Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Reversible RNA Acylation for Control of CRISPR-Cas9 Gene Editing

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Content:

Supporting note 1: Materials and Methods

NAI-N_3 was prepared according to a previously reported procedure (1) and was used as a 2 M solution in dry DMSO. It can also be purchased from Cell Data Sciences. All DNA sequences were purchased from the Integrated DNA Technologies (IDT) and were purified by polyacrylamide gel electrophoresis (PAGE) in denaturing 20% gel. The Molecular Beacon probe was purchased from Sigma Aldrich. GFP-sgRNA (chemically modified on the three last nucleotides at both ends) was purchased from Synthego. Cas9 mRNA (CleanCap Cas9 mRNA, L-7606-100) was purchased from Trilink Biotechnologies. Cas9 nuclease for *in vitro* assays and for CRISPR-Cas9 in cell studies were purchased from Agilent (Cat# 5190-7717) and Synthego (Cas9 nuclease 2NLS, *S. pyogenes*), respectively. Oligonucleotide concentrations were measured using a NanoDrop One microvolume UV-Vis spectrophotometer. Where available, molar concentrations were calculated based on Cy5 absorbance.

Fluorescence studies were performed on a Fluorolog 3-11 instrument (Jobin Yvon-SPEX). All gels were visualized and their fluorescence (Cy5 or SYBR Gold) were recorded using a Typhoon 9500 laser scanner (GE Healthcare). PAGE gel images were analyzed and quantified with Image J software. All quantitative experiments were performed in triplicate, and the results were averaged. All flow cytometry analysis was done on the Scanford instrument in the Stanford Shared FACS Facility.

Supporting note 2: GFP-sgRNA cloaking using NAI-N_3

2M NAI-N_3 solution in DMSO was added to a solution of GFP-sgRNA (1-5 µg) in MOPS buffer (100 mM HEPES, pH 7.5, 6 mM $MgCl_2$, 100 mM NaCl) to a final NAI-N_3 concentration of 200 mM and a total volume of 10 µL. The reaction mixture was incubated at 37 ˚C for 20 minutes, before it was quenched by addition of 90% v:v of 3 M NaOAc followed by 3.75× v:v of ethanol and 1 µL of glycogen (5 mg/mL). The mixture was incubated at -80 ˚C overnight and centrifuged (21000 RCF) for 1 h at 4 ˚C to obtain the RNA pellet. The pellet was washed with 75% ethanol, dried and resuspended in RNase-free water.

As mentioned in the main text, our general approach here was to titrate levels of acylation so as to suppress bioactivity by >95%, but not overacylate the RNA such that recovery of activity is poor upon phosphine treatment.

Supporting note 3: *in vitro* Cas9 DNA cleavage assay

In vitro Cas9 DNA cleavage assay was adapted from the protocol provided by Agilent Technologies (2). In a 10 µL reaction volume, GFP-sgRNA (5 nM) in the presence of Cas9 nuclease (50 nM) and Cy5-labelled target ds-DNA and 1x Cas9 digestion buffer (Agilent) were incubated at 30 °C for 90 minutes. Incubation was then continued at 65 °C for 15 minutes. Upon completion, 1 µL of Proteinase K (New England Biolabs) was added and sample was incubated at 37 °C for 15 minutes. Assay was quenched by addition of 0.1%

Orange G solution in 6.5 M formamide containing 100 mM EDTA and samples were denatured at 95°C before being loaded on 15% denaturing PAGE. Uncleaved DNA substrate and cleavage products were quantified and used to calculate the gene editing efficiency (%) by the formula: (a/b) × 100 where "a" is the sum of the band intensities of the cleavage product and "b" is the sum of band intensities of cleaved and uncleaved DNA.

Supporting note 4: GFP-sgRNA uncloaking using THPP and TPPMS phosphines

To the cloaked GFP-sgRNA (1μM) in phosphate-buffered saline (PBS) buffer (pH: 7.4) was added THPP or TPPMS phosphine solutions (100 mM in DMSO) to reach 1 or 5 mM final phosphine concentrations in 20 μL. The sample was incubated at 37°C for 1 hour, before the reaction was stopped by addition of 90% v:v of 3 M NaOAc followed by 3.75× v:v of ethanol and 1 μL of glycogen (5 mg/mL). The mixture was incubated at -80 ˚C overnight and centrifuged (21000 RCF) for 1 h at 4 ˚C to obtain the RNA pellet. The pellet was washed with 75% ethanol, dried and resuspended in RNase-free water. Note that these phosphines were chosen among several others (data not shown) based on their highest activity in uncloaking, along with good solubility and low toxicity.

Supporting note 5: Engineering the GFP (+) HeLa cells

GFP (+) HeLa cells were engineered using Lentiviral vectors produced in HEK 293Tx cells (ATCC). Briefly, PEI (Polysciences Inc., 24765) was used to transfect HEK cells with lentiviral constructs (GFP Expression Plasmid) and co-transfected with packaging vectors psPAX2 and pMD2.G under standard conditions (Tiscornia et al., 2006 https://www.ncbi.nlm.nih.gov/pubmed/17406239). Cells were grown for 12 hours, cell media changed, and then grown for additional 48 hours. The resulting media was colelcted used to spin-fect HeLa cells (ATCC) in the presence of 10 μg/ml Polybrene (Santa Cruz Biotechnology) at 1000g for 30min. Cells were sequentially selected with 1.5 μg/ml puromycin and 1.5 μg/ml blasticidin beginning 48 hours post-infection, and maintained under selection for 2-3 passages. Resulting GFP positive cells were quantified by flow cytometry.

Supporting note 6: Cellular CRISPR-Cas9 experiments using CARTs

GFP(+) HeLa cells were cultured at 37 °C, 5% CO2, and 95% humidity. Cells were maintained in supplemented DMEM culture medium (10% FBS, Gibco, 1 × Penicillin/Streptomycin, Thermo Fisher Scientific) and were seeded at a concentration of 4×10^4 cells per well in a 24-well plate 16 hours prior to the experiment. Prior to the experiment, cells were rinsed with serum-free media and 400 μL of serum-free media were added to each well. GFP-sgRNA (untreated or cloaked, 120 ng) and Cas9 mRNA (120 ng) were premixed in presence of PBS buffer (pH: 5.5), and were then formulated for CART delivery by being gently

vortexed with the D:A 13:11 CART system in a net 10:1 cation:anion ratio (0.31 μL of 2 mM CART solution in DMSO) shortly before the mixture was added to each well. Plates were gently rocked and were then incubated at 37 °C for 4 hours, before their media replaced with 500 μL of supplemented DMEM culture medium (10% FBS, Gibco, 1 × Penicillin/Streptomycin). In order to uncloak GFP-sgRNAs, a solution of THPP or TPPMS phosphines in water was added 4 hours after transfection to the media of cells earlier transfected with cloaked GFP-sgRNA, to a final phosphine concentrations of 5 mM (THPP) or 1 mM (TPPMS). Cells were subsequently incubated at 37 °C for 17 hours, before their media was changed with supplemented DMEM. Cells were then incubated for 5 days until all GFP protein was depleted and were then analyzed by flow cytometry. GFP expression was measured on a Scanford Cytometer at Blue FL1 channel. Gene editing efficiency was calculated based on the percentage of the cell population shifting from GFP positive gate to GFP negative:

Gene editing efficiency % = [GFP negative/(GFP pos. + GFP Neg.)] *100

Characterization of CART

Protected: 1H NMR (500 MHz, Chloroform-d) δ 7.4-7.25 (m, 5H) 5.15 (s, 2H), 4.33 – 4.21 (m, 66H), 4.10 (td, J = 6.8, 2.6 Hz, 26H), 3.99 (s, 11H), 3.93 (s, 12H), 3.52 (t, J = 11.9 Hz, 26H), 2.88 (d, J = 1.6 Hz, 6H), 1.65 – 1.58 (m, 33H), 1.46 (d, J = 3.1 Hz, 50H), 1.40 (d, J = 1.9 Hz, 47H), 1.32 – 1.25 (m, 94H), 0.87 (t, J = 6.9 Hz, 32H).

GPC Mn=6013 daltons, Mw/Mn= 1.25 (PS calibrated GPC)

Deprotected: 1H NMR (400 MHz, Methanol-d4) δ 7.40-7.25 (m, 5H), 5.1 (s, 2H) 4.58 (q, J = 5.5 Hz, 19H), 4.50 – 4.31 (m, 31H), 4.34 – 4.23 (m, 28H), 4.19 – 4.06 (m, 53H), 3.49 (dt, J = 15.3, 5.4 Hz, 23H), 3.25 – 3.15 (m, 2H), 3.07 (d, J = 12.8 Hz, 6H), 1.64 (d, J = 8.5 Hz, 33H), 1.30 (s, 188H), 1.37 – 1.17 (m, 138H), 0.90 (t, J = 6.6 Hz, 46H).

Instrumentation for GPC: Gel permeation chromatography (GPC) was performed in tetrahydrofuran (THF) at a flow rate of 1.0 mL/min on a Malvern Viscotek VE2001 chromatography system equipped with four 5-μm Waters columns (300 × 7.7 mm) connected in series. The Viscotek VE3580 refractive index (RI) and VE3210 UV/Vis detectors and Viscotek GPCmax autosampler were used, and the number average molecular weights (Mn in g·mol−1) and molecular weight distributions (Mw/Mn) were calibrated using monodisperse polystyrene standards (Polymer Laboratories)

Supporting note 7: Phosphine toxicity assessment

HeLa cells were seeded at a concentration of 10^4 cells per well to 96-well plate (black, flat transparent bottom, Greiner) in supplemented DMEM culture medium (Gibco) supplemented with 10% FBS (Gibco), 100U Pen./Strep. (Gibco) and incubated for 16 h at 37°C and 5% CO_2.HeLa cells were incubated with 1mM

and 10 mM of phosphines THPP and TPPMS for 24h at 37°C and 5% CO_2, in supplemented DMEM culture medium. Control cells were incubated in the supplemented DMEM with addition of nuclease-free water. After the incubation period medium was removed and 100 µl of fresh, not supplemented DMEM medium, without phenol red (Gibco) was added to each well. Medium contained 10 nM SYTOX Green and 5 µM C12-resazurin. Plate was incubated for 15 minutes at 37°C and 5% CO_2 in the dark. Fluorescence of the samples was measured using a microplate reader Tecan Infinite M1000 at excitation 480nm, and emissions 530nm for SYTOX Green, and 575nm for resofurin. All samples were prepared in the quintuplicates.

Deiters has previously reported on toxicity of phosphines in a protein uncaging study (*Nat. Chem*. **2016**, *8*, 1027). In our cellular experiments, we employed 1 mM phosphine; see Fig. S5 for explicit toxicity data at that concentration. In general we note that there is some phosphine toxicity seen at all concentration ranges, but the fluorescence (flow cytometry) assay takes this into account (above) by measuring the whole population after 5 days.

Supporting note 8: Molecular Beacon study

Molecular beacon fluorescence signals were recorded at λ_{ex} = 496 nm and λ_{em} = 520 nm at 52 °C. The molecular beacon was added to molecular beacon buffer (100 mM Tris-HCl, pH 8, 100 mM NaCl, 5 mM $MgCl_2$) to concentration of 80 nM, and fluorescence was recorded to ensure that fluorescence signal does not increase. After 5 min, GFP-sgRNA was added to the molecular beacon a final concentration of 100 nM and final volume of 160 µL, and fluorescence signal was recorded for 200 minutes. The molecular beacon light up signal was calculated by subtracting averaged "dark" molecular beacon fluorescence (before sgRNA addition) from averaged fluorescence of fully fluorescent beacon in the presence of sgRNA (11000 – 12000 seconds range). Averaged fluorescence signal was normalized to that of untreated RNA.

Supporting note 9: Analysis of mutagenesis frequencies in RUNX1 gene using CRISPR-Cas9

HEK293T cells (ATCC) were cultured at 37 °C, 5% CO2, and 95% humidity in supplemented DMEM culture medium (10% FBS, Gibco; 1 × Penicillin/Streptomycin, Gibco) and were seeded at a concentration of 2 x 10^4 cells per well in a 24-well plate. After 16 hours cells the DMEM medium was replaced with 500 µL of fresh serum-free medium. The transfection mixture contained: 0.4 µL of sgRNA[1] (untreated or cloaked, 120 ng, Synthego, 2'OmG2'OmC2'OmAUUUUCAGGAGGAAGCGA-Synthego modified EZ scaffold) and 0.6 µL of Cas9 mRNA (600 ng, TriLink) were premixed in presence of 3.7 µL of PBS buffer (pH: 5.5), and were then formulated for CART delivery by being gently flicked for 1 min. with the D:A 13:11 CART system in a net 10:1 cation:anion ratio (0.28 µL of 2 mM CART solution in DMSO) and immediately added to well. The medium was pipetted three times to mix the transfection mixture well and were incubated at 37 °C for 4 hours, before their media replaced with 500 µL of supplemented DMEM

