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1 Update on the virulence factors of the obligate pathogen *Mycobacterium tuberculosis* and 2 related tuberculosis-causing mycobacteria 3 4 5 Jan MADACKI*, Guillem MAS FIOL** and Roland BROSCH* 6 7 Institut Pasteur, Unit for Integrated Mycobacterial Pathogenomics, CNRS UMR3525, Paris 8 75015, France 9 10 11 correspondence: Jan Madacki (jan.madacki@pasteur.fr) *For or Roland Brosch 12 (roland.brosch@pasteur.fr) ** Present address: Institut Pasteur, Unité de Recherche Yersinia, Paris 75015, France 13 14 15 16 Abstract 17 Over the long course of evolution from a probable environmental reservoir, the pathogen that 18 19 we know today as *Mycobacterium tuberculosis* has become fully capable of adapting to the 20 life inside host cells by evading and modifying their responses to infection. Factors 21 contributing to the success of this pathogen are numerous and thanks to a large body of work 22 accumulated over the past decades, we are closer to understanding the remarkable complexity 23 of tuberculosis pathogenesis. The unique type VII secretion systems and various complex 24 lipids of the cell envelope have emerged as some of the most important and most studied 25 factors in this regard. This review attempts to summarize recent findings on these and other virulence factors, while discussing their evolution in different closely related tuberculosis-26 27 causing bacteria as well, with the aim of exploring the processes which led *M. tuberculosis* to 28 becoming one of the deadliest infections agents. 29 30 31 **1** Introduction 32

With a death toll of estimated 1.3 million HIV-negative people and additional 374 000 deaths among HIV-positive people in the year 2016, tuberculosis (TB) is the leading cause of death by a single infectious agent worldwide (WHO, 2017). Its etiological agent, *Mycobacterium* 36 *tuberculosis*, is a primarily intracellular pathogen that is capable of successfully maintaining 37 its viability within the host, involving a delicate interplay between the immune system and the 38 pathogen's armaments, acquired and trained through thousands of years of host-pathogen 39 interaction (Orgeur and Brosch, 2018). M. tuberculosis is spread by airborne transmission of 40 bacteria-containing droplets from patients with active TB, allowing infection of lung alveoli 41 in new hosts. After the initial stage of innate immune response, presentation of antigens by 42 dendritic cells and recruitment of effector T-cells, characteristic multicellular structures called 43 granulomas are formed with the aim of restricting the spread of the pathogen. In a weakened 44 or immunocompromised host, the thereby established balance between pathogen and host 45 responses may be lost, with subsequent systemic dissemination of the bacilli leading to active 46 disease and new transmission. Besides M. tuberculosis, which is specialized on the human 47 host, some highly related members (more than 99.99 % of genome sequence identity with M. 48 tuberculosis) of the so-called M. tuberculosis complex (MTBC) can cause TB-like disease in 49 other mammalian species, and most of these have kept their traditional names referring to the 50 mammalian species from where they were isolated (Mycobacterium bovis, Mycobacterium 51 microti, Mycobacterium pinnipedii etc.) and these members have been proposed to represent 52 special ecotypes of the tubercle bacilli (Smith et al., 2009).

53 For studying mycobacterial pathogenesis on a cellular level, the use of macrophages 54 has been proven as an invaluable tool, as bacteria are ingested primarily by these host cells 55 upon entry into the alveoli, and events crucial to the disease process occur in their 56 intracellular environment. Inside the infected macrophage, antibacterial mechanisms such as 57 reactive oxygen, nitrogen intermediate production and phagolysosomal fusion are factors that 58 usually function to eliminate pathogens. However, *M. tuberculosis* has evolved efficient ways 59 to counteract some of these host-defense mechanisms. This also can be tested in selected 60 cellular or animal infection models, mostly human or murine cell lines, as well as laboratory 61 mice and guinea pigs, where the ability of a particular strain to multiply and cause lung and 62 spleen pathology can be assessed and quantified over time.

Compared to some other bacterial pathogens, which may produce specific toxins or unique virulence factors, *M. tuberculosis* does not produce a single dominant factor responsible for causing disease, but rather induces pathology by a complex combination of virulence determinants and host responses. Extensive research on mycobacterial virulence, has helped to pinpoint several groups of factors involved in different stages of pathogenesis of TB. A key study of *in vivo* essentiality in the mouse model of infection using transposon site hybridization (TraSH) gave a first rough estimate of the global picture of *M. tuberculosis* 70 virulence factors, by identifying 194 *M. tuberculosis* genes as being specifically required for 71 in vivo growth/survival in mice (Sassetti and Rubin, 2003). When a similar study was done on 72 macrophages, 126 genes were identified, some of them being essential exclusively in the 73 macrophage model (Rengarajan et al., 2005). Moreover, results of a more recent study using 74 deep sequencing, significantly overlapped with the previous one from 2003, but found more 75 than 400 additional genes as being essential in vivo (Zhang et al., 2013). These combined data 76 now provide a framework for the definition of virulence genes of *M. tuberculosis*, whereby 77 the individual genes may still be necessary to be studied in more detail to identify their exact 78 roles and functions in the infection process. A number of genes identified as essential for 79 virulence encode proteins involved in basic metabolism, a fact that makes defining a virulence 80 factor in *M. tuberculosis* an ardent task. On the other hand, factors such as components of the 81 ESX / type VII secretion systems, as well as several types of complex lipids of the cell 82 envelope are standing at the frontline facing the host immune system and the most recent 83 progress in the study of their biological roles and their participation in the virulence of M. 84 tuberculosis constitutes the main focus of this review.

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86 2 Type VII secretion systems

87 The Mycobacterium tuberculosis genome encodes five type VII secretion systems (ESX-1, 88 ESX-2, ESX-3, ESX-4 and ESX-5) which are specialized for transport of selected protein 89 substrates across the cell envelope (Abdallah et al., 2007; Brodin et al., 2004; Gröschel et al., 90 2016). Genes that likely encode core components of the secretion machinery (ESX conserved 91 components, *Ecc*) are conserved in each of the five loci, with *esx-4* having the minimal set of 92 genes encoding the simplest and earliest type VII secretion system, from which the rest 93 probably originated via gene duplication and plasmid-mediated horizontal gene transfer 94 (Dumas et al., 2016; Newton-Foot et al., 2016). A recently published structure of ESX-5 from 95 Mycobacterium xenopi gives insight into the assembly of EccB, EccC, EccD and EccE into a 96 functional complex (Fig. 1) (Beckham et al., 2017). ESX-1 emerged from several independent 97 experimental approaches as one of the most significant for pathogenesis. Comparative 98 genomic studies of *M. tuberculosis* with the attenuated vaccine strain *M. bovis* Bacille 99 Calmette Guérin (BCG), as well as naturally attenuated M. microti have shown that parts of 100 the ESX-1 secretion system are encoded in the regions of difference (RD) 1 (RD1^{BCG} and RD1^{mic}) (Brodin et al., 2002; Gordon et al., 1999; Mahairas et al., 1996). Both RD1^{BCG} and 101 102 RD1^{mic} are notable for encoding secreted proteins ESAT-6 (early secretory antigenic target of 103 6 kDa) and CFP-10 (culture filtrate protein of 10 kDa), also known as EsxA and EsxB,

respectively, which are known for a long time as potent T-cell antigens (Barnes et al., 1992;
Berthet et al., 1998; Sørensen et al., 1995). By constructing an RD1 deletion mutant strain of *M. tuberculosis* H37Rv, as well as knock-in strains of *M. bovis* BCG and *M. microti* it was
confirmed that both the secreted antigens, but also a functional secretion machinery are
necessary for virulence (Hsu et al., 2003; Pym et al., 2002).

109 A considerable amount of research has been conducted in recent years attempting to 110 clarify the fate of *M. tuberculosis* after infection. One of the main strategies of *M.* tuberculosis as an intracellular pathogen, after undergoing phagocytosis by the macrophages, 111 112 is disabling or rather delaying the differentiation of the phagosome into a phagolysosome – 113 thus avoiding the hostile acidic environment optimal for the activity of hydrolytic enzymes. 114 Many factors have been implicated in this process and several pathogen strategies have been 115 described, such as retention of vacuolar H⁺-ATPase (Queval et al., 2017; Wang et al., 2015; 116 Wong et al., 2011), interfering with Rab GTPase recruitment (Via et al., 1997) and evading 117 the toxicity of reactive oxygen and nitrogen species (Voskuil et al., 2011). Surprisingly, there 118 were also indications that *M. tuberculosis* is capable of translocating from these endosomal 119 compartments into the cytosol, probably at later stages of infection (McDonough et al., 1993; 120 van der Wel et al., 2007). In a sophisticated assay using a fluorescent probe sensitive to the 121 beta-lactamase activity localized on the surface of the bacterium and a fluorescence resonance 122 energy transfer (FRET)-based method, contact of M. tuberculosis with the cytoplasm of 123 macrophages was confirmed (Simeone et al., 2012). The ability of *M. tuberculosis* to rupture 124 the phagosomal membrane was subsequently demonstrated in vivo in mouse lungs and by 125 using an inhibitor of phagosomal maturation it was indicated that restriction of phagosomal 126 acidification is a prerequisite of phagosomal rupture (Simeone et al., 2015). Most importantly, 127 phagosomal rupture has been reproducibly linked to ESX-1, as neither *M. tuberculosis* Δ RD1 128 nor *M. bovis* BCG exerted this effect. The observation that ESX-1-deficient strains are unable 129 to cause phagosomal rupture seem to be in concert with previously reported membrane lysing 130 activity of EsxA (D. Houben et al., 2012; Hsu et al., 2003), although its precise function 131 probably needs to be reexamined (Conrad et al., 2017). The consequences of cytosolic contact 132 can be sensing of bacterial components, such as DNA via nucleotidyltransferase cGAS and by 133 AIM-2, which results in type I interferon (IFN) production and activation the inflammasome, 134 respectively (Collins et al., 2015; Wassermann et al., 2015; Watson et al., 2015). It should be 135 mentioned however, that it is not clear from where the DNA that is sensed by cGAS 136 specifically comes from. Apart from mycobacterial DNA that could potentially be released by 137 the bacteria via membrane vesicles or yet unknown mechanisms (Majlessi et al., 2015), the

138 release of mitochondrial DNA into the cytosol has also been suggested (Wiens and Ernst, 139 2016). Lastly, cytosolic contact leads to cell death, as observed in different cellular infection 140 models, promoting bacterial dissemination (Aguilo et al., 2013; Simeone et al., 2012; 141 Augenstreich et al., 2017). Under in vivo conditions however, M. tuberculosis-infected 142 macrophages tend to survive longer (Simeone et al., 2015), although it is tempting to 143 speculate that phagosomal rupture and thereby induced host cell death might play a role 144 during the formation of the caseous lesions inside granulomas, in which the tubercle bacilli 145 find themselves in an extracellular environment.

146 *M. bovis* BCG is to date the only anti-TB vaccine strain licensed, although its efficacy 147 for pulmonary TB in the adolescent and adult population is deficient, prompting urgent efforts 148 for the development of refined or new vaccine strains. As a consequence of the RD1 deletion 149 in BCG strains, phagosomal rupture and subsequent immune signaling events are absent, 150 which seems to limit the protective efficacy of the vaccine. Hence, one of the approaches to 151 develop a more efficient vaccine strain might involve engineering recombinant BCG strains 152 with a functional ESX-1 system, as a BCG strain complemented with the extended RD1 153 region from M. tuberculosis H37Rv did improve this efficacy (Pym et al., 2003), but with 154 safety concerns. As an alternative, a BCG strain harboring the ESX-1 locus of biosafety level 155 2 (BSL-2) species Mycobacterium marinum was prepared (Gröschel et al., 2017), providing 156 desired improvement of protective efficacy in the murine model of infection, with functional 157 phagosomal rupture inducing several host cell responses not present when using parental 158 BCG, and at the same time showing strongly reduced virulence compared to M. bovis BCG 159 ESX-1^{Mtb}. In parallel, reactogenicity to ESX-1-secreted antigens that are absent from BCG 160 has also been suggested to be linked to the improved protection against M. tuberculosis of 161 other vaccine strains, such as the attenuated *M. tuberculosis* vaccine MTBVAC (Aguilo et al., 162 2017), which is presently in late clinical development as a live vaccine (Spertini et al., 2015).

