

## **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  $\geq 2$  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  $\mu\text{M}$  (5) or 2  $\mu\text{M}$  (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**

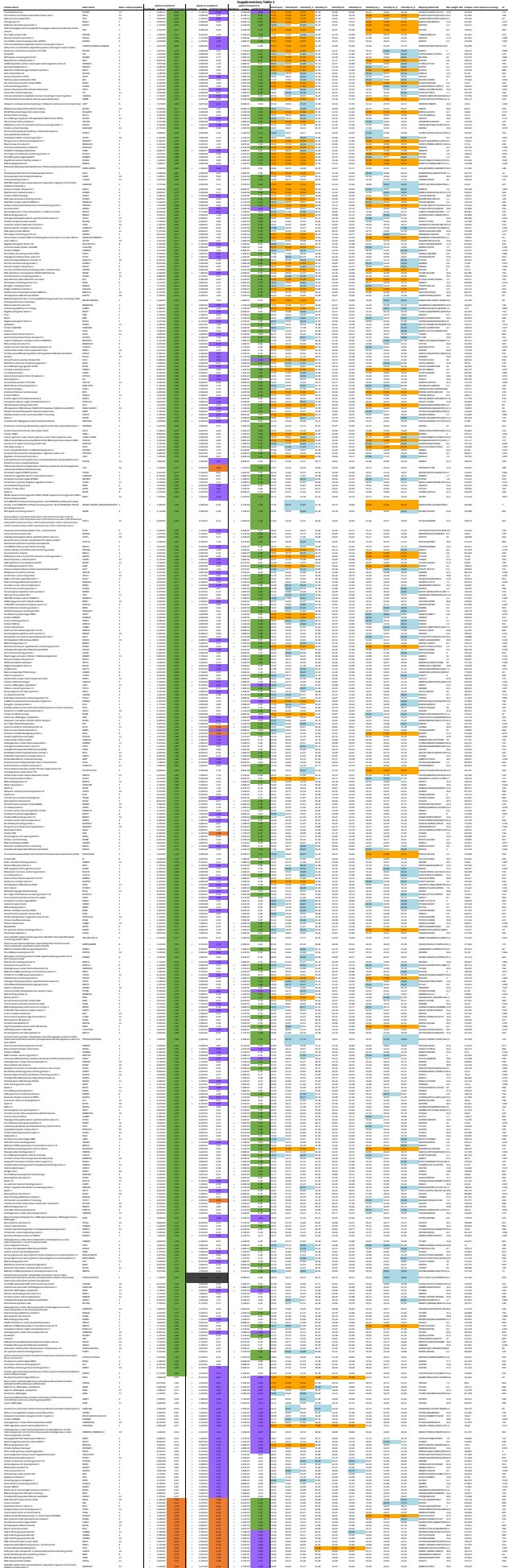
Uncropped and unprocessed versions of Western-blot signals from Figure 6C. Chemiluminescence signals were acquired using the ChemyDoc Touching Imaging System (Bio-Rad).

#### **Supplementary Figure S9A and S9B**

Method used to quantify immunofluorescence from DAPI (**S9A**, blue) and  $\gamma$ 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).

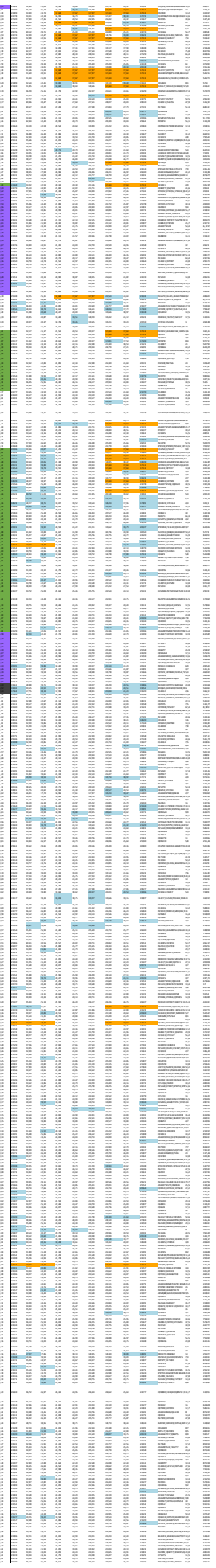




1. Introduction  
2. Methodology  
3. Results  
4. Discussion  
5. Conclusion

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1. Introduction  
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4. Discussion  
5. Conclusion



## Supplementary table 1 Legend

	Statistically enriched with system 2
	Statistically enriched with system 1a
	Statistically enriched with system 8
	missing in all replicates of the condition, imputed with low value
	missing in one or two replicates of the condition, imputed with slsa algorithm
	Manually invalidated (found differentially abundant but contains two imputed values in the condition in which it was found more abundant)



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1000000002	Information Services		
1000000003	Legal Services		
1000000004	Medical Services		
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1000000006	Construction Services		
1000000007	Transportation Services		
1000000008	Security Services		
1000000009	Food Services		
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1000000011	Public Works Services		
1000000012	Fire Services		
1000000013	Police Services		
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Supplementary Table 4.1 Human proteins found enriched on system 1a relatively to system 2

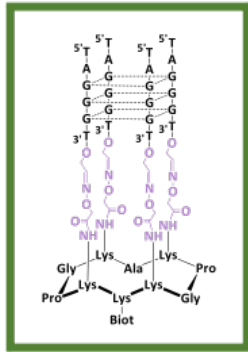
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HUMAN   HGNC=3212   UniProtKB=O43324	EEF1E1	Eukaryotic translation elongation factor 1 epsilon-1;EEF1E1;ortholog
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HUMAN   HGNC=24941   UniProtKB=P51991	HNRNPA3	Heterogeneous nuclear ribonucleoprotein A3;HNRNPA3;ortholog
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HUMAN   HGNC=6215   UniProtKB=Q15046	KARS	Lysine--tRNA ligase;KARS;ortholog
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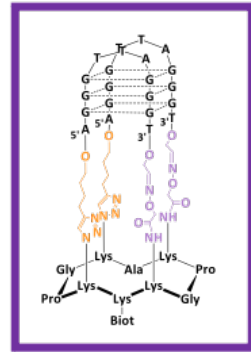




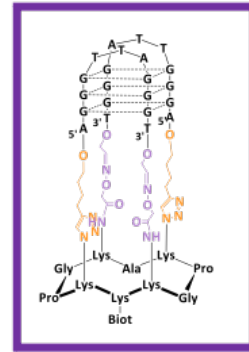
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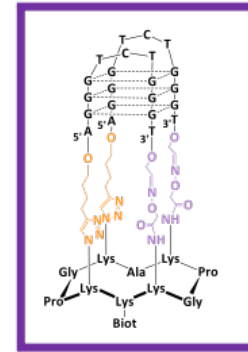
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**1b** ( $3'ATT5'$  extension)  
**RNA G4**



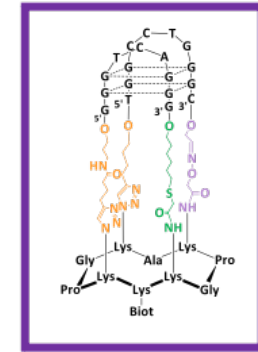
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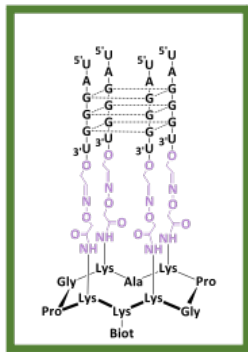
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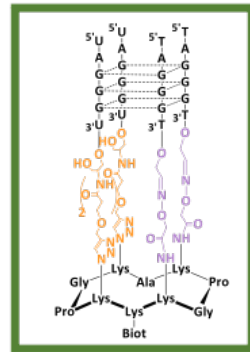
Sequence with TCT loops (antiparallel)  
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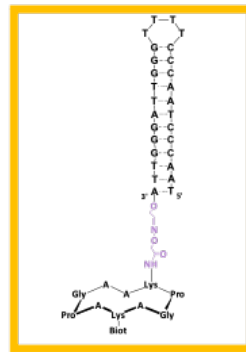
Structure with 2 G4 stacks (antiparallel)  
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Telomeric sequence (parallel)  
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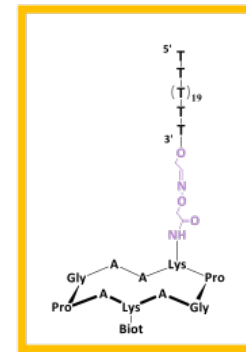


