

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
Δ RD1	$\Delta eccCb^2-espK$	Volkman, HE et al. 2004
M1	<i>espG1::Tn</i>	Gao, LY et al. 2004
M2	<i>espH::Tn</i>	Gao, LY et al. 2004
M3	<i>eccA1::Tn</i>	Gao, LY et al. 2004
M4	<i>eccA1::Tn</i>	Gao, LY et al. 2004
6B10	<i>eccB1::Tn</i>	Champion, MM et al. 2012
F11	<i>eccB1::Tn</i>	This study
Mh3871::Tn	<i>eccCb::Tn</i>	Joshi, SA et al. 2012
Mh Δ esxBA	Δ esxBA	Gao, LY et al. 2004
Mh Δ esxA	Δ esxA	Xu, J et al. 2007
M5	<i>espI::Tn</i>	Gao, LY et al. 2004
17	<i>eccD1::Tn</i>	This study
M6	<i>espJ::Tn</i>	Gao, LY et al. 2004
M7	<i>espK::Tn</i>	Gao, LY et al. 2004
2	<i>espL::Tn</i>	This study
M8	<i>espB::Tn</i>	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 *M. marinum* strains

Protein	Fraction	Δ RD1	<i>espG1</i>	<i>eccA-1</i>	<i>eccA-2</i>	<i>eccB-1</i>	<i>eccB-2</i>	<i>eccCb</i>	Δ <i>esxBA</i>	Δ <i>esxA</i>	<i>espi</i>	<i>eccD1</i>	<i>espJ</i>	<i>espK</i>	<i>espl</i>	<i>espB</i>
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	0.0052	-	-
	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
<i>MMAR_5453 (espJ)</i>	100	100	100	<i>rv3878 (espJ)</i>	33	43	100
<i>MMAR_5455 (espK)</i>	100	100	100	<i>rv3879c (espK)</i>	50	58	99
<i>MMAR_4352</i>	29	38	90	<i>rv3878 (espJ)</i>	41	52	98
<i>MMAR_4351</i>	43	52	100	<i>rv3879c(espK)</i>	46	56	99
<i>MMAR_0197</i>	36	52	33	<i>rv3878(espJ)</i>	41	53	80
<i>MMAR_0199</i>	33	43	99	<i>rv3879c(espK)</i>	33	45	99
<i>MMAR_0200</i>	38	47	38	<i>rv3879c(espK)</i>	29	44	86
<i>MMAR_1328</i>	31	42	69	<i>rv3878(espJ)</i>	51	62	50
<i>MMAR_1331</i>	35	43	99	<i>rv3879c(espK)</i>	30	40	98
