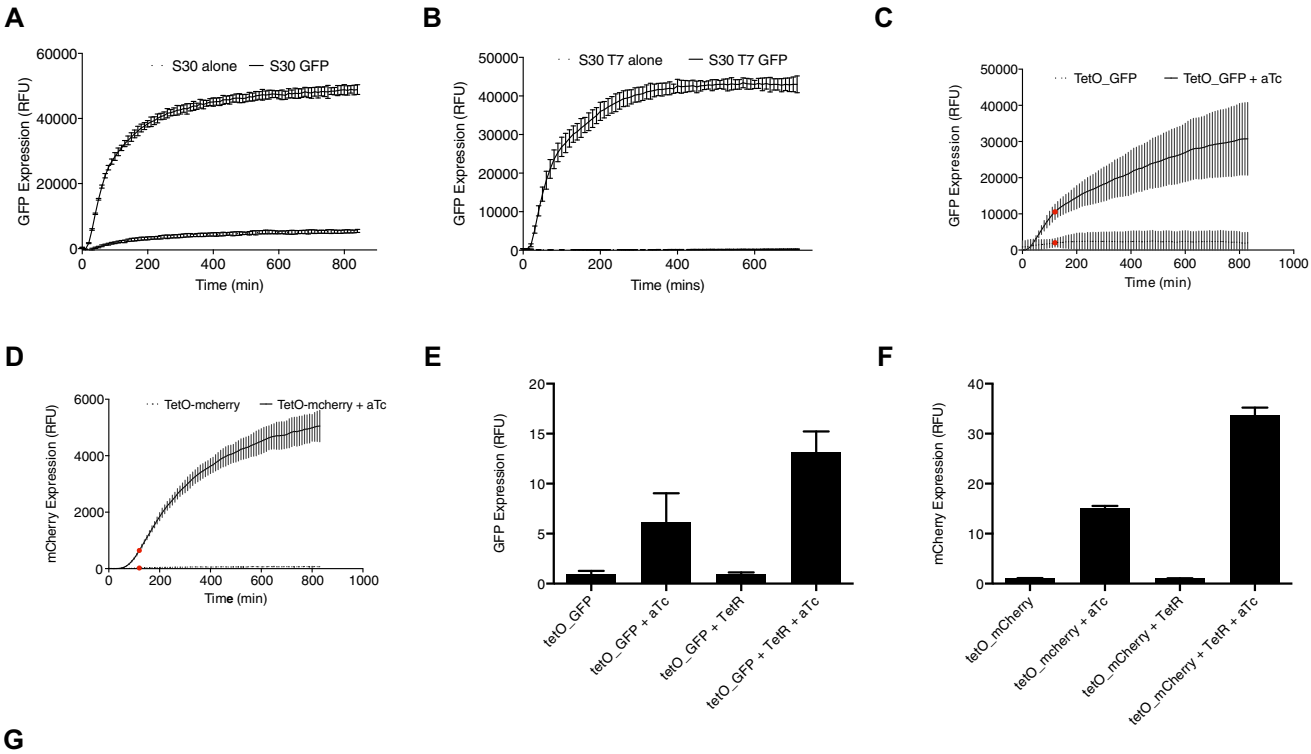
Cell

EXTENDED EXPERIMENTAL PROCEDURES

Experimental Variation

Important considerations for limiting experimental variation include ensuring samples are properly freeze-dried and that trigger RNAs are not degraded. A specific note regarding variation in the results for Figure 5, data presented in Figure 5B and Figures 5D and 5E were done using DNA for sensors prepared by two separate PCR reactions. By design, we did not gel purify sensor DNA so that prototyping could be done rapidly. As a consequence, we did not have absolute control over the concentration of each sensor and concomitantly, control over sensor concentrations between PCR reactions. This led to some variation in the measured fold-change presented in these figures.



