Materials and Methods

A Biological 2-Input Decoder Circuit in Human Cells

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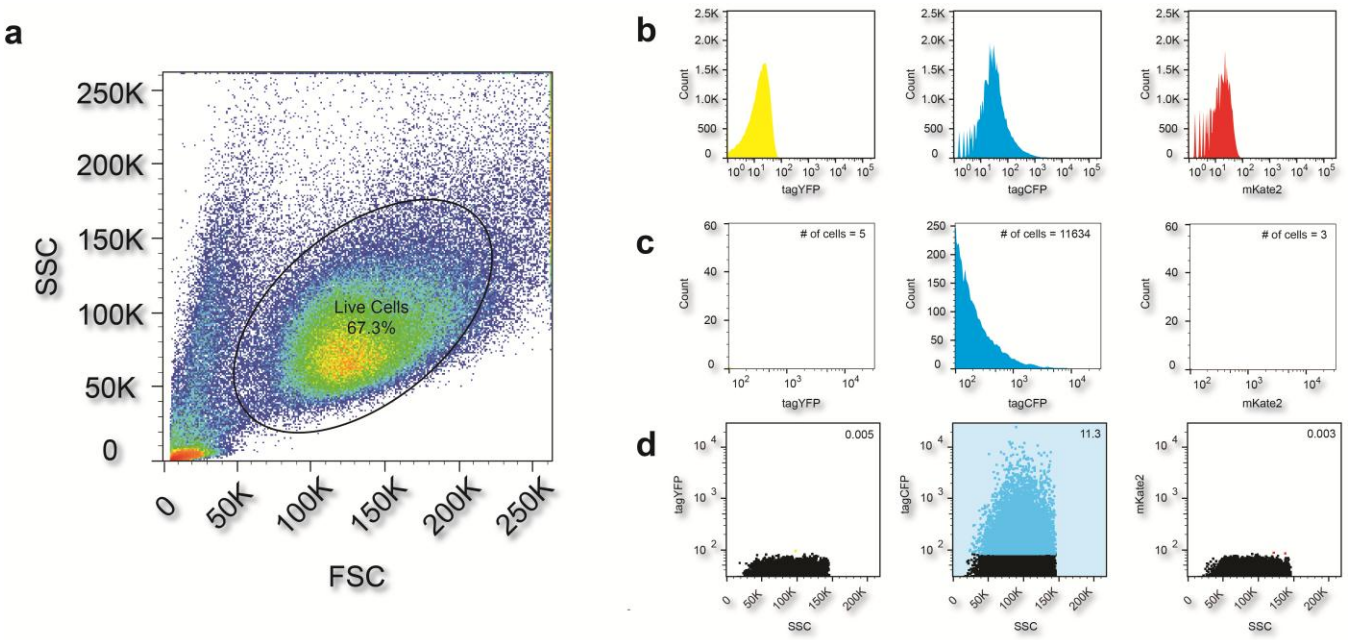
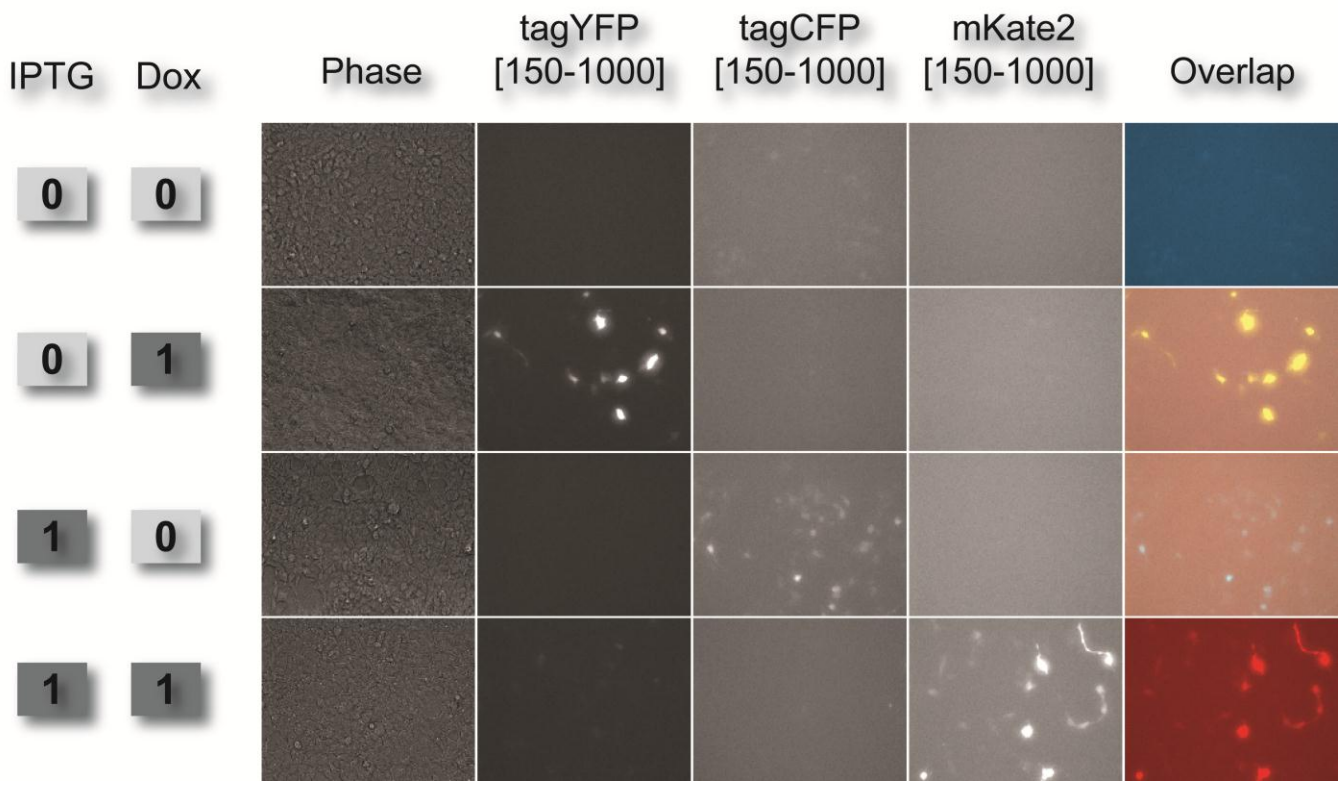
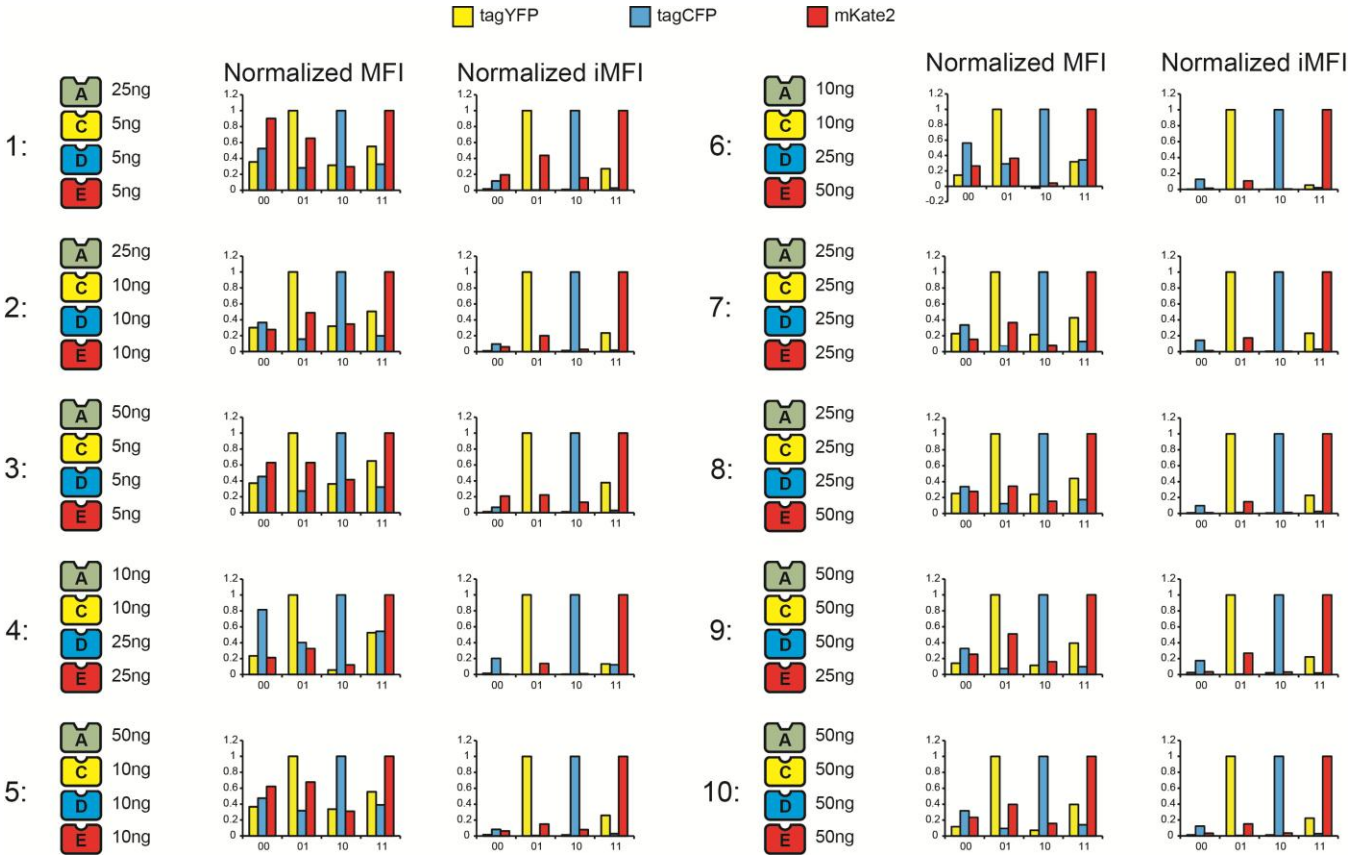
Supplementary Figures



