

Ferroptosis Inducer

Literature Seminar

D3 Miura Kensuke

Outline

- Introduction of Ferroptosis
- Main Paper

nature
chemical biology

ARTICLES

<https://doi.org/10.1038/s41589-018-0031-6>

FINO₂ initiates ferroptosis through GPX4 inactivation and iron oxidation

Michael M. Gaschler^{1,9}, Alexander A. Andia^{2,9}, Hengrui Liu¹, Joleen M. Csuka³, Brisa Hurlocker², Christopher A. Vaiana², Daniel W. Heindel², Dylan S. Zuckerman², Pieter H. Bos³, Eduard Reznik³, Ling F. Ye³, Yulia Y. Tyurina⁴, Annie J. Lin³, Mikhail S. Shchepinov⁵, Amy Y. Chan², Eveliz Peguero-Pereira², Maksim A. Fomich⁷, Jacob D. Daniels⁸, Andrei V. Bekish⁶, Vadim V. Shmanai⁷, Valerian E. Kagan⁴, Lara K. Mahal², K. A. Woerpel^{2*} and Brent R. Stockwell^{1,3*}

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Prof. Brent R. Stockwell



1994: A. B. University of Cornell

**1999: Ph. D. Harvard University
(Prof. Stuart L. Schreiber)**

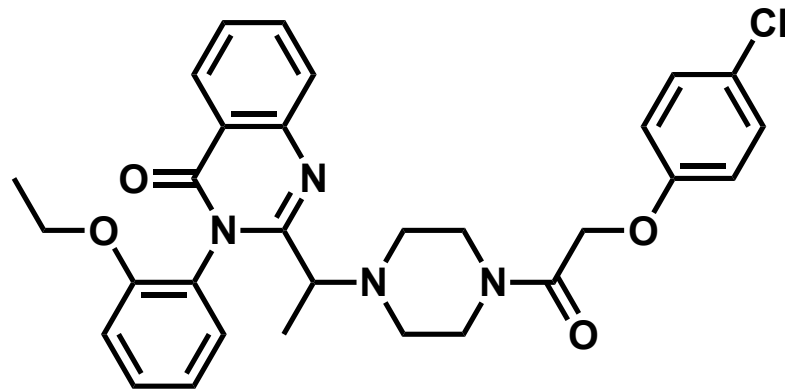
**1999-2003: Research Fellow. Whitehead
Institute (Prof. Stan Fields)**

**2004-: Assistant Professor/ Professor.
University of Columbia**

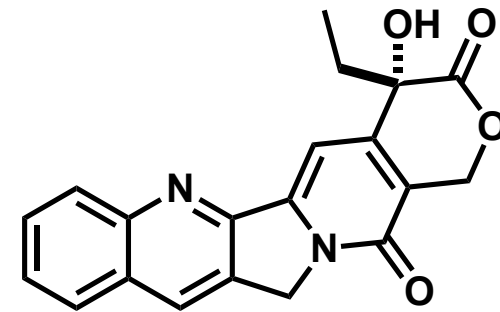
Research topics:

**Probing cell death mechanism with small molecules and genomic
tools, **Ferroptosis****

New Type of Cell Death Induced by Erastin



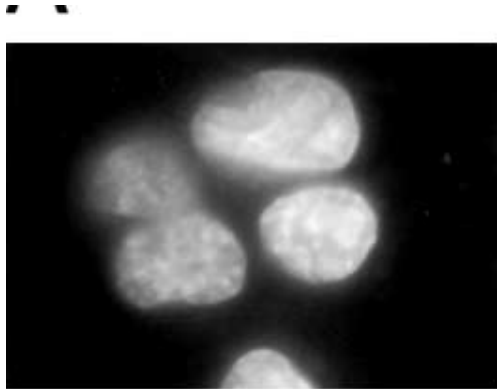
Erastin



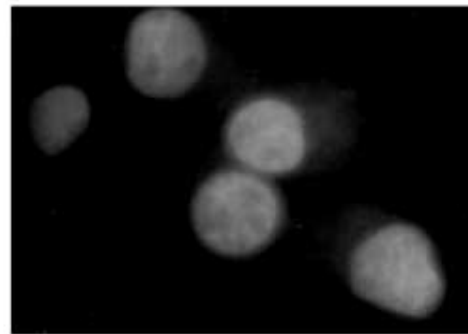
camptothecin

topoisomerase inhibitor inducing apoptosis

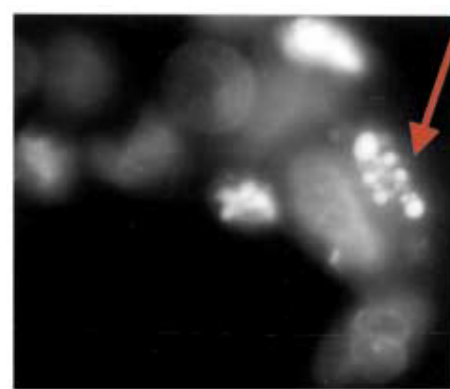
Erastin: identified as a compound having a lethal effect on RAS expressing cancer cells



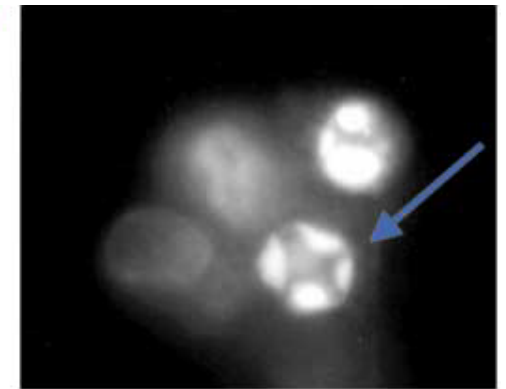
No treatment



erastin



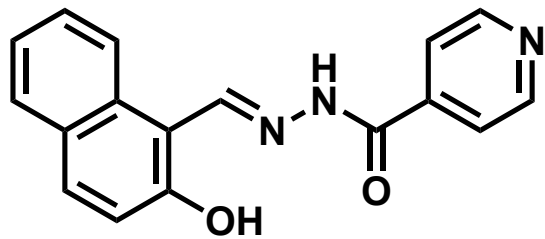
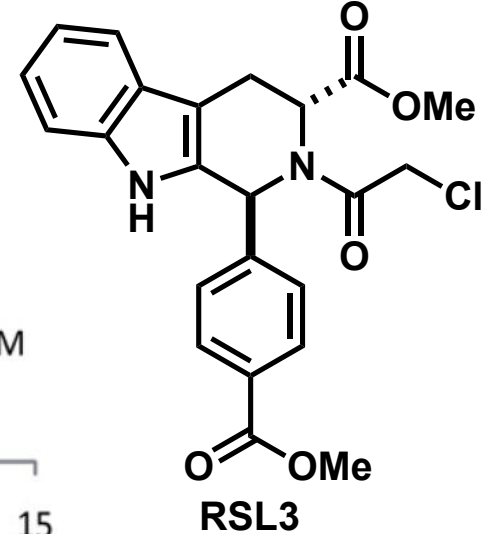
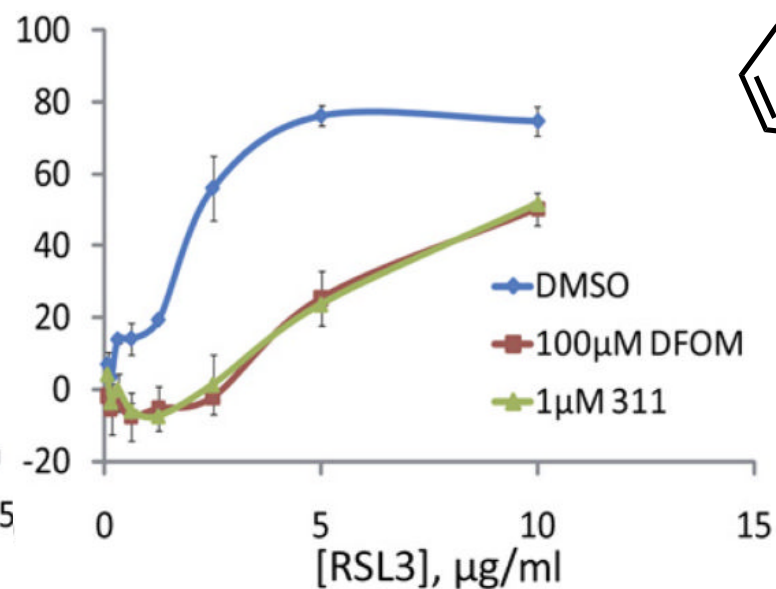
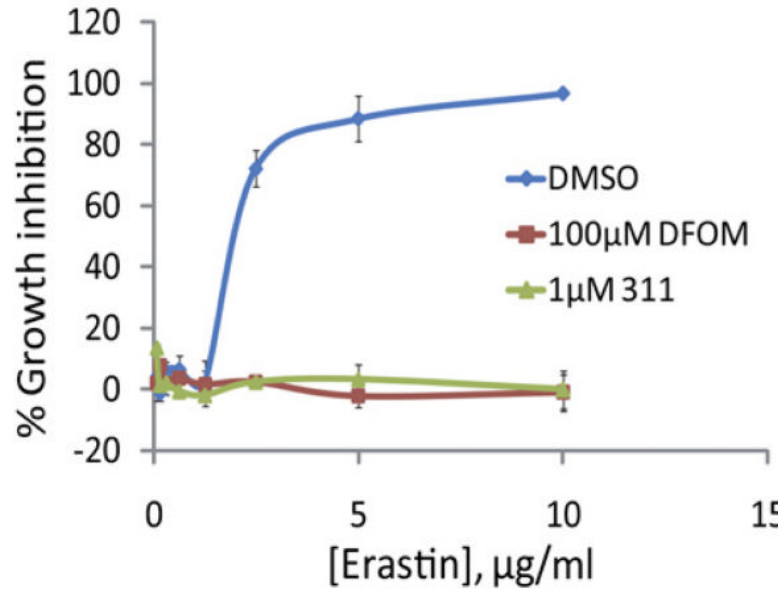
camptothecin



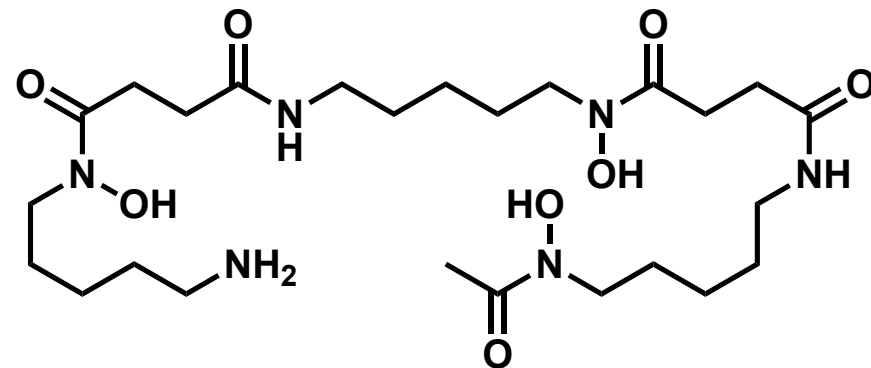
camptothecin

manner of cell death was different from what had been seen before.
no nuclear morphological changes, DNA fragmentation (nonapoptotic)