telomeric DNA/RNA hybrid (parallel)  
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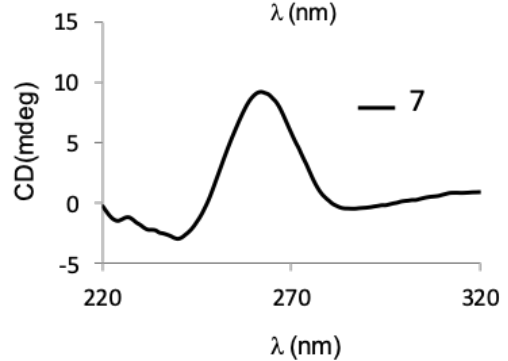
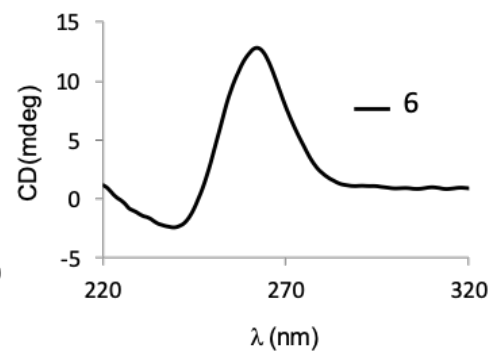
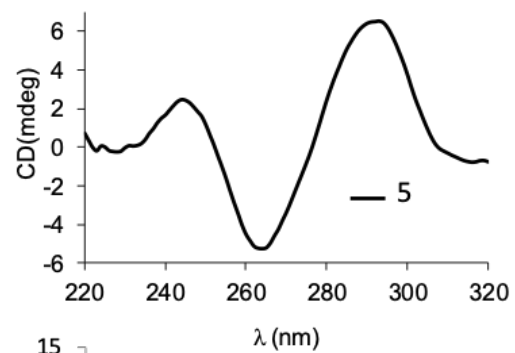
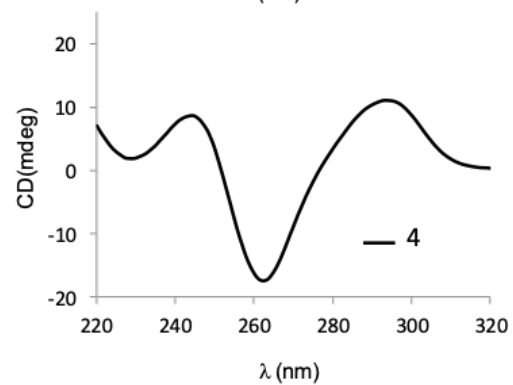
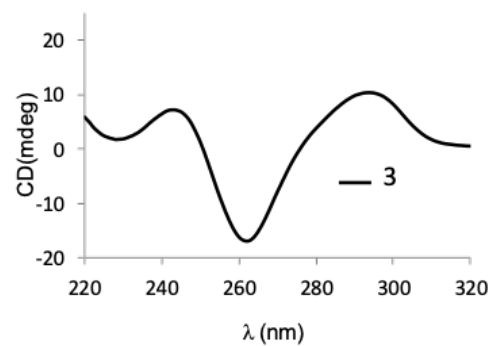
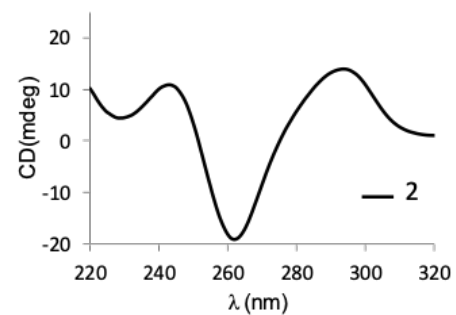
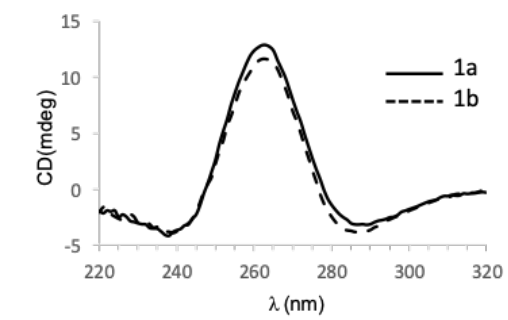


Telomeric sequence (duplex)  
**8**

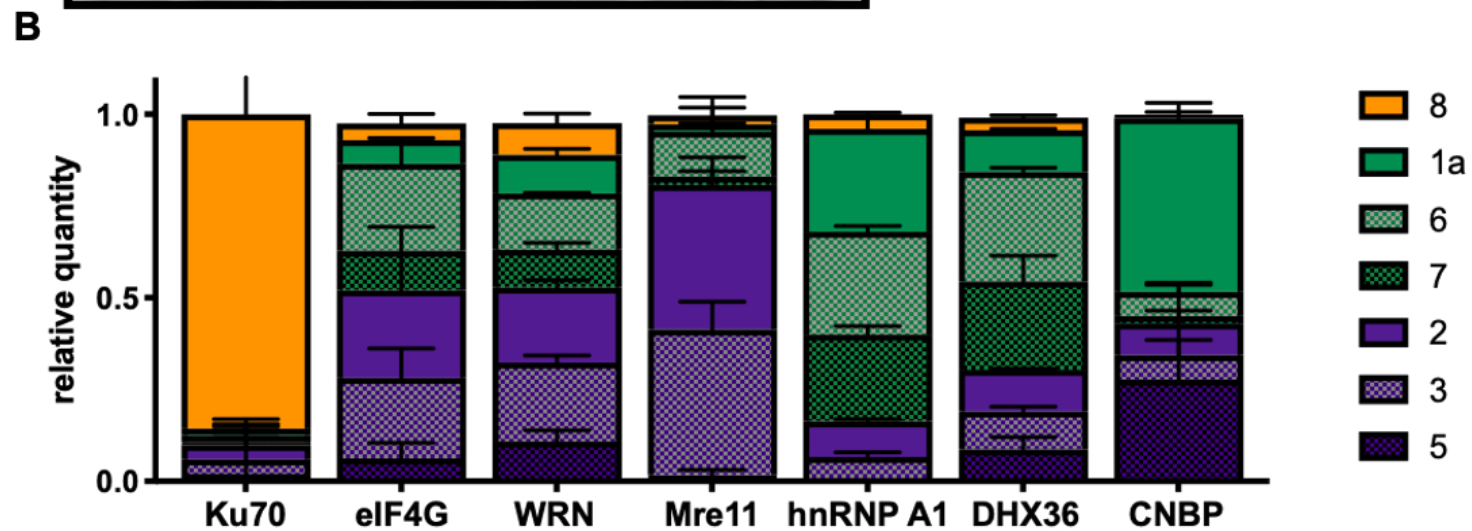
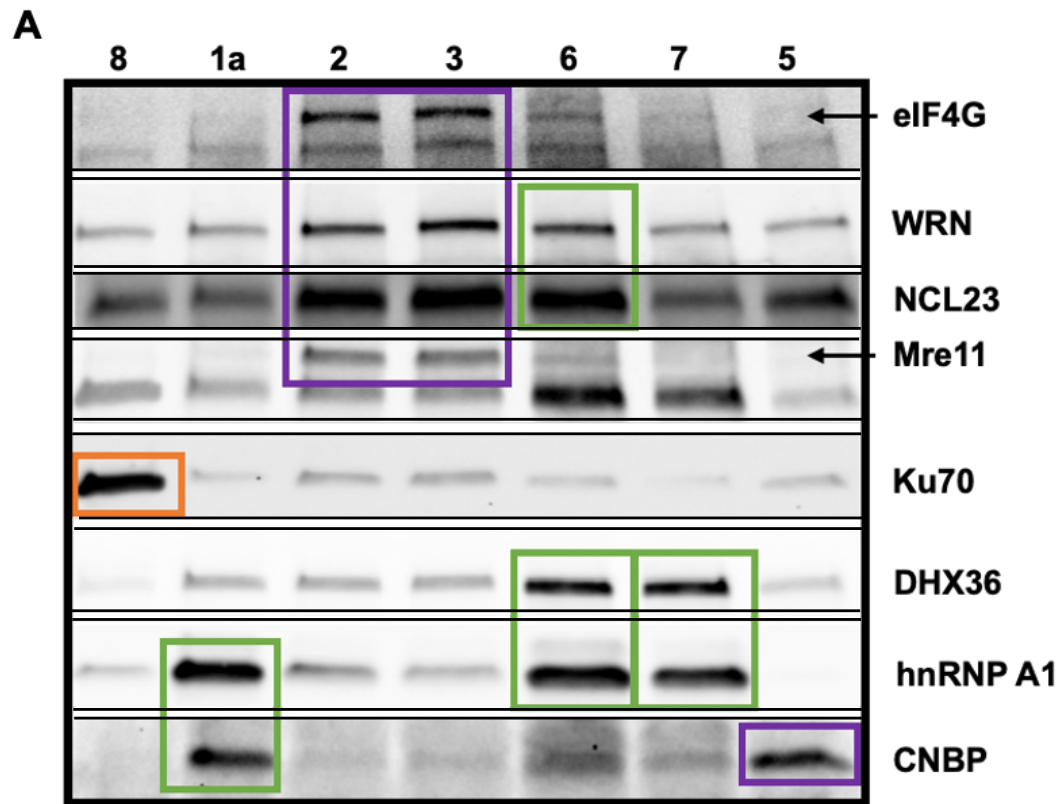
## Control



