

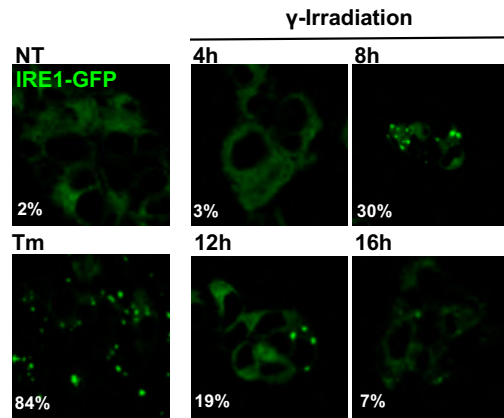
Supplementary Information

Genotoxic stress triggers the selective activation of IRE1 α -dependent RNA decay to modulate the DNA damage response.

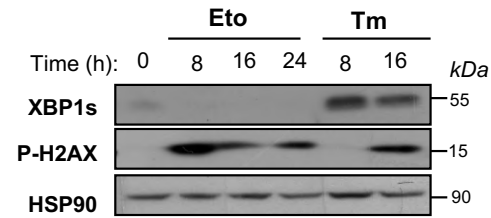
Dufey et al., 2020 Nature Communications

Supplementary figure 1

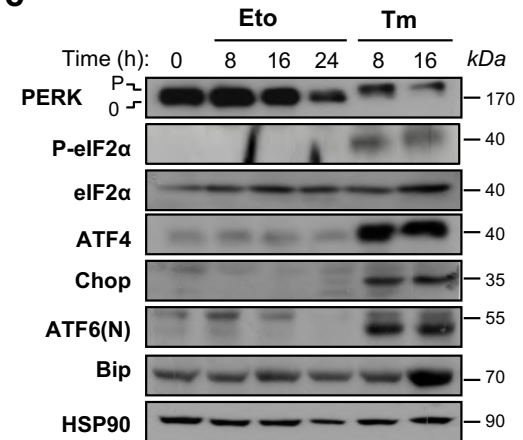
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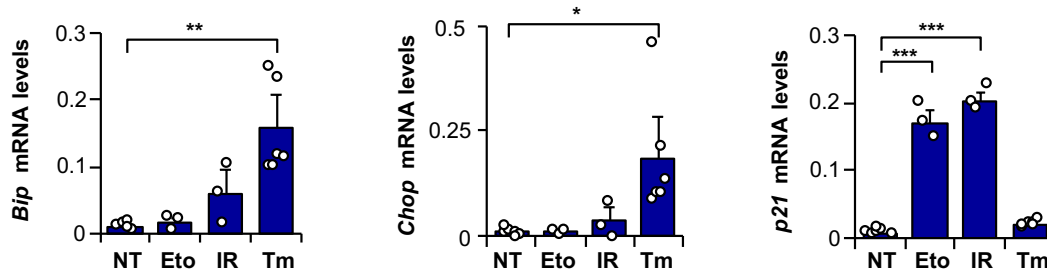
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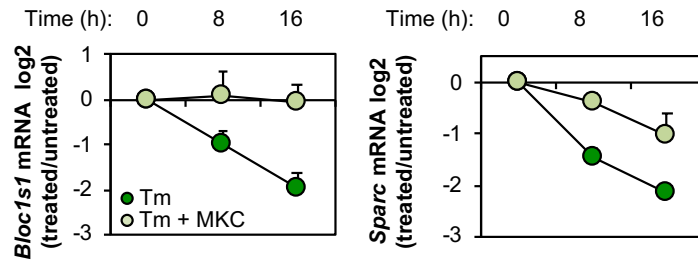
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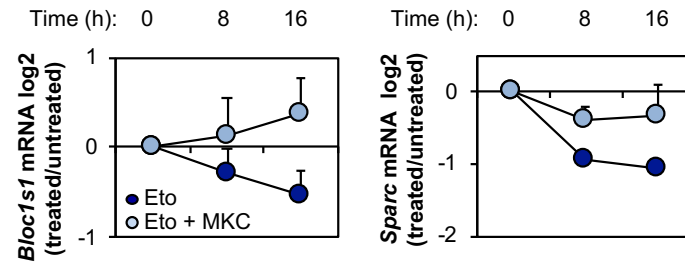
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e



