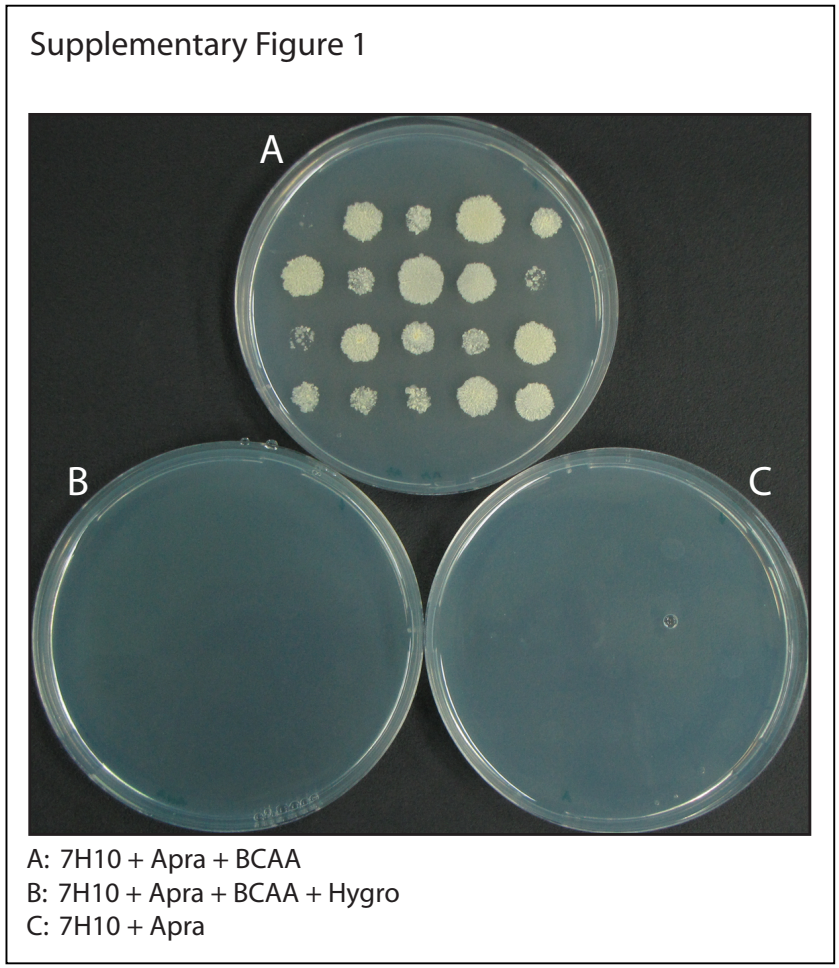
Supplementary Figure 1: Replica plating for confirmation of mc^{2}Δ*ilvH* mutants. Twenty hygromycin sensitive colonies as observed in Figure 4D during replica plating were spotted in replicates on 7H10 agar containing i) only apramycin, ii) apramycin with BCAA (branched chain amino acids) and iii) apramycin with BCAA and hygromycin.



Supplementary Table 1: Bacterial strains and plasmids used in this study

	ANNOTATION	SOURCE
Bacterial Strains		
E. coli DH5α	*Escherichia coli* strain used for cloning	Invitrogen
E. coli DH10B	*Escherichia coli* strain K12 substrain DH10B	Invitrogen
M. smegmatis mc^2155	A mutant of *M. smegmatis* mc^26 which can be efficiently transformed by plasmids	(1)
Vectors		
pENTR/D-TOPO®	*E. coli* cloning vector, pENTR/ Directional TOPO Cloning Kit	Invitrogen
pMV261apra	*E. coli*-mycobacteria shuttle plasmid, source of ***apr^r*** gene cassette	Kind gift from Prof. Jacobs, WR.
pVV16	Shuttle plasmid, Source of ***kan^r***, ***hyg^r***, ***oriE, oriM*** and transcription terminator (***Tt***) cassettes	(TBVRM, Colorado)
pMV361	L5 integration vector, Source of ***int-attP***	(2)
pMC1s	Shuttle plasmid, Source of constitutive $\mathbf{P_{smyc}}$ promoter	(3)
pSE100	Shuttle plasmid, Source of $\mathbf{P_{myc1}}$***tetO*** promoter	(4)
pMC1m	Shuttle plasmid, Source of $\mathbf{P_{imyc}}$***-tetR***	Kind gift from Dr. Ehrt
p2Nil	Recombination vector (non replicating), Source of $\mathbf{P_{hsp60}}$***-sacB***	(5)
$pTrc_{narK2}$-*ung*	pTrc-narK2 vector containing *ung* ORF	(6)
pST-H	Shuttle vector carrying expanded MCS, for constitutive expression of proteins with N- and C- terminal peptide tags (*hyg^r*)	Present Study
pST-HT	pST-H with P_{imyc}*tetR* cloned in SnaBI site	Present Study
pST-KH	Shuttle vector carrying expanded MCS, for constitutive expression of proteins with N- and C- terminal peptide tags (*hyg^r & kan^r*)	Present Study
pST-KHT	pST-KH with P_{imyc}-*tetR* cloned in SnaBI site	Present Study
pST-K	Shuttle vector carrying expanded MCS, for constitutive expression of proteins with N- and C- terminal peptide tags (*kan^r*)	Present Study
pST-KT	pST-K with P_{imyc}-*tetR* cloned in SnaBI site	Present Study
pST-2K	Dual expression shuttle vector for constitutive co-expression of two proteins controlled by independent promoters	Present Study
pST-Ki	pST-K derivative, *oriM* replaced with $attP\text{-}int_{mod}$	Present Study
pST-KiT	pST-KT derivative, *oriM* replaced with $attP\text{-}int_{mod}$	Present Study
pST-KO	Suicide delivery vector containing P_{hsp60}-*sacB (hyg^r)* and expanded MCS	Present Study
$pST\text{-}K_{narK2}$	pST-K shuttle vector with hypoxia inducible P_{narK2} promoter	Present Study

