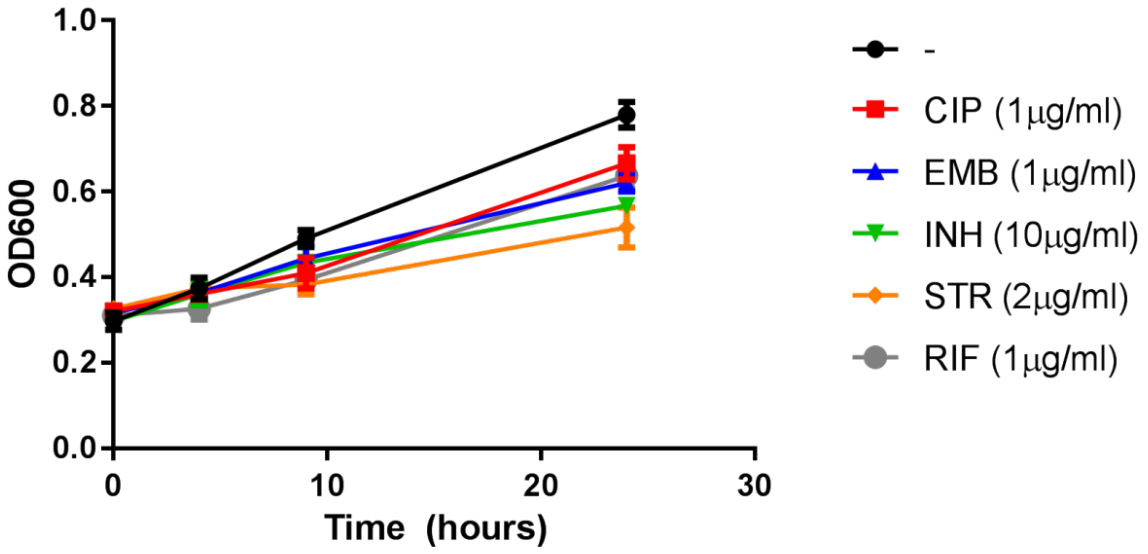

Fig. S1 Cultures stressed with antibiotics show slight growth defect. OD600 values over time of the triplicate of *M. marinum* cultures that were used as basis for RNA isolation. The standard deviation is indicated by the error bars. The control without antibiotic is marked - (black).

