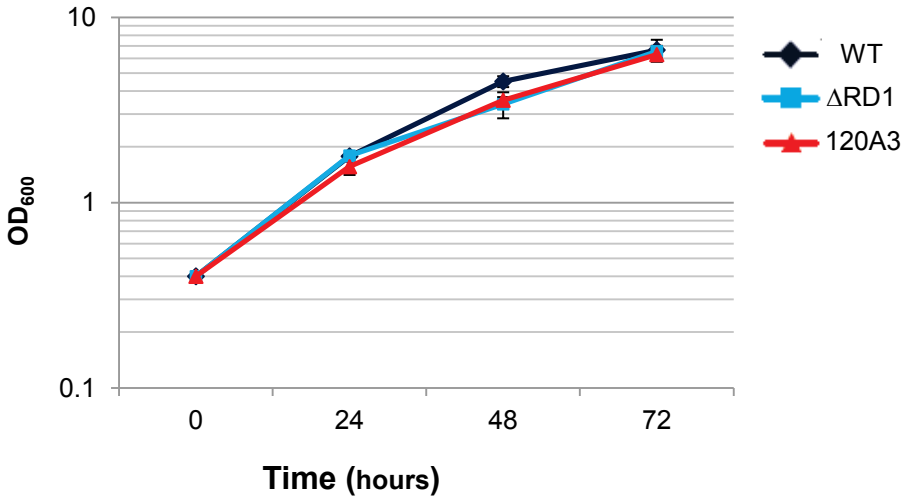


Figure S1: The 120A3 strain does not have a growth defect *in vitro*. Strains were grown until late exponential phase, diluted to an optical density (OD_{600}) of 0.4 and grown for 72 hours in Middlebrook 7H9. Growth curves were generated by OD_{600} measurements and plotted against time.

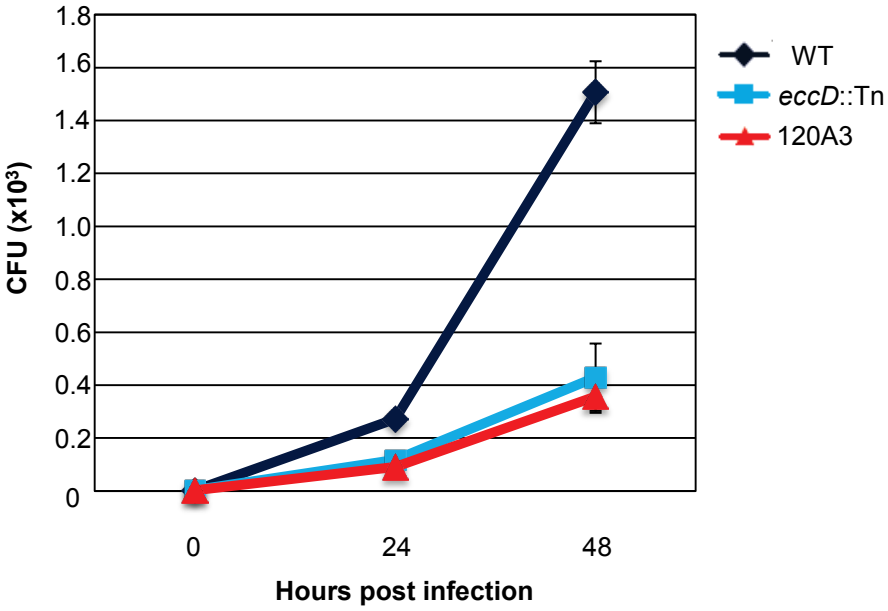


Figure S2: The 120A3 strain is attenuated for growth in RAW246.7 cells. RAW246.7 cells were infected at an MOI of 1 for 2 hours after which cells were treated with gentamicin (100 µg/mL) for two hours. Treated cells were washed and resuspended in fresh media. Bacteria were harvested and plated for Colony Forming Units (CFU) at the indicated time points. Error bars represent the standard deviation; experiments were performed in triplicate.

