

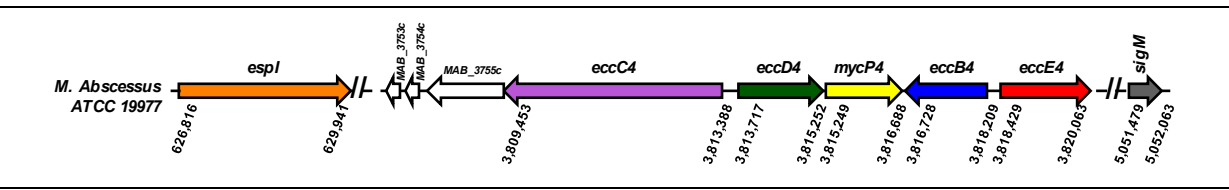
Table S1. List of genes identified by *Tn* library screening, for intra-amoebal and/or intra-macrophage survival rates of less than 50%.

Mutant	<i>M. abscessus</i> ATCC 19977	<i>M. tuberculosis</i> H37Rv	Protein name	Amoeba % survival	MΦ % survival
37F10	MAB_3756c	Rv3447c	EccC - Type VII secretion protein FtsK/SpoIIIE family protein (TA insertion 15bp after 24H3)	9	>50
5A1	MAB_3757	Rv3448	EccD - Type VII secretion protein	9	26
7A1	MAB_3757	Rv3448	EccD - Type VII secretion protein (TA insertion 426bp after 5A1)	9	34
32C9 ^a	MAB_3759c	Rv3450c	EccB - Type VII secretion protein	11	34
3B9	MAB_0364c		ATPase, BadF/BadG/BcrA/BcrD type	12	28
43A3 ^b	MAB_0628		EspI - Secretion protein	13	8
24H3	MAB_3756c	Rv3447c	EccC - Type VII secretion protein FtsK/SpoIIIE family protein	17	>50
55F6	MAB_0855		MmpL - Membrane protein	18	36
14E9 ^c	MAB_3760		EccE* - Type VII secretion protein	25	14
18D2	MAB_3758	Rv3449	MycP - Serine protease	26	>50
13C3	MAB_4938	Rv3911	SigM - RNA polymerase sigma factor	27	>50
30D4	MAB_1027		Unknown	32	>50
2E4	MAB_1118c		Unknown	35	>50
57C10	Intergenic domain (before MAB_4608)		≈ 20 bp before 3-oxoacyl-ACP synthase FabF; β-ketoacyl- ACP synthase II, KASII	41	49
47H4	MAB_2436	Rv1860	ModD	43	18
56C2	MAB_1314		Mesocentin	46	37
22H2	MAB_1421	Rv1278	Unknown	47	24
27F10	MAB_4558c		Membrane protein	47	38
22D4	MYCMA_10265		Unknown	49	31
50C7	MAB_0821		GabT - 4-aminobutyrate aminotransferase	>50	8
29G1	MAB_0431c	Rv3661	Inhibition of morphological differentiation protein	>50	13
50H8	MAB_3787	Rv3399	S-adenosyl-L-methionine-dependent methyltransferase	>50	26
57C7	MAB_4626c	Rv0551c	FadD - Acyl-CoA synthetase	>50	33
24A4	MAB_4302		Transmembrane protein DoxX	>50	33
47F1	MAB_2752	Rv1458c	Spermidine/putrescine ABC transporter ATP-binding protein	>50	33
51H1	MAB_3800c		Glutamate dehydrogenase	>50	34
52B6	MAB_1915		FadD - Fatty-acid-CoA ligase	>50	34
28B7	MAB_2799		MspA	>50	35
58E5	MAB_0090c	Rv3085	EphD - Oxidoreductase	>50	35
29C8	MAB_1911c	Rv3166c	Sugar phospho-transferase, transmembrane protein	>50	36
27A9	Not found		Unknown	>50	36
51H4	MYCMA_08185		Mammalian cell entry protein	>50	37

51H7	<i>MAB_4691c</i>		PstA - Peptide synthetase	>50	38
40A11	<i>MAB_3658c</i>	<i>Rv3305c</i>	N-acyl-L-amino acid amidohydrolase	>50	40
15A11	<i>MAB_3326c</i>	<i>Rv3329</i>	Aminotransferase, class III	>50	41
57G6	Intergenic domain (before <i>MAB_3866c</i>)		≈ 90 nucleotides before a permease	>50	42
22D3	<i>MAB_2426</i>	<i>Rv1851</i>	UreF - Urease accessory protein	>50	42
15C4	<i>MAB_2992c</i>	<i>Rv2689c</i>	23S rRNA methyltransferase	>50	45
39H12	<i>MAB_4593c</i>		Unknown	>50	45
52A12	<i>MAB_4173</i>	<i>Rv3526</i>	Oxygenase	>50	50

The mutants are classified according to their intracellular survival rate. Identical mutants (with the same TA insertion site) isolated several times: ^a duplications are 14B9, 19D4, 32C7, and 25E4; ^b duplications are 13F7 and 2D10; ^c duplication is 15G8. (*) EccE was defined as described in (35).

Table S2. Evaluation of the polar effect of *Tn* insertion. Genes mean normalized expression relative to *sigA*.



	Mabs 43S	24H3 (<i>Tn eccC4</i>)	7A1 (<i>Tn eccD4</i>)
<i>MAB_3755c</i> <i>esx</i>	1.0	1.2	ND
<i>MAB_3756c</i> <i>eccC4</i>	1.0	0.5	ND
<i>MAB_3757</i> <i>eccD4</i>	1.0	ND	0.1
<i>MAB_3758</i> <i>mycP4</i>	1.0	ND	1.9
<i>sigA</i>	1.0	1.0	1.0

Table S3. List of proteins present only in wild-type strain compared to *ΔeccB4* strain (proteome UP000007137) and quantified by using quantification grouping XIC extraction.

