

Supplementary materials

Article information

Interleukin-17 receptor A1 gene knockout causes weight loss and reduction of intestinal metabolism-related genes in the Japanese medaka, *Oryzias latipes*

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* Tables S1-S7 are separately shown in the Excel file as "Supplementary tables".

Fig. S1.

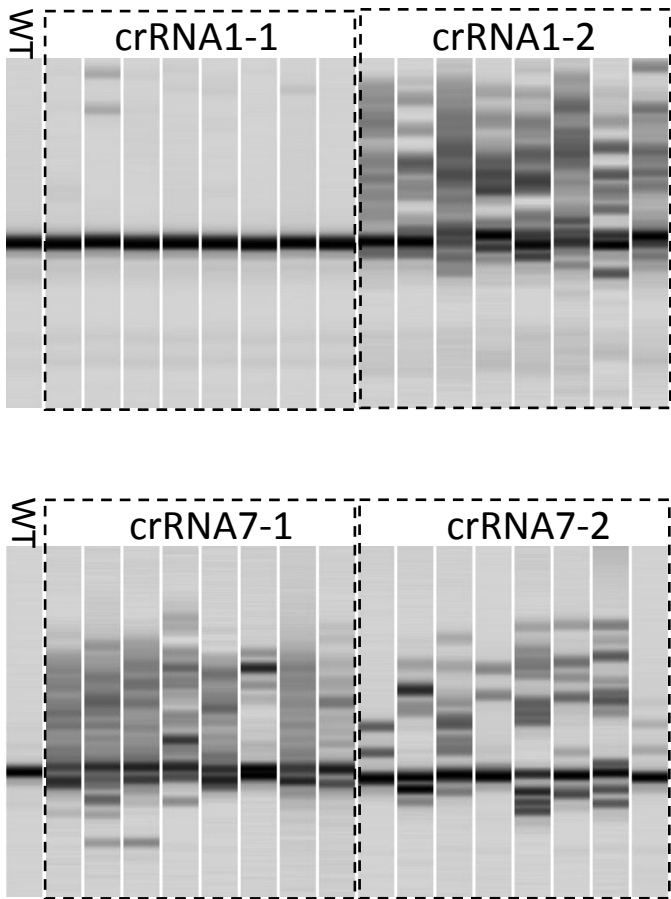


Fig. S1. Efficiency of mutation by each crRNA. The mutation efficiency was evaluated by HMA method. The separated gel lanes were visualized using the MultiNA (SHIMADZU, Japan). The results of HMA examined for the fertilized medaka eggs treated by crRNA1-1, crRNA1-2, crRNA7-1 and crRNA7-2 are shown. Of these, crRNA1-2, crRNA7-1 and crRNA7-2 showed high mutant efficiency.

Fig. S2. The mutation start and end point of mutated IL-17RA1. (A) Overall, we obtained six variants of the mutant. Mutant line B and K were inserted the nucleotide (KO line B: 1 bp inserted, KO line K: 12 bp inserted.). All mutations in KO line started the up-stream of start codon of WT's IL-17RA1 (B) and predicted coding sequence of mutated IL-17RA1 in each KO line are described (C). (D) Two forward primers and a reverse primer used for confirmation of genotype (F1-R1; 286 bp (amplification of the partial region of WT IL-17RA1), F2-R1; 181 bp (amplification of the partial region of mutated IL-17RA1 (line C))).

Fig. S3.

