

Correlation of phenotypic profiles using targeted proteomics identifies mycobacterial Esx-1 substrates

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Table S1: *M. marinum* strains used in this study

Name	Genotype	Reference
M strain	Wild-type <i>M. marinum</i> , parent strain	Gao, LY et al. 2003
ΔRD1	ΔeccCb'-espK	Volkman, HE et al. 2004
M1	espG ₁ ::Tn	Gao, LY et al. 2004
M2	espH::Tn	Gao, LY et al. 2004
M3	eccA ₁ ::Tn	Gao, LY et al. 2004
M4	eccA ₁ ::Tn	Gao, LY et al. 2004
6B10	eccB ₁ ::Tn	Champion, MM et al. 2012
F11	eccB ₁ ::Tn	This study
Mh3871::Tn	eccCb::Tn	Joshi, SA et al. 2012
MhΔesxBA	ΔesxBA	Gao, LY et al. 2004
MhΔesxA	ΔesxA	Xu, J et al. 2007
M5	espI::Tn	Gao, LY et al. 2004
17	eccD ₁ ::Tn	This study
M6	espJ::Tn	Gao, LY et al. 2004
M7	espK::Tn	Gao, LY et al. 2004
2	espL::Tn	This study
M8	espB::Tn	Gao, LY et al. 2004

Table S2: p values for relative differences between protein levels in the WT vs ESX-1-deficient strains. “-” indicates a p value of <.0001. p values were determined by a two tailed Student’s t-test.

ESX-1 deficient <i>M. marinum</i> strains																
Protein	Fraction	ΔRD1	espG1	eccA-1	eccA-2	eccB-1	eccB-2	eccCb	ΔesxBA	ΔesxA	espI	eccD1	espJ	espK	espL	espB
EsxB	CL	-	.0234	-	.0028	-	.0012	.8025	-	-	.0443	.0014	.1334	.4673	.0074	.0008
	CF	-	-	-	-	-	-	-	-	-	-	-	.0007	-	-	-
EsxA	CL	-	.0352	.0034	.0241	.0043	.0152	.0056	-	-	.0777	.9782	.5244	.3558	.0145	.0269
	CF	.0002	-	-	.0003	-	-	-	.0003	.0003	.0012	.0012	.0277	.0065	-	.0004
EspF	CL	-	-	-	-	-	-	.0004	-	-	-	-	.0093	.0052	-	-
	CF	-	-	-	-	-	-	.0006	-	-	.0019	.0003	.0024	-	-	-
EspB	CL	.0022	.0203	.0059	.0165	.0002	.0755	.4230	.1246	.6357	.0013	.2696	.0097	.5332	.1054	.0002
	CF	-	-	-	.0016	-	-	-	-	-	.0028	.0021	.0114	.0007	-	-
EspK	CL	-	.6519	-	-	-	-	.0001	.0001	-	-	.0005	.0003	-	-	.0003
	CF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PPE68	CL	-	.0002	.0008	-	.0014	.0019	.0003	.0001	.0025	.0019	.0059	.3745	.0229	-	.0024
	CF	-	.0010	.0100	.0219	-	-	-	-	-	.0002	.0393	.0123	.7889	-	.0012
EspJ	CL	-	.0009	-	-	-	.0017	.0114	.0023	.0002	.0003	.0030	-	-	-	-
	CF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EsxN	CL	.8178	1.000	.0002	.2012	.0046	.8309	.0224	.0054	.0051	.2073	.0384	.9768	.5303	.0960	.1597
	CF	.0077	.0072	.0010	.0067	.0040	.0027	.8151	.0267	.0085	.0047	.2239	.2526	.0062	.0024	.0130
2929	CL	.0146	.0071	-	.3893	.0002	.0002	.0247	.0027	.0073	.0003	.0264	.0034	.7280	.0005	.0275
	CF	.0172	.2064	.0284	.5141	.6989	.0039	-	.0394	.0171	.0064	-	.0059	.0148	.0003	.0106

<i>M. marinum</i> genes	Comparison with EspJ _{MM} or EspK _{MM}			Best BLAST hit in <i>M. tb</i> H37Rv genome	Comparison with EspJ _{MT} or EspK _{MT}		
	% identity	% similarity	% coverage		% identity	% similarity	% coverage
MMAR_5453 (<i>espJ</i>)	100	100	100	<i>rv3878 (espJ)</i>	33	43	100
MMAR_5455 (<i>espK</i>)	100	100	100	<i>rv3879c (espK)</i>	50	58	99
MMAR_4352	29	38	90	<i>rv3878 (espJ)</i>	41	52	98
MMAR_4351	43	52	100	<i>rv3879c(espK)</i>	46	56	99
MMAR_0197	36	52	33	<i>rv3878(espJ)</i>	41	53	80
MMAR_0199	33	43	99	<i>rv3879c(espK)</i>	33	45	99
MMAR_0200	38	47	38	<i>rv3879c(espK)</i>	29	44	86
MMAR_1328	31	42	69	<i>rv3878(espJ)</i>	51	62	50
MMAR_1331	35	43	99	<i>rv3879c(espK)</i>	30	40	98
MMAR_5425 "orphan espK"	48	62	29	<i>rv3879c(espK)</i>	61	72	75

Table S3. *espK* and *espJ* paralogs in *M. marinum* are found in pairs. *M. marinum* contains several paralogs of both *espJ* and *espK*. All of the *espJ* paralogs are found paired with an *espK* paralog. One *espK* paralog (MMAR_5425) does not have an *espJ* partner nearby. *M. marinum* *espJ* (MMAR_5453) and *espK* (MMAR_5455) are found at the esx-1 locus. MMAR_0197, MMAR_0199, MMAR_0200 are found at a partial duplication of the esx-1 locus in *M. marinum*. MMAR_4352/MMAR_4351 and MMAR_1328/MMAR_1331 are not linked to a known esx locus. *M. marinum* paralogs were identified by using DELTA-BLAST to search the *M. marinum* genome with either MMAR_5453 or MMAR_5455. The TB ortholog was identified as the top hit in a DELTA-BLAST search against the *M. tuberculosis* H37Rv genome. PE/PPE proteins are found at the esx-1 locus (MMAR_5447, MMAR_5448), near the *espK/J* paralog pair at MMAT_4346, and at the partial Esx duplication (MMAR_0183, pe35, ppe68_1 (substrate) and ppe51_2).

