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Supplemental Information

IKKα Kinase Regulates the DNA Damage Response and Drives Chemo-resistance in Cancer

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Figure S1.

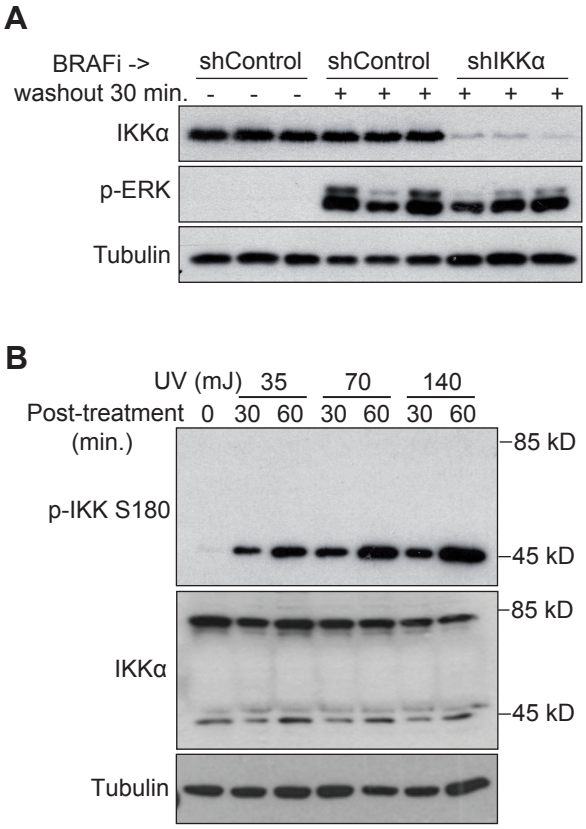
A
BRAFi ->
washout 30 min.
shControl
shControl
shIKKα
- - - + + + + + +
IKKα
p-ERK
Tubulin
B
UV (mJ)
35
70
140
Post-treatment (min.)
0 30 60 30 60 30 60
p-IKK S180
85 kD
45 kD
IKKα
85 kD
45 kD
Tubulin

Fig S1. Related to Figure 1. (**A**) Western blot (WB) analysis of the HT29 cells used in the MS analysis transduced with sh-RNA control or sh-RNA against IKKα. All cell were treated with BRAF inhibitor for 16 hours, washed out (when indicated) and collected 30 minutes after BRAF inhibition release. (**B**) WB analysis of HT29 cells treated with the indicated doses of UV light and collected 30 or 60 minutes after exposure.

Figure S2.

