SUPPLEMENTARY INFORMATION

A transcription activator-like effector induction system mediated by proteolysis

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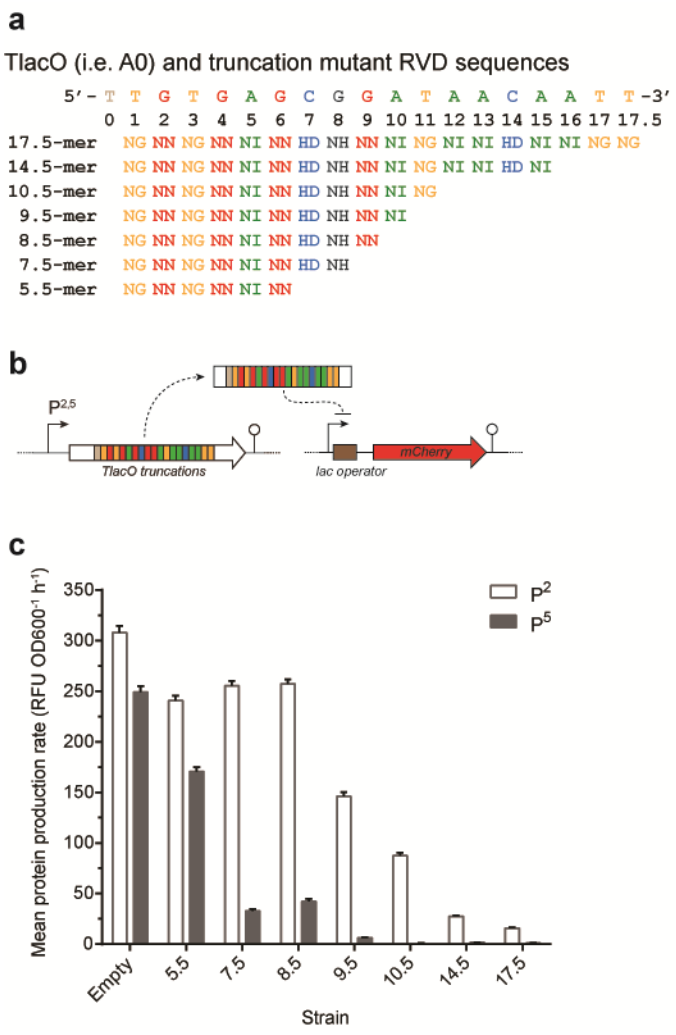
SUPPLEMENTARY RESULTS

Supplementary Figures……Page Numbers

Supplementary Tables

SI Figure 1 Truncation of TALE repeat domain reduces repression. (**a**) Truncated repeat domain variants of a TALE targeted to the lac operator (*i.e.* A0). (**b**) Truncated A0 constructs were constitutively expressed from either a weak (P^2) or strong (P^5) promoter. Expression vectors were transformed into *E. coli* MG1655 *ΔlacI* cells along with the p5Cherry reporter plasmid. (**c**) Mean mCherry protein production rates for the aforementioned strains (n=3). Error bars represent the standard error.

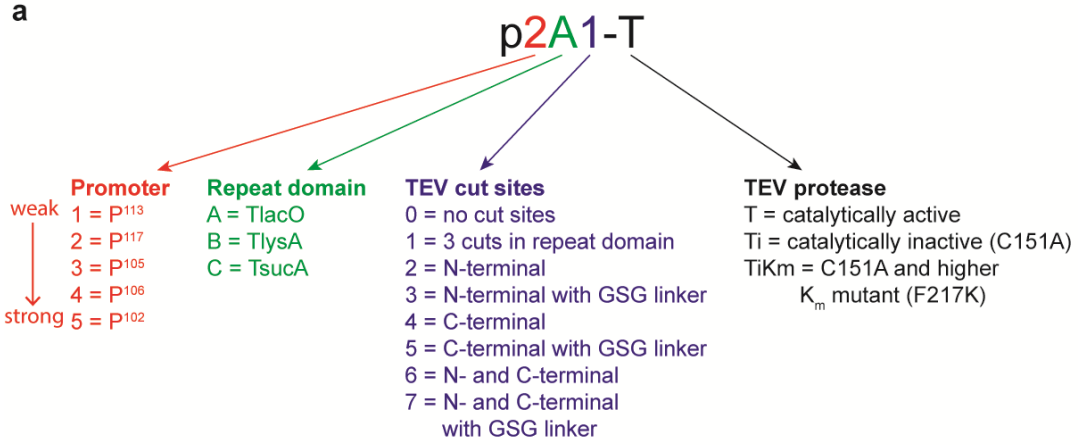
SI Fig. 1


a
TlacO (i.e. A0) and truncation mutant RVD sequences
5′- T T G T G A G C G G A T A A C A A T T -3′
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 17.5
17.5-mer NG NN NG NN NI NN HD NH NN NI NG NI NI HD NI NI NG NG
14.5-mer NG NN NG NN NI NN HD NH NN NI NG NI NI HD NI
10.5-mer NG NN NG NN NI NN HD NH NN NI NG
9.5-mer NG NN NG NN NI NN HD NH NN NI
8.5-mer NG NN NG NN NI NN HD NH NN
7.5-mer NG NN NG NN NI NN HD NH
5.5-mer NG NN NG NN NI NN
b
P2.5
TlacO truncations
lac operator
mCherry
c
Mean protein production rate (RFU OD600-1 h-1)
350
300
250
200
150
100
50
0
P2
P5
Empty
5.5
7.5
8.5
9.5
10.5
14.5
17.5
Strain

SI Figure 2 Nomenclature and primary amino acid sequence of the TALE cut site constructs. (**a**) Legend for the vector nomenclature system used throughout the manuscript. The first number (*i.e.* 1-5) denotes the constitutive promoter used to drive expression of the TALEs targeted to either the *lac* operator (*i.e.* TlacO or A), the *lysA* gene promoter proximal region (*i.e.* TlysA or B), or the *sucA* σ^s promoter-proximal region (*i.e.* TsucA or C). The second number (*i.e.* 0-7) describes the type and location of the TEV protease cut sites, if any, in the TALE construct. T, Ti, and TiKm are used to describe the TEV protease variant present in the vector. All *tev* mutants are regulated by the P^{tet} promoter. (**b**) The amino acid sequence of the different 17.5-mer TlacO constructs sectioned into the three major TALE domains (i.e. N-terminus, repeat domain, and C-terminus). The location, sequence, and name of the different TEV cut sites in each domain are in bold black text. Dashes denote the absence of an amino acid. In the repeat domain, the 12^{th} and 13^{th} amino acids of each 34-amino acid repeat, known as the repeat variable diresidues (*i.e.* RVDs), are spaced apart from the preceding 11 and proceeding 20 amino acids. The color scheme corresponds to the homology models presented in **SI Fig. 3**.

SI Fig. 2

a



b

A0-A7 N-terminus

```
MHHHHHHVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAA
LGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQ
LLKIAKRGGVTAVEAVHAWRNALTGAPLN---------- A0,A1,A4,A5
                          ---ENLYFQS--- A2,A6
                          GSGENLYFQSGSG A3,A7
```

A0-A7 Repeat domain

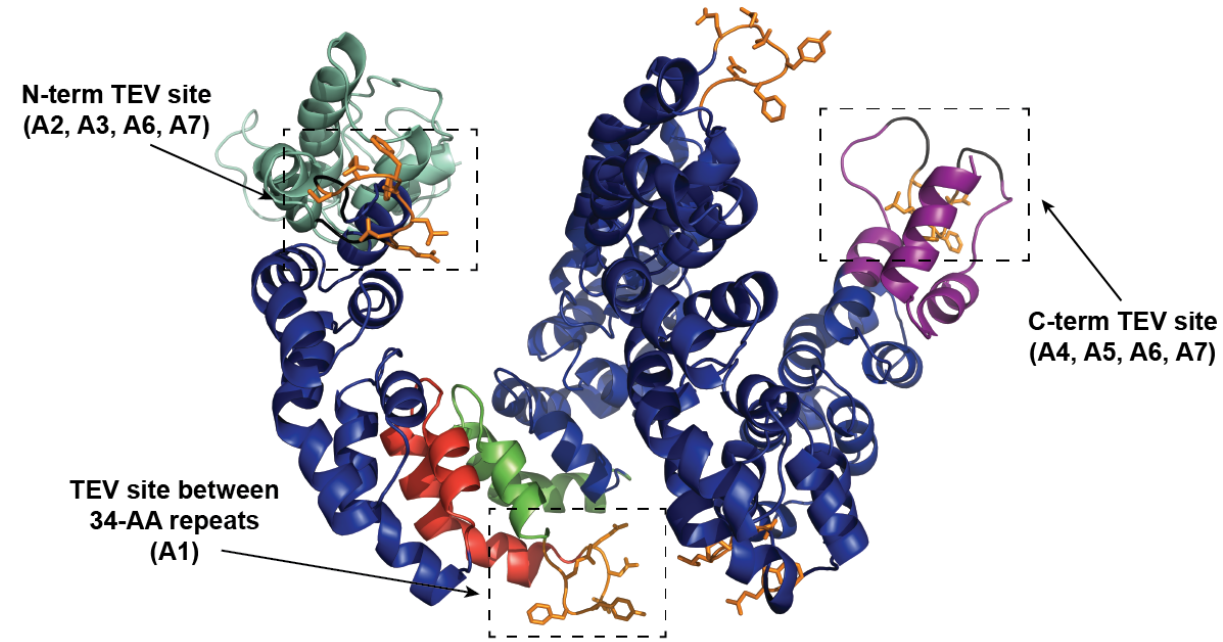
```
1     LTPEQVVAIAS NG GGKQALETVQRLLPVLCQAHG
2     LTPEQVVAIAS NN GGKQALETVQRLLPVLCQAHG
3     LTPEQVVAIAS NG GGKQALETVQRLLPVLCQAHG
4     LTPEQVVAIAS NN GGKQALETVQRLLPVLCQAHG------- A0,A2-A7
                                        ENLYFQG A1
5     LTPEQVVAIAS NI GGKQALETVQRLLPVLCQAHG
6     LTPEQVVAIAS NN GGKQALETVQRLLPVLCQAHG
7     LTPEQVVAIAS HD GGKQALETVQRLLPVLCQAHG
8     LTPEQVVAIAS NH GGKQALETVQRLLPVLCQAHG
9     LTPEQVVAIAS NN GGKQALETVQRLLPVLCQAHG------- A0,A2-A7
                                        ENLYFQG A1
10    LTPEQVVAIAS NI GGKQALETVQRLLPVLCQAHG
11    LTPEQVVAIAS NG GGKQALETVQRLLPVLCQAHG
12    LTPEQVVAIAS NI GGKQALETVQRLLPVLCQAHG
13    LTPEQVVAIAS NI GGKQALETVQRLLPVLCQAHG
14    LTPEQVVAIAS HD GGKQALETVQRLLPVLCQAHG------- A0,A2-A7
                                        ENLYFQG A1
15    LTPEQVVAIAS NI GGKQALETVQRLLPVLCQAHG
16    LTPEQVVAIAS NI GGKQALETVQRLLPVLCQAHG
17    LTPEQVVAIAS NG GGKQALETVQRLLPVLCQAHG
17.5  LTPEQVVAIAS NG GGRPALE
```

