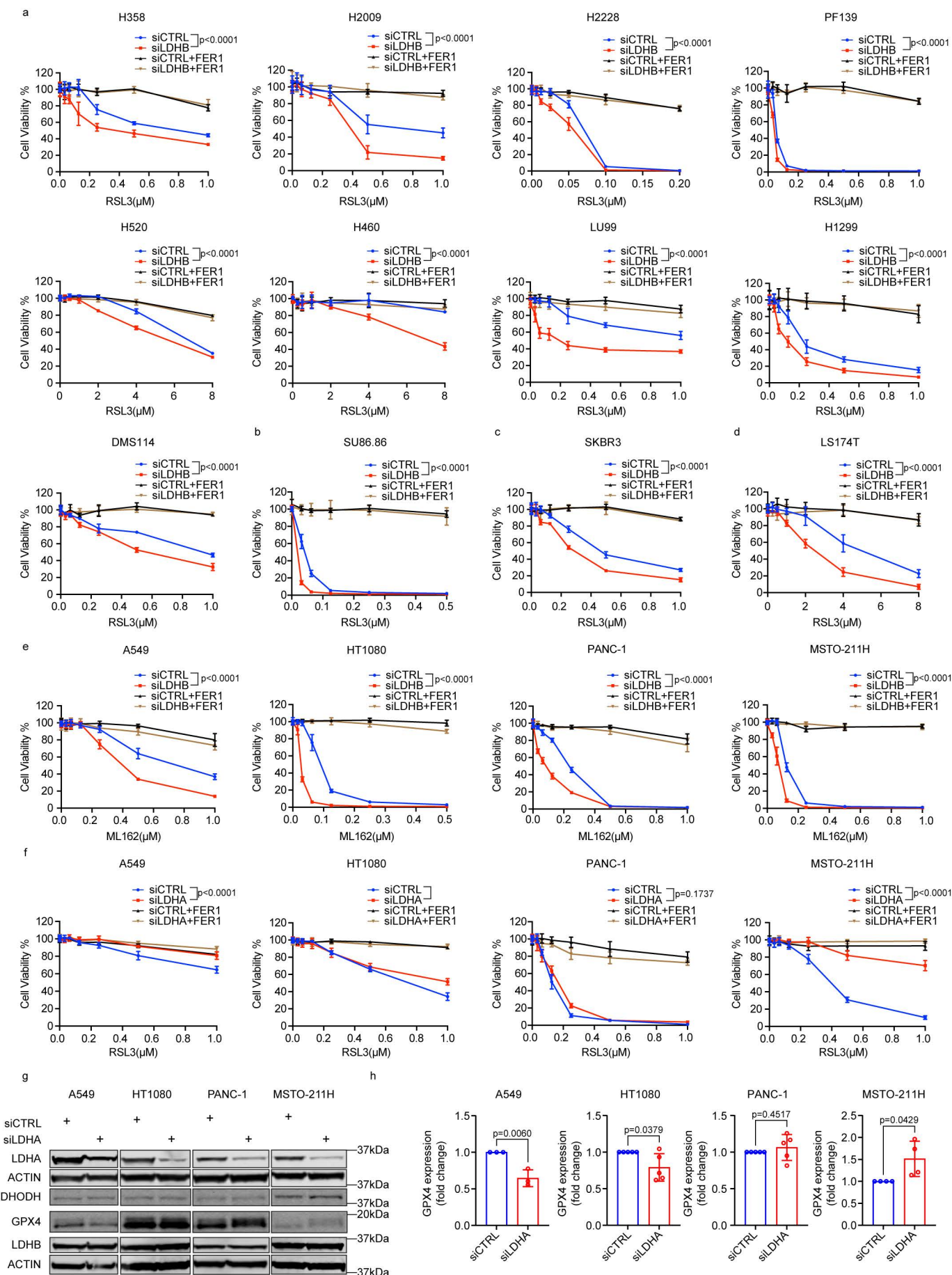