<i>MMAR_5425</i> "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_VSSEPVGEHAQAASAQGGQGMGMPASAGSK.T1	745.3	999.5	12	42.3
	CFP10_GAAGTAAQAAVVR.T2	571.8	785.5	12	33.6	EspA_VSSEPVGEHAQAASAQGGQGMGMPASAGSK.T2	745.3	929.4	12	42.3
	CFP10_GAAGTAAQAAVVR.T3	571.8	714.4	12	33.6	EspA_VSSEPVGEHAQAASAQGGQGMGMPASAGSK.T3	745.3	858.4	12	42.3
	CFP10_TQIDQVESTAGSLQAQWR.T1	673.3	945.5	12	38.7	EspL_IDDGLLKEVGEPEVVSAR.T1	599.7	913.5	12	35
	CFP10_TQIDQVESTAGSLQAQWR.T2	673.3	688.4	12	38.7	EspL_IDDGLLKEVGEPEVVSAR.T2	599.7	814.4	12	35
	CFP10_TQIDQVESTAGSLQAQWR.T3	673.3	888.5	12	38.7	EspL_IDDGLLKEVGEPEVVSAR.T3	599.7	757.4	12	35
	CFP10_ADDEQQALSSQMGF.T1	827.9	999.4	12	46.4	EspL_FQSALDGTINQMNTGNFR.T1	672	967.4	12	38.6
	CFP10_ADDEQQALSSQMGF.T2	827.9	886.4	12	46.4	EspL_FQSALDGTINQMNTGNFR.T2	672	839.4	12	38.6
	CFP10_ADDEQQALSSQMGF.T3	827.9	840.4	12	46.4	EspL_FQSALDGTINQMNTGNFR.T3	672	708.3	12	38.6
	#	ESAT6_LAAAWGGSGSEAYR.T1	465.9	682.3	12	28.3	EspL_GKDDTETVEVINGHQLTAVR.T1	618.6	975.4	12
ESAT6_LAAAWGGSGSEAYR.T2		465.9	538.3	12	28.3	EspL_GKDDTETVEVINGHQLTAVR.T2	618.6	747.3	12	35.9
ESAT6_LAAAWGGSGSEAYR.T3		465.9	883.4	12	28.3	EspL_GKDDTETVEVINGHQLTAVR.T3	618.6	699.4	12	35.9
ESAT6_GVQQNWDSAQELNNSLQNLAR.T1		829.4	915.5	12	46.5	EspL_MEMDPQVAQVLAALAAAR.T1	581.6	912.6	12	34.1
ESAT6_GVQQNWDSAQELNNSLQNLAR.T2		829.4	801.5	12	46.5	EspL_MEMDPQVAQVLAALAAAR.T2	581.6	841.5	12	34.1
ESAT6_GVQQNWDSAQELNNSLQNLAR.T3		829.4	714.4	12	46.5	EspL_MEMDPQVAQVLAALAAAR.T3	581.6	614.4	12	34.1
5455_EspK_GIPRPTGEYAGR.T1		425.2	552.3	13	26.3	CAP_AGIFQGVPEPVAALTK.T1	815	827.5	12	45.7
