

Supplementary Information

Epigenetic differences in the innate response after immune stimulation during zebrafish sex differentiation

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Keywords: gonads, immune, LPS, methylation, reproduction, sex ratio, zebrafish

Supplementary tables

Table S1. Information of the primer sequences, the coordinate location in the *Danio rerio* genome (GRCz11) and the number of CpG sites of three immune related genes used for DNA methylation analysis. Abbreviation: base pairs, bp; transcription start site, TSS.

Gene name	Gene symbol	Reference sequence	Chromosome	Strand	Forward primer	Reverse primer	Amplicon size (bp) and relative position from the TSS (+1)	Total number of CpG in amplicon	Number of CpG before the TSS in amplicon
interleukin 1 β	<i>il1b</i>	NM_212844	10	+	TTTTAAAGGGGTTTGTTTAAATAATAATAA	CAACACTATCTATCTCCACAAACTAC	459 (-182, 277)	9	7
caspase 9	<i>casp9</i>	NM_001007404	23	+	AATATTATTTTGGAAATTTTGAATGTA	AAAACCCCTTTAACAAAACTTAT	441 (-337, 104)	19	15
tumor necrosis factor α	<i>tnfa</i>	NM_212859	19	+	TTTGAAAGTTTTGTGTATATAAATTATT	AATCCAAC TTCCTCCTAAACACT	498 (-409, 89)	7	4

Table S2. Information of primer sequences used for quantitative (qPCR) gene expression. Abbreviation: base pairs, bp.

Gene name	Gene symbol	Accession number	Sequence Forward	Sequence Reverse	Amplicon size (bp)	Efficiency
interleukin 1 β	<i>il1b</i>	NM_212844	TGGACTTCGACGACAAAATG	GTTCACCTCACGCTCTTGGATG	110	2.06
caspase 9	<i>casp9</i>	NM_001007404	CAACATCGACTGCGACAAGC	CATCTCGTGCCTTATGCGTTTAG	101	1.98
tumor necrosis α	<i>tnfa</i>	NM_213190	CAATCCGCTCAATCTGCACG	GTGCCCAGTCTGTCTCCTTC	103	2.10
eukaryotic translation elongation factor 1 alpha 1 α	<i>eef1a1/1</i>	NM_131263	CTGGAGGCCAGCTCAAACAT	ATCAAGAAGAGTAGTACCGCTAGCATTAC	81	1.95
ribosomal protein L13a	<i>rpl13a</i>	NM_212784	TCTGGAGGACTGTAAGAGGTATGC	AGACGCACAATCTTGAGAGCAG	95	1.93

