Supplemental Data

Table S1: Linker region and sugar analyses of cell walls prepared from the *Mtb* CDC1551 and H37Rv mc^{2}6206 WT strains and their corresponding *cpsA1* and *cpsA2* knock-out mutants.
Cell walls from *Mtb* cells grown to log phase were prepared and hydrolyzed with 2 M trifluoroacetic acid (TFA) as described in ref. 44, and alditol acetates derived thereof were subjected to GC/MS. The amounts of each monosaccharide in the samples are expressed relative to Rha set to be constant. Note that the 2 M TFA acid hydrolysis does not fully release the GlcNAc and Mur from the cell wall. In addition, the ratio of MurNAc to MurNAc-6P was determined by LC/MS analysis of the 4 M HCl hydrolysate.

Strain	Rha	Ara	Man	Gal	Glc	GalNAc	Mur	GlcNAc	Mur/ MurNAc-6P
H37Rv mc^{2}6206									
WT	1.00	45.58	0.45	18.75	3.59	1.78	1.35	4.70	9.5
Δ*cpsA1*	1.00	44.56	0.49	17.54	6.74	2.04	1.65	5.15	7.1
Δ*cpsA2*	1.00	45.76	1.25	18.98	5.83	2.90	3.38	7.88	16.0
CDC1551									
WT	1.00	45.77	7.18	22.24	0.88	1.40	6.57	7.03	10.0
Δ*cpsA1*	1.00	66.61	8.46	21.50	3.61	2.82	6.69	11.81	9.30
Δ*cpsA2*	1.00	45.39	8.72	22.75	1.64	1.56	6.89	9.59	12.70

Figure S1: TLC analysis of total lipids extracted from whole *Cgl* cells grown to stationary phase cultures.
Lane 1: *Cgl* WT strain. Lane 2: CGLcKD-0847 strain grown in the presence of 25 µM IPTG. Lane 3: CGLcKD-0847 strain grown in the presence of 1 mM IPTG. TLC plates were developed in the solvent system $CHCl_3/CH_3OH/H_2O$ (65/25/4, by vol.) and revealed by immersion in 10 % H_2SO_4 in ethanol and heating. PG, phosphatidylglycerol; CL, cardiolipin; TDCM, trehalose dicorynomycolates; PIM, phosphatidylinositol mannosides.

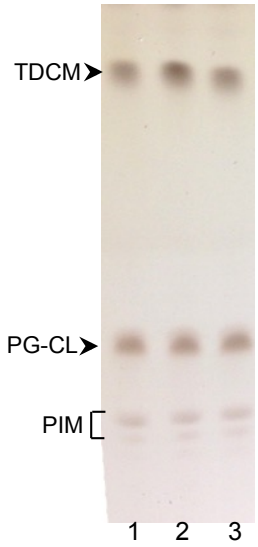
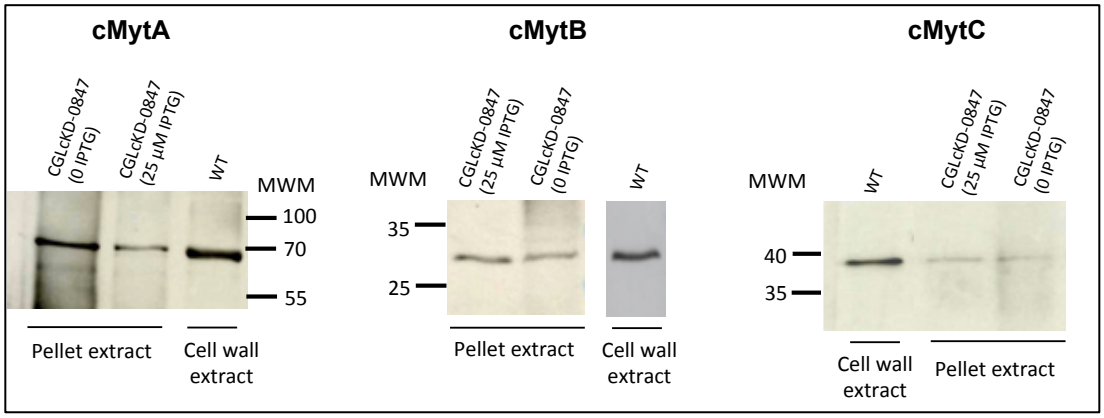


Figure S2: Immunoblot analysis of the material released by CGLcKD-0847 in the culture medium.
Proteins prepared from the material released by CGLcKD-0847 cultured in the presence of 0.025 mM IPTG shown in Fig. 6A were separated by SDS-PAGE, blotted onto a nitrocellulose membrane, and the membrane probed with rabbit polyclonal antibodies directed to the cMytA, cMytB and cMytC mycolyltransferases (Puech *et al.*, 2000; Huc *et al.*, 2013). An anti-rabbit IgG HRP-conjugated antibody (Promega) was used as the secondary antibody. Immune complexes were detected by chemiluminescence using a CDD camera (ImageQant, LAS500, GE Healthcare). The expected sizes of cMytA, cMytB and cMytC are 66.1, 33.5 and 36.6 kDa, respectively. MWM: Molecular weight marker (in kDa).



References:
Huc, E., de Sousa-D'Auria, C., de la Sierra-Gallay, I. L., Salmeron, C., van Tilbeurgh, H., Bayan, N., Houssin, C., Daffé, M., and Tropis, M. (2013) Identification of a mycoloyl transferase selectively involved in O-acylation of polypeptides in Corynebacteriales. *J. Bacteriol.* **195**, 4121-4128

Puech, V., Bayan, N., Salim, K., Leblon, G., and Daffé, M. (2000) Characterization of the in vivo acceptors of the mycoloyl residues transferred by the corynebacterial PS1 and the related mycobacterial antigens 85. *Mol. Microbiol.* **35**, 1026-1041

Figure S3: Growth characteristics of the *cpsA1* and *cpsA2* allelic exchange mutants of *Mtb* CDC1551.
The strains were grown in 7H9-ADC-Tween 80 broth at 37°C with shaking. *Mtb* CDC1551 WT (black line); *Mtb* CDC1551Δ*cpsA1* (red line); *Mtb* CDC1551Δ*cpsA2* (green line).