Supplementary Material

The conserved translation factor LepA is required for optimal synthesis of a porin family in *Mycobacterium smegmatis*

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This PDF contains:
Figure S1 to S3
Table S1 to S5

Other supplementary material for this manuscript includes:
Dataset S1 to S2

Supplementary Figures

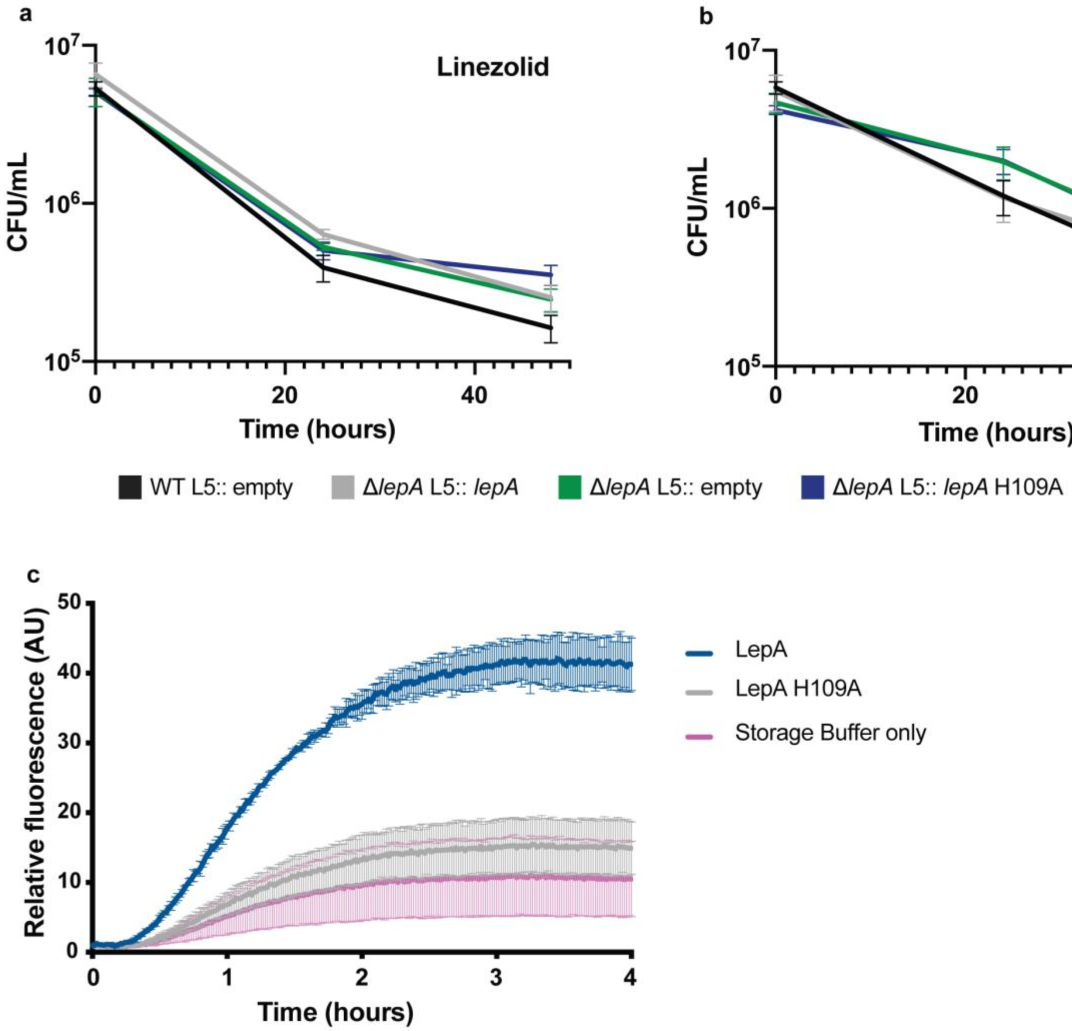

Figure S1. a-b) Msm strains were treated with 10X concentrations of linezolid and isoniazid, and cell survival was measured by colony forming units per mL (CFU/mL) as in Figure 1. Values indicate mean survival with error bars indicating standard deviation of three biological replicates. c) *In vitro* translation was carried out using Venus mRNA with LepA, LepA H109A or storage buffer alone. Curves represent mean relative fluorescence (normalized by fluorescence at t=0) with error bars indicating the range across two technical replicates. Data in a-c) are representative of multiple experiments.

