Supplementary Information

TET1 as a master regulator controlling GPX4-dependent and -independent ferroptosis surveillance in acute myeloid leukemia

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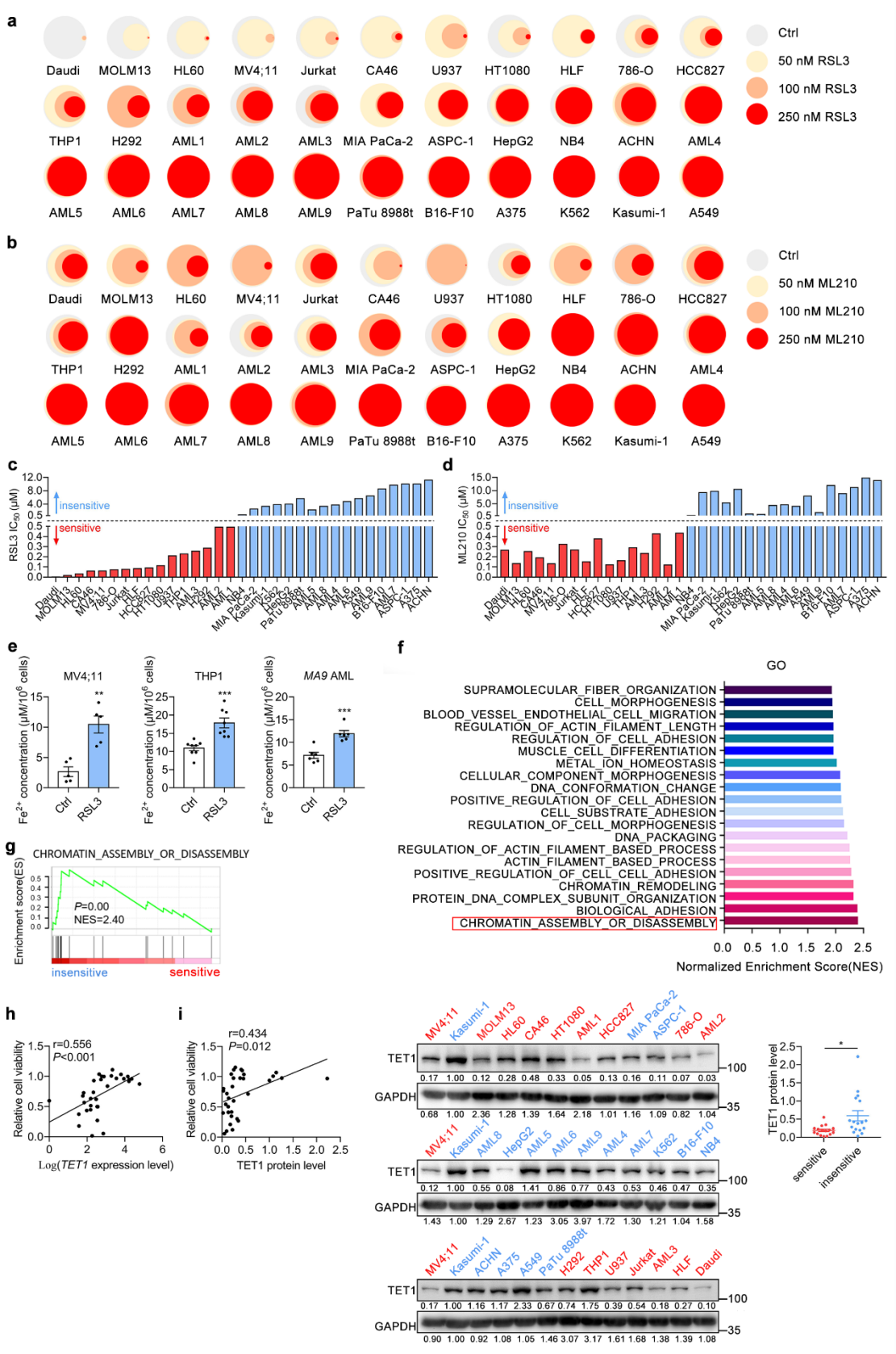
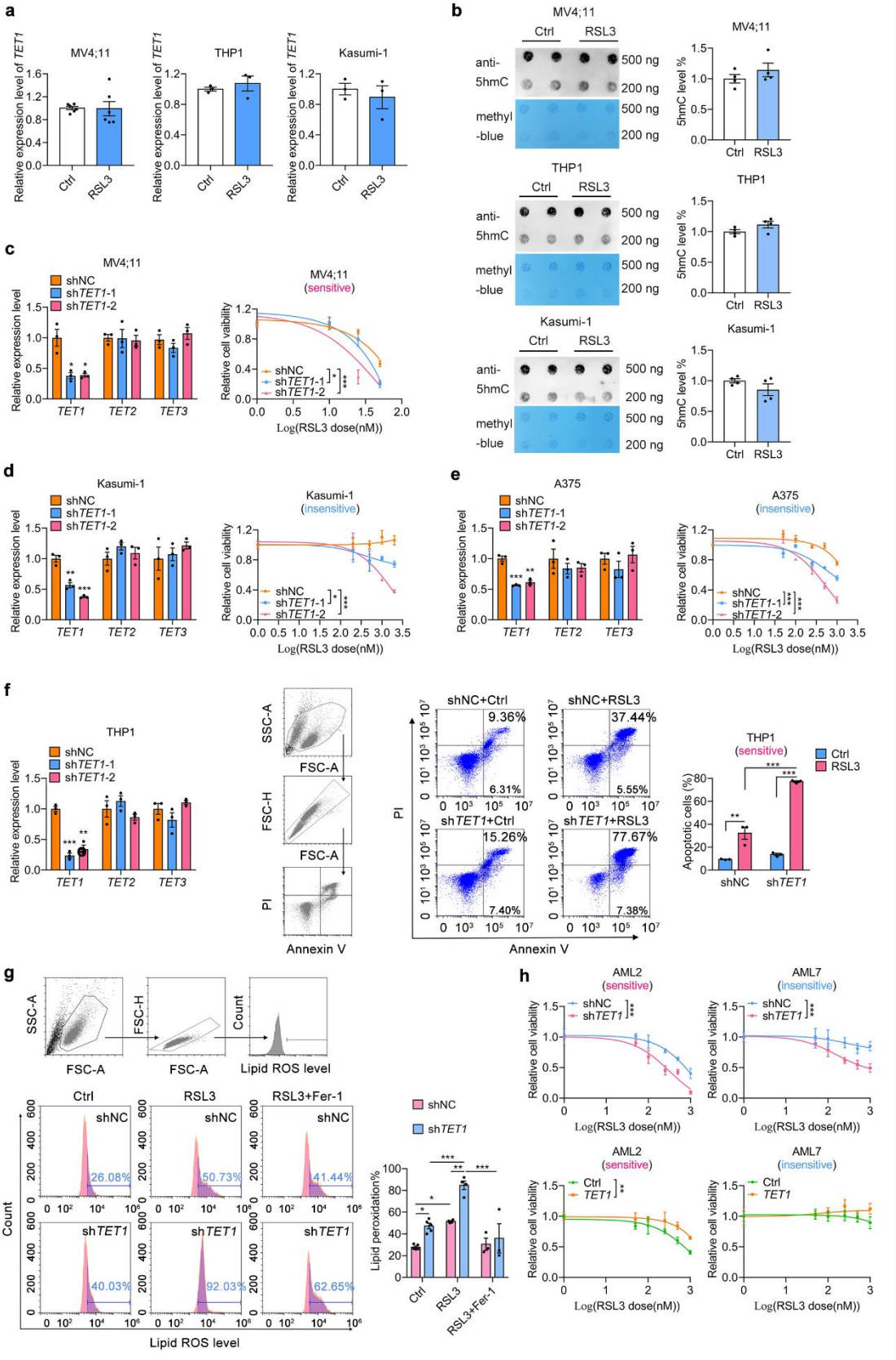


Supplementary Fig. 1. Negative correlation between *TET1* expression and ferroptosis sensitivity in cancer cells. a, **b**, Various vulnerability of cancer cells to

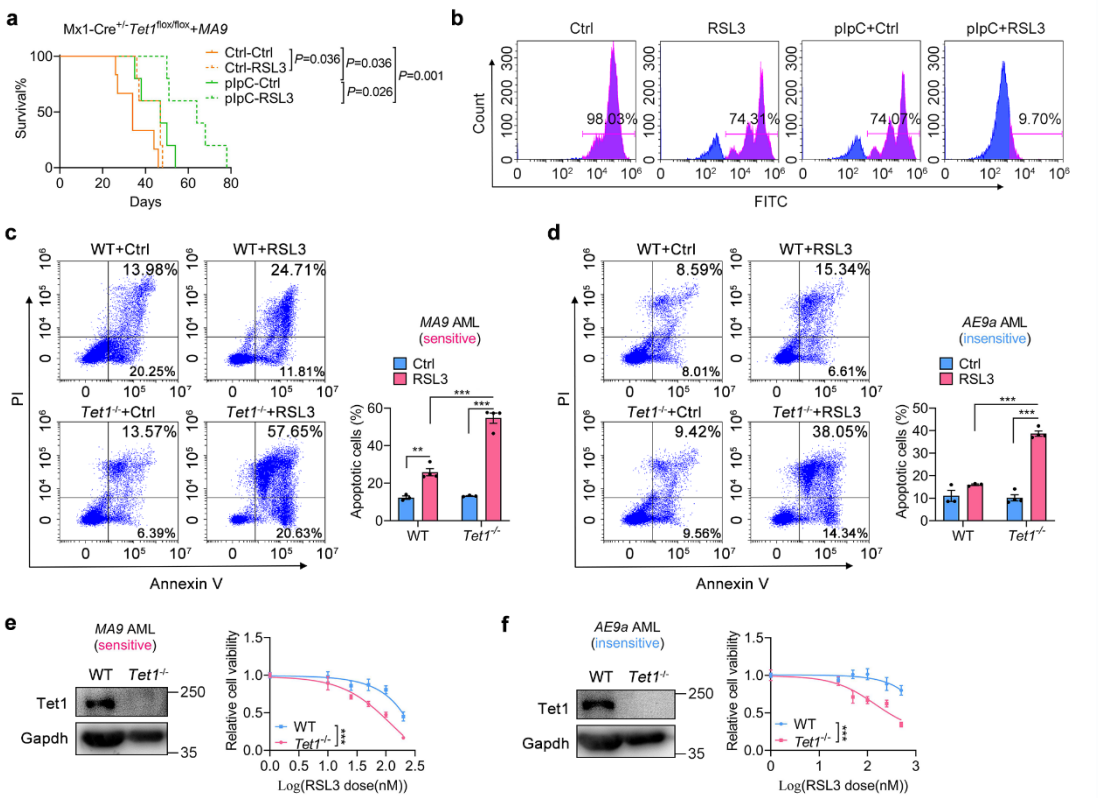
RSL3 or ML210. Shown is relative cell viability of each cancer cell line or primary cell of a 33-cancer cell sample cohort treated with RSL3 (**a**) or ML210 (**b**) at indicated doses for 48 hours. This cohort includes 24 cancer cell lines and 9 primary AML samples. Each color indicates the drug concentration; the circle diameter indicates relative cell viability. n = 3 biologically replicates. **c**, **d**, IC50 values of the 33-cancer cell sample cohort against RSL3 (**c**) or ML210 (**d**). **e**, Shown are Fe^{2+} concentrations of MV4;11 (n = 5 independent experiments), THP1 (n = 8 independent experiments) and mouse *MA9*-AML (n = 6 independent experiments) cells treated with 500 nM RSL3 for 48 hours. Data are presented as mean ± SEM; Statistical significance was determined by unpaired two-tailed t-test. **f**, Gene Ontology (GO) analysis showing gene sets enriched in Kasumi-1 cells (i.e., RSL3 resistant), as compared with MV4;11 cells (i.e., RSL3 sensitive). **g**, The chromatin_assembly_or_disassembly gene set enriched in Kasumi-1 cells (RSL3 resistant), as compared with MV4;11 cells (RSL3 sensitive). Results of GSEA analysis is shown. **h**, Pearson correlation between *TET1* expression level and cell response to ferroptotic inducers. The 33-cancer cell samples were treated with DMSO or 250 nM ML210 for 48 hours. MTT assays were conducted. Expression levels of *TET1* were detected with Q-PCR. n = 3 biologically replicates. **i**, Correlation between TET1 protein level and cellular response to RSL3. Left panel: Shown is Pearson correlation between TET1 protein level and cellular response to RSL3, determined by relative cell viability (RSL3-treated versus control) and basal protein expression levels of TET1 in the 33-sample cohort. Expression levels of TET1 were detected with Western blot (WB). For quantification, band intensity was analyzed using Gel-Pro Analyzer software. The background was subtracted for each band, and the values were normalized to the internal control (GAPDH). The normalized values were then expressed relative to the basal TET1 level of Kasumi-1 cells. The relative expression values are indicated below the respective bands. Average values from at least three independent experiments are included in statistical analysis. Representative Western blots are shown as the middle panels (red: ferroptosis-sensitive, blue:

ferroptosis-insensitive). Statistical analysis of TET1 levels in ferroptosis-sensitive and -insensitive cells is shown in the right panel. Data are presented as mean ± SEM; Statistical significance was determined by unpaired two-tailed t-test. Exact *P* values and source data are provided as a Source Data file.


