Adverse effects of cigarette smoke extract in human cells

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Health effects of tobacco smoking

- Tobacco is the single greatest cause of preventable death globally.
- Tobacco smoking was a definite cause cancer and cardiovascular and pulmonary diseases
- Low levels of exposure, including exposures to second hand tobacco smoke, lead to a rapid and sharp increase in endothelial dysfunction and inflammation.
- There are about 5 million smokers (20%) in **Taiwan**.

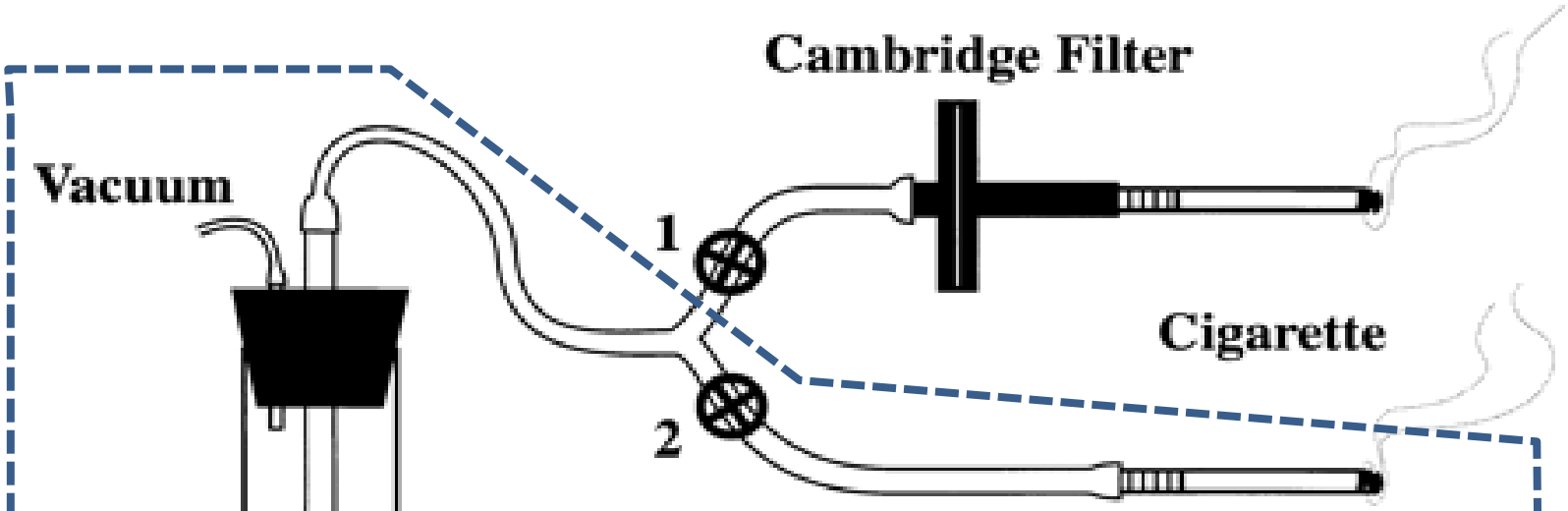
What is harmful in cigarette smoke ?

- Cigarette smoke is a complex and dynamic mixture of more than **7,000** individual chemical constituents. **>100** are toxic and at least **69** cause cancer.
- **Tobacco smoke** is a known human carcinogen.
- The most damaging compounds in tobacco smoke include: Tar, Nicotine, 1,3-butadiene, Volatile chemical (Acrolein, Formaldehyde, Nitrosamines, Benzene), Heavy metal (Arsenic, Cadmium), Toxic gas (Ammonia, Carbon monoxide, Nitrogen oxide), Hydrogen cyanide.

How cigarette smoking causes diseases?

1. Oxidative stress [reactive oxygen (**ROS**) & reactive nitrogen species (**RNS**)]
2. DNA damage [DNA single-strand break, 8-oxo-2'-deoxyguanosine (8-oxodG), DNA-protein cross-links]
3. Inflammation
4. Cell death (apoptosis, necrosis, and necroapoptosis)

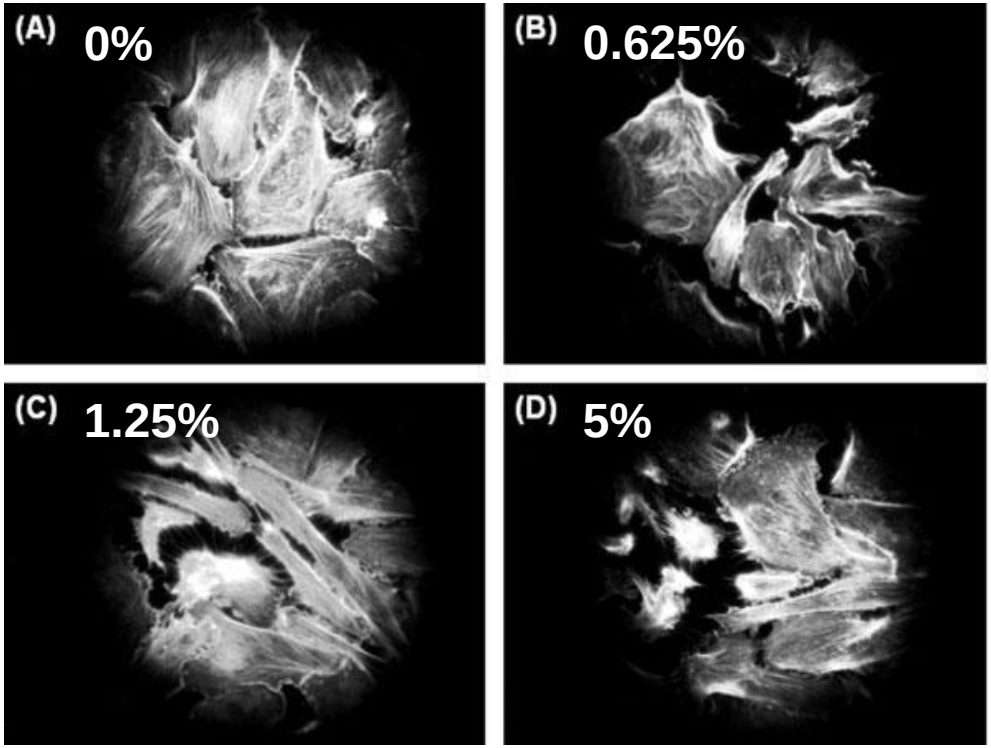
Preparation of whole cigarette smoke extract



Whole cigarette smoke extract (100%) was prepared by bubbling smoke from 3 Marlboro Red cigarettes (5 cm/each) into 15 ml of PBS at a rate of 1 cm/min.

