

## Supplementary Materials

**Table S1.** Primer sequences for qPCR.

| Gene name    | Forward primer (5'-3')   | Reverse primer (5'-3')  |
|--------------|--------------------------|-------------------------|
| Clpro        | CACCCACGCAGATTGGAACT     | TGACGATGGTGCCCACAGAC    |
| Durt1        | TACCACGCCGCCATACCCAA     | GCAGGCTGACTGATACTGAAGGC |
| Chymotrypsin | ACATCAGGTGGAAGAAGCGTC    | GTGTTGAATGTGACGGGCT     |
| Hemocyanin   | TGGTGTGGCTCGCATCAGA      | GCACCCACGACATCAATACCAT  |
| Vmo1         | AAGAGGGGTGACTGGCAAGG     | TCCACTCCAAGTCATCGCC     |
| Hemocyanin A | GAAGTCAAGGCTGGAACGCA     | CATCATTACAGCCTCGTCAGTC  |
| Nodulin-75   | CACCTACACTCCTCCCTGGCA    | TTAGGCAGCGACGAACCTGAA   |
| CD97         | CCTACAAGAACGGTGGAGCG     | TGGGGAAAAGAAATGTTGGGA   |
| Durt2        | TGACATCTTACCTGAACTGCCAA  | AGGCGGTAGTTATCCATCCCA   |
| Iscw         | AAGATGGAATAATAGCCTTACGGC | AGGCGGGAGCAAATCACTG     |
| TLR1         | GCTTGGAGTACACCCAGAA      | TTAACAAAGTCCGTCCAGGTG   |
| TLR2         | GATCCCAGCAGACTCTGAGC     | CAAGATCTGGTGGATGGGGC    |
| SR           | GCACTACTGTCCCTGGCAAT     | GCCCTCACACCATTGAGAA     |
| Mapkk4       | TTCAGTCTGTGTGAGGAGCC     | GGTGGTGGATGGTATCA       |
| Mapkk7       | GTGGCAGTGAAGAAGGTAGAAA   | GCAAACACTCAATGACCAGGCA  |
| Mapkk6       | CGCTCAGATGTGTGGTCACT     | GAAAGTTCCAGGAGGGAGGC    |

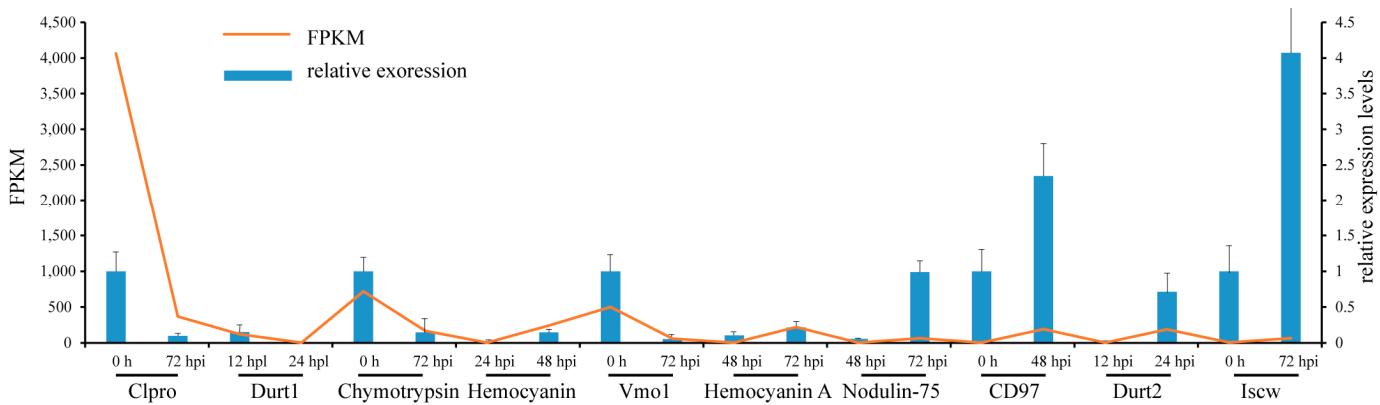
**Table S2.** Statistics of the transcriptome sequencing from blood samples of *P. trituberculatus*.

