

Supporting Information

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SI Methods

To determine the ratio of *Escherichia coli* in the cI and Cro states in a population, we titrated the *E. coli* to yield at least 100 single colonies on lactose indicator plates, and also plated at a 100-fold lower dilution, yielding at least 10,000 colonies.

On the latter plates colonies could not be counted but it was possible to recognize rare colonies of a distinct phenotype. Thus, in Figs. 2–4 and Fig. S6, where it is stated that 100% or 0% of cells have a certain phenotype, the limit of detection is about $1/10^4$.

Fig. S1. Memory element in PAS129.

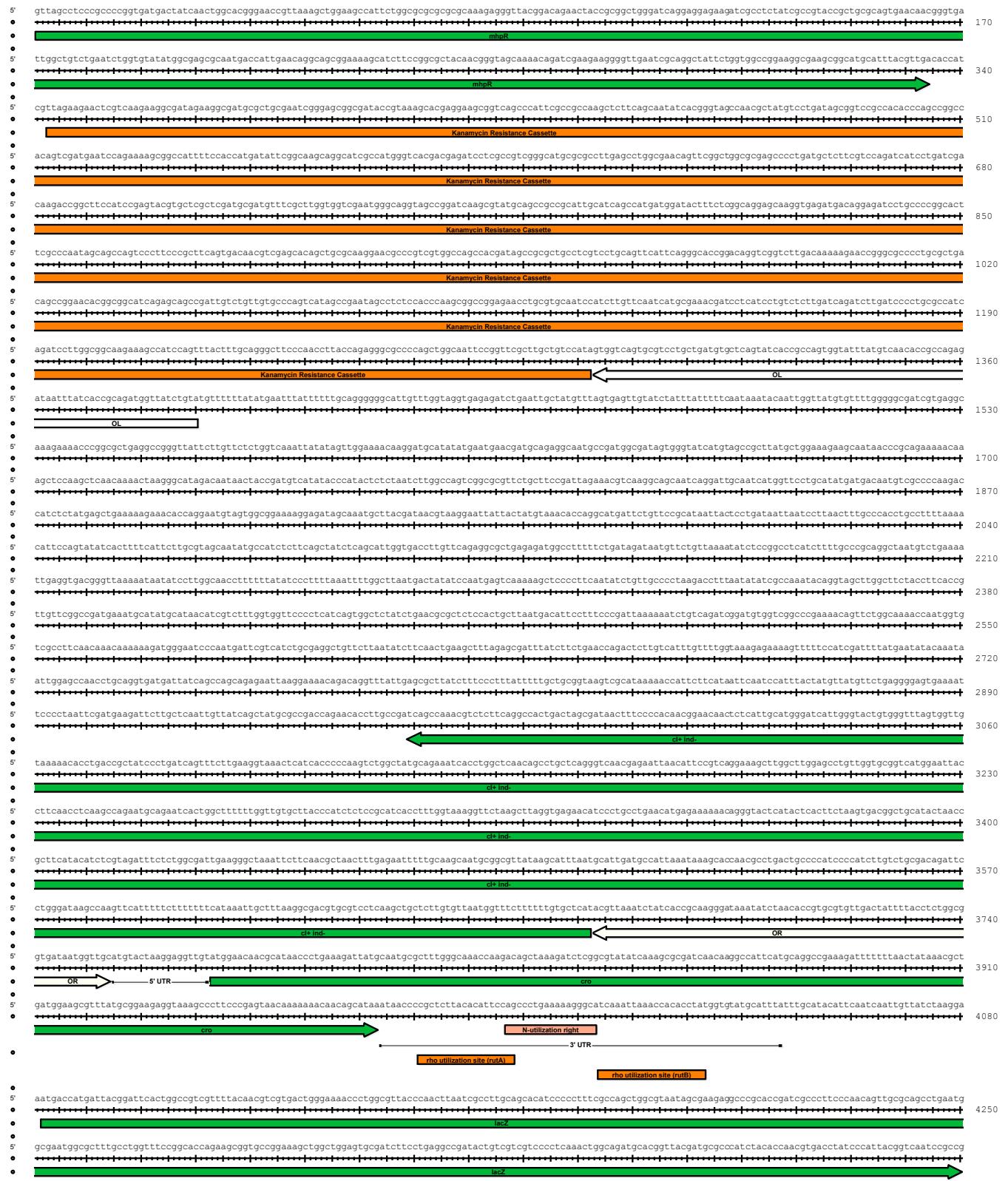


Fig. S2. Memory element in PAS130.

