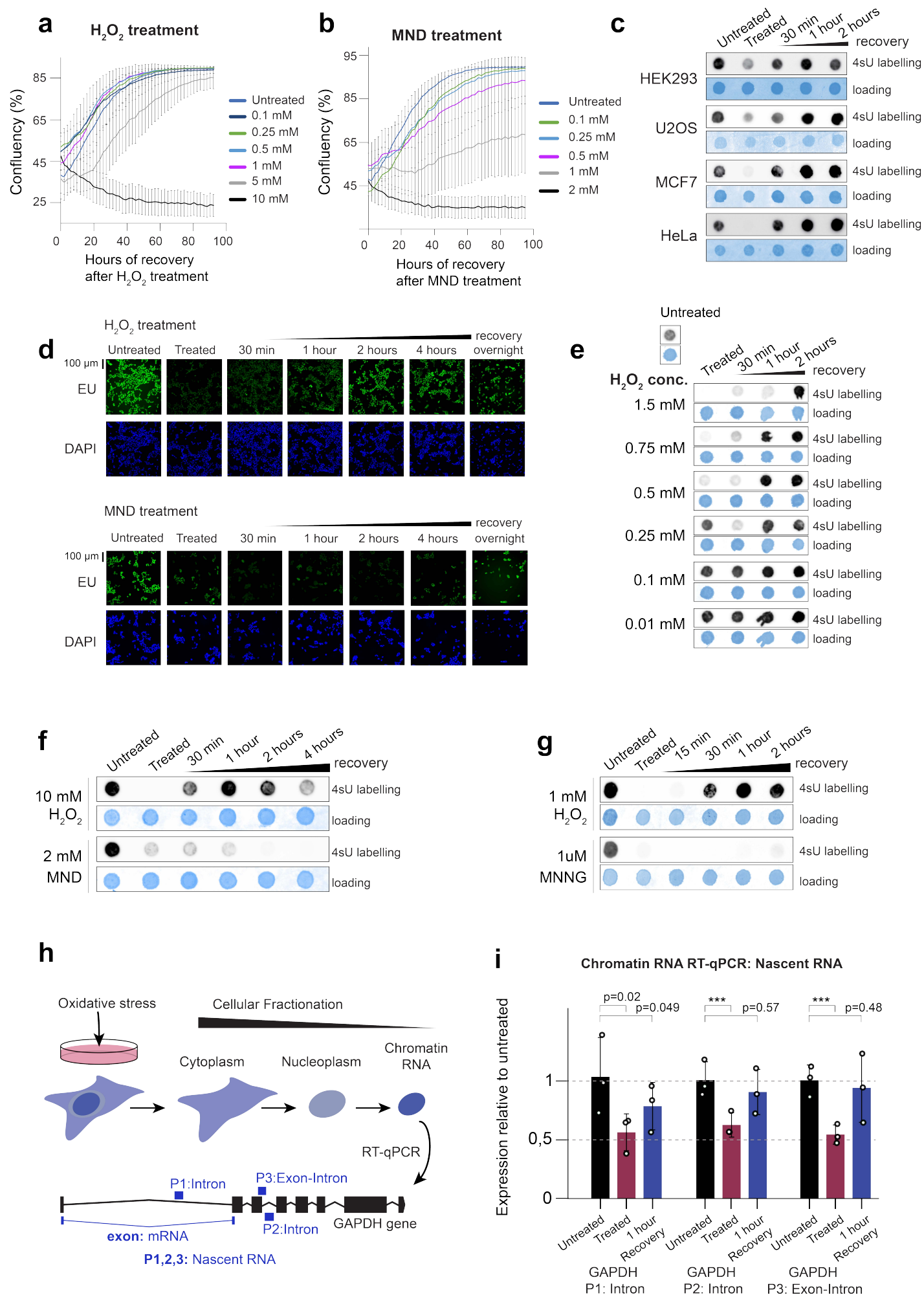


Supplementary Information

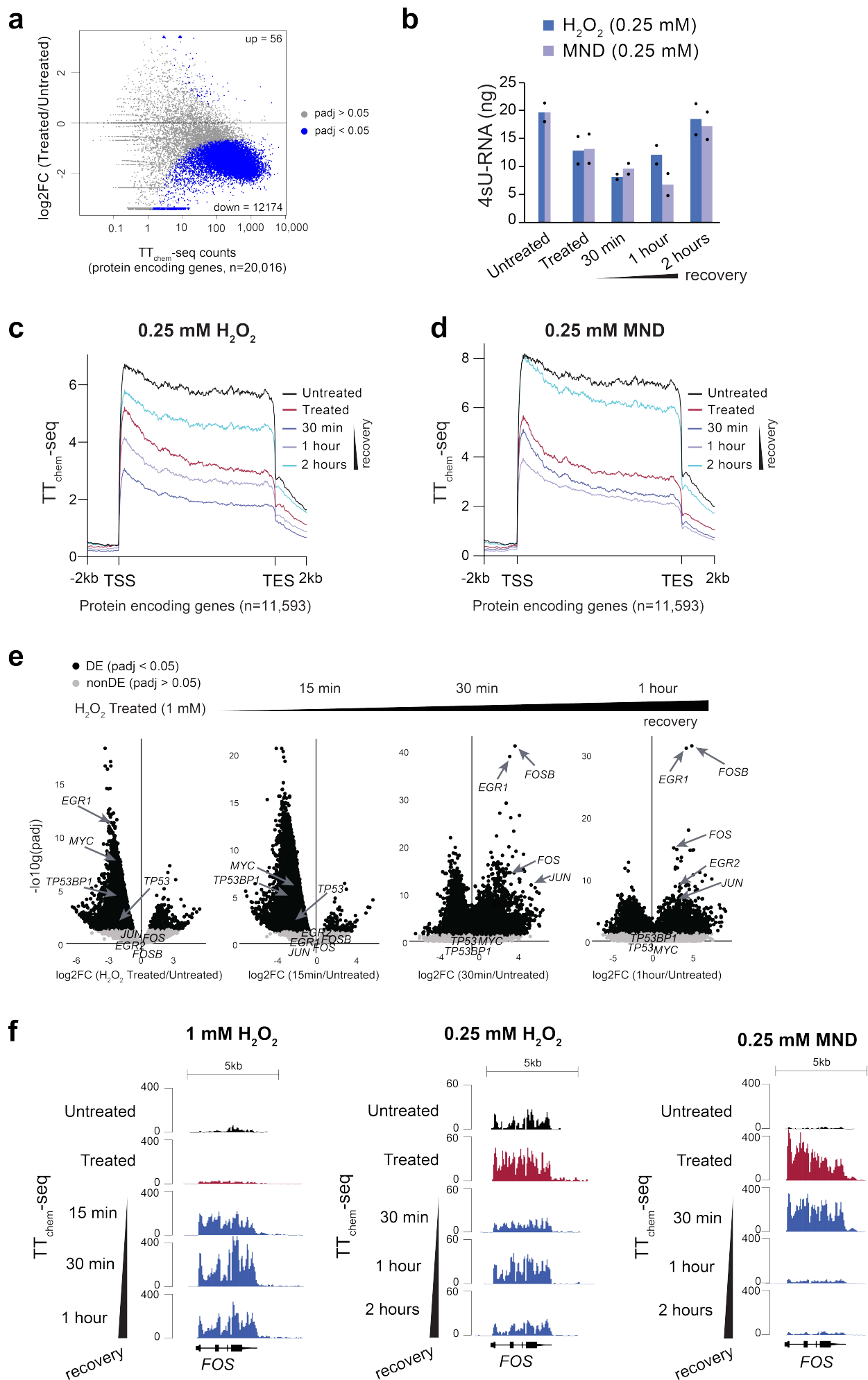
Supplementary Figures and Tables

- **Supplementary Fig. 1. Oxidative stress represses nascent transcription.**
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- **Supplementary Table 2: Oligonucleotides**