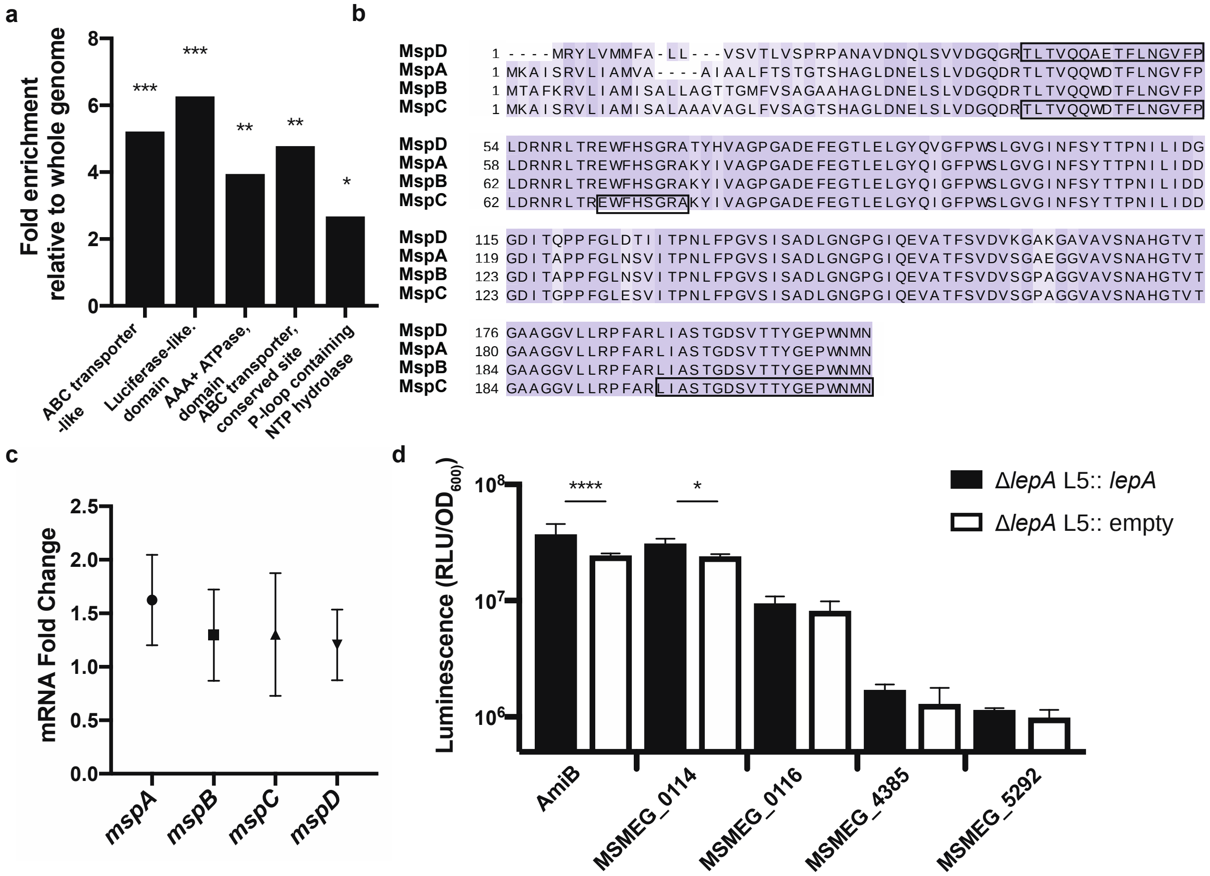

Figure S2. a) Fold-change in enrichment of InterPro functional annotation(1) terms in proteins altered by loss of LepA relative to the total Msm proteome. *** $P<0.001$, ** $P<0.001$, *$P<0.05$, calculated using a modified Fisher's exact test. b) A Clustal Omega alignment of mycobacterial porin family proteins in Msm. Black boxes indicate the 4 unique peptides that mapped to a mycobacterial porin in our proteomics data. Decreasing hue corresponds to decreasing percent identity across the four sequences. c) Changes in porin transcript levels due to LepA, as measured by RT-qPCR. Relative fold change of each mRNA was quantified by normalizing levels of each porin transcript to levels of *sigA* transcript. Points represent the mean of three biological replicates, with error bars depicting standard deviation. Fold changes were not significantly different from 1, as examined by a one-sided Student's t-test. d) Luminescence of candidate reporter fusions in Δ*lepA* deletion mutant and the complemented strain. *** $P<0.001$, * $P<0.05$, computed using a Student's t-test. Data in c-d) are representative of multiple experiments.

