

Supporting information for:

A Toolkit for Rapid Modular Construction of Biological Circuits in Mammalian Cells

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Supporting Information.

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Figure S1

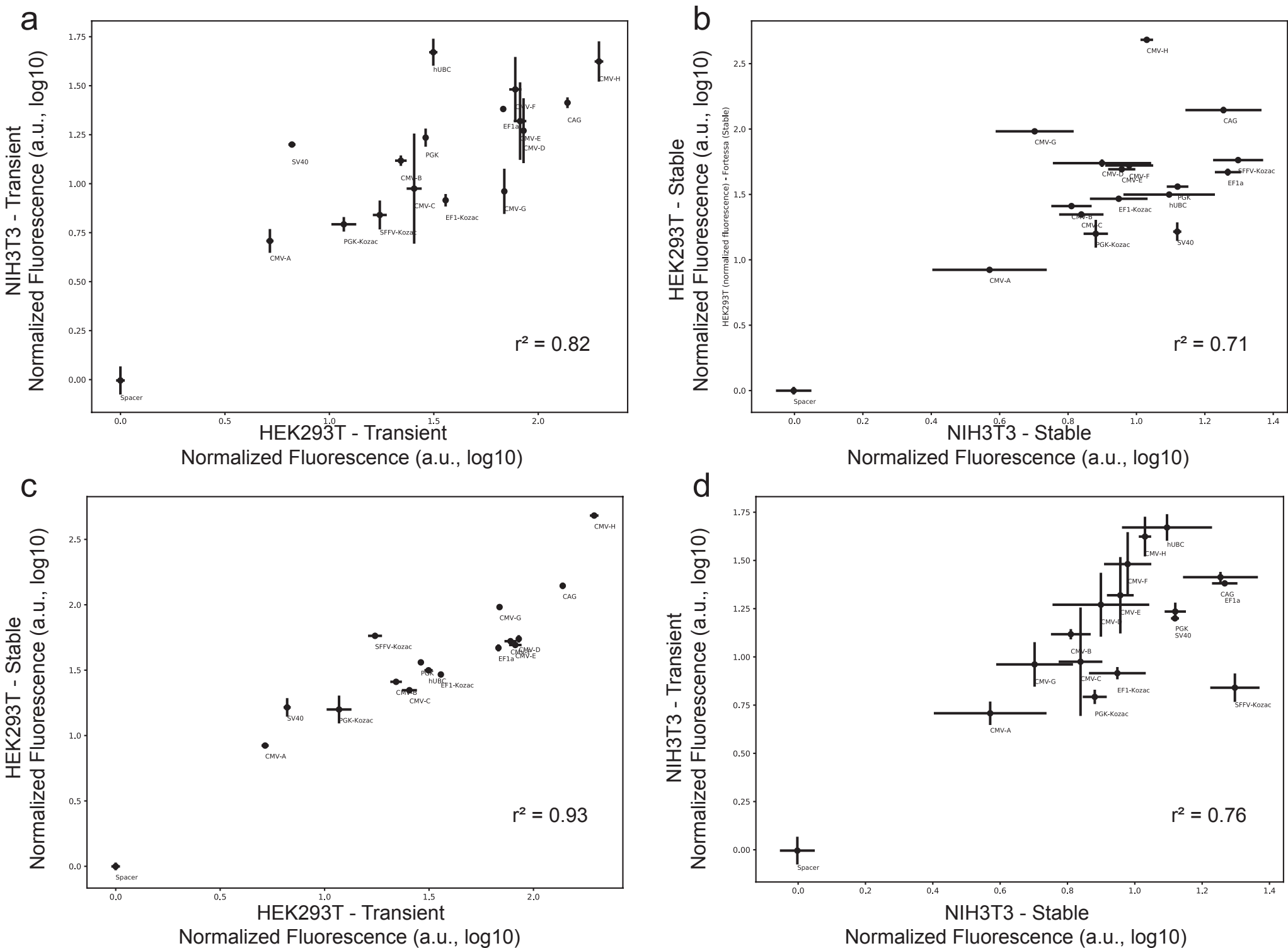


Figure S2

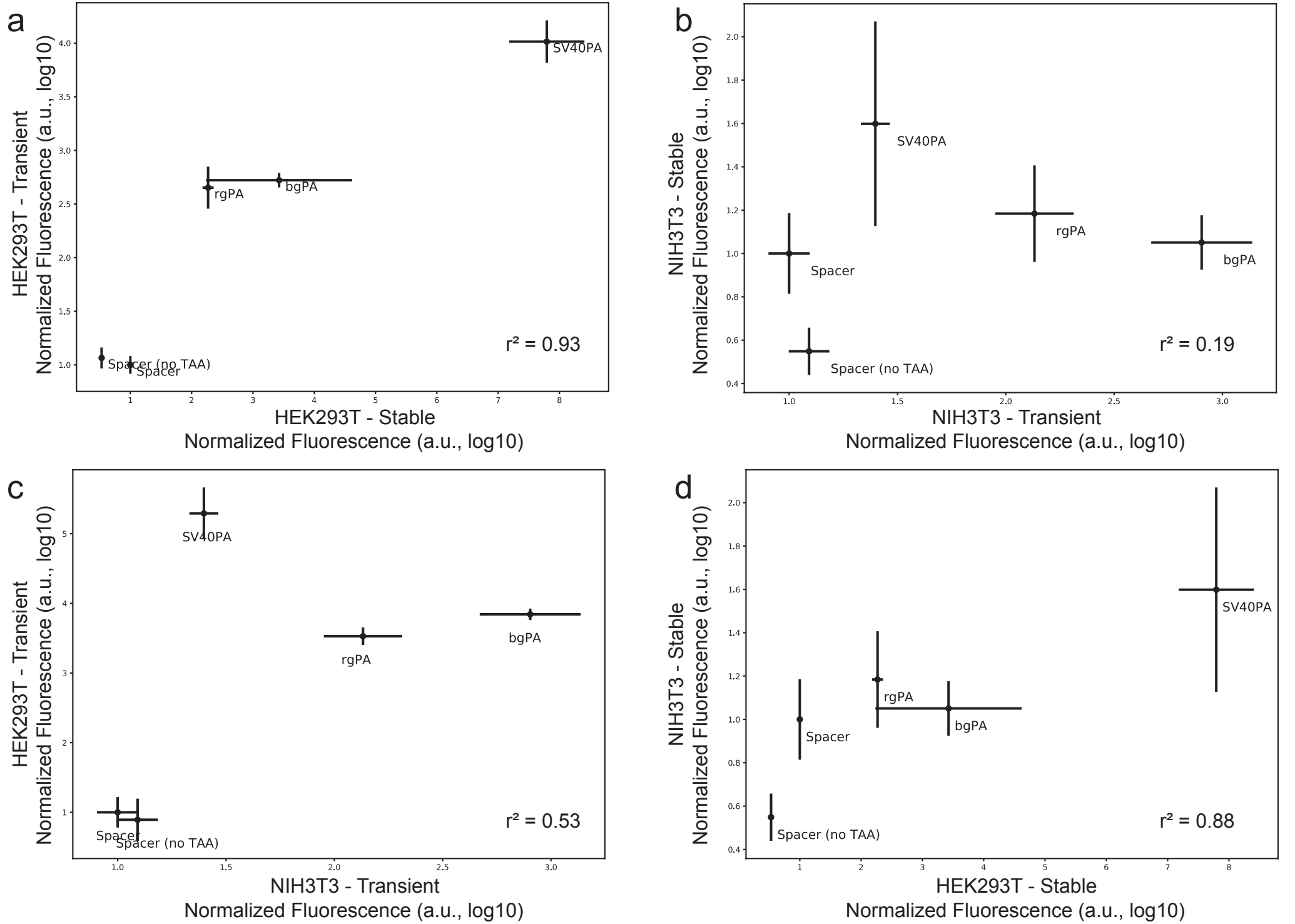
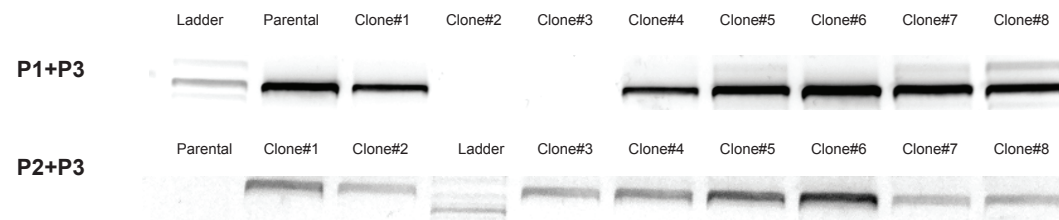


