M. tuberculosis basic biology: how understanding MmpL cell wall lipid transporters can give insight into latent disease

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Overview

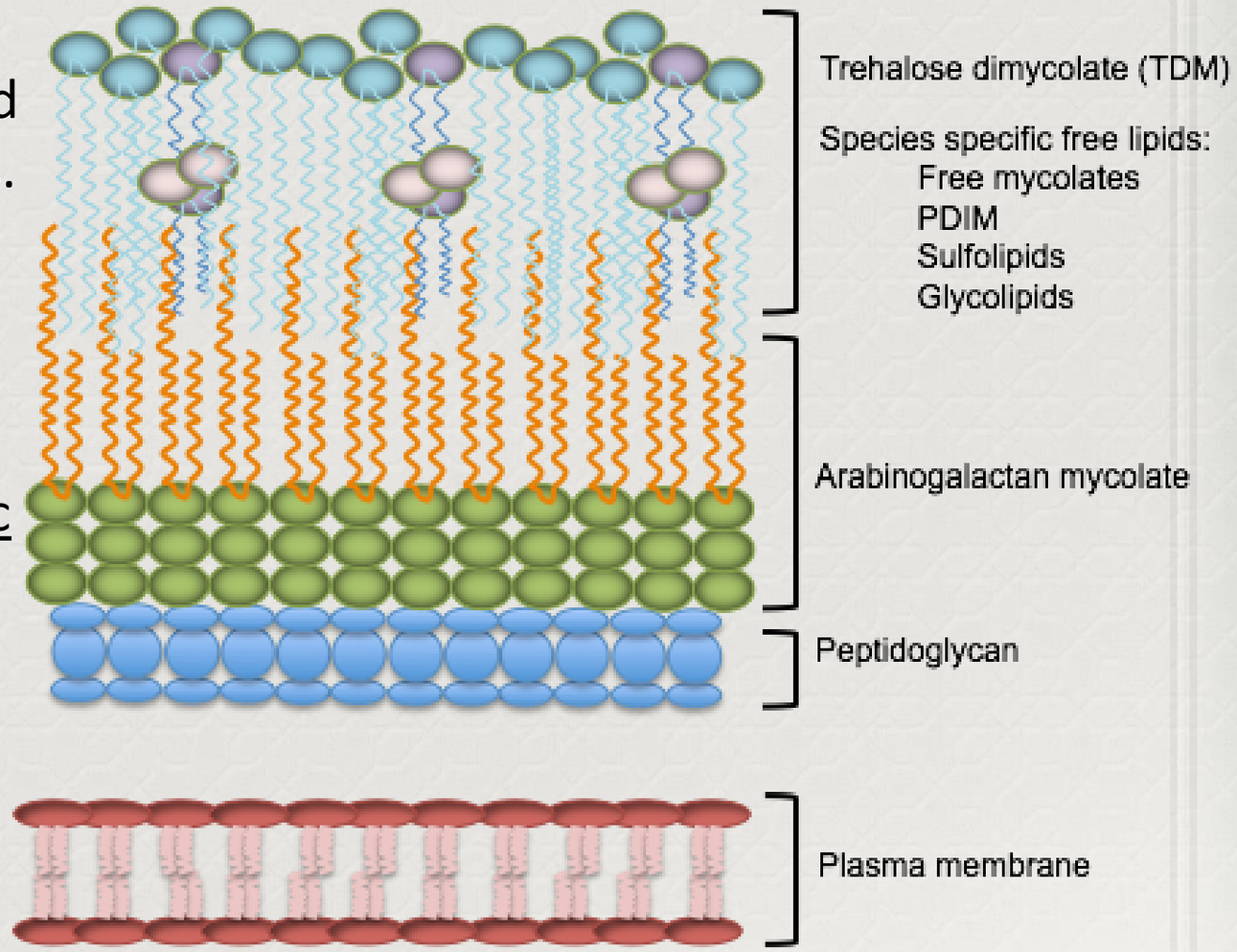
- Introduction: Mycobacterium *spp*., TB disease burden and epidemiology.
- Insights into Mtb cell wall lipids and their transport by MmpL proteins.
- Non-replicating persistence as a therapeutic target.

Mycobacteria

- Most species are non-pathogenic, environmental organisms
- Pathogenic mycobacteria:
  - Species that cause TB in humans and animals are referred to as the mycobacterium complex:
    - *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. canettii*, *M. microti*, *M. caprae*, and *M. pinnipedii*.
  - Environmental isolates can cause disease: ex. *M. avium* complex (MAC)

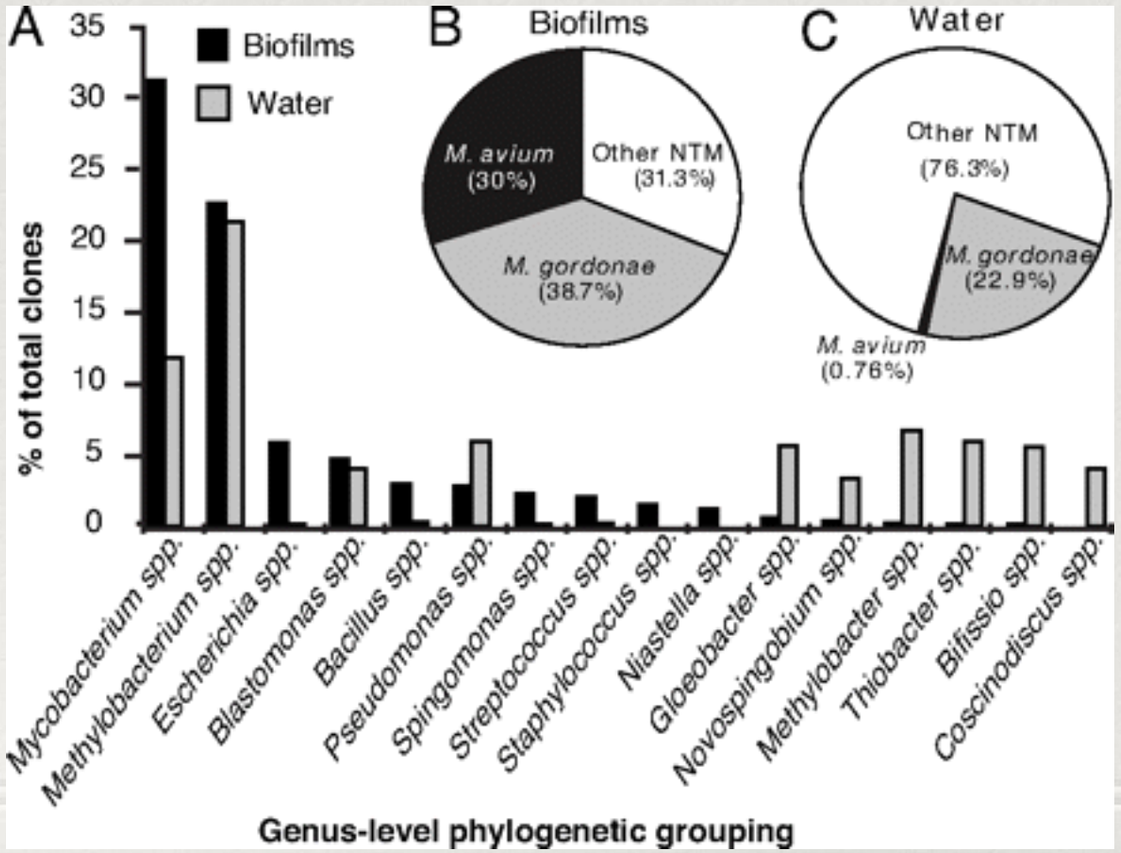
General properties of mycobacteria

- Pathogens exhibit slow growth, vs. rapid growth for most environmental isolates.
- Cell wall considered acid-fast.
- Unique cell wall composition: all mycobacterial cell walls contain Mycolic acids.
- Mycobacteria have additional biologically-active cell wall lipids that are species specific.



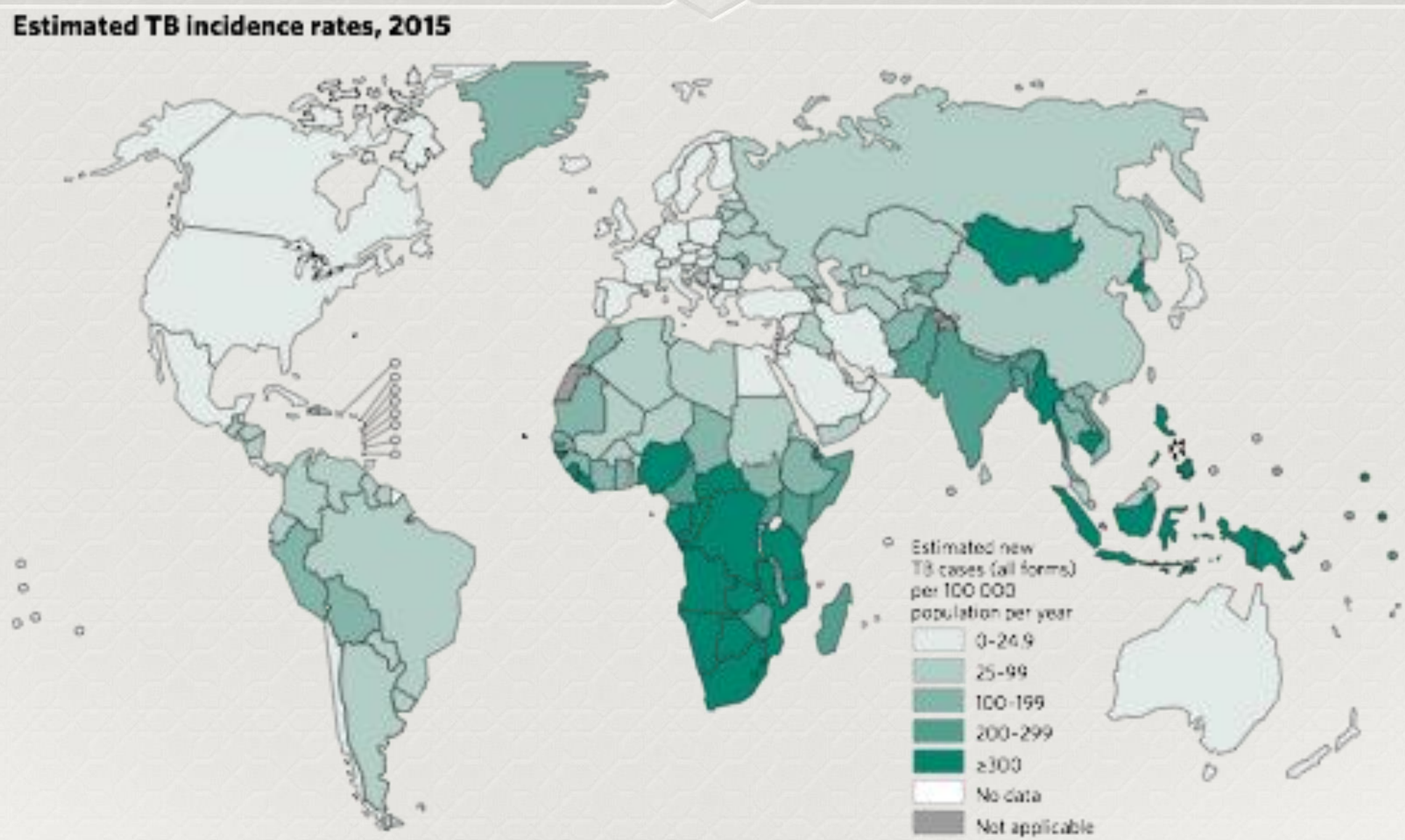
NTM infections are increasingly a public health concern

- Environmental spread, biofilm-associated
- Typically immunocompromised patients



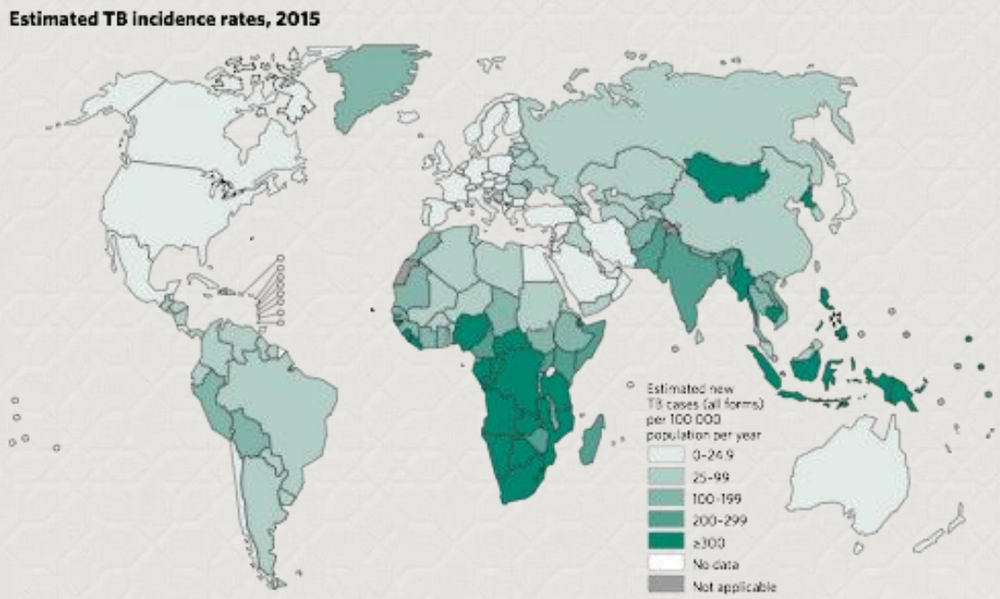
Faze et al., PNAS, 2009. 106 (38) 16393-16399

