

Supplementary Information

“Photocontrol of CRISPR/Cas9 Function by Site-specific Chemical Modification of Guide RNA”

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Materials and Methods

Materials

All chemical reagents were purchased from Energy Chemical. T7 High Yield Transcription Kit, protein K, Cas9 proteinase and Hiscript® III RT SuperMix were purchased from Vazyme. Ni-NTA and Lipofectamine 3000 were purchased from Thermo Fisher. DNase I were purchased from NEB. Opti-men media and fetal bovine serum (FBS) were purchased from Gibco. All DNA sequences and the FAM-labeled DNA substrate used in this study were ordered from Sangon Biotech. The RNA phosphoramidites and CPG were purchased from DNA Chem.

Preparation of gRNA

gRNA was prepared by annealing of crRNA and tracrRNA. Modified or unmodified crRNA was chemically synthesized by a K&A H-8 DNA/RNA Synthesizer using universal CPG as the solid phase support. After the standard procedure for RNA synthesis, the RNA products were cleaved from CPG and treated with ammonium hydroxide (22%) solution at room temperature for 12 hr. After ethanol precipitation, the oligos were dissolved into the mixture of anhydrous DMSO and TEA·3HF (1:1.25(v/v)), and heated to 65 °C for 2.5 hr. Purification was performed on the Agilent 1260 system using the PLRP-S column (250 mm × 4.6 mm, 100Å, 5 μm). The mobile phase was 100 mM TEAA (triethylammonium acetate) with 50% CH₃CN as buffer A and 100 mM TEAA as buffer B. The oligonucleotides were purified using a gradient of 15-60% buffer A over 60 mins at a flow rate of 1mL/min. The purified product was then lyophilized and desalted by the Bio-Spin 6 column (Bio-Rad), and stocked in TEPC-treated water at -80 °C for following experiments.

The tracrRNA was in vitro transcribed by T7 RNA polymerase using the T7 High Yield Transcription Kit (Vazyme) according to manufacturer's instructions. Briefly, the duplex DNA construct containing the T7 promoter and coding sequence of tracrRNA was prepared and purified by commercial PCR kits. The in vitro transcription reaction

was performed by T7 polymerase and incubated for 12 hr at 37 °C. After reaction, the DNA template was digested by DNaseI, and the reaction mixture was subsequently processed by phenol extraction, followed by ethanol precipitation. The RNA product was desalted by the Bio-Spin 6 column (Bio-Rad), and stocked in TEPC-treated water at -80 °C for following experiments. To form the complete gRNA, the tracrRNA was annealed with the equal amount of modified or unmodified crRNA in the buffer (20 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA), and then ready for use in the biochemical and cellular experiments.

(d)Cas9 Protein purification

The coding sequence for Cas9 or dCas9 (D10A and H840A) was cloned into a pET28a expression vector to construct pET28a-dCas9 with an N-terminal hexa-histidine (6xHis) tag. The protein was expressed in *E. coli* strain BL21(DE3) similarly as described.¹ Briefly, BL21(DE3) transformed with pET28a-dCas9 was grown in LB medium supplemented with 50 µg/mL kanamycin at 37 °C to reach OD 0.6. Then, the temperature was cooled down to 16 °C, and the expression was induced with IPTG (0.25 mM) overnight (~16 hr). The protein was purified by Ni-NTA after lysis of cells in 20 mM Tris pH 7.4, 500 mM NaCl (supplemented with 10 mM β-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride). The purified protein was concentrated to ~20 µM in 20 mM Tris-HCl (pH 7.4) with 500 mM NaCl, 1 mM DTT and 5% Glycerol, flash-frozen in liquid nitrogen, and stored at -80 °C.

Gel shift assay for measurement of dCas9 binding ability

To check the binding ability of dCas9, the duplex DNA substrate (see table S1 for sequence information) was labeled by 5'-FAM to monitor the gel shift behavior in the presence of different gRNAs. For evaluation of binding behavior, the modified or unmodified gRNA (960 nM) was premixed with dCas9 protein(800 nM) before addition of FAM-dsDNA (100 nM) in the binding buffer (20 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA). The mixture was incubated at 37°C for 15 min, and then analyzed by electrophoresis in 5% polyacrylamide gel on ice, and imaged by Gel

Image System (Tanon 2500R). For concentration dependent analysis, different concentrations of dCas9 was premixed with 1.2-fold modified or unmodified gRNA before binding to the duplex DNA substrate as performed according to the procedure described above.

Measurement of the duplex melting temperature

To measure the thermostability of RNA/DNA hybrid, 5 μ M modified and unmodified RNA was firstly annealed with the 5 μ M complementary DNA to form the duplex structure. UV absorbance at the 260 nm was recorded by the Shimadzu 2600 UV-Vis spectrophotometer along with the increasing temperature from 25 to 75 $^{\circ}$ C. The heating rate was 0.5 $^{\circ}$ C/min. The measurement buffer was 20 mM sodium cacodylate (pH = 7.0) with 60 mM NaCl. To calculate the melting temperature (T_m), the change of absorbance was normalized, and the T_m values were determined by the temperature with 50% change of absorbance at 260 nm.

Biochemical cleavage assay for measurement of Cas9 nuclease activity

Cleavage assay was performed using a 2-kb PCR amplified DNA substrate. Briefly, 100 nM Cas9, 100 nM modified or unmodified gRNA, and 10 ng/ μ L DNA substrate were incubated in cleavage buffer (20 mM Tris-HCl, 100 mM KCl, 5 mM MgCl₂, 1 mM DTT, 0.1 mM EDTA) at 37 $^{\circ}$ C for designated time points. Reaction was quenched by addition of EDTA (0.5 M), and followed by treatment of proteinase K at 37 $^{\circ}$ C for 10 min. The cleaved product was analyzed by electrophoresis in 1% agarose gel, and imaged by Gel Image System (Tanon 2500R).

To quantify the cleavage rate, a single-exponential equation was utilized to fit the percentage of cleaved product (Eq. 1).

$$[\text{Product}] = A[1 - \exp(-kt)] + \text{Constant} \quad (1)$$

where A is the exponential phase amplitudes; k is the observed rate constant. Eq. 1 is used due to the concentration of Cas9 (100 nM) is much more excessive than the DNA substrate (\sim 7.5 nM, calculated from 10 ng/ μ L 2-kb PCR product).

Photocontrol of Cas9 binding and cleavage activity by caged gRNA

To check the effectiveness of photoactivation, the mixture of caged gRNA and (d)Cas9 was irradiated by 365 nm UV light (3.5 mW/cm^2) for 60 seconds in the binding or cleavage buffer using a UV LED source (uVision™ 365 High Intensity AC Series) before addition of the duplex substrate. The following binding or cleavage assays were performed as described above. For the time dependent decaging experiment, the mixture of caged gRNA and dCas9 were irradiated by 365 nm UV light for 15 s, 30 s, 45 s, and 60 s respectively, before the measurement of the following activity. For the photoactivation through the 405 nm visible light, the samples were irradiated by a 405 nm LED source (HTLD-S20- 100×130-405, 16 mW/cm^2) for designated time periods before the measurement of binding activity.

Cell viability assay

The effect of UV or 405 nm light on HEK-293T cells was determined by CCK-8 kits (Yeasen Biotech Co., Ltd, Shanghai, China) according to the manufacturer's instructions. Briefly, HEK-293T cells were seeded in 96-well plates at the density of 20000 cells/well with or without 100 pmol modified or unmodified RNA transfected. After 6 hours transfection, the cells were subject to 3 minutes of 365/405 nm irradiation by the UV (3.5 mW/cm^2) or 405 nm (16 mW/cm^2) transilluminator. Afterwards, the cells were further cultured for 12 hours, and then 10 μL CCK-8 solution were added to the medium and incubated at 37 °C for another 1 hour. The cells cultured through the same procedure without treatment of UV or 405 nm light were selected as comparison. The absorbance at 450 nm was measured by the Epoch 2 microplate spectrophotometer to determine the relative cell viability.

Gene activation by CRISPRa in HEK-293T cells

First, HEK-293T cells were packaged by lentivirus to generate the stably expressed CRISPRa cell line. Briefly, DNA sequence encoding dCas9 (D10A and H840A) with VP64-p65-Rta (VPR) and EGFP fused to its C-terminus was cloned into pHR-SFFV

(Addgene #79121) lentiviral vector to generate pHR-SFFV-dCas9-VPR-EGFP for constitutive expression of dCas9-VPR-EGFP. To obtain Viral production cells, 1,000,000 cells were seeded into 6-well plates before co-transfection of 1000 ng pMD2.G (Addgene #12259), 2000 ng pCMV-dR8.2 (Addgene #84550) and 3000 ng pHR-SFFVdCas9-VPR-EGFP using 16 μ L P3000 and 7 μ L Lipofectamine 3000. The transfection medium was then replaced by 2 ml fresh medium after 6 hr incubation. 24 hr later, the culture medium was collected and filtered to remove cells, and treated with 10 μ g/ μ L 1.6 μ L polybrene before infecting the freshly seeded 293T cells with a density of 20, 000 cells in 6-plate well. The infected cells were then collected and sorted by flow cytometer (Beckman moFlo XDP) to obtained 293T-dCas9-VPR cells.

