

Supplementary Information

Differential expression of immune receptors in two marine sponges upon exposure to microbial-associated molecular patterns

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Supplementary Tables

Table S1. Annotated genes in *A. aerophoba* reference transcriptome containing conserved domains that characterise PRRs: TIR domain, NACHT domain, and SRCR domain.

Table S2. Annotated genes in *D. avara* reference transcriptome containing conserved domains that characterise PRRs: TIR domain, NACHT domain, and SRCR domain.

Table S3. Differential gene expression analysis comparing *A. aerophoba* samples in MAMP vs control treatment within each time point by edgeR (genes with FDR p-value < 0.05). logFC: log (fold change), negative values denote down-regulation, positive values denote up-regulation in the MAMP treatment compared to control. Log CPM: log (counts per million). FDR: false discovery rate-corrected p-value.

Table S4. Differential gene expression analysis comparing *D. avara* samples in MAMP vs control treatment within each time point by edgeR (genes with FDR p-value < 0.05). logFC: log (fold change), negative values denote down-regulation, positive values denote up-regulation in the MAMP treatment compared to control. Log CPM: log (counts per million). FDR: false discovery rate-corrected p-value.

Table S5. Differentially expressed genes (FDR p-value < 0.005) in *A. aerophoba* that are involved in metabolism, gene expression and other functions.

Table S6. Full annotation of differentially expressed genes (FDR p-value < 0.05) in *A. aerophoba*.

Table S7. Full annotation of differentially expressed genes (FDR p-value < 0.05) in *D. avara*.

Table S5.

Gene Description	Gene IDs	Time	LogFC	FDR
Metabolism				
Long-chain-fatty-acid-CoA ligase	TR142631_c0_g2	3h	4.7	0.003
	TR142631_c0_g9	3h	5.2	0.003
	TR142631_c0_g18	3h	6.7	2.6 e-5
	TR142631_c0_g21	3h	6.1	2.8 e-6
	TR125500_c0_g7	3h	-5.2	4.6 e-5
PP2Cc	TR153708_c0_g1	3h	3.9	2.0 e-4
3-oxoacid CoA-transferase 2A	TR66545_c0_g1	1h	-8.2	6.4 e-4
Acyl-CoA binding domain containing gene	TR150854_c0_g3	5h	-9.3	0.002
Aldehyde dehydrogenase	TR156897_c3_g16	5h	9.5	2.5 e-5
Cytochrome c oxidase assembly factor 3	TR131371_c0_g3	1h	3.5	4.9 e-3
Ubiquinone	TR164188_c2_g2	3h	7.3	0.001
Polysaccharide deacetylase	TR165674_c0_g1	3h	4.4	8.8 e-4
Phosphoglycerate mutase	TR166027_c9_g4	1h;	4.8;	1.5 e-4;
		3h	5.5	4.9 e-4
	TR166027_c9_g6	1h	-5.6	7.1 e-5
Transketolase	TR72158_c0_g1	3h	3.9	2.7 e-4
	TR72158_c0_g3	1h;	-3.9;	0.004;
	TR72158_c0_g4	3h;	-4.9;	1.8 e-4;
		5h	-4.6	2.9 e-4
	TR72158_c0_g5	3h	3.9	4.2 e-4
Transglutaminase	TR167657_c5_g1	3h	7.4	0.003
Adenylosuccinate ligase	TR301111_c0_g1	5h	-8.9	5.2 e-5
Aminotransferase	TR164899_c5_g3	3h	-5.7	8.2 e-5
Potassium channel tetramerization containing domain	TR139584_c0_g2	5h	-7.9	0.001
Ribosomal proteins	TR138971_c2_g2	5h	-8.8	2.6 e-5
	TR154264_c1_g2	1h;	7.7;	0.001;
		3h	7.5	0.001
	TR154264_c1_g5	1h;	-8.2;	1.1 e-4;
		3h;	-8.1;	5.4 e-5;
		5h	-8.7	1.1 e-4
	TR154264_c3_g2	3h	3.4	0.003

Table S5. (cont)

Gene Description	Gene IDs	Time	LogFC	FDR
DNA binding/regulation of transcription				
Pentatricopeptide repeat-containing gene	TR119847_c0_g1	1h; 3h	8.1; 7.8	1.1 e-4; 9.7 e-4
Endonucleases	TR150148_c0_g1	5h	8.0	0.003
	TR173868_c3_g2	3h	5.8	0.004
	TR170028_c2_g3	1h	7.7	4.6 e-3
	TR171176_c2_g2	3h	8.3	1.2 e-8
	TR173961_c1_g1	3h	8.0	2.7 e-4
	TR167214_c1_g2	3h	-5.7	0.004
	TR172196_c2_g5	1h	-10.0	8.5 e-4
Transposases	TR157594_c1_g10	5h	6.0	1.7 e-4
	TR157594_c1_g16	5h	5.6	1.4 e-4
	TR163992_c2_g3	3h	-10.1	5.2 e-9
Polymerase	TR171723_c1_g28	1h; 3h; 5h	7.3; 6.3; 8.9	0.002; 0.003; 4.5 e-3
Transcription factors	TR174280_c6_g4	1h; 3h; 5h	8.6; 7.9; 8.2	1.0 e-4; 9.4 e-5; 0.002
	TR163347_c3_g4	3h	8.3	0.002
Zinc knuckle and retroviral aspartyl protease domain-containing gene	TR168355_c5_g4	3h	7.7	4.2 e-3
Zinc finger protein like	TR170073_c2_g3	3h	-8.2	9.2 e-5
Ependymin-related	TR174135_c4_g21	5h	-7.6	6.8 e-9

