Supplementary Figures

Supplementary Table1

MS-based proteomic analyses of proteins interacting with constrained structures 1a, 2 and the control 8 (duplex).

Supplementary Table2

List of 425 proteins enriched on constrained G4 structures 1a-2 compared to duplex control 8 (fold change ≥ 2 and p-value < 0.05, allowing to reach a false discovery rate (FDR) inferior to 5%).

Supplementary Table3

List of 214 out of 425 proteins enriched on G4 structures that have been described as nucleic acid interacting factors, as indicated by terms from GO Molecular functions data analyses.

Supplementary Table4.1

List of 31 proteins found enriched on system 1a relatively to system 2

Supplementary Table4.2

List of 204 proteins found enriched on system 2 relatively to system 1a

Supplementary Table4.3

List of 190 proteins Common to system 1a and 2

Supplementary Figure S1

Schematic representation of the constrained molecules used in this study

Supplementary Figure S2

CD spectra of G-quadruplex-forming conjugate 1-7 at 1 μ M (5) or 2 μ M (1a, 1b, 2, 3, 4, 6, 7) in 10 mM Tris-HCl, pH 7.4, 20°C in the presence of 100 mM KCl

Supplementary Figure S3

Western-blotting analysis and quantification of molecular interactions of known-G4 interacting factors with constrained G4 structures adopting different topologies (see Supplementary Figure S1). The relative quantity was obtained from the ratio between the signal observed from a

particular construction and the sum of signals from all wells. Digital data were processed and quantified using ImageLab (Biorad) or ImageJ softwares. Green, purple and oranges boxes indicate interaction with Parallel 1a, antiparallel 2 or duplex DNA 8 respectively.

Supplementary Figure S4

Enriched KEGG pathways for the 214 nuclear proteins interacting with nucleic acids found enriched on constrained G4 structures 1a and 2.

Supplementary Figure S5

Most significant pathways and processes covered by constrained G4 interacting proteins. Enriched KEGG pathways of (A) the 190 proteins present in the Common group and (B) the 203 proteins enriched on construction 2 (*i.e.* antiparallel topology with two lateral loops). KEGG analysis of Common group proteins (1a and 2) and proteins enriched on construction 2 showed similar significant enriched pathways compared to those covered by total constrained G4 interacting proteins (see Figure 2A).

Supplementary Figure S6

A. Western-blot detection of NELFE and NELFA proteins immunoprecipitated from a HeLa cell line overexpressing an ectopic Flag-tagged form of the NELF-E protein (Flag-eluted material). **B.** Uncropped and unprocessed versions of Western-blot signals. Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad). When additional signals corresponding to previous hybridizations with other antibodies are present, they are indicated in Italics. Not used lanes for hybridization are indicated by a red square.

Supplementary Figure S7A-E

Uncropped and unprocessed versions of Western-blot signals from Figure 5. Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad). Exposition times are indicated. When additional signals corresponding to previous hybridizations with other antibodies are present, they are indicated in Italics.

Supplementary Figure S8

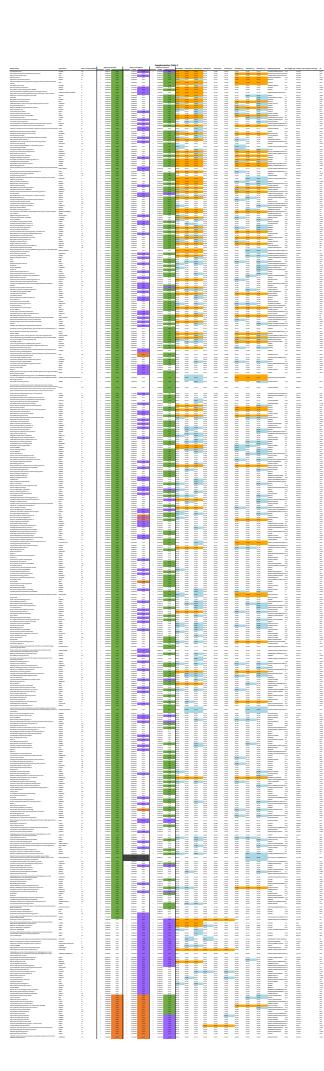
Uncropped and unprocessed versions of Western-blot signals from Figure 6C. Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad).

