Supplemental information

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BRAF^{V600E} lung adenocarcinoma

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In vivo vulnerabilities to GPX4 and HDAC inhibitors in drug-persistent versus drug-resistant BRAF^{V600E} lung adenocarcinoma

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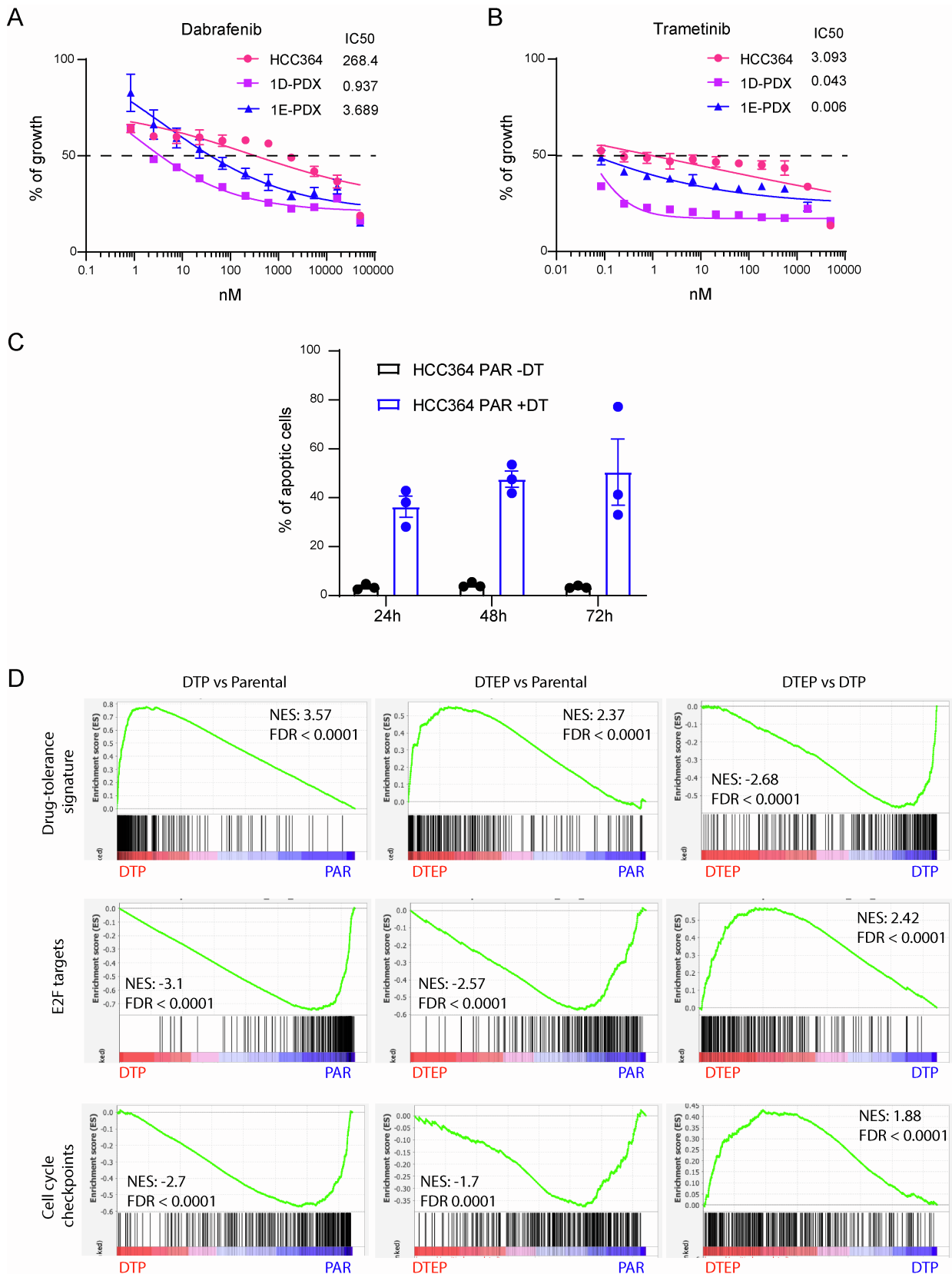
Figure S1: Characterization of HCC364 DTP and DTEP states following D/T treatment, related to Figures 1, 2 and 4. A, B. Cell viability assessment by MTT assay of HCC364, 1D- and 1E-PDX cells treated with serial dilutions of dabrafenib (A) and trametinib (B) for 72 hr. IC50 values are indicated for each condition. n = 3 biological replicates. **C.**

Apoptosis quantification by flow cytometry of HCC364 cells treated with 250/5 nM dabrafenib/trametinib for 24, 48 and 72 hr. n = 3 biological replicates. **D.** Enrichment scores of GSEA in DTP vs Parental, DTEP vs Parental and DTEP vs DTP using the drug tolerance signature and the E2F targets and cell cycle checkpoints gene sets. n = 1 biological replicate. All data are shown as the mean values ± SEM.
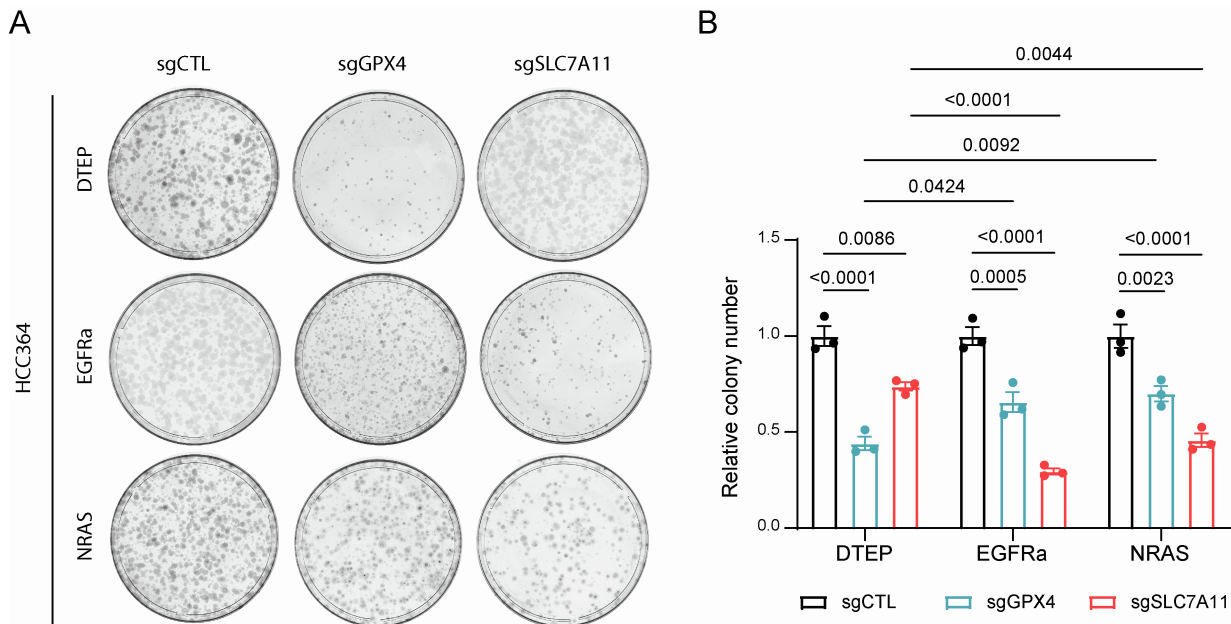
Figure S2: Clonogenic assay following Crispr/Cas9 knock-out of GPX4 and SLC7A11 in DTEP and D/T resistant HCC364 cells, related to Figures 3 and 5. Clonogenic assay of the indicated HCC364 cell line variants cultured with 250/5 nM dabrafenib/trametinib and 1µg/ml doxycycline to induce Crispr/Cas9 mediated knock-out of GPX4 or SLC7A11. An sgRNA targeting LacZ was used as control. Data are shown as the mean values ± SEM. n = 3 biological replicates.