Supplementary figures

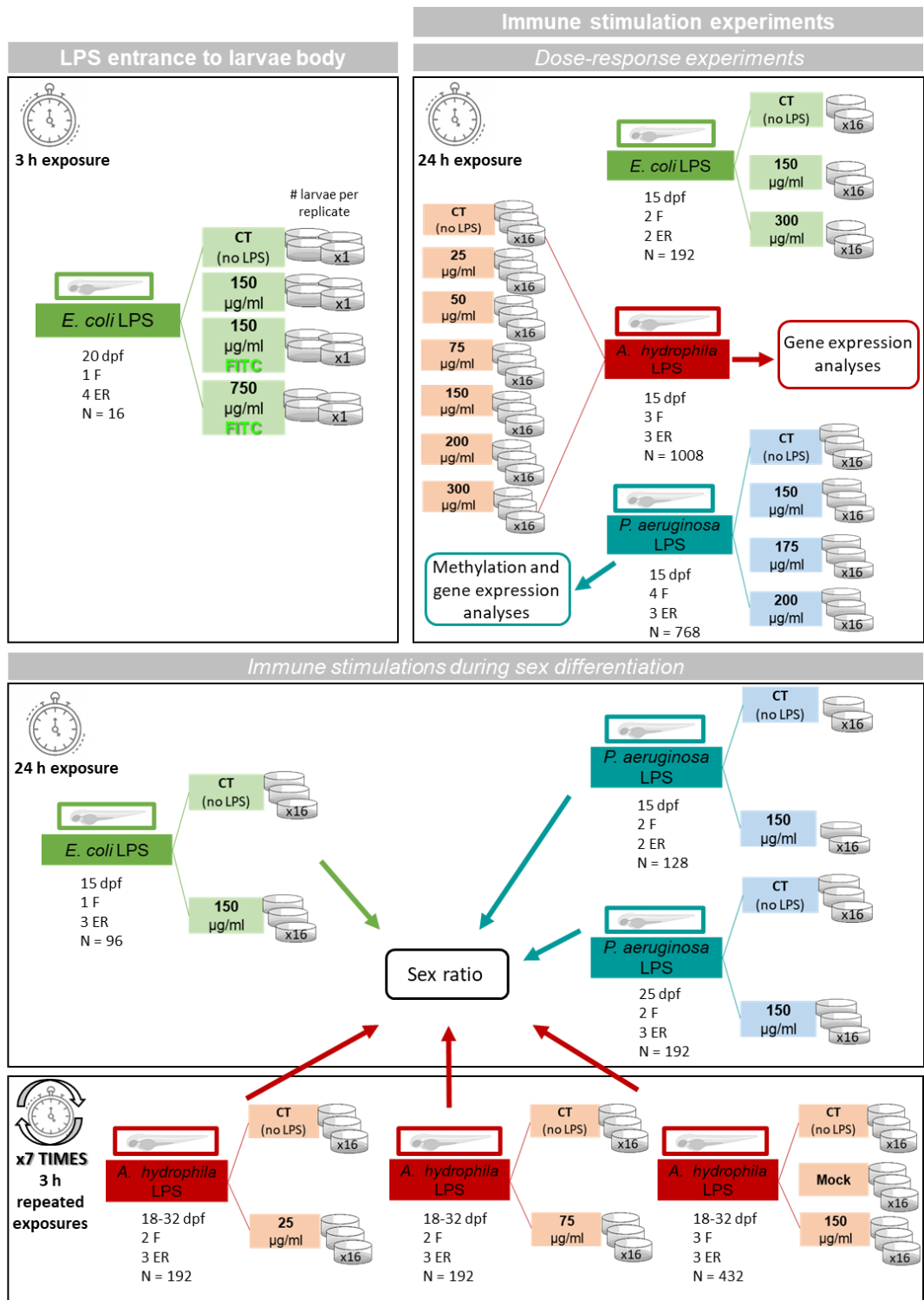


Figure S1. Diagram of all the experiments performed in this study by using zebrafish subjected to LPS bath treatments of three different types of LPS: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Aeromonas hydrophila*. Abbreviations: dpf: days post fertilization; F: number of families; ER: number of experimental replicates; N: total number of initial fish per experiment.

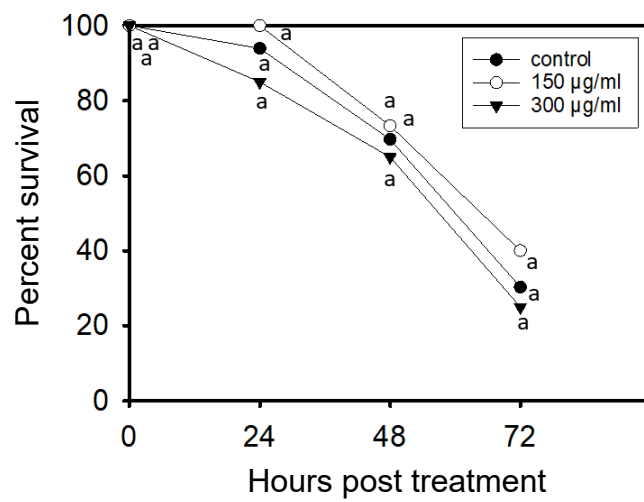
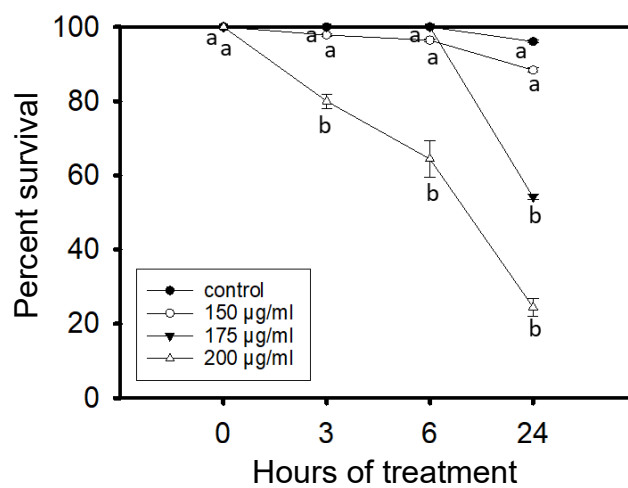
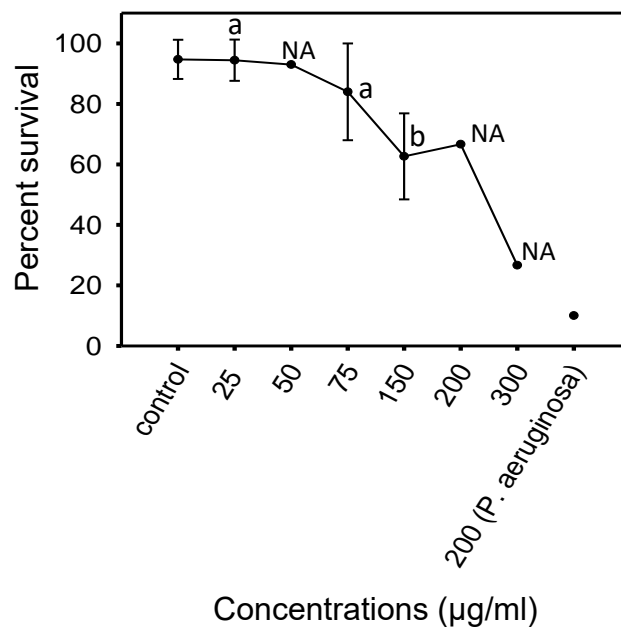
A**B****C**

Figure S2. Dose-response experiments to different LPS strains by immersion. Data show the mean \pm SEM. Statistically significant differences ($P < 0.05$) against the control group are indicated by different letters and were examined by one-way analysis of variance (ANOVA), and Tukey's test was used to perform *post hoc* multiple comparisons. **(A)** Survival of zebrafish along the time (24, 48 and 72 h) after 24 h of exposure to *Escherichia coli* LPS starting at 15 days post fertilization (dpf). Data obtained from two different family pairs. The total number of fish was 192. Positive control of *E. coli* was performed at lethal dose of 300 $\mu\text{g ml}^{-1}$. **(B)** Survival of zebrafish along 24 h of exposure to *Pseudomonas aeruginosa* LPS starting at 15 dpf. Data obtained from four different families. The total number of fish for survival data was 768. Positive control of *P. aeruginosa* was performed at lethal dose of 200 $\mu\text{g ml}^{-1}$. **(C)** Survival of zebrafish after 24 h exposure to *Aeromonas hydrophila* LPS starting at 15 dpf. Data obtained from three different families. The total number of fish was 1008. Positive control of *P. aeruginosa* was performed at lethal dose of 200 $\mu\text{g ml}^{-1}$.