Gene description is based on domain annotation and/or blast results. Supplementary Information provides full information on annotation (including e-values) (Supplementary TableS6) and full DGE results (here we provide rounded log₂ fold change and FDR p-values, full values are reported in Supplementary TableS3). Log FC: log₂ (fold change). Positive values of Log FC denote up-regulated genes and are coloured in orange; negative values of log FC denote down-regulated genes and are coloured in blue. FDR: false discovery rate-corrected p-value.

Supplementary Figures

Figure S1. Components of the Toll-like receptor signalling pathway identified in the reference transcriptome of *A. aerophoba* by KEGG annotation (in red). KEGG: Kyoto Encyclopaedia of Genes and Genomes (Kanehisa et al. 2017). Publication permission granted by Kanehisa laboratories (Ref. 180230).

Figure S2. Components of the Toll-like receptor signalling pathway identified in the reference transcriptome of *D. avara* by KEGG annotation (in red). KEGG: Kyoto Encyclopaedia of Genes and Genomes (Kanehisa et al. 2017). Publication permission granted by Kanehisa laboratories (Ref. 180230).

Figure S3. Number of differentially-expressed genes per time point (FDR p-value < 0.005) for each species as obtained by DESeq2 analysis.

References

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., and Morishima, K. (2017). KEGG: New perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res.* **45**, D353–D361. doi:10.1093/nar/gkw1092.

Figure S1-KEGG map visualization of the Toll-like receptor signalling pathway in the reference transcriptome of *A. aerophoba*

