Supplementary material

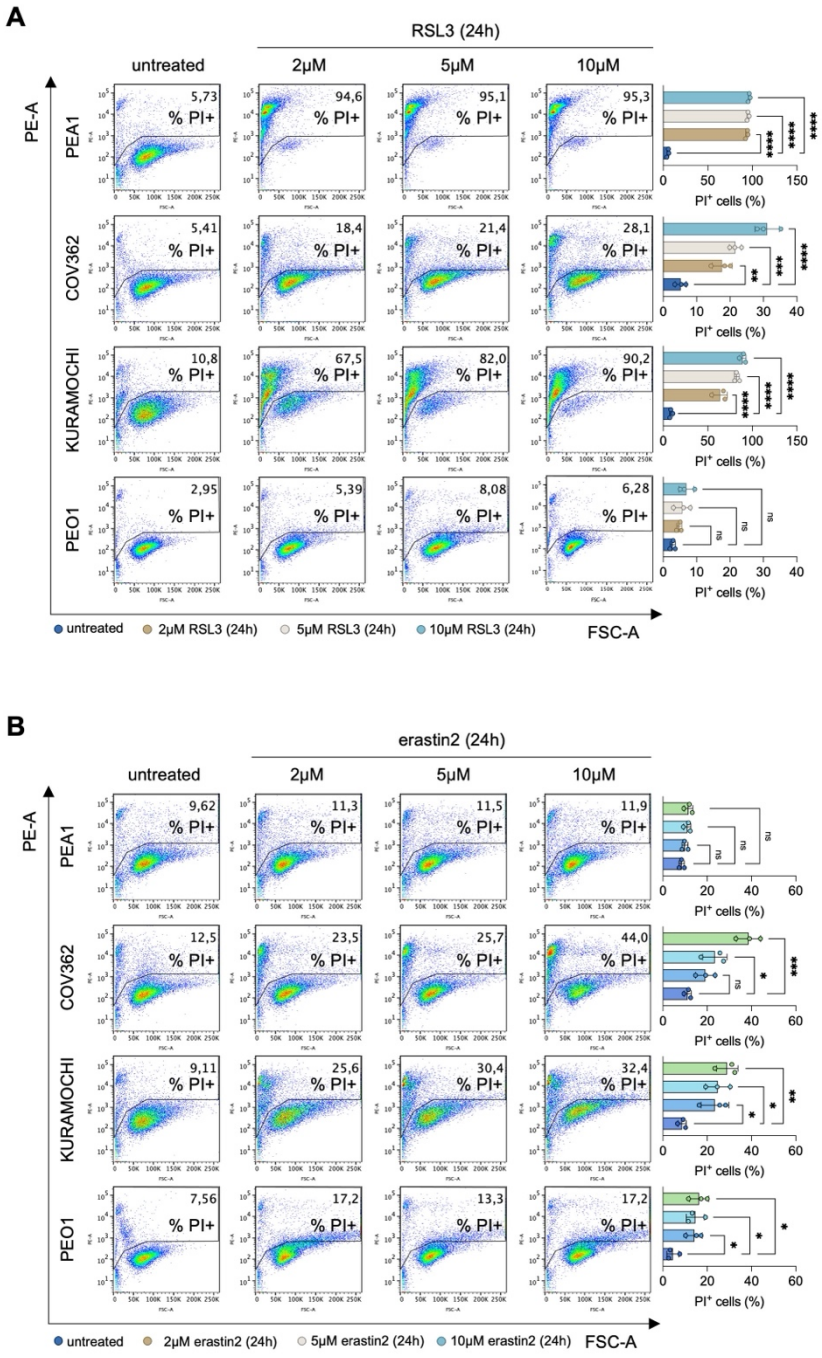

Fig. S1 Iron sensitivity is reproduced by iron dextran and is independent of canonical ferroptosis inducers.

A PI^+ cells (%) by flow cytometry in PEA1, COV362, KURAMOCHI and PEO1 treated with RSL3 (2, 5, 10 μM, 24h). Representative plots with $\%PI^+$; bar graphs (right). Mean ± SD, n=3; one-way ANOVA. **B** PI^+ cells (%) by flow cytometry in the four lines treated with erastin2 (2, 5, 10μM, 24h). Representative plots with $\%PI^+$; bar graphs (right). Mean ± SD, n=3; one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; ns, not significant.

