SUPPORTING INFORMATION

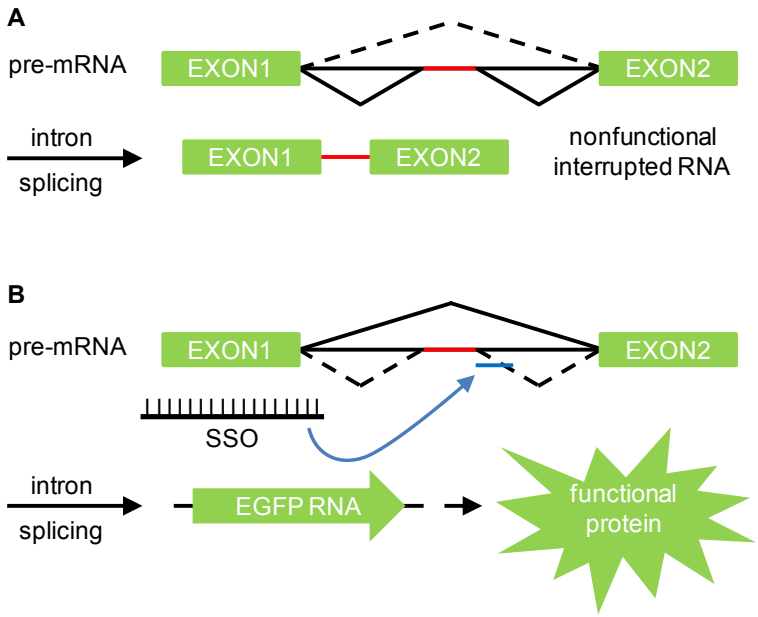
Conditional Control of Alternative Splicing Through Light-Triggered Splice-Switching Oligonucleotides

James Hemphill[1,2], Qingyang Liu[2], Rajendra Uprety[2], Michael Tsang[3], Rudolph L. Juliano[4], and Alexander Deiters[1,2]*

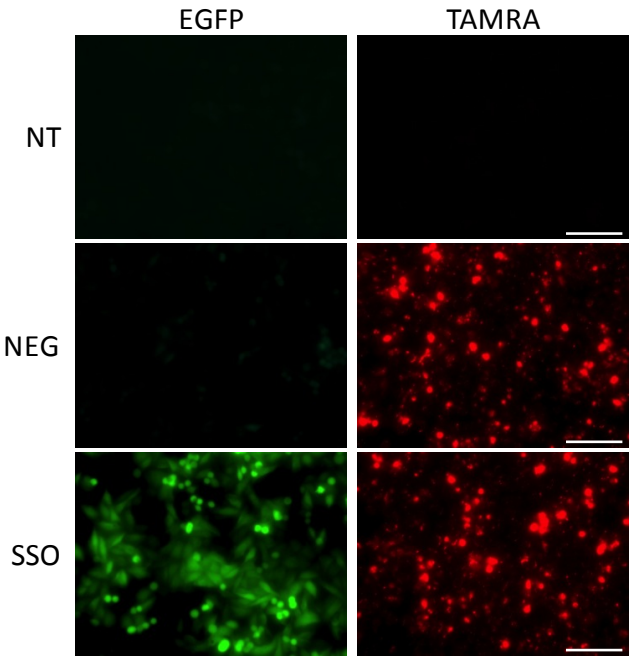
[1]University of Pittsburgh, Department of Chemistry, Pittsburgh, PA 15260. [2]North Carolina State University, Department of Chemistry, Raleigh, NC 27695. [3]University of Pittsburgh, School of Medicine, Department of Developmental Biology, Pittsburgh, PA 15260. [4]University of North Carolina at Chapel Hill, Division of Molecular Pharmaceutics, Eshelman School of Pharmacy, Chapel Hill, NC 27599.

