

Cell-type-specific genome editing with a microRNA-responsive CRISPR–Cas9 switch

Moe Hirosawa^{1,2}, Yoshihiko Fujita¹, Callum J. C. Parr¹, Karin Hayashi¹, Shunnichi Kashida¹, Akitsu Hotta¹, Knut Woltjen^{1,3} and Hirohide Saito^{1,*}

¹Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, ²Graduate School of Medicine, Kyoto University, Kyoto, Japan and ³Hakubi Center for Advanced Research, Kyoto University, Kyoto, Japan

Received December 19, 2016; Revised April 05, 2017; Editorial Decision April 07, 2017; Accepted May 09, 2017

ABSTRACT

The CRISPR–Cas9 system is a powerful genome-editing tool useful in a variety of biotechnology and biomedical applications. Here we developed a synthetic RNA-based, microRNA (miRNA)-responsive CRISPR–Cas9 system (miR-Cas9 switch) in which the genome editing activity of Cas9 can be modulated through endogenous miRNA signatures in mammalian cells. We created miR-Cas9 switches by using a miRNA-complementary sequence in the 5'-UTR of mRNA encoding *Streptococcus pyogenes* Cas9. The miR-21-Cas9 or miR-302-Cas9 switches selectively and efficiently responded to miR-21-5p in HeLa cells or miR-302a-5p in human induced pluripotent stem cells, and post-transcriptionally attenuated the Cas9 activity only in the target cells. Moreover, the miR-Cas9 switches could differentially control the genome editing by sensing endogenous miRNA activities within a heterogeneous cell population. Our miR-Cas9 switch system provides a promising framework for cell-type selective genome editing and cell engineering based on intracellular miRNA information.

INTRODUCTION

The bacterial and archaeal clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) system provided a powerful genome editing tool for a variety of biotechnology and biomedical applications (1,2). The engineered CRISPR–Cas9 system derived from *Streptococcus pyogenes* contains two components: the Cas9 endonuclease and a single guide RNA (sgRNA), which itself is a fusion of a designable CRISPR RNA (crRNA) and an universal trans-activating CRISPR RNA (tracrRNA). The Cas9 complex is recruited to a target DNA sequence by the sgRNA, forming an RNA–DNA

hybrid. Subsequently, the endonuclease activity of Cas9 creates a DNA double strand break (DSB) at the target site and triggers a host DNA repair pathways to induce genomic alterations. To introduce the system into mammalian cells, several delivery approaches for Cas9 and the sgRNA have been tested, including viral vectors, plasmid DNAs, synthetic RNAs, and ribonucleoproteins (RNPs) (3–5). DNA-based delivery systems may induce unwanted side effects. For example, gene therapy using virus vectors may integrate the transgene into host genomic regions randomly, and induce oncogenesis in some cases (6). It has also been reported that plasmid delivery of the CRISPR–Cas9 system may cause genomic integration of the DNA fragment derived from the plasmid at off-target sites (7). Detection of the inserted DNA fragment at off-target sites is difficult, and the insertion might cause problems to host cells. In contrast, RNA-based delivery approaches are proposed to be safer than DNA-based delivery, since the limited expression window for RNA could reduce the risk of off-target mutagenesis while also avoiding the possibility of random genomic integration (3,4,8). Additionally, by using synthetic genetic circuits delivered by modified mRNAs (8), one may be able to control Cas9 protein expression post-transcriptionally by sensing intracellular signals. However, the post-transcriptional regulation of Cas9 activity by employing synthetic mRNA has remained a challenge.

Multicellular organisms consist of various cell types, hence cell type-specific genome editing will be an important tool for restricting genetic modifications to target cells and regulating the cell-fate within sub-populations *in vitro* and *in vivo*. The ideal system is one that modifies the target cells without affecting other cell types. In order to regulate the endonuclease activity of Cas9, several methods have been reported, including optogenetic or small molecule/protein-inducible systems (9–11), a tissue-specific promoter system (12), and transcriptional regulatory circuits using split-Cas9 (13). However, these reports have predominantly used DNA delivery approaches. And cell type-selective genome edit-

*To whom correspondence should be addressed. Tel: +81 75 366 7029; Fax: +81 75 366 7096; Email: hirohide.saito@cira.kyoto-u.ac.jp

ing in a heterogeneous cell population has not been implemented previously.

MicroRNAs (miRNA) are small non-coding RNA that regulates gene expression through translational repression and/or mRNA degradation. The miRNA activities differ in each cell type and can therefore be used as a signature for a given target cell (14). We have previously reported a miRNA-responsive, synthetic mRNA (miRNA switch) that enables the detection and purification of target cells based on endogenous miRNA activities. By inserting a copy of the anti-sense sequence for the target miRNA (anti-miRNA) into the 5'-UTR of the mRNAs, the switch allows for sensitive detection of the target miRNA upon hybridization and subsequent post-transcriptional repression of a downstream protein (15). Additionally, these miRNA switches function with RNA delivery to avoid random genome integration and purify target cell populations with high efficiency and selectivity (15,16).

We hypothesized that a combination of miRNA switch technology with the CRISPR-Cas9 system (i.e. miRNA-responsive Cas9 system: miR-Cas9 switch) would enable the regulation of genome engineering in a miRNA-dependent manner (Figure 1). In the present study, we newly designed a miR-Cas9 switch that controlled Cas9 activity based on an endogenous miRNA expressed in target human cells. Cas9 protein was translated from a miR-Cas9 switch in the absence of the target miRNA (ON-state), but was repressed in its presence (OFF-state). We used this system to attenuate Cas9 activity in HeLa cells, human induced pluripotent stem cells (hiPSCs), iPSC-derived differentiated neuronal cells. In turn, we demonstrate that cell-type selective genome editing can be achieved in heterogeneous cell populations.

MATERIALS AND METHODS

Preparation of template DNA for IVT (*in vitro* transcription)

pAM-L7Ae was prepared according to the same method as pAM-tagBFP described in a previous report (15). The PCR product of tagBFP was inserted into modified pUC19 vector at a multi-cloning site to obtain pA9-tagBFP. The original sources of the genes and plasmid sequences are described in Supplementary Table S6. For the preparation of *Cas9* mRNA, *L7Ae* mRNA and *BFP* mRNA templates, a 5'-UTR without miRNA target sequences (control 5'-UTR) and a 3'-UTR were synthesized by hybridizing oligo-DNAs (oligo-DNAs lists are shown in Supplementary Table S1) followed by elongation; (94°C for 2 min, 13 cycles of 98°C for 10 s and 68°C for 10 s, and hold at 4°C). *Cas9*, *L7Ae* and *BFP* protein-coding regions were amplified by PCR with the appropriate primers (Supplementary Table S4) from pHL-EF1a-SphCas9-iC-A (Addgene, Plasmid #60599), pAM-L7Ae and pA9-tagBFP, respectively. The plasmid DNA was removed following PCR by Dpn I treatment. All PCR products were purified by MinElute PCR purification kit (QIAGEN). The PCR products were then fused to construct a full DNA template for IVT via an additional PCR reaction. We conducted gel extraction when non-specific bands appeared.

For the sgRNA template, a modified protocol (17) was used. Briefly, a forward primer containing the T7 promoter

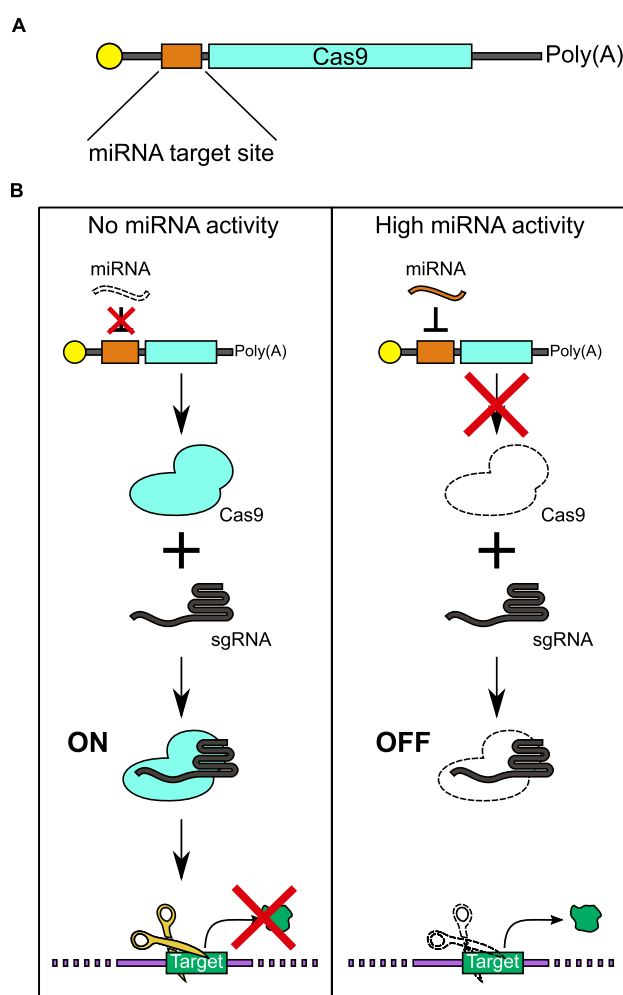


Figure 1. Schematic illustrations of the miR-Cas9 switch. (A) Design of miRNA-responsive *Cas9* mRNA (miR-Cas9 switch). The synthetic mRNA contains an anti-reverse cap analog (yellow), miRNA target site (orange), *Cas9* encoding sequence (cyan), and 120 nucleotide poly(A) tail. The miRNA target site is completely complementary to the miRNA of interest. (B) Overview of the miR-Cas9 switch system. The miR-Cas9 switch and sgRNA are introduced to cells by RNA transfection. *Cas9* protein expressed from the mRNA forms a *Cas9*-sgRNA complex and digests the DNA in the case of no miRNA activity (ON, left). In contrast, in the case of high miRNA activity, interaction between the miRNA and *Cas9* mRNA reduces *Cas9* expression (OFF, right).

sequence immediately followed by the gene-targeting sequence and a reverse primer encoding the remainder of the sgRNA sequence were used.

The complete list of combined primers and template for the PCR reactions is shown in Supplementary Table S4.

Cas9-, *L7Ae*- and *BFP*-mRNAs and sgRNA synthesis and purification

Cas9 mRNAs (with or without miRNA target sequences and with kink-turn motif), *L7Ae* mRNAs (with or without miRNA target sequences) and *BFP* mRNA (without miRNA target sequences) were prepared by using a MEGAscript kit (Ambion). In order to reduce the interferon response caused by long RNA,

pseudouridine-5'-triphosphate and 5-methylcytidine-5'-triphosphate (TriLink Bio Technologies) were used instead of natural rUTP and rCTP, respectively (18). Guanosine-5'-triphosphate was 5-fold diluted with an Anti Reverse Cap Analog (TriLink Bio Technologies) before the IVT reaction. The sgRNA was constructed using a MEGAscript kit (Ambion) according to the instruction manual. Because sgRNA with modified bases may cause downregulation of Cas9 activity, natural rNTPs were used for preparing sgRNA. The template DNA was degraded by TURBO DNase (Ambion), and the mRNAs and sgRNA were purified using a FavorPrep Blood/Cultured Cells total RNA extraction column (Favorgen Biotech) incubated with Antarctic Phosphatase (New England Biolabs) at 37°C for 30 min and then purified again using an RNeasy MinElute Cleanup Kit (QIAGEN). For further purification, sgRNA was electrophoresed, extracted from gel (10% polyacrylamide gel, 8.3 M urea), and ethanol-precipitated.

Cell culture

The heterozygously targeted AAVS1-CAG::GFP human iPSCs 317-12 line (hiPSC-EGFP) (19) was maintained in StemFit (Ajinomoto) on laminin-511 E8 (iMatrix-511, nippi). HeLa-EGFP cells were cultured in DMEM High Glucose (nacalai tesque) supplemented with 10% FBS (JBS) and Hygromycin B (50 µg/ml). Normal HeLa cells were cultured in DMEM High Glucose (nacalai tesque) supplemented with 10% FBS (JBS). All cell lines were cultured at 37°C with 5% CO₂.

RNA transfection

For all assays, HeLa-EGFP and normal HeLa cells were seeded in uncoated 24-well plates, while iPS-EGFP cells were seeded in laminin-coated 24-well plates (all cells were seeded at a density of 5×10^4 cells/well). Appropriate synthetic RNAs were transfected into the cells using a Stemfect RNA transfection kit according to the manufacturer's protocol (Stemgent). The medium was changed 4 h after transfection. Cells were cultured for 24 h (Evaluation of transfection efficiency, Evaluation of gene expression level and Evaluation of Cas9 protein expression level), 48 h (Cell killing assay by targeting Alu1 element) or 72 h (T7E1 assay, Cas9 activity assay, Co-culture assay for cell-type-selective genome editing and Construction of miR-Cas9-ON switch) after transfection. Before analysis, cultured cells were captured by an IX81 microscope with output to a CCD camera (Olympus).

Cas9 activity assay

100 ng Cas9 mRNA, 300 ng sgRNA and 5 pmol miRNA inhibitor/negative control (ThermoFisher, optional) were used. MiRNA negative control (referred to as negative miRNA inhibitor henceforth) was provided by ThermoFisher as mirVana™ miRNA Mimic Negative Control and is a random sequence Anti-miR molecule that has been extensively tested in human cell lines and tissues and validated to not produce identifiable effects on known miRNA function. At 72 h after RNA transfection of miR-Cas9

switch, cells were washed with PBS. HeLa-EGFP cells were treated with 100 µl 0.25% trypsin-EDTA, incubated at 37°C with 5% CO₂ for 5 min, and then 100 µl cultured medium was added. iPS-EGFP cells were treated with 200 µl Accumax (Funakoshi) and incubated at 37°C with 5% CO₂ for 10 min. These cells were collected into 1.5 ml tubes, stained with SYTOX Red dead-cell stain (ThermoFisher), and incubated at room temperature for 15 min with protection from ambient light. Samples were analyzed on an Aria-II (BD Biosciences), Accuri C6 (BD Biosciences) or a LSRFortessa (BD Biosciences) flow cytometer.

From histogram or dot plots (SSC-A versus EGFP), we gated for EGFP negative and positive populations, which correspond to successful or non-successful knock-out of EGFP gene by Cas9, respectively.

Co-culture assay for cell-type-selective genome editing

Before collection, cells were washed with PBS. Cells were treated with 200 µl Accumax (Funakoshi) and incubated at 37°C with 5% CO₂ for 10 min. These cells were collected into 1.5 ml tubes and centrifuged at 1000 rpm and room temperature for 5 min. After supernatant was discarded, 50 µl PBS + PI (BD Biosciences) mixture and 2.5 µl Alexa Fluor® 647 mouse anti-human TRA-1-60 antigen (BD Biosciences) were added and incubated at room temperature for 20 min with protection from ambient light. After incubation, 1 ml PBS was added and centrifuged at 1000 rpm and room temperature for 5 min (2 times). After adding 400 µl PBS, the samples were analyzed on an LSR (BD Biosciences) flow cytometer.

For immunostaining, transfected cells were treated with the following protocol. Cells were washed with PBS (2 times) and treated with 200 µl BD Cytofix™ fixation buffer (BD Biosciences) for 10 min at room temperature. After 10 min, buffer was removed, and cells were washed (2 times). Cells were stained with 200 µl antibody (Alexa Fluor® 647 mouse anti-human TRA-1-60 antigen (BD Biosciences)) diluted in PBS (5 µl antibody/200 µl PBS) for 1 h at room temperature with protection from ambient light. After staining, cells were washed with PBS (2 times). Upon adding 500 µl PBS, cells were captured by the IX81 microscope with output to the CCD camera (Olympus).

Construction of miR-Cas9-ON switch

Harvesting and analysis were done as in Cas9 activity assay above. Briefly, sample cells were collected 72 h after RNA transfection and stained with SYTOX Red dead-cell stain. These samples were analyzed on an Accuri flow cytometer.

Flow cytometry

An Accuri flow cytometer with FL1 (533/30 nm) and FL4 (675/25 nm) filters was used. EGFP and annexin V, Alexa Fluor 488 conjugate were detected with FL1, and SYTOX Red dead-cell stain was detected with FL4. EGFP, SYTOX Red dead-cell stain, PI and Alexa Fluor® 647 mouse anti-human TRA-1-60 antigen were detected with a blue laser (488 nm) and FITC filter (530/30 nm), a red laser (633 nm) and APC filter (660/20 nm), a blue laser and PE filter

(575/25 nm), and a red laser and APC filter, respectively, using Aria-II or LSR flow cytometers.

Statistical analysis

Student's *t* test or Welch's *t* test (Supplementary Figure S3 only) were used for the statistical analysis. The levels of significance are denoted as **P* < 0.05, ***P* < 0.01, ****P* < 0.001, n.s., not significant. Student's *t* test and Welch's *t* test were performed using Excel (Microsoft).

RESULTS

Construction of miR-Cas9 switch

We first constructed *Cas9* mRNA that contains a miRNA-responsive anti-sense sequence (anti-miR) positioned within the 5'-UTR (Figure 1A). When the miRNA is inactive in cells, Cas9 protein will be produced from *Cas9* mRNA and result in endonuclease activity that cleaves a target DNA sequence (Figure 1B, left). In contrast, when the miRNA is active in the cell, the expression from the *Cas9* mRNA will be repressed through the interaction between the anti-miR region of the *Cas9* mRNA and the endogenous miRNA. This interaction will cause post-transcriptional repression of Cas9 expression, reducing the level of DNA cleavage (Figure 1B, right). To evaluate the activity of Cas9, we employed sgRNA_{GFP}, which cleaves an enhanced green fluorescent protein (EGFP) reporter gene integrated into the host cell's genome, and quantified Cas9 activity by assessing the loss of the EGFP fluorescence intensity (20).

We investigated whether the designed miR-Cas9 switch could control Cas9 endonuclease activity in HeLa-EGFP cells that constitutively express EGFP (Figure 2). In this study, we focused on miR-21-5p (miR-21) and miR-302a-5p (miR-302). MiR-21 is highly expressed in many cancer cells including HeLa cells and regulates tumor suppressor genes associated with proliferation, apoptosis and invasion (e.g. PTEN, PDCD4, BCL-2) (21,22). MiR-302 is predominantly expressed in human pluripotent stem cells including hiPSCs (15,23) and regulates pluripotency, proliferation, and cell cycle-related genes (e.g. MBD2, AKT1, CDK4). Indeed, we confirmed that miR-21 and miR-302 are highly expressed in HeLa cells and hiPSCs, respectively (Supplementary Figure S1). Thus, we chose miR-21 and miR-302 as target miRNAs to conduct HeLa cell- or hiPSC-specific genome editing.

We prepared three *Cas9* mRNAs, control *Cas9* mRNA without anti-miR sequence, miR-21-responsive *Cas9* mRNA (miR-21-Cas9 switch), and miR-302-responsive *Cas9* mRNA (miR-302-Cas9 switch). We expected that Cas9 expression from the miR-21-Cas9 switch would be repressed in the presence of endogenous miR-21, resulting in a loss of Cas9 activity in HeLa cells. We co-transfected either control *Cas9* mRNA or miR-21-Cas9 switch and sgRNA_{GFP} into HeLa-EGFP cells. Three days after transfection, we analyzed the EGFP intensity by fluorescence microscopy and flow cytometry. These analyses showed a decrease in EGFP expression in control *Cas9* mRNA-transfected cells compared with untreated cells, indicating successful knock-out of the *EGFP* gene by *Cas9* mRNA

and sgRNA_{GFP} (Figure 2A and B). In contrast, there was no significant loss in EGFP expression in miR-21-Cas9 switch-transfected cells. The fluorescence intensity was similar to that of untreated cells or cells transfected with *Cas9* mRNA and negative control sgRNA_{DMD}, which targets an unrelated genomic region (human Dystrophin gene, DMD) (24) (Figure 2A and B). To investigate the selectivity of our miR-Cas9 switch, we also transfected the miR-302-Cas9 switch, which was designed to respond to miR-302, a miRNA not expressed in HeLa cells (Supplementary Figure S1B). We observed a decrease in EGFP intensity to a level similar to positive control *Cas9* mRNA, suggesting that the insertion of unrelated miRNA-responsive sequences in the 5'UTR region of *Cas9* mRNA does not affect its translation efficiency (Figure 2A and B). We further quantified Cas9 activities based on the fluorescence intensity obtained by flow cytometry analysis (Figure 2C). We observed a decrease in EGFP intensity in control *Cas9* mRNA and miR-302-Cas9 switch transfected cells (~50% of the cell population was EGFP-low), but EGFP intensity from miR-21-Cas9 switch transfected-cells was again similar to that of untreated cells or cells transfected with *Cas9* mRNA and negative control sgRNA_{DMD} (~90% of cells were EGFP-positive). In addition, we confirmed Cas9 protein expression levels from each mRNA by capillary-based immunodetection. We detected Cas9 protein produced from *Cas9* mRNA and miR-302-Cas9 switch but not from miR-21-Cas9 switch (Supplementary Figure S2). These results indicate that Cas9 expression from miR-21-Cas9 switch was regulated by endogenous miR-21 in HeLa cells.

To confirm whether endogenous miR-21 is responsible for controlling Cas9 activity in HeLa-EGFP cells, the miR-21-Cas9 switch was co-transfected with either miR-21 inhibitor or miRNA inhibitor unrelated to miR-21 (negative inhibitor). We observed a decrease in EGFP intensity (i.e. increase of Cas9 activity) in the case of co-transfection with miR-21-Cas9 switch and miR-21 inhibitor, but not with miR-21-Cas9 switch and negative inhibitor (Figure 2D), indicating the rescue of Cas9 activity by inhibiting endogenous miR-21 activity. These results indicated that endogenous miR-21 in HeLa-EGFP cells is indispensable in the repression of Cas9 protein expression from the miR-21-Cas9 switch. Thus, we succeeded in constructing a synthetic RNA-delivered, miRNA-responsive Cas9 system in mammalian cells.

miR-Cas9 switch that targets endogenous Alu1 element

Next, we investigated the versatility of our switch by targeting a different region of the endogenous genome. We focused on the Alu1 element, the most common short interspaced element (SINE) in humans, distributed as thousands of copies throughout the human genome (25). We hypothesized that the cleavage of Alu1 regions by Cas9 would induce genotoxic stress resulting in cell death (Supplementary Figure S3A). We designed sgRNA targeting the Alu1 region (sgRNA_{Alu1}), which exists in >200 000 sites in the human genome (219 114 sites in hg19), and transfected sgRNA_{Alu1} and *Cas9* mRNA in HeLa cells. As expected, the number of dead cells increased in the case of treatment with con-

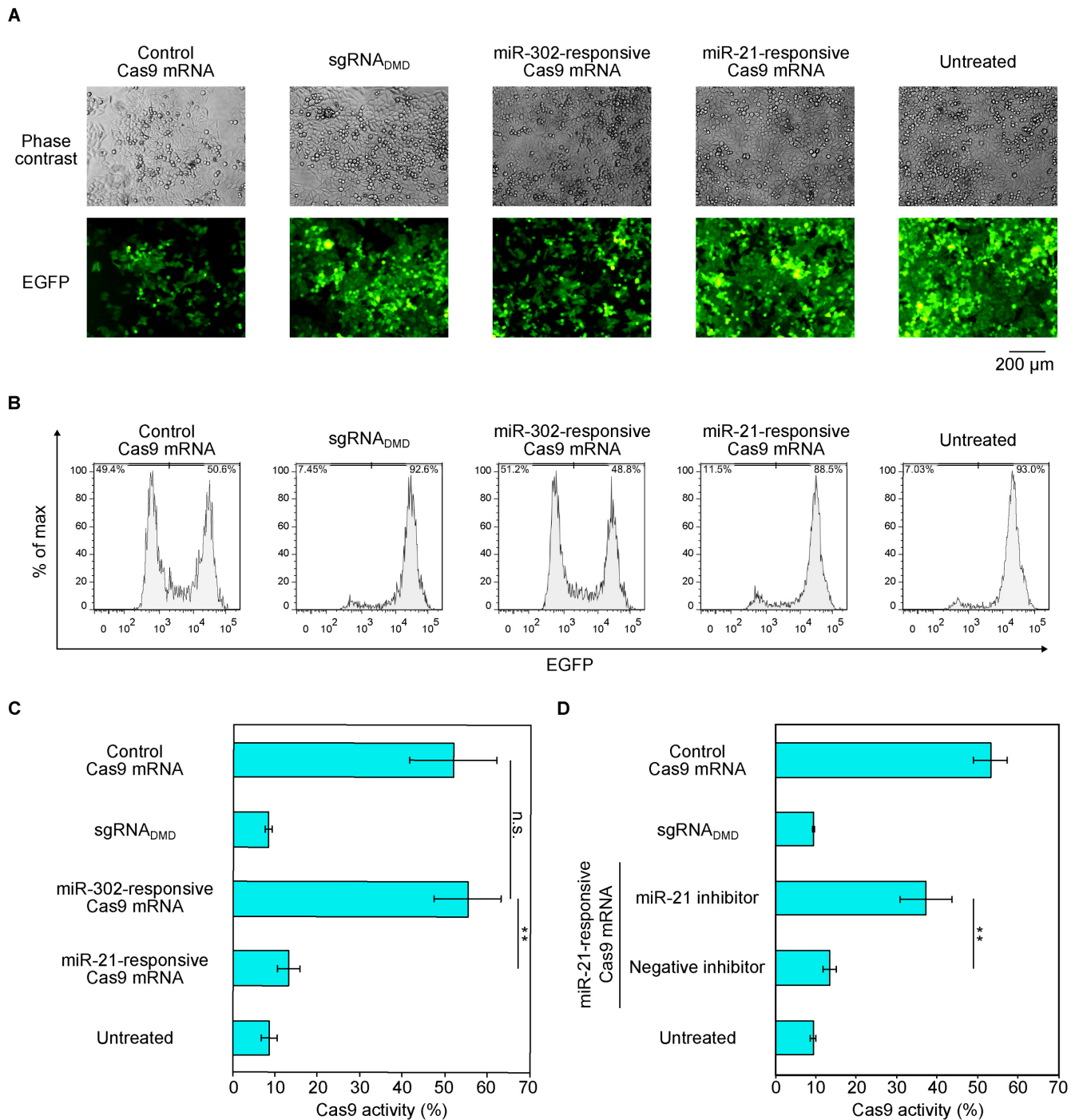


Figure 2. Regulation of Cas9 activity in HeLa cells by miR-21-Cas9 switch. (A) Representative phase contrast and fluorescent microscopic images at 72 h after RNA transfection. Scale bar represents 200 μ m. (B) Representative flow cytometry histograms of EGFP expression. x- and y-axes indicate EGFP intensity and % of max, respectively. (C) Quantification of Cas9 activity from (B). The percentage of EGFP negative cells was defined as Cas9 activity. Cas9 activity was reduced in miR-21-responsive Cas9 mRNA. (D) Verification of endogenous miR-21-dependent Cas9 silencing by using miR-21 inhibitor. The miR-21 inhibitor rescued Cas9 activity in miR-21-responsive Cas9 mRNA, whereas a negative miRNA inhibitor did not. For the negative control, cells were transfected with Cas9 mRNA and a non-targeting sgRNA (sgRNA_{DMD}). Error bars indicate the mean $\pm$ SD ($n = 3$).

trol *Cas9* mRNA and sgRNA_{Alu1}, whereas cells untreated or treated with control *Cas9* mRNA and sgRNA_{DMD} maintained a normal cell phenotype. Importantly, cells treated with miR-21-Cas9 switch and sgRNA_{Alu1} did not increase the number of dead cells, indicating that our miR-Cas9 switch can target endogenous genomic sequences and selectively control cell death signals (Supplementary Figure S3B–D).

miR-302-Cas9 switch modulates genome editing in human iPS cells

To further test our miR-Cas9 switch in different cell types, we next chose a human iPS cell line (201B7) that contains a single copy of the *EGFP* gene at the AAVS1 locus, which is often considered a safe-harbor for transgene targeting (hiPSC-EGFP) (19). We used the miR-302-Cas9 switch to selectively repress Cas9 activity in hiPSCs. Although the miR-302-Cas9 switch did not affect the Cas9 activity in HeLa cells (Figure 2A–C), we observed a repression of Cas9 activity (recovery of EGFP intensity) in hiPSCs transfected with miR-302-Cas9 switch and negative miRNA inhibitor, and de-repression of Cas9 activity in hiPSCs transfected with miR-302-Cas9 switch and miR-302 inhibitor. These results suggest that the suppression is dependent on endogenous miR-302 in hiPSCs (Supplementary Figure S4A).

