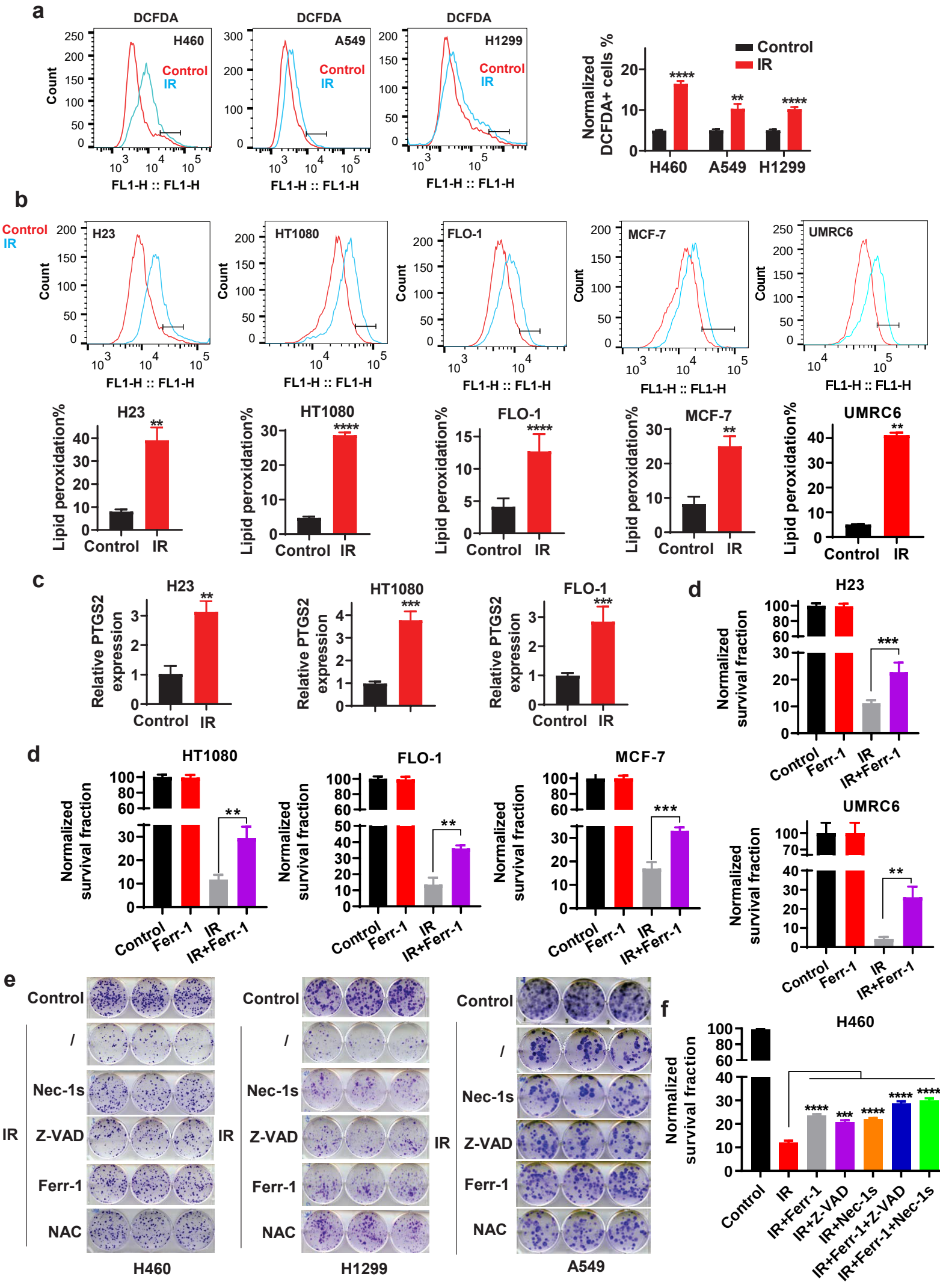
Fig. S1


a
DCFDA
H460
A549
H1299
Control
IR
Count
FL1-H :: FL1-H
Normalized DCFDA+ cells %
b
H23
HT1080
FLO-1
MCF-7
UMRC6
Lipid peroxidation%
c
Relative PTGS2 expression
d
Normalized survival fraction
Ferr-1
IR+Ferr-1
e
Nec-1s
Z-VAD
NAC
f
IR+Z-VAD
IR+Nec-1s
IR+Ferr-1+Z-VAD
IR+Ferr-1+Nec-1s