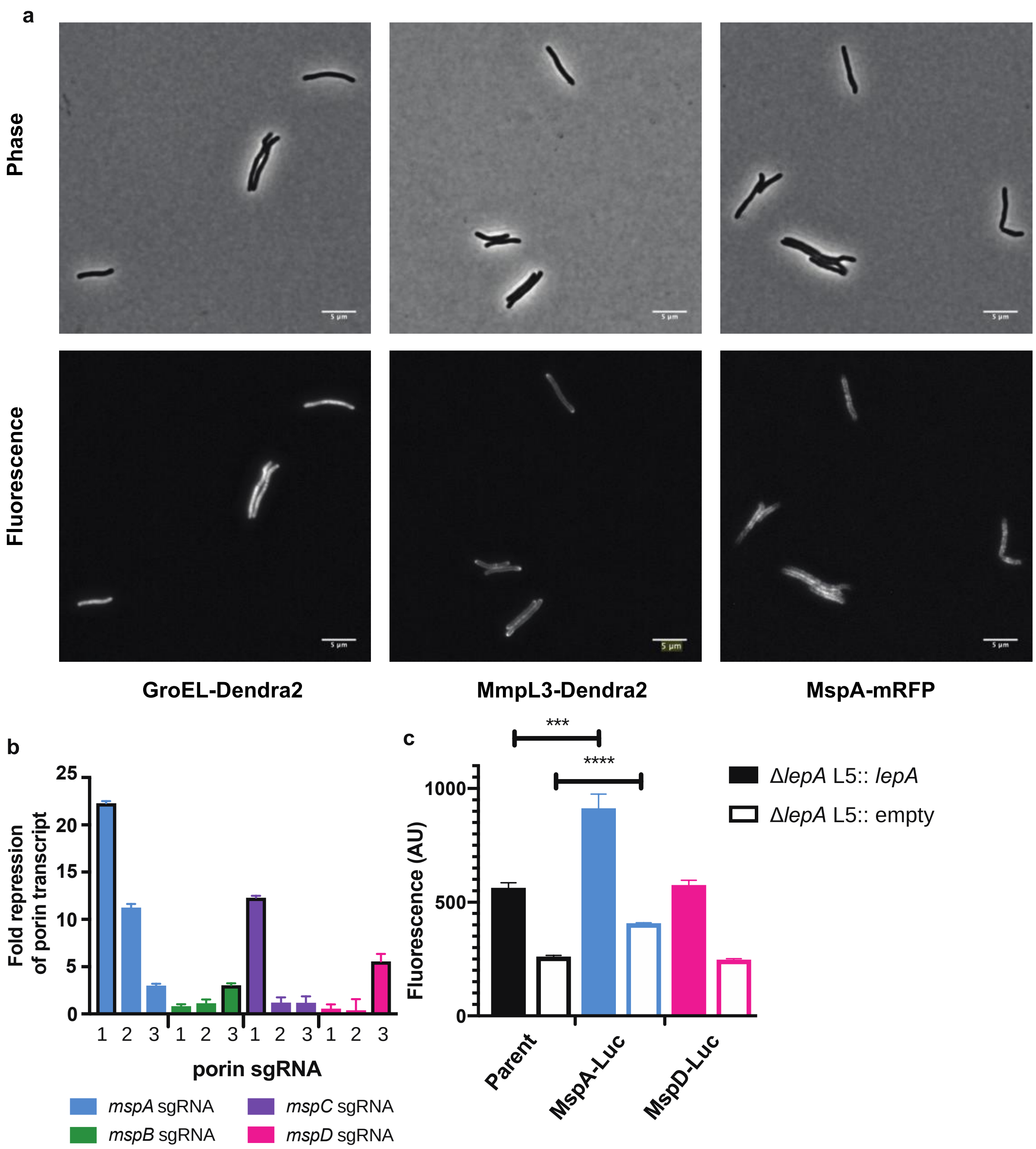

Figure S3. a) Localization of MspA-mRFP in Msm. Scale bar represents 5 microns and applies to all images. Visualization of phase-contrast and fluorescence in GroEL-Dendra2 (left), MmpL3-Dendra2 (middle) and MspA-mRFP expressing strains (right). b) Three sgRNAs with different PAM strengths (2) were chosen to achieve maximal knockdown of each porin. Black lines around a bar indicate selection of that sgRNA for future experiments. Fold repression, quantified using RT-qPCR, indicates the difference in knockdown between induced and uninduced strains. c) Calcein staining of luciferase-porin reporter strains was used to assess porin coding sequence contribution to permeability. ‘Parent’ indicates the complemented strain (filled bar) and Δ*lepA* (open bar), with no merodiploid porin reporter. Each subsequent set of bars indicates derivatives of those two parental strains containing the specified porin reporter used in Figure 2. Bars indicate mean fluorescence with error bars indicating standard deviation around three replicates. ****P<0.0001, ***P<0.001 calculated using a one-sided ANOVA, where complemented strains were compared against one another and Δ*lepA* strains were compared against one another. Data in a-c) are representative of multiple experiments.

