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Supporting Information

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Living Material with Temperature-Dependent Light Absorption

Lealia L. Xiong, Michael A. Garrett, Julia A. Kornfield and Mikhail G. Shapiro**

Supporting information

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Supplementary Table S1. Genetic constructs used in this study.

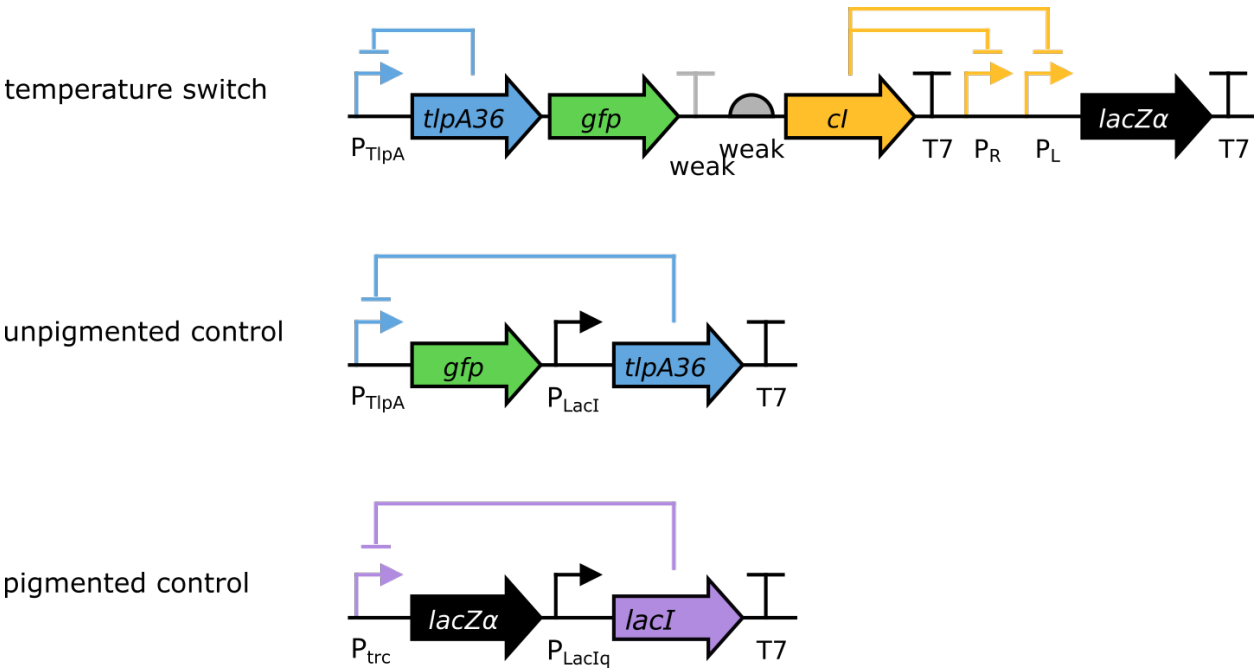
Plasmid	**Purpose**	**Transcriptional Regulator(s)**	**Output Gene Product(s)**
pTSwitch-LacZα	temperature switch	TlpA36, CI	LacZα, mWasabi
pTlpA36-wasabi	unpigmented control	TlpA36	mWasabi
pTrcLacZα	pigmented control	LacIq	LacZα

Supplementary Table S2. Sequences of DNA parts used in this study.

