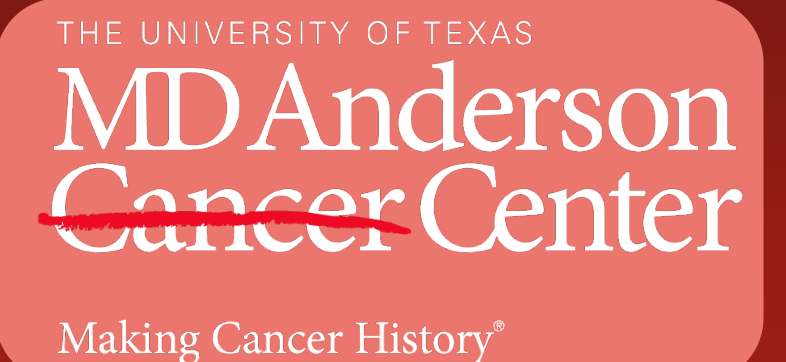


Mitochondrial Respiration Regulates GPX4 Inhibition-Induced Ferroptosis in Acute Myeloid Leukemia

Hiroki Akiyama, Ran Zhao, Yuki Nishida, Lauren B Ostermann, Po Yee Mak, Edward Ayoub, Sujan Piya, Bing Z Carter, Michael Andreeff and Jo Ishizawa

Section of Molecular Hematology and Therapy, Department of Leukemia, University of Texas MD Anderson Cancer Center



Background

Ferroptosis, a form of non-apoptotic cell death regulated by iron-dependent lipid peroxidation, has drawn extensive attention as potential anti-cancer strategy. However, it remains to be explored in hematologic malignancies. We here investigate the molecular mechanisms of ferroptosis in acute myeloid leukemia (AML) and its therapeutic potential with co-targeting of mitochondrial respiration.

Hypothesis

Ferroptosis pathway is a therapeutic vulnerability in AML

- Oxidative stress and iron overload in AML cells
- Induction of cell death that bypasses apoptosis resistance

Materials and Methods

Public datasets: shinyDepMap¹, GEPIA², CRISPR screening for NALM6 cells treated with ClpP agonists³

Cells: Parental AML cell lines, OCI AML3-shGPX4, MOLM13-TP53mut⁴, Kasumi1-shTP53, HL60-Rho0⁵, OCI-AML3-CLPP-Y118A⁶, Primary AML patient samples

Reagents: GPX4 inhibitor; ML210, ClpP agonist; ONC201, lipophilic antioxidants; Liproxstatin-1 (Lip1) and α -Tocopherol (aToc), iron chelator; deferoxamine (DFX), mitochondrial antioxidants; MitoQ and MitoQH2

Flow cytometry: AnnexinV/DAPI cell death assay, C11 BODIPY581/591 and MitoPerOx lipid peroxidation assays, MitoSOX red mitochondrial superoxide indicator,

