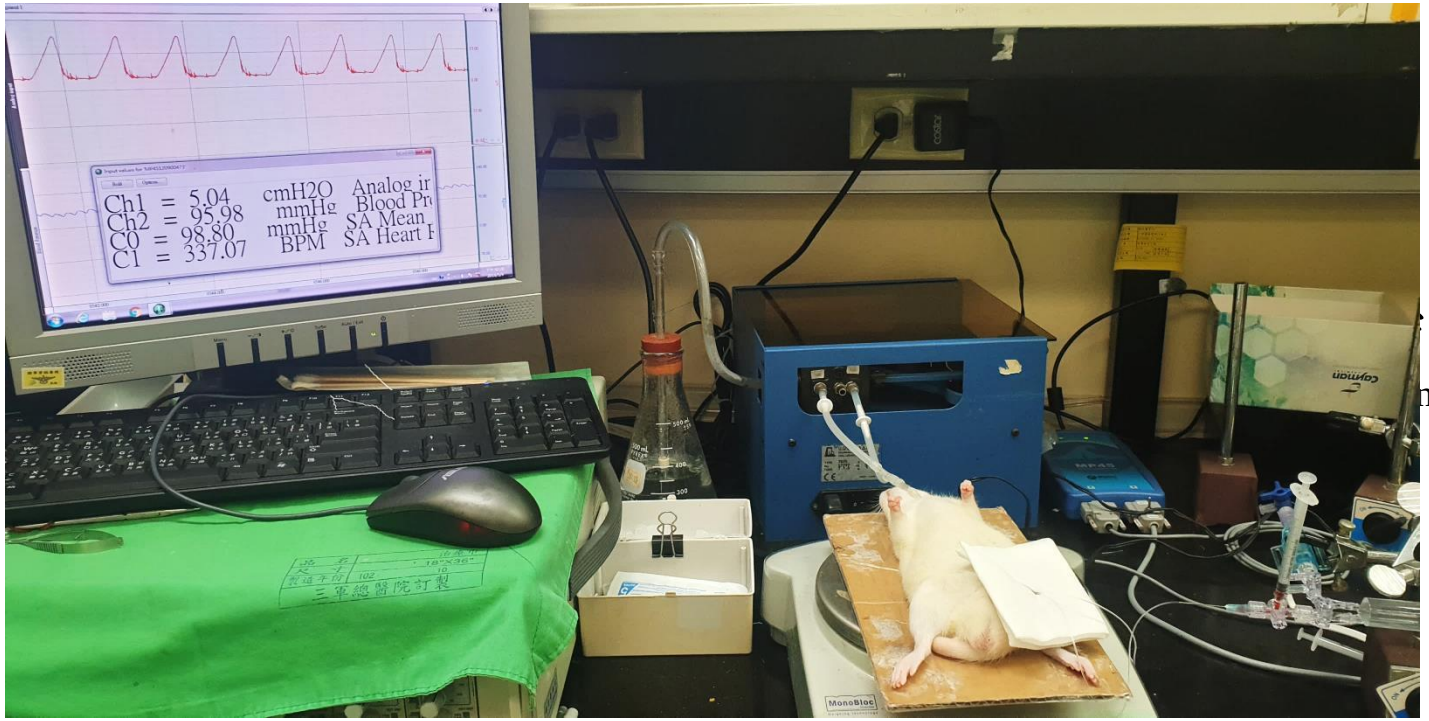

Mechanical stretch
BTP2
CRAC
ROS
ER
GRP78
PERK
ATF6
eIF2α
ATF4
CHOP
ATF6f
XBP-1
NFATc1
Egr3
Egr3
Ca2+ overload
NFAT Activation
(pro) inflammatory cytokine
Endothelial hyperpermeability
Vascular dysfunction
Autophage and apoptosis
Pulmonary edema and injury

Methods

- **Methods:** Male SD rats were exposed to ventilator with normal or high tidal-volume ventilation with intraperitoneal injection of BTP2.

- **BTP2: Calcium release-activated Calcium Channel (CRAC channel) inhibitor**





PEEP = 4 cmH_2O
Respiratory rates(RR) = 66-68/min
BTP2(0.5 mg/kg、1mg/kg、2mg/kg)

BTP2 i.p.

30 min

4 hours

End point
Animal
sacrifice

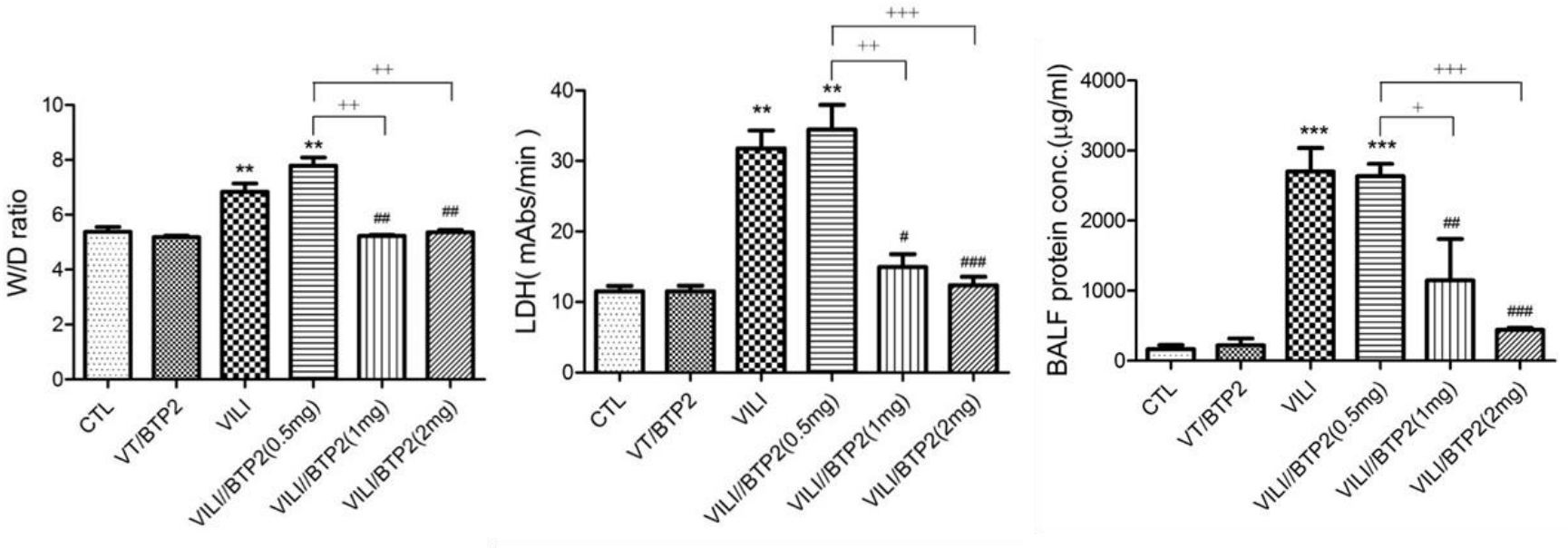
Baseline

V_T = 8-10 ml/kg

VILI

V_T = 22 ml/kg

FIGURE 1 Effect of BTP2 on lung edema.



