Supporting Information

Wei et al. 10.1073/pnas.1018301108

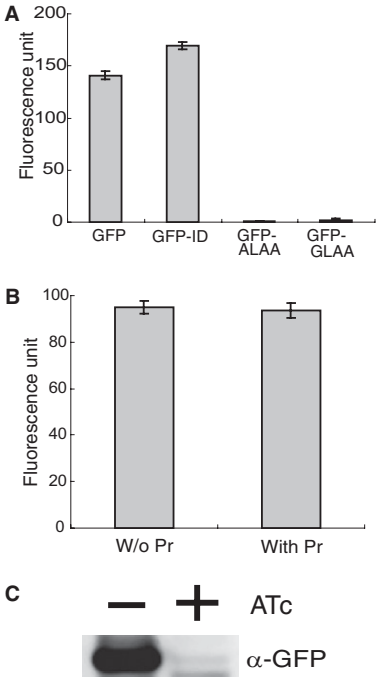

Fig. S1. GFP is conditionally degraded when HIV protease is expressed. (*A*) Fluorescence of WT GFP (GFP), GFP fused to the inducible degradation (ID) tag (GFP-ID), GFP fused to the WT mycobacterial SsrA tag (GFP-ALAA), or a mutated SsrA tag (GFP-GLAA). *Mycobacterium smegmatis* mc^2155 containing pMV261kan::GFP, pMV261kan::GFP-ID, pMV261kan::GFP-mycoSsrA(ALAA), or pMV261kan::GFP-mycoSsrA(GLAA) was cultured overnight from a starting OD_{600} = 0.02 (calculated). Additionally, fluorescence was determined. (*B*) GFP-ID is stable in the absence of protease expression. *M. smegmatis* mc^2155 with pMV261hyg::GFP-ID with or without pMC1s::WT-HIV2Pr was grown from a starting OD_{600} = 0.02 (calculated). Overnight and fluorescence was determined. Values represent mean ± SD of triplicate samples. (*C*) Western blot of the GFP-ID strain grown with or without added anhydrotetracycline (ATc). Western blot analysis was performed as described in *Methods*.

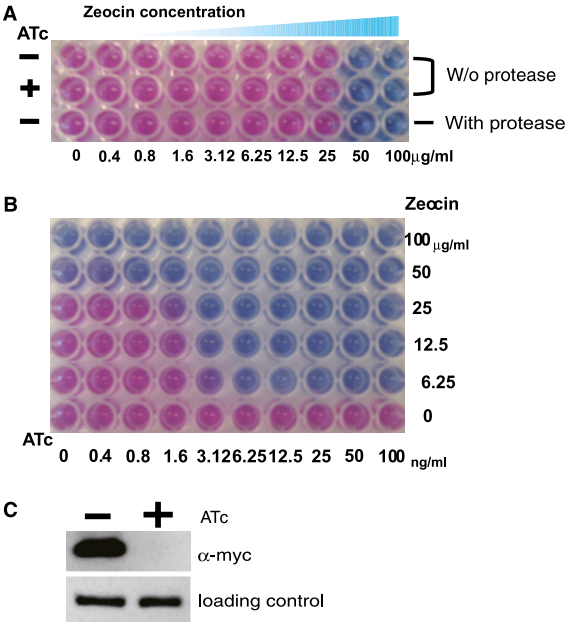

Fig. S2. The Zeocin (Zeo) resistance protein is efficiently degraded using the ID tag. *M. smegmatis* mc^{2}155 containing both pGH1000A::zeo-ID and pMC1s::WT-HIV2Pr was grown in 96-well plates with varying concentrations of Zeo or ATc. Degradation of the Zeo resistance protein results in a decrease in the observed minimal inhibitory concentration (MIC; the concentration of antibiotic necessary to block conversion to a pink color). (*A*) In the absence of protease, the MIC mediated by ID-tagged Zeo protein is equivalent to untagged Zeo protein. (*B*) Low concentration of inducer results in similar levels of Zeo protein degradation. (*C*) Western blot of c-myc epitope-tagged Zeo protein in the strain with or without the induction of ATc.

