Electronic Supplementary Material (ESI) for Chemical Science.

Encoded, Click-Reactive DNA-Binding Domains for Programmable Capture of Specific Chromatin Segments

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Supplementary Information

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MATERIAL AND METHODS

Vector construction

All enzymes were purchased from New England Biolabs. Plasmid maps can be found in the appendix.

Golden gate 2 entry plasmid pAnW1272 was cloned by Gibson assembly[1]. For this, plasmid pAnW891 (based on addgene plasmid #47389) was cut with PacI and AscI to remove the VP64 gene and mixed with the insert mCherry which was amplified from plasmid pTH-NH(G)-mCherry (Addgene #49629) using primers o2731/o2732. For the GG2 entry plasmids containing the different amber codon positions the different amino acid residues on plasmid pAnW1272 were mutated to the amber stop codon by Quickchange site directed mutagenesis using the following primer pairs:

Table S1: Quickchange primer for amber codon positions.

Mutation	Primer pair	Resulting plasmid
V92TAG	o1991/o1992	pAnW1273
Y44TAG	o2811/o2812	pAnW1361
E50TAG	o2817/2818	pAnW1364
K51TAG	o2819/o2820	pAnW1365
S58TAG	o2825/o2826	pAnW1367
L80TAG	o2833/o2834	pAnW1370
G88TAG	o2835/o2836	pAnW1371
I98TAG	o2841/o2842	pAnW1374
A104TAG	o2843/o2844	pAnW1375
S36TAG	o3224/o3225	pAnW1800

TALEs were assembled according to a previously published protocol[2] using all previously described entry plasmids in golden gate 2 reactions, resulting in the following plasmids coding for the SATIII-targeting TALE protein (RVD sequence: NN NI NG NG HD HD NI NG NG HD HD NI NG NG HD HD NI NG NG) followed by a C-terminal mCherry.

Table S2: Generated Plasmids.

Mutation	Entry plasmid	Resulting plasmid
w/o TAG	pAnW1272	pAnW1274
V92TAG	pAnW1273	pAnW1275
Y44TAG	pAnW1361	pAnW1412
E50TAG	pAnW1364	pAnW1415
K51TAG	pAnW1365	pAnW1416
S58TAG	pAnW1367	pAnW1417
L80TAG	pAnW1370	pAnW1419
G88TAG	pAnW1371	pAnW1420
I98TAG	pAnW1374	pAnW1423
A104TAG	pAnW1375	pAnW1424
S36TAG	pAnW1800	pAnW1811

For the in vivo activation assay, the SATIII-TALE as well as a scrambled SATIII-TALE (sc SATIII-TALE) were assembled according to a previously published protocol[2] using the entry plasmid pAnW891 (based on addgene plasmid #47389) in golden gate 2 reactions, resulting in the plasmids pAnW1031 (SATIII-TALE) and pAnW1032 (sc SATIII-TALE). To receive the reporter plasmid pAnW1183 (encoding firefly luciferase under the control of a minimal CMV promoter (minCMV) with a SATIII-TALE binding site upstream of the promoter) the SATIII binding site was introduced via restriction ligation. Specifically, the oligo pair o2481/o2482 containing the SATIII-binding site was hybridized (4µM each) in 1x Cut-Smart buffer (NEB) by heating at 95 °C for 5 min and incubation at RT for 30 min. Plasmid pSF_MinCMV_Fluc (Oxford Genetics) and the hybridized oligonucleotide duplex were digested with each 10 units of *SalI* and *SpeI* and ligated using T4 DNA ligase.

BirA* entry plasmid pAnW2612 was cloned by Gibson assembly[1]. For this, plasmid pAnW750 (addgene plasmid #47389) was cut with PacI and AscI to remove the VP64 gene and mixed with the insert BirA* which was amplified from plasmid pAnW2611 using primers o4203/o4204. For the BirA* based enrichment, the SATIII-TALE as well as a scrambled sc SATIII-TALE were assembled according to a previously published protocol[2], resulting in the following plasmids coding for the SATIII-targeting TALE protein followed by a C-terminal BirA* (pAnW2613) and scSATIII_TALE protein followed by a C-terminal BirA* (pAnW2615).

Live cell imaging of SATIII-TALE amber mutants in HEK293T cells

HEK293T cells were maintained in DMEM (PAN) media supplemented with 1 % penicillin/streptomycin, 10 % FBS (PAN) and 1% L-Glutamine (PAN) at 37 °C, a CO2 level of 5 % and a humidity of ≥95 %. $4x10^5$ cells were cultured in a 3.5 cm dish (ibidi) overnight prior transfection. For transfection Opti MEM (Gibco) and lipofectamin 2000 (Thermo Fisher) were mixed according to the manufacturer's protocol. 1 µg of the $tRNA^{Pyl}$/PylRS-AF pair plasmid pAnW1410 and 1 µg of one SATIII-TALE construct plasmid were mixed together. Transfection mix was added to each plasmid pair and incubated for 20 min at RT. The solution was added to the cells either together with 250 µM TCO (+ncAA) or not (–ncAA) and incubated at 37 °C, a CO_2 level of 5 % and a humidity of ≥95 % for 24 hr. The medium was then changed to HEPES buffered imaging medium for microscopy. For fluorescent live cell imaging, an Olympus IX-81 microscope with a 10x ULPSAPO objective and a MT20 lamp (Olympus) were used. For detection, an EM-CCD camera (C9100-13, Hamamatsu) and a standard filter set were used.

Imaging of reaction of FAM-tetrazine conjugates with TCO-bearing SATIII-TALE amber mutants in HEK293T cells

$4x10^5$ cells were cultured in a 3.5 cm dish (ibidi) overnight prior transfection. The cells were transfected with 1 µg of pAnW1410 ($tRNA^{Pyl}$/PylRS-AF pair) and 1 µg of pAnW1415, pAnW1417 or pAnW1811 (encoding for the SATIII-TALE mCherry fusion protein with different amber codon positions) in the presence of 250 µM TCO or 250µM Boc. The cells were fixed with formaldehyde by adding 37 % formaldehyde drop wise to the cell's medium to a final concentration of 4 % and incubated 20 min at 37 °C. Then, the cells were washed once with a sufficient amount of DPBS and were permeabilized with a sufficient amount of 0.25 % Triton X-100 in DPBS and incubated for 15 min at RT. Afterwards, the cells were washed once with a sufficient amount of DPBS and were covered with 100 nM FAM-tetrazine solution in DBPS and incubated 3 hrs at 37 °C. The cells were washed three times with DPBS and were covered with DPBS for microscopy. For fluorescent imaging of the fixed cells expressing pAnW1415 or pAnW1417, an Olympus IX-81 microscope with a 60x PLAPO/TIRF objective and a MT20 lamp (Olympus) was used. For detection, an EM-CCD camera (C9100-13, Hamamatsu) amd a standard filter set were used. For fluorescent imaging of the fixed cells expressing pAnW1811 an Olympus IX-81 microscope with a 60x UPlanSApo objective and a IX2-UCB lamp were used. For the detection, an ORCA-R^2 – C106000 camera (Hamamatsu) and a standard filter set were used.

Imaging of reactions of biotin-tetrazine with SATIII-TALE_S36 in HEK 293T cells

$4x10^5$ cells were cultured in a 3.5 cm dish (ibidi) overnight prior transfection. The cells were transfected with 1 µg of pAnW1410 ($tRNA^{Pyl}$/PylRS-AF pair) and 1 µg of pAnW1811 (encoding for the SATIII-TALE_S36 mCherry fusion protein) in the presence of 250 µM TCO or 250 µM Boc. After fixation and permeabilization the cells were covered with 100 µM biotin-tetrazine solution in DBPS and incubated 3 hrs at 37 °C. For the fluorescent staining the Cy7-Streptavidin compound was diluted 1:1000 and 500 µL were added to the cells. The cells were washed three times with DPBS and were covered with 1 mL DPBS for microscopy. For fluorescent imaging of the fixed cells, an Olympus IX-81 microscope with a 60x PLAPO/TIRF objective and a MT20 lamp (Olympus) were used. For detection, an EM-CCD camera (C9100-13, Hamamatsu) and a standard filter set were used.

Imaging of differences in reaction rates of three different clickable amino acids in HEK293T cells

$4x10^5$ cells were cultured in a 3.5 cm dish (ibidi) overnight prior transfection. The cells were transfected with 1 µg of pAnW1410 ($tRNA^{Pyl}$/PylRS-AF) and 1 µg of pAnW1811 (encoding for the SATIII-TALE_S36 mCherry fusion protein) in the presence of 250 µM TCO, BCN, SCO or Boc. After fixation and permeabilization the cells were covered with 100 µM biotin-tetrazine solution in DBPS and incubated 5 min, 1 hr or 3 hrs at 37 °C. Then the cells were washed three times with DPBS. For the fluorescent staining, the Cy7-Streptavidin compound was diluted 1:1000 and 500 µL were added to the cells and incubated 1 hr at RT. The cells were washed three times with DPBS and were covered with 1 mL DPBS for microscopy. For fluorescent imaging of the fixed cells, an Olympus IX-81 microscope with a 60x PLAPO/TIRF objective and a MT20 lamp (Olympus) were used. For detection, an EM-CCD camera (C9100-13, Hamamatsu) and a standard filter set were used.