Figure S3: MRM transitions used in this study

N	Q1 (m/z)	Q3 (m/z)	Dwell (ms)	Protein_Peptide_Transition#	CE (v)
1	571.817	943.532	5	CFP10_GAAGTAAQAAVVR.T1	33.6
2	571.817	785.463	5	CFP10_GAAGTAAQAAVVR.T2	33.6
3	571.817	714.426	5	CFP10_GAAGTAAQAAVVR.T3	33.6
4	673.336	945.49	8	CFP10_TQIDQVESTAGSLQAQWR.T1	38.7
5	673.336	688.352	8	CFP10_TQIDQVESTAGSLQAQWR.T2	38.7
6	673.336	888.469	8	CFP10_TQIDQVESTAGSLQAQWR.T3	38.7
7	827.854	999.438	8	CFP10_ADDEQQQALSSQMGF.T1	46.4
8	827.854	886.354	8	CFP10_ADDEQQQALSSQMGF.T2	46.4
9	827.854	840.392	8	CFP10_ADDEQQQALSSQMGF.T3	46.4
10	829.406	915.501	10	ESAT6_GVQQNWDSTAQELNNSLQNLAR.T1	46.5
11	829.406	801.458	10	ESAT6_GVQQNWDSTAQELNNSLQNLAR.T2	46.5
12	829.406	714.426	10	ESAT6_GVQQNWDSTAQELNNSLQNLAR.T3	46.5
13	619.322	956.468	10	EspB_SQPQTVTVDQQEILNR.T1	35
14	619.322	644.373	12	EspB_SQPQTVTVDQQEILNR.T2	35
15	619.322	772.431	12	EspB_SQPQTVTVDQQEILNR.T3	35
16	465.9	682.3	15	ESAT6_LAAAWGGSGSEAYR.Light.T1	20
17	465.9	538.3	15	ESAT6_LAAAWGGSGSEAYR.Light.T2	20
18	465.9	769.35	15	ESAT6_LAAAWGGSGSEAYR.Light.T3	20
19	469.227	692.315	15	ESAT6_LAAAWGGSGSEAYR.Heavy.T1	20
20	469.227	548.262	15	ESAT6_LAAAWGGSGSEAYR.Heavy.T2	20
21	469.227	779.347	15	ESAT6_LAAAWGGSGSEAYR.Heavy.T3	20
22	508.611	556.32	12	GroES_EKPQEGTVVAVGPGR.Light.T1	22
23	508.611	869.436	12	GroES_EKPQEGTVVAVGPGR.Light.T2	22
24	508.611	655.389	12	GroES_EKPQEGTVVAVGPGR.Light.T3	22
25	511.944	566.32	12	GroES_EKPQEGTVVAVGPGR.Heavy.T1	22
26	511.944	869.436	12	GroES_EKPQEGTVVAVGPGR.Heavy.T2	22
27	511.944	665.389	12	GroES_EKPQEGTVVAVGPGR.Heavy.T3	22
28	644.987	951.537	12	EsxN_Ac-TINYQFGDVDAHGALIR.T2	37
29	644.987	737.442	12	EsxN_Ac-TINYQFGDVDAHGALIR.T3	37
30	665.393	903.53	12	EspF_Ac-TGLLNVVPSFLK.T1	36
31	665.393	1016.614	12	EspF_Ac-TGLLNVVPSFLK.T2	36
32	665.393	690.419	12	EspF_Ac-TGLLNVVPSFLK.T3	36
33	949.484	998.479	12	EspB_Ac-SQPQTVTVDQQEILNR.T1	48
34	949.484	820.934	12	EspB_Ac-SQPQTVTVDQQEILNR.T2	48
35	949.484	900.49	12	EspB_Ac-SQPQTVTVDQQEILNR.T3	48
36	630.7	852.469	12	EsxN_TINYQFGDVDAHGALIR.T1	36
37	630.7	951.537	12	EsxN_TINYQFGDVDAHGALIR.T2	36
38	630.7	737.442	12	EsxN_TINYQFGDVDAHGALIR.T3	36
39	425.226	552.283	12	5455_EspK_GIPRPTGEYAGR.T1	26.3
40	425.226	850.405	12	5455_EspK_GIPRPTGEYAGR.T2	26.3
41	425.226	466.241	12	5455_EspK_GIPRPTGEYAGR.T3	26.3
42	526.258	652.254	12	5455_EspK_VLHLFTDVMDAcR.T1	31.3
43	526.258	826.446	12	5455_EspK_VLHLFTDVMDAcR.T2	31.3
44	526.258	925.514	12	5455_EspK_VLHLFTDVMDAcR.T3	31.3
45	471.808	814.515	12	5455_EspK_KAAIASLIR.T1	28.6
46	471.808	743.477	12	5455_EspK_KAAIASLIR.T2	28.6
47	471.808	672.44	12	5455_EspK_KAAIASLIR.T3	28.6
48	552.982	614.874	12	EspB_ADLEPVNPPKPPAAIK.T1	32.6
49	552.982	918.577	12	EspB_ADLEPVNPPKPPAAIK.T2	32.6
50	552.982	735.938	12	EspB_ADLEPVNPPKPPAAIK.T3	32.6
51	483.928	805.42	12	EspB_GHPTLADIVELER.T1	29.2
52	483.928	692.336	12	EspB_GHPTLADIVELER.T2	29.2
53	483.928	645.357	12	EspB_GHPTLADIVELER.T3	29.2
54	662.325	954.442	12	EspB_VAAAGESDFTDLK.T1	38.1
55	662.325	825.399	12	EspB_VAAAGESDFTDLK.T2	38.1
56	662.325	738.367	12	EspB_VAAAGESDFTDLK.T3	38.1
57	595.312	917.505	12	EspF_NSAGTGLQGVTGK.T1	34.8
58	595.312	988.542	12	EspF_NSAGTGLQGVTGK.T2	34.8
59	595.312	1075.574	12	EspF_NSAGTGLQGVTGK.T3	34.8

N	Q1 (m/z)	Q3 (m/z)	Dwell (ms)	Protein_Peptide_Transition#	CE (v)
60	644.387	903.53	12	EspF_TGLLNVVPSFLK.T1	36
61	644.387	1016.614	12	EspF_TGLLNVVPSFLK.T2	36
62	644.387	690.419	12	EspF_TGLLNVVPSFLK.T3	36
63	574.307	989.537	12	EspF_SATNVVSGIGSR.T1	33.7
64	574.307	888.49	12	EspF_SATNVVSGIGSR.T2	33.7
65	574.307	1060.574	12	EspF_SATNVVSGIGSR.T3	33.7
66	608.32	829.453	12	PPE68_GESLPGAGGTLTR.T1	35.4
67	608.32	732.4	12	PPE68_GESLPGAGGTLTR.T2	35.4
68	608.32	942.537	12	PPE68_GESLPGAGGTLTR.T3	35.4
69	753.365	892.416	12	PPE68_LNSLGEAWTGGGSEK.T1	42.7
70	753.365	821.379	12	PPE68_LNSLGEAWTGGGSEK.T2	42.7
71	753.365	1191.564	12	PPE68_LNSLGEAWTGGGSEK.T3	42.7
72	685.85	964.55	12	GROES_YGGEEYLILSAR.T1	39.3
73	685.85	835.5	12	GROES_YGGEEYLILSAR.T2	39.3
74	888.92	1217.58	12	GroEL2_DETTIVEGAGDSDAIAGR.T1	45
75	888.92	1118.51	12	GroEL2_DETTIVEGAGDSDAIAGR.T2	45
76	519.782	838.478	12	EspJ_AEPLAVDPAR.T1	31
77	519.782	741.425	12	EspJ_AEPLAVDPAR.T2	31
78	519.782	628.341	12	EspJ_AEPLAVDPAR.T3	31
79	472.894	680.361	12	EspJ_TASSMSTAADIYAK.T1	28.6
80	472.894	609.324	12	EspJ_TASSMSTAADIYAK.T2	28.6
81	472.894	939.478	12	EspJ_TASSMSTAADIYAK.T3	28.6
82	573.295	1220.622	12	2929_TPDAIAQDIHTTLGEK.Heavy.3T1	30
83	573.295	1149.585	12	2929_TPDAIAQDIHTTLGEK.Heavy.3T2	30
84	573.295	1021.526	12	2929_TPDAIAQDIHTTLGEK.Heavy.3T3	30
85	570.625	1212.622	12	2929_TPDAIAQDIHTTLGEK.Light.3T1	30
86	570.625	1141.585	12	2929_TPDAIAQDIHTTLGEK.Light.3T2	30
87	570.625	1013.526	12	2929_TPDAIAQDIHTTLGEK.Light.3T3	30
88	1137.36	1203.1	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.2y24	50
89	1137.36	1238.4	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.2y25	50
90	1137.36	1369.2	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.2y27	50
91	1137.36	1461.8	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.2y28	50
92	1137.36	1311.7	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.2y26	50
93	1137.36	1477.3	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.y14	50
94	1134.693	1199.1	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.2y24	50
95	1134.693	1234.73	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.2y25	50
96	1134.693	1365.2	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.2y27	50
97	1134.693	1458.2	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.2y28	50
98	1134.693	1308.2	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.2y26	50
99	1134.693	1469.8	20	ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.y14	50
100	1151.2	1203.1	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.2y24	50
101	1151.2	1238.4	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.2y25	50
102	1151.2	1369.2	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.2y27	50
103	1151.2	1461.8	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.2y28	50
104	1151.2	1311.7	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.2y26	50
105	1151.2	1477.3	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Heavy.y14	50
106	1148.6	1199.1	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.2y24	50
107	1148.6	1234.73	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.2y25	50
108	1148.6	1365.2	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.2y27	50
109	1148.6	1458.2	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.2y28	50
110	1148.6	1308.2	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.2y26	50
111	1148.6	1469.8	20	[Ac]ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.Light.y14	50