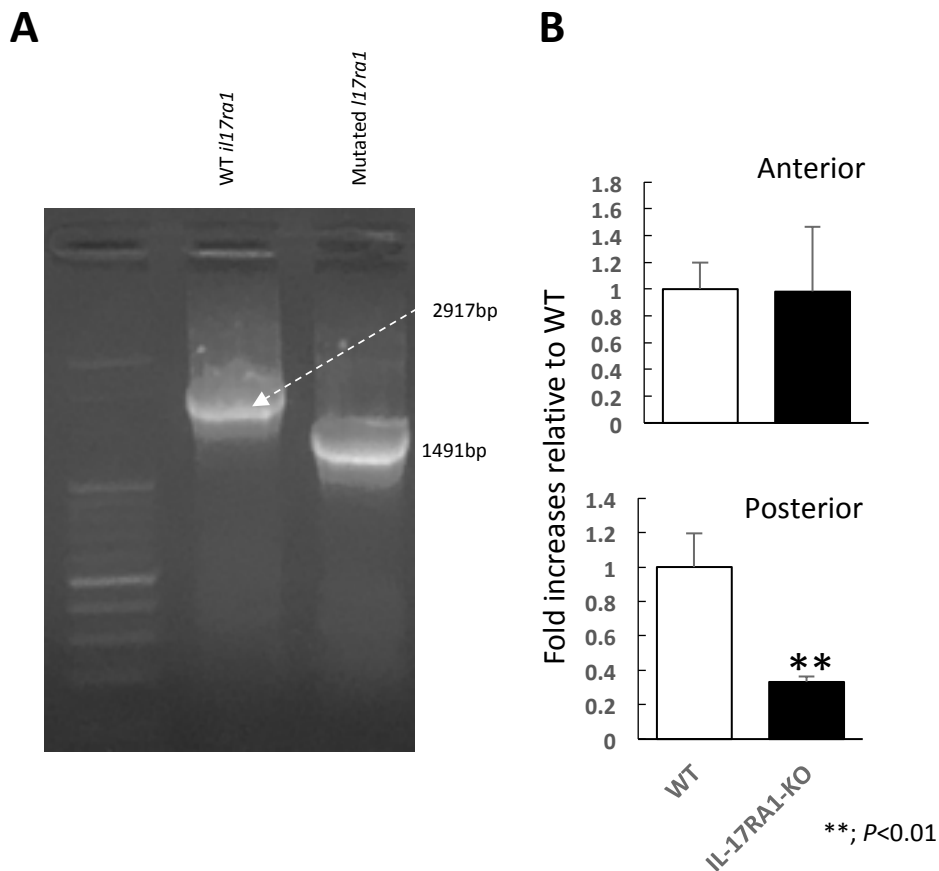


Fig. S3. The ORF comparison of WT and mutated *il17ra1* and its gene expression level in each section of the intestine. (A) The PCR product size of each ORF in WT's and mutated *il17ra1*. The mutated ORF sequence were confirmed by read sequencing and WT's *il17ra1* was not amplified in the cDNA sample of IL-17RA1-KO. (B) The comparison of the gene expression level of *il17ra1* in anterior and posterior intestine of WT and IL-17RA1-KO medaka. ** $P < 0.01$, * $P < 0.05$ (Student's *t*-test). Data are from one experiment with three individual fish ($n = 3$).

Fig. S4.

