

Supplementary Data File

Redox destabilization by ibrutinib promotes ferroptosis in diffuse large B-cell lymphoma (DLBCL)

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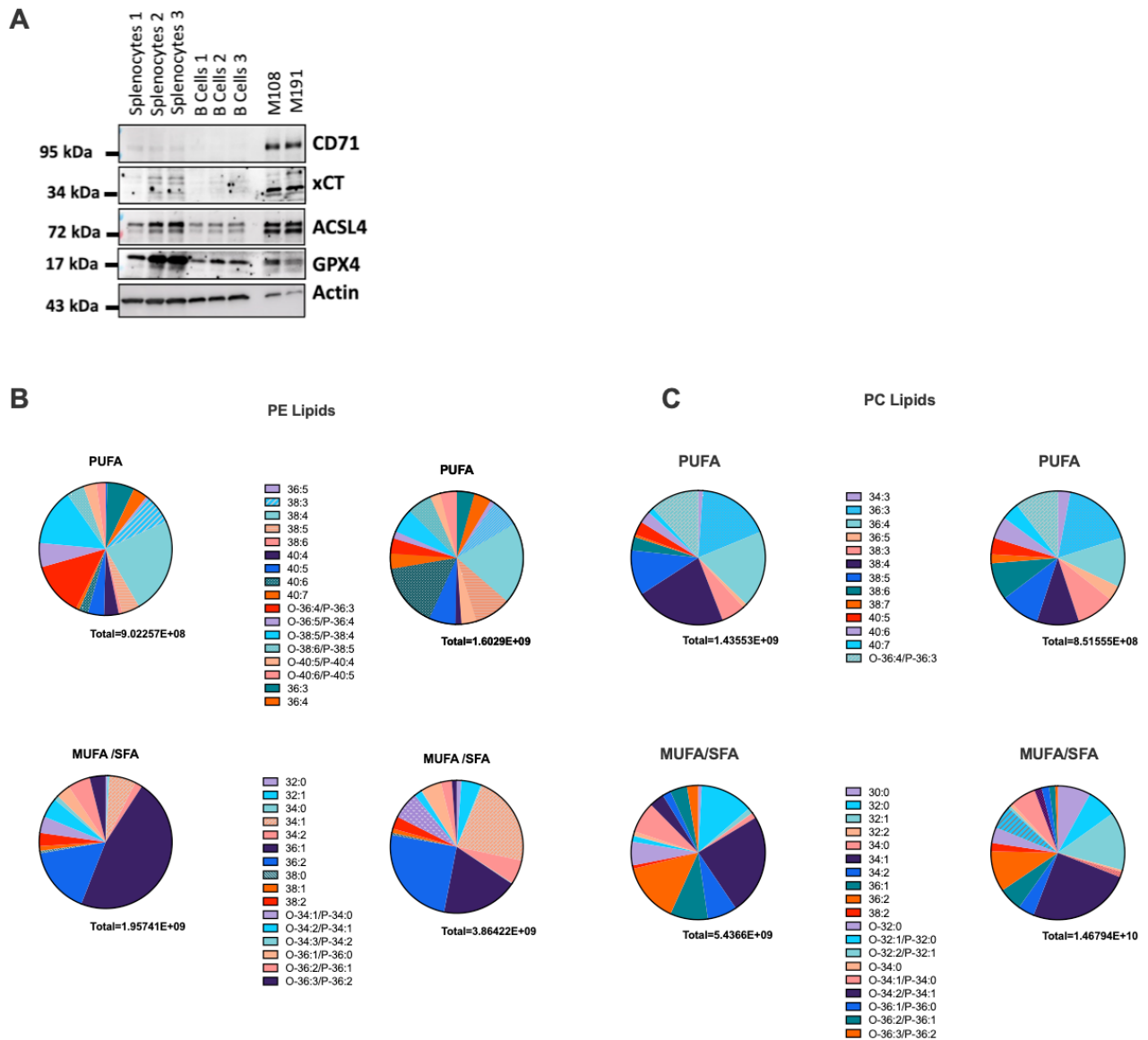
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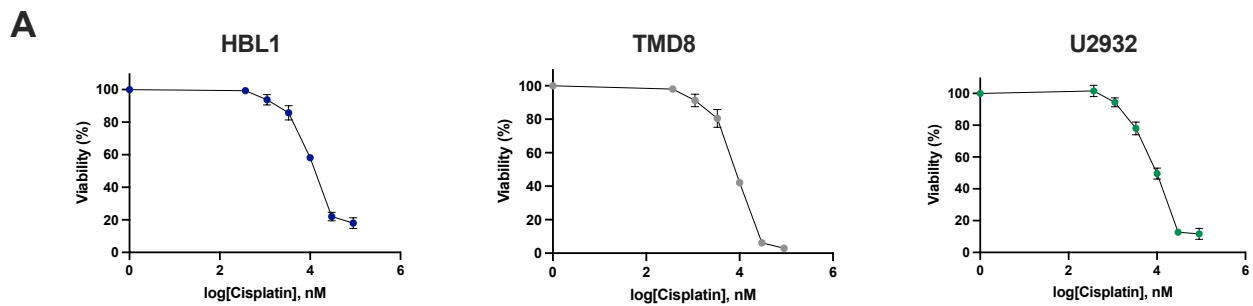
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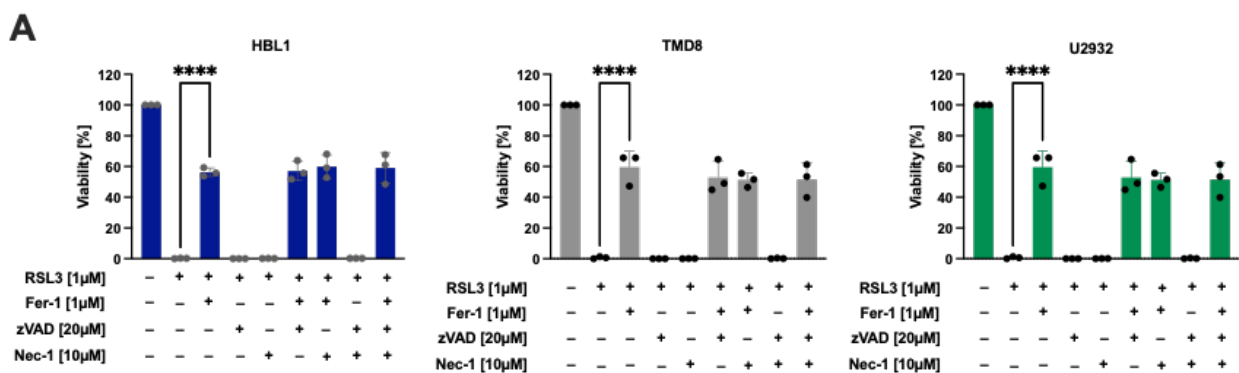


