SUPPLEMENTAL MATERIAL

Differential detergent extraction of *Mycobacterium marinum* cell envelope proteins identifies an extensively modified threonine-rich outer membrane protein with channel activity

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Table S1: primers used in this study

Primer name	Sequence	Restriction site	Resulting plasmid
FtsQ-SpeI-F	cccactagtgtgaccgaagctagcgacg	*SpeI*	pSMT3-FtsQ-HA
FtsQ-BamHI-HA-R	cccggatcctcacgcgtagtccggcacgtcgtacgggtacttcacggtcggcagatcg	*BamHI*	pSMT3-FtsQ-HA
MctB-NheI-F	cccgctagcatgatctcgttacgccaaca	*NheI*	pSMT3-MctB-HA
MctB-BamHI-HA-R	cccggatcctcacgcgtagtccggcacgtcgtacgggtactgagcgaccgtgaccga	*BamHI*	pSMT3-MctB-HA
OmpAMm-NheI-F	cccgctagcgtgggtaccgacgcggg	*NheI*	pSMT3-OmpA-HA
OmpAMm-BamHI-HA-R	cccggatcctcacgcgtagtccggcacgtcgtacgggtagctgaccacaatttcgacac	*BamHI*	pSMT3-OmpA-HA
dppA-NheI-F	cccgctagcatgcggcggatgcgggcc	*NheI*	pSMT3-DppA-HA
dppA-HA-R	ccctcacgcgtagtccggcacgtcgtacgggtaggccttgacgaggttctcg	blunt	pSMT3-DppA-HA
Mm0617-SpeI-F	cccactagtatgagcgagtcgctcggg	*SpeI*	pSMT3-Mm0617-HA pSMT3-Mm0617-His pSMT3-Mm0617ΔC-His
Mm0617-BamHI-HA-R	cccggatcctcacgcgtagtccggcacgtcgtacgggtaggcgcggccgtcgtcgt	*BamHI*	pSMT3-Mm0617-HA
Mm4057-NheI-F	cccgctagcgtgagtgattccacgactct	*NheI*	pSMT3-Mm4057-HA
Mm4057-BglII-HA-R	cccagatcttcacgcgtagtccggcacgtcgtacgggtacggcacgaactccacattg	*BglII*	pSMT3-Mm4057-HA
Mm4366-NheI-F	cccgctagcgtgactggtgagccgcag	*NheI*	pSMT3-Mm4366-HA
Mm4366-BamHI-HA-R	cccggatcctcacgcgtagtccggcacgtcgtacgggtaattcttggtcggcagcggc	*BamHI*	pSMT3-Mm4366-HA
Mm5387-SpeI-F	cccactagtatggaactgatgttggacgc	*SpeI*	pSMT3-Mm5387-HA
Mm5387-BamHI-HA-R	cccggatcctcacgcgtagtccggcacgtcgtacgggtagcctcccggtgcgggttc	*BamHI*	pSMT3-Mm5387-HA
Mm0617-BamHI-His6-R	cccggatcctcagtgatggtgatggtgatgggcccctggcgcctcgg	*BamHI*	pSMT3-Mm0617-His
Mm0617ΔC-BamHI-His6-R	cccggatcctcagtgatggtgatggtgatgtatcacggtcacggtgggg	*BamHI*	pSMT3-Mm0617ΔC-His

Table S2: Proteins identified by nanoLC-MS/MS analysis (supplied as Microsoft Excel-file)
Spectral counts of cell envelope-derived detergent pellet (DP) or detergent supernatant (DS) of three biological replicates (A, B, C). Each identified protein (1433 in total) is annotated and functionally classified using MarinoList. For each protein the number of predicted transmembrane helices (TM), Isoelectric point (pI) and the presence (Y) or absence (N) of a signal peptide (SigP) is described. Proteins are ordered according to the number of spectral counts detected in the detergent solubilized fraction compared to the total number of counts (% extr 3 rep). In an additional sheet, the spectral counts of the proteins are normalized and their fold change (Fc) between detergent pellet and supernatant is calculated with p-values.