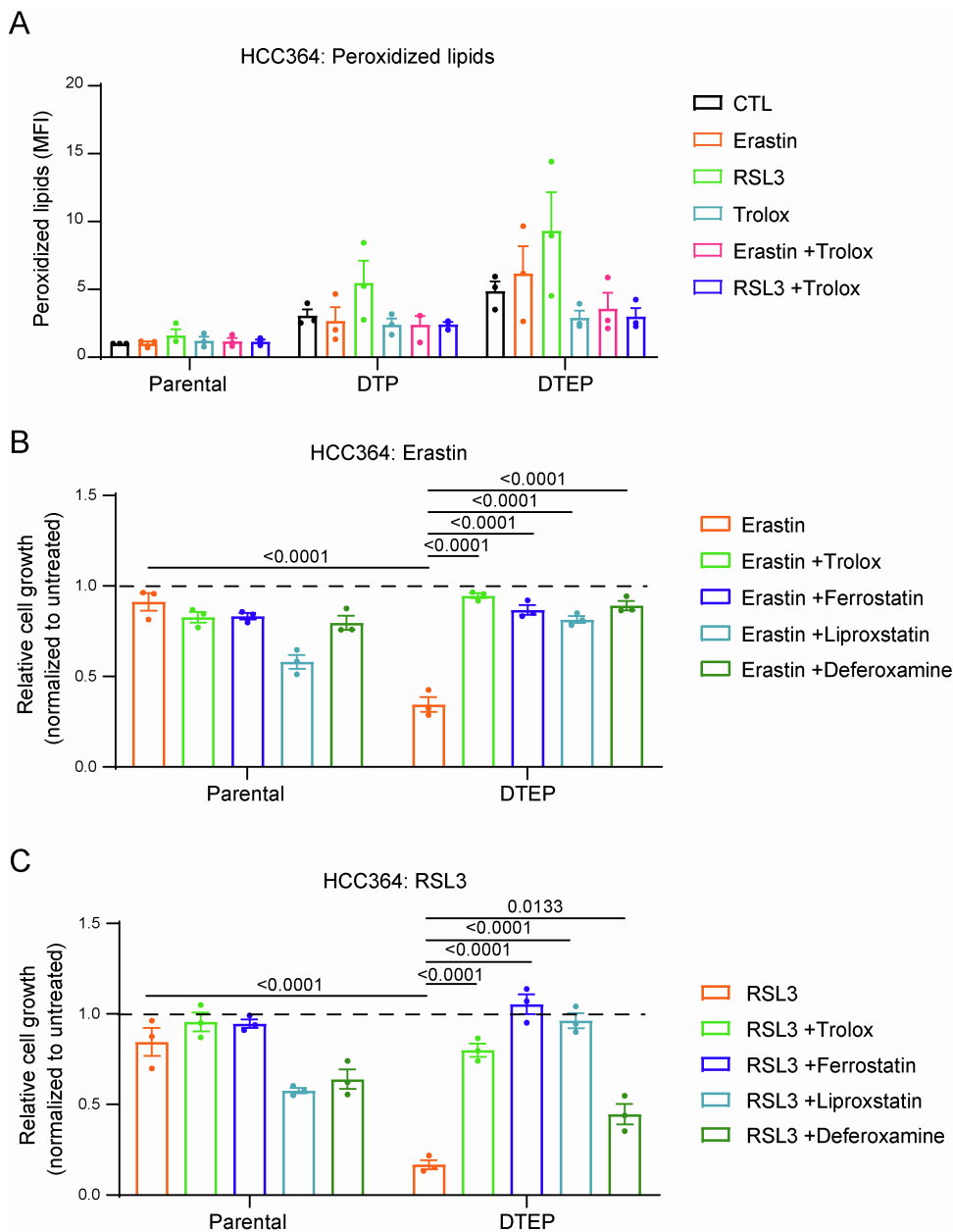
Figure S3: Rescue experiment of RSL3-treated HCC364 DTEPs upon co-incubation with ferroptosis inhibitors, related to Figure 3. A. Intracellular peroxidized lipids content in parental, DTP and DTEP HCC364 cells treated with 500 nM Erastin or 50 nM RSL3 in presence of 10 µM Trolox for 72 hr. Data are shown as the mean values ± SEM. $n = 3$ biological replicates. **B, C.** Cell viability assessment by MTT assay of parental and DTEP HCC364 cells treated with 500 nM Erastin (B) or 50 nM RSL3 (B) in presence of 10 µM Trolox, 200 nM Ferrostatin, 100 nM Liproxstatin, 1 µM Deferoxamine for 72 hr. Data are normalized to untreated cells and shown as the mean values ± SEM. Data were analysed using two-way ANOVA followed by Tukey's multiple comparisons post-test. $n = 3$ biological replicates.