Suppl Fig.1 DLBCL cells lines upregulated mono-unsaturated fatty acids to prevent Ferroptosis activation. **a** Representative western blot of ferroptosis pathway component expression in murine lymphoma cell lines compared to WT Splenocytes and matched B Cells. **b** Phosphatidylethanolamine (PE) lipid species and **c** Phosphatidylcholine(PC) lipid composition measured by lipidomic analysis of n=3 ABC-DLBCL cell lines (HBL1, TMD8, U2932) compared to n=3 healthy donor B Cells isolated from PBMCs from buffy coats. PUFA = Polyunsaturated Fatty Acids. MUFA = Monounsaturated Fatty Acids.

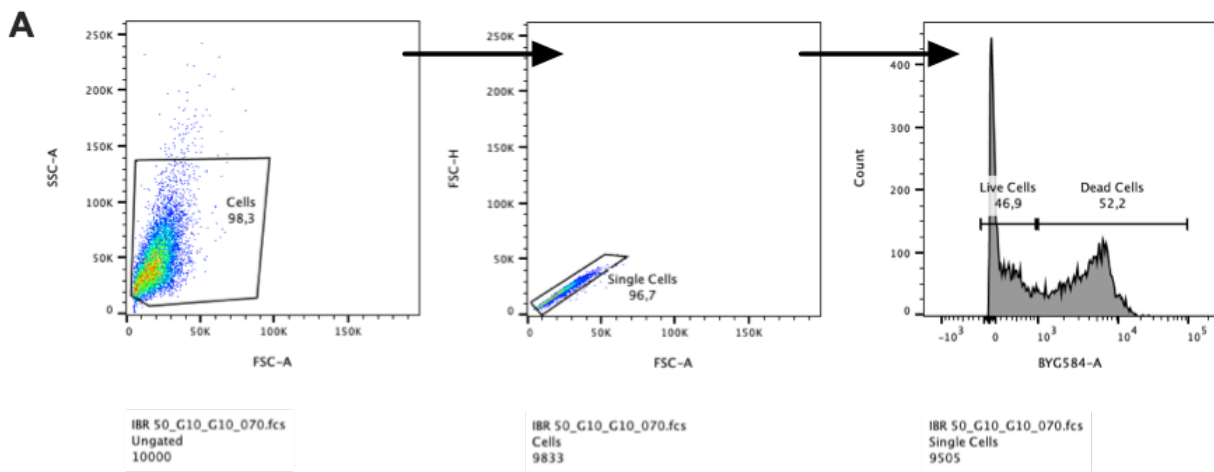


45 **Suppl. Fig. 2 DLBCL are sensitive to cisplatin treatment. a** Viability measurements of human ABC-DLBCL cell lines by CellTiter-Glo® following 48 hours treatment with increasing cisplatin concentrations. Data are mean $\pm$ SD for each cell line of n=3 independent experiments.

