Supplemental Information

Remote control of mammalian cells with heat-triggered gene switches and photothermal pulse trains

Ian C. Miller[1], Marielena Gamboa Castro[1], Joe Maenza[1], Jason P. Weis[1], & Gabriel A. Kwong[1–5,*]

[1] *The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology & Emory University, Atlanta, GA 30332, USA*

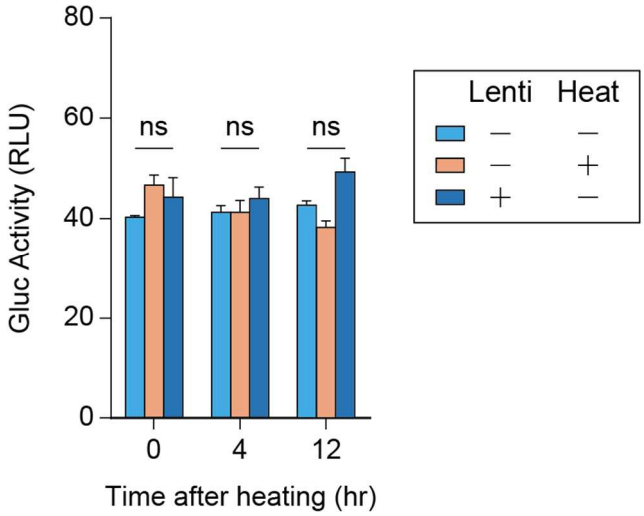
[2] *Institute for Electronics and Nanotechnology, Georgia Institute of Technology, Atlanta, GA 30332, USA*

[3] *Parker H. Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA*

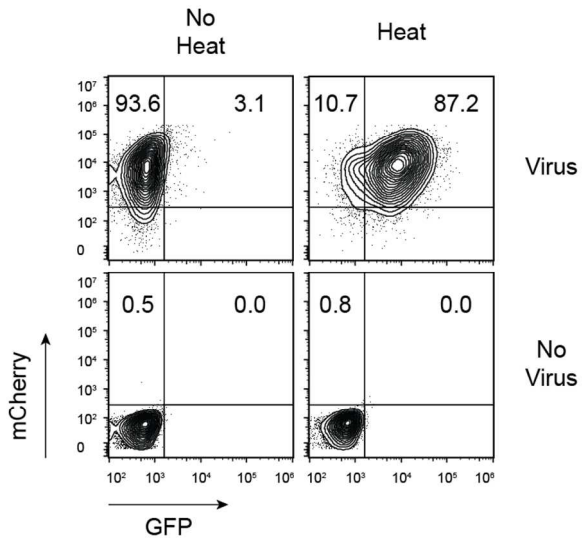
[4] *Integrated Cancer Research Center, Georgia Institute of Technology, Atlanta, GA 30332, USA*

[5] *Georgia Immunoengineering Consortium, Emory University and Georgia Institute of Technology, Atlanta, GA 30332, USA*

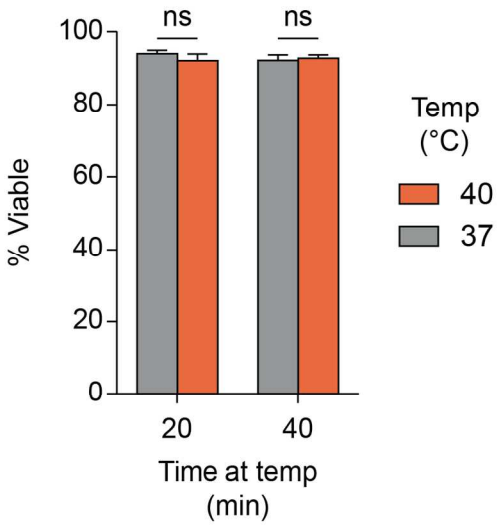
* Corresponding Author: Gabe A. Kwong
Address: Marcus Nanotechnology Building, 345 Ferst Drive, Atlanta, GA 30332, USA
Phone: 404-385-3746
Email: gkwong@gatech.edu



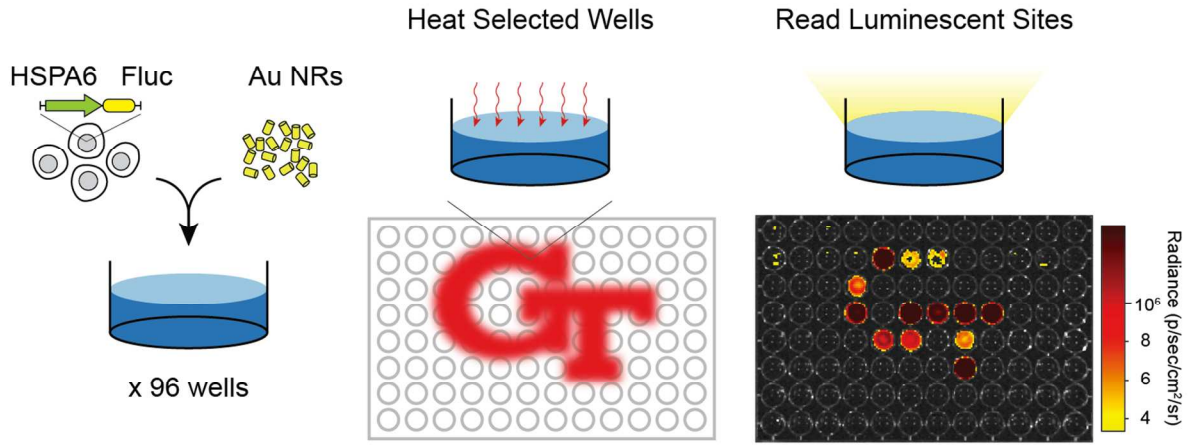
Supplementary Figure 1. Basal activity of HSPA6 switch in Jurkat T cells. T cell radiance after heating for 1 hr at 42 ºC (+) or 37 ºC (–) in cells transduced (+) or untransduced (–) with lentivirus, n = 3, two-way ANOVA and Dunnett's multiple comparison test, error bars = SEM.



Supplementary Figure 2. Heat actuation of engineered Jurkat T cells. Thermal treatments of transduced or untransduced Jurkats containing a heat-activated GFP reporter and a constitutively expressed mCherry reporter under the SFFV promoter. Heating was performed for 15 min at 42 ºC and cells were assayed 24 hrs after heating.



Supplementary Figure 3. Mild hyperthermia is well-tolerated by Jurkat T cells.
Quantification of PI and Annexin V viability stains of Jurkat T cells. Viable = $PI^{-}AnnexV^{-}$ population 24 hr after heat, n = 3, two-way ANOVA with Bonferroni's multiple comparison test, error bars = SEM.



Supplementary Figure 4. Spatially selective activation of thermal switches. Select wells heated in pattern of the Georgia Tech logo using 808 nm laser light. Plate imaged with IVIS Spectrum CT 24 hrs after heating.

Supplementary methods

Switch Construction: HSPA6 promoter sequences had XbaI and XhoI restriction sites added to the 5' and 3' ends respectively during PCR amplification before being digested and ligated into the Lego-C plasmid (Addgene #27348). Annealing sequences for the primers are listed below:

- Forward
  - Constructs i and iv: tcatcttgaattcccacaacacatgg
  - Constructs ii and vi: gatctgaatggaatgttctggattgaaga
  - Construct iii and vii: aattctaccactgaaccaccaatgc
  - Constructs iv and viii: cgaaagttcgcggcgg
- Reverse
  - Constructs i – iv: ggctgaagcttcttgtcgga
  - Constructs v – viii: agtgaggctctccctgcggtttctct