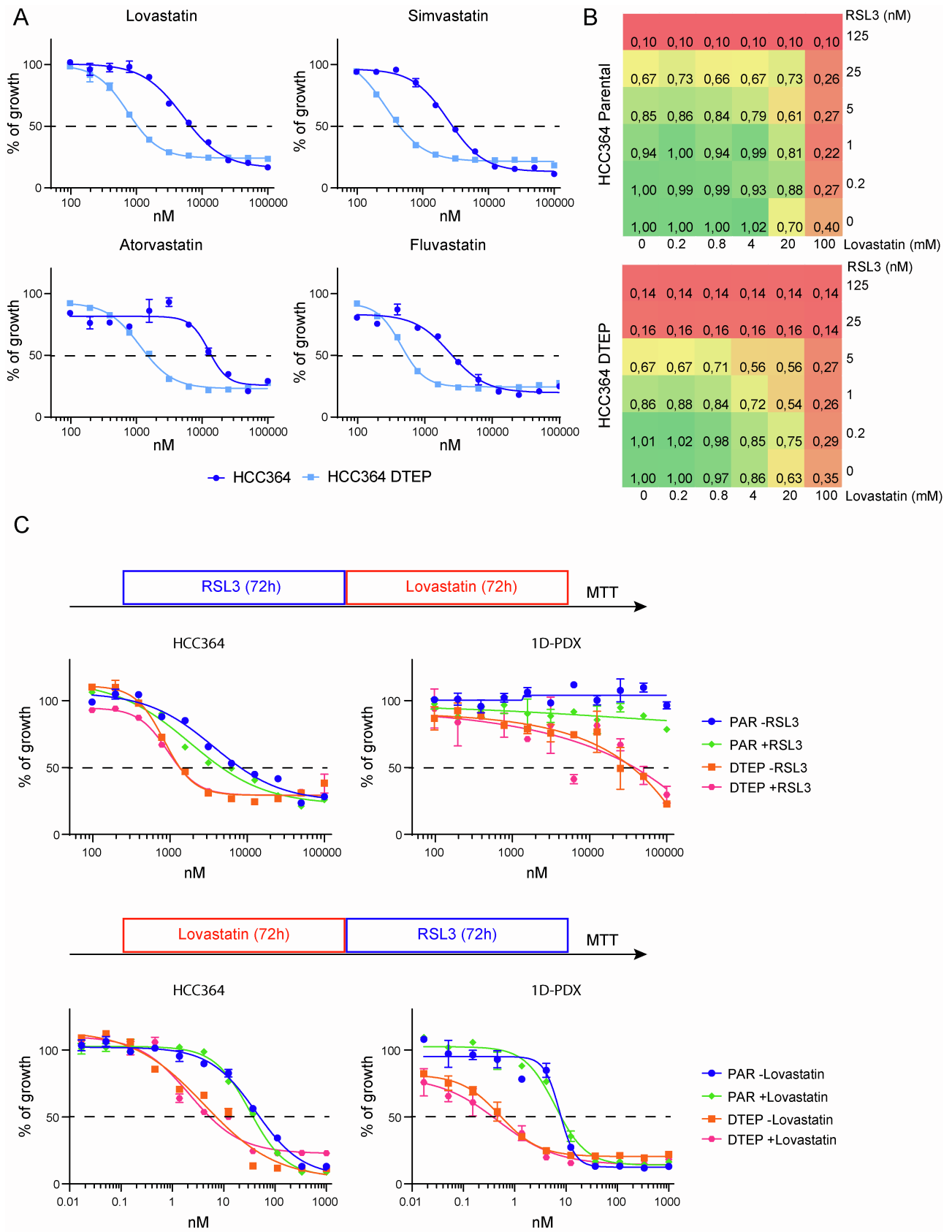
Figure S4: Treatment combinations of the GPX4 inhibitor RSL3 and statins in HCC364 and PDX-derived cell line 1D, related to Figure 3. **A.** Cell viability assessment by MTT assay of parental and DTEP HCC364 cells treated

with serial dilutions of the indicated drugs for 72 hr. n = 3 biological replicates. **B.** Sensitivity matrix comparing parental and DTEP HCC364 cells after 72 hr in presence of the indicated concentrations of RSL3 and Lovastatin. n = 3 biological replicates. **C.** Cell viability assessment by MTT assay of parental and DTEP HCC364 and 1D-PDX cells treated with serial dilutions of Lovastatin (upper panels) or RSL3 (lower panels) for 72 hr after a pretreatment (72 hr) with RSL3 1 nM or Lovastatin 1 µM, respectively. n = 3 biological replicates. All data are shown as the mean values ± SEM of one representative experiment.
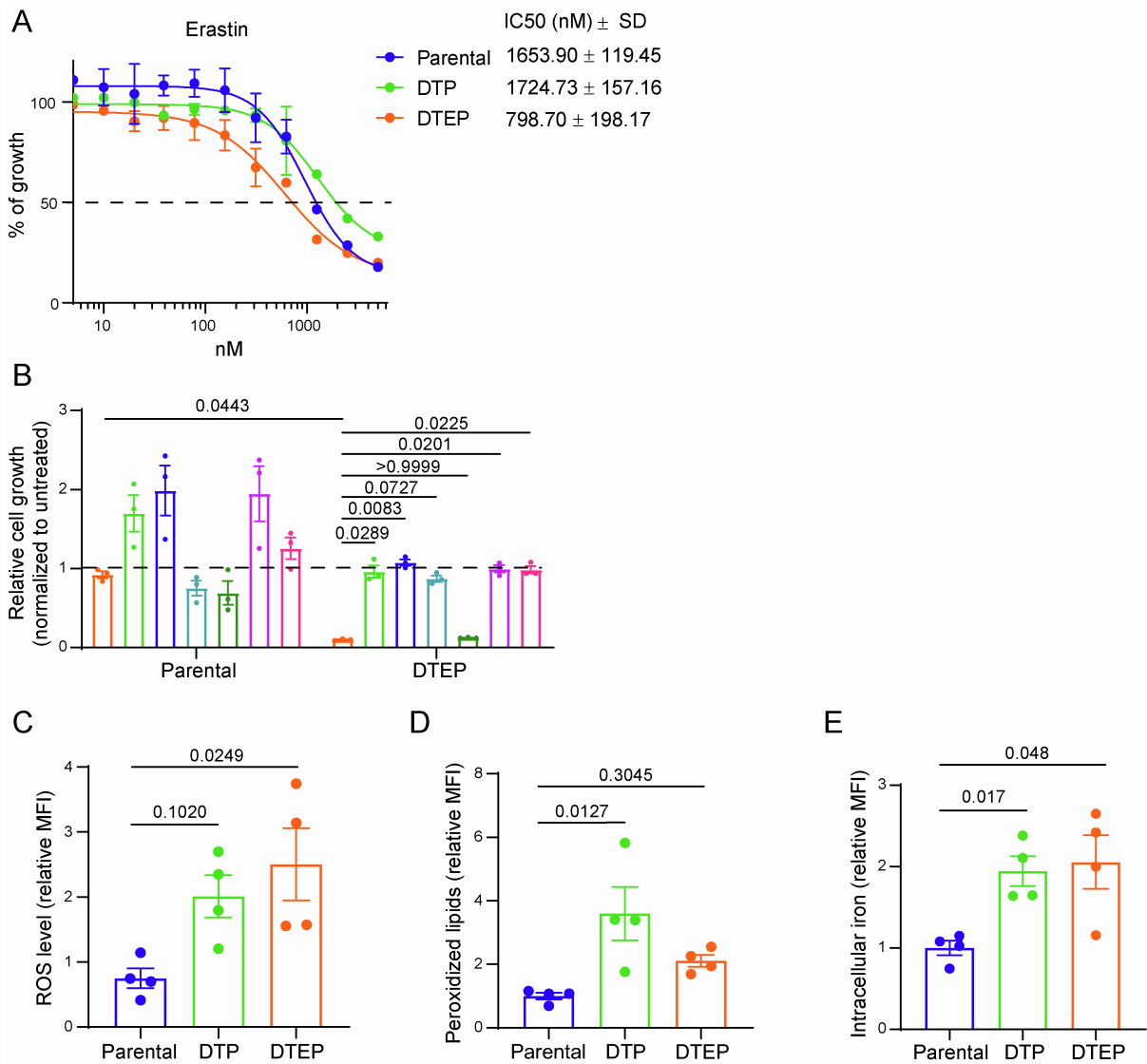
Figure S5: PDX-derived cell line 1D DTP & DTEPs are sensitive to ferroptosis triggers, related to Figure 4. A. Cell viability assessment by MTT assay of parental (PAR), DTP and DTEP 1D cells treated with serial dilutions of Erastin for 72 hr. Data are shown as the mean values ± SEM. IC50 values ± SD are indicated for each condition. $n = 3$ biological replicates. **B.** Cell viability assessment by MTT assay of parental and DTEP 1D cells treated with 500 nM Erastin in presence of 10 µM Trolox, 200 nM Ferrostatin, 100 nM Liproxstatin, 1 µM Deferoxamine, 200 nM selenium (Se) or 2.5 mM N-acetyl-cysteine (NAC) for 72 hr. Data are normalized to untreated cells and shown as the mean values ± SEM. Data were analysed using two-way ANOVA followed by Tukey's multiple comparisons post-test. $n = 3$ biological replicates. **C-E.