HEK-293T cells were cultured in complete media, Gibco™ DMEM (Dulbecco's modification of Eagle medium), 10% (v/v) Gibco™ fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Invitrogen), at 37 °C in a 5% CO₂ incubator. Cells (2.5×10^5 per well) were seeded into 6-well plates, and immediately transfected with 5 μ L 20 μ M modified or unmodified gRNA targeting the ASCL1 or CXCR4 gene using 5.5 μ L Lipofectamine 3000 mixed in 500 μ L Opti-men media (Gibco). The medium was then replaced with a complete DMEM after 6 hr incubation, and the cells were subject to a 3-minute irradiation of 365 nm by the UV (3.5 mW/cm²) or 405 nm (16 mW/cm²) transilluminator. Total RNA from 293T cells was isolated using trizol, chloroform, isopropanol and ethyl alcohol after another 12 hr culture.

RNA extraction and real-time quantitative PCR

To quantify mRNA expression level, 1 μ g total RNA was used to generate cDNA using Hiscript® III RT SuperMix for qPCR according to manufacturer's instructions. Expression levels of GAPDH and target gene ASCL1 and CXCR4 were detected by CFX96 Real-Time System (Bio-Rad) using iTaq™ Universal SYBR® Green Supermix and specific primers (for ASCL1 gene, the primers were 5'-GGAGCTTCTCGACTTCACCA-3' and 5'-AACGCCACTGACAAGAAAGC-3'; for CXCR4 gene, the primers were 5'-CCCTTGGAGTGTGACAGCTT-3' and 5'-

TTGTGGGTGGTTGTGTTCCA-3'), with the following PCR cycle: 95 °C, 10 min; 40 cycles of 95 °C, 15 s and 61 °C, 1 min; 65 °C to 95 °C, 0.5 °C/s. GAPDH was used as the internal control, and the data were normalized to the expression of GAPDH. Relative gene expression was calculated using the Delta-Delta-Ct (ddCt) algorithm.

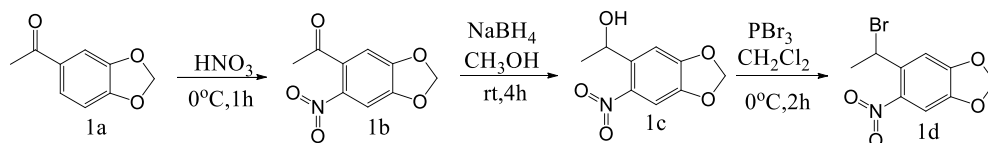
Measurement of average GFP fluorescence by flow cytometry

HEK-293T cells (2.5×10^5 per well) were seeded into 6-well plates and subsequently transfected with 2 μ g of the d2EGFP-Cas9-mCherry plasmid using Lipofectamine 3000 and P3000. Cells were further cultured for 12 hr to allow the expression of GFP, Cas9 and mCherry. Thereafter, the cultured cells were transfected with 5 μ L 20 μ M modified or unmodified gRNA targeting GFP gene using Lipofectamine 3000 and Opti-men media (Gibco). The medium was then replaced with a complete DMEM after 6 hr incubation, and the cells were subject to a 3-minute irradiation of 365 nm by the UV transilluminator (3.5 mW/cm²). Cells were further cultured for 24 hr and collected for the flow cytometer analysis.

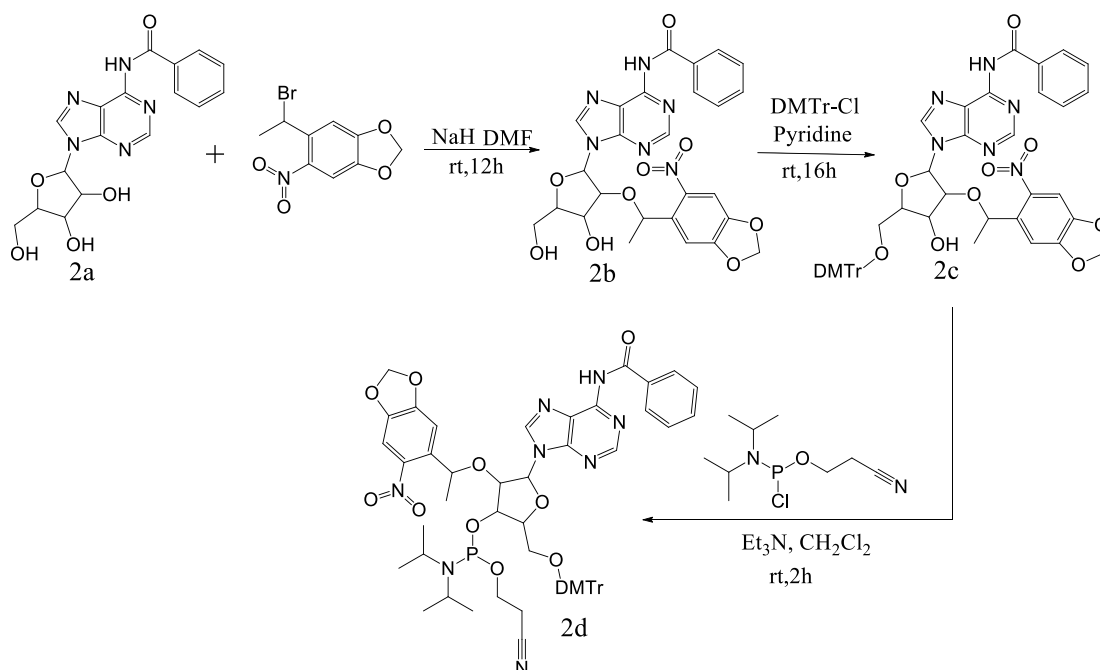
To perform the flow cytometer analysis, cells were centrifuged at 1000 g for 3 min, and resuspended in 1 \times PBS that did not contain calcium or magnesium. ZE5 Cell Analyzer (Bio-rad) was used for fluorescence-activated cell sorting (FACS) analysis with the following settings. The fluorescence signal of GFP was measured using a 488 nm laser and a 530/30 nm filter with a photomultiplier tube (PMT) set at 545 V. The red fluorescence of mCherry was measured with a 561 nm laser and a 670/30 nm filter using a PMT set at 350 V. For each sample, $\sim 2 \times 10^5$ cell events were collected. The average GFP fluorescence was determined from the mCherry positive cells.

Synthesis of rA^{NPOM} (Nitropiperonyloxymethylene) phosphoramidite^{2,3}

Scheme 1



Scheme 2



Compound 1b

3',4'-(Methylenedioxy)acetophenone (1.64 g, 10 mmol, 1.0 eq.)(1a) was dissolved in nitric acid (10 mL, 60~80%) and keep the solution in an ice bath(approximately 0–5 °C) . The reaction was stirred for an additional 1h at 0 °C. The mixture was extracted with CH₂Cl₂ (3×). The combined organic layers were washed with sat. NaHCO₃ , dried over sodium sulfate and concentrated under reduced pressure. The residue was purified via flash column chromatography (SiO₂, 1:4 EtOAc/hexanes) to give the desired product as a light yellow solid (1.78 g, 85%).¹H NMR (400 MHz, CDCl₃) δ 7.56 (s, 1H), 6.77 (s, 1H), 6.20 (s, 2H), 2.51 (s, 3H).

Compound 1c

4',5'-Methylenedioxy-2'-nitroacetophenone (1.05 g, 5 mmol, 1.0 eq.) (1b) was dissolved in CH₃OH (70 mL) and sodium borohydride (380 mg, 10 mmol, 2.0 eq.) was added. The resultant suspension was stirred 4h at room temperature and concentrated under reduced pressure. The mixture was poured into brine, the organic components were extracted with CH₂Cl₂ (3×) and the combined organic layers were dried over sodium sulfate before being concentrated under reduced pressure. The residue was purified via flash column chromatography (SiO₂, 1:3 EtOAc/hexanes) to give the desired product as a yellow solid (0.95 g, 90%) ¹H NMR (400 MHz, CDCl₃) δ 7.47 (d, J = 12.0 Hz, 1H), 7.27 (s, 1H), 6.13 (d, J = 3.2 Hz, 2H), 5.45 (q, J = 6.2 Hz, 1H), 1.53 (d, J = 6.3 Hz, 3H).

Compound 1d

In a round-bottom flask, dissolve 2-nitrobenzylethanol (1.05 g, 5 mmol, 1.0 eq.) (1c) in anhydrous CH₂Cl₂ (10 mL) under nitrogen on an ice bath (approximately 0–5 °C). The phosphorus tribromide (238 μL, 2.5 mmol, 0.5 eq.) was added dropwise. It was stirred at room temperature for 2 h. It was quenched with ice and poured into brine. It was extracted with CH₂Cl₂ (3×), dried over sodium sulfate and concentrated under reduced pressure. The residue was purified via flash column chromatography (SiO₂, 1:5 EtOAc/hexanes) to give the desired product as yellow solid (1.1 g, 80%). ¹H NMR (400 MHz, CDCl₃) δ 7.92 (dd, J = 8.6, 2.3 Hz, 1H), 7.70 (d, J = 2.3 Hz, 1H), 7.37 (s, 1H), 7.29 (d, J = 2.1 Hz, 2H), 6.90 (d, J = 8.6 Hz, 1H), 6.19 – 6.13 (m, 3H), 5.92 (q, J = 6.8 Hz, 1H), 2.06 (d, J = 6.8 Hz, 3H).