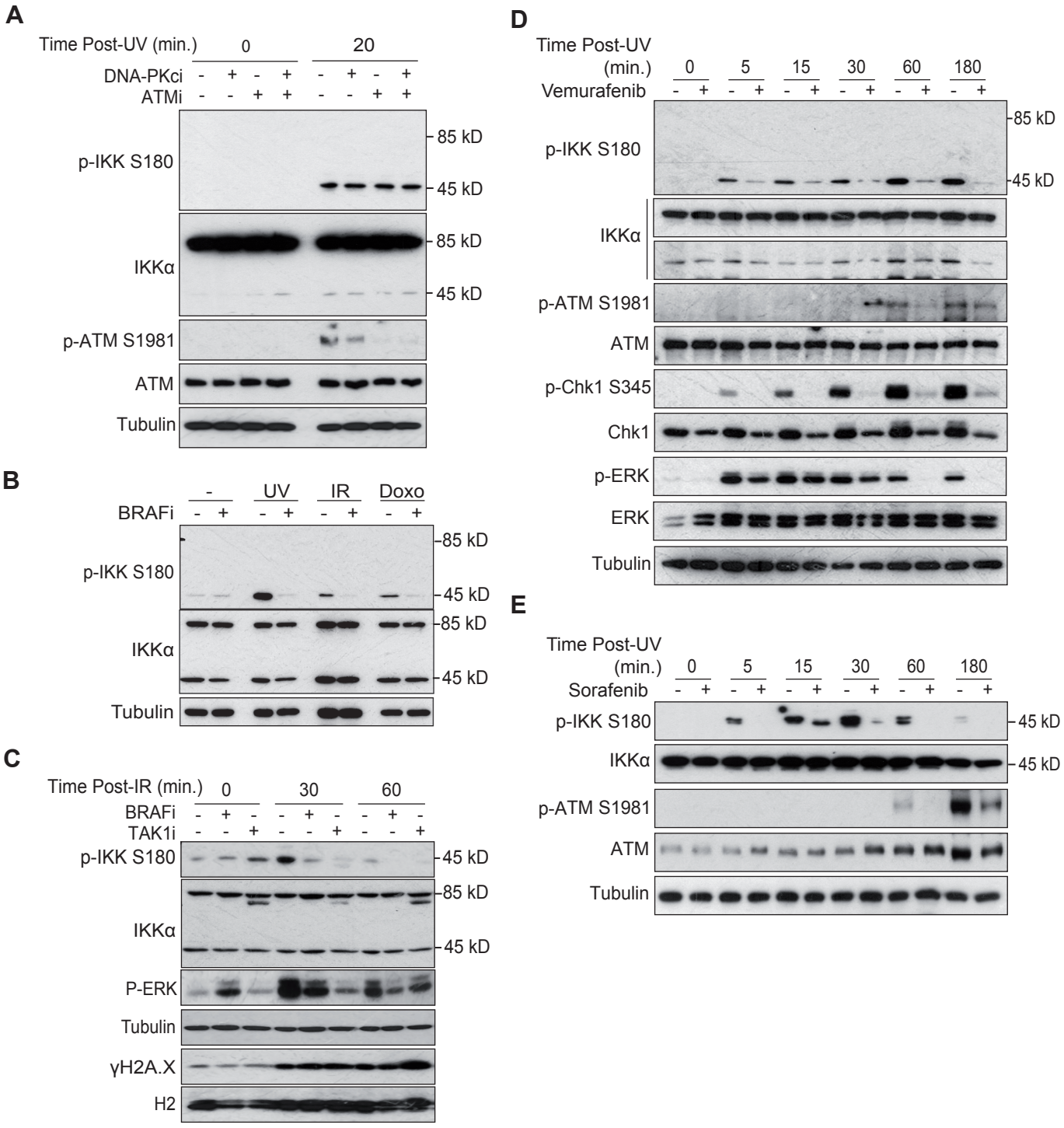
A
Time Post-UV (min.)
0
20
DNA-PKci
ATMi
p-IKK S180
IKKα
p-ATM S1981
ATM
Tubulin
85 kD
45 kD
B
BRAFi
-
UV
IR
Doxo
C
Time Post-IR (min.)
0
30
60
BRAFi
TAK1i
P-ERK
γH2A.X
H2
D
Time Post-UV
(min.)
0
5
15
30
60
180
Vemurafenib
p-Chk1 S345
Chk1
p-ERK
ERK
E
Sorafenib

Fig. S2. Related to Figure 2. (**A**) WB analysis of HT29 cells treated for 16 hours with ATM and/or DNA-PKc inhibitors as indicated. (**B**) WB analysis of HT29 cells pretreated with the BRAF inhibitor AZ628 (16 hours, 10μM) and then exposed to the indicated damaging agents. (**C**) WB analysis of HT29 cells pretreated with the BRAF or TAK1 inhibitors for 16 hours and then exposed to IR for the indicated times. (**D-E**) WB analysis of HT29 cells treated with the BRAF inhibitors vemurafenib (16 hours, 10μM) (**D**) or sorafenib (16 hours, 1μM) **(E)** prior to exposure to UV light for the indicated time points.

Figure S3.

