Cellular heterogeneity mediates inherent sensitivity-specificity tradeoff in cancer targeting by synthetic circuits

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1. Materials and Methods

Plasmid Construction. The constructs used in this work were built by conventional restriction enzyme cloning and/or Gibson assembly (1). The 3rd generation lentiviruses delivery plasmids (Plasmids 22-29) were constructed by Gibson assembly of the different sequences into the pFUGW-H1 empty vector (2) (Plasmid 30). Information about plasmids and key sequences used in this study are given in **Tables S4 and S6**. Full sequences and samples of plasmids can be provided upon request.

Cell Culture and Transfections. Human colorectal tumor cells HCT 116 were obtained from the American Type Culture Collection (ATCC® CCL-247™). Low-passage HEK293T human kidney fibroblasts and WI-38 lung cancer cells T/NEO and T3 cell lines were coming from the laboratory of Professor Varda Rotter. HCT 116 were maintained in RPMI-1640 medium (Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Life Technologies) and 2 mM L-glutamine (Biological Industries, Israel). HEK293T were maintained in Dulbecco's modified Eagle's medium (Biological Industries) supplemented with 10% FBS and 2 mM L-glutamine. WI-38 cells were maintained in Minimal Essential Medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml Penicillin-Streptomycin (Life Technologies) and 1mM Sodium Pyruvate (Biological Industries). All cell lines were grown at 37 °C in a 5% CO2 incubator.

HCT 116, WI-38 and HEK293T cells were transfected with FuGENE®HD Transfection Reagent (Promega, Fitchburg, Wisconsin) at a fixed ratio of 2 µg DNA to 7 µl of reagent, diluted in 100 µl Opti-MEM (Life Technologies). Plasmids were mixed at a fixed amount of 0.5 µg/plasmid and the empty vector pGEM-T-Easy (Promega) was used to reach 2 µg DNA for transfection with less than 4 plasmids. Plasmids compositions for the different experiments are given in **Table S5.** Each transfection was made using 800,000 cells/well in a 6-well plate, and medium was replaced with fresh growth medium 24 h after transfection.

Lenti-Viral Infections. Stably infected cell lines were obtained using a third generation lenti-viral system (3). The pFUGW vector containing the sequence of interest (Plasmids 22 to 29) were co-transfected with the packaging vectors pCMV-dR8.91 (Plasmid 20) and pCMV-VSV-G (4) (Plasmid 21) into HEK293T cells (see above for transfection protocol). The growth medium was collected 48 hours after transfection, filtered with 0.22 µm filter (Millipore, Bedford, MA) and directly used to infect 800,000 target cells in single well of a 6-well plate, thus maintaining the same multiplicity of infection for each virus over all cell lines. Infection media was supplemented with 10 µg/ml polybrene. Each infection was performed with two lentiviruses simultaneously in a 1:1 volume ratio. Growth medium was replaced after 1 day and the infected cells were transferred to a 10 cm plate after 2 days. A second round of infections with two additional plasmids was performed with the same protocol to obtain WI-38 cell lines stably expressing our four constructs: cell-identification constitutive fluorescence, inputs and output of the desired DPI design (see **Table S5** for plasmids composition of each infected cell line). Infection efficiency was assessed by considering the expression level of the fluorescent reporter controlled by the constitutive promoter hUbC, mKate2 (5) for WI-38/T3 cells and CFP for WI-38/T/NEO (**Fig. S7**). T3 and T/NEO cells presented an infection efficiency of 96 ± 1.1 % and 98 ± 0.4 % respectively (mean ± s.d., 9 independent samples of 10 000 cells).We assumed an infection efficiency for the DPI circuit of approx. 88% and 94% for T3 and T/NEO respectively.

Flow Cytometry. HCT 116 cells were prepared for flow cytometry 48h after transfection. Cells were collected, centrifuged for 3 minutes at 3,000 rpm and the supernatant was discarded. The cells pellet was re-suspended in 600 to 900 µl PBS and immediately assayed with a LSRII flow cytometer (BD Biosciences, Franklin Lakes, NJ). A population of 100,000 cells, with positive Sirius (6) level compared to untransfected sample, was recorded per sample in each data set. Experiments were done independently three times with similar conditions and FACS settings (see SI appendix for optical and compensation settings). The 5-WT design was used in all experiments to ensure the reproducibility (**Fig. S4**). Datasets were analyzed using Matlab (The MathWorks, Natick, MA). As fluorescence distributions were roughly log-normal, we computed the median value over cells for simple quantification distributions. In figures, median data were averaged over multiple experiments with error bars representing the standard deviation. A more detailed description of how the Inputs/Output maps and gate profiles were build is given in **SI sections 3 and 4**.

Transfected or infected WI-38/T3 and WI-38/T/NEO cells were mixed at a 1/1 ratio and grown for 4 days. Cells were collected, centrifuged for 3 minutes at 3,000 rpm and the supernatant was discarded. The cell pellet was re-suspended in 500µl PBS and immediately assayed with the LSRII flow cytometer. At least 10,000 cells were recorded per sample in each data set. Experiments were done independently three times with similar conditions and FACS settings, for transfected cells (**Fig. S7**) and infected cells (**Fig. 5**).

Killing Assay of tumor-like cell culture. To generate the tumor-like cell culture assay for each DPI construct, we first deposited a 2 µl sessile drop of T3 cells at 2.10^5 cells/ml in a 8-wells Lab-Tek II chamber (Thermo Scientific, Waltham, MA) and allowing the cells to adhere for 15 mn in the incubator. T/NEO cells, 200 µl at 5.10^4 cells/ml, were then carefully added to the well. After 1 day, growth medium was changed for a fluorescence imaging medium: Leibowitz-15 without phenol red (Life Technologies) supplemented with 10 % FBS, 2mM L-glutamine, 100 U/ml Penicillin-Streptomycin and 1 mM Sodium Pyruvate. After 4 days, a final concentration of 10 µM Ganciclovir (Sigma Aldrich, Saint Louis, MO) was added to the treated wells, and an equivalent volume of PBS to the control wells. After 24 hours, cells were washed once with PBS and imaging medium was replaced. The imaging medium was then replaced every 2 days. The Lab-Tek chambers were imaged daily from day 1 to 18. For some experiments additional images were also taken up to day 35.

Fluorescence Microscopy. Fluorescence images were acquired on an inverted microscope Axiovert 200M (Carl Zeiss GmbH, Germany) equipped with a Neo sCMOS camera (Andor, Belfast, Ireland). We used 10x or 20x magnification objectives to image HCT 116 and WI38 mixtures. A 2x magnification objective was used to image tumor-like cultures. The following filter sets were used to image CFP, YFP and mCher/mKate2 fluorescence respectively (central wavelength and bandwidth in nm for excitation and emission filters): Ex: 436 / 20 nm, Dichroic: 455 DRLP, Em: 480 / 40 nm ; Ex: 500 / 20 nm, Dichroic: 515 DRLP, Em: 535 / 30 nm ; and Ex: 560 / 40 nm, Dichroic: 585 DRLP, Em: 640 / 40 nm. Data were analyzed using ImageJ (NIH, Bethesda, MD) and Matlab (The MathWorks).

2. Four-color flow cytometry calibration

Each color was compensated with the respect to the others to avoid fluorescence crosstalk between channels. We used cell samples expressing a single fluorescent protein: Sirius, CFP, YFP and mCherry. In figure S1 we present the 6 bi-axial graphs after compensation on the Hoechst (Sirius), Pacific Blue (CFP), YFP and mCherry channels for the four colors samples (see Table S1 and S2 for optical and compensation settings, respectively).

Table S1: FACS fluorescence filters settings

Channel	Fluorescent Protein	Laser Excitation Wavelength	Long Pass Filter	Emission Filter (Band Pass)
Hoechst	Sirius	355 nm	-	BP 450/50
Pacific Blue	ECFP	407 nm	-	BP 470/15
YFP	EYFP	488 nm	505nm	BP 550/30
mCherry	mCherry, mKate2	561 nm	600nm	BP 610/20

Table S2: FACS fluorescence compensation setup. Compensation was done with BD FACSDiva (version no. 6.1.3; BD Biosciences) as detailed below.

Fluorochrome	-% Fluorochrome	Spectral Overlap
ECFP	YFP	0.10
mCherry	YFP	4.00
Sirius	YFP	0.00
YFP	ECFP	0.13
mCherry	ECFP	0.00
Sirius	ECFP	0.22
YFP	mCherry	0.10
ECFP	mCherry	0.15
Sirius	mCherry	0.10
YFP	Sirius	0.00
ECFP	Sirius	15.30
mCherry	Sirius	0.00

