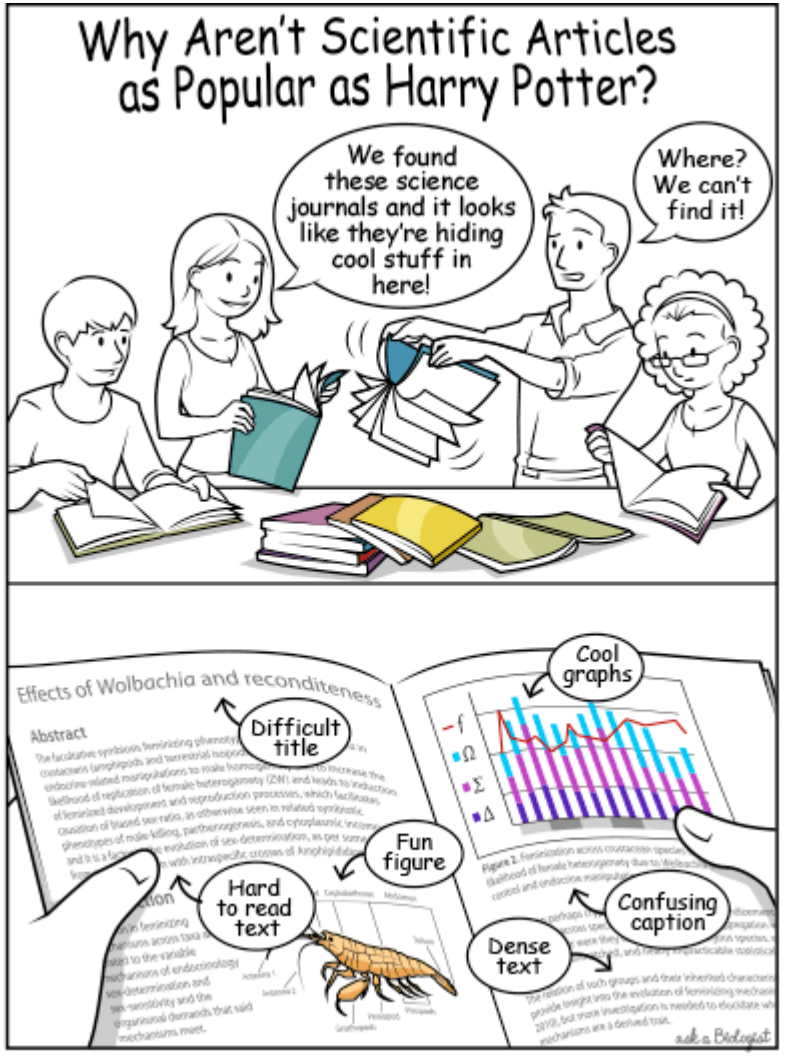
How to Read a Research Article

Brittany Lende

UROP Training



Revised with permission of Adam Beitz

“How to read a scientific paper”

If you’re at the beginning of your career in science, you may be struggling with the same problem. It may help you to familiarize yourself with the 10 Stages of Reading a Scientific Paper:

1. Optimism. “This can’t be too difficult,” you tell yourself with a smile—in the same way you tell yourself, “It’s not damaging to drink eight cups of coffee a day” or “There are plenty of tenure-track jobs.” After all, you’ve been reading *words* for decades. And that’s all a scientific paper is, right? Words?

2. Fear. This is the stage when you realize, “Uh … I don’t think all of these are words.” So you slow down a little. Sound out the syllables, parse the jargon, look up the acronyms, and review your work several times. Congratulations: You have now read the title.

3. Regret. You begin to realize that you should have budgeted much more time for this whole undertaking. Why, oh why, did you think you could read the article in a single bus ride? If only you had more time. If only you had one of those buzzer buttons from workplaces in the 1960s, and you could just press it and say, “Phoebe, cancel my January.” If only there was a compact version of the same article, something on the order of 250 or fewer words, printed in bold at the beginning of the paper…

4. Corner-cutting. Why, what’s this? An abstract, all for me? Blessed be the editors of scientific journals who knew that no article is comprehensible, so they asked their writers to provide, à la ***Spaceballs***, “the short, short version.” Okay. Let’s do this.

5. Bafflement. What the hell? Was that abstract supposed to explain something? Why was the average sentence 40 words long? Why were there so many acronyms? Why did the authors use the word “characterize” five times?

6. Distraction. What if there was, like, a smartphone for ducks? How would that work? What would they use it for? And what was that Paul Simon lyric, the one from “You Can Call Me Al,” that’s been in your head all day? How would your life change if you owned a bread maker? You’d have to buy yeast. Is yeast expensive? You could make your own bread every few days, but then it might go stale. It’s not the same as store-bought bread; it’s just not. Oh, right! “Don’t want to end up a cartoon in a cartoon graveyard.” Is Paul Simon still alive? You should check Wikipedia. Sometimes you confuse him with Paul McCartney or Paul Shaffer. Shame about David Bowie. Can you put coffee in a humidifier?

7. Realization that 15 minutes have gone by and you haven’t progressed to the next sentence.

8. Determination. All righty. Really gonna read this time. Really gonna do it. Yup, yuppers, yup-a-roo, readin’ words is what you do. Let’s just point those pupils at the dried ink on the page, and …

9. Rage. HOW COULD ANY HUMAN BRAIN PRODUCE SUCH SENTENCES?

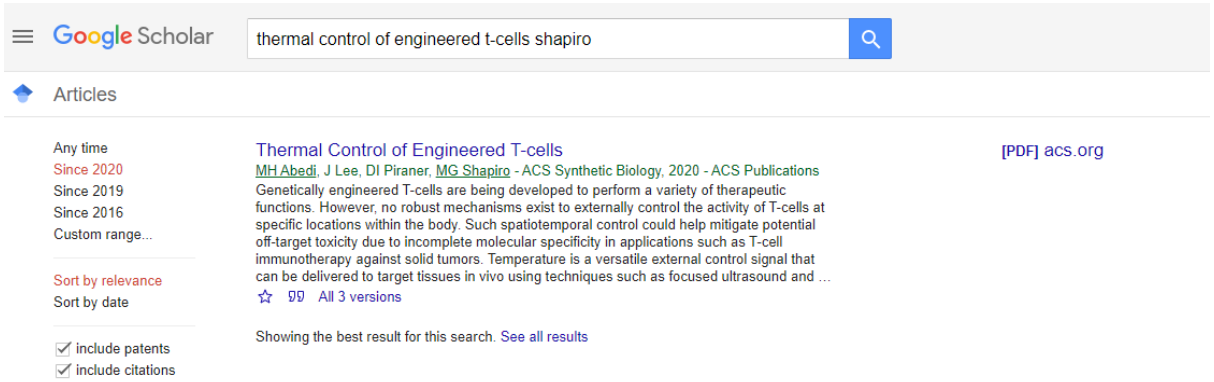
10. Genuine contemplation of a career in the humanities. Academic papers written on nonscientific subjects are easy to understand, right? Right?

https://www.sciencemag.org/careers/2016/01/how-read-scientific-paper

How in depth are you reading?

So you've done your Google Scholar search and you found a paper that might be of interest to your project.

Reading a paper is **time consuming**, so we need to know as soon as possible if we want to read the whole thing!



Reading the whole paper

Section and figure titles

Introduction and Discussion

Abstract and Title

How in depth are you reading?

So you've done your Google Scholar search and you found a paper that might be of interest to your project.

Reading a paper is time consuming, so we need to know as soon as possible if we want to read the whole thing!

Start by breaking down the abstract!

Reading the whole paper

Section and figure titles

Introduction and Discussion

Abstract and Title

Title and Abstract

Background

Motivation

Results/Conclusion

Thermal Control of Engineered T-cells

Mohamad H. Abedi, Justin Lee, Dan I. Piraner, and Mikhail G. Shapiro*

Cite This: *ACS Synth. Biol.* 2020, 9, 1941−1950

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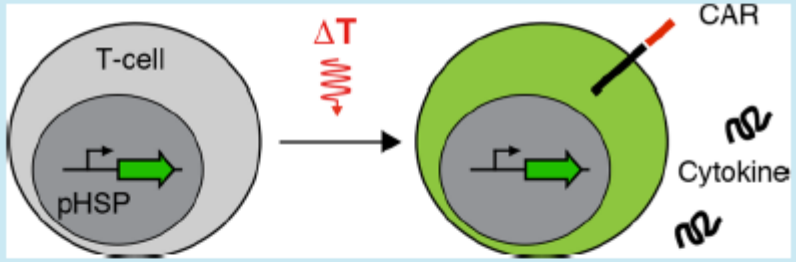
ABSTRACT: Genetically engineered T-cells are being developed to perform a variety of therapeutic functions. However, no robust mechanisms exist to externally control the activity of T-cells at specific locations within the body. Such spatiotemporal control could help mitigate potential off-target toxicity due to incomplete molecular specificity in applications such as T-cell immunotherapy against solid tumors. Temperature is a versatile external control signal that can be delivered to target tissues *in vivo* using techniques such as focused ultrasound and magnetic hyperthermia. Here, we test the ability of heat shock promoters to mediate thermal actuation of genetic circuits in primary human T-cells in the well-tolerated temperature range of 37−42 °C, and introduce genetic architectures enabling the tuning of the amplitude and duration of thermal activation. We demonstrate the use of these circuits to control the expression of chimeric antigen receptors and cytokines, and the killing of target tumor cells. This technology provides a critical tool to direct the activity of T-cells after they are deployed inside the body.



KEYWORDS: *T-cells, CAR, thermal control, mammalian synthetic biology, heat shock promoters, immunotherapy*

Abedi, M.H. *et al.* (2020) Thermal Control of Engineered T-cells. *ACS Synth. Biol.* 9, 1941–1950
doi: 10.1021/acssynbio.0c00238

Title and Abstract

What:

1. Tested heat shock promoters to control gene expression in primary human T-cells
2. Tuned amplitude and duration of expression using different circuit/genetic architecture
3. Expressed a chimeric antigen receptor (CAR) and cytokines from heat shock promoters to show usefulness

Why:

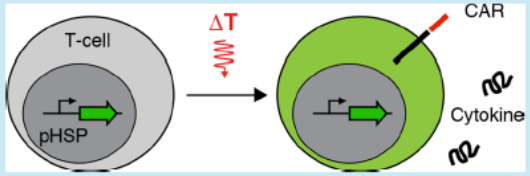
Need to externally control activity of T-cells