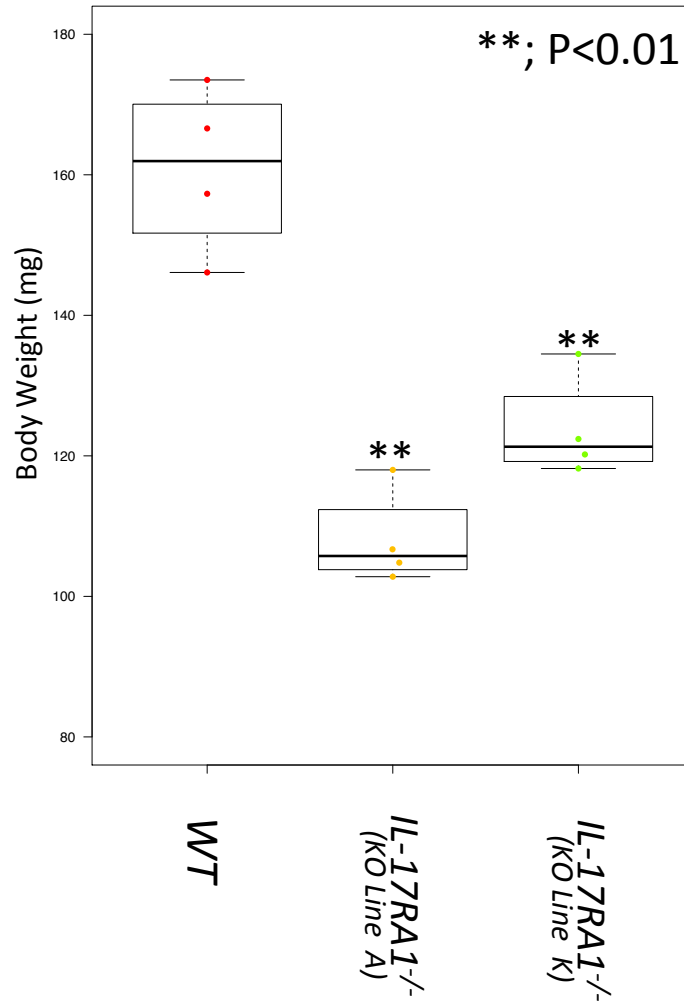


Fig. S4. The Weight losses seen in other lines (KO lines A and K) homologous mutant of IL-17RA1-KO medaka. In 4 months after hatching, body weight were measured (n=4).

Fig. S5.

