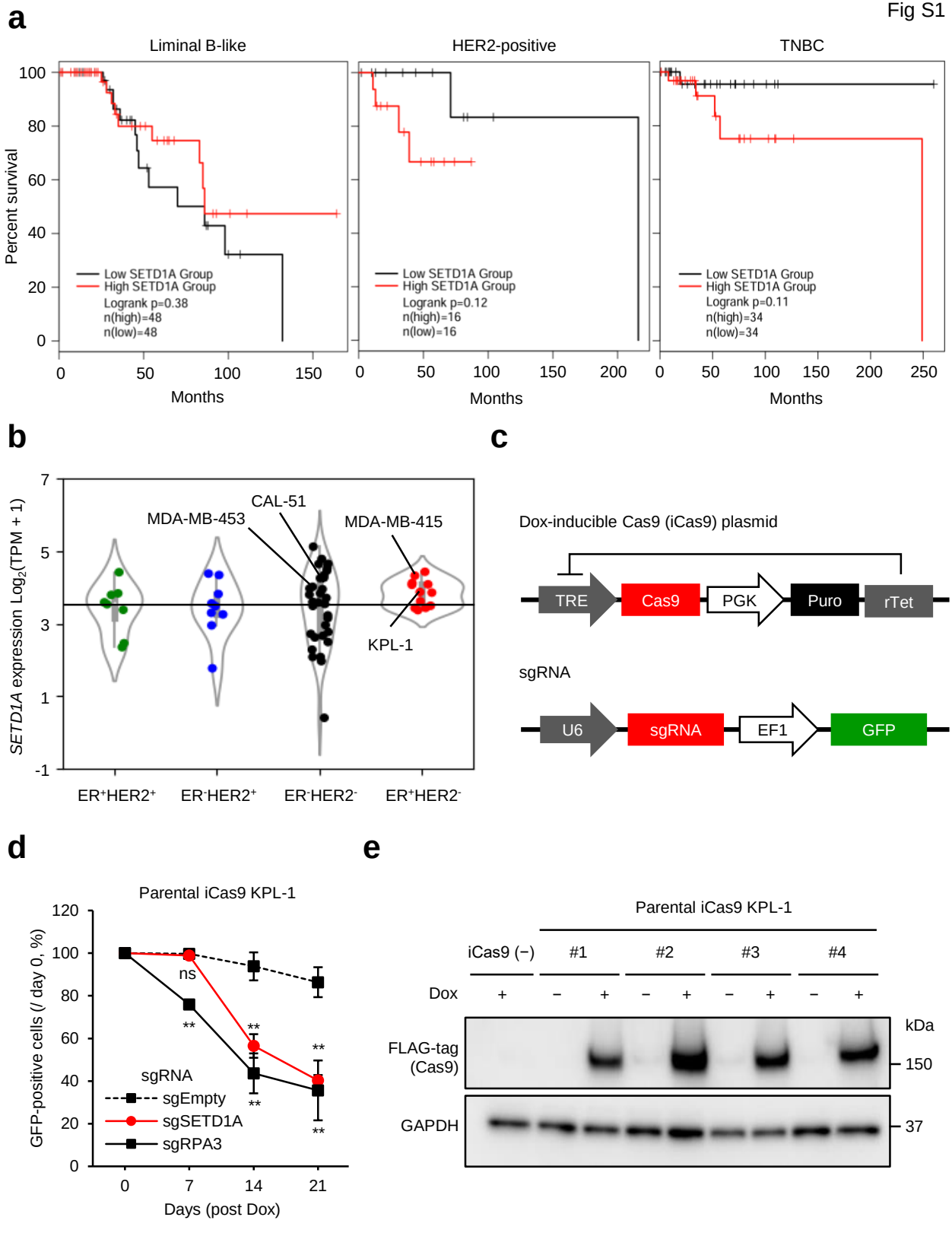
a
Liminal B-like
HER2-positive
TNBC
Percent survival
Low SETD1A Group
High SETD1A Group
Logrank p=0.38
n(high)=48
n(low)=48
Logrank p=0.12
n(high)=16
n(low)=16
Logrank p=0.11
n(high)=34
n(low)=34
Months
b
SETD1A expression Log2(TPM + 1)
MDA-MB-453
CAL-51
MDA-MB-415
KPL-1
ER+HER2+
ER-HER2+
ER-HER2-
ER+HER2-
c
Dox-inducible Cas9 (iCas9) plasmid
TRE
Cas9
PGK
Puro
rTet
sgRNA
U6
sgRNA
EF1
GFP
d
Parental iCas9 KPL-1
GFP-positive cells (/ day 0, %)
ns
sgRNA
sgEmpty
sgSETD1A
sgRPA3
Days (post Dox)
e
Parental iCas9 KPL-1
iCas9 (−)
#1
#2
#3
#4
Dox
FLAG-tag (Cas9)
GAPDH
kDa
150
37

Figure S1. Establishment of a doxycycline-inducible SETD1A knockout breast cancer cell line.