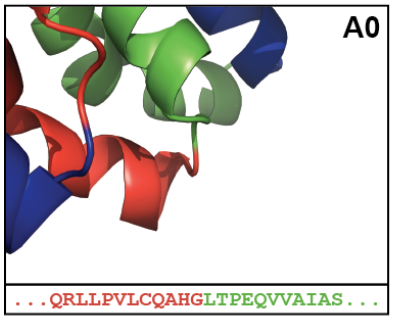
A0-A7 C-terminus

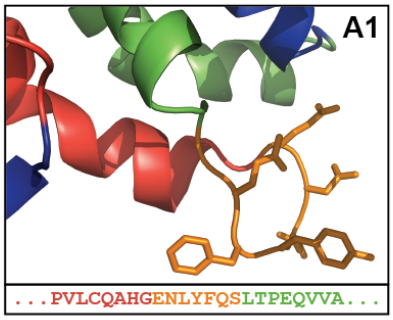
```
SIVAQLSRPDPA---------- A0,A1,A2,A3
            ---ENLYFQS--- A4,A6
            GSGENLYFQSGSG A5,A7
LAALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVADHAQV
VRVLGFFQCHSHPAQAFDDAMTQFGMSDYKDDDDK.
```

SI Figure 3 Homology models of TlacO with various TEV cut site arrangements. The cartoon model at the top is a homology model of a TlacO mutant to illustrate the location of the various TEV cut sites examined in this study. The homology model was generated using ModWeb (https://modbase.compbio.ucsf.edu/modweb/) based on PDB file 3UGM[1]. The panes below the global TlacO model more closely depict the hypothetical conformation of TEV cut sites with and without linkers in the repeat domain (*e.g.* A1), N-terminus (*e.g.* A2 and A3), and C-terminus (*e.g.* A4 and A5), relative to the unmodified A0 construct. The color scheme and displayed amino acid sequences correspond to **SI Fig. 2b**. The interrepeat loop that connects each 34 amino acid repeat was selected as a location to insert the seven amino acid TEV sequence. These loops are located on the outside of the TALE structure, making the insertion of a TEV site in these regions likely accessible to the protease and unlikely to disturb interactions between the RVDs and their cognate DNA base.

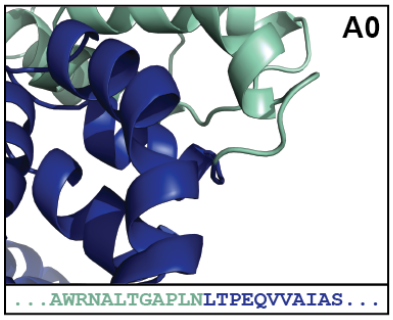
SI Fig. 3

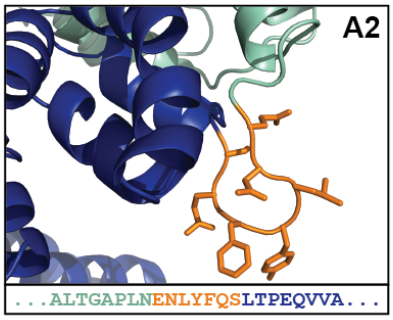


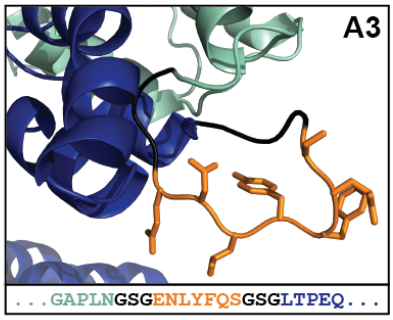


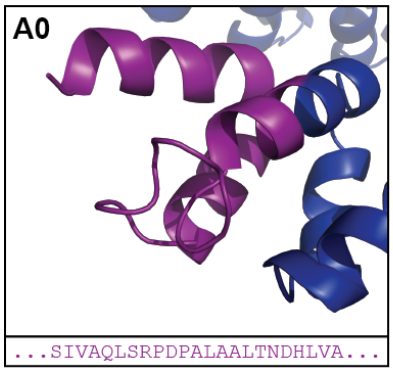


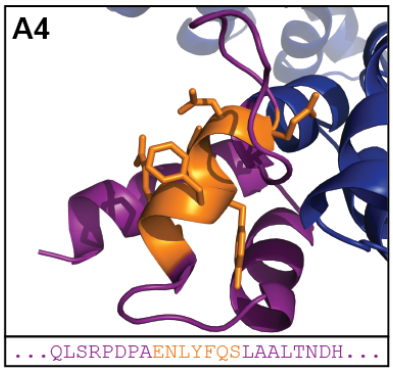
A0 = TlacO1, no TEV sites
A1 = 3 TEV sites between repeats 4/5, 9/10, 14/15
A2 = N-terminal TEV site
A3 = A2 flanked by Gly-Ser-Gly
A4 = C-terminal TEV site
A5 = A4 flanked by Gly-Ser-Gly
A6 = A2 + A4 TEV sites
A7 = A3 + A5 TEV sites

