Conditional protein splicing of the *Mycobacterium tuberculosis* RecA intein in its native host

Ryan F. Schneider, Kelly Hallstrom, Christopher DeMott, Kathleen A. McDonough

Supplemental Tables

Table S1: Strains used in this study.

Strain	**Description**	**Source**
$mc^2$6230	Mtb H37Rv (*ΔpanCD*, *ΔRD1*)	Gift of Dr. William Jacobs, Jr [1]
$mc^2$6230*ΔrecA*	*recA* knockout (*ΔrecA*, *ΔpanCD*, *ΔRD1*)	This Paper
Mycobacterium smegmatis $mc^2$155	Parental (*eptC1*)	Acquired from BEI [2, 3]
Mycobacterium smegmatis ΔrecAX	$mc^2$155 *recAX* knockout (*ΔrecAX*)	Gift from Dr. Keith Derbyshire
E. coli BLR	*ΔrecA* derivative of BL21	Novagen
E. coli BL21	For protein expression and purification	Novagen

Table S2: Plasmids used in this study.

Designation	Description	Source
pMBC409	Single Copy, integrating (AttP) vector to express transcriptional reporters or *recA* complements; Kan^R, Hyg^R, Amp^R	Described in Girardin et al [4]
pMBC1809	mVenus-based *recA* transcriptional reporter; Kan^R, Hyg^R, Amp^R	This Paper
pMBC2149	Full Mtb *recA* gene driven by native Mtb promoters; Kan^R, Hyg^R, Amp^R	This Paper
pMBC2169	Inteinless Mtb *recA* gene driven by native Mtb promoters; Kan^R, Hyg^R, Amp^R	This Paper
pMBC2290	Mtb N-extein driven by native Mtb promoters; Kan^R, Hyg^R, Amp^R	This Paper
pMBC1650	pET28a+ expressing N-extein; Kan^R	This Paper
pMBC1651	pET28A+ expressing intein; Kan^R	This Paper
pMBC1652	pET28a+ expressing C-extein; Kan^R	This paper

Table S3: Primers used in this study.

Designation	Sequence	Description
KM4059	gggggatcctctagatttaagaaggagatatacatatggtgagcaagggcgaggagctg	mVenus forward
KM4138	gggaagctttgatcaccgcggccatg	mVenus reverse
KM4194	tgcagtggatcccgcaccgccgccagg	*recA* promoter forward
KM4195	cctagtggatcccatggtgcctctcctgtg	*recA* promoter reverse
KM4345	agagatatacggcccggagt	*recA* qRT
KM4346	ccgagcttcttggcatagtc	*recA* qRT
KM4290	acgtaaacggccacaagttc	GFPv qRT
KM4291	aagtcgtgctgcttcatgtg	GFPv qRT
KM3727	gggaattcacgcagacccccgatcgg	*recA* NE for protein purification fwd
KM3728	ggaagctttcacttgttcttgacgaccttgac	*recA* NE for protein purification rev
KM3729	gggaattctgcctcgcagagggc	*recA* intein for protein purification fwd
KM3730	ggaagctttcaacagttgtgcacgacaaccc	*recA* intein for protein purification rev
KM3731	gggaattctcgcccccccttcaagca	*recA* CE for protein purification fwd
KM3732	gggagctctcagaagtcgacggggg	*recA* CE for protein purification rev
KM5105	gatatcgtgttgagcagatcgtcggtgatccgga	*recA* full complement fwd
KM5106	gcggccgctcagaagtcgacgggggcgg	*recA* full complement rev
KM5584	gatatcgcctgctcttcgcgctcagaag	*recA* inteinless complement NE fwd
KM5585	caaggtcgtcaagaacaagtgttcgcccccccttcaagcagg	*recA* inteinless complement NE rev
KM5586	gaagggggggcgaacacttgttcttgacgaccttgacccggg	*recA* inteinless complement CE fwd
KM5587	gatatcgacgccgaaaggtcagatccgg	*recA* inteinless complement CE rev
KM6016	aagcttgagcagatcgtcggtgatc	*recA* N-Extein only complement fwd
KM6017	aagcttctacttgttcttgacgaccttgac	*recA* N-Extein only complement rev
KM4413	ttgcaccaggctgtagcgggagtct	Hygromycin resistance cassette fwd
KM4414	ccgctgaccgggaacaccgtgctc	Hygromycin resistance cassette fwd
KM5107	cttgttcttgacgaccttgacccgggt	N-extein reverse (to check KO)