163 Alongside the ESX-1 secretion system, the ESX-5 and ESX-3 systems have also been 164 implicated in virulence of pathogenic mycobacteria. ESX-5 is the most recently evolved type 165 VII secretion system and can be found only in slow growing mycobacterial species (Gey van 166 Pittius et al., 2001). Initially revealed in *Mycobacterium marinum* (Abdallah et al., 2009), the 167 major function of the ESX-5 secretion system appears to be secretion of substrates belonging 168 mostly to the unique families of PE and PPE proteins named after their N-terminal Pro-Glu 169 and Pro-Pro-Glu motifs, respectively (Cole et al., 1998). These large and somewhat 170 mysterious families of proteins comprising about 8 % of the genome of *M. tuberculosis* 171 H37Rv are further divided into subgroups, depending on motifs present in their C-terminal

172 sequence (Cole et al., 1998). The emergence of PE polymorphic GC-rich-repetitive sequence 173 (PE_PGRS) and the PPE major polymorphic tandem repeat (PPE-MPTR) subfamilies 174 represents the most recent evolutionary event among the mycobacterial PE/PPE sequences, 175 and these are thus present only in pathogenic species (Gev van Pittius et al., 2006). 176 Indications that the ESX-5 system is implicated in pathogenesis initially came from research 177 on *M. marinum* (Abdallah et al., 2008; Ramakrishnan et al., 2000; Weerdenburg et al., 2012). 178 Subsequent characterization of different ESX-5 mutants of M. tuberculosis showed that 179 inactivation of the ESX-5 system resulted in a defect in PPE protein export, impaired cell-wall 180 integrity and strong attenuation (Bottai et al., 2012) as well as defects in PE_PGRS export (E. 181 N. G. Houben et al., 2012). In addition, these experiments also revealed that certain ESX-5 182 components were essential for in vitro growth of M. tuberculosis, as certain ESX-5 knock-out 183 mutants could only be obtained when a second copy of selected ESX-5 genes was introduced 184 (Di Luca et al., 2012) or if the cell wall composition was weakened (Ates et al., 2015). 185 Moreover, a mutant in esx-5 region-encoded ppe/pe genes M. tuberculosis Appe25-pe19 has 186 shown attenuation, but still was able to induce strong CD4⁺ T-cell immunity owing to cross-187 reactivity with numerous PE/PPE homologs encoded by other parts of the genome (Sayes et 188 al., 2016, 2012). While the molecular mechanism underlying the attenuation of the $\Delta ppe25$ -189 *pe19* construct remains currently unknown, the interference with the export of certain PE and 190 PPE proteins has also been associated with increase of virulence of selected M. tuberculosis 191 strains. In this respect, interesting observations were made for *M. tuberculosis* strains that had 192 mutated or lost the gene ppe38, whose presence was found to be required for ESX-5-mediated 193 secretion of more than 80 PE PGRS and PPE-MPTR substrates. In the same study it was also 194 found that the ppe38 locus was deleted through IS6110-mediated recombination processes 195 (Gonzalo-Asensio et al., 2018) in most *M. tuberculosis* strains of the so-called "Beijing" 196 family (lineage 2), raising important questions on the role of these PE_PGRS and PPE_MPTR 197 proteins in virulence, but also on the mechanism of their secretion (Ates et al., 2018b). 198 Moreover, the ppe38 locus is also deleted from a series of animal-adapted members of the 199 MTBC, such as *M. bovis*, due to deletion of the RD5 region. Consequently, also the vaccine 200 strain BCG Pasteur does not export the plethora of PE_PGRS and PPE_MPTR proteins, a 201 phenotype which could be repaired by genetic complementation with a copy of the ppe38 202 locus from *M. tuberculosis* in recombinant BCG38 (Ates et al., 2018c). Interestingly, despite 203 the restauration of the PE_PGRS and PPE-MPTR export in such recombinant BCG38 strain, 204 vaccination trials in different mouse infection models have not shown a significant change in 205 the vaccine potential of the recombinant strain, questioning somewhat the specificity of some

of the PPE-MPTR protein components of subunit booster vaccines tested in clinical
vaccination trials (Ates et al., 2018c).

- Investigated to a lesser extent, ESX-3 has been implicated in virulence, aside from its role in iron and zinc acquisition (Serafini et al., 2009; Siegrist et al., 2014) through several PE/PPE proteins (Tufariello et al., 2016) and its secreted substrates, EsxH and EsxG were also found to inhibit phagosomal maturation (Mehra et al., 2013; Portal-Celhay et al., 2017).
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213 **3 Other surface exposed factors**

214 Other components of the cell envelope of *M. tuberculosis* have emerged as important 215 virulence factors, based on their strategic location on the host-pathogen interface. The 216 mycobacterial cell envelope has a complex organization (Brennan and Nikaido, 1995) and 217 consists of a conventional plasma membrane, a layer of peptidoglycan with a covalently 218 attached polysaccharide named arabinogalactan which has its pentaarabinosyl ends esterified 219 by mycolic acids. These latter components, which are characteristic of mycobacteria and 220 associated to the name of this bacterial genus, are very long, 60-90 carbon-atom containing α -221 alkyl β-hydroxy fatty acids which are essential for viability of the mycobacterial cell 222 (Grzegorzewicz et al., 2012a; Portevin et al., 2005; Vilchèze et al., 2000). These mycolic acid 223 residues are the main components of the inner leaflet of the mycobacterial outer membrane, 224 also called the mycomembrane – a unique lipid bilayer, with the outer leaflet consisting of 225 various types of non-covalently bound lipids, such as trehalose mono- and dimycolates 226 (TMM, TDM), phthiocerol dimycocerosates (PDIM), di- and polyacyl trehaloses (DAT, PAT) 227 and sulfolipids (SL), some of which are transported by mycobacterial transporters of the 228 MmpL family to the outer membrane, thereby constituting targets for drug development 229 (Degiacomi et al., 2017; Grzegorzewicz et al., 2012b; Viljoen et al., 2017). The richness of 230 structures of mycobacterial cell envelope lipids has been a subject of research for decades. 231 Some of these lipids are found throughout the Mycobacterium genus, such as trehalose mono-232 and dimycolates, and some, notably phthiocerol dimycocerosates (abbreviated PDM (Brennan 233 and Nikaido, 1995), DIM (Augenstreich et al., 2017) or PDIM (Quigley et al., 2017)) are 234 present only in certain mycobacterial species.

Based on research accumulated over the decades, it became clear that mycobacterial surface lipids and glycoconjugates are important factors for entry into macrophages, stimulating various receptors. As early as the 1950s, TDM has been shown to be an important effector molecule, also receiving the name "cord factor", as it was thought to be the only factor responsible for the characteristic cord-like appearance of *M. tuberculosis* in 240 microscopic preparations (Bekierkunst, 1968; Bloch and Noll, 1955). By stimulating the 241 macrophage inducible C-type lectin (Mincle), TDM causes induction of cytokine and nitric 242 oxide production (Ishikawa et al., 2009) and at the same time TDM may be one of the factors 243 contributing to phagosome maturation delay, which was shown by using TDM-coated beads 244 (Indrigo et al., 2003). TDM is an abundant cell-wall component of all bacteria belonging to 245 the Mycobacterium genus, but subtle modifications of mycolic acid residues, such as 246 cyclopropyl groups which are normally present only in pathogenic species (Barry et al., 1998) 247 (Fig. 2B, C) might be an important factor. Immunomodulatory significance of mycolic acid 248 modifications was confirmed in a laborious study of constructing an M. tuberculosis strain 249 lacking all S-adenosylmethionine dependent methyltransferases responsible for cyclopropyl 250 group modifications of mycolates and a strain additionally lacking oxygenated functional 251 groups (Barkan et al., 2012). Results of using these strains in a murine model of infection 252 show severe attenuation and changes in antigen-specific immune response dependent on the 253 presence of specific modifications. Deletion of the *hma* gene, which is essential for synthesis 254 of both oxygenated mycolate types (methoxy- and ketomycolates) shows that these forms 255 contribute to cell wall integrity, but also seem to be important for specifically inhibiting IL-256 12p40 mediated responses (Dao et al., 2008; Dubnau et al., 2000). Considering the 257 complexity of the biosynthesis of mycolic acids and the apparent interconnectedness of the 258 enzymes in this pathway by protein-protein interactions (Cantaloube et al., 2011; Veyron-259 Churlet et al., 2005), mutations in genes encoding core components of the fatty acid synthase 260 system II (FASII), such as kasB and hadC (Fig. 2A) seem to affect the mycolic acid 261 composition and, consequently, the virulence of respective strains (Bhatt et al., 2007; Slama et 262 al., 2016). Oxygenated mycolates, in particular, are crucial for forming so-called foamy 263 macrophages - differentiated infected macrophages rich in lipid droplets which enable the 264 persistence of the bacilli (Dkhar et al., 2014; Peyron et al., 2008) and recent steps in 265 elucidating the biosynthesis and metabolism of these mycolate classes (Madacki et al., 2018) 266 could provide new clues for studying their impact on the virulence and persistence of 267 mycobacteria.

Contrary to trehalose mycolates, which are common lipids of the mycobacterial cell wall, other identified trehalose containing lipids – di- and polyacyl trehaloses (DAT, PAT) are found exclusively in members of the *M. tuberculosis* complex, and sulfolipids (SL) were suggested to be produced only in *M. tuberculosis* (Daffé and Draper, 1997). The acyl chains of these lipids are synthesized by specialized polyketide synthase systems (PKS), resulting in polymethylated hydrocarbon chains. The specific occurrence of these lipids in pathogenic strains has naturally raised a question of their involvement in tuberculosis pathogenesis, with
rather ambiguous answers when using PKS mutants *in vivo* (Rousseau et al., 2003b, 2003a).
A recent study analyzed multiple deletion strains deficient in DAT, PAT and SL synthesis,
but also in PDIM, which contain methyl branched acyl chains, with the conclusion that all of
the mentioned lipids contribute to phagosome maturation arrest, but with PDIM having a
more dominant role in this process, thus masking the effect of the former (Passemar et al.,
2014).

281 The role of sulfolipids in virulence has been questioned previously, notably by Rousseau et 282 al., 2003b. However, the production of SL was more recently linked to the PhoP/R two 283 component regulatory system, suggesting that PhoP-deficient strains have no or very low 284 expression of genes encoding for proteins involved in SL biosynthesis (Gonzalo-Asensio et 285 al., 2014), with possible implication on virulence. Additionally, using an approach aimed at 286 identifying factors modulating NF-kB-dependent signaling, a transposon mutant defective in 287 SL biosynthesis was identified, showing that SL are Toll-like receptor 2 antagonists (Blanc et 288 al., 2017). Methyl-branched fatty acid-containing lipids of *M. tuberculosis* apparently have a 289 buffering role, as polyketide synthases incorporate toxic propionate, which comes from 290 cholesterol catabolism, a significant energy source for the intracellular bacilli (Lee et al., 291 2013). Apart from their immunogenic properties, PDIM were shown to have an important 292 structural role as components of the mycomembrane, but it was also suggested that their 293 incorporation into the host plasma membrane is able to alter its biophysical properties with 294 downstream effects on phagocytic receptors (Astarie-Dequeker et al., 2009; Camacho et al., 295 2001). Furthermore, it was shown that PDIM are indispensable for efficient phagosomal 296 rupture mediated by ESX-1, a remarkable example of coordinated usage of proteins and 297 lipids, which awaits more mechanistic insights (Augenstreich et al., 2017; Quigley et al., 298 2017). Another group of polymethylated acyl chain containing molecules found in 299 mycobacteria are lipooligosaccharides (LOS), which are, however absent in M. tuberculosis. 300 Interestingly, these surface molecules are present in Mycobacterium canettii strains, which 301 bare the closest resemblance to the progenitor of *M. tuberculosis* (Supply et al., 2013; 302 Boritsch et al., 2016b). It was recently ascertained that LOS are responsible for the 303 characteristic smooth colony morphology of *M. canettii* strains, owing to the presence of an 304 intact dual *pks5* locus, which has apparently undergone homologous recombination resulting 305 in loss of selected pks5 functions and pap-encoded acyltransferase activity in M. tuberculosis 306 during its evolution into a strict pathogen (Boritsch et al., 2016a). This recombination event 307 might have resulted in an evolutionary advantage for *M. tuberculosis*, judging from the

increased fitness and virulence of spontaneous rough morphotype and LOS-deficient *M*. *canettii* K compared to its smooth counterpart. Another group of PKS-synthetized lipids,
phenolic glycolipids (PGL), is found only in a subset of *M. tuberculosis* strains, such as the
hypervirulent Beijing lineage, due to a frameshift mutation in the *pks15/1* gene (Constant et
al., 2002). Although immunomodulatory activity of PGLs from *M. tuberculosis* has been
described (Reed et al., 2004), their reported contribution to the hypervirulent phenotype of the
Beijing strains is not fully understood (Huet et al., 2009; Sinsimer et al., 2008).

315 The abundant mannosylated components of the cell envelope of mycobacteria -316 phosphatidylinositol mannosides (PIMs), lipomannan (LM) and lipoarabinomannan (LAM) 317 were extensively studied for their importance in host-cell recognition - see (Vergne et al., 318 2015) for a recent review. Variations in lipoarabinomannan structure and their impact on its 319 immunogenic properties in vitro have attracted much attention, notably the pathogenic 320 species-specific mannose cap motif, resulting in ManLAM, which was shown to contribute to 321 phagosome maturation arrest (Chatterjee et al., 1992; Fratti et al., 2003). An additional, and 322 unusual, methylthio-D-xylose motif was also characterized in *M. tuberculosis*, binding to one 323 mannose cap per molecule of ManLAM (Joe et al., 2006; Treumann et al., 2002), while a 324 recently discovered genetic locus responsible for its biosynthesis will enable elucidation of its 325 biological role (Angala et al., 2017). Mutant strains of M. bovis BCG and M. tuberculosis 326 producing capless LAM, however, failed to show changes in *in vivo* replication and cytokine 327 production, rather suggesting a redundant function (Afonso-Barroso et al., 2012) which 328 prompts deeper research into the biological functions of this important glycoconjugate.