Fig. S3. Memory element in PAS131.

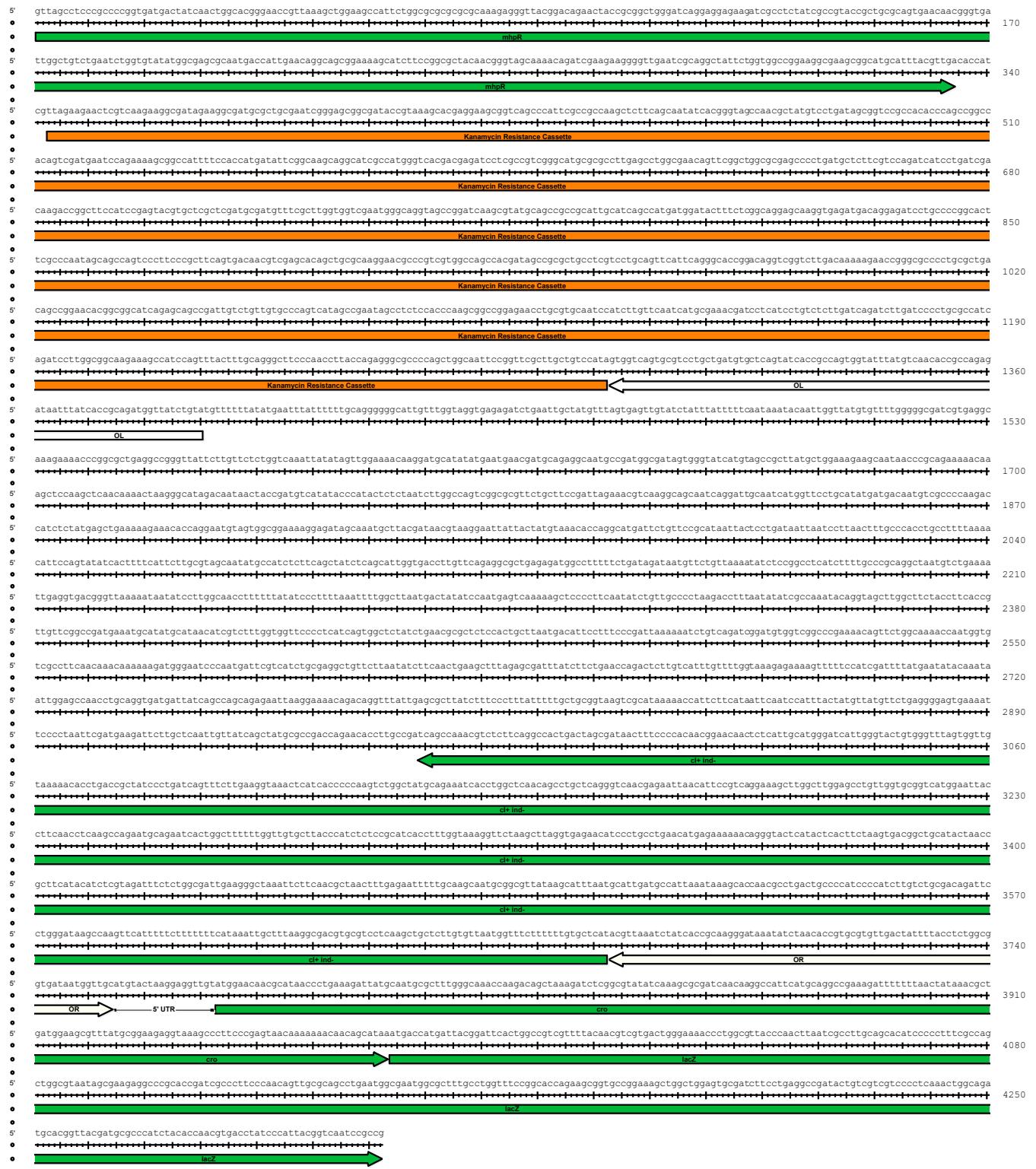


Fig. S4. Memory element in PAS132.

