Supplementary information

Tunable protein degradation in bacteria

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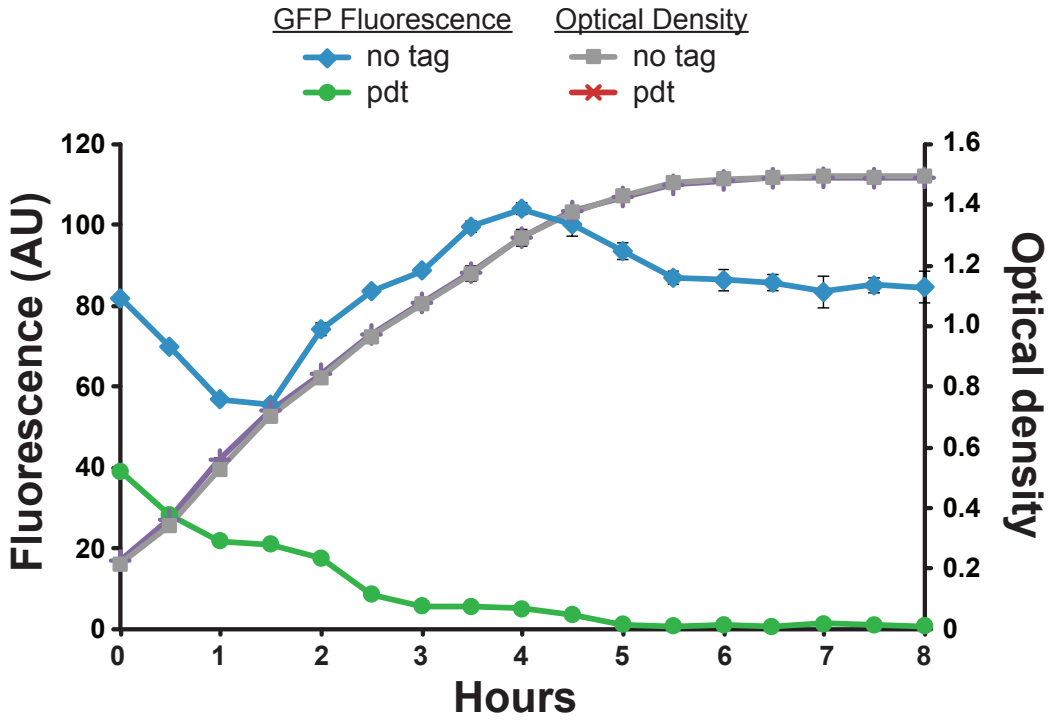
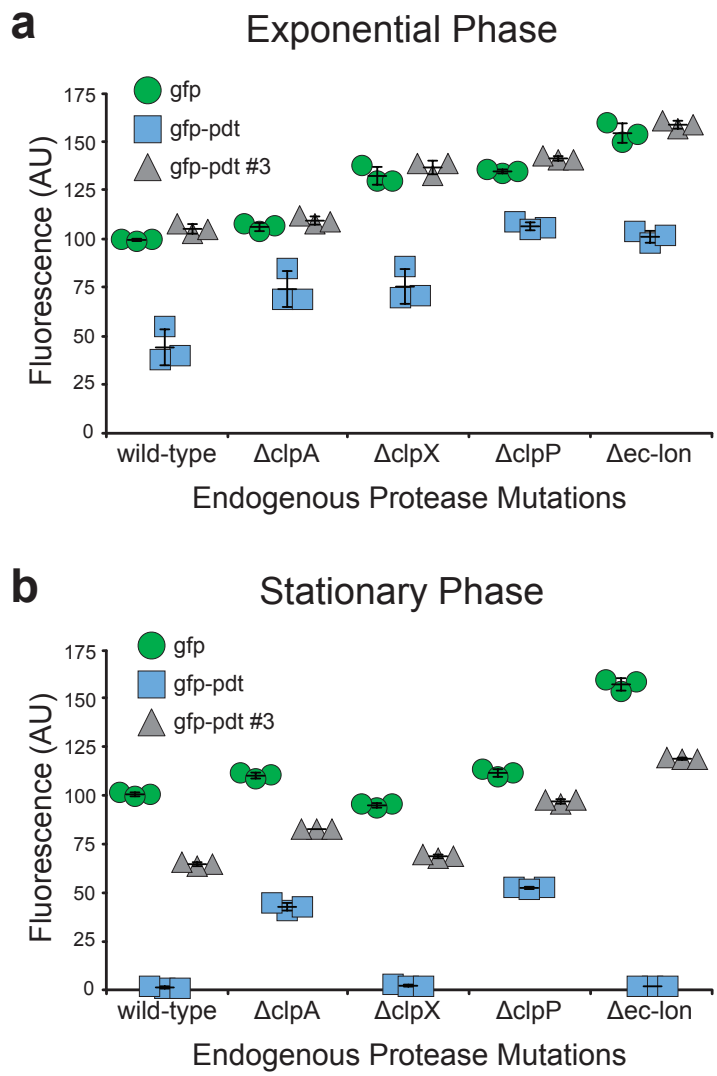
Supplementary Figure 1