Figure S4: Raw data for absolute quantification of EsxA using heavy isotope dilution

Cell Lysate

ESAT6_LAAAWGGSGSEAYR.T1

Sample Name	Light Area	AQUA Area	L/H Ratio	Stats	
120A3 Pellet A	3.46E+05	1.84E+05	1.88E+00	1.73E+00	Average
120A3 Pellet A	5.06E+05	3.00E+05	1.69E+00	0.1428262	StDev
120A3 Pellet A	5.98E+05	3.72E+05	1.61E+00	8.3%	% CV
3871 Pellet A	1.56E+05	4.13E+05	3.77E-01	4.05E-01	Average
3871 Pellet A	1.51E+05	3.76E+05	4.01E-01	0.0295697	StDev
3871 Pellet A	1.52E+05	3.49E+05	4.36E-01	7.3%	% CV
3877 Pellet A	6.71E+05	2.81E+05	2.39E+00	2.33E+00	Average
3877 Pellet A	8.43E+05	3.48E+05	2.42E+00	0.137512	StDev
3877 Pellet A	8.59E+05	3.96E+05	2.17E+00	5.9%	% CV
C3 Pellet A	6.50E+05	4.38E+05	1.48E+00	1.45E+00	Average
C3 Pellet A	6.59E+05	4.60E+05	1.43E+00	0.0337578	StDev
C3 Pellet A	5.86E+05	4.12E+05	1.42E+00	2.3%	% CV
WT Pellet A	4.70E+05	2.76E+05	1.70E+00	1.69E+00	Average
WT Pellet A	6.43E+05	3.86E+05	1.67E+00	0.0206955	StDev
WT Pellet A	7.01E+05	4.11E+05	1.71E+00	1.2%	% CV

ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK2y27

Sample Name	Light Area	AQUA Area	L/H Ratio	Stats	
120A3 Pellet A	4.70E+04	4.25E+04	1.10E+00	8.83E-01	Average
120A3 Pellet A	4.58E+04	5.20E+04	8.81E-01	0.2206008	StDev
120A3 Pellet A	4.19E+04	6.31E+04	6.63E-01	25.0%	% CV
3871 Pellet A	1.59E+04	6.98E+04	2.27E-01	2.27E-01	Average
3871 Pellet A	1.39E+04	5.75E+04	2.41E-01	0.0140277	StDev
3871 Pellet A	1.34E+04	6.30E+04	2.13E-01	6.2%	% CV
3877 Pellet A	3.75E+04	4.89E+04	7.67E-01	8.93E-01	Average
3877 Pellet A	3.83E+04	3.94E+04	9.74E-01	0.1102962	StDev
3877 Pellet A	5.15E+04	5.50E+04	9.37E-01	12.4%	% CV
C3 Pellet A	6.39E+04	8.18E+04	7.81E-01	9.64E-01	Average
C3 Pellet A	5.32E+04	6.82E+04	7.80E-01	0.3184221	StDev
C3 Pellet A	6.49E+04	4.87E+04	1.33E+00	33.0%	% CV
WT Pellet A	5.62E+04	3.31E+04	1.70E+00	1.41E+00	Average
WT Pellet A	5.03E+04	5.36E+04	9.39E-01	0.4128215	StDev
WT Pellet A	5.34E+04	3.33E+04	1.60E+00	29.2%	% CV

ESAT6_AcTEQQWNFAGIEAASSAIQGNVTSISHLLDEGK2y27

Sample Name	Light Area	AQUA Area	L/H Ratio	Stats	
120A3 Pellet A	7.24E+04	8.65E+04	8.36E-01	7.32E-01	Average
120A3 Pellet A	6.42E+04	9.16E+04	7.02E-01	0.0924124	StDev
120A3 Pellet A	7.93E+04	1.20E+05	6.59E-01	12.6%	% CV
3871 Pellet A	3.32E+04	1.08E+05	3.09E-01	2.61E-01	Average
3871 Pellet A	3.25E+04	1.32E+05	2.46E-01	0.0422904	StDev
3871 Pellet A	2.41E+04	1.05E+05	2.28E-01	16.2%	% CV
3877 Pellet A	6.99E+04	8.24E+04	8.48E-01	8.00E-01	Average
3877 Pellet A	6.71E+04	8.63E+04	7.78E-01	0.0421141	StDev
3877 Pellet A	7.63E+04	9.87E+04	7.73E-01	5.3%	% CV
C3 Pellet A	6.73E+04	9.91E+04	6.79E-01	7.17E-01	Average
C3 Pellet A	5.94E+04	8.60E+04	6.91E-01	0.0559386	StDev
C3 Pellet A	5.64E+04	7.22E+04	7.82E-01	7.8%	% CV
WT Pellet A	3.05E+04	7.23E+04	4.21E-01	4.43E-01	Average
WT Pellet A	3.61E+04	7.27E+04	4.96E-01	0.0462078	StDev
WT Pellet A	3.38E+04	8.21E+04	4.12E-01	10.4%	% CV

GroES_EKPQEGTVVAVGPGR.T1

Sample Name	Light Area	AQUA Area	L/H Ratio	Stats	
120A3 Pellet A	5.85E+06	2.42E+06	2.42E+00	2.66E+00	Average
120A3 Pellet A	1.25E+07	4.53E+06	2.77E+00	0.2092599	StDev
120A3 Pellet A	1.47E+07	5.26E+06	2.79E+00	7.9%	% CV
3871 Pellet A	1.75E+07	6.21E+06	2.82E+00	2.81E+00	Average
3871 Pellet A	1.70E+07	6.01E+06	2.83E+00	0.0290051	StDev
3871 Pellet A	1.61E+07	5.79E+06	2.78E+00	1.0%	% CV
3877 Pellet A	1.03E+07	3.82E+06	2.68E+00	2.79E+00	Average
3877 Pellet A	1.71E+07	6.07E+06	2.82E+00	0.0951943	StDev
3877 Pellet A	1.89E+07	6.59E+06	2.87E+00	3.4%	% CV
C3 Pellet A	1.36E+07	6.26E+06	2.17E+00	2.26E+00	Average
C3 Pellet A	1.57E+07	6.96E+06	2.26E+00	0.0883385	StDev
C3 Pellet A	1.54E+07	6.53E+06	2.35E+00	3.9%	% CV
WT Pellet A	7.24E+06	3.36E+06	2.15E+00	2.16E+00	Average
WT Pellet A	1.29E+07	6.12E+06	2.10E+00	0.0629123	StDev
WT Pellet A	1.42E+07	6.40E+06	2.23E+00	2.9%	% CV