Supplementary Tables

Table S1: Minimum inhibitory concentration (MIC) of *lepA* mutant

Drug †	Complemented strain	Deletion mutant
Tetracycline	0.250	0.250
Clarithromycin	0.125	0.125
Chloramphenicol	10	10
Amikacin	0.200	0.200
Erythromycin	0.500	0.500

†Values are averages of three replicates, in μg/ml concentrations of drug.

Table S2: Strain List

Strain Number	Parental Background (Strain number)	Plasmid at L5 Integration Site (Plasmid #)	Plasmid at Tweety Integration Site (Plasmid #)
HR334	Δ*lepA*::ZeoR	None	None
SF177	mc^{2}155	p-empty-KanR(CT242)	None
SF178	Δ*lepA*::ZeoR (HR334)	pP$_{natp}$-*lepA*-KanR(121)	None
SF181	Δ*lepA*::ZeoR (HR334)	p-empty-KanR(CT242)	None
SF290	Δ*lepA*::ZeoR (HR334)	pP$_{natp}$-*lepA*-KanR(121)	pP$_{UV15}$-*mspA*-Nluc-NatR(283)
SF291	Δ*lepA*::ZeoR (HR334)	p-empty-KanR(CT242)	pP$_{UV15}$-*mspA*-Nluc-NatR(283)
SF302	Δ*lepA*::ZeoR (HR334)	pP$_{natp}$-*lepA*-KanR(121)	pP$_{UV15}$-Nluc-NatR(287)
SF303	Δ*lepA*::ZeoR (HR334)	p-empty-KanR(CT242)	pP$_{UV15}$-Nluc-NatR(287)
SF432	Δ*lepA*::ZeoR (HR334)	None	pP$_{natp}$-*lepA*-HygR(417)
SF433	Δ*lepA*::ZeoR (HR334)	None	p-emtpy-HygR(418)
SF434	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA1 (*mspA1*)-KanR(419)	pP$_{natp}$-*lepA*-HygR(417)
SF435	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA2 (*mspA2*)-KanR(420)	pP$_{natp}$-*lepA*-HygR(417)
SF436	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA3 (*mspA3*)-KanR(421)	pP$_{natp}$-*lepA*-HygR(417)
SF437	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA4 (*mspB1*)-KanR(422)	pP$_{natp}$-*lepA*-HygR(417)
SF438	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA5(*mspB2*)-KanR(423)	pP$_{natp}$-*lepA*-HygR(417)
SF439	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA6 (*mspB3)*)-KanR(424)	pP$_{natp}$-*lepA*-HygR(417)
SF440	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA7 (*mspC1*)-KanR(425)	pP$_{natp}$-*lepA*-HygR(417)
SF441	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA8 (*mspC2*)-KanR(426)	pP$_{natp}$-*lepA*-HygR(417)
SF442	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA9 (*mspC3*)-KanR(427)	pP$_{natp}$-*lepA*-HygR(417)
SF443	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA10 (*mspD1*)-KanR(428)	pP$_{natp}$-*lepA*-HygR(417)
SF444	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA11 (*mspD2*)-KanR(429)	pP$_{natp}$-*lepA*-HygR(417)
SF445	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA12 (*mspD3*)-KanR(430)	pP$_{natp}$-*lepA*-HygR(417)
SF388	Δ*lepA*::ZeoR (HR334)	pP$_{natp}$-*lepA*-KanR(SF121)	pP$_{UV15}$-*mspB*(MSMEG_0520)-Nluc-NatR(384)
SF389	Δ*lepA*::ZeoR (HR334)	p-empty-KanR(CT242)	pP$_{UV15}$-*mspB*(MSMEG_0520)-Nluc-NatR(384)
SF390	Δ*lepA*::ZeoR (HR334)	pP$_{natp}$-*lepA*-KanR(121)	pP$_{UV15}$-*mspC*(MSMEG_5483)-Nluc-NatR(383)
SF391	Δ*lepA*::ZeoR (HR334)	p-empty-KanR(CT242)	pP$_{UV15}$-*mspC*(MSMEG_5483)-Nluc-NatR(383)
SF392	Δ*lepA*::ZeoR (HR334)	pP$_{natp}$-*lepA*-KanR(121)	pP$_{UV15}$-*mspD*(MSMEG_6057)-Nluc-NatR(385)
SF393	Δ*lepA*::ZeoR (HR334)	p-empty-KanR(CT242)	pP$_{UV15}$-*mspD*(MSMEG_6057)-Nluc-NatR(385)
SF902	Δ*lepA*::ZeoR (HR334)	pP$_{natp}$-*lepA*-KanR(121)	pP$_{UV15}$-*mspA*-mRFP-NatR(896)
SF903	Δ*lepA*::ZeoR (HR334)	p-empty-KanR(CT242)	pP$_{UV15}$-*mspA*-mRFP-NatR(896)
SF789	Δ*lepA*::ZeoR/Δ*mspA*::HygR	None	None
HR329	Δ*mspA*::ZeoR	None	None
SF447	Δ*lepA*::ZeoR (HR334)	pP$_{UV15\text{-}Tet}$-*dCas9*-sgRNA1 (*mspA1*)-KanR(419)	p-emtpy-HygR(418)