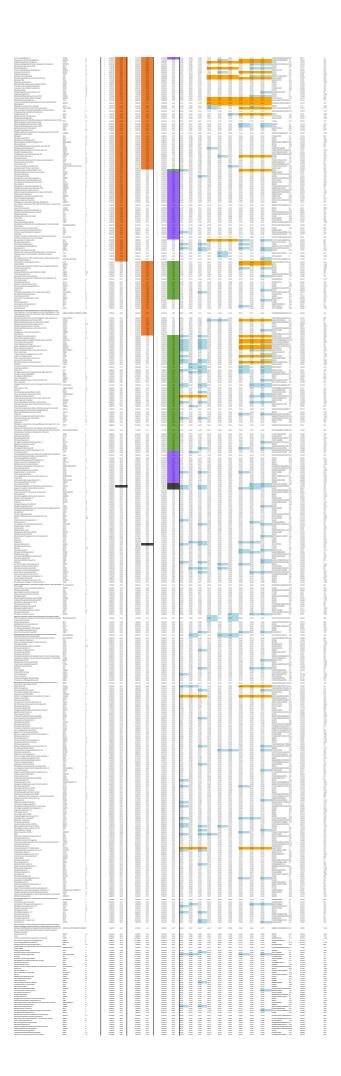
Supplementary Figure S9A and S9B

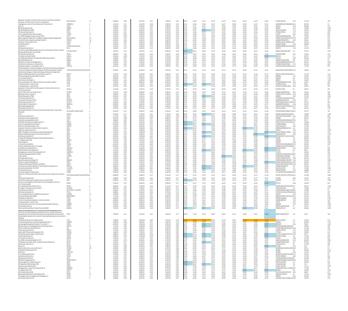
Method used to quantify immunofluorescence from DAPI (**S9A**, blue) and γ H2AX signals (**S9B**, red) from Figure 6C. Raw images were processed as described in Supplementary Figure S9A. Raw and Modified Images used for quantification and for the final figure are shown.

Supplementary Figure S10A-10B

Uncropped and unprocessed versions of Western-blot signals from Supplementary Figure S3A. Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad).







Supplementary table 1 Legend

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Supplementary	Table 4.1 Human proteins f	ound enriched on system :	1a relatively to system 2

