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Heat-triggered remote control of CRISPR-dCas9 for tunable transcriptional modulation

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Abstract

CRISPR-associated proteins (Cas) are enabling powerful new approaches to control mammalian cell functions, yet the lack of spatially defined, noninvasive modalities limit their use as biological tools. Here, we integrate thermal gene switches with dCas9 complexes to confer remote control of gene activation and suppression with short pulses of heat. Using a thermal switch constructed from the heat shock protein A6 (HSPA6) locus, we show that a single heat pulse 3–5°C above basal temperature is sufficient to trigger expression of dCas9 complexes. We demonstrate that dCas9 fused to the transcriptional activator VP64 is functional after heat activation, and depending on the number of heat pulses, drives transcription of endogenous genes *GzmB* and *CCL21* to levels equivalent to that achieved by a constitutive viral promoter. Across a range of input temperatures, we find that downstream protein expression of *GzmB* closely correlates with transcript levels ($R^2=0.99$). Using dCas9 fused with the transcriptional suppressor KRAB, we show that longitudinal suppression of the reporter d2GFP depends on key thermal input parameters including pulse magnitude, number of pulses, and dose fractionation. In living mice, we extend our study using photothermal heating to spatially target implanted cells to suppress d2GFP *in vivo*. Our

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AUTHOR CONTRIBUTIONS

L.G. and G.A.K. conceived of the idea. L.G. and G.A.K. designed the experiments and interpreted results. L.G., E.V.P., H.L., and J.P.M. performed the experiments. L.G. and G.A.K. wrote the manuscript.

SUPPORTING INFORMATION AVAILABLE

Gating strategy for selecting HEK293T cells for d2GFP suppression; kinetic trace of catalytically active Cas9 expression following heating; *GAPDH*, *GzmB*, and *CCL21* transcript levels in WT HEK293T cells; flow cytometry analysis of d2GFP sgRNA knockout efficiency; sgRNA sequences. This material is available free of charge via the internet at <http://pubs.acs.org>.

study establishes a noninvasive and targeted approach to harness Cas-based proteins for modulation of gene expression to complement current methods for remote control of cell function.

Graphical Abstract

RNA-guided endonucleases, which consist of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated proteins (Cas), have transformed genome engineering and are rapidly becoming indispensable tools for biomedical research^{1, 2}. The programmable targeting capacity of catalytically inactive Cas9 (dCas9) has enabled applications beyond genome editing, providing unprecedented new tools to control mammalian cell functions, including regulation of gene expression, epigenetic landscapes, and chromatin structures^{3, 4}. These advances provide new opportunities for *in vivo* therapeutic applications, including recent demonstrations of Cas9 systems for gene therapy in rodent models of disease, such as diabetes, muscular dystrophy, and acute kidney disease^{5, 6}. Despite remarkable progress, hurdles remain that hinder practical applications of Cas technologies as *in vivo* tools and potential clinical therapies; these include off-target and off-tissue effects and the lack of precise methods to deliver or control Cas9 expression in target tissues. Integrating CRISPR technologies with remote-controlled genetic constructs may increase the effectiveness of targeted approaches for controlling synthetic cellular phenotypes *in vivo*.

Toward this end, several inducible systems have been developed to provide the ability to modulate the activity of Cas9 and its variants in living animals^{2, 7}. These include Cas9 systems that rely on chemical triggers by small molecule drugs, such as rapamycin and tamoxifen^{8–10}, to activate and tune Cas9 activity by defined doses and at specific points in time. However, systemic administration of chemical triggers to activate Cas-driven systems are challenging to implement with spatial precision. Alternatively, integrating CRISPR-Cas9 with light-sensitive proteins allows Cas activity to be spatially defined by targeting with visible light^{11–13}. Without invasive or implantable devices for light delivery, however, the efficacy of this approach is limited to superficial targets due to poor light penetration into biological tissue.

Here, we integrate heat as a remote trigger with the rapidly expanding CRISPR toolbox to confer tunable remote control of orthogonal transcriptional commands. In contrast to chemical or optical cues, pulses of heat can be delivered noninvasively with millimeter precision and at depth to anatomical sites by various approaches, such as infrared light¹⁴,

high-intensity focused ultrasound¹⁵, or magnetic particles in alternating magnetic fields¹⁶. Recent control methods based on heat-induction to modulate gene expression^{17–20} include genetically encoded RNA thermometers¹⁷ and temperature-sensitive transcriptional regulators in bacteria¹⁹. In mammalian cells, promoters of the heat shock protein (HSP) family have also been explored for heat-inducible transgene expression^{21–27}. Differences in the arrangement of DNA motifs known as heat shock elements (HSEs), in combination with other regulatory regions, dictate the heat responsiveness each heat-inducible promoter, including its basal activity and inducibility in response to mild hyperthermia^{28–30}. Recently, we engineered mammalian thermal gene switches derived from the human heat shock protein HSP70B' (HSPA6) locus to allow heat-triggered control of target gene expression¹⁸. By truncation analysis, we identified a construct that undergoes a sharp thermal transition in response to mild elevations in temperature (~40–42 °C) while maintaining negligible basal activity at body temperature¹⁸. However, for broad biomedical applications, the ability to activate as well as suppress target genes would be required to fully direct cell function. For example, overexpression of multiple transcription factors (e.g. Oct3/4, Sox2, cMyc, and Klf4³¹) reprogram somatic cells into induced pluripotent stem cells (iPSCs). By contrast, suppression of immune checkpoint pathways (e.g. PD-1) is a promising strategy for enhancing antitumor efficacy of T cell therapies^{32, 33}.

dCas9, which lacks nuclease activity but maintains the ability to bind to genomic DNA via short guide RNAs (sgRNA), can be fused to gene activators or suppressors to modulate the transcriptional activity of downstream targets^{34–38}. To suppress target genes, the Krüppel associated box (KRAB) domain, a repressive chromatin modifier domain of the zinc finger protein Kox1^{34, 39}, is fused to dCas9 to silence expression. By contrast, to turn target genes on, the tetrameric repeat of the herpes simplex viral protein 16 (VP64), an artificial tetrameric repeat of the herpes simplex virus VP16's minimal activation domain DALDDFDLDM, is fused to dCas9 to recruit important cofactors as well as RNA polymerase to activate transcription of target genes⁴⁰. Here, we integrate thermal switches with dCas9-fused transcriptional regulators to allow conditional control of transcriptional activation and suppression by remote heat triggers (Scheme 1).