a
MV4;11
THP1
Kasumi-1
Relative expression level of TET1
Ctrl
RSL3
b
MV4;11
THP1
Kasumi-1
Ctrl
RSL3
anti-5hmC
methyl-blue
500 ng
200 ng
5hmC level %
c
MV4;11
MV4;11 (sensitive)
shNC
shTET1-1
shTET1-2
Relative expression level
Relative cell viability
TET1
TET2
TET3
Log(RSL3 dose(nM))
d
Kasumi-1
Kasumi-1 (insensitive)
e
A375
A375 (insensitive)
f
THP1
THP1 (sensitive)
SSC-A
FSC-A
FSC-H
PI
Annexin V
shNC+Ctrl
shNC+RSL3
shTET1+Ctrl
shTET1+RSL3
9.36%
6.31%
37.44%
5.55%
15.26%
7.40%
77.67%
7.38%
Apoptotic cells (%)
g
Count
Lipid ROS level
Ctrl
RSL3
RSL3+Fer-1
26.08%
50.73%
41.44%
40.03%
92.03%
62.65%
Lipid peroxidation%
h
AML2 (sensitive)
AML7 (insensitive)
shTET1
TET1

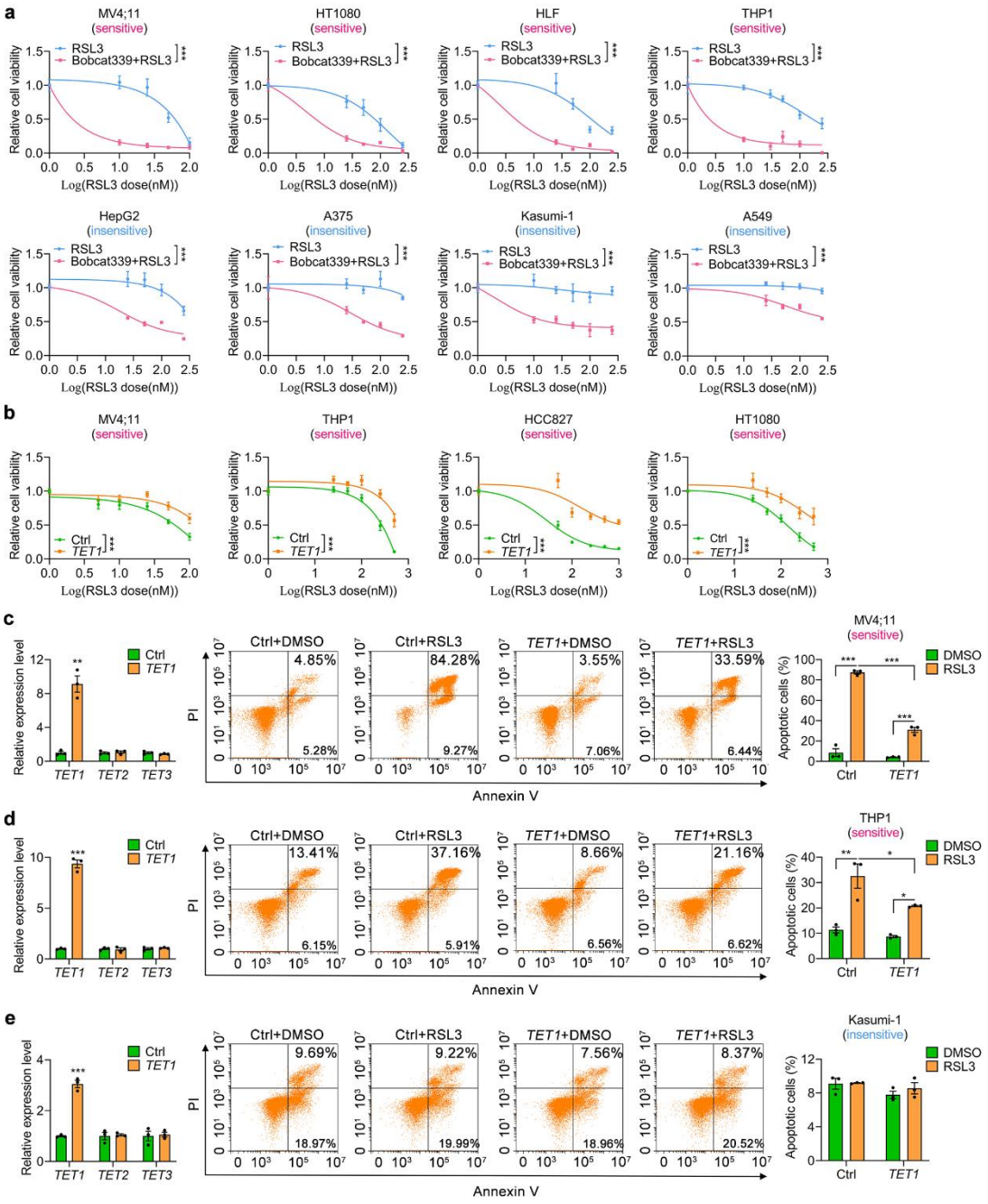
Supplementary Fig. 2. Intervention of *TET1* expression affects ferroptosis sensitivity of AML cells. a, Shown are relative expression levels of *TET1* in MV4;11 (n = 6 independent experiments), THP1 (n = 3 independent experiments) and Kasumi-1 (n = 3 independent experiments) cells treated with 500 nM RSL3 for 48 hours. Expression levels of *TET1* were detected with Q-PCR. **b**, Effects of RSL3 on global 5hmC levels assayed with dot blot analysis. MV4;11, THP1 and Kasumi-1 cells were treated with 500 nM RSL3 or DMSO control for 48 hours. Representative of n = 3 biologically replicates. **c**-**e**, MV4;11 (**c**), Kasumi-1 (**d**) and A375 (**e**) Cells were lentivirally transfected with pLKO.1 vectored *TET1* shRNA (sh*TET1*) or shNC, and then treated with DMSO or RSL3 at indicated doses for 48 hours. Shown are relative *TET1*/*2*/*3* expression levels (left panels) and relative cell viability (right panels) detected with MTT assays. n = 3 biologically replicates. **f**, THP1 cells were lentivirally transfected with pLKO.1 vectored *TET1* shRNA (sh*TET1*) or shNC, and then treated with DMSO or RSL3 for 48 hours. Expression levels of *TET1*/*2*/*3* were detected with Q-PCR (left panels) and cell apoptosis was detected with Annexin V/propidium iodide (PI) staining (right panels). n = 3 biologically replicates. Flow cytometry pre-gating strategy for cell apoptosis analysis in Supplementary Fig. 2f, 3c-d and 4c-e. **g**, Upper panel: Flow cytometry pre-gating strategy for lipid peroxidation analysis in Fig. 1e-f, 4e-f, 5i and Supplementary Fig. 2g, 5l-m, 8j-k, 10k, 10m. Lower panels: Rescue effect of Fer-1 in RSL3 treated THP1 cells. Analysis of lipid peroxidation level in *TET1* knockdown (sh*TET1*) or control (shNC) cells treated with DMSO, 500 nM RSL3 or 500 nM RSL3+ 5 μM Fer-1 for 24 hours was conducted. Statistical results are shown as the right panel (n = 5 independent experiments for Ctrl group; n = 4 independent experiments for RSL3 group; n = 3 independent experiments for RSL3 + Fer-1 group). **h**, Primary AML cells with *TET1* knockdown or overexpression were treated with RSL3 at indicated doses for 48 hours. Relative cell viability was detected with MTT assays. n = 3 biologically replicates. Data are presented as mean ± SEM; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; Statistical significance was determined by unpaired two-tailed t-test (**a**-**b**) and (**c**-**f**, left panels),

or one-way ANOVA with Tukey's multiple comparisons test (**f**, right panel) and (**g**), or two-way ANOVA with Tukey's multiple comparisons test (**c**-**e**, right panels) and (**h**). Exact *P* values and source data are provided as a Source Data file.



Supplementary Fig. 3. *Tet1* deficiency sensitizes murine AML cells to ferroptosis and apoptosis. a, Recipient mice transplanted with Mx1-Cre$^{+/-}$*Tet1*$^{flox/flox}$ *MA9*- AML cells were treated with DMSO control (n = 6 mice) or 14 mg/kg pIpC (n = 5 mice, i.p., every other day, 5 doses in total), 1 mg/kg RSL3 (n = 5 mice, i.p., every other day, 10 doses in total), or pIpC+RSL3 (n = 5 mice). Kaplan-Meier curves are shown. P values were calculated by log-rank test. **b**, BM engraftments of MSCV-PIG-expressed donor cells detected with flow cytometry 35 days post-BMT. **c**, **d**, *MA9* (**c,** n = 3 independent experiments for Ctrl group; n = 4 independent experiments for RSL3 group) or *AE9a* (**d,** n = 3 independent experiments for WT group; n = 4 independent experiments for *Tet1*$^{-/-}$ group)-AML cells of wild-type or *Tet1*$^{-/-}$ mice were treated with DMSO or 250 nM RSL3 for 48 hours. Cell apoptosis was detected with Annexin V/PI staining. **e**, **f**, *Tet1* knockout sensitizes mouse AML cells to RSL3. *MA9* (**e**) or *AE9a* (**f**)-AML cells of wild-type or *Tet1*$^{-/-}$ mice were collected and cultured. Cells were treated with DMSO or RSL3 at indicated doses for 48 hours. Shown are Tet1 expression level (left panels) and relative cell viability (right panel). n = 3 biologically replicates. Data are presented as mean ± SEM; $^{**}P < 0.01$, $^{***}P < 0.001$; Statistical

significance was determined by one-way ANOVA with Tukey's multiple comparisons test (**c**, **d**) or two-way ANOVA with Tukey's multiple comparisons test (**e**, **f**). Exact *P* values and source data are provided as a Source Data file.



Supplementary Fig. 4. TET1 suppression sensitizes cancer cells to ferroptosis and apoptosis. a, Effects of combinational treatment of Bobcat339 and RSL3 in multiple leukemia and solid tumor cell lines. Cells were concurrently treated with DMSO or 30 μM Bobcat339 for 48 hours in the presence of RSL3 at indicated doses. **b**, *TET1* overexpression leads to RSL3 resistance. Cells were lentivirally transfected with *TET1* or control. Transfected cells were treated with DMSO or RSL3 at indicated doses for 48 hours. **c-e**, Influence of *TET1* overexpression on apoptosis. MV4;11 (**c**),

