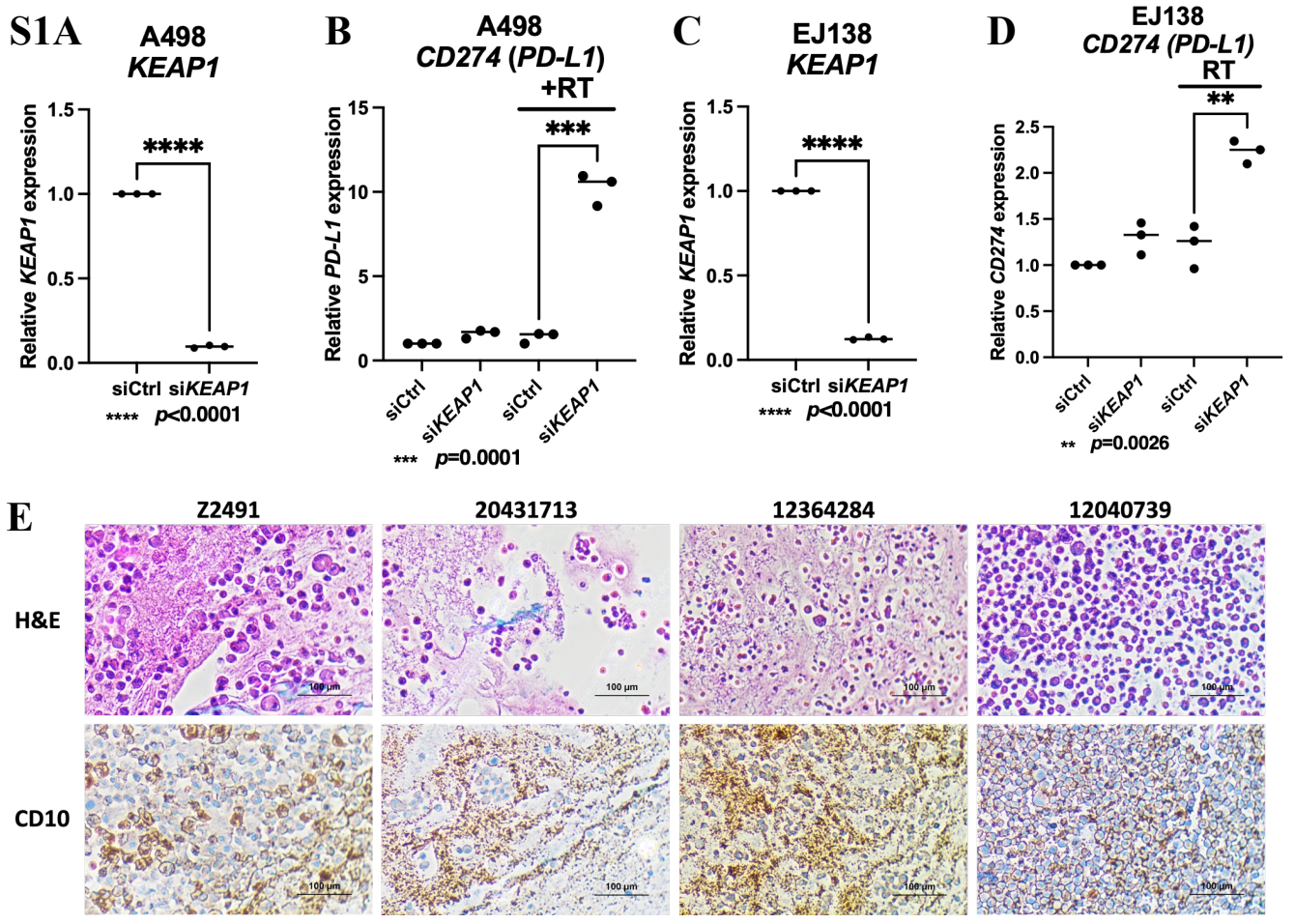
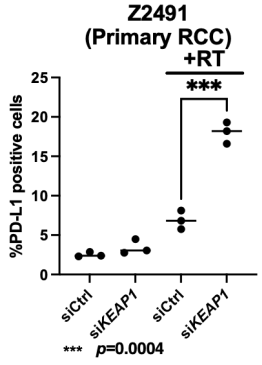
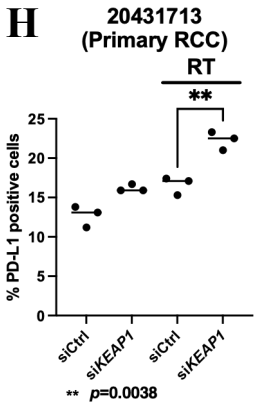

S1A
A498
KEAP1
Relative KEAP1 expression
1.5
1.0
0.5
0.0
****
siCtrl siKEAP1
**** p<0.0001
B
A498
CD274 (PD-L1)
+RT
Relative PD-L1 expression
15
10
5
0
***
siCtrl
siKEAP1
siCtrl
siKEAP1
*** p=0.0001
C
EJ138
KEAP1
Relative KEAP1 expression
1.5
1.0
0.5
0.0
****
siCtrl siKEAP1
**** p<0.0001
D
EJ138
CD274 (PD-L1)
RT
**
Relative CD274 expression
2.5
2.0
1.5
1.0
0.5
0.0
siCtrl
siKEAP1
siCtrl
siKEAP1
** p=0.0026
E
Z2491
20431713
12364284
12040739
H&E
CD10
100 µm
F
Z2491
20431713
12364284
12040739
+RT
SiCtrl
siKEAP1
siCtrl
siKEAP1
KEAP1
PD-L1
GAPDH