Supplement Figure 1. Gating for case '10' of the decoder. (a) Side scatter versus forward scatter gating used to select single cell events. The gate is shown as the ellipse on the scatter plot. A single gate was created based an individual sample and kept constant between all samples of a particular experiment. **(b)** Histograms of the gated single cells from panel a. These histograms are the raw fluorescence intensities of tagYFP, tagCFP, and mKate. **(c)** Histograms for cells above the background fluorescence gate (set at 10^2 au). In the upper-right hand corner of each histogram is the number of cells for each color. In this case ('10'), we observe only tagCFP signal, while tagYFP and mKate2 signals are OFF. **(d)** An alternative representation of the data as a scatter plot of fluorescence intensity versus side scatter. Once again, the only signal present is tagCFP, while tagYFP and mKate2 are OFF. In the upper right hand corner of each scatter plot is the frequency of cells that fall above the background auto-fluorescence gate. The background gate is shown visually on the graph, with black dots falling below this gate, while color dots (yellow, blue, and red) are cell events that fall above the threshold. The MFI of cells above the gate here as well as the frequency of cells above the gate here are used to calculate the iMFI.

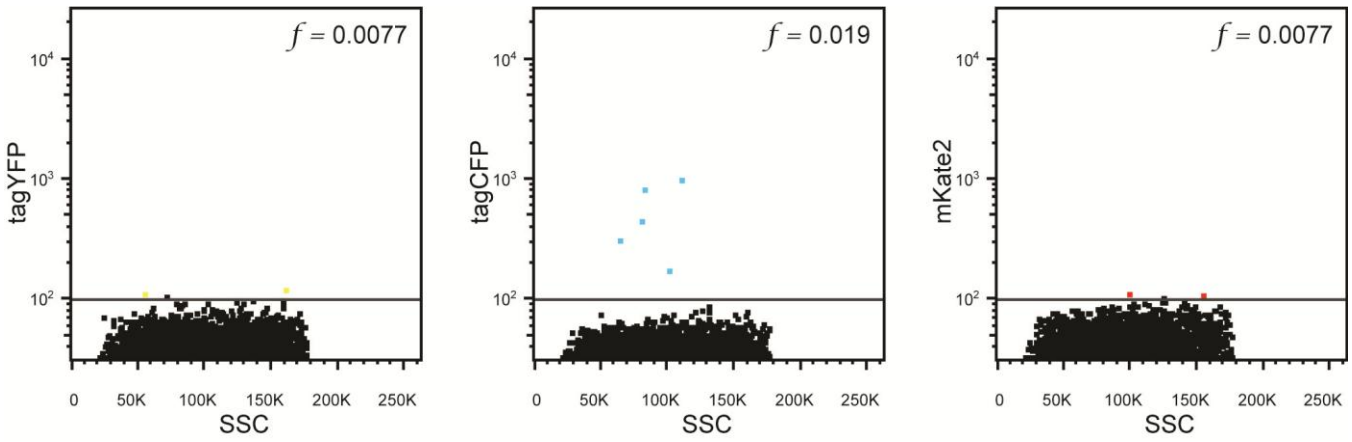


Supplement Figure 2. Fluorescence microscopy data. There are five columns, a bright field showing the live cells, a tagYFP, tagCFP, and mKate2 field, and finally an overlap of the three fluorescence fields. Each row is a different state of the decoder. Each field is normalized to the same intensity, so that images from the same column can be compared with each other.

