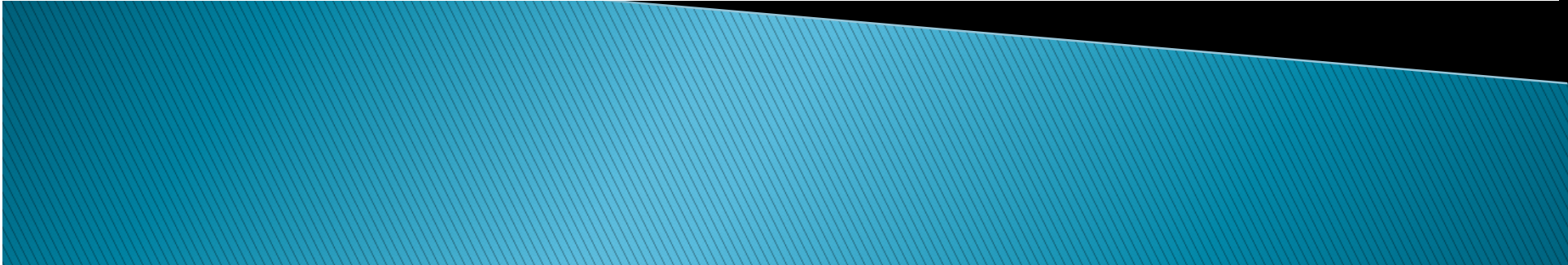


Resveratrol attenuates oxidized LDL-evoked Lox-1 signaling and consequently protects against apoptotic insults to cerebrovascular endothelial cells

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- ▶ B101099139 李佳駿 → introduction
- ▶ B101099047 黃冠閔 → results
- ▶ B101099101 曾士剛 → discussion & conclusion



Introduction

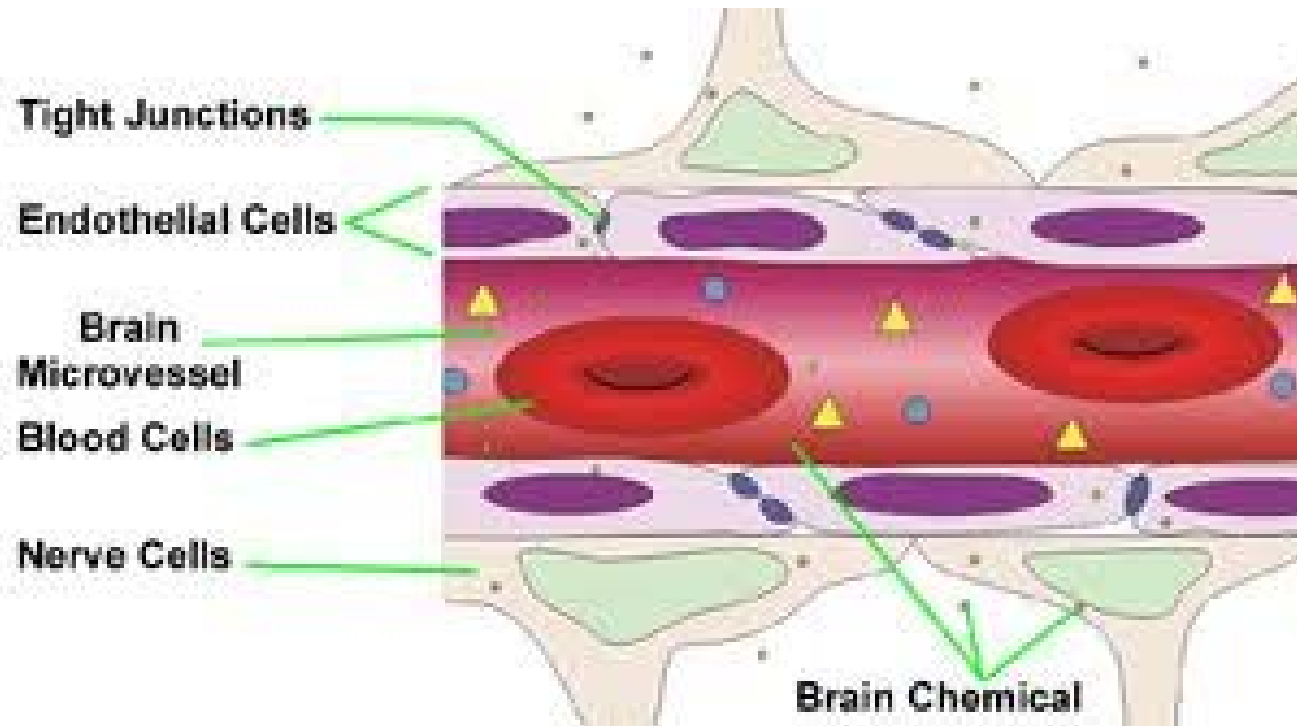


Oxidized low density lipoprotein (OxLDL)

- ▶ More harmful to body comparing with LDL
- ▶ Damage to:
 - Endothelial Cells
 - Cardiac myocytes
 - Smooth muscle cells
 - Platelet endothelium interaction
 - Macrophages
 - Kidney
 - Chondrocytes
 - **BBB: endothelial cell**

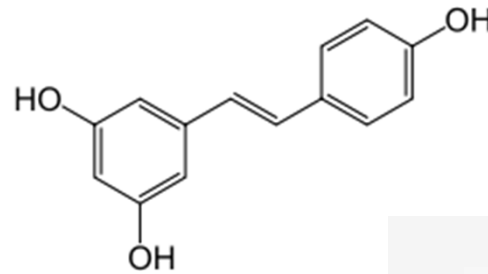


Blood Brain Barrier



- ▶ OxLDL → ECs apoptosis → Homeostatic environment of brain → Brain damage

Resveratrol



- ▶ Life extension *:
 - Worm, fruit fly, short-lived fish
- ▶ Cancer prevention:
 - Skin and GI tumor
- ▶ Cardioprotective effects:
 - eNOS activity ↑
 - Platelet aggregation ↓
 - LDL peroxidation ↓
- ▶ Antidiabetic effects
- ▶ Neuroprotective effects (RIMA)
 - Reversible inhibition of MAO-A
- ▶ Anti-inflammatory effects
 - Arachidonic acid ↓ COX2 ↓



Lox-1: lectin-type oxidized LDL receptor 1

- ▶ binds, internalizes and degrades oxidized low-density lipoprotein
- ▶ regulation of Fas-induced apoptosis
- ▶ atherosclerosis, risk of myocardial infarction, risk of Alzheimer's disease



Materials and Methods

- ▶ Isolation of mouse Cerebrovascular Ecs (CECs):
 - Trypsin, collagenase B, centrifuge
- ▶ LDL and OxLDL isolation
 - Human blood → LDL , + Cu_2SO_4 (48hrs)
- ▶ ROS quantification
 - ROS sensitive Dye and flow cytometry
- ▶ DNA Ladder Analysis
 - Control and drug treated mouse CECs comparison



Method and Material

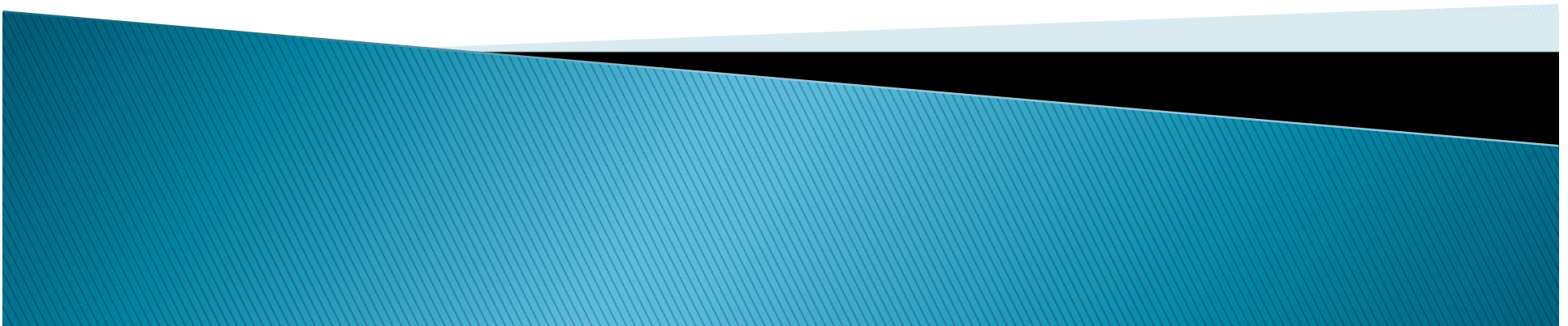
- ▶ Quantification of DNA Fragmentation
 - Anthos 2010 microplate photometer, 450 nm wavelength
- ▶ Analysis of apoptotic Cells
 - Flow cytometry
- ▶ Knockdown and over expression of Lox-1
 - RNAi and siRNA
 - Plasmid with Lox-1 cDNA



- ▶ Immunoblot and RT-PCR
- ▶ Statistical Analysis
 - Student's t test $p < 0.05$



Results



Toxicity of Resveratrol to Mouse Cerebrovascular Endothelial Cells

- ▶ No adverse effect under the concentrations of 0.5, 1, 5, and 10 $\mu\text{mol/L}$ resveratrol for 24, 48, and 72 hours
- ▶ After exposure for 72 hours, resveratrol at 25 $\mu\text{mol/L}$ caused 30% decrease in cell viability
- ▶ When concentration reached 50 $\mu\text{mol/L}$, administration of resveratrol for 48 and 72 hours decreased the viability of mouse CECs by 44% and 59% , respectively

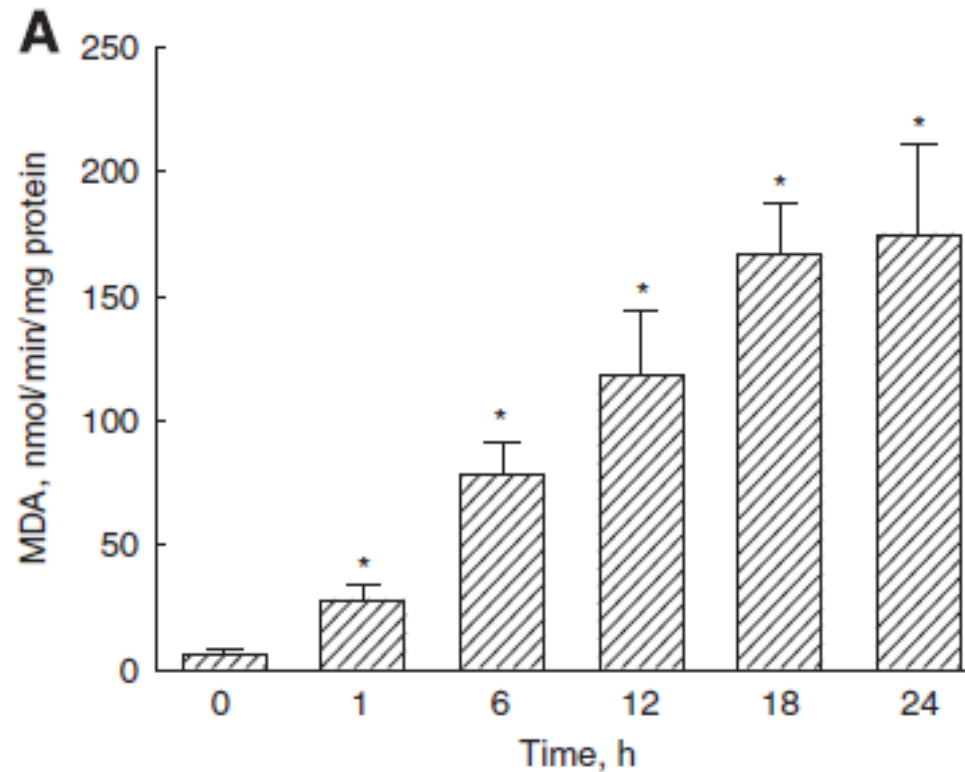
No adverse effect observed below the concentration of 10 $\mu\text{mol/L}$