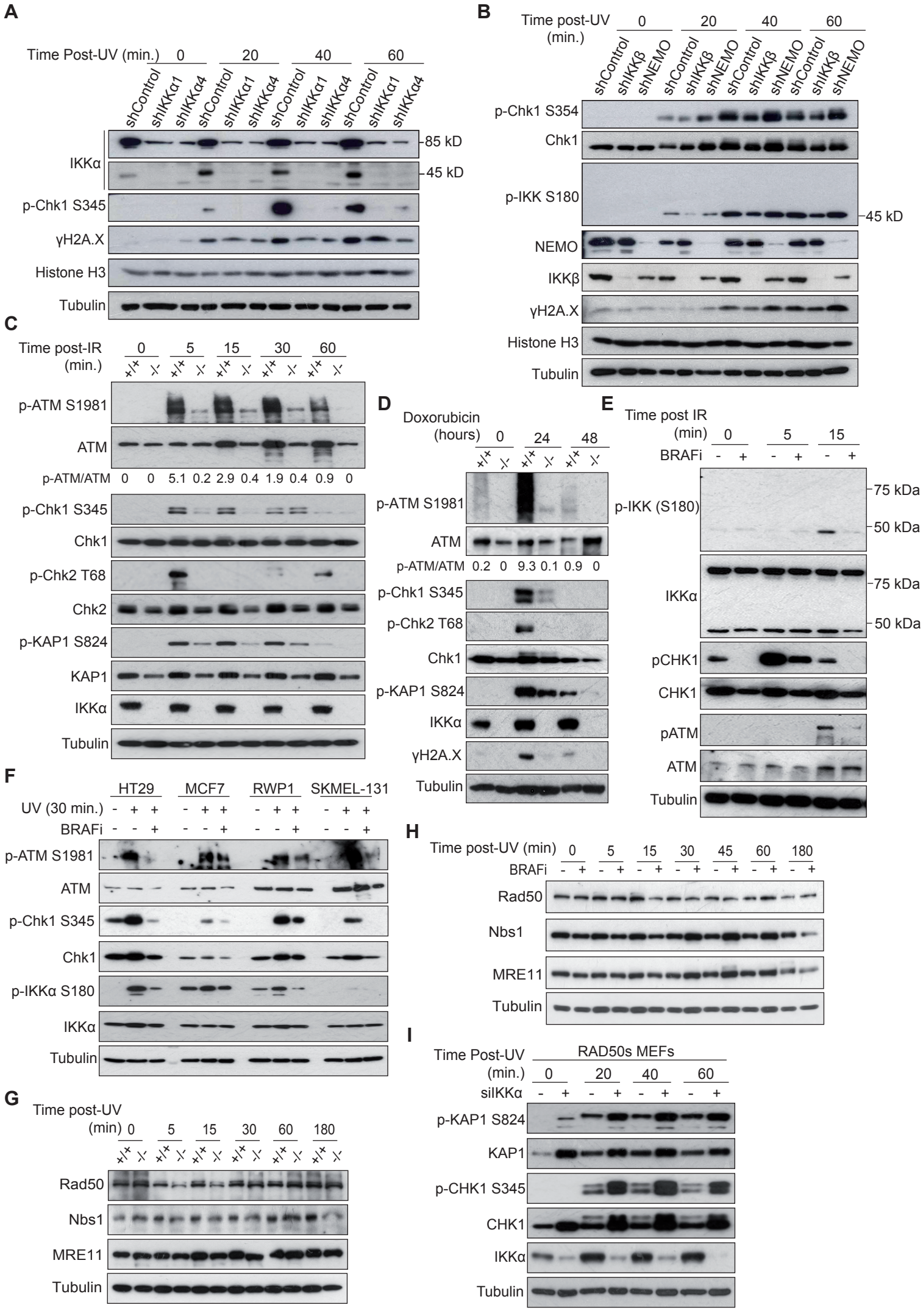
A
Time Post-UV (min.) 0 20 40 60
shControl shIKKα1 shIKKα4 shControl shIKKα1 shIKKα4 shControl shIKKα1 shIKKα4 shControl shIKKα1 shIKKα4
IKKα
85 kD
45 kD
p-Chk1 S345
γH2A.X
Histone H3
Tubulin
B
Time post-UV (min.) 0 20 40 60
shControl shIKKβ shNEMO shControl shIKKβ shNEMO shControl shIKKβ shNEMO shControl shIKKβ shNEMO
p-Chk1 S354
Chk1
p-IKK S180
45 kD
NEMO
IKKβ
γH2A.X
Histone H3
Tubulin
C
Time post-IR (min.) 0 5 15 30 60
+/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/-
p-ATM S1981
ATM
p-ATM/ATM 0 0 5.1 0.2 2.9 0.4 1.9 0.4 0.9 0
p-Chk1 S345
Chk1
p-Chk2 T68
Chk2
p-KAP1 S824
KAP1
IKKα
Tubulin
D
Doxorubicin (hours) 0 24 48
+/+ -/- +/+ -/- +/+ -/-
p-ATM S1981
ATM
p-ATM/ATM 0.2 0 9.3 0.1 0.9 0
p-Chk1 S345
p-Chk2 T68
Chk1
p-KAP1 S824
IKKα
γH2A.X
Tubulin
E
Time post IR (min) 0 5 15
BRAFi - + - + - +
p-IKK (S180)
75 kDa
50 kDa
IKKα
75 kDa
50 kDa
pCHK1
CHK1
pATM
ATM
Tubulin
F
HT29 MCF7 RWP1 SKMEL-131
UV (30 min.) - + + - + + - + + - + +
BRAFi - - + - - + - - + - - +
p-ATM S1981
ATM
p-Chk1 S345
Chk1
p-IKKα S180
IKKα
Tubulin
G
Time post-UV (min) 0 5 15 30 60 180
+/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/-
Rad50
Nbs1
MRE11
Tubulin
H
Time post-UV (min) 0 5 15 30 45 60 180
BRAFi - + - + - + - + - + - + - +
Rad50
Nbs1
MRE11
Tubulin
I
Time Post-UV (min.) RAD50s MEFs 0 20 40 60
siIKKα - + - + - + - +
p-KAP1 S824
KAP1
p-CHK1 S345
CHK1
IKKα
Tubulin

