SUPPLEMENTAL METHODS

Generation of *Mtb* DosR KO strains. A 2,442bp PCR fragment containing *dosR* plus 963bp upstream and 823bp downstream was generated using primers dosR-1 and dosS-1 and cloned into the *Xba*I site of plasmid pcDNA2.1 (Invitrogen). The resulting plasmid was digested with *BbsI* and *BlpI* to replace a 477bp fragment from the *dosR* gene with the 1.6kb Hygromycin resistance cassette obtained from pHint (Garbe *et al*., 1994). Finally, the 3,512bp *Xba*I insert of this plasmid was cloned into the same site of vector pPR23-1 (Pelicic *et al*., 1997) generating the plasmid pPDM15 that was used for transforming *Mtb* in order to generate the *dosR* KO mutants using previously described procedures (Pelicic *et al*., 1997). Confirmation of the *dosR* KO strains was done by PCR using primers Hyg3'-Fd + dosS-D (1,110bp), Hyg5'-rev + Rv3134-A (1,041bp) and devR-antiRev + dosR-A-rev-GK (426bp fragment absent in the KO mutant). Additionally, phenotypic confirmation was performed by quantitative real-time PCR (qRT-PCR) using primers DosR-RT-6-f and DosR-RT-6-r.

Generation of *Mtb* DosT KO strains. A 4.4kb *Bam*HI fragment containing *dosT* was isolated from BAC Rv175 (kindly provided by Dr. Roland Brosch, Institut Pasteur) and ligated with *Bam*HI restricted pBluescript II KS+ (Stratagene) that had previously had its *Eco*RI site removed from the polylinker. The resulting construct was digested with *Eco*RI to remove a 1,374bp fragment internal to *dosT,* filled-in with Platinum *Pfx* polymerase (Invitrogen) and then blunt-end ligated with the 1.6kb Hygromycin resistance cassette. This plasmid was subsequently digested with *Mlu*I and *Sca*I, with the resulting ends of the insert fragment made blunt via treatment with Platinum *Pfx* (Invitrogen). The purified insert was then ligated with pPR23-1 (Pelicic *et al*., 1997) that had previously been digested with *Not*I and *Xba*I, and filled-in with Platinum *Pfx*. The resulting plasmid is referred to as pPR23ΔdosT/Hyg and was used to transform H37Rv (ATCC 27294). All transformation and selection procedures were performed as previously described (Pelicic *et al*., 1997; Snapper *et al*., 1990). Confirmation of the *dosT* KO strains was by PCR using primers Hyg3'-Fd + dosTKO-2R (1,090bp), Hyg5'-rev + dosTKO-1F (1,032bp). Additionally, phenotypic confirmation was performed by qRT-PCR using primers dosTSYBR-R2 and dosTSYBR-F2.

Generation of *Mtb* DosS KO strains. Primers VVdosR-E and Rv3131-D were used to amplify a 2,830bp fragment containing *dosS* from H37Rv genomic DNA. The purified PCR product was restricted with *Xba*I and ligated into the *Xba*I site of pcDNA2.1 (Invitrogen) prior to sequencing. The

965bp *Avr*II-*Kpn*2I fragment contained within the cloned insert was removed and subsequently replaced with a 1.2kb Kanamycin resistance cassette isolated from pMV306 (Stover *et al*., 1991) following digestion with *Nhe*I and *Spe*I. All 4 restriction sites were filled-in prior to blunt-end ligation. Finally, the resulting pVV2 plasmid was then digested with *Xba*I and the purified insert filled-in and blunt-end ligated with pPR23-1 (*Not*I digested, filled-in) to generate pΔdosSv2.0. Genetic confirmation of *Mtb dosS* KO strains was done by PCR using primers Kan3'-Fd + devR-antiRev (1,197bp) and Kan5'-Rev + 3131-F2 (1,459bp). Additionally, phenotypic confirmation was performed by qRT-PCR using primers DosS-F2 and DosS-R2.

Generation of *Mtb* DosR-DosS KO strains. Plasmid pBS#2 (4,044bp *Sal*I-*Sac*I fragment containing Rv3134c/*dosR*/*dosS* from H37Rv genomic DNA cloned into pBluescript II KS+) was digested with *Bbs*I and *Kpn*2I to remove 1,956bp of the *dosR* and *dosS* coding sequence, filled-in with T4 DNA polymerase to generate blunt-ends, dephosphorylated, and then ligated with the Kanamycin resistance cassette as above. The resulting pBS/dosR+S_KO plasmid was digested with *Hind*III and *Sac*I to release the entire insert, which was then filled-in and blunt-end ligated with pPR23-1 (as above) to generate pPR23ΔdosR+S. Genetic confirmation of *dosRdosS* KO strains was done by PCR using primers Kan3'-Fd + dosRa-F (1,603bp), Kan5'-Rev + 3131-F2 (1,028bp) and devR-antiRev + dosS-R1-AF (1,070bp fragment absent in the KO mutant). Phenotypic confirmation was by qRT-PCR using primers DosS-F2 and DosS-R2 and DosR-RT-6-f and DosR-RT-6-r.