Su et al., 1998

CSE induced actin cytoskeleton reorganization in HUVEC



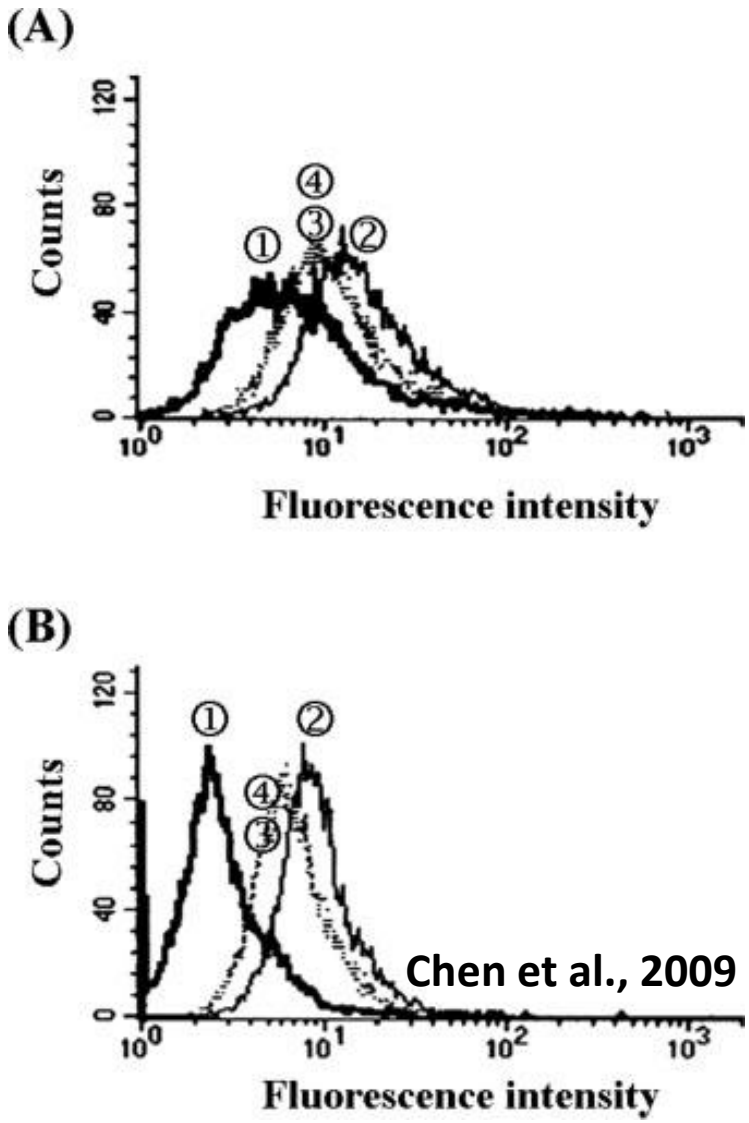
Chen et al., 2009

Exp Design:

1. Cells were exposed or not to various concentrations of CSE for 6 hrs.
2. After treatment, cells were fixed and stained for actin using **rhodamine-labeled phalloidin.**

Magnification for images at x400.

Cytochalasin D (CytD) partially inhibited the surface expression of ICAM-1 and E-selectin

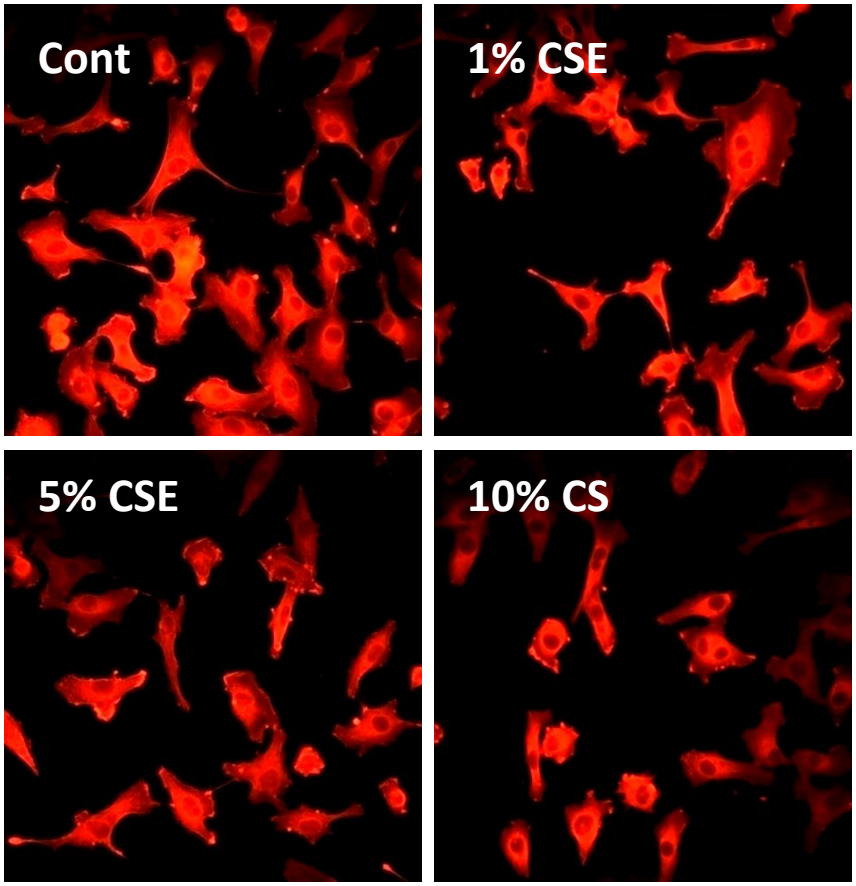


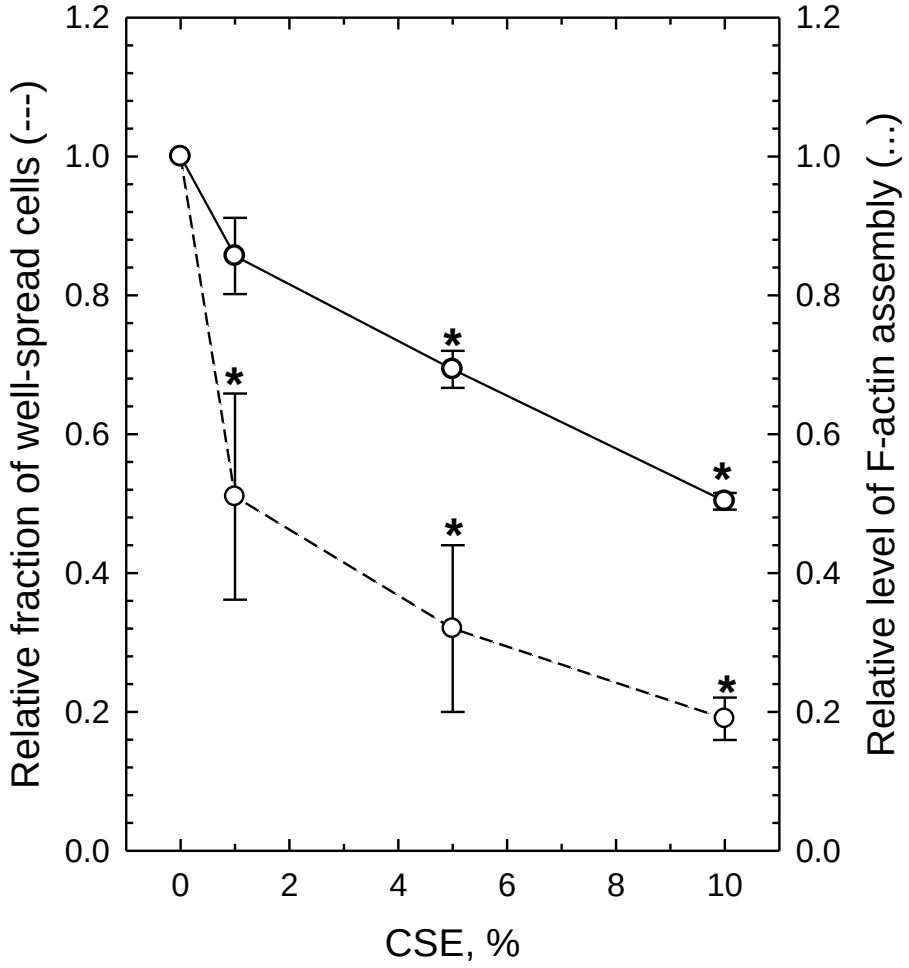
Exp. Design:

1. HUVEC were pretreated with or without CytD (50 nM or 100 nM) for 30 min and were then washed with PBS.
2. Washed cells were incubated with 5% CSE for another 6 hr.
3. After treatment, cells were collected, and the surface expression of ICAM-1 & E-selectin was determined by flow cytometry.

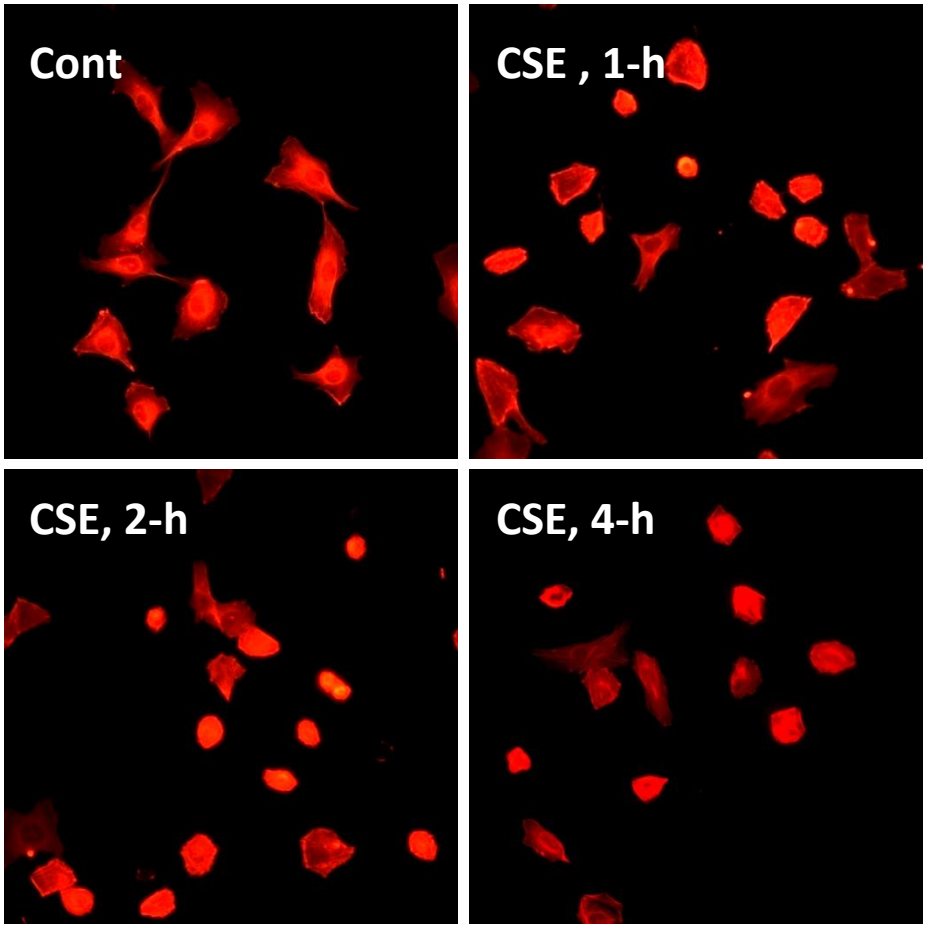
① Control; ②5% CSE for 6 hr; ③ 50 nM CytD + 5% CSE 6 hr; ④100 nM CytD + 5% CSE for 6 hrs.

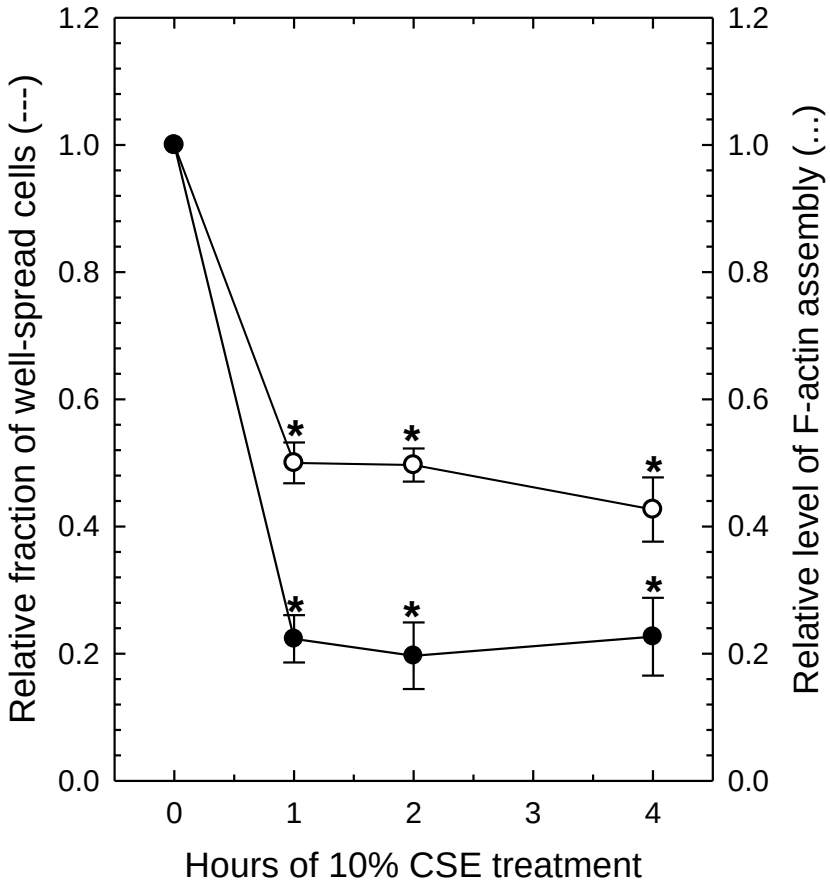
Dose-dependent effect of CSE on cell morphology and actin cytoskeleton organization in EA.h926 cells





Time-dependent effect of CSE on cell morphology and actin cytoskeleton organization in EA.h926 cells