115 THP1 (**d**) and Kasumi-1 (**e**) cells transfected with *TET1* or control lentivirus were
116 treated with RSL3 for 48 hours. Expression levels of *TET1/2/3* were detected with
117 Q-PCR (left panels) and cell apoptosis was detected with Annexin V/PI staining (right
118 panels). Data are presented as mean ± SEM; $n = 3$ biologically independent replicates
119 in (**a**)-(**e**); $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; Statistical significance was
120 determined by two-way ANOVA with Tukey's multiple comparisons test (**a**, **b**), or
121 unpaired two-tailed t-test (**c-e**, left panels), or one-way ANOVA with Tukey's multiple
122 comparisons test (**c-e**, right panels). Exact *P* values and source data are provided as a
123 Source Data file.
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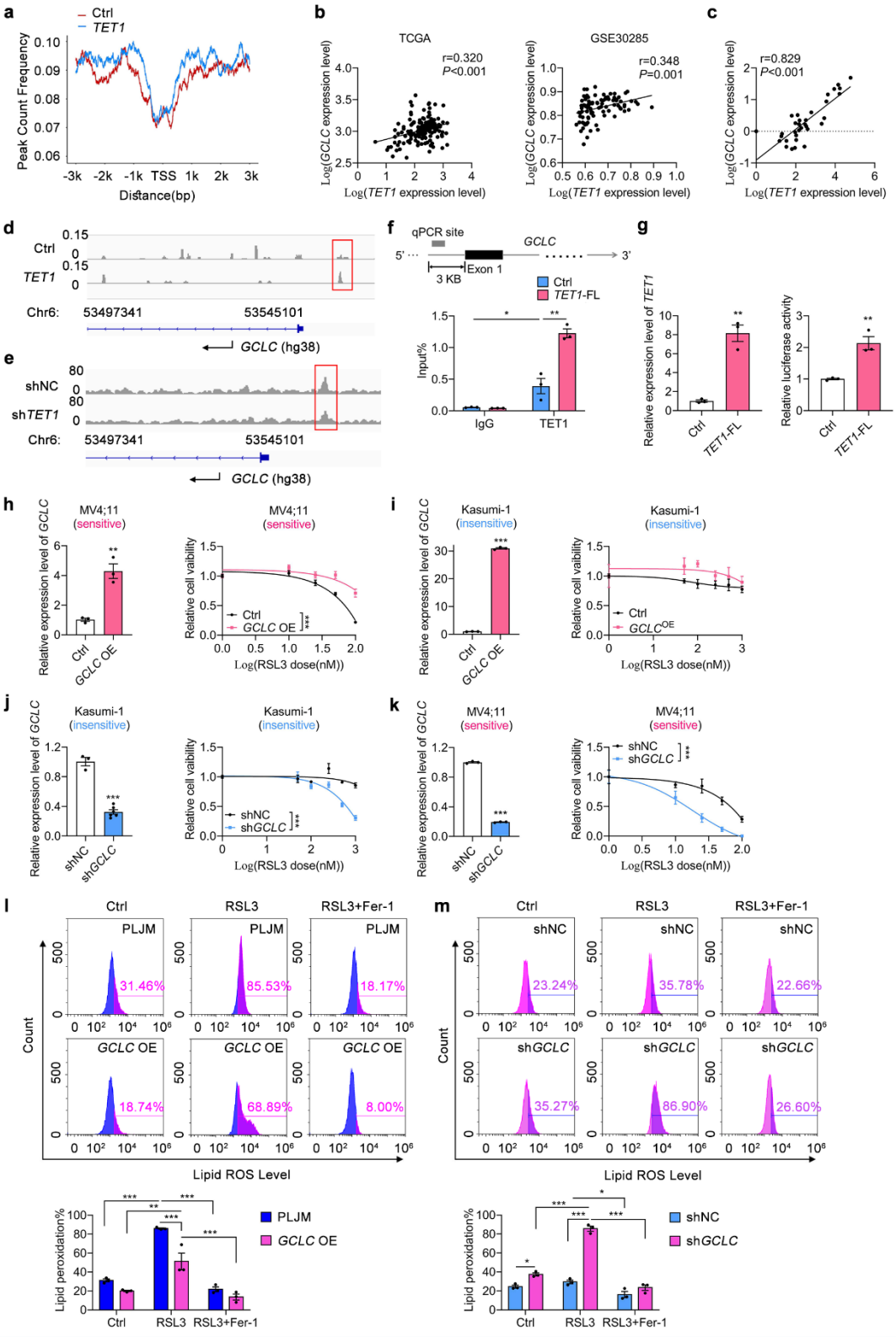
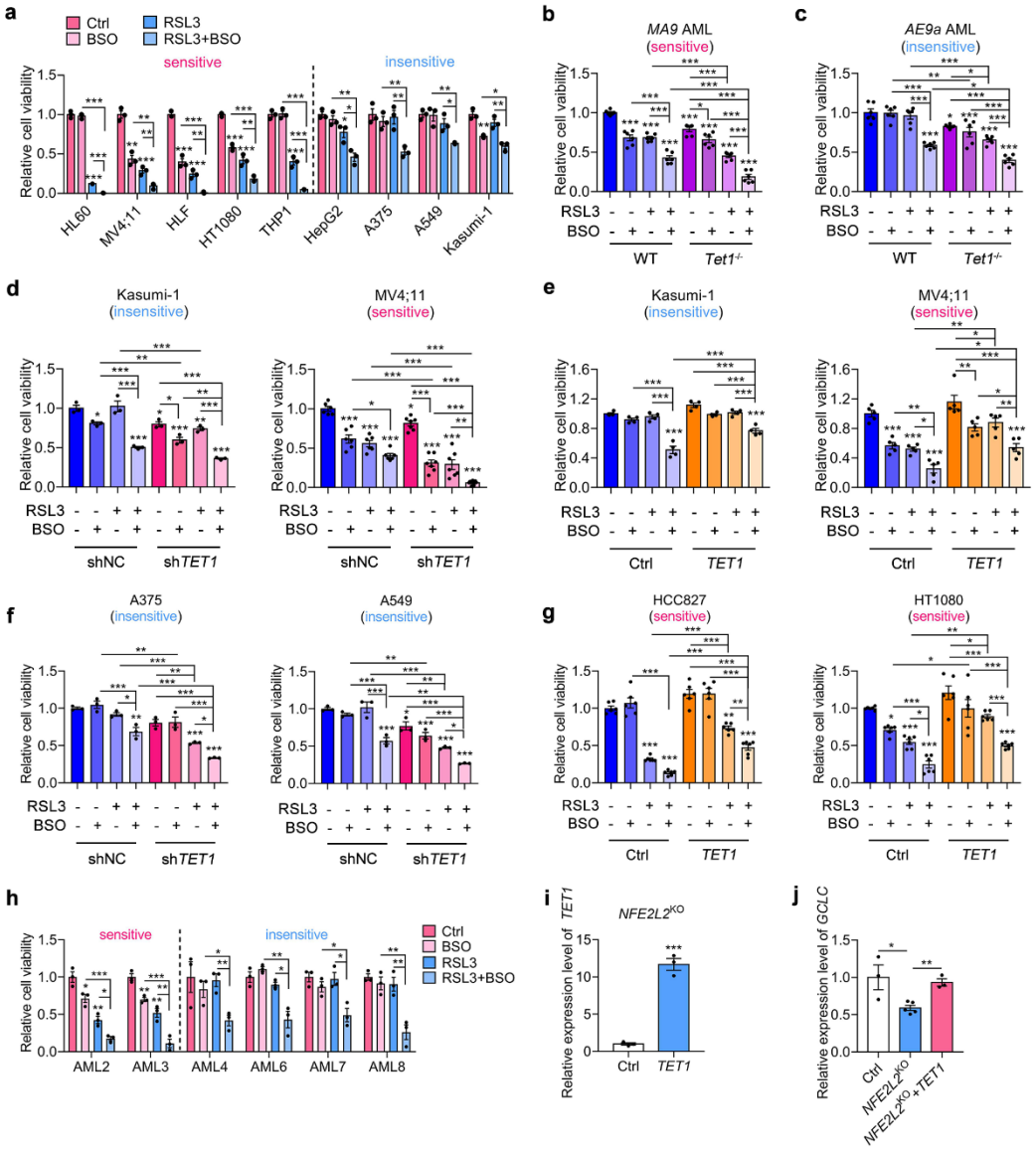


Supplementary Fig. 5. TET1 activates the transcription of *GCLC*. **a**, Genome-wide 5hmC peak abundance in THP1 cells transfected with pLJM1 (Ctrl) or pLJM1-*TET1*. **b**, Pearson correlation between *TET1* and *GCLC* expression levels in

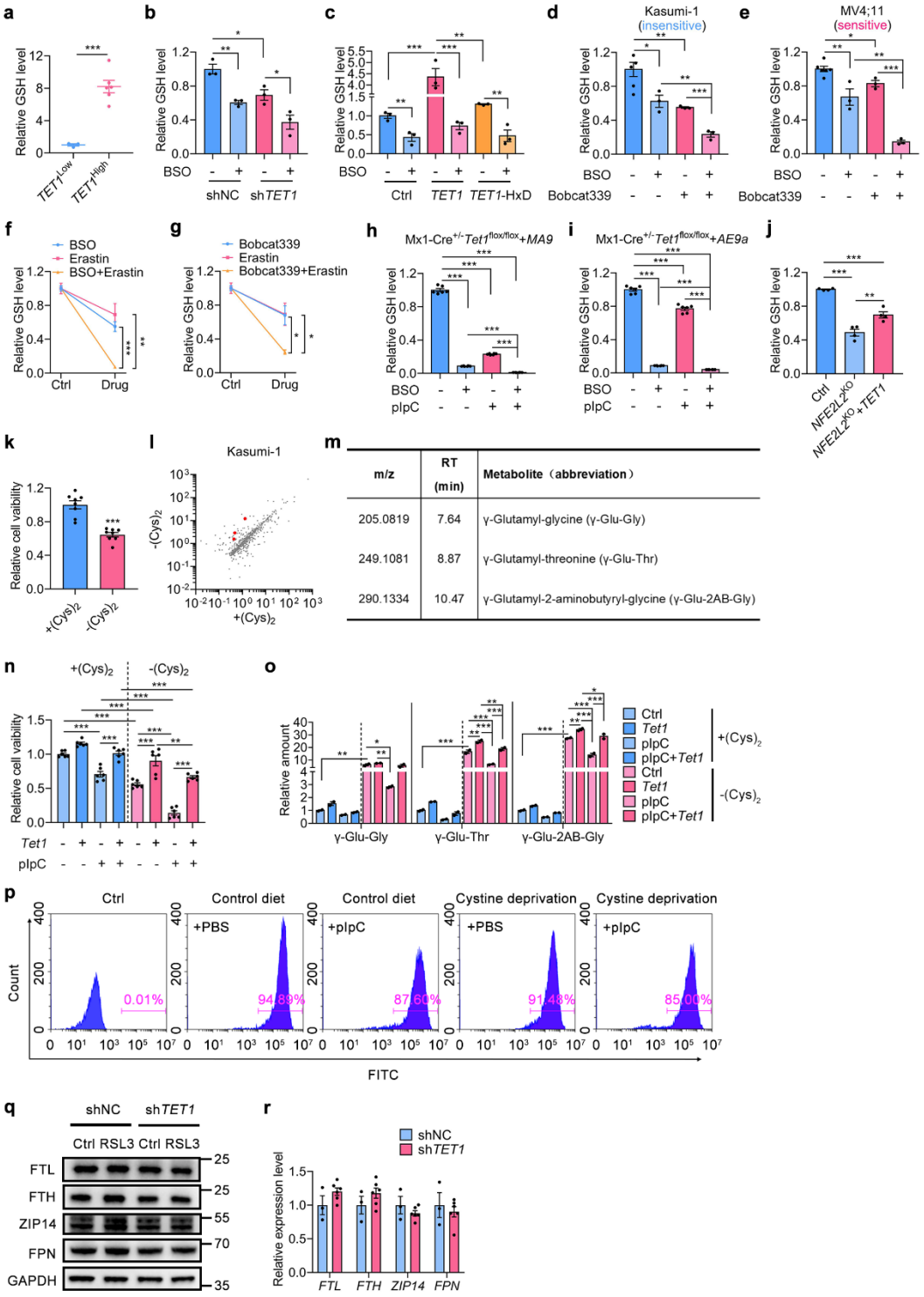
AML was analyzed in TCGA (left panel) and GSE30285 (right panel) datasets. **c**, Pearson correlation between *TET1* and *GCLC* transcription levels in the 33-cancer cell sample cohort. Expression levels were detected with Q-PCR; data were normalized to *TET1* level of Daudi cells and the relative levels were log-transformed. $n = 3$ biologically replicates. **d**, Enriched 5hmC signaling at the promoter region of the *GCLC* gene locus in *TET1* overexpressing THP1 cells. **e**, Genome-wide TET1 occupancy detected with ChIP-seq. The TET1 binding region at the *GCLC* gene locus in Kasumi-1 cells transfected with sh*TET1* or shNC is shown. **f**, Association between full-length TET1 and the *GCLC* gene locus. Upper panel: The predicted TET1 binding site at the promoter region of *GCLC*. Lower panel: ChIP-Q-PCR results showing enrichment of TET1 at the *GCLC* promoter region, which could be promoted by overexpression of full-length *TET1* (*TET1*-FL). Cells were transfected with control or *TET1*-FL lentivirus. IgG was taken as negative control. $n = 3$ biologically replicates. **g**, Overexpression of *TET1*-FL promotes the transcriptional activity of *GCLC*. The pGL3-*GCLC* promoter was co-transfected with control or *TET1*-FL. Shown are relative *TET1* expression levels (left panels) and statistical results of luciferase reporter assays (right panels). $n = 3$ biologically replicates. **h**, **i**, MV4;11 (**h**) or Kasumi-1 (**i**) cells were lentivirally transfected with pLJM1 vectored *GCLC* or the empty vector, and then treated with DMSO or RSL3 at indicated doses for 48 hours. Shown are relative *GCLC* expression levels (left panels) and relative cell viability (right panels). $n = 3$ biologically replicates. **j**, **k**, Kasumi-1 (**j**) or MV4;11 (**k**) cells were lentivirally transfected with pLKO.1 vectored *GCLC* shRNA (sh*GCLC*) or shNC, and then treated with DMSO or RSL3 at indicated doses for 48 hours. Shown are relative *GCLC* expression levels (left panels) and relative cell viability (right panels). $n = 3$ biologically replicates. **l**, Overexpression of *GCLC* reduces lipid peroxidation in MV4;11 cells. Cells lentivirally transfected with pLJM1 vector or pLJM1-*GCLC* were treated with DMSO, 500 nM RSL3, or 500 nM RSL3 + 5 μM Fer-1 for 24 hours. Lipid ROS level was detected with flow cytometry. $n = 3$ biologically replicates. **m**, Knockdown of *GCLC* increases lipid peroxidation in Kasumi-1 cells. Cells

lentivirally transfected with shNC or sh*GCLC* were treated with DMSO, 500 nM RSL3, or 500 nM RSL3 + 5 μM Fer-1 for 24 hours. Lipid ROS level was detected with flow cytometry. $n = 3$ biologically replicates. Data are presented as mean ± SEM; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; Statistical significance was determined by unpaired two-tailed t-test (**f-g**) and (**h-k**, left panels), or two-way ANOVA with Tukey's multiple comparisons test (**h-k**, right panels), or one-way ANOVA with Tukey's multiple comparisons test (**l**, **m**). Exact *P* values and source data are provided as a Source Data file.