5455_EspK_GIPRPTGEYAGR.T2		425.2	850.4	13	26.3	CAP_AGIFQGVPEPVAALTK.T2	815	956.5	12	45.7
5455_EspK_GIPRPTGEYAGR.T3		425.2	466.2	13	26.3	CAP_AGIFQGVPEPVAALTK.T3	815	1112.6	12	45.7
5455_EspK_VLHFLFDVMDAcR.T1		526.3	652.3	13	31.3	CAP_AWIADRPEIAEQLLR.T1	594.3	762.4	12	34.7
5455_EspK_VLHFLFDVMDAcR.T2	526.3	826.4	13	31.3	CAP_AWIADRPEIAEQLLR.T2	594.3	705.9	12	34.7	
5455_EspK_VLHFLFDVMDAcR.T3	526.3	925.5	13	31.3	CAP_AWIADRPEIAEQLLR.T3	594.3	670.4	12	34.7	
5455_EspK_KAAIASLIR.T1	471.8	814.5	13	28.6	CAP_TSSATTITEVR.T1	583.3	977.5	12	34.2	
5455_EspK_KAAIASLIR.T2	471.8	743.5	13	28.6	CAP_TSSATTITEVR.T2	583.3	819.5	12	34.2	
5455_EspK_KAAIASLIR.T3	471.8	672.4	13	28.6	CAP_TSSATTITEVR.T3	583.3	718.4	12	34.2	
EspB_ADLEPVNPPKPPAAIK.T1	553	614.9	13	32.6	EspG_AAAGVLDSAHGR.T1	562.8	755.4	12	33.1	
EspB_ADLEPVNPPKPPAAIK.T2	553	918.6	13	32.6	EspG_AAAGVLDSAHGR.T2	562.8	982.5	12	33.1	
EspB_ADLEPVNPPKPPAAIK.T3	553	735.9	13	32.6	EspG_AAAGVLDSAHGR.T3	562.8	642.3	12	33.1	
EspB_GHPTLADIVELER.T1	483.9	805.4	13	29.2	EspG_YGLTPTTAR.T1	490.3	816.5	12	29.5	
EspB_GHPTLADIVELER.T2	483.9	692.3	13	29.2	EspG_YGLTPTTAR.T2	490.3	646.4	12	29.5	
EspB_GHPTLADIVELER.T3	483.9	645.4	13	29.2	EspG_YGLTPTTAR.T3	490.3	545.3	12	29.5	
EspB_VAAAGESDFDCLK.T1	662.3	954.4	13	38.1	EspG_LYTEIVTNP.K.T1	589.3	901.5	12	34.5	
EspB_VAAAGESDFDCLK.T2	662.3	825.4	13	38.1	EspG_LYTEIVTNP.K.T2	589.3	671.4	12	34.5	
EspB_VAAAGESDFDCLK.T3	662.3	738.4	13	38.1	EspG_LYTEIVTNP.K.T3	589.3	800.5	12	34.5	
EspF_Ac-TGLLNVVPSFLK.T1	665.4	903.5	12	36	EsxN_AAASLEAEHQAIVR.T1	697.9	923.5	12	39.9	
EspF_Ac-TGLLNVVPSFLK.T2	665.4	1016.6	12	36	EsxN_AAASLEAEHQAIVR.T2	697.9	852.5	12	39.9	
