Supp. Figure legends

Sup. Figure 1: DESeq2 statistical analyses.

A. *M. abscessus* transcriptomes in *A. castellanii* 4 and 16 hpi. **B.** *M. abscessus* transcriptome in macrophages 16 hpi**.** **C.** *M. chelonae* transcritome in *A. castellanii* 16 hpi. Hierarchical clustering of raw data (left panel) and transcriptome heatmaps (right panel) are depicted.

Sup. Figure 2: Comparison of *M. abscessus* transcriptomes in *A. castellanii* and in macrophages according to differentially expressed genes fold change.

A. Differentially expressed genes (DEGs) were categorized according to their fold change (FC) expressed in Log_2. Low DEGs depict a FC under |2|, Med DEGs depict a FC between |2| and |4| and High DEGs depict a FC higher than |4|. **B.** Ratio of UP DEGs to DOWN DEGs were represented during co-cultures with A. castellanii (Ac) and macrophages (ϕ).

Sup. Figure 3: Selection of *M. abscessus* genes highly induced in amoebae.

M. abscessus highly induced genes in *A. castellanii* (Ac) were considered (n=77). Genes poorly induced in macrophages (Mϕ) (filter 1) and during *M. chelonae* co-culture with amoeba (filter 2) were conserved (n=45). FC: fold change (Log_2 value).

Sup. Figure 4: Verification of knockout strains growth in culture medium and contribution to virulence in macrophages.

A. KO strains growth in culture medium. The strains were cultured in 7H9 medium supplemented with glycerol 0.1% for seven days. Growth curves were obtained by measuring the cultures optical density each day. **B.** Complementation of *M. abscessus* ΔOP,_3,-4 and 6 strains in macrophages. Macrophages were infected at 10 MOI. Experiments were repeated three times in triplicates. Statistical analyses were performed with GraphPad PRISM6.

Histograms with error bars represent means ± SD. Differences between means were analyzed by ANOVA and the Tukey post-test allowing multiple comparisons to be performed. ns = non-significant. * $p<0.05$. ** $p<0.01$. *** $p<0.001$. **** $p<0.0001$.

Sup. Figure 5: Conservation of *M. abscessus eis* loci in *Mycobacterium tuberculosis* and vice versa.

A. Conservation of *M. abscessus eis1* locus in *M. tuberculosis*. **B.** Conservation of *M. abscessus eis2* locus in *M. tuberculosis*. **C.** Conservation of *M. tuberculosis eis* locus in *M. abscessus*. Bidirectional Best Hit (BBH) search was performed between *M. abscessus* and *M. tuberculosis* genomes with the Opscan software. BBHs are depicted by arrows filled with red, brown or orange. Brown arrows correspond to MmpL-encoding genes. Orange arrows correspond to MmpS-encoding genes. Greys bands link genes or groups of genes conserved in the two species.

