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Supplementary Information for

RORα Is Crucial for Attenuated Inflammatory Response to Maintain Intestinal Homeostasis

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This PDF file includes:

Supplementary Figures and Figure Legends

Figs. S1 to S10

Supplementary Figures and Figure Legends

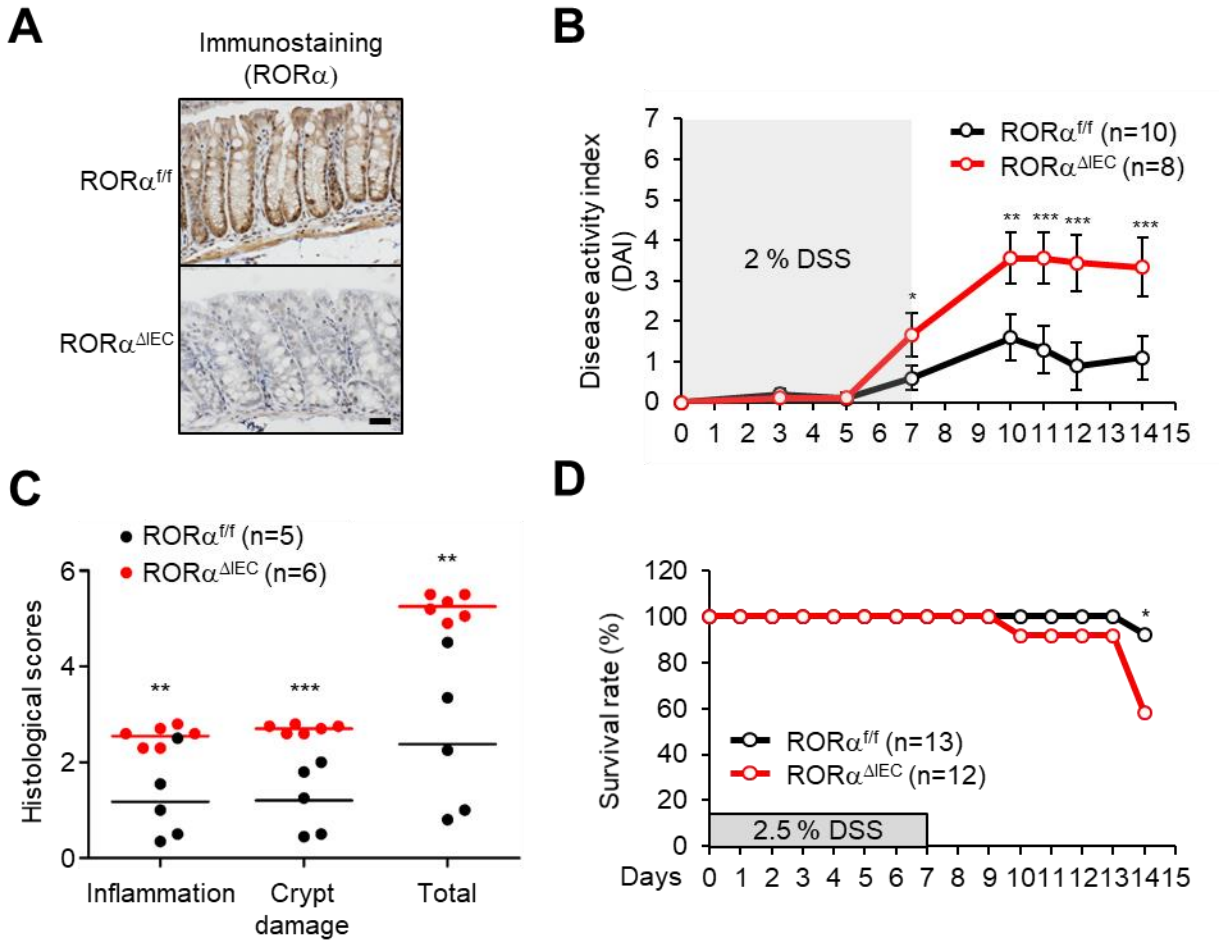

Fig. S1. Colitis severity in RORα$^{\Delta IEC}$ mice with DSS treatment. (**A**) Immunohistochemistry of colon sections from RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice using an anti-RORα antibody. Scale bar, 50 μm. (**B**) Disease activity index (DAI) scores of RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice during described period. (n = 8~10 per group) (**C**) Histological scores of colon sections from RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice after 8 days of 2 % DSS treatment. (n = 5~6 per group) (**D**) The survival curves of DSS-treated RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice. RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice were given 2.5 % DSS in their drinking water for 7 days, then given regular drinking water for an additional 7 days. Viability was monitored daily. Difference in survival was determined with Kaplan-Meier analysis. (n = 12~13 per group); **P*<0.05, ***P*<0.01, ****P*<0.001. Statistical analysis was performed using unpaired *t*-test. (**B** and **C**; mean ± SEM) Data are from three independent experiments.