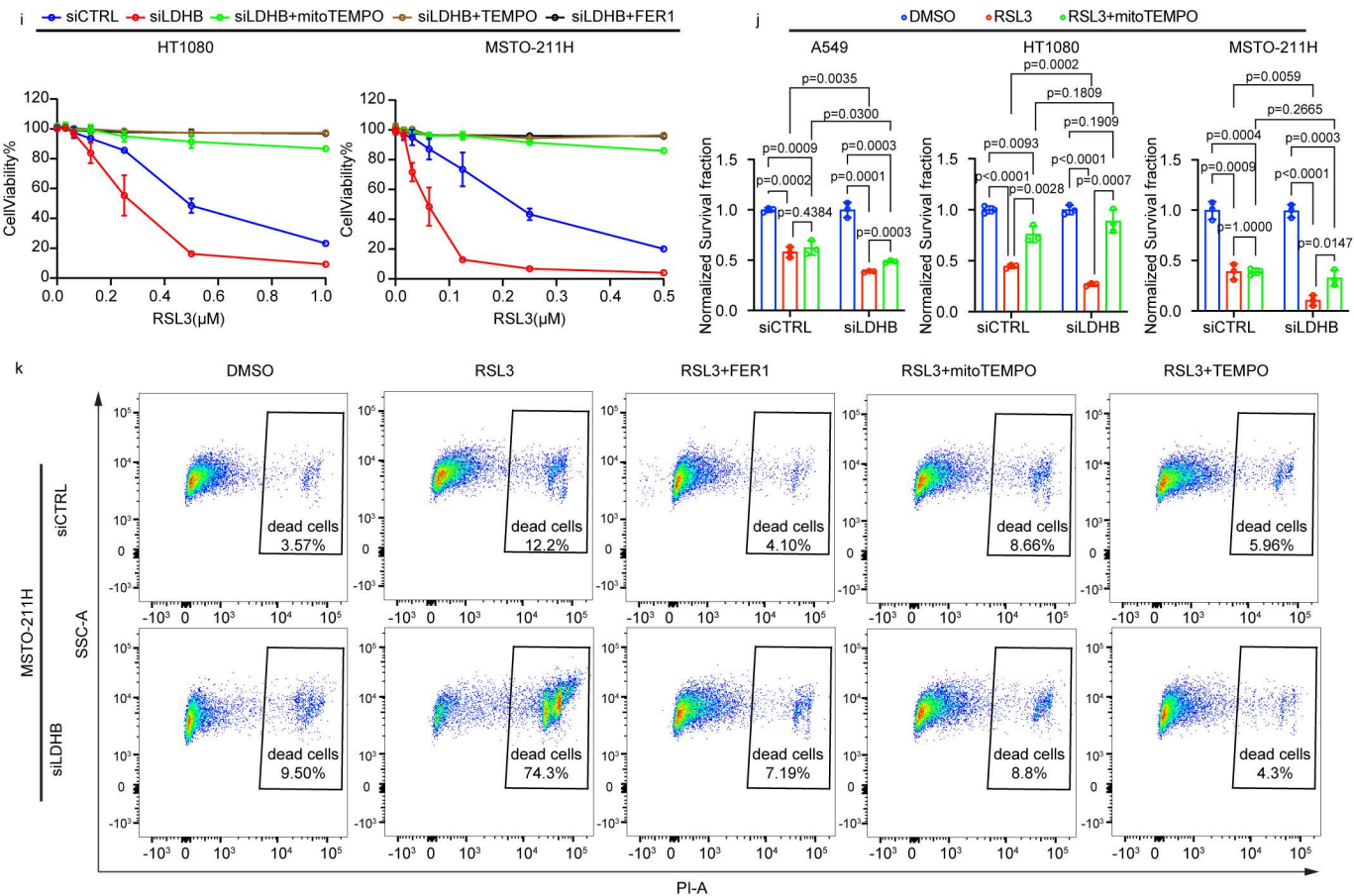