Generation of *Mtb* DosS-DosT KO strains. Once the DosT KO strain (Hyg^R) was generated in H37Rv and confirmed as above, it was transformed with the pΔdosSv2.0 plasmid in order to disrupt *dosS* via insertion of the Kan^R cassette (see above).

Generation of *Mtb* DosR-DosS-DosT KO strains. Once the DosT KO strains (Hyg^R) were generated and confirmed as above, they were subsequently transformed with the pPR23ΔdosR+S plasmid in order to disrupt both *dosR* and *dosS* via insertion of the Kan^R cassette (see above).

Generation of *Mtb* Tgs1 KO strains. A 2,114bp PCR fragment containing the *tgs1* gene plus 736 bp upstream was generated using primers Rv3130-1 and Rv3131-1 and cloned into the *NotI* site of plasmid pcDNA2.1 (Invitrogen). The resulting plasmid was digested with *NheI* and *PshA1* for replacing a 116bp fragment from the *tgs1* gene with the 1.6kb Hygromycin cassette. Finally, the 3,506bp *NotI* insert of this plasmid was cloned into the same site of vector pPR23-1 (Pelicic *et al*.,

1997) generating plasmid pPDM4 that was used for transforming *Mtb* in order to generate the *tgs1* KO mutants using previously described procedures (Pelicic *et al*., 1997). Genetic confirmation of the *tgs1* KO strains was by PCR using primers Hyg3'-Fd + 3130cH3R (1,160bp), Hyg5'-rev + Rv3131-C (1,158bp). Additionally, phenotypic confirmation was performed by qRT-PCR using primers Rv3130c-F-Syb and Rv3130c-R-SyB.

Generation of complemented strains. Complementation of the KO strains generated above was accomplished by electroporation (Snapper *et al*., 1990) with the following plasmids:

pNS3 and pDC4: A 3,359bp fragment containing truncated Rv3134c (from nucleotide 305) and the complete *dosR* and *dosS* genes was cloned into the *HpaI* site of pMV306-Kan. pNS3 contains the 507C and 601C allele from H37Rv, and pDC4 contains the 507G and 601T *dosR* promoter allele from HN878.

pNS5 and pDC5: A 1,883bp fragment containing 392 bp upstream of the Rv3134c gene as well as the complete Rv3134c and *dosR* genes was cloned into the *HpaI* site of pMV306-Kan. pNS5 contains the 507C and 601C allele from H37Rv, and pDC5 contains the 507G and 601T allele from HN878.

pNS6 and pDC6: A 4,049bp fragment containing 392bp upstream of the Rv3134c gene and the complete Rv3134c*, dosR* and *dosS* genes was cloned into the *HpaI* site of pMV306-Kan. pNS6 contains the 507C and 601C allele from H37Rv, and pDC6 contains the 507G and 601T allele from HN878. For generating pNS6-GM and pDC6-GM, the 1.2kb *Nhe*I-*Spe*I fragment (Kanamycin resistance cassette) from pNS6 or pDC6 was replaced with a 1.4kb *Hind*III-*Spe*I fragment from pPR23 containing the Gentamicin resistance cassette (GM).

pAA3: A 4,049bp fragment containing 392bp upstream of the Rv3134c gene and the complete Rv3134c*, dosR* and *dosS* genes was cloned into the *HpaI* site of pMV306-Kan. The *dosR* promoter region contains the 507C H37Rv and 601T HN878 hybrid allele.

pAA6: A 4,049bp fragment containing 392bp upstream the Rv3134c gene and the complete Rv3134c*, dosR* and *dosS* genes was cloned into the *HpaI* site of pMV306-Kan. It contains the 507G and 601T allele from HN878. It also harbors a transversion of T to A at position 3486 (position 161 of the *dosR* gene) for replacing asp54 with glu54.

pAR2: A 3,393bp fragment containing 392bp upstream of the Rv3134c gene and the complete Rv3134c and *dosS* genes (no *dosR*) was cloned into the *HpaI* site of pMV306-Kan. It contains the 507G and 601T allele from HN878.