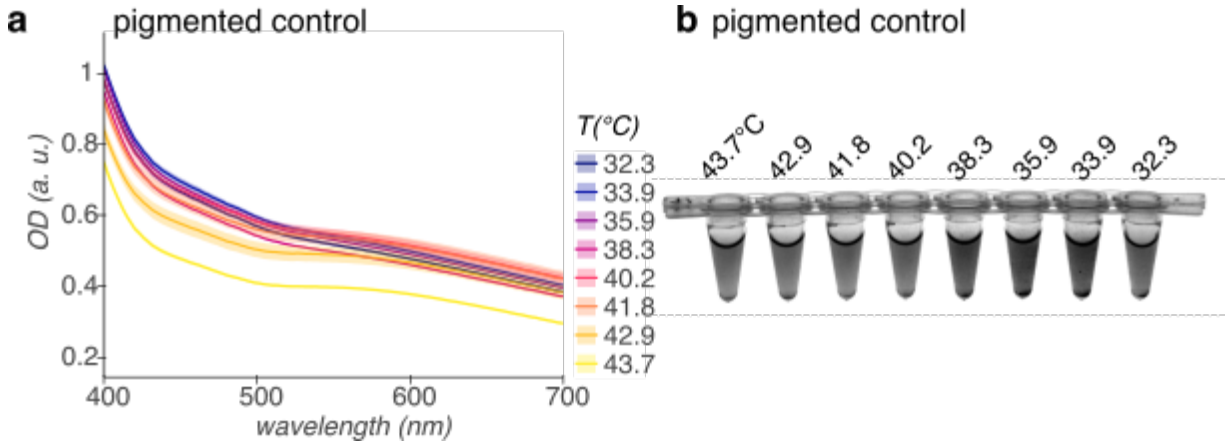
Type	Name	Sequence
promoter	PTlpA	TTTAATTTGTTTGTTAGTTAGTTTATTTGTTGGTTTGTTTGTGTTATA ATAT
promoter	PR	GTGCGTGTTGACTATTTTACCTCTGGCGGTGATAATGGTTGCATGTACTAAG GAGGTTG
promoter	PL	AACCATCTGCGGTGATAAATTATCTCTGGCGGTGTTGACATAAATACCACTG GCGGTGATACTGAGCACATCAGCAGG
promoter	PTrc	TTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAAC AATT
degradation tag	AAV ssrA tag	GCTGCTAACGACGAAAACTACGCTGACGCTTCT
terminator	T7	CTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
terminator	Part:BBa_B1002	CGCAAAAAACCCCGCTTCGGCGGGGTTTTTTCGC
RBS	RBSF	CACCATACACTG
gene	LacZa	CATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAAC CCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCT GGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCA GCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGC CGGAAAGCTGGCTGGAG
gene	mWasabi	ATGGTGAGCAAGGGCGAGGAGACCACAATGGGCGTAATCAAGCCCGACATG AAGATCAAGCTGAAGATGGAGGGCAACGTGAATGGCCACGCCTTCGTGATCG AGGGCGAGGGCGAGGGCAAGCCCTACGACGGCACCAACACCATCAACCTGG AGGTGAAGGAGGGAGCCCCCCTGCCCTTCTCCTACGACATTCTGACCACCGC GTTCAGTTACGGCAACAGGGCCTTCACCAAGTACCCCGACGACATCCCCAAC TACTTCAAGCAGTCCTTCCCCGAGGGCTACTCTTGGGAGCGCACCATGACCT TCGAGGACAAGGGCATCGTGAAGGTGAAGTCCGACATCTCCATGGAGGAGG ACTCCTTCATCTACGAGATACACCTCAAGGGCGAGAACTTCCCCCCCAACGG CCCCGTGATGCAGAAGGAGACCACCGGCTGGGACGCCTCCACCGAGAGGAT GTACGTGCGCGACGGCGTGCTGAAGGGCGACGTCAAGATGAAGCTGCTGCT GGAGGGCGGCGGCCACCACCGCGTTGACTTCAAGACCATCTACAGGGCCAA GAAGGCGGTGAAGCTGCCCGACTATCACTTTGTGGACCACCGCATCGAGATC CTGAACCACGACAAGGACTACAACAAGGTGACCGTTTACGAGATCGCCGTGG CCCGCAACTCCACCGACGGCATGGACGAGCTGTACAAGGGC
gene	TlpA36	ATGCGTCCGGCGACATACGAACCAGAACAGATTATTGAAGCAGGGCTGGCCC TGCAGGCTGAAGGACGGAATATCACCGGGTTCGCACTACGTAACCAGGTGG GTGGCGGCAATCCGACACGTCTCCGCCAGATATGGGACGAATACCAGGCTT CACAGAGCACGGTCGTCACTGAACTCGTTGCCGAGCTGCCAGTGGAAGTGG CTGAAGAAGTGAAGGCCGTCTCCGCCGCGCTGTCCGAACGCATCACCCAGC TGGCGACAGAACTGAATGACAAGGCGGTCCGGGCTGCAGAACGCCGGGTTG CGGAAGTCACGCGTGCTGCCGGTGAACAGACCGCACAGGCAGAGCGGGAGC TGGCCGACGCCGCGCAGACAGTCGACGACCTGGAAGAAAAACTGGTTGAACT GCAGGACAGATATGACAGTTTGACGCTGGCGCTGGAGTCAGAACGTTCACT GCGTCAGCAGCATGATGTGGAGATGGCCCAGCTGAAAGAGCGTCTTGCGGC CGCTGAAGAGAATACCCGTCAGCGAGAGGAACGGTATCAGGAGCAGAGGAC AGTGCTGCAGGATGCGCTTAATGCGGAGCAGGCACAGCACATAAACACGCG GGAAGACCAGCAGAAACGACTGGAGCAAATTTCTGCCGAAGCTAATGCGCGT ACAGAAGAACTGAAGTCTGAACGCGATAAAGTCAATACTCTCCTTACCCGCC TTGAATCGCAGGAAAATGCGCTGGCCTCAGAACGTCAGCAGCATCTGGCCAC CCGCGAAACGCTGCAGCAACGCCTCGAGCAGGCCATCGCTGACACGCAGGC GCGCGCCGGTGAGATTGCACTTGAACGTGACAGAGTCAGCAGCCTCACCGC AAGGCTGGAATCGCAGGAAAAGGCCTCCTCGGAGCAACTGGTGCGTATGGG CAGTGAAATAGCCAGTCTGACAGAGCGTTGCACACAGCTGGAAAACCAGCGT GATGATGCCCGTCTGGAGACGATGGGGGAGAAAGAAACGGTCGCGGCACTG CGTGGTGAGGCTGAAGCCCTGAAGCGTCAGAACCAGTCACTGATGGCGGCG CTTTCAGGCAATAAACAGACCGGTGGCCAGAATGCGT

gene	CI	ATGAGCACAAAAAAGAAACCATTAACACAAGAGCAGCTTGAGGACGCACGTC GCCTTAAAGCAATTTATGAAAAAAAGAAAAATGAACTTGGCTTATCCCAGGAA TCTGTCGCAGACAAGATGGGGATGGGGCAGTCAGGCGTTGGTGCTTTATTT AATGGCATCAATGCATTAAATGCTTATAACGCCGCATTGCTTGCAAAAATTCT CAAAGTTAGCGTTGAAGAATTTAGCCCTTCAATCGCCAGAGAAATCTACGAG ATGTATGAAGCGGTTAGTATGCAGCCGTCACTTAGAAGTGAGTATGAGTACC CTGTTTTTTCTCATGTTCAGGCAGGGATGTTCTCACCTGAGCTTAGAACCTT TACCAAAGGTGATGCGGAGAGATGGGTAAGCACAACCAAAAAAGCCAGTGAT TCTGCATTCTGGCTTGAGGTTGAAGGTAATTCCATGACCGCACCAACAGGCT CCAAGCCAAGCTTTCCTGACGGAATGTTAATTCTCGTTGACCCTGAGCAGGC TGTTGAGCCAGGTGATTTCTGCATAGCCAGACTTGGGGGTGATGAGTTTAC CTTCAAGAAACTGATCAGGGATAGCGGTCAGGTGTTTTTACAACCACTAAAC CCACAGTACCCAATGATCCCATGCAATGAGAGTTGTTCCGTTGTGGGGAAAG TTATCGCTAGTCAGTGGCCTGAAGAGACGTTTGGCTGA