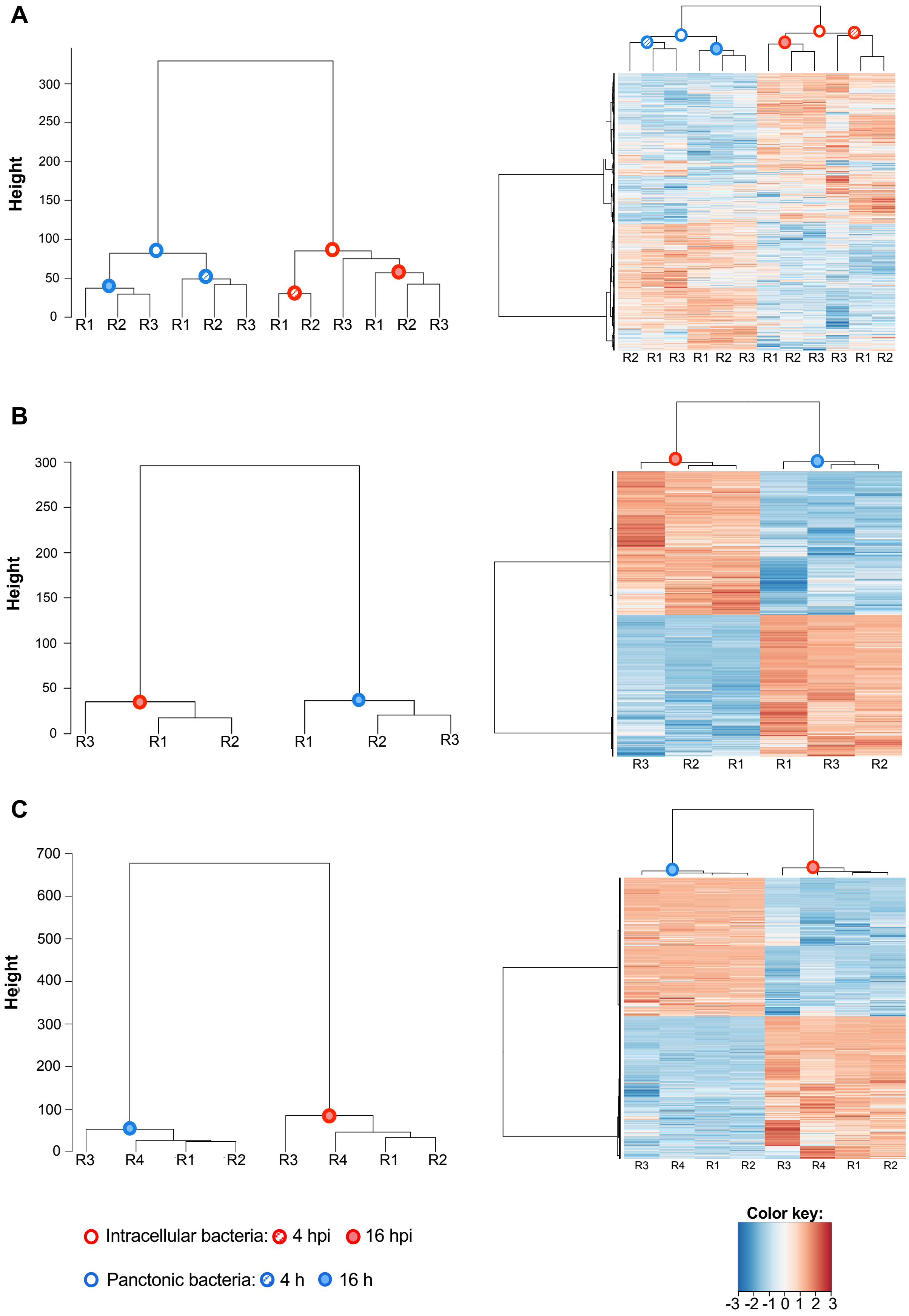
Sup. Figure 6: Expression of *M. abscessus eis* genes in macrophages 4 and 16 hpi. $Eis1_{MAB}$ (left panel) and $eis2_{MAB}$ (right panel) expression in macrophages was measured twice in triplicates by quantitative-real time PCR by normalization with *sigA* housekeeping gene.

Sup. Figure 7: Intracellular phenotypes uncontrolled by *M. abscessus eis2* genes.

A. Cell death. Macrophage death following infection with *M. abscessus* was assessed with the Dead Cell Apoptosis Kit with Annexin V FITC and PI for flow cytometry. **B.** Cell autophagy was measured Premo Autophagy Tandem Sensor RFP-GFP-LC3B Kit. At least 40 cells per condition were analyzed by confocal microscopy. The number of autophagic particules per cell (left panel) and acidification of autophagosomes (right panel) was determined with the Fiji software. **C.** Phagosomal acidification was assessed as previously described (Roux *et al.*, 2016).