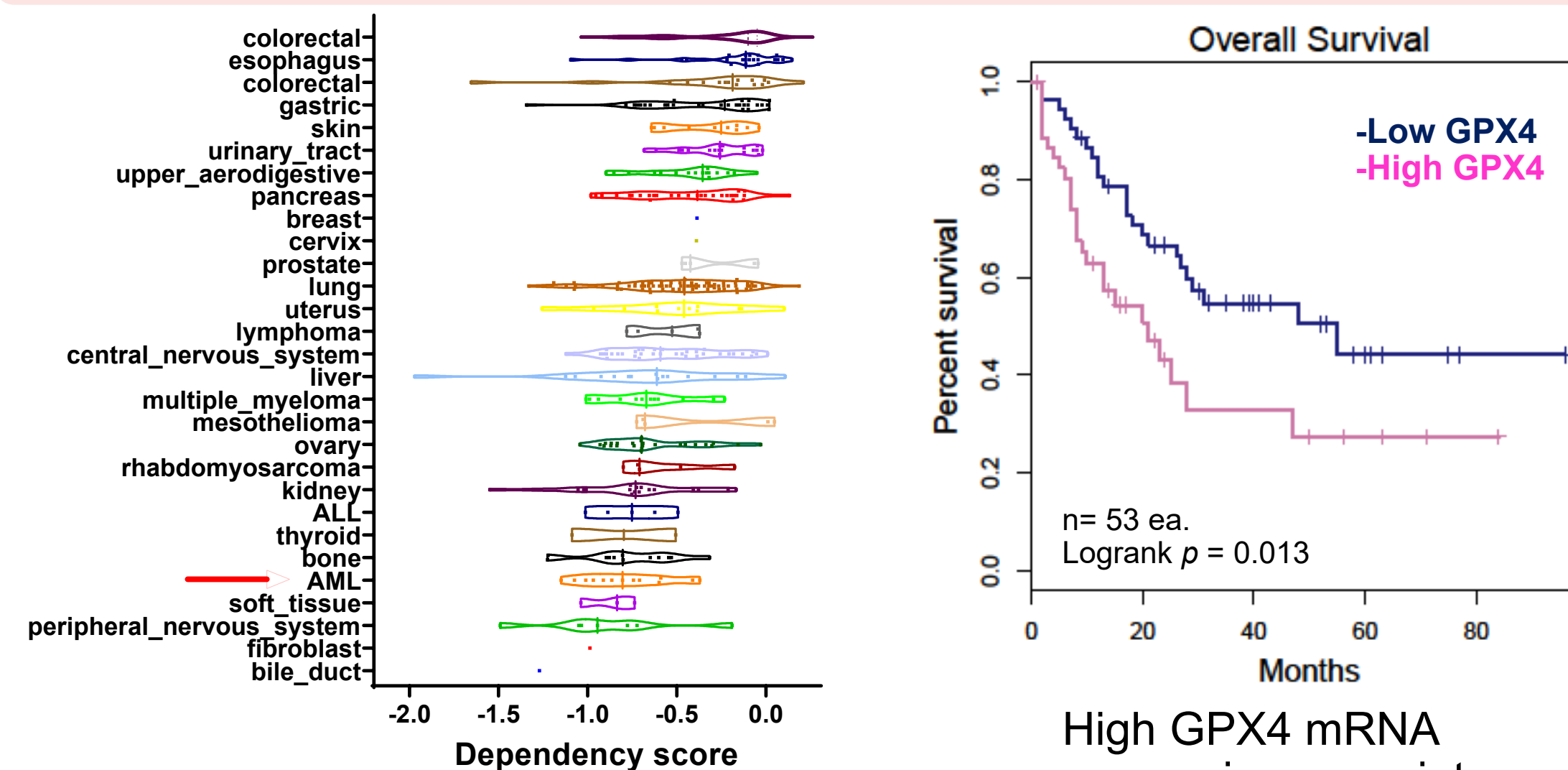
Western blot

Transmission electron microscopy

References

1. Shimada, K., et al. eLife 2021;10:e57116
2. Tang, Z., et al. Nucleic Acids Research 2017;45, 98–102.
3. Jacques, S., et al. Genetics 2020;214:1103–1120.
4. Boettcher, S., et al. Science 2019;365(6453):599–604.
5. Pelicano, H., et al. Journal of Biological Chemistry 2003;278(39):37832–37839.
6. Ishizawa, J., et al. Cancer Cell 2019;35(5):721–737.e9.