Gene & Synonyms	Delta-ECCB4/WT						Total Peptides	MW (kDa)	Description
	Ratio	Log2	n-value	Coeff. var.	Dist. pept. used	Pept. used			
MAB 1372	0.6496537	-0.6222572	0.3922764	0.9680881	5	35	35	50.8	Probable sugar ABC transporter, sugar-binding protein LcaY
MAB 4240c	1.0072634	0.010441	0.9965026	1.838688	5	32	32	104.9	Putative membrane protein, MmpL
MAB 0719	0.5701225	-0.810656	0.063134	0.3943692	3	24	24	58.0	Putative oligopeptide ABC transporter, solute-binding protein
MAB 2821c	0.8232974	-0.2820924	0.8288937	0.6759251	3	22	22	42.8	Coenzyme A biosynthesis bifunctional protein CnaBC
MAB 1340	0.931499	-0.1023739	0.9447592	0.8373199	4	20	20	27.4	Cytokinin riboside 5'-monophosphate phosphoribidylase
MAB 3014	0.7173365	-0.4792781	0.8498491	1.6954196	3	19	19	49.1	Putative FAD-dependent pyridine nucleotide-disulphide oxidoreductase, similar to mercuric reductases protein
MAB 2736c	1.2064554	0.2707746	0.6827613	0.6648359	3	18	18	58.6	Probable macrolide ABC transporter, ATP-binding protein
MAB 0056c	1.1497825	0.2013609	0.9067549	1.2044549	2	16	16	144.6	ATP-dependent helicase HrpA
MAB 0985	1.0448903	0.0633514	0.9504132	0.4838856	1	16	16	55.7	Putative monoxygenase EihA
MAB 2977	0.7467379	-0.4213261	0.8730468	1.9224785	2	16	16	53.4	Uncharacterized protein
MAB 1418	0.5503971	-0.8614551	0.2486619	0.6398666	2	16	16	18.3	Uncharacterized protein
MAB 0855	0.9945128	-0.0079382	0.9964129	0.2966129	2	15	15	111.2	Putative membrane protein, MmpL family
MAB 1160	0.7240453	-0.465848	0.4917118	0.4292955	2	15	15	45.3	Uncharacterized protein
xseA.MAB 12	0.7167715	-0.4804149	0.9016702	2.6266578	2	15	15	44.3	Exodeoxyribonuclease 7 large subunit
MAB 0574c	1.0325039	0.0461472	0.9797785	1.2574889	2	14	14	25.0	Uncharacterized protein
MAB 4204	0.6260738	-0.6755954	0.6534113	1.1160815	2	14	14	73.3	Amine oxidase
MAB 3285	0.4865464	-1.0383806	0.4917118	1.0530711	1	14	14	19.0	Uncharacterized protein
MAB 2533	0.6194847	-0.6908593	0.3288841	0.3431699	1	12	12	9.9	Uncharacterized protein
MAB 4180c	0.5885759	-0.7646995	0.6817488	1.4375098	2	12	12	49.5	Hypothetical MCE-family protein
MAB 4336	0.5552355	-0.8488284	0.5133653	0.356738	2	12	12	77.0	Probable acyl-CoA dehydrogenase FadE
MAB 2502	0.9249418	-0.1125655	0.9623828	0.6517624	2	11	11	38.0	Uncharacterized protein
MAB 4337	0.6903825	-0.5345323	0.5669508	0.9169274	2	11	11	33.8	Putative hydrolase, alpha/beta fold
MAB 3759c	0.1405161	-2.8311928	0.2323173	1.3772418	2	10	15	52.0	Uncharacterized protein
MAB 2368	1.6615752	0.7325516	0.6199509		1	8	8	32.2	Uncharacterized protein
MAB 0118c	1.5779745	0.6580739	0.4847307		1	8	8	23.0	Superoxide dismutase
MAB 0636c	1.5396859	0.6226361	0.4665977		1	8	8	100.2	Putative cation antiporter NADH dehydrogenase subunit
MAB 3373c	1.379172	0.4638024	0.5510935		1	8	8	31.8	Uncharacterized protein
MAB 4906	1.3353332	0.4171998	0.115377		1	8	8	20.9	Putative transcriptional regulator, PadR family
MAB 2106c	1.2798472	0.3559716	0.7958945		1	8	8	37.1	Probable conserved lipoprotein LpplL
MAB 2610	1.2385437	0.3086448	0.9038505		1	8	8	46.9	Putative aminotransferase
MAB 1812c	1.1876447	0.2481033	0.8766006		1	8	8	11.3	Uncharacterized protein
MAB 4086	1.1707961	0.2274899	0.8691924		1	8	8	27.2	Possible TetR-family transcriptional regulator
MAB 4465	1.1650177	0.2203518	0.6086966		1	8	8	49.6	Uncharacterized protein
MAB 1917	1.1587741	0.2125993	0.7923269		1	8	8	49.5	Diacylglycerol O-acyltransferase
MAB 1570	1.1027819	0.1411475	0.8895544		1	8	8	31.1	Uncharacterized protein
MAB 1633	1.0588792	0.082538	0.9466742		1	8	8	33.3	Putative DNA polymerase III delta subunit
MAB 3776	1.0170017	0.0243221	0.9723147		1	8	8	17.8	Uncharacterized protein
MAB 0948c	1.0101027	0.0145019	0.9902276		1	8	8	52.2	Uncharacterized protein
MAB 0527c	0.9773886	-0.0329559	0.9739455		1	8	8	43.3	Possible monoxygenase (Luciferase-like)
MAB 0291	0.9563647	-0.0643672	0.9525471		1	8	8	67.2	Uncharacterized protein
MAB 3423	0.9361515	-0.0951861	0.9675525		1	8	8	55.9	Probable cytochrome P450
MAB 3952	0.9345314	-0.0976849	0.9485283		1	8	8	39.5	Possible O-succinylbenzoic acid-CoA ligase MenE
MAB 1414c	0.896895	-0.156989	0.9238506		1	8	8	68.6	Probable ABC transporter, ATP-binding protein
MAB 2220c	0.8455928	-0.241965	0.4969699		1	8	16	32.0	Uncharacterized protein
MAB 2499c	0.832265	-0.2648852	0.8872955		1	8	8	36.7	Putative hydrolase, alpha/beta fold
MAB 4682c	0.8231909	-0.2824547	0.8451906		1	8	8	10.3	Uncharacterized protein
MAB 4719c	0.8113779	-0.3015154	0.8646435		1	8	8	73.2	Uncharacterized protein
MAB 4796	0.7992279	-0.3233212	0.8766006		1	8	8	35.4	Uncharacterized protein
MAB 4635	0.7822857	-0.3542325	0.8664635		1	8	8	44.9	Probable NADH:flavin oxidoreductase/NADH oxidase
MAB 3908c	0.779623	-0.3591514	0.5363251		1	8	8	42.1	Uncharacterized protein