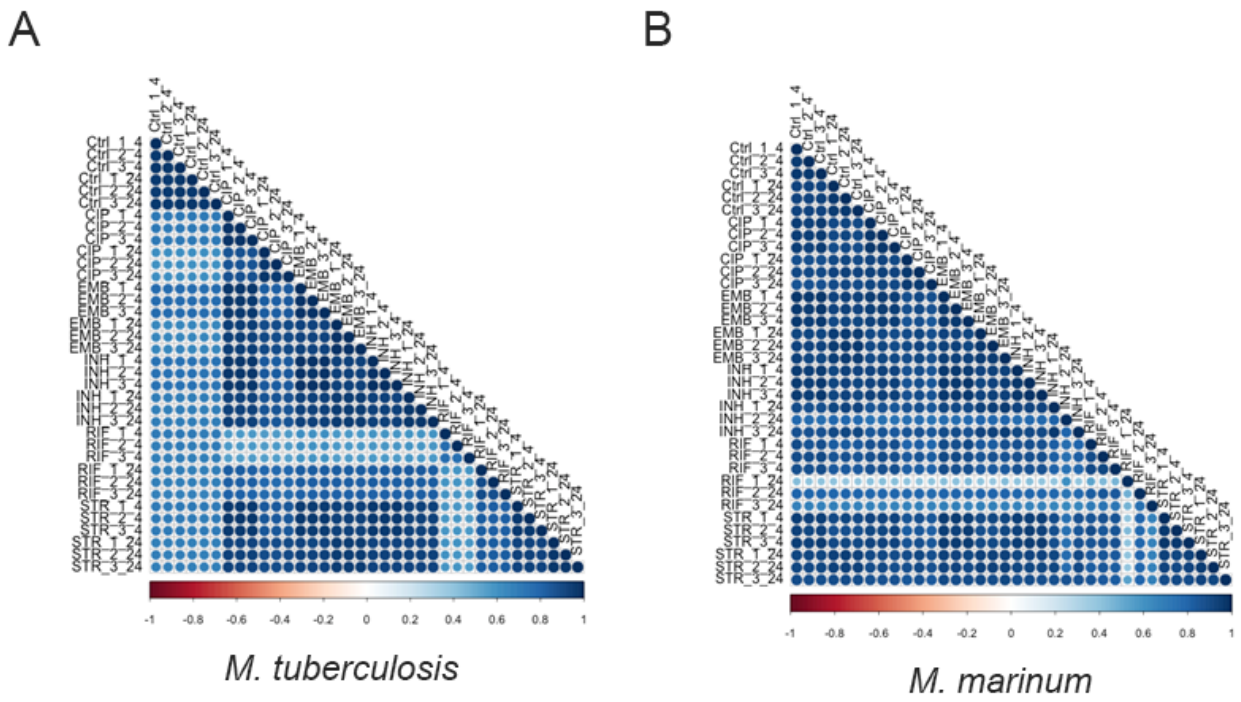

Fig. S2 The RNA sequencing results are highly reproducible. The reproducibility of the data for the triplicate of RNA samples is indicated for both A) *M. tuberculosis* and B) *M. marinum*. Correlation between the samples is indicated by the colored scale bar. A positive correlation is visualized by a dark blue color.

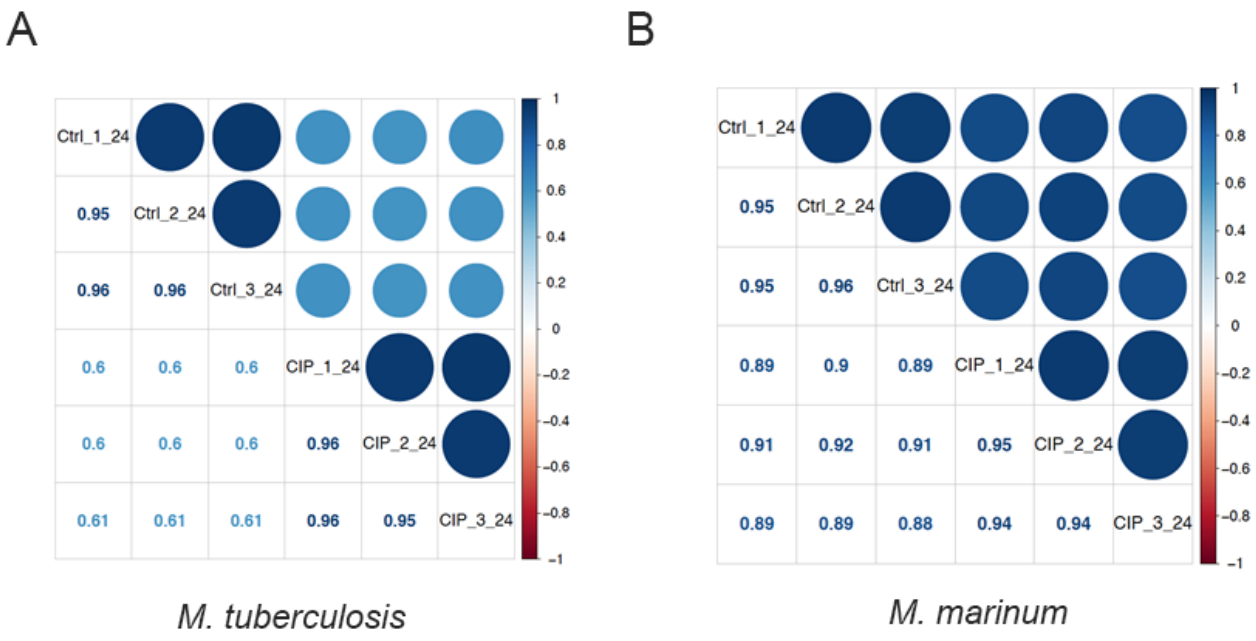

Fig.. S3 The transcriptional responses for ciprofloxacin are reproducible. The reproducibility of the data for the triplicate samples for ciprofloxacin for both A) *M. tuberculosis* and B) *M. marinum.* A positive correlation is visualized by a blue color. A scale bar indicating the correlation values is placed next to the figure. Both control (CTRL) as well as ciprofloxacin (CIP) samples are included.