GPX4 is a potential therapeutic target in AML with prognostic relevance

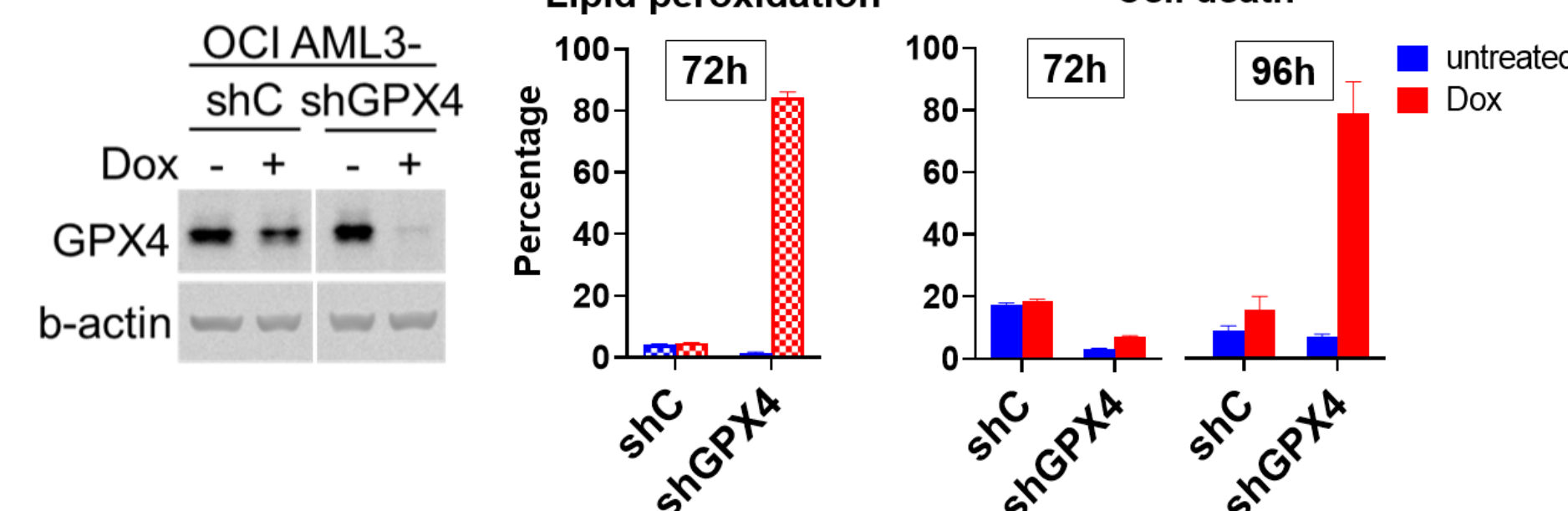


AML cell lines are one of the cell lines highly dependent (low dependency score) on GPX4 among those on DepMap (shinyDepMap)

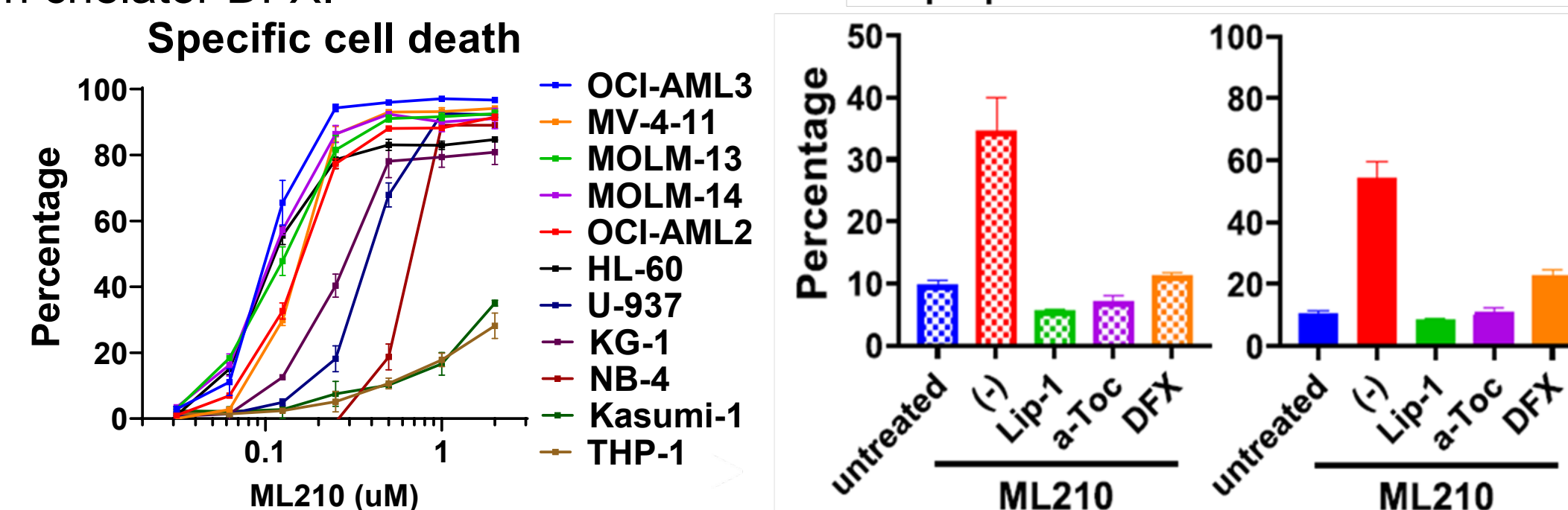
High GPX4 mRNA expression associates with shorter survival in AML patients (TCGA)