MAB 0795	0.7777886	-0.3625501	0.493441		1	8	8	27.6	Uncharacterized protein
MAB 0138	0.7752029	-0.3673541	0.7414211		1	8	8	22.4	Putative transcriptional regulator, TetR family
MAB 3583c	0.7451463	-0.4244044	0.1530895		1	8	8	16.3	Uncharacterized protein
MAB 3382c	0.7374432	-0.4393961	0.8498491		1	8	8	27.6	Probable enoyl-CoA hydratase/isomerase
MAB 0654	0.7114393	-0.4911875	0.4300089		1	8	8	33.1	Uncharacterized protein
MAB 2855c	0.7080441	-0.4980889	0.4888575		1	8	8	41.8	Uncharacterized protein
MAB 2184	0.7038277	-0.5067059	0.6501019		1	8	8	33.2	Uncharacterized protein
MAB 4762	0.6663275	-0.5856966	0.1928121		1	8	8	68.5	Uncharacterized protein
MAB 0704c	0.6572947	-0.6053877	0.6381113		1	8	8	37.2	Uncharacterized protein
MAB 1650c	0.6388738	-0.6463971	0.5275136		1	8	8	53.7	Uncharacterized protein
MAB 0865c	0.6367983	-0.6520662	0.697375		1	8	8	49.1	Probable sugar-transport, intracellular membrane protein
MAB 2241	0.6330318	-0.6596501	0.7095013		1	8	8	49.6	Putative nitrate ABC transporter, periplasmic protein
MAB 0955c	0.6236522	-0.6811864	0.6277257		1	8	16	50.6	Probable sensor histidine kinase PrbB
MAB 0708	0.6205949	-0.6882762	0.192952		1	8	8	65.5	Uncharacterized protein
MAB 1938c	0.6055386	-0.7237091	0.4995978		1	8	8	22.8	Putative transcriptional regulator, TetR family
MAB 2751	0.5991847	-0.7389274	0.245589		1	8	8	63.6	Uncharacterized protein
MAB 1489	0.5872276	-0.7680084	0.4917118		1	8	8	64.2	Probable acyl-CoA ligase FadD
MAB 3344	0.5803566	-0.7849886	0.1662721		1	8	8	66.3	Probable glycosyl transferase
MAB 4179c	0.5654113	-0.8226273	0.2070673		1	8	8	43.1	Uncharacterized protein
MAB p02	0.5467716	-0.8709898	0.7081268		1	8	8	15.6	Uncharacterized protein
MAB 0888c	0.5317647	-0.91114	0.2323173		1	8	8	42.4	Putative fumarylacetoacetase
MAB 2151c	0.5292579	-0.9179571	0.2182385		1	8	8	52.4	Putative cytochrome P450
MAB 4151c	0.5237772	-0.9329749	0.2240795		1	8	8	37.9	Hypothetical MCE-family protein
MAB 0988c	0.5029013	-0.9848128	0.0797285		1	8	8	16.6	Probable membrane protein, MmpS
MAB 4088c	0.4898363	-1.0296283	0.3107978		1	8	8	15.1	Uncharacterized protein
MAB 4460	0.4838248	-1.0474433	0.0597673		1	8	8	23.8	Uncharacterized protein
MAB 3114	0.4792158	-1.0612526	0.3408009		1	8	8	44.7	Uncharacterized protein
MAB 4387	0.4672615	-1.097698	0.4071086		1	8	8	17.9	Uncharacterized protein
MAB 2112	0.4630488	-1.1107638	0.0359669		1	8	8	32.0	Uncharacterized protein
MAB p15c	0.4187231	-1.2559317	0.0597673		1	8	8	119.4	TraA/ATP-dependent exonuclease/relaxase
MAB 3790	0.4016635	-1.3159407	0.1818956		1	8	8	56.9	Uncharacterized protein
MAB 3855c	0.3895	-1.3603046	0.0130044		1	8	8	18.9	Putative lipoprotein LpPC
MAB 4546c	0.3740554	-1.4186762	0.240118		1	8	8	26.1	Putative TetR-family transcriptional regulator
MAB 1993	1.5941057	0.6274743	0.259769		1	7	7	35.4	Methylenetetrahydrofolate reductase
MAB 1399	1.2364216	0.3061708	0.8442147		1	7	7	45.1	Probable peptidase
his1.MAB 264	1.1478122	0.1988866	0.9426841		1	7	7	12.5	Phosphoribosyl-AMP cyclohydrolase
MAB 3466c	0.9018817	-0.1489899	0.8691924		1	7	7	5.4	Uncharacterized protein
MAB 4325c	0.49549	-1.0130722	0.3658655		1	7	7	36.6	Uncharacterized protein
MAB 0965	0.4763755	-1.0698388	0.2323173		1	7	7	39.4	Putative lipase
MAB 4703c	0.4661445	-1.1011508	0.552147		1	7	7	103.2	Probable membrane protein, MmpL
MAB 0107c	0.7943708	-0.3321154	0.822221		1	6	6	16.4	4-hydroxy-4-methyl-2-oxoglutarate aldolase
MAB 1314	0.5754183	-0.797317	0.6989942		1	6	6	23.5	Uncharacterized protein
MAB 4383c	0.2181577	-2.196557	0.0359669		1	6	6	15.3	Putative membrane protein, MmpS
MAB 4531	1.1635206	0.2184968	0.9447592		1	5	5	16.0	Uncharacterized protein
MAB 3366	0.7721405	-0.3730648	0.8930716		1	5	5	44.8	Possible glycosyl transferase
MAB 4306	0.4388274	-1.1882746	0.1762696		1	5	5	28.8	Putative transglutaminase-like protein
MAB 4511c	0.1436602	-2.7992676	0.2601116		1	5	5	44.0	Putative Mce family protein
MAB 0664	0.1143057	-3.1293005	0.3844635		1	5	5	9.8	PE family protein
MAB 2102	1000	1000			1	4	4	48.4	Probable peptidase
MAB 2934	2.1097025	1.0770396	0.4555747		1	4	4	25.7	Probable Asp/Glu racemase
MAB 3901	1.9740702	0.9811733	0.7617026		1	4	4	19.6	Peptidase C56 PfpI
MAB 0299c	0.4734197	-1.0788084	0.5554235		1	4	4	12.0	Uncharacterized protein
MAB 4141	0.3683975	-1.4410173	0.3533305		1	4	4	55.1	PE/PPE family protein
MAB 2270c	0.001	-1000			1	4	4	43.4	Putative peroxidase
MAB 3970c	0.001	-1000			1	3	3	12.1	Uncharacterized protein
MAB 3354	0.001	-1000			1	2	2	37.8	Probable acyl-L-acyl-carrier protein] desaturase DesA1
MAB 2287	0.001	-1000			1	1	1	27.7	Putative metal dependent phosphohydrolase
MAB 2989	0.001	-1000			1	1	1	26.2	Chloramphenicol acetyltransferase
MAB 1820c	0.001	-1000			1	1	1	21.8	Putative resolvase