** Intracellular ROS level (C), peroxidized lipids content (D) and iron level (E) in parental, DTP and DTEP 1D cells. Data were analysed using one-way ANOVA followed by Dunnett's multiple comparisons post-test and are shown as the mean values ± SEM. $n = 4$ biological replicates.
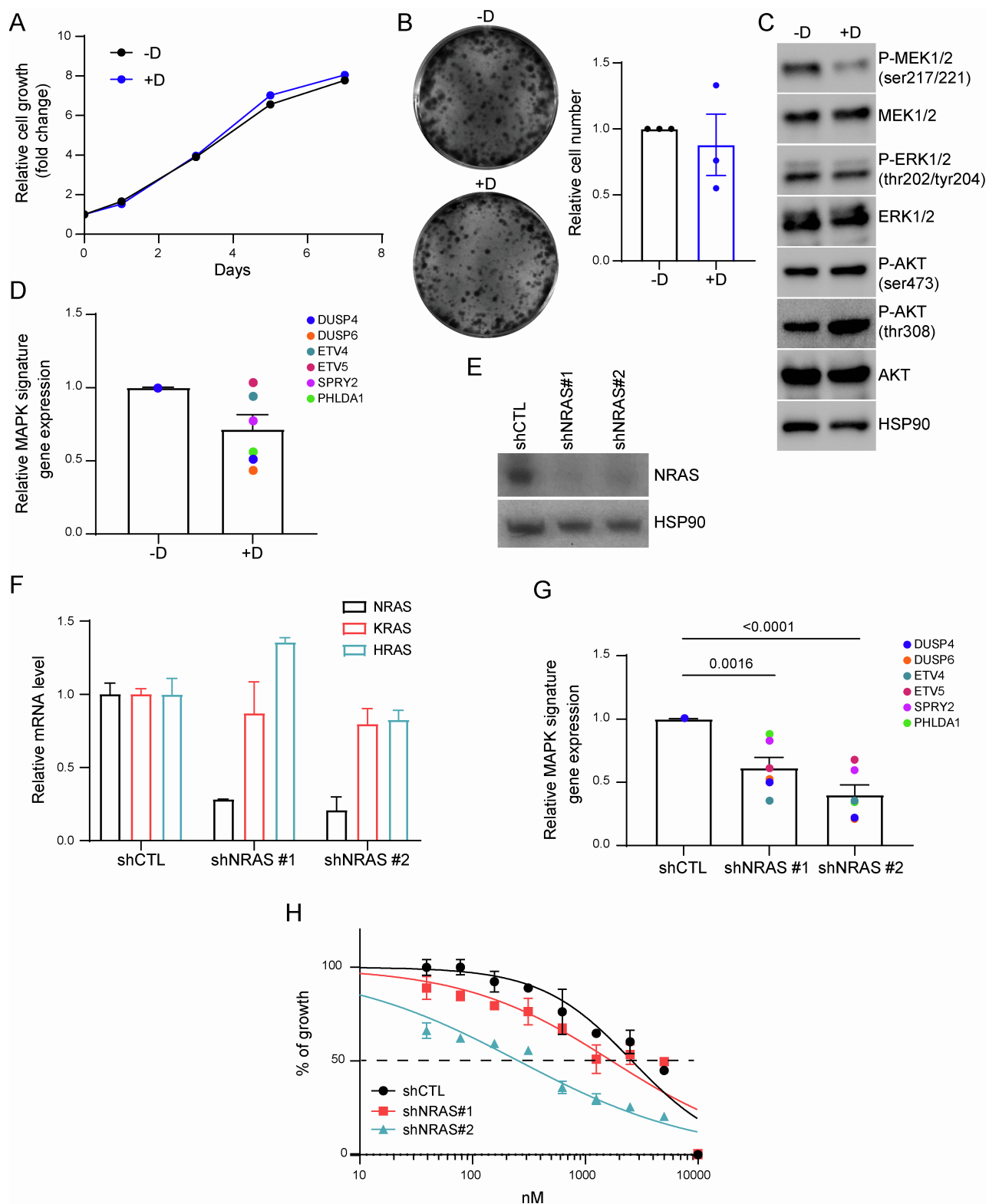
**Figure S6: An acquired *NRAS^Q61K* mutation drives D/T resistance in DFCI471 cells, related to Figure 5. A.** Growth curves of DFCI471 cells in presence of 1 μM dabrafenib. Data are shown as the mean values ± SEM of one representative experiment. *n* = 3 biological replicates. **B.** Clonogenic assay of DFCI471 cells cultured with 1 μM dabrafenib. Data are shown as the mean values ± SEM. *n* = 3 biological replicates. **C.** Immunoblot of phospho-MEK1/2, -ERK1/2 and -AKT in DFCI471 cells cultured in presence of 1μM dabrafenib (D) for 48 hr. HSP90 was used

as a loading control. *n* = 2 biological replicates. **D.** Quantitative RT-PCR analysis of relative MAPK pathway target genes (DUSP4, DUSP6, ETV4, ETV5, SPRY2, PHLDA1) mRNA levels in DFCI471 cells cultured in presence of 1μM dabrafenib (+D) for 48 hr. Data are shown as the mean values ± SEM. *n* = 3 biological replicates. **E.** Immunoblot of NRAS in DFCI471 upon NRAS shRNAs (shNRAS #1 and #2) mediated knock-down. *n* = 2 biological replicates. **F and G.