Supplemental Figures for

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Figure S1

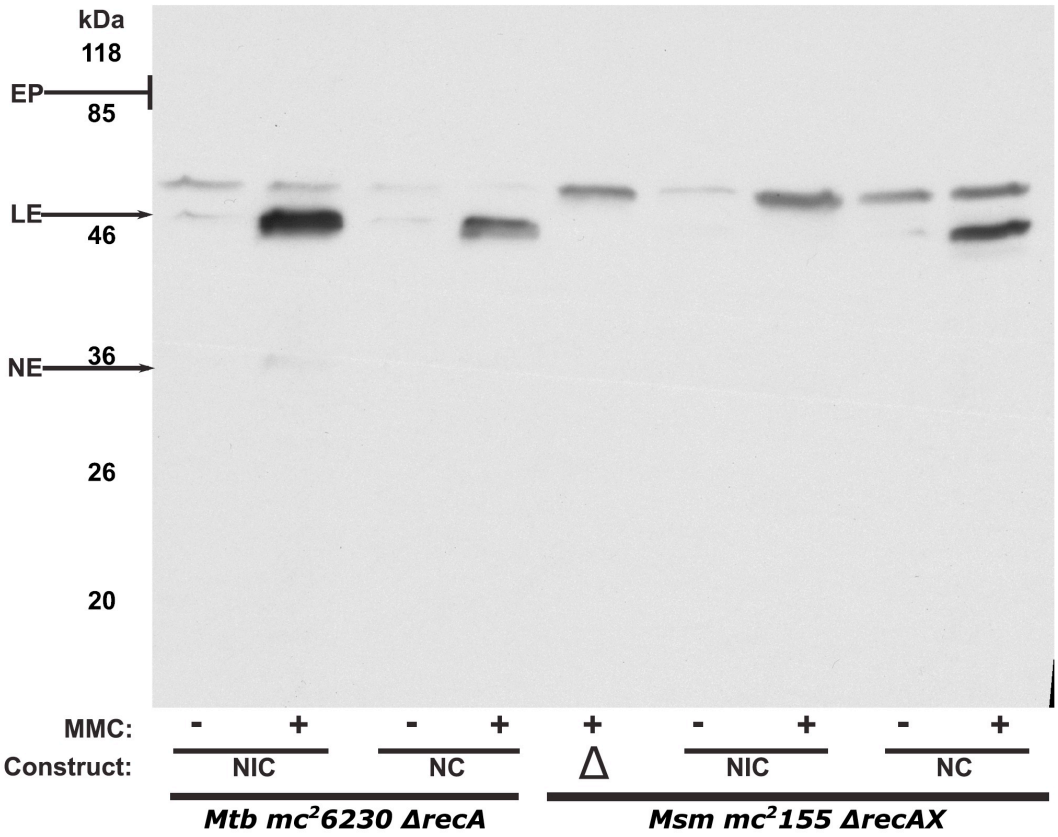

Figure S1: RecA precursor was not detected in other mycobacteria. *M. tuberculosis ΔrecA* and *M. smegmatis ΔrecAX* were complemented with intein-containing *recA* (NIC) or inteinless *recA* (NC) alleles driven by their native mycobacterial promoters. Cells were grown to mid-log and treated for 3 days with Mitomycin C to induce *recA* transcription and RecA production. Protein from cultures was harvested for western blotting against the N-extein, which detects ligated exteins (LE) and the N-extein (NE) as well as precursor (see Figure 1B). EP: expected size of precursor RecA, which was not detected.