Discovery of Ferroptosis



iron chelator 311



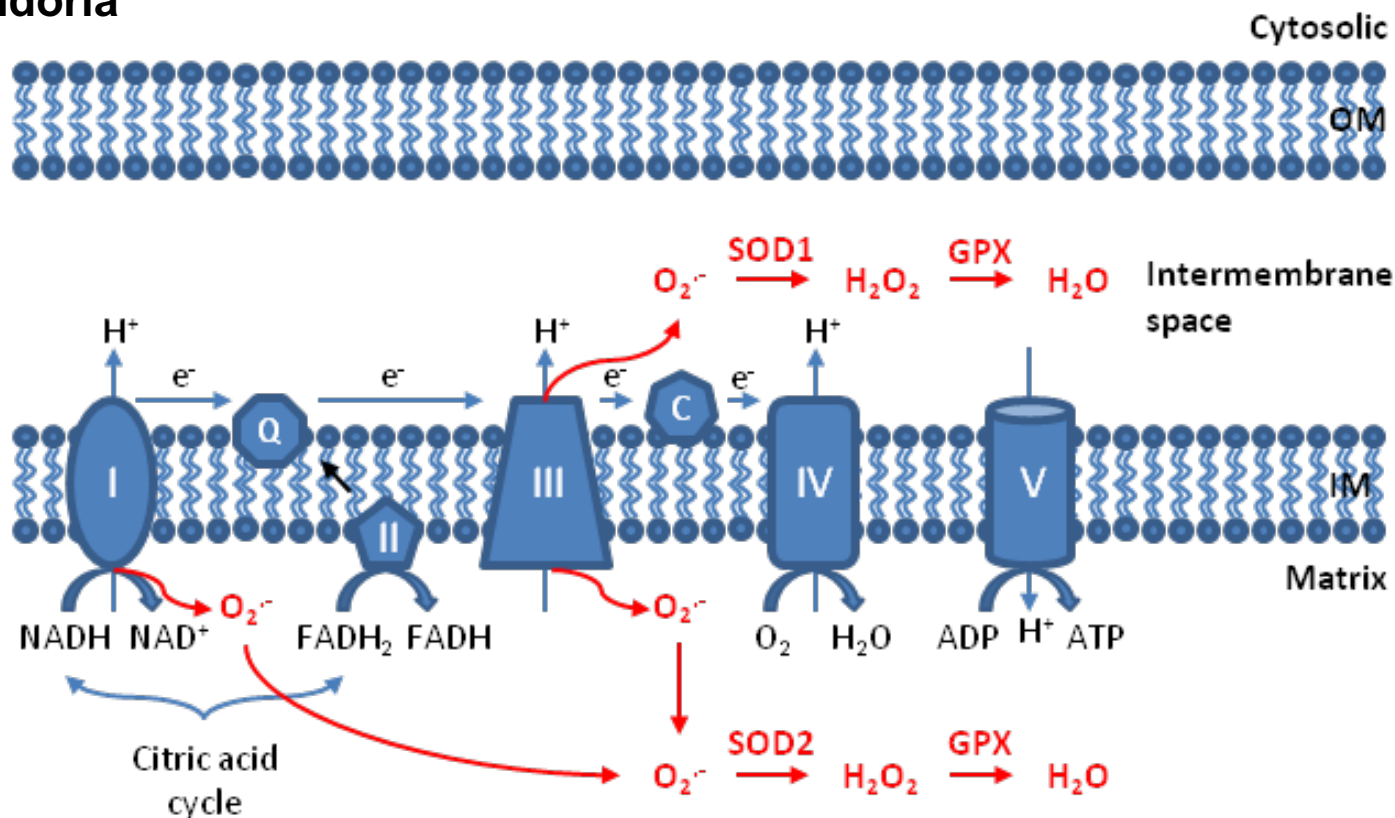
Deferoxamine (DFOM) iron chelator

Cell death induced by erastin and RSL3 was inhibited by iron chelators.
→ Ferroptosis ("Ferr" + "optosis")

- 1) Lemmon, A. M.; Schlessinger, J. *Cell*, **2010**, 141, 1117
- 2) Volinsky, N.; Kholodenko, B. N. *Cold Spring Harb. Perspect. Biol.* **2013**, 5, a009043

Reactive Oxygen Species (ROS)

mitochondria



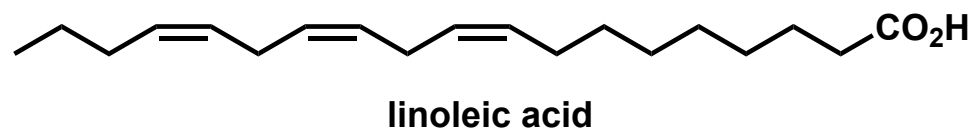
iron-mediated generation of ROS: Fenton reaction



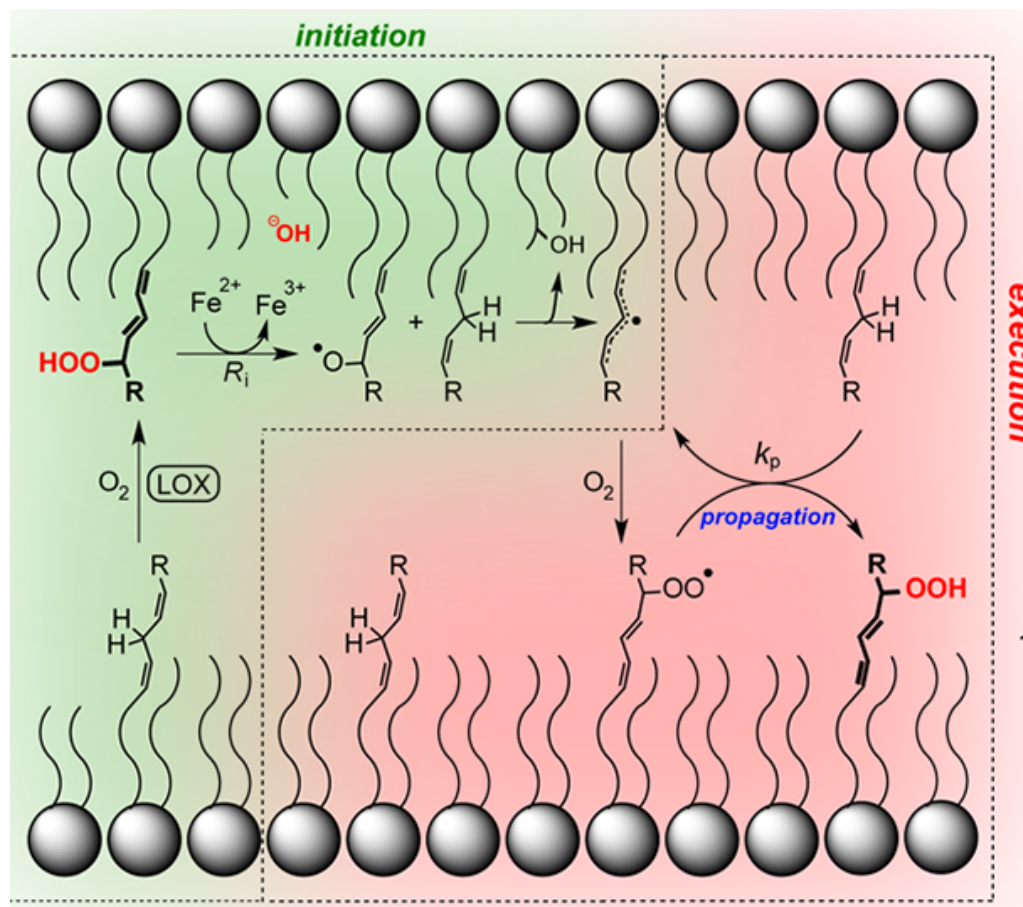
ROS generate lipid peroxidation products

Lipid Oxidation

substrate: polyunsaturated fatty acid (PUFA)

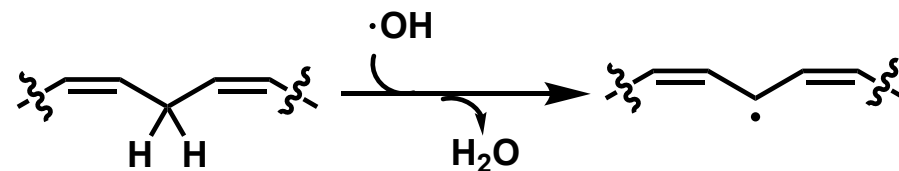


Lipid oxidation

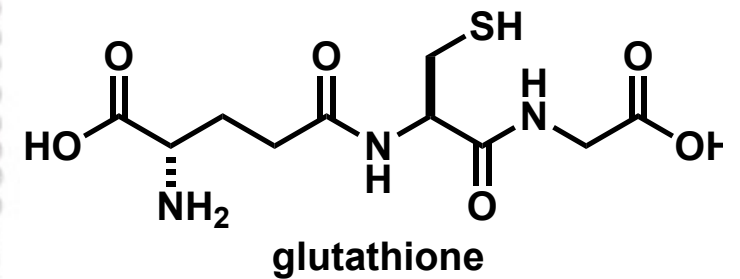
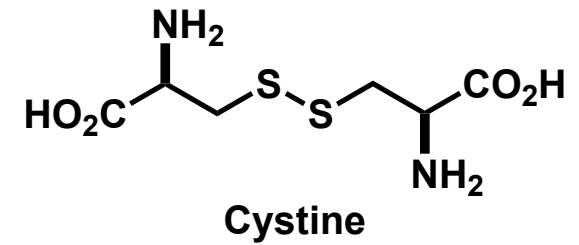
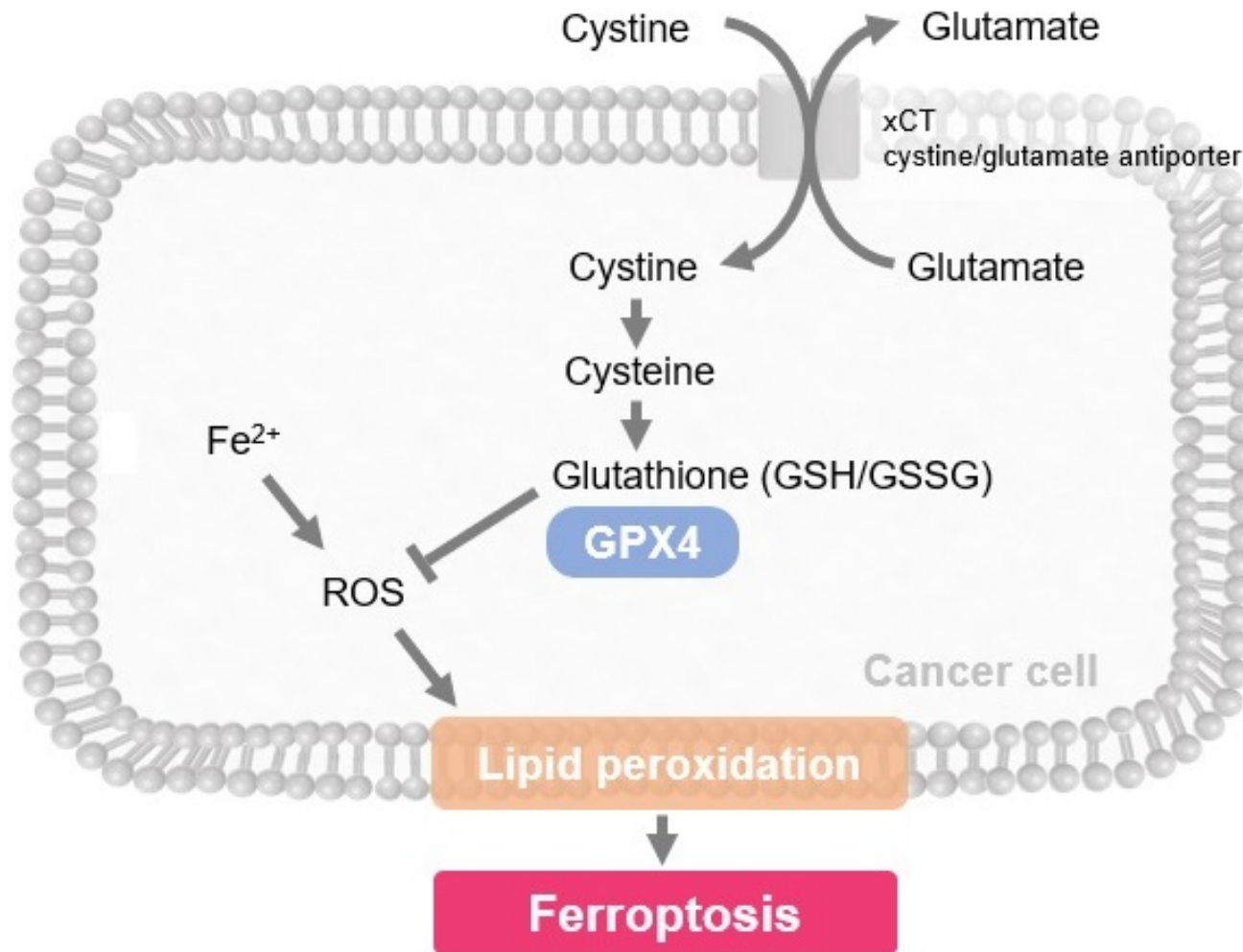


initiation of lipid oxidation was conducted by ROS or lipoxygenase (LOX)