Table S4. List of proteins present only in wild-type strain compared to *ΔeccB4* strain (proteome UP0000038470) and quantified by using quantification grouping XIC extraction.

120/166 proteins	Delta-ECCB4/WT					Total Peptides	MW (kD)	Description	
	Ratio	Log2	p-value	Coeff. var.	Dist. pept. used				
AA00U0XHK9	0.6516033	-0.6179341	0.4095446	0.9694194	5	35	35	50.8	Probable sugar ABC transporter, sugar-binding protein LpaY
AA00U1A6G4	0.8251966	-0.2771900	0.8350575	0.6780384	3	22	22	42.8	Coenzyme A biosynthesis bifunctional protein CoaBC
AA00U0ZYQ4	1.3023717	0.381412	0.9179987	2.2820302	3	21	29	106.8	Membrane protein mmp10
AA00U1DWS8	0.9258249	-0.0956895	0.9417481	0.8365823	4	20	20	27.4	Cytokinin riboside 5'-monophosphate phosphoribohydrolase
AA00U0ZFD4	1.2092983	0.2747901	0.6572382	0.6708797	3	18	18	58.6	ABC transporter
AA00U0ZFE5	1.1976146	0.2601639	0.8385813	0.8409254	2	16	16	21.6	Putative transcriptional regulator, TetR family
AA00U1AV71	1.152064	0.2042208	0.9179987	1.2044549	2	16	16	144.2	ATP-dependent helicase HrpA
AA00U1A2Y2	1.1232806	0.1677184	0.9320953	1.0041597	2	16	16	25.8	L-hydroxy-tetrahydrodipicolinate reductase
AA00U1ADX3	1.0469636	0.0662113	0.9420111	0.4838856	1	16	16	55.7	Putative monooxygenase EthA
AA00U1A421	0.7482196	-0.4184663	0.8781956	1.9224785	2	16	16	53.4	Hydrolase
AA00U1AQ08	0.669483	-0.5788808	0.4465197	0.4718889	2	16	16	56.1	D,D-dipeptide-binding periplasmic protein ddaA
AA00U1A4L4	1.4984728	0.1174199	0.9589852	1.3032107	2	15	15	57.4	Uncharacterized protein containing a von Willebrand factor type A (VWA) domain
AA00U0YSW0	0.7253176	-0.4635153	0.4888152	0.4294923	2	15	15	44.5	Uncharacterized protein
AA00U1A3F3	0.988442	-0.0167717	0.9982928	1.9815678	2	13	13	49.1	Putative FAD-dependent pyridine nucleotide-disulphide oxidoreductase, similar to mercuric reductases protein
AA00U0ZY15	0.3376304	-1.5664833	0.2664075	1.4703604	2	13	13	30.3	Uncharacterized protein
AA00U1AXX2	0.6210686	-0.6871754	0.3327292	0.3442104	1	12	12	11.5	Conserved exported protein of uncharacterized function
AA00U0ZYZ7	0.591489	-0.7575768	0.6858724	1.4405969	2	12	12	49.6	Hypothetical MCE-family protein
AA00U0W TG6	0.1407043	-2.829261	0.2378297	1.3786172	2	10	15	52.0	ESX-4 secretion system protein eccB4
AA00U1APU1	1.7021308	0.7673385	0.1005493		1	8	8	25.0	Uncharacterized protein
AA00U1T2V0	1.6648723	0.754115	0.6273841		1	8	8	32.2	Segregation and condensation protein A
AA00U1AEF5	1.6429364	0.7162767	0.744083		1	8	8	15.9	Probable molybdenum biosynthesis protein Mog
AA00U1AVW0	1.5811056	0.6609338	0.4656354		1	8	8	23.0	Superoxide dismutase
AA00U1A870	1.5296279	0.6131808	0.7276229		1	8	8	30.1	Putative short chain dehydrogenase/reductase
AA00U0ZAZ3	1.3556631	0.4389987	0.6934538		1	8	8	22.0	Possible transposase fusion protein
AA00U1BK86	1.3379829	0.4200597	0.4112906		1	8	8	26.9	Paik family transcriptional regulator
AA00U1AG97	1.2823868	0.588831	0.7837886		1	8	8	36.4	Probable conserved lipoprotein LplL
AA00U1A0Z5	1.2691444	0.3438562	0.7060058		1	8	8	40.5	Putative dihydrodipicolinate reductase
AA00U1B373	1.2014456	0.2647713	0.6709206		1	8	8	48.8	Putative manganese-dependent inorganic pyrophosphatase
AA00U0XGZ3	1.1975807	0.2601290	0.589263		1	8	8	38.0	Glutamate 5-kinase
AA00U1AVB6	1.1953024	0.2573757	0.9320953		1	8	8	38.1	Topoisomerase IB
AA00U1AL6	1.1826384	0.2428208	0.6720268		1	8	8	31.2	ESX1 C-terminal conserved region
AA00U0ZXY2	1.1731193	0.232497	0.8556901		1	8	8	26.9	Possible TetR family transcriptional regulator
AA00U0WM26	1.1673294	0.2321172	0.616595		1	8	8	49.6	Uncharacterized protein
AA00U1ASW6	1.165761	0.2212721	0.8486992		1	8	8	26.4	ABC transporter, ATP-binding protein
AA00U1A7K6	1.1610734	0.2154592	0.7785398		1	8	8	49.4	Diacylglycerol O-acyltransferase
AA00U1AQ08	1.1267126	0.1721195	0.8154307		1	8	8	45.2	Probable aminotransferase/cysteine desulfurase
AA00U1TK9	1.1248312	0.1697086	0.9179987		1	8	8	16.4	AsnC family transcriptional regulator
AA00U1AC36	1.119779	0.163214	0.9179987		1	8	8	39.4	Uncharacterized protein
AA00U0ZFE4	1.048109	0.0677888	0.9179987		1	8	8	36.8	UDP-glucose 4,6-dehydratase
AA00U0ZYZ9	1.0358238	0.0507786	0.9557204		1	8	8	40.9	Probable glycylglycyl transferase
AA00U0ZYY9	1.0190197	0.027182	0.9697378		1	8	8	17.8	Uncharacterized protein
AA00U1B2F8	1.0069365	0.0099727	0.9552523		1	8	8	51.4	rRNA modification GTPase MnmE
AA00U1AE7	0.9609903	-0.0587581	0.9677545		1	8	8	43.1	Flavin-dependent monooxygenase, oxycarenase subunit HsaA
AA00U1A1Z9	0.9598224	-0.0615074	0.9585042		1	8	8	39.6	Uncharacterized protein
AA00U0ZAD0	0.9363858	-0.0948785	0.9434306		1	8	8	39.4	Possible O-succinylbenzoic acid-CoA ligase MenE
AA00U0ZYP6	0.8859952	-0.1746292	0.9309936		1	8	8	55.0	Probable aldehyde dehydrogenase
AA00U1ARA8	0.8780524	-0.187621	0.6868324		1	8	8	54.0	Ecopolysaccharide biosynthesis polyphosphoryl glycosylphosphotransferase
AA00U1B4D4	0.8677466	-0.2046543	0.9179323		1	8	8	57.7	Primase C terminal 1 (PriC-T1)
AA00U0ZX17	0.8670776	-0.205767	0.9275237		1	8	8	31.1	Uncharacterized protein
AA00U1AHX6	0.8472707	-0.2391051	0.4888152		1	8	8	37.0	Uncharacterized protein
AA00U1BE33	0.814133	-0.2490791	0.8253962		1	8	8	45.8	N-isopropylamide isopropyl amidohydrolase
AA00U0ZWX1	0.8738224	-0.2795948	0.8411385		1	8	8	10.4	Uncharacterized protein
AA00U1ARX7	0.8138427	-0.2971782	0.6112286		1	8	8	22.9	Pyridoxone-carboxylate peptidase