Compound 2b/2c

Dissolve *N*⁶-benzoyladenine (372 mg, 1 mmol) in 5 ml dry DMF in a round-bottom flask in an ice bath (approximately 0–5 °C). To the stirring solution, add sodium hydride (225 mg, 60% (w/v) in oil). Stir the resulting solution for 45 min under nitrogen. Add 410 mg (1.5 mmol) of 2-nitrobenzylbromide (*o*-nitro-BnBr) (1 d) in 2 ml of dry DMF

using a syringe. Stir the reaction mixture at room temperature under nitrogen for 12 h. It was extracted with EtOAc (3×), dried over sodium sulfate. Collect the resulting yellow precipitate by vacuum filtration and dry in vacuo. The yield is assumed to be quantitative for the next step in the preparation. Dissolve the yellow precipitate, and 12 mg (0.1 mol%) of DMAP and 355 mg (1.1 mmol) of DMTrCl in 5 ml of dry pyridine. Stir the reaction mixture at room temperature for 12 h under nitrogen. The residue was purified via flash column chromatography (SiO₂, 1:2 EtOAc/hexanes) to give the desired product as a light yellow solid (113 mg, 20%). *m/z* calcd. [M+H]⁺ 867.29, found 867.27. ¹H NMR (400 MHz, CDCl₃) δ 9.26 (d, *J* = 17.5 Hz, 1H), 8.53 (s, 1H), 8.06 (d, *J* = 6.1 Hz, 3H), 7.61 (t, *J* = 7.3 Hz, 1H), 7.53 (t, *J* = 7.4 Hz, 2H), 7.40 (d, *J* = 7.5 Hz, 2H), 7.30 (d, *J* = 8.7 Hz, 4H), 7.26 – 7.20 (m, 4H), 6.84 – 6.77 (m, 4H), 6.57 (s, 1H), 6.07 (d, *J* = 6.8 Hz, 2H), 5.91 (s, 1H), 5.37 (q, *J* = 6.0 Hz, 1H), 4.94 (dd, *J* = 15.3, 9.6 Hz, 1H), 4.49 (d, *J* = 10.4 Hz, 1H), 4.32 (s, 1H), 3.83 – 3.77 (m, 6H), 3.75 (d, *J* = 9.1 Hz, 1H), 3.46 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.36 (dd, *J* = 10.4, 4.0 Hz, 1H), 3.09 (s, 1H), 1.55 (dd, *J* = 16.4, 6.2 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 164.62 (s), 158.55 (s), 152.53 (s), 151.92 (s), 151.35 (s), 149.47 (s), 147.18 (s), 144.32 (s), 142.15 (s), 141.74 (s), 135.65 (d, *J* = 11.0 Hz), 133.56 (s), 132.89 (s), 129.98 (d, *J* = 6.6 Hz), 128.91 (s), 128.36 – 128.31 (m), 128.31 – 127.76 (m), 126.97 (s), 123.28 (s), 113.17 (s), 105.47 (s), 104.37 (s), 103.25 (s), 86.59 (d, *J* = 13.2 Hz), 84.85 (s), 80.23 (s), 74.81 (s), 70.18 (s), 63.30 (s), 55.24 (s), 30.21 (s), 23.81 (s).

Compound 2d

Dissolve 2c (434 mg 0.6 mmol) in dry CH₂Cl₂ (5 ml) under nitrogen in round-bottom flask. Add diisopropylethylamine (0.53 ml, 3 mmol) and 2-cyanoethyl-*N,N*-diisopropylaminochlorophosphite (0.2 ml, 1.3 mmol) to the reaction using a syringe and allow the reaction to proceed for 2 h under nitrogen. The residue was purified via flash column chromatography (SiO₂, 1:4 EtOAc/hexanes, Et₃N 1%) to give the desired product as a light yellow solid (480 mg, 75%). *m/z* calcd. [M+Na]⁺ 1089.40, found 1089.42. ¹H NMR (400 MHz, CDCl₃) δ 9.23 (s, 1H), 8.50 (d, *J* = 13.4 Hz, 1H), 8.14 (s, 1H), 8.06 (d, *J* = 7.2 Hz, 2H), 7.63 (t, *J* = 7.1 Hz, 1H), 7.54 (t, *J* = 7.4 Hz, 2H), 7.42 (d,

$J = 7.3$ Hz, 2H), 7.31 (d, $J = 8.4$ Hz, 4H), 7.27 – 7.21 (m, 4H), 6.80 (d, $J = 7.9$ Hz, 4H), 6.65 (d, $J = 13.7$ Hz, 1H), 6.15 (d, $J = 5.4$ Hz, 1H), 6.10 (s, 1H), 5.95 (s, 1H), 5.43 (q, $J = 5.8$ Hz, 1H), 4.67 (t, $J = 4.8$ Hz, 1H), 4.61 (d, $J = 12.0$ Hz, 1H), 4.40 (d, $J = 3.4$ Hz, 1H), 3.97 (dt, $J = 14.6, 7.2$ Hz, 1H), 3.87 (d, $J = 6.6$ Hz, 1H), 3.80 (s, 6H), 3.68 (dd, $J = 16.4, 6.8$ Hz, 2H), 3.50 (dd, $J = 10.3, 3.8$ Hz, 1H), 3.35 (dd, $J = 10.4, 4.3$ Hz, 1H), 2.66 (t, $J = 5.9$ Hz, 2H), 1.52 (d, $J = 6.1$ Hz, 3H), 1.24 (d, $J = 6.5$ Hz, 6H), 1.14 (d, $J = 6.7$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 158.49 (s), 152.21 (d, $J = 32.2$ Hz), 151.10 (s), 149.46 (s), 147.10 (s), 144.31 (s), 142.37 (d, $J = 5.2$ Hz), 136.21 (s), 135.64 (s), 133.73 (s), 132.81 (s), 130.07 (s), 128.89 (s), 128.24 (s), 127.82 (s), 126.89 (s), 123.27 (s), 117.62 (s), 113.12 (s), 105.85 (s), 104.41 (s), 103.14 (s), 87.63 (s), 86.55 (s), 83.85 (s), 78.31 (s), 73.47 (s), 71.08 (d, $J = 15.7$ Hz), 62.61 (s), 60.40 (s), 58.42 (d, $J = 18.5$ Hz), 55.21 (s), 43.15 (d, $J = 12.4$ Hz), 31.52 (s), 30.14 (s), 24.63 (d, $J = 6.6$ Hz), 23.48 (s), 21.05 (s), 20.29 (d, $J = 6.6$ Hz), 14.21 (s). ^{31}P NMR (162 MHz, CDCl_3) δ 149.22 – 148.68 (m), 9.66 (d, $J = 12.1$ Hz).

Supporting Figures

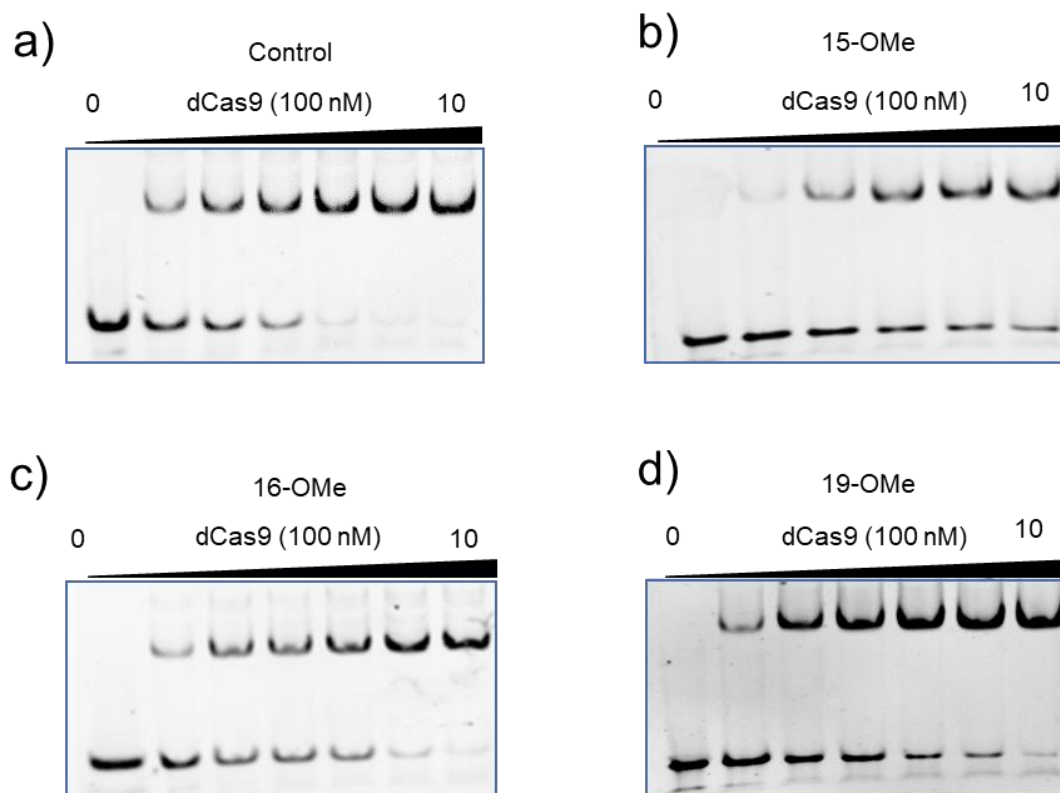


Figure S1. Binding abilities of dCas9 with unmodified (**a**), 15-methoxy (**b**), 16-methoxy (**c**), and 19-methoxy (**d**) modified gRNA. The concentrations of dCas9 in **a**, **c** and **d** were 0, 200, 300, 400, 600, 800, and 1000 nM, respectively; the concentrations in **b** were 0, 200, 400, 600, 800, and 1000 nM, respectively.