Figure S2

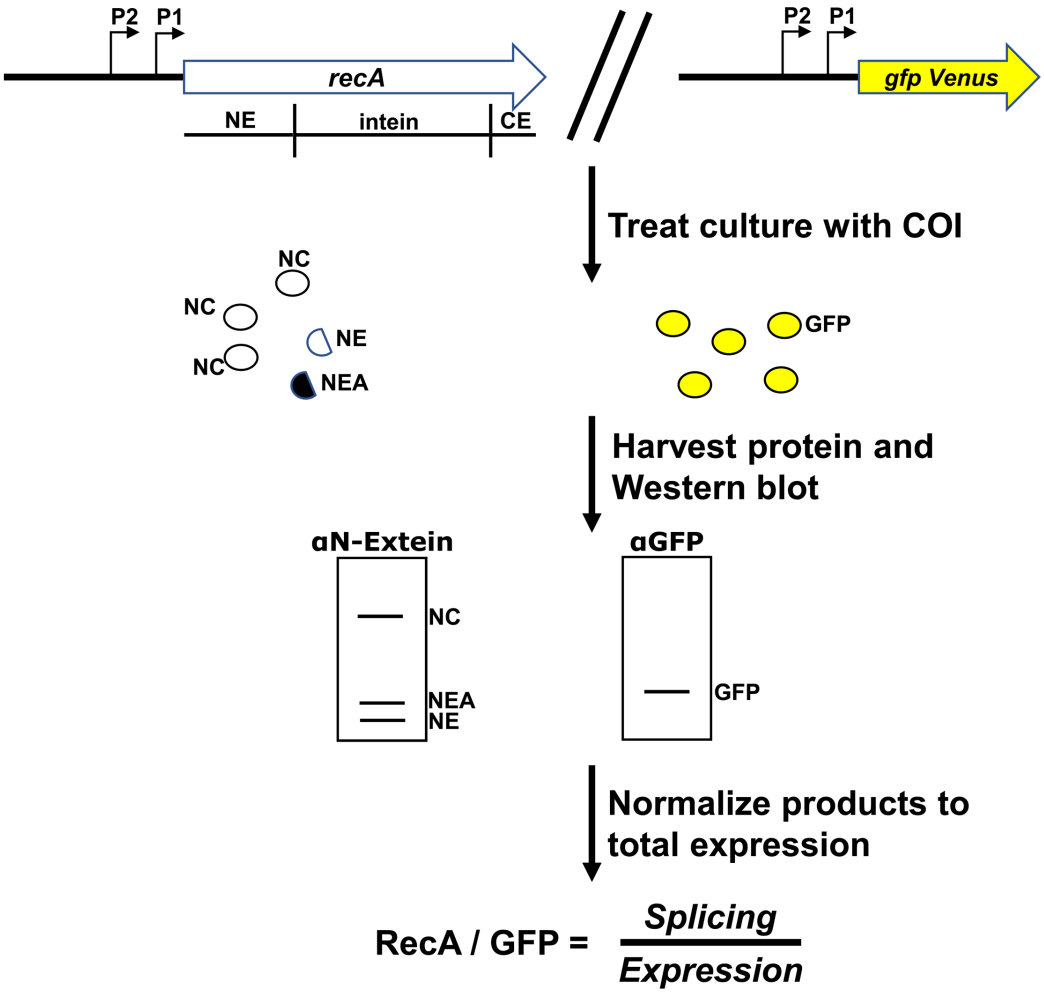

Figure S2: Schematic of experimental setup and analysis. Mtb auxotroph mc^{2}6230 harboring a native *recA* allele and a *gfp* allele driven by the native Mtb *recA* promoters is grown to the desired phase and treated with conditions of interest (COI). If treatment induces *recA* production, RecA products and GFP will be made at a higher level compared to untreated. After treatment, total protein lysate is extracted and subjected to western blotting targeting the N-extein (left), GFP (right) or Intein (not shown). Western blots are quantitated by densitometry and the ratio of RecA product to GFP is calculated to normalize for changes in transcriptional expression.

Figure S3

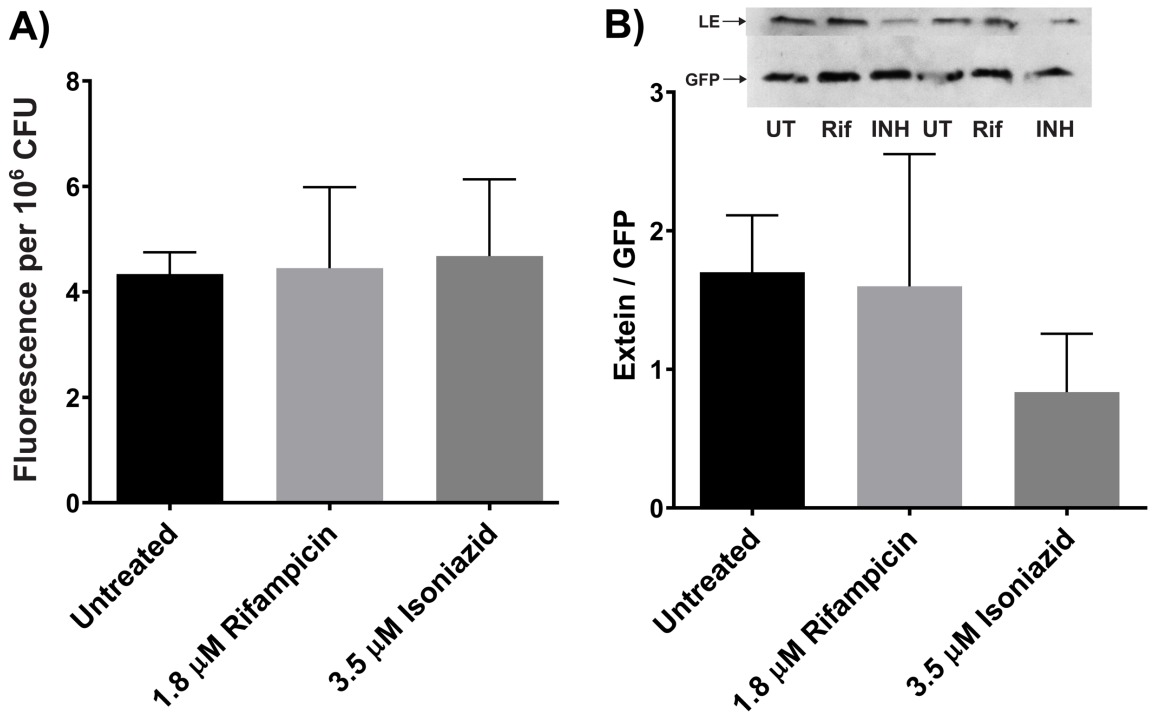

Figure S3: First-line therapeutics rifampicin and Isoniazid do not induce the *recA* transcription or RecA splicing. Mtb auxotrophic strain $mc^2$6230 harboring our *recA* transcriptional reporter was grown to mid-log phase and then treated with rifampicin or isoniazid. (A) *recA* transcription in response to the two therapeutics as read by our GFP based transcriptional reporter system. (B) Western blot of RecA production in response to rifampicin or isoniazid. Error bars represent standard deviation of two biological repeats.