Table S3: Shortlist of putative MOMPs (supplied as Microsoft Excel-file). See the main text for the selection criteria**.**

Figure S1. Detergent solubilisation of cell envelopes of *M. bovis* BCG and that of *M. marinum* used for nanoLC-MS/MS analysis and supervised clustering. (A) Immunoblots with equal amounts of pellet (DP) and supernatant (DS) fractions of *M. bovis* BCG cell envelope (CE) after solubilization with PBS or n-octyl-β-D-glucopyranoside (OBG), probed with antisera recognizing the inner membrane protein FtsH and MOM proteins MctB and OmpATb. (B) Three replicates (A, B, C) of cell envelopes of *M. marinum* E11 were treated with 1% n-Octyl-β-D-glucopyranoside, detergent pellet and detergent supernatant fractions were loaded in a 1:1 ratio on SDS-PAGE and stained by CBB. The entire lanes were cut out, trypsinized and subjected to nanoLC-MS/MS analysis. (C) Supervised clustering of fold change in identified proteins by nanoLC-MS/MS (p-value <0.05) between the detergent pellet (DP) and detergent supernatant (DS).

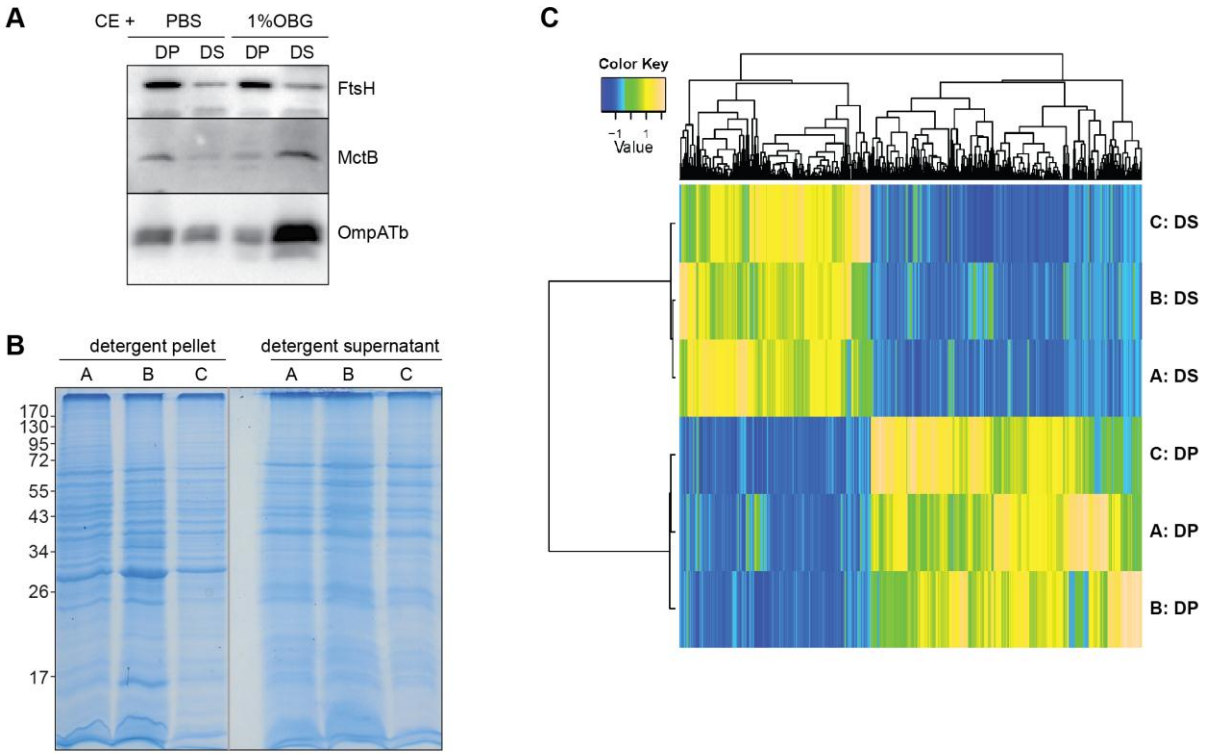

Figure S2. Expression levels of HA-labelled candidate MOMPs. (A) Schematic representation of MOMP candidate proteins, showing putative transmembrane domain (TM), predicted beta-strand rich- (β) or alpha helical (α) domains and threonine rich (T-rich) or proline-alanine rich (PA-rich) stretches. (B)Immunoblot of total lysates of strains expressing HA-tagged marker and candidate MOMPs under the control of an *hsp60* promoter. Proteins were detected with antiserum against the HA epitope and are indicated with an asterisk. An antiserum against GroEL was used to assure equal loading.