Plasmid Constructs		
pENTR-*oriE*	pENTR construct, *E. coli* origin of replication	Present Study
pENTR-*oriM*	pENTR construct, pAL5000 based mycobacterial origin of replication	Present Study
pENTR-hyg^{r}_{mod}	pENTR construct, modified hygromycin resistance gene	Present Study
pENTR-*EMH*	pENTR construct, containing *oriE*, *oriM*, hyg^{r}_{mod}	Present Study
pENTR-*ESH*	pENTR-*EMH* derivative, *oriM* is replaced with P_{hsp60}-*sacB*	Present Study
pENTR-$EMHP_{smyc}$	pENTR construct, *oriE*, *oriM*, hyg^{r}, P_{smyc} promoter	Present Study
pENTR-$EMHP_{myc1}tetO$	pENTR construct, *oriE*, *oriM*, hyg^{r}, $P_{myc1}tetO$ promoter	Present Study
p$EMHP_{myc1}tetO$	pENTR sequence excised out of pENTR-$EMHP_{myc1}tetO$	Present Study
pST-K-*pknK*	*M. tuberculosis pknK* cloned into XbaI-HindIII sites of the pST-K vector	Present Study
pST-KT-*pknK*	*M. tuberculosis pknK* cloned in XbaI-HindIII sites of the pST-KT vector	Present Study
pST-Ki-*pknK*	*M. tuberculosis pknK* cloned in XbaI-HindIII sites of the pST-Ki vector	Present Study
pST-KiT-*pknK*	*M. tuberculosis pknK* cloned in XbaI-HindIII sites of the pST-KiT vector	Present Study
pST-2K-*virS*	*M. tuberculosis virS* cloned in HindIII site of second MCS of pST-2K vector	Present Study
pST-2K-*pknK*-*virS*	*M. tuberculosis* H37Rv *pknK* cloned in XbaI site of first MCS and *virS* cloned in HindIII site of second MCS, respectively.	Present Study
pST-2K-*pknK*-K55M-*virS*	*M. tuberculosis pknK* kinase inactive mutant cloned in XbaI site of first MCS and *virS* cloned in HindIII site of second MCS, respectively.	Present Study
pST-2K-*pknB KD*	*M. tuberculosis pknB* kinase domain cloned between NdeI-NotI sites of first MCS of pST-2K vector	Present Study
pST-2K-*garA*	*M. tuberculosis garA* is cloned in HindIII site of second MCS of pST-2K vector	Present Study
pST-2K-*pknB KD*-*garA*	*M. tuberculosis pknB* kinase domain cloned in NdeI-NotI sites of first MCS and *garA* in HindIII site of second MCS, respectively.	Present Study
pQE-5'-apr-3'-*ilvH*	Apr^{r} sequence cloned in between 5' and 3' Flank sequences of *ilvH* in PstI-NotI sites of pQEII vector	Present Study
pST-KO-5'-apr-3'-*ilvH*	*5'-apr-3'ilvH* cassette excised from pQEII with SmaI-NotI and cloned into corresponding sites in pST-KO	Present Study
pMV-gfp_{aav}	gfp_{aav} cloned in pMV shuttle vector	
pST-K_{narK2}-gfp_{aav}	pST-K shuttle vector with hypoxia inducible P_{narK2} promoter and gfp_{aav} cloned between EcoRI-HindIII sites	Present Study

