Supplementary Information

Supplemental Table S1. Clinicopathological characterisitcs of 109 GC patients

Characterisitcs	Number of patients (%)
Patients	109
Gender	
Male	68 (62.39%)
Female	41 (37.61%)
Age (years)	26 to 84, mean 54.78
Tumor size (cm)	3.2 to 12.5, mean 6.36
Lymph node metastasis (N stage)	
N0	1 (0.92%)
N1	29 (26.61%)
N2	37 (33.94%)
N3	42 (38.53%)
Depth of invasion (T stage)	
T1	0 (0.00%)
T2	3 (2.75%)
T3	18 (16.51%)
T4a	84 (77.06%)
T4b	4 (3.67%)
Histology	
well and morderately	54 (49.54%)
Poorly and others	55 (50.46%)
Perineural Invasion	
Negative	70 (64.22%)
Positive	39 (35.78%)
Lymphovascular invasion	
Negative	66 (60.55%)
Positive	43 (39.45%)

Supplementary Table S2. Clinicopathological characterisitcs of 52 GC patients

Characterisitcs	Number of patients (%)
Patients	52
Gender	
Male	33 (63.46%)
Female	19 (36.53%)
Age (years)	27 to 73, mean 52.32
Tumor size (cm)	5.6 to 13.8, mean 8.36
Tumor (pre-NAC,CT)	
T4A	38 (73.07%)
T4B	14 (26.92%)
Lymph node (pre-NAC CT)	
N0	1 (1.92%)
N1	4 (7.69%)
N2	41 (78.85%)
N3	6 (11.54%)
TNM stage (pre-NAC CT)	
III	48 (92.31%)
IVA	4 (7.69%)

Supplementary Table S3. Primer sequence used in this study.

PCR Primers		Enzyme
Gene	sequence	
β-Actin-F	5'-GGGAAATCGTGCGTGACATTAAG-3'	
β-Actin -R	5'-TGTGTTGGCGTACAGGTCTTTG-3'	
SOX13-F	5'- CTATGTCCCTCGTAGCCT-3’	
SOX13-R	5'- AGCACCACCAACTCCC -3’	
SCAF1-F	5'- CACCAACTAAACTGACCTCCG-3’	
SCAF1-R	5'- GGGCACACCATCAGCTTTCT-3’	
Cox7a2-F	5'-CTCGGAGGTAGTTCCGGTTC-3’	
Cox7a2-R	5'-TCTGCCCAATCTGACGAAGAG-3’	
NADK-F	5'-CACAATGGGCTGGGTGAGAA-3’	
NADK-R	5'-TGGACAGGTAGGAGGAGGG-3’	
NADK2-F	5'-GCTCTACAGTCCGGAAGAACC -3’	
NADK2-R	5'-GCATCCCAACAACGAGAACG -3’	
SCAF1-F (mice)	5'- GTTTAGCAGTTTCACGCAGAAG-3’	
SCAF1-R (mice)	5'-GGCAAATATGATAGGTGGTGCT-3’	
Primers for SCAF1 promoter construct:		
(-1443/+60) SCAF1 sense:	5’-TATAGATTCCGTAACGGGGCAGGGAG-3’	KpnI
(-926/+60) SCAF1:	5’-TATAGATTCCGCTATGGGGTCGGGCTTCTA -3’	KpnI
(-323/+60) SCAF1	5’-TATAGATTCCCTCCTGTTCCGCCACCC-3’	KpnI
(-103/+60) SCAF1:	5’- TATAGATTCCACGTCGCCGTGCTCGGTACT -3’	KpnI
Antisense:	5’- ATATAGATCTGACTCATCTTCTTCCTCCAT -3’	HindIII
Primers for SCAF1 promoter site-directed mutagenesis:		
binding site 3 mutation sense:	5’-GGGTCCTTAGGAGAAAATactcCTGGAGATGTGGACTCC-3’	
binding site 3 mutation antisense:	5’-GGAGTCCACATCTCCAGGagtaTTTTCTCCTAAGGACCC-3’	
binding site 2 mutation sense:	5’-CTGGAGTGCAATGGgaCGATCTTGGCCCAC -3’	
binding site 2 mutation antisense:	5’-GTGGGCCAAGATCGTccCATTGCACTCCAG -3’	
binding site 1 mutation sense:	5’-CCCATGTGTCCCATTacCTTCTGGGAAACCG -3’	
binding site 1 mutation antisense:	5’-CGGTTTCCCAGAAGGtaATGGGACACATGGG -3’	
Primers used for ChIP in the SCAF1 promoter:		
binding site 3 sense (-1287~-1277):	5’-GGGTCCTTAGGAGAAAA-3’	
binding site 3 antisense (-1287~-1277):	5’-CCGGCTCCCTCCTTTA-3’	
binding site 2 sense (-840~-830):	5’-GGAGTTTCCGTCTTGTAG-3’	
binding site 2 antisense	5’-CGGGAGGCTGAGGTAG-3’	

(-840～830):	
binding site 1 sense (-153～143):	5’-GCGACAGAGGCGGTGC-3’
binding site 1 antisense (-153～143):	5’-ACGGCGACGTGAAGGC-3’
Vector construction	
shRNA-SOX13-1	5’- CCGGCCTGCAAACCAGTGGAGTATC CTCGAGGATACTCCACTGGTTTGCAGGTTTTTTG -3’
shRNA-SOX13-2	5’- CCGGACCTCAGCCTTTAGGGCTTATCT CGAGATAAGCCCTAAAGGCTGAGGTTTTTTTG -3’
siRNA-NADK-1	5’- UGAAUGAGGUGGUGAUUGA-3’
siRNA-NADK-2	5’- CGCCAGCGAUGAAAGCUUU-3’
siRNA-NADK-3	5’- GAAGACGGCGUGCACAAU-3’
siRNA-NADK2-1	5’- GCAAUUGCUUCGAUGAUGA-3’
siRNA-NADK2-2	5’- GAGAAUUGGUAGAGAAAGU-3’
siRNA-NADK2-3	5’- UGUGAAAGCUGGACGGAUA-3’
shRNA-NC	5’- CAACAAGATGAAGAGCACCAA -3'
Lv-SOX13-F	5’-TATAAAGCTTCTGAGTAGATGTCCATGAGG-3’
Lv-SOX13-R	5’-ATATTCTAGAGGATCAGTCTGTGAGCACCA-3’
sg-SCAF1(human)	5’-GGTGTGGCAAATATGATAGG-3’
sg-SCAF1(mice)	5’-GGTGACGATAGCCCCCGCC-3’

Supplementary Table S4. Information on antibodies.

Antibody	IHC	WB	CHIP	Specificity	Company (catalog number)
SOX13	1:100	1:1000	1:50	Rabbit polyclonal	proteintech (18902-1-AP)
COX7A2L/SCAF1	1:100	1:1000	/	Rabbit Polyclonal	proteintech (11416-1-AP)
NADK	/	1:1000	/	Rabbit polyclonal	Abcam (ab233261)
NADK2	/	1:1000	/	Rabbit monoclonal	Abcam (ab181028)
ACSL4	/	1:1000	/	Rabbit monoclonal	Abcam (ab155282)
SLC7A11	/	1:1000	/	Rabbit monoclonal	Abcam (ab175186)
GPX4	/	1:1000	/	Rabbit monoclonal	Abcam (ab125066)
E-cadherin	/	1:800	/	Mouse monoclonal	Abcam (ab1416)
Vimentin	/	1:1000	/	Rabbit monoclonal	Abcam (ab92547)
SDHA	/	1:1000	/	Mouse monoclonal	Abcam (ab14715)
MTCO1	/	1:1000	/	Mouse monoclonal	Abcam (ab14705)
NDUFS2	/	1:1000	/	Mouse monoclonal	Abcam (ab110249)
UQCRC2	/	1:1000	/	Mouse monoclonal	Abcam (ab14745)
Trim25	/	1:1000	/	Rabbit monoclonal	Abcam (ab167154)
FBXO28	/	1:800		Rabbit polyclonal	Abcam (ab154068)
TRAF2	/	1:1000		Rabbit monoclonal	Abcam (ab126758)
β-Actin	/	1:2000	/	Rabbit polyclonal	Abcam (ab8227)

Supplementary Table S5. Correlation between SOX13/SCAF1 immunohistochemical scores in 109 GC samples or normal tissue samples. Statistical significance is determined by Pearson correlation analysis.

Tumor	r=0.406	p<0.001
Normal	r=0.162	p=0.214

Supplementary Table S6. Correlation between SOX13/SCAF1 coexpression values and Tumor regression Grade. Statistical significance is determined by the nonparametric Mann-Whitney Wilcoxon test.

SOX13/SCAF1 coexpression value	Tumor Regression Grade		p
	≤2	≥3	
Low	22	5	0.006
High	11	14	

Supplementary **Table S7.** Correlation between 4-HNE staining and Tumor regression Grade. Statistical significance is determined by the nonparametric Mann-Whitney Wilcoxon test.

4-HNE staining	Tumor Regression Grade		p
	⩽2	⩾3	
Low	10	16	0.003
High	23	3	

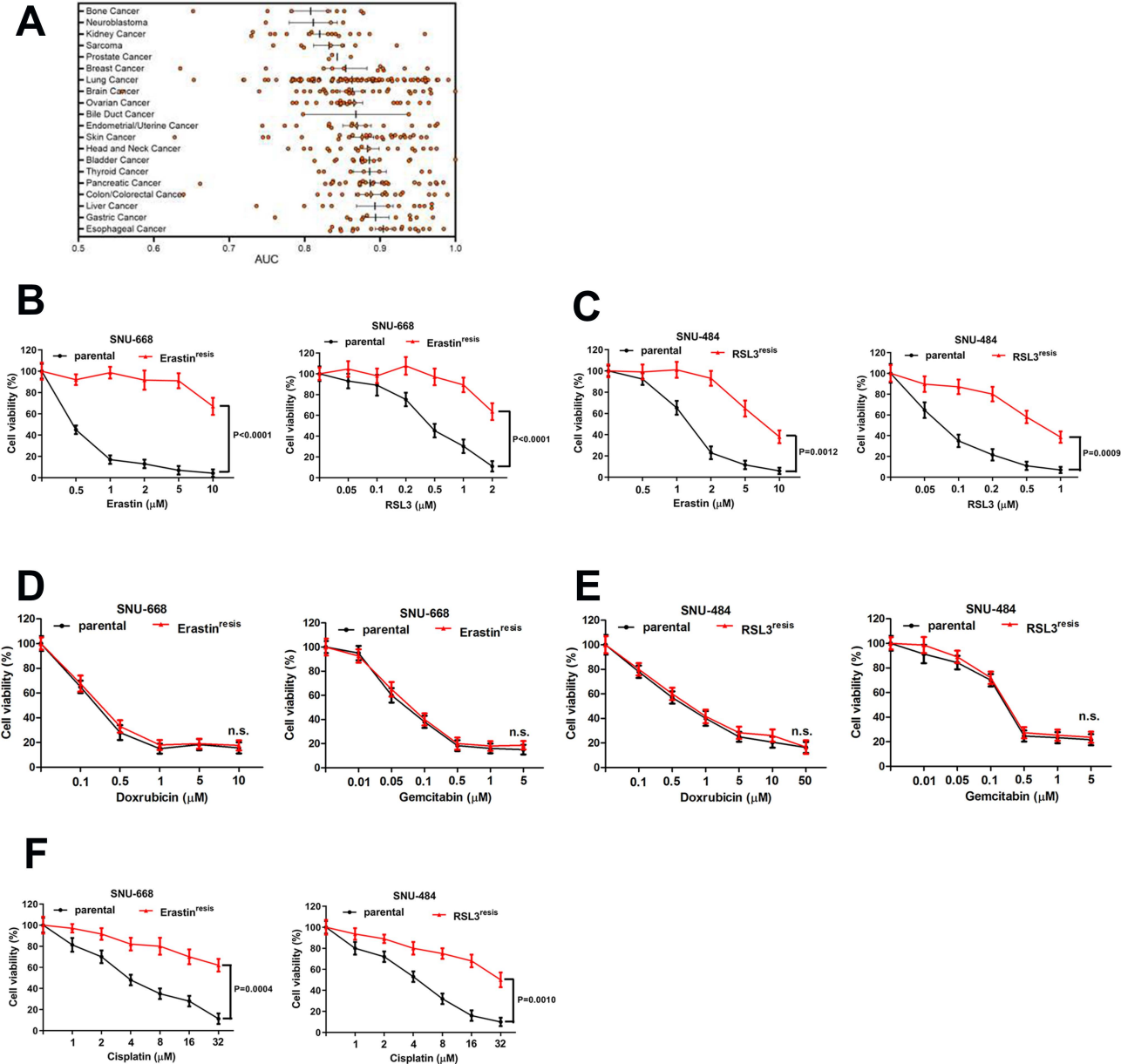
Supplementary Table S8. Correlation between 4-HNE staining and SOX13/SCAF1 IHC scores. Statistical significance is determined by the nonparametric Mann-Whitney Wilcoxon test.

4-HNE staining	SOX13/SCAF1 IHC scores		p
	Low	High	
Low	9	17	0.025
High	18	8	

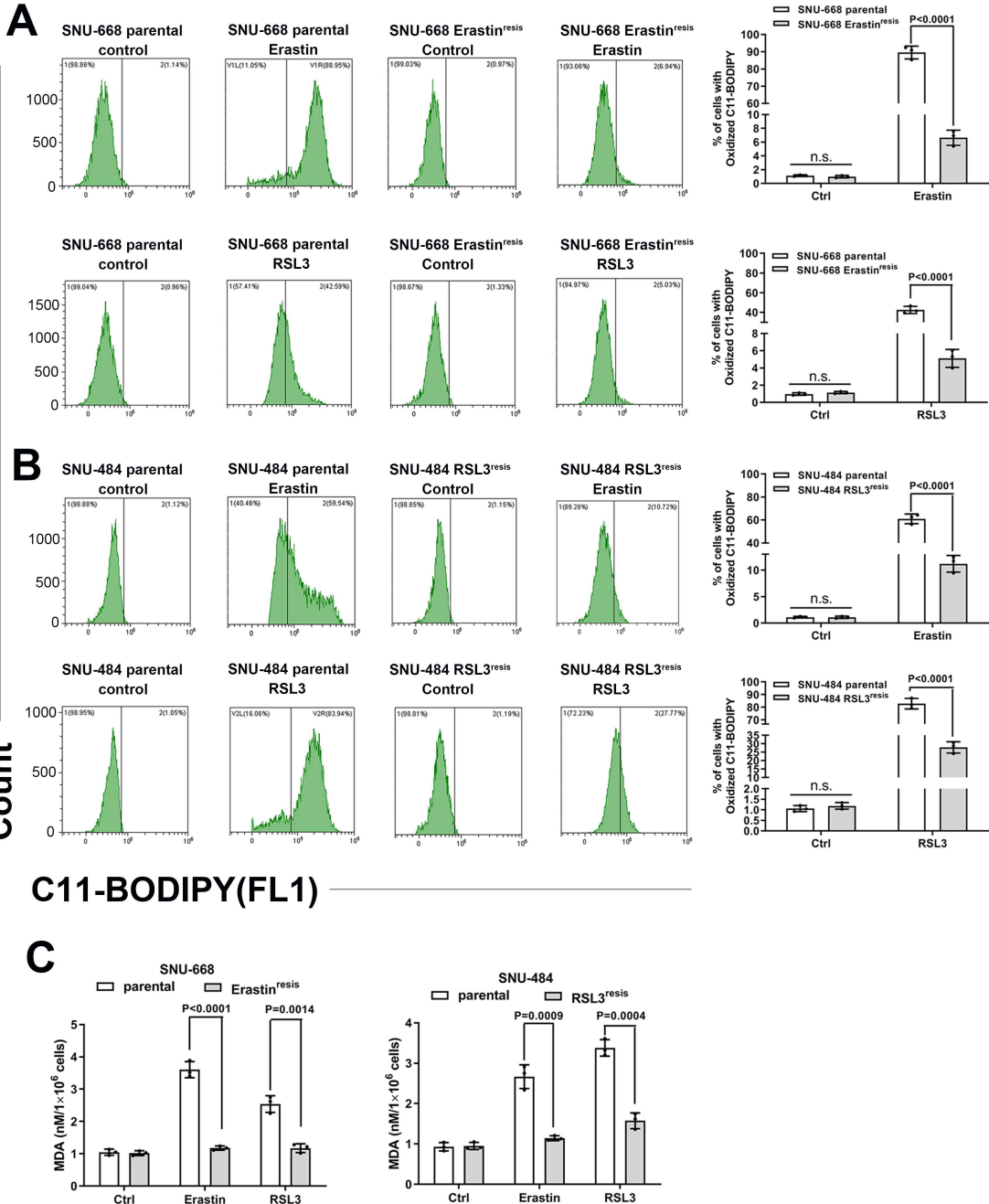
Supplementary Table S9. The 40 compounds with the highest scores.

