

## Additional Information

### **Impact of *Tilapia* hepcidin 2-3 dietary supplementation on the gut microbiota profile and immunomodulation in the grouper (*Epinephelus lanceolatus*)**

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## Supplementary Figure legends

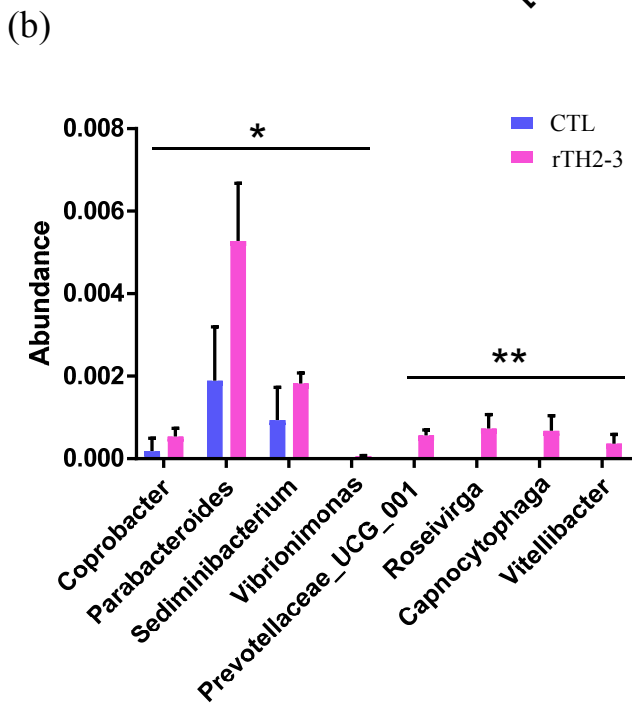
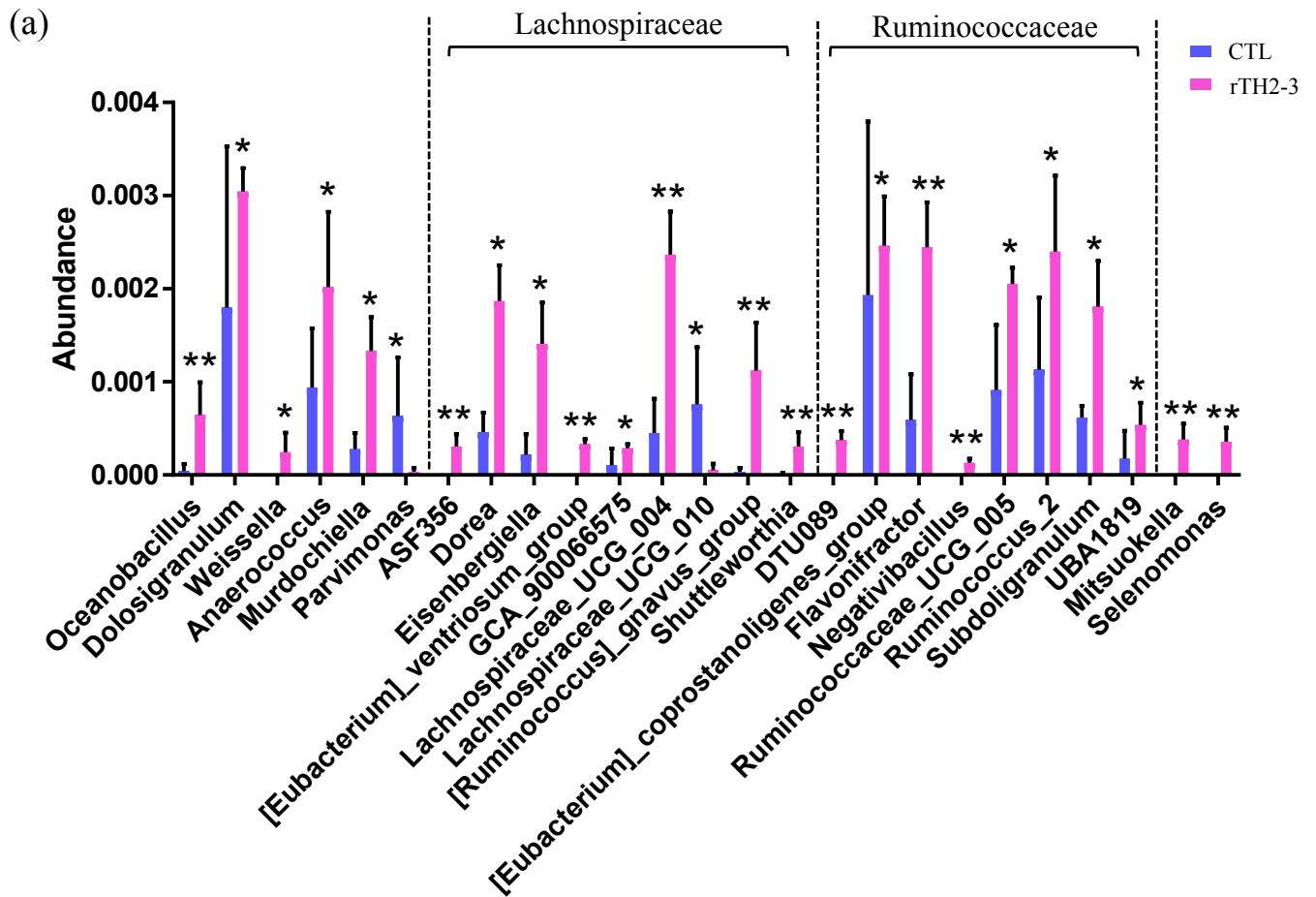
**Supplementary Figure 1.** Abundance analyses at the genus level. **(a, b)** Bacteria from the phyla *Firmicutes* (a) and *Bacteroidetes* (b) were analyzed for their abundance in regular diet and rTH2-3-supplemented groups. Specific bacteria belonging to the families *Lachnospiraceae* and *Ruminococcaceae* are indicated in (a). Results are presented as mean  $\pm$  SD (n = 3 in each group), \* $p$  < 0.05; \*\* $p$  < 0.01 between groups, analyzed by STAMP, Welch's t-test.