Oligonucleotide	Sequence (5' → 3')
SSO	GUUAUUCUUUAGAAUGGUGC
NEG	GU**A**AUU**A**UUUA**U**AAU**C**GU**C**C
LASSO	G**U***UAU**U***CUU**U***AGAA**U***GGUGC
LDSSO	GUUAU-CUUUAGA-UGGUGC
sox31 SSO	AGCCCUUUUCUCAAAACAAACCUGU
sox31 LASSO	AGCCC**U***UU**U***C**U***CAAAACAAACC**U***GU

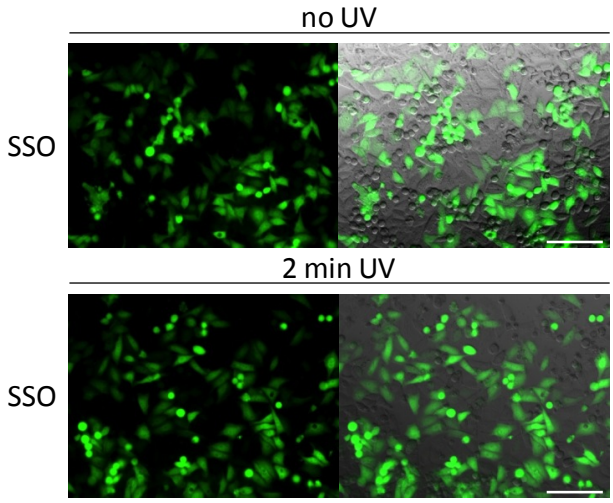
Supporting Table 1. Oligonucleotide sequences[1,2] used in the investigation of photochemically regulated splice-switching. All oligonucleotides contain phosphorothioate backbone linkages and 2'OMe modified nucleobases. Mismatched base pairs are shown as red. NPOM-caged 2'OMe uridines are in bold and indicated with an asterisk (*). Light-cleavable linker substitutions are indicated with a dash (-).



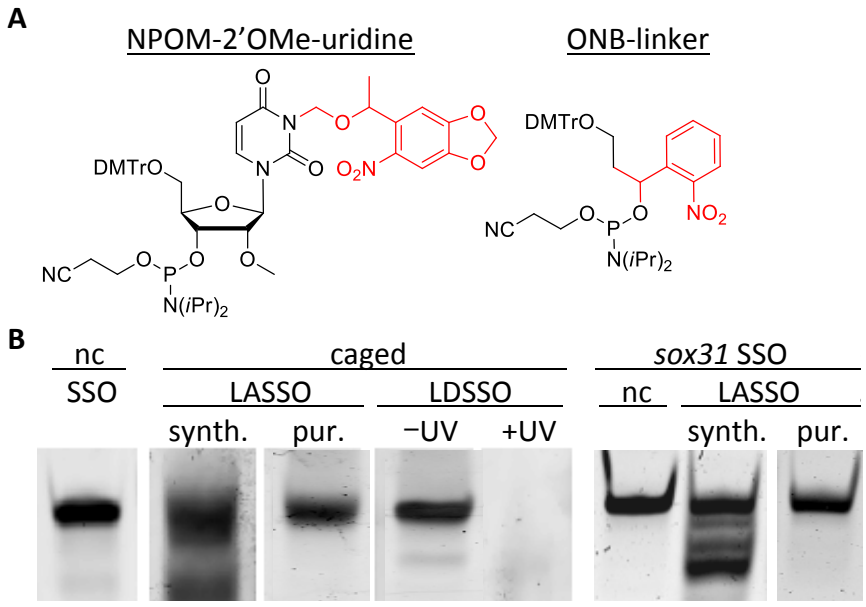
Supporting Figure 1. Schematic of splice-switching oligonucleotide function for pre-mRNA processing and regulation of gene expression through alternative splicing (AS). Active splicing pathways are indicated with solid black lines and AS pathways are indicated with dashed lines. The mutant intron is shown in red, and the activated SSO is shown in blue. A) In the absence of the SSO, a mutant intron from the pre-mRNA is present in the mRNA sequence and disrupts formation of a functional gene product. B) The SSO binds to the target site, blocks the spliceosome from interacting with the target site, and creates an AS pathway that will remove the mutant intron from the mRNA allowing for the expression of functional protein (e.g., EGFP).