RESULTS AND DISCUSSION

To establish a thermal dCas9 transcriptional modulator, we cloned dCas9-VP64 and KRAB-dCas9 under control of our previously described thermal gene switch⁴¹ into HEK293T cells. This thermal switch is a 1,349 bp construct derived from the human HSPA6 promoter with over a 2.5-fold greater inducibility than the wildtype clone⁴¹. Upon thermal treatment, monomers of heat shock transcription factor 1 (HSF1) undergo a conformational change that exposes hydrophobic interfaces to form trimers. HSF1 trimers then translocate to the nucleus, bind to heat shock response elements (HREs), and initiate transcription¹⁸. We first sought to determine how temperature and duration of heat treatments affect dCas9 expression (Figure 1). In wild type and sorted transduced cells (Supplementary Figure 1), we quantified basal expression of KRAB-dCas9 protein at 37°C and found statistically identical levels by ELISA (Figure 1b). By contrast, 24 hours after a 30 min heat treatment at 42°C, transduced cells produced KRAB-dCas9 to levels 5.7-fold over unheated cells (** $p < 0.01$). We further measured KRAB-dCas9 expression by flow cytometry across distinct

temperatures (37–42°C, Figure 1c) and heat pulse durations (0–60 min, Figure 1d), which were selected to activate HSF1 while maintaining cellular thermal tolerance and reversibility^{18, 42}. As anticipated, increasing temperatures to values above 37°C resulted in a sharp thermal transition that significantly increased dCas9 expression at temperatures greater than 41°C (****p < 0.0001) by greater than 7-fold (Figure 1c), consistent with thermal switch control of reporter genes previously reported¹⁸. We observed a similar increase in thermal response as heating durations were progressively extended from 0 to 60 min while maintaining a constant trigger temperature of 42°C (Figure 1d). Heat activation by as few as 15 minutes significantly elevated dCas9 expression (Figure 1d, ****p < 0.0001).

To determine whether multiple heat treatments would affect the ability of cells to express Cas9 longitudinally, we quantified catalytically active Cas9 lacking fusion proteins by intracellular staining and flow cytometry following 30-minute pulses of heat at 42°C (Supplementary Figure 2). Separate heat treatments at days 0 and 5 each led to transient Cas9 expression that peaked within ~18 hours at ~45% and 69% of heated cells respectively, while unheated cells maintained low expression (<5.4%) throughout 10 days in culture. To determine whether longitudinal control can be extended to dCas9 fused to transcriptional regulators, we monitored KRAB-dCas9 expression (Figure 1e) and in contrast to unheated controls which showed minimal levels of dCas9 (<0.43%), the percentage of KRAB-dCas9 expressing cells peaked at 36.1% 16 hours after heating and returned to basal levels by day 3 (t_{1/2}=17 hrs, Figure 1e). A second pulse of heat delivered on day 4 showed similar thermal response kinetics, demonstrating that KRAB-dCas9 expression can be activated by heat multiple times (Figure 1e). Our data showed that rapid and transient expression of Cas proteins can be sustained for several days with the delivery of discrete, short pulses of heat.

We next explored thermal modulation to activate target gene transcription. We designed sgRNA backbones containing MS2 RNA aptamers that bind to the MS2:P65:HSF1 (MPH) complex, a multidomain activator protein that increases activation efficiency⁴³, and four sgRNAs that target ~–400 to –50 bp window relative to the TSS of either human *GzmB* or *CCL21*^{35–37, 43}. The plasmid encoding the MPH complex was co-delivered along with dCas9-VP64 under control of our thermal switch (Figure 2a, Supplementary Table 1). By qRT-PCR, we found that a single heat treatment (42°C, 30 min) significantly enhanced *GzmB* (**p<0.01) and *CCL21* (*p<0.05) mRNA expression by ~4-fold by 72 hrs compared to transduced controls kept at 37°C (Figure 2b). Cells treated with two additional heating cycles 24 hrs apart increased *GzmB* (****p<0.0001) and *CCL21* (***p<0.001) expression levels to ~8-fold, which was statistically identical to levels achieved by a constitutive CMV promoter (Figure 2b). These data demonstrated that thermal control of dCas9-VP64 activates target genes to levels comparable to a strong viral promoter⁴⁴.

Subjecting cells to heat shock activates several competing response mechanisms that can lead to changes in transcription⁴⁵. At elevated temperatures, mRNA degradation rates, including the deadenylation by exonucleases, may be increased by Arrhenius kinetics⁴⁶ and the bound occupancy rate of RNA polymerase to DNA is reduced, leading to transient transcriptional suppression⁴⁷. By contrast, mRNA stability is increased during heat shock by binding to HSPs, which enhance transcript longevity and translational efficiency⁴⁸. Therefore, we sought to determine whether these opposing response mechanisms would

have a net effect on mRNA transcript levels independent of dCas9-VP64 activity. We found that neither mRNA levels of the endogenous housekeeping gene *GAPDH* (Supplementary Figure 3) nor *GzmB* activated by constitutively expressed dCas9-VP64 under a CMV promoter (gray bars, Figure 2c) statistically changed in response to temperatures ranging from 40–42°C. Endogenous mRNA levels of *GzmB* and *CCL21* were also undetectable in heated and unheated wildtype cells (Supplementary Figure 3). We further sought to correlate *GzmB* mRNA levels produced by thermal activation of dCas9-VP64 to protein levels, because we postulated that if significant mRNA degradation occurs with elevated temperatures, downstream protein translation would likewise be affected. Across the range of temperatures tested (37, 40–42°C), dCas9-VP64 activity increased *GzmB* mRNA by ~3.7-fold at 42°C compared to unheated controls (****p<0.0001) (purple bars, Figure 2c). By ELISA, *GzmB* protein levels were upregulated by ~1.9- and 6.5-fold at 41°C and 42°C respectively (purple bars, Figure 2d). In control cells where *GzmB* expression was under the constitutive CMV promoter (gray bars), no significant elevation in *GzmB* protein was detected except a ~1.3-fold upregulation at 42°C (***p=0.002), which we attributed to potential increased mRNA stability due to HSP activity. Across the range of temperatures tested (37, 40–42°C), we observed a high correlation ($R^2 = 0.99$, Figure 2e) between *GzmB* protein and mRNA levels from dCas9-VP64 activity. Collectively, we interpreted these results as support that transient heat treatments did not affect *GzmB* mRNA stability and downstream protein production.