Macrophages were infected at 10 (C) or 50 MOI (A and B). Histograms with error bars represent means ± SD. Differences between means were analyzed by ANOVA and the Tukey post-test allowing multiple comparisons to be performed. ns = non-significant. * $p<0.05$. ** $p<0.01$. *** $p<0.001$. **** $p<0.0001$.

Supp. Figure 1



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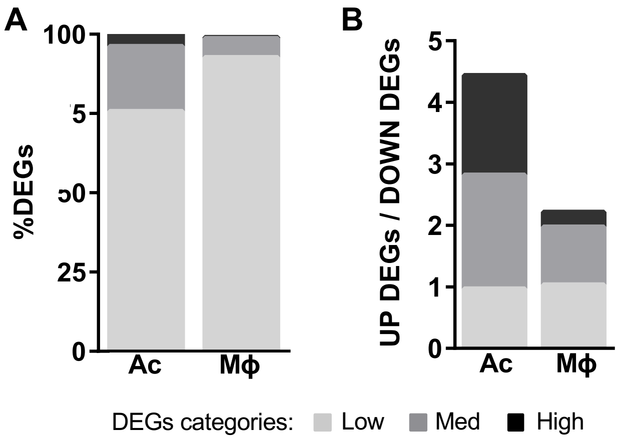

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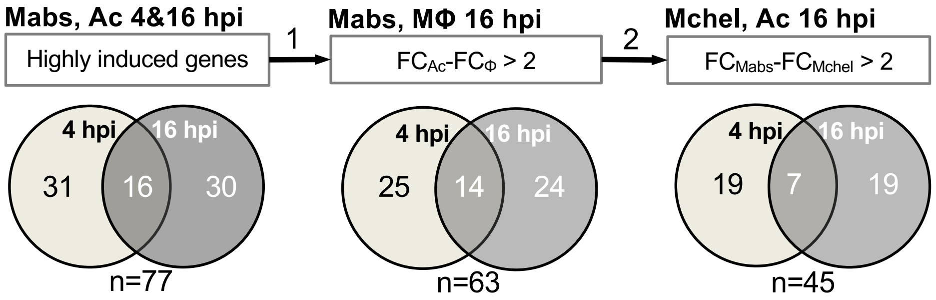

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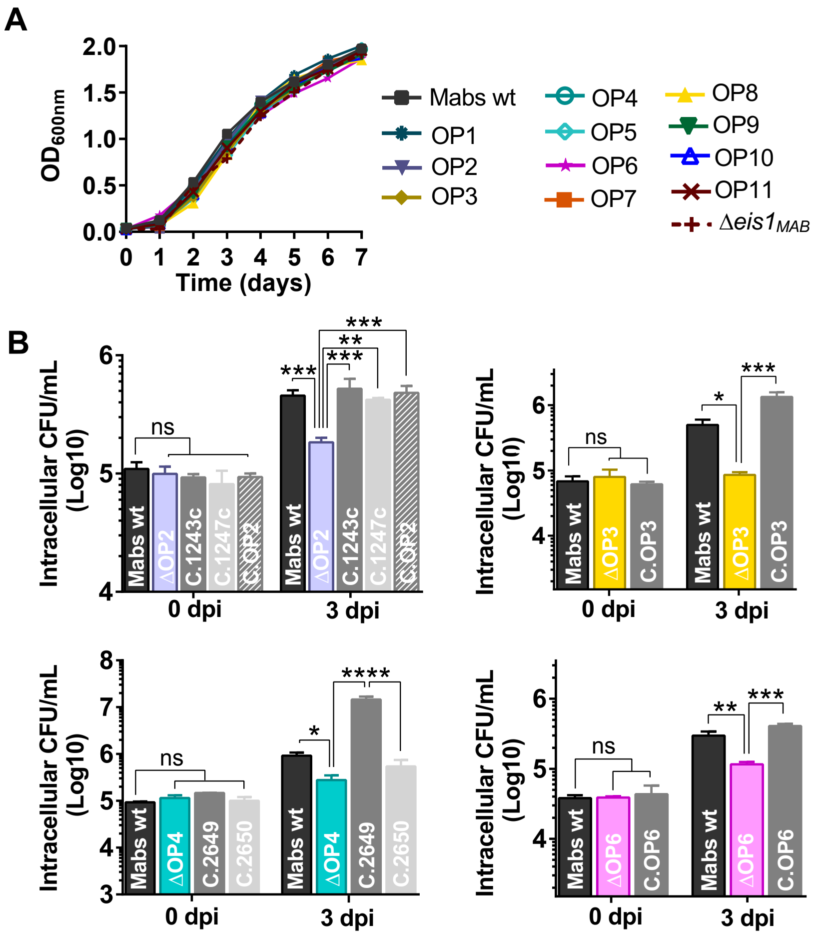