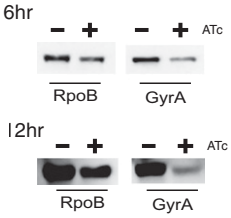

Fig. S3. Degradation of RNA polymerase β-subunit (RpoB) or gyrase A subunit (GyrA) fused to the ID tag. Western blot analysis of lysates carrying ID tag fusions to RpoB or GyrA in the presence or absence of inducer (ATc) was performed using α-c-myc antibody to visualize ID-tagged proteins. Cultures were 1:100 diluted in the presence or absence of ATc. Samples were collected after 6 or 12 h.

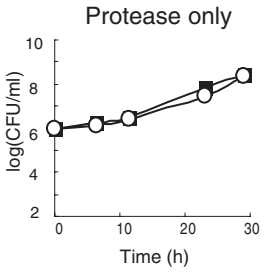

Fig. S4. Growth of *M. smegmatis* mc^{2}155 pMC1s::WT-HIV2Pr with or without added ATc. cfu values represent the mean ± SD of triplicate samples.

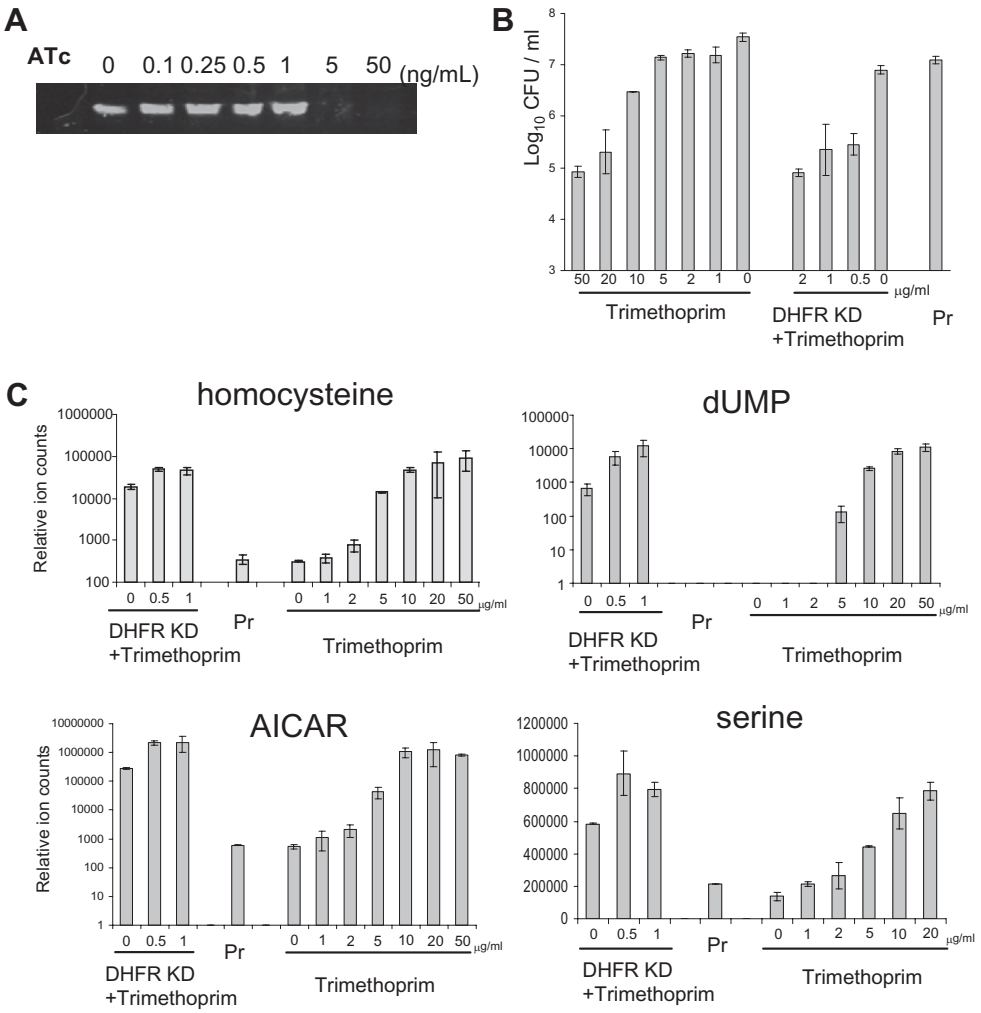

Fig. S5. Metabolomic profile of dihydrofolate reductase (DHFR) depletion and trimethoprim-treated *M. smegmatis*. (*A*) Western analysis of DHFR-depleted cells grown on nitrocellulose filters; 5 ng/mL inducer (ATc) are sufficient to deplete DHFR below the level of detection. (*B*) cfu of overnight cultures grown on nitrocellulose filter. Prs are cells expressing the HIV-2 protease in a WT background. Except Pr, the rest of the cultures are in a background where DHFR has been ID-tagged and a HIV-2 protease may be expressed *in trans*. DHFR-KD + trimethroprim cells are induced and depleted for DHFR in the presence of different concentrations of trimethoprim. Trimethroprim cells are not deleted for DHFR but treated with various concentrations of trimethoprim.

Table S1. Plasmids used in this work

	Relevant genotype/sequence	Source or reference
pMC1s	Integrated mycobacterial *E. coli* shuttle plasmid (L5 site) has TetR repressor; Kan^R	1
pMC1s::WT-HIV2Pr*	Integrating plasmid (L5 site), tet-inducible, contains codon-optimized HIV-2 protease; Kan^R	This work (GenBank no. GU459075)
pMV261	Episomal plasmid; Kan^R	2
pMV261kan::GFP†	Derived from pMV261 and contains GFP from GFPmut3; Kan^R	This work (GenBank no. GU459078)
pMV261kan::GFP-ID†	Derived from pMV261 and contains GFP with ID tag; Kan^R	This work (GenBank no. GU459077)
pMV261kan::GFP-mycoSsrA(ALAA)†	Derived from pMV261 and contains GFP with WTTB SsrA tag; Kan^R	This work (GenBank no. GU459081)