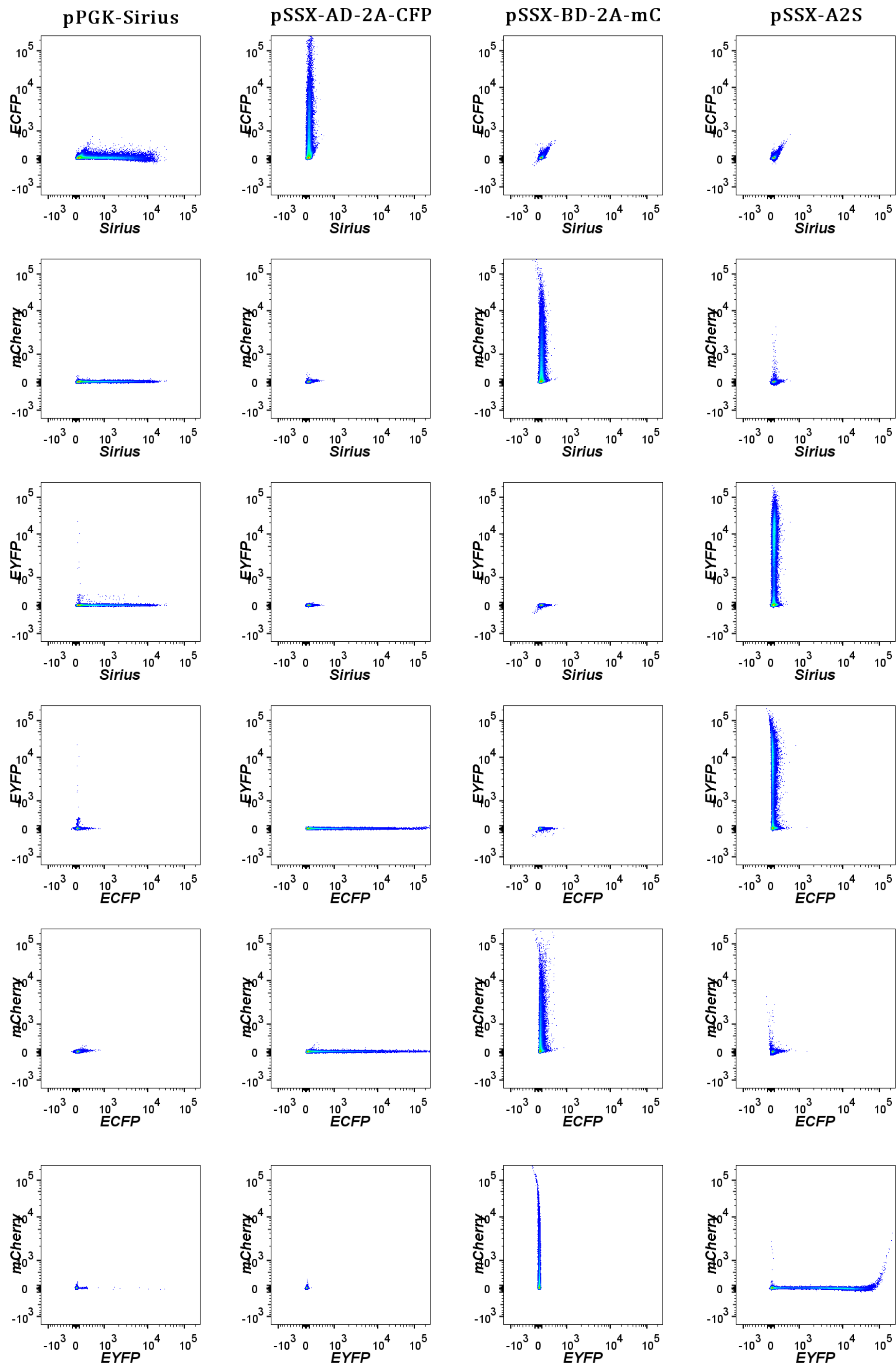
Fig. S1: Fluorescent signals crossovers following compensations for a typical FACS experiment.

pPGK-Sirius: Sirius fluorescence only, pPGK-Sirius (plasmid 01).

pSSX-AD-2A-CFP: CFP fluorescence only, pSSX1-NLS-VP16AD-DocS-2A-CFP (plasmid 05).

pSSX-BD-2A-mC: mCherry fluorescence only, pSSX1-Gal4BD-Coh2-2A-mCherry (plasmid 14).

pSSX-A2S : YFP fluorescence only, pSSX1-NLS-VP16AD-DocS (plasmid 02) + pSSX1-Gal4BD-Coh2 (plasmid 03) + p14x-UAS-YFP (plasmid 19).



pPGK-Sirius
pSSX-AD-2A-CFP
pSSX-BD-2A-mC
pSSX-A2S
ECFP
mCherry
EYFP
Sirius
ECFP
EYFP

3. Input-Output (I/O) response function map of raw data and data corrected by constitutive Sirius expression

We measured single cell fluorescence levels for the 3x3 promoter pairs (CycD1/CycD1, CycD1/SSX1, CycD1/H2A1, SSX1/CycD1, SSX1/SSX1, SSX1/H2A1, H2A1/CycD1, H2A1/SSX1 and H2A1/H2A1). For each promoter pair, $1x10^5$ cells were recorded using their Sirius levels as a reporter for positive transfection. We assumed that each Sirius-positive cell was co-transfected with the four plasmids with equal probability. Samples from 3 experiments were pooled together. To build the map, the 2D-input space was discretized in 48x48 squares following an hyperbolic sine scale. Output and constitutive values of cells in input regions containing more than 100 cells were averaged (**Fig. S2**). Matlab contour plot was used to render a smoother plot of the map.

In raw data, both constitutive and output signals were graded and increased concomitantly with the input level (**Fig. S2**). We assumed that the graded constitutive signal accounted for a cell-dependent link between the four reporters expression levels, probably due to variations in plasmid copy number after transfection, and variability in cell size or overall metabolism. To reduce this extrinsic noise at the single-cell level, inputs and output fluorescence values were divided by the Sirius fluorescence value and then multiplied by the mean Sirius value in the sample. Sirius level was kept as is. I/O maps were then plotted in the corrected inputs space with either corrected output or raw constitutive values (**Fig S3**). After correction of inputs and output by the constitutive level, the input space was indeed less stretched and the map of the constitutive signal was no more graded and more homogenous.

Depending on the promoter identity, similar input values can generate different YFP output, even after correcting for transfection variability. Because the measurements were done after 48hr, we hypothesize that variations in expression dynamics affect the promoter output. Synchrony in expression of the two inputs can result in higher output for a given apparent AD/BD value, as observed for symmetric promoter pairs SSX1/SSX1 and H2A1/H2A1. The input reporter proteins do not fully take this into account because they accumulate in the cytoplasm regardless of expression dynamics in the nucleus.

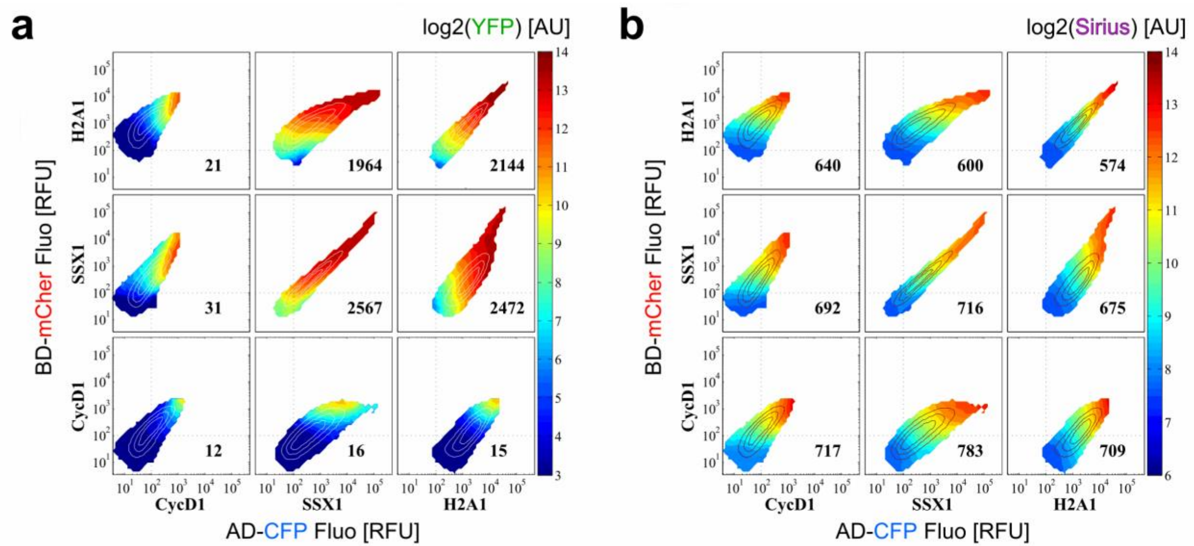


Fig. S2: Raw data of output levels for distinct promoter pairs. (a) YFP expression level (DPI output) for the 5-WT design. **(b)** Sirius expression level (constitutive) for the 5-WT design. Each promoter pair is plotted separately in the full input space (x=AD-CFP; y= BD-mCher). The median output is written in the bottom right corner. White or brown lines indicate the cell density, 70% of the cells are included within these regions. Three independent experiments on HCT 116 cells (3x10^5 cells per promoter pair).

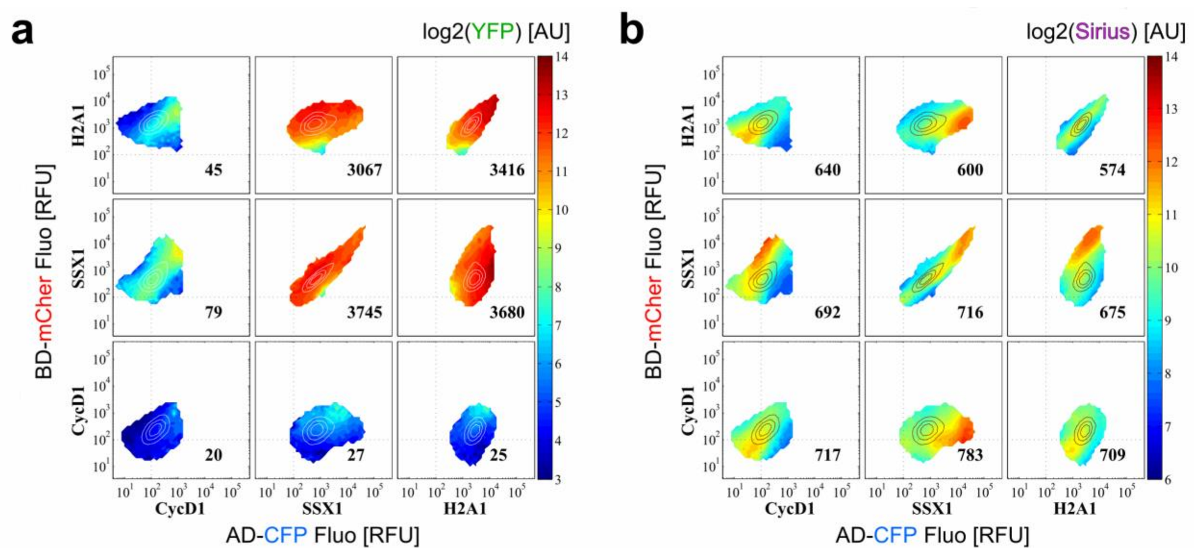


Fig. S3: Data in corrected input space for distinct promoter pairs. (a) YFP expression level (DPI output) for the 5-WT design after correction (see text above). **(b)** Sirius expression level (constitutive) for the 5-WT design without correction. Each promoter pair is plotted in the corrected full input space (x=AD-CFP; y= BD-mCher). The median output is written in the bottom right corner. White or brown lines indicate the cell density, 70% of the cells are included within these regions. Three independent experiments on HCT 116 cells (3x10^5 cells per promoter pair).