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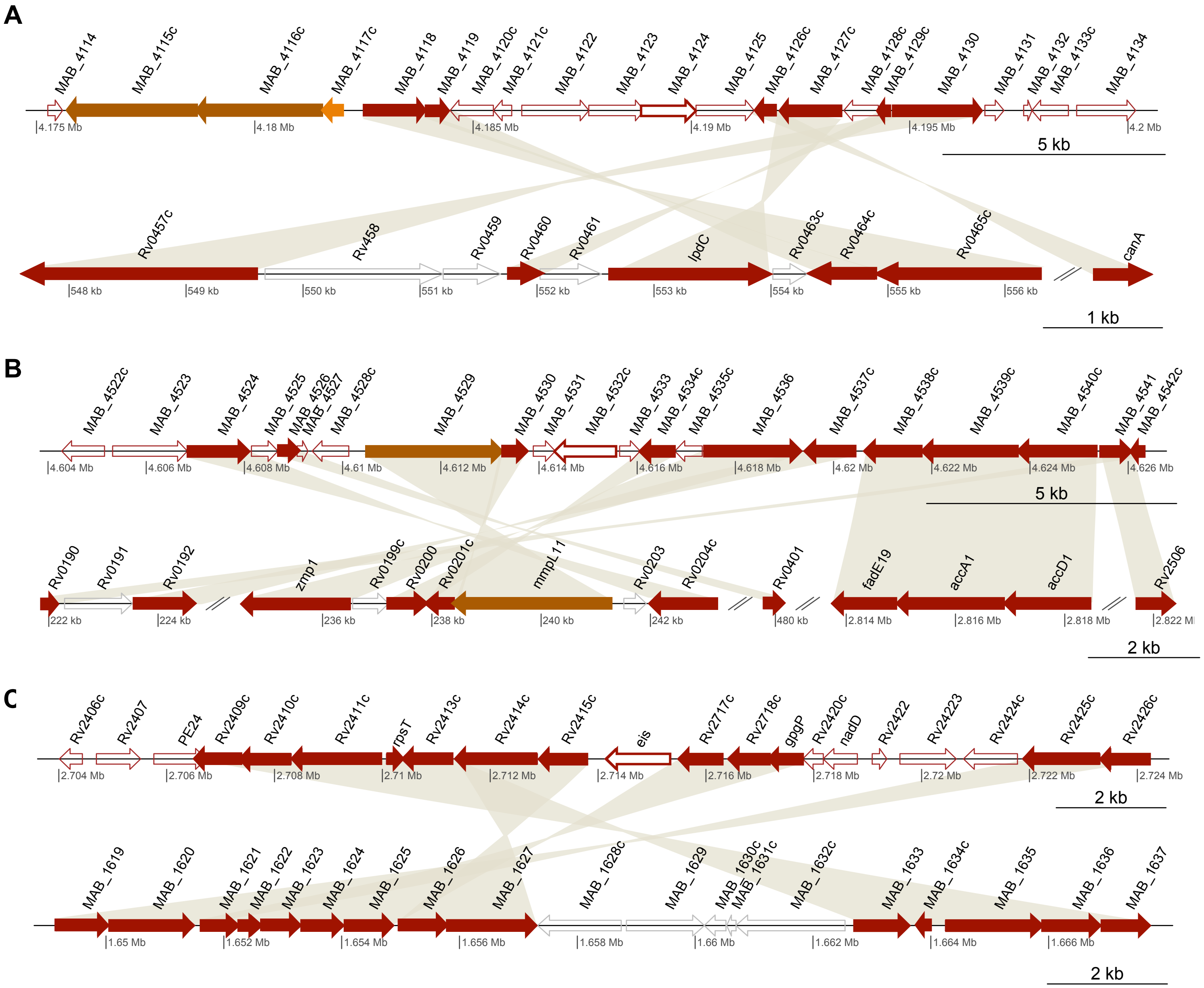
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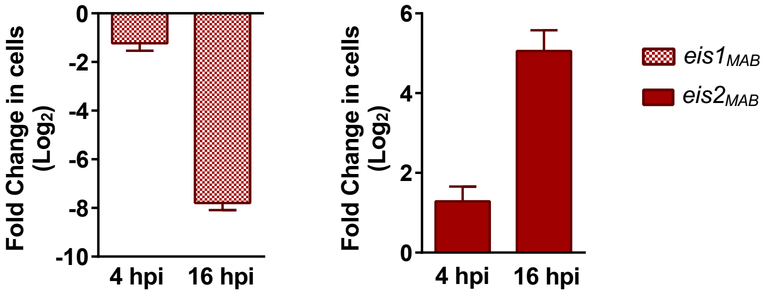
Supp. Figure 6