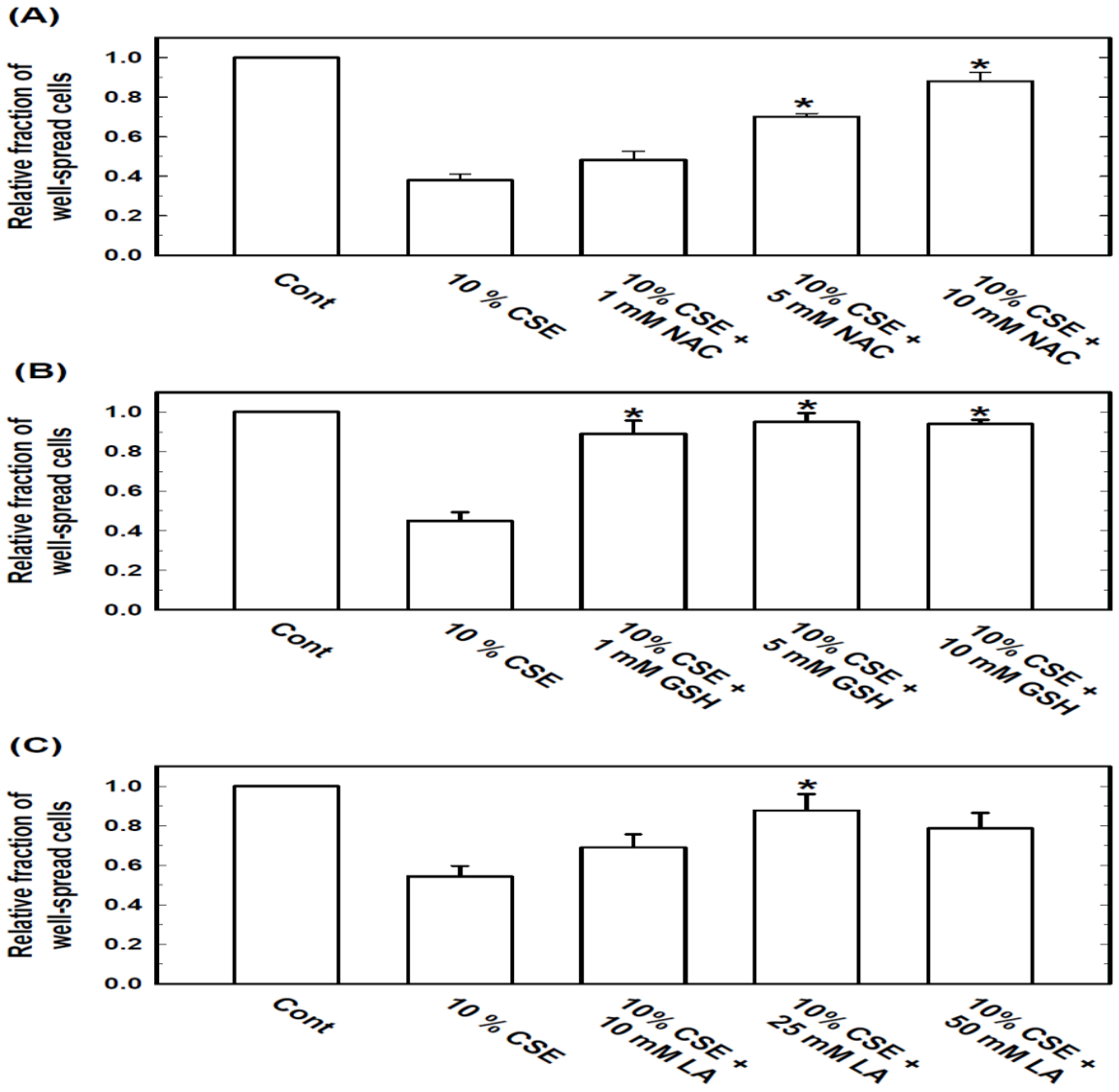




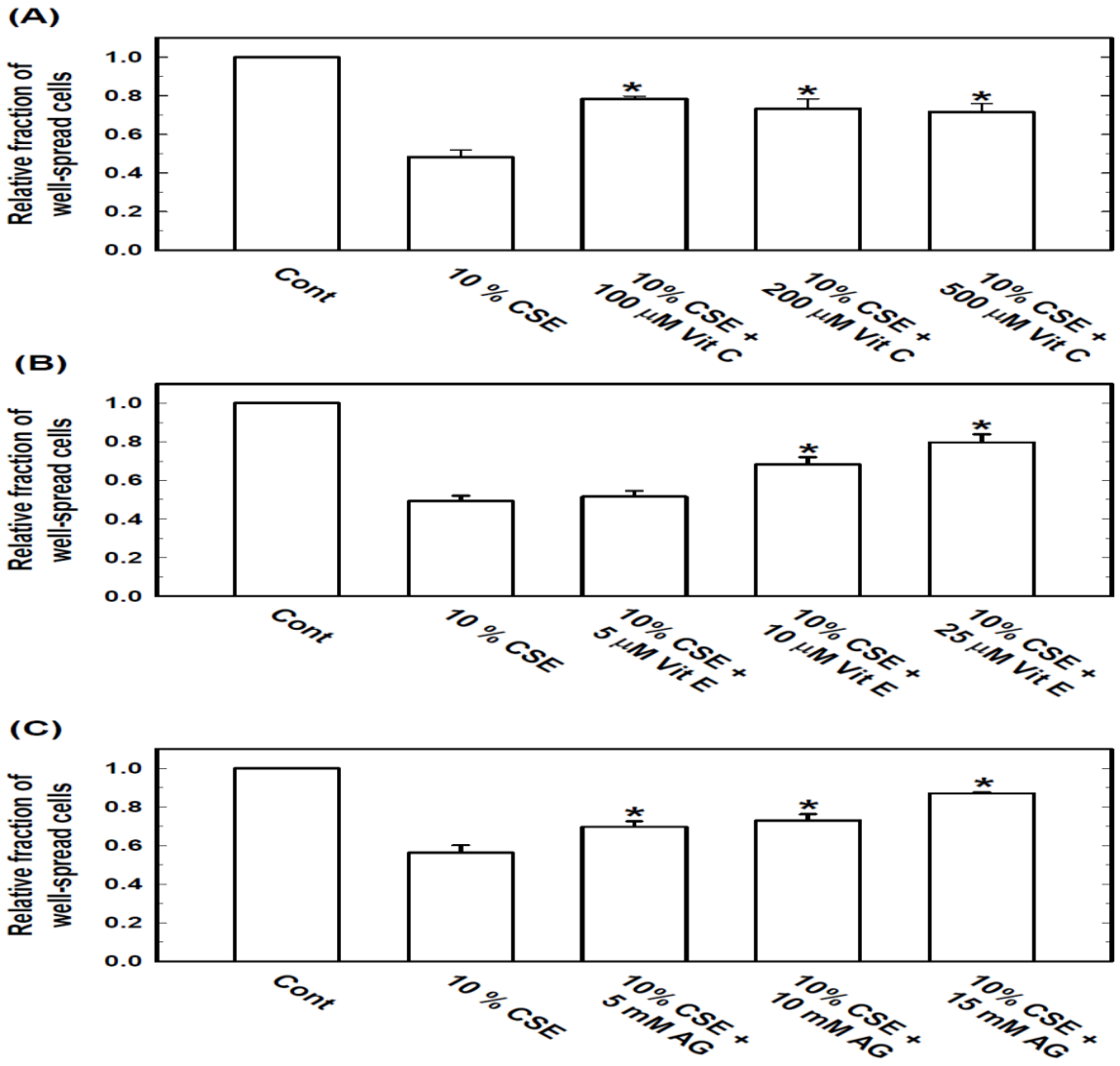
Actin cytoskeleton reorganization may be linked to CSE-induced pre-inflammation gene expression

