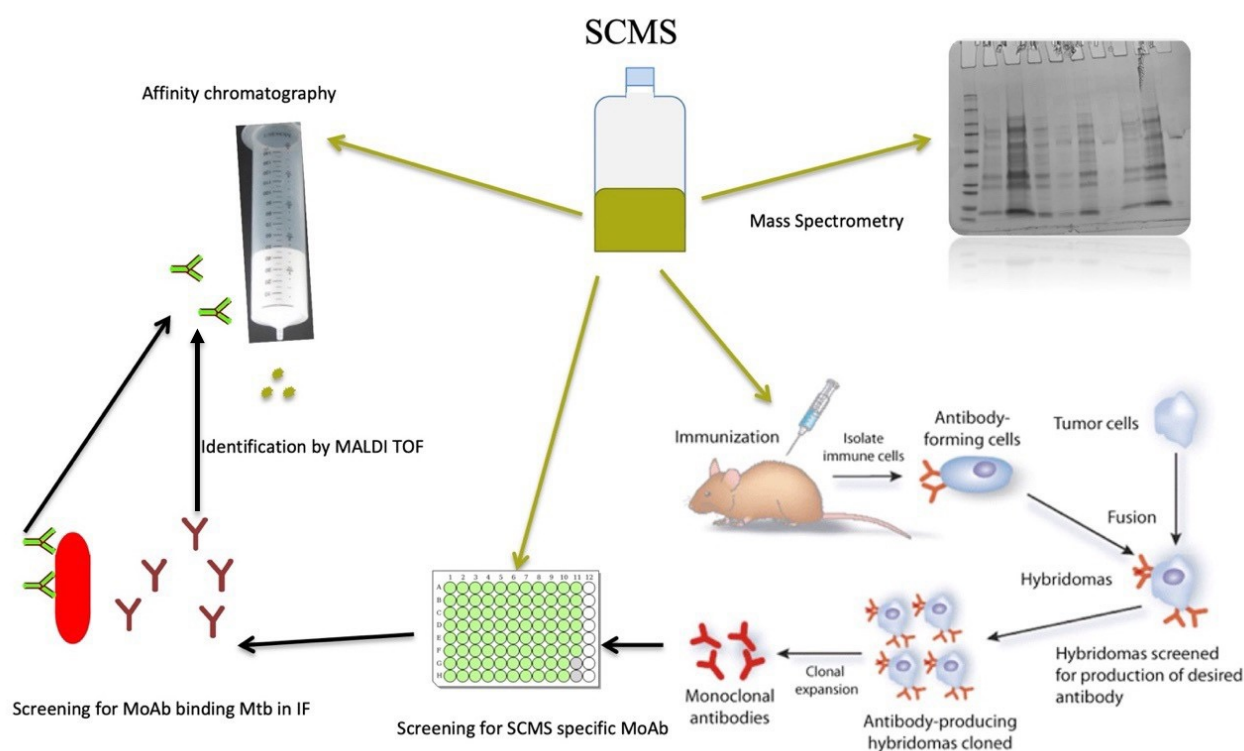


Supplementary Material

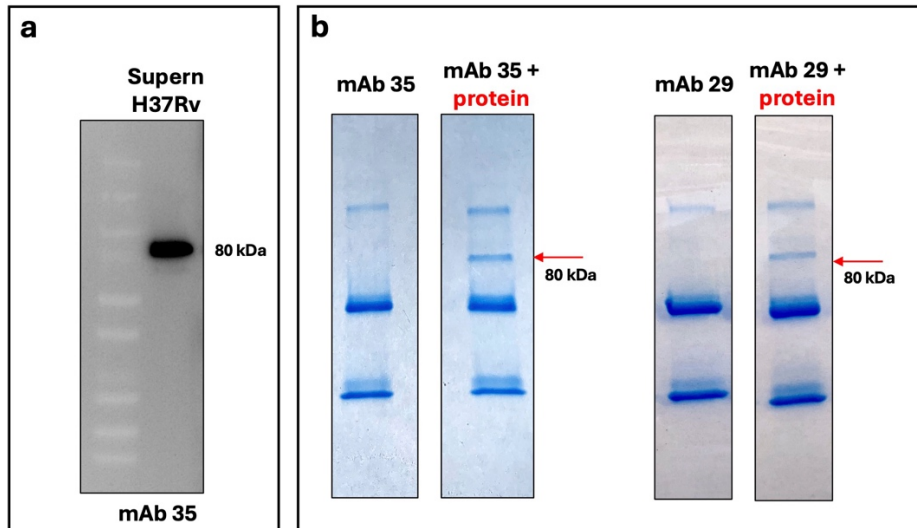
1 Supplementary Figures and Tables

1.1 Supplementary Figures

Supplementary Figure S1: Scheme of the strategy followed to obtain monoclonal antibodies (MoAb) specific for released Mtb proteins. Sterile culture medium supernatants (SCMS); Immunization of mice with SCMS, production of MoAbs specific for SCMS components. Selection of MoAbs binding SCMS and recognizing Mtb cells analyzed by immunofluorescence (IF) by flow cytometry. Immunoprecipitation and characterization of antigens recognized by MoAbs by LC MALDI.



Supplementary Figure S2. Immunoprecipitation of identified antigen. **a)** WB analysis after SDS-PAGE of H37Rv SCMS by using mAb M35 as primary antibody. **b)** SDS-PAGE of isolated mAbs and (M35 and M29) and immuno-precipitation complex of protein (from the SCMS) and purified mAbs (M35 + protein and M29 + protein). Red arrows indicate the 80 kDa protein identified.



Supplementary Figure S3. A) MetE sequence comparison between Mtb H37Rv (query) and *E. coli* BL21 (subject), performed by BLASTP NCBI tool. The full length (aa 1-759) comparison shows amino acid sequence identity of 48%, while comparison of the N-terminal region (aa 1-420), reveal 38% sequence identities. (+) indicates functional equivalence, amino acid not identical but with similar characteristics. **B)** Clustal Omega