Number	Catalog Number	Docking score	Number	Catalog Number	Docking score
1	T1085L	-13.589	21	T1352	-10.461
2	T1663	-12.578	22	T0278	-10.369
3	T0278	-12.344	23	T4987	-10.339
4	T5584	-12.303	24	T4726	-10.334
5	T6654	-12.100	25	T2720	-10.262
6	T4897	-11.943	26	T3795	-10.260
7	T4706	-11.742	27	T0148L	-10.222
8	TMA2440	-11.595	28	T0853L	-10.211
9	TMS1830	-11.543	29	T2133	-10.121
10	T4S1578	-11.419	30	T1723	-10.095
11	T2971	-11.405	31	T5022	-10.049
12	T0278	-11.388	32	T4718	-9.996
13	T2812	-11.266	33	T0966	-9.958
14	T2529	-11.163	34	T5075	-9.942
15	T4969	-11.006	35	T3910	-9.899
16	T1192	-10.940	36	T2727	-9.898
17	T5075	-10.852	37	T5022	-9.866
18	T4076	-10.699	38	T4901	-9.856

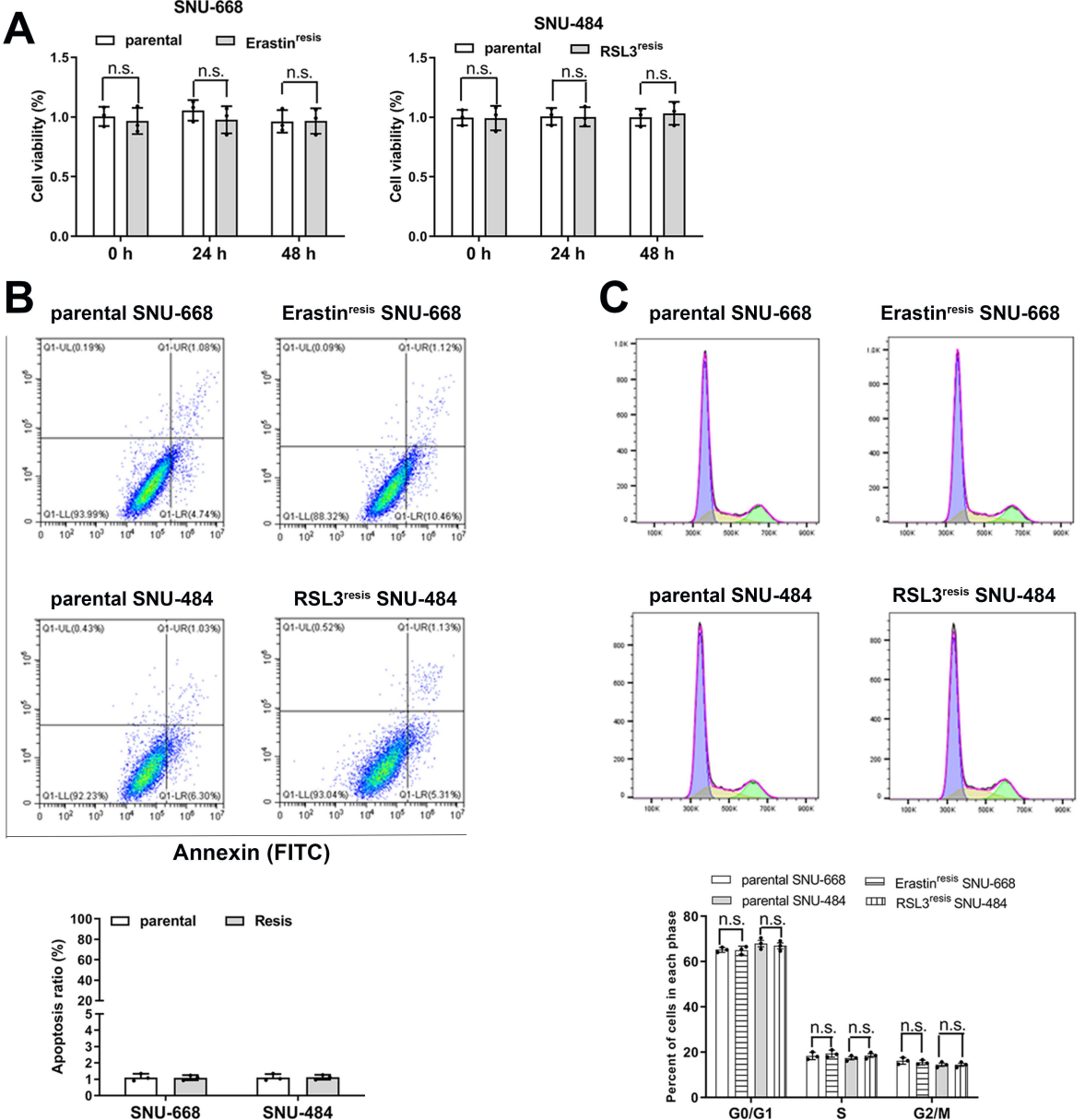
19	T1352	-10.521	39	T1658	-9.837
20	T2185	-10.465	40	T2001	-9.814


A
Bone Cancer
Neuroblastoma
Kidney Cancer
Sarcoma
Prostate Cancer
Breast Cancer
Lung Cancer
Brain Cancer
Ovarian Cancer
Bile Duct Cancer
Endometrial/Uterine Cancer
Skin Cancer
Head and Neck Cancer
Bladder Cancer
Thyroid Cancer
Pancreatic Cancer
Colon/Colorectal Cancer
Liver Cancer
Gastric Cancer
Esophageal Cancer
0.5 0.6 0.7 0.8 0.9 1.0
AUC
B
SNU-668
parental
Erastin^resis
Cell viability (%)
P<0.0001
Erastin (μM)
SNU-668
parental
Erastin^resis
Cell viability (%)
P<0.0001
RSL3 (μM)
C
SNU-484
parental
RSL3^resis
Cell viability (%)
P=0.0012
Erastin (μM)
SNU-484
parental
RSL3^resis
Cell viability (%)
P=0.0009
RSL3 (μM)
D
SNU-668
parental
Erastin^resis
Cell viability (%)
n.s.
Doxrubicin (μM)
SNU-668
parental
Erastin^resis
Cell viability (%)
n.s.
Gemcitabin (μM)
E
SNU-484
parental
RSL3^resis
Cell viability (%)
n.s.
Doxrubicin (μM)
SNU-484
parental
RSL3^resis
Cell viability (%)
n.s.
Gemcitabin (μM)
F
SNU-668
parental
Erastin^resis
Cell viability (%)
P=0.0004
Cisplatin (μM)
SNU-484
parental
RSL3^resis
Cell viability (%)
P=0.0010
Cisplatin (μM)

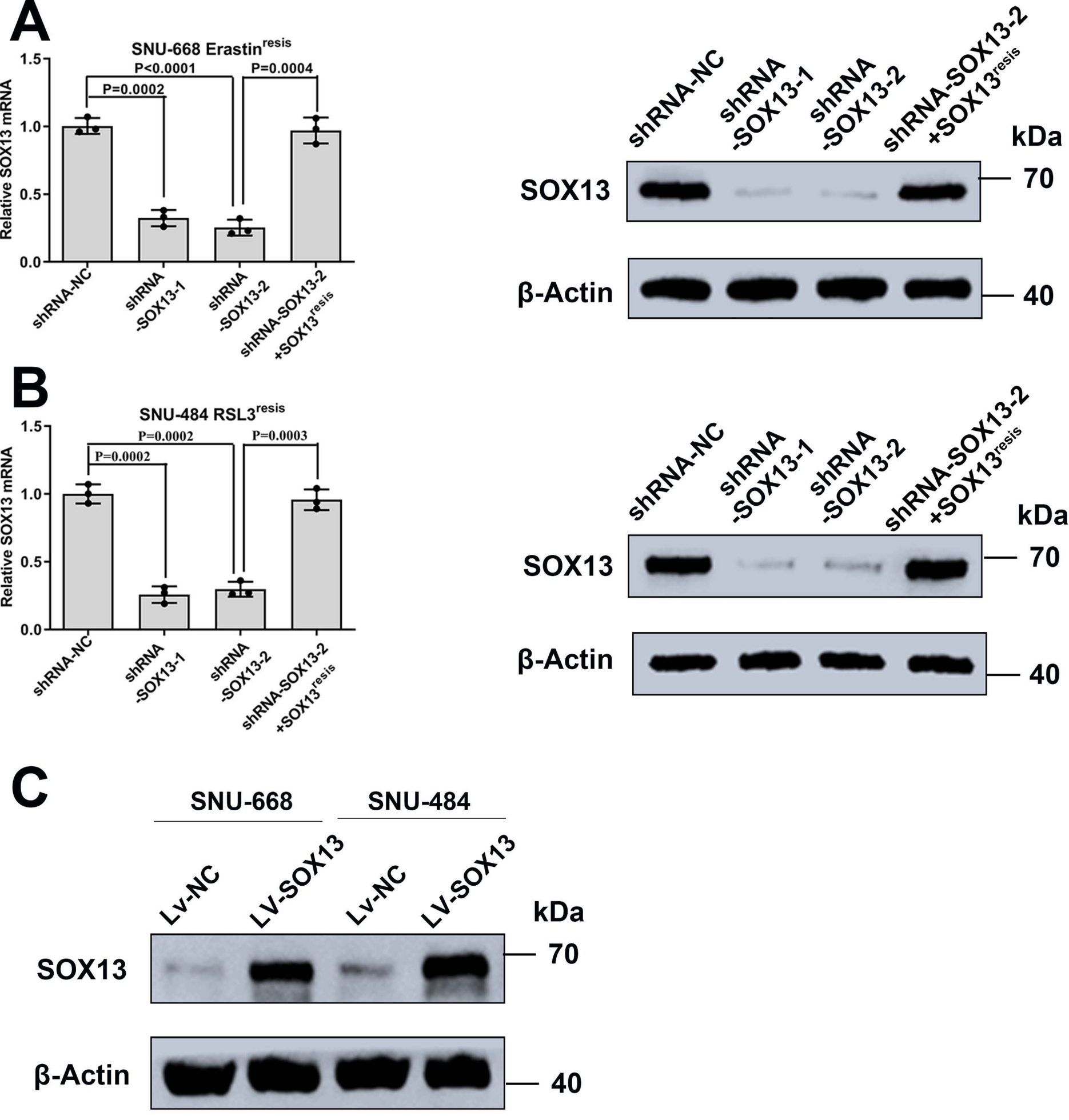
Supplementary Figure 1. (A) The sensitivity of 20 tumor types to Erastin was analyzed using the DepMap database. AUC = Area Under Curve. Each dot represents a cell line from indicated cancer type. Cell lines with higher AUC values are more resistant to Erastin. (B,C) Relative viability of parental or Erastin^{resis} SNU-668 cells (B) or RSL3^{resis} SNU-484 (C) cells treated with different concentrations of the ferroptosis inducers Erastin or RSL3 for 24 h (n=3 independent experiments). Data are presented as mean values ± SD. (D,E) Relative viability of parental or Erastin^{resis} SNU-668 cells (D) or RSL3^{resis} SNU-484 (E) cells treated with different concentrations of the apoptosis inducers doxrubicin and gemcitabine for 24 h (n=3 independent experiments). Data are presented as mean values ± SD. (F) Relative viability of parental or Erastin^{resis} SNU-668 cells or RSL3^{resis} SNU-484 cells treated with different concentrations of cisplatin for 24 h. Cell viability was evaluated with a CellTiter-Glo luminescent cell viability assay (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (B-F) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
SNU-668 parental control
SNU-668 parental Erastin
SNU-668 Erastin^resis Control
SNU-668 Erastin^resis Erastin
SNU-668 parental control
SNU-668 parental RSL3
SNU-668 Erastin^resis Control
SNU-668 Erastin^resis RSL3
SNU-668 parental
SNU-668 Erastin^resis
P<0.0001
% of cells with Oxidized C11-BODIPY
n.s.
Ctrl
Erastin
RSL3
B
SNU-484 parental control
SNU-484 parental Erastin
SNU-484 RSL3^resis Control
SNU-484 RSL3^resis Erastin
SNU-484 parental control
SNU-484 parental RSL3
SNU-484 RSL3^resis Control
SNU-484 RSL3^resis RSL3
SNU-484 parental
SNU-484 RSL3^resis
Count
C11-BODIPY(FL1)
C
SNU-668
parental
Erastin^resis
P<0.0001
P=0.0014
MDA (nM/1×10^6 cells)
Ctrl
Erastin
RSL3
SNU-484
parental
RSL3^resis
P=0.0009
P=0.0004

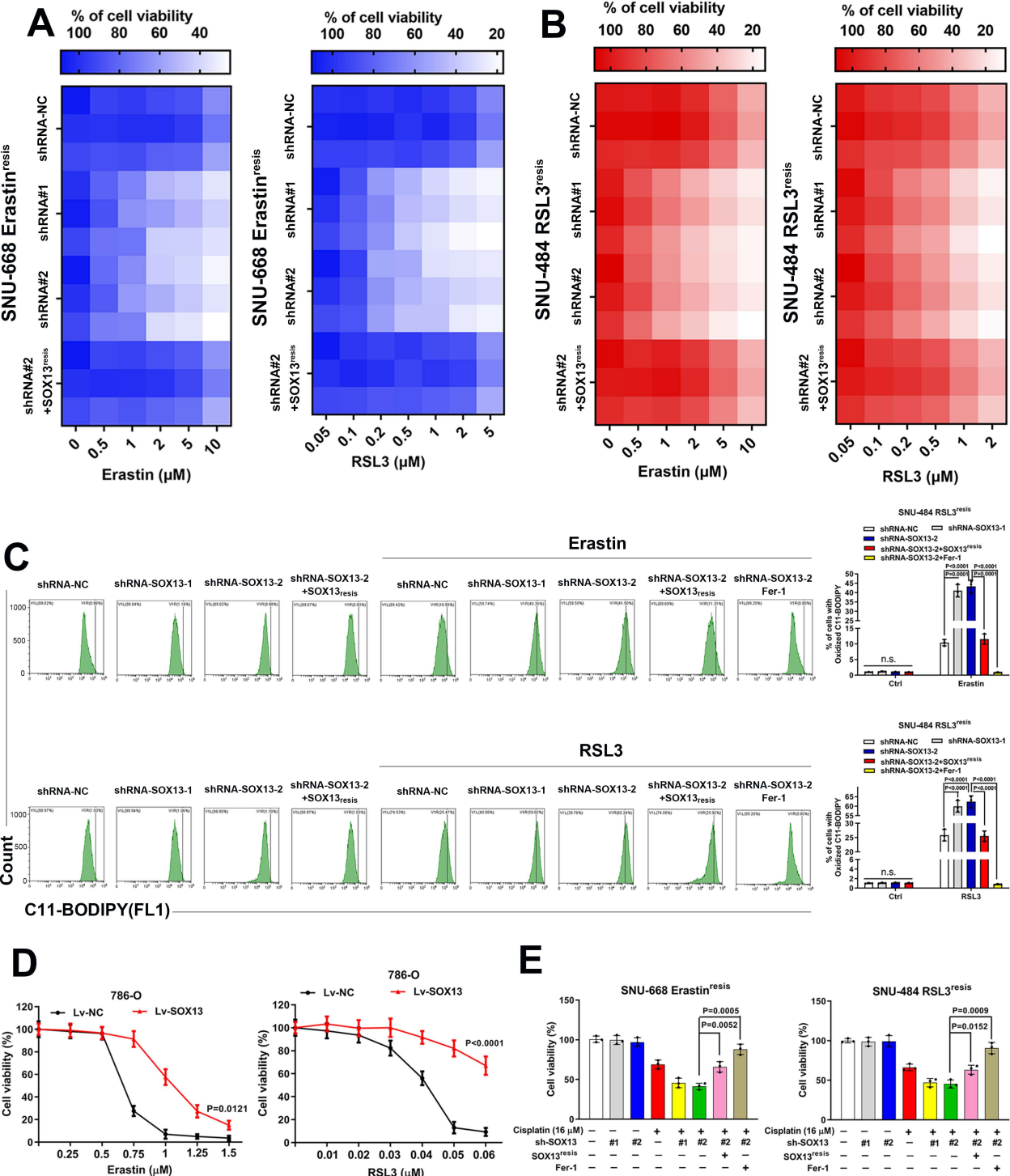
Supplementary Figure 2. (A-C) Parental or Erastin^{resis} SNU-668 cells or RSL3^{resis} SNU-484 cells treated with Erastin (0, 2 μM) or RSL3 (0, 0.5 μM) for 24 h (n=3 independent experiments). (A,B) Representative histogram plot for fluorescence of oxidized BODIPY-C11. (C) Intracellular MDA were assayed with ELISA. Data are presented as mean values ± SD. Statistical significance in (A-C) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
SNU-668
parental
Erastin^resis
Cell viability (%)
n.s.
0 h
24 h
48 h
SNU-484
parental
RSL3^resis
B
parental SNU-668
Erastin^resis SNU-668
parental SNU-484
RSL3^resis SNU-484
PI
Annexin (FITC)
C
parental SNU-668
Erastin^resis SNU-668
parental SNU-484
RSL3^resis SNU-484
parental
Resis
Apoptosis ratio (%)
SNU-668
SNU-484
Percent of cells in each phase
G0/G1
S
G2/M

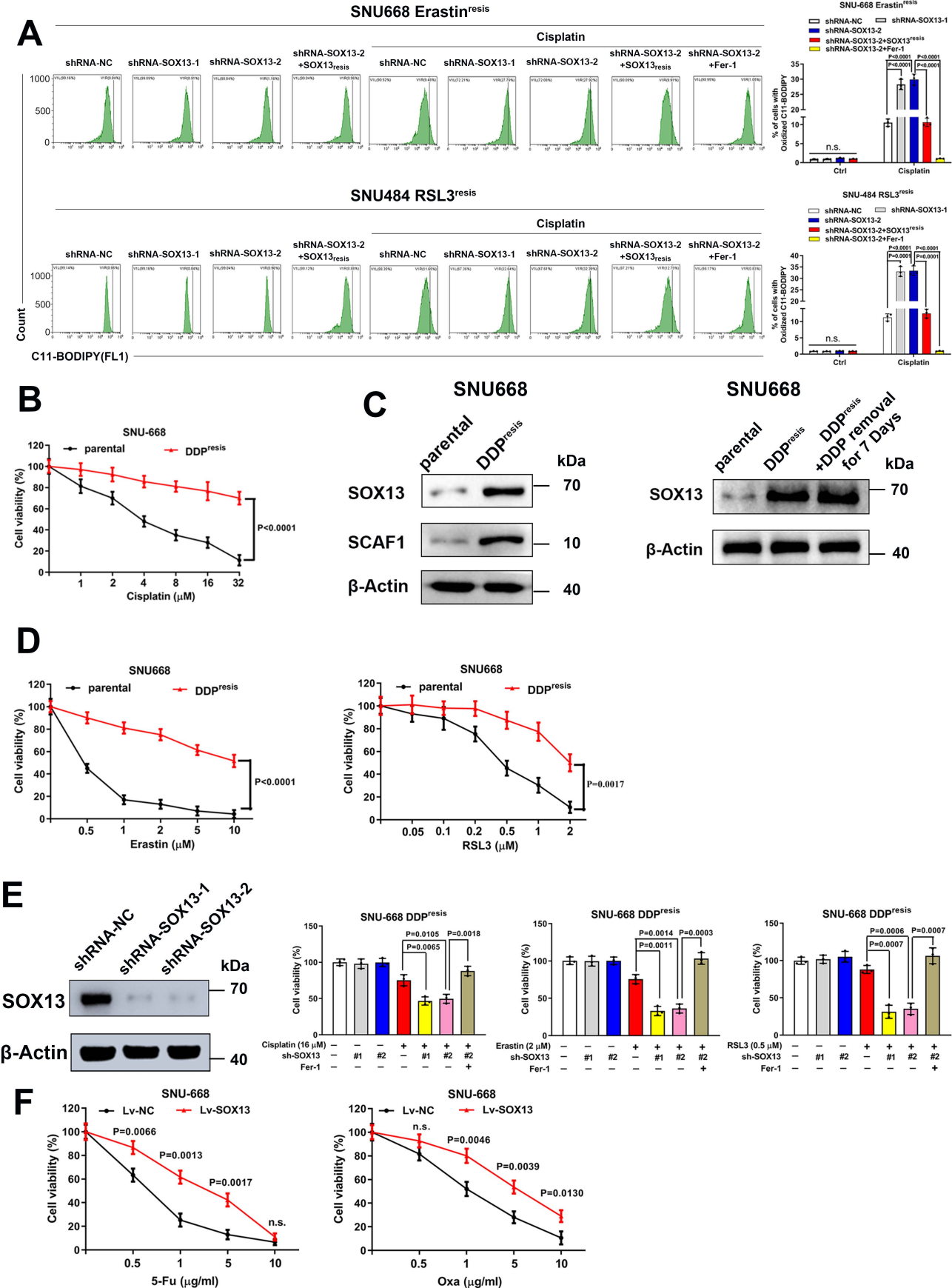
Supplementary Figure 3. (A) Relative viability of parental or Erastin^{resis} SNU-668 cells or RSL3^{resis} SNU-484 cells cultured after 24 h or 48 h (n=3 independent experiments). (B) Annexin V/PI staining and flow cytometry analysis assessing apoptosis in parental or Erastin^{resis} SNU-668 cells or RSL3^{resis} SNU-484 cells (n=3 independent experiments). (C) Cell cycle analysis in parental or Erastin^{resis} SNU-668 cells or RSL3^{resis} SNU-484 cells determined the relative cell numbers in each cell-cycle phase with propidium iodide staining (n=3 independent experiments). Statistical significance in (A-C) is determined by two-tailed unpaired *t*-test. Data are presented as mean values ± SD. Source data are provided as a Source Data file.