Figure S3

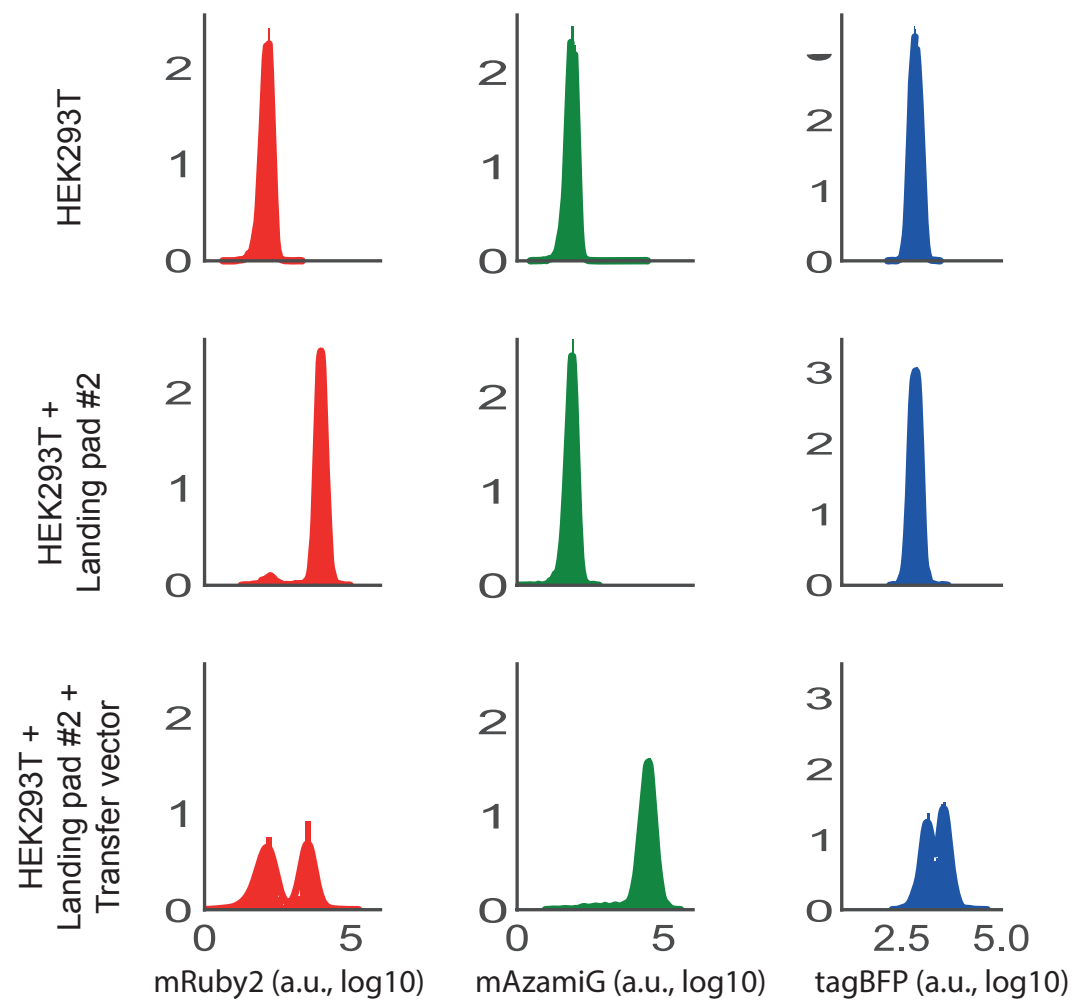
a

Primer Name	Primer Sequence
P1	CATCTCTCCTCCCTCACCCA
P2	AGGGCTCCGTTAATGGTCAC
P3	ATCCCTCCTCTCTGAACCCC

