

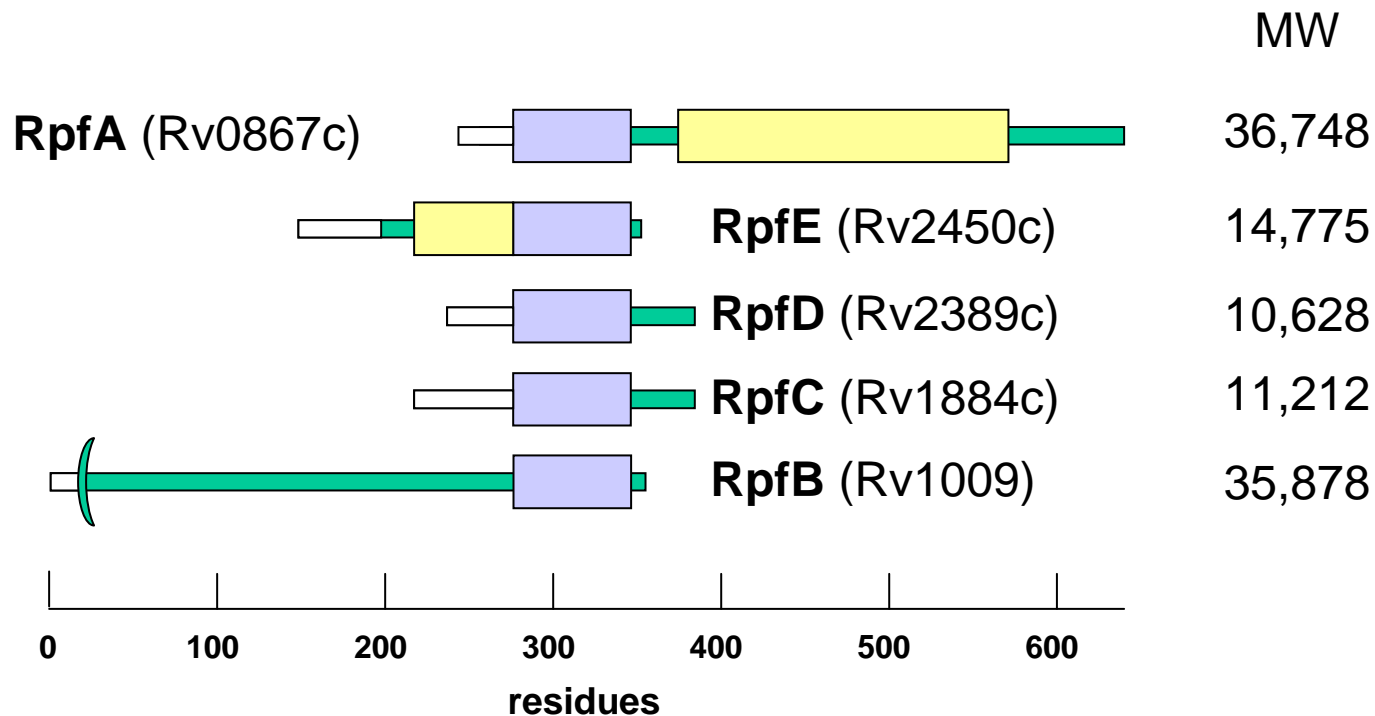
**Refined models for the studying  
vaccination against TB infection**