Supplementary Fig. 6. BSO treatment sensitizes cancer cells to RSL3-induced ferroptosis. a, Synergistic effect of RSL3 and BSO in suppressing cell viability. Shown is relative cell viability of HL60, MV4;11, HLF, HT1080, THP1, HepG2, A375, A549 and Kasumi-1 cells treated with DMSO, 250 μM BSO and/or 250 nM RSL3 for 48 hours (n = 3 independent experiments). **b**, **c**, Relative cell viability of wild type and *Tet1*$^{-/-}$ *MA9* (**b**) or *AE9a* (**c**)-AML cells treated with DMSO, BSO and/or RSL3 for 48 hours (n = 6 independent experiments). **d**, Relative cell viability of Kasumi-1 (left panel, n = 3 independent experiments) and MV4;11 (right panel, n = 6 independent experiments for shNC group; n = 7 independent experiments for sh*TET1* group) cells with *TET1* knockdown treated with DMSO, BSO and/or RSL3 for 48

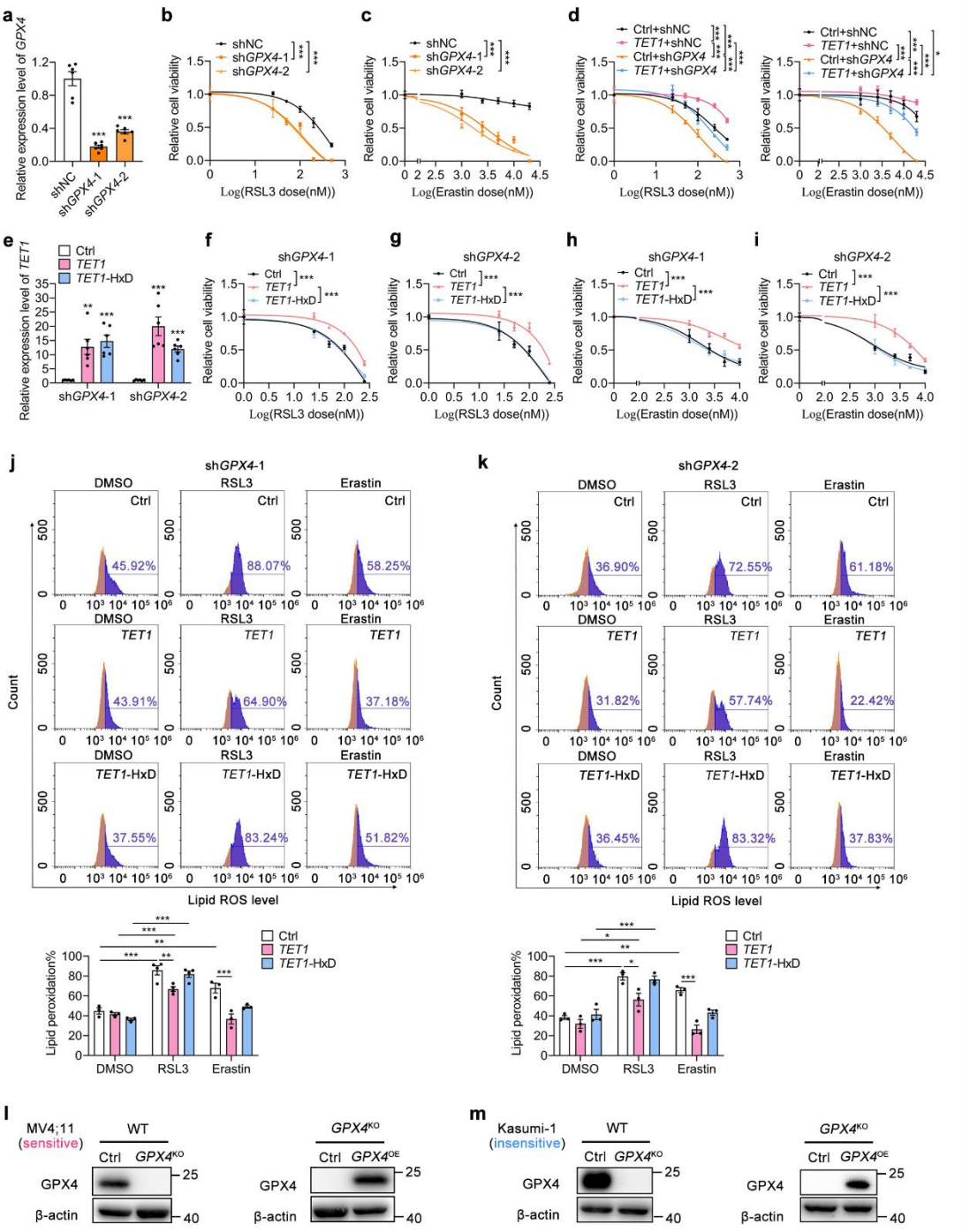
hours. **e**, Relative cell viability of Kasumi-1 (left panel, n = 4 independent experiments) and MV4;11 (right panel, n = 5 independent experiments) cells with *TET1* overexpression treated with DMSO, BSO and/or RSL3 for 48 hours. **f**, BSO treatment enlarges sh*TET1* induced vulnerability of A375 (left panel) or A549 (right panel) cells to RSL3. Cells were lentivirally transfected with shNC or sh*TET1*, and treated with DMSO, 100 nM RSL3 alone or in combination with 100 μM BSO for 48 hours. Relative cell viability is shown. n = 3 biologically replicates. **g**, BSO treatment restores the sensitivity of *TET1* overexpressed solid tumor cells to RSL3. HCC827 (left panel) or HT1080 (right panel) cells were lentivirally transfected with pLJM1 vector or pLJM1-*TET1*. Cells were treated with DMSO, 100 nM RSL3 alone or in combination with 100 μM BSO for 48 hours. Relative cell viability is shown. n = 6 biologically replicates. **h**, Combined treatment with BSO and RSL3 dramatically suppresses primary AML cell viability. Shown is relative cell viability of 6 primary AML cells treated with DMSO, 250 μM BSO and/or 250 nM RSL3 for 48 hours (n = 3 independent experiments). **i**, *NFE2L2* knockout THP1 cells were lentivirally transfected with control or *TET1*, and *TET1* expression level was detected with Q-PCR (n = 3 independent experiments). **j**, Expression levels of *GCLC* in WT or *NFE2L2* knockout THP1 cells transfected with control or *TET1* lentivirus were detected with Q-PCR (n = 3 independent experiments for Ctrl and $NFE2L2^{KO}$ + *TET1* group; n = 5 independent experiments for $NFE2L2^{KO}$ group). Data are presented as mean ± SEM; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; Statistical significance was determined by unpaired two-tailed t-test (**a**, **i-j**), or two-way ANOVA with Tukey's multiple comparisons test (**b-g**). Exact P values and source data are provided as a Source Data file.



Supplementary Fig. 7. Cystine starvation induced glutamate-derived γ-glutamyl-peptide accumulation in Kasumi-1 cells. a, Relative GSH levels of primary AML cells (blue: *TET1*^{low} cells, i.e. AML1-3; red: *TET1*^{high} cells, i.e. AML4-9). GSH levels were detected with the GSH/GSSG ratio detection kit. Shown are GSH readouts normalized to the average level of *TET1*^{low} cells. **b**, Knockdown of

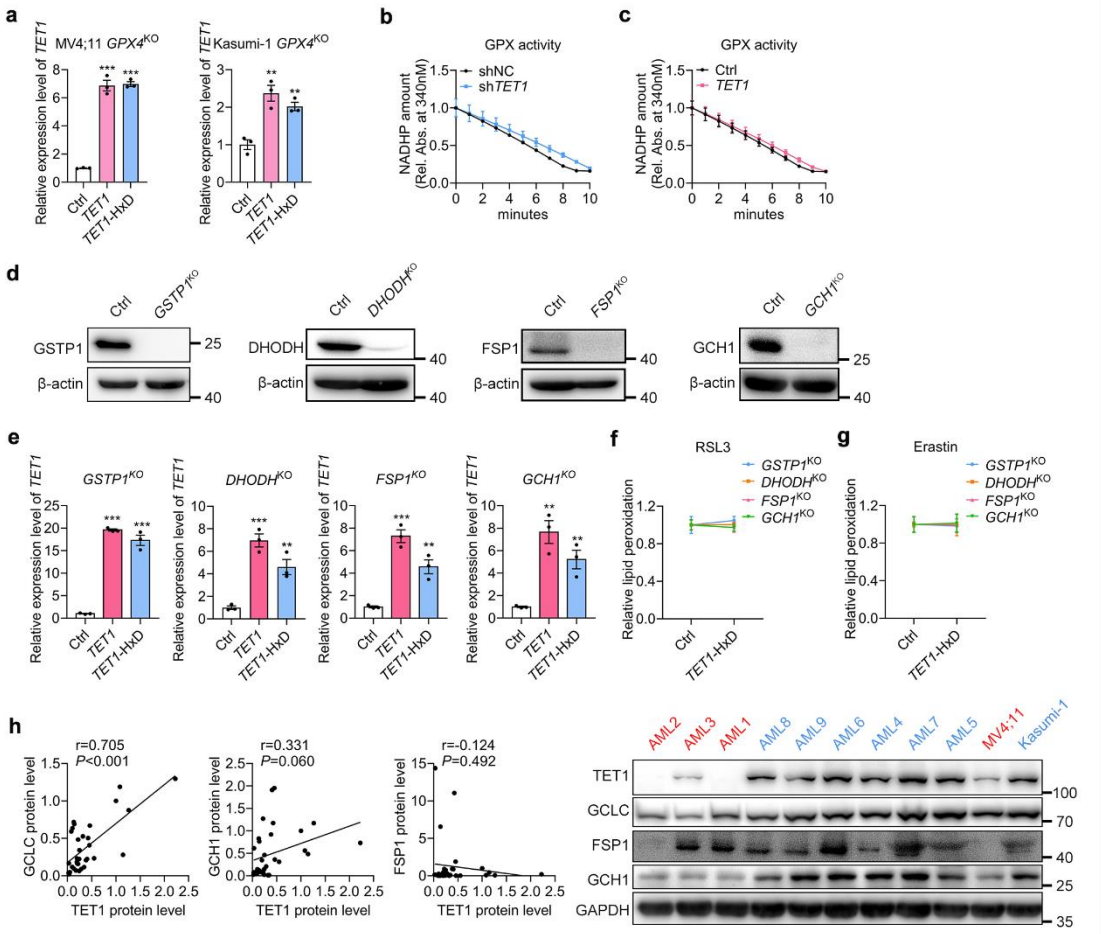
TET1 reduces GSH production in THP1 cells. Cells transfected with sh*TET1* or shNC were treated with 100 μM BSO or DMSO for 24 hours. GSH levels were detected with the GSH/GSSG ratio detection kit. Shown are GSH readouts normalized to control (i.e., shNC/BSO-) (n = 3 independent experiments). **c**, Overexpression of *TET1* promotes GSH synthesis. THP1 cells transfected with pLJM1-*TET1*, *TET1*-HxD or empty vector control. Cells were treated with 100 μM BSO or DMSO for 24 hours. GSH levels were detected with the GSH/GSSG ratio detection kit. Shown are GSH readouts normalized to control (i.e., Ctrl/BSO-) (n = 3 independent experiments). **d**, **e**, Synergistic effect of BSO and Bobcat339 in suppressing GSH production. Kasumi-1 (**d**) and MV4;11 (**e**) cells were treated with DMSO control, 50 μM BSO and/or 30 μM Bobcat339 for 24 hours. GSH levels were detected with the GSH/GSSG ratio detection kit. Shown are GSH readouts normalized to control (i.e., BSO-/Bobcat339-). (n = 6 independent experiments for control group; n = 3 independent experiments for other groups). **f**, **g**, THP1 cells were treated with 5 μM Erastin or DMSO control, together with 100 μM BSO (**f**), 30 μM Bobcat339 (**g**) or DMSO for 24 hours. GSH levels were detected with the GSH/GSSG ratio detection kit. Shown are GSH readouts normalized to control (i.e., BSO-/Erastin-, or Bobcat339-/Erastin-). n = 3 biologically replicates. **h**, **i**, BM cells of Mx1-Cre$^{+/-}$*Tet1*$^{flox/flox}$ mice were transfected with *MA9* (**h**) or *AE9a* (**i**), and were treated with or without pIpC treatment (i.e., *Tet1* depletion). These cells were treated with DMSO or 100 μM BSO for 24 hours. GSH levels were detected with the GSH/GSSG ratio detection kit. Shown are GSH readouts normalized to control (i.e., BSO-/pIpC-) (n = 6 independent experiments). **j**, WT or *NFE2L2* knockout THP1 cells were transfected with control or *TET1* lentivirus. GSH levels were detected with the GSH/GSSG ratio detection kit. Shown are GSH readouts normalized to control (n = 4 independent experiments). **k**, AML cells were cultured under cystine-replete or -starved conditions for 48 hours. Relative cell viability was detected with MTT assays (n = 8 independent experiments). **l**, Scatterplot comparison of non-targeted metabolomics features in Kasumi-1 cells cultured under cysteine-replete (+Cys_2) and