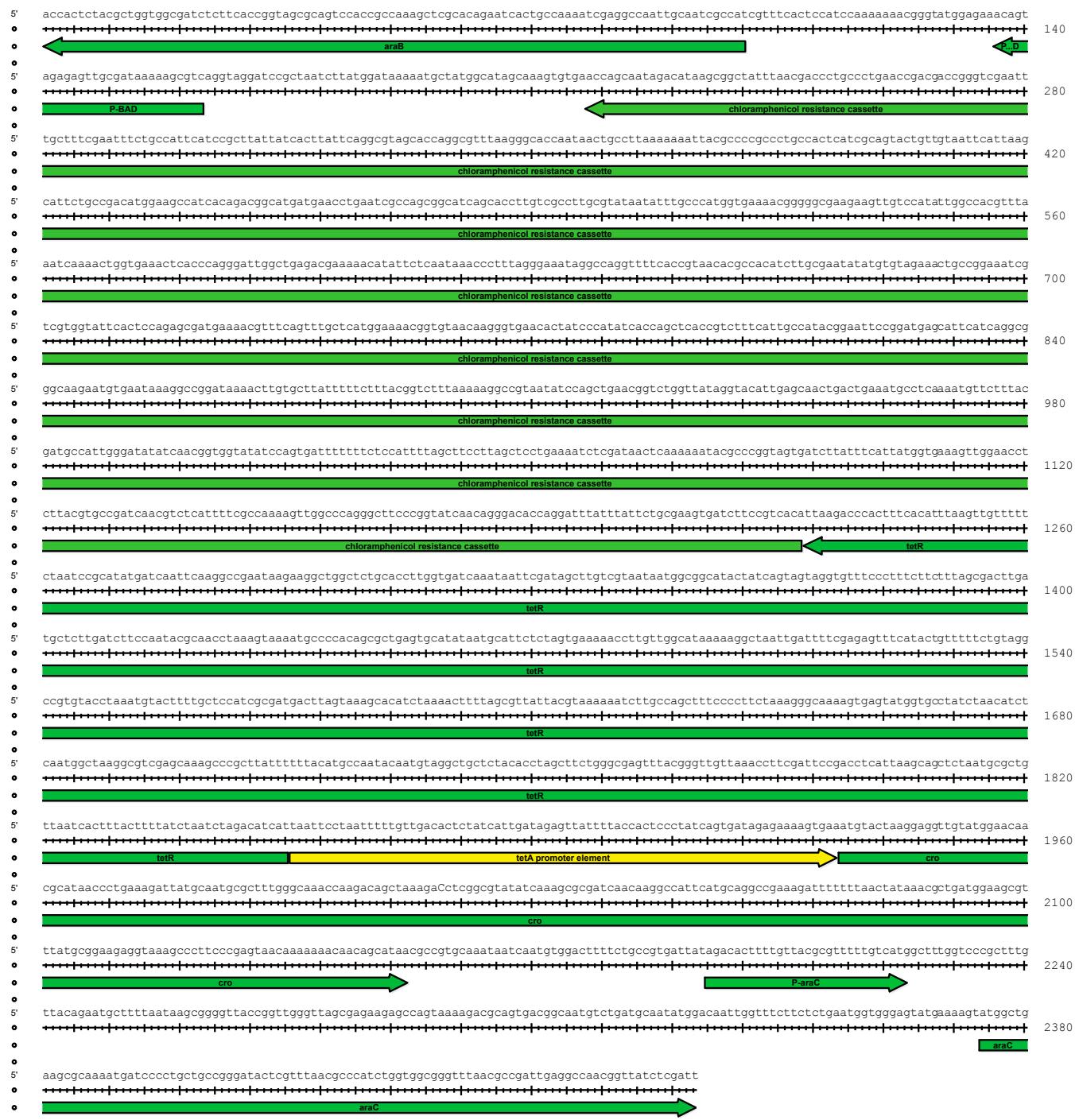


Fig. S5. The tetP-cro trigger element used in this study.

