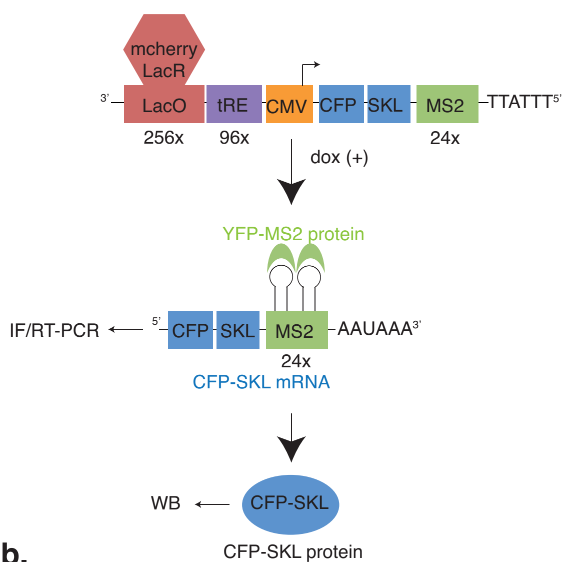
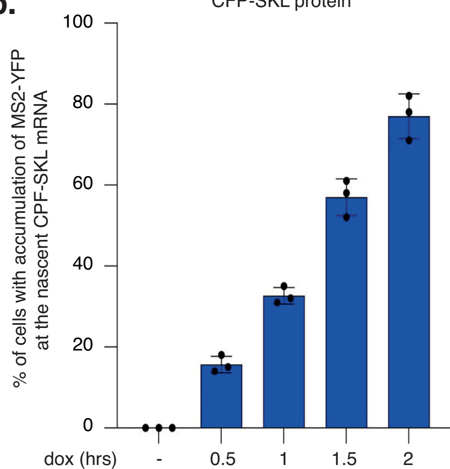
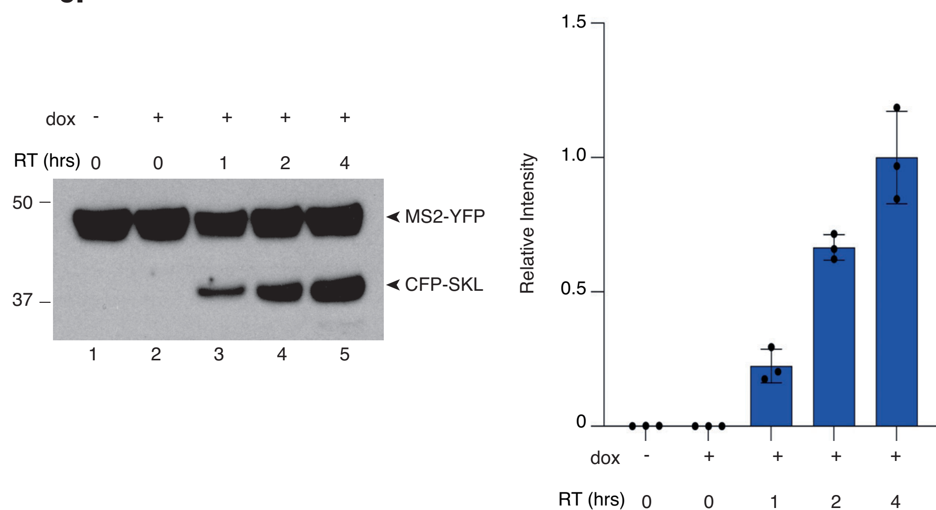
a.



b.



c.



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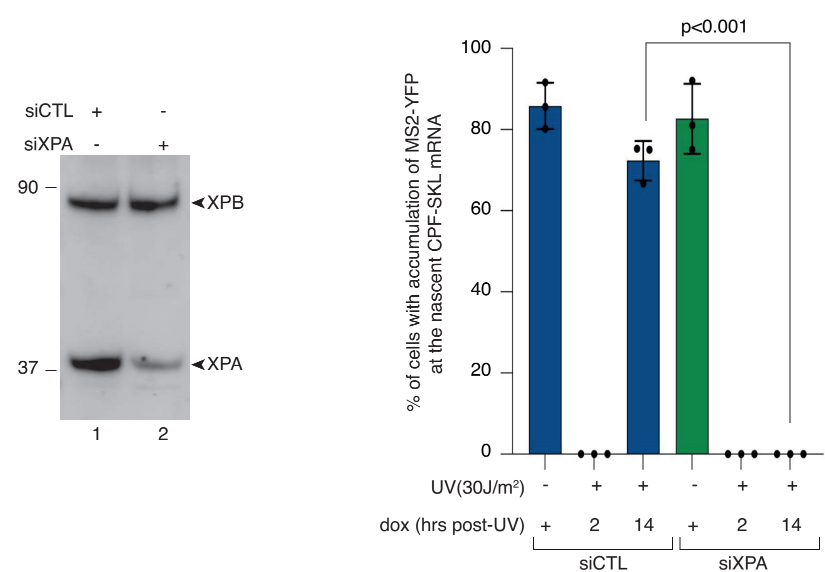