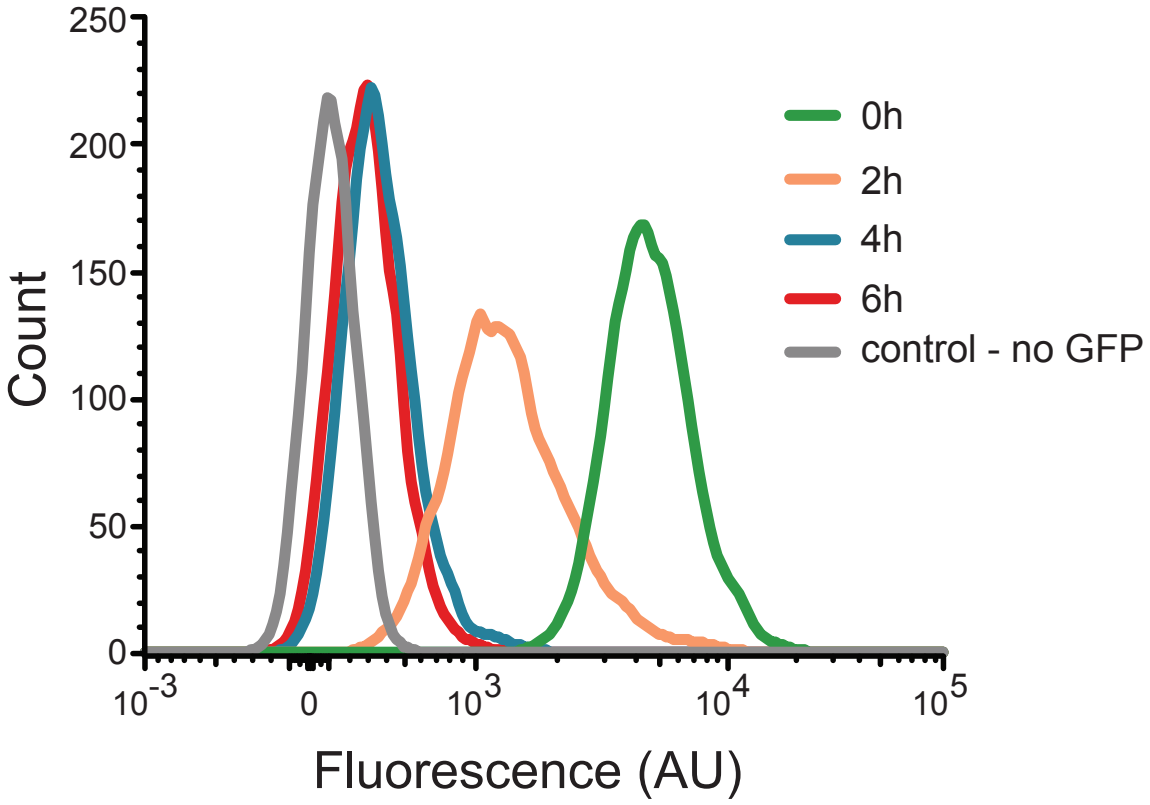
Supplementary Figure 1. GFP-pdt growth phase dependent degradation. GFP-pdt expressed from the constitutive P_{lacIq} promoter showed reduced GFP fluorescence in exponential and stationary phase growth. GFP fluorescence was measured by flow cytometry and optical density (600 nm) was measured by microplate reader.