- Cytoskeletal reorganization in skeletal muscle differentiation: from cell morphology to gene expression (Formigli et al., 2007).
- Actin has been shown to be an important regulator in RNA polymerase II transcription (Visa & Percipalle, 2010; Louvet E & Percipalle, 2009).
- The cytoplasmic dynamics of the actin cytoskeleton have been shown to regulate the subcellular localization of some transcription factors, such as MRTF-A and MRTF-B (Zheng et al., 2009).

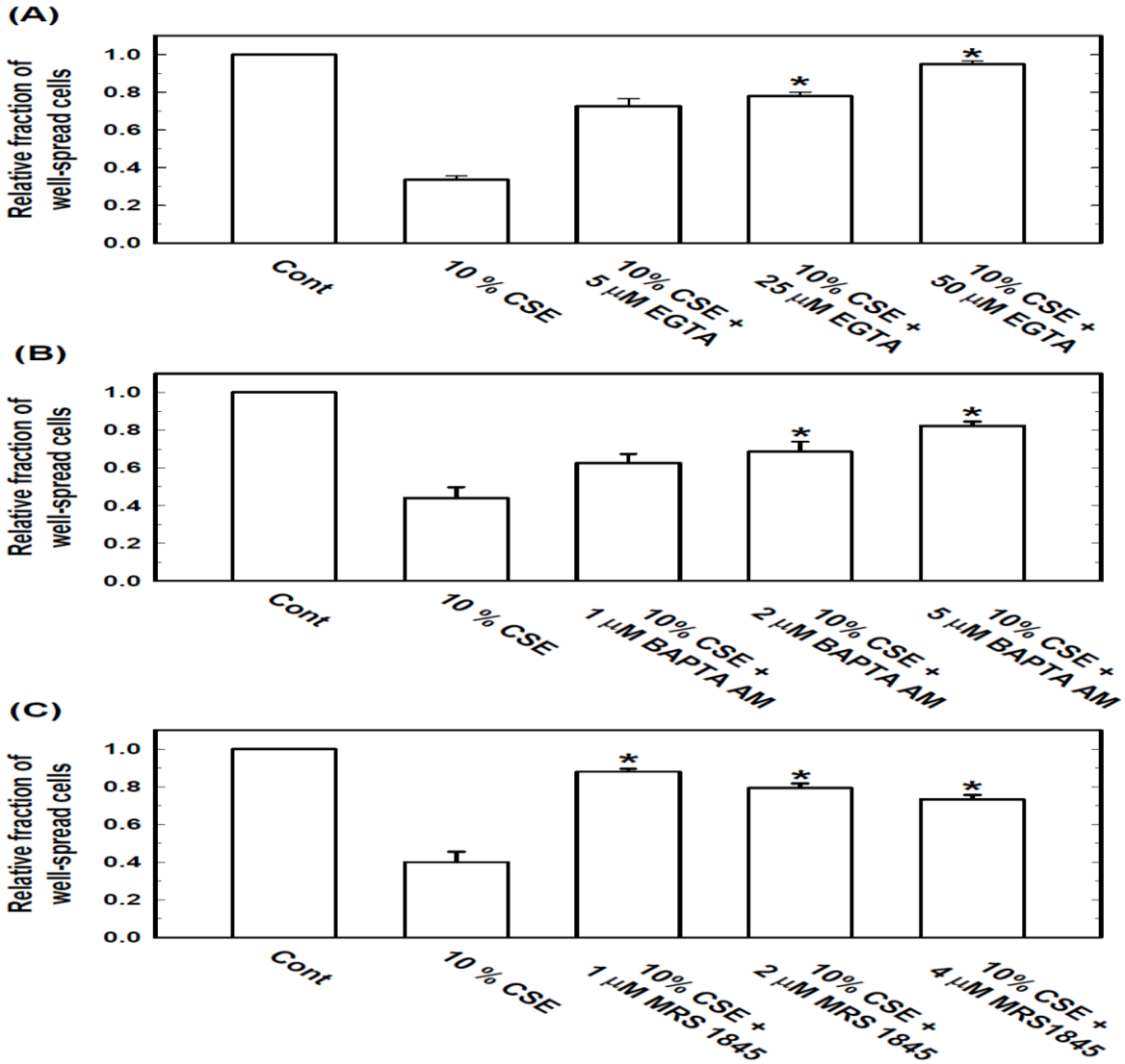
Effects of thiol-containing antioxidants on CSE induced cell shrinkage in EA.hy926 cells



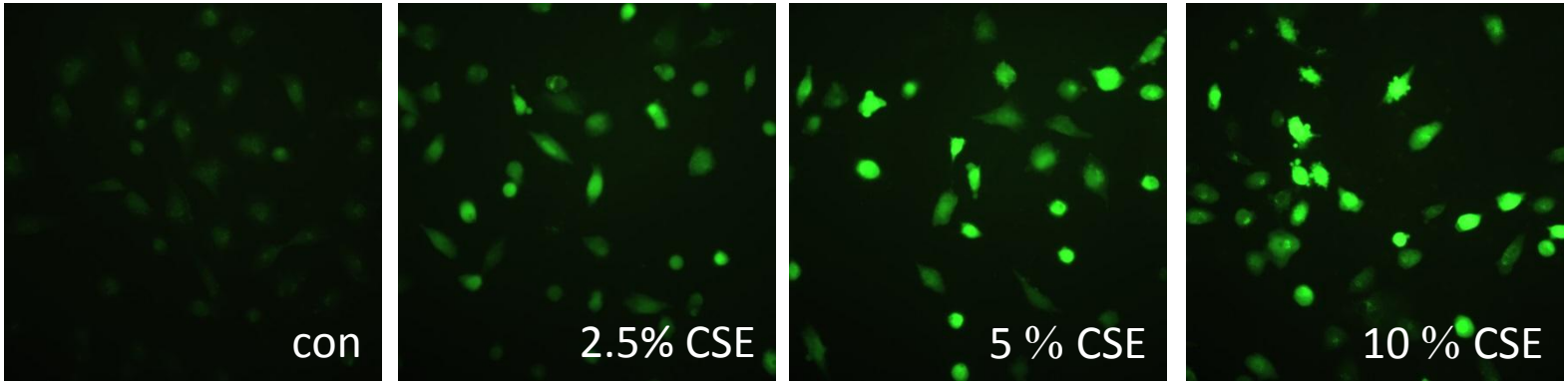
Effects of non-thiol-containing antioxidants on CSE induced cell shrinkage in EA.hy926 cells