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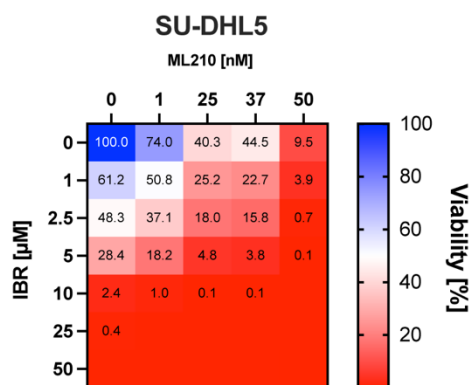
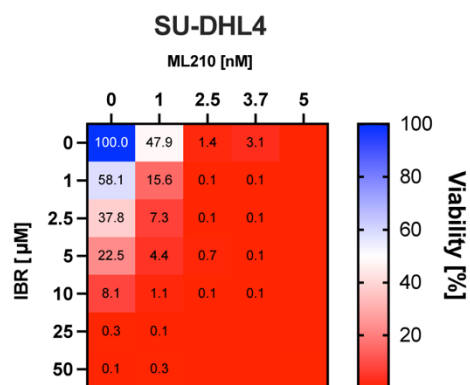
55 **Suppl. Fig. 3 DLBCL are sensitive to RSL3 induced ferroptosis. a** Viability measurements of human ABC-DLBCL cell lines by CellTiter-Glo® following 48 hours treatment indicated concentrations of RSL3 in combination with Fer-1 and/or zVAD and/or Nec-1s. Data are mean $\pm$ SD for each cell line of n=3 independent experiments. *=p value 0.05, **=p value 0.01, ***= p value 0.001, ****= p value 0.0001.



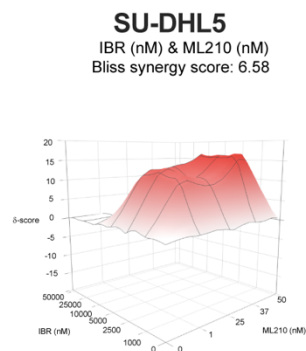
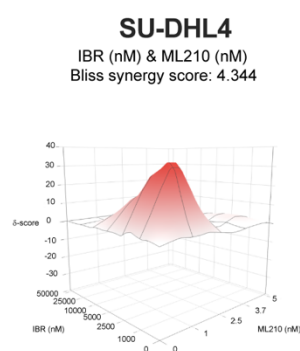
Suppl. Fig. 4 Gating strategy of Flow cytometric analysis. a Gating Strategy for flow cytometric analysis.

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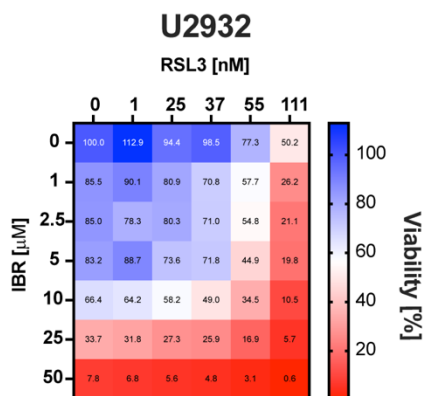
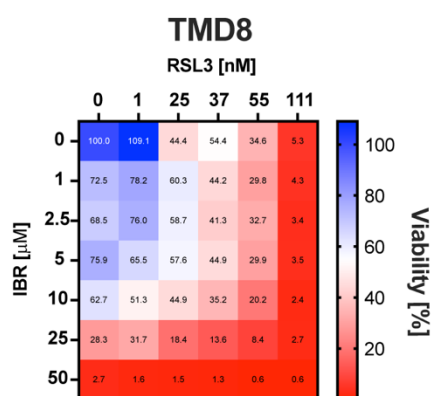
A



