

Virulence determinants of *Mycobacterium tuberculosis* – special focus on ESX / type VII secretion

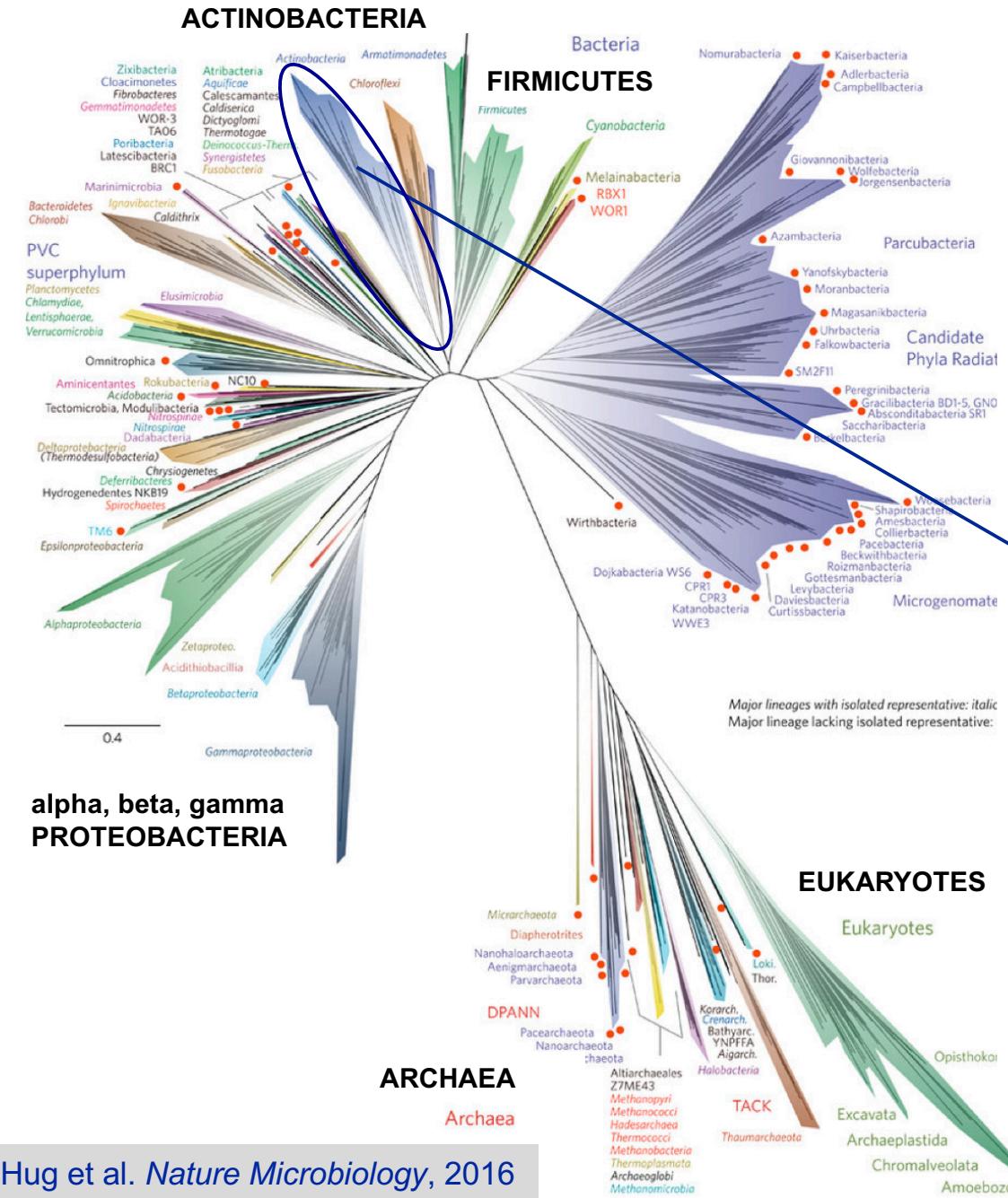
or Tuberculosis: From the genome to the cell

Roland Brosch

Unit for Integrated Mycobacterial Pathogenomics

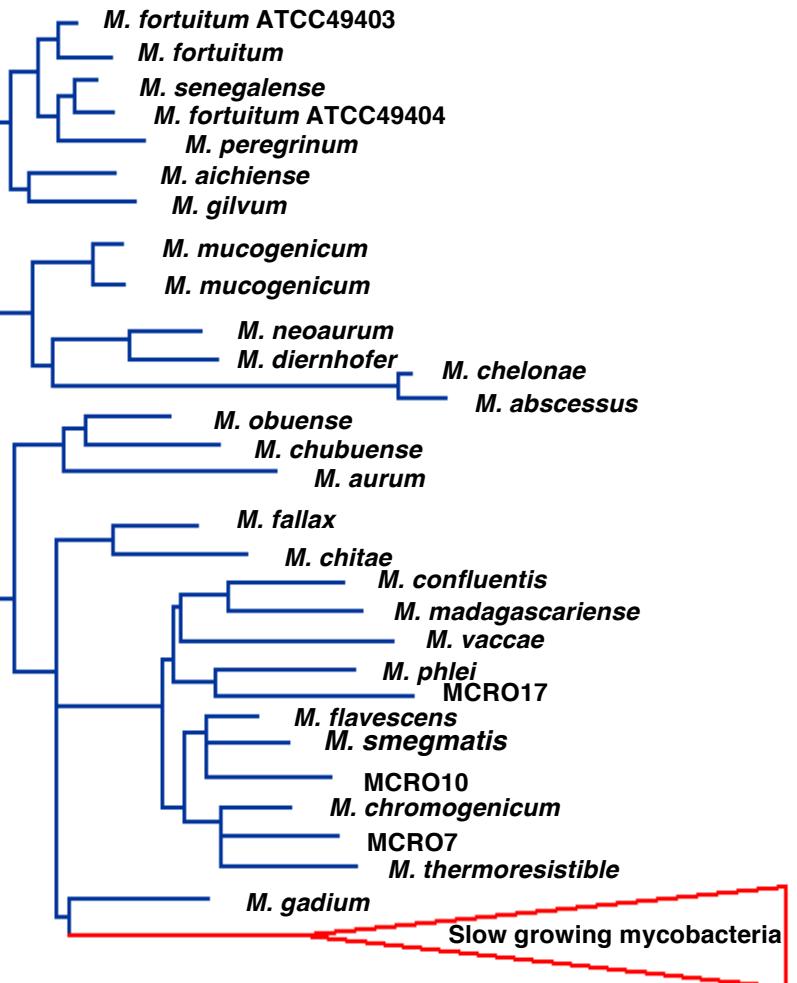
Bioinformatics and Genome Analyses
Institut Pasteur Tunis, Tunisia. September 18 – December 15, 2017



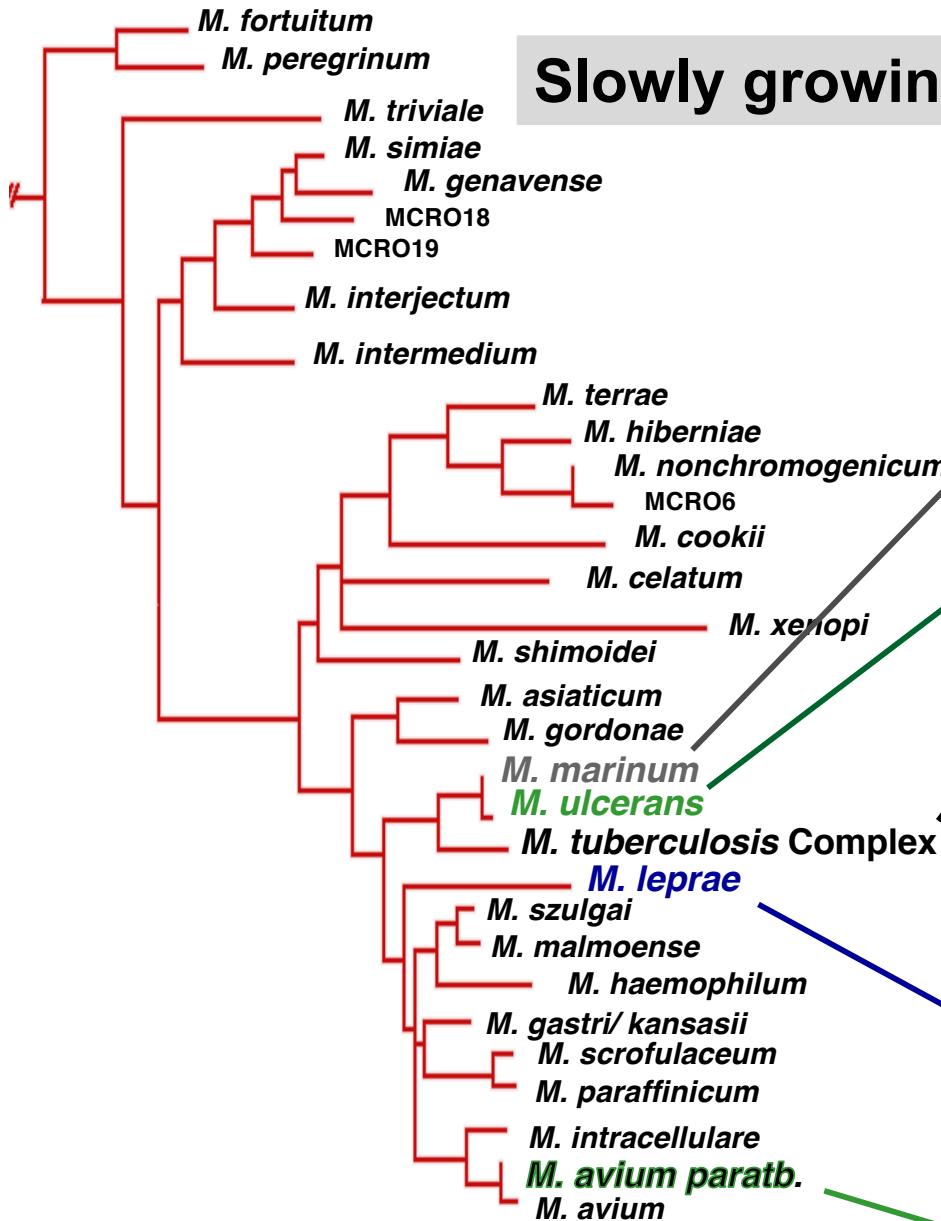


Mycobacteria are part of the phylum Actinobacteria

consists of fast-growers and slow-growers

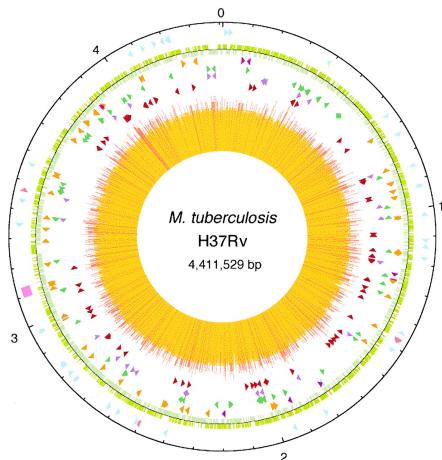


16S rRNA tree - Springer et al., J. Clin. Microbiol., (1996)



Slowly growing mycobacteria harbour pathogens

- fish pathog & human opportun. pathogen
 - aquatic mycobacterium,
 - **etiological agent of Buruli ulcer**
 - aquatic mycobacterium,
 - produces mycolactone
- M. canettii***
- **etiological agent of human tuberculosis (TB)**
 - ~ 10 million new cases
 - 1.8 million deaths
 - 2 billion latently infected
 - Synergy with HIV epidemic
 - MDR and XDR strains
- M. tuberculosis***
- agent of bovine TB
 - \$ 3 billion loss for world agriculture
- M. africanum***
- M. microti***
- M. pinnipedii***
- M. bovis***
- M. bov. BCG***
- etiological agent of leprosy
 - reductive evolution
- Johne's disease**



Cole ST, Brosch R, Parkhill J, et al., (1998), *Nature* 393, 537-544.

Genome of *M. tuberculosis* H37Rv

First mycobacterial genome sequenced

4,4 Mb

GC content 65.6%

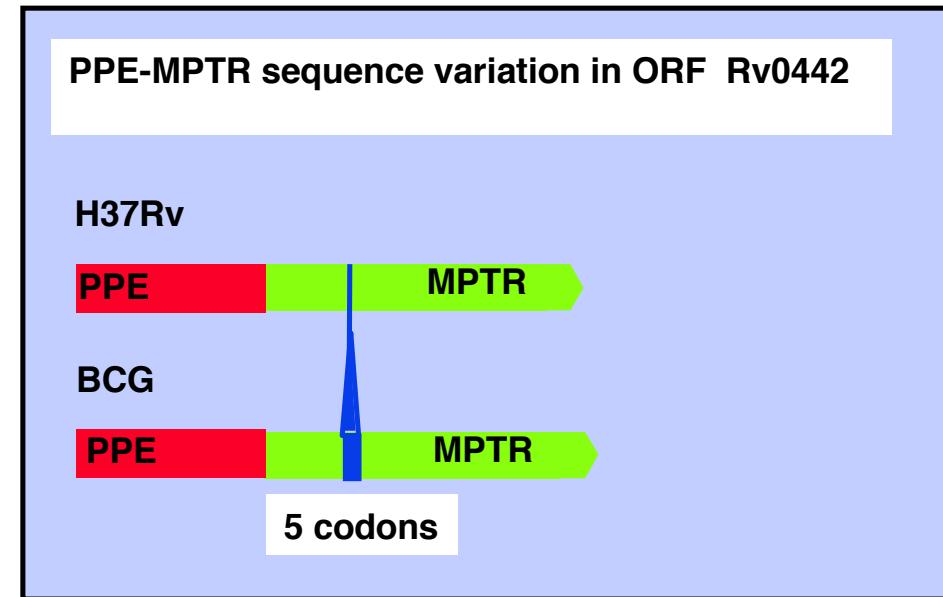
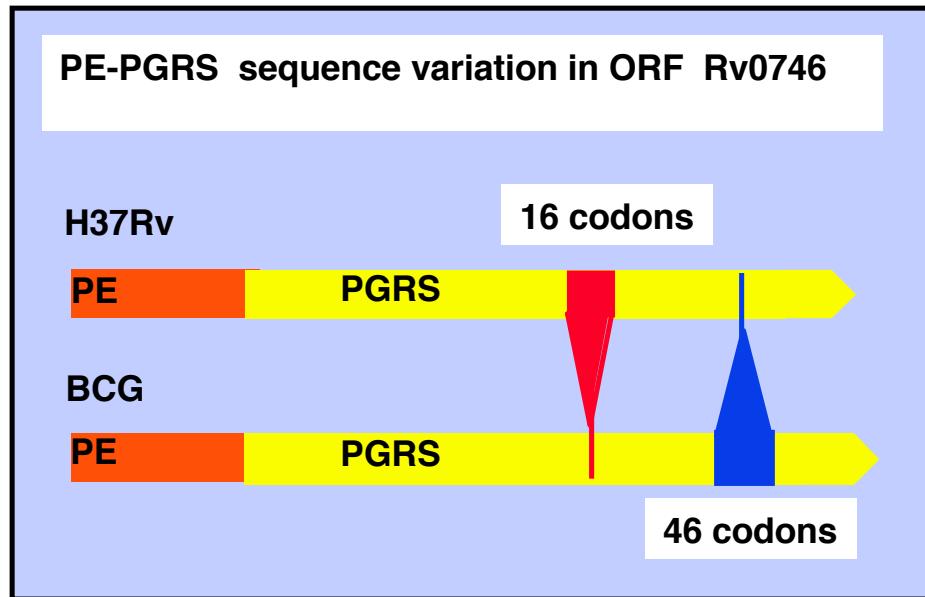
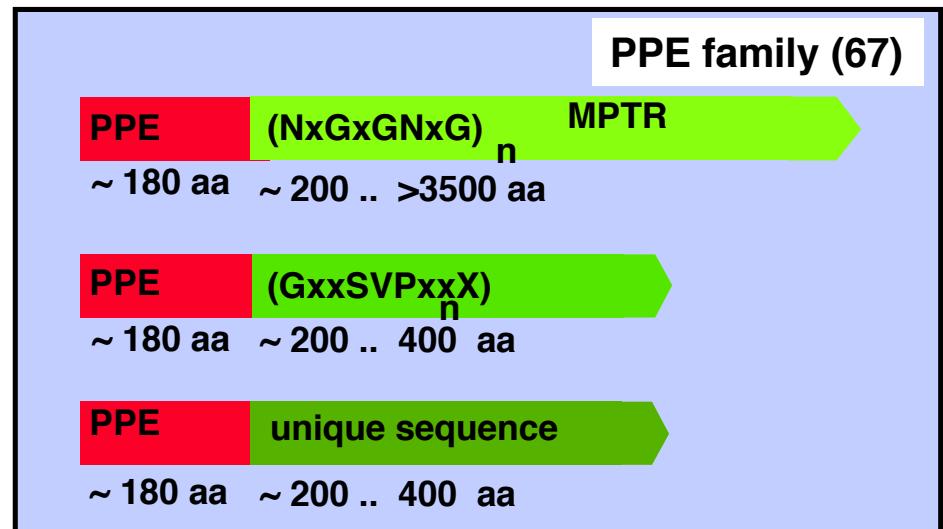
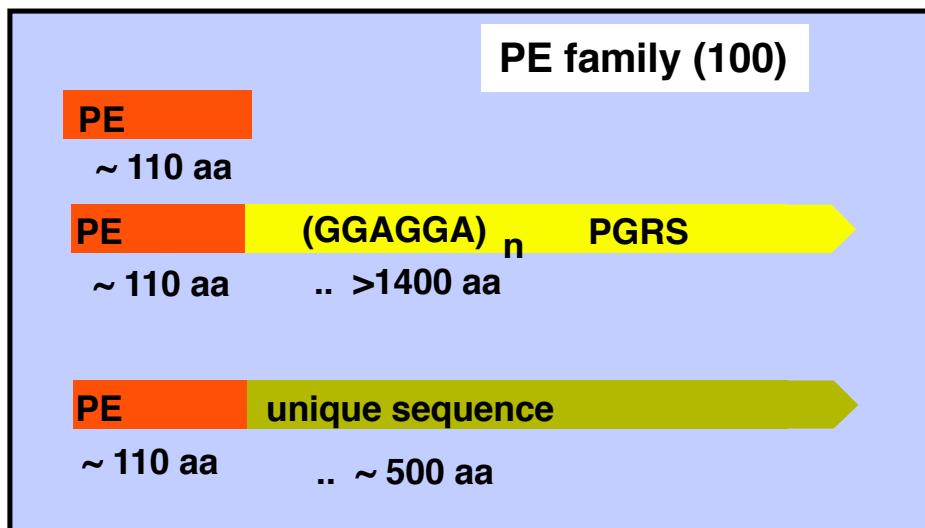
Genes for aerobic, micro-aerophilic and anaerobic growth

Abundance of genes involved in lipid metabolism

Eukaryotic-like Serine/Threonine Protein Kinases

Novel gene/protein families e.g. PE/PPE, ESAT-6,

Genome highlights → The PE and PPE protein families



Use of genomics for getting new insights into the biology of the tuberculosis agent

Key questions:

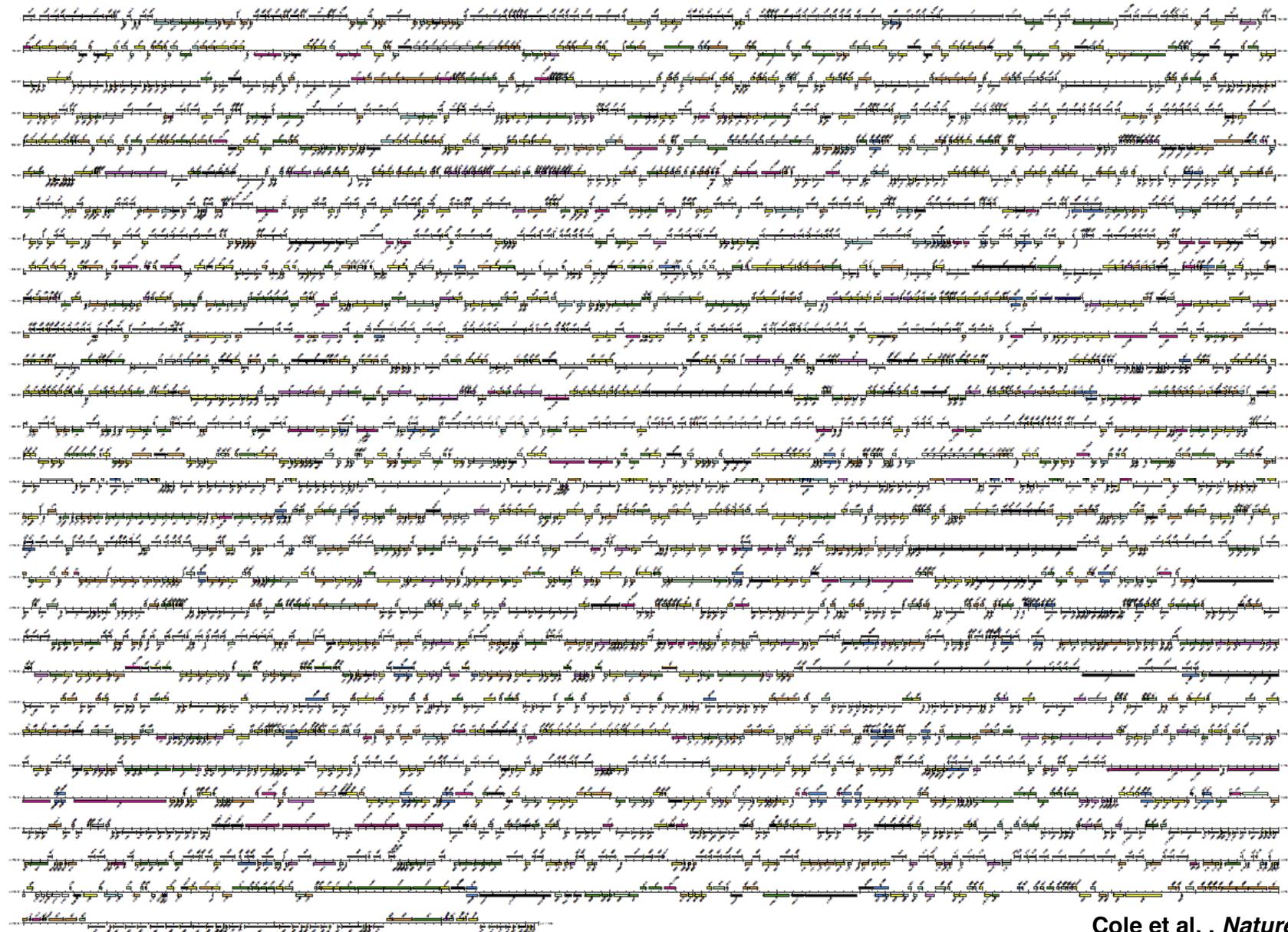
- identification of new drug targets
- identification of new virulence genes/
mechanisms → host pathogen interaction/
vaccines

Use of genomics for getting new insights into the biology of the tuberculosis agent

Key questions:

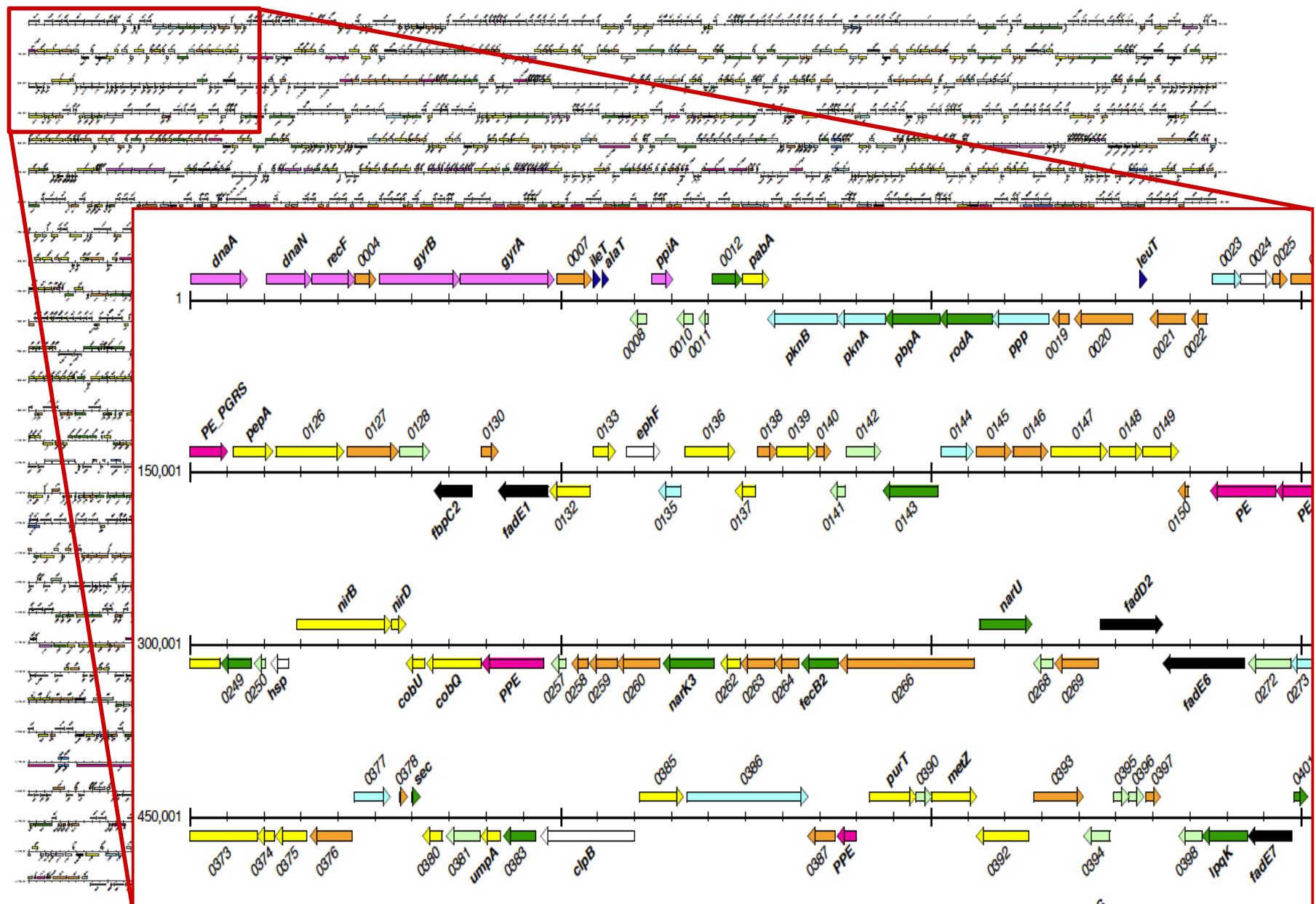
- **identification of new drug targets**
- **identification of new virulence genes/ mechanisms → host pathogen interaction**

M. tuberculosis H37Rv: ~ 4000 genes annotated

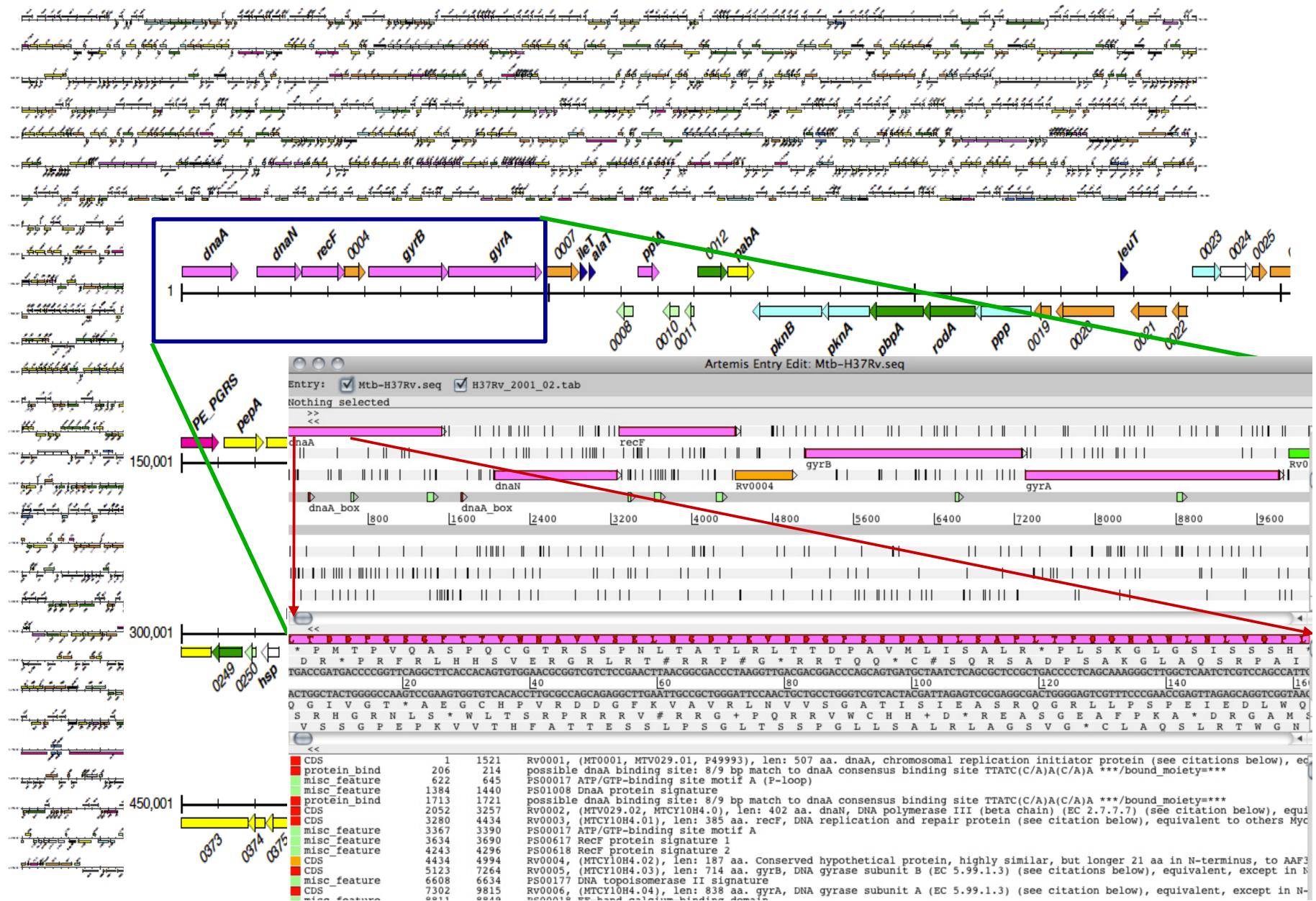


Cole et al., *Nature* 1998

M. tuberculosis H37Rv: zoom-in



M. tuberculosis H37Rv: zoom-in



Broad classification of *M. tuberculosis* genes

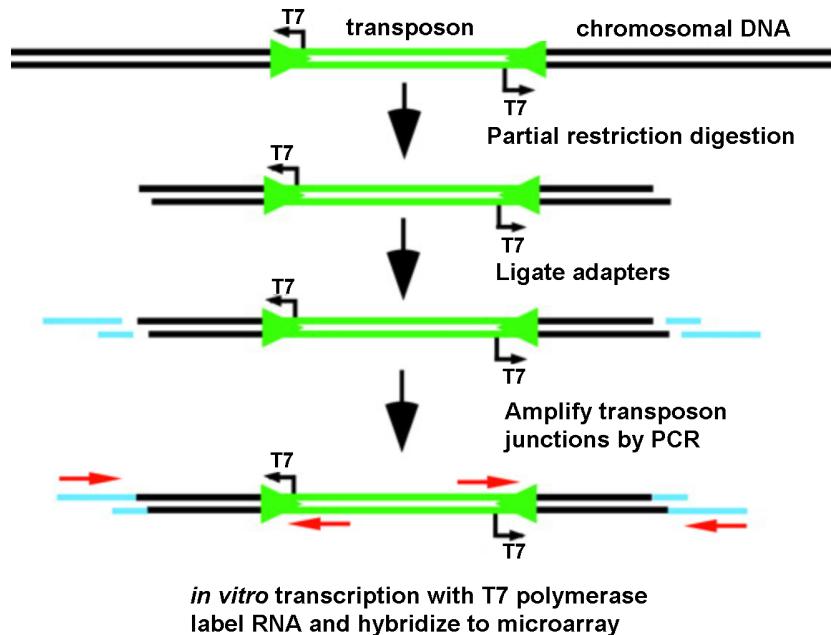
Class	Function	Number of genes	Total length (kb)	% total coding
1	Lipid metabolism	225	372	9.3
2	Information pathways	207	243	6.1
3	Cell wall and cell processes	517	620	15.5
4	Stable RNAs	50	10	0.2
5	Insertion sequences and phages	137	100	2.5
6	PE and PPE proteins	167	283	7.1
7	Intermediary metabolism and respiration	877	985	24.6
8	Proteins of unknown function	607	396	9.9
9	Regulatory proteins	188	162	4.0
10	Conserved hypothetical proteins	911	739	18.4
0	Virulence, detoxification, adaptation	91	95	2.4
potentially non-coding sequences			434	