Figure S1: MRM transitions monitored in this study

Transition Name/ID	Q1 (m/z)	Q3 (m/z)	Dwell (ms)	CE (V)	Transition Name/ID	Q1 (m/z)	Q3 (m/z)	Dwell (ms)	CE (V)
* CFP10_GAAGTAAQAAVVR.T1	571.8	943.5	12	33.6	EspA_VSSEPVGEHAQAAASAQGGQMGGMHPASAGSK.T1	745.3	999.5	12	42.3
CFP10_GAAGTAAQAAVVR.T2	571.8	785.5	12	33.6	EspA_VSSEPVGEHAQAAASAQGGQMGGMHPASAGSK.T2	745.3	929.4	12	42.3
CFP10_GAAGTAAQAAVVR.T3	571.8	714.4	12	33.6	EspA_VSSEPVGEHAQAAASAQGGQMGGMHPASAGSK.T3	745.3	858.4	12	42.3
CFP10_TQIDQVESTAGSLQAQWR.T1	673.3	945.5	12	38.7	EspL_IJDDGLLKEVGPVEVSAR.T1	599.7	913.5	12	35
CFP10_TQIDQVESTAGSLQAQWR.T2	673.3	688.4	12	38.7	EspL_IJDDGLLKEVGPVEVSAR.T2	599.7	814.4	12	35
CFP10_TQIDQVESTAGSLQAQWR.T3	673.3	888.5	12	38.7	EspL_IJDDGLLKEVGPVEVSAR.T3	599.7	757.4	12	35
CFP10_ADDEQQQALSSQMGF.T1	827.9	999.4	12	46.4	EspL_FQSALDGTLNQMNTGNFR.T1	672	967.4	12	38.6
CFP10_ADDEQQQALSSQMGF.T2	827.9	886.4	12	46.4	EspL_FQSALDGTLNQMNTGNFR.T2	672	839.4	12	38.6
CFP10_ADDEQQQALSSQMGF.T3	827.9	840.4	12	46.4	EspL_FQSALDGTLNQMNTGNFR.T3	672	708.3	12	38.6
# ESAT6_LAAAWGGSGSEAYR.T1	465.9	682.3	12	28.3	EspL_GKDDDTETVETINGHQWLTAJR.T1	618.6	975.4	12	35.9
ESAT6_LAAAWGGSGSEAYR.T2	465.9	538.3	12	28.3	EspL_GKDDDTETVETINGHQWLTAJR.T2	618.6	747.3	12	35.9
ESAT6_LAAAWGGSGSEAYR.T3	465.9	883.4	12	28.3	EspL_GKDDDTETVETINGHQWLTAJR.T3	618.6	699.4	12	35.9
ESAT6_GVQQNWQDSTAELNNSLQLNLR.T1	829.4	915.5	12	46.5	EspL_MEMDPQVAQVLALAR.T1	581.6	912.6	12	34.1
ESAT6_GVQQNWQDSTAELNNSLQLNLR.T2	829.4	801.5	12	46.5	EspL_MEMDPQVAQVLALAR.T2	581.6	841.5	12	34.1
ESAT6_GVQQNWQDSTAELNNSLQLNLR.T3	829.4	714.4	12	46.5	EspL_MEMDPQVAQVLALAR.T3	581.6	614.4	12	34.1
S455_EspK_GIPRPTGEYAGR.T1	425.2	552.3	13	26.3	CAP_AGIFQGVPEGAVAALT.K1	815	827.5	12	45.7
S455_EspK_GIPRPTGEYAGR.T2	425.2	850.4	13	26.3	CAP_AGIFQGVPEGAVAALT.K2	815	956.5	12	45.7
S455_EspK_GIPRPTGEYAGR.T3	425.2	466.2	13	26.3	CAP_AGIFQGVPEGAVAALT.K3	815	1112.6	12	45.7
S455_EspK_VLHLFTDVMDAcR.T1	526.3	652.3	13	31.3	CAP_AWIADRPEIAEQLLR.T1	594.3	762.4	12	34.7
S455_EspK_VLHLFTDVMDAcR.T2	526.3	826.4	13	31.3	CAP_AWIADRPEIAEQLLR.T2	594.3	705.9	12	34.7
S455_EspK_VLHLFTDVMDAcR.T3	526.3	925.5	13	31.3	CAP_AWIADRPEIAEQLLR.T3	594.3	670.4	12	34.7
S455_EspK_KAAIASLIR.T1	471.8	814.5	13	28.6	CAP_TSATTITEVR.T1	583.3	977.5	12	34.2
S455_EspK_KAAIASLIR.T2	471.8	743.5	13	28.6	CAP_TSATTITEVR.T2	583.3	819.5	12	34.2
S455_EspK_KAAIASLIR.T3	471.8	672.4	13	28.6	CAP_TSATTITEVR.T3	583.3	718.4	12	34.2
EspB_ADLEPVNPCKPPAAIK.T1	553	614.9	13	32.6	EspG_AAAGVLDLSAHR.T1	562.8	755.4	12	33.1
EspB_ADLEPVNPCKPPAAIK.T2	553	918.6	13	32.6	EspG_AAAGVLDLSAHR.T2	562.8	982.5	12	33.1
EspB_ADLEPVNPCKPPAAIK.T3	553	735.9	13	32.6	EspG_AAAGVLDLSAHR.T3	562.8	642.3	12	33.1
EspB_GHPTLADIVELER.T1	483.9	805.4	13	29.2	EspG_YGLPTTAR.T1	490.3	816.5	12	29.5
EspB_GHPTLADIVELER.T2	483.9	692.3	13	29.2	EspG_YGLPTTAR.T2	490.3	646.4	12	29.5
EspB_GHPTLADIVELER.T3	483.9	645.4	13	29.2	EspG_YGLPTTAR.T3	490.3	545.3	12	29.5
EspB_VAAAGESDFTDLK.T1	662.3	954.4	13	38.1	EspG_LYTEIVTNPK.T1	589.3	901.5	12	34.5
EspB_VAAAGESDFTDLK.T2	662.3	825.4	13	38.1	EspG_LYTEIVTNPK.T2	589.3	671.4	12	34.5
EspB_VAAAGESDFTDLK.T3	662.3	738.4	13	38.1	EspG_LYTEIVTNPK.T3	589.3	800.5	12	34.5
EspF_Ac-TGLLNVVPFLK.T1	665.4	903.5	12	36	ExsN_AASLEAEHQAVR.T1	697.9	923.5	12	39.9
EspF_Ac-TGLLNVVPFLK.T2	665.4	1016.6	12	36	ExsN_AASLEAEHQAVR.T2	697.9	852.5	12	39.9
EspF_Ac-TGLLNVVPFLK.T3	665.4	690.4	12	36	ExsN_AASLEAEHQAVR.T3	697.9	1052.5	12	39.9
EspF_NSAGTGLQGVGTGK.T1	595.3	917.5	12	34.8	ExsN_AQAASLEAHQAVR.T1	531.9	923.5	12	31.6
EspF_NSAGTGLQGVGTGK.T2	595.3	988.5	12	34.8	ExsN_AQAASLEAHQAVR.T2	531.9	697.9	12	31.6
EspF_NSAGTGLQGVGTGK.T3	595.3	1075.6	12	34.8	ExsN_AQAASLEAHQAVR.T3	531.9	662.4	12	31.6
EspF_SATNVVGIGSR.T1	574.3	989.5	12	33.7	5443_LLAEAQEELDR.T1	643.8	989.5	12	37.2