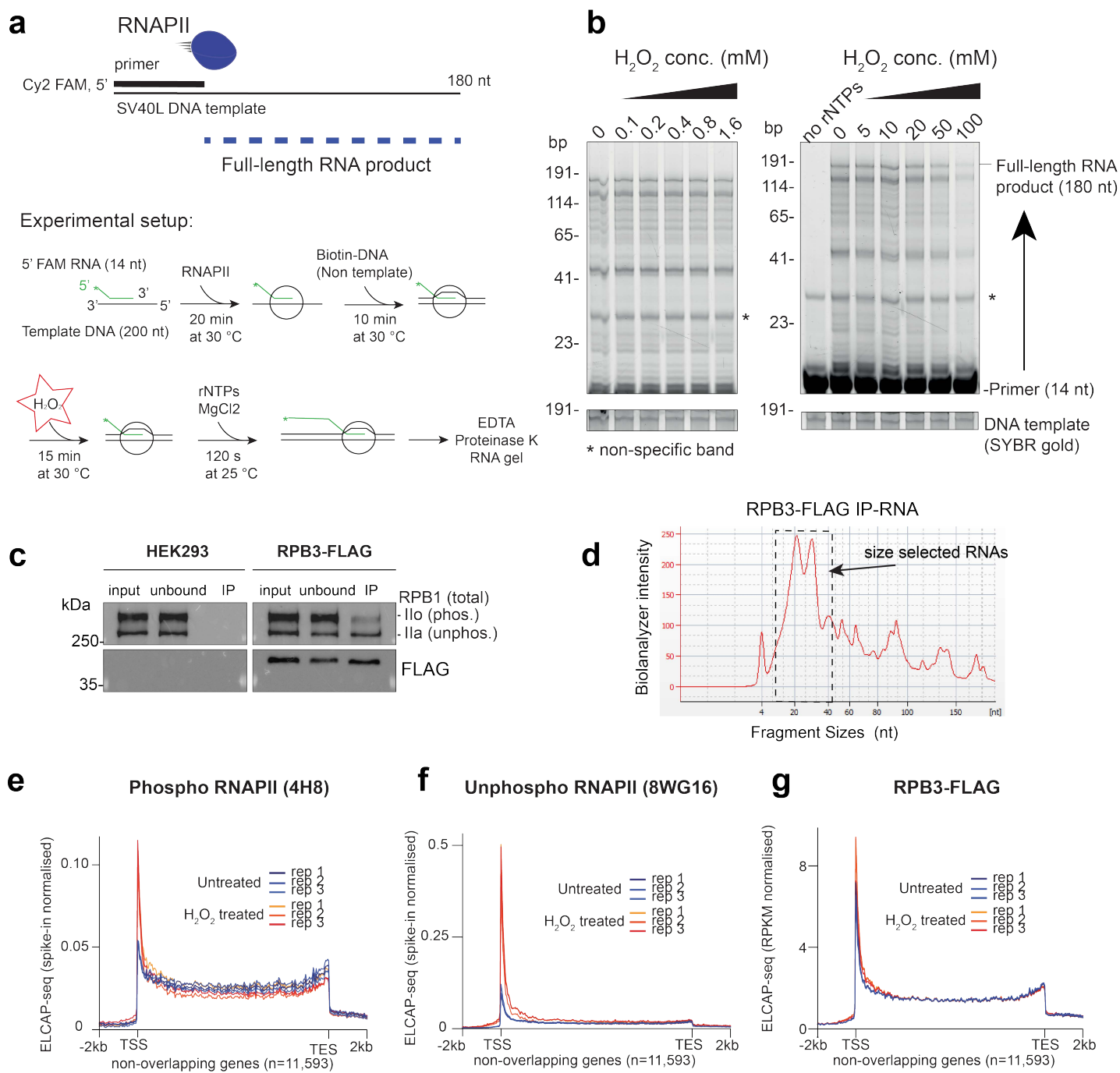
Supplementary Figure 1

Supplementary Fig. 1. Oxidative stress represses nascent transcription. **a-b** Growth assay of HEK293 cells monitored by live cell imaging (IncuCyte). Cells were treated for 15 min with indicated doses of H₂O₂ (d) or MND (e), followed by media replenishment. Each timepoint is the mean of three biological replicates (n=3) and error bars represent ± 1 standard deviation **c** Nascent transcription levels (4sU dot blot) in HEK293, U2OS, MCF7 and HeLa cells treated for 15 min with 1 mM H₂O₂ and following media replenishment. Staining with methylene blue serves as a loading control. (SD) **d** Representative images of global nascent transcription (EU immunofluorescence) from experiments in Fig. 1c and 1d of WT HEK293 cells following oxidative stress conditions. All image intensities are displayed with same exposure as the untreated control. **e** Nascent RNA levels (4sU dot blot) of cells treated with decreasing H₂O₂ doses for 15 min. Staining with methylene blue serves as a loading control. **f** Nascent RNA levels (4sU dot blot) cells treated for 15 min with 10 mM H₂O₂ or 2 mM MND with indicated recovery times after media replenishment. Staining with methylene blue serves as a loading control. **g** Nascent RNA levels (4sU dot blot) cells treated for 15 min with 1 mM H₂O₂ or 1 μ M MNNG with indicated recovery times after media replenishment. Staining with methylene blue serves as a loading control. **h** Schematic of RNA chromatin extraction and RT-PCR primer pair position over *GAPDH* gene features used to amplify nascent RNA. **i** Relative expression of nascent *GAPDH* RNA transcript in HEK293 cells treated for 15 min with 1 mM H₂O₂ and cells treated and harvested 1 hour following media replenishment. All are shown relative to untreated cells. Significance was assessed with two-sided student t-test (star represents significance of with ***; exact P >0.001 are labelled on the plot)