***Alexander S. Apt***

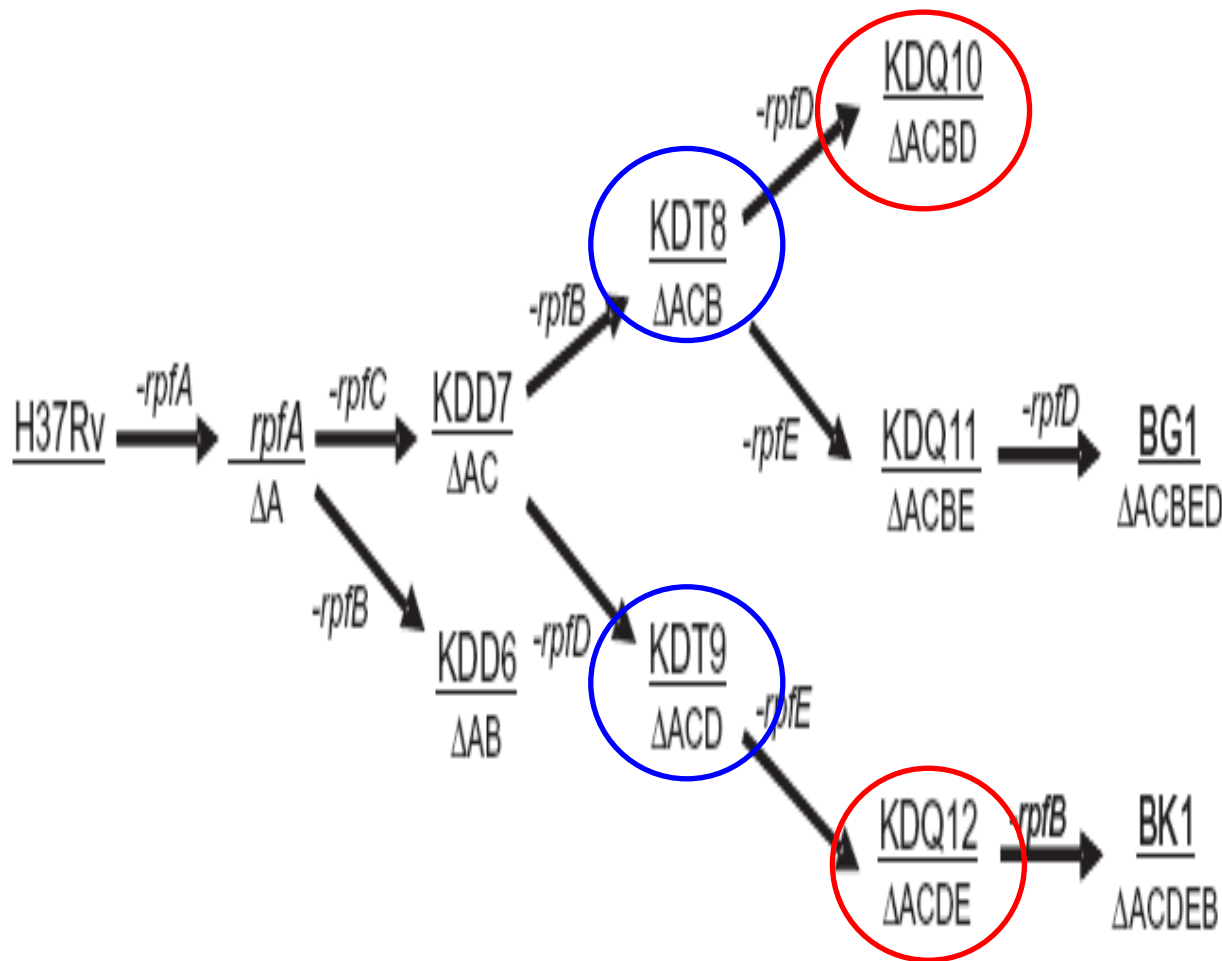
***Russian TB Cluster Coordinator***

**Central Institute for Tuberculosis, Moscow**

## The structure of Rpf-like proteins from *Mycobacterium tuberculosis*



- signal sequence
- Rpf-like segment
- proline / alanine-rich segment



**Fig. 1.** Stepwise deletion of *rpfA-E* genes in *M. tuberculosis* H37Rv. The arrows represent in-frame deletions introduced by allelic exchange mutagenesis to produce the strains whose names are underlined. For the sake of simplicity, the mutant strains are referred to throughout the text according to their abbreviated genotypes, which are given beneath the strain names and are named according to the order in which the *rpf*-like genes were deleted.

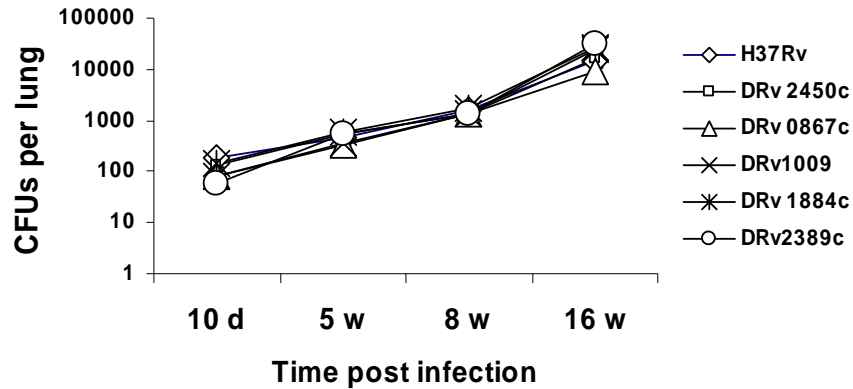
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**The resuscitation-promoting factors of *Mycobacterium tuberculosis* are required for virulence and resuscitation from dormancy but are collectively dispensable for growth *in vitro***