Correction Factor vs GroES

120A3 is	1.23E+00	of WT
3871 is	1.30E+00	of WT
3877 is	1.29E+00	of WT
C3 is	1.05E+00	of WT
WT is	1.00E+00	of WT

Peptide	Stock	Dil	Load (ul)	*25ul resusp 2ul load pmol/tube	Oncolumn*
GroES	130uM	500	5	1.3	0.13
E6internal	142uM	500	5	1.42	0.142

Absolute Quant	in fmol/800ng lysate				**E6i corrected**	Units to Table fmol /ug CF	
Sample Name	ESAT6i		GroES		**to GroES**		
120A3 Pellet A	2.67E+02	244.97	314.15	345.50	199.11	248.89	82.93%
120A3 Pellet A	2.39E+02	20.28	359.50	27.20	+/-		
120A3 Pellet A	2.28E+02	0.08	362.85	0.08	22.7	28.44	
3871 Pellet A	5.36E+01	57.47	366.25	365.31	44.18	55.22	18.40%
3871 Pellet A	5.69E+01	4.20	368.52	3.77	+/-		
3871 Pellet A	6.19E+01	0.07	361.16	0.01	3.3	4.07	
3877 Pellet A	3.40E+02	330.54	348.80	362.64	255.97	319.96	106.61%
3877 Pellet A	3.44E+02	19.53	366.47	12.38	+/-		
3877 Pellet A	3.08E+02	0.06	372.64	0.03	17.5	21.83	
C3 Pellet A	2.11E+02	205.37	282.65	293.85	196.27	245.34	81.75%
C3 Pellet A	2.03E+02	4.79	293.29	11.48	+/-		
C3 Pellet A	2.02E+02	0.02	305.60	0.04	8.9	11.17	
WT Pellet A	2.41E+02	240.09	280.00	280.82	240.09	300.12	100.00%
WT Pellet A	2.37E+02	2.94	273.09	8.18	+/-		
WT Pellet A	2.42E+02	1.2%	2.89E+02	2.9%	7.6	9.48	

Culture Filtrate

ESAT6_LAAAWGGSGSEAYR.T1

Sample Name	Light Area	AQUA Area	L/H Ratio	Stats	
Blank	0	0.00E+00	0		
120A3 Soup A	6.40E+04	2.01E+05	0.318051	0.318935	Average
120A3 Soup A	7.68E+04	2.44E+05	0.315353	0.004097	StDev
120A3 Soup A	8.40E+04	2.60E+05	0.323403	1.3%	%CV
3871 Soup A	2.16E+04	2.27E+05	0.095368	0.085801	Average
3871 Soup A	1.85E+04	2.39E+05	0.077193	0.009126	StDev
3871 Soup A	2.03E+04	2.39E+05	0.084841	10.6%	%CV
3877 Soup A	2.90E+04	1.74E+05	0.166648	0.156514	Average
3877 Soup A	3.11E+04	2.10E+05	0.147834	0.00949	StDev
3877 Soup A	2.96E+04	1.91E+05	0.15506	6.1%	%CV
C3 Soup A	1.45E+05	2.46E+05	0.591779	0.594692	Average
C3 Soup A	1.43E+05	2.59E+05	0.550965	0.045253	StDev
C3 Soup A	1.56E+05	2.43E+05	0.641331	7.6%	%CV
WT Soup A	1.49E+06	1.41E+05	10.56188	9.863688	Average
WT Soup A	1.75E+06	1.86E+05	9.424422	0.611337	StDev
WT Soup A	1.77E+06	1.85E+05	9.604764	6.2%	%CV

ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.2y27

Sample Name	Light Area	AQUA Area	L/H Ratio	Stats	
Blank	0	0	0		
120A3 Soup A	1.97E+04	5.72E+04	0.344514	0.2533245	Average
120A3 Soup A	1.68E+04	6.75E+04	0.249408	0.0892958	StDev
120A3 Soup A	1.22E+04	7.32E+04	0.166052	35.2%	%CV
3871 Soup A	4.83E+03	8.53E+04	0.056607	0.0478069	Average
3871 Soup A	3.44E+03	7.99E+04	0.043104	0.007627	StDev
3871 Soup A	3.95E+03	9.05E+04	0.04371	16.0%	%CV
3877 Soup A	4.17E+03	5.21E+04	0.08005	0.0520932	Average
3877 Soup A	1.78E+03	6.36E+04	0.028025	0.0262295	StDev
3877 Soup A	3.21E+03	6.66E+04	0.048204	50.4%	%CV
C3 Soup A	5.50E+04	7.90E+04	0.695977	0.6103617	Average
C3 Soup A	4.93E+04	7.62E+04	0.647422	0.1089782	StDev
C3 Soup A	4.73E+04	9.71E+04	0.487687	17.9%	%CV
WT Soup A	3.49E+05	6.16E+04	5.670237	4.9288776	Average
WT Soup A	3.50E+05	7.10E+04	4.938689	0.7463135	StDev
WT Soup A	3.66E+05	8.76E+04	4.177707	15.1%	%CV

Ac-ESAT6_[Ac]TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK.2y27

Sample Name	Light Area	AQUA Area			
Blank	0	0			
120A3 Soup A	1.27E+04	1.02E+05	1.24E-01	0.148104529	Average
120A3 Soup A	1.88E+04	9.86E+04	1.91E-01	0.037257597	StDev
120A3 Soup A	1.66E+04	1.29E+05	1.29E-01	25.2%	%CV
3871 Soup A	7.13E+03	1.38E+05	5.17E-02	0.048154323	Average
3871 Soup A	6.23E+03	1.39E+05	4.48E-02	0.00349742	StDev
3871 Soup A	7.65E+03	1.60E+05	4.80E-02	7.3%	%CV
3877 Soup A	1.08E+04	8.87E+04	1.22E-01	0.097547533	Average
3877 Soup A	1.07E+04	1.13E+05	9.44E-02	0.023311354	StDev
3877 Soup A	1.01E+04	1.33E+05	7.60E-02	23.9%	%CV
C3 Soup A	1.94E+04	1.49E+05	1.31E-01	0.148038931	Average
C3 Soup A	2.13E+04	1.40E+05	1.52E-01	0.015845233	StDev
C3 Soup A	2.31E+04	1.43E+05	1.62E-01	10.7%	%CV
WT Soup A	1.83E+05	8.17E+04	2.24E+00	2.251047839	Average
WT Soup A	1.84E+05	8.53E+04	2.16E+00	0.096295554	StDev
WT Soup A	1.89E+05	8.04E+04	2.35E+00	4.3%	%CV