EspF_SATNVVGIGSR.T2	574.3	888.5	12	33.7	5443_LLAEAQEELDR.T2	643.8	1060.5	12	37.2
EspF_SATNVVGIGSR.T3	574.3	1060.6	12	33.7	5443_LLAEAQEELDR.T3	643.8	860.4	12	37.2
PPE68_GESLPGAGGTLTR.T1	608.3	829.5	12	35.4	5443_VVANMLAGLGVIAEPK.T1	528	954.6	12	31.4
PPE68_GESLPGAGGTLTR.T2	608.3	732.4	12	35.4	5443_VVANMLAGLGVIAEPK.T2	528	713.4	12	31.4
PPE68_GESLPGAGGTLTR.T3	608.3	942.5	12	35.4	5443_VVANMLAGLGVIAEPK.T3	528	1067.6	12	31.4
PPE68_MLWHAMPPELNLTAR.T1	556.3	770.3	12	32.8	5443_LLETNEGLR.T1	522.8	931.5	12	31.1
PPE68_MLWHAMPPELNLTAR.T2	556.3	897.5	12	32.8	5443_LLETNEGLR.T2	522.8	818.4	12	31.1
PPE68_MLWHAMPPELNLTAR.T3	556.3	800.4	12	32.8	5443_LLETNEGLR.T3	522.8	689.4	12	31.1
PPE68_LNSLGEAWTGGSKE.T1	753.4	892.4	12	42.7	5443_DGRTPFGQEAMDTLLAR.T1	665	974.4	12	38.2
PPE68_LNSLGEAWTGGSKE.T2	753.4	821.4	12	42.7	5443_DGRTPFGQEAMDTLLAR.T2	665	890.5	12	38.2
PPE68_LNSLGEAWTGGSKE.T3	753.4	1191.6	12	42.7	5443_DGRTPFGQEAMDTLLAR.T3	665	819.4	12	38.2
GroES_EKPQEGTVVAVGPGR.T1	508.6	869.4	12	30.4	5446_LGDVNETQIJD.R1	630.3	974.5	12	36.5
GroES_EKPQEGTVVAVGPGR.T2	508.6	556.3	12	30.4	5446_LGDVNETQIJD.R2	630.3	875.4	12	36.5
GroES_EKPQEGTVVAVGPGR.T3	508.6	968.5	12	30.4	5446_LGDVNETQIJD.R3	630.3	761.4	12	36.5
GROES_YGGEYLILSAR.T1	685.8	964.5	12	39.3	5446_VVAEMQAVMR.T1	567.3	935.4	12	33.4
GROES_YGGEYLILSAR.T2	685.8	835.5	12	39.3	5446_VVAEMQAVMR.T2	567.3	864.4	12	33.4
GroEL_AEINSDSDYDR.T1	707.3	971.4	12	37	5446_VVAEMQAVMR.T3	567.3	735.4	12	33.4
GroEL_AEINSDSDYDR.T2	707.3	1100.4	12	37	5446_VGSIAMYR.T1	448.7	797.4	12	27.4
MMAR292_TPDIAIAQDIHTTGEK.T1	855.4	1212.6	12	45	5446_VGSIAMYR.T2	448.7	740.4	12	27.4
MMAR292_TPDIAIAQDIHTTGEK.T2	855.4	1141.6	12	45	5446_VGSIAMYR.T3	448.7	653.3	12	27.4
GroEL2_DETTIVEGAGDSDAIAGR.T1	888.9	1217.6	12	45	EspR_GPHTSAEVIAALK.T1	431.9	779.4	12	26.6
GroEL2_DETTIVEGAGDSDAIAGR.T2	888.9	1118.5	12	45	EspR_GPHTSAEVIAALK.T2	431.9	680.3	12	26.6
EspI_AEPLAVDPAR.T1	519.8	838.5	12	31	EspR_GPHTSAEVIAALK.T3	431.9	515.4	12	26.6
EspI_AEPLAVDPAR.T2	519.8	741.4	12	31	EspR_SQGLSTQAQQEIVER.T1	837.4	972.5	12	46.9
EspI_AEPLAVDPAR.T3	519.8	628.3	12	31	EspR_SQGLSTQAQQEIVER.T2	837.4	901.5	12	46.9
EspI_TASSMSTAADIYAK.T1	472.9	680.4	12	28.6	EspR_SQGLSTQAQQEIVER.T3	837.4	773.4	12	46.9
EspI_TASSMSTAADIYAK.T2	472.9	609.3	12	28.6	EspR_TNPSTATMAALANFFR.T1	571.6	909.5	12	33.6
EspI_TASSMSTAADIYAK.T3	472.9	939.5	12	28.6	EspR_TNPSTATMAALANFFR.T2	571.6	838.5	12	33.6
EspE_APIDAGSNTGQNEGTLT.T1	857.9	989.5	12	47.9	EspR_TNPSTATMAALANFFR.T3	571.6	654.3	12	33.6
EspE_APIDAGSNTGQNEGTLT.T2	857.9	1103.5	12	47.9	5444_ATNQLVLLSGLQLPQHVPVNLTSAR.T1	853.5	1193.6	12	47.7
EspE_APIDAGSNTGQNEGTLT.T3	857.9	1190.6	12	47.9	5444_ATNQLVLLSGLQLPQHVPVNLTSAR.T2	853.5	1016.1	12	47.7
EspE_MIDGVYK.T1	413.2	694.4	12	25.7	5444_ATNQLVLLSGLQLPQHVPVNLTSAR.T3	853.5	1072.6	12	47.7
EspE_MIDGVYK.T2	413.2	581.3	12	25.7	EccE_JADGLASNGVDAVCGR.T1	787.9	1183.6	12	44.4
EspE_MIDGVYK.T3	413.2	466.3	12	25.7	EccE_JADGLASNGVDAVCGR.T2	787.9	1105.5	12	44.4
EspH_AANMSESALAEEIFVIADLAR.T1	741	904.5	12	42.1	EccE_JADGLASNGVDAVCGR.T3	787.9	1034.5	12	44.4
EspH_AANMSESALAEEIFVIADLAR.T2	741	946.4	12	42.1	EspE_LLEDLLSVHCPDLEADVVSAGYR.T1	857.8	1173.1	12	47.9
EspH_AANMSESALAEEIFVIADLAR.T3	741	757.5	12	42.1	EspE_LLEDLLSVHCPDLEADVVSAGYR.T2	857.8	937.9	12	47.9
EspH_YEVDTYSR.T1	516.7	869.4	12	30.8	EccE_YLVASATR.T1	440.7	604.3	12	27
EspH_YEVDTYSR.T2	516.7	740.4	12	30.8	EccE_YLVASATR.T2	440.7	505.3	12	27
EspH_YEVDTYSR.T3	516.7	641.3	12	30.8	EccE_YLVASATR.T3	440.7	717.4	12	27
EspH_AAQHTFMVEAMASELSDTEEEGALLR.T1	989.1	1146.6	12	54.5	* CFP-10 is synonymous with EsxB				
EspH_AAQHTFMVEAMASELSDTEEEGALLR.T2	989.1	886.4	12	54.5	# ESAT-6 is synonymous with EsxA				
EspH_AAQHTFMVEAMASELSDTEEEGALLR.T3	989.1	916.5	12	54.5					