AA00U1AMZ3	0.8109656	-0.3028741	0.8603658		1	8	8	137.2	Uncharacterized protein
AA00U1AEH7	0.8008138	-0.3204613	0.884889		1	8	8	35.4	Alpha/beta hydrolase
AA00U0ZQ0	0.7967938	-0.327216	0.8384338		1	8	8	41.1	Putative Mce family protein
AA00U1AL63	0.7793819	-0.3594903	0.83833		1	8	8	26.7	Uncharacterized protein
AA00U1ATX3	0.7676741	-0.3649471	0.7396307		1	8	8	22.4	Putative transcriptional regulator, TetR family
AA00U1AJ27	0.7389065	-0.4365362	0.8486992		1	8	8	27.8	Probable enol-CoA hydratase/isomerase
AA00U1AVG2	0.7363624	-0.4415122	0.3056219		1	8	8	32.0	Putative pyruvate, phosphate dikinase regulatory protein
AA00U1APD9	0.712851	-0.4883276	0.4239446		1	8	8	33.1	Membrane protein
AA00U1AMS0	0.7119051	-0.4902431	0.3217986		1	8	8	24.3	Acid phosphatase
AA00U1B073	0.7084984	-0.4971634	0.7252589		1	8	8	11.8	Uncharacterized protein
AA00U1B3H5	0.7084984	-0.4971634	0.7252589		1	8	8	68.1	Heavy metal transcriptional P-urea ATPase
AA00U1AXQ3	0.7060639	-0.5021293	0.7895929		1	8	8	51.4	rRNA/rDNA cytosine-C5-methylase
AA00U1AFS1	0.7052243	-0.503846	0.6708058		1	8	8	33.2	Cullin, a subunit of E3 ubiquitin ligase
AA00U0ZVK0	0.700045	-0.5144801	0.5168702		1	8	8	51.2	Probable aldehyde dehydrogenase
AA00U1AAM6	0.6946548	-0.5256319	0.399609		1	8	8	46.5	Uncharacterized protein
AA00U1B80	0.6826891	-0.5560993	0.3729399		1	8	8	46.0	Prophage integrase
AA00U1AL9	0.6676497	-0.5828865	0.1939099		1	8	8	65.8	ABC transporter
AA00U1A58	0.6601415	-0.643372	0.527385		1	8	8	53.8	Putative secreted protein
AA00U1A3N1	0.637631	-0.6492063	0.7041882		1	8	8	49.1	Probable sugar-transport integral membrane protein
AA00U1A797	0.6067402	-0.7208493	0.493833		1	8	8	22.7	Putative transcriptional regulator, TetR family
AA00U0ZW29	0.591578	-0.7573596	0.0513261		1	8	8	34.0	Putative hydrolase, alpha-beta fold
AA00U1ARV1	0.5815082	-0.7821287	0.1825567		1	8	8	66.3	Mannosyltransferase yycA
AA00U1ATU3	0.5712956	-0.8076089	0.1664263		1	8	8	37.7	Uncharacterized protein
AA00U0ZM5	0.5666333	-0.8197671	0.2013371		1	8	8	43.2	Uncharacterized protein
AA00U1B30	0.5633255	-0.8279594	0.1327773		1	8	8	17.1	Single-stranded DNA-binding protein
AA00U1ALX1	0.5377925	-0.8948785	0.0279107		1	8	8	33.6	Pseudouridine synthase
AA00U1BPT3	0.5248165	-0.930115	0.3378297		1	8	8	37.5	Hypothetical MCE-family protein
AA00U1A2H3	0.522485	-0.936385	0.47399		1	8	8	13.6	Uncharacterized protein
AA00U1A8T5	0.507781	-0.9778436	0.032732		1	8	8	21.4	Uncharacterized protein
AA00U1AF41	0.506239	-0.9819529	0.077768		1	8	8	16.1	Probable membrane protein, MmpS
AA00U1B30	0.4978637	-1.0001721	0.1852297		1	8	8	139.0	ATP-dependent helicase/deoxyribonuclease subunit B
AA00U1AWK0	0.464268	-1.069702	0.4922793		1	8	8	89.8	Probable phosphotetrase
AA00U1AFZ5	0.4639676	-1.0709309	0.0341203		1	8	8	31.9	Uncharacterized protein
AA00U1AJM3	0.4561322	-1.1324729	0.493833		1	8	8	65.4	ABC transporter, transmembrane region
AA00U1BOT1	0.4493854	-1.1539748	0.2218203		1	8	8	69.3	N-acetylmuramoyl-L-alanine amidase domain-containing protein SAOUHSC_02979
AA00U1AV37	0.4077229	-1.2943056	0.0126321		1	8	8	42.0	Eflux transporter, RND family, MFP subunit
AA00U0ZXM6	0.3902729	-1.3744471	0.0126321		1	8	8	19.0	Putative lipoprotein LipC
AA00U0ZYT1	0.3747976	-1.4158163	0.2469415		1	8	8	27.3	HTH-type transcriptional regulator
AA00U1AD19	0.3624978	-1.4639558	0.0893864		1	8	8	64.7	Fibronectin-binding protein A-N-terminus (FbpA)
AA00U1AC13	0.3025407	-1.7247991	0.2931233		1	8	8	21.7	Two-component system response regulator
AA00U1AGN3	1.6040163	0.6818888	0.2585021		1	7	7	35.4	Methylenetetrahydrofolate reductase
AA00U1A8K3	1.2594988	0.3097569	0.8330575		1	7	7	45.2	Probable peptidase
AA00U1ALH4	1.190576	0.2515598	0.8330575		1	7	7	29.3	Phosphomethylpyrimidine kinase
AA00U1AE18	0.4793371	-1.0608874	0.738235		1	7	7	41.8	Putative ligase
AA00U1AYD9	1.685964	0.7532591	0.8183341		1	6	14	20.6	Fimbrial isopeptide formation D2 domain
AA00U1AUV6	1.3823432	0.4671158	0.8411385		1	6	6	60.4	Putative 3-keetopirid-1-dehydrogenase of fumarate reductase/succinate dehydrogenase
AA00U0ZYS5	1.2743253	0.3497336	0.9019967		1	6	6	15.4	Uncharacterized protein
AA00U1AVS9	0.801601	-0.3190438	0.8330575		1	6	6	16.4	L-hydroxy-L-methyl-2-oxoglutarate aldolase
AA00U0ZXP3	0.6437311	-0.63547	0.8272281		1	6	6	76.9	Probable acyl-CoA dehydrogenase FadE
AA00U1BYE9	0.5574775	-0.8430161	0.2359601		1	6	6	46.6	Acyl-CoA dehydrogenase
AA00U0ZG2	0.428444	-1.2228215	0.2680524		1	6	6	73.3	Amine oxidase
AA00U1A400	0.379926	-1.3962096	0.1825567		1	6	6	27.6	Putative short-chain dehydrogenase/reductase
AA00U1B3W2	0.3425718	-1.545218	0.4664508		1	6	6	79.7	ABC-type amino acid transport system, permease and periplasmic component
AA00U0ZW57	0.2194704	-2.1879017	0.0326167		1	6	6	15.4	Putative membrane protein, MmpS
AA00U0ZVF7	1.1693681	0.2257292	0.939024		1	5	5	16.1	Uncharacterized protein
AA00U1ALV6	0.8313736	0.2878231	0.43738		1	5	5	23.6	Chromosomal partition ATPase protein
AA00U0IYL5	0.7774957	-0.363935	0.9089657		1	5	5	44.8	Glycerol synthase
AA00U1BKT2	0.4403966	-1.1831248	0.1822436		1	5	5	28.9	Putative transglutaminase-like protein
AA00U0ZVV2	0.1435321	-2.800552	0.2637772		1	5	5	44.1	Putative Mce family protein
AA00U1ARH8	0.1152733	-3.1168695	0.4020695		1	5	5	9.8	PE family protein
AA00U1AG29	1000	1000			1	4	4	48.3	Succinyl-diaminopimelate desuccinylase
AA00U1AR2	2.1220613	1.48858							

