



Shielding of Phospholipid Oxidation by 5,7-unsaturated Sterol Metabolites

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What is ferroptosis?

Regulation of membrane redox state

Survival



Selenoprotein
Glutathione Peroxidase 4

Ferroptosis is a terminal process driven by lipid peroxidation

GPX4 a central regulator of membrane redox state



Ursini et al, 1982

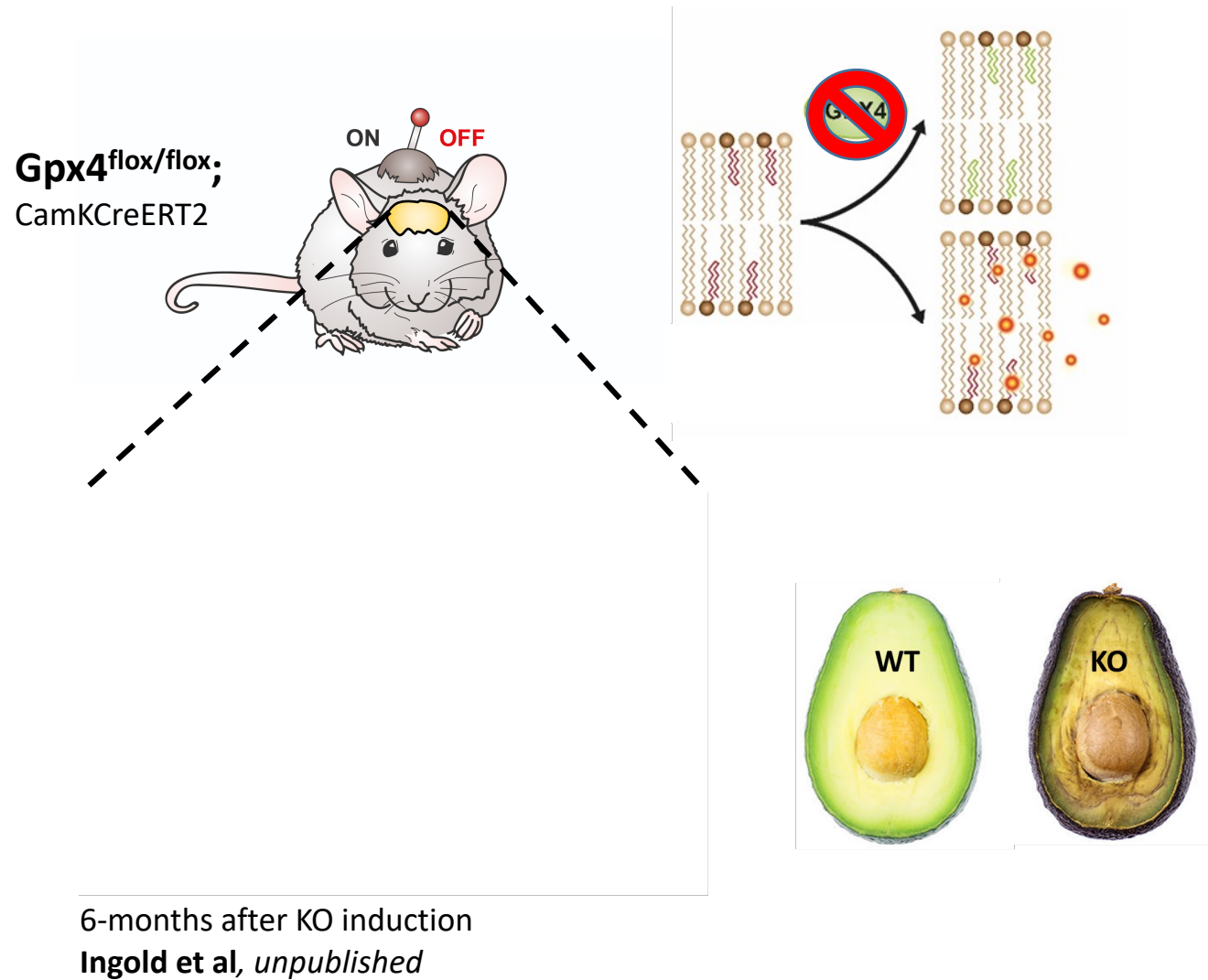


LIPID PEROXIDATION

- Is a **selenoprotein**
- One out of 8 glutathione peroxidases in mammals
- Has a **cytosolic**, mitochondrial and a nuclear form
- The **only** enzyme efficiently reducing phospholipid hydroperoxides (PLOOH)
- Utilizes, but it **not restricted** to, GSH as a substrate
- The central regulator of **ferroptosis**

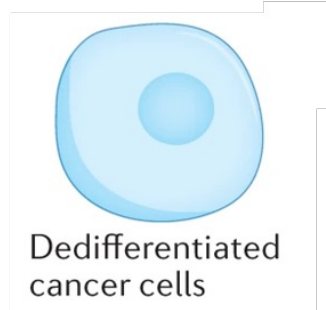
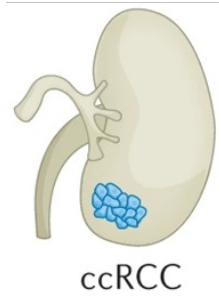
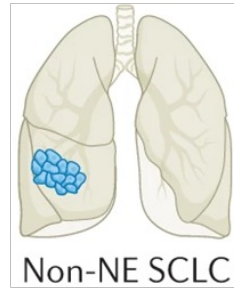
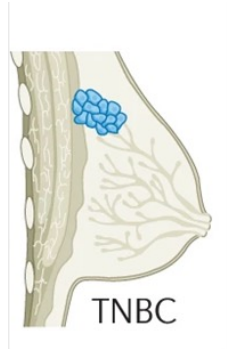
How relevant is GPX4 for life?

Prevention of lipid peroxidation – *in vivo* relevance



Phospholipid damage is actively taking place (and repaired)

Lipid peroxidation - cancer progression and therapy



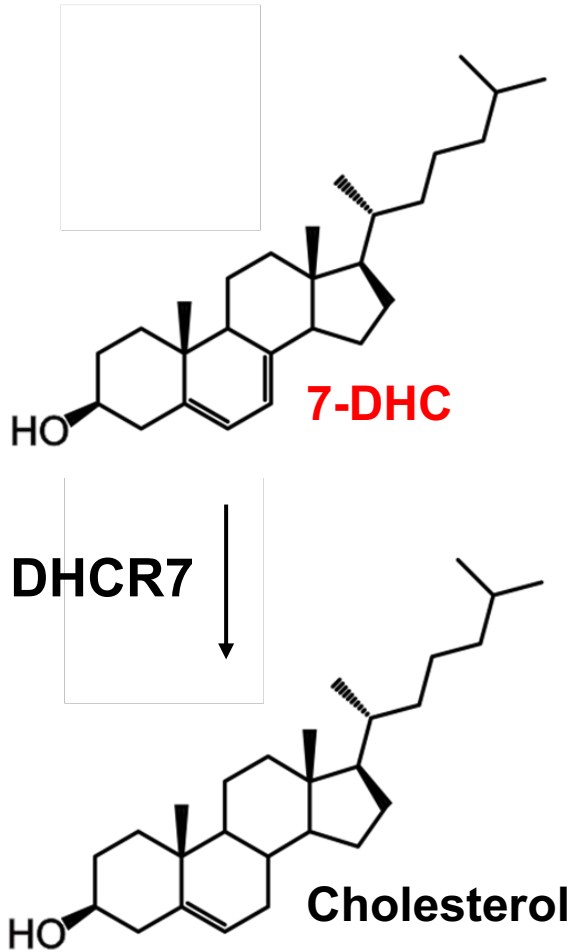
Bebber et al., 2021, *Nat Comm*; Doll et al., 2017, *Nat Chem Bio*. Vishwanathan et al., 2018, *Nature*. Zou et al., 2020, *Nat Comm*. Lei et al., *Nat Rev Cancer*, 2022. Alborzinia et al., 2023, *Embo Mol Med*. Alborzinia et al., 2022, *Nat Cancer*.

GPX4 inhibition induces rapid cell death in specific cancer lineages/st

Identification of factors regulating ferroptosis

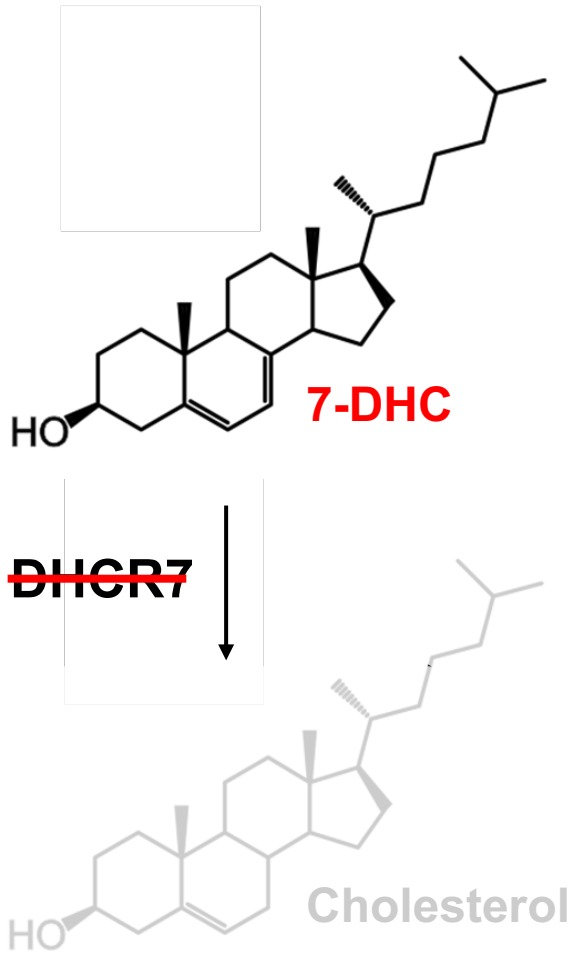
DHCR7 is a yet unidentified regulator of ferroptosis