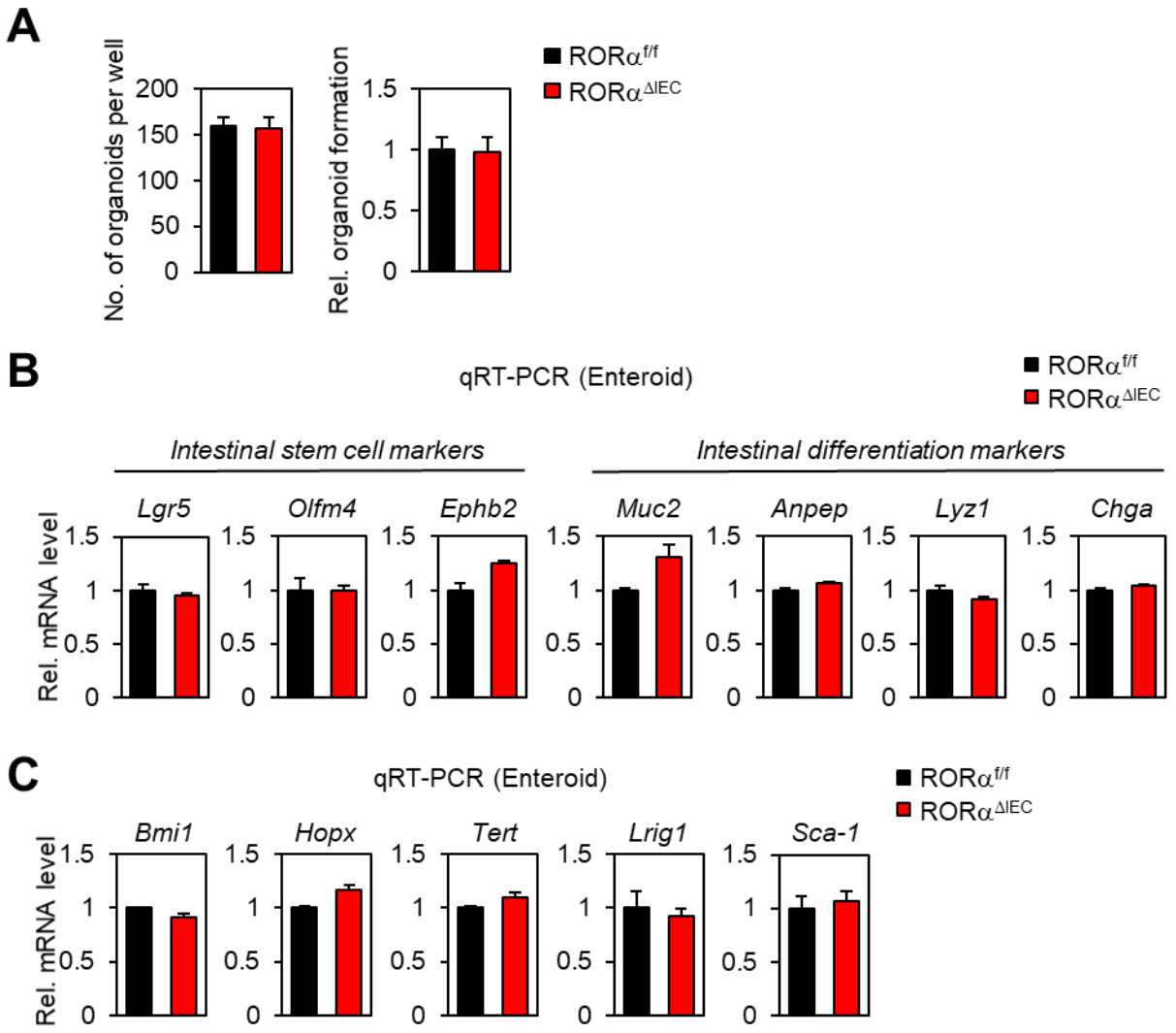

Fig. S2. Regenerative capacity of RORα KO organoids is normal compared with those of WT organoids. (**A**) Intestinal organoid formation efficiency from RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice. 1000-crypts were seeded, and live organoids were counted using microscopy at day 4 of culture. (**B**) mRNA levels of intestinal stem cell markers and differentiation markers in colon organoids from RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice. (**C**) mRNA levels reserve stem cell markers in colon organoids from RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice. Statistical analysis was performed using unpaired *t*-test. (**A**, **B** and **C**; mean ± SEM) Data are from three independent experiments.