multiple sequence alignment. Amminoacidic sequence of Mtb H37Rv Rv1133c/MetE protein (NCBI accession number CCP43887.1) was compared to that of BCG (NCBI accession number A1KHS4), *M. abscessus* (NCBI accession number WP_005112521), *M. avium* (NCBI accession number WP_009975418) and *M. chimaera* (NCBI accession number AOS91244). (*) indicates conserved amminoacid; (:) indicates the alignment has strongly similar residues; (.) indicates the residues have weakly similar property.

A

Query	16	GSPRIGPRELKRATEGYWAGRTSRSELEAVAATLRRDTWSALAAAGLDSVPVNTFSYYD	75
Sbjct	9	G PR+G RRELK+A E YWAG ++R EL AV LR W AG+D +PV F++YD	68
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Sbjct	69	+L T++LLG +PPR DG +D F RG + A EMTKWF+TNYHY+V	127
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Sbjct	128	PE F L ++L E+ EAL G +PV++GP+T+L L K + L ++	187
Query	189	VPVYSELLSLLADGGAQWVQFDEPALVTDLSPDAPALAEAVYTALCSVSNRPAIYVATYF	248
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Sbjct	541	QSLTDKPVKGMLTGPVTIL WSF R+D A Q+ALA+RDE DL++AGI +IQ+DE	600
Query	607	PALRELLPLRRADQAEYLRWAVGAFRLATSGVSDATQIHTHLCYSEFGEVIGAIADLDAD	666
Sbjct	601	PALRE LPLRR+D YL+W V AFR+ + D TQIHTH+CY EF +++ +IA LDAD	660
Query	667	VTSIEAARSHMEVLDDLNAIGFANGVGPVYDIHSPRVPSAEEMADSLRAALRAVPAERL	726
Sbjct	661	V +IE +RS ME+L+ + N +GPGVYDIHSP VPS E + L+ A + +PAERL	720
Query	727	WVNPDCGLKTRNVDEVTSASLHNMVAAAREVRAG	759
Sbjct	721	WVNPDCGLKTR E A+L NMV AA+ +R G	753