A
SNU-668 Erastin^resis
P<0.0001
P=0.0004
P=0.0002
Relative SOX13 mRNA
1.5
1.0
0.5
0.0
shRNA-NC
shRNA -SOX13-1
shRNA -SOX13-2
shRNA-SOX13-2 +SOX13^resis
shRNA-NC
shRNA -SOX13-1
shRNA -SOX13-2
shRNA-SOX13-2 +SOX13^resis
kDa
SOX13
70
β-Actin
40
B
SNU-484 RSL3^resis
P=0.0002
P=0.0003
P=0.0002
Relative SOX13 mRNA
1.5
1.0
0.5
0.0
shRNA-NC
shRNA -SOX13-1
shRNA -SOX13-2
shRNA-SOX13-2 +SOX13^resis
shRNA-NC
shRNA -SOX13-1
shRNA -SOX13-2
shRNA-SOX13-2 +SOX13^resis
kDa
SOX13
70
β-Actin
40
C
SNU-668
SNU-484
Lv-NC
LV-SOX13
Lv-NC
LV-SOX13
kDa
SOX13
70
β-Actin
40

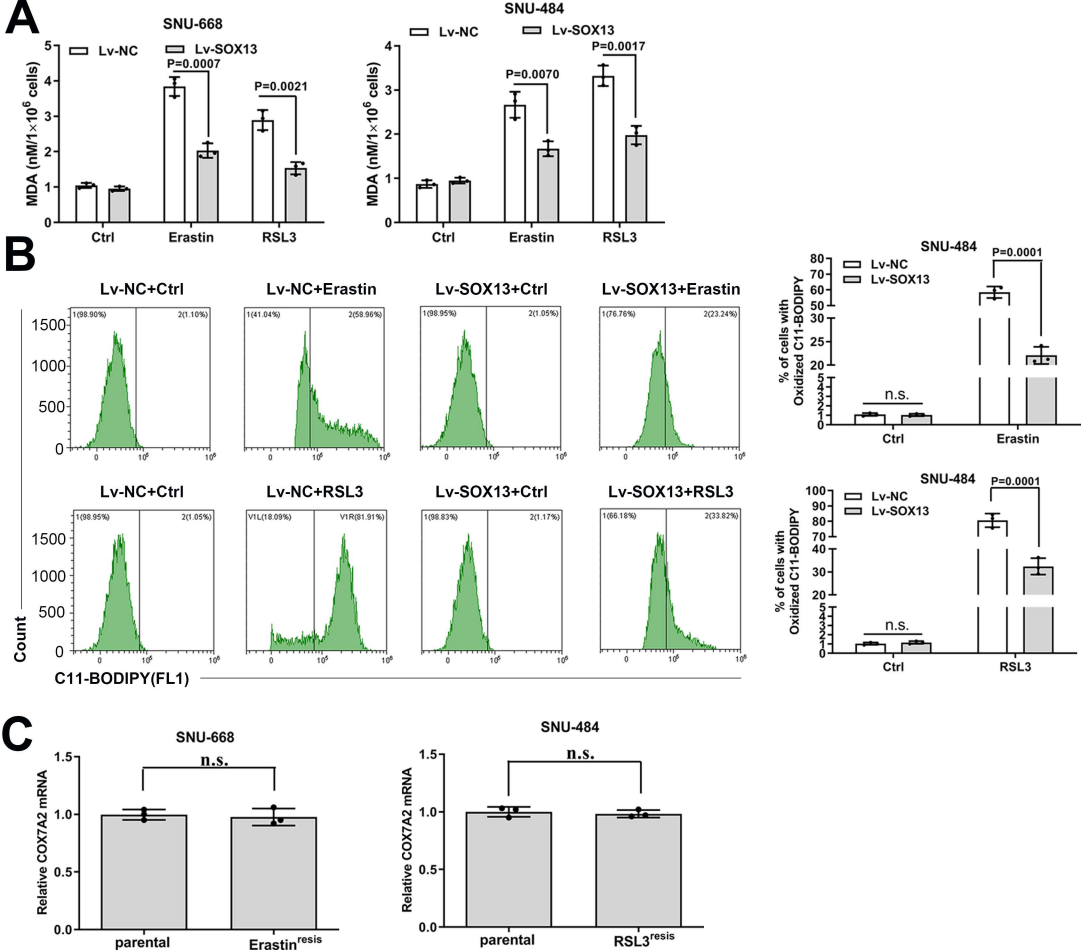
Supplementary Figure 4. SOX13 expression was detected in Erastin^{resis} SNU-668 cells (A) or RSL3^{resis} SNU-484 cells (B) by western blot after transduction of lentiviruses encoding SOX13 shRNA-1 or shRNA-2 or a scrambled shRNA and/or resistant SOX13 cDNA (SOX13^{resis}) (n=3 independent experiments). Data are presented as mean values ± SD. (C) SOX13 expression was detected in parental SNU-668 and SNU-484 cells by western blot after transfection of lentivirus harboring the human SOX13 sequence or the empty vector (n=3 independent experiments). Statistical significance in (A,B) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
% of cell viability
100 80 60 40
SNU-668 Erastin^resis
shRNA-NC
shRNA#1
shRNA#2
shRNA#2 +SOX13^resis
0 0.5 1 2 5 10
Erastin (μM)
% of cell viability
100 80 60 40 20
0.05 0.1 0.2 0.5 1 2 5
RSL3 (μM)
B
% of cell viability
100 80 60 40 20
SNU-484 RSL3^resis
0 0.5 1 2 5 10
Erastin (μM)
0.05 0.1 0.2 0.5 1 2
RSL3 (μM)
C
Erastin
RSL3
shRNA-NC
shRNA-SOX13-1
shRNA-SOX13-2
shRNA-SOX13-2 +SOX13resis
shRNA-SOX13-2 Fer-1
Count
C11-BODIPY(FL1)
SNU-484 RSL3^resis
% of cells with Oxidized C11-BODIPY
n.s.
P<0.0001
Ctrl
Erastin
RSL3
D
786-O
Lv-NC
Lv-SOX13
Cell viability (%)
P=0.0121
Erastin (μM)
P<0.0001
RSL3 (μM)
E
SNU-668 Erastin^resis
SNU-484 RSL3^resis
Cell viability (%)
P=0.0005
P=0.0052
P=0.0009
P=0.0152
Cisplatin (16 μM)
sh-SOX13
SOX13^resis
Fer-1

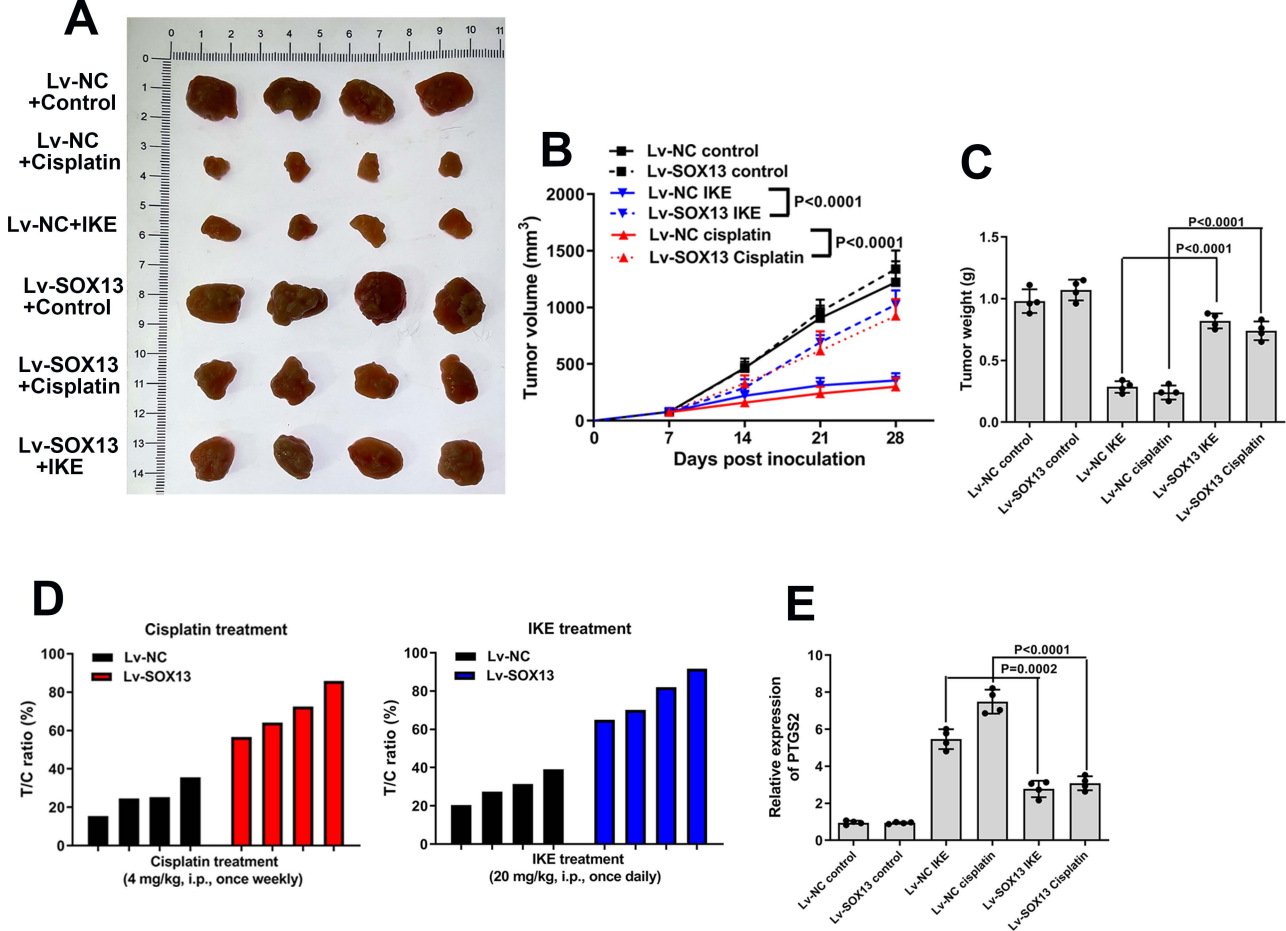
Supplementary Figure 5. (A-C) Effect of knockdown of SOX13 and SOX13 re-expression on sensitivity of Erastin^{resis} SNU-668 cells (A) or RSL3^{resis} SNU-484 cells (B) to the ferroptosis inducers RSL3 or Erastin. The heatmap shows altered cell viability. Cells were treated with lentiviruses encoding SOX13 shRNA-1 or shRNA-2 or a scrambled shRNA alone and/or resistant SOX13 cDNA (SOX13^{resis}), or in combination with various concentrations of RSL3 or Erastin for 24 hours. The heatmap shows altered cell viability. (C) SNU-484 RSL3^{resis} cells were treated with Erastin (2 μM) or RSL3 (0.5 μM) for 24 hours. Lipid peroxidation was determined with a lipid peroxidation C11-BODIPY assay in SNU-484 RSL3^{resis} cells (n=3 independent experiments), and a representative flow cytometry histogram plot is presented. Data are presented as mean values ± SD. (D) 786-O cells were treated with lentivirus harboring the human SOX13 sequence or the empty vector. Cells were treated with different concentrations of the ferroptosis inducers Erastin or RSL3 for 24 h (n=3 independent experiments). Data are presented as mean values ± SD. (E) Effect of SOX13 downregulation on cisplatin sensitivity in the absence or presence of Fer-1 (1 μM) in Erastin^{resis} SNU-668 cells or RSL3^{resis} SNU-484 cells (n=3 independent experiments). The cells were treated with cisplatin (16 μM) for 24 hours. Data are presented as mean values ± SD. Statistical significance in (C-E) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
SNU668 Erastin^resis
Cisplatin
shRNA-NC
shRNA-SOX13-1
shRNA-SOX13-2
shRNA-SOX13-2 +SOX13resis
shRNA-SOX13-2 +Fer-1
SNU-668 Erastin^resis
shRNA-SOX13-2+SOX13^resis
shRNA-SOX13-2+Fer-1
% of cells with Oxidized C11-BODIPY
n.s.
Ctrl
Cisplatin
SNU484 RSL3^resis
SNU-484 RSL3^resis
Count
C11-BODIPY(FL1)
B
SNU-668
parental
DDP^resis
Cell viability (%)
P<0.0001
Cisplatin (μM)
C
SNU668
kDa
SOX13
SCAF1
β-Actin
70
10
40
+DDP removal for 7 Days
D
Erastin (μM)
RSL3 (μM)
P=0.0017
E
SNU-668 DDP^resis
P=0.0105
P=0.0018
P=0.0065
P=0.0014
P=0.0003
P=0.0011
P=0.0006
P=0.0007
Cisplatin (16 μM)
Erastin (2 μM)
RSL3 (0.5 μM)
sh-SOX13
Fer-1
F
Lv-NC
Lv-SOX13
P=0.0066
P=0.0013
P=0.0017
P=0.0046
P=0.0039
P=0.0130
5-Fu (μg/ml)
Oxa (μg/ml)

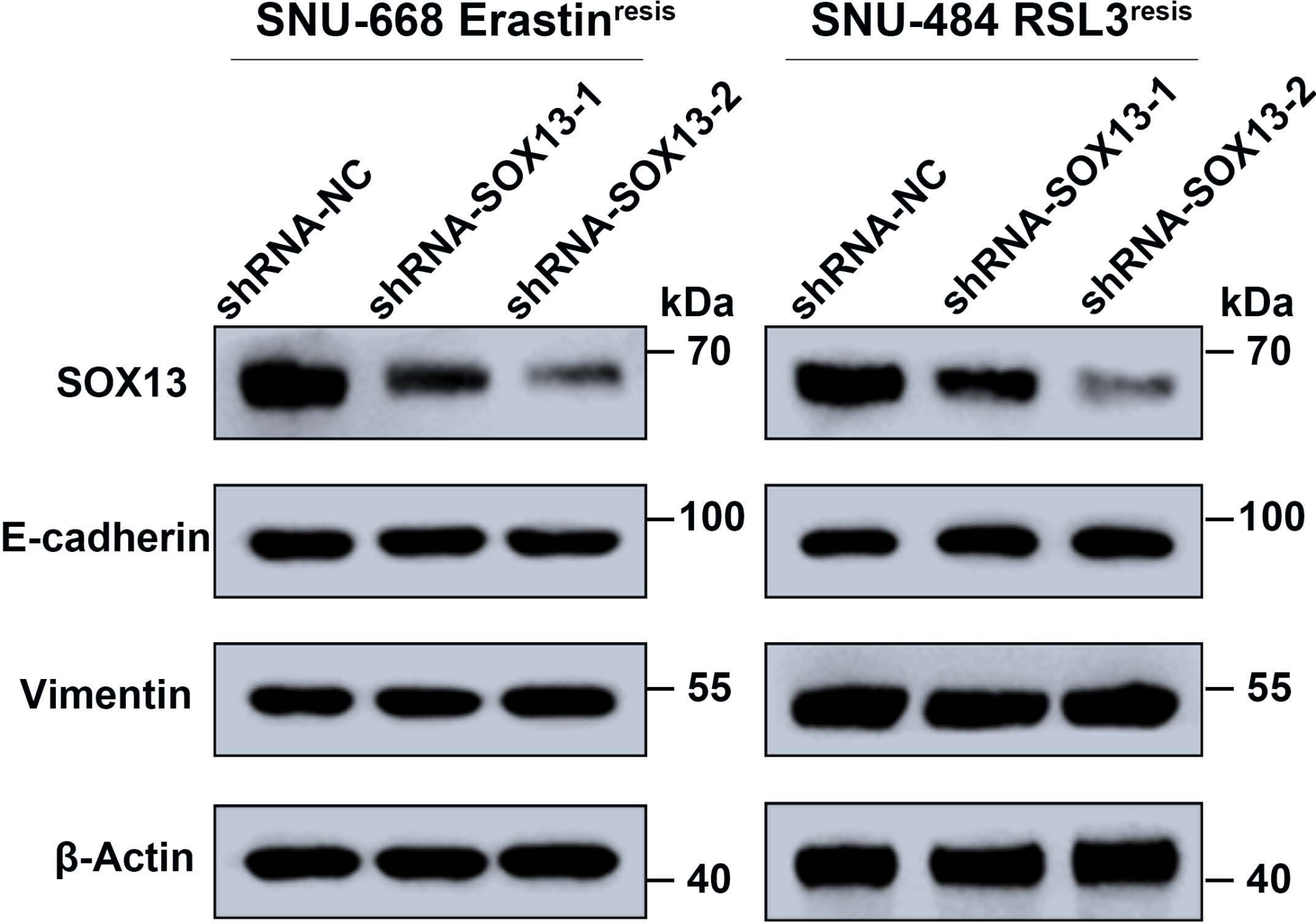
Supplementary Figure 6. (A) Effect of SOX13 downregulation and SOX13 re-expression on cisplatin (16 μM) sensitivity in the absence or presence of Fer-1 (1 μM) in SNU-668 Erastin^{resis} and SNU-484 RSL3^{resis} cells. Cells were treated with cisplatin (16 μM) for 24 hours. Lipid peroxidation was determined with a lipid peroxidation C11-BODIPY assay (n=3 independent experiments) and a representative flow cytometry histogram plot is presented. Data are presented as mean values ± SD. (B) Relative viability of parental or DDP^{resis} SNU-668 cells treated with different concentrations of cisplatin for 24 h (n=3 independent experiments). Data are presented as mean values ± SD. (C) SOX13 and SCAF1 were determined by immunoblotting assay and normalized to β-actin (n=3 independent experiments). (D) Relative viability of parental or DDP^{resis} SNU-668 cells treated with different concentrations of Erastin or RSL3 for 24 h (n=3 independent experiments). Data are presented as mean values ± SD. (E) Effect of SOX13 downregulation on Erastin (2 μM), RSL3 (0.5 μM) or Cisplatin (16 μM) sensitivity in the absence or presence of Fer-1 (1 μM) in DDP^{resis} SNU-668 cells (n=3 independent experiments). The cells were treated with FINs or cisplatin for 24 hours. Data are presented as mean values ± SD. (F) SNU-668 cells were treated with lentivirus harboring the human SOX13 sequence or the empty vector. Cells were treated with different concentrations of 5-Fu or oxaliplatin for 24 h. Cell viability was evaluated with a CellTiter-Glo luminescent cell viability assay (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (A,B,D-F) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
SNU-668
Lv-NC
Lv-SOX13
P=0.0007
P=0.0021
MDA (nM/1×10^6 cells)
Ctrl
Erastin
RSL3
SNU-484
Lv-NC
Lv-SOX13
P=0.0070
P=0.0017
MDA (nM/1×10^6 cells)
Ctrl
Erastin
RSL3
B
Lv-NC+Ctrl
Lv-NC+Erastin
Lv-SOX13+Ctrl
Lv-SOX13+Erastin
Lv-NC+Ctrl
Lv-NC+RSL3
Lv-SOX13+Ctrl
Lv-SOX13+RSL3
Count
C11-BODIPY(FL1)
SNU-484
Lv-NC
Lv-SOX13
P=0.0001
% of cells with Oxidized C11-BODIPY
n.s.
Ctrl
Erastin
SNU-484
Lv-NC
Lv-SOX13
P=0.0001
% of cells with Oxidized C11-BODIPY
n.s.
Ctrl
RSL3
C
SNU-668
n.s.
Relative COX7A2 mRNA
parental
Erastin^resis
SNU-484
n.s.
Relative COX7A2 mRNA
parental
RSL3^resis