EspF_Ac-TGLLNVVPSFLK.T3	665.4	690.4	12	36	EsxN_AAASLEAEHQAIVR.T3	697.9	1052.5	12	39.9	
EspF_NSAGTGLQGVGTGK.T1	595.3	917.5	12	34.8	EsxN_AQAASLEAEHQAIVR.T1	531.9	923.5	12	31.6	
EspF_NSAGTGLQGVGTGK.T2	595.3	988.5	12	34.8	EsxN_AQAASLEAEHQAIVR.T2	531.9	697.9	12	31.6	
EspF_NSAGTGLQGVGTGK.T3	595.3	1075.6	12	34.8	EsxN_AQAASLEAEHQAIVR.T3	531.9	662.4	12	31.6	
EspF_SATNVVSGIGSR.T1	574.3	989.5	12	33.7	5443_LLAEAQEELDR.T1	643.8	989.5	12	37.2	
EspF_SATNVVSGIGSR.T2	574.3	888.5	12	33.7	5443_LLAEAQEELDR.T2	643.8	1060.5	12	37.2	
EspF_SATNVVSGIGSR.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_VVANMLAGLVIAEPK.T1	528	954.6	12	31.4	
PPE68_GESLPGAGGTLTR.T2	608.3	732.4	12	35.4	5443_VVANMLAGLVIAEPK.T2	528	713.4	12	31.4	
PPE68_GESLPGAGGTLTR.T3	608.3	942.5	12	35.4	5443_VVANMLAGLVIAEPK.T3	528	1067.6	12	31.4	
PPE68_MLWHAMPPELNTAR.T1	556.3	770.3	12	32.8	5443_LLETNEGLR.T1	522.8	931.5	12	31.1	
PPE68_MLWHAMPPELNTAR.T2	556.3	897.5	12	32.8	5443_LLETNEGLR.T2	522.8	818.4	12	31.1	
PPE68_MLWHAMPPELNTAR.T3	556.3	800.4	12	32.8	5443_LLETNEGLR.T3	522.8	689.4	12	31.1	
PPE68_LNSLGEAWTGGGSEK.T1	753.4	892.4	12	42.7	5443_DGRTPDFGQEAAMDLLAR.T1	665	974.4	12	38.2	
PPE68_LNSLGEAWTGGGSEK.T2	753.4	821.4	12	42.7	5443_DGRTPDFGQEAAMDLLAR.T2	665	890.5	12	38.2	
PPE68_LNSLGEAWTGGGSEK.T3	753.4	1191.6	12	42.7	5443_DGRTPDFGQEAAMDLLAR.T3	665	819.4	12	38.2	
GroES_EKPQEGTVAVGPGR.T1	508.6	869.4	12	30.4	5446_LGDVNETQIDR.T1	630.3	974.5	12	36.5	
GroES_EKPQEGTVAVGPGR.T2	508.6	556.3	12	30.4	5446_LGDVNETQIDR.T2	630.3	875.4	12	36.5	
GroES_EKPQEGTVAVGPGR.T3	508.6	968.5	12	30.4	5446_LGDVNETQIDR.T3	630.3	761.4	12	36.5	