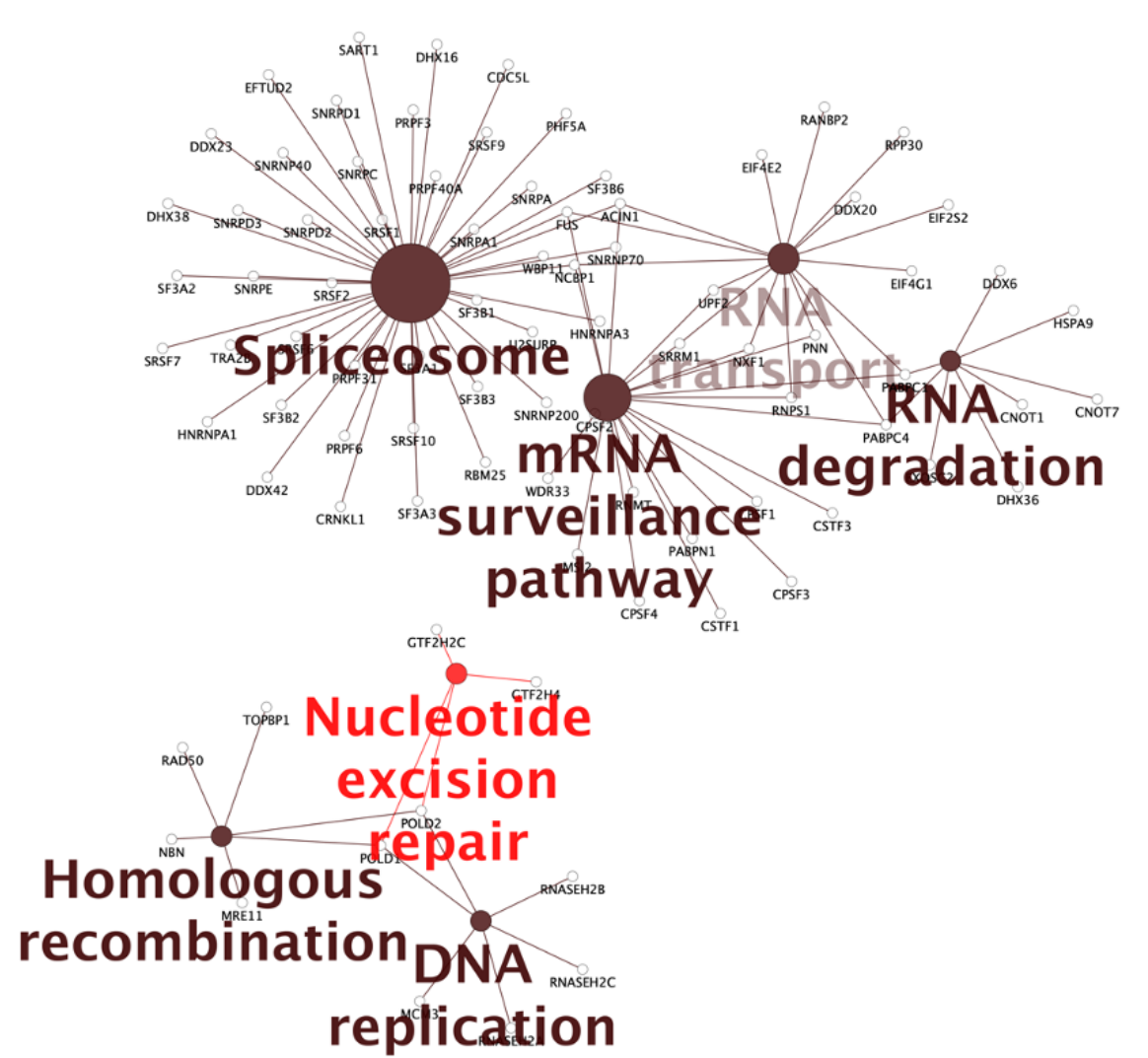
Poly T (single strand)  
**CP-T23**



Supplementary Figure S2



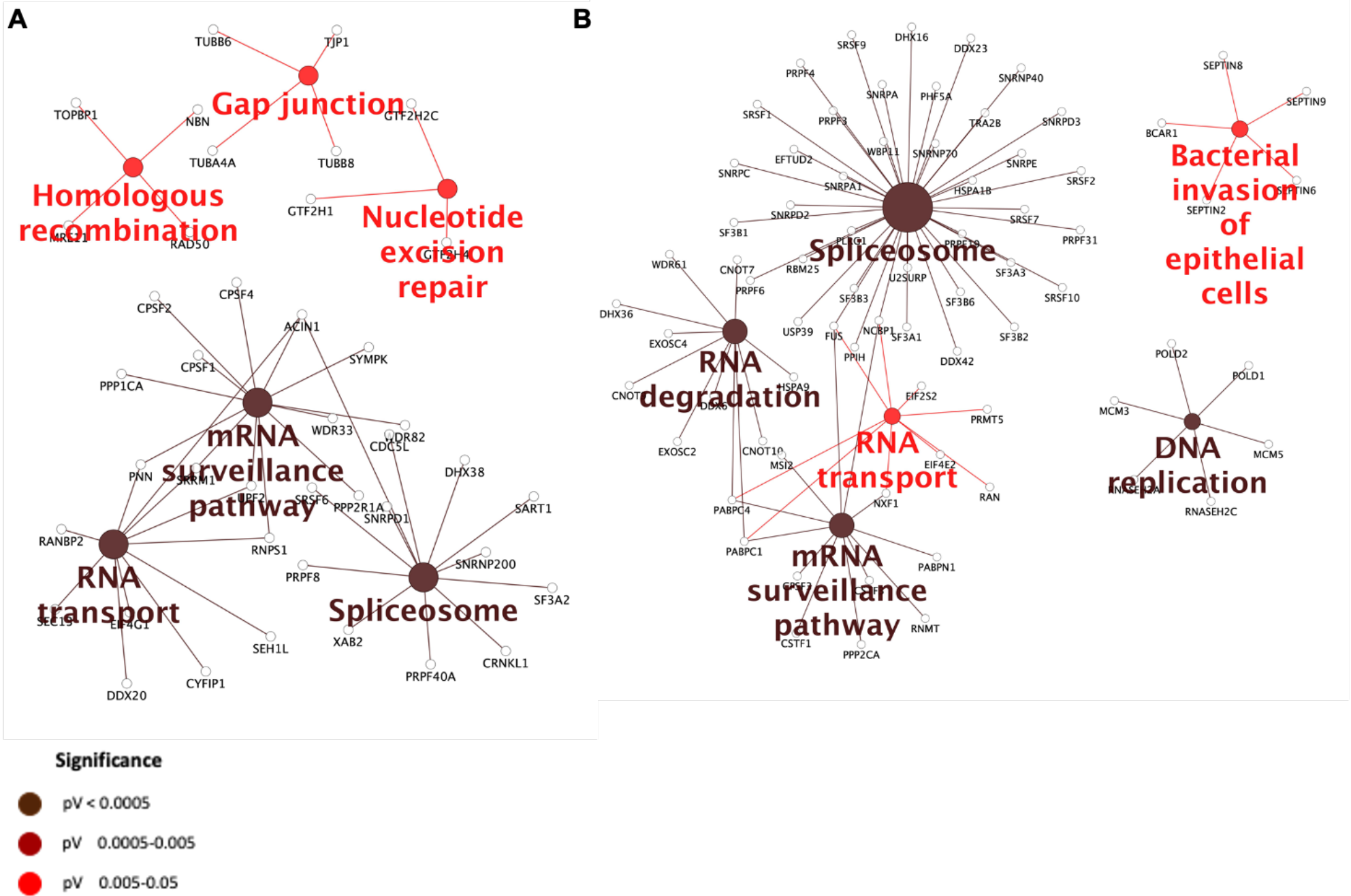
Supplementary Figure S3



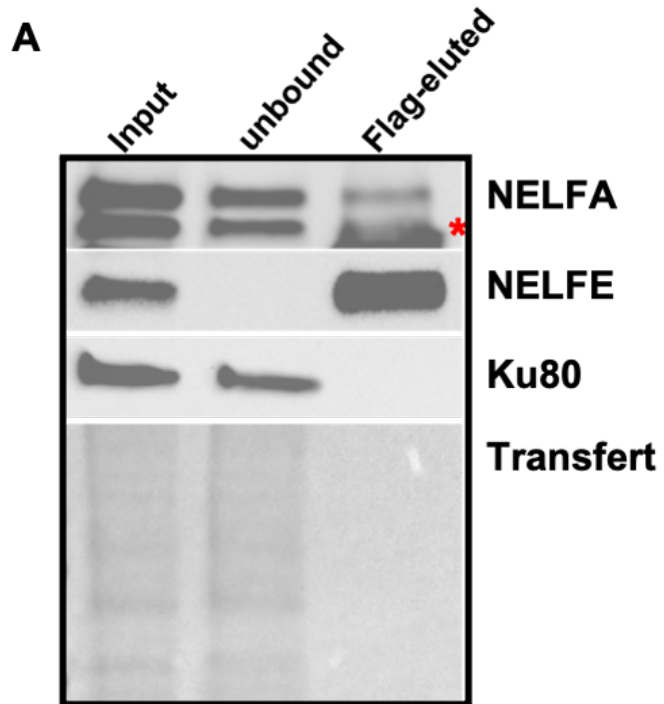
**Significance**

- pV < 0.0005
- pV 0.0005-0.005
- pV 0.005-0.05

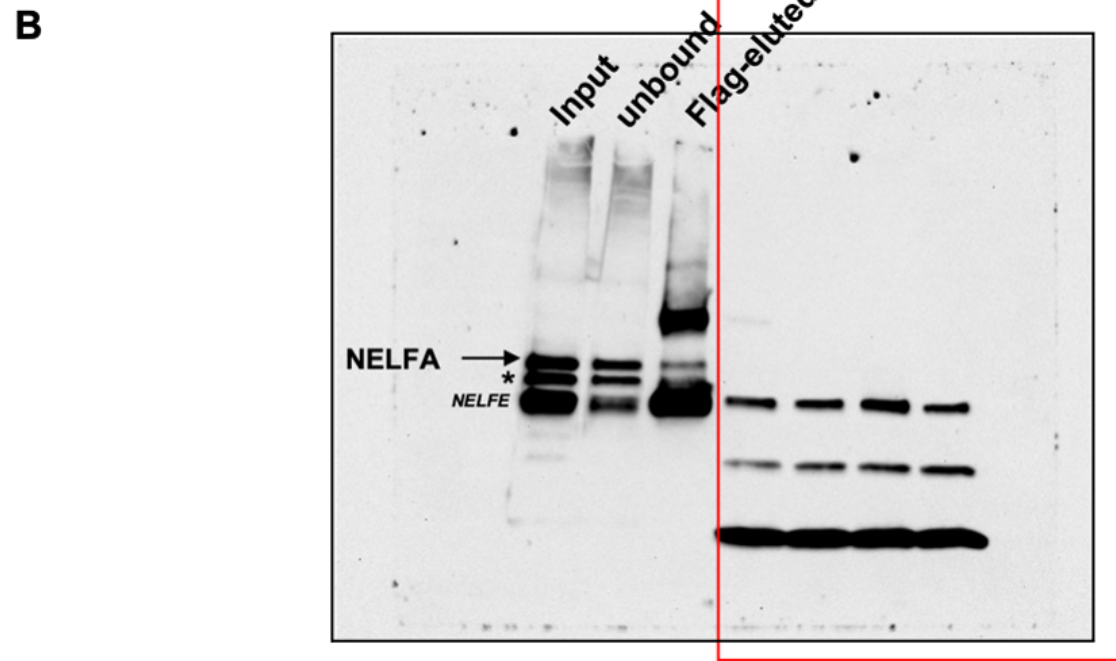
Supplementary Figure S4