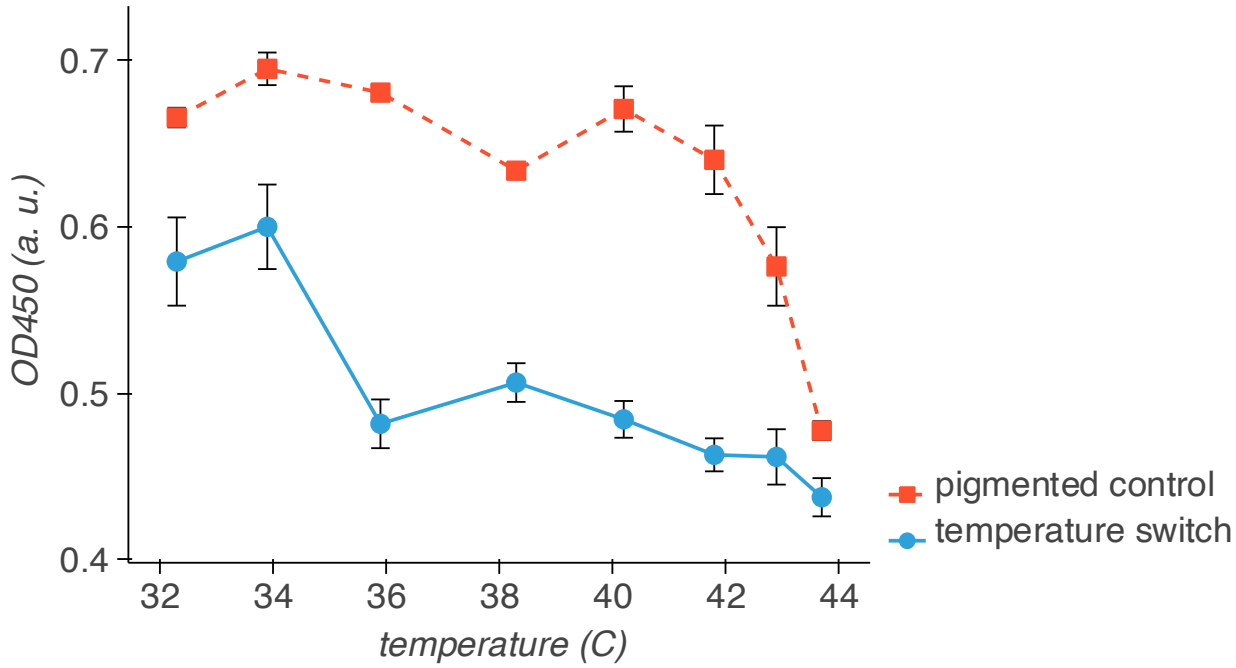
SUPPLEMENTARY FIGURES S1-S11



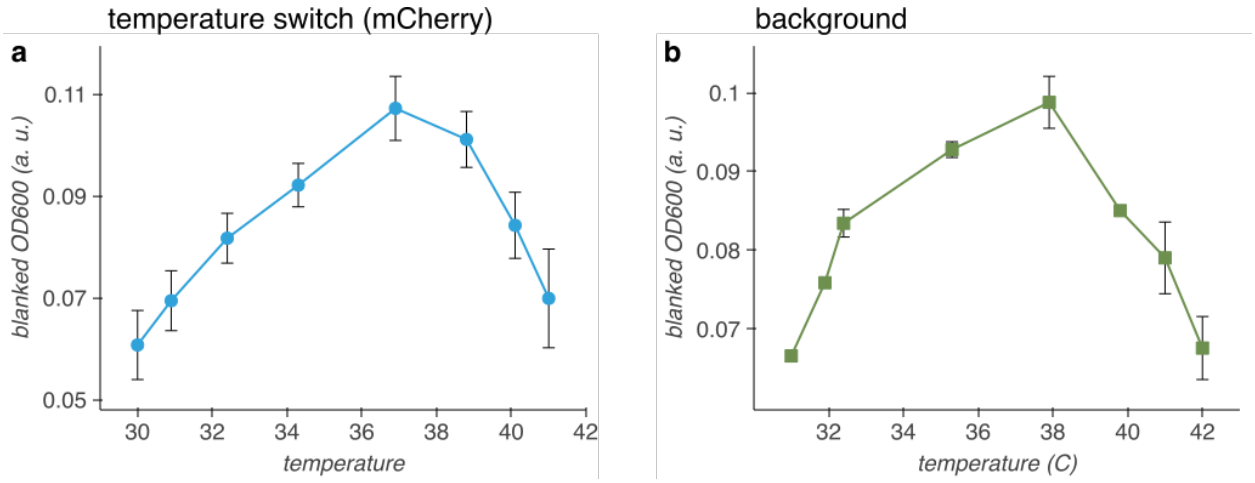
Supplementary figure S1. Circuit diagrams of temperature switch contruct for low-temperature pigmentation, unpigmented control, and pigmented control. The unpigmented control construct encodes mWasabi green fluorescent protein (GFP) under the control of TlpA36. Below 36°C, TlpA36 represses the expression of GFP by binding to the P_{TlpA} promoter. Above 36°C, TlpA36 loses repressor function, so GFP is expressed from the P_{TlpA} promoter. The pigmented control construct encodes LacZα peptide under the control of the *lac* repressor (LacI). LacI represses the expression of LacZα by binding to the P_{trc} promoter. Isopropyl β-D-1-thiogalactopyranoside (IPTG) binds to LacI and causes an allosteric change in its shape, causing LacI to lose its ability to bind to the P_{trc} promoter. Thus, IPTG induces the expression of LacZα from this contruct.