DHCR7 and lipid peroxidation



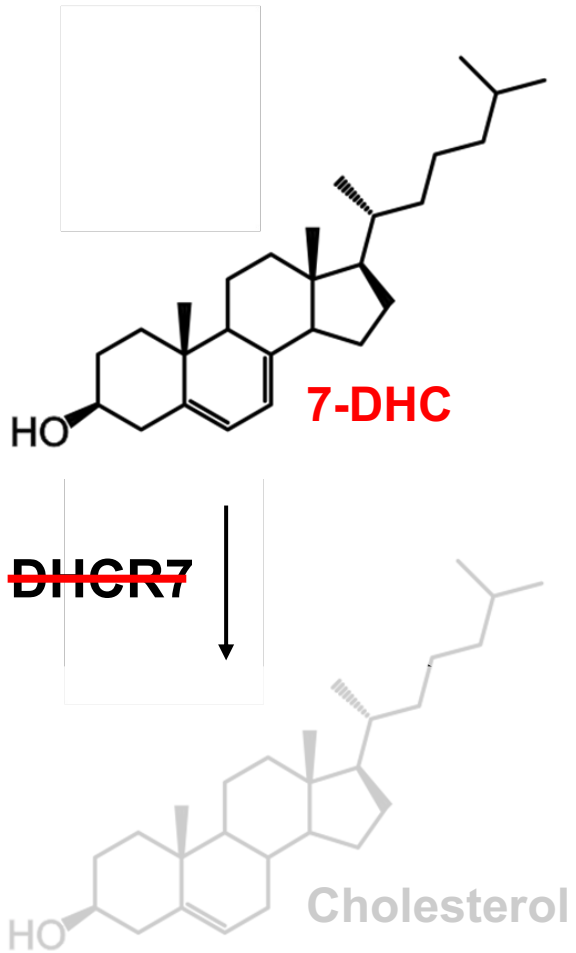
- DHCR7 catalyses the last step in cholesterol biosynthesis
- DHCR7 loss is responsible for the metabolic syndrome Smith Lemli Optiz Syndrome (SLOS)
- 7-DHC accumulation is associated to loss of membrane redox homeostasis and increased lipid peroxidation
- Antioxidants, such as vitamin E, ameliorate SLOS symptoms in model organisms

Generation of DHCR7 deficient cellular models



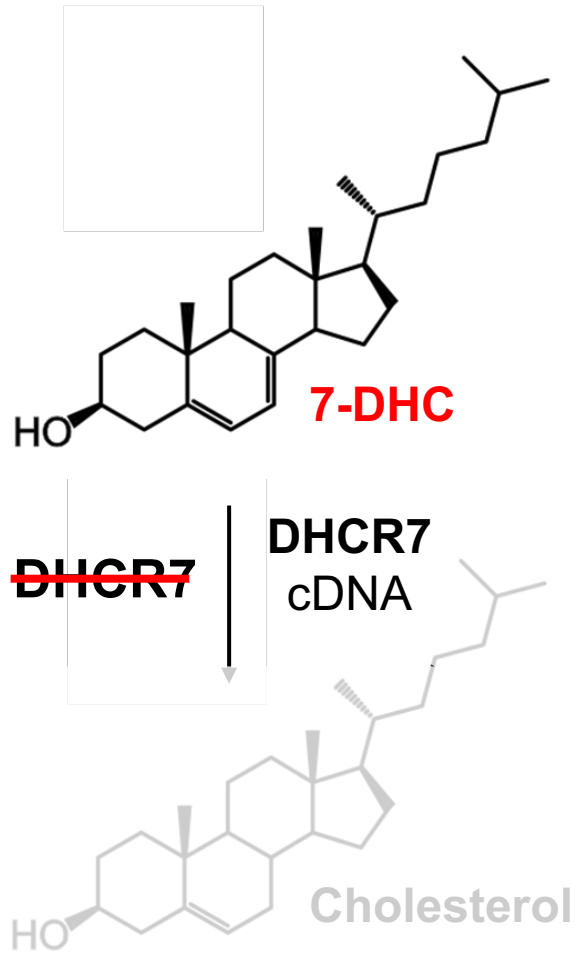
Loss of DHCR7 leads to the accumulation of 7-DHC

Consequence of DHCR7 loss on the response to GPX4 inhibitors



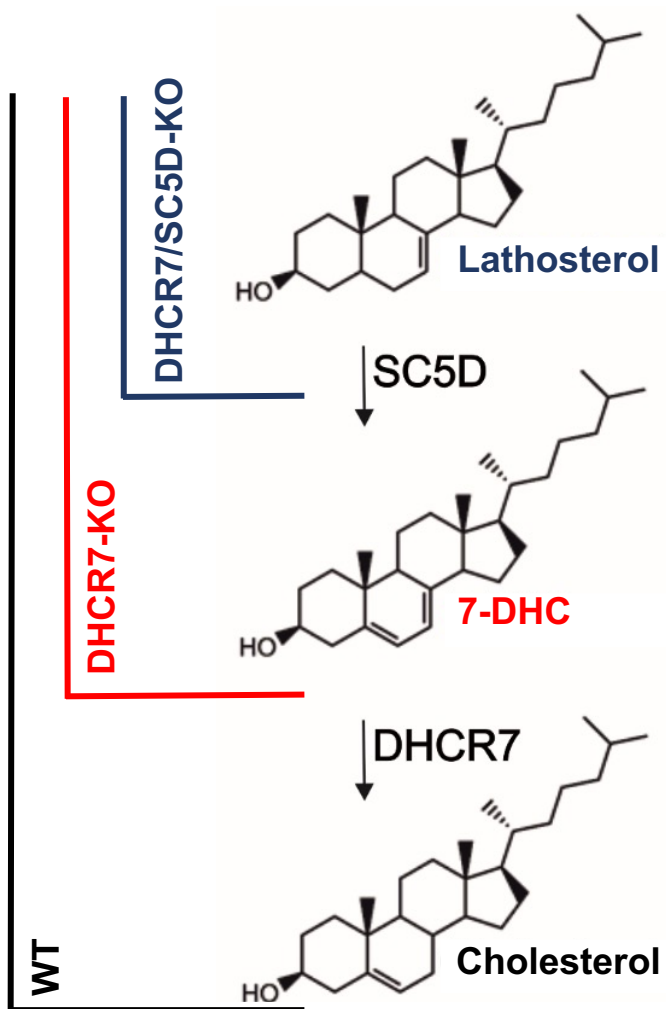
DHCR7 loss results in increased resistance to GPX4 inhibitors

DHCR7 reconstitution and response to GPX4 inhibitors



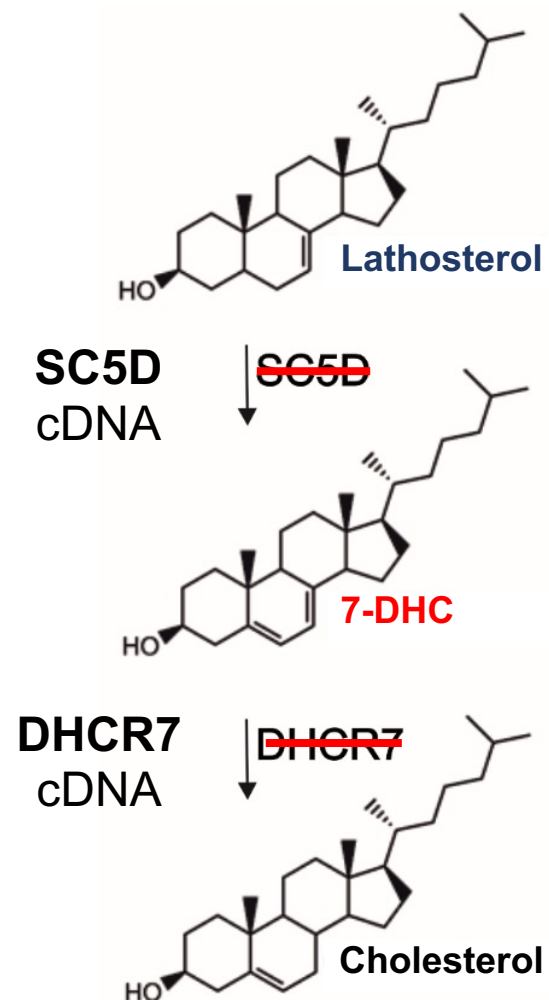
Re-expression of DHCR7 re-sensitizes cells to GPX4i induced cell death

Impact of 7-DHC accumulation on cell death induced by GPX4i



7-DHC accumulation contributes to resistance to ferroptosis

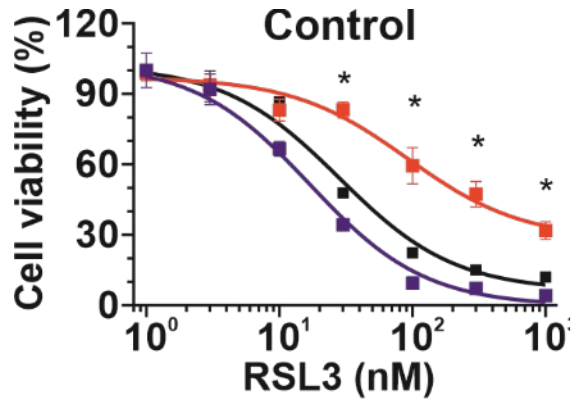
Restoring DHCR7 and SC5D in double deficient cells