Imaging of SATIII-TALE_mCherry and HSF1 in HEK293T and HeLa cells

Tissue-culture plates were coated with PLL for HEK293T cells by adding a sufficient amount of 0.01 % PLL to the plates. After 1 h incubation at 37 °C the plates were washed once with DPBS and were directly used for passaging of cells. $4x10^5$ cells were cultured in a 3.5 cm PLL coated dishes (ibidi) overnight prior transfection. For transfection of HEK293T cells Opti MEM (Gibco) and Lipofectamin 2000 (Thermo) and for HeLa cells Opti MEM (Gibco) and XtremeGene (SigmaAldrich) were mixed according to the manufacturer's protocol. The cells were transfected with 1 µg pAnW1410 ($tRNA^{Pyl}$/PylRS-AF) and 1 µg of pAnW1811 (encoding for the SATIII-TALE_S36 mCherry fusion protein) in the presence of 250 µM TCO. After fixation and permeabilization the cells were blocked in 1 mL DPBS+ 1% BSA + 0.05 % Tween-20 for 30 min at RT. Staining was done by adding 500 µL DPBS with 1:500 diluted HSF1 (rabbit) antibody. After 2 h incubation at RT, cells were washed three times with DPBS-T (0.05 % Tween-20) and the secondary antibody fluorescein goat anti-rabbit (1:2000 diluted) was added. After 1 hr at RT cells were washed again two times with DPBS-T and once with DPBS and then stored in 2 mL DPBS for microscopy. For fluorescent imaging of the fixed cells, an Olympus IX-81 microscope with a 60x PLAPO/TIRF objective and a MT20 lamp (Olympus) were used. For detection, an EM-CCD camera (C9100-13, Hamamatsu) and a standard filter set were used.

Imaging of SATIII-TALE_BirA* and HSF1_mCherry in HEK293T cells

$4x10^5$ cells were cultured in a 3.5 cm PLL coated dishes (ibidi) overnight prior transfection. For transfection of HEK293T cells Opti MEM (Gibco) and Lipofectamin 2000 (Thermo) were mixed according to the manufacturer's protocol. The cells were transfected with 1 µg pAnW_HSF1 (HSF1_mCherry) and 1 µg of pAnW2613 (encoding for the SATIII-TALE_BirA* fusion protein) or 1 µg of pAnW2615 (encoding for the scSATIII-TALE_BirA* fusion protein). After fixation and permeabilization the cells were blocked in 1 mL DPBS+ 1% BSA + 0.05 % Tween-20 for 30 min at RT. Staining was done by adding 500 µL DPBS with 1:500 diluted HA (rabbit) antibody. After 2 h incubation at RT, cells were washed three times with DPBS-T (0.05 % Tween-20) and the secondary antibody fluorescein goat anti-rabbit (1:2000 diluted) was added. After 1 hr at RT cells were washed again two times with DPBS-T and once with DPBS and then stored in 2 mL DPBS for microscopy. For fluorescent imaging of the fixed cells, an Olympus IX-81 microscope with a 60x PLAPO/TIRF objective and a MT20 lamp (Olympus) were used. For detection, an EM-CCD camera (C9100-13, Hamamatsu) and a standard filter set were used.

Image analysis

Microscopic images were analyzed and processed with ImageJ (NIH, Bethesda) and prepared for presentation using PowerPoint. Image manipulations were restricted to background subtraction, adjustment of brightness levels, cropping, scaling and false color-coding using LUT tables.

Click-mediated enrichment of genomic loci for qPCR

$2.5x10^6$ HEK293T cells (cultured on PLL coated plates) or $1.5x10^5$ HeLa cells were cultured in 10 cm TC-plates (Sarstedt) overnight prior transfection. Cells were transfected with 6 µg (HEK293T) or 4.5 µg (HeLa) of pAnW1410 ($tRNA^{Pyl}$/PylRS-AF) and 6 µg /4.5 µg of pAnW1811 (encoding for the SATIII-TALE_S36 mCherry fusion protein) in presence of 250 µM TCO or 250 µM Boc. Cells were either exposed to heat stress by incubating them at 44 °C for 1 h or were incubated an additional hour at 37 °C. For crosslinking 37 % formaldehyde was added to 1 % final concentration into the culture medium of the target cells and incubated at 37 °C for 10 min. The crosslinking reaction was stopped by adding 1.25 M glycine solution to 127 mM final concentration and an incubation at room temperature for 10 min. The solution was discarded and the cells were washed with 3 mL DPBS. The cells were permeabilized by adding 3 mL 0.25 % Triton X-100 in DPBS and incubated at room temperature for 10 min. After washing the cells with 3 mL DBPS, 10 mL 100 µM biotin-tetrazine in DPBS were added to the cells and incubated over night at 4 °C. Next day the cells were collected by centrifugation at 2000 rpm and 4 °C for 8 min. The cell pellet was washed once with 10 mL DPBS and was frozen at −80 °C. The chromatin was prepared based on previous published protoco[3]. Specifically, the crosslinked pellet was suspended in 10 mL cell lysis buffer (CLB) and incubated on ice for 10 min. The cell suspension was centrifuged at 2000 rpm and 4 °C for 8 min and the supernatant was discarded. The cell pellet was again suspended in 10 mL nuclear lysis buffer (NLB) and incubated on ice for 10 min, while the cell suspension was vortexed every 3 minutes. After centrifugation at 2000 rpm and 4 °C for 8 min the supernatant was discarded and the pellet was washed with 10 mL DPBS. The suspension was centrifuged at 2000 rpm and 4 °C for 10 min and the chromatin containing pellet was suspended in 800 µL modified lysis buffer 3 (MLB3). The chromatin was sheared by sonication using the Bioruptor Pico and the following conditions: HEK293T cells: 1. 5 sec on, 90 sec off, 7 cycle; 2. 10 sec on, 90 sec off, 7 cycle; 3. 20 sec on, 90 sec off, 6 cycle; HeLa: 30 sec on, 30 sec off, 10 cycle. After chromatin preparation, the DNA amount was determined by qPCR. For the click-mediated enrichment the same DNA amount of each sample was mixed with 2.125-fold amount of 1.47 % Triton X-100 in MLB3 and added to the streptavidin-conjugated Dynabeads (Thermo Fisher) (blocked with 0.1 % BSA). After a rotation step at RT overnight, the tube was placed in a magnet rack and the supernatant was discarded. The beads were washed twice with 900 µL Low Salt Buffer (LSB), High Salt Buffer (HSB), LiCl Buffer and TBS with 0.1 % IGEPAL. Afterwards the DNA was eluted via reverse crosslinking. Specifically the beads were suspended in 85 µL ddH2O and 4 µL 5 M NaCl and incubated at 65 °C overnight. After adding 1 µL of 10 mg/mL RNase A and an incubation step at 37 °C for 45 min, 2 µL of 0.5 M EDTA (pH 8.0), 8 µL of 0.5 M Tris-HCl (pH 6.8) and 1 µL Proteinase K were added and incubated at 45 °C for 1.5 h. Afterwards, the beads were separated by a magnetic rack and the supernatant containing the DNA was purified using PCR purification kit (Macherey Nagel) according to the manufacture's guidelines. For qPCR analysis 4 µL of the purified DNA were pipetted in a PCR workstation into a 384 well plate. A reaction mixture containing the 2x GoTaq master mix, forward primer, reverse primer and ddH2O was prepared and added to the sample without mixing. The plate was tightly sealed and centrifuged at RT for 1 min at 2500 rpm. Afterwards the qPCR was performed on the CFX384 Touch real-time PCR detection system.