	Managed IDa	
Gene ID	Mapped IDs	Gene Name
HUMAN HGNC=10648 UniProtKB=Q12904	AIMP1	Aminoacyl tRNA synthase complex-interacting multifunctional protein 1;AIMP1;ortholog
HUMAN HGNC=20609 UniProtKB=Q13155	AIMP2	Aminoacyl tRNA synthase complex-interacting multifunctional protein 2;AIMP2;ortholog
HUMAN HGNC=11393 UniProtKB=014965	AURKA	Aurora kinase A;AURKA;ortholog
HUMAN HGNC=18673 UniProtKB=Q96JB5	CDK5RAP3	CDK5 regulatory subunit-associated protein 3;CDK5RAP3;ortholog
HUMAN HGNC=2678 UniProtKB=P14868	DARS	AspartatetRNA ligase, cytoplasmic;DARS;ortholog
HUMAN HGNC=3061 UniProtKB=P23919	DTYMK	Thymidylate kinase;DTYMK;ortholog
HUMAN HGNC=3212 UniProtKB=O43324	EEF1E1	Eukaryotic translation elongation factor 1 epsilon-1;EEF1E1;ortholog
HUMAN HGNC=3418 UniProtKB=P07814	EPRS	Bifunctional glutamate/prolinetRNA ligase; EPRS; or tholog
HUMAN HGNC=21109 UniProtKB=Q96EK7	FAM120B	Constitutive coactivator of peroxisome proliferator-activated receptor gamma;FAM120B;ortholog
HUMAN HGNC=26773 UniProtKB=Q52LJ0	FAM98B	Protein FAM98B;FAM98B;ortholog
HUMAN HGNC=5031 UniProtKB=P09651	HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1;HNRNPA1;ortholog
HUMAN HGNC=5033 UniProtKB=P22626	HNRNPA2B1	Heterogeneous nuclear ribonucleoproteins A2/B1;HNRNPA2B1;ortholog
HUMAN HGNC=24941 UniProtKB=P51991	HNRNPA3	Heterogeneous nuclear ribonucleoprotein A3;HNRNPA3;ortholog
HUMAN HGNC=5330 UniProtKB=P41252	IARS	IsoleucinetRNA ligase, cytoplasmic;IARS;ortholog
HUMAN HGNC=6215 UniProtKB=Q15046	KARS	LysinetRNA ligase;KARS;ortholog
HUMAN HGNC=6512 UniProtKB=Q9P2J5	LARS	LeucinetRNA ligase, cytoplasmic;LARS;ortholog
HUMAN HGNC=6701 UniProtKB=P30533	LRPAP1	Alpha-2-macroglobulin receptor-associated protein;LRPAP1;ortholog