b



c



d

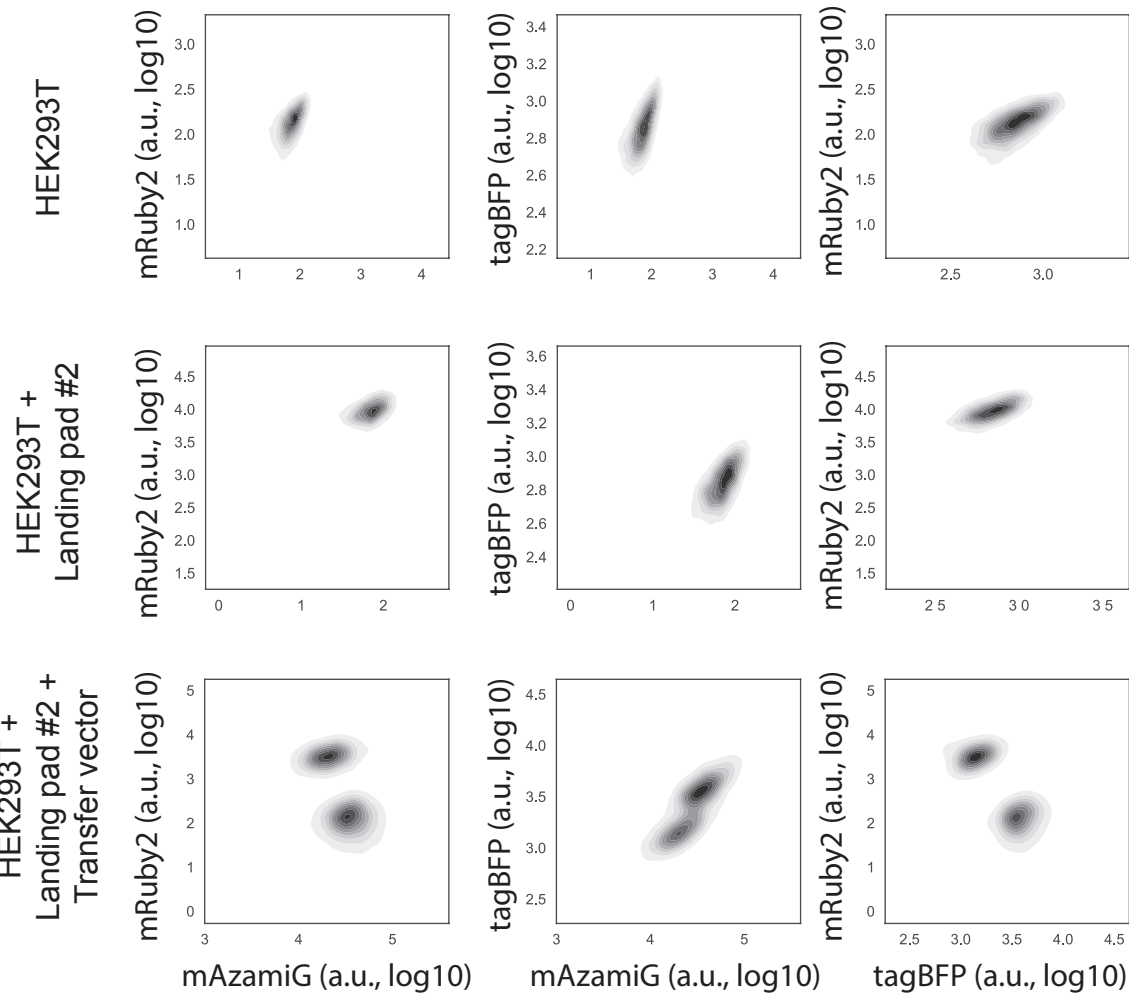


Figure S4.

a

Classification report

Predicted label	tagBFP	218	2	12	127
	non targeting	23	193	164	47
	mScarlet	2	19	71	5
	tagBFP + mScarlet	10	8	18	81
		tagBFP	non targeting	mScarlet	tagBFP + mScarlet
True label					