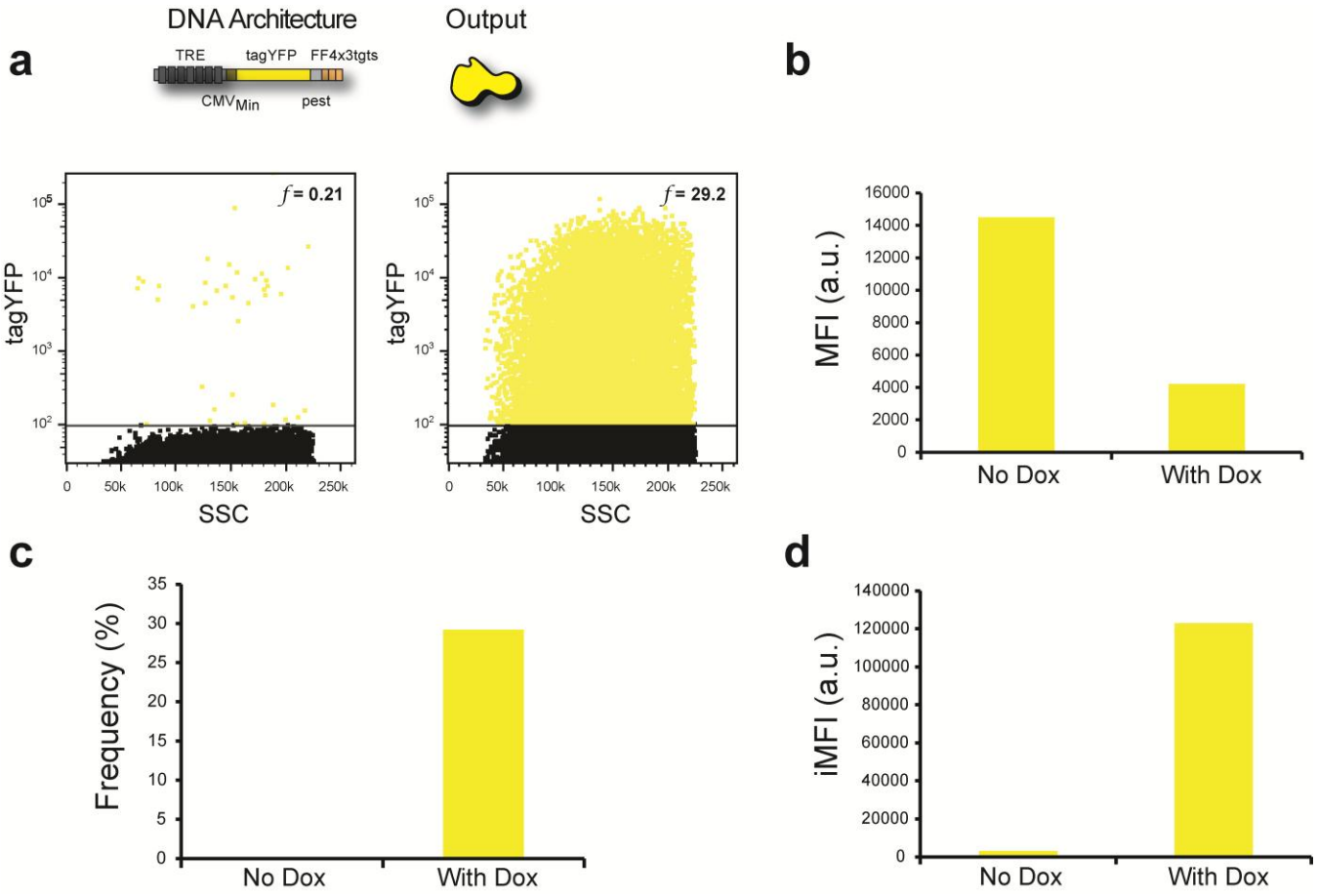


Supplement Figure 3. Titration experiments. Several different mass and molar ratios of circuit nodes displayed.

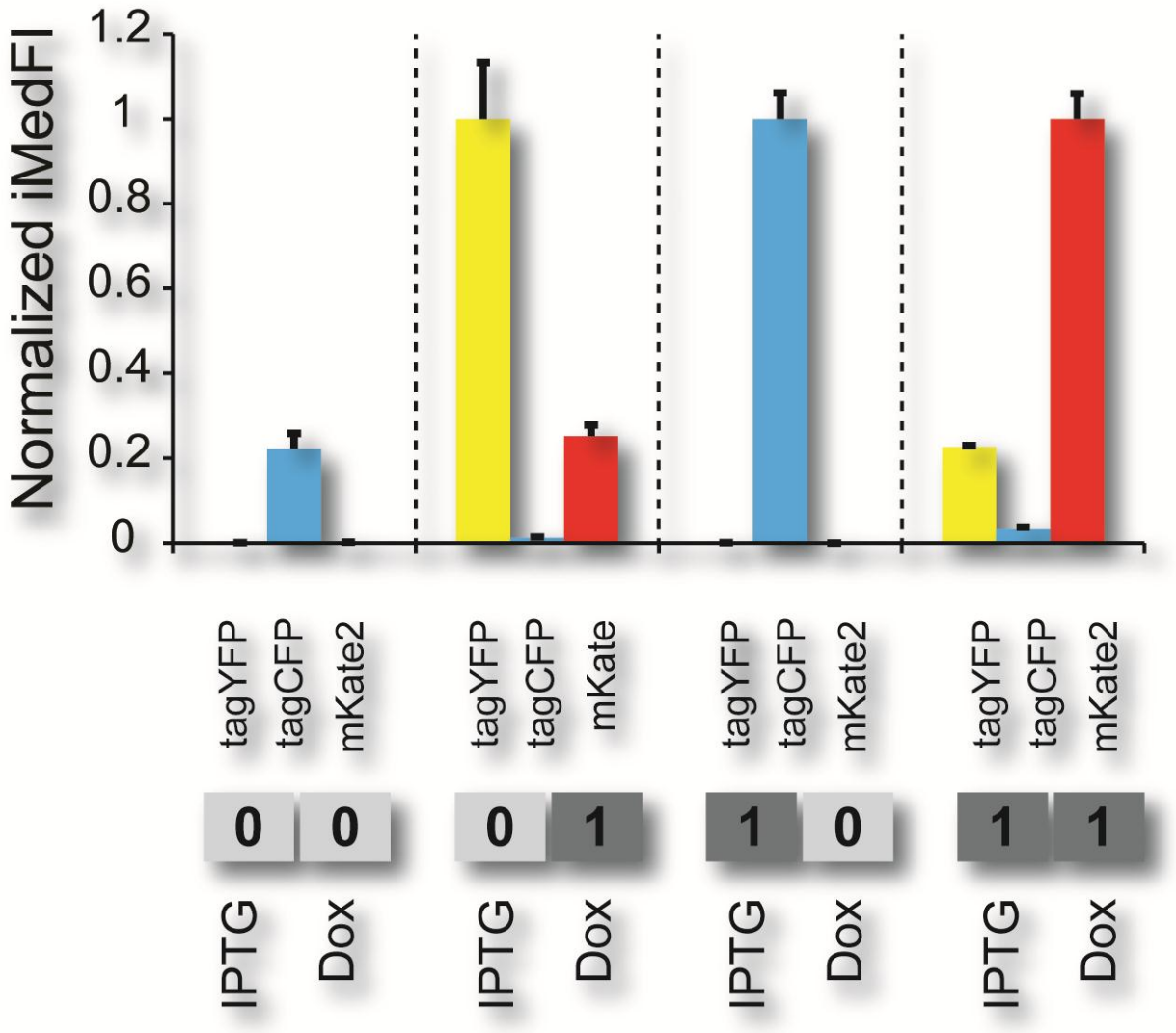
Each transfection state has both the normalized MFI and iMFI shown.