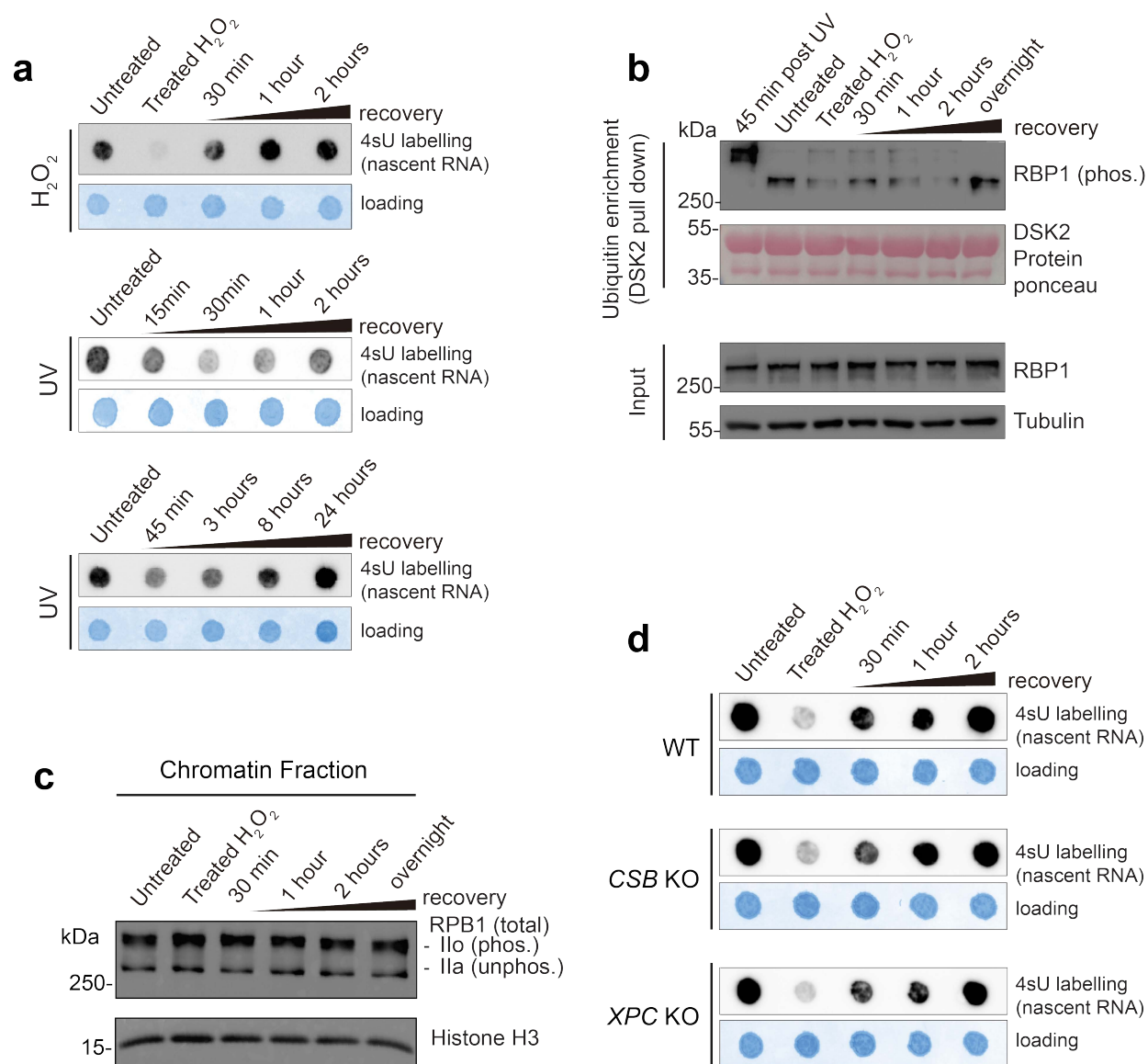
Supplementary Figure 2

Supplementary Fig. 2. The transcriptional response to varying doses of ROS. **a** Scatterplot of log₂ fold-change between the mean TT_{chem}-seq normalised (with spike-in) read counts per protein encoding-genes of three biological replicates of H₂O₂ treated cells compared to untreated cells, relative to mean read counts for all protein coding genes (n = 20,016). Blue dots represent genes with a false discovery rate (FDR) corrected q-value < 0.05 measured with Wald test. **b** 4sU labelled RNA yield measured after streptavidin pull-down for duplicates of untreated HEK293 cells, treated for 15 min with 0.25 mM H₂O₂ or 0.25 mM MND and indicated recovery times. **c-d** Metagene analysis of TT_{chem}-seq data for non-overlapping coding genes (n=11,593) of untreated and indicated recovery timepoint following 0.25 mM H₂O₂ treatment of HEK293 cells. (b) or 0.25 mM MND. (c) Lines is the mean of spike-in normalised read counts between two biological replicates. **e** Scatterplots of fdr p-adjusted value (-log₁₀ padj) of comparison between the means of triplicates TT_{chem}-seq spike-in normalised signals of H₂O₂ treated or recovery cells compared to untreated cells, relative to log₂ fold-change for all annotated genomic features (n = 60,649). Genes in black have Wald test FDR adjusted p < 0.05 and in grey p > 0.05. Immediate early genes *EGR1*, *EGR2*, *FOS*, *FOSB*, *TP53*, *TP53BP1* and *MYC* are highlighted. **f** Single-gene view of TT_{chem}-seq data for the immediate early stress response genes *FOS* for experimental conditions in Figure 3a and Supplementary Figure 2c-d.