Supplementary Figure 2



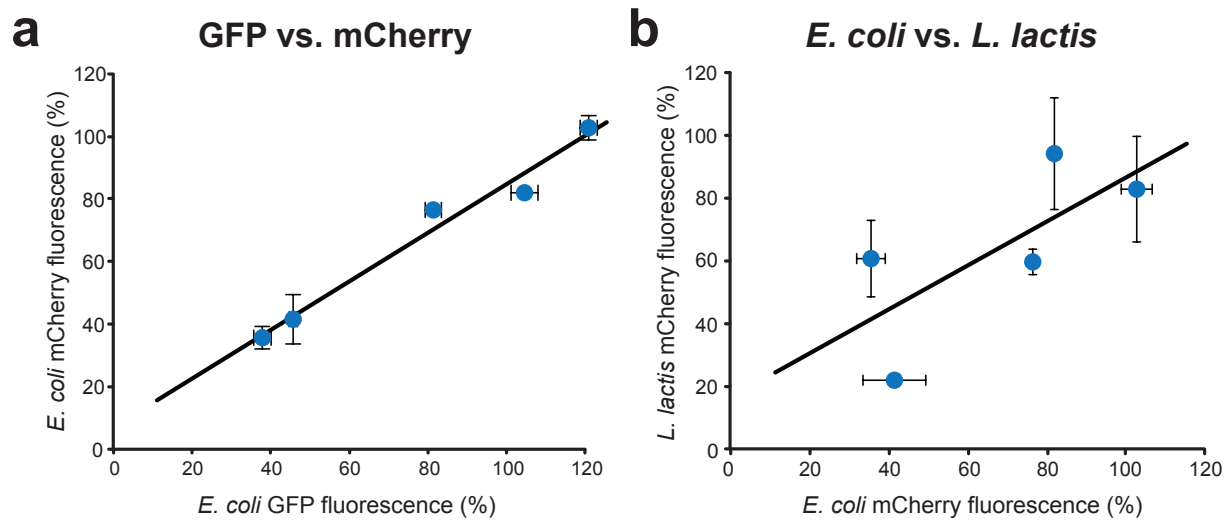
Supplementary Figure 2. GFP-pdt degradation by endogenous *E. coli* proteases. (a) Dot plot of GFP and GFP-pdt levels in *E. coli* strains containing an in-frame deletion of the indicated *E. coli* protease gene. GFP, GFP-pdt, and GFP-pdt#3 were constitutively expressed from the P_{lacIq} promoter, and fluorescence was measured by flow cytometry. Optical density of exponential and stationary phase cells was approximately 0.3 and 1.6 respectively. Fluorescence units are arbitrary, with untagged GFP set to 100 for both the exponential phase and stationary phase conditions. Error bars show the standard deviation of three biological replicates.

Supplementary Figure 3



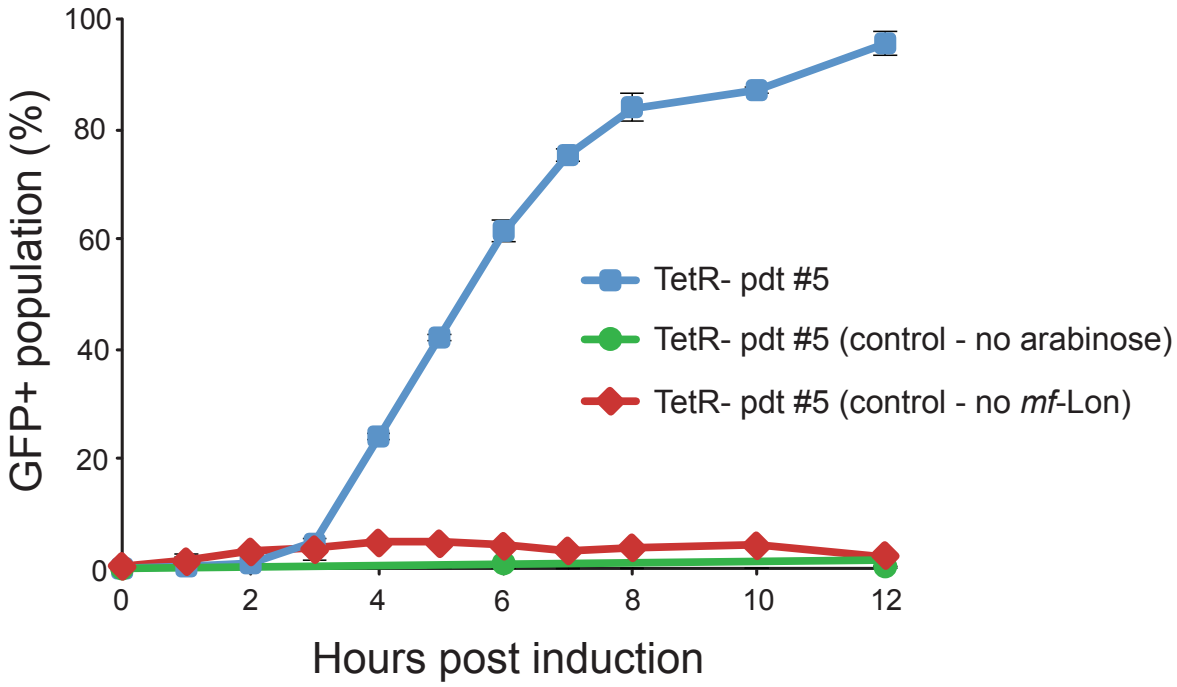
Supplementary Figure 3. Population-level degradation dynamics. Cells in exponential phase growth that constitutively express GFP-pdt#3 were induced to express *mf*-Lon (50 ng/ml ATc), and GFP fluorescence was measured by flow cytometry at the indicated time post induction. The histogram plot shows a monomodal shift in the cell population over time. Cells that do not express GFP are shown in grey (control- no GFP).