(a) Kaplan-Meier curves show the overall survival of luminal B-like, HER2-positive, and triple-negative breast cancer patients with high and low *SETD1A* expression levels. The data were obtained from the TCGA database and visualized using GEPIA2.

(b) The mRNA levels (TPM) of *SETD1A* in 67 breast cancer cell lines from the DepMap database (Expression Public 23Q2) are shown. ER^+HER2^+, n = 8; ER^-HER2^+, n = 9; ER^-HER2^-, n = 33; ER^+HER2^-, n = 12. The horizontal black line indicates the average *SETD1A* expression level across all 67 cell lines (3.44, ($\log_2$(TPM + 1)) .

(c) Schematic models of the iCas9 plasmid and sgRNA constructs used in this study.

(d) sgRNAs with a GFP reporter were used in a competitive growth assay in parental iCas9 KPL-1 breast cancer cells. The percentage of GFP-positive sgRNA-expressing iCas9 KPL-1 cells was analyzed every 7 days after Dox treatment. Representative data from two independent experiments with three biological replicates each are shown. Statistical differences compared with Empty sgRNA-expressing iCas9 KPL-1 cells (dotted line) at each time point are indicated.

(e) Western blot analysis was performed using single-cell-cloned iCas9-expressing KPL-1 cells at 4 days post-Dox. Clone #1 was used for the following studies.

ns, no significance; $^{**}P < 0.01$.

a

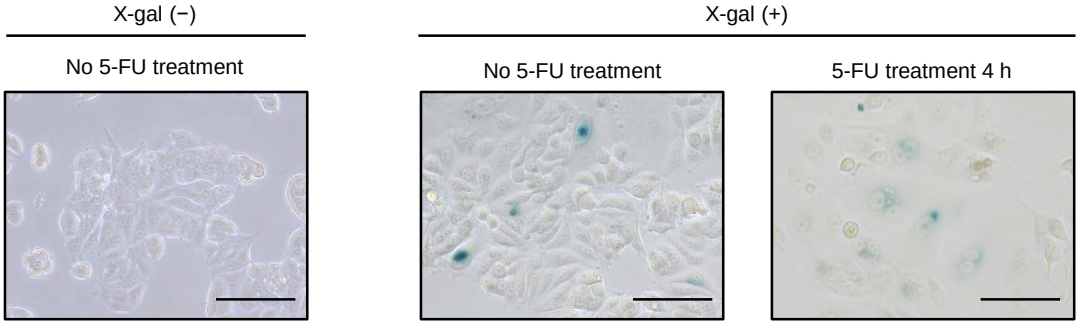

Figure S2. 5-FU treatment induces β-galactosidase-positive cellular senescence in KPL-1 breast cancer cells.

Representative images from senescence-associated β-galactosidase staining of iCas9 KPL-1 cells are shown. Scale = 100 μm.