| Sample   | Raw_reads  | Clean_reads | Q20 (%) | Q30 (%) | Mapped to genome (%) | Coverage depth |
|----------|------------|-------------|---------|---------|----------------------|----------------|
| 0h_1     | 45,218,876 | 44,011,870  | 97.46   | 93.1    | 88.54%               | 7.97           |
| 0h_2     | 37,195,138 | 36,084,876  | 97.69   | 93.63   | 88.79%               | 8.27           |
| 0h_3     | 45,525,094 | 44,600,106  | 97.89   | 94.35   | 88.59%               | 8.01           |
| Ls_12h_1 | 46,859,006 | 45,828,352  | 98.17   | 94.92   | 87.23%               | 8.24           |
| Ls_12h_2 | 45,279,026 | 44,062,912  | 98.06   | 94.5    | 87.70%               | 7.93           |
| Ls_12h_3 | 42,583,858 | 41,359,654  | 98.08   | 94.65   | 86.33%               | 7.43           |
| Ls_24h_1 | 47,453,074 | 46,231,682  | 97.92   | 94.35   | 84.48%               | 8.31           |
| Ls_24h_2 | 45,837,770 | 44,449,438  | 98.01   | 94.57   | 84.96%               | 8.00           |
| Ls_24h_3 | 44,245,524 | 43,263,644  | 97.93   | 94.29   | 85.09%               | 7.78           |
| Ls_48h_1 | 46,152,442 | 45,084,506  | 98.13   | 94.79   | 85.96%               | 8.11           |
| Ls_48h_2 | 49,249,936 | 48,575,640  | 97.65   | 93.72   | 87.32%               | 8.74           |
| Ls_48h_3 | 45,747,746 | 45,034,808  | 97.61   | 93.64   | 87.06%               | 8.11           |
| Ls_72h_1 | 45,811,612 | 44,415,824  | 97.65   | 93.74   | 86.42%               | 7.99           |
| Ls_72h_2 | 41,454,254 | 40,265,614  | 97.8    | 94.12   | 86.06%               | 7.24           |
| Ls_72h_3 | 45,655,424 | 44,919,668  | 97.54   | 93.63   | 85.94%               | 8.08           |
| Vp_12h_1 | 43,997,912 | 43,196,466  | 97.35   | 93.14   | 86.61%               | 7.77           |
| Vp_12h_2 | 38,543,930 | 37,381,920  | 97.52   | 93.57   | 86.82%               | 6.73           |
| Vp_12h_3 | 43,404,780 | 42,550,502  | 97.59   | 93.71   | 86.88%               | 7.65           |
| Vp_24h_1 | 44,069,140 | 43,047,190  | 97.56   | 93.43   | 85.74%               | 7.75           |