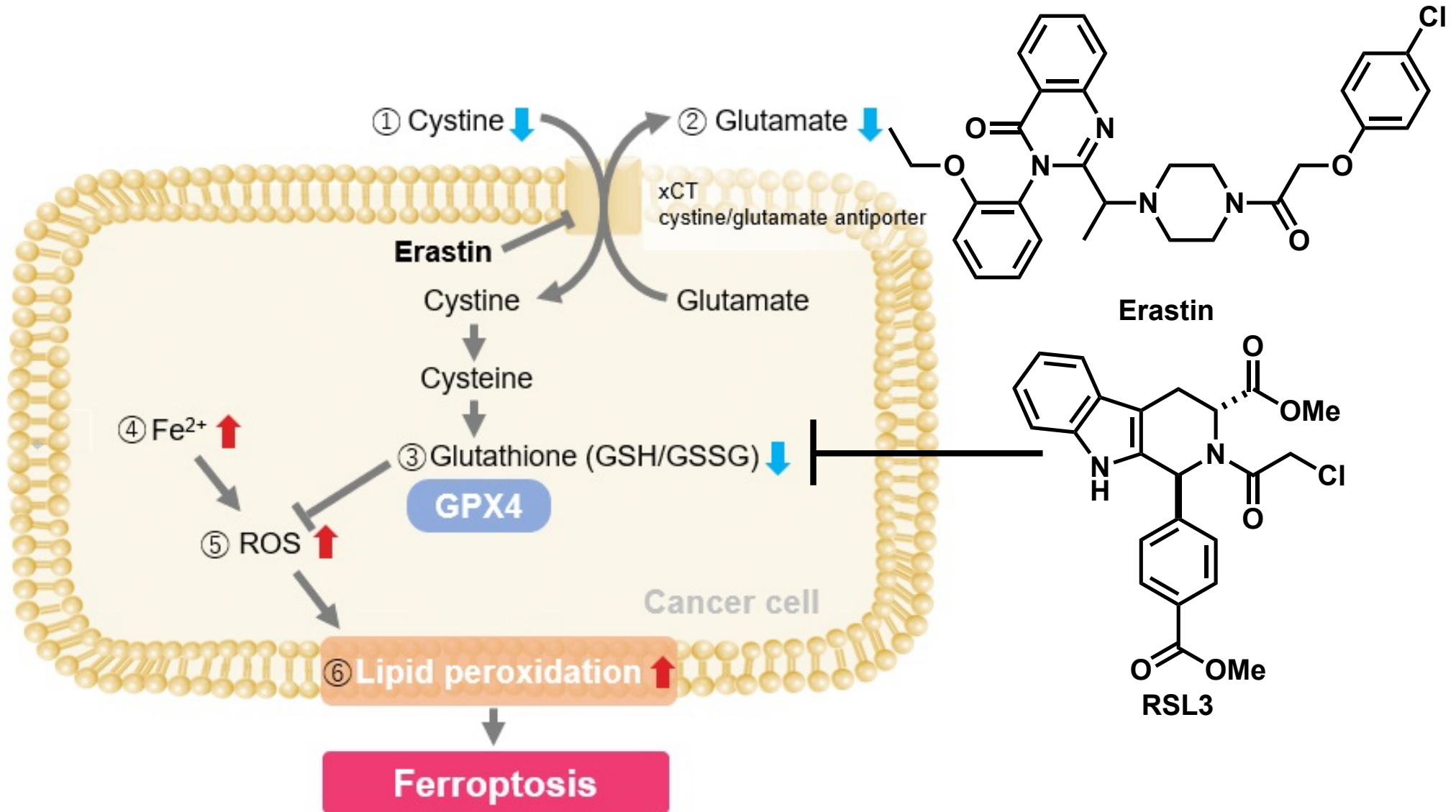
initiation of lipid oxidation by ROS



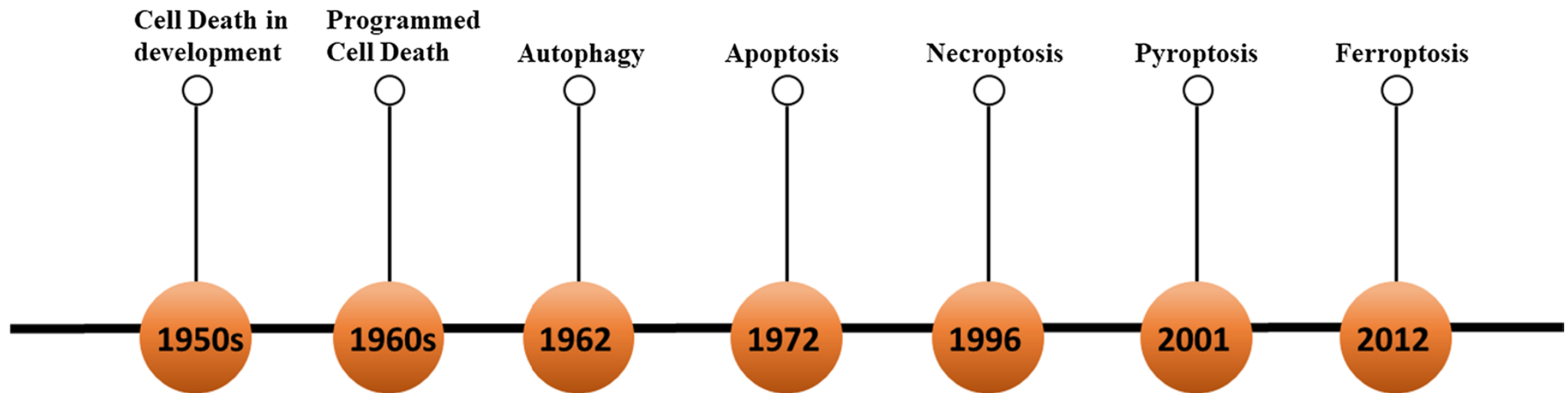
Regulatory System of Ferroptosis



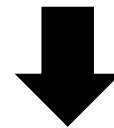
Mode of Action of Erastin and RSL3



Programed Cell Death for Cancer Therapy



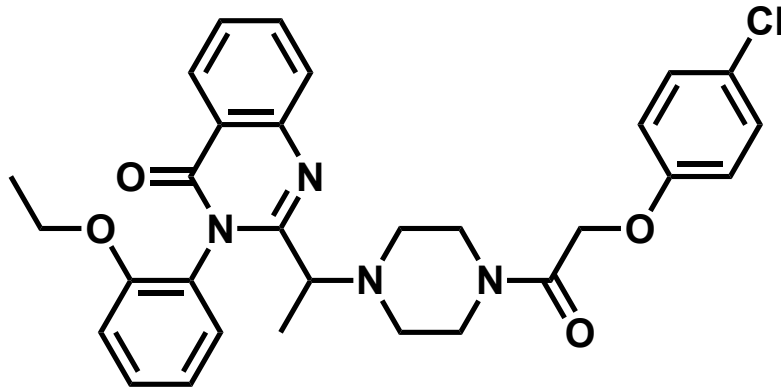
Targeting other forms of non-apoptotic cell death has become a new treatment approach to eliminate cancer cells and reduce the drug resistance of cancer cells



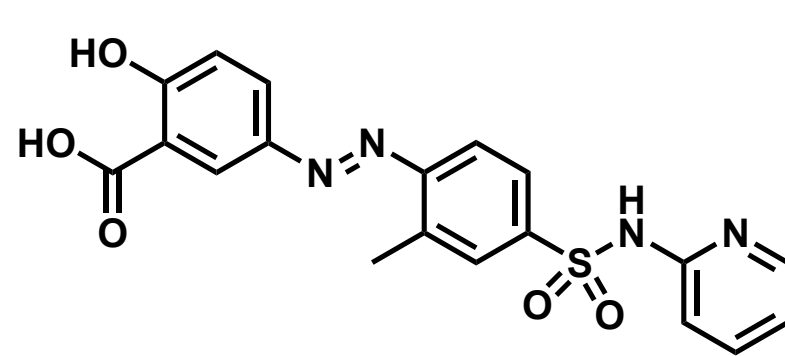
Ferroptosis has a potential for cancer treatments