SUPPLEMENTARY FIGURE LEGENDS

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b-c Total ROS levels assessment in sg Control (sg C), sg ACSL4-2, and sg ACSL4-3 A549 (b) and H460 (c) cell lines at 24 h after exposure to 6 Gy of ionizing radiation. Bar graph showing IR-induced relative fold changes of total ROS by DCFDA staining in the indicated cells. Error bars are mean ± SD, n = 3 independent repeats. *P* values calculated using two-tailed unpaired Student *t* test.

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h Clonogenic survival assays in sg C and sg KEAP1-1 H1299 cells that were pretreated with 5 μM ferrostatin-1 or DMSO for 24 h followed by exposure to 6 Gy of ionizing radiation. Error bars are mean ± SD, n = 3 independent repeats. *P* values calculated using two-tailed unpaired Student *t* test.

i Western blotting analysis of KEAP1 and SLC7A11 expression in sg C, sg KEAP1-1, and sg KEAP1-2 H23 cell lines.

j Lipid peroxidation assessment in sg C, sg KEAP1-1, and sg KEAP1-2 H23 cells at 24 h after exposure to 2 Gy of ionizing radiation. Bar graph showing IR-induced relative fold changes of lipid peroxidation by C11-BODIPY staining in the indicated cells. Error bars are mean ± SD, n = 3 independent repeats. *P* values calculated using two-tailed unpaired Student *t* test.

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a Representative immunofluorescence images showing staining of phospho-H2AX foci (red) and

nuclei counterstained with DAPI (blue) in the indicated cells with or without exposure to IR.

b The numbers of phospho-H2AX foci per nucleus were counted on the basis of immunofluorescence at 30 min and 24 h after exposure to 6 Gy of ionizing radiation in UMRC6 cells with stable expression of EV and GPX4. Error bars are mean ± SD, n = 50 independent repeats. *P* values calculated using two-tailed unpaired Student *t* test.

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Supplementary Figure 6. Ferroptosis is involved in ionizing radiation-induced tumor suppression *in vivo*.

a Treatment schema for H460 xenograft, A549 xenograft, and lung cancer patient-derived xenograft mice.

b Representative images of A549 xenograft tumors with the indicated treatments at experimental endpoints.

c Representative images of hematoxylin and eosin and immunohistochemical staining (Ki67, phospho-H2AX, cleaved caspase-3, and 4-HNE) of H460 xenograft tumors with the indicated treatments. Scale bars, 50 μm (insert).

d-g Immunochemistry scoring of Ki67 (d), phospho-H2AX (e), cleaved caspase-3 (f), and 4-HNE staining (g). Error bars are mean ± SD, n = 6 randomly selected magnification fields. *P* values calculated using two-tailed unpaired Student's *t* test.

Supplementary Table 1. Correlations between clinical pathological characteristics, radiotherapy response, and 4-HNE staining in patients with esophageal cancer.

Supplementary Table 2. The list of primer sequences used in this manuscript.