pMV261kan::GFP-mycoSsrA(GLAA)†	Derived from pMV261 and contains GFP with mutated TB ssrA tag (A to G); Kan^R	This work (GenBank no. GU459076)
pMV261hyg::GFP-ID‡	Derived from pMV261 and contains GFP with ID tag; Hyg^R	This work (GenBank no. GU459074)
pGH1000A	Integrating plasmid (Giles site); Hyg^R	Gift from Graham Hatfull (3)
pGH1000A::zeo-ID§	Derived from pGH1000A and contains Zeocin resistance gene with ID tag; Hyg^R	This work (GenBank no. GU459079)
pBluescriptKS+	Cloning plasmid for general purpose; Amp^R	Stratagene
pBKS::zeo-ID-hyg¶	Derived from pBluescriptKS+ and contains Zeocin resistance gene with ID tag and hygromycin resistance gene cassette; Amp^R and Hyg^R	This work (GenBank no. GU459080)
pUC57::alr-design‖	Synthesized flanking DNA sequence of alr in pUC57; Amp^R	This work
pUC57::dfrA-design‖	Synthesized flanking DNA sequence of dfrA in pUC57; Amp^R	This work
pUC57::inhA-design‖	Synthesized flanking DNA sequence of inhA in pUC57; Amp^R	This work
pUC57::kasA-design‖	Synthesized flanking DNA sequence of kasA in pUC57; Amp^R	This work
pUC57::alr-ID-hyg	ID-hyg DNA cassette inserted into pUC57::alr-design; Amp^R and Hyg^R	This work
pUC57::dfrA-ID-hyg	ID-hyg DNA cassette inserted into pUC57::dfrA-design; Amp^R and Hyg^R	This work
pUC57::inhA-ID-hyg	ID-hyg DNA cassette inserted into pUC57::inhA-design; Amp^R and Hyg^R	This work
pUC57::kasA-ID-hyg	ID-hyg DNA cassette inserted into pUC57::kasA-design; Amp^R and Hyg^R	This work
pNit-1	Nitrile-inducable, episomal mycobacterial *E. coli* shuttle plasmid; Kan^R	4
pNit::ET	Derived from pNit-1, recombinase from phage che9, *sacBR* from *Bacillus* for counter selection; Kan^R	This work (GenBank no. GU459073)

*pMC1s::WT-HIV2Pr is an integrating tetracycline(tet)-inducible plasmid (1) that encodes the codon-optimized HIV-2 protease (GenBank no. GU459075). The original HIV-2 protease sequence was a gift from Phyllis Kanki and Nzovu (Harvard School of Public Health, Boston) We subsequently synthesized a codon-optimized version to improve expression in mycobacteria.