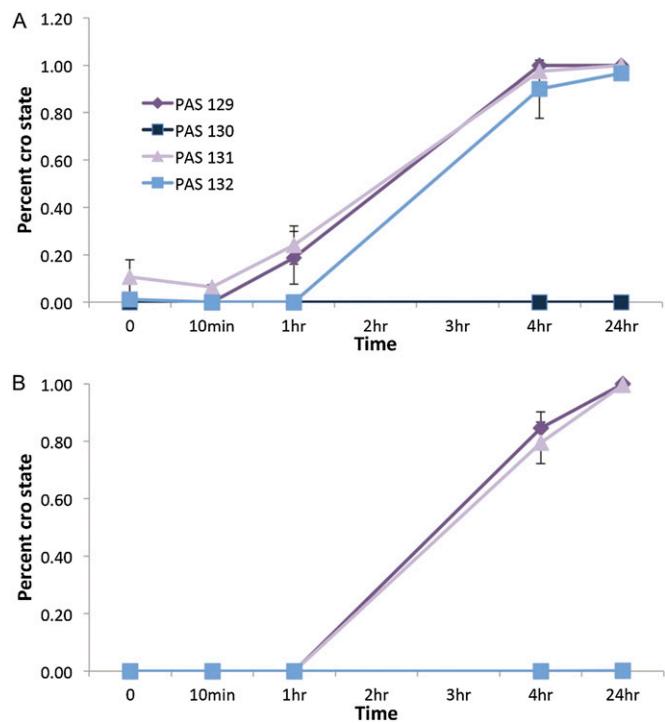


Fig. S6. Identification of a memory element with optimal switching properties. (A) Memory elements 11–14, which were integrated into strains PAS129–PAS132, were evaluated for switching in response to anhydrotetracycline (ATC). (B) PAS129–PAS132 were evaluated for switching in response to an incubation temperature of 42 °C, without ATC. For both panels, points represent the means \pm SD of three or more independent samples.