Resveratrol Suppresses oxLDL and ROS Production

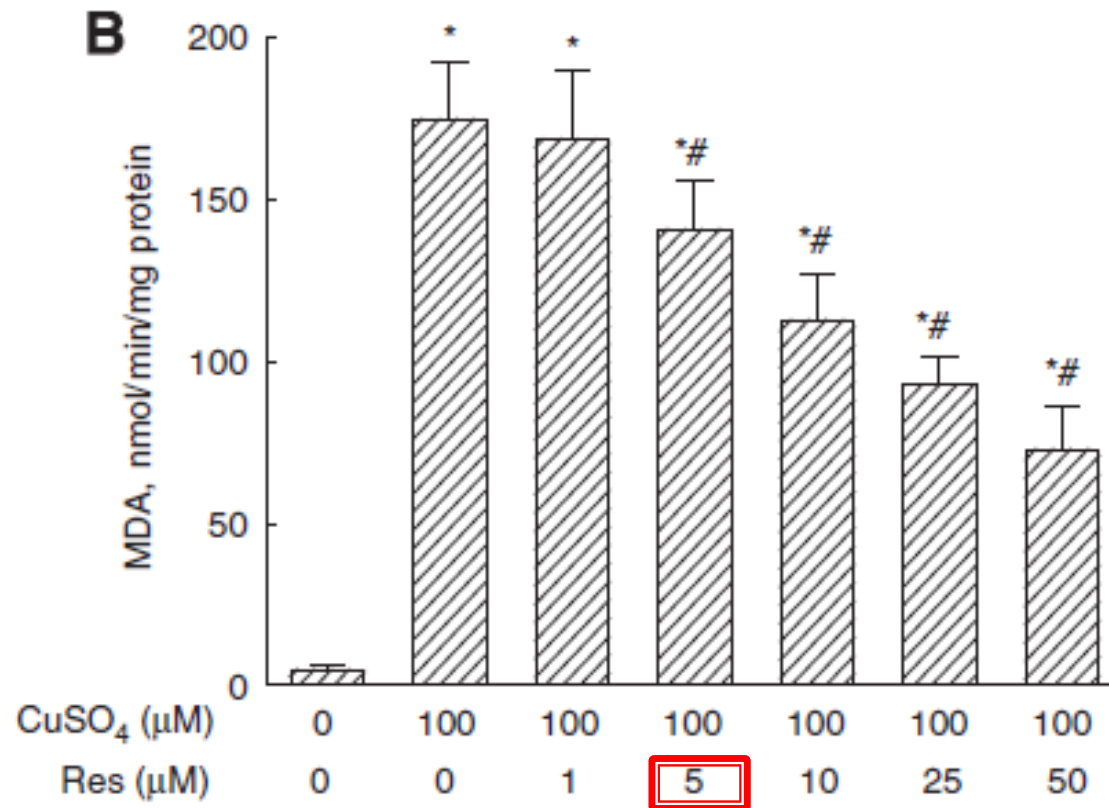


Positive correlation of Concentration of oxLDL and reaction time



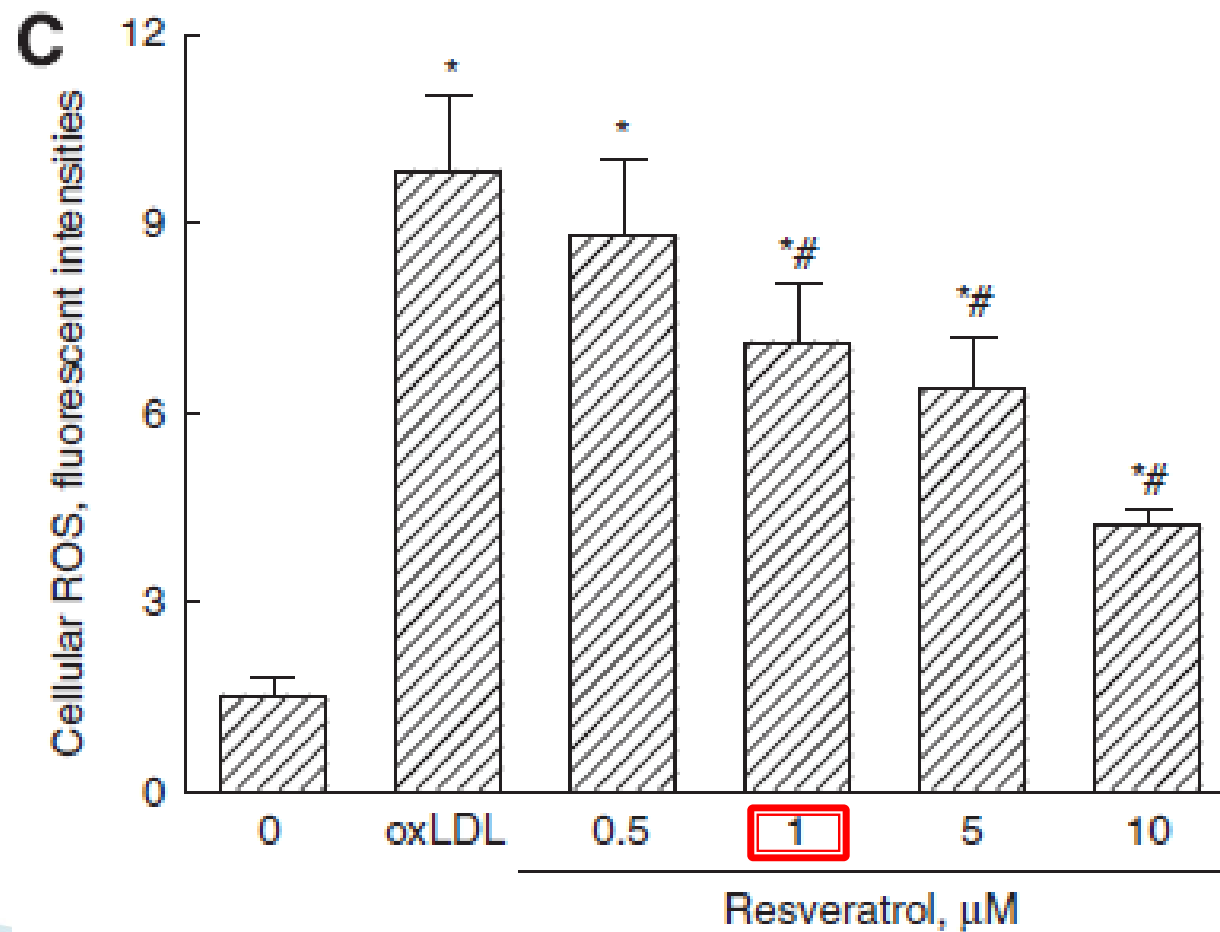
LDL was reacted with copper sulfate for 1, 6, 12, 18, and 24 hours
Formation of MDA (which implies the increase of oxLDL)
increase as reaction time prolonged

Negative correlation of oxLDL concentration and Resveratrol concentration in vitro