Fig. S3. Related to Figure 3. (**A**) WB analysis of HT29 cells transduced with shRNA control or 2 different IKKα-specific shRNAs, exposed to UV and collected at the indicated time points. (**B**) WB analysis of HT29 cells transduced with sh-RNA control or specific shRNAs against IKKβ and NEMO exposed to UV light and collected at the indicated time points. (**C-D**) WB analysis of *IKKα* WT *(*+/+) and *IKKα* KO *(*-/-) treated with IR (2Gy) **(C)** or doxorubicin (4 μg/ml) (**D**) and collected at the indicated time points. (**E**) WB analysis of HT29 cells pretreated for 16 hours with AZ628 and then exposed to IR as indicated. (**F**) WB analysis of different cancer cell lines pretreated as in E and then exposed to UV light. (**G-H**) WB analysis of the indicated MRN elements in *IKKα* WT (+/+) and KO (-/-) control or UV-treated MEFs **(G)** or BRAF inhibitor-treated HT29 cells **(H)**. (**I**) WB analysis of control and *IKKα*-knocked down RAD50s MEFs treated as indicated.

Figure S4.

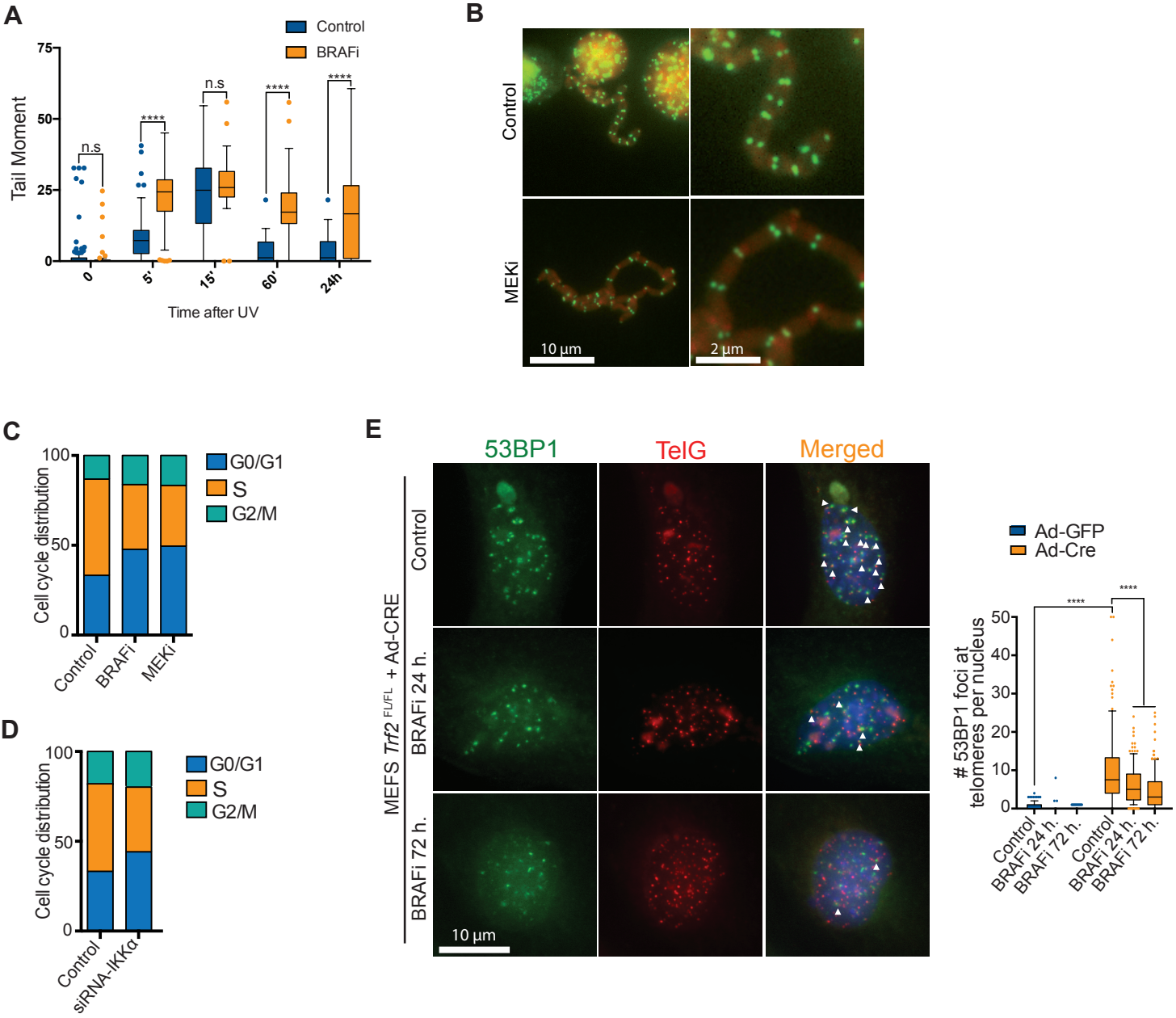
A
Control
BRAFi
Tail Moment
75
50
25
0
n.s
****
n.s
****
****
0
5'
15'
60'
24h