Cole et al., Nature, 1998

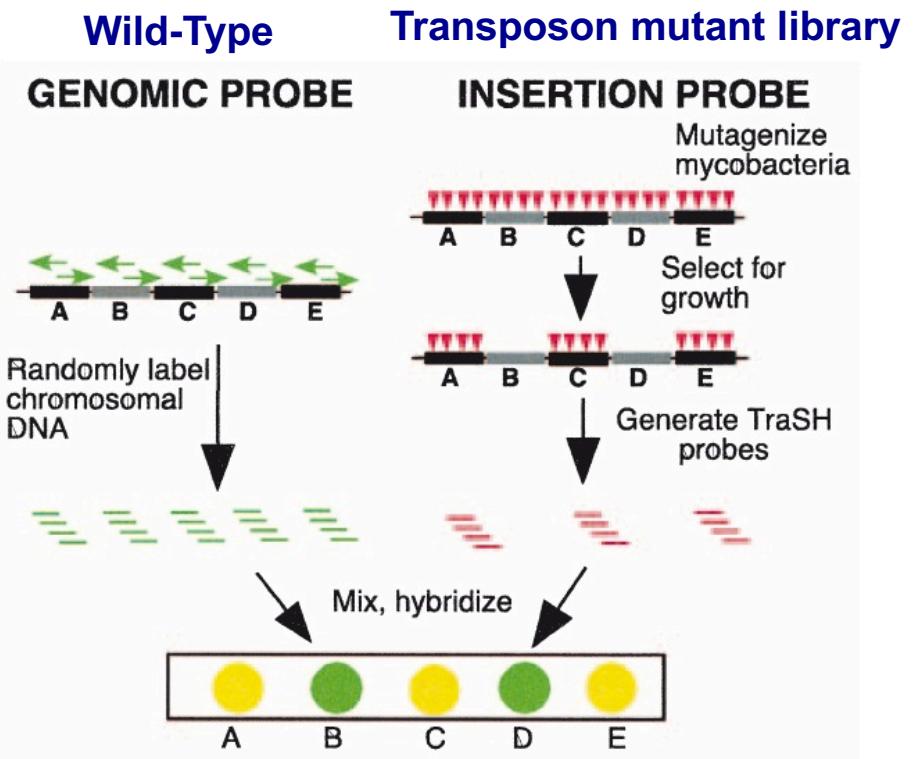
How to identify the essential genes of *M. tuberculosis* ?

the power of high density transposon mutation screens:

Transducing Phage phiMycoMarT7
Himar1 mariner transposon
~100 000 mutants



Sassetti et al. PNAS, 2001



Sassetti et al. Mol. Microbiol 2003

Alternative method: postgenomic prediction using Bayesian statistical analysis (Markov chain Monte Carlo)
Lamichhane et al., PNAS, 2003

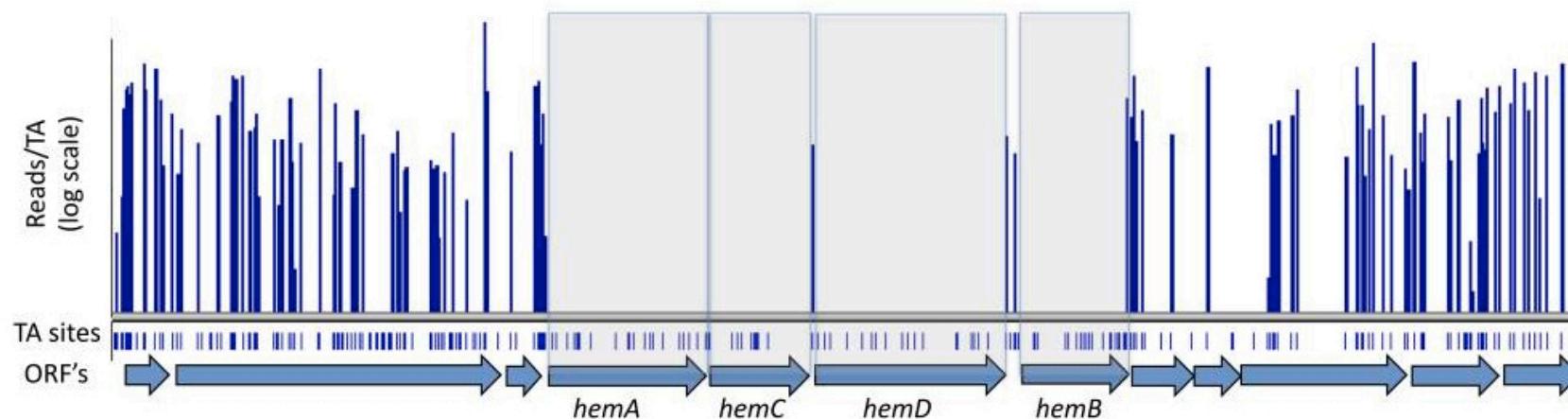
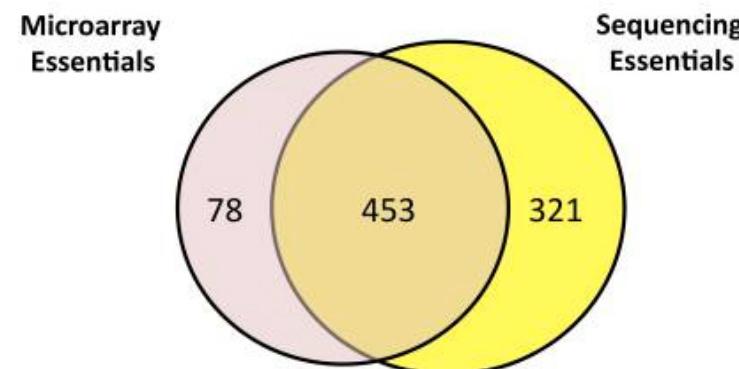
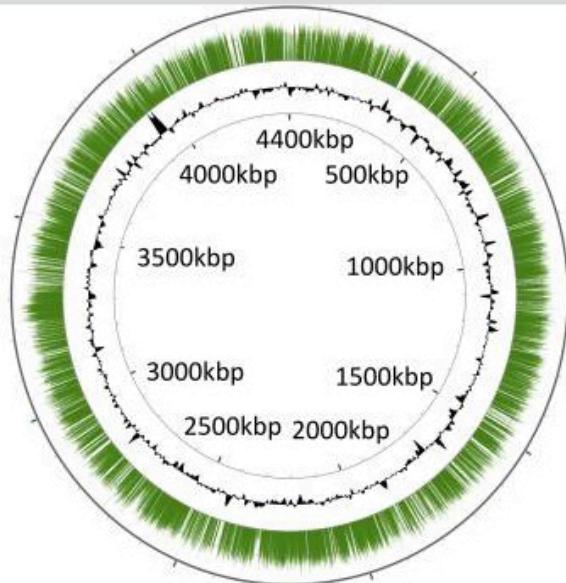
Transposon site hybridization (TraSH) predicts ~ 600 essential genes (*in vitro*)

Functional classification	<i>In vitro</i>	
	No. of genes	Percent of category
Lipid metabolism	30	14.9
Carbohydrate transport and metabolism	24	22.4
Inorganic ion transport and metabolism	8	8.0
Cell envelope biogenesis, outer membrane	32	29.4
Amino acid transport and metabolism	80	43.0
Transcription	15	11.6
Coenzyme metabolism	38	32.8
DNA replication, recombination and repair	19	17.4
Translation, ribosomal structure	76	59.4
Signal transduction mechanisms	12	15.6
Secretion	8	36.4
Energy production and conversion	31	16.3
Cell division and chromosome partitioning	8	34.8
Posttranslational modification, chaperones	27	34.2
Nucleotide transport and metabolism	25	38.5
Unknown	181	8.0
Total	614	15.7

Sassetti et al., Mol. Microbiol, 2003

Sassetti & Rubin, PNAS, 2003

High density transposon mutation screens & new generation sequencing (NGS)



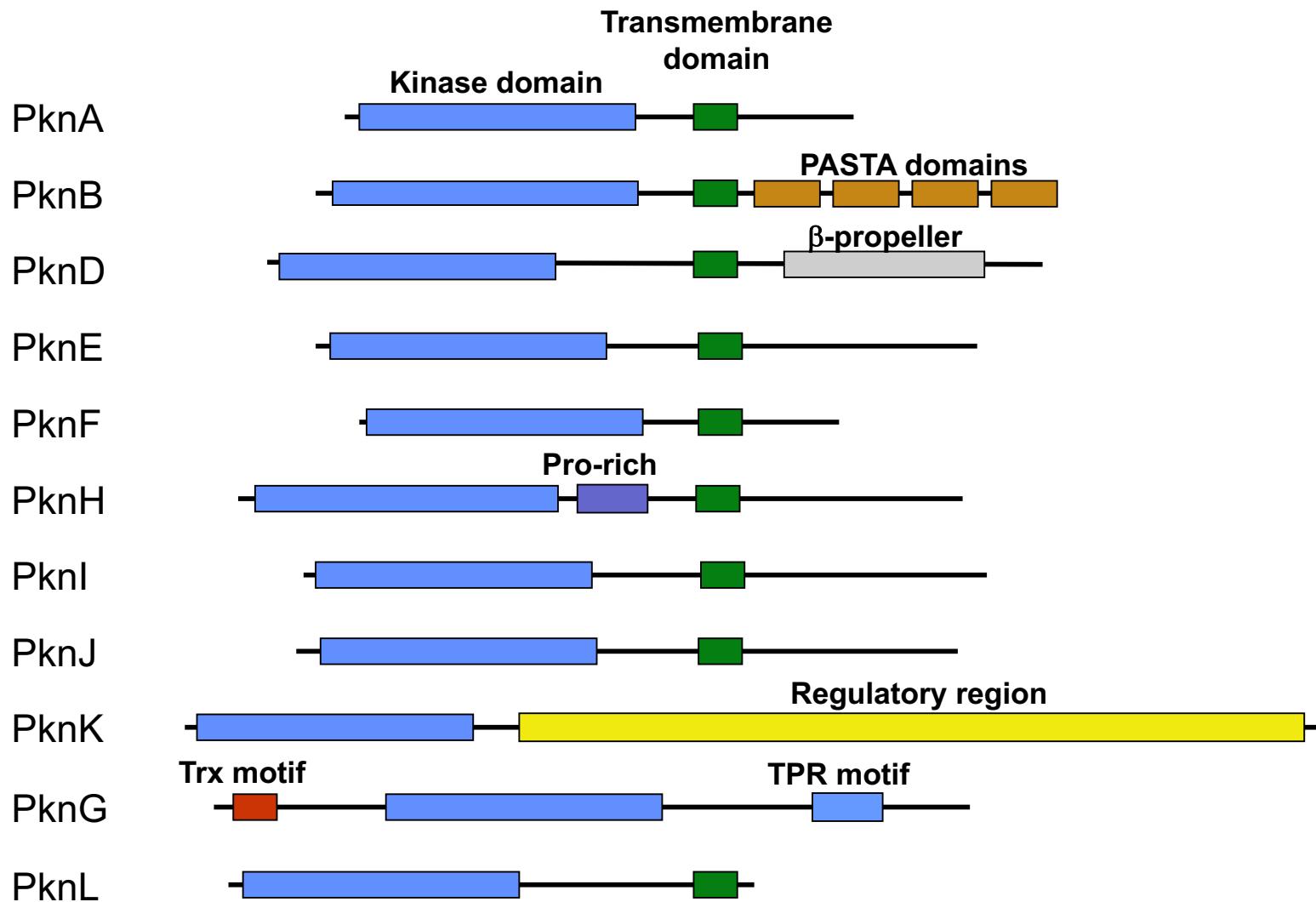
Example for an essential gene: *pknB* ...

... encoding the serine/threonine protein kinase PknB

Locus	Gene Name	Totals TA's	Total Insertions	Expected Run of Unoccupied Sites	Max Run of Unoccupied Sites	p value	in vitro essential (Sassetti et al 2003)
Rv0001	dnaA	32	1	4,4	31	0,000	essential
Rv0002	dnaN	31	0	4,3	31	0,000	no-data
Rv0003	recF	35	11	4,5	7	0,131	non-essential
Rv0004	-	8	2	2,4	6	0,062	non-essential
Rv0005	gyrB	43	3	4,8	40	0,000	essential
Rv0006	gyrA	46	2	4,9	44	0,000	no-data
Rv0007	-	11	4	2,8	5	0,160	no-data
Rv0008c	-	4	3	1,3	1	0,632	non-essential
Rv0009	ppiA	7	7	2,2	0	0,969	non-essential
Rv0010c	-	10	6	2,7	3	0,467	non-essential
Rv0011c	-	3	0	0,9	3	0,172	non-essential
Rv0012	-	16	11	3,4	3	0,635	non-essential
Rv0013	trpG	15	1	3,3	14	0,000	no-data
Rv0014c	pknB	24	0	3,9	24	0,000	essential
Rv0015c	pknA	16	1	3,4	15	0,000	essential
Rv0016c	pbpA	36	10	4,5	7	0,134	non-essential
Rv0017c	rodA	27	11	4,1	7	0,102	non-essential
Rv0018c	ppp	25	7	4,0	12	0,003	non-essential
Rv0019c	-	13	8	3,1	2	0,804	non-essential

Griffin et al., PLoS Pathog, 2011

M. tub. Serine Threonine Proteine Kinases (STPK)



However, testing of kinase inhibitors with IC₅₀ values in the nanomolar range showed no MIC → presently no inhibitors

All new anti-TB drug candidates were identified by microbial whole cell screening

- **some examples: TMC207 (Bedaquiline), Q203**

- Genome sequencing identified target of Bedaquiline (TMC207)

Scienceexpress

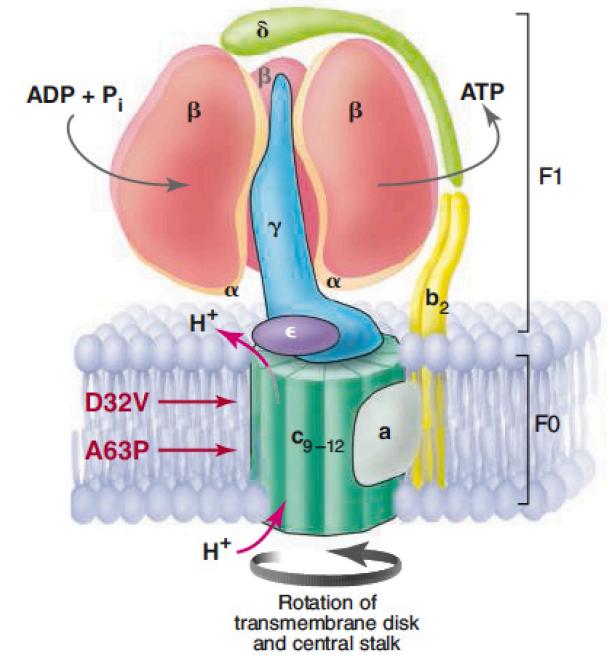
Research Article

A Diarylquinoline Drug Active on the ATP Synthase of *Mycobacterium tuberculosis*

Koen Andries,^{1,*} Peter Verhasselt,¹ Jerome Guillemont,² Hinrich W. H. Göhlmann,¹ Jean-Marc Neefs,¹ Hans Winkler,¹ Jef Van Gestel,¹ Philip Timmerman,¹ Min Zhu,³ Ennis Lee,⁴ Peter Williams,⁴ Didier de Chaffoy,¹ Emma Huitric,⁵ Sven Hoffner,⁵ Emmanuelle Cambau,⁶ Chantal Truffot-Pernot,⁶ Nacer Lounis,^{6,†} Vincent Jarlier⁶

Genome sequencing of drug resistant mutants (*M. smegmatis* & *Mtb*) identified point mutations in *atpE*, a gene encoding part of the F0 subunit of ATP synthase.

Andries et al., Science. 2005, 307:214-5.



A chink in the mycobacterial armor. Model of the mycobacterial ATP synthase showing the position of mutations that confer resistance to the diarylquinoline drug

Whole genome sequencing identified target of Q203, an Imidazopyridine amide (IPA) compound

Q203 blocks *M. tuberculosis* growth by targeting the respiratory cytochrome *bc₁* complex.

Q203 targets *qcrB* (Rv2196) encoding cytochrome *b* subunit) and triggers a rapid ATP depletion in *M. tuberculosis*.

Whole genome sequencing identified mutation in *qcrB*, leading to a single AA sunstitution in the cytochrome *b* subunit of Q203-resistant spontaneous mutants of *M. tuberculosis*

Pathe et al., Nat Med. 2013 Sep;19(9):1157-60.

Use of genomics for getting new insights into the biology of the tuberculosis agent

Key questions:

- identification of new drug targets
- identification of new virulence genes/
mechanisms → host pathogen interaction

Advances in mycobacterial genetics -- identification of virulence factors

Tools for gene inactivation

pAL5000 de *M. fortuitum* counter selective marker (thermosensitive ori), *sacB*.

erp (*Rv3810*)

Berhet, F.-X. et al., Science, 1998

phoP (*Rv0757*)

Perez E. et al., Mol. Microbiol, 2001

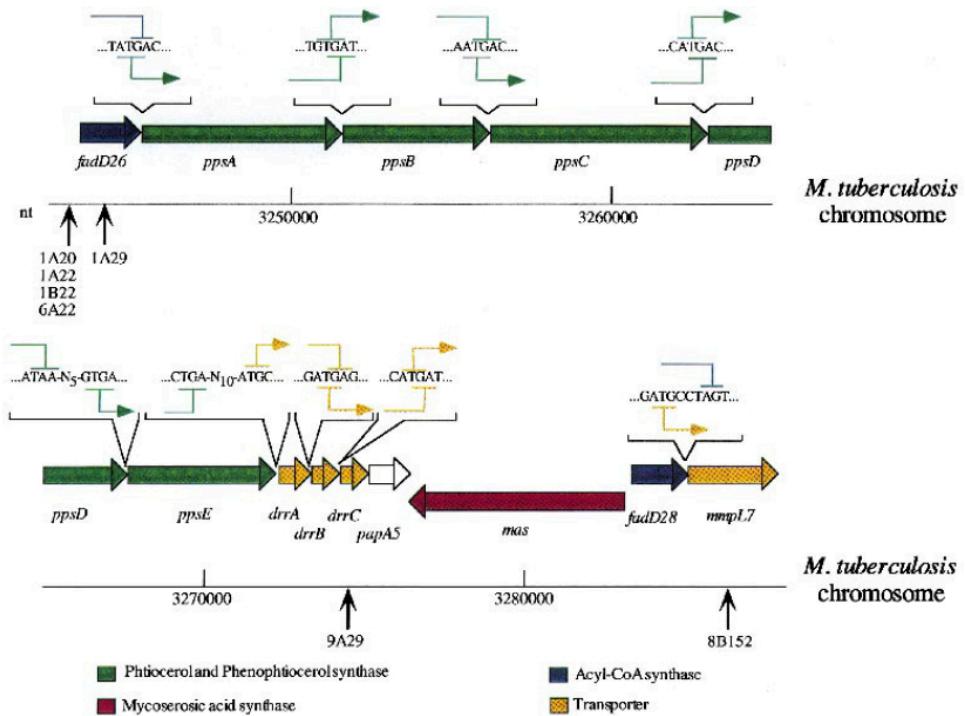
Thermosensitive phages

leuC

Hondalus et al., Infect Immun, 2000

Bardarov et al., PNAS, 1997

STM (Signature Tagged Mutagenesis)

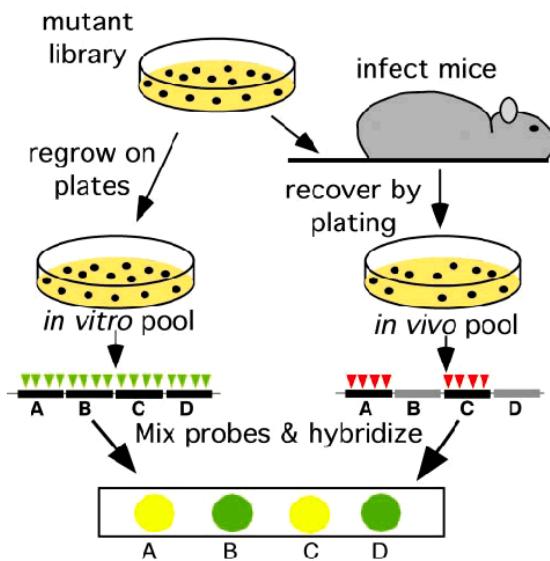


Defect in the synthesis of phthiocerol dimycoserosate (PDIM) involved in permeability of the cell envelope and its structure

Camacho et al., Mol. Microbiol, 1999

Cox JS, et al., Nature, 1999

Genetic requirements for mycobacterial survival during infection



TraSH (Transposon site hybridization) using phage MycoMarT7 and micro-arrays

Functional classification	<i>In vivo</i>	
	No. of genes	Percent of category
Lipid metabolism	15	7.5
Carbohydrate transport and metabolism	9	8.4
Inorganic ion transport and metabolism	8	8.0
Cell envelope biogenesis, outer membrane	8	7.3
Amino acid transport and metabolism	8	4.3
Transcription	7	5.4
Coenzyme metabolism	7	6.0
DNA replication, recombination and repair	5	4.6
Translation, ribosomal structure	5	3.9
Signal transduction mechanisms	4	5.2
Secretion	3	13.6
Energy production and conversion	3	1.6
Cell division and chromosome partitioning	2	8.7
Posttranslational modification, chaperones	2	2.5
Nucleotide transport and metabolism	1	1.5
Unknown	107	4.7
Total	194	5.0

Sassetti & Rubin, PNAS, 2003

Example for gene cluster Rv3868-3882 ...

...

... neighboring the genes encoding ESAT-6

	A	B	C	D	E	F	G
1	Table 3. Genes predicted to be required for <i>in vivo</i> survival						
2	Name	Rv#	Week 1 Ratio	Week 1 P value	Week 2 Ratio	Week 2 P value	Week 4 R
3							
184	Rv3869c	Rv3869c	0,347	0,02023332	0,300	0,1540300	0,371
185	pirG	Rv3810	0,425	9,26E-04	0,39	0,004832614	0,549
186	Rv3855	Rv3855	0,405	0,00204324	0,461	0,01836709	0,396
187	Rv3864	Rv3864	0,591	0,0707385	0,525	0,016921503	0,231
188	Rv3868	Rv3868	0,465	0,033339594	0,351	0,04903753	0,395
189	Rv3869	Rv3869	0,305	0,001679109	0,294	2,80E-04	0,227
190	Rv3870	Rv3870	0,505	2,95E-04	0,427	4,18E-04	0,593
191	Rv3871	Rv3871	0,237	0,04236548	0,189	0,017277295	0,394
192	Rv3872	Rv3872	0,386	1,02E-04	0,296	6,30E-04	0,144
193	Rv3873	Rv3873	0,288	1,91E-05	0,44	0,001109353	0,271
194	Rv3876	Rv3876	0,331	5,59E-05	0,343	2,32E-04	0,539
195	Rv3877	Rv3877	0,133	0,001835569	0,191	0,001382477	0,168
196	Rv3882c	Rv3882c	0,132	4,13E-07	0,308	6,06E-04	0,381
197	Rv3910	Rv3910	0,194	1,80E-08	0,364	1,81E-05	0,182
198							
199							

Sassetti & Rubin, PNAS, 2003

Analysis of the proteome of *Mycobacterium tuberculosis* in silico

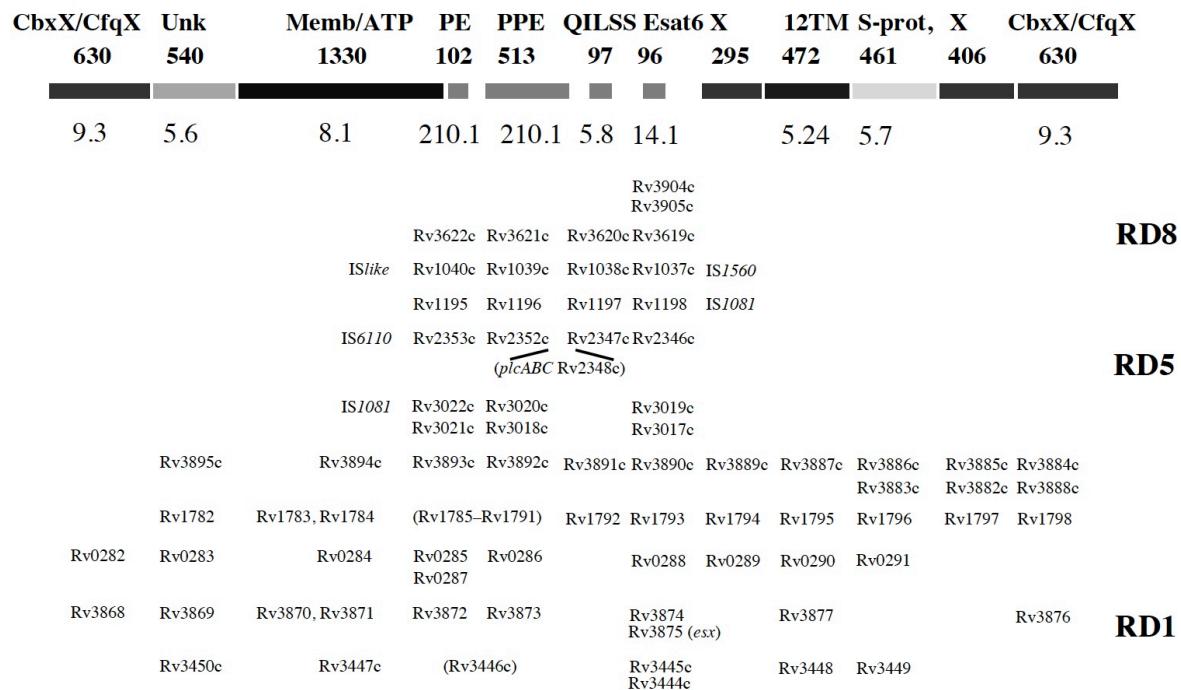
F. Tekaia*, S. V. Gordon[†], T. Garnier[†], R. Brosch[†], B. G. Barrell[‡], S. T. Cole[†]

*Unité de Génétique Moléculaire des Levures, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France

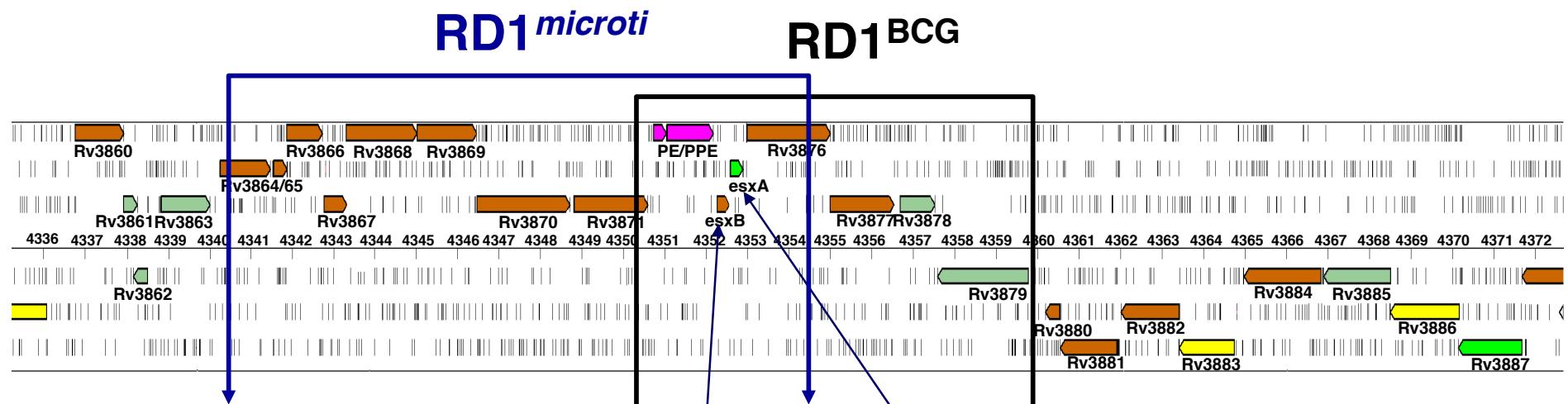
[†]Unité de Génétique Moléculaire Bactérienne, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France

[‡]Sanger Centre, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, UK

The ESAT6 family: Organization of genomic loci



Region of Difference 1 (RD1) - regions absent from mycobacterial vaccine strains



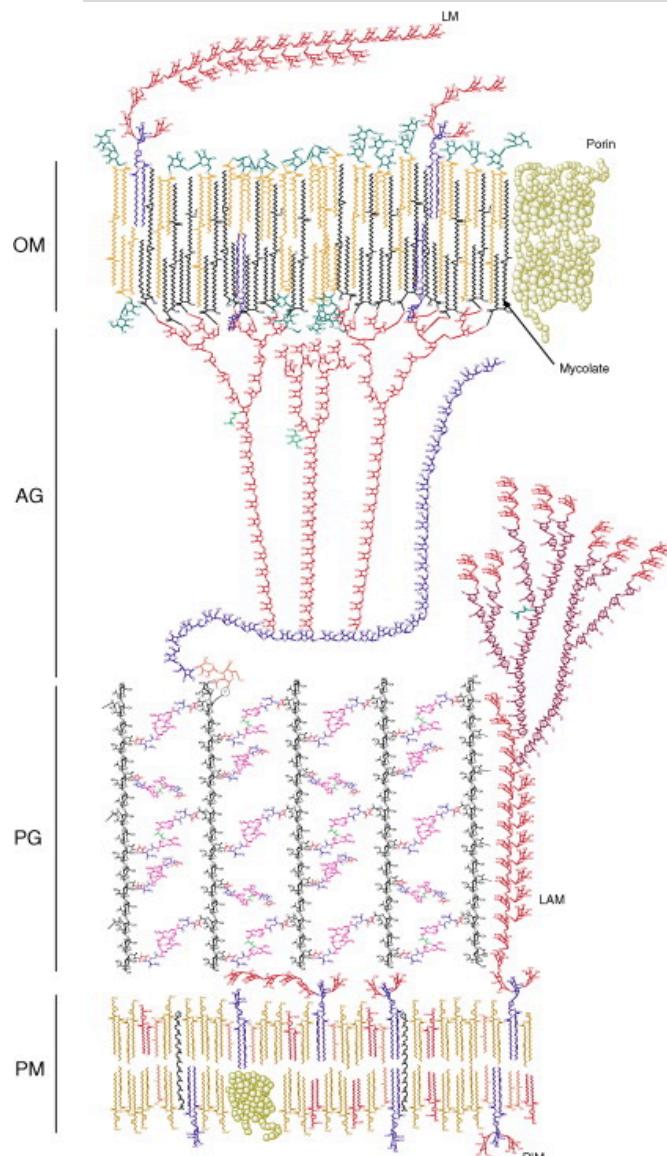
**ESAT-6 (6kD early secreted antigenic target)
(EsxA)**

- > found in culture filtrate of *M. tub.*
- > triggers strong IFN- γ response
- > used for TB diagnostics

**CFP-10 (10 kD culture
(EsxB) filtrate protein)**

Mahairas *et al.*, 1996 J Bact;
Gordon *et al.*, 1999 Mol Micro;
Behr *et al.*, 1999 Science;
Brosch *et al.*, 2002 PNAS.