Only cells able to produce 7-DHC are protected from ferroptosis

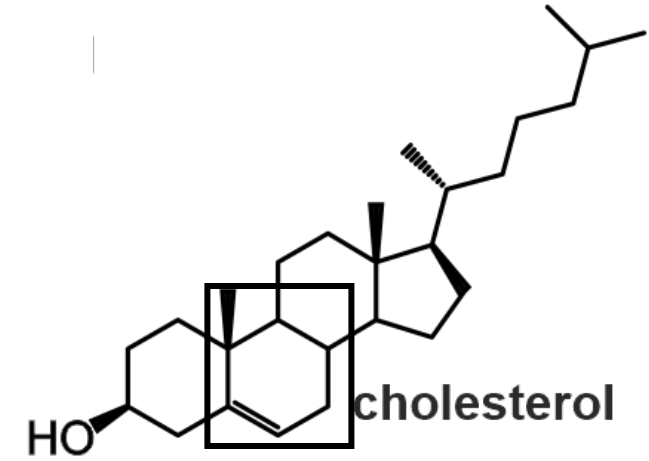
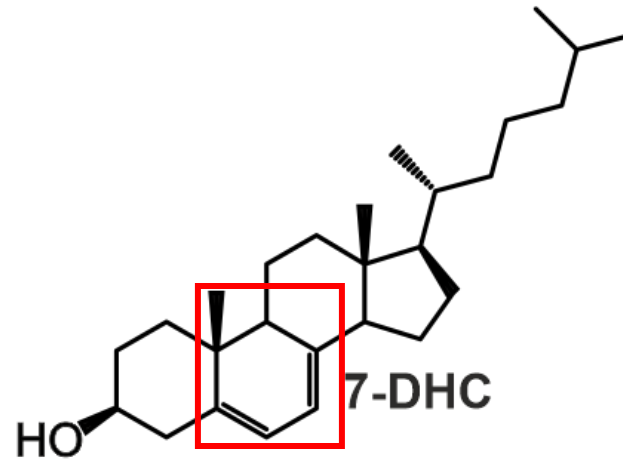
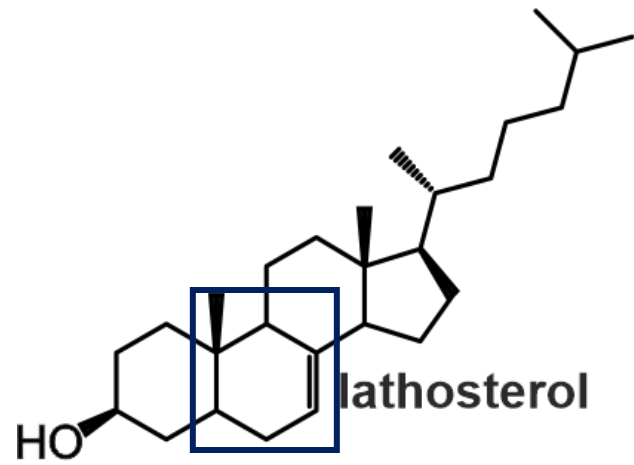
Sterol supplementation and resistance to GPX4i

■ WT ■ DHCR7-KO ■ DHCR7/SC5D-KO



Cells able to build up 7-DHC are protected from ferroptosis

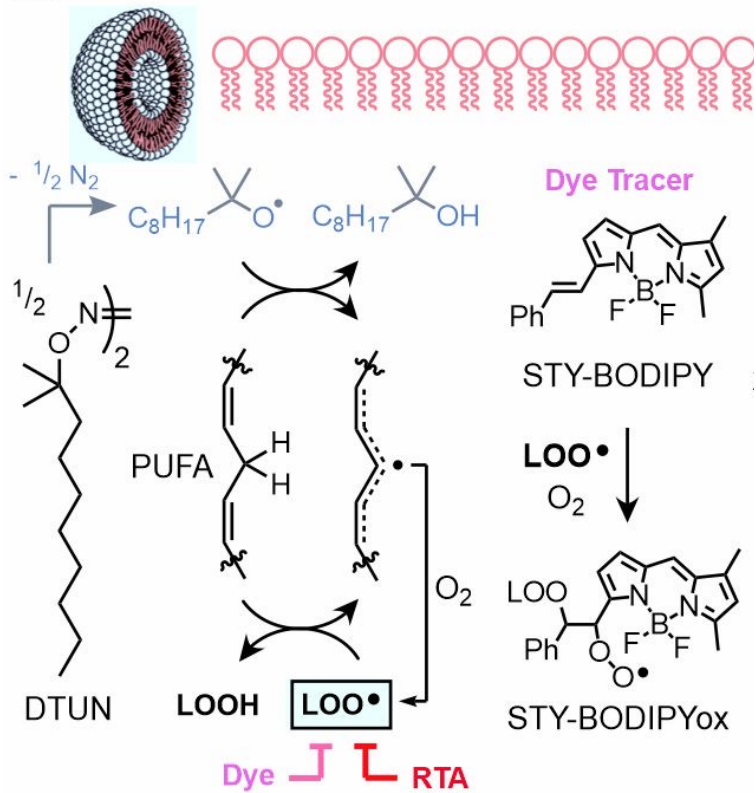
Where is the difference?



7-DHC is 200x more „oxidizable“ than cholesterol/lathosterol

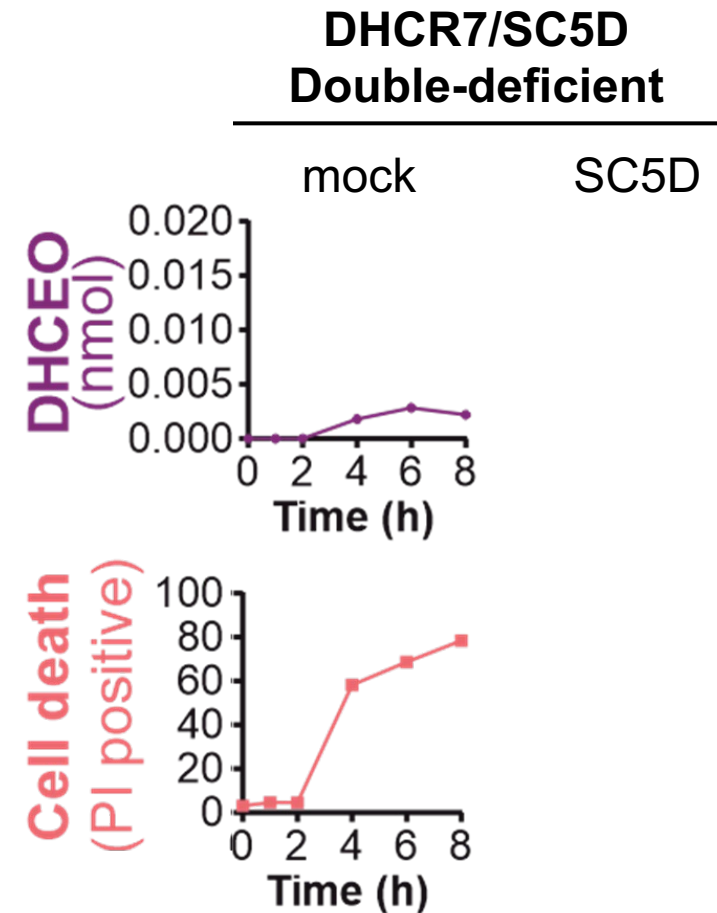
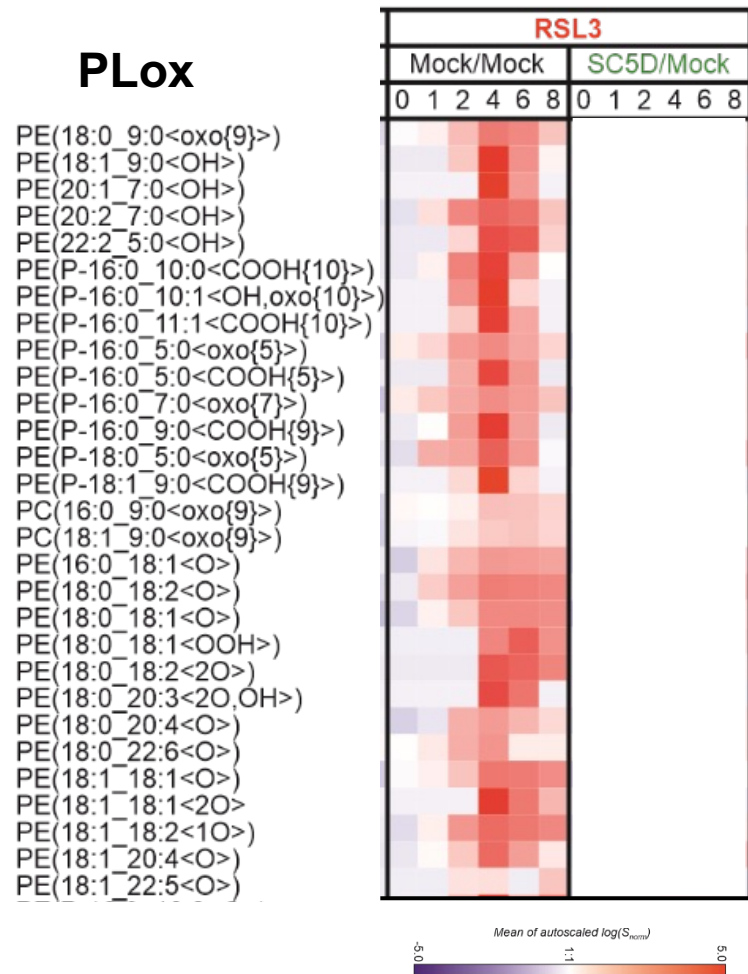
Oxidation rate constants: studies by Porter and colleagues (reviewed in Lamberson et al., Chem Phys Lipids 2017)