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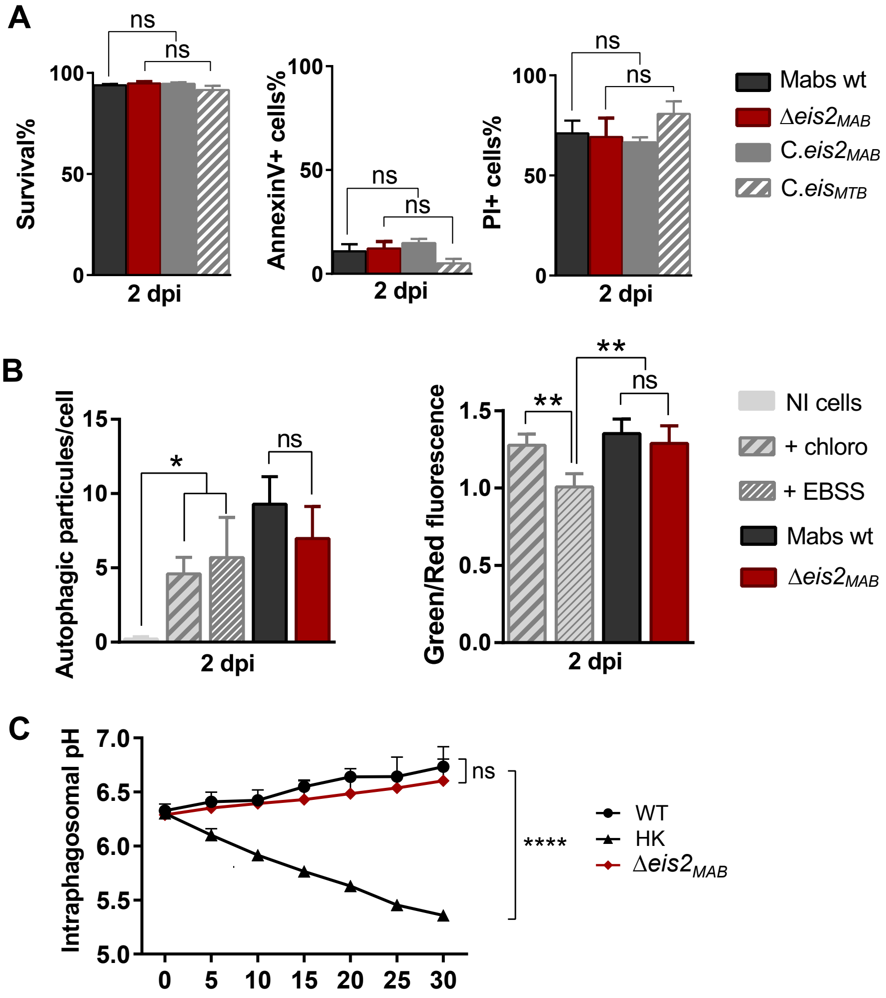

