Genetically Encoded Protein Thermometer Enables Precise Electrothermal Control of Transgene Expression

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Body temperature is maintained at around 37 °C in humans, but may rise to 40 °C or more during high-grade fever, which occurs in most adults who are seriously ill. However, endogenous temperature sensors, such as ion channels and heat-shock promoters, are fully activated only at noxious temperatures above this range, making them unsuitable for medical applications. Here, a genetically encoded protein thermometer (human enhanced gene activation thermometer; HEAT) is designed that can trigger transgene expression in the range of 37–40 °C by linking a mutant coiled-coil temperature-responsive protein sensor to a synthetic transcription factor. To validate the construct, a HEAT-transgenic monoclonal human cell line, FeverSense, is generated and it is confirmed that it works as a fever sensor that can temperature- and exposure-time-dependently trigger reporter gene expression in vitro and in vivo. For translational proof of concept, microencapsulated designer cells stably expressing a HEAT-controlled insulin production cassette in a mouse model of type-1 diabetes are subcutaneously implanted and topical heating patches are used to apply heat corresponding to a warm sensation in humans. Insulin release is induced, restoring normoglycemia. Thus, HEAT appears to be suitable for practical electrothermal control of cell-based therapy, and may also have potential for next-generation treatment of fever-associated medical conditions.

1. Introduction

Since metabolic reaction networks are highly temperature sensitive, most living systems have evolved to function in ecosystems with a narrow temperature range, while having the ability to survive short-term environmental temperature fluctuations.[1,2] Endothermic species such as humans actively control their body temperature to remain within a very narrow range, enabling them to be largely independent of daily and seasonal temperature shifts.[3,4] For this purpose, the hypothalamus acts as central thermostat by integrating temperature inputs from cold- and heat-sensitive neuronal fibers that collect information from thermal sensors, such as cold-activated (TRPA1, TRPM8) and heat-activated (TRPV1, TRPM2, TRPM3) channels,[5–8] across the entire body. The hypothalamus adjusts heat production through muscle contraction (shivering) or heat dissipation (perspiration) as required via a range of hormone-based closed-loop control circuits.[3,6] The hypothalamus also actively increases the body temperature as a defence mechanism to contain viral, bacterial and parasitic infections, such as influenza, meningitis, COVID-19 and malaria,[9–12] a process known as pyrexia or fever. This transient increase of the body’s homothermal temperature by a few degrees Celsius is one of the most common medical signs and occurs in about 75% of adults who are seriously ill.[13] At the cellular level, thermal emergency situations are attenuated by a panoply of chaperone molecules[14,15] and heat-shock promoters, which manage unfolded protein responses in a feed-forward control manner.[14,16] The hypothalamus also actively increases the body temperature as a defence mechanism to contain viral, bacterial and parasitic infections, such as influenza, meningitis, COVID-19 and malaria,[9–12] a process known as pyrexia or fever. This transient increase of the body’s homothermal temperature by a few degrees Celsius is one of the most common medical signs and occurs in about 75% of adults who are seriously ill.[13] At the cellular level, thermal emergency situations are attenuated by a panoply of chaperone molecules[14,15] and heat-shock promoters, which manage unfolded protein responses in a feed-forward control manner.[14,16]
recognizes the body temperature of its host upon infection and adapts by the expression of virulence factors that promote its survival and persistence inside human cells. The cytoplasmic protein TlpA encoded on a virulence plasmid of *S. typhimurium* was one of the first documented temperature-sensing gene regulators. TlpA forms a C-terminal α-helical coiled-coil motif and a sequence-specific DNA-binding domain acting as an autoregulatory repressor. TlpA has recently been successfully engineered to control green-fluorescent protein (GFP) expression in *Escherichia coli* maintained in culture or injected into nude mice. Although TlpA-GFP fusion proteins could be used to visualize subcellular thermoregulation in living mammalian cells, attempts to achieve TlpA-mediated temperature-responsive gene expression have been unsuccessful.

In addition, previous studies have found that low-temperature induction systems based on viral replicons or the bacterial Rhea repressor are not functional in mammals. Even the cold-sensation channel hTRPM8 was insensitive in mice kept at low ambient temperatures, since these warm-blooded animals can maintain their body temperature in cold environments. Furthermore, although native heat-shock promoters appear to be functional in mammalian cells as well as in mice, their activation requires noxious temperatures associated with tissue damage upon prolonged exposure, and their use involves sophisticated equipment such as magnetic resonance imaging (MRI)-guided high-intensity-focused ultrasound or magnetic nanoparticles which are suspected to elicit adverse health effects. Thus, the quest for an orthogonal, genetically encoded mammalian protein thermometer capable of programming transgene expression in human cells is ongoing.

To meet this need, we have designed a human enhanced gene activation thermometer (HEAT) that enables precise, robust and reversible temperature-adjustable gene expression within the pathophysiologically relevant temperature range of 37–40 °C. Using an in vivo mouse model of type-1 diabetes, we confirmed that subcutaneously implanted microencapsulated designer cells stably expressing a HEAT-controlled insulin production cassette can restore normoglycemia in response to topical warming with electrothermal patches.