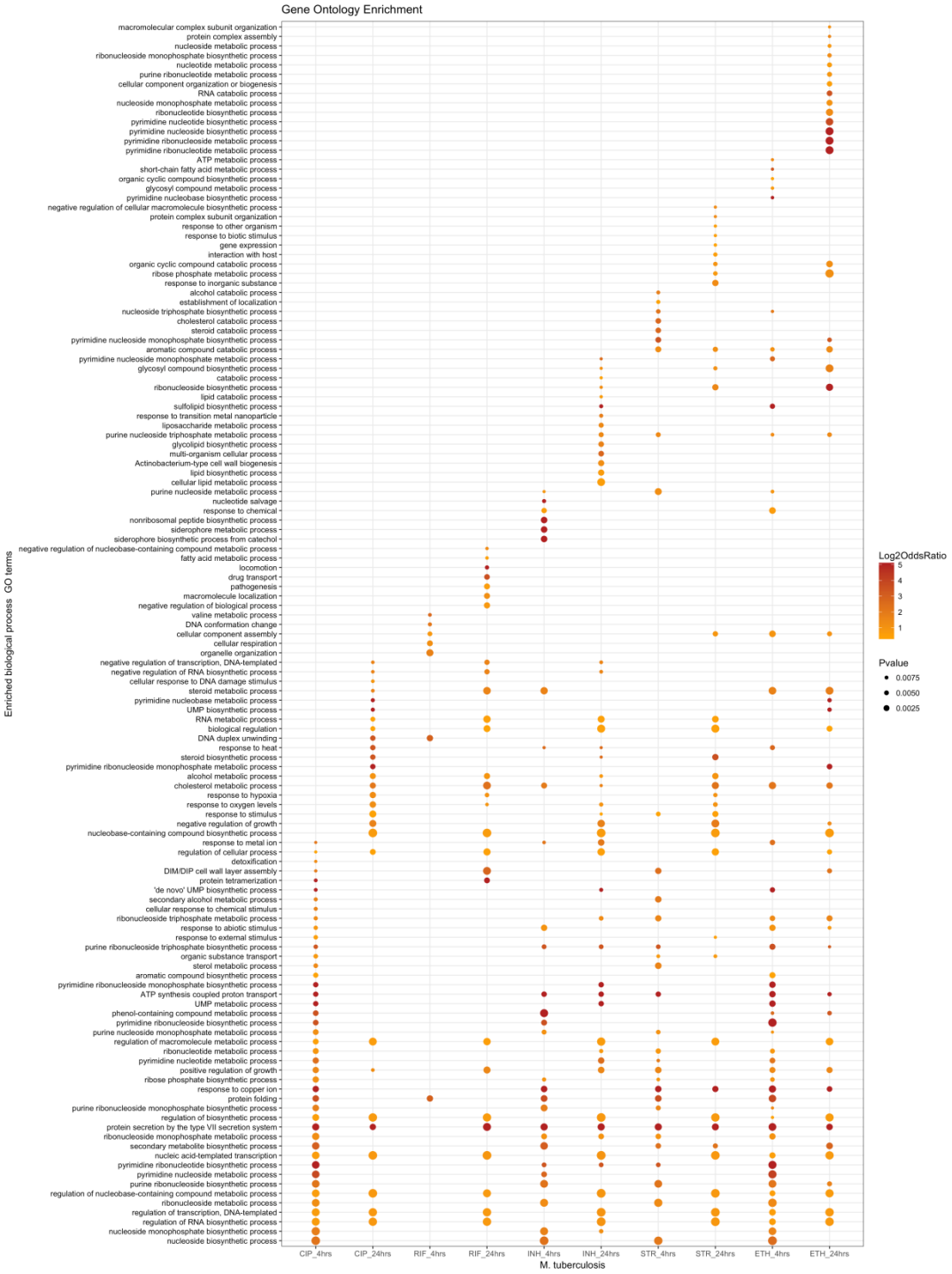

Fig. S4 *M. tuberculosis* does not show a specific stress fingerprint for each type of antibiotic. The stress fingerprint of *M. tuberculosis* visualized per antibiotic for selected GO terms. The type of gene product that a gene encodes determines the GO-term. The size of the dot indicates the p-value.