†pMV261kan::GFP (GenBank no. GU459078), pMV261kan::GFP-ID (GenBank no. GU459077), pMV261kan::GFP-mycoSsrA(ALAA) (GenBank no. GU459081), and pMV261kan::GFP-mycoSsrA(GLAA) (GenBank no. GU459076) are pMV261(2)-based episomal plasmids in which the GFP open-reading frame is fused with different tags.

‡pMV261hyg::GFP-ID (GenBank no. GU459074) is a pMV261hyg-based plasmid in which the GFP open-reading frame is fused with the ID tag.

§pGH1000A::zeo-ID (GenBank no. GU459079) is a pGH1000A-based plasmid in which Zeocin resistance gene is fused with the ID tag.

¶pBKS::zeo-ID-hyg (GenBank no. GU459080) is a pBluescript plasmid with zeo-ID and a hygromicin resistance gene used for generating substrate PCR for recombineering.

‖pUC57::alr-design, pUC57::dfrA-design, pUC57::inhA-design, and pUC57::kasA-design are plasmids containing synthesized DNA fragments that correspond to the flanking region of target genes. DNA fragments containing ID-hyg (ID tag with hygromycin cassette) from pBKS::zeo-ID-hyg were digested and inserted into each of them with appropriate restriction enzymes. The sequence of synthesized DNA fragments is listed in Table S3.

1. Guo XV, et al. (2007) Silencing *Mycobacterium smegmatis* by using tetracycline repressors. *J Bacteriol* 189:4614–4623.
2. Stover CK, et al. (1991) New use of BCG for recombinant vaccines. *Nature* 351:456–460.
3. Morris P, Marinelli LJ, Jacobs-Sera D, Hendrix RW, Hatfull GF (2008) Genomic characterization of mycobacteriophage Giles: Evidence for phage acquisition of host DNA by illegitimate recombination. *J Bacteriol* 190:2172–2182.
4. Pandey AK, et al. (2009) Nitrile-inducible gene expression in mycobacteria. *Tuberculosis (Edinb)* 89:12–16.

Table S2. Primers used in this work

Name	Sequence (5′ to 3′)
gyrA-mycF1	GACGAGGCGGCCGAGTCGATCAGCGAATCCGACGCGGACACCGCCGAGTCACCCGAGGCGGAGCAGAAGCTGATCTCGGA
gyrA-mycF2	CGCTTGATGAACCTGGCCGAGGGCGACACACTGATTGCCATCGCCCGCAACGCGGACGAGGACGAGGCGGCCGAGT
gyrA-hygR1	TTGGGTGAACTCACCTACAGCTCCTTAGCTCGGGCCGAGGACCTTTCGGGGACCTCAGCGGATCAGCTAGAGGGGCGTC
gyrA-hygR2	CCCGGACCCGTGCCGTTGGTGGCGCCCGGCCGGTCCCCCGCGCGCGGGTATCCCGGCTCGTTGGGTGAACTCACCTACAG
rpoB-mycF1	GCCGCGAACCTGGGAATCAACCTGTCGCGCAACGAATCCGCGTCCGTCGAGGATCTCGCGGAGCAGAAGCTGATCTCGGA
rpoB-mycF2	TCCAGCGACGGCGCGGCGATCGAGATGCGTGACGGTGACGACGAGGACCTGGAGCGCGCTGCCGCGAACCTGGGAATC
rpoB-hygR1	GTAACTCCCTTTCCCCTTGCGGGTGTTGAAACTTGACTACTGAGGCGGTCTTCGGACGAGGATCAGCTAGAGGGGCGTC
rpoB-hygR2	GATGTCGTCCGCGGTCGCGAGACCGATGCGGAGTTCATCGAAGAAGTTGACGTCTAGCACGTAACTCCCTTTCCCCTTGC
dfrAF	TGCGATGGCACTGGCGCGGG
dfrAR	GCCTTCCCAATCCCGCTCGTA
alrF	GGACGCGGACGACGTGGCC
alrR	ACGCCGTCGTCGGTGGTGATC
inhAF	CGGTGCGCTGGGCGAC
inhAR	TCGCGACGATCAGGTCCC
kasAF	GGTGCGCTGGAATCGATCC
kasAR	CCGGCAGGTTGTACTCCTC