Class of Ferroptosis Inducer

Class 1: System Xc⁻ Inhibitors

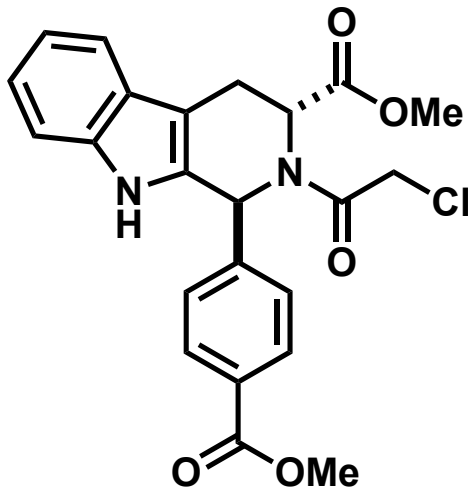


Erasitine

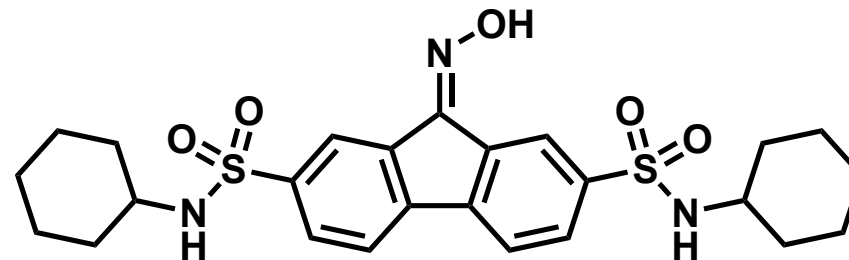


Sulfasalazine

Class 2: GPX4 inhibitors

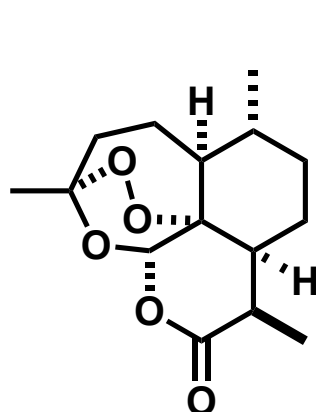


RSL3
covalent inhibitor

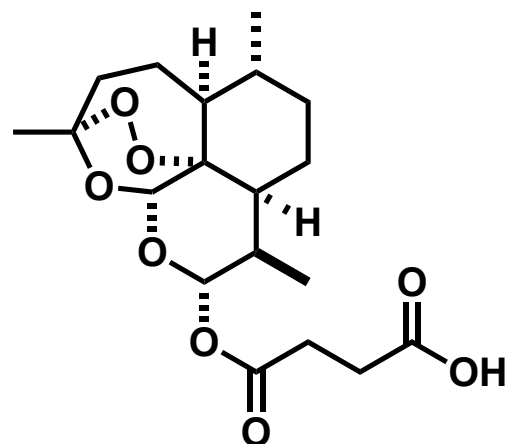


FIN56
inducing degradation of GPX4

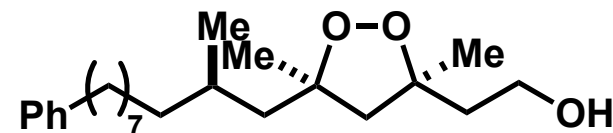
Natural Product Inducing Ferroptosis



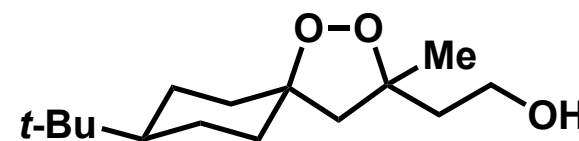
Artemisinin



Artemisinin

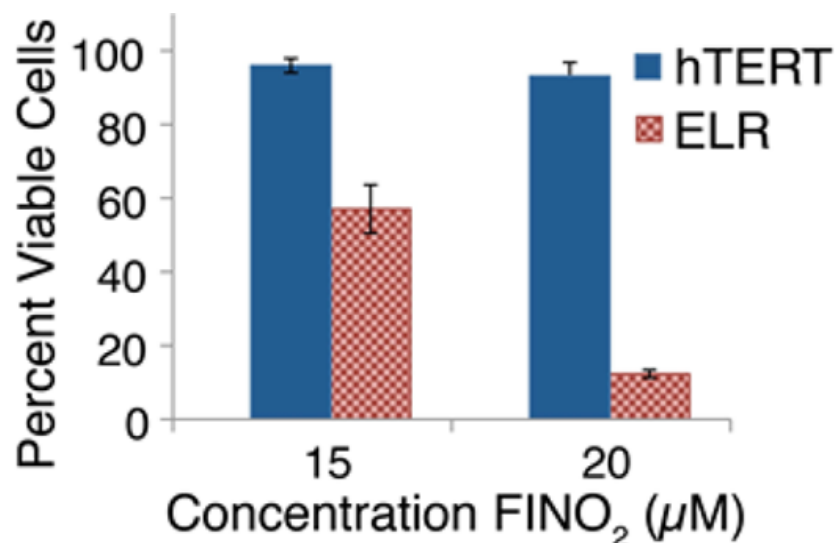


Plakinic acid J



FINO₂

analogues plakinic acid J



FINO₂ selectively induces cell death in Bj-eLR cancer cells compared to the isogenic, non cancerous BJ-hTERT

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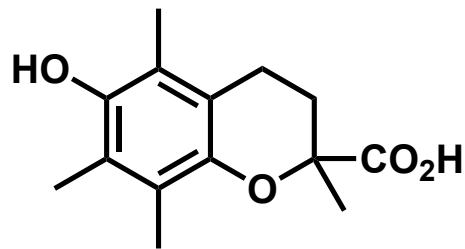
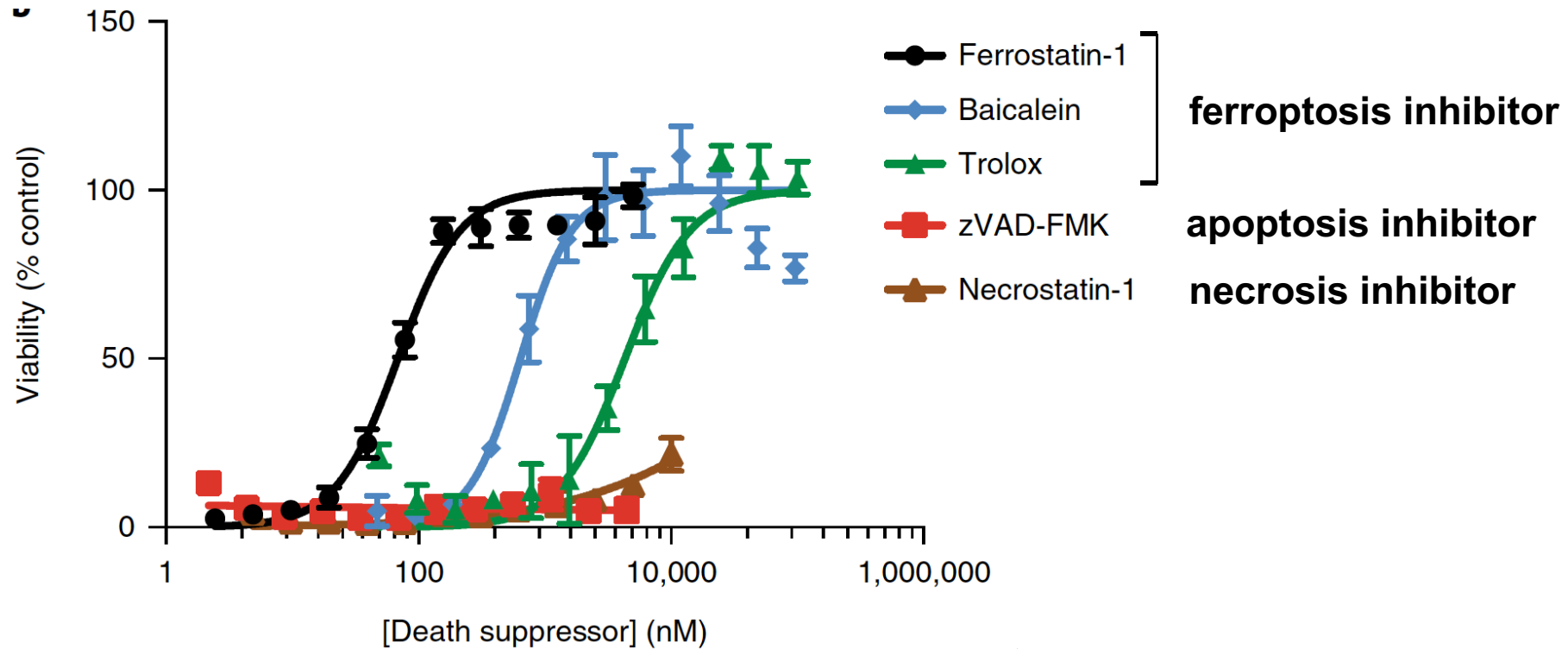
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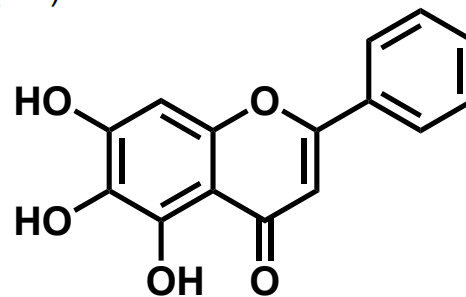
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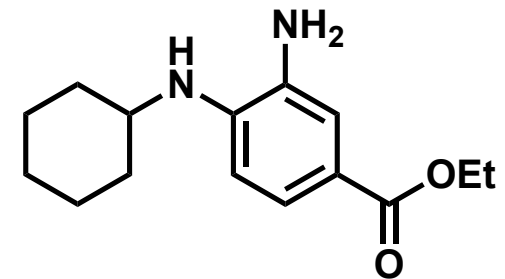
Ferroptosis Induced by FINO2



Trolox



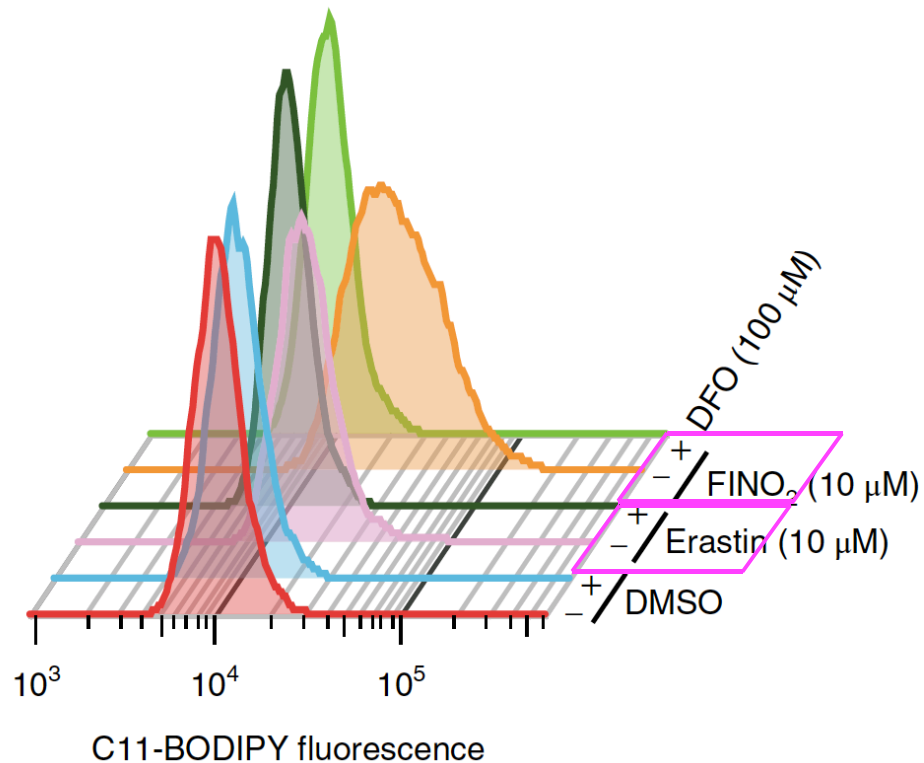
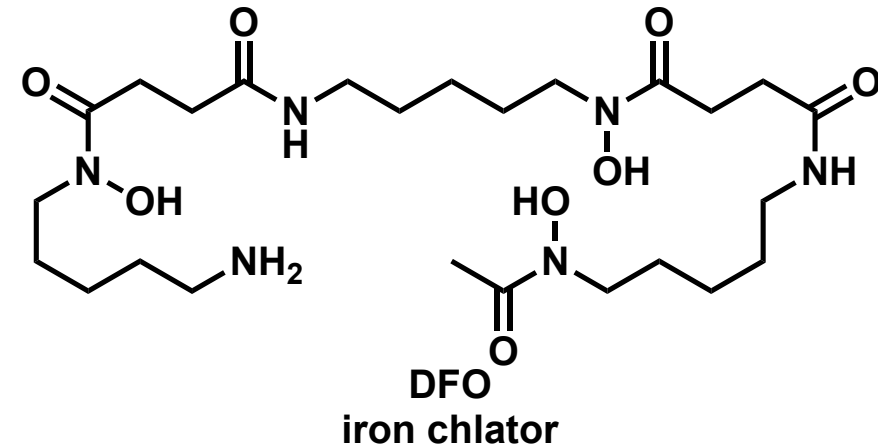
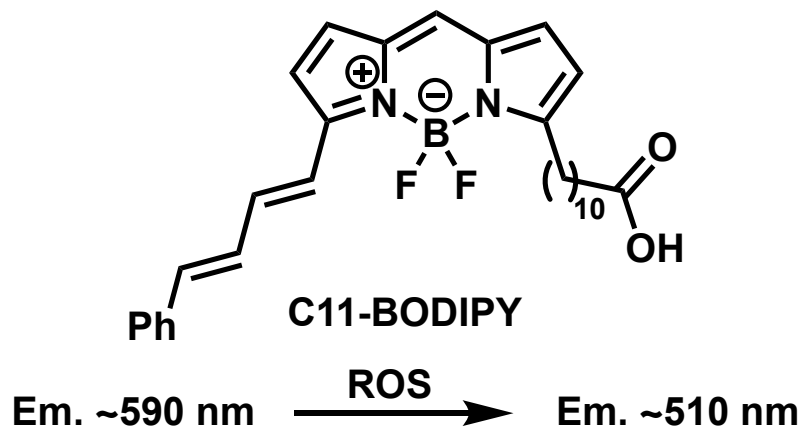
Baicalein