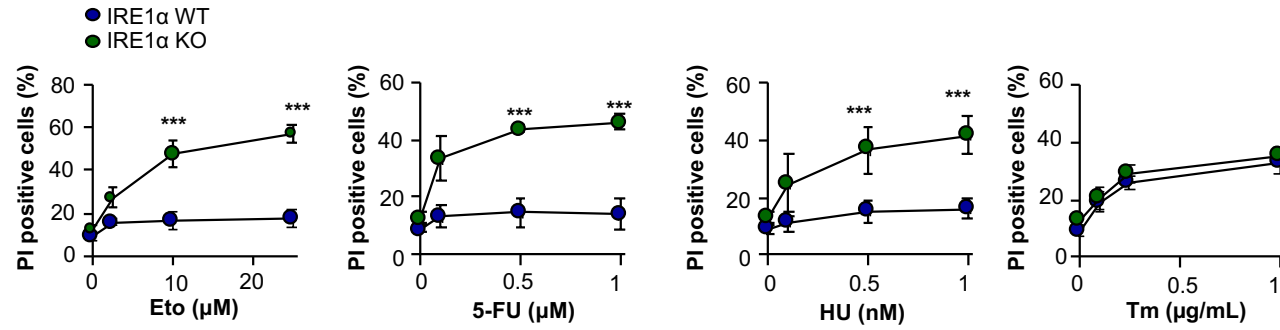
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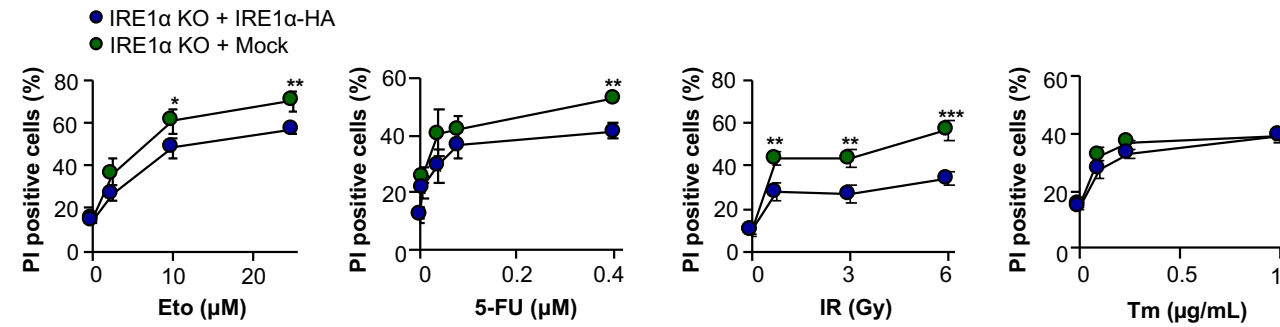
Supplementary Figure 1. DNA damage triggers the selective activation of IRE1 α -dependent RNA decay in the absence of an ER stress signature. **a** TREX IRE1-3F6H-GFP cells were exposed to 25 Gy of ionizing radiation (IR), and then fixed at indicated time points post-IR to quantify the percentage of cells containing IRE1 α -GFP foci (bottom left numbers). Treatment with 500 ng/mL tunicamycin (Tm) for 4h was used as positive control. **b** WT MEF cells were treated with 10 μ M etoposide (Eto) at indicated time points and the expression levels of XBP1s and phosphorylated H2AX (P-H2AX) detected by western blot. Hsp90 was monitored as loading control. Treatment with 500 ng/mL Tm was used as positive control. **c** WT MEF cells were treated with 10 μ M Eto or 500 ng/mL Tm at indicated time points. Then, the expression levels of indicated ER stress proteins monitored by western blot. Hsp90 was monitored as loading control. **d** *HSPA5 (BiP)*, *DDIT3 (Chop)* and *CDKN1A (p21)* mRNA were quantified by real-time PCR in cells treated with 25 Gy of IR, 10 μ M Eto or 500 ng/mL Tm for 16 h (n = 3-6). **e** and **f** WT MEF cells were treated with 10 μ M Eto, 500 ng/mL of Tm, or in combination with the IRE1 α RNase inhibitor 25 μ M MKC-8866 for 16h. *Bloc1s1* and *Sparc* mRNA levels were monitored by real-time PCR and normalized with the expression levels of *Rpl19* mRNA (n = 4). In all panels, data is shown as mean $\pm$ s.e.m.; * p < 0.05, ** p < 0.01 and *** p < 0.001, based on **(d)** One-way ANOVA followed Tukey's test. Data is provided as a Source Data file.