2. Results

2.1. Thermocontrol by Endogenous Heat-Sensing Mechanisms

Inspired by the use of the human cold-sensitive channel TRPM8 to program successful treatment of experimental muscle atrophy in response to cooling sensation, we tested the mammalian heat-sensitive channels TRPV1 and TRPV4 for temperature-responsive activation of synthetic calcium-dependent signaling cascades (Figure S1a, Supporting Information). However, constitutively expressed TRPV1 or TRPV4 showed only modest fold induction, even at 44 °C, which represents a noxious temperature (Figure S1b, Supporting Information). With a goal to achieve innocuous temperature induction, we placed either the native human HSP70B promoter sequence or the tandem HSPA1A-derived heat-shock elements upstream of a minimal eukaryotic promoter following established in vitro designs. Both synthetic heat-shock promoters were strongly induced at 44 °C, but showed only marginal induction below 43 °C (Figure S1c,d, Supporting Information). Wild-type mice, subcutaneously implanted with engineered cells for heat-shock promoter-driven SEAP (human placental secreted alkaline phosphatase) expression, whose skin surface temperature was increased to 44 °C by infrared illumination or topical heat patches, showed only modestly increased blood SEAP levels (Figure S1e, Supporting Information), suggesting that the animals’ homeostatic control compensated for external heating of the skin. These results suggest that orthogonal temperature-inducible transgene regulation remains challenging in living animals and likely requires genetically encoded temperature sensors to operate much closer to the homeostatic temperature.

2.2. Toward the Design of an Orthogonal Mammalian Protein Thermometer

Since endogenous thermosensors such as channels and heat-shock promoters did not provide acceptable temperature sensitivity, we considered other avenues to engineer an orthogonal mammalian protein thermometer. Capitalizing on the heat-inducible DNA-binding ability of TlpA, which contributes to infection-associated temperature adaptation of *S. typhimurium*, we fused a modified TlpA variant (TlpA39) to the VP16 transactivation domain of the *Herpes simplex* virus. This resulted in a synthetic mammalian transcription factor (pAna117, PhCMV-TlpA39-VP16-pAbGH) binding to chimeric promoters containing a TlpA-specific operator sequence fused to a minimal promoter (pAna115, O62pA-pmin-SEAP-pA6GH) in a temperature-repressible manner. However, this classical gene switch topology was unable to activate SEAP expression beyond background in human cells (37 °C, 1.6 ± 0.3 U/L; 40 °C, 2.0 ± 0.3 U/L), so we next tested a split transcription factor design. The *E. coli* tetacycline-dependent repressor (TetR) was C-terminally fused (pAna126, PhCMV-TetR-TlpA39-pA6GH) and VP16 was N-terminally fused to TlpA (pAna117, PhCMV-TlpA39-VP16-pA6GH). Dimerization of TlpA’s coiled-coil domains at 37 °C reconstitutes a tetracycline-dependent transactivator (TA) variant (TetR-TlpA39-TlpA39-VP16-pA6GH), which binds to and activates standard TA-dependent tetracycline-responsive promoters (PhCMV-pA6GH) (Figure 1a,b). At 40 °C, the coiled-coil domains unfold and the TA variant splits, thereby shutting off PhCMV-pA6GH-driven target-gene expression (Figure 1). Although this prototypic mammalian thermometer design was in principle responsive between 37 °C and 40 °C, its overall leakiness was high, limiting the induction-fold to 2.5 (Figure 1b). In addition, this OFF-type device can only be shut down at elevated temperatures, which would be problematic for potential clinical applications.

2.3. Design and Validation of the Human Enhanced Gene Activation Thermometer (HEAT)

Instead of reconstituting a synthetic transcription factor via TlpA-derived coiled-coil domain-mediated heterodimerization in a
high-temperature-repressible manner, we next directly linked a single TlpA domain C-terminally to the tetracycline-dependent transactivator (tTA) via a triple VP16-derived F-type minimal transactivation domain (pBS707, P_{hCMV}-TlpA_{39}-FFF-TetR-VP16-pA_{GHC})(Figure 1c). In this system, the TlpA coiled-coil domains lock tTA in an inactive homodimerized configuration at 37 °C (Figure 1c). At higher temperatures, however, the TlpA moiety adopts an unfolded monomeric state, releasing functional tTA, which can bind to and activate classical tTA-dependent tetracycline-responsive promoters (Figure 1c). Indeed, as expected, the TlpA-FFF-tTA thermometer remained inactive at 37 °C and provided fully induced P_{hCMV} driven SEAP expression in human HEK-293 cells at 40 °C (Figure 1d). While the viability of isogenic cultures constitutively expressing SEAP was comparable at 37 °C and at 40 °C, the overall viable cell count was lower at 40 °C, indicating that proliferation of human HEK-293 cells was lower at elevated temperature (Figure S2a,b, Supporting Information). Despite this, the TlpA-FFF-tTA-mediated P_{hCMV} was repressed at 37 °C but induced at 40 °C, reaching SEAP values comparable to those driven by either native tTA or the isogenic constitutive promoter P_{hCMV} (Figure S2c, Supporting Information).