–deplete conditions (-Cys_2) for 12 hours. **m**, The LC-MS peaks highly accumulated under cysteine starvation were identified and annotated. **n**, **o**, Mouse Mx1-$Cre^{+/-}$-*Tet1*$^{flox/flox}$-*MA9*-AML cells with or without pIpC treatment (i.e., *Tet1* depletion) were transfected with control or *Tet1* retrovirus. Cells were cultured under cystine-replete or -starved conditions for 48 hours. (**n**) Relative cell viability is shown (n = 6 independent experiments). (**o**) Quantitation of $^{13}C_5$,$^{15}N_2$-Gln tracing into the indicated γ-glutamyl-peptides is shown (n = 2). **p**, The proportions of FITC positive cells (i.e., MSCV-PIG expressed donor independent experiments cells) were analyzed with flow cytometry. **q**, Expression levels of FTL, FTH, ZIP14 and FPN in Kasumi-1 cells infected with shNC or sh*TET1*. Cells were treated with DMSO or 500 nM RSL3 for 24 hours. Results of WB are shown. Samples from a single experiment were analyzed with proteins FTL, ZIP14, FPN, and GAPDH on one gel and FTH on a parallel gel. Three times experiments were repeated independently with similar results. **r**, Relative gene expression levels of *FTL*, *FTH*, *ZIP14* and *FPN* in the above cells were detected with Q-PCR (n = 3 independent experiments for shNC group; n = 6 independent experiments for sh*TET1* group). Data are presented as mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001; Statistical significance was determined by unpaired two-tailed t-test (**a**-**k**) and (**r**), or two-way ANOVA with Tukey's multiple comparisons test (**n**, **o**). Exact P values and source data are provided as a Source Data file.



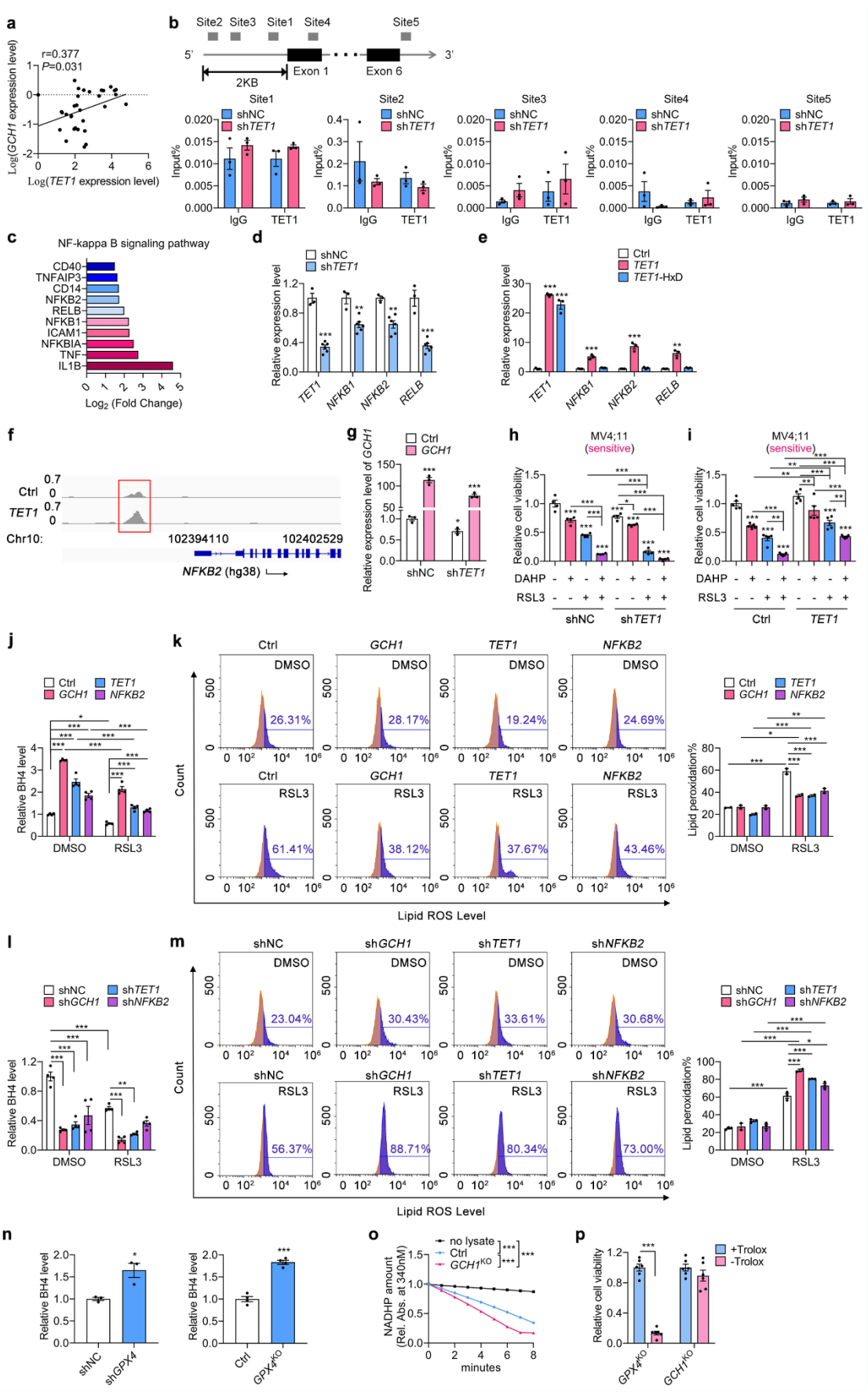
Supplementary Fig. 8. TET1 induces GPX4-independent ferroptotic resistance. a, THP1 cells were lentivirally transfected with pLKO.1 vectored *GPX4* shRNA (sh*GPX4*-1 and sh*GPX4*-2) or shNC. Expression levels of *GPX4* were detected with Q-PCR (n = 6 independent experiments). **b**, **c**, THP1 cells were lentivirally transfected with shNC, sh*GPX4*-1 or sh*GPX4*-2, and then treated with DMSO or RSL3 (**b**), Erastin (**c**) at indicated doses for 48 hours. Shown is relative cell viability.

n = 3 biologically replicates. **d**, *TET1*-overexpressing cells were lentivirally transfected with shNC or sh*GPX4*, and then treated with DMSO, RSL3 (left panel), or Erastin (right panel) at indicated doses for 48 hours. Shown is relative cell viability. *n* = 3 biologically replicates. **e**, *GPX4* knockdown cells were lentivirally transfected with pLJM1-neo, *TET1* or *TET1*-HxD. Expression levels of *TET1* were detected with Q-PCR (*n* = 6 independent experiments). **f-i**, *GPX4* knockdown cells were lentivirally transfected with pLJM1-neo, *TET1* or *TET1*-HxD, and then treated with DMSO, RSL3 (**f**, **g**), or Erastin (**h**, **i**) at indicated doses for 48 hours. Shown is relative cell viability. *n* = 3 biologically replicates. **j**, **k**, *GPX4* knockdown cells were lentivirally transfected with pLJM1-neo, *TET1* or *TET1*-HxD, and treated with DMSO, 500 nM RSL3 or 10 μM Erastin for 24 hours. Shown are statistical results of analysis of lipid peroxidation level (lower panels). For **j** panels, *n* = 3 independent experiments for DMSO and Erastin group; *n* = 4 independent experiments for RSL3 group. For **k** panels, *n* = 3 independent experiments for all groups. **l**, **m**, *GPX4* gene knockout efficiency (left panels) and overexpression efficiency (right panels) in MV4;11 (**l**) or Kasumi-1 (**m**) cells were detected with Western blot. Three times experiments were repeated independently with similar results. Data are presented as mean ± SEM; **P* < 0.05, ***P* < 0.01, ****P* < 0.001; Statistical significance was determined by unpaired two-tailed t-test (**a**, **e**), or two-way ANOVA with Tukey's multiple comparisons test (**b-d**, **f-i**), or one-way ANOVA with Tukey's multiple comparisons test (**j**, **k**). Exact *P* values and source data are provided as a Source Data file.



Supplementary Fig. 9. The TET1/GCH1 ferroptotic surveillance axis in cancer. a, Relative expression levels of *TET1* in *GPX4* knockout MV4;11 (left panels) or Kasumi-1 cells (right panels) transfected with pLJM1-neo (Ctrl), *TET1* or *TET1*-HxD. **b**, GPX activity in THP1 cells lentivirally transfected with shNC or sh*TET1*. **c**, GPX activity in THP1 cells lentivirally transfected with control or *TET1*. **d**, Expression levels of GSTP1, DHODH, FSP1, GCH1 in THP1 cells with or without *GSTP1*, *DHODH*, *FSP1*, *GCH1* knockout were detected with WB. DHODH and β-actin were run in parallel on two separate gels, with both sets of samples originating from the same experiment. Similarly, FSP1 and β-actin were also processed in parallel on different gels, all from the same experimental run. **e**, Relative expression levels of *TET1* in *GSTP1*, *DHODH*, *FSP1*, *GCH1* knockout THP1 cells transfected with pLJM1-neo (Ctrl), *TET1* or *TET1*-HxD. **f**, **g**, The impacts of *TET1*-HxD on lipid peroxidation level in *GSTP1*, *DHODH*, *FSP1*, *GCH1* knockout THP1 cells in the

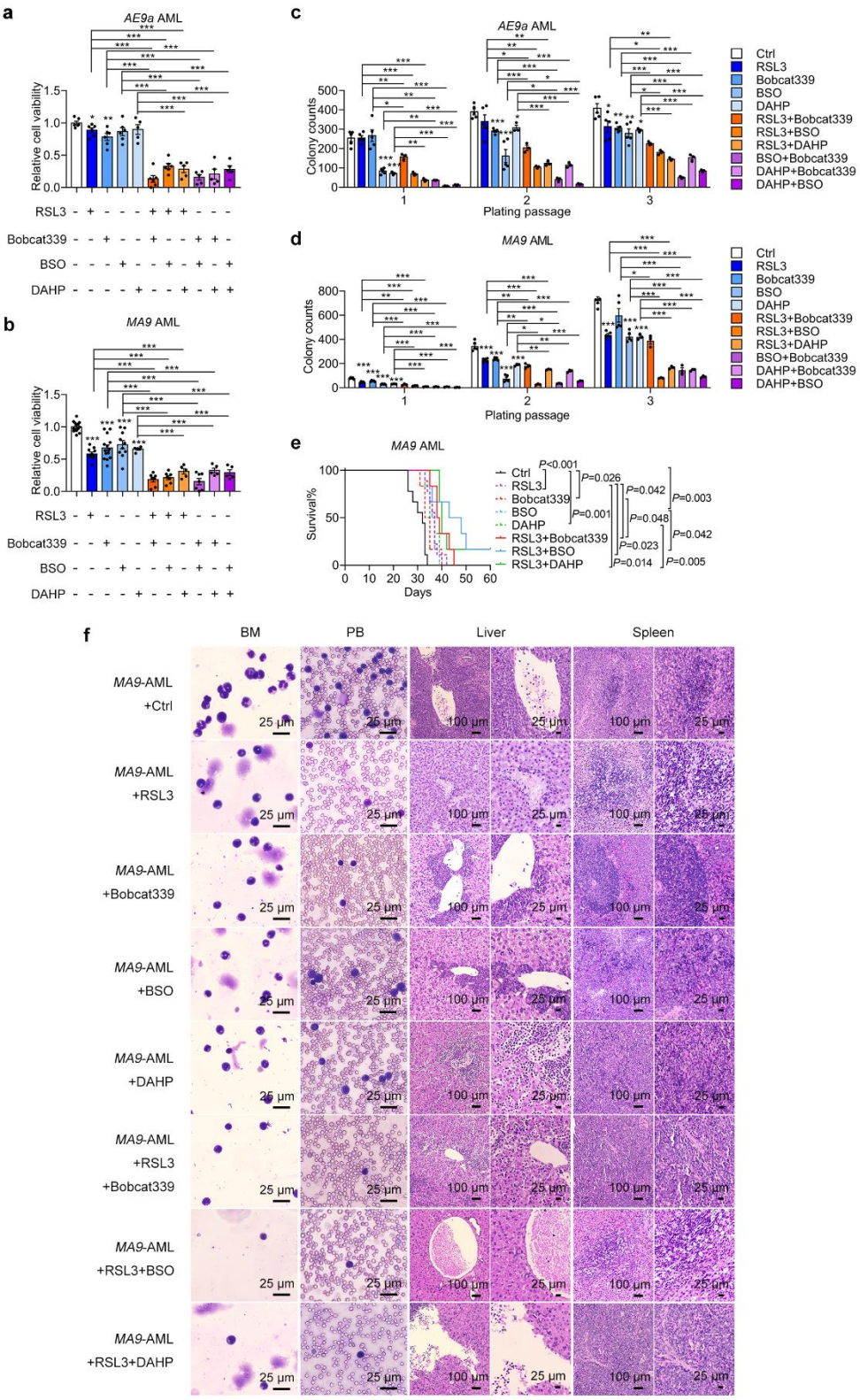
presence of RSL3 (**f**) or Erastin (**g**) for 24 hours. **h**, Pearson correlation between TET1 and GCLC, GCH1 or FSP1 protein levels in the 33-sample cancer cell cohort. Left and middle panels: Expression levels of TET1, GCLC, GCH1 and FSP1 were detected with WB and data were normalized to the basal expression levels of the above proteins in Kasumi-1 cells. The WB slides are shown as right panels (red: ferroptosis-sensitive; blue: ferroptosis-insensitive). Samples from a single experiment were analyzed with proteins TET1, GAPDH, and GCH1 on one gel and proteins GCLC and FSP1 on a parallel gel. Data are presented as mean ± SEM; $n = 3$ biologically independent replicates in (**a**)-(**h**); $^{**}P < 0.01$, $^{***}P < 0.001$; Statistical significance was determined by unpaired two-tailed t-test (**a**, **e**), or two-way ANOVA with Tukey's multiple comparisons test (**b**, **c**), or one-way ANOVA with Tukey's multiple comparisons test (**f**, **g**). Exact *P* values and source data are provided as a Source Data file.