(A) Lung wet/dry ratio
(B) LDH level
(C) Protein concentrations in bronchoalveolar lavage fluid (BALF)

Fig. 2

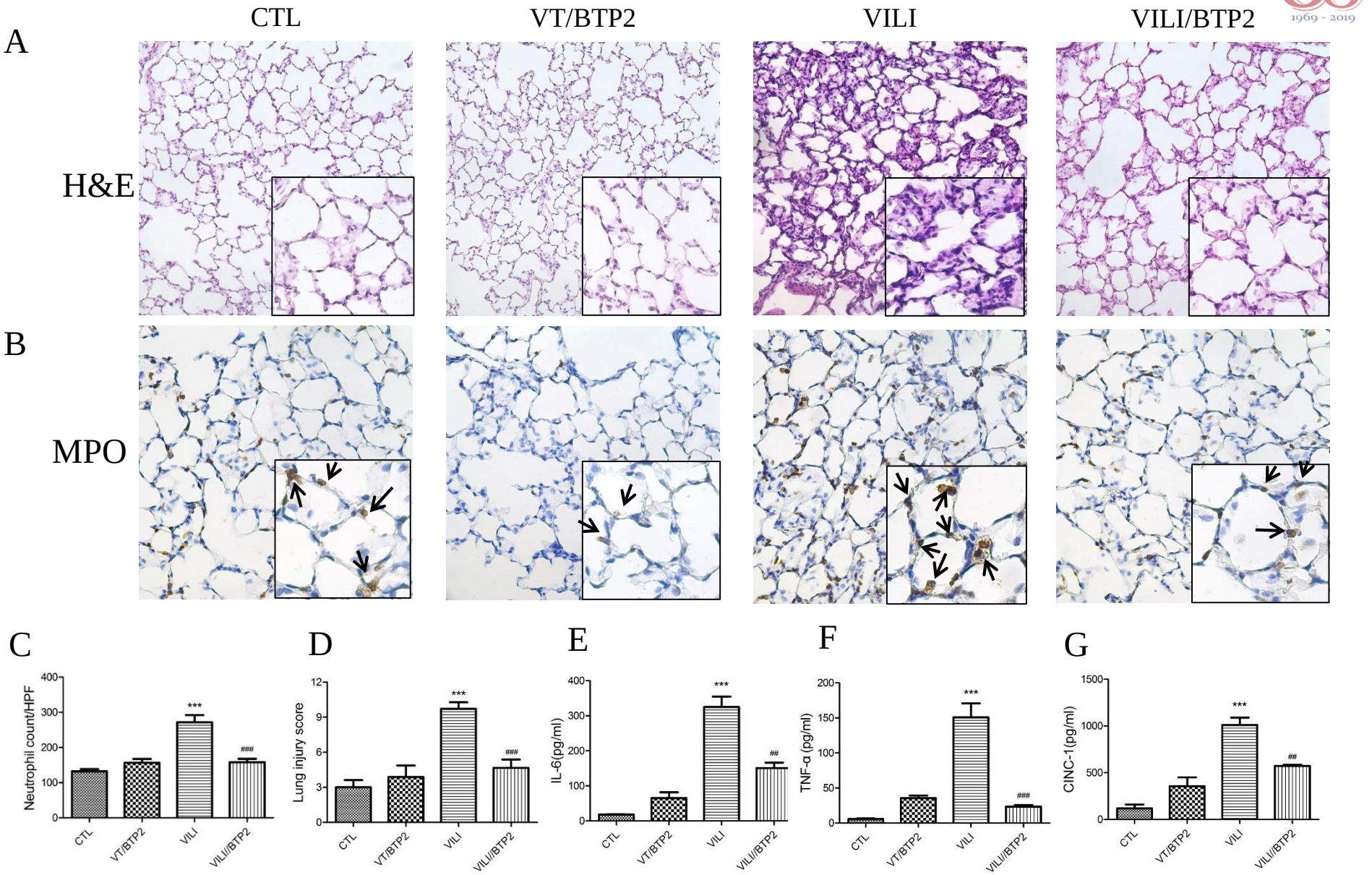


FIGURE 2 Assessment of BTP2 treatment on lung inflammatory.

