

## Supplementary material for

### Successful production of antibodies against extra cytoplasmic loops of the *Mycobacterium tuberculosis* ABC transporter Rv1819c

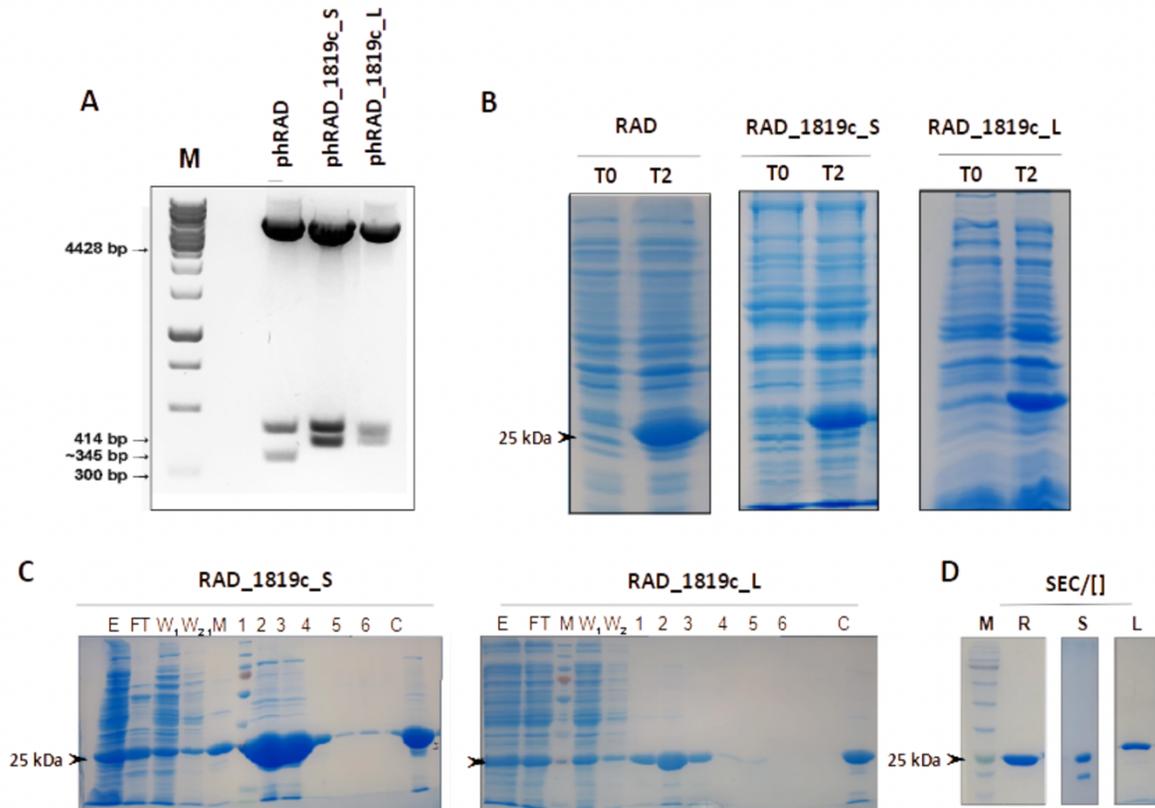
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#### Supplementary Table

**Table S1. Oligonucleotides designed for cloning the selected peptide sequences into the ABC transporters used in this work.**

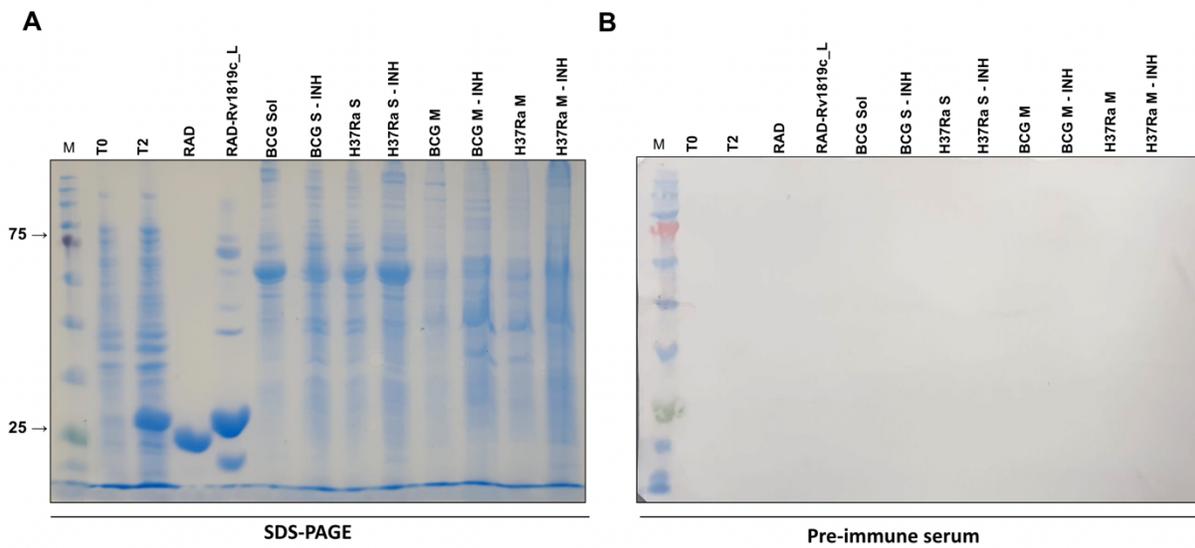
Oligo nucleotides	Sequences (5'-3')
Rv1819_2C F	GGCGGCGGGCTTAAGGGTATTGCAAGTGGTGATGGTAC
Rv1819_2C R	CGTTCACCACTACCATGGCACTTTGAATTCCTCCGCC
Rv1819_4 F	GGCGGCGGGCTTAAGCGTCTGTTTGCAGGTCAAATTGATTTTGG
Rv1819_4 R	CGTCCAGTTTAACTAAAACCACTACACCCAGAATTCCTCCGCC
Rv1819_2L F1	GGCGGCGGGCTTAAGCAAAAAGCATTGAAGGTATTGCAAGTGGTGATGGTACCG
Rv1819_2L F2	TGAAACGTAGTGGTGTGCGT
Rv1819_2L R1	CCGCCTCCCTTAAGACGCACCACTACGTTTCACGGTACCATCACCCTTGCAA
Rv1819_2L R2	TACCTTCAAATGCTTTTT