** Quantitative RT-PCR analysis of relative NRAS, KRAS and HRAS mRNA levels (F) and of relative MAPK pathway target genes (DUSP4, DUSP6, ETV4, ETV5, SPRY2, PHLDA1) mRNA levels (G) in NRAS knock-down DFCI471 cells. Data are shown as the mean values ± SEM. *n* = 3 biological replicates. **H.** Cell viability assessment by MTT assay of DFCI471 cells upon NRAS shRNAs (shNRAS #1 and #2) mediated knock-down treated with serial dilutions of Dabrafenib for 72 hr. Data are shown as the mean values ± SEM. *n* = 3 biological replicates.
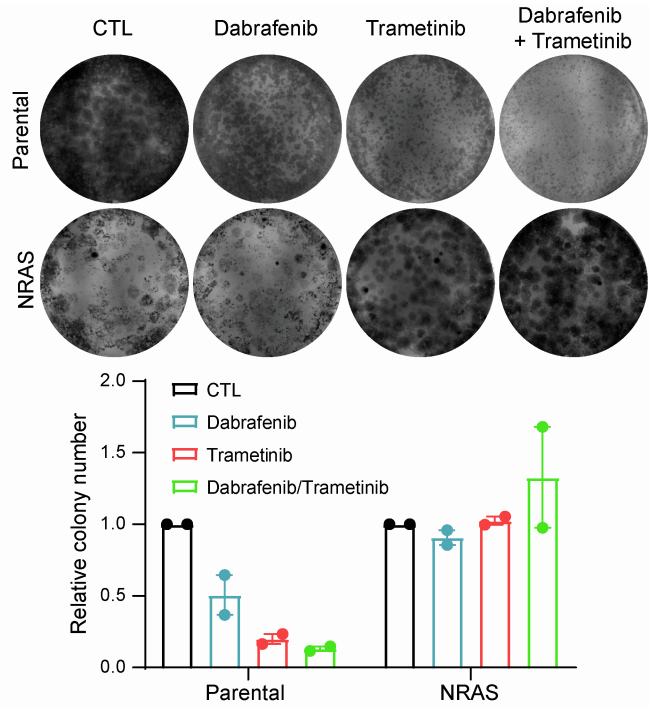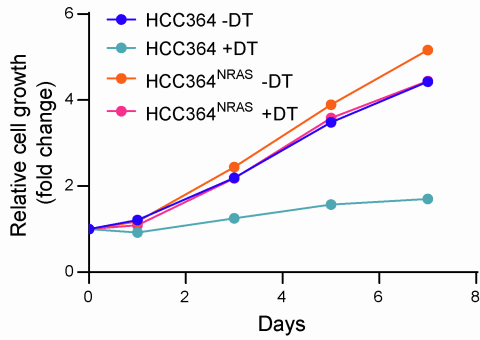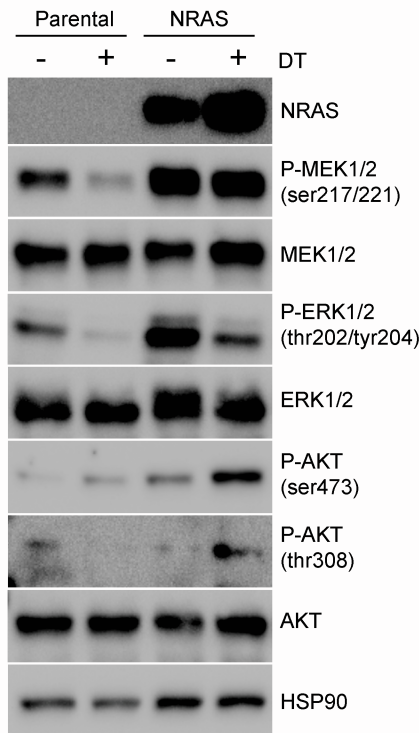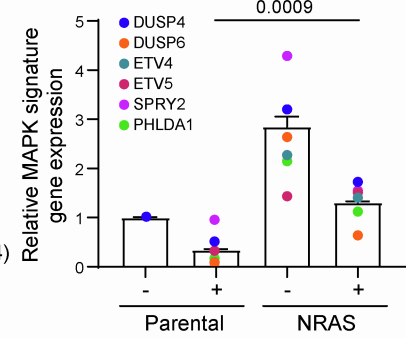
Figure S7: Acquired mutations resulting in MAPK reactivation drive D/T resistance in HCC364 cells, related to Figure 5. A. Cell viability ass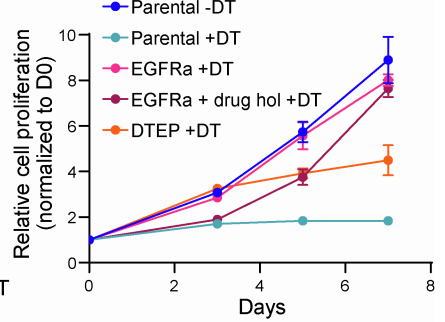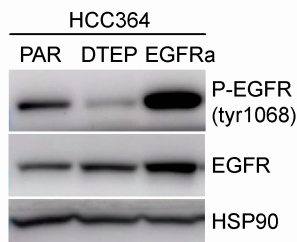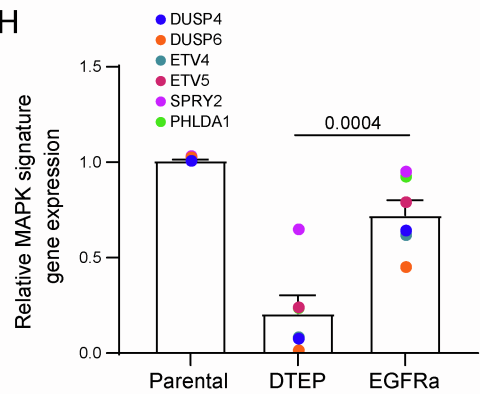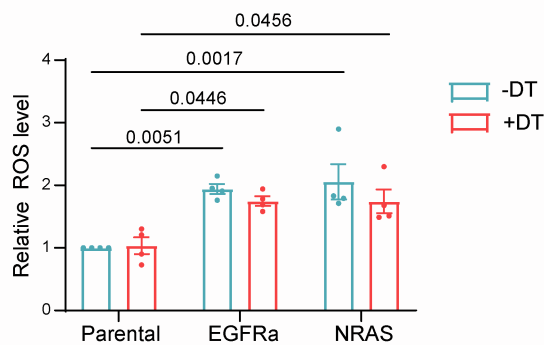essment by MTT assay of HCC364 and HCC364^{NRAS} cells treated with serial dilutions of dabrafenib or trametinib for 72 hr. IC50 values are indicated for each condition. n = 3 biological replicates. **B.** Clonogenic assay of HCC364 and HCC364^{NRAS} cells treated with 250 nM dabrafenib, 5 nM trametinib or 250/5 nM dabrafenib/trametinib. Data are shown as the mean values ± SEM. n = 2 biological replicates. **C.** Growth curves of HCC364 and HCC364^{NRAS} cells in presence of 250/5 nM dabrafenib/trametinib (+DT). Data are shown as the mean values ± SEM of one representative experiment. n = 3 biological replicates. **D.