Figure S4

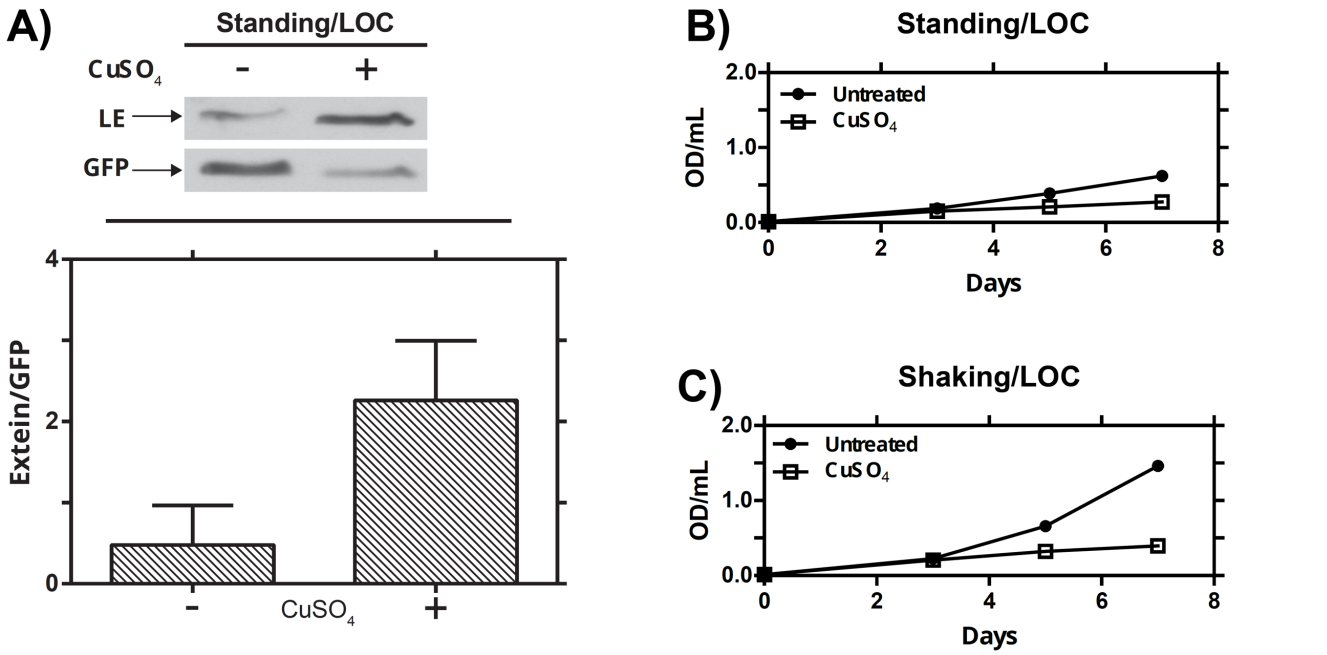

Figure S4: Growth does not affect copper-induced splicing. Mtb cultures starting at OD0.035 were grown in the presence or absence of copper for seven days either standing or shaking in low oxygen conditions supplemented with carbon dioxide (LOC). Copper was added at the time cultures were started. A) Representative western blot with quantitative analysis of RecA ligated extein (LE) produced in response to copper in standing LOC. Error bars represent standard deviation of three biological repeats. B) Optical density of cultures grown in standing LOC conditions with copper absent (black circle) or present (open square). C) Optical densities of cultures grown in shaking LOC conditions with copper absent (black circle) or present (open square).

Figure S5

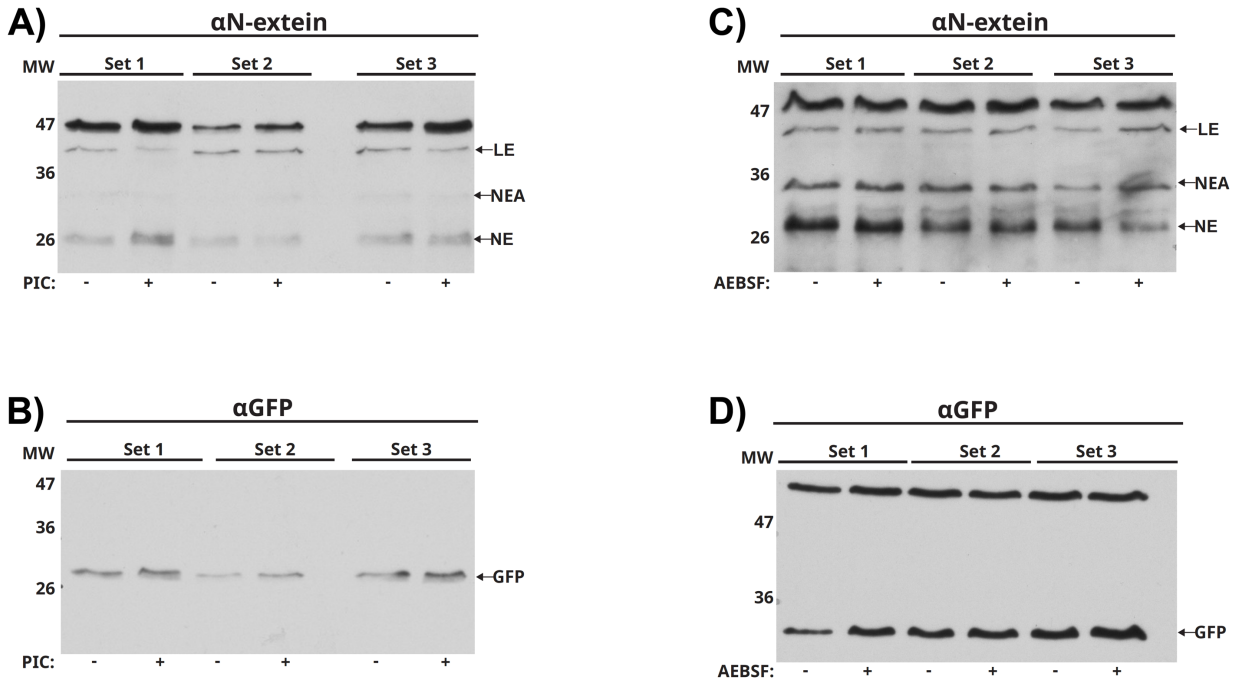

Figure S5: Treatment with protease inhibitors does not affect levels of RecA or intein. Mtb mc^{2}6230 harboring a native *recA* allele and a *gfp* allele driven by the native Mtb *recA* promoters was grown to (A, B) mid-log phase before treating with 1:1000 dilution of Sigma Aldrich's protease inhibitor cocktail for 24hrs in triplicate. (A) Western blot analysis targeting the N-extein. (B) Western blot analysis targeting GFP. (C-D) Mtb mc^{2}6230 harboring a native *recA* allele and a *gfp* allele driven by the native *recA* promoters was grown to late-log phase and treated with AEBSF for 48hrs in triplicate. Western blot analysis targeted the N-extein (C) or GFP (D).