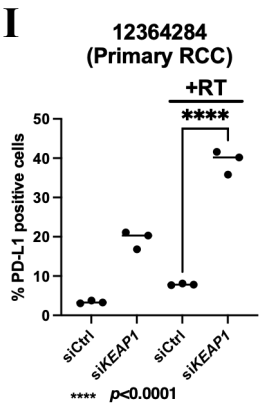
S1G



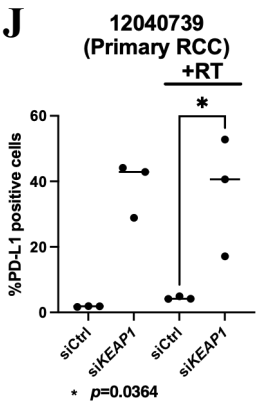
H



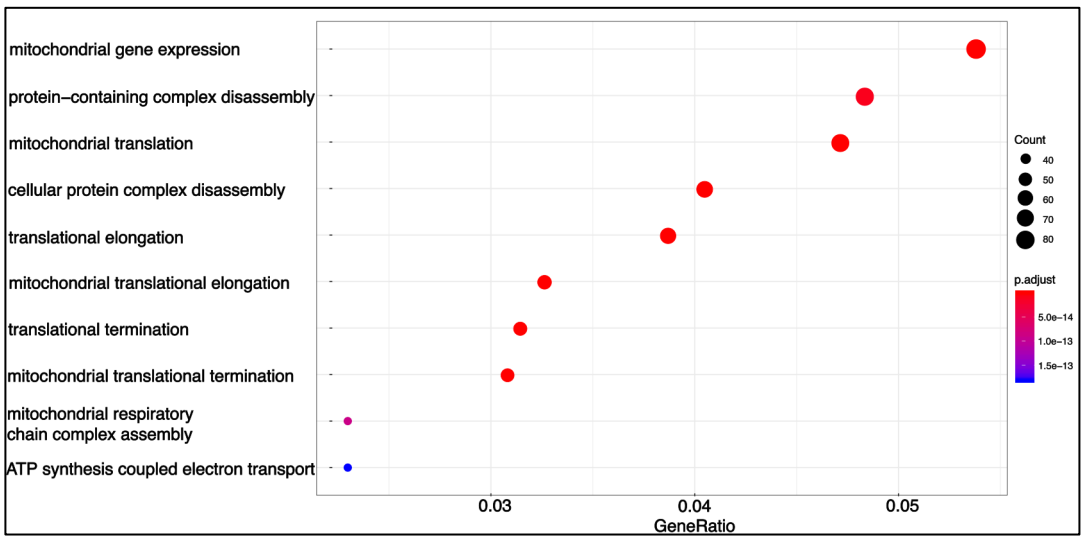
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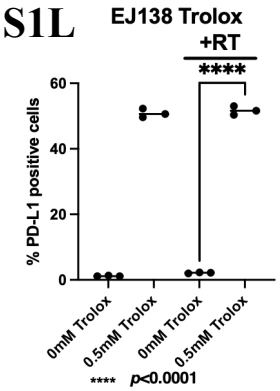
J