Supplementary Figure 4



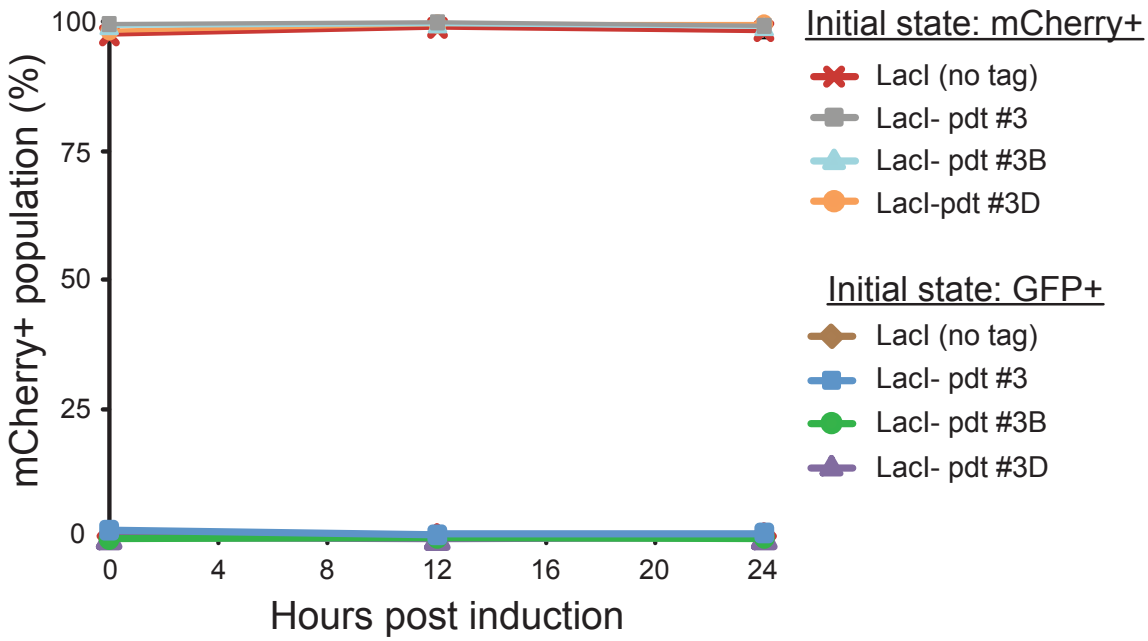
Supplementary Figure 4. Pdt number variant comparisons. Comparative analysis of pdt number variants on GFP and mCherry. Fluorescence was measured by flow cytometry without *mf*-Lon induction and is presented as a percent of the fluorescence of the untagged protein target. **(a)** Pdt number variant correlation between GFP and mCherry in *E. coli*. The pdt variants, listed in order of increasing percent mCherry fluorescence, are pdt#1, pdt, pdt#2, pdt#3, pdt#5. The displayed linear regression is y=0.78x + 0.07 with an R^2 value of 0.96. **(b)** Pdt number variant correlation between *L. lactis* and *E. coli*. The pdt number variants, listed in order of increasing percent fluorescence in *E. coli*, are pdt#1, pdt, pdt#2, pdt#3, pdt#5. The displayed linear regression is y=0.70x + 0.16 with an R^2 value of 0.43. In both figures, linear regression was performed using the mean of the x-axis variable and all observed data from the y-axis variable. Error bars show the standard deviation of three biological replicates.