SF448	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA2 (*mspA2*)-KanR(420)	p-emtpy-HygR(418)
SF449	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA3 (*mspA3*)-KanR(421)	p-emtpy-HygR(418)
SF450	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA4 (*mspB1*)-KanR(422)	p-emtpy-HygR(418)
SF451	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA5(*mspB2*)-KanR(423)	p-emtpy-HygR(418)
SF452	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA6 (*mspB3)*)-KanR(424)	p-emtpy-HygR(418)
SF453	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA7 (*mspC1*)-KanR(425)	p-emtpy-HygR(418)
SF454	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA8 (*mspC2*)-KanR(426)	p-emtpy-HygR(418)
SF455	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA9 (*mspC3*)-KanR(427)	p-emtpy-HygR(418)
SF456	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA10 (*mspD1*)-KanR(428)	p-emtpy-HygR(418)
SF457	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA11 (*mspD2*)-KanR(429)	p-emtpy-HygR(418)
SF458	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA12 (*mspD3*)-KanR(430)	p-emtpy-HygR(418)
SF502	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA null-KanR(CT295)	p-natp-*lepA*-HygR(417)
SF503	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA null-KanR(CT295)	p-emtpy-HygR(418)
SF504	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA1-sgRNA6-sgRNA7-KanR(484)	p-natp-*lepA*-HygR(417)
SF505	Δ*lepA*::ZeoR (HR334)	pP$_{\text{UV15-Tet}}$-*dCas9*-sgRNA1-sgRNA6-sgRNA7-KanR(484)	p-emtpy-HygR(418)
SF740	BL21 *E.coli*/pET26-P$_{\text{T7}}$-6xHisTag-*lepA*-KanR	None	None
SF1037	Δ*lepA*::ZeoR (HR334)	pPnatp-lepA H109A-KanR(1036)	none
SF1038	Δ*lepA*::ZeoR (HR334)	pPnatp-lepA H109A-KanR(1036)	pP$_{\text{UV15-Tet}}$-*mspA*-Nluc-NatR(283)
SF1039	Δ*lepA*::ZeoR (HR334)	pPnatp-lepA H109A-KanR(1036)	pP$_{\text{UV15-Tet}}$-*mspB*(MSMEG_0520)-Nluc-NatR(384)
SF1040	Δ*lepA*::ZeoR (HR334)	pPnatp-lepA H109A-KanR(1036)	pP$_{\text{UV15-Tet}}$-*mspC*(MSMEG_5483)-Nluc-NatR(383)
SF1041	Δ*lepA*::ZeoR (HR334)	pPnatp-lepA H109A-KanR(1036)	pP$_{\text{UV15-Tet}}$-*mspA* recode (AT-rich)-Nluc-NatR(555)
SF1042	Δ*lepA*::ZeoR (HR334)	pPnatp-lepA H109A-KanR(1036)	pP$_{\text{UV15-Tet}}$-*mspA* recode (AT-rich)-Nluc-NatR(555)
SF583	Δ*lepA*::ZeoR (HR334)	pPnatp-lepA H109A-KanR(1036)	pP$_{\text{UV15-Tet}}$-*mspA* recode (AT-rich)-Nluc-NatR(555)
WT	mc^{2}155	None	None
MmpL3-Dendra*	WT	pP$_{\text{myc1-tetO}}$-MSMEG_0250-Dendra-ApR	None
GroEL-Dendra*	WT	pP$_{\text{myc1-tetO}}$-MSMEG_0880-Dendra-ApR	None
FT150	BL21 *E.coli*/pET26-P$_{\text{T7}}$-6xHisTag-*lepA*H109A-KanR	None	None