Tuberculosis is a global health concern



Source - WHO report 2016

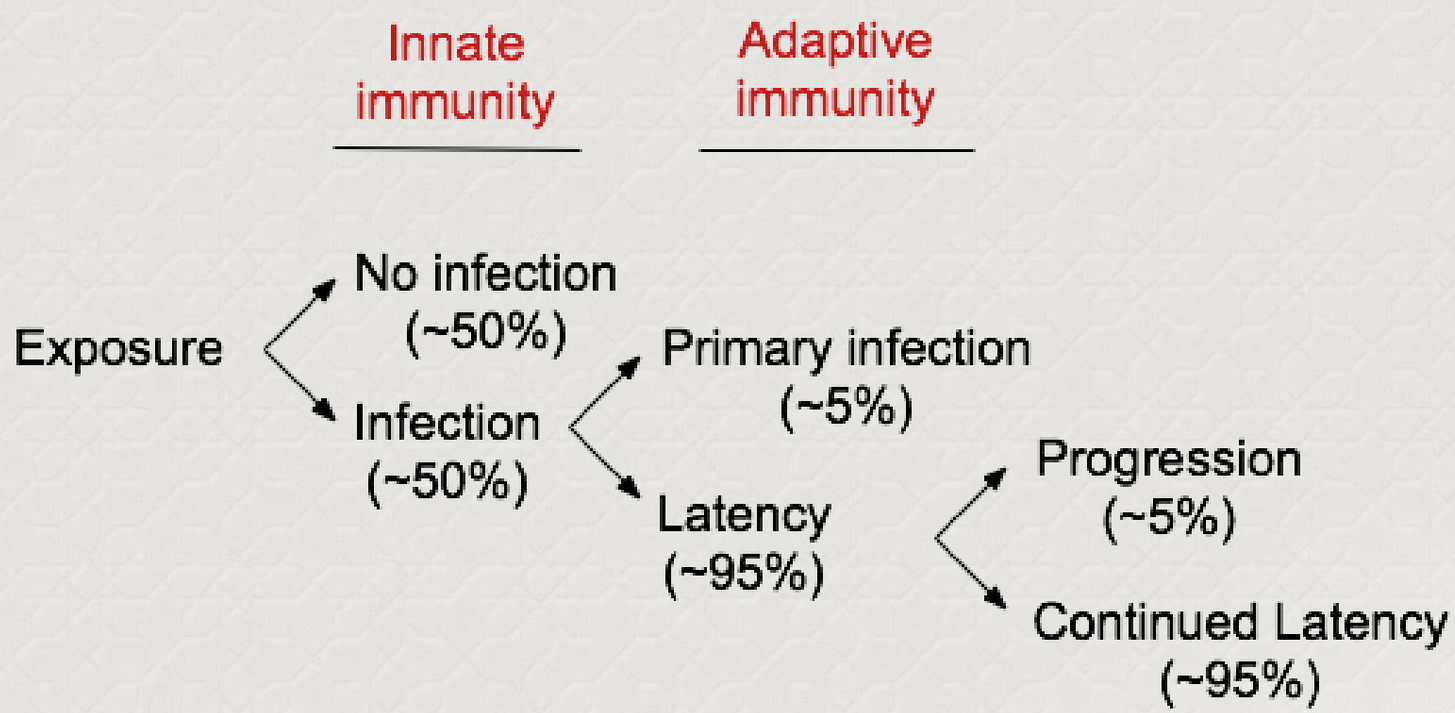
Tuberculosis is a global health concern



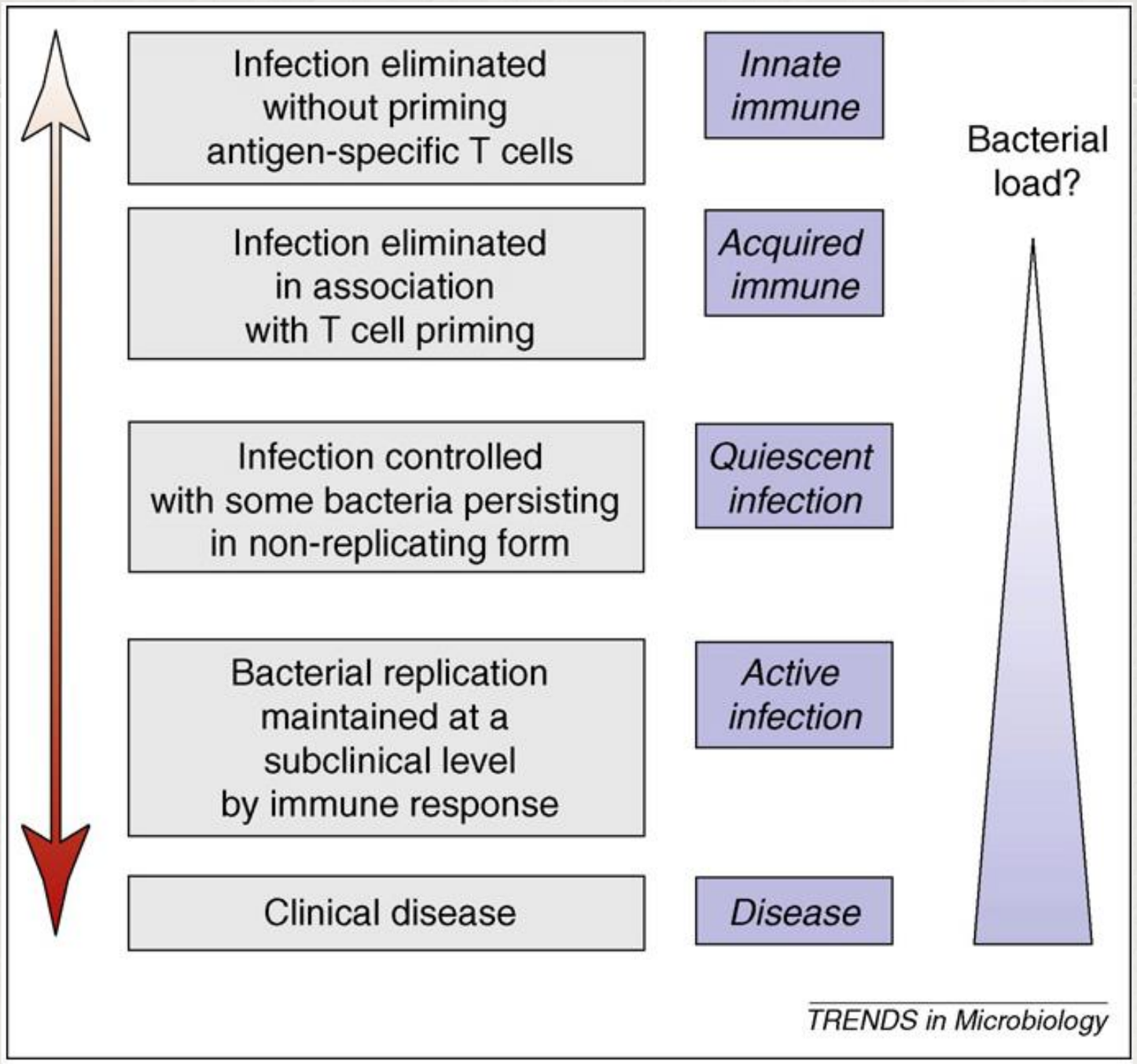
- Roughly one third of world population infected
- 9 million new cases reported annually
- 2 million deaths annually
- Southeast Asia accounts for nearly half (45.6%) of the global burden in terms of TB incidence even though it comprises only a quarter of the world's population. (WHO report, 2017)

Mtb pathogenesis

- Terminology:
- Active disease
- Latent infection
- Progression (Reactivation)

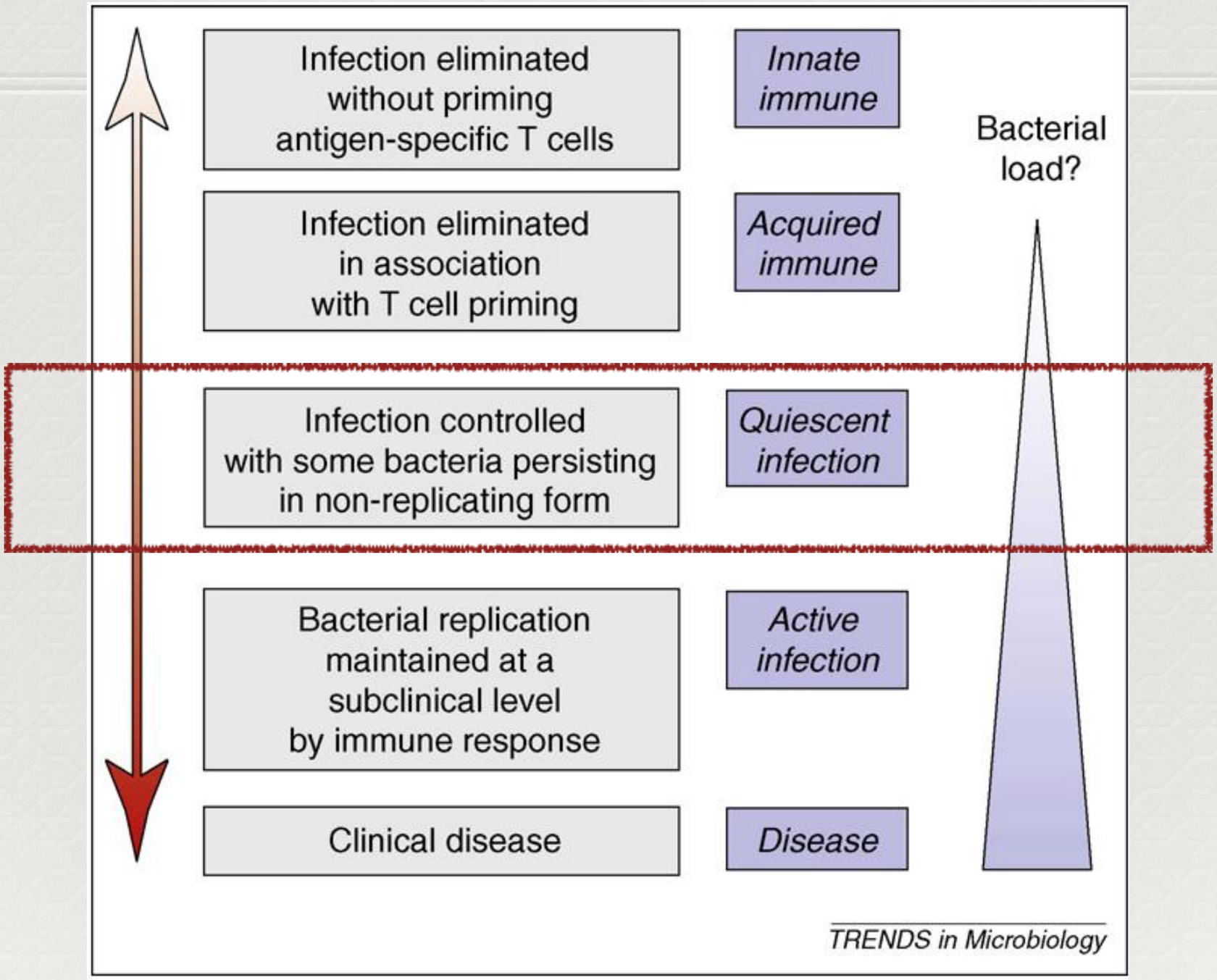


Mtb pathogenesis - disease spectrum



Young et al , Trends in Microbiology 17: 183-188

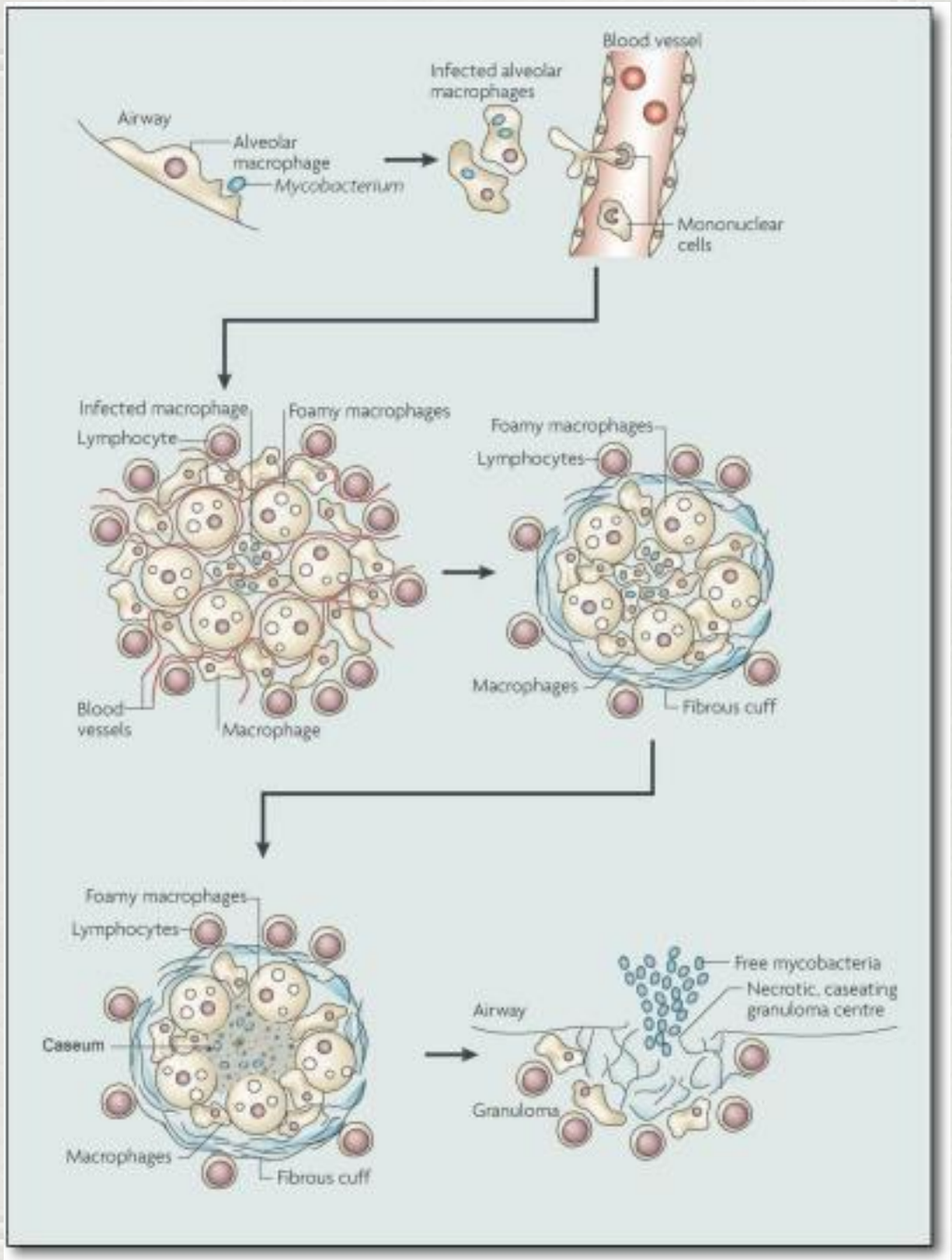
Mtb pathogenesis - disease spectrum



Young et al , Trends in Microbiology 17: 183-188

Understanding the molecular pathogenesis of Mtb

- Studies from infected humans and animal models informed the following model of disease:
  - Mtb infects and multiplies within the host macrophages.
  - The acquired immune response is associated with the arrival of lymphocytes, eventually a granuloma forms.
  - Human granulomas have a organized, stratified structure.
  - Mtb persists in a largely inactive or quiescent form.



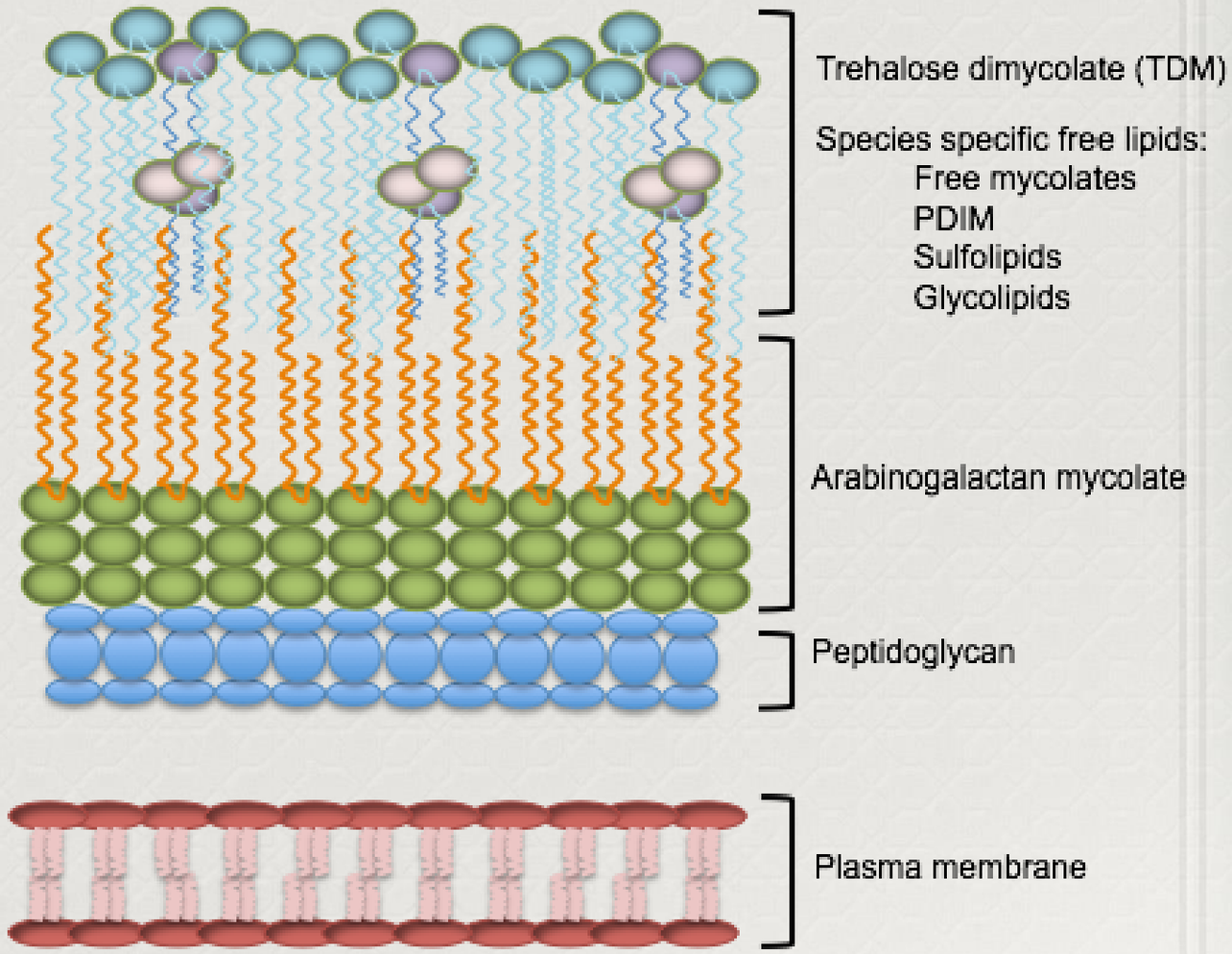
Russell DG, Barry CE 3rd, Flynn JL. Science. 2010 May 14;328(5980):852-6.

