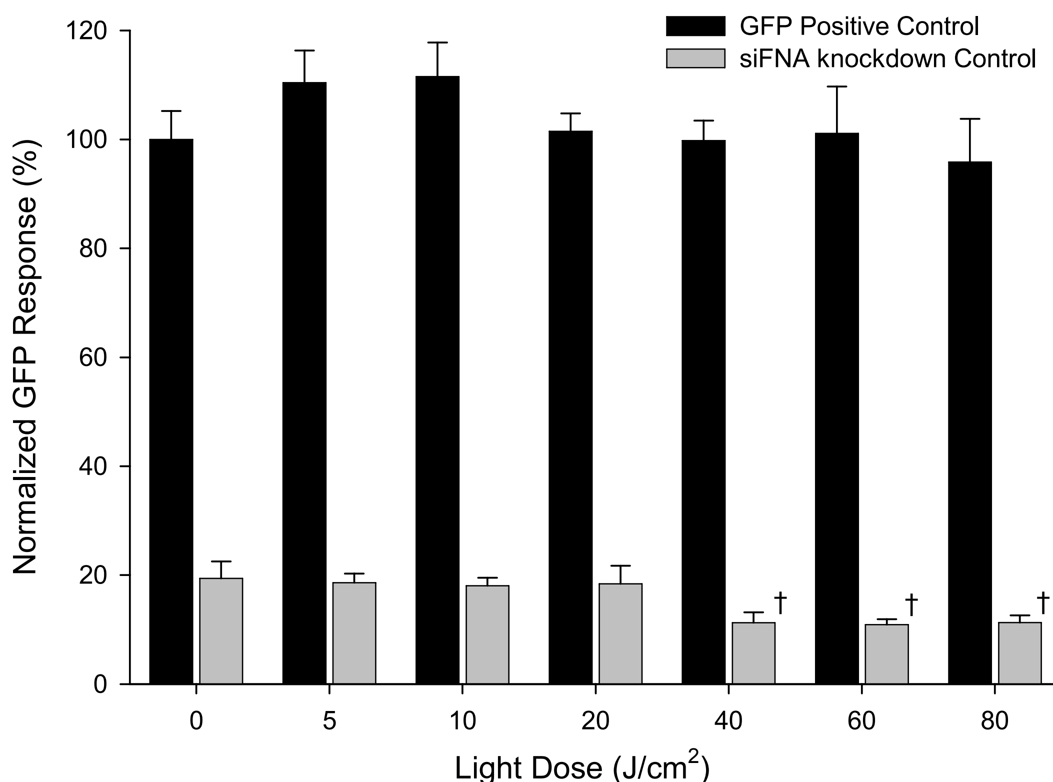
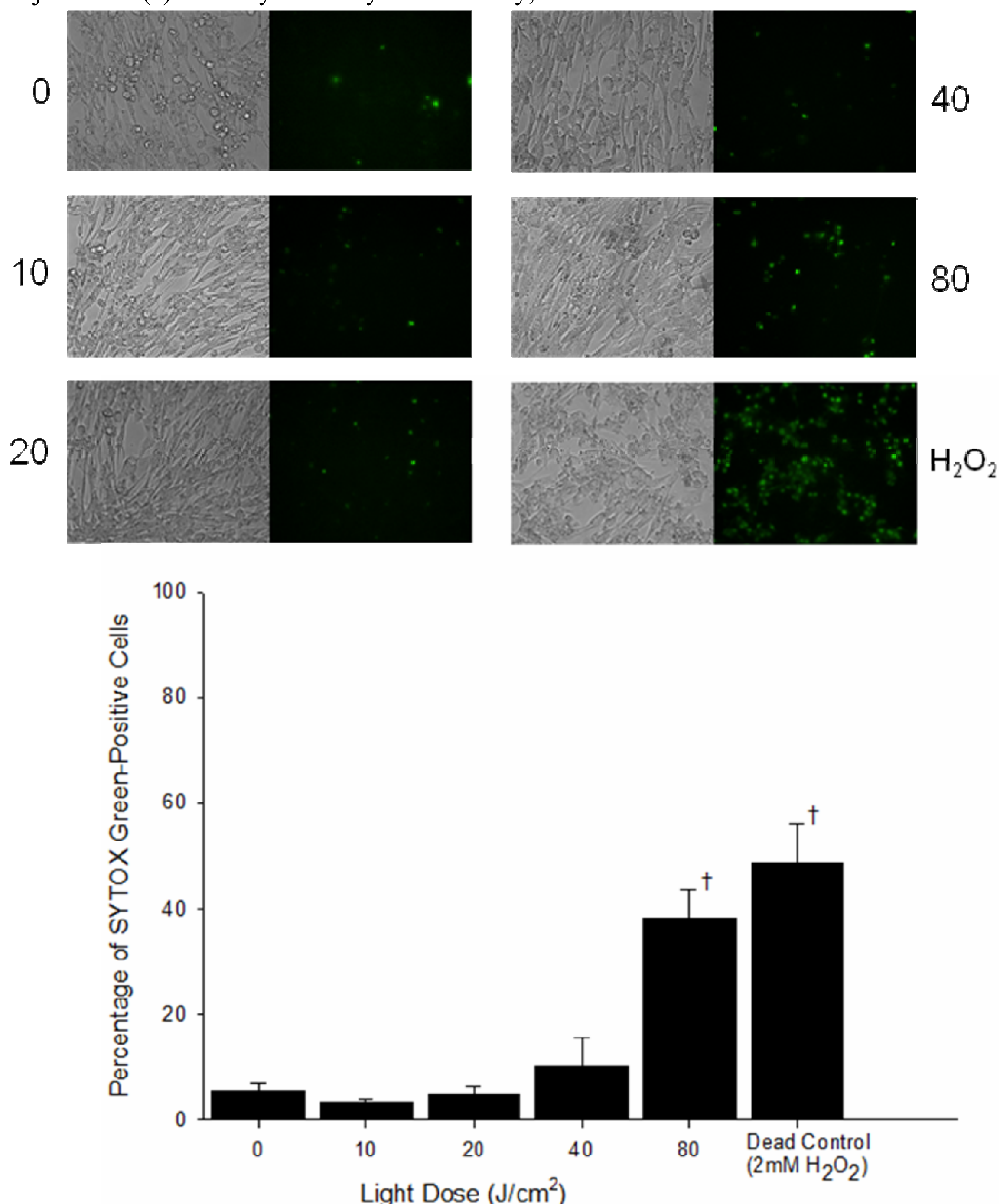