Ferrostatin-1

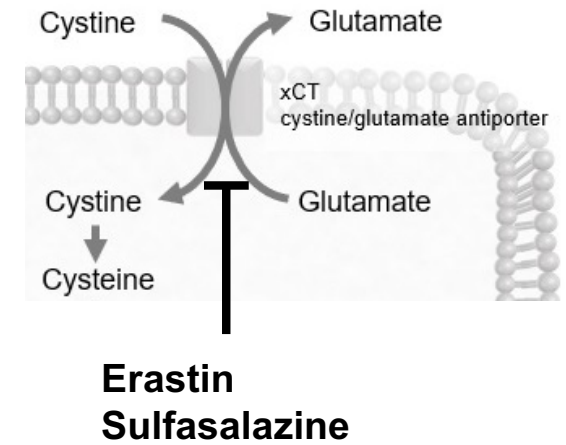
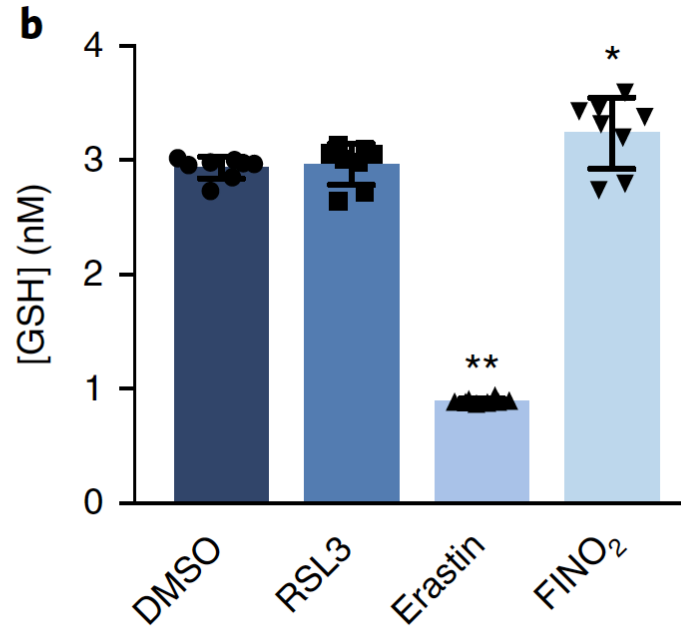
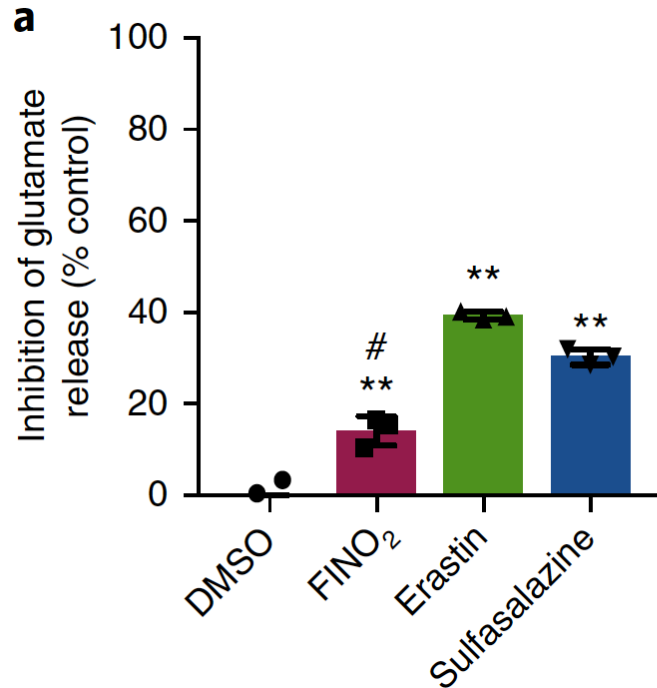
suppression of lethality of FINO2 by ferroptosis inhibitor
→induction of ferroptosis by FINO2

Ferroptosis Induced by FINO2 (2)

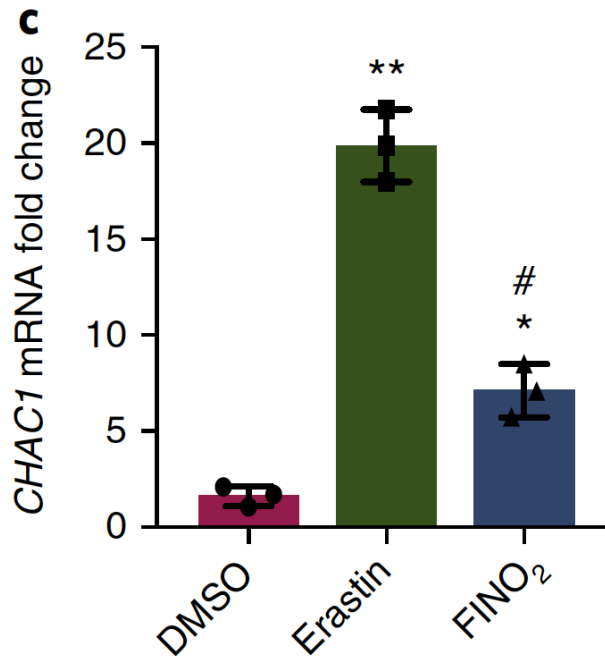


suppression of oxidation by iron chelator
 → iron dependency of lipid peroxidation

Mechanistic Study of FINO2 Comparing Erastin



FINO2 is not a system Xc⁻ inhibitor and does not deplete GSH



CHAC1:
gene encoding GSH specific γ -glutamylcyclotransferase enzyme

Induction of different transcriptional responses than erastin

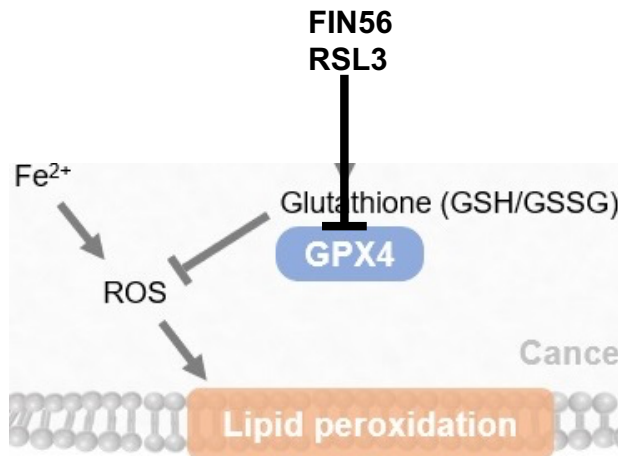
FINO2 does not display the characteristic functional or genetic hallmarks of a class 1 ferroptosis inducer

Inhibition of GPX4 by FINO2

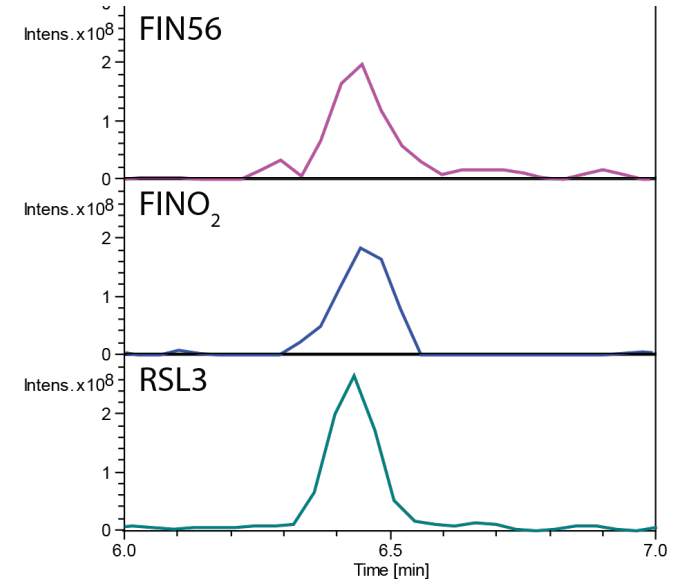
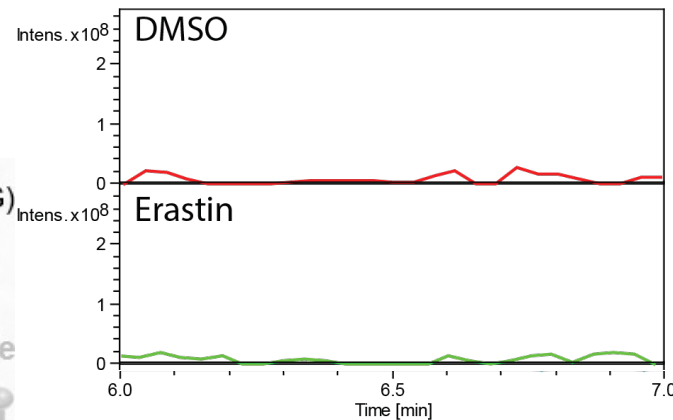
1. preincubation with compounds
 2. cell lysis
 3. incubation with PCOOH and GSH
1. extracted with choroform/MeOH
 2. LC-MS analysis for quantification of PCOOH



HT-1080 cells



PCOOH: phosphatidylcholine hydroperoxide



PCOOH was detected in the case of incubation with GPX4 inhibitor
→ FINO2 inhibited the activity of GPX4