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M.abscessus      lgldvlvhgpependmvqyfaeqldgffatqngwvqsygsrcvrppilygdvarqqpmtv 533
M.tuberculosis  lgldvlvhgpependmvqyfaeqldgffatqngwvqsygsrcvrppilygdvsrrpamtv 540
BCG              lgldvlvhgpependmvqyfaeqldgffatqngwvqsygsrcvrppilygdvsrrpamtv 540
M.avium          lgldvlvhgpependmvqyfaeqldgffatqngwvqsygsrcvrppilygdvvrthpmtv 533
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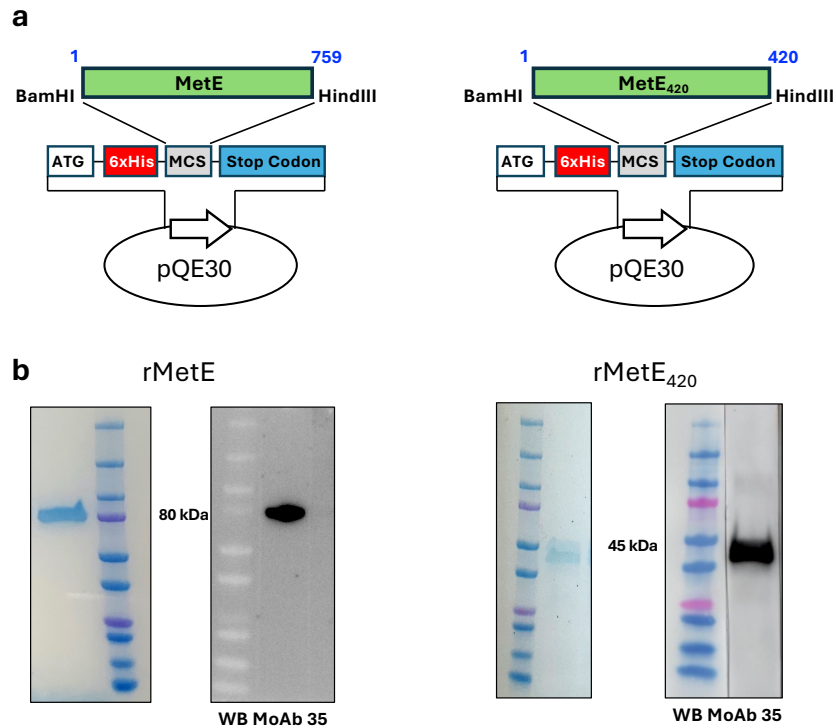
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M. tuberculosis	ewityaqsltdkpvkvgmltgpvtlilawsfvrddqpladtangvalairdetvdlqsagia	600
BCG	ewityaqsltdkpvkvgmltgpvtlilawsfvrddqpladtangvalairdetvdlqsagia	600
M. avium	ewityaqsltdkpvkvgmltgpvtlilawsfvrddqpladtangvalairdetvdlqaagia	593
M. chimaera	ewakyaqsltdkpvkvgmltgpvtlilawsfvrddqpladtangvalairdetvdlqsagia	593
	* * * * *	

M.abscessus	iqvdepalrellplrdseqayldwvavgafrlstsgvsdatqinhthlcysfegveigai	653
M.tuberculosis	viqvdepalrellplrradgaeylrwavgafrlatsgvsdatqinhthlcysfegveigai	660
BCG	viqvdepalrellplrradgaeylrwavgafrlatsgvsdatqinhthlcysfegveigai	660
M.avium	viqvdepalrellplrradqeeylrwavgafrlatsgvsdstqinhthlcysfegveigai	653
M.chimaera	viqvdepalrellplrradqddylrwavgafrlatsgvsdstqinhthlcysfegfdvigi	653
	***** : : * ***** * ***** : *****	