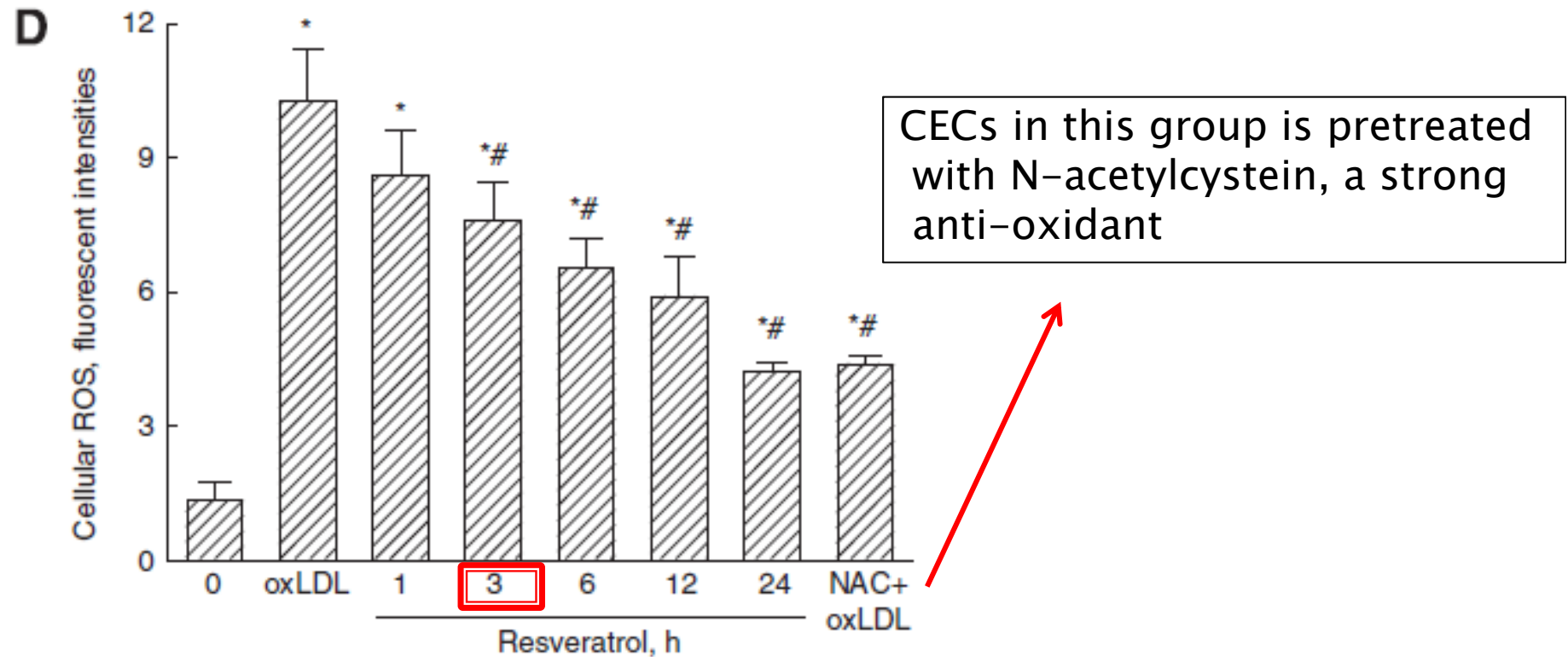


Level of oxLDL drop as the concentration of resveratrol increase

Negative correlation of Cellular ROS and Resveratrol concentration

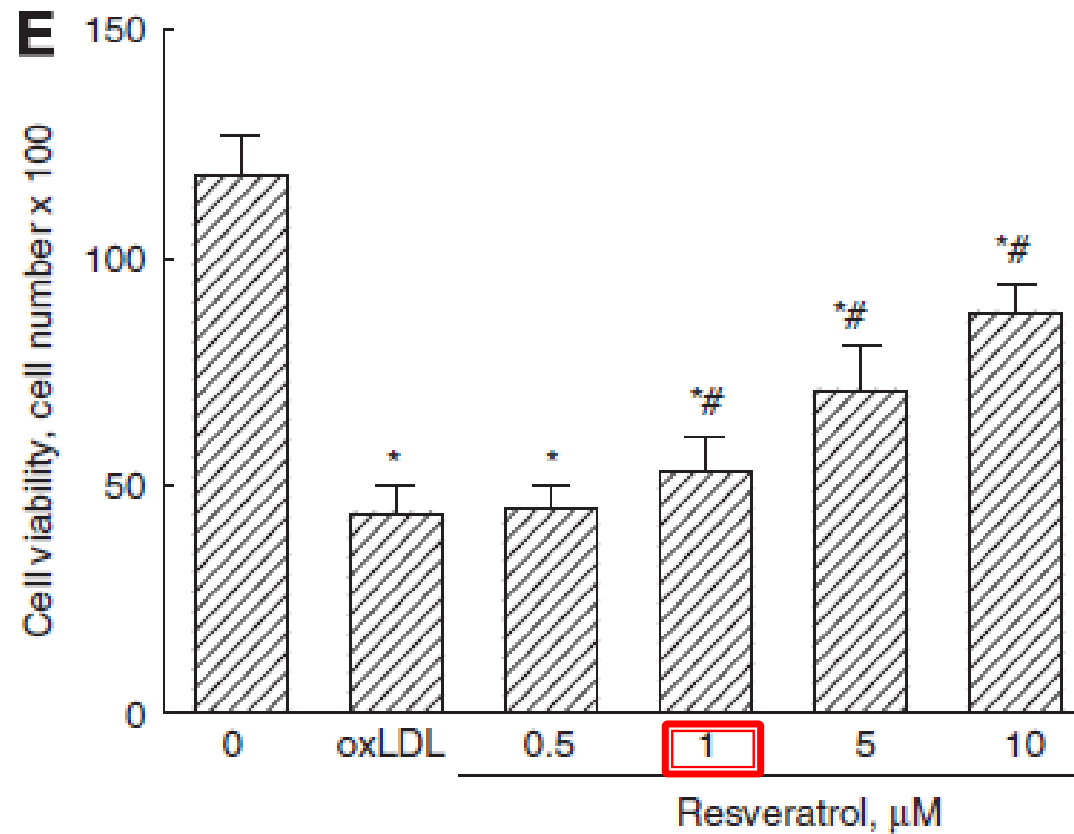


Negative correlation of Cellular ROS and the reaction time

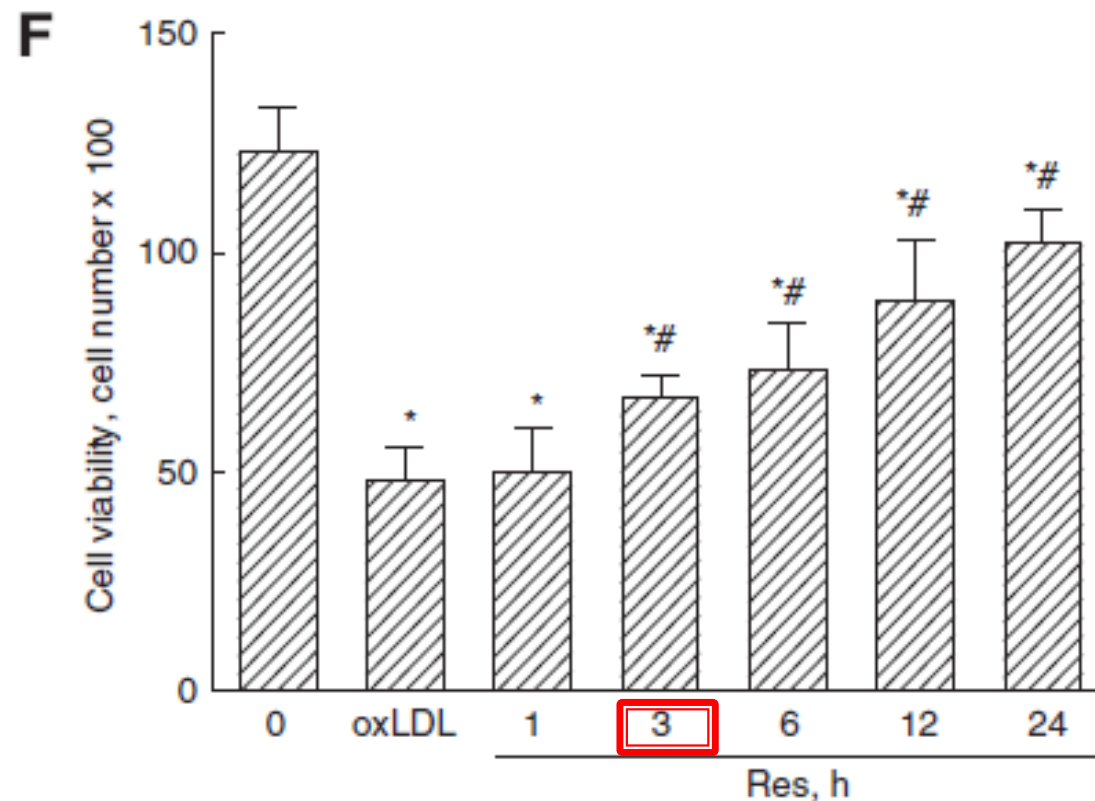


Concentration of resveratrol is 10μmol/L

Positive correlation of Cell viability and the concentration of resveratrol



Positive correlation of Cell viability and the reaction time



Concentration of resveratrol is 10 μ mol/L

Table 1 Effects of pretreatment and posttreatment with resveratrol (Res) on protection against oxLDL-induced death of mouse CECs

<i>Treatments</i>	<i>Cell viability (OD values at 550 nm)</i>
Control	1.46 ± 0.08
oxLDL	$0.70 \pm 0.07^*$
Pretreatment with Res	1.38 ± 0.04
Pretreatment with Res+oxLDL	$0.85 \pm 0.09^*$
Posttreatment with Res	1.41 ± 0.08
Posttreatment with Res+oxLDL	$1.07 \pm 0.08^{*,\#}$

No significant difference oxLDL group and pretreatment with Res+oxLDL Pretreatment is not protective

Cell viability in post treatment with Res+oxLDL group is significantly less than control, yet higher than pretreatment with Res+oxLDL group

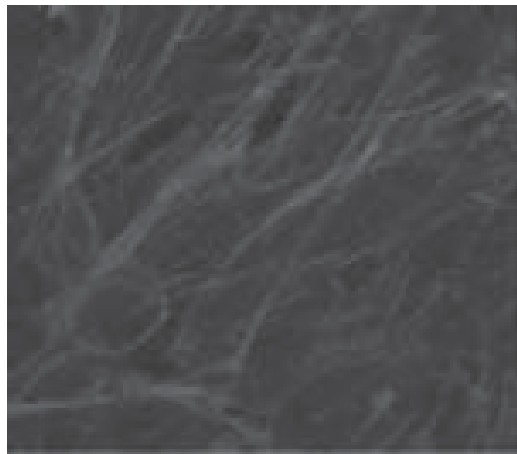
Protection Against oxLDL-Induced Insults to Mouse CECs by Resveratrol Occurs via an Antiapoptotic Mechanism