** Immunoblot of NRAS, phospho-MEK1/2, -ERK1/2 and -AKT in HCC364 and HCC364^{NRAS} cells cultured in presence of 250/5 nM D/T (DT) for 48 hr. HSP90 was used as a loading control. n = 2 biological replicates. **E.** Quantitative RT-PCR analysis of relative MAPK pathway target genes (DUSP4, DUSP6, ETV4, ETV5, SPRY2, PHLDA1) mRNA levels in HCC364 and HCC364^{NRAS} cells cultured in presence of 250/5 nM D/T for 48 hr. Data were analysed using one-way ANOVA followed by Tukey's multiple comparisons post-test and are shown as the mean values ± SEM. n = 3 biological replicates. **F.** HCC364^{EGFRa} were derived spontaneously from HCC364-DTEPs after continuous D/T (250/5 nM) treatment for 70 weeks. Growth curves of parental, DTEP and EGFRa HCC364 cells in presence of 250/5 nM D/T. EGFRa cells undergone a drug holiday for 3 weeks and were rechallenged with D/T to confirm the genetic adaptation of these cells to the treatment (EGFRa + drug hol +DT). Data are shown as the mean values ± SEM of one representative experiment. n = 3 biological replicates. **G.** Immunoblot of phospho-EGFR in parental, DTEP and EGFRa HCC364 cells. HSP90 was used as a loading control. n = 3 biological replicates. **H.** Quantitative RT-PCR analysis of relative MAPK pathway target genes (DUSP4, DUSP6, ETV4, ETV5, SPRY2, PHLDA1) mRNA levels in parental, DTEP and EGFRa HCC364 cells. Data are shown as the mean values ± SEM. n = 3 biological replicates. **I.** Intracellular ROS level in HCC364 and HCC364^{NRAS} cells cultured in presence of 250/5 nM D/T (+DT) for 72 hr. Data were analysed using two-way ANOVA followed by Tukey's multiple comparisons post-test and are shown as the mean values ± SEM. n = 4 biological replicates.