To explore the use of KRAB-dCas9 for transcriptional suppression (Figure 3a), we sought to design sgRNAs to suppress the activity of the reporter gene d2GFP. For recombinant genes that lack introns, sgRNA design guidelines for CRISPR knockout or interference are similar. To increase the likelihood that a frameshift mutation will produce a nonfunctional protein, a gene knockout sgRNA is targeted to an early upstream exon⁴⁹, while sgRNAs for gene suppression show maximum efficacy when they are targeted within a ~50–100bp window just downstream of the TSS³⁵. Given the overlap in design criteria, we screened four d2GFP-targeting guides (A1, A2, A3, and A4) with catalytically active Cas9, which we chose in order to assess sgRNA potency independent of the kinetics associated with transient target gene suppression characteristic of KRAB-dCas9 (Supplementary Figure 4, Supplementary Table 2). The top performing sgRNA (A3, 5'-GACCAGGATGGGCACCACCC-3'), which has also been used by other labs in conjunction with the CRISPR interference platform to suppress GFP^{34, 50, 51}, recognized a sequence 100–120 bp downstream of the TSS and knocked out d2GFP in greater than 70% of the population.

To test thermal control of transcriptional suppression, we explored how different heat pulse characteristics (temperature, number, and pulse width) affect d2GFP expression. With increasing temperatures, we observed that for all heated samples (41, 41.5 and 42°C), d2GFP suppression peaked at day 2 followed by a gradual decrease in suppression until full recovery of reporter expression by day 6 (Figure 3b). Using 100% d2GFP expression as the baseline for area-under-the-curve (AUC) quantification, we found that total suppression of d2GFP went from ~2% to ~20% of maximum AUC as temperature increased from 37 to 42°C (Figure 3c), consistent with earlier data which showed that dCas9 expression was higher with increased temperatures (Figure 1b). To determine the effect of the number of heat pulses, we treated cells with 0–3 total pulses spread across three days (Figure 3d) and

found that d2GFP suppression increased from ~10% (1×) to ~25% (3×) by AUC analysis (Figure 3e). This resulted in improved maximum suppression (30% versus 42%) and increased persistence of d2GFP suppression (~5 and 9 days). Lastly, inspired by dose fractionation in radiation oncology that is used to modulate the therapeutic efficacy of total radiation dose⁵², we analyzed the effect of fractioning a 60-min thermal treatment over 24 hours (2 × 30 min, 3 × 20 min, or 6 × 10 min) on target gene suppression. A thermal dose fractioned into six 10-min pulses resulted in an d2GFP suppression AUC of 57.0%, while three 20-min pulses and two 30-min pulses in AUCs of 49.2% and 45.0%, respectively (Figure 3f). We observed that the highest fractioned thermal treatment (i.e., 6 × 10 min) led to the least potent total suppression (% max AUC = 17.4%) compared to thermal treatments delivered using 20- or 30-min pulse widths (% max AUC = 25.0%, 28.9% respectively) (Figure 3g). We attribute these differences to induction of thermotolerance whereby heat pulses delivered in close proximity after cells have been primed become less effective, as has been shown in classic studies on thermotolerance⁵³. Altogether, our data showed that thermal pulse modulation can be used to tune key kinetic features of d2GFP suppression by KRAB-dCas9.

After demonstrating transcriptional modulation *in vitro*, we next set out to implement this system for remote thermal control of gene expression *in vivo*. Spatially controlled delivery of heat is routinely employed in thermal medicine to improve treatment efficacy of drugs and can be accomplished by various modalities such as plasmonic photothermal heating^{14–16}. To implement this system *in vivo*, we subcutaneously implanted tissue phantoms in the rear flank of nude mice (Figure 4a). These tissue phantoms comprised Matrigel implants seeded with plasmonic gold nanorods (AuNRs), which absorb near infrared (NIR) light and convert the resonant energy to heat¹⁴, and d2GFP+ HEK293T cells containing d2GFP-targeting sgRNAs and the thermal switch driving KRAB-dCas9. Using a NIR laser, we maintained skin temperatures at 44°C for 30 min once every 24 hrs for 3 consecutive days (Figure 4b). Recovery of engineered cells following the last thermal treatment revealed that heated cells significantly decreased d2GFP expression compared to cells extracted from unheated phantoms (*** $p < 0.001$). Using NIR light and plasmonic gold nanorods as a method to locally deliver heat, we demonstrate remote control of transcriptional activity in mammalian cells at distinct anatomical sites (Figure 4c). While the use of photothermal heating limits this *in vivo* demonstration to superficial tissues a few millimeters deep, the use of alternate heating modalities that noninvasively penetrate deep tissue, such as high intensity focused ultrasound, would expand the use of this platform for use in deep tissue.

Here, we developed a tunable, heat-triggered platform to regulate mammalian cell transcription by remote control. Our data demonstrate that discrete pulses of heat selectively trigger expression of KRAB-dCas9 or dCas9-VP64, which then reversibly activate or suppress target genes depending on the strength and duration of thermal inputs. In optogenetic systems, Cas constructs are constitutively expressed but lack activity until photo-illumination induces assembly of nonfunctional split proteins or transactivators into functional dimers². Dimerization by light occurs rapidly but is also immediately reversible, thereby requiring continuous light illumination to sustain functional Cas activity (i.e. the ON state)^{11, 12}. By comparison, the functional ON state under thermal control is transiently

delayed as dCas9-fused transcriptional modulators are expressed at negligible levels at basal temperatures. However, the activity of Cas proteins can be sustained longitudinally by delivering discrete pulses of heat (30 minute or less) without the need for a continuous input signal. In applications that require rapid OFF kinetics, the use of destabilized Cas proteins may enable basal expression levels to be restored promptly⁵⁴.