Fig. 3

Lung injury: Autophagy

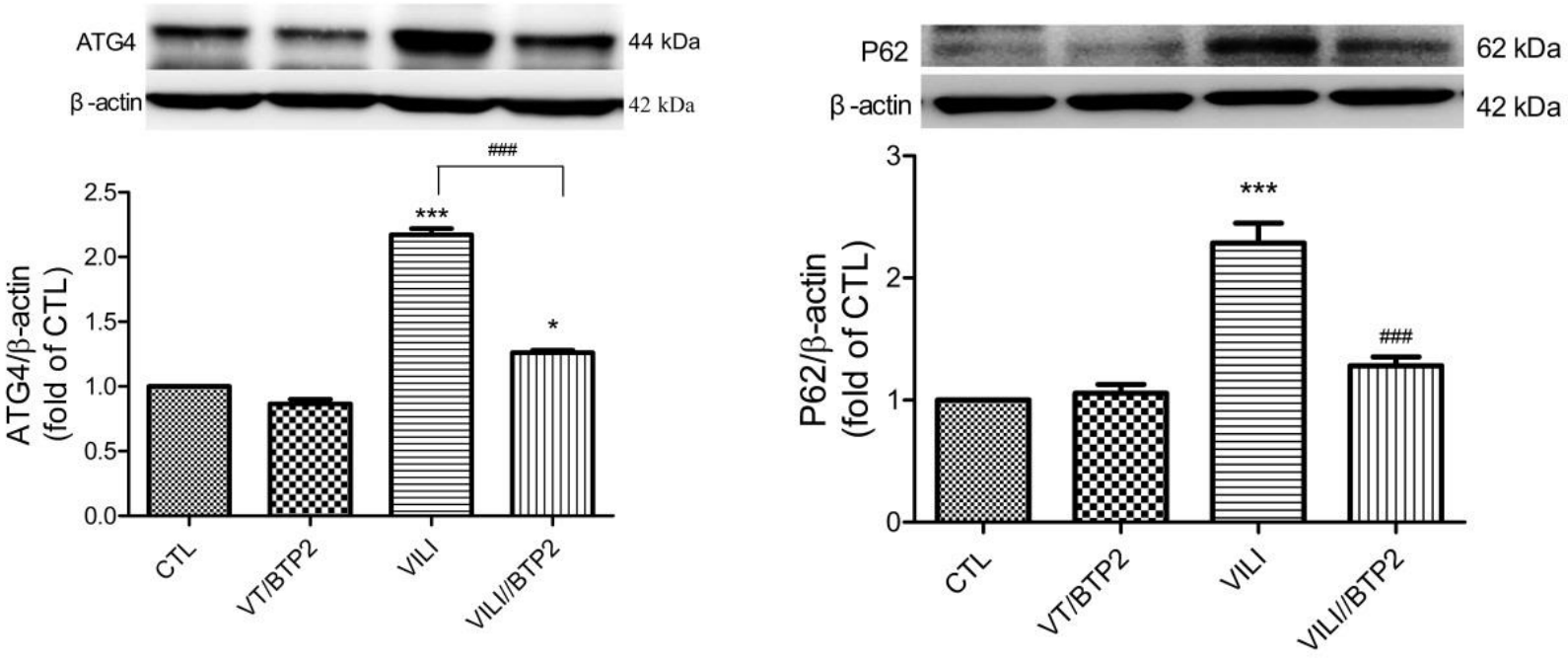
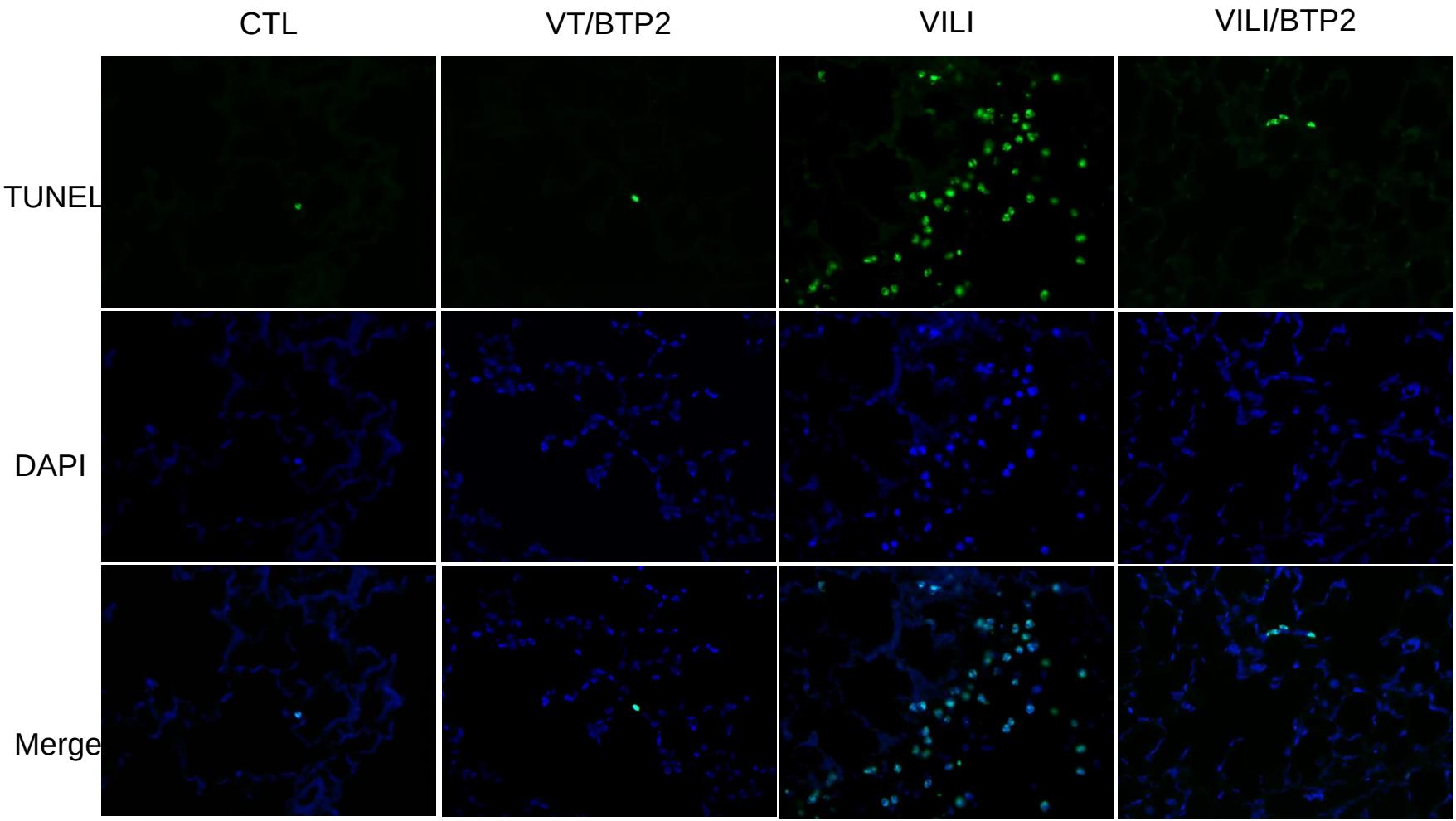