4. Gates profiles and sigmoidal fittings of parameters

To obtain output probability profiles for the activation gate for increasing concentration of activation domain AD (**Fig. 2E, left**) or concentration of binding domain BD (**Fig. 2E, right**) we selected cells with high BD or high AD values respectively (10^3 to 10^4 RFU). We then computed the output probability distribution at increasing input values with an arcsin spacing (for AD or BD, respectively). Distribution for all inputs were concatenated to obtain a matrix with output probability as column and for increasing input along rows. The probability of output was defined for each input range and not for the overall profile, the sum of probability being equal to 1 along each vertical line. A contour plot was used to smoothen the profile representation, but data used for further calculation were not modified.

For each input the median output was also computed to obtain the median profile, and we averaged the profiles of 3 independent experiments to obtain the final profiles shown in grey in **Fig. 2 E** (mean ± s.d.). Activation gate parameters given in **Table S3** were obtained by sigmoidal fittings of the 3 experiments.

Response function maps for the different experiments with 5-WT (**Fig. S4**) , as well as gate profiles for increasing sTF Binding Sites (**Fig. S5**) and for two-hybrid mutants (**Fig. S6**) are given below.

Table S3: Parameters of AND gates for the 5-WT design. Minima, maxima, activation thresholds and Hill coefficients were obtained from sigmoidal fittings of the median output, along the DocS-VP16AD-2A-CFP axis (AD) and along the Coh2-Gal4BD-2A-mCher axis (BD) :
$O(I) = O_{max} \cdot \left(\frac{I^n}{I^n + Th^n}\right) + O_{min}$, with *O* the output intensity, *I* the input intensity, *Th* the gating threshold and *n* the Hill coefficient. Mean ± s.d., 3 independent experiments.

Design	Output min (RFU)	Output max (RFU)	Threshold (RFU)	Hill coefficient
5-WT along AD	31.4 ± 10	3210 ± 238	510 ± 56	5.6 ± 1.1
5-WT along BD	-13.3 ± 13	3527 ± 206	574 ± 29	4.8 ± 1.2

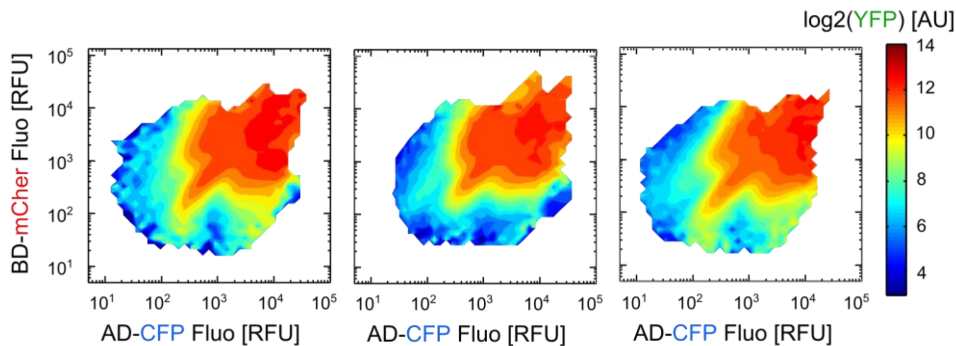


Fig. S4 : Response function map of 5-WT design with HCT 116 cells for 3 different experiments. Corrected data from all the 3x3 promoter pairs presented in Fig. S2 were pooled together to build the Input/Output response function map (9.10^5 cells per experiment).

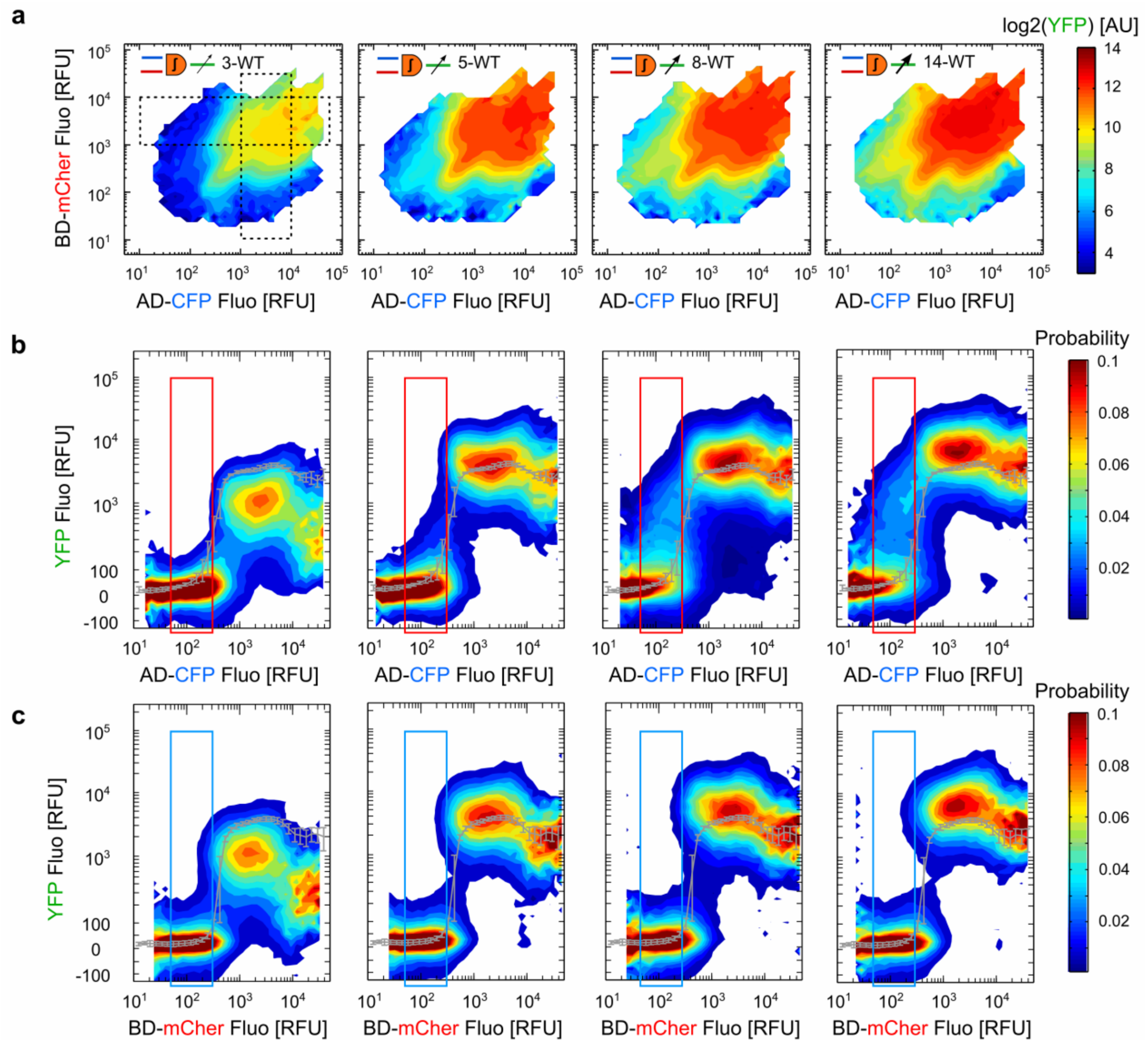


Fig. S5 : Response function maps and output probability profiles for increasing binding repeats and WT DocS/Coh2. (a) I/O maps for 3-WT, 5-WT, 8-WT and 14-WT (9.10^5 cells per map, single experiment). **(b)** Output probability profiles along the AD axis at fixed high BD (horizontal dashed box in a), for 3-WT, 5-WT, 8-WT and 14-WT. The red dashed box indicates the region used for reduced distrisbution in **Fig. 3F** (red box shown in **Fig. 3D**). In all the figures the grey line is the average median profile of 5-WT for 3 experiments, and is given as reference. **(c)** Output probability profiles along the BD axis at fixed high AD (vertical dashed box in a), for 3-WT, 5-WT, 8-WT and 14-WT. The blue dashed box indicates the region used for reduced distrisbution in **Fig. 3E** (blue box shown in **Fig. 3D**). In all the figures the grey line is the average median profile of 5-WT for 3 experiments, and is given as reference.