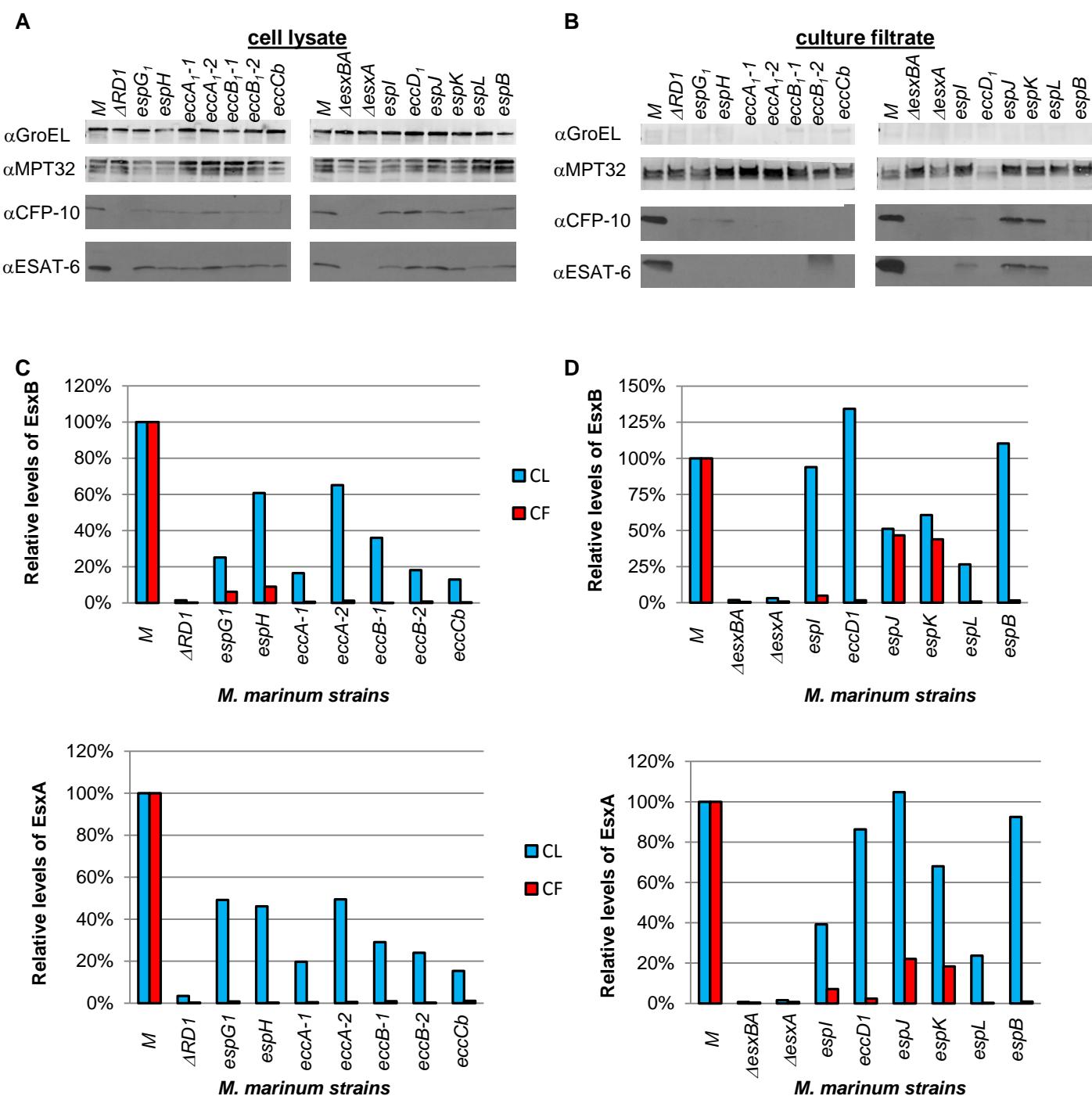


Figure S2: Western blot analysis for the production and secretion of EsxA and EsxB. **A)** Western blot analysis of cell lysates and **B)** culture filtrates generated from WT *M. marinum* M strain and the ESX-1-deficient strains bearing transposon insertions or deletions in the extended RD1 locus. MPT32 served as a loading control for the CFs. GroEL, a cytosolic protein, served as a loading control for the CLs and a lysis control for the CFs. Western blot images were quantified using Image J. **C)** Quantification of the blots in panel A **D)** Quantification of the blots in panel B. CL proteins were normalized to GroEL. CF proteins were normalized to MPT-32. All results were normalized to the wild-type levels to allow for comparison between gels.