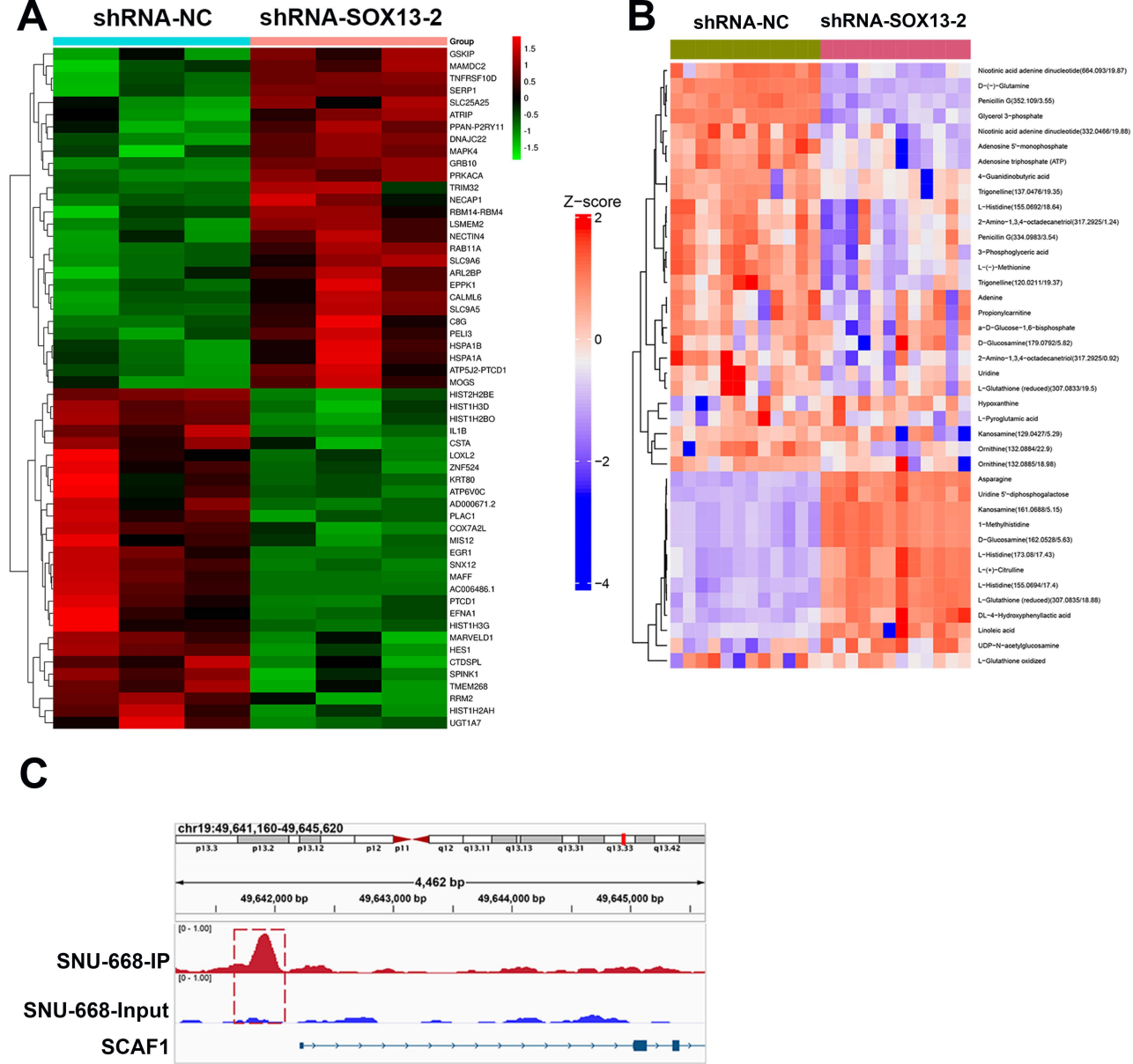
Supplementary Figure 7. (A,B) SNU-668/SNU-484 Cells were treated with lentivirus harboring the human SOX13 sequence or the empty vector, or in combination with Erastin (2 μM) or RSL3 (0.5 μM) for 24 hours. (A) ELISA was utilized to assess the intracellular MDA level (n=3 independent experiments). Data are presented as mean values ± SD. (B) Lipid peroxidation was determined with a lipid peroxidation C11-BODIPY assay (n=3 independent experiments), and a representative flow cytometry histogram plot is presented. Data are presented as mean values ± SD. (C) COX7A2 mRNA abundance in resistant versus parental cell lines of SNU-668 and SNU-484 (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (A-C) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
Lv-NC +Control
Lv-NC +Cisplatin
Lv-NC+IKE
Lv-SOX13 +Control
Lv-SOX13 +Cisplatin
Lv-SOX13 +IKE
B
Lv-NC control
Lv-SOX13 control
Lv-NC IKE
Lv-SOX13 IKE
P<0.0001
Lv-NC cisplatin
Lv-SOX13 Cisplatin
P<0.0001
Tumor volume (mm3)
Days post inoculation
C
P<0.0001
P<0.0001
Tumor weight (g)
Lv-NC control
Lv-SOX13 control
Lv-NC IKE
Lv-NC cisplatin
Lv-SOX13 IKE
Lv-SOX13 Cisplatin
D
Cisplatin treatment
Lv-NC
Lv-SOX13
T/C ratio (%)
Cisplatin treatment
(4 mg/kg, i.p., once weekly)
IKE treatment
Lv-NC
Lv-SOX13
T/C ratio (%)
IKE treatment
(20 mg/kg, i.p., once daily)
E
P<0.0001
P=0.0002
Relative expression of PTGS2
Lv-NC control
Lv-SOX13 control
Lv-NC IKE
Lv-NC cisplatin
Lv-SOX13 IKE
Lv-SOX13 Cisplatin

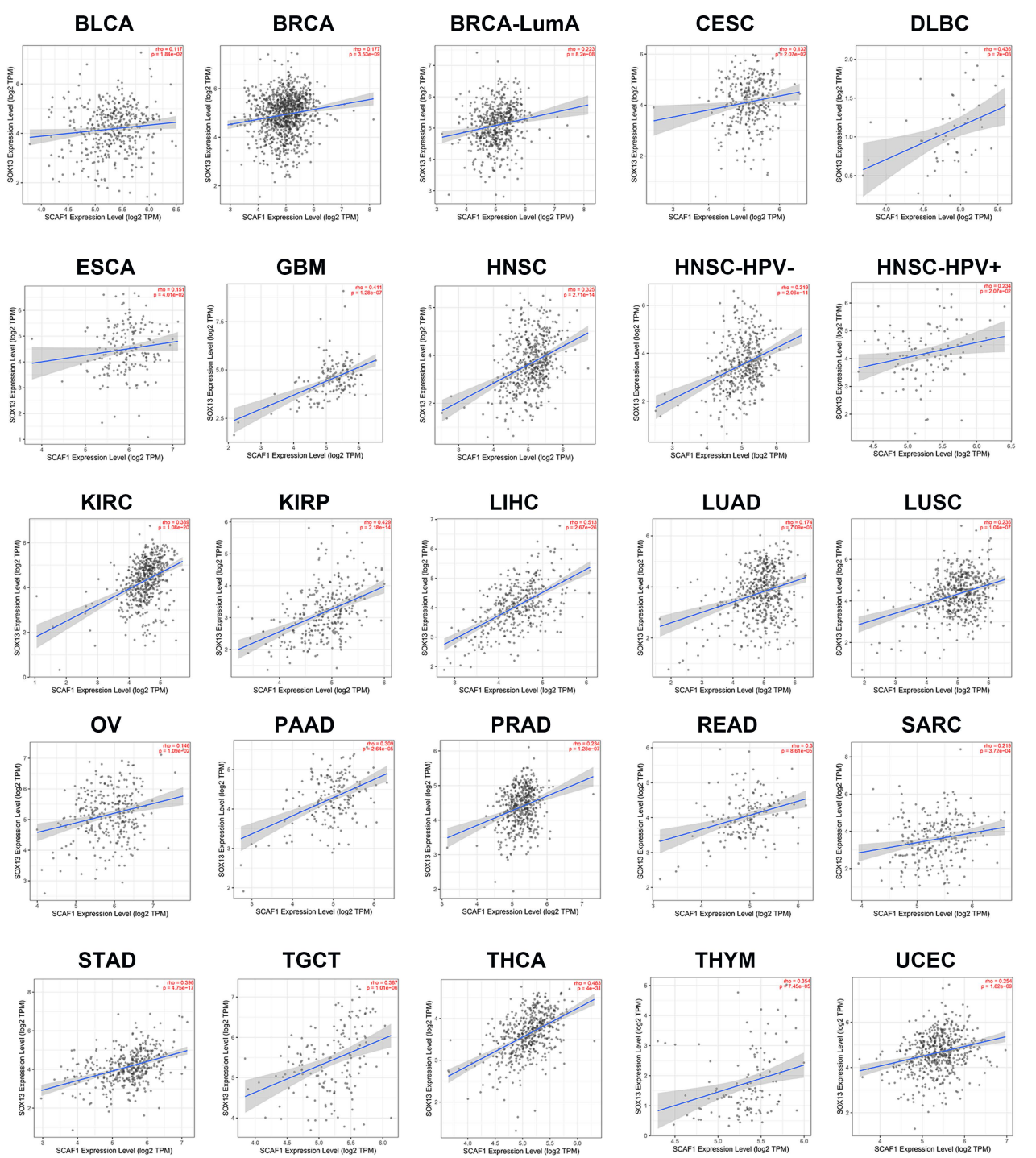
Supplementary Figure 8. SNU-484-SOX13-CDX tumors and SNU-484-NC-CDX tumors were treated with cisplatin and IKE. Representative images of tumors formed (A), tumor growth curves (B), tumor weights (C), T/C ratio (D) and PTGS2 (E) expression analysis are shown. Treatment with IKE (20 mg/kg, i.p., once daily), cisplatin (4 mg/kg, i.p., once weekly) or PBS (100 μl, i.p., once daily) started on Day 7 and lasted for 3 consecutive weeks (n=4 mice per group). T/C% = $T_{RTV}/C_{RTV} \times 100\%$; T_{RTV}, relative tumor volume after treatment; C_{RTV}, relative tumor volume of control group. Data are presented as mean values ± SD. Statistical significance in (B,C,E) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


SNU-668 Erastin^resis
SNU-484 RSL3^resis
shRNA-NC
shRNA-SOX13-1
shRNA-SOX13-2
shRNA-NC
shRNA-SOX13-1
shRNA-SOX13-2
kDa
kDa
SOX13
70
70
E-cadherin
100
100
Vimentin
55
55
β-Actin
40
40

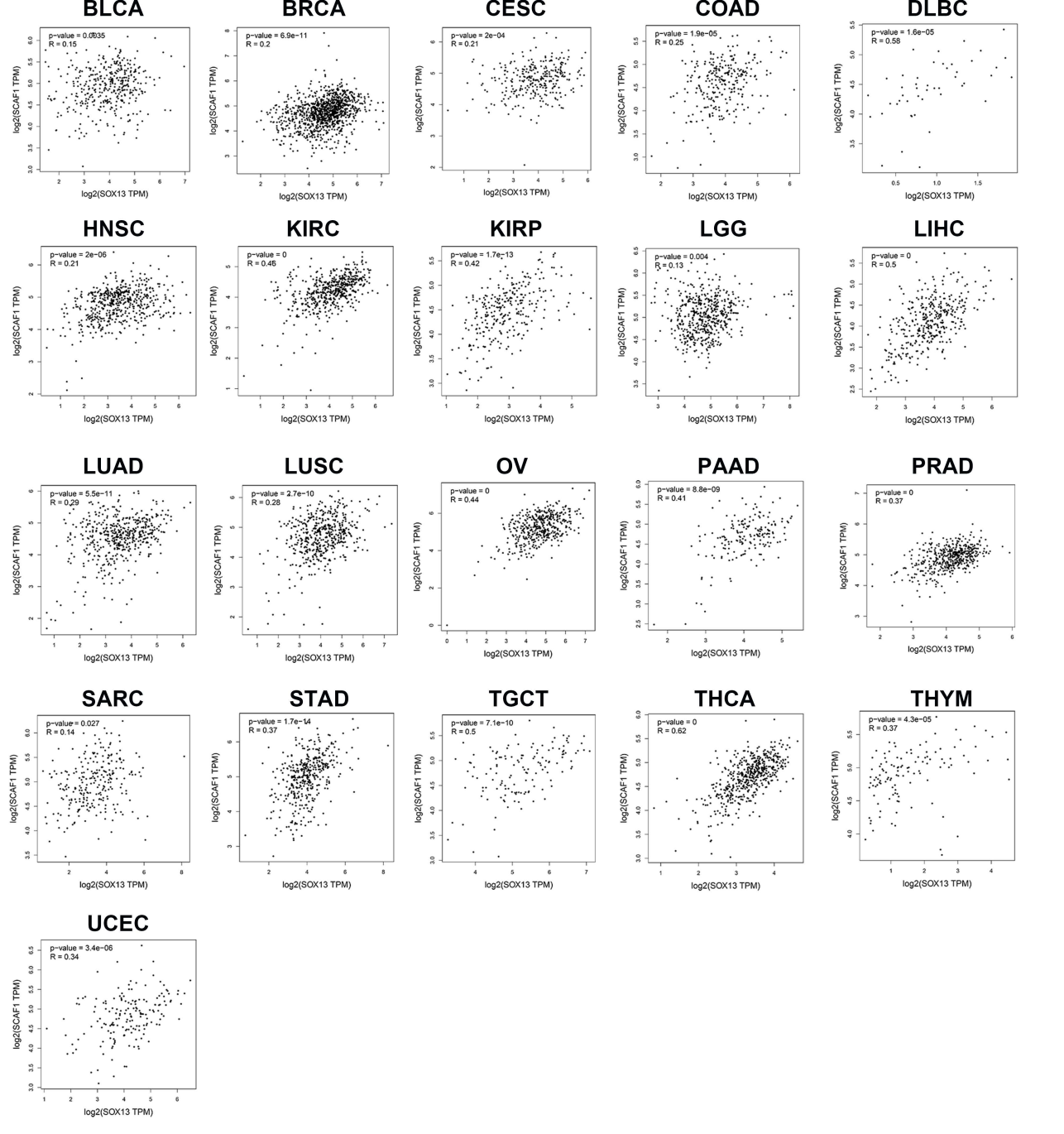
Supplementary Figure 9. Effect of SOX13 knockdown on E-cadherin and Viementin protein abundance in FINs resistant cells (n=3 independent experiments).


A
shRNA-NC
shRNA-SOX13-2
Group
GSKIP
MAMDC2
TNFRSF10D
SERP1
SLC25A25
ATRIP
PPAN-P2RY11
DNAJC22
MAPK4
GRB10
PRKACA
TRIM32
NECAP1
RBM14-RBM4
LSMEM2
NECTIN4
RAB11A
SLC9A6
ARL2BP
EPPK1
CALML6
SLC9A5
C8G
PELI3
HSPA1B
HSPA1A
ATP5J2-PTCD1
MOGS
HIST2H2BE
HIST1H3D
HIST1H2BO
IL1B
CSTA
LOXL2
ZNF524
KRT80
ATP6V0C
AD000671.2
PLAC1
COX7A2L
MIS12
EGR1
SNX12
MAFF
AC006486.1
PTCD1
EFNA1
HIST1H3G
MARVELD1
HES1
CTDSPL
SPINK1
TMEM268
RRM2
HIST1H2AH
UGT1A7
1.5
1
0.5
0
-0.5
-1
-1.5
Z-score
2
0
−2
−4
B
shRNA-NC
shRNA-SOX13-2
Nicotinic acid adenine dinucleotide(664.093/19.87)
D−(−)−Glutamine
Penicillin G(352.109/3.55)
Glycerol 3−phosphate
Nicotinic acid adenine dinucleotide(332.0466/19.88)
Adenosine 5''−monophosphate
Adenosine triphosphate (ATP)
4−Guanidinobutyric acid
Trigonelline(137.0476/19.35)
L−Histidine(155.0692/18.64)
2−Amino−1,3,4−octadecanetriol(317.2925/1.24)
Penicillin G(334.0983/3.54)
3−Phosphoglyceric acid
L−(−)−Methionine
Trigonelline(120.0211/19.37)
Adenine
Propionylcarnitine
a−D−Glucose−1,6−bisphosphate
D−Glucosamine(179.0792/5.82)
2−Amino−1,3,4−octadecanetriol(317.2925/0.92)
Uridine
L−Glutathione (reduced)(307.0833/19.5)
Hypoxanthine
L−Pyroglutamic acid
Kanosamine(129.0427/5.29)
Ornithine(132.0884/22.9)
Ornithine(132.0885/18.98)
Asparagine
Uridine 5''−diphosphogalactose
Kanosamine(161.0688/5.15)
1−Methylhistidine
D−Glucosamine(162.0528/5.63)
L−Histidine(173.08/17.43)
L−(+)−Citrulline
L−Histidine(155.0694/17.4)
L−Glutathione (reduced)(307.0835/18.88)
DL−4−Hydroxyphenyllactic acid
Linoleic acid
UDP−N−acetylglucosamine
L−Glutathione oxidized
C
chr19:49,641,160-49,645,620
p13.3
p13.2
p13.12
p12
p11
q12
q13.11
q13.13
q13.31
q13.33
q13.42
4,462 bp
49,642,000 bp
49,643,000 bp
49,644,000 bp
49,645,000 bp
[0 - 1.00]
SNU-668-IP
[0 - 1.00]
SNU-668-Input
SCAF1

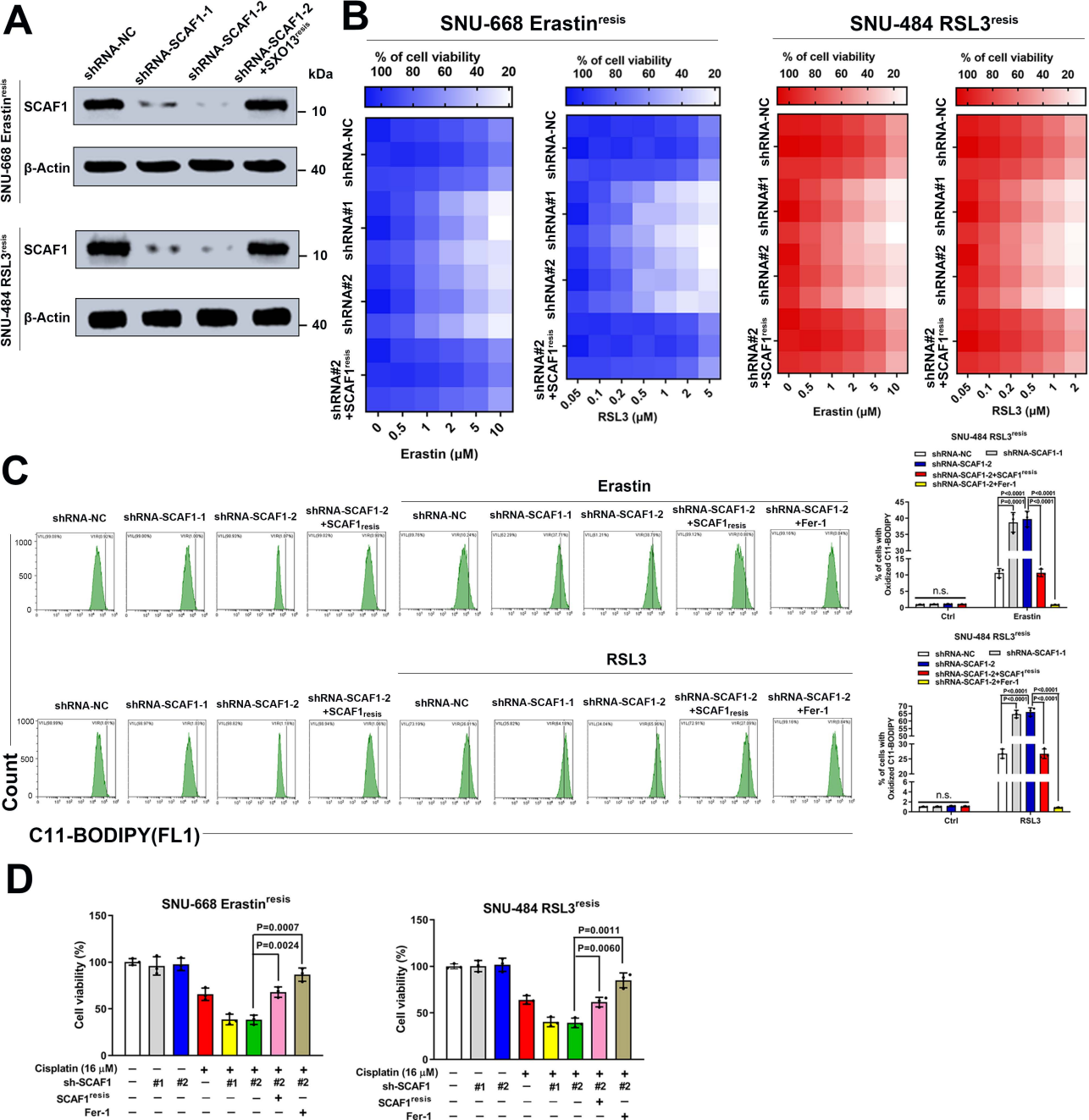
Supplementary Figure 10. (A) Heatmap of the top 56 dysregulated genes (>3-fold; $p < 0.05$) in response to SOX13 knockdown (n=3 independent experiments). (B) Metabolic profile changes from control to SOX13-downregulated Erastin^{resis} SNU-668 cells (n= 12 independent experiments). (C) Binding profiles and peak calling records of SOX13 in the SCAF1 promoter. Significant peak ($P < 1 \times 10^{-11}$) is highlighted by a red box with dotted line.