culture medium. In order to uncloak sgRNAs, to cells earlier transfected with cloaked sgRNA was added supplemented medium containing THPP or TPPMS phosphines in concentration 1 mM and 5 mM. Cells were incubated for next 17 hours, before the medium was replaced with a fresh supplemented DMEM. Cells were then incubated for 5 days. The 5th day cells were harvested in PBS by scraping and total DNA isolated using Quick-DNA Miniprep Kit (Zymo Research) according to producer's protocol. To investigate mutagenesis frequencies generated in RUNX1 gene by sgRNA, we used T7 endonuclease I assay. In 50 µL volume PCR reaction was performed by mixing 100 ng of total DNA with 2.5 µL of primers mix (10mM, forward: CCAGCACAACTTACTCGCACTTGAC ; reverse: CATCACCAACCCACAGCCAAGG; IDT), and 25 µL of Phusion High-Fidelity PCR Master Mix with HF Buffer (New England Biolabs) and using program: 98°C for 30 sec.; 30 times: 98°C for 10 sec., 59°C for 30 sec, 72°C for 30 sec.; 72°C for 5 min., 4°C forever. The reaction product was purified using DNA Clean & Concentrator Kit (Zymo research) and concentration measured by NanoDrop spectrophotometer. The renaturation was performed on the mixture of 100 ng of control RUNX1 PCR product from HEK293T cells and 100 ng of DNA product achieved form the CRISPR experimental conditions by heating to 95°C for 2 min. and decreasing temperature to 25°C in rate of 2°C per min. The annealing was followed by digestion with T7 endonuclease I (10U, New England Biolabs) for 15 min. at 37°C. The reaction was quenched by addition of 0.75 µL of 0.5 M EDTA (pH 8, Invitrogen). The reaction products (with addition of 4 µL of 6xDNA Loading Dye, Thermo Scientific) were analyzed in 1.5% agarose gel (65 mA, ~ 1.5 h, 1×TBE pH 8.3, Sigma Aldrich), visualized with Gel Doc™ XR+ System (SYBR Gold filter) and quantified using ImageJ software.

Supporting note 10: Analysis of disruptive effect of cloaking on sgRNA/Cas9 complex by electrophoresis mobility shift assays (EMSA)

The Cas9/sgRNA ribonucleoprotein complex was formed by incubating 20 µmoles of Cas9 NLS protein (Synthego) with 15.5 µmoles of sgRNA (untreated or cloaked, Synthego, 2'OmG2'OmC2'OmAUUUUCAGGAGGAAGCGA-Synthego modified EZ scaffold) in 10 µL reaction buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, and 0.1% Tween 20, pH 8.0) for 15 min at 37 °C as described previously.[2] To the reaction was added 2 µL of Novex Hi-Density TBE Sample Buffer (5x, Thermo Fisher Scientific) and the ribonucleoprotein complex was analyzed in 6% TBE gel (Invitrogen) in 1×TBE buffer(pH 8.3, Sigma Aldrich, 15 mA, r, for ~ 1.5 h) next to GeneRuler 1kb Plus DNA Ladder (Thermo Fisher Scientific). Gel was stained in 1×SYBR Gold (10000×conc., Invitrogen) in 1×TBE buffer for 5 min. at room temperature with constant rocking in the dark and was imaged on a Typhoon FLA 9500 biomolecular imager (green laser, GE Healthcare Life Science). Afterwards, the gel was stained in 100 ml of Coomassie Blue R-250 staining mixture (50% methanol, 10% acetic acid, 40% water) for 2 h, destained for 4 h (5% methanol, 7.5% acetic acid, 87.5% water) and pictured by a mobile camera.

Table S1. List of all the oligonucleotides and target sequences used in this study

Identifier	Sequence
GFP-sgRNA	5'- CAACUACAAGACCCGCGCCGGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC U**UUU**-3'
GFP-sgRNA *in cellulo*	5'- 2'Om C2'Om A2'Om ACUACAAGACCCGCGCCG - Synthego modified EZ scaffold-3'
GFP-DNA-S	5'-Cy5- CCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCG CCGAGGTGAAGTTCGAGGGC -3'
GFP-DNA- AS	5'- GCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTCGTCCTTGAA GAAGATGGTGCGCTCCTGG-3'
Molecular Beacon (MB)	5'-[6FAM]-CGCGTTACTGCCCTGTGGGGCAAGCCCCGCG-[DABC]-3'
sgRNA used in MB study	5'- **GGG**CUUGCCCCACAGGGCAGUAAGUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCAC CGAGUCGGUGCU**UUU**-3'

Bold nucleotides denote 2'-O-Methyl modifications, with phosphorothioate linkages in between.

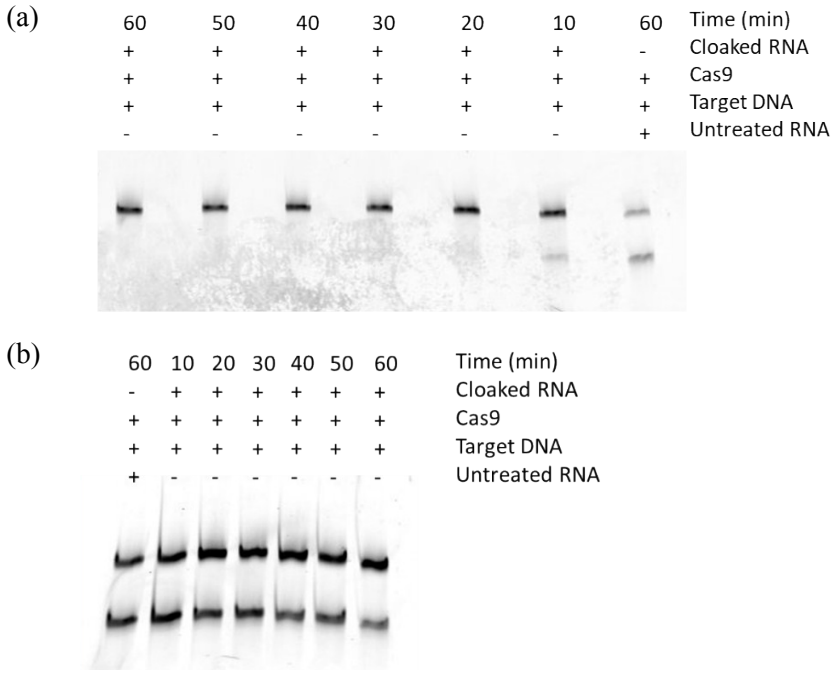
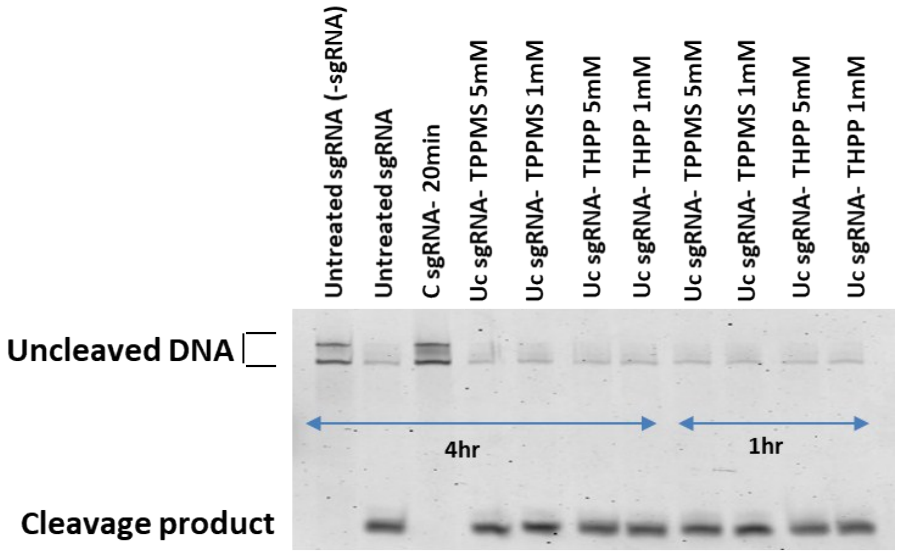