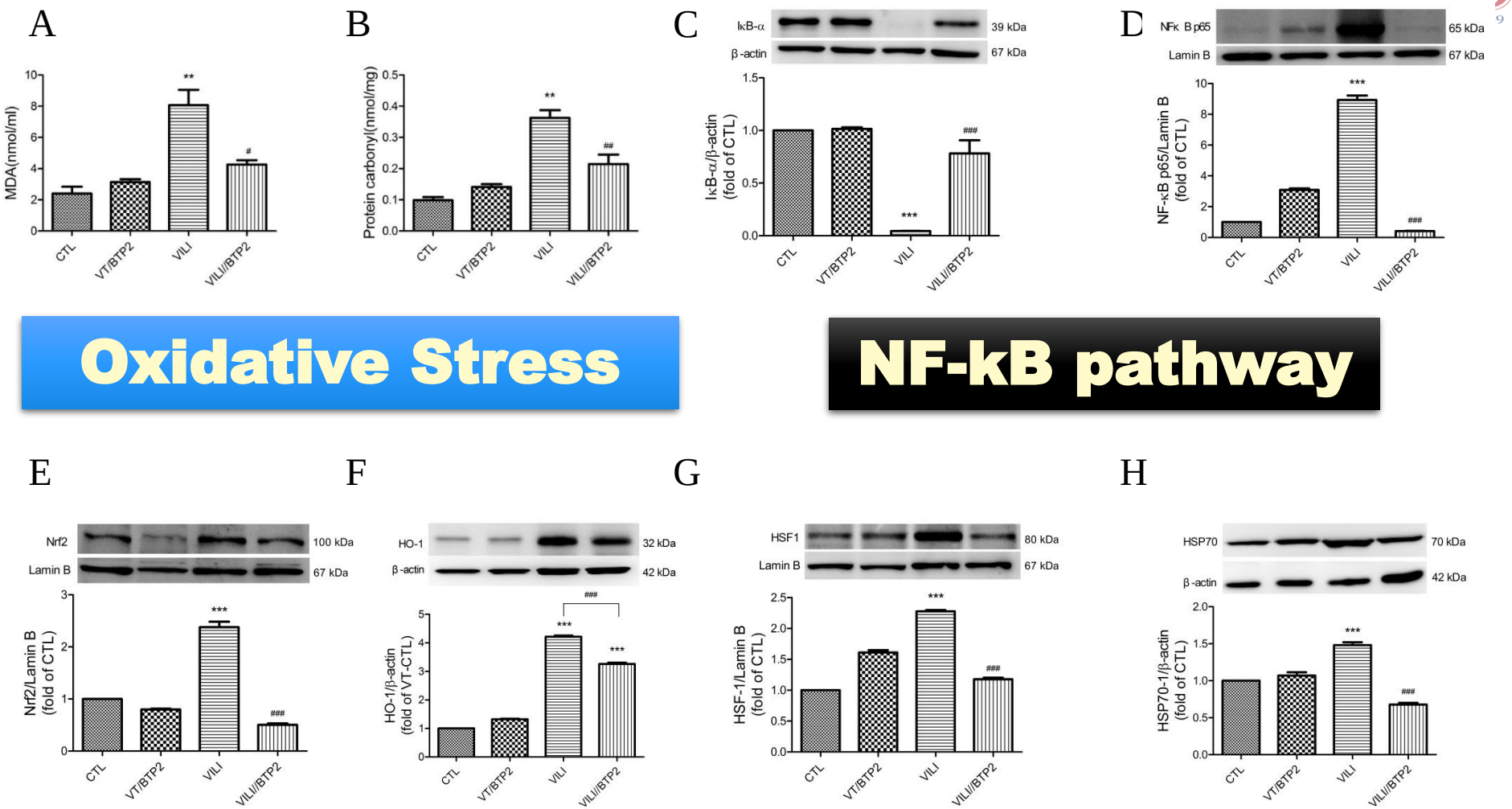
Fig. 3

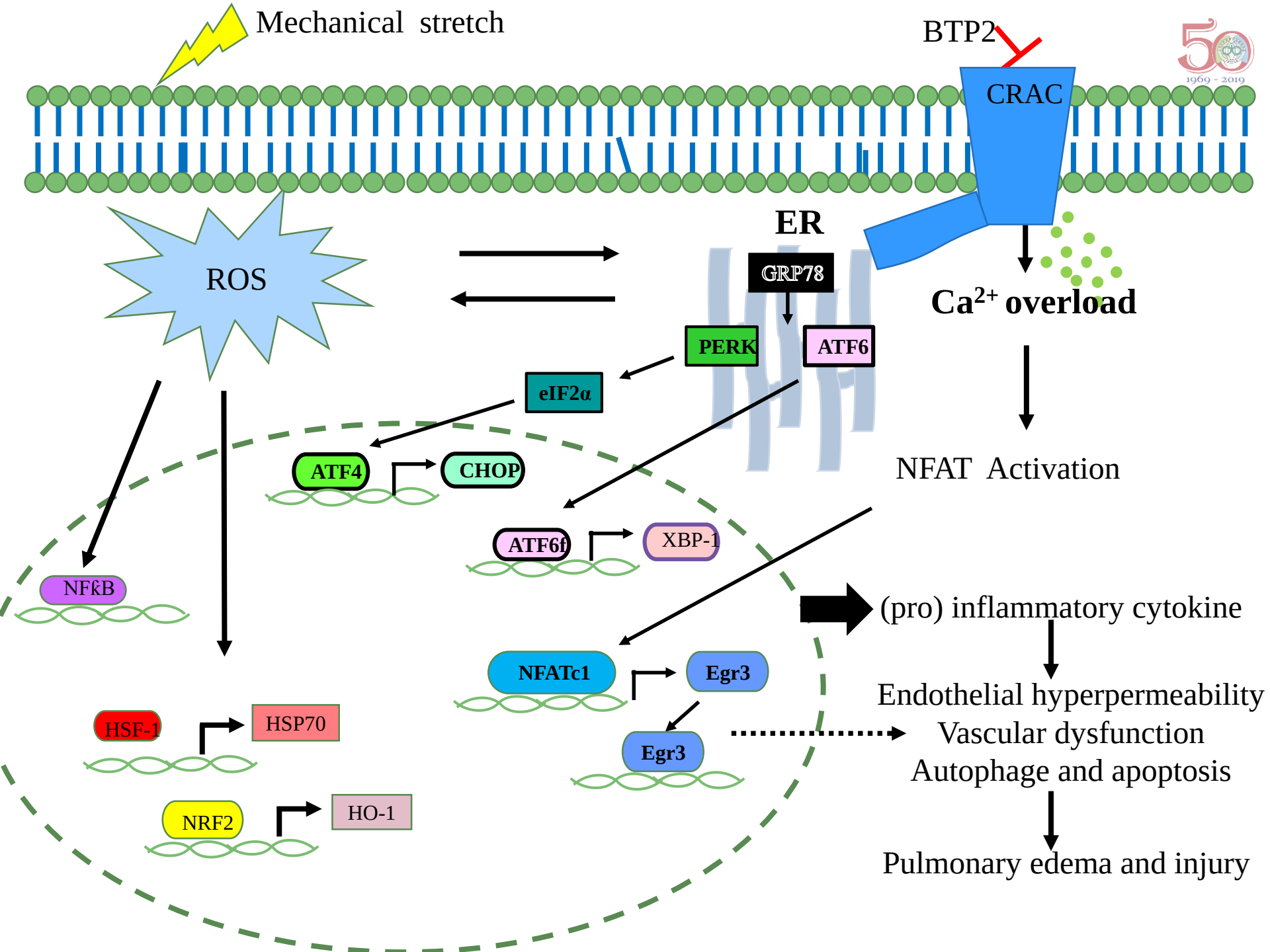
Lung injury: Apoptosis



TUNEL assay

Fig. 4


A
MDA(nmol/ml)
CTL
VT/BTP2
VILI
VILI/BTP2
B
Protein carbonyl(nmol/mg)
C
IκB-α
β-actin
39 kDa
67 kDa
IκB-α/β-actin (fold of CTL)
D
NFκ B p65
Lamin B
65 kDa
67 kDa
NF-κB p65/Lamin B (fold of CTL)
Oxidative Stress
NF-kB pathway
E
Nrf2
Lamin B
100 kDa
67 kDa
Nrf2/Lamin B (fold of CTL)
F
HO-1
β-actin
32 kDa
42 kDa
HO-1/β-actin (fold of VT-CTL)
G
HSF1
Lamin B
80 kDa
67 kDa
HSF-1/Lamin B (fold of CTL)
H
HSP70
β-actin
70 kDa
42 kDa