BLCA
BRCA
BRCA-LumA
CESC
DLBC
ESCA
GBM
HNSC
HNSC-HPV-
HNSC-HPV+
KIRC
KIRP
LIHC
LUAD
LUSC
OV
PAAD
PRAD
READ
SARC
STAD
TGCT
THCA
THYM
UCEC
SOX13 Expression Level (log2 TPM)
SCAF1 Expression Level (log2 TPM)

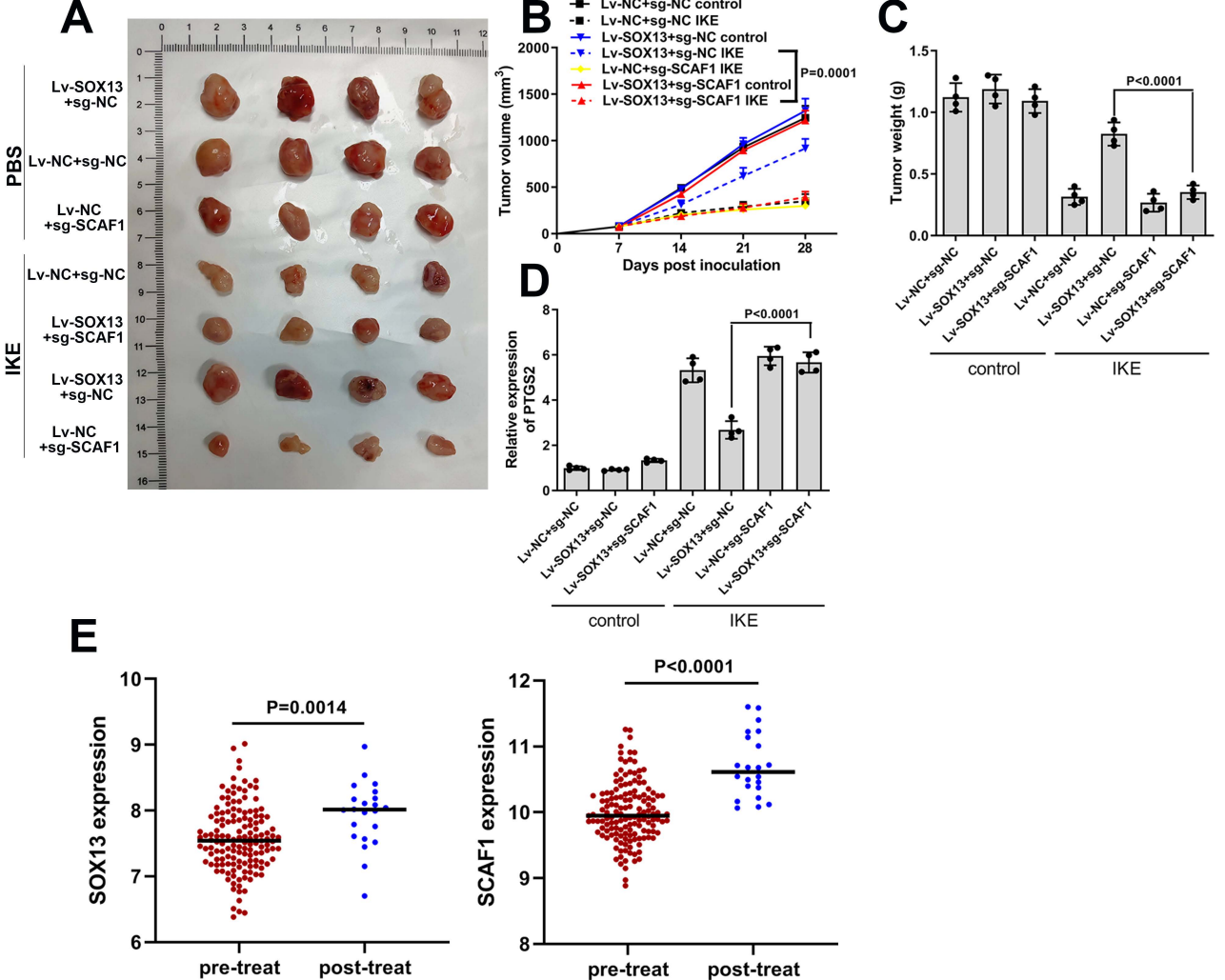
Supplementary Figure 11. Scatterplots of correlations between SOX13 expression and SCAF1 expression adjusted by purity according to TIMER (http://cistrome.org/TIMER/) in BLCA (bladder urothelial carcinoma, n=408), BRCA (breast invasive carcinoma, n=1100), BRCA-LumA (breast invasive carcinoma luminal A type, n=568), CESC (Cervical squamous cell carcinoma and endocervical adenocarcinoma, n=306), DLBC (Lymphoid Neoplasm Diffuse Large B-cell Lymphoma, n=48), ESCA (Esophageal carcinoma, n=185), GBM (Glioblastoma multiforme, n=153), HNSC (Head and Neck squamous cell carcinoma, n=522), HNSC-HPV- (Head and Neck squamous cell carcinoma HPV negative, n=422), HNSC-HPV+ (Head and Neck squamous cell carcinoma HPV positive, n=98), KIRC (Kidney renal clear cell carcinoma, n=533), KIRP (Kidney renal papillary cell carcinoma, n=290), LIHC (Liver hepatocellular carcinoma, n=371), LUAD (Lung adenocarcinoma, n=515), LUSC (Lung squamous cell carcinoma, n=501), OV (Ovarian serous cystadenocarcinoma, n=303), PAAD (Pancreatic adenocarcinoma, n=179), PRAD (Prostate adenocarcinoma, n=498), READ (Rectum adenocarcinoma, n=166), SARC (Sarcoma, n=260), STAD (Stomach adenocarcinoma, n=415), TGCT (Testicular Germ Cell Tumors, n=150), THCA (Thyroid carcinoma, n=509), THYM (Thymoma, n=120), UCEC (Uterine Corpus Endometrial Carcinoma, n=545).


BLCA
p-value = 0.0035
R = 0.15
BRCA
p-value = 6.9e-11
R = 0.2
CESC
p-value = 2e-04
R = 0.21
COAD
p-value = 1.9e-05
R = 0.25
DLBC
p-value = 1.6e-05
R = 0.58
HNSC
p-value = 2e-06
R = 0.21
KIRC
p-value = 0
R = 0.46
KIRP
p-value = 1.7e-13
R = 0.42
LGG
p-value = 0.004
R = 0.13
LIHC
p-value = 0
R = 0.5
LUAD
p-value = 5.5e-11
R = 0.29
LUSC
p-value = 2.7e-10
R = 0.28
OV
p-value = 0
R = 0.44
PAAD
p-value = 8.8e-09
R = 0.41
PRAD
p-value = 0
R = 0.37
SARC
p-value = 0.027
R = 0.14
STAD
p-value = 1.7e-14
R = 0.37
TGCT
p-value = 7.1e-10
R = 0.5
THCA
p-value = 0
R = 0.62
THYM
p-value = 4.3e-05
R = 0.37
UCEC
p-value = 3.4e-06
R = 0.34
log2(SCAF1 TPM)
log2(SOX13 TPM)

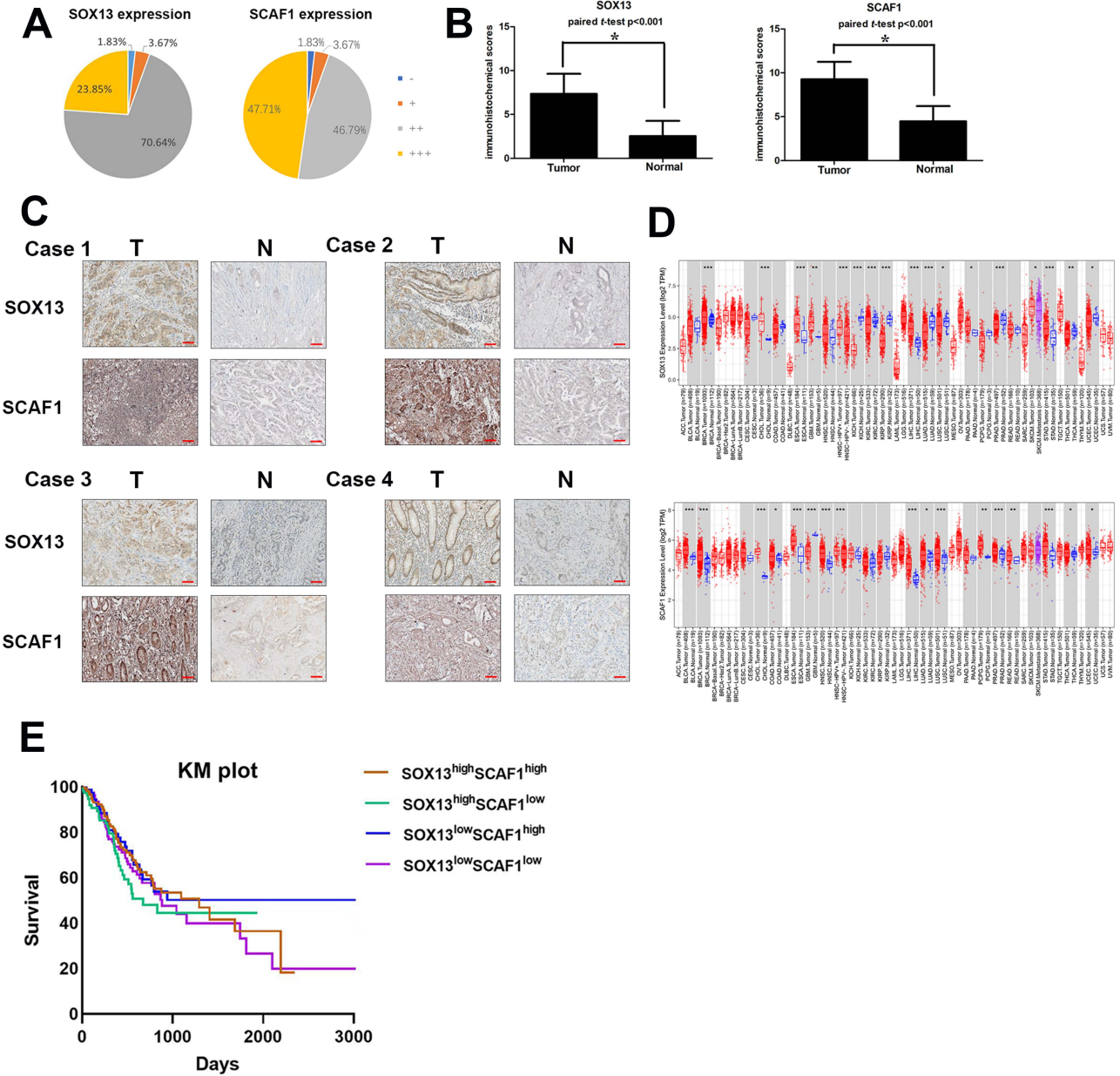
Supplementary Figure 12. Correlation results between SOX13 and SCAF1 according to GEPIA (http://gepia.cancer-pku.cn/) in BLCA, BRCA, CESC, COAD, DLBC, HNSC, KIRC, KIRP, LGG (Brain Lower Grade Glioma), LIHC, LUAD, LUSC, OV, PAAD, PRAD, SARC, STAD, TGCT, THCA, THYM, UCEC.


A
shRNA-NC
shRNA-SCAF1-1
shRNA-SCAF1-2
shRNA-SCAF1-2 +SXO13resis
kDa
SNU-668 Erastinresis
SCAF1
β-Actin
10
40
SNU-484 RSL3resis
B
SNU-668 Erastinresis
SNU-484 RSL3resis
% of cell viability
100 80 60 40 20
shRNA-NC
shRNA#1
shRNA#2
shRNA#2 +SCAF1resis
0 0.5 1 2 5 10
Erastin (μM)
0.05 0.1 0.2 0.5 1 2 5
RSL3 (μM)
C
Erastin
RSL3
shRNA-NC
shRNA-SCAF1-1
shRNA-SCAF1-2
shRNA-SCAF1-2 +SCAF1resis
shRNA-SCAF1-2 +Fer-1
Count
C11-BODIPY(FL1)
SNU-484 RSL3resis
% of cells with Oxidized C11-BODIPY
n.s.
P<0.0001
Ctrl
Erastin
RSL3
D
SNU-668 Erastinresis
SNU-484 RSL3resis
Cell viability (%)
P=0.0007
P=0.0024
P=0.0011
P=0.0060
Cisplatin (16 μM)
sh-SCAF1
SCAF1resis
Fer-1

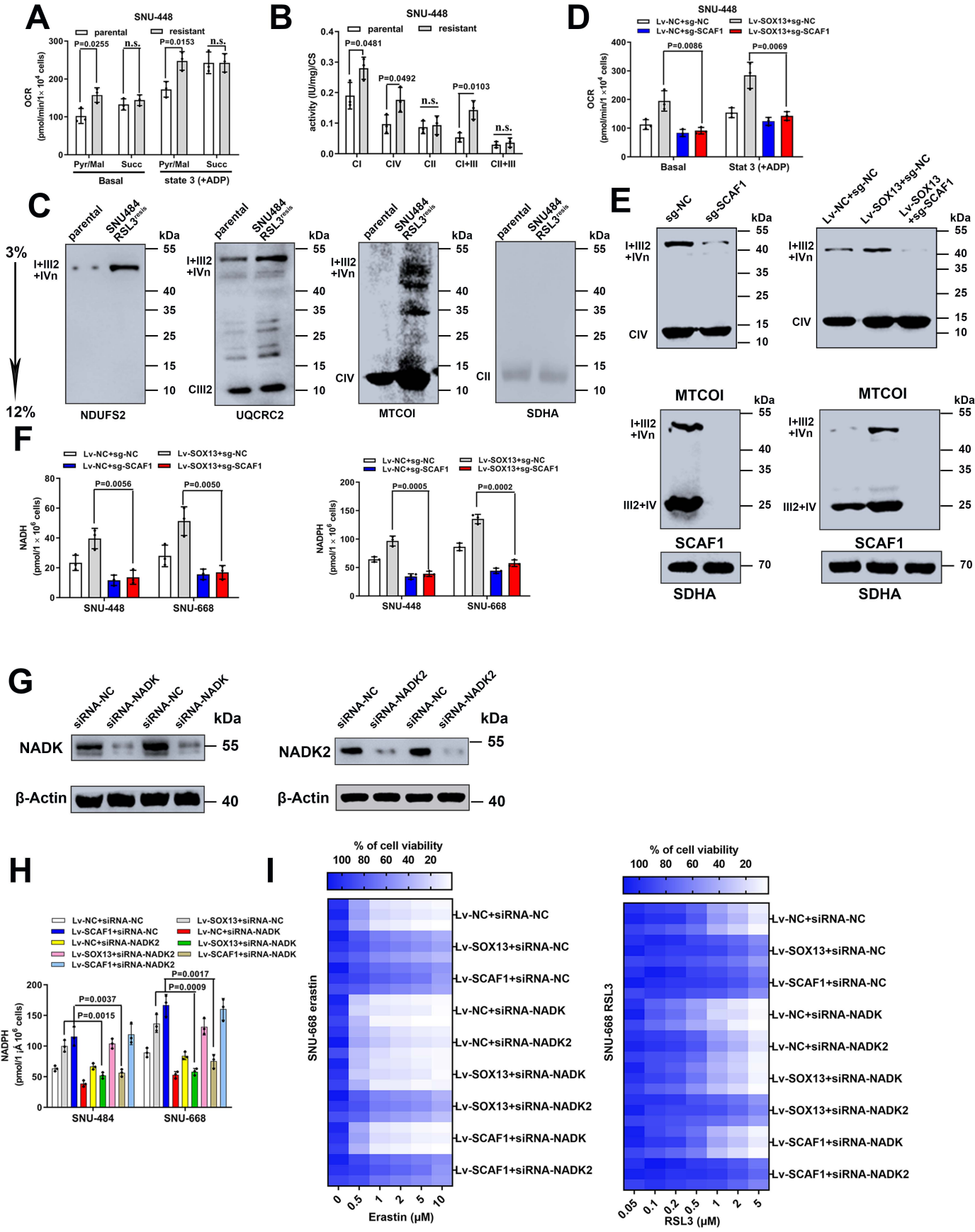
Supplementary Figure 13. (A-D) SNU-668 Erastin^{resis} cells or SNU-484 RSL3^{resis} cells were transducted of lentiviruses encoding SCAF1 shRNA-1 or shRNA-2 or a scrambled shRNA and/or resistant SOX13 cDNA (SOX13^{resis}). (A) SCAF1 was determined by immunoblotting assay and normalized to β-actin (n=3 independent experiments). (B,C) Effect of knockdown of SCAF1 and SCAF1 re-expression on sensitivity of SNU-668 Erastin^{resis} cells or SNU-484 RSL3^{resis} cells to the ferroptosis inducers RSL3 or Erastin. Cells transfected with desired vector were treated with various concentrations of RSL3 or Erastin for 24 hours. (B) The heatmap shows altered cell viability. (C) SNU-484 RSL3^{resis} cells transfected with desired vector were treated with Erastin (2 μM) or RSL3 (0.5 μM) for 24 hours. Lipid peroxidation was determined with a lipid peroxidation C11-BODIPY assay (n=3 independent experiments), and a representative flow cytometry histogram plot is presented. Data are presented as mean values ± SD. (D) Effect of SCAF1 downregulation on cisplatin sensitivity in the absence or presence of Fer-1 (1 μM) in Erastin^{resis} SNU-668 cells or RSL3^{resis} SNU-484 cells. The cells were treated with cisplatin (16 μM) for 24 hours. Cell viability was evaluated with a CellTiter-Glo luminescent cell viability assay (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (C,D) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
PBS
IKE
Lv-SOX13 +sg-NC
Lv-NC+sg-NC
Lv-NC +sg-SCAF1
Lv-NC+sg-NC
Lv-SOX13 +sg-SCAF1
Lv-SOX13 +sg-NC
Lv-NC +sg-SCAF1
B
Lv-NC+sg-NC control
Lv-NC+sg-NC IKE
Lv-SOX13+sg-NC control
Lv-SOX13+sg-NC IKE
Lv-NC+sg-SCAF1 IKE
Lv-SOX13+sg-SCAF1 control
Lv-SOX13+sg-SCAF1 IKE
P=0.0001
Tumor volume (mm3)
Days post inoculation
C
Tumor weight (g)
P<0.0001
control
IKE
D
Relative expression of PTGS2
P<0.0001
control
IKE
E
SOX13 expression
P=0.0014
pre-treat
post-treat
SCAF1 expression
P<0.0001
pre-treat
post-treat

Supplementary Figure 14. (A-D) SOX13-overexpressing CDX tumors (Lv-SOX13), SCAF1 KO-CDX tumors (sg-SCAF1) and control SNU-668-CDX tumors were treated with IKE 7 days after injection. Representative images of tumors formed (A), tumor growth curves (B), tumor weights (C), and PTGS2 expression (D) analysis are shown (n=4 mice per group). Data are presented as mean values ± SD. (E) SOX13 and SCAF1 expression levels were significantly increased after acquiring resistance to CF combination chemotherapy. Data are presented as mean values ± SD. Two-tailed unpaired t-test (B-D) or two-tailed paired t-test test (E). Source data are provided as a Source Data file.