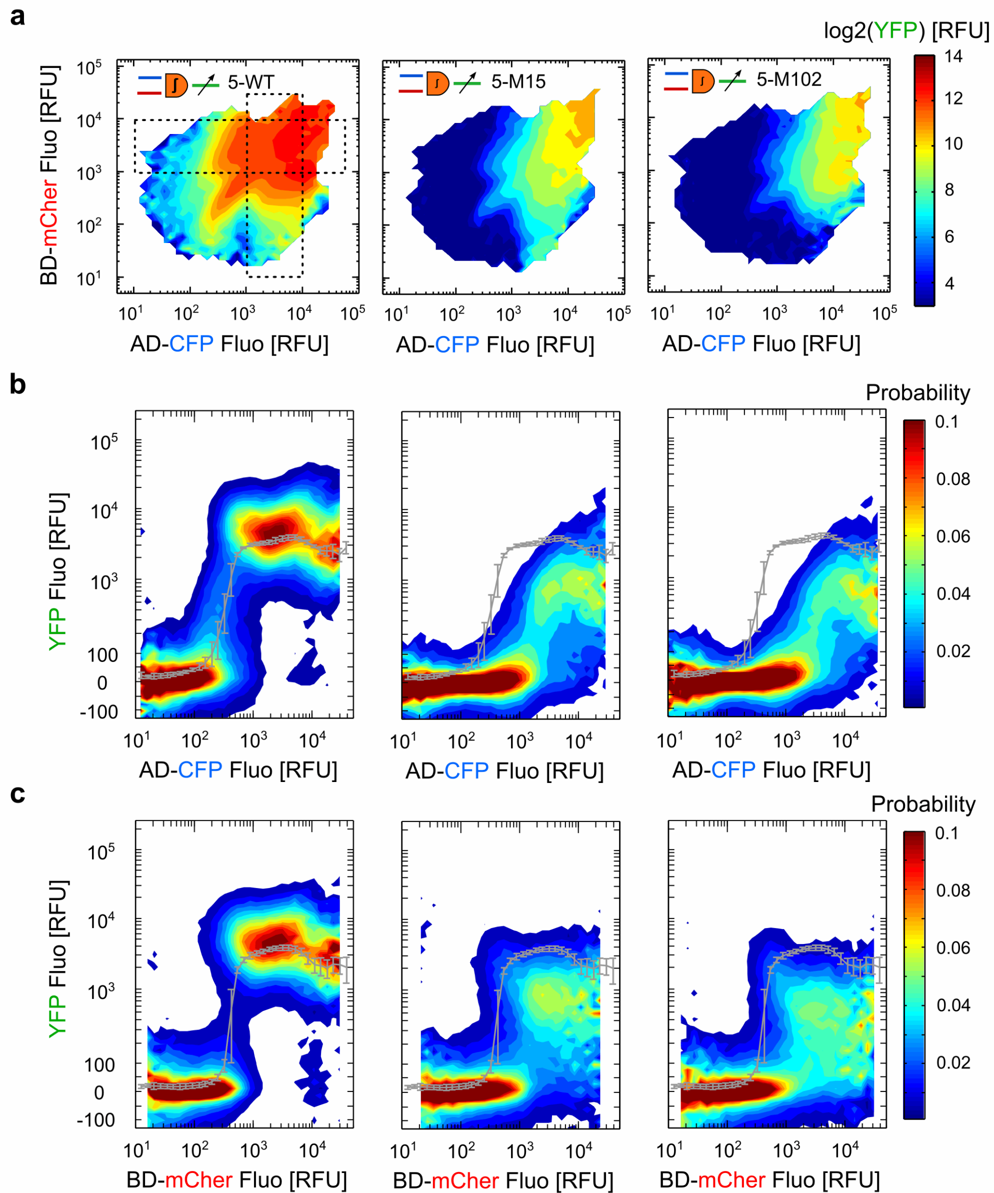


Fig. S6 : Response function maps and output probability profiles for 5 binding repeats with WT or weak affinity mutants of the DocS/Coh2. (a) I/O maps for 5-WT, 5-M15 and 5-M102 (9.10^5 cells per map, single experiment). **(b)** Output probability profiles along the AD axis at fixed high BD (horizontal dashed box in a), for 5-WT, 5-M15 and 5-M102. In all the figure the grey line is the average median profile of 5-WT for 3 experiments, and is given as reference. **(c)** Output probability profiles along the BD axis at fixed high AD (vertical dashed box in a), for 5-WT, 5-M15 and 5-M102. In all the figure the grey line is the average median profile of 5-WT for 3 experiments, and is given as reference.

5. Reproducibility of the SSX1-H2A1 5-WT design with transfected WI38 cells:

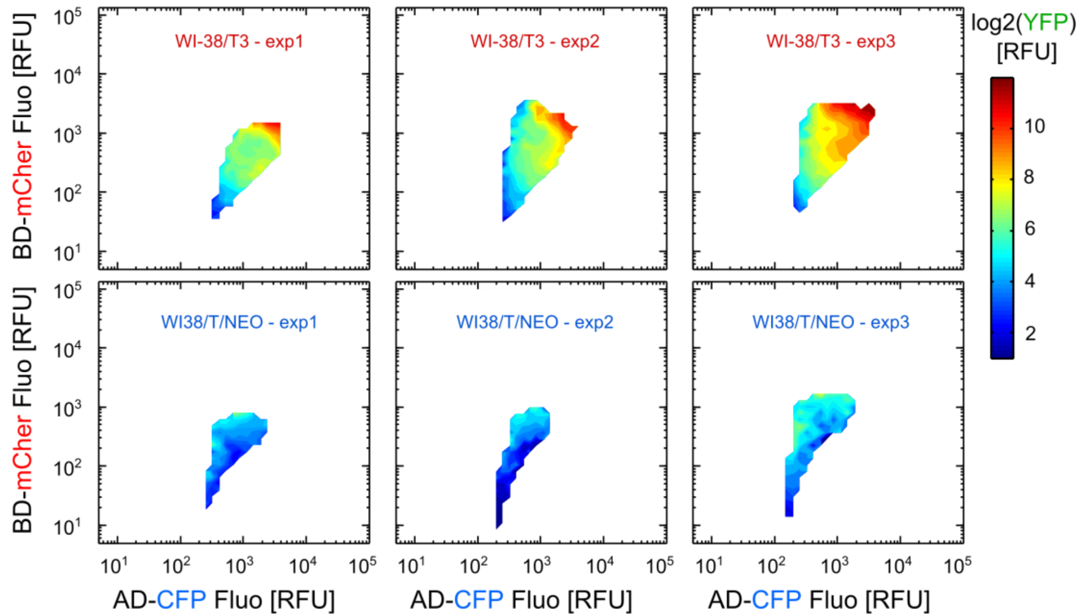


Fig. S7 : Response function maps of WI-38 cells for the SSX1/H2A1 promoter pair of the 5-WT design. I/O maps obtained with T3 cells (top row) and T/NEO cells (bottom) transfected with the 5-WT DPI for the SSX1/H2A1 promoter pair (transfection with plasmids 11,15,17 and 31). Collected data ($3x10^4$ cells per cell type and per experiment) were selected and corrected according to constitutive Sirius level. We used a constitutive CMV promoter (plasmid 31), instead of the PGK promoter (plasmid 1) used with HCT 116 cells, due to the low constitutive expression level obtained with PGK promoter.

6. Infection efficiency

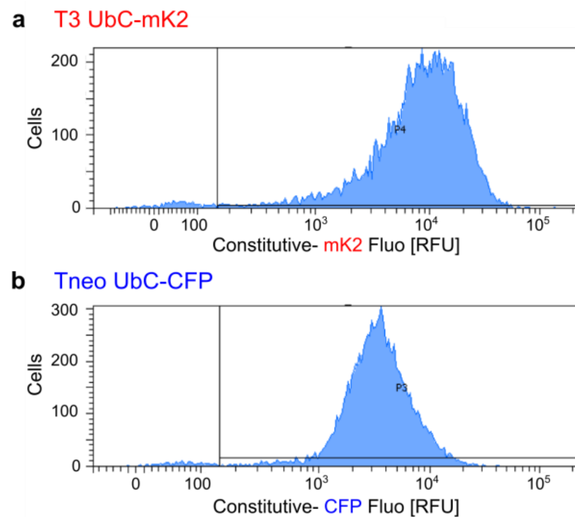


Fig. S8 : Lentiviral infection efficiency on T3 and T/NEO cells. (a) Distribution of mKate2 fluorescence in T3 cells infected by pFUGW-UbC-mK2, pFUGW-SSX1-NLS-VP16AD-DocS, pFUGW-H2A1-Gal4BD-Coh2 and pFUGW-3x-UAS-TK1-2A-YFP. **(b)** Distribution of CFP fluorescence in T/NEO cells infected by pFUGW-UbC-CFP, pFUGW-SSX1-NLS-VP16AD-DocS, pFUGW-H2A1-Gal4BD-Coh2 and pFUGW-5x-UAS-TK1-2A-YFP.

7. Receiver Operating Characteristic curves

A Receiver Operating Characteristic curves (ROC) illustrates the performance of a binary classifier with an increasing discrimination threshold (here the killing threshold based on YFP output). True positive rate (namely sensitivity) is plotted as a function of false positive rate (namely 1-specificity) for all the possible value of killing threshold. The ROC curve of a non-discriminating classifier is a straight line from (0;0) to (1;1). A perfect classifier will have points on (0;1).

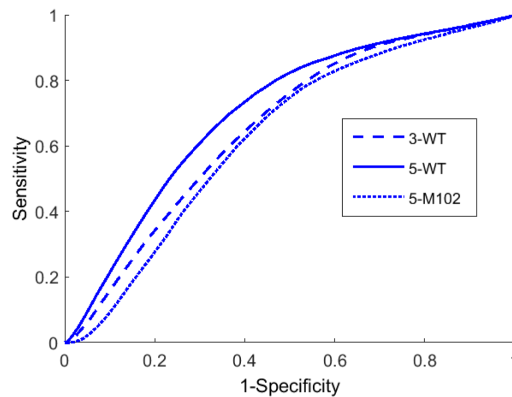


Fig. S9 : ROC curves for the different designs. Sensitivity and specificity of the design were calculated from FACS measurement of infected T3 and T/NEO cell mixtures, as shown in **Fig. 5E** (1.5 10^4 cells per design, 3 independent experiments). Values of area under the ROC curves are given in the main text.