Table S3. Sequence of synthesized DNA fragments for pUC57::alr-design, pUC57::dfrA-design, pUC57::inhA-design, and pUC57::kasA-design

	Sequence
alr-design	GGACGCGGACGACGTGGCCGTAGGTGACGACGCCATCCTGTTCGGCCCGGGCG CCAACGGCGAACCGACCGCGCAGGACTGGGCCGAACTGCTCGACACCATTCA CTACGAGGTCGTCACGAGCCCGCGCGGACGCGTCACGCGCACGTATCTTCCGG CAGGACAACAAGATggatccCGCctcgagGCGCGCGATGGCTTGCCGGCGTGGCC GGGCTGGCCGCTGTCGGCACCGTGGCAGGTGTGTCGATCGCGCGCTCGCTCA CCTTGCGGGTGAGCAAGGAAGACCCCTACGCCGGTGAGGATTTCGAGCTGCT CGACGCCGATCGCAGCTCGGTGATCACCACCGACGACGGCGT
dfrA-design	TGCGATGGCACTGGCGCGGGCCGACCGCTGTGAGGTCACCGAGGTCGACAT CGCCCTGACGCCACTCGACGGTGATGCGCGCGCACCGGTGCTCGACGAC TCGTGGGTCGCGACGACGGGTGAGTGGCAGACCAGTACGTCAGGCCT GCGTTTCCGGTTCTGCAGCTACCGGCGCggatccCGCctcgagCGAACTTCCAGC GAGACCACCTCCAGGCTCGGTACATTGTTCCGGGGTCAGCAGGAGAGGTGC TCCGCCCATGACTGAGTCTTCGATTCGTGTCGTACTGCGTGAGCGCGCGAGT CTGCAGCCCAATGACACCGCGTTCACATTCGTTGATTACGAGCGGGATT GGGAAGGC
inhA-design	GGCGGTGCGCTGGGCGACGAGGCCGGCCAGCAGATGCAGCTGCTCGAAGA GGGCTGGGATCAGCGCGCGCCGCTGGGCTGGAACATGAAGGACCCGACGC CCGTCGCCAAGACCGTGTGCGCACTGCTGTCGGACTGGCTGCCGGCCACCA CCGGCACCGTGATCTACGCCGACGGCGGCGCCAGCACGCAGCTG TTGggatccCGCctcgagCCGTGTCGTTTGACGCCTTGCTGCTGCTGTCGTTCGG CGGGCCGGAAGCTCCCGAGCAGGTGATGCCGTTCTTGGAGAACGTCACCA GGGGCCGCGGAATCCCCAGGGAGCGGCTGGAATCGGTGGCCGAGCACT ATCTGCACTTCGGCGGGGTGTCACCGATCAACGGCATCAACC GGGACCTGATCGTCGCGA
kasA-design	GGTGCGCTGGAATCGATCCTCACGGTCCTGGCTCTCCGCGACGGCGTCATCCC CCCGACGCTGAACTACGAGACCCCGGATCCCGAGATCGATCTCGATATCGTT GCAGGTGAGCCTCGGTACGGCGAATACAAGTACGCCATCAACAACTCGTTC GGGTTCGGCGGCCACAATGTGGCTCTGGCATTCGGGCGTTACagatctGCTCAGctc gagGTAAAGGAAAGAATCGCGTAAATATGGCGGCACTGTCCACGGGGAACGGG CTTCCCAACGTGGTCGTCACCGGCGTTGCGATGACGACAGCGCTGGCGACGGA TGCGGAAAGCACCTGGAAGAAGCTGCTCGACGGCCAGAGCGGTATCCGTAC GCTCACGGATCCGTTCGTCGAGGAGTACAACCTGCCGG

The underlined sequences are designed restriction enzyme sites.