SUPPORTING INFORMATION

A cleavage-responsive stem-loop hairpin for assaying guide RNA activity

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Experimental Methods

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EXPERIMENTAL METHODS

Materials. GUIDER cleavage assay samples were prepared using Corning Molecular Biology Grade Water, Thermo Scientific 50mM MgCl. GUIDER probes (GUIDER-1, GUIDER-2, GUIDER-3, GUIDER(AA-TT), GUIDER(CC-GG), GUIDER-overhang) were purchased from Integrated DNA Technologies (IDT). Guide RNAs were synthesized as reported in the literature.[7] In short, overlap PCR was performed using designed T7 and L2 DNA molecules purchased from IDT that afforded a double stranded sgRNA template. The DNA was transcribed to the desired single-guide RNA (sgRNA) product using a MEGAscript T7 High Yield Transcription Kit. Cas9 protein was obtained from MacroLab at UC Berkeley.

All single-stranded GUIDER DNA solutions were heated in Bio-Rad Thermocycler, unless otherwise noted. Samples were loaded in a Falcon 384-well plate and the fluorescence emission of the corresponding samples was measured in Tecan Infinite M200 instrument. PAGE analysis was conducted in Mini-Protean TGX Precast Gels (4-15%, 12 well comb, 20µl) from Bio-Rad with Thermo Scientific, using GeneRuler 100 bp or 50 bp and Invitrogen Gel Loading Buffer, with 1X Corning TBE Buffer. Gels were stained in Life Technologies SYBR Gold nucleic acid gel stain**.**

Mouse PCSK9 gene target (*mpcsk9*) were amplified from cDNA purchased from Sino Biological (China) using M13 Forward (-41) and M13 Forward (-48) primer purchased from IDT. PCR amplification was performed using a Thermo Scientific Phusion High-Fidelity PCR Kit, and ran in C1000 Touch™ Thermal Cycler from Bio-Rad. PCR products were purified using QlAquick PCR Purification Kit from Qiagen (Germany).

GUIDER folding: All stock GUIDER solutions (100µM) were diluted to 500 nM in a 2mM $MgCl_2$ solution, unless otherwise noted, and then heated in a thermocycler to 90°C for two minutes with -0.2 min^{-1} incremental cooling over the 2 min period down to 20°C, unless otherwise noted.

GUIDER nucleation sequence, loop sequence, and fluorophore position optimization for increased GUIDER stability: We were able to compare the relative propensity of the variant GUIDER DNA molecules to fold into the desired hairpin structure by determining the percent fluorescent quenching of the various GUIDER solutions after heating and cooling. Single-

stranded GUIDER DNA molecules there were identical with the exception of their nucleation sequences, being either AA-TT or CC-GG (GUIDER(AA-TT) and GUIDER CC-GG, respectively) were purchased from IDT. Solutions of GUIDER were prepared to 500 nM final concentration and fluorescence of these solutions were measured prior to and after thermocycler annealing. Similarly, we purchased GUIDER-overhang from IDT, where the four quenching guanines were not base paired. Solutions prepared to a final concentration of 500 nM and fluorescence of these solutions were measured prior to and after thermocycler annealing. Experiments were conducted in duplicates and standard error were calculated for n=2.

In vitro cleavage assay with GUIDER: Four samples were prepared to the final volume of 10 µl containing: only GUIDER-1, GUIDER-1 and sgRNA-1, GUIDER-1 and Cas9, and GUIDER-1, sgRNA-1, and Cas9, where 300 mg of all components were maintained in every condition and were supplemented with MgCl (final concentration of 1mM). Incubating the respective sgRNA with Cas9 at 37°C for 15 min completed complexation of sgRNAs and Cas9. After incubation, heated GUIDER solutions were added into each sample respectively, and the samples were further incubated at 37°C for 1 h.

After one hour, 4 µl of Invitrogen Gel Loading Buffer was added into each sample respectively, and samples were transfered into Mini-Protean TGX Precast Gels (4-15%, 12 well comb, 20µl) with an addition lane loaded with 50 bp ladder. The gel was run under 170 V for 30 min, and was then stained in 40µl molecular grade mixed with 4µl SYBR Gold stain for 20 minutes and imaged using a BioRad gel imager.

PCSK9 PCR from cDNA: PCSK9 gene was amplified from cDNA using 50µl molecular grade water, 16µl GC Buffer, 1.6 µl dNTP mix, 4µl 10µM M13 Forward primer, 4 10µM M13 Reverse primer, 1µl of cDNA, and 0.8 µl of Phusion. The total volume was 80µl, and the final solution was separated into 4 tubes, with 20 µl of mixture in each. The PCR mixtures were then run in thermocycler with the protocol as follows: 98°C for 30 sec, 98°C for 10 sec, 61°C for 10 sec, 72°C for 10 sec (repeat for 38 cycles) and then holding at 72°C for 2 minutes prior to cooling to 4°C. The products were then purified using the PCR purification kit.

PCSK9 gRNA screen: In the wells of a 384-welled plate, samples of 500 nM GUIDER were incubated with 300 ng of sgRNA, Cas9, or RNP (prepared by incubating sgRNA to Cas9 at 37°C for 15 min). All wells were supplemented with 1mM MgCl (final concentration). Samples were incubated for 30 min at 37°C, after which time they were allowed to cool to room temperature and then analyzed using a plate reader. Fluorescence emission at 520 nm were collected, with excitation at 490 nm. Experiments were conducted in duplicates and standard error were calculated for n=2.