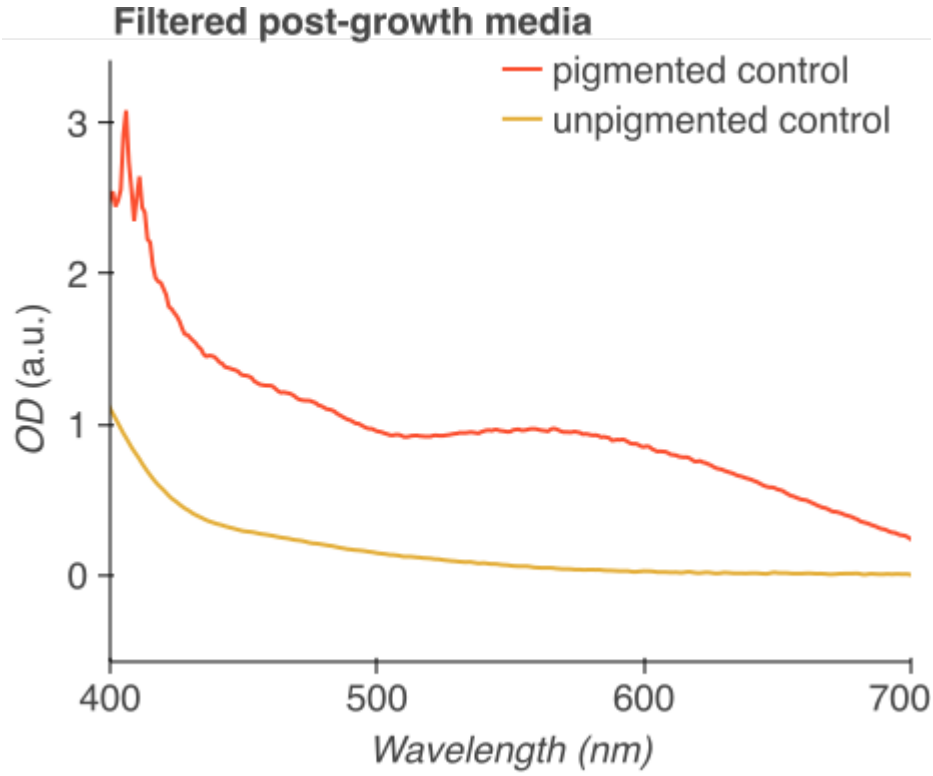
Supplementary figure S2. Visible light optical density (OD) spectra **(a)** and representative white light transillumination image **(b)** of cultures of *E. coli* containing a pigmented control construct encoding IPTG-inducible LacZα after 24 h growth in pigment-induction media at temperatures ranging from 43.7°C to 32.3°C. n = 2 biological replicates; shading represents +/- standard error of the mean.



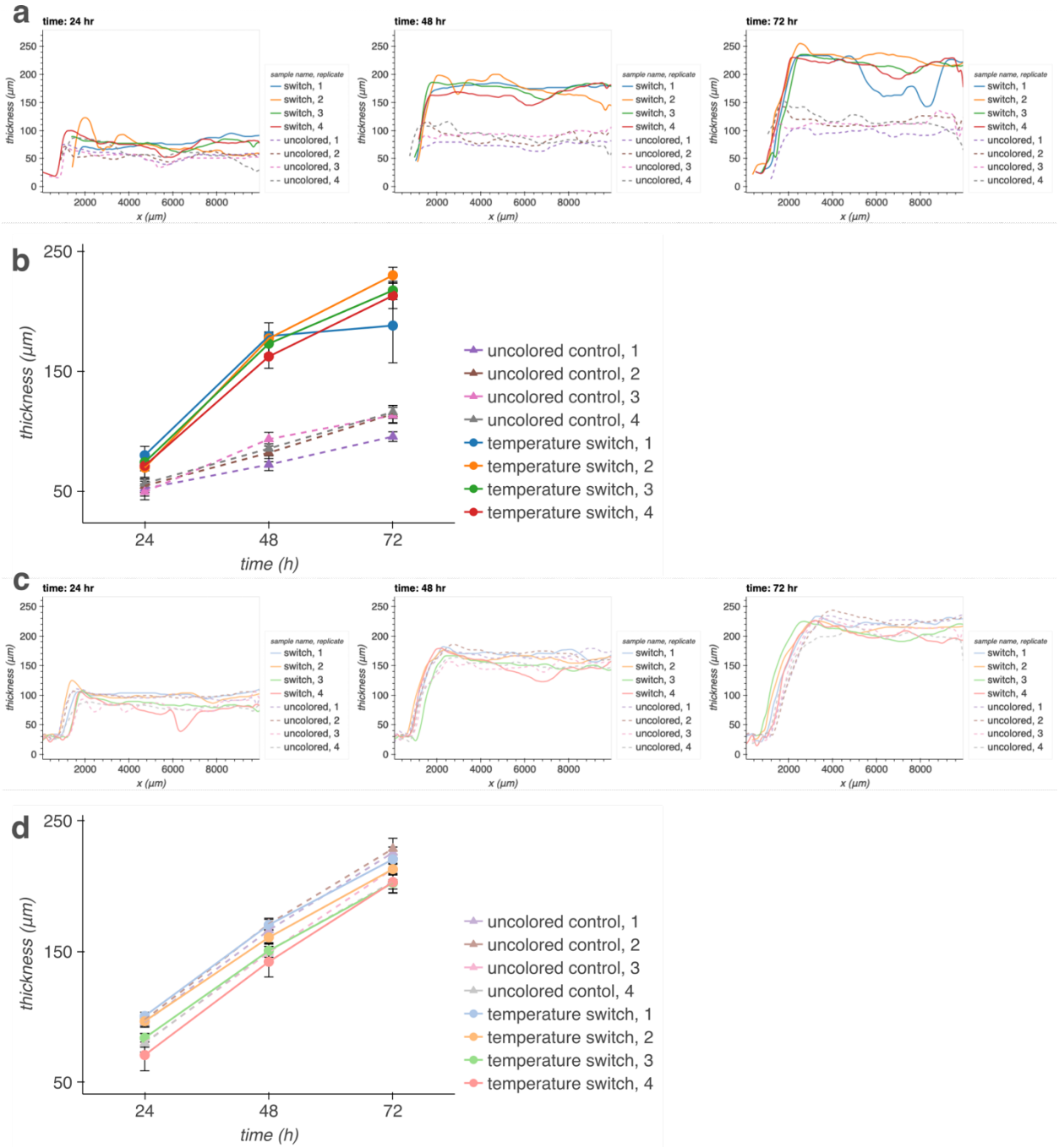
Supplementary figure S3. Optical density at 450 nm of cultures of *E. coli* containing the temperature switch construct (n = 4) or the pigmented control construct encoding IPTG-inducible LacZα (n = 2) after 24 h growth in pigment-induction media at temperatures ranging from 43.7°C to 32.3°C. 450 nm is within the visible light spectrum, but avoids the maximum excitation wavelength of mWasabi, 493 nm.