Click-mediated enrichment of genomic loci for proteomics

$2.5x10^6$ HEK293T cells (cultured on PLL coated plates) or $1.5x10^5$ HeLa cells were cultured in 10 cm TC-plates (Sarstedt) overnight prior transfection. Cells were transfected with 6 µg (HEK293T) or 4.5 µg (HeLa) of pAnW1410 ($tRNA^{Pyl}$/PylRS-AF) and 6 µg /4.5 µg of pAnW1811 (encoding for the SATIII-TALE_S36 mCherry fusion protein) in presence of 250 µM TCO or 250 µM Boc. For the off-target amber suppression background, cells were transfected with 6 µg/4.5 µg pAnW1872 (empty vector: EV; expressing mCherry only) and 6 µg /4.5 µg pAnW1410 ($tRNA^{Pyl}$/PylRS-AF) in the presence of TCO. Cells were either exposed to heat stress by incubating them at 44 °C for 1 h or were incubated an additional hour at 37 °C. For crosslinking 37 % formaldehyde was added to 1 % final concentration into the culture medium of the target cells and incubated at 37 °C for 10 min. The crosslinking reaction was stopped by adding 1.25 M glycine solution to 127 mM final concentration and an incubation at room temperature for 10 min. The solution was discarded, and the cells were washed with 3 mL DPBS. The cells were permeabilized by adding 3 mL 0.25 % Triton X-100 in DPBS and incubated at room temperature for 10 min. After washing the cells with 3 mL DBPS, 10 mL 0.001 % avidin in DBPS was added to cells and incubated one hour at room temperature. Again, three washing steps were performed with 3 mL DPBS and 10 mL 0.001 % biotin in DPBS was added and the cells were incubated one hour at room temperature. The cells were washed once with 3 mL DPBS before 10 mL 100 µM biotin-tetrazine in DPBS were added and the cells were incubated over night at 4 °C. Next day the cells were collected by centrifugation at 2000 rpm and 4 °C for 8 min. The cell pellet was washed once with 10 mL DPBS and was frozen at −80 °C.After pervious described chromatin preparation, the protein amount was determined by using the Pierce “BCA Protein Assay” Kit according to the manufacture’s guidelines. For the click-mediated enrichment the same protein amount of each sample was mixed with 2.125-fold amount of 1.47 % Triton X-100 in MLB3 and added to the streptavidin-conjugated Dynabeads (Thermo Fisher) (blocked with 0.1 % BSA). After a rotation step at RT overnight, the tube was placed in a magnet rack and the supernatant was discarded. The beads were washed twice with 900 µL Low Salt Buffer (LSB), High Salt Buffer (HSB), LiCl Buffer and three times with 900 µL DPBS. The detergent free beads were resuspended in 50 µL Denaturing/Reducing Buffer and incubated shaking for 30 min at RT. After adding 5.55 µL of Alkylation Buffer the beads were incubated shaking for 30 min at RT. The first digestion step was performed by adding 2 µg of LysC solved in nuclease free water and an shaking incubation for 1 hour at 37 °C. For the second digestion step 165 µL Tris pH 7.5 containing 1 µg trypsin were added to the bead solution and shaked again for 1 hour at 37 °C. The supernatant was separated from the beads using a magnetic rack and 2µg of trypsin solved in 50 mM acetic acid were added to the supernatant. The reaction mixture was incubated shaking overnight at 37 °C. For proteomic analysis the digestion was stopped by adding 2 µL TFA and the peptides were purified using stage tips (Thermo Fisher). The peptide solution was dryed in a speedvac for 1.5 hours at 30 °C and send for proteomic analysis.

Proteomics analysis - Nano-HPLC/MS/MS

After tryptic digestion and purification, the protein fragments were analyzed by nano-HPLC-MS/MS by using an UltimateTM 3000 RSLC nano-HPLC system, and a Q ExactiveTM Plus Hybrid Quadrupole-Orbitrap or an Orbitrap Fusion Lumos Tribrid mass spectrometer equipped with a nano-spray source (all from ThermoFisher Scientific). Briefly, the lyophilized tryptic peptides were suspended in 20 □L 0.1% TFA and 3 of the samples were injected onto and enriched on a C18 PepMap 100 column (5 □m, 100 Å, 300 □m ID * 5 mm, Thermo Scientific) using 0.1% TFA, at a flow rate of 30 µL/min, for 5 min (Plus) or 5.5 min (Lumos), respectively. Subsequently, the peptides were separated on a C18 PepMap 100 column (3 □m, 100 Å, 75 □m ID * 50 cm) using a linear gradient, starting with 95% solvent A/5% solvent B and increasing to 30.0% solvent B in 90 min (Plus) or increasing to 20% solvent B in 110 min and further increasing to 32% solvent B in 20 min (Lumos), with a flow rate of 300 nL/min (solvent A: water containing 0.1% formic acid; solvent B: acetonitrile containing 0.1% formic acid). The nano-HPLC apparatus was coupled online with the mass spectrometer using a standard coated Pico Tip emitter (ID 20, Tip-ID 10, New Objective, Woburn, MA, USA). Signals in the mass range of m/z 300 to 1650 were acquired at a resolution of 70,000 (Plus) or 60,000 (Lumos) for full scan, followed by up to ten (Plus) or fifteen (Lumos) high-energy collision-dissociation (HCD) MS/MS scans of the most intense at least doubly charged ions at a resolution of 17,500 (Plus) or 15,000 (Lumos).

Protein identification and relative quantification were performed by using MaxQuant[4] v.1.6.3.4, including the Andromeda search algorithm and searching the human reference proteome of the UniProt database in parallel with the TALE- and the mCherry sequences. Briefly, an MS/MS ion search was performed for enzymatic trypsin cleavage, allowing two missed cleavages. Carbamidomethylation was set as a fixed protein modification, and oxidation of methionine and acetylation of the N-terminus were set as variable modifications. The mass accuracy was set to 20 ppm for the first search, and to 4.5 ppm for the second search. The false discovery rates for peptide and protein identification were set to 0.01. Only proteins for which at least two peptides were quantified were chosen for further validation.

Relative quantification of proteins was performed by using the label-free quantification algorithm implemented in MaxQuant. All experiments were performed in technical triplicates. Statistical data analysis was performed using Perseus[5] v.1.6.2.3. Label-free quantification (LFQ) intensities were log-transformed (log2) and replicates were grouped together. Proteins had to be quantified at least three times in at least one of the two groups used for a dedicated t-test to be retained for further analysis. Missing values were imputed using small normal distributed values (width 0.3, down shift 1.8), and a t-test (false discovery rate of 0.05, s0=0.8) was performed. Proteins that were statistically significant outliers were considered as hits.

BirA* based enrichment of genomic loci for qPCR

$2.5x10^6$ HEK293T cells (cultured on PLL coated plates) overnight prior transfection. Cells were transfected with 6 µg of pAnW2613 (coding for the SATIII-TALE_BirA* fusion protein) or 6 µg of pAnW2615 (coding for the scSATIII-TALE_BirA* fusion protein) in presence or absence of 50 µM biotin. For crosslinking 37 % formaldehyde was added to 1 % final concentration into the culture medium of the target cells and incubated at 37 °C for 10 min. The crosslinking reaction was stopped by adding 1.25 M glycine solution to 127 mM final concentration and an incubation at room temperature for 10 min. Next day the cells were collected by centrifugation at 2000 rpm and 4 °C for 8 min. The cell pellet was washed once with 10 mL DPBS and was frozen at −80 °C. After pervious described chromatin preparation, the DNA amount was determined by qPCR. For the click-mediated enrichment the same DNA amount of each sample was mixed with 2.125-fold amount of 1.47 % Triton X-100 in MLB3 and added to the streptavidin-conjugated Dynabeads (Thermo Fisher) (blocked with 0.1 % BSA). After a rotation step at RT overnight, the tube was placed in a magnet rack and the supernatant was discarded. The beads were washed twice with 900 µL Low Salt Buffer (LSB), High Salt Buffer (HSB), LiCl Buffer and TBS with 0.1 % IGEPAL. Afterwards the DNA was eluted via reverse crosslinking. Specifically, the beads were suspended in 85 µL ddH2O and 4 µL 5 M NaCl and incubated at 65 °C overnight. After adding 1 µL of 10 mg/mL RNase A and an incubation step at 37 °C for 45 min, 2 µL of 0.5 M EDTA (pH 8.0), 8 µL of 0.5 M Tris-HCl (pH 6.8) and 1 µL Proteinase K were added and incubated at 45 °C for 1.5 h. Afterwards, the beads were separated by a magnetic rack and the supernatant containing the DNA was purified using PCR purification kit (Macherey Nagel) according to the manufacture's guidelines. For qPCR analysis 2 µL of the purified DNA were pipetted in a PCR workstation into a 384 well plate. A reaction mixture containing the 2x GoTaq master mix, forward primer, reverse primer and ddH2O was prepared and added to the sample without mixing. The plate was tightly sealed and centrifuged at RT for 1 min at 2500 rpm. Afterwards the qPCR was performed on the CFX384 Touch real-time PCR detection system.