HUMAN HGNC=6898 UniProtKB=P56192	MARS	MethioninetRNA ligase, cytoplasmic;MARS;ortholog
HUMAN HGNC=7095 UniProtKB=015344	MID1	E3 ubiquitin-protein ligase Midline-1;MID1;ortholog
HUMAN HGNC=7804 UniProtKB=P23511	NFYA	Nuclear transcription factor Y subunit alpha;NFYA;ortholog
HUMAN HGNC=7827 UniProtKB=Q9BPW8	NIPSNAP1	Protein NipSnap homolog 1;NIPSNAP1;ortholog
HUMAN HGNC=7857 UniProtKB=P30419	NMT1	Glycylpeptide N-tetradecanoyltransferase 1;NMT1;ortholog
HUMAN HGNC=17946 UniProtKB=Q9Y314	NOSIP	Nitric oxide synthase-interacting protein;NOSIP;ortholog
HUMAN HGNC=8604 UniProtKB=095340	PAPSS2	Bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase 2;PAPSS2;ortholog
HUMAN HGNC=9077 UniProtKB=P53350	PLK1	Serine/threonine-protein kinase PLK1;PLK1;ortholog
HUMAN HGNC=9658 UniProtKB=P29350	PTPN6	Tyrosine-protein phosphatase non-receptor type 6;PTPN6;ortholog
HUMAN HGNC=9751 UniProtKB=P47897	QARS	GlutaminetRNA ligase;QARS;ortholog
HUMAN HGNC=9870 UniProtKB=P54136	RARS	ArgininetRNA ligase, cytoplasmic;RARS;ortholog
HUMAN HGNC=32310 UniProtKB=Q8IXT5	RBM12B	RNA-binding protein 12B;RBM12B;ortholog
HUMAN HGNC=17688 UniProtKB=P78346	RPP30	Ribonuclease P protein subunit p30;RPP30;ortholog
HUMAN HGNC=11303 UniProtKB=076094	SRP72	Signal recognition particle subunit SRP72;SRP72;ortholog
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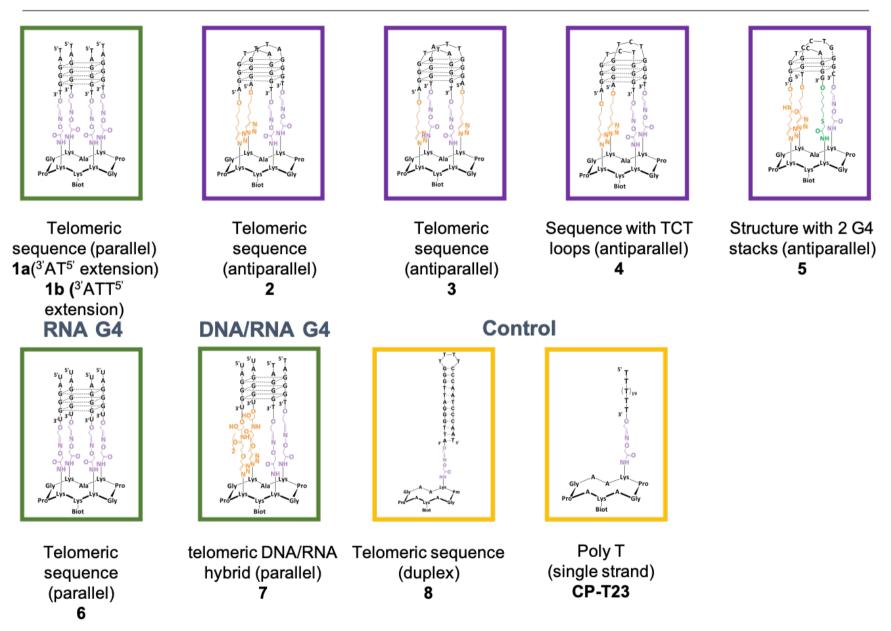
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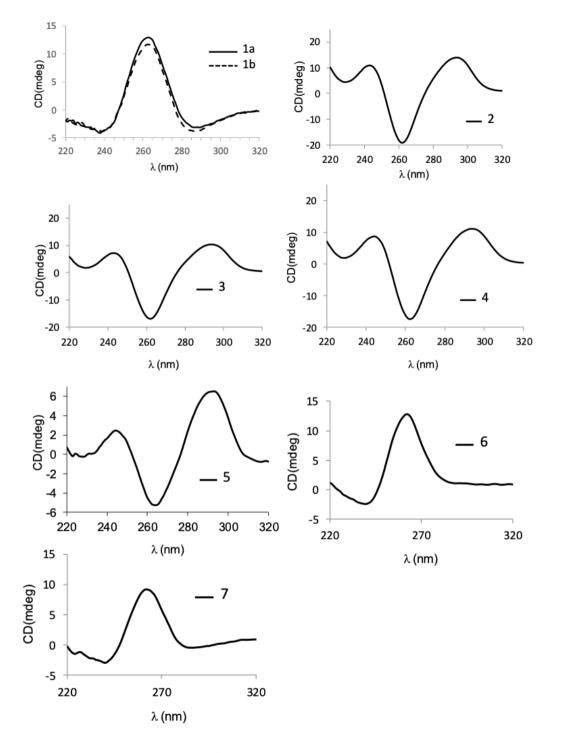
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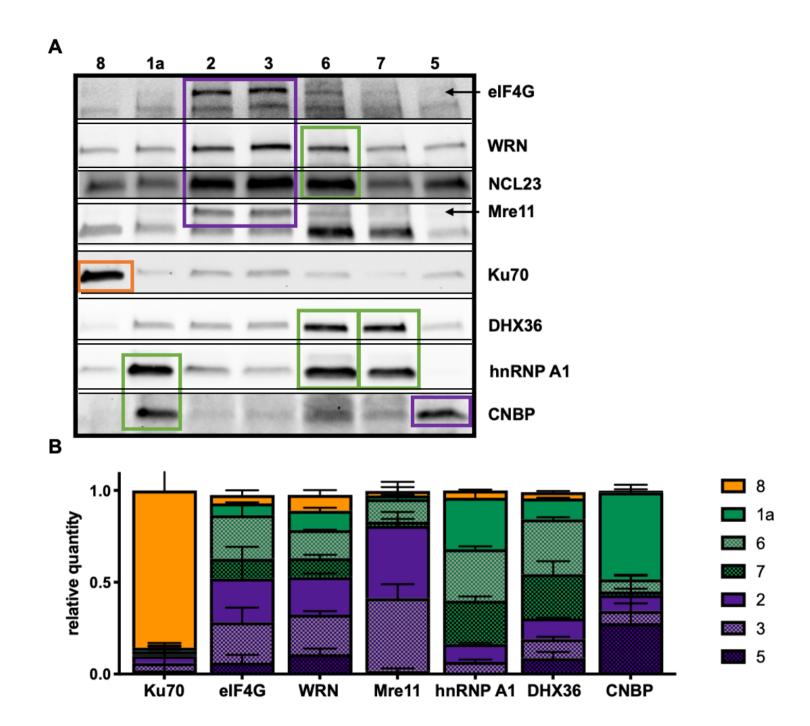
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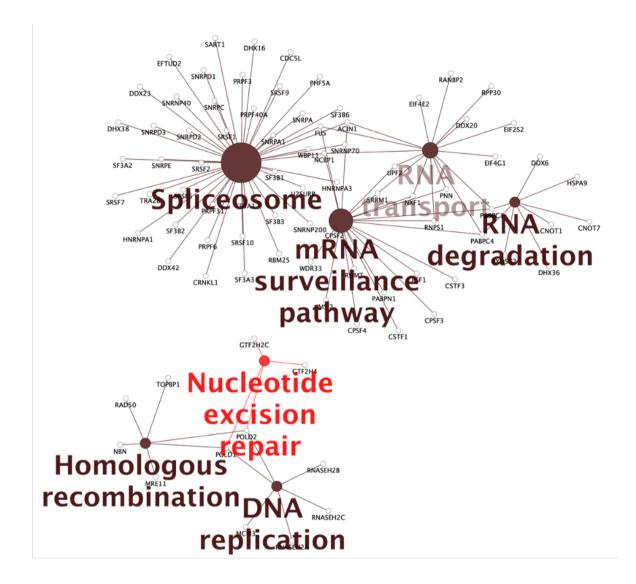