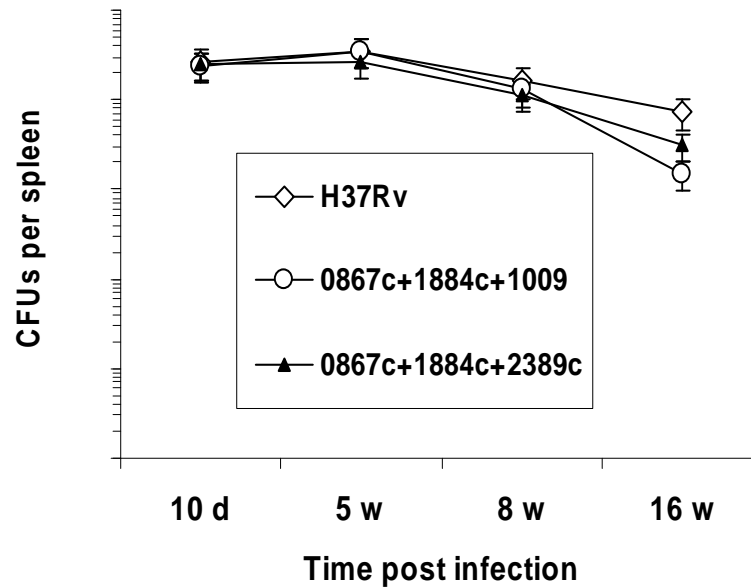
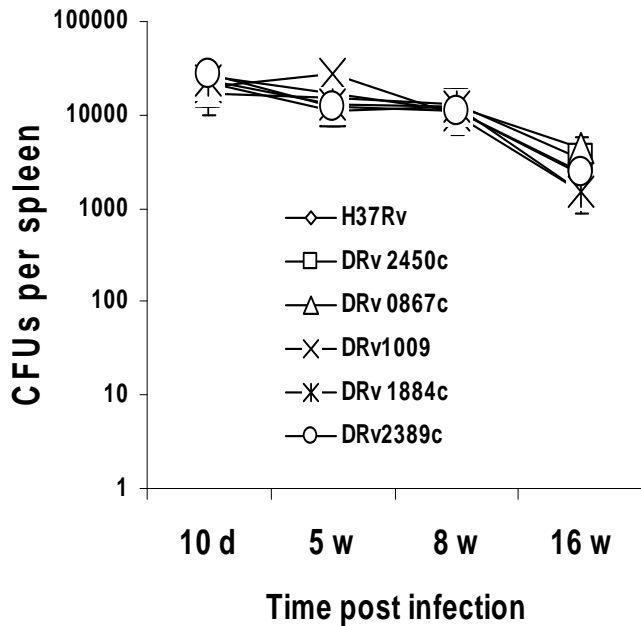
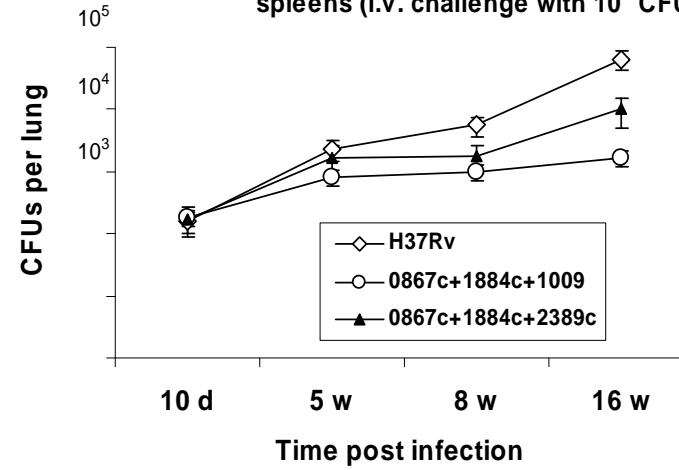
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## Attenuation of Rpf-KO strains: growth in B6 mice

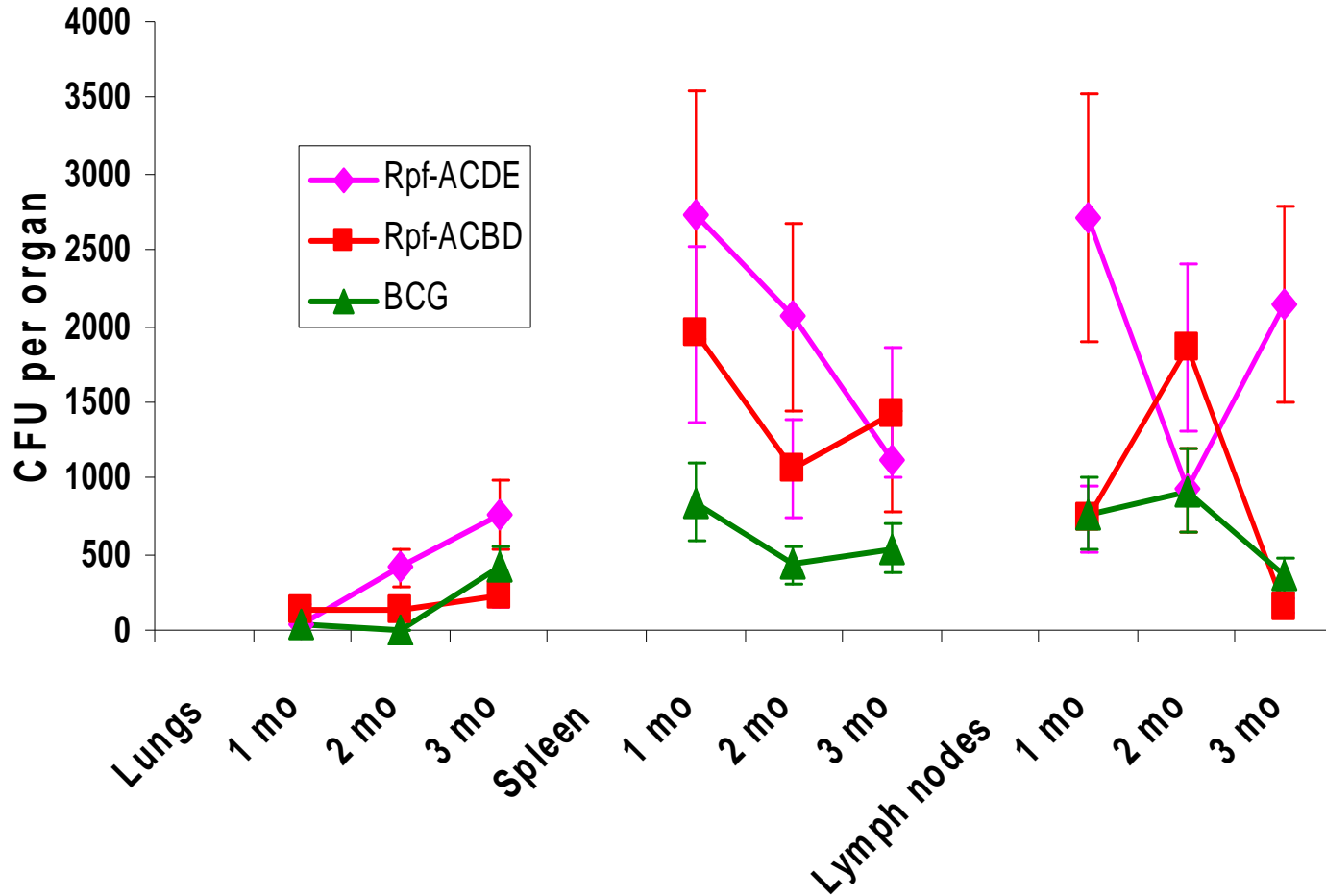
**Multiplication of single KO and wild-type strains in lungs and spleens (i.v. challenge with  $10^4$  CFUs)**