Figure S6

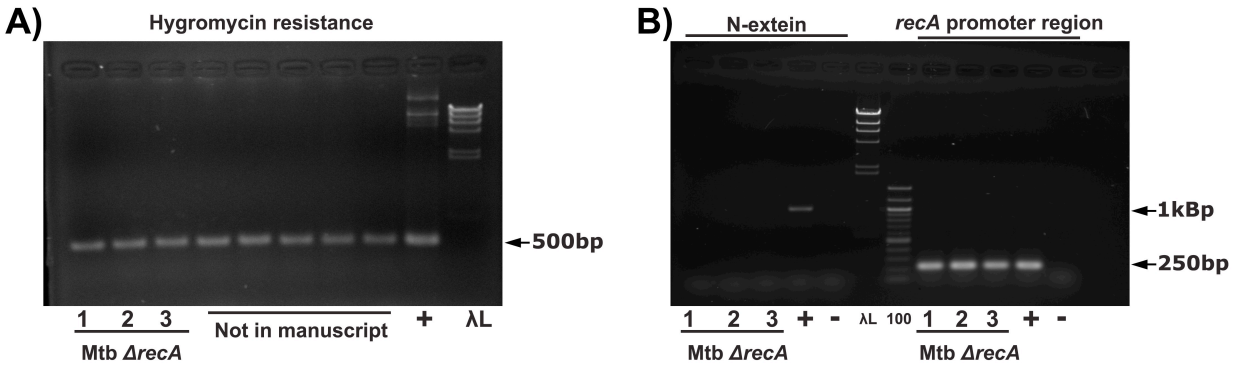

Figure S6: PCR confirming knockout of the *recA* gene in mc^{2}6230. (A) PCR targeting a ~500bp section of the hygromycin resistance cassette encoded on the phagemid (negative water only control not shown). (B) PCR targeting either the *recA* promoter region + N-extein (~1kb) or the *recA* promoter region (~230bp). The positive control for PCRs was heat-killed H37Rv and the negative control was water only. λL: NEB lambda phage DNA digested with HindIII. 100: NEB's 100bp ladder.

References for supplemental information:

1. Sambandamurthy, V.K. *et al. Mycobacterium tuberculosis* DeltaRD1 DeltapanCD: a safe and limited replicating mutant strain that protects immunocompetent and immunocompromised mice against experimental tuberculosis. *Vaccine* **24**, 6309-6320 (2006).
2. Snapper, S.B., Melton, R.E., Mustafa, S., Kieser, T. & Jacobs, W.R., Jr. Isolation and characterization of efficient plasmid transformation mutants of *Mycobacterium smegmatis*. *Mol Microbiol* **4**, 1911-1919 (1990).
3. Panas, M.W. *et al.* Noncanonical SMC protein in *Mycobacterium smegmatis* restricts maintenance of *Mycobacterium fortuitum* plasmids. *Proc Natl Acad Sci U S A* **111**, 13264-13271 (2014).
4. Girardin, R.C. & McDonough, K.A. Small RNA Mcr11 requires the transcription factor AbmR for stable expression and regulates genes involved in the central metabolism of *Mycobacterium tuberculosi*s. *Mol Microbiol* **113**, 504-520 (2020).

Supplementary compilation of full gel images used for western blots shown in:

Conditional protein splicing of the *Mycobacterium tuberculosis* RecA intein in its native host
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Figure 1B (Right)
αN-Extein