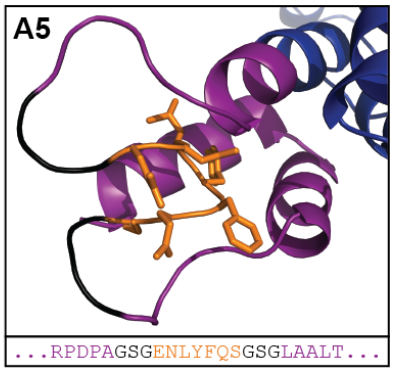






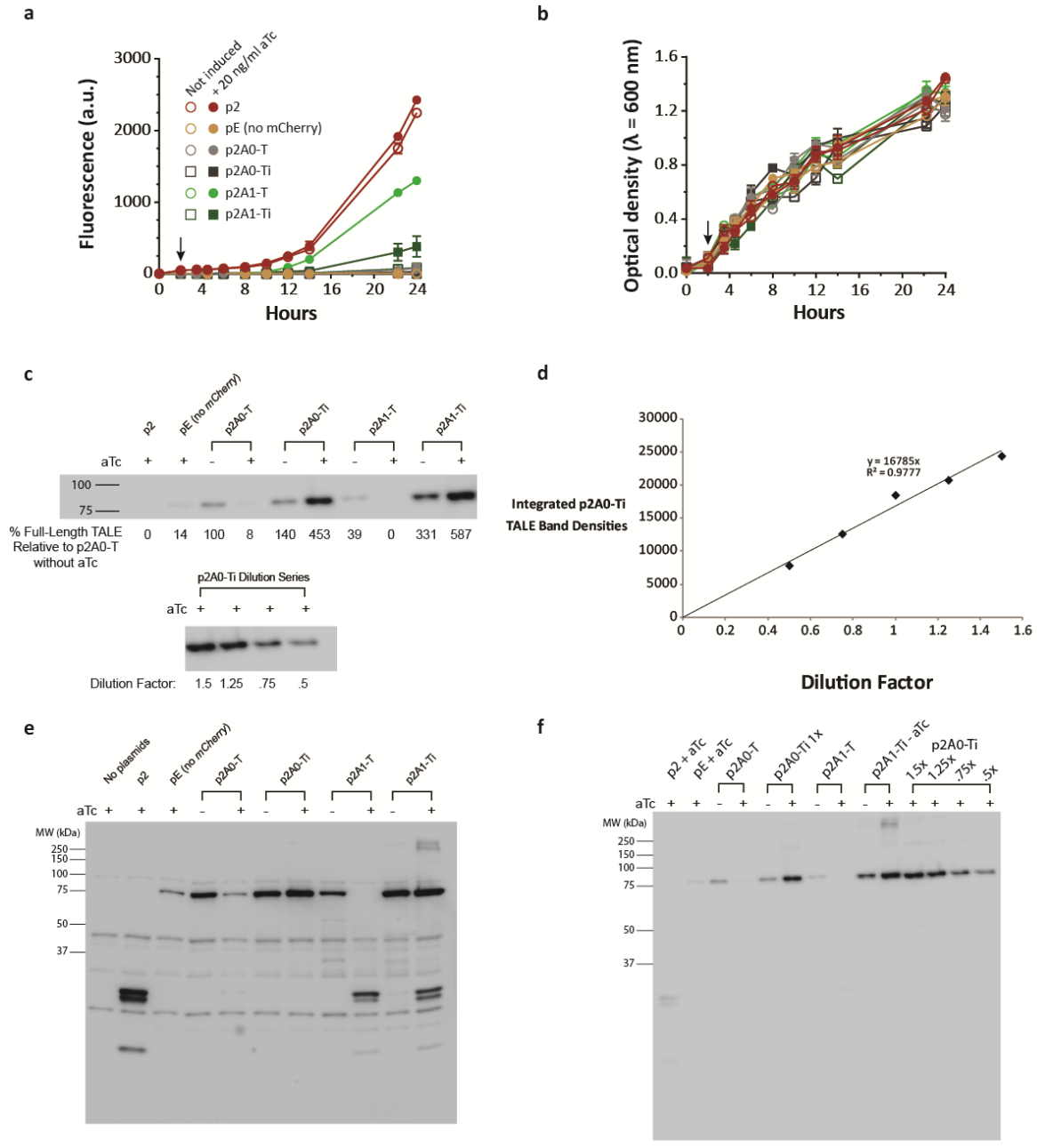






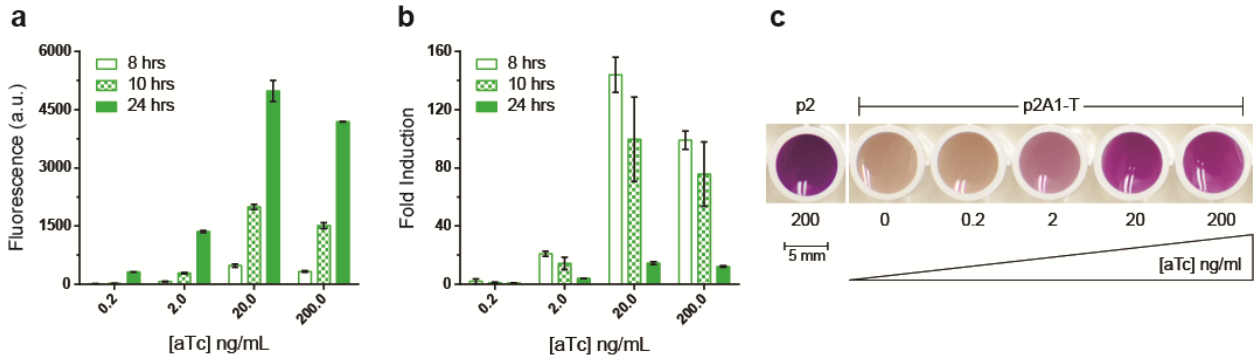
SI Figure 4 Time course (**a**) growth and (**b**) fluorescence data (n=3) associated with **Fig. 2** as well as a semi-quantitative full-length TALE Western blot. The pE strain (○, ●) contains a null reporter vector. Induction of *tev* protease with aTc (20 ng/ml) 2.5 hours after inoculation, denoted by (↓), results in de-repression of the *mCherry* reporter gene in strain p2A1-T (●) expressing the A1 TALE and an active protease. Error bars represent the standard deviation. (**c**) Repetition of the Western blot from **Fig. 2e** using the same samples but a reduced number of cells loaded per lane in order to achieve a linear correlation between full-length TALE integrated band intensity (as determined in ImageJ) and abundance. Surprisingly, this revealed that our TALE without TEV cut sites appears to be degraded in the presence of TEV protease (p2A0-T + aTc vs. p2A0-T – aTc), though to a lesser extent than when TEV cut sites are incorporated (p2A1-T + aTc vs. p2A1-T – aTc). Note that active TEV protease is required to observe this phenomenon in both cases, and that no full-length TALE band was detected in the p2A1-T + aTc lane as well as in the negative control p2 lane. A dilution series of the sample with the most TALE was included on this blot. (**d**) A plot of integrated band intensities from the p2A0-Ti dilution series versus the dilution factors confirmed that signal (integrated band density) was proportional to the amount of protein loaded. All data shown is from cultures grown at 30°C. (**e**) Full Western blot from which **Fig. 2e** was derived. (**f**) Full Western blot from which **SI Fig. 4c** was derived. Dilution factors are indicated next to p2A0-Ti + aTc lanes.

SI Fig. 4


a
Fluorescence (a.u.)
Hours
Not induced
+ 20 ng/ml aTc
p2
pE (no mCherry)
p2A0-T
p2A0-Ti
p2A1-T
p2A1-Ti
b
Optical density (λ = 600 nm)
Hours
c
aTc
% Full-Length TALE Relative to p2A0-T without aTc
0 14 100 8 140 453 39 0 331 587
p2A0-Ti Dilution Series
Dilution Factor: 1.5 1.25 .75 .5
d
Integrated p2A0-Ti TALE Band Densities
y = 16785x
R² = 0.9777
Dilution Factor
e
No plasmids
MW (kDa)
f
p2 + aTc
pE + aTc
p2A0-Ti 1x
p2A1-Ti - aTc
1.5x
1.25x
.75x
.5x

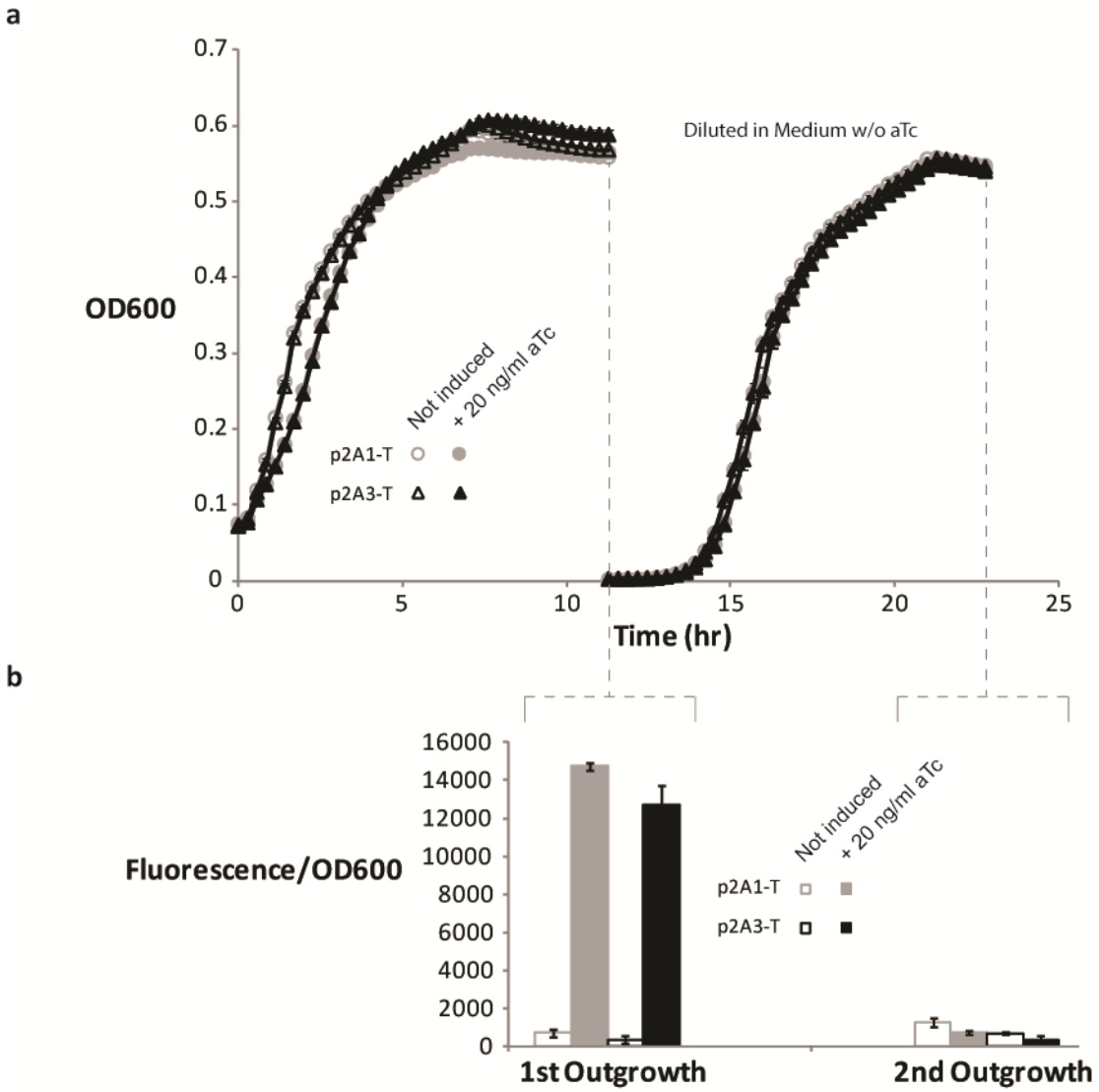
SI Figure 5 Titration of *tev* protease expression. (**a**) Mean mCherry fluorescence values of *E. coli* MG1655 *ΔlacI* cells containing the p2A1-T expression and p5Cherry reporter vectors at 8, 10, and 24 hours after inoculation (n=3). Cultures were induced 1.5 hours after inoculation with 0.2, 2, 20, or 200 ng/ml aTc. (**b**) Fold induction values derived from the ratio of the induced to uninduced state for each of the aTc concentrations examined at 8, 10, and 24 hours (n=3). All error bars represent the standard deviation. (**c**) Photograph of the p2 control sample and p2A1-T cells from the 24 hour time point following centrifugation and resuspension in 300 ul of media.