Supplementary Figure 2

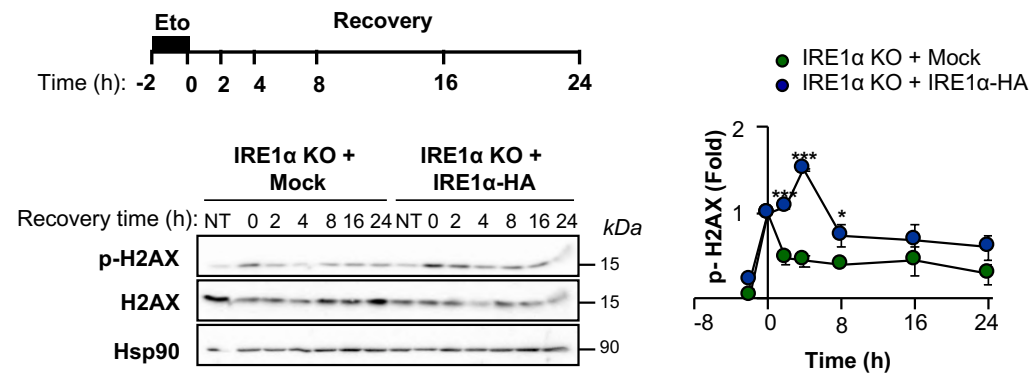
a



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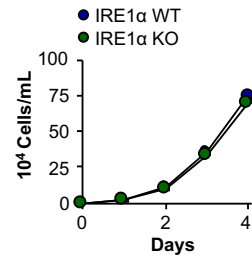
c



Supplementary Figure 2. IRE1 α deficiency reduces the survival of cells under DNA damage and alters the phosphorylation of the histone H2AX. **a** WT and IRE1 α KO MEF cells were treated with indicated concentration of etoposide (Eto), 5-fluorouracil (5-FU), hydroxyurea (HU) and tunicamycin (Tm). After 24h, cell viability was analyzed by propidium iodide (PI) staining and FACS analysis (n = 3). **b** IRE1 α KO (Mock) and reconstituted cells with an IRE1 α -HA expression vector were treated with indicated concentrations of Eto, 5-FU, gamma irradiation (Gy), or Tm. After 24h cell viability was analyzed after PI staining and FACS analysis (n = 3). **c** Diagram of the experimental setting (upper panel). IRE1 α KO (Mock) and IRE1 α -HA reconstituted cells were pre-incubated with 1 μ M Eto for 2 h and washed three times with PBS and fresh cell culture media was added. The decay of phosphorylated H2AX (P-H2AX) was monitored over time by western blot (middle panel). Quantification of the levels of P-H2AX in cells stimulated with Eto (bottom panel). Hsp90 was monitored as loading control (n = 3). In all panels, data is shown as mean $\pm$ s.e.m.; * p < 0.05, ** p < 0.01 and *** p < 0.001, based on (a,b,c) two-way ANOVA followed Bonferroni's test. Data is provided as a Source Data file.

Supplementary Figure 3

a



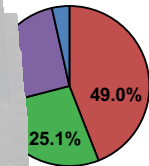
b

IRE1α KO + IRE1α-HA
IRE1α KO + Mock

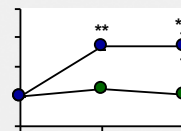
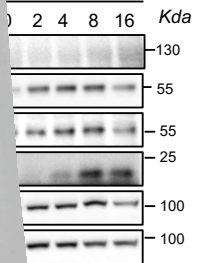
Cells/mL