Chromatin Immunoprecipitation (ChIP)

$1.5x10^5$ HeLa cells were cultured in 10 cm TC-plates (Sarstedt) overnight prior transfection. Cells were transfected with or 4.5 µg pAnW1410 ($tRNA^{Pyl}$/PylRS-AF) and 4.5 µg pAnW1811 (encoding for the SATIII-TALE_S36 mCherry fusion protein) in presence of 250 µM TCO. Cells were either exposed to heat stress by incubating them at 44 °C for 1 h or were incubated an additional hour at 37 °C. For crosslinking 37 % formaldehyde was added to 1 % final concentration into the culture medium of the target cells and incubated at 37 °C for 10 min. The crosslinking reaction was stopped by adding 1.25 M glycine solution to 127 mM final concentration and an incubation at room temperature for 10 min. the cells were collected by centrifugation at 2000 rpm and 4 °C for 8 min. The cell pellet was washed once with 10 mL DPBS and was frozen at −80 °C. After pervious described chromatin preparation, 160 µL fragmented chromatin were mixed with 340 µL of 1.47 % Triton X-100 in MLB3 and added to the HSF1-antibody (Cell Signaling) conjugated Protein G Dynabeads (Thermo Fisher) (blocked with 0.1 % BSA). After a rotation step at 4 °C overnight, the tube was placed in a magnet rack and the supernatant was discarded. The beads were washed twice with 900 µL Low Salt Buffer (LSB), High Salt Buffer (HSB), LiCl Buffer and TBS with 0.1 % IGEPAL. Afterwards the DNA was eluted via reverse crosslinking. Afterwards, the beads were separated by a magnetic rack and the supernatant containing the DNA was purified using PCR purification kit (Macherey Nagel) according to the manufacture's guidelines. For qPCR analysis 4 µL of the purified DNA were pipetted in a PCR workstation into a 384 well plate. A reaction mixture containing the 2x GoTaq master mix, forward primer, reverse primer and ddH2O was prepared and added to the sample without mixing. The plate was tightly sealed and centrifuged at RT for 1 min at 2500 rpm. Afterwards the qPCR was performed on the CFX384 Touch real-time PCR detection system.

***In vivo* reporter activation assay in HEK293T cells**

1.6 x 10^4 cells were cultured in a 96 well plate overnight prior transfection. The cells were transfected with 25 ng of the luciferase reporter plasmid pAnW1183 (encoding the TALE binding site and a minCMV promoter upstream of a firefly luciferase gene) and 175 ng of the TALE plasmid pAnW1031 (encoding the SATIII-TALE-VP64 fusion constructs) or pAnW1032 (encoding the sc SATIII-TALE-VP64 fusion constructs). 48 hr after transfection, each well was washed with 20 µl of DPBS. 40 µl of lysis buffer (100 mM NaH2PO4 and 0.2 % Triton X-100) was added to each well and mixed vigorously. The plate was then incubated on ice for 20 min. After incubation, 20 µL of the lysis solution from each well was combined with each 90 µL of Bright-Glo in a second 96 well plate. The plate was quickly spun down and the luminescence was immediately measured on a TECAN M1000 plate reader (wavelength 380-600 nM). Ratio of luminescence from each sample to that of a sample transfected with TALE-VP64 and the luciferase reporter plasmid bearing the TALE binding-site was plotted as relative luminescence. The error bars represent standard errors from duplicate experiments.

SUPPLEMENTARY TABLES

Antibodies

Table S3: Primary Antibodies

Epitope	Description	Company (Cat. #)
HSF1	Rabbit	Cell Signaling Technologies (4356S)

Table S4: Secondary Antibodies

Description	Company (Cat. #)
Fluorescein goat anti-rabbit IgG (H+L)	Invitrogen (F2765)

Table S5: Oligonucleotides for cloning.

All oligonucleotides were synthesized by Sigma-Aldrich.

Name	Description	Sequence
o1991	Quick Change Primer V92TAG fw	GGGACCGTCGCTTAGACGTATCAGCACATAATCACGGCG
o1992	Quick Change Primer V92TAG rv	GATTATGTGCTGATACGTCTAAGCGACGGTCCCTAACGC
o2471	Gibson Primer mCherry into pAnW891 fw	AAACGCAAAGTTGGGCGCGCCGTGAGCAAGGGCGAGGAGCTGTTC
o2472	Gibson Primer mCherry into pAnW891 rv	GAACGTCGTACGGGTAGTTAATCTTGTACAGCTCGTCCATGCCGAG
o2481	SATIII-TALE binding site fw for hybridization and restriction (SalI and SpeI)	TTTTGTCGACTGATTCCATTCCATTCCATTACTAGTTTTT
o2482	SATIII-TALE binding site rv for hybridization and restriction (SalI and SpeI)	AAAAACTAGTAATGGAATGGAATGGAATCAGTCGACAAAA
o2811	Quick Change Primer Y44TAG fw	CTACGCACGCTCGGCTAGAGTCAGCAGCAG
o2812	Quick Change Primer Y44TAG rv	CTGCTGACTCTAGCCGAGCGTGCGTAGATC
o2817	Quick Change Primer E50TAG fw	CAGCAGCAGCAATAGAAGATCAAACCGAAG
o2818	Quick Change Primer E50TAG rv	CACCTTCGGTTTGATCTTCTATTGCTGCTG
o2819	Quick Change Primer K51TAG fw	CAGCAGCAGCAAGAGTAGATCAAACCGAAG
o2820	Quick Change Primer K51TAG rv	CACCTTCGGTTTGATCTACTCTTGCTGCTG
o2825	Quick Change Primer S58TAG fw	CCGAAGGTGCGTTAGACAGTGGCGCAGCAC
o2826	Quick Change Primer S58TAG rv	CTGCGCCACTGTCTAACGCACCTTCGGTTT
o2833	Quick Change Primer L80TAG fw	CGCACATCGTTGCGTAGAGCCAACACCCGG
o2834	Quick Change Primer L80TAG rv	GGTGTTGGCTCTACGCAACGATGTGCGCGT
o2835	Quick Change Primer G88TAG fw	CCCGGCAGCGTTATAGACCGTCGCTGTCAC

o2836	Quick Change Primer G88TAG rv	ACAGCGACGGTCTATAACGCTGCCGGGTGT
o2841	Quick Change Primer I98TAG fw	CGTATCAGCACATATAGACGGCGTTGCCAG
o2842	Quick Change Primer I98TAG rv	GCAACGCCGTCTATATGTGCTGATACGTGA
o2843	Quick Change Primer A104TAG fw	GGCGTTGCCAGAGTAGACACACGAAGACAT
o2844	Quick Change Primer A104TAG rv	TCTTCGTGTGTCTACTCTGGCAACGCCGTG
o3224	Quick Change Primer S36TAG fw	GAAGGTGGGCCGCGGATAGGTGGATCTACG
o3225	Quick Change Primer S36TAG rv	GTGCGTAGATCCACCTATCCGCGGCCCACC
o3387	Insert GG Cassette deletion on pAnW1272 fw	GGAGGTGGCGGTGGCAGCGGG
o3388	Insert GG Cassette deletion on pAnW1272 rv	CGCGCCCGCTGCCACCGCCACCTCCGC

Table S6: qPCR Primer.

Locus	**Name**	**Sequence (5' → 3')**
SATIII	o2419_fw	AATCAACCCGAGTGCAATCGAATGGAATCG
	o2420_rv	TCCATTCCATTCCTGTACTCGG
MGMT	o1459_fw	GCCTGACCCGGATG
	o1457_rv	GAAAAGGTACGGGCCA

SUPPLEMENTARY FIGURES

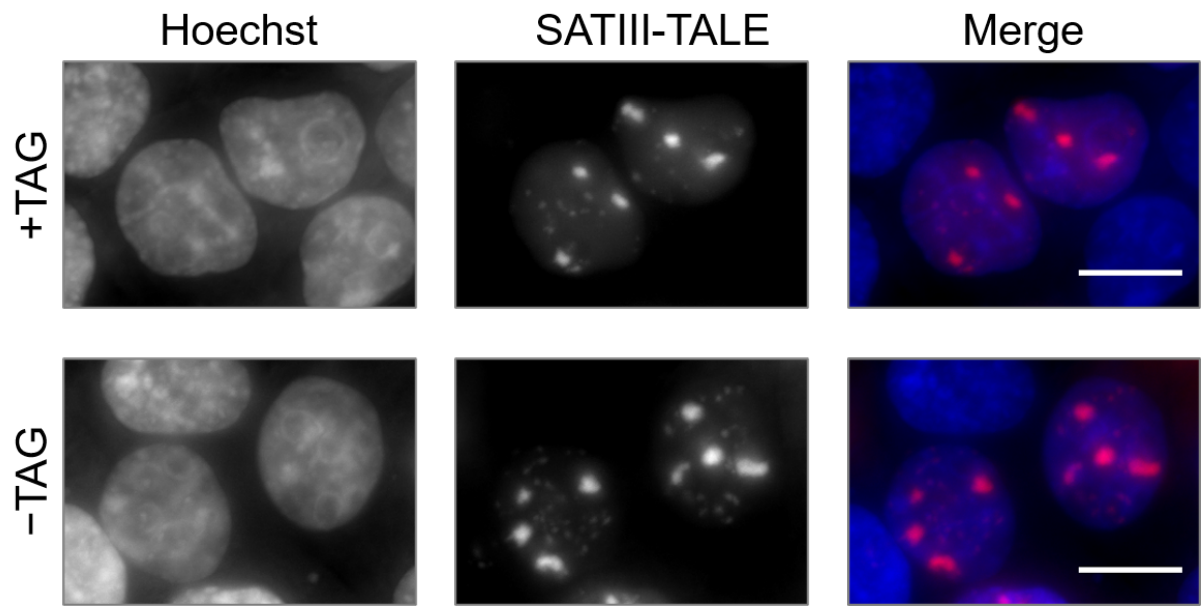

Figure S1. Nuclear localization of SATIII-TALE.