Supplemental Figure 1a: GFP expression in cells exposed to various doses of 365 nm light. Dark bars represent GFP positive controls at various light doses, while light bars represent siFNA knockdown cultures exposed to various light doses. No significant difference can be found by photoexposing positive controls up to 80 J/cm^2 ($p \leq 0.05$). A small decrease in GFP expression was significant (denoted with †, $p \leq 0.05$) at light doses $\geq$ 40 J/cm^2 relative to non-photoexposed cultures. These results indicate that light dosing in this range does not have noteworthy effect on GFP expression or silencing activity.



Supplemental Figure 1b: Fluorescent microscopy (top) and flow cytometry (bottom) assessment of 365nm light effects on BHK cells. Cells seeded and photoexposed as described in the transfection protocol were incubated with 2mM SYTOX Green (Invitrogen) membrane-impermeant stain for 15 minutes prior to fluorescent analysis. The percentage of SYTOX-positive cells, indicating compromised membrane integrity, was not different from no light treatment until doses > 40 J/cm^2 (denoted with †, $p \leq 0.05$).

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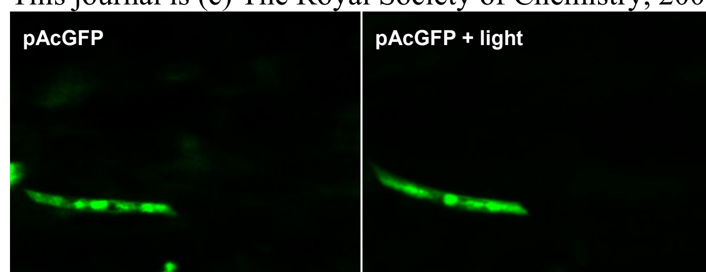
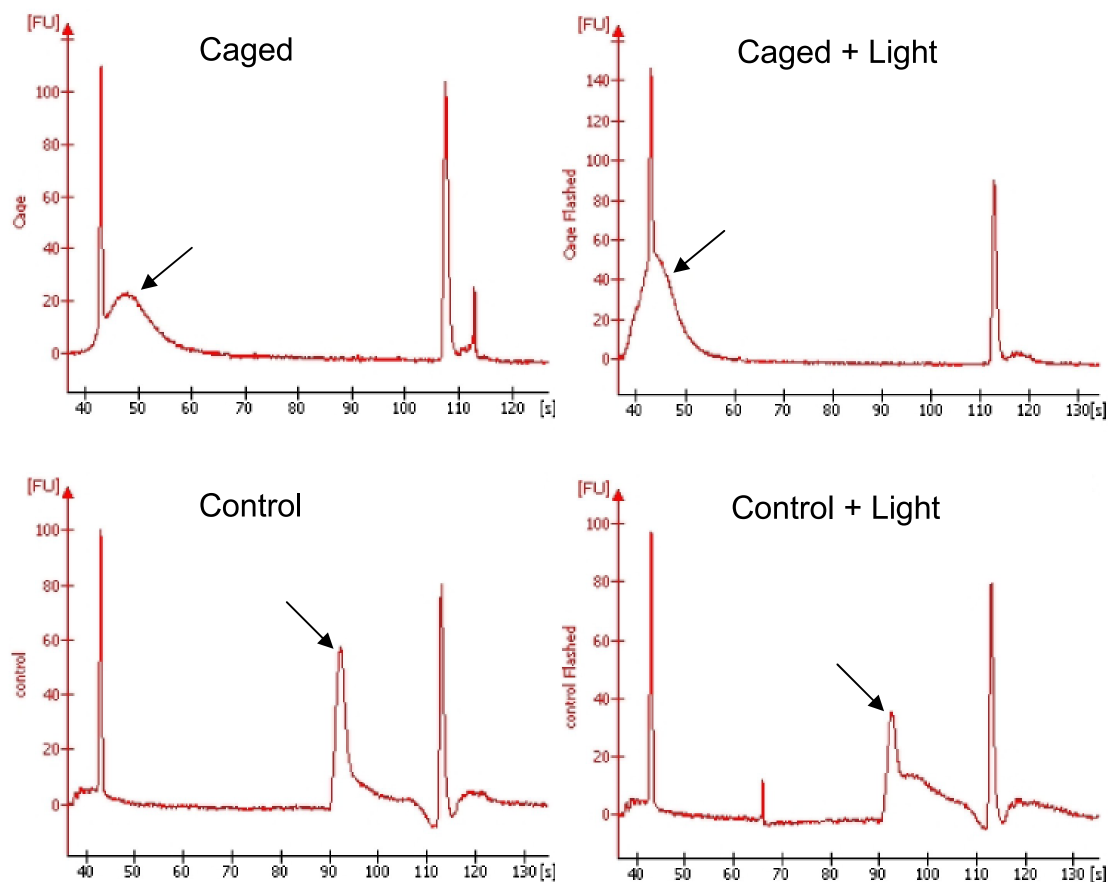


Supplemental Figure 2: GFP expression is unaffected by UVA light exposure (40 J/cm^2)



Supplemental Figure 3: Capillary electrophoresis of a process control versus a caged 700bp double-stranded RNA. Products of the caging process consist of smaller fragments of RNA, as illustrated by a quicker migration relative to control RNA. Light exposure of cage products further increased their mobility, suggesting DMNPE photocleavage. All samples elute (denoted by arrow) between two standard markers (15bp, 1500bp).

Supplementary Material (ESI) for Molecular BioSystems