Supplementary Figure 5



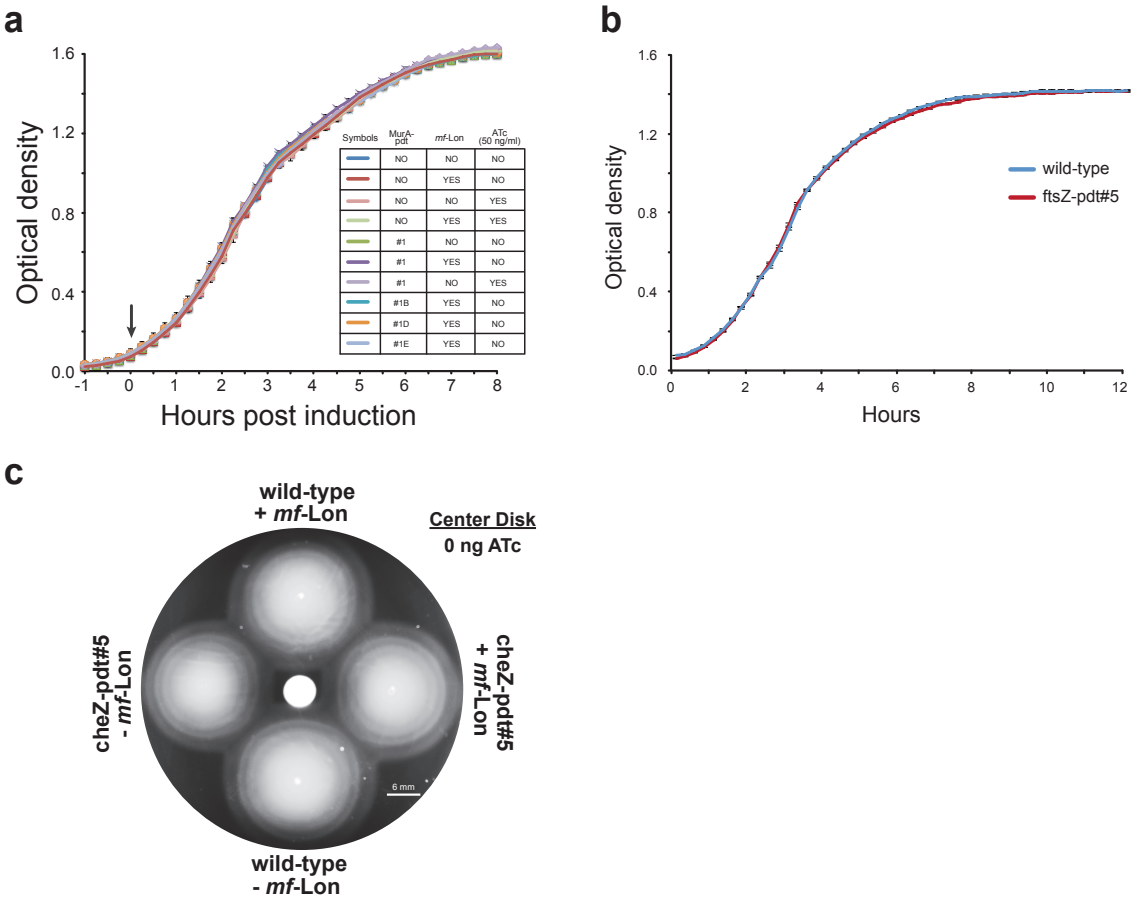
Supplementary Figure 5. Protease driven toggle switch using TetR-pdt variants. Cells containing the toggle switch, based on pKDL071R9, with the indicated *tetR-pdt* fusion were switched to the mCherry+ state with 500 μM IPTG and allowed to stabilize for 12 hours in non-inducing media. Expression of *mf*-Lon was induced with 1 mM arabinose at 0 hours, and the cells were monitored by flow cytometry for GFP and mCherry expression according to the parameters shown in **Figure 3b**. 96% of cells containing a *tetR-pdt#5* fusion switched to the GFP+ state within 12 hours of *mf*-Lon induction, while cells that did not contain the *mf*-Lon expression plasmid (control- no *mf*-Lon) or were not induced (control- no arabinose) did not switch. Error bars show the standard deviation of three biological replicates.