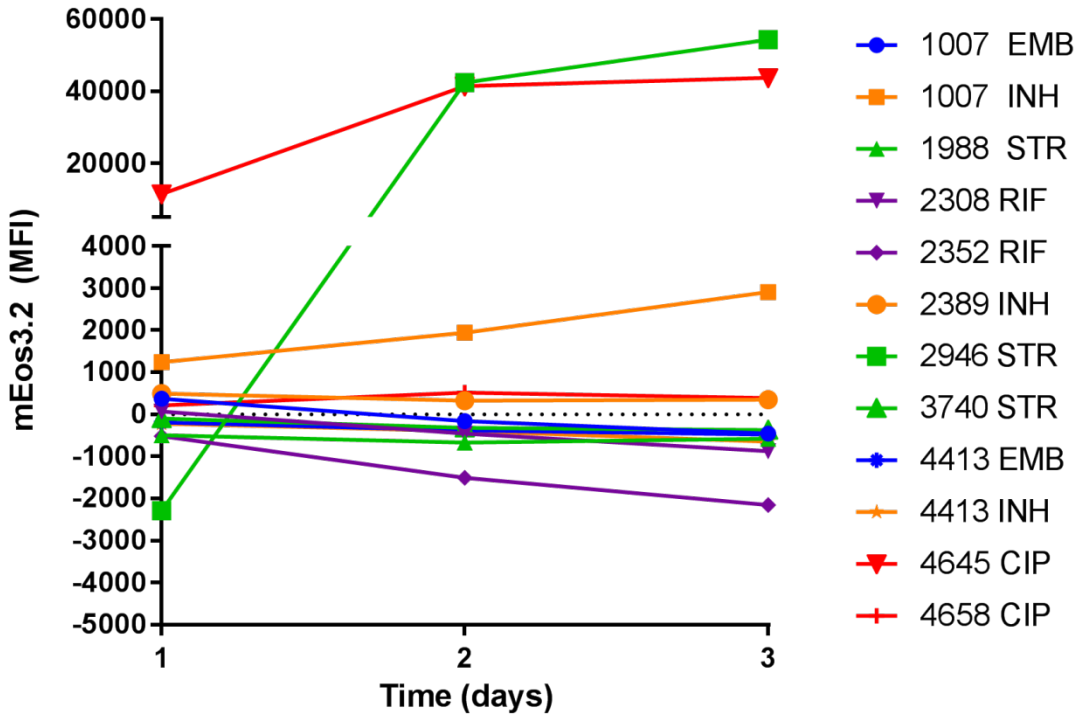

Fig. S5 Fluorescence responses of the ten stress reporter strains in *M. marinum*. Ten stress reporters were monitored over the course of three days. The mean fluorescence intensity (MFI) of the treated stress reporters was corrected by deducting the corresponding untreated stress reporter induction levels. Mean fluorescence intensity (mEos3.2 levels) is indicated on the Y-axis. The numbers indicate the gene number of the promoter used, the colors indicate the type of antibiotic: ciprofloxacin (red), ethambutol (blue), isoniazid (orange), streptomycin (green) and rifampicin (purple).

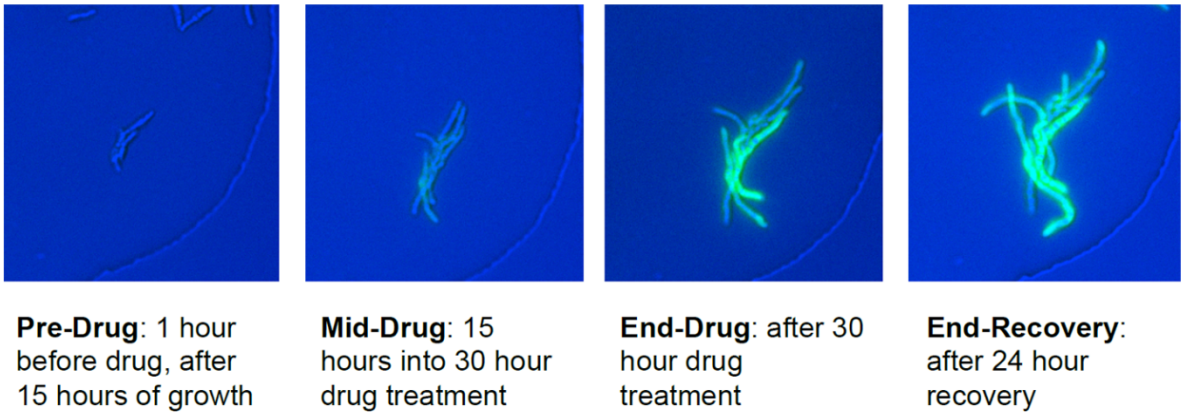

Fig. S6 Overview of the different phases of the time-lapse experiment of the CIP-rep strain. There is a clear induction of CIP-rep during the time-lapse experiment. The still images were obtained from supplemental movie 1. The panels shown indicate the stage of the time-lapse experiment. Over time there is a strong increase in mEos3.2 positivity.