Cotransfection of PylRS_AF and SATIII-TALES36TCO (+TAG) or cotransfection of PylRS_AF and SATIII-TALE_wt + TCO (–TAG) fused to mCherry in HEK293T cells with Hoechst staining (scale bar: 10 μm).

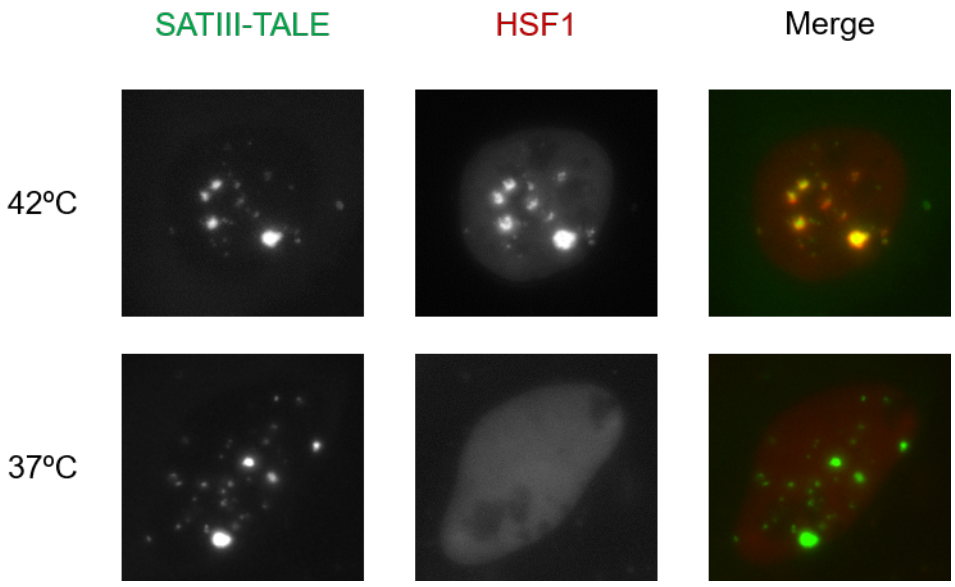

Figure S2. Colocalization of SATIII-TALE and HSF1.

Cotransfection of SATIII-TALE fused to mClover3 and HSF1-mCherry in U2OS cells at either 37ºC or after heatshock treatment at 42ºC for 4 hours.

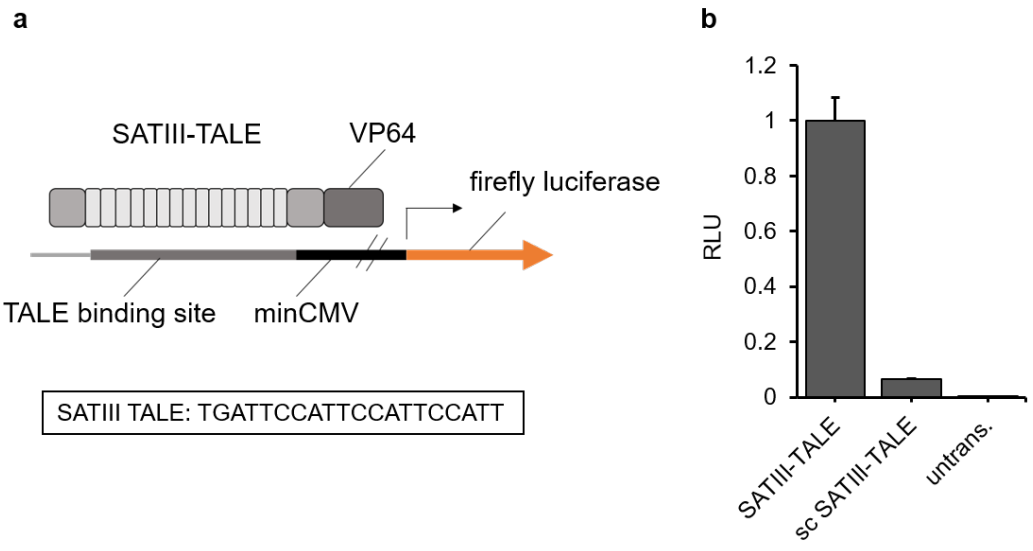

Figure S3. ***In vivo*** **reporter activation assay for studying TALE affinity.**

a) Schematic overview of the in vivo reporter activation assay. b) Relative luminescence units (RLU) plotted for different TALE constructs. SATIII-TALE fused to VP64; sc SATIII-TALE: scrambled SATIII-TALE containing the same RVDs but in a different order; untransfected HEK293T cells.

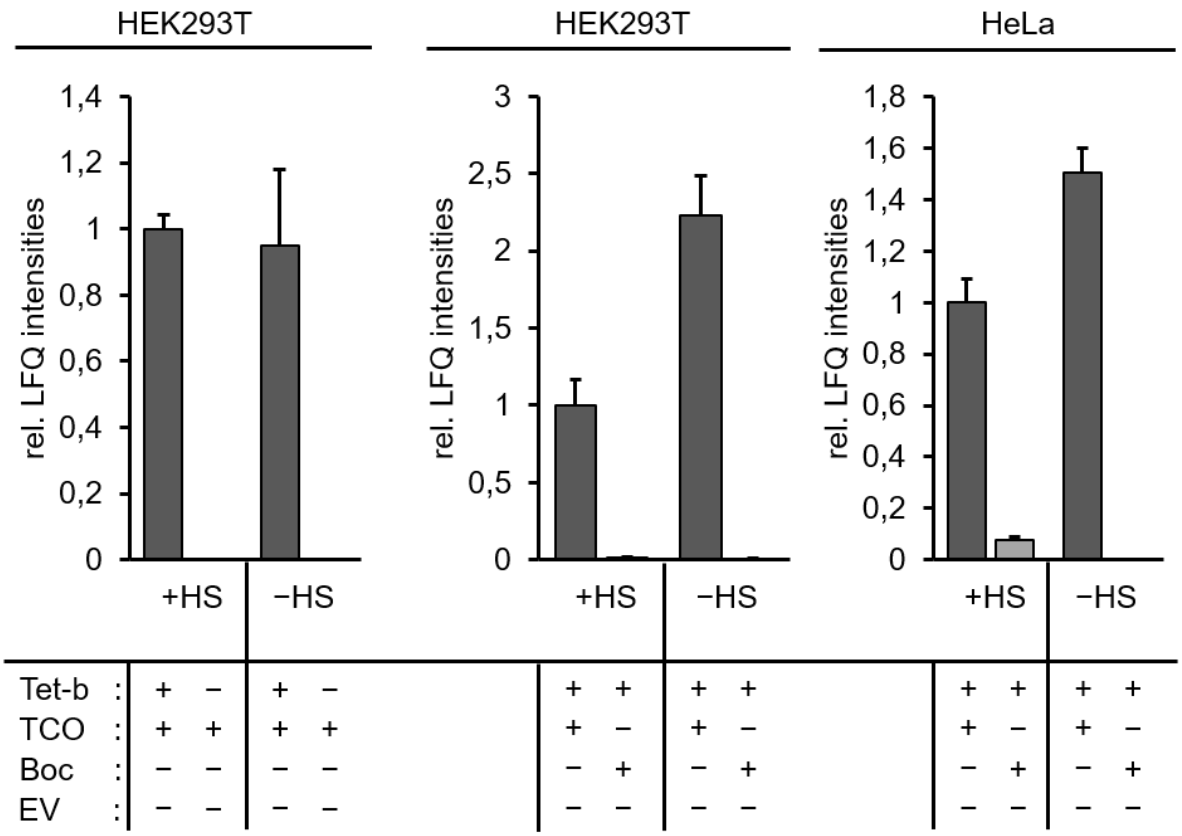

Figure S4. MS analysis of click-mediated enrichment of the SATIII-TALE.

Click-mediated MS enrichment of SATIII-TALE_S36TCO in presence or absence of Tet-b., SATIII-TALE_S36Boc in HEK293T and HeLa cells. under heat shock (1 h at 42 °C (left diagram)/44 °C (middle, right diagram)) or non-heat shock (1 h at 37 °C) conditions.