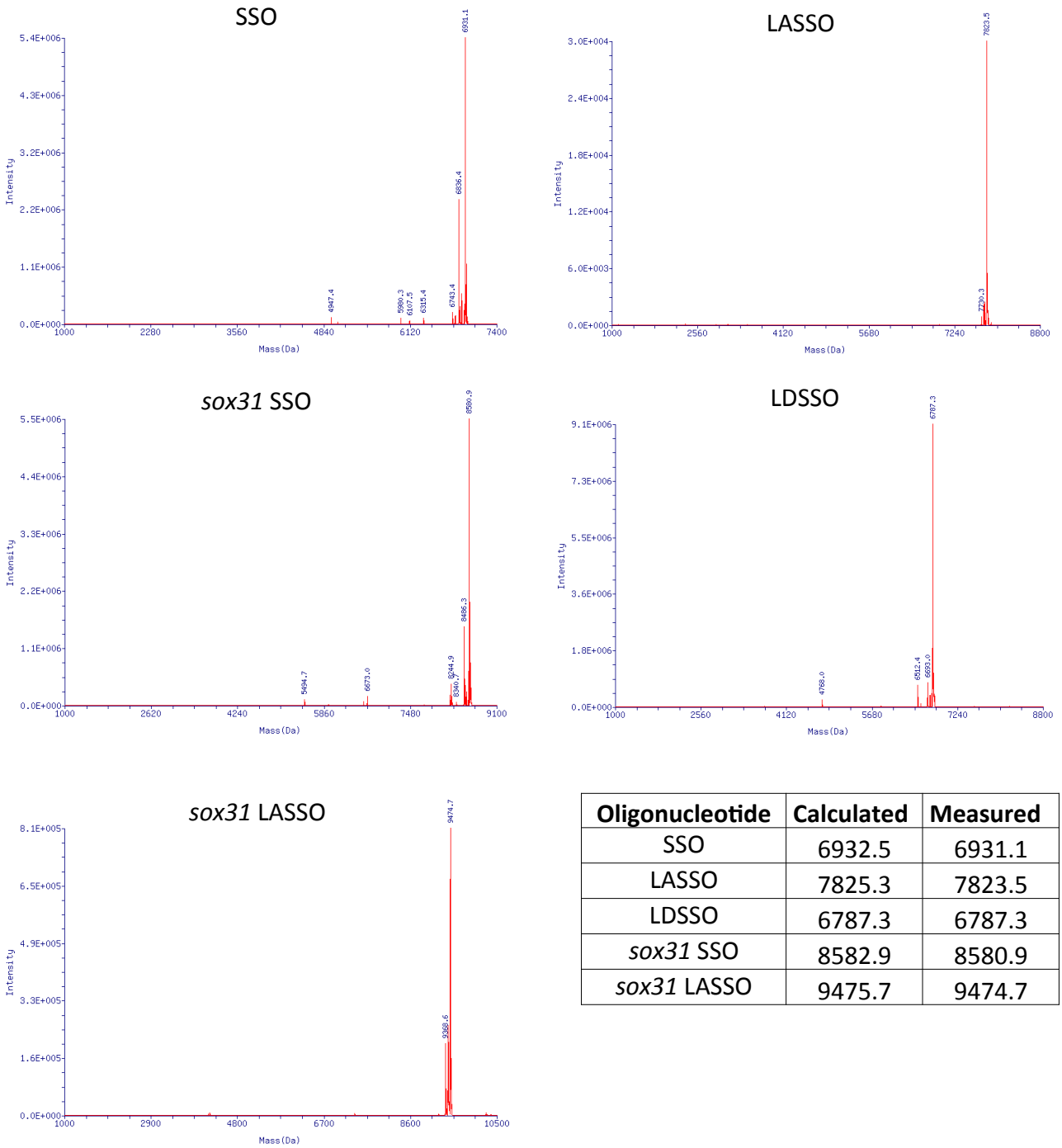
Supporting Figure 2. Confirmation of HeLa:EGFP654 reporter function using noncaged TAMRA labeled control oligonucleotides. HeLa:EGFP654 cells were passaged into 96-well plates and transfected with SSOs (50 nM) using X-tremeGENE siRNA reagent (Roche). The EGFP and TAMRA fluorescence were observed at 24 hours on 20X magnification. TAMRA fluorescence shows successful transfection of the labeled oligos. The negative control oligo (NEG) does not induce EGFP expression, while the noncaged SSO shows high levels of EGFP expression. NT = no oligo treatment. Scale bars indicate 0.2 mm.