Time after UV
B
Control
MEKi
10 µm
2 µm
C
Cell cycle distribution
100
50
0
G0/G1
S
G2/M
Control
BRAFi
MEKi
D
Cell cycle distribution
100
50
0
G0/G1
S
G2/M
Control
siRNA-IKKα
E
53BP1
TelG
Merged
MEFS Trf2 FL/FL + Ad-CRE
Control
BRAFi 24 h.
BRAFi 72 h.
10 µm
Ad-GFP
Ad-Cre
53BP1 foci at telomeres per nucleus
50
40
30
20
10
0
****
****
Control
BRAFi 24 h.
BRAFi 72 h.
Control
BRAFi 24 h.
BRAFi 72 h.

Fig. S4. Related to Figure 4. (**A**) Comet Assay of HT29 cells treated with AZ628 10μM (BRAFi) at the indicated time points after UV light (130mJ) (**B**) Representative images of FISH analysis from control and MEK-inhibited *Trf2* deficient cells using a telomeric probe (in green). (**C-D**) Cell cycle profiles of Trf2 deficient cells treated as indicated (C) or transfected with specific siRNA against IKKα (D). (**E**) IF followed by telomeric FISH of *Trf2* deficient MEFs control or treated with BRAF inhibitor 24 or 72 hours. Quantification of 53BP1 colocalizing with telomeres is shown in the right panel. For statistical analysis in A, two-way ANOVA was used and the p-values are indicated as ****p<0.0001.

Figure S5.