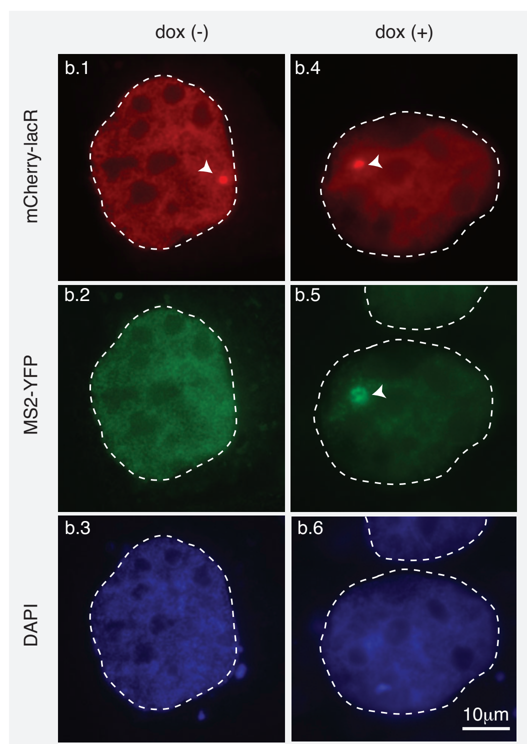

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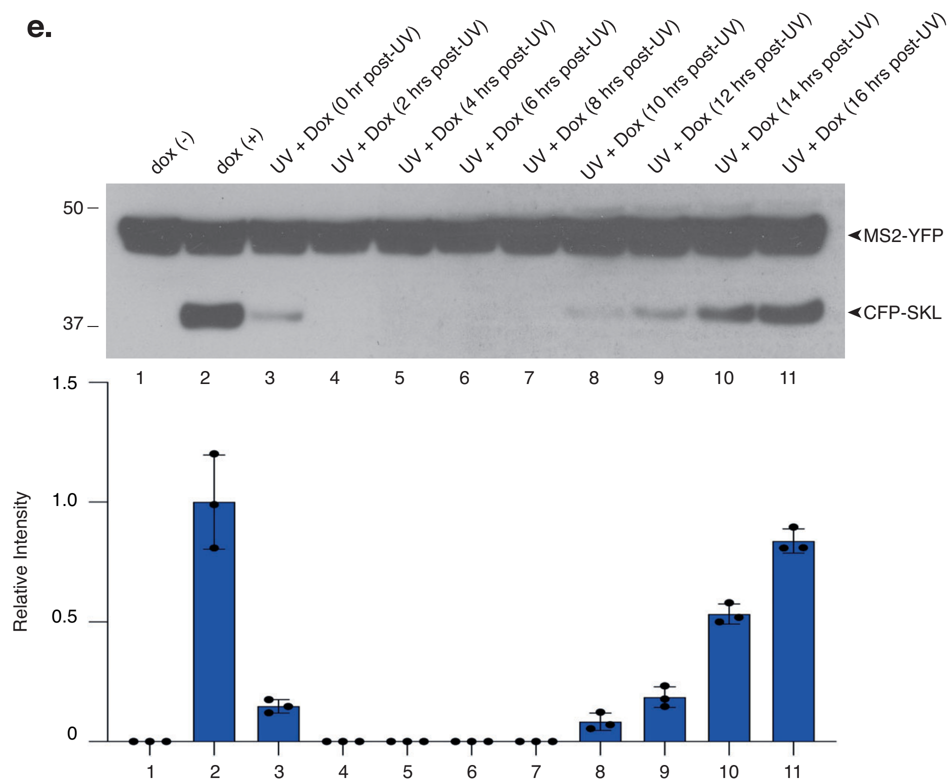


Supplementary Figure 1: The CFP-SKL reporter assay

a. 256 copies of lacO array provide examination of the reporter through a mCherry protein fused to lacR. Tetracycline response elements (TREs) control expression of the unique *CFP-SKL* mRNA transcript upon dox treatment. *CFP-SKL* transcription is detected through the binding of YFP-MS2 and encoded CFP-SKL protein that can be detected by WB.

b. U-2 OS were treated with dox as indicated and *CFP-SKL* mRNA expression was quantified. Results are expressed as % of cells showing YFP-MS2 accumulation at a single locus (n= 3x20 cells, biological triplicates). Bars represent mean values of three different experiments (Biological triplicates) (+/- SD). Representative confocal images of MS2-YFP accumulation at *CFP-SKL* is shown. The reporter locus is detected with a mCherry-LacR fusion construct.