Supplementary Fig. 1 Silencing of LDHB induces mitochondrial lipid peroxidation in cancer cells. mRNA expression of PTGS2 in A549, H358 siRNAs cells, n=3 independent replicates (**a**). Measurement of total lipid peroxidation in A549, H460 siRNAs after 72 h of transfection, n=6 (A549), or 5 (H460) independent replicates (**b, c**). Total lipid peroxidation assessed by immunofluorescence in A549 siRNAs cells after 72 h of transfection (**d**, scale bar, 20µm). Mitochondrial lipid peroxidation assessed in malignant pleural mesothelioma (**e**), pancreatic cancer (**f**), breast cancer (**g**), lung cancer (**h**) siRNAs cells after 72 h of transfection. n=3-4 independent replicates. Colocalization analysis by immunofluorescence in HT1080, MSTO-211H siRNAs after 72 h of transfection (**i, j**, scale bar, 20µm). Transmission electron microscopy images of H460 siRNAs cells after 72 h of transfection (**k**). Analysis of cell viability of siRNAs cells after normalization to siCTRL group, n=3 independent replicates (**l-p**). Cellular ROS analysis in A549, HT1080, MSTO-211H, PANC-1 cells after 72 h of transfection with siRNAs and 100 µM TBHP (tert-butyl-hydroperoxide) treated samples as positive control, n=3-8 independent replicates (**q, r**). Ferroptotic cells (in green and red box) shown by bright field microscope images of A549 siRNAs and counted by Fiji (**s**, scale bar, 20µm). Quantitative analysis of colony numbers of H358, H460 siCTRL and siLDHB cells at day 10 after treatment with DMSO or 2 µM ferrostatin-1 (FER1) for three days after 24 h of transfection, n=3-6 independent replicates (**t**). Data are presented as mean \pm SD. Unpaired, two-tailed t test. *ns* no significant difference, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. Source data are provided as a Source Data file.