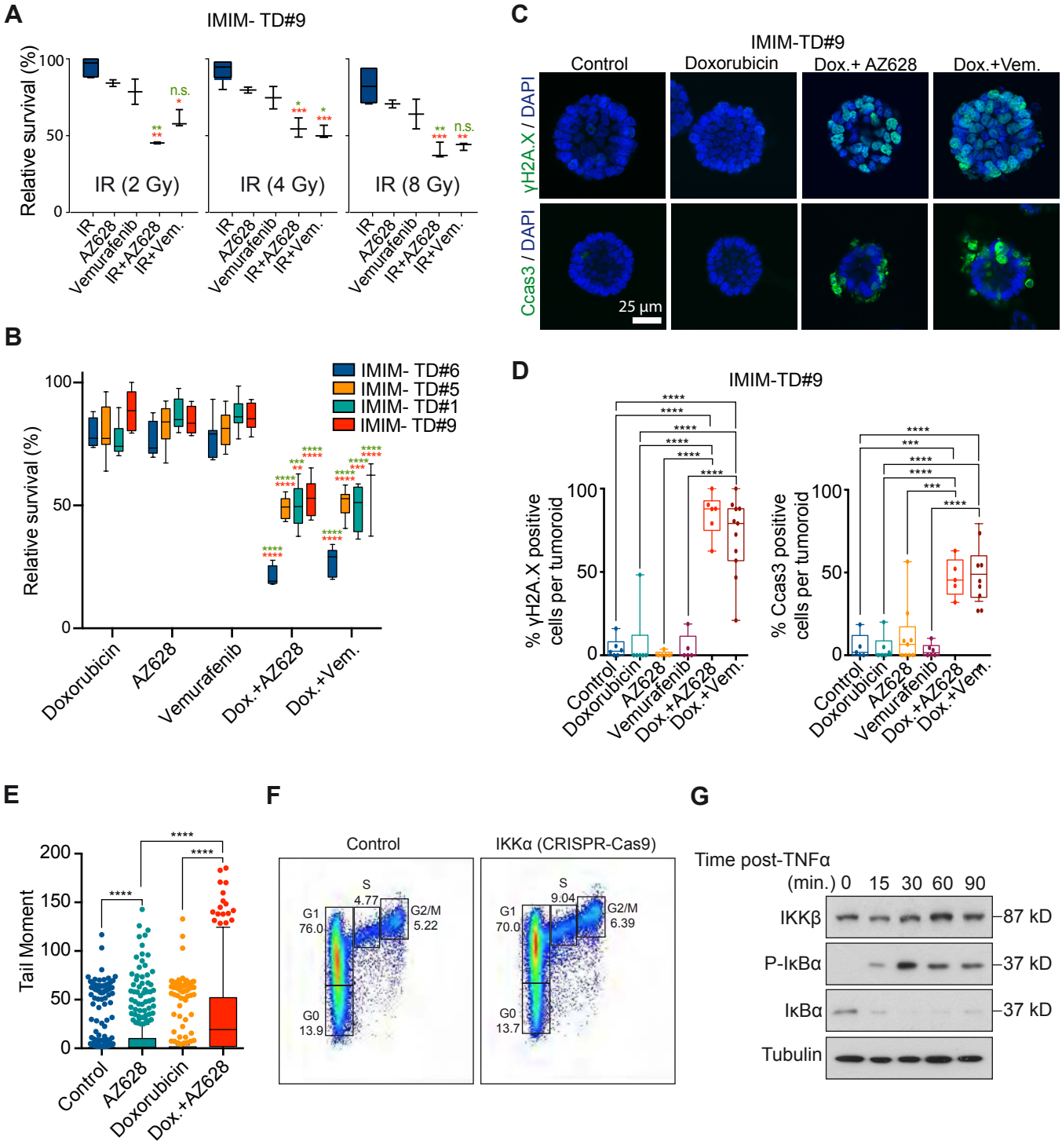
A
IMIM- TD#9
Relative survival (%)
IR (2 Gy)
IR (4 Gy)
IR (8 Gy)
IR
AZ628
Vemurafenib
IR+AZ628
IR+Vem.
n.s.
B
IMIM- TD#6
IMIM- TD#5
IMIM- TD#1
IMIM- TD#9
Relative survival (%)
Doxorubicin
AZ628
Vemurafenib
Dox.+AZ628
Dox.+Vem.
C
IMIM-TD#9
Control
Doxorubicin
Dox.+ AZ628
Dox.+Vem.
γH2A.X / DAPI
Ccas3 / DAPI
25 μm
D
IMIM-TD#9
% γH2A.X positive cells per tumoroid
% Ccas3 positive cells per tumoroid
Control
Doxorubicin
AZ628
Vemurafenib
Dox.+AZ628
Dox.+Vem.
E
Tail Moment
Control
AZ628
Doxorubicin
Dox.+AZ628
F
Control
IKKα (CRISPR-Cas9)
S
4.77
G1
76.0
G2/M
5.22
G0
13.9
S
9.04
G1
70.0
G2/M
6.39
G0
13.7
G
Time post-TNFα
(min.) 0 15 30 60 90
IKKβ
P-IκBα
IκBα
Tubulin
87 kD
37 kD
37 kD