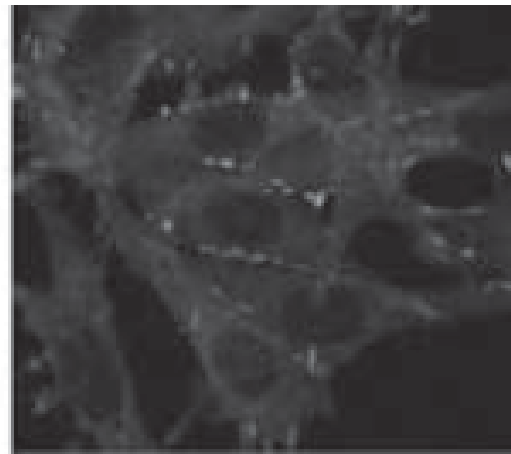
Expression of ZO-1 and Occludin in CECs

A

ZO-1



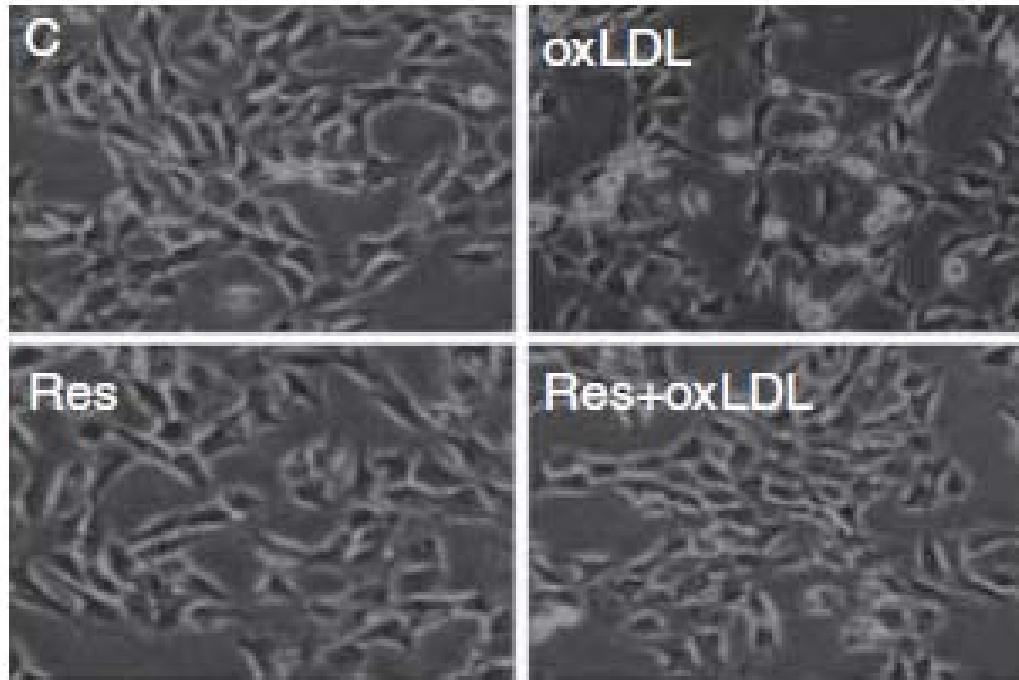
Occludin



These two proteins are essential for the formation of tight junction

Resveratrol protect CECs from oxLDL-induced morphological change

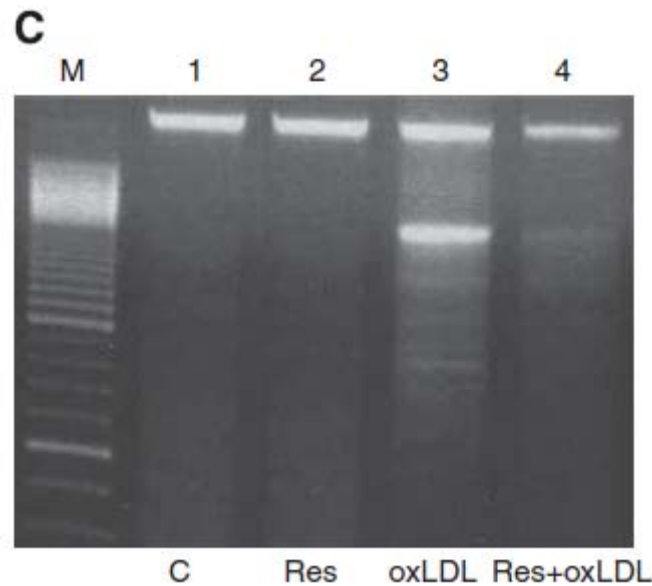
B



Apoptotic changes
(shrinkage of cell)
induced by oxLDL

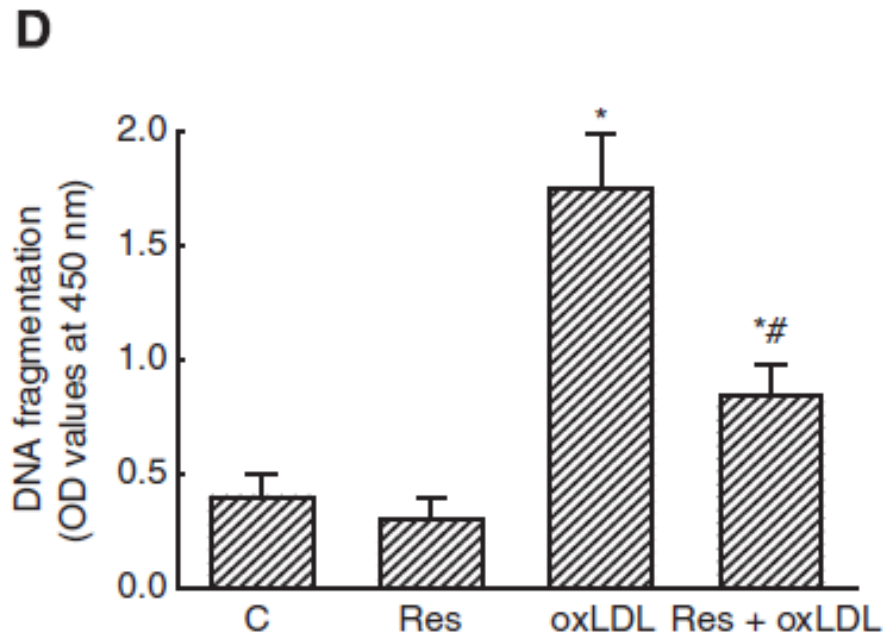


Resveratrol protect CECs from oxLDL-induced DNA fragmentation



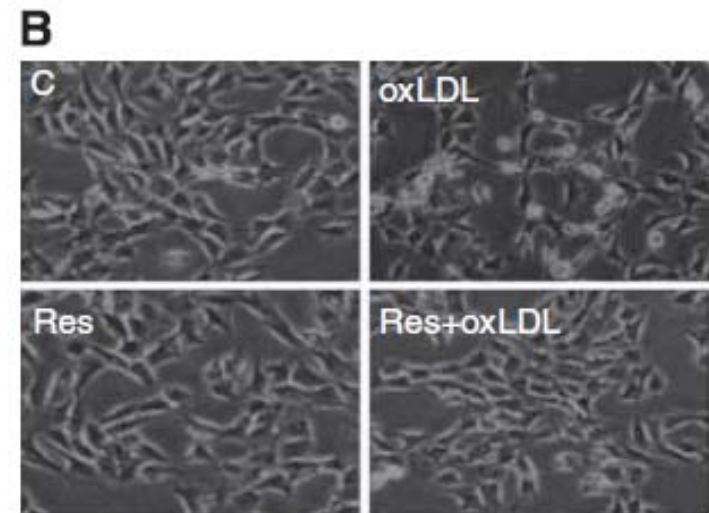
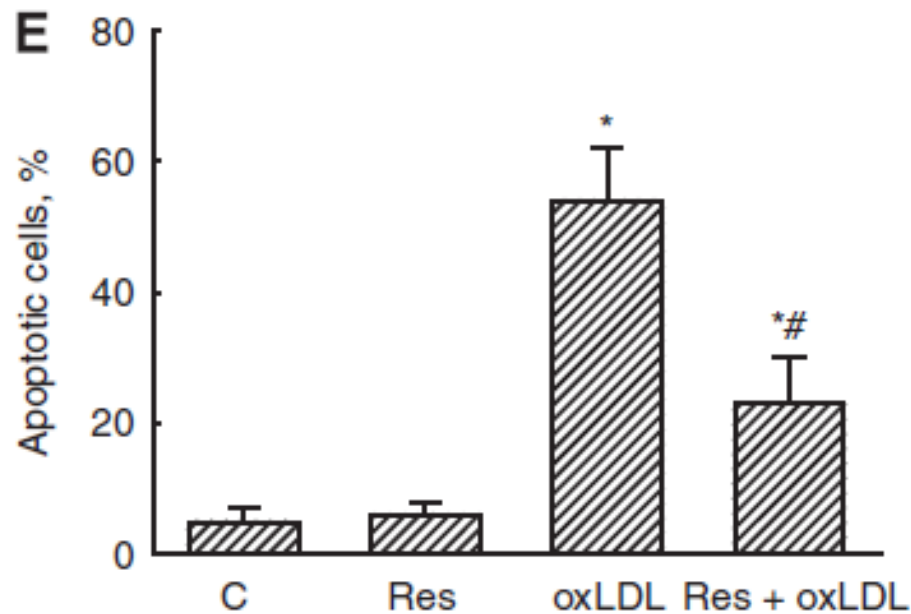
Fragmentation of DNA
(formation of DNA
ladder) induced by
oxLDL

Resveratrol protect CECs from oxLDL-induced DNA fragmentation



Fragmentation of DNA increase
OD values at 450nm

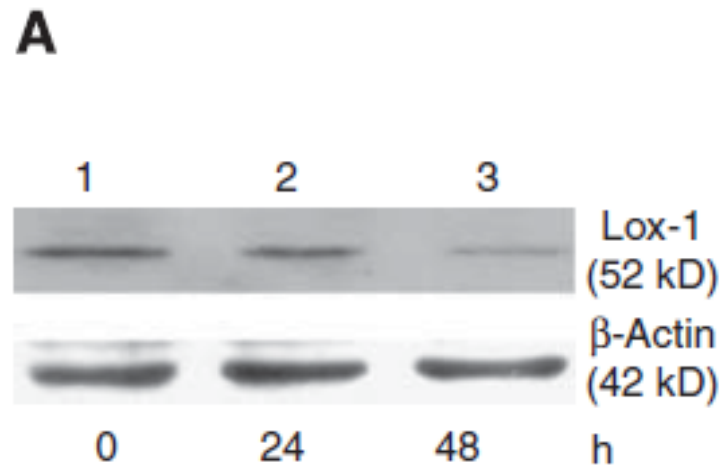
Resveratrol protect CECs from oxLDL-induced apoptosis



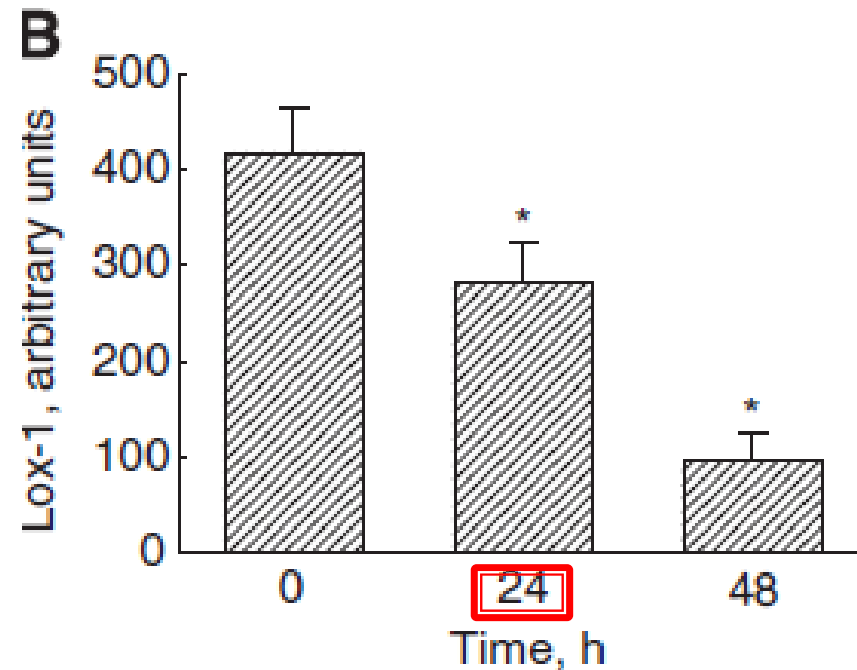
The Lox-1 Receptor Participates in Resveratrol-Involved Cell Protection