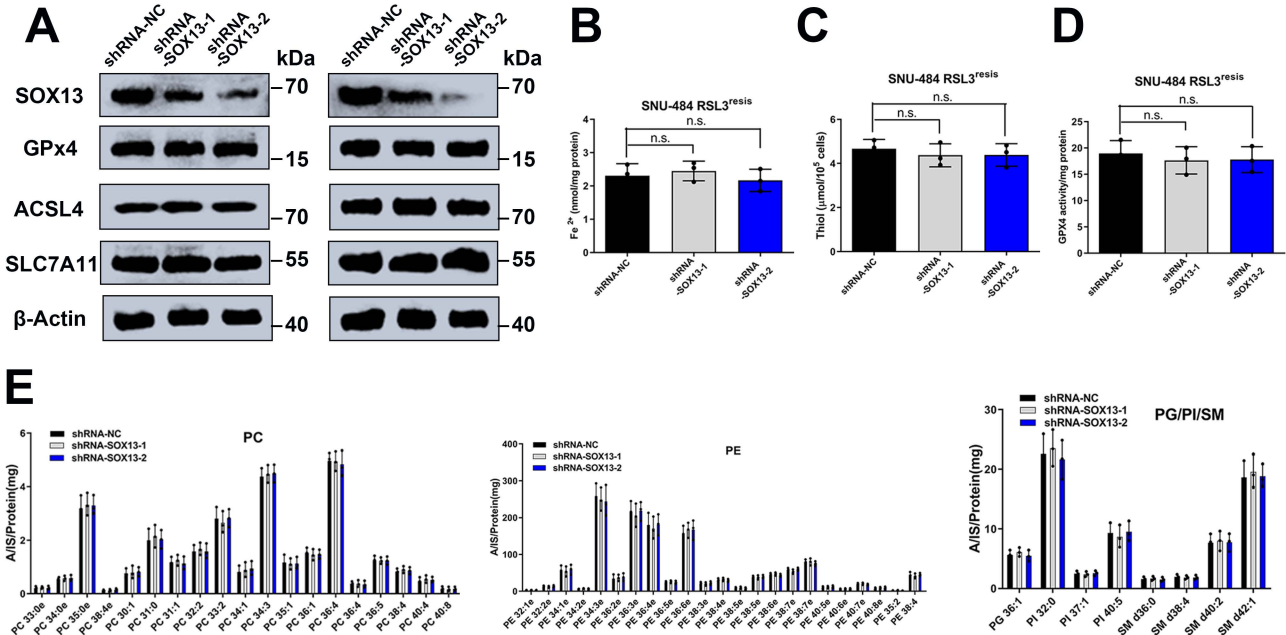

A
SOX13 expression
1.83%
3.67%
23.85%
70.64%
SCAF1 expression
1.83%
3.67%
47.71%
46.79%
-
+
++
+++
B
SOX13
paired t-test p<0.001
immunohistochemical scores
Tumor
Normal
SCAF1
paired t-test p<0.001
immunohistochemical scores
Tumor
Normal
C
Case 1
T
N
Case 2
T
N
SOX13
SCAF1
Case 3
T
N
Case 4
T
N
SOX13
SCAF1
D
SOX13 Expression Level (log2 TPM)
SCAF1 Expression Level (log2 TPM)
E
KM plot
SOX13highSCAF1high
SOX13highSCAF1low
SOX13lowSCAF1high
SOX13lowSCAF1low
Survival
Days

Supplementary Figure 15. (A) Immunohistochemical staining of SOX13/SCAF1 in 109 GC samples and matched normal stomach tissues. The expression was scored as negative (-), low (+), intermediate (++), or high (+++). (B) SOX13/SCAF1 immunohistochemical scores in 109 GC tissues and matched normal stomach tissues (paired *t* test, $p<0.0001$). Data are presented as mean values ± SD. (C) Representative immunohistochemical staining images for SOX13/SCAF1 are presented. Scale bar: 50 μm. (D) Normalized expression of SOX13 and SCAF1 across different tumor types from TIMER data showing high overall expression in STAD. (E) Kaplan–Meier analysis of concurrent SOX13 and SCAF1 expression with overall survival in patients receiving surgery only according to data from TCGA-STAD. Two-tailed paired t-test (B) or two-sided log-rank test (E).

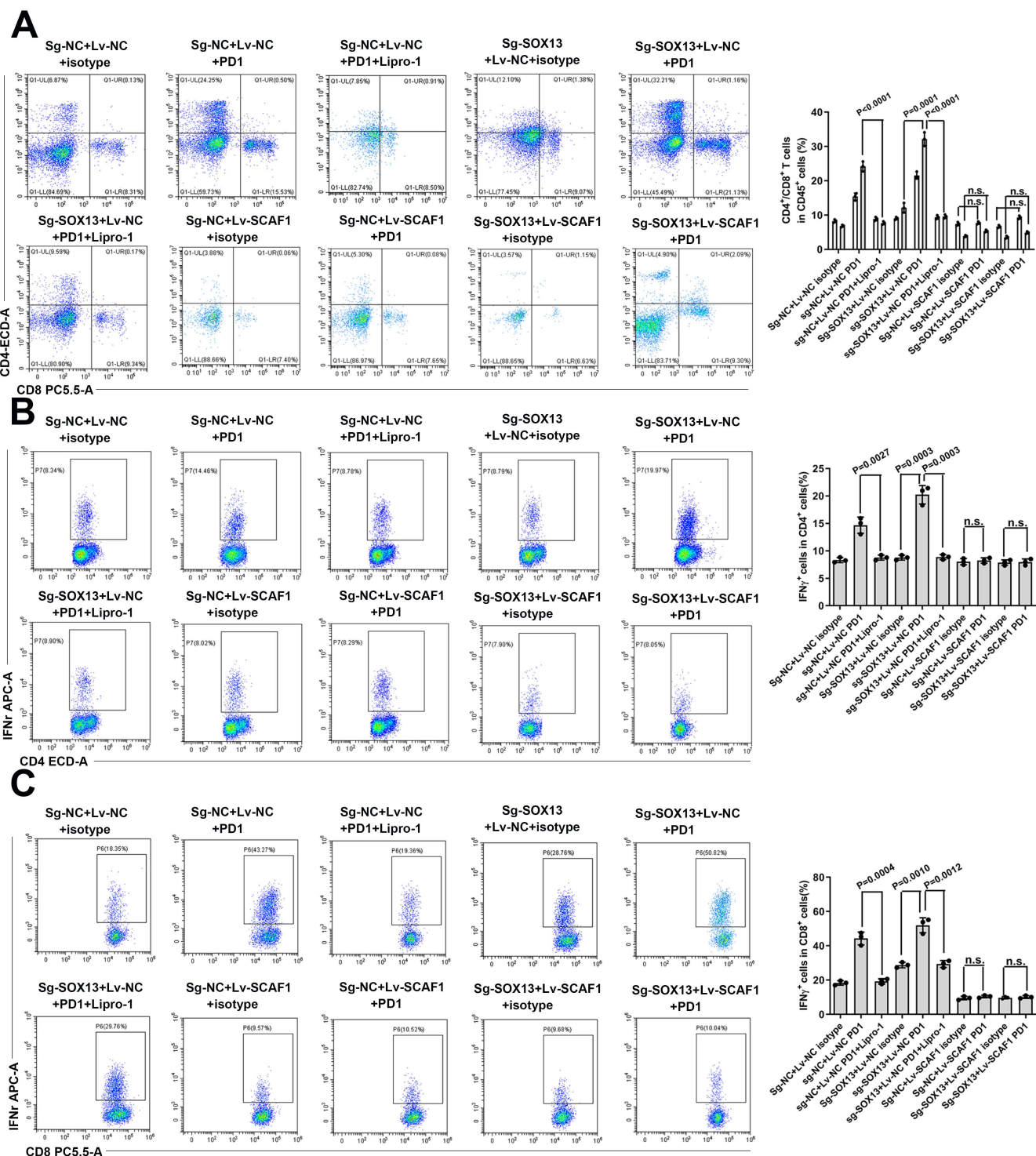

A
SNU-448
parental
resistant
OCR (pmol/min/1× 10^4 cells)
P=0.0255
n.s.
P=0.0153
n.s.
Pyr/Mal
Succ
Pyr/Mal
Succ
Basal
state 3 (+ADP)
B
SNU-448
parental
resistant
activity (IU/mg)/CS
P=0.0481
P=0.0492
n.s.
P=0.0103
n.s.
CI
CIV
CII
CI+III
CII+III
D
SNU-448
Lv-NC+sg-NC
Lv-SOX13+sg-NC
Lv-NC+sg-SCAF1
Lv-SOX13+sg-SCAF1
OCR (pmol/min/1 ×10^4 cells)
P=0.0086
P=0.0069
Basal
Stat 3 (+ADP)
C
parental
SNU484 RSL3res
kDa
3%
12%
I+III2 +IVn
CIII2
CIV
CII
NDUFS2
UQCRC2
MTCOI
SDHA
E
sg-NC
sg-SCAF1
Lv-NC+sg-NC
Lv-SOX13+sg-NC
Lv-SOX13 +sg-SCAF1
kDa
I+III2 +IVn
CIV
MTCOI
III2+IV
SCAF1
SDHA
F
Lv-NC+sg-NC
Lv-NC+sg-SCAF1
Lv-SOX13+sg-NC
Lv-SOX13+sg-SCAF1
NADH (pmol/1 × 10^6 cells)
P=0.0056
P=0.0050
SNU-448
SNU-668
NADPH (pmol/1 × 10^6 cells)
P=0.0005
P=0.0002
SNU-448
SNU-668
G
siRNA-NC
siRNA-NADK
siRNA-NADK2
kDa
NADK
NADK2
β-Actin
55
40
H
Lv-NC+siRNA-NC
Lv-SCAF1+siRNA-NC
Lv-NC+siRNA-NADK2
Lv-SOX13+siRNA-NADK2
Lv-SOX13+siRNA-NC
Lv-NC+siRNA-NADK
Lv-SOX13+siRNA-NADK
Lv-SCAF1+siRNA-NADK
Lv-SCAF1+siRNA-NADK2
NADPH (pmol/1 Å 10^6 cells)
P=0.0037
P=0.0015
P=0.0017
P=0.0009
SNU-484
SNU-668
I
% of cell viability
100 80 60 40 20
SNU-668 erastin
Lv-NC+siRNA-NC
Lv-SOX13+siRNA-NC
Lv-SCAF1+siRNA-NC
Lv-NC+siRNA-NADK
Lv-NC+siRNA-NADK2
Lv-SOX13+siRNA-NADK
Lv-SOX13+siRNA-NADK2
Lv-SCAF1+siRNA-NADK
Lv-SCAF1+siRNA-NADK2
0 0.5 1 2 5 10
Erastin (μM)
SNU-668 RSL3
0.05 0.1 0.2 0.5 1 2 5
RSL3 (μM)

Supplementary Figure 16. (A) Oxygen consumption analysis was performed in isolated mitochondria of parental and resistant SNU-484 cells using pyruvate-malate or succinate as specific substrates, respectively (n=3 independent experiments). Data are presented as mean values ± SD. (B) Mitochondrial enzymatic activities of CI, CIV, CII, CI+III, and CII+III normalized to citrate synthase (CS) levels in parental and resistant SNU-484 cells (n=3 independent experiments). Data are presented as mean values ± SD. (C) Western blot analysis of the indicated proteins, including CI, CIII, CIV, and CII, after blue native PAGE (BN-PAGE) of digitonin-solubilized mitochondria from parental and resistant SNU-484 cells (n=3 independent experiments). (D) Oxygen consumption rates of isolated mitochondria from parental SNU-484 cells with SOX13 overexpression (Lv-SOX13) alone, SCAF1 KO (sg-SCAF1) alone or the two in combination (n=3 independent experiments). Data are presented as mean values ± SD. (E) SC levels in parental SNU-484 cells with SOX13 overexpression (Lv-SOX13) alone, SCAF1 KO (sg-SCAF1) alone or the two in combination (n=3 independent experiments). (F) The increased NADH/NADPH production in parental cells induced by SOX13 overexpression was attenuated with SCAF1 silencing (n=3 independent experiments). Data are presented as mean values ± SD. (G) NADK and NADK2 protein expression levels were measured by Western blot analysis (n=3 independent experiments). β-Actin served as a loading control. (H) The cellular NADPH level was significantly decreased with silencing of the cytosolic enzyme NADK; however, downregulation of the mitochondrial enzyme NADK2 had no such effects (n=3 independent experiments). Data are presented as mean values ±

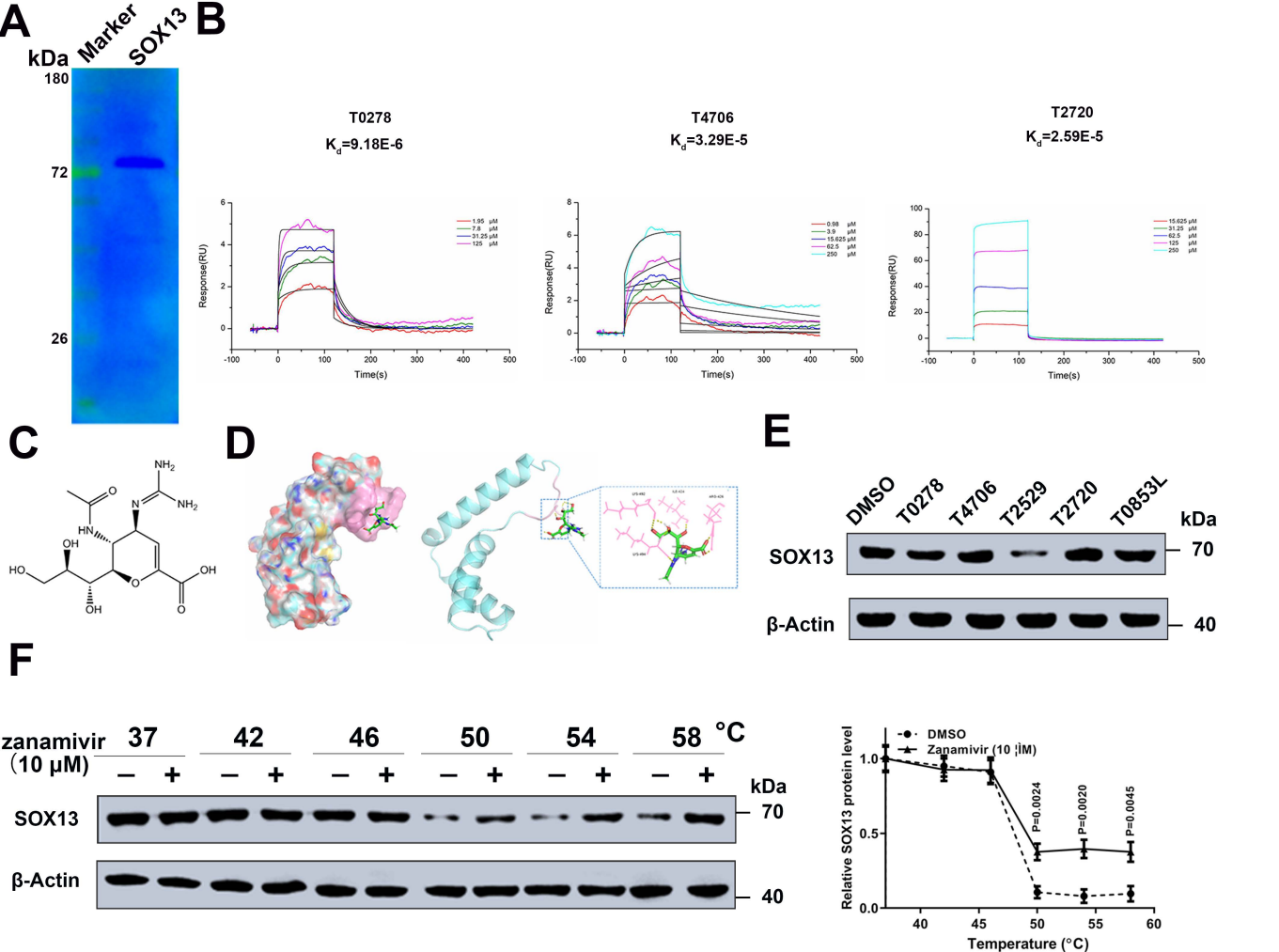
SD. (I) The survival benefit of SOX13/SCAF1-overexpressing parental SNU-668 cells was largely eliminated with NADK silencing. The cells were exposed to RSL3 (0.5 μM) or Erastin (2 μM) for 24 hours. Statistical significance in (A,B,D,F,H) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
shRNA-NC
shRNA -SOX13-1
shRNA -SOX13-2
kDa
SOX13
GPx4
ACSL4
SLC7A11
β-Actin
70
15
70
55
40
B
SNU-484 RSL3resis
Fe 2+ (nmol/mg protein)
n.s.
C
SNU-484 RSL3resis
Thiol (μmol/10^5 cells)
n.s.
D
SNU-484 RSL3resis
GPX4 activity/mg protein
n.s.
E
PC
PE
PG/PI/SM
A/IS/Protein(mg)
shRNA-NC
shRNA-SOX13-1
shRNA-SOX13-2
PC 33:0e
PC 34:0e
PC 35:0e
PC 36:4e
PC 30:1
PC 31:0
PC 31:1
PC 32:2
PC 33:2
PC 34:1
PC 34:3
PC 35:1
PC 36:1
PC 36:4
PC 36:5
PC 38:4
PC 40:4
PC 40:8
PE 32:1e
PE 32:2e
PE 34:1e
PE 34:2e
PE 34:3e
PE 36:2e
PE 36:3e
PE 36:4e
PE 36:5e
PE 36:6e
PE 38:5e
PE 38:4e
PE 38:5e
PE 38:6e
PE 38:7e
PE 38:7e
PE 40:5e
PE 40:6e
PE 40:7e
PE 40:8e
PE 35:2
PE 36:4
PG 36:1
PI 32:0
PI 37:1
PI 40:5
SM d36:0
SM d38:4
SM d40:2
SM d42:1