*****These strains were a kind gift from the Mycobacterial Systems Resource (PI, K. Derbshire)

Table S3: Plasmid List

Number	Contents	Selection	Parental Vector	Notes
121	pP$_{natp}$-*lepA*-KanR	Kan	CT94	
283	pP$_{rpsA}$-*mspA*(MSMEG_0965)-Nanoluciferase(Nluc)-NatR	Nat	CT250	
287	pP$_{rpsA}$-Nluc-NatR	Nat	CT250	
383	pP$_{rpsA}$-*mspC*(MSMEG_5483)-Nluc-NatR	Nat	CT250	
384	pP$_{rpsA}$-*mspB*(MSMEG_0520)-Nluc-NatR	Nat	CT250	
385	pP$_{rpsA}$-*mspD*(MSMEG_6057)-Nluc-NatR	Nat	CT250	
417	pP$_{natp}$-*lepA*-HygR	Hyg	CT204	
418	p-emtpy-HygR	Hyg	CT204	
419	pP$_{rpsA}$-Tet-dCas9-sgRNA1 (*mspA*1)-KanR	Kan	CT295	
420	pP$_{rpsA}$-Tet-dCas9-sgRNA2 (*mspA*2)-KanR	Kan	CT295	
421	pP$_{rpsA}$-Tet-dCas9-sgRNA3 (*mspA*3)-KanR	Kan	CT295	
422	pP$_{rpsA}$-Tet-dCas9-sgRNA4 (*mspB*1)-KanR	Kan	CT295	
423	pP$_{rpsA}$-Tet-dCas9-sgRNA5(*mspB*2)-KanR	Kan	CT295	
424	pP$_{rpsA}$-Tet-dCas9-sgRNA6 (*mspB*3)-KanR	Kan	CT295	
425	pP$_{rpsA}$-Tet-dCas9-sgRNA7 (*mspC*1)-KanR	Kan	CT295	
426	pP$_{rpsA}$-Tet-dCas9-sgRNA8 (*mspC*2)-KanR	Kan	CT295	
427	pP$_{rpsA}$-Tet-dCas9-sgRNA9 (*mspC*3)-KanR	Kan	CT295	
428	pP$_{rpsA}$-Tet-dCas9-sgRNA10 (*mspD*1)-KanR	Kan	CT295	
429	pP$_{rpsA}$-Tet-dCas9-sgRNA11 (*mspD*2)-KanR	Kan	CT295	
430	pP$_{rpsA}$-Tet-dCas9-sgRNA12 (*mspD*3)-KanR	Kan	CT295	
711	pET26-P$_{T7}$-6xHisTag-*lepA*-KanR	Kan	pET26	
741	pET21-P$_{T7}$-Venus-AmpR	Amp	pET21	
896	pP$_{rpsA}$-*mspA*-mRFP-NatR	Nat	CT250	
CT204	pP$_{TB21}$-eGFP-*wag31*-HygR	Hyg	Lab vector	Backbone of cloning vector for Tweety-integrating *lepA* alleles
CT242	p-empty-KanR	Kan	CT94	
CT250	pP$_{rpsA}$-eGFP-NatR	Nat	Lab vector	Backbone of cloning vector for Tweety-integrating porin alleles
CT295	pP$_{rpsA}$-Tet-dCas9-sgRNA null-KanR	Kan	Courteous gift of Jeremy Rock	
CT94	pP$_{rpsA}$-Tet-eGFP-KanR	Kan	Lab vector	Backbone of cloning vector for L5-integrating *lepA* alleles

1036	pP$_{natp}$- *lepA*H109A-KanR	Kan	Lab vector	Backbone of cloning vector for L5-integrating *lepA* alleles
FT150	pET26-P$_{T7}$-6xHisTag-*lepA*H109A-KanR	Kan	Lab vector	