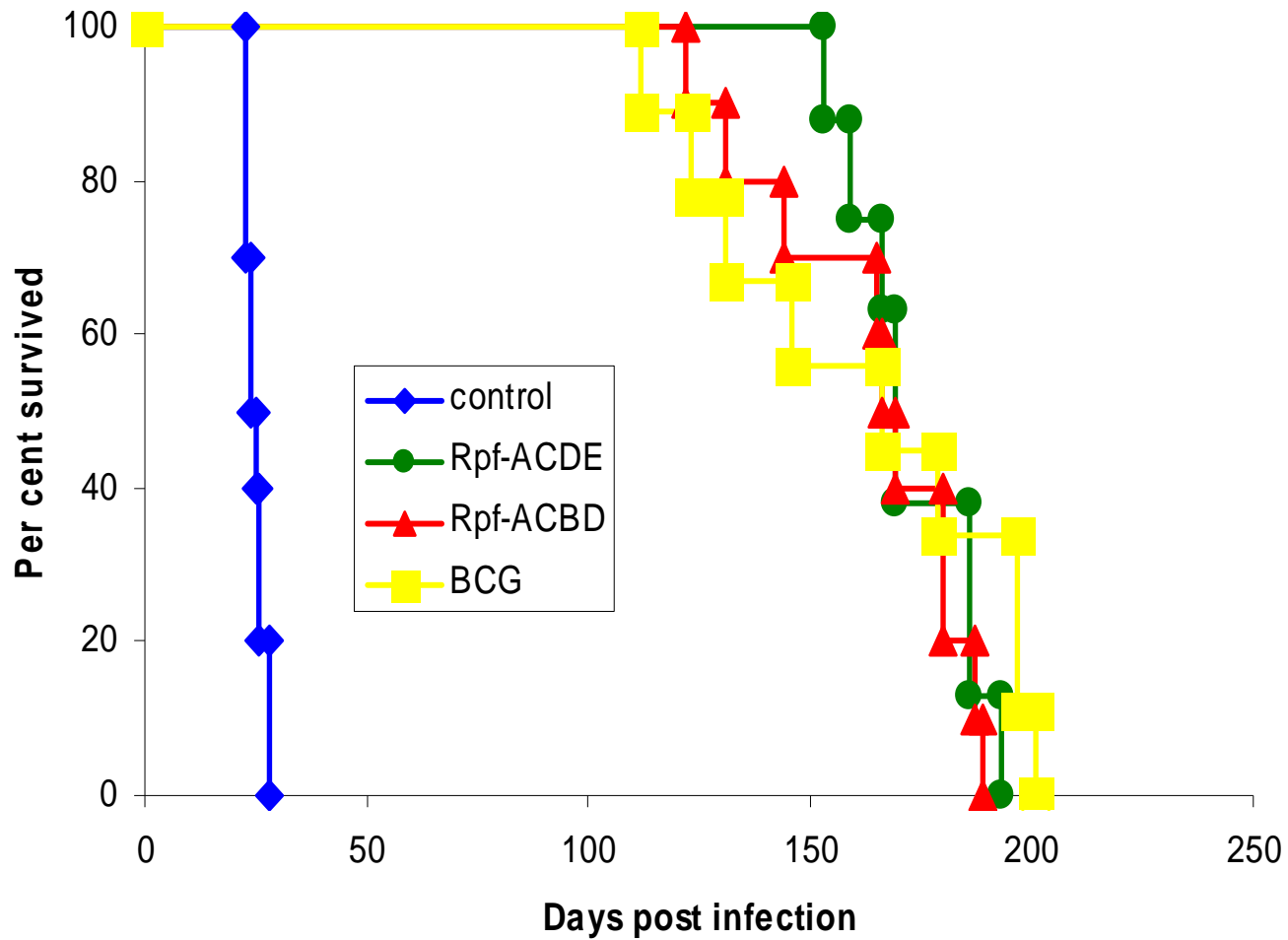
**Multiplication of triple KO (Rv 0867c+1884c+1009; Rv 0867c+1884c+2389c) and wild-type strains in lungs and spleens (i.v. challenge with  $10^4$  CFUs)**