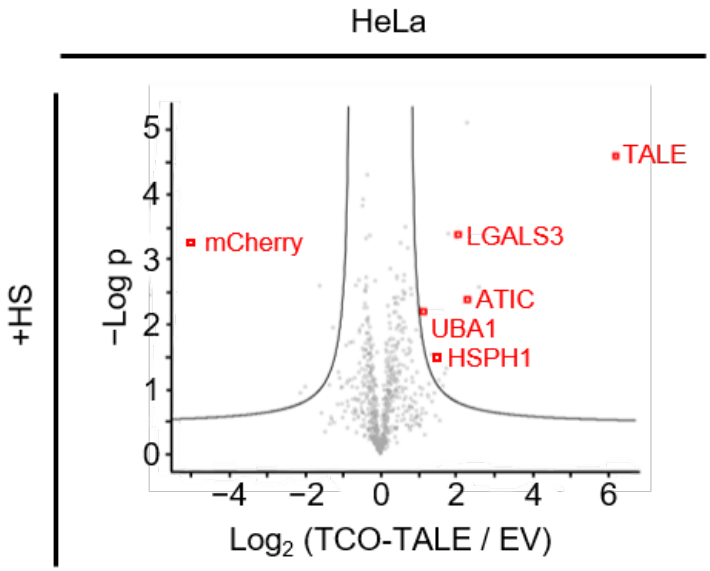

Figure S5. Proteomic analysis of SATIII loci under heat-shock conditions.

Volcano plots depicting the enrichment and significance (FDR: 0.05) of the identified proteins for SATIII-TALE_S36TCO in heat shocked HeLa cells (biological triplicates) .

References

[1] D. G. Gibson, L. Young, R.-Y. Chuang, J. C. Venter, C. A. Hutchison, H. O. Smith, *Nat Methods* **2009**, *6*, 343.

[2] T. Cermak, E. L. Doyle, M. Christian, L. Wang, Y. Zhang, C. Schmidt, J. A. Baller, N. V. Somia, A. J. Bogdanove, D. F. Voytas, *Nucleic Acids Res* **2011**, *39*, e82.

[3] T. Fujita, H. Fujii, *Biochem Biophys Res Commun* **2013**, *439*, 132.

[4] J. Cox, M. Mann, *Nat Biotechnol* **2008**, *26*, 1367.

[5] S. Tyanova, T. Temu, P. Sinitcyn, A. Carlson, M. Y. Hein, T. Geiger, M. Mann, J. Cox, *Nat Methods* **2016**, *13*, 731.

[6] I. Nikić, G. Estrada Girona, J. H. Kang, G. Paci, S. Mikhaleva, C. Koehler, N. V. Shymanska, C. Ventura Santos, D. Spitz, E. A. Lemke, *Angew Chem Int Ed Engl* **2016**, *55*, 16172.

APPENDIX

Sequence SATIII

TTCCATTCCATTCCTGTACTCGGGTTGATTCCATTCCATTCCATTCCAATCCATGCCATTCC
ACTCGTGTTGATTCCATTCTTTCCATTCCATTCAAGTTGAATCCATTCCATTGCATTCCATT
CATTCGATTCCATTCGATTGCACTCGGGTTGATT

Figure S6. SATIII Sequence.

SATIII Sequence with SATIII-TALE binding site (blue).

Plasmid maps

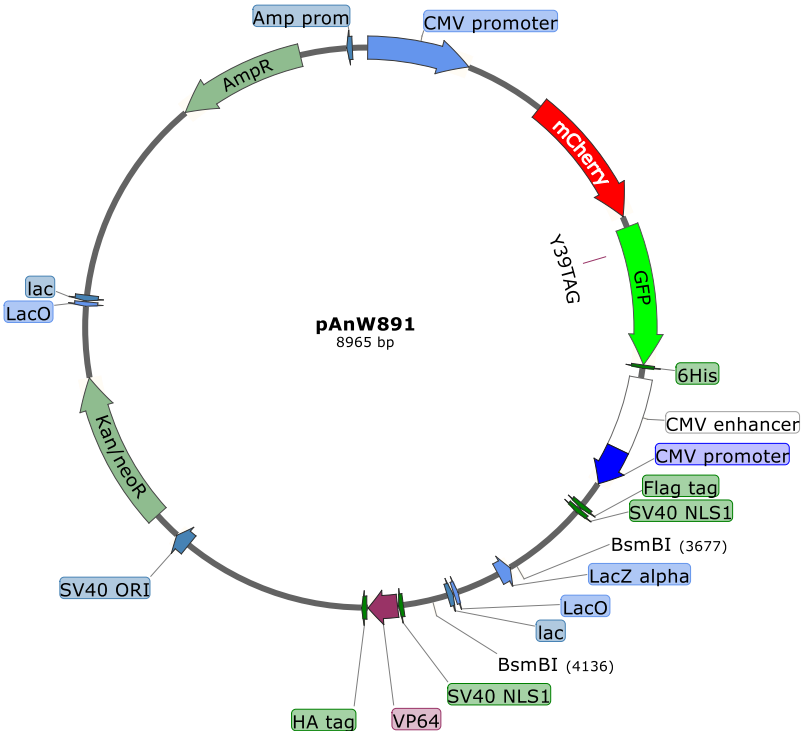

Figure S7. Plasmid map of pAnW891.

Golden-Gate entry plasmid with CMV promoter, C-terminal VP64 domain.

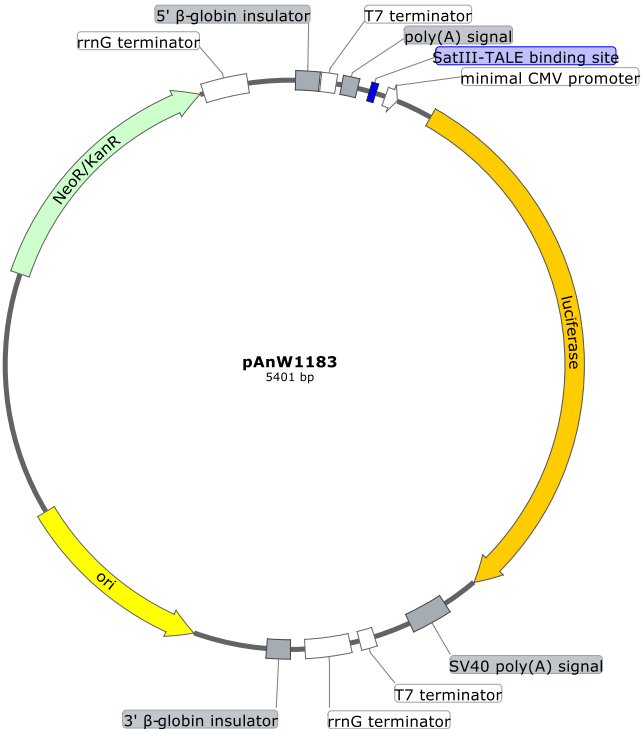

Figure S8. Plasmid map of pAnW1183.

Luciferase reporter plasmid with SATIII-TALE binding site.

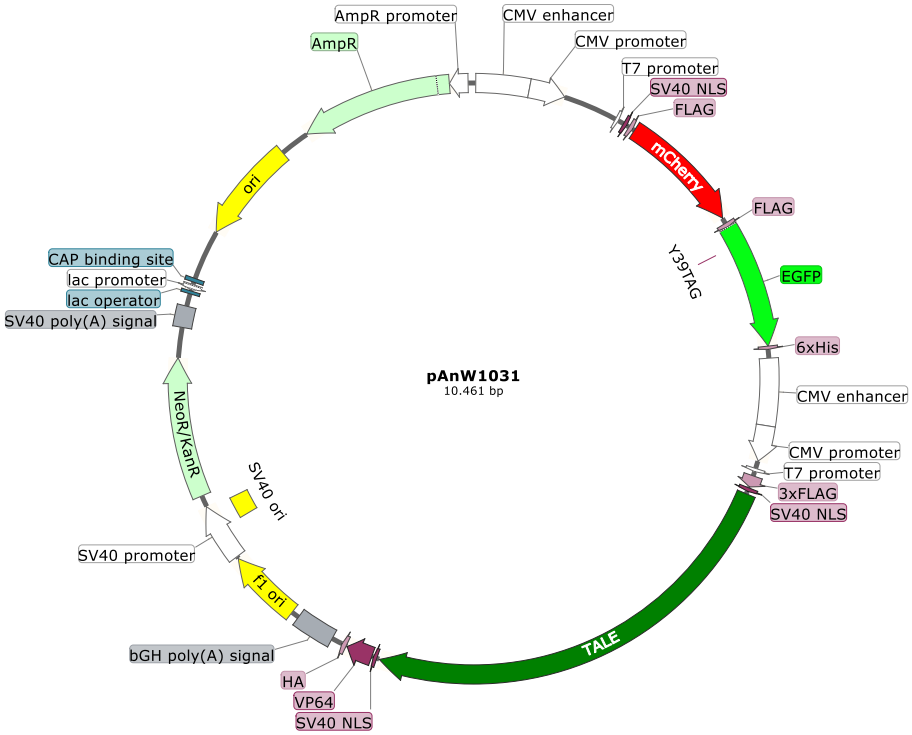

Figure S9. Plasmid map of pAnW1031.