Supplementary Figure S2

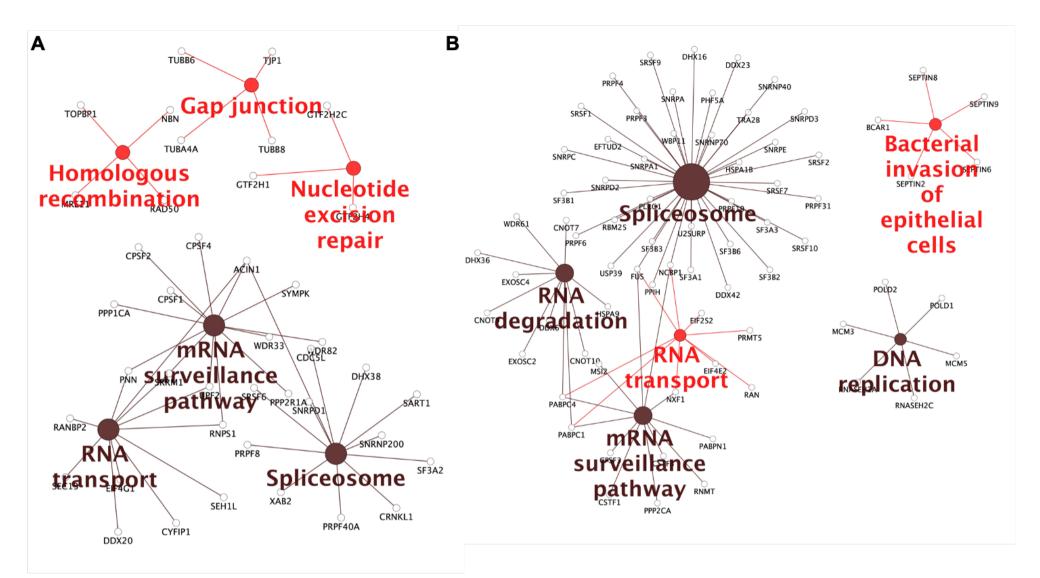


Supplementary Figure S3



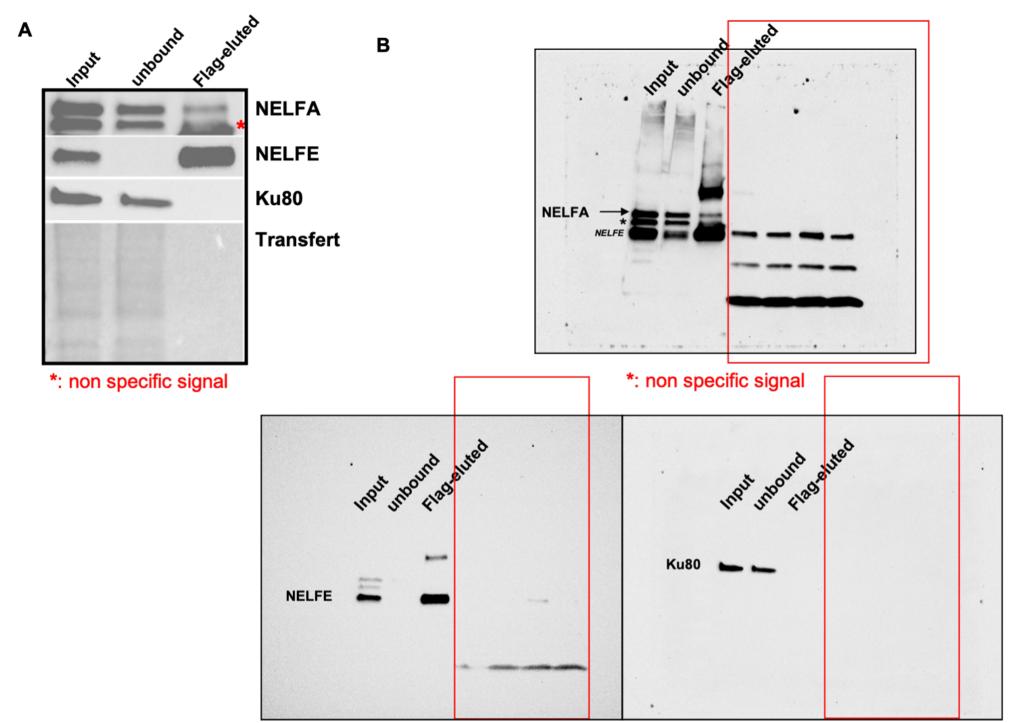
Significance

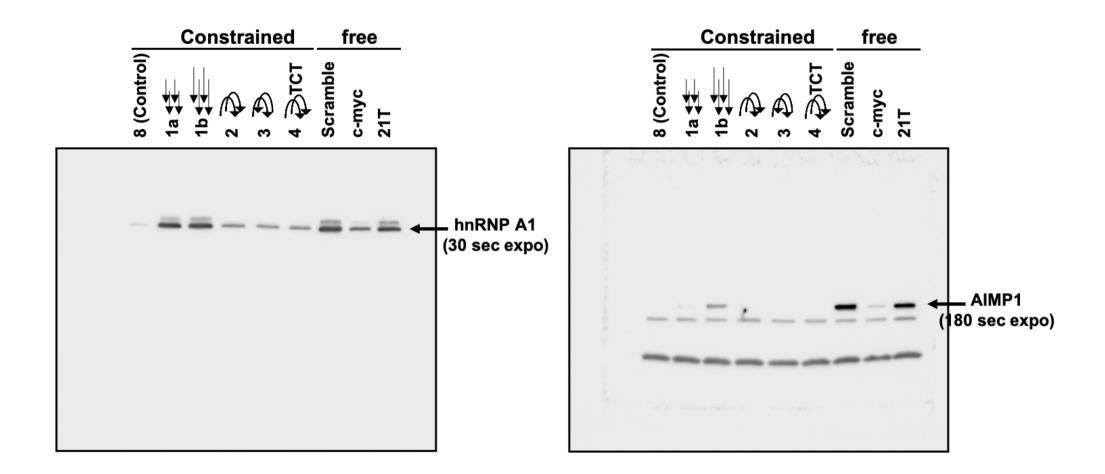
- pV < 0.0005</p>