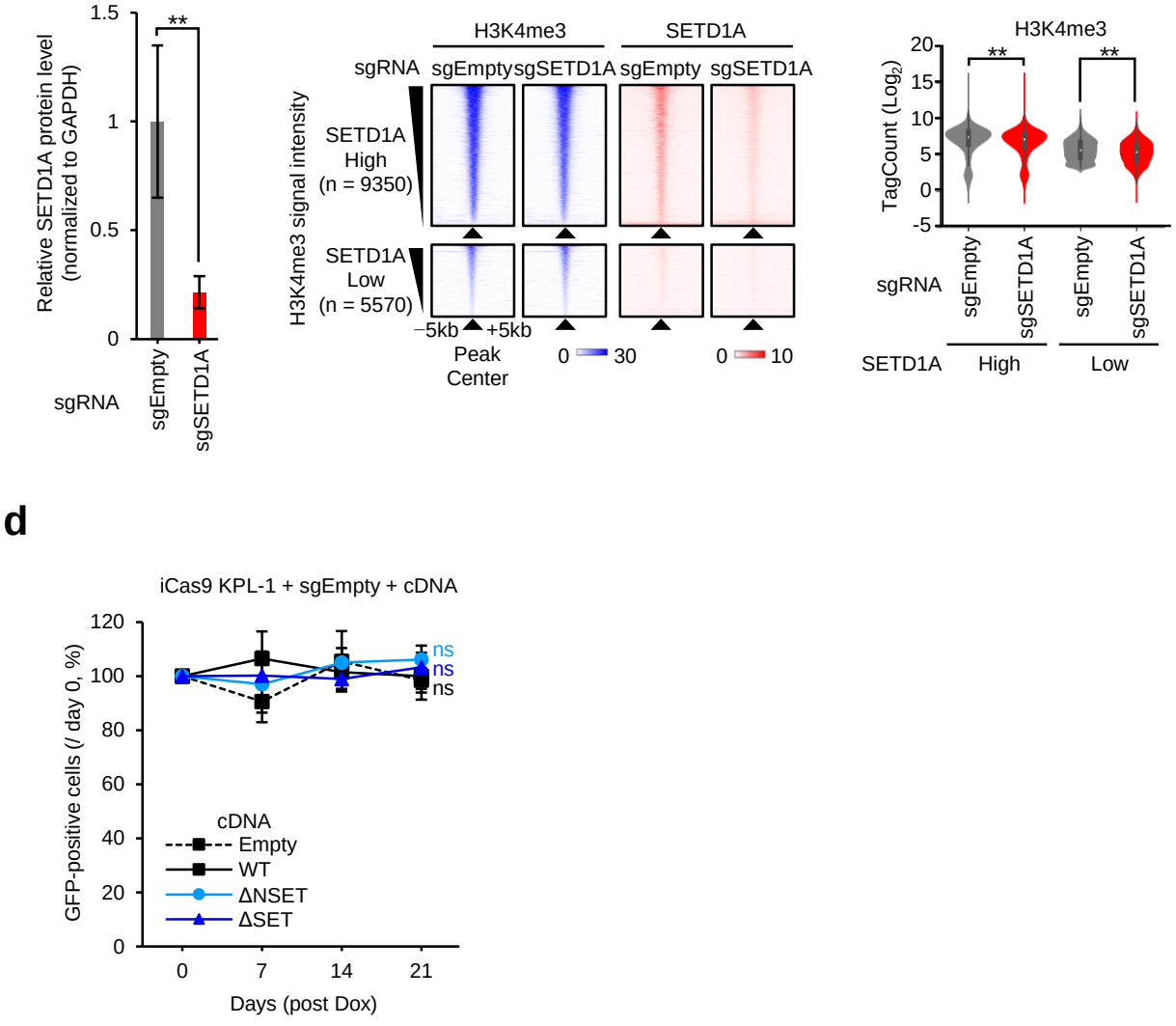
a
Relative SETD1A protein level (normalized to GAPDH)
1.5
1
0.5
0
**
sgRNA
sgEmpty
sgSETD1A
b
H3K4me3
SETD1A
sgRNA
sgEmpty
sgSETD1A
sgEmpty
sgSETD1A
H3K4me3 signal intensity
SETD1A High (n = 9350)
SETD1A Low (n = 5570)
−5kb
+5kb
Peak Center
0
30
0
10
c
H3K4me3
TagCount (Log2)
20
15
10
5
0
-5
**
**
sgRNA
sgEmpty
sgSETD1A
sgEmpty
sgSETD1A
SETD1A
High
Low
d
iCas9 KPL-1 + sgEmpty + cDNA
GFP-positive cells (/ day 0, %)
120
100
80
60
40
20
0
ns
ns
ns
cDNA
Empty
WT
ΔNSET
ΔSET
0
7
14
21
Days (post Dox)

Figure S3. Reduction of H3K4me3 in SETD1A-deficient cells is not associated with the chromatin distribution of SETD1A.

(a) Western blot analysis was performed using sgRNA-expressing iCas9 KPL-1 cells at 7 days post-Dox. The band intensities of SETD1A were normalized to GAPDH. Three biological replicates were used in this analysis.

(b) ChIP intensities of H3K4me3 (blue) and SETD1A (red) in sgRNA-expressing iCas9 KPL-1 cells are depicted as heat maps. Data were analyzed at SETD1A high or low regions within ± 5 kb from the H3K4me3 peak center, respectively. SETD1A High (Log_2 Tagcount ≥ 3) and SETD1A Low (Log_2 Tagcount < 3) regions were defined based on SETD1A ChIP-seq data.

(c) H3K4me3 Log_2 Tagcount in sgRNA-expressing iCas9 KPL-1 cells at SETD1A-high and SETD1A-low regions are shown.

(d) Competitive growth assay performed in cDNA-transduced iCas9 KPL-1 cells with Empty sgRNA infection. The percentage of GFP-positive cells was analyzed every 7 days post-Dox. Representative data from three biological replicates are shown. Statistical differences versus Empty vector-transduced cells (dotted line) at each time point are shown.

ns, no significance; $^{**}P < 0.01$.