Mycobacterial cell envelope— General features



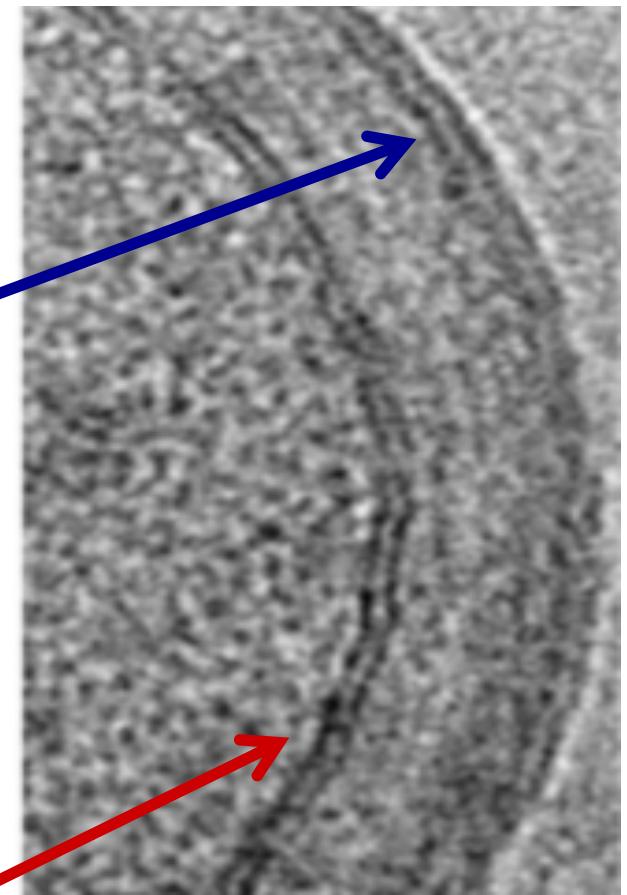
Capsule

Extractable lipids
Mycolic-acid layer
(outer membrane)

Arabinogalactan

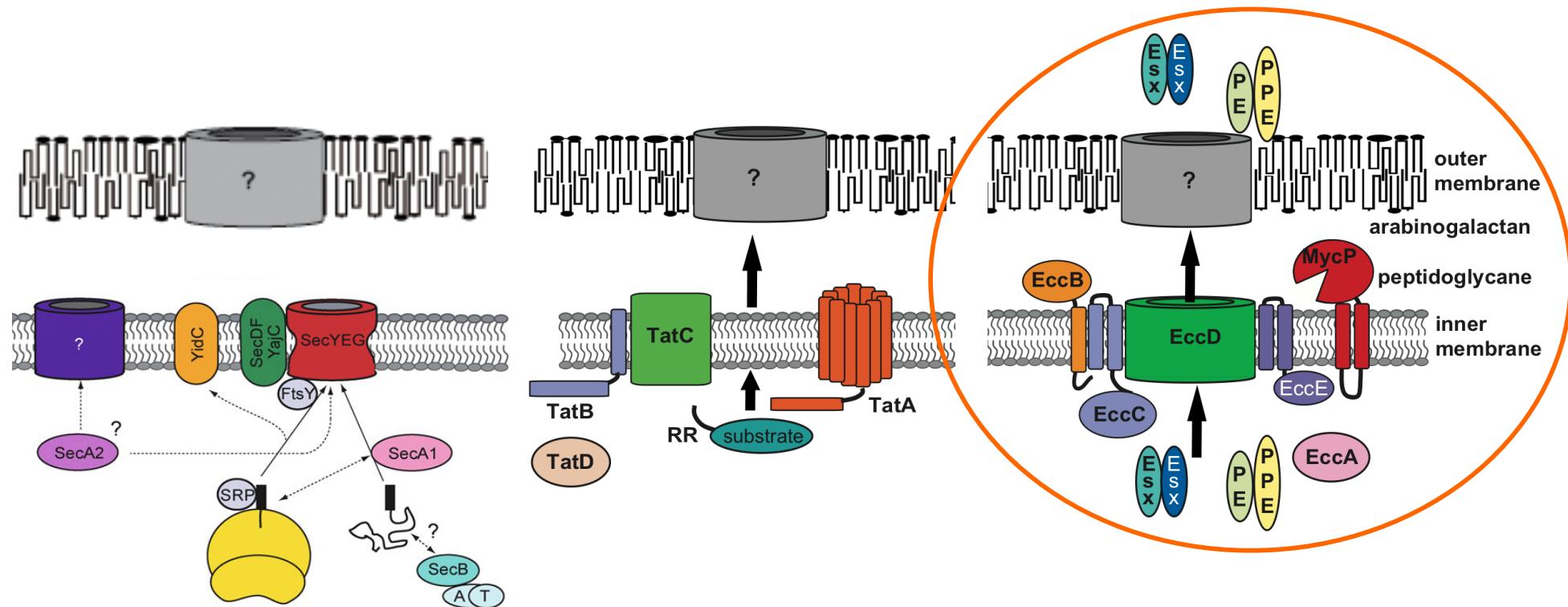
Peptidoglycan

Plasma membrane



Minnikin *et al.*, 1982
Brennan & Nikaido, 1995
Zuber *et al.*, 2008
Hoffmann *et al.*, 2008
Sani *et al.*, 2010

Mycobacteria harbour several protein export systems

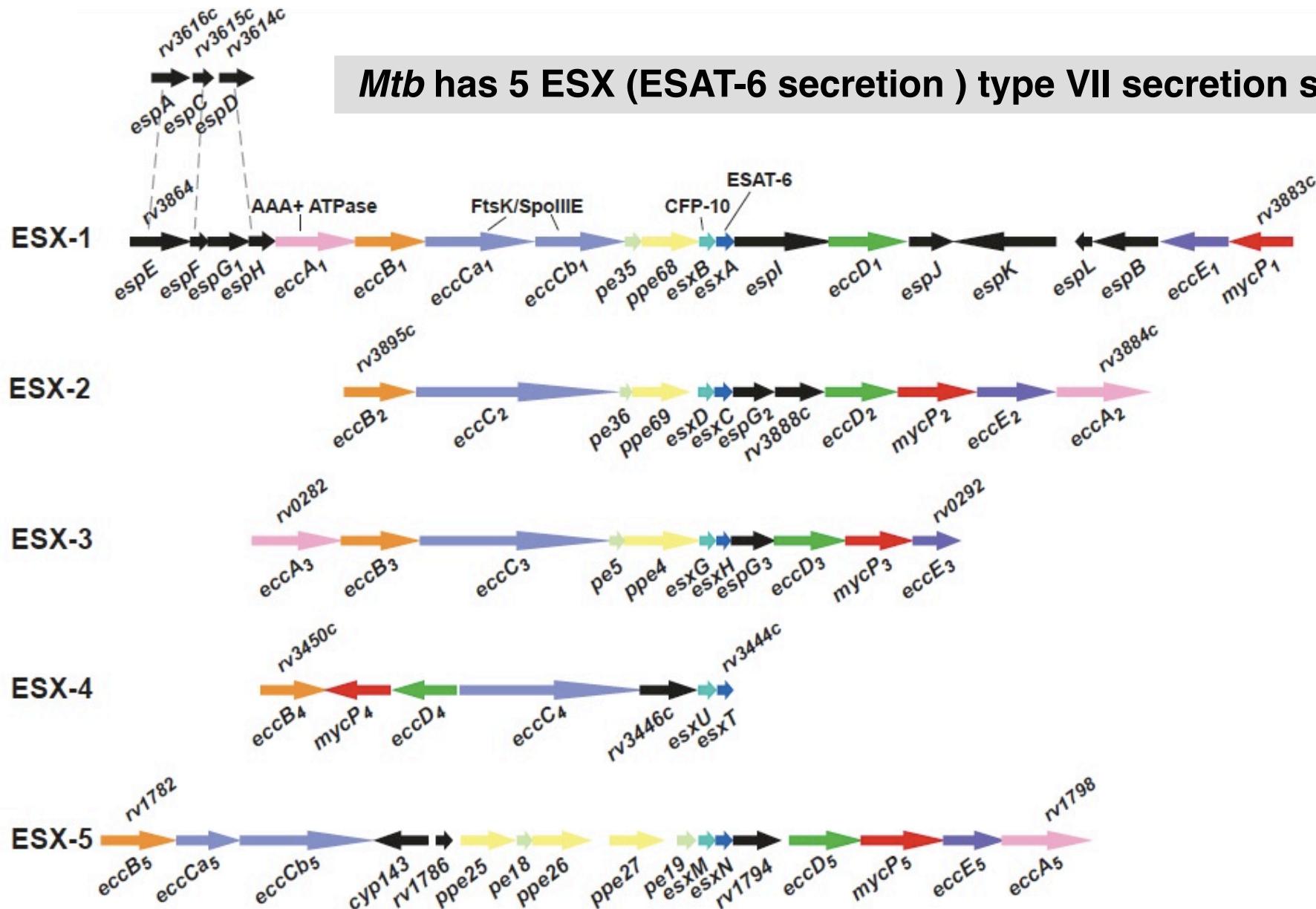


General SecA export pathway

N-term signal sequence - SecA1
no N-term signal sequence - SecA2

**Twin Arginine
Translocation
(TAT)
pathway**

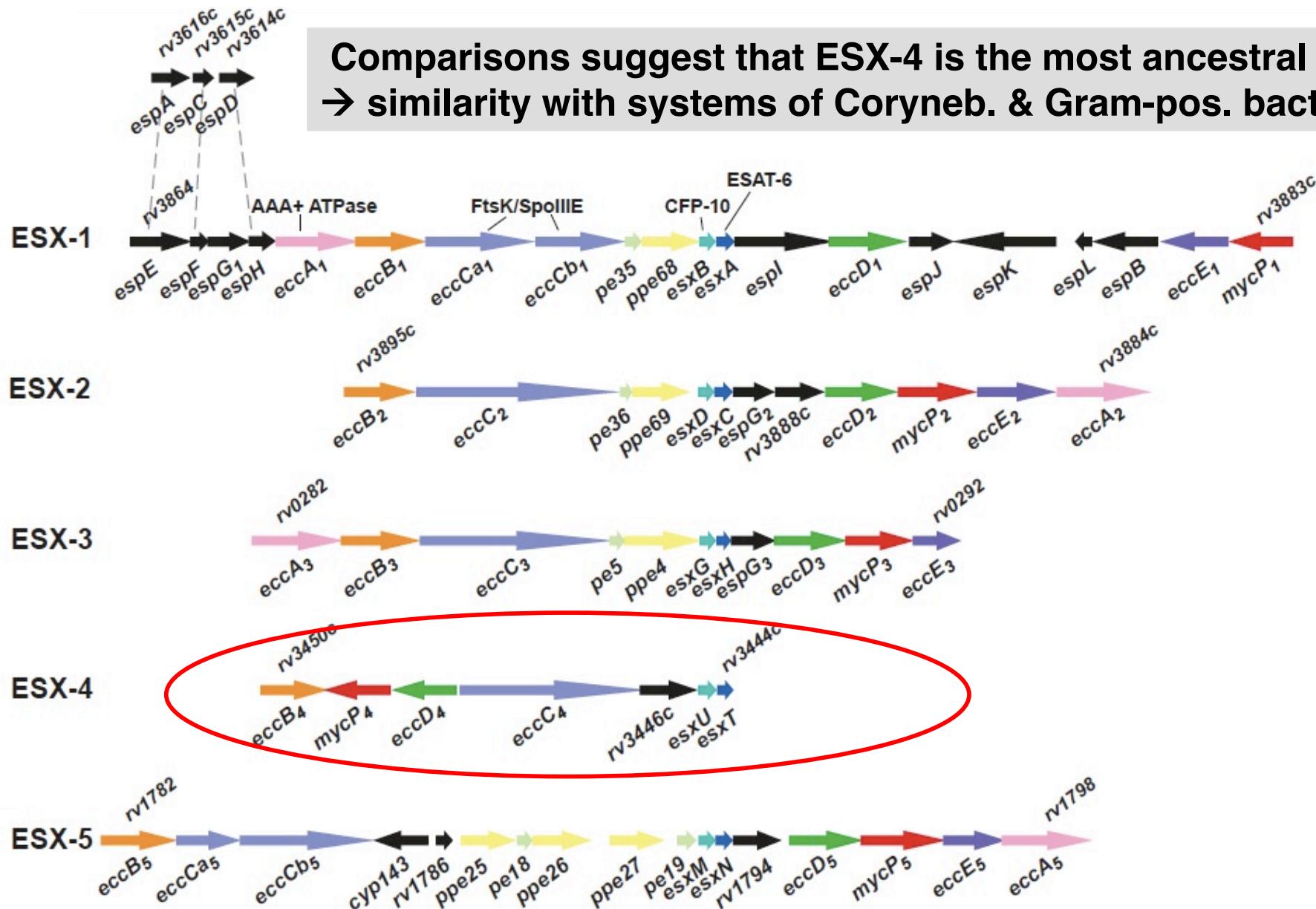
**ESX / Type VII
secretion
(T7S)
pathway**



Cole et al., Nature, 1998

Tekaia et al., Tuber Lung Dis, 1999

Gey van Pittius et al., Genome Biol., 2001

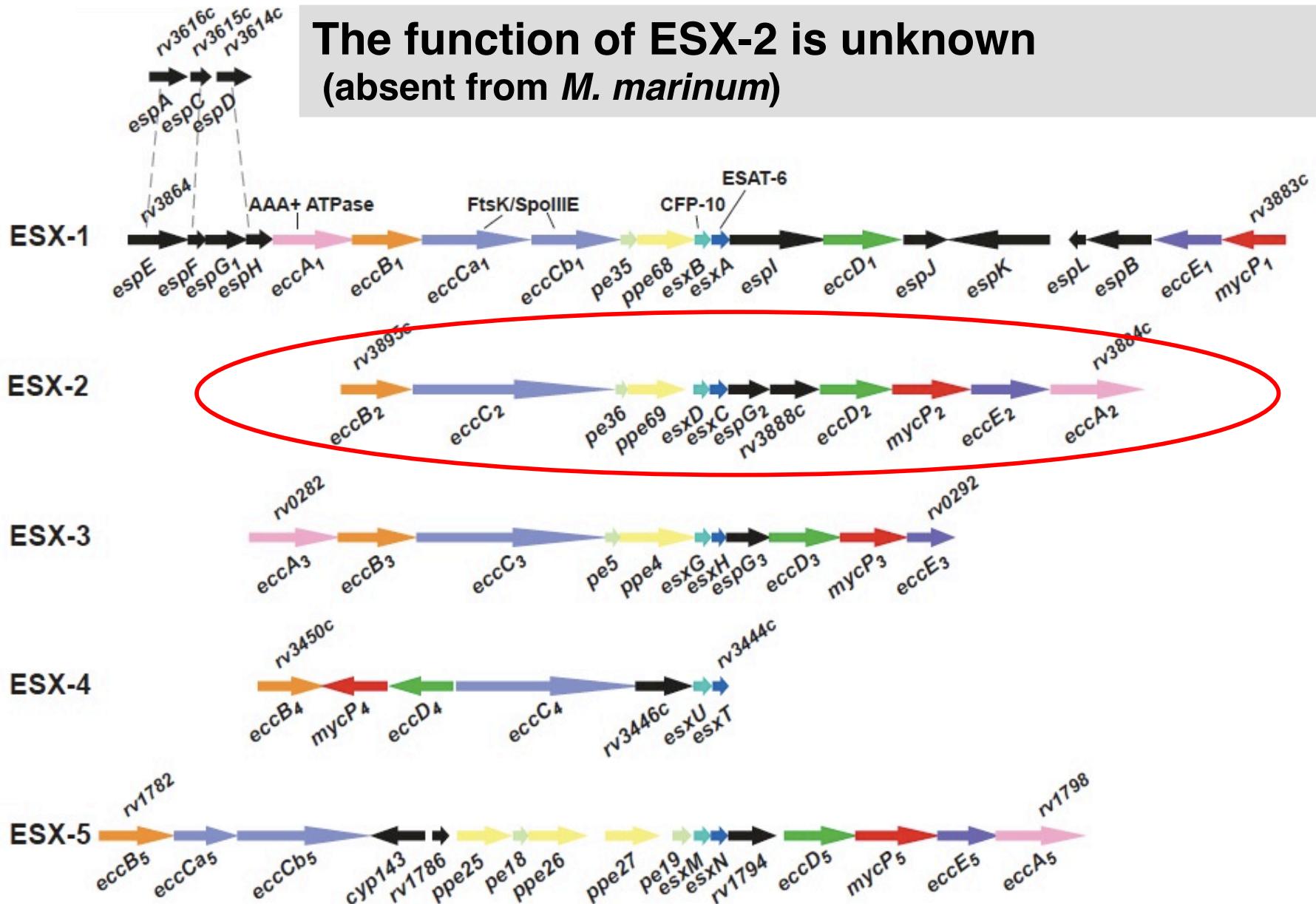


Cole et al., Nature, 1998

Tekaia et al., Tuber Lung Dis, 1999

Gey van Pittius et al., Genome Biol., 2001

**Comparisons suggest that ESX-4 is the most ancestral T7SS
→ similarity with systems of Coryneb. & Gram-pos. bacteria**

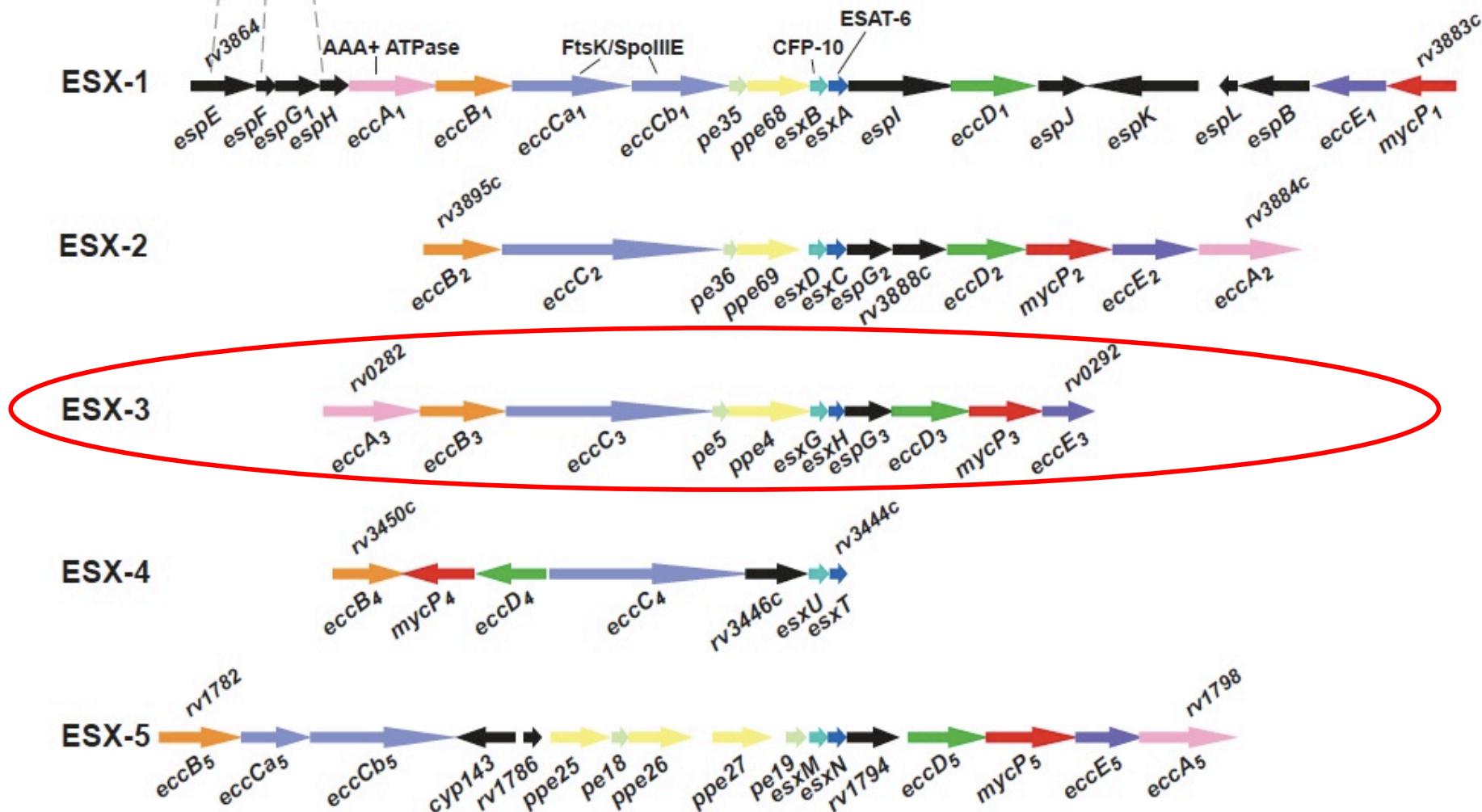


Cole et al., Nature, 1998

Tekaia et al., Tuber Lung Dis, 1999

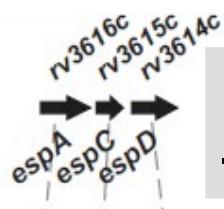
Gey van Pittius et al., Genome Biol., 2001

**ESX-3 is involved in iron and zinc acquisition
→ in *M. tuberculosis* essential for *in vitro* growth**

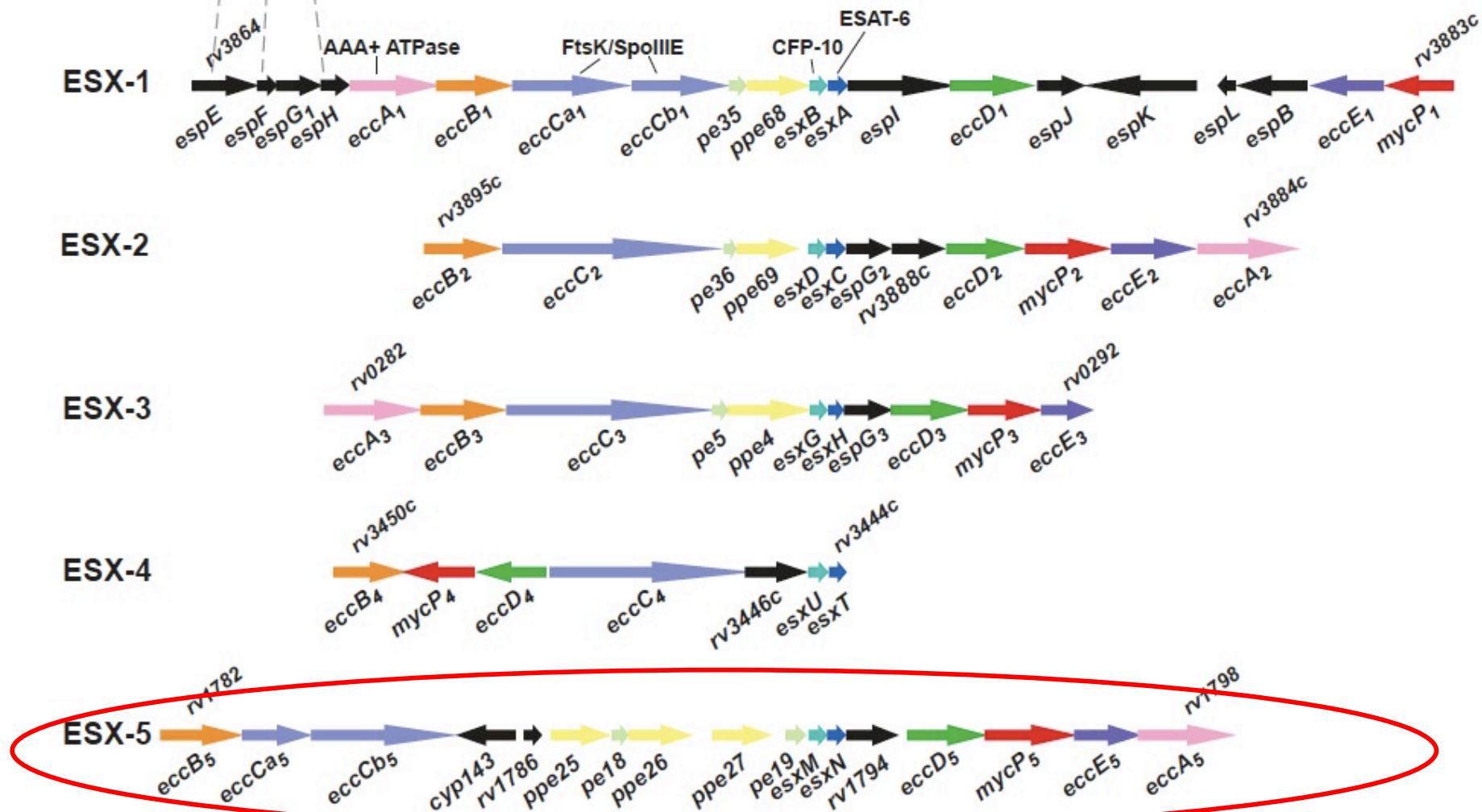


Sigrist et al., PNAS, 2009, & Mbio 2014

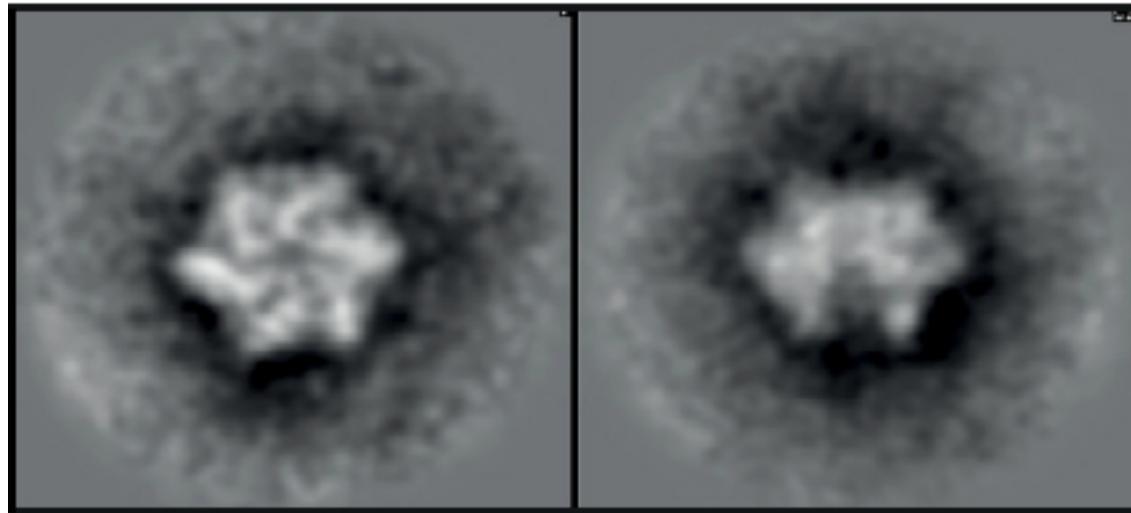
Serafini et al., J Bact, 2009



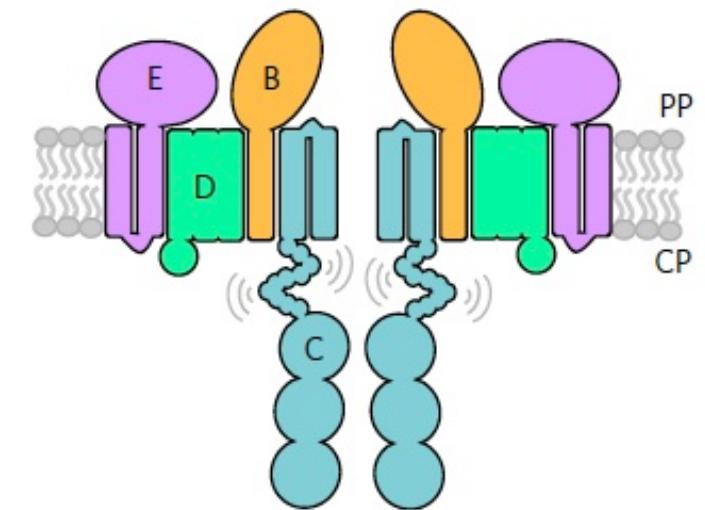
**ESX-5 only present in slow-growing, pathogenic mycob.
→ involved in PE & PPE protein secretion and virulence**



The refined structure of the ESX systems as revealed by Single Particle Analysis



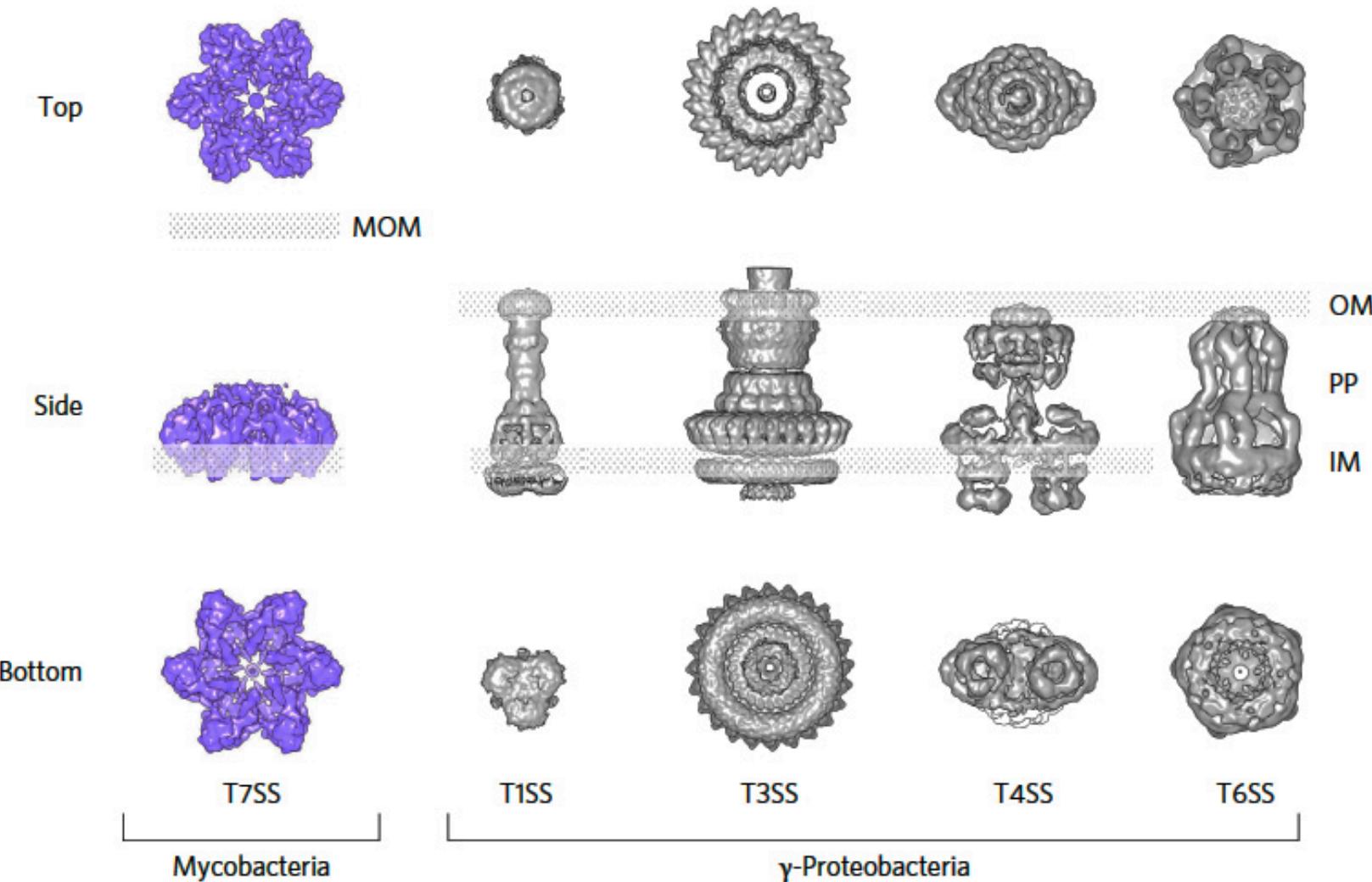
Membrane complex from *Mycobacterium xenopi*
ESX-5 orthologue
@ 13 Å resolution by electron microscopy



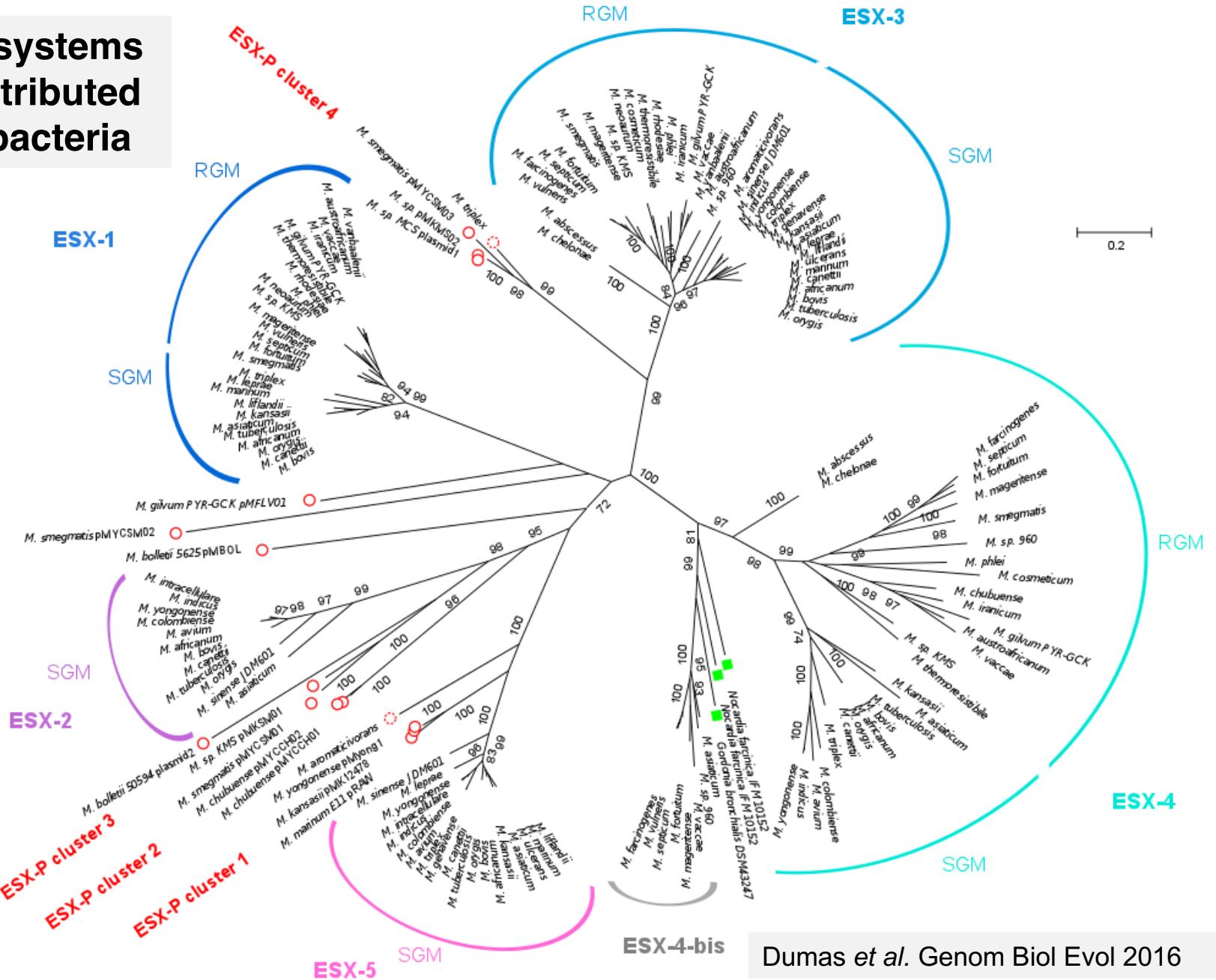
six-fold symmetry

Beckham, Ciccarelli, Bunduc et al., *Nat Microbiol*, 2017

The structure of the ESX system – different to other systems

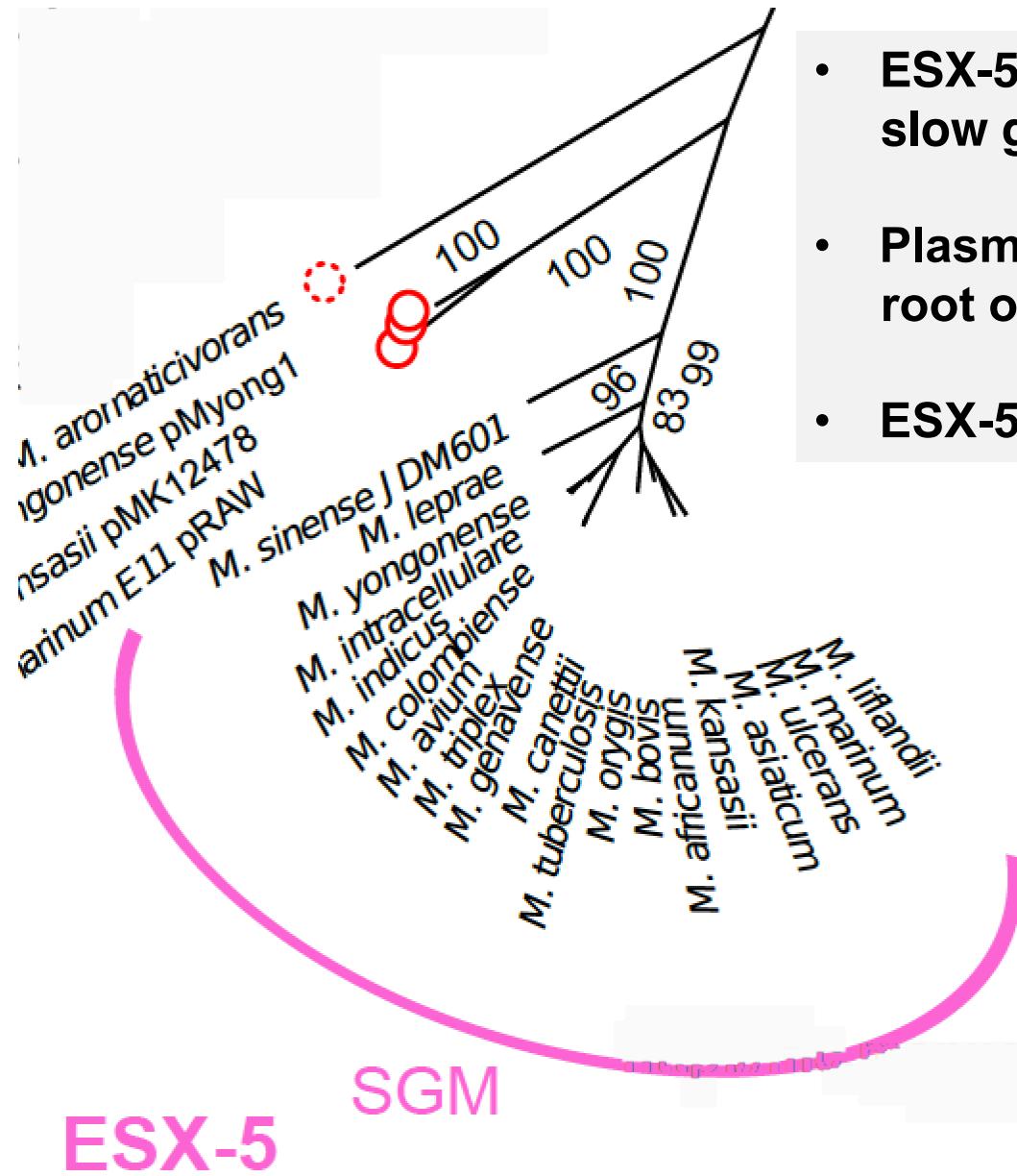


**ESX/Type VII systems
are widely distributed
among mycobacteria**

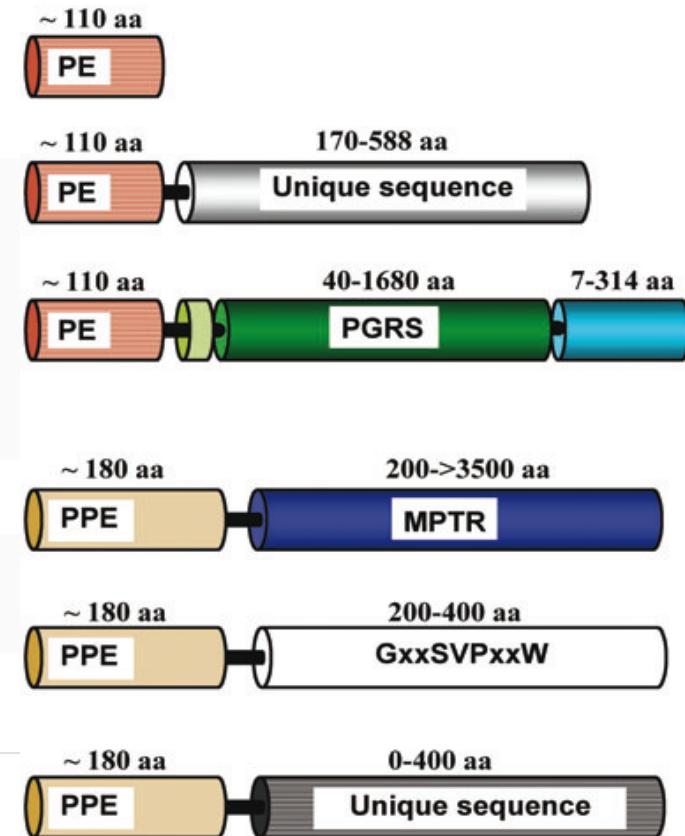


Tree based on EccB/EccC/MycP amino-acid sequences

Dumas et al. Genom Biol Evol 2016



- **ESX-5 systems only exist in slow growing mycobacteria (SGM)**
- **Plasmid-born ESX cluster 4 branch at the root of chromosomal ESX-5**
- **ESX-5 secretes PE and PPE proteins**