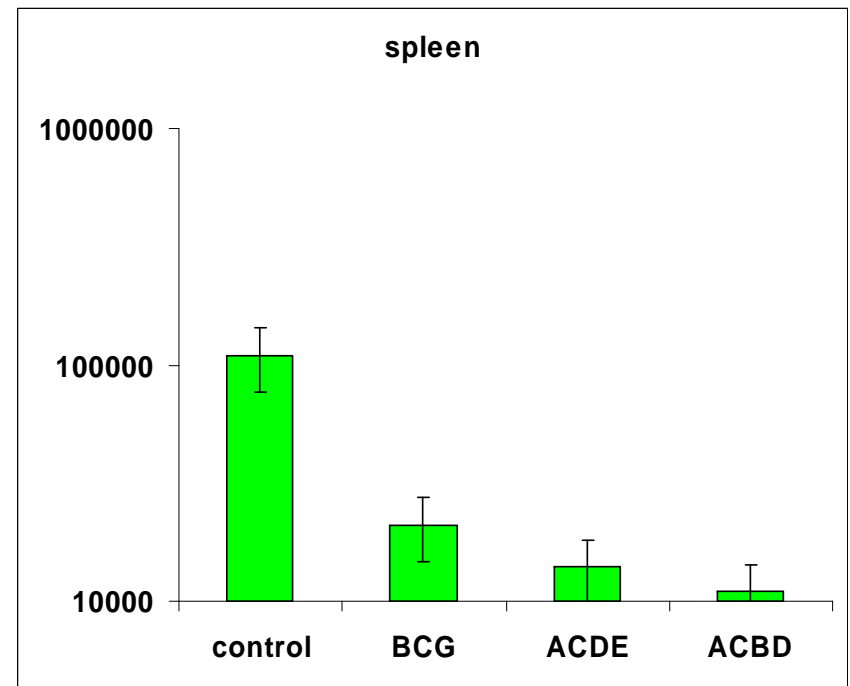
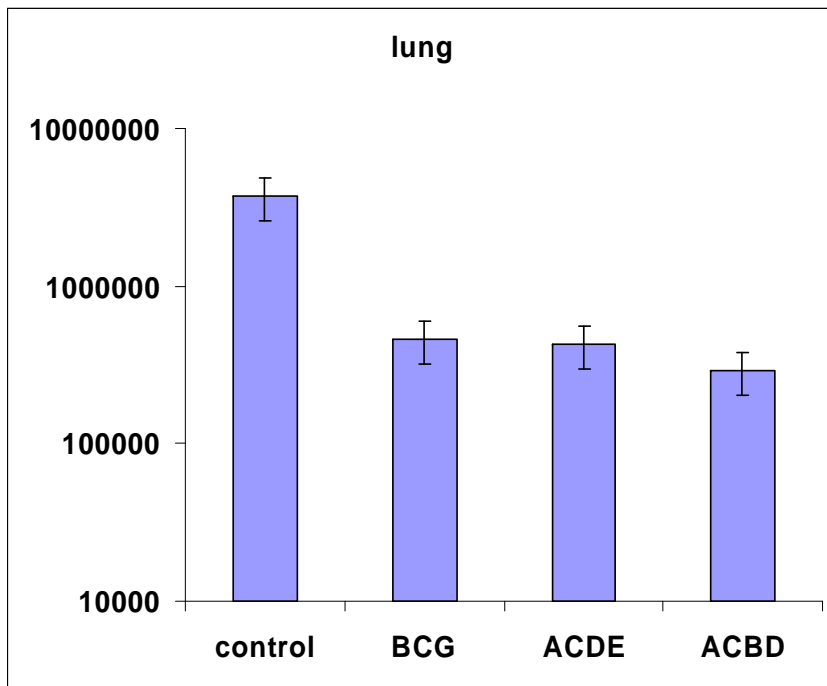
## Dissemination and persistence of vaccine strains following subcutaneous vaccination ( $10^7$ CFU/mouse)