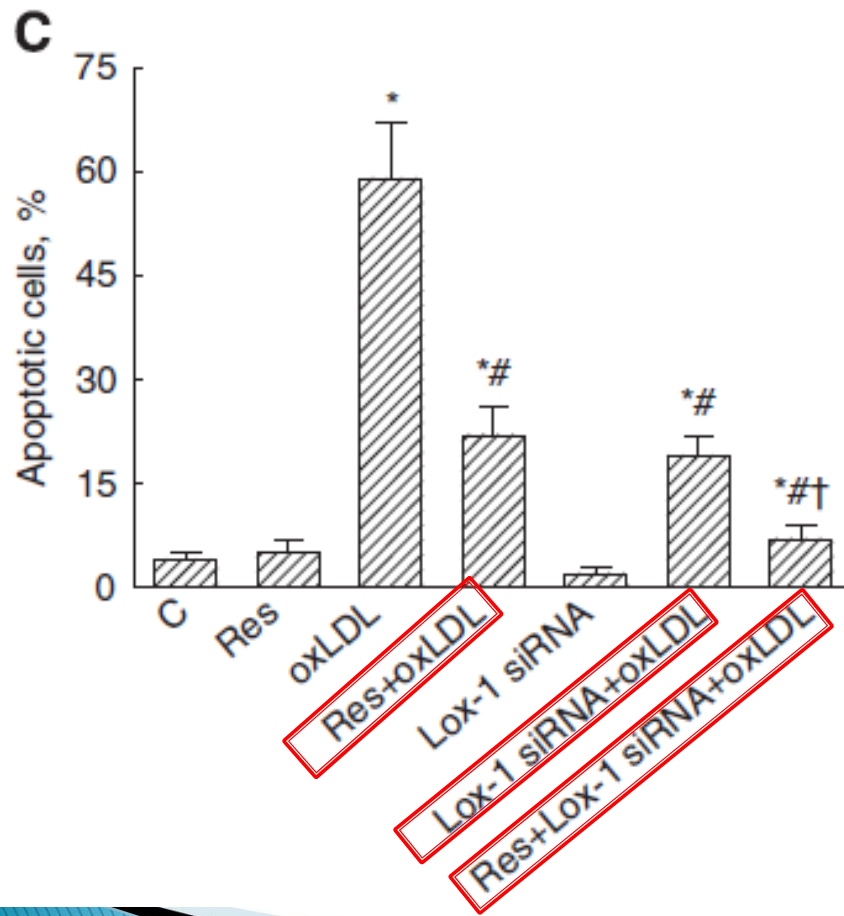
Application of Lox-1 siRNA decrease levels of Lox-1 receptor



β -actin serve as control

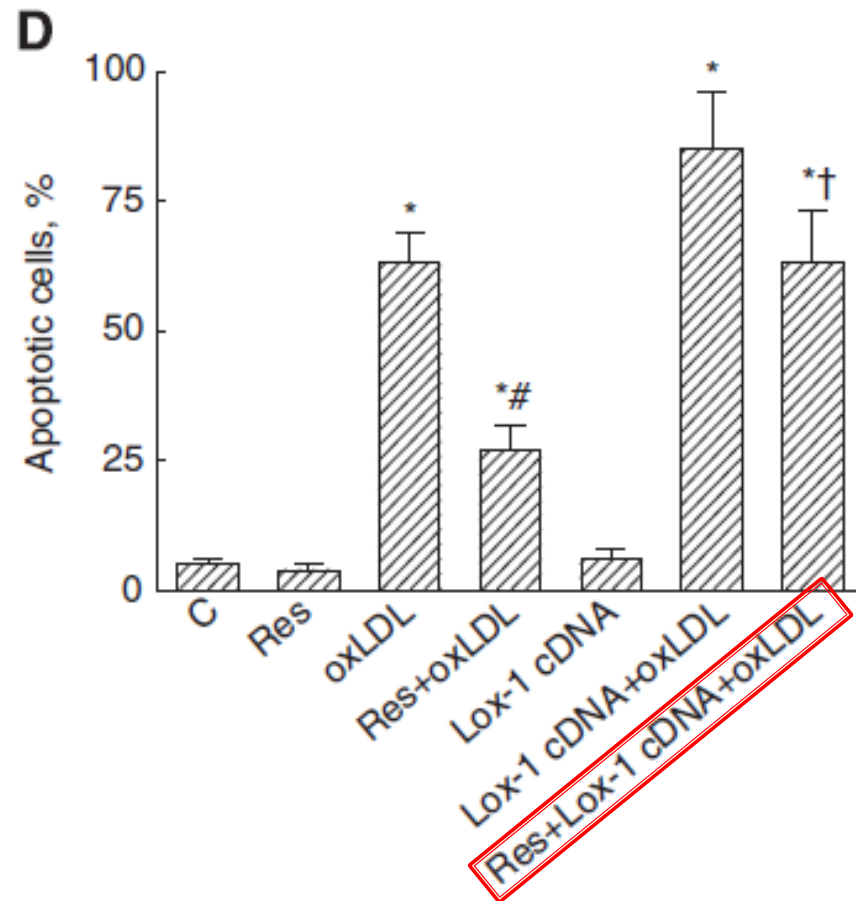


Resveratrol and Lox-1 siRNA protect CECs from oxLDL-induced apoptosis



More effect if co-treated with Resveratrol and Lox-1 siRNA

Resveratrol effectiveness decrease as Lox-1 receptor over-expressed

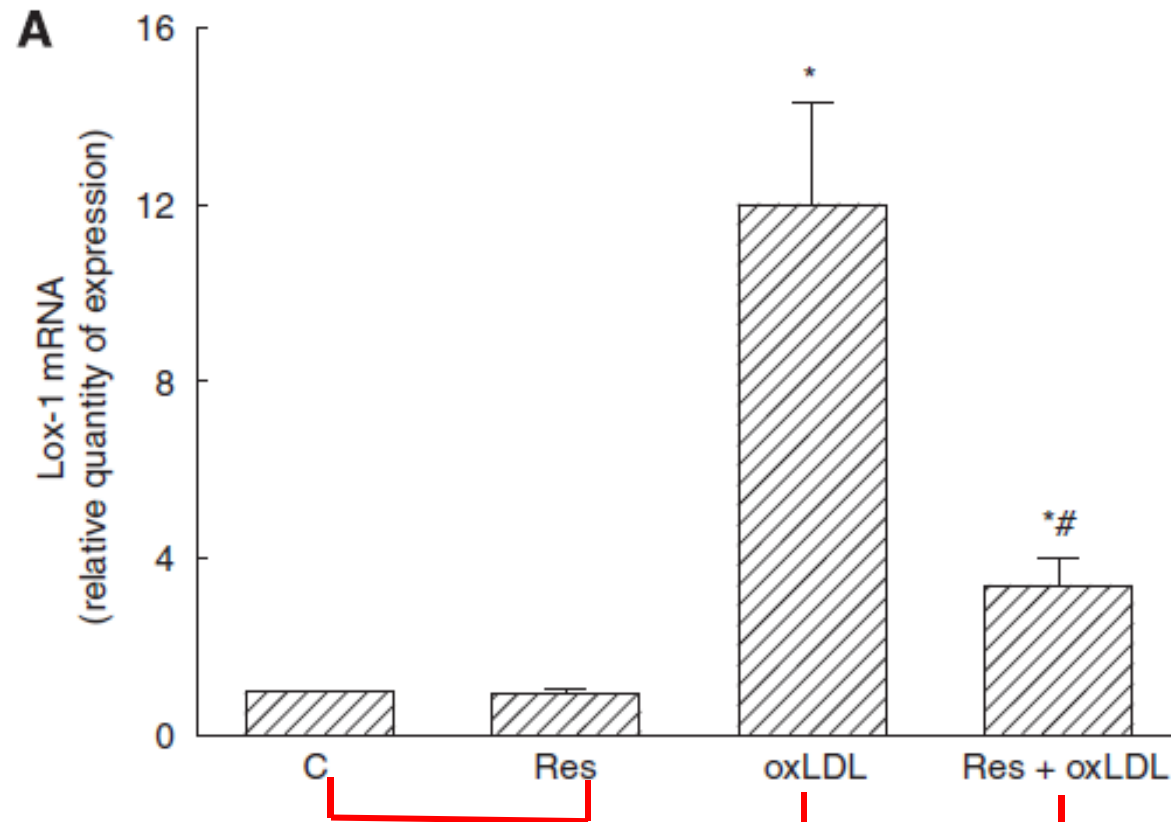


Lox-1 cDNA can increase the Production of Lox-1 receptor

Resveratrol Can Inhibit oxLDL- Induced Lox-1 mRNA and Protein Expressions



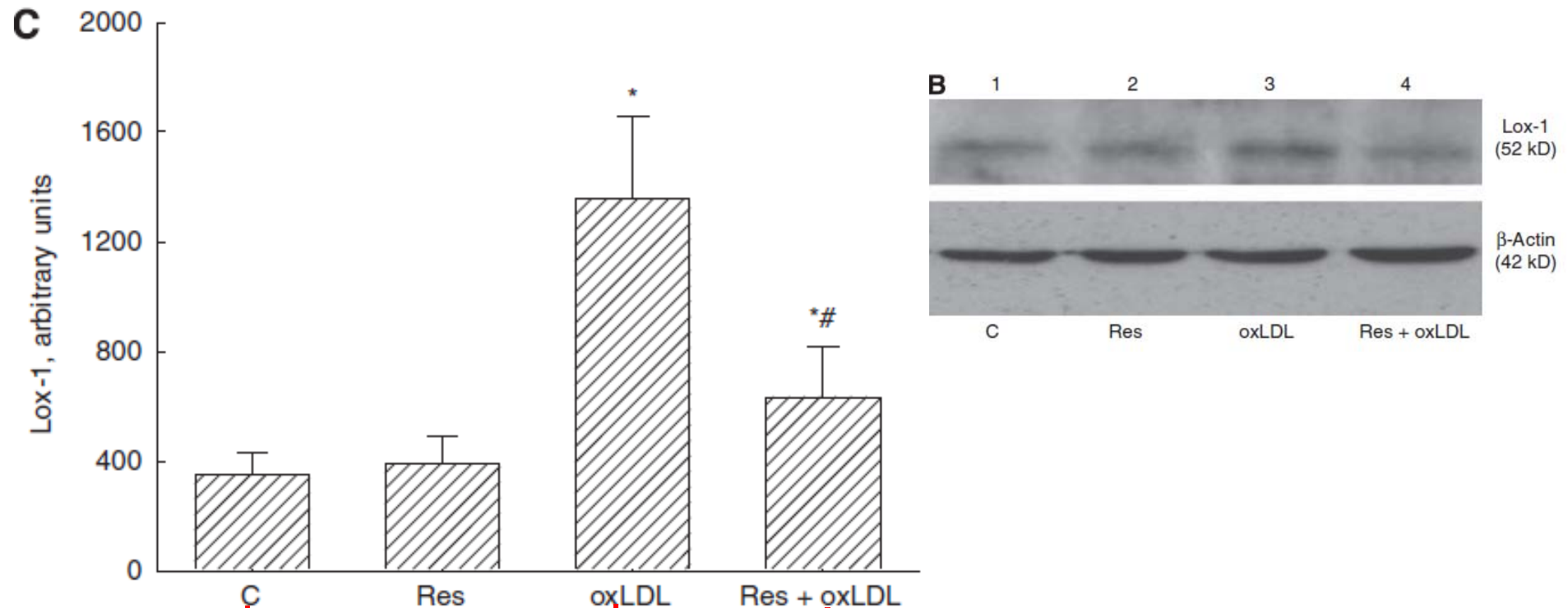
Resveratrol and the production of Lox-1 mRNA