Features and tasks (ESX-5 deletion mutant)

Molecular Microbiology (2012) ■

doi:10.1111/j.1365-2958.2012.08001.x

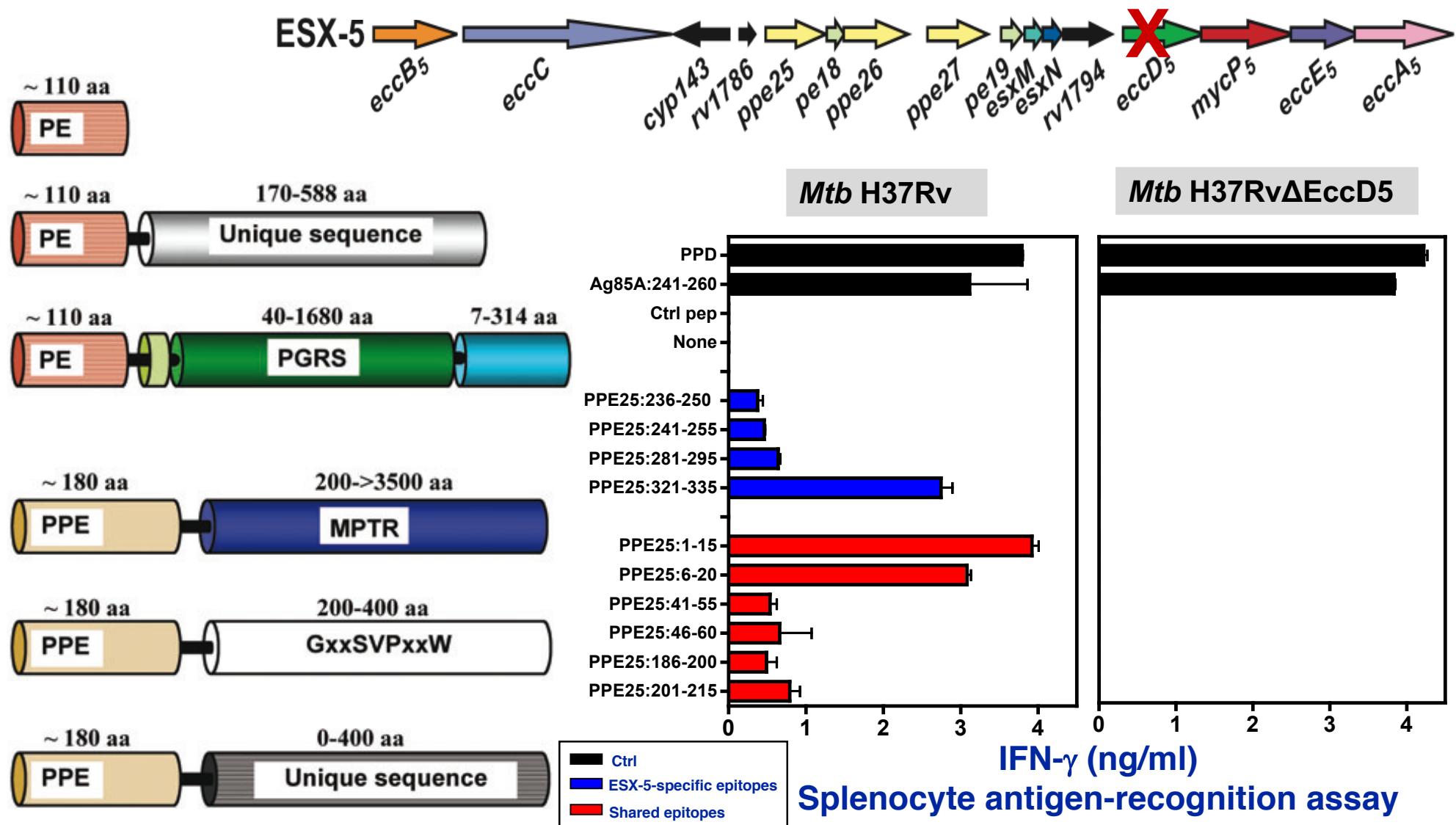
Disruption of the ESX-5 system of *Mycobacterium tuberculosis* causes loss of PPE protein secretion, reduction of cell wall integrity and strong attenuation

Darla Bottal,^{1*} Marlagrazia Di Luca,¹
Laleh Majlessi,^{2,3} Wafa Frigui,⁴ Roxane Simeone,⁴
Fadel Sayes,^{2,3} Wilbert Bitter,⁵ Michael J. Brennan,⁶
Claude Leclerc,^{2,3} Giovanna Batoni,¹ Mario Campa,¹
Roland Brosch⁴ and Semih Esin¹

and in the severe combined immune-deficient mouse infection model. Altogether these findings indicate an essential role of ESX-5 for transport of PPE proteins, cell wall integrity and full virulence of *M. tuberculosis*, thereby opening interesting new perspectives for the

▪ESX-5 deletion mutant strain (*M.tb*ΔEccD5)

ESX-5-secreted PE & PPE proteins are recognized by the immune system

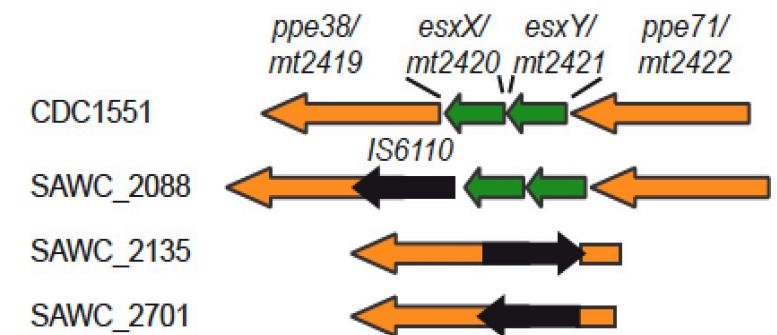
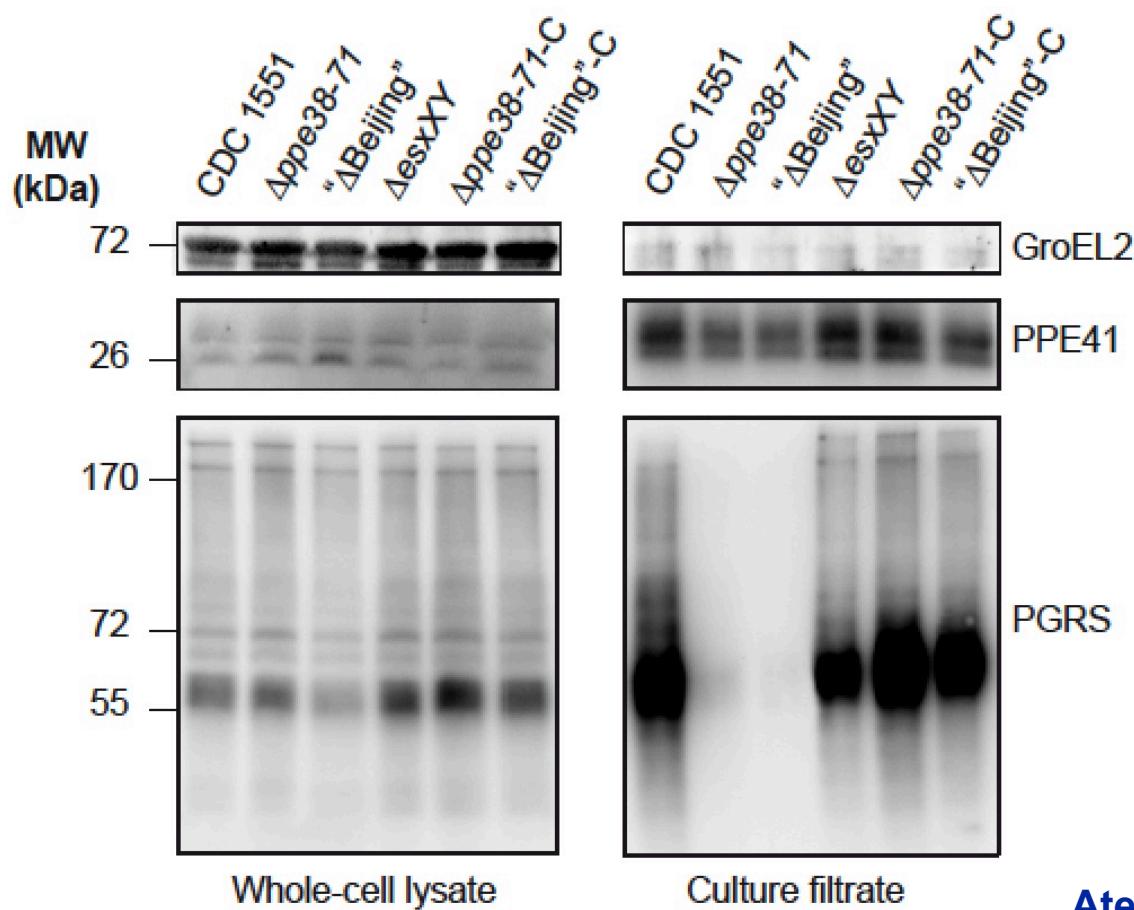


Cole et al., Nature, 1998

Bottai et al., Mol Microbiol, 2012

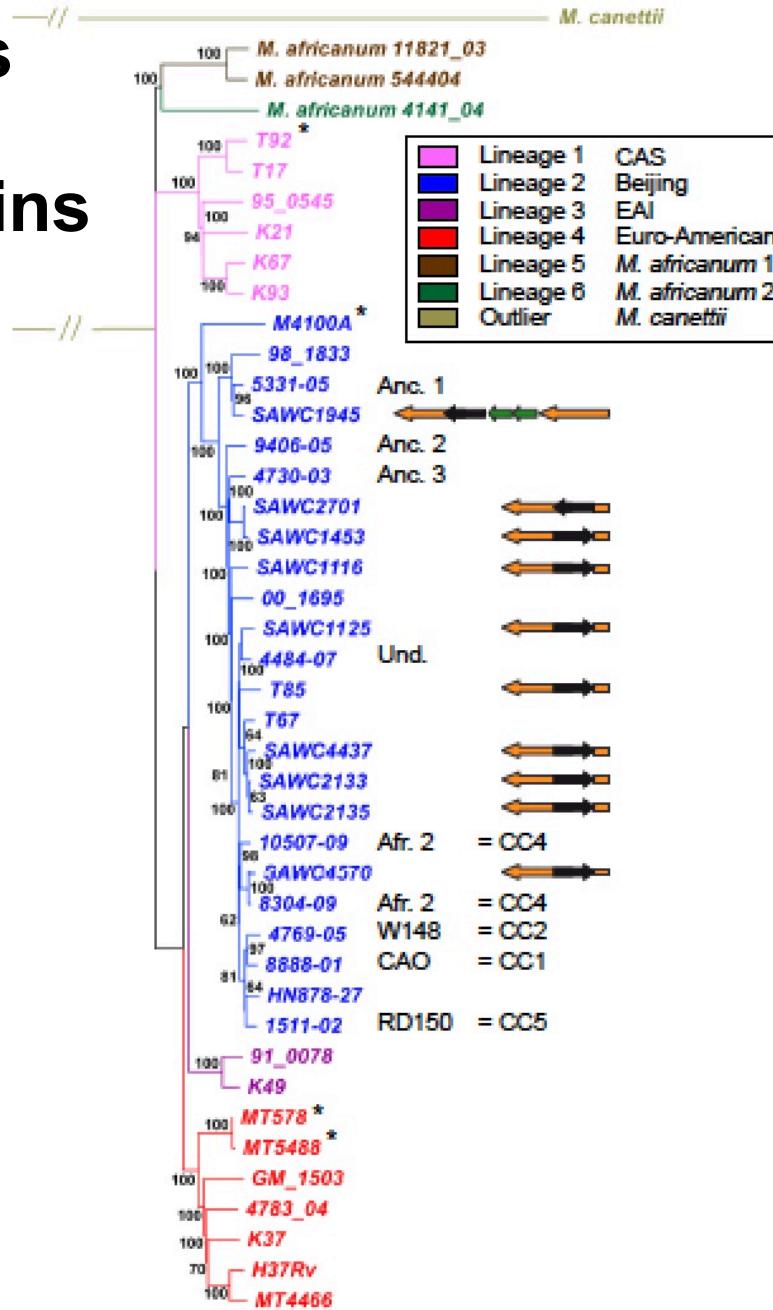
Sayes et al., Cell Host & Microbe, 2012

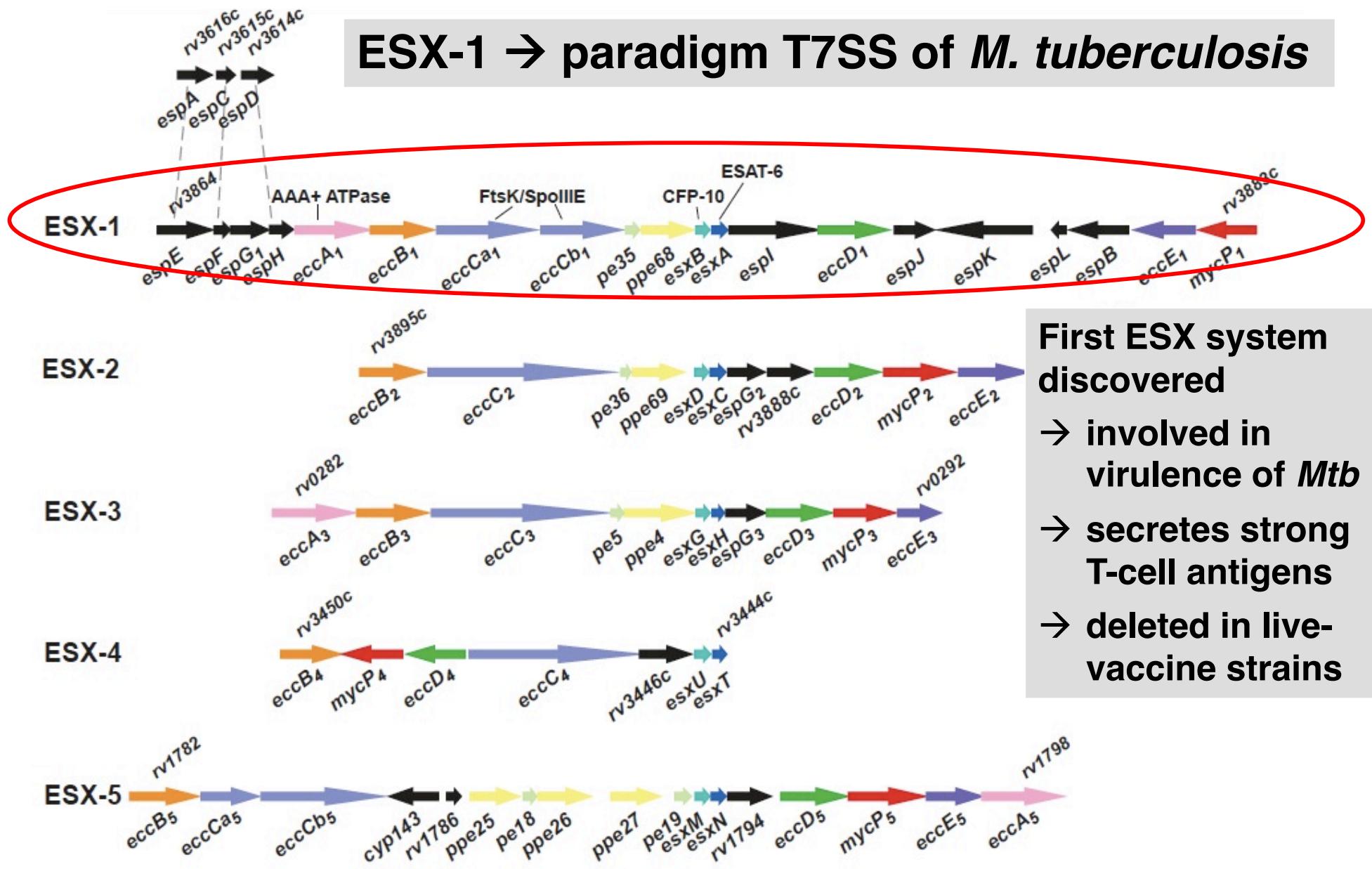
PPE38 is essential for the export/secretion of PE_PGRS and PPE_MPTR proteins



Ates et al., Nat Microbiol, in press 2018

M. tuberculosis Beijing strains lack export/secretion of PE_PGRS & PPE_MPTR proteins

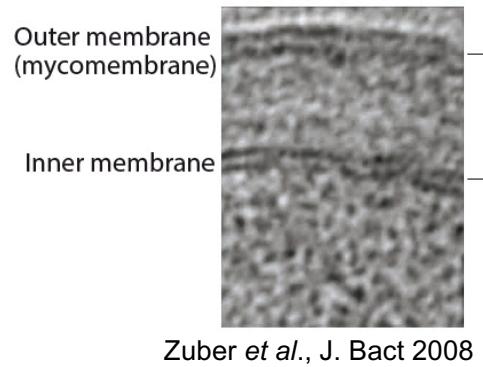




Mahairas et al., J Bact 1996

Pym et al., Mol Microbiol 2002 & Nat Med 2003

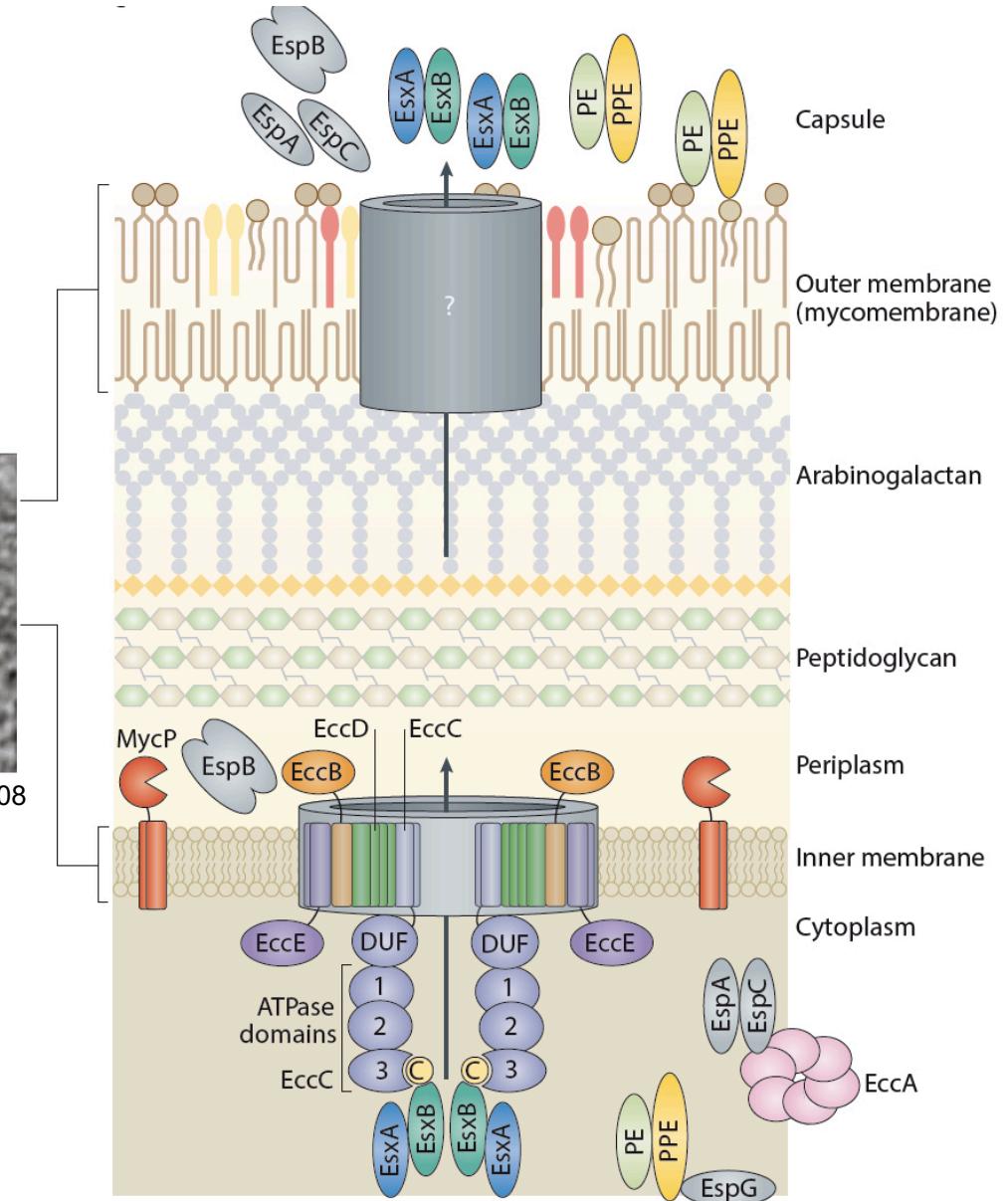
ESX-1 → paradigm
type VII secretion system
of tubercle bacilli



Zuber et al., J. Bact 2008

Ecc = ESX conserved component

Esp = ESX-1 secretion-associated protein



Gröschel et al., *in press*, 2016

ESX-1

RGM

SGM

M. gilvum PYR-GCK pMFLV01

M. austroafricanum
M. vanbaalenii
M. vaccae
M. iranicum
M. gilvum PYR-GCK
M. thermoresistibile
M. modestiae
M. phlei
M. neoaurum
M. sp. KMS
M. magentense
M. vulnens
M. septicum
M. fortuitum
M. smegmatis
M. triplex
M. leprae
M. marinum
M. liflandii
M. kansasii
M. asiaticum
M. tuberculosis
M. africanum
M. orygis
M. canettii
M. bovis

ESX-1 systems exist in
Rapid- & Slow-Growing
(RGM & SGM)
mycobacteria

→ Functions in DNA Transfer

→ Functions in host-
pathogen interaction

→ Functions in DNA Transfer

→ ESX-I system is essential for Distributive Conjugative Transfer (DCT) in *M. smegmatis*

→ ESX-1 likely also plays a role in a similar type of mosaic transfer of large DNA fragments from donor to recipient strains of *Mycobacterium canettii*

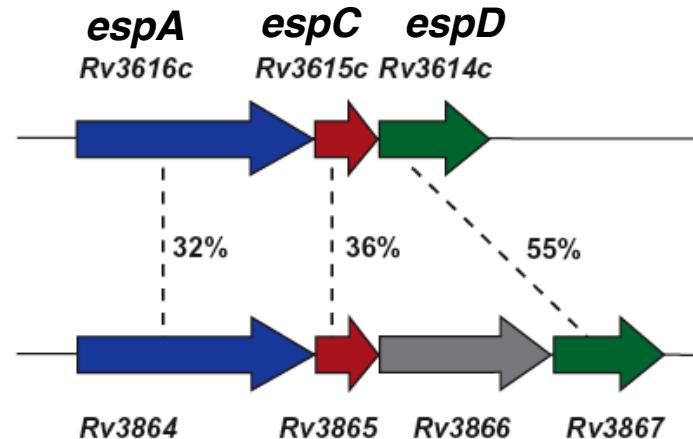
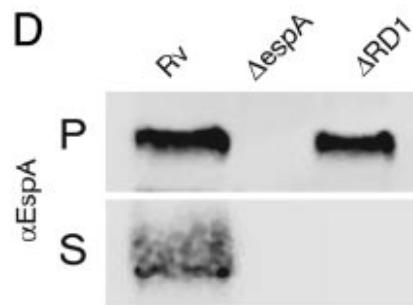
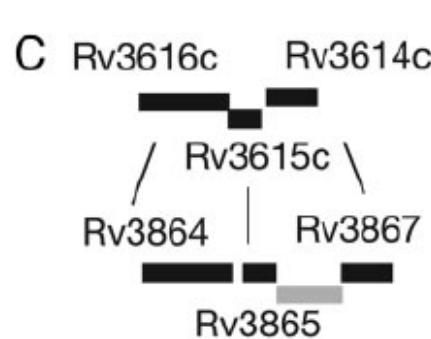


Key experimental evidence of chromosomal DNA transfer among selected tuberculosis-causing mycobacteria

Eva C. Boritsch^a, Varun Khanna^b, Alexandre Pawlik^a, Nadine Honoré^a, Victor H. Navas^c, Laurence Ma^d, Christiane Bouchier^d, Torsten Seemann^e, Philip Supply^f, Timothy P. Stinear^g, and Roland Brosch^{a,1}

^aInstitut Pasteur (IP), Unit for Integrated Mycobacterial Pathogenomics, 75015 Paris, France; ^bIP, Hub Bioinformatique et Biostatistique, C3BI, Unité de Services et de Recherche, USR 3756, IP CNRS, 75015 Paris, France; ^cIP, Lymphocyte Cell Biology Unit, 75015 Paris, France; ^dIP, PF1-Plate-Forme Génomique, 75015 Paris, France; ^eVictorian Life Sciences Computation Initiative, University of Melbourne, Carlton, VIC 3053, Australia; ^fINSERM U1019, CNRS UMR 8204, Center for Infection and Immunity, Institut Pasteur de Lille, Université de Lille, 59000 Lille, France; and ^gDepartment of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, VIC 3000, Australia

Non-RD1 gene cluster *espA*, *espC* & *espD* (*rv3616c-rv3614c*) is co-dependent for secretion of ESAT-6 and CFP-10



Esp = ESX-1 secretion-associated protein

EspACD cluster is regulated by at least 2 different regulators:

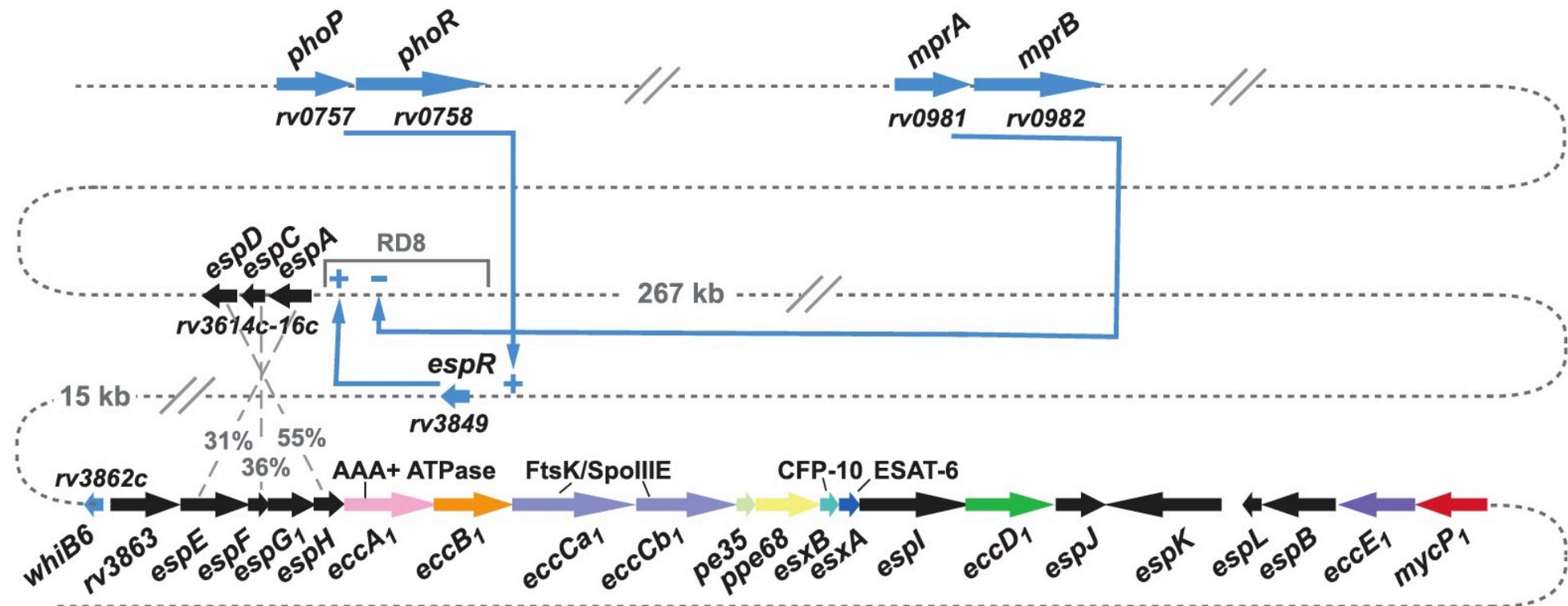
EspR (Rv3849)
and
two component regulator
PhoP (Rv0757) →

Indirect regulation of
ESAT-6 secretion

Raghavan et al, 2008, Nature
Frigui et al., 2008, PLoS Pathog.