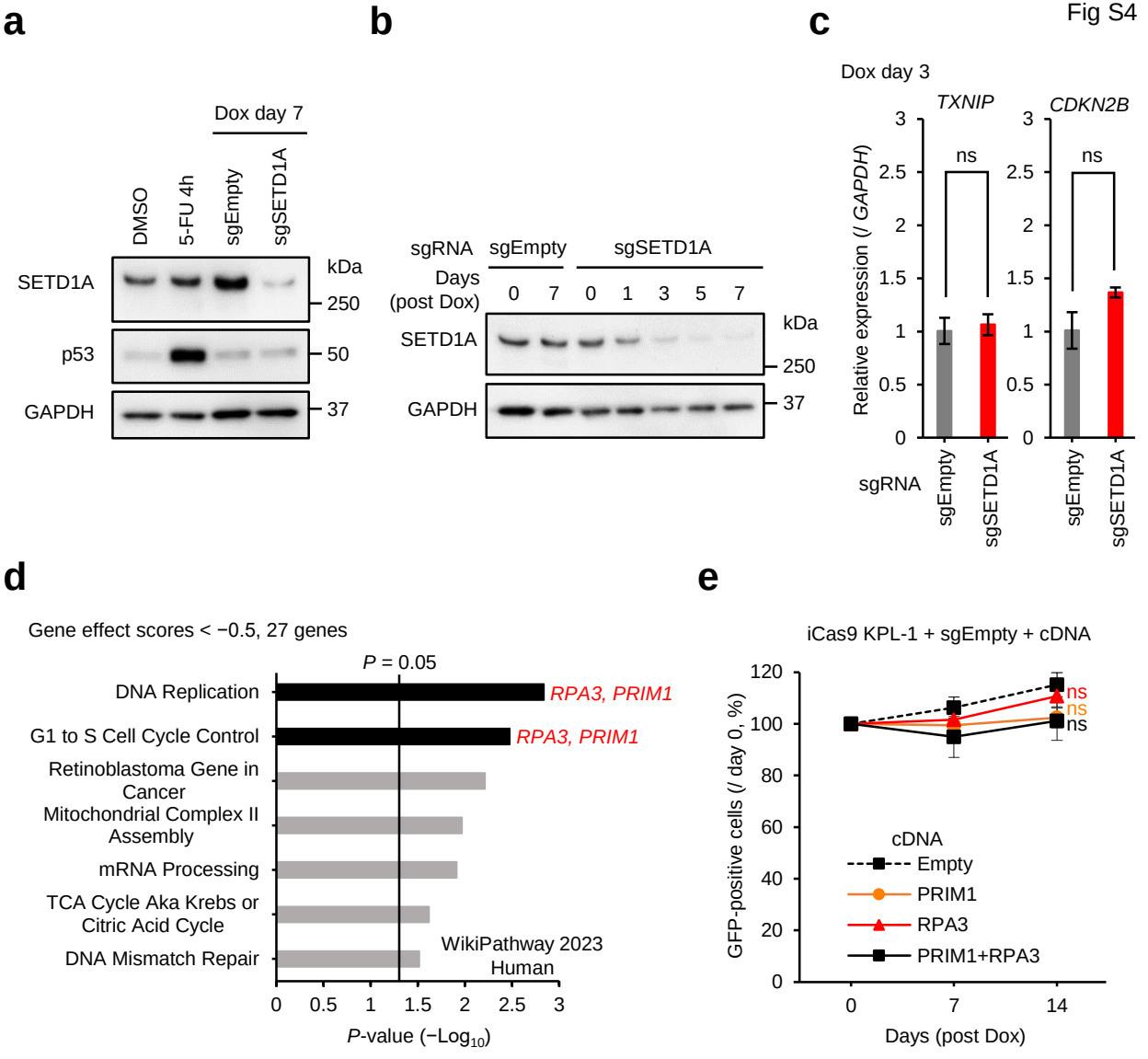
a
Dox day 7
DMSO
5-FU 4h
sgEmpty
sgSETD1A
SETD1A
p53
GAPDH
kDa
250
50
37
b
sgRNA
sgEmpty
sgSETD1A
Days (post Dox)
0 7 0 1 3 5 7
SETD1A
GAPDH
kDa
250
37
c
Dox day 3
TXNIP
CDKN2B
ns
Relative expression (/ GAPDH)
sgRNA
sgEmpty
sgSETD1A
d
Gene effect scores < −0.5, 27 genes
P = 0.05
DNA Replication
G1 to S Cell Cycle Control
Retinoblastoma Gene in Cancer
Mitochondrial Complex II Assembly
mRNA Processing
TCA Cycle Aka Krebs or Citric Acid Cycle
DNA Mismatch Repair
RPA3, PRIM1
RPA3, PRIM1
WikiPathway 2023 Human
P-value (−Log10)
e
iCas9 KPL-1 + sgEmpty + cDNA
GFP-positive cells (/ day 0, %)
cDNA
Empty
PRIM1
RPA3
PRIM1+RPA3
ns
Days (post Dox)

Figure S4. *RPA3* and *PRIM1* are functional downstream targets of SETD1A in KPL-1 breast cancer cells.

(a) Western blot analysis was performed using iCas9 KPL-1 breast cancer cells and sgRNA-expressing iCas9 KPL-1 cells, respectively. iCas9 KPL-1 cells were analyzed 4 h after DMSO or 5-FU treatment. sgRNA-expressing iCas9 KPL-1 cells were analyzed at 7 days post-Dox treatment.

(b) Western blot analysis was performed using sgRNA-expressing iCas9 KPL-1 cells at the indicated time points after Dox treatment. Representative data from two independent experiments are shown.