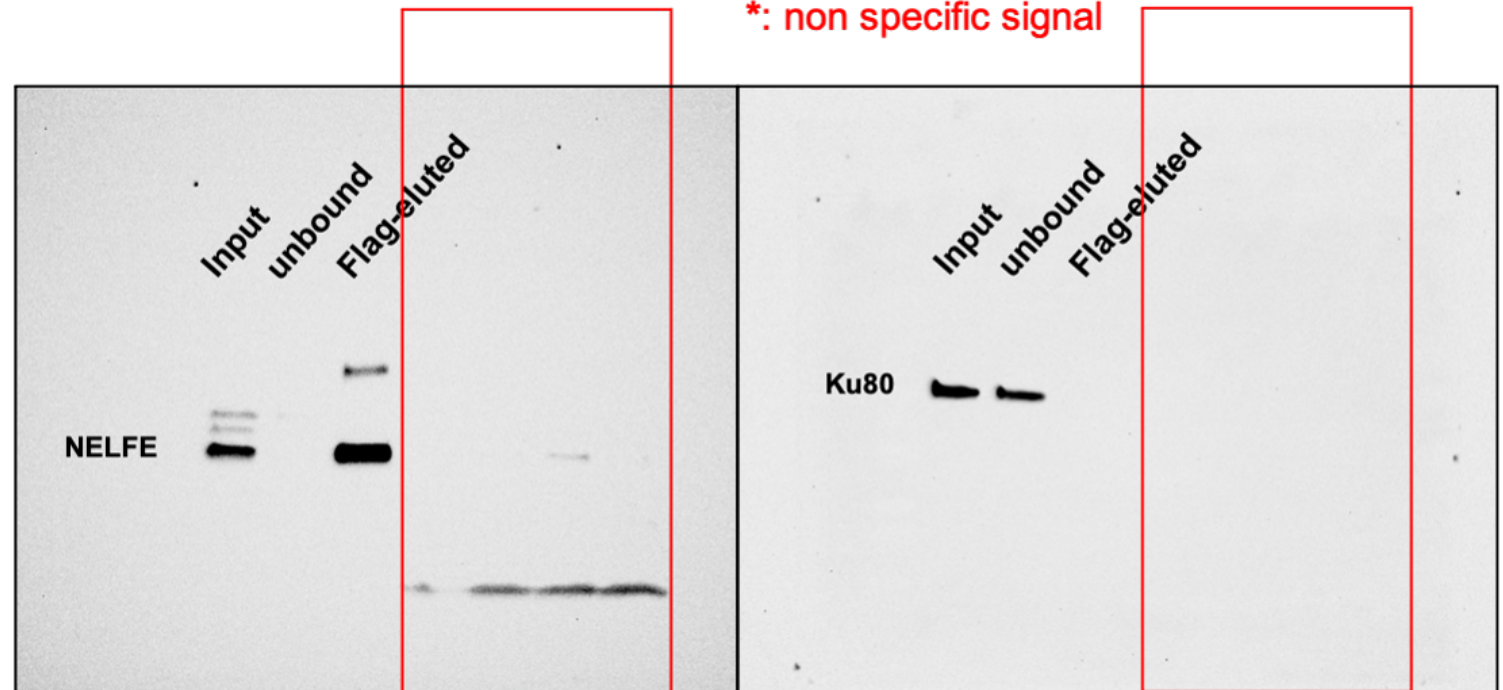
Supplementary Figure S5



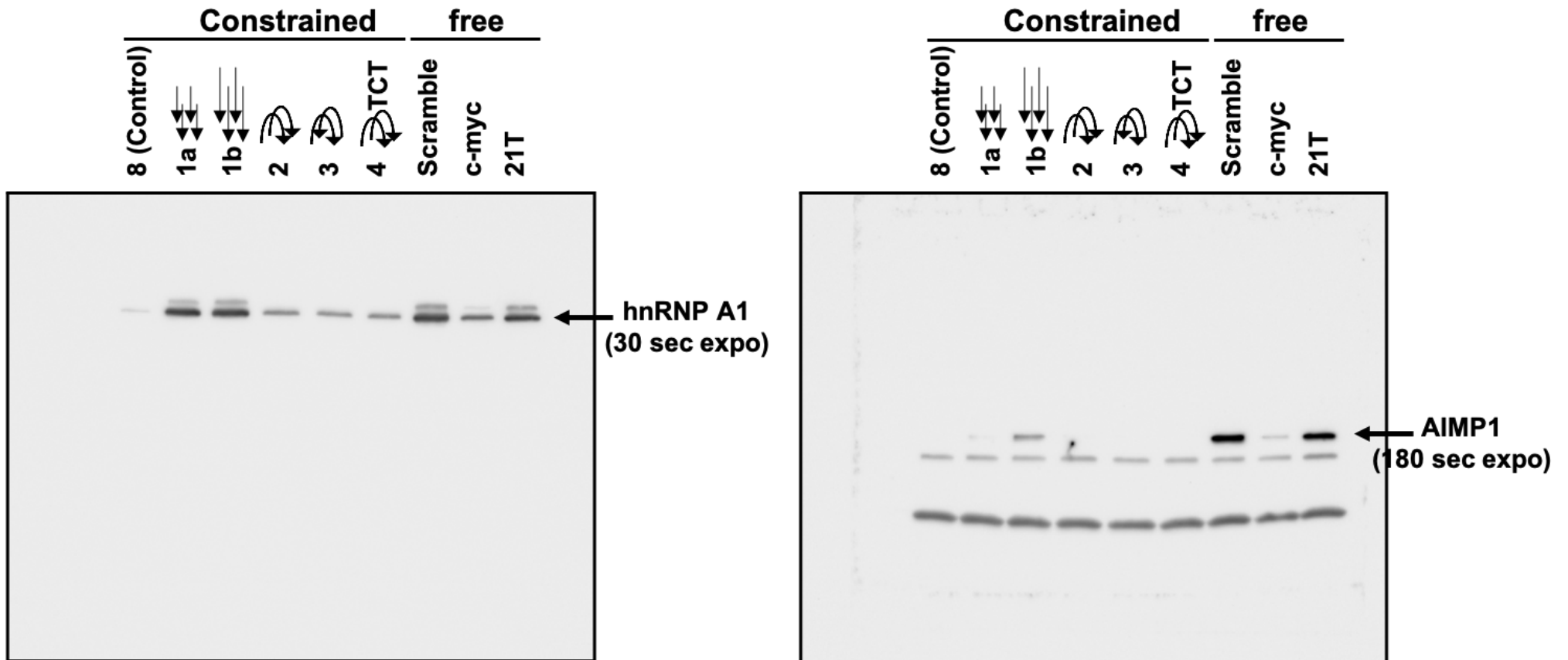
\*: non specific signal



\*: non specific signal



Supplementary Figure S6



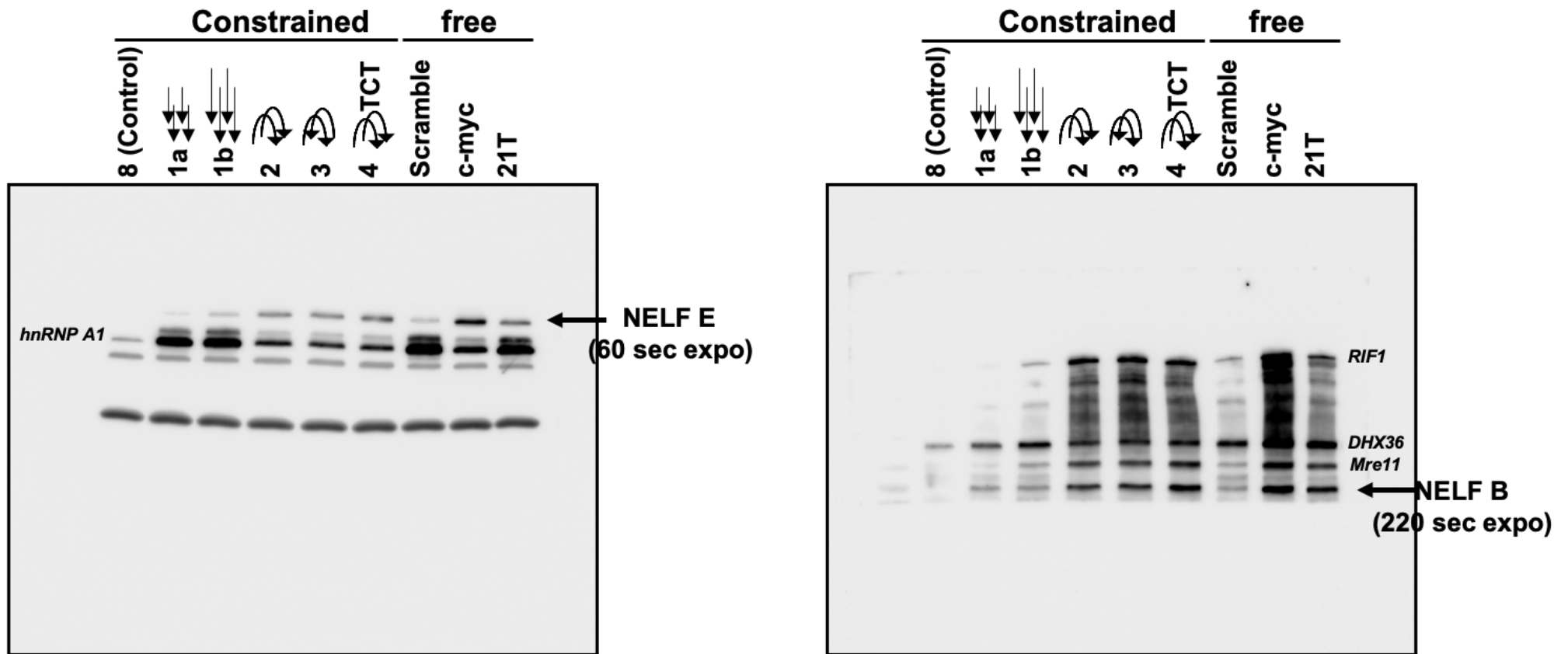
Uncropped and unprocessed versions of western-blot signals from Figure 5.

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*