Fortune et al., (2005) PNAS,
MacGurn et al., (2005), Mol. Microbiol

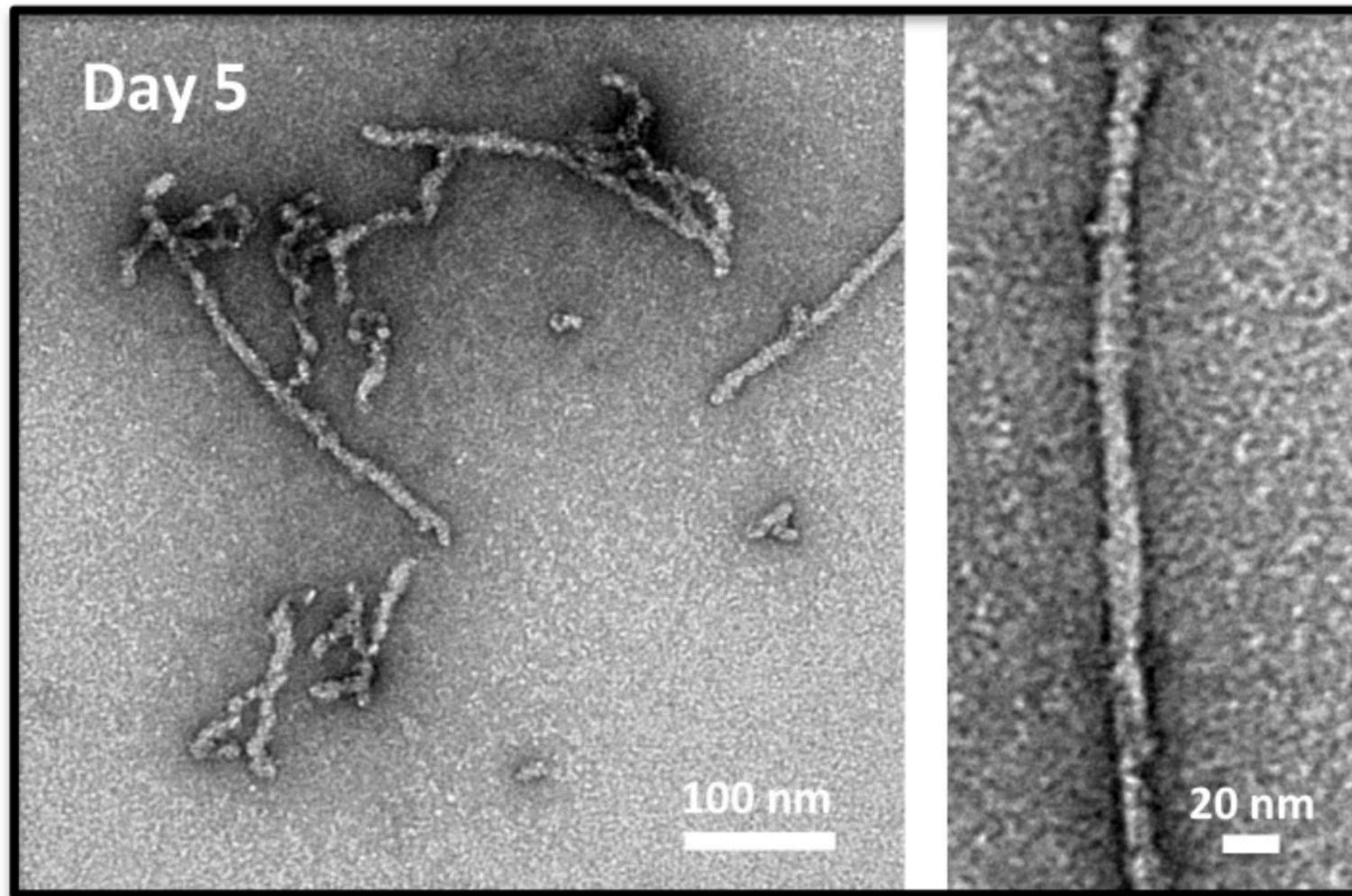
Secretion of ESAT-6 is co-dependent on EspA/C



Ates & Brosch Mol Microbiol 2017

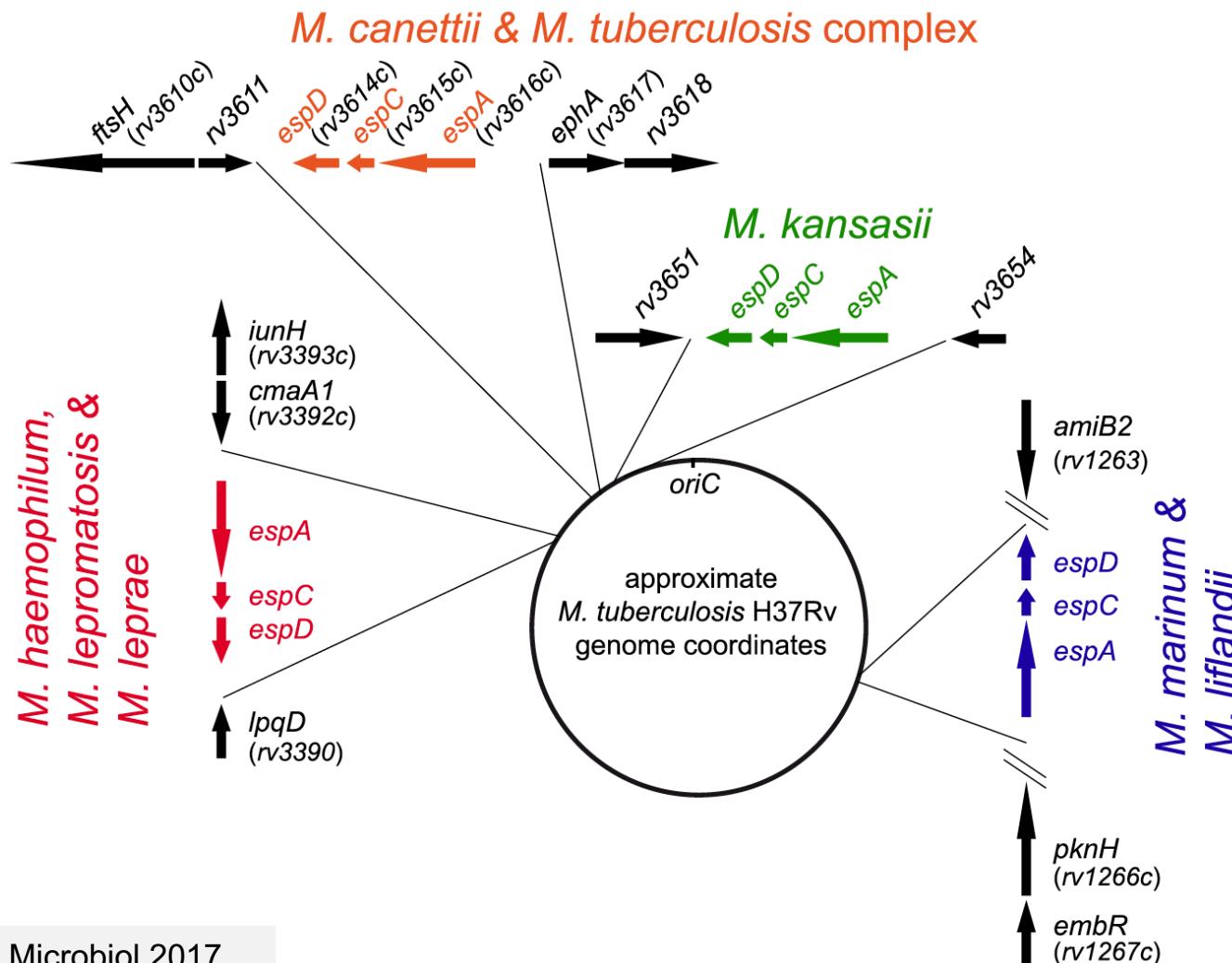
Majlessi et al., Immunol Rev 2015

EspC forms a secretion needle-like structure



Lou et al., Mol Microbiol 2017

EspACD locus has been independently acquired in different pathogenic mycobacteria → pathogenicity island ?

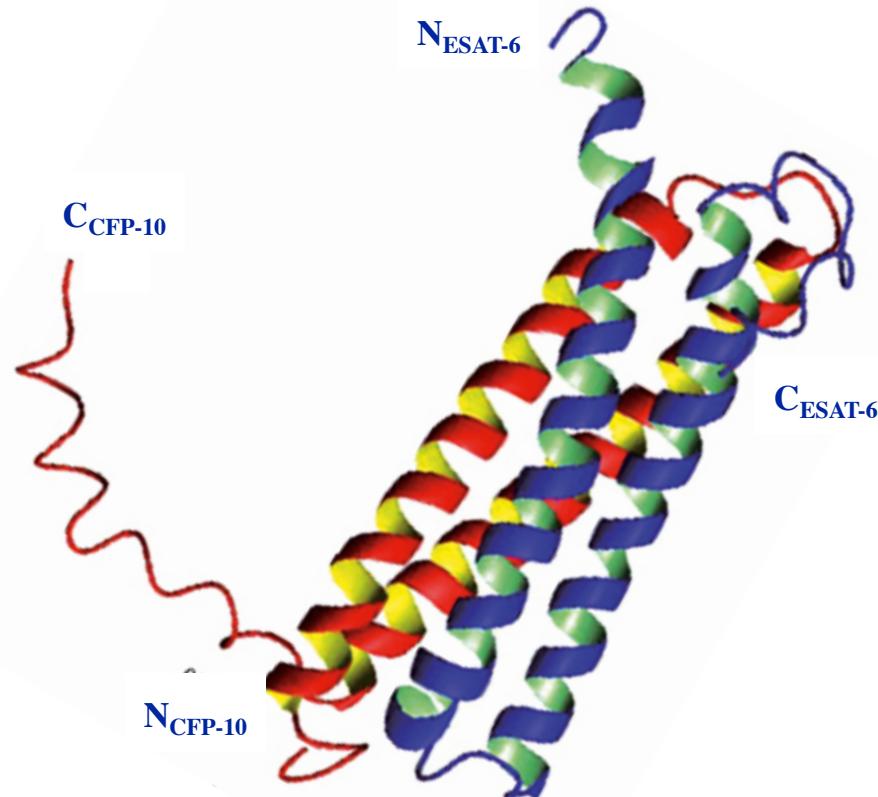


What is the biological function of ESAT-6 / CFP-10 in Mtb?

Secreted effector proteins of the ESX-1 / T7S system

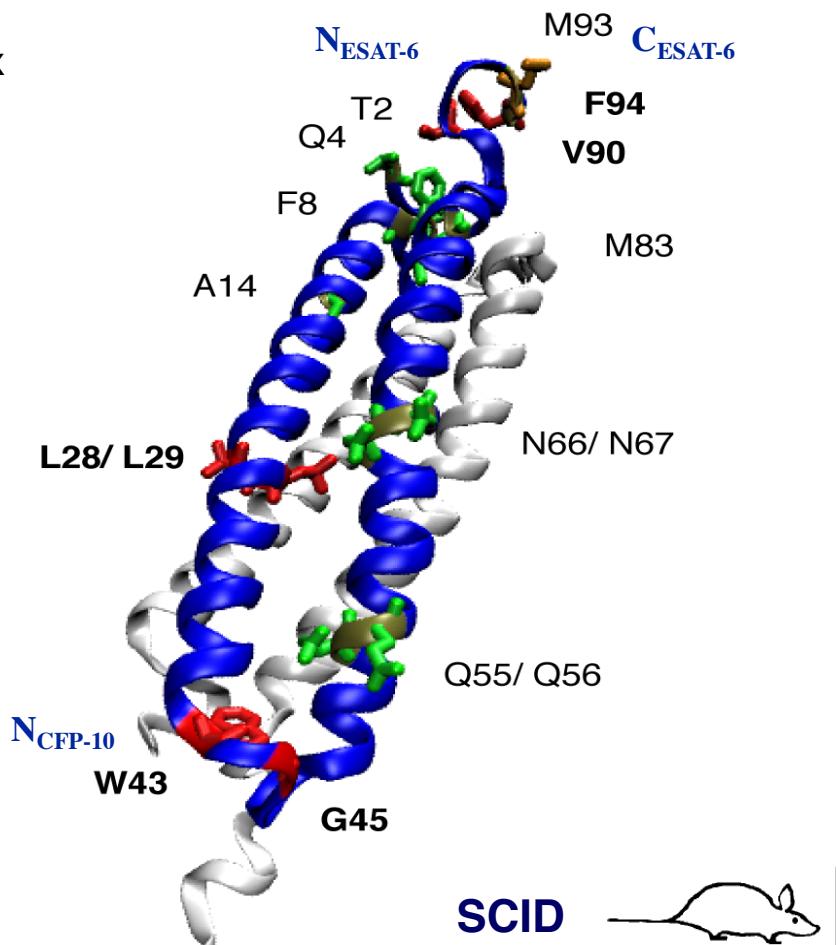
ESAT-6 and CFP-10 form a 1:1 heterodimeric complex

(Renshaw *et al.*, JBC, 2002)



Nuclear Magnetic Resonance
Determined solution structure

Renshaw *et al.*, EMBO J., (2005) 24:2491-8



**Strain attenuated
Virulence retained**

Brodin *et al.*, (2005) J. Biol. Chem.,

What is the biological function of ESAT-6/CFP-10 in *Mtb*?

ESAT-6 disrupts artificial lipid bilayers

CFP-10

ESAT-6

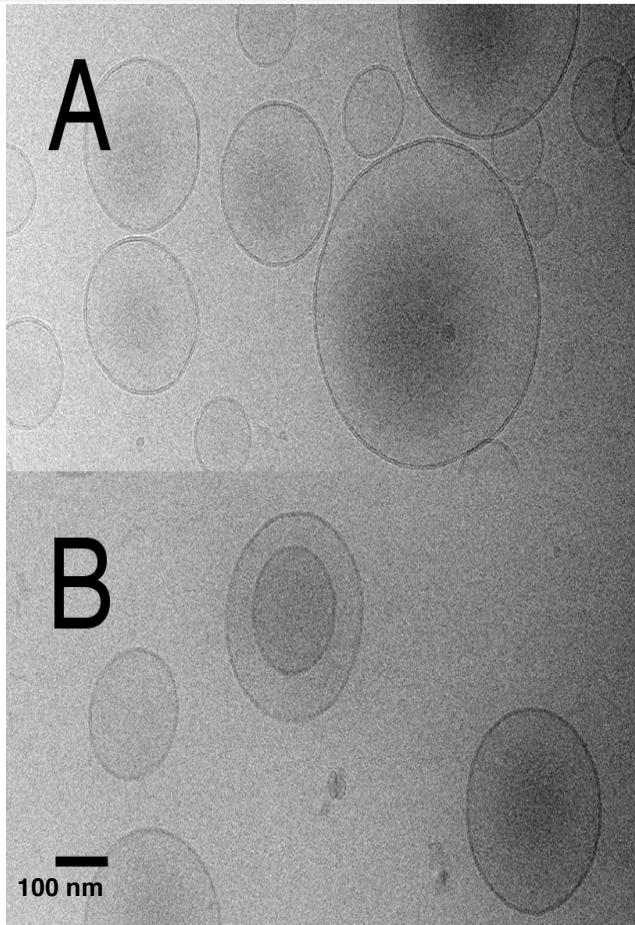


Hsu et al., PNAS, 2003

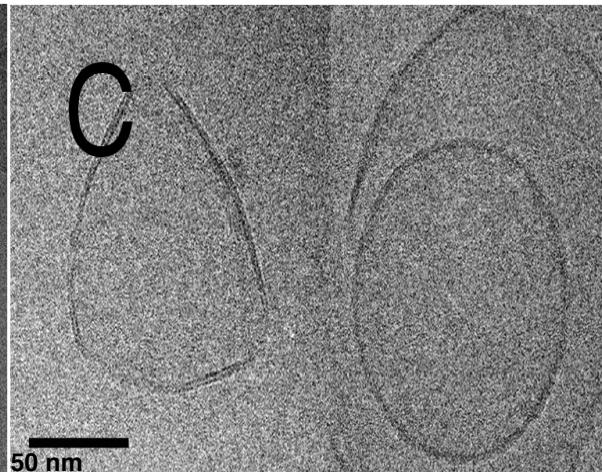
What is the biological function of ESAT-6/CFP-10 in *Mtb*?

ESAT-6 lyses liposomes

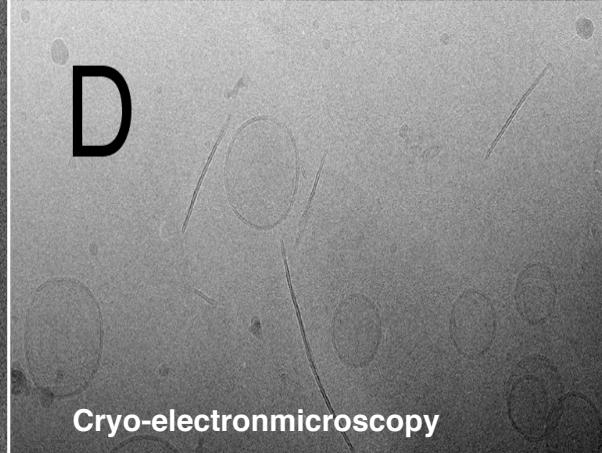
A)
Liposomes
only



B)
Liposomes +
CFP-10 (EsxB)



C)
Liposomes +
ESAT-6 (EsxA)



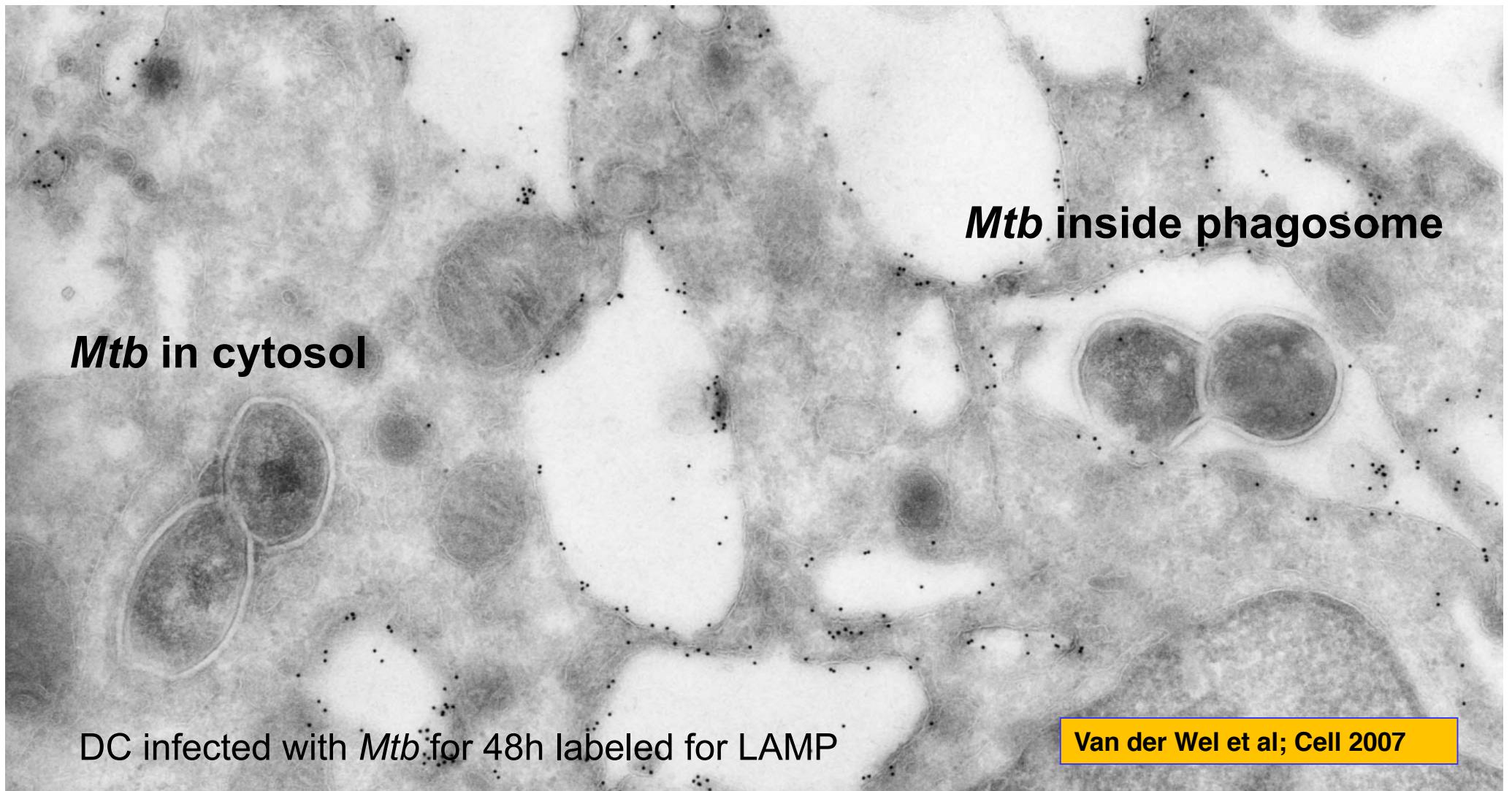
D)
Liposomes +
ESAT-6 (EsxA)

Liposome composition:

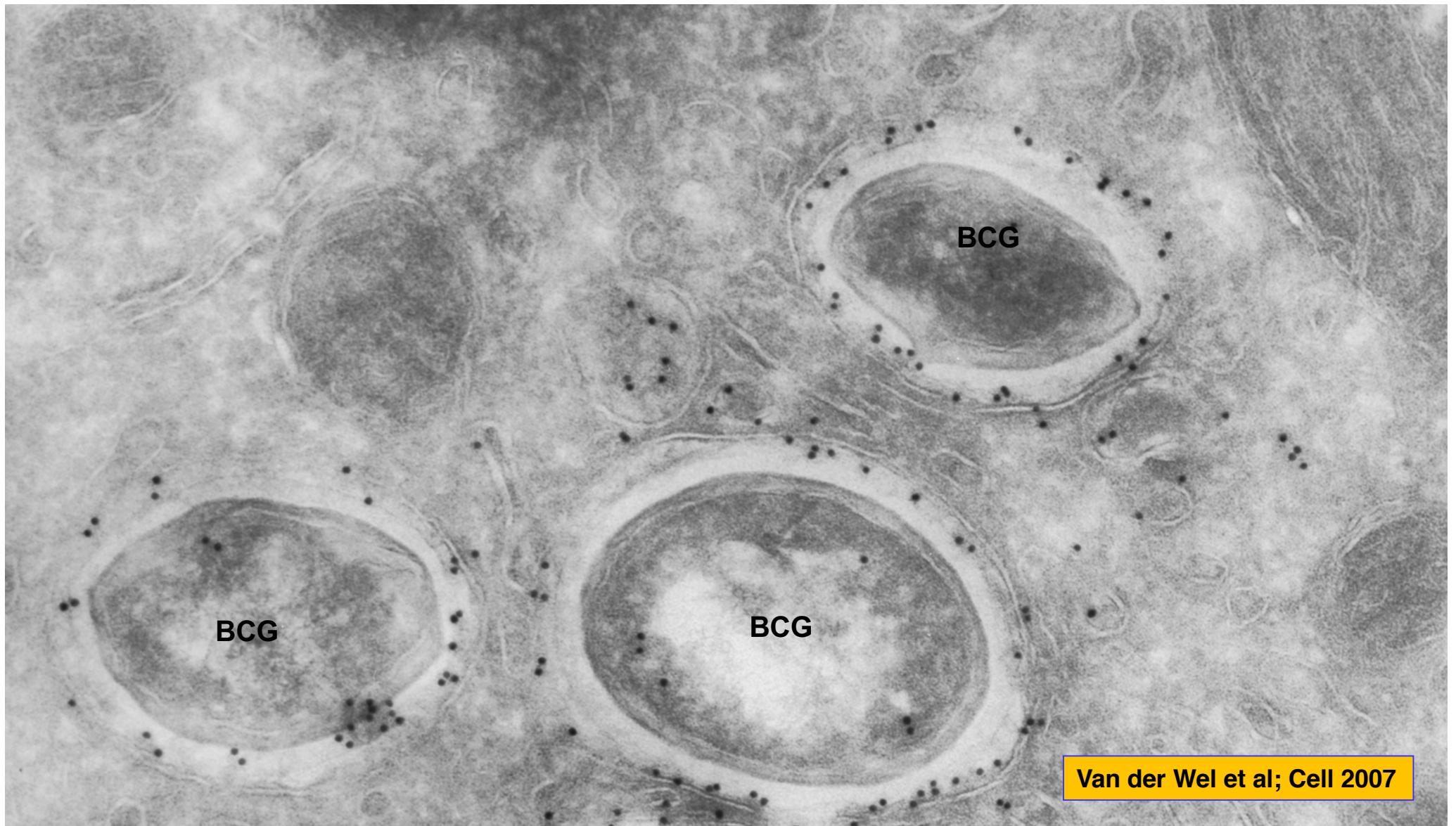
Dimyristoyl-phosphatidyl-choline (DMPC) (4 parts),
Dimyristoyl-phosphatidyl-glycerol (DMPG)(1 part),
cholesterol (1 part)

de Jonge et al., (2007) J. Bact

***M. tuberculosis* seems to translocate to the cytosol of infected dendritic cells and macrophages at later timepoints of infection**

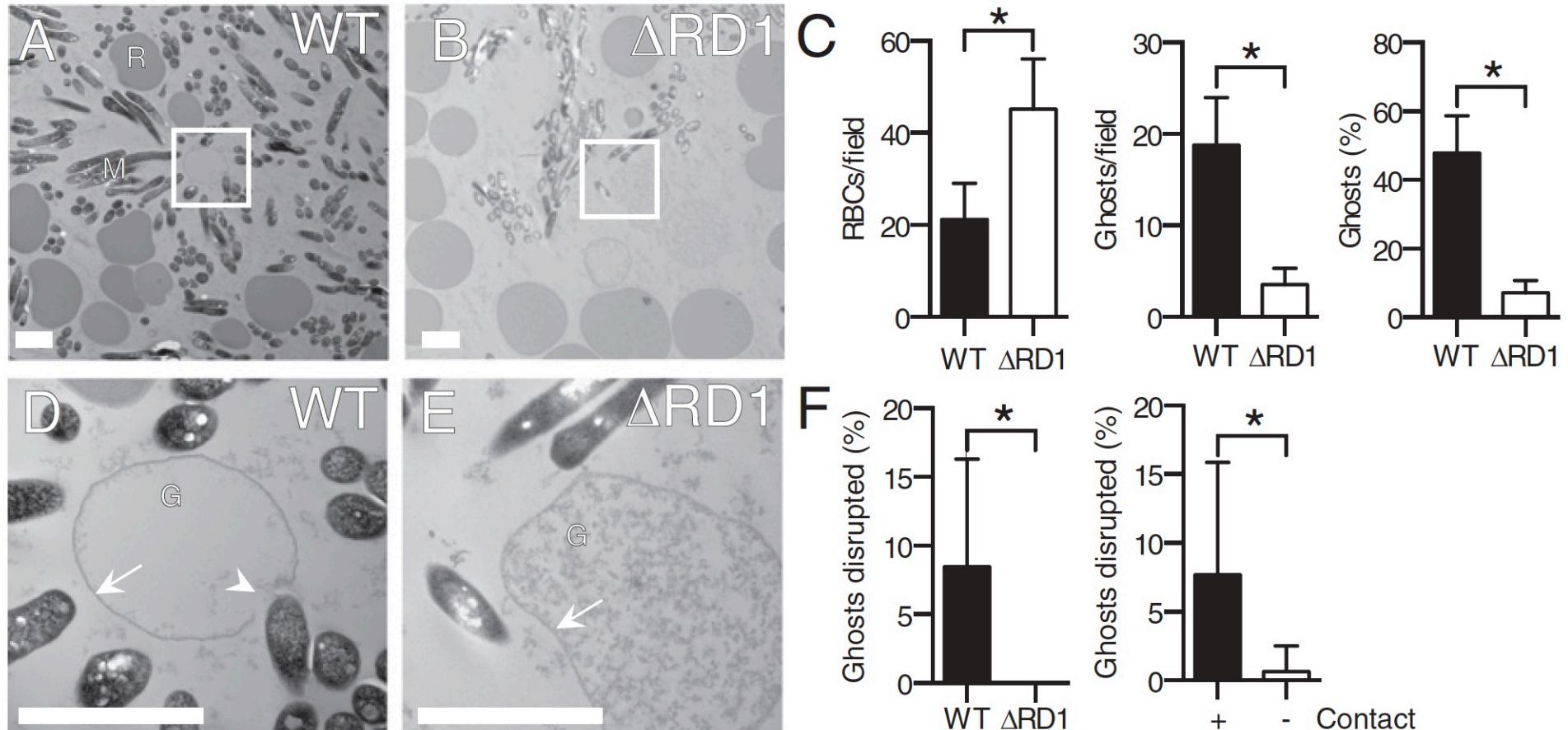


DC infected with *BCG* labeled for LAMP-1 remain phagolysosomal

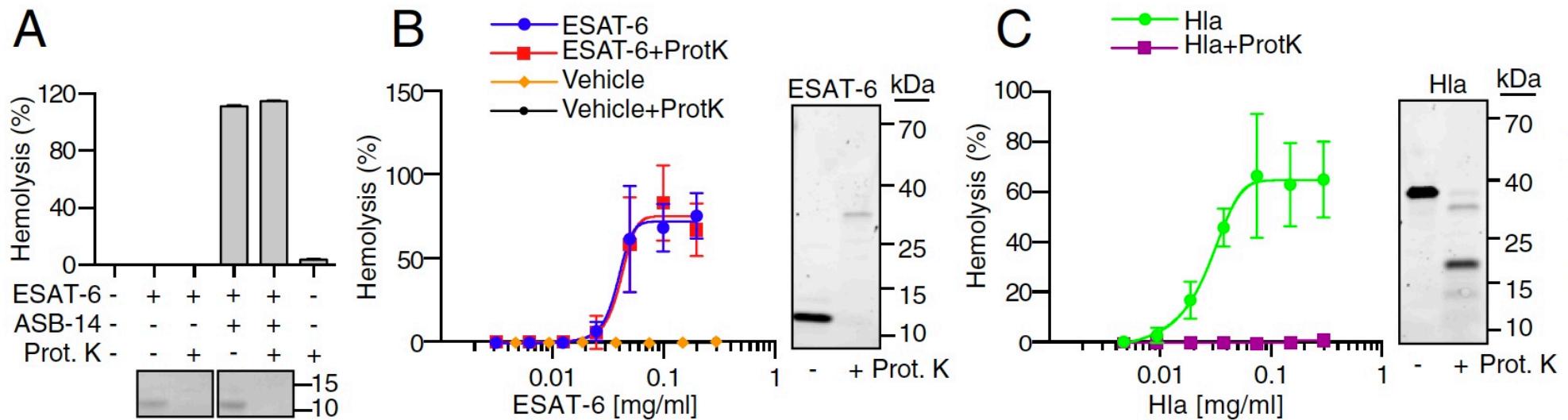


Van der Wel et al; Cell 2007

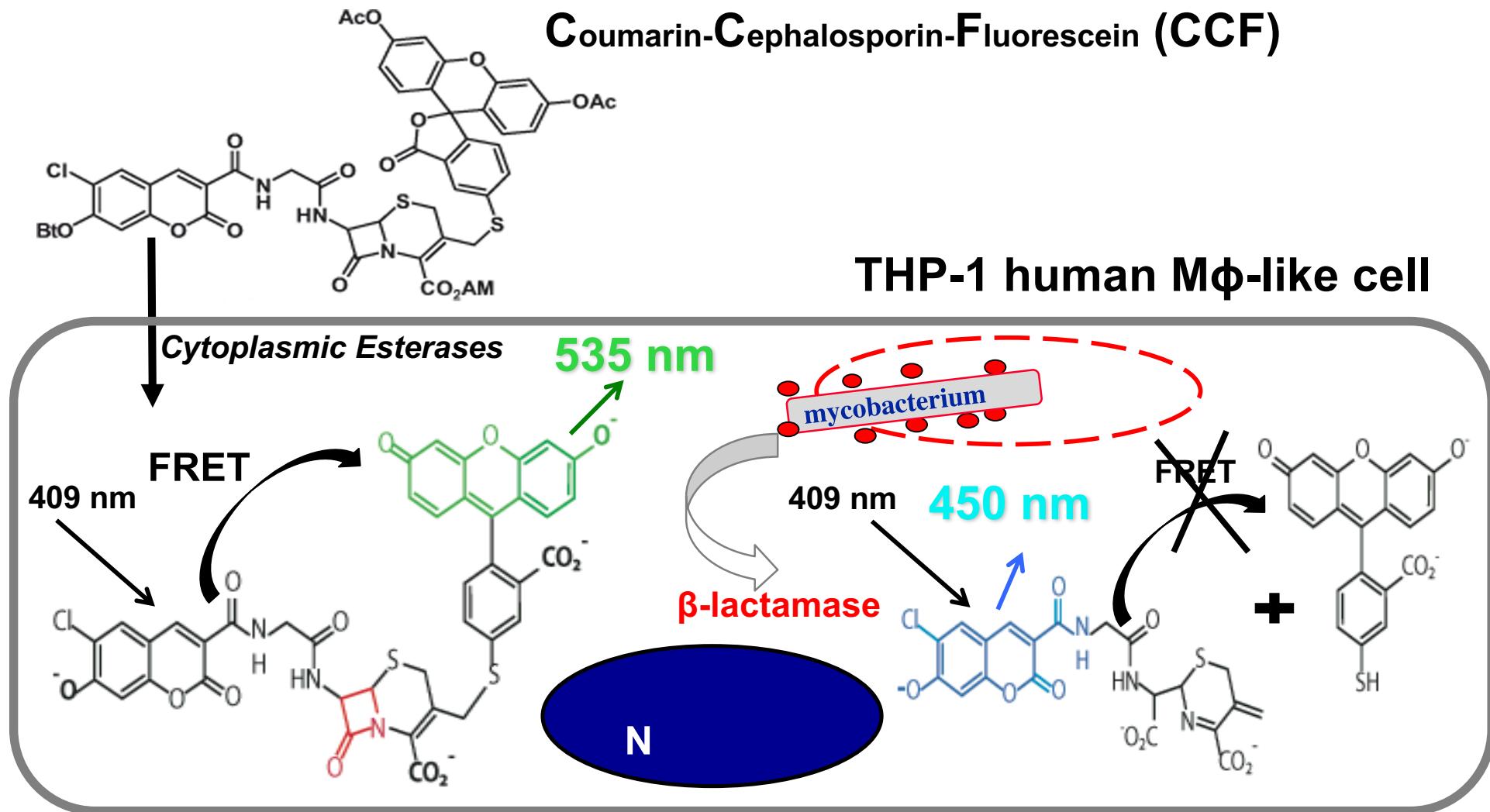
ESX-1-mediated membrane disruptions are contact dependent