HSP70-1/β-actin (fold of CTL)
Stress Response Proteins
NRF2/HO-1 and HSF-1/HSP70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BTP2
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ATF4
CHOP
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NFATc1
Egr3
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HSF-1
HSP70
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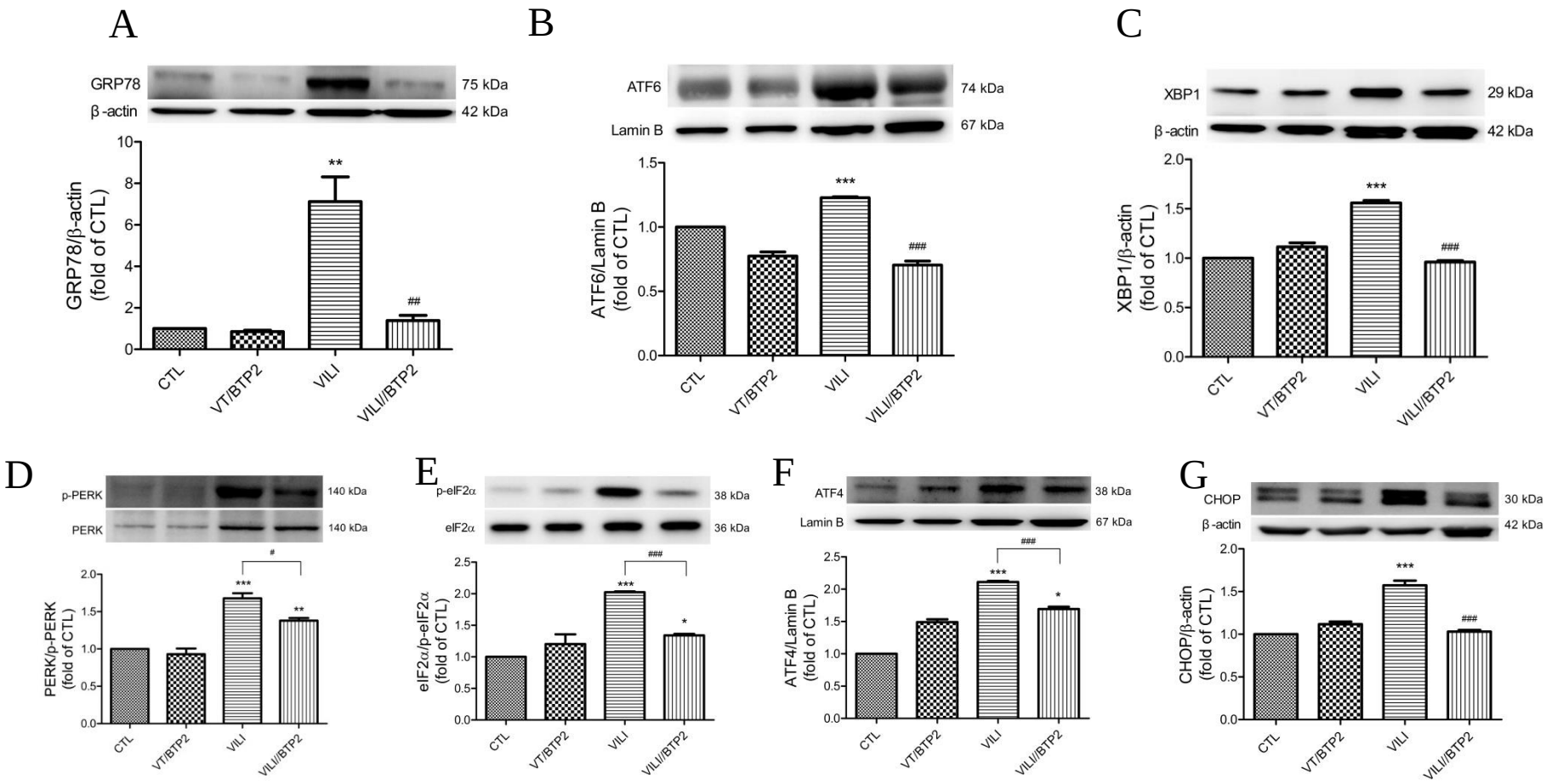
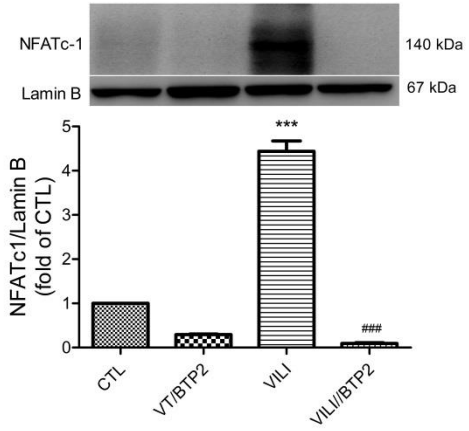
HO-1
Endothelial hyperpermeability
Vascular dysfunction
Autophage and apoptosis
Pulmonary edema and injury

