Time-resolved analysis of DNA-protein interactions in living cells by UV laser pulses

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Abstract

Immunoprecipitation assay

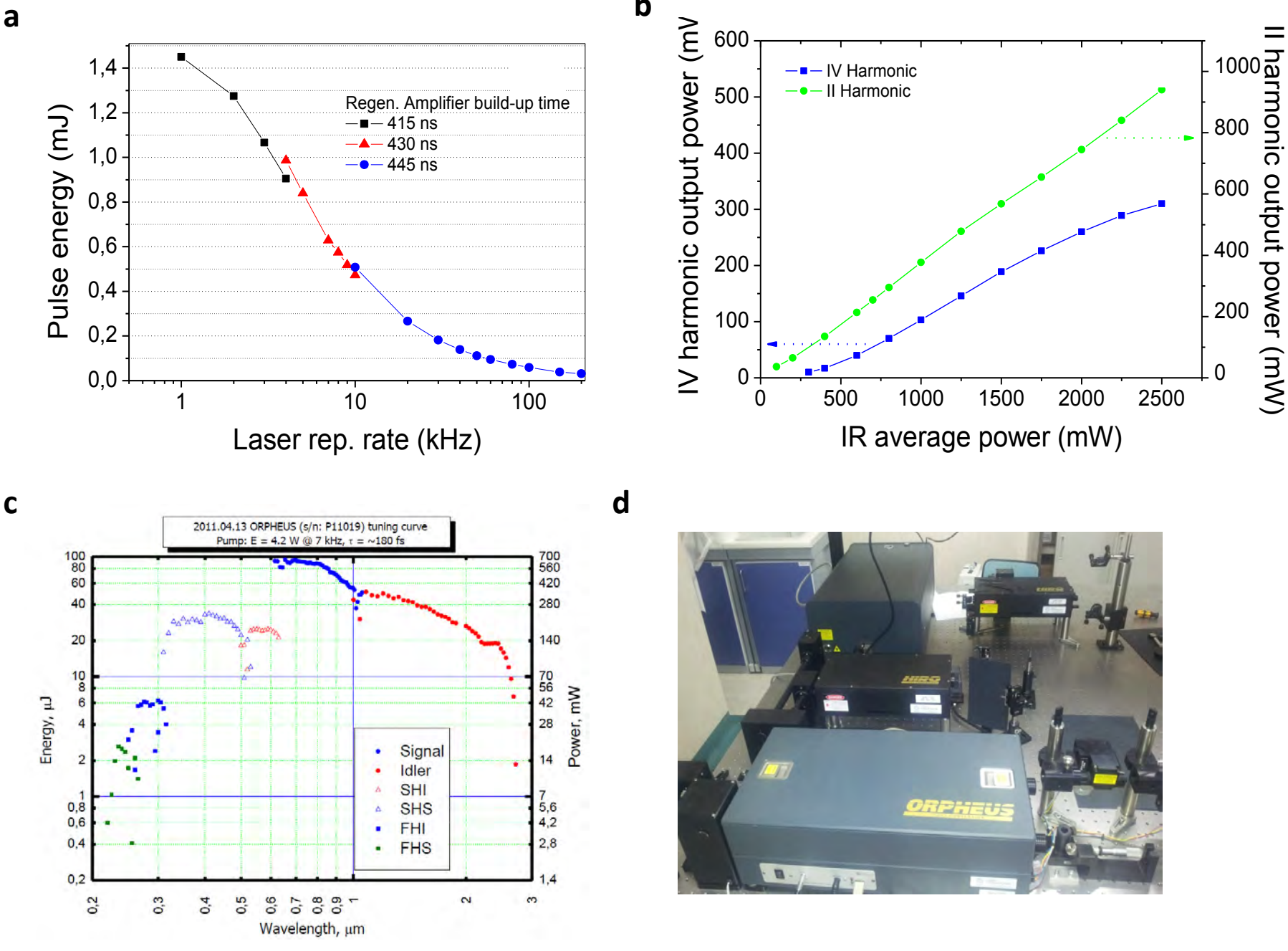
MDA-ERα-GFP extracts were incubated in NP-40 (0.5%), Tris-HCl pH 8.0 (20 mM), NaCl, (150 mM), PMSF (1 mM), 10% glycerol, EDTA (1 mM) and 1X protease inhibitor mix (Sigma) for 20' on ice. Cell debris was removed and the soluble incubated with anti-GFP and anti-ERα overnight at 4°C. The immune complexes were isolated by adding agarose-protein A/G Plus (Santa-Cruz) for 2h at 4°C. Proteins were then eluted, resuspended and analyzed by Western blot.