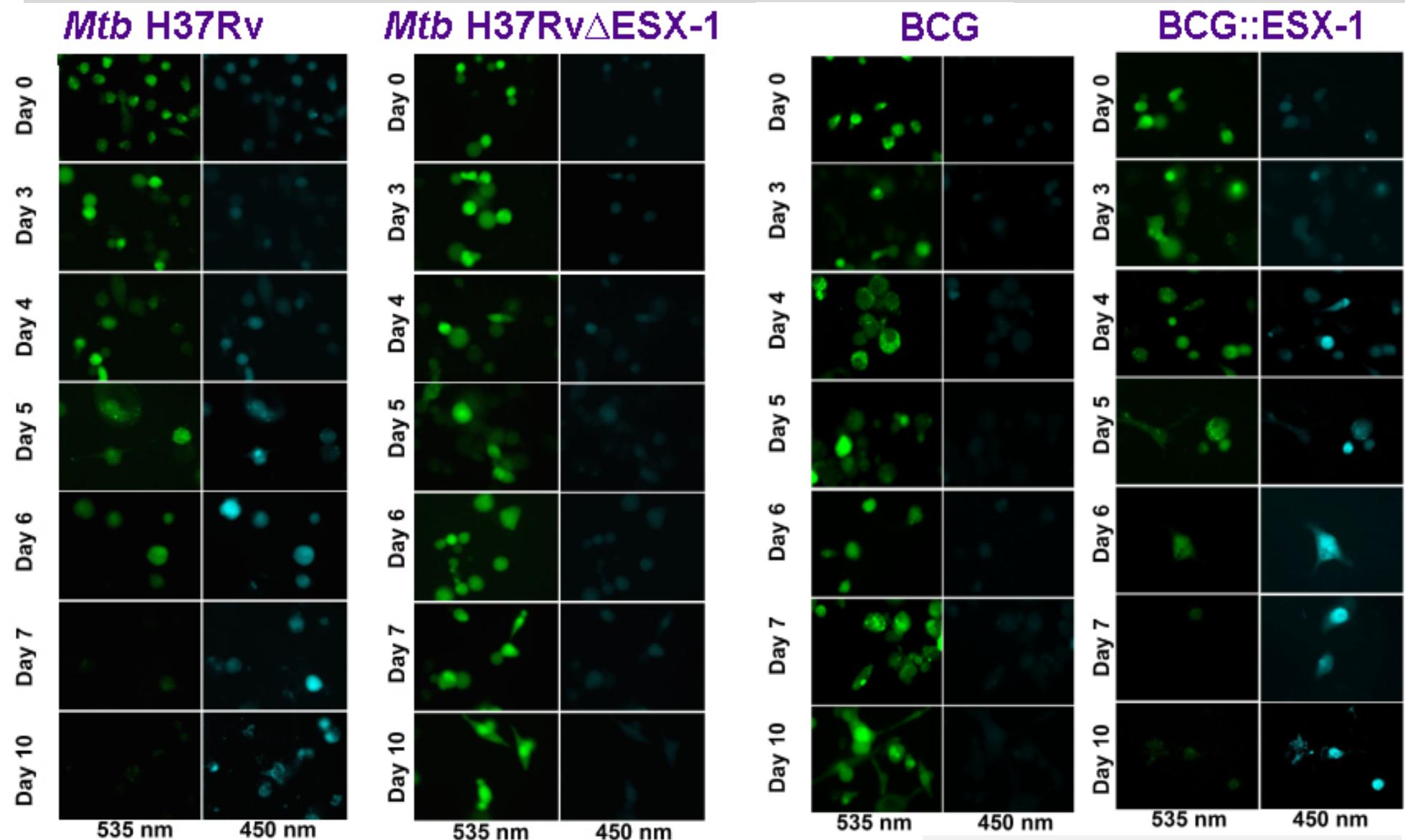
Lytic activity of recombinant ESAT-6 seems to be due to detergent ASB-14



Confirmation of phagosomal rupture and cytosolic access of *Mtb* by Fluorescence Resonance Energy Transfer-based assay



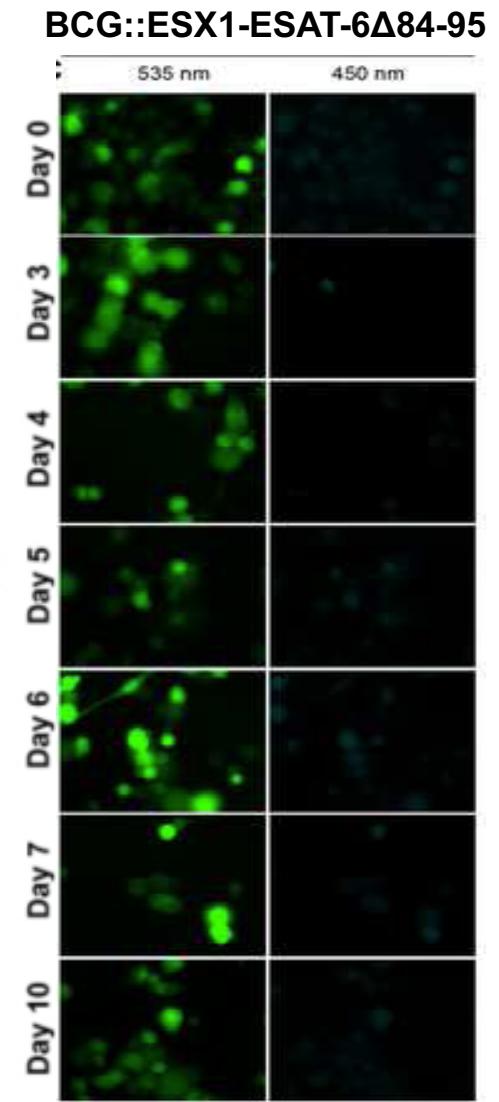
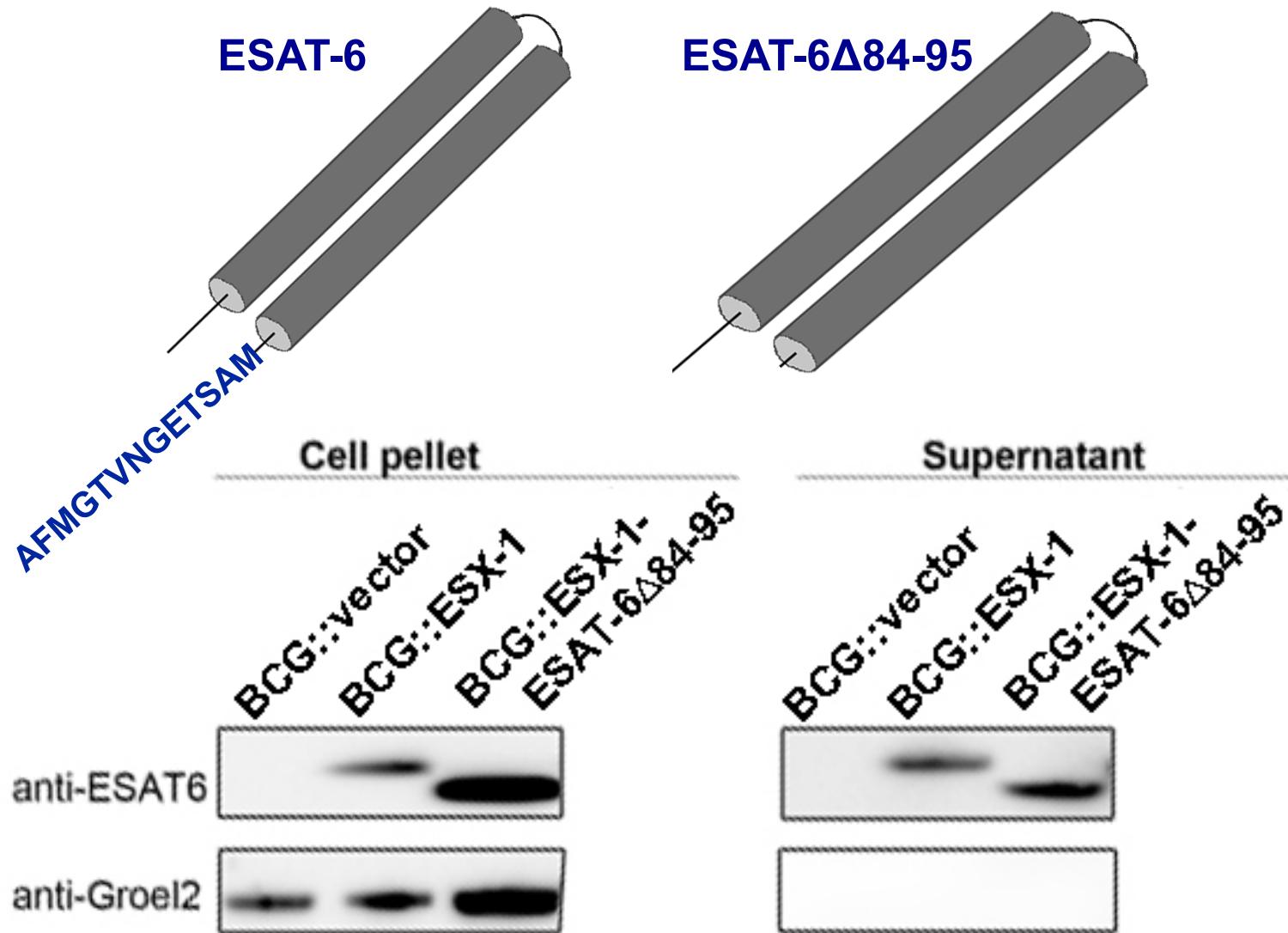
Rupture assay shows cytosolic contact of *Mtb* and BCG::ESX-1



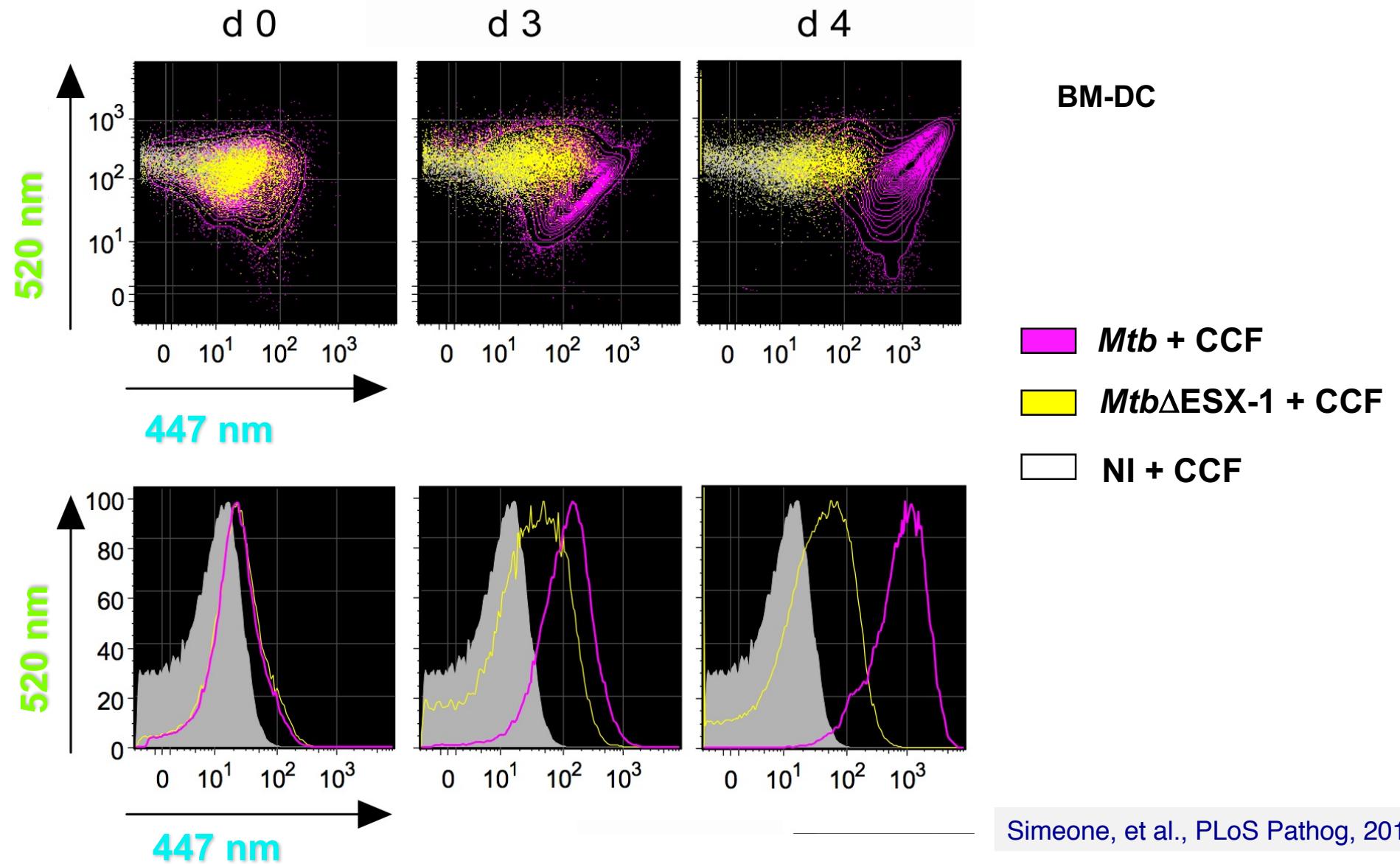
Simeone, et al., PLoS Pathog, 2012

Truncated ESAT-6 Δ 84-95 is secreted ... but does not cause rupture

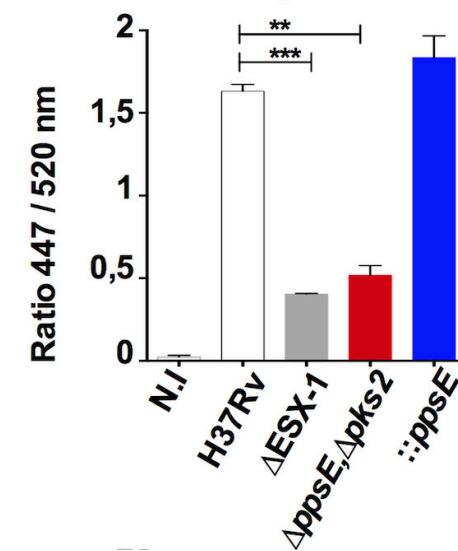
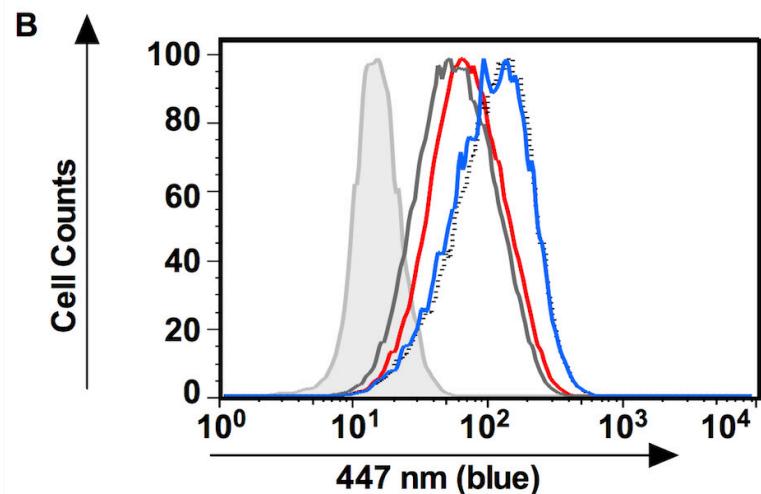
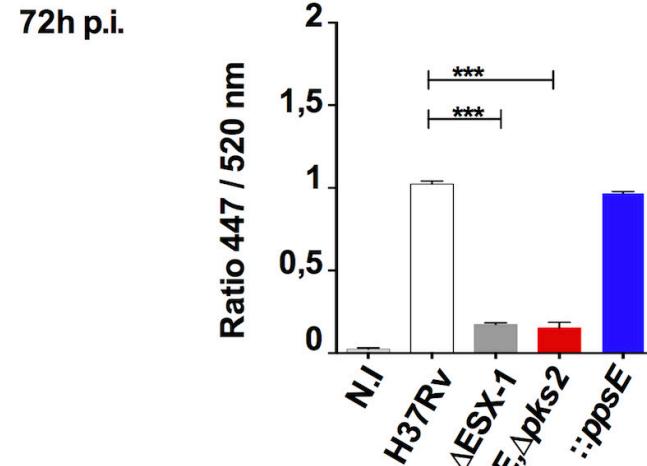
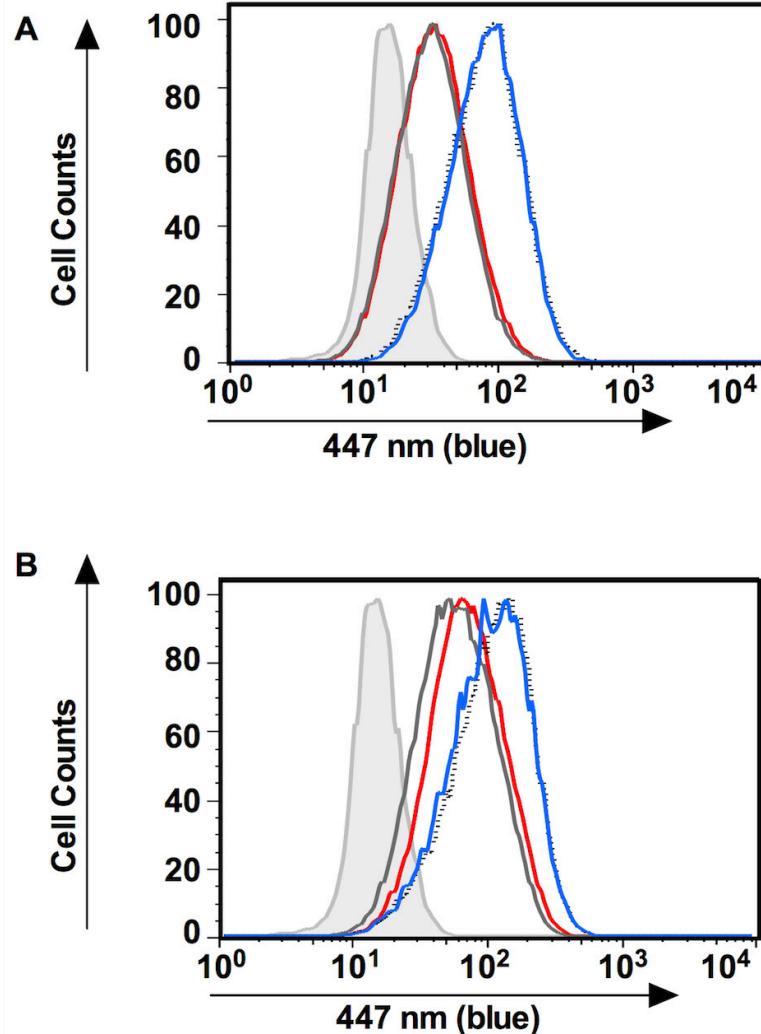
→ underlining importance of ESAT-6 for cytosolic translocation

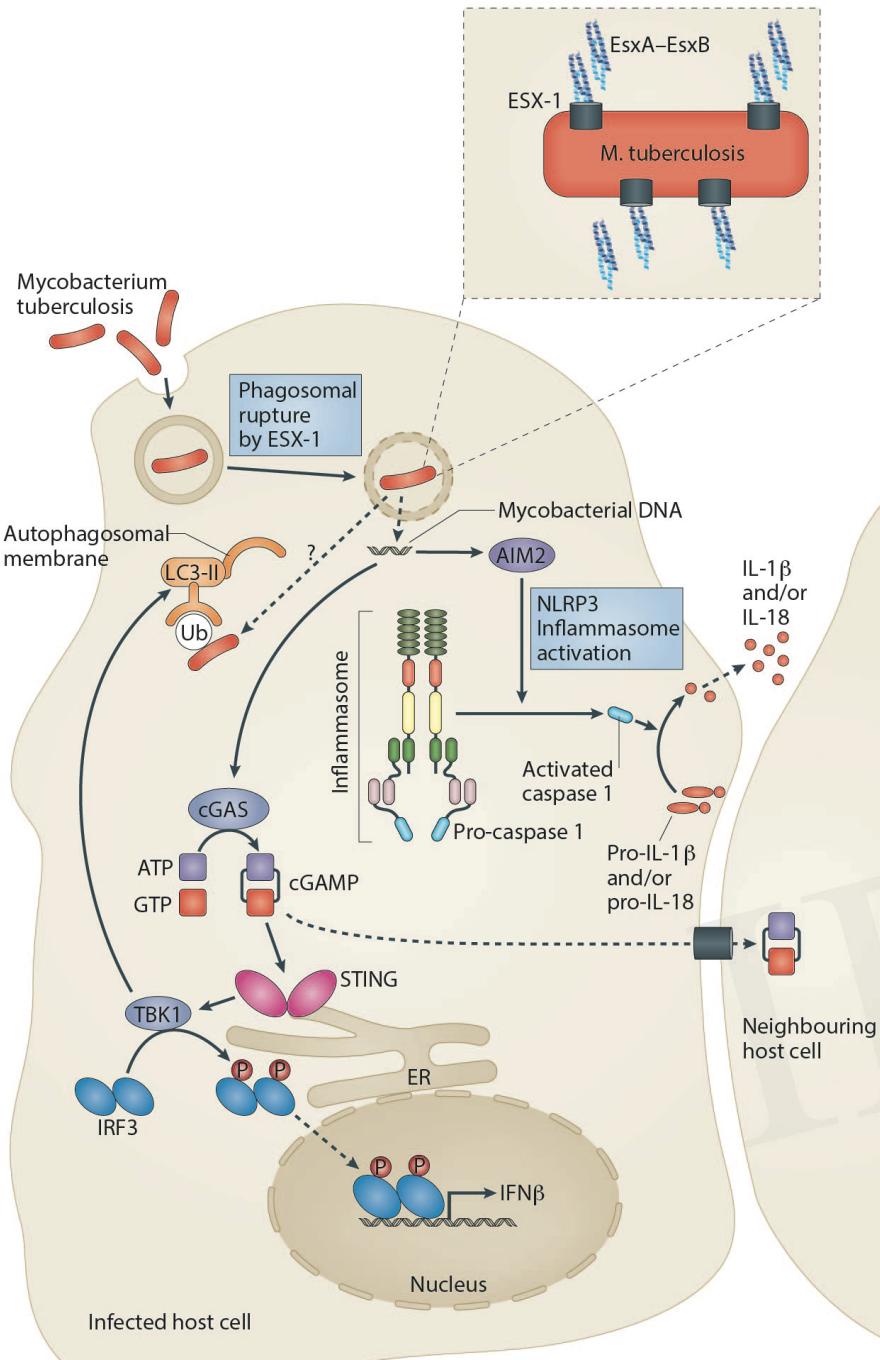


FRET rupture assay was adapted for use with Flow Cytometry
→ for testing new host cell types and parameters



ESX-1 and Phthiocerol dimycocerosate (DIM/PDIM) act in concert to cause phagosomal rupture





ESX-1-induced cytosolic access is essential for cGAS-mediated signalling

→ Secretion of type I interferons (IFN- β)

→ Inflammasome activation causing secretion of interleukin IL1- β & IL-18

Kupz et al., JCI, 2016

Collins, et al. (2015) CH&M

Wassermann, et al. (2015) CH&M

Watson, et al. (2015) CH&M

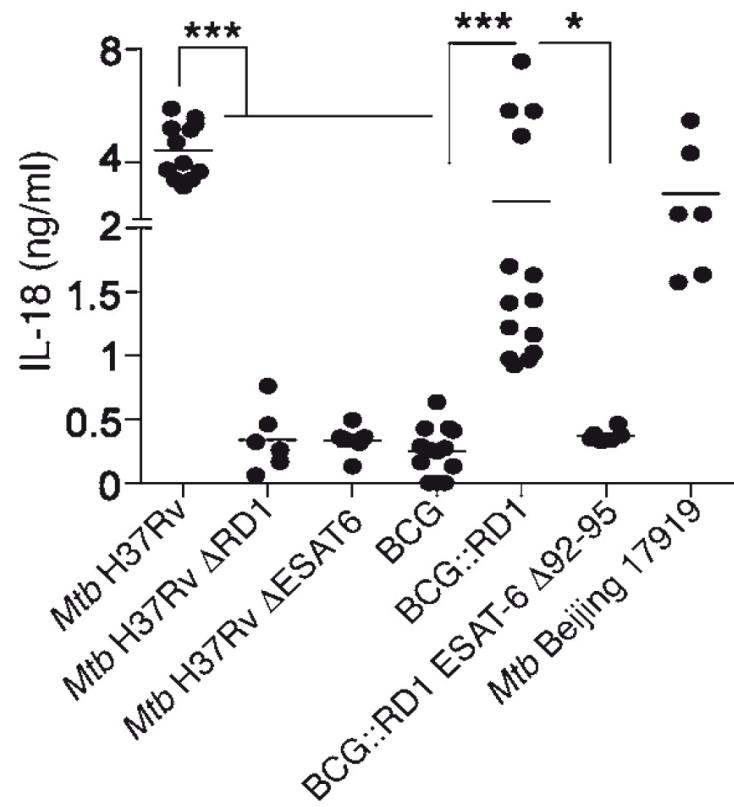
reviewed in Gröschel et al. Nat Rev Microbiol, 2016,

cGAS = cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase

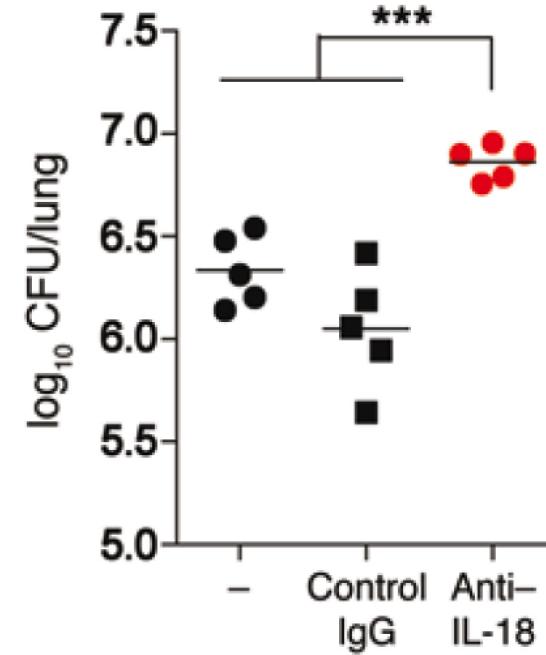
ESX-1 is also required for IFN- γ secretion by *Mtb* antigen-independent memory CD8 $^{+}$ T cells & NK cells

Kupz *et al.*, Journal of Clinical Investigation, 2016

This IFN- γ secretion requires **IL-18** → dependent on functional ESX-1

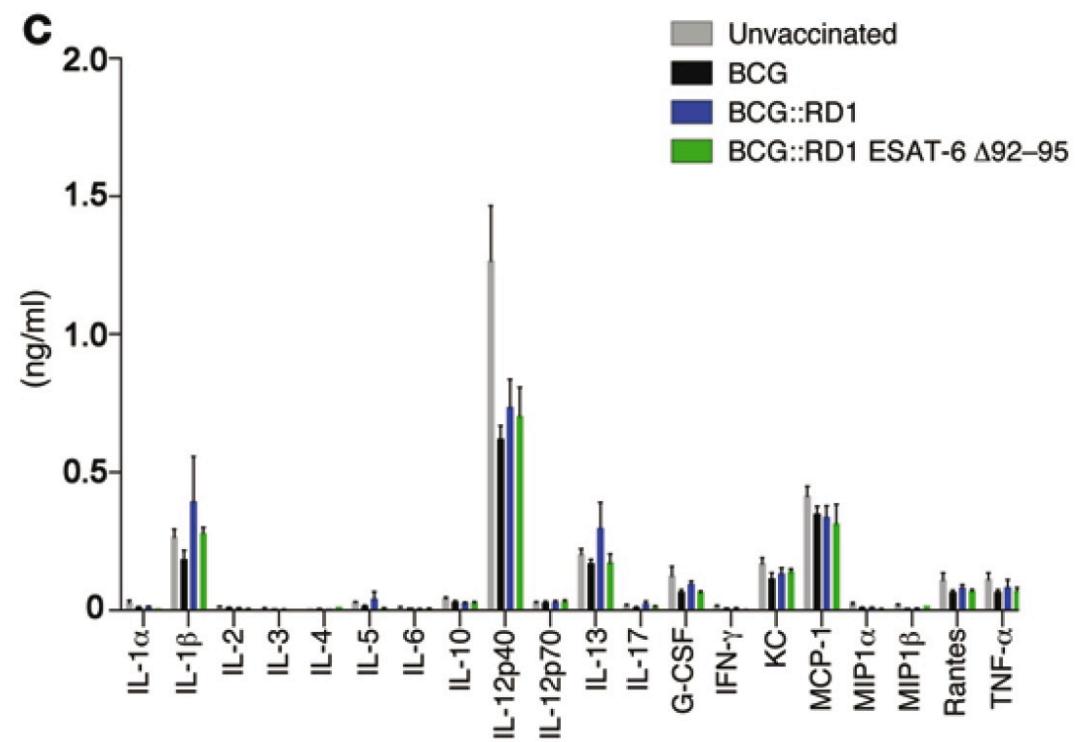
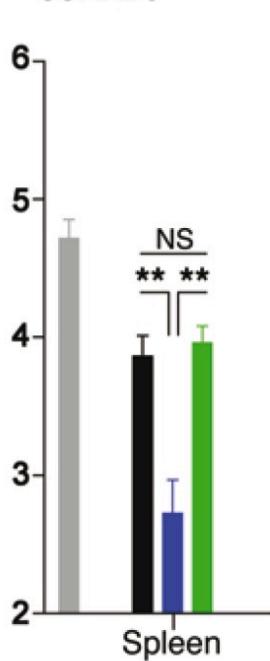
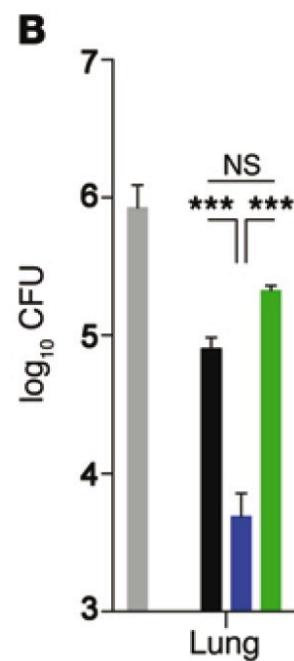
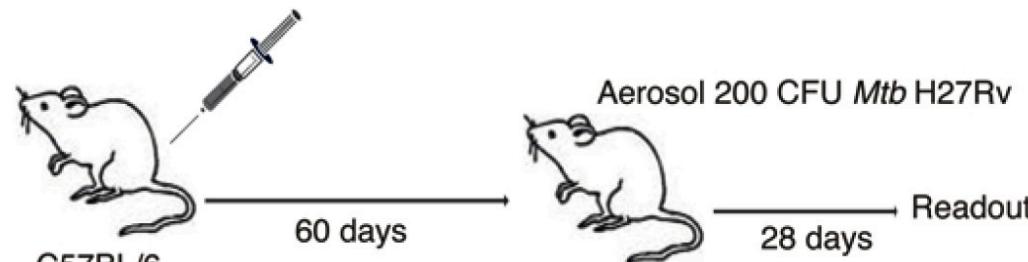


Effect could be blocked by Anti-IL-18



ESX-1 proficient and functional strain protects mice better than BCG

A i.v. 1×10^7 CFU BCG, BCG::RD1, BCG::RD1 ESAT-6 Δ 92–95



Main objectives and aims are:

- The development of attenuated TB vaccines that are more protective than current BCG

The Rational is :

- to increase protective efficacy of a vaccine by triggering **cytosolic pattern recognition** in the host cell
- currently used TB vaccines are unable to induce such responses

The widely used vaccines in the history of TB vaccination:

BCG (Bacille Calmette & Guérin)

- *Mycobacterium bovis* → BCG
230 passages
- ~ 3 billion doses used worldwide
- good protection against childhood TB, but limited impact on TB pandemic



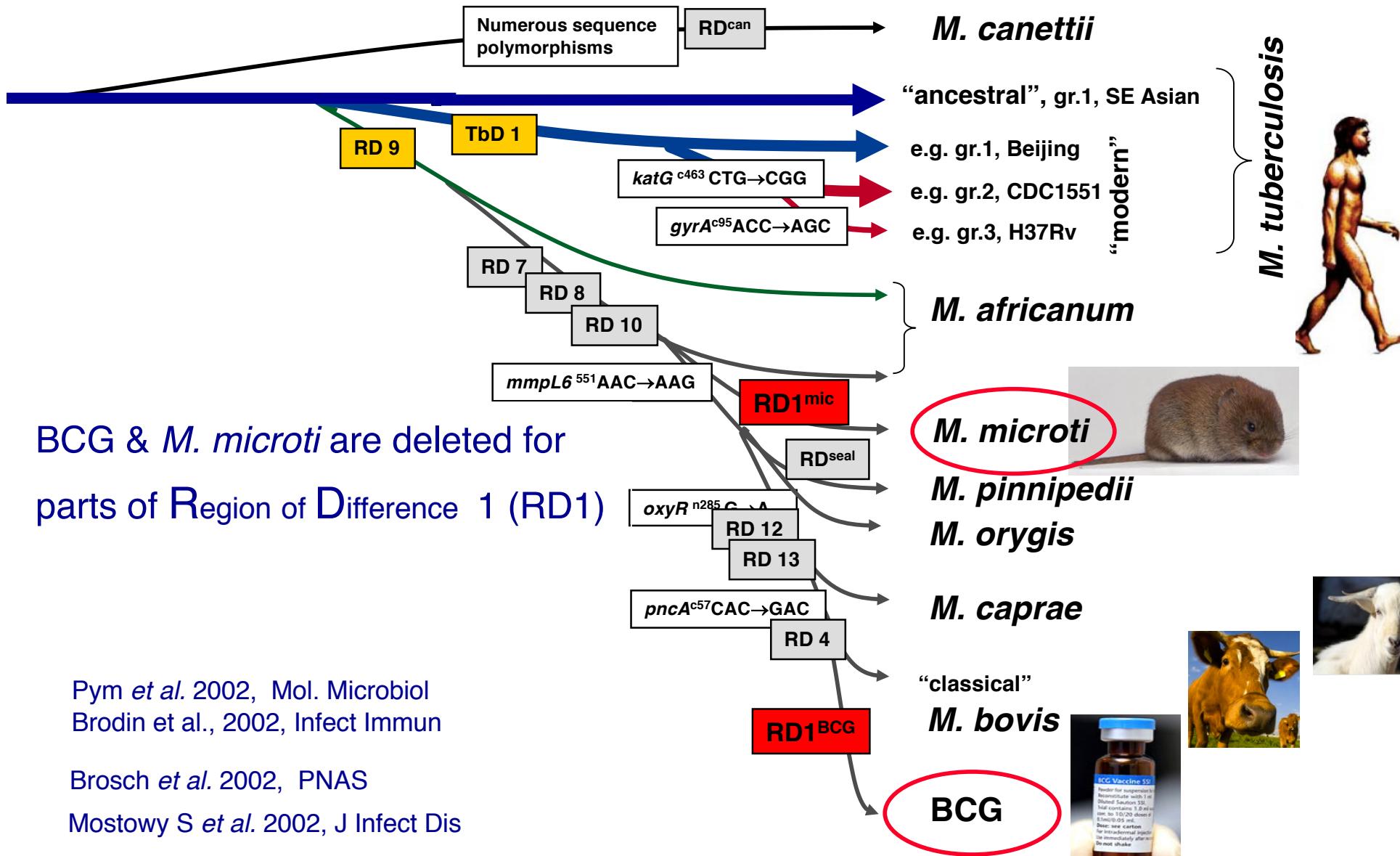
Calmette & Guérin

Mycobacterium microti (vole bacillus)