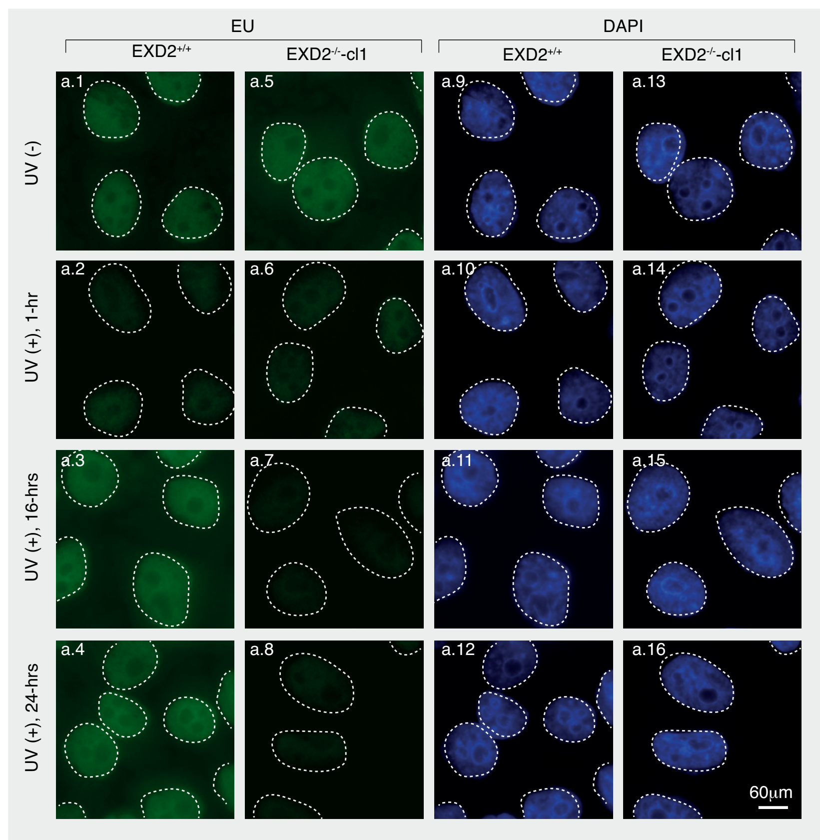
c. Immuno-blot for CFP-SKL and YFP-MS2 in U-2 OS cells treated with dox and subsequently let to recover in dox-free medium. Extracts were immuno-blotted with anti-GFP. CFP-SKL signals were normalized with YFP-MS2 signals (+/-SEM). Three independent immunoblots were performed (technical triplicates). Source data are provided as a Source Data file.

d. U-2 OS cells were transfected with siCTL or siXPA for 24 hours and with a construct expressing mCherry-lacR for 24 hours before UV irradiation (30J/m^2) and subsequent 2-hour pulse-incubation with dox. **Left panel**; cell extracts were immunoblotted against XPB and XPA. **Right panel**; Newly transcribed *CFP-SKL* were detected at the reporter locus. Quantification of the transcribing locus was done and results are expressed as % of cells showing YFP-MS2 accumulation at a single locus (n= 3x20 cells, biological triplicates). Bars represent mean values of three different experiments (Biological triplicates) (+/- SD). One-way ANOVA with post-hoc Tukey adjustment comparisons were used to determine the p-values**.** Source data are provided as a Source Data file.

e. U-2 OS cells were UV-irradiated (30 J/m^2) and pulse-incubated for 2 hours with dox. Cells were allowed to recover for 4 hours in the absence of dox. Extracts were immuno-blotted with anti-GFP antibody. Lane 1; cells were not treated with dox. Lane 2; cells were treated with dox for 2 hours before to recover 4 hours in the absence of dox. CFP-SKL signals were normalized

with YFP-MS2 signals (+/-SEM). Three independent immunoblots were performed (technical triplicates). Source data are provided as a Source Data file.

a.



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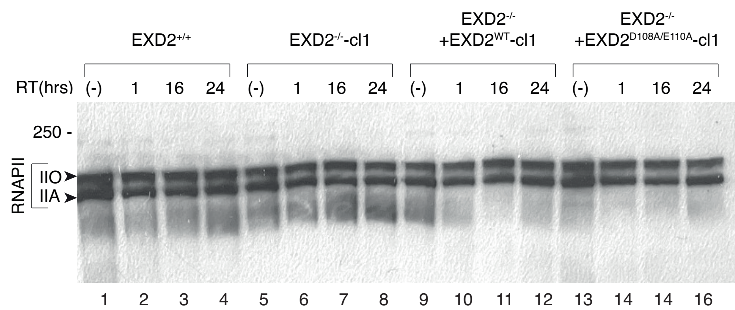

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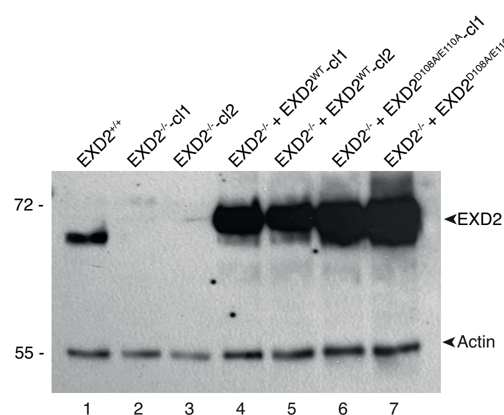