IRE1α-HA

ETO



IRE1α KO



SMC1

p-KAP1

KAP1

GAPDH

130

100

100

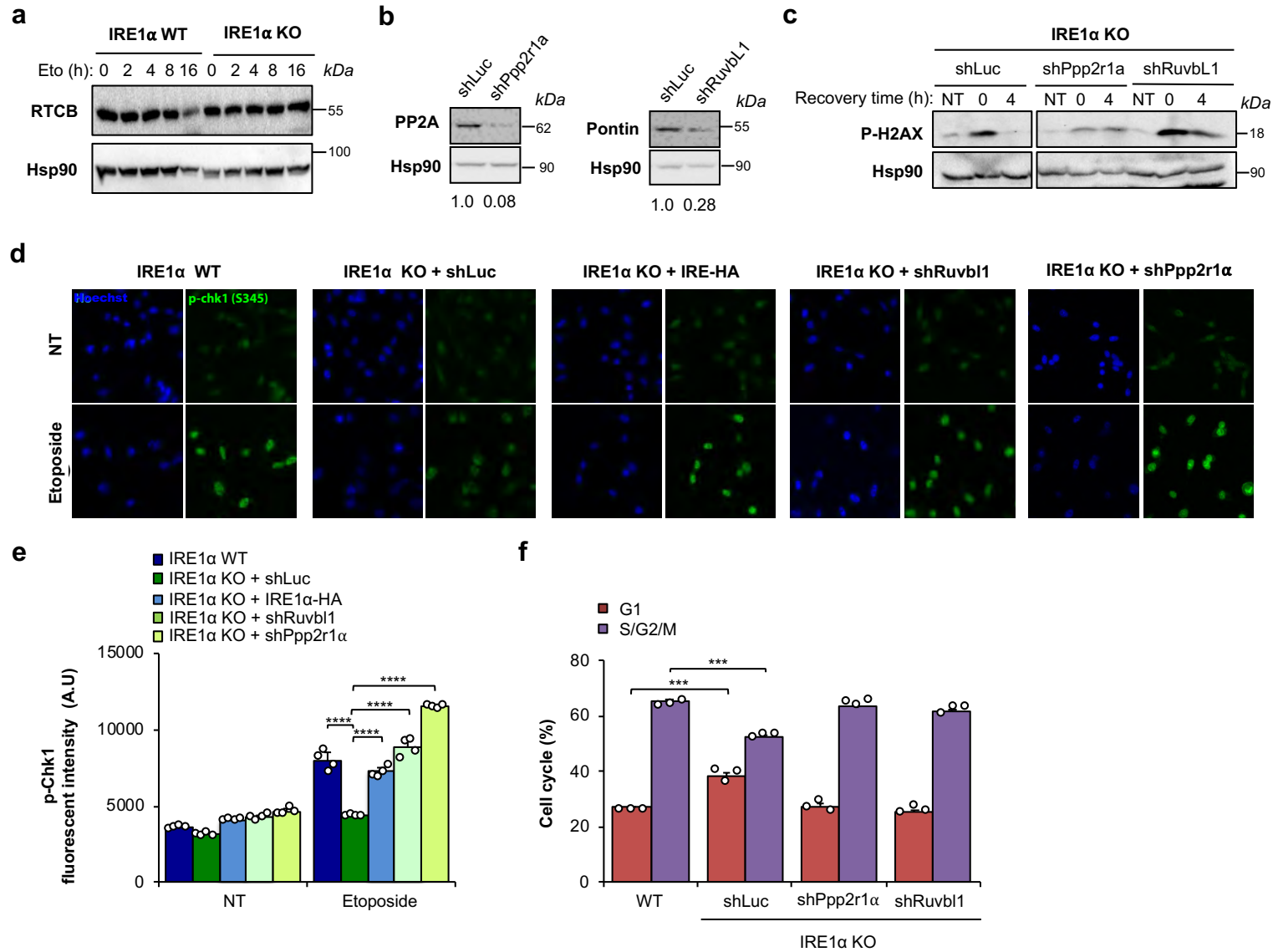
35

and in WT and
cells with an
treated with
centage of cells in
I Eto at indicated
on of CHK1 and
with an IRE1α-HA

time points. An
CHK2 phosphorylation was

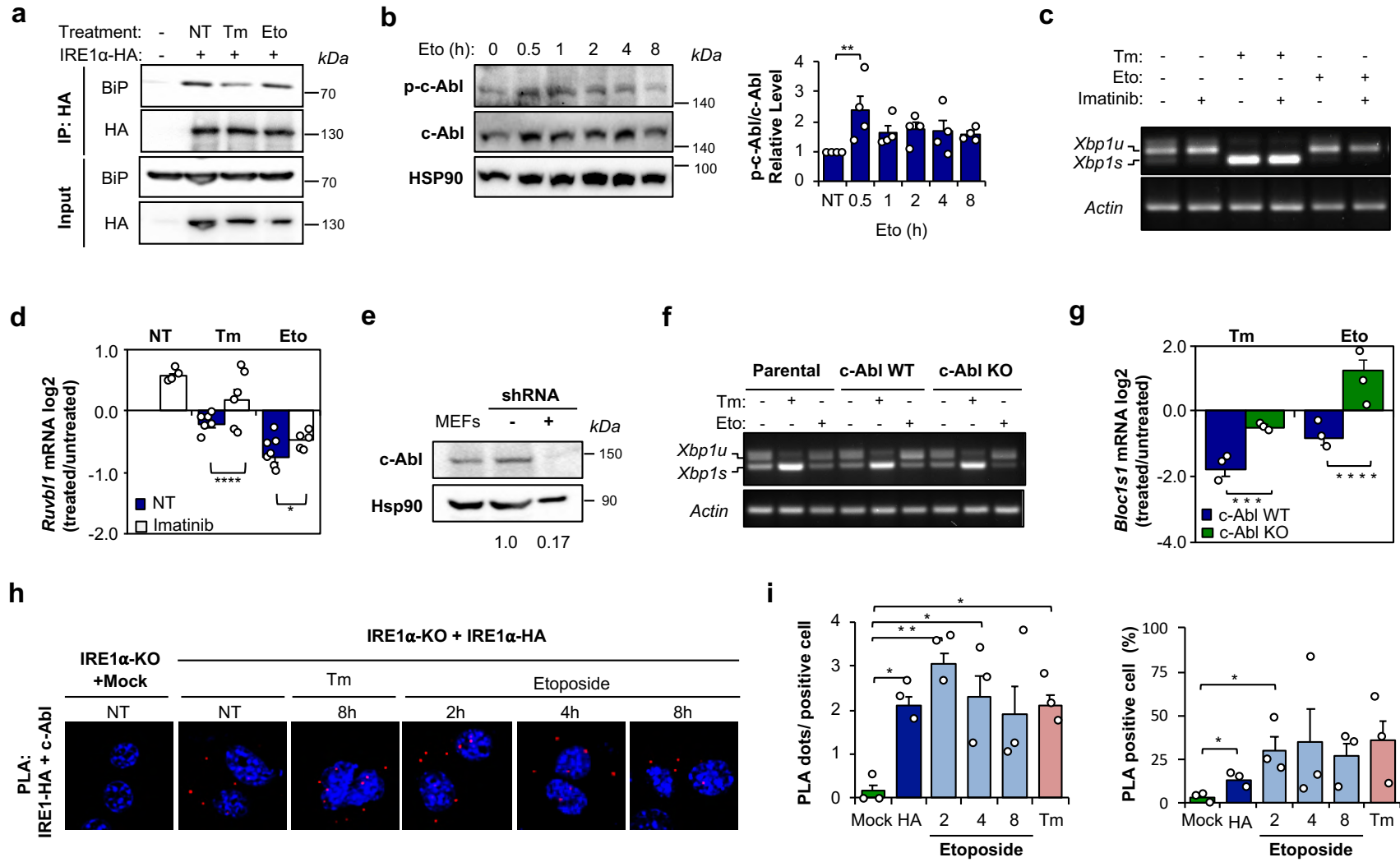
were treated with 10 μ M Eto (8h). An expression level of indicated proteins was monitored by western blot analysis. **e** WT and IRE1 α KO MEF cells were treated with 10 μ M Eto at indicated time points. An expression level of indicated proteins was monitored by western blot analysis. In all panels, data is shown as mean $\pm$ s.e.m.; * p < 0.05, ** p < 0.01 and *** p < 0.001, based on **(a,c)** two-way ANOVA followed Bonferroni's test. Data is provided as a Source Data file.

