Supporting information

for

Light-mediated *in cell* downregulation of G-quadruplex-containing genes using a photo-caged ligand.

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Experimental procedures

Synthesis: Commercial reagents were used as received unless otherwise noted. 4,5-Dimethoxy-2-nitrobenzyl bromide, *N*,*N*-Diidopropylethylamine was purchased from *Aldrich*. NMR spectra were acquired on a Bruker® DRX-500 instrument using deuterated solvents as detailed and at ambient probe temperature (300 K). Notation for the ^{1}H NMR spectral splitting patterns includes: singlet (*s*), doublet (*d*), triplet (*t*), broad (*br*) and multiplet/overlapping peaks (*m*). Signals are quoted as δ values in ppm, coupling constants (*J*), are quoted in Hertz and approximated to the nearest 0.5. Data analysis for the nuclear magnetic resonance (NMR) spectra was performed using MestReNova® software. Mass spectra were recorded on a Micromass® Q-Tof (ESI) spectrometer. High performance liquid chromatography (HPLC) purification was carried out by using a Varian Pursuit C18, 5 µ column (250 × 21.2 mm) and a gradient elution with H2O/ acetonitrile (MeCN) containing 0.1% TFA at a flow rate of 12.0 ml/ min. Tandem HPLC-MS analysis was performed on a *Bruker* amaZon X Ion Trap MS and chromatographed by a *Dionex* UltiMate 3000 UHPLC system equipped with a diode array detector and a column oven. HPLC analyses were performed on a Kinetex C18 column (*Phenomenex*, 50 × 2.1 mm, 2.6 µm) using the following solvent system: solvent A, 0.1 % Formic acid in water; solvent B, CH_3CN; flow rate of 1 mL.min^{-1}; using the following gradient (0 min: 2% of B → 1.5 min: 5% of B → 2.5 min: 95% of B → 3.5 min: 95 of B). The column temperature was maintained at 30 °C.

In vitro photo-deprotection of **[C]-PDS**: 500 µL of a 100 µM solution of [**C]-PDS** in a 10 mM PBS buffer pH 7.0 supplemented with 70 mM KCl, in a quartz cell (*l* = 1 cm). The cell was then placed 3 cm from the UV lamp (*Spectroline* ENF-280C/FE, AC

230V, 8W, long wave UV-365nm). The sample was irradiated through the cell while the solution was stirred.

FRET-melting studies: 100 μM stock solutions of oligonucleotides were prepared in molecular biology grade DNase-free water. Further dilutions were carried out in 60 mM potassium cacodylate buffer, pH 7.4. FRET experiments were carried out with a 200 nM oligonucleotide concentration. All labeled DNA oligonucleotides were supplied by Eurogentec® Ltd. Three dual fluorescently labeled DNA oligonucleotides were used in these experiments: SRC (5'-FAM-GGG CGG CGG GCT GGG CGG GG-TAMRA-3'), H-Telo (5′-FAM-GGG TTA GGG TTA GGG TTA GGG-TAMRA-3′), C-myc (5′-FAM-TGA GGG TGG GTA GGG TGG GTA A- TAMRA-3′). The donor fluorophore was 6-carboxyfluorescein (FAM) and the acceptor fluorophore was 6-carboxytetramethylrhodamine (TAMRA). The dual-labeled oligonucleotides were annealed at a concentration of 400 nM by heating at 95 °C for 10 min followed by slow cooling to RT. 96-well plates were prepared by addition of 50 μl of the annealed DNA solution to each well, followed by 50 μl solution of **[C]-PDS**. Measurements were made in triplicate with an excitation wavelength of 483 nm and a detection wavelength of 533 nm.

Cell culture: MRC5-SV40 cells were cultured in T-75 flasks in Phenol Red free Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum and split at 70–80% confluency using trypsin EDTA. The MRC5-SV40 cells were a gift from the laboratory of Prof Steve Jackson.