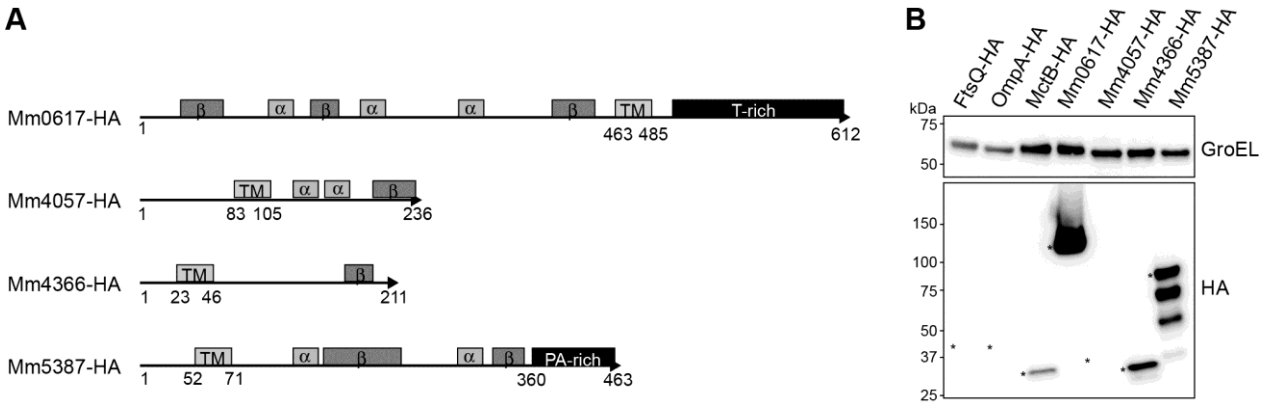

Figure S3. MMAR_0617-His purification and oligomerization. (A) CBB-stained SDS-PAGE gel of Ni-NTA purification in 0.1% Triton X-100 using cell envelopes without (-) and with (+) MMAR_0617-His (indicated with *) or MMAR_0617ΔC-His (indicated with <). (B) Immunoblot after blue native PAGE analysis of different fractions after Ni-NTA purification and subsequent gel filtration chromatography of MMAR_0617-His in the presence of 0.1% or 1% Triton X-100, probed with an anti-His antibody. (C) Immunoblot and absorbance measurements (280 nm) of the gel filtration analysis of MMAR_0617-His in 1% Triton X-100 buffer. Fractions in which marker proteins were found in a separate run using the same buffer are indicated.