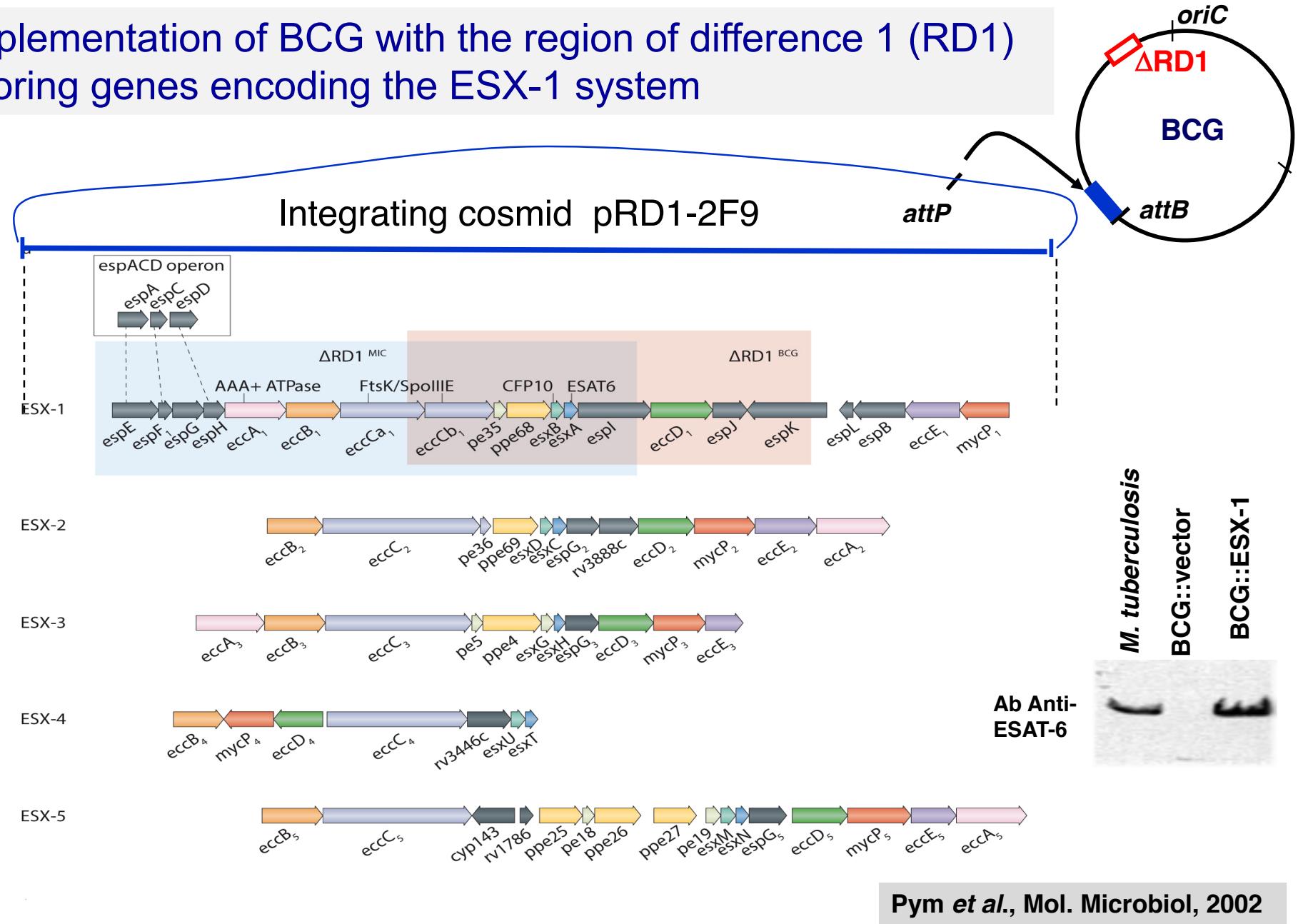
- less well known
- used as attenuated vaccine in the 1960s in the UK and former Czechoslovakia



BCG & *M. microti* are derived from the animal lineage of tubercle bacilli



Complementation of BCG with the region of difference 1 (RD1) harboring genes encoding the ESX-1 system

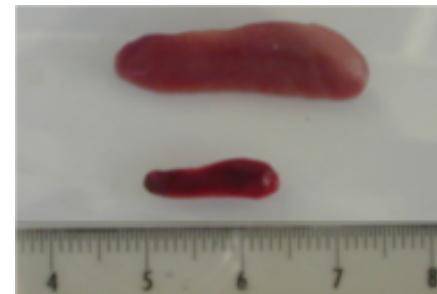


BCG::ESX-1 differs in biological features from BCG

- BCG::ESX1 is more virulent than BCG in SCID mice

BCG::ESX1

BCG::vector-control



Severe Combined
Immuno-Deficient

- BCG::ESX-1 is more persistant than BCG, but still much less virulent than *M. tuberculosis* in immuno-competent mice (BALB/c)

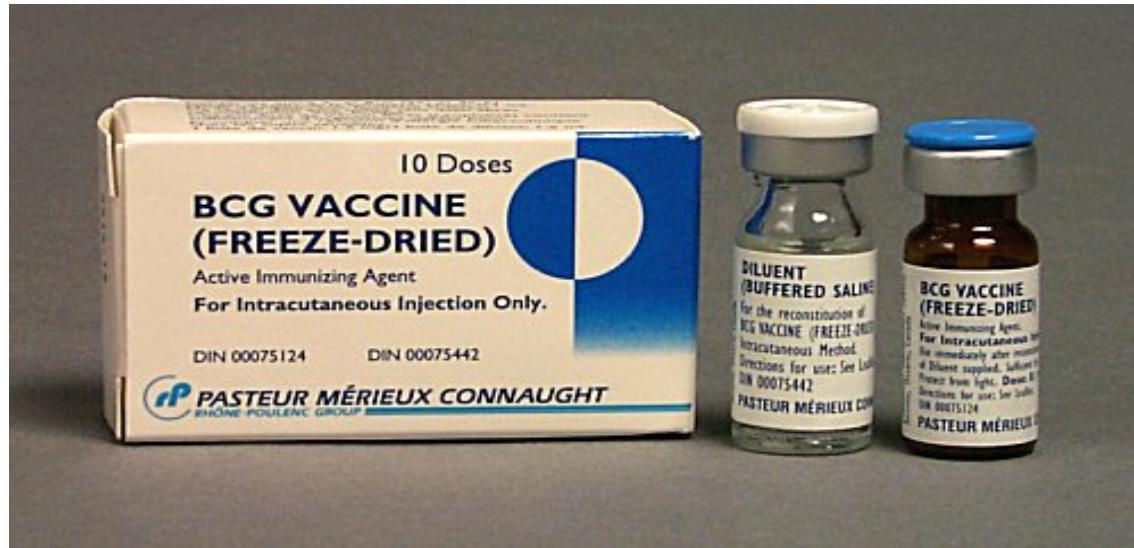
Pym et al., Mol. Microbiol, 2002

- BCG::ESX-1 protects better than BCG against disseminated TB in mice & guinea pigs



Pym et al., Nat. Medicine, 2003

However → How to make a safe & efficient BCG::ESX-1 vaccine ?

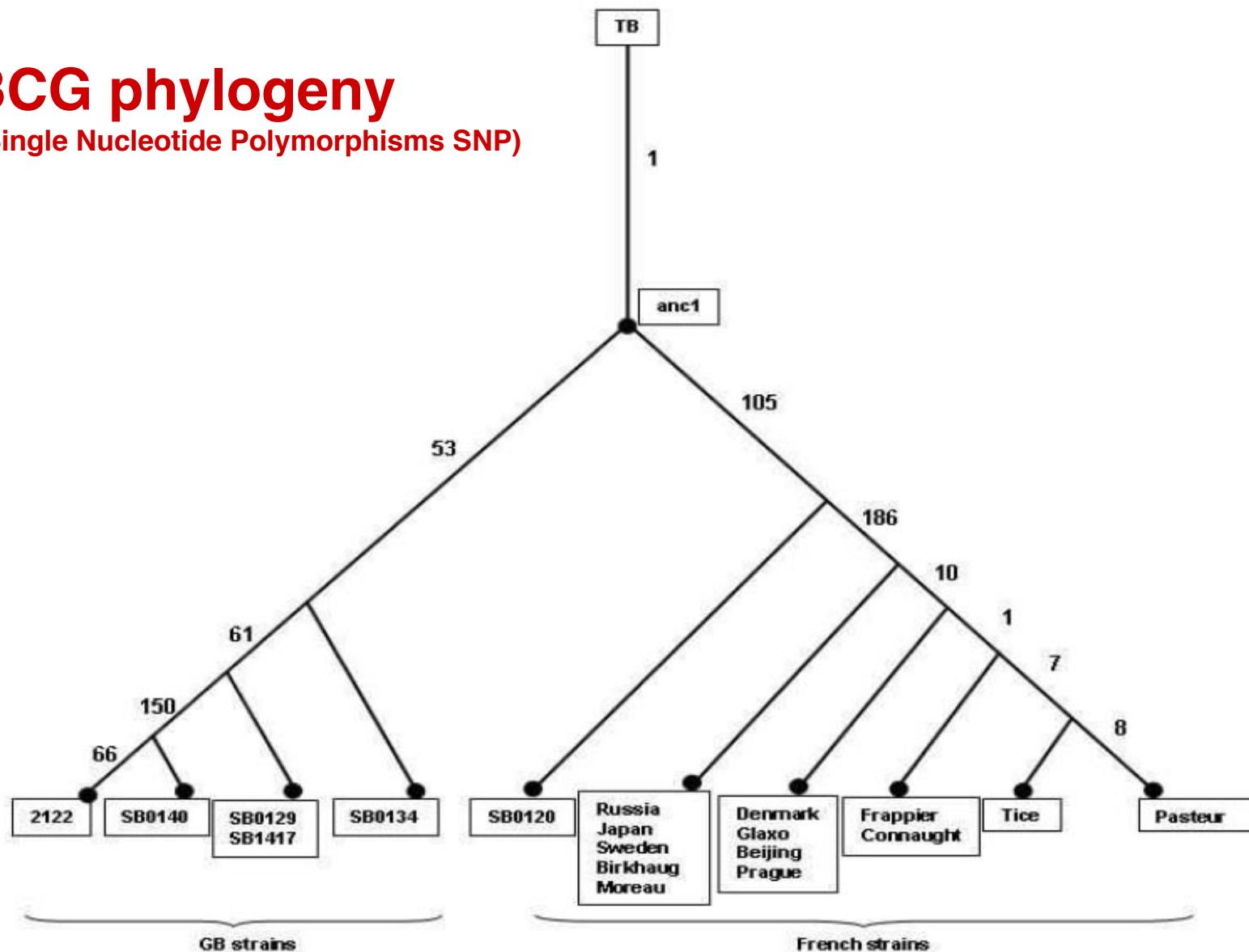


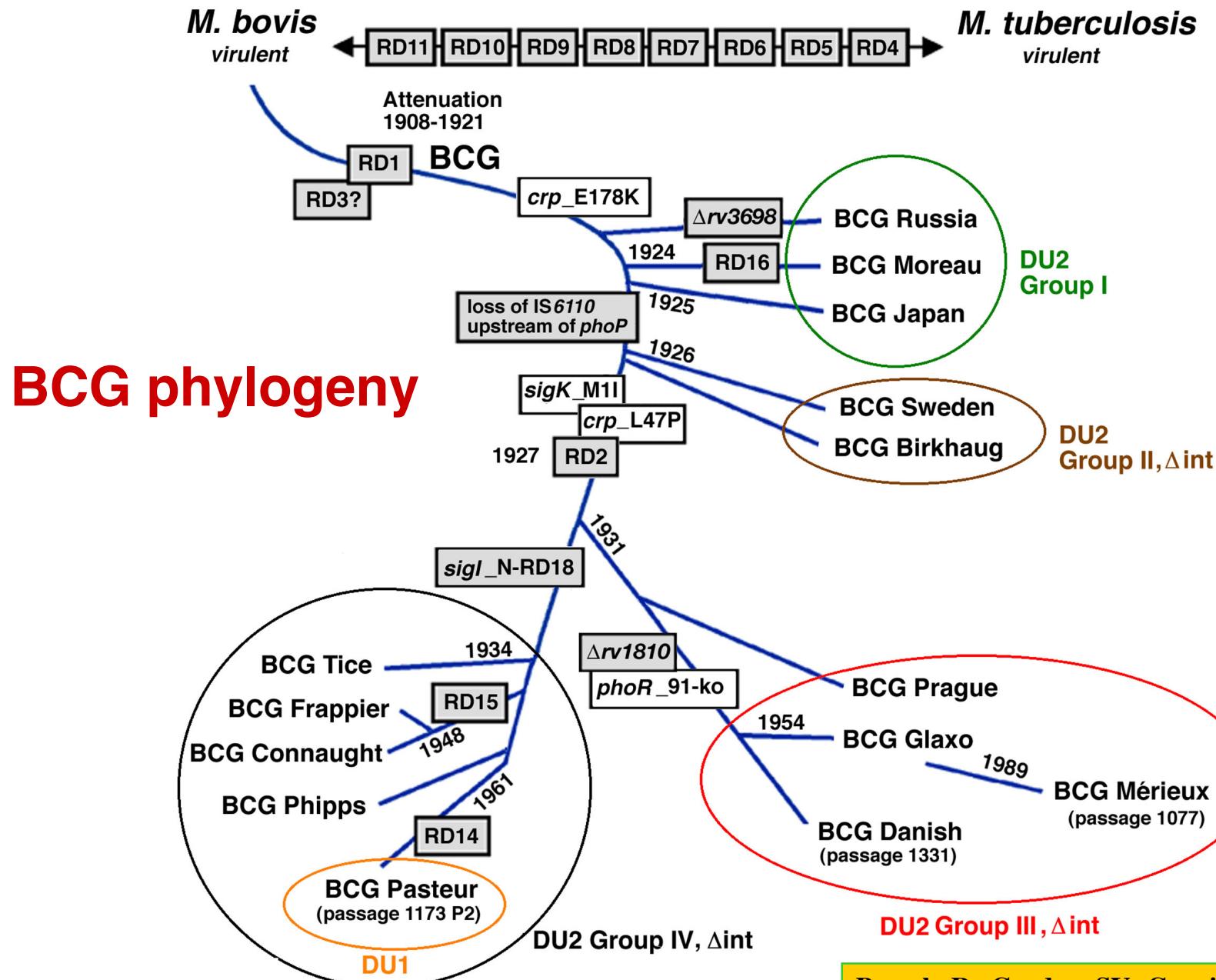
Rational:

→ Use an ESX-1 system from a BSL 2 organism instead of that from *Mtb*

BCG phylogeny

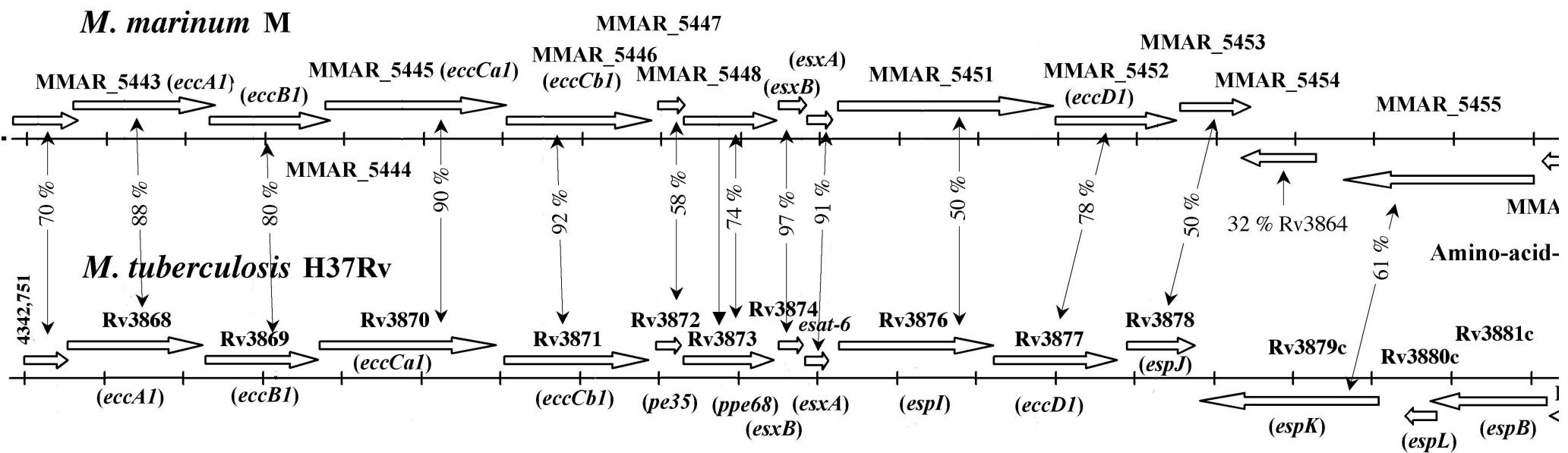
(based on Single Nucleotide Polymorphisms SNP)





Brosch, R., Gordon, SV., Garnier, T., et al., PNAS (2007)

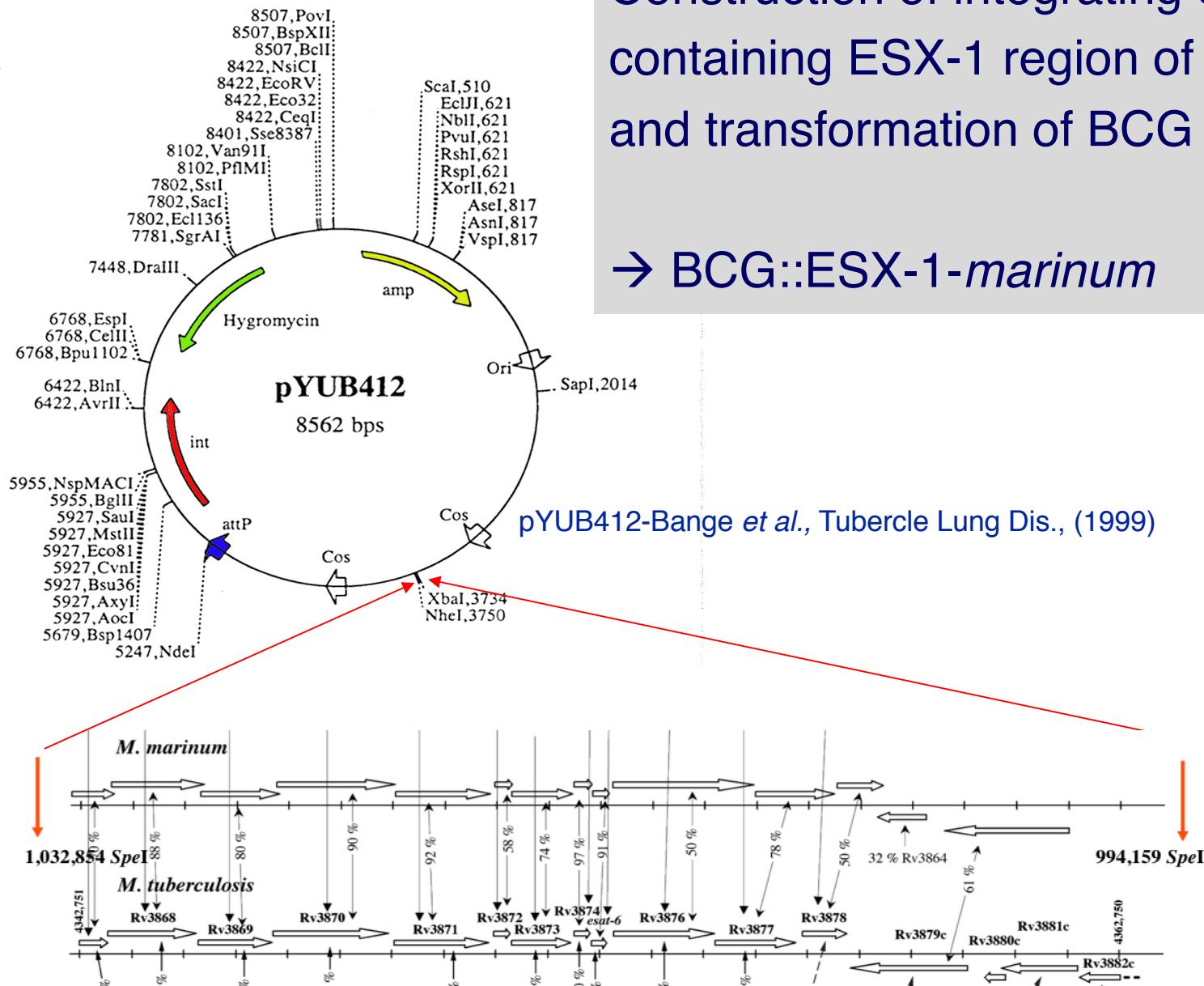
ESX-1 system from *Mycobacterium marinum* (BSL2) shows large similarity to ESX-1 system of *M. tuberculosis* (BSL-3)



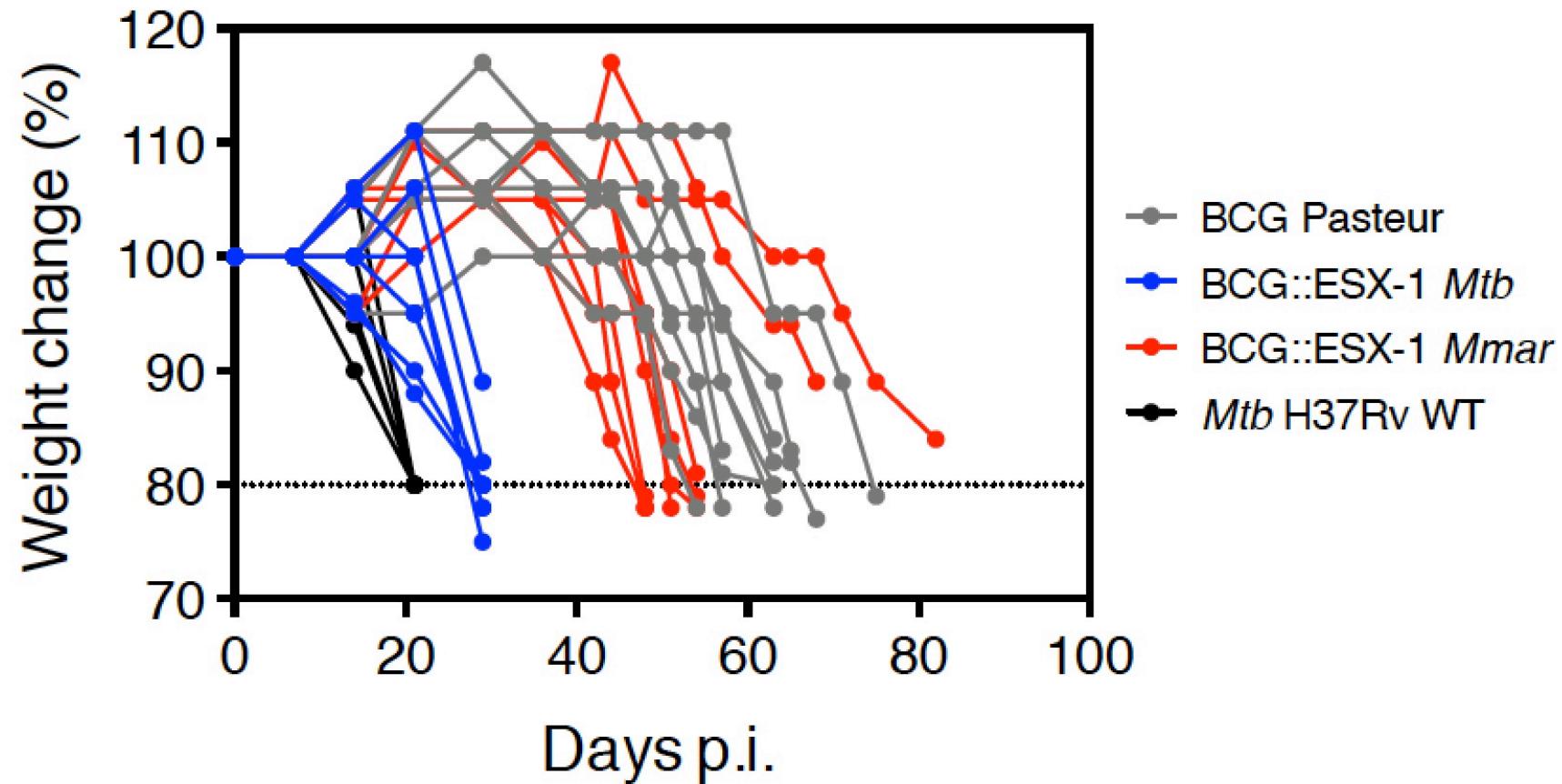
M. marinum was reported to escape the phagosome in an ESX-1 dependent way
 (Stamm et al., JEM, 2003)

Construction of integrating cosmid containing ESX-1 region of *M. marinum* and transformation of BCG

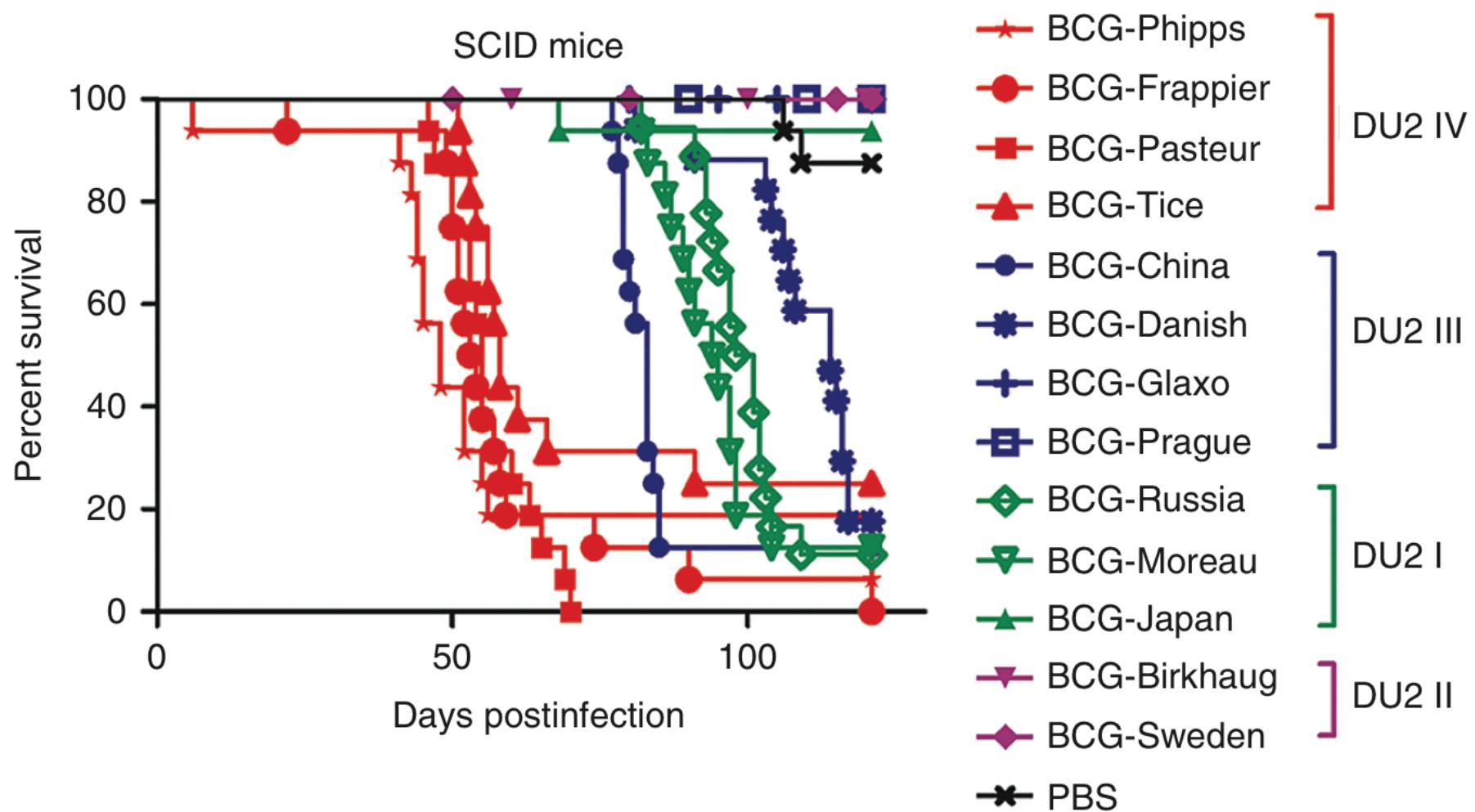
→ BCG::ESX-1-marinum



BCG::ESX-1-*marinum* features low virulence - similar to BCG Pasteur
(much less than BCG::ESX-1-*Mtb*)



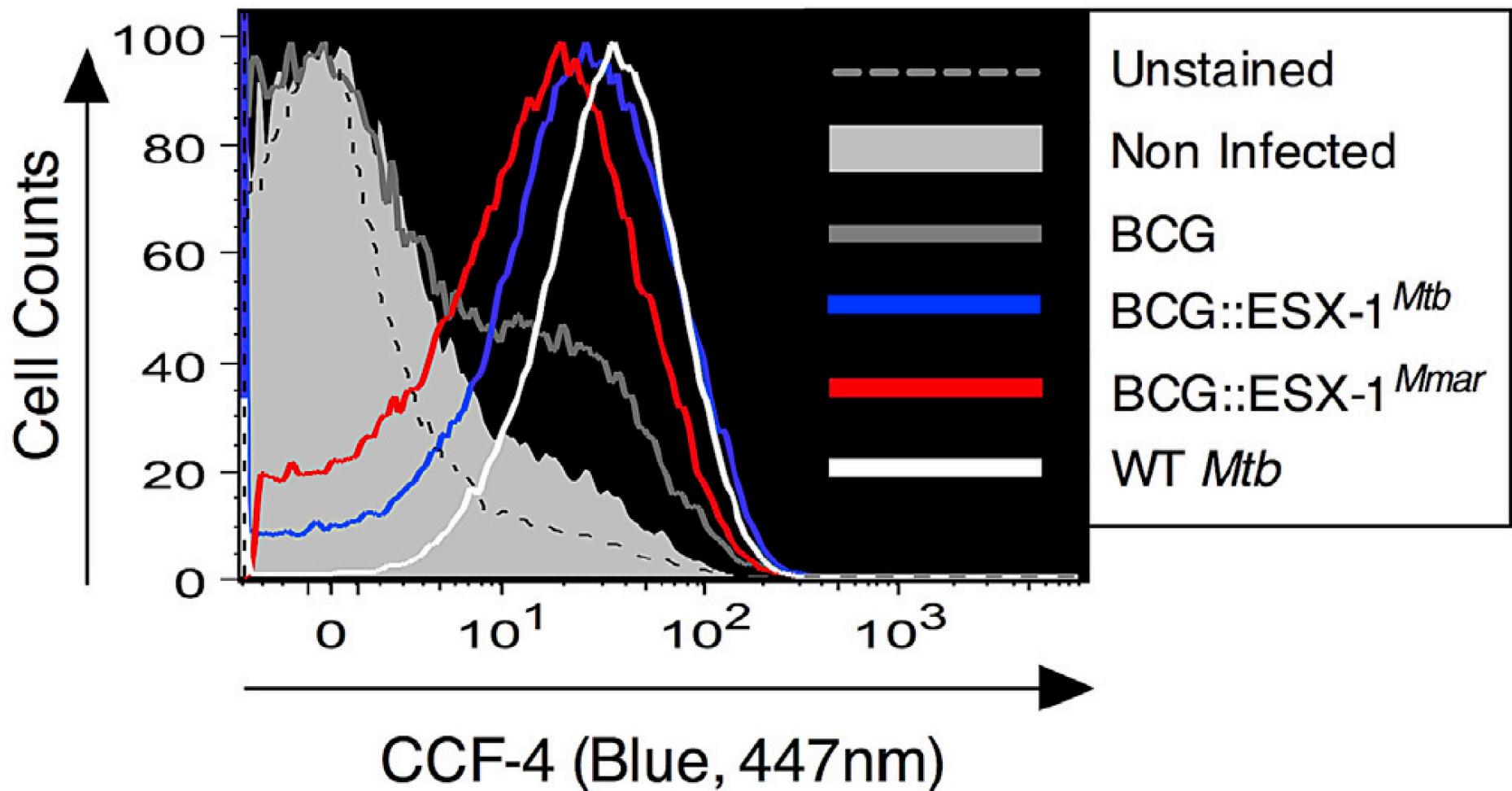
Recent study by team of Jun Liu (Toronto) shows extensive variation in time-to-death experiments ranging from ~ 45 to 120 d for BCG strains