SI Fig. 5


a
Fluorescence (a.u.)
6000
4500
3000
1500
0
8 hrs
10 hrs
24 hrs
0.2
2.0
20.0
200.0
[aTc] ng/mL
b
Fold Induction
160
120
80
40
0
8 hrs
10 hrs
24 hrs
0.2
2.0
20.0
200.0
[aTc] ng/mL
c
p2
p2A1-T
200
0
0.2
2
20
200
5 mm
[aTc] ng/ml

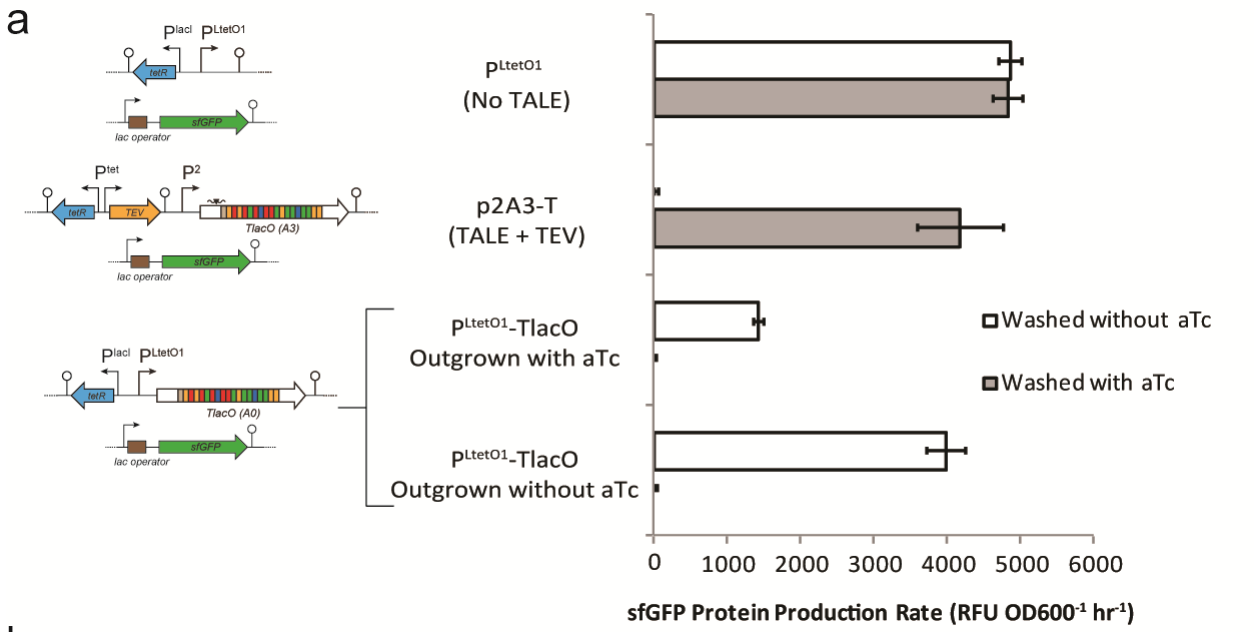
SI Figure 6 Induction by proteolytic TALE degradation is reversible. MG1655 ΔlacI was transformed with the reporter plasmid p5sfGFP and either p2A1-T or p2A3-T. An empty vector control strain was created by transforming MG1655 ΔlacI with pE and P^{LtetO1} (growth data not shown). Each of these strains was outgrown in LB medium with kanamycin and ampicillin in duplicate from an initial OD_{600} of ~.03 for 1.5 hours. Following this initial outgrowth, each culture received a final concentration of 20 ng/mL aTc or a corresponding amount of 50% v/v ethanol. Three 100 μL portions of each culture were then transferred to a 96 well plate which was incubated at 37°C with shaking and periodic OD_{600}/sfGFP fluorescence measurements in a Tecan m200 plate reader. After ~11 hours of growth, each replicate in this 96 well plate was subcultured by serially diluting 1000-fold in fresh LB medium with appropriate antibiotics but without aTc (*i.e.* no further TEV induction) in a second 96 well plate. This second plate was then returned to the plate reader for another ~11 hour incubation with the same conditions as previously described. (**a**) Growth curves for both the initial cultures and their subcultures. Growth data for the subcultures was appended to the initial growth curve. (**b**) Mean fluorescence of each culture normalized to optical density after subtracting away the average fluorescence of the empty-vector control at the end of both incubation periods (n=3). Growth in medium without aTc restored repression of the sfGFP reporter. All error bars represent the standard deviation.

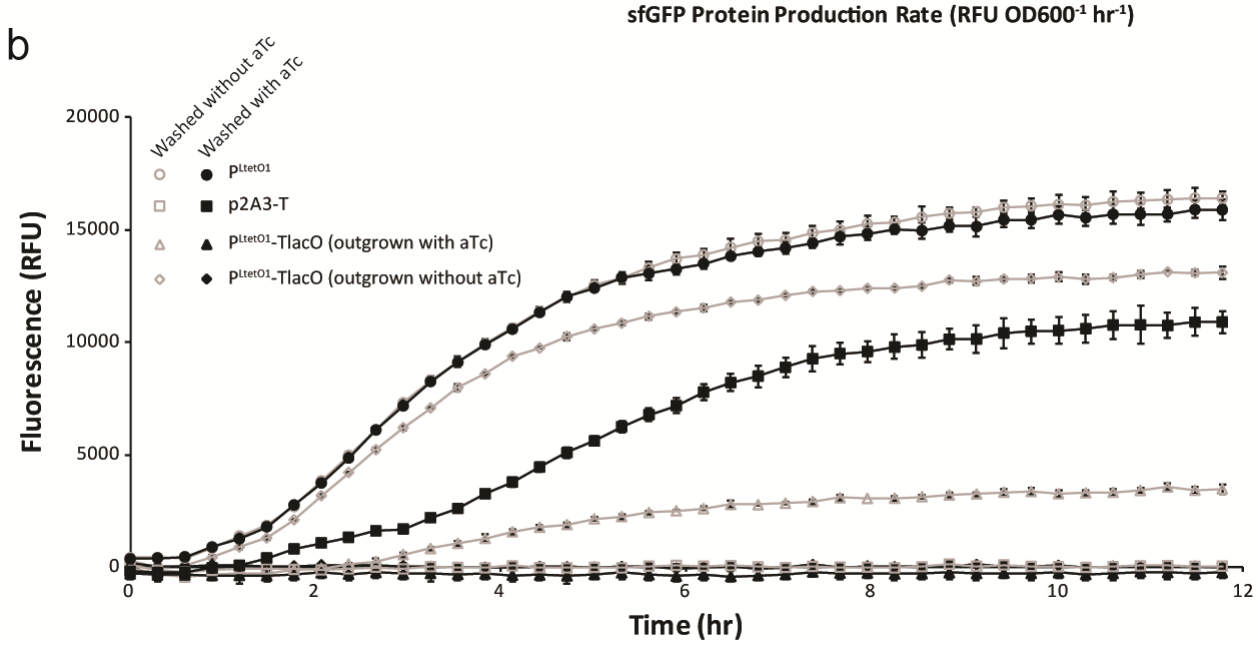
SI Figure 6


a
OD600
Time (hr)
Diluted in Medium w/o aTc
Not induced
+ 20 ng/ml aTc
p2A1-T
p2A3-T
b
Fluorescence/OD600
1st Outgrowth
2nd Outgrowth

SI Figure 7 Comparison of induction by TALE degradation to induction by dilution of TALE through growth. (**a**) MG1655 ΔlacI was transformed with the reporter plasmid p5sfGFP and either p2A3-T, a vector harboring an aTc inducible copy of TlacO (P^{LtetO1}-TlacO), or an empty-vector control (P^{LtetO1}). Each of these strains was outgrown in LB medium with kanamycin and ampicillin from an initial OD_{600} of ~.03 for 1.5 hours. The strain harboring P^{LtetO1}-TlacO was outgrown either with or without 20 ng/mL aTc. Following this initial outgrowth, the cultures were split into two equal volumes, harvested by centrifugation, and washed twice in LB medium with (grey bars) or without (white bars) 20 ng/mL aTc and appropriate antibiotics. After normalizing the OD_{600} of each culture to ~0.2 by dilution with the correct medium, the resuspensions were aliquoted in triplicate into a 96 well plate and fluorescence and optical density measurements were taken over a period of 24 hours as described in the Methods section. Protein production rates were then calculated from the resulting data (n=3), with error bars representing the standard error. As can be seen below, diluting the intracellular concentration of TALE by washing away its inducer (P^{LtetO1}-TlacO Outgrown with aTc, white bar) resulted in a protein production rate about one-third that of the samples where induction was mediated by proteolytic cleavage (p2A3-T, grey bar). This indicates that proteolytic cleavage and subsequent degradation of the TALE significantly enhances the strength of induction relative to simple dilution of the repressor despite an increase of cell density by an order of magnitude while growing in the plate reader (data not shown). (**b**) Time course fluorescence data for the experiment described in (**a**) (n=3). Error bars represent the standard deviation.