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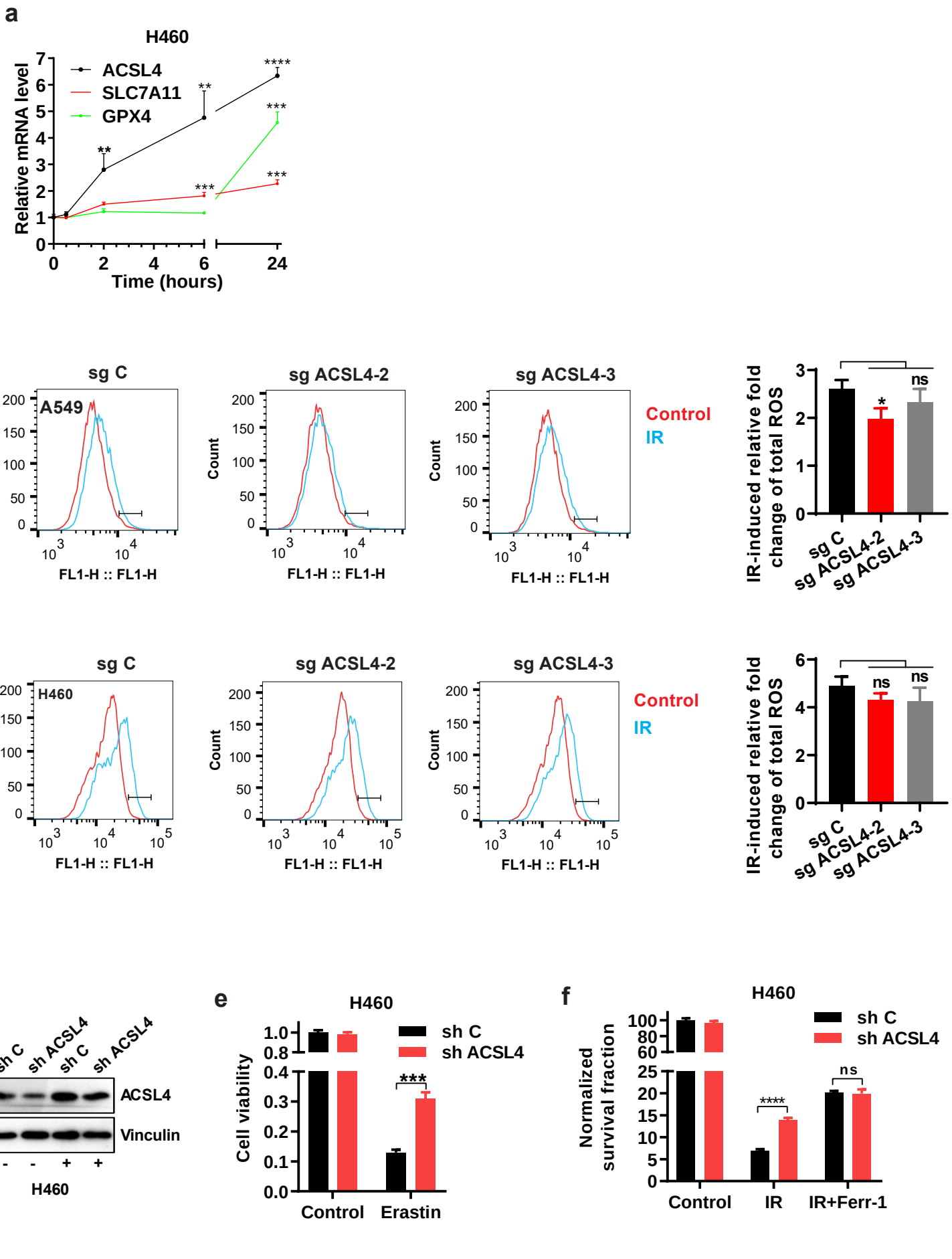
Fig. S2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A549
Count
FL1-H :: FL1-H
Control
IR
IR-induced relative fold change of total ROS
ns
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sg ACSL4-3
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H460
d
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ACSL4
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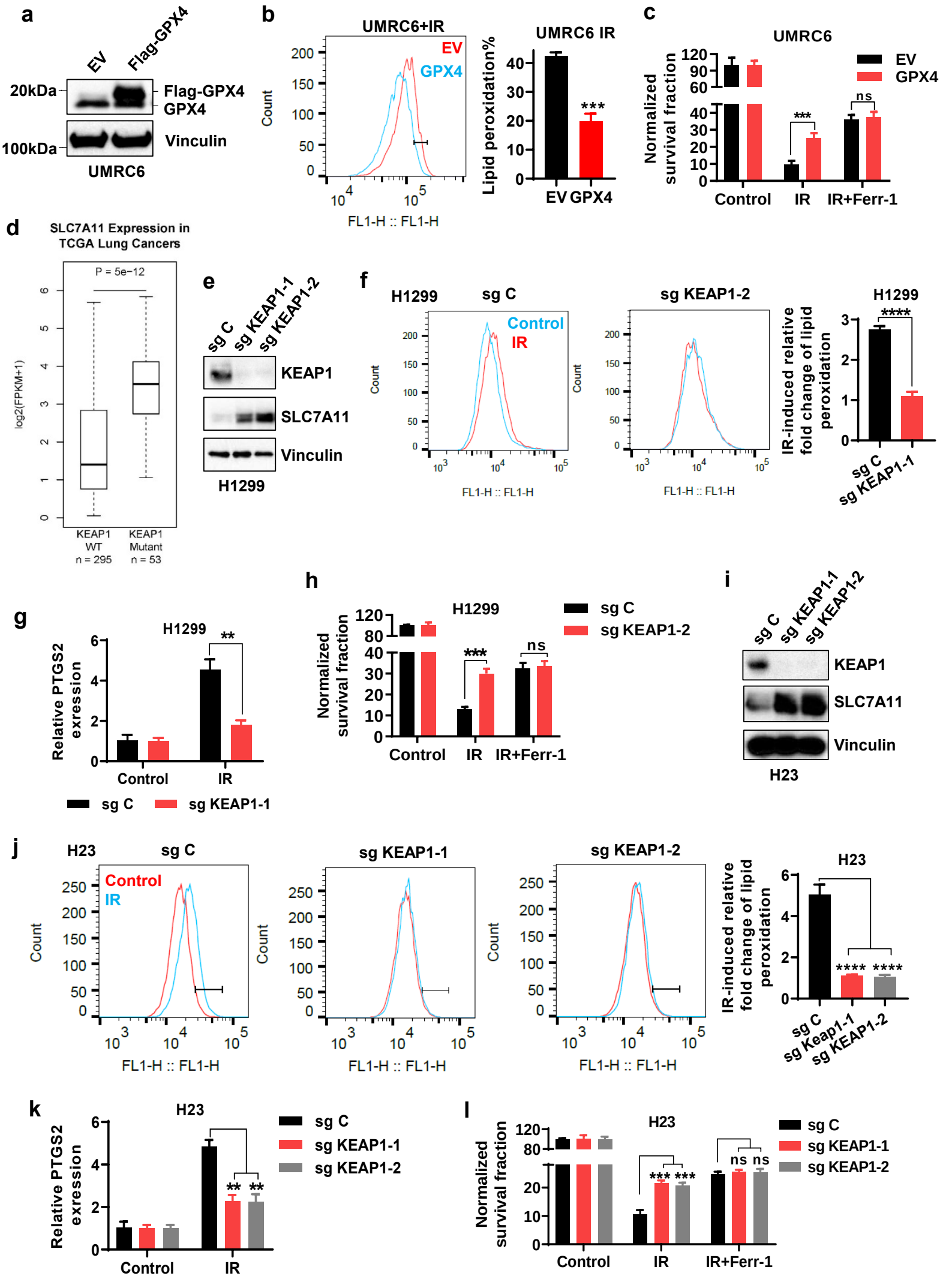