b

Confusion matrix

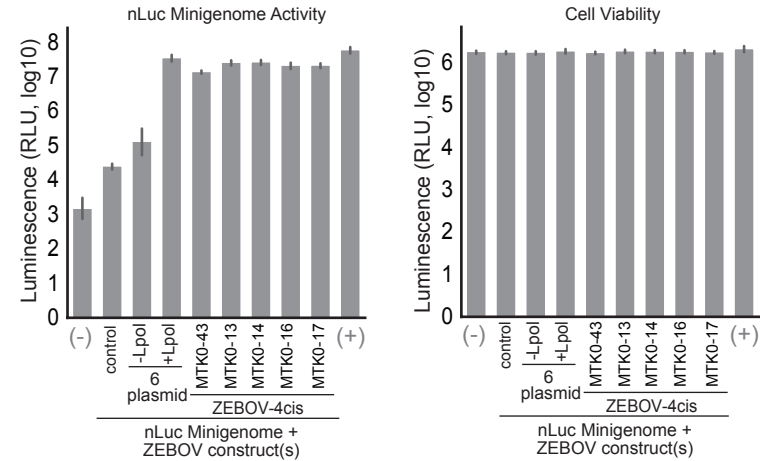
non targeting	0.45	0.87	0.59
mScarlet	0.73	0.27	0.39
tagBFP	0.61	0.86	0.71
tagBFP + mScarlet	0.69	0.31	0.43
Average	0.62	0.58	0.53
	Precision	Recall	f1 score

Figure S5.

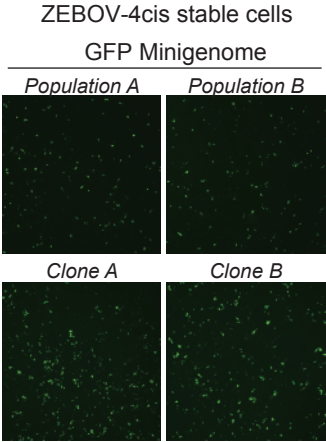
a

Donor Vector	Donor Name	ZEBOV-4cis	
		Colonies Screened	Colonies Correct
MTK0-43	PiggyBac Transposon	9	9
MTK0-13	PhiC31 Integrase	4	4
MTK0-14	BxB1 Integrase	4	3
MTK0-16	PhiC31 tagBFP	4	4
MTK0-17	BxB1 tagBFP	4	4

b



c



Supplementary Fig. 1. **Comparison of constitutive promoters across cell lines and delivery methods.** (a) The mAzamiGreen expression from transient transfection and PiggyBac integration of each promoter was assessed in HEK293T and (b) NIH3T3 cells. (c) mAzamiGreen expression of each promoter was compared between HEK293T and NIH3T3 in transient expression and (d) PiggyBac integration. Each point represents the mean of four biological replicates and error bars represent the standard deviation across replicates.

Supplementary Fig. 2. **Comparison of impact of 3' UTRs across cell lines and delivery methods.** (a) The mAzamiGreen expression from transient transfection and PiggyBac integration delivery of each 3' UTR was assessed in HEK293T and (b) NIH3T3 cells. (c) mAzamiGreen expression of each 3' UTR was compared between HEK293T and NIH3T3 in transient expression and (d) PiggyBac integration. Each point represents the mean of four biological replicates and error bars represent the standard deviation across replicates.

Supplementary Fig. 3. **Generation of landing pads for human cell lines.** (a) PCR products from landing pad genotyping. P1+P3 indicate presence of WT hAAVS1 locus and P2+P3 indicate presence of BxB1 landing pad in hAAVS1 locus. mRuby2, mAzamiGreen and tagBFP expression in populations of parental, Landing pad and Landing Pad with Transfer vector HEK293T cells. (b) mRuby2, mAzamiGreen and tagBFP expression in populations of parental, Landing pad #2 and Landing Pad #2 with Transfer vector HEK293T cells. In this clone, both wild type alleles of hAAVS1 locus were replaced by the landing pad construct, showing two populations as measured by fluorescence upon integration of the transfer vector and suggesting these two populations had one or two copies that integrated into the genome. mRuby2

expression indicates presence of hAAVS1 landing pad. mAzamiGreen and tagBFP expression indicates precise integration of transfer vector in hAAVS1 landing pad.

Supplementary Fig. 4. **Building a linear classifier to distinguish target sgRNA knockdown populations. (a)** Classification report of linear classifier. **(b)** Confusion matrix of linear classifier.