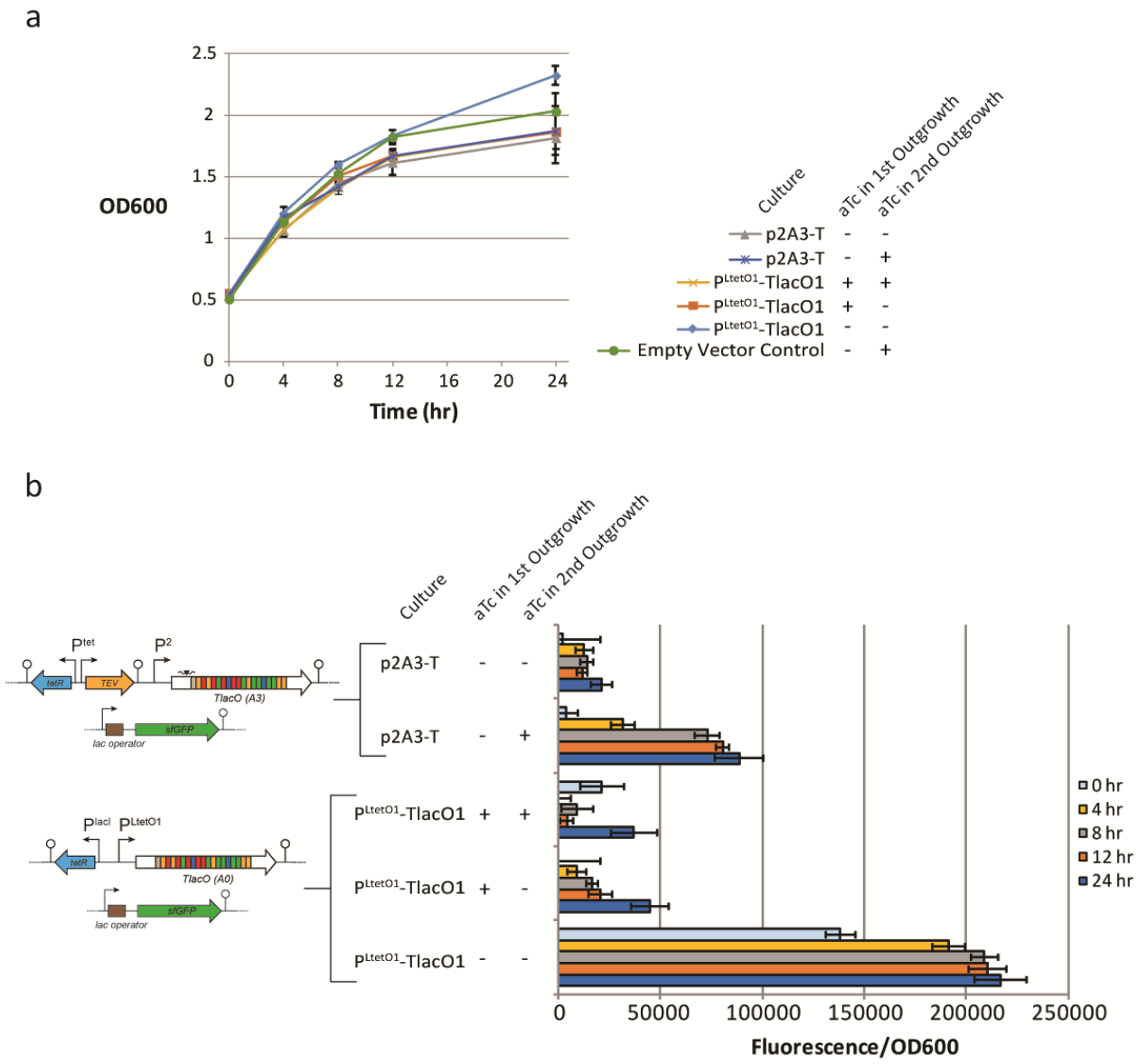
SI Figure 7


a
PlacI PLtetO1
tetR
sfGFP
lac operator
Ptet P2
tetR
TEV
TlacO (A3)
sfGFP
lac operator
PlacI PLtetO1
tetR
TlacO (A0)
sfGFP
lac operator
PLtetO1
(No TALE)
p2A3-T
(TALE + TEV)
PLtetO1-TlacO
Outgrown with aTc
PLtetO1-TlacO
Outgrown without aTc
Washed without aTc
Washed with aTc
0
1000
2000
3000
4000
5000
6000
sfGFP Protein Production Rate (RFU OD600-1 hr-1)


b
Washed without aTc
Washed with aTc
PLtetO1
p2A3-T
PLtetO1-TlacO (outgrown with aTc)
PLtetO1-TlacO (outgrown without aTc)
Fluorescence (RFU)
20000
15000
10000
5000
0
0
2
4
6
8
10
12
Time (hr)

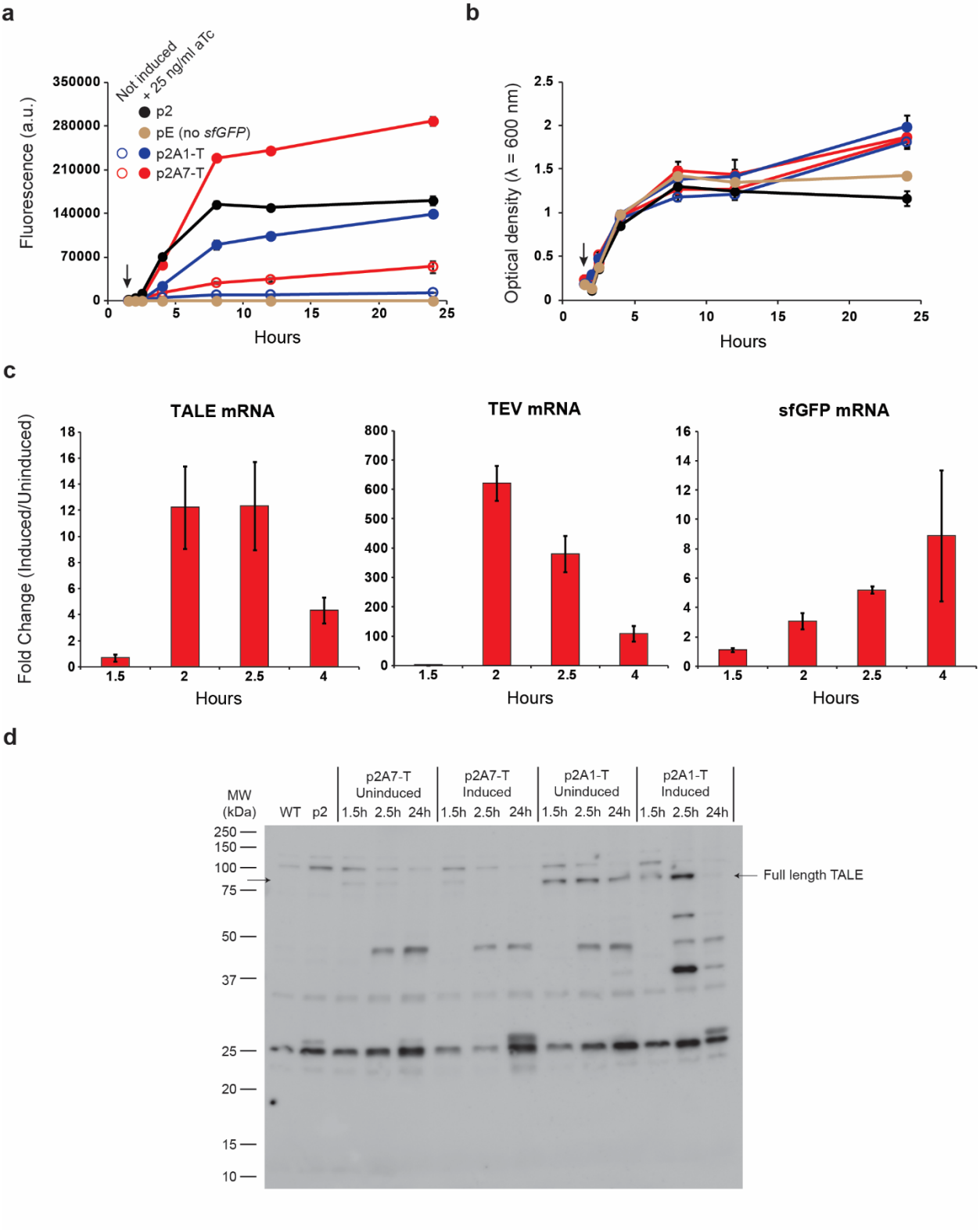
SI Figure 8 Induction by proteolytic TALE degradation during stationary phase. MG1655 ΔlacI was transformed with the reporter plasmid p5sfGFP and either p2A3-T or P^{LtetO1}-TlacO1 (see **SI Fig. 7**). An empty vector control strain was created by transforming MG1655 ΔlacI with pE and P^{LtetO1}. Each of these strains was outgrown in triplicate in LB medium with kanamycin and ampicillin from an initial OD_{600} of ~0.03 for 6 hours. The strain harboring P^{LtetO1}-TlacO1 was outgrown either with or without 20 ng/mL aTc. Following this initial outgrowth, the cultures were split into two equal volumes, harvested by centrifugation, and washed twice in LB medium with (+) or without (-) 20 ng/mL aTc and appropriate antibiotics. These cultures were then grown at 37°C for an additional 24 hours in shake tubes, and samples were periodically withdrawn from each culture and diluted ten-fold to take OD_{600} and sfGFP fluorescence measurements in a Tecan m200 plate reader. (**a**) Growth curves following washes and resuspension (n=3). All cultures doubled approximately twice over the course of this experiment. (**b**) Fluorescence of each culture normalized to optical density after subtracting away the average fluorescence of the empty-vector control (n=3). The cultures where induction was mediated by proteolytic cleavage (p2A3-T -/+) recovered more than 30% of the positive control's fluorescence (P^{LtetO1}-TlacO1 -/-) after 8 hours, while the system mediated by dilution (P^{LtetO1}-TlacO1 +/-) only recovered 8% after the same period of time. All error bars represent the standard deviation.

SI Figure 8


a
OD600
Time (hr)
Culture
aTc in 1st Outgrowth
aTc in 2nd Outgrowth
p2A3-T - -
p2A3-T - +
PLtetO1-TlacO1 + +
PLtetO1-TlacO1 + -
PLtetO1-TlacO1 - -
Empty Vector Control - +
b
Ptet
P2
tetR
TEV
TlacO (A3)
lac operator
sfGFP
PlacI
PLtetO1
TlacO (A0)
0 hr
4 hr
8 hr
12 hr
24 hr
Fluorescence/OD600

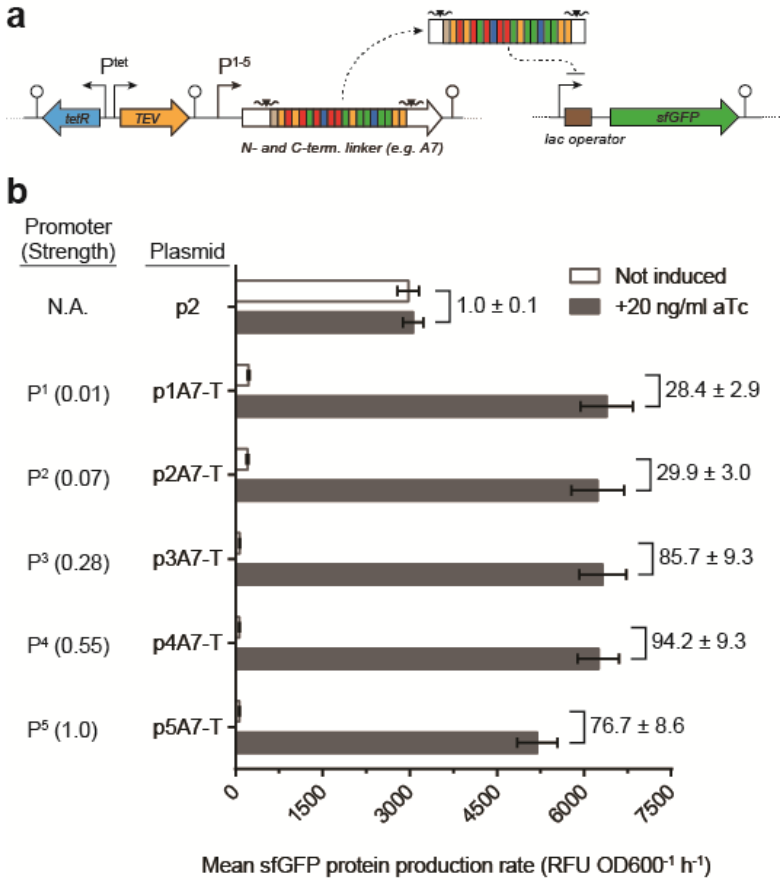
SI Figure 9 Characterization of the p2A7-T system. (**a**) Mean sfGFP fluorescence values of *E. coli* MG1655 *ΔlacI* cells containing the p2, p2A1-T, or p2A7-T expression vectors and the p5sfGFP reporter vector. The pE strain (○, ●) contains a null reporter vector. Cells were grown in 250 ml shake flasks at 37°C and sampled at each time point to determine OD_{600} and fluorescence values in a plate reader (n=3). Induction of *tev* with aTc (25 ng/ml) occurred 1.5 hours after inoculation, denoted as (↓), and results in de-repression of the *sfGFP* reporter gene in strains carrying both p2A1-T (●) and p2A7-T (●). (**b**) Observed average growth of strains in shake flasks over time monitored by turbidity measurements (n=3). (**c**) Fold change (*i.e.* the ratio of the induced to the uninduced mRNA levels) for the A7 TALE, TEV protease, and sfGFP mRNAs prior to induction (*i.e.* 1.5 hours) and at three time points (*i.e.* 2, 2.5, and 4 hours) after induction of *tev* with aTc (n=3). See Methods for a description of RNA isolation and quantitative PCR methodology. (**d**) Western blot probed with an anti-FLAG primary antibody to observe full length A1 and A7 TALEs in both induced and uninduced cultures at 1.5, 2.5, and 24 hours after inoculation. The arrow denotes the band corresponding to the respective full length TALE. Error bars throughout the figure represent the standard deviation.