Consequence of 7-DHC accumulation on liposomal oxidation



7-DHC inhibits the propagation of (phospho)lipid peroxidation

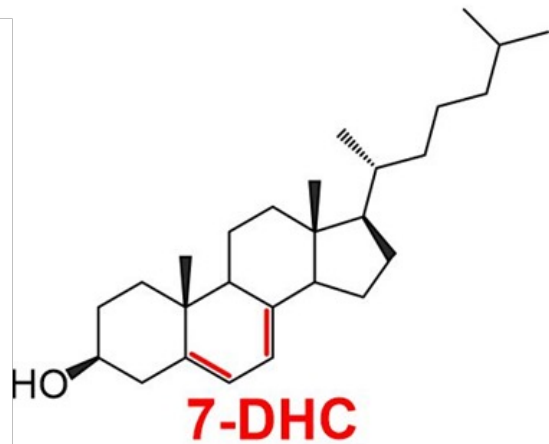
Consequence of 7-DHC accumulation on lipidomic oxidation



7-DHC oxidation shields PL from oxidation and prevent cell death

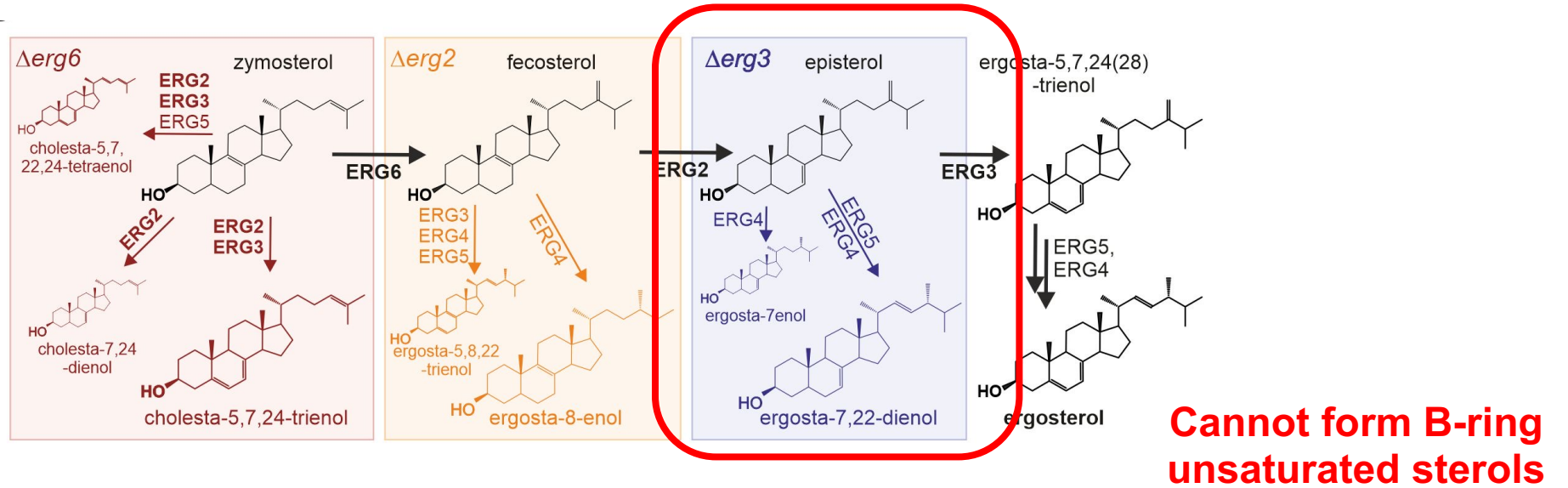


Impact of 5,7- unsaturated sterols on ferroptosis



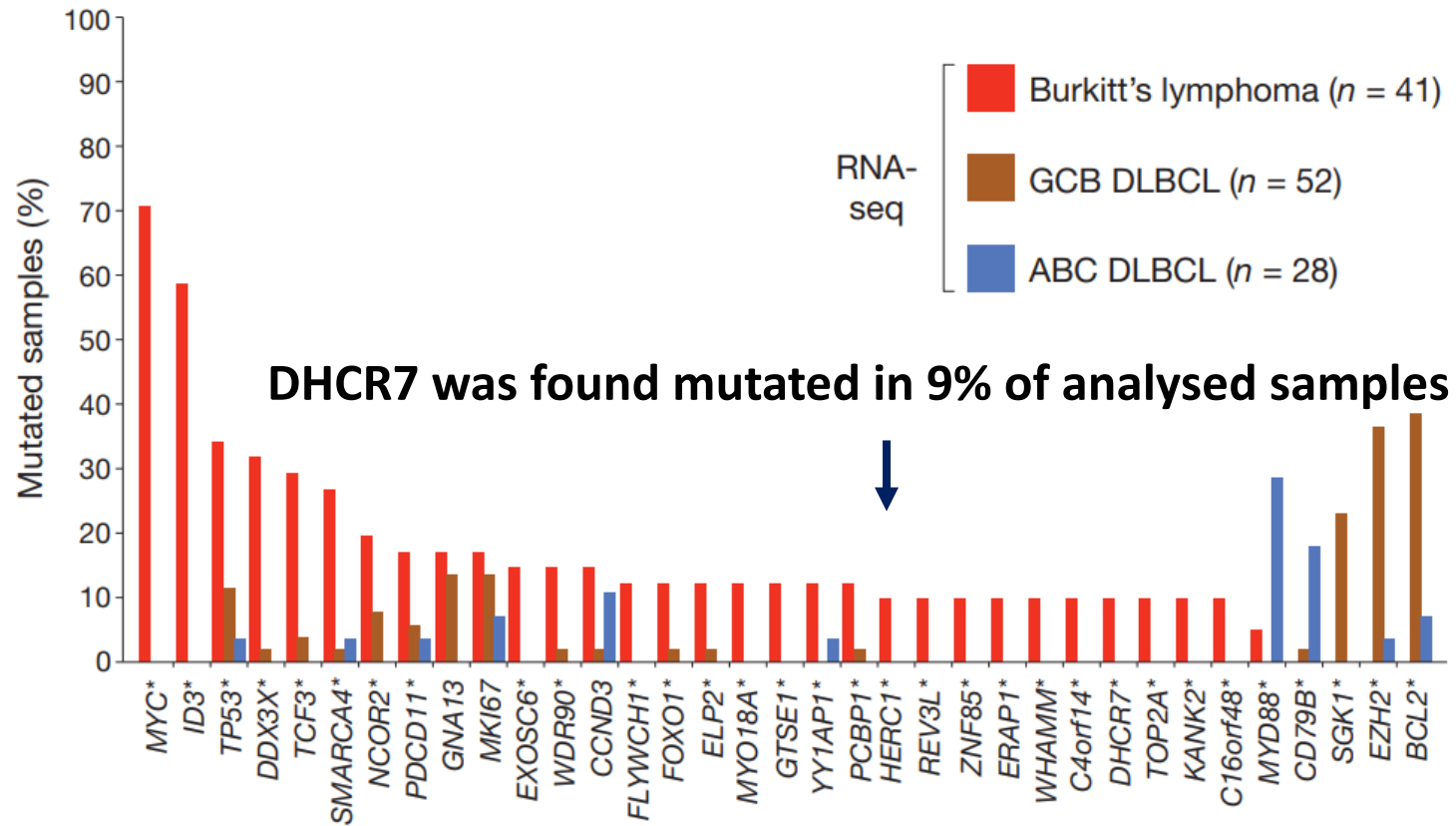
B-ring unsaturated sterols are potent inhibitors of ferroptosis

Impact of unsaturated sterols on yeast tolerance to PUFAs



B-ring unsaturated sterols buffer PUFA mediated toxicity in yeast

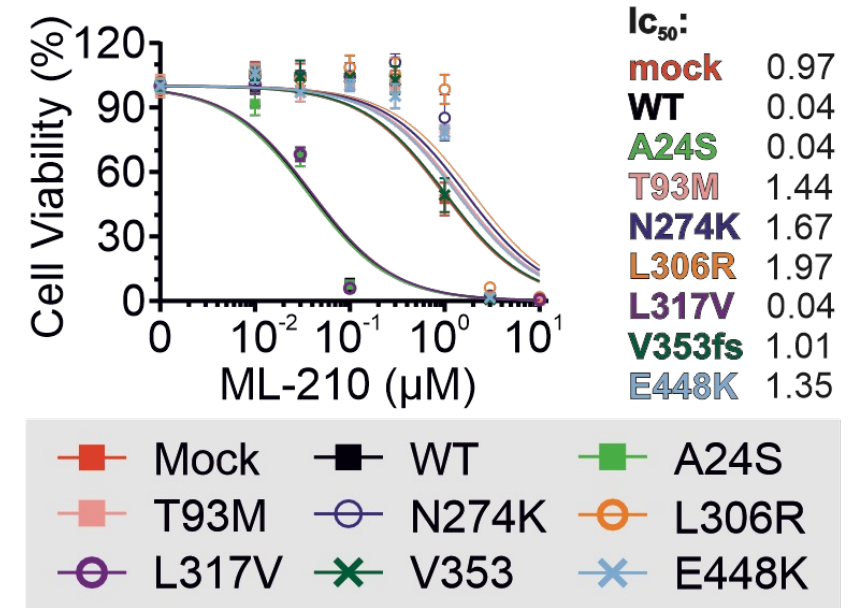
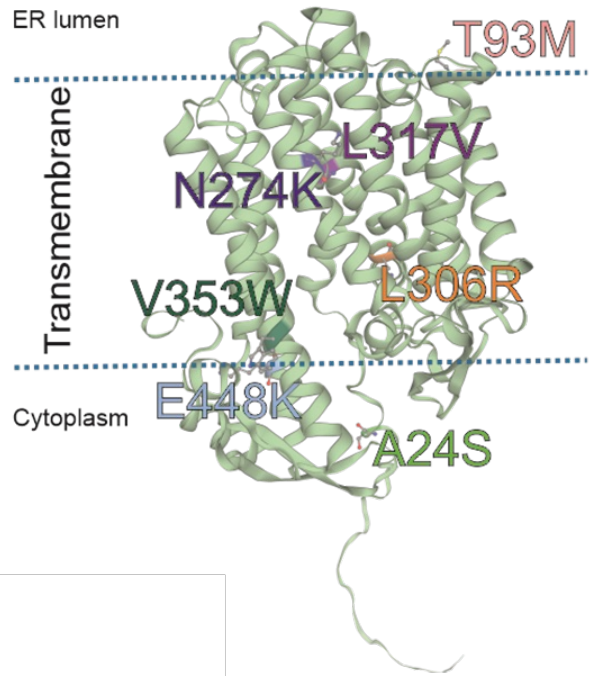
DHCR7 role in cancer progression?