Supplementary Fig. 5. **Generation and quality control of multicistronic constructs for Zaire ebolavirus ribonucleoproteins. (a)** Multicistronic construct cloning efficiency. ZEBOV multicistronic construct containing 4 viral ORFs separated by P2A elements (ZEBOV-4cis) was BsmBI assembled directly into donor vectors for genome engineering. Specifically, ZEBOV-4cis was generated in donor vectors for PiggyBac transposon (MTK0-43), PhiC31 Integrase (MTK0-13, MTK0-16 tagBFP), and BxbI Integrase (MTK0-14, MTK0-17 tagBFP). The number of bacterial colonies screened and positive for correct construct by size (NotI digestions) is indicated. **(b)** Luminescence measurements of ZEBOV minigenome activity and cell viability. HEK293T cells were transfected with ZEBOV nLuc minigenome in combination with pCAGGs empty control plasmid (control), the ZEBOV 6 plasmids (with or without Lpol: +Lpol, -Lpol), or with ZEBOV-4cis in various part 0 donor vectors. Positive (+) and negative (-) controls include transfection of only pCAGGS-nLuc plasmid or pCAGGs empty plasmid, respectively. Nano luciferase activity was measured two days post transfection. Bar plots represent the mean of biological replicates (n=2). **(c)** GFP analysis of minigenome activity in stable cells. ZEBOV-4cis stable populations and clones were transfected with a T7-driven ZEBOV minigenome construct encoding the eGFP reporter along with T7 polymerase. After 2 days cells were imaged for GFP detection (Leica, 4X).

Supplementary Table 1

[illegible]

[illegible]

Supplementary Table 2

Figure	Target Locus	Protospacer Sequence	Cas9 Species (Part Plasmid)	PAM Sequence	Forward Oligo	Reverse Oligo	Backbone Vector	Source
3	hAAVS1 (site 1)	GAGCCACATTAACCGGCCCT	S. pyogenes (MTK3_025)	GGG	5' TGTTTGGAGCCACATTAACCGGCCCTG	5' TAAACAGGGCCGGTTAATGTGGCTCCA	MTK234_002	This study
3	hAAVS1 (site 2)	GAGCCACATTAACCGGCCCT	S. pyogenes (MTK3_025)	TGG	5' TGTTTGATTCCCAGGGCCGGTTAATGG	5' TAAACCATTAACCGGCCCTGGGAATCA	MTK234_002	This study
3	hAAVS1 (site 3)	GGGGCCACTAGGGACAGGAT	S. pyogenes (MTK3_025)	TGG	5' TGTTTGGGGGCCACTAGGGACAGGATG	5' TAAACATCCTGTCCCTAGTGGCCCCCA	MTK234_002	This study
4	tagBFP	CTACAACGTCAAGATCAGAG	S. pyogenes (MTK3_025)	GGG	5' TGTTTGCTACAACGTCAAGATCAGAGG	5' TAAACCTCTGATCTTGACGTTGTAGCA	MTK234_002	This study
4	mScarlet	CCACAACGAAGATTATACCG	S. pyogenes (MTK3_025)	TGG	5' TGTTTGCCACAACGAAGATTATACCGG	5' TAAACCGGTATAATCTTCGTTGTGGCA	MTK234_002	This study
4	human non-targeting	ACGGAGGCTAAGCGTCGCAA	S. pyogenes (MTK3_025)		5' TGTTTGACGGAGGCTAAGCGTCGCAAG	5' TAAACTTGCGACGCTTAGCCTCCGTCA	MTK234_002	Doench, J. G. <i>et al.</i> (2016)
5	TRE	TACGTTCTCTACTGATA	S. pyogenes (MTK3_025)	GGG	5' TGTTTGACGTTCTCTATCACTGATAG	5' TAAACTATCAGTGATAGAGAACGTACA	MTK234_002	Gao, Y. <i>et al.</i> (2016)
5	TRE	GTTACTCCCTATCAGTGATA	S. aureus (MTK3b_019)	AGGAGT	5' TGTTTGGTTACTCCCTATCAGTGATAG	5' ATAACTATCACTGATAGGGAGTAACCA	MTK234_050	Gao, Y. <i>et al.</i> (2016)
5	UAS	GAGCACTGTCTCCGAACGT	S. pyogenes (MTK3_025)	CGG	5' TGTTTGGAGCACTGTCTCCGAACGTG	5' TAAACACGTTCGGAGGACAGTGCTCCA	MTK234_002	This study
5	UAS	GAACGTCGGAGCACTGTCCT	S. aureus (MTK3b_019)	CCGAAC	5' TGTTTGAACGTCGGAGCACTGTCCTG	5' ATAACAGGACAGTGCTCCGACGTTCCA	MTK234_050	This study