M.abscessus	adldadvtsieaarshmevldldlnaigfnsnvpgpvdyihsprvpsteematslrealka	713
M.tuberculosis	adldadvtsieaarshmevldldlnaigfngvpgpvdyihsprvpssaemadslraalra	720
BCG	adldadvtsieaarshmevldldlnaigfngvpgpvdyihsprvpssaemadslraalra	720
M.avium	adldadvtsleaaarshmevldldnavgfnsnvpgpvdyihsprvpstaieaeslraalra	713
M.chimaera	adldadvtsieaarshmevldldlnaigfnsnvpgpvdyihsprvpsteemaeslraalra	713
	*****.*****.*****.***.*****.*****.***.*****.*****	

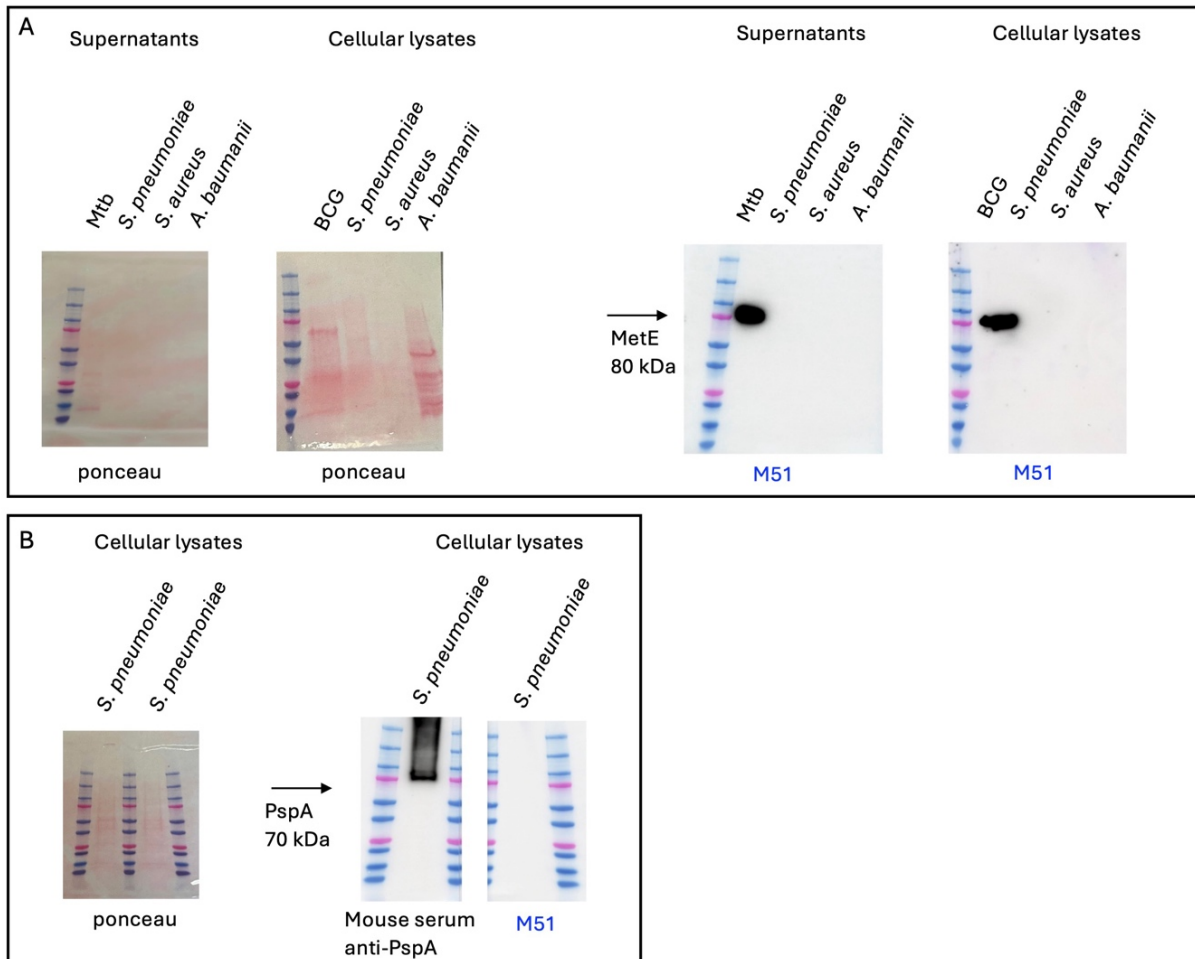
[illegible]