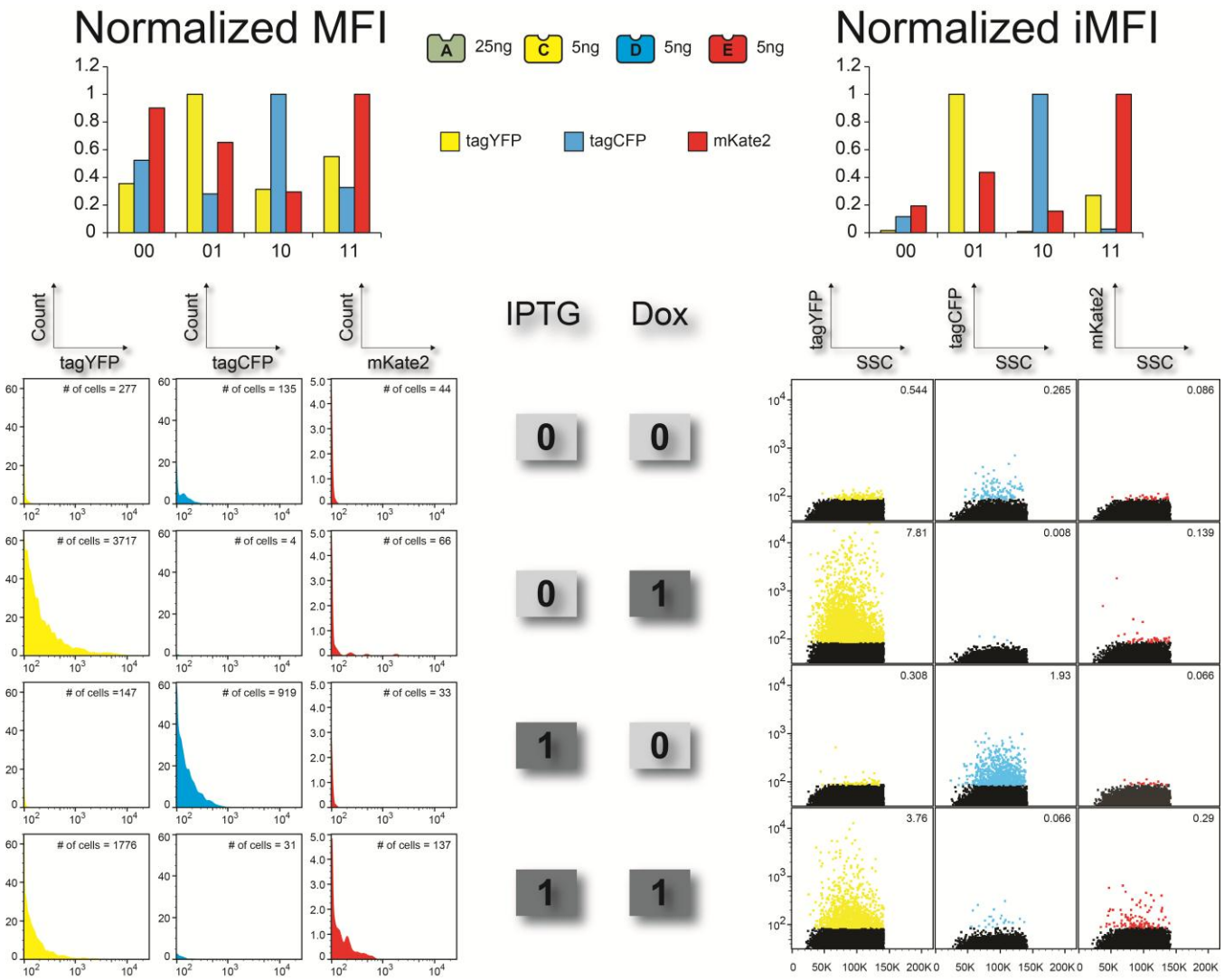
Supplement Figure 4. Untransfected cells for setting the background gate. A scatter plot for untransfected mammalian cells used to set a threshold for background fluorescence. The black horizontal line is the threshold and was set at 100 a.u. for all fluorescence fields.



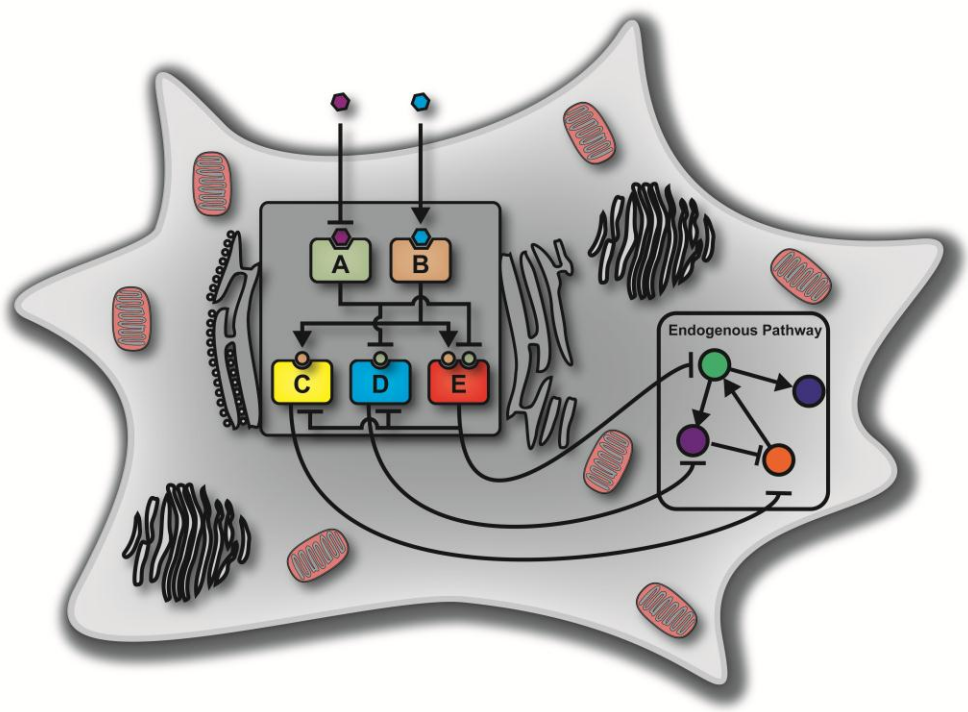
Supplement Figure 5. Control experiment for iMFI. (a) Flow cytometry data of tagYFP expression above background fluorescence mammalian cells transfected with node C of the decoder. There are two states: the "OFF" state with the absence of doxycycline and the "ON" state with the presence of doxycycline. **(b)** The mean fluorescence intensity is higher for the OFF state due to leaky cells. **(c)** Frequency of tagYFP expression in mammalian cells above background fluorescence. **(d)** Integrated mean fluorescence intensity of tagYFP expression in mammalian cells above background fluorescence.