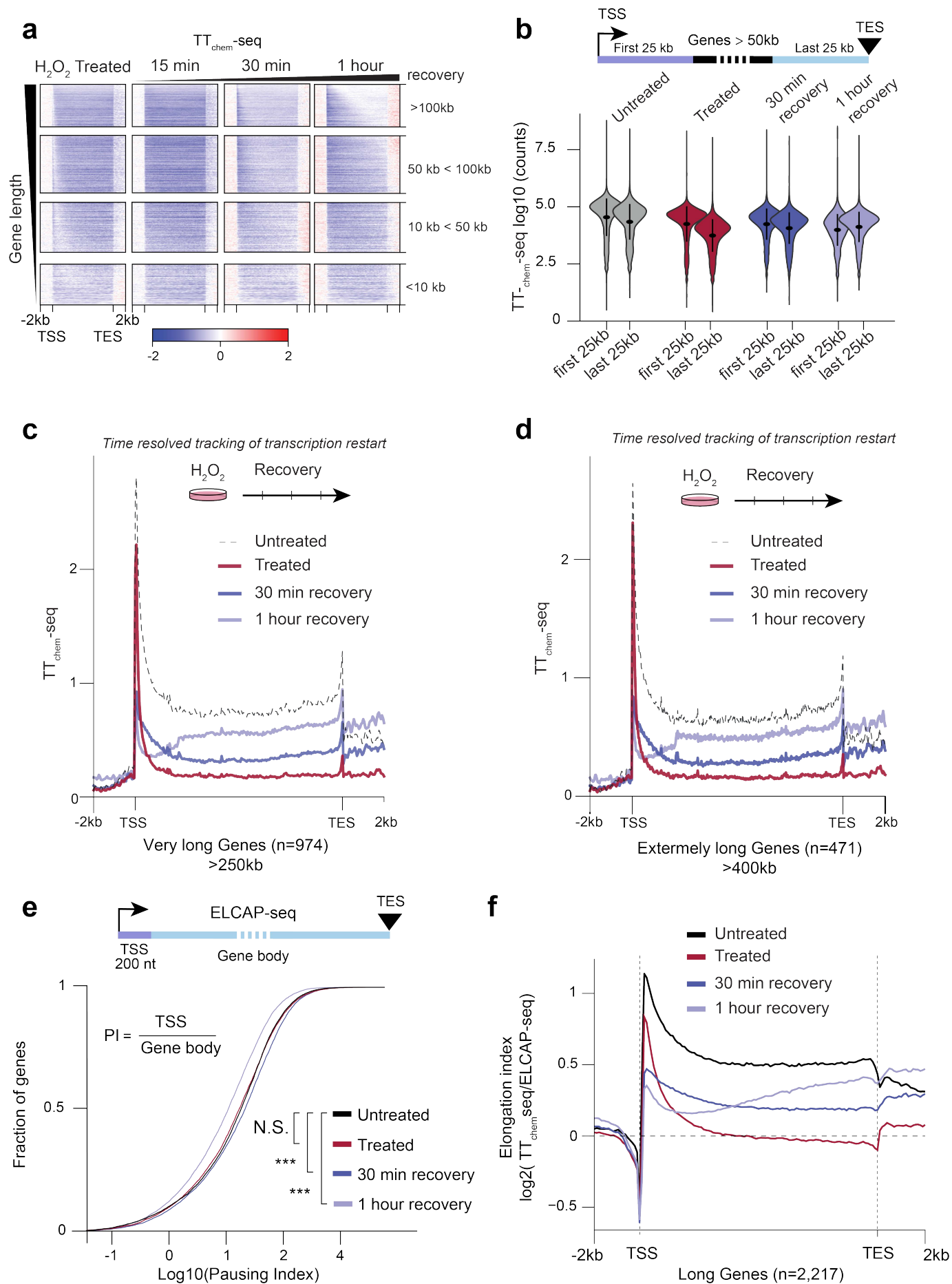


Supplementary Fig. 3. *In vitro* RNAPII activity after H₂O₂ exposure and RNAPII ELCAP-seq. a

Setup used for RNAPII *in vitro* transcription assay. RNAPII complexes purified from pig thymus is loaded on the FAM-labelled primer bound to SV40L DNA template resulting in various sized RNA fragments up to 180 nt. **b** Reconstituted *in vitro* transcription assay performed with RNAPII complexes treated with indicated H₂O₂ concentrations for 15 min. The resulting transcripts from the *in vitro* transcription assay were resolved on 8 M Urea TBE gels and visualised by Cy2 fluorescence. **c** Verification of RNAPII IP used in the ELCAP protocol from HEK293-RPB3-FLAG cell line. Western blot analysis of total RNAPII using either a RBP1 antibody (D8L4Y) or FLAG antibody. Input, unbound, and IP fractions were resolved. **d** Bioanalyzer spectrum of RNA extracted from RPB3-FLAG IPs and used as input for ELCAP-seq. Detected RNA intensity is shown for different size fragments (nt). Dotted line represents the region size-selected for library preparation. **e-g** Metagene analysis of ELCAP-seq signal for phosphorylated RNAPII (e), unphosphorylated RNAPII (f) and RPB3-FLAG RNAPII (g) in untreated cells and H₂O₂ treated cells for a set of protein-encoding genes (non-overlapping $\pm$ 2 kb, n = 11,593). Signal is represented for each biological replicate and counts normalised to *Drosophila* spike-in (e-f) or library size (g)).



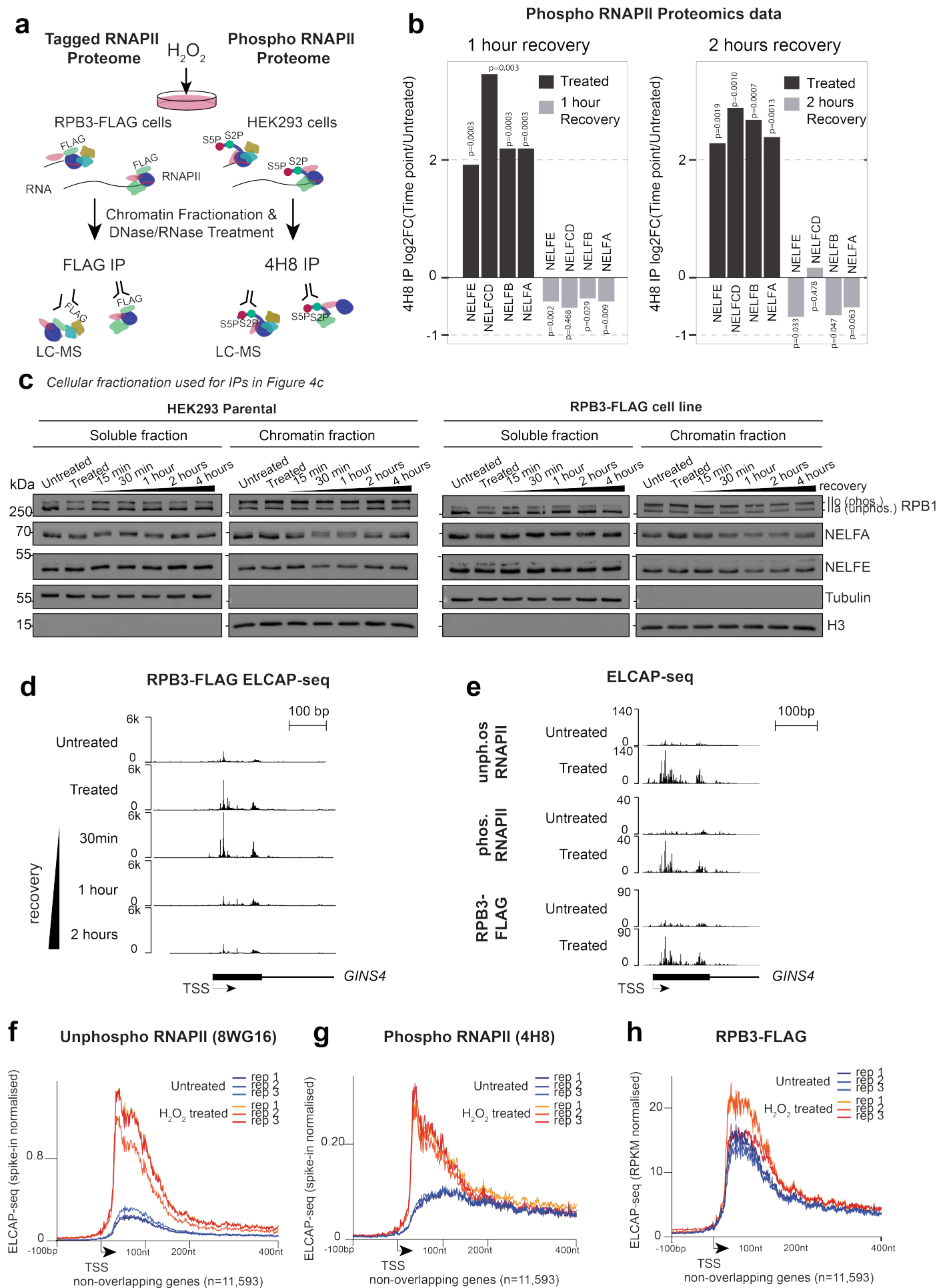
Supplementary Fig. 4. The transcriptional response to oxidative stress is distinct from the UV-induced transcriptional response and not dependent on NER factors CSB or XPC. **a** Nascent transcription levels (4sU dot blot) in HEK293 cells treated with 1 mM H₂O₂ at indicated times after media washout or recovery time following 15 J/m² UV-C (256 nm) irradiation. Staining with methylene blue serves as a loading control. **b** Western blot of phosphorylated RNAPII (4H8) in DSK2-enriched proteins fractions (ubiquitin enriched) for HEK293 cells 45 min after 20 J/m² UV-C or 1 mM H₂O₂ treatment and indicated times after media replenishment. Ponceau represents DSK2 protein loading control (top). Input fraction was probed with a pan RPB1 phosphorylated antibody (4H8) and tubulin for loading control (bottom). **c** Western blot of total RNAPII (D8L4Y) level of chromatin-enriched cellular fraction after 1 mM H₂O₂ treatment and indicated recovery time after media replenishment. (Same experiment as shown in Figure 1i). Histone H3 serves as a loading control. **d** Nascent transcription levels (4sU dot blot) in WT (HEK293), *CSB* KO and *XPC* KO cells treated with 1 mM H₂O₂ and indicated times after media replenishment. Staining with methylene blue serves as a loading control.