(c) RNA was extracted from sgRNA-expressing iCas9 KPL-1 cells at 3 days post-Dox treatment. The mRNA levels of *TXNIP* and *CDKN2B* were quantified by RT-qPCR. Three biological replicates were used in this experiment.

(d) Pathway analysis of 27 genes with gene effect scores (from DepMap Public 23Q2+Score) < −0.5 was performed using the Enrichr tool. The vertical black line indicates *P*-value = 0.05.

(e) Competitive growth assay was performed using cDNA-transduced iCas9 KPL-1 cells with Empty sgRNA infection. The percentage of GFP-positive cells was analyzed every 7 days after Dox treatment. Three biological replicates were used in this experiment. Statistical differences versus Empty vector-transduced cells (dotted line) at each time point are shown.

ns, no significance.

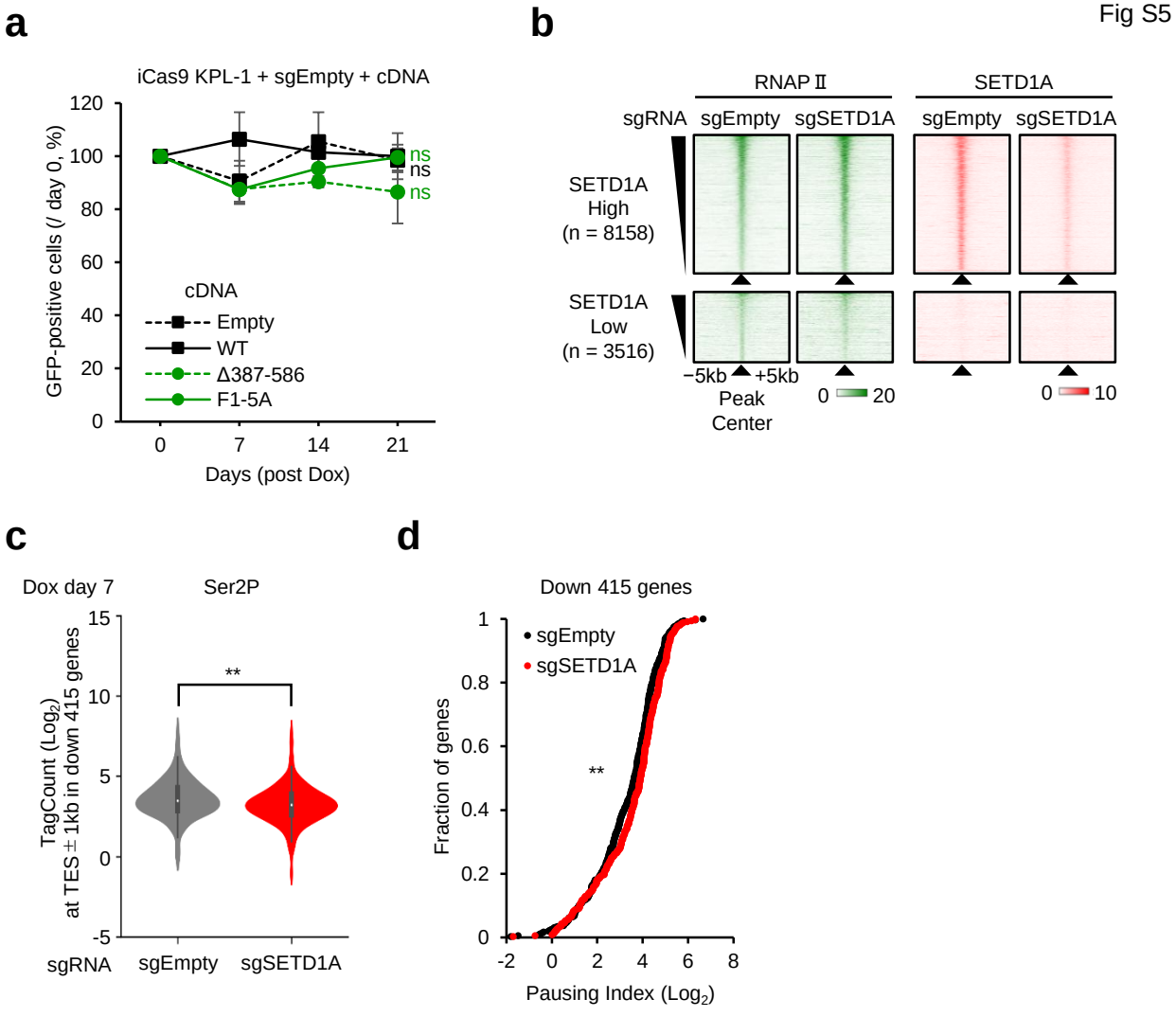
a
iCas9 KPL-1 + sgEmpty + cDNA
GFP-positive cells (/ day 0, %)
120
100
80
60
40
20
0
ns
ns
ns
cDNA
Empty
WT
Δ387-586
F1-5A
0
7
14
21
Days (post Dox)
b
RNAP Ⅱ
SETD1A
sgRNA
sgEmpty
sgSETD1A
sgEmpty
sgSETD1A
SETD1A
High
(n = 8158)
SETD1A
Low
(n = 3516)
−5kb
+5kb
Peak
Center
0
20
0
10
c
Dox day 7
Ser2P
TagCount (Log2)
at TES ± 1kb in down 415 genes
15
10
5
0
-5
**
sgRNA
sgEmpty
sgSETD1A
d
Down 415 genes
sgEmpty
sgSETD1A
Fraction of genes
1
0.8
0.6
0.4
0.2
0
**
-2
0
2
4
6
8
Pausing Index (Log2)