Supplementary Fig. 10. TET1 activates the transcription of *NFKB2* and *GCH1*. a,

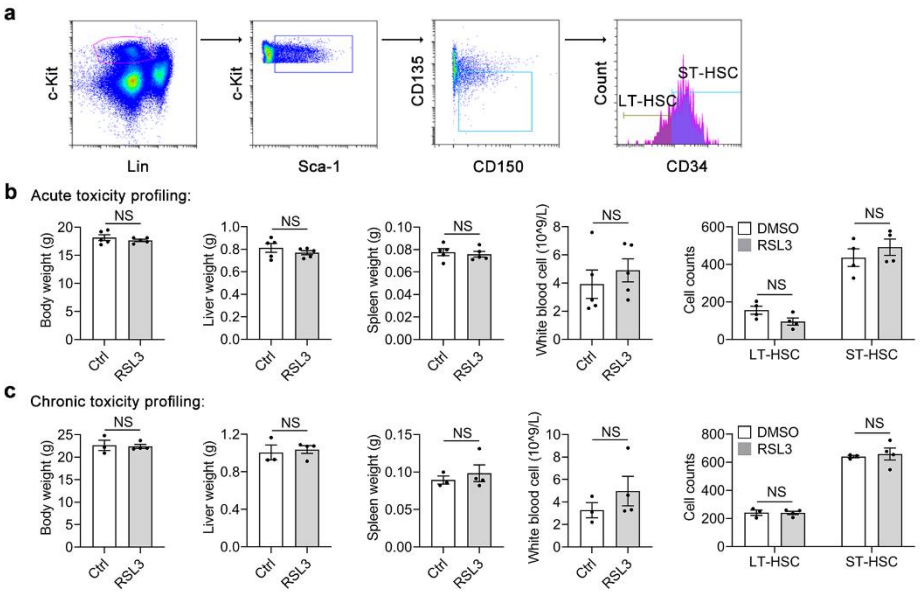
Pearson correlation between *TET1* and *GCH1* gene expression levels in the 33-sample cancer cell cohort. Expression levels were detected with Q-PCR. Data were normalized to the basal gene expression levels of Daudi cells and the expression levels were log-transformed. n = 3 biologically replicates. **b**, Upper panel: Genomic sites designed for ChIP-Q-PCR analysis to identify the potential association between TET1 and the *GCH1* gene locus. Lower panels: ChIP-Q-PCR results showing no remarkable enrichment of TET1 or IgG at the *GCH1* promoter or other regions. Kasumi-1 cells were transfected with shNC or sh*TET1* lentivirus. n = 3 biologically replicates. **c**, The top 10 genes of the NFκB signaling pathway gene set expressed at higher level in *TET1* overexpressing THP1 cells than in control cells. **d**, Expression levels of *TET1*, *NFKB1*, *NFKB2* and *RELB* in MV4;11 cells transfected with shNC or sh*TET1* lentivirus were detected with Q-PCR (n = 3 independent experiments for shNC group; n = 6 independent experiments for sh*TET1* group). **e**, Expression levels of *TET1*, *NFKB1*, *NFKB2* and *RELB* in MV4;11 cells transfected with pLJM1, *TET1* or *TET1*-HxD were detected with Q-PCR (n = 3 independent experiments). **f**, The enriched 5hmC signaling at the promoter region of the *NFKB2* gene locus in *TET1* overexpressing THP1 cells. **g**, Relative expression levels of *GCH1* in *TET1* knockdown or control cells (shNC) transfected with pLJM1-neo (Ctrl) or *GCH1* (n = 3 independent experiments). **h**, **i**, The impacts of *TET1* knockdown (**h**) or *TET1* overexpression (**i**) on cell viability in the presence of DMSO, DAHP and/or RSL3. MV4;11 cells were transfected with shNC/sh*TET1* (**h**), or pLJM1/*TET1* (**i**), and were concurrently treated with DMSO, RSL3 or/and DAHP for 48 hours. Cell viability was detected by MTT. For **h** panel, n = 4 independent experiments for all groups; For **i** panel, n = 5 independent experiments for all groups. **j**, Relative BH4 levels of MV4;11 cells transfected with pLJM1, *GCH1*, *TET1* or *NFKB2*. Cells were treated with DMSO or 250 nM RSL3 for 24 hours (n = 4 independent experiments). **k**, Flow cytometry analysis of lipid peroxidation levels in MV4;11 cells transfected with pLJM1, *GCH1*, *TET1* or *NFKB2* treated with DMSO or 500 nM RSL3 for 24 hours. Statistical results are shown (right panels, n = 2 independent experiments). **l**, Relative

BH4 levels of MV4;11 cells transfected with shNC, sh*GCH1*, sh*TET1* or sh*NFKB2*. Cells were treated with DMSO or 250 nM RSL3 for 24 hours (n = 4 independent experiments). **m**, Flow cytometry analysis of lipid peroxidation levels in MV4;11 cells transfected with shNC, sh*GCH1*, sh*TET1* or sh*NFKB2* treated with DMSO or 500 nM RSL3 for 24 hours. Statistical results are shown (right panels, n = 3 independent experiments for shNC, sh*TET1* and sh*NFKB2* group; n = 2 independent experiments for sh*GCH1* group). **n**, Effects of GPX4 on BH4 levels. BH4 levels were detected with ELISA. Left panel: Relative BH4 levels of THP1 cells transfected with shNC or sh*GPX4* (n = 3 independent experiments); Right panel: Relative BH4 levels of WT or *GPX4* knockout THP1 cells (n = 4 independent experiments). **o**, GPX activity was detected with cellular glutathione peroxidase assay kit in THP1 cells with or without *GCH1* knockout. n = 3 biologically replicates. **p**, Relative cell viability of *GPX4*$^{-/-}$, *GCH1*$^{-/-}$ THP1 cells with or without 100 μM Trolox suppliance for 24 hours (n = 6 independent experiments). Data are presented as mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001; Statistical significance was determined by unpaired two-tailed t-test (**b**, **d**, **e**, **g**, **n** and **p**), or two-way ANOVA with Tukey's multiple comparisons test (**h**-**m** and **o**). Exact P values and source data are provided as a Source Data file.

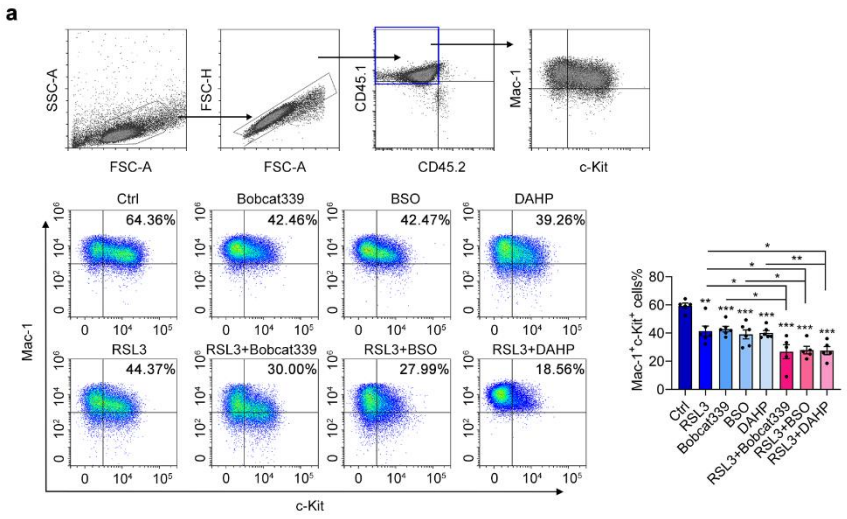


Supplementary Fig. 11. Therapeutic effects of RSL3 + Bobcat339/BSO/DAHP combinational administration in murine AML. a, **b,** Effects of Bobcat339, BSO and DAHP in sensitizing murine *AE9a*-AML (**a**) or *MA9*-AML (**b**) cells to RSL3

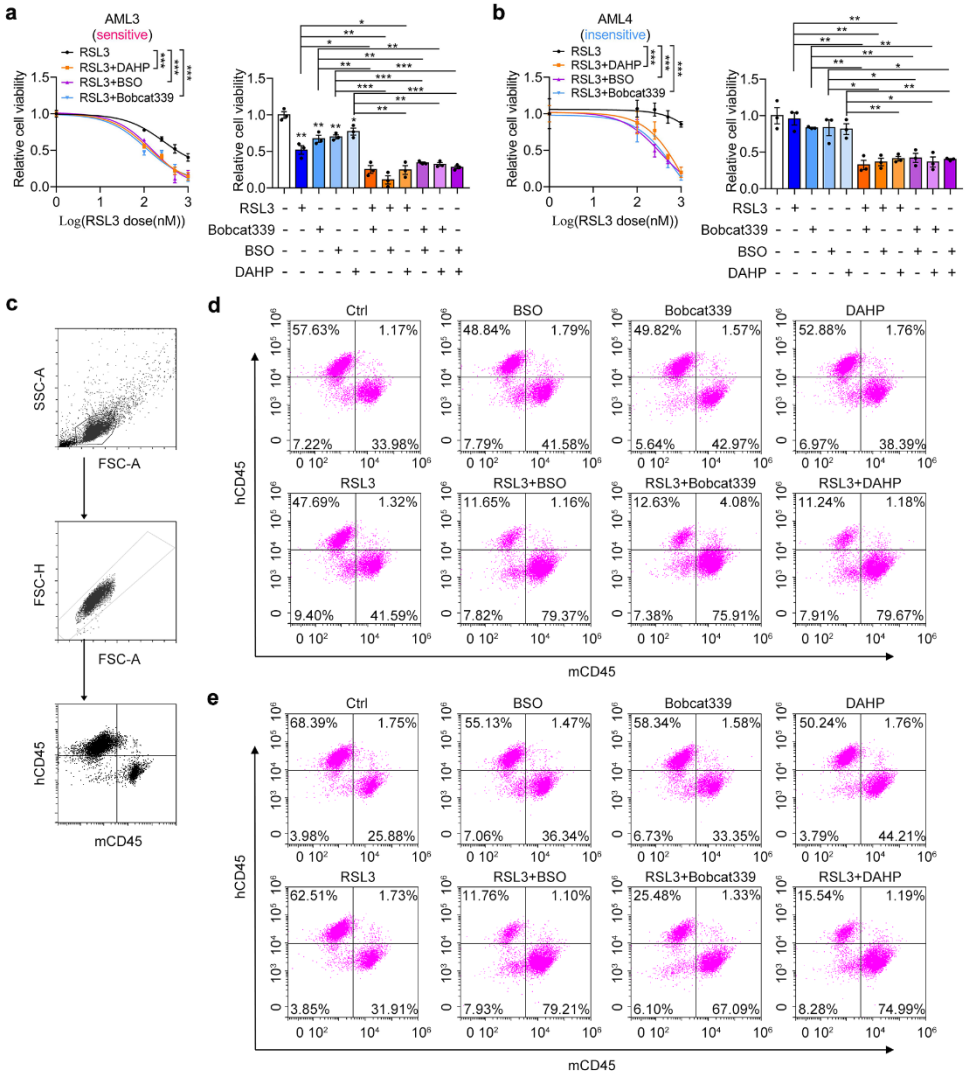
induced ferroptosis. Cells were treated with DMSO or 250 nM RSL3 alone, or in combination with 100 μM BSO, 30 μM Bobcat339 and/or 500 μM DAHP for 48 hours. Relative cell viability is shown. For **a** panel: $n = 6$ independent experiments for Ctrl, RSL3, Bobcat339, BSO, RSL3 + Bobcat339 and RSL3 + BSO group; $n = 5$ independent experiments for DAHP, RSL3 + DAHP, BSO + Bobcat339, Bobcat339 + DAHP and BSO + DAHP group. For **b** panel: $n = 14$ independent experiments for Ctrl and Bobcat339 group; $n = 10$ independent experiments for RSL3 and BSO group; $n = 5$ independent experiments for DAHP, RSL3 + DAHP, Bobcat339 + DAHP and BSO + DAHP group; $n = 7$ independent experiments for RSL3 + Bobcat339, RSL3 + BSO and BSO + Bobcat339 group. **c**, **d**, Bobcat339, BSO and DAHP impairs the colony forming capacity of AML cells. CFAs were conducted with mouse BM HSPCs retrovirally transfected with *AE9a* (**c**) or *MA9* (**d**). Shown are colony counts of each round of plating ($n = 5$ independent experiments for Ctrl, RSL3, Bobcat339 and BSO group; $n = 3$ independent experiments for other groups). **e**, Combinational administration of RSL3 and Bobcat339, BSO or DAHP prolongs survival of *MA9*-AML mice. AML mice were treated with DMSO control ($n = 9$ mice), 1 mg/kg RSL3 ($n = 9$ mice), 10 mg/kg Bobcat339 ($n = 6$ mice), 200 mg/kg BSO ($n = 6$ mice), 50 mg/kg DAHP ($n = 6$ mice), RSL3+Bobcat339 ($n = 6$ mice), RSL3+BSO ($n = 6$ mice) or RSL3+DAHP ($n = 6$ mice), i.p., once every other day for two weeks. Kaplan-Meier curves are shown. *P* values were calculated by log-rank test. **f**, Wright-Giemsa staining of mouse PB and BM, or H&E staining of mouse spleen and liver (along with the enlarged view) of *MA9*-AML mice treat with control, Bobcat339, BSO, DAHP alone or combined with RSL3. Three mice per group euthanized at day 25. Data are presented as mean ± SEM; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; Statistical significance was determined by unpaired two-tailed t-test. Exact *P* values and source data are provided as a Source Data file.