Supplementary Table 3

Part	Forward Primer	Reverse Primer	Destination Vector	Notes
	1 5' gcatCGTCTCcatcGGTCTCaCCCTCGTCTCaNNNN	5' gcatCGTCTCaGGTCTCaCGTT	MTK0_027	NNNN is barcode for connector, see supplemental table 4
1 (reverse connectors)	5' gcatCGTCTCcatcGGTCTCaCCCT	5' gcatCGTCTCaGGTCTCaCGTTCGTCTCINNNN	MTK0_027	NNNN is barcode for connector, see supplemental table 4
	2 5' gcatCGTCTCcatcGGTCTCaAACG	5' gcatCGTCTCaGGTCTCaCATA	MTK0_027	
	3 5' gcatCGTCTCcatcGGTCTCaTATG	5' gcatCGTCTCaGGTCTCaGGAT	MTK0_027	
3a	5' gcatCGTCTCcatcGGTCTCaTATG	5' gcatCGTCTCaGGTCTCaAGAA	MTK0_027	
3b	5' gcatCGTCTCcatcGGTCTCaTTCT	5' gcatCGTCTCaGGTCTCaGGAT	MTK0_027	
	4 5' gcatCGTCTCcatcGGTCTCaATCCTaa	5' gcatCGTCTCaGGTCTCaCAGC	MTK0_027	
4a	5' gcatCGTCTCcatcGGTCTCaATCC	5' gcatCGTCTCaGGTCTCaGCCA	MTK0_027	remove first 3 bases (taa) for multicistronic use
4b	5' gcatCGTCTCcatcGGTCTCaTCGG	5' gcatCGTCTCaGGTCTCaCAGC	MTK0_027	
	5 5' gcatCGTCTCcatcGGTCTCaGCTG	5' gcatCGTCTCaGGTCTCaTGACGTCTCINNNN	MTK0_027	NNNN is barcode for connector, see supplemental table 4
5 (reverse connectors)	5' gcatCGTCTCcatcGGTCTCaGCTGCGTCTCaNNNN	5' gcatCGTCTCaGGTCTCaTGTA	MTK0_027	NNNN is barcode for connector, see supplemental table 4
	6 5' gcatCGTCTCcatcGGTCTCaTACA	5' gcatCGTCTCaGGTCTCaACTC	MTK0_027	
	7 5' gcatCGTCTCcatcGGTCTCaGAGT	5' gcatCGTCTCaGGTCTCaTCGG	MTK0_027	
	8 5' gcatCGTCTCcatcGGTCTCaCCGA	5' gcatCGTCTCaGGTCTCaAGGG	MTK0_027	
8a	5' gcatCGTCTCcatcGGTCTCaCCGA	5' gcatCGTCTCaGGTCTCaATTG	MTK0_027	
8b	5' gcatCGTCTCcatcGGTCTCaCAAT	5' gcatCGTCTCaGGTCTCaAGGG	MTK0_027	
234	5' gcatCGTCTCcatcGGTCTCaAACG	5' gcatCGTCTCaGGTCTCaCAGC	MTK0_027	
234 (SpCas9 sgRNA)	5' TGTTTGNNNNNNNNNNNNNNNNNNNG	5' TAAACN'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'CA	MTK234_002	N(20) are guide specific sequence, N' is reverse complement
234 (SaCas9 sgRNA)	5 TGTTTGNNNNNNNNNNNNNNNNNNNG	5' ATAACN'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'N'CA	MTK234_050	N(20) are guide specific sequence, N' is reverse complement

Supplementary Table 4

Connector	Overhang
LS, LS'	5' CTGA
L1, R1	5' CCAA
L2, R2	5' GATG
L3, R3	5' GTTC
L4, R4	5' GGTA
L5, R5	5' AAGT
L6, R6	5' CCCT
L7, R7	5' GCGG
L8, R8	5' TTTA
RE, RE'	5' AGCA