SI Fig. 9


a
Not induced
+ 25 ng/ml aTc
p2
pE (no sfGFP)
p2A1-T
p2A7-T
Fluorescence (a.u.)
Hours
b
Optical density (λ = 600 nm)
Hours
c
TALE mRNA
TEV mRNA
sfGFP mRNA
Fold Change (Induced/Uninduced)
Hours
d
MW (kDa)
WT
p2
p2A7-T Uninduced
p2A7-T Induced
p2A1-T Uninduced
p2A1-T Induced
1.5h 2.5h 24h
Full length TALE

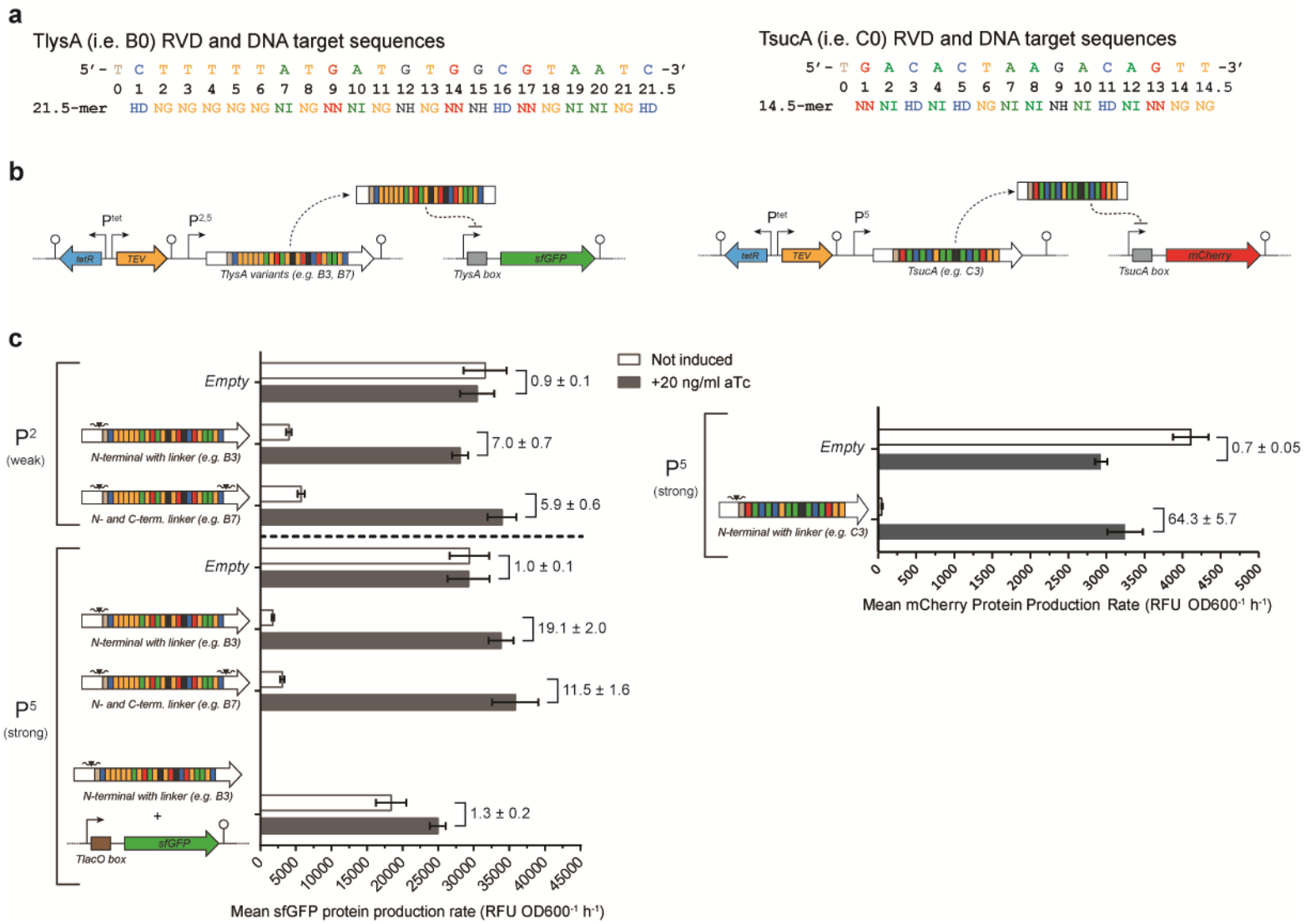
SI Figure 10 Tuning the repression and induction of A7-T system. (**a**) Schematic of the system used to examine the different constitutive promoters driving the A7 TALE. (**b**) Mean sfGFP protein production rates for each of the constitutive promoters (P^{1-5}) used to express A7 (n=3). Promoter strengths relative to the strongest promoter P^5 are shown in the far left column and are derived from the iGEM distrubtion (http://parts.igem.org/Promoters/Catalog/Anderson). Bracketed values indicate the fold induction. Error bars represent standard error.

SI Fig. 10



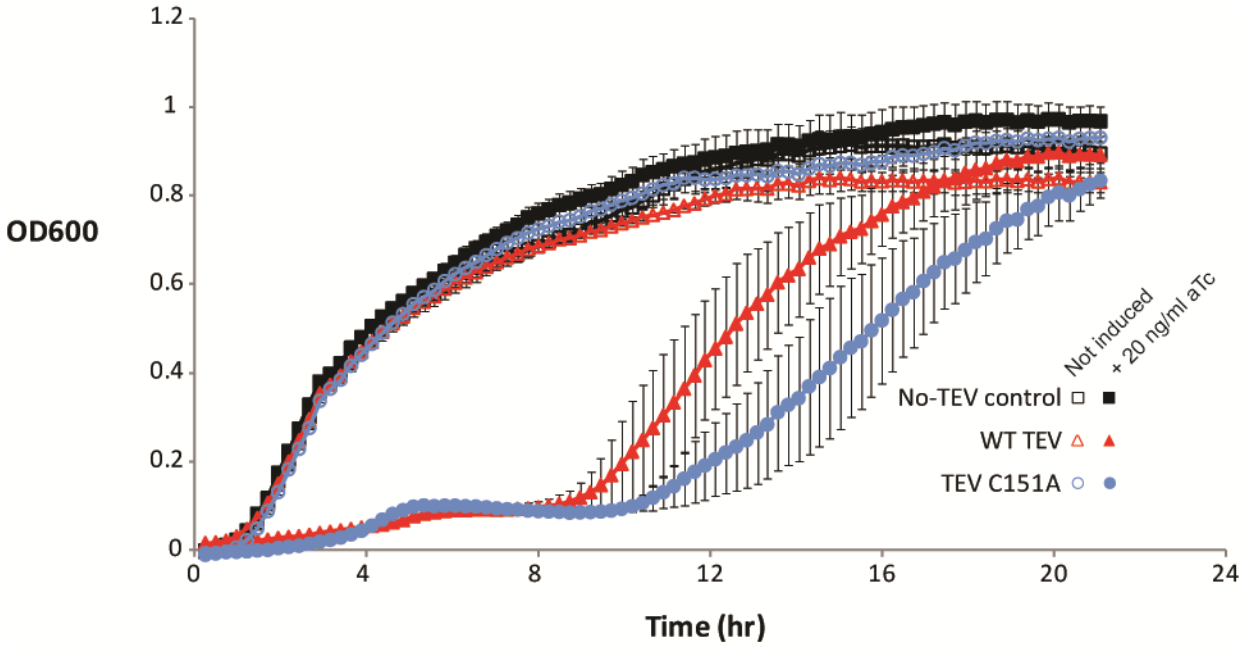
SI Figure 11 Design and verification of the TlysA and TsucA constructs with TEV cut sites. (**a**) The *lysA* and *sucA* gene promoter proximal DNA sequences and the corresponding RVD identities. (**b**) Schematic of the TALE-TEV system used to verify that the B3, B7, and C3 TALE constructs are capable of both repression and de-repression of a plasmid-based reporter gene. The B3 and B7 TALEs were expressed from either a weak (i.e. P^2) or strong (i.e. P^5) constitutive promoter, while TALE C3 was only expressed from P^5. The *lysA* DNA target sequence was substituted for the *lac* operator originally present in the p5sfGFP reporter to make the reporter p5lysAsfGFP. Likewise, the sucA DNA target sequence was substituted into p5mCherry to make p5sucAmCherry. (**c**) Mean protein production rates from the reporter vectors for B3-T, B7-T, and C3-T constructs. Additionally, the p5B3-T vector was co-transformed with the p5sfGFP reporter plasmid to demonstrate the fidelity of TALE binding to its cognate DNA target sequence (n=3). Note that the empty vector control cultures (*i.e.* no TALE) harboring p5sucAmCherry exhibited a significant lag phase during growth (data not shown). Fold induction values ± standard deviation are bracketed. Error bars represent the standard error.