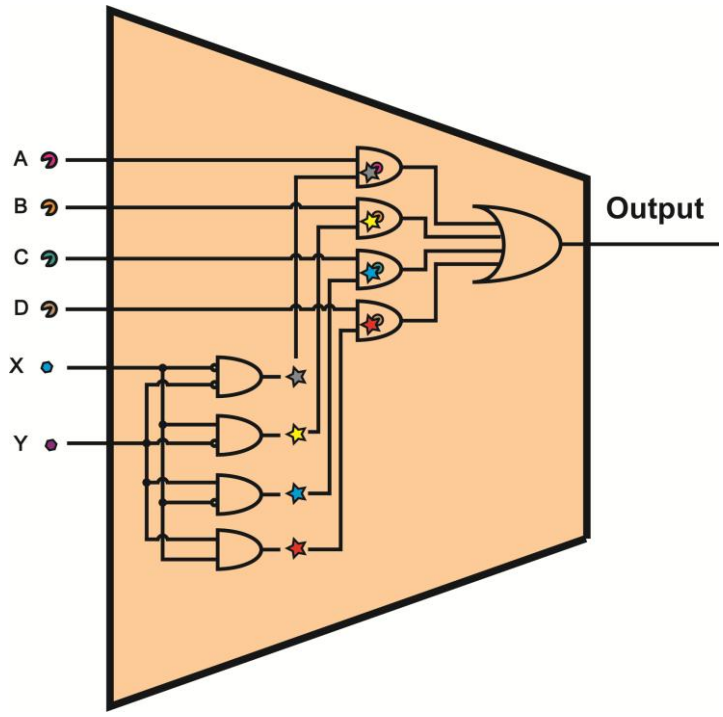
Supplement Figure 6. Integrated median fluorescence intensity (iMedFI). The same experiment as in Figure 3d (main text), but the integrated median fluorescence intensity was calculated instead of the integrated mean fluorescence intensity. Similar to the iMFI, the iMedFI shows at least 4-5 fold change between all ON and OFF states.



Supplement Figure 7. A side-by-side comparison of MFI and iMFI for condition 1 of Supplement Figure 2. On the left, the MFI and the corresponding histograms for the four states. On the right, the iMFI and the corresponding scatter plots for the four states. Each flow cytometry panel contains the number of cells or the frequency.



Supplement Figure 8. An illustration of the decoder circuit interfaced with an endogenous cellular pathway. Depending on the state of perturbation, the decoder can interface a variety of nodes within a given pathway.



Supplement Figure 9. A biological multiplexer. The decoder module decodes the combination of inputs (X, Y) and depending on the response generated, a line A, B, C, or D is chosen as the output line.

Enzymes and reagents

Phusion DNA polymerase (Thermo Scientific, catalog number: F-530S), Q5 High-Fidelity DNA polymerase (New England Biolabs, catalog number: M0493S), and dNTPs (New England Biolabs, catalog number N0447S) were used in various PCR amplification reactions. Endonuclease restriction enzymes and T4 DNA Ligase (catalog number: M0202S) were purchased from New England Biolabs and used in digestion and ligation reactions respectively. Calf intestinal alkaline phosphatase (New England Biolabs, catalog number: M0290S) was used for de-phosphorylation reactions. Doxycycline (Sigma-Aldrich, product number: D9891) was used in transfection of the synthetic biological circuit. Isopropyl β-D-1-thiogalactopyranoside (Sigma-Aldrich, product number: I6758) was used in transfection of the synthetic biological circuit.

Recombinant DNA Constructs

CMV_LacI-KRAB_FF5X3tgts_FF6X3tgts: A CMV_LacI-KRAB_FF5X3tgts_FF6X3tgts was used as a decoder node 2 [1].