**Supplementary Figure 2.** PICRUSt classification of KEGG Orthology (KO). **(a–g)** Predicted functional genes in the categories of environmental information processing (a), genetic information (b), human diseases (c), immune system (d), metabolism (e–g) at KEGG level 3 are shown for the gut microbiota. Results are presented as mean  $\pm$  SEM (n = 3 in each group).

**Supplementary Table 1.** Primer sequences used for quantitative real-time PCR.

| Genes (Predicted)               | Sequence   | Reference species            |
|---------------------------------|--|------------------------------|
| <i><math>\beta</math>-Actin</i> | F: TGTGTCATCTTCTCCCTGT<br>R: GAGAGGTATCCTGACTCTGAAGTA        | <i>Oryzias latipes</i>       |
| <i>Lysozyme</i>                 | F: GACATTCAGTCCAGCCTAGAGTCC<br>R: CCCGACTCCCCTGGCTC          | <i>Epinephelus coioides</i>  |
| <i>C6</i>                       | F: TTGAGGCGTCACAGCGA<br>R: GGCCTGTTCTCTCAGGA                 | <i>Amphiprion ocellaris</i>  |
| <i>C7</i>                       | F: CGATCACAGCTCCAACC<br>R: AAGAGCCACAGAGGAGT                 | <i>Labrus bergylta</i>       |
| <i>CCL1</i>                     | F: ATCTCAGTCTCAGGTTTCATTG<br>R: TCTCCATCATCACATCCACT         | <i>Epinephelus coioides</i>  |
| <i>Tlr-5</i>                    | F: AGAAATGGTGATTCAAGACCC<br>R: TCAAATGTGAATTTCCATCTGATTTCTAT | <i>Epinephelus bruneus</i>   |
| <i>Transferrin</i>              | F: GCAACATGGGCTAGTGGTGT<br>R: AATGCGAACAACCTCATTGC           | <i>Epinephelus coioides</i>  |
| <i>Il-1<math>\beta</math></i>   | F: TAAACCTCTGTGTGGCG<br>R: CACTGACCTGCACAGAC                 | <i>Epinephelus coioides</i>  |
| <i>Il-8</i>                     | F: CATGATGGAAGCCATGAG<br>R: GTCAGCGTGTGAGGAAT                | <i>Oplegnathus fasciatus</i> |
| <i>Mhc-I<math>\alpha</math></i> | F: TCTCCTGGTGTTACTGTCATAG<br>R: CGCTTCTGCTTCTCGTG            | <i>Epinephelus coioides</i>  |
| <i>Mhc-II</i>                   | F: CATGGCCTCACCTCTAC<br>R: TGTGGGATTAGAACTGGACA              | <i>Sebastes schlegelii</i>   |