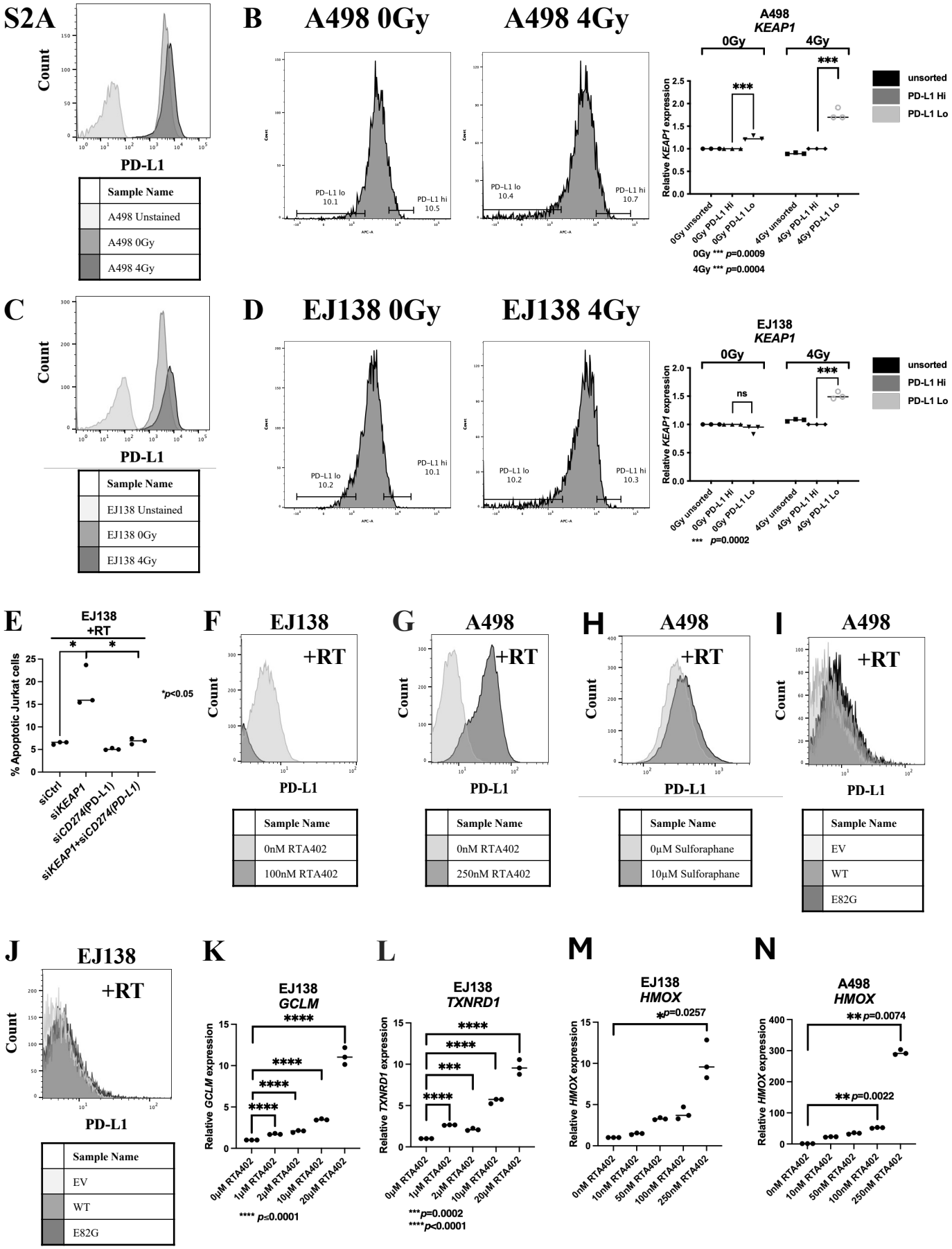
S1K



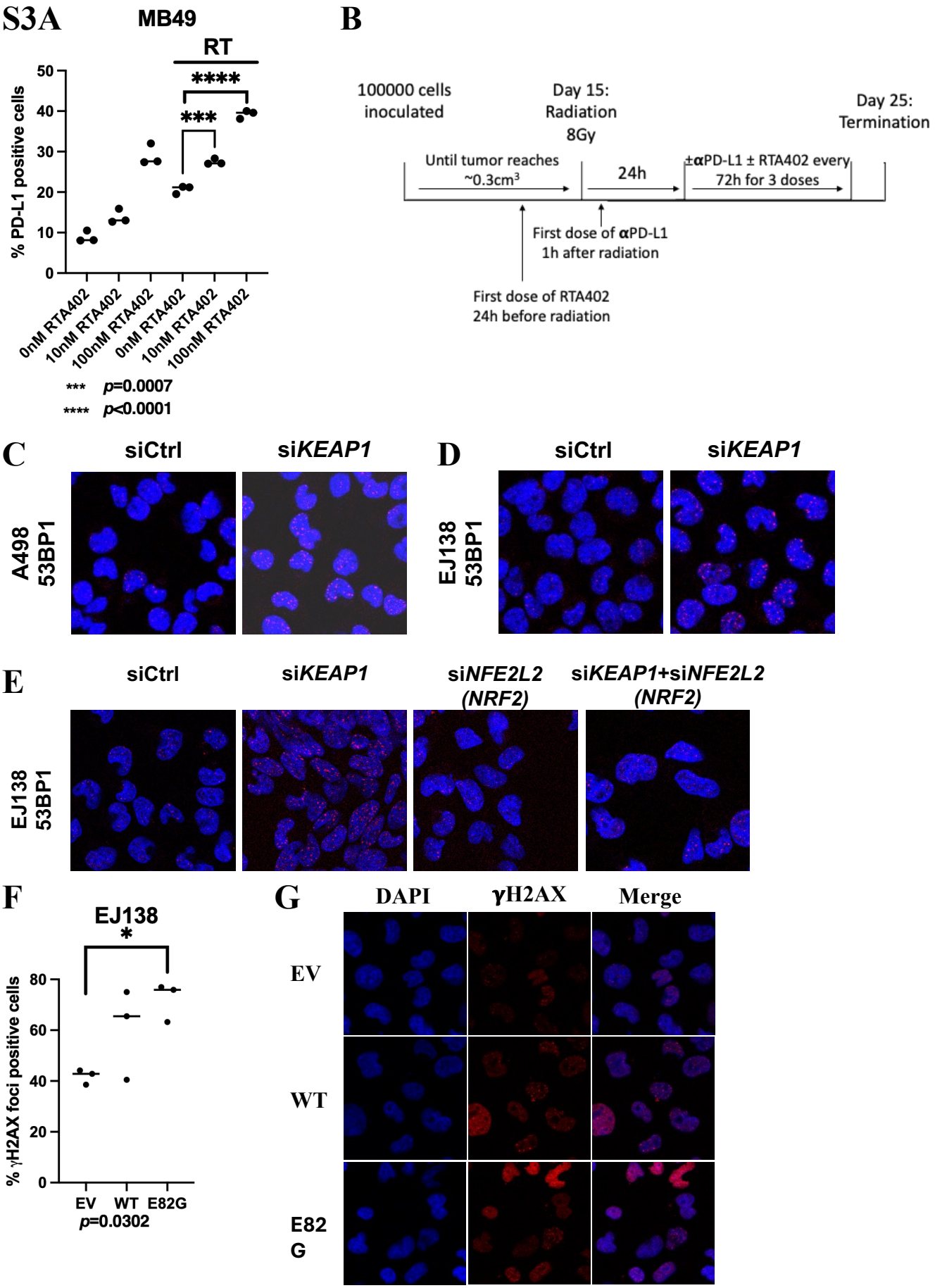
S1L



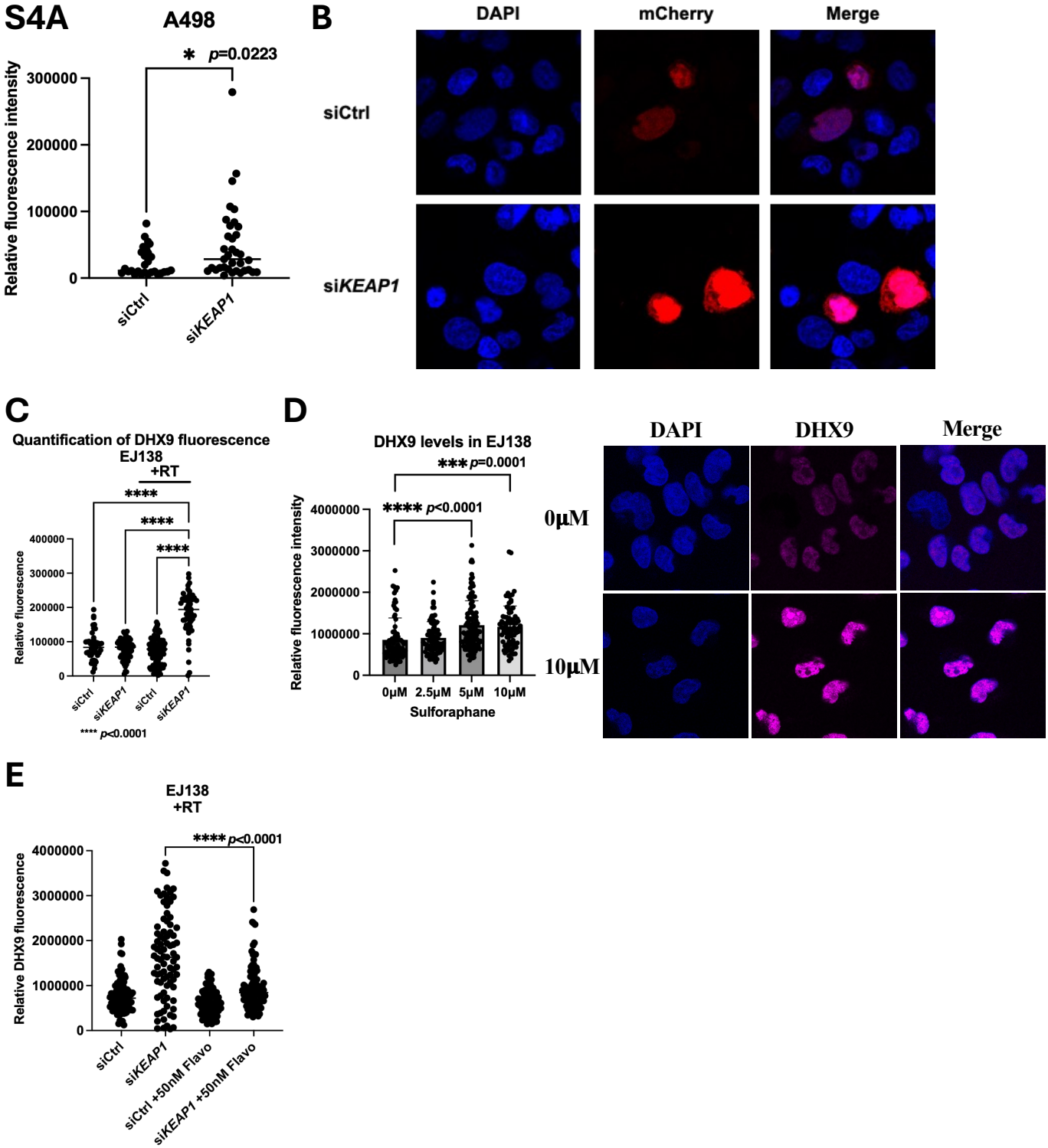
S1A) Quantitative real-time PCR (qPCR) analysis in A498 cells showing decrease in *KEAP1* expression after *KEAP1* silencing by siRNA. B) qPCR analysis of *CD274* (PD-L1) expression in A498 cells treated with and without RT, with and without si*KEAP1*. C) qPCR analysis in EJ138 cells showing decrease in *KEAP1* expression after *KEAP1* silencing by siRNA. D) qPCR analysis of *CD274* (PD-L1) expression in EJ138 cells treated with and without RT, with and without si*KEAP1*. (Unpaired t-test was used to determine statistical significance in S1A-D). E) Hematoxylin and eosin, as well as CD10 IHC stains of patient-derived RCC cell lines Z2491, 20431713, 12364284 and 12040739. F) Western blot of KEAP1, PD-L1 and GAPDH in patient-derived RCC cell lines Z2491, 20431713, 12364284 and 12040739 treated with and without RT, with and without si*KEAP1*. G-J) Flow cytometry experiment measuring PD-L1 expression in patient-derived RCC cell lines Z2491, 20431713, 12364284 and 12040739 treated with and without RT, with and without si*KEAP1* (unpaired t-test). K) Plot showing enriched pathways that lead to PD-L1 upregulation when lost in the CRISPR screen. L) Flow cytometry experiment measuring PD-L1 expression in bladder cancer cells EJ138 following treatment with 4Gy of radiation and 0.5mM of Trolox (unpaired t-test).