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Overview of experimental workflow

1) Treatment of paper to block non-specific interactions

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2) Cut paper into 2 mm discs

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3) Apply cell-free system and DNA elements described below to paper (1.8 ul)
   Figures 1 - 4: DNA copy of gene circuit
   Figure 5: No gene circuit
   Figure 6: DNA copy of T3 and/or T7-dependent GFP

↓

4) Flash freeze and freeze-dry overnight

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5) Rehydrate with nuclease-free water plus elements described below to a volume of 1.8 ul.
   Figures 1 - 4: Water, inducer or trigger RNA
   Figure 5: Water or DNA for sensor +/- RNA trigger
   Figure 6: Water or DNA copy of the T3 and/or T7 cascade

Figure S1. Related to Figure 1

(A) GFP expression from freeze-dried pellets of S30 cell extract.
(B) GFP expression from freeze-dried pellets of S30 T7 cell extracts.
(C) Time course expression of tetO-regulated GFP on freeze-dried paper discs.
(D) Time course expression of tetO-regulated mCherry on freeze-dried paper discs.
(E) aTc induction of tetO-GFP in the absence and presence of TetR supplementation. Fold change was calculated by comparing each induced reaction to the matching uninduced reaction.
(F) aTc induction of tetO-mCherry in the absence and presence of TetR supplementation. Error bars represent SD. Fold change was calculated by comparing each induced reaction to the matching uninduced reaction. RFU, relative fluorescence units.
(G) Overview of experimental workflow that describes the order of operations for Figures 1, 2, 3, 4, 5, and 6.