Supplementary Figure 4



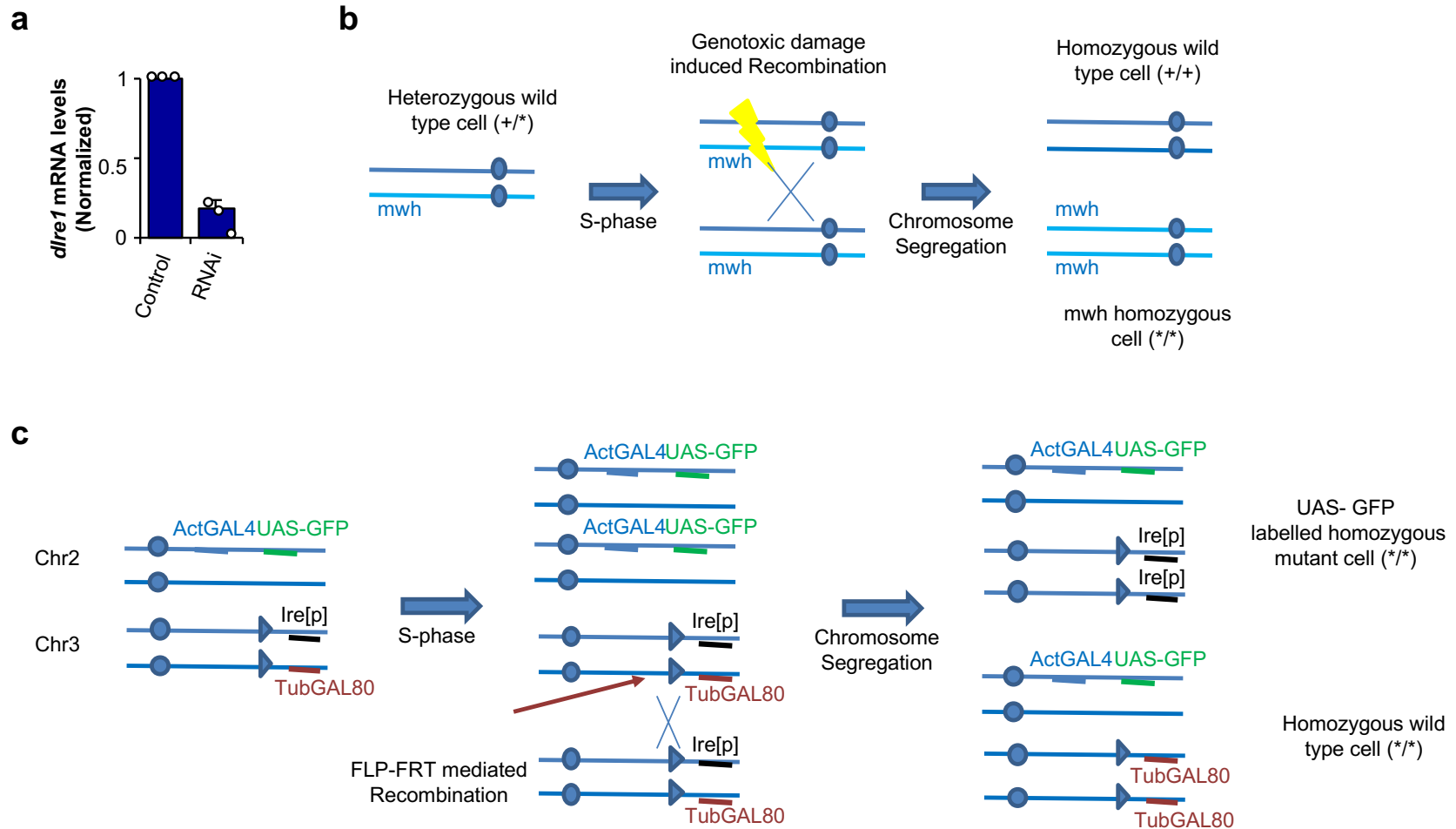
Supplementary Figure 4. Knocking down *Ppp2r1a* or *Ruvb1* in IRE1 α KO cells augmented the phosphorylation of H2AX under DNA damage. **a** WT and IRE1 α KO MEF cells were treated with 10 μ M Etoposide (Eto) at indicated time points. An expression level of RTCB was monitored by western blot analysis. **b** IRE1 α KO cells were stably transduced with shRNA constructs to target the *Ppp2r1a*, *Ruvb1* or Luciferase (Luc) mRNA. Expression levels of PP2A and Pontin were determined by Western blot analysis to the indicated proteins. Hsp90 was monitored as loading control. **c** IRE1 α KO MEF cells were stably transduced with lentiviruses expressing shRNAs against the *Ppp2r1a* (*shPpp2r1a*), *RuvbL1* (*shRuvbL1*) or luciferase as control (*shLuc*). Cells were incubated with 1 μ M Eto for 4h. Then, cells were washed three times with PBS and fresh culture media was added. The expression levels of phosphorylated-H2AX were monitored by western blot. Hsp90 was monitored as loading control. **d-e** MEF WT, MEF IRE1 α KO reconstituted cells with an IRE1 α -HA and IRE1 α KO MEF cells stably transduced with lentiviruses expressing shRNAs against the *Ppp2r1a* (*shPpp2r1a*), *RuvbL1* (*shRuvbL1*) or luciferase as control (*shLuc*), were treated 8 hours with 10 μ M Eto. Then immunofluorescence analysis was performed to detect the CHK1 phosphorylation. The results were graphed as arbitrary units (A.U) of green intensity (n = 4). **f** MEF WT and IRE1 α KO MEF cells stably transduced with lentiviruses expressing shRNAs against the *Ppp2r1a* (*shPpp2r1a*), *RuvbL1* (*shRuvbL1*) or luciferase as control (*shLuc*) were treated with 10 μ M Eto and cell cycle was analyzed by propidium iodide (PI) staining. Quantification of the percentage of cells in G1 and S/G2/M phases is shown (n=3). Representative images of three independent experiments. In all panels, data is shown as the mean $\pm$ s.e.m.; * p < 0.05, ** p < 0.01 and *** p < 0.001, based on (**e,f**) two-way ANOVA followed Bonferroni's test. Data is provided as a Source Data file.