Figure S5. SETD1A knockout increases RNAPII intensity in SETD1A-high regions.

(a) Competitive growth assay was performed using cDNA-transduced iCas9 KPL-1 cells with Empty sgRNA infection. The percentage of GFP-positive cells was analyzed every 7 days post-Dox. Three biological replicates were used in this experiment. Statistical differences versus Empty vector-transduced cells (black dotted line) at each time point are shown.

(b) ChIP intensities of RNAPII (green) and SETD1A (red) in sgRNA-expressing iCas9 KPL-1 cells are shown as heat maps. Data were analyzed at SETD1A high or low regions within ± 5 kb from the RNAPII peak center, respectively.

(c) ChIP-seq analysis of Ser2P was performed using sgRNA-expressing iCas9 KPL-1 cells at 7 days post-Dox treatment. Ser2P Tagcount at TES ± 1 kb in 415 SETD1A-downregulated genes are shown.

(d) The pausing index (PI) was calculated for the 415 SETD1A-downregulated genes using RNAPII ChIP-seq data.

ns, no significance; $^{**}P < 0.01$.

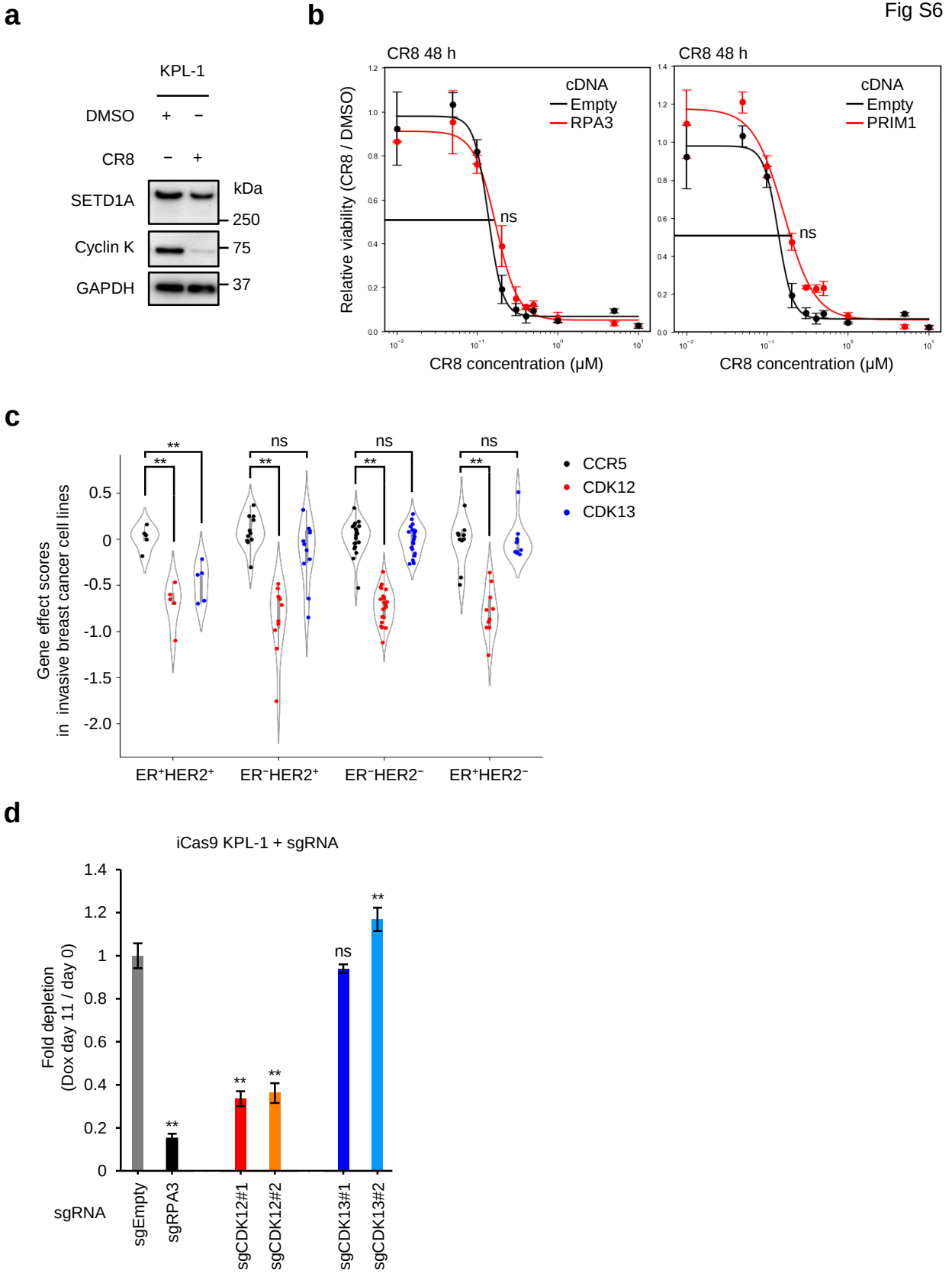
a
KPL-1
DMSO + −
CR8 − +
kDa
SETD1A
250
Cyclin K
75
GAPDH
37
b
CR8 48 h
cDNA
Empty
RPA3
Relative viability (CR8 / DMSO)
ns
CR8 concentration (μM)
CR8 48 h
cDNA
Empty
PRIM1
ns
CR8 concentration (μM)
c
Gene effect scores in invasive breast cancer cell lines
**
**
ns
ns
ns
CCR5
CDK12
CDK13
ER+HER2+
ER−HER2+
ER−HER2−
ER+HER2−
d
iCas9 KPL-1 + sgRNA
Fold depletion (Dox day 11 / day 0)
**
**
**
ns
**
sgRNA
sgEmpty
sgRPA3
sgCDK12#1
sgCDK12#2
sgCDK13#1
sgCDK13#2