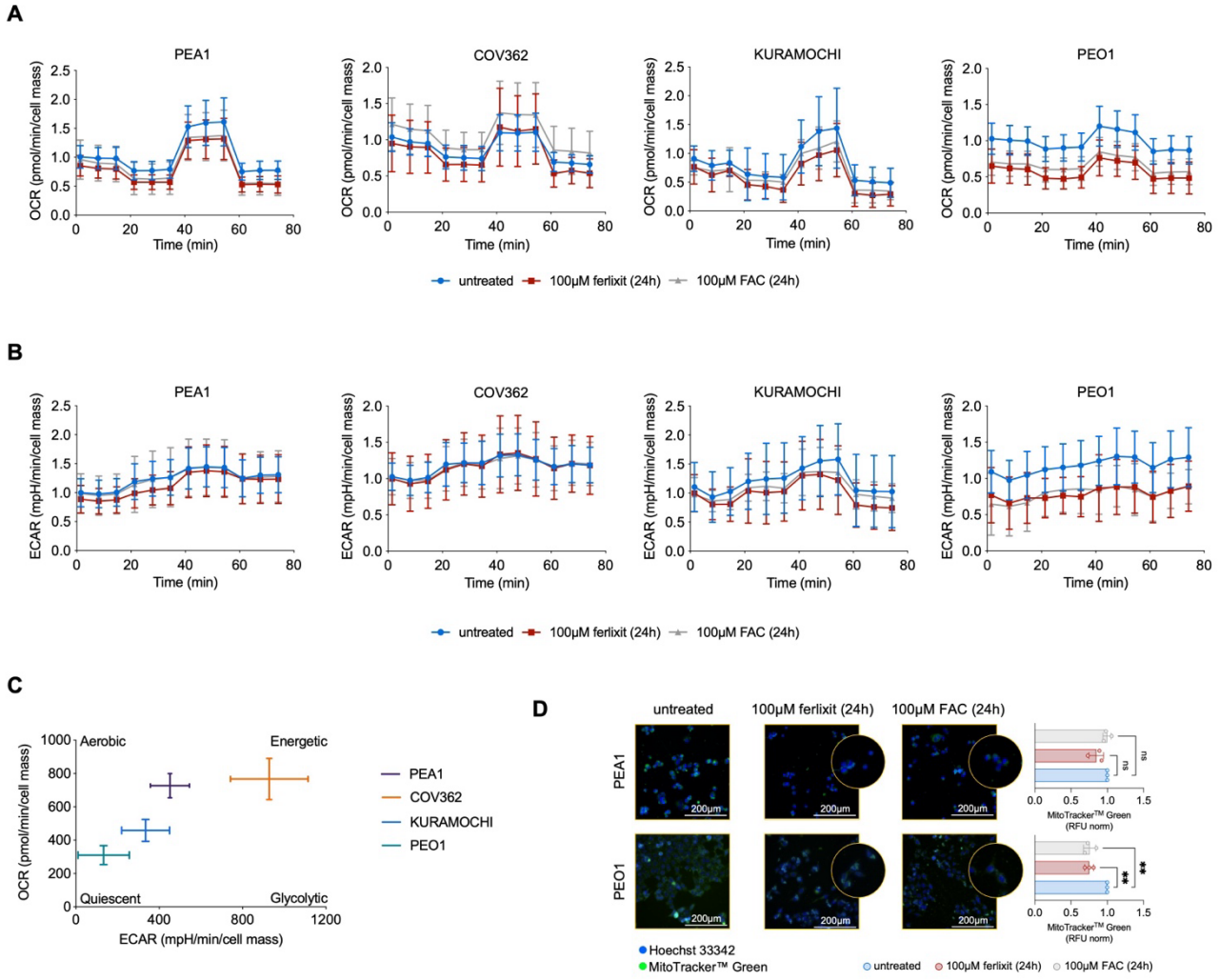

Fig. S2 Iron loading impairs mitochondrial respiration without affecting glycolysis.
A Oxygen consumption rate (OCR) profiles over time from the Seahorse XF Cell Mito Stress Test in PEA1, COV362, KURAMOCHI and PEO1, untreated or treated with 100μM ferlixit or FAC (24h), following sequential injection of oligomycin, FCCP and rotenone/antimycin A; values normalised to protein content (SRB) and expressed relative to the untreated baseline. Mean ± SD, n=3. **B** Extracellular acidification rate (ECAR) profiles over time for the same lines and conditions, normalised to protein content (SRB) and expressed relative to the untreated baseline. Mean ± SD, n=3. Iron loading did not appreciably alter glycolytic acidification. **C** Basal energetic map (OCR versus ECAR) of untreated PEA1, COV362, KURAMOCHI and PEO1, defining their resting metabolic phenotype. Mean ± SD, n=3. **D** Mitochondrial mass assessed by MitoTracker™ Green (green) with Hoechst 33342 (blue, nuclei); representative fluorescence images. Scale bars, 200μm. Graphs: MitoTracker™ Green fluorescence normalised to untreated, with individual data points shown. Mean ± SD, n=3; one-way ANOVA. *p<0.05, **p<0.01; ns, not significant.