Thermal Control of Engineered T-cells

Mohamad H. Abedi, Justin Lee, Dan I. Piraner, and Mikhail G. Shapiro*

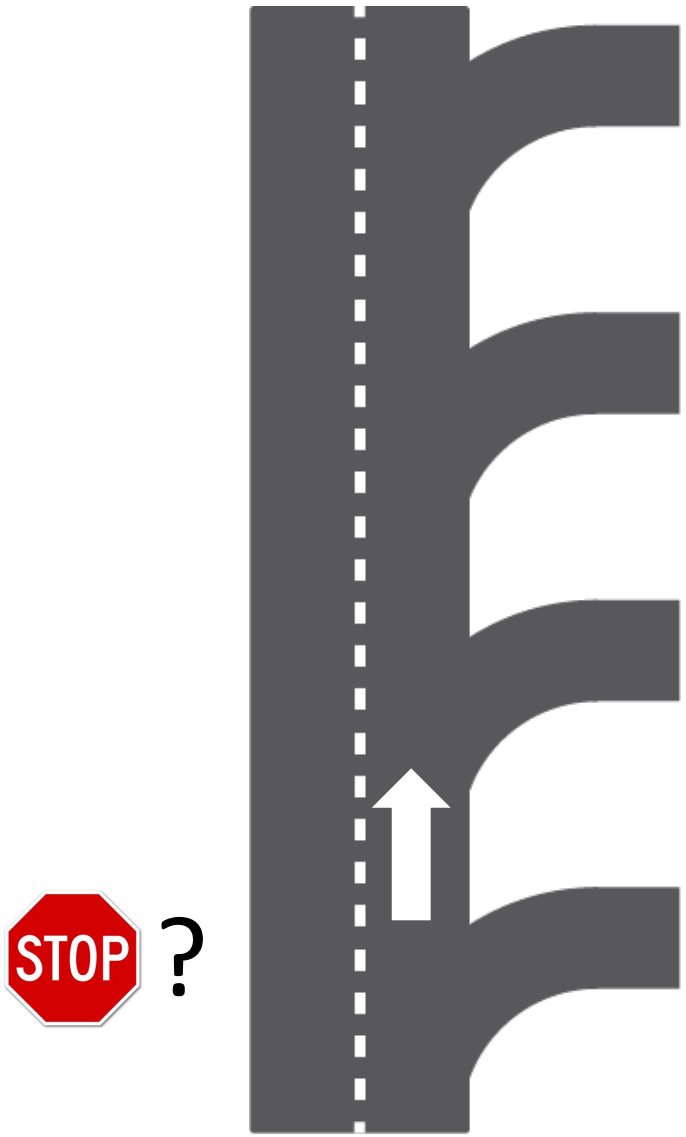
Cite This: *ACS Synth. Biol.* 2020, 9, 1941−1950 | Read Online

ABSTRACT: Genetically engineered T-cells are being developed to perform a variety of therapeutic functions. However, no robust mechanisms exist to externally control the activity of T-cells at specific locations within the body. Such spatiotemporal control could help mitigate potential off-target toxicity due to incomplete molecular specificity in applications such as T-cell immunotherapy against solid tumors. Temperature is a versatile external control signal that can be delivered to target tissues *in vivo* using techniques such as focused ultrasound and magnetic hyperthermia. Here, we test the ability of heat shock promoters to mediate thermal actuation of genetic circuits in primary human T-cells in the well-tolerated temperature range of 37−42 °C, and introduce genetic architectures enabling the tuning of the amplitude and duration of thermal activation. We demonstrate the use of these circuits to control the expression of chimeric antigen receptors and cytokines, and the killing of target tumor cells. This technology provides a critical tool to direct the activity of T-cells after they are deployed inside the body.



KEYWORDS: *T-cells, CAR, thermal control, mammalian synthetic biology, heat shock promoters, immunotherapy*

Still Interested?



Reading the whole paper

Section and figure titles

Introduction and Discussion

Abstract and Title

Introduction

Unlike small molecule and biologic therapies, cells have a natural ability to navigate, persist, and proliferate within the body, providing the potential for more targeted and sustained disease treatment. This potential is enhanced by the capacity of cells to probe, process, and respond to their environment and carry out a wide range of sophisticated behaviors, which can be engineered using the tools of synthetic biology.[1] Among the cell types being developed for therapy, T-cells are one of the most promising due to their central roles in cancer, infectious disease, and autoimmune disorders, along with their relative ease of isolation, genetic modification, and re-engraftment. For example, this potential has been realized in T-cells engineered to express modularly targeted chimeric antigen receptors (CARs), allowing them to specifically eradicate cancers such as lymphomas bearing the CD19 antigen.[2−5] Unfortunately, it has been challenging to translate these successful results into solid tumors, where CAR T-cells encounter a more immunosuppressive environment[6] and the risk of sometimes fatal on-target off-tumor toxicity due to the presence of tumor-overexpressed epitopes in healthy tissues.[7,8] Likewise, emerging approaches in which T-cells are used to treat autoimmune disease through local immunosuppression carry the risk of reducing important immune system activity outside the target tissues.[9] Existing strategies seeking to reduce off-target toxicity use additional target recognition elements[10,11] or chemically triggered kill switches.[12−14] However, it can be difficult to ensure perfect recognition solely through molecular markers, and premature termination of T-cell therapy using kill-switches turns off their beneficial therapeutic action.

Here we describe a cellular engineering approach to regulate the activity of therapeutic T-cells with greater specificity through a combination of molecular and physical actuation. This approach is designed to take advantage of the ability of technologies such as focused ultrasound (FUS) and magnetic hyperthermia to noninvasively deposit heat at precise locations in deep tissue.[15−18] By engineering thermal bioswitches that allow T-cells to sense small changes in temperature and use them as inputs for the actuation of genetic circuits, we enable these penetrant forms of energy to spatially control T-cell activity. Our approach is based on heat shock promoters (pHSP), which have been shown to drive gene expression in response to FUS-delivered heating,[19−21] but have not been tested in primary human T-cells. This is important because the behavior of pHSPs varies greatly between cell types and cellular states. In this study, we screen a library of pHSPs in primary T-cells and engineer gene circuits providing transient and sustained activation of gene expression in T-cells in response to brief thermal stimuli within the well-tolerated temperature range of 37−42 °C.[22−24] Our circuits incorporate feed-forward amplification, positive feedback, and recombinase-based state switches. We demonstrate the use of these circuits to control the secretion of a therapeutic cytokine, expression of a CAR, and killing of target tumor cells.

Received: May 1, 2020
Published: July 30, 2020

Introduction

What did the authors actually do?

1. Screened a library of heat shock promoters in primary human T-cells
2. Implemented circuits with feed-forward amplification, positive feedback, and recombinase-based switches to control duration of expression
3. Applied to control expression of a cytokine, CAR, and to kill target tumor cells

Unlike small molecule and biologic therapies, cells have a natural ability to navigate, persist, and proliferate within the body, providing the potential for more targeted and sustained disease treatment. This potential is enhanced by the capacity of cells to probe, process, and respond to their environment and carry out a wide range of sophisticated behaviors, which can be engineered using the tools of synthetic biology.[1] Among the cell types being developed for therapy, T-cells are one of the most promising due to their central roles in cancer, infectious disease, and autoimmune disorders, along with their relative ease of isolation, genetic modification, and re-engraftment. For example, this potential has been realized in T-cells engineered to express modularly targeted chimeric antigen receptors (CARs), allowing them to specifically eradicate cancers such as lymphomas bearing the CD19 antigen.[2−5] Unfortunately, it has been challenging to translate these successful results into solid tumors, where CAR T-cells encounter a more immunosuppressive environment[6] and the risk of sometimes fatal on-target off-tumor toxicity due to the presence of tumor-overexpressed epitopes in healthy tissues.[7,8] Likewise, emerging approaches in which T-cells are used to treat autoimmune disease through local immunosuppression carry the risk of reducing important immune system activity outside the target tissues.[9] Existing strategies seeking to reduce off-target toxicity use additional target recognition elements[10,11] or chemically triggered kill switches.[12−14] However, it can be difficult to ensure perfect recognition solely through molecular markers, and premature termination of T-cell therapy using kill-switches turns off their beneficial therapeutic action.

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Discussion

DISCUSSION

Our results demonstrate that engineered bioswitch circuits using pHSP can provide control of T-cell therapy with mild hyperthermia. While it has been previously shown that light-switchable proteins could also confer spatiotemporal control over T-cell activity,[34] light has poor penetration into tissues, limiting the utility of such tools. On the other hand, temperature can be elevated at arbitrary depth and with high spatial precision using noninvasive methods such as FUS or magnetic hyperthermia.[15−18]

Our study showed that temperatures in the well-tolerated range of 37−42 °C[35,36] can provide control over T-cell function, including the synthesis and release of a cytokine and the CAR-mediated killing of cancer cells *in vitro*, with minimal baseline activity. In future studies, this performance must be characterized and optimized in the *in vivo* setting. In particular, it will be useful to optimize the thermal requirements of ultrasound activation. While thermal tissue damage is not a major concern in tumor therapy (where it can be synergistic), damage to healthy tissues in nontumor applications could be detrimental.[35,36] It would also be desirable to shorten the FUS treatment duration to substantially less than the 1 h heat pulse used in this study. Further promoter engineering, protein engineering, and thermal pulse optimization could broaden the range of applications for this technology.

Despite their name, pHSPs can respond to a variety of stimuli such as heat, hypoxia, heavy metals, cytokines, and cell division.[37,38] Therefore, the context in which these promoters are being used must be carefully considered. In this work, we capitalized on nonthermal pHSP induction by the T-cell receptor pathway to generate sustained killing circuits. In other contexts where the promiscuous responsiveness of pHSPs presents an unexploitable hindrance, it may be desirable to develop thermal response mechanisms based on orthogonal molecular bioswitches.[22,23]

Discussion

Original Claim:

- Heat shock promoters successfully control gene expression with **minimal baseline activity**

Future Studies:

- Optimize *in vivo* (therefore the study only did *in vitro* experiments)
- Optimize using the translational technology for heating

Limitation of Data:

- Heat shock promoters are not specifically activated by elevated temperature
  - They utilized this promiscuous behavior (it's a feature not a bug), but it could hinder other applications

DISCUSSION

Our results demonstrate that engineered bioswitch circuits using pHSP can provide control of T-cell therapy with mild hyperthermia. While it has been previously shown that light-switchable proteins could also confer spatiotemporal control over T-cell activity,[34] light has poor penetration into tissues, limiting the utility of such tools. On the other hand, temperature can be elevated at arbitrary depth and with high spatial precision using noninvasive methods such as FUS or magnetic hyperthermia.[15−18]

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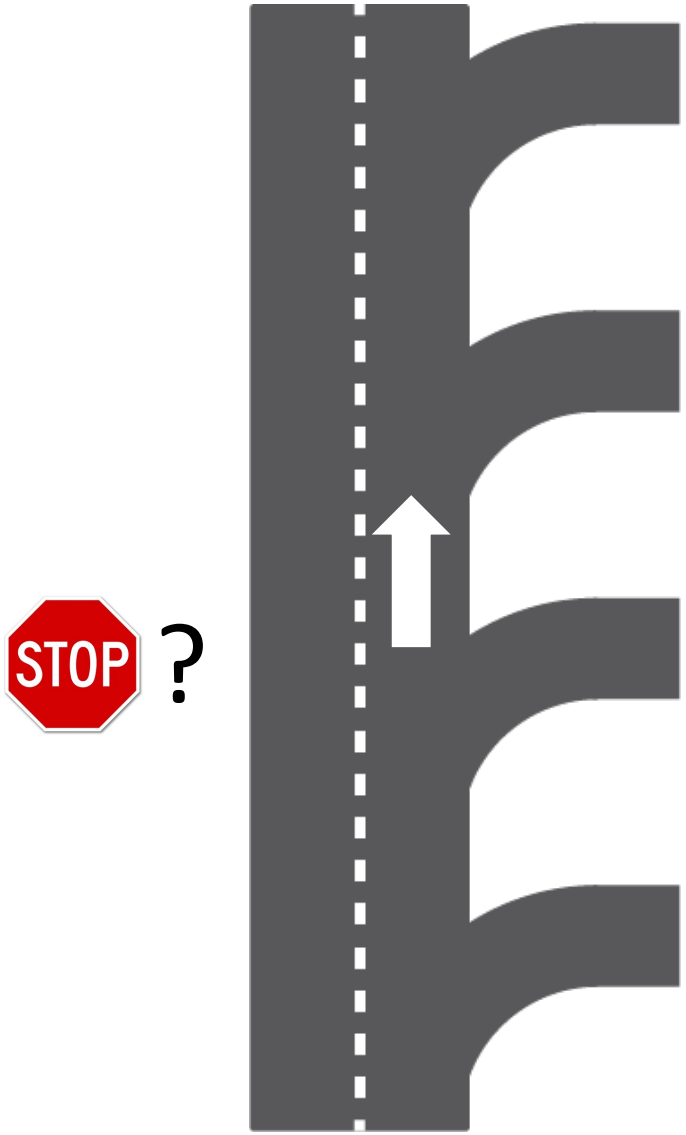