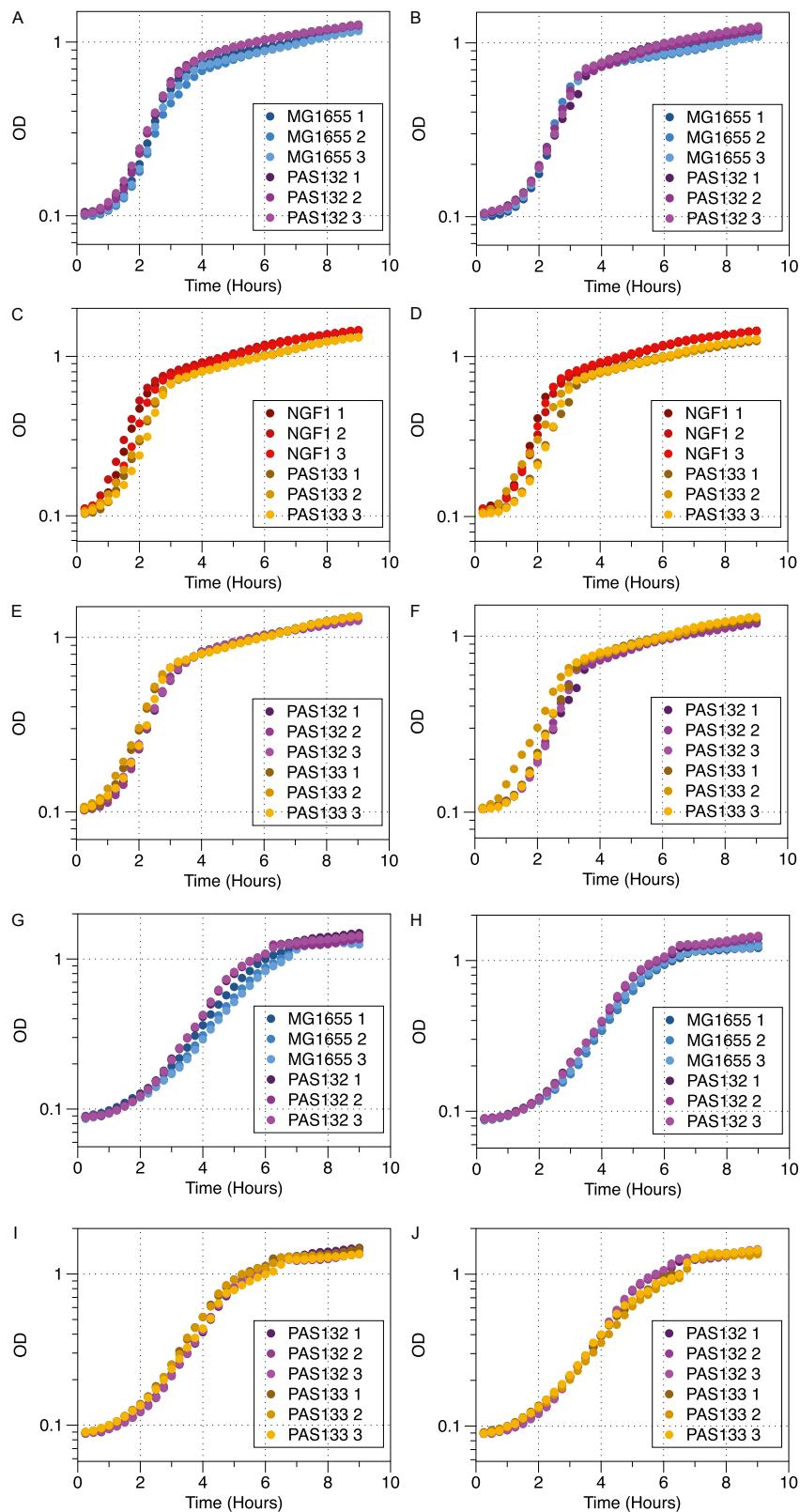


Fig. S7. Growth curves of engineered strains in brain-heart infusion (BHI) media. (A) Growth of MG1655 and PAS132 in BHI media without ATC. (B) Growth of MG1655 and PAS132 in BHI media with ATC. (C) Growth of NGF1 and PAS133 in BHI media without ATC. (D) Growth of NGF1 and PAS133 in BHI media with ATC. (E) Growth of PAS132 and PAS133 in BHI media without ATC. (F) Growth of PAS132 and PAS133 in BHI media with ATC. (G) Growth of MG1655 and PAS132 in M9 glucose + casamino acids media without ATC. (H) Growth of MG1655 and PAS132 in M9 glucose + casamino acids media with ATC. (I) Growth of PAS132 and PAS133 in M9 glucose + casamino acids media without ATC. (J) Growth of PAS132 and PAS133 in M9 glucose + casamino acids media with ATC.