Exemplary plasmid map for TALE without amber codon and with C-terminal VP64 domain and CMV promoter.

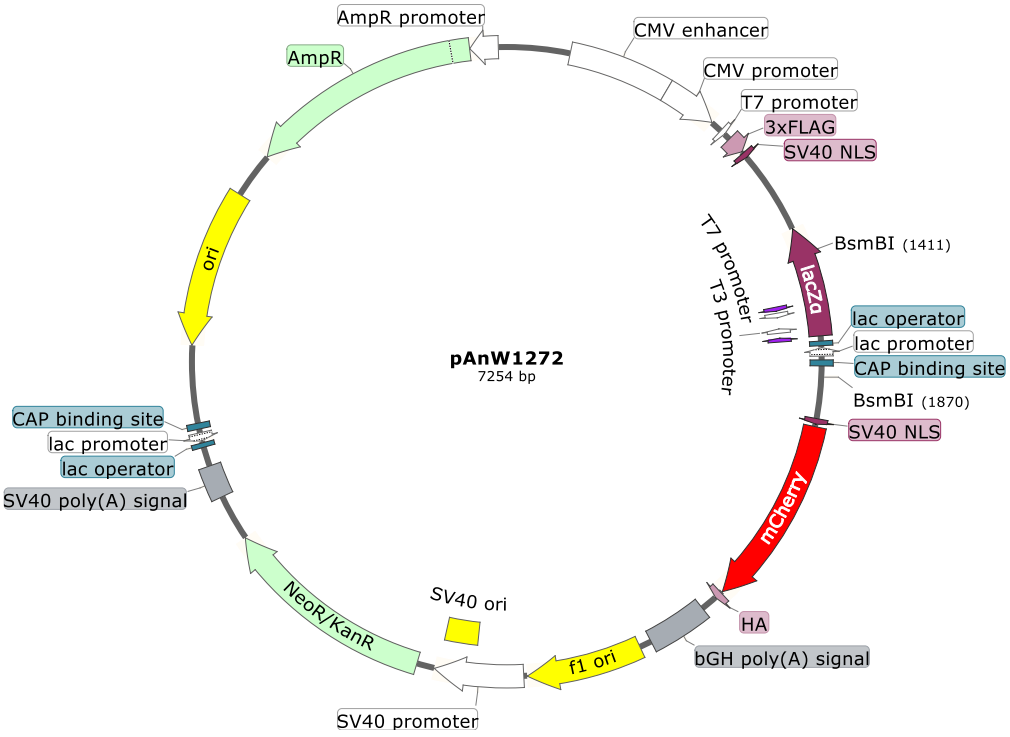

Figure S10. Plasmid map of pAnW1272.

Golden-Gate entry plasmid with CMV promoter, C-terminal mCherry.

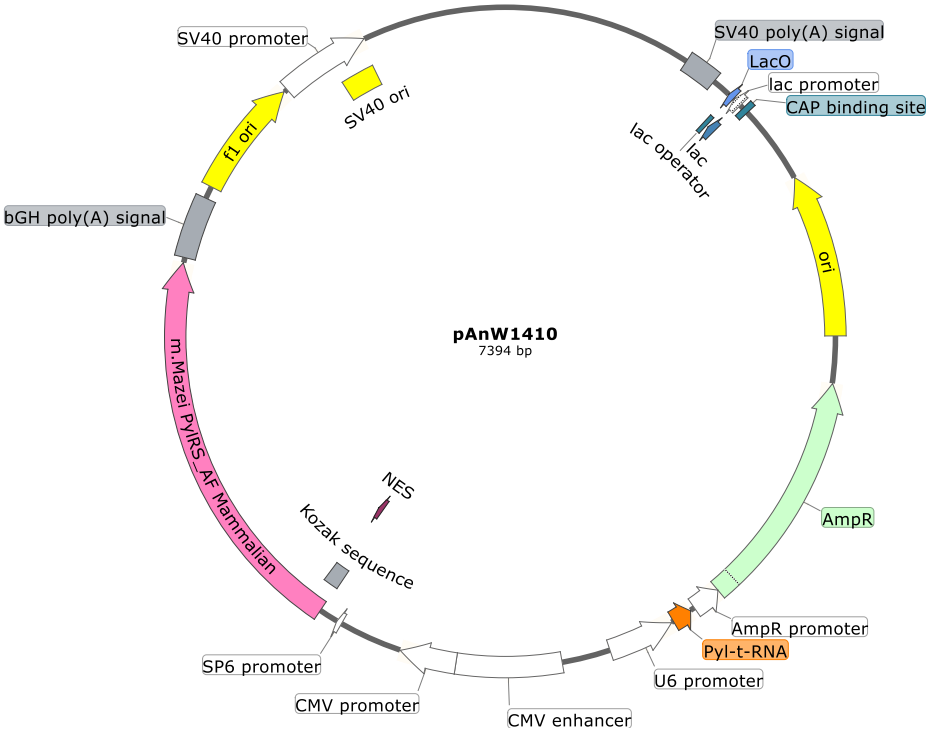

Figure S11. Plasmid map of pAnW1410.

Encoding for $tRNA^{Pyl}$ and PylRS-AF + nuclear export signal (NES)[6]

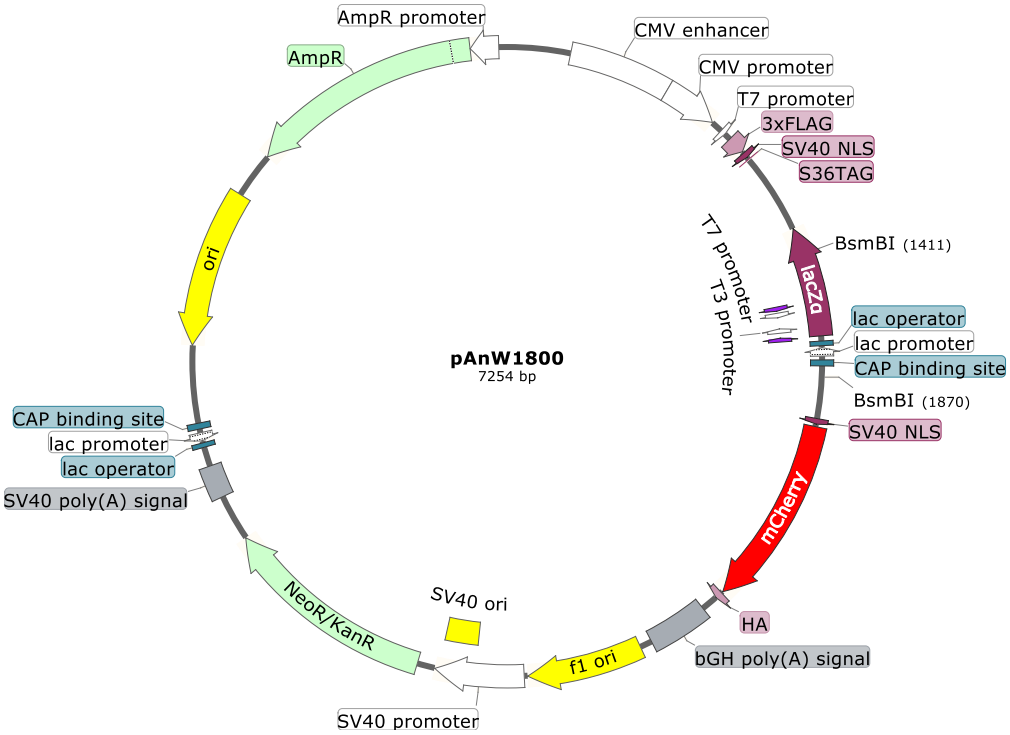

Figure S12. Plasmid map of pAnW1800.

Exemplary Golden-Gate entry plasmid with amber codon (S36TAG), CMV promoter, C-terminal mCherry.