We also performed T7EI assays to investigate whether the change in EGFP intensity observed in the flow cytometry analysis was caused by mutations in the target *EGFP* gene due to the Cas9 activity. Treating the DNA products from genomic PCR with T7 Endonuclease I, we observed the target cleaved bands in cells transfected with *Cas9* mRNA, or with miR-302-Cas9 switch and miR-302 inhibitor (Supplementary Figure S5). However, we did not observe these bands in cells treated with miR-302-Cas9 switch and negative miRNA inhibitor. The percentage of insertions and deletions (indels) at the *EGFP* gene as assessed by T7EI analysis showed similar trends as the flow cytometry analysis (Supplementary Figure S4A versus Supplementary Figure S5B), indicating that the loss of EGFP fluorescence intensity correlated with Cas9 nuclease activity. Moreover, we performed DNA sequencing and observed mutations at the target *EGFP* region in cells transfected with control *Cas9* mRNA and miR-302-Cas9 switch with miR-302 inhibitor, but did not observe them with miR-302-Cas9 switch and negative miRNA inhibitor (Supplementary Figure S6). These results confirm that miR-302-Cas9 switch repressed Cas9-mediated genome engineering by detecting endogenous miR-302 in hiPSCs. Thus, we selectively controlled the activity of Cas9, in two different cell-types (HeLa and hiPSC cells) by using miR-21- and miR-302-Cas9 switches, respectively. In addition, these two switches had negligible effect on endogenous mRNA expressions regulated by miR-21 or miR-302 (Supplementary Figure S7).

The Cas9 activity in hiPSCs was slightly lower (~30%) than that in HeLa cells (~50%), even though the sgRNA and mRNA transfection efficiency was high in both cells (~90% efficiency for both cell types, Supplementary Figure S8) (16). To further increase the Cas9 activity and miR-302 sensitivity in hiPSCs, we engineered sgRNA and miRNA-responsive elements embedded in the 5'UTR of *Cas9*

mRNA (Supplementary Text). We redesigned sgRNA_{GFP} by removing the first tandem GG sequences (these do not hybridize with the target EGFP sequence), in order to increase the binding efficiency between the sgRNA and the target sequence (26). To enhance the miRNA binding, we inserted four tandem copies of anti-miR-302 sequences (instead of one copy) in the 5'-UTR region of the switch (4× miR-302-Cas9 switch). The resulting switch increased Cas9 activity (~60%) and responded to endogenous miR-302 to modulate Cas9 production, because miR-302 inhibitor recovered Cas9 activity (Figure 3). Thus, our miRNA-responsive Cas9 switch could be fine-tuned the balance between Cas9 activity and miRNA sensitivity.

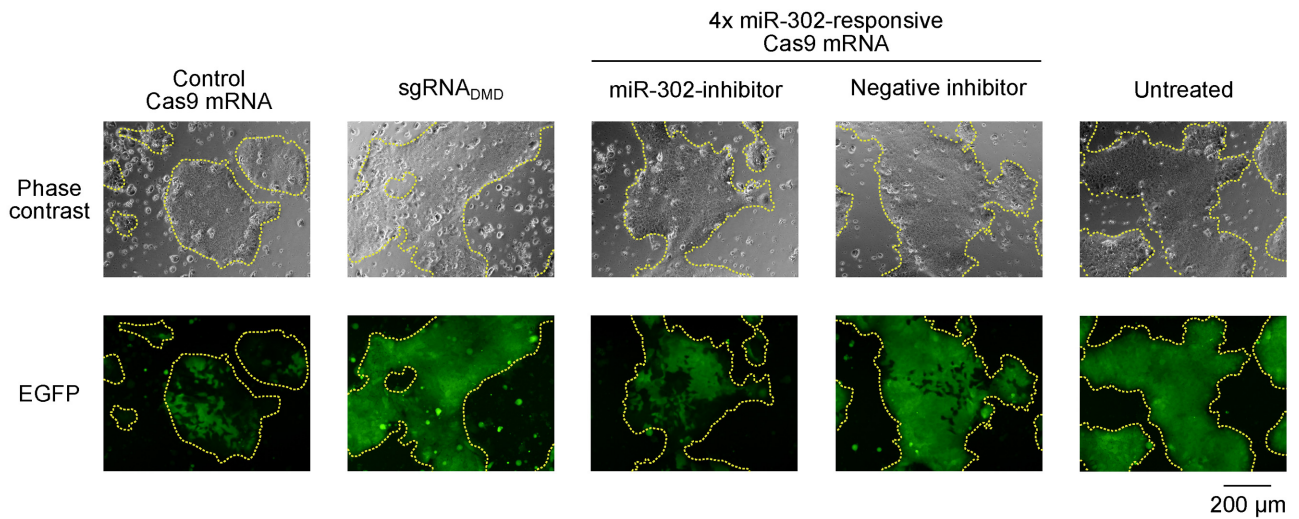
miR-302-Cas9 switch distinguishes hiPSCs and differentiated neurons

The activities of miRNAs dynamically change during differentiation. For example, miR-302 is highly expressed in undifferentiated hiPSCs but repressed to basal level in mid-brain dopaminergic (mDA) neuronal cells differentiated from hiPSCs (16) (Supplementary Figure S1B, iPS versus mDA). We expected that miR-302-Cas9 switch could modulate Cas9 activity before and after differentiation and cleave a target gene in differentiated neurons but not in hiPSCs. Therefore, we differentiated hiPSCs into mDA neurons (16) and transfected the miR-302-Cas9 switch and sgRNA_{2GFP} into these cells. As expected, in contrast to the results from hiPSCs, we observed Cas9 activity that was similar between control *Cas9* mRNA and miR-302-responsive *Cas9* mRNA (Supplementary Figure S9).

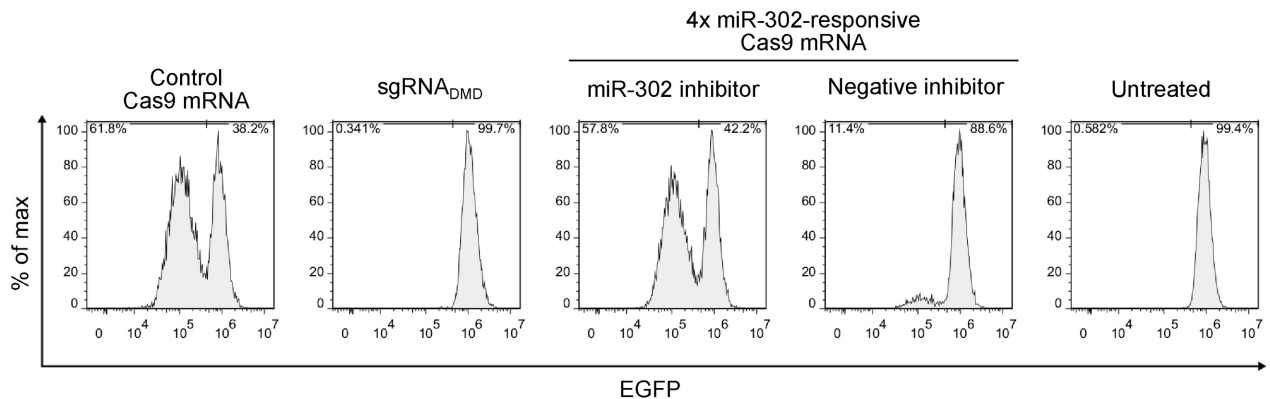
Cell-type selective genome editing in mixed cell populations

We next investigated whether miR-Cas9 switch could distinguish cells in mixed heterogeneous cell populations. Because the translation from the miR-21- and miR-302-responsive *Cas9* mRNAs was selectively repressed in HeLa cells and hiPSCs, respectively, we applied our system to distinguish these two cell types. We mixed HeLa-EGFP and hiPS-EGFP cells in a dish and aimed to knockout HeLa-EGFP cells but not hiPS-EGFP cells, by employing the 4× miR-302-Cas9 switch (Figure 4A). To distinguish the two cell types, we stained the mixed cells with the TRA-1-60 antibody (a cell surface marker specific to undifferentiated pluripotent stem cells) for flow cytometry analysis. When control (non-regulated) *Cas9* mRNA was transfected into the cell mixture, EGFP negative (knocked-out) cells were observed in both HeLa cells and hiPSCs. In contrast, when the 4× miR-302-Cas9 switch was transfected into the mixed cell population, the EGFP negative population was mainly observed in HeLa cells but not in hiPSCs (Figure 4B–D). This result showed that the miR-302-responsive *Cas9* mRNA was preferentially translated to produce Cas9 in HeLa cells compared with hiPSCs, facilitating the genome editing activity in HeLa but preventing it in hiPSCs. Thus, our miRNA-responsive Cas9 system distinguishes different cell types within a heterogeneous cell population.

A



B



C

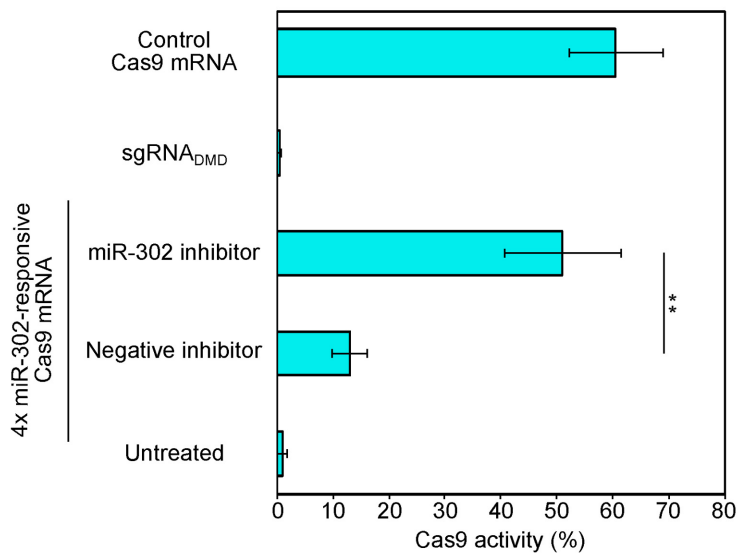


Figure 3. Regulation of Cas9 activity in iPS cells by 4x miR-302-Cas9 switch and improved sgRNA. (A) Representative phase contrast and fluorescent microscopic images at 72 h after RNA transfection. iPS-EGFP cells formed colonies (yellow dashed lines). Scale bar represents 200 μm . (B) Representative flow cytometry histograms of EGFP expression. x- and y-axes indicate EGFP intensity and % of max, respectively. (C) Quantification of Cas9 activity from (B). A miR-302 inhibitor rescued Cas9 activity expressed from miR-302-responsive *Cas9* mRNA, whereas a negative miRNA inhibitor did not. To improve this system, 4x miR-302-responsive Cas9 mRNA (4 copies of miR-302 target site were tandemly inserted into 5'-UTR) and $sgRNA_{2GFP}$ (removal of GG from 5' terminal) were used. Negative control is Cas9- $sgRNA_{DMD}$ that targets *DMD* gene. Error bars indicate the mean $\pm$ SD ($n = 3$).

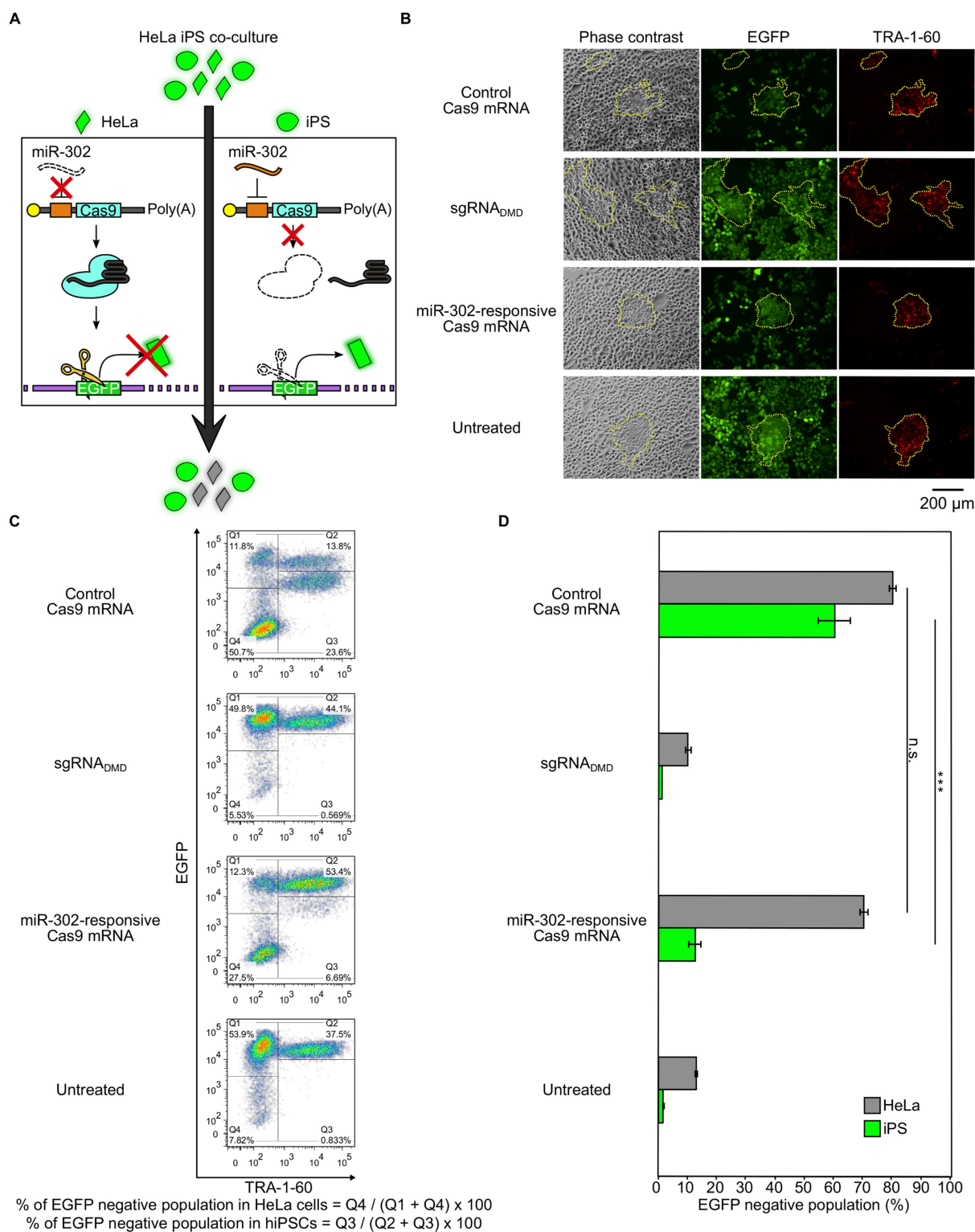


Figure 4. Cell-type-selective genome editing in a mixed cell population. (A) Schematic illustration of cell-type-selective gene knockout in a mixed cell population. HeLa-EGFP and iPS-EGFP cells are mixed (HeLa/iPS co-culture), and 4× miR-302-responsive *Cas9* mRNA is used. Because miR-302 activity is high in iPS cells, but not in HeLa cells, Cas9 protein is selectively produced in HeLa cells (low EGFP intensity). (B) Representative fluorescent microscopic images at 72 h after RNA transfection. Phase contrast, EGFP and TRA-1-60 indicate phase contrast, EGFP fluorescent images and Alexa Fluor 647 signal, respectively. Areas in the yellow dashed lines indicate TRA-1-60 positive hiPSCs. Scale bar represents 200 μm. (C) Representative dot plots. x- and y-axes indicate TRA-1-60 and EGFP, respectively. Q1 and Q4 indicate HeLa cells (TRA-1-60 negative). Q2 and Q3 indicate hiPSCs (TRA-1-60 positive). (D) EGFP knockout efficiencies of hiPSCs and HeLa cells in a co-culture condition. Bar graphs show EGFP negative populations. Gray and light green boxes show HeLa and hiPSCs, respectively. 4× miR-302-responsive *Cas9* mRNA was used for the experiments. Negative control is Cas9-sgRNA_{DMD} that targets *DMD* gene. Error bars indicate the mean ± SD ($n = 3$).

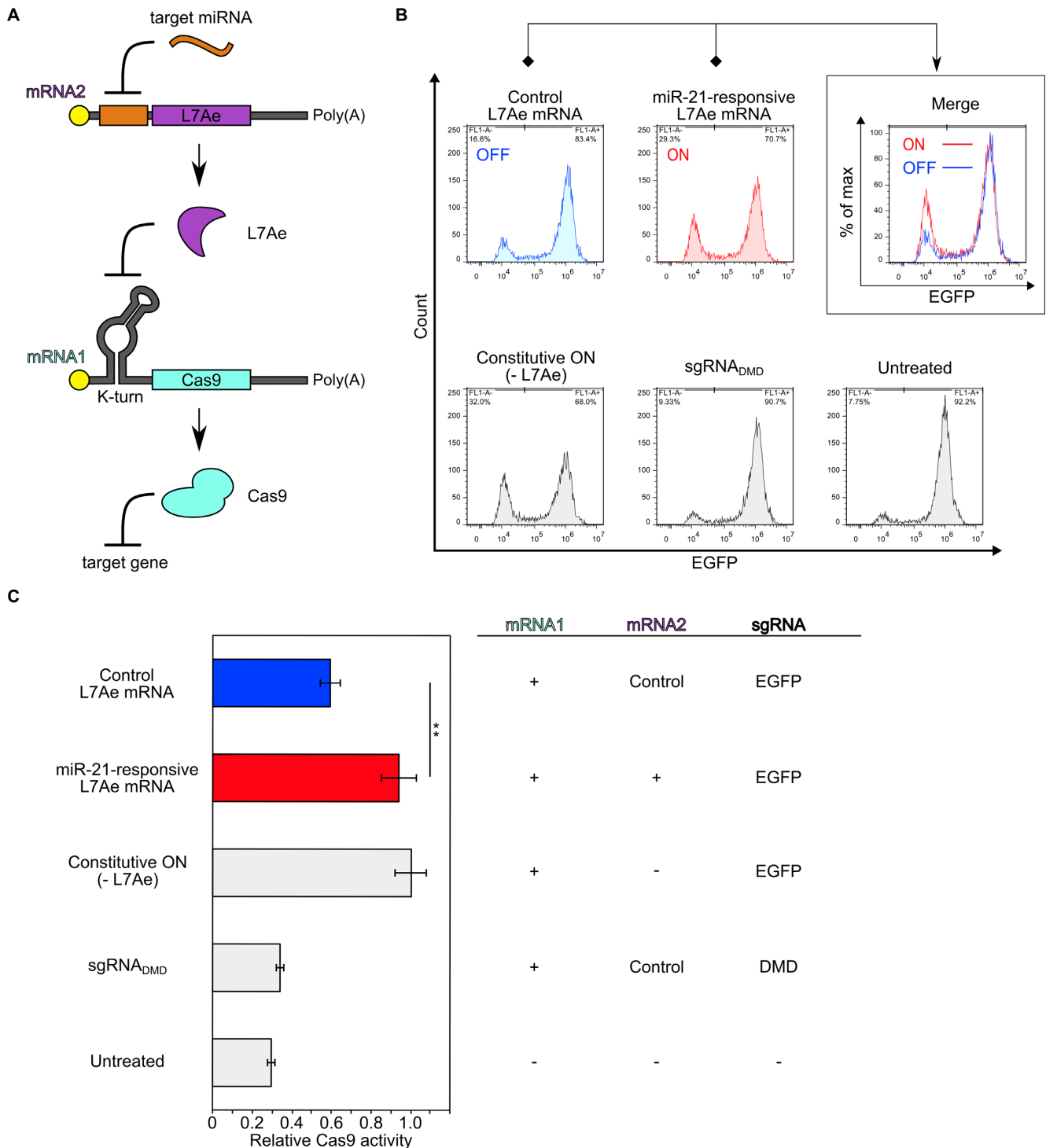


Figure 5. Construction of miR-Cas9-ON switch. (A) Mechanism of the ON system. The expressions of L7Ae from mRNA2 and Cas9 protein from mRNA1 are suppressed by target miRNA (here, miR-21) and L7Ae, respectively. L7Ae suppresses the translation of Cas9 protein by interacting with its binding motif (K-turn), and miR-21 suppresses the translation of L7Ae by interacting with its anti-sense region, resulting in Cas9 translation. (B) Flow cytometry histograms of EGFP expression. The x- and y-axes indicate EGFP intensity and cell number, respectively. An increase of the EGFP negative population was observed in miR-21-responsive L7Ae mRNA (ON) compared with control L7Ae mRNA (OFF). This experiment was repeated three times and representative histograms are shown. Control L7Ae mRNA (L7Ae mRNA without miRNA-responsive element). Constitutive ON (without L7Ae mRNA). (C) Quantification of relative Cas9 activity from (B). An increase of the EGFP negative population was observed in miR-21-responsive L7Ae mRNA (ON) compared with control L7Ae mRNA (OFF). Each Cas9 activity was normalized by Constitutive ON (- L7Ae). Control L7Ae mRNA (L7Ae mRNA without miRNA-responsive element). Constitutive ON (without L7Ae mRNA). Negative control is Cas9-sgRNA_{DMD} that targets DMD gene. Error bars indicate the mean $\pm$ SD ($n = 3$).

miR-Cas9-ON switch that enhances Cas9 production by sensing target miRNA

Thus far, our miR-Cas9 switch system was able to achieve potent Cas9 repression in target cells. In order to address Cas9 activation in a cell-type specific manner, we constructed a miR-Cas9-ON switch, which up-regulates the translation of *Cas9* mRNA by responding to a target miRNA (Figure 5). We mimicked the topology of a cell-classifier circuit that is composed of two mRNA species (*Cas9*-coding mRNA1 and RNA-binding protein (*L7Ae*)-coding mRNA2) (8). We designed the 5'-UTR sequence of each mRNA to contain either the *L7Ae*-binding kink-turn RNA motif (mRNA1) or the miR-21-responsive antisense sequence (mRNA2). In the absence of the target miRNA activity, *L7Ae* will be produced from mRNA2 and repress the translation of *Cas9* from mRNA1 through the *L7Ae*-kink-turn RNA interaction. In contrast, in the presence of the target miRNA, *L7Ae* translation is repressed, which in turn de-represses *Cas9* translation (Figure 5A). As designed, the co-transfection of both mRNAs and the sgRNA increased the level of *Cas9* activity by responding to miR-21 in HeLa cells compared with untreated cells or cells transfected with mRNA1 and control *L7Ae* mRNA without miR-21-responsive element (Figure 5B, C). Thus, this system can selectively increase the level of *Cas9* activity in target cells, by sensing the miRNA. Thus, both miR-Cas9-OFF and miR-Cas9-ON switches can be constructed to detect endogenous miRNA signatures in mammalian cells, expanding the possibilities for selective genome editing.