S2A
Count
PD-L1
Sample Name
A498 Unstained
A498 0Gy
A498 4Gy
B
A498 0Gy
A498 4Gy
PD-L1 lo 10.1
PD-L1 hi 10.5
PD-L1 lo 10.4
PD-L1 hi 10.7
A498 KEAP1
0Gy
4Gy
Relative KEAP1 expression
unsorted
PD-L1 Hi
PD-L1 Lo
0Gy unsorted
0Gy PD-L1 Hi
0Gy PD-L1 Lo
4Gy unsorted
4Gy PD-L1 Hi
4Gy PD-L1 Lo
0Gy *** p=0.0009
4Gy *** p=0.0004
C
Count
PD-L1
Sample Name
EJ138 Unstained
EJ138 0Gy
EJ138 4Gy
D
EJ138 0Gy
EJ138 4Gy
PD-L1 lo 10.2
PD-L1 hi 10.1
PD-L1 lo 10.2
PD-L1 hi 10.3
EJ138 KEAP1
ns
*** p=0.0002
E
EJ138 +RT
% Apoptotic Jurkat cells
*p<0.05
siCtrl
siKEAP1
siCD274(PD-L1)
siKEAP1+siCD274(PD-L1)
F
EJ138
+RT
Count
PD-L1
Sample Name
0nM RTA402
100nM RTA402
G
A498
+RT
Count
PD-L1
Sample Name
0nM RTA402
250nM RTA402
H
A498
+RT
Count
PD-L1
Sample Name
0μM Sulforaphane
10μM Sulforaphane
I
A498
+RT
Count
PD-L1
Sample Name
EV
WT
E82G
J
EJ138
+RT
Count
PD-L1
Sample Name
EV
WT
E82G
K
EJ138 GCLM
Relative GCLM expression
0μM RTA402
1μM RTA402
2μM RTA402
10μM RTA402
20μM RTA402
**** p≤0.0001
L
EJ138 TXNRD1
Relative TXNRD1 expression
0μM RTA402
1μM RTA402
2μM RTA402
10μM RTA402
20μM RTA402
***p=0.0002
****p<0.0001
M
EJ138 HMOX
*p=0.0257
Relative HMOX expression
0nM RTA402
10nM RTA402
50nM RTA402
100nM RTA402
250nM RTA402
N
A498 HMOX
**p=0.0074
**p=0.0022
Relative HMOX expression
0nM RTA402
10nM RTA402
50nM RTA402
100nM RTA402
250nM RTA402