Fig. S3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***
EV GPX4
c
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EV
GPX4
Normalized survival fraction
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sg C
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sg KEAP1-2
KEAP1
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Vinculin
H1299
f
H1299
sg C
Control
IR
sg KEAP1-2
Count
FL1-H :: FL1-H
IR-induced relative fold change of lipid peroxidation
****
sg C
sg KEAP1-1
g
H1299
**
Relative PTGS2 expression
Control
IR
sg C
sg KEAP1-1
h
H1299
sg C
sg KEAP1-2
Normalized survival fraction
***
ns
Control
IR
IR+Ferr-1
i
sg C
sg KEAP1-1
sg KEAP1-2
KEAP1
SLC7A11
Vinculin
H23
j
H23
sg C
Control
IR
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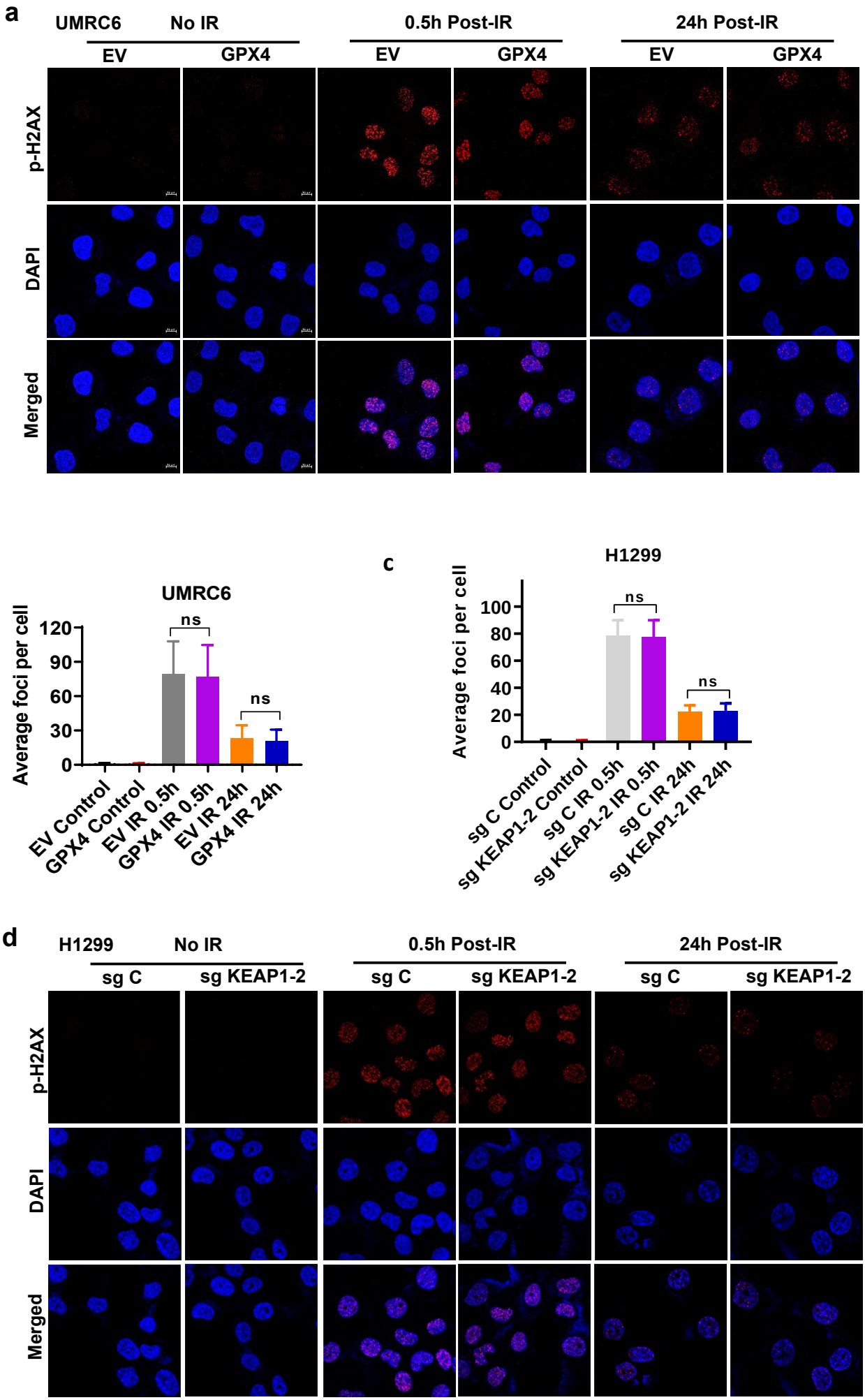
sg KEAP1-2
Count
FL1-H :: FL1-H
IR-induced relative fold change of lipid peroxidation
****
sg C
sg Keap1-1
sg KEAP1-2
k
H23
Relative PTGS2 expression
**
sg C
sg KEAP1-1
sg KEAP1-2
Control
IR
l
H23
Normalized survival fraction
***
ns
sg C
sg KEAP1-1
sg KEAP1-2
Control
IR
IR+Ferr-1