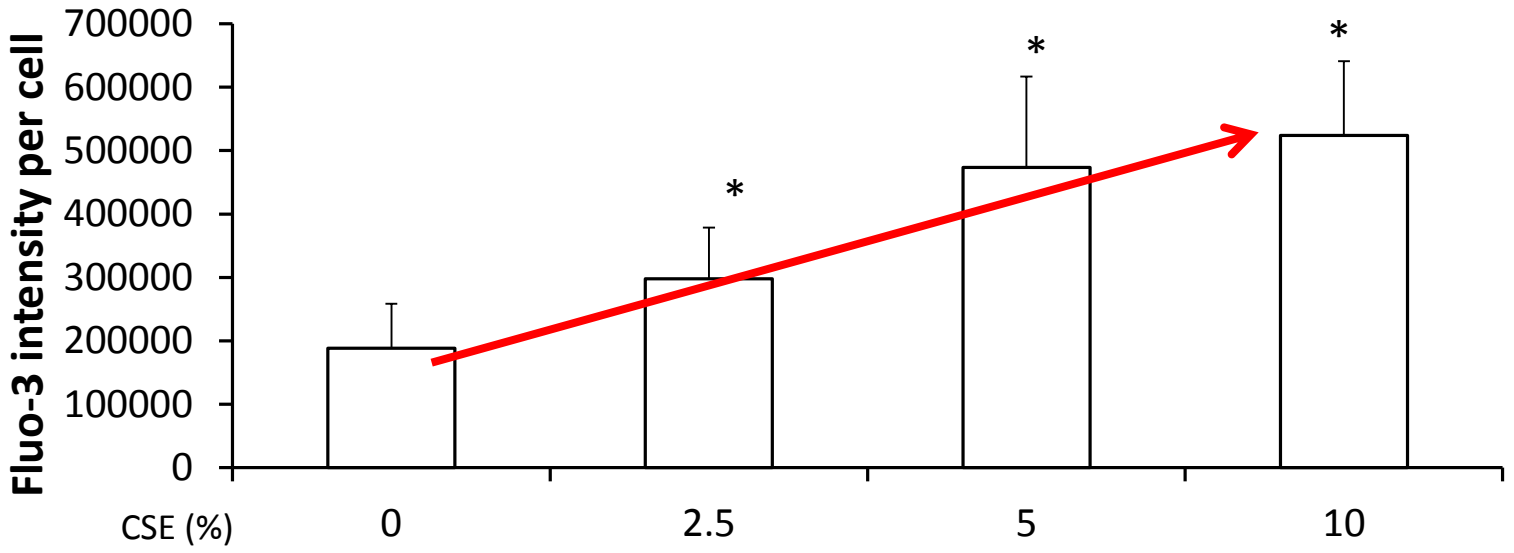


Effects of Ca^{2+} chelators & Ca^{2+} channel blocker on CSE induced cell shrinkage in EA.hy926 cells

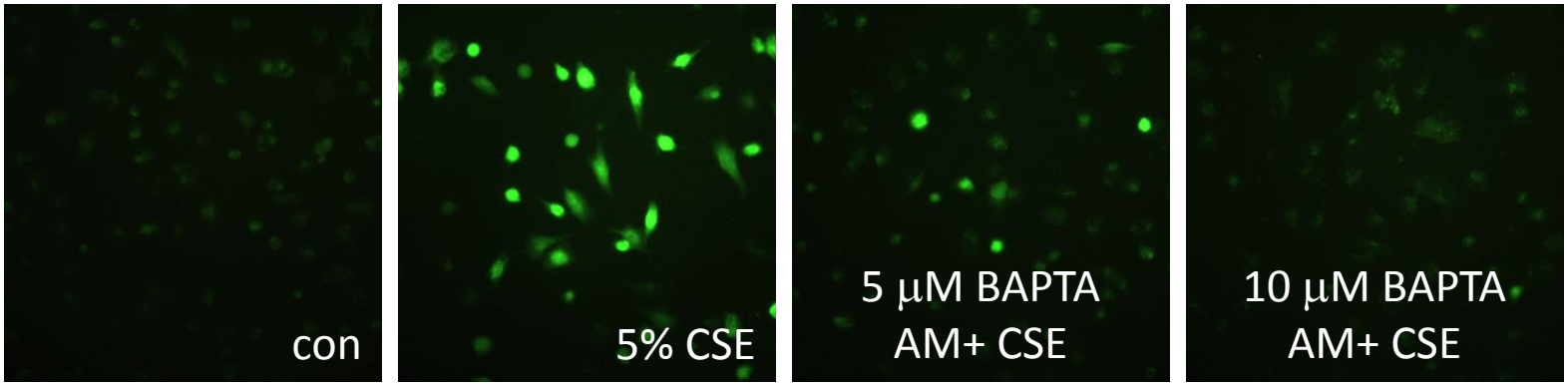


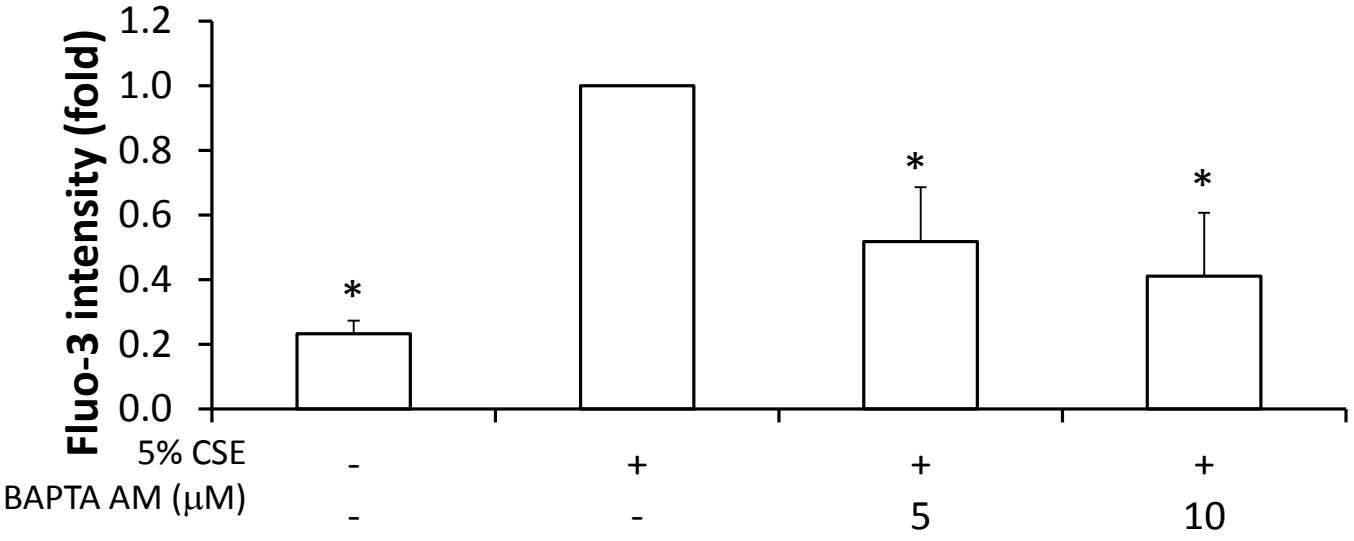
CSE increases the free intracellular calcium level using Fluo-3/AM