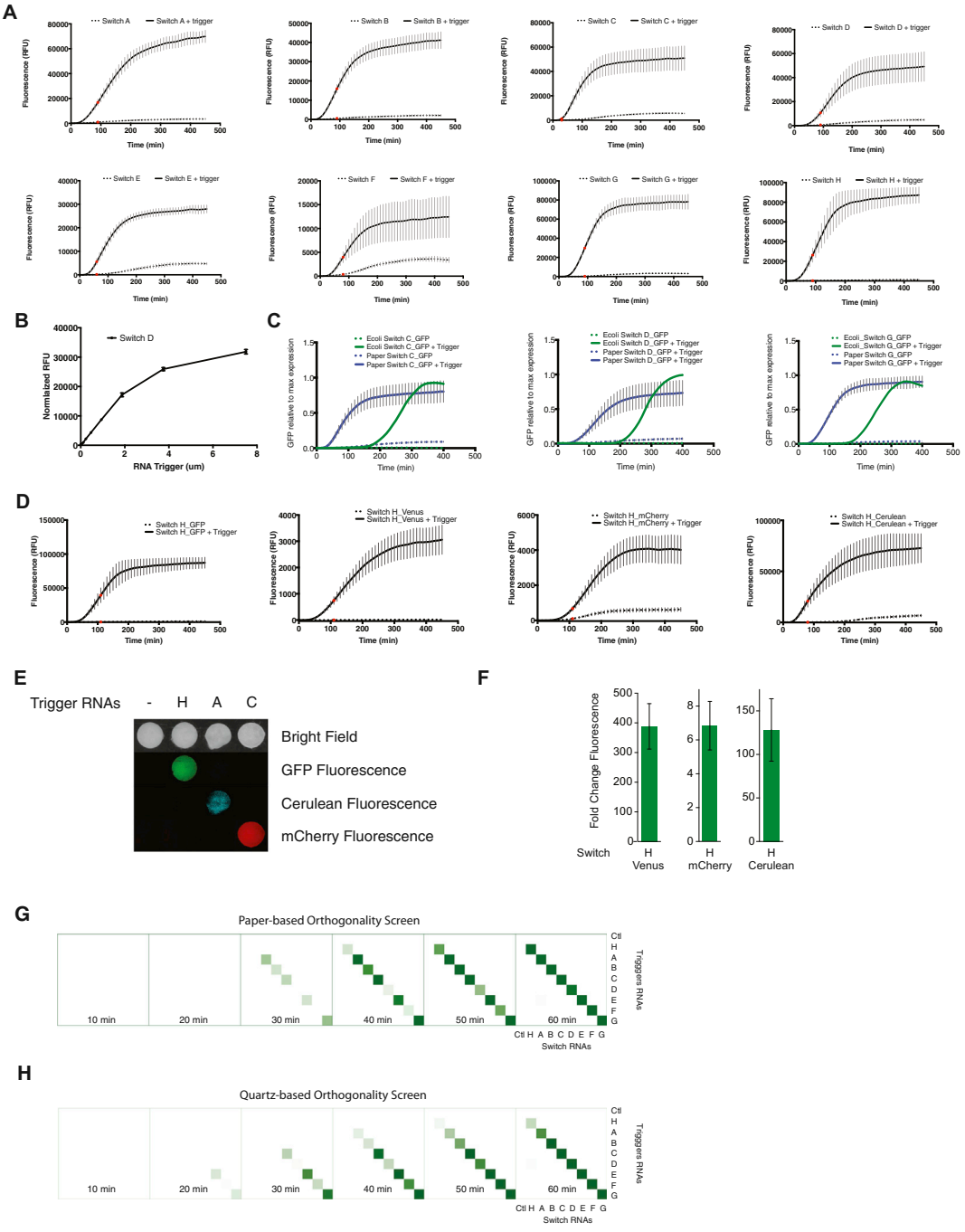

Figure S2. Related to Figure 2

(A) Paper-based regulation of GFP expression from toehold switches A – H in freeze-dried paper-based reactions. The red dots indicate the time point from which maximum fold change calculations reported in Figure 2C were taken. RNA trigger concentration 5 uM.

(B) Titration of RNA trigger for toehold switch D in solution phase reactions. Values presented are the average of data at the 360 min time point.

(C) Comparison of toehold switch induction in *E. coli* and on freeze-dried, paper-based reactions. Dynamic data collected from paper and *E. coli*-based reactions of toehold switches C, D and E.

(D) Paper-based regulation of GFP, Venus, mCherry and Cerulean fluorescent proteins by toehold switch H over a time course. The red dots indicate the time point from which fold change calculations reported in Figure S2F were taken.

(E) Images of quartz microfiber discs each embedded with three toehold switches (switch H_GFP, switch A_Cerulean and switch C_mCherry). The four discs carrying these switches were rehydrated and incubated with either water or trigger RNAs for H, A or C. The individual activation of these switches was imaged using the green, blue and red fluorescence channels.

(legend continued on next page)

(F) Maximum fold change measurement of Venus, mCherry and Cerulean expression from toehold switches H during the first two hours of incubation.
(G) Heat map of GFP expression during the first 60 min of the orthogonality screen for GFP expressing toehold switches on paper.
(H) Heat map of GFP expression during the first 60 min of the orthogonality screen for GFP expressing toehold switches on quartz microfiber. Values presented are the average of either triplicate or quadruplicate data. Error bars represent SD. RFU, relative fluorescence units.