8. Pixel analysis of fluorescence microscopy and effect of T3 to T/NEO cells proximity

Killing assays with mixed culture of T/NEO and T3 cells resulted in an enhanced killing of T/NEO cells compared to tumor like culture (**Fig. S10a**). We monitored during one week in culture the constitutive (CFP and mKate2 fluorescence for T/Neo and T3 respectively, **Fig. S10b**) and output level (YFP fluorescence, **Fig. S10c**). Distributions of fluorescence are given in percent of pixels having a given values, extracted from 4 images (2 independent samples, approx. 2000 cells). Pixels were assigned to T/NEO or T3 based on their exclusive constitutive level: CFP > 200 & mKate2 < 150 for T/NEO pixels, and mKate2 > 150 & CFP < 200 for T3 pixels. In co-culture, distribution of output for T/NEO cells evolved with time and became comparable to the distribution observed with T3 cells after 7 days (solid lines in **c**). This effect was not observed when cells were cultured separately (dashed lines in **c**). Distributions of the constitutive signals remained roughly unchanged when cells were mixed (CFP for T/NEO and mKate2 for T3 in **b**).

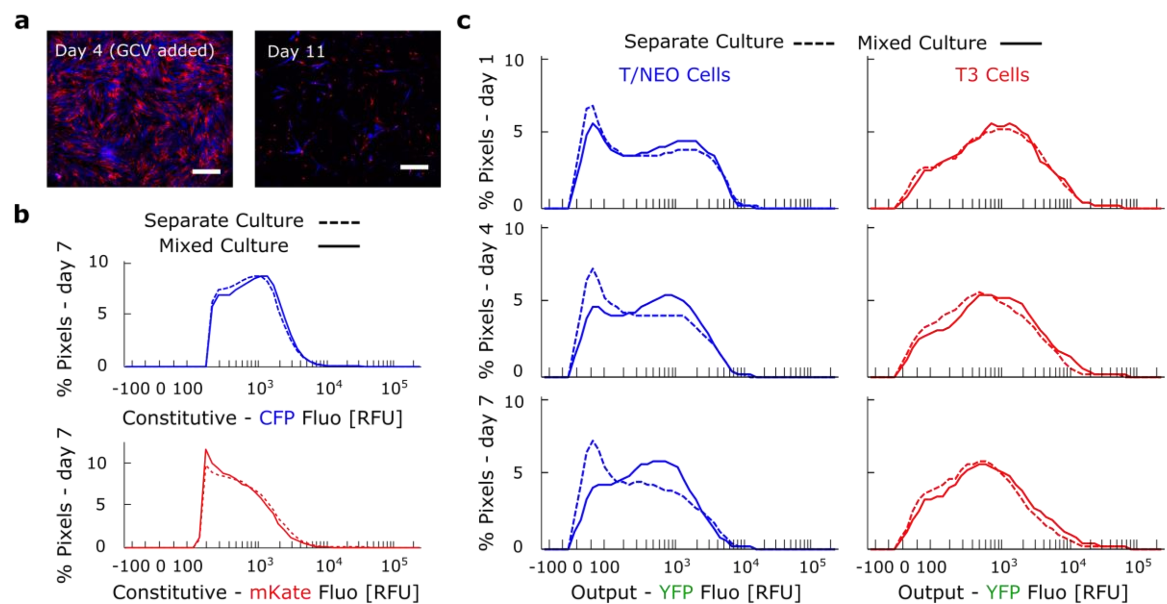


Fig. S10 : Circuit output is increased in T/NEO cells in contact with T3 cells. a) Fluorescence images of mixed T/NEO - T3 co-culture. A 10 µM GCV treatment is done at day 4 (**left**) and observed at day 11 (**right**). Scale bar 500 µm. **b)** Distributions of pixel fluorescence for the constitutive CFP (T/NEO) and mKate2 (T3) at day 7. **c**) Distributions of pixel fluorescence for the output (YFP) for T/NEO (blue) and T3 cells (red) at day 1, 4 and 7.

9. List of Plasmids used

Table S4: Constructs names and short descriptions. Full sequences and samples of plasmids can be provided upon request.

Plasmid n°	Full name	Description
Plasmid 01	pPGK-Sirius	Constitutive Sirius expression regulated by PGK promoter
Plasmid 02	pSSX1-NLS-VP16AD-DocS	Wild Type activation input regulated by SSX1 promoter – no reporter
Plasmid 03	pSSX1-Gal4BD-Coh2	Binding input regulated by SSX1 promoter – no reporter
Plasmid 04	pCycD1-NLS-VP16AD-DocS-2A-CFP	Wild Type activation input and CFP regulated by CycD1promoter
Plasmid 05	pSSX1-NLS-VP16AD-DocS-2A-CFP	Wild Type activation input and CFP regulated by SSX1 promoter
Plasmid 06	pH2A1-NLS-VP16AD-DocS-2A-CFP	Wild Type activation input and CFP regulated by H2A1promoter
Plasmid 07	pCycD1-NLS-VP16AD-DocS15-2A-CFP	Mutant activation input and CFP regulated by CycD1promoter
Plasmid 08	pSSX1-NLS-VP16AD-DocS15-2A-CFP	Mutant activation input and CFP regulated by SSX1 promoter
Plasmid 09	pH2A1-NLS-VP16AD-DocS15-2A-CFP	Mutant activation input and CFP regulated by H2A1promoter
Plasmid 10	pCycD1-NLS-VP16AD-DocS102-2A-CFP	Mutant activation input and CFP regulated by CycD1promoter
Plasmid 11	pSSX1-NLS-VP16AD-DocS102-2A-CFP	Mutant activation input and CFP regulated by SSX1 promoter
Plasmid 12	pH2A1-NLS-VP16AD-DocS102-2A-CFP	Mutant activation input and CFP regulated by H2A1promoter
Plasmid 13	pCycD1-Gal4BD-Coh2-2A-mCherry	Binding input and mCherry regulated by CycD1 promoter
Plasmid 14	pSSX1-Gal4BD-Coh2 -2A-mCherry	Binding input and mCherry regulated by SSX1 promoter
Plasmid 15	pH2A1-Gal4BD-Coh2 -2A-mCherry	Binding input and mCherry regulated by H2A1 promoter
Plasmid 16	p3x-UAS-YFP	3 repeats output
Plasmid 17	p5x-UAS-YFP	5 repeats output
Plasmid 18	p8x-UAS-YFP	8 repeats output
Plasmid 19	p14x-UAS-YFP	14 repeats output
Plasmid 20	pCMV-ΔR8.91	Lentivirus proteins Addgene #2221
Plasmid 21	pCMV-VSV-G	Lentivirus envelope Addgene #8454
Plasmid 22	pFUGW-hUbC-CFP	Constitutive CFP (Lentivirus)
Plasmid 23	pFUGW-hUbC-mKate2	Constitutive mKate2 (Lentivirus)
Plasmid 24	pFUGW-SSX1-NLS-VP16AD-DocS	WT activation input regulated by SSX1 promoter
Plasmid 25	pFUGW-SSX1-NLS-VP16AD-DocS102	Mutant activation input regulated by SSX1 promoter
Plasmid 26	pFUGW-H2A1-Gal4BD-Coh2	Binding input regulated by H2A1 promoter
Plasmid 27	pFUGW-3x-UAS-TK1-2A-YFP	3 repeats output with HSV-TK1 and YFP
Plasmid 28	pFUGW-5x-UAS-TK1-2A-YFP	5 repeats output with HSV-TK1 and YFP
Plasmid 29	pFUGW-14x-UAS-TK1-2A-YFP	14 repeats output with HSV-TK1 and YFP
Plasmid 30	pFUGW-H1	pFUGW-H1 empty vector Addgene #25870
Plasmid 31	pcDNA3-Sirius	Constitutive Sirius expression regulated by CMV promoter Addgene#51957

Table S5: Plasmid used for experiments shown in the different figures

Figure	Cell type	Design	Plasmids #	Inputs Promoters (AD, BD)	DocS derivative	Binding site repeats	Output
2 b, c	HCT 116	5-WT	1,4,13,17	CycD1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,4,15,17	CycD1, H2A1	WT	5	YFP
	HCT 116	5-WT	1,6,13,17	H2A1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,6,15,17	H2A1, H2A1	WT	5	YFP
2 d, e and S2-4	HCT 116	5-WT	1,4,13,17	CycD1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,4,14,17	CycD1, SSX1	WT	5	YFP
	HCT 116	5-WT	1,4,15,17	CycD1, H2A1	WT	5	YFP
	HCT 116	5-WT	1,5,13,17	SSX1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,5,14,17	SSX1, SSX1	WT	5	YFP
	HCT 116	5-WT	1,5,15,17	SSX1, H2A1	WT	5	YFP
	HCT 116	5-WT	1,6,13,17	H2A1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,6,14,17	H2A1, SSX1	WT	5	YFP
	HCT 116	5-WT	1,6,15,17	H2A1, H2A1	WT	5	YFP
3 a, b, c	HCT 116	3-WT	1,6,15,16	H2A1, H2A1	WT	3	YFP
	HCT 116	5-WT	1,6,15,17	H2A1, H2A1	WT	5	YFP
	HCT 116	8-WT	1,6,15,18	H2A1, H2A1	WT	8	YFP
	HCT 116	14-WT	1,6,15,19	H2A1, H2A1	WT	14	YFP
3 d, e, f and S5	HCT 116	3-WT	1,4,13,16	CycD1, CycD1	WT	3	YFP
	HCT 116	3-WT	1,4,14,16	CycD1, SSX1	WT	3	YFP
	HCT 116	3-WT	1,4,15,16	CycD1, H2A1	WT	3	YFP
	HCT 116	3-WT	1,5,13,16	SSX1, CycD1	WT	3	YFP
	HCT 116	3-WT	1,5,14,16	SSX1, SSX1	WT	3	YFP
	HCT 116	3-WT	1,5,15,16	SSX1, H2A1	WT	3	YFP
	HCT 116	3-WT	1,6,13,16	H2A1, CycD1	WT	3	YFP
	HCT 116	3-WT	1,6,14,16	H2A1, SSX1	WT	3	YFP
	HCT 116	3-WT	1,6,15,16	H2A1, H2A1	WT	3	YFP
	HCT 116	5-WT	1,4,13,17	CycD1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,4,14,17	CycD1, SSX1	WT	5	YFP
	HCT 116	5-WT	1,4,15,17	CycD1, H2A1	WT	5	YFP
	HCT 116	5-WT	1,5,13,17	SSX1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,5,14,17	SSX1, SSX1	WT	5	YFP
	HCT 116	5-WT	1,5,15,17	SSX1, H2A1	WT	5	YFP
	HCT 116	5-WT	1,6,13,17	H2A1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,6,14,17	H2A1, SSX1	WT	5	YFP
	HCT 116	5-WT	1,6,15,17	H2A1, H2A1	WT	5	YFP
	HCT 116	8-WT	1,4,13,18	CycD1, CycD1	WT	8	YFP
	HCT 116	8-WT	1,4,14,18	CycD1, SSX1	WT	8	YFP
	HCT 116	8-WT	1,4,15,18	CycD1, H2A1	WT	8	YFP
	HCT 116	8-WT	1,5,13,18	SSX1, CycD1	WT	8	YFP
	HCT 116	8-WT	1,5,14,18	SSX1, SSX1	WT	8	YFP
	HCT 116	8-WT	1,5,15,18	SSX1, H2A1	WT	8	YFP
	HCT 116	8-WT	1,6,13,18	H2A1, CycD1	WT	8	YFP