GPX4 inhibition induces ferroptosis in AML cells

GPX4 knockdown by doxycycline-inducible shRNA induces lipid peroxidation followed by cell death in AML cells



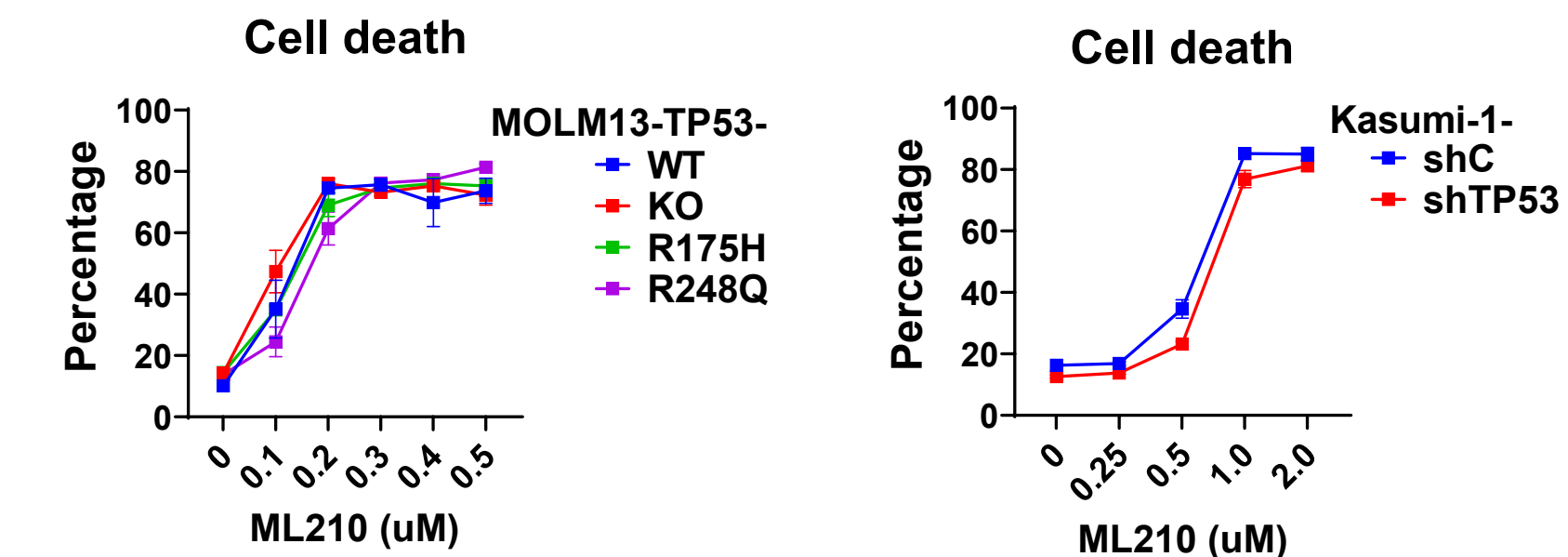
GPX4 inhibition by ML210 induces lipid peroxidation and cell death in AML cell lines. The pharmacologic effects are blocked by lipophilic antioxidants and the iron chelator DFX.