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Supplemental Methods 1. 700bp dsRNA transcripts were prepared using a TurboScript™ T7 Transcription Kit (Genlantis, San Diego, CA) according the manufacturer instructions. Product generation was verified by PAGE separation. RNA transcripts were suspended in 100 µL of 10 mmol Bis-Tris buffer (pH 5.5) at 1 µg/µL, (150 µM). 4,5-Dimethoxy-2-nitroacetophenone hydrazone precursor (5 mg, 22 µmol) (Molecular Probes, Inc., Eugene, OR) was oxidized with MnO_2 (50 mg, 557 µmol) in 1.0 mL DMSO at room temperature under agitation for 20 minutes. MnO_2 was removed by filtration through an acetonitrile-wetted Celite (Sigma Aldrich, St. Louis, MO) syringe filter. The activated diazo-cage solution was mixed with the RNA aqueous solution to achieve a 2:1 acetonitrile:aqueous buffer and 300 molar equivalents of the active diazo compound. A process control sample without nucleic acid and a process control sample without active caging compound were simultaneously run with experimental samples in order to rule out downstream effects resulting from caging reaction processing. The reaction solutions were agitated at 4°C for 18 hours. Excess cage compound was removed by size exclusion chromatography through a gravity column of preswollen (1:2 acetonitrile:water) solvent-resistant LH-20 sephadex (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). The product was further purified by 4 X 500 µL washes through a YM-3 nitrocellulose spin filter (Millipore, Billerica, MA) to remove any residual cage compound, buffer, and organic solvent. The products were dried *in vacuo*, and resuspended in TE buffer (10 mM Tris, 1mM EDTA, pH 8.0) made with nuclease free water at a concentration of 500 ng/µL.

Caged and process control RNA samples were further diluted to 50 ng/µL with nuclease-free water and either protected from light or exposed to 40 J/cm^2 of 365 nm light. Caged and photo-released products were evaluated by capillary electrophoresis using the DNA 1000 reagent kit for the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA) lab-on-a-chip system the according to manufacturer instructions. Results indicate that caging results in fragmentation of the 700bp transcript (Supplement. Fig. 4).