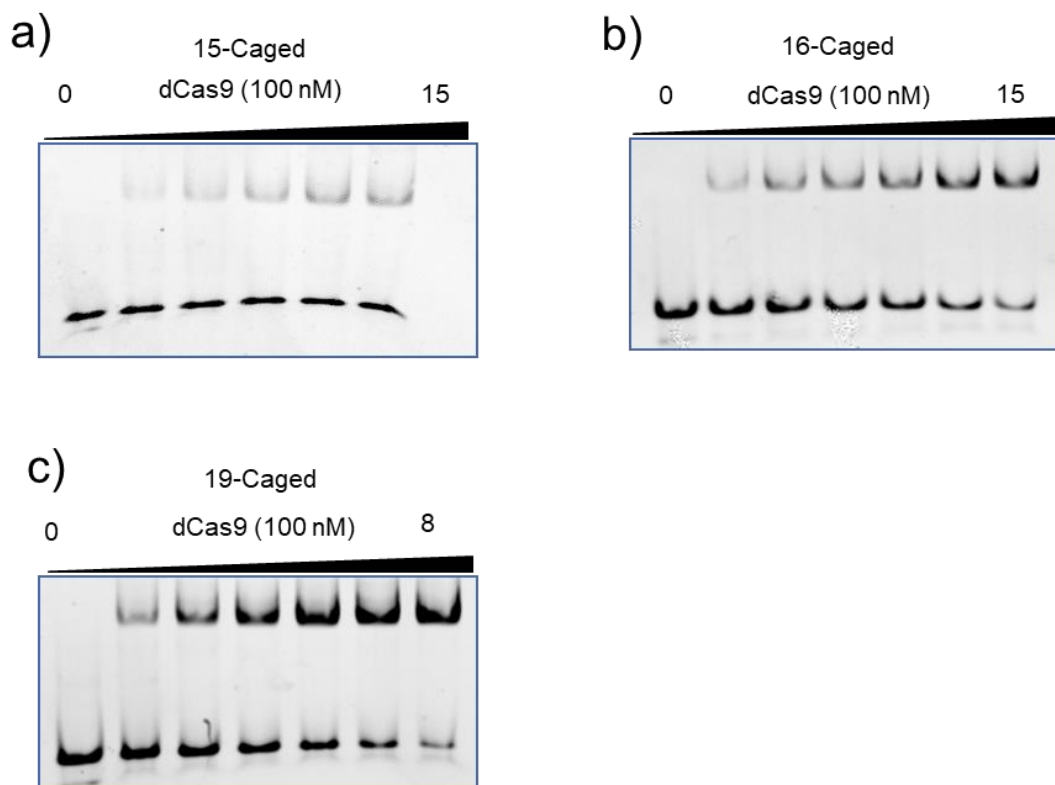


Figure S2. Binding abilities of dCas9 with 15-caged (a), 16-caged (b), and 19-caged (c) gRNA. a) The concentrations of dCas9 were 0, 300, 600, 900, 1200, and 1500 nM for the 15-caged gRNA, respectively. b) The concentrations of dCas9 were 0, 300, 600, 900, 1200, and 1500 nM for the 16-caged gRNA, respectively. c) The concentrations of dCas9 were 0, 200, 300, 400, 500, 600, 700 and 800 nM for the 19-caged gRNA, respectively.

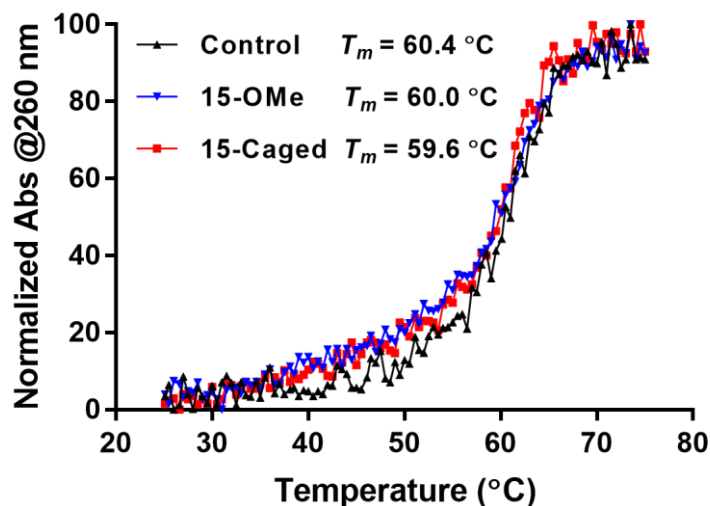


Figure S3. The duplex stability of RNA/DNA hybrid with different modifications at the 2'-OH of RNA. The sequence was selected from the target region (20 nt) of the gRNA Seq-ASCL1 as described in Figure 1b. The control sample was the unmodified RNA/DNA hybrid; the 15-OMe sample represented the duplex with substitution of 2'-OH by the methoxy group at the 15th position of RNA; the 15-Caged sample represented the duplex with modification of the ribose by the photolabile group at the 15th position of RNA.

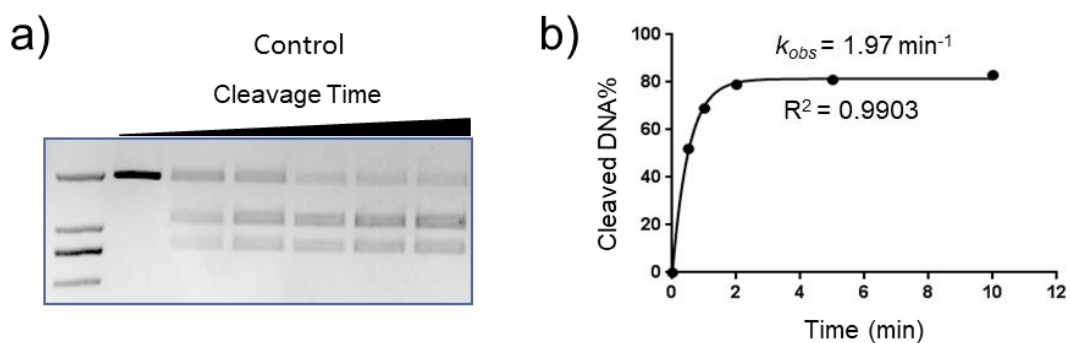


Figure S4. Cleavage activities of Cas9 with unmodified gRNA. **a)** The cleavage gel for unmodified gRNA. Time points were 0, 0.5, 1, 2, 5, and 10 min, respectively. The control samples indicated the cleavage activity of Cas9 with unmodified gRNA. The length of duplex DNA substrate was 2 kb, and the cleaved products were 1.2 kb and 0.8 kb dsDNA, respectively. **b)** The observed kinetic rate for cleavage activity with unmodified gRNA.

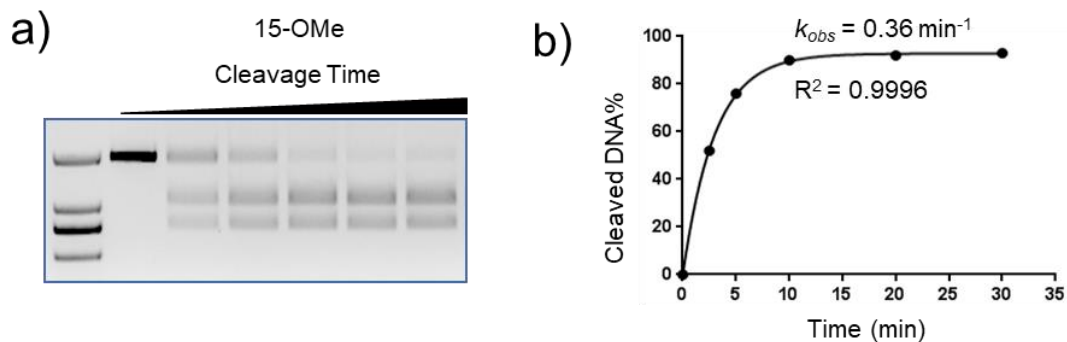


Figure S5. Cleavage activities of Cas9 with 15-methoxy modified gRNA. **a)** The time points were 0, 2.5, 5, 10, 20 and 30 min, respectively. **b)** The observed kinetic rate for cleavage activity with 15-methoxy modified gRNA.