Supporting Figure 3. Splice-switch driven EGFP expression with UV irradiation. HeLa:EGFP654 cells were transfected with the noncaged SSO (50 nM) using X-tremeGENE siRNA reagent (Roche), then UV irradiated on a transilluminator (365 nm, 25 W), and imaged for EGFP fluorescence on 20X magnification 24 hours after UV exposure. No significant decrease of EGFP expression is observed with exposure up to 2 minutes and the HeLa:EGFP654 reporter system for SSO activity is not responsive to UV irradiation in the absence of light-controlled SSOs. Scale bars indicate 0.2 mm.

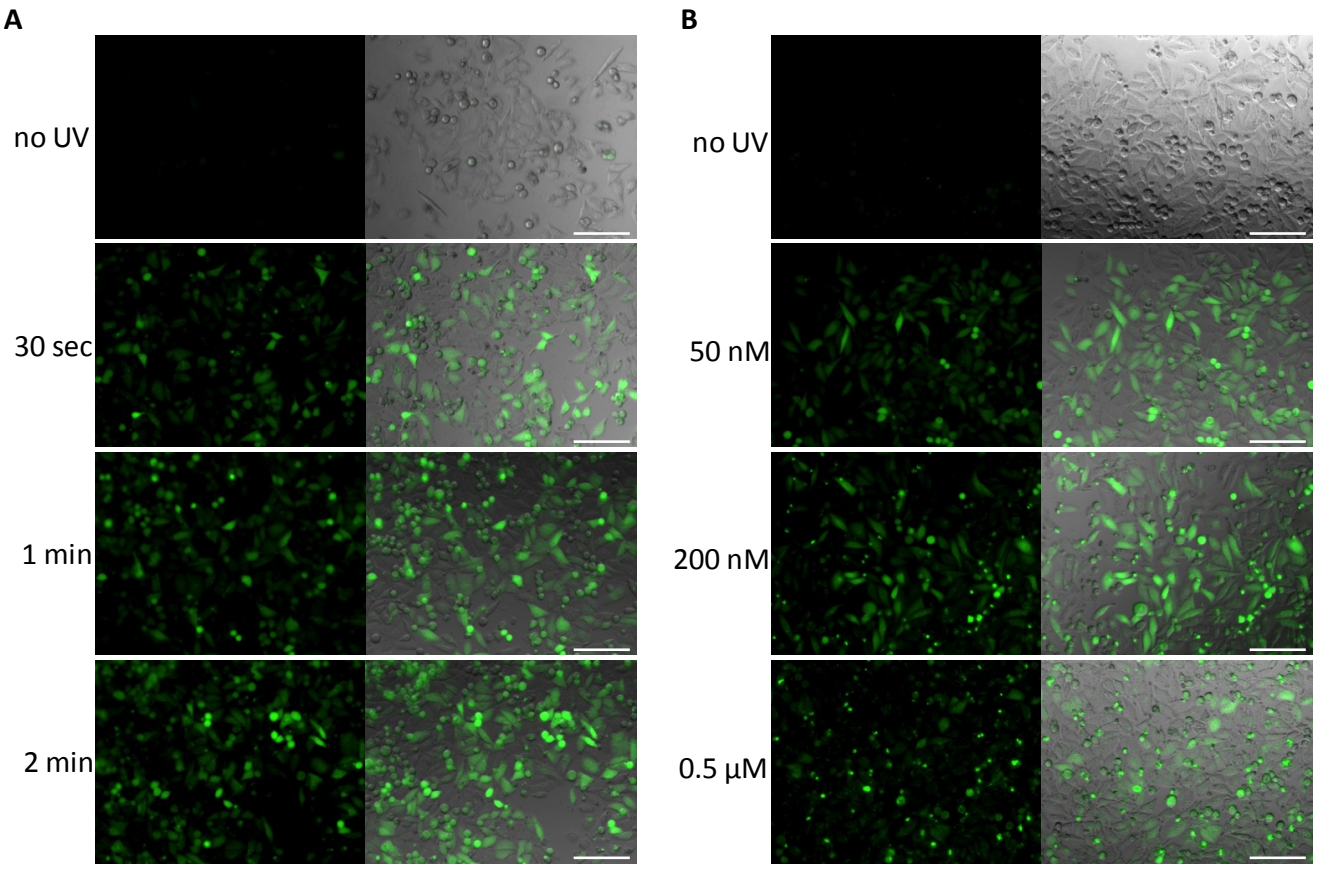


Supporting Figure 4. A) Chemical structures of the two specialized phosphoramidites used for the synthesis of photochemically regulated oligonucleotides. The NPOM caging group is removed with UV irradiation to restore native uridine and base hybridization, while the ONB linker is a photo-cleavable backbone linker that allows for UV-dependent oligonucleotide degradation. B) Gel analysis of 10 pmol of each oligonucleotide synthesis. All syntheses were performed on an ABI 394 DNA/RNA synthesizer. The abbreviation "nc" stands for noncaged. The LASSO synthesis contained major truncation products and required gel purification. The abbreviations "synth." and "pur." represent the synthesis and purification products, respectively. The LDSSO synthesis had significantly less truncation products and required no gel purification. Additionally, UV dependent cleavage of the full length LDSSO oligo was demonstrated with a 2 min irradiation (UV transilluminator, 365 nm, 25 W). The *sox31* LASSO synthesis also contained major truncation products and required gel purification.