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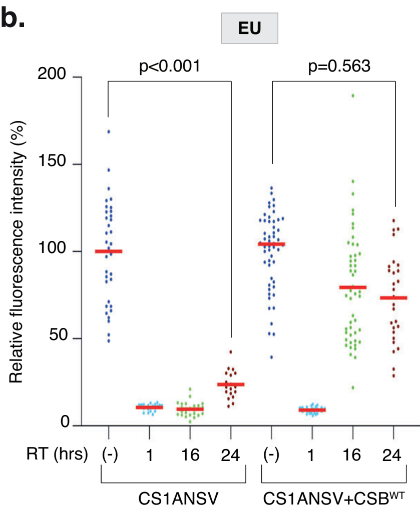

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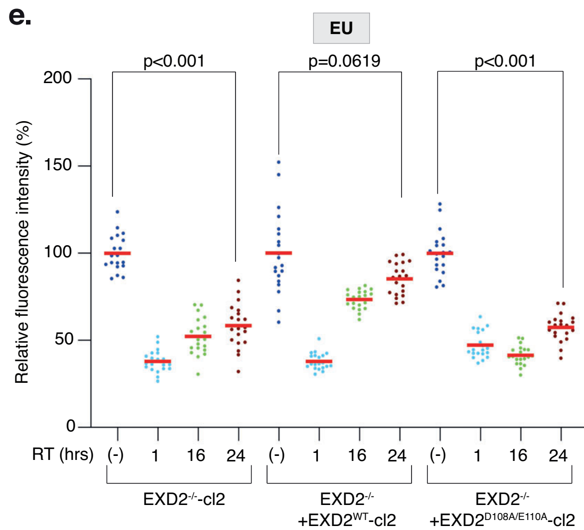

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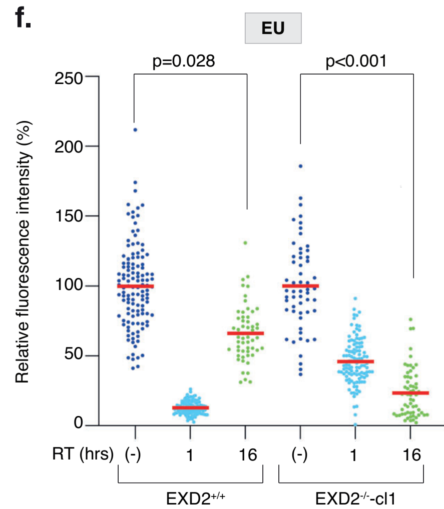


Supplementary Figure 2: Lack of EXD2 induces inhibition of RRS

a. Representative confocal images of $EXD2^{+/+}$ and $EXD2^{-/-}$-cl1. Cells were mock or UV-irradiated ($15J/m^2$) and mRNA was labelled with EU 1, 16 and 24 hours after UV-irradiation. Images of the cells were obtained with the same microscopy system and constant acquisition parameters.

b. CS1ANSV and CS1ANSV+CSB^{WT} were UV-irradiated ($15J/m^2$) and mRNA was labelled with EU at 1, 16 and 24 hours after UV-irradiation. EU signal was quantified by ImageJ and relative integrated density normalized to mock-treated level set to 100% are reported on the graph (n=at least 20 cells per condition). Red bars indicate mean integrated density. One-way ANOVA with post-hoc Tukey adjustment comparisons were used to determine the p-values**.** Source data are provided as a Source Data file.