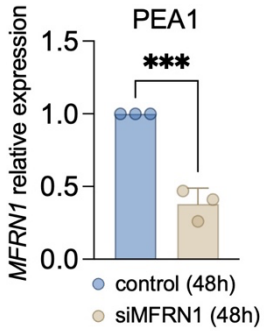

Fig. S3 Validation of MFRN1 knockdown in PEA1 cells.
qRT-PCR of MFRN1 mRNA in PEA1 cells 48h after transfection with control or MFRN1-targeting siRNA (siMFRN1), normalised to GAPDH and expressed relative to control. Mean ± SD, n=3; two-tailed unpaired Student's t-test. ***p<0.001.

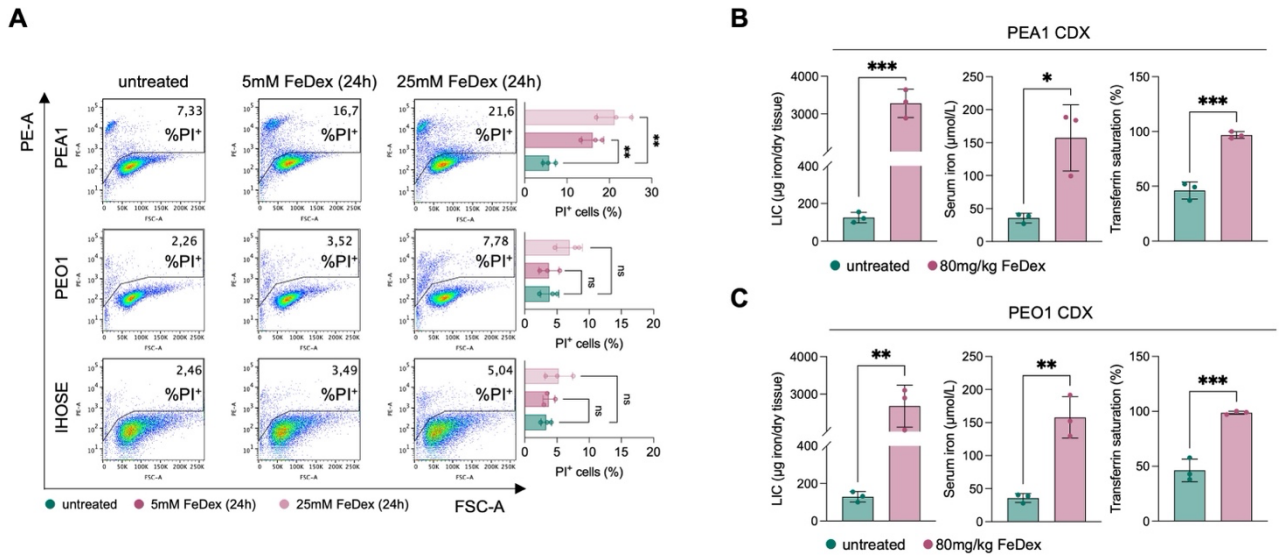

Fig. S4 FeDex increases systemic iron and alters tumour iron-handling proteins *in vivo*.
A PI^+ cells (%) by flow cytometry in PEA1, PEO1 and IHOSE untreated or treated with iron dextran (FeDex, 5 or 25mM, 24h). Representative plots with $\%PI^+$ indicated; bar graphs (right). Mean ± SD, n=3; one-way ANOVA. **B, C** Liver iron content (LIC), serum iron and transferrin saturation in PEA1 (A) and PEO1 (B) xenograft-bearing SCID mice, untreated or treated with FeDex (80mg/kg). Mean ± SD, n=3 mice per group; two-tailed unpaired Student's t-test. Mean ± SD, n=[3-4] per group; two-tailed unpaired Student's t-test. *p<0.05, **p<0.01, ***p<0.001; ns, not significant.