Supp. Table 1: Differentially expressed genes identified with the *DEseq2* package.

Transcriptomes	**Down DEGs**	**Up DEGs**	**Tot**	**CDS cover**
Mabs, Ac 4 hpi	1218	1355	2573	56%
Mabs, Ac 16 hpi	1095	1122	2217	49%
Mabs, Mϕ hpi	1646	1671	3317	73%
Mchel, Ac 16 hpi	1186	1949	3835	80%

Supp. Table 2: List of *M. abscessus* genes highly induced in Ac only.

Mma gene	Encoded protein	IP5 analysis (IPR)	Mabs BBH	MTB BBH	MAV BBH	FC Ac, 4 hpi	FC Ac, 16 hpi
Highly induced genes 4 hpi							
MYCMA_RS01100	Hypothetical protein	No IPR	*MAB_4664*			5.71	0.00
MYCMA_RS08980	Glyoxalase		*MAB_3056c*			5.32	1.81
MYCMA_RS10865	Hypothetical protein	Magnesium transporter MgtE (006669)	*MAB_2717c*		*MAV_2122*	5.26	1.44
MYCMA_RS14505	MULTISPECIES: 3-methyl-2-oxobutanoate hydroxymethyltransferase		*MAB_1916c*	*panB*	*panB*	5.22	1.89
MYCMA_RS17210	Copper oxidase		*MAB_1271c*			4.99	2.71
MYCMA_RS10860	Hypothetical protein	No IPR	-			4.99	1.26
MYCMA_RS11360	Monooxygenase		*MAB_2607*			4.93	0.00
MYCMA_RS10755	Hypothetical protein	No IPR	*MAB_2738c*			4.84	2.22
MYCMA_RS17970	Hypothetical protein	No IPR	*MAB_1117c*			4.49	2.33
MYCMA_RS07655	MULTISPECIES: alkylhydroperoxidase		*MAB_3311c*			4.48	3.25
MYCMA_RS02005	Short-chain dehydrogenase		*MAB_4478c*		*MAV_1797*	4.45	1.84
MYCMA_RS06830	Nitroreductase		*MAB_3461c*			4.38	2.11
MYCMA_RS20755	Membrane protein	Transport accessory protein MmpS (008693)	*MAB_0477*			4.31	2.77
MYCMA_RS09135	Hypothetical protein	Virulence factor BrkB (017039)	*MAB_3025*	*Rv2707*	*MAV_3600*	4.29	1.90
MYCMA_RS11655	Hypothetical protein	No IPR	-			4.23	1.87
MYCMA_RS08985	Pyridoxamine 5'-phosphate oxidase		*MAB_3055c*			4.16	0.00
MYCMA_RS11985	Sulfite reductase subunit alpha		*MAB_2492*			4.13	2.55
MYCMA_RS12840	Hypothetical protein	No IPR	*MAB_2313*			4.07	3.46
MYCMA_RS03450	Acyl-CoA dehydrogenase		*MAB_4158*	*fadE26*	*MAV_0652*	4.05	2.31
Highly induced genes 4 & 16 hpi							
MYCMA_RS18820	Sugar translocase		-			6.52	6.22
MYCMA_RS11145	MULTISPECIES: MmpS family protein		*MAB_2649*			4.73	6.82