GroES_EKPQEGTVVAVGPGR.T1

Sample Name	Light Area	AQUA Area	L/H		
Blank	0	0	0		
120A3 Soup A	5.69E+06	5.06E+06	1.125148	1.035007	Average
120A3 Soup A	7.48E+06	8.14E+06	0.919975	0.104827	StDev
120A3 Soup A	7.72E+06	7.28E+06	1.059898	10.1%	%CV
3871 Soup A	7.99E+06	7.32E+06	1.091803	1.086017	Average
3871 Soup A	8.59E+06	7.89E+06	1.088638	0.007451	StDev
3871 Soup A	8.33E+06	7.73E+06	1.07761	0.7%	%CV
3877 Soup A	3.28E+06	4.67E+06	0.701863	0.663679	Average
3877 Soup A	4.47E+06	6.75E+06	0.662372	0.037548	StDev
3877 Soup A	4.65E+06	7.43E+06	0.626801	5.7%	%CV
C3 Soup A	4.28E+06	7.24E+06	0.590802	0.581168	Average
C3 Soup A	4.26E+06	7.22E+06	0.590412	0.016349	StDev
C3 Soup A	4.21E+06	7.49E+06	0.562291	2.8%	%CV
WT Soup A	1.90E+06	4.66E+06	0.406565	0.382868	Average
WT Soup A	2.53E+06	6.86E+06	0.368989	0.020623	StDev
WT Soup A	2.77E+06	7.44E+06	0.37305	5.4%	%CV

Correction

120 is	2.7033013	of WT
3871 is	2.8365317	of WT
3877 is	1.7334411	of WT
C3 is	1.5179345	of WT
WT is	1	of WT

Peptide	Stock	Dil	Load (ul)	*25ul resusp 2ul load pmol/tube	Oncolumn*
GroES	130uM	500	5	1.3	0.13
E6interal	142uM	500	5	1.42	0.142

Absolute Quant		in fmol/800ng CF			E6i Normalized	fmol /ug CF	
Sample Name	ESAT6i	Stats	GroES	Stats	to GroES		
120A3 Soup A	45.1632024	45.288835	146.26928	134.5509547	16.75	20.94	1.20%
120A3 Soup A	44.7801314	0.5817843	119.5968	13.62747737	+/-		
120A3 Soup A	45.9231717	1.3%	137.78678	10.1%	1.71	2.14	
3871 Soup A	13.5423026	12.183703	141.93443	141.1822098	4.30	5.37	0.31%
3871 Soup A	10.9614035	1.2958369	141.52295	0.968631416	+/-		
3871 Soup A	12.0474037	10.6%	140.08925	0.7%	0.46	0.57	
3877 Soup A	23.6639495	22.224997	91.242236	86.27826913	12.82	16.03	0.92%
3877 Soup A	20.9924798	1.3476457	86.108396	4.881247886	+/-		
3877 Soup A	22.0185632	6.1%	81.484175	5.7%	1.06	1.33	
C3 Soup A	84.03256	84.446216	76.804309	75.55189479	55.63	69.54	3.97%
C3 Soup A	78.2370656	6.4259716	76.753499	2.125393685	+/-		
C3 Soup A	91.0690222	7.6%	73.097877	2.8%	4.51	5.64	
WT Soup A	1499.78663	1400.6437	52.853465	49.77283024	1400.64	1750.80	100.00%
WT Soup A	1338.26789	86.809807	47.968522	2.680937071	+/-		
WT Soup A	1363.87656	6.2%	48.496503	5.4%	115.01	143.76	

eg 20.9 +/- 2.14

Surface Proteins

ESAT6_LAAAWGGSGSEAYR.T1

Sample Name	Light Area	AQUA Area	L/H Ratio	Stats	
120A3 Washate 1	1.84E+05	1.81E+05	1.02E+00	8.09E-01	**Average**
120A3 Washate 1	3.28E+05	4.75E+05	6.89E-01	0.1799281	**StDev**
120A3 Washate 1	3.35E+05	4.64E+05	7.23E-01	22.2%	**% CV**
C3 Washate 1	9.64E+04	7.72E+05	1.25E-01	1.28E-01	**Average**
C3 Washate 1	1.00E+05	7.96E+05	1.26E-01	0.0047814	**StDev**
C3 Washate 1	9.96E+04	7.46E+05	1.34E-01	3.7%	**% CV**
WT Washate 1	2.35E+05	3.71E+05	6.34E-01	5.96E-01	**Average**
WT Washate 1	2.89E+05	5.10E+05	5.65E-01	0.0349079	**StDev**
WT Washate 1	3.27E+05	5.56E+05	5.88E-01	5.9%	**% CV**

ESAT6_TEQQWNFAGIEAASSAIQGNVTSISHLLDEGK2y27

Sample Name	Light Area	AQUA Area	L/H Ratio	Stats	
120A3 Washate 1	5.90E+04	2.53E+05	2.33E-01	2.09E-01	**Average**
120A3 Washate 1	7.18E+04	3.20E+05	2.25E-01	0.0340442	**StDev**
120A3 Washate 1	6.09E+04	3.57E+05	1.70E-01	16.3%	**% CV**
C3 Washate 1	9.09E+03	1.73E+05	5.24E-02	6.29E-02	**Average**
C3 Washate 1	8.08E+03	1.38E+05	5.86E-02	0.0132045	**StDev**
C3 Washate 1	8.33E+03	1.07E+05	7.77E-02	21.0%	**% CV**
WT Washate 1	2.95E+04	1.02E+05	2.89E-01	2.76E-01	**Average**
WT Washate 1	3.92E+04	1.51E+05	2.60E-01	0.0147746	**StDev**
WT Washate 1	4.42E+04	1.58E+05	2.80E-01	5.3%	**% CV**

ESAT6_AcTEQQWNFAGIEAASSAIQGNVTSISHLLDEGK2y27

Sample Name	Light Area	AQUA Area	L/H Ratio	Stats	
120A3 Washate 1	1.24E+05	1.53E+05	8.14E-01	5.44E-01	**Average**
120A3 Washate 1	1.13E+05	2.58E+05	4.36E-01	0.23531564	**StDev**
120A3 Washate 1	1.15E+05	3.00E+05	3.82E-01	43.2%	**% CV**
C3 Washate 1	2.42E+04	2.41E+05	1.00E-01	6.77E-02	**Average**
C3 Washate 1	1.28E+04	2.11E+05	6.05E-02	0.02977108	**StDev**
C3 Washate 1	7.44E+03	1.76E+05	4.23E-02	44.0%	**% CV**
WT Washate 1	1.57E+04	1.37E+05	1.15E-01	1.24E-01	**Average**
WT Washate 1	2.21E+04	1.54E+05	1.44E-01	0.01733869	**StDev**
WT Washate 1	1.79E+04	1.58E+05	1.13E-01	14.0%	**% CV**