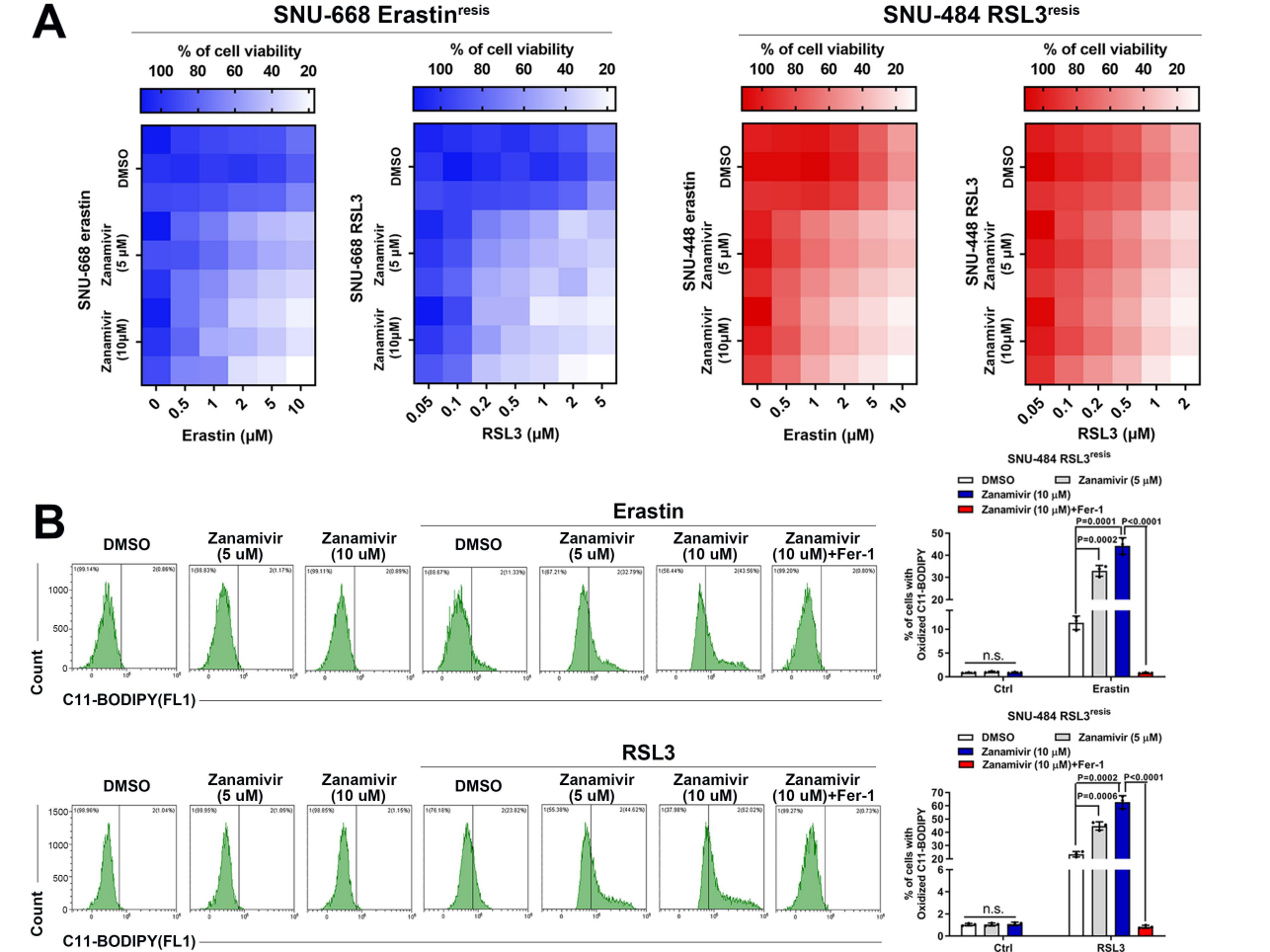
Supplementary Figure 17. (A-E) Effect of SOX13 knockdown on the expression of GPX4, SLC7A11, and ACSL4 (A), cellular divalent iron levels (B), system Xc^- (C) and GPX4 activity (D) or cellular phospholipid composition (E). (A) GPX4, SLC7A11, and ACSL4 protein expression levels were measured by Western blot analysis (n=3 independent experiments). β-Actin served as a loading control. Cellular divalent iron levels (B), system Xc^- (C) and GPX4 activity (D) were determined as described in the Materials and methods in SNU-484 $RSL3^{resis}$ cells (n=3 independent experiments). Data are presented as mean values ± SD. (E) Lipidomic profile of SNU-484 $RSL3^{resis}$ (control or SOX13 downregulated) cells. The data represents the mean value of the ratio of the area of analyte (A)/internal standard (IS)/protein (mg). Each group has three replicates, and results were representative of three independent experiments. Data are presented as mean values ± SD. Statistical significance in (B-E) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
Sg-NC+Lv-NC +isotype
Sg-NC+Lv-NC +PD1
Sg-NC+Lv-NC +PD1+Lipro-1
Sg-SOX13 +Lv-NC+isotype
Sg-SOX13+Lv-NC +PD1
Sg-SOX13+Lv-NC +PD1+Lipro-1
Sg-NC+Lv-SCAF1 +isotype
Sg-NC+Lv-SCAF1 +PD1
Sg-SOX13+Lv-SCAF1 +isotype
Sg-SOX13+Lv-SCAF1 +PD1
CD4-ECD-A
CD8 PC5.5-A
CD4+/CD8+ T cells in CD45+ cells (%)
P<0.0001
P=0.0001
P<0.0001
n.s.
B
IFNr APC-A
CD4 ECD-A
IFNγ+ cells in CD4+ cells(%)
P=0.0027
P=0.0003
P=0.0003
C
IFNr APC-A
CD8 PC5.5-A
IFNγ+ cells in CD8+ cells(%)
P=0.0004
P=0.0010
P=0.0012
Sg-NC+Lv-NC isotype
sg-NC+Lv-NC PD1
sg-NC+Lv-NC PD1+Lipro-1
Sg-SOX13+Lv-NC isotype
sg-SOX13+Lv-NC PD1
sg-SOX13+Lv-NC PD1+Lipro-1
Sg-NC+Lv-SCAF1 isotype
Sg-NC+Lv-SCAF1 PD1
Sg-SOX13+Lv-SCAF1 isotype
Sg-SOX13+Lv-SCAF1 PD1

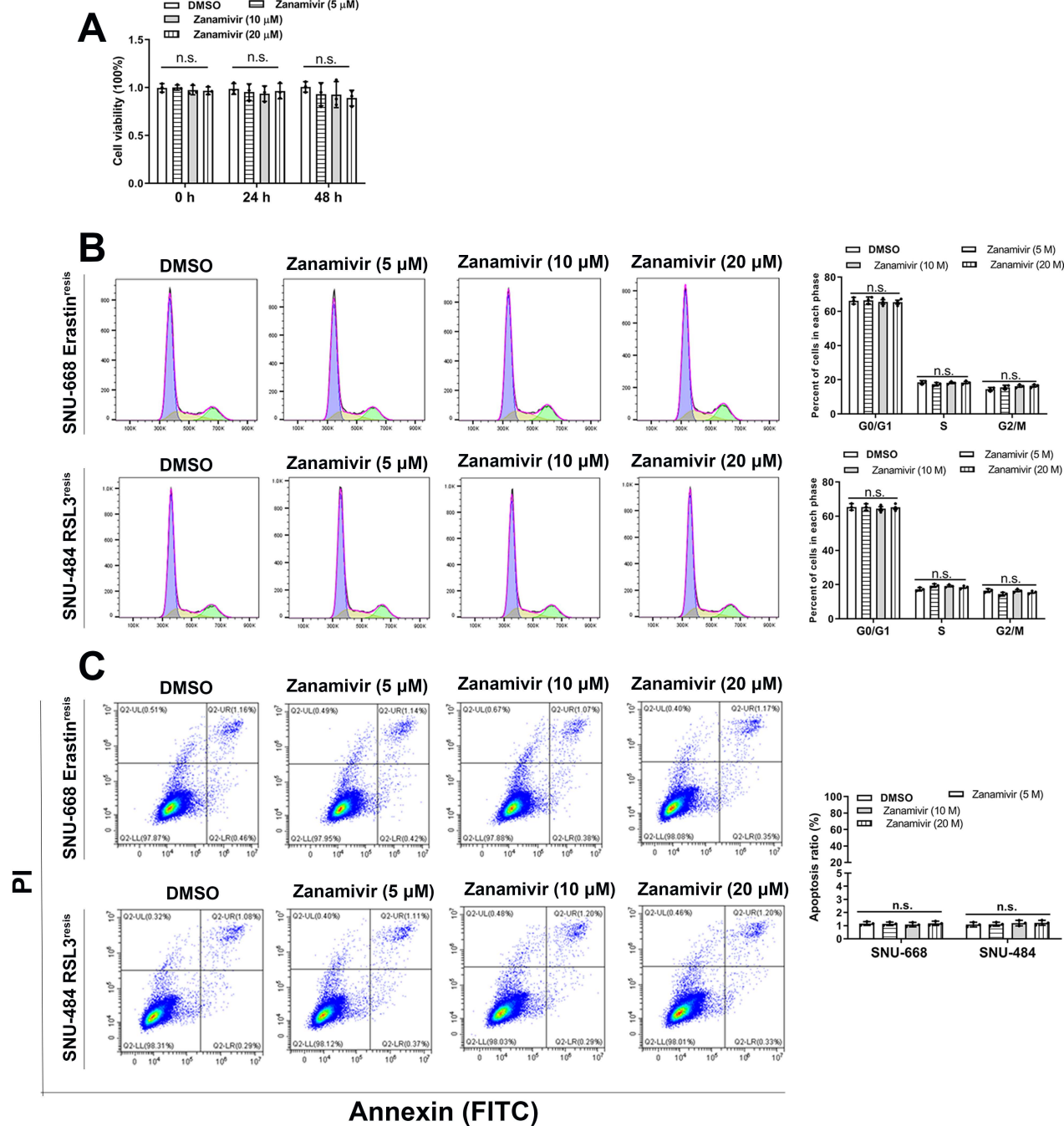
Supplementary Figure 18. (A-C) Representative flow cytometry analysis and quantification of the indicated $CD8^+$ and $CD4^+$ TILs in YTH16 (m)-derived xenografts with the treatment described above (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (A-C) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
kDa
Marker
SOX13
180
72
26
B
T0278
K_d=9.18E-6
T4706
K_d=3.29E-5
T2720
K_d=2.59E-5
Response(RU)
Time(s)
C
D
E
DMSO
T0278
T4706
T2529
T2720
T0853L
kDa
SOX13
70
β-Actin
40
F
zanamivir (10 μM)
37
42
46
50
54
58
°C
SOX13
β-Actin
70
40
DMSO
Zanamivir (10 μM)
Relative SOX13 protein level
P=0.0024
P=0.0020
P=0.0045
Temperature (°C)

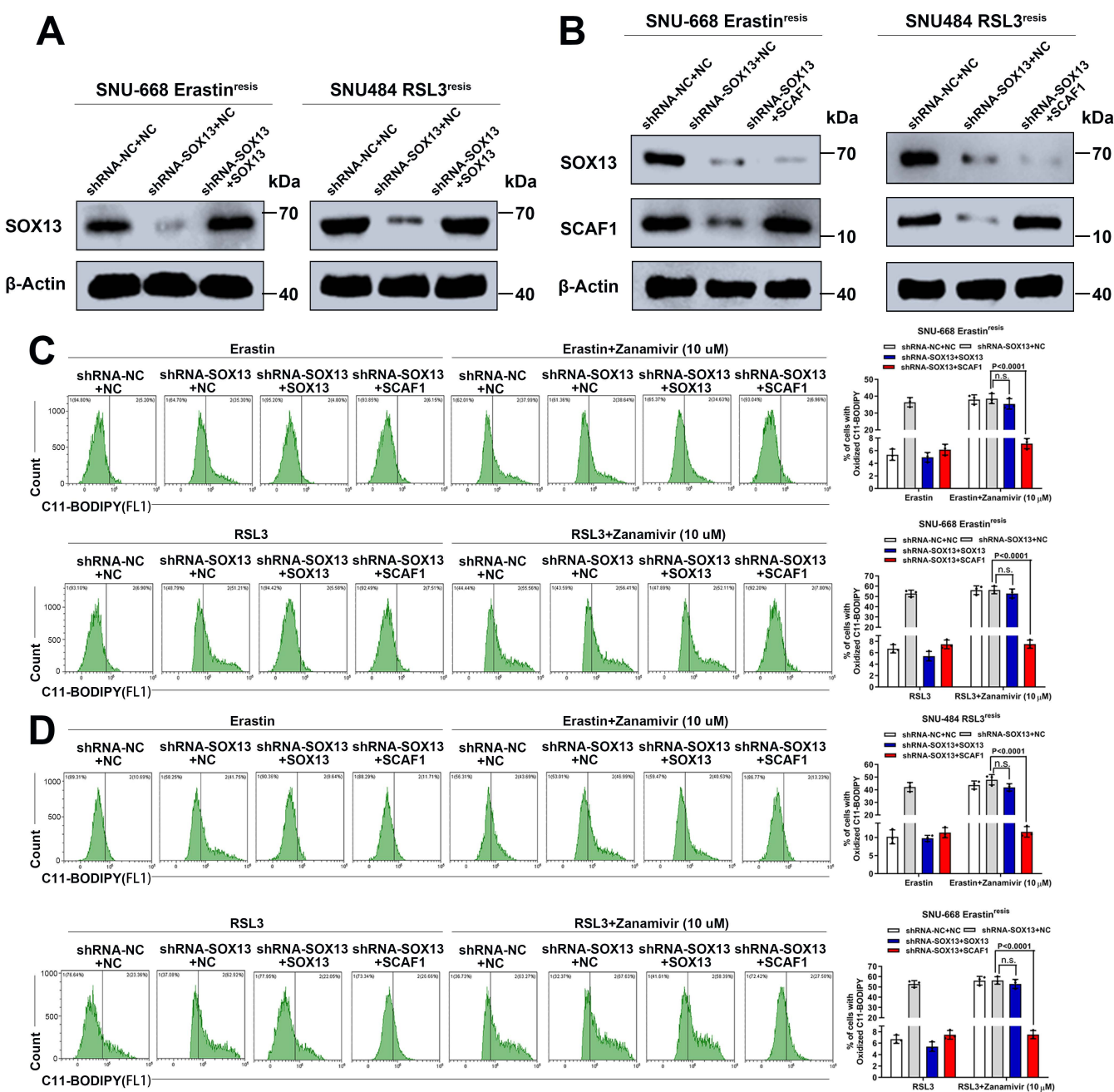
Supplementary Figure 19. (A) Coomassie blue staining showing the purity of recombinant human SOX13 protein. (B) SPR analysis results showing the binding of the SOX13 protein to several other small molecules (T0278, T4706 and T2720). (C) Chemical structures of zanamivir. (D) Computational molecular docking analysis to investigate the interaction of zanamivir binding to SOX13. The green molecule represents zanamivir. (E) Western blot analysis of the expression of SOX13 in Erastin^{resis} SNU-668 cells treated with different compounds (10 μM) for 36 h (n=3 independent experiments). (F) Melt curves of SOX13 protein in CETSA in Erastin^{resis} SNU-668 cells treated with zanamivir or vehicle for 1 h. The graph shows the quantification of SOX13 protein versus temperature points based on Western blot analyses (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (F) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
SNU-668 Erastin^resis
% of cell viability
100 80 60 40 20
SNU-668 erastin
DMSO
Zanamivir (5 μM)
Zanamivir (10μM)
0 0.5 1 2 5 10
Erastin (μM)
SNU-668 RSL3
0.05 0.1 0.2 0.5 1 2 5
RSL3 (μM)
SNU-484 RSL3^resis
SNU-448 erastin
SNU-448 RSL3
B
Erastin
RSL3
DMSO
Zanamivir (5 uM)
Zanamivir (10 uM)
Zanamivir (10 uM)+Fer-1
Count
C11-BODIPY(FL1)
SNU-484 RSL3^resis
DMSO
Zanamivir (5 μM)
Zanamivir (10 μM)
Zanamivir (10 μM)+Fer-1
% of cells with Oxidized C11-BODIPY
P=0.0001
P<0.0001
P=0.0002
n.s.
Ctrl
Erastin
P=0.0002
P<0.0001
P=0.0006
RSL3

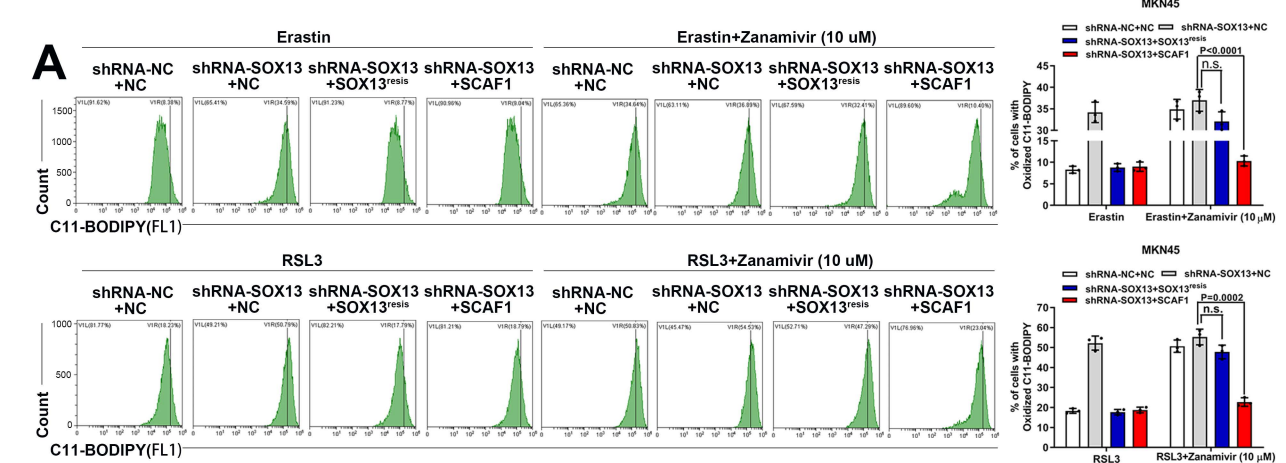
Supplementary Figure 20. (A) The Erastin^{resis} SNU-668 cells or RSL3-resistant SNU-484 cells were pretreated with zanamivir (0 μM, 5 μM, 10 μM) for 12 h and then exposed to various concentrations of RSL3 or Erastin for 24 hours. The heatmap shows altered cell viability. (B) The RSL3^{resis} SNU-484 cells cells were pretreated with zanamivir (0 μM, 5 μM, 10 μM) for 12h and then exposed to Erastin (2 μM) or RSL3 (0.5 μM) for 24 hours. Lipid peroxidation was determined using a lipid peroxidation C11-BODIPY assay (n=3 independent experiments), and representative flow cytometry histogram plot is presented. Data are presented as mean values ± SD. Statistical significance in (B) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
DMSO
Zanamivir (5 μM)
Zanamivir (10 μM)
Zanamivir (20 μM)
Cell viability (100%)
n.s.
0 h
24 h
48 h
B
DMSO
Zanamivir (5 μM)
Zanamivir (10 μM)
Zanamivir (20 μM)
SNU-668 Erastin^resis
SNU-484 RSL3^resis
Percent of cells in each phase
G0/G1
S
G2/M
C
PI
Annexin (FITC)
Apoptosis ratio (%)
SNU-668
SNU-484