SI Fig. 11


a
TlysA (i.e. B0) RVD and DNA target sequences
5′- T C T T T T T A T G A T G T G G C G T A A T C -3′
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 21.5
21.5-mer HD NG NG NG NG NG NI NG NN NI NG NH NG NN NH HD NN NG NI NI NG HD
TsucA (i.e. C0) RVD and DNA target sequences
5′- T G A C A C T A A G A C A G T T -3′
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 14.5
14.5-mer NN NI HD NI HD NG NI NI NH NI HD NI NN NG NG
b
Ptet
P2.5
tetR
TEV
TlysA variants (e.g. B3, B7)
TlysA box
sfGFP
Ptet
P5
tetR
TEV
TsucA (e.g. C3)
TsucA box
mCherry
c
Not induced
+20 ng/ml aTc
P2
(weak)
Empty
0.9 ± 0.1
N-terminal with linker (e.g. B3)
7.0 ± 0.7
N- and C-term. linker (e.g. B7)
5.9 ± 0.6
P5
(strong)
Empty
1.0 ± 0.1
N-terminal with linker (e.g. B3)
19.1 ± 2.0
N- and C-term. linker (e.g. B7)
11.5 ± 1.6
N-terminal with linker (e.g. B3)
+
sfGFP
TlacO box
1.3 ± 0.2
0 5000 10000 15000 20000 25000 30000 35000 40000 45000
Mean sfGFP protein production rate (RFU OD600-1 h-1)
P5
(strong)
Empty
0.7 ± 0.05
N-terminal with linker (e.g. C3)
64.3 ± 5.7
0 500 1000 1500 2000 2500 3000 3500 4000 4500 5000
Mean mCherry Protein Production Rate (RFU OD600-1 h-1)

SI Figure 12 Induction of TEV protease upon inoculation causes a growth defect due to the burden of protein expression. Growth data for MG1655 ΔlacI transformed with a plasmid harboring either wild-type TEV protease or the catalytically inactive C151A mutant demonstrates that induction with 20 ng/mL aTc immediately following inoculation causes a lag in growth (n=3). This growth phenotype is independent of whether the TEV protease is catalytically active. These plasmids have the same origin of replication, kanamycin resistance marker, and aTc inducible TEV protease cassette as used throughout the paper, though these plasmids do not contain a TALE. Error bars represent the standard deviation.

SI Figure 12


OD600
1.2
1
0.8
0.6
0.4
0.2
0
0
4
8
12
16
20
24
Time (hr)
Not induced
+ 20 ng/ml aTc
No-TEV control
WT TEV
TEV C151A

Supplemental Table 1: Strains used in this study

STRAINS

Strain	Description	Reference
MG1655	F- *λ-* *ilvG- rfb-50 rph-1*	Blattner et al. 1997[2]
MG1655 ΔlacI	F- *λ-* *ilvG- rfb-50 rph-1 ΔlacI*	Politz et al. 2013
MG1655 *lacIZYA*::P^{trc}-*sfGFP*	F- *λ-* *ilvG- rfb-50 rph-1 lacIZYA::P^{trc}- sfGFP*	This study
MG1655 *lacIZYA::P^{trc}-mCherry*	F- *λ-* *ilvG- rfb-50 rph-1 lacIZYA::P^{trc}- mCherry*	This study
DH5α	F⁻ Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17 (rK⁻, mK⁺) *pho*A *sup*E44 *λ*⁻ *thi*-1 *gyr*A96 *rel*A1	Life Technologies

Supplemental Table 2: Plasmids used in this study

EXPRESSION PLASMIDS

Cloning vectors for building expression vectors

Plasmid	Description	Reference
pA0	Full length *TlacO* (17.5 repeats); Kan^R; ColE1 origin	Politz et al. 2013
pA0-NI	pA0 with an NI RVD as the half-mer repeat	This study
pA0-HD	pA0 with an HD RVD as the half-mer repeat	This study
pA0-NN	pA0 with an NN RVD as the half-mer repeat	This study
pA0-NH	pA0 with an NH RVD as the half-mer repeat	This study
pA0-NK	pA0 with an NK RVD as the half-mer repeat	This study
pA0-5.5	Truncated *TlacO* with 5.5 repeats nearest the N-terminus	This study
pA0-7.5	Truncated *TlacO* with 7.5 repeats nearest the N-terminus	This study
pA0-8.5	Truncated *TlacO* with 8.5 repeats nearest the N-terminus	This study
pA0-9.5	Truncated *TlacO* with 9.5 repeats nearest the N-terminus	This study
pA0-10.5	Truncated *TlacO* with 10.5 repeats nearest the N-terminus	This study
pA0-14.5	Truncated *TlacO* with 14.5 repeats nearest the N-terminus	This study
pRK793	Catalytic domain of the tobacco etch virus protease fused to the maltose binding protein via an autoprocessing site for self-cleavage; TEV protease is the S219V variant and has an N-terminal His-tag; Amp^R or Cam^R	Kapust et al. 2001
pBbB2K-GFP	Divergent P^{tet} promoter driving *gfp* and *tetR* expression; Kan^R	Lee et al. 2011
pA1	pA0; *TlacO* with the 7 amino acid TEV recognition sequence inserted between the $4^{th}/5^{th}$, $9^{th}/10^{th}$, and $14^{th}/15^{th}$ repeats	This study
pA2	pA0; *TlacO* with a TEV sequence in the N-terminus	This study
pA3	pA0; *TlacO* with a TEV sequence flanked on both sides by Gly-Ser-Gly in the N-terminus	This study
pA4	pA0; *TlacO* with a TEV sequence in the C-terminus	This study
pA5	pA0; *TlacO* with a TEV sequence flanked on both sides by Gly-Ser-Gly in the C-terminus	This study

pA6	pA0; *TlacO* with a TEV sequence in the N- and C-termini	This study
pA7	pA0; *TlacO* with a TEV sequence flanked on both sides by Gly-Ser-Gly in the N- and C-termini	This study

Empty expression vectors

Plasmid	Description	Reference
pBT-2	Empty vector; Kan^R; pBBR1 origin	Lynch et al. 2006
J23113	P^{113} Constitutive promoter; Amp^R; ColE1 origin	iGEM parts registry
J23117	P^{117} Constitutive promoter; Amp^R; ColE1 origin	iGEM parts registry
J23105	P^{105} Constitutive promoter; Amp^R; ColE1 origin	iGEM parts registry
J23106	P^{106} Constitutive promoter; Amp^R; ColE1 origin	iGEM parts registry
J23102	P^{102} Constitutive promoter; Amp^R; ColE1 origin	iGEM parts registry
p2	pBT-2 containing P^{117} promoter	This study
p3	pBT-2 containing P^{105} promoter	This study
p4	pBT-2 containing P^{106} promoter	This study
p5	pBT-2 containing P^{102} promoter	This study
P^{LtetO1}	P^{LtetO1} promoter; Kan^R; pBBR1 origin	This study
pBAD24	Empty vector control; pMB1 origin, Amp^R	Guzman et al. 1995[3]
pBAD33	Empty vector control; P15A origin, Cm^R	Guzman et al. 1995[3]

Vectors for constitutive expression of TALE variants and LacI

Plasmid	Description	Reference
p2LacI	p2 with constitutive expression of *lacI*	Politz et al. 2013
p3LacI	p3 with constitutive expression of *lacI*	This study
p4LacI	p4 with constitutive expression of *lacI*	This study
p5LacI	p5 with constitutive expression of *lacI*	This study
p2A0	p2 with constitutive expression of full length *TlacO* from pA0	This study
p2A0-5.5	p2A0 expressing a truncated *TlacO* with 5.5 repeats	This study
p2A0-7.5	p2A0 expressing a truncated *TlacO* with 7.5 repeats	This study
p2A0-8.5	p2A0 expressing a truncated *TlacO* with 8.5 repeats	This study
p2A0-9.5	p2A0 expressing a truncated *TlacO* with 9.5 repeats	This study
p2A0-10.5	p2A0 expressing a truncated *TlacO* with 10.5 repeats	This study
p2A0-14.5	p2A0 expressing a truncated *TlacO* with 14.5 repeats	This study
p5A0	p5 with constitutive expression of full length *TlacO* from pA0	This study
p5A0-5.5	p5A0 expressing a truncated *TlacO* with 5.5 repeats	This study
p5A0-7.5	p5A0 expressing a truncated *TlacO* with 7.5 repeats	This study
p5A0-8.5	p5A0 expressing a truncated *TlacO* with 8.5 repeats	This study
p5A0-9.5	p5A0 expressing a truncated *TlacO* with 9.5 repeats	This study
p5A0-10.5	p5A0 expressing a truncated *TlacO* with 10.5 repeats	This study
p5A0-14.5	p5A0 expressing a truncated *TlacO* with 14.5 repeats	This study
p2A1	p2 with constitutive expression of *TlacO* containing three TEV sites from pA1	This study
p5A1	p5 with constitutive expression of *TlacO* containing three TEV sites from pA1	This study

p2A7	p2 with constitutive expression of *TlacO* containing N- and C-terminal TEV sites each flanked by Gly-Ser-Gly	This study
p5A7	p5 with constitutive expression of *TlacO* containing N- and C-terminal TEV sites each flanked by Gly-Ser-Gly	This study
p5A3-15A	5A3 cassette from p5A3-T cloned into the pBAD33 backbone	This study