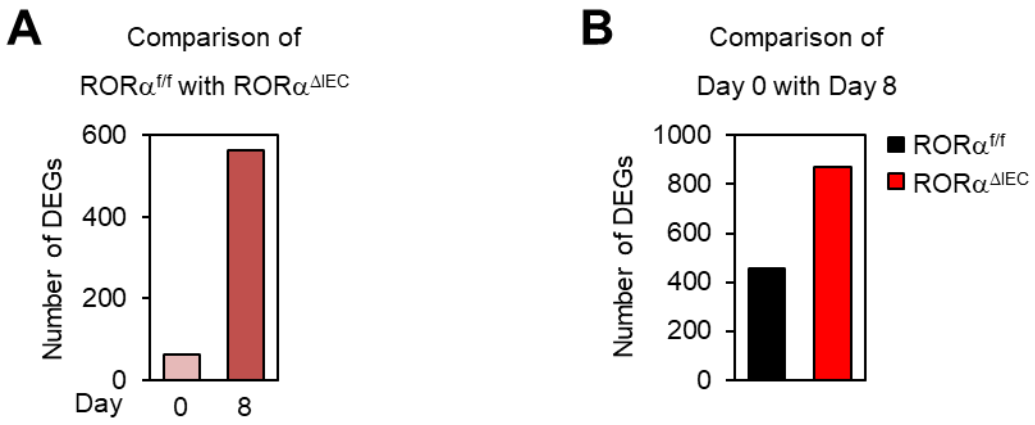

Fig. S3. The number of DEGs from RNA-sequencing analysis of RORα$^{f/f}$ and RORα$^{\Delta IEC}$ IECs. DEGs are identified by either comparing the gene expression between two genotypes or days of DSS treatment. (Adjusted p-value ≤ 0.05 and $|\log2FC| \geq 1$) (**A**) The number of DEGs generated by comparing RORα$^{f/f}$ IECs with RORα$^{\Delta IEC}$ IECs on Day 0 and Day 8, respectively. (**B**) The number of DEGs generated by comparing gene expression on Day 0 with that on Day 8 in each genotype, respectively.

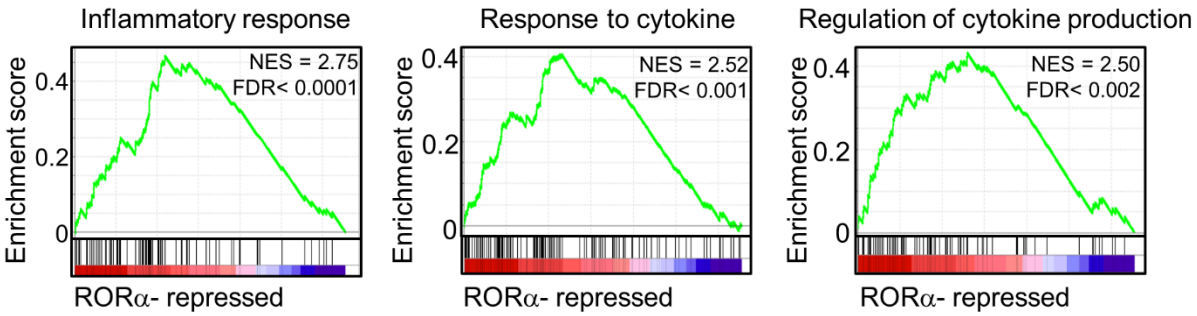

Fig. S4. Representative gene set enrichment analysis (GSEA) results. The gene sets involved in inflammatory response, response to cytokine and regulation of cytokine production are significantly enriched according to the phenotypic labels.

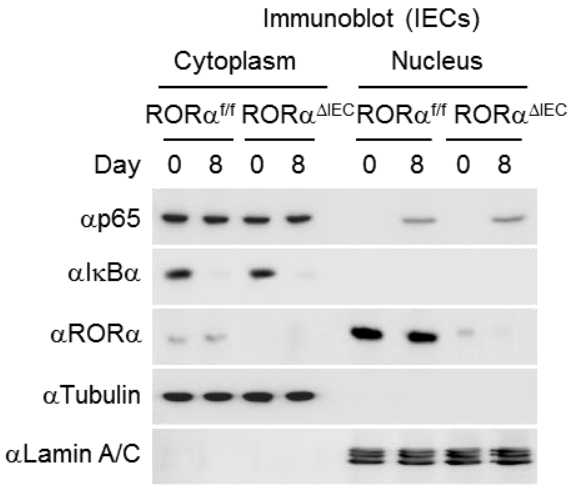

Fig. S5. Immunoblot analysis of IECs from RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice with the indicated antibodies after 8 days (DSS 5 days + water 3 days) of 2 % DSS (n = 6 per group). Tubulin was used as a loading control for cytoplasmic proteins and Lamin A/C was used for nuclear proteins.