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Significance

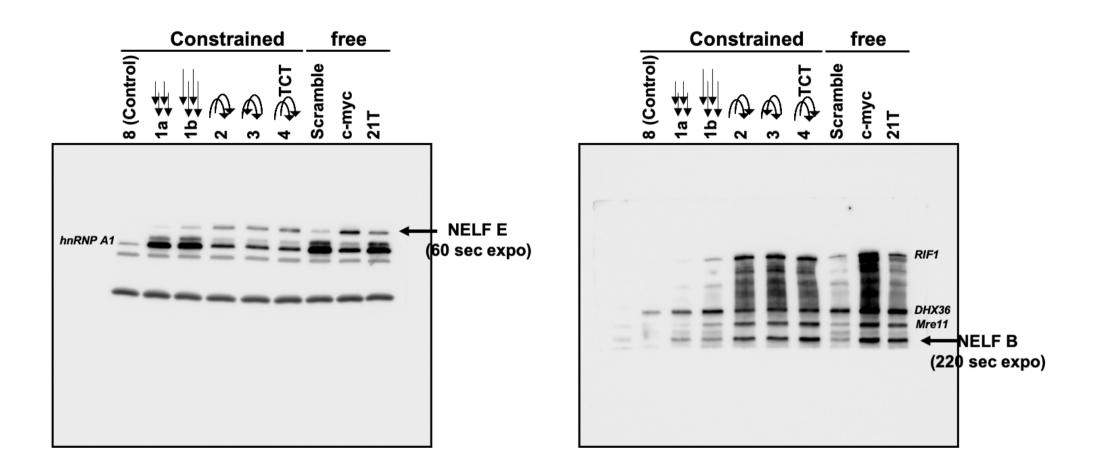
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- pV 0.0005-0.005
- pV 0.005-0.05