2.4. Detailed Characterization of HEAT Performance In Vitro

Performance profiling in different cell types confirmed 37 °C-OFF, 40 °C-ON thermocontrol by HEAT in a variety of rodent and human cell lines, suggesting that HEAT would be broadly applicable for basic and translational research, and would be compatible with biopharmaceutical manufacturing (CHO-K1, BHK-21, HEK-293) as well as major tissues, including liver (Hep G2).
colon (Caco-2), muscle (C2C12) and connective tissue (HT-1080) (Figure S3, Supporting Information). In particular, HEAT is able to program gene expression in human mesenchymal stem cells (hMSCs) and also in patient-derived induced pluripotent stem cells (iPSCs), which are currently in the limelight as candidates for the next wave of cell-based therapies.\(^\text{[18,39]}\) Importantly, HEAT was specifically temperature-inducible, both by exposure to 40 °C and by a heat-shock pulse, and was orthogonal to other physiological inducers (Figure S4a,b, Supporting Information). In comparison, the best-performing heat-shock promoter did not show significant induction upon incubation at 40 °C and was activated by the anti-cancer medication luminespib (Figure S4c,d, Supporting Information).

HEAT showed highly temperature-sensitive induction of SEAP expression in the pathophysiological range of 37–40 °C (Figure 2a and distinctive temperature-programmable expression kinetics (Figure 2b, Figure S5, Supporting Information). Additionally, HEAT fully retained the tetracycline responsiveness of its tTA moiety, as SEAP expression by HEAT-transgenic cells could be dose-dependently shut down at 40 °C by exposure to increasing concentrations of the clinically licensed antibiotic doxycycline (Figure 2c,d). This demonstrates the dual-input responsiveness of the HEAT device to both small-molecular and physical cues and furthermore suggests that doxycycline interventions could serve as both an additional layer of adjustability and as a safety trigger to transiently override the thermometer in emergency situations (Figure 2d). These characteristics would be important assets in potential future clinical applications. Further, when we replaced HEAT's tTA domain with its reverse tTA (rtTA) variant (pAB659, P<sub>hCMV</sub>-TlpA<sub>rtTA</sub>-FF-FrtetR-VP16-P<sub>AdCMV</sub>), HEAT provided AND-gate-type temperature and doxycycline inducibility (Figure 2d). HEAT was also fully compatible with the melanopsin-based light switch (Figure 2e,f)\(^\text{[40]}\) and could be multiplexed to enable independent, interference-free, traceless control of two sets of transgenes using nonmolecular physical cues (Figure 2f).

### 2.5. HEAT Acts as a Fever Thermometer in Mice

In humans, the body's temperature set point is around 37 °C and fevers are not typically higher than 40.5 °C.\(^\text{[9,13]}\) Therefore, a molecular fever sensor has to react to quite small changes in body temperature (Figure 2a) and provide precise temperature-responsive induction kinetics (Figure 2b). To validate HEAT's fever response in vivo, we generated HEAT-transgenic monoclonal human cell lines (Figure S6, Supporting Information). The best-in-class temperature-inducible clonal cell line, FeverSense, showed finely graded temperature induction profiles between 37 and 40 °C with sub-degree precision 3a. Also, temperature-inducible SEAP induction kinetics could be precisely programmed by employing different high-temperature exposure times, indicating that FeverSense would be able to sense and react to the severity as well as the duration of a febrile state (Figure 3b). In addition, the FeverSense actuation profile was rapidly reversible, showing baseline SEAP expression upon cooling from 40 to 37 °C (Figure 3c).

In contrast to humans, infectious febrile responses in mice are of shorter duration and involve a lower temperature rise,\(^\text{[41]}\) which sets additional performance challenges for the HEAT device in an experimental model.\(^\text{[42]}\) To validate HEAT's fever response in vivo, we implanted microencapsulated FeverSense cells into mice and either maintained the animals at room temperature (21 °C) or exposed them to an ambient temperature of 36 °C, which increases their core body temperature, thereby simulating fever.\(^\text{[42–44]}\) Indeed, while animals kept at room temperature maintained a standard homeostatic body temperature of around 36 °C and showed only basal SEAP expression, mice kept in the hot environment exhibited a body temperature above 38 °C, corresponding to a mild fever state.\(^\text{[41]}\) and showed increased circulating SEAP level in their bloodstream, demonstrating that HEAT-transgenic FeverSense cells were able to sense and react to fever (Figure 3d).