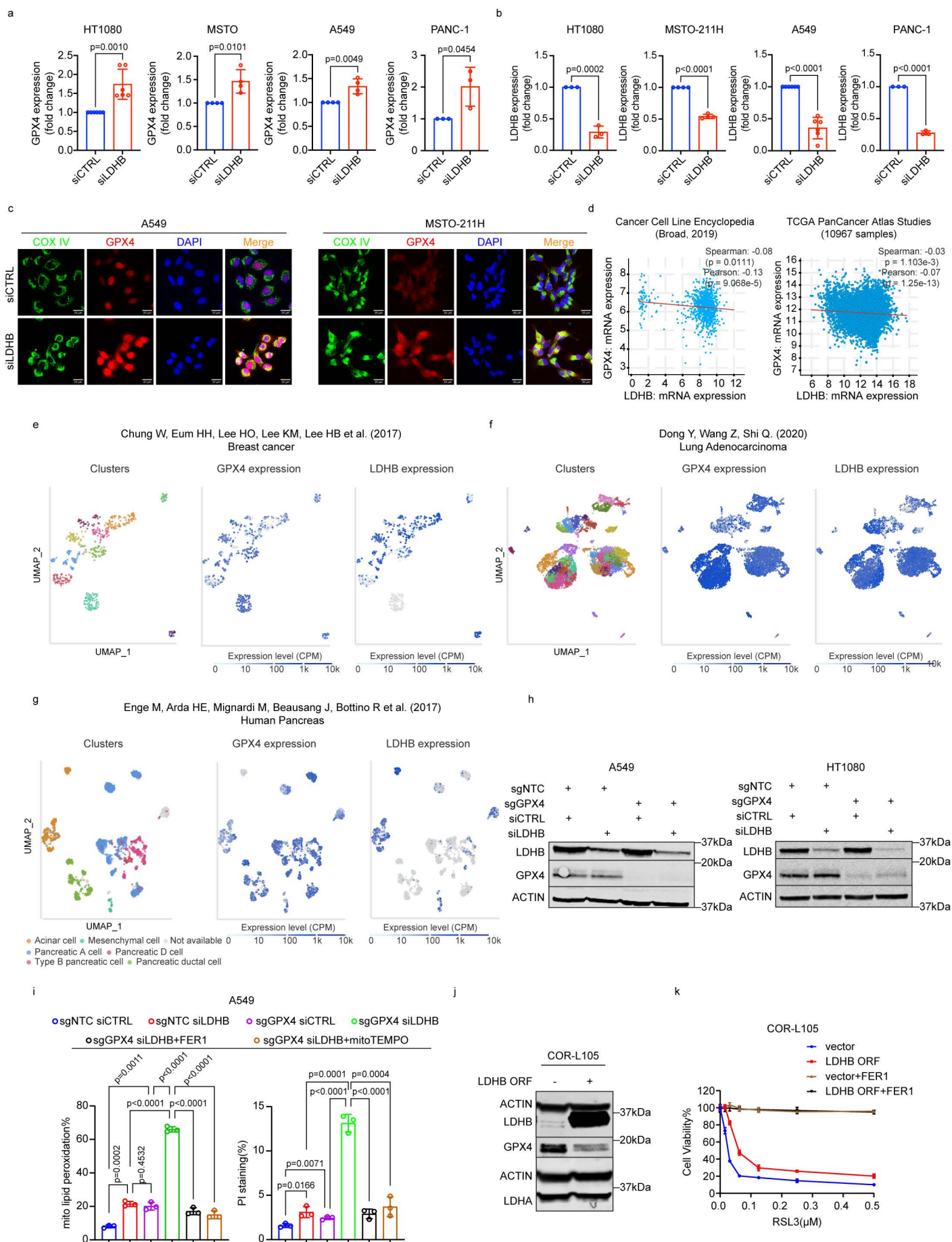
Supplementary Figure 2



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Supplementary Fig. 2 LDHB suppresses mitochondria-associated ferroptosis in cancer cells. Cell viability of siRNAs cells treated with DMSO or GPX4 inhibitors (RSL3 or ML162) alone or in combination with 5 μ M FER1 for 48 h, following pretreatment with 5 μ M FER1 for 24 h, n=3 (**a, b, d, e**), or 4 independent replicates (**c**). Western blot analysis of the expression of LDHA, GPX4, DHODH, LDHB, and the statistical analysis for GPX4 expression after normalizing to corresponding control in A549 (n=3), HT1080 (n=5), PANC-1 (n=5), MSTO-211H (n=4) siRNAs cells, n indicates independent replicates (**g, h**). Assessment of cell viability by APH assay in HT1080, MSTO-211H siRNAs cells treated with DMSO or RSL3 alone or in combination with 20 μ M mitoTEMPO, 20 μ M TEMPO, 5 μ M FER1 for 48 h after pretreatment with vehicle, 20 μ M mitoTEMPO, 20 μ M TEMPO, 5 μ M FER1 for 24 h, n=3 independent replicates (**i**). Clonogenic survival assay of A549, HT1080 and MSTO-211H siRNAs cells treated with DMSO or RLS3 alone (at the same dose as in Fig.2e) or in combination with 20 μ M mitoTEMPO for 48 h after pretreatment with vehicle or 20 μ M mitoTEMPO for 24 h and then grown in fresh medium for additional 7 days. The data were normalized to DMSO groups, n=3 independent replicates (**j**). Analysis of PI staining by flow cytometer in MSTO-211H cells after 72 h of transfection with siRNAs treated with DMSO or 250 nM RSL3 alone or in combination with 5 μ M FER1, 20 μ M mitoTEMPO, 20 μ M TEMPO for 24 h. n=3 independent replicates (**k**). Data are presented as mean \pm SD. Two-way ANOVA (**a-f**), Unpaired, two-tailed t test (**h, j**). *ns* no significant difference, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. Source data are provided as a Source Data file.