MYCMA_RS19885	Short-chain dehydrogenase		*MAB_0646c*	*Rv0068*	*MAV_4710*	5.67	5.27
MYCMA_RS01105	Hypothetical protein	No IPR	*MAB_4663*			6.16	4.18
MYCMA_RS00455	Hypothetical protein	Twin-arginine translocation pathway, signal sequence (006311)	*MAB_4791c*			5.17	4.96
MYCMA_RS21900	TQXA domain-containing protein		*MAB_0219*		*MAV_2053*	5.33	4.73
MYCMA_RS07540	MULTISPECIES: ATP-binding protein		*MAB_3325c*			5.03	4.75
Highly induced genes 16 hpi							
MYCMA_RS13965	(Fe-S)-cluster assembly protein		*MAB_2020c*			2.77	4.93
MYCMA_RS17325	Hypothetical protein	No IPR	*MAB_1244c*			3.11	5.03
MYCMA_RS06800	MerR family transcriptional regulator		-			2.71	4.00
MYCMA_RS15995	TetR family transcriptional regulator		*MAB_1518*		*MAV_4046*	1.52	4.01
MYCMA_RS18825	MarR family transcriptional regulator		*MAB_0925c*			3.20	5.04
MYCMA_RS17330	Hypothetical protein	ABC transporter, FecCD/TroCD-like	*MAB_1243c*			2.86	4.97
MYCMA_RS17315	Hypothetical protein	Alkaline shock protein Asp23 (05531)	*MAB_1247c*			3.43	5.10
MYCMA_RS02820	MULTISPECIES: molecular chaperone		*MAB_4273c*	*dnaK*	*dnaK*	2.63	4.87
MYCMA_RS12505	Activator of HSP90 ATPase		*MAB_2387*			3.12	4.99
MYCMA_RS20640	Carboxymuconolactone decarboxylase		-			0.00	7.63
MYCMA_RS10645	YrbE family protein		-			0.00	4.71
MYCMA_RS16000	GlcNAc transferase		*MAB_1517c*		*MAV_4048*	0.00	4.31
MYCMA_RS17320	Hypothetical protein	No IPR	*MAB_1246c*			2.07	4.27
MYCMA_RS09170	GntR family transcriptional regulator		*MAB_3018*	*Rv0586*	*MAV_4554*	0.00	5.71
MYCMA_RS10650	Membrane protein	No IPR	-			0.00	4.17
MYCMA_RS01300	TetR family transcriptional regulator		*MAB_4625*			2.55	4.30
MYCMA_RS10655	Membrane protein	No IPR	-			0.00	4.05
MYCMA_RS02345	MULTISPECIES: alkyl hydroperoxide		*MAB_4408c*	*ahpC*	*MAV_2839*	3.46	4.04
MYCMA_RS14940	Transposase		-			3.22	4.03

Supp. Table 3 : List of *M. abscessus* genes highly induced in Mϕ or Ac 16 hpi.