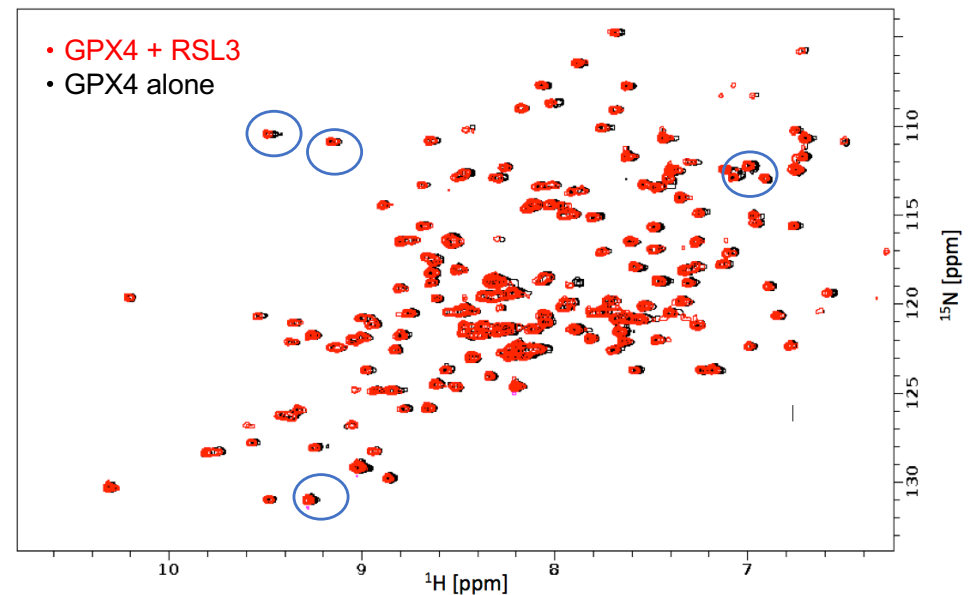
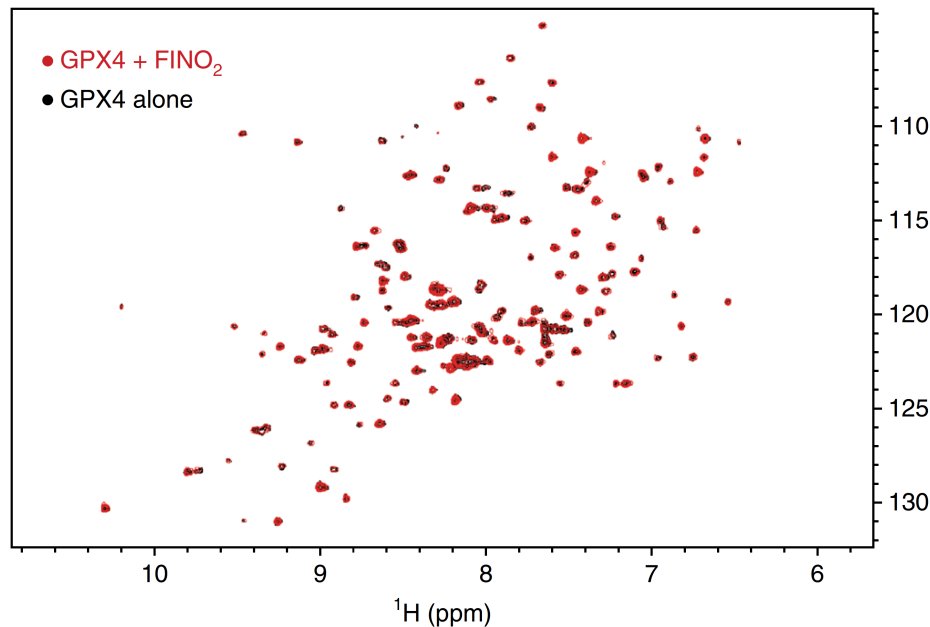
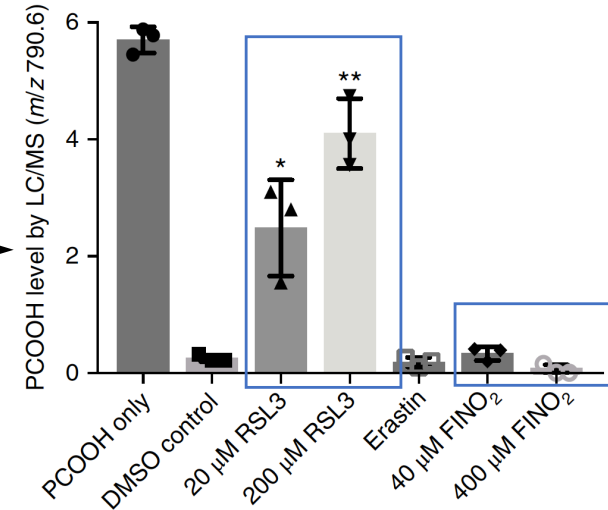
Indirect Interaction with GPX4 (1)

1. cell lysis
2. preincubation with compounds
3. incubation with PCOOH and GSH
4. extracted with choroform/MeOH
5. LC-MS analysis for quantification of PCOOH



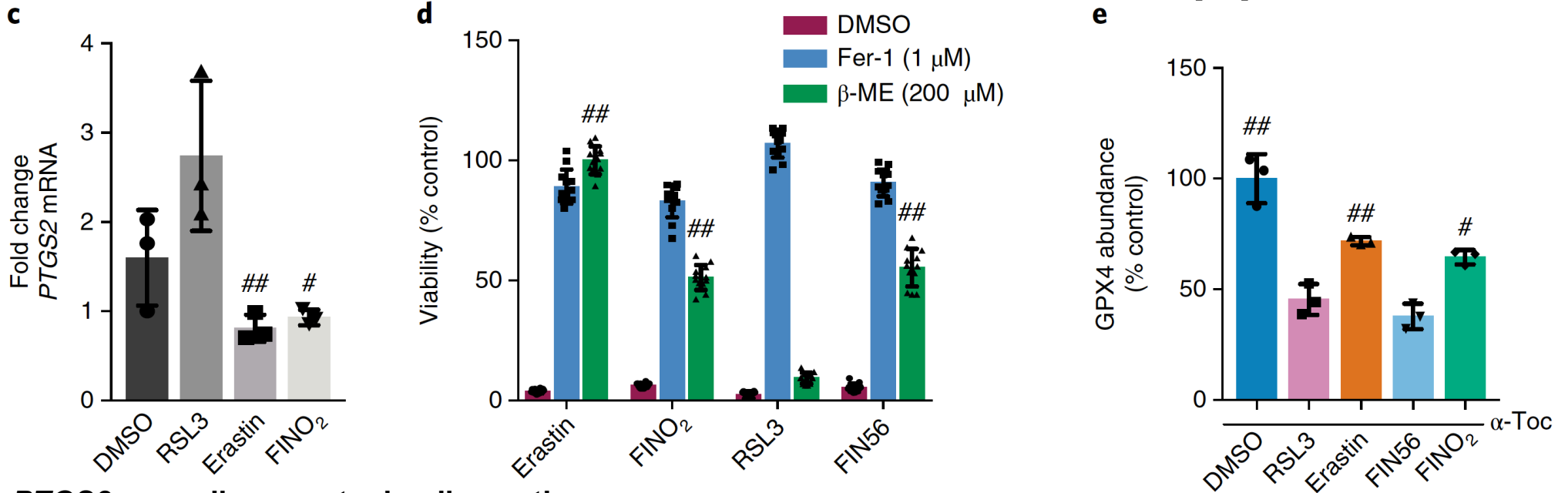
HT-1080 cells

*cell lysis was conducted prior to preincubation with compounds

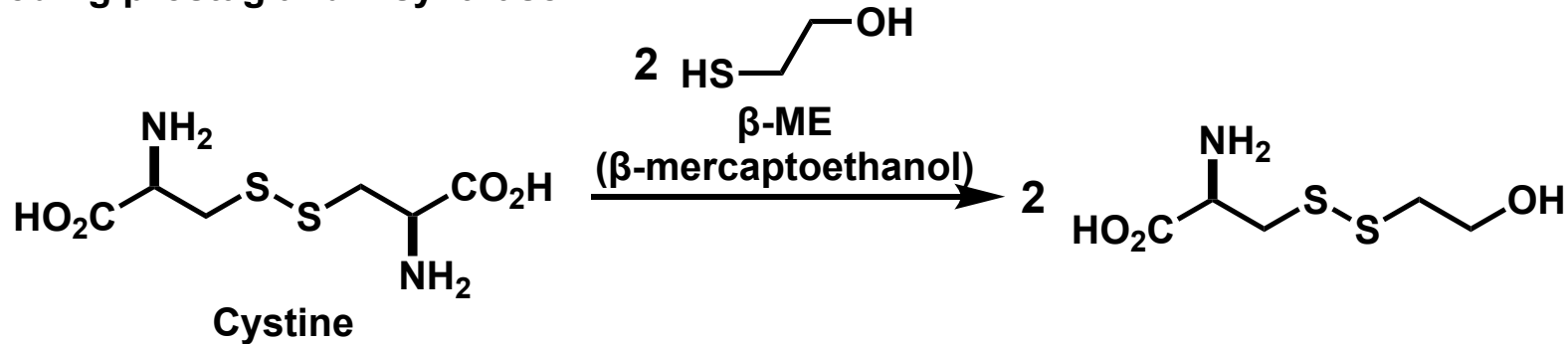


HSQC spectrum of GPX4 did not change with FONO2→indirect inhibition of GPX4

Indirect Interaction with GPX4 (2)



PTGS2: encoding prostaglandin synthase



bypassing xc⁻ system and increasing intracellular cysteine availability

FINO2 induces ferroptosis in different mechanism other than calss 1 and class2 ferroptosis inducer

SAR study was conducted to determine functional group required for inducing ferroptosis

SAR Study of FINO₂ (1)

SAR study was conducted to determine functional group required for inducing ferroptosis



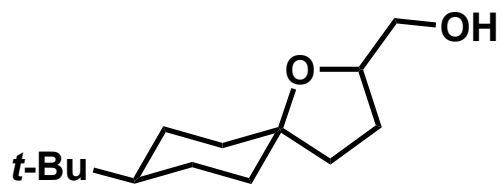
>100 μ M, all cells



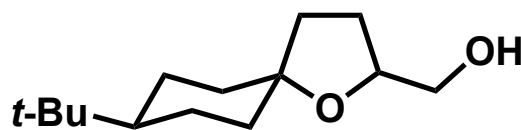
>100 μ M, all cells



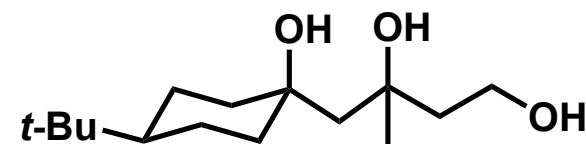
>100 μ M, all cells



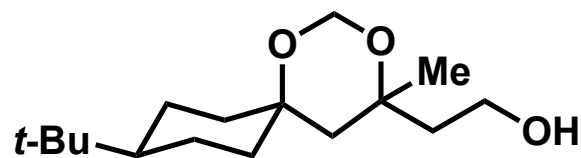
>100 μ M, all cells



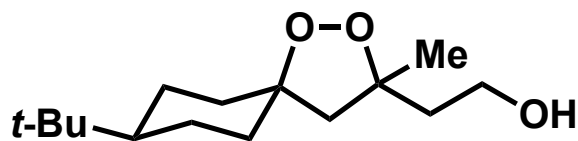
>100 μ M, all cells



>100 μ M, all cells



>100 μ M, all cells



FINO₂

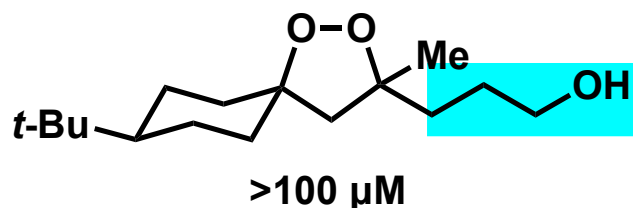
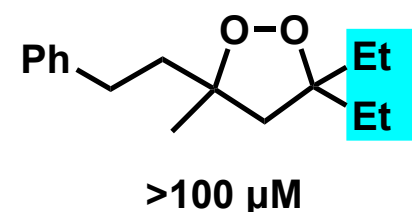
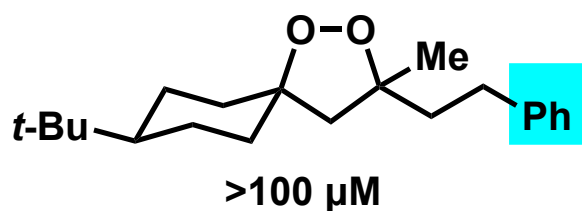
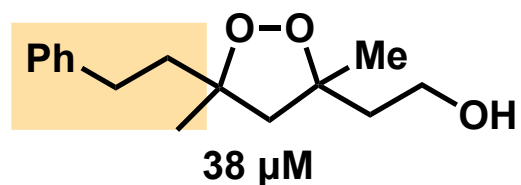
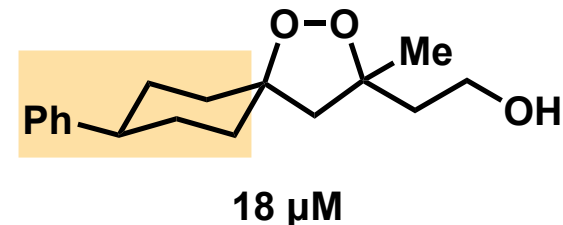
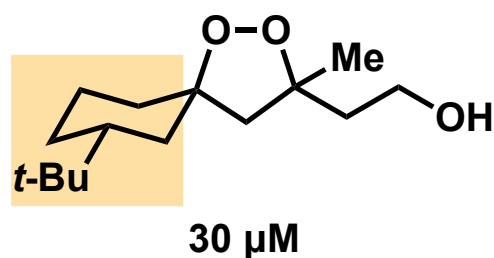
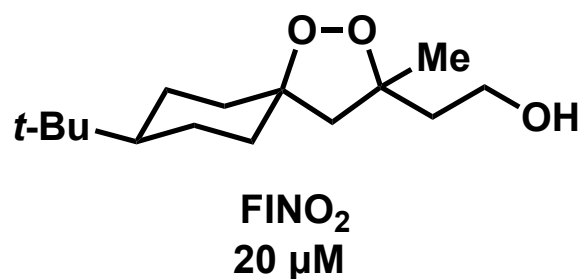
20 μ M, CHK-1