Supplementary Figure 5

Supplementary Fig. 5. Nascent transcription dynamics upon recovery from oxidative stress. a

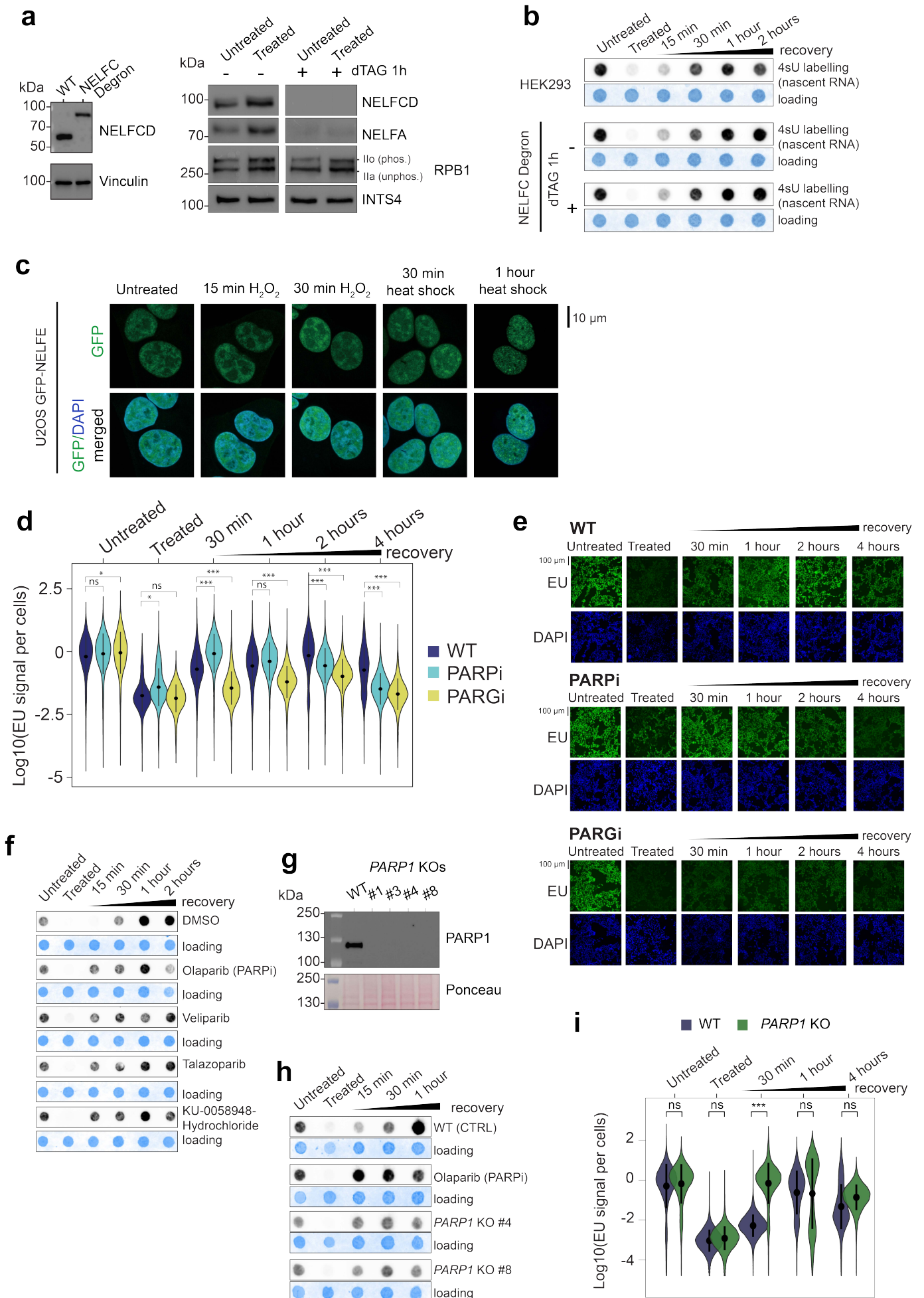
Heatmaps of TT_{chem}-seq signal (normalised to spike-in) in HEK293 cells. The signal is shown as the log₂ fold-change for indicated treatment relative to untreated control. Heatmaps are scaled between TSS and TES (± 2 kb). Genes are sorted by decreasing sized and separated in four size thresholds. **b** Quantification of TT_{chem}-seq signal for the first 25 kb and the last 25 kb of non-overlapping coding genes > 50 kb ($n = 5,875$). **c-d** Metagene analysis of TT_{chem}-seq data for very long genes (>250 kb, $n=974$) (c) and extremely long genes (>400 kb, $n=471$) following H₂O₂ treatment and indicated recovery time points in HEK293 cells. The line represents the mean of spike-in normalised read counts from three biological replicates. **e** Pausing index (log₁₀ ratio of TSS signal (first 150 nt) relative to remaining gene body signal) of ELCAP-seq signal for all non-overlapping coding genes ($n = 17,266$). Distribution comparison measured by two-sided Wilcoxon-test, N.S. (p-value > 0.05), *** (p-value < 0.001). **f** Metagene analysis of elongation index, the signal is the log₂ fold-change of TT_{chem}-seq read counts (normalised to spike-in) signal over RPKM ELCAP-seq signal for respective conditions plotted for long coding genes (> 90 kb, $n=2,217$).



Supplementary Figure 6

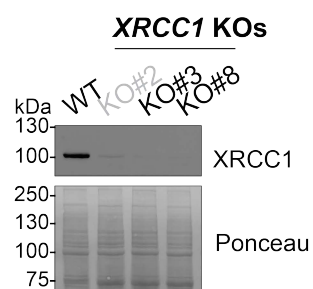
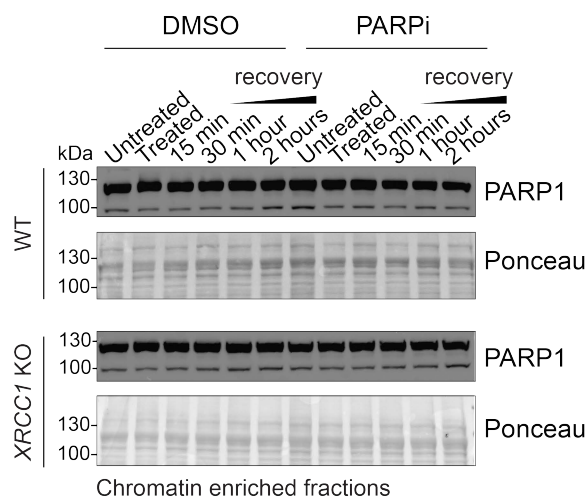
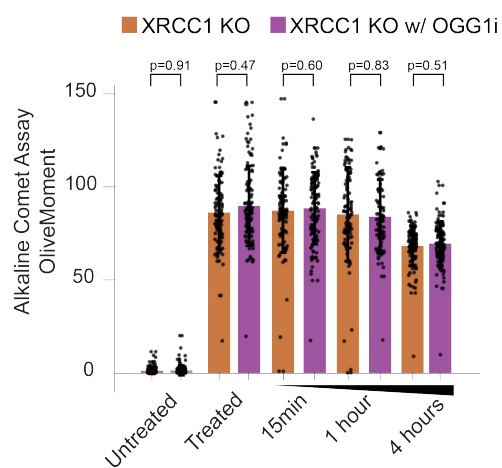
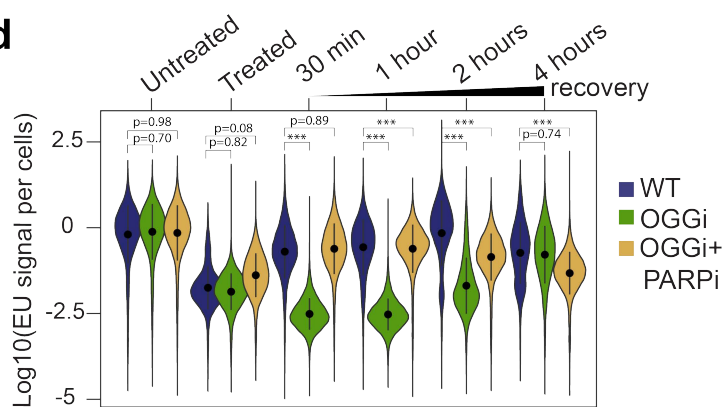
Supplementary Fig. 6. NELF-mediated regulation of RNAPII following oxidative stress. a