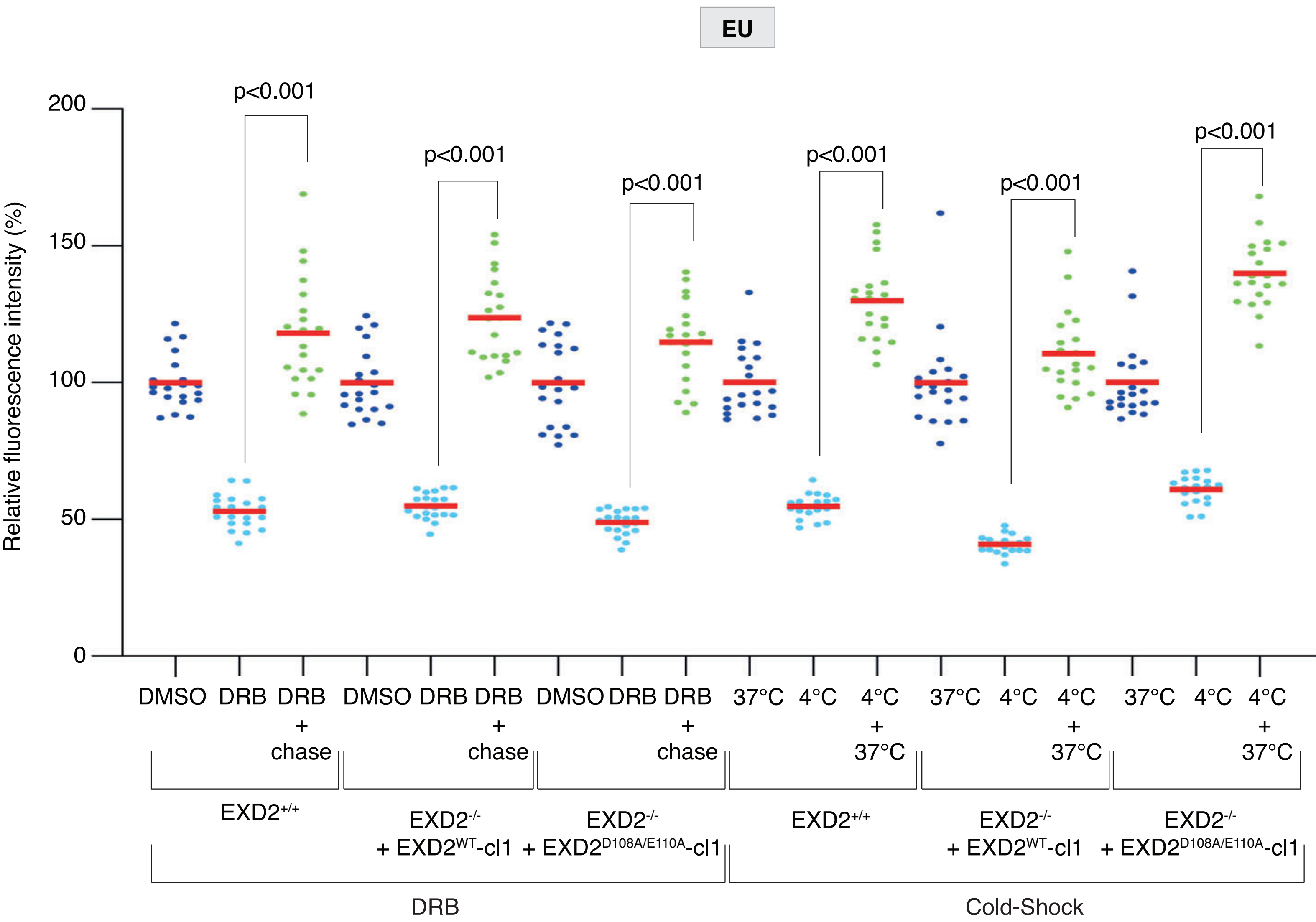
c. HeLa were mock or UV-irradiated ($15J/m^2$) and let to recover for the indicated times before lysis. Protein lysates were immuno-blotted for RPB1 subunit of RNAPII. Molecular mass of the proteins is indicated on the left (kDa). RT; recovery time. IIO, phosphorylated form of RPB1. IIA non-phosphorylated form. Source data are provided as a Source Data file.

d. Protein lysates were immuno-blotted for proteins as indicated. Molecular mass of the proteins is indicated (kDa). Source data are provided as a Source Data file.

e. HeLa cells were mock or UV-irradiated ($15J/m^2$) and mRNA was labelled with EU 1, 16 and 24 hours after UV-irradiation. EU signal was quantified by ImageJ and relative integrated density normalized to mock-treated level set to 100% are reported on the graph (n=at least 20 cells per condition). Red bars indicate mean integrated density. One-way ANOVA with post-hoc Tukey adjustment comparisons were used to determine the p-values**.** Source data are provided as a Source Data file.

f. $EXD2^{+/+}$ and $EXD2^{-/-}$-cl 1 cells were grown to 80% confluence and further incubated 24 hours in medium containing 0.5% FCS. Cells were mock or UV-irradiated ($15J/m^2$) and mRNA was labelled with EU at 1 and 16 hours after UV-irradiation. EU signal was quantified by ImageJ and relative integrated density normalized to mock-treated level set to 100% are reported on the graph (n= at least 50 cells per condition). Red bars indicate mean integrated density. One-

way ANOVA with post-hoc Tukey adjustment comparisons were used to determine the p-values. Source data are provided as a Source Data file.


EU
p<0.001
p<0.001
p<0.001
p<0.001
p<0.001
p<0.001
Relative fluorescence intensity (%)
200
150
100
50
0
DMSO
DRB
DRB + chase
DMSO
DRB
DRB + chase
DMSO
DRB
DRB + chase
37°C
4°C
4°C + 37°C
37°C
4°C
4°C + 37°C
37°C
4°C
4°C + 37°C
EXD2+/+
EXD2-/- + EXD2WT-cl1
EXD2-/- + EXD2D108A/E110A-cl1
EXD2+/+
EXD2-/- + EXD2WT-cl1
EXD2-/- + EXD2D108A/E110A-cl1
DRB
Cold-Shock

Supplementary Figure 3: EXD2 is not involved is RRS after DRB or cold-shock treatment