Reading the Whole Paper

Section and figure titles

Introduction and Discussion

Abstract and Title

Section and Figure Titles

1. Evaluating Candidate pHSPs in Primary Cells
2. Thermal Parameters for pHSP Activation
3. Genetic Circuits for Amplified and Sustained Thermal Activation
4. Temperature-Activated Cytokine Release
5. Dependence of pHSP-Drive Circuits on T-Cell Activation
6. Autosustained Thermally Induced CAR Expression and Tumor Cell Killing

Section and Figure Titles

Claim 1: Screened a library of heat shock promoters in primary human T-cells

1. Evaluating Candidate pHSPs in Primary Cells
2. Thermal Parameters for pHSP Activation

Claim 2: Implemented circuits with feed-forward amplification, positive feedback, and recombinase-based switches to control duration of expression

3. Genetic Circuits for Amplified and Sustained Thermal Activation

Claim 3: Applied genetic circuits to control expression of a cytokine, CAR, and to kill target tumor cells

4. Temperature-Activated Cytokine Release
5. Dependence of pHSP-Driven Circuits on T-Cell Activation
6. Autosustained Thermally Induced CAR Expression and Tumor Cell Killing

Experimental design figures

Figure 1a: Evaluating Candidate pHSPs in Primary Cells

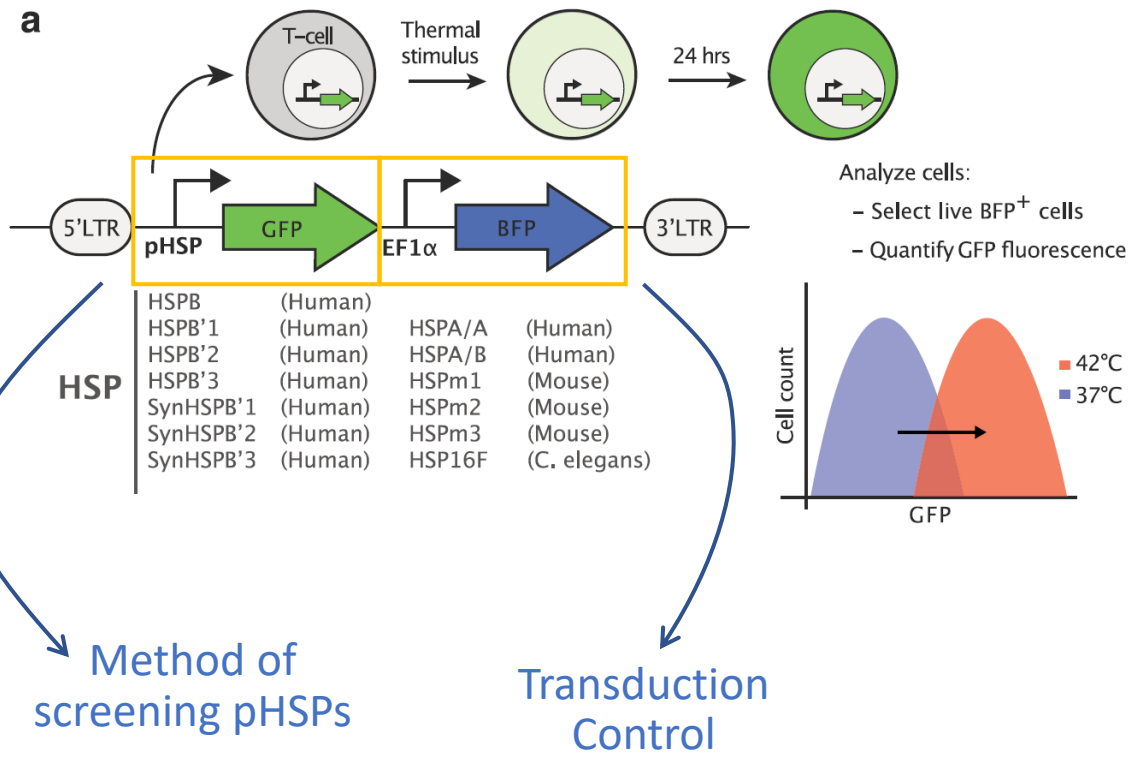
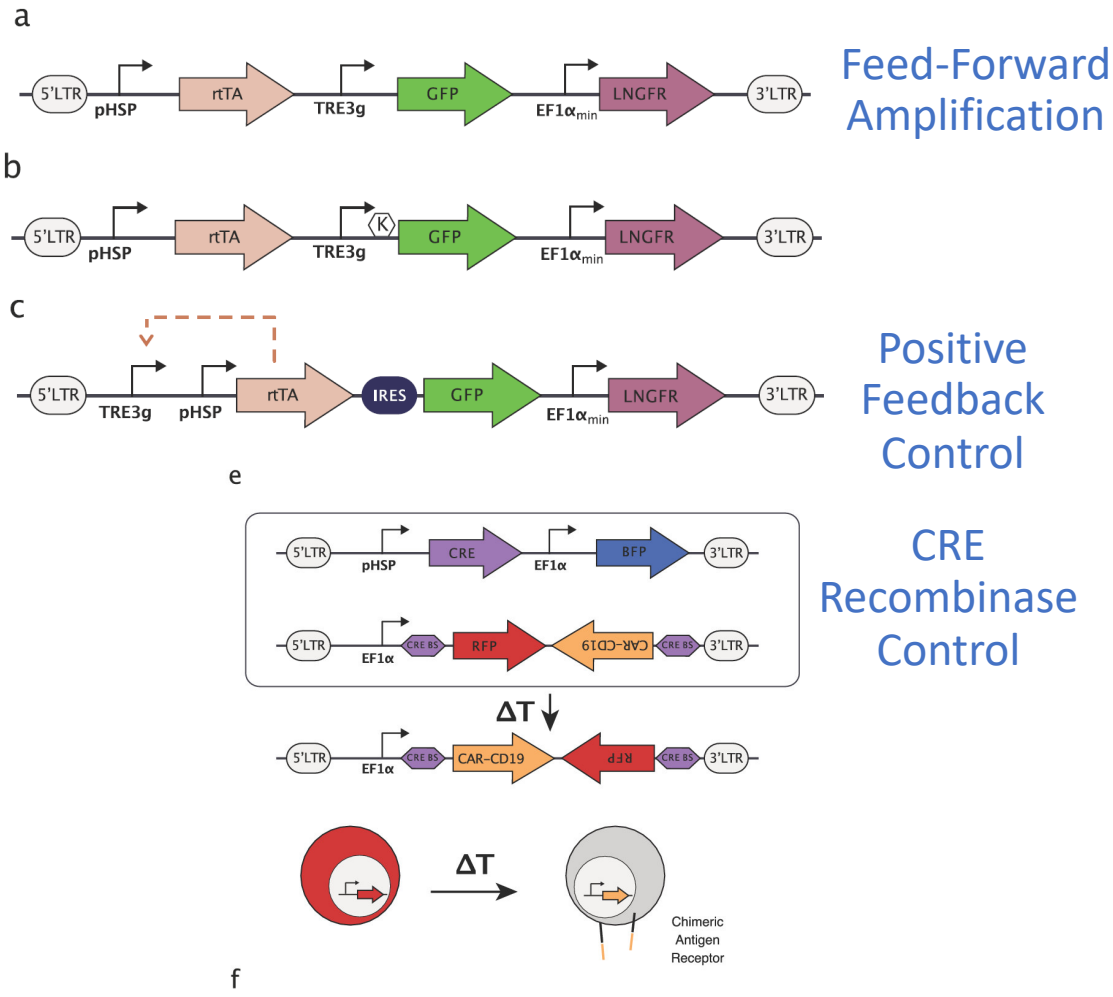