Figure	Cell type	Design	Plasmids #	Inputs Promoters (AD, BD)	DocS derivative	Binding site repeats	Output
3 d, e, f and S5	HCT 116	8-WT	1,6,14,18	H2A1, SSX1	WT	8	YFP
	HCT 116	8-WT	1,6,15,18	H2A1, H2A1	WT	8	YFP
	HCT 116	14-WT	1,4,13,19	CycD1, CycD1	WT	14	YFP
	HCT 116	14-WT	1,4,14,19	CycD1, SSX1	WT	14	YFP
	HCT 116	14-WT	1,4,15,19	CycD1, H2A1	WT	14	YFP
	HCT 116	14-WT	1,5,13,19	SSX1, CycD1	WT	14	YFP
	HCT 116	14-WT	1,5,14,19	SSX1, SSX1	WT	14	YFP
	HCT 116	14-WT	1,5,15,19	SSX1, H2A1	WT	14	YFP
	HCT 116	14-WT	1,6,13,19	H2A1, CycD1	WT	14	YFP
	HCT 116	14-WT	1,6,14,19	H2A1, SSX1	WT	14	YFP
	HCT 116	14-WT	1,6,15,19	H2A1, H2A1	WT	14	YFP
4 b	HCT 116	5-WT	1,6,15,17	H2A1, H2A1	WT	5	YFP
	HCT 116	5-M15	1,9,15,17	H2A1, H2A1	M15	5	YFP
	HCT 116	5-M102	1,12,15,17	H2A1, H2A1	M102	5	YFP
4 c,d	HCT 116	5-M102	1,10,13,17	CycD1, CycD1	M102	5	YFP
	HCT 116	5-M102	1,10,14,17	CycD1, SSX1	M102	5	YFP
	HCT 116	5-M102	1,10,15,17	CycD1, H2A1	M102	5	YFP
	HCT 116	5-M102	1,11,13,17	SSX1, CycD1	M102	5	YFP
	HCT 116	5-M102	1,11,14,17	SSX1, SSX1	M102	5	YFP
	HCT 116	5-M102	1,11,15,17	SSX1, H2A1	M102	5	YFP
	HCT 116	5-M102	1,12,13,17	H2A1, CycD1	M102	5	YFP
	HCT 116	5-M102	1,12,14,17	H2A1, SSX1	M102	5	YFP
	HCT 116	5-M102	1,12,15,17	H2A1, H2A1	M102	5	YFP
S6	HCT 116	5-WT	1,4,13,17	CycD1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,4,14,17	CycD1, SSX1	WT	5	YFP
	HCT 116	5-WT	1,4,15,17	CycD1, H2A1	WT	5	YFP
	HCT 116	5-WT	1,5,13,17	SSX1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,5,14,17	SSX1, SSX1	WT	5	YFP
	HCT 116	5-WT	1,5,15,17	SSX1, H2A1	WT	5	YFP
	HCT 116	5-WT	1,6,13,17	H2A1, CycD1	WT	5	YFP
	HCT 116	5-WT	1,6,14,17	H2A1, SSX1	WT	5	YFP
	HCT 116	5-WT	1,6,15,17	H2A1, H2A1	WT	5	YFP
	HCT 116	5-M15	1,7,13,17	CycD1, CycD1	M15	5	YFP
	HCT 116	5-M15	1,7,14,17	CycD1, SSX1	M15	5	YFP
	HCT 116	5-M15	1,7,15,17	CycD1, H2A1	M15	5	YFP
	HCT 116	5-M15	1,8,13,17	SSX1, CycD1	M15	5	YFP
	HCT 116	5-M15	1,8,14,17	SSX1, SSX1	M15	5	YFP
	HCT 116	5-M15	1,8,15,17	SSX1, H2A1	M15	5	YFP
	HCT 116	5-M15	1,9,13,17	H2A1, CycD1	M15	5	YFP
	HCT 116	5-M15	1,9,14,17	H2A1, SSX1	M15	5	YFP
	HCT 116	5-M15	1,9,15,17	H2A1, H2A1	M15	5	YFP
	HCT 116	5-M102	1,10,13,17	CycD1, CycD1	M102	5	YFP
	HCT 116	5-M102	1,10,14,17	CycD1, SSX1	M102	5	YFP

Figure	Cell type	Design	Plasmids #	Inputs Promoters (AD, BD)	DocS derivative	Binding site repeats	Output
S6	HCT 116	5-M102	1,10,15,17	CycD1, H2A1	M102	5	YFP
	HCT 116	5-M102	1,11,13,17	SSX1, CycD1	M102	5	YFP
	HCT 116	5-M102	1,11,14,17	SSX1, SSX1	M102	5	YFP
	HCT 116	5-M102	1,11,15,17	SSX1, H2A1	M102	5	YFP
	HCT 116	5-M102	1,12,13,17	H2A1, CycD1	M102	5	YFP
	HCT 116	5-M102	1,12,14,17	H2A1, SSX1	M102	5	YFP
	HCT 116	5-M102	1,12,15,17	H2A1, H2A1	M102	5	YFP
S7	T/NEO - WI38	5-WT	1,5,15,31	SSX1, H2A1	WT	5	YFP
	T3-WI38	5-WT	1,5,15,31	SSX1, H2A1	WT	5	YFP
5	T/NEO-WI38	5-M102	20,21,22,25,26,28	SSX1, H2A1	M102	5	TK1-HSV, YFP
	T/NEO - WI38	3-WT	20,21,22,24,26,27	SSX1, H2A1	WT	3	TK1-HSV, YFP
	T/NEO-WI38	5-WT	20,21,22,24,26,28	SSX1, H2A1	WT	5	TK1-HSV, YFP
	T3-WI38	5-M102	20,21,23,25,26,28	SSX1, H2A1	M102	5	TK1-HSV, YFP
	T3-WI38	3-WT	20,21,23,24,26,27	SSX1, H2A1	WT	3	TK1-HSV, YFP
	T3-WI38	5-WT	20,21,23,24,26,28	SSX1, H2A1	WT	5	TK1-HSV, YFP
6	T/NEO-WI38	5-M102	20,21,22,25,26,28	SSX1, H2A1	M102	5	TK1-HSV, YFP
	T/NEO - WI38	3-WT	20,21,22,24,26,27	SSX1, H2A1	WT	3	TK1-HSV, YFP
	T/NEO-WI38	5-WT	20,21,22,24,26,28	SSX1, H2A1	WT	5	TK1-HSV, YFP
	T/NEO - WI38	No output	20,21,22,24,26	SSX1, H2A1	WT	-	No
	T3-WI38	5-M102	20,21,23,25,26,28	SSX1, H2A1	M102	5	TK1-HSV, YFP
	T3-WI38	3-WT	20,21,23,24,26,27	SSX1, H2A1	WT	3	TK1-HSV, YFP
	T3-WI38	5-WT	20,21,23,24,26,28	SSX1, H2A1	WT	5	TK1-HSV, YFP
	T3-WI38	No output	20,21,23,24,26	SSX1, H2A1	WT	-	No