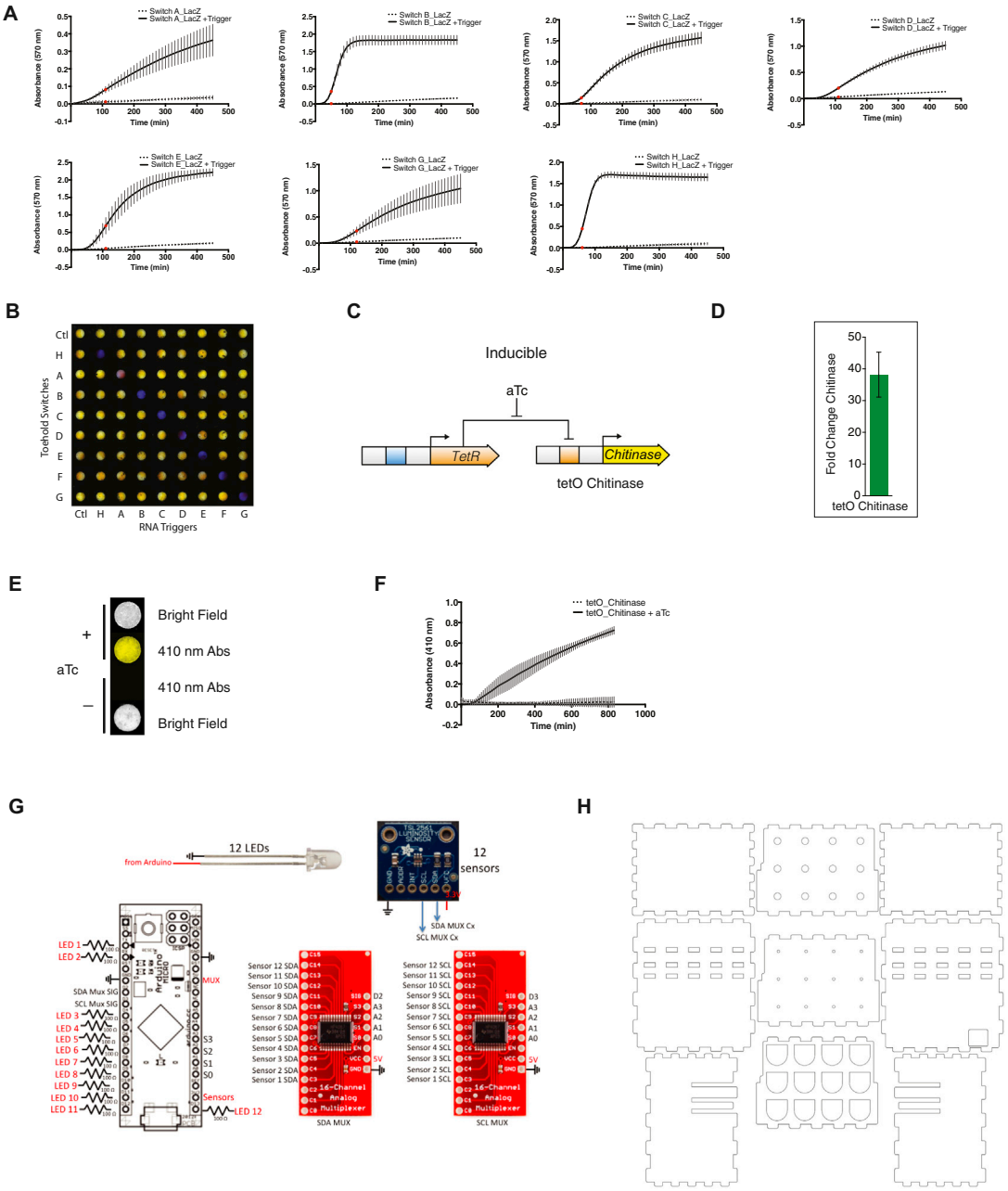

Figure S3. Related to Figure 3

(A) Regulation of LacZ toehold switch colorimetric response from freeze-dried, paper-based reactions over time. Colorimetric response was quantified as a measure of absorbance at 570 nm. The red dots indicate the time point from which the fold change LacZ rate calculations reported in Figure 3C were taken. The rate was calculated as $S_n = (T_{n+1}-T_n)/10$, where T is the normalized data at a time point (T_n) and the time point 10 min later (T_{n+1}), and S_n is the slope reported for T_n.

(B) Composite image of orthogonality screen of LacZ colorimetric toehold switch reactions on paper discs arrayed in a 384-well plate.

(C) Schematic of tetO regulation for *chitinase*.

(D) Maximum fold change of the colorimetric tetO_chitinase output at 420 min.

(E) Bright field and 410 nm absorbance images of the tetO_chitinase system embedded into paper after overnight incubation, ± aTc induction. Chitinase expression leads to the cleavage of a colorless precursor to generate a yellow product, which was quantified by monitoring absorbance at 410 nm.

(F) Time course evolution of the paper-based colorimetric reaction in Figure S3D as measured by 410 nm absorbance.

(G) Components used in the fabrication of the in-house optical electronic reader. 570 nm LED light source and luminosity sensors are coordinated to the Arduino Micro through two multiplexers.

(H) Line drawings used to cut the reader housing from black acrylic using a laser cutter. Values presented are the average of either triplicate or quadruplicate data. Error bars represent SD.