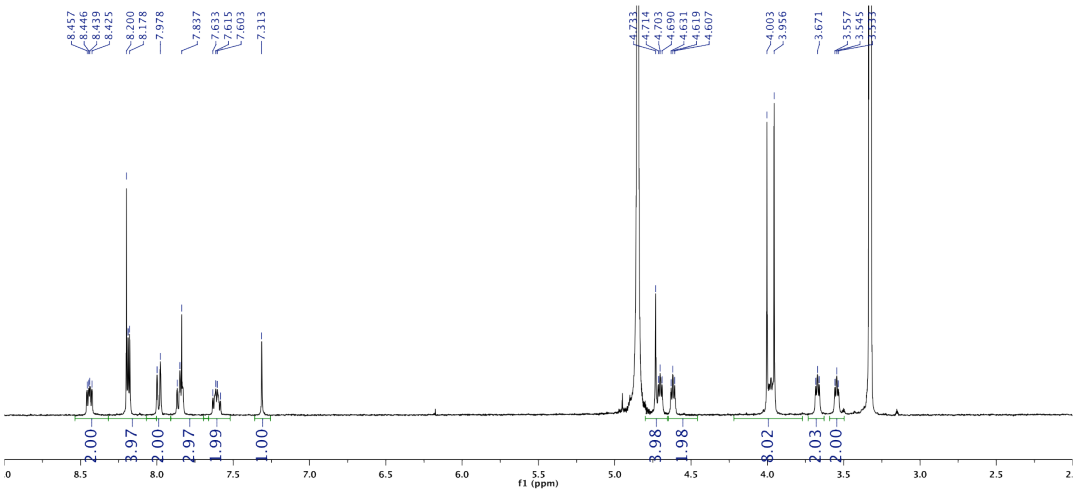
Luminescent cell viability assay: IG50 values of growth inhibition were determined using the cell viability assay CellTiter-GloTM (Promega®). Cells were plated in 96 well plates at a density of 5000 cells per well in 100 μL of media and incubated for 24 h. Compounds were added in serial dilutions (in a range between 0–40 μM) at a volume of 100 μl per well at the respective concentrations. The plates were then placed 3 cm apart from the UV lamp (*Spectroline* ENF-280C/FE, AC 230V, 8W, long wave UV-365nm) and irradiated for the indicated times. Cell viability was measured after 72 h using the manufacturer's protocol. All measurements were made in triplicate.

Gene expression / mRNA analysis: MRC5-SV40 cells were plated in 96 well plates at a density of 10 000 cells per well in 100 μL of media. The cells were either treated or untreated with 2 μM **[C]-PDS**. Total RNA was purified from each sample using an RNeasy kit from Qiagen following the manufacturer's protocol. cDNA synthesis of 1 μg of total RNA was performed with the *Thermo Scientific* Maxima Reverse Transcriptase kit using oligo(dT) primers following the manufacturer's protocol for GC-rich RNA. For quantitative Real-Time PCR (qRT-PCR) analyses, 1/30th of each reaction was used. The following gene specific Quantitect primer assays (Qiagen, cat. number included) were used in our analysis: *B2M* (QT00088935), *ALAS1* (QT0073122), *MYC* (QT00035406), *NOTCH1* (QT01005109), *RET* (QT00047985), *SRC* (QT00039326). RT-PCR analysis was performed using Power SYBR® green (*Applied Biosystems*) for detection with an CFX96™ Real-Time system (*BioRad*). All measurements were made in technical and biological triplicate.

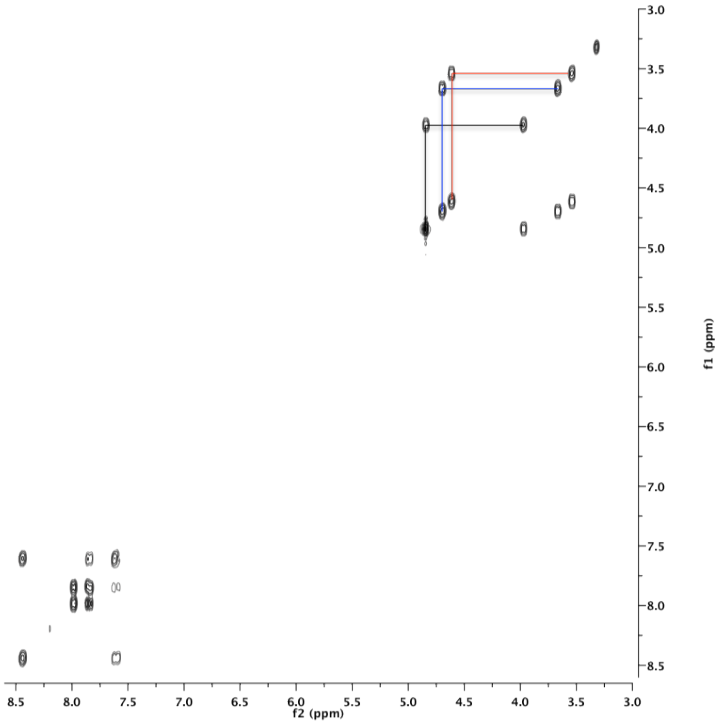
Synthesis and characterisation of [C]-PDS

PDS was synthesized as reported in: Rodriguez, R.; Müller, S.; Yeoman, J. A.; Trenteseaux, C.; Rio, J.-F.; Balasubramanian, S. *J. Am. Chem. Soc.* **2008**, *130*, 15758.