Figure 3: Genetic Circuits for Amplified and Sustained Thermal Activation



Experimental design figures

Figure 4: Temperature-Activated Cytokine Release

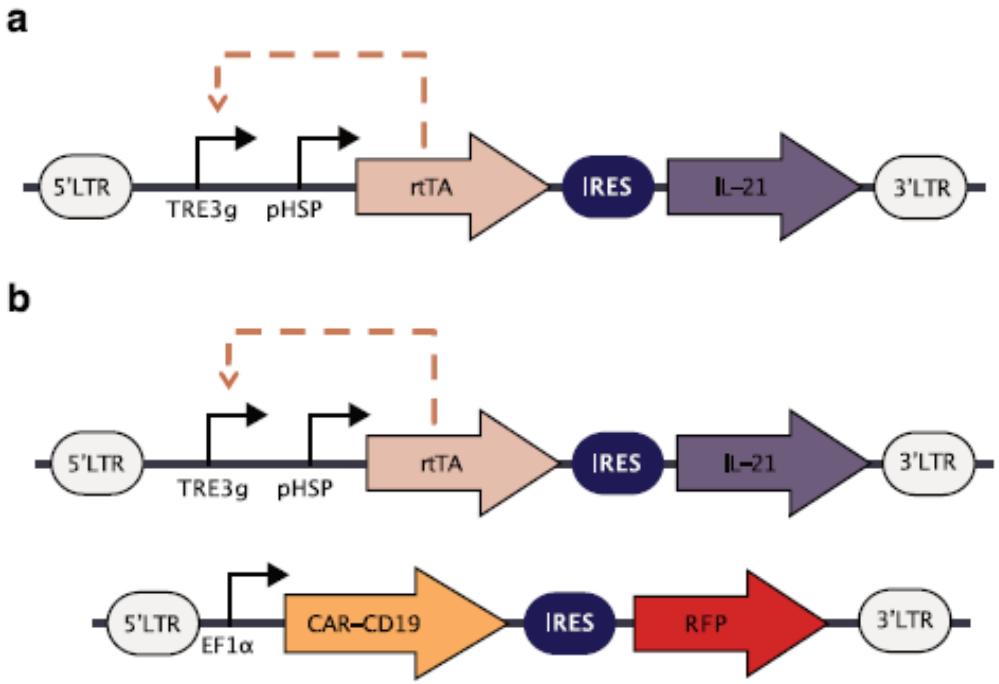
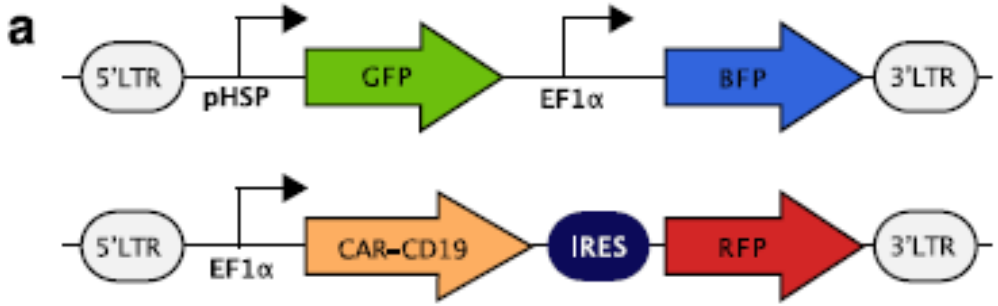
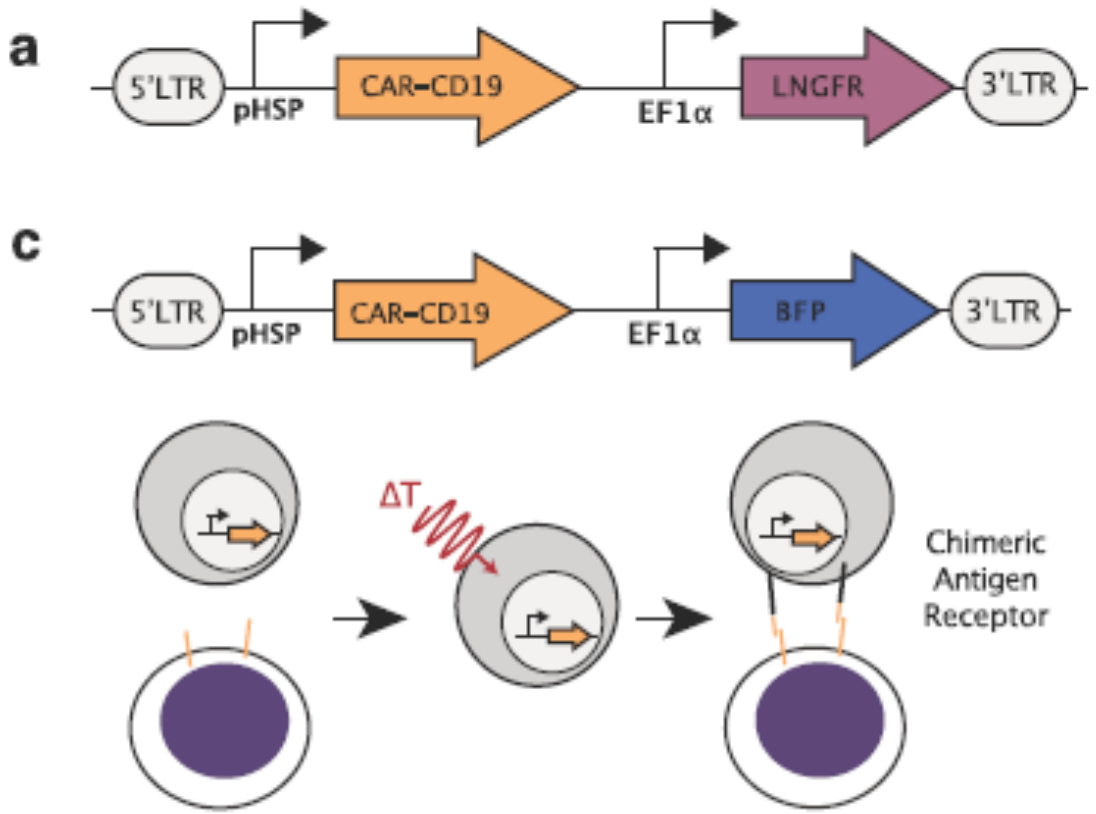


Figure 5: Dependence of pHSP-driven circuits on T-cell activation



Experimental design figures

Figure 6: Autosustained Thermally Induced CAR Expression and Tumor Cell Killing



Finally…

?

Reading the whole paper

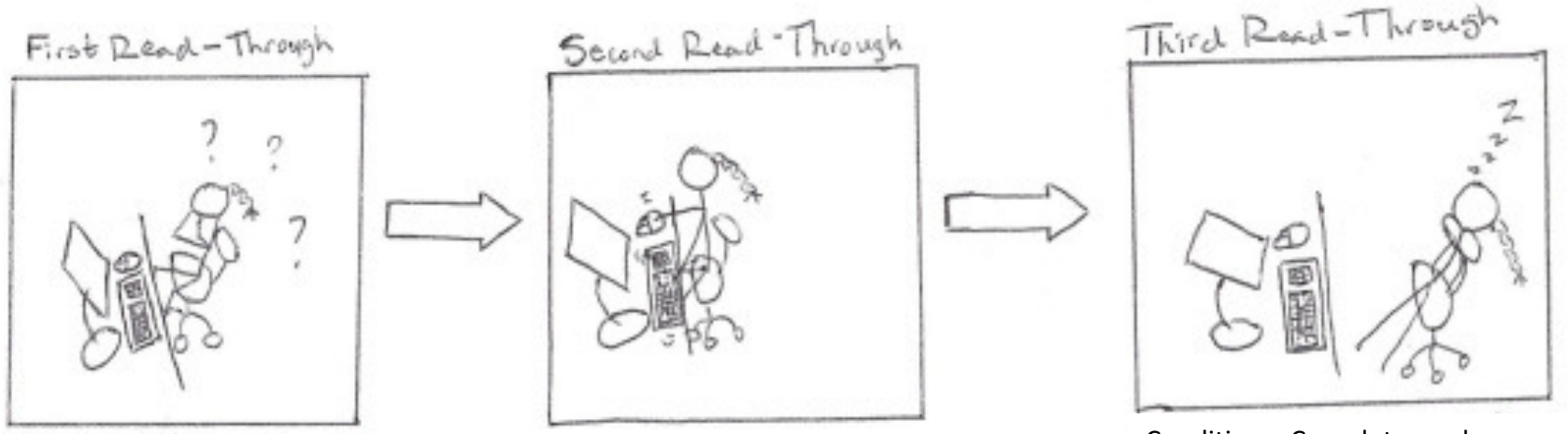
Section and figure titles

Introduction and Discussion

Abstract and Title

Reading the paper: Critically examining data and claims

- Finally read through each section
- Review the data
- Examine the experimental methods
- Do the results back up the article's claims?



Conditions: 4 windows, each with 10+ tabs open, to define words, explain methods, and check citations.

Conditions: Furiously cross-checking results and conclusions to verify the article's claims.

Conditions: Complete read-through to make sure nothing was missed. Hard work! Time for a nap.

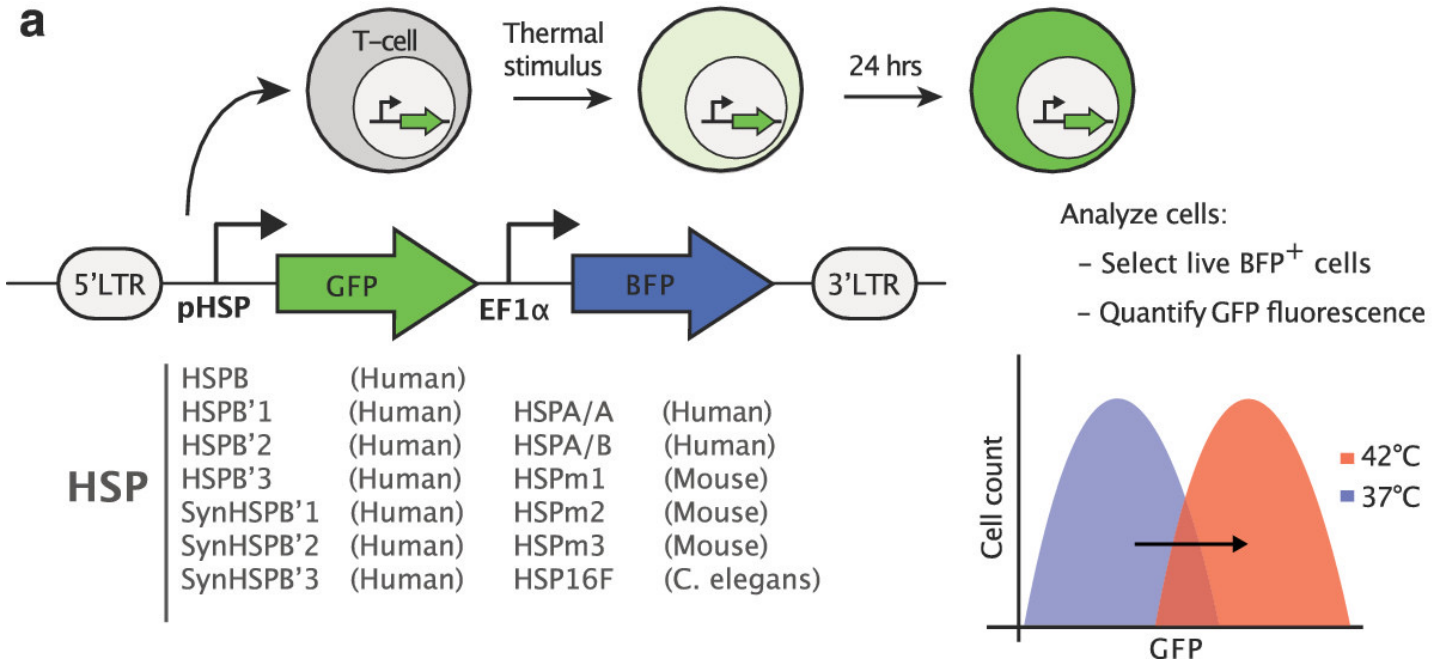
https://twowomensci.wordpress.com/2018/02/16/how-to-read-a-scientific-paper-part-3/

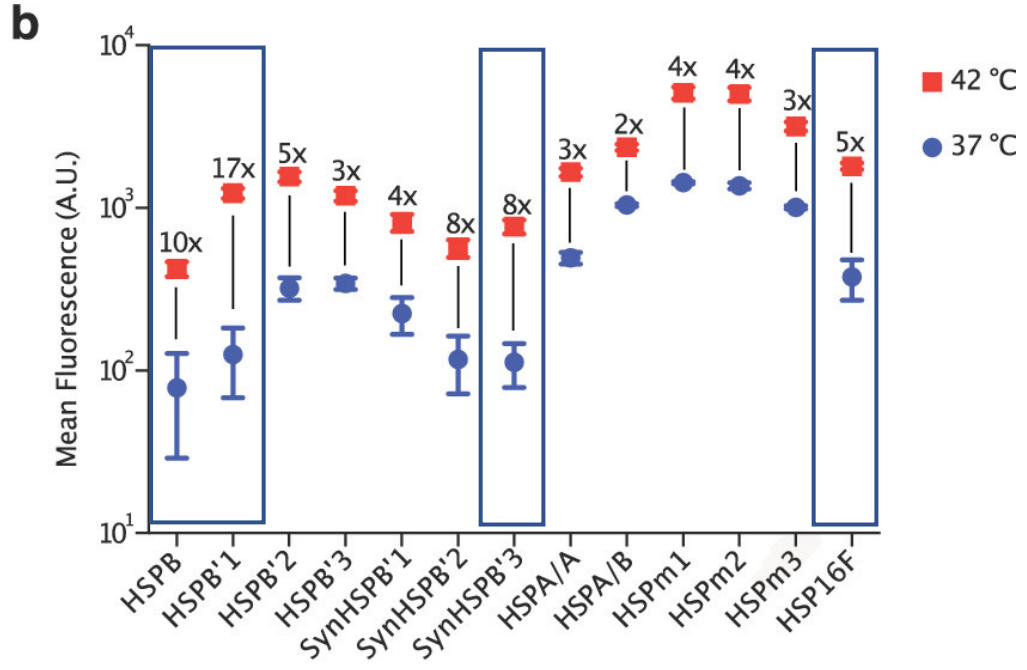
Section: Evaluating Candidate pHSPs in Primary Cells

Claim 1: Screened a library of heat shock promoters in primary human T-cells

Section Objective: "To enable thermal control of T-cell activity, we required a pHSP with robust switching behavior in primary human T-cells."