By using thermal switches to control dCas9-VP64 or KRAB-dCas9 expression, we show that heat pulse modulation can control the level of activation or suppression of target genes. We also demonstrate that elevated temperatures do not alter mRNA levels of the target genes we studied, and that there is a high correlation between *GzmB* mRNA and protein levels, showing that transient and mild heating do not impact mRNA stability and downstream synthesis of protein of *GzmB*. We anticipate that heat-triggered transcriptional control can be expanded to broad gene targets without potential confounding effects due to heat treatment itself. The exception would be the heat shock proteins (HSPs) since their expression is responsive to mild hyperthermia⁵⁵ or other target genes that contain heat responsive elements within their promoter. The efficiency of the dCas9-sgRNA complex may be limited by DNA accessibility governed by chromatin structure⁵⁶, and thus may vary by cell type⁵⁷, similar to the limitations faced by current CRISPR platforms and other gene-editing tools. Additionally, global chromatin architecture is conserved during heat shock⁵⁸, and therefore access to genes by dCas9 complexes will likely be similar between heated and unheated cells.

Lastly, we demonstrate that transcriptional activity can be modulated by remote thermal control in living mice. In comparison to optogenetic and small-molecule based systems, local delivery of heat can be achieved for superficial as well as deep target sites by clinically available platforms such as focused ultrasound. We anticipate that thermal control of Cas transcriptional modulators will allow future biomedical applications focused on remote multi-gene control (e.g., transcription factors, immune checkpoint pathways) to enhance cell therapies such as with the use of ‘dead’ sgRNAs⁵⁹. This may be achieved, for instance, by viral (e.g. AAV⁶⁰) or non-viral (e.g. lipid nanoparticle⁶¹) delivery of sgRNAs and Cas constructs directly *in vivo*^{62–65}, or by modifying therapeutic cells *ex vivo* prior to adoptive transfer^{66, 67} to ultimately achieve site-specific control of dCas9-mediated transcriptional activity using thermal cues. Applications include local control of genome wide gain-of-function or loss-of-function screens such as recent studies that identified new targets to enhance anti-tumor activity of adoptive T cell therapies^{66, 67}. These future advances could provide a multiplexed approach to remotely interrogate mammalian biology and modulate synthetic cellular phenotypes directly *in vivo*.

MATERIALS AND METHODS

Thermal Switch Construction

The promoter of the HSPA6 gene (Uniprot P17066) was amplified from human genomic DNA (Clontech #636401) from –1231 bp to +119 bp relative to the transcriptional start site, as previously described¹⁸. The dCas9 variants (Addgene 47107, 21916) were placed under the control of the heat shock promoter via restriction enzyme cloning using AgeI and XhoI

in LeGO-C. For d2GFP suppression studies, the mCherry reporter was replaced with Thy1.1 (Uniprot P01831).

In Vitro Heating Assays

HEK293T cells stably transduced with heat-triggered circuits (HSPA6- KRAB dCas9-IRES-mCherry SFFV Thy 1.1 or HSPA6-eSpCas9-IRES-mCherry SFFV Thy 1.1) and a d2GFP-targeting guide (5'-GACCAGGATGGGCACCACCC-3') were heated in a thermal cycler (42°C, 30 min, unless otherwise noted). At the indicated timepoints, cells were either reheated, analyzed by flow cytometry for d2GFP expression (BD Accuri C6), or fixed, stained (Biolegend, 1°: anti-Cas9 Clone 7A9, 2°: FITC anti-mouse IgG1 Clone RMG1-1 antibodies), and analyzed by flow cytometry for dCas9 or Cas9 expression. To quantify basal KRAB-dCas9 protein levels, cell lysates were collected 24 hrs post-heating (42°C for 30 min) by treating samples with RIPA Lysis Buffer (Sigma 20-188) supplemented with complete protease inhibitor cocktail (Sigma 11697498001) for 30 min on ice. Samples were centrifuged at 5000×g for 5 min and supernatants were analyzed with a Cas9 ELISA Kit following the manufacturer's instructions (Cell Biolabs PRB-5079).

For activation, sgRNA-coding plasmids targeting CCL21 (5'→3': GGTA GCTGGGAATAGAAGGA, GAGGGGAAGGGTATGGATCC, GAGACAGTCATGGTGTTCCTCA, GACATAAAATTTGGCAGCTG, GCGTAGTGAGGAGACAGTCA) or GzmB (5'→3': GGCACCCAGAGGACGTCATC, GAGAGGACGTCATCAGGCAG, GTCAGCTGTGGGTGATGATG, GACTCTGAGTCATCAGCTGT, GCTGCTCTGGGCTGAATAGG) were pooled and transfected at a 1:1:1 mass ratio with HSPA6- dCas9-VP64-SFFV-mCherry and MPH. For constitutive expression controls, the plasmid pcDNA-dCas9-VP64 (Addgene #47107) was used. Cells were transfected with Lipofectamine 2000 according to the manufacturer's instructions. 24 hrs post transfection, cells were heated for 30 min at indicated temperatures. mRNA was collected at t=72 hrs (RNeasy Plus Mini Kit). cDNA was synthesized from 0.4 µg of total cellular RNA (RT2 First Strand Kit). TaqMan qPCR probes Hs00188051_m1, Hs00171076_m1, Hs03929097_g1) were used in 10 µL reactions. Relative levels of cDNA were detected using QuantStudio™ 6 Flex Real-Time PCR System. Raw data was normalized to GAPDH levels and untreated (37°C) controls using the $\Delta\Delta C_t$ method.