Fig. S4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Merged
b
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90
60
30
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c
H1299
Average foci per cell
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80
60
40
20
0
ns
ns
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24h Post-IR
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DAPI
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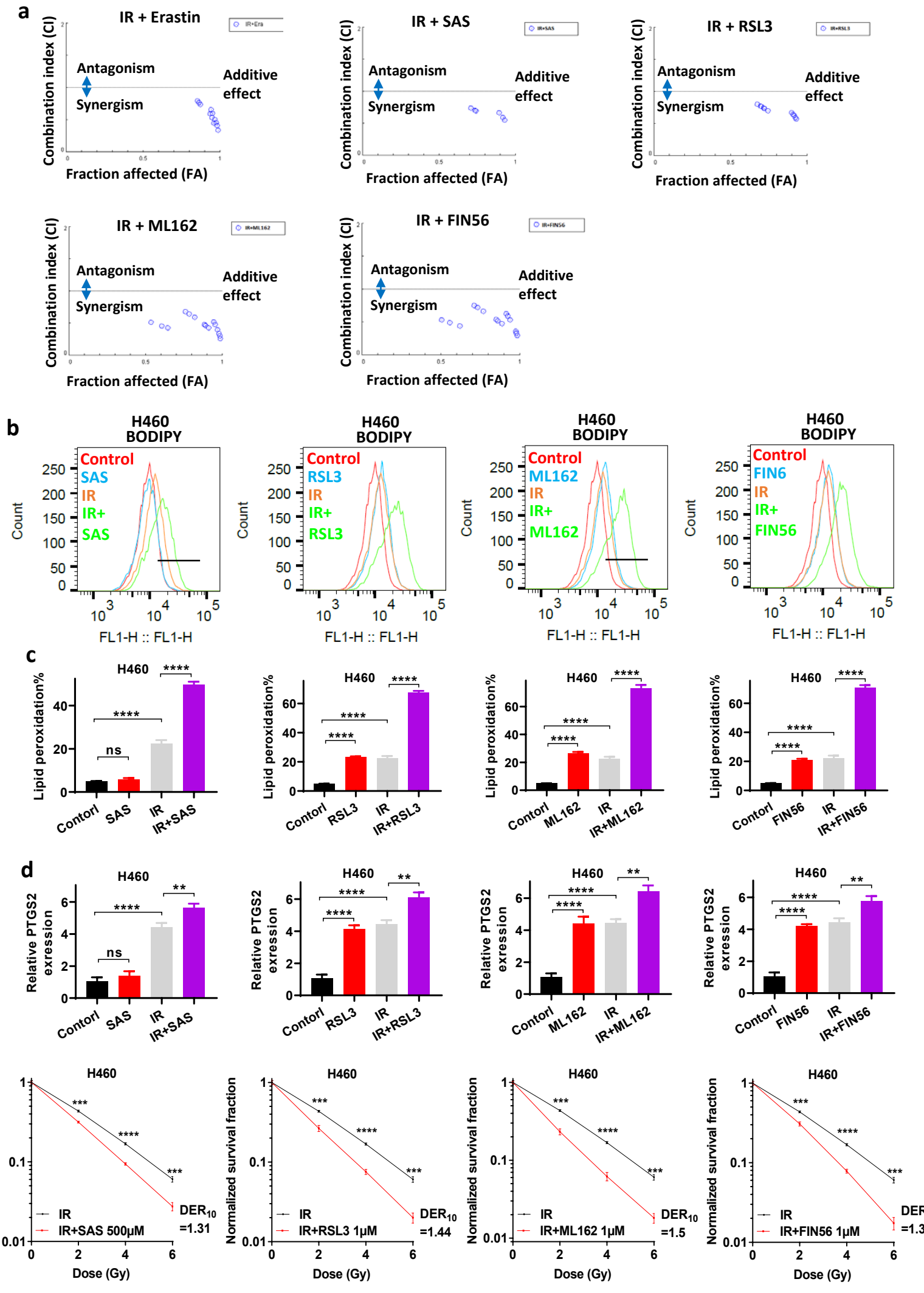
Fig. S5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IR + Erastin
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IR + RSL3
IR + ML162
IR + FIN56
Combination index (CI)
Antagonism
Synergism
Additive effect
Fraction affected (FA)
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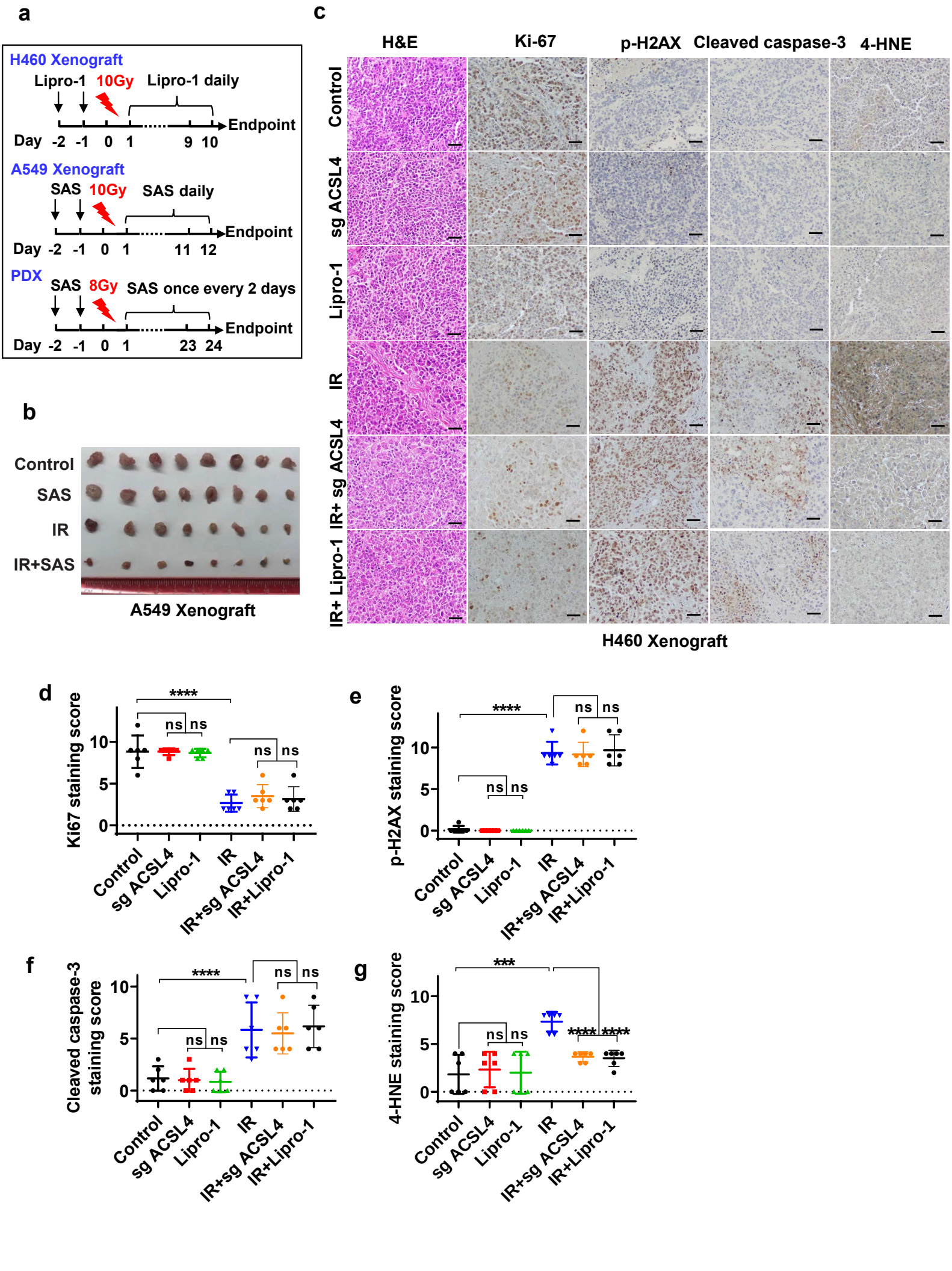
H460 BODIPY
Control
SAS
IR
IR+ SAS
RSL3
IR+ RSL3
ML162
IR+ ML162
FIN6
FIN56
IR+ FIN56
Count
FL1-H :: FL1-H
c
H460
Lipid peroxidation%
Contorl
SAS
IR+SAS
RSL3
IR+RSL3
ML162
IR+ML162
FIN56
IR+FIN56
ns
d
Relative PTGS2 exression
e
Normalized survival fraction
Dose (Gy)
IR
IR+SAS 500μM
IR+RSL3 1μM
IR+ML162 1μM
IR+FIN56 1μM
DER10 =1.31
DER10 =1.44
DER10 =1.5
DER10 =1.39

Fig. S6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A549 Xenograft
SAS
10Gy
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Endpoint
Day -2 -1 0 1 11 12
PDX
SAS
8Gy
SAS once every 2 days
Endpoint
Day -2 -1 0 1 23 24
b
Control
SAS
IR
IR+SAS
A549 Xenograft
c
H&E
Ki-67
p-H2AX
Cleaved caspase-3
4-HNE
Control
sg ACSL4
Lipro-1
IR
IR+ sg ACSL4
IR+ Lipro-1
H460 Xenograft
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Ki67 staining score
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Cleaved caspase-3 staining score
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Control
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Lipro-1
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