Method: "…, we decided to systematically evaluate the activity of 13 different pHSPs in response to a 1-h incubation at 42 °C."





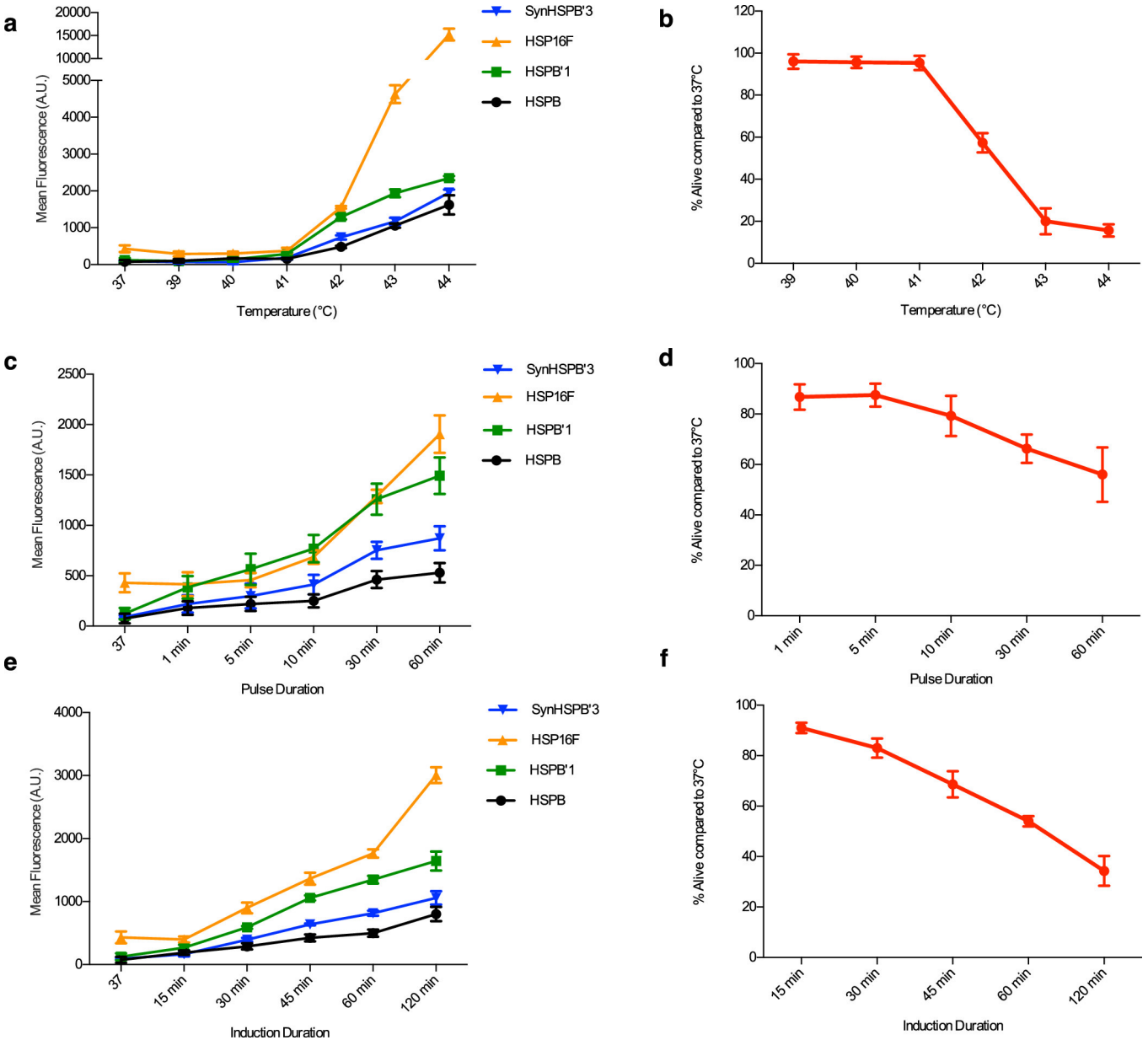
Section: Thermal Parameters for pHSP Activation

Claim 1: Screened a library of heat shock promoters in primary human T-cells

Section Objective: "To search for temperatures that provide rapid induction with minimal thermal burden to the cells,…"

Method:

1. "…, we incubated pHSP-transduced T-cells at temperatures ranging from 37 to 44 °C for 1 h."
2. "To reduce the effect of thermal exposure on cell viability, we tested a pulsatile heating scheme with a 50% duty cycle"
3. "We also investigated continuous stimulation durations ranging from 15 to 120 min."



Did they support their claim?

Claim 1: Screened a library of heat shock promoters in primary human T-cells

1. Evaluating Candidate pHSPs in Primary Cells
2. Thermal Parameters for pHSP Activation

Claim 2: Implemented circuits with feed-forward amplification, positive feedback, and recombinase-based switches to control duration of expression

3. Genetic Circuits for Amplified and Sustained Thermal Activation

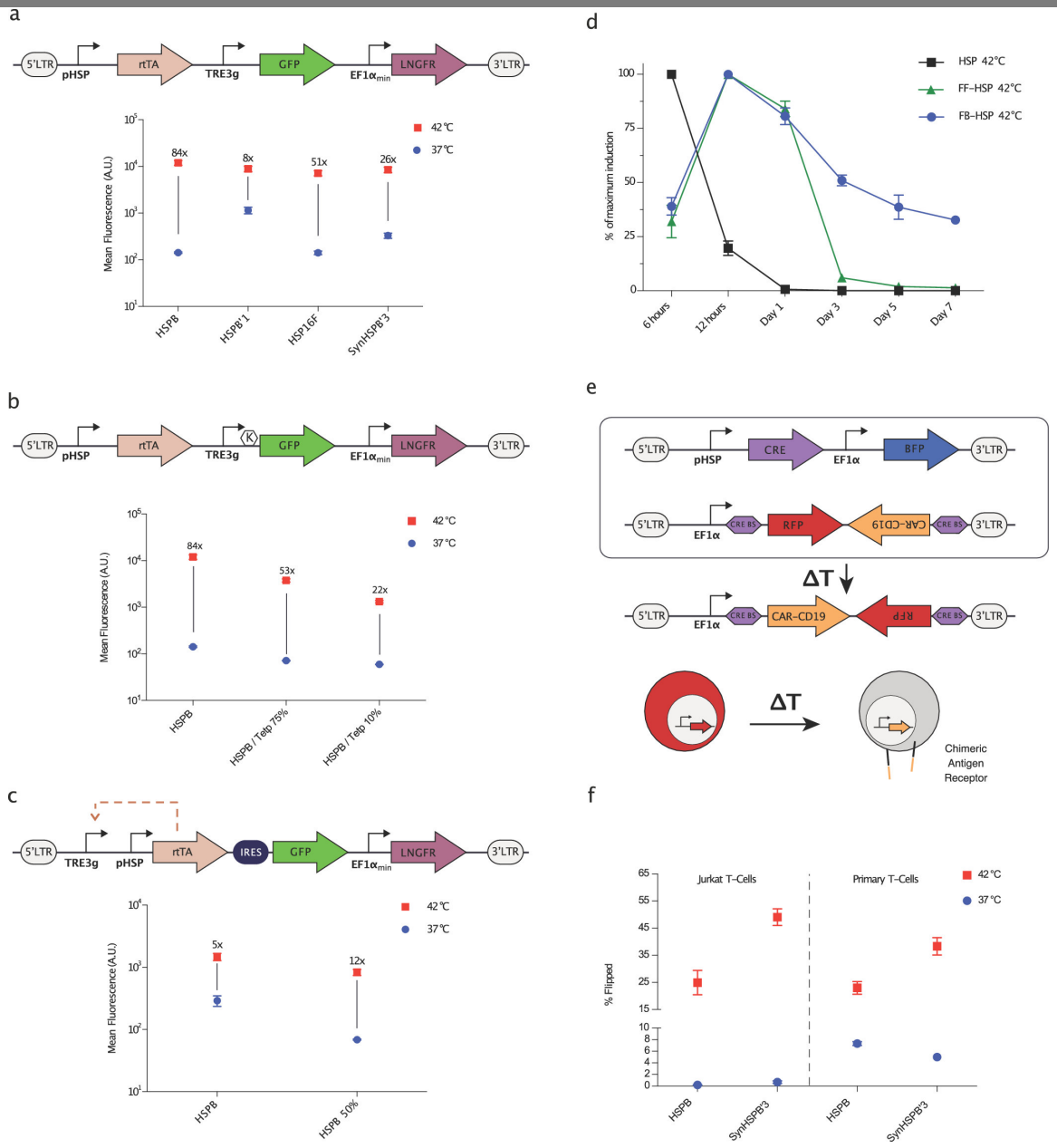
Claim 3: Applied to control expression of a cytokine, CAR, and to kill target tumor cells

4. Temperature-Activated Cytokine Release
5. Dependence of pHSP-Driven Circuits on T-Cell Activation
6. Autosustained Thermally Induced CAR Expression and Tumor Cell Killing

Section: Genetic Circuits for Amplified and Sustained Thermal Activation

Claim 2: Implemented circuits with feed-forward amplification, positive feedback, and recombinase-based switches to control duration of expression

Section Objective: "To enable the use of pHSPs in T-cell therapy applications, it is useful to amplify the output of pHSP-driven circuits. "