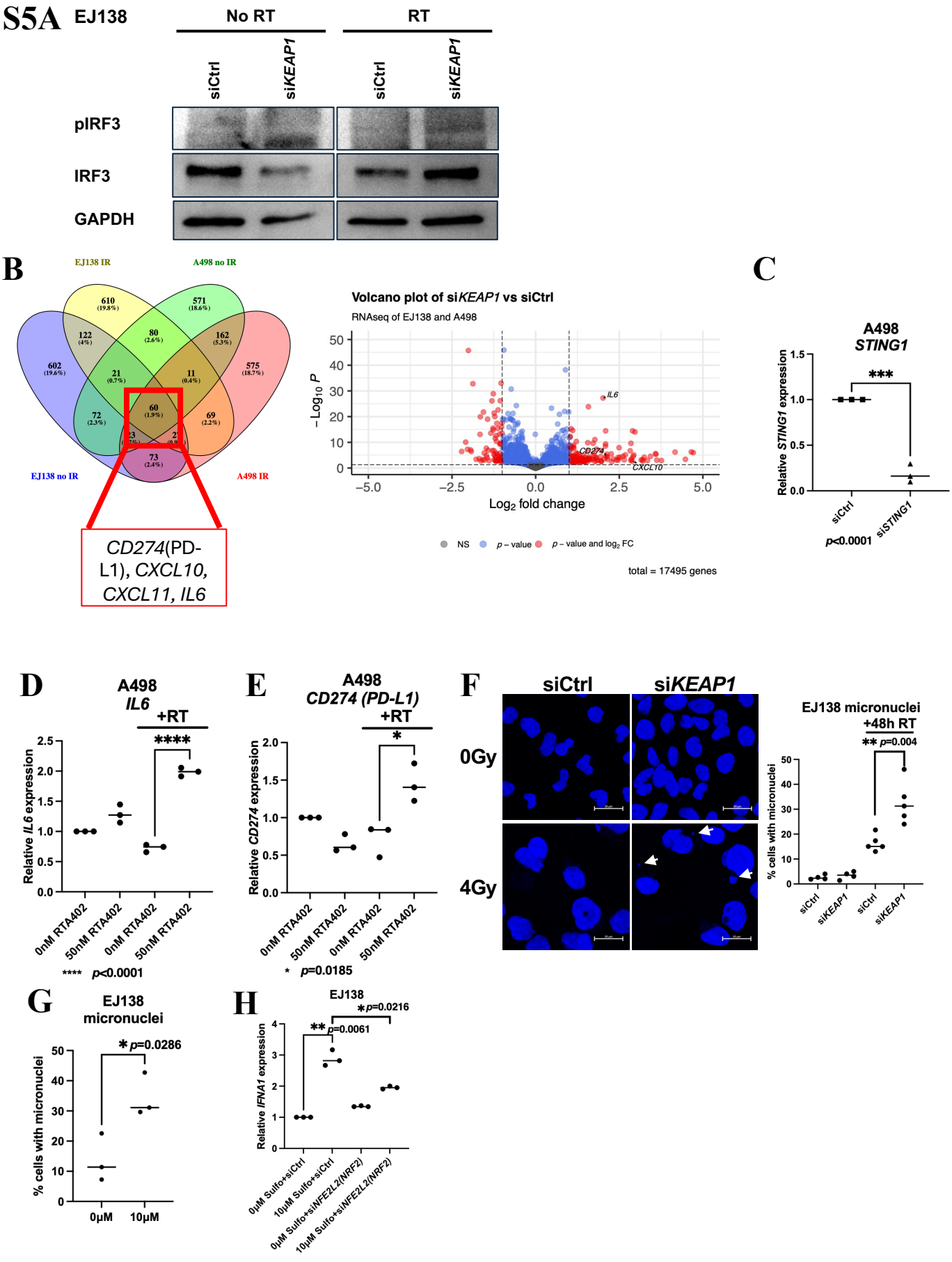
S2A) Flow cytometry plot of unstained A498 cells and A498 cells with or without RT treatment B) Flow cytometry plot showing the sorting gates for the top and bottom 10% PD-L1 expressing A498 cells with or without RT treatment, collected for quantitative real-time PCR analysis of *KEAP1* expression. C) Flow cytometry plot of unstained EJ138 cells and EJ138 cells with or without RT treatment. D) Flow cytometry plot showing the sorting gates for the top and bottom 10% PD-L1 expressing EJ138 cells with or without RT treatment, collected for quantitative real-time PCR analysis of *KEAP1* expression. E) Flow cytometry analysis comparing apoptosis levels in PI-stained Jurkat cells co-cultured with EJ138 cells treated with RT and siCtrl, si*KEAP1*, si*CD274* (PD-L1) or si*KEAP1*+si*CD274* (PD-L1). (Unpaired t-test was used to determine statistical significance in S2B, D and E). F) Representative PD-L1 flow cytometry plots of EJ138 treated with RT, with and without RTA402 treatment, relating to Figure 2C. G) Representative PD-L1 flow cytometry plots of A498 treated with RT, with and without RTA402 treatment, relating to Figure 2D. H) Representative PD-L1 flow cytometry plots of A498 treated with RT, with and without Sulforaphane treatment, relating to Figure 2E. I) Representative PD-L1 flow cytometry plots of A498 treated with RT, transfected with either Empty Vector (EV), or plasmid encoding Wild-Type NRF2 (WT) or E82G NRF2 mutant (E82G), relating to Figure 2G. J) Representative PD-L1 flow cytometry plots of EJ138 treated with RT, transfected with either Empty Vector (EV), or plasmid encoding Wild-Type NRF2 (WT) or E82G NRF2 mutant (E82G), relating to Figure 2H. K-N) Quantitative real-time PCR analysis of NRF2 target genes *GCLM* (K), *TXNRD1* (L) and *HMOX* (M) in EJ138 cells and A498 (N) treated with increasing concentrations of RTA402. (Unpaired t-test was used to determine statistical significance in S2K-N).


S3A
MB49
RT
****
***
% PD-L1 positive cells
50
40
30
20
10
0
0nM RTA402
10nM RTA402
100nM RTA402
0nM RTA402
10nM RTA402
100nM RTA402
*** p=0.0007
**** p<0.0001
B
100000 cells inoculated
Day 15: Radiation 8Gy
Day 25: Termination
Until tumor reaches ~0.3cm3
24h
±αPD-L1 ± RTA402 every 72h for 3 doses
First dose of αPD-L1 1h after radiation
First dose of RTA402 24h before radiation
C
siCtrl
siKEAP1
A498 53BP1
D
siCtrl
siKEAP1
EJ138 53BP1
E
siCtrl
siKEAP1
siNFE2L2 (NRF2)
siKEAP1+siNFE2L2 (NRF2)
EJ138 53BP1
F
EJ138
*
% γH2AX foci positive cells
80
60
40
20
0
EV
WT
E82G
p=0.0302
G
DAPI
γH2AX
Merge
EV
WT
E82 G

S3A) Flow cytometry measuring PD-L1 positivity in MB49 cells with increasing doses of RTA402 treatment with and without RT (unpaired t-test). B) Experiment design for C57BL/6 mice inoculated with MB49 tumors and treated with RT, PD-L1 inhibitor and RTA402. C) Representative images of A498 cells stained with 53BP1, relating to Figure 3C. D) Representative images of EJ138 cells stained with 53BP1, relating to Figure 3D. E) Representative images of EJ138 cells stained with 53BP1, relating to Figure 3E. F) Comparison of % **γ**H2AX positive cells in EJ138 cells transfected with either Empty Vector (EV), or plasmid encoding wild type NRF2 or E82G NRF2 with RT (One-way ANOVA, followed by Dunnett's test to correct for multiple comparisons). G) Representative images of EJ138 cells stained with **γ**H2AX.