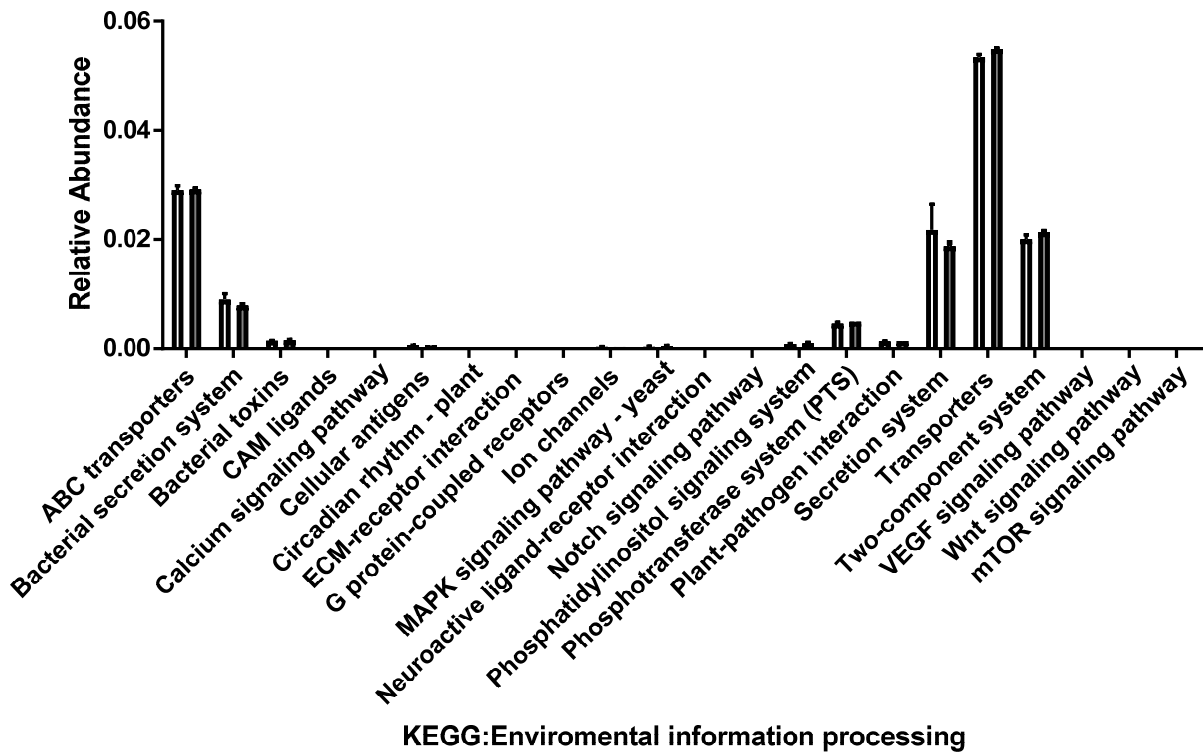
# Supplementary Figure 1



# Supplementary Figure 2

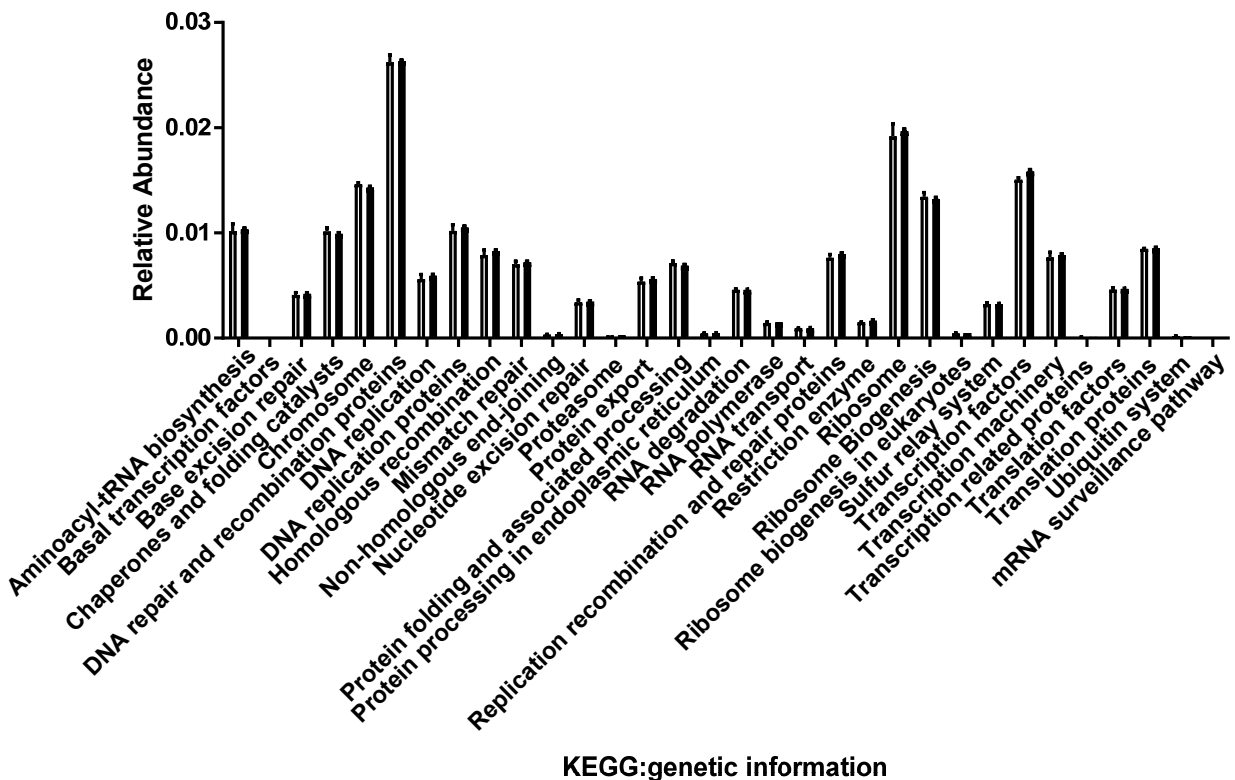
(a)