329 Although the protein composition of the mycomembrane is still largely unexplored, 330 interesting insights are beginning to emerge from their studies. One of the few characterized 331 integral proteins of the mycomembrane, CpnT is a channel forming protein with an N-332 terminal exotoxin domain, which confers the ability of *M. tuberculosis* to induce necrosis of 333 infected cells (Danilchanka et al., 2014; Sun et al., 2015). Upon exerting its necrotizing 334 activity, CpnT does not appear to induce vast cytokine production, and is thus suggested to 335 allow "quiet" cellular escape and dissemination of the bacilli (Maueröder et al., 2016). 336 Alternative approaches for dissecting the mycobacterial cell envelope and defining the composition of the native mycomembrane in rapidly growing mycobacteria have suggested 337 338 that the outer membrane fraction might also contain Antigen 85 complex proteins (see below) 339 and members of the large Mce (mammalian cell entry) protein family (Chiaradia et al., 2017). 340 Finally, other potential candidates that might be localized in the outer membrane are the 341 EspACD proteins, which are associated to the ESX-1 type VII system (Orgeur and Brosch,

342 2018) (Fig. 1). EspC, for example was shown to form a filamentous structure in the
343 mycobacterial cell envelope, which could theoretically be embedded in the mycomembrane
344 layer (Lou et al., 2017).

345 Several important secreted proteins independent from type VII secretion systems (Fig. 346 1) have also been described as virulence factors. For example, the three mycolyl transferases 347 that are exported by the Twin- arginine translocation (TAT) system catalyze the attachment of 348 mycolate residues onto arabinogalactan, as well as the synthesis of TDM (Belisle et al., 1997; 349 Jackson et al., 1999; Katti et al., 2008). These major secreted immunogens also known as the 350 Antigen 85 complex, are also found associated to the mycobacterial cell surface (Wiker and 351 Harboe, 1992). These antigens have previously been associated to altered vaccination 352 efficacy (Horwitz et al., 1995; Ndiaye et al., 2015), but more recent studies have found that 353 the level of export and the availability of the Ag85 proteins for generating immune responses 354 is influenced by the PhoP/R two component regulatory system (Sayes et al., 2018; Solans et 355 al., 2014), and the use of Ag85 as immunogens might not be generally applicable for 356 protection against all *M. tuberculosis* strains, as important, strain lineage-dependent variation 357 of immune recognition might exist (Sayes et al., 2018). Another notable example is the 358 protein tyrosine phosphatase PtpA, directly binding to vacuolar H⁺-ATPase, thus being one of 359 the main inhibitors of phagosome acidification (Wong et al., 2011). However, as mentioned 360 before, the inhibition of phagosomal acidification and maturation by mycobacteria seems to 361 be more complex, like recently found by the screening of host-factors that impact this process 362 (Queval et al., 2017) as well as the identification of specific proteins (SapM, PknG) that are 363 exported via the SecA2 mediated general secretory pathway (GSP) (Fig. 1) and also impact 364 acidification/maturation of mycobacteria-containing phagosomes (Zulauf et al., 2018).

365

366 4 Miscellaneous factors

367 A considerable number of factors that were found to be indispensable for proper process of 368 pathogenesis do not necessarily fall into the category of surface-exposed effectors directly 369 interacting with the host. The above-mentioned preferential utilization of host-cell cholesterol, 370 but also triacylglycerols by intracellular M. tuberculosis relies on several enzymes required 371 for their degradation, their absence causing severe attenuation (Crowe et al., 2018, 2017; 372 Daniel et al., 2011; Pandey and Sassetti, 2008; Singh et al., 2017; VanderVen et al., 2015). In 373 this context, members of the Mce family of proteins should again be mentioned, as they are 374 thought to import cholesterol or other hydrophobic substrates such as fatty acids and were 375 shown to be implicated in virulence of tubercle bacilli (Marjanovic et al., 2010; Nazarova et 376 al., 2017; Senaratne et al., 2008; Shimono et al., 2003; Ekiert et al., 2017). Also, research of 377 gene and posttranslational regulation has also pointed out the importance of DosR, WhiB, 378 PhoP (Geiman et al., 2006; Smith et al., 2017), adenylyl cyclases (Samanta et al., 2017; 379 Shleeva et al., 2017), and protein kinase pknG (Khan et al., 2017; Rieck et al., 2017), and new 380 insights on manipulation of small molecules interfering with cytosolic surveillance are 381 emerging (Dey et al., 2015, 2017). Finally, in our selection of recently identified and/or 382 confirmed virulence factors of *M. tuberculosis* that are presented in this review, we also 383 would like to highlight a dedicated gene cluster, named *moaA1-D1*. The proteins encoded by 384 this cluster have been recently found to enable the tubercle bacillus to respire nitrate and to 385 survive oxygen depletion, a feature which seems particularly important in hypoxic 386 granulomas in the host (Levillain et al., 2017). Strikingly, this gene cluster, which is involved 387 in the biosynthesis of the molybdenum cofactor seems to have been acquired horizontally 388 together with its hypoxia-responsive transcriptional regulator by a recent common ancestor of 389 the tubercle bacilli from plasmids of environmental bacteria of another phylum (β-390 proteobacteria Burkholderia vietnamiensis) (Levillain et al., 2017) (Fig. 3A). The specific 391 acquisition by tubercle bacilli is underlined by the absence of the *moaA1-D1* cluster in closely 392 related mycobacteria (M. marinum, Mycobacterium kansasii) (Fig. 3B) that have been used as 393 model organisms for studying the evolution and emergence of *M. tuberculosis* (Stinear et al., 394 2008; Wang et al., 2015). Apart from the MTBC strains, the moaA1-D1 genetic locus is also 395 present in *M. canettii* strains, although it is not complete in all the strains 396 (http://genolist.pasteur.fr/CanettiiList/), which suggests that after the acquisition of the 397 moaA1-D region via horizontal transfer, parts of the locus were lost in a subgroup of M. 398 canettii strains. However, in the MTBC this locus is highly conserved, suggesting that the 399 associated function in nitrate respiration (Williams et al., 2014) is important and a matter of 400 selection, apparently giving MTBC members some advantages for the survival in mammalian 401 hosts (Levillain et al., 2017).

402

In conclusion, as briefly shown in this review by selected examples, the evolution of *M*. *tuberculosis* towards pathogenicity was a complex process involving a multitude of adaptative steps, some of which appear ancient while others are likely more recent. *M. tuberculosis* is a highly adapted, professional human pathogen which needs to cause disease in the human lungs for efficient spread. Thus, the co-evolution of humans and *M. tuberculosis* has resulted in an intimate host-pathogen relationship. How long it really has been, this is a question of debate, estimations range from 70000 years (Comas et al., 2013) to 6000 years (Bos et al., 410 2014), whereby the latter hypothesis becomes more and more accepted as it is based on the 411 analysis of ancient DNA samples and less on estimations derived from genome comparisons 412 of extant mycobacterial strains (Kay et al., 2015; Mokrousov, et al., 2017). However, it should 413 also be repeated here that the last common ancestor of the MTBC was likely a strain that 414 resembled M. canettii, which underwent a phenomenal clonal expansion by becoming a 415 dedicated pathogen. It is well known that the MTBC besides M. tuberculosis also harbors 416 very closely related relatives that infect selected other mammalian species than humans as 417 their preferential hosts. Interestingly, these animal-adapted species seem to have evolved from 418 a sublineage of the *M. tuberculosis* complex, which is characterized by the deletion of the 419 region RD9 (Brosch et al., 2002; Orgeur and Brosch, 2018), and also comprises the two M. 420 africanum lineages L5 and L6, which cause tuberculosis in humans in West Africa (Ates et 421 al., 2018a; de Jong et al., 2010; Otchere et al., 2018). As such, M. africanum strains seem to 422 be closely related to the common ancestor of animal strains and it will be of particular interest 423 to determine which molecular factors have been involved in the adaptation processes of these 424 tubercle bacilli to new animal hosts (Gonzalo-Asensio et al., 2014; Malone et al., 2018; 425 Orgeur and Brosch, 2018). One surprising feature in this respect is the long-time known 426 finding that *M. tuberculosis* in contrast to *M. bovis* does not cause disease in cattle, which was 427 recently re-established in an advanced cattle infection model (Villarreal-Ramos et al., 2018). 428 These are just another few examples of how complex the relationship between tubercle bacilli 429 and their various hosts are. Similar processes have certainly also contributed to the emergence 430 of *M. tuberculosis* strains from probably less virulent and much less prevalent ancestor pools 431 (resembling *M. canettii* strains) into the professional human pathogens that they represent 432 today. Without doubt, such factors make up the highly complex mosaic scaffold of 433 mycobacterial virulence, for which we have tried to highlight some of the key players that we 434 consider having an important impact on virulence. This said, there is certainly much more 435 room for studying further virulence factors of *M. tuberculosis*, as well as host immunity 436 factors that have not been covered in this review, but which can also contribute strongly to 437 advance or restrict disease progression, and thereby influence the outcome of infection. The 438 particularities of TB as disease in relation to the host and the extremely wide, and often 439 specific distribution of certain *M. tuberculosis* strain families in the global human population 440 make the infection dynamics associated to TB very complex. Research on TB-causing 441 mycobacterial pathogens will therefore need to continue for a better understanding of the 442 evolutionary adaptation processes that made *M. tuberculosis* such a powerful and devastating 443 pathogen and for finding new possible solutions to counteract this situation.

444

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454 **References**

- Abdallah, A.M., Gey van Pittius, N.C., DiGiuseppe Champion, P.A., Cox, J., Luirink, J.,
 Vandenbroucke-Grauls, C.M.J.E., Appelmelk, B.J., Bitter, W., 2007. Type VII
 secretion mycobacteria show the way. Nat. Rev. Microbiol. 5, 883–891.
 https://doi.org/10.1038/nrmicro1773
- Abdallah, A.M., Savage, N.D.L., Zon, M. van, Wilson, L., Vandenbroucke-Grauls, C.M.J.E.,
 Wel, N.N. van der, Ottenhoff, T.H.M., Bitter, W., 2008. The ESX-5 Secretion System
 of *Mycobacterium marinum* Modulates the Macrophage Response. J. Immunol. 181,
 7166–7175. https://doi.org/10.4049/jimmunol.181.10.7166
- Abdallah, A.M., Verboom, T., Weerdenburg, E.M., Gey van Pittius, N.C., Mahasha, P.W., 463 464 Jiménez, C., Parra, M., Cadieux, N., Brennan, M.J., Appelmelk, B.J., Bitter, W., 2009. 465 PPE and PE_PGRS proteins of Mycobacterium marinum are transported via the type 466 ESX-5. VII secretion system Mol. Microbiol. 73. 329-340. 467 https://doi.org/10.1111/j.1365-2958.2009.06783.x
- Afonso-Barroso, A., Clark, S.O., Williams, A., Rosa, G., Nóbrega, C., Silva-Gomes, S., ValeCosta, S., Ummels, R., Stoker, N., Movahedzadeh, F., der Ley, P., Sloots, A., Cot, M.,
 Appelmelk, B.J., Puzo, G., Nigou, J., Geurtsen, J., Appelberg, R., 2012.
 Lipoarabinomannan mannose caps do not affect mycobacterial virulence or the
 induction of protective immunity in experimental animal models of infection and have
 minimal impact on in vitro inflammatory responses. Cell. Microbiol. 15, 660–674.
 https://doi.org/10.1111/cmi.12065
- Aguilo, J.I., Alonso, H., Uranga, S., Marinova, D., Arbués, A., Martino, A., Anel, A.,
 Monzon, M., Badiola, J., Pardo, J., Brosch, R., Martin, C., 2013. ESX-1-induced
 apoptosis is involved in cell-to-cell spread of *Mycobacterium tuberculosis*. Cell.
 Microbiol. 15, 1994–2005. https://doi.org/10.1111/cmi.12169
- Aguilo, N., Gonzalo-Asensio, J., Alvarez-Arguedas, S., Marinova, D., Gomez, A.B., Uranga,
 S., Spallek, R., Singh, M., Audran, R., Spertini, F., Martin, C., 2017. Reactogenicity to
 major tuberculosis antigens absent in BCG is linked to improved protection against *Mycobacterium tuberculosis*. Nat Commun. 8, 16085.
 https://doi.org/10.1038/ncomms16085.
- Angala, S. kumar, McNeil, M.R., Shi, L., Joe, M., Pham, H., Zuberogoitia, S., Nigou, J.,
 Boot, C.M., Lowary, T.L., Gilleron, M., Jackson, M., 2017. Biosynthesis of the
 Methylthioxylose Capping Motif of Lipoarabinomannan in *Mycobacterium tuberculosis*. ACS Chem. Biol. 12, 682–691.
 https://doi.org/10.1021/acschembio.6b01071