Schmitz et al., *Nature*, 2012
Bonfiglio et al., *EBioMed*, 2023

DHCR7 mutation have been reported in Burkitt's Lymphoma and Neuroblastoma

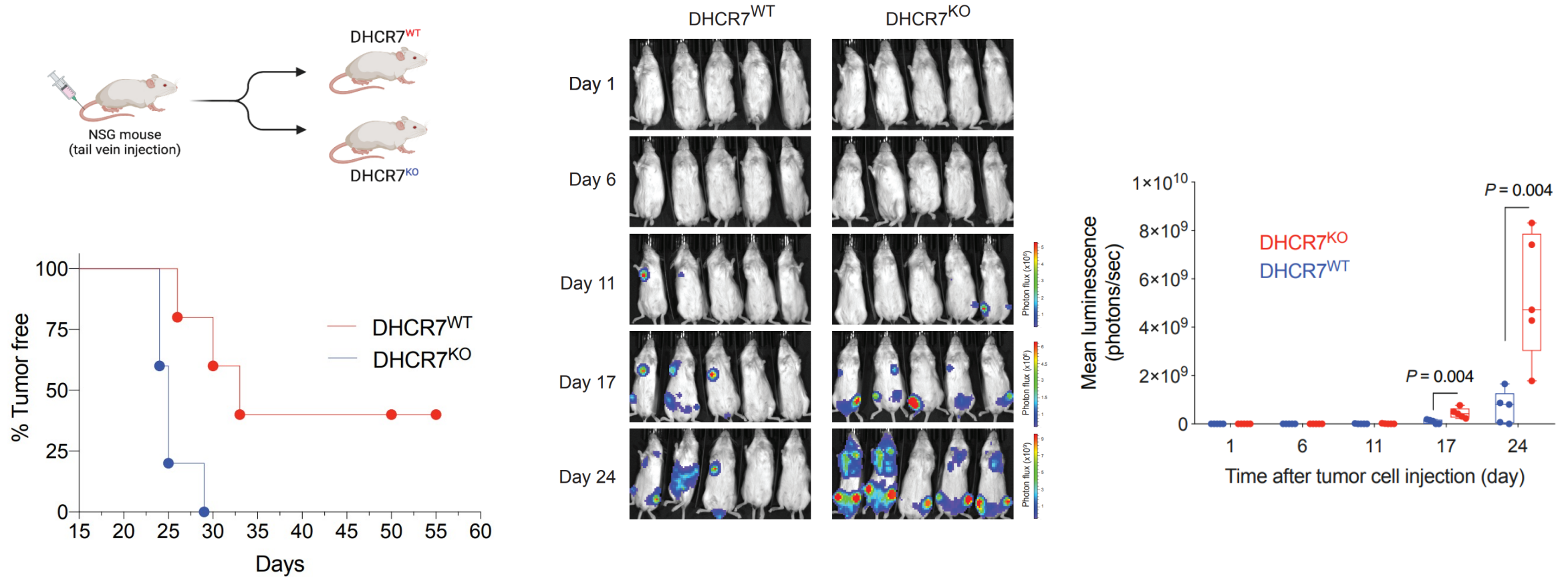
Functional characterization of DHCR7 mutations



DHCR7 mutations reported in NB and BL are loss of function

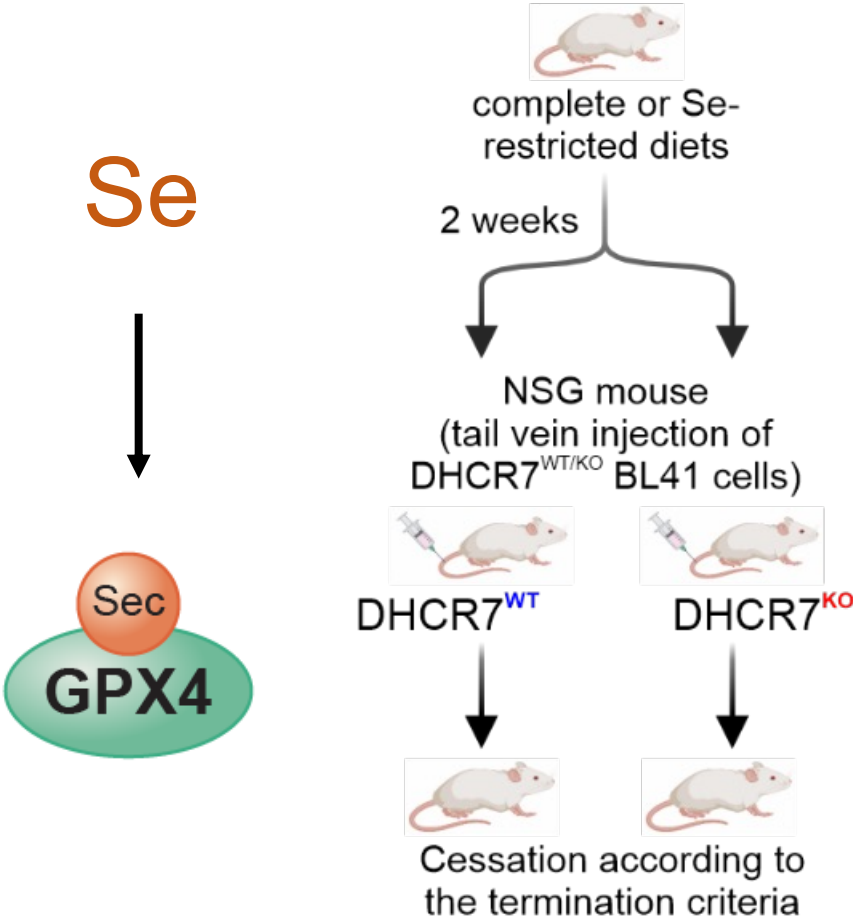
Consequence of DHCR7 loss on the growth of BL *in vivo*

BL41



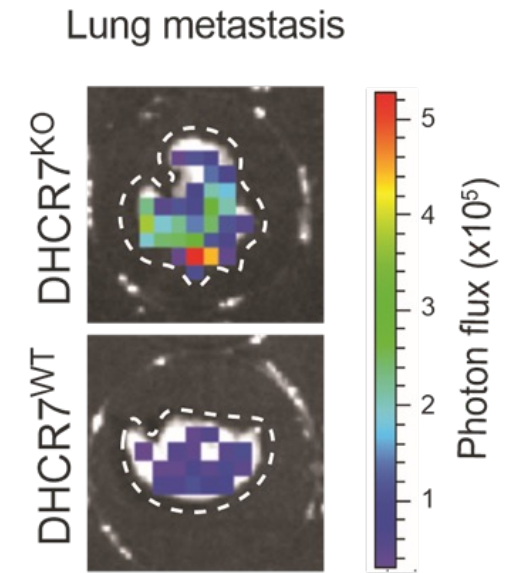
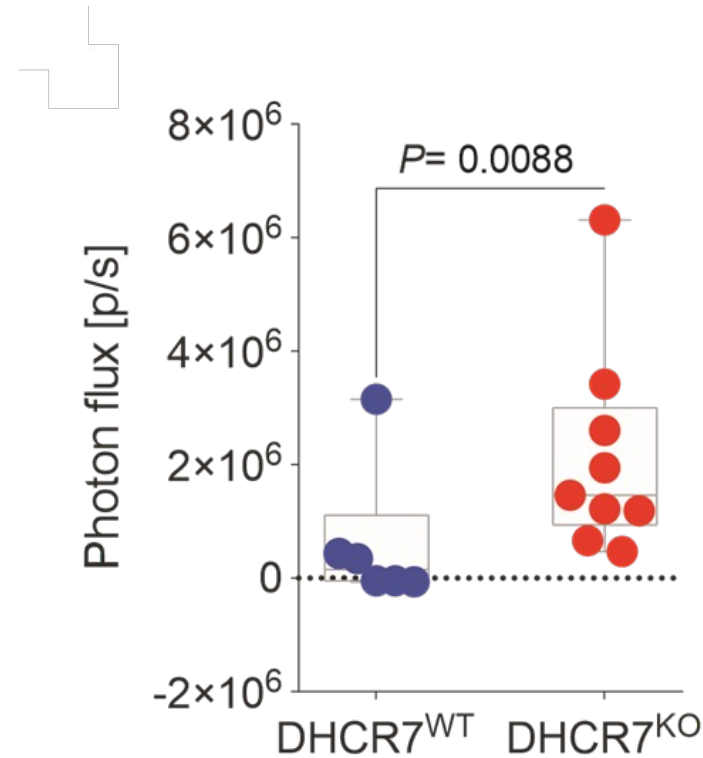
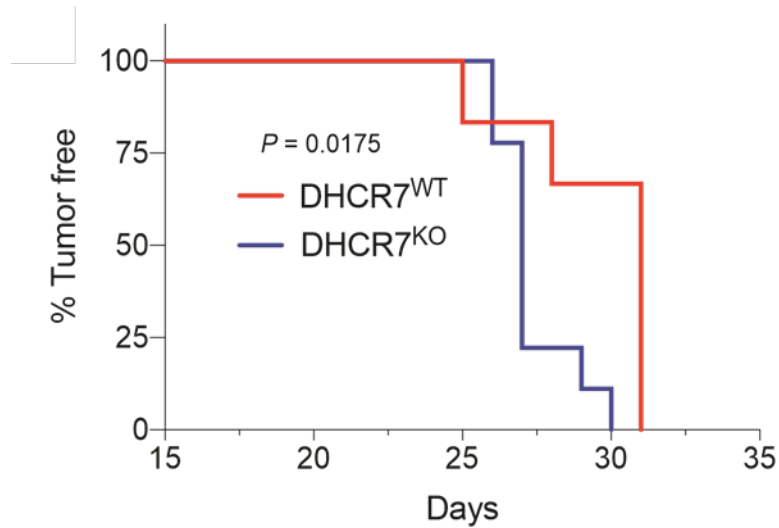
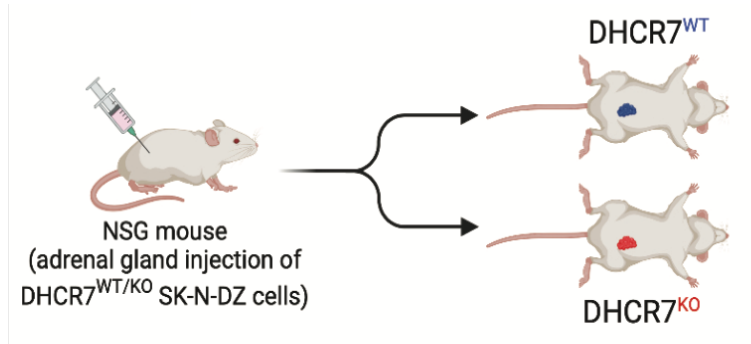
Loss of DHCR7 leads to a more aggressive phenotype in BL41 cells