In vivo suppression of d2GFP

All animal studies were approved by the IACUC at Georgia Tech. AuNRs were functionalized with PEG as previously described(7). 0.5 µg AuNRs and 2.5×10⁵ cells mixed in 100 µL Matrigel (8 mg/mL) and injected subcutaneously into Nu/J mice (Jackson). At t = 4, 5, & 6 d, sites were heated with a laser (Coherent λ=808 nm, ~9.5 A/cm²). The surface temperature of heated implants was held at 44 ± 1°C for 20 min and monitored using a FLIR 450sc thermal camera. Cells were recovered by incubating implants in Opti-MEM (0.5 mg/mL Liberase DL, 0.1 mg/mL DNase I) for 30 min at 37°C and were analyzed by flow cytometry (BD Fusion).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Pickar-Oliver A, and Gersbach CA (2019) The next generation of CRISPR-Cas technologies and applications, *Nat. Rev. Mol. Cell Biol* 20, 490–507. [PubMed: 31147612]
2. Nunez JK, Harrington LB, and Doudna JA (2016) Chemical and Biophysical Modulation of Cas9 for Tunable Genome Engineering, *ACS Chem. Biol* 11, 681–688. [PubMed: 26857072]
3. Bae T, Hur JW, Kim D, and Hur JK (2019) Recent trends in CRISPR-Cas system: genome, epigenome, and transcriptome editing and CRISPR delivery systems, *Genes Genomics* 41, 871–877. [PubMed: 31119685]
4. Adli M (2018) The CRISPR tool kit for genome editing and beyond, *Nat. Commun* 9, 1911. [PubMed: 29765029]
5. Liao H-K, Hatanaka F, Araoka T, Reddy P, Wu M-Z, Sui Y, Yamauchi T, Sakurai M, O'Keefe DD, Nunez-Delicado E, Guillen P, Campistol JM, Wu C-J, Lu L-F, Esteban CR, and Izpisua Belmonte JC (2017) In Vivo Target Gene Activation via CRISPR/Cas9-Mediated Trans-epigenetic Modulation, *Cell* 171, 1495–1507.e15. [PubMed: 29224783]
6. Komor AC, Badran AH, and Liu DR (2017) CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes, *Cell* 168, 20–36. [PubMed: 27866654]
7. Zhu H, Zhang L, Tong S, Lee CM, Deshmukh H, and Bao G (2018) Spatial control of in vivo CRISPR-Cas9 genome editing via nanomagnets, *Nat. Biomed. Eng* 3, 126–136. [PubMed: 30944431]
8. Liu KI, Ramli MNB, Woo CWA, Wang Y, Zhao T, Zhang X, Yim GRD, Chong BY, Gowher A, Chua MZH, Jung J, Lee JHJ, and Tan MH (2016) A chemical-inducible CRISPR-Cas9 system for rapid control of genome editing, *Nat. Chem. Biol* 12, 980. [PubMed: 27618190]
9. Zetsche B, Volz SE, and Zhang F (2015) A split-Cas9 architecture for inducible genome editing and transcription modulation, *Nat. Biotechnol* 33, 139. [PubMed: 25643054]
10. Maji B, Moore CL, Zetsche B, Volz SE, Zhang F, Shoulders MD, and Choudhary A (2016) Multidimensional chemical control of CRISPR-Cas9, *Nat. Chem. Biol* 13, 9. [PubMed: 27820801]
11. Polstein LR, and Gersbach CA (2015) A light-inducible CRISPR-Cas9 system for control of endogenous gene activation, *Nat. Chem. Biol* 11, 198. [PubMed: 25664691]
12. Nihongaki Y, Kawano F, Nakajima T, and Sato M (2015) Photoactivatable CRISPR-Cas9 for optogenetic genome editing, *Nat. Biotechnol* 33, 755. [PubMed: 26076431]
13. Shao J, Wang M, Yu G, Zhu S, Yu Y, Heng BC, Wu J, and Ye H (2018) Synthetic far-red light-mediated CRISPR-dCas9 device for inducing functional neuronal differentiation, *Proc. Natl. Acad. Sci* 115, E6722–E6730. [PubMed: 29967137]
14. Chatterjee DK, Diagaradjane P, and Krishnan S (2011) Nanoparticle-mediated hyperthermia in cancer therapy, *Ther. Delivery* 2, 1001–1014.
15. Partanen A, Yarmolenko PS, Viitala A, Appanaboyina S, Haemmerich D, Ranjan A, Jacobs G, Woods D, Enholm J, Wood BJ, and Dreher MR (2012) Mild hyperthermia with magnetic resonance-guided high-intensity focused ultrasound for applications in drug delivery, *Int. J. Hyperthermia* 28, 320–336. [PubMed: 22621734]