Immunofluorescence experiment

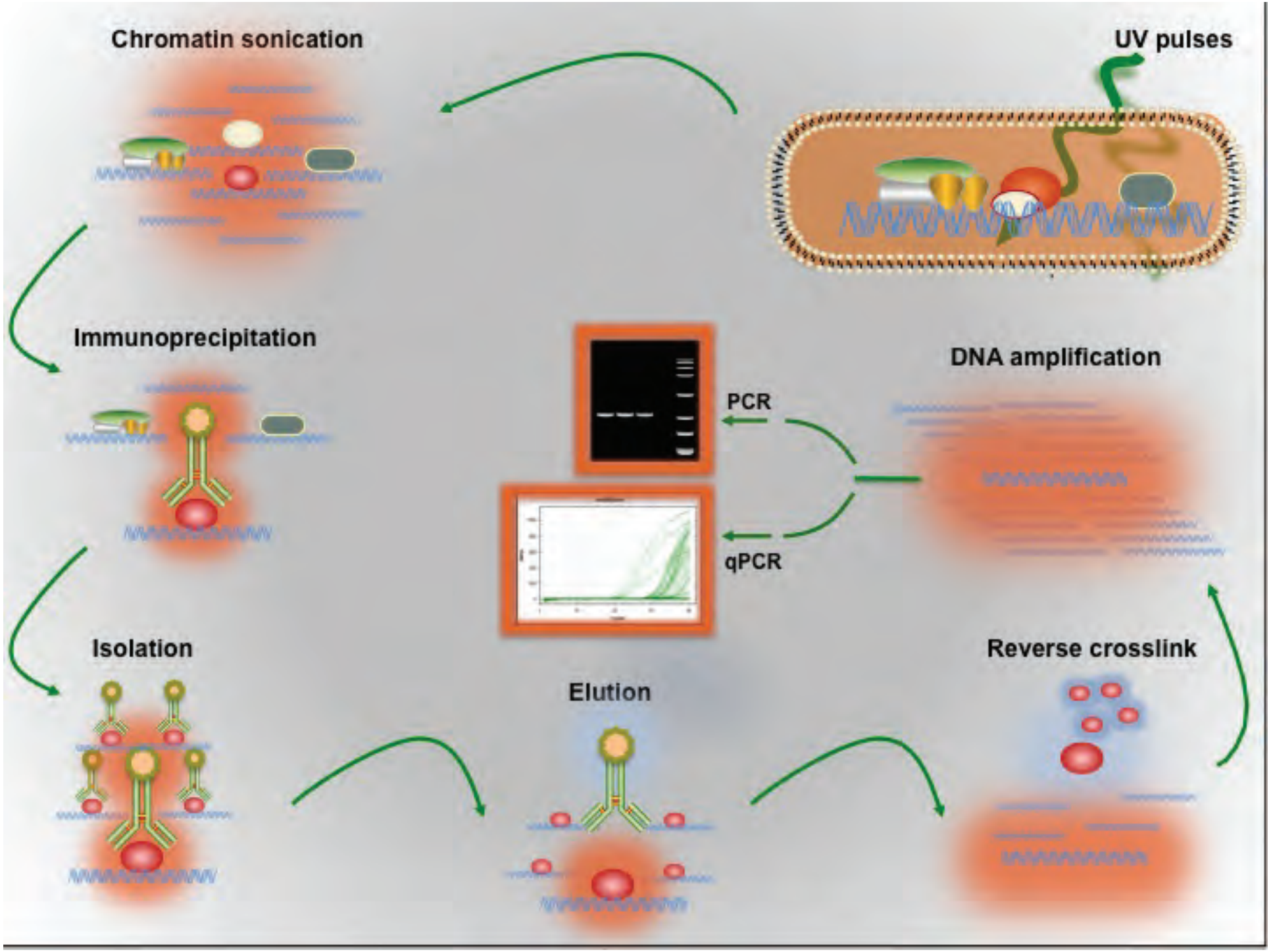
MDA-ERα-GFP cells were grown on gelatin-coated slides and then incubated with anti-ERα (Santa Cruz) according to manufacture's instructions. Nuclei were stained with Hoechst for 10 minutes; images were analyzed by fluorescence microscope (Zeiss).