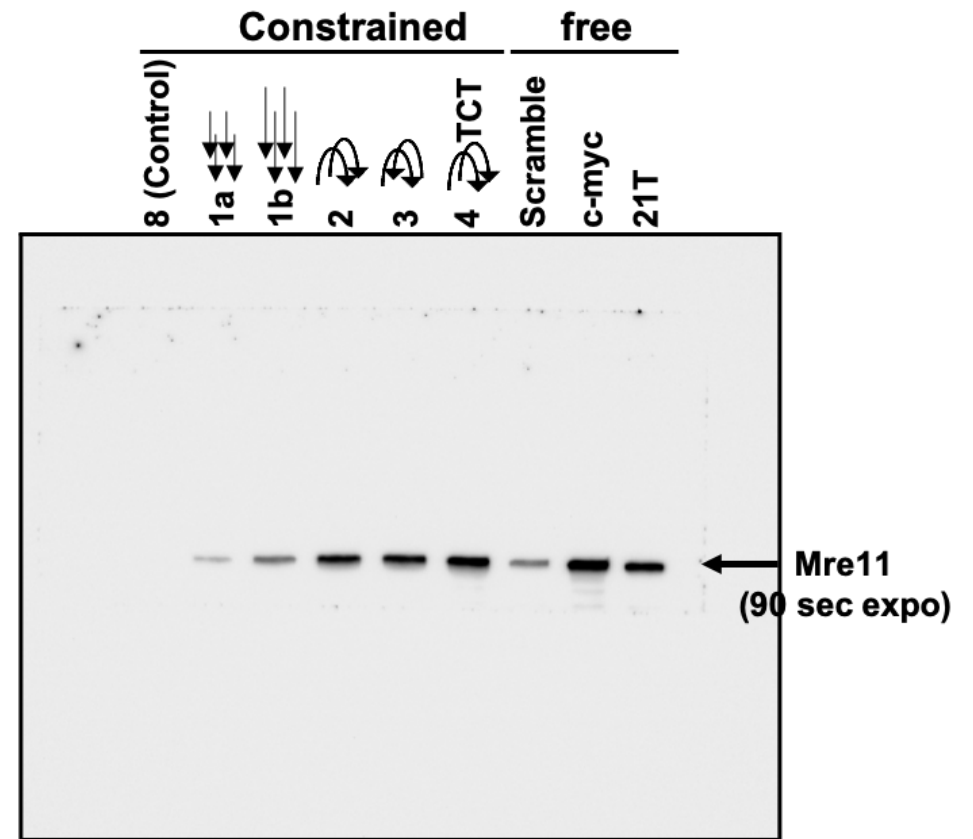
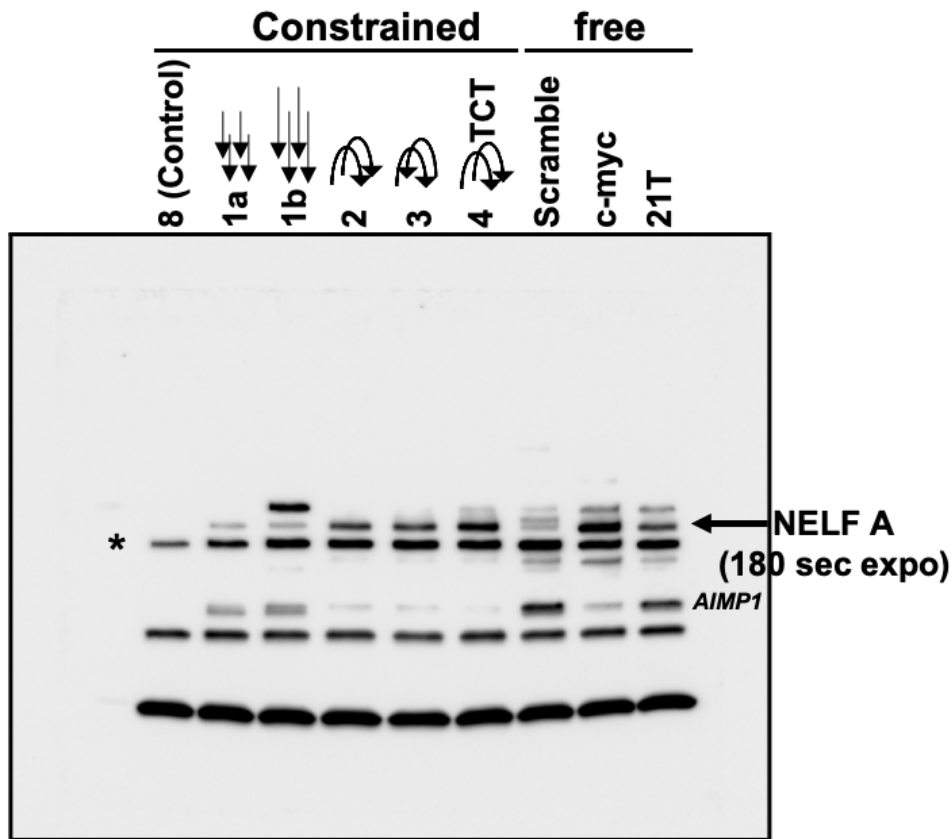


Uncropped and unprocessed versions of western-blot signals from Figure 5.

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



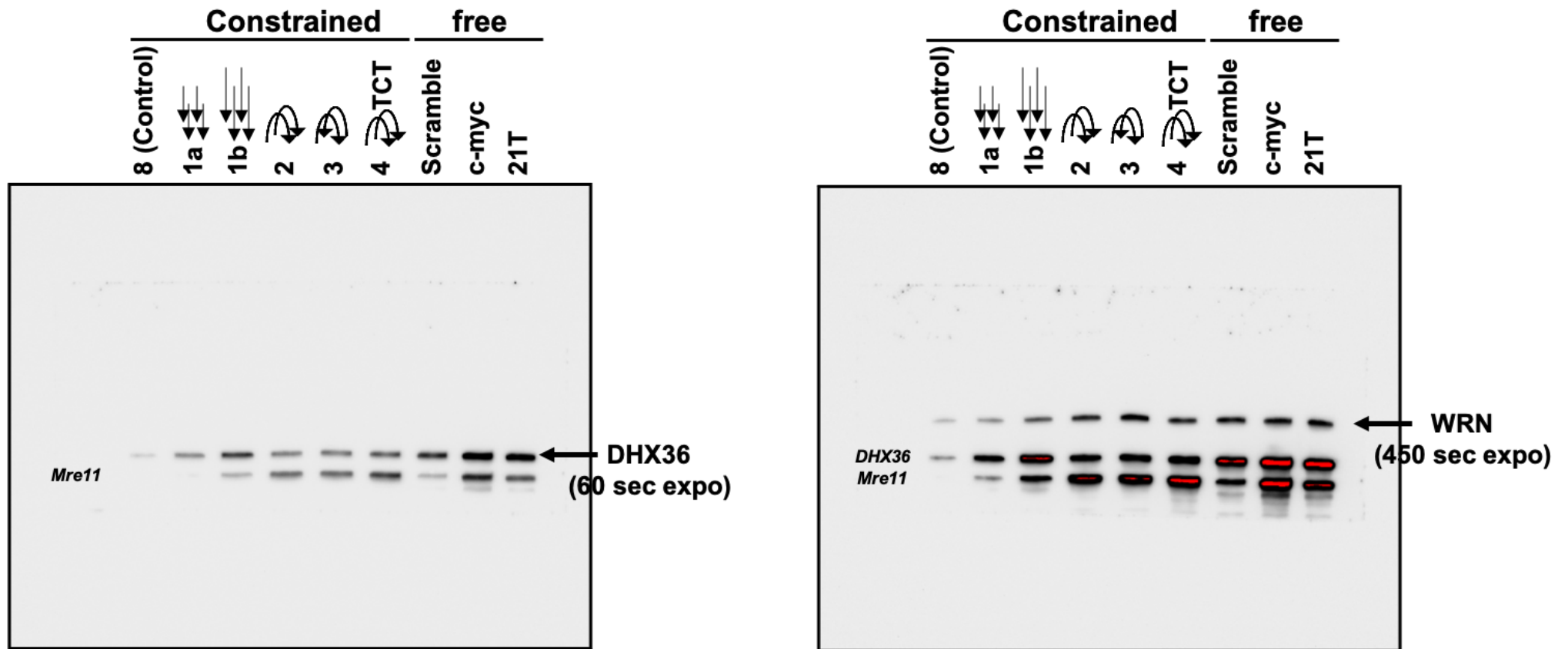
\*: non-specific signal

Uncropped and unprocessed versions of western-blot signals from Figure 5.