Consequence of DHCR7 loss on the growth of BL *in vivo*



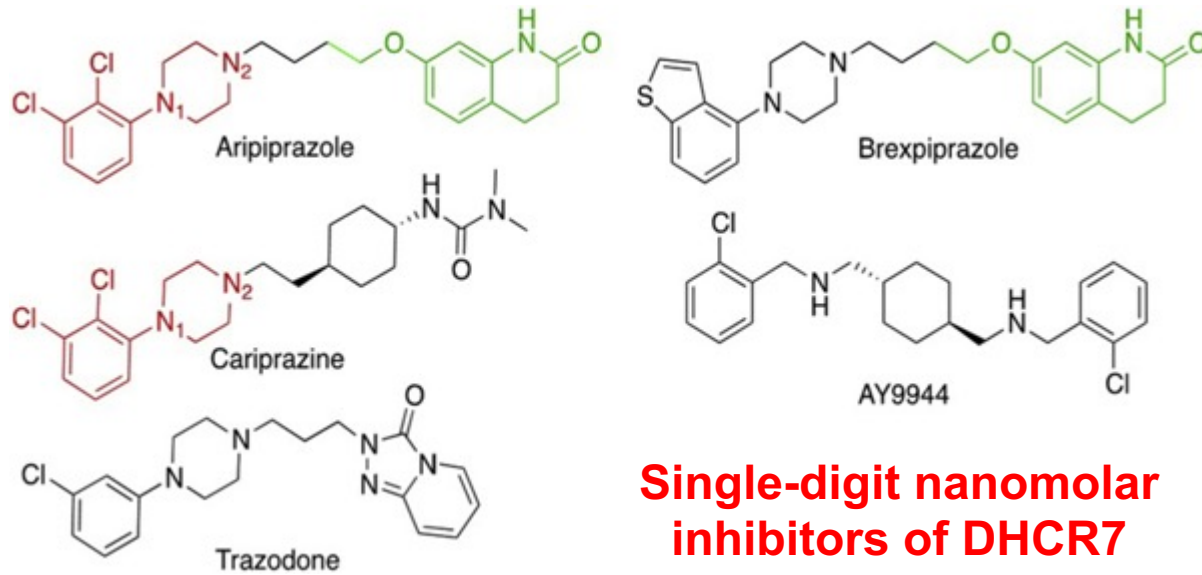
7-DHC promotes growth by suppressing lipid peroxidation

Consequence of DHCR7 loss on the orthotopic NB xenografts



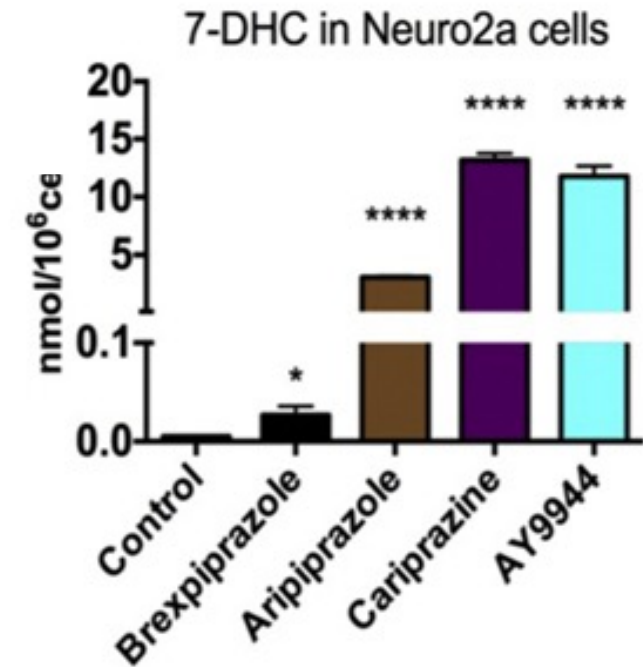
7-DHC increases metastatic capacity in NB orthotopic xenografts

DHCR7 pharmacological inhibition



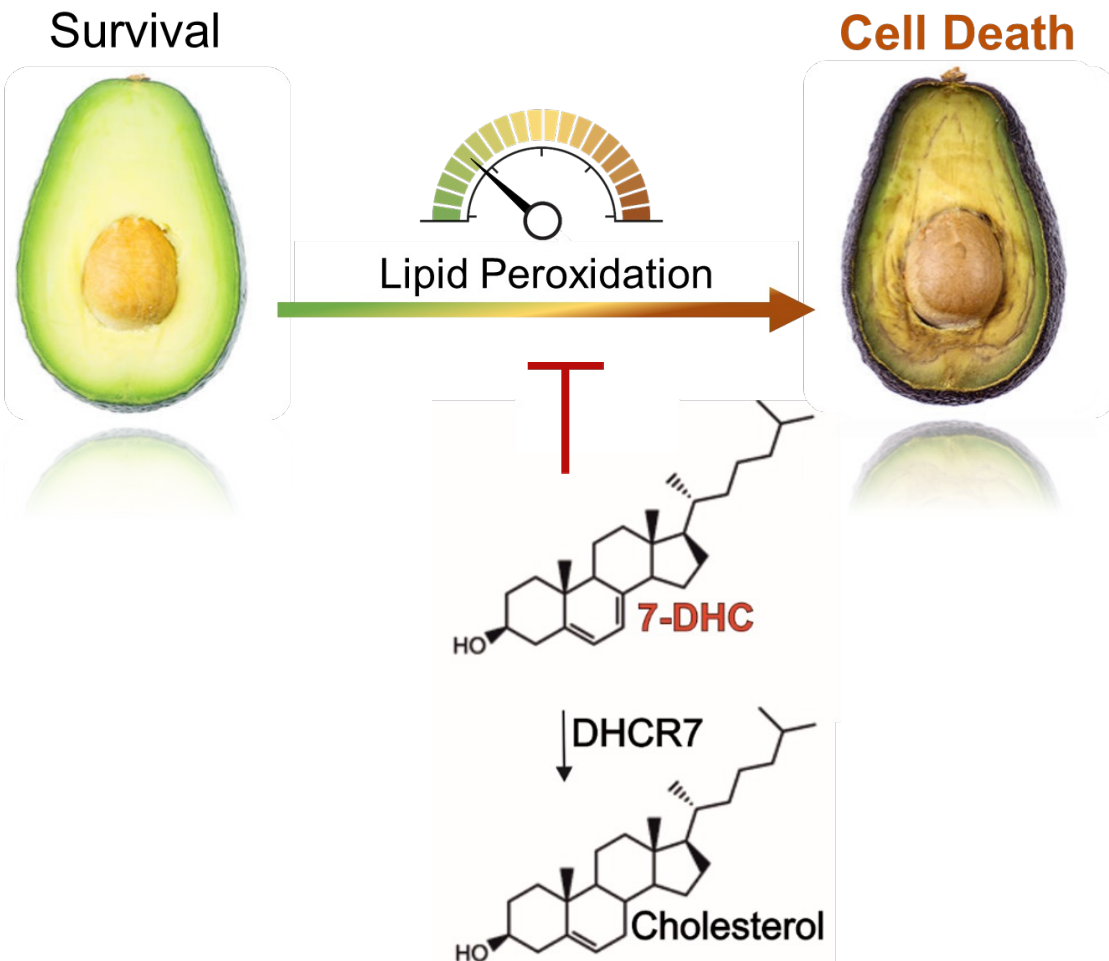
Single-digit nanomolar inhibitors of DHCR7

- Psychotropic medication – some of which are prescribed over 20 million times a year in the US, sometimes off-label as a sleep aid.
- Studies of patients on cariprazine (as confirmed by the presence of the drug and its metabolites in plasma) have elevated plasma levels of 7-DHC.