In our Label-free protein quantification approach to build up both Tables S3 and S4, we used the Quantification grouping XIC extraction, and the option Matching between runs from MaxQuant, which is an option forcing the match between runs. The information of the runs regarding the identifications from one run will be used to screen another run looking for peptide peaks closely located in the same retention time range. Match between runs is used to boost number of identifications, meaning for peptides, which are present in several samples, but not identified via the MS/MS data search in all of them, can still be identified via matching between runs. In the case of EccB₄, which was not identified in the LC-MS/MS search of the Δ -eccB₄ sample, the option match between runs could extract in the 4 replicates only one peptide (and not the same one) in 3 of the 4 replicates. In our MS criteria, 1 peptide is not enough to identify a protein but with this option we could have quantifications and not only absent/present values.

Table S5. Primers used in RT-qPCR.

<i>sigA F</i>		TCCGAGAAAGACAAGGCTTC
<i>sigA R</i>		CCAGCTCGACTTCCTCTCG
<i>MYCMA_05485 F</i>	<i>esx</i>	GTCCCGTCTCGCGCCCTGTG
<i>MYCMA_05485 R</i>	<i>esx</i>	ACCGCACCGTCAACGTCGCA
<i>MYCMA_2064 F</i>	<i>eccC4</i>	GCCATCGGTGTGCCGGATGC
<i>MYCMA_2064 R</i>	<i>eccC4</i>	CACCACTTCGGCGGGTCGCA
<i>MYCMA_2065 F</i>	<i>eccD4</i>	AACGGCGGAAAAGCCCACGG
<i>MYCMA_2065 R</i>	<i>eccD4</i>	GGGTGCCTCGGTCTGCGGG
<i>MYCMA_2066 F</i>	<i>mycP4</i>	GTCGTCGTGGTGGTGGCAGC
<i>MYCMA_2066 R</i>	<i>mycP4</i>	GCCGCCGAGACTGAATTTGGACG

Fig. S1. Visual first round of screening of the transposon library for reduced intracellular survival in amoeba and evaluation of mycobacterial adhesion to and internalization in MΦ.

(A) Examples of mutants identified are highlighted with a white circle and are named according to the plate number and location in the 96-well plates of the original library. For comparison a non-selected mutant is shown with a black circle. (B) The *in vitro* growth of the selected mutants with a reduced ability to survive in amoeba was next checked. Growth was assessed by measuring the OD of the cultures at 600 nm every 2 days. (C) Adhesion assay (left): The different mycobacterial strains were added to a 24-wells plate containing J774.2 MΦ cells. The plate was centrifuged to allow the bacteria to reach the monolayer and incubated 30 min. on ice. After 2 washes, the cells were lysed and the number of bacteria that had adhered to the MΦ were enumerated by CFU counting. Internalization assay (right): J774.2 MΦ cells were infected with the different mycobacterial strains during 1 hr at a MOI 10:1 at 37°C. After 3 washes to eliminated extracellular mycobacteria, the number of internalized bacteria were enumerated by CFU counting. Histograms and error bars represent means \pm SD that are representative data of three independent experiments. Statistical analysis: Unpaired *t* test using GraphPad prism program, ns non-significant.