Supplementary Figure S4. Cloning and expression of recombinant MetE and MetE₄₂₀ proteins. A) expression vectors pQE30 construction. B) SDS-PAGE of recombinant MetE and MetE₄₂₀ with the respective WB analysis performed by using mAb M35.



Supplementary Figure S5. WB analysis of the presence of MetE in other interesting bacteria.

A) SDS-PAGE ponceau colored of culture filtrate supernatant and bacterial pellet lysates, and WB analysis performed with mAb M51. **B)** SDS-PAGE ponceau colored of *S. pneumoniae* bacterial pellet lysates, and WB analysis performed with a mouse serum specific for PspA (pneumococcal surface protein A) as positive control, compared to WB with M51, in order to confirm the good quality of sample.



1.2 Supplementary tables

Supplementary table S1. Primers used for cloning and recombinant proteins production and for qRT-PCR analysis.

Primers for cloning	Sequence 5'-3'
MetEFw	GGACAGGGATCCACCCAGCCTGTACGTCGTCAACCC
MetERv	GCTCGTAAGCTTGCCCGCGCGCACCTCCC
MetE₄₂₀Rv	GTCTTGAAGCTTGCGGCGCTGGGCGGCATTG
Primers for qRT-PCR	Sequence 5'-3'
16S-S	GCAGCAGTGGGGAATATTGCACAA
16S-AS	TCCACCTACCGTCAATCCGAGAGA
acr-S	CCGAGCGCACCGAGCAGAAG
acr-AS	GCCTTAATGTCGTCCTCGTCAGCA
metE2-S	ACCACGACGATCGGCTCCTA
metE2-AS	ACTCGGCCTCGTCGATCTCA

Supplementary table S2. The 10 most significant proteins of Mtb H37Rv growth medium identified through nRP-LC-MS/MS analysis