## Supplementary Figures

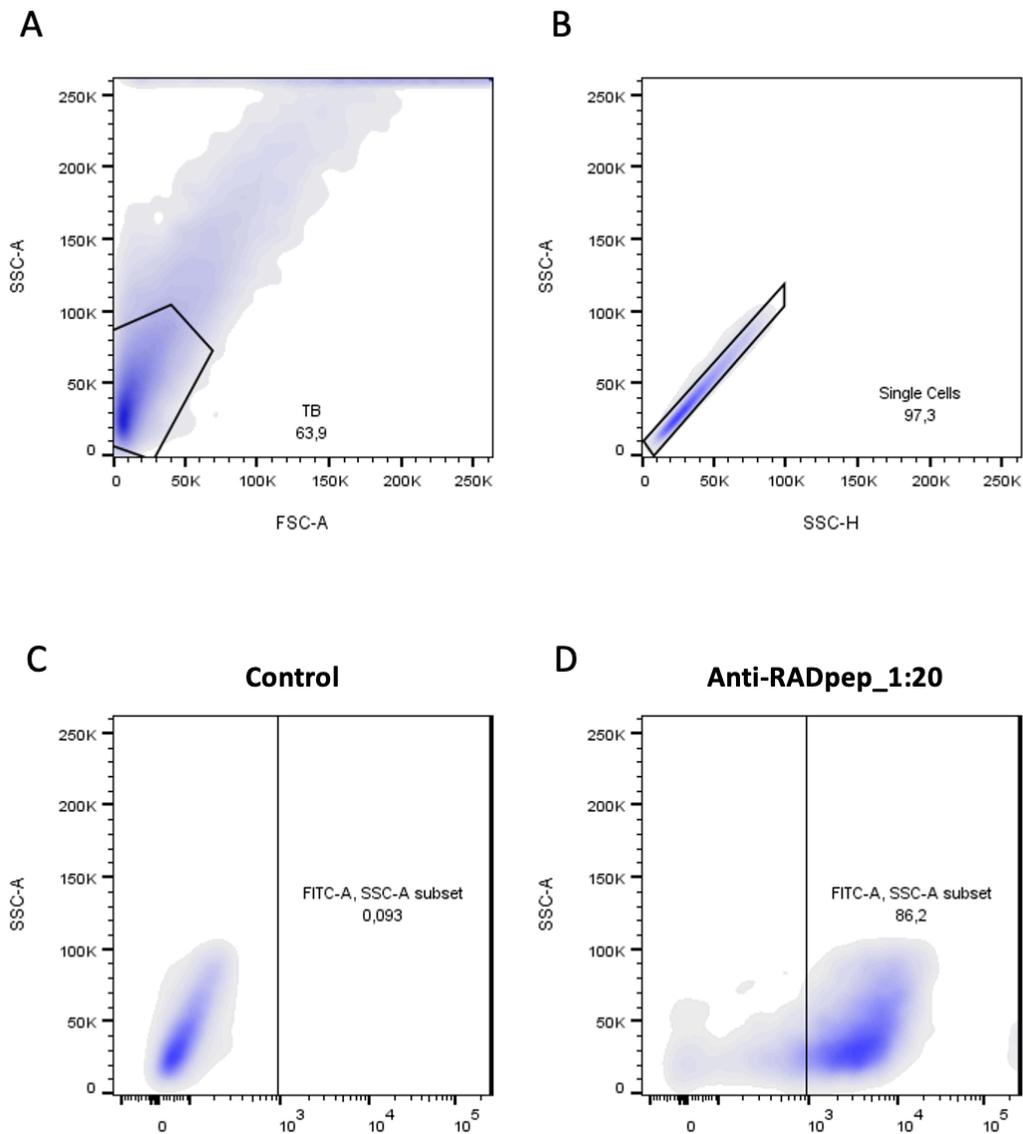


**Figure S1. Production of the recombinant proteins.** **A** Restriction analysis of the phRAD constructs. Electrophoresis in 1.5% agarose gel. Digestion of the vectors with *Hind*III generates two small fragments of 414 and 300 bp in the phRAD. In the presence of the inserts the 300 bp fragment is shifted for a higher molecular mass. **B** Expression assays of the proteins RAD, RAD\_1819c\_S and RAD\_1819c\_L. The cellular extracts before (T0) and after (T2) induction of *E. coli* BL21(DE3)/pUBS520 transformed with the corresponding vectors of interest are shown in the 12% SDS-PAGE. Induction of the cells was carried out for 3 h at 37°C after induction with 0.4 mM IPTG showing the uninduced (T0) and induced (T2) fractions for each protein. **C** Ni-IMAC purification of RAD\_1819c\_S and RAD\_1819c\_L in 50 mM Tris HCl and 150 mM NaCl. M: molecular mass marker, E: total extract after lysis, F: Soluble fraction after centrifugation, FT: Soluble fraction after centrifugation. **D** SEC analysis of purified proteins. M: molecular mass marker, R: Recombinant protein, S: Soluble fraction, L: Lysate.

Flowthrough, W<sub>1</sub> and W<sub>2</sub>: Washes 1 and 2, respectively with 10 mM and 20 mM imidazole, 1-6: eluted fractions with 400 mM Imidazole. **D** Proteins purified by SEC on a HiLoad 16/60 Superdex 200 column. R: RAD, S: RAD\_1819c\_S and L: RAD\_1819c\_L.