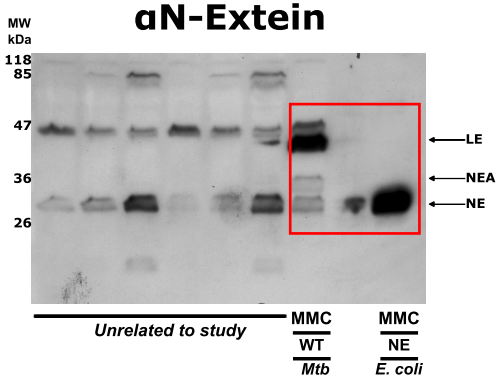

Figure 1C
αN-Extein

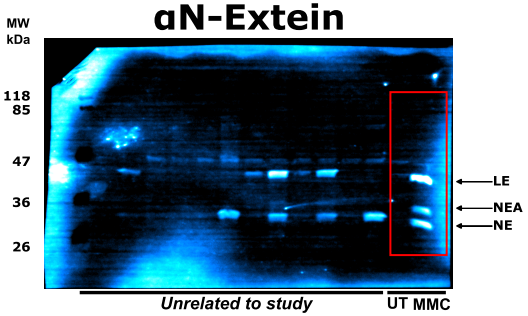

Figure 1C
αC-Extein

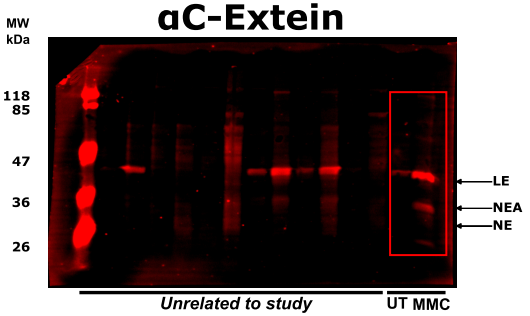

Figure 2A
αIntein

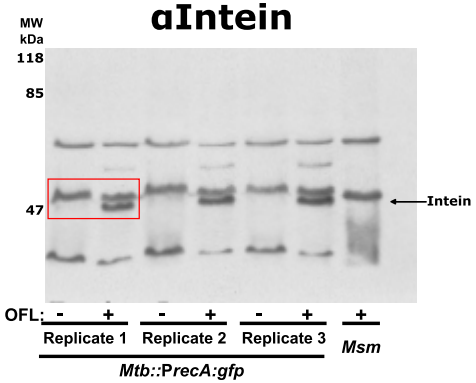

Figure 2B
αN-Extein

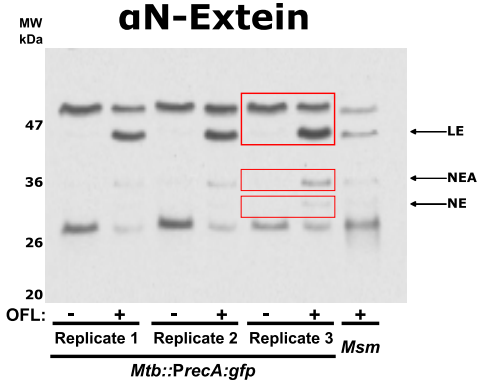

Figure 2A,B
αGFP

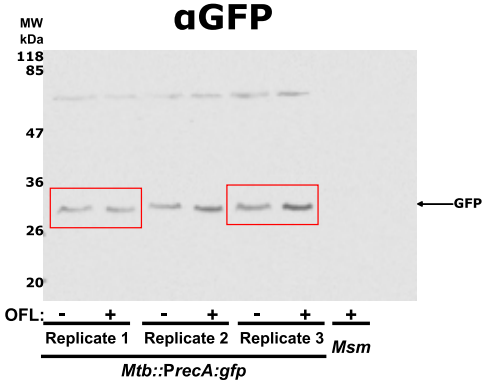

Figure 2C
αIntein

Figure 2D
αN-Extein

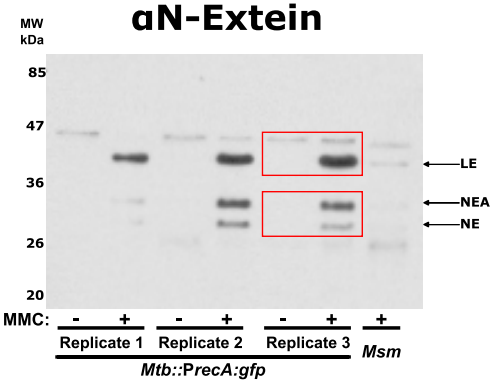

Figure 2C,D
αGFP

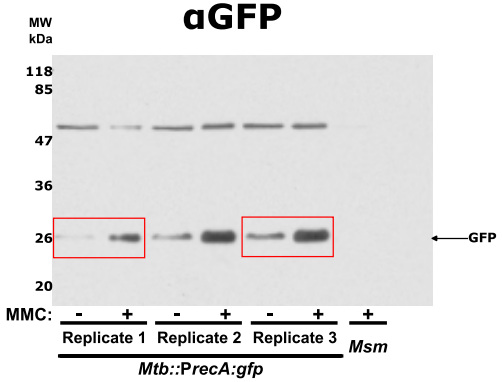

Figure 3A
αIntein

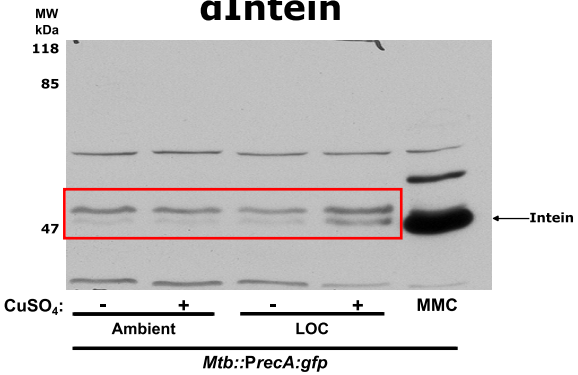