Supplementary Figure 5



Supplementary Figure 5. ER stress and DNA damage synergize to induce RIDD without affecting XBP1 mRNA splicing. **a** IRE1 α KO cells were reconstituted with IRE1 α -HA and treated with 500 ng/ml tunicamycin (Tm) for 4 h or 10 μ M etoposide (Eto) for 16h then protein extract were prepared in 1% NP-40 buffer. IRE1 α -HA was immunoprecipitated (IP) and analyzed by western blot the presence of BiP in the isolated protein complexes. **b** c-Abl phosphorylation was monitored in WT MEFs cells treated with 10 μ M Eto at indicated time points followed by western blot analysis (n = 4). **c** IRE1 α WT MEF cells were treated with 10 μ M Eto, 500 ng/mL tunicamycin (Tm) or in combination with c-Abl inhibitor Imatinib 10 μ M for 8h, to measuring the *Xbp1* mRNA splicing by PCR. **d** WT MEF cells were treated with 10 μ M Eto, 500 ng/mL of Tm, or in combination with the c-Abl inhibitor Imatinib 10 μ M for 8 h. *Ruvbl1* mRNA levels were monitored by real-time PCR (n = 4-7). **e** MEFs cells were transfected with shRNA scramble or shRNA against *c-Abl*, and through WB we measured the protein knockdown, using Hsp90 as loading control. **f** MEF WT (Parental), MEF CRISPR-Control and MEF CRISPR-Abl cells were treated with 10 μ M Eto, 500 ng/mL Tm for 8h, to measuring the *Xbp1* mRNA splicing by PCR. **g** MEF CRISPR-Control and MEF CRISPR-Abl cells were treated with 10 μ M Eto or 500 ng/mL of Tm for 8h. *Bloc1s1* mRNA levels were monitored by real-time PCR and normalized with the expression levels of *Rpl19* mRNA (n=3). **h-i** IRE1 α KO (Mock) and reconstituted cells with an IRE1 α -HA (HA) were treated with 10 μ M Eto at indicated time points and stained with a proximity ligation assay (PLA) using an anti-HA or anti-c-Abl antibodies and analyzed by confocal microscopy. Left panel: Number of dots per cell analyzed and percentage of PLA positive cells were quantified (n = 3). In all panels, data is shown as mean $\pm$ s.e.m.; * p < 0.05, ** p < 0.01 and *** p < 0.001, based on **(b,i)** One-way ANOVA followed Tukey's test, **(d)** two-tailed unpaired t-Student's test, **(g)** two-way ANOVA followed Bonferroni's test. Data is provided as a Source Data file.