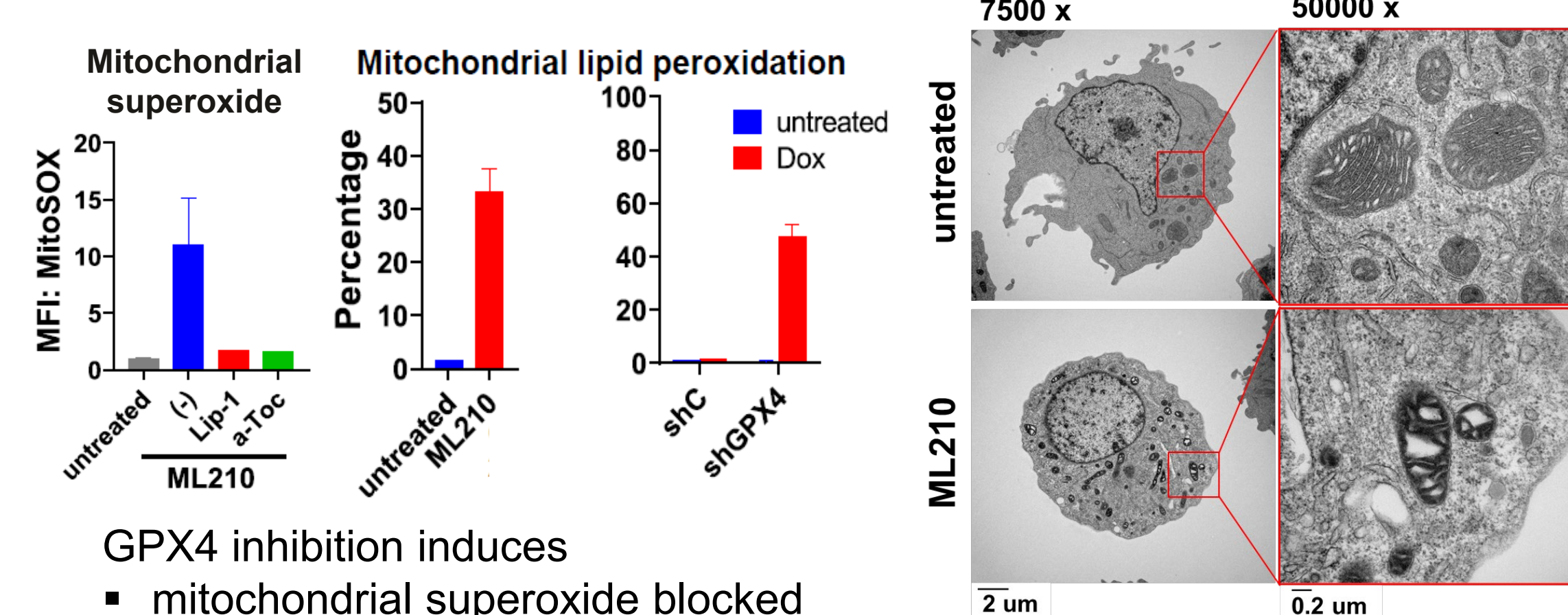
Results

Anti-leukemia effect of GPX4 inhibition is independent of TP53 mutational status

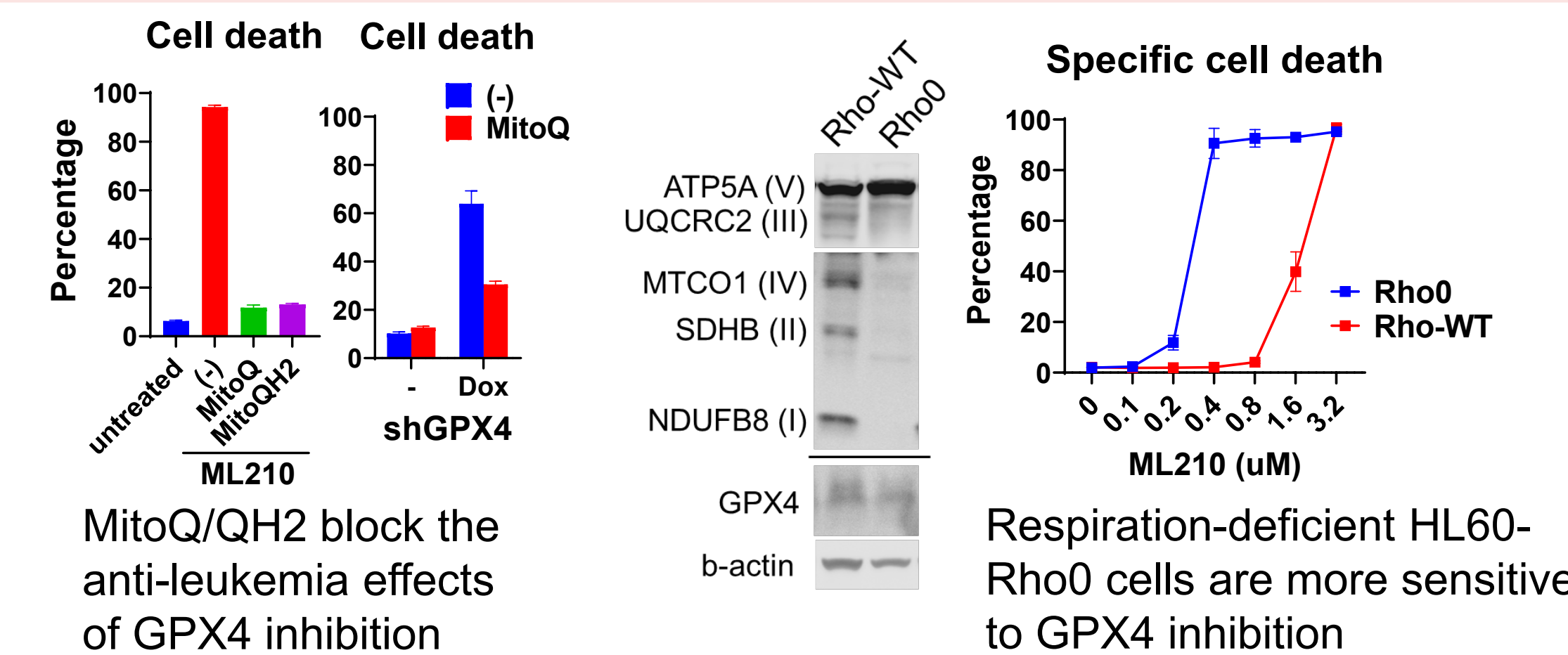
TP53 modulations in MOLM13 (CRISPR-mediated gene editing) or Kasumi-1 (knockdown of mutant p53) do not change the ML210 sensitivity