A. Correlations between Esx-1-associated proteins in the CL

EspF	1**	EspG1	1**	EspH		EccA1		EccCb		PPE68		EsxB		EsxA		EspJ		EspK		EspL		EspB		2929 ^S		EsxN ⁵	
EspG1	0.8845**		1**																								
EspH	0.8587**		0.7920**		1**																						
EccA1	0.8319**		0.9200**		0.7719**		1**																				
EccCb	0.7152**		0.8365**		0.7455**		0.8599**		1**																		
PPE68	0.9546**		0.9052**		0.8530**		0.8702**		0.8160**		1**																
EsxB	0.519*		0.6680**		0.4528		0.6879**		0.4786		0.5528*		1**														
EsxA	0.7449**		0.7966**		0.5708*		0.7910**		0.5751*		0.7460**		0.9294**														
EspJ	0.3759		0.5096*		0.3778		0.3678		0.3353		0.3951		0.1023		0.1593		1**										
EspK	0.479		0.4946		0.1921		0.5497*		0.4343		0.5472*		0.4253		0.5175*		0.4745		1**								
EspL	0.8143**		0.8475**		0.8961*		0.7891**		0.7207**		0.7962**		0.5213*		0.5861*		0.6106*		0.3697		1**						
EspB	0.8297**		0.7838**		0.8359**		0.8155**		0.6895**		0.7605**		0.4100		0.5460*		0.5156*		0.3759		0.8890**		1**				
2929 ^S	0.6553 **		0.6195*		0.7626**		0.7196**		0.5618*		0.6610**		0.6039*		0.6066*		0.1368		0.3465		0.7363**		0.5927*		1**		
EsxN ⁵	0.334		0.2862		0.4433		0.2950		0.2052		0.283		-0.0752		-0.0403		0.7223**		0.3482		0.6182*		0.5642*		0.4424		

B. Significance of correlations between Esx-1-associated proteins in the CL

EspF	6.084x10 ⁻²¹⁷	EspG1	5.381x10 ⁻⁰⁶	EspH	4.789x10 ⁻²²⁴	EccA	2.056x10 ⁻⁰⁵	EccA1	4.536x10 ⁻⁰⁵	EccCb	9.202x10 ⁻²²²	PPE68	1.946x10 ⁻⁰⁵	EccB	2.020x10 ⁻²²²	EccCb	8.447x10 ⁻²²⁵	EsxB	6.076x10 ⁻²²¹	EsxA	1.207x10 ⁻²²	EspJ	2.603x10 ⁻²¹⁸	EspK	1.136x10 ⁻²²¹	EspL	1.841x10 ⁻²²⁵	EspB	7.010x10 ⁻⁰⁵	2929 ^S	EsxN ⁵
EspG1	5.381x10 ⁻⁰⁶		0.0003		4.562x10 ⁻²³⁰																										
EspH	0.0003																														
EccA1	0.2750		0.9004**		-0.2560		1**																								
PPE68	0.5005*		0.025		0.4536		0.0261		1**																						
EsxB	0.9243**		0.2246		0.5424*		0.1786		0.6994**		1**																				
EsxA	0.9475**		0.286		0.4705		0.2273		0.6069*		0.9865**		1**																		
EspJ	0.8405**		0.2106		0.0561		0.1595		0.3830		0.7942**		0.8537**		1**																
EspK	0.7754**		0.1989		-0.0260		0.1606		0.3829		0.7547**		0.8129**		0.9854**		1**														
EspL	0.0263		0.7466**		-0.273		0.8889**		-0.052		-0.071		-0.055		-0.15		-0.158		1**												
EspB	0.9107**		0.1865		0.2728		0.1645		0.4578		0.9006**		0.9189**		0.9042**		0.8711**		-0.118		1**		2929 ^S								
2929 ^S	0.0112		0.5867*		-0.329		0.7319**		-0.083		-0.082		-0.069		-0.117		-0.101		0.8763**		-0.0810		1**								
EsxN ⁵	0.465		0.6341**		-0.013		0.7870**		0.2266		0.4209		0.4286		0.2862		0.2771		0.7493**		0.3565		0.8138**		1**						