Supplementary Fig. 12. Short-term and long-term toxicity effects of RSL3 in mice. a, Flow cytometry pre-gating strategy for LT-HSC/ST-HSC sorting in Supplementary Fig. 12b, c. **b**, **c**, Acute and long-term toxicity evaluation of RSL3 in C57BL/6 mice. Mice were treated with DMSO (Ctrl), 2 mg/kg RSL3, i.p. once every day for 14 days. Shown are body weight, liver and spleen weights, white cell counts, and BM LT-HSC/ST-HSC proportions. Data was collected 24 hours post the last i.p. injection (Acute, $n = 5$ mice) (**b**) or 200 days post the last i.p. (Long-term, $n = 3$ mice for Ctrl group; $n = 4$ mice for RSL3 group) (**c**). Data are presented as mean ± SEM; NS: no significance; Statistical significance was determined by unpaired two-tailed t-test. Exact *P* values and source data are provided as a Source Data file.

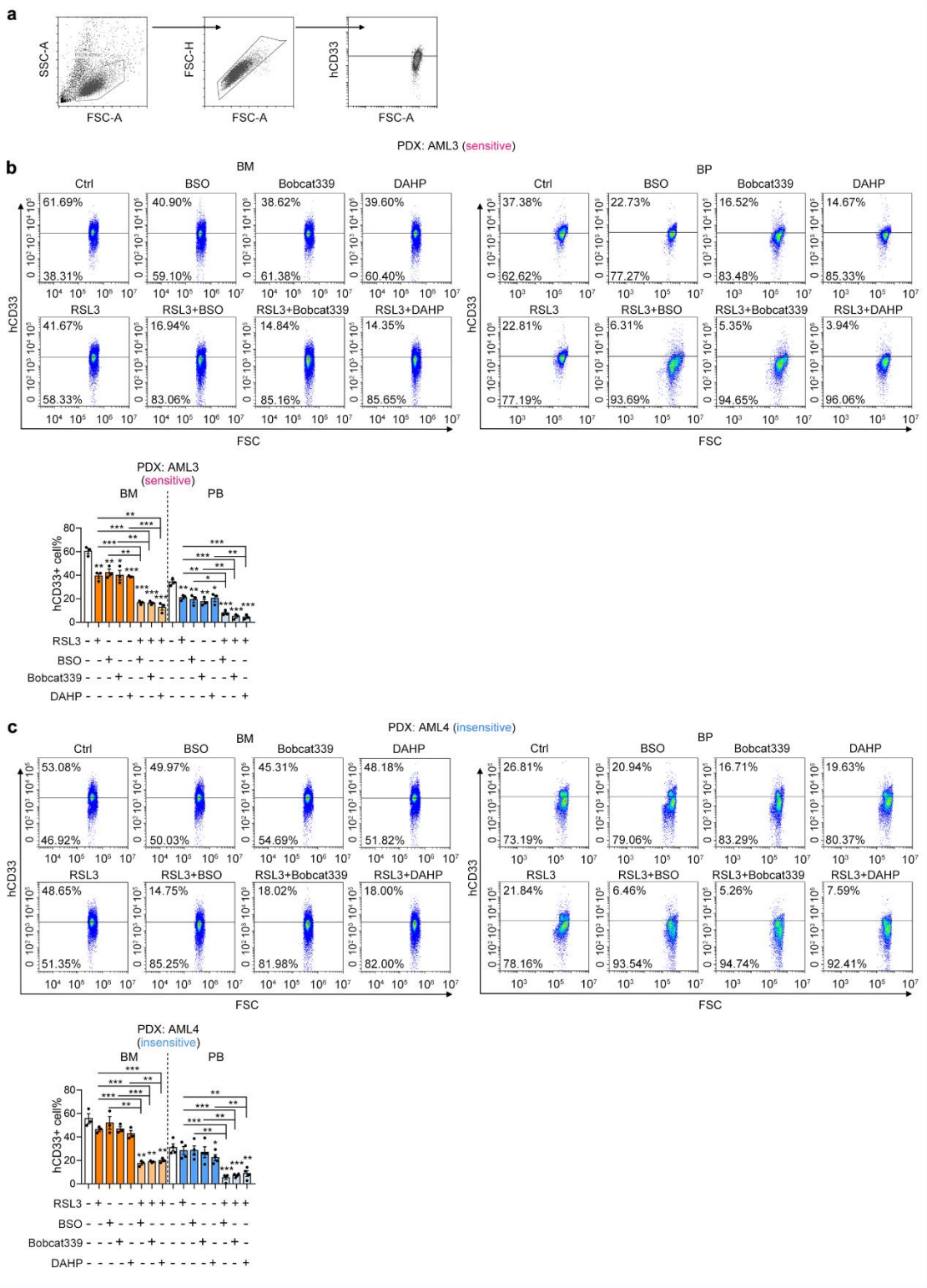


Supplementary Fig. 13. Therapeutic efficacy of RSL3 + Bobcat339/BSO/DAHP combinational administration against LICs. a, Upper panels: Flow cytometry pre-gating strategy for LIC sorting. Lower panels: Flow cytometry analysis of *MA9*-AML mouse BM LIC proportions on day 25 post-BMT. Left panels: Shown are proportions of c-Kit$^+$ and Mac1$^+$ (CD11b$^+$) cells. Right panels: Statistical results are shown (n = 5 mice for Ctrl, RSL3 + Bobcat339, RSL3 + BSO and RSL3 + DAHP group; n = 6 mice for RSL3, Bobcat339, BSO and DAHP group). Statistical significance was determined by unpaired two-tailed t-test. Data are presented as mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001; Exact P values and source data are provided as a Source Data file.



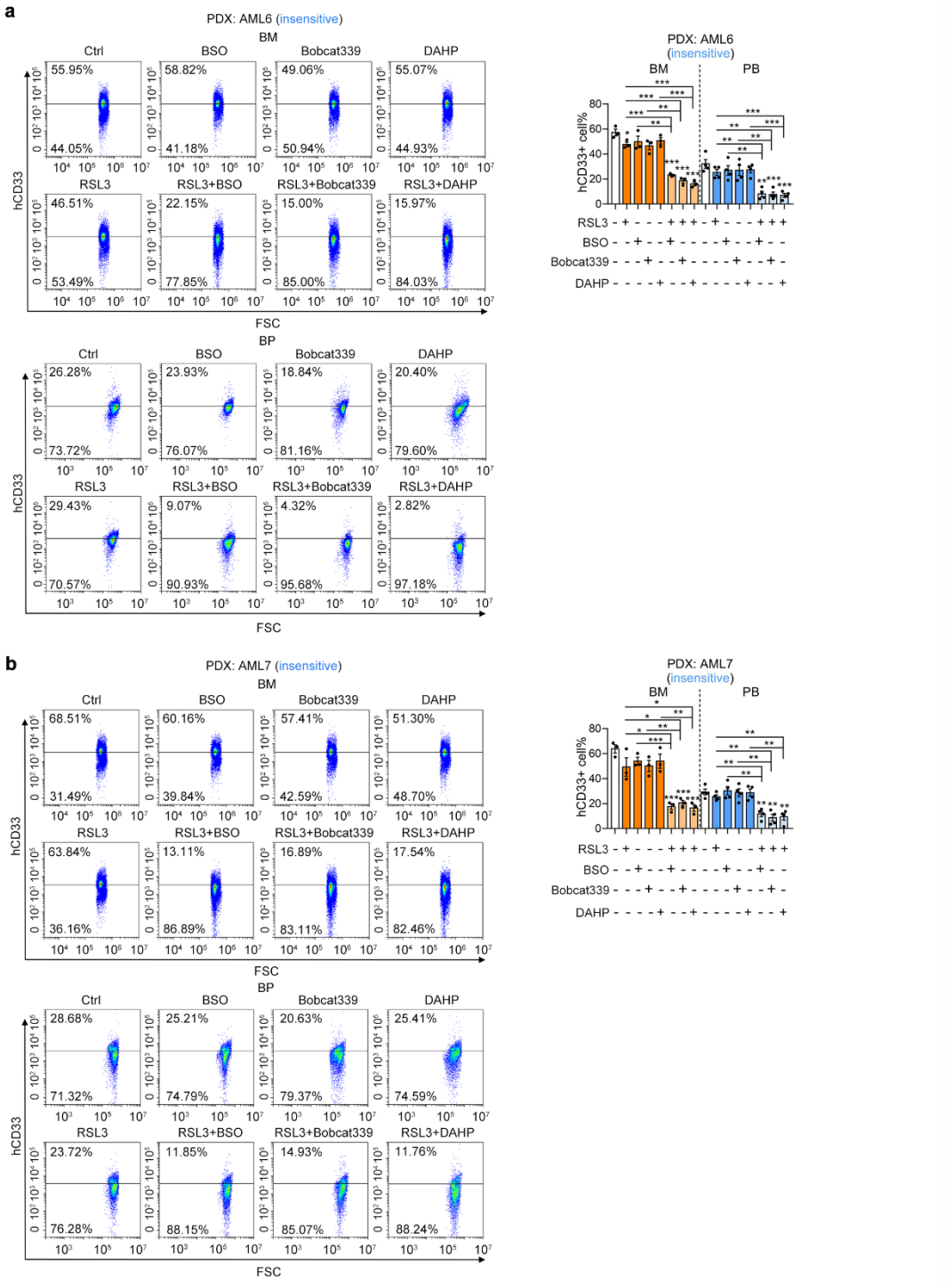
Supplementary Fig. 14. Therapeutic effects of RSL3 + Bobcat339/BSO/DAHP combinational administration in human AML. a, **b**, Synergistic effect of Bobcat339, BSO and DAHP in enhancing RSL3 induced cell death of primary AML cells. AML cells collected directly from two individual AML patients, i.e., AML3 (**a**) and AML4 (**b**), were treated with RSL3 at indicated concentrations in the presence of DMSO, 500 μM BSO, 30 μM Bobcat339 or 500 μM DAHP for 48 hours. Left panels: RSL3 dose curve; right panels: Relative cell viability at a 500 nM fixed RSL3 dose. *n* = 3 biologically replicates. **c**, Flow cytometry pre-gating strategy for human $CD45^+$ donor cells sorting in Supplementary Fig. 14d-e, 18c-d. **d**, **e**, Combinational administration of RSL3 and Bobcat339, BSO or DAHP suppresses BM engraftment of AML4 (**d**) or AML7 (**e**) cells in the PDX models. Proportions of human $CD45^+$

donor cells and mouse $CD45^+$ cells in BM were detected with flow cytometry 100 days post xeno-BMTs. Data are presented as mean ± SEM; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; Statistical significance was determined by two-way ANOVA with Tukey's multiple comparisons test (**a**-**b**, left panel), or unpaired two-tailed t-test (**a**-**b**, right panel). Exact *P* values and source data are provided as a Source Data file.