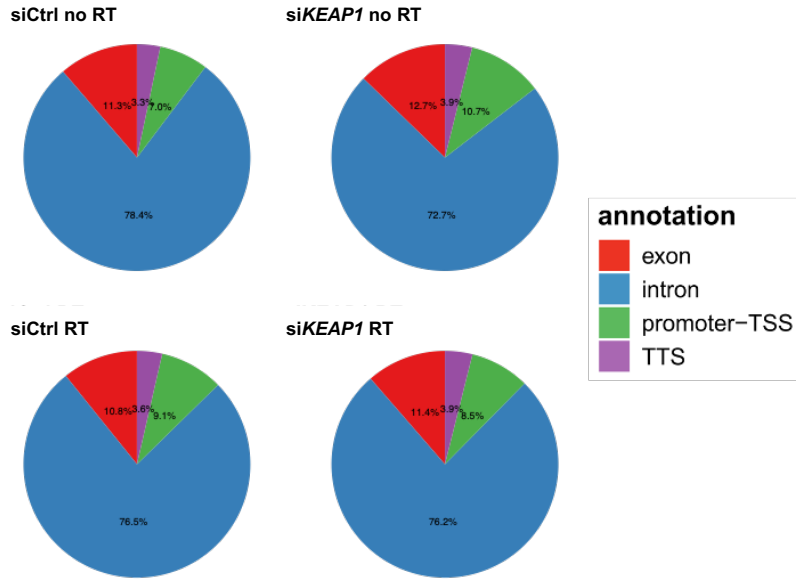

S4A
A498
* p=0.0223
Relative fluorescence intensity
300000
200000
100000
0
siCtrl
siKEAP1
B
DAPI
mCherry
Merge
siCtrl
siKEAP1
C
Quantification of DHX9 fluorescence
EJ138
+RT
****
****
****
Relative fluorescence
400000
300000
200000
100000
0
siCtrl
siKEAP1
siCtrl
siKEAP1
**** p<0.0001
D
DHX9 levels in EJ138
*** p=0.0001
**** p<0.0001
Relative fluorescence intensity
4000000
3000000
2000000
1000000
0
0μM 2.5μM 5μM 10μM
Sulforaphane
DAPI
DHX9
Merge
0μM
10μM
E
EJ138
+RT
**** p<0.0001
Relative DHX9 fluorescence
4000000
3000000
2000000
1000000
0
siCtrl
siKEAP1
siCtrl +50nM Flavo
siKEAP1 +50nM Flavo

S4A) Comparison of relative mCherry-mutantRNaseH1 fluorescence intensity in A498 cells transfected with either siCtrl or si*KEAP1*, as a readout of R-loop levels in cells (unpaired t-test). B) Representative images of A498 cells transfected with mCherry-mutantRNaseH1 showing increased mCherry fluorescence intensity with *KEAP1* silencing. C) Comparison of DHX9 fluorescence in EJ138 cells transfected with either siCtrl or si*KEAP1* with or without RT (unpaired t-test). D) Comparison of DHX9 fluorescence in EJ138 cells treated with increasing doses of sulforaphane 4h after RT (left) and representative images of DHX9 fluorescence in EJ138 cells with or without sulforaphane treatment 4h after RT (right) (unpaired t-test). E) Comparison of DHX9 fluorescence in EJ138 cells treated with RT and transfected with either siCtrl or si*KEAP1* with or without 50nM flavopiridol (unpaired t-test).