Understanding the molecular pathogenesis of Mtb

- Barriers:
  - Biosafety Level 3 organism that grows slowly and was historically genetically intractable
    - Alternatives: BSL1/2 mycobacteria *M. smegmatis*, *M. marinum*
  - Animal models - TB is a human disease and Mtb is exquisitely adapted to its host
    - Non-human primates vs. mice.
    - Latent vs. active disease.
    - Alternatives: In vitro cell culture models - primary macrophages, DCs , autologous T-cells

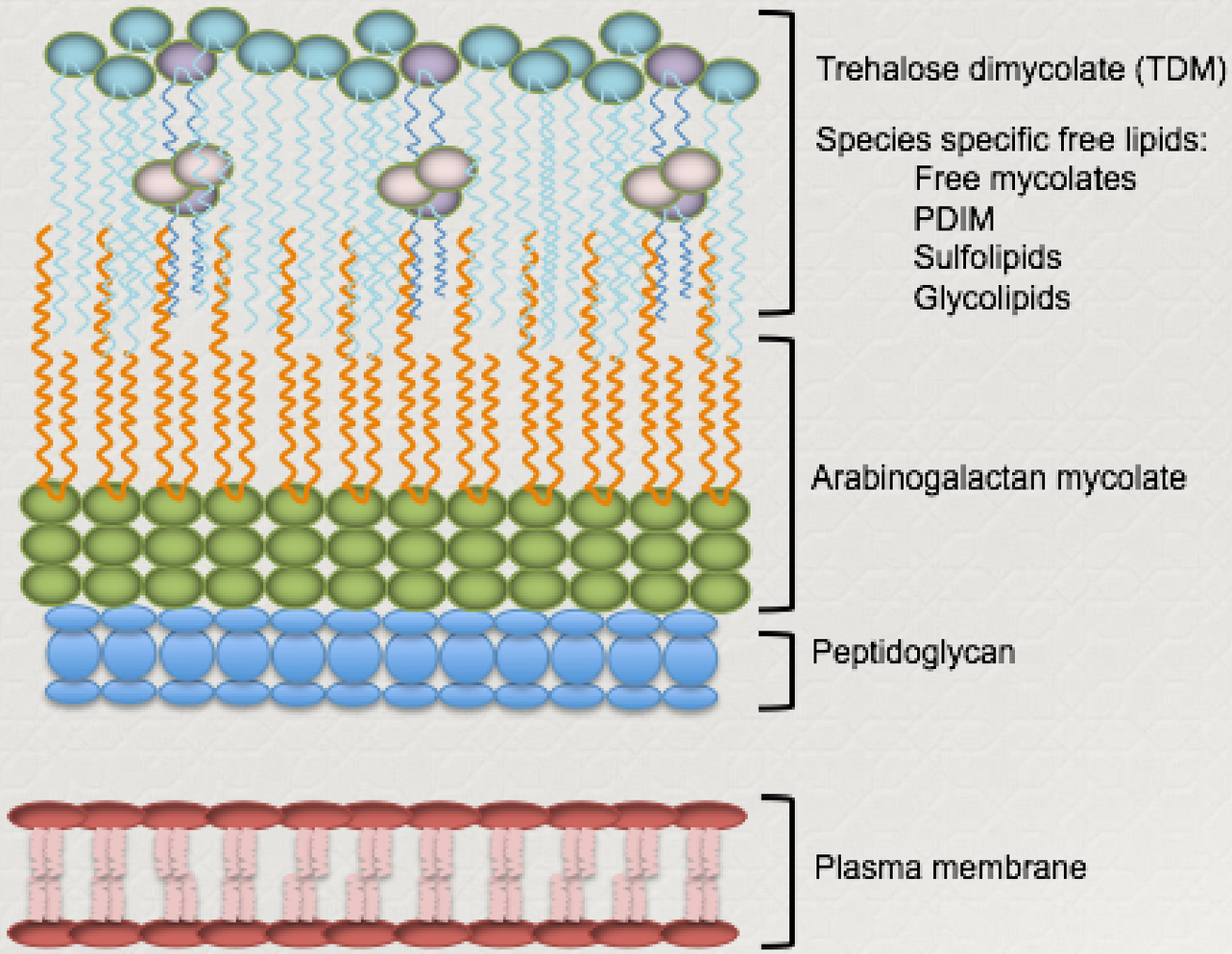
Mycobacterial cell wall

- The mycobacterial cell wall contributes to intrinsic resistance to antibiotics and host environments.
- Mycolic acids are 60-80% of cell wall mass.
  - Immunomodulatory
  - Biosynthesis is target of front-line anti-TB drugs INH and EMB
  - Contribute to the extracellular matrix in biofilms



Mycobacterial cell wall

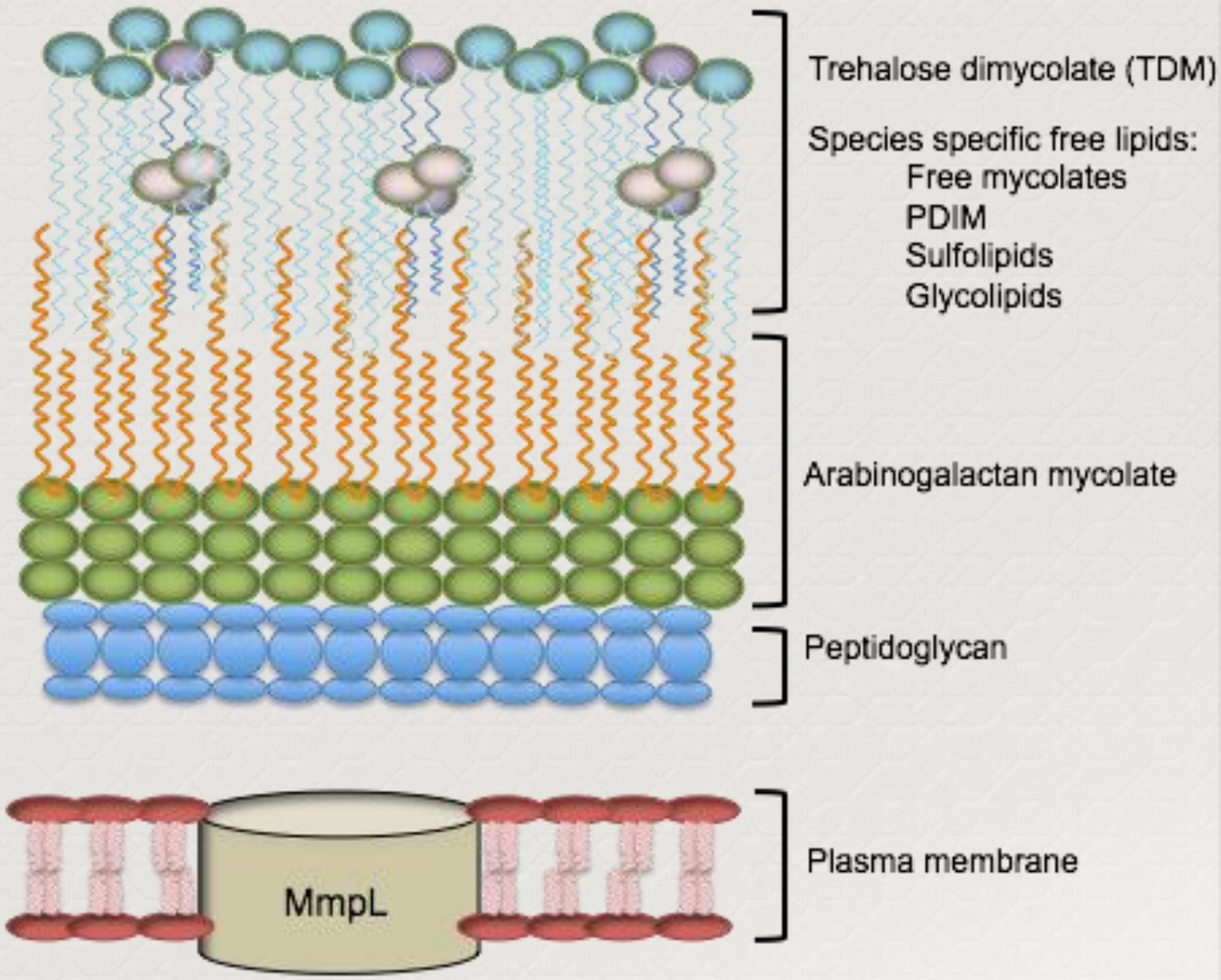
- There are still unanswered questions about cell wall biosynthesis (!!)



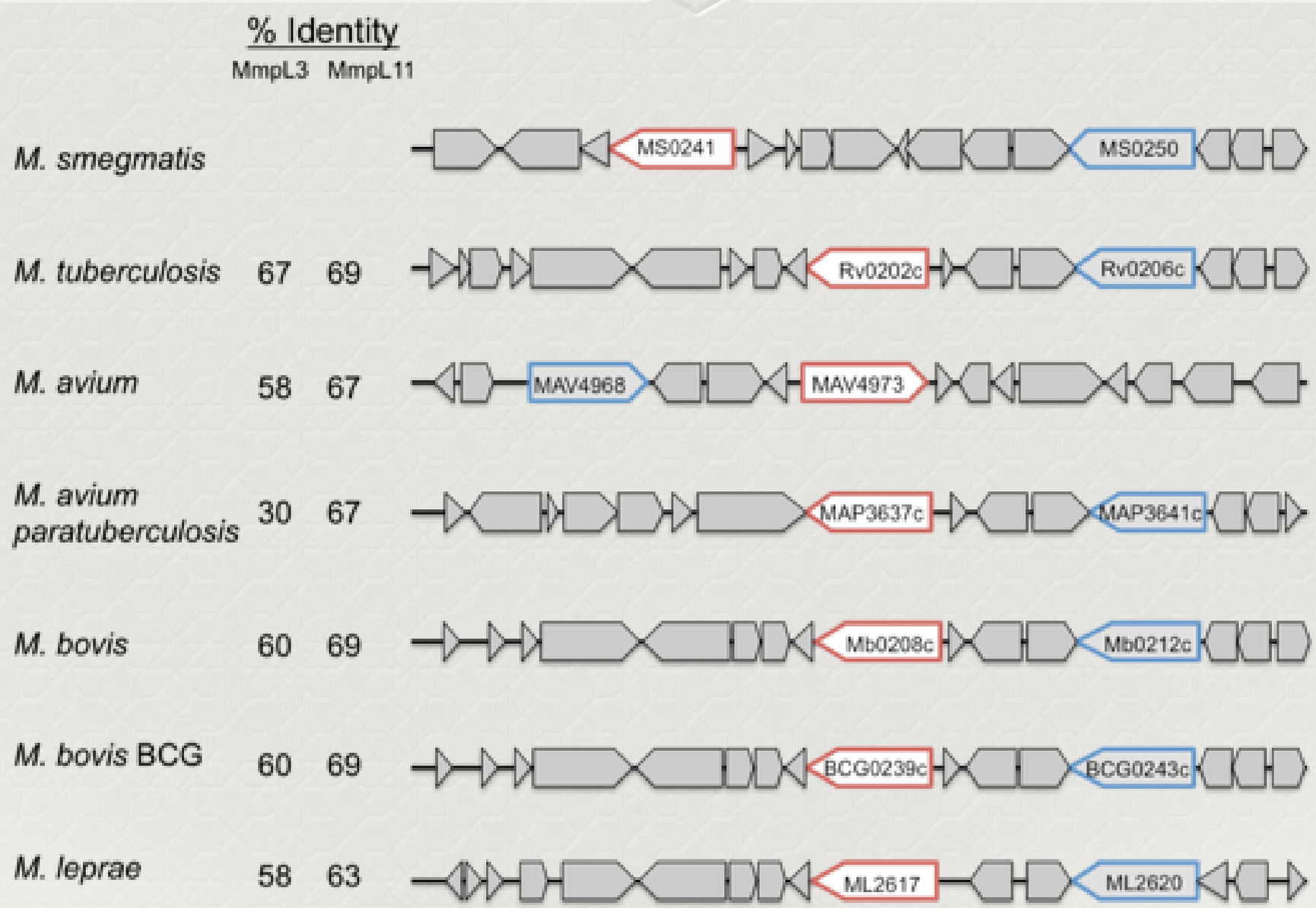
MmpL transporters

- Involved in lipid/siderophore transport:
  - MmpL3 - TMM
  - MmpL4/5 - mycobactin
  - MmpL7 - PDIM
  - MmpL8 - sulfolipid
  - MmpL10 - DATs

- MmpL3 is essential
- Others are required for *M. tuberculosis* virulence.

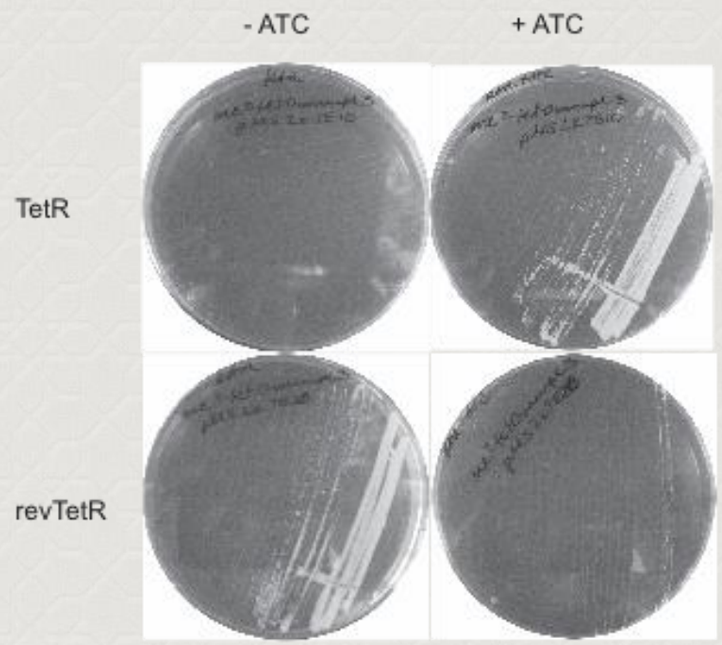


MmpL3 and MmpL11 are genetically conserved throughout mycobacteria



Inhibition of MmpL3 results in reduced *M. smegmatis* viability

A



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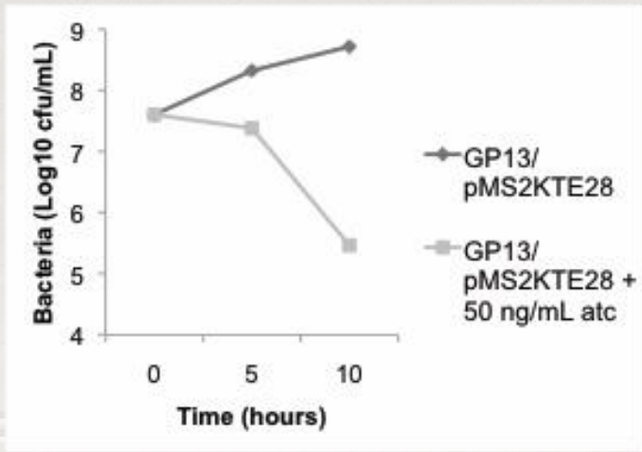
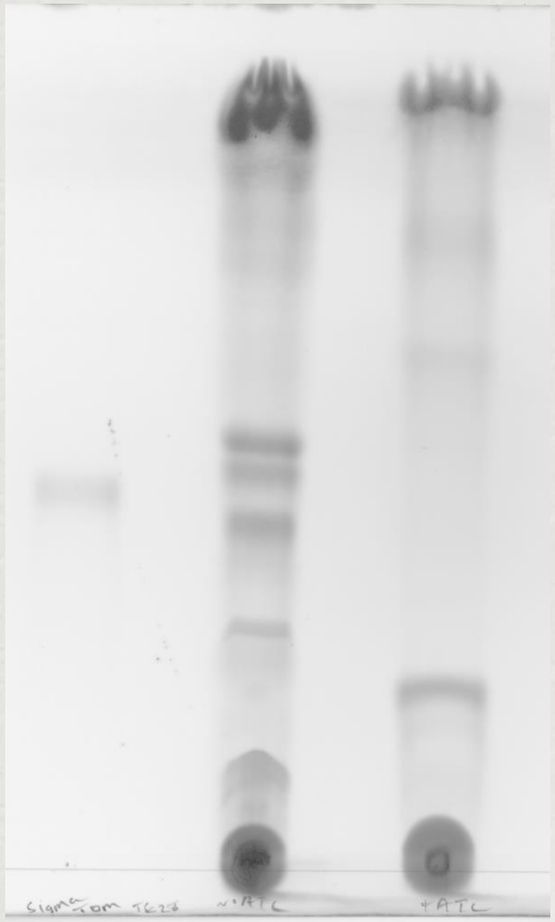
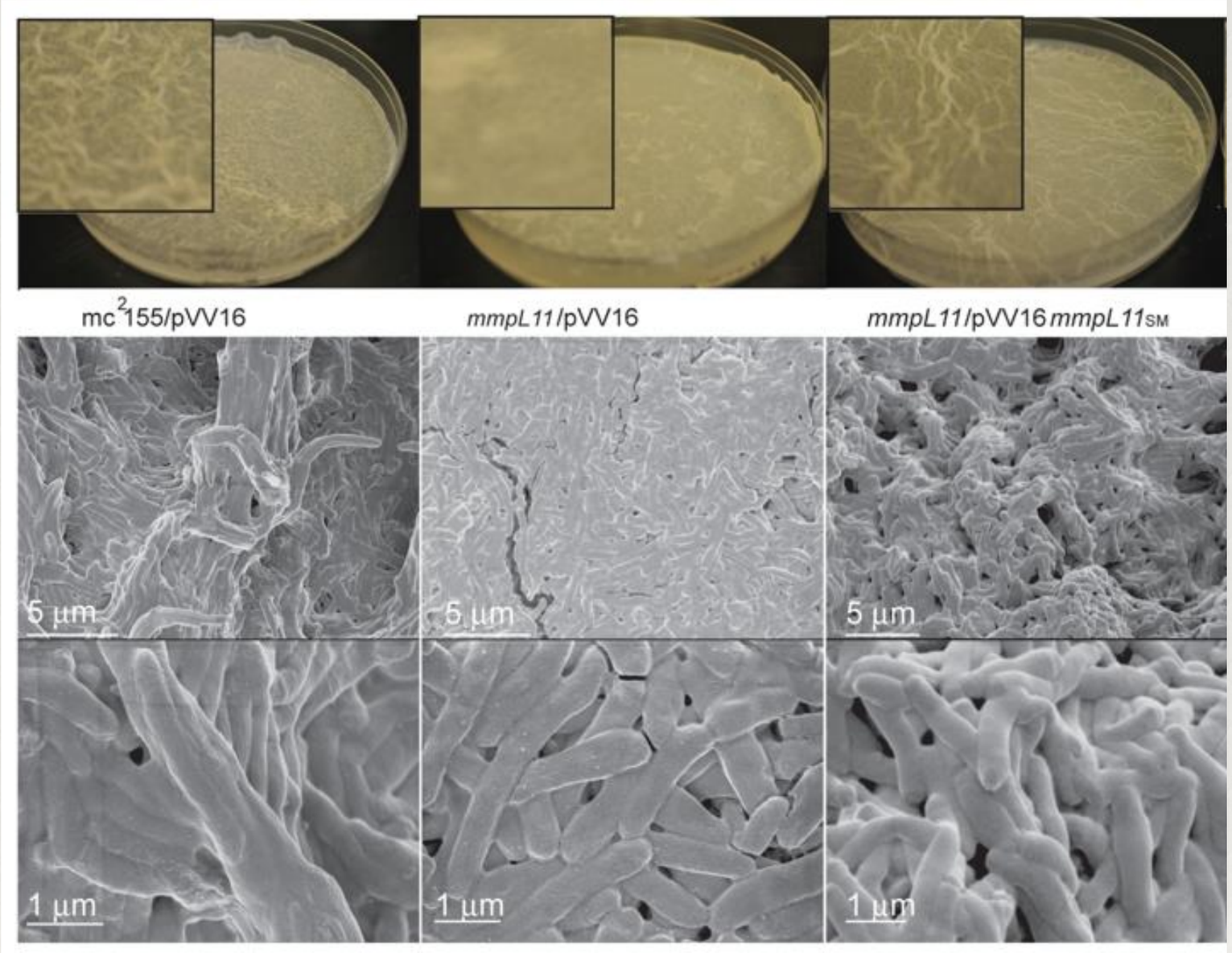