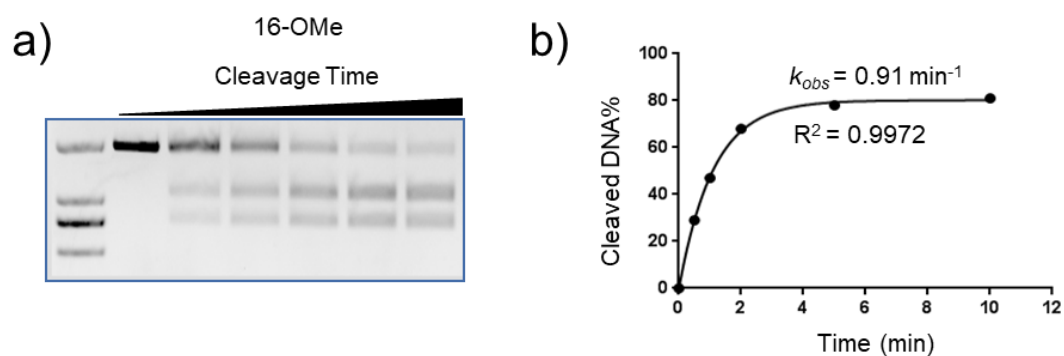


Figure S6. Cleavage activities of Cas9 with 16-methoxy modified gRNA. **a)** The time points were 0, 0.5, 1, 2, 5 and 10 min, respectively. **b)** The observed kinetic rate for cleavage activity with 16-methoxy modified gRNA.

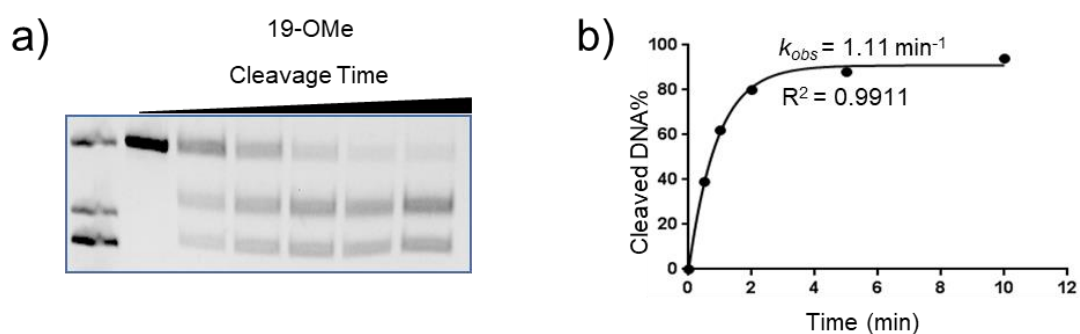


Figure S7. Cleavage activities of Cas9 with 19-methoxy modified gRNA. **a)** The time points were 0, 0.5, 1, 2, 5, and 10 min, respectively. **b)** The observed kinetic rate for cleavage activity with 19-methoxy modified gRNA.

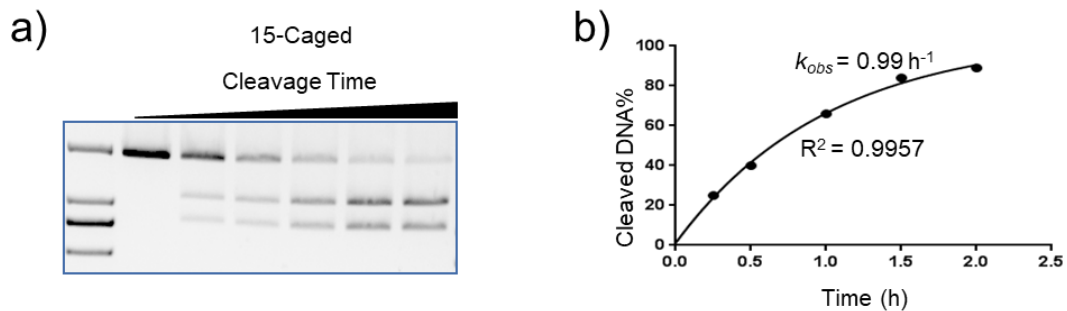


Figure S8. Cleavage activities of Cas9 with 15-Caged gRNA. **a)** The time points were 0, 0.25, 0.5, 1, 1.5 and 2 h, respectively. **b)** The observed kinetic rate for cleavage activity with 15-Caged gRNA.

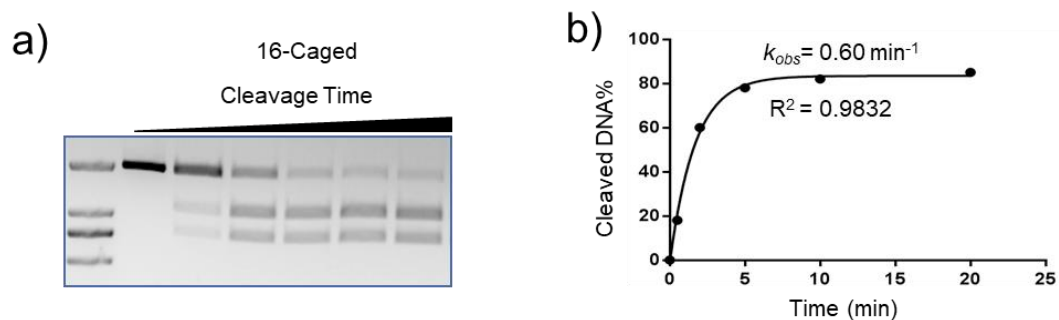


Figure S9. Cleavage activities of Cas9 with 16-Caged gRNA. **a)** The time points were 0, 0.5, 2, 5, 10 and 20 min, respectively. **b)** The observed kinetic rate for cleavage activity with 16-Caged gRNA.

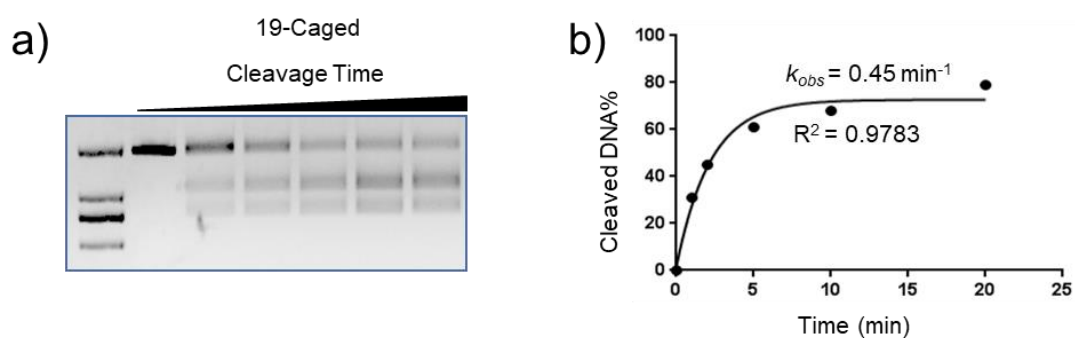


Figure S10. Cleavage activities of Cas9 with 19-Caged gRNA. **a)** The time points were 0, 1, 2, 5, 10 and 20 min, respectively. **b)** The observed kinetic rate for cleavage activity with 19-Caged gRNA.

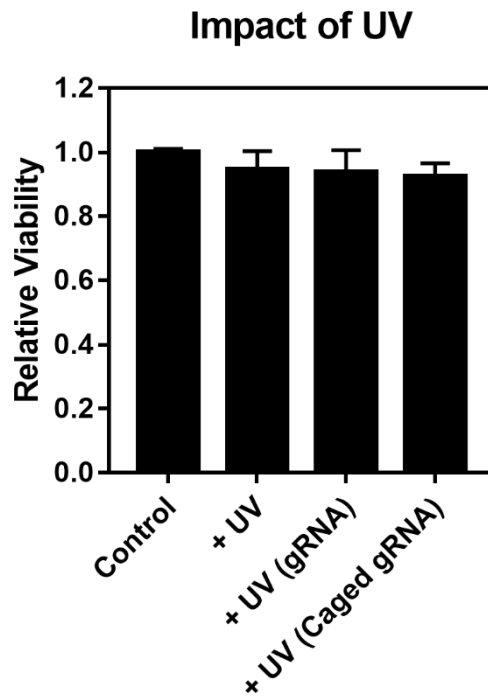


Figure S11. Impact of UV light on the cell viability. The control sample indicated the HEK-293T cells without treatment of UV light; the “+ UV” sample indicated the HEK-293T cells with 3-min UV light exposure (3.5 mW/cm^2). The rest two samples indicated the HEK-293T cells with 3-min UV light exposure after transfection of gRNA or caged gRNA with comparison of unirradiated samples. The 3-min UV exposure caused a slightly negative impact on the cell viability. Error bars indicated the standard deviations derived from three independent biological replicates.