Section: Genetic Circuits for Amplified and Sustained Thermal Activation

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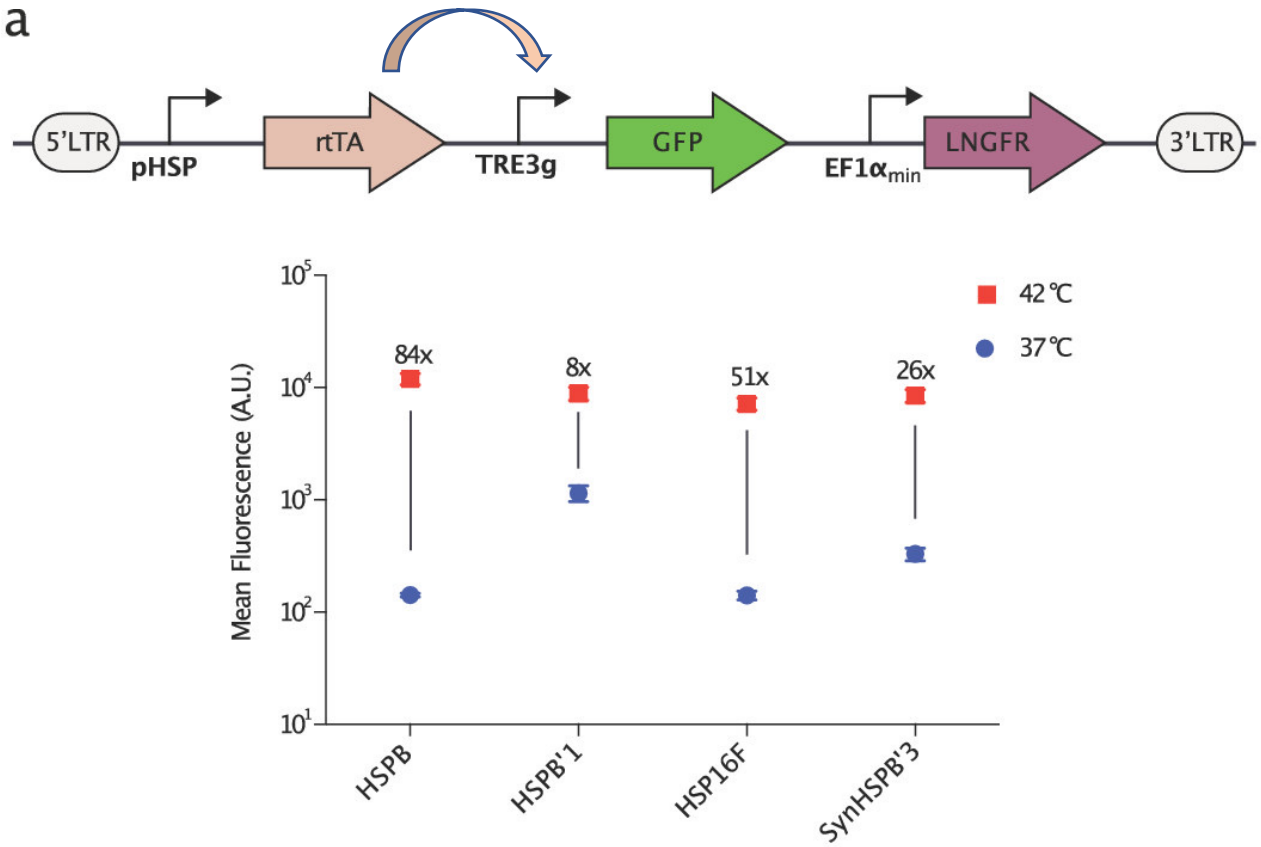
Section Objective: "To enable the use of pHSPs in T-cell therapy applications, it is useful to amplify the output of pHSP-driven circuits. "

Method:

1. "We implemented a feed-forward amplification circuit in which the pHSP drives an rtTA transactivator, which produces stronger transcriptional activation tunable with doxycycline."



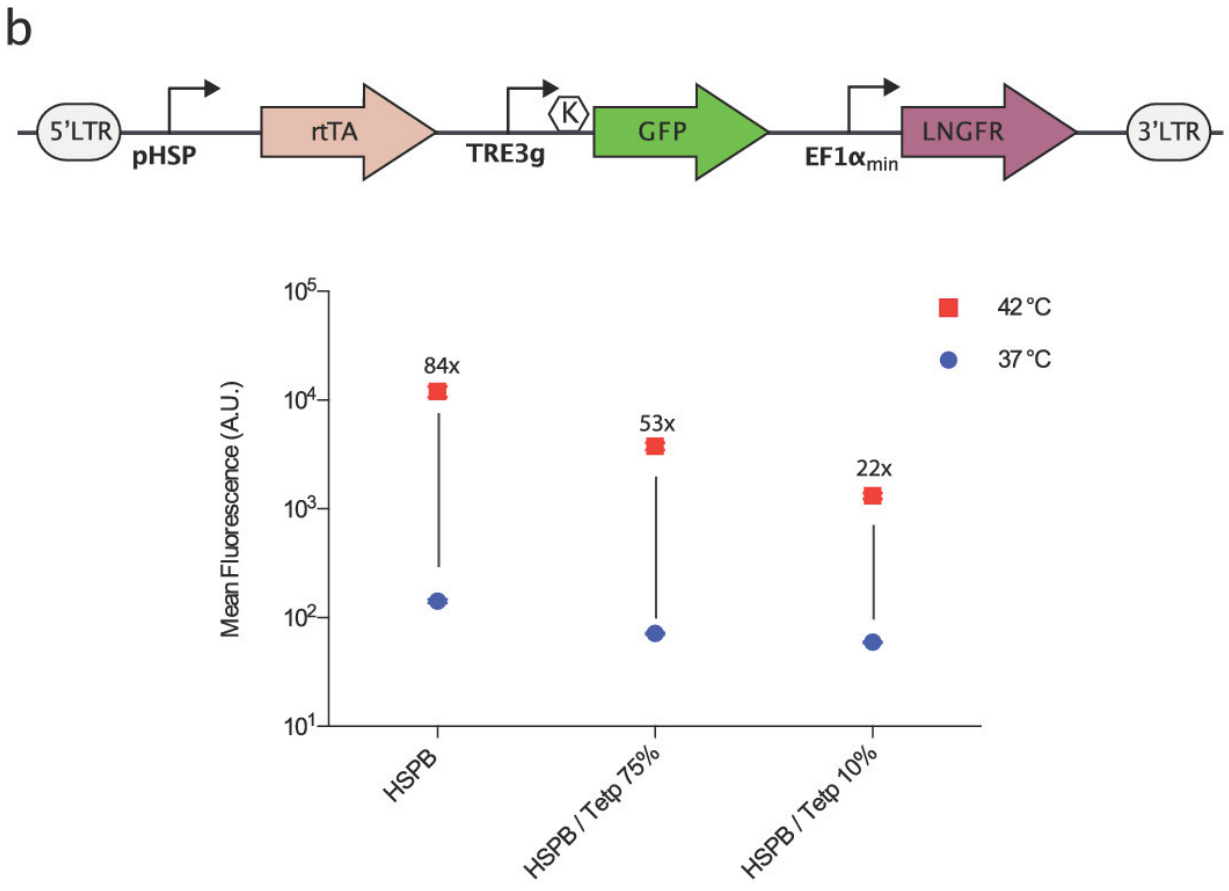
Section: Genetic Circuits for Amplified and Sustained Thermal Activation

Claim 2: Implemented circuits with feed-forward amplification, positive feedback, and recombinase-based switches to control duration of expression

Section Objective: "To enable the use of pHSPs in T-cell therapy applications, it is useful to amplify the output of pHSP-driven circuits. "

Method:

2. "To further tune the performance of the HSPB amplifier circuit, we designed constructs with reduced translation of the GFP by varying the Kozak sequence or inserting a micro-open reading frame upstream"



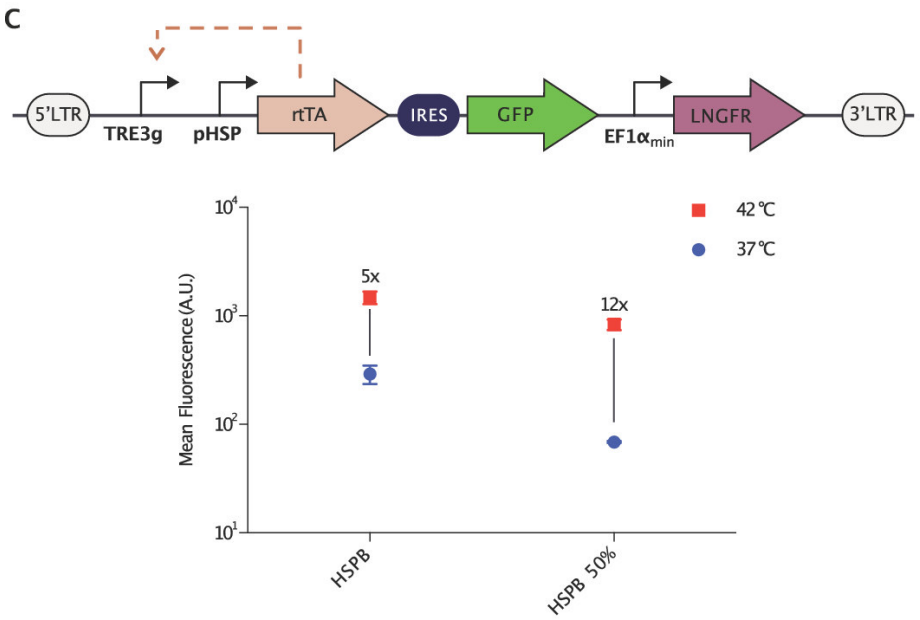
Section: Genetic Circuits for Amplified and Sustained Thermal Activation

Claim 2: Implemented circuits with feed-forward amplification, positive feedback, and recombinase-based switches to control duration of expression

Section Objective: "In some therapeutic scenarios, it is critical to prolong the therapeutic action of T-cells following thermal induction."

Method:

3. "We established a positive feedback amplifier circuit by rearranging the elements of our feed-forward amplifier such that rtTA could drive its own expression in the presence of doxycycline"



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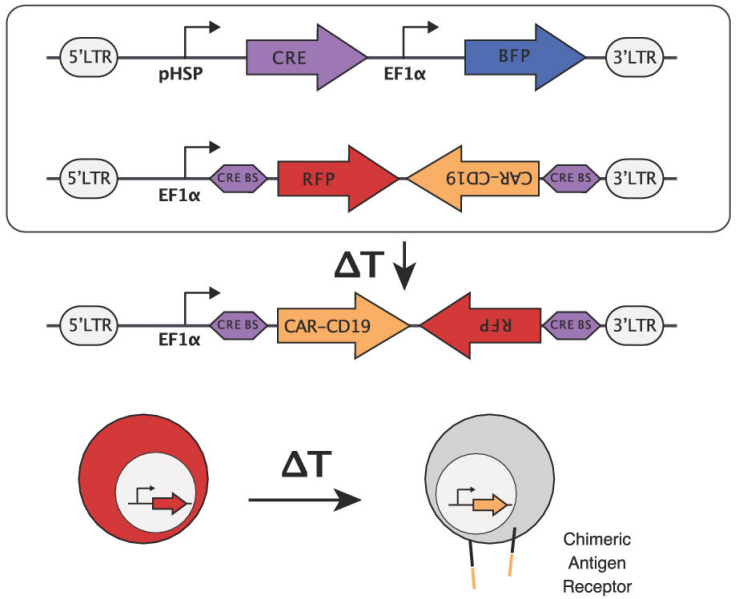
Claim 2: Implemented circuits with feed-forward amplification, positive feedback, and recombinase-based switches to control duration of expression

Section Objective: "In some therapeutic scenarios, it is critical to prolong the therapeutic action of T-cells following thermal induction."

Method:

4. "To establish a permanent thermal switch, we tested gene circuits in which we placed the expression of CRE recombinase under the control of candidate pHSPs"

e



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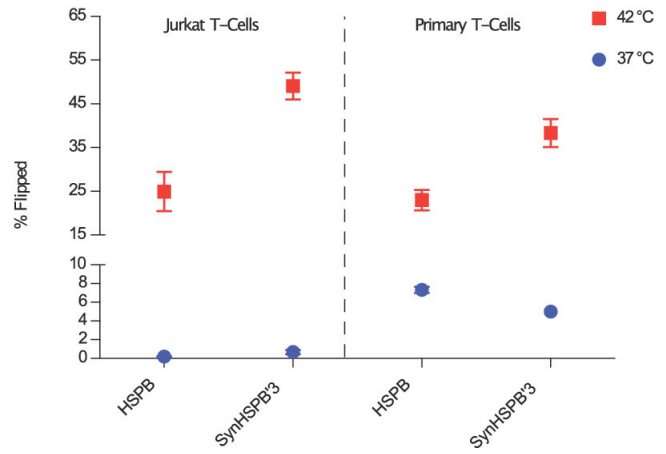


Did they support their claim?