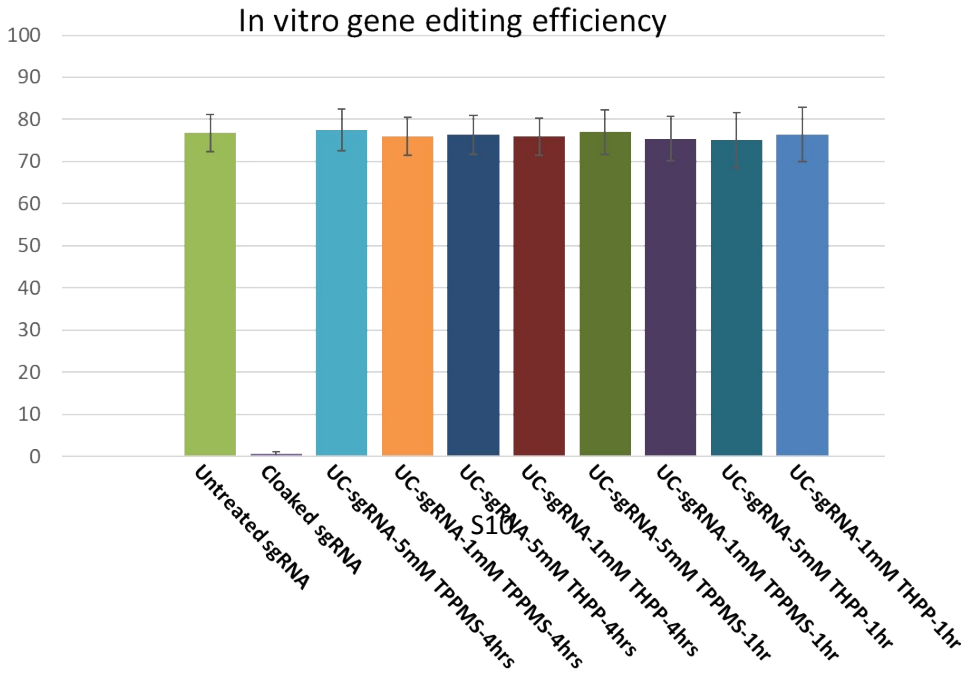
Figure S1. Optimization of cloaking time through cleavage assay: PAGE analysis of *in vitro* Cas9 DNA cleavage assay using Cy5 labelled DNA target, as well as sgRNAs that were cloaked with 200 mM NAI-N_3 in MOPS SHAPE buffer (a) or water (b) at 37 °C for 10, 20, 30, 40, 50, and 60 minutes. sgRNA cloaked for 20 minutes in buffer provides the minimum amount of cloaking required to inhibit Cas9 DNA cleavage >90%.



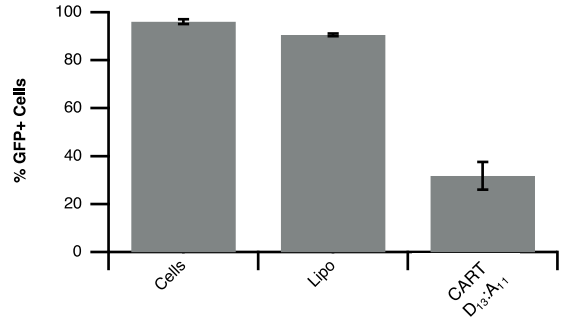
(a)

(b)

Figure S2. Optimization of sgRNA uncloaking time through Cas9 cleavage assay. Cloaked sgRNA (20 min, 200 mM NAI-N_3) was incubated for 1 or 5 hours with THPP or TPPMS at 1 or 5 mM phosphine concentrations at 37 °C. The uncloaked sgRNA was then precipitated and further used in *in vitro* Cas9 DNA cleavage assay. (a) PAGE analysis of the Cas9 in vitro assays using the cloaked and uncloaked sgRNAs at different conditions. Two DNA bands are visible because of an alternate conformation of the DNA. (b) Bar graph shows means of three independent *in vitro* Cas9 DNA cleavage experiments. Error bars represent ± s.d.



(a)



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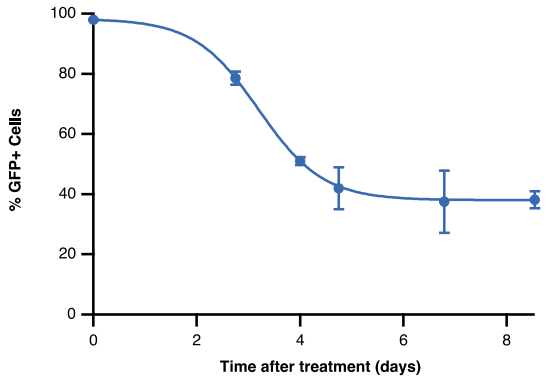
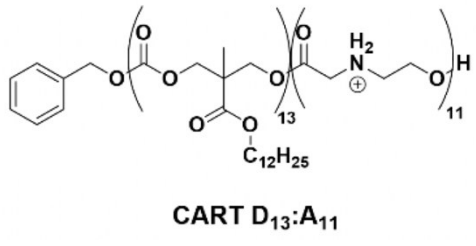
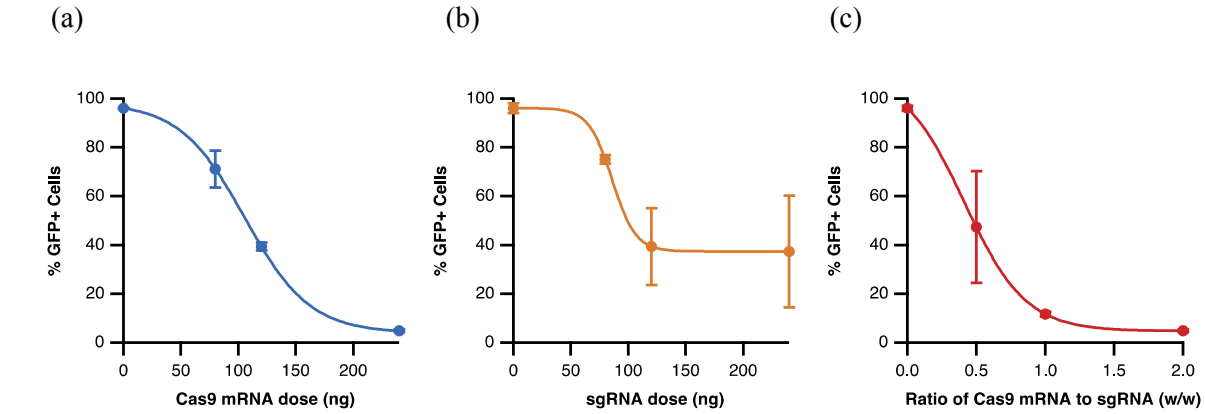