- Astarie-Dequeker, C., Guyader, L.L., Malaga, W., Seaphanh, F.-K., Chalut, C., Lopez, A.,
 Guilhot, C., 2009. Phthiocerol dimycocerosates of *M. tuberculosis* participate in
 macrophage invasion by inducing changes in the organization of plasma membrane
 lipids. PLOS Pathog. 5, e1000289. https://doi.org/10.1371/journal.ppat.1000289
- Ates, L.S., Dippenaar, A., Sayes, F., Pawlik, A., Bouchier, C., Ma,L., Warren, R.M.,
 Sougakoff, W., Majlessi, L., van Heijst, J.W.J., Brossier,F., Brosch, R. 2018a.
 Unexpected genomic and phenotypic diversity of *Mycobacterium africanum* Lineage 5
 affects drug resistance, protein secretion and immunogenicity. Genome Biol Evol,
 10,1858-1874, https://doi.org/10.1093/gbe/evy145.
- Ates, L.S., Dippenaar, A., Ummels, R., Piersma, S.R., Woude, A.D. van der, Kuij, K. van der,
 Chevalier, F.L., Mata-Espinosa, D., Barrios-Payán, J., Marquina-Castillo, B.,
 Guapillo, C., Jiménez, C.R., Pain, A., Houben, E.N.G., Warren, R.M., Brosch, R.,
 Hernández-Pando, R., Bitter, W., 2018b. Mutations in *ppe38* block PE_PGRS
 secretion and increase virulence of *Mycobacterium tuberculosis*. Nat. Microbiol. 3,
 181–188. https://doi.org/10.1038/s41564-017-0090-6
- Ates, L.S., Sayes, F., Frigui, W., Ummels, R., Damen, M.P.M., Bottai, D., Behr, M.A., van
 Heijst, J.W.J., Bitter, W., Majlessi, L., Brosch, R., 2018c. RD5 deletion-mediated lack
 of PE_PGRS and PPE-MPTR 1 export in BCG vaccine strains results in strong
 reduction of antigenic repertoire but little impact on protection. PLoS Pathog 14,
 e1007139, https://doi.org/10.1371/journal.ppat.1007139
- 509 Ates, L.S., Ummels, R., Commandeur, S., van de Weerd, R., van der Weerd, R., Sparrius, M., 510 Weerdenburg, E., Alber, M., Kalscheuer, R., Piersma, S.R., Abdallah, A.M., Abd El Ghany, M., Abdel-Haleem, A.M., Pain, A., Jiménez, C.R., Bitter, W., Houben, 511 E.N.G., 2015. Essential role of the ESX-5 secretion system in outer membrane 512 513 permeability of pathogenic mycobacteria. PLoS Genet. 11, e1005190. 514 https://doi.org/10.1371/journal.pgen.1005190
- Augenstreich, J., Arbues, A., Simeone, R., Haanappel, E., Wegener, A., Sayes, F., Le
 Chevalier, F., Chalut, C., Malaga, W., Guilhot, C., Brosch, R., Astarie-Dequeker, C.,
 2017. ESX-1 and phthiocerol dimycocerosates of *Mycobacterium tuberculosis* act in
 concert to cause phagosomal rupture and host cell apoptosis. Cell. Microbiol. 19, n/an/a. https://doi.org/10.1111/cmi.12726
- Barkan, D., Hedhli, D., Yan, H.-G., Huygen, K., Glickman, M.S., 2012. *Mycobacterium tuberculosis* lacking all mycolic acid cyclopropanation is viable but highly attenuated and hyperinflammatory in mice. Infect. Immun. 80, 1958–1968. https://doi.org/10.1128/IAI.00021-12
- Barnes, P.F., Mehra, V., Rivoire, B., Fong, S.J., Brennan, P.J., Voegtline, M.S., Minden, P.,
 Houghten, R.A., Bloom, B.R., Modlin, R.L., 1992. Immunoreactivity of a 10-kDa
 antigen of *Mycobacterium tuberculosis*. J. Immunol. 148, 1835–1840.
- Barry, C.E. III, Lee, R.E., Mdluli, K., Sampson, A.E., Schroeder, B.G., Slayden, R.A., Yuan,
 Y., 1998. Mycolic acids: structure, biosynthesis and physiological functions. Prog.
 Lipid Res. 37, 143–179. https://doi.org/10.1016/S0163-7827(98)00008-3
- Beckham, K.S.H., Ciccarelli, L., Bunduc, C.M., Mertens, H.D.T., Ummels, R., Lugmayr, W.,
 Mayr, J., Rettel, M., Savitski, M.M., Svergun, D.I., Bitter, W., Wilmanns, M.,
 Marlovits, T.C., Parret, A.H.A., Houben, E.N.G., 2017. Structure of the mycobacterial
 ESX-5 type VII secretion system membrane complex by single-particle analysis. Nat.
 Microbiol. 2, 17047. https://doi.org/10.1038/nmicrobiol.2017.47
- Bekierkunst, A., 1968. Acute granulomatous response produced in mice by trehalose-6,6 dimycolate. J. Bacteriol. 96, 958–961.

- Belisle, J.T., Vissa, V.D., Sievert, T., Takayama, K., Brennan, P.J., Besra, G.S., 1997. role of
 the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. Science 276,
 1420–1422. https://doi.org/10.1126/science.276.5317.1420
- Berthet, F.-X., Rasmussen, P.B., Rosenkrands, I., Andersen, P., Gicquel, B., 1998. A *Mycobacterium tuberculosis* operon encoding ESAT-6 and a novel low-molecularmass culture filtrate protein (CFP-10). Microbiology 144, 3195–3203.
 https://doi.org/10.1099/00221287-144-11-3195
- 544 Bhatt, A., Fujiwara, N., Bhatt, K., Gurcha, S.S., Kremer, L., Chen, B., Chan, J., Porcelli, S.A., 545 Kobayashi, K., Besra, G.S., Jacobs, W.R., 2007. Deletion of kasB in Mycobacterium 546 tuberculosis causes loss of acid-fastness and subclinical latent tuberculosis in 547 Acad. Sci. immunocompetent mice. Proc. Natl. 104. 5157-5162. 548 https://doi.org/10.1073/pnas.0608654104
- 549 Blanc, L., Gilleron, M., Prandi, J., Song, O., Jang, M.-S., Gicquel, B., Drocourt, D., 550 Neyrolles, O., Brodin, P., Tiraby, G., Vercellone, A., Nigou, J., 2017. Mycobacterium 551 tuberculosis inhibits human innate immune responses via the production of TLR2 552 antagonist glycolipids. Proc. Natl. Acad. Sci. 114, 11205-11210. 553 https://doi.org/10.1073/pnas.1707840114
- Bloch, H., Noll, H., 1955. Studies on the virulence of tubercle bacilli. The effect of cord
 factor on murine tuberculosis. Br. J. Exp. Pathol. 36, 8–17.
- Boritsch, E.C., Frigui, W., Cascioferro, A., Malaga, W., Etienne, G., Laval, F., Pawlik, A., Le
 Chevalier, F., Orgeur, M., Ma, L., Bouchier, C., Stinear, T.P., Supply, P., Majlessi, L.,
 Daffé, M., Guilhot, C., Brosch, R., 2016a. *pks5*-recombination-mediated surface
 remodelling in *Mycobacterium tuberculosis* emergence. Nat. Microbiol. 1, 15019.
 https://doi.org/10.1038/nmicrobiol.2015.19
- Boritsch, E.C., Khanna, V., Pawlik, A., Honore, N., Navas, V.H., Ma, L., Bouchier, C.,
 Seemann, T., Supply, P., Stinear, T.P., Brosch, R., 2016b. Key experimental evidence
 of chromosomal DNA transfer among selected tuberculosis-causing mycobacteria.
 Proc Natl Acad Sci U S A. 113, 9876-9881. doi: 9810.1073/pnas.1604921113.
- Bos, K.I., Harkins, K.M., Herbig, A., Coscolla, M., Weber, N., Comas, I., Forrest, S.A.,
 Bryant, J.M., Harris, S.R., Schuenemann, V.J., Campbell, T.J., Majander, K., Wilbur,
 A.K., Guichon, R.A., Steadman, D.L.W., Cook, D.C., Niemann, S., Behr, M.A.,
 Zumarraga, M., Bastida, R., Huson, D., Nieselt, K., Young, D., Parkhill, J., Buikstra,
 J.E., Gagneux, S., Stone, A.C., Krause, J., 2014. Pre-Columbian mycobacterial
 genomes reveal seals as a source of New World human tuberculosis. Nature 514, 494–
 497. https://doi.org/10.1038/nature13591
- Bottai, D., Di Luca, M., Majlessi, L., Frigui, W., Simeone, R., Sayes, F., Bitter, W., Brennan,
 M.J., Leclerc, C., Batoni, G., Campa, M., Brosch, R., Esin, S., 2012. Disruption of the
 ESX-5 system of *Mycobacterium tuberculosis* causes loss of PPE protein secretion,
 reduction of cell wall integrity and strong attenuation. Mol. Microbiol. 83, 1195–1209.
 https://doi.org/10.1111/j.1365-2958.2012.08001.x
- 577 Brennan, P.J., Nikaido, H., 1995. The Envelope of Mycobacteria. Annu. Rev. Biochem. 64,
 578 29–63. https://doi.org/10.1146/annurev.bi.64.070195.000333
- Brodin, P., Eiglmeier, K., Marmiesse, M., Billault, A., Garnier, T., Niemann, S., Cole, S.T.,
 Brosch, R., 2002. bacterial artificial chromosome-based comparative genomic analysis
 identifies *Mycobacterium microti* as a natural ESAT-6 deletion mutant. Infect. Immun.
 70, 5568–5578. https://doi.org/10.1128/IAI.70.10.5568-5578.2002
- Brodin, P., Rosenkrands, I., Andersen, P., Cole, S.T., Brosch, R., 2004. ESAT-6 proteins:
 protective antigens and virulence factors? Trends Microbiol. 12, 500–508.
 https://doi.org/10.1016/j.tim.2004.09.007

- Brosch, R., Gordon, S.V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., Garnier,
 T., Gutierrez, C., Hewinson, G., Kremer, K., Parsons, L.M., Pym, A.S., Samper, S.,
 Soolingen, D. van, Cole, S.T., 2002. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proc. Natl. Acad. Sci. 99, 3684–3689.
 https://doi.org/10.1073/pnas.052548299
- 591 Camacho, L.R., Constant, P., Raynaud, C., Lanéelle, M.-A., Triccas, J.A., Gicquel, B., Daffé, 592 M., Guilhot, C., 2001. Analysis of the phthiocerol dimycocerosate locus of 593 Mycobacterium tuberculosis: evidence that this lipid is involved in the cell wall 594 permeability barrier. Biol. Chem. 276, 19845-19854. J. 595 https://doi.org/10.1074/jbc.M100662200
- Cantaloube, S., Veyron-Churlet, R., Haddache, N., Daffé, M., Zerbib, D., 2011. The
 Mycobacterium tuberculosis FAS-II dehydratases and methyltransferases define the
 specificity of the mycolic acid elongation complexes. PLOS ONE 6, e29564.
 https://doi.org/10.1371/journal.pone.0029564
- Chatterjee, D., Lowell, K., Rivoire, B., McNeil, M.R., Brennan, P.J., 1992.
 Lipoarabinomannan of *Mycobacterium tuberculosis*. Capping with mannosyl residues
 in some strains. J. Biol. Chem. 267, 6234–6239.https://doi.org/10.1046/j.14625822.2001.00110.x
- 604 Chiaradia, L., Lefebvre, C., Parra, J., Marcoux, J., Burlet-Schiltz, O., Etienne, G., Tropis, M.,
 605 Daffé, M., 2017. Dissecting the mycobacterial cell envelope and defining the
 606 composition of the native mycomembrane. Sci Rep. 7, 12807.
 607 https://doi.org/10.1038/s41598-017-12718-4.
- Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S.V., 608 609 Eiglmeier, K., Gas, S., Barry, C.E., Tekaia, F., Badcock, K., Basham, D., Brown, D., 610 Chillingworth, T., Connor, R., Davies, R., Devlin, K., Feltwell, T., Gentles, S., 611 Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Krogh, A., McLean, J., Moule, S., Murphy, L., Oliver, K., Osborne, J., Quail, M.A., Rajandream, M.-A., Rogers, J., 612 613 Rutter, S., Seeger, K., Skelton, J., Squares, R., Squares, S., Sulston, J.E., Taylor, K., 614 Whitehead, S., Barrell, B.G., 1998. Deciphering the biology of Mycobacterium 615 tuberculosis from the complete genome sequence. Nature 393, 537-544. 616 https://doi.org/10.1038/31159
- 617 Collins, A.C., Cai, H., Li, T., Franco, L.H., Li, X.-D., Nair, V.R., Scharn, C.R., Stamm, C.E.,
 618 Levine, B., Chen, Z.J., Shiloh, M.U., 2015. Cyclic GMP-AMP synthase is an innate
 619 immune DNA sensor for *Mycobacterium tuberculosis*. Cell Host Microbe 17, 820–
 620 828. https://doi.org/10.1016/j.chom.2015.05.005
- Comas, I., Coscolla, M., Luo, T., Borrell, S., Holt, K.E., Kato-Maeda, M., Parkhill, J., Malla,
 B., Berg, S., Thwaites, G., Yeboah-Manu, D., Bothamley, G., Mei, J., Wei, L.,
 Bentley, S., Harris, S.R., Niemann, S., Diel, R., Aseffa, A., Gao, Q., Young, D.,
 Gagneux, S., 2013. Out-of-Africa migration and Neolithic coexpansion of *Mycobacterium tuberculosis* with modern humans. Nat. Genet. 45, 1176–1182.
 https://doi.org/10.1038/ng.2744
- Conrad, W.H., Osman, M.M., Shanahan, J.K., Chu, F., Takaki, K.K., Cameron, J.,
 Hopkinson-Woolley, D., Brosch, R., Ramakrishnan, L., 2017. Mycobacterial ESX-1
 secretion system mediates host cell lysis through bacterium contact-dependent gross
 membrane disruptions. Proc. Natl. Acad. Sci. U S A. 114, 1371–1376.
 https://doi.org/10.1073/pnas.1620133114
- Constant, P., Perez, E., Malaga, W., Lanéelle, M.-A., Saurel, O., Daffé, M., Guilhot, C., 2002.
 Role of the *pks15/1* Gene in the biosynthesis of phenolglycolipids in the
 Mycobacterium tuberculosis complex: evidence that all strains synthesize glycosylated
 p-hydroxybenzoic methyl esters and that strains devoid of phenolglycolipids harbor a