Supplementary Figure 3

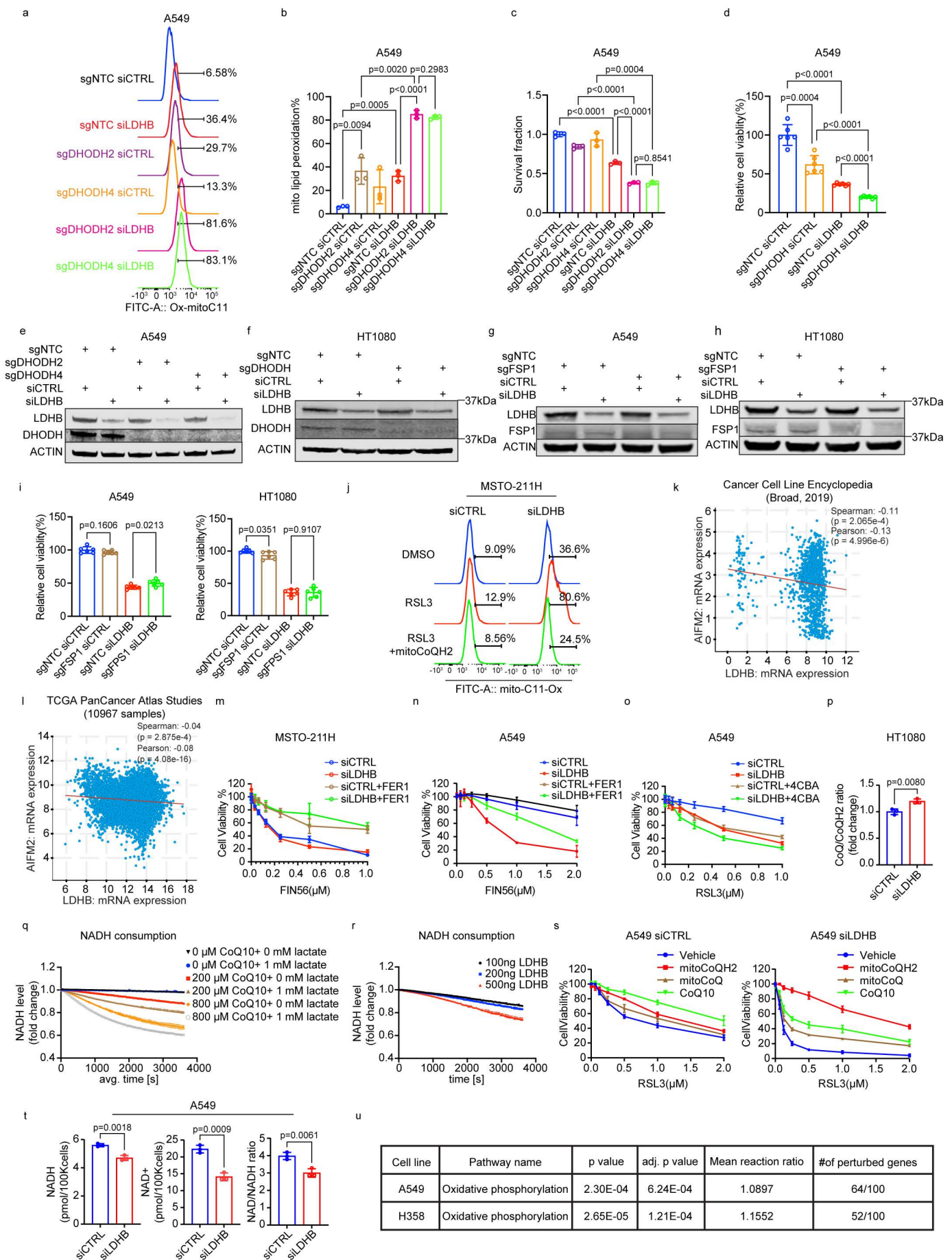


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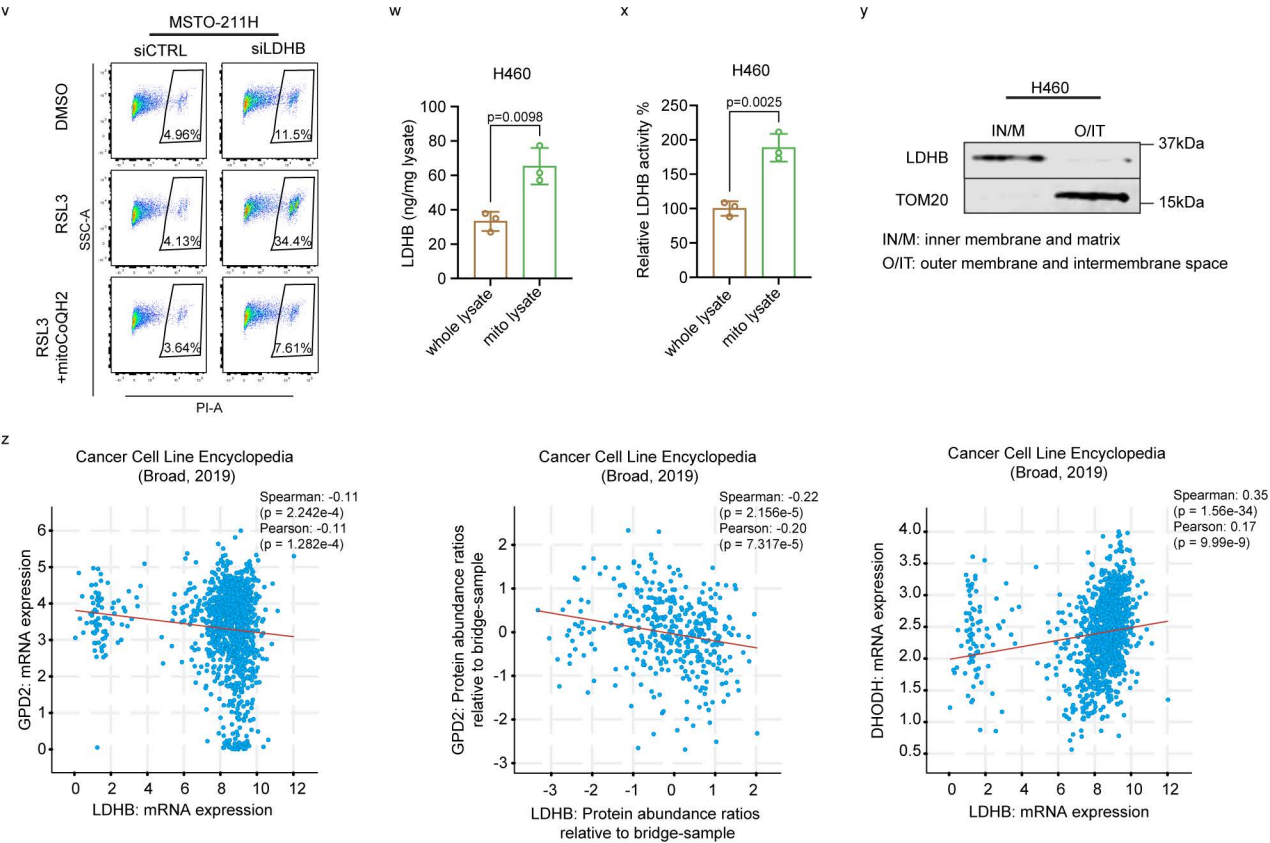
Supplementary Fig. 3 LDHB acts in parallel with GPX4 to suppress mitochondria-associated ferroptosis.