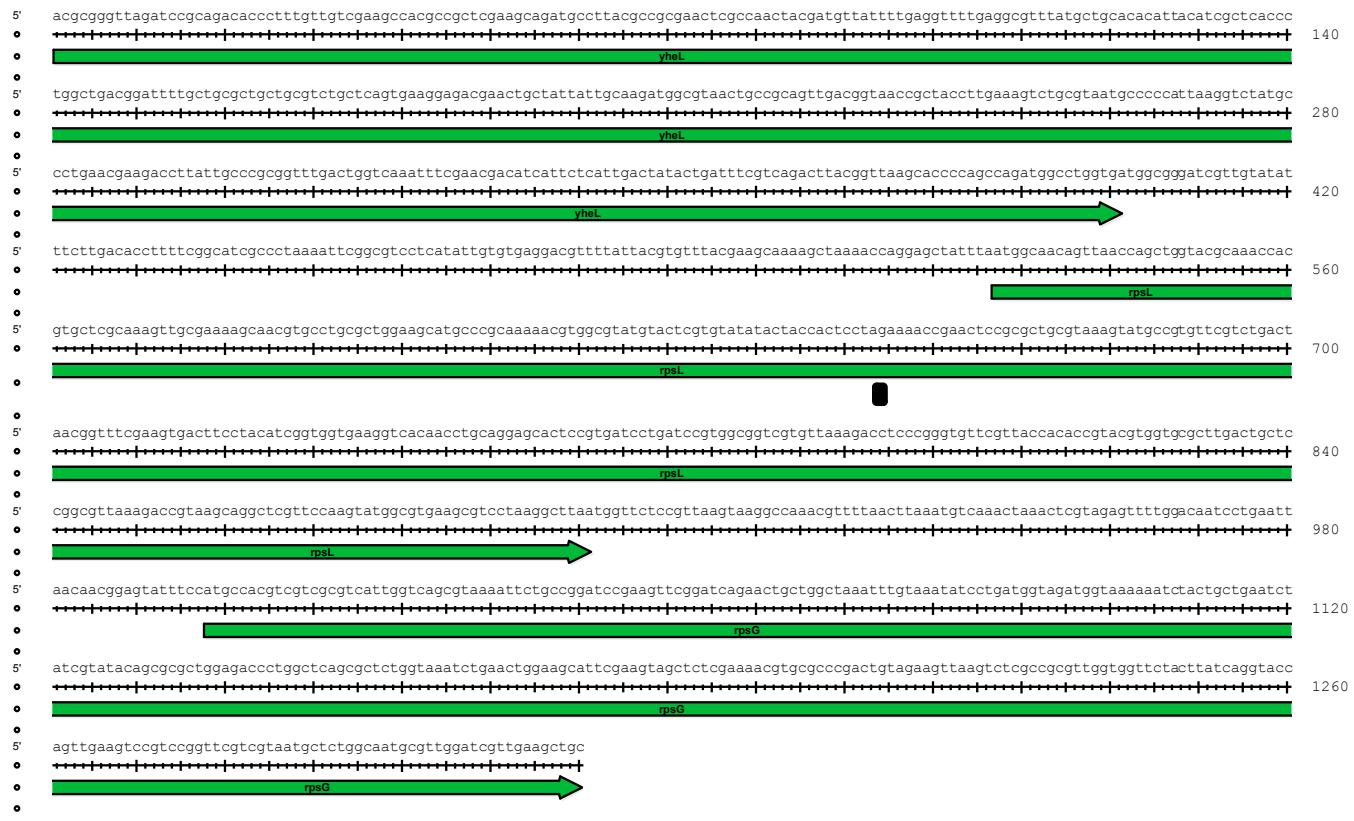


Fig. S8. Sequence of the *rpsL* mutation. The *rpsL* gene of PAS132 and MG1655 was amplified using 5'-CCA GCC AGA TGG CCT GG-3' and 5'-GAC GCG ACG ACG TGG C-3' primers, then sequenced. The sequences were compared using Lasergene software to identify the A430G mutation that resulted in a Lys42Arg mutation.

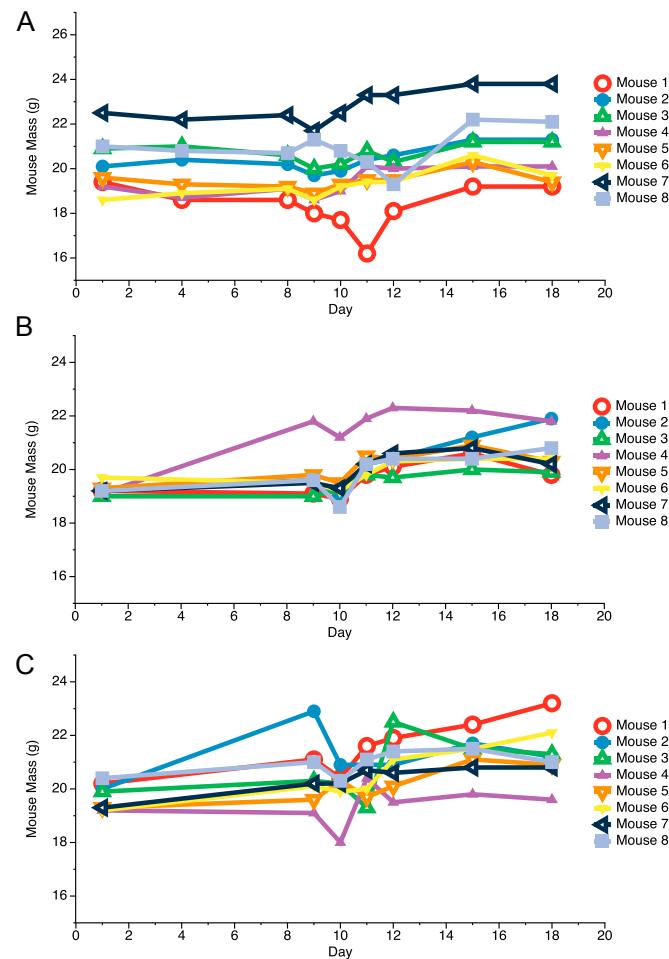


Fig. S9. Monitoring mouse weights. Mice were weighed on the indicated days to monitor their health. A drop in total body mass >20% would indicate that there was a potential health concern. From day 1 to day 18 of all in vivo experiments, all of the mice showed a net gain in total body mass. This gain indicated that administering two drugs, streptomycin and ATC, as well as our engineered bacteria, did not adversely affect mouse health. (A) In vivo experiment #1 corresponds to data presented in Fig. 3B. (B) In vivo experiment #2 corresponds to data presented in Fig. 3D. (C) In vivo experiment #3 corresponds to data presented in Fig. 4. Points represent the mass of an individual mouse on the specified day.