TDM

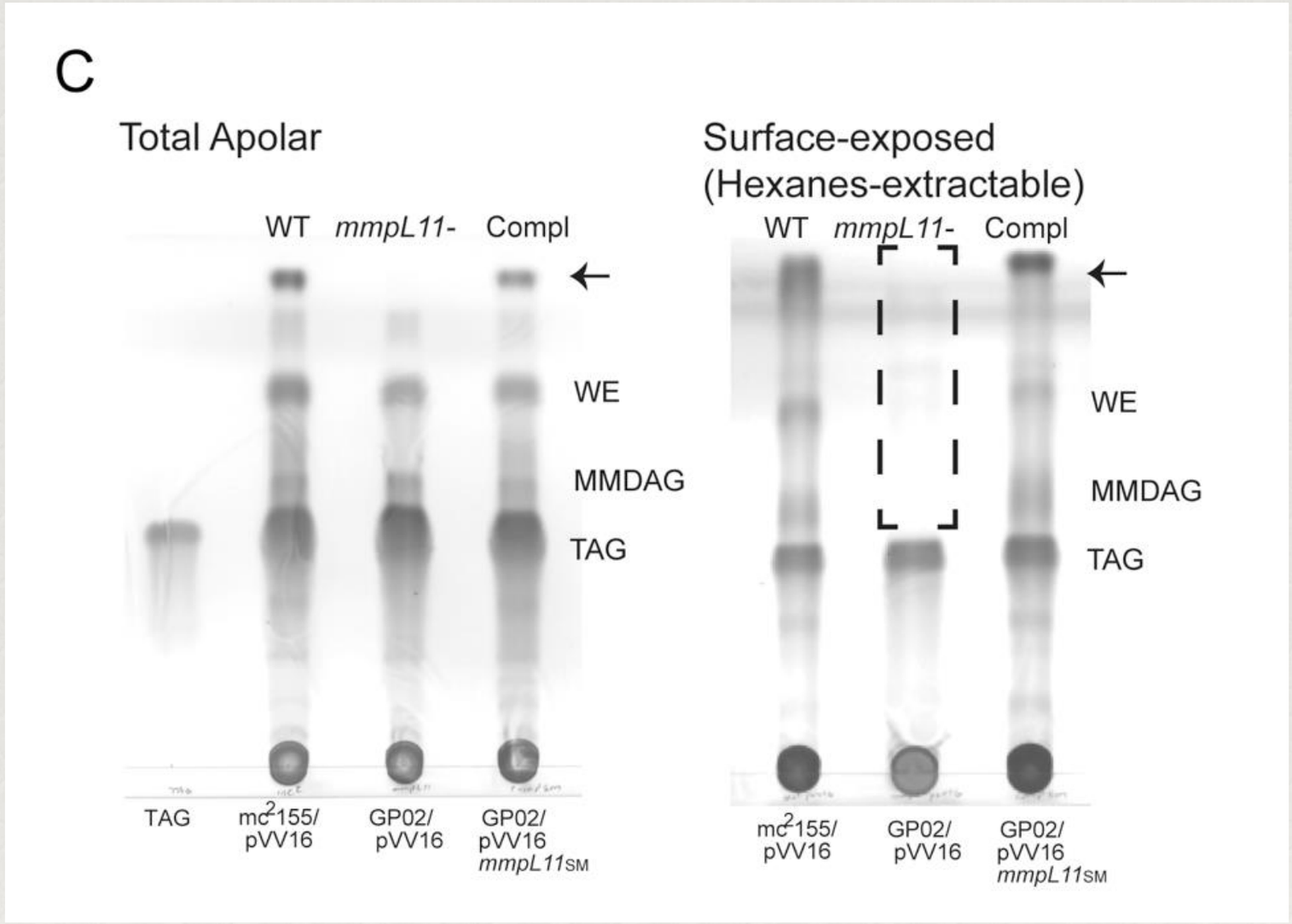
TDM -atc +atc

M. smegmatis mmpL11 mutant has reduced biofilm formation



Pacheco et al., 2013

M. smegmatis mmpL11 mutant lacks MMDAG and wax ester mycolate on the bacterial surface



Pacheco et al., 2013

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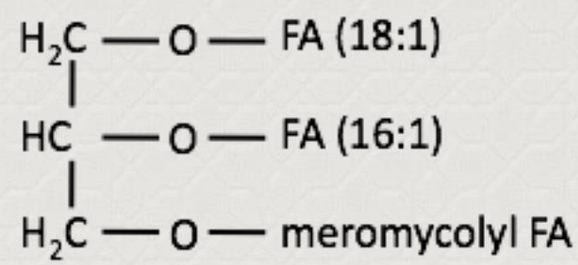
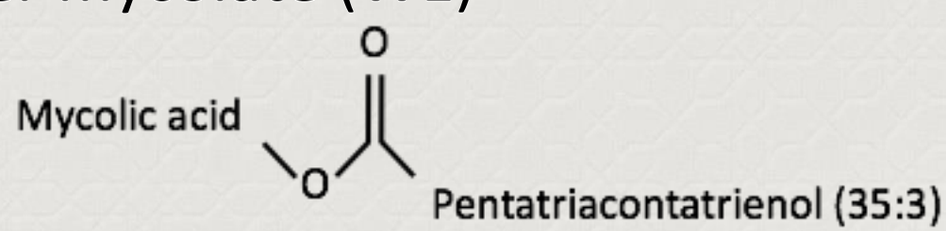
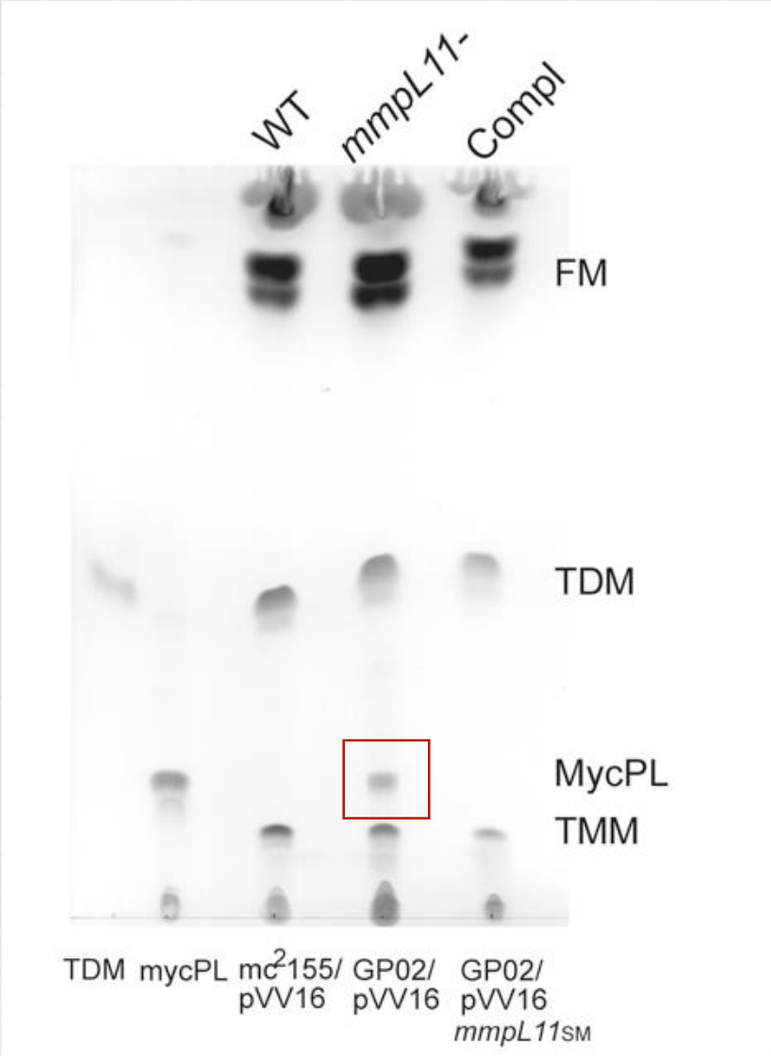
Meromycolyl diacylglycerol (MMDAG)

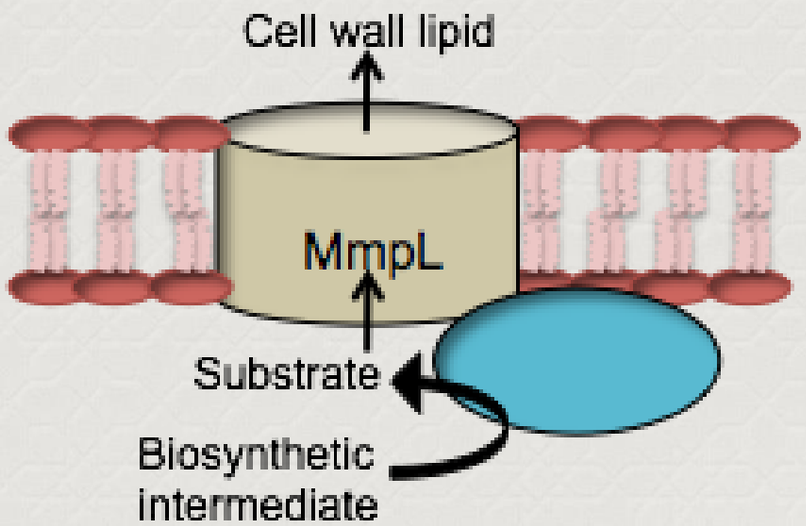


Wax ester mycolate (WE)



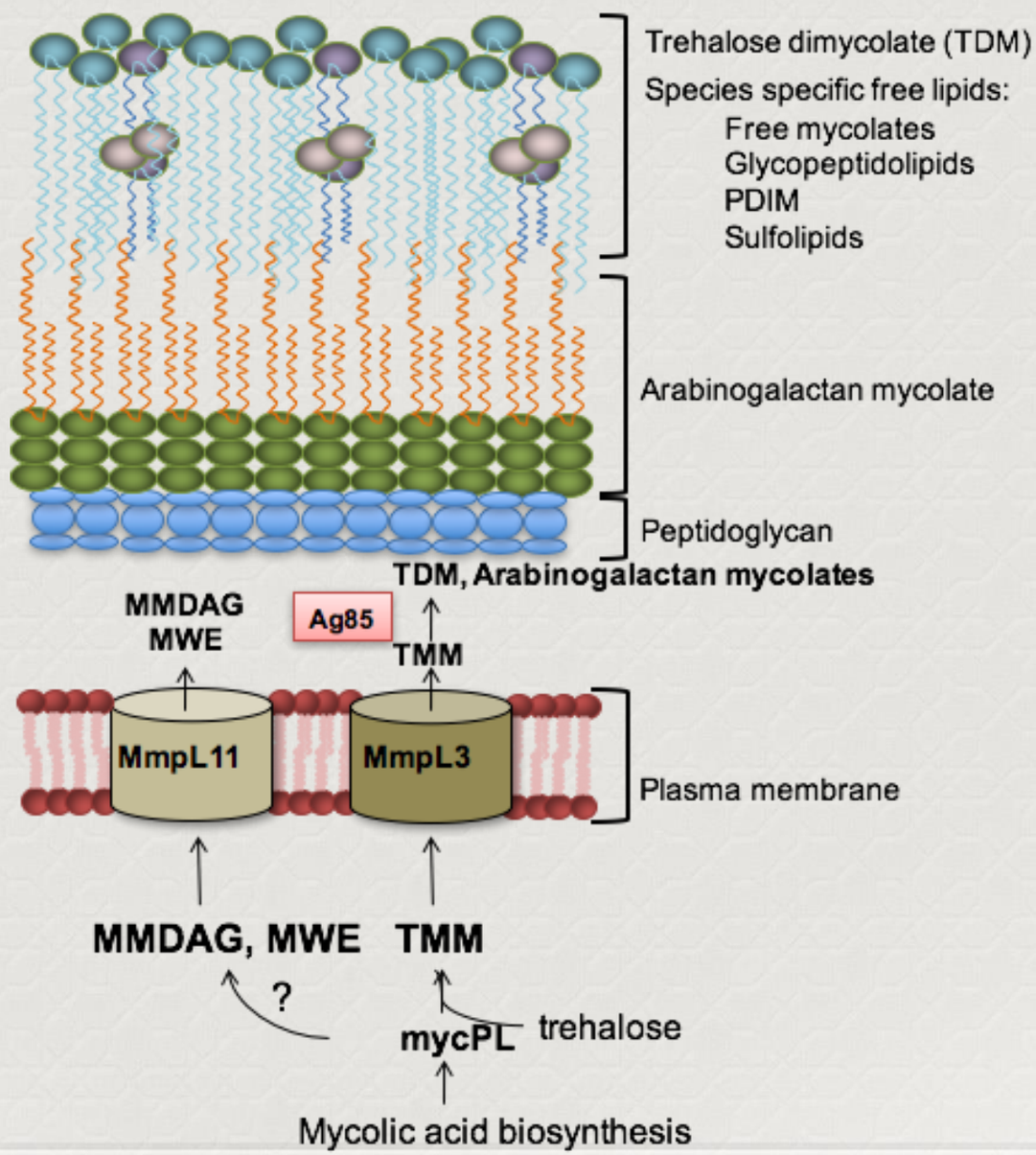
M. smegmatis mmpL11 mutant accumulates mycPL



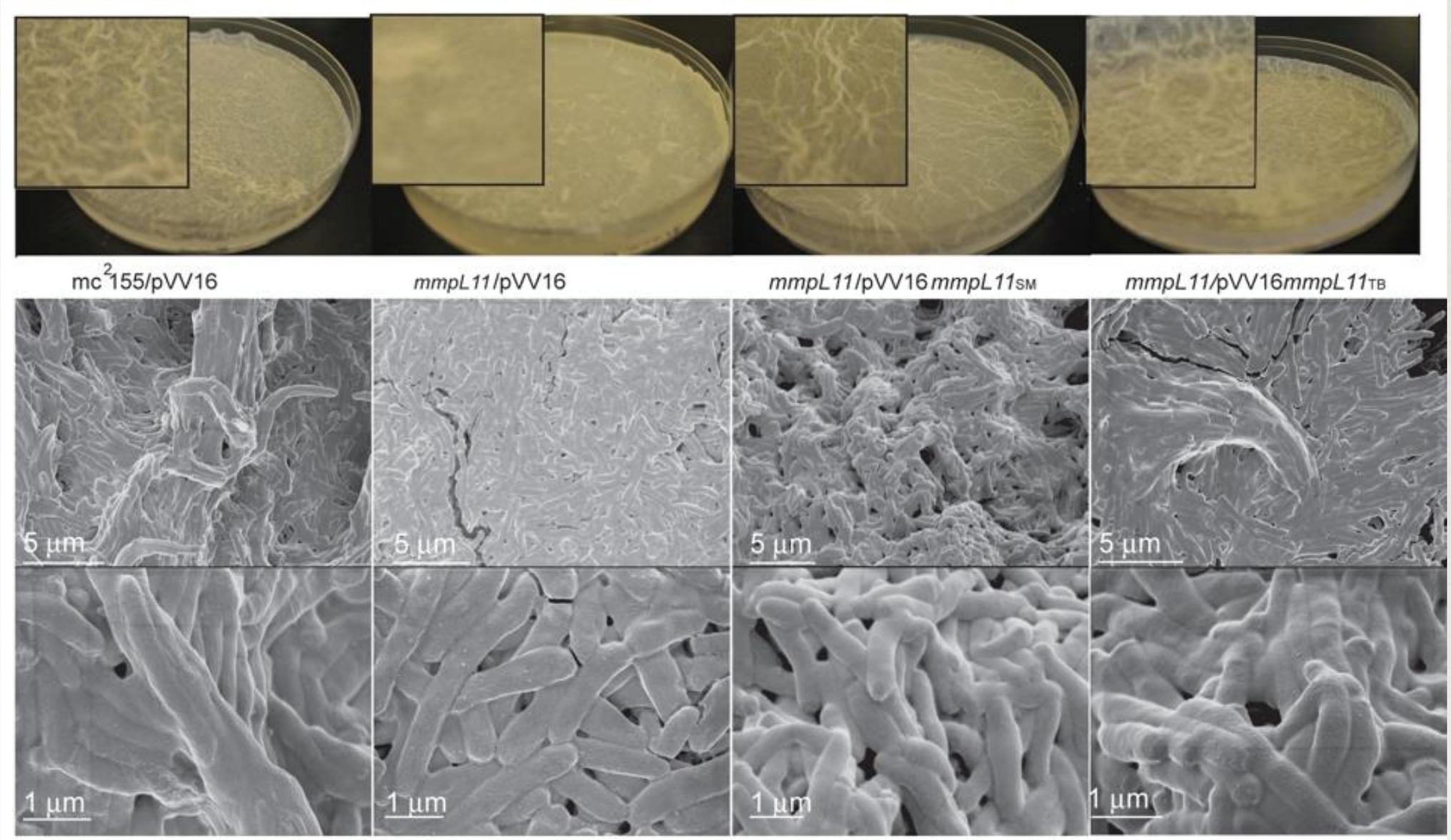


Pacheco et al., 2013

MmpL11 transports mycolate-containing lipids in *M. smegmatis*



What is the role of MmpL11 in Mtb?