16. Chang D, Lim M, Goos JACM, Qiao R, Ng YY, Mansfeld FM, Jackson M, Davis TP, and Kavallaris M (2018) Biologically Targeted Magnetic Hyperthermia: Potential and Limitations, *Front. Pharmacol* 9, 831. [PubMed: 30116191]
17. Kortmann J, and Narberhaus F (2012) Bacterial RNA thermometers: molecular zippers and switches, *Nat. Rev. Microbiol* 10, 255. [PubMed: 22421878]
18. Miller IC, Gamboa Castro M, Maenza J, Weis JP, and Kwong GA (2018) Remote Control of Mammalian Cells with Heat-Triggered Gene Switches and Photothermal Pulse Trains, *ACS Synth. Biol* 7, 1167–1173. [PubMed: 29579381]
19. Piraner DI, Abedi MH, Moser BA, Lee-Gosselin A, and Shapiro MG (2016) Tunable thermal bioswitches for in vivo control of microbial therapeutics, *Nat. Chem. Biol* 13, 75. [PubMed: 27842069]
20. Richter F, Fonfara I, Bouazza B, Schumacher CH, Bratovi M, Charpentier E, and Möglich A (2016) Engineering of temperature- and light-switchable Cas9 variants, *Nucleic Acids Res.* 44, gkw930.
21. Vekris A, Maurange C, Moonen C, Mazurier F, De Verneuil H, Canioni P, and Voisin P (2000) Control of transgene expression using local hyperthermia in combination with a heat-sensitive promoter, *J. Gene Med* 2, 89–96. [PubMed: 10809142]
22. Vilaboa N, Fenna M, Munson J, Roberts SM, and Voellmy R (2005) Novel Gene Switches for Targeted and Timed Expression of Proteins of Interest, *Mol. Ther* 12, 290–298. [PubMed: 15925546]
23. Huang Q, Hu JK, Lohr F, Zhang L, Braun R, Lanzen J, Little JB, Dewhirst MW, and Li C-Y (2000) Heat-induced Gene Expression as a Novel Targeted Cancer Gene Therapy Strategy, *Cancer Res.* 60, 3435–3439. [PubMed: 10910053]
24. Tang Q. s., Zhang D. s., Cong X. m., Wan M. l., and Jin L. q. (2008) Using thermal energy produced by irradiation of Mn-Zn ferrite magnetic nanoparticles (MZF-NPs) for heat-inducible gene expression, *Biomaterials* 29, 2673–2679. [PubMed: 18396332]
25. Deckers R, Quesson B, Arsaut J, Eimer S, Couillaud F, and Moonen CTW (2009) Image-guided, noninvasive, spatiotemporal control of gene expression, *Proc. Natl. Acad. Sci* 106, 1175–1180. [PubMed: 19164593]
26. Siddiqui F, Li C-Y, LaRue SM, Poulson JM, Avery PR, Pruitt AF, Zhang X, Ullrich RL, Thrall DE, Dewhirst MW, and Hauck ML (2007) A phase I trial of hyperthermia-induced interleukin-12 gene therapy in spontaneously arising feline soft tissue sarcomas, *Mol. Cancer Ther* 6, 380–389. [PubMed: 17237297]
27. Andersson HA, Kim Y-S, O'Neill BE, Shi Z-Z, and Serda RE (2014) HSP70 promoter-driven activation of gene expression for immunotherapy using gold nanorods and near infrared light, *Vaccines (Basel, Switz.)* 2, 216–227.
28. Jaeger AM, Makley LN, Gestwicki JE, and Thiele DJ (2014) Genomic Heat Shock Element Sequences Drive Cooperative Human Heat Shock Factor 1 DNA Binding and Selectivity, *J. Biol. Chem* 289, 30459–30469. [PubMed: 25204655]
29. Kroeger PE, and Morimoto RI (1994) Selection of new HSF1 and HSF2 DNA-binding sites reveals difference in trimer cooperativity, *Mol. Cell. Biol* 14, 7592–7603. [PubMed: 7935474]
30. Yamamoto N, Takemori Y, Sakurai M, Sugiyama K, and Sakurai H (2009) Differential recognition of heat shock elements by members of the heat shock transcription factor family, *FEBS J.* 276, 1962–1974. [PubMed: 19250318]
31. Takahashi K, and Yamanaka S (2006) Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors, *Cell* 126, 663–676. [PubMed: 16904174]
32. Mardiana S, Solomon BJ, Darcy PK, and Beavis PA (2019) Supercharging adoptive T cell therapy to overcome solid tumor-induced immunosuppression, *Sci. Transl. Med* 11, eaaw2293. [PubMed: 31167925]
33. Rupp LJ, Schumann K, Roybal KT, Gate RE, Ye CJ, Lim WA, and Marson A (2017) CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells, *Sci. Rep* 7, 737. [PubMed: 28389661]
34. Gilbert Luke A., Larson Matthew H., Morsut L, Liu Z, Brar Gloria A., Torres Sandra E., Stern-Ginossar N, Brandman O, Whitehead Evan H., Doudna Jennifer A., Lim Wendell A., Weissman