Mma gene	**Encoded protein**	**IP5 analysis (IPR)**	**Mabs gene**	**MTB BBH**	**MAV BBH**	**FC M**ϕ	**FC Ac**
MYCMA_RS01880	Hypothetical protein	No IPR	*MAB_4509c*	-	-	5.78	5.11
MYCMA_RS01765	Hypothetical protein	N-acetyltransferase Eis (016181)	*MAB_4532c*	-	-	5.37	2.09
MYCMA_RS13035	MFS transporter		*MAB_2273*	-	-	5.26	4.15
MYCMA_RS17085	Acyltransferase		*MAB_1297c*	-	*MAV_4113*	4.86	4.91
MYCMA_RS08590	Transcriptional regulator		*MAB_3134c*	-	-	4.54	1.55
MYCMA_RS08595	Hemin transporter		*MAB_3133c*	-	-	4.36	1.14
MYCMA_RS06600	MULTISPECIES: transcriptional regulator		*MAB_3508c*	*whiB7*	*MAV_4142*	4,24	4,37
MYCMA_RS02565	Acetyltransferase		*MAB_4324c*	-	-	4.18	0.00
MYCMA_RS05440	Membrane protein	EamA domain (000620)	*MAB_3762*	-	-	3.96	4.49
MYCMA_RS12630	ABC transporter		*MAB_2355c*	-	-	3.92	1.76
MYCMA_RS17930	GNAT family acetyltransferase		*MAB_1125c*	-	-	3.89	1.62
MYCMA_RS05315	Hypothetical protein	No IPR	*MAB_3786c*	-	-	3.85	0.90
MYCMA_RS08600	Membrane protein	No IPR	*MAB_3132c*	*Rv2620c*	*MAV_3498*	3.83	0.00
MYCMA_RS19730	Membrane protein	EamA domain (000620)	*MAB_0677c*	-	-	3.83	0.00
MYCMA_RS22615	Esterase		*MAB_0078*	-	*MAV_3025*	3.82	2.92
MYCMA_RS13590	DEAD/DEAH box helicase		*MAB_2158c*	-	*MAV_2956*	3.81	4.38
MYCMA_RS11580	Universal stress protein UspA		-	-	-	3.78	0.00
MYCMA_RS06595	Hypothetical protein	No IPR	*MAB_3509c*	-	-	3.72	3.35
MYCMA_RS22620	IclR family transcriptional regulator		*MAB_0077*	-	*MAV_3024*	3.69	2.54
MYCMA_RS08355	Membrane protein	EamA domain (000620)	*MAB_3180*	-	*MAV_0095*	3.68	-1.60
MYCMA_RS04790	DNA-binding response regulator		*MAB_3891c*	*devR*	*MAV_4109*	3.62	-1.02
MYCMA_RS19020	Hypothetical protein	FAD//NAD(P)-binding domain superfamily (I036188)	*MAB_0857*	-	-	3.55	2.73
MYCMA_RS10510	Transporter		*MAB_2780c*	-	-	3.47	0.83
MYCMA_RS13570	Hypothetical protein	No IPR	-	-		3.45	0.00
MYCMA_RS16570	MFS transporter		*MAB_1409c*	*Rv1258c*	*MAV_1406*	3.44	2.59
MYCMA_RS22575	Taurine catabolism dioxygenase		*MAB_0086*	*Rv3406*	-	3.41	5.90
MYCMA_RS19295	Membrane protein		*MAB_0766*	-	-	3.36	0.00
MYCMA_RS11690	Transcriptional regulator		*MAB_2562c*	*Rv0081*	*MAV_5108*	3.32	0.00
MYCMA_RS21395	Catalase		*MAB_0351*	-	*katA*	3.27	0.00
MYCMA_RS00530	SAM-dependent methyltransferase		-	-	-	3.26	0.00

MYCMA_RS07025	Hypothetical protein	2 isopropylmate synthase LeuA, allosteric (dimerization) domain superfamily (036230)	*MAB_3424c*	-	*MAV_3928*	3.25	3.06
MYCMA_RS19455	Hypothetical protein	No IPR	*MAB_0733*	-	-	3.22	1.62
MYCMA_RS06265	Alkane 1-monooxygenase		*MAB_3598c*	*alkB*	*MAV_4215*	3.14	2.51
MYCMA_RS15610	Guanylate cyclase	Papain-like cysteine peptidase superfamily (IPR038765)	*MAB_1591*	*Rv1118c*	*MAV_1249*	3.14	0.00
MYCMA_RS14880	ABC transporter		*MAB_1846*	-	-	3.09	2.68
MYCMA_RS14875	Peptidase		*MAB_1847*	-	-	3.04	0.87
MYCMA_RS02705	MULTISPECIES: aminotransferase AlaT		*MAB_4294*	*aspC*	*MAV_4818*	3.04	1.54
MYCMA_RS19450	Hypothetical protein	Leukocidin/porin MspA superfamily (036435)	*MAB_0734*	-	-	3.00	3.15