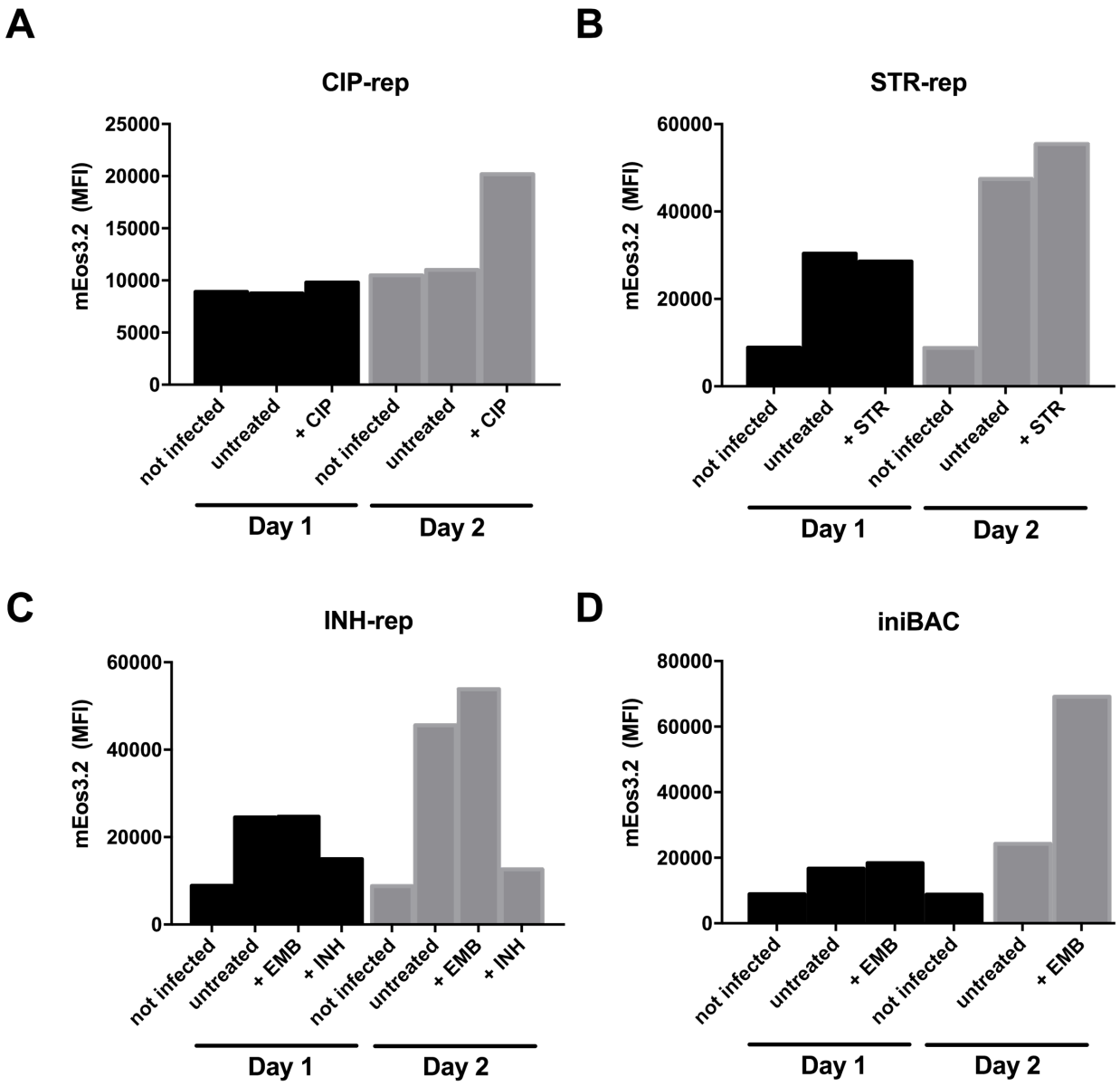

Fig. S7 The CIP-rep is a functional stress reporter in RAW macrophages. Mean fluorescence intensities of stress reporters was analyzed in a RAW macrophage cell infection model over two days of infection. Bars represent 1 representative measurement of 30,000 cells per time point. (A) RAW cells infected with the CIP-rep strain tested with 1x MIC (1µg/ml) ciprofloxacin (CIP). (B) RAW cells infected with the STR-rep strain were treated with 1x MIC (2µg/ml) streptomycin (STR) (C) RAW cells infected with the INH-rep strain were treated with 1x MIC (1µg/ml) ethambutol (EMB) and 1x MIC (10 µg/ml) isoniazid (INH) and (D) the previously published *iniBAC* reporter was used as a positive control for induction with EMB (15).