In Fluorescence Experiments

Cells, irradiated and not were gently lysed to preserve the integrity of nuclear membranes. Nuclei were then sonicated and the chromatin was recovered. The protein ERα-GFP linked to DNA (on ERE sequences) was purified and the quantity of GFP signal was measured by a multimode reader Infinite M200 Pro (Tecan) at 485 nm and 520 nm.

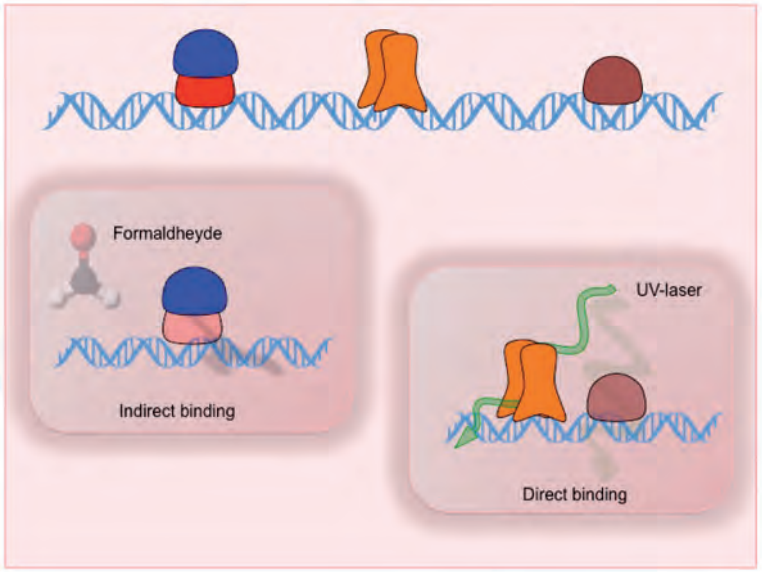


Supplementary Figure S1: Pharos-based laser parameter settings



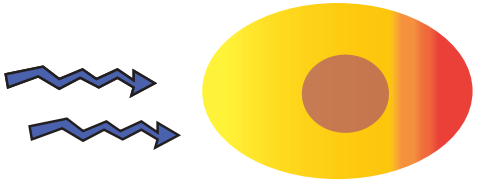
Supplementary Figure S2: Chromatin immunoprecipitation scheme.

a



b

UV laser irradiation of cells



1% formaldehyde crosslinking (positive control)

Isolation of nuclei

RNase treatment, 1 h @ 4 ^{0} C

Sonicate- 50% duty cycle,10 min

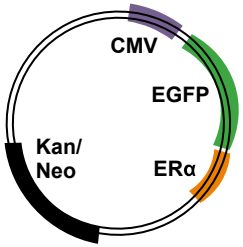
DNase treatment, urea denaturation

FASP - trypsin + reduction + alkylation

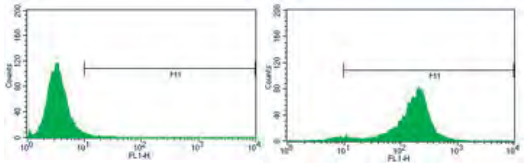
Thermo Scientific Q Exactive hybrid quadrupole-Orbitrap mass spectrometer

Supplementary Figure S3: (a) Formaldehyde- and UV laser-mediated bindings; (b) MS/MS procedure scheme.

Stable trasfection

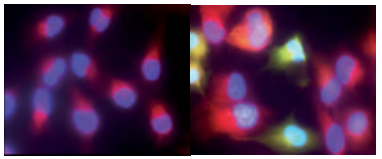


Transfection efficiency evaluation



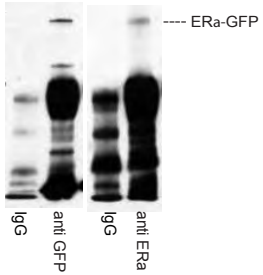
Parental MDA-MB231 cells

Transfected MDA-MB231 cells



ERa signal

ERa GFP signal



Nucleus

ERα-GFP RNAm!