- Jonathan S., and Qi Lei S. (2013) CRISPR-Mediated Modular RNA-Guided Regulation of Transcription in Eukaryotes, *Cell* 154, 442–451. [PubMed: 23849981]
35. Gilbert Luke A., Horlbeck Max A., Adamson B, Villalta Jacqueline E., Chen Y, Whitehead Evan H., Guimaraes C, Panning B, Ploegh Hidde L., Bassik Michael C., Qi Lei S., Kampmann M, and Weissman Jonathan S.(2014) Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation, *Cell* 159, 647–661. [PubMed: 25307932]
36. Cheng AW, Wang H, Yang H, Shi L, Katz Y, Theunissen TW, Rangarajan S, Shivalila CS, Dadon DB, and Jaenisch R (2013) Multiplexed activation of endogenous genes by CRISPR-on, an RNA-guided transcriptional activator system, *Cell Res.* 23, 1163–1171. [PubMed: 23979020]
37. Maeder ML, Linder SJ, Cascio VM, Fu Y, Ho QH, and Joung JK (2013) CRISPR RNA-guided activation of endogenous human genes, *Nat. Methods* 10, 977. [PubMed: 23892898]
38. Perez-Pinera P, Kocak DD, Vockley CM, Adler AF, Kabadi AM, Polstein LR, Thakore PI, Glass KA, Ousterout DG, Leong KW, Guilak F, Crawford GE, Reddy TE, and Gersbach CA (2013) RNA-guided gene activation by CRISPR-Cas9-based transcription factors, *Nat. Methods* 10, 973. [PubMed: 23892895]
39. Margolin JF, Friedman JR, Meyer WK, Vissing H, Thiesen HJ, and Rauscher FJ 3rd. (1994) Kruppel-associated boxes are potent transcriptional repression domains, *Proc. Natl. Acad. Sci* 91, 4509–4513. [PubMed: 8183939]
40. Seipel K, Georgiev O, and Schaffner W (1992) Different activation domains stimulate transcription from remote ('enhancer') and proximal ('promoter') positions, *EMBO J* 11, 4961–4968. [PubMed: 1464321]
41. Miller IC, Gamboa Castro M, Maenza J, Weis JP, and Kwong GA (2018) Remote Control of Mammalian Cells with Heat-Triggered Gene Switches and Photothermal Pulse Trains, *ACS Synth. Biol* 7, 1167–1173. [PubMed: 29579381]
42. Chu KF, and Dupuy DE (2014) Thermal ablation of tumours: biological mechanisms and advances in therapy, *Nat. Rev. Cancer* 14, 199. [PubMed: 24561446]
43. Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H, Nureki O, and Zhang F (2014) Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex, *Nature* 517, 583. [PubMed: 25494202]
44. Damdindorj L, Karnan S, Ota A, Hossain E, Konishi Y, Hosokawa Y, and Konishi H (2014) A Comparative Analysis of Constitutive Promoters Located in Adeno-Associated Viral Vectors, *PLoS One* 9, e106472. [PubMed: 25170953]
45. Mahat DB, Salamanca HH, Duarte FM, Danko CG, and Lis JT (2016) Mammalian Heat Shock Response and Mechanisms Underlying Its Genome-wide Transcriptional Regulation, *Mol. Cell* 62, 63–78. [PubMed: 27052732]
46. Fabre A-L, Colotte M, Luis A, Tuffet S, and Bonnet J (2014) An efficient method for long-term room temperature storage of RNA, *Eur. J. Hum. Genet* 22, 379–385. [PubMed: 23860045]
47. Cardiello JF, Goodrich JA, and Kugel JF (2018) Heat Shock Causes a Reversible Increase in RNA Polymerase II Occupancy Downstream of mRNA Genes, Consistent with a Global Loss in Transcriptional Termination, *Mol. Cell. Biol* 38, e00181–00118.
48. Kishor A, Tandukar B, Ly YV, Toth EA, Suarez Y, Brewer G, and Wilson GM (2013) Hsp70 Is a Novel Posttranscriptional Regulator of Gene Expression That Binds and Stabilizes Selected mRNAs Containing AU-Rich Elements, *Mol. Cell. Biol* 33, 71–84. [PubMed: 23109422]
49. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen TS, Heckl D, Ebert BL, Root DE, Doench JG, and Zhang F (2014) Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells, *Science* 343, 84–87. [PubMed: 24336571]
50. Retallack H, Di Lullo E, Arias C, Knopp KA, Laurie MT, Sandoval-Espinosa C, Mancina Leon WR, Krencik R, Ullian EM, Spatazza J, Pollen AA, Mandel-Brehm C, Nowakowski TJ, Kriegstein AR, and DeRisi JL (2016) Zika virus cell tropism in the developing human brain and inhibition by azithromycin, *Proc. Natl. Acad. Sci* 113, 14408–14413. [PubMed: 27911847]
51. Zhao Y, Dai Z, Liang Y, Yin M, Ma K, He M, Ouyang H, and Teng C-B (2014) Sequence-specific inhibition of microRNA via CRISPR/CRISPRi system, *Sci. Rep.* 4, 3943. [PubMed: 24487629]
52. Bentzen SM (2010) Fractionation Effects in Clinical Practice, Leibel and Phillips Textbook of Radiation Oncology (Third Edition) 40–54.

53. Urano M (1986) Kinetics of Thermotolerance in Normal and Tumor Tissues: A Review, *Cancer Res.* 46, 474–482. [PubMed: 3510074]
54. Senturk S, Shirole NH, Nowak DG, Corbo V, Pal D, Vaughan A, Tuveson DA, Trotman LC, Kinney JB, and Sordella R (2017) Rapid and tunable method to temporally control gene editing based on conditional Cas9 stabilization, *Nat. Commun* 8, 14370–14370. [PubMed: 28224990]
55. Lindquist S (1986) THE HEAT-SHOCK RESPONSE, *Annu. Rev. Biochem* 55, 1151–1191. [PubMed: 2427013]
56. Daer RM, Cutts JP, Brafman DA, and Haynes KA (2017) The Impact of Chromatin Dynamics on Cas9-Mediated Genome Editing in Human Cells, *ACS Synth. Biol* 6, 428–438. [PubMed: 27783893]
57. Marstrand TT, and Storey JD (2014) Identifying and mapping cell-type-specific chromatin programming of gene expression, *Proc. Natl. Acad. Sci* 111, E645–E654. [PubMed: 24469817]
58. Ray J, Munn PR, Vihervaara A, Lewis JJ, Ozer A, Danko CG, and Lis JT (2019) Chromatin conformation remains stable upon extensive transcriptional changes driven by heat shock, *Proc. Natl. Acad. Sci* 116, 19431–19439. [PubMed: 31506350]
59. Dahlman JE, Abudayyeh OO, Joung J, Gootenberg JS, Zhang F, and Konermann S (2015) Orthogonal gene knockout and activation with a catalytically active Cas9 nuclease, *Nat. Biotechnol* 33, 1159. [PubMed: 26436575]
60. Lau C-H, and Suh Y (2017) In vivo genome editing in animals using AAV-CRISPR system: applications to translational research of human disease, *F1000Res* 6, 2153–2153. [PubMed: 29333255]
61. Finn JD, Smith AR, Patel MC, Shaw L, Youniss MR, van Heteren J, Dirstine T, Ciullo C, Lescarbeau R, Seitzer J, Shah RR, Shah A, Ling D, Growe J, Pink M, Rohde E, Wood KM, Salomon WE, Harrington WF, Dombrowski C, Strapps WR, Chang Y, and Morrissey DV (2018) A Single Administration of CRISPR/Cas9 Lipid Nanoparticles Achieves Robust and Persistent In Vivo Genome Editing, *Cell Rep.* 22, 2227–2235. [PubMed: 29490262]
62. Yin H, Song C-Q, Dorkin JR, Zhu LJ, Li Y, Wu Q, Park A, Yang J, Suresh S, Bizhanova A, Gupta A, Bolukbasi MF, Walsh S, Bogorad RL, Gao G, Weng Z, Dong Y, Koteliensky V, Wolfe SA, Langer R, Xue W, and Anderson DG (2016) Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo, *Nat. Biotechnol* 34, 328–333. [PubMed: 26829318]
63. Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, Zetsche B, Shalem O, Wu X, Makarova KS, Koonin EV, Sharp PA, and Zhang F (2015) In vivo genome editing using *Staphylococcus aureus* Cas9, *Nature* 520, 186–191. [PubMed: 25830891]
64. Swiech L, Heidenreich M, Banerjee A, Habib N, Li Y, Trombetta J, Sur M, and Zhang F (2015) In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9, *Nat. Biotechnol* 33, 102–106. [PubMed: 25326897]
65. Zuris JA, Thompson DB, Shu Y, Guilinger JP, Bessen JL, Hu JH, Maeder ML, Joung JK, Chen Z-Y, and Liu DR (2015) Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo, *Nat. Biotechnol* 33, 73–80. [PubMed: 25357182]
66. Ye L, Park JJ, Dong MB, Yang Q, Chow RD, Peng L, Du Y, Guo J, Dai X, Wang G, Errami Y, and Chen S (2019) In vivo CRISPR screening in CD8 T cells with AAV-Sleeping Beauty hybrid vectors identifies membrane targets for improving immunotherapy for glioblastoma, *Nat. Biotechnol* 37, 1302–1313. [PubMed: 31548728]
67. Pan D, Kobayashi A, Jiang P, Ferrari de Andrade L, Tay RE, Luoma AM, Tsoucas D, Qiu X, Lim K, Rao P, Long HW, Yuan G-C, Doench J, Brown M, Liu XS, and Wucherpennig KW (2018) A major chromatin regulator determines resistance of tumor cells to T cell-mediated killing, *Science* 359, 770–775. [PubMed: 29301958]