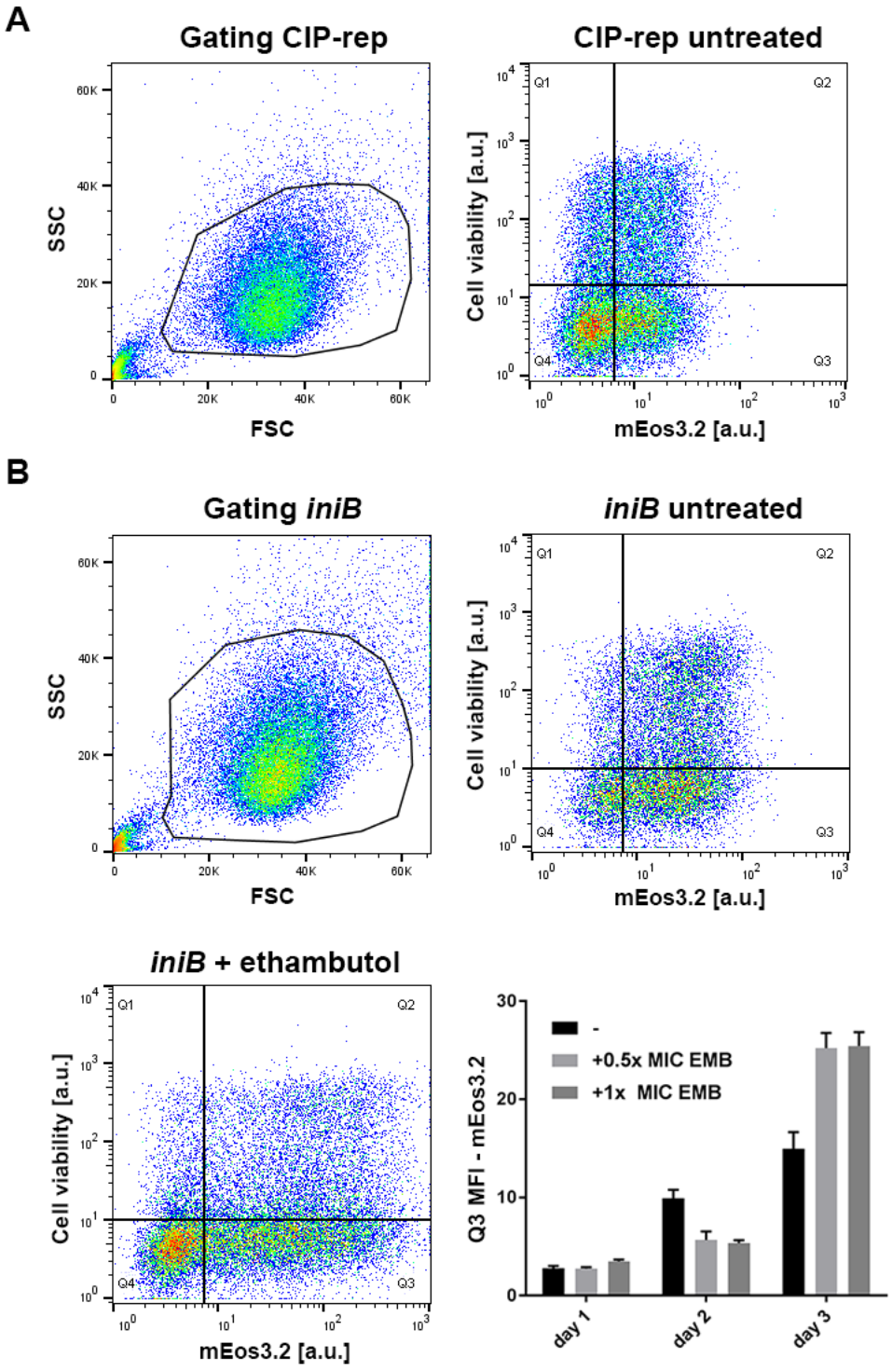
A
Gating CIP-rep
SSC
FSC
CIP-rep untreated
Cell viability [a.u.]
mEos3.2 [a.u.]
Q1
Q2
Q3
Q4
B
Gating iniB
iniB untreated
iniB + ethambutol
Q3 MFI - mEos3.2
-
+0.5x MIC EMB
+1x MIC EMB
day 1
day 2
day 3

Fig. S8 The gating strategy for *MMAR_4645* and quantification of the *iniB* control. All cells were stained with a viability marker (on Y-axis in a.u.). (A) The left panel shows the gate used to select for the RAW macrophages that were included for analysis in the right panel. In this panel quadrants were drawn for the sample labeled *4645* untreated to distinguish cells by viability (Y-axis) and fluorescence (X-axis). In total, Q3 counted 6590 events. (B) The gating strategy for the *iniB* controls as well as plots that shows the fluorescence in induction comparing *iniB* untreated (Q3: 6121 events) to *iniB* + ethambutol (Q3: 9515 events) day 3 post infection and post treatment. A quantification of the data is also provided in the graph, clearly showing that *iniB* is induced over time upon treatment with both 0.5x MIC (0.5 μg/ml) and 1x MIC EMB (1 μg/ml). Error bars represent the s.d. Each bar represents three independent samples.