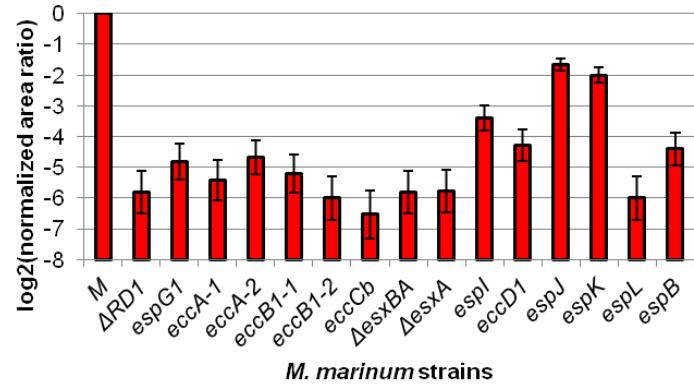
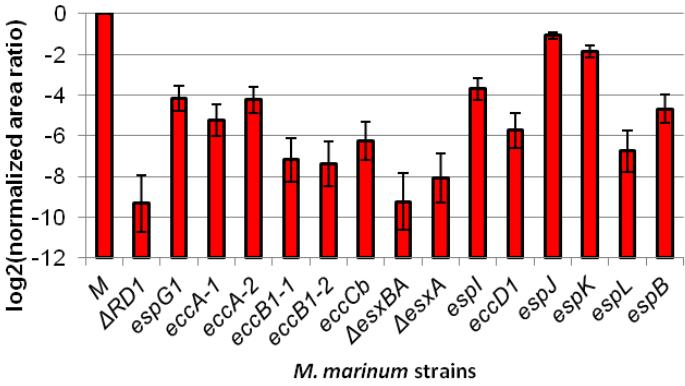
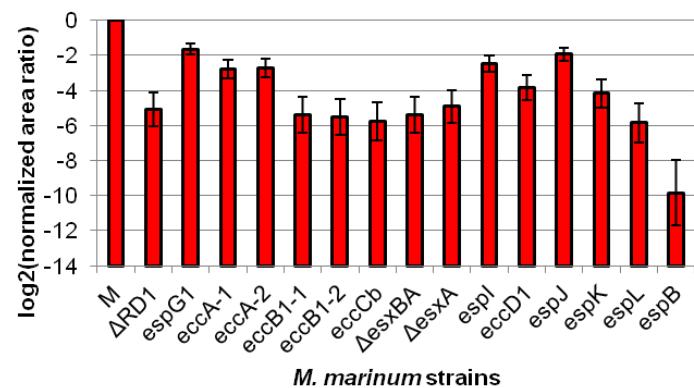
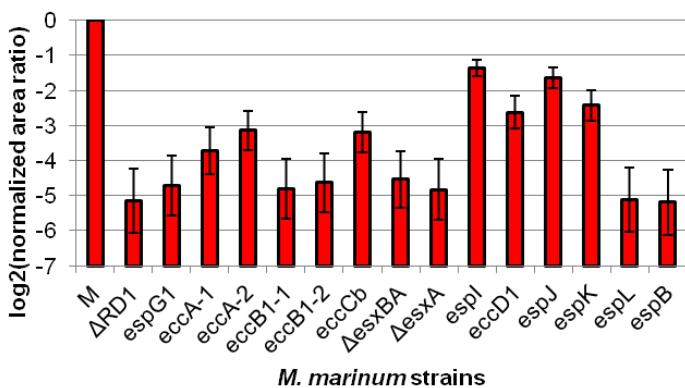
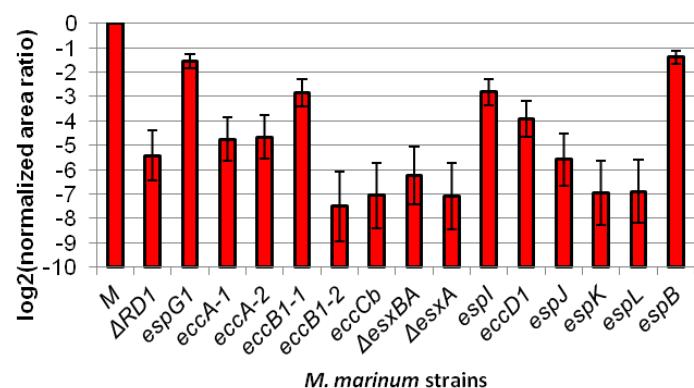
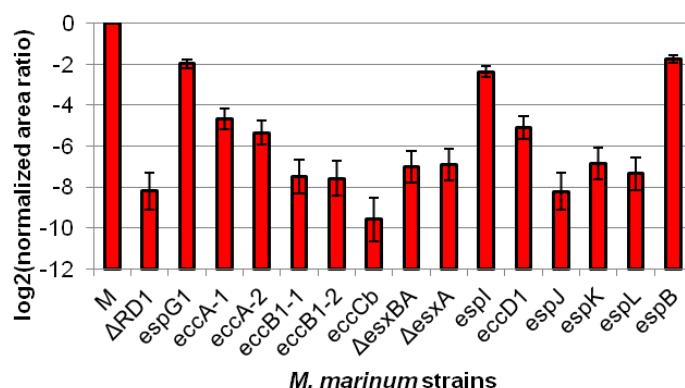
C. Correlations between Esx-1-associated proteins in the CF

EspF	1**	EspG1	1**	EspH		EccA1		PPE68		EsxB		EsxA		EspJ		EspK		EspL		EspB		2929 ^S		EsxN ⁵		
EspG1	0.3230		1**																							
EspH	0.4631		-0.1470		1**																					
EccA1	0.2750		0.9004**		-0.2560		1**																			
PPE68	0.5005*		0.025		0.4536		0.0261		1**																	
EsxB	0.9243**		0.2246		0.5424*		0.1786		0.6994**		1**															
EsxA	0.9475**		0.286		0.4705		0.2273		0.6069*		0.9865**		1**													
EspJ	0.8405**		0.2106		0.0561		0.1595		0.3830		0.7942**		0.8537**		1**											
EspK	0.7754**		0.1989		-0.0260		0.1606		0.3829		0.7547**		0.8129**		0.9854**		1**									
EspL	0.0263		0.7466**		-0.273		0.8889**		-0.052		-0.071		-0.055		-0.15		-0.158		1**							
EspB	0.9107**		0.1865		0.2728		0.1645		0.4578		0.9006**		0.9189**		0.9042**		0.8711**		-0.118		1**		2929 ^S		EsxN ⁵	
2929 ^S	0.0112		0.5867*		-0.329		0.7319**		-0.083		-0.082		-0.069		-0.117		-0.101		0.8763**		-0.0810		1**			
EsxN ⁵	0.465		0.6341**		-0.013		0.7870**		0.2266		0.4209		0.4286		0.2862		0.2771		0.7493**		0.3565		0.8138**		1**	

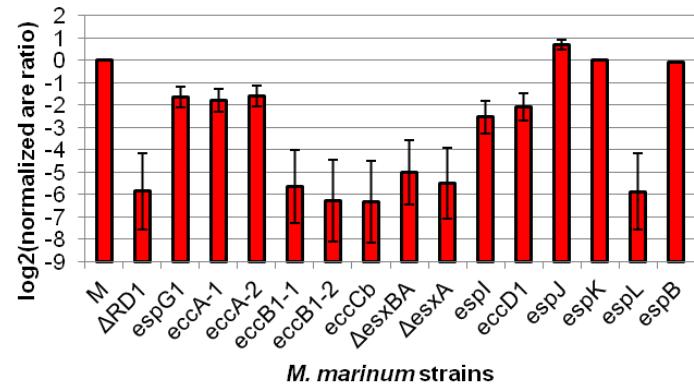
D. Significance of correlations between Esx-1-associated proteins in the CF