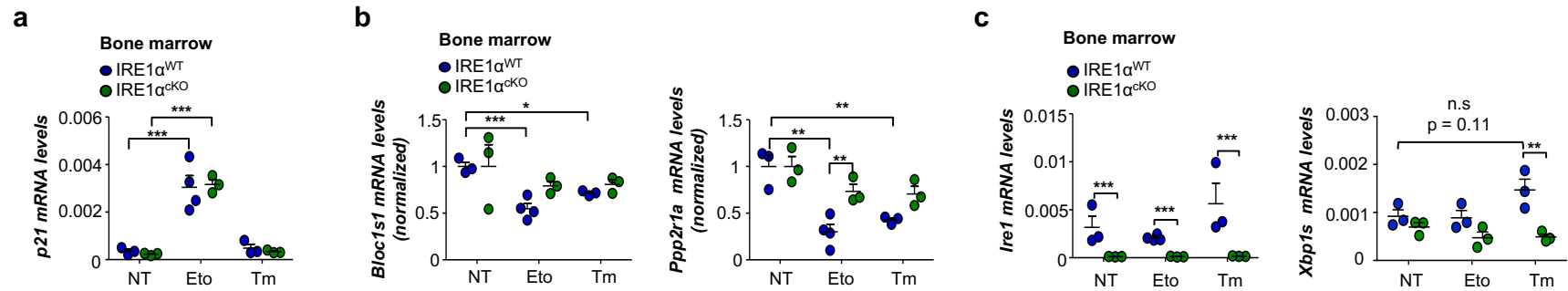
Supplementary Figure 6



Supplementary Figure 6. Study of IRE1 α function on the DNA damage response in *D. melanogaster*. **a** *D. melanogaster* IRE1 α homologue (*dlre1*) was knocked down by expressing a specific RNAi construct under the control of tubulin-GAL4 driver using the UAS-GAL4 system. The levels of *dlre1* mRNA were evaluated from control (GFP-IR;;Tub-GAL4) and *dlre1* RNAi expressing larvae (Ire-IR;;

Tub-GAL4) by real-time RT-PCR (n = 3). **b** Scheme of the SMART assay. The case of the *mwh*¹ mutation is presented, however an equivalent scenario applies for the *flr*³ mutation in the homolog chromosome. *mwh*¹ and *flr*³ are recessive mutations, therefore heterozygous cells display no phenotype. DNA breaks induced by the genotoxic agents favor mitotic recombination between homolog chromosomes, this generates one cell which receives both wild type alleles (+/+, no phenotype) and the other inherits both mutant alleles (*/*, *mwh*¹ phenotype). **c** Scheme of the MARCM assay used. The act-GAL4 driver (Actin-Gal4) and the UAS-GFP transgenes are located in the second chromosome. The Tub-GAL80 construct coding for a competitive inhibitor of the GAL4 transcription factor and the FRT80A recombination site are located in left arm of the third chromosome (3L). *dlre1* null mutation and the same FRT site are present in the homolog chromosome. FLP induction promotes the recombination between FRT sites in dividing cells. This stochastic event during mitosis generates a descendant inheriting the two Tub-GAL80 alleles, while the other segregates both *dlre1* mutant alleles. The lack of Tub-GAL80 in *dlre1* mutant cells allows Gal4 activation of GFP expression. *Source data are provided as a Source Data file.*

Supplementary Figure 7



Supplementary Figure 7. Deletion of IRE1 α in bone marrow alters the DDR under genotoxic stress. Mouse IRE1 α was conditionally deleted in the liver and bone marrow using the MxCre and LoxP system (IRE1 α^{CKO}). Mice were intraperitoneally injected with 50 mg/Kg etoposide (Eto) or 1 mg/Kg tunicamycin (Tm) and sacrificed 6 hours later. **a** Total mRNA levels of *p21* were measured in the bone marrow by real-time PCR and normalized to the expression levels of actin. **b** *Bloc1s1* and *Ppp2r1a* mRNA expression levels were monitored by RT-PCR in samples indicated in a (n = 3-4 mice per group). **c** IRE1 α mRNA levels and *Xbp1* mRNA splicing was monitored by RT-PCR in the same samples indicated in a. In all panels, data is shown as the mean $\pm$ s.e.m.; * p < 0.05, ** p < 0.01 and *** p < 0.001, based on (a,b,c) two-way ANOVA followed Bonferroni's test. Data is provided as a Source Data file.