UbC_2xLacO2_tagCFP_FF4X3tgts: CMV_tagCFP was purchased from Evrogen (catalog number: FP112). The pNEBR-R1 vector was purchased from New England BioLabs (catalog number: N8079). The pEXPR-IBA15 vector was purchased from IBA GmbH-Solutions For Life Sciences (catalog number: 2-1915-000). The pCMV_CuO_RFP_t2A_copGFP vector was purchased from System Biosciences (catalog number: QM350A). The UbC sequence was cloned out of the pNEBR-R1 using NheI and AscI restrictions enzyme sites and cloned into the pEXPR-IBA15 vector using NheI and MluI restriction enzyme sites. This produced the UbC vector. The CuO sequence was PCR amplified from the pCMV_CuO_RFP_t2A_copGFP vector using primers **P1** and **P2** and cloned into the UbC_mKate2_pest [2] vector using NheI and KpnI restriction enzyme sites. This produced the UbC-CuO vector (unpublished data). The Kozak_tagCFP sequence was PCR amplified from the CMV_tagCFP vector using primers **P3** and **P4** and cloned into the CMV_tagYFP_pest vector using BspEI and NheI restriction enzyme sites. This produced CMV_tagCFP_pest vector. The tagCFP_pest sequence was PCR amplified from the CMV_tagCFP_pest vector using primers **P5** and **P6** and cloned into the UbC_CuO vector with the KpnI restriction enzyme site. This produced the UbC_CuO_tagCFP_pest vector. A 2xLacO_tagCFP_pest sequence was PCR amplified from the UbC_CuO_tagCFP_pest vector using primers **P7** and **P8** and cloned into the UbC_CuO_tagCFP_pest vector NheI and KpnI restriction enzyme sites. The primers from the previous PCR (**P7** and **P8**) contained an overhang with two consecutive lac operator sequences upstream to the sequence that bound the UbC_CuO_tagCFP_pest vector. This produced the UbC_2xLacO_tagCFP_pest vector. Primers **P9** and **P10** were annealed together and cloned into the UbC_2xLacO_tagCFP_pest vector using KpnI and BamHI restriction enzyme sites. This produced the UbC_2xLacO_tagCFP_pest_FF4X3tgts vector. The UbC_2xLacO-tagCFP sequence was PCR amplified from the UbC_2xLacO_tagCFP_pest_FF4X3tgts vector using the primers **P11 and P12** and cloned into a EF1α_FF3X3tgts_FF4X3tgts vector using ClaI and KpnI restriction enzymes. This produced the UbC_2xLacO_tagCFP_FF3X3tgts_FF4X3tgts vector.

TRE_CMV$_{min}$_2xLacO_mKate2_pest_miR-FF4: The pTRE3G vector was purchased from Clontech (catalog number: 631332). The pmKate2-C vector was purchased from Evrogen (catalog number: FP181). The pmKate2-C vector was first transformed into dam$^-$/dcm$^-$ competent *E. coli* (New England Biolabs, catalog number: C2925) to free the XbaI restriction site. The pest sequence was PCR amplified from Switchgear Genomics luciferase reporter system for SPERPINE1 (catalog number: S721729) using primers using primers **P13** and **P14** and cloned into the pmKate2-C vector using the BspEI and XbaI restriction enzyme sites. This produced CMV_mKate2_pest. A miR-FF4 sequence was PCR amplified from the p234-PCMV-Neo-FF4 [3] vector using primers **P15** and **P16** and cloned into the CMV_mKate2_pest vector using ClaI and EcoRI restriction enzyme sites. This produced CMV_mKate2_pest_miR-FF4 vector. The mKate2_pest_miR-FF4 sequence was PCR amplified from the CMV_mKate2_pest_miR-FF4 with Primers **P17** and **P18** and cloned into the pTRE3G vector using BamHI and EcoRV restriction enzymes sites. This produced TRE_CMV$_{min}$_mKate2_pest_miR-FF4. Primers **P19** and **P20** were annealed together and cloned into the TRE_CMV$_{min}$_mKate2_pest_FF4 vector using BglII and BamHI restriction enzyme sites. This produced the TRE_CMV$_{min}$_2xLacO_mKate_pest_FF4 vector.

TRE_CMV$_{min}$_YFP_pest_FF3X1tgts_FF4X3tgts: The Tet-On-3G System Bidirectional vector (pTRE3G) was purchased from Clontech (catalog number 631332). pCMV_YFP-C was purchased from Evrogen (catalog number: FP131). The pest sequence was PCR amplified from Switchgear Genomics luciferase reporter system for SPERPINE1 (catalog number: S721729) using primers **P21** and **P22** and cloned into pCMV_YFP-C vector using BglII and SalI restriction enzyme sites. This generated the pCMV_tagYFP_pest vector. The pCMV_tagYFP_PEST was PCR amplified from above plasmid using primers **P23** and **P24** and cloned into EF1α_FF3X3tgts_FF4X3tgts vector using ClaI restriction enzyme sites. This generated CMV_tagYFP_pest_FF3x3tgts_FF4x3tgts vector. The tagYFP_pest_FF3X3tgts_FF4X3tgts sequence was PCR amplified from CMV_tagYFP_pest_FF3X1tgts-FF4X3tgts vector using the primers **P25** and **P26** and cloned into the pTRE3G vector using EcoRI and KpnI restriction enzyme sites. This produced the TRE_CMVmin_YFP_pest_FF3X1tgt_FF4X3tgts vector.