Figure 1 |. Heat-triggered gene switch enables dynamic, tunable control of dCas9 expression.

(a) Following exposure to distinct thermal triggers, which are tunable by the modulation of temperature, heating duration, the number of pulses delivered, and time interval between heating doses, cells transduced with a thermal switch dynamically express dCas9 variants. (b) KRAB-dCas9 protein expression in wild-type (WT) cells and in cells transduced with the thermal switch as quantified by ELISA 24 hrs post-heating at 42°C for 30 min ($n=3$, mean $\pm$ s.d. one-way ANOVA, $**p<0.01$). Intracellular staining of KRAB dCas9 expression after (c) increasing activating temperature from 37°C to 42°C or (d) heating duration from 0 to 60 min ($n=3$, mean $\pm$ s.d. one-way ANOVA, $****p<0.0001$) as measured by flow cytometry (Inset: representative flow cytometry scatter plot of dCas9-KRAB expression for cells heated at 42°C). (e) Kinetic trace of KRAB-dCas9 expression following 30 min of heating at 42°C treated at 0 and 4 or 5 d ($n=3$, mean $\pm$ s.d., blue trace=37°C, red trace = 42°C).

Figure 2 |. Modulation of endogenous gene activation by heat-triggered dCas9-VP64.

(a) DNA constructs for thermal control of transcriptional activation of endogenous genes. A plasmid coding for the MS2-P65-HSF1 (MPH) transcriptional activation complex, which binds to MS2 loops included within the sgRNA scaffold⁴³, is included to enhance target gene upregulation. Guide RNAs (sgRNAs) are constitutively expressed via the human U6 (hU6) promoter, while the thermal switch controls the expression of dCas9-VP64. (b) Endogenous gene activation 72 hrs after heating for 30 min one time (1×) at $t = 0$ or three times (3×) at $t = 0$, 1 d, and 2 d at 42°C in cells with HSPA6 thermal gene switch control of dCas9-VP64 (blue, orange, and red bars). dCas9-VP64 driven expression of target genes driven by a constitutive CMV promoter is depicted by gray bars ($n = 4-6$, mean $\pm$ s.d., one-way ANOVA, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). (c) Fold mRNA expression of *GzmB* 3 days following a 30 min thermal treatment at the indicated temperatures. dCas9-VP64

expression is controlled by either the HSPA6 thermal switch (purple bars) or the constitutive CMV promoter (gray bars) ($n = 3-4$, mean $\pm$ s.d., one-way ANOVA, **** $p < 0.0001$). (d) Fold protein expression as measured by ELISA of the same conditions ($n = 3-4$, mean $\pm$ s.d., one-way ANOVA, *** $p < 0.001$, **** $p < 0.0001$). (e) Correlation plot between fold *GzmB* mRNA (qRT-PCR) and protein expression (ELISA) controlled by the A6 thermal switch at the indicated temperatures (error bars = mean $\pm$ s.d., and are smaller than displayed data points along the y-axis).

Figure 3 | Thermal control of KRAB-dCas9 using tunable heat triggers enables target gene suppression

(a) DNA constructs for thermal control of KRAB-dCas9 and downstream d2GFP suppression. Guide RNAs (sgRNAs) are constitutively expressed via the human U6 (hU6) promoter, while d2GFP is constitutively expressed via the spleen focus-forming virus (SFFV) promoter. The thermal switch controls the expression of KRAB-dCas9. Kinetic trace of d2GFP suppression following (b) one 30 min heat treatment at the indicated temperatures, (d) 0–3 heating treatments at 42°C for 30 min, or (f) a total heating time of 60 min at 42°C split by pulses of different widths ($n = 3$, error bars show s.d. and are smaller than displayed data points) (c, e, g) Total suppression of d2GFP achieved by each thermal treatment as quantified by the area under the curve (AUC) of the kinetic traces (c, e, g) with a baseline set to $y = 100\%$ d2GFP+ ($n = 3$, error bars show s.d., **** $p < 0.0001$).

Figure 4 |. Remote control of gene suppression by heat-triggered KRAB-dCas9 *in vivo*. Engineered cells were subcutaneously implanted with gold nanorods (AuNRs) and subsequently heated using an NIR laser. (a) Thermal image of a mouse undergoing laser-induced plasmonic heating (H = Heated, UH = Unheated). (b) Engineered cells were heated locally *in vivo* to a skin temperature of 44°C using a NIR laser at t=4, 5, and 6 d. This temperature was chosen to compensate for lower temperatures at the core of the implant due to NIR-light scattering by tissue and heat diffusion. Inset: a representative thermal trace showing skin temperature of 3 × 3-pixel ROI centered on implant site. (c) MFI of d2GFP in HEK293T cells recovered from UH (37°C) & H (44°C) implants (n=6–9, mean ± s.d., unpaired t-test, ***p<0.001).

Scheme 1 |.

dCas9 expression is triggered by short pulses of heat to enable remote and targeted modulation of downstream transcriptional activity.