

Supporting Information

Impact of methylthioxylose substituents on the biological activities of lipomannan and lipoarabinomannan in *Mycobacterium tuberculosis*

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Table S1. Monosaccharide composition of LM from WT *Mtb* H37Rv, the *mtxT* mutant and the *mtxT* complemented mutant (*MtbΔmtxT*/pMV306H-*mtxT* [*MtbΔmtxT* comp]).

The reported values are averages \pm SD of three technical repeats and show relative distribution in %. No statistically significant differences were found between WT and mutant or complemented mutant LM pursuant to the Student's *t*-test.

	Ino	Manp
WT H37Rv	2.6 \pm 0.2	97.4 \pm 0.2
<i>ΔmtxT</i>	2.3 \pm 0.2	97.7 \pm 0.2
<i>ΔmtxT</i> comp	3.5 \pm 1.3	96.5 \pm 1.3

Table S2. Glycosyl linkage analysis of per-*O*-methylated LM from WT *Mtb* H37Rv, the *mtxT* mutant and the *mtxT* complemented mutant (*MtbΔmtxT*/pMV306H-*mtxT* [*MtbΔmtxT* comp]).

The reported values are averages \pm SD of three technical repeats and show relative distribution in %. Asterisks denote statistically significant differences between WT and mutant or complemented mutant LM pursuant to the Student's *t*-test; **p*<0.05.

	t-Manp	6-Manp	2,6-Manp	2-Manp
WT H37Rv	44.8 \pm 0.3	22.5 \pm 1.8	29.5 \pm 2.2	3.2 \pm 0.3
<i>ΔmtxT</i>	42.4 \pm 1.0*	25.7 \pm 0.4*	28.2 \pm 1.0	3.7 \pm 0.3
<i>ΔmtxT</i> comp	42.6 \pm 0.2*	19.7 \pm 2.0	33.7 \pm 2.1	4.0 \pm 0.3*

Table S3. Monosaccharide composition of LAM from WT *Mtb* H37Rv, the *mtxT* mutant and the *mtxT* complemented mutant (*MtbΔmtxT*/pMV306H-*mtxT* [*MtbΔmtxT* comp]).

The reported values are averages \pm SD of three technical repeats and show relative distribution in %. Asterisks denote statistically significant differences between WT and mutant or complemented mutant LAM pursuant to the Student's *t*-test; **p*<0.05.

	Araf	Ino	Manp	Araf/Manp
WT H37Rv	47.3 \pm 0.5	0.7 \pm 0.1	52.0 \pm 0.6	0.9 \pm 0.0
<i>ΔmtxT</i>	50.1 \pm 0.9*	1.0 \pm 0.2	48.9 \pm 1.0*	1.0 \pm 0.0*
<i>ΔmtxT</i> comp	48.0 \pm 1.0	1.7 \pm 0.5*	50.3 \pm 0.8	1.0 \pm 0.0

Table S4. Glycosyl linkage analysis of per-*O*-methylated LAM from WT *Mtb* H37Rv, the *mtxT* mutant and the *mtxT* complemented mutant (*Mtb* Δ *mtxT*/pMV306H-*mtxT* [*Mtb* Δ *mtxT* comp]).

The reported values are averages \pm SD of three technical repeats and show relative distribution in %. Asterisks denote statistically significant differences between WT and mutant or complemented mutant LAM pursuant to the Student's *t*-test; **p*<0.05.

	t-Araf	2-Araf	5-Araf	3,5-Araf	t-Manp	6-Manp	2,6-Manp	2-Manp
WT H37Rv	1.0 \pm 0.0	4.9 \pm 0.2	34.3 \pm 1.4	8.6 \pm 0.1	21.3 \pm 1.0	6.2 \pm 0.0	13.7 \pm 0.2	10.0 \pm 0.4
Δ <i>mtxT</i>	0.8 \pm 0.0	4.9 \pm 0.6	35.1 \pm 2.6	10.0 \pm 0.4*	18.6 \pm 0.3	7.3 \pm 0.6	14.4 \pm 0.8	8.9 \pm 0.4
Δ <i>mtxT</i> comp	0.7 \pm 0.2	5.1 \pm 0.6	32.5 \pm 2.2	9.0 \pm 0.5	20.7 \pm 0.8	6.7 \pm 0.5	14.5 \pm 0.7	10.8 \pm 0.8

Figure S1: 2D ^1H - ^{13}C HMQC NMR spectrum of LAM purified from the *Mtb capA* transposon mutant.

Red asterisks indicate the expected positions of the signals typifying the MTX motif based on the LM spectrum presented in Fig. 4A.