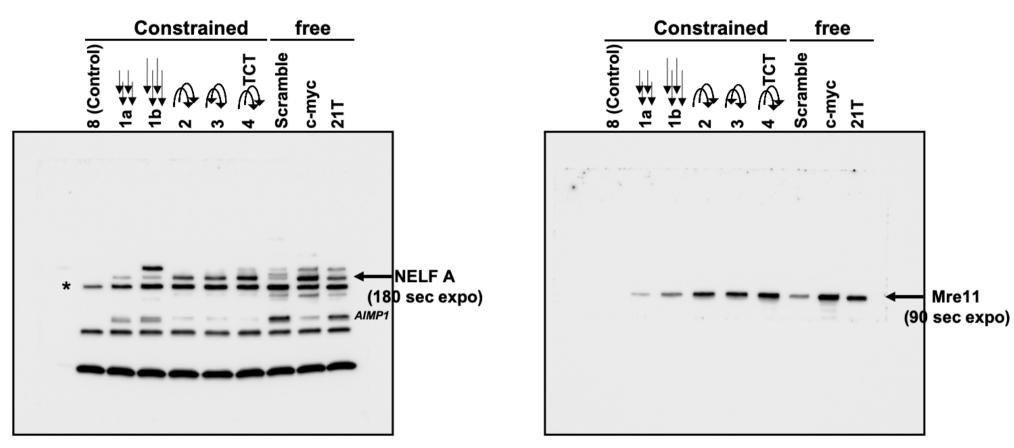
Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad).

Exposition times are indicated.



Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad).

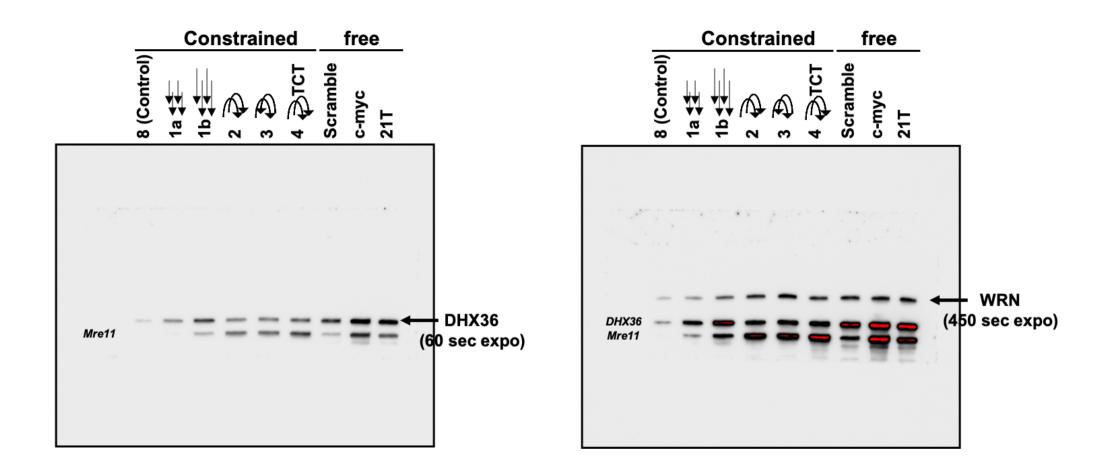
Exposition times are indicated.



*: non-specific signal

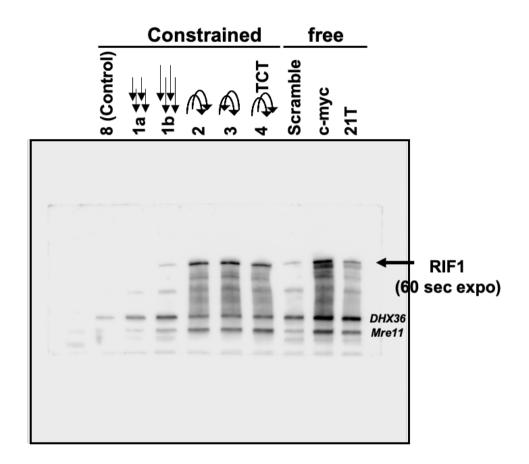
Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad).

Exposition times are indicated.



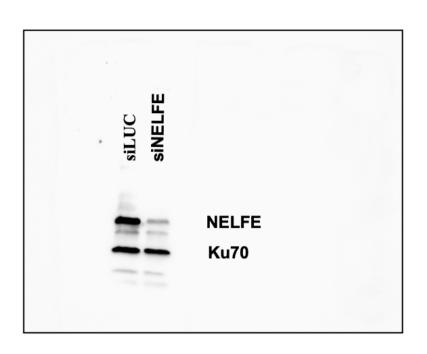
Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad).

Exposition times are indicated.



Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad).

Exposition times are indicated.



Uncropped and unprocessed versions of western-blot signals from Figure 6C. Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad).