10. Table S6: Key sequences used in this study

Name	Sequence
PGK promoter	AATTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGGCAGTCTGGAGCATGCGCTTTAGCAGCCCCGCTGGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACATTCCACATCCACCGGTAGGCGCCAACCGGCTCCGTTCTTTGGTGGCCCCTTCGCGCCACCTTCTACTCCTCCCCTAGTCAGGAAGTTCCCCCCCGCCCCGCAGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGCACGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAATGGAAGCGGGTAGGCCTTTGGGGCAGCGGCCAATAGCAGCTTTGCTCCTTCGCTTTCTGGGCTCAGAGGCTGGGAAGGGGTGGGTCCGGGGGCGGGCTCAGGGGCGGGCTCAGGGGCGGGGCGGGCGCCCGAAGGTCCTCCGGAGGCCCGGCATTCTGCACGCTTCAAAAGCGCACGTCTGCCGCGCTGTTCTCCTCTTCCTCATCTCCGGGCCTTTCGACCTGCA
hUbC promoter	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTCACGGCGAGCGCTGCCACGTCAGACGAAGGGCGCAGGAGCGTTCCTGATCCTTCCGCCCGGACGCTCAGGACAGCGGCCCGCTGCTCATAAGACTCGGCCTTAGAACCCCAGTATCAGCAGAAGGACATTTTAGGACGGGACTTGGGTGACTCTAGGGCACTGGTTTTCTTTCCAGAGAGCGGAACAGGCGAGGAAAAGTAGTCCCTTCTCGGCGATTCTGCGGAGGGATCTCCGTGGGGCGGTGAACGCCGATGATTATATAAGGACGCGCCGGGTGTGGCACAGCTAGTTCCGTCGCAGCCGGGATTTGGGTCGCGGTTCTTGTTTGTGGATCGCTGTGATCGTCACTTGGTGAGTTGCGGGCTGCTGGGCTGGCCGGGGCTTTCGTGGCCGCCGGGCCGCTCGGTGGGACGGAAGCGTGTGGAGAGACCGCCAAGGGCTGTAGTCTGGGTCCGCGAGCAAGGTTGCCCTGAACTGGGGGTTGGGGGGAGCGCACAAAATGGCGGCTGTTCCCGAGTCTTGAATGGAAGACGCTTGTAAGGCGGGCTGTGAGGTCGTTGAAACAAGGTGGGGGGCATGGTGGGCGGCAAGAACCCAAGGTCTTGAGGCCTTCGCTAATGCGGGAAAGCTCTTATTCGGGTGAGATGGGCTGGGGCACCATCTGGGGACCCTGACGTGAAGTTTGTCACTGACTGGAGAACTCGGGTTTGTCGTCTGGTTGCGGGGGCGGCAGTTATGCGGTGCCGTTGGGCAGTGCACCCGTACCTTTGGGAGCGCGCGCCTCGTCGTGTCGTGACGTCACCCGTTCTGTTGGCTTATAATGCAGGGTGGGGCCACCTGCCGGTAGGTGTGCGGTAGGCTTTTCTCCGTCGCAGGACGCAGGGTTCGGGCCTAGGGTAGGCTCTCCTGAATCGACAGGCGCCGGACCTCTGGTGAGGGGAGGGATAAGTGAGGCGTCAGTTTCTTTGGTCGGTTTTATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGGTTTTGAACTATGCGCTCGGGGTTGGCGAGTGTGTTTTGTGAAGTTTTTTAGGCACCTTTTGAAATGTAATCATTTGGGTCAATATGTAATTTTCAGTGTTAGACTAGTAAAGCTTCTGCAGGTCGACTCTAGAAAATTGTCCGCTAAATTCTGGCCGTTTTTGGCTTTTTTGTTAGAC
CyclinD1 promoter	GGAACCTTCGGTGGTCTTGTCCCAGGCAGAGGGGACTAATATTTCCAGCAATTTAATTTCTTTTTTAATTAAAAAAAATGAGTCAGAATGGAGATCACTGTTTCTCAGCTTTCCATTCAGAGGTGTGTTTCTCCCGGTTAAATTGCCGGCACGGGAAGGGAGGGGGTGCAGTTGGGGACCCCCGCAAGGACCGACTGGTCAAGGTAGGAAGGCAGCCCGAAGAGTCTCCAGGCTAGAAGGACAAGATGAAGGAAATGCTGGCCACCATCTTGGGCTGCTGCTGGAATTTTCGGCATTTATTTTATTTTATTTTTTGAGCGAGCGCATGCTAAGCTGAAATCCCTTTAACTTTTAGGGTTACCCCCTTGGGCATTTGCAACGACGCCCCTGTGCGCCGGAATGAAACTTGCACAGGGGTTGTGTGCCCGGTCCTCCCCGTCCTTGCATGCTAAATTAGTTCTTGCAATTTACACGTGTTAATGAAAATGAAAGAAGATGCAGTCGCTGAGATTCTTTGGCCGTCTGTCCGCCCGTGGGTGCCCTCGTGGCGTTCTTGGAAATGCGCCCATTCTGCCGGCTTGGATATGGGGTGTCGCCGCGCCCCAGTCACCCCTTCTCGTGGTCTCCCCAGGCTGCGTGTGGCCTGCCGGCCTTCCTAGTTGTCCCCTACTGCAGAGCCACCTCCACCTCACCCCCTAAATCCCGGGGGACCCACTCGAGGCGGACGGGGCCCCCTGCACCCCTCTTCCCTGGCGGGGAGAAAGGCTGCAGCGGGGCGATTTGCATTTCTATGAAAACCGGACTACAGGGGCAACTCCGCCGCAGGGCAGGCGCGGCGCCTCAGGGATGGCTTTTGGGCTCTGCCCCTCGCTGCTCCCGGCGTTTGGCGCCCGCGCCCCCTCCCCCTGCGCCCGCCCCCGCCCCCCTCCCGCTCCCATTCTCTGCCGGGCTTTGATCTTTGCTTAACAACAGTAACGTCACACGGGGCGCGCCCCTCAGC

SSX1 promoter	GGGTAGCCAGATCATGGCTCACTGCAACCTCGTACTCCTGGGCTCAAGCTATCCTCCTACC TCAGCCTCCTGAGTAACGGACTACAGGCACACCACCCCACCTCGCTAATTTTATTTATTTTT TTTGTAGAGAAAAGAGACAGGGTATTGCTCTGTTGCCCAGGGTGGGGTGCAGTGGCATG ATCATGGCTCACTGCAACCTCTGCCTCCCAGGTTCAAGTGATCCTCCAGCTGTGGCCTCCC TAAGTGCTGGGATTACAACCGTGAGCCGCCGCACCGGCCCAAATTTCTTACGTCACTACA GAGTTCCTAGGAAAAAATCCCATACCTGAAAAAGATAGAAACTGACAGGAAGGATTTGA GATGATGACCTGCTTCATATACACTCCTTATTAAAACTGGATAACAATGCACCACCGAGGA GGTGGGGAGGGATAGGAAAAATGAAAAGAGAAAATCAGCGCATGCGTACTCTGATTTG GGAAGACTCCAAAGAGAAAATCAGAGCATGCGTACTCTGAACTTGGAGTAGCCAATCCC AGGGGATGCTTTAGGCGGGAAAGTCAGAGTTTCTGCCTCCATTTTGAGAAGGTTCTGTCC CTAGAGCCTAGACTGATAGACCCCACATCAGCTTGGCTTGTCCCGCCTACTGTTCTGACTT CTGATTGGCCAGATGGAGTTCACTAACTGCCCTGATTGGTCCATCATCCTGGAGCAATGA CATTGCAGAATATTTTCTCCTCCTCCAGCCACACTTTGTCACCAACTGCTGCCAACTCGCCA CCACTGCTGCCGACCTCGCAACCACTGCTTTGTCTCTGGCGCGCCCCTCAGC
H2A1 promoter	GGTGAGTTAATAGCTCTGCGCGGAGGGAGGGGATGGAGAGGGGGTCCTTGATCGCCTC CCAACATTACTGAGCTACATCACCTGAGCAGTTTCTACCCCGATTTCTGTAACGAGCAGTT TTTCTCGTTCTGTGCCGGCGCGCGCGCACACACACACACACACACACACACACACGGC TCAGATTCGGCCGCACCCGGCAACCGCTAGGGTGCATGGAGACACATTAGGTTACATAAC CCTTCACCGTGTTCGAAACCCTTTCTTGATCGTGTGGGTGGCTCTGAAAAGAGCCTTTGGG TTCAGGACGCCGAGGAACGCCTCACTTGGAGCTGGTGTACTTGGTGACAGCCTTGGTGCC CTCGGACACGGCGTGCTTGGCCAGCTCGCCGGGCAGCAGCAGGCGAACGGCCGTCTGCA CTTCGCGGGACGTGATGGTGGAGCGCTTGTTGTAGTGTGCCAGGCGGGAGGCCTCGCTG GCGATGCGCTCGAAGATGTCATTGACGAAGGAGTTCATGATGCCCATGGCCTTGGACGA GATGCCGGTGTCGGGGTGCACCTGCTTCAGCACCTTGTACACGTAGATAGAATAGCTCTC CTTGCGGCCGCGCTTGCGCTTCTTGCCGTCCTTCTTCTGTGCCTTGGTGACAGCCTTTTTAG AACCCTTCTTGGGCGCAGGAGCCGATTTGGACGGGTCTGGCATGATGGCTGAGTCTCTCC AAACAGAAACGCGCGGCGCTCGGAGTAACTCTATTTGTACGTTTTGTATTCAAATGAAGG CTCAGGATTTGCTCACTTCTGATTGGATCAAACGTTGTTCTACGTCATCGCTGGGAAAGGA ATACGCAAATTAGGAGTGCCAGGTTCTTTTTCTGATTGGCTACCATAGCCATCCAATCGAA CGCCGCGGTCTAGCCTACCTCTGTACCATACATAAGGGCTCGCTGGCCTTCACTGCCCTCT TGTTTTTAGTCTCGCTTTTCGGTTGCCGTTGTCTTTTTTCCTTGACTCGGAAGGCGCGCCCC TCAGC
VP16AD	TCGACGGCCCCCCCGACCGATGTCAGCCTGGGGGACGAGCTCCACTTAGACGGCGAGGA CGTGGCGATGGCGCATGCCGACGCGCTAGACGATTTCGATCTGGACATGTTGGGGGACG GGGATTCCCCGGGTCCGGGA
Gal4BD	ATGAAGCTACTGTCTTCTATCGAACAAGCATGCGATATTTGCCGACTTAAAAAGCTCAAGT GCTCCAAAGAAAAACCGAAGTGCGCCAAGTGTCTGAAGAACAACTGGGAGTGTCGCTAC TCTCCCAAAACCAAAAGGTCTCCGCTGACTAGGGCACATCTGACAGAAGTGGAATCAAG GCTAGAAAGACTGGAACAGCTATTTCTACTGATTTTTCCTCGAGAAGACCTTGACATGATT TTGAAAATGGATTCTTTACAGGATATAAAAGCATTGTTAACAGGATTATTTGTACAAGATA ATGTGAATAAAGATGCCGTCACAGATAGATTGGCTTCAGTGGAGACTGATATGCCTCTAA CATTGAGACAGCATAGAATAAGTGCGACATCATCATCGGAAGAGAGTAGTAACAAAGGT CAAAGACAGTTGACTGTATCG
DocS-WT	TCTACTAAATTATACGGCGACGTCAATGATGACGGAAAAGTTAACTCAACTGACGCTGTA GCATTGAAGAGATATGTTTTGAGATCAGGTATAAGCATCAACACTGACAATGCCGATTTG AATGAAGACGGCAGAGTTAATTCAACTGACTTAGGAATTTTGAAGAGATATATTCTCAAA GAAATAGATACATTGCCGTACAAGAAC
DocS15	TCTACTAAATTATACGGCGACGTCAATGATGACGGAAAAGTTAACTCAACTGACGCTGTA GCATTGAAGAGATATGTTTTGAGATCAGGTATAAGCATCAACACTGACAATGCCGCTTTG AATGAAGACGGCAGAGTTAATTCAACTGCCTTAGGAATTTTGAAGAGATATATTCTCAAA GAAATAGATACATTGCCGTACAAGAAC