Next, to simulate a high-grade fever, we repeatedly exposed the animals to 39.5 °C for short periods\(^\text{[45]}\) driving their skin temperatures above 39 °C (Figure 3e). This treatment increased the blood SEAP levels compared to those of animals with a body temperature of 36 °C or 38 °C (Figure 3f). Overall, these results show that HEAT was able to detect and differentiate between different body temperatures in the range from normal to high-grade fever, and was able to program a proxy biopharmaceutical response accordingly. To provide additional insight into the clinical potential of the system, we demonstrated that HEAT-transfected bone-marrow-derived hMSCs are not affected by the increased temperature (Figure S7a, Supporting Information) and that the system is functional upon stable integration of the components into the genome of these cells (Figure S7b,c, Supporting Information). When encapsulated in a protective alginate matrix the obtained induction for the stable hMSCs was comparable to that of the FeverSense cells (Figure S8a,b, Supporting Information). Furthermore, subcutaneous implantation in mice enabled control over gene expression by using an electronic heatpad (Figure S9, Supporting Information).

### 2.6. Noninvasive Heat-Patch-Controlled Insulin Release Restores Glucose Homeostasis in Experimental Type-1 Diabetes

In order to further expand the potential applications of HEAT, we considered the fact that touching human skin with objects having a temperature between 39 and 40 °C triggers a pleasant sensation of warmth.\(^\text{[46]}\) Since this is within the working range of HEAT, we examined whether warming local skin tissue to this temperature with an electrothermal skin patch would be effective for percutaneous HEAT-mediated remote-control of biopharmaceutical protein production, release and systemic delivery by designer cells. Electrothermal skin patches are particularly attractive for therapeutic interventions using cell-based therapies, as they enable traceless control in the absence of any molecular cues or co-factors, can be discretely and comfortably worn during therapy, and provide a direct link between wearable electronics and therapeutic gene expression. Therefore, as a translational proof-of-concept, we first linked HEAT sensing to expression of insulin and produced stable monoclonal human cell lines that provide temperature-activated release of this peptide hormone (Figure S10, Supporting Information). The best-in-class cell line showed precise temperature-adjustable insulin release in the warm-sensation range, even when microencapsulated in...
**Figure 2.** Characterization of the human enhanced gene activation thermometer (HEAT) as a genetically encoded temperature-operated controller for cellular therapy. 

a) The temperature–response relationship of HEAT (pBS707) for transcription from a p\textsubscript{HCMV-1}–controlled SEAP expression vector in transfected HEK-293 cells was characterized by incubating the cells at the indicated temperatures from 37 to 40 °C (in the physiological fever range).

b) The time–response relationship of HEAT (pBS707) for transcription from a p\textsubscript{HCMV-1}–controlled SEAP reporter vector in transfected HEK-293 cells was characterized by incubating the cells for the indicated duration at 40 °C and then returning them to 37 °C for the rest of the 24 h period.

c) Integration of a small-molecule safety switch into the HEAT system is enabled by the TetR DNA binding domain, which loses affinity for its recognition sequence upon binding of doxycycline. This prevents the activation of target genes even at 40 °C. Exchange of TetR for the reverse TetR DNA binding domain results in an AND-gate logic circuit where both the temperature stimulus and doxycycline are necessary for the activation of target genes.

d) Doxycycline dose-dependent relationship of SEAP reporter gene expression at 37 and 40 °C from cells transfected with a p\textsubscript{HCMV-1}–controlled reporter vector and HEAT (p\textsubscript{HCMV-TIPa39-FFF-TetR-VP16-pAbGH}, pBS707) or a TipA39–rtTA (p\textsubscript{HCMV-TIPa39-FFF-rTetR-VP16-pAbGH}, pAB659; rTetR – reverse...
clinically licensed semi-permeable alginate to protect them from the mouse immune system (Figure 4a).

To enable precise transdermal control, we designed a companion electrothermal skin patch that was operated by a compact electronic controller (Figure 4b). With its skin-contact surface of 1 cm² and its small and light-weight (0.5 g) design, the patch could be easily strapped to the skin immediately above a subcutaneously placed implant (Figure 4b). Type-1-diabetic mice subcutaneously implanted with microencapsulated HEAT-transgenic insulin-producing cells and equipped with heating patches showed continued hyperglycemia without any improvement of their diabetic state when the patch was switched off, suggesting that the engineered cells do not show any physiologically relevant leakiness (Figure 4c). However, fasting blood-glucose levels rapidly decreased upon activation of the heating patch, confirming the effectiveness of warmth-mediated remote-control of insulin release by the implanted cells (Figure 4c). The HEAT device was not only able to restore normoglycemia in the type-1-diabetic mice, but also provided sufficient insulin to rapidly attenuate postprandial blood-glucose spikes simulated in glucose-tolerance tests (Figure 4d). In contrast, untreated mice and treated animals with the heating patch switched off retained extreme hyperglycemia. These results indicate that the HEAT-based traceless electrothermal pectunomatous programming of cell implants is robust and reliable to correct one of the most difficult-to-control endocrine conditions.