Correction Factor Gro ES

120A3	1.30E+00 of WT
C3	2.62E-01 of WT
WT	1.00E+00 of WT

GroES_EKPQEGTVVAVGPGR.T1

Sample Name	Light Area	AQUA Area	L/H Ratio	Stats	
120A3 Washate 1	1.84E+05	6.00E+06	3.06E-02	2.56E-02	**Average**
120A3 Washate 1	3.28E+05	1.43E+07	2.28E-02	0.0043214	**StDev**
120A3 Washate 1	3.35E+05	1.43E+07	2.34E-02	16.9%	**% CV**
C3 Washate 1	9.64E+04	2.06E+07	4.69E-03	5.16E-03	**Average**
C3 Washate 1	1.00E+05	1.84E+07	5.46E-03	0.0004092	**StDev**
C3 Washate 1	9.96E+04	1.87E+07	5.33E-03	7.9%	**% CV**
WT Washate 1	2.35E+05	1.12E+07	2.10E-02	1.97E-02	**Average**
WT Washate 1	2.89E+05	1.56E+07	1.85E-02	0.0012459	**StDev**
WT Washate 1	3.27E+05	1.66E+07	1.96E-02	6.3%	**% CV**

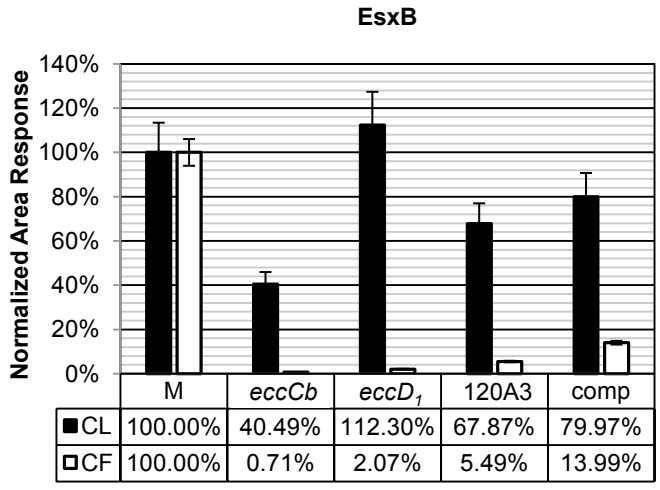
Peptide	Stock	Dil	Load (ul)	*25ul resusp 2ul load pmol/tube	Oncolumn*
GroES	130uM	500	5	1.3	0.130
E6internal	142uM	500	5	1.42	0.142

Absolute Quant	in fmol/50ul washate eqv.				**E6i Normalized**	nor to BCA**
Sample	ESAT6i		GroES		**to GroES**	
120A3 Washate 1	144.28	114.91	3.98	3.33	88.23	56.96
120A3 Washate 1	97.82	25.55	2.97	0.56	+/-	1.55
120A3 Washate 1	102.62	0.22	3.05	0.17	24.62	
C3 Washate 1	17.72	18.18	0.61	0.67	69.38	284.35
C3 Washate 1	17.86	0.68	0.71	0.05	+/-	0.24
C3 Washate 1	18.96	0.04	0.69	0.08	6.08	
WT Washate 1	90.00	84.57	2.72	2.56	84.57	150.22
WT Washate 1	80.29	4.96	2.40	0.16	+/-	0.56
WT Washate 1	83.43	0.06	2.55	0.06	7.30	

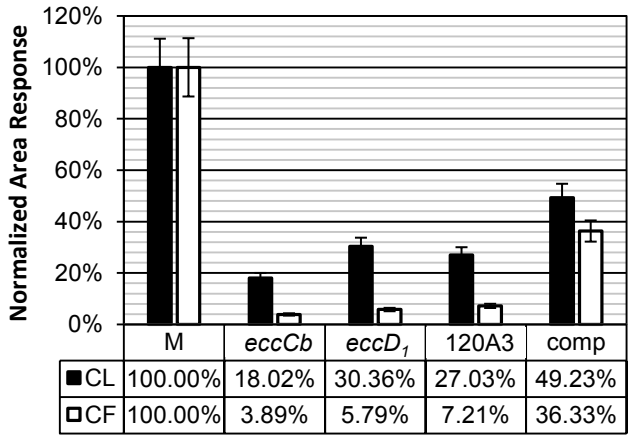
** FM 2/10/2014 BCA Data

Yellow box is fmol protein per 1/2 washate (100ul total)
Light green is fmol error (Propagated error of ESAT6 and GroES)

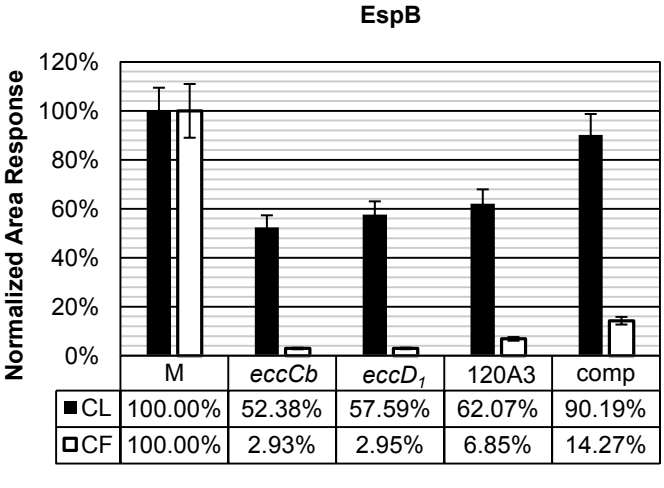
Relative Quantitation



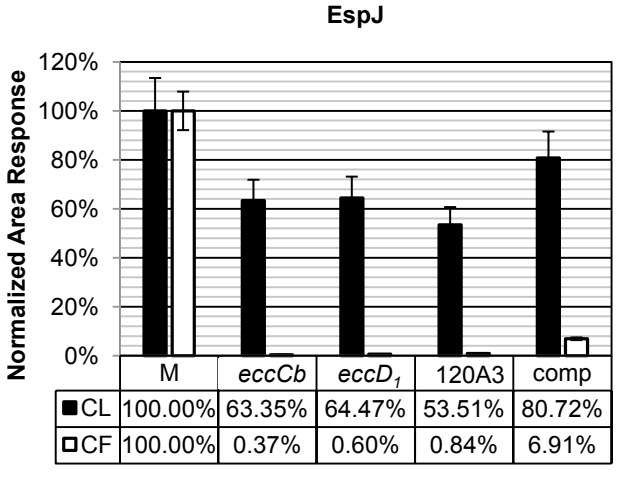
	M	*eccCb*	*eccD*$_1$	120A3	comp
CL	100.00%	40.49%	112.30%	67.87%	79.97%
CF	100.00%	0.71%	2.07%	5.49%	13.99%