Supplementary Table 2: List of primers used in the study

Name	Description	Sequence
OriE F (Forward)	*E.coli* ori F - HpaI, SpeI	CACCGTTAACACTAGTTCCACTGAGCGTCAG
OriE R (Reverse)	*E. coli* ori R-SnaBI, MluI	ATGTACGTAACGCGTTGCGCTCGGTCGTTC
OriM F	*M. tuberculosis* ori F- SnaBI	CACCTACGTAGAGCCCACCAGCTCCGTAAG
OriM R	*M. tuberculosis* ori R- KpnI	CCAGGTACCGCCACGGATGCCACCACAAGC
Hyg^r F	Hygromycin resistance F- KpnI	CACCGGTACCTTGATCCGGCATGAGATTATC
Hyg^r R	Hygromycin resistance R- ScaI	ACTAGTACTGATCCGGGGGGCGTCAGGCGC
Hyg^r-EcoRI F	EcoRI site mutagenesis primer F	CTGCGGAACGACCAGGAGTTCTGGGAGCCGCTG
Hyg^r-EcoRI R	EcoRI site mutagenesis primer R	CAGCGGCTCCCAGAACTCCTGGTCGTTCCGCAG
TetR F	Tet repressor- SnaBI	CACCTACGTAAATATTGGATCGTCGCACCGGG
TetR R	Tet repressor- SnaBI	GTATACGTAGCCCATGGTCATTAAGACCCAC
TetR-Bam,Kpn&XbaI F	BamHI, KpnI and XbaI site mutagenesis F	CGACCCTCCTGCCACGGATCGGAGGAATCACTTCGCAATGTCAAGATTAG
TetR-Bam,Kpn&XbaI R	BamHI, KpnI and XbaI site mutagenesis R	CTAATCTTGACATTGCGAAGTGATTCCTCCGATCCGTGGCAGGAGGGTCG
TetR-HindIII F	HindIII site mutagenesis primer F	CGT AAA CTC GCC CAG AAA CTT GGT GTA GAG CAG
TetR-HindIII R	HindIII site mutagenesis primer R	CTGCTCTACACCAAGTTTCTGGGCGAGTTTACG
TetR-ApaI F	ApaI site mutagenesis primer F	GGCATGTAAAAAATAAGCGAGCCCTGCTCGACGCC
TetR-ApaI R	ApaI site mutagenesis primer R	GGCGTCGAGCAGGGCTCGCTTATTTTTTACATGCC
TetR-SnaBI F	SnaBI site mutagenesis primer F	CTGGCAAGATTTTTTGCGTAATAACGCTAAAAG
TetR-SnaBI R	SnaBI site mutagenesis primer R	CTTTTAGCGTTATTACGCAAAAAATCTTGCCAG
TetR-NdeI F	NdeI site mutagenesis primer F	GGCCTTGAATTGATCATCTGCGGATTAGAAAAAC
TetR-NdeI R	NdeI site mutagenesis primer R	GTTTTTCTAATCCGCAGATGATCAATTCAAGGCC
attP-Integrase F	Integrase gene along with attP site F- SnaBI	CACCTACGTATGCGTTGCAACCGCGTATGC
attP-Integrase R	Integrase gene along with attP site R- KpnI/Acc65I	CGAGGTACCTGGTTCTTCGCGTACTGACAAG
Int-BamHI F	BamHI site mutagenesis primer F	CAGCGCAGCGGGAAGATCCAAGCCTCATA
Int-BamHI R	BamHI site mutagenesis primer R	TATGAGGCTTGGATCTTCCCGCTGCGCTG
Int-PstI F	PstI site mutagenesis primer F	TGAGGTACTACGCGTTGCAGACCTACGACA
Int-PstI R	PstI site mutagenesis primer R	TGTCGTAGGTCTGCAACGCGTAGTACCTCA
Int-NdeI F	NdeI site mutagenesis primer F	GTCTGCCGACCACAAATGGGCCGGTCAAGA
Int-NdeI R	NdeI site mutagenesis primer R	TCTTGACCGGCCCATTTGTGGTCGGCAGAC
P_{myc1}*tetO* F	P_{myc1}/P_{smyc} promoter with/without tet operator sequence primer F- PvuII, ScaI	CACCCAGCTGAGTACTAATATTGGATCGTCGGCACCGTCAC
P_{myc1}*tetO* R	P_{myc1}/P_{smyc} promoter with/without tet operator sequence primer R- HpaI, ApaI & SphI	GTTAACGGGCCCGCATGCGGATCGTGCTCATTTCGGGCGGCG
Oligomer-A F	RBS, Hexa His-tag, NdeI overhang (oh) DNA oligomer F	CGGAGGAATCACTTCGCAATGCACCACCACCACCACCA
Oligomer-A R	RBS, Hexa His-tag, NdeI oh DNA oligomer R	TATGGTGGTGGTGGTGGTGCATTGCGAAGAGATTCCTCCGCATG
Oligomer-B F	MCS primer containing NdeI, BamHI, EcoRV, XbaI, NotI, PvuII and ApaI sites F	TATGGGATCCGATATCTCTAGAGCGGCCGCACAGCTGGGGCC
Oligomer-B R	MCS primer containing NdeI, BamHI, EcoRV,	CCAGCTGTGCGGCCGCTCTAGAGATATCGGATCCCA