Resveratrol alone doesn't decrease Lox-1 mRNA production

Lox-1 mRNA production decreased Significantly comparing these to group

Resveratrol and the production of Lox-1 protein



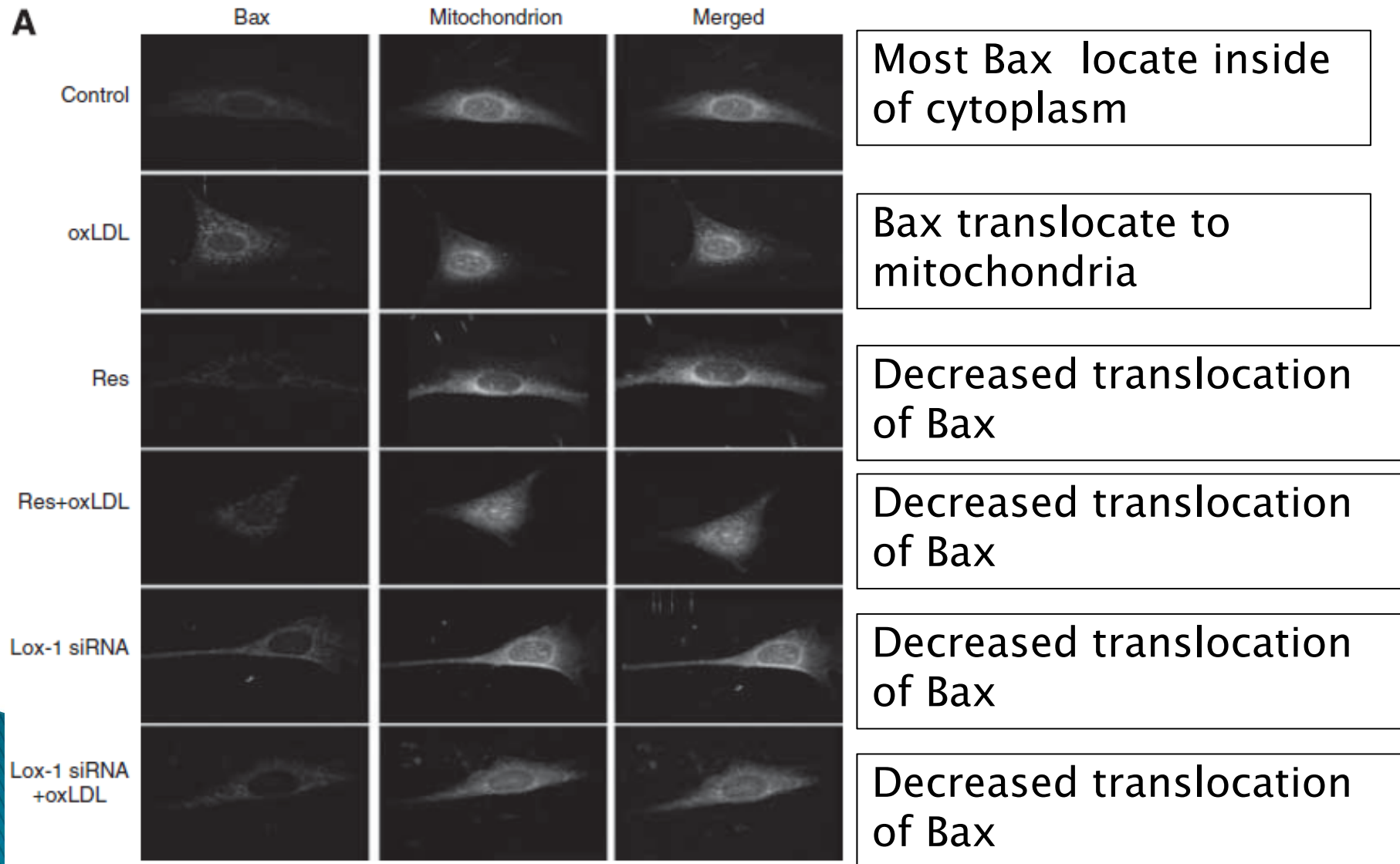
Resveratrol alone doesn't decrease Lox-1 protein production

Lox-1 protein production decreased Significantly comparing these to group

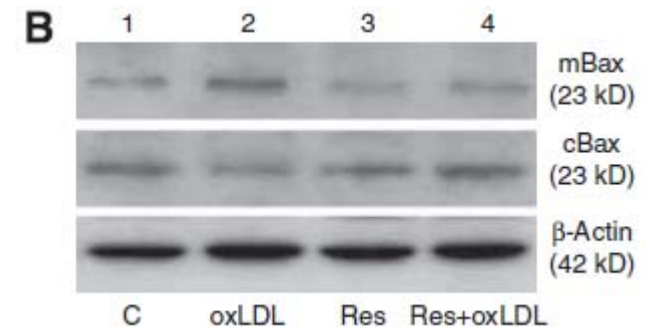
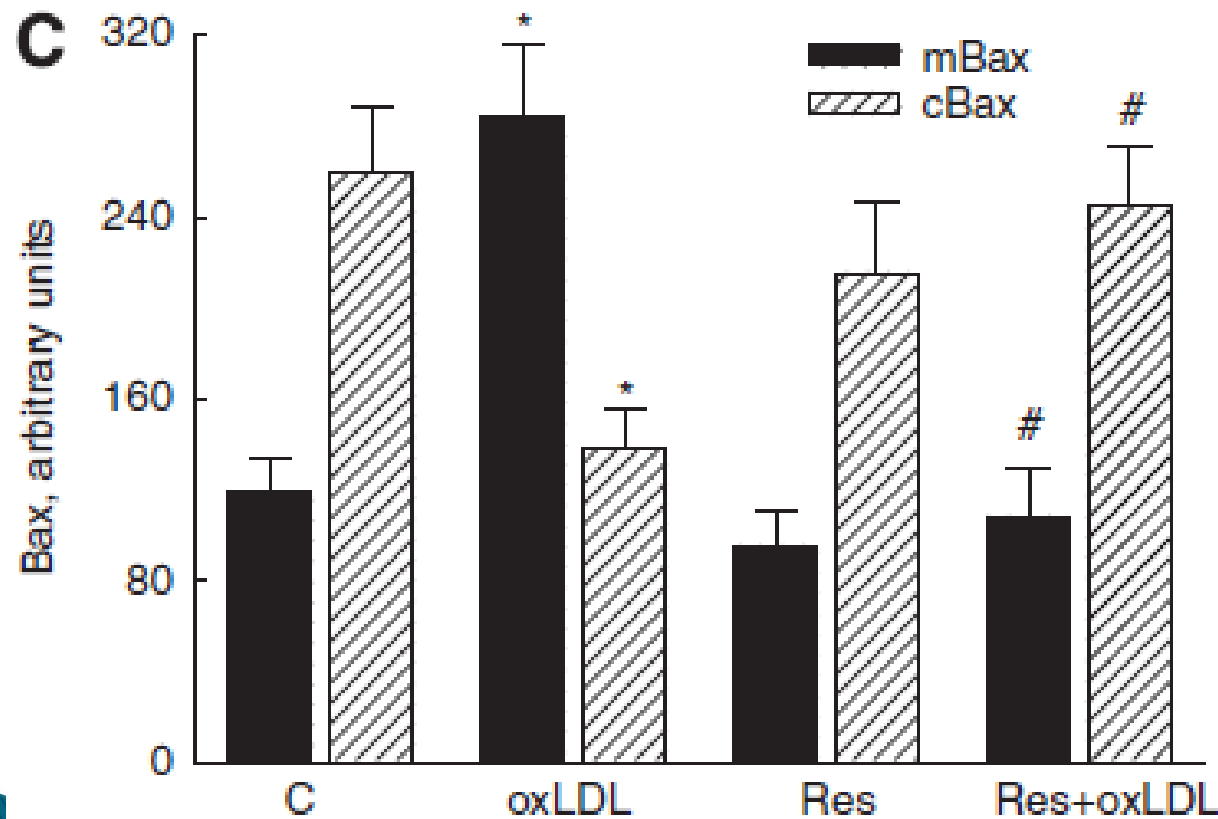
Resveratrol's Protection May Be Involved in Lox-1 Receptor-Mediated Bax Translocation



oxLDL induce translocation of Bax from cytoplasm into mitochondria



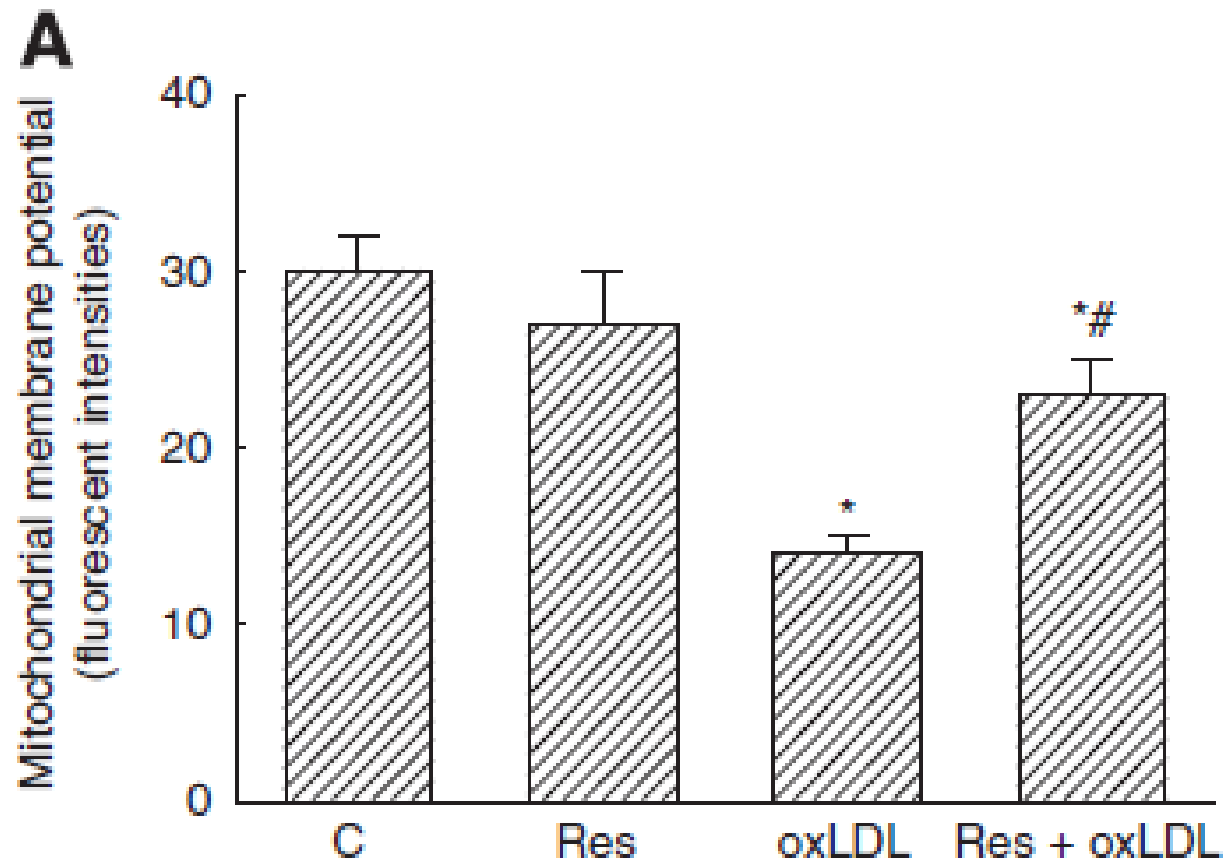
Affect of resveratrol on oxLDL induce translocation of Bax from cytoplasm into mitochondria