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Exposition times are indicated.

When additional signals corresponding to previous hybridizations with other antibodies are present they are indicated in Italics



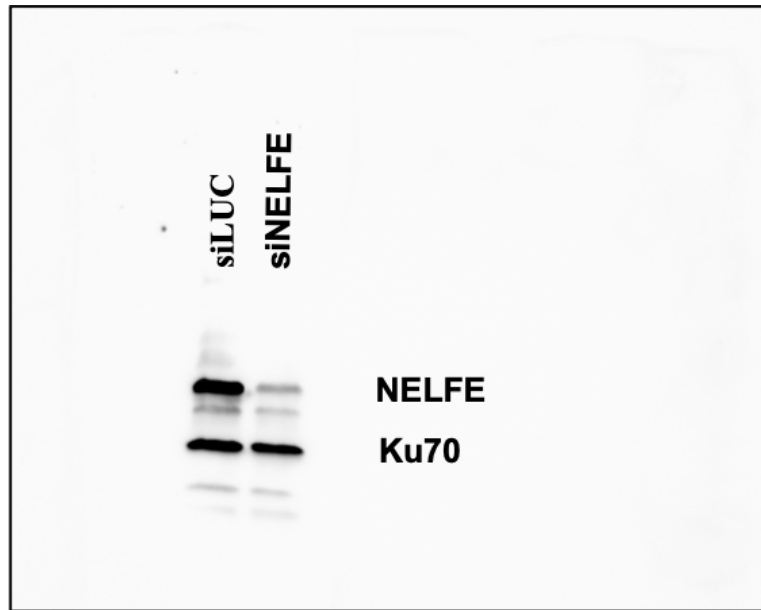
Uncropped and unprocessed versions of western-blot signals from Figure 5.

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*





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

### Modification of images:

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

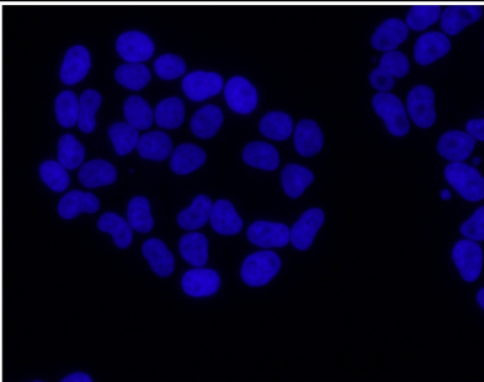
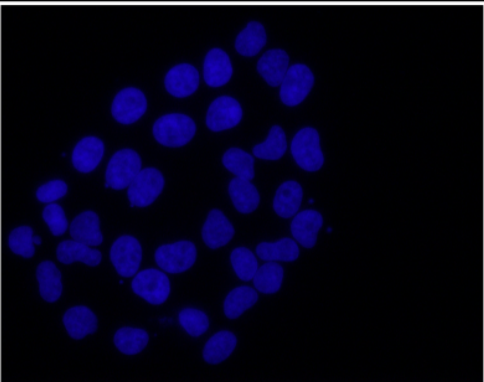
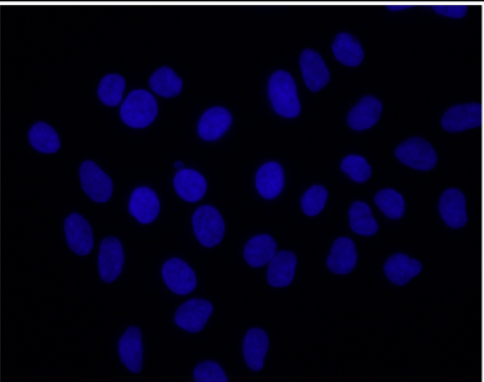
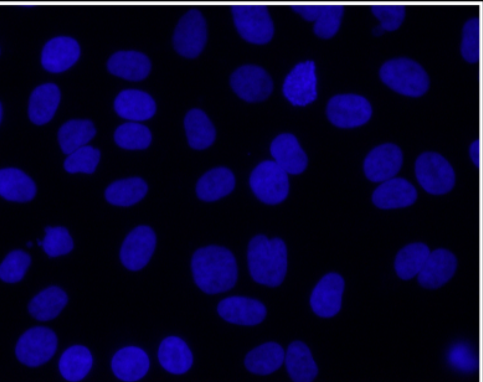
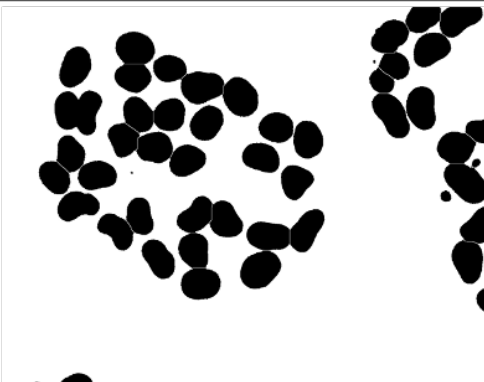
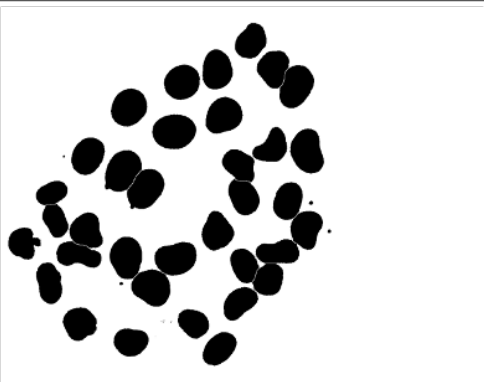
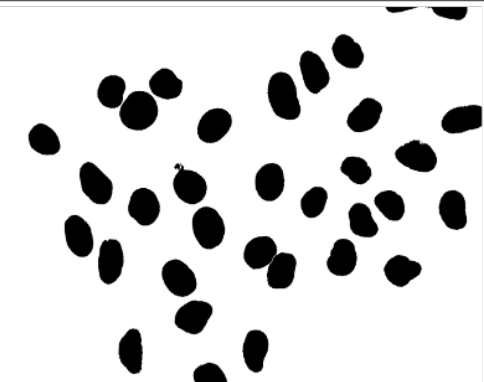
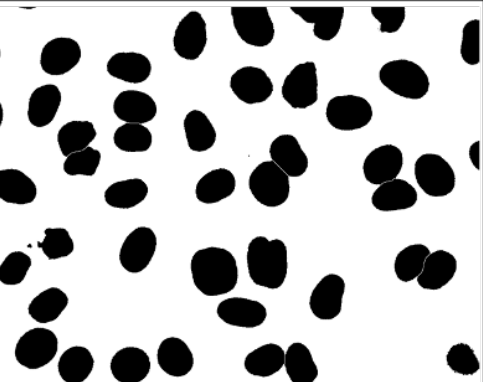
→  $\gamma$ H2AX (p. 3): removal of background noise without brightness and contrast modification