DISCUSSION

In this study, we developed a miRNA-responsive Cas9 switch system (miR-Cas9 switch) that is capable of performing cell-type selective genome editing by detecting an endogenous miRNA signature, *via* a synthetic RNA delivery approach. We can repress or activate *Cas9* expression in the presence or absence of the target miRNA, respectively, by employing our miR-Cas9-OFF switch. Our method can selectively control *Cas9* activity that cleaved either an exogenous reporter gene (EGFP) integrated into the genome or an endogenous repetitive genomic sequence (Alu1) by detecting endogenous miRNA signatures: miR-21-5p and miR-302a-5p in HeLa and hiPSCs, respectively (Figures 2 and 3). We also demonstrated the application of our miR-Cas9 switch for the selective induction of genome editing in HeLa cells within a mixed (hiPSCs and HeLa) cell population (Figure 4). Additionally, by employing a cell classifier circuit with an RNA-binding protein (*L7Ae*), we constructed a miR-Cas9-ON switch, which up-regulates *Cas9* activity when the target miRNA is present in the cell (Figure 5).

We believe that our miR-Cas9 switch could provide a promising framework to engineer genomes of target cells for the following reasons: (1) RNA delivery approaches are valuable for CRISPR-Cas9 systems, as synthetic RNA may avoid potentially harmful genomic integration (8). (2) Our system can be used as a cell-type selective genome-editing tool that responds to endogenous miRNA signatures in target cells. Unlike non-cell autonomous regulation

systems (e.g. small chemicals or light), *Cas9* activity is controllable dependent on cell-state (miRNA activity). In fact, we demonstrated cell-type-selective genome editing capability in a heterogeneous cell population (Figure 4), although further improvement of the performance of the OFF and ON switches (e.g. avoiding translation of *Cas9* protein from kink-turn-*Cas9* mRNA in the presence of *L7Ae*) is important to reduce leaky expression of *Cas9* in non-target cells. This improvement could be achieved by employing switches that detect multiple miRNAs (15). (3) MiRNA activities dynamically change with development and cell reprogramming. Thus, by choosing an appropriate miRNA signature that represents the target cell type or the cell state, we could selectively control genome editing activity in a variety of cell types. To reach this potential, it will be important to expand our knowledge about active and distinctive miRNA signature(s) in different cell types (15). (4) Finally, our technology should be applicable to other *Cas9* variants (e.g. orthologs, engineered *Cas9*/d*Cas9* proteins (27,28)) and other miRNAs in a plug-and-play manner. Additionally, we believe that our miR-Cas9 switch could have important medical applications. For example, we could maintain or purify target cells (e.g. iPS cells) but selectively eliminate non-target cells by targeting repetitive elements (e.g. Alu1) using miR-Cas9 switch. Also, in order to develop a safe and selective genome editing system *in vivo*, we may be able to avoid unexpected off-target effects in non-target cells (e.g. germ cells), by using germ cell-specific miRNA-responsive switch. The transient and conditional expression of our system using synthetic RNA could provide genome editing as a potentially 'one-shot therapy' to treat numerous genetic diseases. In conclusion, our miR-Cas9 switch combined with a RNA-delivery approach can selectively engineer genomes of target cells by detecting an endogenous miRNA signature, with potential benefits for synthetic biology, genome editing field and future therapeutic applications.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

We thank Dr Hongmei Lisa Li (Kyoto University), Dr Kanae Mitsunaga (Kyoto University) and Saito laboratory members for kind advice about the experimental conditions, data analysis and discussion. We also thank Dr Peter Karagiannis (Kyoto University) and Ms Yukiko Nakagawa & Miho Nishimura for critical reading of the manuscript and administrative support, respectively.

FUNDING

JSPS KAKENHI [15H05722, 24104002, 15H02376 to H.S. and 15H05581 to A.H.]; Naito Foundation (to H.S.); Canon Foundation (to H.S.); Nakatani Foundation (to H.S.); JSPS Fellows [16J10715 to M.H.]. Funding for open access charge: JSPS KAKENHI [15H05722 to H.S.].

Conflict of interest statement. Kyoto University has filed a patent application broadly relevant to this work. M. Hiro-sawa and H. Saito are the investigators of record listed on the patent application.

REFERENCES

- Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L.A. *et al.* (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science*, **339**, 819–823.
- Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., DiCarlo, J.E., Norville, J.E. and Church, G.M. (2013) RNA-guided human genome engineering via Cas9. *Science*, **339**, 823–826.
- Liang, X., Potter, J., Kumar, S., Zou, Y., Quintanilla, R., Sridharan, M., Carte, J., Chen, W., Roark, N., Ranganathan, S. *et al.* (2015) Rapid and highly efficient mammalian cell engineering via Cas9 protein transfection. *J. Biotechnol.*, **208**, 44–53.
- Yin, H., Song, C.-Q., Dorkin, J.R., Zhu, L.J., Li, Y., Wu, Q., Park, A., Yang, J., Suresh, S., Bizhanova, A. *et al.* (2016) Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. *Nat. Biotechnol.*, **34**, 328–333.
- Nelson, C.E., Hakim, C.H., Ousterout, D.G., Thakore, P.I., Moreb, E.A., Rivera, R.M.C., Madhavan, S., Pan, X., Ran, F.A., Yan, W.X. *et al.* (2016) In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. *Science*, **351**, 403–407.
- Thomas, C.E., Ehrhardt, A. and Kay, M.A. (2003) Progress and problems with the use of viral vectors for gene therapy. *Nat. Rev. Genet.*, **4**, 346–358.
- Kim, S., Kim, D., Cho, S.W., Kim, J. and Kim, J.-S. (2014) Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. *Genome Res.*, **24**, 1012–1019.
- Wroblewska, L., Kitada, T., Endo, K., Siciliano, V., Stillo, B., Saito, H. and Weiss, R. (2015) Mammalian synthetic circuits with RNA binding proteins for RNA-only delivery. *Nat. Biotechnol.*, **33**, 839–841.
- Hemphill, J., Borchardt, E.K., Brown, K., Asokan, A. and Deiters, A. (2015) Optical Control of CRISPR/Cas9 Gene Editing. *J. Am. Chem. Soc.*, **137**, 5642–5645.
- Zetsche, B., Volz, S.E. and Zhang, F. (2015) A split-Cas9 architecture for inducible genome editing and transcription modulation. *Nat. Biotechnol.*, **33**, 139–142.
- Liu, Y., Zhan, Y., Chen, Z., He, A., Li, J., Wu, H., Liu, L., Zhuang, C., Lin, J., Guo, X. *et al.* (2016) Directing cellular information flow via CRISPR signal conductors. *Nat. Methods*, **13**, 938–944.
- Liu, Y., Zeng, Y., Liu, L., Zhuang, C., Fu, X., Huang, W. and Cai, Z. (2014) Synthesizing AND gate genetic circuits based on CRISPR–Cas9 for identification of bladder cancer cells. *Nat. Commun.*, **5**, 5393.
- Ma, D., Peng, S. and Xie, Z. (2016) Integration and exchange of split dCas9 domains for transcriptional controls in mammalian cells. *Nat. Commun.*, **7**, 13056.
- Mullokandov, G., Baccarini, A., Ruza, A., Jayaprakash, A.D., Tung, N., Israelow, B., Evans, M.J., Sachidanandam, R. and Brown, B.D. (2012) High-throughput assessment of microRNA activity and function using microRNA sensor and decoy libraries. *Nat. Methods*, **9**, 840–846.
- Miki, K., Endo, K., Takahashi, S., Funakoshi, S., Takei, I., Katayama, S., Toyoda, T., Kotaka, M., Takaki, T., Umeda, M. *et al.* (2015) Efficient detection and purification of cell populations using synthetic MicroRNA switches. *Cell Stem Cell*, **16**, 699–711.
- Parr, C.J.C., Katayama, S., Miki, K., Kuang, Y., Yoshida, Y., Morizane, A., Takahashi, J., Yamanaka, S. and Saito, H. (2016) MicroRNA-302 switch to identify and eliminate undifferentiated human pluripotent stem cells. *Sci. Rep.*, **6**, 32532.
- Bassett, A.R., Tibbit, C., Ponting, C.P. and Liu, J.-L. (2013) Highly efficient targeted mutagenesis of *Drosophila* with the CRISPR/Cas9 system. *Cell Rep.*, **4**, 220–228.
- Warren, L., Manos, P.D., Ahfeldt, T., Loh, Y., Li, H., Lau, F., Ebina, W., Mandal, P.K., Smith, Z.D., Meissner, A. *et al.* (2010) Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell*, **7**, 618–630.
- Ocegüera-Yáñez, F., Kim, S.-I., Matsumoto, T., Tan, G.W., Xiang, L., Hatani, T., Kondo, T., Ikeya, M., Yoshida, Y., Inoue, H. *et al.* (2016) Engineering the AAVS1 locus for consistent and scalable transgene expression in human iPSCs and their differentiated derivatives. *Methods*, **101**, 43–55.
- Fu, Y., Foden, J.A., Khayter, C., Maeder, M.L., Reyon, D., Joung, J.K. and Sander, J.D. (2013) High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat. Biotechnol.*, **31**, 822–826.
- Feng, Y. and Tsao, C. (2016) Emerging role of microRNA-21 in cancer (review). *Biochem. Rep.*, **5**, 395–402.
- Xie, Z., Wroblewska, L., Prochazka, L., Weiss, R. and Benenson, Y. (2011) Multi-Input RNAi-based logic circuit for identification of specific cancer cells. *Science*, **333**, 1307–1311.
- Subramanyam, D., Lamouille, S., Judson, R.L., Liu, J.Y., Bucay, N., Derynck, R. and Belloch, R. (2011) Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. *Nat. Biotechnol.*, **29**, 443–448.
- Li, H.L., Fujimoto, N., Sasakawa, N., Shirai, S., Ohkame, T., Sakuma, T., Tanaka, M., Amano, N., Watanabe, A., Sakurai, H. *et al.* (2015) Precise correction of the dystrophin gene in duchenne muscular dystrophy patient induced pluripotent stem cells by TALEN and CRISPR–Cas9. *Stem Cell Rep.*, **4**, 143–154.
- Cordaux, R. and Batzer, M.A. (2009) The impact of retrotransposons on human genome evolution. *Nat. Rev. Genet.*, **10**, 691–703.
- Cho, S.W., Kim, S., Kim, Y., Kweon, J., Kim, H.S., Bae, S. and Kim, J.-S. (2014) Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. *Genome Res.*, **24**, 132–141.
- Kleinstiver, B.P., Prew, M.S., Tsai, S.Q., Topkar, V. V., Nguyen, N.T., Zheng, Z., Gonzales, A.P.W., Li, Z., Peterson, R.T., Yeh, J.-R.J. *et al.* (2015) Engineered CRISPR–Cas9 nucleases with altered PAM specificities. *Nature*, **523**, 481–485.
- Slaymaker, I.M., Gao, L., Zetsche, B., Scott, D.A., Yan, W.X. and Zhang, F. (2016) Rationally engineered Cas9 nucleases with improved specificity. *Science*, **351**, 84–88.

Supplementary Information

Cell-type-specific genome editing with a microRNA-responsive CRISPR-Cas9 switch

Moe Hirosawa^{1,2}, Yoshihiko Fujita¹, Callum J.C Parr¹, Karin Hayashi¹, Shunnichi Kashida¹, Akitsu Hotta¹, Knut Woltjen^{1,3}, and Hirohide Saito*¹.

¹ Department of Life Science Frontiers, Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan.

² Graduate School of Medicine, Kyoto University, Kyoto, Japan.

³ Hakubi Center for Advanced Research, Kyoto University, Kyoto, Japan.

This file includes:

Supplementary Text

Supplementary Methods

Supplementary Figures S1 to S10

Supplementary Table S1: Primers and oligo DNAs for RNAs

Supplementary Table S2: Primers for T7E1 assay

Supplementary Table S3: qPCR primers used in this study

Supplementary Table S4: List of PCR reactions

Supplementary Table S5: RNA sequences used in this study

Supplementary Table S6: Plasmid sequences used in this study

Supplementary Table S7: Transfection tables for all experiments in this study

Supplementary References

Supplementary Text

Engineering miR-Cas9 switch and sgRNA that target EGFP

We noticed that Cas9 activity from control Cas9 mRNA was originally lower in hiPSCs than HeLa cells, although we observed a difference in Cas9 activity using control Cas9 mRNA and miR-302-Cas9 switches in hiPSCs (Supplementary Figure S4A). Our initial sgRNA_{GFP} contained an additional GG sequence at the 5' region to facilitate *in vitro* transcription by T7 RNA polymerase. A previous report showed that the extra nucleotides at the 5' end could affect mutation frequencies at on-target and off-target sites (1). To check the effect of the GG sequences in sgRNA_{GFP}, we removed them from sgRNA_{GFP} (sgRNA2_{GFP}) and found that sgRNA2_{GFP} with Cas9 mRNA showed higher Cas9 activity compared with the original sgRNA_{GFP} (Supplementary Figure S4A vs Supplementary Figure S4B). However, we also observed leaky expression of Cas9 in cells transfected with miR-302-Cas9 switch, sgRNA2_{GFP}, and negative miRNA inhibitor (Supplementary Figure S4B).

We therefore aimed to improve the sensitivity of miR-Cas9 switches to avoid leaky Cas9 expression in the target cells (Supplementary Figure S4B). We inserted four copies of the anti-miR sequence in the 5'-UTR region of the switch (4x miR-302-Cas9 switch) and found that this switch improved sensitivity to the target miR-302 and reduced leaky Cas9 expression in iPSCs (Figure 3A).

We also tuned the amount of miR-Cas9 switch and sgRNA. Consequently, we optimized conditions and improved Cas9 activity with lower leaky expression in the presence of miR-302 in hiPSCs (Supplementary Figure S4C).

Supplementary Methods

Evaluation of miRNA expression level

Relative miRNA expression level was quantified using TaqMan[®] MicroRNA Cells-to-C_T[™] Kit (Ambion). Cell lysate containing total RNA was reverse transcribed using has-miR-21-5p (Assay ID: 000397), 302a-5p (Assay ID: 002381) and RNU6B (Assay ID: 001093) TaqMan probes (Applied Biosystems). Quantitative PCR (qPCR) was performed with the TaqMan probes, and samples were analyzed using a StepOne Plus Real-Time PCR System (Applied Biosystems). Target miRNA was normalized to the the small RNA RNU6B and analyzed by $\Delta\Delta C_t$ method.

Evaluation of Cas9 protein expression level

After PBS wash, cells were lysed by adding 50 μ L M-PER cocktail (contains M-PER[®] Mammalian Protein Extraction Reagent (ThermoFisher), protease inhibitor, PMSF). After shaking for 5 min, lysate was collected into a 1.5 mL tube and centrifuged at 12400 rpm at 4°C for 10 min. The supernatant was collected and used in a BCA assay using Pierce[™] BCA Protein Assay Kit (ThermoFisher) following the manufacturer's protocol. The protein in the cell lysates were diluted to 0.5 mg/mL and analyzed using automated capillary electrophoresis-based immunoblotting with the Wes (ProteinSimple) following the manufacturer's protocol. Cas9 antibody (Active Motif) and GAPDH antibody (Santa Cruz) were

diluted 1 : 50 and 1 : 100, respectively. To detect primary antibody, anti-mouse and anti-rabbit antibodies (ProteinSimple) were used.

Cell killing assay by targeting Alu1 element

We collected all cell samples at 48 h after RNA transfection. After washing with PBS, normal HeLa cells were treated with 100 μ L 0.25% trypsin-EDTA, incubated at 37°C with 5% CO₂ for 5 min, and then 100 μ L cultured medium was added. After the cell suspension was collected, it was centrifuged at 1000 rpm at room temperature for 5 min. The supernatant was discarded and the remaining cell pellet was washed with PBS and centrifuged (1000 rpm) at room temperature for 5 min. After aspirating supernatant, cell samples were stained with 2.5 μ L annexin V, Alexa Fluor 488 conjugate (Life Technologies) and 0.5 μ L SYTOX Red dead-cell stain in 50 μ L Annexin-binding buffer 5x (Life Technologies). Samples were analyzed on an Accuri C6 flow cytometer (BD Biosciences).

T7E1 assay

For the T7E1 assay, 100 ng Cas9 mRNA and 300 ng sgRNA were used. Genomic DNA was extracted before PCR. The target site was amplified by nested PCR using the appropriate primers (Supplementary Table S2). The first reaction condition was 94°C for 2 min, 20 cycles of 98°C for 10 sec, 60°C for 30 sec, and 68°C for 30 sec, and 72°C for 3 min and finally 4°C for ∞ . The second reaction condition was 94°C for 2 min, 35 cycles of 98°C for 10 sec, 60°C for 30 sec, and

68°C for 15 sec, and 72°C for 3 min and 4°C for ∞ . The PCR amplicon was purified by MinElute PCR purification Kit (QIAGEN). Then, denaturing and re-annealing reactions were conducted at 95°C for 5 min, 95°C to 85°C at -2.0°C/sec, 85°C to 25°C at -0.1°C/sec and finally 4°C for ∞). This fragment was then digested by T7 Endonuclease I. The resulting products were analyzed by electrophoresis in a 5% polyacrylamide gel and were visualized by staining with SYBR Green mixture (I+II =1:1).

Insertions and deletions (Indels) were determined by the formula, $100 \times (1 - \sqrt{1 - (b + c)/(a + b + c)})$, where **a** is the integrated intensity of the undigested PCR product, and **b** and **c** are the integrated intensities of each cleavage product.

Sequencing

For phosphorylation of PCR amplicon (T7E1 second PCR product), primers (T7E1_EGFP Fwd_ORF and T7E1_EGFP Rev_Univ., sequences are shown in Supplementary Table S2) were phosphorylated by T4PNK (TAKARA) and ATP (Ambion). Amplicon was phosphorylated by PCR reaction using the phosphorylated primers and cloned in pUC19 vector at the SmaI site. The purified plasmids were sequenced by using M13 FwdNew primer, T7E1 Fwd primer or T7E1 Rev primer as the sequence primer. Sequencing was conducted by using Applied Biosystems 3500xL Genetic analyzer (Applied Biosystems).

Evaluation of gene expression level

To collect total RNA, cells were treated with Trizol (ThermoFisher) following the manufacturer's protocol. To eliminate genomic DNA, samples were treated with TURBO DNase Inactivation Kit (Ambion) following the manufacturer's protocol. To measure the mRNA level, 250 or 300 ng of total RNA were reverse-transcribed by ReverTra Ace[®] qPCR RT Master Mix (TOYOBO) following the manufacturer's protocol. Quantitative PCR (qPCR) was performed using THUNDERBIRD[®] SYBR[®] qPCR Mix (TOYOBO) following the manufacturer's protocol, and the samples were analyzed using a StepOne Plus Real-Time PCR System (Applied Biosystems). Primers for qPCR are listed in Supplementary Table S3. Target mRNA was normalized to GAPDH and analyzed by the $\Delta\Delta C_t$ method.

Cy5-labelling of sgRNA

Cy5 labelled sgRNA were prepared by ligation using T4 RNA ligase (Ambion) and pCp-Cy5 (Jane Bioscience). Cy5 labelling was performed with 200 pmol purified sgRNA using 40 U T4 RNA ligase, 4 nmol pCp-Cy5 and 10% DMSO in 40 μ L at 16°C for 48 h. Cy5-labelled sgRNA was purified using an RNeasy MinElute Cleanup Kit (QIAGEN).

Evaluation of transfection efficiency

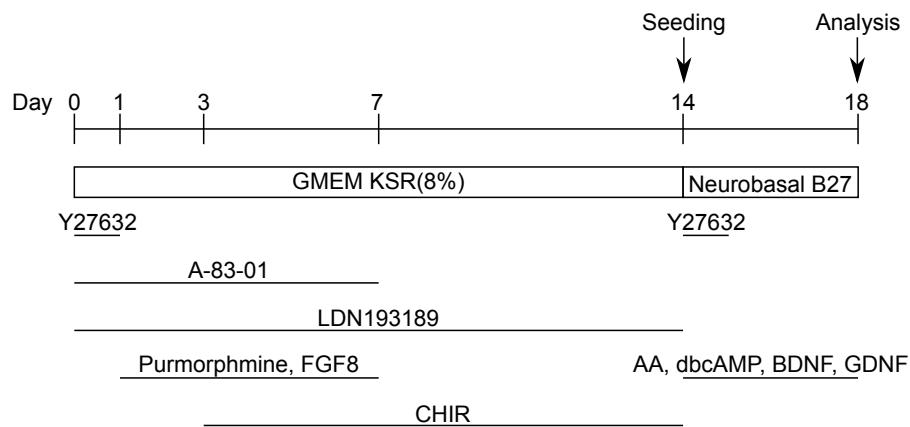
To measure the relative transfection efficiency, 100 ng *BFP* mRNA and 300 ng Cy5-labeled sgRNA were transfected into HeLa-EGFP cells or iPS-EGFP cells. At 24 h after RNA transfection, cells were washed with PBS three times. These

cells were harvested as described in Cas9 activity assay and analyzed on a LSR flow cytometer.

Neuron differentiation assay

Differentiated mDA-EGFP cells were derived from iPS-EGFP cells. Briefly, differentiation was started from a single cell suspension of iPS-EGFP cells in day 0 differentiation medium with ROCK inhibitor and seeded at 5×10^6 cells/well in laminin-coated 6-well plates. The differentiating cells were cultured at 37°C with 5% CO₂. At least 24 h later, the medium was changed to day 1 medium, and the medium was changed daily. For the assays, cells were passaged at day 14 in 24-well plates at a density of 2.0×10^5 cells/well.