Fig. 5

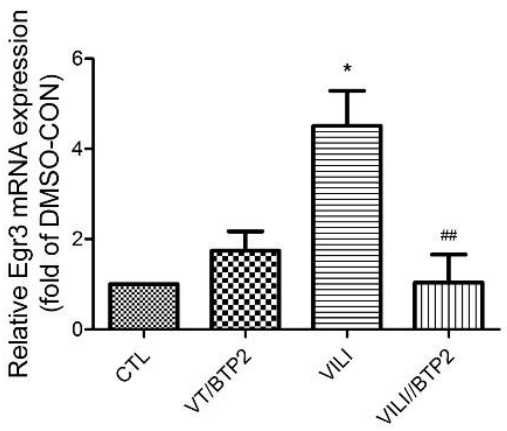


Lung injury: ER stress

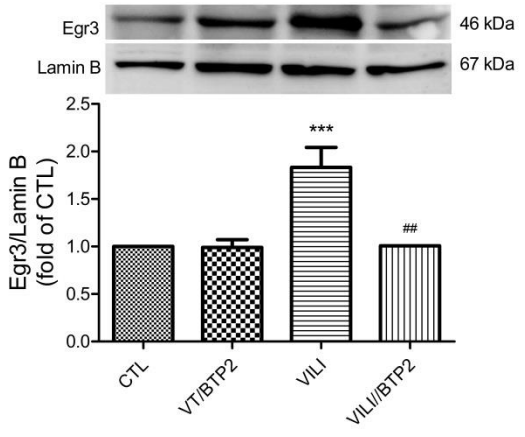
A



B

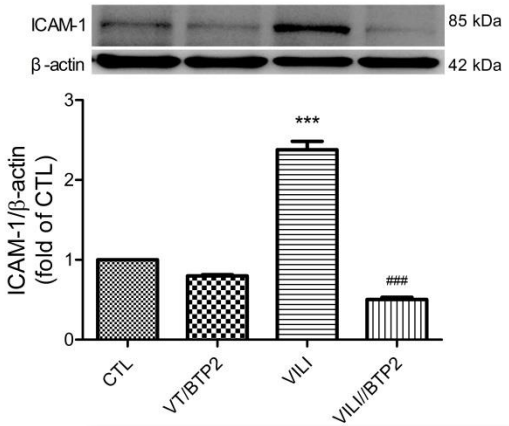


C

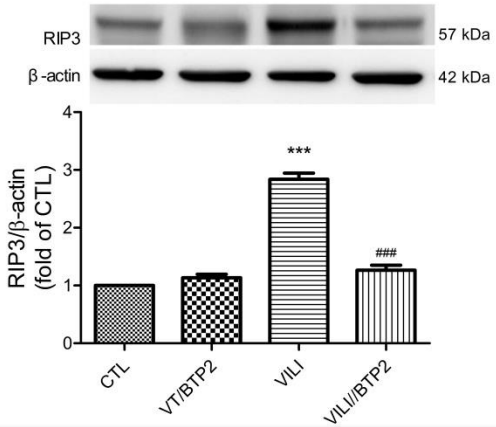


NFATc-1/Egr-3 pathway

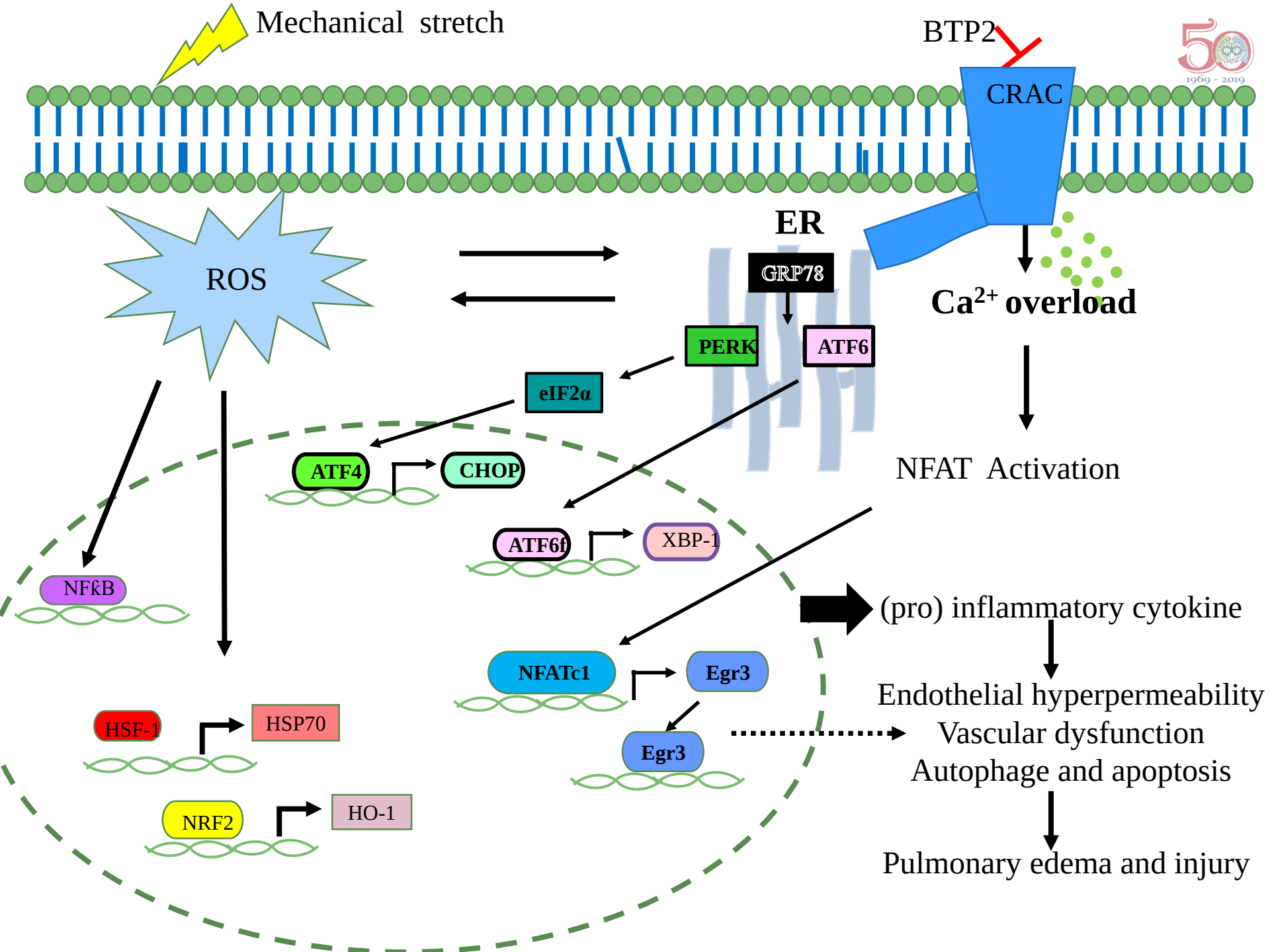
D



E



Endothelial cells injury markers