	XbaI, NotI, PvuII and ApaI oh R	
Oligomer-C F	MCS primer containing ApaI oh, EcoRI, PstI, PmlI, PacI, HindIII, stop and HpaI sites F	CGAATTCCTGCAGCACGTGTTAATTAAGCTTTAAGTT
Oligomer-C R	MCS primer containing ApaI oh, EcoRI, PstI, PmlI, PacI, HindIII, stop and HpaI sites F	AACTTAAAGCTTAATTAACACGTGCTGCAGGAATTCGGGCC
Oligomer-D F	FLAG-tag DNA oligomer F	AGCTTGACTACAAGGACGACGACGACAAGTAAA
Oligomer-D R	FLAG-tag DNA oligomer R	AGCTTTTACTTGTCGTCGTCGTCCTTGTAGTCA
Oligomer-E F	Transcription terminator DNA oligomer F	GGCCGCATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATAG
Oligomer-E R	Transcription terminator DNA oligomer R	GGCCCTATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATGC
pST-2K-MCS1 F	MCS1 oligomer containing NdeI oh, BamHI, EcoRV, XbaI, NotI, stop, PvuII and ApaI sites F	TATGGGATCCGATATCTCTAGAGCGGCCGCATAACAGCTGCAGGGGCC
pST-2K-MCS1 R	MCS1 oligomer containing NdeI oh, BamHI, EcoRV, XbaI, NotI, stop, PvuII and ApaI sites R	CCTGCAGCTGTTATGCGGCCGCTCTAGAGATATCGGATCCCA
pST-2K-MCS2 F	MCS2 oligomer containing ApaI oh, RBS, EcoRI, PstI, PmlI, PacI, HindIII, stop, HpaI oh F	CGGAGGAATCACTTCGCAATGGAATTCCTGCAGCACGTGTTAATTAAGCTTTAAGTT
pST-2K-MCS2 R	MCS2 oligomer containing ApaI oh, RBS, EcoRI, PstI, PmlI, PacI, HindIII, stop, HpaI oh R	AACTTAAAGCTTAATTAACACGTGCTGCAGGAATTCCATTGCGAAGTGATTCCTCCGGGCC
pST-KO-MCS F	pST-KO vector MCS DNA oligomer F	CTAGCCATGGATCCCGGGCCCTGCAGCGGCCGCTCTAGATTAATTAA
pST-KO-MCS R	pST-KO vector MCS DNA oligomer R	CTAGTTAATTAATCTAGAGCGGCCGCTGCAGGGCCCGGGATCCATGG
PknB KD F	PknB forward primer F- NdeI	CGAGATAGCCATATGACCACCCCTTCCCACCTGTCC
PknB KD R	PknB kinase domain (1-330 aa) R- HindIII	GCGGCCGCAAGCTTAGCCCACCGAACCGATGCTGCG
GarA F	GarA primer F- NotI	CACCGCGGCCGCACATATGGTAACGGACATGAACCCGG
GarA R	GarA primer R- PvuI	CGATCGACATCGCGGCCGCTTCGGGGCTATCGGGTG
P_{hsp60}-*sacB* F	Hsp60 promoter with SacB gene primer F	CACCGCATGCGTGACCACAACGACGCGCCC
P_{hsp60}-*sacB* R	Hsp60 promoter with SacB gene primer R	ACTGCATGCGGTTAGGAATACGGTTAGCC
IlvH F	IlvH primer F	CACCCATATGAGTAACGGAACCCCCACCCAC
IlvH R	IlvH primer R	GGTGCGGCCGCTTACTTCGCTGCGCCGATGCC
IlvH 5' Flank F	IlvH knockout 5’ Flank F	CACCCTGCAGGATCGCGCTGGGCACGCGGTTCG
IlvH 5' Flank R	IlvH knockout 5’ Flank R	GGTAGTACTGCGCCGACCGCGAGCGACTGG
$Apra^r$-F	Apramycin res F	CACCAGTACTACGTAGTTATCGAATTCCTGC
$Apra^r$-R	Apramycin res R	GATAGTACTTGGATCCCCCGTGTTGCCC
IlvH 3' Flank F	IlvH knockout 3’ Flank F	CACCAGTACTCCTGGAGCCGTATGGCATCCG
IlvH 3' Flank R	IlvH knockout 3’ Flank R	GGTGCGGCCGCATCGGGTGCTCGGCGTTGGCC

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