pdosT-wt and pdosT-mut: A 1,722bp fragment containing *dosT* was cloned into the *Bal*I-*Hind*III sites of pMV361-GM. pdosT-wt contains the wild type *dosT* gene of H37Rv and pdosT-mut corresponds to *dosT* from HN878 which contains a frame-shift mutation at position G775.

pN-FLAGdosT-WT and pN-FLAGdosT-MUT: A 1,742bp fragment containing *dosT* was cloned into pMV361-Kan. It also harbors the Flag-epitope sequence (5'-GACTACAAGGACGACGAT GACAAG-3') inserted at the 5'end of *dosT* for generating a N-terminal Flag-DosT fusion. Expression is driven by the *hsp60* promoter. pN-FLAGdosT-WT contains the wild-type *dosT* allele from H37Rv and pN-FLAGdosT-MUT corresponds to the gene from HN878 with a frame-shift mutation at position G775.

pC-FLAGdosT-WT and pC-FLAGdosT-MUT: A 1,742bp fragment containing *dosT* was cloned into pMV361-Kan. It also harbors a Flag-epitope sequence (5'-GACTACAAGGACGACGAT GACAAG-3') located at the 3'end of *dosT* for generating a C-terminal DosT-Flag fusion. Expression is driven by the *hsp60* promoter. pC-FLAGdosT-WT contains the wild-type *dosT* allele from H37Rv and pC-FLAGdosT-MUT corresponds to the gene from HN878 with a frame-shift mutation at position G775.

Genetic complementation of the wild-type or KO strains was initially confirmed by standard PCR using the appropriate primers: Genta-Fd + Genta-rev (377bp), Hyg-Fd + Hyg-R (389bp) or KanF + KanR (480bp). Further confirmation was through qRT-PCR as described in the main body of the text.

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TABLE S1: Primers used in this study

Name	Sequence	R.E. site
3130cH3R	5'-CGCCCAGTTACTACCGTGG-3'	
3131-F2	5'-ATGCTGCGGCAGGTCAGTG-3'	
devR-antiRev	5'-TC**GAATTC**GTGGTAAAGGTCTTCTTGGTCG-3'	*Eco*RI
dosR-1	5'-TGC**TCTAGA**TTGCGTCTGTCATCGGTCGAT-3'	*Xba*I
dosRa-F	5'-CCTAACGTCGATTGCGCAC-3'	
dosR-A-rev-GK	5'-TCTCGGCGGCACCGCGCA-3'	
DosR-RT-6-f	5'-CTGCGCTGTCTGATCCTCAC-3'	
DosR-RT-6-r	5'-CAGCGCCCACATCTTTGAC-3'	
dosS-1	5'-TGC**TCTAGA**ACTGTTTCAATCACCAGCAGCT-3'	*Xba*I
dosS-C	5'-GGCATTCGCGACTGCGATAC-3'	
dosS-D	5'-AACAATGGAAGCCAQCAGCGCT-3'	
dosS-F1-AF	5'-CGGGCTGCTCATCGAAGA-3'	
DosS-F2	5'-TCGCCCGTGACCTCCAT-3'	
DosS-R2	5'-CTGCAAAGCCAGGCCAAT-3'	
dosTKO-1F	5'-GAGAACGCGGTGCGCTAC-3'	
dosTKO-2R	5'-GTTCCAGTACTAGCTGGGAC-3'	
dosTSYBR-F2	5'-CCGATGCGGACCTTCCT-3'	
dosTSYBR-R2	5'-GGTCAAGTAAAGATTGCCGAACA-3'	
Genta-Fd	5'-ATCGTCACCGTAATCTGCTTGC-3'	
Genta-rev	5'-ATCATTCGCACATGTAGGCTCG-3'	
Hyg-Fd	5'-CATTCCGAGGTCTTCCCGGA-3'	
Hyg-R	5'-TGCAGGAAGGTGAAGGCGAG-3'	
Hyg3'-Fd	5'-GGAACTGGCGCAGTTCCTCT-3'	
Hyg5'-rev	5'-TGTGGACCTCGACGACCTG-3'	
KanF	5'-ATGAGCCATATTCAACGGGAAA-3'	
KanR	5'-CAAACCGTTATTCATTCGTGAT-3'	
Kan3'-Fd	5'-AGGATCAGATCACGCATCTTC-3'	
Kan5'-rev	5'-TCTTGTGCAATGTAACATCAGAG-3'	
Rv3130-1	5'-AAGGAAAAAA**GCGGCCGC**CCGCTTACTGATCGCCACCA-3'	*Not*I
Rv3130c-F-SyB	5'-GCGGCGACTCACCTGCTA-3'	
Rv3130c-R-SyB	5'-GCGAAGCTGTCGCTCATACTT-3'	