EspF	1.007x10 ⁻²²⁴	EspG1	6.841x10 ⁻²³¹	EspH	0.5866	EccA1	1.314x10 ⁻²¹⁷	PPE68	9.521x10 ⁻²³⁵	EsxB	2.553x10 ⁻²²²	EsxA	2.348x10 ⁻²³³	EspJ	3.089x10 ⁻²¹⁷	EspK	2.592x10 ⁻²²⁵	EspL	3.704x10 ⁻¹²	EspB	3.235x10 ⁻²¹⁹	2929 ^S	EsxN ⁵			
EspG1	0.9267		6.841x10 ⁻²³¹		0.5866		1.314x10 ⁻²¹⁷																			
EspH	0.0708																									
EccA1	0.3026		2.002x10 ⁻⁰⁶		0.3381		9.521x10 ⁻²³⁵																			
PPE68	0.0483		0.9267		0.0776		0.9236		2.553x10 ⁻²²²																	
EsxB	3.124x10 ⁻⁰⁷		0.4030		0.0299		0.5080		2.567x10 ⁻⁰³		2.348x10 ⁻²³³															
EsxA	2.562x10 ⁻⁰⁸		0.2830		0.0659		0.3972		0.0127*		2.132x10 ⁻¹²		3.089x10 ⁻²¹⁷													
EspJ	4.576x10 ⁻⁰⁵		0.4336		0.8364		0.5553		0.1432		0.0002		2.592x10 ⁻²²⁵		2.065x10 ⁻²²³											
EspK	0.0004		0.4602		0.9247		0.5524		0.1432		0.0007		0.0001		3.704x10 ⁻¹²		3.235x10 ⁻²¹⁹									
EspL	0.9230		0.0009		0.3067		4.150x10 ⁻⁰⁶		0.8475		0.7925		0.8401		0.5788		0.5599		1.325x10 ⁻²³⁶							
EspB	9.558x10 ⁻⁰⁷		0.4891		0.3067		.5427		0.0746		1.974x10 ⁻⁰⁶		4.994x10 ⁻⁰⁷		1.534x10 ⁻⁰⁶		1.123x10 ⁻⁰⁵		0.6623		5.758x10 ⁻²²¹		2929 ^S			
2929 ^S	0.9671		0.0169		0.2130		1.267E-03		0.7592		0.7613		0.7996		0.6670		0.7109		8.538x10 ⁻⁰⁶		0.7662		9.376x10 ⁻²¹⁹		EsxN ⁵	
EsxN ⁵	0.0696		0.0083		0.9625		2.976E-04		0.3986		0.1045		0.0976		0.2826		0.2989		0.0008		0.1753		0.0001		7.819x10 ⁻²²⁸	

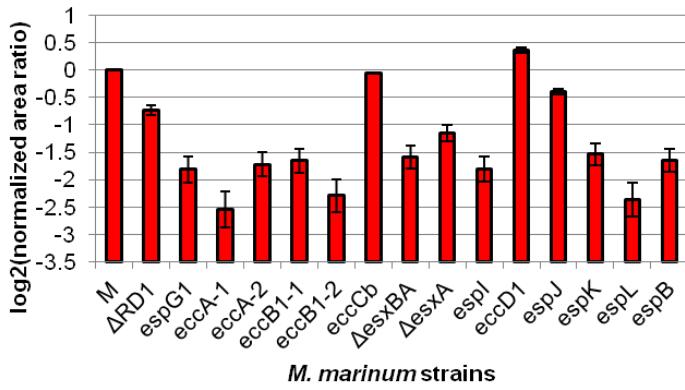
Figure S3: Pearson correlation coefficients and the significance of correlation for all proteins in this study in the CL and CF. Correlation coefficients for Esx-1 associated proteins and controls in the cell lysates (**A**), and the significance of these correlations (**B**) are shown. Correlation coefficients for proteins in the culture filtrates (**C**) and the significance of these correlations (**D**) are shown. Pearson's correlation coefficients (r) were calculated using the area ratios of Esx-1-associated and control (EsxN and MMAR_2929) proteins. The p-values computed by fitting a linear model using R. Shaded grey indicates correlation coefficients that were considered significant; * $p \leq 0.05$, ** $p \leq 0.01$. 5 and S superscripts refer to proteins secreted by the Esx-5 and Sec secretion systems, respectively.

A **EsxA****B** **EsxB****C** **EspB****D** **EspF****E** **EspK****F** **EspJ**

PPE68



EsxN



MMAR_2929

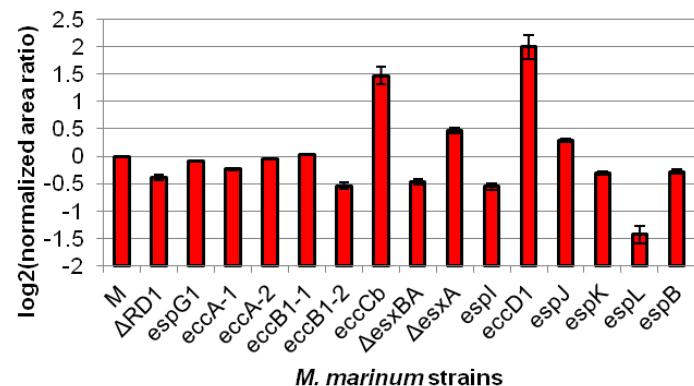


Figure S4: Log 2 transformed normalized area ratios for protein levels in the culture filtrates.

The normalized area ratio data for secreted proteins in the culture filtrate presented in Figures 2 and 3 were log2 transformed in Microsoft Excel and plotted. Error bars represent the average propagated standard error and were calculated as described in the materials and methods section **A) EsxA** **B) EsxB** **C) EspB** **D) EspF** **E) EspK** **F) EspJ** **G) PPE68** **H) EsxN** **I) MMAR_2929**.