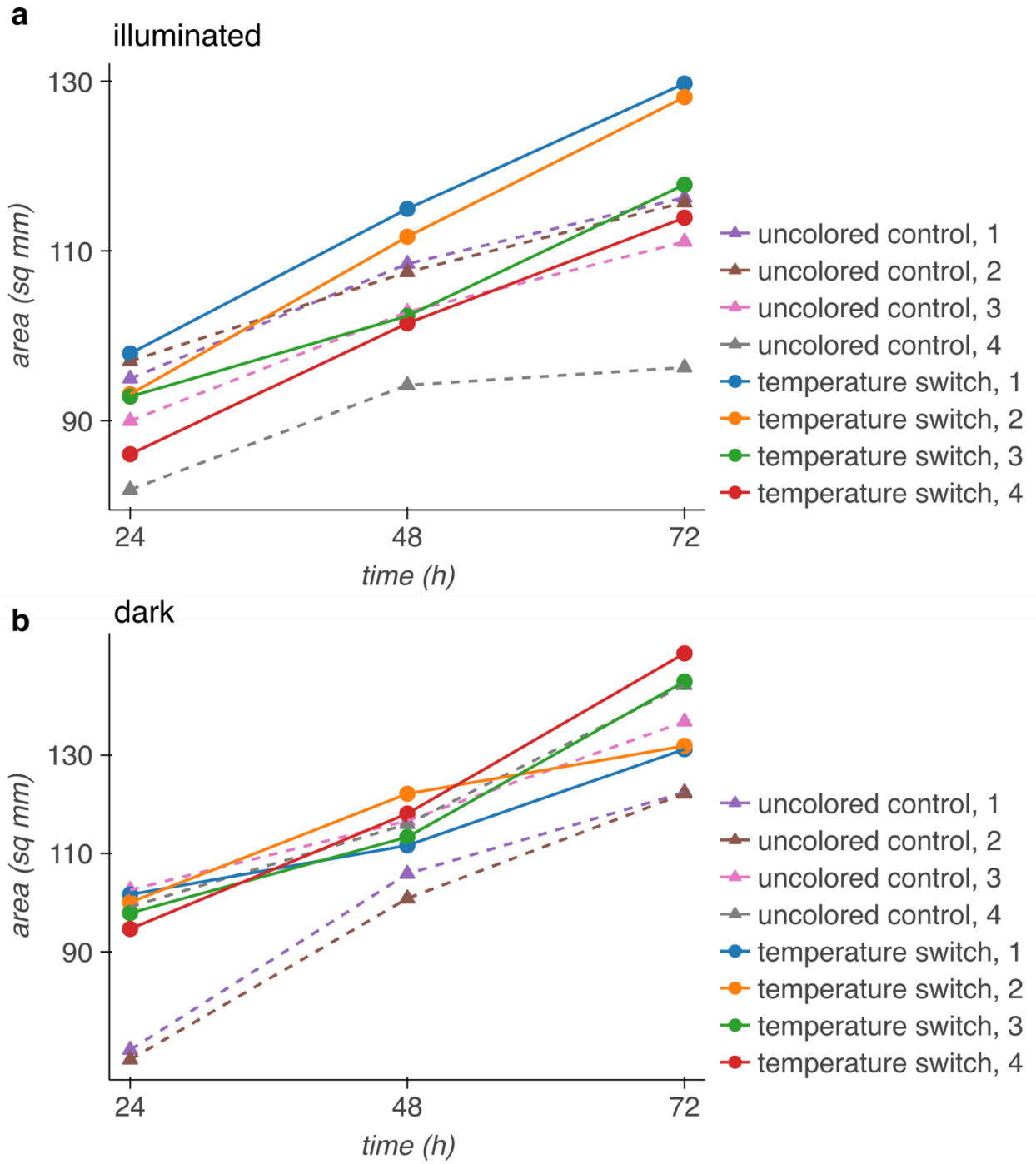
Supplementary figure S4. Cell density of *E. coli* grown for 12 hours at temperatures ranging from 31°C to 42°C, measured by optical density at 600 nm (OD600). **(a)** OD600 of cultures of DH10B *E. coli* containing a variant of the temperature switch circuit wherein *mCherry* replaces *lacZα*. $n = 4$ biological replicates; error bars represent +/- standard error of the mean. **(b)** OD600 of cultures of DH10B *E. coli* containing a construct encoding a nonfluorescent mutant of mWasabi (S71T, G73A) under the control of TlpA. At these temperatures, TlpA is always functional, repressing the expression of the nonfluorescent mWasabi. $n = 2$ biological replicates; error bars represent +/- standard error of the mean.



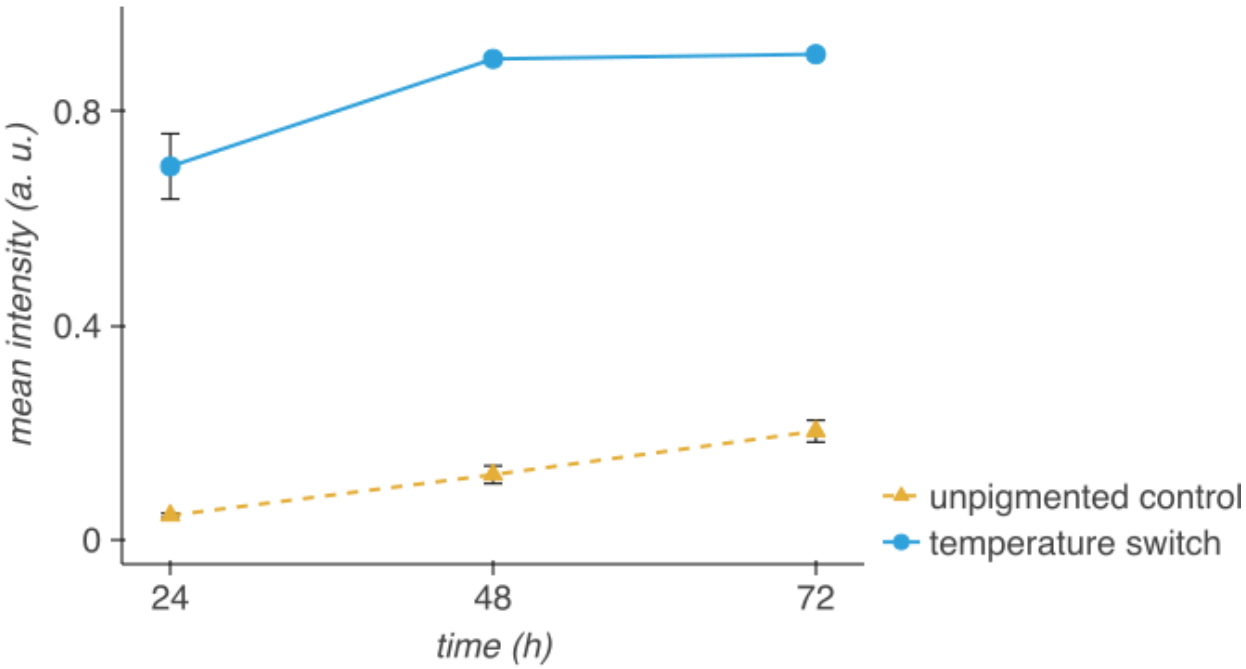
Supplementary figure S5. Visible light optical density (OD) spectra of media from *E. coli* cultures grown for 23 h at 30°C, 250 rpm with 300 μg/mL S-gal, 1 h after removing cells by passing through a 0.22 μm filter and adding 500 μg/mL ferric ammonium citrate. Black pigment forms visibly in media from *E. coli* containing a pigmented control circuit, resulting in increased optical density from 450 nm to 700 nm compared with media from *E. coli* containing an unpigmented control circuit. Spectra measured using a NanoDrop 2000/2000c Spectrophotometer (ThermoFisher) in cuvette mode. Constructs differ from main text slightly: Unpigmented control encodes nonfluorescent mWasabi mutant under the control of TlpA (TlpA represses product at 30°C). Pigmented control encodes IPTG-inducible full-length LacZ. Temperature switch construct lacks AAV ssrA tag on LacZα.