Quantitative analysis of GPX4 and LDHB expression in HT1080, MSTO-211H, A549, PANC-1 siRNAs cells by Western blot. **(a, b)**. The colocalization of GPX4 and COX IV was investigated by immunofluorescence in A549 and MSTO-211H siRNAs cells after 72 hours of transfection **(c)**, scale bar, 20μm). Correlation analysis of mRNA expression of LDHB and GPX4 in 1739 cancer cell lines and 10967 patient samples with cBioPortal **(d)**. UMAP plot of single cells from breast cancer, lung adenocarcinoma patients and human pancreas and analysis of GPX4 and LDHB expression with the Single Cell Expression Atlas **(e-g)**. Immunoblot analysis of LDHB, GPX4 and ACTIN in A549, HT1080 sgNTC and sgGPX4 cells after 72 h transfection with siRNAs **(h)**. Analysis of mitochondrial lipid peroxidation and PI staining in A549 sgNTC, sgDHODH cells after 72 h of transfection with siRNAs, n=3 independent replicates **(i)**. Immunoblot analysis of LDHB, GPX4, LDHA and ACTIN in COR-L105 and COR-L105 LDHB ORF cells **(j)**. Cell viability assay of COR-L105 cells and COR-L105 LDHB ORF cells treated with RSL3 with or without 5 μM FER1 for 48 h after pretreatment with 5 μM FER1 for 24h, n=3 independent replicates **(k)**. Tumor volume **(l)**, weight **(m)**, representative immunohistochemistry (IHC) images **(n)**, scale bar, 50μm) of A549 shCTRL and shLDHB xenograft tumors treated with solvent or RLS3, n=4-8 tumors from different mice. H-score analysis of 4HNE and LDHB, n=6-11 random fields from different tumors **(o)**. Representative IHC images and H-score analysis of A549 **(p, q)**, scale bar, 50μm) and HT1080 **(r, s)**, scale bar, 50μm) sgNTC shCTRL, sgNTC shLDHB, sgGPX4 shCTRL, sgGPX4 shLDHB xenograft tumors. n=10-12 random fields from different tumors. Data are presented as mean ± SD **(a, b, i, k, m, o, q, s)** or mean ± SEM **(l)**. Two-way ANOVA **(l)** or Unpaired, two-tailed t test **(a, b, i, k, o, q, s)**. *ns* no significant difference, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. Source data are provided as a Source Data file.