11 μ M, BJ-eLR

23 μ M, BJ-hTERT

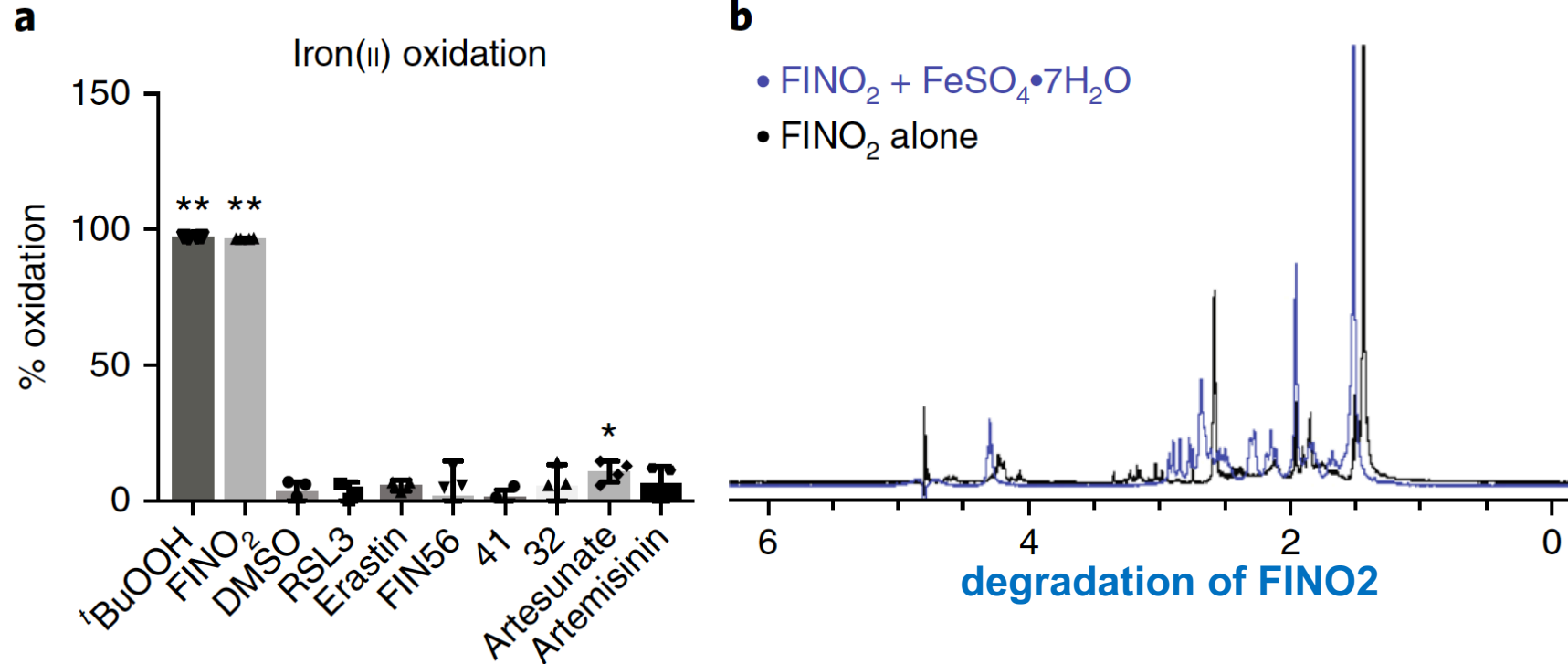
The activities of nonperoxide derivatives were significantly lower than FINO₂.
→The peroxide moiety was essential for the induction of ferroptosis.

SAR Study of FINO₂ (2)

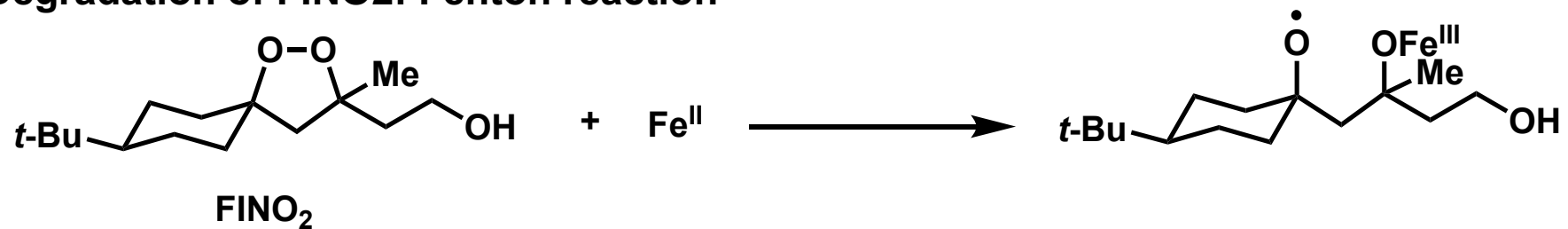


- *t*-Bu group and spirobicyclic core structure is not required
- The hydroxy portion of FINO₂ must be present and have a specific spatial relationship to the peroxide.

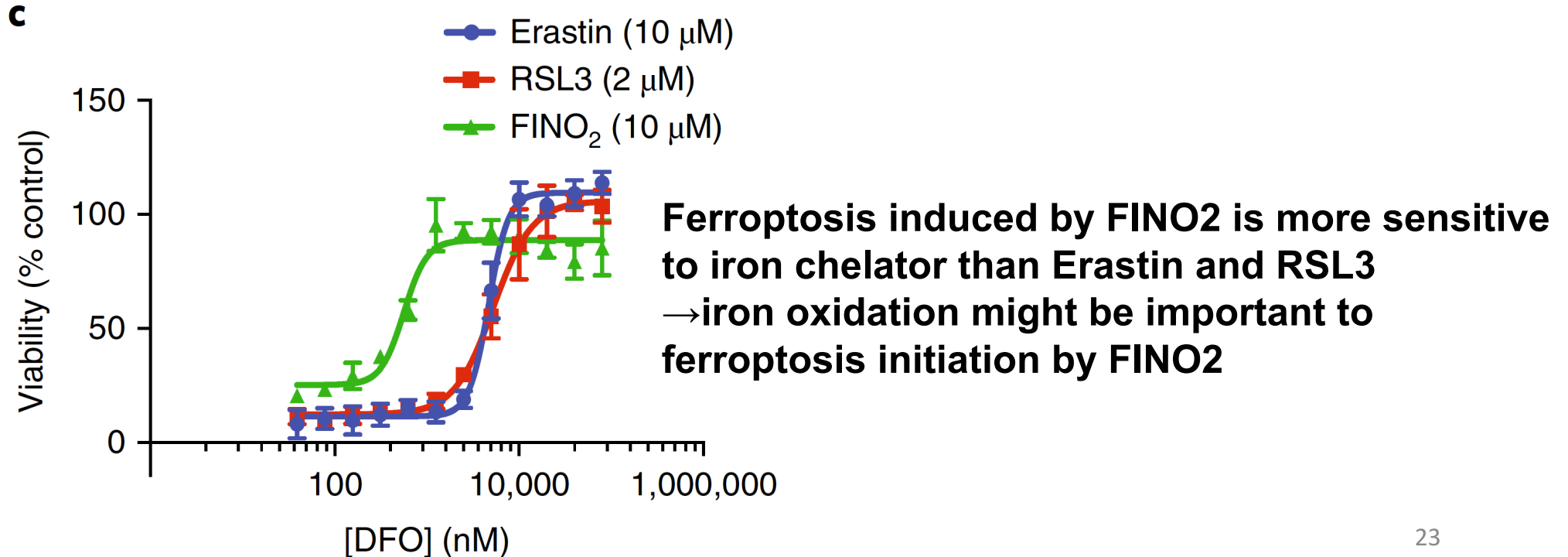
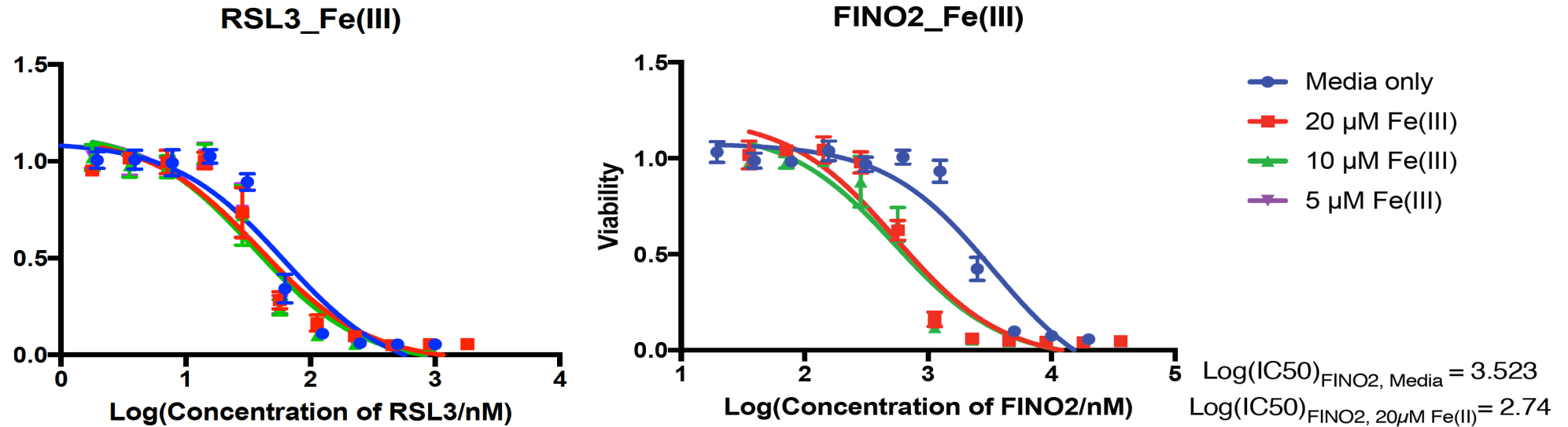
Direct Oxidation of Fe(II) (1)