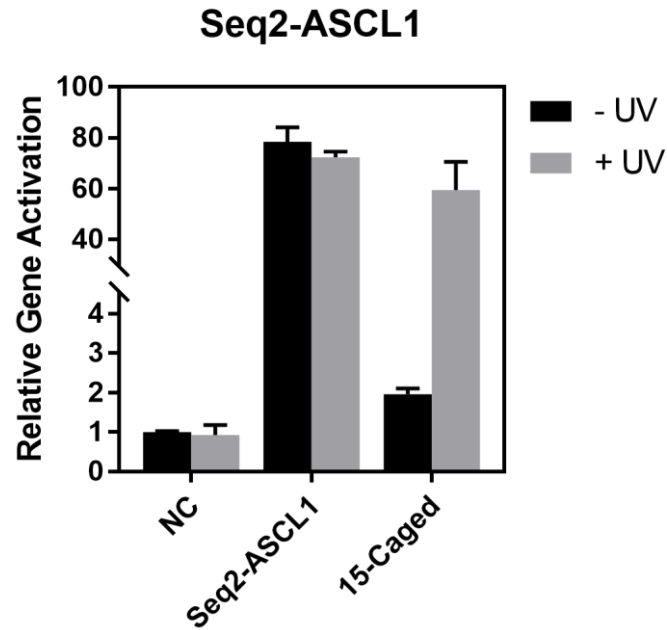


Figure S12. Optical control of CRISPR activation by 15th-caged gRNA to target another different sequence (Seq2-ASCL1) for regulation of ASCL1 gene. The control sample (Seq2-ASCL1) indicated the unmodified gRNA; “NC” indicated the effect of gRNA that cannot target any genes in HEK293T cells. The light-irradiation caused the effect of transcriptional activation changing from ~2-fold to ~60-fold with a ~30-fold dynamic range for the 15th-caged gRNA. The guide region for Seq2-ASCL1 was 5'-GGGGGAGUUUAGGGAGUGGG-3'. Error bars indicated the standard deviations derived from three independent biological replicates.

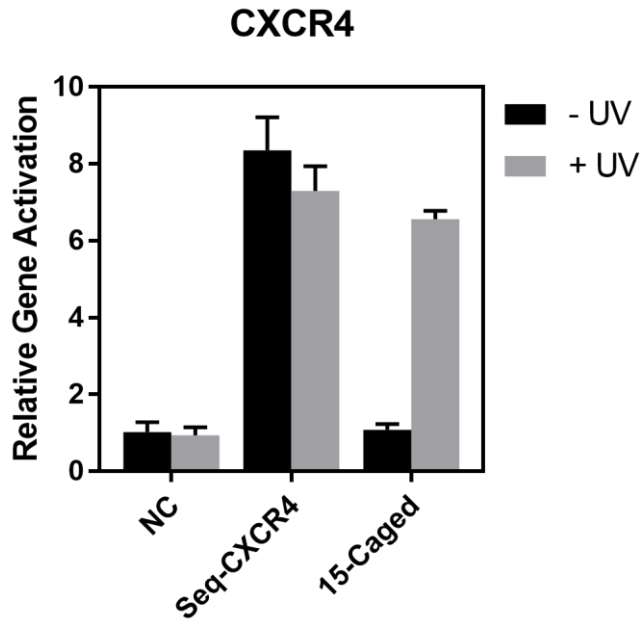


Figure S13. Optical control of CRISPR activation by 15th-caged gRNA to target the CXCR4 gene. The control sample (Seq-CXCR4) indicated the unmodified gRNA; “NC” indicated the effect of gRNA that cannot target any genes in HEK293T cells. Notably, the CRISPR activation effect for this CXCR4 gene was less efficient than the ASCL1 gene, but the 15th-caged gRNA could still achieve an efficient optical control of gene activation. The guide region for Seq-CXCR4 was 5'-UGGGGCGGGGAGGGAGAAGG-3'. Error bars indicated the standard deviations derived from three independent biological replicates.

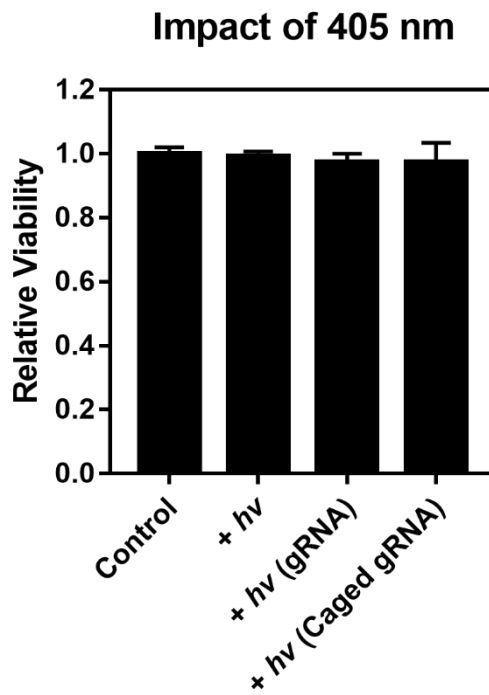


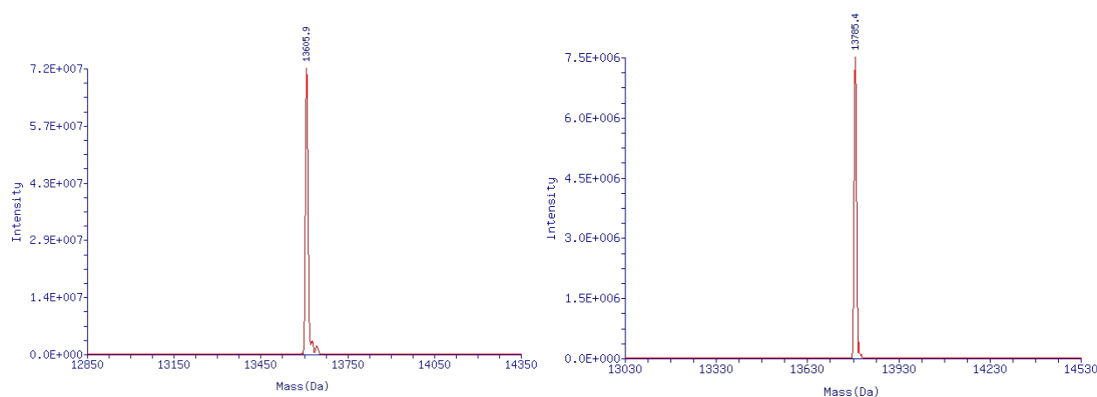
Figure S14. Impact of 405 nm light on the cell viability. The control sample indicated the HEK-293T cells without treatment of 405 nm light; the “+ $h\nu$ ” sample indicated the HEK-293T cells with 3-min 405 nm light exposure (16 mW/cm^2). The rest two samples indicated the HEK-293T cells with 3-min 405 nm light exposure after transfection of gRNA or caged gRNA with comparison of unirradiated samples. The 3-min 405 nm light exposure caused a negligible effect on the cell viability. Error bars indicated the standard deviations derived from three independent biological replicates.

Table for sequence information

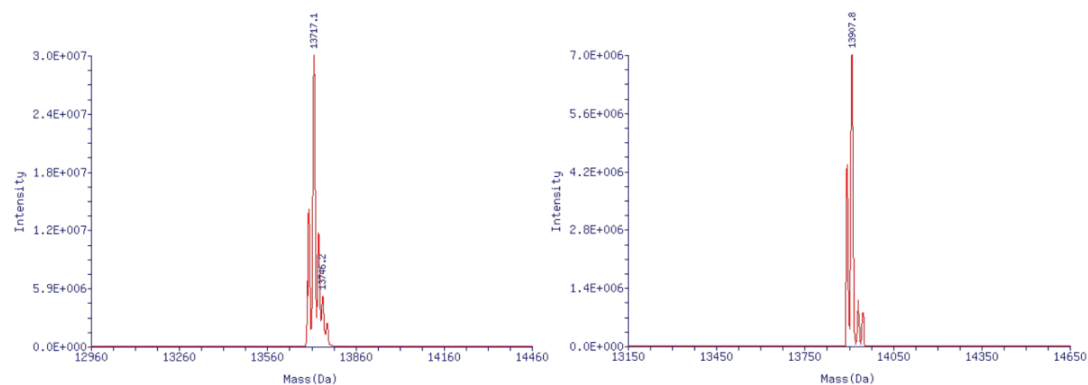
Table S1. RNA and DNA sequences used in this study.

Name	Sequence (5'-3')	
RNA Sequences related to Seq-ASCL1	Unmodified crRNA	AAU GGA GAG UUU GCA AGG AG GUU UAA GAG CUA UGC UGU UUU G
	13-OMe	AAU GGA GAG UUU G(OMe) CA AGG AG GUU UAA GAG CUA UGC UGU UUU G
	14-OMe	AAU GGA GAG UUU GC(OMe) A AGG AG GUU UAA GAG CUA UGC UGU UUU G
	15-OMe	AAU GGA GAG UUU GCA (OMe) AGG AG GUU UAA GAG CUA UGC UGU UUU G
	16-OMe	AAU GGA GAG UUU GCA A(OMe) GG AG GUU UAA GAG CUA UGC UGU UUU G
	17-OMe	AAU GGA GAG UUU GCA AG(OMe) G AG GUU UAA GAG CUA UGC UGU UUU G
	18-OMe	AAU GGA GAG UUU GCA AGG(OMe) AG GUU UAA GAG CUA UGC UGU UUU G
	19-OMe	AAU GGA GAG UUU GCA AGG A(OMe) G GUU UAA GAG CUA UGC UGU UUU G
	20-OMe	AAU GGA GAG UUU GCA AGG AG(OMe) GUU UAA GAG CUA UGC UGU UUU G
	15-Caged	AAU GGA GAG UUU GCA(NPOM) AGG AG GUU UAA GAG CUA UGC UGU UUU G
	16-Caged	AAU GGA GAG UUU GCA A(NPOM) GG AG GUU UAA GAG CUA UGC UGU UUU G
	19-Caged	AAU GGA GAG UUU GCA AGG A(NPOM) G GUU UAA GAG CUA UGC UGU UUU G
DNA sequences used for binding analysis	FAM-GGG CCT TGC AGT GGG CGC AAT GGA GAG TTT GCA AGG AGC GGC GCT TTG GT CG GCA TGG C	
	GCC ATG CC GAC CAA AGC GCC GCT CCT TGC AAA CTC TCC ATT GCG CCC ACT GCA AGG CCC	
The crRNA sequence for Seq2-ASCL1	GGGGGAGUUUAGGGAGUGGG GUU UAA GAG CUA UGC UGU UUU G	
The crRNA sequence for the CXCR4 gene	UGGGGCGGGGAGGGAGAAGG GUU UAA GAG CUA UGC UGU UUU G	
The crRNA sequence for the dsGFP gene	CAGCGUGUCCGGCGAGGGCG GUU UAA GAG CUA UGC UGU UUU G	