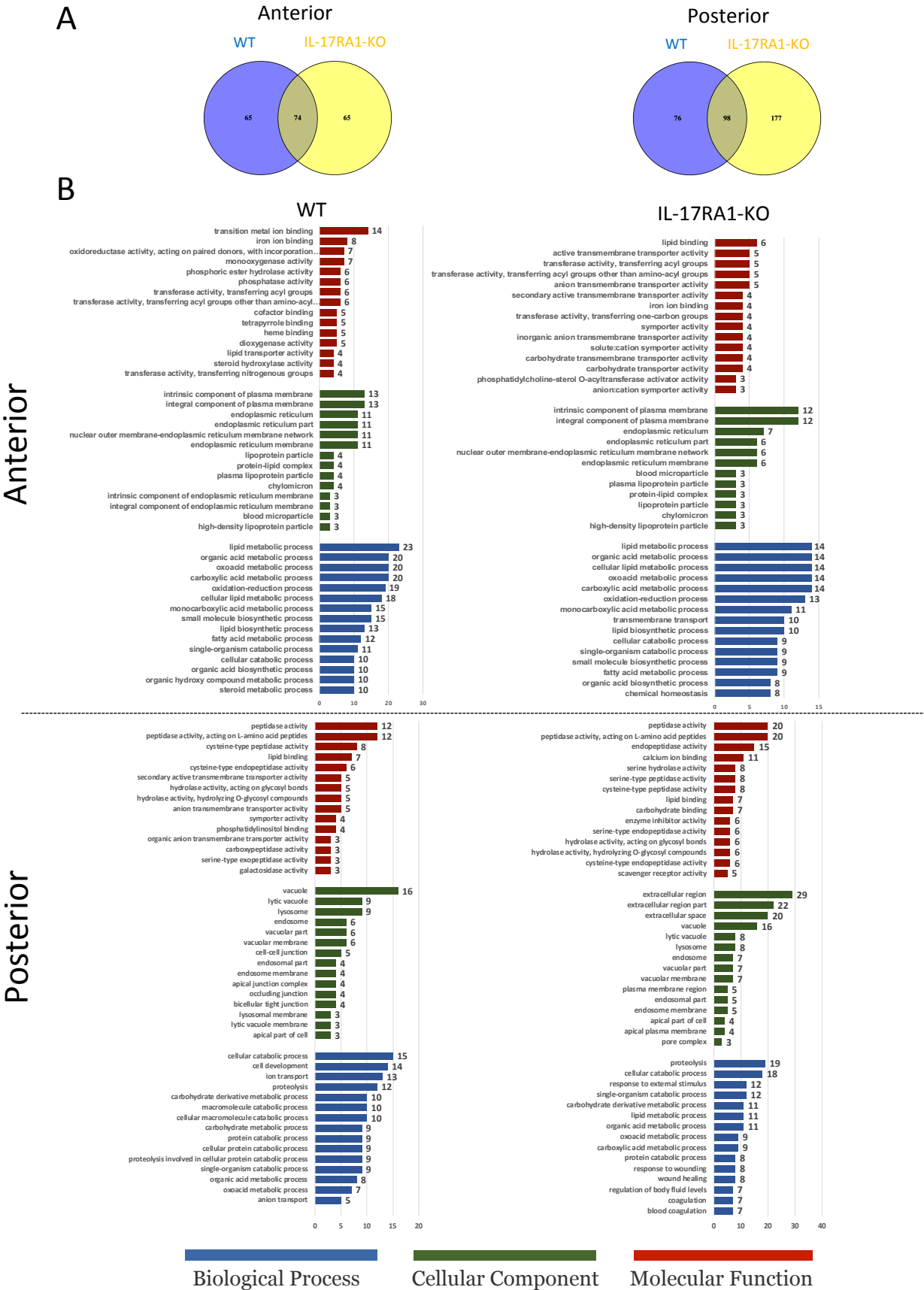


Fig. S5. Enrichment of gene ontology (GO) classification of the differently expressed gene (DEG) when comparing between anterior and posterior intestine. Significant different genes ($P<0.05$) were extracted as DGEs. (A) In WT's comparison of anterior and posterior intestine, and IL-17RA1-KO's comparison of anterior and posterior intestine, Anterior 74 genes and posterior 98 genes were consensus DEGs in both of WT and KO. (B) Anterior intestine develops the expression of lipid metabolism-related genes.

Fig. S6.

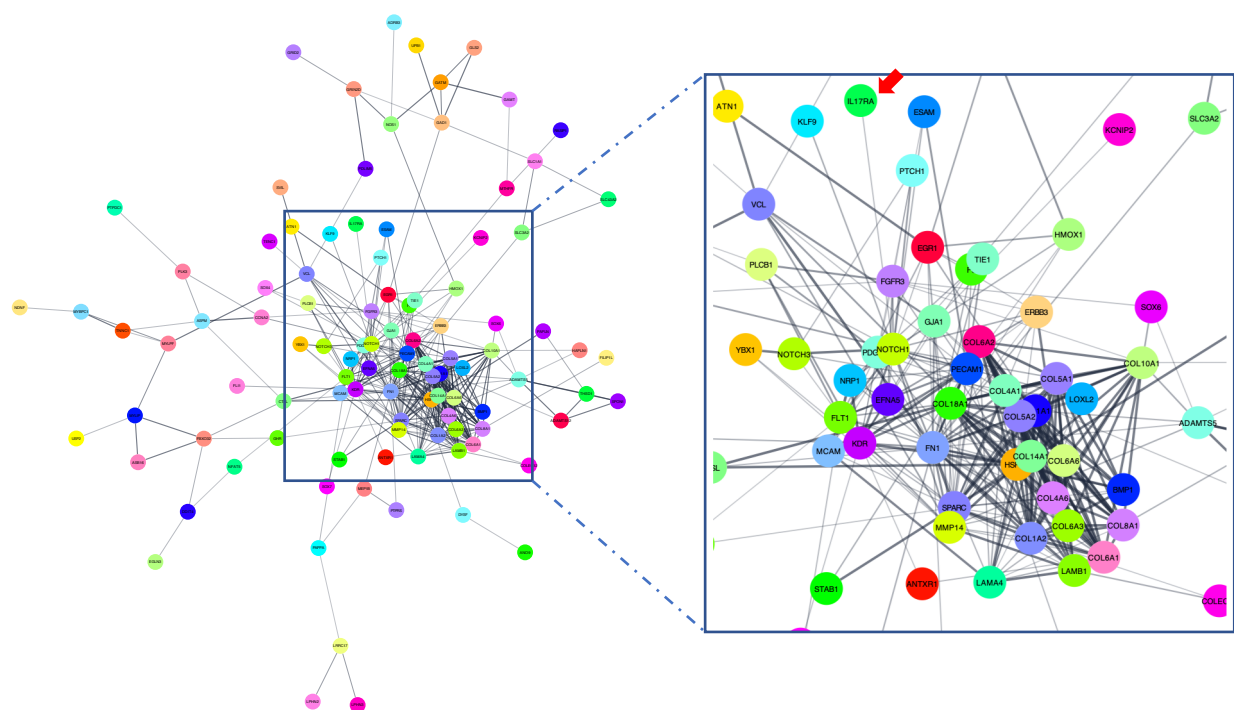


Fig. S6. The prediction of the interaction among DEGs up-regulated in IL-17RA1-KO and IL-17RA. Interaction network of these genes as defined by Cytoscape’s software STRING. Of 167 DEGs, 102 genes formed the most complex cluster and contained IL-17RA. Red arrow shows IL-17RA.

Fig. S7.