## Survival curves of vaccinated and control mice



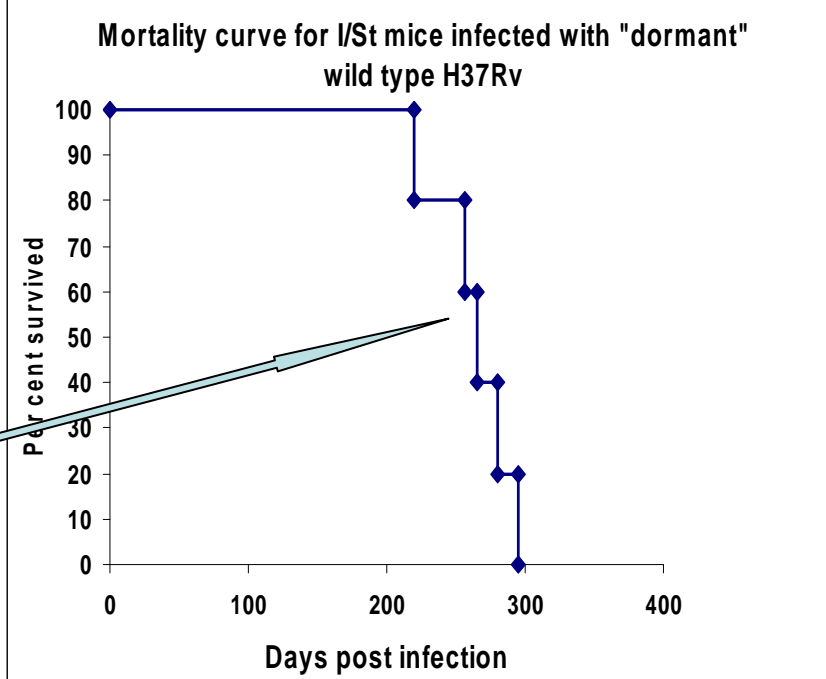
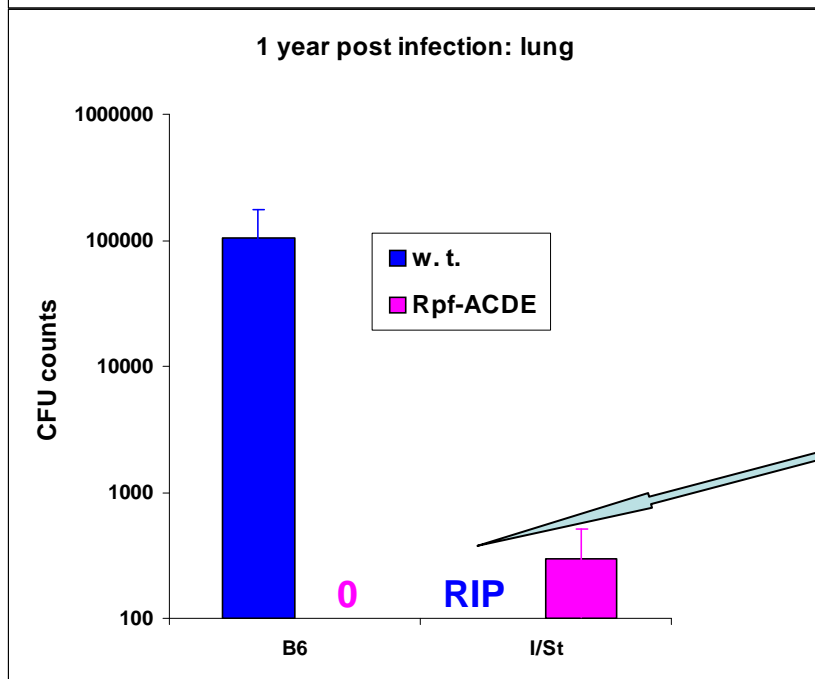
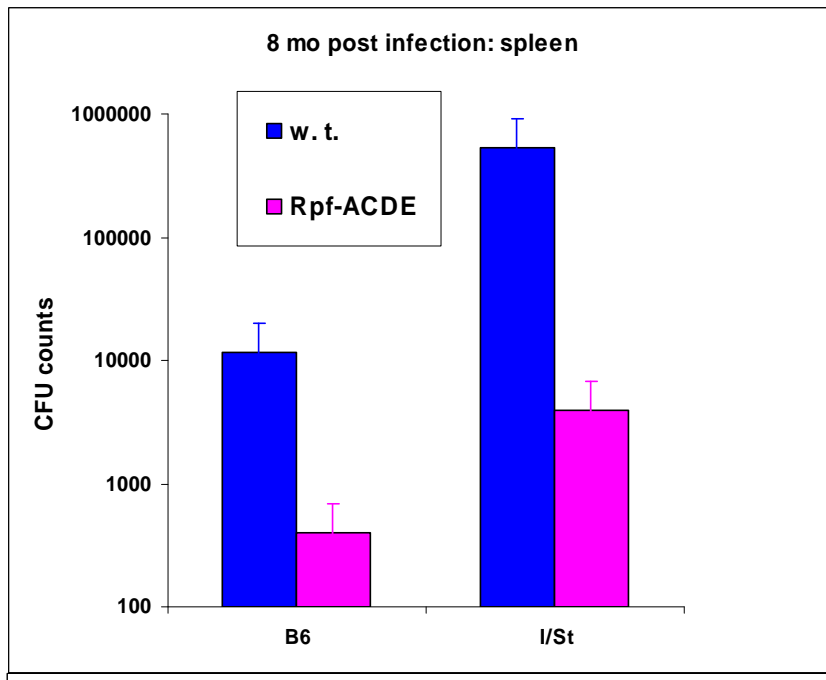
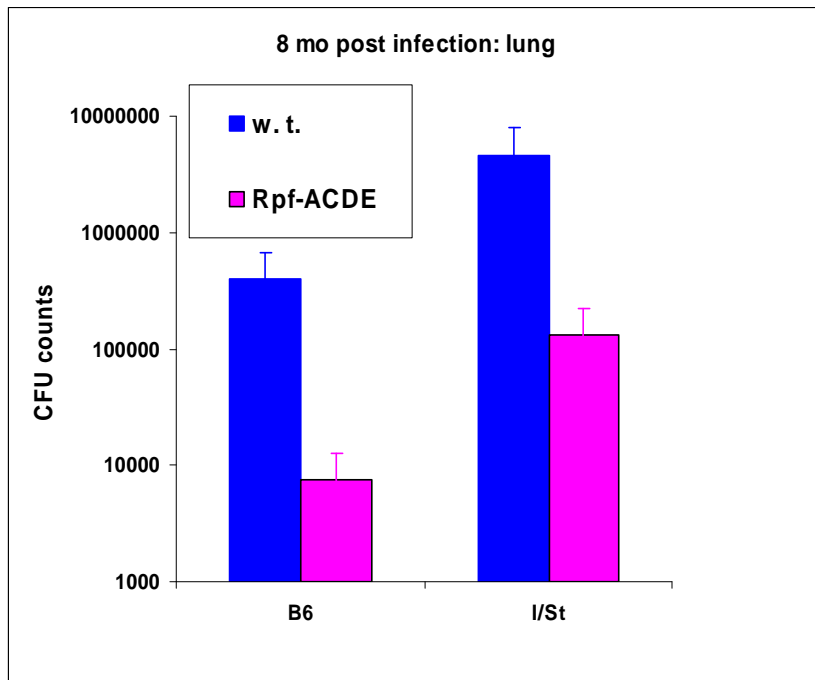
# CFU counts in organs of vaccinated and control mice at week 5 post-challenge