GROES_YGGEYLLSAR.T1	685.8	964.5	12	39.3	5446_VVAEMQAVMR.T1	567.3	935.4	12	33.4	
GROES_YGGEYLLSAR.T2	685.8	835.5	12	39.3	5446_VVAEMQAVMR.T2	567.3	864.4	12	33.4	
GroEL_AEIENSDDSDYDR.T1	707.3	971.4	12	37	5446_VVAEMQAVMR.T3	567.3	735.4	12	33.4	
GroEL_AEIENSDDSDYDR.T2	707.3	1100.4	12	37	5446_VGSIAMYR.T1	448.7	797.4	12	27.4	
MMAR2929_TPDIAIQDIHTLGEK.T1	855.4	1212.6	12	45	5446_VGSIAMYR.T2	448.7	740.4	12	27.4	
MMAR2929_TPDIAIQDIHTLGEK.T2	855.4	1141.6	12	45	5446_VGSIAMYR.T3	448.7	653.3	12	27.4	
GroEL2_DETTIVEGAGSDAIAGR.T1	888.9	1217.6	12	45	EspR_GPHTSAEVIAALK.T1	431.9	779.4	12	26.6	
GroEL2_DETTIVEGAGSDAIAGR.T2	888.9	1118.5	12	45	EspR_GPHTSAEVIAALK.T2	431.9	680.3	12	26.6	
EspJ_AEPLAVDPAR.T1	519.8	838.5	12	31	EspR_GPHTSAEVIAALK.T3	431.9	515.4	12	26.6	
EspJ_AEPLAVDPAR.T2	519.8	741.4	12	31	EspR_SQGLSTQAQQEIVER.T1	837.4	972.5	12	46.9	
EspJ_AEPLAVDPAR.T3	519.8	628.3	12	31	EspR_SQGLSTQAQQEIVER.T2	837.4	901.5	12	46.9	
EspJ_TASSMSTAADIYAK.T1	472.9	680.4	12	28.6	EspR_SQGLSTQAQQEIVER.T3	837.4	773.4	12	46.9	
EspJ_TASSMSTAADIYAK.T2	472.9	609.3	12	28.6	EspR_TNPSTATMAALANFFR.T1	571.6	909.5	12	33.6	
EspJ_TASSMSTAADIYAK.T3	472.9	939.5	12	28.6	EspR_TNPSTATMAALANFFR.T2	571.6	838.5	12	33.6	
EspE_APIDAGSNTGGNEGTLT.T1	857.9	989.5	12	47.9	EspR_TNPSTATMAALANFFR.T3	571.6	654.3	12	33.6	
EspE_APIDAGSNTGGNEGTLT.T2	857.9	1103.5	12	47.9	5444_ATNQLYVLLSGQLHPVYNLSAR.T1	853.5	1193.6	12	47.7	
EspE_APIDAGSNTGGNEGTLT.T3	857.9	1190.6	12	47.9	5444_ATNQLYVLLSGQLHPVYNLSAR.T2	853.5	1016.1	12	47.7	
EspE_MIDGVYK.T1	413.2	694.4	12	25.7	5444_ATNQLYVLLSGQLHPVYNLSAR.T3	853.5	1072.6	12	47.7	