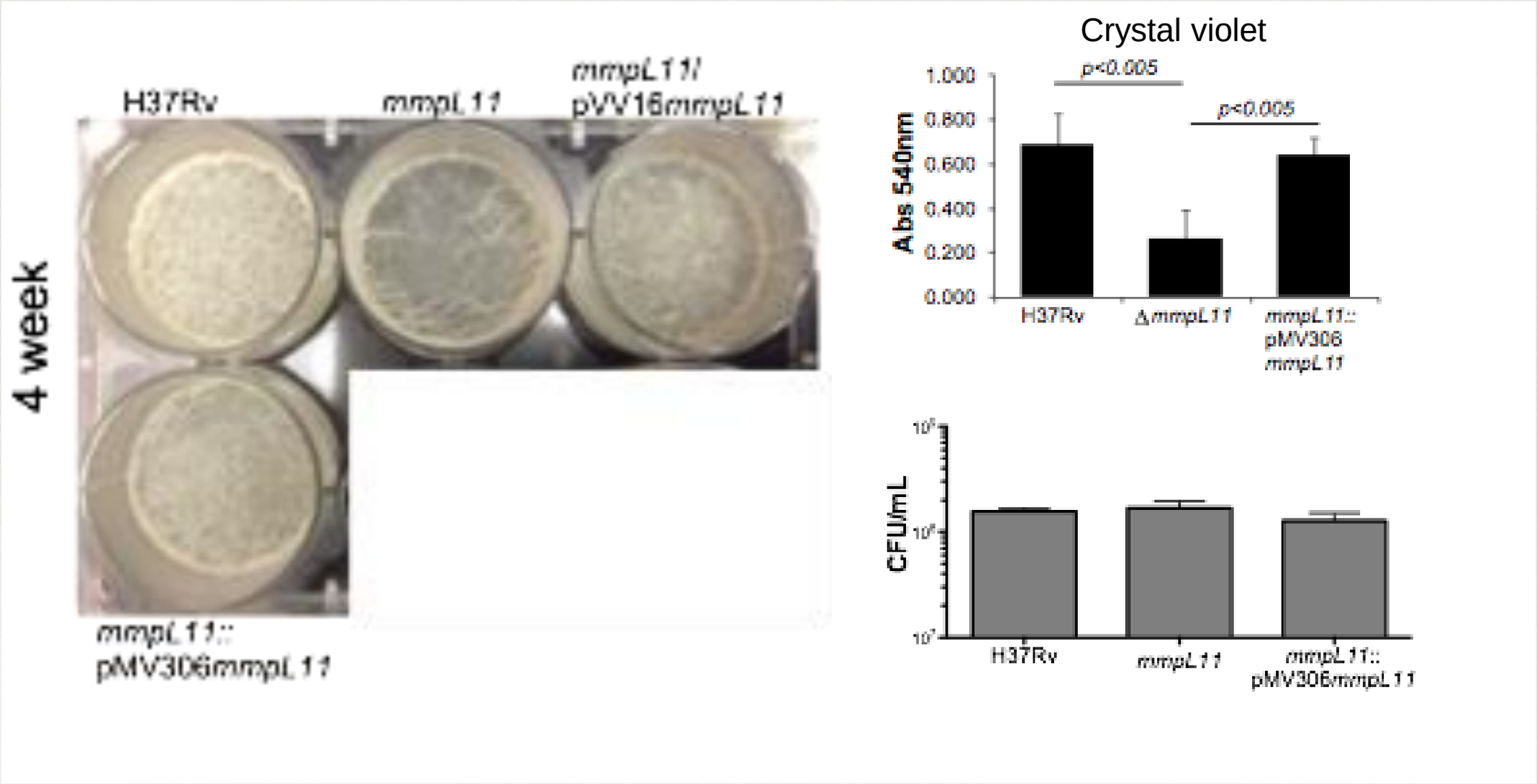


Pacheco et al., 2013

What is the role of MmpL11 in Mtb?

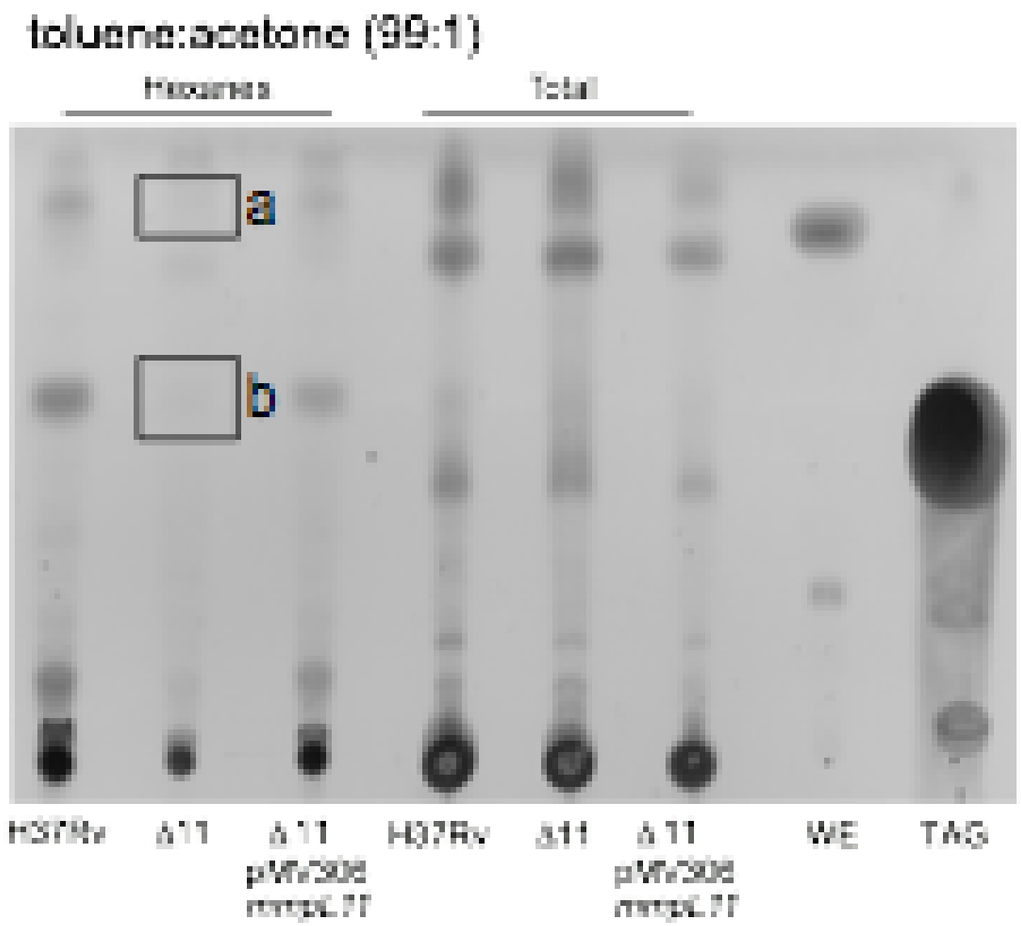
- We hypothesized that:
  - MmpL11 also transports mycolic acid-containing lipids in *M. tuberculosis*.
  - The *M. tuberculosis mmpL11* mutant has altered cell wall properties including biofilm formation.

Mtb *mmpL11* mutant has reduced biofilm formation



Wright et al., 2017

Mtb *mmpL11* mutant lacks LC-TAG and wax ester mycolate on the bacterial surface



Wright et al., 2017

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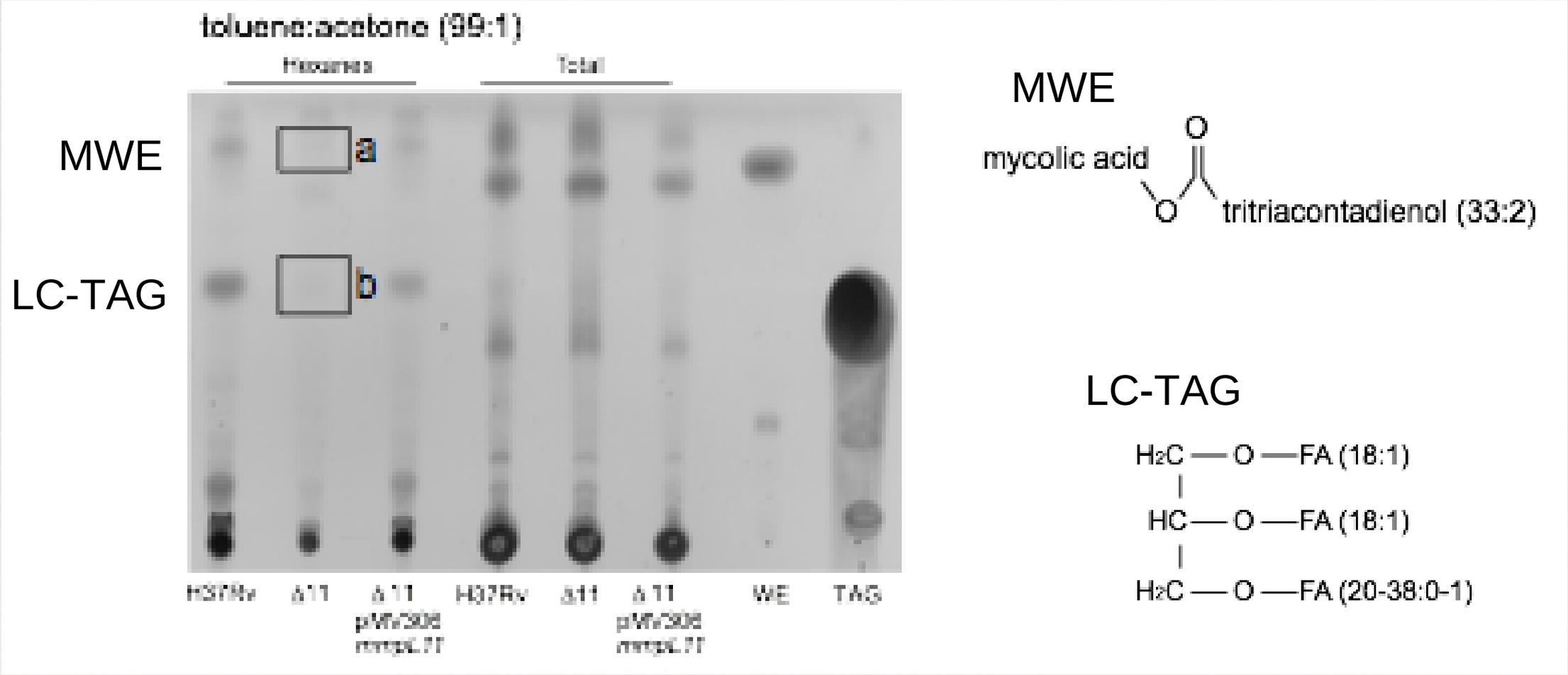
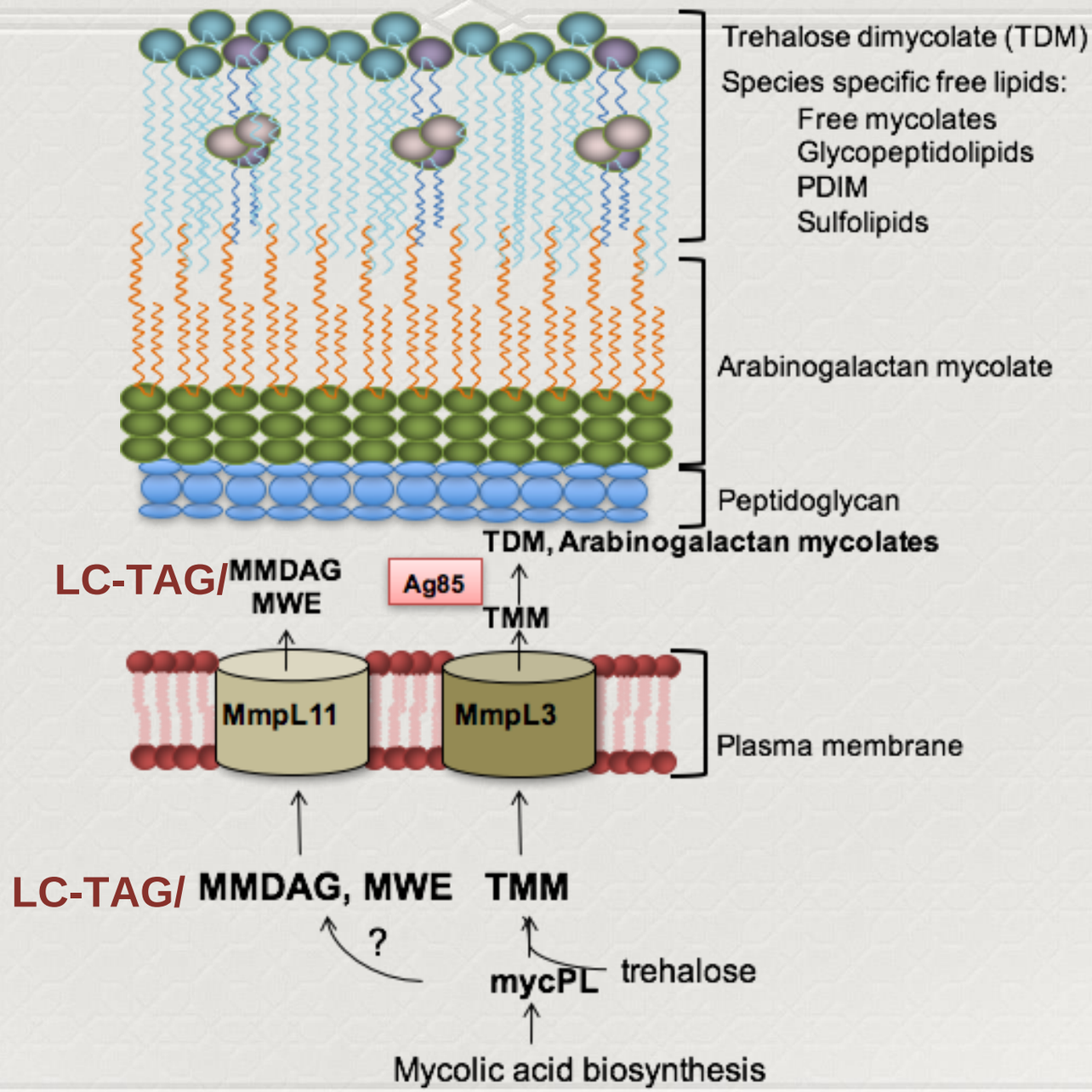
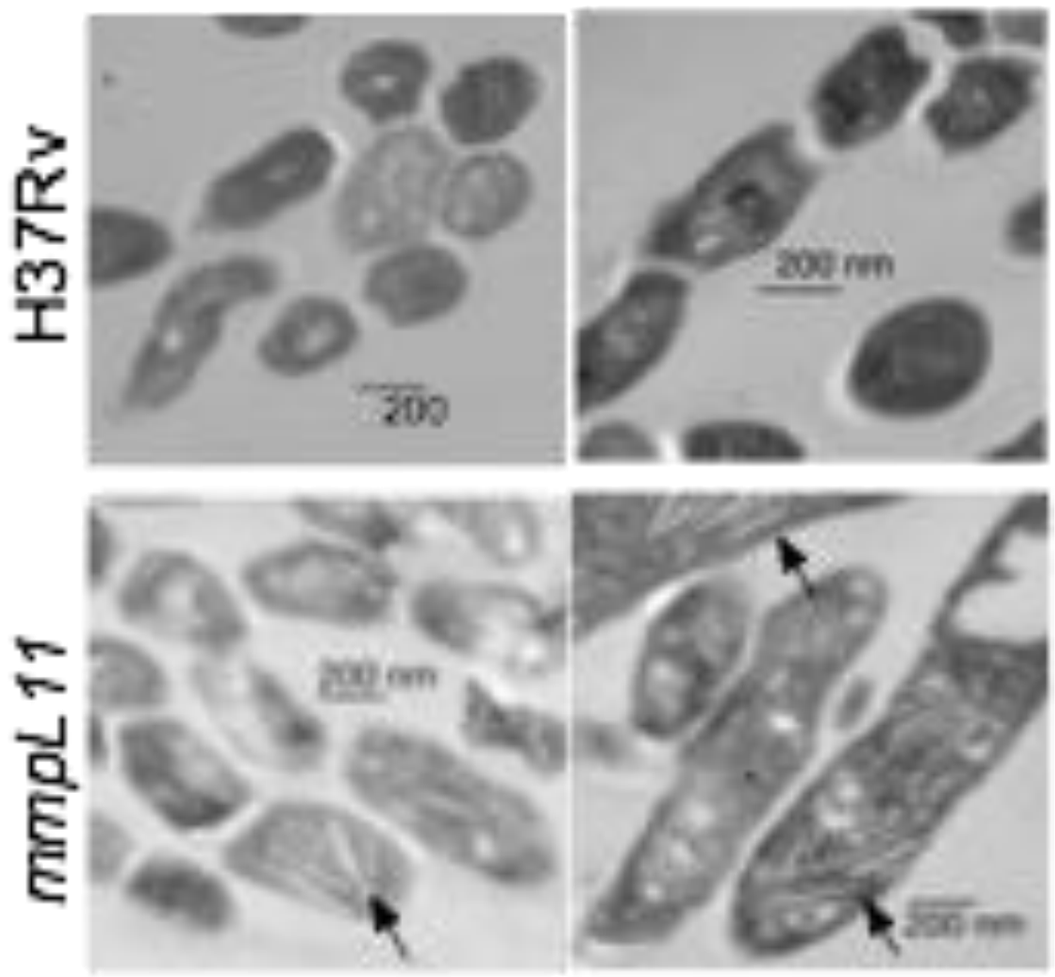