In vitro PCSK9 cleavage assay (with sgRNA-1, sgRNA-2, and sgRNA-3): A 2kb region of DNA corresponding to the cDNA for the mouse PCSK9 gene was obtained from Sino Biological and amplified as described above. 300 ng of sgRNA per reaction was heated in the thermocycler to 95°C for 5 minutes, and then cooled to 20°C at a rate of -1°C /min. 400 ng of Cas9 was added to reactions containing Cas9, MgCl to a final concentration of 2 mM, and molecular grade water to a final volume of 10 µL, allowing for the volume of cleavage template to be added (1µL). Solutions were incubated at 37°C for 15 min, and then 400 ng of cleavage template was added and the solutions were further incubated for 1 hr. Then, 4 µl of Invitrogen Gel Loading Buffer was added into each sample respectively, and samples were run into Mini-Protean TGX Precast Gels (4-15%, 12 well comb, 20µl) at 200 V for 20 min.

SUPPORTING FIGURES

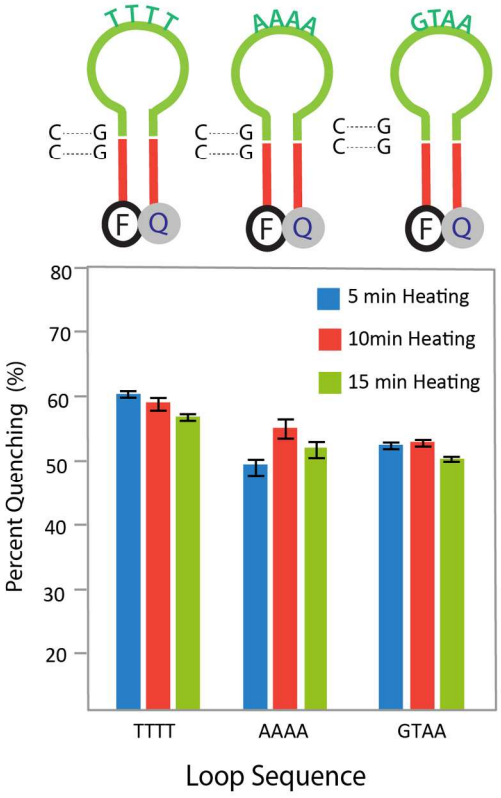

Figure S1. Loop sequence does not affect GUIDER hairpin stability. The calculated percent quenching of 3 GUIDER(GG-CC) hairpins are shown in the plot, where percent quenching is calculated as a percent difference between the fluorescence emission at 520 nm of the single-stranded GUIDER DNA molecule (denoted as "unfolded") and the final folded GUIDER hairpin (denoted as "folded") afforded after heating. A pictorial representation of each GUIDER hairpin is represented above the corresponding percent quenching values of the plot. Error bars in **c** represent s.d. where n = 3.

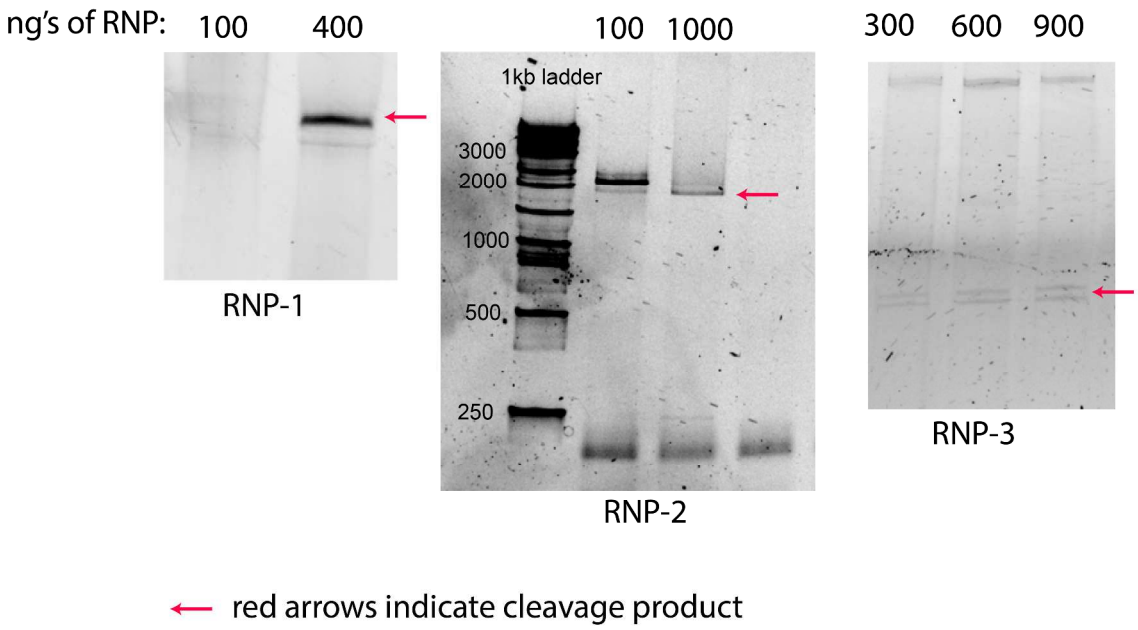

Figure S2. *In vitro* cleavage assay to measure sgRNA efficacy. In vitro cleavage assay performed to evaluate the efficacy of sgRNA-1, sgRNA-2, and sgRNA-3. RNP-1, RNP-2, and RNP-3 were serially diluted to determine the minimum amount required to afford complete cleavage of the *mpcsk9* DNA target. *Mpcsk9* cleavage template was incubated with each RNP for 1 h at 37ºC and then analyzed by PAGE. Red arrows highlight the resulting cleavage bands.