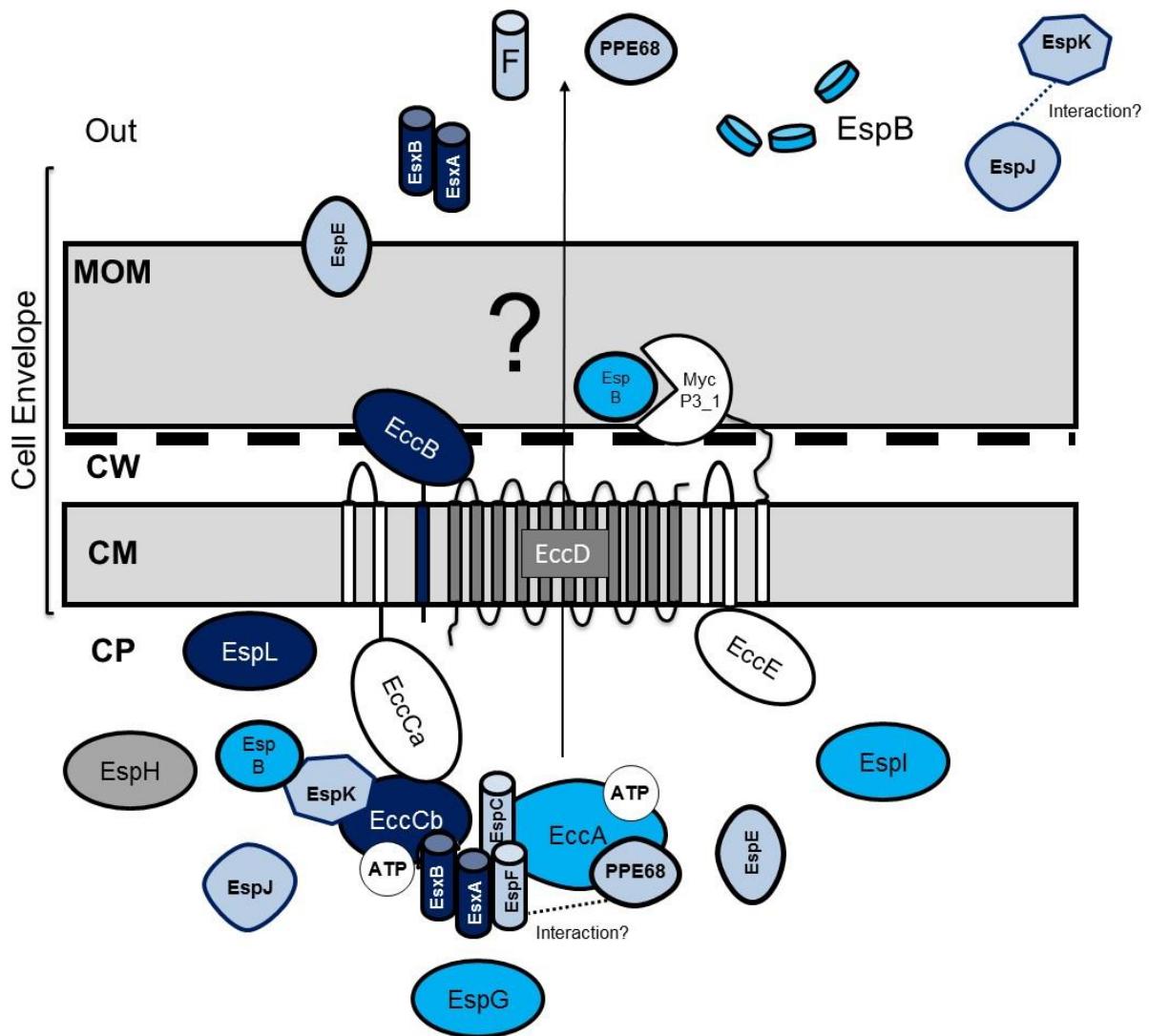


Figure S5. A model of Esx-1 export in *M. marinum*. The Esx-1 exporter promotes the translocation of substrates across the cytoplasmic membrane (CM) of *M. marinum*. The mechanism of transport across the MOM (mycolate outer membrane) is not known, and is indicated with a question mark. The membrane complex includes EccCb, EccD and EccE. EccCa, EccCb and EccA are all AAA ATPase proteins. Substrates include EsxA, EsxB, EspF, EspC (in *M. tb*), EspE, and EspK, EspJ and PPE68, which were defined in this study. Our findings indicate that EspL, EccB, EccCb, EsxA and EsxB (Dark Blue) are required for the export of all esx-1 encoded substrates (light blue). In the absence of EspB, EspG, EccA and Espl (medium blue) the levels of all Esx-1 proteins measured in this study were reduced. We propose that these proteins function as chaperones for esx-1. Proteins in white were not measured in this study. Potential interactions based on the correlation coefficients, which need further testing, are indicated by dotted lines (EspK and EspJ, EspF, PPE68).

Figure S6. The R code used to calculate the significance of correlation

```
# Read in data, compute and save Pearson's correlation scores
data_pel <- read.csv("pellet_data.csv", sep=",", head=T, row.names=1)
cor_data_pel <- as.matrix(cor(data_pel, use="everything", method="pearson"))
write.table(cor_data_pel, "corr_pellet.csv", sep=",")

data_sup <- read.csv("supernatant_data.csv", sep=",", head=T, row.names=1)
cor_data_sup <- as.matrix(cor(data_sup, use="everything", method="pearson"))
write.table(cor_data_sup, "corr_supernatant.csv", sep=",")

# Function to extract p-values from fitting linear models
lmp <- function (modelobject) {
  if (class(modelobject) != "lm") stop("Not an object of class 'lm' ")
  f <- summary(modelobject)$fstatistic
  p <- pf(f[1],f[2],f[3],lower.tail=F)
  attributes(p) <- NULL
  return(p)
}

# Fitting linear models and output a matrix of p-values
a = 1
b = 1
pvalsout_pel = NULL

for (a in 1:length(data_pel[,])) {
  for (b in 1:length(data_pel[,])) {
    pvals = lmp(lm(data_pel[,a] ~ data_pel[,b]))
    pvalsout_pel = c(pvalsout_pel, pvals)
    b = b + 1
  }
  a = a + 1
}

output_pel = matrix(pvalsout_pel, nrow = length(data_pel[,]), ncol =
length(data_pel[,]), byrow=T)
write.table(output_pel, "p-values_pellet.csv", sep = ",")

c = 1
d = 1
pvalsout_sup = NULL

for (c in 1:length(data_sup[,])) {
  for (d in 1:length(data_sup[,])) {
    pvals = lmp(lm(data_sup[,c] ~ data_sup[,d]))
    pvalsout_sup = c(pvalsout_sup, pvals)
    d = d + 1
  }
  c = c + 1
}

output_sup = matrix(pvalsout_sup, nrow = length(data_sup[,]), ncol =
length(data_sup[,]), byrow=T)
write.table(output_sup, "p-values_supernatant.csv", sep = ",")
```