Wright et al., 2017

MmpL11 transports LC-TAG and mycolate wax ester in Mtb



The Mtb *mmpL11* mutant accumulates inclusions



Wright et al., 2017

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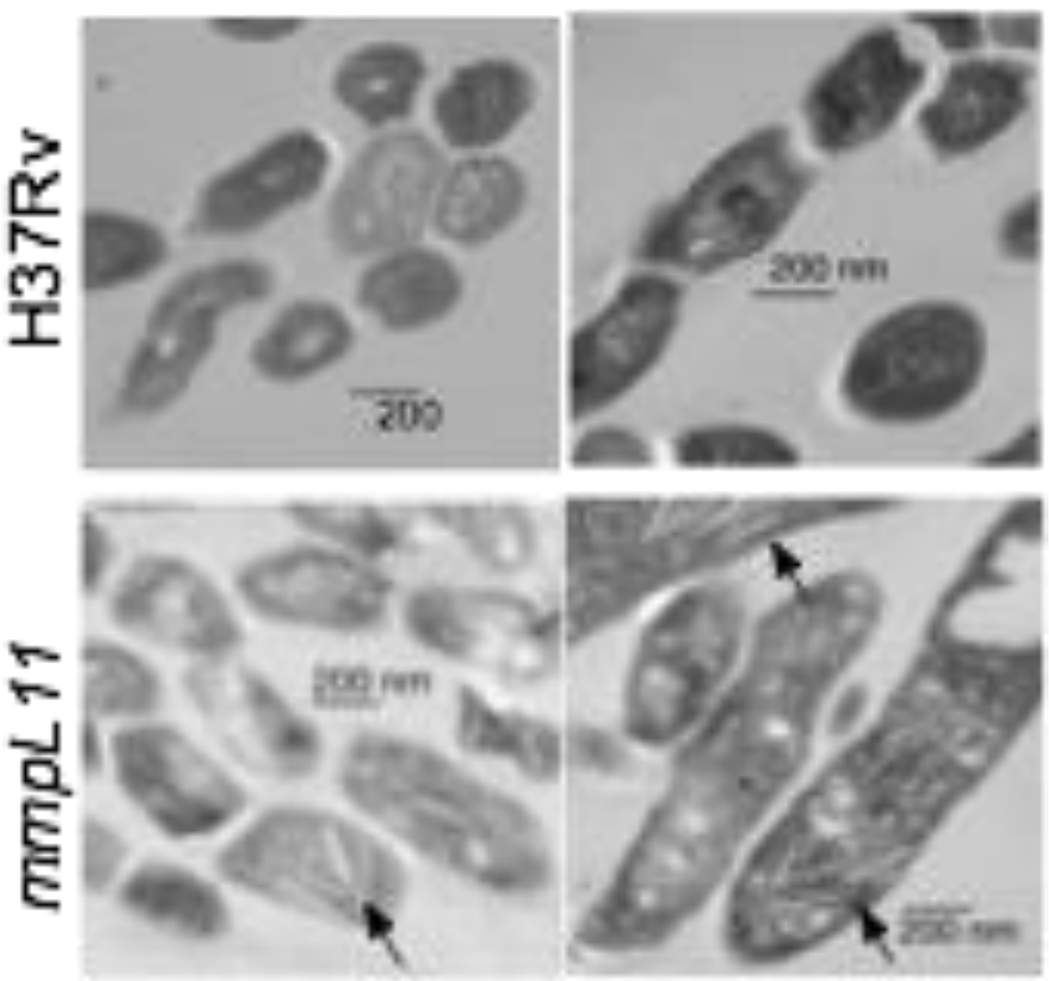
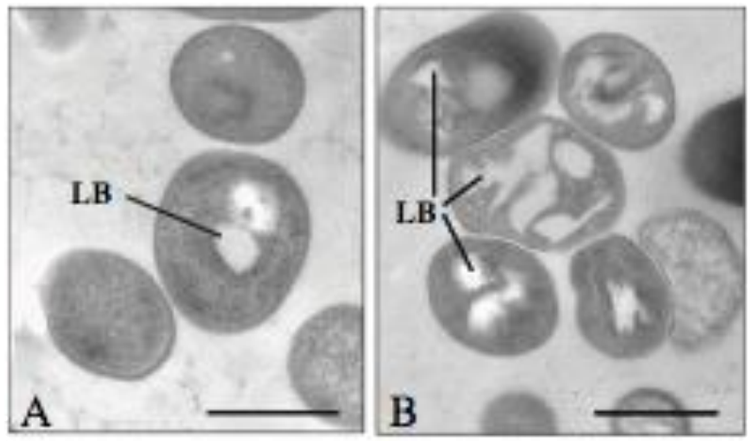


M. kansasii

Control +INH



Kremer, et al. 2005

Wright et al., 2017

MmpL11 is required for Mtb virulence

TABLE 3. Median survival and *P* values (by the log rank test) of different groups of mice

Strain with which mice were infected	Median survival (days)[a]	*P* value
M. tuberculosis H37Rv	265	
M. tuberculosis mmpL1::hyg	214	0.0005
M. tuberculosis mmpL2::hyg	177	<0.0001
M. tuberculosis mmpL4::hyg	Undef.	<0.0001
M. tuberculosis mmpL5::hyg	276	0.9081
M. tuberculosis mmpL6::hyg	235	0.0433
M. tuberculosis mmpL7::hyg	Undef.	<0.0001
M. tuberculosis mmpL8::hyg	328	<0.0006
M. tuberculosis mmpL9::hyg	302	0.2482
M. tuberculosis mmpL10::hyg	252	0.5444
M. tuberculosis mmpL11::hyg	398	<0.0001
M. tuberculosis mmpL12::hyg	217	0.0029

[a] Data were analyzed as described in the legend to Fig. 5B. Undef., undefined.

Domenech et al., 2005 (C57Bl6)

MmpL11 is required for Mtb virulence- Why?

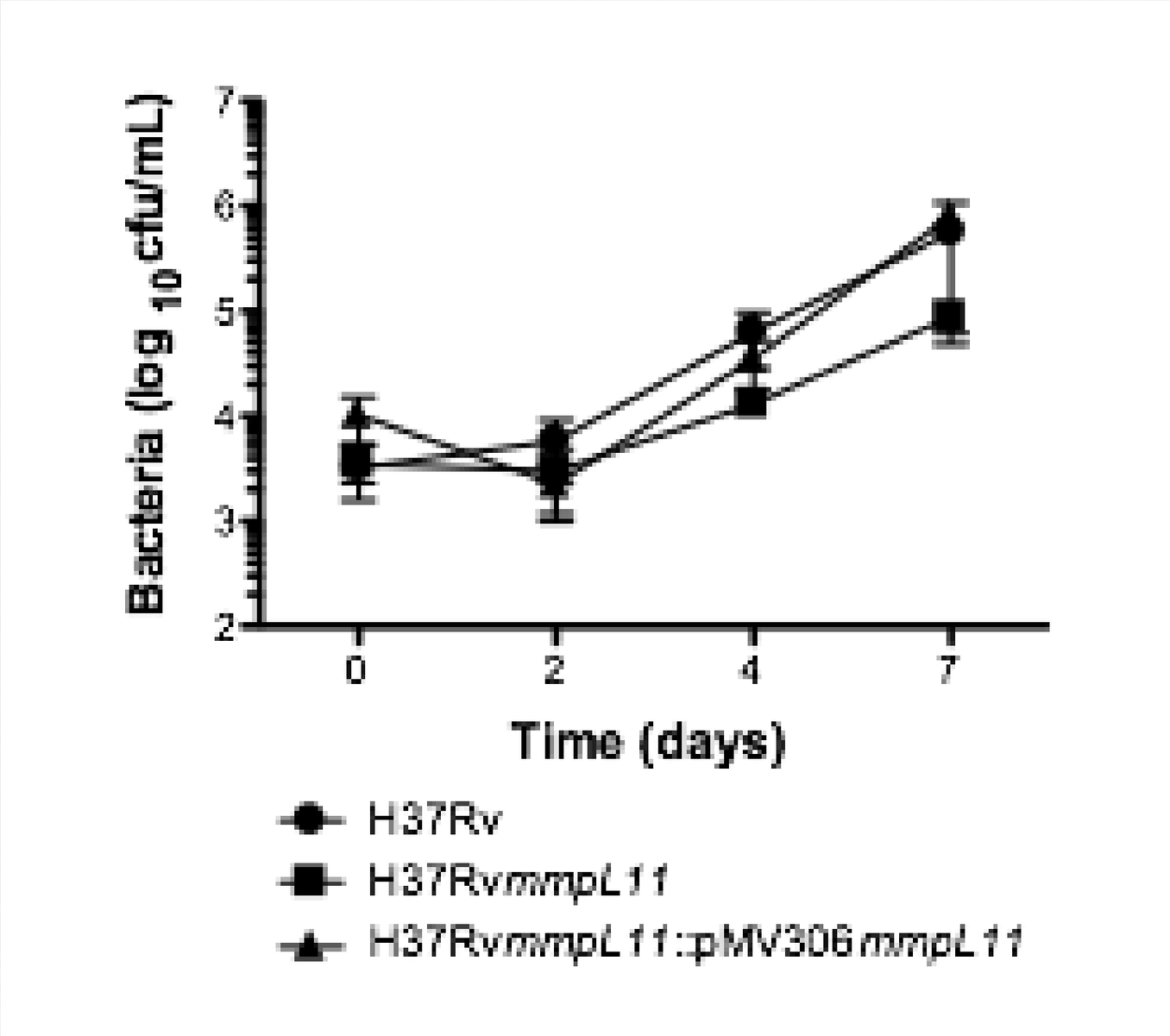
- The Mtb *mmpL11* mutant may not survive in the context of adaptive immune pressure.
- The *mmpL11* mutant may lack “storage lipids” required to establish a persistent phenotype.

Wax Ester Synthesis is Required for *Mycobacterium tuberculosis* to Enter *In Vitro* Dormancy

Tatiana D. Sirakova, Chirajyoti Deb, Jaiyanth Daniel, Harminder D. Singh, Hedia Maamar, Vinod S. Dubey, Pappachan E. Kolattukudy*

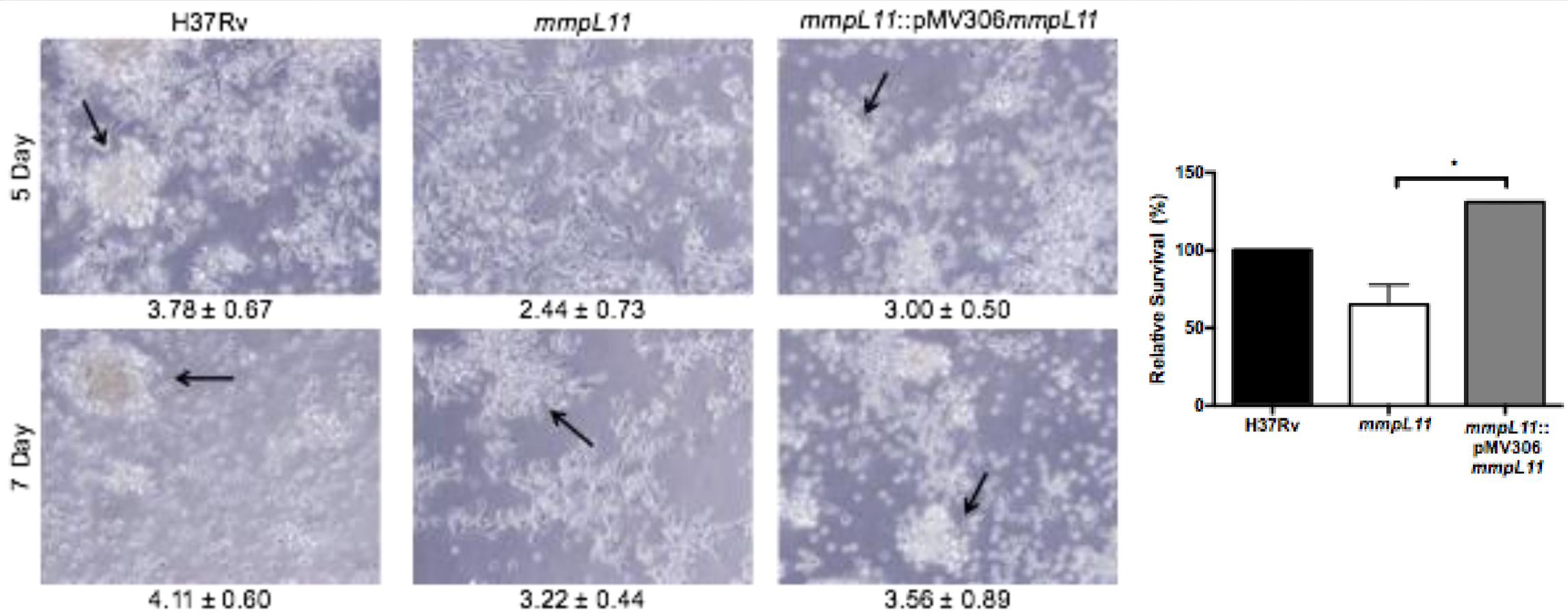
- Nutrient acquisition may be impaired.