Degradation of FINO₂: Fenton reaction

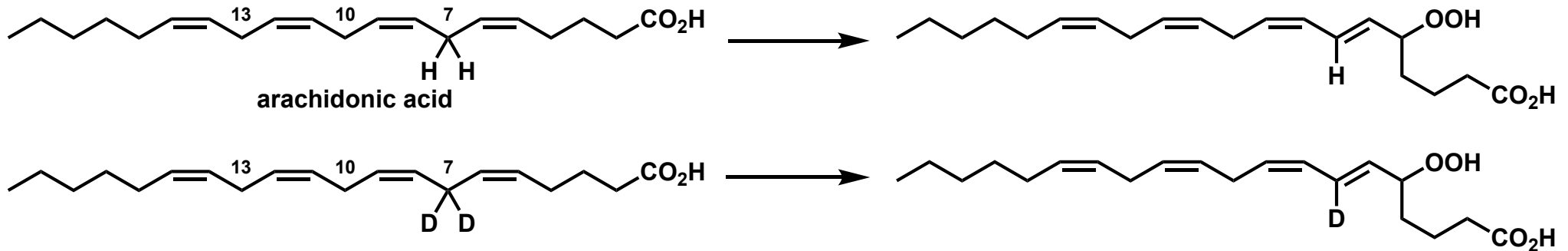


Direct Oxidation of Fe(II) (2)

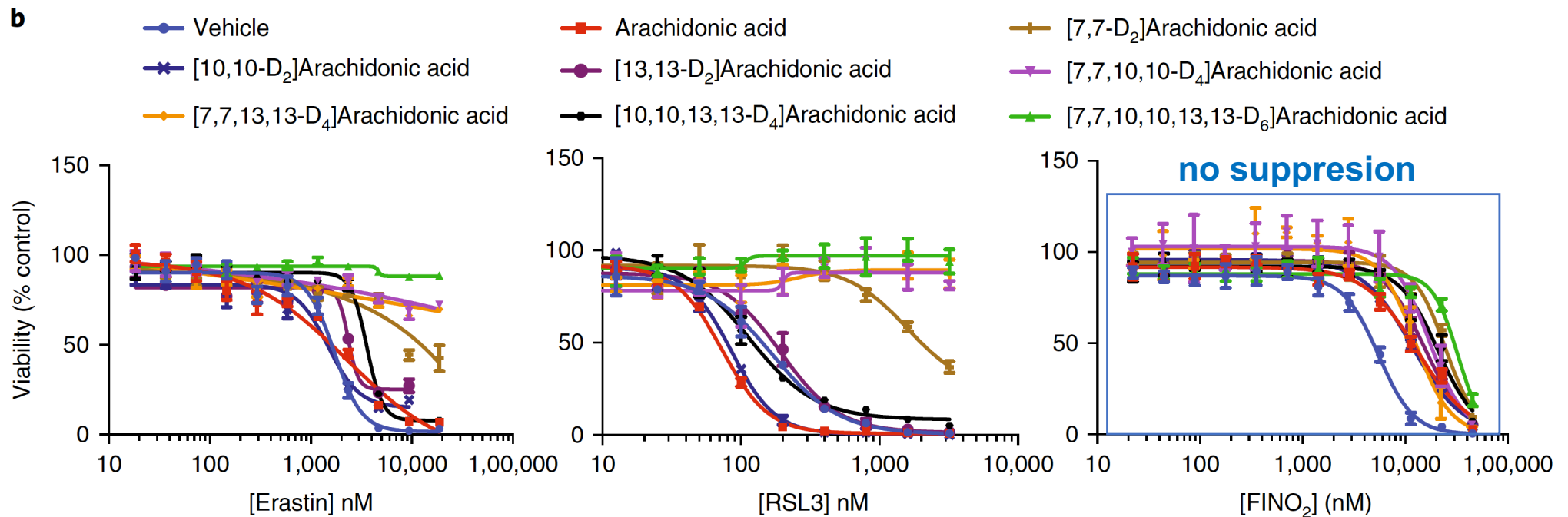


Lipid Oxidation by FINO2

Deuteration at bis-allylic position → kinetic isotope effect in lipoxygenase enzymes



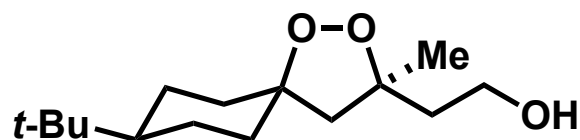
suppression of ferroptosis → dependent on oxidation by ALOX



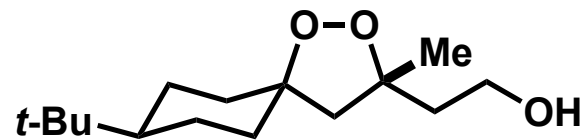
Ferroptosis induced by FINO2 does not require ALOX peroxidation

Remaining Question

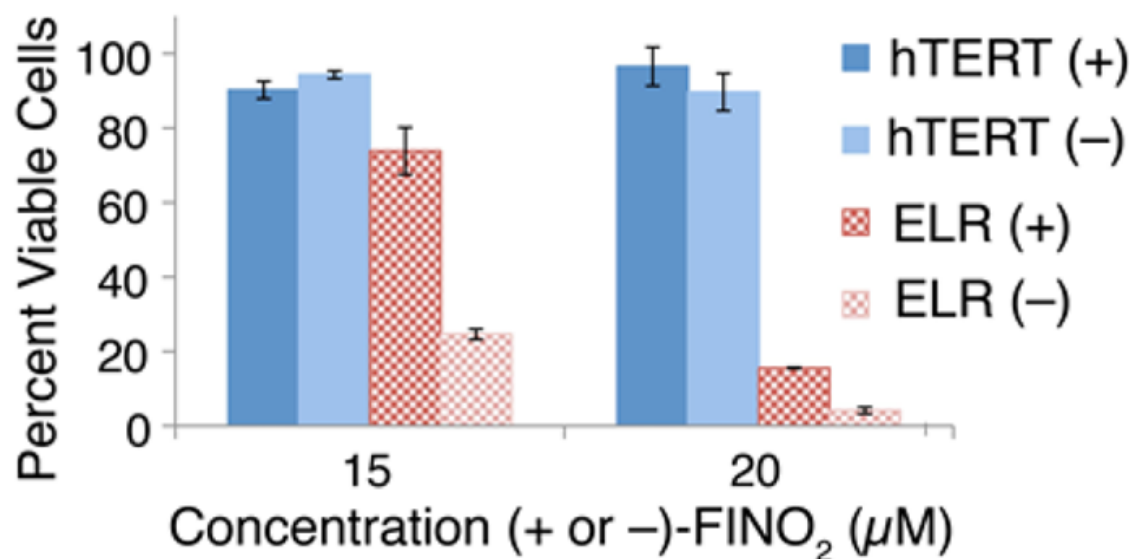
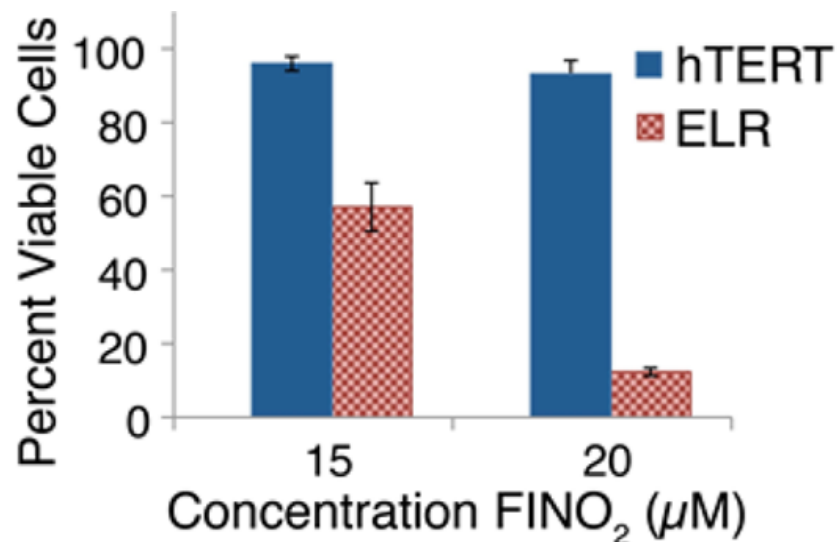
Mechanism of indirect GPX4 inactivation



(-)-FINO₂



(+)-FINO₂



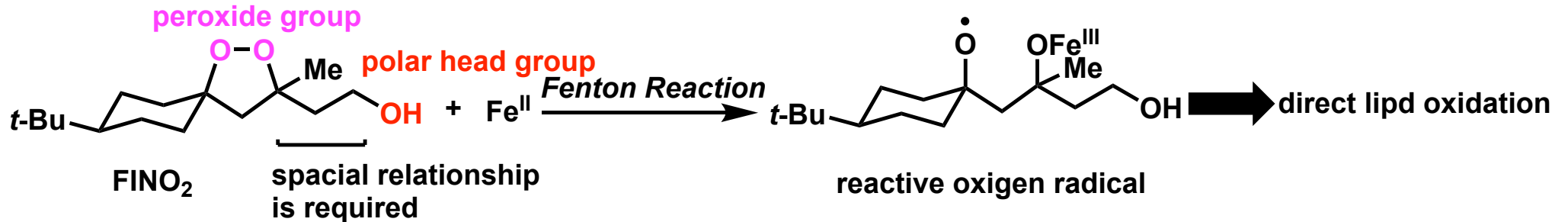
The increased activity of one enantiomer

→FINO2 might have a specific protein interaction necessary for its activity

Summary

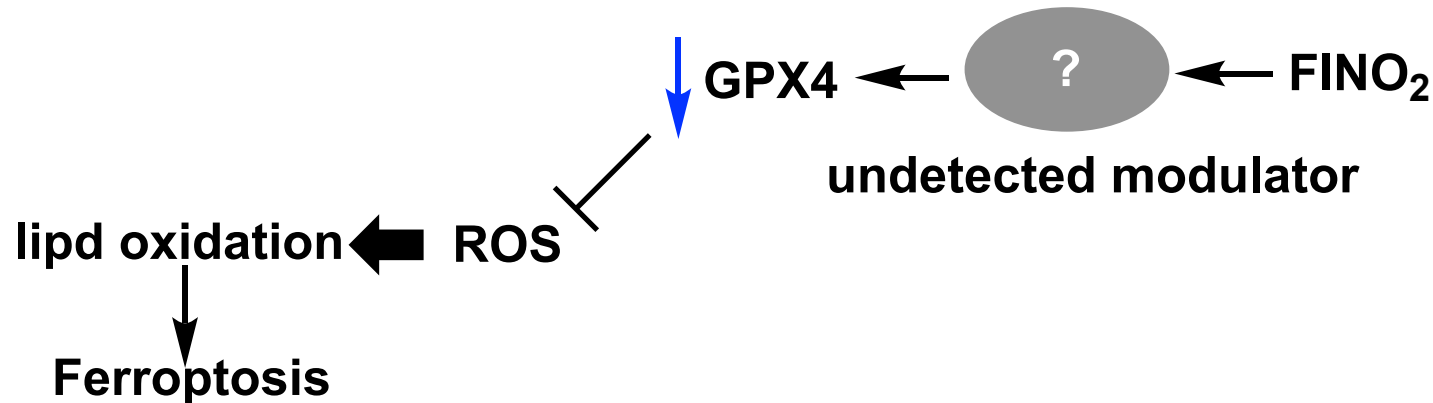
FINO₂ induces ferroptosis by

1. direct oxidation of ferroptosis-relevant substrates



2. indirect GPX4 inactivation

- no degradation of GPX4
- neither an allosteric nor active site ligand of GPX4



- FINO₂ is a new class of ferroptosis inducer
- Undetected targets of FINO₂ may be important regulators of ferroptosis