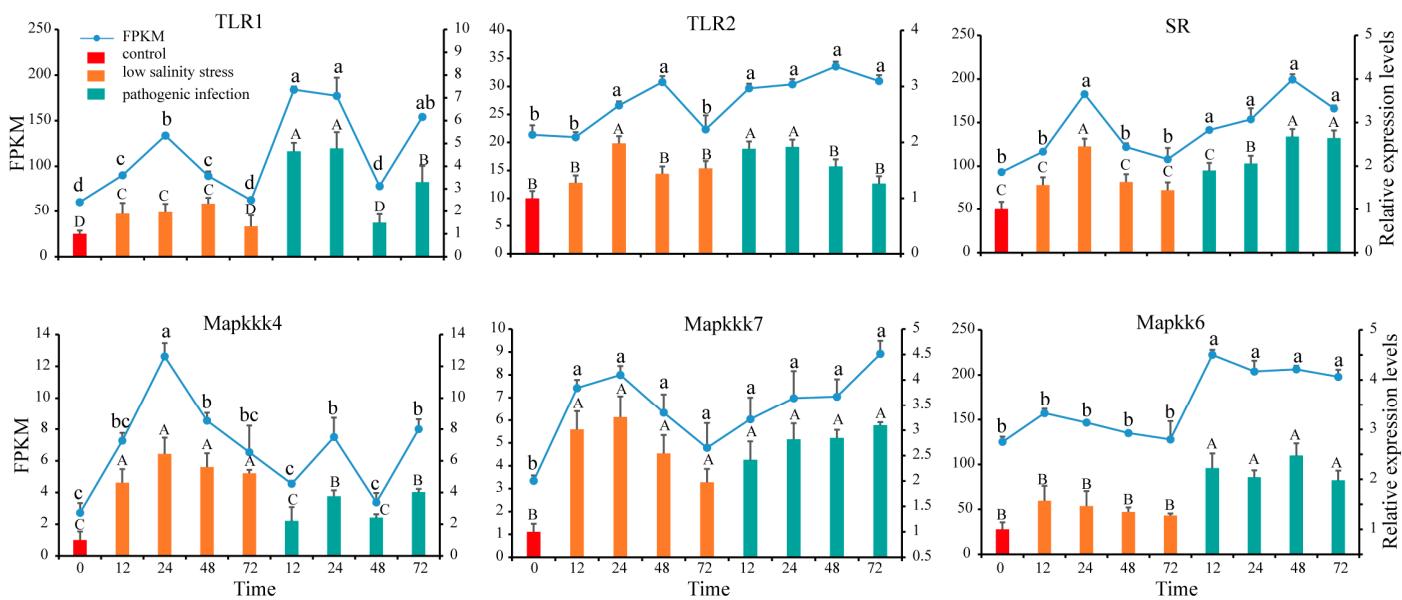
|          |            |            |       |       |        |      |
|----------|------------|------------|-------|-------|--------|------|
| Vp_24h_2 | 38,601,836 | 37,812,006 | 97.24 | 92.7  | 85.78% | 6.80 |
| Vp_24h_3 | 42,987,796 | 41,768,994 | 97.64 | 93.8  | 87.00% | 7.52 |
| Vp_48h_1 | 46,401,016 | 45,466,782 | 97.7  | 93.83 | 86.12% | 8.18 |
| Vp_48h_2 | 42,439,408 | 41,578,678 | 97.74 | 93.98 | 86.02% | 7.48 |
| Vp_48h_3 | 47,674,034 | 46,769,312 | 97.68 | 93.88 | 85.73% | 8.42 |
| Vp_72h_1 | 46,008,204 | 45,190,418 | 97.43 | 93.35 | 85.65% | 8.13 |
| Vp_72h_2 | 43,930,664 | 43,097,034 | 97.42 | 93.3  | 85.56% | 7.75 |
| Vp_72h_3 | 40,381,368 | 39,615,108 | 97.55 | 93.58 | 86.07% | 7.12 |

**Table S3.** KEGG of DEGs with opposite trends in expression patterns post pathogen infection and low salinity stress.

|                 | KEGG ID  | Description                              | BgRatio | padj     | Gene name  | Count |
|-----------------|----------|--|---------|----------|--|-------|
| Vp_up & ls_down | dme04310 | Wnt signaling pathway                    | 64/2203 | 0.001662 | receptor tyrosine kinase   | 2     |
|                 | dme04150 | mTOR signaling pathway                   | 73/2203 | 0.06519  | Frizzled-4   | 1     |
|                 | dme00760 | Nicotinate and nicotinamide metabolism   | 16/2203 | 0.053146 | Frizzled-4   | 1     |
|                 | dme00260 | Glycine, serine and threonine metabolism | 25/2203 | 0.053146 | Cytosolic purine 5'-nucleotidase   | 1     |
|                 | dme00513 | Various types of N-glycan biosynthesis   | 25/2203 | 0.053146 | sarcosine dehydrogenase  | 1     |
| Vp_down & ls_up | dme00510 | N-Glycan biosynthesis                    | 31/2203 | 0.053146 | alpha-1,3-mannosyl-glycoprotein<br>4-beta-N-acetylglucosaminyltransferase C-like | 1     |
|                 | dme00240 | Pyrimidine metabolism                    | 33/2203 | 0.053146 | alpha-1,3-mannosyl-glycoprotein<br>4-beta-N-acetylglucosaminyltransferase C-like | 1     |
|                 | dme00230 | Purine metabolism                        | 72/2203 | 0.09492  | Cytosolic purine 5'-nucleotidase   | 1     |