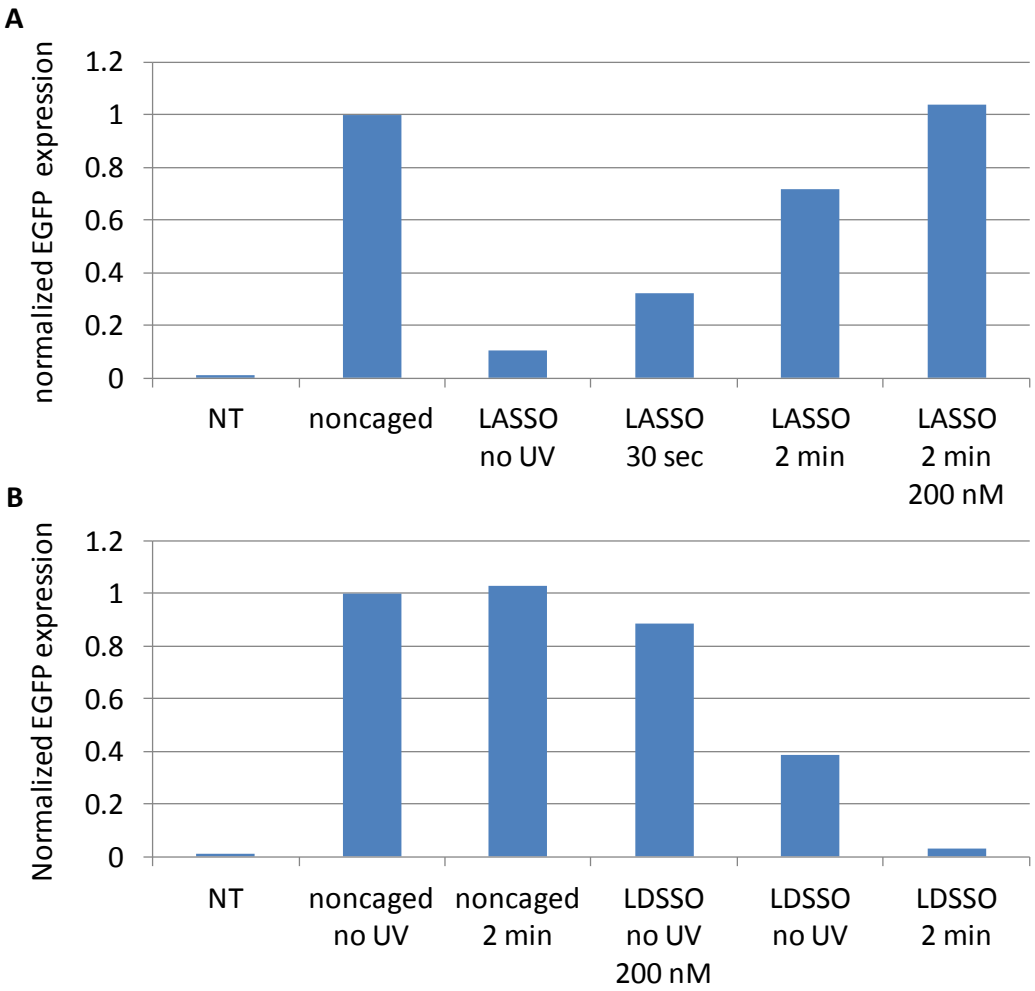


Oligonucleotide	Calculated	Measured
SSO	6932.5	6931.1
LASSO	7825.3	7823.5
LDSSO	6787.3	6787.3
sox31 SSO	8582.9	8580.9
sox31 LASSO	9475.7	9474.7

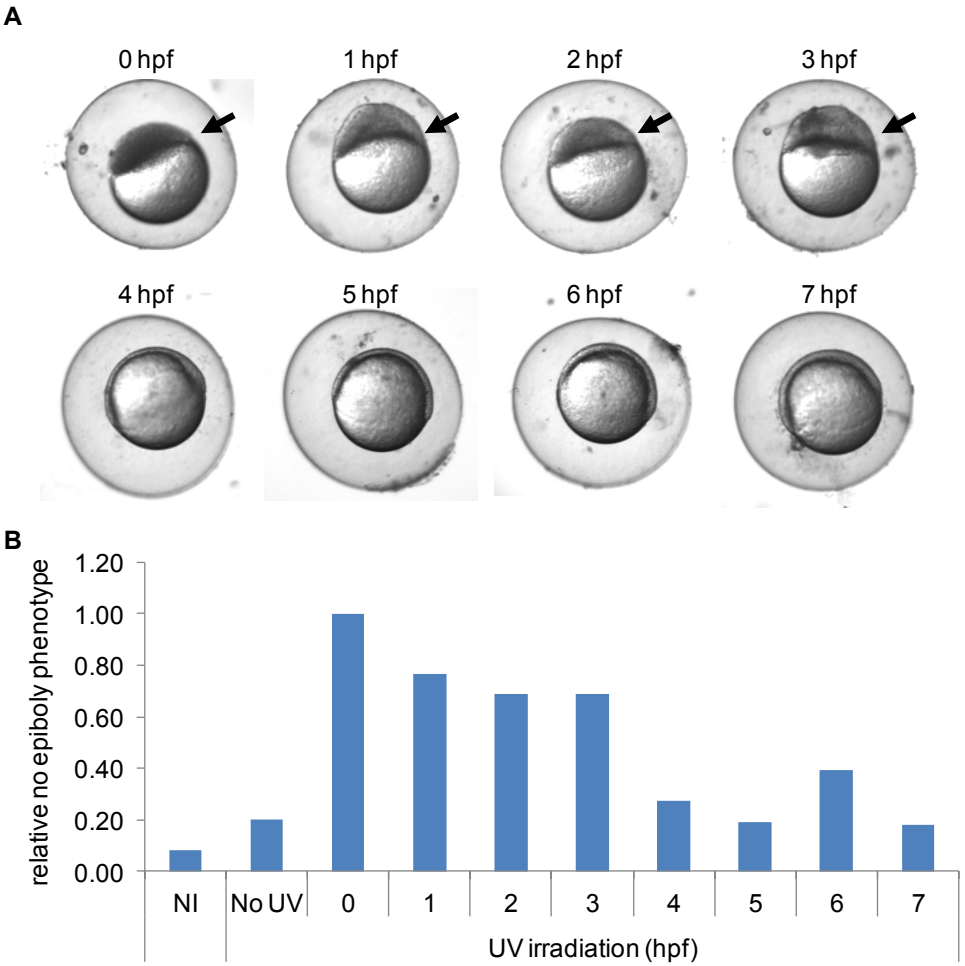
Supporting Figure 5. ESI mass spectra of synthesized oligonucleotides and tabulated results of calculated and measured masses (in Da). No photolysed products (loss of 1-4 NPOM groups or linker cleavage) were detected.