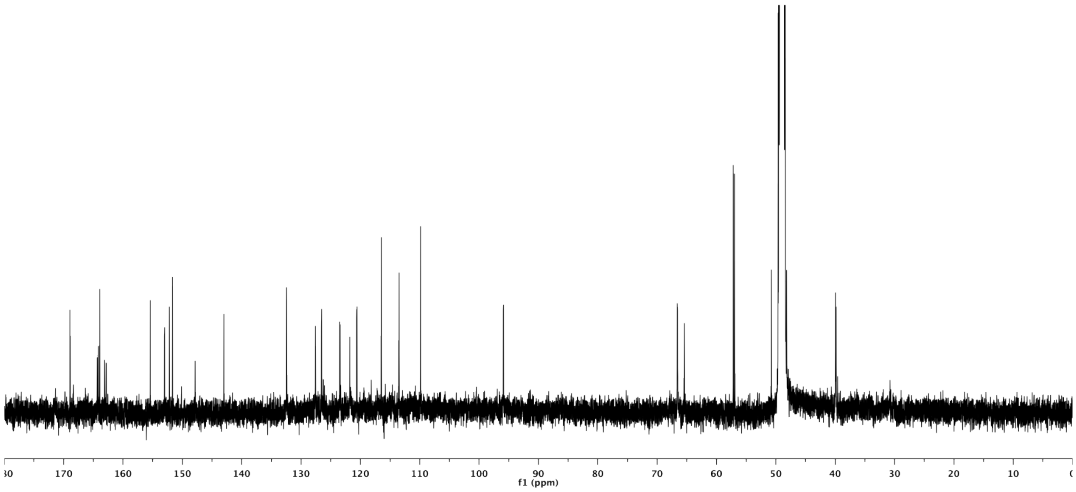
PDS (20 mg, 33.6 µmol) was mixed in DMF (5 mL) with 1 eq 4,5-Dimethoxy-2-nitrobenzyl bromide (5.88 mg). TEA (100 µL, 260 mmol) was added and the mixture was stirred at RT overnight. The major compound was then purified by HPLC (Gradient: 10 % ACN in H_2O containing 0.1 % TFA to 90 % ACN over 20 min) to recover **[C]-PDS** as a white powder (22.7 mg, 28.6 µmol, 85.1 %) after the solvents have been freeze-dried. ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.44 (*dd*, *J* = 7.2, 4.4 Hz, 2H), 8.20 (*s*, 2H), 8.17 (*d*, *J* = 4 Hz, 2H), 7.98 (*d*, *J* = 8.4 Hz, 2H), 7.85 (*t*, *J* = 7.2 Hz, 2H), 7.83 (*s*, 1H), 7.61 (*dd*, *J* = 7.2, 12.0 Hz, 2H), 7.31 (*s*, 1H), 4.84 (*t*, *J* = 4.8 Hz, 2H), 4.73 (*s*, 2H), 4.70 (*t*, *J* = 4.4 Hz, 2H), 4.62 (*t*, *J* = 4.8 Hz, 2H), 4.00 (*s*, 3H), 3.97 (*t*, *J* = 4.8 Hz, 2H), 3.95 (*s*, 3H), 3.67 (*t*, *J* = 4.4 Hz, 2H), 3.54 (*t*, *J* = 4.8 Hz, 2H); ^{1}H NMR (125 MHz, CD_3OD) δ_C 168.9, 164.3, 164.2, 163.9, 163.1, 162.8, 155.4, 153.0, 152.9, 151.6, 147.9, 142.9, 132.4, 127.6, 126.5, 123.4, 121.8, 120.6, 116.5, 113.5, 109.8, 95.9, 66.6, 65.4, 57.2, 56.9, 50.7, 48.2, 39.9, 39.8; HRMS (ES) calculated for $C_{40}H_{41}N_9O_9.H^+$: 792.3027, found 792.2349.