**#2: for figure**

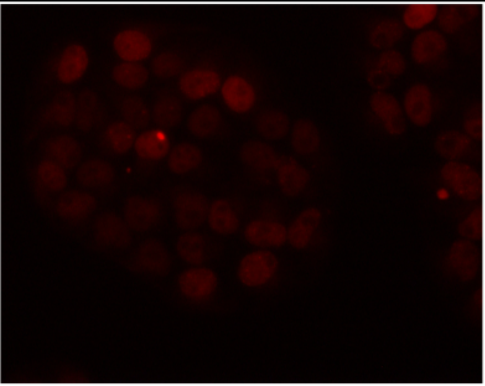
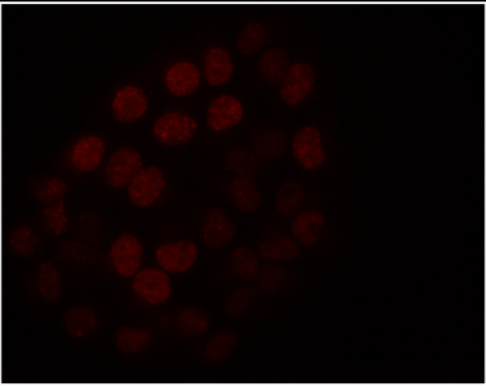
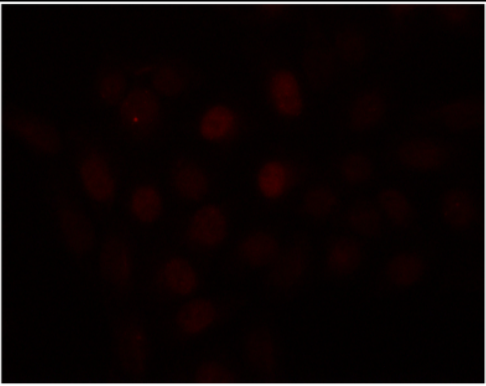
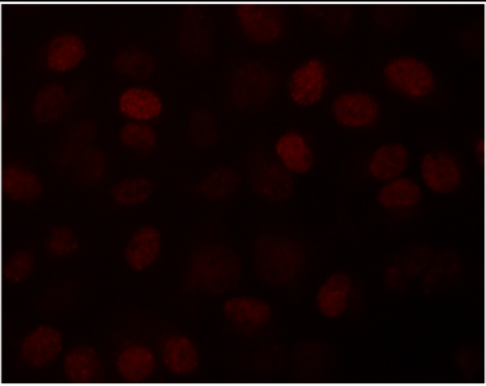

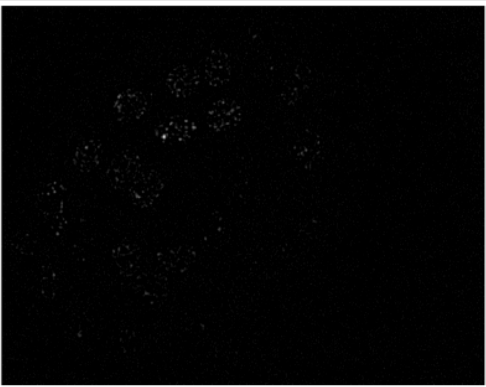

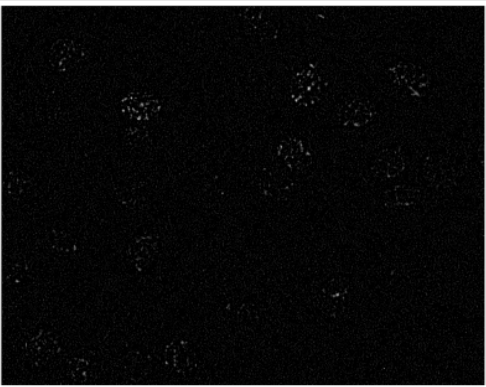
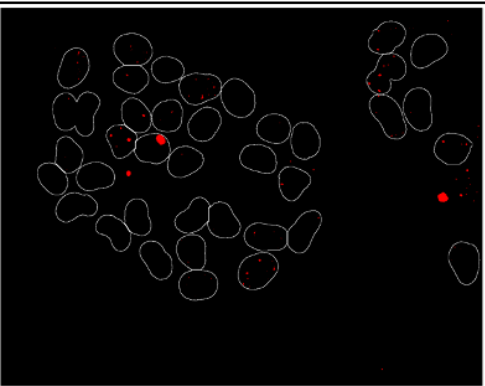
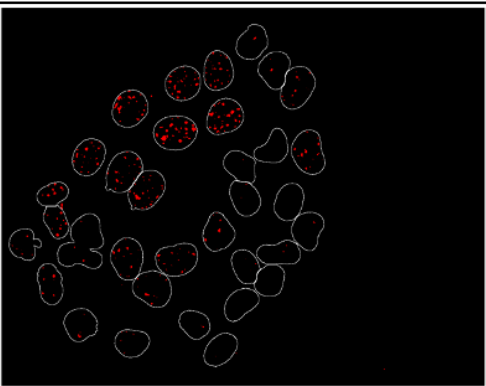
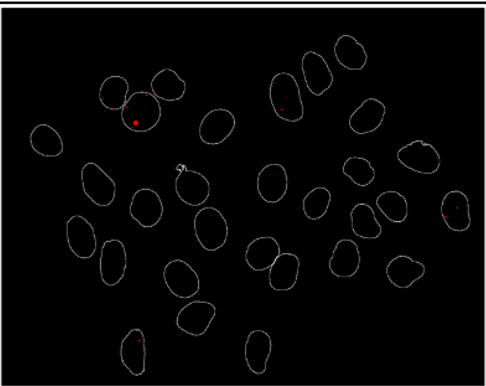
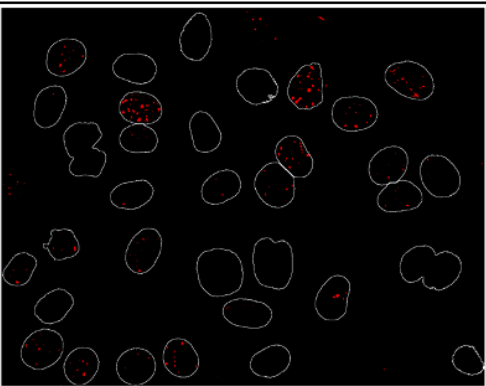
→ Overlay of nuclei edges (from #1 modification of DAPI) over  $\gamma$ H2AX staining (from #1 modification of  $\gamma$ H2AX with modified brightness and contrast) (p. 3 only)

✓ 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

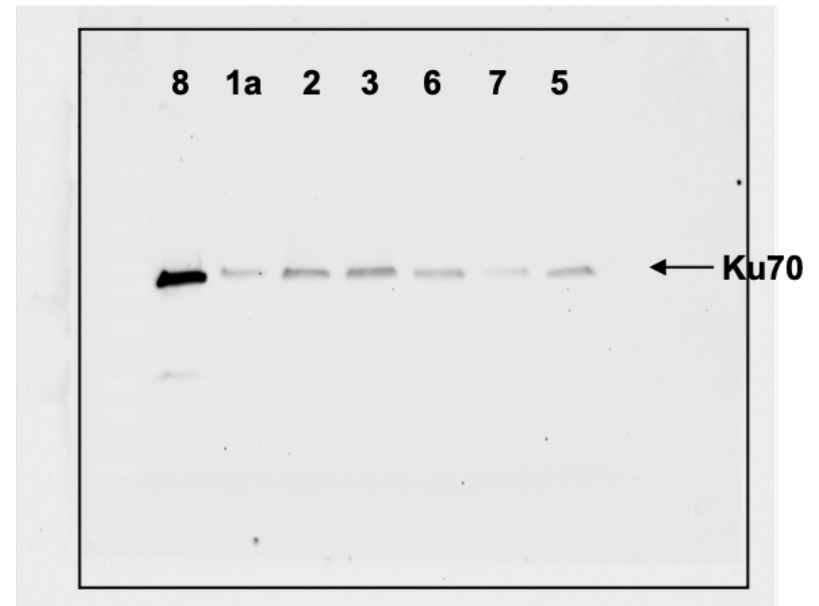
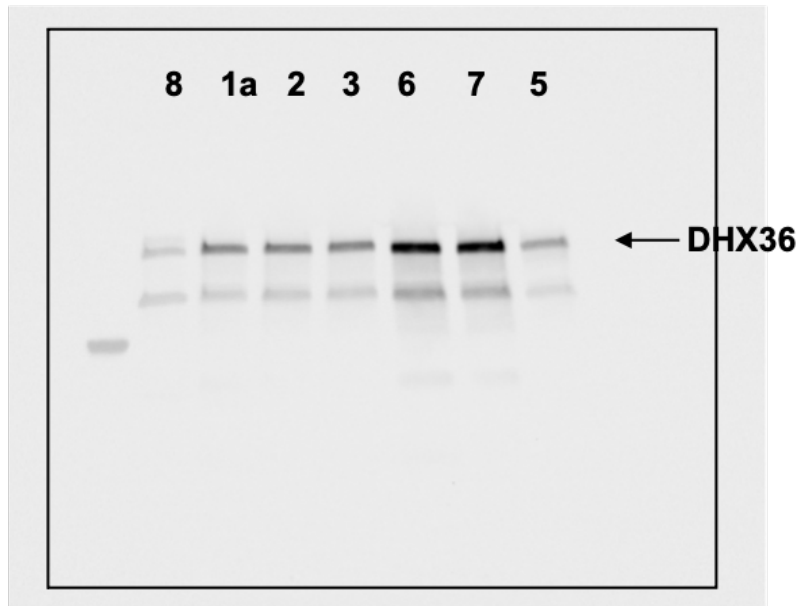
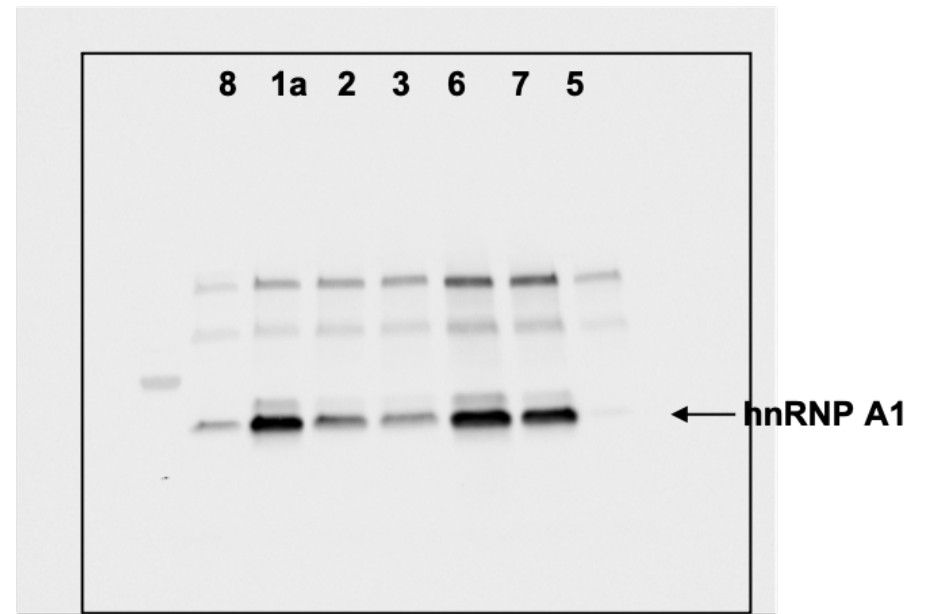
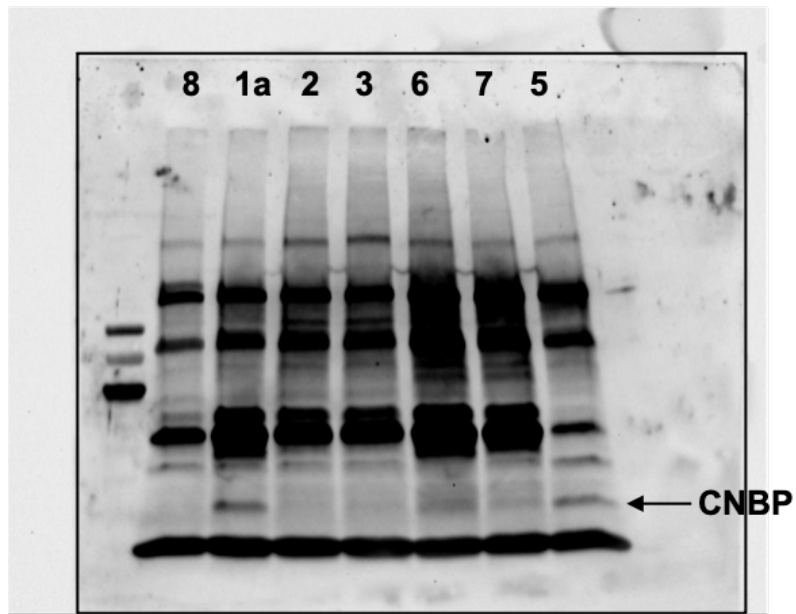
	siLUC NT	siLUC PDS	siNELFE NT	siNELFE PDS
# Images	7	19	34	48
Raw images				
Modified imgs for quantif.				