EspE_MIDGVYK.T2	413.2	581.3	12	25.7	EccE_IADGLASNGVDVAVCGR.T1	787.9	1183.6	12	44.4	
EspE_MIDGVYK.T3	413.2	466.3	12	25.7	EccE_IADGLASNGVDVAVCGR.T2	787.9	1105.5	12	44.4	
EspH_AANMSESALEAEEFVIADLAR.T1	741	904.5	12	42.1	EccE_IADGLASNGVDVAVCGR.T3	787.9	1034.5	12	44.4	
EspH_AANMSESALEAEEFVIADLAR.T2	741	946.4	12	42.1	EspE_LLEDLLSVHCPDLEADVVSAGYR.T1	857.8	1173.1	12	47.9	
EspH_AANMSESALEAEEFVIADLAR.T3	741	757.5	12	42.1	EccE_LLEDLLSVHCPDLEADVVSAGYR.T3	857.8	937.9	12	47.9	
EspH_YEVDYTSR.T1	516.7	869.4	12	30.8	EccE_YLVSATR.T1	440.7	604.3	12	27	
EspH_YEVDYTSR.T2	516.7	740.4	12	30.8	EccE_YLVSATR.T2	440.7	505.3	12	27	
EspH_YEVDYTSR.T3	516.7	641.3	12	30.8	EccE_YLVSATR.T3	440.7	717.4	12	27	
EspH_AAQHTFMVEAMASELSEDETEEGALLR.T1	989.1	1146.6	12	54.5						
EspH_AAQHTFMVEAMASELSEDETEEGALLR.T2	989.1	886.4	12	54.5						
EspH_AAQHTFMVEAMASELSEDETEEGALLR.T3	989.1	916.5	12	54.5						

* CFP-10 is synonymous with EsxB

ESAT-6 is synonymous with EsxA

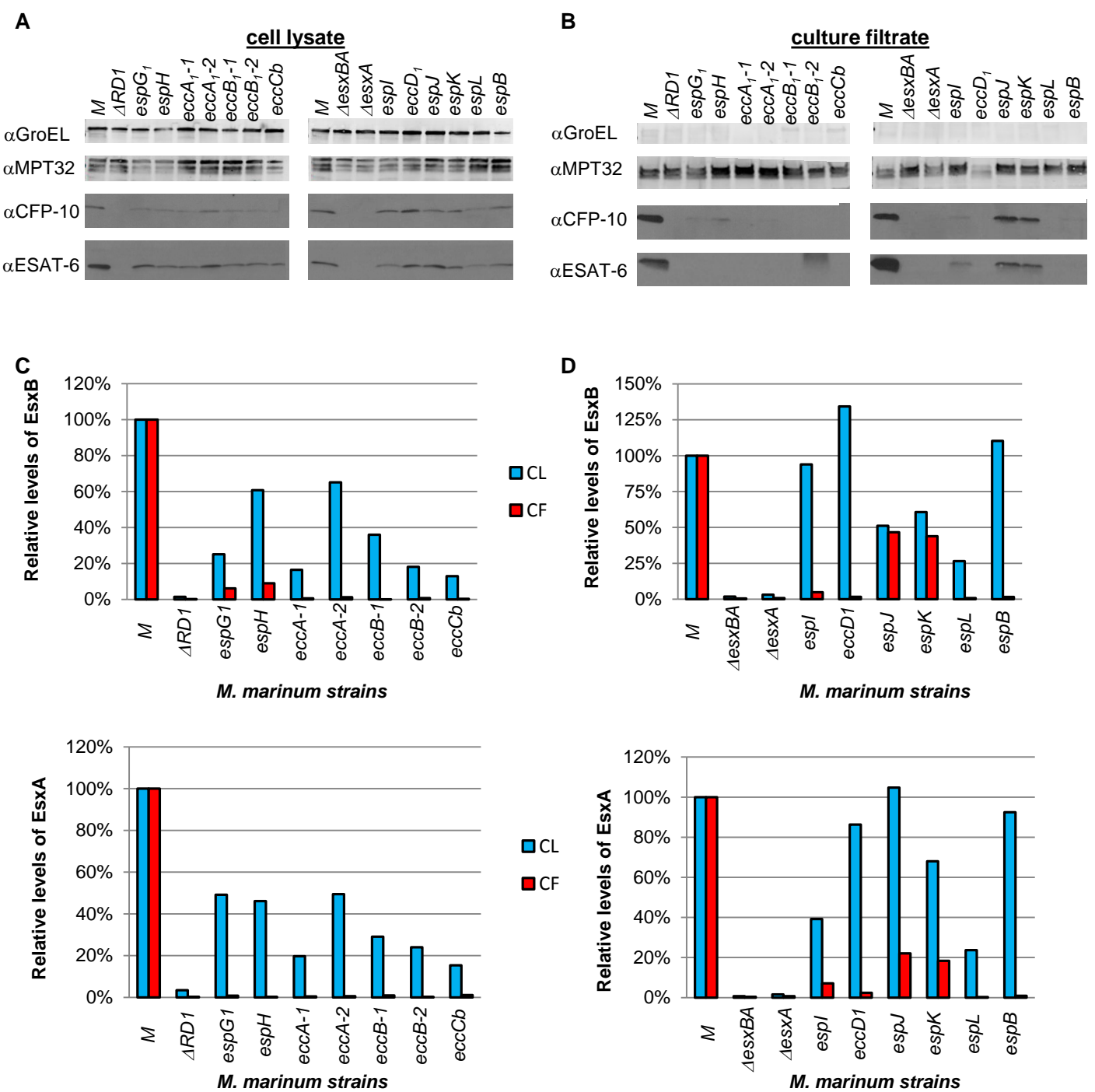
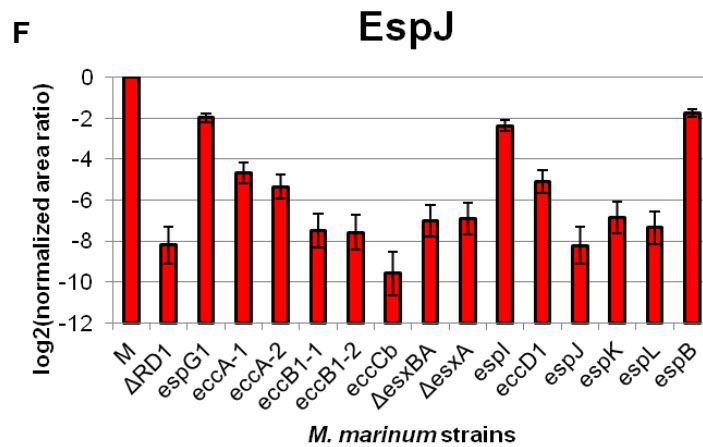
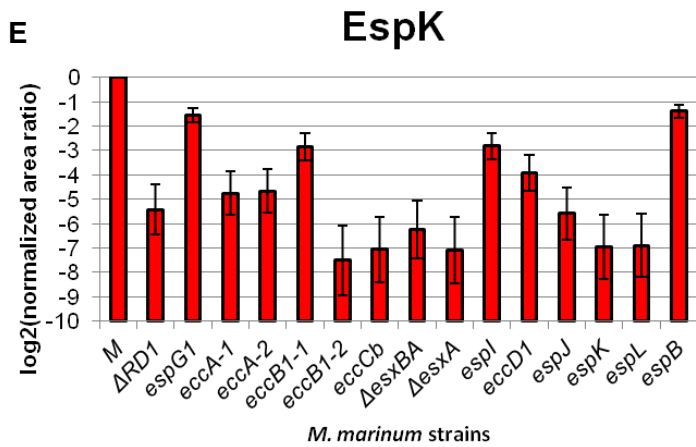
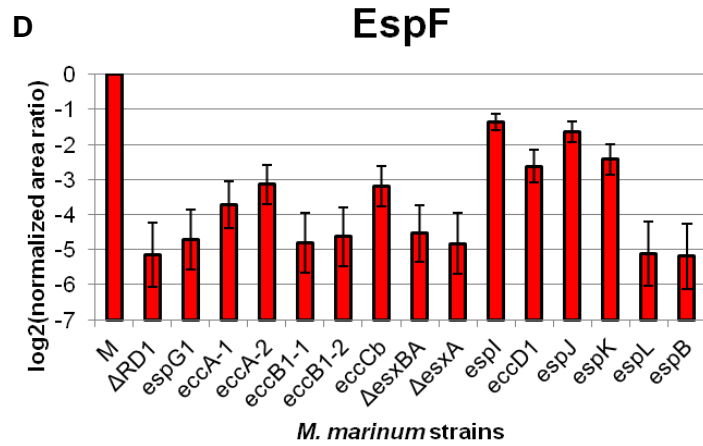
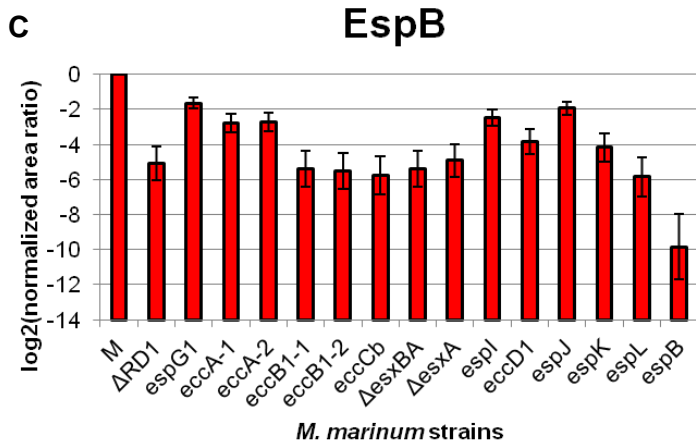
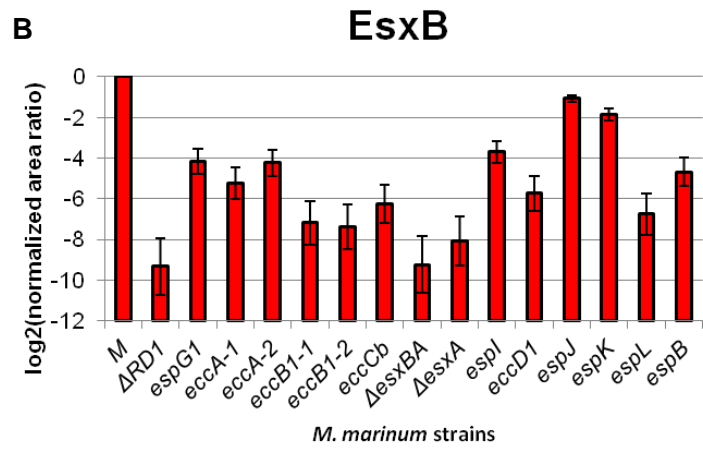
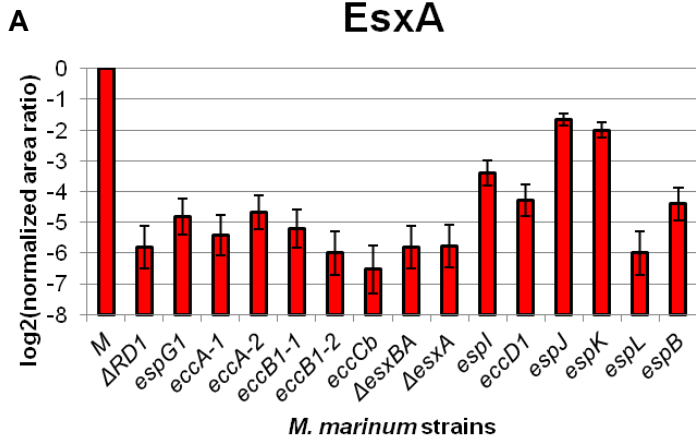


Figure S2: Western blot analysis for the production and secretion of EsxA and EsxB. **A)** Western blot analysis of cell lysates and **B)** culture filtrates generated from WT *M. marinum* M strain and the ESX-1-deficient strains bearing transposon insertions or deletions in the extended RD1 locus. MPT32 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.