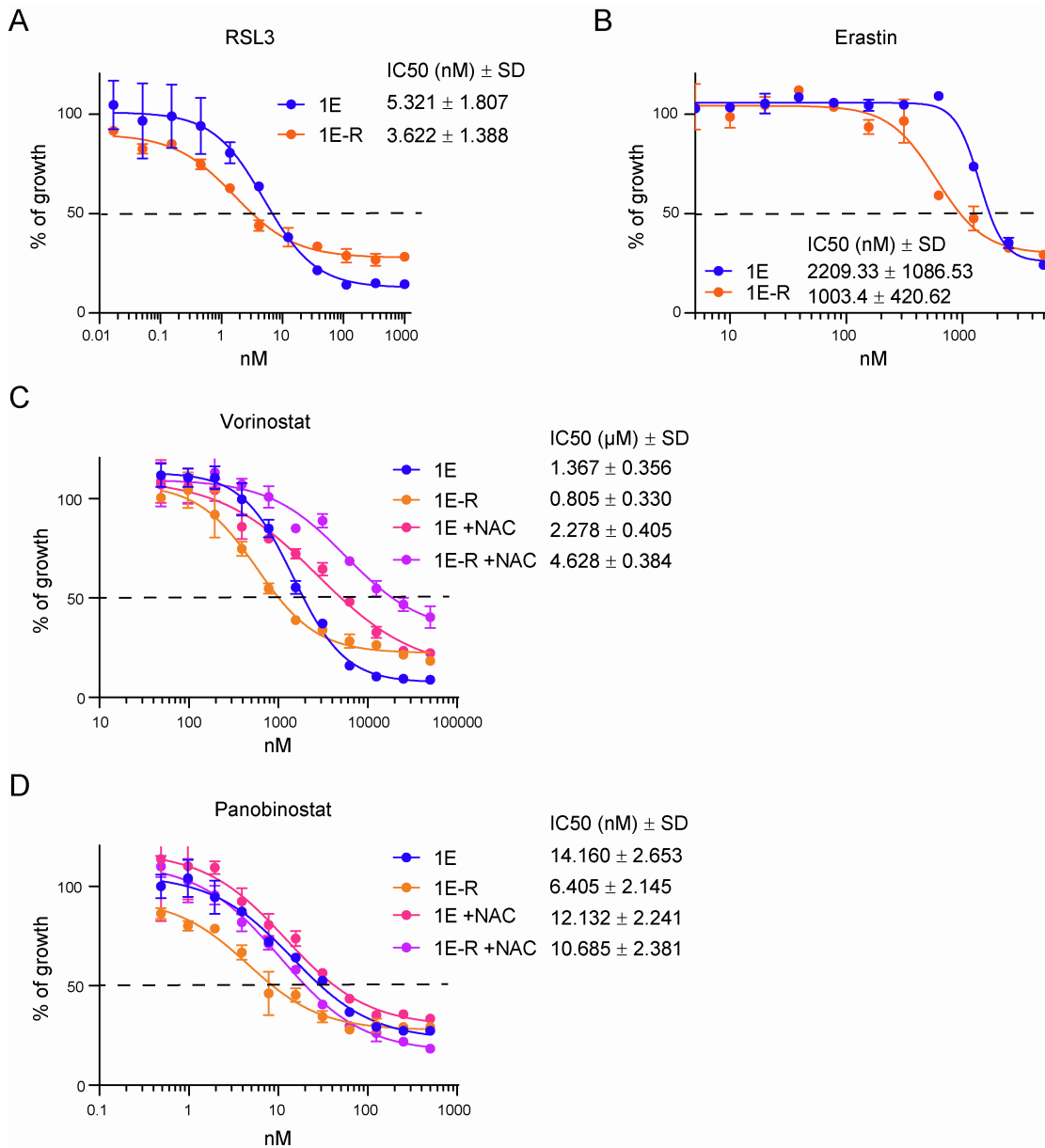
Figure S8: D/T resistant PDX-derived cell line 1E are insensitive to ferroptosis triggers and sensitive to HDAC inhibition, related to Figure 6. A, B. Cell viability assessment by MTT assay of 1E and 1E-R cells treated with serial dilutions of RSL3 (A) and Erastin (B) for 72 hr. n = 3 biological replicates. **C, D.** Cell viability assessment by MTT assay of 1E and 1E-R cells treated with serial dilutions of vorinostat (C) and panobinostat (D) in presence of 2.5 mM NAC for 72 hr. n = 3 biological replicates. All data are shown as the mean values ± SEM. IC50 values ± SD are indicated for each condition.
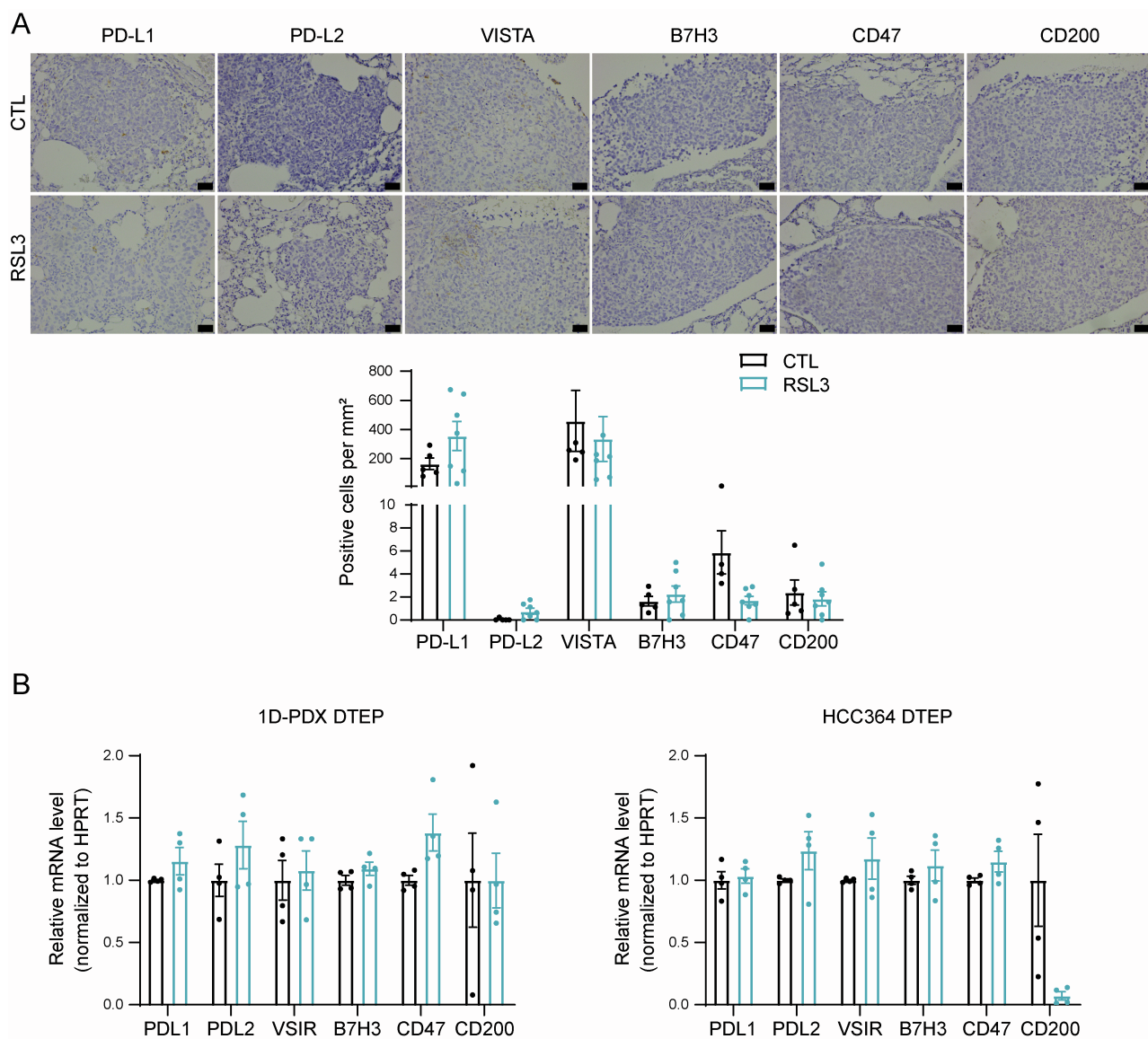
Figure S9: *In vitro* **&** *in vivo* **evaluation of various immune-checkpoint related proteins at the DTEP stage upon GPX4 inhibition, related to Figure 4. A.** Representative PD-L1, PD-L2, VISTA, B7H3, CD47 and CD200 immunostaining in lung sections of mice treated with RSL3 (upper panel). Scale bars represent 50 μm. Quantification of immune checkpoint proteins staining scores (per mice) in 1D tumours from mice treated with vehicle or with RSL3 (lower panel). *n* = 5 or 7 mice per group. **B.** Quantitative RT-PCR analysis of relative immune checkpoint genes (*PDL1, PDL2, VSIR, B7H3, CD47 and CD200*) mRNA levels in DTEP 1D-PDX and HCC364 cells cultured in presence of RSL3 1 nM for 48 hr. Data are shown as the mean values ± SEM. *n* = 4 biological replicates.