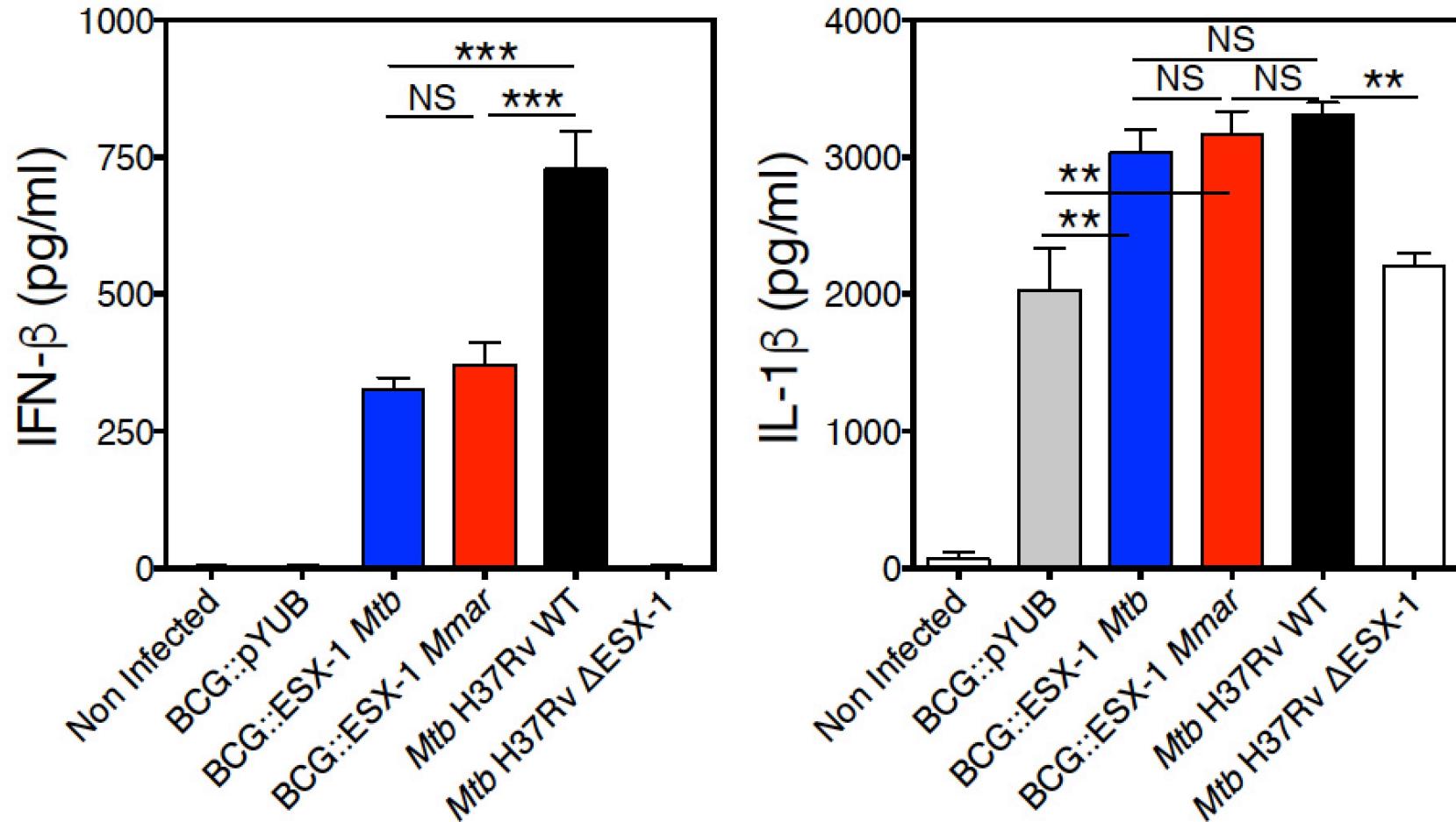
commented by Bottai & Brosch, Mol Ther, 2016

Zhang et al. Molecular Therapy, 2016

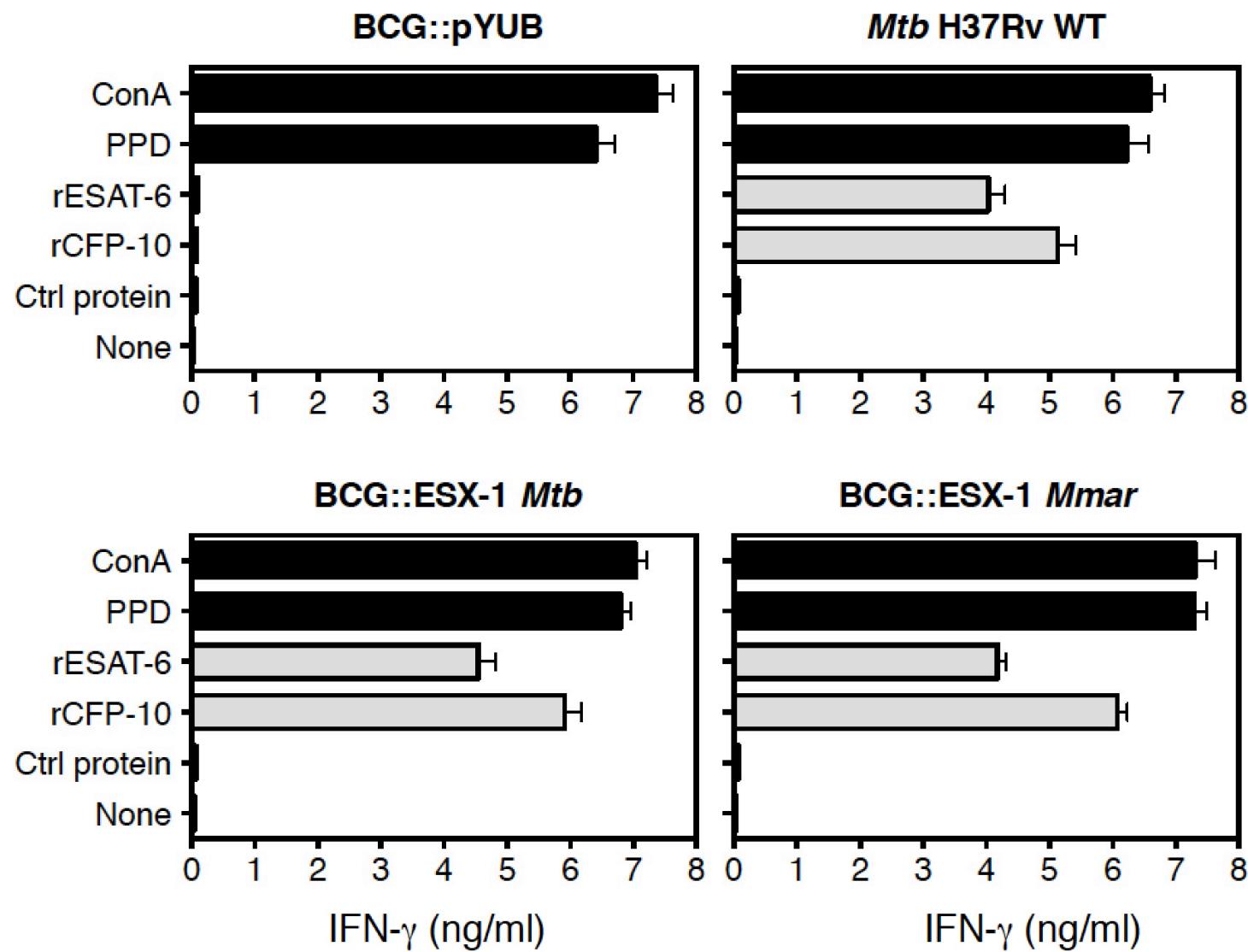
BCG::ESX-1-*marinum* induces phagosomal rupture similar to BCG::ESX-1-*mtb*



BCG::ESX-1-*marinum* induces IFN- β and IL1- β responses similar to BCG::ESX-1-*mtb*

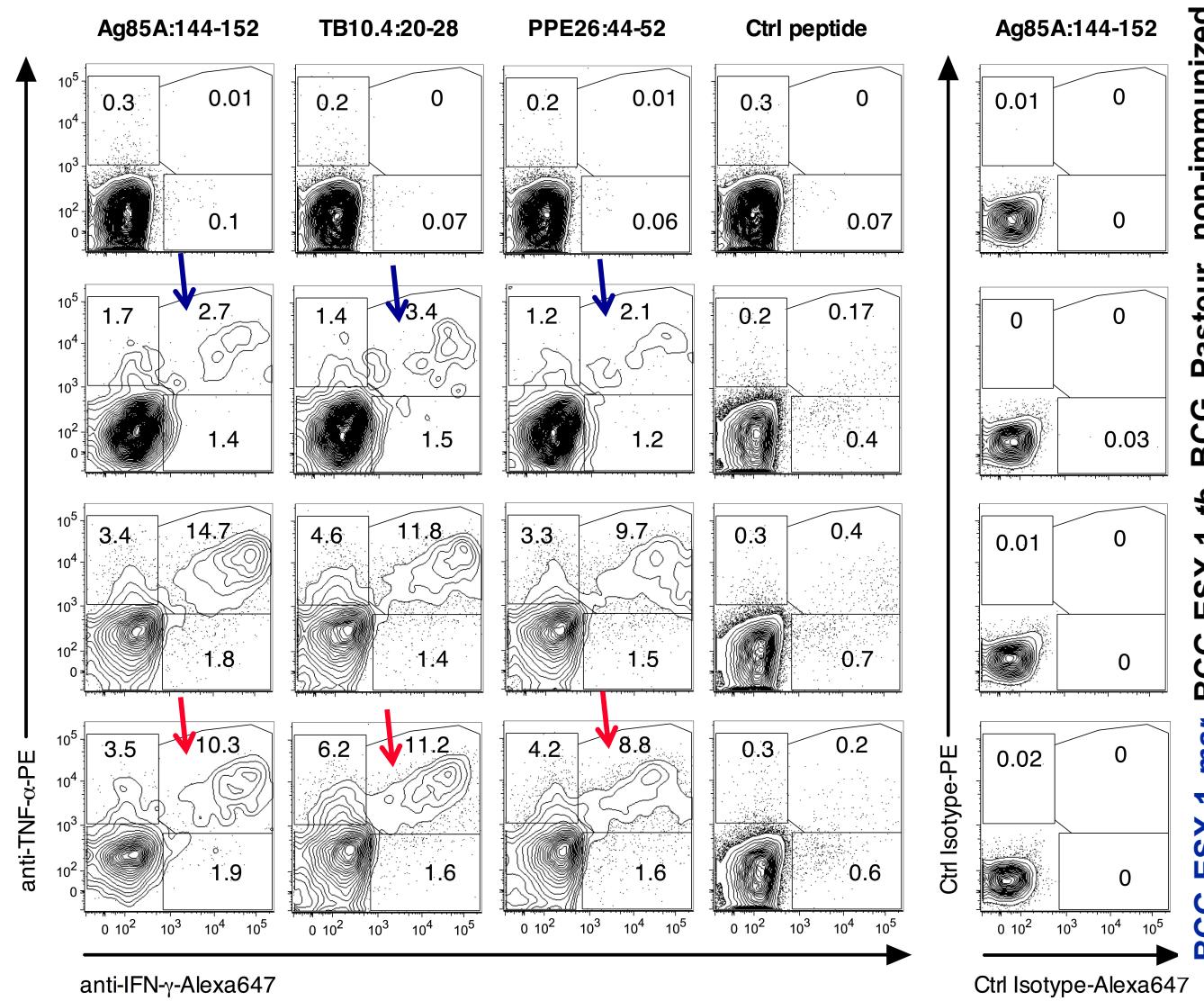


BCG::ESX-1-*marinum* induces Th1 T-cell responses against ESX-1 antigens



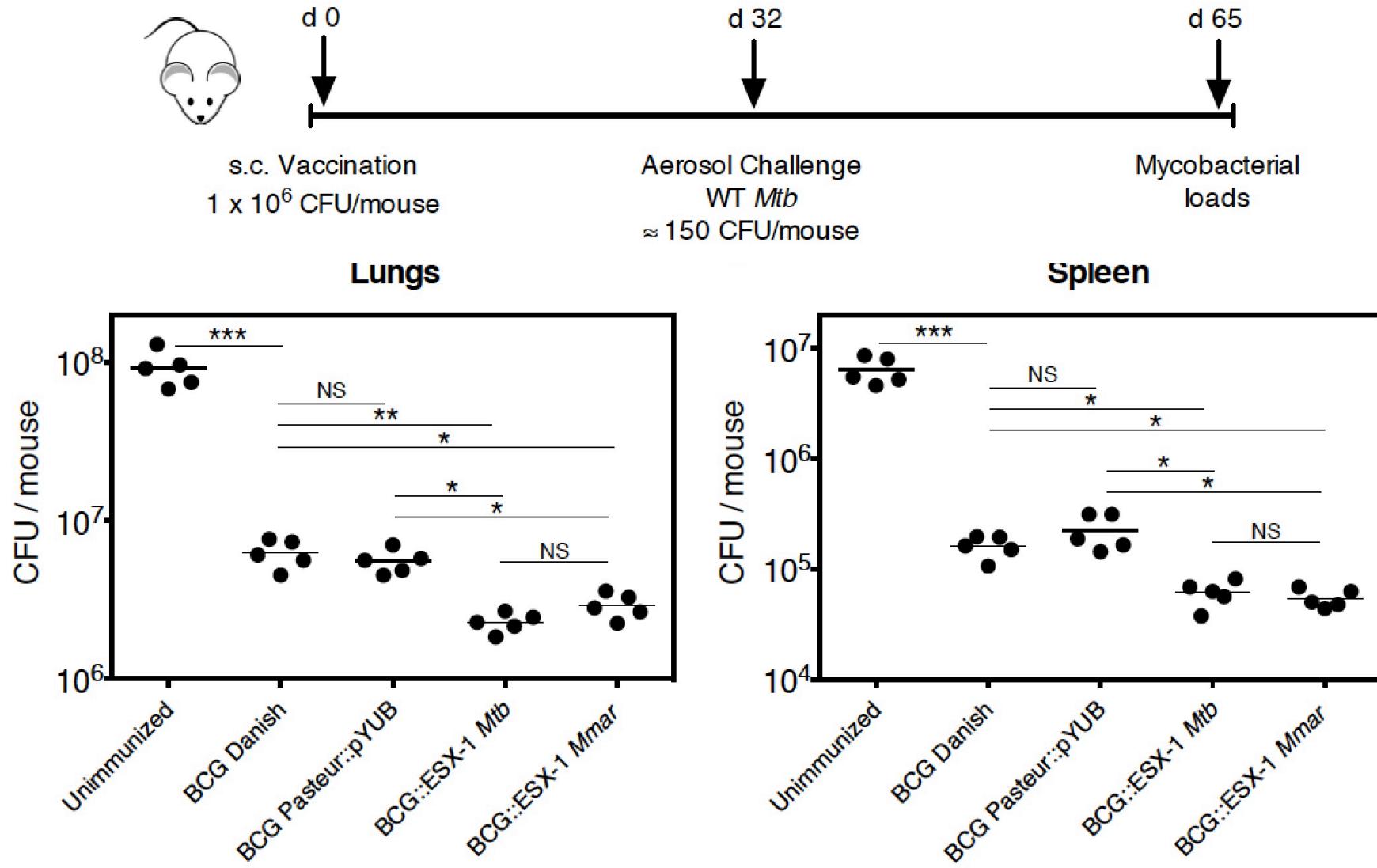
Expression of ESX-1 induces improved CD8⁺ T-Cell responses by vaccine candidates

Balb/C s.c. immunisation (10^6) → 4 weeks (3 mice per group) stimulation in vitro prior ICS



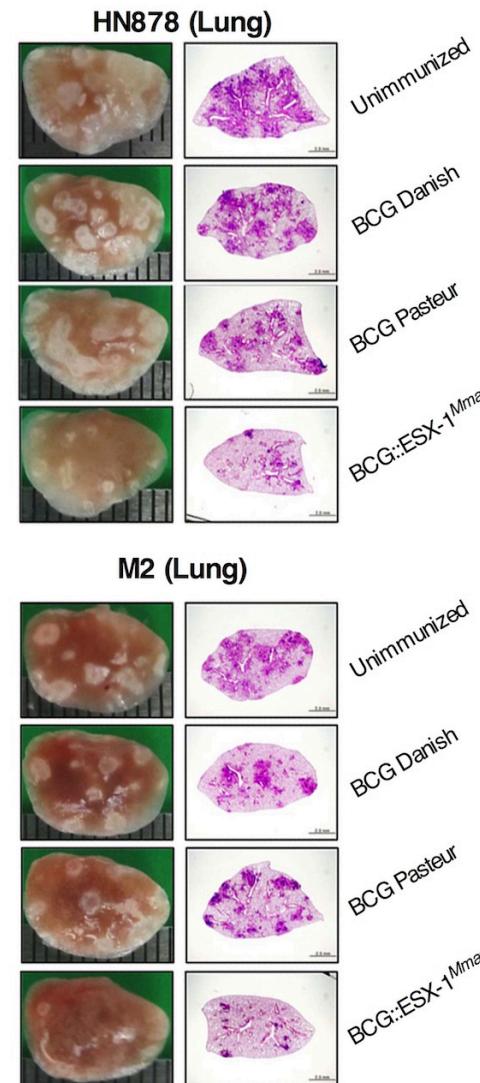
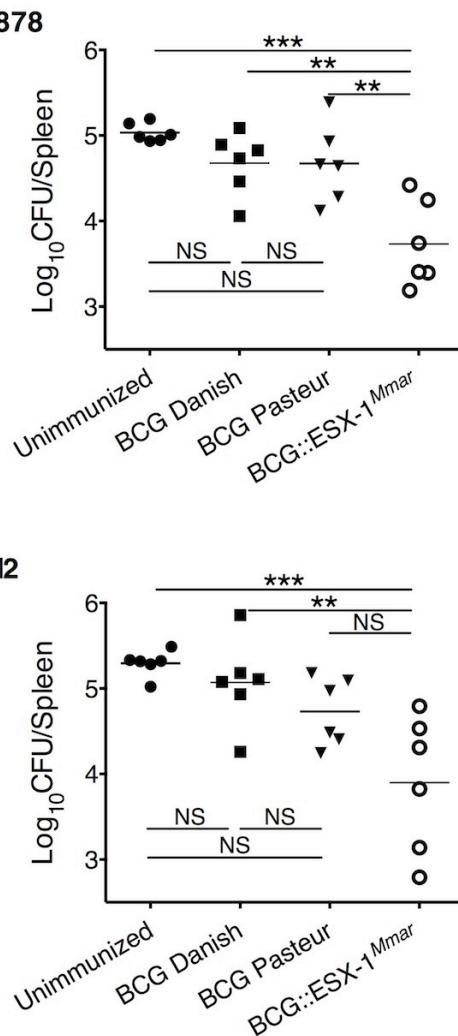
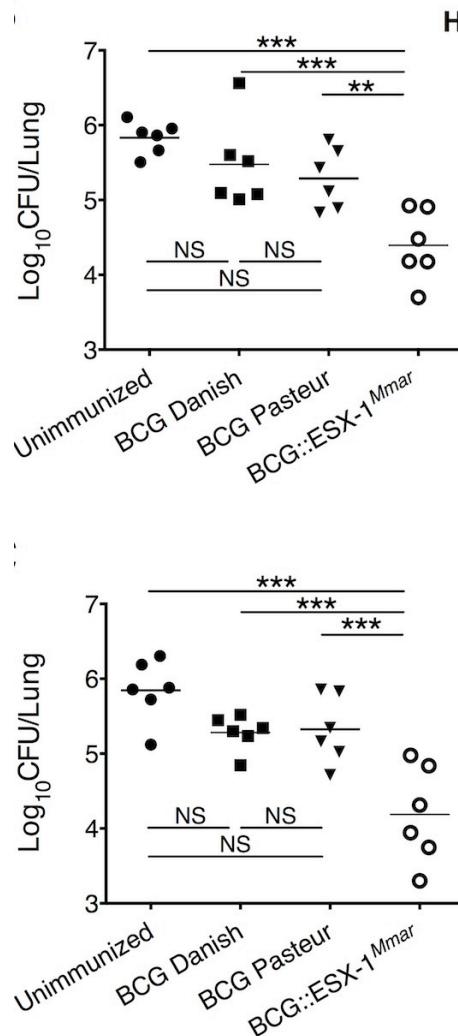
Gröschel et al, Cell Rep, 2017

BCG::ESX-1-marinum protects better than BCG against an *Mtb* challenge



Significantly improved protective efficacy for ESX-1 proficient vaccines in mice

Challenge *Mtb* HN878 & M2



Conclusion & perspectives I:

- Mycobacterial genomics prepared the way for post-genomic techniques
- Genomics has allowed to identify drug targets
- Comparative genomics allowed to identify gene clusters involved in virulence (RD1)
- Comparative genomics allowed to identify horizontal gene transfer of ESX-1 involved gene cluster (*espACD*)

Conclusion & perspectives II:

- ESX-1 mediated **cytosolic access** is a key event during mycobacterial infection
 - it changes numerous parameters concerning innate & adaptive immune responses
- Heterologously-expressed ESX-1 from *M. marinum* renders recombinant BCG more protective in mice
- Promising concept → worth to be considered for the development of next generation whole mycobacterial vaccines

The UPMI team



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MAJLESSI



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SIMEONE



Wafa
FRIGUI



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PAWLIK



Ludovic
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Roland
BROSCH



Fadel
SAYES



Mickael
ORGEUR



Jan
MADACKI



Florence
BROSSIER (50%)

Contrat d'interface pour
hospitaliers



Alexandre
GIRAUD GATINEAU



Nadine
HONORE



Alessandro
CASCIOPERO



Fabien
LE CHEVALIER



Eva
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Priscille BRODIN

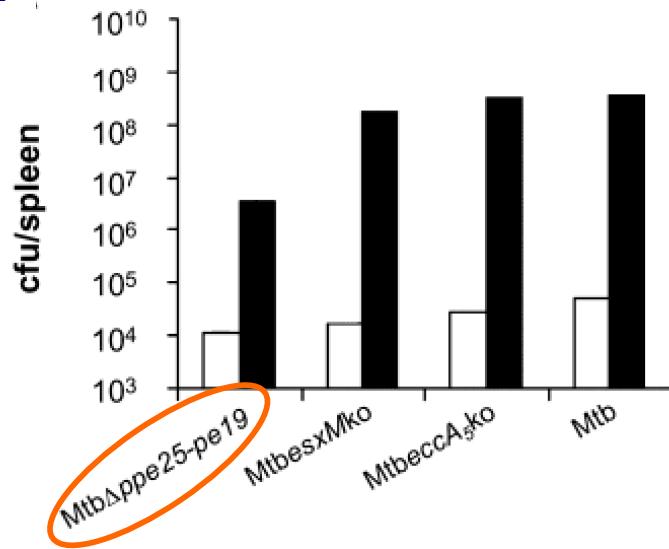
Philip SUPPLY

(Institut Pasteur Lille)

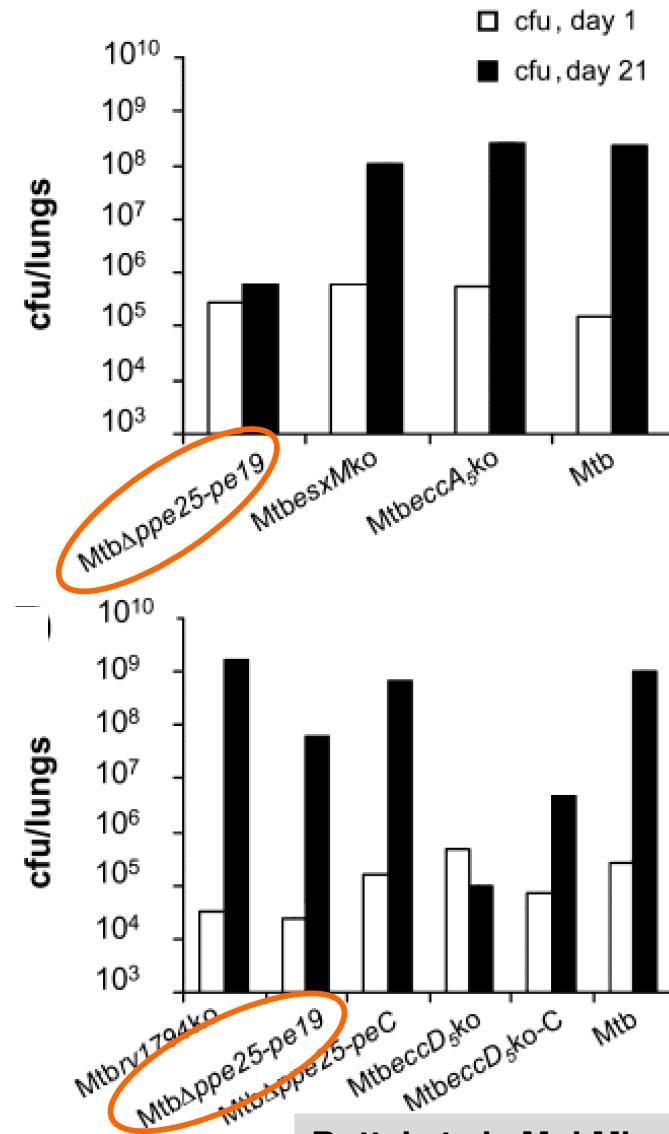
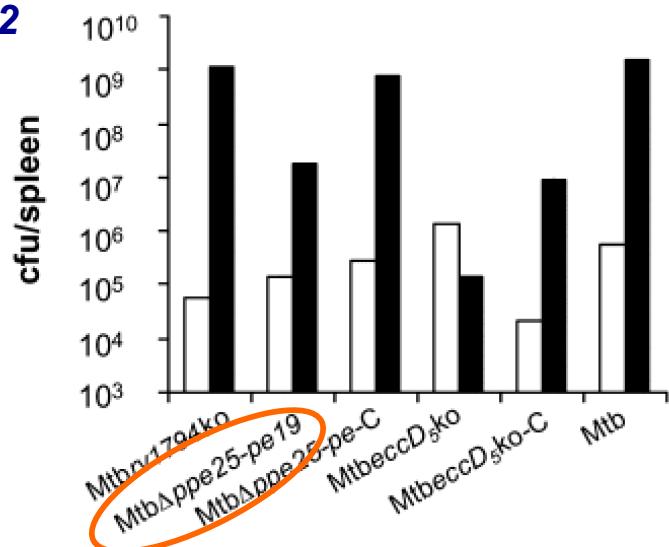


***Mtb* Δ ppe25-pe19 is attenuated in SCID mice**

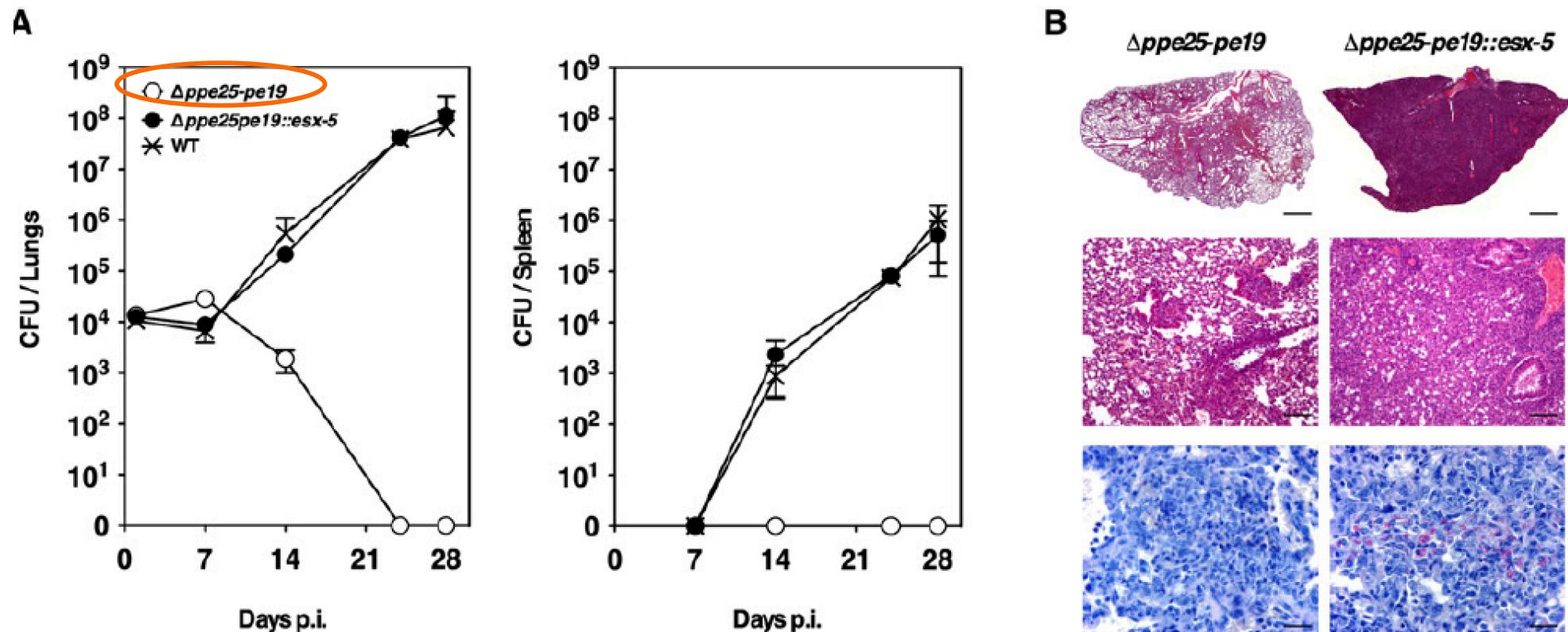
Experiment 1



Experiment 2

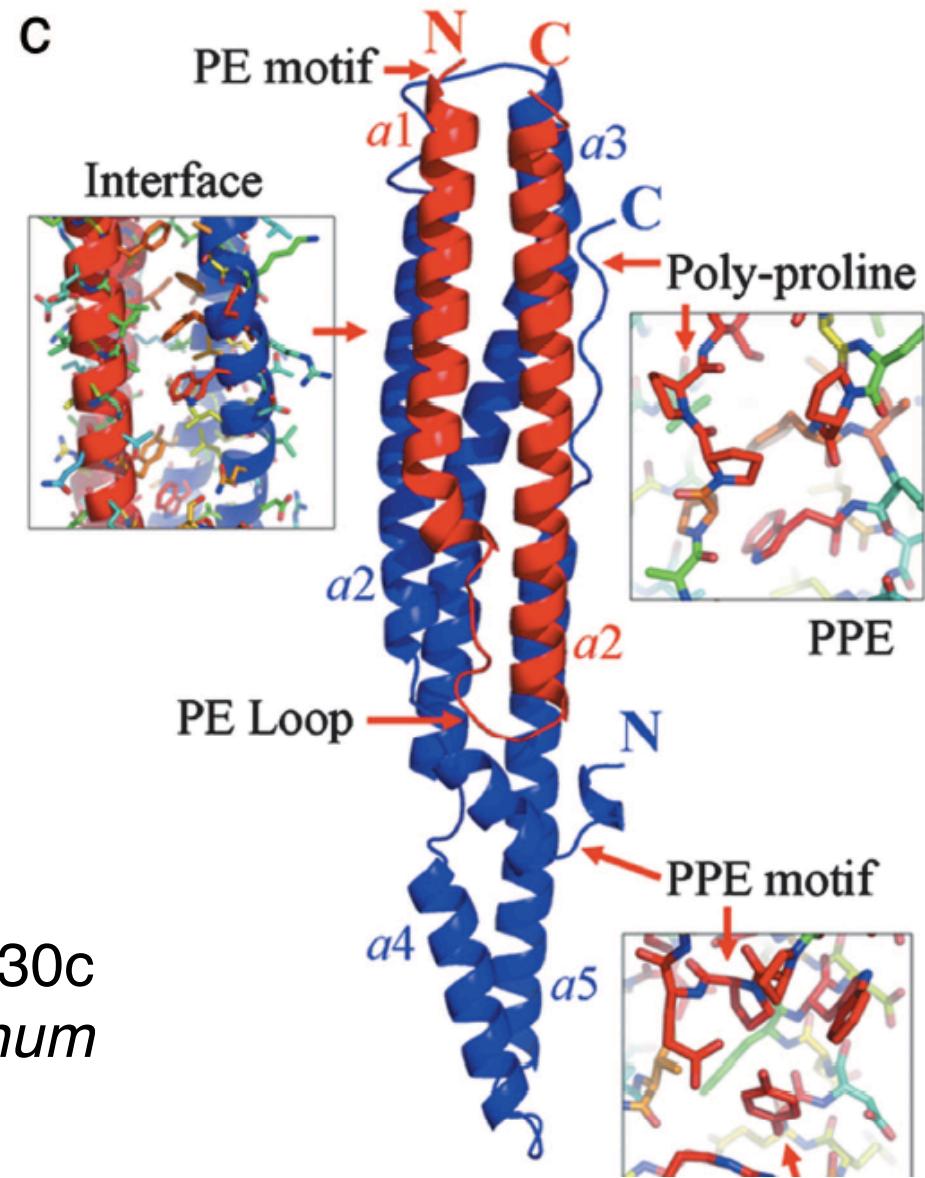


MtbΔppe25-pe19 is attenuated in immunocompetent C57BL/6 mice



PE25 and PPE41 proteins form four-helical bundle

PE25 (Rv2431c) and PPE41 Rv2430c
are secreted via ESX-5 in *M. marinum*



Abdallah et al., 2006, Mol. Microbiol.

Strong et al., 2006, PNAS:103: 8060-5