Figure S6. CR8, a cyclin K degrader, recapitulates the phenotype of SETD1A deletion.

(a) Western blot analysis was performed using KPL-1 cells. Proteins were extracted from KPL-1 cells treated with DMSO or CR8 at the IC50 concentration for 48 h.

(b) cDNA-transduced iCas9 KPL-1 cells were treated with DMSO or CR8 for 48 h. IC50 values were a compared using bootstrap-based hypothesis testing method with 1,000 iterations to assess significance. Differences were evaluated under the null hypothesis of no difference in IC50. Representative data from two independent experiments with three biological replicates each are shown.

(c) Gene effect scores (from DepMap Public 25Q2+Score) in 47 breast cancer cell lines are shown. The non-essential gene CCR5 was used as a control. $ER^{+}HER2^{+}$, n = 5; $ER^{-}HER2^{+}$, n = 11; $ER^{-}HER2^{-}$, n = 21; $ER^{+}HER2^{-}$, n = 10.

(d) Competitive growth assay performed in iCas9 KPL-1 cells infected with sgRNAs. The percentage of RFP-positive cells was analyzed at day 0 and day 11 after Dox treatment. Representative data from three biological replicates are shown. Statistical differences compared with Empty sgRNA-expressing cells (grey) are indicated.

ns, no significance; $*P < 0.05$; $**P < 0.01$.