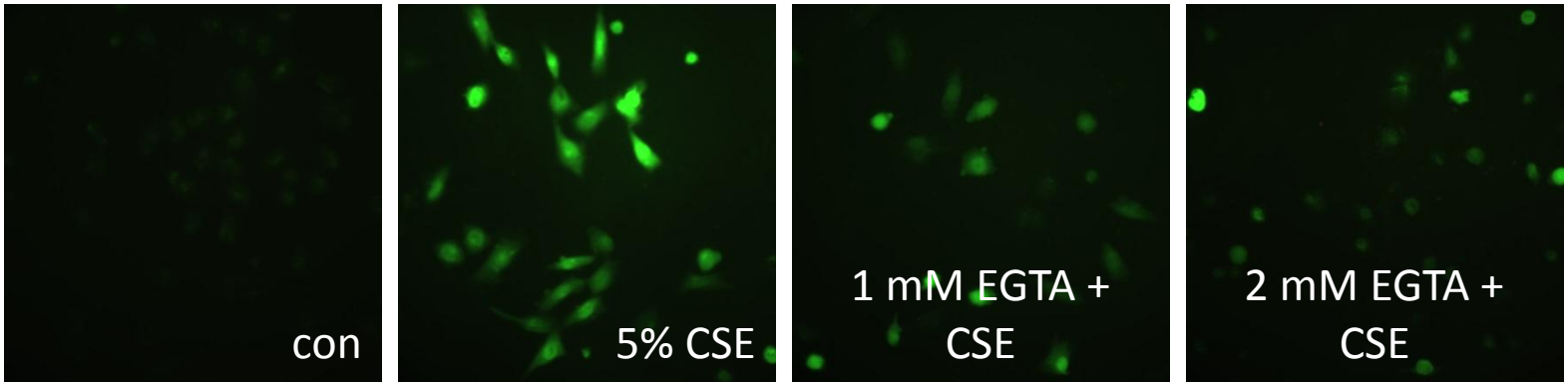


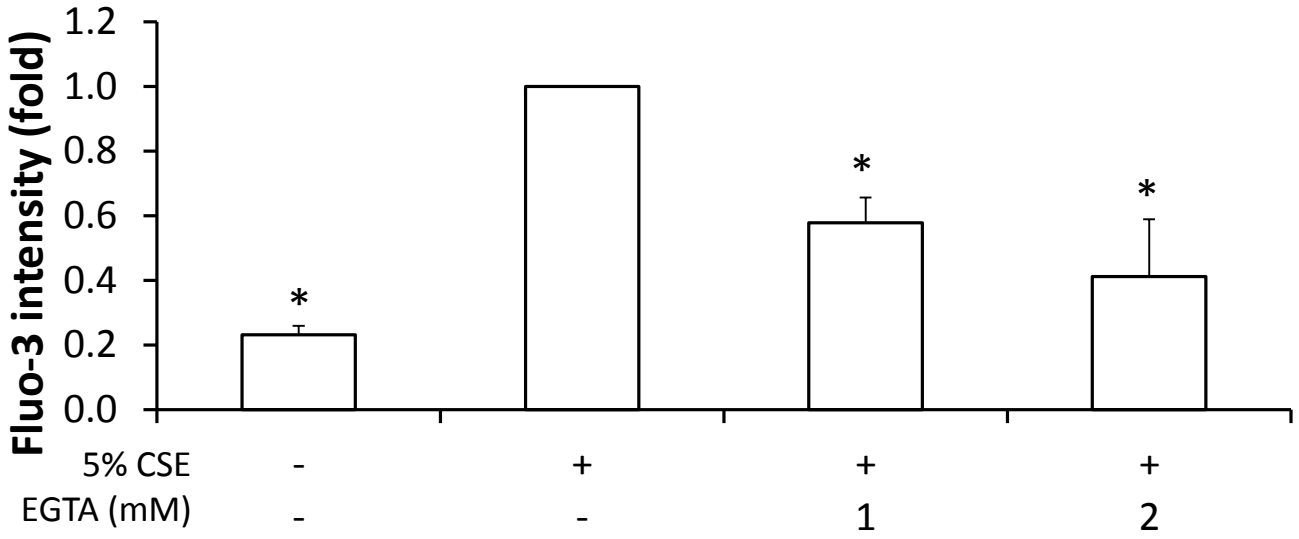
Cell-permeable Ca^{2+} chelator blocks CSE-induced intracellular Ca2+ increase in Ca^{2+}-free medium