- 636 frameshift mutation in the pks15/1 gene. J. Biol. Chem. 277, 38148–38158.
 637 https://doi.org/10.1074/jbc.M206538200
- Crowe, A.M., Casabon, I., Brown, K.L., Liu, J., Lian, J., Rogalski, J.C., Hurst, T.E., Snieckus,
 V., Foster, L.J., Eltis, L.D., 2017. catabolism of the last two steroid rings in *Mycobacterium tuberculosis* and other bacteria. mBio 8, e00321-17.
 https://doi.org/10.1128/mBio.00321-17
- 642 Crowe, A.M., Workman, S.D., Watanabe, N., Worrall, L.J., Strynadka, N.C.J., Eltis, L.D.,
 643 2018. IpdAB, a virulence factor in *Mycobacterium tuberculosis*, is a cholesterol ring644 cleaving hydrolase. Proc. Natl. Acad. Sci. 201717015.
 645 https://doi.org/10.1073/pnas.1717015115
- Daffé, M., Draper, P., 1997. The envelope layers of mycobacteria with reference to their
 pathogenicity, in: Poole, R.K. (Ed.), Advances in microbial physiology. Academic
 Press, pp. 131–203. https://doi.org/10.1016/S0065-2911(08)60016-8
- Daniel, J., Maamar, H., Deb, C., Sirakova, T.D., Kolattukudy, P.E., 2011. *Mycobacterium tuberculosis* uses host triacylglycerol to accumulate lipid droplets and acquires a dormancy-like phenotype in lipid-loaded macrophages. PLOS Pathog. 7, e1002093. https://doi.org/10.1371/journal.ppat.1002093
- Danilchanka, O., Sun, J., Pavlenok, M., Maueröder, C., Speer, A., Siroy, A., Marrero, J.,
 Trujillo, C., Mayhew, D.L., Doornbos, K.S., Muñoz, L.E., Herrmann, M., Ehrt, S.,
 Berens, C., Niederweis, M., 2014. An outer membrane channel protein of *Mycobacterium tuberculosis* with exotoxin activity. Proc. Natl. Acad. Sci. 111, 6750–
 6755. https://doi.org/10.1073/pnas.1400136111
- Dao, D.N., Sweeney, K., Hsu, T., Gurcha, S.S., Nascimento, I.P., Roshevsky, D., Besra, G.S.,
 Chan, J., Porcelli, S.A., Jacobs, W.R., 2008. Mycolic acid modification by the *mmaA4*gene of *M. tuberculosis* modulates IL-12 production. PLoS Pathog. 4.
 https://doi.org/10.1371/journal.ppat.1000081
- de Jong, B.C., Antonio, M., Gagneux, S., 2010. *Mycobacterium africanum*—review of an
 important cause of human tuberculosis in West Africa. PLoS Negl. Trop. Dis. 4, e744.
 https://doi.org/10.1371/journal.pntd.0000744
- Degiacomi, G., Benjak, A., Madacki, J., Boldrin, F., Provvedi, R., Palù, G., Kordulakova, J.,
 Cole, S.T., Manganelli, R., 2017. Essentiality of *mmpL3* and impact of its silencing on *Mycobacterium tuberculosis* gene expression. Sci. Rep. 7, 43495.
 https://doi.org/10.1038/srep43495
- Dey, B., Dey, R.J., Cheung, L.S., Pokkali, S., Guo, H., Lee, J.-H., Bishai, W.R., 2015. A
 bacterial cyclic dinucleotide activates the cytosolic surveillance pathway and mediates
 innate resistance to tuberculosis. Nat. Med. 21, 401–406.
 https://doi.org/10.1038/nm.3813
- Dey, R.J., Dey, B., Zheng, Y., Cheung, L.S., Zhou, J., Sayre, D., Kumar, P., Guo, H.,
 Lamichhane, G., Sintim, H.O., Bishai, W.R., 2017. Inhibition of innate immune
 cytosolic surveillance by an *M. tuberculosis* phosphodiesterase. Nat. Chem. Biol. 13,
 210–217. https://doi.org/10.1038/nchembio.2254
- Di Luca, M., Bottai, D., Batoni, G., Orgeur, M., Aulicino, A., Counoupas, C., Campa, M.,
 Brosch, R., Esin, S., 2012. The ESX-5 Associated *eccB5-eccC5* locus is essential for *Mycobacterium tuberculosis* viability. PLOS ONE 7, e52059.
 https://doi.org/10.1371/journal.pone.0052059
- Dkhar, H.K., Nanduri, R., Mahajan, S., Dave, S., Saini, A., Somavarapu, A.K., Arora, A.,
 Parkesh, R., Thakur, K.G., Mayilraj, S., Gupta, P., 2014. *Mycobacterium tuberculosis*keto-mycolic acid and macrophage nuclear receptor TR4 modulate foamy biogenesis
 in granulomas: A case of a heterologous and noncanonical ligand-receptor pair. J.
 Immunol. 193, 295–305. https://doi.org/10.4049/jimmunol.1400092

- Dubnau, E., Chan, J., Raynaud, C., Mohan, V.P., Lanéelle, M.-A., Yu, K., Quémard, A.,
 Smith, I., Daffé, M., 2000. Oxygenated mycolic acids are necessary for virulence of *Mycobacterium tuberculosis* in mice. Mol. Microbiol. 36, 630–637.
 https://doi.org/10.1046/j.1365-2958.2000.01882.x
- Dumas, E., Christina Boritsch, E., Vandenbogaert, M., Vega, R. de la, C, R., Thiberge, J.-M.,
 Caro, V., Gaillard, J.-L., Heym, B., Girard-Misguich, F., Brosch, R., Sapriel, G., 2016.
 mycobacterial pan-genome analysis suggests important role of plasmids in the
 radiation of type VII secretion systems. Genome Biol. Evol. 8, 387–402.
 https://doi.org/10.1093/gbe/evw001
- Ekiert, D.C., Bhabha, G., Isom, G.L., Greenan, G., Ovchinnikov, S., Henderson, I.R., Cox,
 J.S., Vale, R.D. 2017. architectures of lipid transport systems for the bacterial outer
 membrane. Cell. 169(2):273-285.e17. doi: 10.1016/j.cell.2017.03.019.
- Fratti, R.A., Chua, J., Vergne, I., Deretic, V., 2003. *Mycobacterium tuberculosis* glycosylated
 phosphatidylinositol causes phagosome maturation arrest. Proc. Natl. Acad. Sci. 100,
 5437–5442. https://doi.org/10.1073/pnas.0737613100
- Geiman, D.E., Raghunand, T.R., Agarwal, N., Bishai, W.R., 2006. Differential gene expression in response to exposure to antimycobacterial agents and other stress conditions among seven *Mycobacterium tuberculosis whiB*-like genes. Antimicrob.
 Agents Chemother. 50, 2836–2841. https://doi.org/10.1128/AAC.00295-06
- Gey van Pittius, N.C., Gamieldien, J., Hide, W., Brown, G.D., Siezen, R.J., Beyers, A.D.,
 2001. The ESAT-6 gene cluster of *Mycobacterium tuberculosis* and other high G+C
 Gram-positive bacteria. Genome Biol. 2, research0044.1-research0044.18.
- Gey van Pittius, N.C., Sampson, S.L., Lee, H., Kim, Y., van Helden, P.D., Warren, R.M.,
 2006. Evolution and expansion of the *Mycobacterium tuberculosis* PE and PPE
 multigene families and their association with the duplication of the ESAT-6 (esx) gene
 cluster regions. BMC Evol. Biol. 6, 95. https://doi.org/10.1186/1471-2148-6-95
- 712 Gonzalo-Asensio, J., Malaga, W., Pawlik, A., Astarie-Dequeker, C., Passemar, C., Moreau, 713 F., Laval, F., Daffé, M., Martin, C., Brosch, R., Guilhot, C., 2014. Evolutionary 714 history of tuberculosis shaped by conserved mutations in the PhoPR virulence 715 regulator. Proc. Natl. Acad. Sci. 111. 11491-11496. 716 https://doi.org/10.1073/pnas.1406693111
- Gonzalo-Asensio, J., Pérez, I., Aguiló, N., Uranga, S., Picó, A., Lampreave, C., Cebollada, 717 718 A., Otal, I., Samper, S., Martín, C., 2018. New insights into the transposition 719 mechanisms of IS6110 and its dynamic distribution between Mycobacterium 720 Complex lineages. Genet. tuberculosis PLOS 14, e1007282. https://doi.org/10.1371/journal.pgen.1007282 721
- Gordon, S.V., Brosch, R., Billault, A., Garnier, T., Eiglmeier, K., Cole, S.T., 1999.
 Identification of variable regions in the genomes of tubercle bacilli using bacterial artificial chromosome arrays. Mol. Microbiol. 32, 643–655.
 https://doi.org/10.1046/j.1365-2958.1999.01383.x
- Gröschel, M.I., Sayes, F., Shin, S.J., Frigui, W., Pawlik, A., Orgeur, M., Canetti, R., Honoré,
 N., Simeone, R., van der Werf, T.S., Bitter, W., Cho, S.-N., Majlessi, L., Brosch, R.,
 2017. Recombinant BCG expressing ESX-1 of *Mycobacterium marinum* combines
 low virulence with cytosolic immune signaling and improved TB protection. Cell Rep.
 18, 2752–2765. https://doi.org/10.1016/j.celrep.2017.02.057
- Gröschel, M.I., Sayes, F., Simeone, R., Majlessi, L., Brosch, R., 2016. ESX secretion
 systems: mycobacterial evolution to counter host immunity. Nat. Rev. Microbiol. 14,
 677–691. https://doi.org/10.1038/nrmicro.2016.131
- Grzegorzewicz, A.E., Korduláková, J., Jones, V., Born, S.E.M., Belardinelli, J.M., Vaquié,
 A., Gundi, V.A.K.B., Madacki, J., Slama, N., Laval, F., Vaubourgeix, J., Crew, R.M.,

- Gicquel, B., Daffé, M., Morbidoni, H.R., Brennan, P.J., Quémard, A., McNeil, M.R.,
 Jackson, M., 2012a. A common mechanism of inhibition of the *Mycobacterium tuberculosis* mycolic acid biosynthetic pathway by isoxyl and thiacetazone. J. Biol.
 Chem. 287, 38434–38441. https://doi.org/10.1074/jbc.M112.400994
- Grzegorzewicz, A.E., Pham, H., Gundi, V.A.K.B., Scherman, M.S., North, E.J., Hess, T.,
 Jones, V., Gruppo, V., Born, S.E.M., Korduláková, J., Chavadi, S.S., Morisseau, C.,
 Lenaerts, A.J., Lee, R.E., McNeil, M.R., Jackson, M., 2012b. Inhibition of mycolic
 acid transport across the *Mycobacterium tuberculosis* plasma membrane. Nat. Chem.
 Biol. 8, 334–341. https://doi.org/10.1038/nchembio.794
- Horwitz, M.A., Lee, B.W., Dillon, B.J., Harth, G., 1995. Protective immunity against
 tuberculosis induced by vaccination with major extracellular proteins of *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci. U. S. A. 92, 1530–1534.
- 748 Houben, D., Demangel, C., van Ingen, J., Perez, J., Baldeón, L., Abdallah Abdallah, M., 749 Caleechurn, L., Bottai, D., van Zon, M., de Punder, K., van der Laan, T., Kant, A., 750 Bossers-de Vries, R., Willemsen, P., Bitter, W., van Soolingen, D., Brosch, R., van der 751 Wel, N., Peters, P., J., 2012. ESX-1-mediated translocation to the cytosol controls 752 mycobacteria. Microbiol. virulence of Cell. 14, 1287-1298. 753 https://doi.org/10.1111/j.1462-5822.2012.01799.x
- Houben, E.N.G., Bestebroer, J., Ummels, R., Wilson, L., Piersma, S.R., Jiménez, C.R.,
 Ottenhoff, T.H.M., Luirink, J., Bitter, W., 2012. Composition of the type VII secretion
 system membrane complex. Mol. Microbiol. 86, 472–484.
 https://doi.org/10.1111/j.1365-2958.2012.08206.x
- Hsu, T., Hingley-Wilson, S.M., Chen, B., Chen, M., Dai, A.Z., Morin, P.M., Marks, C.B.,
 Padiyar, J., Goulding, C., Gingery, M., Eisenberg, D., Russell, R.G., Derrick, S.C.,
 Collins, F.M., Morris, S.L., King, C.H., Jacobs, W.R., 2003. The primary mechanism
 of attenuation of bacillus Calmette–Guérin is a loss of secreted lytic function required
 for invasion of lung interstitial tissue. Proc. Natl. Acad. Sci. 100, 12420–12425.
 https://doi.org/10.1073/pnas.1635213100
- Huet, G., Constant, P., Malaga, W., Lanéelle, M.-A., Kremer, K., Soolingen, D. van, Daffé,
 M., Guilhot, C., 2009. A Lipid Profile Typifies the Beijing Strains of *Mycobacterium tuberculosis*: identification of a mutation responsible for a modification of the
 structures of phthiocerol dimycocerosates and phenolic glycolipids.. J. Biol. Chem.
 284, 27101–27113. https://doi.org/10.1074/jbc.M109.041939
- Indrigo, J., Hunter, R.L., Actor, J.K., 2003. Cord factor trehalose 6,6'-dimycolate (TDM)
 mediates trafficking events during mycobacterial infection of murine macrophages.
 Microbiology 149, 2049–2059. https://doi.org/10.1099/mic.0.26226-0
- Ishikawa, E., Ishikawa, T., Morita, Y.S., Toyonaga, K., Yamada, H., Takeuchi, O., Kinoshita,
 T., Akira, S., Yoshikai, Y., Yamasaki, S., 2009. Direct recognition of the
 mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J. Exp. Med.
 206, 2879–2888. https://doi.org/10.1084/jem.20091750
- Jackson, M., Raynaud, C., Lanéelle, M.-A., Guilhot, C., Laurent-Winter, C., Ensergueix, D.,
 Gicquel, B., Daffé, M., 1999. Inactivation of the antigen 85C gene profoundly affects
 the mycolate content and alters the permeability of the *Mycobacterium tuberculosis*cell envelope. Mol. Microbiol. 31, 1573–1587. https://doi.org/10.1046/j.13652958.1999.01310.x
- 781 Joe, M., Sun, D., Taha, H., Completo, G.C., Croudace, J.E., Lammas, D.A., Besra, G.S., Lowary, T.L., 2006. the 5-deoxy-5-methylthio-xylofuranose residue in mycobacterial 782 lipoarabinomannan. absolute stereochemistry, linkage position, conformation, and 783 784 immunomodulatory activity. J. Am. Chem. Soc. 5059-5072. 128, 785 https://doi.org/10.1021/ja057373q