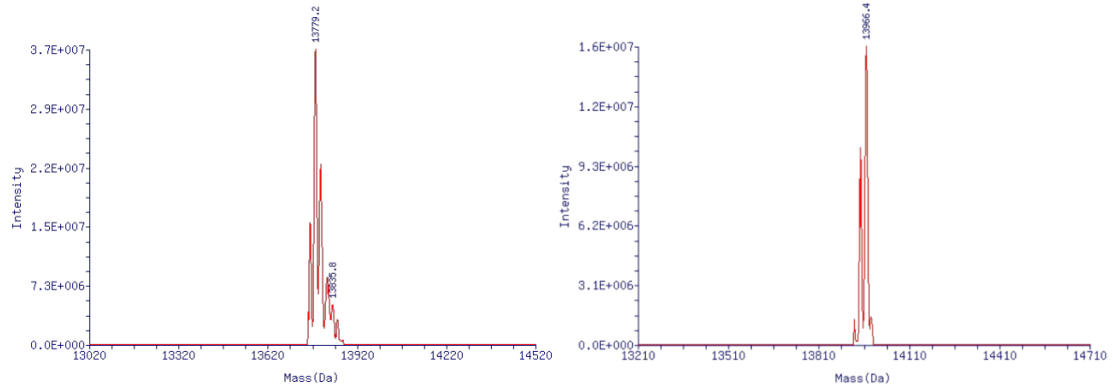
ESI spectra



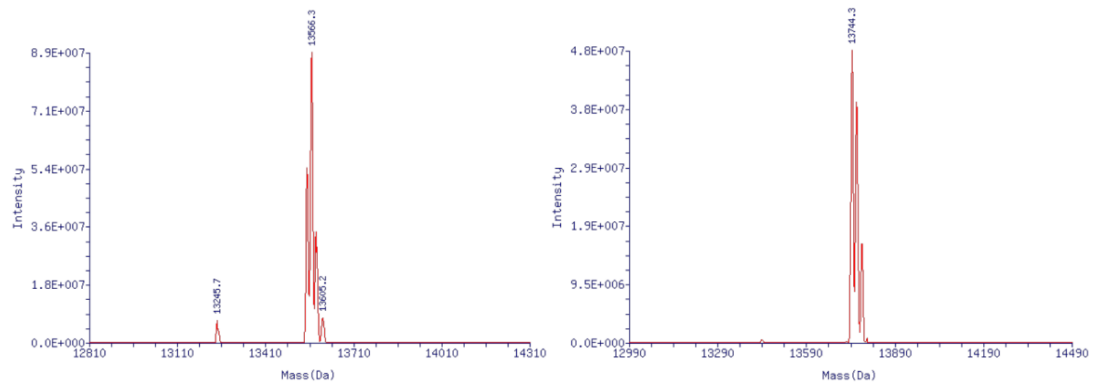
ESI Spec-1. Representative ESI spectra of methoxy modified (left panel) and photocaged crRNA (right panel) for Seq-ASCL1. For the methoxy modified, calculated mass: 13608.5 Da; observed mass: 13605.9 Da. For the caged, calculated mass: 13787.5 Da; observed mass: 13785.4 Da.



ESI Spec-2. ESI spectra of unmodified (left panel) and photocaged (right panel) crRNA for Seq2-ASCL1. Multiple peaks were caused by different ionization states. For the unmodified, calculated mass $[M+Na]^+$: 13721.6 Da; observed mass: 13717.1 Da. For the caged, calculated mass $[M+Na]^+$: 13914.6 Da; observed mass: 13907.8 Da.



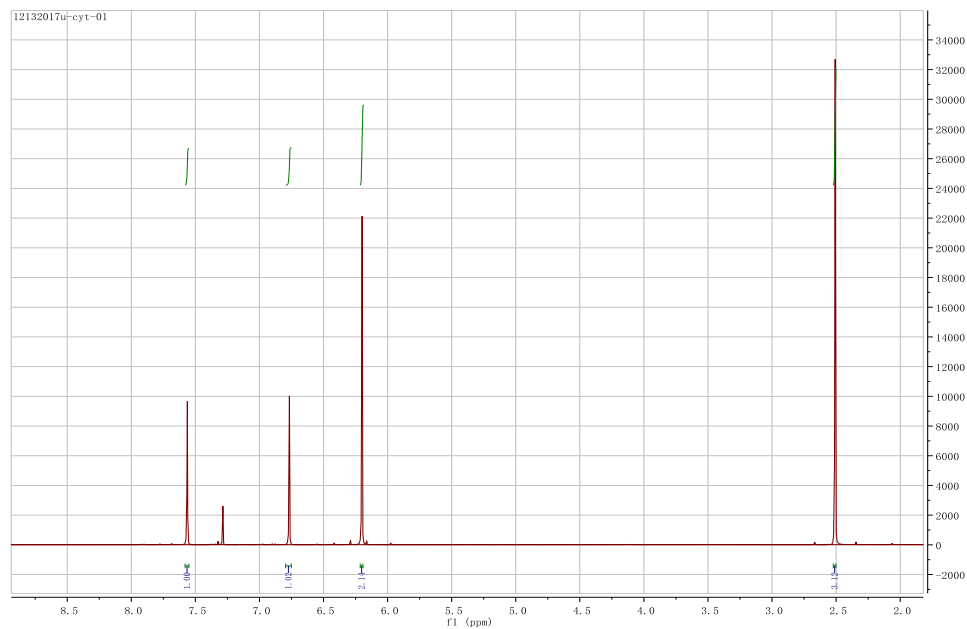
ESI Spec-3. ESI spectra of unmodified (left panel) and photocaged (right panel) crRNA to target the CXCR4 gene. Multiple peaks were caused by different ionization states. For the unmodified, calculated mass $[M+Na]^+$: 13782.3 Da; observed mass: 13779.2 Da. For the caged, calculated mass $[M+Na]^+$: 13974.3 Da; observed mass: 13966.4 Da.



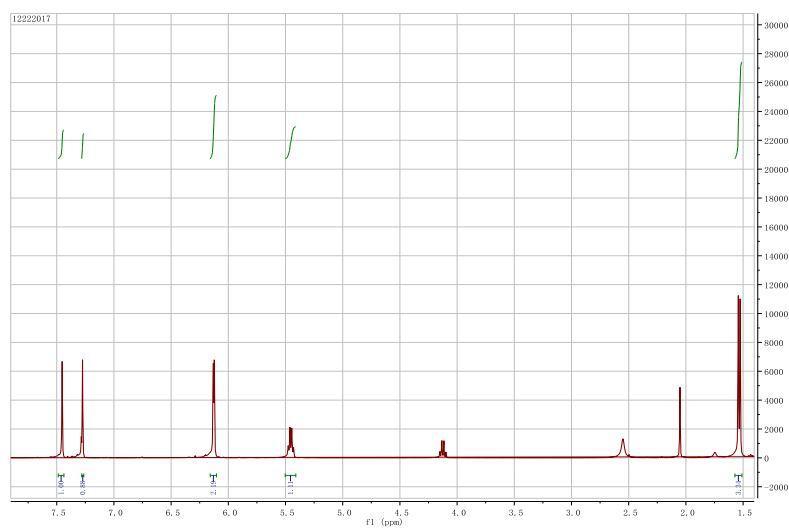
ESI Spec-4. ESI spectra of unmodified (left panel) and photocaged (right panel) crRNA to target the GFP gene. Multiple peaks were caused by different ionization states. For the unmodified, calculated mass $[M+Na]^+$: 13575.1 Da; observed mass: 13566.3 Da. For the caged, calculated mass 13745.1 Da; observed mass: 13744.3 Da.