3. Discussion

Next-generation cell-based therapies require sophisticated gene switches programming precise, adjustable and reversible trigger-inducible therapeutic gene expression in order to deliver therapeutic proteins at effective doses in a timely manner.[59] Many trigger-inducible gene switches responsive to small-molecular compounds have been developed and used to modulate the activity of synthetic transcription factors, cell-surface receptors and membrane channels,[28] or to activate chimeric promoters[48] and synthetic signaling cascades[49] in order to produce and release therapeutic proteins.[39,48] Trigger compounds include drugs,[50] anti-infectives,[47] vitamins,[51] food additives[52] and metabolites.[48] But, despite these advances, issues of cytotoxicity, pharmacodynamics, bioavailability, accumulation, and pleiotropism of the trigger compounds and co-factors for a long time remained as major challenges impeding real-world medical applications.[53] These challenges were largely overcome with the advent of traceless control inputs such as light,[50] magnetic fields,[11,54] radio waves[35,36] and electric currents.[57] Nevertheless, many of these traceless control circuits require sophisticated stimulation hardware, complex chemical co-factors or harmful inorganic nanoparticles.[31,33] Also, traceless control systems are responsive to the orthogonal input, but are typically unable to sense and react to endogenous signals. In the present work, we have engineered HEAT as a temperature sensitive control device working in the physiological range: it can respond either to endogenous fever or to external heating at a level corresponding to a sensation of warmth in humans. HEAT offers excellent control parameters, providing robust, reversible and precisely adjustable trigger-inducible ON-type expression profiles, and does not require any co-factor or sophisticated equipment. As a generalized expression system, HEAT should be applicable for improved control of multiple diseases by the therapeutic delivery of hormones (such as desmopressin for the treatment of nocturia), peptides (such as glp1 for type 2 diabetes) or antibodies (such as anti-IL6R in inflammatory conditions). From the viewpoint of potential clinical applications, HEAT actuation can be fine-tuned or completely overridden in an emergency by clinically licensed tetracycline drugs. Owing to the bacterial origin of its components, it might be necessary to incorporate an immunological mask for this transcriptional factor[58] in the case of applications where no protective barrier would shield the engineered cells.

Our translational proof-of-concept study demonstrated that simple electrothermal induction using topical skin patches was effective to percutaneously program insulin release by subcutaneously implanted insulin-releasing cells and to restore normoglycemia in experimental type-1 diabetes in mice. We chose this model because type-1 diabetes is one of the most common and difficult-to-control metabolic disorders, with dramatically increasing prevalence worldwide.[59] Importantly, HEAT was able not only to restore fasting blood-glucose levels to the normal range, but also to attenuate short-term postprandial blood-glucose spikes, restoring near wild-type kinetics. With wearable electronic devices profiling blood-glucose levels noninvasively on the horizon,[60] noninvasive closed-loop control of diabetes by wearable electronic devices combining optogenetics and theremoelectronics seems to be within reach. Moreover, as HEAT is compatible with optogenetic devices, it could find application for interference-free, independent traceless electronic remote control of different sets of biopharmaceuticals.

HEAT offers key advantages over previously reported temperature-responsive sensors: it is activated by simple topical warming at a temperature that is perceived as pleasant by humans, and coupling the treatment with a positive sensation could improve patient compliance; it can precisely sense and discriminate fever-range temperatures between 37 and 40 °C with sub-degree precision enabling the detection of physiological temperature changes; it quickly returns to baseline activity upon a reduction in temperature, whereas endogenous transcription factors (such as HSF1 or NFAT) show prolonged activity after stimulation. Previously reported high-temperature sensors have required an excess of 40 °C for activation, and such...
Figure 3. Fever-sensing cells and their performance in murine models of elevated body temperature. a) Temperature–response relationship for FeverSense cells, which stably express TlpA39-FFF-tTA (\(P_{\text{E}}\text{EF}_{1\alpha}\)-TlpA39-FFF-TetR-VP16-pAbGH, pBS786) and SEAP from a \(P_{\text{CMV}}*-1\) promoter (pBS828). b) Time-dependent induction of SEAP expression in FeverSense cells over a fixed period of 48 h. At the start of the 48 h measurement period, cells were subjected to a 40 °C stimulation of the indicated duration and were then cultured at 37 °C for the remaining time. c) Characterization of the reversibility of SEAP reporter gene expression by FeverSense cells stably expressing TlpA39-FFF-tTA (\(P_{\text{E}}\text{EF}_{1\alpha}\)-TlpA39-FFF-TetR-VP16-pAbGH, pBS786) and a SEAP reporter gene expression vector controlled by a \(P_{\text{CMV}}*-1\) promoter. Cells were subjected to two temperature profiles consisting of incubation periods at each temperature: 40–37–40 °C or 37–40–37 °C, and SEAP accumulation was quantified in the culture supernatant every 24 h. d) Subcutaneous implant containing FeverSense designer cells can differentiate between normal and elevated body temperatures. Microencapsulated engineered cells were injected into the back of Swiss albinomice. SEAP reporter protein was quantified in serum samples (\(n = 7\) per group, 1 outlier excluded per group) collected before and after housing one of the groups at 36 °C for 48 h. e) Skin surface temperature measurement of c57bl/6 mice at the end of a representative 2 h cycle at 39.5 °C in a model consisting of 4 cycles in 9 h d−1 to simulate high fever episodes. f) FeverSense cells differentiate between homeostatic temperature, prolonged low-grade fever, and high-grade fever models in c57bl/6 mice. Microencapsulated designer cells were injected subcutaneously on the back of the mice, which were then either maintained at 21 °C as a room temperature control (\(n = 8\)), maintained at 36 °C as a prolonged low fever simulation (\(n = 10\)), or placed at 39.5 °C for four cycles of 2 h interspaced by 15 min recovery periods for a total of 9 h to simulate severe fever episodes (\(n = 10\)). Data in (a)–(c) are mean ± SD of \(n = 3\) biologically independent samples, representative of \(n = 3\) independent experiments. Small error bars covered by the symbol are not shown. Data in (d)–(f) are shown as bar graphs of mean ± SEM, overlaid with the individual data points. Statistical analysis in (d) was done with a two-tailed t-test and in (e) and (f) with one-way ANOVA for multiple comparisons: *\(p < 0.05\), **\(p < 0.01\), ***\(p < 0.001\), ****\(p < 0.0001\).
temperatures represent a life-threatening medical emergency associated with pleiotropic heat-shock responses.\cite{13,16} Since hypothalamus-coordinated thermostatic activities act to restore a normal core body temperature, which would be outside the sensors’ sensitivity and permissive temperature range,\cite{13,61} these sensors are operational only transiently if at all in vivo. Emerging technologies such as short-duration focused IR-light-induced temperature excursions following the incorporation of gold nanorods\cite{62,63} could increase the efficiency of thermal stimulation and benefit the clinical translatability of both heat-shock promoters and temperature-sensing transcription factors.