Schematic of mDA neuron differentiation assay



8GMK

Glasgow's MEM (Thermo FisherScientific)
1X Non-essential amino acids (Thermo FisherScientific)
8% knockout-serum (Thermo FisherScientific)
100 µM sodium pyruvate (Thermo FisherScientific)
100 µM β-mercaptoethanol (Wako)

Neurobasal B27

Neurobasal medium (Thermo FisherScientific)
2% B27 supplement (Thermo FisherScientific)
2 mM L-glutamine

Day 0

8GMK
10 µM Y26732 (Wako)
100 nM LDN-193189 (Stemgent)
500 nM A-83-01 (Wako)

Day 14

Neurobasal B27
10 µM Y26732
200 µM Abscorbic acid (Sigma Aldrich)
400 µM dbcAMP (Sigma Aldrich)
20 ng/mL Human recombinant BDNF (R&D)
10 ng/mL Human recombinant GDNF (R&D)

Day 1-2

8GMK
100 nM LDN-193189
500 nM A-83-01
2 µM Purmorphamine (Calbiochen)
100 ng/mL Human recombinant FGF-8 (Wako)

Day 15-

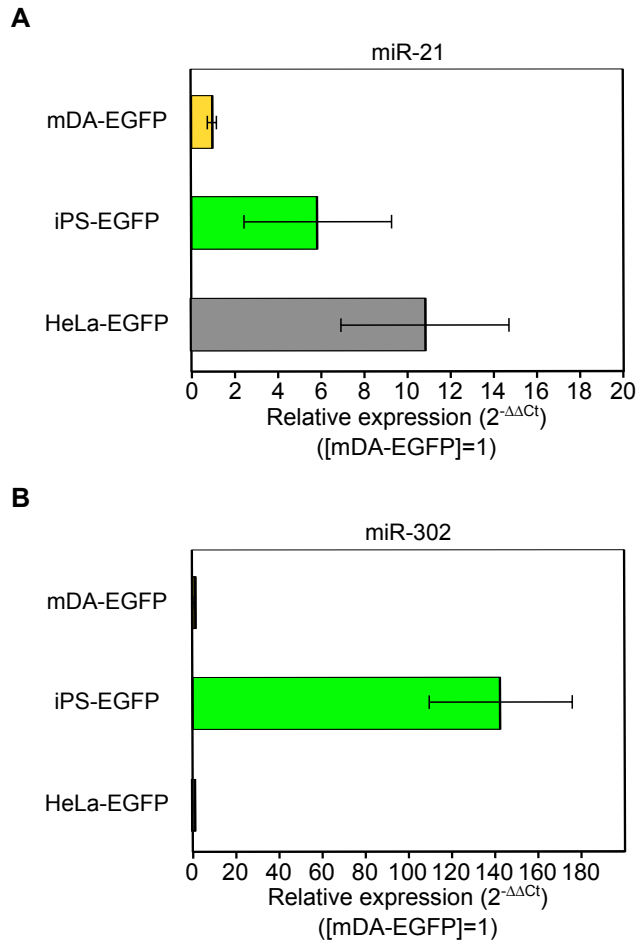
Neurobasal B27
200 µM Abscorbic acid (Sigma Aldrich)
400 µM dbcAMP (Sigma Aldrich)
20 ng/mL Human recombinant BDNF (R&D)
10 ng/mL Human recombinant GDNF (R&D)

Day 3-6

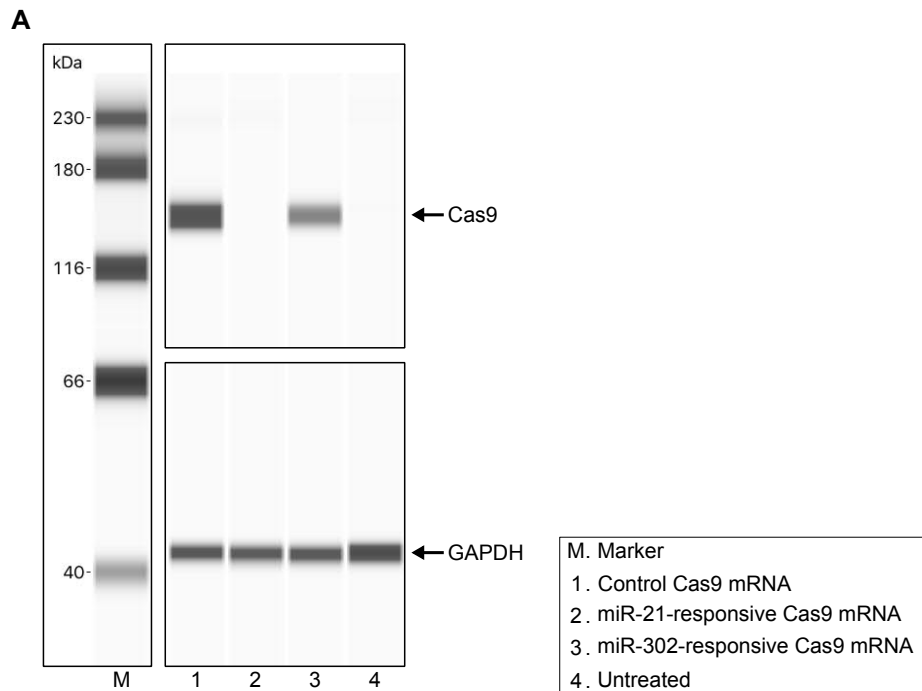
8GMK
100 nM LDN-193189
500 nM A-83-01
2 µM Purmorphamine
100 ng/mL Human recombinant FGF-8
30 µM CHIR99021 (Stemgent)

Day 7-13

8GMK
100 nM LDN-193189
30 µM CHIR99021

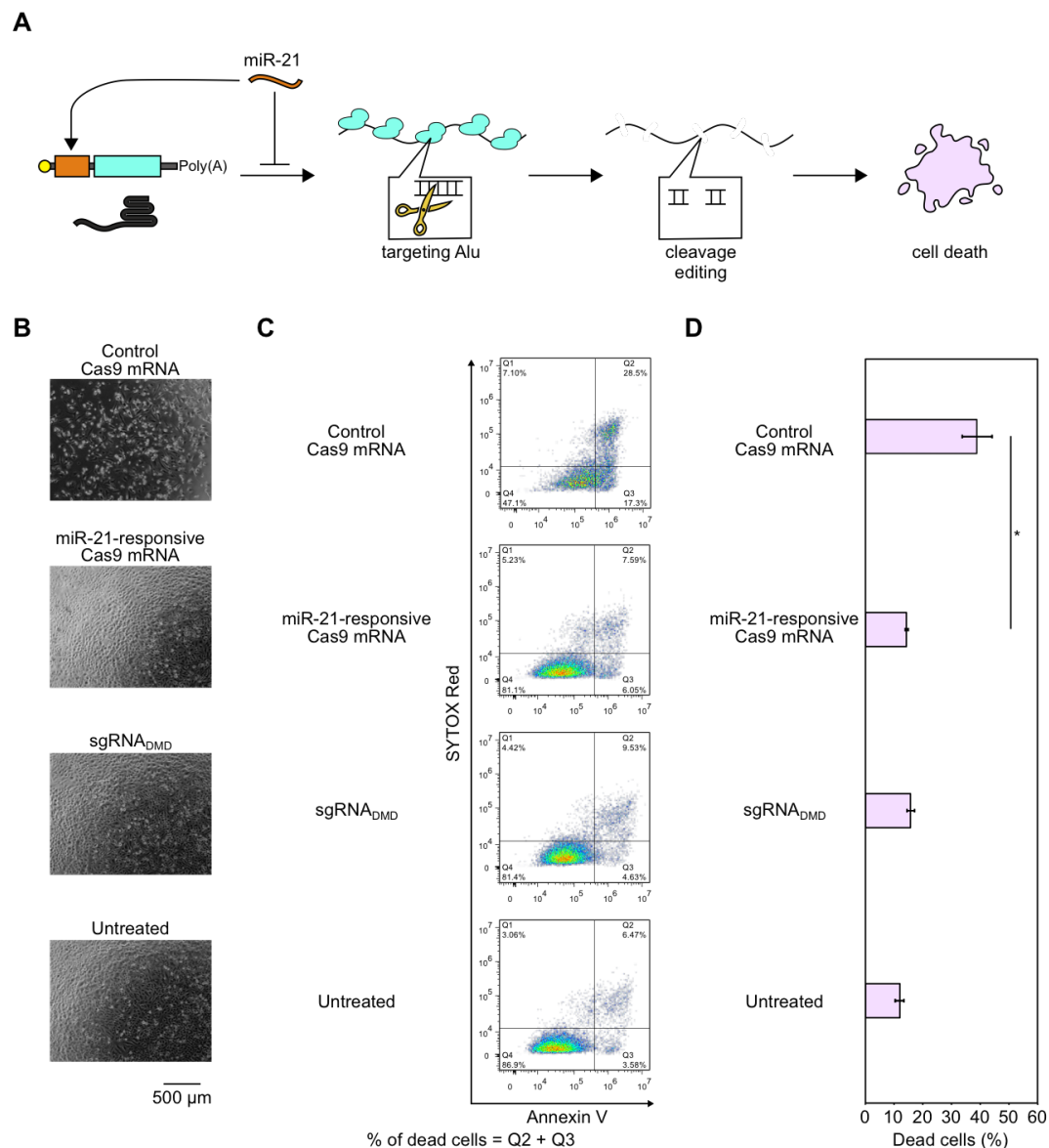


Supplementary Figure S1. Analysis of endogenous miRNA expression by qPCR. (A) Representative bar graph of miR-21 expression in HeLa cells, iPS cells and mDA neurons (Day 14). (B) Representative bar graph of miR-302 expression levels in HeLa cells, iPS cells and mDA neurons (Day 14). Each miRNA expression was normalized with RNU6B expression ($2^{-\Delta\Delta C_t}$) and then each normalized miRNA expression ($2^{-\Delta\Delta C_t}$) was normalized again with mDA neurons (*i.e.*, $2^{-\Delta\Delta C_t}_{\text{mDA neurons}} = 1$). Error bars indicate the mean $\pm$ SD ($n = 3$).

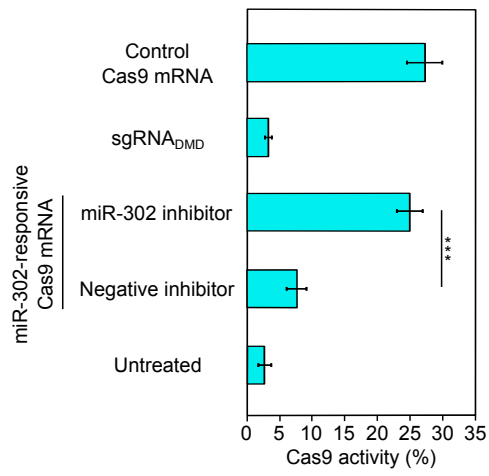
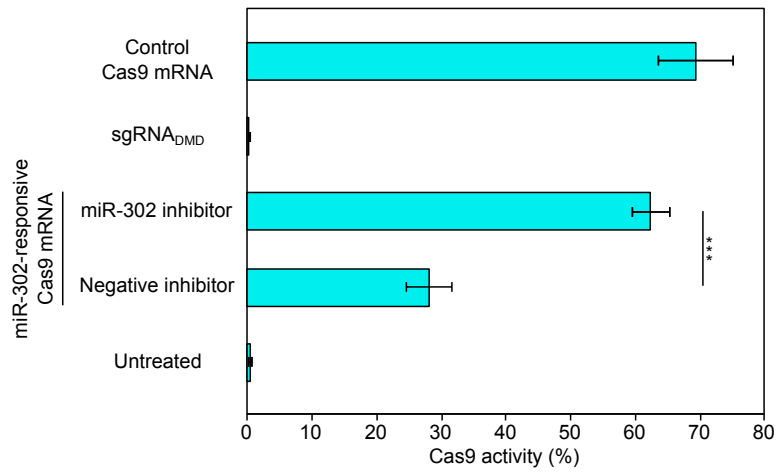
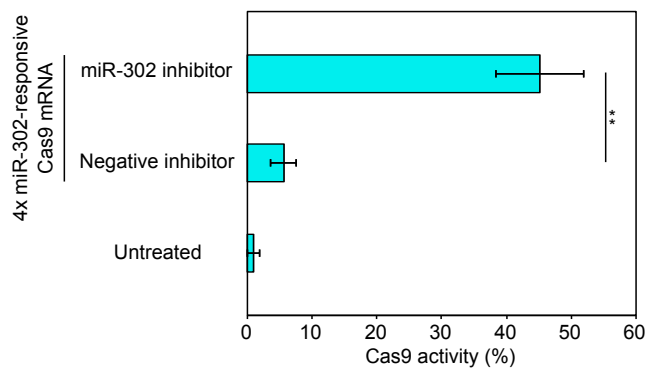


Supplementary Figure S2. Cas9 protein expression levels in HeLa cells.

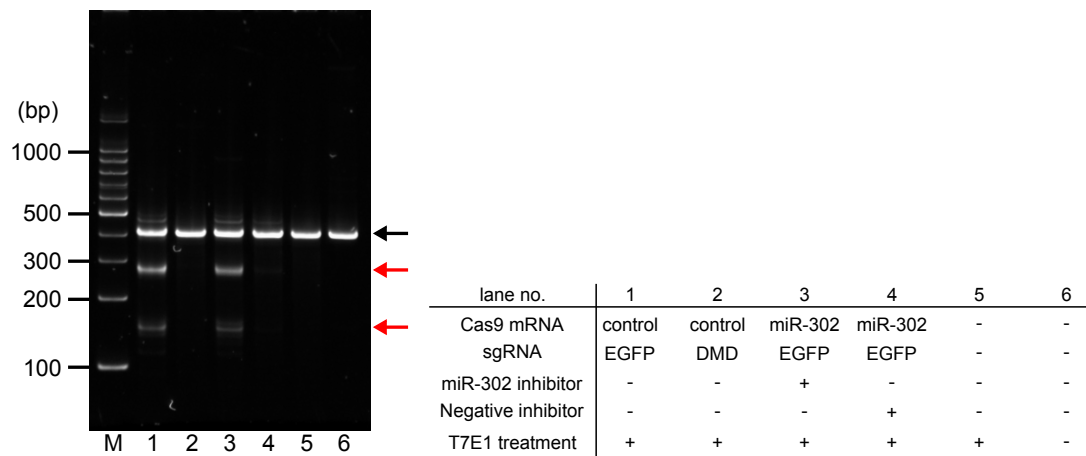
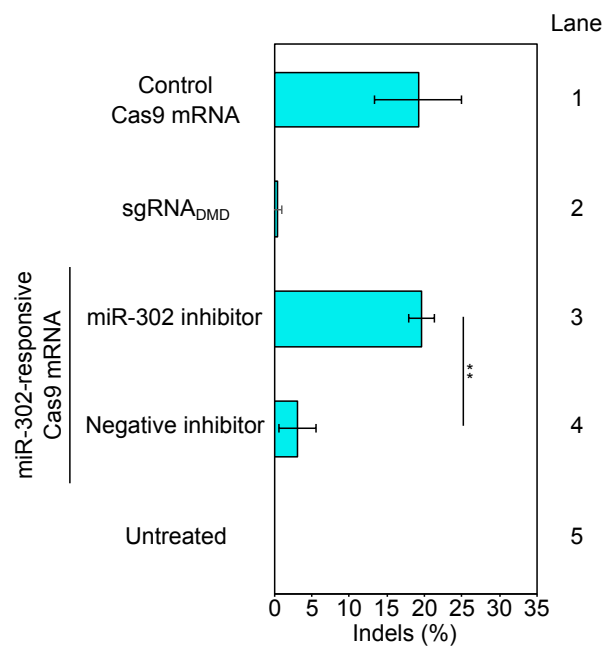
Representative virtual blot of Simple Western analysis at 24 h after RNA transfection. Cas9 protein expression levels were detected by virtual blot-like images of the capillary electrophoresis. GAPDH was used as a loading control. Lanes: M, molecular weight marker; 1, control *Cas9* mRNA; 2, miR-21-responsive *Cas9* mRNA; 3, miR-302-responsive *Cas9* mRNA; 4, untreated cells. The experiment was repeated three times. A representative image is shown.



Supplementary Figure S3. miR-Cas9 switch targeting Alu. **(A)** Schematic illustration of the system targeting Alu. The Cas9-sgRNA complex targeting a common Alu sequence cleaves genomic DNA at multiple locations and induces cell death. **(B)** Representative microscopic images at 48 h after RNA transfection. The scale bar represents 500 μ m. **(C)** Representative dot plots of the cells at 48 h after RNA transfection. Positive populations of Annexin V (x-axis) and SYTOX Red (y-axis) indicate dead cells. Representative images are shown from three independent experiments. **(D)** Dead cells were calculated by counting Q2 + Q3 cells shown in **(C)**. Negative control targets the *DMD* gene. Error bars indicate the mean $\pm$ SD ($n = 3$).

A**B****C**

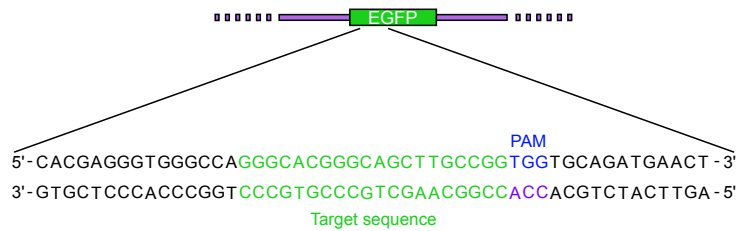
Supplementary Figure S4. Improvement of Cas9 activity in hiPSCs by engineering sgRNA and miR-302-Cas9 switch. (A) First evaluation of miR-302-responsive Cas9 mRNA in hiPSCs. A miR-302 inhibitor rescued Cas9 activity expressed from miR-302-responsive Cas9 mRNA, whereas a negative miRNA inhibitor did not. However, the Cas9 activity was low (25 - 30%, Control Cas9 mRNA). (B) Improvement of Cas9 activity by changing sgRNA (removal of GG from 5' terminal, sgRNA2_{GFP}) in hiPS-EGFP cells. In comparison with data using the original sgRNA (sgRNA_{GFP}) shown in (A), Cas9 activity increased about 40-50%, though leaky expression of Cas9 was observed (Negative inhibitor). (C) Optimization of 4x miR-302-responsive Cas9 mRNA and sgRNA2_{GFP}. Final condition was 50 ng 4x miR-302-responsive Cas9 mRNA and 300 ng sgRNA2_{GFP}. High Cas9 activity (45%, miR-302 inhibitor) and reduced leaky expression (8%, Negative inhibitor) were observed. Error bars indicate the mean $\pm$ SD (n = 3).

A**B**

Supplementary Figure S5. T7E1 assay for hiPSCs with miR-302-Cas9 switch.

(A) Representative gel image of T7E1 assay. Top band (black arrow, 436 bp) indicates the target amplicon. The lower two bands (red arrows, 286 bp and 150 bp) indicate digested fragments by T7 endonuclease I. Lanes: M, molecular weight marker (100-bp ladder); 1, control *Cas9* mRNA; 2, negative control (targeting DMD); 3, miR-302-responsive *Cas9* mRNA + miR-302 inhibitor; 4, miR-302-responsive *Cas9* mRNA + negative miRNA inhibitor; 5, untreated cells with T7 endonuclease I; and 6, untreated cells without T7 endonuclease I. The samples are summarized in the table to the right. **(B)** Quantification of *Cas9* activity (indels) from **(A)**. No digestion products were detected in the untreated sample. Error bars indicate the mean $\pm$ SD ($n = 3$).

A



B

Control Cas9 mRNA

5' - AGTTCATCTGCACCA^{CCG} GCAAGCTGCCCGTGCCCTGGCCCCACCCTCGTG - 3' WT 15/18

5' - AGTTCATC - - C - - - - - GCAAGCTGCCCGTGCCCTGGCCCCACCCTCGTG - 3' Δ9 1/18

5' - AGTTCATCTGCACCA^{CCG} TGCAGATGACCTGAAGTTCATCTGCAAGCTGCCCGTGCCCTGGCCCCACCCTCGTG - 3' +23 1/18

5' - AGTTCATCTGCACCA^{CCG} TGCCCTGGCCCA^{CCCTC} GCAAGCTGCCCGTGCCCTGGCCCCACCCTCGTG - 3' +17 1/18

miR-302-responsive Cas9 mRNA + miR-302 inhibitor

5' - AGTTCATCTGCACCA^{CCG} CAAGCTGCCCGTGCCCTGGCCCCACCCTCGTG - 3' WT 16/18

5' - AGTTCATCTGCACCA^{CCG} GAAGCTGCCCGTGCCCTGGCCCCACCCTCGTG - 3' +1 1/18

5' - AGTTCATCTGCACCA^{CC} - G CAAGCTGCCCGTGCCCTGGCCCCACCCTCGTG - 3' Δ1 1/18

miR-302-responsive Cas9 mRNA + Negative inhibitor

5' - AGTTCATCTGCACCA^{CCG} GAAGCTGCCCGTGCCCTGGCCCCACCCTCGTG - 3' WT 19/19

sgRNA_{DMD}

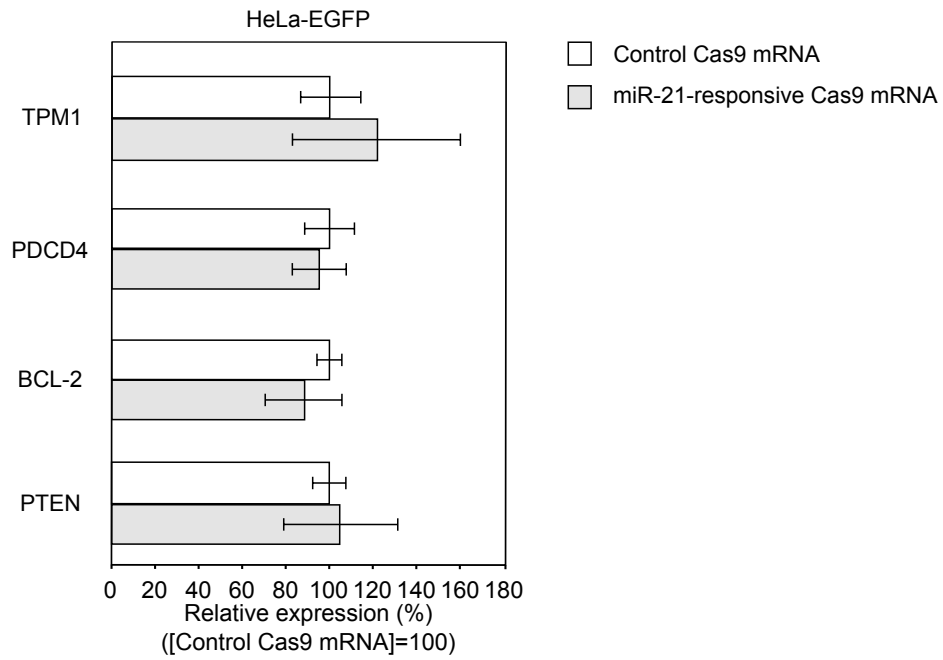
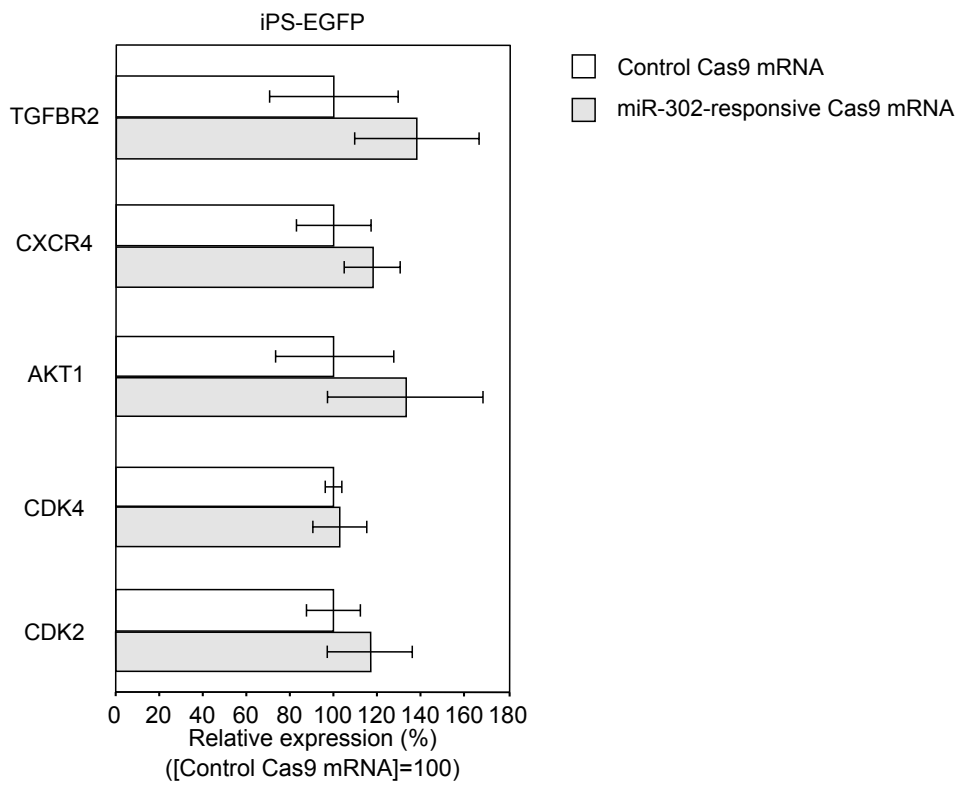
5' - AGTTCATCTGCACCA^{CCG} GAAGCTGCCCGTGCCCTGGCCCCACCCTCGTG - 3' WT 10/10

Untreated

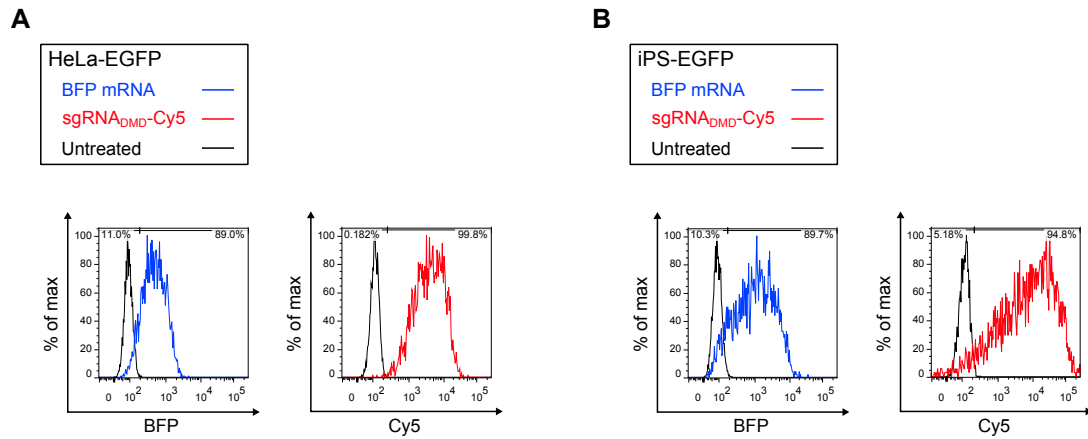
5' - AGTTCATCTGCACCA^{CCG} GAAGCTGCCCGTGCCCTGGCCCCACCCTCGTG - 3' WT 9/9

Supplementary Figure S6. Confirmation of DNA sequencing in T7E1 assays.

(A) Illustration of the target sequence. Target sequence is green letters. Protospacer adjacent motif (PAM) sequence and complimentary PAM sequence are written as blue and purple letters, respectively. (B) Observed sequences in Supplementary Figure S5. Red letters and dashes indicate the identified mutations. The sgRNA targeting sequence and complimentary PAM sequence are written as green and purple letters, respectively. The size of the insertions and deletions are shown to the right of each mutated sequence (+, insertions; Δ, deletions). Numbers on the right indicate the numbers of mutated clones and all analyzed clones. Each sequence was read by forward and reverse primers.