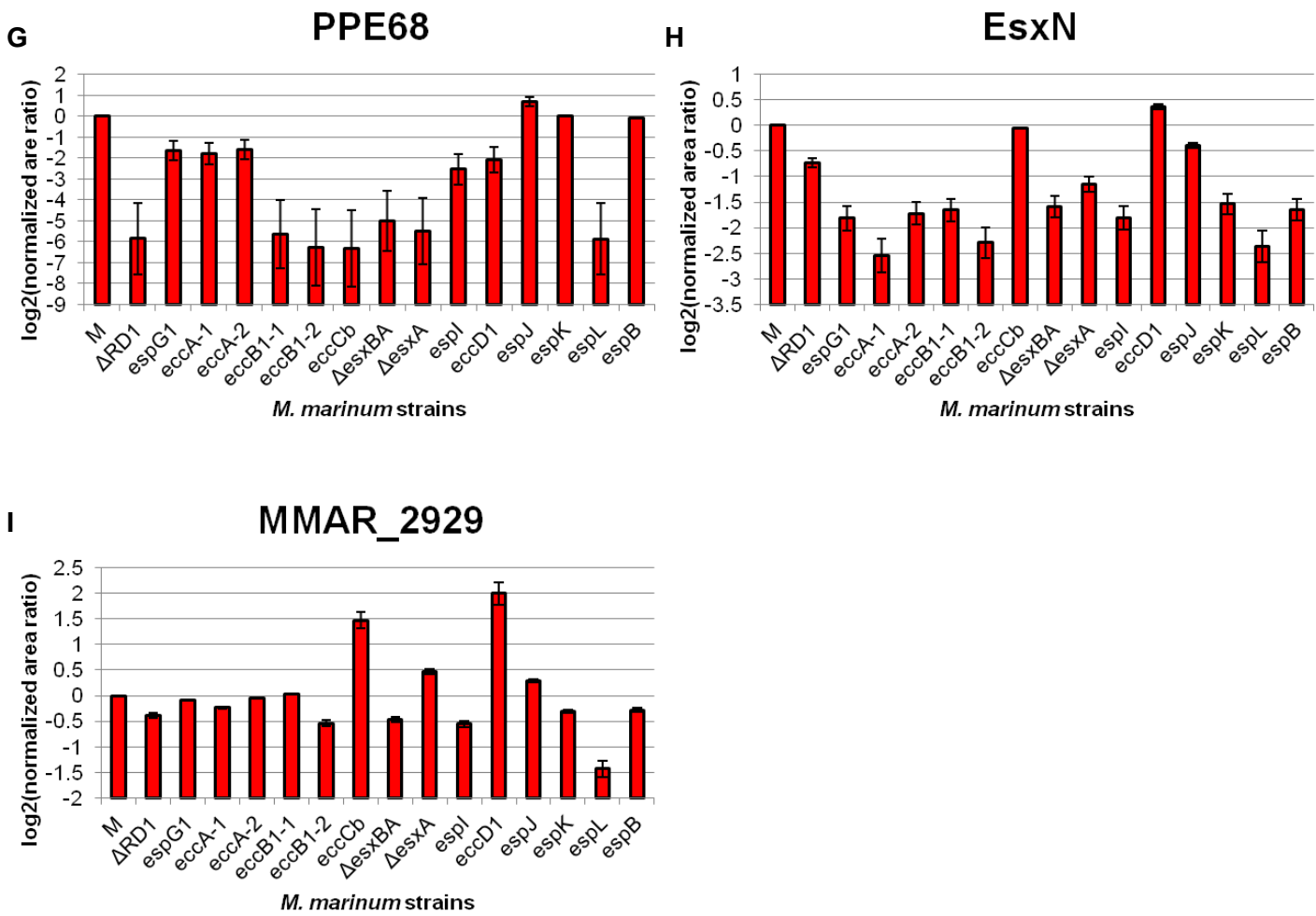


Figure S4: Log 2 transformed normalized area ratios for protein levels in the culture filtrates.