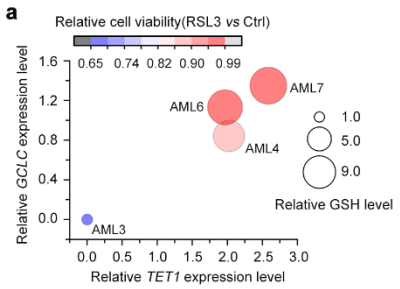
Supplementary Fig. 15. Combined treatment with RSL3 and Bobcat339/BSO/DAHP suppresses the infiltration of AML3/4 cells. a, Flow cytometry pre-gating strategy for human CD33$^+$ donor cells sorting in Supplementary Fig. 15b-c, 16a-b. **b**, **c**, Combinational administration of RSL3 and Bobcat339, BSO

443 or DAHP suppresses the engraftment and infiltration of AML3 (**b**, $n = 3$ mice) or
444 AML4 (**c**, $n = 3$ mice for BM group; $n = 4$ mice for PB group) cells in PDX models.
445 Proportions of human $CD33^+$ donor cells in BM and peripheral blood were detected
446 with flow cytometry 100 days post xeno-BMTs (upper panels). Statistical analysis is
447 shown in the lower panel. Data are presented as mean ± SEM; $*P < 0.05$, $**P < 0.01$,
448 $***P < 0.001$; Statistical significance was determined by unpaired two-tailed t-test.
449 Exact *P* values and source data are provided as a Source Data file.
450

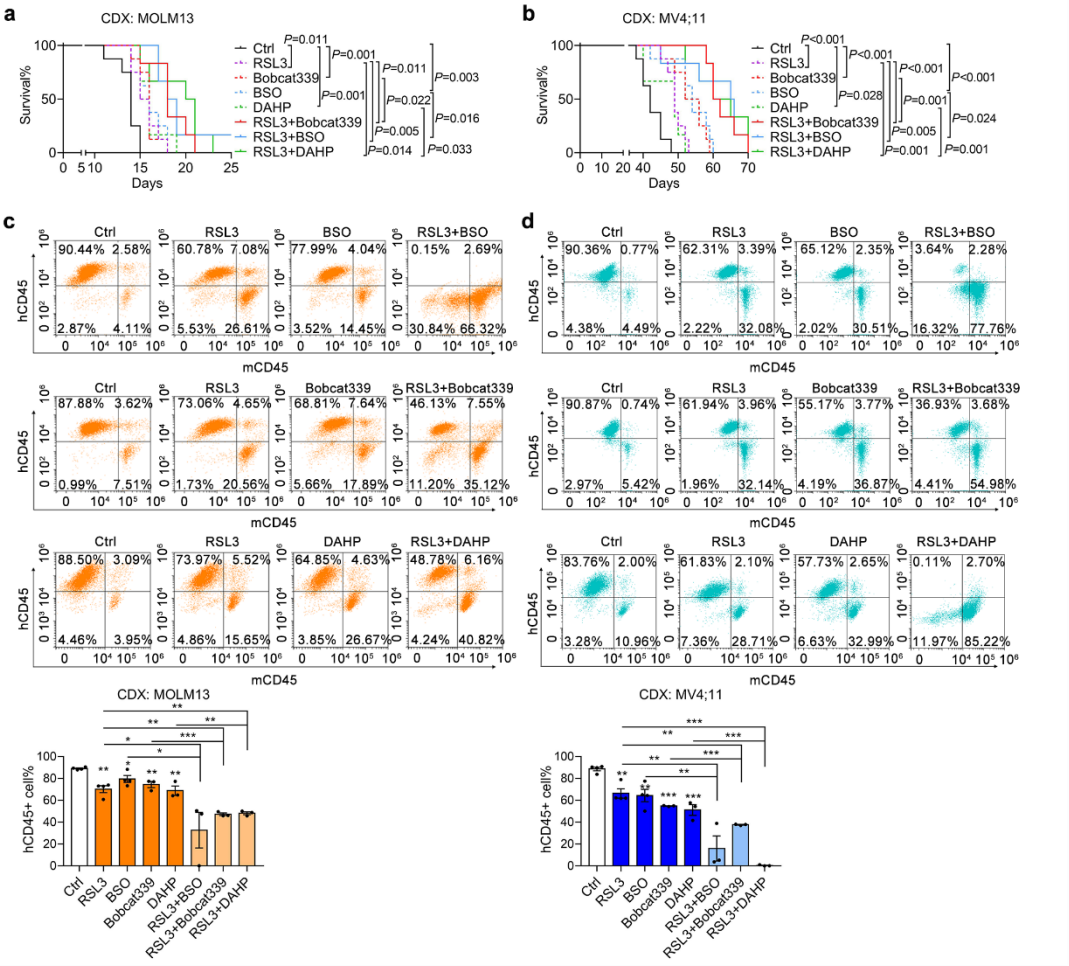


Supplementary Fig. 16. Combined treatment with RSL3 and Bobcat339/BSO/DAHP suppresses the infiltration of AML6/7 cells. a-b, Combinational administration of RSL3 and Bobcat339, BSO or DAHP suppresses the engraftment and infiltration of AML6 (**a**) or AML7 (**b**) cells in PDX models. Proportions of human CD33$^+$ donor cells in BM and peripheral blood were detected

457 with flow cytometry 100 days post xeno-BMTs (left panels). Statistical analysis is
458 shown in the right panel (n = 3 mice for BM group; n = 4 mice for PB group). Data
459 are presented as mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001; Statistical
460 significance was determined by unpaired two-tailed t-test. Exact P values and source
461 data are provided as a Source Data file.
462



Supplementary Fig. 17. Positive correlation of *TET1* expression, GCLC expression and GSH levels in human AML cells. a, Correlation among *TET1* expression, *GCLC* expression, GSH levels and RSL3 sensitivity. BM cells from recipient mice at 100 days post-xeno-BMT were collected and the $hCD45^+$ populations were isolated. Expression levels of *TET1* and *GCLC* in AML patient-derived cell samples (AML3, AML4, AML6 and AML7) isolated from recipient mice were measured by Q-PCR, and normalized to the expression levels in AML3 cells. GSH levels were determined with the GSH/GSSG ratio detection kit, with the circle area representing the relative GSH level normalized to that of AML3 cells. For cell viability assessment, cells were treated with 250 nM RSL3 or DMSO as a control for 48 hours, and viability was evaluated using MTT assays. The color scale indicates relative cell viability, calculated as the ratio of the RSL3-treated group to the control group. $n = 3$ biologically replicates.



Supplementary Fig. 18. Targeting the TET1/GCL and TET1/GCH1 axes inhibits the progression of AML in CDX models. a, **b**, Combinational administration of RSL3 and Bobcat339, BSO or DAHP suppressed human AML pathogenesis in MOLM13 (**a**) and MV4;11 (**b**) derived CDX models. NSG recipients were treated with DMSO as control (n = 8 mice) or 5 mg/kg RSL3 (n = 8 mice), 10 mg/kg Bobcat339 (n = 8 mice), 200 mg/kg BSO (n = 8 mice), 50 mg/kg DAHP (n = 6 mice), RSL3+Bobcat339 (n = 6 mice), RSL3+BSO (n = 6 mice) or RSL3+DAHP (n = 6 mice), i.p, once every other day for two weeks after the onset of leukemia. Kaplan-Meier curves are shown. P values were calculated by log-rank test. **c**, **d**, Combinational administration of RSL3 and Bobcat339, BSO or DAHP suppresses engraftment of MOLM13 (**c**) or MV4;11 (**d**) cells in the CDX models. BM engraftment of donor cells 2 weeks (MOLM13) or 7 weeks (MV4;11) post-BMT detected by flow cytometry. Statistical analysis is shown in the lower panel (n = 4

mice for Ctrl, RSL3 and BSO group; $n = 3$ mice for Bobcat339, DAHP, RSL3 + BSO, RSL3 + Bobcat339 and RSL3 + DAHP group). Data are presented as mean ± SEM; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; Statistical significance was determined by unpaired two-tailed t-test. Exact P values and source data are provided as a Source Data file.

498 **Supplementary Table 1. IC_{50} of cancer cell lines to ferroptosis inducers.**

Cell line	RSL3 IC_{50} (nM)	ML210 IC_{50} (nM)	
Daudi	1.77	264.2	Sensitive
MOLM13	16.02	132.5	
HL60	31.54	250	
CA46	59.49	189.8	
MV4;11	59.87	131.9	
786-O	73.49	321.5	
Jurkat	76.42	266.6	
HLF	84.18	148.9	
HCC827	90.08	376.2	
HT1080	113.3	120.6	
U937	208.4	160.9	
THP1	228.1	289.7	
AML3	253.3	233.6	
H292	285.7	426.1	
AML2	492.8	119.1	
AML1	493.3	433.2	
NB4	734.7	576.7	Resistant
MIA PaCa-2	2.48×10^3	9.26×10^3	
Kasumi-1	3.31×10^3	9.73×10^3	
K562	3.8×10^3	5.37×10^3	
HepG2	4.0×10^3	1.05×10^4	
PaTu 8988t	5.62×10^3	1.08×10^3	
AML5	2.21×10^3	991.7	
AML8	3.30×10^3	4.32×10^3	
AML4	3.72×10^3	4.62×10^3	
AML6	4.74×10^3	4.03×10^3	
A549	5.63×10^3	7.91×10^3	
AML9	6.40×10^3	1.62×10^3	
B16-F10	8.51×10^3	1.2×10^4	
AML7	9.71×10^3	8.89×10^3	
ASPC-1	1.0×10^4	1.12×10^4	
A375	1.0×10^4	1.48×10^4	
ACHN	1.12×10^4	1.38×10^4	

499

500

Supplementary Table 2. Information of AML patients (AML1-9).

Sample ID	Patient ID	Molecular abnormalities	FAB	R/R
AML1	P20190127A	WT1, NRAS, JAK2/3, CEBPA	M5	No
AML2	P20181024A	MLL-AF9, TP53	M5	Yes
AML3	P20200608	DNMT3A, FLT3-ITD, NPM1	M5	Yes
AML4	P20180914B	WT1, MLL-AF4	/	No
AML5	P20190114	MLL-AF9	M5b	Yes
AML6	P20181023	IDH1, NPM1, FLT3-ITD	M2a	No
AML7	P20190123	MLL-AF9	M5	No
AML8	P20211228	WT1	M5	Yes
AML9	P20210513A	FLT3-ITD	M2a	No

Additional information: A total of 9 AML patients were included in this study. The cohort had a median age of 45 years (range, 27 to 78). Among them, male patients constituted the majority, with a total of 7 cases (77.8%), while there were 2 female patients (22.2%).

507 **Supplementary Table 3. The sequence of primers.**

Target gene	Forward	Reverse
TET1	GCTATACACAGAGCTCACAGAG	GAAGCTCCACAAGTCTCTGGA
GAPDH	AATCCCATCACCATCTTCCAG	AAATGAGCCCCAGCCTTC
GPX4	GAGGCAAGACCGAAGTAAACTAC	CCGAACTGGTTACACGGGAA
GCLC	GGAGGAAACCAAGCGCCAT	CTTGACGGCGTGGTAGATGT
NFKB2	AGAGGCTTCCGATTTCGATATGG	GGATAGGTCTTTCGGCCCTTC
NFKB1	GAAGCACGAATGACAGAGGC	GCTTGGCGGATTAGCTCTTTT
RELB	TTCCGAGCCCGTCTATGACAA	CGTCTTGAACACAATGGCAATC
GCH1	ACGAGCTGAACCTCCCTAAC	GAACCAAGTGATGCTCACACA
Tet1	GCTGGATTGAAGGAACAGGA	GTCTCCATGAGCTCCCTGAC
β-actin	TGAACCCTAAGGCCAACCGTGAAA	CAGGATGGCGTGAGGGAGAGCATAG
TET2	CCCTTCTCCGATGCTTTCT	TGGGTTATGCTTGAGGTGTTC
TET3	AAGACACCTCGCAAGTTCC	GTTGGTCACCTGGTTCTGATA
FTL	CAGCCTGGTCAATTTGTACCT	GCCAATTCGCGGAAGAAGTG
FTH	CCCCCATTTGTGTGACTTCAT	GCCCGAGGCTTAGCTTTCATT
ZIP14	AAGGCCCTACTCAACCACCT	CGACTGCTCGCTGAAATTGTG
FPN	TGGATGGGTTCTCACTTCCTG	GTCAATCCTTCGTATTGTGGCAT

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