Experimental setup used for RNAPII interactome experiments for capture of either epitope-tagged RPB3-FLAG RNAPII complexes or phosphorylated RNAPII complexes. **b** Log2 fold-change of mass spectrometry average intensities over three technical replicates for individual NELF subunits. Comparison of protein enrichment between treated/1 hour recovery (left) or treated/2 hours recovery (right) over untreated measured by FDR corrected two-sided unpaired SMAR t-test, * exact P values are labelled on the plot. **c** Western blot analysis of soluble and chromatin protein fractions (input fraction for experiment shown in Figure 4c) of HEK293 cells (left), and RPB3-FLAG HEK293 cells (right) before and after H₂O₂ treatment and recovery time. Total RPB1 probed with D8L4Y antibody, anti-NELFA, anti-NELFE and anti-H3. **d** Single-gene view of ELCAP-seq data in RPB3-FLAG cells for the *GINS4* transcription start site following oxidative stress and recovery timepoints. **e** Single-gene view of ELCAP-seq data from 4H8 and 8WG16 IP in HEK293 cells and FLAG IP in RPB3-FLAG cells for the *GINS4* transcription start site in untreated and H₂O₂ treated conditions **f-h** Metagene analysis of ELCAP-seq signal of triplicates from unphosphorylated RNAPII with 8WG16 immunoprecipitation (f), phosphorylated RNAPII with 4H8 immunoprecipitation (g) and RPB3-FLAG tagged RNAPII with FLAG immunoprecipitation (h). The signal is centred on the TSS of non-overlapping coding genes (n=11,593) in untreated control and H₂O₂ treated samples.



Supplementary Figure 7

Supplementary Fig. 7. Role of NELF and PARylation in RNAPII transcription recovery. **a** Left: Western blot of WT and NELFCD-degron cells probed with anti-NELFCD and vinculin (loading control). The lower molecular weight band corresponds to WT NELFC, and the higher band to degron-tagged NELFCD. Right: Western blot of chromatin-enriched fractions from NELFCD-degron cells with or without 1 hour pre-treatment with 250 nM dTAG-v1, under untreated and H₂O₂-treated conditions. Blots were probed for NELFCD, NELFA, total RNAPII (D8L4Y), and INTS4 (loading control). **b** Nascent RNA levels measured by 4sU dot blot in HEK293 cells (top) and NELFCD-degron cells (bottom, $\pm$ 1 hour 250 nM dTAG-v1 pre-treatment) following 1 mM H₂O₂ treatment and recovery at indicated timepoints. Methylene blue staining serves as a loading control. **c** Fluorescence microscopy of U2OS GFP-NELFE cells showing GFP and merged GFP/DAPI signals following 15 min and 30 min H₂O₂ treatment, and 30 min or 1 hour heat shock (43 °C). **d** Nascent transcription quantified by 5-ethynyluridine (EU) incorporation and single-cell immunofluorescence (EU assay). EU signal per cell was normalised to DAPI and log₁₀-transformed. HEK293 cells treated with DMSO (WT), PARPi, or PARGi were exposed to 1 mM H₂O₂ and labelled with EU for 30 min. Statistical significance was determined using a linear mixed-effects model (***P < 0.001, exact P > 0.001 are labelled on the plot). **e** Representative EU immunofluorescence images from (d). Images were acquired using identical exposure settings relative to untreated controls. **f** Nascent RNA levels (4sU dot blot) in HEK293 cells pre-treated with 10 μ M Olaparib, Veliparib, Talazoparib, or KU-0058948-HCl, followed by 1 mM H₂O₂ treatment for 15 min and recovery at indicated timepoints. Methylene blue staining serves as a loading control. **g** Western blot validation of HEK293 *PARP1* knockout clones. Ponceau S staining indicates protein loading. Clones KO#4 and KO#8 were used in parallel experiments. **h** Nascent RNA levels (4sU dot blot) in HEK293 cells pre-treated with 10 μ M Olaparib or *PARP1* knockout cells, following 1 mM H₂O₂ treatment for 15 min and recovery at indicated timepoints. Methylene blue staining serves as a loading control. **i** Nascent transcription quantified by EU incorporation and single-cell immunofluorescence (EU assay) in HEK293 *PARP1* KO#8 cells treated with H₂O₂ for 30 min and labelled for 30 min. EU signal was normalised to DAPI and log₁₀-transformed. Statistical significance was determined by two-sided linear mixed-effects model (***P < 0.001, exact P > 0.001 are labelled on the plot).

a**b****c****d**

Supplementary Fig. 8. Role of XRCC1 and OGG1 in RNAPII transcription recovery. **a** Western blot validation of HEK293 *XRCC1* KOs. Protein loading is represented by ponceau S staining. The two clones KO#3 and KO#8 were used in parallel for experiment with *XRCC1* KOs. **b** Western blot of from HEK293 and HEK293 *XRCC1* KO#3 cells chromatin-enriched cellular fractions either pre-treated for 1 hour with DMSO or PARPi and treated with H₂O₂ and indicated recovery after media washout in WT HEK293 (top) and *XRCC1* KO#3 cells (bottom). Protein loading is represented by ponceau S staining. **c** Quantification of DNA strand breaks measured by alkaline comet assays in HEK293 *XRCC1* KO#3 cells pre-treated for 1 hour with DMSO or OGG1i and treated for 15 min with H₂O₂ (not treated; 'Untreated') and indicated timepoints following media replenishment. Data show individual Olive tail moments for > 50 cells per conditions. Statistical significance was determined by linear mixed-effect model (LME) analysis (star represents significance with ***; P values < 0.001, *; P values < 0.05, ns; P values > 0.05). **d** Nascent transcription levels quantified by 5-Ethynyluridine (EU) labelling and single cell quantification by immunofluorescence microscopy (EU-assay). Per cell EU signal is normalised to DAPI and log10 scaled. DMSO (WT), OGG1i (same experiment as in Figure 7a) and OGG1i together with PARPi treated HEK293 cells were treated with H₂O₂ for 30 min and EU-labelled for 30 min. Statistical significance was determined by two-sided linear mixed-effect model (LME) analysis (exact P values are labelled on the plot).