(c)



CART D_{13}:A_{11}

Figure S3. Initial evaluation of CRISPR/Cas9-mediated knockdown of GFP expression. (a) sgRNA/Cas9 mRNA transfection using CARTs compared to Lipofectamine (Lipo). GFP (+) HeLa cells were treated with 120 ng Cas9 mRNA and untreated sgRNA either formulated with Lipo according to the manufacturer's instructions, or with CART D_{13}:A_{11} at a 10:1 charge ratio, for 4 hours. GFP expression was assayed by flow cytometry 5 days after treatment and compared to untreated GFP (+) HeLa cells. (b) Optimization of reading time for determining CRISPR/Cas9 editing efficiency by flow cytometry. GFP (+) HeLa cells were treated with 120 ng Cas9 mRNA and untreated sgRNA formulated with CART D_{13}:A_{11} at a 10:1 charge ratio, for 4 hours. GFP expression was assayed by flow cytometry over the course of 9 days following treatment and compared to untreated GFP (+) HeLa cells. All points represent the average of three experiments; error bars show the standard deviations. (c) Chemical structure of charge-altering releasable transporters (CARTs) used for the delivery of Cas9 mRNA and sgRNA.



(d)

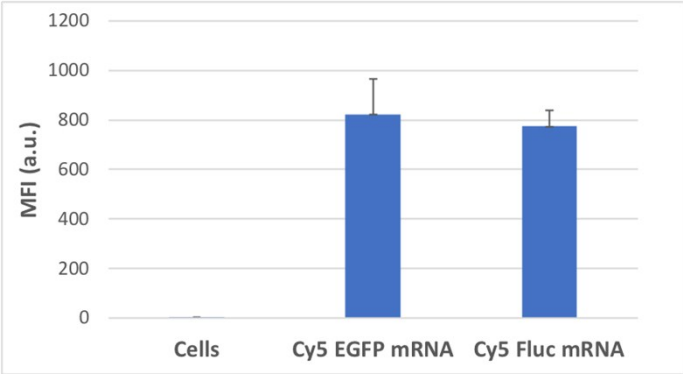


Figure S4. Optimizing Cas9 mRNA/sgRNA ratio for CRISPR-mediated GFP knockdown. 9a) Effect of Cas9 mRNA dose on GFP knockdown when sgRNA dose was held constant at 120 ng. (b) Effect of sgRNA dose on GFP knockdown when Cas9 mRNA dose was held constant at 120 ng. (c) Effect of the mass ratio of Cas9 mRNA to sgRNA on GFP knockdown at a constant total oligonucleotide dose. For all experiments, GFP (+) HeLa cells were treated with Cas9 mRNA and sgRNA co-formulated with CART D_{13}:A_{11} at a 10:1 charge ratio, for 4 hours. GFP expression was assayed by flow cytometry 5 days after treatment and compared to untreated GFP (+) HeLa cells. All points represent the average of three experiments; error bars correspond to the standard deviation. (d) Testing the effect of mRNA length on transfection efficiency by CARTs. Fluorescent-labeled eGFP mRNA (996 nt) and fLuc mRNA (1929 nt) were compared for intracellular concentrations by flow cytometry after CART delivery. HeLa cells were treated with CART $\mathbf{D}_{13}$:$\mathbf{A}_{11}$ formulated with Cy5 Fluc mRNA or EGFP mRNA at a 10:1 charge ratio (dose of 1ng mRNA per 1000 cells). After 8 hours, cells were assayed by flow cytometry for Cy5 fluorescence using the APC channel. Data shown consists of triplicate measurements and normalized to the background fluorescence of cells alone.

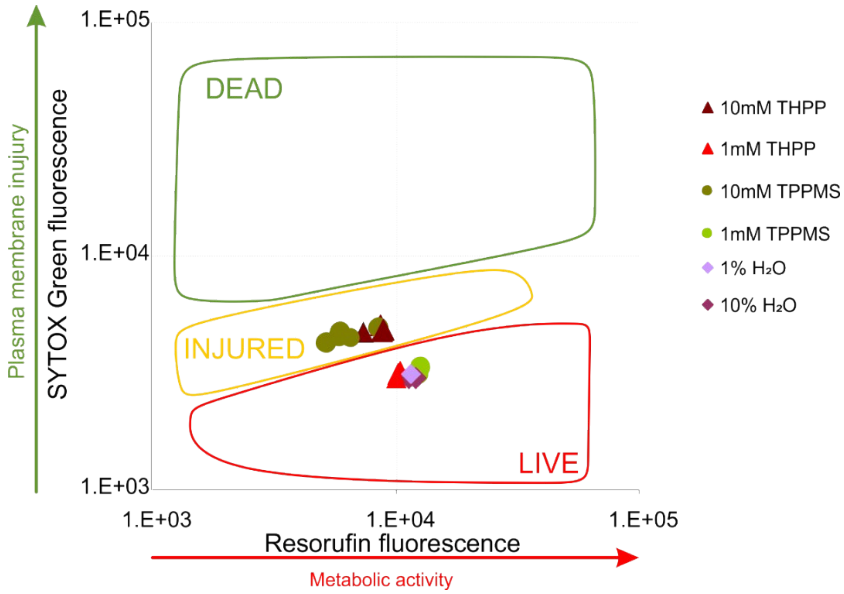


Figure S5. Phosphine toxicity assessment. Hela cells were incubated with 1 mM and 10 mM phosphines THPP and TPPMS for 24 h in a supplemented DMEM. The low concentration of phosphines (1 mM) does not injure cellular membrane and does not decrease metabolic activity of cells. The increase of phosphine concentration to 10 mM induces a slight injury of cellular membrane and a drop of metabolic activity.