Korade, Z., et al., 2021. Translational Psychiatry

Concluding remarks



- Ferroptosis is initiated by inhibiting enzymatic systems involved in the repair of peroxidized phospholipids
- Endogenous metabolites can have membrane antioxidant activity and suppress the process of lipid peroxidation
- 7-DHC accumulation contributes to resistance to ferroptosis
- B-ring unsaturated sterols efficiently shield phospholipids from (phospho)lipid peroxidation
- Specific cancer entities could benefit from highjacking this primitive mechanism of „ferroptosis“ suppression?
- Some psychotropic FDA-approved are potent DHCR7 inhibitors -> influence growth/metastasis?

Thank you for your attention

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Currently recruiting postdocs

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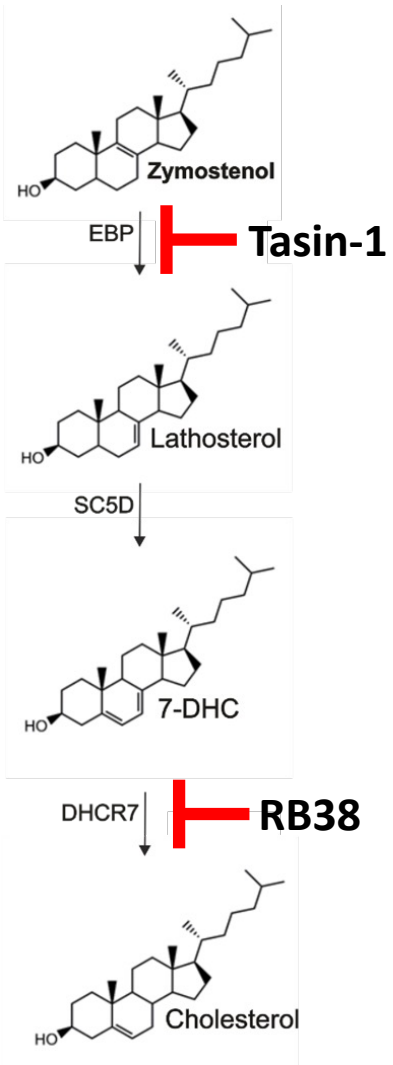


Captivated by the topic? Fascinated by CRISPR-based and pharmacological screens targeting ferroptosis?

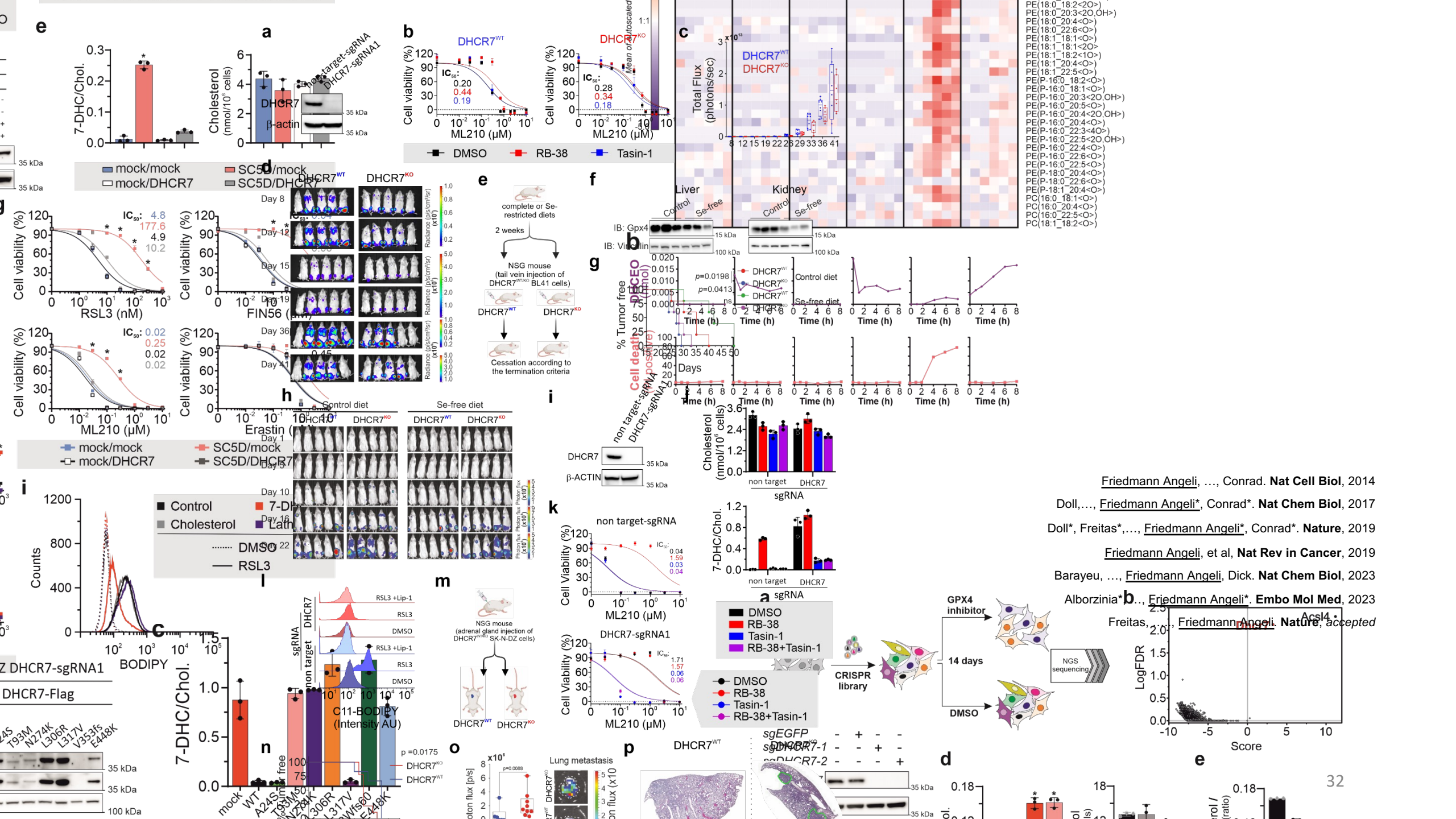
Join our team!

We're actively seeking PhD candidates and Postdocs to contribute to groundbreaking research in this field.

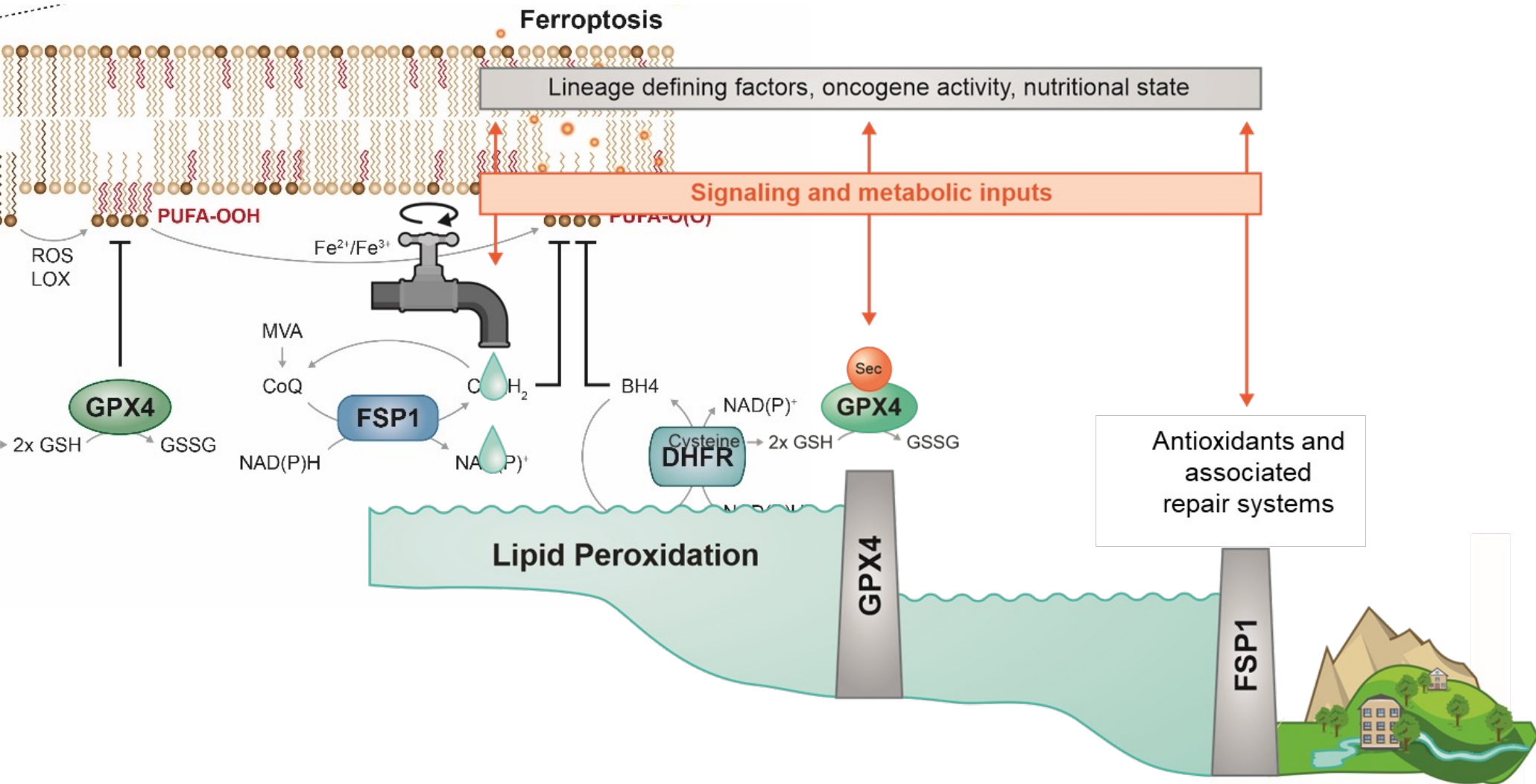
Consequence of DHCR7 inhibition on BL growth *in vitro*