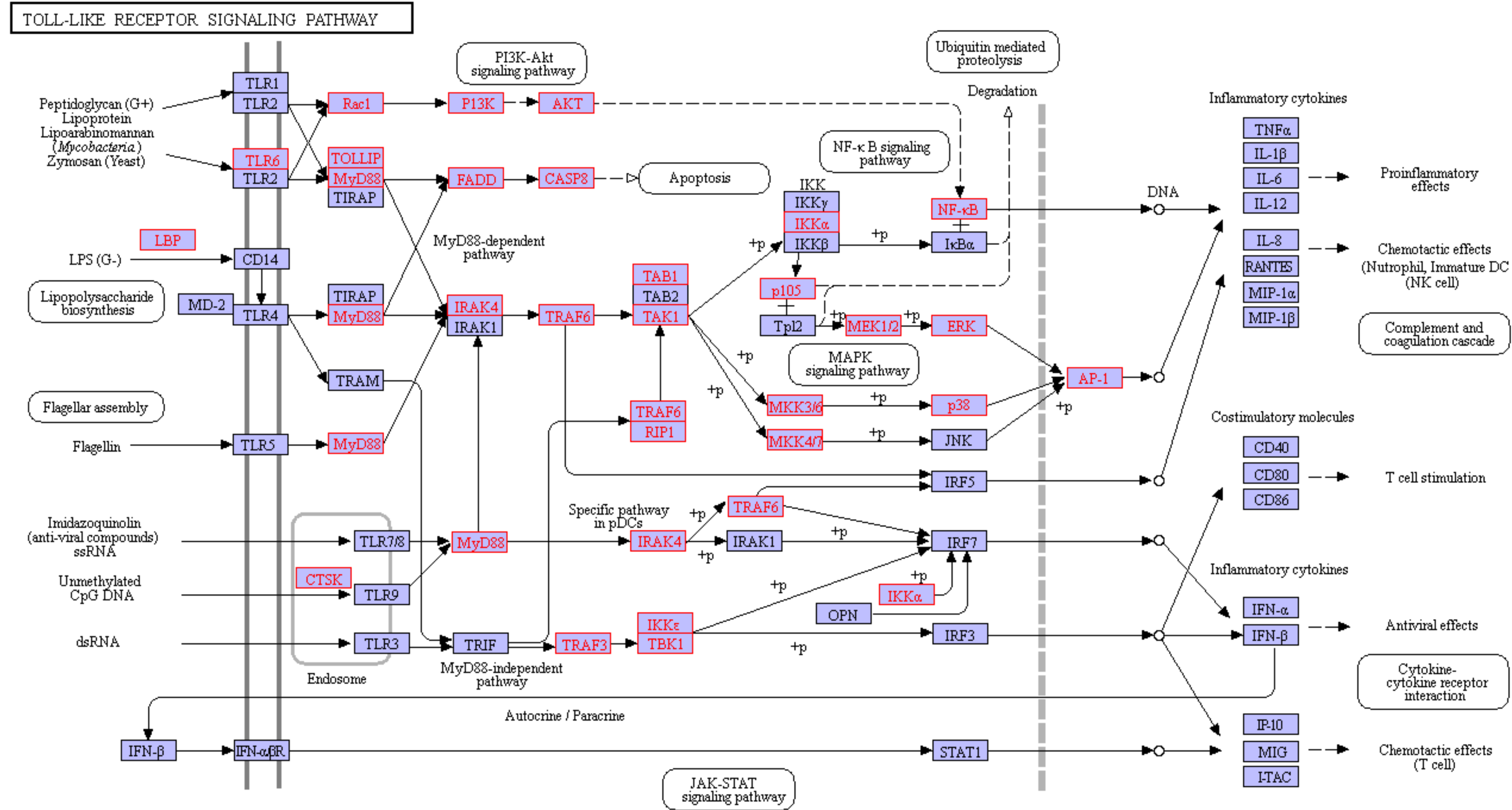


Figure S2- KEGG map visualization of the Toll-like receptor pathway in the reference transcriptome of *D. avara*

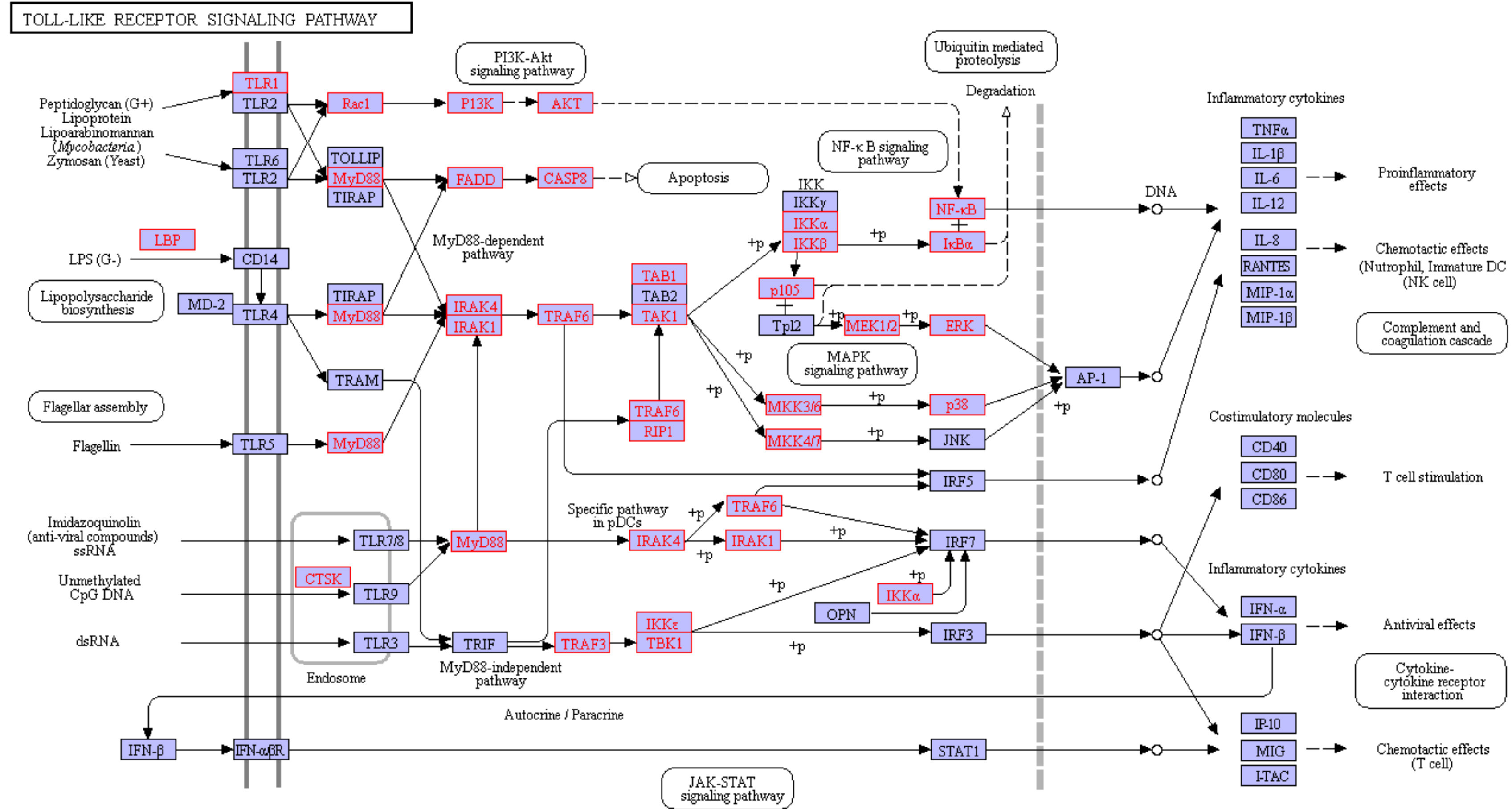


Figure S3-Number of DEGs
(FDR p-value < 0.005) as
identified in DESeq2

Number of DEGs

1h

3h

5h

A. aerophoba

D. avara

— down-regulated — up-regulated