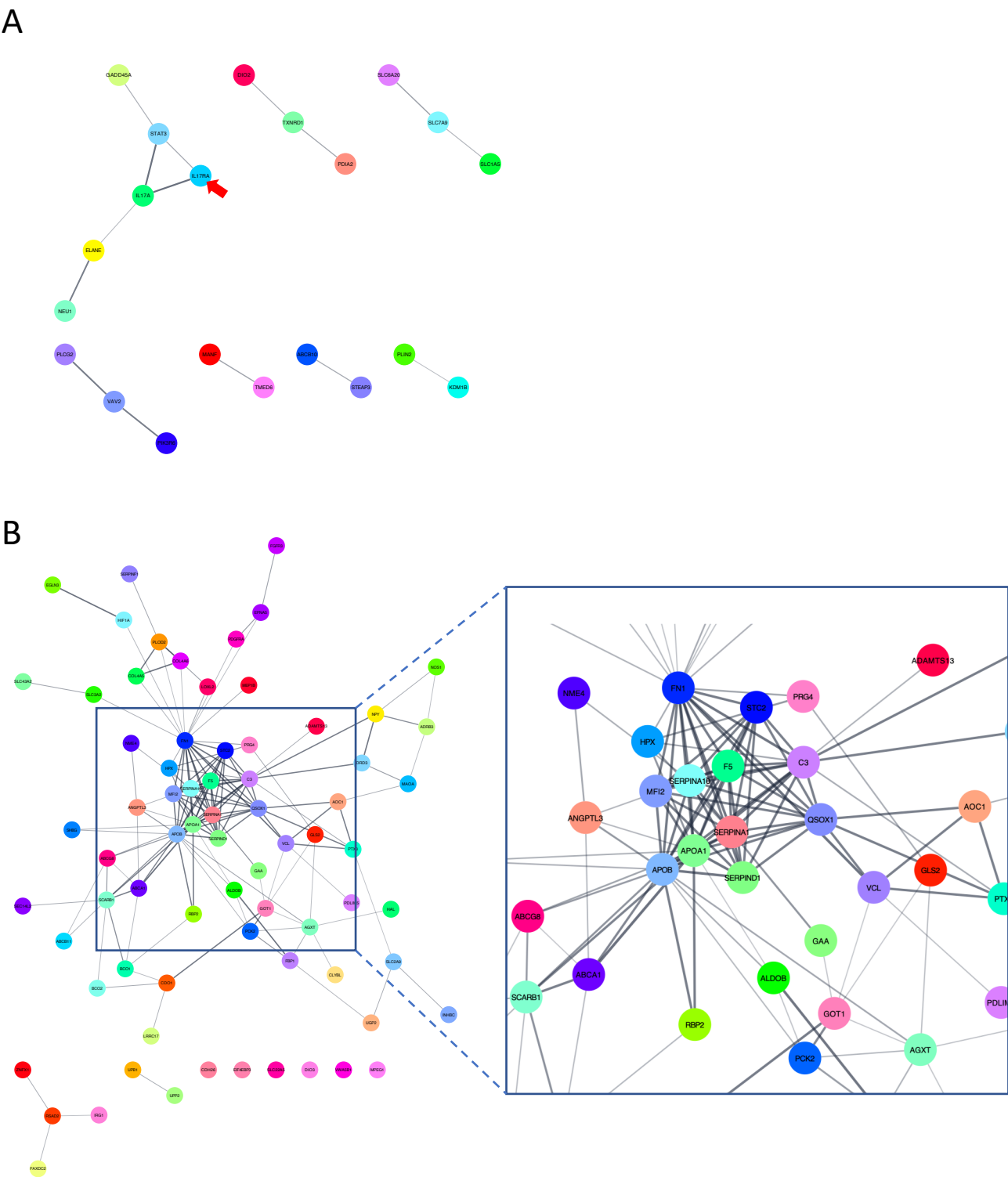


Fig. S7. The prediction of the interaction between posterior DEGs in IL-17RA1-KO and IL-17RA. Interaction network of these genes as defined by software STRING in Cytoscape. (A) down-regulated DEGs in posterior intestine of IL-17RA1-KO. IL-17RA containing cluster contains IL-17 signaling related genes such as IL-17A/F1 ligands and transcriptional factor STAT3. Red arrow shows IL-17RA. (B) Up-regulated DEGs in the posterior intestine of IL-17RA1-KO. Up-regulated DEGs in the posterior intestine of IL-17RA1-KO did not form same cluster with IL-17RA.