Figure 3A
αGFP

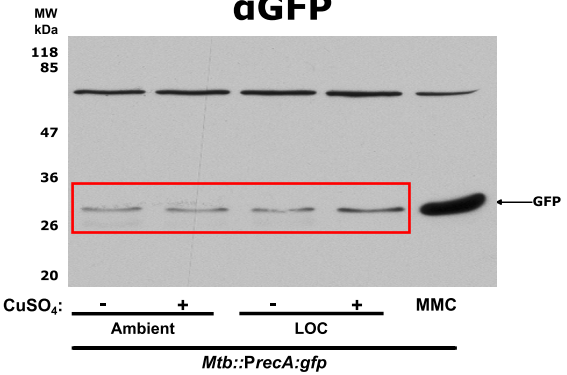

Figure 3B
αN-Extein

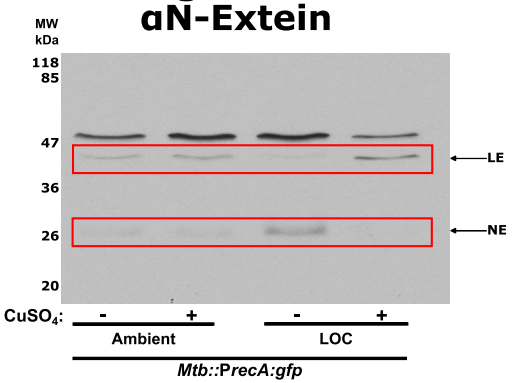

Figure 3B
αGFP

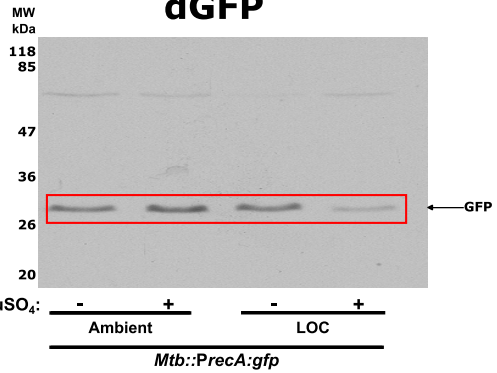

Figure 3C
αIntein

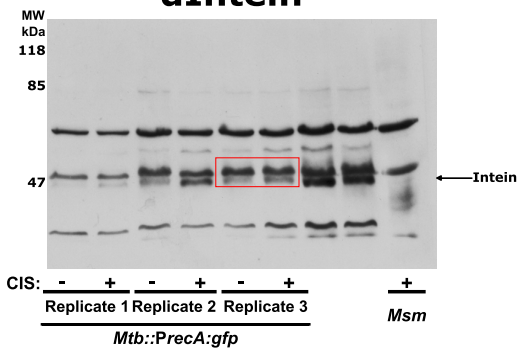

Figure 3D
αN-Extein

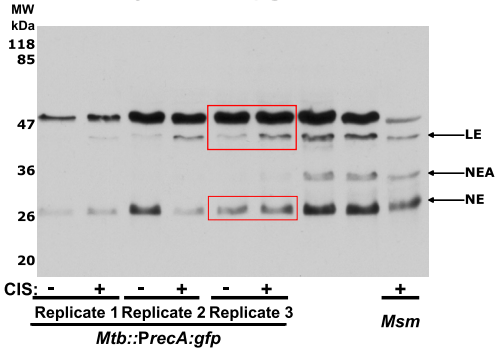

Figure 3C,D
αGFP

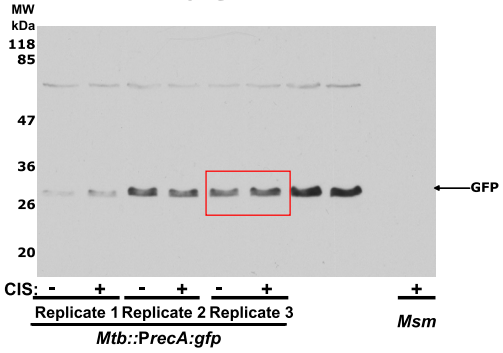

Figure 4A aIntein

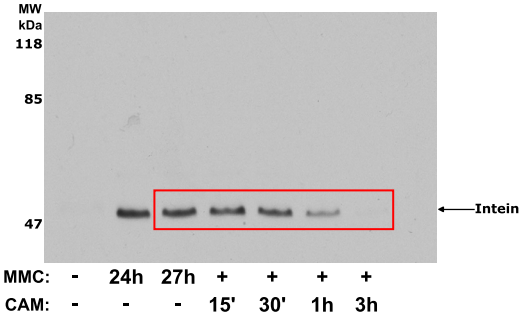

Figure 4A aGFP

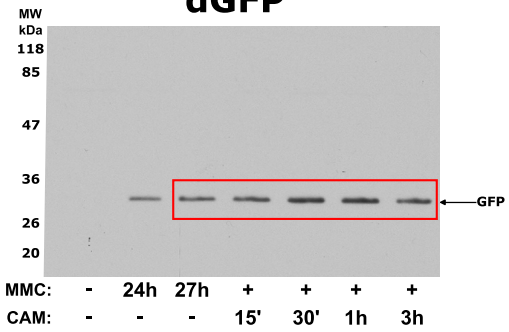

Figure 4B aN-Extein

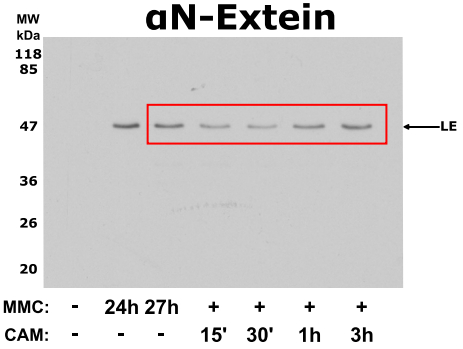

Figure 4B aGFP