Name	Sequence	R.E. site
Rv3131-1	5’-AAGGAAAAAA**GCGGCCGC**TGAGCTCGCGCAGATATTCCT-3’	*Not*I
Rv3131-C	5’ TCTGAT**GCCGGC**TCGTTGCG-3’	*Nae*I
Rv3131-D	5’-TGC**TCTAGA**TGGCGCAGGCTGTCTTGGAC-3’	*Xba*I
Rv3134-A	5’-GATAGGTGAGATTCATTCTCGC-3’	
Rv3134c-M-F	5’-GGTCAAGATCGAAACGGAGG-3’	
sigA1-F	5’-TCGCGCCTACCTCAAACAG-3’	
sigA1-R	5’-CGTACAGGCCAGCCTCGAT-3’	
VVdosR-E	5’-TGC**TCTAGA**ACGAGGTGGTGCGTCGTGGT-3’	*Xba*I

Bold underlined sequences correspond to restriction enzyme sites.

Fig. S1

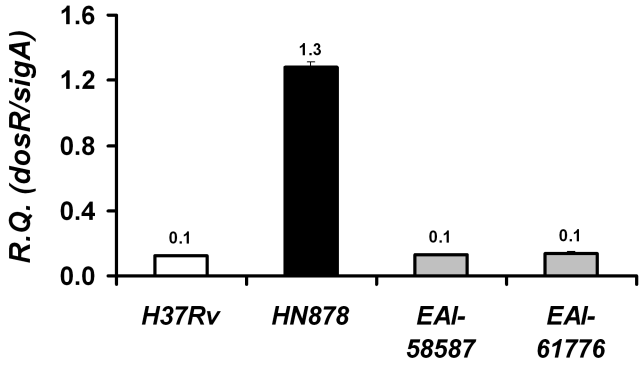

Figure S1. *Mtb* strains with only the C507G SNP display a level of *dosR* expression that is equivalent to H37Rv. qRT-PCR of *dosR* expression analyzed in H37Rv (Euro-American or Lineage 4), HN878 (Beijing lineage), and 2 independent isolates of the East-African/Indian lineage (or Lineage 3). Results are shown as relative quantities (R.Q.) using *sigA* as the normalizing gene. The error bars represent standard deviation as indicated in the Materials and Methods.

Fig. S2

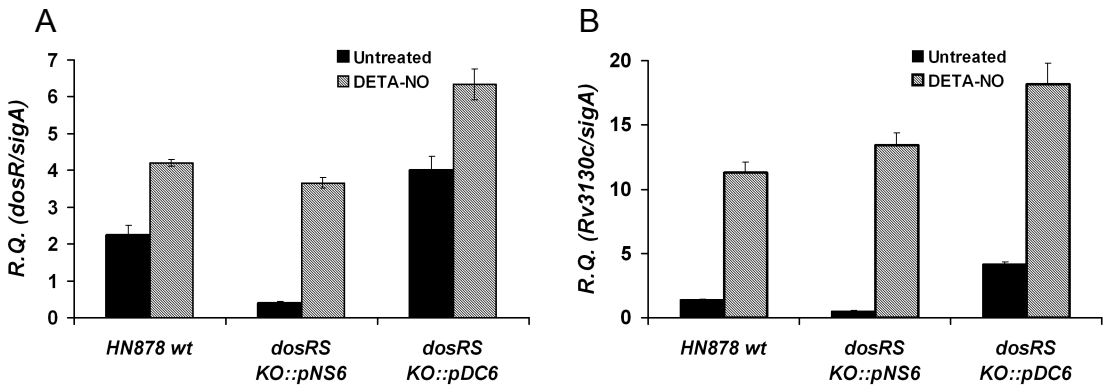

Figure S2. Complementation of the HN878 *dosRS* KO restores DosR regulon expression.
A and **B**. Prior to carrying out the mouse experiments indicated in Fig. 5, qRT-PCR of *dosR* (**A**) and Rv3130c (**B**) expression was compared between HN878 wild-type, HN878 *dosRS* KO, and the latter complemented with either pNS6 or pDC6 (see Fig. 2 for a complete description). Results are presented as relative quantities (R.Q.) using *sigA* as the normalizing gene. Standard deviations are shown.