Fig. S8.

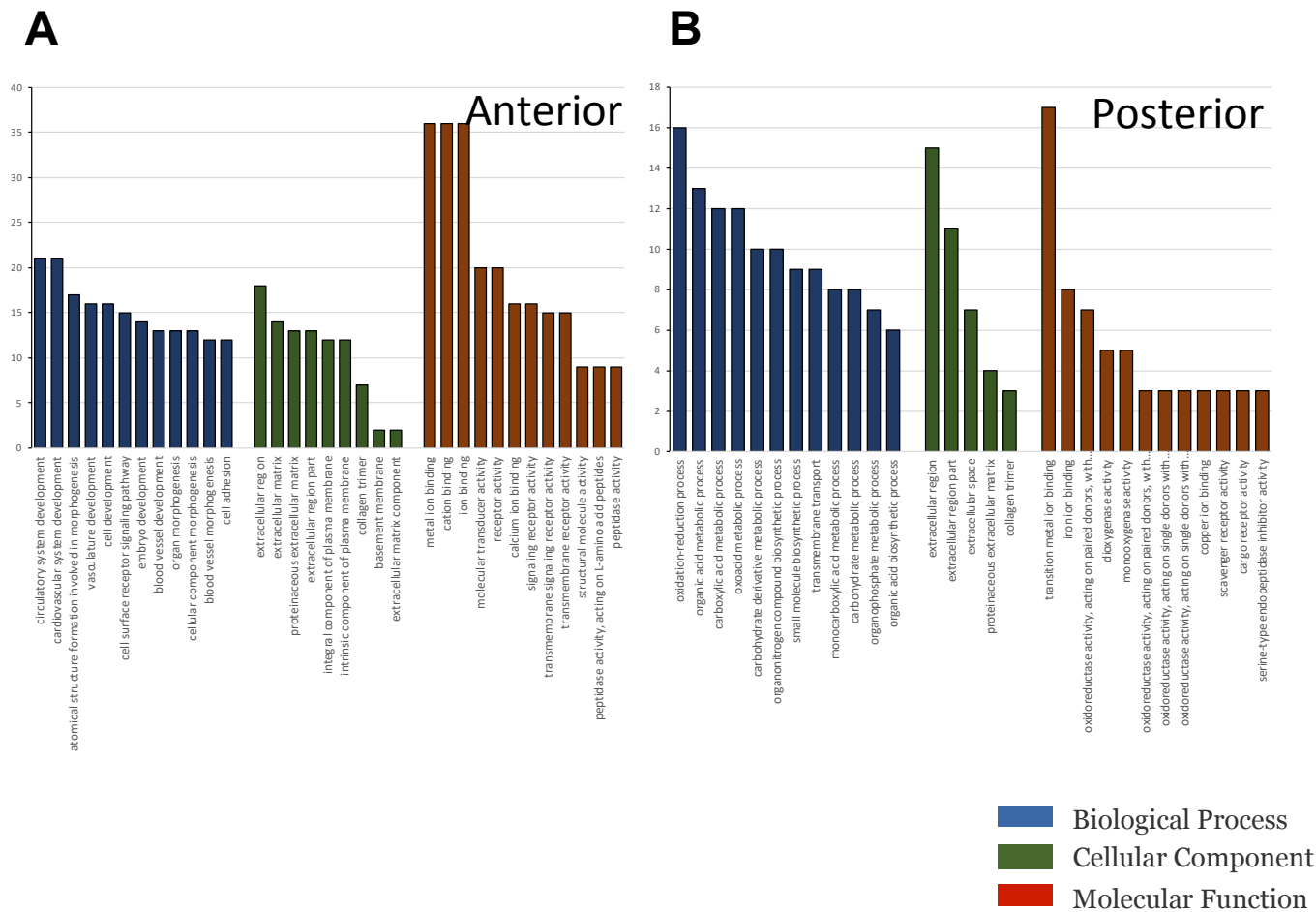


Fig. S8. Top 12 classification of gene ontology (GO) enrichment of up-regulated DEGs in anterior and posterior intestine of IL-17RA1-KO. (A) GO term in anterior intestine and (B) posterior intestine.