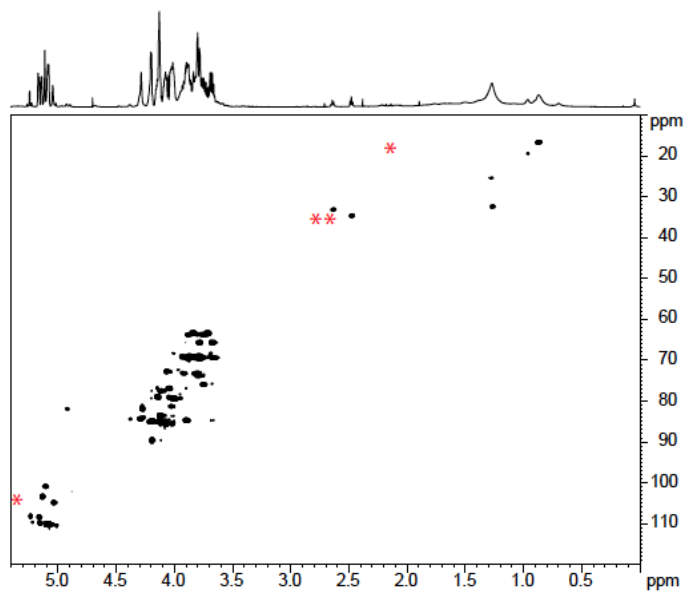
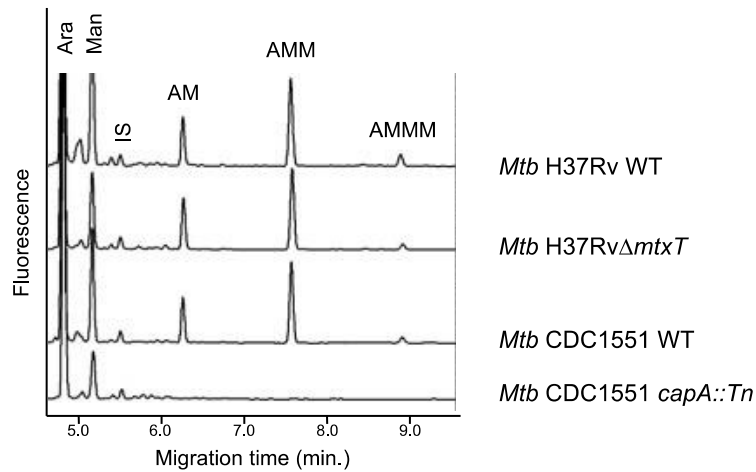


Figure S2: Quantification of manno oligosaccharide caps in the LAM from WT *Mtb* CDC1551, WT *Mtb* H37Rv, the *Mtb* H37Rv *mtxT* mutant and the *Mtb* CDC1551 *capA* transposon mutant.

(A) Capillary electrophoresis cap profile of LAM prepared from the different strains.

(B) Abundance of the different cap motifs per LAM molecule. IS, internal standard, mannoheptose-APTS; AM, Manp-(α 1 \rightarrow 5)-Ara-APTS (mono-mannoside cap); AMM, Manp-(α 1 \rightarrow 2)-Manp-(α 1 \rightarrow 5)-Ara-APTS (di-mannoside cap); AMMM, Manp-(α 1 \rightarrow 2)-Manp-(α 1 \rightarrow 2)-Manp-(α 1 \rightarrow 5)-Ara-APTS (tri-mannoside cap). The results are shown as averages and standard deviations from two technical replicates. Statistical differences between mutant strains and corresponding wild-type parent (*Mtb* H37Rv or *Mtb* CDC1551) were obtained using a two-way ANOVA with Tukey's multiple comparisons test with, **** $p \leq 0.001$. The *capA* transposon mutant is devoid of mannoside capping as expected.

(A)



(B)

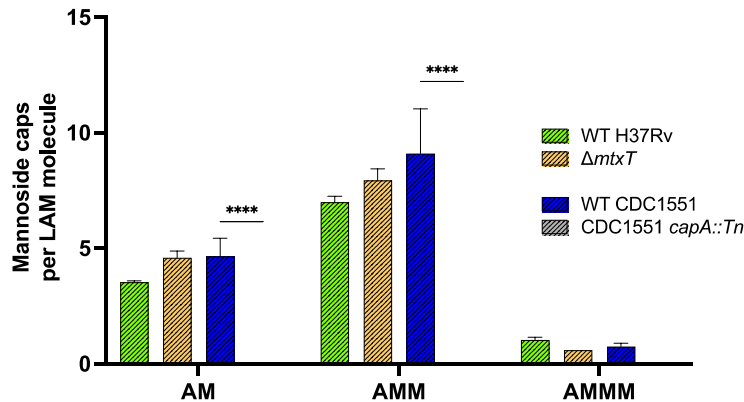


Figure S3: Evaluation of chemokine and cytokine secretion by THP-1 cells treated with purified LM and LAM from wild-type *Mtb* H37Rv, the *Mtb* H37Rv *mtxT* mutant and the complemented *mtxT* mutant.

THP-1 cells were treated with purified LAM or LM (10 $\mu\text{g mL}^{-1}$) from either WT *Mtb* H37Rv, *Mtb* Δ *mtxT* and *Mtb* Δ *mtxT*/pMV306H-*mtxT* for a total of 48 h in the absence of LPS. Supernatants were analyzed for the indicated cytokines/chemokines using Luminex® multiplex assay. Statistical differences were obtained using a two-way ANOVA with Tukey's multiple comparisons test with, **** $p \leq 0.0001$; *** $p \leq 0.005$; ** $p \leq 0.01$ and * $p \leq 0.05$.