DRB: $EXD2^{+/+}$, $EXD2^{-/-}$ + $EXD2^{WT}$-cl1 and $EXD2^{-/-}$ + $EXD2^{D108A/E110A}$-cl1 were treated with DRB (100 μM) for 1 hour before chase and cells were let to recover for 30 minutes. mRNA was labelled with EU before (DMSO), during (DRB) or after the chase (DRB + chase) of DRB treatment. **Cold-Shock**: $EXD2^{+/+}$, $EXD2^{-/-}$ + $EXD2^{WT}$-cl1 and $EXD2^{-/-}$ + $EXD2^{D108A/E110A}$-cl1 were incubated at 4°C for 15 minutes and let to recover at 37°C for 30 minutes. mRNA was labelled with EU before (37°C), after 15 minutes at 4°C (4°C) or after the recovery at 37°C (4°C+37°C). For both experiments, EU signal was quantified by ImageJ and relative integrated densities normalized to mock-treated level set to 100% are reported on the graph (n= at least 20 cells per condition). Red bars indicate mean integrated density. One-way ANOVA with post-hoc Tukey adjustment comparisons were used to determine the p-values. Source data are provided as a Source Data file.

EXD2$^{+/+}$ EXD2$^{-/-}$-cl1

RT(hrs) (-) 4 8 24 (-) 4 8 24

20 - γH2AX

72 - EXD2

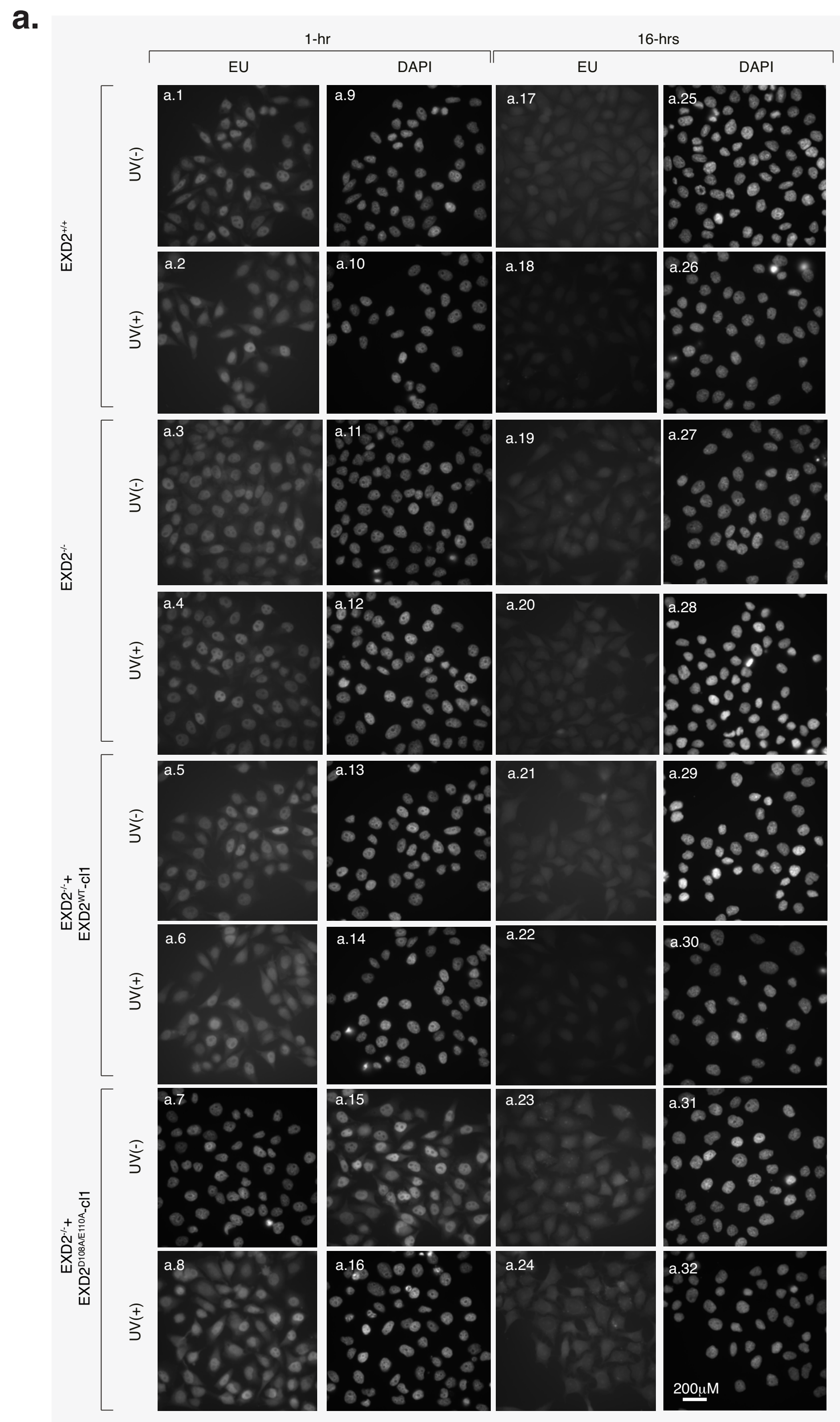
140 - Vinculin

1 2 3 4 5 6 7 8

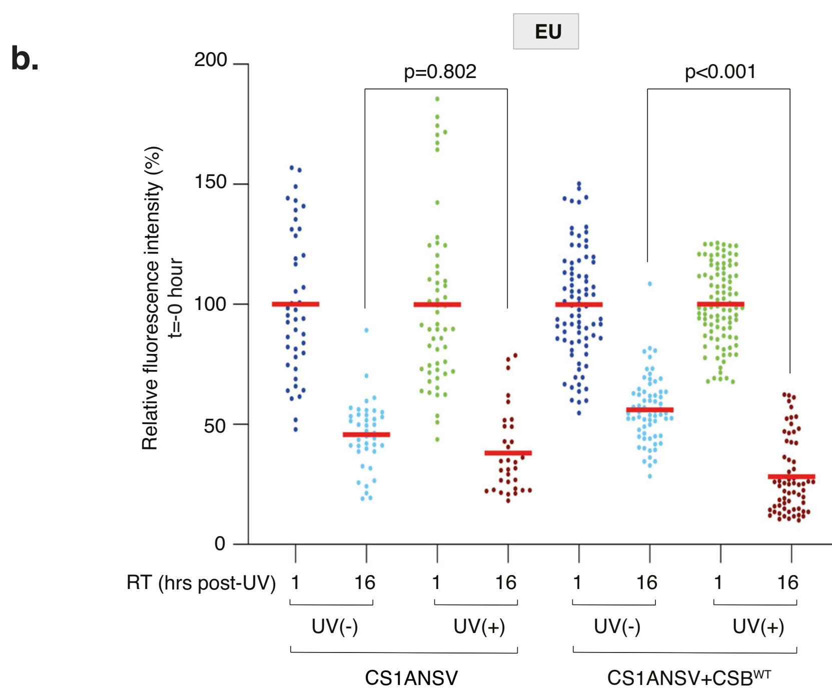
Supplementary Figure 4: NER is not affected by the lack of EXD2