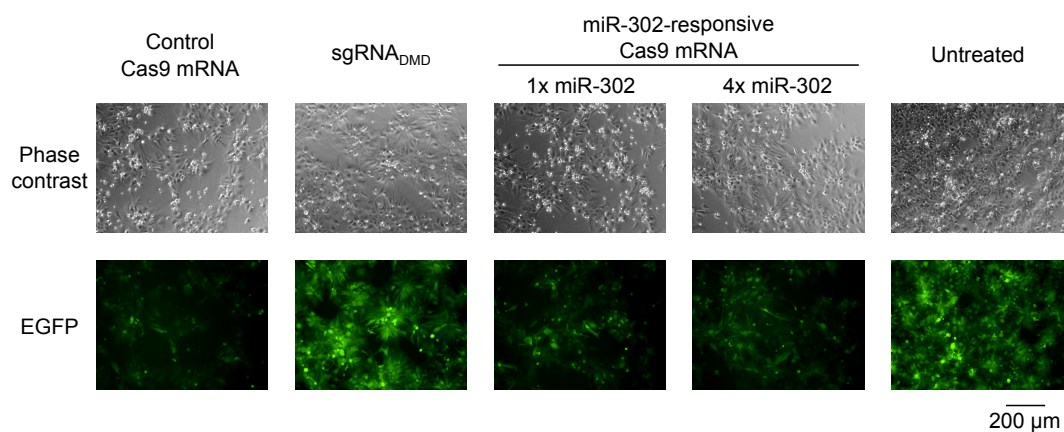
A**B**

Supplementary Figure S7. Effect of miRNA-responsive Cas9 mRNA on endogenous gene expression in HeLa and iPS cells. (A) The effect of miR-21-responsive Cas9 mRNA in HeLa cells was evaluated by qPCR. (B) The effect of miR-302-responsive Cas9 mRNA in iPS cells was evaluated by qPCR. Target mRNA levels were normalized with *GAPDH* mRNA (target gene expression) and then each target gene expression was normalized with each control Cas9 mRNAs. Error bars indicate the mean $\pm$ SD (n = 6).

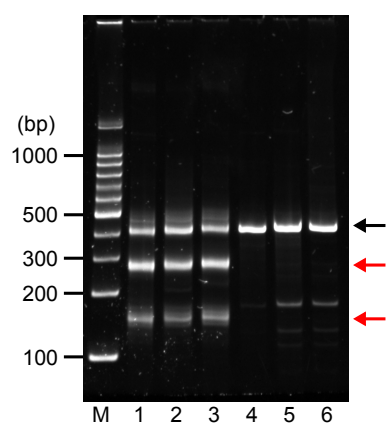


Supplementary Figure S8. Relative transfection efficiencies in HeLa and iPS cells. (A) Relative transfection efficiencies in HeLa cells. Representative flow cytometry histograms of *BFP* mRNA (blue) and Cy5-labeled sgRNA (red). (B) Relative transfection efficiencies in iPS cells. Representative flow cytometry histograms of *BFP* mRNA (blue) and Cy5-labeled sgRNA (red). x- and y-axes indicate BFP or Cy5 intensity and % of max, respectively. These experiments were repeated three times.

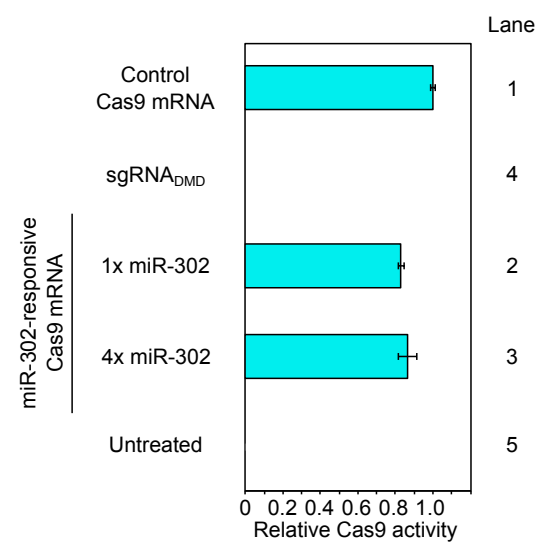
A



B



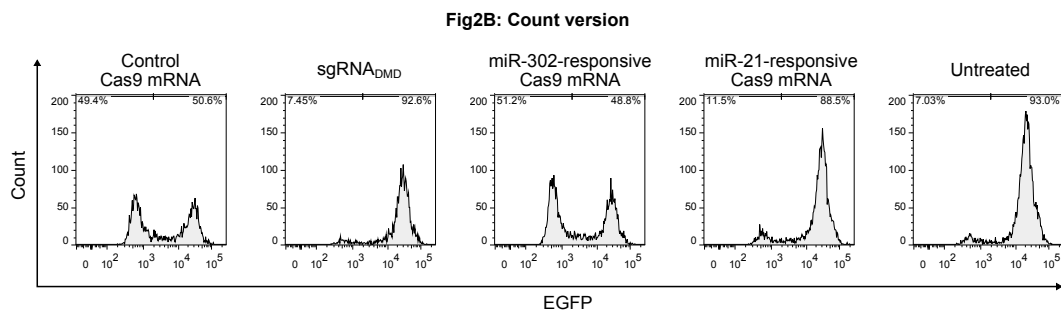
C



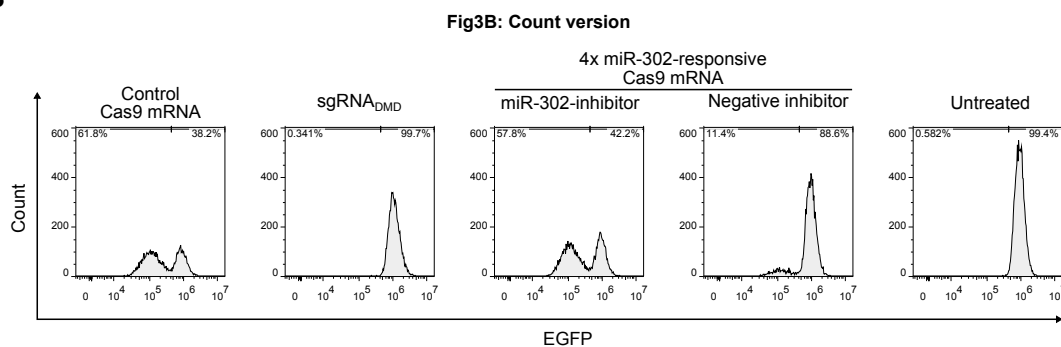
lane no.	1	2	3	4	5	6
Cas9 mRNA	control	1x miR-302	4x miR-302	control	-	-
sgRNA	EGFP	EGFP	EGFP	DMD	-	-
T7E1 treatment	+	+	+	+	+	-

Supplementary Figure S9. miR-302-Cas9 switch in mDA neuron. (A) Representative fluorescent microscopic images at 72 h after RNA transfection. Phase contrast and EGFP indicate phase contrast and EGFP fluorescent images, respectively. Scale bar represents 200 μ m. **(B)** Representative gel image from a T7E1 assay using mDA-EGFP cells derived from hiPS-EGFP cells. Top band (black arrow, 436 bp) indicates the target amplicon. The below two bands (red arrows, 286 bp and 150 bp) indicate digested fragments by T7 endonuclease I. Lanes: M, molecular weight marker (100-bp ladder); 1, control Cas9 mRNA; 2, miR-302-responsive Cas9 mRNA; 3, 4x miR-302-responsive Cas9 mRNA; 4, negative control (targeting DMD); 5, untreated cells with T7 endonuclease I; and 6, untreated cells without T7 endonuclease I. The samples are summarized in the table below. **(C)** Quantification of relative Cas9 activity from **(B)**. Each Cas9 activity was normalized by control Cas9 mRNA (lane no.1). Error bars indicate the mean $\pm$ SD (n = 3).

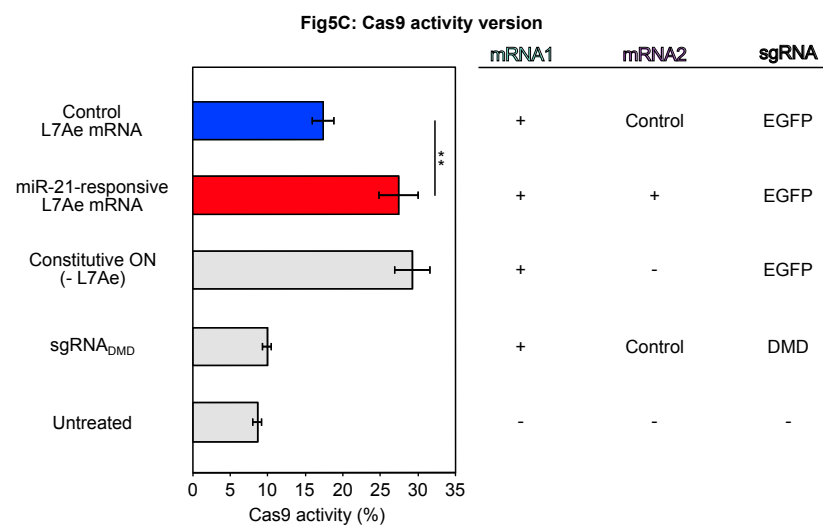
A



B



C



Supplementary Figure S10. Unnormalized raw data. (A) and (B) Cell counts from Figure 2B and 3B. x- and y-axes indicate EGFP intensity and cell number, respectively. **(C)** Quantification of Cas9 activity from Figure 5B.

Supplementary Table S1: Primers and oligo DNAs for RNAs

1. miRNA target sites and RNA motifs are written as orange and red letters, respectively.
2. sgRNA target sequences are written as green letters.
3. All primers were designed manually.

Primer/Oligo DNA name	Sequence (5' -> 3')
TAP_T7_G3C fwd primer	CAGTGAATTGTAATACGACTCACTATAGGGC
IVT_5prime_UTR primer	CAGTGAATTGTAATACGACTCACTATAGGGCGAATTAAGAGAG AAAAGAAGAGTAAGAAGAAATATAAGACACCGGTCGCCACCAT G
Rev5UTR primer	CATGGTGGCGACCGGTGTCTTATATTTCTTCTTACTC
SphcCas9 ORF fwd primer	CACCGGTCGCCACCATGGATAAGAAATACAGCATTGGAC
SphcCas9 ORF rev primer	GCCCCGCAGAAGGTCTAGACTATCACACCTTCCTCTTCTTCTT GG
L7Ae_IVTfwd	CACCGGTCGCCACCATGTACGTGAGATTTGAGGTTCTG
Tag BFP Fwd	CACCGGTCGCCACCATGGGATCCAGCGAG
TAP_IVTrev	GCCCCGCAGAAGGTCTAGACTATCACTCGAGATGCATATGAGA TC
IVT_3prime_UTR primer	TCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTT CTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGA GTAGG
Fwd3UTR primer	TCTAGACCTTCTGCGGGGC
Rev3UTR2T20	TTTTTTTTTTTTTTTTTTTCTACTCAGGCTTTATTCAAAGACCA AG
GCT7pro_5UTR2	GCTAATACGACTCACTATAGGTTCTTAATCGCGGATCC
5UTRtemp_T302a-5p	CGACTCACTATAGGTTCCGCGATCGCGGATCCAGCAAGTACAT CCACGTTTAAGTAGATCCACCGGTCGCCACCATG
5UTRtemp_T21-5p	CGACTCACTATAGGTTCCGCGATCGCGGATCCCAACATCAGT CTGATAAGCTAAGATCACACCGGTCGCCACCATG

Primer/Oligo DNA name	Sequence (5' -> 3')
5UTRtemp_4x302a-5p	CGACTCACTATAGGTTCCGCGATCGCGGATCCAGCAAGTACAT CCACGTTTAAGTAGCAAGTACATCCACGTTTAAGTAGCAAGTAC ATCCACGTTTAAGTAGCAAGTACATCCACGTTTAAGTAGATCAC ACCGGTCGCCACCATG
CGCT7G2	CGCTAATACGACTCACTATAGG
5UTR4ntmoxCDr0sp	CGCTAATACGACTCACTATAGGATCCGTGATCGGAAACGTGAG ATCCACCTCAGATCCGCTAGGACACCCGCAGATCGAG
Spacer5UTRFwd	ACACCCGCAGATCGAGAAGAAGGCGAATTAAGAGAGAAAAGA AGAGTAAGAAGAAATATAAGACACCGGTCGCCACCATG
3UTR120A	TT TT TTTTTTTTTTTTTTTTTTTTTTTTTCTACTCAGGCTTTATTCA
T7-sgRNA fwd primer (DMD)	GAAATTAATACGACTCACTATAGGTATCTTACAGGAAGTCCGTT TTAGAGCTAGAAATAGCAAG
T7-sgRNA fwd primer (EGFP)	GAAATTAATACGACTCACTATAGGGGGCACGGGCAGCTTGCC GGGTTTTAGAGCTAGAAATAGCAAG
T7-sgRNA fwd primer (EGFP ΔGG)	GAAATTAATACGACTCACTATAGGGGGCACGGGCAGCTTGCCGG GTTTTAGAGCTAGAAATAGCAAG
T7-sgRNA fwd primer (Alu1)	GAAATTAATACGACTCACTATAGGGCCTGTAATCCCAGCACTTT GTTTTAGAGCTAGAAATAGCAAG
sgRNA+85 rev primer	AAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACT AGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC

Supplementary Table S2: Primers for T7E1 assay

1. T7E1_EGFP Fwd_L and T7E1_EGFP Rev_univ. were designed using Primer3Plus*.
2. Other primers were designed manually.

Primer/Oligo DNA name	Sequence (5' -> 3')
T7E1_EGFP Fwd_L	CAGCCATTGCCTTTTATGG
T7E1_EGFP Rev_Univ.2	GTGTTCTGCTGGTAGTGGTCGGCGAG
T7E1_EGFP Fwd_ORF	TGAGCAAGGGCGAGGAGCTGTTCAC
T7E1_EGFP Rev_Univ.	TTGTAGTTGTACTCCAGCTTGTGC
M13 FwdNew primer	GTTTTCCCAGTCACGAC

*Primer3Plus: <http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>

Supplementary Table S3: qPCR primers used in this study

1. Primers were designed according to the given reference (except for CXCR4 Fwd & PDCD4 Rev).
2. CXCR4 Fwd was redesigned using Primer3Plus* to minimize amplicon size.
3. PDCD4 Rev's 3' terminal base was replaced (C to G).

Target gene		Sequence (5' -> 3')	Length (nt)	Tm (°C)	GC%	Amplicon (bp)	Ref.
PTEN	Fwd	CAAGATGATGTTTGAAACT ATTCCAATG	28	55	32	77	(2)
	Rev	CCTTTAGCTGGCAGACCA CAA	21	59	52		
BCL2	Fwd	TCCGCATCAGGAAGGCTA GA	20	59	55	113	(3)
	Rev	AGGACCAGGCCTCCAAGC T	19	62	63		
PDCD4	Fwd	TATGATGTGGAGGAGGTG GATGTGA	25	61	48	96	(4)
	Rev	CCTTTCATCCAAAGGCAA AACTACAG	26	58	42		
TPM1	Fwd	GCCGACGTAGCTTCTCTG AAC	21	59	57	160	(5)
	Rev	TTTGGGCTCGACTCTCAA TGA	21	58	48		
CDK2	Fwd	GCTAGCAGACTTTGGACT AGCCAG	24	61	54	85	(3)
	Rev	AGCTCGGTACCACAGGGT CA	20	61	60		
CDK4	Fwd	CTGGTGTTTGAGCATGTA GACC	22	57	50	102	(3)
	Rev	AAACTGGCGCATCAGATC CTT	21	59	48		

Target gene		Sequence (5' -> 3')	Length (nt)	Tm (°C)	GC%	Amplicon (bp)	Ref.
AKT1	Fwd	GCACAAACGAGGGGAGTACAT	21	58	52	113	(3)
	Rev	CCTCACGTTGGTCCACATC	19	56	58		
CXCR4	Fwd	TCATCCTGGCCTTCATCAGT	20	57	50	112	(6)
	Rev	ATCCAGACGCCAACATAGAC	20	55	50		
TGFB2	Fwd	GTAGCTCTGATGAGTGCAATGAC	23	57	48	132	(7)
	Rev	CAGATATGGCAACTCCCATGTG	21	56	52		

*Primer3Plus: <http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>

Supplementary Table S4: List of PCR reactions

No.	Name	Type	Templates	Forward primer	Reverse primer	Additional oligos
1	5'-UTR (control)	UTR	IVT_5prime_UTR primer	TAP_T7_G3C fwd primer	Rev5UTR primer	
2	3'-UTR	UTR	IVT_3prime_UTR primer	Fwd3UTR primer	Rev3UTR2T20	
3	ORF PCR (Cas9)	ORF	pHL-EF1a-SphcCas9-iC-A	SphcCas9 ORF fwd primer	SphcCas9 ORF rev primer	
4	ORF PCR (L7Ae)	ORF	pAM_L7Ae	L7Ae_IVTfwd	TAP_IVTrev	
5	ORF PCR (BFP)	ORF	pA9-tagBFP	Tag BFP Fwd	TAP_IVTrev	
6	Control Cas9 mRNA Template	IVT template	ORF PCR (Cas9), 5'-UTR (control), 3'-UTR	TAP_T7_G3C fwd primer	3UTR120A	
7	miR-21-responsive Cas9 mRNA Template	IVT template	ORF PCR (Cas9), 5UTRtemp_T21-5p, 3'-UTR	GCT7pro_5UTR2	3UTR120A	
8	miR-302-responsive Cas9 mRNA Template	IVT template	ORF PCR (Cas9), 5UTRtemp_T302a-5p, 3'-UTR	GCT7pro_5UTR2	3UTR120A	
9	4x miR-302-responsive Cas9 mRNA Template	IVT template	ORF PCR (Cas9), 5UTRtemp_4x302a-5p, 3'-UTR	GCT7pro_5UTR2	3UTR120A	
10	Kt-Cas9 mRNA Template	IVT template	ORF PCR (Cas9), 5UTR4ntmoxCDr0sp, 3'-UTR	CGCT7G2	3UTR120A	Spacer5UTRFwd
11	Control L7Ae mRNA Template	IVT template	ORF PCR (L7Ae), 5'-UTR (control), 3'-UTR	TAP_T7_G3C fwd primer	3UTR120A	
12	miR-21-responsive L7Ae mRNA Template	IVT template	ORF PCR (L7Ae), 5UTRtemp_T21-5p, 3'-UTR	GCT7pro_5UTR2	3UTR120A	
13	BFP mRNA Template	IVT template	ORF PCR (BFP), 5'-UTR (control), 3'-UTR	TAP_T7_G3C fwd primer	3UTR120A	
14	sgRNA _{DMD} Template	IVT template		T7-sgRNA fwd primer (DMD)	sgRNA+85 rev primer	
15	sgRNA _{GFP} Template	IVT template		T7-sgRNA fwd primer (EGFP)	sgRNA+85 rev primer	
16	sgRNA _{2GFP} Template	IVT template		T7-sgRNA fwd primer (EGFP ΔGG)	sgRNA+85 rev primer	
17	sgRNA _{Alu1} Template	IVT template		T7-sgRNA fwd primer (Alu1)	sgRNA+85 rev primer	
18	T7E1 assay First PCR product	T7E1 PCR template	Genomic DNA	T7E1_EGFP Fwd_L	T7E1_EGFP Rev_Univ.2	
19	T7E1 assay Second PCR product	For T7E1 assay and sequencing	T7E1 assay First PCR product	T7E1_EGFP Fwd_ORF	T7E1_EGFP Rev_Univ.	

Supplementary Table S5: RNA sequences used in this study

1. The 5' terminus of the mRNA is capped with ARCA.
2. The protein coding regions are shown as bold letters.
3. The start and stop codons are underlined.
4. miRNA target sites and RNA motifs are written as orange and red letters, respectively.
5. sgRNA target sequences are written as green letters.

Control Cas9 mRNA

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGACACCGGUCGCCACCAU
GGAUAAGAAAUACAGCAUUGGACUGGACAUUGGGACAAACUCCGUGGGAUGGGCCGU
GAUUACAGACGAUAACAAAGUGCCUCAAAGAAGUUAAGGUGCUGGGCAACACCGA
UAGACACAGCAUCAAGAAAAAUCUGAUUGGAGCCCUGCUGUUCGACUCCGGCGAGAC
AGCUGAAGCAACUCGGCUGAAAAGAACUGCUCGGAGAAGGUUAUACCCGCCGAAAGAA
UAGGAUCUGCUACCUGCAGGAGAUUUUCAGCAACGAAAUGGCCAAGGUGGACGAUAG
UUUCUUUCACCGCCUGGAGGAAUCAUUCUGGUCGAGGAAGAUAAAGAAACACGAGCG
GCAUCCCAUCUUUGGCAACAUUGUGGACGAGGUCGCUUAUCACGAAAAGUACCCUAC
CAUCUAUCAUCUGAGGAAGAAACUGGUGGACUCCACAGAUAAAGCAGACCUGCGCCU
GAUCUAUCUGGCCCUGGCUCACAUGAUUAAGUUCGGGGCCAUUUUCUGAUCGAGGG
GGAUCUGAACCCAGACAAUUCUGAUGUGGACAAGCUGUUAUCCAGCUGGUCCAGAC
AUACAAUCAGCUGUUUGAGGAAAACCCCAUUAUUGCAUCUGGCGUGGACGAAAAGC
CAUCCUGAGUGCCAGACUGUCUAAGAGUCGGAGACUGGAGAACCUGAUCGCUCAGCU
GCCAGGGGAAAAGAAAAACGGCCUGUUUGGGAAUCUGAUUGCACUGUCACUGGGACU
GACUCCCAACUUAAGAGCAAUUUUGAUCUGGCCGAGGACGCUAAACUGCAGCUGUC
CAAGGACACCUAUGACGAUGACCUGGAUAACCUGCUGGCUCAGAUCGGGGAUCAGUA
CGCAGACCUGUUCUGGCCGCUAAGAAUCUGUCUGACGCCAUCCUGCUGAGUGAUUAU
UCUGCGCGUGAACACCGAGAUUACAAAAGCCCCCUGUCAGCUAGCAUGAUCAAGAG
AUAUGACGAGCACCAUCAGGAUCUGACCCUGCUGAAGGCUCUGGUGAGGCAGCAGCU
GCCUGAGAAGUACAAGGAAUCUUCUUUGAUCAGUCUAAGAACGGAUACGCCGGCUA
UAUUGACGGCGGGGCUAGUCAGGAGGAGUUCUACAAGUUUAUCAAACCCAUUCUGGA
GAAGAUGGAUGGCACAGAGGAACUGCUGGUGAAACUGAAUCGGGAAGACCUGCUGAG
GAAGCAGCGCACUUUUGAUAAACGGAAGCAUCCUCACCAGAUUCAUCUGGGAGAGCU
GCACGCAAUCCUGAGGCGCCAGGAAGACUUCUACCCAUUUCUGAAGGAUAACAGGGA

GAAGAUCGAAAAAUUCUGACAUUCCGCAUCCCCUACUAUGUGGGCCCUCUGGCAAG
AGGCAACAGCCGGUUUGCCUGGAUGACUCGAAAUCUGAGGAAACAAUCACUCCCUG
GAACUUCGAGGAAGUGGUCGAUAAGGGCGCUUCCGCACAGUCUUUCAUUGAGCGGAU
GACAAACUUCGACAAGAACCUGCCAAACGAAAAAGUGCUGCCCAAGCACUCUCUGCU
GUACGAGUAUUUCACAGUCUAUAACGAACUGACUAAGGUGAAAUACGUCACCGAGGG
GAUGAGAAAGCCUGCCUCCUGAGUGGAGAACAGAAGAAAGCUAUCGUGGACCUGCU
GUUUAAAACCAUAGGAAGGUGACAGUCAAGCAGCUGAAAGAGGACUAUUUCAAGAA
AAUUGAAUGUUUCGAUUCUGUGGAGAUAGUGGCGUCGAAGACAGGUUUAACGCCUC
CCUGGGGACCUACCACGAUCUGCUGAAGAUCAUUAAGGAUAAAGACUUCUGGACAA
CGAGGAAAAUGAGGAUAUCCUGGAAGACAUUGUGCUGACCCUGACACUGUUUGAGGA
UAGGGAAAUGAUCGAGGAACGCCUGAAGACCUAUGCCCAUCUGUUCGAUGACAAAGU
GAUGAAACAGCUGAAGCGACGGAGAUACACAGGAUGGGGGCCGACUGUCUCGGAAGCU
GAUCAUUGGGAUUCGCGACAAACAGAGUGGAAAGACCAUCCUGGACUUUCUGAAAUC
AGAUGGCUUCGCCAACCGGAACUUAUGCAGCUGAUUCACGAUGACAGCCUGACAUI
CAAAGAGGAUAUCCAGAAGGCACAGGUGUCCGGGCAGGGAGACUCUCUGCACGAGCA
UAUCGCAAACCUGGCCGGCAGCCCUGCCAUAAGAAAGGGAUUCUGCAGACCGUGAA
GGUGGUGGACGAGCUGGUGAAAGUCAUGGGAAGACAUAAGCCAGAAAACAUCGUGAU
UGAGAUGGCCAGGGAAAAUCAGACCACACAGAAAGGCCAGAAAGACUCAAGGGAGCG
CAUGAAAAGAAUCGAGGAAGGAUUAAGGAACUGGGCAGCCAGAUCCUGAAAGAGCA
CCCCGUGGAAAACACACAGCUGCAGAAUGAGAAGCUGUAUCUGUACUAUCUGCAGAA
UGGACGCGAUUAUGUACGUGGACCAGGAGCUGGAUAUUAACCGACUGUCCGAUUACGA
CGUGGAUCAUAUCGUCCCACAGUCAUUCUGAAAGAUGACAGCAUUGACAAUAAGGU
GCUGACCCGCUCUGACAAAAACCGAGGCAAGAGUGAUAAUGUCCCCUCAGAGGAAGU
GGUCAAGAAAAUGAAGACUACUGGAGGCAGCUGCUGAAUGCCAAACUGAUCACACA
GCGAAAGUUUGAUAAACUGACUAAAGCUGAGCGGGGAGGCCUGAGUGAACUGGACAA
AGCAGGCUUCAUUAAGCGACAGCUGGUGGAGACACGGCAGAUACAAAGCACGUCGC
CCAGAUUCUGGAUUAAGAAUGAACACUAAGUACGAUGAGAAUGACAAACUGAUCAG
AGAAGUGAAGGUCAUUAACCCUGAAGUCAAAACUGGUGAGCGACUUUCGGAAAGAUUU
CCAGUUUUUAUAAAGGUCAGAGAGAUCAACAACUACCACCAUGCUCAUGACGCAUACCUG
AACGCAGUGGUCGGCACAGCCCUGAUUAAGAAAUAACCUAAACUGGAGUCCGAGUUC
GUGUACGGGGACUAUAAGGUGUACGAUGUCAGAAAAAUGAUCGCCAAGUCUGAGCAG
GAAAUUGGCAAAGCCACUGCUAAGUAUUUCUUUUACAGUAACAUCAUGAAUUUCUUUA
AGACUGAGAUACCCUGGCAAAUGGGGAAAUCCGAAAGCGGCCACUGAUUGAGACUA