Fig.S1

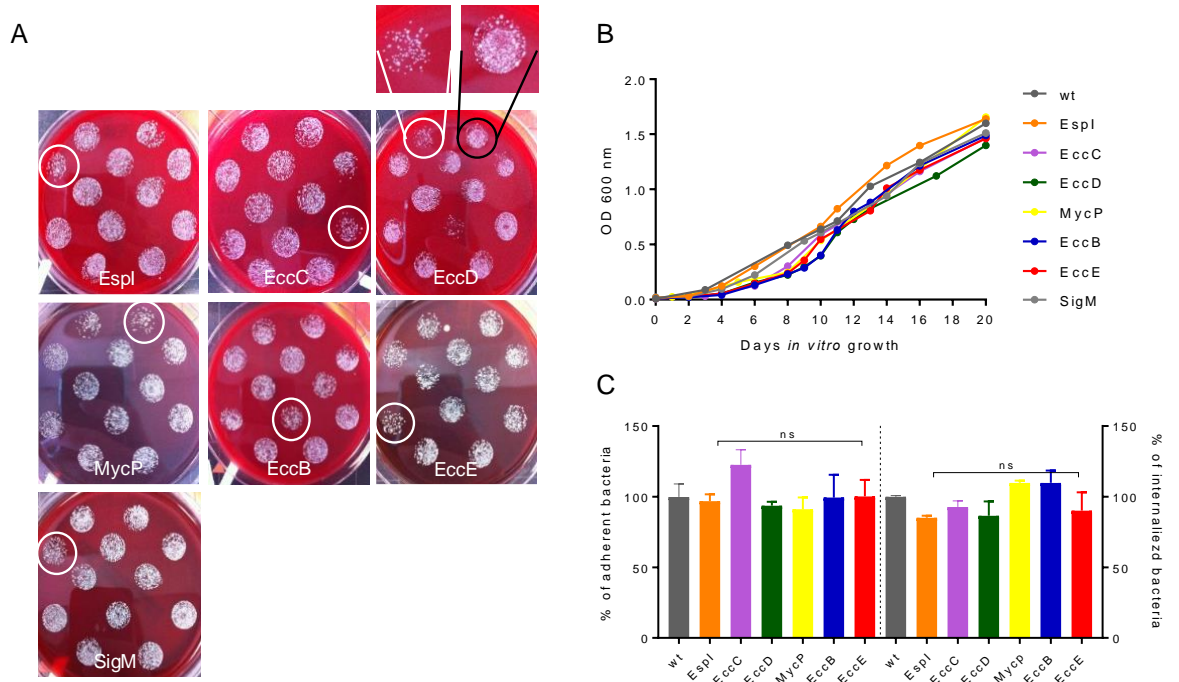


Fig. S2. Construction of the *M. abscessus* KO mutant strain $\Delta eccB_4$ by homologous recombination. (A) *M. abscessus* $\Delta eccB_4$ KO mutant was constructed by first preparing the Allelic Exchange Substrate (AES) fragment, which contains an antibiotic resistance cassette (the zeocin resistance gene *ble* of *Streptoalloteichus hindustanus*) flanked by 1,000 bp of the DNA upstream and downstream of the target gene *eccB₄*. Homologous recombination with *M. abscessus* chromosome occurred upon electroporation of the AES fragment into *M. abscessus* wild-type (wt) containing the recombineering plasmid pJV53K, which was lost after plating the colonies in media without kanamycin. (B) PCR was performed to check the deletion of *eccB₄* by homologous recombination. Primers are identified by arrows (primers F and R). (C) *In vitro* growth curves estimated by spectrophotometry (OD 600_{nm}) of *M. abscessus* MabsCIP (wt), $\Delta eccB_4$, and *C.ΔeccB₄* strains.

Fig.S2

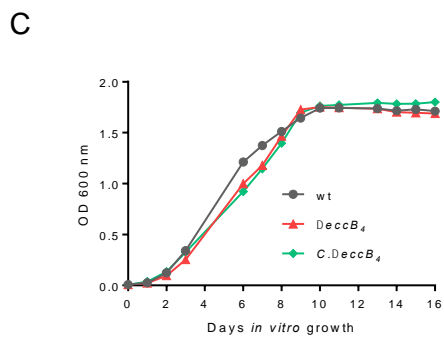
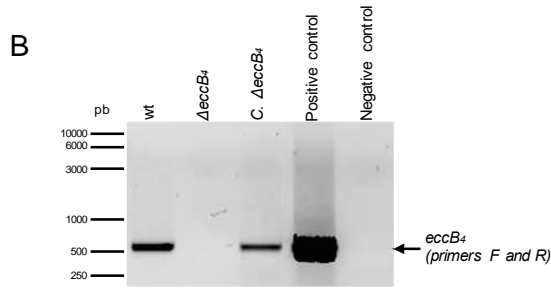
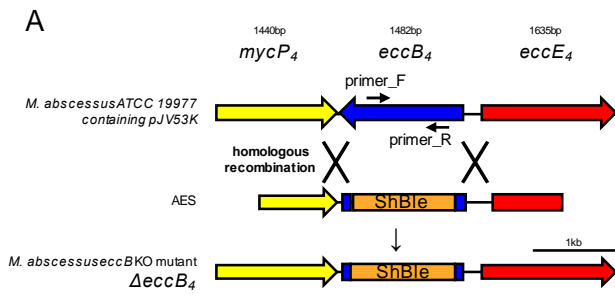


Fig. S3. Evaluation of mycobacterial adhesion and internalization on amoeba (A, C) or MΦ (B, D). (A, B) The different mycobacterial strains wild-type (wt), $\Delta eccB_4$ and $C.\Delta eccB_4$ were added to a 24-wells plate containing *A. castellanii* amoeba or J774.2 MΦ cells. The plate was centrifuged to allow the bacteria to reach the monolayer and incubated 30 min on ice. After 2 washes, the cells were lysed and the number of bacteria which have adhered to the amoeba or MΦ were enumerated evaluating CFU. (C, D) *A. castellanii* amoeba or J774.2 MΦ cells were infected with wild-type (wt), $\Delta eccB_4$ and $C.\Delta eccB_4$ during 1 h at a MOI 10:1 at 37°C. After 3 washes to eliminated extracellular mycobacteria, the number of internalized bacteria was evaluated counting CFU. Histograms and error bars represent means \pm SD that are representative data of three independent experiments. Statistical analysis: Unpaired *t* test using GraphPad prism program, ns non-significant.