Supplementary Figure 6



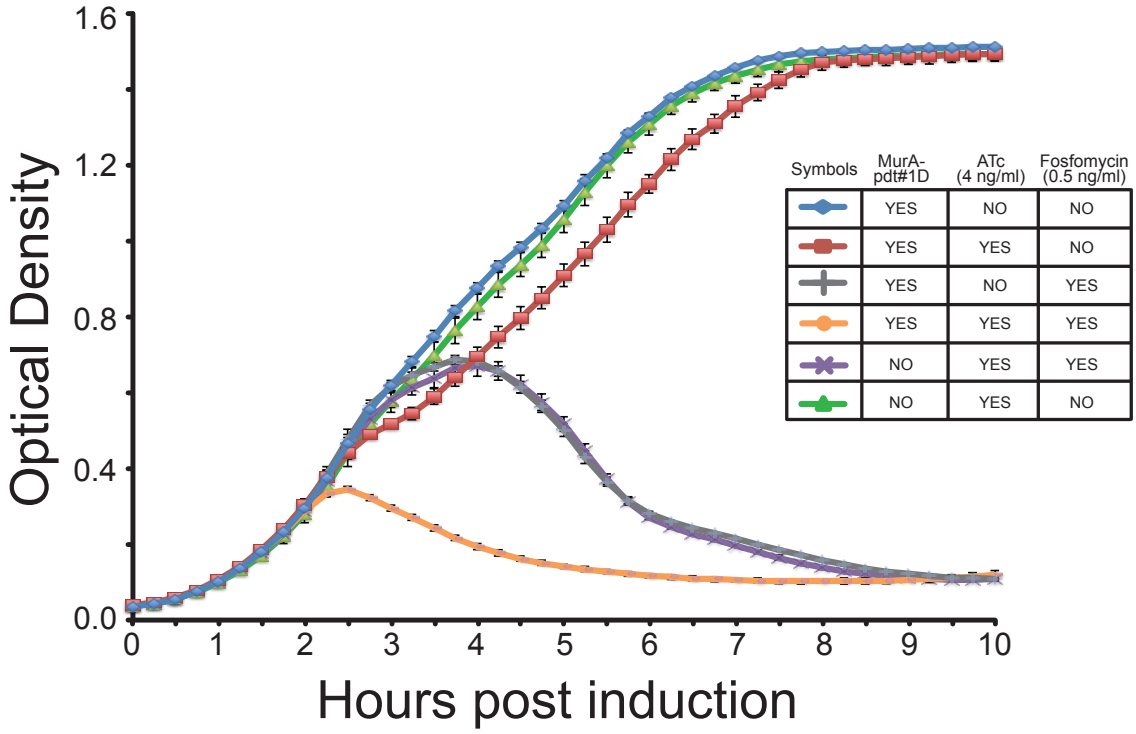
Supplementary Figure 6. Bistability of the protease-inducible toggle switch. Cells containing a toggle switch with the indicated *lacI-pdt* fusion were induced with ATc or IPTG into the GFP+ state or mCherry+ state, respectively as indicated. The cells were then moved into non-inducing media and monitored over time for their toggle switch state using the fluorescence parameters shown in **Figure 3b**. Error bars show the standard deviation of three biological replicates.