Supplementary Table 1

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-FLAG (IP/ELCAP:1µg/sample, WB: 0.2 µg/mL)	Sigma	Cat#F1804; RRID:AB_262044
RPB1 D8L4Y (total, N-terminal) (WB: 1µg/mL)	Cell Signaling	Cat#14958S; RRID:AB_2687876
RPB1 4H8 (raised against S5-P, recognizes multiple phosphorylated forms) (IP/ELCAP:1µg/sample, WB: 0.5 µg/mL)	Francis Crick Institute Cell Services	4H8
RPB1, serine 5 phosphorylated (WB: 0.2 µg/mL)	Kind gift from Dirk Eick	3E8
RPB1 8WG16, (raised against unphosphorylated CTD) (IP/ELCAP:2µg/sample, WB: 1 µg/mL)	Francis Crick Institute Cell Services	8WG16
monoclonal anti-NELFCD (D5G6W) (WB: 1 µg/mL)	Cell signaling	Cat#12265,RRID:AB_2797862
recombinant anti-NELFE (IP:1µg/sample, WB: 1µg/mL)	Abcam	CAT#ab170104,RRID:AB_2827280
polyclonal anti-NELFA (WB: 1 µg/mL)	Thermo fisher scientific	Cat#A301-910A,RRID:AB_1524101
polyclonal anti-GFP (IF: 2 µg/mL)	Abcam	Cat#ab290, RRID:AB_2313768
Horse Anti-Mouse IgG Antibody (H+L), HRP (WB: 0.2 µg/mL)	Vector Laboratories	Cat#PI-2000; RRID:AB_2336177
Goat Anti-rabbit IgG Antibody (H+L), HRP (WB: 0.2 µg/mL)	Vector Laboratories	Cat#PI-1000; RRID:AB_2336198
Goat Anti-rat IgG Antibody (H+L), HRP (WB: 0.2 µg/mL)	Jackson ImmunoResearch Labs	Cat#112-035-003,RRID:AB_2338128
Tubulin (WB: 0.2 µg/mL)	Abcam	Cat#ab15246; RRID:AB_301787
Histone 3 (WB: 0.1 µg/mL)	Abcam	Cat#ab18521; RRID:AB_732917

monoclonal anti-poly-ADP-ribose binding reagent (WB: 1 µg/mL)	Novus Biologicals	Cat#MABE1031, RRID:AB_2665467
anti-phospho-Histone H2AX (Ser139, JBW301) (IF: 2 µg/mL)	Merck	Cat#05-636,RRID:AB_309864
Goat anti-Mouse IgG, Alexa Fluor™ 594 (IF: 1 µg/mL)	ThermoFisher Scientific	Cat#A11005,RRID:AB_2534073
Goat anti-Rabbit IgG,Alexa Fluor™ 488 (IF: 1 µg/mL)	ThermoFisher Scientific	Cat#A11008,RRID:AB_143165
polyclonal anti-PARP1 (WB: 1 µg/mL)	Cell Signaling	Cat#9542S,RRID:AB_2160739
Bacterial and virus strains		
NEB 5-alpha Competent E. coli	NEB	C2988J
One Shot™ ccdB Survival™ 2 T1R Competent Cells	ThermoFisher Scientific	A10460
One Shot™ Stbl3™ Chemically Competent E. coli	ThermoFisher Scientific	C7373-03
Chemicals, peptides, and recombinant proteins		
dTAG-V1	Tocris Bioscience	6914
Trizol Reagent	Cell Signaling	15596026
UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v)	Invitrogen	15593031
DENARASE	c-Lecta	20804
2-Propanol	Sigma-Aldrich	I9516
Deoxynucleotide (dNTP) Solution Set	NEB	N0446S
DMEM High Glucose w/L-Glutamine w/ Sodium Pyruvate	biowest	L0104
FBS	Thermo Fisher Scientific	10270106
Poly-D-Lysine	Gibco	A3890401
Hydrogen peroxide solution 30 % (w/w)	Merck	H1009
Methylnitronitrosoguanidine (MNNG)	MedchemExpress	HY-128612
Menadione Sodium Bisulfite	Sigma-Aldrich	M5750

Olaparib (PARP1i)	MedchemExpress	HY-10162
Talazoparib	MedchemExpress	HY-16106
Veliparib	MedchemExpress	HY-10129
KU-0058948 HCl	Axon	2108829-86-1
PDD 00017273 (PARGi)	MedchemExpress	HY-108360
TH5487 (OGG1i)	MedchemExpress	HY-125276
Gateway LR Clonase II Enzyme Mix	ThermoFisher Scientific	11791020
Doxycycline (DOX)	Clontech	8634-1
4-thiouridine (4sU)	Glentham Life Sciences	GN6085
4-thiouracil (4tU)	Sigma-Aldrich	440736
Lipofectamine™ 3000	ThermoFisher Scientific	L3000015
5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB)	Sigma-Aldrich	D1916
Puromycin	Cayman chemicals	13884
QuickExtract DNA Extraction Solution	Epicentre Technologies	SS000035-D2
5-Ethynyl-uridine (5-EU)	Jena Bioscience	CLK-N002
MTSEA biotin-XX linker	Biotium	BT90066
EZ-Link™ HPDP-Biotin	ThermoFisher Scientific	21341
Dynabeads™ Protein G	Invitrogen	10004D
Alexa Fluor™ 488 Azide	ThermoFisher Scientific	A10266
HRP-conjugated streptavidin	ThermoFisher Scientific	N100
Protease Inhibitor Cocktail	Sigma-Aldrich	P2714
Sodium orthoVanadate (Na ₃ VO ₄)	Sigma-Aldrich	S6508
Sodium fluoride (NaF)	Sigma-Aldrich	S1504
B-glycerolphosphate	Sigma-Aldrich	G5422

Bio-Rad Protein Assay Dye Reagent Concentrate	Biorad	5000001
Citifluor™ Mounting media	EMS	17970-25
Ponceau S solution	Sigma-Aldrich	PI-2000-1
Clarity ECL Western Blotting Substrates	Biorad	1705061
DNAse I, RNAse-free	Thermo Fisher	EN0521
HOT FIREPol® EvaGreen® qPCR Mix Plus	Solis BioDyne	08-25-00001-5
SeaPlaque® GTG Agarose	Thermo Fisher	50111
IGEPAL® CA-630	Sigma	I8896
Critical commercial assays		
µMACS Streptavidin Kit	Miltenyi	130-074-101
Bioanalyzer Small RNA Analysis	Agilent	5067-1548
Qubit RNA HS Assay Kit	Thermo Fisher	Q32852
Qubit RNA BR Assay Kit	Thermo Fisher	Q10210
RNeasy MinElute kit	QIAGEN	74204
NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®	NEB	E7645
NEBNext® Ultra™ II RNA Library Prep Kit for Illumina®	NEB	E7760
NEBNext® Small RNA Library Prep Set for Illumina® (Multiplex Compatible)	NEB	E7300
NEBNext® Multiplex Oligos for Illumina® (Unique Dual Index UMI Adaptors DNA Set 1)	NEB	E7395
Quick-RNA MicroPrep	Zymo	R1050
OxiSelect™ 10-Well Comet Assay Slides	Cell Biolabs Inc	STA-853
Deposited data		
Genome-wide data are available under SRA	This manuscript	SRA: PRJNA1275866
Mass Spectrometry data are available under ProteinXchange	This manuscript	PXD067831,PXD067969,
Experimental models: Cell lines		
T-REx™ HeLa (HeLa)	kind gift of Jesper Svejstrup	R71407