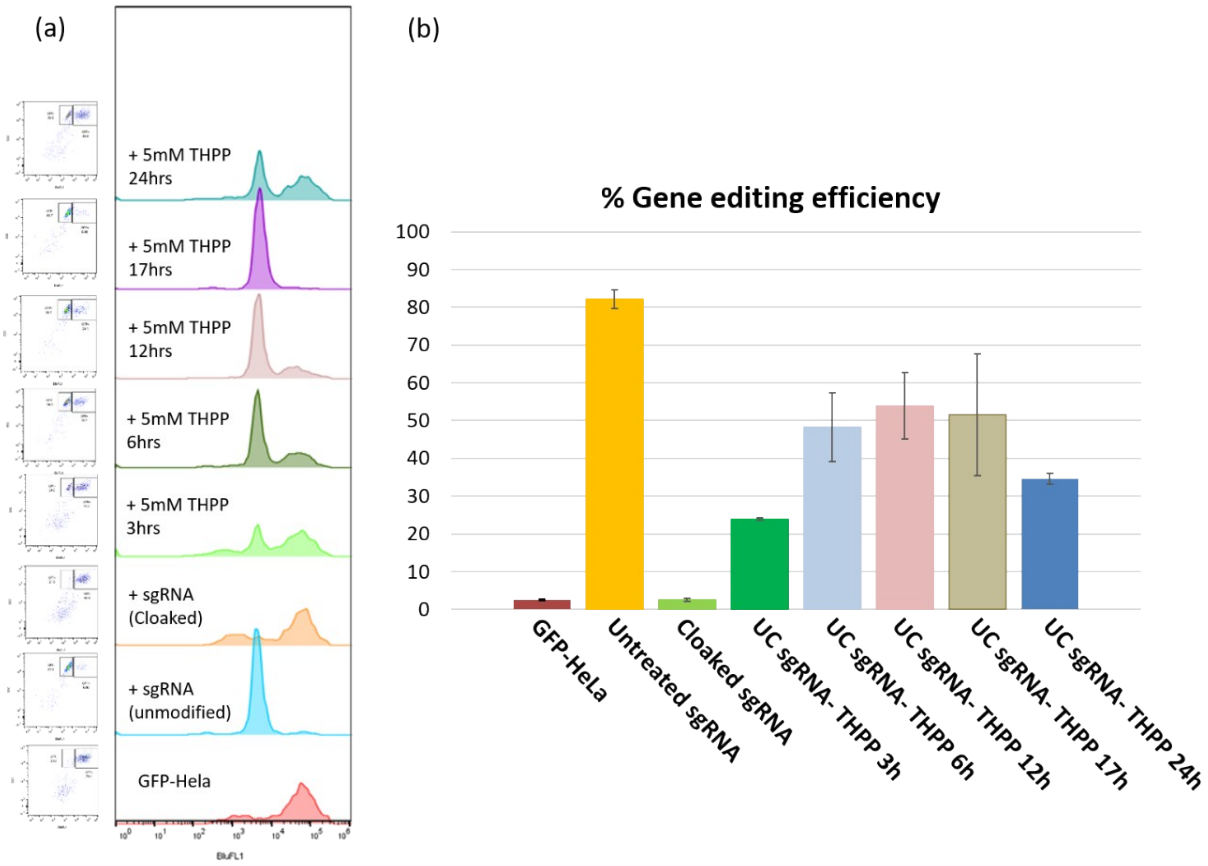


Figure S6. Uncloaking optimization by varying phosphine incubation time. (a) Flow cytometric analysis of GFP (+) HeLa cells transfected with untreated/cloaked sgRNA and Cas9 mRNA using CART system, and their in-cell uncloaking using 5 mM THPP delivered 6 hours post transfection. Cells were incubated with phosphine for 3,6,12,17, or 24 hours 5 days prior to the FACS analysis. Samples incubated with phosphine for 12-17 hours showed the highest sgRNA uncloaking and hence gene editing efficiencies. (b) Bar graph shows means gene editing efficiency in three independent in cell CRISPR-Cas9 experiments. Error bars represent ± s.d.

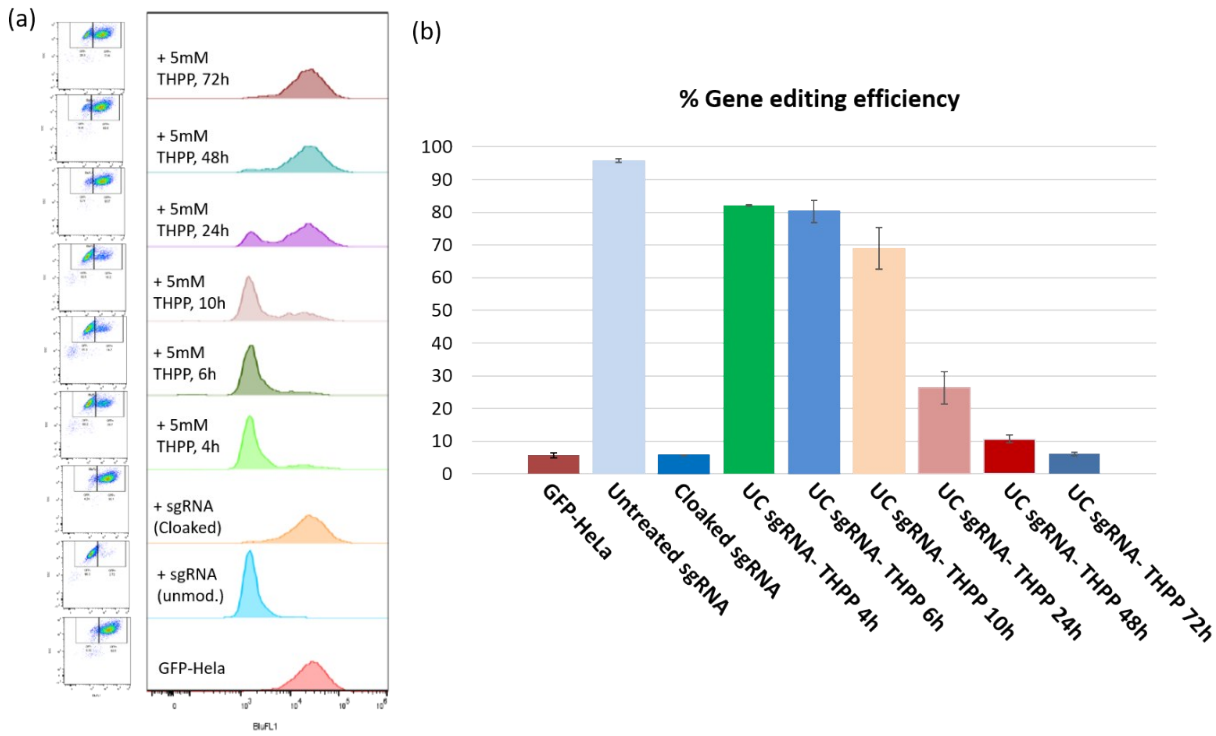


Figure S7. Uncloaking optimization by varying phosphine addition time. (a) Flow cytometric analysis of GFP (+) HeLa cells transfected with untreated/cloaked sgRNA and Cas9 mRNA using CART system, and their in-cell uncloaking using 5 mM THPP delivered at 4, 6, 10, 24, 48, 72 hours post transfection. Cells were incubated with phosphine for 17 hours, 5 days prior to the FACS analysis. Samples that were incubated with phosphine 4-6 hours post transfection showed the highest sgRNA uncloaking and hence gene editing efficiencies. (b) Bar graph shows means gene editing efficiency in three independent in cell CRISPR-Cas9 experiments. Error bars represent ± s.d.