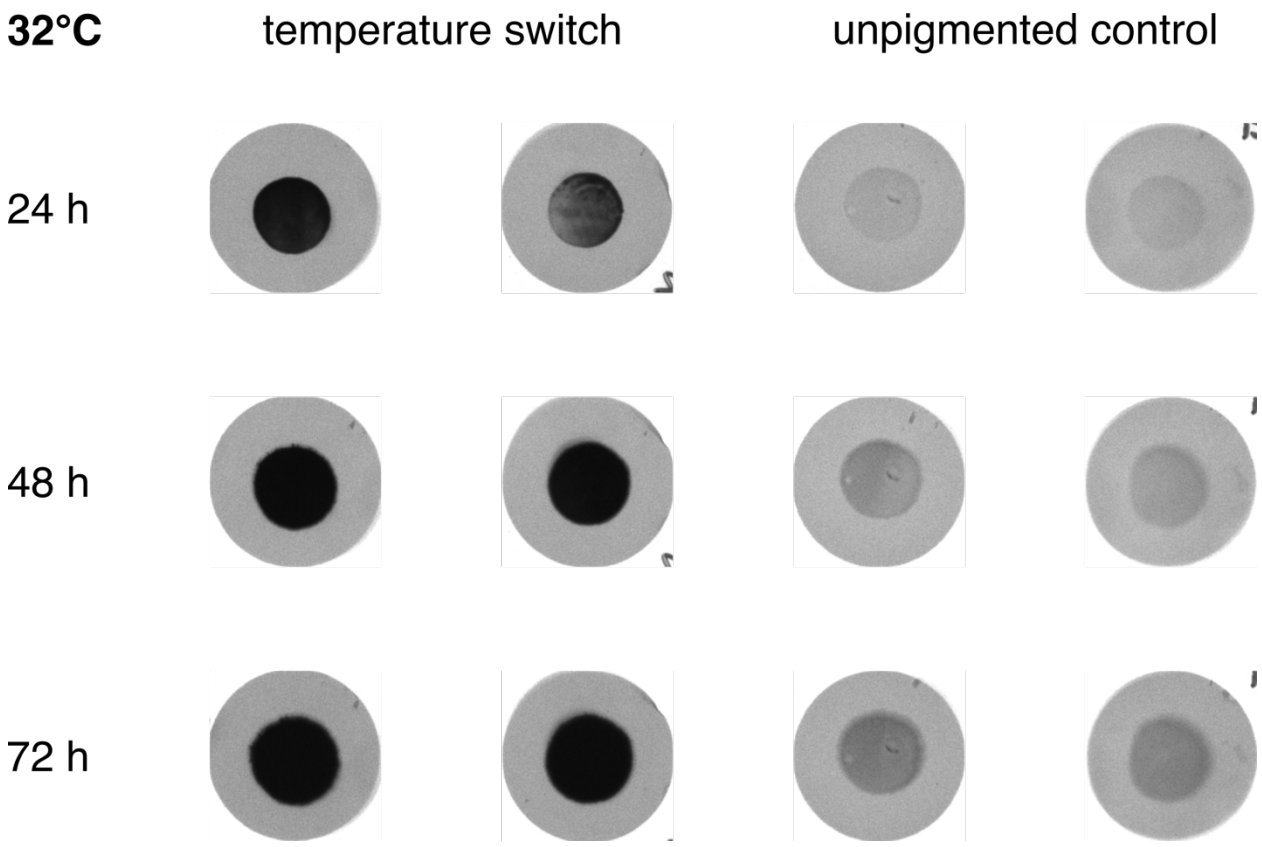
Supplementary figure S6. (**a, c**) Thicknesses over time measured across full OCT cross-section for each patch of *E. coli* containing either the temperature switch construct or an unpigmented control construct encoding heat-inducible GFP, grown at 32°C with (**a**) or without (**c**) illumination. (**b, d**) Mean thickness and standard deviation between x = 3.5 mm and x = 9.0 mm (avoiding the edges of the patch) of each patch, grown with (**b**) or without (**d**) illumination over time.



Supplementary figure S7. Area of patches of *E. coli* containing either the temperature switch construct or an unpigmented control construct encoding heat-inducible GFP, grown at 32°C with (**a**) or without (**b**) illumination over time.