MCF7	kind gift of René Villadsen	N/A
HEK293 Flp-In™ T-REx™ (HEK293)	ThermoFisher Scientific	R78007
Flp-In T-REx U2OS cells (U2OS)	kind gift of Michael Lisby	N/A
HEK293 Flp-In™ T-REx_FKPB12F36V-NELFCD (NELFC degron)	This manuscript	N/A
U2OS Flp-In™ T-REx_GFP-NELFE (GFP-NELFE)	This manuscript	N/A
HEK293 Flp-In™ T-REx_XRCC1 KO_3 (XRCC1 KO#3)	This manuscript	N/A
HEK293 Flp-In™ T-REx_XRCC1 KO_8 (XRCC1 KO#8)	This manuscript	N/A
HEK293 Flp-In™ T-REx_RPB3-3xFLAG (RPB3-FLAG)	This manuscript	N/A
HEK293 Flp-In™ T-REx_CSB KO (CSB KO)	kind gift of Jesper Svejstrup ¹	N/A
HEK293 Flp-In™ T-REx_XPC KO (XPC KO)	This manuscript	N/A
HEK293 Flp-In™ T-REx_PARP1 KO_4 (PARP1 KO#4)	This manuscript	N/A
HEK293 Flp-In™ T-REx_PARP1 KO_8 (PARP1 KO#8)	This manuscript	N/A
Experimental models: Organisms/strains		
<i>S. cerevisiae</i> , W303a MATa ura3 leu2-3, 112 his3-11,15 trp1-1 ade2-1 can1-100	Kind gift from Jesper Svejstrup	N/A
Oligonucleotides		
	See Table S2	
Recombinant DNA		
pOG44	ThermoFisher Scientific	V600520
pDG458	Addgene	RRID: Addgene_100900
pSpCas9(BB)-2A-Puro (pX459) V2.0	Addgene	RRID: Addgene_48138
pFRT/TO/GFP_DEST	Gift from Jesper Svejstrup	N/A
pIRESpuro-RPB3-HA	Gift from Jesper Svejstrup	N/A
pDEST/TO/FRT/3xFLAG	This manuscript	N/A

pENTR4-RPB3	This manuscript	N/A
pENTR4-NELFE	This manuscript	N/A
pDONR-HA1/NELFCD/FKBP/PURO/HA2	This manuscript	N/A
pDEST/TO/FRT/RPB3-FLAG	This manuscript	N/A
pDEST/TO/GFP-NELFE	This manuscript	N/A
pDG458-XRCC1-sg1&2	This manuscript	N/A
pDG458-NELFCD-sg1&2	This manuscript	N/A
pX459-XPC-gRNA1	This manuscript	N/A
pX458-PARP1-gRNA1	This manuscript	N/A
Software and algorithms		
SAMtools	Danecek et al. ²	https://www.htslib.org/
deepTools	Ramirez et al. ³	https://deeptools.readthedocs.io/en/latest/content/about.html
BEDtools	Quinlan and Hall ⁴	https://bedtools.readthedocs.io/en/latest/
TrimGalore	Babraham Bioinformatics	https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/
FijiImageJ	Schindelin et alNIH	https://imagej.net/software/fiji/
IGB	NIH	https://www.bioviz.org/
Illustrator	Adobe	https://www.adobe.com/

STAR v2.7.11	Dobin et al ⁵	https://github.com/alexdobin/STAR
SubreadFeatureCounts	Liao et al ⁶	https://subread.sourceforge.net/
UMItools	Smith et al ⁷	https://umi-tools.readthedocs.io/en/latest/index.html
CAGEfightR	Thordberg et al ⁸	https://www.bioconductor.org/packages/devel/bioc/vignettes/CAGEfightR/inst/doc/Introduction_to_CAGEfightR.html
R (v4.3.2)	R Core Team	N/A
scanR High-Content Screening System	Olympus	https://evidentscientific.com/en/products/high-content-screening/scanr
Other		
HybondTM-N+ membranes	Cytiva	RPN203B
Nitrocellulose membranes	GE Healthcare Life Sciences	Cat#10600002

Supplementary Table 2

Oligonucleotides		
XRCC1_sgRNA#1_F	CACCTGCAGGACACGACATGGCGG	This manuscript
XRCC1_sgRNA#1_R	AAACCCGCCATGTCGTGTCCTGCA	This manuscript
XRCC1_sgRNA#2_F	ACCGAGCCCACGACGTTGACATGCGT	This manuscript
XRCC1_sgRNA#2_R	TAAACGCATGTCAACGTCGTGGGCT	This manuscript
NELFCD_sgRNA#1_F	CACCATTTCAGTGAGCTTTAACG	This manuscript
NELFCD_sgRNA#1_R	AAACCGTTAAAGCTCACTGCAAAT	This manuscript
NELFCD_sgRNA#2_F	ACCGTGAAAGGGTTTTCCACAACGT	This manuscript
NELFCD_sgRNA#2_R	TAAACGTTGTGGAAAAACCCTTTCA	This manuscript
NELFCD-HomologyArm-1_fwd	ctataggcgcaattgggtacATGTTGGAGATGGGTGGTG	This manuscript
NELFCD-HomologyArm-1_rev	gcccagccaccgccaccGTTACCATGATGAAGTTAGATTG	This manuscript
NELFCD-Degron_fwd	aacttcacatggtgaacGGTGGCGGTGGCTCGGGC	This manuscript
NELFCD-Degron_rev	tggaggatgctctaaattaGGCACCGGGCTTGCGGGTC	This manuscript
NELFCD-HomologyArm-2_fwd	tgaccgcaagcccggtgcctaatttagcatcctccagag	This manuscript
NELFCD-HomologyArm-2_rev	CAGGAATTCGATATCAAGCTatattggatggcgaggacag	This manuscript
NELFCD-genotyping-fwd	TCACCTGAGGTTGGGAGTTC	This manuscript
NELFCD-genotyping-rev	GGATCGTGACCAGCACCTAT	This manuscript
Custom ELCAP-seq 3' Adapter	5'-App-NNNNNNAGATCGGAAGAGCACACGTCT-3'	This manuscript
GAPDH exon_F	AGCCACATCGCTCAGACA	This manuscript
GAPDH exon_R	CCCAATACGACCAAATC	This manuscript
GAPDH intron1_F	TGACCTTTCTGTAGCTGGGG	This manuscript
GAPDH intron1_R	GAGTGGGAGCACAGGTAAGT	This manuscript
GAPDH intron 2_F	GAAATGTGCTTTGGGGAGGC	This manuscript
GAPDH intron 2_R	GGGACAGGACCATATTGAGGG	This manuscript
GAPDH intron-exon_F	GAACGGGAAGCTTGTCATCA	This manuscript
GAPDH intron-exon_R	TCCTAGTTGCCTCCCCAAAG	This manuscript