The Mtb *mmpL11* mutant infects and replicates in macrophages



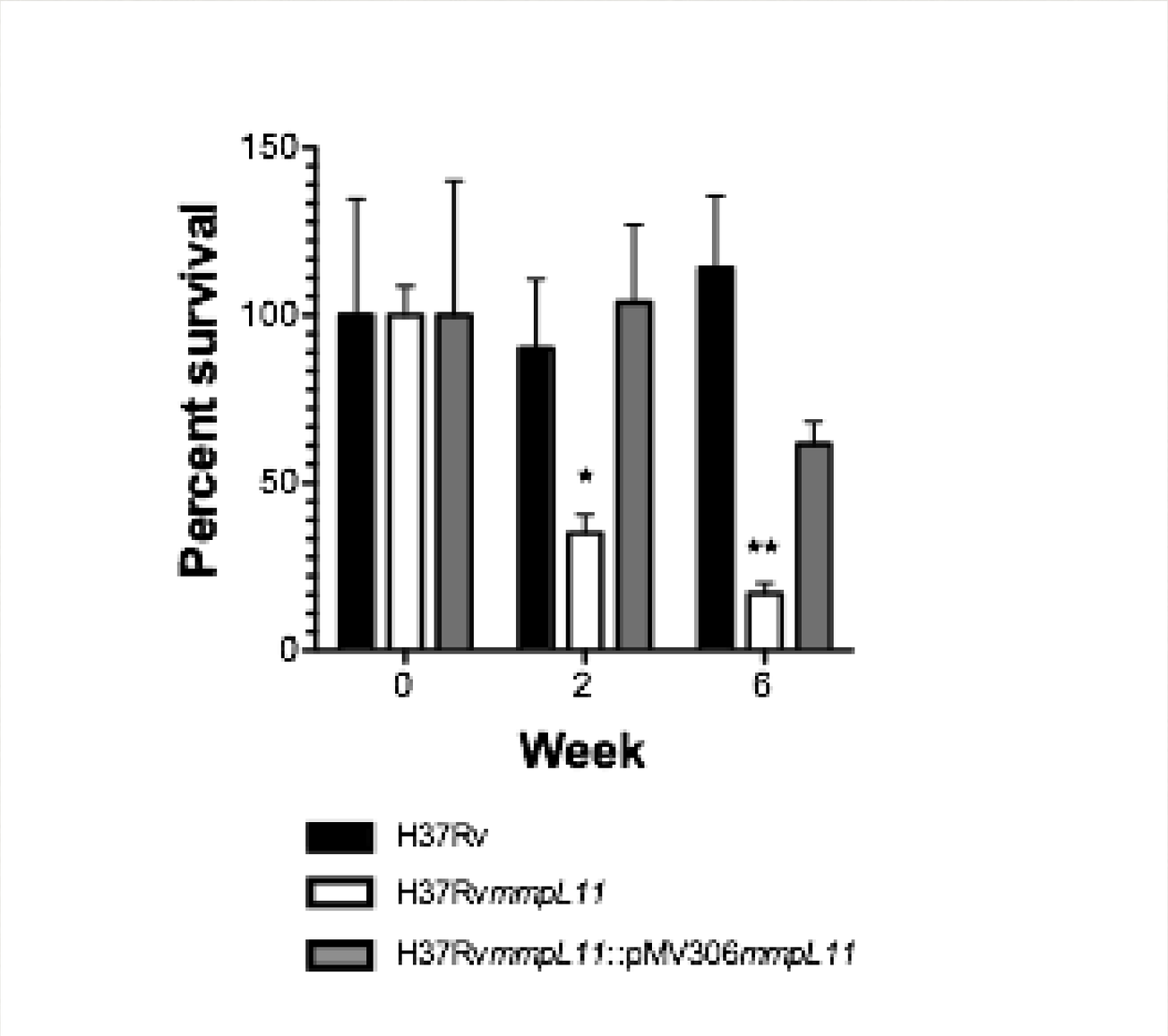
Wright et al., 2017

The Mtb *mmpL11* mutant has reduced survival in an in vitro granuloma model



Wright et al., 2017

The Mtb *mmpL11* mutant has reduced survival in an in vitro dormancy model

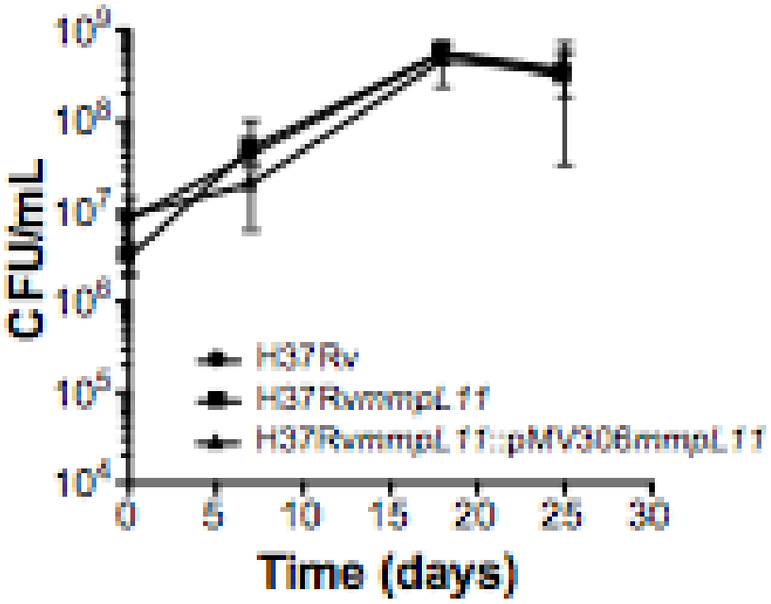


Wright et al., 2017

The Mtb *mmpL11* mutant forms small colonies on agar plates

7H10 agar

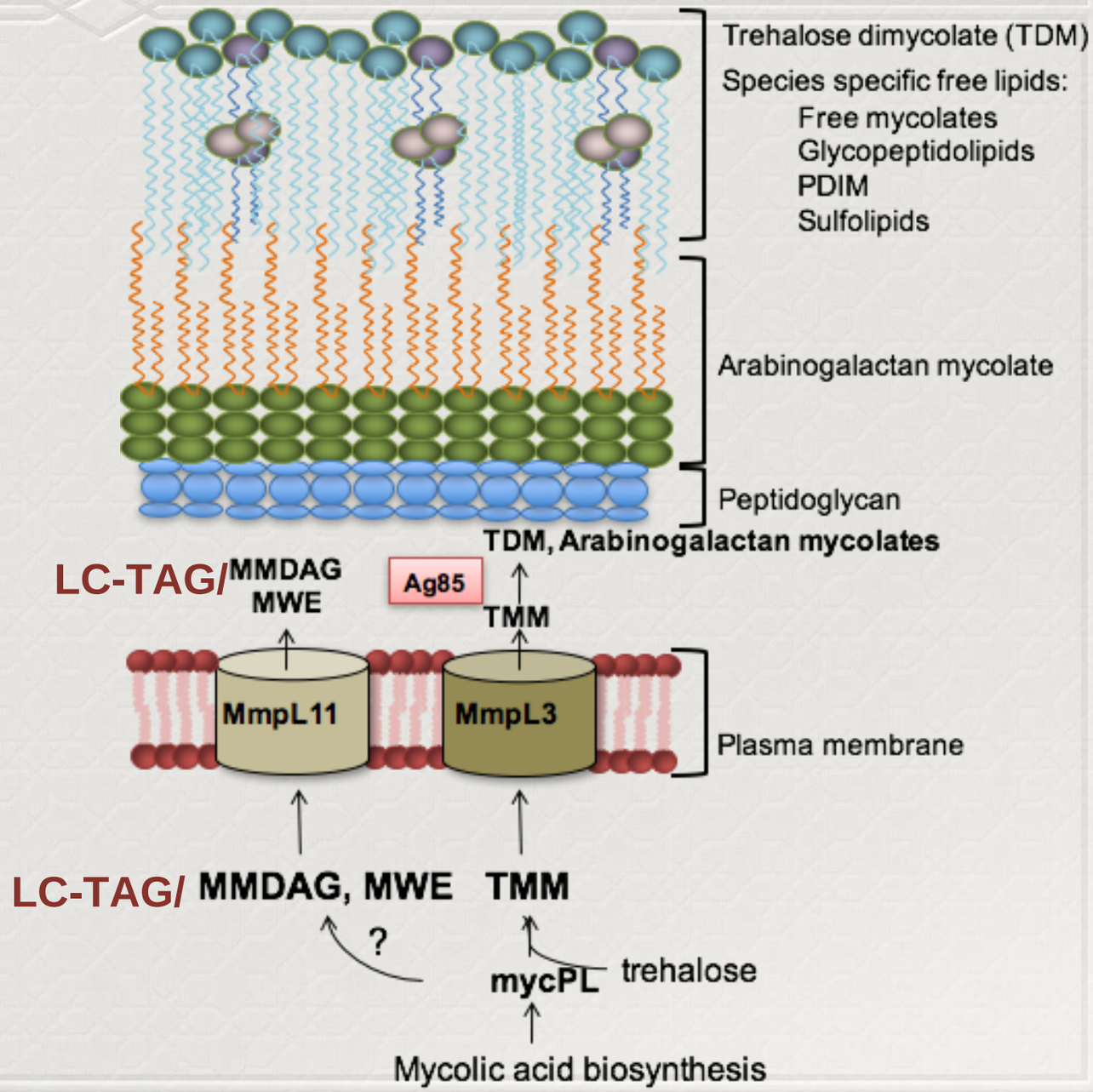
Sauton's broth



Wright et al., 2017

Conclusions

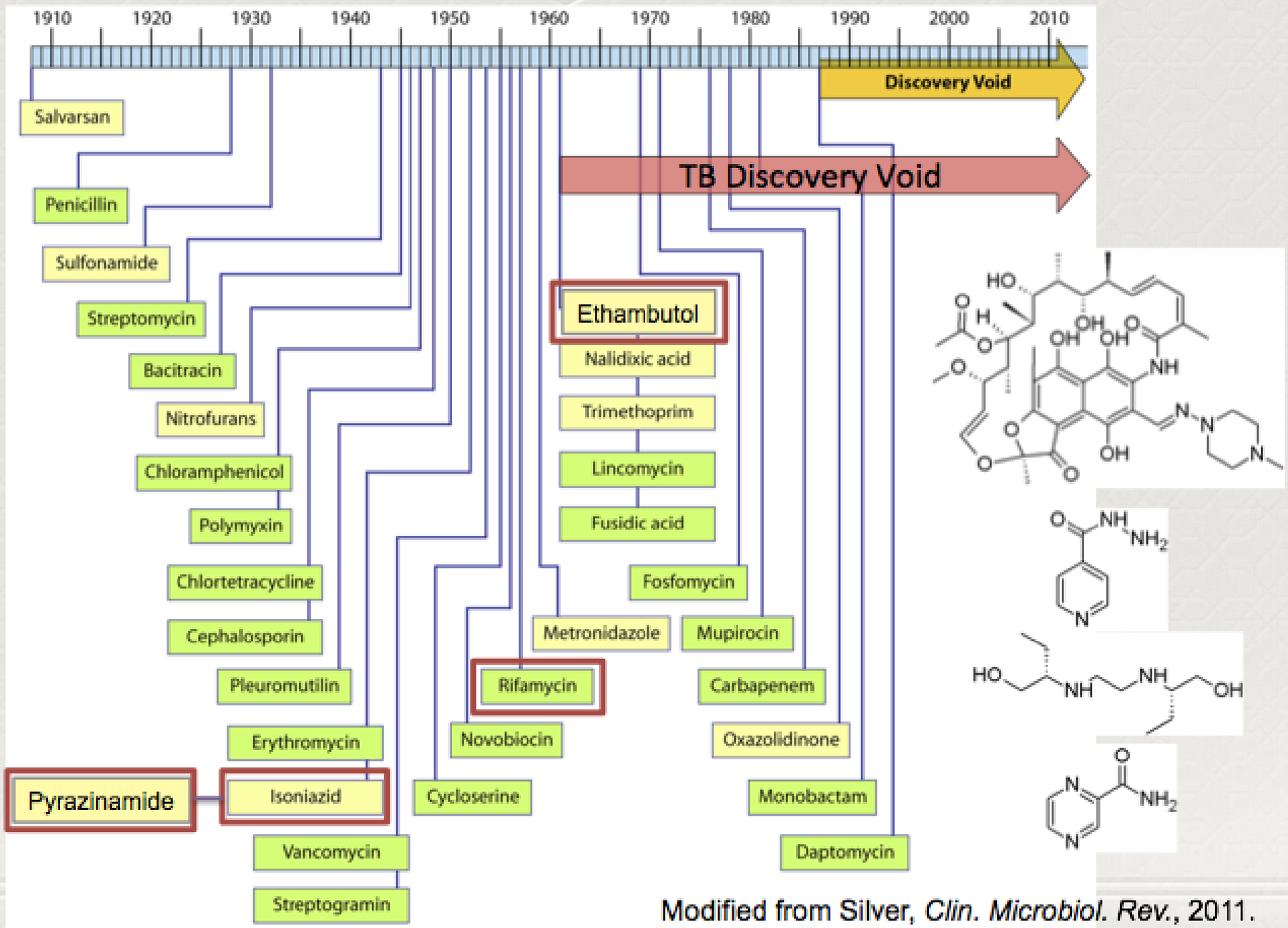
- MmpL3 is needed for Mtb replication.
- MmpL11 and its substrates are necessary for Mtb non-replicating persistence.



Non-replicating persistence as a therapeutic target

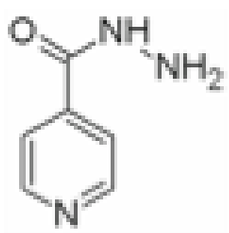
- Most people with TB have latent infections where the bacteria are not replicating.
- Non-replicating persistent (dormant) Mtb are phenotypically-resistant to first-line Mtb drugs.
- What pathways are still functional in non-replicating persistent bacteria? Can we target them to develop new therapeutics?

Shortfall in antibiotic interventions

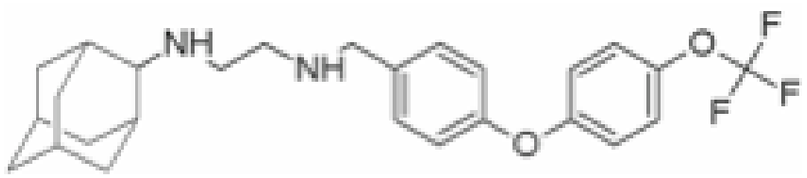


Modified from Silver, *Clin. Microbiol. Rev.*, 2011.

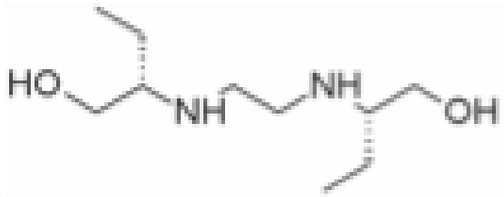
Translating basic cell wall physiology into novel TB therapeutics



Isoniazid (INH)



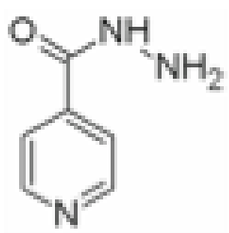
TBL-140



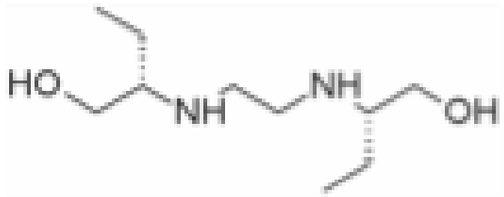
Ethambutol (EMB)

Foss et al., 2016

Translating basic cell wall physiology into novel TB therapeutics



Isoniazid (INH)



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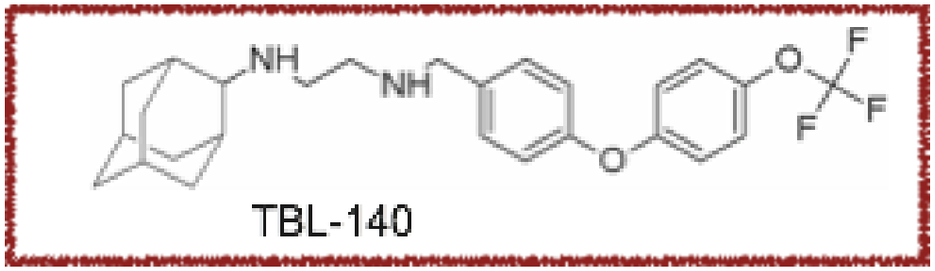


Foss et al., 2016

TBL-140 has similar potency to Ethambutol

Antibiotic	**MIC_{99} *M. tuberculosis***
Isoniazid	50 ng/mL
Rifampicin	25 ng/mL
Ethambutol	2 ug/mL
DA5	6.4 ug/mL
TBL-140	**0.58 ug/mL**