Fig. S3

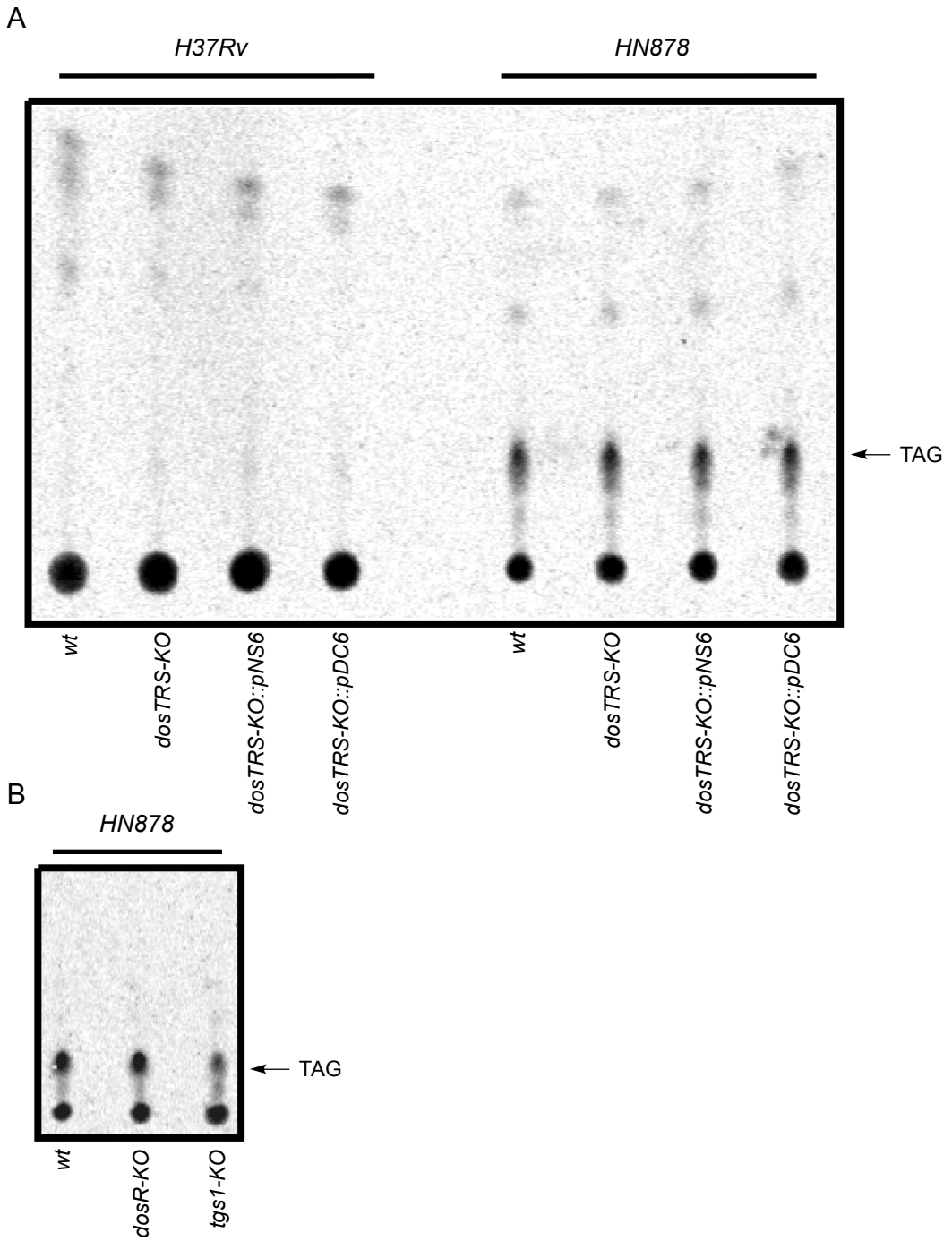

Figure S3. Constitutive *dosR* overexpression is not the underlying cause of the TAG accumulation seen in Beijing strains.

A. Thin Layer Chromatography (TLC) of [14C(U)] glycerol labeled apolar lipids extracted from wild-type, *dosTRS* triple KO, and pNS6 and pDC6 complemented strains of the latter (see Fig. 2 for a complete description) prepared in both Beijing and non-Beijing (H37Rv) backgrounds. Samples were run three times in hexanes/ethyl acetate (98:2, v/v). The position of the triacylglycerides is shown by an arrow.

B. TLC of [14C (U)] glycerol labeled apolar lipids prepared from wild-type HN878, HN878 *dosR* KO and HN878 *tgs1* KO strains is shown. Plates were run three times in hexanes/ethyl acetate (98:2, v/v). The arrow indicates the relative position of the labeled triacylglycerides.