EXD2$^{+/+}$ and EXD2$^{-/-}$-cl1 were mock or UV-irradiated (15J/m^2) and let to recover for the indicated times before lysis. Protein lysates were immuno-blotted for EXD2, γH2AX or Vinculin. Molecular mass of the proteins is indicated (kDa). RT; recovery time. Source data are provided as a Source Data file.

a.



b.



Supplementary Figure 5: mRNAs under synthesis are degraded by EXD2 following UV irradiation

a. Representative confocal images of $EXD2^{+/+}$, $EXD2^{-/-}$, $EXD2^{-/-}$ + $EXD2^{WT}$-cl1 and $EXD2^{-/-}$ + $EXD2^{D108A/E110A}$-cl1 showing newly synthetized mRNA (1 hour or 16 hours after mock-treatment or UV irradiation at 15J/m^2). Images of the cells were obtained with the same microscopy system and constant acquisition parameters.

b. CS1ANSV and CS1ANSV+CSB^{WT} were treated as indicated in Figure 4a, upper panel, and EU signals were quantified using ImageJ and normalized to the value obtained at 1 hour set to 100%. Values are reported on the graph (n= at least 50 cells). Red bars indicate mean integrated density. *RT*; recovery time. One-way ANOVA with post-hoc Tukey adjustment comparisons were used to determine the p-values. Source data are provided as a Source Data file.

a.

EXD2-GFP

EXD2-GFP+DAPI

Flavo (-)

c.1

c.2

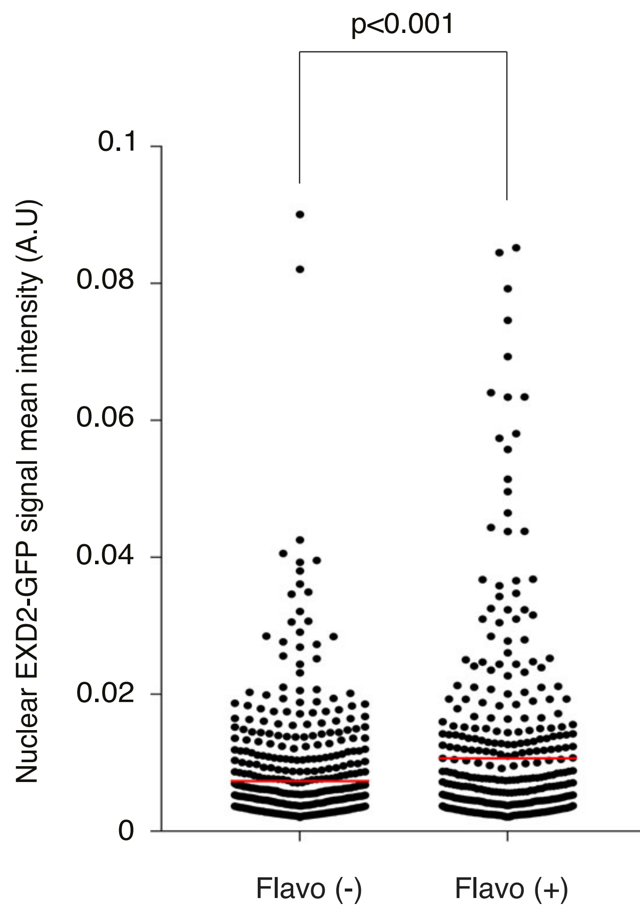
Flavo (+)

c.3

c.4

20uM

b.