Owing to its reversibility, HEAT may well be suited to monitor relapsing fever episodes in real-time and could possibly be set up for closed-loop therapeutic interventions. Considering its sub-degree sensitivity, HEAT may in principle also be further refined to sense and react to transient sub-fever body temperature deviations that occur during the circadian rhythm\cite{64} as well as fertility cycles\cite{65} or changes related to sleep disturbances, caloric restriction,\cite{66} alcohol consumption,\cite{67} strenuous exercise\cite{68} or a wide range of psychological factors.\cite{69} Wearables that continuously record body temperatures in real-time have recently come into the limelight as a replacement for classic thermometry at
random time points in order to monitor immunologic responses to viral infections and to track the spread of SARS-CoV-2. An improvement in sensitivity could further benefit applications deep in the body, where heat delivery is more efficiently counteracted by the body’s homeostatic control of temperature. Genetically encoded protein thermometers such as HEAT that can directly, precisely and dynamically interface warm sensation or pyrexia with in-situ actuation of biopharmaceutical production could provide the missing link between medical conditions associated with elevated temperature and preventive, chronic and acute interventions using cell-based personalized medicine.

4. Experimental Section

Plasmid Construction: Mammalian gene expression plasmids were constructed by standard molecular cloning techniques using restriction endonucleases (New England Biolabs, Ipswich, Massachusetts, USA; high-fidelity enzymes were used whenever possible), followed by ligation with T4 DNA ligase (New England Biolabs, cat. no. M0202L). Before ligation the plasmid backbones were dephosphorylated with Antarctic phosphatase (New England Biolabs, cat. no. M0289L). PCR reactions were performed using Phusion High-Fidelity DNA Polymerase (New England Biolabs, cat. no. M0530L) or Q5 High-Fidelity DNA Polymerase (New England Biolabs, cat. no. M0491L). Details of all restriction enzymes and primers used for each plasmid are presented in Table S1 (Supporting Information). The plasmids were amplifed in XL10 Gold chemically competent cells (New England Biolabs, cat. no. C2992) and DNA was extracted using a plasmid miniprep kit (Zymo Research, Irvine, California, USA, cat. no. D4054) or, for larger-scale preparation, a ZymoPURE II Plasmid Midiprep Kit (Zymo Research, cat. no. D4200). Operator binding sites in reporter plasmids were introduced using custom oligonucleotides (Sigma Aldrich, Haverhill, UK) designed as complementary forward and reverse strands, which, upon annealing, form double-stranded fragments with overhangs suitable for restriction endonuclease-based cloning. For this, 10 × 10^−6 m oligonucleotide mixture (S × 10^-6 m of each strand) was phosphorylated with T4 polynucleotide kinase (New England Biolabs, cat. no. M0202L) before incubation in 0.05% trypsin-EDTA (Life Technologies, Carlsbad, California, USA, cat. no. 02886010) solution in 2x SEAP buffer (20 μL of heat-inactivated serum (FBS; Sigma-Aldrich, Saint Louis, USA, cat. no. F7524, lot no. 022M3395) and penicillin (100 U/ml) and streptomycin (100 μg/ml) solution (Sigma-Aldrich, cat. no. P433) at 37 °C under a humidified atmosphere containing 5% CO₂. Passaging of pre-confluent HEK-293 cultures was done by detaching cells through incubation in 0.05% trypsin-EDTA (Life Technologies, Carlsbad, California, USA, cat. no. 25300054) for 5 min at 37 °C. Cells were transferred to 10 mL cell culture medium and centrifuged for 1 min at 200 x g, then the supernatant was discarded and the cells were resuspended in fresh medium at a standard cell density (1.5 × 10^5 cells/mL), before reseeding them in cell culture plates. Cell number and viability were quantified using an electric field multichannel cell counting device (Cyto Count