- Katti, M.K., Dai, G., Armitige, L.Y., Marrero, C.R., Daniel, S., Singh, C.R., Lindsey, D.R.,
 Dhandayuthapani, S., Hunter, R.L., Jagannath, C., 2008. The Δ*fbpA* mutant derived
 from *Mycobacterium tuberculosis* H37Rv has an enhanced susceptibility to
 intracellular antimicrobial oxidative mechanisms, undergoes limited phagosome
 maturation and activates macrophages and dendritic cells. Cell. Microbiol. 10, 1286–
 1303. https://doi.org/10.1111/j.1462-5822.2008.01126.x
- Kay, G.L., Sergeant, M.J., Zhou, Z., Chan, J.Z., Millard, A., Quick, J., Szikossy, I., Pap, I.,
 Spigelman, M., Loman, N.J., Achtman, M., Donoghue, H.D., Pallen, M.J. 2015.
 Eighteenth-century genomes show that mixed infections were common at time of peak
 tuberculosis in Europe.Nat Commun. 6:6717. doi: 10.1038/ncomms7717.
- Khan, M.Z., Bhaskar, A., Upadhyay, S., Kumari, P., Rajmani, R.S., Jain, P., Singh, A.,
 Kumar, D., Bhavesh, N.S., Nandicoori, V.K., 2017. Protein kinase G confers survival
 advantage to *Mycobacterium tuberculosis* during latency-like conditions. J. Biol.
 Chem. 292, 16093–16108. https://doi.org/10.1074/jbc.M117.797563
- Lee, W., VanderVen, B.C., Fahey, R.J., Russell, D.G., 2013. Intracellular *Mycobacterium tuberculosis* exploits host-derived fatty acids to limit metabolic stress. J. Biol. Chem.
 288, 6788–6800. https://doi.org/10.1074/jbc.M112.445056
- Levillain, F., Poquet, Y., Mallet, L., Mazères, S., Marceau, M., Brosch, R., Bange, F.-C.,
 Supply, P., Magalon, A., Neyrolles, O., 2017. Horizontal acquisition of a hypoxiaresponsive molybdenum cofactor biosynthesis pathway contributed to *Mycobacterium tuberculosis* pathoadaptation. PLOS Pathog. 13, e1006752.
 https://doi.org/10.1371/journal.ppat.1006752
- Lou, Y., Rybniker, J., Sala, C., Cole, S.T., 2017. EspC forms a filamentous structure in the
 cell envelope of *Mycobacterium tuberculosis* and impacts ESX-1 secretion. Mol
 Microbiol. 103, 26-38. https://doi.org/10.1111/mmi.13575
- Madacki, J., Laval, F., Grzegorzewicz, A., Lemassu, A., Záhorszká, M., Arand, M., McNeil,
 M., Daffé, M., Jackson, M., Lanéelle, M.-A., Korduláková, J., 2018. Impact of the
 epoxide hydrolase EphD on the metabolism of mycolic acids in mycobacteria. J. Biol.
 Chem. 293, 5172–5184. https://doi.org/10.1074/jbc.RA117.000246
- Mahairas, G.G., Sabo, P.J., Hickey, M.J., Singh, D.C., Stover, C.K., 1996. Molecular analysis
 of genetic differences between *Mycobacterium bovis* BCG and virulent *M. bovis*. J.
 Bacteriol. 178, 1274–1282. https://doi.org/10.1128/jb.178.5.1274-1282.1996
- Majlessi, L., Prados-Rosales, R., Casadevall, A., Brosch, R., 2015. Release of mycobacterial
 antigens. Immunol. Rev. 264, 25–45. https://doi.org/10.1111/imr.12251
- Malone, K.M., Rue-Albrecht, K., Magee, D.A., Conlon, K., Schubert, O.T., Nalpas, N.C.,
 Browne, J.A., Smyth, A., Gormley, E., Aebersold, R., MacHugh, D.E., Gordon, S.V.
 2018. Comparative 'omics analyses differentiate *Mycobacterium tuberculosis* and *Mycobacterium bovis* and reveal distinct macrophage responses to infection with the
 human and bovine tubercle bacilli. Microb Genom. doi: 10.1099/mgen.0.000163.
- Marjanovic, O., Miyata, T., Goodridge, A., Kendall, L.V., Riley, L.W., 2010. Mce2 operon
 mutant strain of *Mycobacterium tuberculosis* is attenuated in C57BL/6 mice.
 Tuberculosis 90, 50–56. https://doi.org/10.1016/j.tube.2009.10.004
- Maueröder, C., Chaurio, R.A., Dumych, T., Podolska, M., Lootsik, M.D., Culemann, S.,
 Friedrich, R.P., Bilyy, R., Alexiou, C., Schett, G., Berens, C., Herrmann, M., Munoz,
 L.E., 2016. A blast without power cell death induced by the tuberculosis-necrotizing
 toxin fails to elicit adequate immune responses. Cell Death Differ. 23, 1016–1025.
 https://doi.org/10.1038/cdd.2016.4
- McDonough, K.A., Kress, Y., Bloom, B.R., 1993. Pathogenesis of tuberculosis: interaction of
 Mycobacterium tuberculosis with macrophages. Infect. Immun. 61, 2763–2773.

- Mehra, A., Zahra, A., Thompson, V., Sirisaengtaksin, N., Wells, A., Porto, M., Köster, S.,
 Penberthy, K., Kubota, Y., Dricot, A., Rogan, D., Vidal, M., Hill, D.E., Bean, A.J.,
 Philips, J.A., 2013. *Mycobacterium tuberculosis* type VII secreted effector EsxH
 targets host ESCRT to impair trafficking. PLOS Pathog. 9, e1003734.
 https://doi.org/10.1371/journal.ppat.1003734
- Mokrousov, I., Shitikov, E., Skiba, Y., Kolchenko, S., Chernyaeva, E., Vyazovaya, A.2017.
 Emerging peak on the phylogeographic landscape of *Mycobacterium tuberculosis* in
 West Asia: Definitely smoke, likely fire. Mol Phylogenet Evol. 116:202-212. doi: 10.1016/j.ympev.2017.09.002.
- Nazarova, E.V., Montague, C.R., La, T., Wilburn, K.M., Sukumar, N., Lee, W., Caldwell, S.,
 Russell, D.G., VanderVen, B.C., 2017. Rv3723/LucA coordinates fatty acid and
 cholesterol uptake in *Mycobacterium tuberculosis*. eLife 6, e26969.
 https://doi.org/10.7554/eLife.26969
- 848 Ndiaye, B.P., Thienemann, F., Ota, M., Landry, B.S., Camara, M., Dièye, S., Dieye, T.N., 849 Esmail, H., Goliath, R., Huygen, K., January, V., Ndiaye, I., Oni, T., Raine, M., 850 Romano, M., Satti, I., Sutton, S., Thiam, A., Wilkinson, K.A., Mboup, S., Wilkinson, 851 R.J., McShane, H., 2015. Safety, immunogenicity, and efficacy of the candidate 852 tuberculosis vaccine MVA85A in healthy adults infected with HIV-1: a randomised, 853 placebo-controlled, phase 2 trial. Lancet Respir. Med. 3. 190-200. 854 https://doi.org/10.1016/S2213-2600(15)00037-5
- Newton-Foot, M., Warren, R.M., Sampson, S.L., van Helden, P.D., Gey van Pittius, N.C.,
 2016. The plasmid-mediated evolution of the mycobacterial ESX (Type VII) secretion
 systems. BMC Evol. Biol. 16, 62. https://doi.org/10.1186/s12862-016-0631-2
- Orgeur, M., Brosch, R., 2018. Evolution of virulence in the *Mycobacterium tuberculosis* complex. Curr. Opin. Microbiol., Host-pathogen interactions: Evolution and regulation
 of host pathogen interactions 41, 68–75. https://doi.org/10.1016/j.mib.2017.11.021
- Otchere, I.D., Coscolla, M., Sanchez-Buso, L., Asante-Poku, A., Brites, D., Loiseau, C.,
 Meehan, C., Osei-Wusu, S., Forson, A., Laryea, C., Yahayah, A.I., Baddoo, A., Ansa,
- 863 G.A., Aboagye, S.Y., Asare, P., Borrell, S., Gehre, F., Beckert, P., Kohl, T.A., N'Dira,
- 864 S., Beisel, C., Antonio, M., Niemann, S., de Jong, B.C., Parkhill, J., Harris, S.R.,
- 865 Gagneux, S., Yeboah-Manu, D., 2018. Comparative genomics of Mycobacterium
- africanum Lineage 5 and Lineage 6 from Ghana suggests distinct ecological niches.
 Sci Rep. 8, 11269. https://doi.org/10.1038/s41598-018-29620-2.
- 868 Pandey, A.K., Sassetti, C.M., 2008. Mycobacterial persistence requires the utilization of host
- 869 cholesterol. Proc. Natl. Acad. Sci. 105, 4376–4380. https://doi.org/10.1073/pnas.0711159105
- Passemar, C., Arbués, A., Malaga, W., Mercier, I., Moreau, F., Lepourry, L., Neyrolles, O.,
 Guilhot, C., Astarie-Dequeker, C., 2014. Multiple deletions in the polyketide synthase
 gene repertoire of *Mycobacterium tuberculosis* reveal functional overlap of cell
 envelope lipids in host–pathogen interactions. Cell. Microbiol. 16, 195–213.
 https://doi.org/10.1111/cmi.12214
- Peyron, P., Vaubourgeix, J., Poquet, Y., Levillain, F., Botanch, C., Bardou, F., Daffé, M., 875 876 Emile, J.-F., Marchou, B., Cardona, P.-J., Chastellier, C. de, Altare, F., 2008. foamy 877 macrophages from tuberculous patients' granulomas constitute a nutrient-rich 878 reservoir for М. tuberculosis persistence. PLOS Pathog. 4, e1000204. 879 https://doi.org/10.1371/journal.ppat.1000204
- Portal-Celhay, C., Tufariello, J.M., Srivastava, S., Zahra, A., Klevorn, T., Grace, P.S., Mehra,
 A., Park, H.S., Ernst, J.D., Jacobs Jr, W.R., Philips, J.A., 2017. *Mycobacterium tuberculosis* EsxH inhibits ESCRT-dependent CD4⁺ T-cell activation. Nat. Microbiol.
 2, 16232. https://doi.org/10.1038/nmicrobiol.2016.232