ACGGCGAGACAGGAGAAAUCGUGUGGGACAAAGGAAGAGAUUUUGCUACCGUGAGGA
AGGUCCUGAGCAUGCCCCAAGUGAAUAUUGUCAAGAAAACAGAGGUGCAGACUGGGG
GAUUCAGUAAGGAAUCAAUUCUGCCUAAACGCAACUCCGAUAAGCUGAUCGCCCCGAA
AGAAAGACUGGGACCCCAAGAAGUAUGGCGGGUUCGACUCCCCAACUGUGGCUUACU
CUGUCCUGGUGGUCGCAAAGGUGGAGAAGGGAAAAAGCAAGAAACUGAAAUCCGUCA
AGGAACUGCUGGGCAUCACCAUUAUGGAGCGCAGCUCCUUCGAAAAGAAUCCUAUCG
AUUUUCUGGAGGCCAAAGGCUAUAAGGAAGUGAAGAAAGACCUGAUCAUCAAGCUGC
CAAAGUACUCACUGUUUGAGCUGGAAAACGGGAGAAAGAGGAUGCUGGCAAGCGCCG
GGGAGCUGCAGAAAGGAAAUGAACUGGCCUGCCUCCAAGUACGUGAACUCCUGU
AUCUGGCUAGCCACUACGAGAAGCUGAAAGGGUCCCCUGAGGAUAACGAACAGAAAC
AGCUGUUUGUGGAGCAGCACAAGCAUUAUCUGGACGAGAUCAUUGAACAGAUUAGCG
AGUUCUCCAAAAGAGUGAUCCUGGCUGACGCAAUCUGGAUAAGGUCCUGAGCGCAU
ACAACAAACACCGGGAUAAAGCCAAUCAGAGAGCAGGCCGAAAAUAUCAUUCAUCUGU
UCACUCUGACCAACCUGGGAGCCCCCGCAGCCUUCAAGUAUUUUGACACUACCAUCGA
UCGCAAACGAUACACAAGCACUAAGGAGGUGCUGGACGCUACCCUGAUUCAUCAGAG
CAUUACUGGCCUGUAUGAAACAAGGAUUGACCUGUCUCAGCUGGGCGGCGACUCCGG
AGCUGACCCCAAGAAGAAGAGGAAGGUGUGAUAGUCUAGACCUUCUGCGGGGCUUGC
CUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCUCUUGGUCUUUGAAUAAA
GCCUGAGUAGGAA
AAA
AAAAAA

miR-21-responsive Cas9 mRNA

GGUUCCUUAUUCGCGGAUCCUCAACAUCAGUCUGAUAAAGCUAAGA UCCACCGGUCGCCA
CCAUGGAUAAGAAAUACAGCAUUGGACUGGACAUUGGGACAAACUCCGUGGGGAUGGG
CCGUGAUUACAGACGAAUACAAAGUGCCUUCAAAGAAGUUCAAGGUGCUGGGCAACA
CCGAUAGACACAGCAUCAAGAAAAAUCUGAUUGGAGCCCUGCUGUUCGACUCCGGCG
AGACAGCUGAAGCAACUCGGCUGAAAAGAACUGCUCGGAGAAGGUUAUACCCGCCGAA
AGAAUAGGAUCUGCUACCUGCAGGAGAUUUUCAGCAACGAAAUUGGCCAAGGUGGACG
AUAGUUUCUUUACCGCCUGGAGGAUCAUUCUGGUCGAGGAAGAUAAAGAAACACG
AGCGGCAUCCCAUCUUUGGCAACAUUGUGGACGAGGUCGCUUAUCACGAAAAGUACC
CUACCAUCUAUCAUCUGAGGAAGAAACUGGUGGACUCCACAGAUAAAGCAGACCUGC
GCCUGAUCUAUCUGGCCCGUGGCUCACAUGAUUAAGUUCGGGGCCAUUUUCUGAUCG
AGGGGGAUCUGAACCCAGACAAUUCUGAUGUGGACAAGCUGUUAUCCAGCUGGUCC
AGACAUACAAUCAGCUGUUUGAGGAAAACCCCAUUAUUGCAUCUGGCGUGGACGCAA
AAGCCAUCCUGAGUGCCAGACUGUCUAAGAGUCGGAGACUGGAGAACCUGAUCGCUC
AGCUGCCAGGGGAAAAGAAAAACGGCCUGUUUGGGAUUCUGAUUGCACUGUCACUGG
GACUGACUCCCAACUUCAAGAGCAAUUUUGAUCUGGCCGAGGACGCUAAACUGCAGC
UGUCCAAGGACACCUAUGACGAUGACCUGGAUAACCUGCUGGCUCAGAUCCGGGAUC
AGUACGCAGACCUGUUCUGGCCGCUAAGAAUCUGUCUGACGCCAUCCUGCUGAGUG
AUAUUCUGCGCGUGAACACCGAGAUUACAAAAGCCCCCUGUCAGCUAGCAUGAUCA
AGAGAU AUGACGAGCACCAUCAGGAUCUGACCCUGCUGAAGGCUCUGGUGAGGCAGC
AGCUGCCUGAGAAGUACAAGGAAAUCUUCUUUGAUCAGUCUAAGAACGGAUACGCCG
GCUAU AUUGACGGCGGGGCUAGUCAGGAGGAGUUCUACAAGUUUAUCAAACCCAUUC
UGGAGAAGAUGGAUGGCACAGAGGAACUGCUGGUGAAACUGAAUCGGGAAGACCUGC
UGAGGAAGCAGCGCACUUUUGAUAAACGGAAGCAUCCUCACCAGAUUCAUCUGGGAG
AGCUGCACGCAAUCCUGAGGCGCCAGGAAGACUUCUACCCAUUUCUGAAGGAUAACA
GGGAGAAGAUCGAAAAAAUUCUGACAUUCCGCAUCCCCUACUAUGUGGGCCCUCUGG
CAAGAGGCAACAGCCGGUUUGCCUGGAUGACUCGCAAUUCUGAGGAAACAAUCACUC
CCUGGAACUUCGAGGAAGUGGUCGAUAAGGGCGCUUCCGCACAGUCUUUCAUUGAGC
GGAUGACAAACUUCGACAAGAACCUGCCAAACGAAAAAGUGCUGCCCAAGCACUCUC
UGCUGUACGAGUAUUUCACAGUCUAUAACGAACUGACUAAGGUGAAAUAACGUCACCG
AGGGGAUGAGAAAGCCUGCCUUCUGAGUGGAGAACAGAAGAAAGCUAUCGUGGACC
UGCUGUUUAAAACCAAUAGGAAGGUGACAGUCAAGCAGCUGAAAGAGGACUAUUUCA
AGAAAAUUGAAUGUUUCGAUUCUGUGGAGAUCAUGGGCGUCGAAGACAGGUUUAACG

CCUCCCUGGGGACCUACCACGAUCUGCUGAAGAUCAUUAAGGAUAAAGACUCCUGG
ACAACGAGGAAAAUGAGGAUAUCCUGGAAGACAUUGUGCUGACCCUGACACUGUUUG
AGGAUAGGGAAAUGAUCGAGGAACGCCUGAAGACCUAUGCCCAUCUGUUCGAUGACA
AAGUGAUGAAACAGCUGAAGCGACGGAGAUACACAGGAUGGGGGCCGACUGUCUCGGA
AGCUGAUCAAUGGGAUUCGCGACAAACAGAGUGGAAAGACCAUCCUGGACUUUCUGA
AAUCAGAUGGGCUUCGCCAACCGGAACUUCAUGCAGCUGAUUCACGAUGACAGCCUGA
CAUUCAAGAGGAUAUCCAGAAGGCACAGGUGUCCGGGCAGGGAGACUCUCUGCACG
AGCAUAUCGCAAACCUGGCCGGCAGCCCUGCCAUCAAGAAAGGGAUUCUGCAGACCG
UGAAGGUGGUGGACGAGCUGGUGAAAGUCAUGGGAAGACAUAAAGCCAGAAAACAUCG
UGAUUGAGAUGGCCAGGGAAAAUCAGACCACACAGAAAGGCCAGAAGAACUCAAGGG
AGCGCAUGAAAAGAAUCGAGGAAGGAUUUAAGGAACUGGGCAGCCAGAUCUGAAAG
AGCACCCCGUGGAAAACACACAGCUGCAGAAUGAGAAGCUGUAUCUGUACUAUCUGC
AGAAUGGACGCGAUUAUGUACGUGGACCAGGAGCUGGAUAUUAACCGACUGUCCGAUU
ACGACGUGGAUCAUAUCGUCCCACAGUCAUCCUGAAAGAUGACAGCAUUGACAAUA
AGGUGCUGACCCGCUUCUGACAAAAACCGAGGCAAGAGUGAUAAUGUCCCCUCAGAGG
AAGUGGUCAAGAAAAUGAAGAACUACUGGAGGCAGCUGCUGAAUGCCAAACUGAUCA
CACAGCGAAAGUUUGAUAAACCUGACUAAAGCUGAGCGGGGAGGCCUGAGUGAACUGG
ACAAAGCAGGCUUCAUUAAGCGACAGCUGGUGGAGACACGGCAGAUACAAAGCACG
UCGCCCAGAUUCUGGAUUAAGAAUGAACACUAAGUACGAUGAGAAUGACAAACUGA
UCAGAGAAGUGAAGGUCAUUACCCUGAAGUCAAAACUGGUGAGCGACUUUCGGAAAG
AUUUCAGUUUUUAUAAGGUCAGAGAGAUCAACAACUACCACCAUGCUCAUGACGCAUA
CCUGAACGCAGUGGUCGGCACAGCCCUGAUUAAGAAAUACCCUAAACUGGAGUCCGA
GUUCGUGUACGGGGACUUAAGGUGUACGAUGUCAGAAAAAUGAUCGCCAAGUCUGA
GCAGGAAAUUGGCAAAGCCACUGCUAAGUAUUUCUUUUACAGUAACAUCAUGAAUUUC
UUUAAGACUGAGAUACCCUGGCAAUGGGGAAAUCCGAAAGCGGCCACUGAUUGAG
ACUAACGGCGAGACAGGAGAAAUCGUGUGGGACAAAGGAAGAGAUUUUGCUACCGUG
AGGAAGGUCCUGAGCAUGCCCCAAGUGAAUAUUGUCAAGAAAACAGAGGUGCAGACU
GGGGGAUUCAGUAAGGAAUCAAUUCUGCCUAAACGCAACUCCGAUAAGCUGAUCGCC
CGAAAGAAAGACUGGGACCCCAAGAAGUAUGGCGGGUUCGACUCCCCAACUGUGGCU
UACUCUGUCCUGGUGGUCGCAAAGGUGGAGAAGGGAAAAAGCAAGAAACUGAAAUCC
GUCAAGGAACUGCUGGGCAUCACCAUUAUGGAGCGCAGCUCCUUCGAAAAGAAUCCU
AUCGAUUUUCUGGAGGCCAAAGGCUAUAAGGAAGUGAAGAAAGACCUGAUCAUCAAG
CUGCCAAAGUACUCACUGUUUGAGCUGGAAAACGGGAGAAAGAGGAUGCUGGCAAGC

GCCGGGGAGCUGCAGAAAGGAAAUGAACUGGCCCUGCCCUCCAAGUACGUGAACUUC
CUGUAUCUGGCUAGCCACUACGAGAAGCUGAAAGGGUCCCCUGAGGAUAACGAACAG
AAACAGCUGUUUGUGGAGCAGCACAAGCAUUAUCUGGACGAGAUCAUUGAACAGAUU
AGCGAGUUCUCCAAAAGAGUGAUCCUGGCUGACGCAAUCUGGAUAAGGUCCUGAGC
GCAUACAACAAACACCGGGAUAAGCCAAUCAGAGAGCAGGCCGAAAAUAUCAUUCAU
CUGUUCACUCUGACCAACCUGGGAGCCCCCGCAGCCUUCAAGUAUUUUGACACUACCA
UCGAUCGCAAACGAUACACAAGCACUAAGGAGGUGCUGGACGCUACCCUGAUUCAUC
AGAGCAUUACUGGCCUGUAUGAAACAAGGAUUGACCUGUCUCAGCUGGGCGGCGACU
CCGGAGCUGACCCCAAGAAGAAGAGGAAGGUGUGAUAGUCUAGACCUUCUGCGGGGC
UUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCUCUUGGUCUUUGAA
UAAAGCCUGAGUAGGAAA
AAA
AAAAAAAAAA

miR-302-responsive Cas9 mRNA

GGUUCCUUAUUCGCGGAUCCAGCAAGUACAUCCACGUUUAAGUAGA UCCACCGGUCGC
CACCAUGGAUAAGAAAUACAGCAUUGGACUGGACAUUGGGACAAACUCCGUGGGGAUG
GGCCGUGAUUACAGACGAAUACAAAGUGCCUUCAAAGAAGUUCAAGGUGCUGGGCAA
CACCGAUAGACACAGCAUCAAGAAAAAUCUGAUUGGAGCCCUGCUGUUCGACUCCGG
CGAGACAGCUGAAGCAACUCGGCUGAAAAGAACUGCUCGGAGAAGGUUAUACCCGCCG
AAAGAAUAGGAUCUGCUACCUGCAGGAGAUUUUCAGCAACGAAAUGGCCAAGGUGGA
CGAUAGUUUCUUUCACCGCCUGGAGGAUCAUUCUGGUCGAGGAAGAUAAAGAAACA
CGAGCGGCAUCCCAUCUUUGGCAACAUUGUGGACGAGGUCGCUUAUCACGAAAAGUA
CCCUACCAUCUAUCAUCUGAGGAAGAAACUGGUGGACUCCACAGAUAAAGCAGACCU
GCGCCUGAUCUAUCUGGCCCUGGCUCACAUGAUUAAGUUCGGGGCCAUUUUCUGAU
CGAGGGGGGAUCUGAACCCAGACAAUUCUGAUGUGGACAAGCUGUUAUCCAGCUGGU
CCAGACAUACAAUCAGCUGUUUGAGGAAAACCCCAUUAUGCAUCUGGCGUGGACGC
AAAAGCCAUCCUGAGUGCCAGACUGUCUAAGAGUCGGAGACUGGAGAACCUGAUCGC
UCAGCUGCCAGGGGAAAAGAAAAACGGCCUGUUUGGGAUCUGAUUGCACUGUCACU
GGGACUGACUCCCAACUUCAAGAGCAAUUUUGAUCUGGCCGAGGACGCUAAACUGCA
GCUGUCCAAGGACACCUAUGACGAUGACCUGGAUAACCUGGCUGGCUCAGAUCGGGGA
UCAGUACGCAGACCUGUUCUGGCCGCUAAGAAUCUGUCUGACGCCAUCCUGCUGAG
UGAUAUUCUGCGCGUGAACACCGAGAUUACAAAAGCCCCCUGUCAGCUAGCAUGAU
CAAGAGAUUAGACGAGCACCAUCAGGAUCUGACCCUGCUGAAGGCUCUGGUGAGGCA
GCAGCUGCCUGAGAAGUACAAGGAAAUCUUCUUUGAUCAGUCUAAGAACGGAUACGC
CGGCUAUUUGACGGCGGGGCUAGUCAGGAGGAGUUCUACAAGUUUAUCAAAACCCAU
UCUGGAGAAGAUGGAUGGCACAGAGGAACUGCUGGUGAAACUGAAUCGGGAAGACCU
GCUGAGGAAGCAGCGCACUUUUGAUAAACGGAAGCAUCCUCACCAGAUUCAUCUGGG
AGAGCUGCACGCAAUCCUGAGGCGCCAGGAAGACUUCUACCCAUUUCUGAAGGAUAA
CAGGGAGAAGAUCGAAAAAAUUCUGACAUUCGCAUCCCCUACUAUGUGGGCCCUCU
GGCAAGAGGCAACAGCCGGUUUGCCUGGAUGACUCGCAAUCUGAGGAAACAAUCAC
UCCUGGAACUUCGAGGAAGUGGUCGAUAAGGGCGCUUCCGCACAGUCUUUCAUUGA
GCGGAUGACAAACUUCGACAAGAACCUGCCAAACGAAAAAGUGCUGCCCAAGCACUC
UCUGCUGUACGAGUAUUUCACAGUCUAUAACGAACUGACUAAGGUGAAAUAACGUCAC
CGAGGGGAUGAGAAAGCCUGCCUCCUGAGUGGAGAACAGAAGAAAGCUAUCGUGGA
CCUGCUGUUUAAAACCAAUAGGAAGGUGACAGUCAAGCAGCUGAAAGAGGACUAUUU
CAAGAAAAUUGAAUGUUUCGAUUCUGUGGAGAUCAGUGGCGUCGAAGACAGGUUUA

CGCCUCCCUGGGGACCUACCACGAUCUGCUGAAGAUCAUUAAGGAUAAAGACUCCU
GGACAACGAGGAAAAUGAGGAUAUCCUGGAAGACAUUGUGCUGACCCUGACACUGUU
UGAGGAUAGGGAAAUGAUCGAGGAACGCCUGAAGACCUAUGCCCAUCUGUUCGAUGA
CAAAGUGAUGAAACAGCUGAAGCGACGGAGAUACACAGGAUGGGGCGACUGUCUCG
GAAGCUGAUCAAUGGGAUUCGCGACAAACAGAGUGGAAAGACCAUCCUGGACUUUCU
GAAAUCAUGAUGGCUUCGCCAACCGGAACUUCAUGCAGCUGAUUCACGAUGACAGCCU
GACAUUCAAGAGGAUAUCCAGAAGGCACAGGUGUCCGGGCAGGGAGACUCUCUGCA
CGAGCAUAUCGCAAACCUGGCCGGCAGCCCUGCCAUAAGAAAGGGAUUCUGCAGAC
CGUGAAGGUGGUGGACGAGCUGGUGAAAGUCAUGGGAAGACAUAAAGCCAGAAAACAU
CGUGAUUGAGAUGGCCAGGGAAAAUCAGACCACACAGAAAGGCCAGAAGAACUCAAG
GGAGCGCAUGAAAAGAAUCGAGGAAGGAAUUAAGGAACUGGGCAGCCAGAUCUGAA
AGAGCACCCCGUGGAAAACACACAGCUGCAGAAUGAGAAGCUGUAUCUGUACUAUCU
GCAGAAUGGACGCGAUUUGUACGUGGACCAGGAGCUGGAUAUUAACCGACUGUCCGA
UUACGACGUGGAUCAUAUCGUCCACAGUCAUUCUGAAAGAUGACAGCAUUGACAA
UAAGGUGCUGACCCGCUCUGACAAAAACCGAGGCAAGAGUGAUAAUGUCCCUUGAGA
GGAAGUGGUCAAGAAAAUGAAGAACUACUGGAGGCAGCUGCUGAAUGCCAAACUGAU
CACACAGCGAAAGUUUGAUAAACCUGACUAAAGCUGAGCGGGGAGGCCUGAGUGAACU
GGACAAAGCAGGCUUCAUUAAGCGACAGCUGGUGGAGACACGGCAGAUACAAAAGCA
CGUCGCCCAGAUUCUGGAUUAAGAAUGAACACUAAGUACGAUGAGAAUGACAAACU
GAUCAGAGAAGUGAAGGUCAUUAACCCUGAAGUCAAAACUGGUGAGCGACUUUCGGAA
AGAUUUCAGUUUUUAUAAAGGUCAGAGAGAUCAACAACUACCACCAUGCUCAUGACGCA
UACCUGAACGCAGUGGUCGGCACAGCCCUGAUUAAGAAAUAACCUAAACUGGAGUCC
GAGUUCGUGUACGGGGACUAUAAGGUGUACGAUGUCAGAAAAAUGAUCGCCAAGUCU
GAGCAGGAAAUUGGCAAAGCCACUGCUAAGUAUUUCUUUUACAGUAACAUCAUUGAAU
UUCUUUAAGACUGAGAUACCCUGGCAAUUGGGGAAAUCCGAAAGCGGCCACUGAUU
GAGACUAACGGCGAGACAGGAGAAAUCGUGUGGGACAAAGGAAGAGAUUUUGCUACC
GUGAGGAAGGUCCUGAGCAUGCCCCAAGUGAAUAUUGUCAAGAAAACAGAGGUGCAG
ACUGGGGGGAUUCAGUAAGGAAUCAAUUCUGCCUAAACGCAACUCCGAUAAGCUGAUC
GCCCCGAAAGAAAGACUGGGACCCCAAGAAGUAUGGCGGGUUCGACUCCCCAACUGUG
GCUUACUCUGUCCUGGUGGUCGAAAGGUGGAGAAGGGGAAAAAGCAAGAAACUGAAA
UCCGUCAAGGAACUGCUGGGCAUCACCAUUAUGGAGCGCAGCUCCUUCGAAAAGAAU
CCUAUCGAUUUUCUGGAGGCCAAAGGCUAUAAGGAAGUGAAGAAAGACCUGAUCAUC
AAGCUGCCAAAGUACUCACUGUUUGAGCUGGAAAACGGGAGAAAGAGGAUGCUGGCA

AGCGCCGGGGAGCUGCAGAAAGGAAAUGAACUGGCCCUGCCCUGCAAGUACGUGAAC
UUCCUGUAUCUGGCUAGCCACUACGAGAAGCUGAAAGGGUCCCCUGAGGAUAACGAA
CAGAAACAGCUGUUUGUGGAGCAGCACAAAGCAUUAUCUGGACGAGAUCAUUGAACAG
AUUAGCGAGUUCUCCAAAAGAGUGAUCCUGGCUGACGCAAUCUGGAUAAGGUCCUG
AGCGCAUACAACAAACACCGGGAUAAAGCCAAUCAGAGAGCAGGCCGAAAAUAUCAUU
CAUCUGUUCACUCUGACCAACCUGGGAGCCCCCGCAGCCUUAAGUAUUUUGACACUA
CCAUCGAUCGCAAACGAUACACAAGCACUAAGGAGGUGCUGGACGCUACCCUGAUUC
AUCAGAGCAUUACUGGCCUGUAUGAAACAAGGAUUGACCUGUCUCAGCUGGGCGGCG
ACUCCGGAGCUGACCCCAAGAAGAAGAGGAAGGUGUGAUAGUCUAGACCUUCUGCGG
GGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCUCUUGGUCUUU
GAAUAAAGCCUGAGUAGGAAA
AA
AAAAAAAAAAAAA