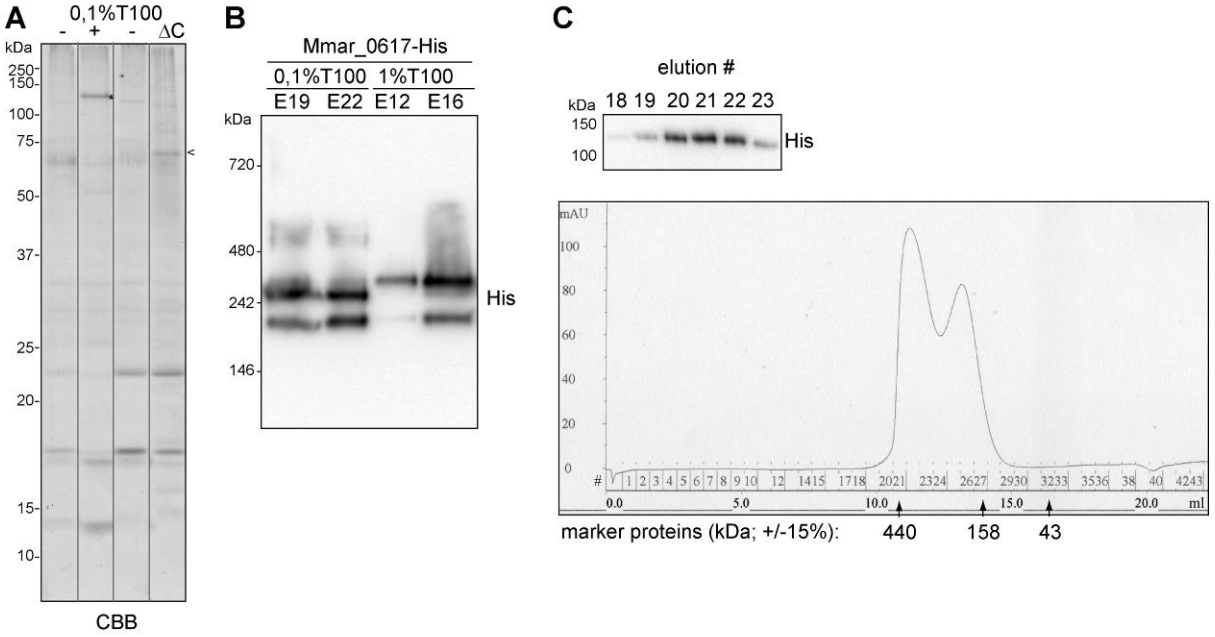

Figure S4. Immunoprecipitation and channel activity of HA-labelled MMAR_0617. (A) Immunoblot analysis of fractions obtained by immunoprecipitation using beads coated with anti-HA and Triton X-100-solubilized cell envelopes of *M. marinum* expressing MMAR_0617-HA. Unbound proteins (FT), the three washing steps (W_{1-3}) and eluted proteins (E) are shown in a 1:4:4 ratio. In addition, the eluate (HA) is compared to the Ni-NTA purification of MMAR_0617-His (His) by CBB staining. (B) Single channel activity recordings in lipid bilayers of purified MMAR0617-HA.

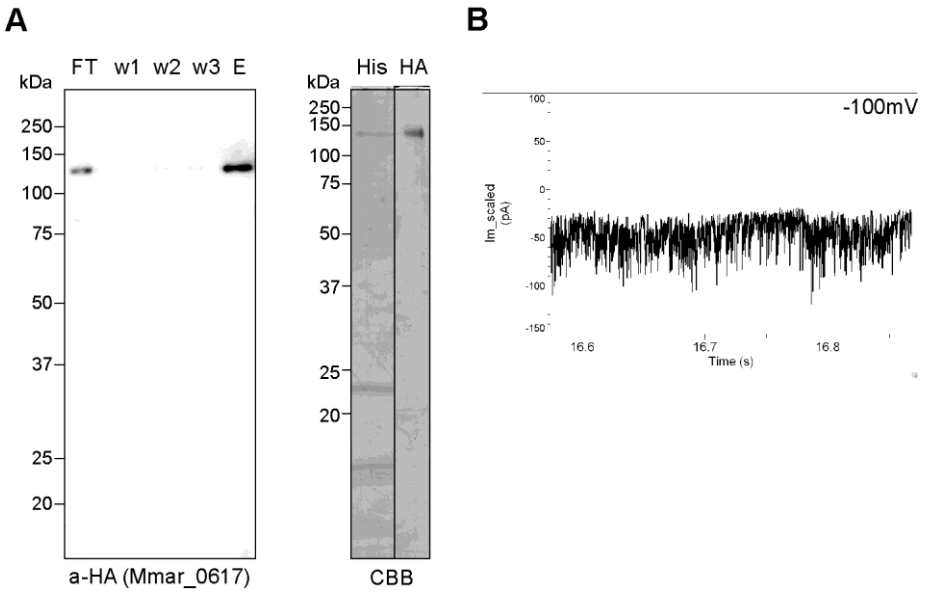

Figure S5. Additional channel activities of MMAR_0617-His after a alternative Ni-NTA purification procedure. Typical ion current recordings through a single channel showing larger conductance of ~4-10 nS measured at 100 mV observed in MMAR_0617-His purification of 0.1 % Triton X-100. Channel closes in several conductance states. Experimental conditions were 1 M KCl, 10 mM HEPES, pH 7.4 at room temperature.