NMR spectra

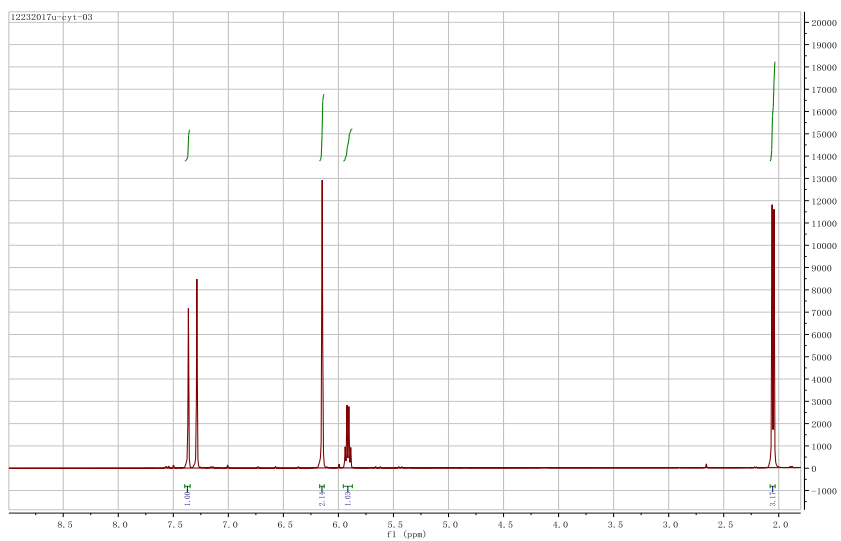
^1H NMR of compound 1b



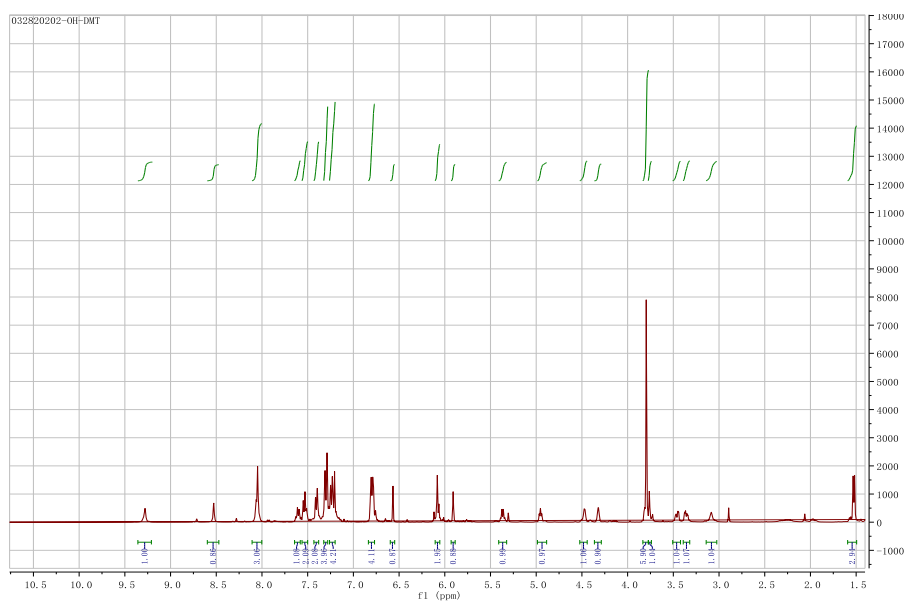
^1H NMR of compound 1c



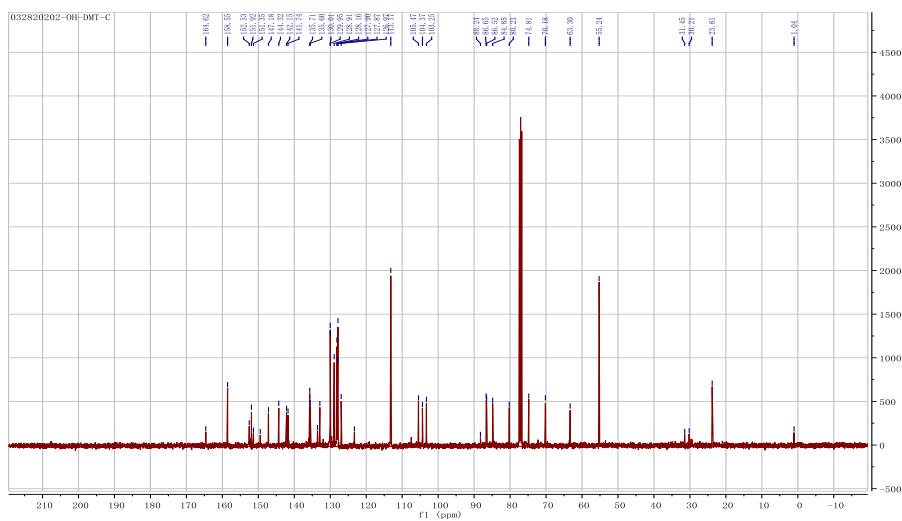
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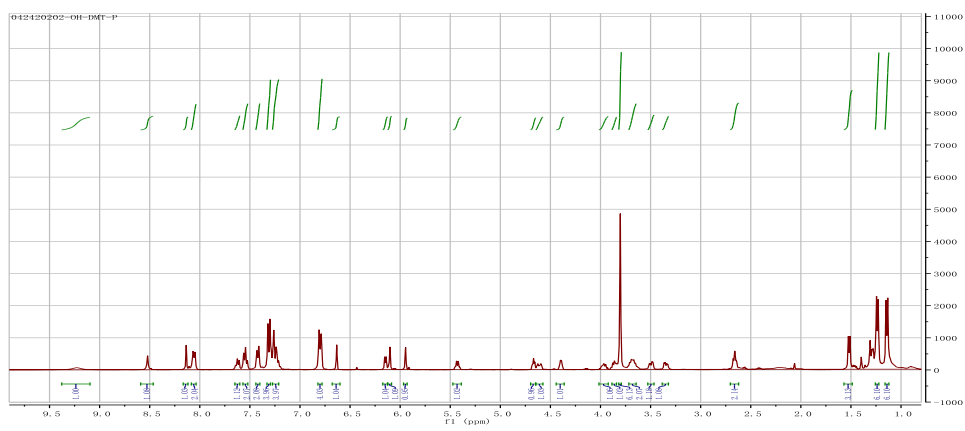
^1H NMR of compound 2c



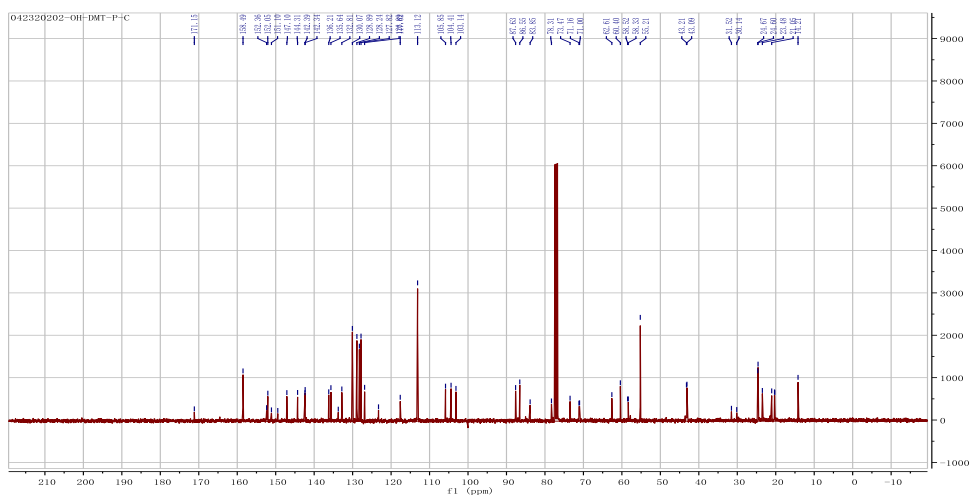
^{13}C NMR of compound 2c



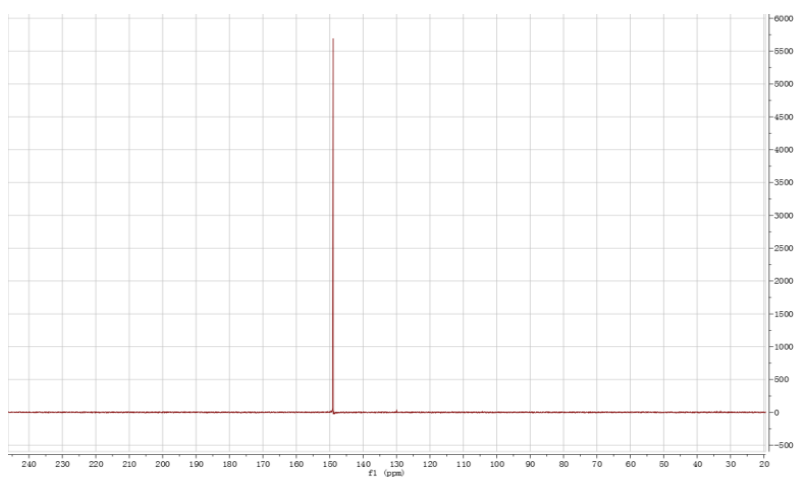
^1H NMR of compound 2d



^{13}C NMR of compound 2d



^{31}P NMR of compound 2d



References:

1. M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, and J. A. Doudna, *Science*, 2012, 337,816-821.
2. E.Ohtsuka, S.Tanaka and M. Ikehara, *Chem Pharm Bull* 1977, 25, 949-959.
3. S. G. Chaulk and A. M. MacMillan, *Nat Protoc*, 2007, 2, 1052-1057.