AAAAA

CMV Promoter

ERα-GFP

ERE

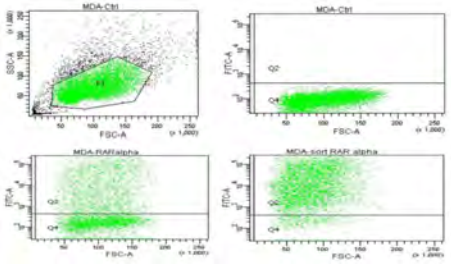
ERα-GFP proteins

Cytosol

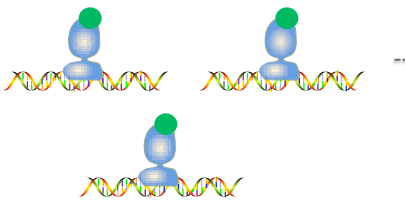
Growth of trasfected cells disomogenuos population

UV laser

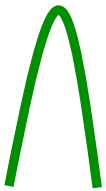
Sorting of GFP cells omogenous population



Extraction and purification of DNA-protein complexeses

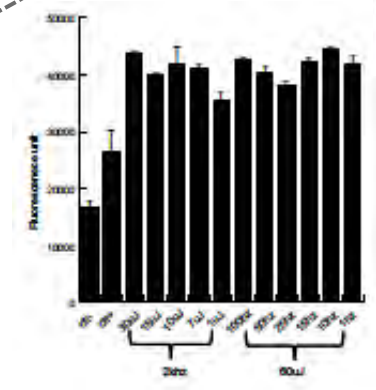


absorbance



450nm

Peaks intensity is proportional to GFP amount



Supplementary Figure S4: Generation of MDA-MB-231 breast cancer cells, negative for ERα and stably expressing GFP- ERα fusion protein

RR (Hz)	Pulse energy (µJ)													Total energy (Hz*µJ)
1	60										✔			60
10	60										✔			600
6	150		✔								✔			900
7,2	125		✔								✔			900
9	100		✔								✔			900
15	60		✔								✔			900
60	15							✔			✔			900
10	140				✔									1400
25	60										✔			1500
15	125				✔									1875
2000	1										✔			2000
50	60										✔			3000
30	125			✔	✔						✔			3750
30	140													4200
50	100			✔			✔							5000
48	125		✔								✔			6000
40	150		✔								✔			6000
60	100		✔								✔			6000
100	60		✔								✔			6000
60	125			✔	✔						✔			7500
1000	8				✔									8000
100	110				✔									11000
200	60			✔										12000
100	125			✔	✔						✔			12500
100	140				✔									14000
2000	**7**	✔	✔	✔	✔		✔	✔	✔	✔	✔	✔	✔	✔	14000
2000	8										✔			16000
2000	10		✔								✔			20000
200	100		✔								✔			20000
160	125		✔											20000
134	150		✔								✔			20100
200	125				✔	✔	✔	✔			✔			25000
2000	15			✔	✔	✔	✔	✔	✔	✔	✔	✔		30000
20000	2				✔									40000
5000	8				✔									40000
7000	7					✔			✔		✔			49000
400	125			✔	✔						✔			50000
2000	30		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔		60000
480	125			✔	✔	✔	✔	✔			✔	✔		60000
400	150		✔		✔	✔	✔	✔			✔	✔		60000
600	100		✔		✔	✔	✔	✔			✔	✔		60000
500	125				✔									62500
480	150		✔											72000
2000	60			✔	✔	✔	✔	✔	✔	✔	✔	✔		120000
1000	125			✔	✔						✔			125000
20000	8	✔		✔	✔	✔	✔	✔			✔			160000
2000	90				✔	✔	✔	✔	✔	✔	✔	✔		180000
2000	125	✔		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	250000
2000	140				✔									280000
20000	14				✔									280000
2000	150				✔									300000

ChIP
photos and counts
PI
Fluorescence
WB
Comet assay
eosin-ematoxylin coloration
ROS
Caspase
Dimers
Cell cycle
WGA staining
Phalloidin staining

Supplementary Table S1: Different irradiation conditions used.

Supplementary Table S2: Different irradiation conditions used.

RR (Hz)	Pulse energy (µJ)													260nm	300nm	Total energy (Hz*µJ)
2000	1		✔											✔	✔	2000
2000	3		✔											✔	✔	6000
2000	1		✔											✔	✔	8000
2000	1	✔	✔	✔	✔	✔	✔	✔			✔				✔	✔	14000
7000	7		✔			✔								✔	✔	49000

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