Supplementary Figure 8. Functional networks altered upon deleting Ern1 in the liver after etoposide treatment. **a** Heatmap representation of UPR, ER stress signaling (ERS) and apoptosis/DDR related mRNAs derived from WT or IRE1 α ^{KO} mice after 6h or 16h of etoposide treatment as described in Figure 6a. **b** Reactome pathway enrichment analysis associated to WT (left panel) and IRE1 α ^{KO} (right panel) mice after 6h (upper panel) and 16h (lower panel) of tunicamycin treatment. Briefly, functional pathway analysis was carried out using ClueGO v2.3.2 plugin in Cytoscape 3.2.0 software, using Reactome (v09.11.2016) enrichment pathway database in default mode. Only pathways with a significant pValue ($P < 0.05$ with Bonferroni correction) were displayed. **c** Heatmap of IRE1 α -related gene signature in WT or IRE1 α ^{ckKO} after 6h or 16h of tunicamycin treatments. *Data is provided as a Source Data file.*

Supplementary Table 1. Primers used in the study

mRNA	Forward	Reverse
PCR		
mXBP1S	AAGAACACGCTTGGGAATGG	CTGCACCTGCTGCGGAC
m β -Actin	ACCACCATGTACCCAGGCA	CTCAGGAGGAGCAATGATCTTGAT
qPCR		
mCDKN1A (p21)	CGAGAACGGTGGAACTTTGAC	CCAGGGCTCAGGTAGACCTT
m β -Actin	TACCACCATGTACCCAGGCA	CTCAGGAGGAGCAATGATCTTGAT
mXBP1S	GAGTCCGCAGCAGGTG	GTGTCAGAGTCCATGGGA
mSPARC	GTGGAAATGGGAGAATTTGAG GA	CTCACACACCTTGCCATGTTT
mBLOC1S1	TCCCGCCTGCTCAAAGAAC	GAGGTGATCCACCAACGCTT
mPPP2R1A	GACGGTGACGATTCGCTCTAT	CTGGTCCGTTCAACCCCAAG
mRUVBL1	TTGGGTTGCGGATAAAGGAGA	GGCTGATAGTTTTGCCGTACC
mRPL19	CTGATCAAGGATGGGCTGAT	GCCGCTATGTACAGACACGA
dXBP1	CCTTGGATCTGCCGCAGGGTA TAC	AGAGGGCCACAACCTTTCCAGAGTGA
dSPARC	ATTGAGCGCGAGAAGAGATTG CC	ACTCCGGAATGCACACGCATTT
dMYC	TCGCAAAATCTCCTCTTCGG	GGGACACTTGAGCAACTGAA
dRPL32	AGCGCACCAAGCACTTCATA	GTGCGCTTGTTGATCCGTAA

* m= Mouse; d= *D. melanogaster*

Supplementary Table 2. Cleavage assay. Oligonucleotides sequence used were:

Oligonucleotide	Sequence
Hppp2r1a-CF1	AGCCGAATTCTAATACGACTCACTATAGGGAATCTTGGTCGCTA
Hppp2r1a-CR840	TAGCAGGATCCACCATGTAGCGAACACGCCAAGACTT
Hppp2r1a-CF1336	AGCCGAATTCTAATACGACTCACTATAGGGCATTGTGGAGCTGGCTGA GGA
Hppp2r1a-CR1919	TAGCAGGATCCCACCAGAGGCCAGTGTTTGC
hRuvbL1-CF1290	AGCCGAATTCTAATACGACTCACTATAGGGACTCAGTGCAGCTGCTGA CC
hRuvbL1-CR1650	TAGCAGGATCCTGTTCTGAAAAGTTTATTTTGA

Supplementary Table 3. Fly strains.

Experiment	Flies
SMART Assays	w [*] , Nub-GAL4, Ire1-IR; flr ³ /mwh ¹
	w [*] , Nub-GAL4; flr ³ /mwh ¹
Survival and pPCR Assays	Ire-IR; tub-GAL4
	GFP-IR; tub-GAL4
Immunohistochemistry Analysis	w [*] , Nub-GAL4; Ire1-IR
	w [*] , Nub-GAL4; GFP-IR
MARCM Mosaic Analysis	yw, eyFLP; act<y+<;Gal4, UAS-GFP; FRT82B tubGal80/ Ire1[f02170], FRT82B
	yw,ey-FLP;act<y+<Gal4, UAS-GFP; FRT82B tub-Gal80/FRT82B