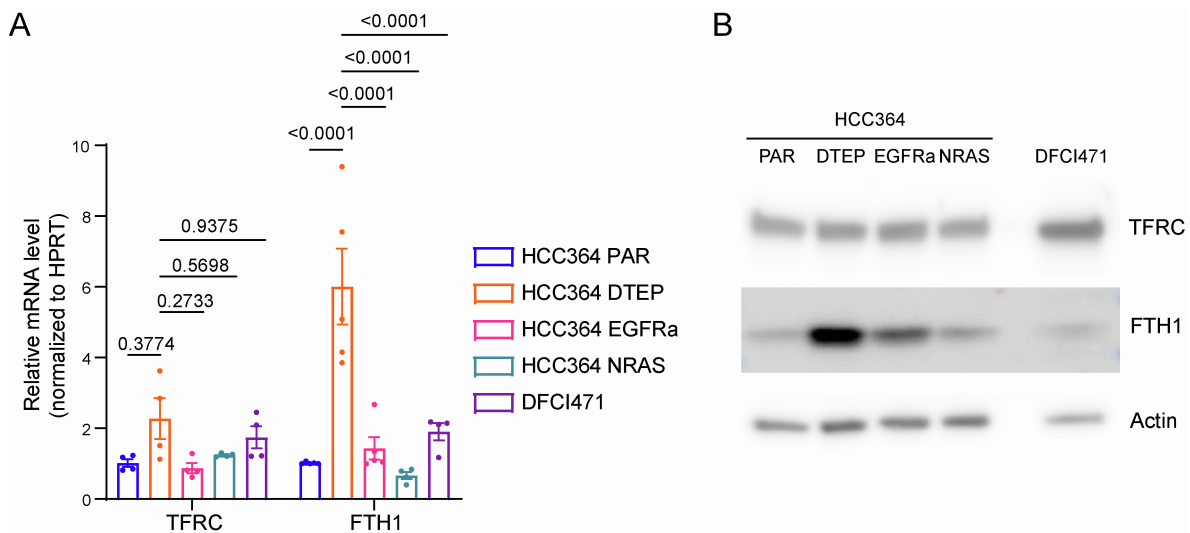
Figure S10: Evaluation of TFRC & FTH1 expression in DTEP and acquired resistance settings, related to Figures 3 and 5. A. Quantitative RT-PCR analysis of relative TFRC and FTH1 mRNA levels in parental and DTEP HCC364, HCC364^{EGFRa}, HCC364^{NRAS} and DFCI471 cells. Data were analysed using one-way ANOVA followed by Tukey's multiple comparisons post-test and are shown as the mean values ± SEM. $n = 4$ biological replicates. **B.** Immunoblot of TFRC and FTH1 in parental and DTEP HCC364, HCC364^{EGFRa}, HCC364^{NRAS} and DFCI471 cells. Actin was used as a loading control. $n = 2$ biological replicates.

Table S1: Primer sequences used for RT-PCR. Related to STAR Methods.

Human gene	Forward primer	Reverse primer
SLC7A11	5′- AGCACATAGCCAATGGTGAC-3′	5′-GCTGGCTGGTTTTACCTCAA-3′
DUSP4	5′- GGCGGCTATGAGAGGTTTTCC-3′	5′-TGGTCGTGTAGTGGGGTCC -3′
DUSP6	5′- GAAATGGCGATCAGCAAGACG-3′	5′- CGACGACTCGTATAGCTCCTG-3′
ETV4	5′- CAGTGCCTTTACTCCAGTGCC-3′	5′- CTCAGGAAATTCCGTTGCTCT-3′
ETV5	5′- CAGTCAACTTCAAGAGGCTTGG-3′	5′- TGCTCATGGCTACAAGACGAC-3′
SPRY2	5′-CCTACTGTCGTCCCAAGACCT-3′	5′- GGGGCTCGTGCAGAAGAAT-3′
PHLDA1	5′- GAAGATGGCCCATTCAAAAGCG-3	5′- GAGGAGGCTAACACGCAGG-3′
TFRC	5′-GGCTACTTGGGCTATTGTAAAGG-3′	5′-CAGTTTCTCCGACAACTTTCTCT-3′
FTH1	5′-TGAAGCTGCAGAACCAACGAGG-3′	5′-GCACACTCCATTGCATTCAGCC-3′
NRAS	5′- TGAGAGACCAATACATGAGGACA-3′	5′- CCCTGTAGAGGTTAATATCCGCA-3′
KRAS	5′- GGACTGGGGAGGGCTTTCT-3′	5′- GCCTGTTTTGTGTCTACTGTTCT-3′
HRAS	5′- GACGTGCCTGTTGGACATC-3′	5′- CTTCACCCGTTTGATCTGCTC-3′
PDL1	5′-TGGCATTTGCTGAACGCATTT-3′	5′-TGCAGCCAGGTCTAATTGTTTT-3′
PDL2	5′-ATTGCAGCTTCACCAGATAGC-3′	5′-AAAGTTGCATTCCAGGGTCAC-3′
VSIR (VISTA)	5′-ACGCCGTATTCCCTGTATGTC-3′	5′-TTGTAGAAGGTCACATCGTGC-3′
B7H3	5′-TGTCTCATTGCACTGCTGGT-3′	5′-TGTCTTGGAGCCTTCTCCCT-3′
CD47	5′-AGAAGGTGAAACGATCATCGAGC-3′	5′-CTCATCCATACCACCGGATCT-3′
CD200	5′-ACGTCTGTTACCAGCATCCTC-3′	5′-CTTAAAGTCGGTCACAGTCCC-3′

Table S2: sgRNA sequences for CRISPR/Cas9 editing. Related to STAR Methods

Human gene	Forward primer	Reverse primer
GPX4	5′-CACCGTTTCCGCCAAGGACATCGAC-3′	5′-AAACGTCGATGTCCTTGGCGGAAAC-3′
SLC7A11	5′-CACCGGCAACATAGAATAACCTGAT-3′	5′-AAACATCAGGTTATTCTATGTTGCC-3′
LACZ Ctrl	5′-CACCGAGACGATCCGCTGGCCGTTA-3′	5′-AAACTAACGGCCAGCGGATCGTCTC-3′