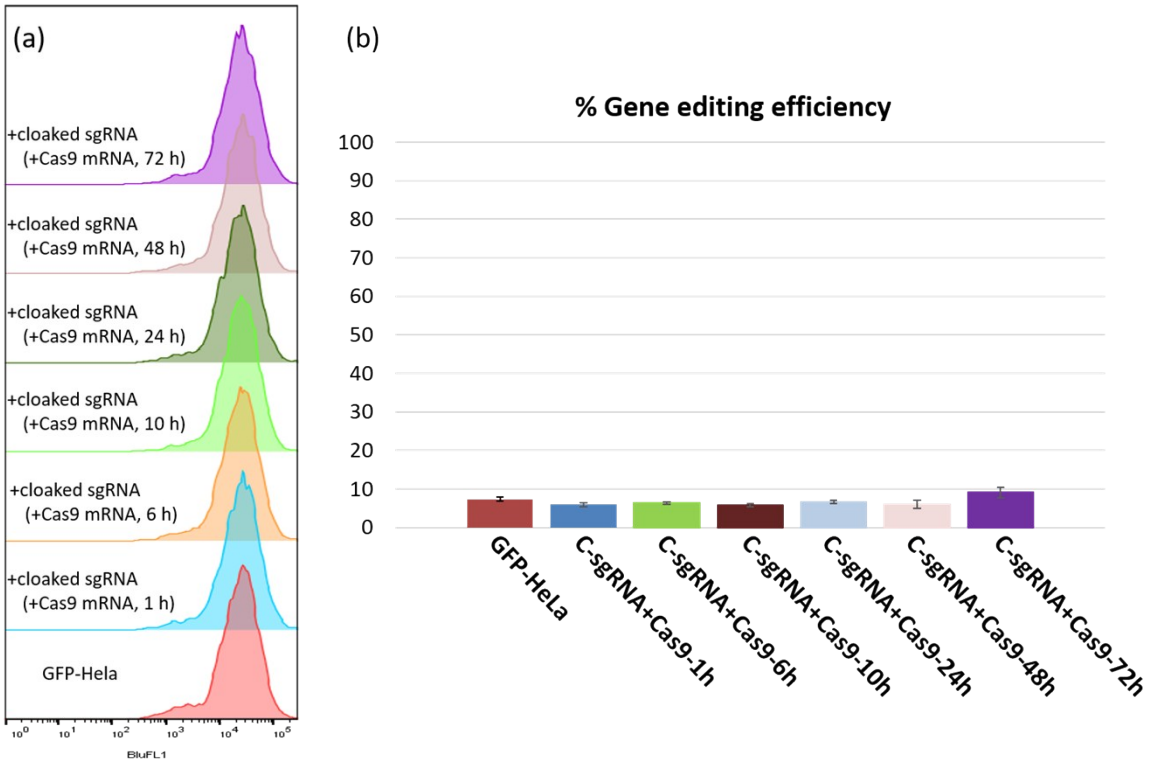


Figure S8. Control experiments for cloaked sgRNA stability in cell. (a) Flow cytometric analysis of GFP (+) HeLa cells transfected with cloaked sgRNA followed by Cas9 mRNA using CART system. Cas9 mRNA was delivered at 1, 6, 10, 24, 48, 72 hours post sgRNA transfection. Cells were incubated with phosphine for 17 hours, 5 days prior to the FACS analysis. Samples did not show any sign of self-uncloaking over the period of 72 hours. (b) Bar graph shows means gene editing efficiency in three independent in cell CRISPR-Cas9 experiments. Error bars represent ± s.d.