[C]-PDS ^{1}H NMR spectrum

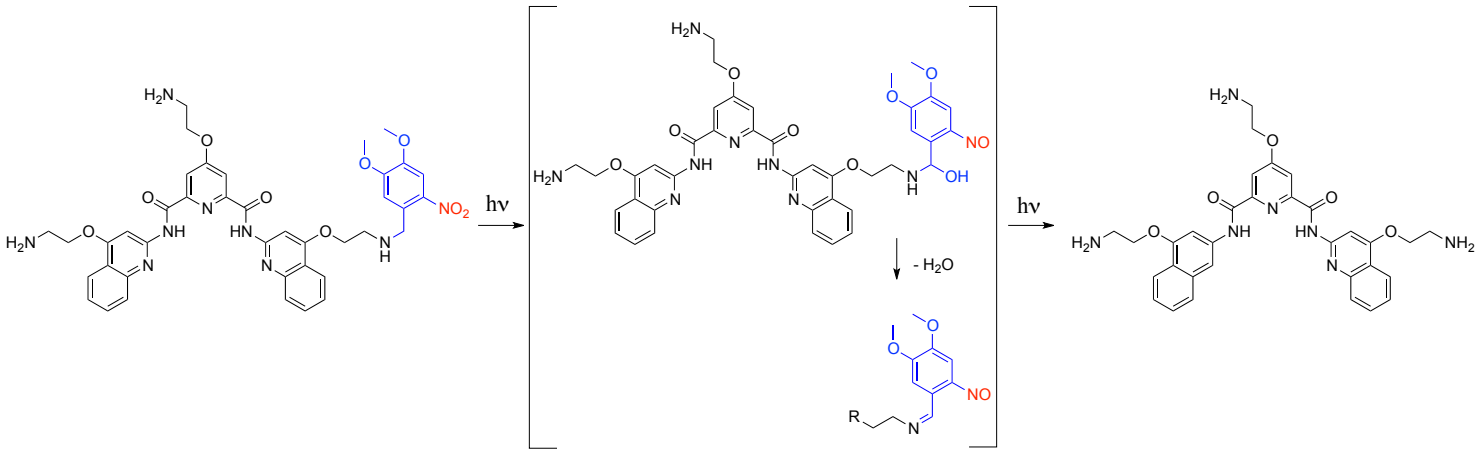


[**C]-PDS** COSY NMR spectrum. The three highlighted correlations show the non-equivalence of the three side-chains of **[C]-PDS** therefore supporting the structure of the isomer reported in **Scheme 1**.



[**C]-PDS** ^{13}C NMR spectrum

Kinetic of photo-deprotection of [C]-PDS:



[C]-PDS
M_{th} = 791.3
M_{exp} = 791.4

Intermediate
M_{th} = 791.3; M_{th} - H_2O = 773.3
M_{exp} = 791.4, 773.4

PDS
M_{th} = 595.2
M_{exp} = 595.4

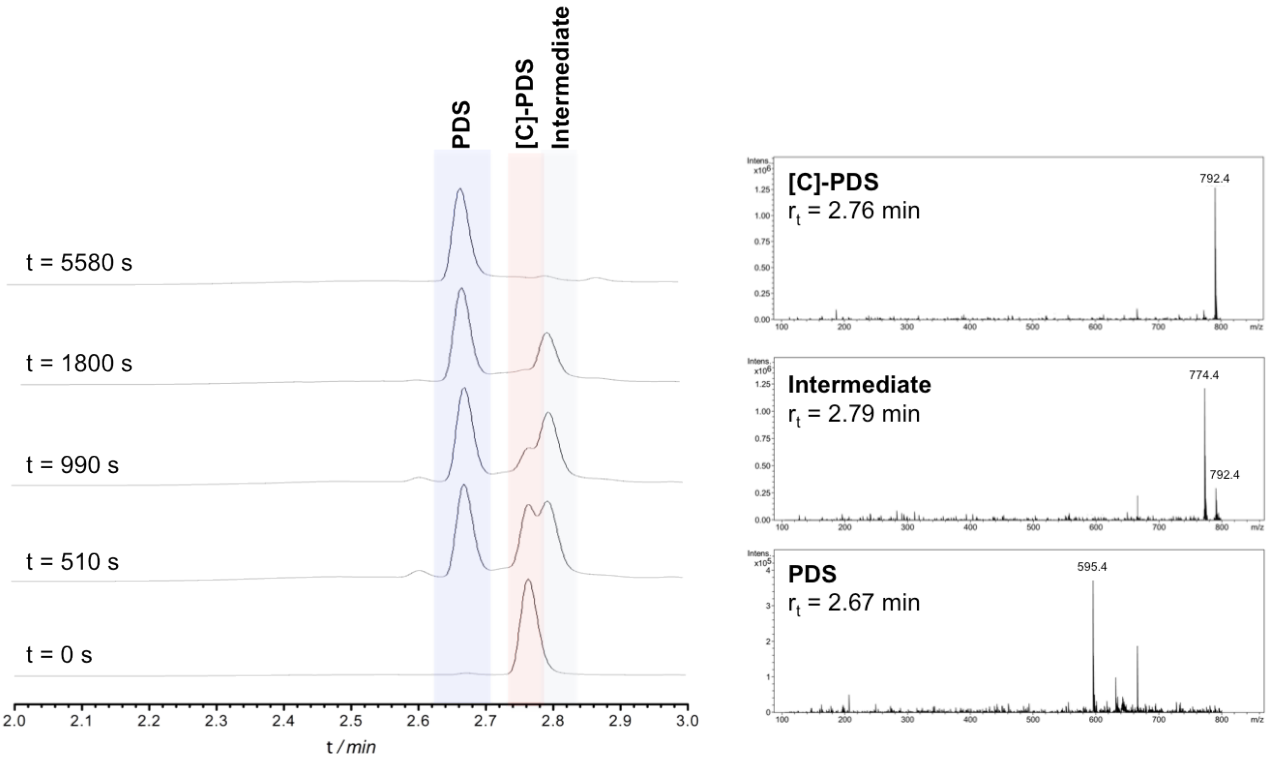


Figure S1. Kinetic of photo-deprotection of [**C**]**-PDS** followed by tandem HPLC-MS analysis.

Cell viability assay in presence of PDS and [C]-PDS.

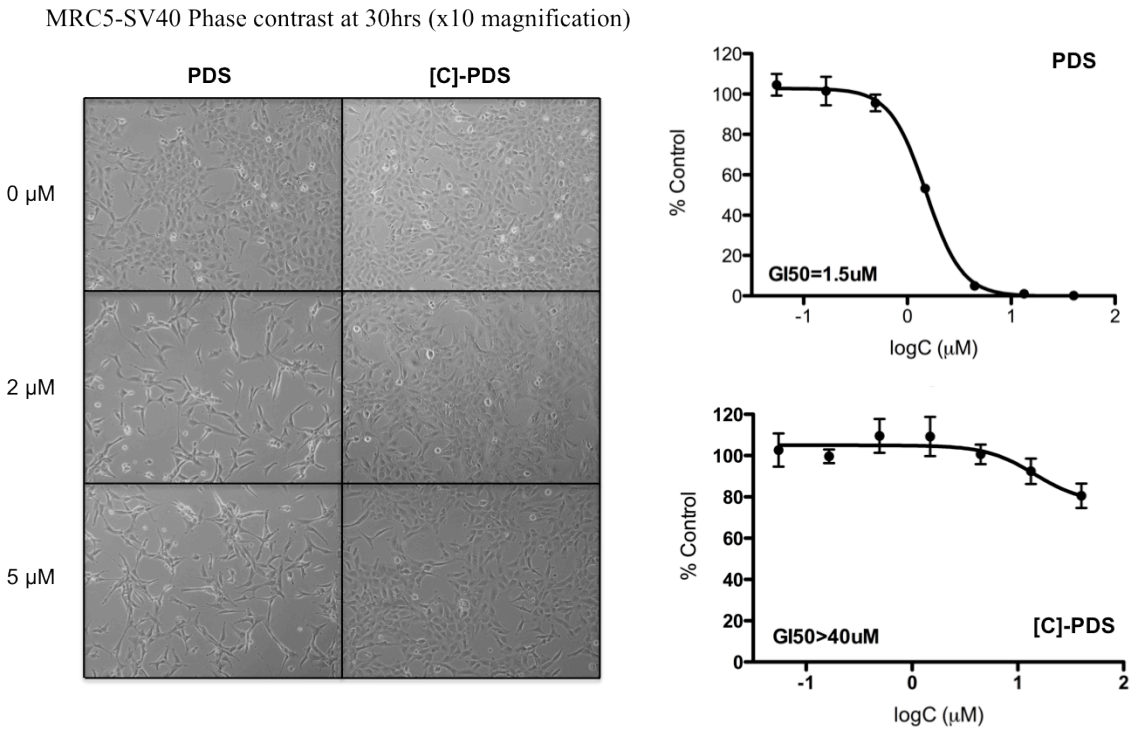


Figure S2. MRC5-SV40 cell viability assay for the determination of **PDS** and **[C]-PDS** GI 50. Cell viability was measured 72 h after incubation with small molecules.