 The normalized area ratio data for secreted proteins in the culture filtrate presented in Figures 2 and 3 were log₂ transformed in Microsoft Excel and plotted. Error bars represent the average propagated 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.**

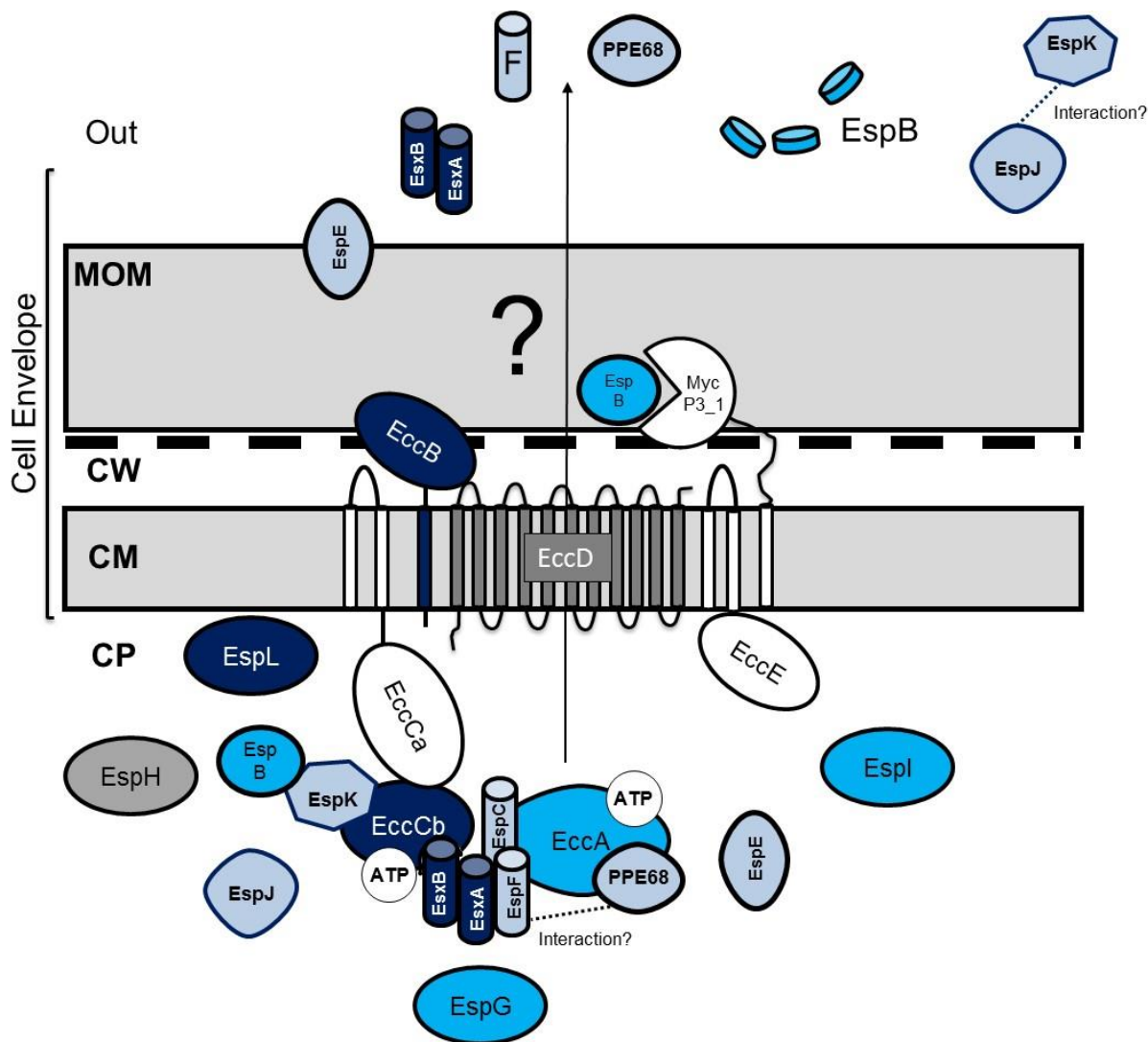


Figure S5. A model of Esx-1 export in *M. marinum*. The Esx-1 exporter promotes the translocation of substrates across the cytoplasmic membrane (CM) of *M. marinum*. The mechanism of transport across the MOM (mycolate outer membrane) is not known, and is indicated with a question mark. The membrane complex 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 EspI (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[1,])){
  for (b in 1:length(data_pel[1,])){
    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[1,]), ncol =
length(data_pel[1,]), 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[1,])){
  for (d in 1:length(data_sup[1,])){
    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[1,]), ncol =
length(data_sup[1,]), byrow=T)
write.table(output_sup, "p-values_supernatant.csv", sep = ",")
```