Fig. S5. Related to Figure 5. **(A-B)** Quantification of cell viability by CellTiter-Glo 3D cell viability assay in the indicated tumoroids after 72 hours of treatment at different doses of γ-irradiation (A) or drug combinations (B). (**C-D**) Representative images of γH2A.X and cleaved caspase 3 IF in a representative tumoroid treated as indicated (C) and quantification of the percent of positive cells from 20 tumoroids per condition counted (D). (**E**) Comet Assay quantification from tumoroid IMIM-TD#9 treated with the indicated treatments for 24h. (**F**) Cell cycle profiles of the same tumoroid after CRISPR-Cas9-mediated IKKα depletion. (**G**) WB analysis of IKKα-depleted IMIM-TD#9 treated with TNFα as indicated. Statistical analysis in A, B, D and E was performed by unpaired t-test. In A and B comparison of compound treatments with single IR/Dox or BRAF inhibitors are shown in red and green, respectively. p-values are indicated as n.s.=non-significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. In the legends Vem, vemurafenib; Dox, doxorubicin and IR, γ-radiation.

Figure S6.

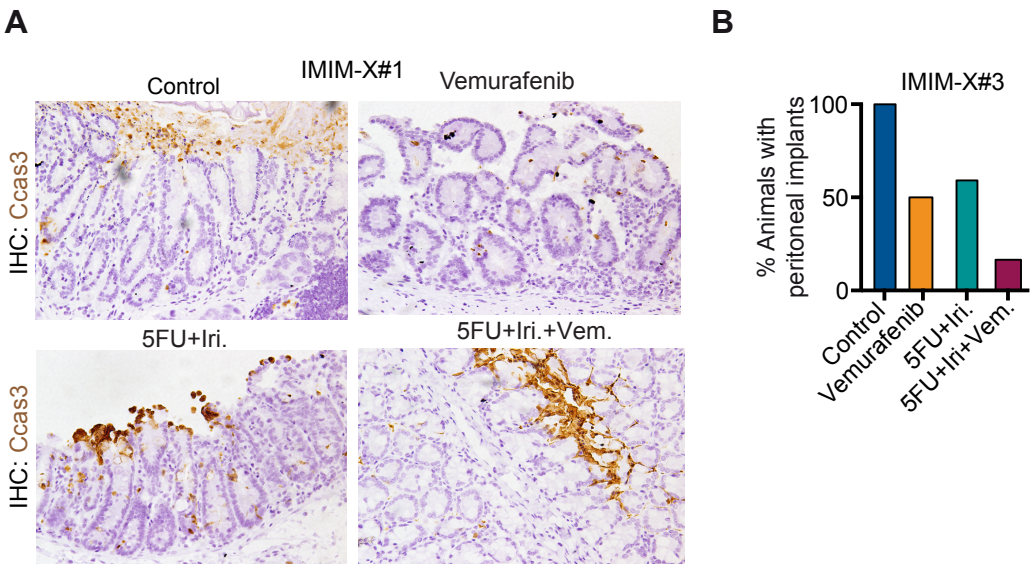
A
IMIM-X#1
Control
Vemurafenib
IHC: Ccas3
5FU+Iri.
5FU+Iri.+Vem.
IHC: Ccas3
B
IMIM-X#3
% Animals with peritoneal implants
100
50
0
Control
Vemurafenib
5FU+Iri.
5FU+Iri+Vem.

Fig. S6. Related to Figure 6. (**A**) Cleaved caspase 3 staining of normal colonic tissue from the indicated treated animals. (**B**) Graph indicating the percent of animals with detectable peritoneal implant in each group of treatment. In the figure: Vem, vemurafenib; 5FU, 5-fluorouracil; Dox, doxorubicin and Iri, irinotecan.