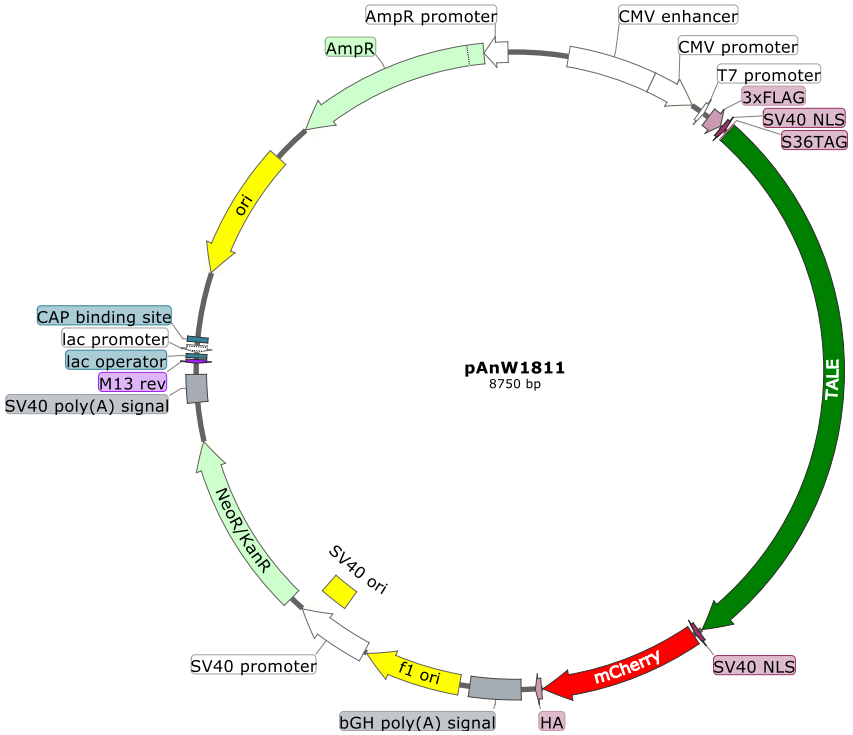

Figure S13. Plasmid map of pAnW1811.

Exemplary plasmid map for TALE with amber codon (S36TAG), C-terminal mCherry and CMV promoter.

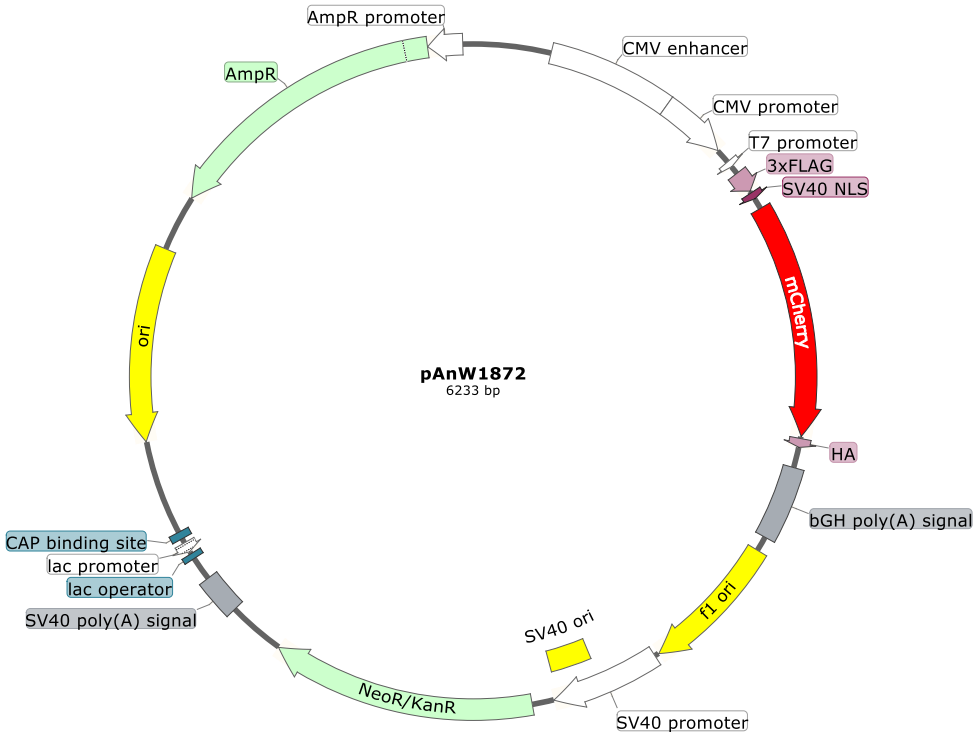

Figure S14. Plasmid map of pAnW1872.

Empty vector control, expressing only mCherry.

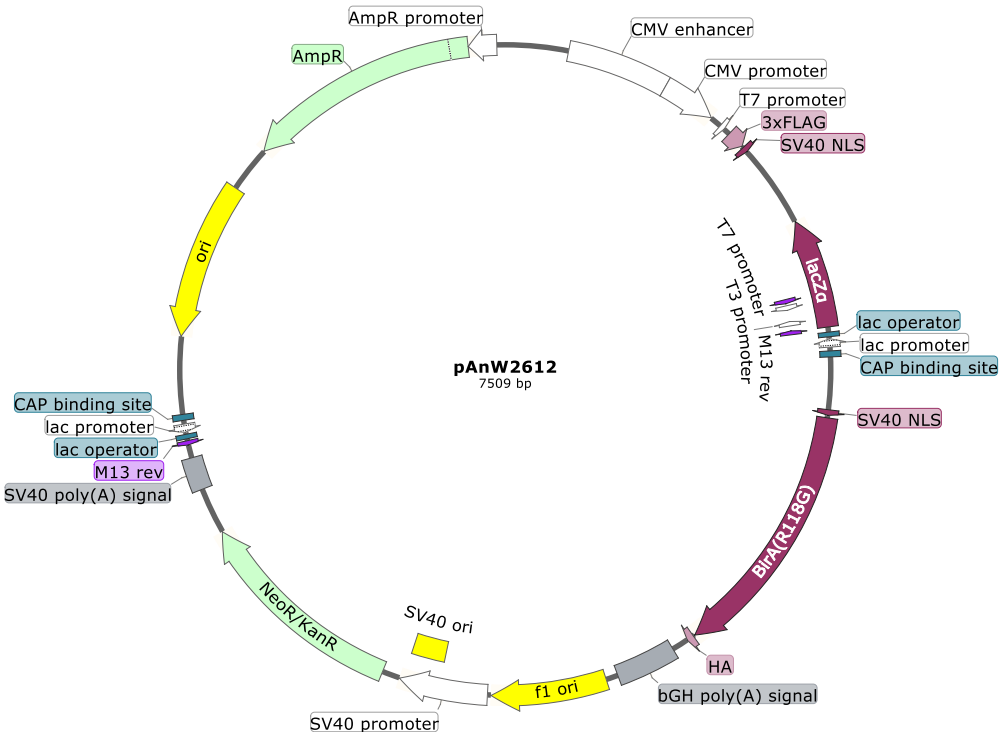

Figure S15. Plasmid map of pAnW2612.

Golden-Gate entry plasmid with CMV promoter, C-terminal BirA*.

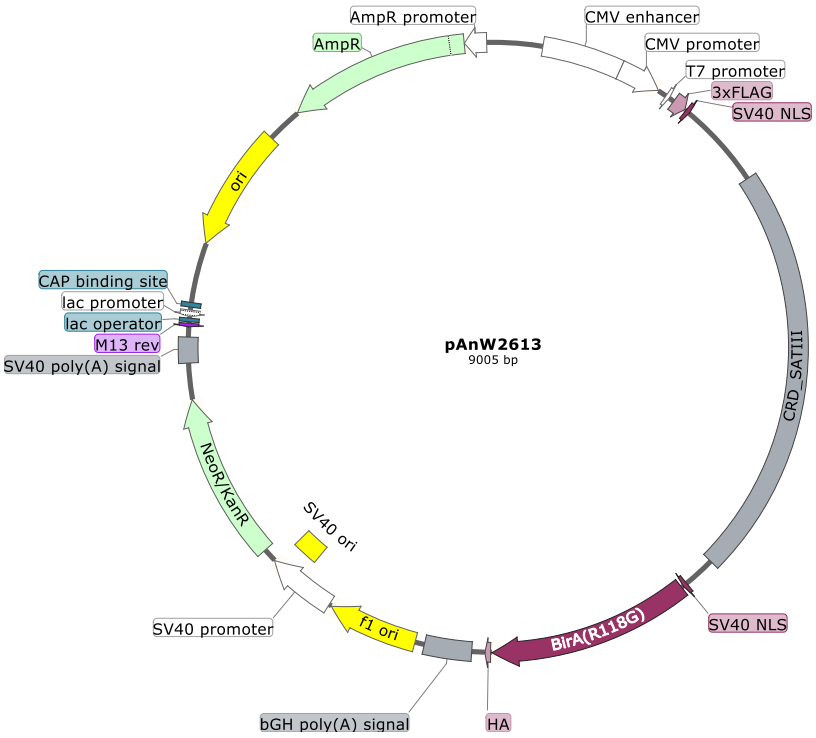

Figure S16. Plasmid map of pAnW2613.

Exemplary plasmid map for TALE with C-terminal BirA* and CMV promoter.