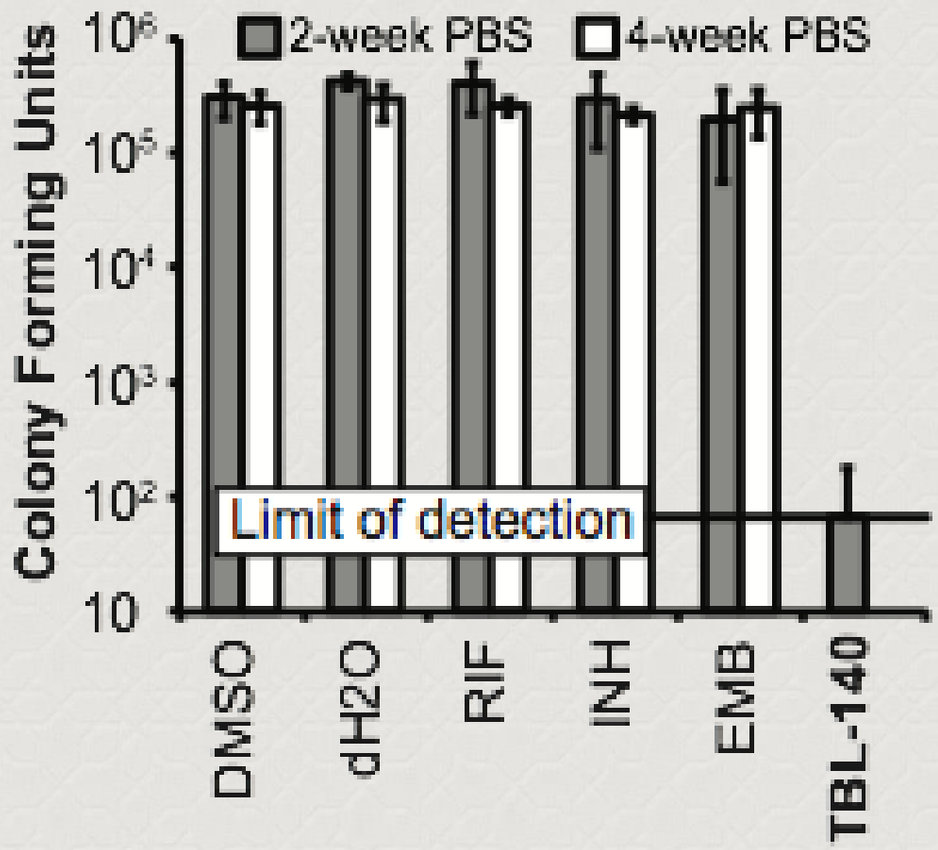
Foss et al., 2016

TBL-140 is effective against drug-resistant Mtb

Strain S – sensitive R – resistant	Rifampicin (ng/mL)	Isoniazid (ng/mL)	PSP2-140 (µg/mL)
T21 (INH – S)	12.5	50	0.58
T27 (INH – R)	12.5	>800	0.58
Beijing 26 (RIF – S)	12.5	25	1.2
Beijing 22 (RIF – R)	>400	25	1.2
Haarlem 23 (RIF – S)	12.5	25	1.2
Haarlem 9 (RIF – R)	>400	25	1.2

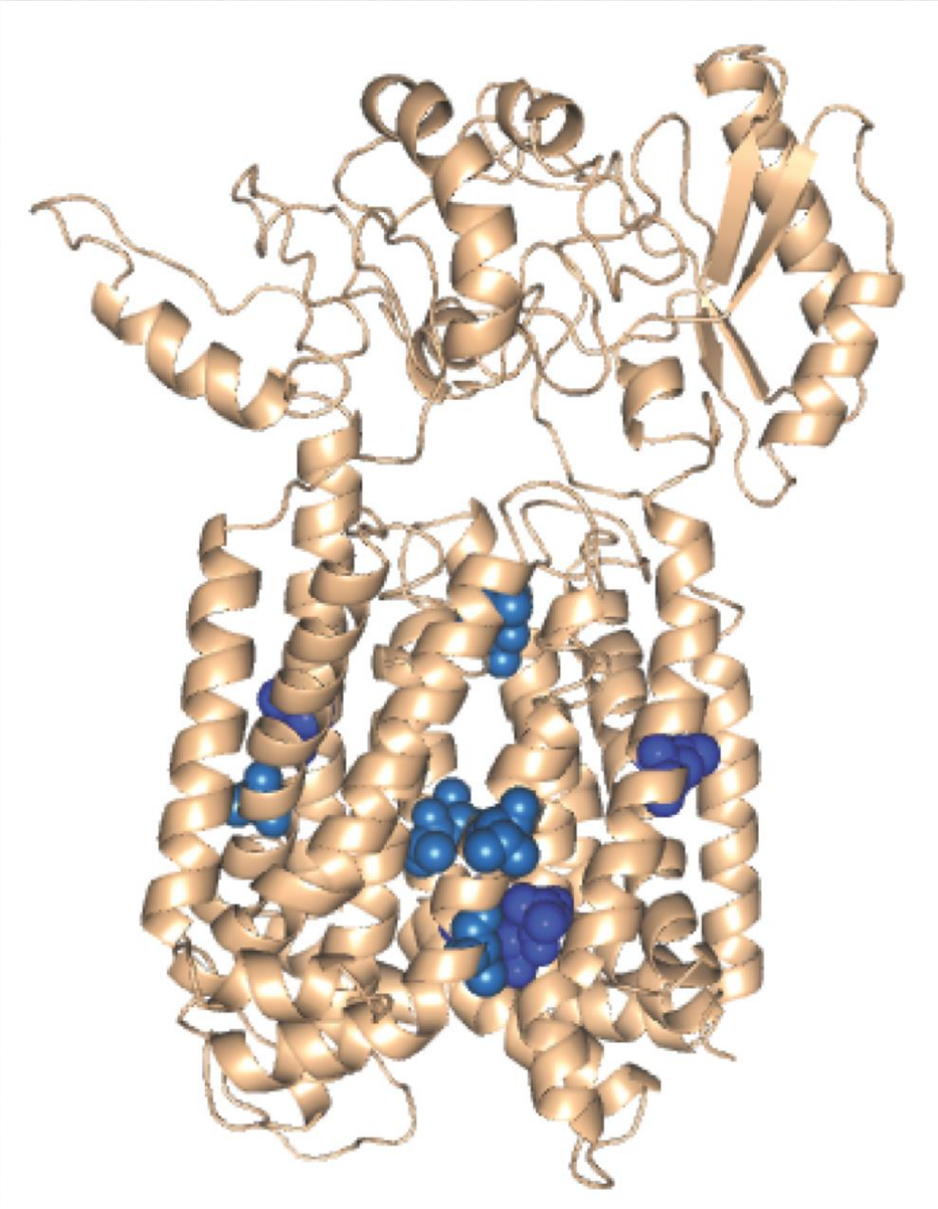
Foss et al., 2016

TBL-140 targets non-replicating persistent Mtb



Foss et al., 2016

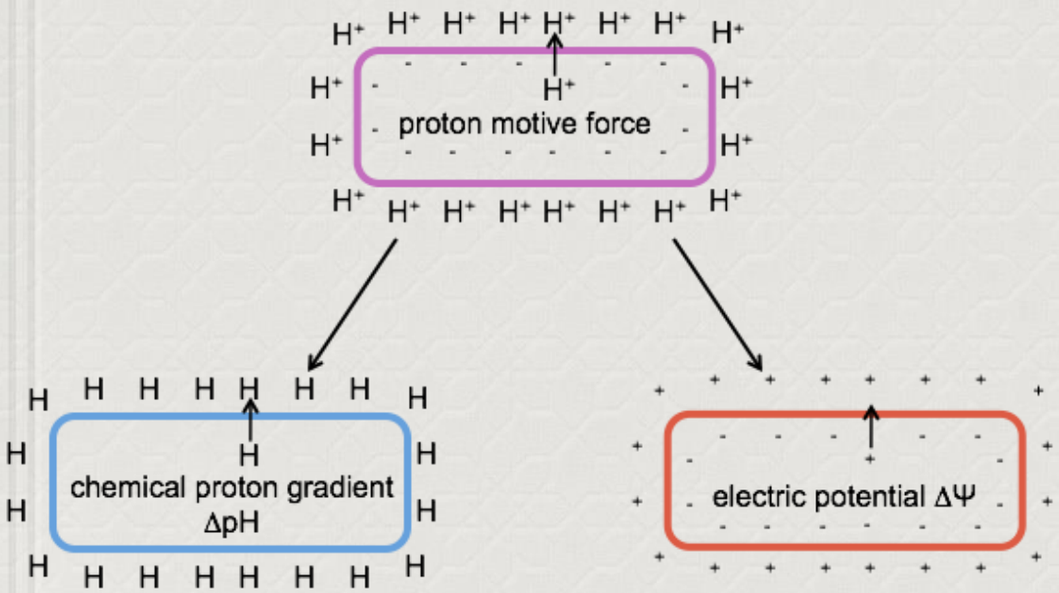
Genetic analysis indicates TBL-140 targets MmpL3

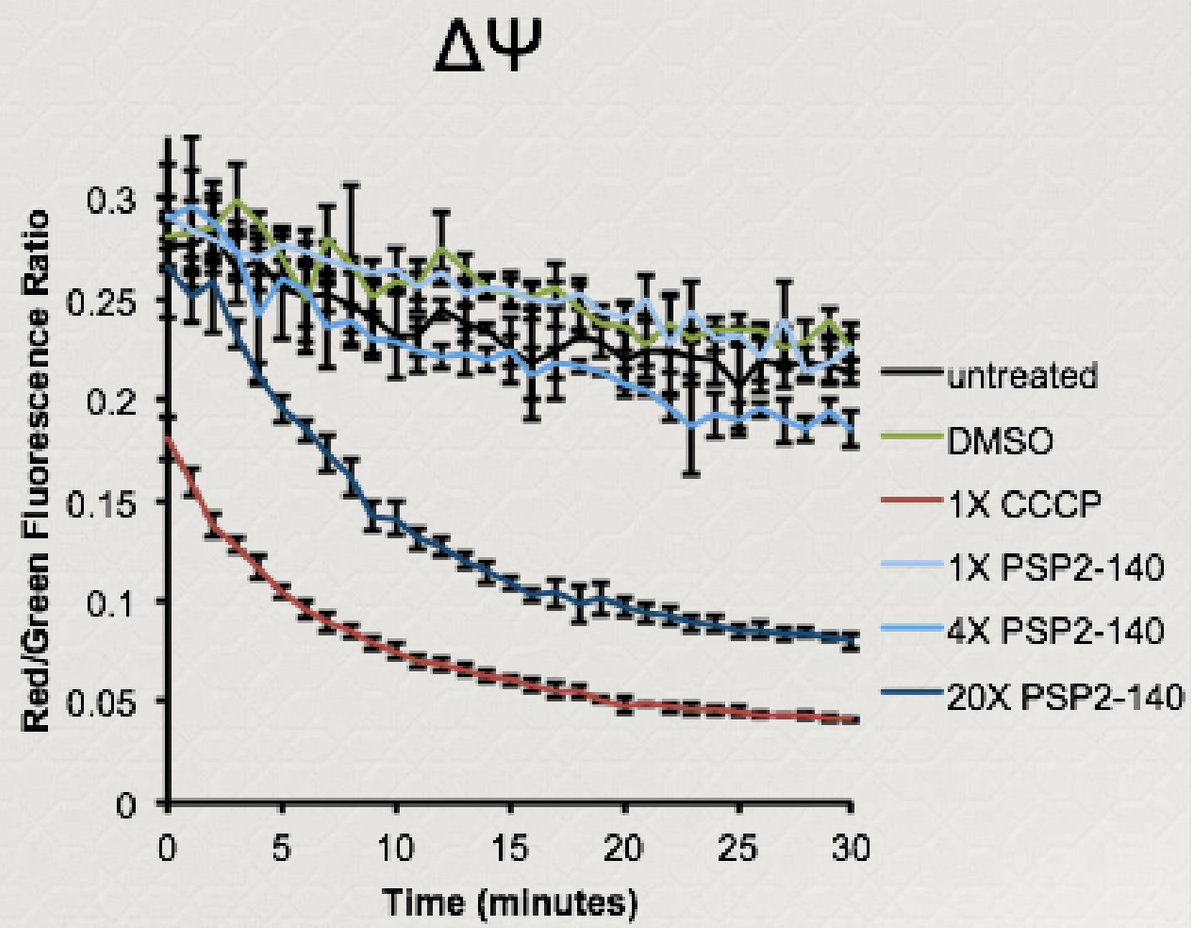


Resistant Isolates	Base	Codon	Amino Acid	TBL-140 MIC (µg/mL)
H37Rv	NA	NA	NA	1.2
TBL-140 #X3	566T	CTG→CGG	L189R	9.2
TBL-140 #16	1700T	CTG→CCG	L567P	9.2
TBL-140 #13, 14, 17	1927G	GTG→ATG	V643M	9.2
TBL-140 #12	1932C	TTC→TTG	F644L	9.2

Foss et al., 2016

PSP2-140 targets mycobacterial PMF



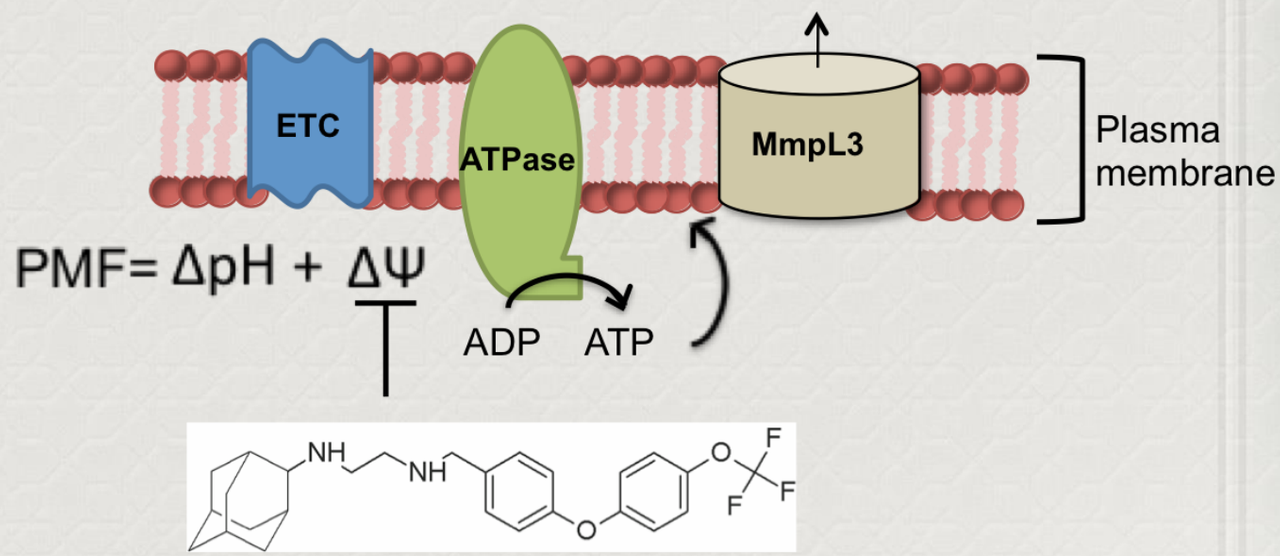


M. bovis BCG

Foss et al., 2016

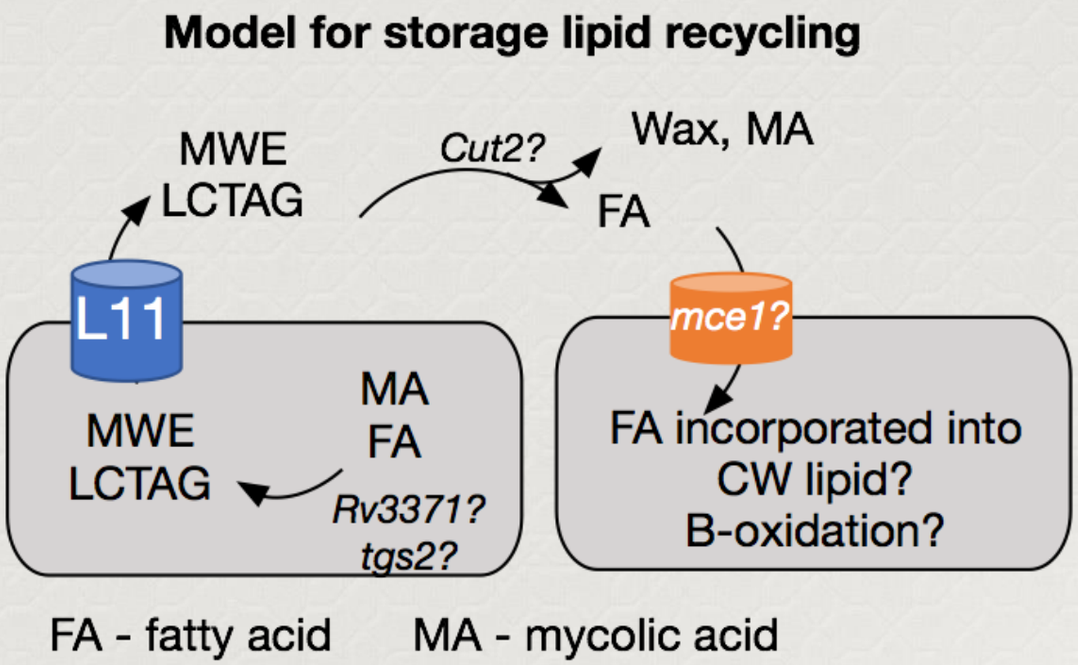
Conclusions

- TBL-140 targets the PMF and can kill non-replicating persistent Mtb.
- Unfortunately TBL-140 is “doomed” for other pharmacological reasons.



Future directions

- Identify pathways required for Mtb survival or resuscitation from non-replicating persistence.
  - Screen for bacterial mutants with defects.
  - Characterize targeted mutants that might play a role in storage lipid recycling



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