Supplementary Figure S9A

	siLUC NT	siLUC PDS	siNELFE NT	siNELFE PDS
# Images	7	19	34	48
Raw images				
Modified imgs for quantif.				
Modified imgs for figure				

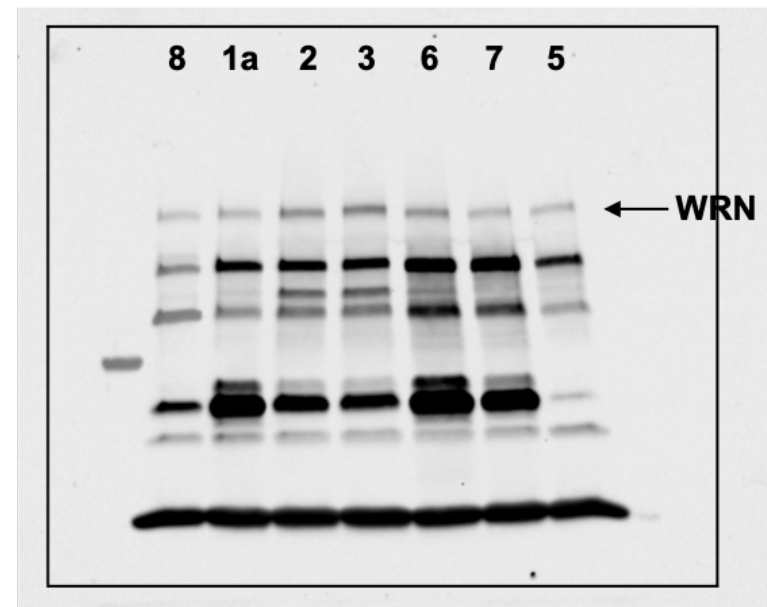
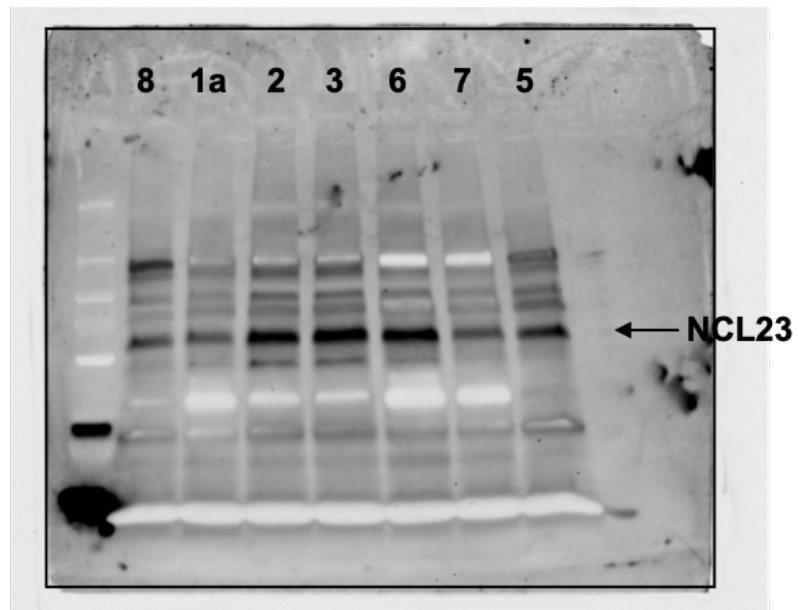
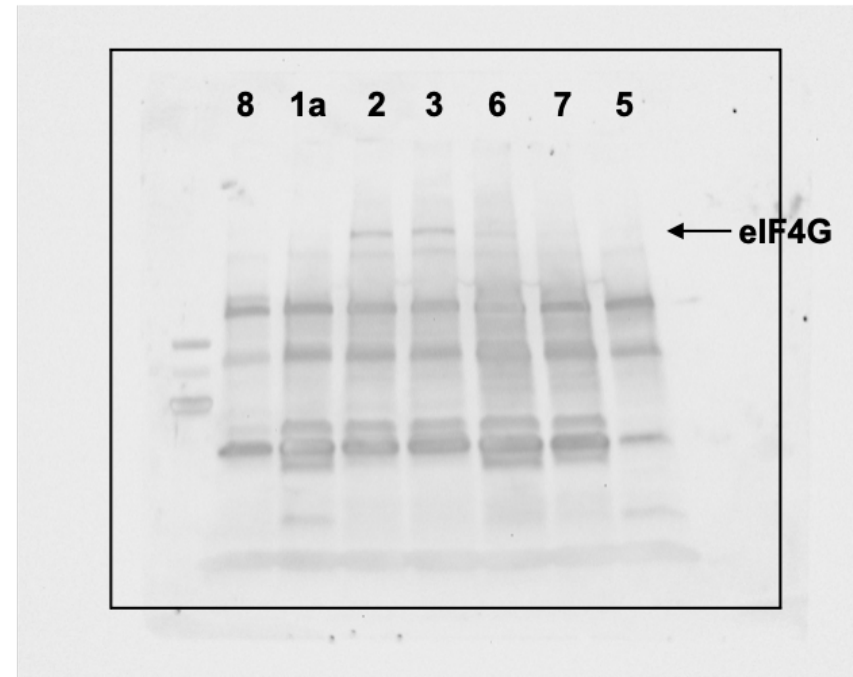
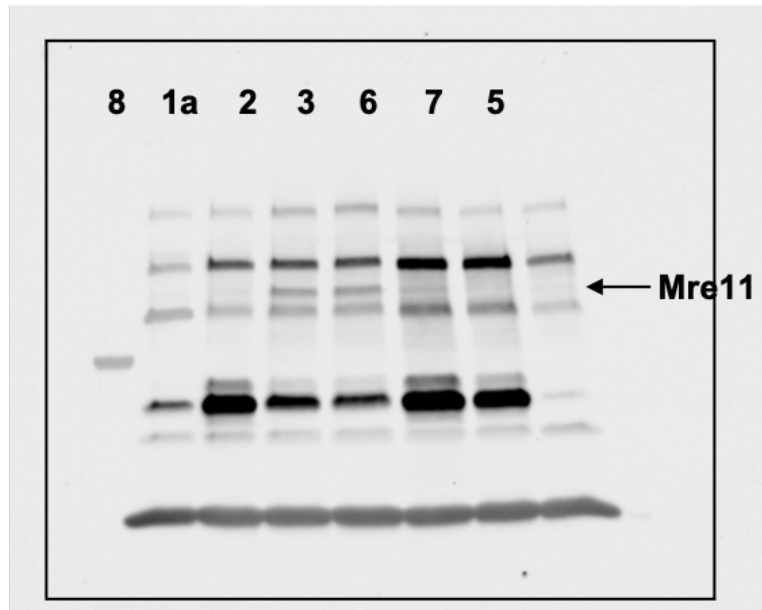
Supplementary Figure S9B





**Supplementary Figure S10A**





Supplementary Figure S10B