Claim 1: Screened a library of heat shock promoters in primary human T-cells

1. Evaluating Candidate pHSPs in Primary Cells
2. Thermal Parameters for pHSP Activation

Claim 2: Implemented circuits with feed-forward amplification, positive feedback, and recombinase-based switches to control duration of expression

3. Genetic Circuits for Amplified and Sustained Thermal Activation

Claim 3: Applied to control expression of a cytokine, CAR, and to kill target tumor cells

4. Temperature-Activated Cytokine Release
5. Dependence of pHSP-Driven Circuits on T-Cell Activation
6. Autosustained Thermally Induced CAR Expression and Tumor Cell Killing

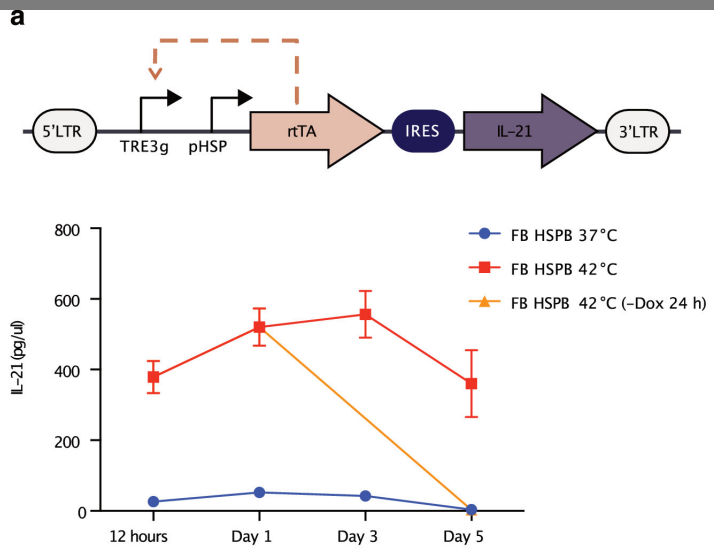
Section: Temperature-Activated Cytokine Release

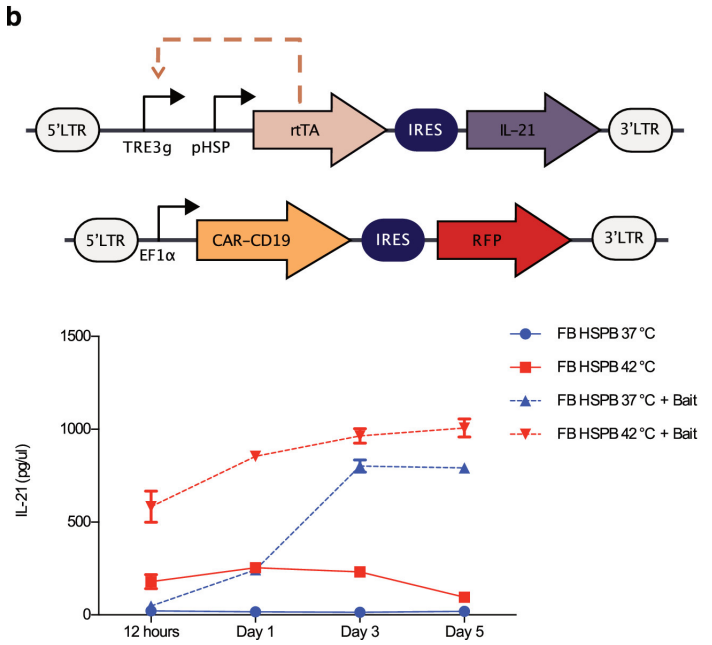
Claim 3: Applied to control expression of a cytokine, CAR, and to kill target tumor cells

Section Objective: "To demonstrate the ability of our positive feedback circuit to sustain a therapeutically relevant function after thermal induction, we connected its output to the production of a cytokine"

Method:

1. We incorporated human IL-21 in place of GFP in our positive feedback circuit. Without thermal induction, primary T-cells transduced with this circuit produced minimal IL-21.
2. In some scenarios, it would be useful for cytokine release to be triggered from a T-cell constitutively expressing a CAR, allowing the cytokine to locally boost immune activation during CAR-directed killing. To test this possibility, we cotransduced primary T-cells with our positive IL-21 circuit and a constitutively expressed anti-CD19 CAR.





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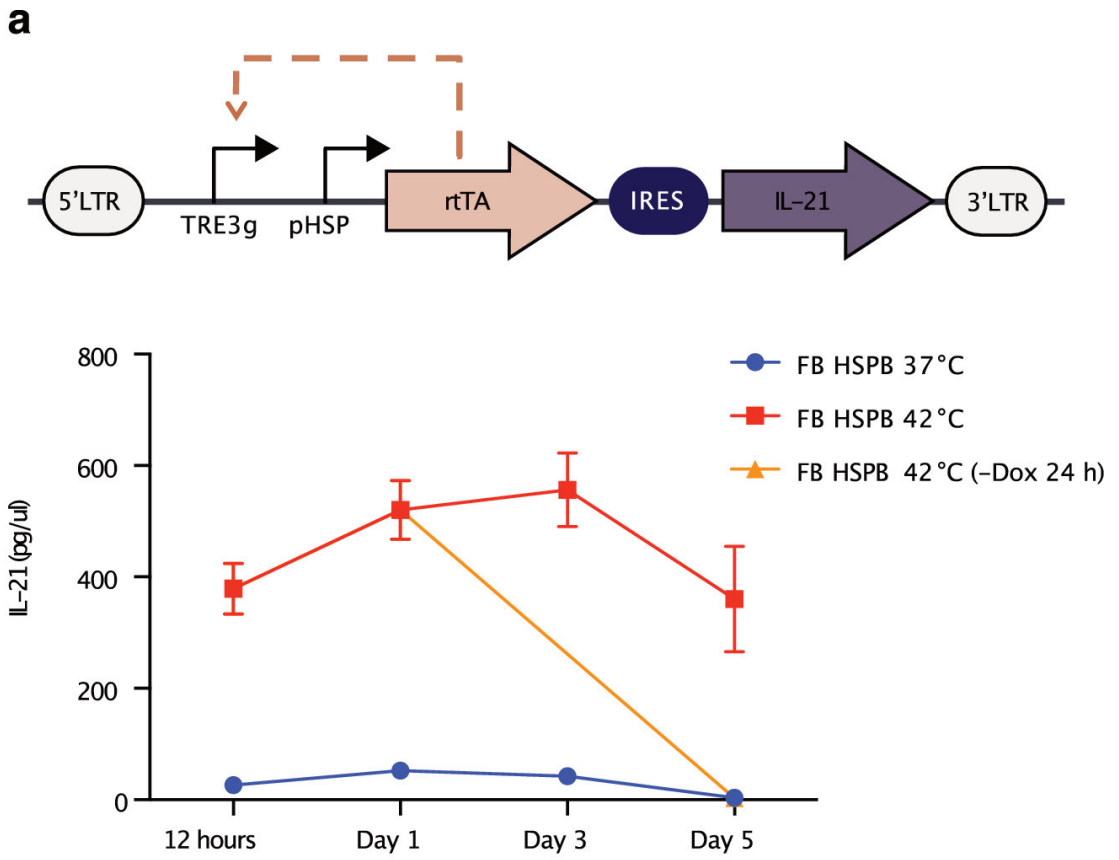

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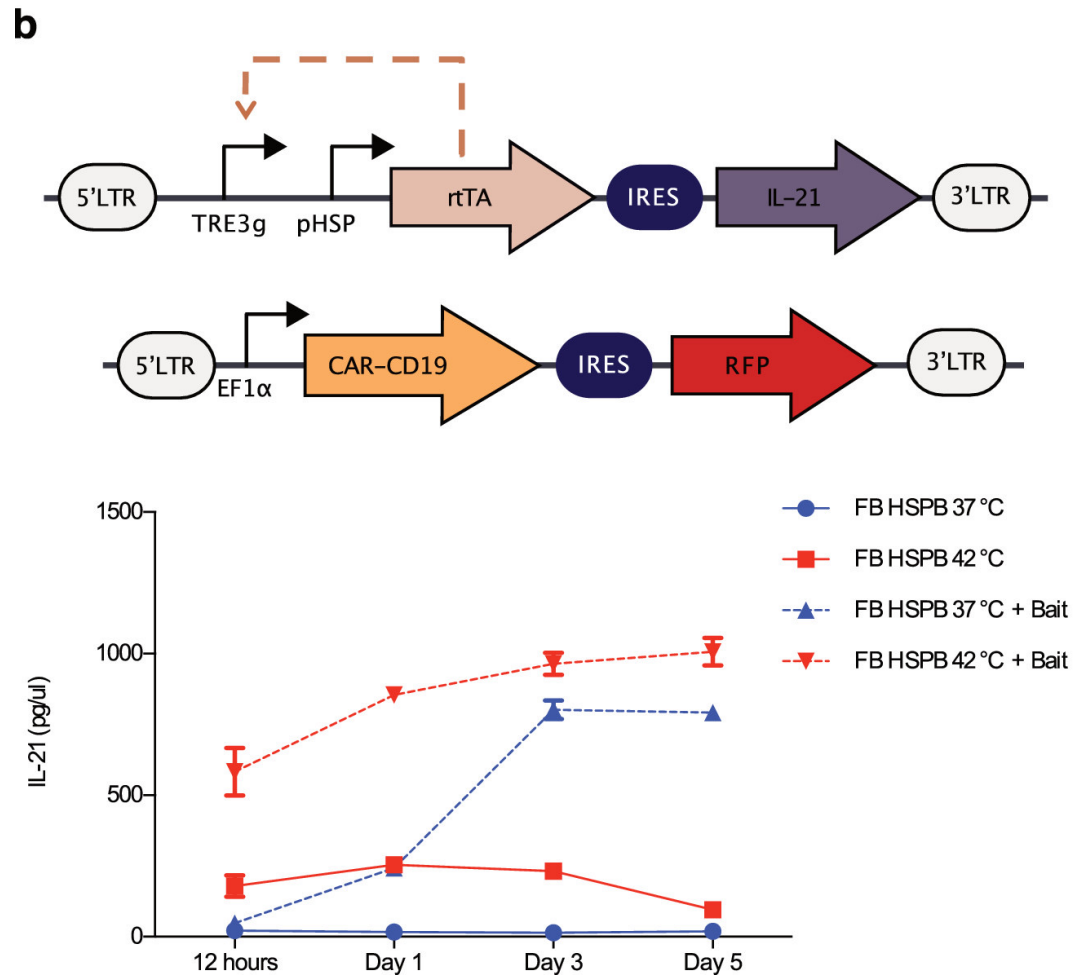
2. In some scenarios, it would be useful for cytokine release to be triggered from a T-cell **constitutively** expressing a CAR, allowing the cytokine to locally boost immune activation during CAR-directed killing. To test this possibility, we co-transduced primary T-cells with our positive IL-21 circuit and a constitutively expressed anti-CD19 CAR.