Table S1 All primers used in this study

Primer name	Sequence 5' > 3'	Amplicon (bp)
Mmar_2352FW	TGGCGGCCGCTCTAGA*GGCGCCGAGAACTCGAAC*	1577
Mmar_2352RV	GTGATTCCTCGGATCC*ACCGCCGAACCAGAATGC*	
Mmar_2308FW	TGGCGGCCGCTCTAGA*AGCACCAGCGCCAGC*	432
Mmar_2308RV	GTGATTCCTCGGATCC*GGTCAGCCAAGTTACCTGCAAC*	
Mmar_4645FW	TGGCGGCCGCTCTAG*ACCGAGGGGAACAAAAGGCA*	1032
Mmar_4645RV	GTGATTCCTCGGATCC*GAAAGTCTCCTTGCTGTTGGTTTCC*	
Mmar_4658FW	TGGCGGCCGCTCTAG*AGGCTGTCACCATCCCCAC*	282
Mmar_4658RV	GTGATTCCTCGGATCC*GCCGACACGGTGCCT*	
Mmar_2389FW	TGGCGGCCGCTCTAG*AGCGCTGCGCCCTCCA*	382
Mmar_2389RV	GTGATTCCTCGGATCC*TCTCGCGCACGATCCATCC*	
Mmar_1988FW	TGGCGGCCGCTCTAG*AGTGACCGCCGCGCGTG*	282
Mmar_1988RV	GTGATTCCTCGGATCC*CAGATCAGGTTACGTGCCGC*	
Mmar_3740FW	TGGCGGCCGCTCTAG*AGGCCGTGTGCGTACACAC*	282
Mmar_3740RV	GTGATTCCTCGGATCC*GGGCTCCGACTCTGGCAC*	
Mmar_2946FW	TGGCGGCCGCTCTAG*AGTCAGCTGCTGCAGCCT*	332
Mmar_2946RV	GTGATTCCTCGGATCC*AATCCCTGCTCCTCAGTTCG*	
Mmar_1178FW	TGGCGGCCGCTCTAG*AGCCGTCGCTGCGGCGG*	332
Mmar_1178RV	GTGATTCCTCGGATCC*GCTTGCTCCCATCGGATTTGG*	
Mmar_5163FW	TGGCGGCCGCTCTAG*AACCGCCAACTCCAGCG*	282
Mmar_5163RV	GTGATTCCTCGGATCC*GGCTTCGAATCAACACCAGGTC*	
Mmar_1007FW	TGGCGGCCGCTCTAG*AAACCGGCGATCGCGAC*	282
Mmar_1007RV	GTGATTCCTCGGATCC*AAGTTTCACTGTACTCTGAAACCTTAGTAGATTT*	
Mmar_4413FW	TGGCGGCCGCTCTAG*AGCCCGCCGTTGCCTCA*	532
Mmar_4413RV	GTGATTCCTCGGATCC*GCTGGCACCGCCTTTG*	

[a] Primer sequences used for cloning of reporter constructs

[b] The promoter specific sequence is italicized

Table S2 List of *M. marinum* stress reporter constructs

Gene	Induced in Mmar by (antibiotic):	Max fold induction compared to NT	H37Rv orthologue	Induced in Mtb by (antibiotic):	Gene product name
MMAR_0614[*]	EMB4+24 INH4+24	91 328.55	*Rv0341*	EMB4+24 INH4+24	IniB
MMAR_1007	INH24	2	*Rv0678*	INH4+24	MmpR5
MMAR_1988	STR4+24 RIF4+24	10.33 4.85	*Rv2725c*	All antibiotics 4+24	HflX
MMAR_2308	RIF4+24	19.15	-	-	MMAR_2308
MMAR_2352	RIF4+24	8.51	*Rv1517*	-	MMAR_2352
MMAR_2389	INH4+24	5.97	*Rv1592c*	-	MMAR_2389
MMAR_2946	STR4+24	11.55	-	-	MMAR_2946
MMAR_3740	STR4+24	39.12	*Rv2416c*	All antibiotics 4+24	Eis
MMAR_4413	EMB4+24 INH4+24	2 5.46	*Rv1057*	EMB4 INH4+24	MMAR_4413
MMAR_4645	CIP4+24	47.83	*Rv0887c*	-	MMAR_4645
MMAR_4658	CIP4+24	93.7	-	-	MMAR_4658

[a] Selected genes for stress reporter construction.

[b] Indicated are the MMAR gene numbers, the H37Rv orthologue (if present), the antibiotic and time point that was found to upregulate the gene in *M. marinum*.

[c] The fold inductions were calculated by dividing the transcript levels of the antibiotic induced sample to the untreated samples.

[d] * indicates a previously constructed reporter (1).