CTL  
rTH2

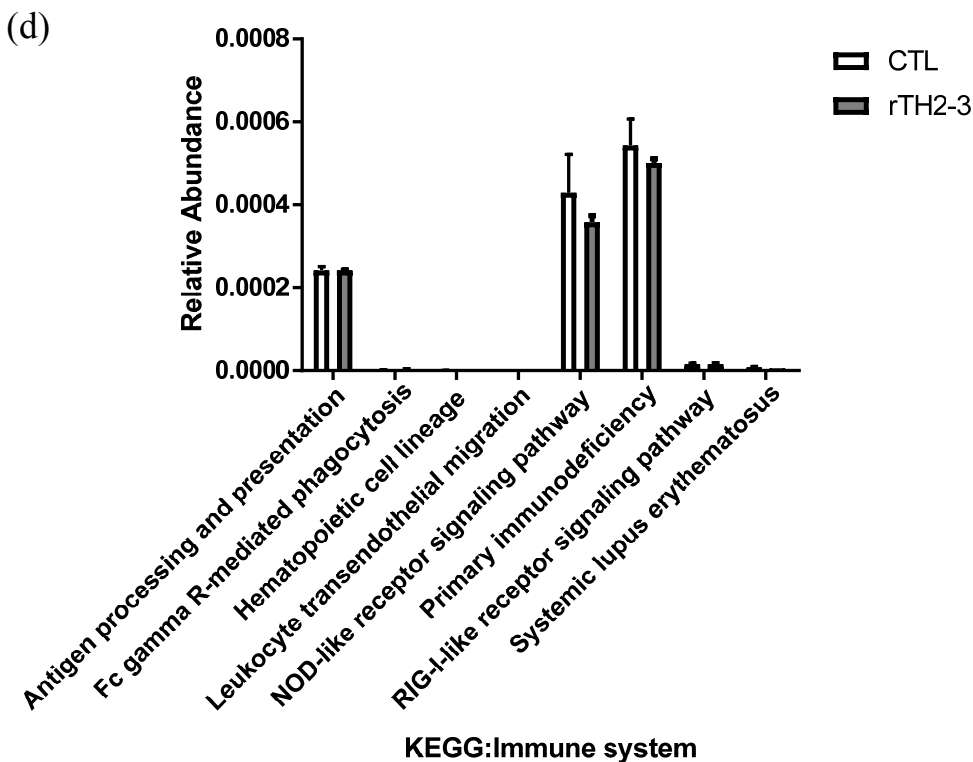
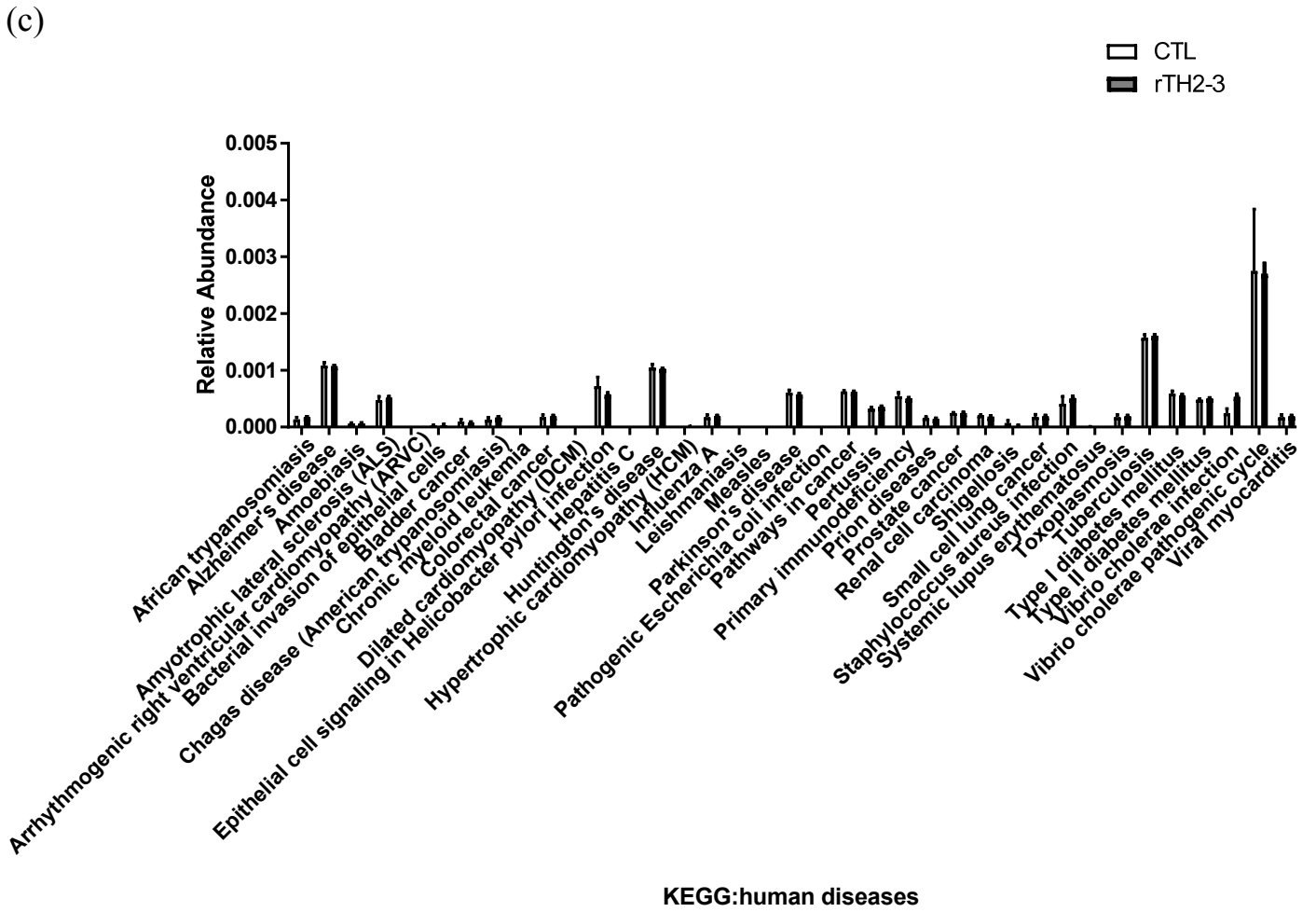


(b)

CTL  
rTH2-3



# Supplementary Figure 2





# Supplementary Figure 2

(g)