Majority	GGCGXXXXXXXXACATGCA-CTGCAGAAGCTAACAGGAAC									
	18	28	38	48	58	68	78	88		
Escherichia coli MG1655 16S	AAATTGAGAGTGTTCATGCCATGGCTCAGATGGACGGCCGACCTAACACATGCA-GAGCTGAACTGGAGAAC	88								
	PAS 132								-GGCGCGAGCTAACATGCA-CTGCAGAAGCTAACAGGAAC	39
	PAS 133								-AACG 4	
Majority	AGCTTGCTTCTTGGTACGGTGGCGGGGGTGAATGCTGGAACTCTGGCTATGGAGGGGGATAACTACTGGA									
	188	180	131	130	140	150	160			
Escherichia coli MG1655 16S	AGCTTGGCTTCTTGGTACGGTGGCGGGGGTGAATGCTGGAACTCTGGCTATGGAGGGGGATAACTACTGGA	168								
	PAS 132								-AGCTTGGCTTCTTGGTACGGTGGCGGGGGTGAATGCTGGAACTCTGGCTATGGAGGGGGATAACTACTGGA	119
	PAS 133								-AGCTTGGCTTCTTGGTACGGTGGCGGGGGTGAATGCTGGAACTCTGGCTATGGAGGGGGATAACTACTGGA	84
Majority	AACGGTAGCTAACCCATAACGGCTCACAGGACCAAGGGGGACCTTGGCTGGCTTGGCATGATGCCCCAGATG									
	170	188	198	208	218	220	230	240		
Escherichia coli MG1655 16S	AAACGGTAGCTAACCCATAACGGCTCACAGGACCAAGGGGGACCTTGGCTGGCTTGGCATGATGCCCCAGATG	240								
	PAS 132								-AAACGGTAGCTAACCCATAACGGCTCACAGGACCAAGGGGGACCTTGGCTGGCTTGGCATGATGCCCCAGATG	199
	PAS 133								-AAACGGTAGCTAACCCATAACGGCTCACAGGACCAAGGGGGACCTTGGCTGGCTTGGCATGATGCCCCAGATG	164
Majority	GGATTAGCTAGTTGGGGTACGGCTCACCTGGCGACCCCTACTGGCTGGCATGATGACCGGGATCACCTGGA									
	250	268	270	280	290	300	310	320		
Escherichia coli MG1655 16S	GGATTAGCTAGTTGGGGTACGGCTCACCTGGCGACCCCTACTGGCTGGCATGATGACCGGGATCACCTGGA	320								
	PAS 132								-GGATTAGCTAGTTGGGGTAAAGCCCTACTGGCGACCCCTACTGGCTGGCATGATGACCGGGATCACCTGGA	279
	PAS 133								-GGATTAGCTAGTTGGGGTAAAGCCCTACTGGCGACCCCTACTGGCTGGCATGATGACCGGGATCACCTGGA	244
Majority	ACTGAGACGGCTCACAGCTACGGGGAGCAGTGGGGATAATCTTCAACATGGCGAACCTGGTACGGCATG									
	330	340	350	360	370	380	390	400		
Escherichia coli MG1655 16S	ACTGAGACGGCTCACAGCTACGGGGAGCAGTGGGGATAATCTTCAACATGGCGAACCTGGTACGGCATG	400								
	PAS 132								-ACTGAGACGGCTCACAGCTACGGGGAGCAGTGGGGATAATCTTCAACATGGCGAACCTGGTACGGCATG	359
	PAS 133								-ACTGAGACGGCTCACAGCTACGGGGAGCAGTGGGGATAATCTTCAACATGGCGAACCTGGTACGGCATG	324
Majority	CGCGTGTATAAGAAAGCCCTGGGGTGTAAAGTACTTACGGGGAGGAGGGGATAATTAACCTTGTCTT									
	410	420	430	440	450	460	470	480		
Escherichia coli MG1655 16S	CGCGTGTATAAGAAAGCCCTGGGGTGTAAAGTACTTACGGGGAGGAGGGGATAATTAACCTTGTCTT	480								
	PAS 132								-CGCGTGTATAAGAAAGCCCTGGGGTGTAAAGTACTTACGGGGAGGAGGGGATAATTAACCTTGTCTT	439