Supplementary Figure 4

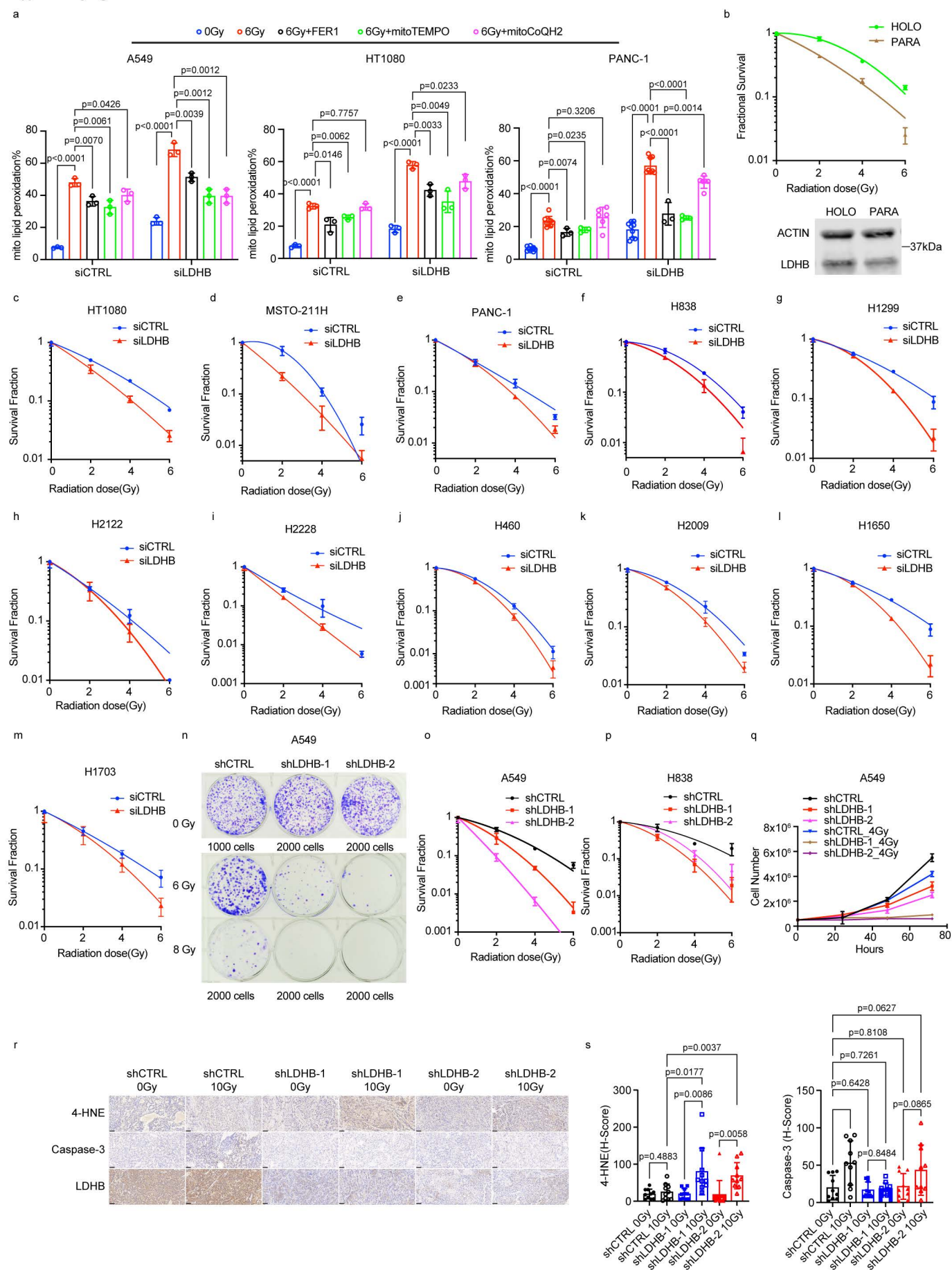


Supplementary Figure 4



Supplementary Fig. 4 LDHB suppresses ferroptosis by regulating the reduction of CoQH2 in mitochondria. Analysis of mitochondrial lipid peroxidation (**a, b**), survival fraction (**c**), relative cell viability (**d**) in A549 sgNTC and sgDHODH cells after 72 h of transfection with siRNAs. n=3-6 independent replicates. Analysis of the expression of LDHB, DHODH or FSP1 in A549 and HT1080 sgNTC, sgDHODH or sgFSP1 cells after 72 h of transfection with siRNAs (**e-h**). The analysis of relative cell viability in A549 and HT1080 sgNTC, sgFSP1 cells after 72 h of transfection with siRNAs, n=3 independent repeats (including 2 technical repeats of each independent repeat) (**i**). Assessment of mitochondrial lipid peroxidation in MSTO-211H cells after 72 h of transfection with siRNAs treated with DMSO or RSL3 alone or in combination with 500 nM mitoCoQH2 for 1 h (**j**). Correlation analysis of LDHB and AIFM2 in cancer cell lines and patient samples using cBioPortal (**k, l**). Cell viability in siRNAs cells treated with DMSO or FIN56 (**m, n**) or RSL3 (**o**) alone or in combination with 5 μ M FER1 (**m, n**) or 5 mM 4CBA (**o**) for 48 h, following pretreatment with 5 μ M FER1 (**m, n**) or 5 mM 4CBA (**o**) for 24 h. n=3 independent replicates. CoQ/CoQH2 ratio in HT1080 siRNAs cells (**p**). NADH consumption assay (A340 nm) in TBS buffer with or without lactate at different concentrations of CoQ10 (**q**), and different LDHB concentration without lactate (**r**). Cell viability of A549 siRNA transfected cells treated with DMSO or RSL3 alone or in combination with 100 nM mitoCoQH2 or 100 nM mitoCoQ or 10 μ M CoQ10, for 48 h, following pretreatment with vehicle, 100 nM mitoCoQH2 or 100 nM mitoCoQ or 10 μ M CoQ10 for 24 h, n=3 independent replicates (**s**). Measurement of NAD and NADH in A549 cells after 72 hours of transfection with RNAs (**t**). Transcriptomics-driven metabolic pathway analysis (TDPA) of the RNA seq data from A549 and H358 siCTRL and siLDHB cells (**u**). Flow cytometer plot of PI staining in MSTO-211H transfected cells after treatment with 0.75 μ M RSL3 for 6 h (**v**). Measurement of LDHB protein (**w**) and activity (**x**) in H460 whole and mitochondrial lysate. Immunoblot analysis of LDHB expression in inner and outer mitochondrial membrane (**y**). Correlation analyses of LDHB, GPD2, DHODH in cancer cell lines using cBioPortal (**z**). Data are presented as mean \pm SD. Unpaired, two-tailed t test. *ns* no significant difference, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. Source data are provided as a Source Data file.