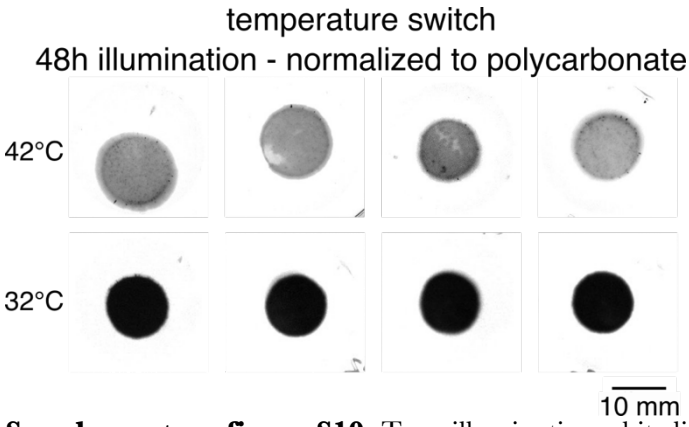


Supplementary figure S8. Mean pixel intensity of patches of *E. coli* containing either the temperature switch construct or an unpigmented control construct encoding heat-inducible GFP, grown at 32°C under illumination, over time. Data was quantified from a series of transillumination. Images were normalized so that the polycarbonate membranes have a mean intensity of 0 and opaque black plastic has a mean intensity of 1. n = 4 biological replicates; error bars represent +/- standard error of the mean.

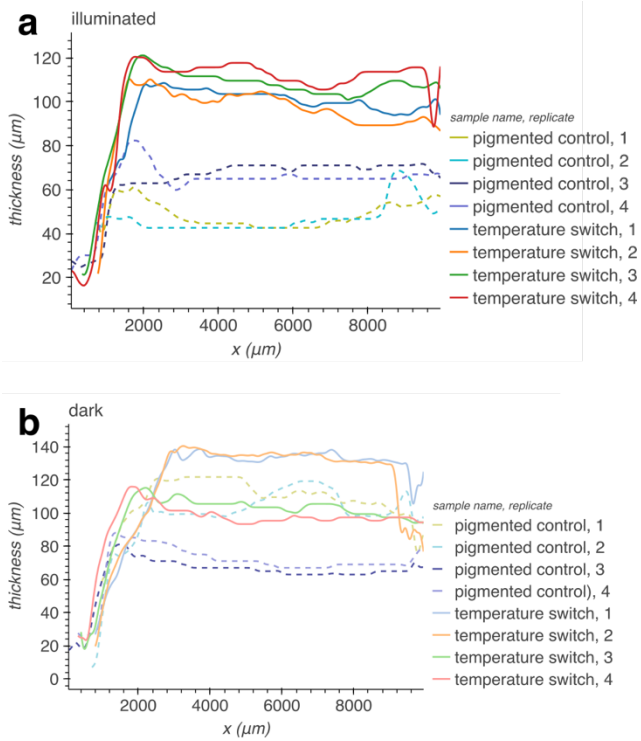


Supplementary figure S9. Representative transillumination white light images of patches of *E. coli* containing the temperature switch construct or the unpigmented control construct on polycarbonate membranes after 24 h, 48 h, and 72 h of growth in the illuminated growth chamber with pigment-induction media at 32°C.

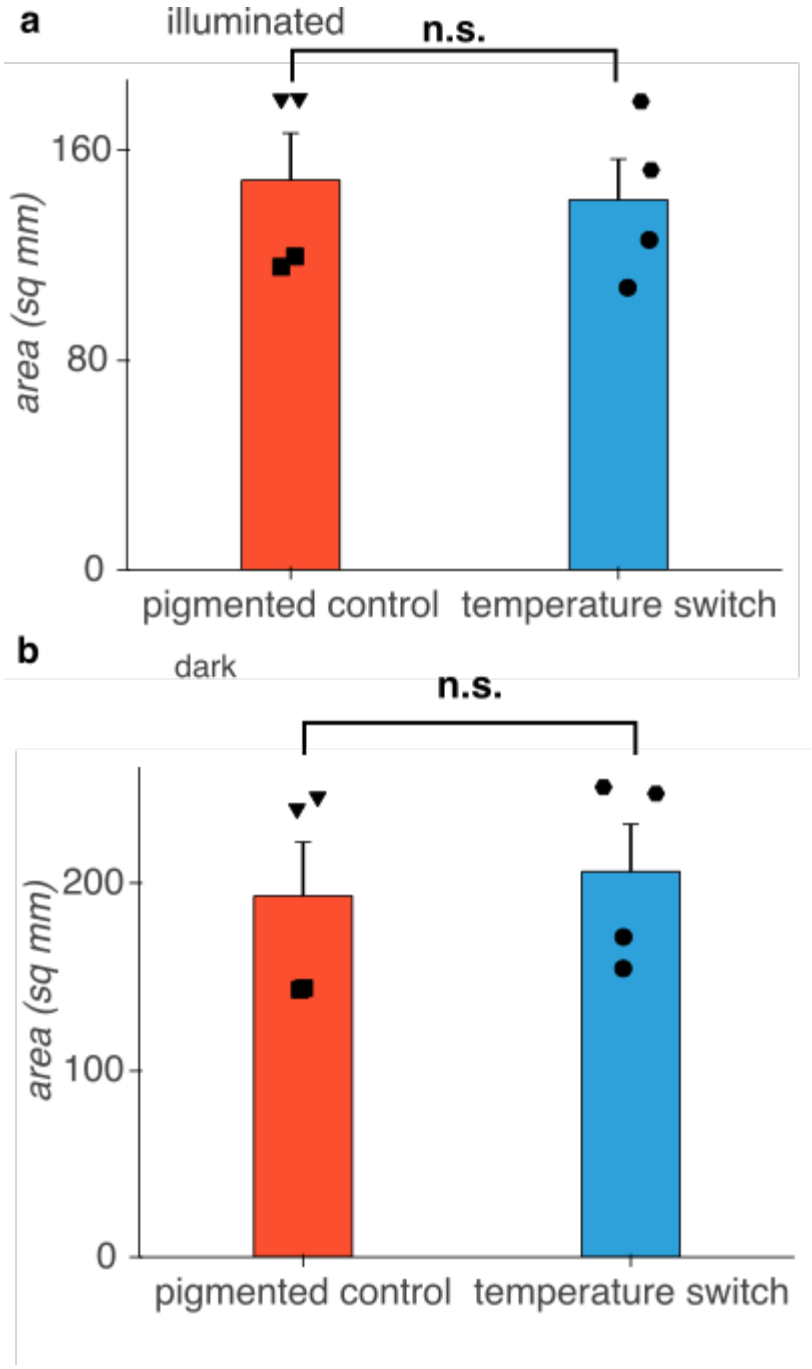
r



Supplementary figure S10. Transillumination white light images of patches of *E. coli* containing the temperature switch construct on polycarbonate membranes after 48 h growth in the illuminated growth chamber with pigment-induction media at 42°C and 32°C, normalized so that the polycarbonate membranes have a mean intensity of 0 and opaque black plastic has a mean intensity of 1.