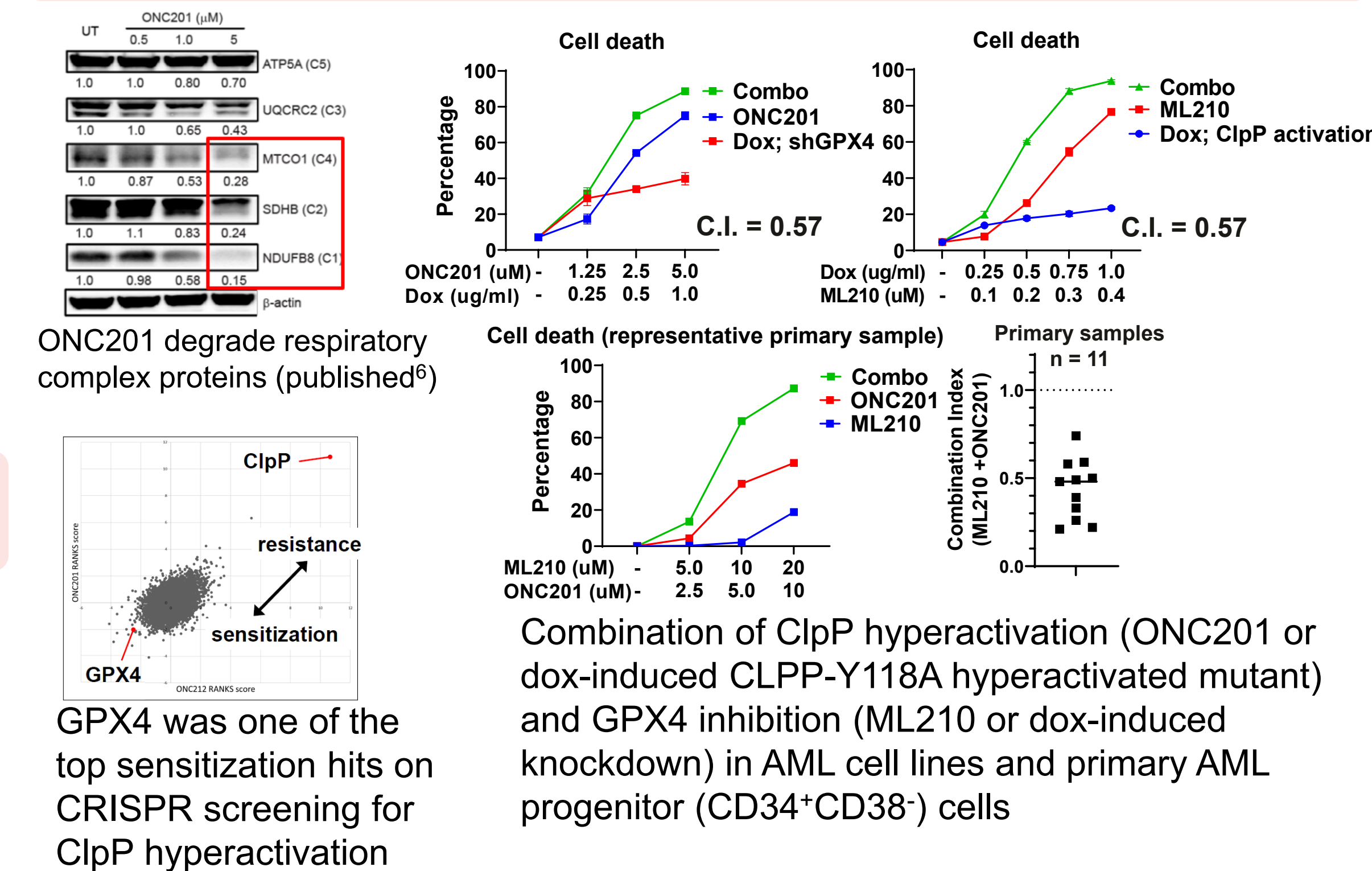
GPX4 inhibition induces lipid-associated oxidative stress and ultrastructural alterations in mitochondria



Mitochondrial respiration protects AML cells from ferroptosis



ClpP-mediated degradation of mitochondrial respiratory complex proteins⁶ sensitizes AML cells to ferroptosis



Summary

- GPX4 inhibition induces ferroptosis in AML cells
- Mitochondrial respiration protects AML cells from GPX4-mediated ferroptosis
- ClpP-mediated degradation of mitochondrial respiratory complex proteins and GPX4 inhibition synergistically exerts anti-leukemia effects
- Studies are in progress to determine the molecular mechanisms and the *in vivo* efficacy of the combination

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