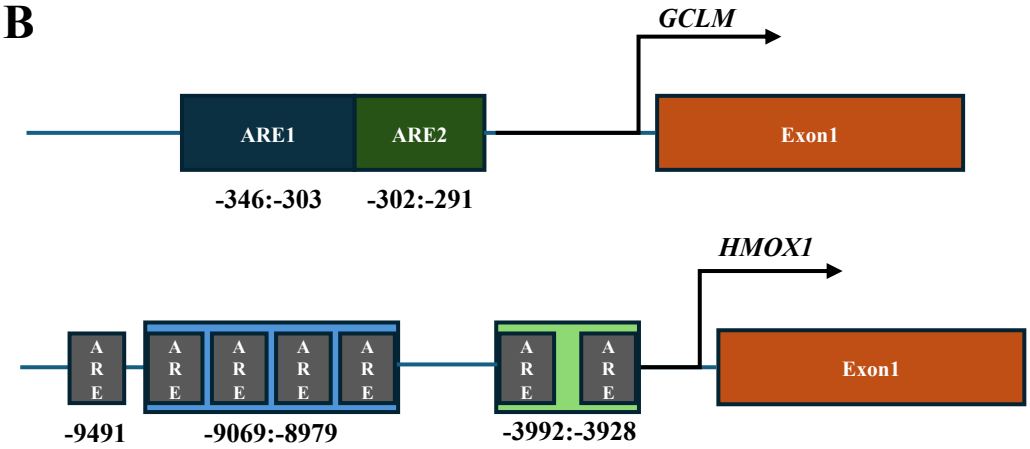

S5A
EJ138
No RT
RT
siCtrl
siKEAP1
siCtrl
siKEAP1
pIRF3
IRF3
GAPDH
B
EJ138 IR
A498 no IR
610 (19.8%)
571 (18.6%)
122 (4%)
80 (2.6%)
162 (5.3%)
602 (19.6%)
21 (0.7%)
11 (0.4%)
575 (18.7%)
72 (2.3%)
60 (1.9%)
69 (2.2%)
73 (2.4%)
EJ138 no IR
A498 IR
CD274(PD-L1), CXCL10, CXCL11, IL6
Volcano plot of siKEAP1 vs siCtrl
RNAseq of EJ138 and A498
−Log10 P
Log2 fold change
IL6
CD274
CXCL10
NS
p – value
p – value and log2 FC
total = 17495 genes
C
A498
STING1
Relative STING1 expression
***
siCtrl
siSTING1
p<0.0001
D
A498
IL6
+RT
****
Relative IL6 expression
0nM RTA402
50nM RTA402
0nM RTA402
50nM RTA402
**** p<0.0001
E
A498
CD274 (PD-L1)
+RT
*
Relative CD274 expression
0nM RTA402
50nM RTA402
0nM RTA402
50nM RTA402
* p=0.0185
F
siCtrl
siKEAP1
0Gy
4Gy
EJ138 micronuclei
+48h RT
** p=0.004
% cells with micronuclei
siCtrl
siKEAP1
siCtrl
siKEAP1
G
EJ138
micronuclei
* p=0.0286
% cells with micronuclei
0μM
10μM
H
EJ138
* p=0.0216
** p=0.0061
Relative IFNA1 expression
0μM Sulfo+siCtrl
10μM Sulfo+siCtrl
0μM Sulfo+siNFE2L2(NRF2)
10μM Sulfo+siNFE2L2(NRF2)

S5A) Western blot of pIRF3, IRF3 and GAPDH in EJ138 cells transfected with either siCtrl or si*KEAP1* with and without RT. B) Left: Venn diagram showing that the top genes upregulated in EJ138 and A498 cells after *KEAP1* silencing, with or without radiation treatment are *CD274* (PD-L1), *IL6*, *CXCL10* and *CXCL11*. Yellow: EJ138 cells after radiation treatment. Blue: EJ138 cells without radiation treatment. Green: A498 cells after radiation treatment. Red: A498 cells without radiation treatment. Right: Volcano plot showing the genes of interest plotted against *p*-value and log2FC (Wald test). C) Quantitative real time PCR analysis of *STING1* in A498 cells transfected with either siCtrl or si*STING1*. D-E) Quantitative real time PCR analysis of *IL6* (D) and *CD274* (PD-L1) (E) in A498 cells after treatment with 0nM or 50nM of RTA402, with or without radiation treatment. (Unpaired t-test was used to determine statistical significance in S5C-E) F) Representative images of micronuclei (left) and quantification of micronuclei in EJ138 cells treated with either siCtrl or si*KEAP1*, with or without radiation treatment (right). G) Comparison of micronuclei levels in EJ138 cells treated with or without sulforaphane treatment 48h after RT (unpaired t-test). H) Quantitative real time PCR analysis of *IFNA1* in EJ138 cells treated with RT and transfected with either siCtrl or si*NFE2L2* (siNRF2), with and without 10μM sulforaphane treatment (unpaired t-test).

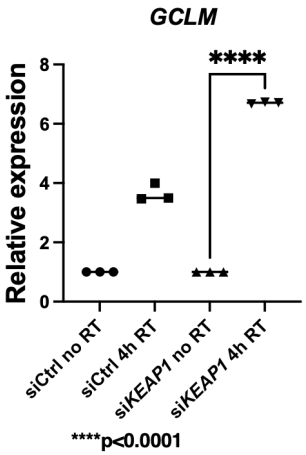
S6A


siCtrl no RT
siKEAP1 no RT
siCtrl RT
siKEAP1 RT
11.3%
3.3%
7.0%
78.4%
12.7%
3.9%
10.7%
72.7%
10.8%
3.6%
9.1%
76.5%
11.4%
3.9%
8.5%
76.2%
annotation
exon
intron
promoter−TSS
TTS

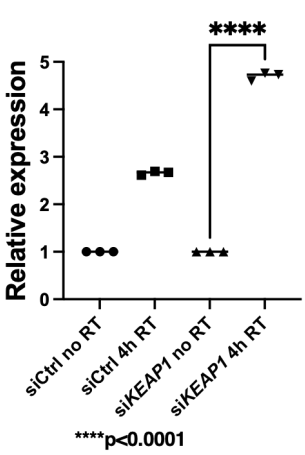
B


GCLM
ARE1
ARE2
Exon1
-346:-303
-302:-291
HMOX1
ARE
Exon1
-9491
-9069:-8979
-3992:-3928

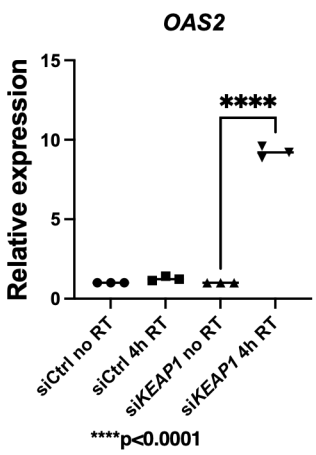
C


GCLM
Relative expression
****
siCtrl no RT
siCtrl 4h RT
siKEAP1 no RT
siKEAP1 4h RT
****p<0.0001

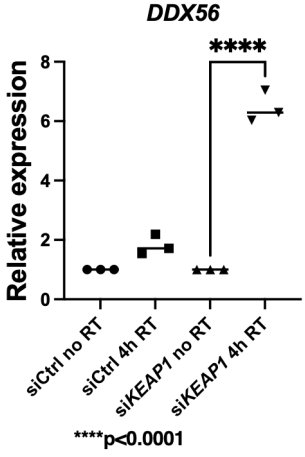
D


HMOX1
Relative expression
****
siCtrl no RT
siCtrl 4h RT
siKEAP1 no RT
siKEAP1 4h RT
****p<0.0001