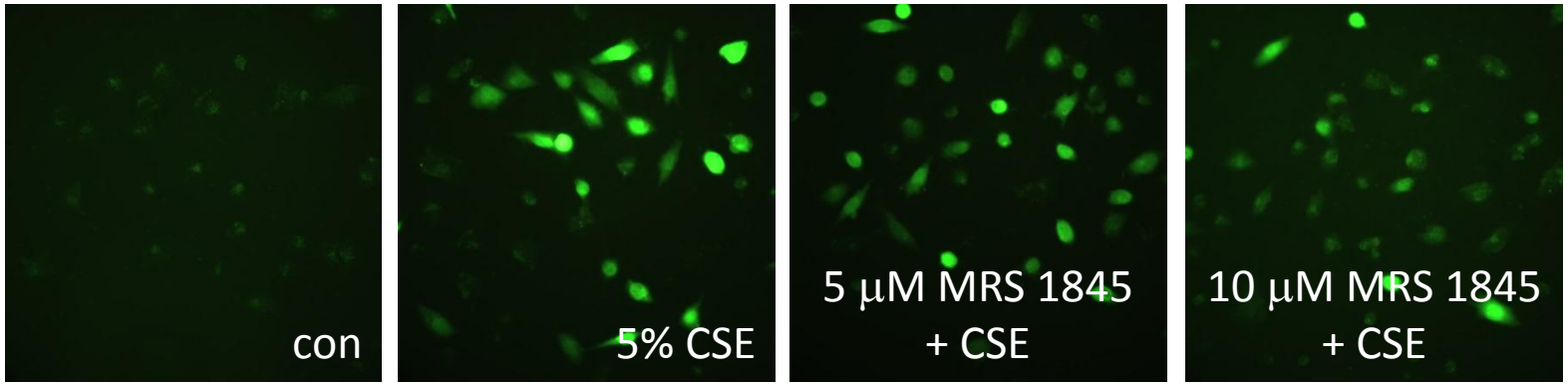


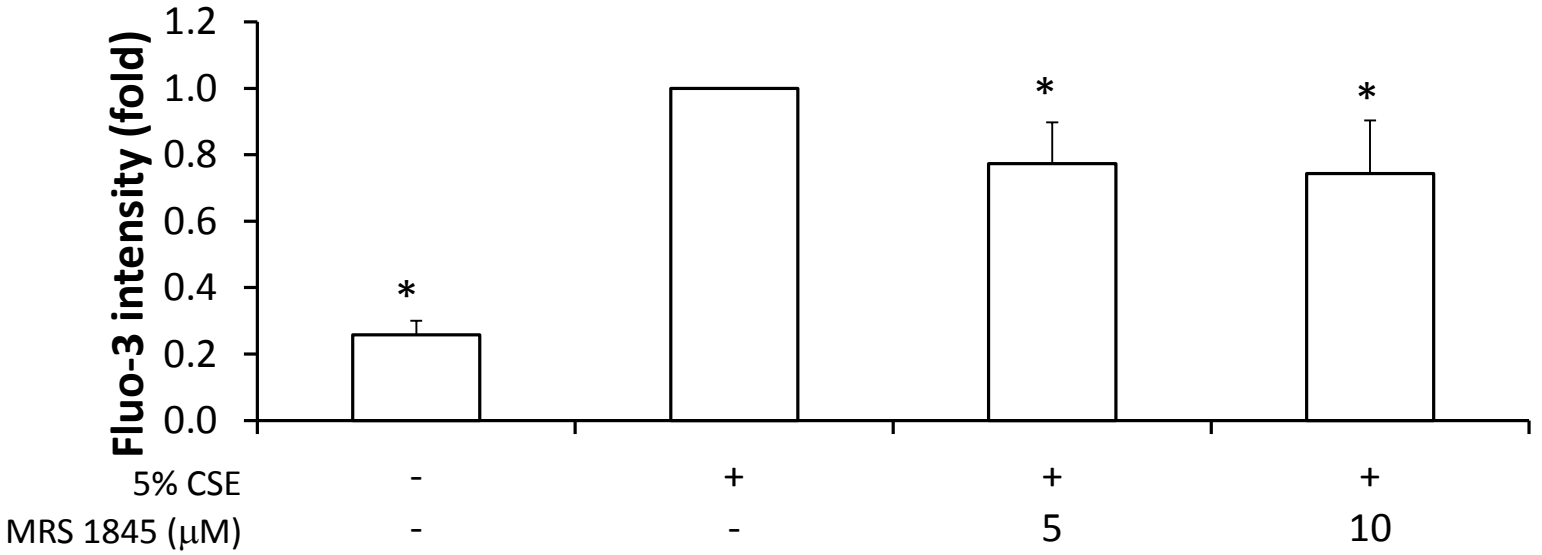
A non-permeable Ca^{2+} chelator blocks CSE-induced intracellular Ca^{2+} increase in normal medium



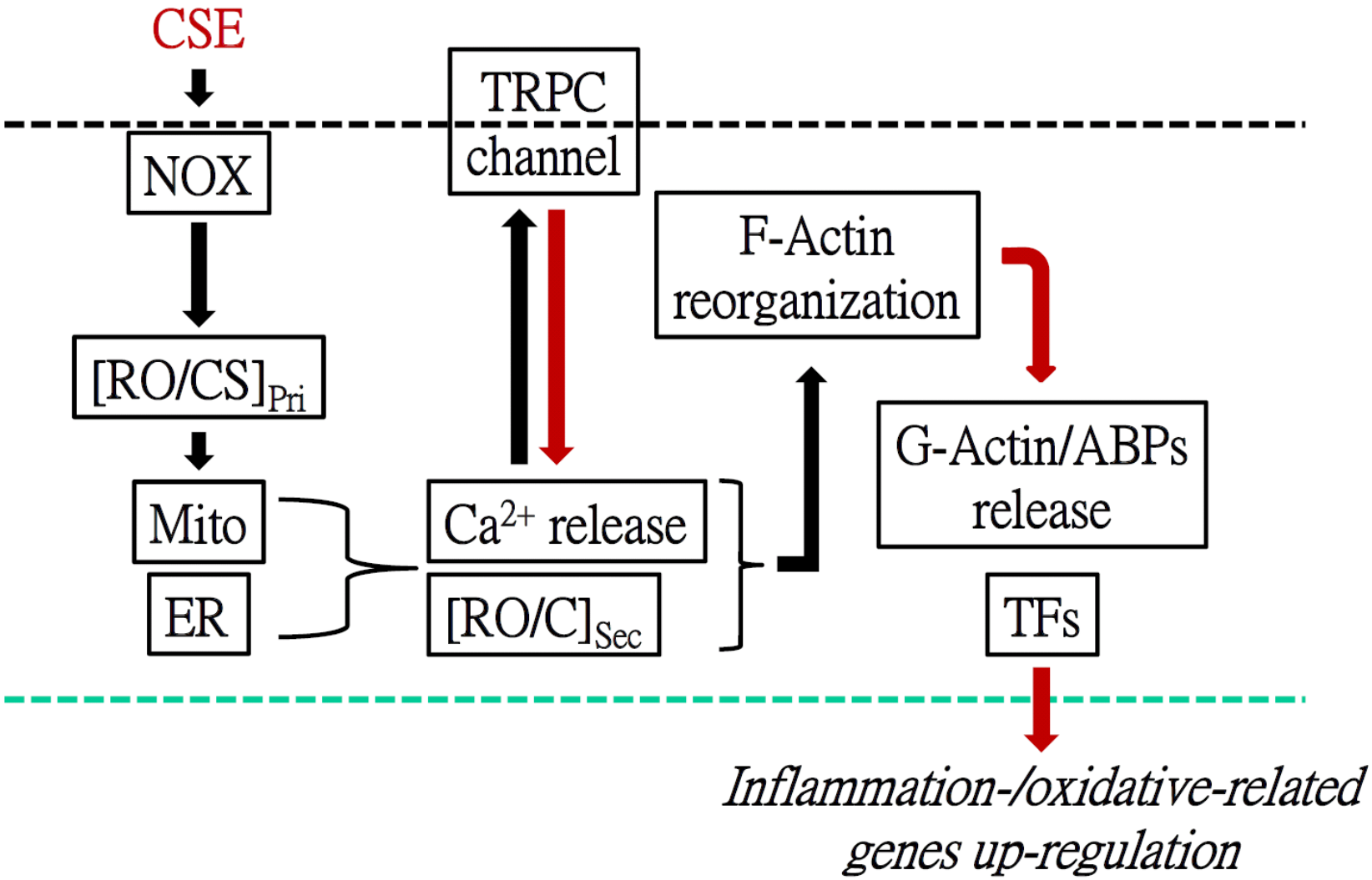


Transient receptor potential canonical (TRPC) channels inhibitor blocks CSE-induced intracellular Ca^{2+} increase in normal medium





Conclusion


CSE
NOX
[RO/CS]Pri
Mito
ER
TRPC channel
Ca2+ release
[RO/C]Sec
F-Actin reorganization
G-Actin/ABPs release
TFs
Inflammation-/oxidative-related genes up-regulation

Acknowledgments

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