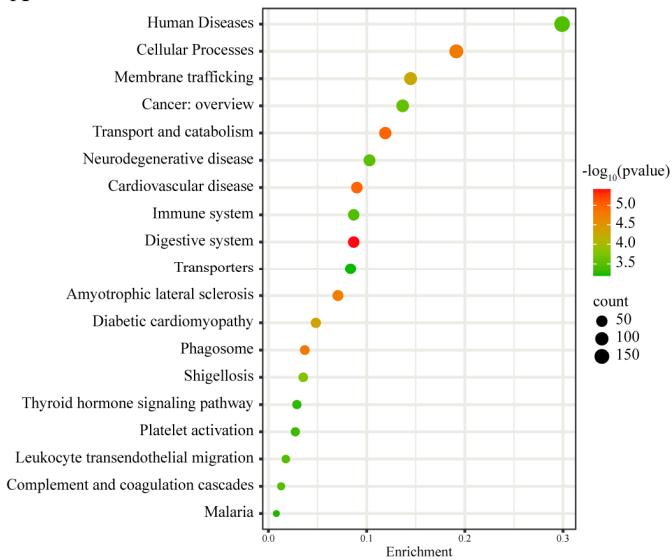


**Figure S1.** qPCR validation of transcriptome data. The RT-qPCR data was presented as the mean  $\pm$  S.D.

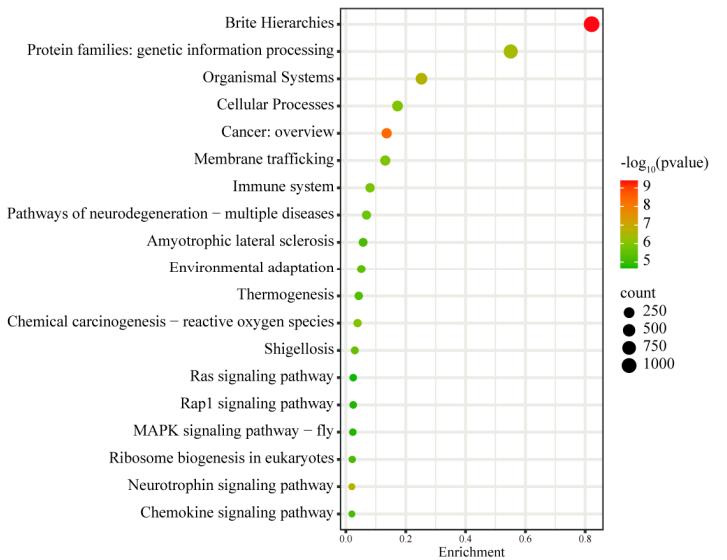


**Figure S2.** qPCR validation of genes in the Toll and IMD signaling pathways. TLR1, Toll-like receptors 1; TLR2, Toll-like receptors 2; SR, scavenger receptor; Mapkk4, mitogen-activated protein kinase kinase 4; Mapkk7, mitogen-activated protein kinase kinase 7; Mapkk6, mitogen-activated protein kinase kinase 6. Different lowercase letters indicate significant differences ( $P < 0.05$ ) in RNA-seq; different uppercase letters indicate significant differences ( $P < 0.05$ ) in qPCR.

A



B



**Figure S3.** KEGG annotation of DEGs under low salinity and *V. parahaemolyticus*. (A) KEGG annotation of shared DEGs under low salinity stress. (B) KEGG annotation of shared DEGs under pathogen stress.