Supplementary Figure S8

Modification of images:

#1: for quantification → DAPI (p. 2): selection of nuclei

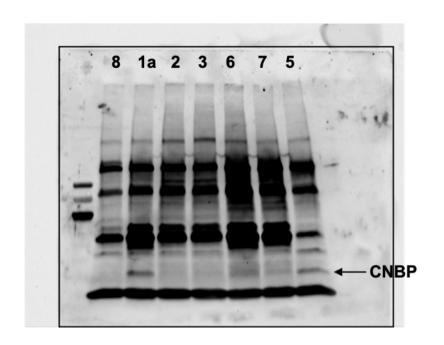
→ γH2AX (p. 3): removal of background noise without brightness and contrast modification

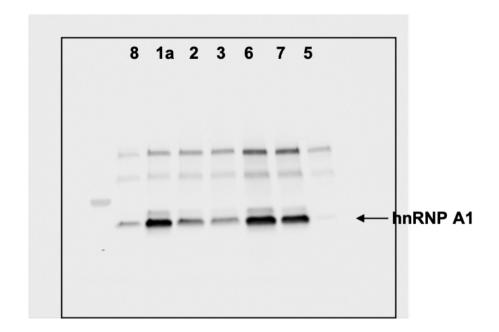
#2: for figure \rightarrow Overlay of nuclei edges (from #1 modification of DAPI) over γ H2AX staining (from #1 modification of γ H2AX with modified brightness and contrast) (p. 3 only)

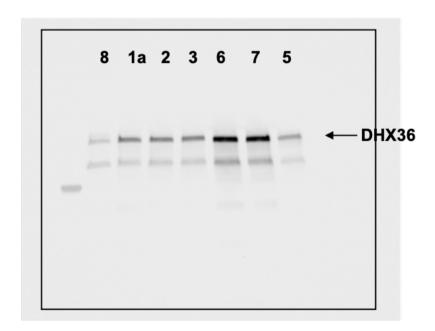
- ✓ Same background noise removal for all images, for all conditions of treatment and for all experiments (n=3)
- ✓ Same brightness and contrast applied for all images representative of each conditions

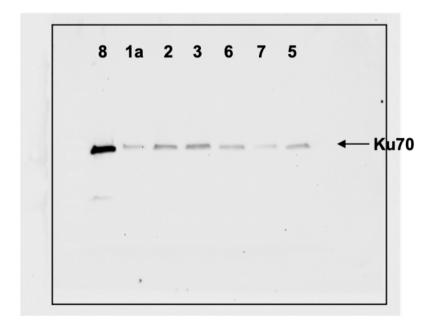
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Raw images				
Modified imgs for quantif.				

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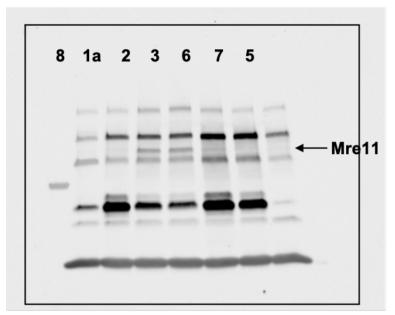


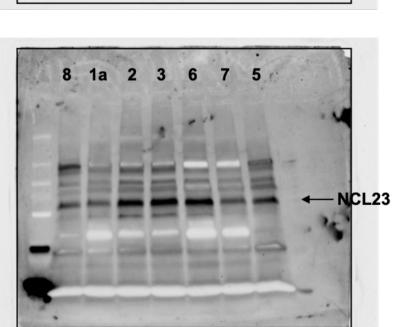


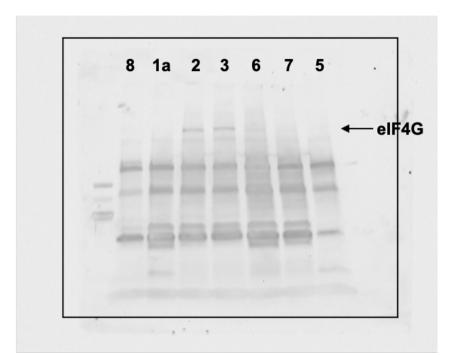


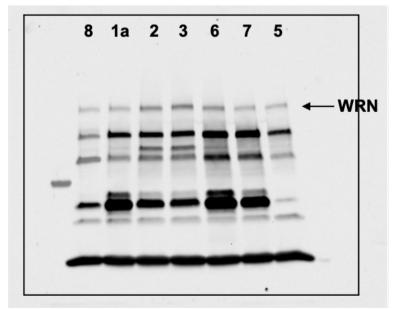


Supplementary Figure S10A









Supplementary Figure S10B