Primers List

Primer ID	Sequence	Function
P1	CAGTACGCTAGCCGCAAATGGGCAAGCTTGCC	Forward primer for cumate operator
P2	CAGTACGGTACCACCAAACTTGGACCTGGGAG	Reverse primer for cumate operator
P3	CAGTACGCTAGCACCGGTCGCCACCATGAGCGGGGG	Forward primer for tagCFP
P4	CAGTACTCCGGAGCGGTACAGCTCGTCCATG	Reverse primer for tagCFP
P5	CAGTACGGTACCCGCCACCATGAGCGGGGGCGAGGAG	Forward primer for tagCFP-pest
P6	CAGTACGGTACCTTAGACGTTGATCCTGGCGCTGGCG	Reverse primer for tagCFP pest
P7	CAGTACGCTAGCAATTGTGAGCGCTCACAATTCCACAACCTAGAATTGTGAGCGCTCACAATTCCACAACCTAGCGCCACCATGAGCGGGGGCGAGGAG	Forward primer for 2xLacO-tagCFP-pest
P8	CAGTACGGTACCTTAGACGTTGATCCTGGCGCTGGC	Reverse primer for 2xLacO -tagCFP-pest
P9	CCCGCTTGAAGTCTTTAATTAAACCGCTTGAAGTCTTTAATTAAACCGCTTGAAGTCTTTAATTAAAG	Forward primer for miR-FF4x3tgts
P10	GATCCTTTAATTAAAGACTTCAAGCGGTTTAATTAAAGACTTCAAGCGGTTTAATTAAAGACTTCAAGCGGGGTAC	Reverse primer for miR-FF4x3tgts
P11	CAGTACATCGATATACGCGCCGGCCTCCGCGCCGGGT	Forward primer for UbC-2xLacO-tagCFP
P12	CAGTACGGTACCTTAGCGGTACAGCTCGTCCATGCCGTGG	Reverse primer for UbC-2xLacO-tagCFP
P13	CAGTACTCCGGATCTCACGGCTTCCCTCCCGAGGTGG	Forward primer for pest domain
P14	CAGTACTCTAGATTAGACGTTGATCCTGGCGCTGGCG	Reverse primer for pest domain
P15	CAGTACTCTAGAGCGGCCGCAAGCCTTGTTAAGTGCT	Forward primer for miR-FF4
P16	CAGTACTCTAGACGGTCGAACGCGTCGCCGCGTGTTT	Reverse primer for miR-FF4
P17	CAGTCGGGATCCCGCCACCATGGTGAGCGAGCTGATT	Forward primer for mKate-pest-miR-FF4
P18	CAGTCGGATATCGCGTGTTTAAACGCATTAGTCTTCC	Reverse primer for mKate-pest-miR-FF4
P19	GATCTAATTGTGAGCGCTCACAATTCCACAACCTAGAATTGTGAGCGCTCACAATTCCACAACCTAGG	Forward primer for 2xLacO
P20	GATCCCTAGGTTGTGGAATTGTGAGCGCTCACAATTCTAGGTTGTGGAATTGTGAGCGCTCACAATTA	Reverse primer for 2xLacO

P21	CAGTACAGATCTTCTCACGGCTTCCCTCCCGAGGTGG	Forward primer for pest domain
P22	CAGTACGTCGACTTAGACGTTGATCCTGGCGCTGGCG	Reverse primer for pest domain
P23	CAGTACATCGATTAGTTATTAATAGTAATCAATTACG	Forward primer for pCMV-tagYFP-pest
P24	CAGTACATCGATGTTAAGATACATTGATGAGTTTGGAC	Reverse primer for pCMV-tagYFP-pest
P25	CAGTACGAATTCCGCCACCATGGTTAGCAAAGGCGAG	Forward primer for tagYFP-pest-FF3x3tgts-FF4x3tgts
P26	CCAGGTACCTTATCTAGATCCGGTGGATCC	Reverse primer for tagYFP-pest-FF3x3tgts-FF4x3tgts

Biological Parts

Biological Component	Description
CMV	The Cytomegalovirus immediate-early promoter which causes strong constitutive transcription of the gene downstream.
rtTA	The Tet-On 3G protein that binds the Tet Respone Element (TRE) when bound by doxycycline and therefore activates transcription of the gene downstream of the TRE site.
LacI-KRAB	A DNA-binding protein fused with the Krüppel associated box (KRAB) that binds the operator sequence of the lac operon therefore preventing transcription of the gene downstream of the lac operon sequence.
KRAB	The Krüppel associated box (KRAB) is a transcriptional repression domain.
IPTG	Isopropyl β-D-1-thiogalactopranoside (IPTG) is a molecular compound that binds to the lac repressor protein (LacI), and releases the tetrameric repressor from the lac operator sequence and allowing transcription of the gene downstream of the lac operator sequence.
Doxycycline	A tetracycline antibiotic that binds the Tet-On 3G protein, rtTA, causing a conformational change, allowing the complex to bind the Tet Responsive Element (TRE). These events activate transcription of the gene downstream of the TRE site.
TRE	A DNA sequence that consists of 7 repeats of the 19 base pair bacterial tet-operator sequence. The synthetic site allows the binding of the protein rtTA, which drives transcription of the gene downstream of the TRE site.
CMV_{min}	A 59 nucleotide sequence of CMV promoter sequence that only drives gene transcription when the adjacent enhancer region is bound by the activating transcription factor (in this case rtTA and the Tet Response Element).
LacO	The DNA sequence known as the lac operator which is bound by the lac repressor (LacI).
tagYFP	Yellow Fluorescent Protein
tagCFP	Cyan Fluorescent Protein
mKate2	Far-red Fluorescent Protein
miR-FF4	A synthetic micro RNA produced from miR-30.
FF4X3tgts	Perfect complimentary microRNA targets that the synthetic microRNA miR-FF4 bind.

References

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2. Li, Y., Moore, R., Guinn, M., and Bleris, L. (2012) Transcription activator-like effector hybrids for conditional control and rewiring of chromosomal transgene expression. *Sci. Rep. 2*.

3. Leisner, M., Bleris, L., Lohmueller, J., Xie, Z., and Benenson, Y. (2010) Rationally designed logic integration of regulatory signals in mammalian cells. *Nat Nano. 5*, 666-670.