- Portevin, D., Sousa-D'Auria, C. de, Montrozier, H., Houssin, C., Stella, A., Lanéelle, M.-A.,
 Bardou, F., Guilhot, C., Daffé, M., 2005. The acyl-AMP ligase FadD32 and AccD4containing acyl-CoA carboxylase are required for the synthesis of mycolic acids and
 essential for mycobacterial growth identification of the carboxylation product and
 determination of the acyl-CoA carboxylase components. J. Biol. Chem. 280, 8862–
 887 8874. https://doi.org/10.1074/jbc.M408578200
- Pym, A.S., Brodin, P., Brosch, R., Huerre, M., Cole, S.T., 2002. Loss of RD1 contributed to
 the attenuation of the live tuberculosis vaccines *Mycobacterium bovis* BCG and *Mycobacterium microti*. Mol. Microbiol. 46, 709–717. https://doi.org/10.1046/j.13652958.2002.03237.x
- Pym, A.S., Brodin, P., Majlessi, L., Brosch, R., Demangel, C., Williams, A., Griffiths, K.E.,
 Marchal, G., Leclerc, C., Cole, S.T., 2003. Recombinant BCG exporting ESAT-6
 confers enhanced protection against tuberculosis. Nat. Med. 9, 533–539.
 https://doi.org/10.1038/nm859
- 898 Queval, C.J., Song, O.-R., Carralot, J.-P., Saliou, J.-M., Bongiovanni, A., Deloison, G., 899 Deboosère, N., Jouny, S., Iantomasi, R., Delorme, V., Debrie, A.-S., Park, S.-J., 900 Gouveia, J.C., Tomavo, S., Brosch, R., Yoshimura, A., Yeramian, E., Brodin, P., 901 2017. Mycobacterium tuberculosis controls phagosomal acidification by targeting 902 **CISH-mediated** signaling. Cell Rep. 20. 3188-3198. 903 https://doi.org/10.1016/j.celrep.2017.08.101
- 904Quigley, J., Hughitt, V.K., Velikovsky, C.A., Mariuzza, R.A., El-Sayed, N.M., Briken, V.,9052017. The Cell wall lipid PDIM contributes to phagosomal escape and host cell exit of906Mycobacteriumtuberculosis.907https://doi.org/10.1128/mBio.00148-17
- Ramakrishnan, L., Federspiel, N.A., Falkow, S., 2000. Granuloma-specific expression of
 mycobacterium virulence proteins from the glycine-rich PE-PGRS family. Science
 288, 1436–1439. https://doi.org/10.1126/science.288.5470.1436
- Reed, M.B., Domenech, P., Manca, C., Su, H., Barczak, A.K., Kreiswirth, B.N., Kaplan, G.,
 Iii, C.E.B., 2004. A glycolipid of hypervirulent tuberculosis strains that inhibits the
 innate immune response. Nature 431, 84–87. https://doi.org/10.1038/nature02837
- Rengarajan, J., Bloom, B.R., Rubin, E.J., 2005. Genome-wide requirements for
 Mycobacterium tuberculosis adaptation and survival in macrophages. Proc. Natl.
 Acad. Sci. 102, 8327–8332. https://doi.org/10.1073/pnas.0503272102
- 917 Rieck, B., Degiacomi, G., Zimmermann, M., Cascioferro, A., Boldrin, F., Lazar-Adler, N.R., 918 Bottrill, A.R., Chevalier, F. le, Frigui, W., Bellinzoni, M., Lisa, M.-N., Alzari, P.M., 919 Nguyen, L., Brosch, R., Sauer, U., Manganelli, R., O'Hare, H.M., 2017. PknG senses 920 amino acid availability to control metabolism and virulence of Mycobacterium 921 tuberculosis. PLOS e1006399. Pathog. 13, 922 https://doi.org/10.1371/journal.ppat.1006399
- 923 Rousseau, C., Neyrolles, O., Bordat, Y., Giroux, S., Sirakova, T.D., Prevost, M., Kolattukudy, 924 P.E., Gicquel, B., Jackson, M., 2003a. Deficiency in mycolipenate- and mycosanoate-925 derived acyltrehaloses enhances early interactions of Mycobacterium tuberculosis with 926 host cells. Cell. Microbiol. 5, 405-415. https://doi.org/10.1046/j.1462-927 5822.2003.00289.x
- Rousseau, C., Turner, O.C., Rush, E., Bordat, Y., Sirakova, T.D., Kolattukudy, P.E., Ritter,
 S., Orme, I.M., Gicquel, B., Jackson, M., 2003b. Sulfolipid deficiency does not affect
 the virulence of *Mycobacterium tuberculosis* H37Rv in mice and guinea pigs. Infect.
 Immun. 71, 4684–4690. https://doi.org/10.1128/IAI.71.8.4684-4690.2003

- Samanta, S., Singh, A., Biswas, P., Bhatt, A., Visweswariah, S.S., 2017. Mycobacterial
 phenolic glycolipid synthesis is regulated by cAMP-dependent lysine acylation of
 FadD22. Microbiology 163, 373–382. https://doi.org/10.1099/mic.0.000440
- Sassetti, C.M., Rubin, E.J., 2003. Genetic requirements for mycobacterial survival during
 infection. Proc. Natl. Acad. Sci. 100, 12989–12994.
 https://doi.org/10.1073/pnas.2134250100
- Sayes, F., Blanc, C., Ates, L.S., Deboosere, N., Orgeur, M., Le Chevalier, F., Gröschel, M.I.,
 Frigui, W., Song, O.-R., Lo-Man, R., Brossier, F., Sougakoff, W., Bottai, D., Brodin,
 P., Charneau, P., Brosch, R., Majlessi, L., 2018. Multiplexed quantitation of
 intraphagocyte *Mycobacterium tuberculosis* secreted protein effectors. Cell Rep. 23,
 1072–1084. https://doi.org/10.1016/j.celrep.2018.03.125
- Sayes, F., Pawlik, A., Frigui, W., Gröschel, M.I., Crommelynck, S., Fayolle, C., Cia, F.,
 Bancroft, G.J., Bottai, D., Leclerc, C., Brosch, R., Majlessi, L., 2016. CD4+ T cells
 recognizing PE/PPE antigens directly or via cross reactivity are protective against
 pulmonary *Mycobacterium tuberculosis* infection. PLOS Pathog. 12, e1005770.
 https://doi.org/10.1371/journal.ppat.1005770
- 948 Sayes, F., Sun, L., Di Luca, M., Simeone, R., Degaiffier, N., Fiette, L., Esin, S., Brosch, R., 949 Bottai, D., Leclerc, C., Majlessi, L., 2012. strong immunogenicity and cross-reactivity 950 of Mycobacterium tuberculosis ESX-5 type VII secretion -encoded PE-PPE proteins 951 Host Microbe predicts vaccine potential. Cell 11, 352-363. 952 https://doi.org/10.1016/j.chom.2012.03.003
- Senaratne, R.H., Sidders, B., Sequeira, P., Saunders, G., Dunphy, K., Marjanovic, O., Reader,
 J.R., Lima, P., Chan, S., Kendall, S., McFadden, J., Riley, L.W., 2008. *Mycobacterium tuberculosis* strains disrupted in *mce3* and *mce4* operons are attenuated in mice. J.
 Med. Microbiol. 57, 164–170. https://doi.org/10.1099/jmm.0.47454-0
- Serafini, A., Boldrin, F., Palù, G., Manganelli, R., 2009. Characterization of a *Mycobacterium tuberculosis* ESX-3 conditional mutant: Essentiality and rescue by iron and zinc. J. Bacteriol. 191, 6340–6344. https://doi.org/10.1128/JB.00756-09
- Shimono, N., Morici, L., Casali, N., Cantrell, S., Sidders, B., Ehrt, S., Riley, L.W., 2003.
 Hypervirulent mutant of *Mycobacterium tuberculosis* resulting from disruption of the
 mce1 operon. Proc. Natl. Acad. Sci. 100, 15918–15923.
 https://doi.org/10.1073/pnas.2433882100
- Shleeva, M.O., Kondratieva, T.K., Demina, G.R., Rubakova, E.I., Goncharenko, A.V., Apt,
 A.S., Kaprelyants, A.S., 2017. overexpression of adenylyl cyclase encoded by the *Mycobacterium tuberculosis* Rv2212 gene confers improved fitness, accelerated
 recovery from dormancy and enhanced virulence in mice. Front. Cell. Infect.
 Microbiol. 7. https://doi.org/10.3389/fcimb.2017.00370
- Siegrist, M.S., Steigedal, M., Ahmad, R., Mehra, A., Dragset, M.S., Schuster, B.M., Philips,
 J.A., Carr, S.A., Rubin, E.J., 2014. Mycobacterial Esx-3 requires multiple components
 for iron acquisition. mBio 5, e01073-14. https://doi.org/10.1128/mBio.01073-14
- Simeone, R., Bobard, A., Lippmann, J., Bitter, W., Majlessi, L., Brosch, R., Enninga, J., 2012.
 Phagosomal Rupture by *Mycobacterium tuberculosis* Results in Toxicity and Host Cell Death. PLOS Pathog. 8, e1002507. https://doi.org/10.1371/journal.ppat.1002507
- Simeone, R., Sayes, F., Song, O., Gröschel, M.I., Brodin, P., Brosch, R., Majlessi, L., 2015.
 Cytosolic access of *Mycobacterium tuberculosis*: critical impact of phagosomal acidification control and demonstration of occurrence *in vivo*. PLOS Pathog. 11, e1004650. https://doi.org/10.1371/journal.ppat.1004650
- Singh, K.H., Jha, B., Dwivedy, A., Choudhary, E., N, A.G., Ashraf, A., Arora, D., Agarwal,
 N., Biswal, B.K., 2017. Characterization of a secretory hydrolase from *Mycobacterium*

- *tuberculosis* sheds critical insight into host lipid utilization by *M. tuberculosis*. J. Biol.
 Chem. 292, 11326–11335. https://doi.org/10.1074/jbc.M117.794297
- Sinsimer, D., Huet, G., Manca, C., Tsenova, L., Koo, M.-S., Kurepina, N., Kana, B.,
 Mathema, B., Marras, S.A.E., Kreiswirth, B.N., Guilhot, C., Kaplan, G., 2008. The
 phenolic glycolipid of *Mycobacterium tuberculosis* differentially modulates the early
 host cytokine response but does not in itself confer hypervirulence. Infect. Immun. 76,
 3027–3036. https://doi.org/10.1128/IAI.01663-07
- Slama, N., Jamet, S., Frigui, W., Pawlik, A., Bottai, D., Laval, F., Constant, P., Lemassu, A.,
 Cam, K., Daffé, M., Brosch, R., Eynard, N., Quémard, A., 2016. The changes in
 mycolic acid structures caused by *hadC* mutation have a dramatic effect on the
 virulence of *Mycobacterium tuberculosis*. Mol. Microbiol. 99, 794–807.
 https://doi.org/10.1111/mmi.13266
- Smith, L.J., Bochkareva, A., Rolfe, M.D., Hunt, D.M., Kahramanoglou, C., Braun, Y.,
 Rodgers, A., Blockley, A., Coade, S., Lougheed, K.E.A., Hafneh, N.A., Glenn, S.M.,
 Crack, J.C., Le Brun, N.E., Saldanha, J.W., Makarov, V., Nobeli, I., Arnvig, K.,
 Mukamolova, G.V., Buxton, R.S., Green, J., 2017. Cmr is a redox-responsive
 regulator of DosR that contributes to *M. tuberculosis* virulence. Nucleic Acids Res.
 45, 6600–6612. https://doi.org/10.1093/nar/gkx406
- Smith, N.H., Hewinson, R.G., Kremer, K., Brosch, R., Gordon, S.V., 2009. Myths and
 misconceptions: the origin and evolution of *Mycobacterium tuberculosis*. Nat. Rev.
 Microbiol. 7, 537–544. https://doi.org/10.1038/nrmicro2165
- Solans, L., Gonzalo-Asensio, J., Sala, C., Benjak, A., Uplekar, S., Rougemont, J., Guilhot, C.,
 Malaga, W., Martín, C., Cole, S.T., 2014. The PhoP-dependent ncRNA Mcr7
 modulates the TAT secretion system in *Mycobacterium tuberculosis*. PLOS Pathog.
 1005 10, e1004183. https://doi.org/10.1371/journal.ppat.1004183
- Sørensen, A.L., Nagai, S., Houen, G., Andersen, P., Andersen, A.B., 1995. Purification and characterization of a low-molecular-mass T-cell antigen secreted by *Mycobacterium tuberculosis*. Infect. Immun. 63, 1710–1717.
- Spertini, F., Audran, R., Chakour, R., Karoui, O., Steiner-Monard, V., Thierry, A.C., Mayor,
 C.E., Rettby, N., Jaton, K., Vallotton, L., Lazor-Blanchet, C., Doce, J., Puentes, E.,
 Marinova, D., Aguilo, N., Martin, C., 2015. Safety of human immunisation with a
 live-attenuated *Mycobacterium tuberculosis* vaccine: a randomised, double-blind,
 controlled phase I trial. Lancet Respir Med. 3:953-962. http://doi.org/910.1016/S22132600(1015)00435-X.
- 1015 Stinear, T.P., Seemann, T., Harrison, P.F., Jenkin, G.A., Davies, J.K., Johnson, P.D.,
- Abdellah, Z., Arrowsmith, C., Chillingworth, T., Churcher, C., Clarke, K., Cronin, A.,
 Davis, P., Goodhead, I., Holroyd, N., Jagels, K., Lord, A., Moule, S., Mungall, K.,
- 1018 Norbertczak, H., Quail, M.A., Rabbinowitsch, E., Walker, D., White, B., Whitehead,
- S., Small, P.L., Brosch, R., Ramakrishnan, L., Fischbach, M.A., Parkhill, J., and Cole,
 S.T., 2008. Insights from the complete genome sequence of *Mycobacterium marinum*on the evolution of *Mycobacterium tuberculosis*. Genome Res. 18:729-741.
- 1022 https://doi.org/10.1101/gr.075069.107
- Sun, J., Siroy, A., Lokareddy, R.K., Speer, A., Doornbos, K.S., Cingolani, G., Niederweis,
 M., 2015. The tuberculosis necrotizing toxin kills macrophages by hydrolyzing NAD.
 Nat. Struct. Mol. Biol. 22, 672–678. https://doi.org/10.1038/nsmb.3064
- Supply, P., Marceau, M., Mangenot, S., Roche, D., Rouanet, C., Khanna, V., Majlessi, L.,
 Criscuolo, A., Tap, J., Pawlik, A., Fiette, L., Orgeur, M., Fabre, M., Parmentier, C.,
 Frigui, W., Simeone, R., Boritsch, E.C., Debrie, A.-S., Willery, E., Walker, D., Quail,
 M.A., Ma, L., Bouchier, C., Salvignol, G., Sayes, F., Cascioferro, A., Seemann, T.,
 Barbe, V., Locht, C., Gutierrez, M.-C., Leclerc, C., Bentley, S.D., Stinear, T.P.,