Supplementary Figure 7



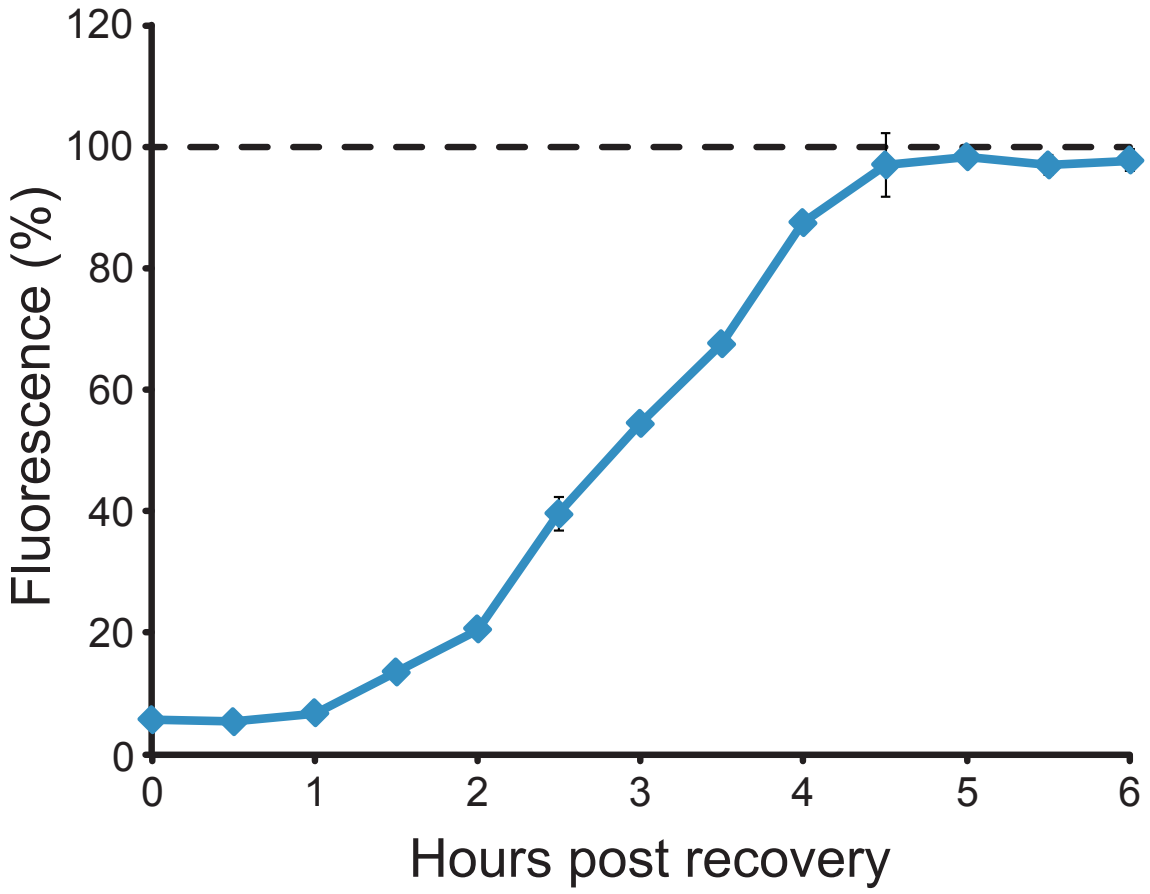
Supplementary Figure 7. Endogenous protein degradation controls. (a) Growth of *murA-pdt* cells in the absence of *mf*-Lon induction. The growth rate of cells containing the indicated pdt variants, with or without *mf*-Lon and with or without ATc induction as indicated, are indistinguishable from wild-type cells as measured by optical density (600 nm). **(b)** The growth rate of *E. coli* containing *ftsZ-pdt#5* was indistinguishable from wild-type cells in the absence of *mf*-Lon induction. **(c)** Disk diffusion assay on a chemotactic motility plate showing inducible loss of chemotactic motility due to pdt-dependent CheZ degradation. In the absence of ATc induction, all cells show wild-type chemotactic motility. Cells were stabbed into the chemotaxis plate and the plate was imaged after 18 hours at 30°. Scare bar is 6 mm.

Supplementary Figure 8



Supplementary Figure 8. Growth curve for MurA hypersensitivity assay. Cell growth following simultaneous addition of ATc and fosfomycin, as indicated. For cells that contain *murA-pdt#1D*, exposure to ATc and fosfomycin (orange) causes a larger growth defect than exposure to only ATc (red) or only fosfomycin (grey). Data for **Figure 4e** was taken at 4 hours after ATc and fosfomycin induction.

Supplementary Figure 9



Supplementary Figure 9. GFP recovery. Cells containing the GFP-pdt#3 fusion were induced with ATc (50 ng/ml) for 6 hours to cause *mf*-Lon-mediated GFP degradation and were then moved into media without ATc and measured every 30 minutes for 6 hours. Fluorescence was measured by flow cytometry and is presented as a percent of the fluorescence of cells not exposed to ATc. Full recovery of GFP-pdt#3 levels occurs within 4.5 hours of ATc removal.