Counter and Analyzer Model TT, Roche Diagnostics GmbH, Rotkreuz, Switzerland). HeLa cells (HeLa, ATCC: CCL-2), bone-marrow-derived immortalized mesenchymal stem/stromal cells (hMSC-TERT) cells (HEPG2 cells (HEPG2, ATCC: HB-8065), baby hamster kidney cells (BHK-21, ATCC: CCL-10), HT-1080 cells (HT-1080, ATCC: CCL-121), Hana3a (Hana3a, RRID:CVCL-RW32), C2C12 cells (C2C12, ATCC: CRL-1772), and Caco-2 cells (Caco2, ATCC: HTB-37) were cultivated as described for HEK-293. INS-1E cells (RRID:CVCL-0351) were cultivated in RPMI-1640 medium supplemented with 10% (v/v) FBS. Chinese hamster ovary cells (CHO-K1, ATCC: CCL-61) were cultivated in Ham’s F-12K (Kainh’s) medium (Thermo Fisher Scientific, Gibco F-12K, cat. no. 21127-022). All plasmid transfections were conducted using 1:4 DNA:PEI mixture (polyethylenimine, MW 40000; Polyscience Inc., Warrington, USA, cat. no. 24765) with a total DNA amount of 350 ng cm⁻², followed by incubation at room temperature over 15 min. After 1:500 dilution, the supernatant was discarded and the cells were resuspended in fresh Adv. Sci. 2021, 8, 201813

SEAP Quantification: Human placentalsecretedalkalinephosphatase (SEAP) concentration in the cell culture medium was determined by means of light-absorbance time-course measurement. For this, 20 μL of culture supernatant was mixed with 80 μL of ddH₂O and heat-inactivated for 30 min at 65 °C. Then, 80 μL of 2x SEAP buffer (20 × 10⁻³ M MgCl₂, 1 × 10⁻³ M MgOCl₂, 21% (v/v) diethanolamine, pH 9.8) and 20 μL of 120 × 10⁻³ m para-nitrophenyl phosphate (Fisher Scientific Acros Organics, Geel, Belgium, cat. no. 128861000) solution in 2x SEAP buffer were added to each well and the absorbance at 405 nm was measured at 37 °C using a Tecan M1000 plate reader (Tecan Group Ltd., Maennedorf, Switzerland). SEAP concentrations in serum were quantified using the 96-well SEAP kit (Roche Diagnostics, RRID:CVCL-0351) and had a total area of 1 cm². The system temperature was regulated by means of a JUMO cTRON compact controller (JUMO GmbH, Fulda, Germany) operated with a self-optimizing proportional-integral-derivative (PID) algorithm.

Temperature Treatment of Engineered Cells: Heat-inducible transgene expression in cultured human cells was achieved by placing parafilm-sealed multi-well cell culture dishes on a preheated Thermomixer Comfort heating block (Eppendorf AG, Hamburg, Germany) set at the required temperature. Long-term temperature treatment was done by cultivation of the cells in an incubator heated to the required temperature. In reversibility experiments, SEAP concentrations were probed 3 h after returning the cells to 37 °C to account for temperature transfer in the culture plates and accumulated mRNA.
added to each well, and incubation was continued at ct. for 10 min. Lumin-
escence was measured using a Tecan M1000 plate reader and concentra-
tions were calculated from a standard curve.

**NanoLuciferase Quantification**: The concentration of NanoLuc in cell culture supernatants was measured by using the Nano-Glo® NanoLuc Assay System (Promega, Madison, Wisconsin, USA; cat. no. N1110). In brief, 7.5 μL from each sample was mixed with 7.5 μL buffer/substrate mix (50:1) in 384 well plates (Greiner Bio One, Kremsmünster, Austria; cat. no. 781076), and incubated at room temperature (21 °C) for 10 min. Lumin-
escence was measured with a Tecan M1000 plate reader (Tecan Group Ltd., Switzerland).

**Insulin Quantification**. Recombinant mINS levels in culture super-
natants of engineered cells were quantified using a mouse insulin ELISA kit according to the manufacturer’s instructions (Merckodia, Uppsala, Swe-
den; cat. no. 10-1247-01). Optical density was measured at 450 nm on a Tecan M1000 plate reader and the corresponding concentrations were cal-
culated in Prism 7 (GraphPad Software Inc., San Diego, California, USA) using a cubic-spline regression based on the measured absorbances of manufacturer-provided standard solutions.