Supplemental data file 1

MMAR_1007

AACCGGCGATCGCGACCACAACCAGAATGAGCAGCGGTATCCACGCACGCTTGAGAATGCCAAT
CATCGTCTTCCCCGAGGAATCTTGCAGATGGGCTTGCCGACCCGAAGCGGTCCGCTTCGATCGC
CTTGCCCGGTGGTGAAGCCTGGAGCGCTCATCGGTGGCTTCCCCTCCTGCGCATGAAGTTGTTG
TAGACCTATCGGGCAGTCTAGATGAAATCTACTAAGGTTTCAGAGTACAGTGAAACTTGTG

MMAR_2946

GTCAGCTGCTGCAGCCTCTCCGGCTTGGCGCCGCGAAGCCCCTTCAAGCGCCTGCCCAGACGCA
CGGCGTCACGGGTGCTCAGACCGTCGAGACGGCTGCGCAGCTGCGCAACGGACAGATCGGCCA
CTCCGGTCAGGATAGGCGGTGCCGCGTTGCCCCTCGATAGCGCGGTCCGCGGATCTGACCGGGC
ACCGAAAATACGTTGCCAGCTACCTGATTTGGAGCCATCATGCTAAGGGCTTGCAGCCGGGCCT
TGGCGAGAGCCGGCGCTTGGGCTGACGAACTGAGGAGCAGGGATTGTG

MMAR_4645

CCGAGGGGAACAAAAGGCAGCAAGAAGGCACTGCCAGTGACGACCGTTGCAGCGACGTTCTGTC
GTGAGTGAACTGATCTTGCCAGCCGGATAGCGCACCAGAAACGCGATCAAAATGTCCATAAGCA
TTGCAAGACAGCACTTATCACTTATTCGCAGCCATCCCGAGTTAGTGCAGTTCAAGGTTTTTCCG
CTGCTGCCCAATGCCTTTCGAATTAGACAATCGGTGAGCGATGCCATCAGCATCCTCGTTTCGCC
CAGGCTTCTGACTTCGGCGCATTTTCGGGTCATCCTATGCACCAACATATCTCGTACATAACGAA
CCTGAACACCAGTCGCTTTCACCCTCTCGGACAACATTCGCATCCGACGCCGGAGCCCGAACAC
CAAAGCGACACCAGTCCGCGATCGTGGATGTTCGGCCACCAAGTGTTGACATATCGCCAGGAAG
TGCTAACGGAGCGCAATCCCCTCGCAAATCGCGTCAAAATCAGCGTGTTTCGTGACGGAATAGT
ACCCGTGTCGCGATCCAATCGTATTCGCGCCGATGCCGAAAAGTGCTCTCGCAGAGATGAAATC
GTAGTCAACGGCGCCGTGTCTAGCGCTTTGGCGTCCACCAGGTCACTTGACGATCACTCGCCCA
TCACGGTCAGACAGTCACGGTGGACGCATCGCAACACCGGCCCGCGGCCGGGAGCCGGCCGCA
GCCAGCAACCGGGGGCGAACCAGGGGCGGTCGGGAACCGAGGCGAACCGCACCGGGCGACGGC
CACCGCCCGTCGGCTCATCCGCGCCTCATCCGCGCCTGTCTGCGCCTGATCTGCGCCTGATCTG
CGCCTGATCCAAGAGTCCCGCACAGGCGACCAGCGCCCGTCCGGCCACAGGCCACCGGGGCTTG
TCGGTGCCCCGCGGTTAAATGACGAACGCAGCGATGAGTTTGTGTCGCGGCTTGGGTCAGTACC
GGTGAGCCCATTCGGGAAACCAACAGCAAGGAGACTTTCATG

Sequence of cloned promoter regions
Putative -10 (in *Mtb* TANNNT)
5' UTR
ATG - Methionine Start Codon

For *MMAR_1007* and *MMAR_4645*, regions were based on data available for *M. tuberculosis* orthologues *rv0678* and *rv0887c* respectively from Cortes *et al*. (2). For *MMAR_2946* these sequences are unknown. *MMAR_1007* does not have an SD sequence (2).

Supplemental movie 1: CIP-rep is strongly induced in response to ciprofloxacin treatment

Supplemental movie 2: *iniBAC* is strongly induced in response to isoniazid treatment

Supplemental references

1. Boot M, Sparrius M, Jim KK, Commandeur S, Speer A, van de Weerd R, Bitter W. 2016. *iniBAC* induction is vitamin B12- and MutAB-dependent in *Mycobacterium marinum*. J Biol Chem, **291:**19800–19812. https://doi.org/10.1074/jbc.M116.724088

2 Cortes T, Schubert OT, Rose G, Arnvig KB, Comas I, Aebersold R, Young DB. Genome-wide mapping of transcriptional start sites defines an extensive leaderless transcriptome in *Mycobacterium tuberculosis*. Cell Rep, **5**:1121-1131. https://doi.org/10.1016/j.celrep.2013.10.031