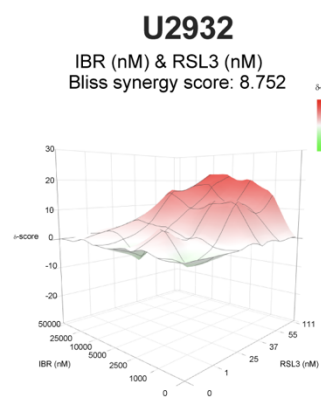
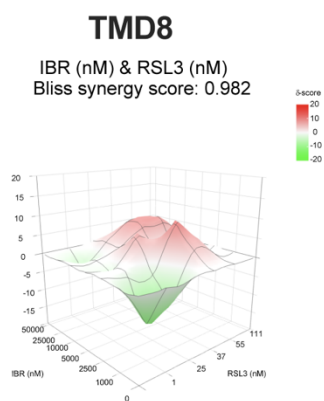
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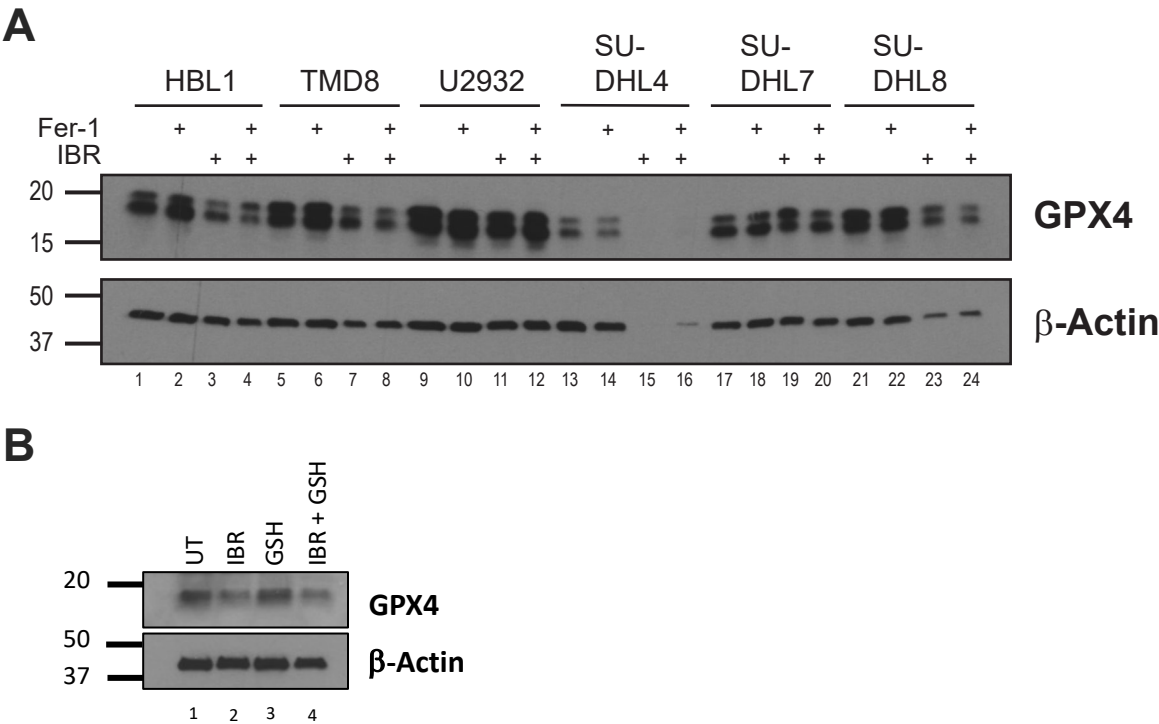


D



Suppl. Fig. 5 GPX4 inhibition treatment has an additive effect in combination with Ibrutinib.

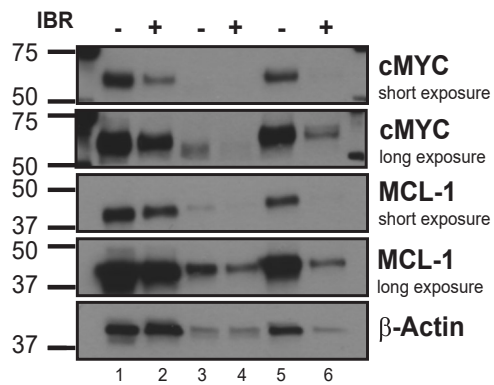
a-d Cell viability of the indicated human **a** GCB-DLBCL **c** ABC-DLBCL cell lines following 48 hours treatment with increasing concentrations of **a** ML210 or **c** RSL3 in combination with IBR as determined by CellTiter-Glo®. Heatmap color code indicates viability levels of each sample. **b,d** BLISS synergy scores were determined using <https://synergyfinder.fimm.fi>.



Suppl. Fig. 6 Ibrutinib downregulates GPX4 protein levels independently of Glutathione.

a Western blot analysis of indicated DLBCL cell lines following 48h of treatment with IBR [10 μ M] and/or Fer-1 [1 μ M]. **b** Western blot analysis of U2932 cells treated with IBR [25 μ M] and/or GSH [2 mM].

A



75 **Suppl. Fig. 7 Ibrutinib downregulates translation of short-lived proteins. a** Replicates of western blot of U2932 cells untreated or treated with IBR [25 μ M] in the presence of Fer-1 [1 μ M] for 24h.