Influence of UV light on MRC5-SV40 cells viability.

Irradiation time (min)	Number of viable cells	p-value
0	211004 ± 1779	
8	204076 ± 792	ns
15	197554 ± 6291	ns
30	195456 ± 11513	ns

Table S1. Number of viable cells at 72 h after irradiation for different irradiation times in the absence of any added molecule. p-values have been calculated to check the relevance of changes in quantity of cells. ns : not statically relevant.

Influence of UV light on gene expression.

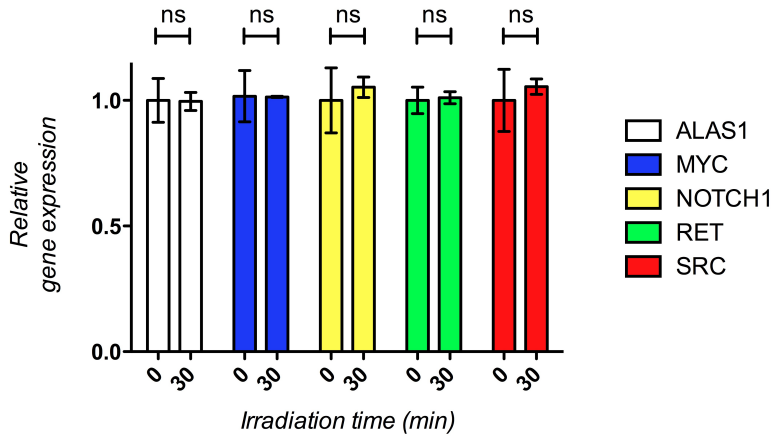


Figure S3. Relative gene expression of untreated MRC5-SV40 cells for 0 or 30 min of 365nm irradiation. The expression data for each gene is normalized to the housekeeping gene *B2M*. These results are the combination of biological triplicate and error bars represent the standard deviation. ns: not statically relevant.

Light-mediated downregulation of mRNAs of quadruplex-containing genes.

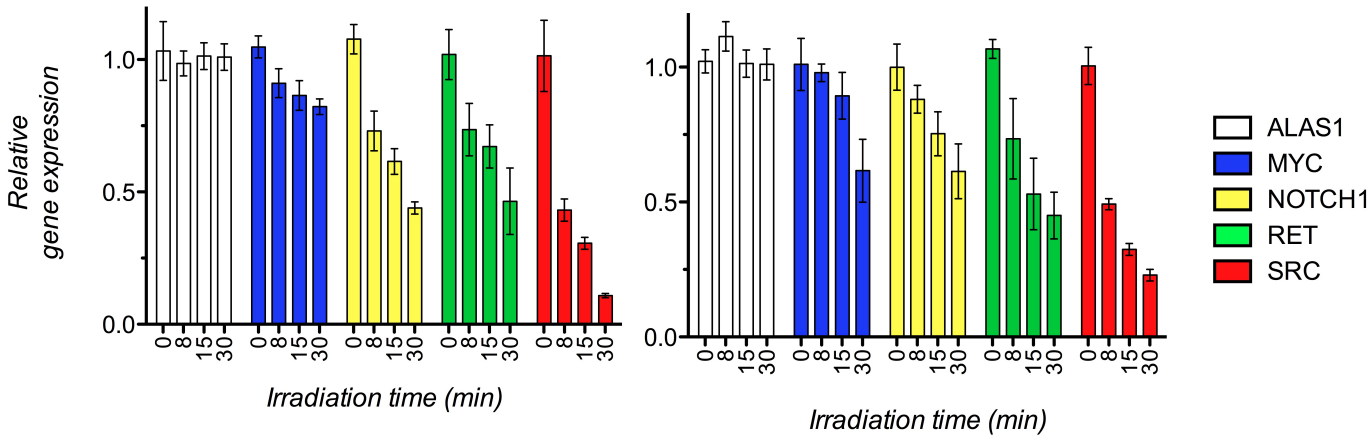


Figure S4. Three independent replicates showing the light-mediated down regulation of mRNAs of quadruplex-containing genes by 2 µM [**C**]**-PDS** in MRC5-SV40 cells for different time of 365 nm irradiation. The expression data for each gene is normalized to the housekeeping gene *B2M*. These results are the combination of technical triplicates and error bars represent the standard deviation.