Table S4: Primer List

Number	Name	Sequence	Purpose (vector number or description)
P315	CT133_nat_prom_4556_F	TTTGCGTTTAATACTGCATGCACTgaatcgagcatatgccagcgg	121
P422	4556_R_redo	CTAGGGTCCCCAATTAATTAGCTAAtcacttcttgggcttgtcgccc	121
P626	UV15-mspA_F	cttaattaagaaggagatatacatatgaaggcaatcagtcgggtgctg	283;896
P627	mspA-Nluc_R	ggctcacgtctgtTCCAGAACCgttcatgttccagggttcgccg	283;944
P628	mspA-Nluc_F	aaccctggaacatgaacGGTTCTGGAacagacgtgagccgaaagattcg	283
P629	Nluc-Ct250-R	GGGTCCCCAATTAATTAGCTAATCACAGGTCTTCCTCGCTGATCAGCTTCTGCTCggcgaggatgcgctcgc	283;287;383;384;385
P632	UV15-Nluc_F	gcttaattaagaaggagatatacatatgacagacgtgagccgaaagattcg	287
P844	Nluc-F	gtcttcaccctggaggacttcgtcg	383;384;385
P772	UV15_MspC_F	cttaattaagaaggagatatacatatgaaggcaatcagtcgggtgctg	384
P773	MspC_Nluc_R	cctccagggtgaagacTCCAGAACCgttcatgttccagggttcgccg	384
P774	UV15_MspB_F	ttaattaagaaggagatatacatatgacggcattcaagcgggtgct	383
P775	MspB_Nluc_R	cctccagggtgaagacTCCAGAACCgttcatattccaaggctcgccgta	383
P776	UV15_MspD_F	ttaattaagaaggagatatacatgtgcgctacctcgtcatgatg	385
P777	MspD_Nluc_R	tccagggtgaagacTCCAGAACCgttcatgttccagggctcgcc	385
P842	CT204_lepA_F	actgtttaaactctagaaatattattgcgggtgcgttgttcgc	417
P843	CT204_lepA_R	tcgtcgccaccaatccccatatgTCGTACGCTAGTTAACTACGTCGACA	417
P807	mspA_R	AAACAATGGTGTGTTCCCCCTGGAC	419 (Figure 3a,Supp. Figure 2d#1)
P808	mspA_F	GGGAGTCCAGGGGGAACACACCATT	419 (Figure 3a,Supp. Figure 2d #1)
P826	MspA_R2	AAACGTCGACGTCTCCGGCGCCGAGGGT	420(Supp. Figure 2d #2)
P827	MspA_F2	GGGAACCCTCGGCGCCGGAGACGTCGAC	420(Supp. Figure 2d #2)
P828	MspA_R3	AAACCTGGACCGCAACCGTCTTACCCGT	421(Supp. Figure 2d #3)
P829	MspA_F3	GGGAACGGGTAAGACGGTTGCGGTCCAG	421(Supp. Figure 2d #3)
P811	mspB_R	AAACTGGTCGCTGGGTGTGGGCATC	422(Supp. Figure 2d #1)
P812	mpsB_F	GGGAGATGCCCACACCCAGCGACCA	422(Supp. Figure 2d #1)

P830	MspB_R2	AAACGTCGACGTCTCGGGTCCTGC	423(Supp. Figure 2d #2)
P831	MspB_F2	GGGAGCAGGACCCGAGACGTCGAC	423(Supp. Figure 2d #2)
P832	MspB_R3	AAACGTTGCTCGCAGGCACCACGGGAAT	424(Figure 3a,Supp. Figure 2d #3)
P833	MspB_F3	GGGAATTCCCGTGGTGCCTGCGAGCAAC	424(Figure 3a,Supp. Figure 2d #3)
P813	mspC_R	AAACGTTGGCTGCGGCCGTCGCGGGGT	425(Figure 3a, Supp. Figure 2d #1)
P814	mspC_F	GGGAACCCCGCGACGGCCGCAGCCAAC	425(Figure 3a, Supp. Figure 2d #1)
P834	MspC_R2	AAACGTCGACGTCTCGGGTCCCGCAGGC	426(Supp. Figure 2d #2)
P835	MspC_F2	GGGAGCCTGCGGGACCCGAGACGTCGAC	426(Supp. Figure 2d #2)
P836	MspC_R3	AAACTCGACGACGGTGACATCACCGGT	427(Supp. Figure 2d #3)
P837	MspC_F3	GGGAACCGGTGATGTCACCGTCGTCGA	427(Supp. Figure 2d #3)
P809	mspD_R	AAACTGGTCATTGGGCGTCGGCATC	428(Supp. Figure 2d #1)
P810	mspD_F	GGGAGATGCCGACGCCCAATGACCA	428(Supp. Figure 2d #1)
P838	MspD_R2	AAACGTGGACGTGAAGGGCGCGAAAGGAGC	429(Supp. Figure 2d #2)
P839	MspD_F2	GGGAGCTCCTTTCGCGCCCTTCACGTCCAC	429(Supp. Figure 2d #2)
P840	MspD_R3	AAACTCGACGGAGGCGACATCACC	430(Supp. Figure 2d #3)
P841	MspD_F3	GGGAGGTGATGTCGCCTCCGTCGA	430(Supp. Figure 2d #3)
P1139	MspA_mRFP_R	CTTGATGACGTCCTCGGAGGAGGCGTTCATGTTCCAGGGTTCGCCGT	896
P1144	mRFP_CT250_R	GGTCCCCAATTAATTAGCTAAAGCTTCAGCGGCCCTCGGCGCGCTCGTACT	896
P1143	mRFP_F	GCCTCCTCCGAGGACGTCATCAA	896
P1042	pET26_venus_F	actttaagaaggagatatacatATGGTCTCGAAGGGCGAGGAGCTG	741
P1043	pET26_venus_R	gtggtggtggtggtgctcgatcaGTGATGGTGATGGTGATGACACTTGTACAGC	741
P845	mspA_F1_RT	CAGATGTGATCCTCTTAGATCTCC	RT-qPCR of porins
P846	mspA_R1_RT	CTCAGGCCCACCTGTTT	RT-qPCR of porins
P847	mspA_F2_RT	TAGATCTCCGAAGTCTCTGAA	RT-qPCR of porins
P848	mspA_R2_RT	GTTCTCCCTAACTGTATCGC	RT-qPCR of porins
P849	mspB_F1_RT	ATTCAAGCGGGTGCTGAT	RT-qPCR of porins
P850	mspB_R1_RT	ACGAGGCTCAATTCGTTGT	RT-qPCR of porins
P851	mspB_F2_RT	ACGGGAATGTTCGTGAGTG	RT-qPCR of porins