	Accession		Score	Coverage	# Unique Peptides	# Peptides	MW [kDa]
1	A5U3S7	Rv1908c Catalase-peroxidase OS=Mycobacterium tuberculosis (strain ATCC 25177 / H37Ra) GN=katG PE=3 SV=1 - [KATG_MYCTA]	543.92	54.73	23	23	80.6
2	P9WMK0	Rv2031c Alpha-crystallin OS=Mycobacterium tuberculosis (strain CDC 1551 / Oshkosh) GN=hspX PE=1 SV=1 - [ACR_MYCTO]	492.08	40.28	4	4	16.2
3	P9WN38	Rv2220 Glutamine synthetase 1 OS=Mycobacterium tuberculosis (strain CDC 1551 / Oshkosh) GN=glnA1 PE=3 SV=1 - [GLNA1_MYCTO]	353.32	51.67	12	12	53.5
4	A5U1I0	Rv1133c 5-methyltetrahydropteroyltri glutamate-homocysteine methyltransferase OS=Mycobacterium tuberculosis (strain ATCC 25177 / H37Ra) GN=metE PE=3 SV=1 - [METE_MYCTA]	348.74	47.83	20	20	81.5
5	A5U893	Rv3418c 10 kDa chaperonin OS=Mycobacterium tuberculosis (strain ATCC 25177 / H37Ra) GN=groS PE=3 SV=1 - [CH10_MYCTA]	346.47	58.00	5	5	10.8
6	O53166	Rv1475c Aconitate hydratase A OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) GN=acn PE=1 SV=1 - [ACNA_MYCTU]	316.26	46.55	23	23	102.4
7	P9WNZ4	Rv1094 Putative acyl-[acyl-carrier-protein] desaturase desA2 OS=Mycobacterium tuberculosis (strain CDC 1551 / Oshkosh) GN=desA2 PE=3 SV=1 - [DESA2_MYCTO]	284.47	49.09	9	9	31.3
8	P9WPD4	Rv0896 Citrate synthase 1 OS=Mycobacterium tuberculosis (strain CDC 1551 / Oshkosh) GN=gltA2 PE=3 SV=1 - [CISY1_MYCTO]	279.21	50.58	12	12	47.9
9	P9WGU0	Rv0934 Phosphate-binding protein PstS 1 OS=Mycobacterium tuberculosis (strain CDC 1551 / Oshkosh) GN=pstS1 PE=3 SV=1 - [PSTS1_MYCTO]	256.96	40.64	8	8	38.2
10	A5TYT6	Rv0211 Phosphoenolpyruvate carboxykinase [GTP] OS=Mycobacterium tuberculosis (strain ATCC 25177 / H37Ra) GN=pckG PE=1 SV=1 - [PCKG_MYCTA]	255.54	43.07	16	16	67.2

Supplementary table S3A. LC-MALDI analysis performed on the 80 kDa antigen immuno-precipitated by mAb M35.

	Proteinpilot Unused Score	Proteinpilot Total Score	% of Coverage Sequence	Species	UniProtKB Accession Number
1	58.27	58.27	47.8	MYCTU	P9WK07
2	35.7	35.7	52.9	MOUSE	P01869
3	12.02	12.02	83	MOUSE	P01837
4	8	8	20.2	HUMAN	P04264
5	7.19	7.19	19.1	BOVIN	P02769
6	6.6	6.6	26.2	MOUSE	P01633
7	4.02	4.02	15.3	HUMAN	P35527
8	4	4	19.7	BOVIN	P34955
9	3.41	3.41	58.5	HUMAN	Q8IUE6
10	3.34	3.34	35.5	DANRE	Q5BJA5
11	2.85	2.85	17	HUMAN	P13645
12	2.6	2.6	33.6	MOUSE	P97430
13	2.01	2.01	15.9	YEAST	Q12263
14	2	2	10.9	CHAGB	Q2GXM1
15	2	2	16.1	HUMAN	P35908
16	2	2	34	MORAP	Q9HDF5
17	2	2	11.2	ENTFA	Q833N6
18	2	2	8.6	BOVIN	P12763
19	2	2	5.1	RHOS5	A4WU98
20	2	2	38	MYCTU	P9WNK5
21	2	2	6.4	TRYBB	P12865
22	1.85	1.85	6.5	ACRMI	B3EWZ5
23	1.82	1.82	4.4	DROME	Q9VYN8
24	1.57	1.57	10.2	RABIT	P04221
25	1.39	1.39	56.7	STRM5	B4SRB3

Protein Name		Number of Unique Peptides (C.I. 95%)
1	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) GN=metE PE=1 SV=1	37
2	Ig gamma-1 chain C region, membrane-bound form OS=Mus musculus GN=Ighg1 PE=1 SV=2	42
3	Ig kappa chain C region OS=Mus musculus PE=1 SV=1	11