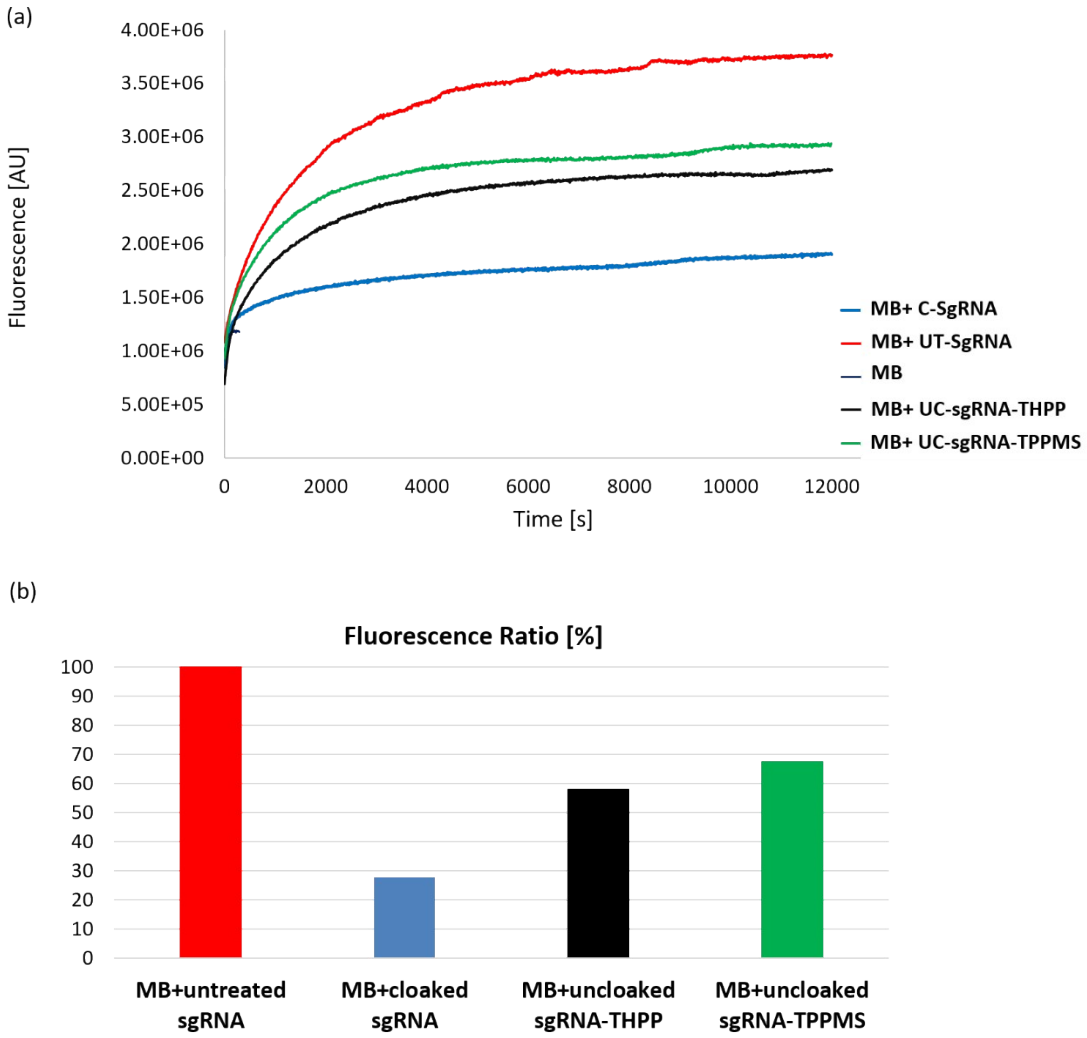


Figure S9. Molecular beacon assay. Experiments showing that cloaking reduces the ability of sgRNA to hybridize to a DNA complement containing sequence from the targeted segment of GFP. (a) Plot of effect of cloaking/uncloaking on MB fluorescence. Increase of fluorescence of MB upon its incubation with untreated sgRNA (red), cloaked sgRNA (blue), signal after uncloaking with THPP (5 mM, 1h, 37 °C; black) and TPPMS (1 mM, 1h, 37 °C; green); (b) Bar graph shows MB fluorescence increase upon addition of sgRNA (values normalized to MB+ untreated sgRNA). Note that these are data from the 1h time point (see part (a) where reactions are not yet complete. Also note that air oxidation of phosphines is likely to compete with full uncloaking in some cases.

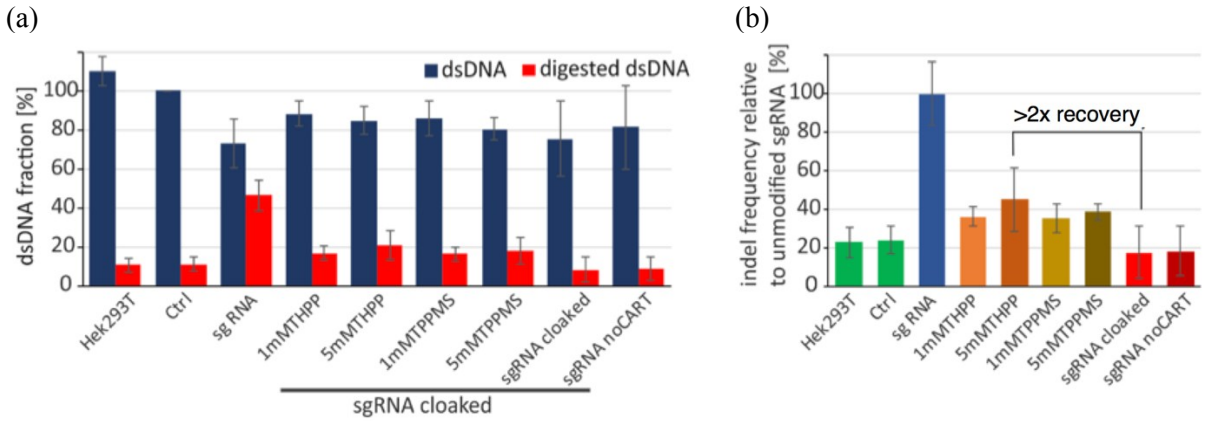


Figure S10. Testing cloaking-controlled activity of CRISPR/Cas9 targeted to the endogenous gene *RUNX1*. (a) Shown is percent modification determined by T7 endonuclease I assay in *RUNX1* DNA sequence; error bars represent s.d. for n = 3. Blue bars present undigested fraction of DNA by T7 endonuclease and red bars represent digested fraction of dsDNA that is equivalent of indels frequency after editing of *RUNX1*. From left: HEK293T Ctrl – annealed and treated with TE7 control DNA strands; Ctrl – HEK293T Ctrl and Ctrl experiment DNA; sgRNA – cells treated with Cas9/sgRNA/CART mixture; 1 mM THPP – cells treated with Cas9/cloaked sgRNA/CART mixture and incubated with 1 mM THPP for 17 h; 5 mM THPP – cells treated with Cas9/cloaked sgRNA/CART mixture and incubated with 5 mM THPP for 17 h; 1 mM TPPMS– cells treated with Cas9/cloaked sgRNA/CART mixture and incubated with 1 mM TPPMS for 17 h; 5 mM TPPMS – cells treated with Cas9/cloaked sgRNA/CART mixture and incubated with 5 mM TPPMS for 17 h; sgRNA – cells treated with Cas9/cloaked sgRNA/CART; sgRNA no CART - – cells treated with Cas9/sgRNA mixture. (b) Plot of frequency of indels relative to unmodified sgRNA treatment, showing >2x recovery of activity after 5 mM THPP treatment.

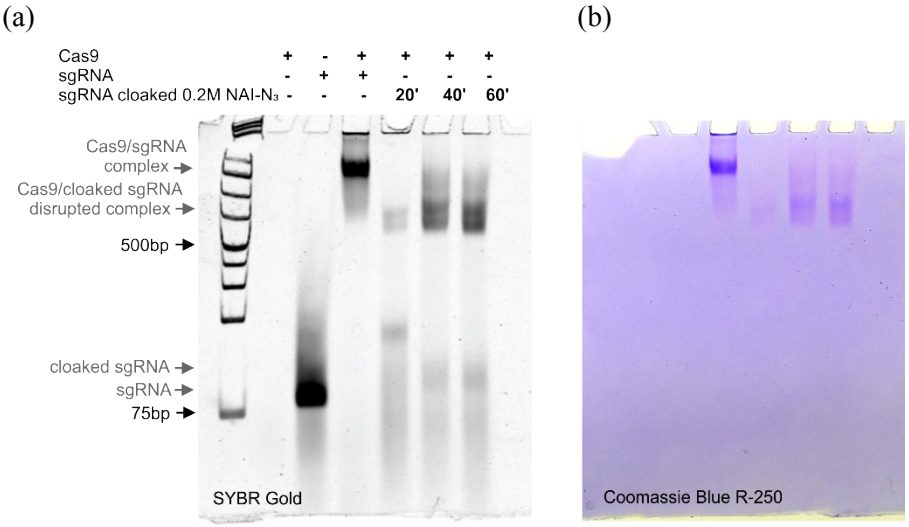


Figure S11. Cloaking perturbs Cas9/sgRNA ribonucleoprotein complex *in vitro*. (a) Gel stained with SYBRGold to observe nucleic acids. (b) Gel stained with Coomassie Blue R-250 to observe proteins. Lanes in the gel from left: GeneRuler 1kb Plus DNA Ladder; Cas 9 protein; sgRNA, sgRNA/Cas9 complex;

cloaked by 0.2 M NAI-N_3 for 20 min. sgRNA in complex with Cas9; cloaked by 0.2 M NAI-N_3 for 40 min. sgRNA in complex with Cas9; cloaked by 0.2 M NAI-N_3 for 60 min. sgRNA in complex with Cas9.

References

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