4x miR-302-responsive Cas9 mRNA

GGUUCCUUAUUCGCGGAUCCAGCAAGUACAUCCACGUUUAAGUAGCAAGUACAUCCACG
UUUAAGUAGCAAGUACAUCCACGUUUAAGUAGCAAGUACAUCCACGUUUAAGUAGAUC
ACCCGGUCGCCACCAUGGAUAAGAAUACAGCAUUGGACUGGACAUUGGGACAAACU
CCGUGGGGAUGGGCCGUGAUUACAGACGAAUACAAAGUGCCUUCAAAGAAGUUCAAGG
UGCUGGGCAACACCGAUAGACACAGCAUCAAGAAAAAUCUGAUUGGAGCCCUGCUGU
UCGACUCCGGCGAGACAGCUGAAGCAACUCGGCUGAAAAGAACUGCUCGGAGAAGGU
AUACCCGCCGAAAGAAUAGGAUCUGCUACCUGCAGGAGAUUUUCAGCAACGAAAUUG
CCAAGGUGGACGAUAGUUUCUUCACCGCCUGGAGGAAUCAUUCUGGUCGAGGAAG
AUAAGAAACACGAGCGGCAUCCCAUCUUUGGCAACAUUGUGGACGAGGUCGCUUAUC
ACGAAAAGUACCCUACCAUCUAUCAUCUGAGGAAGAAACUGGUGGACUCCACAGAU
AAGCAGACCUGCGCCUGAUCUAUCUGGCCUGGCUCACAUGAUUAAGUUCGGGGCC
AUUUUCUGAUCGAGGGGGGAUCUGAACCCAGACAAUUCUGAUGUGGACAAGCUGUUA
UCCAGCUGGUCCAGACAUACAAUCAGCUGUUUGAGGAAAACCCCAUUAUGCAUCUG
GCGUGGACGCAAAAGCCAUCCUGAGUGCCAGACUGUCUAAGAGUCGGAGACUGGAGA
ACCUGAUCGCUCAGCUGCCAGGGGAAAAGAAAAACGGCCUGUUUGGGAUCUGAUUG
CACUGUCACUGGGACUGACUCCCAACUUAAGAGCAAUUUUGAUCUGGCCGAGGACG
CUAAACUGCAGCUGUCCAAGGACACCUAUGACGAUGACCUGGAUAACCUGCUGGCUC
AGAUCGGGGAUCAGUACGCAGACCUGUUCUGGCCGCUAAGAAUCUGUCUGACGCCA
UCCUGCUGAGUGAUUUCUGCGCGUGAACACCGAGAUUACAAAAGCCCCCUGUCAG
CUAGCAUGAUCAAGAGAUUUGACGAGCACCAUCAGGAUCUGACCUGCUGAAGGCUC
UGGUGAGGCAGCAGCUGCCUGAGAAGUACAAGGAAUUCUUCUUUGAUCAGUCUAAGA
ACGGAUACGCCGGCUAUUUGACGGCGGGGCUAGUCAGGAGGAGUUCUACAAGUUUA
UCAAACCCAUUCUGGAGAAGAUGGAUGGCACAGAGGAACUGCUGGUGAAACUGAAUC
GGGAAGACCUGCUGAGGAAGCAGCGCACUUUUGAUAAACGGAAGCAUCCUCACCAGA
UUAUCUGGGAGAGCUGCACGCAUCCUGAGGCGCCAGGAAGACUUCUACCCAUUUC
UGAAGGAUAACAGGGAGAAGAUCGAAAAAAUUCUGACAUUCCGCAUCCCUACUAUG
UGGGCCCUCUGGCAAGAGGCAACAGCCGGUUUGCCUGGAUGACUCGCAAAUCUGAGG
AAACAAUCACUCCUGGAACUUCGAGGAAGUGGUCGAUAAGGGCGCUUCCGCACAGU
CUUUCAUUGAGCGGAUGACAAACUUCGACAAGAACCUGCCAAACGAAAAAGUGCUGC
CCAAGCACUCUCUGCUGUACGAGUAUUUCACAGUCUAUAACGAACUGACUAAGGUGA
AAUACGUCACCGAGGGGAUGAGAAAGCCUGCCUCCUGAGUGGAGAACAGAAGAAAG
CUAUCGUGGACCUGCUGUUUAAAACCAUAGGAAGGUGACAGUCAAGCAGCUGAAAG

AGGACUAUUUCAAGAAAAUUGAAUGUUUCGAUUCUGUGGAGAUCAUGGGCGUCGAAG
ACAGGUUUAACGCCUCCCUGGGGACCUACCACGAUCUGCUGAAGAUAUUAAGGAUA
AAGACUCCUGGACAACGAGGAAAAUGAGGAUAUCCUGGAAGACAUUGUGCUGACCC
UGACACUGUUUGAGGAUAGGGAAAUGAUCGAGGAACGCCUGAAGACCUAUGCCCAUC
UGUUCGAUGACAAAGUGAUGAAACAGCUGAAGCGACGGAGAUACACAGGAUGGGGGCC
GACUGUCUCGGAAGCUGAUCAAUGGGAUUCGCGACAAACAGAGUGGAAAGACCAUCC
UGGACUUUCUGAAAUCAGAUGGCUUCGCCAACCGGAACUUCAUGCAGCUGAUUCACG
AUGACAGCCUGACAUUCAAGAGGAUAUCCAGAAGGCACAGGUGUCCGGGCAGGGAG
ACUCUCUGCACGAGCAUAUCGCAAACCUGGCCGGCAGCCCUGCCAUCAAGAAAGGGA
UUCUGCAGACCGUGAAGGUGGUGGACGAGCUGGUGAAAGUCAUGGGAAGACAUAAAGC
CAGAAAACAUCGUGAUUGAGAUGGCCAGGGAAAAUCAGACCACACAGAAAGGCCAGA
AGAACUCAAGGGAGCGCAUGAAAAGAAUCGAGGAAGGAAUUAAGGAACUGGGCAGCC
AGAUCUGAAAGAGCACCCCGUGGAAAACACACAGCUGCAGAAUGAGAAGCUGUAUC
UGUACUAUCUGCAGAAUGGACGCGAUUUGUACGUGGACCAGGAGCUGGAUAUUAACC
GACUGUCCGAUUACGACGUGGAUCAUAUCGUCCCACAGUCAUUCUGAAAGAUGACA
GCAUUGACAAUAAGGUGCUGACCCGCUCUGACAAAAACCGAGGCAAGAGUGAUAAUG
UCCCCUCAGAGGAAGUGGUCAAGAAAAUGAAGAAUACUGGAGGCAGCUGCUGAAUG
CCAAACUGAUCACACAGCGAAAGUUUGAUAAACCUGACUAAAGCUGAGCGGGGAGGCC
UGAGUGAACUGGACAAAGCAGGCUUCAUUAAGCGACAGCUGGUGGAGACACGGCAGA
UCACAAAGCACGUCGCCCAGAUUCUGGAUUAAGAAUGAACACUAAGUACGAUGAGA
AUGACAAACUGAUCAGAGAAGUGAAGGUCAUUACCCUGAAGUCAAAACUGGUGAGCG
ACUUUCGGAAAGAUUUCAGUUUUUAUAAAGGUCAGAGAGAUCAACAACUACCACCAUGC
UCAUGACGCAUACCUGAACGCAGUGGUCGGCACAGCCCUGAUUAAGAAAUAACCUAA
ACUGGAGUCCGAGUUCGUGUACGGGGACUAUAAGGUGUACGAUGUCAGAAAAAUGAU
CGCCAAGUCUGAGCAGGAAAUUGGCAAAGCCACUGCUAAGUAUUUCUUUUACAGUAA
CAUCAUGAAUUUCUUUAAGACUGAGAUACCCUGGCAAUUGGGGAAAUCCGAAAGCG
GCCACUGAUUGAGACUAACGGCGAGACAGGAGAAAUCGUGUGGGACAAAGGAAGAGA
UUUUGCUACCGUGAGGAAGGUCCUGAGCAUGCCCCAAGUGAAUAUUGUCAAGAAAAC
AGAGGUGCAGACUGGGGGAUUCAGUAAGGAUCAAUUCUGCCUAAACGCAACUCCGA
UAAGCUGAUCGCCGAAAGAAAGACUGGGACCCCAAGAAGUAUGGCGGGUUCGACUC
CCCAACUGUGGCUUACUCUGUCCUGGUGGUCGCAAAGGUGGAGAAGGGAAAAAGCAA
GAAACUGAAAUCCGUCAAGGAACUGCUGGGCAUCACCAUUAUGGAGCGCAGCUCCUU
CGAAAAGAAUCCUAUCGAUUUUCUGGAGGCCAAAGGCUAUAAGGAAGUGAAGAAAGA

CCUGAUCAUCAAGCUGCCAAAGUACUCACUGUUUGAGCUGGAAAACGGGAGAAAGAG
GAUGCUGGCAAGCGCCGGGGAGCUGCAGAAAGGAAAUGAACUGGCCCUGCCCUCCAA
GUACGUGAACUCCUGUAUCUGGCUAGCCACUACGAGAAGCUGAAAGGGUCCCCUGA
GGAUAACGAACAGAAACAGCUGUUUGUGGAGCAGCACAAAGCAUUAUCUGGACGAGAU
CAUUGAACAGAUUAGCGAGUUCUCCAAAAGAGUGAUCCUGGCUGACGCAAUCUGGA
UAAGGUCCUGAGCGCAUACAACAAACACCGGGAUAAGCCAAUCAGAGAGCAGGCCGA
AAAUAUCAUUAUCUGUUCACUCUGACCAACCUGGGAGCCCCCGCAGCCUUAAGUAU
UUUGACACUACCAUCGAUCGCAAACGAUACACAAGCACUAAGGAGGUGCUGGACGCU
ACCCUGAUUCAUCAGAGCAUUACUGGCCUGUAUGAAACAAGGAUUGACCUGUCUCAG
CUGGGCGGCGACUCCGGAGCUGACCCCAAGAAGAAGAGGAAGGUGUGAUAGUCUAGA
CCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCU
CUUGGUCUUUGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA
AAAAAAAAAAAAAAAAAAAAAAAAAAAA

Kt-Cas9 mRNA

GGAUCCGUGAUCGGAAACGUGAGAUCACCUCAGAUCCGCUAGGACACCCGCAGAUCC
AGAAGAAGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGC
CACCAUGGAUAAGAAUACAGCAUUGGACUGGACAUUGGGACAAACUCCGUGGGAUG
GGCCGUGAUUACAGACGAAUACAAAGUGCCUUCAAAGAAGUUCAAGGUGCUGGGCAA
CACCGAUAGACACAGCAUCAAGAAAAAUCUGAUUGGAGCCCUGCUGUUCGACUCCGG
CGAGACAGCUGAAGCAACUCGGCUGAAAAGAACUGCUCGGAGAAGGUUAUACCCGCCG
AAAGAAUAGGAUCUGCACCGCAGGAGAUUUUCAGCAACGAAAUGGCCAAGGUGGA
CGAUAGUUUCUUUCACCGCCUGGAGGAAUCAUUCUGGUCGAGGAAGAUAAAGAAACA
CGAGCGGCAUCCCAUCUUUGGCAACAUUGUGGACGAGGUCGCUUAUCACGAAAAGUA
CCCUACCAUCUAUCAUCUGAGGAAGAAACUGGUGGACUCCACAGAUAAAGCAGACCU
GCGCCUGAUCUAUCUGGCCUGGCUCACAUGAUUAAGUUCGGGGCCAUUUUCUGAU
CGAGGGGGAUUCUGAACCCAGACAAUUCUGAUGUGGACAAGCUGUUAUCCAGCUGGU
CCAGACAUACAAUCAGCUGUUUGAGGAAAACCCCAUUAUGCAUCUGGCGUGGACGC
AAAAGCCAUCCUGAGUGCCAGACUGUCUAAGAGUCGGAGACUGGAGAACCUGAUCGC
UCAGCUGCCAGGGGAAAAGAAAAACGGCCUGUUUGGGAAUCUGAUUGCACUGUCACU
GGGACUGACUCCCAACUUAAGAGCAAUUUUGAUCUGGCCGAGGACGCUAAACUGCA
GCUGUCCAAGGACACCUAUGACGAUGACCUGGAUAACCUGCUGGCUCAGAUCGGGGA
UCAGUACGCAGACCUGUUCUGGCCGCUAAGAAUCUGUCUGACGCCAUCCUGCUGAG
UGAUAUUCUGCGCGUGAACACCGAGAUUACAAAAGCCCCCUGUCAGCUAGCAUGAU
CAAGAGAUUAGACGAGCACCAUCAGGAUCUGACCCUGCUGAAGGCUCUGGUGAGGCA
GCAGCUGCCUGAGAAGUACAAGGAAAUCUUCUUUGAUCAGUCUAAGAACGGAUACGC
CGGCUAUAUUGACGGCGGGGCUAGUCAGGAGGAGUUCUACAAGUUUAUCAAACCCAU
UCUGGAGAAGAUGGAUGGCACAGAGGAACUGCUGGUGAAACUGAAUCGGGAAGACCU
GCUGAGGAAGCAGCGCACUUUUGAUAAACGGAAGCAUCCCUACCCAGAUUCAUCUGGG
AGAGCUGCACGCAAUCCUGAGGCGCCAGGAAGACUUCUACCCAUUUCUGAAGGAUAA
CAGGGAGAAGAUCGAAAAAAUUCUGACAUUCGCAUCCCCUACUAUGUGGGCCCUCU
GGCAAGAGGCAACAGCCGGUUUGCCUGGAUGACUCGCAAUCUGAGGAAACAAUCAC
UCCUGGAACUUCGAGGAAGUGGUCGAUAAGGGCGCUUCCGCACAGUCUUUCAUUGA
GCGGAUGACAAACUUCGACAAGAACCUGCCAAACGAAAAAGUGCUGCCCAAGCACUC
UCUGCUGUACGAGUAUUUCACAGUCUAUAACGAACUGACUAAGGUGAAAUACGUCAC
CGAGGGGAUGAGAAAGCCUGCCUUCUGAGUGGAGAACAGAAGAAAGCUAUCGUGGA
CCUGCUGUUUAAAACCAAUAGGAAGGUGACAGUCAAGCAGCUGAAAGAGGACUAUUU

CAAGAAAUUGAAUGUUUCGAUUCUGUGGAGAUCAUGGGCGUCGAAGACAGGUUUAA
CGCCUCCCUGGGGACCUACCACGAUCUGCUGAAGAUAUUAAGGAUAAAGACUCCU
GGACAACGAGGAAAAUGAGGAUAUCCUGGAAGACAUUGUGCUGACCCUGACACUGUU
UGAGGAUAGGGAAAUGAUUCGAGGAACGCCUGAAGACCUAUGCCCAUCUGUUCGAUGA
CAAAGUGAUGAAACAGCUGAAGCGACGGAGAUACACAGGAUGGGGCGGACUGUCUCG
GAAGCUGAUAUUGGGAUUCGCGACAAACAGAGUGGAAAGACCAUCCUGGACUUUCU
GAAAUCAUGGCUUCGCCAACCGGAACUUCAUGCAGCUGAUUCACGAUGACAGCCU
GACAUUCAAGAGGAUAUCCAGAAGGCACAGGUGUCCGGGCAGGGAGACUCUCUGCA
CGAGCAUAUCGCAAACCUGGCCGGCAGCCUGCCAUAAGAAAGGGAUUCUGCAGAC
CGUGAAGGUGGUGGACGAGCUGGUGAAAGUCAUGGGAAGACAUAAAGCCAGAAAACAU
CGUGAUUGAGAUGGCCAGGGAAAAUCAGACCACACAGAAAGGCCAGAAGAACUCAAG
GGAGCGCAUGAAAAGAAUCGAGGAAGGAUUUAAGGAACUGGGCAGCCAGAUCUGAA
AGAGCACCCCGUGGAAAACACACAGCUGCAGAAUGAGAAGCUGUAUCUGUACUAUCU
GCAGAAUGGACGCGAUUUGUACGUGGACCAGGAGCUGGAUAUUAACCGACUGUCCGA
UUACGACGUGGAUCAUAUCGUCCACAGUCAUUCUGAAAGAUGACAGCAUUGACAA
UAAGGUGCUGACCCGCUCUGACAAAAACCGAGGCAAGAGUGAUAAUGUCCCCUCAGA
GGAAGUGGUCAAGAAAAUGAAGAACUACUGGAGGCAGCUGCUGAAUGCCAAACUGAU
CACACAGCGAAAGUUUGAUAAACCUGACUAAAGCUGAGCGGGGAGGCCUGAGUGAACU
GGACAAAGCAGGCUUCAUUAAGCGACAGCUGGUGGAGACACGGCAGAUACAAAGCA
CGUCGCCCAGAUUCUGGAUUAAGAAUGAACACUAAGUACGAUGAGAAUGACAAACU
GAUCAGAGAAGUGAAGGUCAUUAACCCUGAAGUCAAAACUGGUGAGCGACUUUCGGAA
AGAUUUCAGUUUUUAUAAAGGUCAGAGAGAUCAACAACUACCACCAUGCUCAUGACGCA
UACCUGAACGCAGUGGUCGGCACAGCCCUGAUUAAGAAAUAACCUAAACUGGAGUCC
GAGUUCGUGUACGGGGACUAUAAGGUGUACGAUGUCAGAAAAAUGAUCGCCAAGUCU
GAGCAGGAAAUUGGCAAAGCCACUGCUAAGUAUUUCUUUUACAGUAACAUCAUGAAU
UUCUUUAAGACUGAGAUACCCUGGCAAUUGGGGAAAUCCGAAAGCGGCCACUGAUU
GAGACUAACGGCGAGACAGGAGAAAUCGUGUGGGACAAAGGAAGAGAUUUUGCUACC
GUGAGGAAGGUCCUGAGCAUGCCCCAAGUGAAUAUUGUCAAGAAAACAGAGGUGCAG
ACUGGGGGGAUUCAGUAAGGAAUCAAUUCUGCCUAAACGCAACUCCGAUAAGCUGAUC
GCCCAGAAAGAAAGACUGGGACCCCAAGAAGUAUGGCGGGUUCGACUCCCCAACUGUG
GCUUACUCUGUCCUGGUGGUCGCAAAGGUGGAGAAGGGAAAAAGCAAGAAACUGAAA
UCCGUCAAGGAACUGCUGGGCAUCACCAUUAUGGAGCGCAGCUCCUUCGAAAAGAAU
CCUAUCGAUUUUCUGGAGGCCAAAGGCUAUAAGGAAGUGAAGAAAGACCUGAUAUC

AAGCUGCCAAAGUACUCACUGUUUGAGCUGGAAAACGGGAGAAAGAGGAUGCUGGCA
AGCGCCGGGGAGCUGCAGAAAGGAAAUGAACUGGCCCUGCCCUGCAAGUACGUGAAC
UUCCUGUAUCUGGCUAGCCACUACGAGAAGCUGAAAGGGUCCCCUGAGGAUAACGAA
CAGAAACAGCUGUUUGUGGAGCAGCACAAGCAUUAUCUGGACGAGAUCAUUGAACAG
AUUAGCGAGUUCUCCAAAAGAGUGAUCCUGGCUGACGCAAUCUGGAUAAGGUCCUG
AGCGCAUACAACAAACACCGGGGAUAAGCCAAUCAGAGAGCAGGCCGAAAAUAUCAU
CAUCUGUUCACUCUGACCAACCUGGGAGCCCCCGCAGCCUUAAGUAUUUUGACACUA
CCAUCGAUCGCAAACGAUACACAAGCACUAAGGAGGUGCUGGACGCUACCCUGAUUC
AUCAGAGCAUUAUCUGGCCUGUAUGAAACAAGGAUUGACCUGUCUCAGCUGGGCGGCG
ACUCCGGAGCUGACCCCAAGAAGAAGAGGAAGGUGUGAUAGUCUAGACCUUCUGCGG
GGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCUCUUGGUCUUU
GAAUAAAGCCUGAGUAGGAAA
AA
AAAAAAAAAAAAA

L7Ae mRNA

GGGCGAAUUAAGAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACCAU
GUACGUGAGAUUUUGAGGUUCCUGAGGACAUGCAGAACGAAGCUCUGAGUCUGCUGGA
GAAGGUUAGGGAGAGCGGUAAGGUAAAGAAAGGUACCAACGAGACGACAAAGGCUGU
GGAGAGGGGACUGGCAAAGCUCGUUUACAUCGCAGAGGAUGUUGACCCGCCUGAGAU
CGUUGCUCaucugccccuccucugcgaggagaagaaugugccguacauuuacguuAA
AAGCAAGAACGACCUUGGAAGGGCUGUGGGCAUUGAGGUGCCAUGCGCUUCGGCAGC
GAUAAUCAACGAGGGAGAGCUGAGAAAGGAGCUUGGAAGCCUUGUGGAGAAGAUUAA
AGGCCUUCAGAAGAGAUCUCAUAUGCAUCUCGAGUGAUAGUCUAGACCUUCUGCGGG
GCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCUCUUGGUCUUUG
AAUAAAGCCUGAGUAGGAAA
AA
AAAAAAAAAAAA

miR-21-responsive *L7Ae mRNA*

GGUUCCUUAUUCGCGGAUCCUCAACAUCAGUCUGAUAGCUAAGAUCACACCGGUCGCC
ACCAUGUACGUGAGAUUUUGAGGUUCCUGAGGACAUGCAGAACGAAGCUCUGAGUCUG
CUGGAGAAGGUUAGGGAGAGCGGUAAGGUAAAGAAAGGUACCAACGAGACGACAAAG
GCUGUGGAGAGGGGACUGGCAAAGCUCGUUUACAUCGCAGAGGAUGUUGACCCGCCU
GAGAUCGUUGCUCaucugccccuccucugcgaggagaagaaugugccguacauuuac
GUUAAAAGCAAGAACGACCUUGGAAGGGCUGUGGGCAUUGAGGUGCCAUGCGCUUCG
GCAGCGAUAAUCAACGAGGGAGAGCUGAGAAAGGAGCUUGGAAGCCUUGUGGAGAAG
AUUAAAGGCCUUCAGAAGAGAUCUCAUAUGCAUCUCGAGUGAUAGUCUAGACCUUCUG
CGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCUCUUGGUC
UUUGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA
AAAAAAAAAAAA

Tag *BFP* mRNA

GGGCGAAUUAAGAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACCAU
GGAUCCAGCGAGCUGAUUAAGGAGAACAUGCACAUGAAGCUGUACAUGGAGGGCAC
CGUGGACAACCAUCACUUCAAGUGCACAUCCGAGGGCGAAGGCAAGCCCUACGAGGG
CACCCAGACCAUGAGAAUCAAGGUGGUCGAGGGCGGCCUCUCCCCUUCGCCUUCGA
CAUCCUGGCUACUAGCUUCCUCUACGGCAGCAAGACCUUCAUCAACCACACCCAGGGC
AUCCCCGACUUCUUCAAGCAGUCCUUCCUGAGGGCUUCACAUGGGAGAGAGUCACC
ACAUACGAAGACGGGGGCGUGCUGACCGCUACCCAGGACACCAGCCUCCAGGACGGC
UGCCUCAUCUACAACGUCAAGAUCAGAGGGGUGAACUUCACAUCCAACGGCCCUGUG
AUGCAGAAGAAAACACUCGGCUGGGAGGCCUUCACCGAGACGCUGUACCCCGCUGAC
GGCGGCCUGGAAGGCAGAAACGACAUGGCCCUGAAGCUCGUGGGCGGGAGCCAUCUG
AUCGCAAACAUCAAGACCACAUAUAGAUCCAAGAAACCCGCUAAGAACCUCAGAUGC
CUGGCGUCUACUUGUGGACUACAGACUGGAAAGAAUCAAGGAGGCCAACACGAGA
CCUACGUCGAGCAGCACGAGGUGGCAGUGGCCAGAUACUGCGACCUCUCCUAGCAAAC
UGGGGCACAGAUCUCAUAUGCAUCUCGAGUGAUAGUCUAGACCUUCUGCGGGGCUUG
CCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCUCUUGGUCUUUGAAUAA
AGCCUGAGUAGGAAA
AAA
AAAAAA

sgRNA_{GFP}

GGGGGCACGGGCAGCUUGCCGGGUUUUAGAGCUAGAAUAGCAAGUUAAAAUAAGGCU
AGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUU

sgRNA2_{GFP}

GGGCACGGGCAGCUUGCCGGGUUUUAGAGCUAGAAUAGCAAGUUAAAAUAAGGCUAG
UCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUU

sgRNA_{DMD}

GGUAUCUUACAGGAACUCCGUUUUAGAGCUAGAAUAGCAAGUUAAAAUAAGGCUAGUC
CGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUU

sgRNA_{Alu1}

GGGCCUGUAAUCCAGCACUUUGUUUAGAGCUAGAAUAGCAAGUUAAAAUAAGGCUA
GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGG

Supplementary Table S6: Plasmid sequences used in this study

1. The protein coding regions (Cas9, L7Ae or BFP) are shown as bold letters.
2. The start and stop codons are underlined.
3. L7Ae was cloned from L7Ae plasmid provided by A. Huttenhofer (Innsbruck Medical University, Biocenter) and T.S. Rozhdestvensky (University of Muenster).
4. BFP was cloned from pTagBFP-actin (Evrogen).

pHL-EF1a-SphcCas9 (Addgene, Plasmid #60599)