- Brisse, S., Médigue, C., Parkhill, J., Cruveiller, S., Brosch, R., 2013. Genomic
 analysis of smooth tubercle bacilli provides insights into ancestry and pathoadaptation
 of *Mycobacterium tuberculosis*. Nat. Genet. 45, 172–179.
 https://doi.org/10.1038/ng.2517
- Treumann, A., Xidong, F., McDonnell, L., Derrick, P.J., Ashcroft, A.E., Chatterjee, D.,
 Homans, S.W., 2002. 5-methylthiopentose: a new substituent on lipoarabinomannan in *Mycobacterium tuberculosis*. J. Mol. Biol. 316, 89–100.
 https://doi.org/10.1006/jmbi.2001.5317
- Tufariello, J.M., Chapman, J.R., Kerantzas, C.A., Wong, K.-W., Vilchèze, C., Jones, C.M.,
 Cole, L.E., Tinaztepe, E., Thompson, V., Fenyö, D., Niederweis, M., Ueberheide, B.,
 Philips, J.A., Jacobs, W.R., 2016. Separable roles for *Mycobacterium tuberculosis*ESX-3 effectors in iron acquisition and virulence. Proc. Natl. Acad. Sci. 113, E348–
 E357. https://doi.org/10.1073/pnas.1523321113
- 1044 van der Wel, N., Hava, D., Houben, D., Fluitsma, D., Zon, M. van, Pierson, J., Brenner, M., 1045 Peters, P.J., 2007. M. tuberculosis and M. leprae translocate from the phagolysosome 1046 myeloid cells. to the cytosol in Cell 129, 1287-1298. 1047 https://doi.org/10.1016/j.cell.2007.05.059
- 1048 VanderVen, B.C., Fahey, R.J., Lee, W., Liu, Y., Abramovitch, R.B., Memmott, C., Crowe, A.M., Eltis, L.D., Perola, E., Deininger, D.D., Wang, T., Locher, C.P., Russell, D.G., 1049 1050 2015. novel inhibitors of cholesterol degradation in Mycobacterium tuberculosis reveal how the bacterium's metabolism is constrained by the intracellular 1051 1052 environment. PLOS Pathog. 11. e1004679. 1053 https://doi.org/10.1371/journal.ppat.1004679
- 1054 Vergne, I., Gilleron, M., Nigou, J., 2015. Manipulation of the endocytic pathway and
 1055 phagocyte functions by *Mycobacterium tuberculosis* lipoarabinomannan. Front. Cell.
 1056 Infect. Microbiol. 4. https://doi.org/10.3389/fcimb.2014.00187
- 1057 Veyron-Churlet, R., Bigot, S., Guerrini, O., Verdoux, S., Malaga, W., Daffé, M., Zerbib, D.,
 1058 2005. the biosynthesis of mycolic acids in *Mycobacterium tuberculosis* relies on
 1059 multiple specialized elongation complexes interconnected by specific protein–protein
 1060 interactions. J. Mol. Biol. 353, 847–858. https://doi.org/10.1016/j.jmb.2005.09.016
- Via, L.E., Deretic, D., Ulmer, R.J., Hibler, N.S., Huber, L.A., Deretic, V., 1997. Arrest of
 mycobacterial phagosome maturation is caused by a block in vesicle fusion between
 stages controlled by rab5 and rab7. J. Biol. Chem. 272, 13326–13331.
 https://doi.org/10.1074/jbc.272.20.13326
- Vilchèze, C., Morbidoni, H.R., Weisbrod, T.R., Iwamoto, H., Kuo, M., Sacchettini, J.C.,
 Jacobs, W.R., 2000. Inactivation of the *inhA*-encoded fatty acid synthase II (FASII)
 enoyl-acyl carrier protein reductase induces accumulation of the FASI end products
 and cell lysis of *Mycobacterium smegmatis*. J. Bacteriol. 182, 4059–4067.
 https://doi.org/10.1128/JB.182.14.4059-4067.2000
- 1070 Viljoen, A., Dubois, V., Girard-Misguich, F., Blaise, M., Herrmann, J.-L., Kremer, L., 2017.
 1071 The diverse family of MmpL transporters in mycobacteria: from regulation to
 1072 antimicrobial developments. Mol. Microbiol. 104, 889–904.
 1073 https://doi.org/10.1111/mmi.13675
- 1074 Villarreal-Ramos, B., Berg, S., Whelan, A., Holbert, S., Carreras, F., Salguero, F.J., Khatri,
 1075 B.L., Malone, K., Rue-Albrecht, K., Shaughnessy, R., Smyth, A., Ameni, G., Aseffa,
 1076 A., Sarradin, P., Winter, N., Vordermeier, M., Gordon, S.V. 2018. Experimental
 1077 infection of cattle with *Mycobacterium tuberculosis* isolates shows the attenuation of
 1078 the human tubercle bacillus for cattle. Sci Rep. 8, 894. doi: 10.1038/s41598-0171079 18575-5.

- 1080 Voskuil, M.I., Bartek, I.L., Visconti, K., Schoolnik, G.K., 2011. The response of
 1081 Mycobacterium tuberculosis to reactive oxygen and nitrogen species. Front.
 1082 Microbiol. 2. https://doi.org/10.3389/fmicb.2011.00105
- Wang, J., Li, B.-X., Ge, P.-P., Li, J., Wang, Q., Gao, G.F., Qiu, X.-B., Liu, C.H., 2015. *Mycobacterium tuberculosis* suppresses innate immunity by coopting the host ubiquitin system. Nat. Immunol. 16, 237–245. https://doi.org/10.1038/ni.3096
- Wassermann, R., Gulen, M.F., Sala, C., Perin, S.G., Lou, Y., Rybniker, J., Schmid-Burgk, 1086 J.L., Schmidt, T., Hornung, V., Cole, S.T., Ablasser, A., 2015. Mycobacterium 1087 tuberculosis differentially activates cGAS- and inflammasome-dependent intracellular 1088 1089 immune responses through ESX-1. Cell Host Microbe 17, 799-810. 1090 https://doi.org/10.1016/j.chom.2015.05.003
- Watson, R.O., Bell, S.L., MacDuff, D.A., Kimmey, J.M., Diner, E.J., Olivas, J., Vance, R.E.,
 Stallings, C.L., Virgin, H.W., Cox, J.S., 2015. The cytosolic sensor cGAS detects
 Mycobacterium tuberculosis DNA to induce type I interferons and activate autophagy.
 Cell Host Microbe 17, 811–819. https://doi.org/10.1016/j.chom.2015.05.004
- Weerdenburg, E.M., Abdallah, A.M., Suman, M., de Punder, K., van der Wel, N., Bird, S.,
 Appelmelk, B.J., Bitter, W., van der Sar, A.M., 2012. ESX-5-deficient *Mycobacterium marinum* is hypervirulent in adult zebrafish. Cell. Microbiol. 14, 728–739.
 https://doi.org/10.1111/j.1462-5822.2012.01755.x
- 1099 WHO Global tuberculosis report, 2017. World Health Organization, Geneva.
- Wiens, K.E., Ernst, J.D., 2016. the mechanism for type I interferon induction by
 Mycobacterium tuberculosis is bacterial strain-dependent. PLOS Pathog. 12,
 e1005809. https://doi.org/10.1371/journal.ppat.1005809
- Wiker, H.G., Harboe, M., 1992. The antigen 85 complex: a major secretion product of Mycobacterium tuberculosis. Microbiol. Rev. 56, 648–661.
- Williams, M., Mizrahi, V., Kana, B.D., 2014. Molybdenum cofactor: A key component of *Mycobacterium tuberculosis* pathogenesis? Crit. Rev. Microbiol. 40, 18–29. https://doi.org/10.3109/1040841X.2012.749211
- Wang, J., McIntosh, F., Radomski, N., Dewar, K., Simeone, R., Enninga, J., Brosch, R.,
 Rocha, E.P., Veyrier, F.J., and Behr, M.A., 2015. Insights on the emergence of
 Mycobacterium tuberculosis from the analysis of *Mycobacterium kansasii*. Genome
 Biol Evol. 7:856-70. https://doi.org/10.1093/gbe/evv035
- Wong, D., Bach, H., Sun, J., Hmama, Z., Av-Gay, Y., 2011. *Mycobacterium tuberculosis*protein tyrosine phosphatase (PtpA) excludes host vacuolar-H+–ATPase to inhibit
 phagosome acidification. Proc. Natl. Acad. Sci. 108, 19371–19376.
 https://doi.org/10.1073/pnas.1109201108
- Zhang, Y.J., Reddy, M.C., Ioerger, T.R., Rothchild, A.C., Dartois, V., Schuster, B.M.,
 Trauner, A., Wallis, D., Galaviz, S., Huttenhower, C., Sacchettini, J.C., Behar, S.M.,
 Rubin, E.J., 2013. Tryptophan biosynthesis protects mycobacteria from CD4 T-cellmediated killing. Cell 155, 1296–1308. https://doi.org/10.1016/j.cell.2013.10.045
- Zulauf, K.E., Sullivan, J.T., Braunstein, M., 2018. The SecA2 pathway of *Mycobacterium tuberculosis* exports effectors that work in concert to arrest phagosome and autophagosome maturation. PLOS Pathog. 14, e1007011.
 https://doi.org/10.1371/journal.ppat.1007011
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- 1128 **Figure legends:**
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Figure 1: Schematic representation of three types of secretion systems present in mycobacteria – the general Sec secretory pathway, the TAT pathway and the ESX / type VII secretion systems. The three-dimensional structure of ESX-5 system from *Mycobacterium xenopi* as determined by electron microscopy and single particle analysis (Beckham et al., 2017) is also shown.

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Figure 2: Functional groups are attached to the main (meromycolic) hydrocarbon chain of mycolic acids likely during its elongation by fatty acid synthase sytem II (FASII) (A) in two distinct positions relative to the carboxyl group (B) – distal and proximal – resulting in dicyclopropylated α -mycolates and oxygenated methoxy- and ketomycolates (C). The mycolic acid modifying enzymes (in orange) are connected to the core enzymes of the FASII system (in blue) through protein-protein interactions.

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1143 Figure 3: Example of a genomic locus (moaA1-D1 locus) acquired by tuberculosis-causing 1144 mycobacteria: (A) Jensen-Shannon divergence profiles of the *M.tuberculosis* genomic region 1145 harboring the Moco-1 island and its genomic context. The red curve represents the divergence 1146 between the signature of the whole *M. tuberculosis* H37Rv genome and that of the sliding 1147 windows (5-kb long, 100-bp step) over the region. The blue curve represents the divergence 1148 between the signature of the Burkholderia vietnamiensis G4 plasmid pBVIE01 and that of the 1149 sliding windows over the region, as described in detail by Levillain et al., 2017. Image 1150 adapted from Ref.(Levillain et al., 2017); (B) genomic region of the horizontally transferred 1151 moaA1-D1 locus in M. tuberculosis and its flanking regions containing conserved 1152 mycobacterial genes in comparison to the orthologous regions in Mycobacterium marinum M 1153 and Mycobacterium kansasii ATCC 12478. Note that M. marinum M shows the minimal gene 1154 content in this genomic region, while in *M. tuberculosis* the moaA1 -D1 gene cluster showing 1155 similarity to the *B. vietnamiensis* G4 plasmid pBVIE01 encoded genes has been inserted in 1156 addition to mycobacterial genes involved in molybdenum cofactor biosynthesis. In M. 1157 kansasii ATCC 12478, genes with different functions, including one encoding a putative 1158 polyketide synthase, are present in the genomic locus. Percentages on grey lines represent 1159 amino acid identity values between gene products of respective strains. Color coding of genes 1160 the in TubercuList CanettiiList refers to scheme used or

- 1161 (http://genolist.pasteur.fr/CanettiiList/). Mtb = M. tuberculosis; vs. = versus; B. vientn. =
- *Burkholderia vietnamiensis;* HGT = Horizontal Gene Transfer.