Supporting Figure 6. Optimization of photochemical LASSO activation in HeLa:EGFP654 cells. A) First, UV irradiation times were analyzed for increased EGFP expression with transfection of the LASSO (50 nM) using X-tremeGENE siRNA reagent (Roche), followed by different irradiation times (UV transilluminator, 365 nm, 25 W) and imaging after 24 hours (20X magnification). Two minutes of UV irradiation displayed the largest increase in EGFP expression. B) The 2 min exposure time was then used across a range of SSO transfection concentrations, and the highest intensity of EGFP expression was observed with a 200 nM oligonucleotide concentration. A higher concentration of the SSO (0.5 µM) increased cell death and was detrimental to splice-switching activity. Scale bars indicate 0.2 mm.



Supporting Figure 7. Optimization for flow cytometry quantification of photochemically regulated splice-switching with A) LASSO and B) LDSSO oligonucleotides. HeLa:EGFP654 cells were transfected in 6-well plates with the different SSOs (50 nM unless otherwise indicated), irradiated with a UV transilluminator (365 nm, 25 W), and analyzed for EGFP expression after 24 hours using a FACSCalibur (Becton-Dickinson) flow cytometer (20,000 gating events). The gated EGFP positive cells were normalized relative to the noncaged control. Nontreated cells (NT) have no background EGFP expression. The noncaged, positive control SSO shows high EGFP expression and is not detrimentally affected by a 2 minute UV exposure. The LASSO is inactive in the absence of UV irradiation, and (as expected) UV exposure activated EGFP expression – with longer irradiation times showing higher levels of EGFP expression. Activation optimizations for the LASSO, as demonstrate by cellular micrographs (**Supporting Figure 5**), were confirmed with 2 minutes UV at 200 nM exhibiting EGFP expression at levels similar to the noncaged control. The LDSSO was active in the absence of UV, with 200 nM showing similar levels of EGFP expression as the noncaged SSO control. The LDSSO splice-switch regulation is fully deactivated with UV irradiation as observed by the inhibition of EGFP expression after a 2 min light exposure.



Supporting Figure 8. *sox31* LASSO light-activation time points. Zebrafish embryos were injected with the *sox31* LASSO (5 ng) and UV irradiated (365 nm, 2 min) at 1-7 hpf. A) At 8 hpf, the embryos were imaged to demonstrate the majority phenotype and B) the frequency of embryos exhibiting a *no epiboly phenotype* was determined. N = 17-22.

REFERENCES

(1) Resina, S.; Kole, R.; Travo, A.; Lebleu, B.; Thierry, A. R. *J Gene Med* **2007**, *9*, 498.

(2) Hu, S. N.; Yu, H.; Zhang, Y. B.; Wu, Z. L.; Yan, Y. C.; Li, Y. X.; Li, Y. Y.; Li, Y. P. *FEBS Lett* **2012**, *586*, 222.