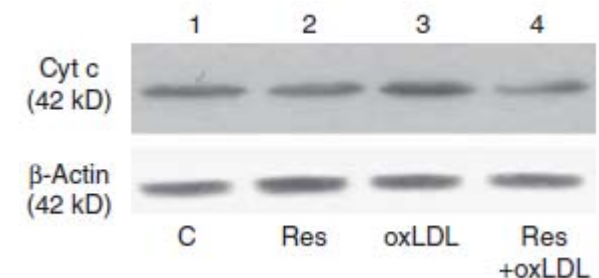
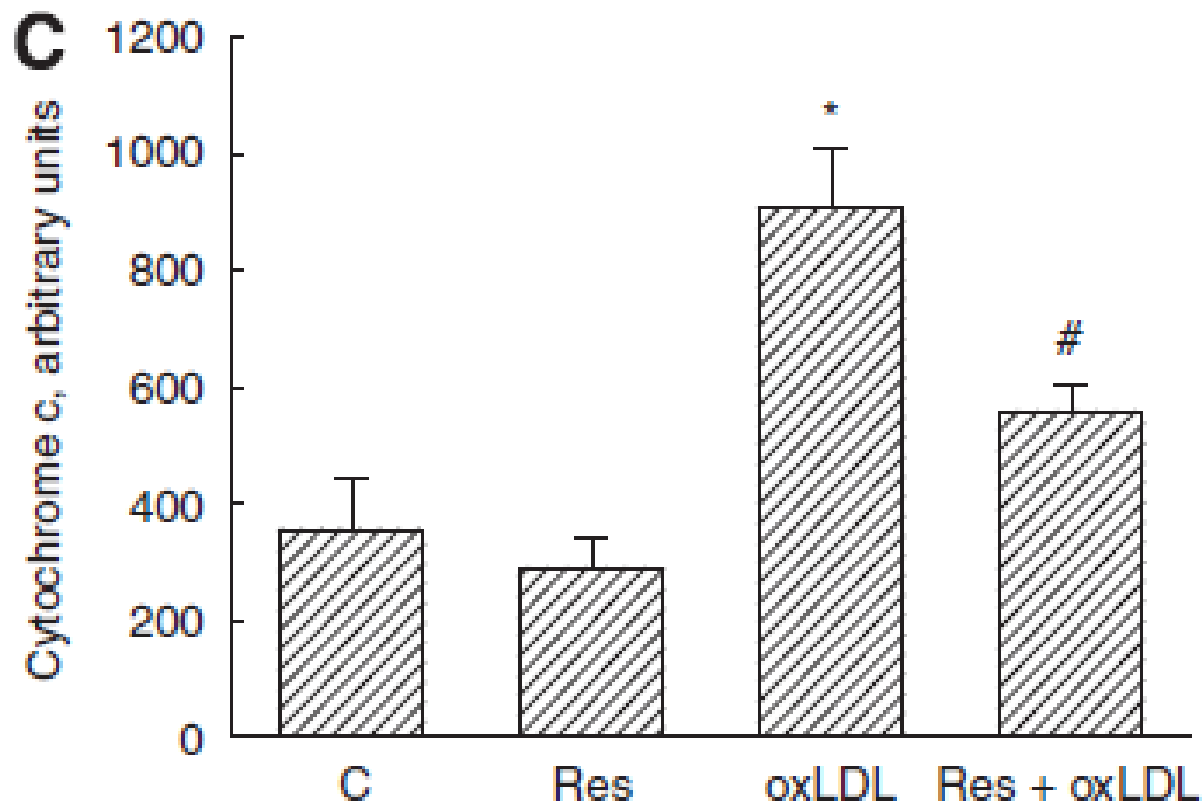
Resveratrol Sequentially Suppresses Activation of a Mitochondria–Cytochrome c– Caspase Protease Pathway



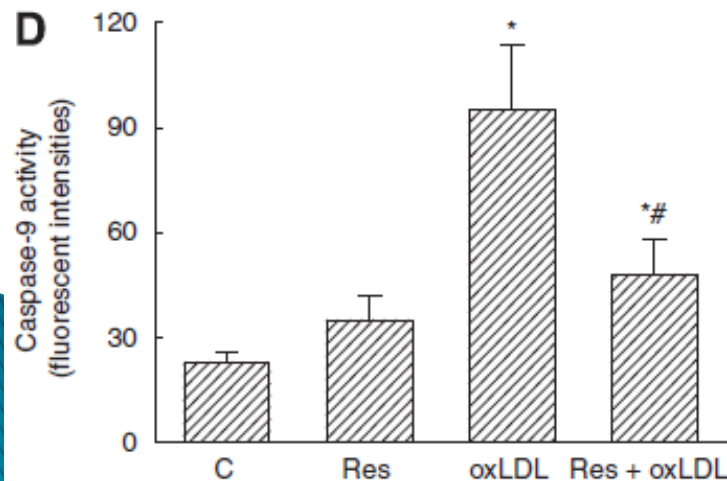
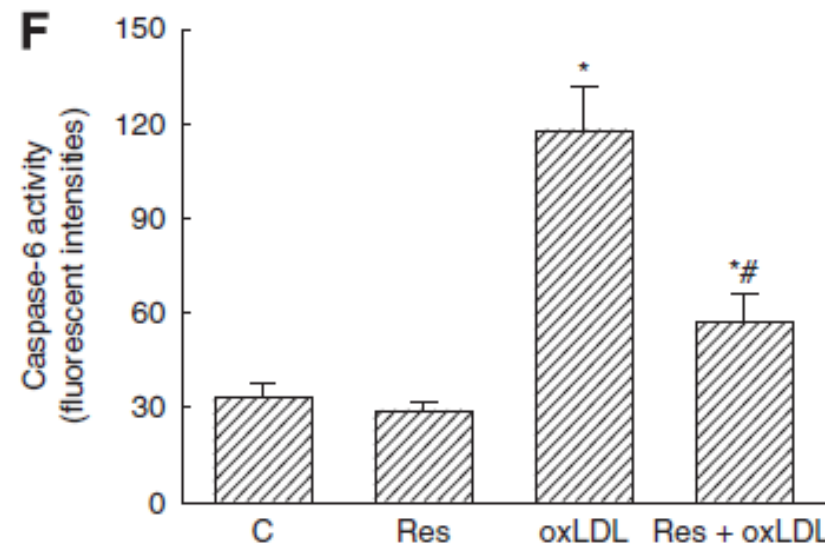
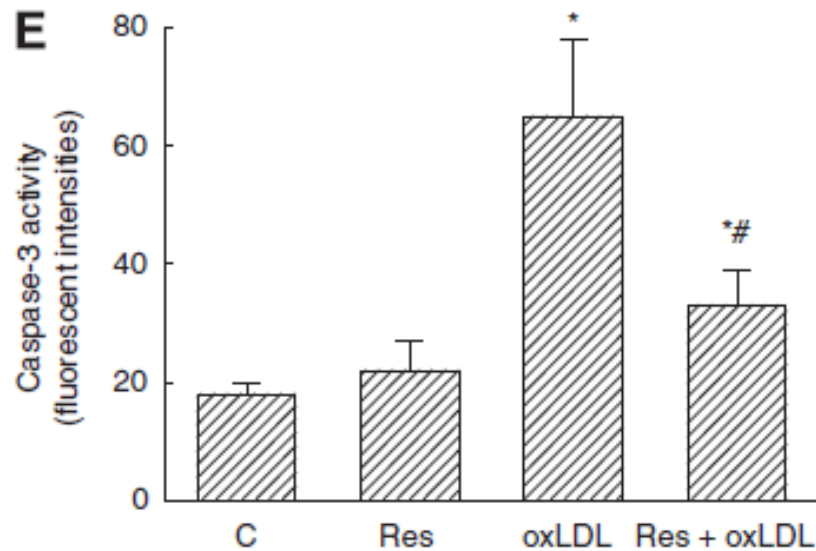
Resveratrol decrease oxLDL-induced mitochondrial membrane potential change



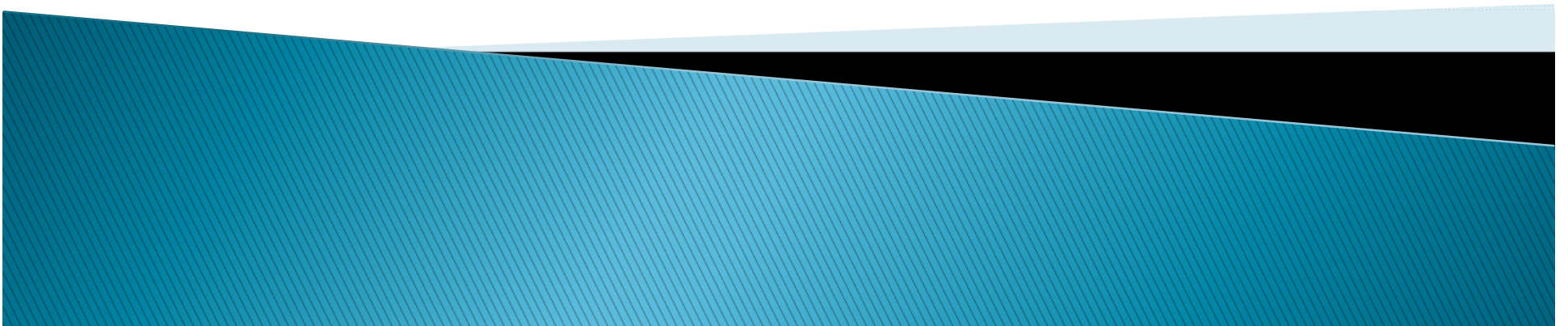
Resveratrol and the production of Cytochrome c