**Generation of Monoclonal Stable Cell Lines**: Polyclonal cell lines were generated by co-transfection of a hyperactive Sleeping Beauty transposase (SB100x) expression vector in a 1:20 (weight/weight) ratio with the in-
tended integration vectors containing SB8 recognition sites and encoding both a resistance marker and a fluorophore. The medium was exchanged 12 h after transfection and cells were incubated for 48 h before the ad-
dition of selection medium containing antibiotics corresponding to the resistance encoded by the integrated vectors. The following antibiotic con-
centrations were used during selection: zeocin 100 μg mL⁻¹, puromycin 3 μg mL⁻¹, blasticidin 8 μg mL⁻¹. The cells were maintained in selection medium for three passages and then those with the highest expression lev-
els were selected by means of fluorescence-activated cell sorting (FACS) according to the signal intensity of the encoded fluorophores. Monoclonal cell populations were obtained by restrictive dilution at a ratio of 0.3 cells per well in a 96-well format, followed by 2 weeks expansion before func-
tional analysis.

**Animal Experiments**: Temperature-sensitive designer-cell implants were produced by encapsulating transgenic HEK-293 cells into coher-
tent alginate-poly-(l-lysine) beads (Na-alginate, Buechi Labortechnik AG, Flawil, Switzerland; cat. no. L8662-25G) using an Inotech Encapsulator Research Unit IE-50R (Buechi Labortechnik AG, Flawil, Switzerland) with the following parameters: 200 μm nozzle, vibration frequency 1000 Hz, 900 V for bead dispersion, 20 mL syringe operated at a flow rate of 400 units. 500 μL of serum-free DMEM containing 5x10⁶ microencapsulated transgenic cells was microencapsulated into the back of mice (Janvier Labs, Le Genest-
Saint-Ise, France). Treated animals were kept for 24 h before experiments were conducted. Low-grade persistent hyperthermia was used to simul-
ulate fever with a body temperature of 38 °C according to an established protocol.[43, 44] In brief, animals were given a 500 μL i.p. injection of physi-
ological saline, and kept in cages in a ventilated incubator set to 36 °C. Access to food and water was provided ad libitum. To ensure that dehydra-
tion did not occur, mice received oral gavage of 200 μL water twice daily. For simulation of high-grade fever, immobilized mice were sequentially subjected to an environmental temperature of 39.5 °C for 2 h followed by a 15 min resting time at room temperature (21 °C) for a total of 9 h d⁻¹ as described in an established protocol.[45] Before the initial stimulation, mice received i.p. injections of 500 μL physiological saline and oral gav-
age of 200 μL during the subsequent rest phases. Skin temperature was recorded at the implant site with a Uni-T UT305H infrared thermometer, which provides an accurate estimate of the body’s core temperature.[46] For extracorporeal transcutaneous temperature control of designer cell implants in mice, the custom-designed heating patch was fixed on the shaved backs of mice and adjusted to two degrees higher temperature than the measured temperature in the implants, to account for tempera-
ture dissipation through the skin. Type-1 diabetes was induced in mice as previously described.[57] Mice were fasted for 12 h overnight and in-
jected with 60 mg kg⁻¹ of streptozotocin (Sigma-Aldrich, cat. no. S0130) for 5 d. Fasting blood glucose was measured after 8 h of food restric-
tion using a clinically licensed glucometer (Contour Next, Bayer Health-
care, Germany). Postprandial glycemic excursions were simulated by i.p. injection of 1.5 g kg⁻¹ glucose, and blood glucose was profiled every 15 to 30 min for the following 2 h. For analysis of SEAP levels, blood serum was collected using Microtainer serum-separating tubes according to the manufacturer’s protocol (Becton Dickinson, cat. no. 365967) and quantified as described above. All experiments involving animals were performed according to the directive of the European Community Coun-
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cense no. 69266309) at the Institut Universitaire de Technologie of the Université Claude Bernard Lyon 1, F-696226, Villeurbanne Cedex, France.

**Statistical Analysis**: Details of data presentation, sample size (n), sta-
tistical analysis and significance of differences are given in the figure cap-
tions. Statistical evaluation was conducted by using an unpaired Student’s two-tailed t-test for comparing two sets of data and one-way ANOVA for multiple comparisons as implemented in Prism GraphPad 7 (GraphPad Software Inc., San Diego, California, USA).

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Author Contributions**

B.A.S. and M.F. conceived the project and designed the experiments. B.A.S., A.P.T., M.M., A.B., G.C., S.X., and K.K. conducted the experiments. B.A.S., A.P.T., and M.F. analyzed the data and wrote the manuscript.

**Data Availability Statement**

Detailed vector information is provided in Table S1 (Supporting Informa-
tion). All vectors and data are freely available upon request.

**Keywords**

diabetes, gene regulation, gene switches, synthetic biology, temperature

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