Accumulation of 7-DHC favours the outgrowth of B-cell lymphomas

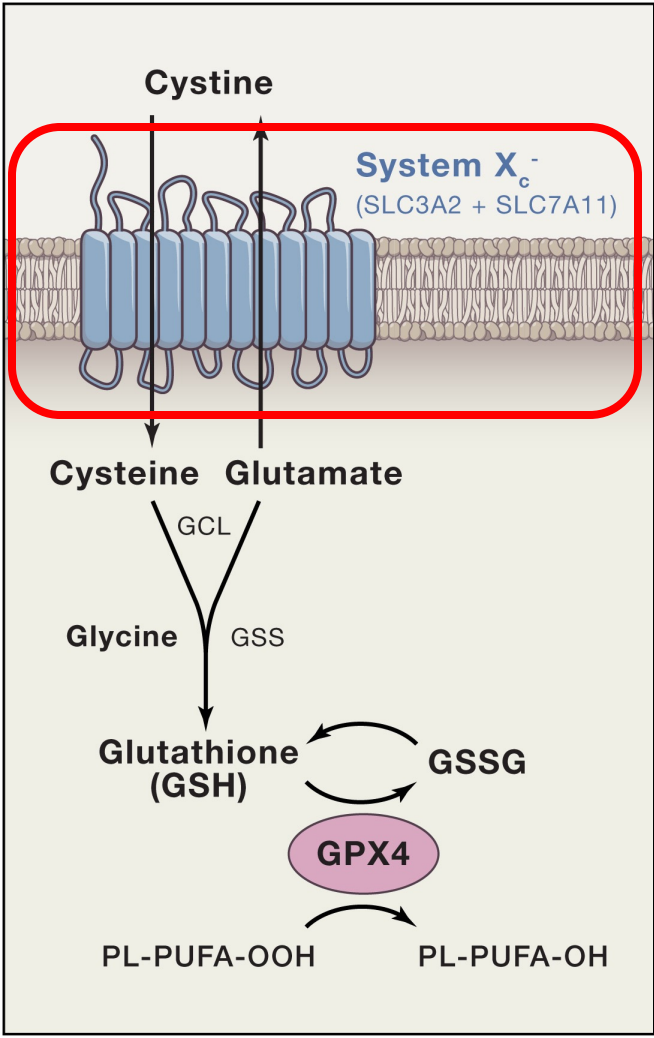


Regulation of lipid peroxidation



GPX4i triggers endogenous lipid peroxidation and cell death

Early works on Burkitt's Lymphoma



BL and other B-Cell lymphoma have markedly low activity of system Xc-

Int. J. Cancer: 55, 485–491 (1993)
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UICC Publication of the International Union Against Cancer
Publication de l'Union Internationale Contre le Cancer

IRRADIATED FIBROBLASTS PROTECT BURKITT LYMPHOMA CELLS FROM APOPTOSIS BY A MECHANISM INDEPENDENT OF BCL-2

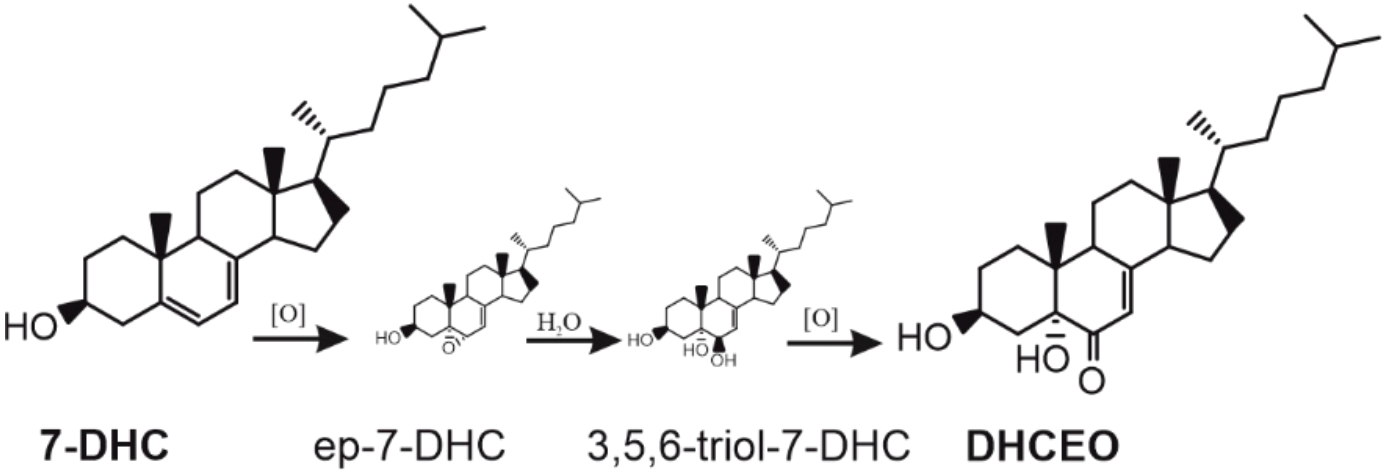
Martin H. FALK^{1,4}, Lothar HÜLTNER², Anne MILNER³, Christopher D. GREGORY³ and Georg W. BORNKAMM¹
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TABLE I – CRITICAL CELL NUMBER PER WELL FOR OUTGROWTH OF COLONIES

Cell lines	Without feeder	With feeder	Ratio without feeder:with feeder
BL, EBV-negative:			
BL02	10 ^{3.6}	10 ^{0.3}	2,000
BL40	10 ^{3.6}	10 ⁰	4,000
BL41	10 ^{4.6}	10 ¹	4,000
BL70	10 ^{4.6}	10 ^{0.6}	10,000

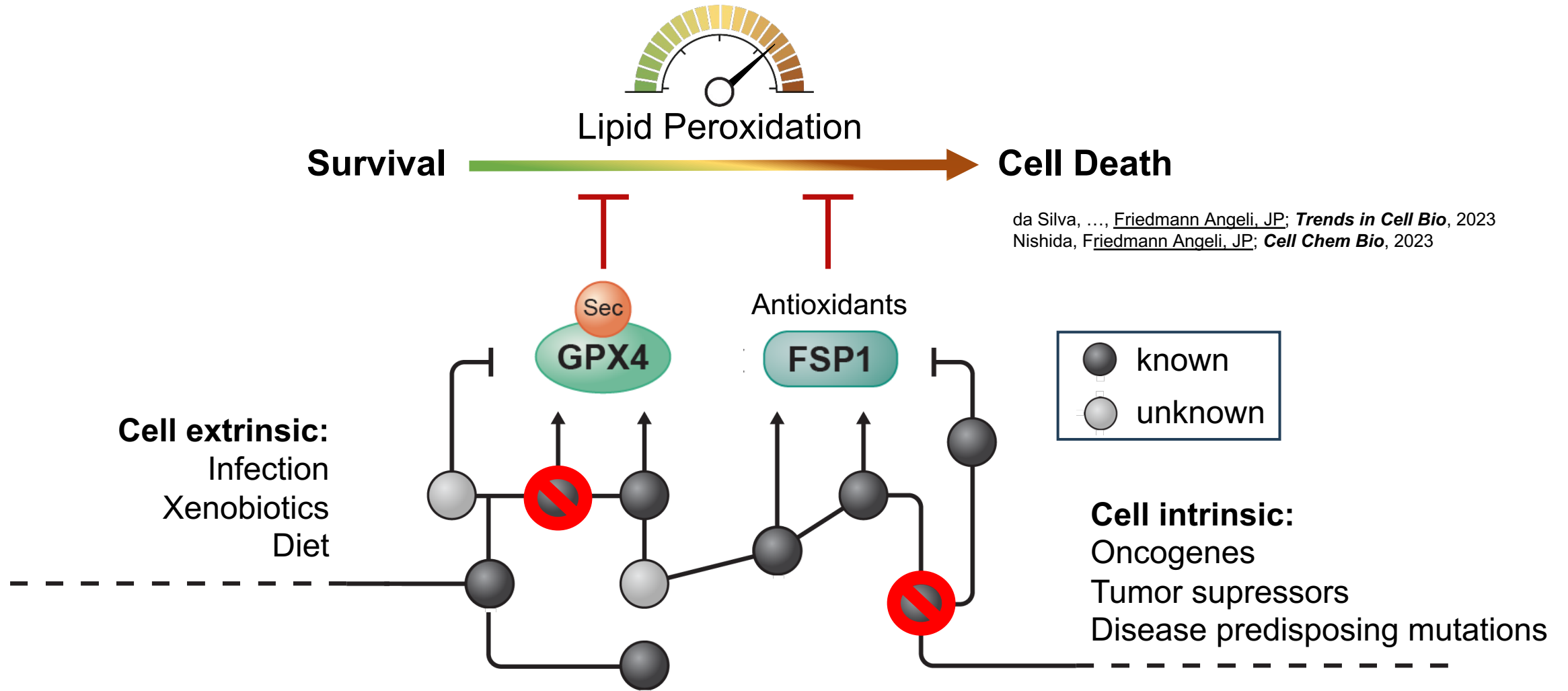
BL are inherently sensitive to lipid peroxidation mediated cell death

Quantification of 7-DHC oxidation products during ferroptosis



DHCEO accumulates in cells upon GPX4i

Our vision



Understanding these pathways will unravel fundamental processes regulating cellular membrane redox homeostasis