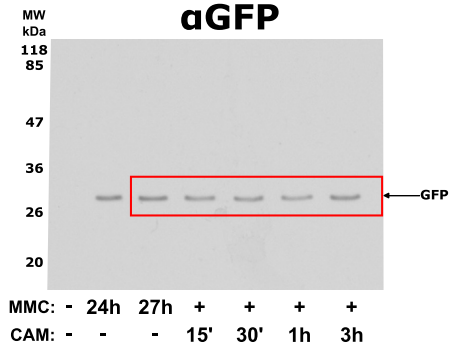

Figure 4D aN-extein

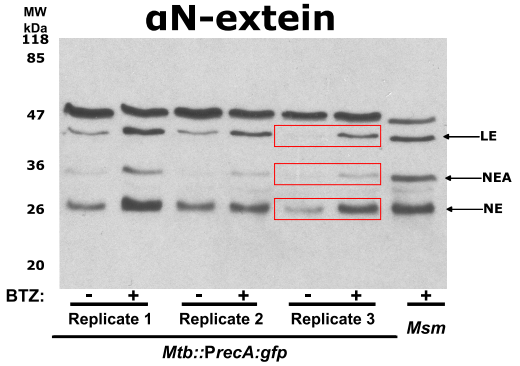

Figure4C aIntein

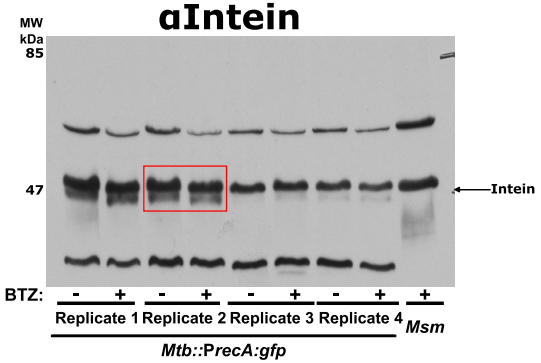

Figure 4C aGFP

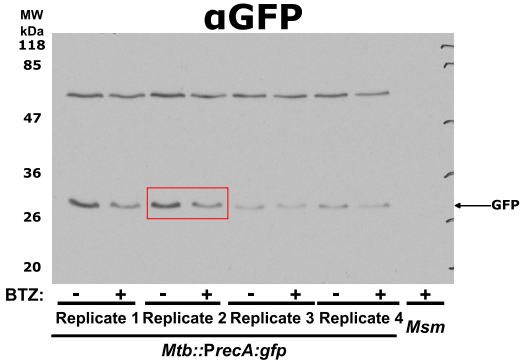

Figure 4D aGFP

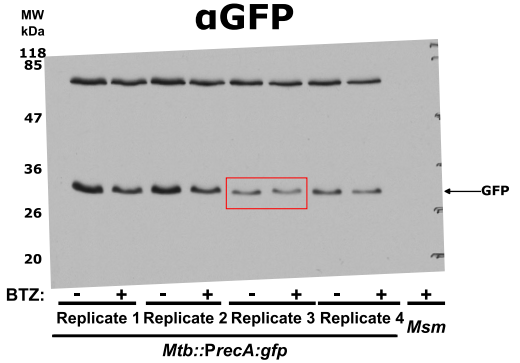

Figure 5D Inset
αLexA

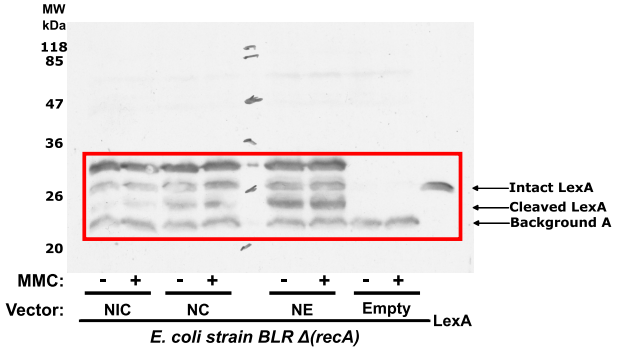

Figure S3B
αN-Extein

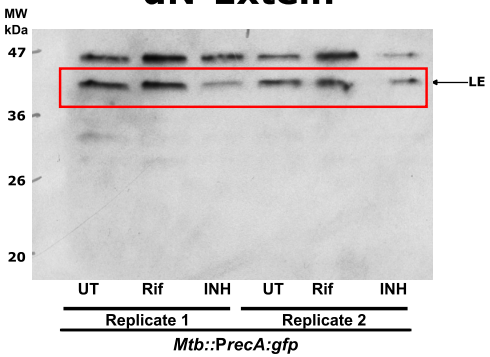

Figure S3B
αGFP

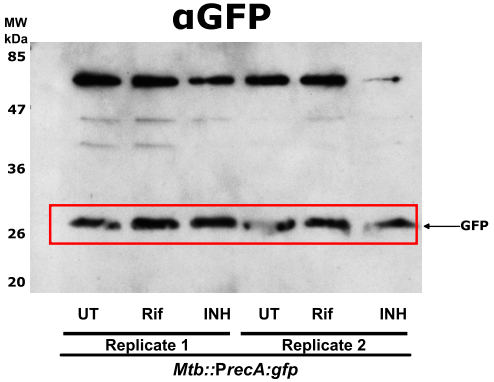

Figure S4A
αN-Extein

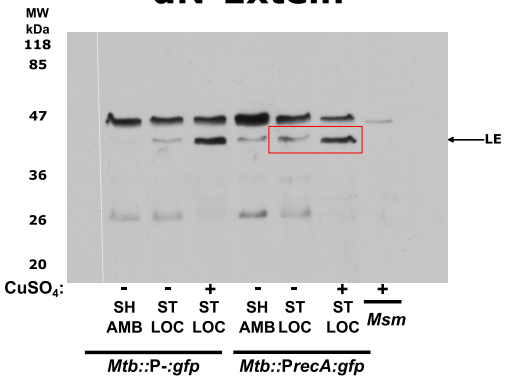

Figure S4A
αGFP