4	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6	4	
5	Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4	4	
6	Ig kappa chain V19-17 OS=Mus musculus GN=Igk-V19-17 PE=1 SV=1	8	
7	Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3	2	
8	Alpha-1-antiproteinase OS=Bos taurus GN=SERPINA1 PE=1 SV=1	2	
9	Histone H2A type 2-B OS=Homo sapiens GN=HIST2H2AB PE=1 SV=3	2	
10	Histone H2B 1/2 OS=Danio rerio GN=zgc:112234 PE=2 SV=3	2	
11	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6	1	
12	Antileukoproteinase OS=Mus musculus GN=Slpi PE=1 SV=1	1	
13	Serine/threonine-protein kinase GIN4 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=GIN4 PE=1 SV=1	1	
14	COPII coat assembly protein SEC16 OS=Chaetomium globosum (strain ATCC 6205 / CBS 148.51 / DSM 1962 / NBRC 6347 / NRRL 1970) GN=SEC16 PE=3 SV=1	1	
15	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2	1	
16	Histone H4 OS=Mortierella alpina GN=H4.1 PE=3 SV=3	1	
17	UDP-N-acetylmuramate--L-alanine ligase OS=Enterococcus faecalis (strain ATCC 700802 / V583) GN=murC PE=3 SV=1	1	
18	Alpha-2-HS-glycoprotein OS=Bos taurus GN=AHSG PE=1 SV=2	1	
19	Cysteine--tRNA ligase OS=Rhodobacter sphaeroides (strain ATCC 17025 / ATH 2.4.3) GN=cysS PE=3 SV=1	1	
20	ESAT-6-like protein EsxB OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) GN=esxB PE=1 SV=1	1	
21	Bloodstream-specific protein 2 OS=Trypanosoma brucei brucei GN=BS2 PE=3 SV=1	1	
22	MAM and LDL-receptor class A domain-containing protein 1 (Fragment) OS=Acropora millepora PE=1 SV=1	1	
23	Teneurin-a OS=Drosophila melanogaster GN=Ten-a PE=1 SV=2	1	
24	Ig mu chain C region membrane-bound form OS=Oryctolagus cuniculus PE=2 SV=2	1	
25	Carbon storage regulator homolog OS=Stenotrophomonas maltophilia (strain R551-3) GN=csrA PE=3 SV=1	1	

Supplementary table S3B. LC-MALDI analysis performed on the 80 kDa antigen immuno-precipitated by mAb M29

	Protein Pilot Unused Score	Protein Pilot Total Score	% of Coverage Sequence	Species	UniProtKB Accession Number
1	44.04	44.04	42.3	MYCTU	P9WK07
2	32	32	49.1	MOUSE	P01869
3	12.03	12.03	89.7	MOUSE	P01837
4	6	6	19.8	HUMAN	P06312
5	2	2	22.3	ORYSJ	Q8S857
6	2	2	29.8	DANRE	Q5BJA5
7	2	2	4.9	PONAB	Q5NVH5
8	2	2	8.8	GEOKA	Q5L3S9

Protein Name		Number of Unique Peptides (C.I. 95%)
1	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) OX=83332 GN=metE PE=1 SV=1	25
2	Ig gamma-1 chain C region, membrane-bound form OS=Mus musculus OX=10090 GN=Ighg1 PE=1 SV=2	31
3	Immunoglobulin kappa constant OS=Mus musculus OX=10090 GN=Igkc PE=1 SV=2	15
4	Immunoglobulin kappa variable 4-1 OS=Homo sapiens OX=9606 GN=IGKV4-1 PE=1 SV=1	5
5	Probable histone H2A variant 2 OS=Oryza sativa subsp. japonica OX=39947 GN=Os10g0418000 PE=2 SV=1	1
6	Histone H2B 1/2 OS=Danio rerio OX=7955 GN=zgc:112234 PE=2 SV=3	1
7	Serum albumin OS=Pongo abelii OX=9601 GN=ALB PE=2 SV=2	1
8	33 kDa chaperonin OS=Geobacillus kaustophilus (strain HTA426) OX=235909 GN=hslO PE=3 SV=2	1