# Interplay between mycobacterial and host genetics: resuscitation of growth in genetically susceptible and resistant mice

## Model:

- *M. tuberculosis* H37Rv w. t. and Rpf-ACDE-KO mutant
- Anaerobic liquid cultures, rotation at 37°C for 140 days
- No colonies on agar formed by either strain
- Centrifugation and washing with saline
- Intratracheal injection in genetically susceptible I/St and resistant B6 mice,  $5 \times 10^3$  cells per mouse (concentration estimated by direct microscopy)
- Monitoring of CFU counts in organs and survival



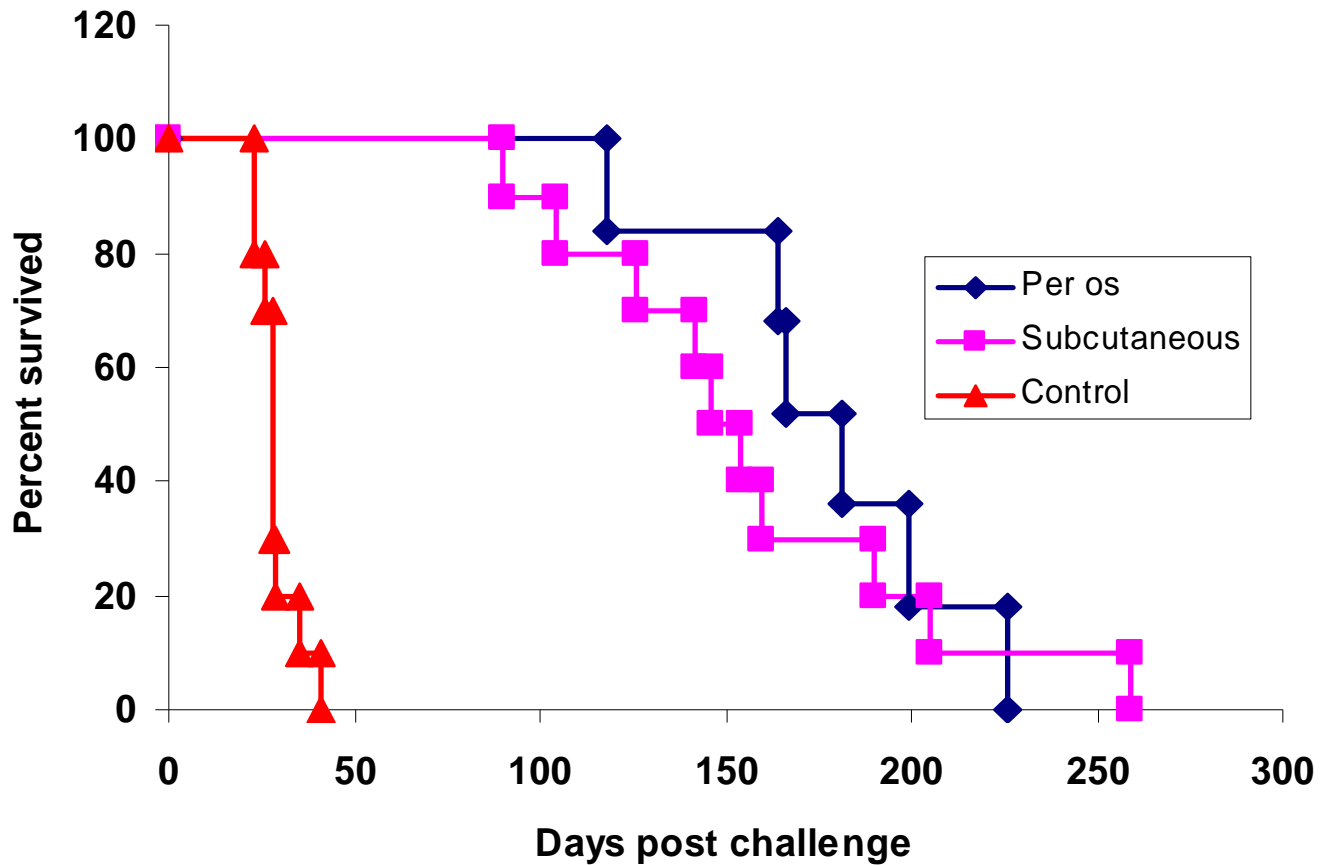
# Conclusions and suggestions

- Multiple Rpf-KO mutants are reasonable live vaccine candidates, with Rpf-ACBD – KO strain providing somewhat better protection than ACDE.
- Potential advantage of RPF-KO-based vaccines over BCG is that priming with the former allows real boosting with products encoded by genes located in RD1. BCG can not initiate primary responses against these important molecules.
- The degree of attenuation (safety) of live vaccine candidates should be tested in animals genetically susceptible to TB.
- Partial redundancy of Rpf products suggests that introduction of additional mutation (s) diminishing virulence via different mechanisms into potential vaccine candidate (e.g., ACBD) may be considered.
- Once created, such novel strains should be tested with respect to its safety and vaccination capacity.
- **The whole issue looks like a good ground for collaboration**