Resveratrol and the production of Caspase-3, Caspase-6 and caspase-9



Discussion



About the concentration of this study

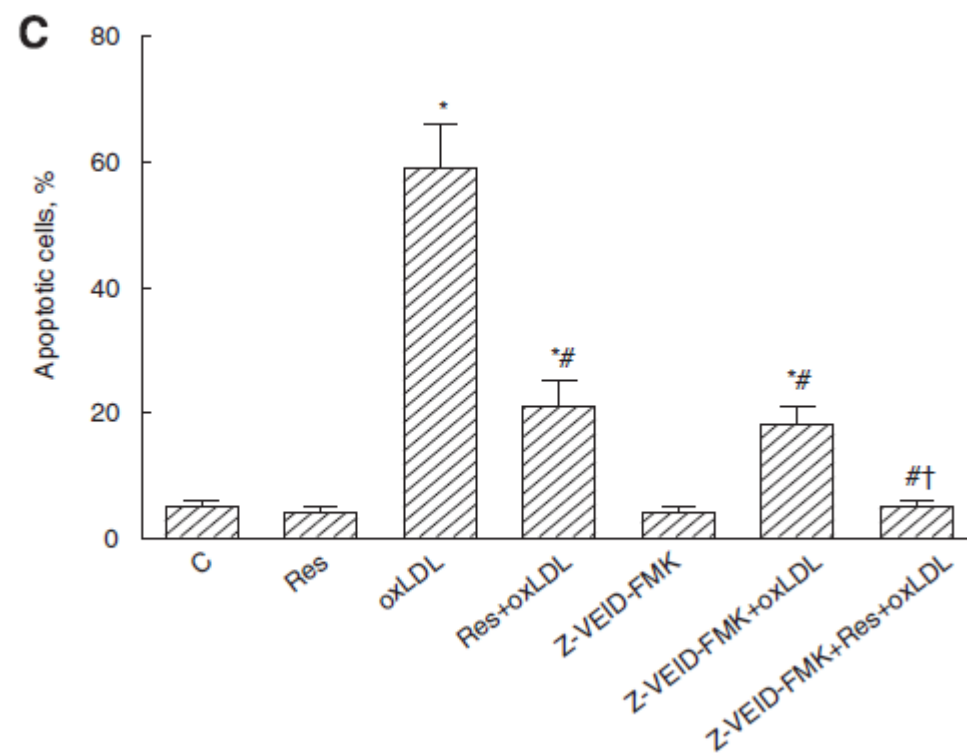
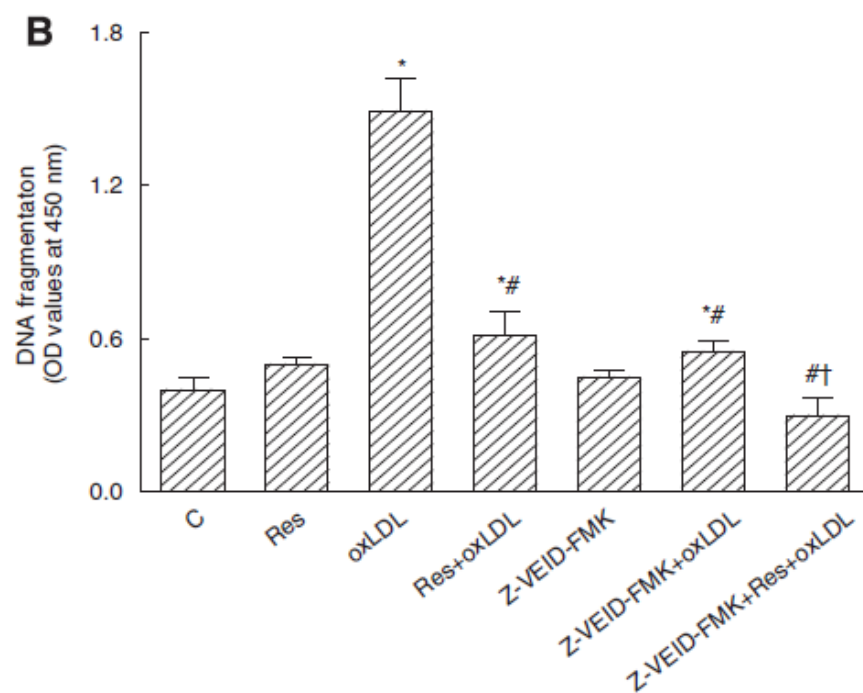
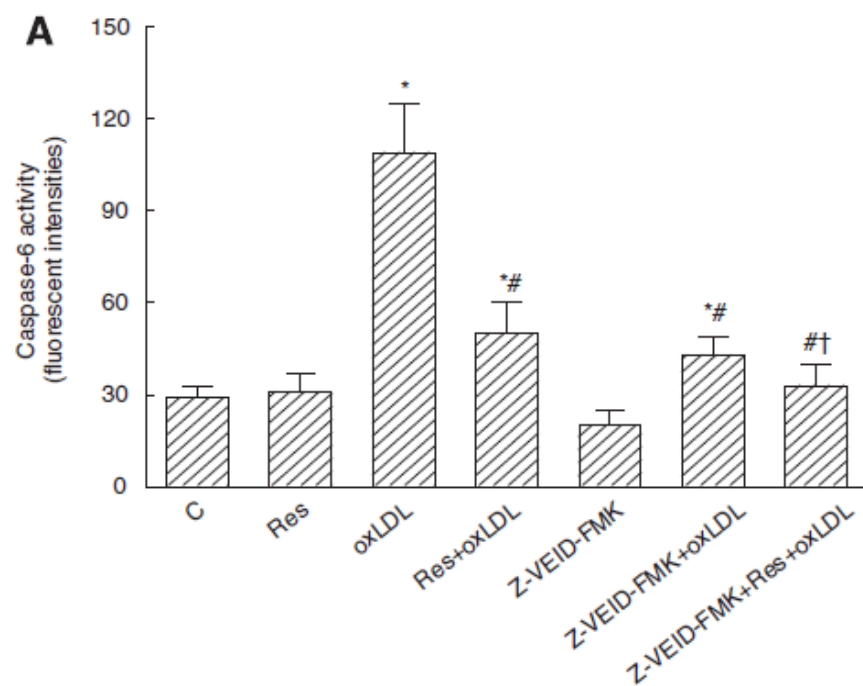
- ▶ According to the previous research, when resveratrol is about $4\mu\text{mol/L}$, it has antitumor activity.
- ▶ Thus, the concentration of $1\sim 10\mu\text{mol/L}$ may be higher than it can be in vivo applied. However, the first experiment of this study has showed that it is within the safe range.



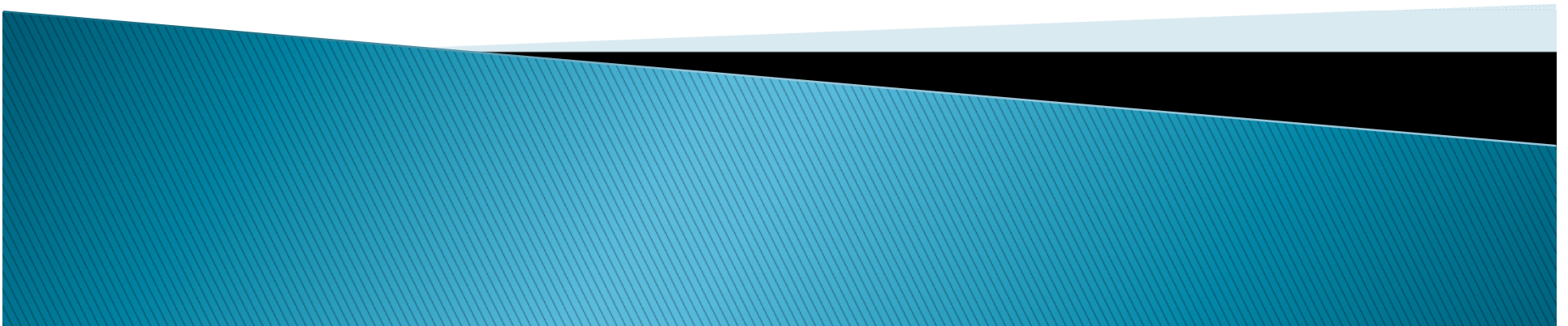
Resveratrol, CECs, and oxLDL

- ▶ Resveratrol protects CECs from oxLDL-induced damage of CECs via an anti-apoptotic mechanism.
- ▶ Resveratrol decreases alterations of the fragmentation of genomic DNA, reduces sub-G1 phase arrested CECs, reduces the translocation of Bax proteins, has synergistic effects with Z-VEID-FMK, and suppresses the overexpression of LOX-1.





Conclusion



- ▶ This study shows resveratrol can protect CECs from oxLDL-induced apoptotic insults via the way mentioned previously.



Thanks for your attetion!!

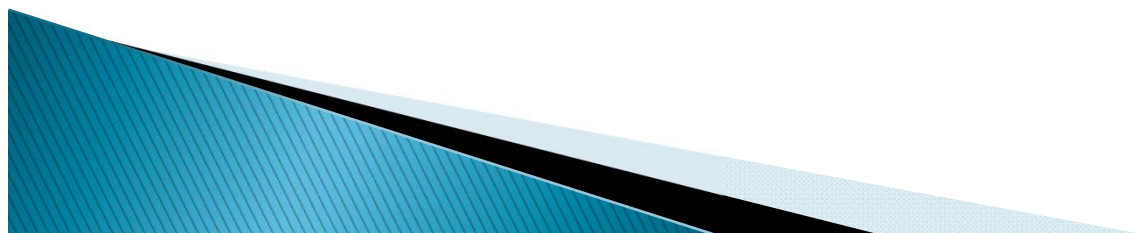


Questions?



B101099135 劉冠宏

- ▶ Q:紅酒要喝多少才可以達到研究中的效果？
A:根據北醫另外一位老師的研究，大約要十公升。
- ▶ Q:把受傷的細胞就回來，會不會反而癌化？
A:Resveratrol是在源頭就吸收了自由基，進而拯救細胞，而不是在細胞受損後才讓它不死。
- ▶ Resveratrol中的苯環可以吸收電子，吸收自由基



B101099069 謝綦

- ▶ Q: Z-VEID FMK的作用？

A: 它是一種caspase-6的抑制劑。

- ▶ Q: 為什麼是這個而不是用其他的藥呢？

A: 根據前人的研究，這個是最常用的，因為它是細胞凋亡前的最後一個caspase，有caspase-6就一定會有細胞凋亡，有3或9則不一定，所以抑制caspase-6可以抑制凋亡，所以選這個藥。



組員的提問

- ▶ 曾士剛：為什麼老師不把定量後的數據做計算，得到更多的推論？(例如把oxLDL的量和resveratrol的量相除，得到最適比例等等)
- ▶ 連老師：這個實驗的假設比較是要實驗定性的，之所以定量是可以協助我們進行差異比較，這樣就可以說明我們的假設是否成立，這麼一點數據的數據計算，並沒辦法多說明什麼，不如留在下次實驗，一次做的更完整些。