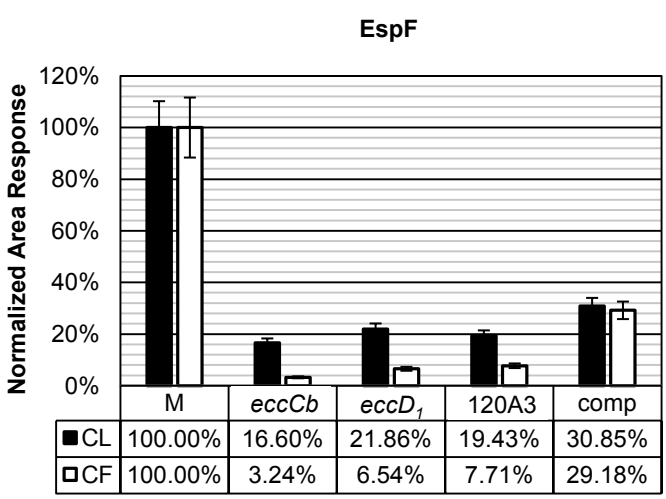
	M	*eccCb*	*eccD*$_1$	120A3	comp
CL	100.00%	18.02%	30.36%	27.03%	49.23%
CF	100.00%	3.89%	5.79%	7.21%	36.33%



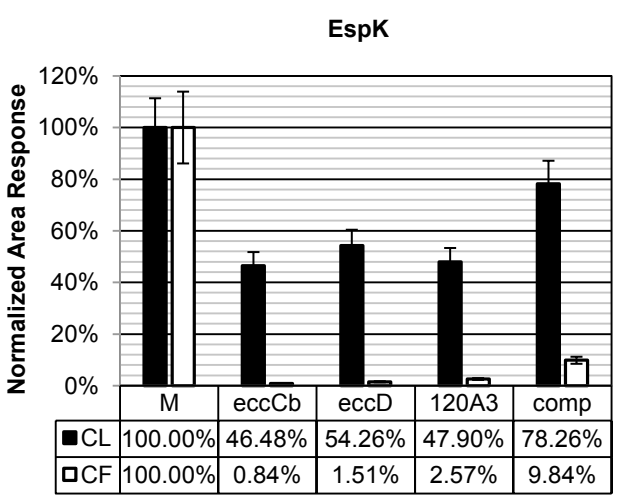
	M	*eccCb*	*eccD*$_1$	120A3	comp
CL	100.00%	52.38%	57.59%	62.07%	90.19%
CF	100.00%	2.93%	2.95%	6.85%	14.27%



	M	*eccCb*	*eccD*$_1$	120A3	comp
CL	100.00%	63.35%	64.47%	53.51%	80.72%
CF	100.00%	0.37%	0.60%	0.84%	6.91%



	M	*eccCb*	*eccD*$_1$	120A3	comp
CL	100.00%	16.60%	21.86%	19.43%	30.85%
CF	100.00%	3.24%	6.54%	7.71%	29.18%



	M	eccCb	eccD	120A3	comp
CL	100.00%	46.48%	54.26%	47.90%	78.26%
CF	100.00%	0.84%	1.51%	2.57%	9.84%

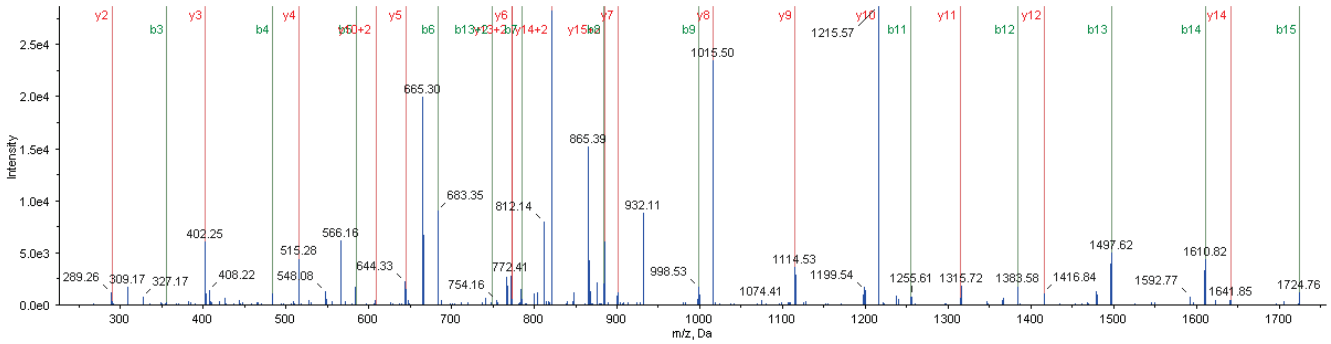
Figure S5: MMAR_0039 is required for the export of several Esx-1 substrates. Relative levels of EsxB, PPE68, EspB, EspJ, EspF and EspK in CL and CF fractions generated from the *eccCb*, *eccD*$_1$, 120A3, and complemented strains as compared to the WT (M) strain. Error bars represent the %CV.

Figure S6: Spectra demonstrating Nα- terminal acetylation of EspB and EspF: Shown here are the annotated MS/MS spectra demonstrating NT-Acetylation of EspB (A&B), andEspF (C&D) substrates in *M. marinum*. Shown here for clarity only the y and b-type ions are annotated.

EspB

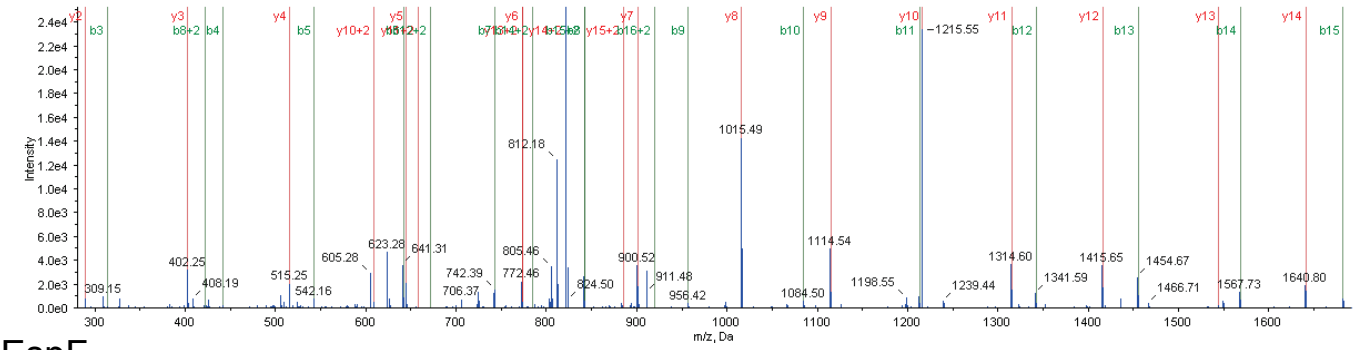
Ac-SQPQTVTVDQQEILNR. m/z 949.4848 $[M+2H]^{2+}$ Δ0.0010