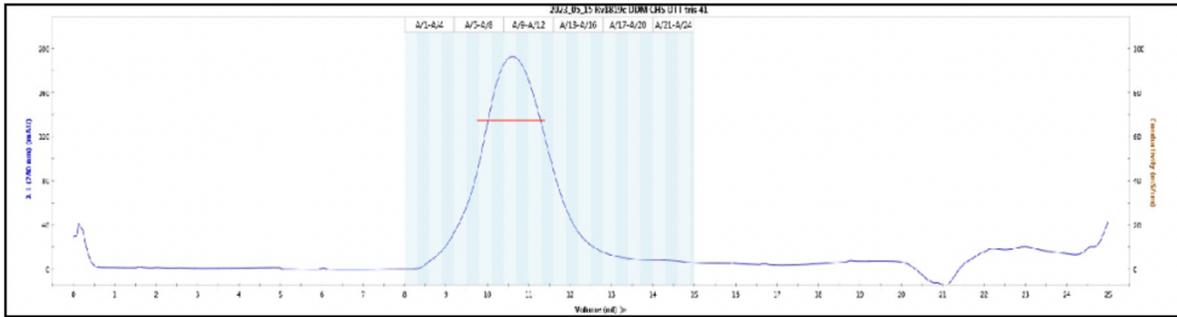


**Figure S2. Western blotting for detection of the Rv1819c transporter in extracts of *M. bovis* BCG Moreau and *M. tuberculosis* H37Ra by  $\alpha$ -RAD\_Rv1819c\_S and  $\alpha$ -RAD\_Rv1819c\_L. A** 12% SDS-PAGE (mirror) containing 35  $\mu$ g/well of the cellular protein extracts and purified proteins. **B** Western blotting of the samples with the pre-immune serum.

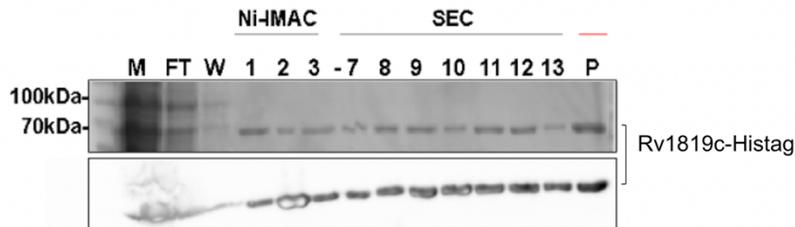


**Figure S3. Flow cytometry scatterplots showing a representative gating strategy. A** *Mycobacterium tuberculosis* H37Rv cell population; **B** gating on single cells using area and height; **C** and **D** gating FITC positive cells subset.

**A**



**B**



**Figure S4. Purification of the ABC Transporter Rv1819c.** **A** Chromatogram of SEC purification of the Rv1819c-His8 construct, showing the eluted fractions of the protein. **B** 12% polyacrylamide gel and Western blotting of samples obtained during Ni-IMAC and SEC. M: Membrane fraction; FT: Flow through; W: Wash; 1-3: Ni-IMAC Elutions; 7-13: SEC Elutions; P: Pool of elutions 8-11 used in subsequent assays.