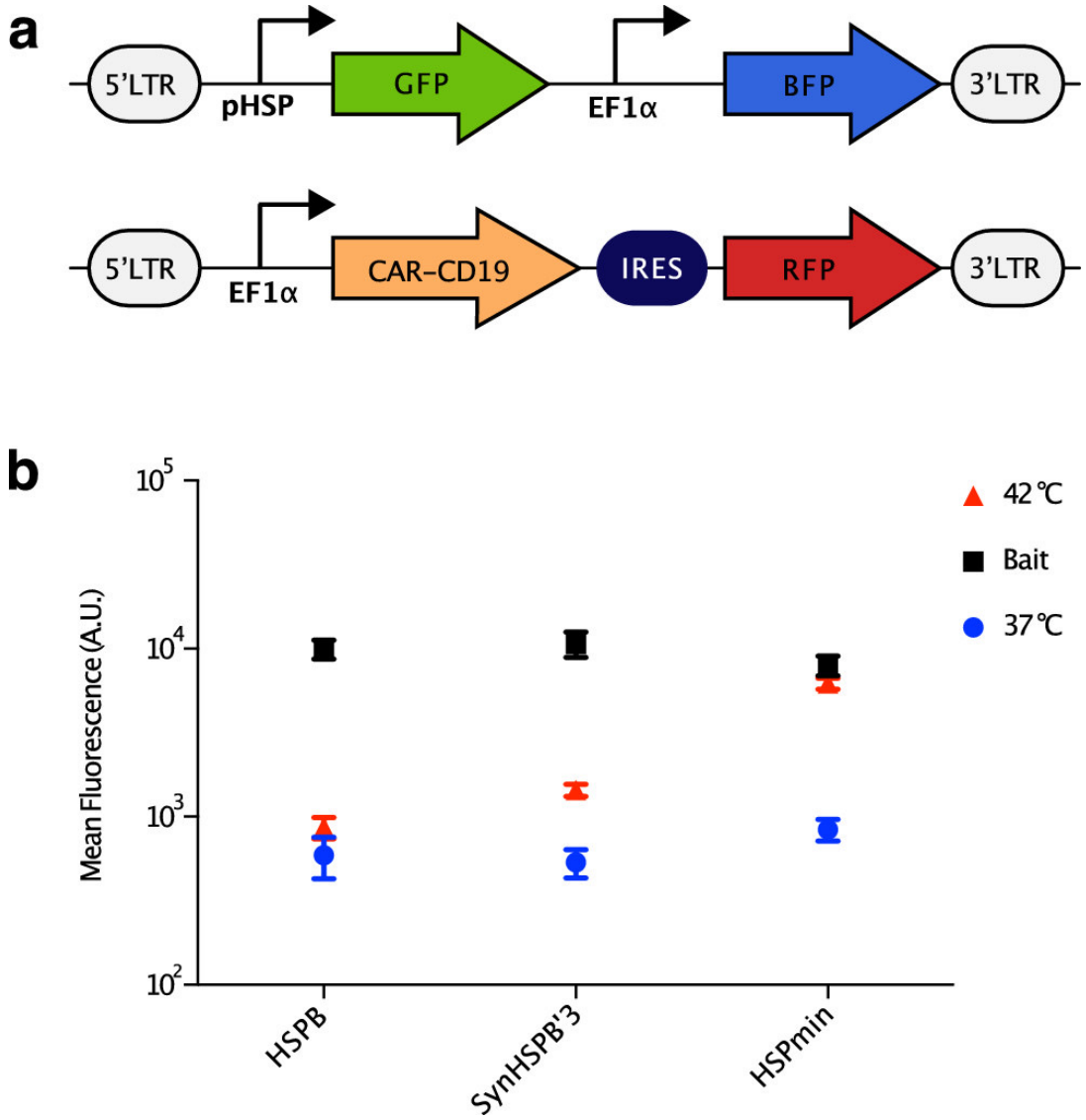
Section: Dependence of pHSP-Drive Circuits on T-Cell Activation

Claim 3: Applied genetic circuits to control expression of a cytokine, CAR, and to kill target tumor cells

Section Objective: **"**To directly examine the possibility that pHSPs are turned on in response to CAR-driven T-cell activation, … "

Method:

"…we tested the expression of pHSP-driven GFP in constitutively CAR-expressing T-cells upon exposure to a thermal stimulus *or* bait cells. "



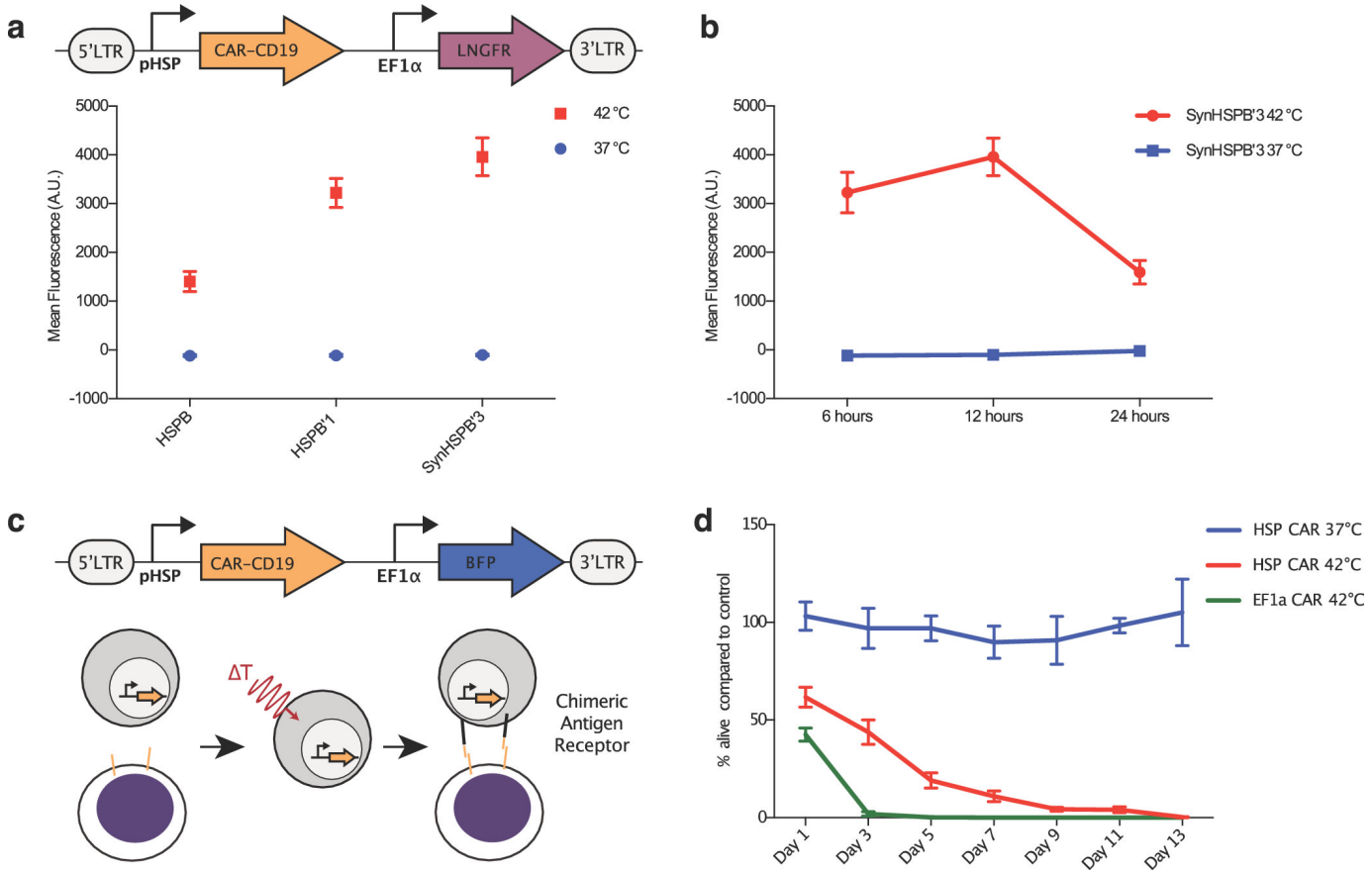
Section: Autosustained Thermally Induced CAR Expression and Tumor Cell Killing

Claim 3: Applied genetic circuits to control expression of a cytokine, CAR, and to kill target tumor cells

Section Objective: "We hypothesized that placing CAR expression under the control of a pHSP would result in T-cells with no initial CAR expression or activity, even in the presence of target cells. Upon thermal induction, CAR would become transiently expressed. If the CAR target is present in the vicinity of T-cells, these cells would become activated, driving sustained expression of additional CAR from the pHSP and target cell killing."

Method:

- Incubated T-cells with bait cells under 37 C or 42 C conditions (Fig 6C/D)



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Summarize for Later

Summary:

Highlight:

Weakness:

Open Questions:

Summarize for Later

Summary: Heat shock promoters were screened and chosen that exhibit low baseline activity and high-fold induction. Incorporation of heat shock promoters into circuits increases the magnitude and/or duration of expression. Heat shock promoters are also activated by T-cell activation, and are utilized to create autosustaining activation circuits for killing bait cells.

Highlight: Heat shock promoters in T-cells exhibit 2-17 fold induction, and are also activated by T-cell activation.

Weakness: All T-cell studies were done only with bait cells, not directly showing off-target effects. Also, the method of heating for translational therapies that was specifically mentioned (focused ultrasound) was not used.

Open Questions: How might these circuits function *in vivo*? How can we better probe off target effects? Can the auto-sustained circuit be externally stopped as the dox-inducible feedback/forward loops can?

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Words of Wisdom

- Reading scientific papers is a skill that requires **practice** and **patience**
- **It gets easier** to read papers over time!
- **Google** is your friend
- Make **vocab** lists
- Don’t worry if you don’t understand every single thing right away
- Do what works best for **you**!

The task:
- [ ] Read paper A

EXPECTATION:
- [x] ~~Read paper A~~

REALITY:
- [x] ~~Read paper A~~
- [ ] Read paper B mentioned in paper A
- [ ] Read paper C mentioned in paper A
- [ ] Read paper D mentioned in paper A
- [ ] Read paper E mentioned in paper A
- [ ] Read paper F mentioned in paper A

Side Note: Where to manage citations?

- **Zotero:** Zotero is an excellent free, open-source citation manager. After downloading Zotero from https://www.zotero.org/, it should prompt you to install the Zotero Connector, a browser plugin that lets you download paper citations with one-click. If not, download the connector here. We also have a shared Zotero group, to accumulate citations when writing manuscripts.

  Several helpful plugins can be downloaded; the recommended ones are:

Recommended Zotero plugins

Addon name	Description
ZotFile	Enables useful file operations, such as extracting annotations from a marked-up PDF, transferring new papers to a tablet for annotation, and auto-file renaming.
Zutilo	Enables helpful tagging operations, such as the ability to copy/paste tags or easily add paper relationships.
Better Bibtex	If you plan to use LaTeX, install this plugin before exporting to BibTeX. This addon makes nice-looking, stable citation keys that do not change on export.

Downloading Zotero plugins through Firefox

Since Zotero is built on modified Firefox, Zotero plugins appear similar to Firefox plugins. If downloading these plugins through Firefox, you will need to explicitly right click->download target; left-clicking on download links will attempt to install the Zotero plugin as a Firefox plugin, which will fail.

See https://gallowaylabmit.github.io/protocols/en/latest/bootcamp/iap/day_0_setup.html

Next time…

How to review a paper

Reading the whole paper

Section and figure titles

Introduction and Discussion

Abstract and Title