Mechanical stretch
BTP2
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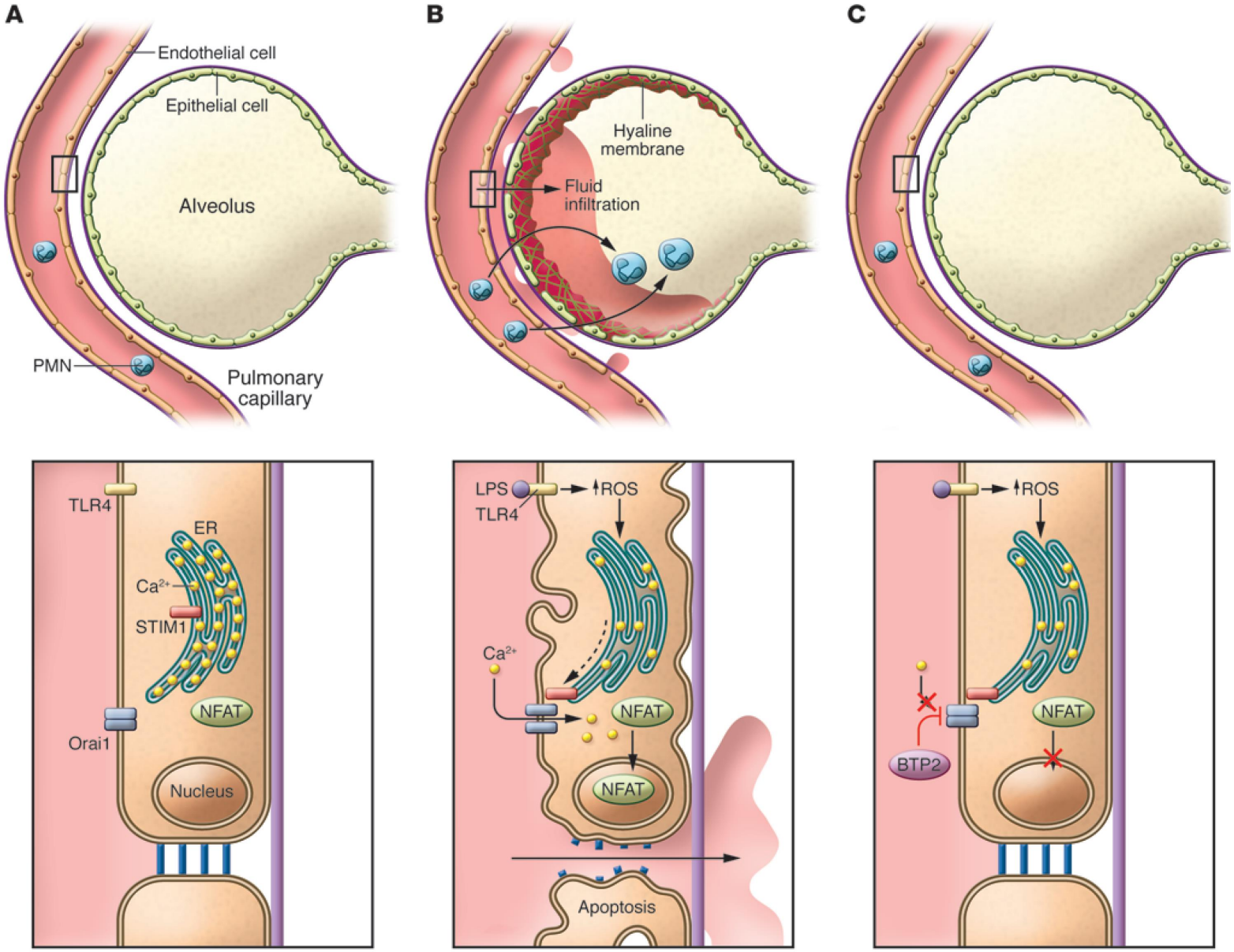
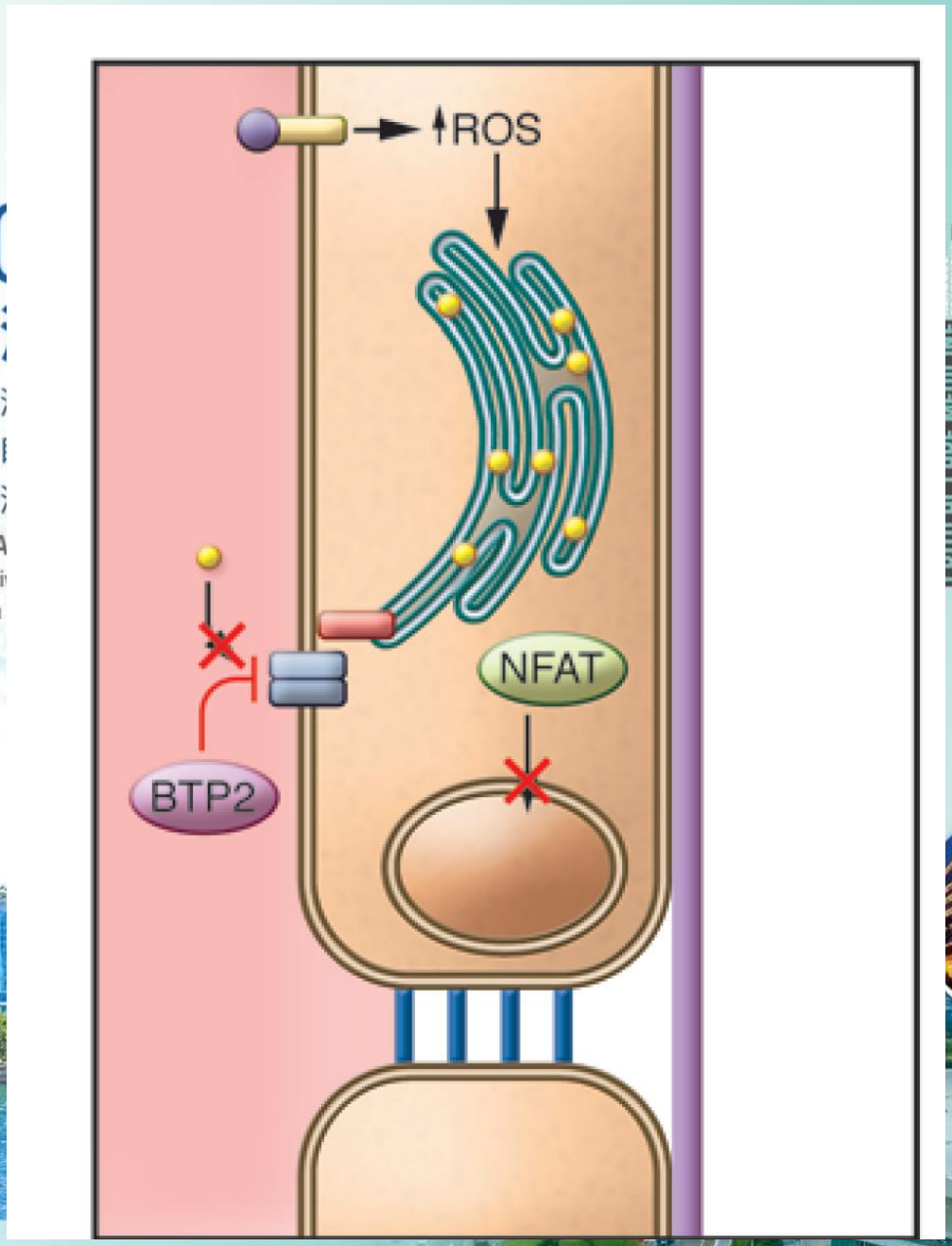
NFκB
(pro) inflammatory cytokine
NFATc1
Egr3
Egr3
HSF-1
HSP70
NRF2
HO-1
Endothelial hyperpermeability
Vascular dysfunction
Autophage and apoptosis
Pulmonary edema and injury