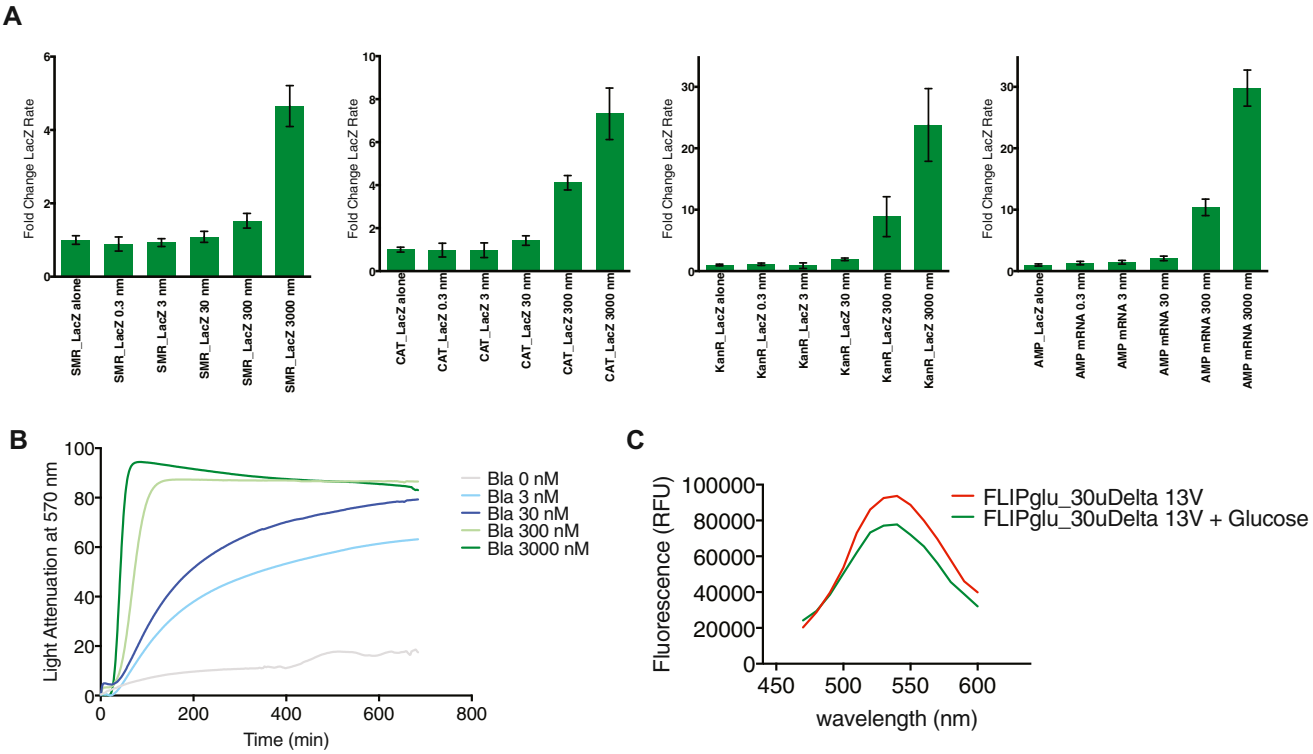

Figure S4. Related to Figure 4

(A) Titration of full-length target mRNA for paper-based antibiotic resistance gene mRNA sensors with LacZ output. Summary of rate-based fold change for LacZ mRNA sensors for kanamycin, spectinomycin, chloramphenicol and ampicillin resistance gene (Plate reader, Abs 570 nm). Error bars represent SD.

(B) Time course data for the ampicillin resistance sensor from the in-house electronic optical reader. Values presented are the average of either triplicate or quadruplicate data.

(C) Fluorescence spectrum of FRET-based glucose nanosensor in the presence and absence of 10 mM glucose. Using freeze-dried, cell-free expression in HeLa cell extracts, the 528 nm fluorescence peak of the nanosensor in solution is suppressed in the presence of glucose as previously reported (Takanaga and Frommer, 2010). RFU, relative fluorescence units.

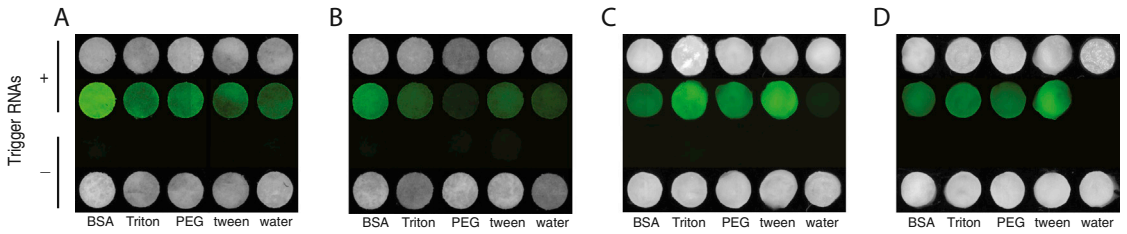

Figure S5. Pretreating Matrix Materials with Blocking Agents Improves the Efficiency of GFP Expression, Related to Experimental Procedures

(A) Toehold switch H_GFP reactions on 2 mm paper discs that have been blocked with 5% BSA, 5% Triton X-100, 5% PEG 8k, 5% Tween-20 or washed with water.

(B–D) (B) Toehold switch B_GFP on paper discs treated as above, (C) Toehold switch H_GFP on 2 mm quartz microfiber discs treated as above and (D) Toehold switch B_GFP on quartz microfiber discs treated as above.