P852	mspB_R2_RT	GCTGCACGGTCAGAGTT	RT-qPCR of porins
P853	mspC_F1_RT	GAACGTAAGAACAGAGTGTGGAG	RT-qPCR of porins
P854	mspC_R1_RT	CACCGATGTTGTGGTGTAGG	RT-qPCR of porins
P855	mspC_F2_RT	TTTAATTCGCGCCACATGAC	RT-qPCR of porins
P856	mspC_R2_RT	GGGTCCACCGATGTTGT	RT-qPCR of porins
P857	mspD_F1_RT	CGTCATGATGTTCGCTCTACTC	RT-qPCR of porins
P858	mspD_R1_RT	TTGAGGAATGTCTCGGCTTG	RT-qPCR of porins
P859	mspD_F2_RT	CGCTACCTCGTCATGATGTT	RT-qPCR of porins
P860	mspD_R2_RT	AATCAGCTCAGCGTGGTC	RT-qPCR of porins
SF37	RTPCR_sigA_F	gactacaccaagggctacaag	RT-qPCR of porins
SF38	RTPCR_sigA_R	ttgatcacctcgaccatgtg	RT-qPCR of porins
P1017	pET28_LepA_myco_R	agtggtggtggtggtggtgctcgactacttcttgggcttgtcgcccgc	711
P1018	pET28_LepA_myco_F	ttaactttaagaaggagatataccatgggcagcagccatcatcatcatcatcaccgcttgacccacgctcaccag	711
P1018	pET28_LepA_myco_F	ttaactttaagaaggagatataccatgggcagcagccatcatcatcatcatcaccgcttgacccacgctcaccag	711
P1236	lepA_H109A_F	GTGCTGCACCTGATCGACACCCCGGGCGCCGTCGACTTCACCTACGAGGTGTCGC	GTPase mutant (1036)
P1237	lepA_H109A_R	GCGACACCTCGTAGGTGAAGTCGACGGCGCCCGGGGTGTCGATCAGGTGCAGCAC	GTPase mutant (1036)

Note: underlined basepairs indicate sequence-specific region of sgRNA

Table S5: Recombinant DNA

mRFP gene
GCCTCCTCCGAGGACGTCATCAAGGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCCAGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGC
Nanoluciferase gene
ATGGTCTTCACCCTGGAGGACTTCGTCGGCGACTGGCGTCAGACCGCAGGCTACAACTTGGACCAGGTGCTGGAACAGGGGGGCGTGTCCTCGCTCTTCCAGAACCTCGGCGTGTCCGTGACCCCGATCCAGCGGATCGTCTTGTCGGGCGAAAACGGACTGAAGATCGACATCCACGTCATCATCCCATACGAAGGCCTGAGCGGTGACCAGATGGGCCAGATCGAAAAGATCTTCAAGGTCGTCTACCCCGTTGATGACCACCACTTCAAGGTGATCCTGCACTACGGTACTCTGGTCATCGACGGCGTCACCCCAAACATGATCGACTACTTCGGGCGTCCTTACGAAGGCATCGCCGTGTTCGACGGCAAGAAAATCACCGTCACCGGCACGTTGTGGAACGGCAACAAGATCATCGACGAGCGCCTCATCAACCCCGACGGATCGCTGCTGTTCCGGGTCACCATCAACGGCGTCACCGGTTGGCGCTTGTGCGAGCGCATCCTCGCCTGA

Additional dataset S1 (separate file)

Quantitative proteomics of wildtype, *lepA* deletion mutant, and complemented strain

Additional dataset S2 (separate file)

RNA-sequencing data for wildtype, *lepA* deletion mutant, and complemented strain

Supplementary Material References

1. Huang da W, Sherman BT, Lempicki RA. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44-57.
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