Supplementary Table 1. Pdt identification and characterization

Name	PDT amino acid sequence*	AA 13-15	AA 24-27	GFP (uninduced)	st. dev.	GFP (induced)	st. dev.
no tag				100%	0%	93%	1%
pdt	AANKNEENTNEVPTFMLNAGQANYAFA	PTF	YAFA	46%	1%	1%	1%
pdt#1	AANKNEENTNEVPTFMLNAGQAN**RLQL**	PTF	RLQL	38%	2%	1%	1%
pdt#2	AANKNEENTNEVPTFMLNAGQAN**YLSQ**	PTF	YLSQ	81%	2%	2%	0%
pdt#3	AANKNEENTNEVPTFMLNAGQAN**RRRV**	PTF	RRRV	105%	3%	5%	0%
pdt#4	AANKNEENTNEVPTFMLNAGQAN**HAQP**	PTF	HAQP	110%	7%	5%	0%
pdt#5	AANKNEENTNEVPTFMLNAGQAN**RARQ**	PTF	RARQ	121%	2%	4%	1%
pdt#6	AANKNEENTNEVPTFMLNAGQAN**ICRL**	PTF	ICRL	71%	3%	2%	0%
pdt#7	AANKNEENTNEVPTFMLNAGQAN**FTQQ**	PTF	FTQQ	91%	7%	27%	2%
pdt#8	AANKNEENTNEVPTFMLNAGQAN**VVRR**	PTF	VVRR	96%	5%	13%	1%
pdt#9	AANKNEENTNEVPTFMLNAGQAN**RICR**	PTF	RICR	99%	3%	9%	1%
pdt#10	AANKNEENTNEVPTFMLNAGQAN**RQRH**	PTF	RQRH	102%	4%	30%	2%
pdt#11	AANKNEENTNEVPTFMLNAGQAN**YRTP**	PTF	YRTP	153%	2%	20%	2%
pdt#2A	AANKNEENTNEV**FKL**MLNAGQAN**YLSQ**	FKL	YLSQ	85%	7%	7%	0%
pdt#2B	AANKNEENTNEV**RAI**MLNAGQAN**YLSQ**	RAI	YLSQ	92%	6%	15%	1%
pdt#2C	AANKNEENTNEV**AQP**MLNAGQAN**YLSQ**	AQP	YLSQ	91%	3%	23%	1%
pdt#2D	AANKNEENTNEV**APN**MLNAGQAN**YLSQ**	APN	YLSQ	104%	8%	42%	1%
pdt#2E	AANKNEENTNEV**PDG**MLNAGQAN**YLSQ**	PDG	YLSQ	97%	4%	59%	1%
pdt#3A	AANKNEENTNEV**FKL**MLNAGQAN**RRRV**	FKL	RRRV	120%	3%	27%	2%
pdt#3B	AANKNEENTNEV**RAI**MLNAGQAN**RRRV**	RAI	RRRV	100%	3%	38%	2%
pdt#3C	AANKNEENTNEV**AQP**MLNAGQAN**RRRV**	AQP	RRRV	115%	5%	54%	4%
pdt#3D	AANKNEENTNEV**APN**MLNAGQAN**RRRV**	APN	RRRV	104%	3%	66%	1%
pdt#3E	AANKNEENTNEV**PDG**MLNAGQAN**RRRV**	PDG	RRRV	112%	2%	87%	1%
pdt#5A	AANKNEENTNEV**FKL**MLNAGQAN**RARQ**	FKL	RARQ	130%	13%	27%	1%
pdt#5B	AANKNEENTNEV**RAI**MLNAGQAN**RARQ**	RAI	RARQ	119%	2%	41%	3%
pdt#5C	AANKNEENTNEV**AQP**MLNAGQAN**RARQ**	AQP	RARQ	133%	3%	62%	2%
pdt#5D	AANKNEENTNEV**APN**MLNAGQAN**RARQ**	APN	RARQ	137%	1%	87%	1%
pdt#5E	AANKNEENTNEV**PDG**MLNAGQAN**RARQ**	PDG	RARQ	140%	8%	115%	4%

* mutated amino acids are in bold