DocS102	TCTACTAAATTATACGGCGACGTCAATGATGACGGAAAAGTTAACTCAACTGCCGCTGTA GCATTGAAGAGATATGTTTTGAGATCAGGTATAAGCATCAACACTGACAATGCCGATTTG AATGAAGACGGCAGAGTTAATTCAACTGCCTTAGGAATTTTGAAGAGATATATTCTCAAA GAAATAGATACATTGCCGTACAAGAAC
Coh2	GTGGTAGTAGAAATTGGCAAAGTTACGGGATCTGTTGGAACTACAGTTGAAATACCTGTA TATTTCAGAGGAGTTCCATCCAAAGGAATAGCAAACTGCGACTTTGTGTTCAGATATGAT CCGAATGTATTGGAAATTATAGGGATAGATCCCGGAGACATAATAGTTGACCCGAATCCT ACCAAGAGCTTTGATACTGCAATATATCCTGACAGAAAGATAATAGTATTCCTGTTTGCGG AAGACAGCGGAACAGGAGCGTATGCAATAACTAAAGACGGAGTATTTGCAAAAATAAGA GCAACTGTAAAATCAAGTGCTCCGGGCTATATTACTTTCGACGAAGTAGGTGGATTTGCA GATAATGACCTGGTAGAACAGAAGGTATCATTTATAGACGGTGGTGTTAACGTT
NLS	CCAAAAAAGAAGAGAAAGGTAGAT
2A peptide	GGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACC CTGGACCT
3xUAS promoter+ lateADEp	CGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGACTCGAGCGGAGTACTGTCCTCCG AAGAC**GCTAGCGGGGGGCTATAAAAGGGGGTGGGGGCGTTCGTCCTCACTCT**
5xUAS promoter+ lateADEp	CGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGACTCGAGCGGAGTACTGTCCTCCG ATCGGAGTACTGTCCTCCGCGAATTCCGGAGTACTGTCCTCCGAAGAC**GCTAGCGGGGG** **GCTATAAAAGGGGGTGGGGGCGTTCGTCCTCACTCT**
8xUAS promoter+ lateADEp	CGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGACTCGAGCGGAGTACTGTCCTCCG ATCGGAGTACTGTCCTCCGCGAATTCCGGAGTACTGTCCTCCGAAGACGCTAGACGGAGT ACTGTCCTCCGAGCGGAGTACTGTCCTCCGACTCGAGCGGAGTACTGTCCTCCG**GCTAGC** **GGGGGGCTATAAAAGGGGGTGGGGGCGTTCGTCCTCACTCT**
14xUAS promoter+ lateADEp	CGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGACTCGAGCGGAGTACTGTCCTCCG ATCGGAGTACTGTCCTCCGCGAATTCCGGAGTACTGTCCTCCGAAGACGCTAGACGGAGT ACTGTCCTCCGAGCGGAGTACTGTCCTCCGACTCGAGCGGAGTACTGTCCTCCGGCTAGA CGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGACTCGAGCGGAGTACTGTCCTCCG GCTAGACGGAGTACTGTCCTCCGAGCGGAGTACTGTCCTCCGACTCGAGCGGAGTACTGT CCTCCG**GCTAGCGGGGGGCTATAAAAGGGGGTGGGGGCGTTCGTCCTCACTCT**
lateADEp	**GCTAGCGGGGGGCTATAAAAGGGGGTGGGGGCGTTCGTCCTCACTCT**
TK1	ATGGCTTCGTACCCCTGCCATCAACACGCGTCTGCGTTCGACCAGGCTGCGCGTTCTCGC GGCCATAGCAACCGACGTACGGCGTTGCGCCCTCGCCGGCAGCAAGAAGCCACGGAAGT CCGCCTGGAGCAGAAAATGCCCACGCTACTGCGGGTTTATATAGACGGTCCTCACGGGAT GGGGAAAACCACCACCACGCAACTGCTGGTGGCCCTGGGTTCGCGCGACGATATCGTCT ACGTACCCGAGCCGATGACTTACTGGCAGGTGCTGGGGGCTTCCGAGACAATCGCGAAC ATCTACACCACACAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGT GGTAATGACAAGCGCCCAGATAACAATGGGCATGCCTTATGCCGTGACCGACGCCGTTCT GGCTCCTCATATCGGGGGGGAGGCTGGGAGCTCACATGCCCCGCCCCCGGCCCTCACCCT CATCTTCGACCGCCATCCCATCGCCGCCCTCCTGTGCTACCCGGCCGCGCGATACCTTATG GGCAGCATGACCCCCCAGGCCGTGCTGGCGTTCGTGGCCCTCATCCCGCCGACCTTGCCC GGCACAAACATCGTGTTGGGGGCCCTTCCGGAGGACAGACACATCGACCGCCTGGCCAA ACGCCAGCGCCCCGGCGAGCGGCTTGACCTGGCTATGCTGGCCGCGATTCGCCGCGTTTA CGGGCTGCTTGCCAATACGGTGCGGTATCTGCAGGGCGGCGGGTCGTGGCGGGAGGAT TGGGGACAGCTTTCGGGGACGGCCGTGCCGCCCCAGGGTGCCGAGCCCCAGAGCAACG CGGGCCCACGACCCCATATCGGGGACACGTTATTTACCCTGTTTCGGGCCCCCGAGTTGC TGGCCCCCAACGGCGACCTGTACAACGTGTTTGCCTGGGCCTTGGACGTCTTGGCCAAAC GCCTCCGTCCCATGCACGTCTTTATCCTGGATTACGACCAATCGCCCGCCGGCTGCCGGG ACGCCCTGCTGCAACTTACCTCCGGGATGGTCCAGACCCACGTCACCACCCCCGGCTCCAT ACCGACGATCTGCGACCTGGCGCGCACGTTTGCCC

Sirius	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGG ACGGCGACGTAAACGGCCACAGGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCAC CTACGGCAAGCTGACCCTGAAGCTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCC CACCCTCGTGACCACCCTGCAATTCGGCGTGCTGTGCTTCGCCCGCTACCCCGACCACATG AAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGTACCATC TTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACAC CCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGG GGCACAAGCTGGAGTACAACGGGATAAGCTCAAACGTATATATCACCGCCGACAAGCAG AAGAACGGCATCAAGGCCCACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCA GCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGA CAACCACTACCTGAGCGTCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGATC ACATGGTCCTGCTGGAGTCCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTG TACAAGTAA
CFP	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGG ACGGCGACGTAAACGGCCACAGGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCAC CTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCC CACCCTCGTGACCACCCTGACCTGGGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACAT GAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGTACCAT CTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACA CCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTG GGGCACAAGCTGGAGTACAACTACATCAGCCACAACGTCTATATCACCGCCGACAAGCA GAAGAACGGCATCAAGGCCCACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGC AGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCG ACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGAT CACATGGTCCTGAAGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCT GTACAAGTAA
YFP	ATGAGCAGCGGCGCCCTGCTGTTCCACGGCAAGATCCCCTACGTGGTGGAGATGGAGGG CGATGTGGATGGCCACACCTTCAGCATCCGCGGTAAGGGCTACGGCGATGCCAGCGTGG GCAAGGTGGATGCCCAGTTCATCTGCACCACCGGCGATGTGCCCGTGCCCTGGAGCACCC TGGTGACCACCCTGACCTACGGCGCCCAGTGCTTCGCCAAGTACGGCCCCGAGCTGAAG GATTTCTACAAGAGCTGCATGCCCGATGGCTACGTGCAGGAGCGCACCATCACCTTCGAG GGCGATGGCAATTTCAAGACCCGCGCCGAGGTGACCTTCGAGAATGGCAGCGTGTACAA TCGCGTGAAGCTGAATGGCCAGGGCTTCAAGAAGGATGGCCACGTGCTGGGCAAGAATC TGGAGTTCAATTTCACCCCCCACTGCCTGTACATCTGGGGCGATCAGGCCAATCACGGCC TGAAGAGCGCCTTCAAGATCTGCCACGAGATCGCCGGCAGCAAGGGCGATTTCATCGTG GCCGATCACACCCAGATGAATACCCCCATCGGCGGCGGCCCCGTGCACGTGCCCGAGTAC CACCACATGAGCTACCACGTGAAGCTGAGCAAGGATGTGACCGATCACCGCGATAATAT GAGCCTGACGGAGACCGTGCGCGCCGTGGATTGCCGCAAGACCTACCTG
mCherry	ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAA GGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAG GGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCT GCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAA GCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGA GCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGC AGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGA GGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCAC TACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGC CTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGA ACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAG

mKate2	ATGGTGTCTAAGGGCGAAGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGTACGGCAGCAAAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCTGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCGAGATGCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAAGCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACTTGAAGACCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACAGAAGACTGGAAAGAATCAAGGAGGCCGACAAAGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAACTGGGGCACAAACTTAATTGA

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