	PAS 133								-CGCGTGTATAAGAAAGCCCTGGGGTGTAAAGTACTTACGGGGAGGAGGGGATAATTAACCTTGTCTT	484
Majority	GACGTTCACCCGAGAAGCACCCGCTAACCTGGTCCACAGCGGCCGCTAACATGGGGTCAAGCGCTTAATCGGAA									
	510	580	590	600	610	620	630	640		
Escherichia coli MG1655 16S	GACGTTCACCCGAGAAGCACCCGCTAACCTGGTCCACAGCGGCCGCTAACATGGGGTCAAGCGCTTAATCGGAA	560								
	PAS 132								-GACGTTCACCCGAGAAGCACCCGCTAACCTGGTCCACAGCGGCCGCTAACATGGGGTCAAGCGCTTAATCGGAA	519
	PAS 133								-GACGTTCACCCGAGAAGCACCCGCTAACCTGGTCCACAGCGGCCGCTAACATGGGGTCAAGCGCTTAATCGGAA	484
Majority	TTCAGTGGCCATTAACGGCACAGCGGGCTTGTAGTCAGTGTGAATACCCGGGCTCACCTGGAACTGATCTGA									
	570	580	590	600	610	620	630	640		
Escherichia coli MG1655 16S	TTCAGTGGCCATTAACGGCACAGCGGGCTTGTAGTCAGTGTGAATACCCGGGCTCACCTGGAACTGATCTGA	640								
	PAS 132								-TTCAGTGGCCATTAACGGCACAGCGGGCTTGTAGTCAGTGTGAATACCCGGGCTCACCTGGAACTGATCTGA	599
	PAS 133								-TTCAGTGGCCATTAACGGCACAGCGGGCTTGTAGTCAGTGTGAATACCCGGGCTCACCTGGAACTGATCTGA	564
Majority	TACTGGCAAGCTGGCTTGGCTGGAGGGGG-TAGAATTCTGGCTGGAGGGGGTCAAGCTGGCTGGAGGGGG									
	650	660	670	680	690	700	710	720		
Escherichia coli MG1655 16S	TACTGGCAAGCTGGCTTGGCTGGAGGGGG-TAGAATTCTGGCTGGAGGGGGTCAAGCTGGCTGGAGGGGG	719								
	PAS 132								-TACTGGCAAGCTGGCTTGGCTGGAGGGGG-TAGAATTCTGGCTGGAGGGGGTCAAGCTGGCTGGAGGGGG	678
	PAS 133								-TACTGGCAAGCTGGCTTGGCTGGAGGGGG-TAGAATTCTGGCTGGAGGGGGTCAAGCTGGCTGGAGGGGG	644
Majority	CGGTGG-CGAGGGCCGCCCTGGAGAACGACTGAGCTGGCTGGAGGGGGAACAGGGTAGATACCT									
	730	740	750	760	770	780	790	800		
Escherichia coli MG1655 16S	CGGTGG-CGAGGGCCGCCCTGGAGAACGACTGAGCTGGCTGGAGGGGGAACAGGGTAGATACCT	798								
	PAS 132								-CGGTGG-CGAGGGCCGCCCTGGAGAACGACTGAGCTGGCTGGAGGGGGAACAGGGTAGATACCT	758
	PAS 133								-CGGTGG-CGAGGGCCGCCCTGGAGAACGACTGAGCTGGCTGGAGGGGGAACAGGGTAGATACCT	723

Fig. S10. Alignment of the 16S Sequence of PAS132 and PAS133 with MG1655. The gene encoding the 16S ribosomal subunits of PAS132 and PAS133 were amplified by PCR, then sequenced (1). The sequences were aligned against the reference sequence of MG1655 using Lasergene software. A phylogenetic tree was constructed comparing the reference sequences of the indicated bacteria using Lasergene software.

1. Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173(2):697-703.