A



SQPQTVTVDQQEILNR. m/z 928.4788 $[M+2H]^{2+}$ Δ0.0004

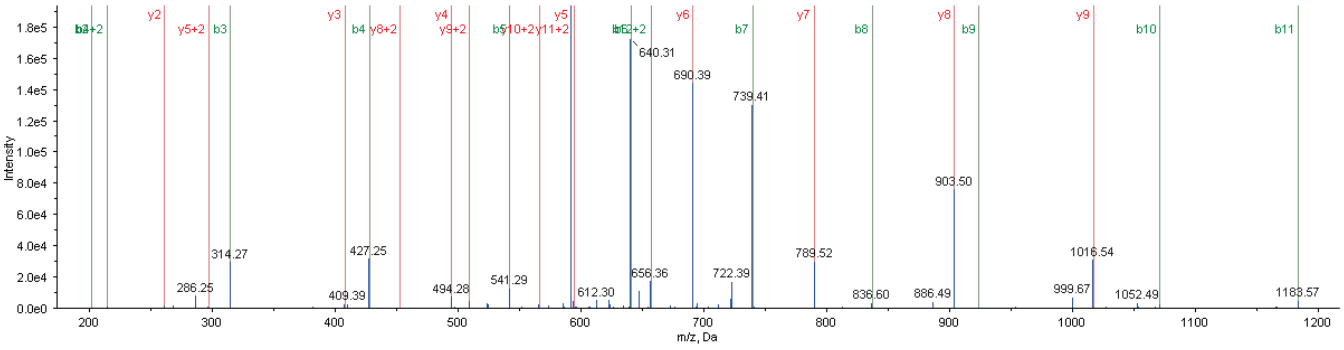
B



EspF

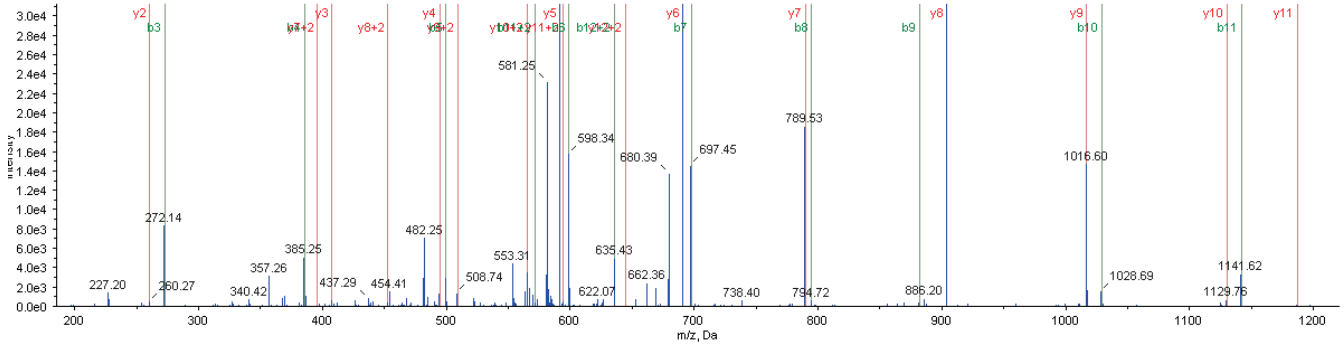
Ac-TGLLNVVPSFLK. m/z 665.3924 $[M+2H]^{2+}$ Δ0.0002

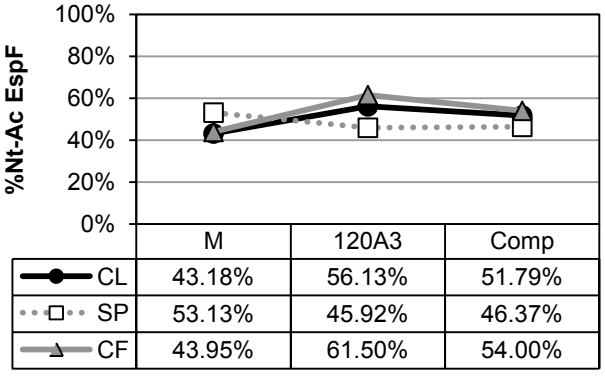
C



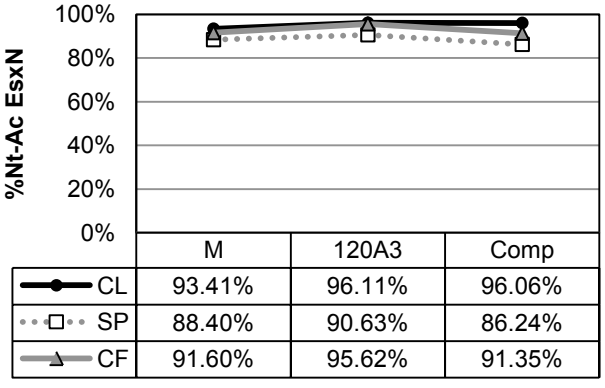
TGLLNVVPSFLK. m/z 644.3872 $[M+2H]^{2+}$ Δ0.0000

D





	M	120A3	Comp
CL	43.18%	56.13%	51.79%
SP	53.13%	45.92%	46.37%
CF	43.95%	61.50%	54.00%



	M	120A3	Comp
CL	93.41%	96.11%	96.06%
SP	88.40%	90.63%	86.24%
CF	91.60%	95.62%	91.35%

Figure S7: Analysis of Nα-terminal Ac EspF and EsxN peptides in the CL, CF and SP. Percentage of N-α terminal acetylation of EspF and EsxN in CL, CF and SP from *M. marinum* WT (M),120A3 and complemented strains. % acetylation was calculated as the area ratio (N-t Ac peptide)/(area ratio Nt-Ac peptide + area ratio unacetylated Nt peptide) * 100. The differences in % acetylation of EspF for all fractions between the WT and 120A3 strains were significantly different from each other using a student's t-test ($p<0.05$). The % acetylation in the complemented strain was also significantly different from the WT strain ($p< 0.05$). For EsxN, the changes between the WT and 120A3 strain was significant for the CL and CF ($p<0.05$). ANOVA indicates there is no difference between the % acetylation at the cell surface ($p=.4864$). The % acetylation of EsxN in the CF in the WT and complemented strains were not significantly different ($p=0.72$).