Supplementary Figure 6: EXD2 ^{WT}-GFP localization in U-2 OS cell

a. Representative confocal images of U-2 OS cells stably expressing EXD2^{WT}-GFP with or without Flavopiridol treatment. Images of the cells were obtained with the same microscopy system and constant acquisition parameters.

b. Quantification of the EXD2-GFP nuclear signal in U-2 OS cells treated or not with Flavopiridol (n= at least 400 cells). Red bars indicate mean integrated density. One-way ANOVA with post-hoc Tukey adjustment comparisons were used to determine the p-values. Source data are provided as a Source Data file.

Supplementary Table 1: Reagents and chemicals

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
ACTIN 1/1000	Abcam	AB209856
CPD 1/500	Cosmo Bio	CAC-NM-DND-001
ATP5A 1/1000	ProteinTech Europe	66037-1-IG
EXD2 1/2000	Sigma-Aldrich	HPA005848
FLAG M2 1/1000	Sigma-Aldrich	F1804
GAPDH 1/2000	Abcam	AB8245
GFP 1/2000	Amsbio	TP401
GFP (PLA)	Roche	11814460001
GST 1/1000	Abcam	ab19256
γH2AX 1/1000	Abcam	ab22551
Histone H3 1/5000	Abcam	ab1791100
RPB1 1/5000	Homemade IGBMC	7C2
RNA Pol II (PLA)	Bethyl	A300-653A
TFIIEα 1/1000	Homemade, IGBMC	2A1
Vinculin 1/1000	Sigma Aldrich	V9131
XPA 1/500	SCBT	SC-853
6-4PP 1/1000	Cosmo Bio	CAC-NM-DND-002
Chemicals, peptides, and recombinant proteins		
Actinomycin D	Sigma Aldrich	A-1410
DRB	Merck Chemicals	287891
Et743	PharmaMar	(Feuerhahn et al., 2011)
LightCycler 480 SYBR Green I Master	Roche	04887352001
MitoTracker Red CMXRos	Invitrogen	M7512
Streptavidin magnetic beads	Fisher Scientific SAS	88816
TriReagent	Molecular Research Center	TR118
Vectashield	VectorLaboratories	H-1000-10
Critical commercial assays		
Click-iT EU Alexa Fluor 488 Imaging Kit	ThermoFisher	C10329
Click-iT EdU Alexa Fluor 488 Imaging Kit	ThermoFisher	C10337
Lipofectamine RNAiMAX Transfection Reagent	Fisher Scientific SAS	13778030
SuperScript IV kit	Invitrogen	18090050
X-tremeGene DNA Transfection Reagent	Roche	063657870001
Qproteome Mitochondria Isolation Kit	Qiagen	37612
Experimental models: Cell lines		
U-2 OS and U-2 OS pTuner 263		(Nagy et al., 2016)
CS1ANSV and CS1ANSV+CSB		(Kristensen et al., 2013)
HeLa		Nieminuszczy et al., 2019)
HeLa EXD2$^{-/-}$-cl1 and 2		(Nieminuszczy et al., 2019)

HeLa EXD2$^{-/-}$+EXD2^{WT}-cl1 and 2		(Nieminuszczy et al., 2019)
HeLa EXD2$^{-/-}$+EXD2$^{D108A/E110A}$-cl1 and 2		(Nieminuszczy et al., 2019)
U-2 OS^{GFP}		(Nieminuszczy et al., 2019)
U-2 OS$^{EXD2-GFP}$		(Nieminuszczy et al., 2019)
XP4PA-SV		(Donnio et al., 2019)
XPC HeLa Silencix		(Le May et al., 2010)
Oligonucleotides		
F: GCAGAAGAACGGCATCAAGG	*CFP-SKL*	This paper
R: GTCCATGCCGAGAGTGATCC	*CFP-SKL*	This paper
F: CTTCCCCGATGAGAACTTCAAACT	*CycB*	This paper
R: CACCTCCATGCCCTCTAGAACTTT	*CycB*	This paper
siXPF	Dharmacon	019946-00
siXAB2	Dharmacon	004914-01
siEXD2	Dharmacon	020899-02
siCTL	Dharmacon	001810-02
siXPA	Dharmacon	005067-01
siCSB	Dharmacon	004888-00
siCSA	Dharmacon	011008-00
siUSP33	Dharmacon	006081-00
siBPTF	Dharmacon	004025-00
siYLPM1	Dharmacon	032696-00
Recombinant DNA		
mCherry-LacR		(Nagy et al., 2016)
Software and algorithms		
GraphPad Prism	GraphPad Software Inc.	
ImageJ	NIH	
CellProfiler	Broad Institut	CellProfiler
Other		
Leica DM 4000 B equipped with a CoolSnap FX monochrome camera	Leica	

Supplementary References

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