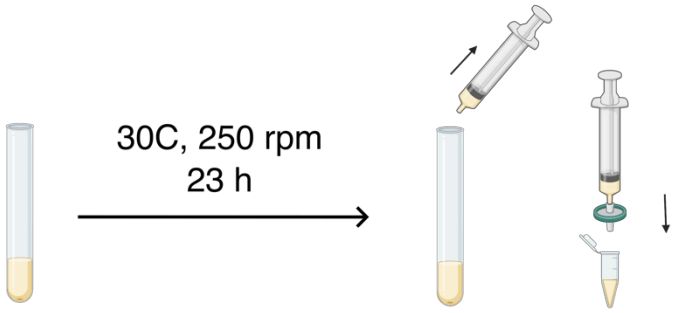


Supplementary figure S11. Thicknesses measured across full OCT cross-section for each patch of *E. coli* containing either the temperature switch construct or a pigmented control construct encoding IPTG-inducible LacZα, grown at 42°C for 48 h with **(a)** or without **(b)** illumination. Replicates 1 and 2 for both the pigmented control and the temperature switch construct were coated on Whatman Nuclepore polycarbonate membranes. Replicates 3 and 4 were coated on Sartorius polycarbonate membranes.



Supplementary figure S12. Area of patches of *E. coli* containing either the temperature switch construct or a pigmented control construct encoding IPTG-inducible LacZα, grown at 42°C with (**a**) or without (**b**) illumination for 48 h. Inverted triangle and hexagon markers indicate patches coated onto Whatman Nucleopore polycarbonate membranes; square and circle markers indicate patches coated onto Sartorius polycarbonate membranes.

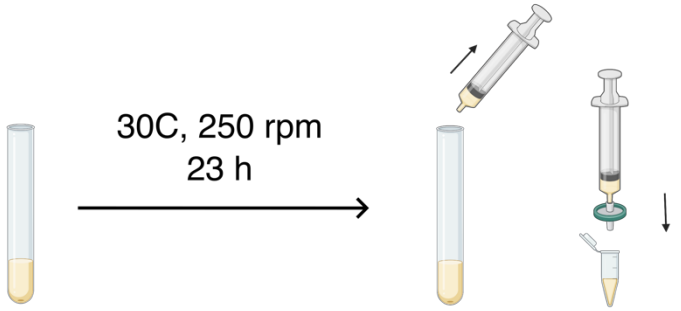
a



- S-gal

filtered media
+ S-gal
+ Fe^{3+}

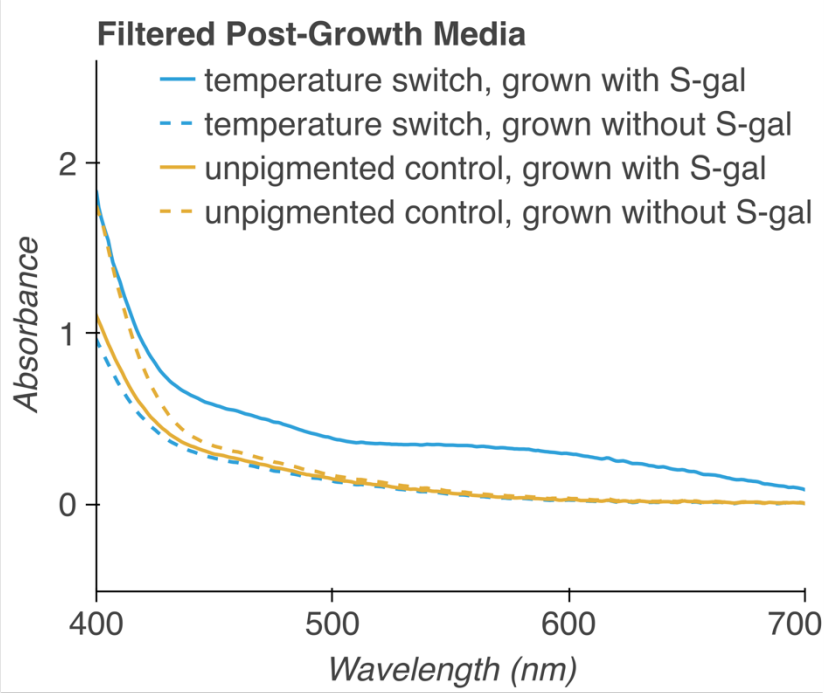
b



+ S-gal

filtered media
+ Fe^{3+}

c



Supplementary figure S13. Enzymatic cleavage of S-gal occurs intracellularly before extracellular coordination with ferric iron to form pigment. **(a)** DH10B *E. coli* were grown in LB media supplemented with 100 μg/mL ampicillin at 30°C, 250 rpm for 23 h. The cultures were passed through a 0.22 μm filter to remove the cells; then, 300 μg/mL S-gal and 500 μg/mL ferric ammonium citrate were added to the filtered media. **(b)** DH10B *E. coli* were grown in LB media supplemented with 100 μg/mL ampicillin and 300 μg/mL S-gal at 30°C, 250 rpm for 23 h. The cultures were passed through a 0.22 μm filter to remove the cells; then, 500 μg/mL ferric ammonium citrate were added to the filtered media. **(c)** Visible light optical density spectra of cultures of *E. coli* 1 h after filtering and adding either S-gal or S-gal and ferric ammonium citrate. The temperature switch sample grown with S-gal exhibits increased optical density from 450 nm to 700 nm compared with the unpigmented control sample grown with and without S-gal and the temperature switch sample grown without S-gal. This suggests that β-galactosidase cleaves S-gal intracellularly and at least some of the cyclohexenoesculetin product is exported from the cell, where it coordinates with ferric iron to form the light-absorptive pigment. Spectra measured using a NanoDrop 2000/2000c Spectrophotometer (ThermoFisher) in cuvette mode. Constructs differ from main text slightly: Unpigmented control encodes nonfluorescent mWasabi mutant under the control of TlpA (TlpA represses product at 30°C). Pigmented control encodes IPTG-inducible full-length LacZ. Temperature switch construct lacks AAV ssrA tag on LacZα.