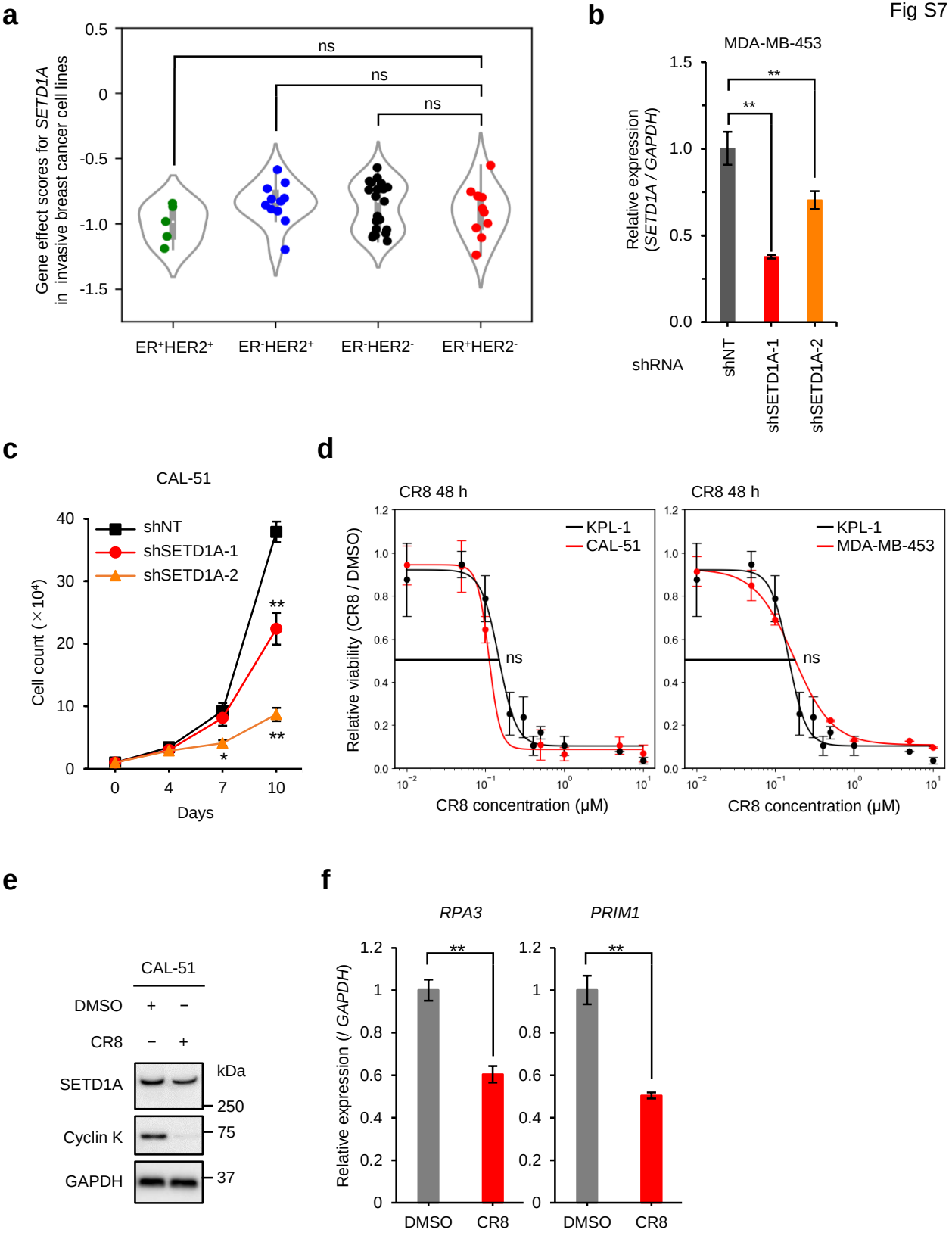
a
Gene effect scores for SETD1A in invasive breast cancer cell lines
0.5
0
-0.5
-1.0
-1.5
ns
ns
ns
ER+HER2+
ER-HER2+
ER-HER2-
ER+HER2-
b
MDA-MB-453
Relative expression (SETD1A / GAPDH)
1.5
1.0
0.5
0.0
**
**
shRNA
shNT
shSETD1A-1
shSETD1A-2
c
CAL-51
Cell count (×10⁴)
40
30
20
10
0
shNT
shSETD1A-1
shSETD1A-2
**
**
*
0
4
7
10
Days
d
CR8 48 h
Relative viability (CR8 / DMSO)
KPL-1
CAL-51
ns
CR8 concentration (μM)
CR8 48 h
KPL-1
MDA-MB-453
ns
CR8 concentration (μM)
e
CAL-51
DMSO + −
CR8 − +
kDa
SETD1A
250
Cyclin K
75
GAPDH
37
f
RPA3
PRIM1
Relative expression (/ GAPDH)
1.2
1
0.8
0.6
0.4
0.2
0
**
**
DMSO
CR8
DMSO
CR8

Figure S7. CR8, a cyclin K degrader, effectively inhibits cell proliferation in TNBC cells.

(a) Gene effect scores (from DepMap Public 25Q2+Score) for *SETD1A* in 47 breast cancer cell lines are shown. ER^+HER2^+, n = 5; ER^-HER2^+, n = 11; ER^-HER2^- (TNBC), n = 21; ER^+HER2-, n = 10.

(b) Relative expression levels of *SETD1A* in shRNA-expressing MDA-MB-453 TNBC cells were quantified by RT-qPCR at 4 days after puromycin selection. Representative data from two independent experiments with three biological replicates are shown.

(c) Cell numbers were counted in shRNA-expressing CAL-51 TNBC cells at the indicated time points after puromycin selection. This experiment was repeated twice with three biological replicates per experiment. Statistical differences versus shNT-expressing CAL-51 cells at each time point are shown.

(d) Two TNBC cell lines, CAL-51 (left) and MDA-MB-453 (right), were treated with DMSO or CR8 for 48 h. Data for KPL-1 are reused from Figure 6b. IC50 values were statistically compared using a bootstrap-based hypothesis testing with 1,000 iterations under the null hypothesis of no IC50 difference. This experiment was repeated twice with three biological replicates per experiment.

(e) Western blot analysis was performed using CAL-51 TNBC cells treated with DMSO or CR8 at the IC50 concentration for 48 h.

(f) Relative expression levels of *RPA3* and *PRIM1* in CAL-51 TNBC cells were quantified by RT-qPCR. Cells were treated with DMSO or CR8 at the IC50 concentration for 48 h. Representative data from two independent experiments with three biological replicates are shown.

ns, no significance; $*P < 0.05$; $**P < 0.01$.