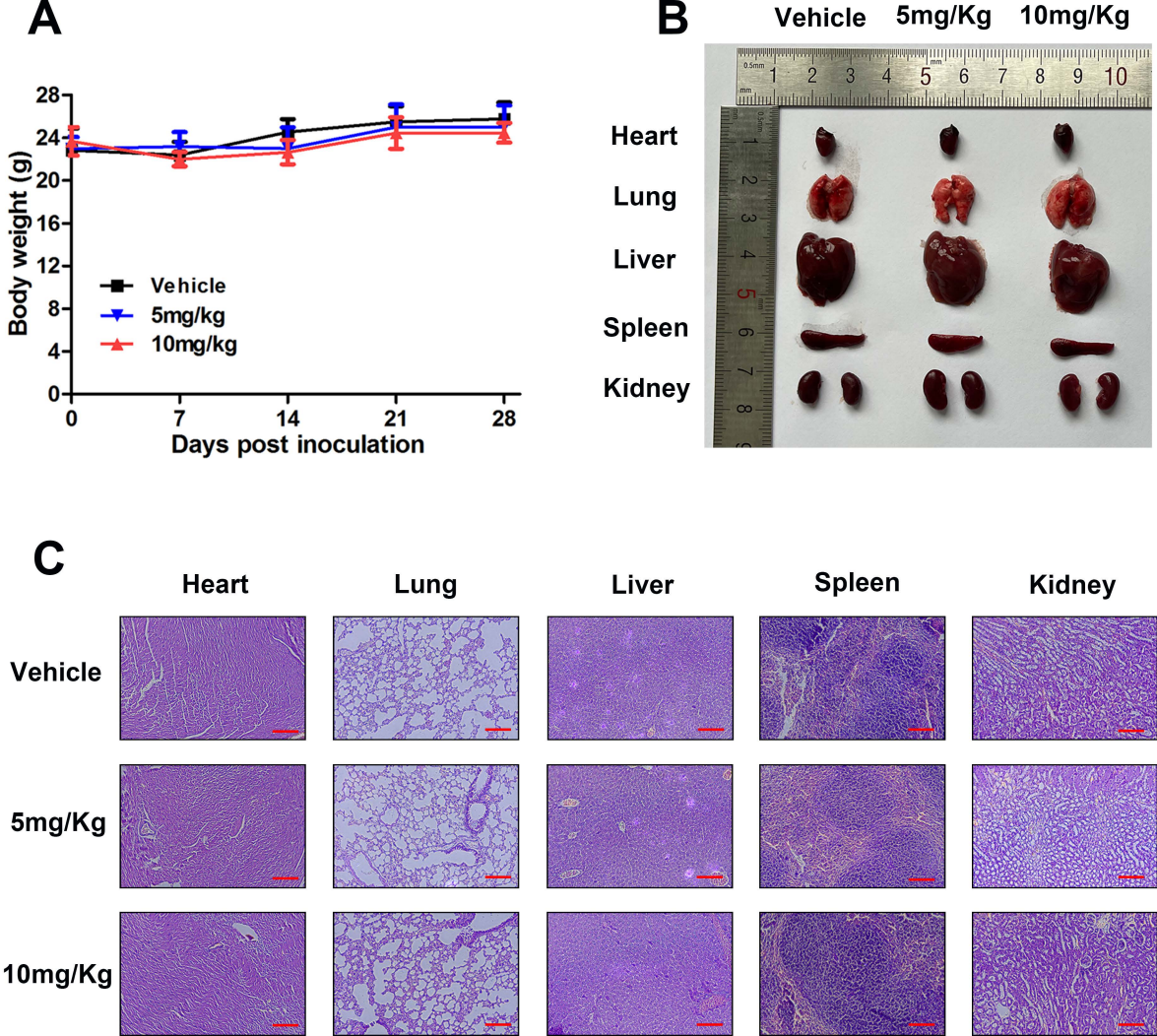
Supplementary Figure 21. (A-C) The Erastin^{resis} SNU-668 cells or RSL3^{resis} SNU-484 cells were pretreated with zanamivir (0 μM, 5 μM, 10 μM, 20 μM) for 48 h (A) or 36 h (B-C). (A) Relative viability was determined in Erastin^{resis} SNU-668 cells (n=3 independent experiments). Data are presented as mean values ± SD. (B) Cell cycle analysis determined the relative cell numbers in each cell-cycle phase with propidium iodide staining (n=3 independent experiments). Data are presented as mean values ± SD. (C) Annexin V/PI staining and flow cytometry analysis assessing apoptosis (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (A-C) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
SNU-668 Erastin^resis
SNU484 RSL3^resis
shRNA-NC+NC
shRNA-SOX13+NC
shRNA-SOX13 +SOX13
kDa
SOX13
β-Actin
70
40
B
SNU-668 Erastin^resis
SNU484 RSL3^resis
shRNA-NC+NC
shRNA-SOX13+NC
shRNA-SOX13 +SCAF1
kDa
SOX13
SCAF1
β-Actin
70
10
40
C
Erastin
Erastin+Zanamivir (10 uM)
RSL3
RSL3+Zanamivir (10 uM)
shRNA-NC +NC
shRNA-SOX13 +NC
shRNA-SOX13 +SOX13
shRNA-SOX13 +SCAF1
Count
C11-BODIPY(FL1)
SNU-668 Erastin^resis
shRNA-NC+NC
shRNA-SOX13+NC
shRNA-SOX13+SOX13
shRNA-SOX13+SCAF1
P<0.0001
n.s.
% of cells with Oxidized C11-BODIPY
Erastin
Erastin+Zanamivir (10 μM)
RSL3
RSL3+Zanamivir (10 μM)
D
Erastin
Erastin+Zanamivir (10 uM)
RSL3
RSL3+Zanamivir (10 uM)
Count
C11-BODIPY(FL1)
SNU-484 RSL3^resis
SNU-668 Erastin^resis
P<0.0001
n.s.
% of cells with Oxidized C11-BODIPY
Erastin
Erastin+Zanamivir (10 μM)
RSL3
RSL3+Zanamivir (10 μM)

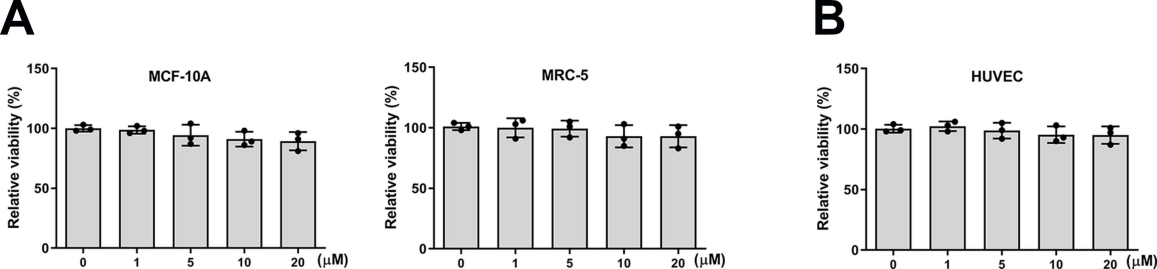
Supplementary Figure 22. (A,B) Wild-type SOX13 (A) or SCAF1 (B) were re-overexpressed in SOX13-deficient FINs resistant cells. SOX13, and SCAF1 protein expression levels were measured by Western blot analysis (n=3 independent experiments). (C,D) Wild-type SOX13 or SCAF1 were re-overexpressed in SOX13-deficient Erastin^{resis} SNU-668 cells (C) or RSL3^{resis} SNU-484 cells (D). Cells were pretreated with zanamivir (0 μM, 10 μM) for 12h and exposed to Erastin (2 μM) or RSL3 (0.5 μM) for 24 hours. The sensitivity of cells to FINs was evaluated with a lipid peroxidation C11-BODIPY assay (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (C,D) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Supplementary Figure 22. (A,B) Wild-type SOX13 (A) or SCAF1 (B) were re-overexpressed in SOX13-deficient FINs resistant cells. SOX13, and SCAF1 protein expression levels were measured by Western blot analysis (n=3 independent experiments). (C,D) Wild-type SOX13 or SCAF1 were re-overexpressed in SOX13-deficient Erastinresis SNU-668 cells (C) or RSL3resis SNU-484 cells (D). Cells were pretreated with zanamivir (0 μM, 10 μM) for 12h and exposed to Erastin (2 μM) or RSL3 (0.5 μM) for 24 hours. The sensitivity of cells to FINs was evaluated with a lipid peroxidation C11-BODIPY assay (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (C,D) is determined by two-tailed unpaired t-test. Source data are provided as a Source Data file.


A
Erastin
Erastin+Zanamivir (10 uM)
shRNA-NC +NC
shRNA-SOX13 +NC
shRNA-SOX13 +SOX13resis
shRNA-SOX13 +SCAF1
V1L(91.62%)
V1R(8.38%)
V1L(65.41%)
V1R(34.59%)
V1L(91.23%)
V1R(8.77%)
V1L(93.96%)
V1R(6.04%)
V1L(65.36%)
V1R(34.64%)
V1L(63.11%)
V1R(36.89%)
V1L(67.59%)
V1R(32.41%)
V1L(89.60%)
V1R(10.40%)
Count
C11-BODIPY(FL1)
MKN45
shRNA-NC+NC
shRNA-SOX13+NC
shRNA-SOX13+SOX13resis
shRNA-SOX13+SCAF1
P<0.0001
n.s.
% of cells with Oxidized C11-BODIPY
Erastin
Erastin+Zanamivir (10 μM)
RSL3
RSL3+Zanamivir (10 uM)
V1L(81.77%)
V1R(18.23%)
V1L(49.21%)
V1R(50.79%)
V1L(82.21%)
V1R(17.79%)
V1L(81.21%)
V1R(18.79%)
V1L(49.17%)
V1R(50.83%)
V1L(45.47%)
V1R(54.53%)
V1L(52.71%)
V1R(47.29%)
V1L(76.96%)
V1R(23.04%)
P=0.0002
RSL3
RSL3+Zanamivir (10 μM)

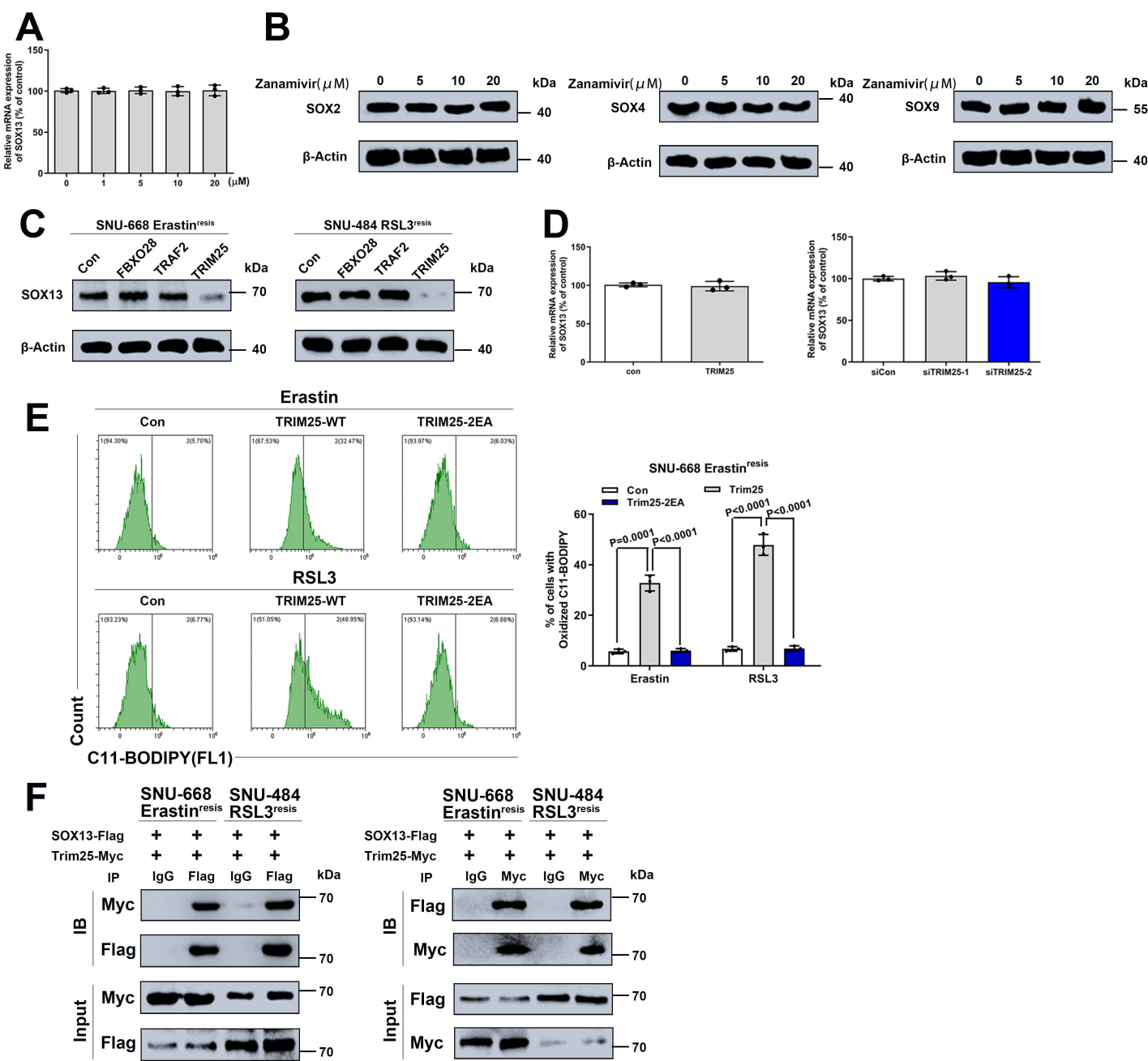
Supplementary Figure 23. Wild-type SOX13 or SCAF1 were re-overexpressed in SOX13-deficient MKN45 cells. Cells were pretreated with zanamivir (0 μM, 10 μM) for 12h and exposed to Erastin (2 μM) or RSL3 (0.5 μM) for 24 hours. The sensitivity of cells to FINs was evaluated with a lipid peroxidation C11-BODIPY assay (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (A) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
Body weight (g)
28
24
20
16
12
8
4
0
0
7
14
21
28
Days post inoculation
Vehicle
5mg/kg
10mg/kg
B
Vehicle
5mg/Kg
10mg/Kg
Heart
Lung
Liver
Spleen
Kidney
C
Heart
Lung
Liver
Spleen
Kidney
Vehicle
5mg/Kg
10mg/Kg

Supplementary Figure 24. (A) Body weights of mice in 28 days of treatment with vehicle or zanamivir (5 mg/kg and 10 mg/kg). (B) The size and appearance of heart, liver, spleen, lung and kidney of each group in Erastin^{resis} SNU-668 xenograft model. (C) HE staining of heart, liver, spleen, lung and kidney of each group. Scale bars: 200 μm (100× magnification).


A
MCF-10A
Relative viability (%)
150
100
50
0
0
1
5
10
20
(μM)
MRC-5
Relative viability (%)
150
100
50
0
0
1
5
10
20
(μM)
B
HUVEC
Relative viability (%)
150
100
50
0
0
1
5
10
20
(μM)

Supplementary Figure 25. Toxicity of zanamivir in non-tumorigenic epithelial and endothelial cells. (A) Zanamivir has little effect on the viability of non-tumorigenic mammary and pulmonary epithelial cells, MCF-10A and MRC-5 (n=3 independent experiments). (B) Zanamivir has no effect on the viability of human umbilical vein endothelial cells HUVEC (n=3 independent experiments). Data are presented as mean values ± SD. Statistical significance in (A,B) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.


A
Relative mRNA expression of SOX13 (% of control)
150
100
50
0
0
1
5
10
20
(μM)
B
Zanamivir(μM)
0
5
10
20
kDa
SOX2
40
β-Actin
40
Zanamivir(μM)
0
5
10
20
kDa
SOX4
40
β-Actin
40
Zanamivir(μM)
0
5
10
20
kDa
SOX9
55
β-Actin
40
C
SNU-668 Erastin^resis
Con
FBXO28
TRAF2
TRIM25
kDa
SOX13
70
β-Actin
40
SNU-484 RSL3^resis
Con
FBXO28
TRAF2
TRIM25
kDa
70
40
D
Relative mRNA expression of SOX13 (% of control)
150
100
50
0
con
TRIM25
Relative mRNA expression of SOX13 (% of control)
150
100
50
0
siCon
siTRIM25-1
siTRIM25-2
E
Erastin
Con
TRIM25-WT
TRIM25-2EA
1(84.30%)
2(5.70%)
1(67.53%)
2(32.47%)
1(93.97%)
2(6.03%)
RSL3
Con
TRIM25-WT
TRIM25-2EA
1(93.23%)
2(6.77%)
1(51.05%)
2(48.95%)
1(93.14%)
2(6.86%)
Count
C11-BODIPY(FL1)
SNU-668 Erastin^resis
Con
Trim25
Trim25-2EA
% of cells with Oxidized C11-BODIPY
60
40
20
0
P=0.0001
P<0.0001
P<0.0001
P<0.0001
Erastin
RSL3
F
SNU-668 Erastin^resis
SNU-484 RSL3^resis
SOX13-Flag
Trim25-Myc
IP
IgG
Flag
IgG
Flag
kDa
IB
Myc
70
Flag
70
Input
Myc
70
Flag
70
SNU-668 Erastin^resis
SNU-484 RSL3^resis
SOX13-Flag
Trim25-Myc
IP
IgG
Myc
IgG
Myc
kDa
IB
Flag
70
Myc
70
Input
Flag
70
Myc
70

Supplementary Figure 26. (A) qRT-PCR results showing the mRNA expression of SOX13 in Erastin^{resis} SNU-668 cells treated with zanamivir (0 μM, 5 μM, 10 μM, and 20 μM) for 36 h (n=3 independent experiments). Data are presented as mean values ± SD. (B) The Erastin^{resis} SNU-668 cells were pretreated with zanamivir (0 μM, 5 μM, 10 μM, 20 μM) for 36 h. SOX2, SOX4, and SOX9 protein expression levels were measured by Western blot analysis and normalized to β-Actin (n=3 independent experiments). (C) Western blot analysis of SOX13 expression in Erastin^{resis} SNU-668 cells or RSL3^{resis} SNU-484 cells overexpressing TRIM25, FBXO28 or TRAF2 (n=3 independent experiments). (D) qRT-PCR results showing the expression level of SOX13 in TRIM25-overexpressing and TRIM25-knockdown Erastin^{resis} SNU-668 cells (n=3 independent experiments). Data are presented as mean values ± SD. (E) TRIM25-WT, TRIM25-2EA or vector control were overexpressed in Erastin^{resis} SNU-668 cells. The cells were treated with zanamivir (10 μM) for 12h before exposed to Erastin (2 μM) or RSL3 (0.5 μM) for 24 hours. Lipid peroxidation was determined utilizing a lipid peroxidation C11-BODIPY assay (n=3 independent experiments), and representative flow cytometry histogram plot is presented. Data are presented as mean values ± SD. (F) Immunoprecipitation results showing the interaction between SOX13 and TRIM25 (n=3 independent experiments). Statistical significance in (A,D,E) is determined by two-tailed unpaired *t*-test. Source data are provided as a Source Data file.