Species	CRAC channel inhibitory activity	Selectivity	Proposed mechanism of action
Lanthanides	Complete blockade at submicromolar concentration range [63]	Block other cationic ion channels, such as voltage-gated calcium channels and TRP channels [70,71]	Directly block ORAI1 [69]
SKF-96365	IC_{50}: 12 μM (I_{CRAC}) [72]	Suppresses voltage-gated calcium channels, nonselective cation channels and cyclic AMP-gated Cl^- channels [72,73]	Has not yet been fully clarified
2-APB	Activates at low micromolar and inhibits at high micromolar [75,76]	Affect the activities of potassium channels, SERCA pumps, heat-gated recombinant TRPV1, TRPV2 and TRPV3 channels [90–92]	Might act on STIM1 multimerization, STIM1–ORAI1 interaction or the ORAI channel itself [85,86]
DPB162-AE	IC_{50}: 200 nM [93]	Relatively selective [93,95–97]	Probably acts directly on the coupling interface between SOAR and ORAI1 [93,94]
BTP2	Inhibited thapsigargin-induced Ca^{2+} influx in Jurkat T cells with an IC_{50} of 100 nM [101]	Activates TRPM4 channels and inhibits the activities of TRPC3 and TRPC5 channels [105,106]	Has not yet been fully clarified
GSK-7975A	IC_{50}: 4 μM (I_{CRAC} in HEK293 cells) [111]	Potently blocks TRPV6 channels [111,113]	May act by altering the ORAI pore geometry [111]
Synta 66	IC_{50}: 1.4 μM (I_{CRAC} in RBL cells) [114,115]	Relatively selective [115]	Has not yet been fully clarified
ML-9	Reversibly inhibit SOCE with an IC_{50} of approximately 10 μM [116,117]	Inhibits MLCK [116]	Might target STIM1 [117]
DES	IC_{50}: 0.6 μM (I_{CRAC} in RBL cells) [118,119]	Activates estrogen receptors [118]	Might act on the extracellular regions on CRAC channel [118,119]
CAI	IC_{50}: ~0.5 μM (I_{CRAC} in HEK293 cells) [120,121]	Not very selective	Reduces the production of IP3 and depolarizes mitochondria [123–125]
RO2959	IC_{50}: 400 nM (I_{CRAC} in RBL-2H3 cells) [126]	Relatively selective [126]	Has not yet been fully clarified
Linoleic acid	Inhibit antigen- or thapsigargin-mediated SOCE in mast cells by acute addition at micromolar concentrations [127]	Has not yet been examined	Inhibits SOCE by affecting STIM1 oligomerization and subsequent STIM1/ORAI1 coupling [127]
1-phenyl-3-(1-phenylethyl) urea	Inhibits Ca^{2+} influx with IC_{50} of ~3 μM in HEK293 cells [128]	Has not yet been examined	Targets ORAI1 [128]

Future Med. Chem. (2016) 8(7), 817–832



J Clin Invest. 2013;123:1015-8


ROS
NFAT
BTP2

1969 - 2019

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暨台灣胸腔外科醫學會、台灣呼吸治療學會、
台灣睡眠醫學學會聯合學術會議
暨台灣胸腔暨重症加護醫學會第17屆第3次會員大會

2019 Annual Congress of Taiwan Society of Pulmonary and Critical Care Medicine
And Taiwan Society of Thoracic Surgeons, Taiwan Society for Respiratory Therapy,
Taiwan Society of Sleep Medicine Joint Conference

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恭請指教