E

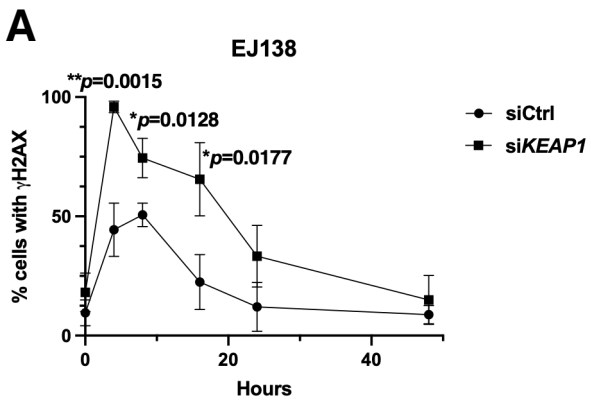

OAS2
Relative expression
****
siCtrl no RT
siCtrl 4h RT
siKEAP1 no RT
siKEAP1 4h RT
****p<0.0001

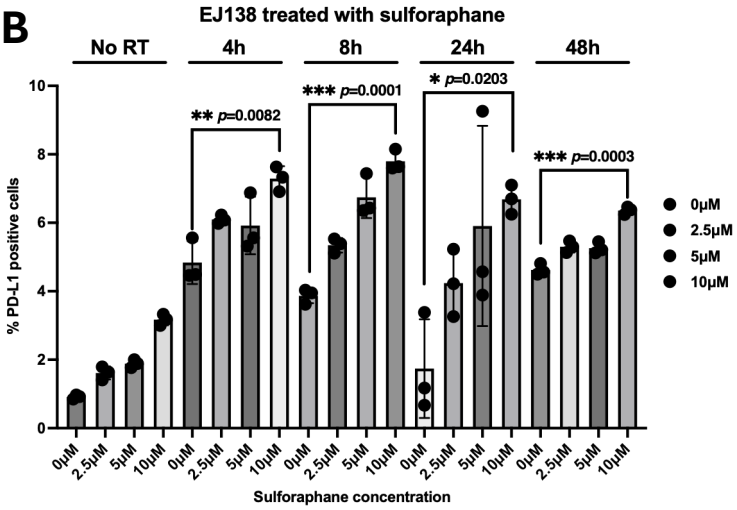
F

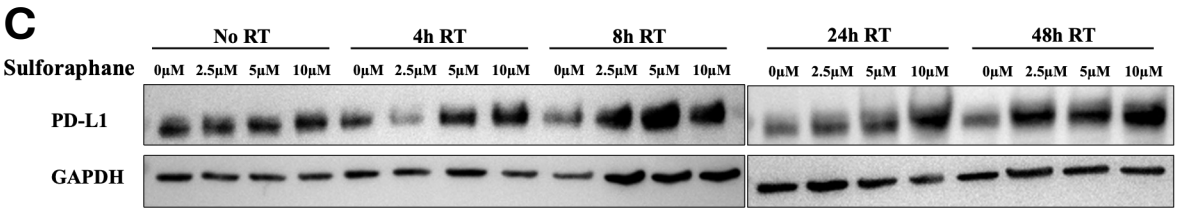

DDX56
Relative expression
****
siCtrl no RT
siCtrl 4h RT
siKEAP1 no RT
siKEAP1 4h RT
****p<0.0001

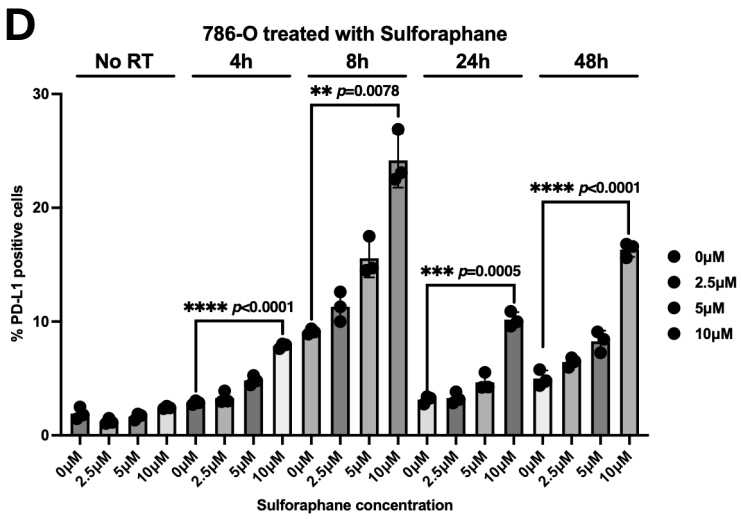
S6A) Pie chart showing R-loop ratio at different gene structural elements. B) Schematic of NRF2 target genes *GCLM* and *HMOX* showing ARE (Antioxidant Response Element) site(s). C-F) Quantitative real time PCR analysis of *GCLM* (B), *HMOX1* (C), *OAS2* (D) and *DDX56* (E). (Unpaired t-test was used to determine statistical significance in S6B-E).

S7


A
EJ138
**p=0.0015
*p=0.0128
*p=0.0177
siCtrl
siKEAP1
% cells with γH2AX
Hours


B
EJ138 treated with sulforaphane
No RT
4h
8h
24h
48h
** p=0.0082
*** p=0.0001
* p=0.0203
*** p=0.0003
% PD-L1 positive cells
0μM
2.5μM
5μM
10μM
Sulforaphane concentration


C
No RT
4h RT
8h RT
24h RT
48h RT
Sulforaphane
0μM 2.5μM 5μM 10μM
PD-L1
GAPDH


D
786-O treated with Sulforaphane
No RT
4h
8h
24h
48h
** p=0.0078
**** p<0.0001
*** p=0.0005
**** p<0.0001
% PD-L1 positive cells
0μM
2.5μM
5μM
10μM
Sulforaphane concentration

S7A) Comparison of γH2AX foci in EJ138 cells transfected with either control siRNA or si*KEAP1*, with no RT and at 4h, 8h, 16h, 24h and 48h post RT (unpaired t-test). B) Comparison of %PD-L1 positive EJ138 cells treated with increasing concentrations of sulforaphane with no RT and at 4h, 8h, 16h, 24h and 48h post RT with flow cytometry (unpaired t-test). C) Western blot of PD-L1 and GAPDH in 786-O cells treated with increasing concentrations of sulforaphane with no RT and at 4h, 8h, 16h, 24h and 48h post RT. D) Comparison of %PD-L1 positive 786-O cells treated with increasing concentrations of sulforaphane with no RT and at 4h, 8h, 16h, 24h and 48h post RT with flow cytometry (unpaired t-test).