Supplementary Figure 5





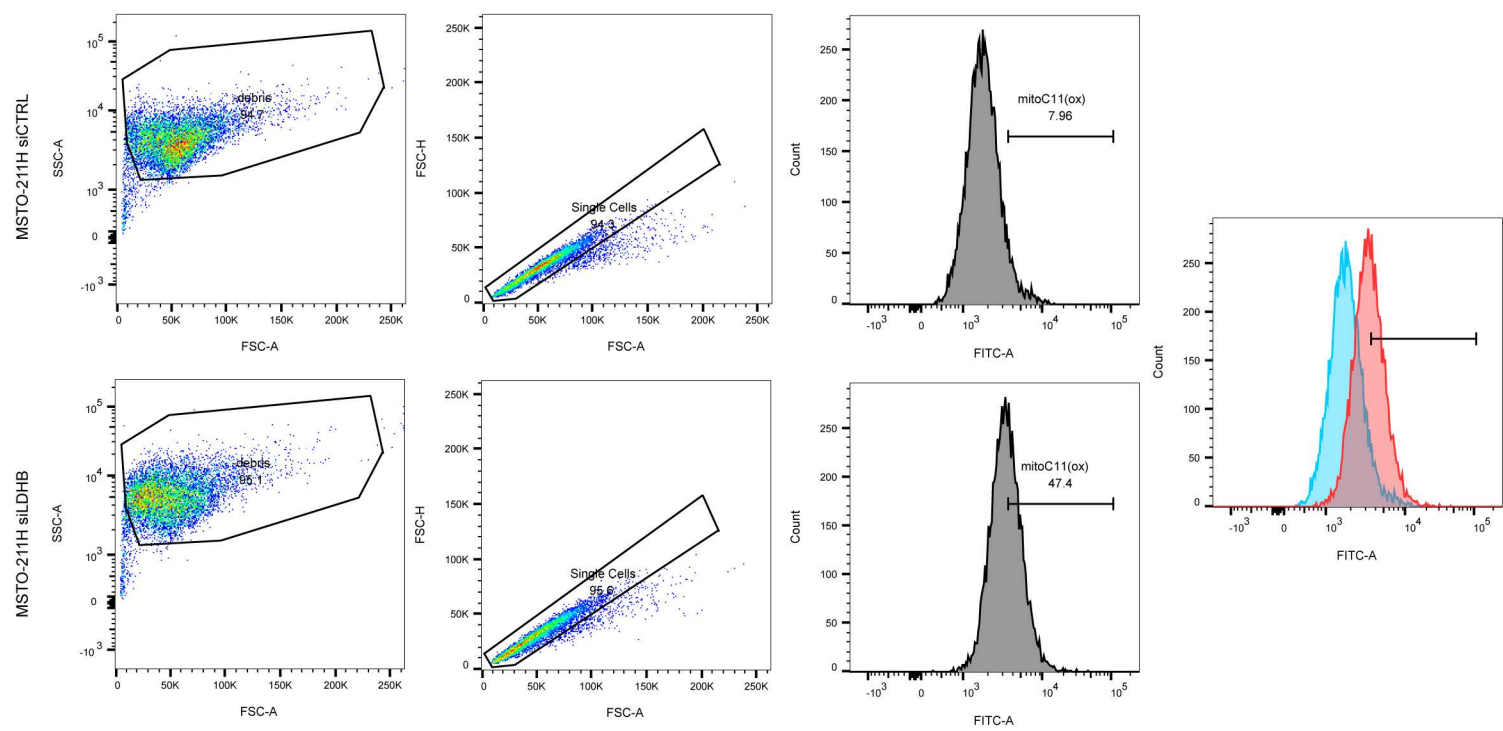
Low expression cohort (months)	High expression cohort (months)
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88 **Supplementary Fig. 5 Inhibition of LDHB sensitizes tumor cells to radiotherapy by enhancing**
89 **mitochondria-associated ferroptosis.** Analysis of mitochondrial lipid peroxidation in A549, HT1080, PANC-
90 1 siRNAs cells after 24 h of 6Gy irradiation with or without 5 μ M FER1, 20 μ M mitoTEMPO, 500 nM
91 mitoCoQH2 treatment. n=3 independent replicates **(a)**. Clonogenic survival curve of HOLO and PARA cells
92 after irradiation with doses from 0 to 6 Gy **(b)**. Immunoblot analysis of LDHB and ACTIN in HOLO and PARA
93 cells **(b)**. Clonogenic survival curve of siCTRL and siLDHB cells after irradiation with doses from 0 to 6 Gy
94 **(c-m)**. Images of the clonogenic assay of A549 shCTRL and shLDHB cells irradiated from 0 Gy to 8 Gy **(n)**.
95 Clonogenic survival curve of A549 and H838 shCTRL and shLDHB cells after irradiation with doses from 0 to
96 6 Gy **(o, p)**. Cell number of A549 shCTRL and shLDHB cells after exposure to 4 Gy at different time points
97 **(q)**. Representative IHC images and H-score analysis of 4-HNE and caspase-3 in A549 shCTRL and shLDHB
98 xenograft tumors after treatment with a single dose of local IR, n=10 random fields from different tumors **(r, s,**
99 **scale bar, 50 μ m)**. Schematic representation of the plan for local irradiation of xenografts and orthotopic lung
100 tumors **(t, u)**. Kaplan-Meier median survival analysis of high and low LDHB groups in patients with lung cancer
101 who received radiotherapy **(v)**. Data are presented as mean \pm SD. Unpaired, two-tailed t test. * $P < 0.05$, ** $P <$
102 0.01 , *** $P < 0.001$, **** $P < 0.0001$. Source data are provided as a Source Data file.

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Supplementary Figure 6



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105 **Supplementary Fig. 6 Flow cytometry gating strategy for analyzing lipid peroxidation.** Representative
106 flow cytometry gating strategy for lipid peroxidation analysis.

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