GAAAAGTGCCACCTGACGTCGCTAGCggttaaaccgggcatcgctctagactagtcggccgctgcagAAGC
TTTGCAAAGATGGATAAAGTTTTAAACAGAGAGGAATCTTTGCAGCTAATGGACCTTCTAGGT
CTTGAAAGGAGTGGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCA
CAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGG
CGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTTCCCGAGGGTGGG
GGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCG
CCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTATG
GCCCTTGCGTGCCCTTGAATTACTTCCACGCCCTGGCTGCAGTACGTGATTCTTGATCCCG
AGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCG
CCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCGCGTGCGAATCTGGTG
GCACCTTCGCGCCTGTCTCGCTGCTTTGATAAGTCTCTAGCCATTTAAATTTTTGATGACC
TGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTG
GTATTTTCGTTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGT
TCGGCGAGGCGGGGCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAG
CTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCGGTGTATCGCCCCGCCCTGGGCG
GCAAGGCTGGCCCGGTGCGCACCAAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCC
TGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCA
CCCACACAAAGGAAAAGGGCCTTCCGTCCTCAGCCGTGCTTCATGTGACTCCACGGAG
TACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTGCTCTTAG
GTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCACACTGAGTGGGTGGAGACTGAAG
TTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGAATTTGCCCTTTTTGAGTTTGGATCTT
GGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTCTTCCATTTAGGTGTCGTGA
GAATTAGCGGTGGCGGCCATCACAAAGTTTGTACAAAAAGCAGGCTGGCGCCGGAACCAA
TTCAGTCGACC**ATGGATAAGAAATACAGCATTGGACTGGACATTGGGACAAACTCCGTGG**

GATGGGCCGTGATTACAGACGAATACAAAGTGCCTTCAAAGAAGTTCAAGGTGCTGGGC
AACACCGATAGACACAGCATCAAGAAAAATCTGATTGGAGCCCTGCTGTTCTGACTCCGGC
GAGACAGCTGAAGCAACTCGGCTGAAAAGAACTGCTCGGAGAAGGTATACCCGCCGAA
AGAATAGGATCTGCTACCTGCAGGAGATTTTCAGCAACGAAATGGCCAAGGTGGACGATA
GTTTCTTTCACCGCCTGGAGGAATCATTCTGGTCGAGGAAGATAAGAAACACGAGCGG
CATCCCATCTTTGGCAACATTGTGGACGAGGTGCTTATCACGAAAAGTACCCTACCATCT
ATCATCTGAGGAAGAACTGGTGGACTCCACAGATAAAGCAGACCTGCGCCTGATCTATC
TGGCCCTGGCTCACATGATTAAGTTCCGGGGCCATTTTCTGATCGAGGGGGATCTGAACC
CAGACAATTCTGATGTGGACAAGCTGTTTCATCCAGCTGGTCCAGACATACAATCAGCTGT
TTGAGGAAAACCCCATTAATGCATCTGGCGTGGACGCAAAAGCCATCCTGAGTGCCAGA
CTGTCTAAGAGTCGGAGACTGGAGAACCTGATCGCTCAGCTGCCAGGGGAAAAGAAAAA
CGGCCTGTTTGGGAATCTGATTGCACTGTCACTGGGACTGACTCCCAACTTCAAGAGCAA
TTTTGATCTGGCCGAGGACGCTAAACTGCAGCTGTCCAAGGACACCTATGACGATGACCT
GGATAACCTGCTGGCTCAGATCGGGGATCAGTACGCAGACCTGTTCTGGCCGCTAAGA
ATCTGTCTGACGCCATCCTGCTGAGTGATATTCTGCGCGTGAACACCGAGATTACAAAAG
CCCCCTGTCAGCTAGCATGATCAAGAGATATGACGAGCACCATCAGGATCTGACCCTGC
TGAAGGCTCTGGTGAGGCAGCAGCTGCCTGAGAAGTACAAGGAAATCTTCTTTGATCAGT
CTAAGAACGGATACGCCGGCTATATTGACGGCGGGGCTAGTCAGGAGGAGTTCTACAAGT
TTATCAAACCCATTCTGGAGAAGATGGATGGCACAGAGGAACTGCTGGTGAACTGAATC
GGGAAGACCTGCTGAGGAAGCAGCGCACTTTTGATAACGGAAGCATCCCTCACCAGATT
CATCTGGGAGAGCTGCACGCAATCCTGAGGCGCCAGGAAGACTTCTACCCATTTCTGAA
GGATAACAGGGAGAAGATCGAAAAAATTCTGACATTCCGCATCCCCTACTATGTGGGCCC
TCTGGCAAGAGGCAACAGCCGGTTTGCCTGGATGACTCGCAAATCTGAGGAAACAATCA
CTCCCTGGAACCTTCGAGGAAGTGGTCGATAAGGGCGCTTCCGCACAGTCTTTCATTGAGC
GGATGACAACTTCGACAAGAACCTGCCAAACGAAAAAGTGCTGCCCAAGCACTCTCTG
CTGTACGAGTATTTACAGTCTATAACGAACTGACTAAGGTGAAATACGTCACCGAGGGG
ATGAGAAAGCCTGCCTTCCTGAGTGGAAGACAGAAGAAAGCTATCGTGGACCTGCTGTT
TAAACCAATAGGAAGGTGACAGTCAAGCAGCTGAAAGAGGACTATTTCAAGAAAATTGA
ATGTTTCGATTCTGTGGAGATCAGTGGCGTCGAAGACAGGTTTAACGCCTCCCTGGGGAC
CTACCACGATCTGCTGAAGATCATTAAGGATAAAGACTTCCTGGACAACGAGGAAAATGA
GGATATCCTGGAAGACATTGTGCTGACCCTGACACTGTTTGAGGATAGGGAAATGATCGA
GGAACGCCTGAAGACCTATGCCCATCTGTTCGATGACAAAGTGATGAAACAGCTGAAGC
GACGGAGATACACAGGATGGGGCCGACTGTCTCGGAAGCTGATCAATGGGATTCGCGAC

AAACAGAGTGGAAGACCATCCTGGACTTTCTGAAATCAGATGGCTTCGCCAACCGGAA
CTTCATGCAGCTGATTACGATGACAGCCTGACATTCAAAGAGGATATCCAGAAGGCACA
GGTGTCCGGGCAGGGAGACTCTCTGCACGAGCATATCGCAAACCTGGCCGGCAGCCCT
GCCATCAAGAAAGGGATTCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAAGTCAT
GGGAAGACATAAGCCAGAAAACATCGTGATTGAGATGGCCAGGGAAAATCAGACCACAC
AGAAAGGCCAGAAGAACTCAAGGGAGCGCATGAAAAGAATCGAGGAAGGAATTAAGGA
ACTGGGCAGCCAGATCCTGAAAGAGCACCCCGTGGAACACACAGCTGCAGAATGAG
AAGCTGTATCTGTACTATCTGCAGAATGGACGCGATATGTACGTGGACCAGGAGCTGGAT
ATTAACCGACTGTCCGATTACGACGTGGATCATATCGTCCCACAGTCATTCTGAAAGATG
ACAGCATTGACAATAAGGTGCTGACCCGCTCTGACAAAAACCGAGGCAAGAGTGATAAT
GTCCCCTCAGAGGAAGTGGTCAAGAAAATGAAGAACTACTGGAGGCAGCTGCTGAATGC
CAAAGTATCACACAGCGAAAGTTTGATAACCTGACTAAAGCTGAGCGGGGAGGCCTGA
GTGAACTGGACAAAGCAGGCTTCATTAAGCGACAGCTGGTGGAGACACGGCAGATCACA
AAGCACGTCGCCCAGATTCTGGATTCAAGAATGAACACTAAGTACGATGAGAATGACAAA
CTGATCAGAGAAGTGAAGGTCATTACCCTGAAGTCAAAACTGGTGAGCGACTTTCGGAA
AGATTTCCAGTTTTATAAGGTCAGAGAGATCAACAACTACCACCATGCTCATGACGCATAC
CTGAACGCAGTGGTCGGCACAGCCCTGATTAAGAAATACCCTAACTGGAGTCCGAGTTC
GTGTACGGGGACTATAAGGTGTACGATGTCAGAAAAATGATCGCCAAGTCTGAGCAGGAA
ATTGGCAAAGCCACTGCTAAGTATTTCTTTTACAGTAACATCATGAATTTCTTTAAGACTGA
GATCACCCCTGGCAAATGGGGAAATCCGAAAGCGGCCACTGATTGAGACTAACGGCGAGA
CAGGAGAAATCGTGTGGGACAAAGGAAGAGATTTTGCTACCGTGAGGAAGGTCCTGAGC
ATGCCCCAAGTGAATATTGTCAAGAAAACAGAGGTGCAGACTGGGGGATTAGTAAGGA
ATCAATTCTGCCTAAACGCAACTCCGATAAGCTGATCGCCCCGAAAGAAAGACTGGGACCC
CAAGAAGTATGGCGGGTTCGACTCCCCAACTGTGGCTTACTCTGTCCTGGTGGTCGCAAA
GGTGGAGAAGGGAAAAAGCAAGAACTGAAATCCGTCAAGGAACTGCTGGGCATCACC
ATTATGGAGCGCAGCTCCTTCGAAAAGAATCCTATCGATTTTCTGGAGGCCAAAGGCTATA
AGGAAGTGAAGAAAGACCTGATCATCAAGCTGCCAAAGTACTCACTGTTTGAGCTGGAA
AACGGGAGAAAGAGGATGCTGGCAAGCGCCGGGGAGCTGCAGAAAGGAAATGAACTG
GCCCTGCCCTCCAAGTACGTGAACTTCCTGTATCTGGCTAGCCACTACGAGAAGCTGAAA
GGGTCCCCTGAGGATAACGAACAGAAACAGCTGTTTGTGGAGCAGCACAAAGCATTATCT
GGACGAGATCATTGAACAGATTAGCGAGTTCTCCAAAAGAGTGATCCTGGCTGACGCAA
ATCTGGATAAGGTCCTGAGCGCATACAACAAACACCGGGATAAGCCAATCAGAGAGCAG
GCCGAAAATATCATTCATCTGTTCACTCTGACCAACCTGGGAGCCCCCGCAGCCTTCAAG

TATTTTGACACTACCATCGATCGCAAACGATACACAAGCACTAAGGAGGTGCTGGACGCT
ACCCTGATTCATCAGAGCATTACTGGCCTGTATGAAACAAGGATTGACCTGTCTCAGCTG
GGCGGCGACTCCGGAGCTGACCCCAAGAAGAAGAGGAAGGTG**TGA****GACCTCTAGACCC**
 AGCTTTCTTGTACAAAGTGGTGATCTAGCTAAAACGCGGCCTCGAATCGAATTCCGCCCCC
 CCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGT
 GTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCCGGA
 AACCTGGCCCTGTCTTCTTGACGAGCATTCTAGGGGTCTTTCCCCTCTCGCCAAAGGAAT
 GCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACA
 ACGTCTGTAGCGACCCTTTCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTG
 CGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGT
 TGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGG
 CTGAAGGATGCCCAGAAGGTACCCCATTTGATGGGATCTGATCTGGGGCCTCGGTGCACAT
 GCTTTACATGTGTTTAGTCGAGGTTAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTG
 GTTTTCCTTTGAAAAACACGATGATAATATGGCCACAACCATGGTGAGCAAGGGCGAGGAG
 GATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGA
 ACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCACCCA
 GACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGT
 CCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACT
 ACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACG
 GCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAG
 GTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATG
 GGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGAT
 CAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCT
 ACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACA
 TCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCC
 ACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAAGCGCTCTAGGCTTAAGTATACCTCG
 AGTTAAATTCACCTCCTCAGGTGCAGGCTGCCTATCAGAAGGTGGTGGCTGGTGTGGCCAAT
 GCCCTGGCTCACAATAACCACTGAGATCTTTTTCCCTCTGCCAAAATTATGGGGACATCAT
 GAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTG
 TTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAACATCA
 GAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCCATATGCTGGCTGCCATGAACAAAG
 GTTGGCTATAAAGAGGTGATCAGTATATGAAACAGCCCCCTGCTGTCCATTCTTATCCATA
 GAAAAGCCTTGACTTGAGGTTAGATTTTTTTTTATATTTTGTGTTATTTTTTTCTTTAACA

TCCCTAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAG
TCATAGCTGTCCCTCTTCTCTTATGGAGATCCCTCGACTTAATTAAACCGGTCAGCTGCATTA
ATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTCGCTATTGGGCGCTCTTCCGCTTCCTC
GCTCACTGACTCGCTGCGCTCGGTCTGCTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAA
GGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAA
GGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTC
CGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACA
GGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGA
CCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCA
TAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTG
CACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA
ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG
CGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAG
AAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTA
GCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCA
GATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACG
CTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTC
ACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTG
GTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTT
ATCCATAGTTGCCTGACTCCCCGTCTGTAGATAACTACGATACGGGAGGGCTTACCATCTG
GCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAAT
AAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCAT
CCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCA
ACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTC
AGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGG
TTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCGAGTGTTATCACTCATG
GTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACT
GGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCC
CGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGA
AAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTA
ACCCACTCGTGACCCAACTGATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGGGTGAG
CAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAAT
ACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGA

TACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCC

pAM-L7Ae

TAATACGACTCACTATAGGGCGAATTCAGGAGCTCATATGCTGCAGGTCGACACCATGGGAT
CCATGTACGTGAGATTTGAGGTTCTGAGGACATGCAGAACGAAGCTCTGAGTCTGCTGG
AGAAGGTTAGGGAGAGCGGTAAGGTAAAGAAAGGTACCAACGAGACGACAAAGGCTGT
GGAGAGGGGACTGGCAAAGCTCGTTTACATCGCAGAGGATGTTGACCCGCCTGAGATCG
TTGCTCATCTGCCCCCTCCTCTGCGAGGAGAAGAATGTGCCGTACATTTACGTTAAAAGCA
AGAACGACCTTGGAAGGGCTGTGGGCATTGAGGTGCCATGCGCTTCGGCAGCGATAATC
AACGAGGGAGAGCTGAGAAAGGAGCTTGGAAGCCTTGTGGAGAAGATTAAAGGCCTTC
AGAAGAGATCTCATATGCATCTCGAGTGATAGTCTAGACAAGCTTGAGTATTCTATAGTGTC
ACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCA
CAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGT
GAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCGCTTTCAGTCGGGAAACCTGTGC
TGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGC
TCTTCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCTGTTTCGGCTGCGGCGAGCGGTA
TCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGA
ACATGTGAGCAAAAAGGCCAGCAAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTG
GCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCG
CTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGC
GTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCA
AGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACT
ATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAA
CAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAAC
TACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGG
AAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTT
GTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTC
TACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTAT
CAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATA
TATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATC
TGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGA
GGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCC
AGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAAC
TTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAG

TTAATAGTTT GCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGT CACGCTCGTCGTTT
GGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTT
GTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCA
GTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGA
TGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACC
GAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAA
GTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGA
GATCCAGTTTCGATGTAACCCACTCGTGACCCAACTGATCTTCAGCATCTTTTACTTTTACC
AGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCG
ACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTT
ATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGC
GCACATTTCCCCGAAAAGTGCCACCTGATGCGGTGTGAAATACCGCACAGATGCGTAAGGA
GAAATACCGCATCAGGAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGT
TAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAAT
AGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGT
GGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACC
ATCACCCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAG
GGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGG
AAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGT
AACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCATTCGCCATTCAGG
CTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCG
AAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGA
CGTTGTAAAACGACGGCCAGTGAATTG

pA9-tagBFP

TCGCGCGTTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCA
CAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGT
GTTGGCGGGTGTCTGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTG
CACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCC
ATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTAT
TACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGT
TTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCGAGCTCATATGCTGCAGGTC
GACACC**ATGGGATCCAGCGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGA**
GGGCACCGTGGACAACCATCACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTAC
GAGGGCACCCAGACCATGAGAATCAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTT
CGACATCCTGGCTACTAGCTTCCTCTACGGCAGCAAGACCTTCATCAACCACACCCAGGG
CATCCCCGACTTCTTCAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCAC
ATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGC
CTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCACATCCAACGGCCCTGTGATGCAG
AAGAAAACACTCGGCTGGGAGGCCTTACCCGAGACGCTGTACCCCGCTGACGGCGGCC
TGGAAGGCAGAAACGACATGGCCCTGAAGCTCGTGGGCGGGAGCCATCTGATCGCAAA
CATCAAGACCACATATAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCTGGCGTCTA
CTATGTGGACTACAGACTGGAAAGAATCAAGGAGGCCAACAACGAGACCTACGTCGAGC
AGCACGAGGTGGCAGTGGCCAGATACTGCGACCTCCCTAGCAAACCTGGGGCACAGATCT
CATATGCATCTCGAGTGA**TAGTCTAGACAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCT**
GTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTA
AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCT
TTCCAGTCGGGAAACCTGTCTGCGCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGA
GGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTC
GTTCCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAAT
CAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA
AAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAA
TCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCC
CCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCC
GCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTT
CGGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTACGCCCCGACC
GCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCA

CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAG
TTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCT
GCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACC
GCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTC
AAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAA
GGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAATGAA
GTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAG
TGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCTG
TG TAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCG
AGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGA
GCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAG
CTAGAGTAAGTAGTTTCGCCAGTTAATAGTTTTCGCAACGTTGTTGCCATTGCTACAGGCATC
GTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCTAACGATCAAGGC
GAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTT
GTCAGAAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCT
TACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCT
GAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGC
GCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACCTCT
CAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCAACTGATCT
TCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCG
CAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTCAATATT
ATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAA
TAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACC
ATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

Supplementary Table S7: Transfection tables for all experiments in this study

Figure 2A-C					
	Control Cas9 mRNA	sgRNA _{DMD} (Negative control)	miR-302-responsive Cas9 mRNA	miR-21-responsive Cas9 mRNA	Untreated
Control Cas9 mRNA	100 ng	100 ng			
miR-302-responsive Cas9 mRNA			100 ng		
miR-21-responsive Cas9 mRNA				100 ng	
sgRNA _{GFP}	300 ng		300 ng	300 ng	
sgRNA _{DMD}		300 ng			

Figure 2D					
	Control Cas9 mRNA	sgRNA _{DMD} (Negative control)	miR-21-responsive Cas9 mRNA + miR-21 inhibitor	miR-21-responsive Cas9 mRNA + negative inhibitor	Untreated
Control Cas9 mRNA	100 ng	100 ng			
miR-21-responsive Cas9 mRNA			100 ng	100 ng	
sgRNA _{GFP}	300 ng		300 ng	300 ng	
sgRNA _{DMD}		300 ng			
miR-21 inhibitor			5 pmol		
negative inhibitor				5 pmol	

Figure 3					
	Control Cas9 mRNA	sgRNA _{DMD} (Negative control)	4x miR-302-responsive Cas9 mRNA + miR-302 inhibitor	4x miR-302-responsive Cas9 mRNA + negative inhibitor	Untreated
Control Cas9 mRNA	100 ng	100 ng			
4x miR-302-responsive Cas9 mRNA			100 ng	100 ng	
sgRNA _{2GFP}	300 ng		300 ng	300 ng	
sgRNA _{DMD}		300 ng			
miR-302 inhibitor			5 pmol		
negative inhibitor				5 pmol	

Figure 4B-D				
	Control Cas9 mRNA	sgRNA _{DMD} (Negative control)	4x miR-302-responsive Cas9 mRNA	Untreated
Control Cas9 mRNA	50 ng	50 ng		
4x miR-302-responsive Cas9 mRNA			50 ng	
sgRNA _{2GFP}	150 ng		150 ng	
sgRNA _{DMD}		150 ng		

Figure 5B, C					
	Control L7Ae mRNA	miR-21-responsive L7Ae mRNA	Constitutive ON (-L7Ae)	sgRNA _{DMD} (Negative control)	Untreated
Kt-Cas9 mRNA	5 ng	5 ng	5 ng	5 ng	
sgRNA _{2GFP}	150 ng	150 ng	150 ng		
sgRNA _{DMD}				150 ng	
Control L7Ae mRNA	15 ng			15 ng	
miR-21-responsive L7Ae mRNA		15 ng			

Supplementary Figure S2				
	Control Cas9 mRNA	miR-21-responsive Cas9 mRNA	miR-302-responsive Cas9 mRNA	Untreated
Control Cas9 mRNA	100 ng			
miR-21-responsive Cas9 mRNA		100 ng		
miR-302-responsive Cas9 mRNA			100 ng	
sgRNA _{GFP}	300 ng	300 ng	300 ng	

Supplementary Figure S3B-D				
	Control Cas9 mRNA	miR-21-responsive Cas9 mRNA	sgRNA _{DMD} (Negative control)	Untreated
Control Cas9 mRNA	10 ng		10 ng	
miR-21-responsive Cas9 mRNA		10 ng		
sgRNA _{Alu1}	300 ng	300 ng		
sgRNA _{DMD}			300 ng	

Supplementary Figure S4A, S5, S6B					
	Control Cas9 mRNA	sgRNA _{DMD} (Negative control)	miR-302-responsive Cas9 mRNA + miR-302 inhibitor	miR-302-responsive Cas9 mRNA + negative inhibitor	Untreated
Control Cas9 mRNA	100 ng	100 ng			
miR-302-responsive Cas9 mRNA			100 ng	100 ng	
sgRNA _{GFP}	300 ng		300 ng	300 ng	
sgRNA _{DMD}		300 ng			
miR-302 inhibitor			5 pmol		
negative inhibitor				5 pmol	

Supplementary Figure S4B					
	Control Cas9 mRNA	sgRNA _{DMD} (Negative control)	miR-302-responsive Cas9 mRNA + miR-302 inhibitor	miR-302-responsive Cas9 mRNA + negative inhibitor	Untreated
Control Cas9 mRNA	100 ng	100 ng			
miR-302-responsive Cas9 mRNA			100 ng	100 ng	
sgRNA _{2GFP}	300 ng		300 ng	300 ng	
sgRNA _{DMD}		300 ng			
miR-302 inhibitor			5 pmol		
negative inhibitor				5 pmol	

Supplementary Figure S4C			
	4x miR-302-responsive Cas9 mRNA + miR-302 inhibitor	4x miR-302-responsive Cas9 mRNA + negative inhibitor	Untreated
4x miR-302-responsive Cas9 mRNA	50 ng	50 ng	
sgRNA _{2GFP}	300 ng	300 ng	
miR-302 inhibitor	5 pmol		
negative inhibitor		5 pmol	

Supplementary Figure S7A			
	Control Cas9 mRNA	miR-21-responsive Cas9 mRNA	Untreated
Control Cas9 mRNA	100 ng		
miR-21-responsive Cas9 mRNA		100 ng	
sgRNA _{GFP}	300 ng	300 ng	

Supplementary Figure S7B			
	Control Cas9 mRNA	miR-302-responsive Cas9 mRNA	Untreated
Control Cas9 mRNA	100 ng		
miR-302-responsive Cas9 mRNA		100 ng	
sgRNA _{GFP}	300 ng	300 ng	

Supplementary Figure S8		
	BFP + Cy5	Untreated
BFP mRNA	100 ng	
sgRNA _{DMD} -Cy5	300 ng	

Supplementary Figure S9					
	Control Cas9 mRNA	sgRNA _{DMD} (Negative control)	1x miR-302-responsive Cas9 mRNA	4x miR-302-responsive Cas9 mRNA	Untreated
Control Cas9 mRNA	100 ng	100 ng			
miR-302-responsive Cas9 mRNA			100 ng		
4x miR-302-responsive Cas9 mRNA				100 ng	
sgRNA2 _{GFP}	100 ng		100 ng	100 ng	
sgRNA _{DMD}		100 ng			

Supplementary References

1. Cho,S.W., Kim,S., Kim,Y., Kweon,J., Kim,H.S., Bae,S. and Kim,J.-S. (2014) Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. *Genome Res.*, **24**, 132–141.
2. Shen,Y.H., Zhang,L., Gan,Y., Wang,X., Wang,J., Lemaire,S.A., Coselli,J.S. and Wang,X.L. (2006) Up-regulation of PTEN (Phosphatase and Tensin Homolog Deleted on Chromosome Ten) Mediates p38 MAPK Stress Signal-induced Inhibition of Insulin Signaling A CROSS-TALK BETWEEN STRESS SIGNALING AND INSULIN SIGNALING IN RESISTIN-TREATED HUMAN ENDOTHELIAL CELL. *J. Biol. Chem.*, **281**, 7727–7736.
3. Kang,J., Perry,J.K., Pandey,V., Fielder,G.C., Mei,B., Qian,P.X., Wu,Z.S., Zhu,T., Liu,D.X. and Lobie,P.E. (2009) Artemin is oncogenic for human mammary carcinoma cells. *Oncogene*, **28**, 2034–2045.
4. Frankel,L.B., Christoffersen,N.R., Jacobsen,A., Lindow,M., Krogh,A. and Lund,A.H. (2008) Programmed Cell Death 4 (PDCD4) Is an Important Functional Target of the MicroRNA miR-21 in Breast Cancer Cells. *J. Biol. Chem.*, **283**, 1026–1033.
5. Wang,J., Guan,J., Lu,Z., Jin,J., Cai,Y., Wang,C. and Wang,F. (2015) Clinical and tumor significance of tropomyosin-1 expression levels in renal cell carcinoma. *Oncol. Rep.*, **33**, 1326–1334.
6. Schutyser,E., Su,Y., Yu,Y., Gouwy,M., Zaja-Milatovic,S., Damme,J. Van and Richmond,A. (2007) Hypoxia enhances CXCR4 expression in human microvascular endothelial cells and human melanoma cells. *Eur Cytokine Netw.*, **18**, 59–70.
7. James,J.M., Nalbandian,A. and Mukouyama,Y. (2013) TGF β signaling is required for sprouting lymphangiogenesis during lymphatic network development in the skin. *Development*, **140**, 3903–3914.