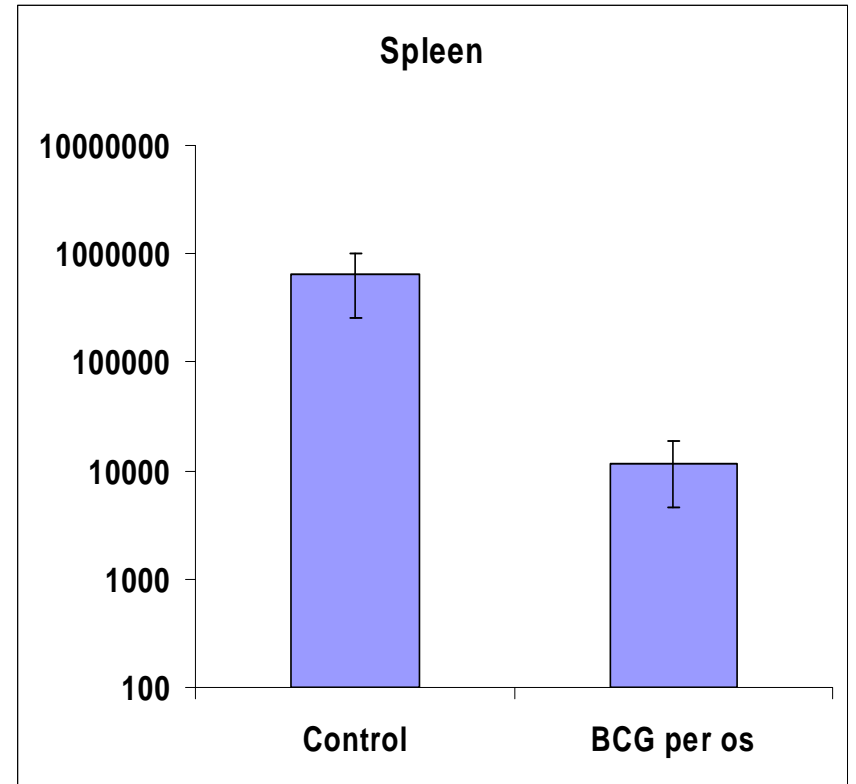
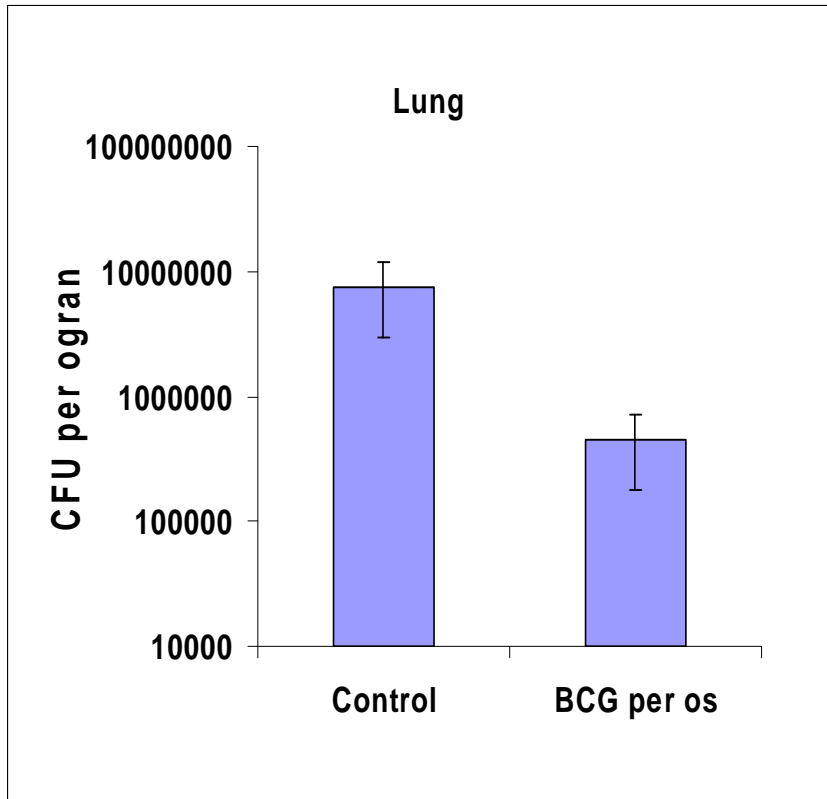
**BCG vaccination *per os***  
**(extractions from ISTC**  
**project #3257)**

# BCG vaccination *per os* is effective when applied to newborns

Survival time post vaccination of 8-days-old mice with  $2.5 \times 10^4$  BCG



# Protection by CFU counts



# Oral BCG vaccination does not work in adult mice