Fig.S3

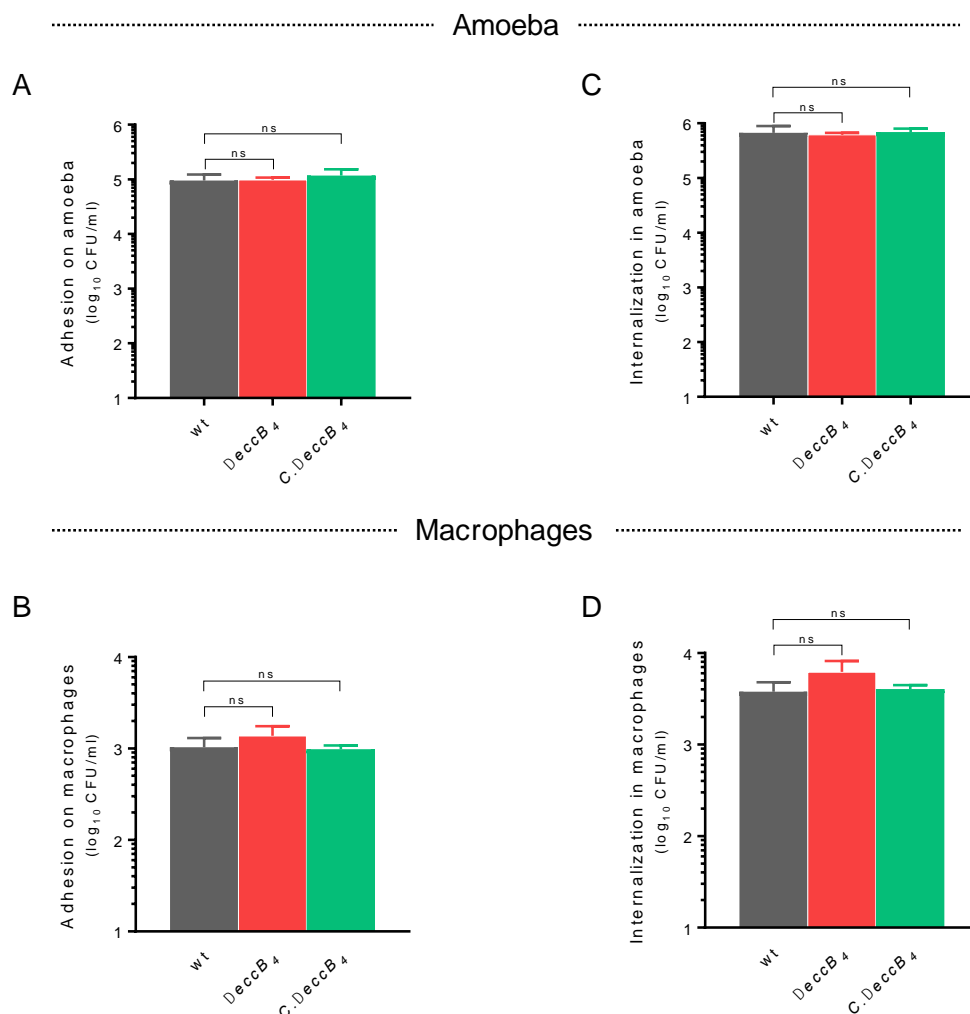


Fig S4. Systematic thin layer liquid chromatographic (TLC) analysis reveal no changes in cell wall lipid compositions between *M. abscessus* $\Delta eccB_4$ mutants and wild type strains. (A) TLC analyses of apolar and polar lipids. Solvent systems used were as follows: A: petroleum ether / ethyl acetate 98:2 run thrice (first dimension), petroleum ether / acetone 98:2 (second dimension); B: petroleum ether acetone 92:8 run thrice (first dimension), toluene / acetone 95:5 (second dimension); C: chloroform / methanol 96:4 (first dimension), toluene acetone 80:20 (second dimension); D: chloroform / methanol / water 100:14:0.8 (first dimension), chloroform / acetone / methanol / water 50:60:2.5:3 (second dimension); E: chloroform / methanol / water 60:30:6 (first dimension), chloroform / acetic acid / methanol / water 40:25:3:6 (second dimension). (B) TLC analyses of methyl esterified fatty acids and mycolic acids. The solvent system was petroleum ether / acetone 95:5.

Fig.S4

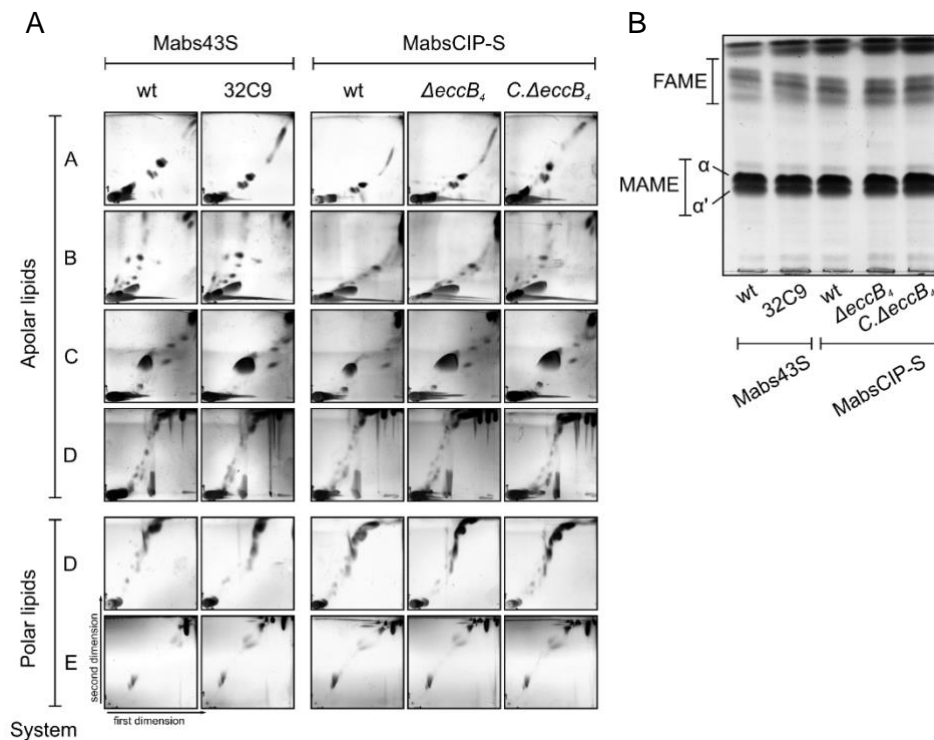
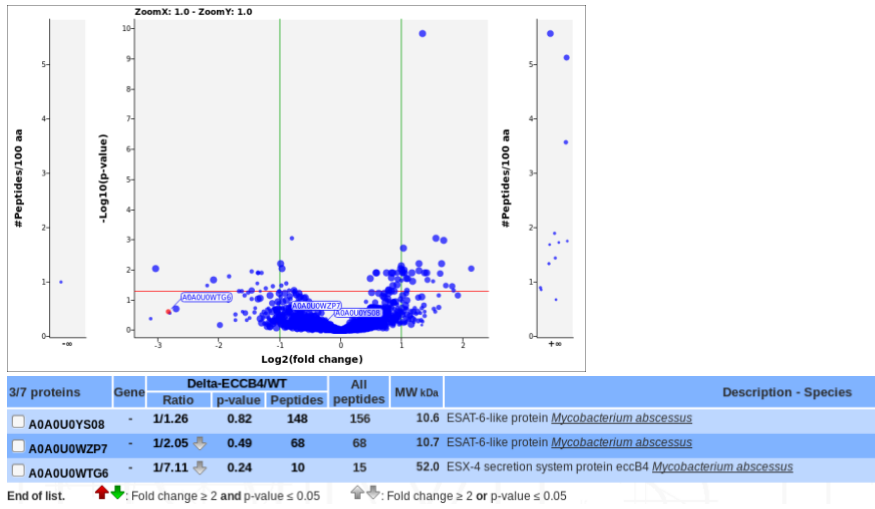


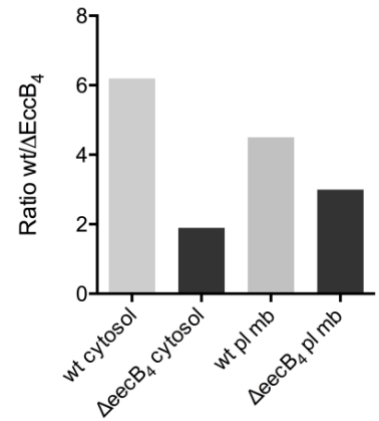
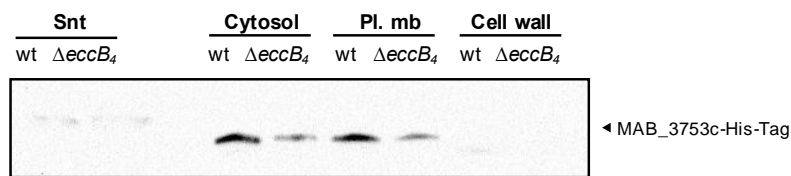
Fig S5. EccB₄-dependant secretome (A) Proteomic analysis of concentrated supernatants from wild-type (wt) and $\Delta eccB_4$ mutants. The figure represents the volcano plots for finding significant and not significant proteins using *t* test. The below table represents the results concerning EccB₄, EsxT and EsxU (referred as ESAT-6 like proteins). A fold-change ≥ 2 was found for EccB₄ and EsxT although the fold change was considered non significant. (B) Left: His-EsxT (*Mab_3753c*) detection using a Histidine Tag antibody in different bacterial compartments: cytosol, plama membrane (Pl. mb) and cell wall, and in the supernatant (Snt) of wild-type (wt), $\Delta eccB_4$ mutant. Right: quantification of His-Esx-T band intensity (ImageJ software). (C) Red Ponceau stain of the different bacterial compartments (as in B).

Fig. S5

A



B



C