Vectors for expression of TlacO and TEV protease variants

Plasmid	Description	Reference
p2A0-T	p2A0 with P^{tet} regulation of catalytically active TEV protease	This study
p2A1-T	p2A1 with P^{tet} regulation of catalytically active TEV protease	This study
p2A0-Ti	p2A0 with P^{tet} regulation of catalytically inactive TEV protease	This study
p2A1-Ti	p2A1 with P^{tet} regulation of catalytically inactive TEV protease	This study
p5A0-T	p5A0 with P^{tet} regulation of catalytically active TEV protease	This study
p5A1-T	p5A1 with P^{tet} regulation of catalytically active TEV protease	This study
p5A0-Ti	p5A0 with P^{tet} regulation of catalytically inactive TEV protease	This study
p5A1-Ti	p5A1 with P^{tet} regulation of catalytically inactive TEV protease	This study
p2A0-MT	p2A0 with P^{tet} regulation of catalytically active TEV protease fused to maltose binding protein (see pRK793)	This study
p2A1-MT	p2A1 with P^{tet} regulation of catalytically active TEV protease fused to maltose binding protein (see pRK793)	This study
p2A0-MTi	p2A0 with P^{tet} regulation of catalytically inactive TEV protease fused to maltose binding protein (see pRK793)	This study
p2A1-MTi	p2A1 with P^{tet} regulation of catalytically inactive TEV protease fused to maltose binding protein (see pRK793)	This study
p5A0-MT	p5A0 with P^{tet} regulation of catalytically active TEV protease fused to maltose binding protein (see pRK793)	This study
p5A1-MT	p5A1 with P^{tet} regulation of catalytically active TEV protease fused to maltose binding protein (see pRK793)	This study
p5A0-MTi	p5A0 with P^{tet} regulation of catalytically inactive TEV protease fused to maltose binding protein (see pRK793)	This study
p5A1-MTi	p5A1 with P^{tet} regulation of catalytically inactive TEV protease fused to maltose binding protein (see pRK793)	This study
p2A2-T	p2 expression of pA2 (i.e. N-terminal TEV site) with P^{tet} regulation of active TEV protease	This study
p2A3-T	p2 expression of pA3 (i.e. N-terminal TEV site with flanking G-S-G) with P^{tet} regulation of active TEV protease	This study
p2A4-T	p2 expression of pA4 (i.e. C-terminal TEV site) with P^{tet} regulation of active TEV protease	This study
p2A5-T	p2 expression of pA5 (i.e. C-terminal TEV site with flanking G-S-G) with P^{tet} regulation of active TEV protease	This study
p2A6-T	p2 expression of pA6 (i.e. N-, C-terminal TEV sites) with P^{tet} regulation of active TEV protease	This study
p2A7-T	p2 expression of pA7 (i.e. N-, C-terminal TEV sites with flanking G-S-G) with P^{tet} regulation of active TEV protease	This study
p2A2-Ti	p2A2 with P^{tet} regulation of catalytically inactive TEV protease	This study

p2A3-Ti	p2A3 with P^{tet} regulation of catalytically inactive TEV protease	This study
p2A4-Ti	p2A4 with P^{tet} regulation of catalytically inactive TEV protease	This study
p2A5-Ti	p2A5 with P^{tet} regulation of catalytically inactive TEV protease	This study
p2A6-Ti	p2A6 with P^{tet} regulation of catalytically inactive TEV protease	This study
p2A7-Ti	p2A7 with P^{tet} regulation of catalytically inactive TEV protease	This study
p2A1-TiKm	p2A1-Ti, except the protease has an ~10-fold higher Km	This study
p2A7-TiKm	p2A7-Ti, except the protease has an ~10-fold higher Km	This study
p1A7-T	p1 (i.e. P^{113}) expression of pA7 (i.e. N-, C-terminal TEV sites with flanking G-S-G) with P^{tet} regulation of active TEV protease	This study
p3A7-T	p3 (i.e. P^{105}) expression of pA7 (i.e. N-, C-terminal TEV sites with flanking G-S-G) with P^{tet} regulation of active TEV protease	This study
p4A7-T	p4 (i.e. P^{106}) expression of pA7 (i.e. N-, C-terminal TEV sites with flanking G-S-G) with P^{tet} regulation of active TEV protease	This study
p5A7-T	p5 (i.e. P^{102}) expression of pA7 (i.e. N-, C-terminal TEV sites with flanking G-S-G) with P^{tet} regulation of active TEV protease	This study
p5A3-T	p5 (i.e. P^{102}) expression of pA7 (i.e. N-terminal TEV sites with flanking G-S-G) with P^{tet} regulation of active TEV protease	This study
P^{LtetO1}-TlacO1	P^{LtetO1} (aTc inducible) expression of A0 (no cut sites)	This study
pTEV	pBT-2 with catalytically active TEV protease expressed from the aTc inducible promoter P^{tet}	This study

Vectors for expression of TlysA and TsucA with and without expression of TEV protease

Plasmid	Description	Reference
pB0	Full length *TlysA* (21.5 repeats); Kan^R; ColE1 origin	This study
p2B0	p2 with constitutive expression of full length *TlysA* from pB0	This study
p5B0	p5 with constitutive expression of full length *TlysA* from pB0	This study
p2B7	p2 with constitutive expression of *TlysA* containing N- and C-terminal TEV sites each flanked by Gly-Ser-Gly	This study
p5B7	p5 with constitutive expression of *TlysA* containing N- and C-terminal TEV sites each flanked by Gly-Ser-Gly	This study
p2B3-T	p2 expression of *TlysA* containing N-terminal TEV sites with flanking G-S-G with P^{tet} regulation of active TEV protease	This study
p2B7-T	p2 expression of *TlysA* containing N-, C-terminal TEV sites with flanking G-S-G with P^{tet} regulation of active TEV protease	This study
p5B3-T	p5 expression of *TlysA* containing N-terminal TEV sites with flanking G-S-G with P^{tet} regulation of active TEV protease	This study
p5B7-T	p5 expression of *TlysA* containing N-, C-terminal TEV sites with flanking G-S-G with P^{tet} regulation of active TEV protease	This study
p5C3-T	p5 expression of TsucA containing N-terminal TEV sites with flanking G-S-G with P^{tet} regulation of active TEV protease	This study

REPORTER PLASMIDS

Plasmid	Description	Reference
pE	pMSB-6; Amp^R; pMB1 origin; Empty vector	Politz et al. 2013
ptrcCherry	Amp^R; pMB1 origin; P^{trc}-mCherry	Politz et al. 2013

ptrcCherry2	ptrcCherry with *lacI* removed	Politz et al. 2013
ptrcScramble	ptrcCherry2 with scrambled *lac* operator in P^{trc}	This study
ptrcRandom	ptrcCherry2 with randomized *lac* operator in P^{trc}	This study
p5mCherry	ptrcCherry2 with P^{102} from J23102 substituted for P^{trc}	This study
p4mCherry	ptrcCherry2 with P^{106} from J23106 substituted for P^{trc}	This study
p3mCherry	ptrcCherry2 with P^{105} from J23105 substituted for P^{trc}	This study
p5sfGFP	p5mCherry with superfolder GFP substituted for mCherry	This study
p5lysAsfGFP	p5sfGFP with a 23 bp sequence of the *lysA* 5′-UTR substituted for the *lac* operator	This study
p5sucAmCherry	p5mCherry with a 16 bp sequence from the *sucA* 5′-UTR substituted for the *lac* operator	This study

PLASMIDS FOR CHROMOSOMAL ENGINEERING

Plasmid	Description	Reference
pMP004-sfGFP-thyA	P^{trc}-*sfGFP-thyA* cassette, R6Kγ origin, Amp^R	This study
pMP004-mCherry-thyA	P^{trc}-*mCherry-thyA* cassette, R6Kγ origin, Amp^R	This study
pKD46	λ red recombination vector; Amp^R	Datsenko et al. 2000

Supplemental Table 3: Select list of primers used in this study

OLIGONUCLEOTIDES

qPCR primers and primers for making standard curve templates

Primer	Sequence	Description
MC119	GTCACTACTCTGACCTATGG	qPCR, sfGFP-L
MC120	TCCTGTACATAACCTTCG	qPCR, sfGFP-R
MC121	GAGAAGAACTTTTCACTGGAG	Standard curve, sfGFP-L
MC122	GCAGCAGTTACAAACTCAA	Standard curve, sfGFP-R
MC127	CATATTGTTGCACTGAGC	qPCR, TALE Nterm-L
MC128	ACCTGACCACTGTTTACC	qPCR, TALE Nterm-R
MC129	ATCATCACCATGTTGATCTG	Standard curve, TALE Nterm-L
MC130	CACCGGTCAGTGCATTA	Standard curve, TALE Nterm-R
MC135	CATGTCTAGCATGGTGTC	qPCR, TEV-L
MC136	AGTTGATACTAATGGACTGC	qPCR, TEV-R
MC137	CATCATCATCATCATCATGG	Standard curve, TEV-L
MC138	ACGATTCATGAGTTGAGTCG	Standard curve, TEV-R
MC143	GTAGAGATCTGGAGGAATAC	qPCR, rrsA-L
MC144	GACTACCAGGGTATCTAATC	qPCR, rrsA-R
MC145	GATCATGGCTCAGATTGAA	Standard curve, rrsA-L
MC146	CCCTACGGTTACCTTGTTAC	Standard curve, rrsA-R
MC147	ACGACCGAACAGTTAATC	qPCR, cysG-L
MC148	AGTCCTTTCAGTGTCAGC	qPCR, cysG-R
MC149	GTGGATCATTTGCCTATATTTT	Standard curve, cysG-L
MC150	TGGTTGGAGAACCAGTTC	Standard curve, cysG-R

Primers for engineering MG1655 P^{trc}-*sfGFP*::*lacIZYA*

Primer	Sequence	Description
rMP019	CGTCGCAGCCCACAGCAACACGTTTCCTGAGGAACCATGAAACAGTATTTAGAACTGATG	See Methods
rMP020	GCACACTGGCGTCGGCTCTGGCAGGATGTTTCGTAATTAGATAGCCACCGGCGC	See Methods
rMP145	CGCTGGATGTTGATGCAATGG	See Methods
rMP150	CTATGGCTCGCCATCAGGAT	See Methods
rMP084	gatggccttttgcgtttctacaaactcttATTATAAAAATTGCCTGATACGCTGCGCTT	See Methods

References from Supplemental Information

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2. Blattner, F.R. et al. The complete genome sequence of Escherichia coli K-12. *Science* **277**, 1453-1462 (1997).
3. Guzman, L.M., Belin, D., Carson, M.J. & Beckwith, J. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *Journal of Bacteriology* **177**, 4121-4130 (1995).