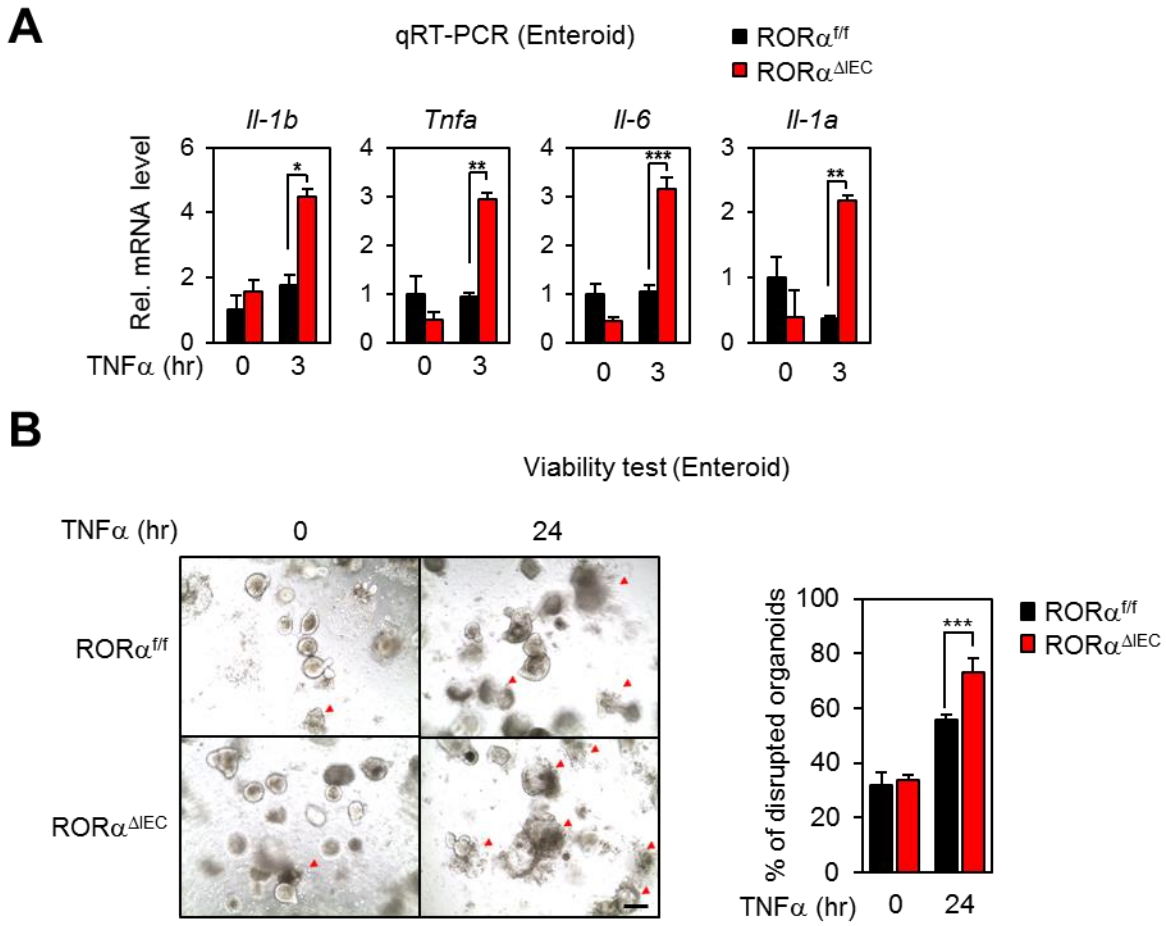

Fig. S6. RORα KO organoids are highly susceptible to TNFα treatment. (**A**) Relative mRNA level measured in colon organoids from RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice. Colon organoids were treated with TNFα (100ng/ml) for 3 hrs. (**B**) Representative microscopic images showing colon organoids treated with TNFα (100ng/ml) for 24 hrs. The number of live and disrupted organoids was counted after the treatment. (n = 2 per groups). **P*<0.05, ***P*<0.01, ****P*<0.001. Statistical analysis was performed using unpaired *t*-test. (**A** and **B**; mean ± SEM) Data are from three independent experiments.

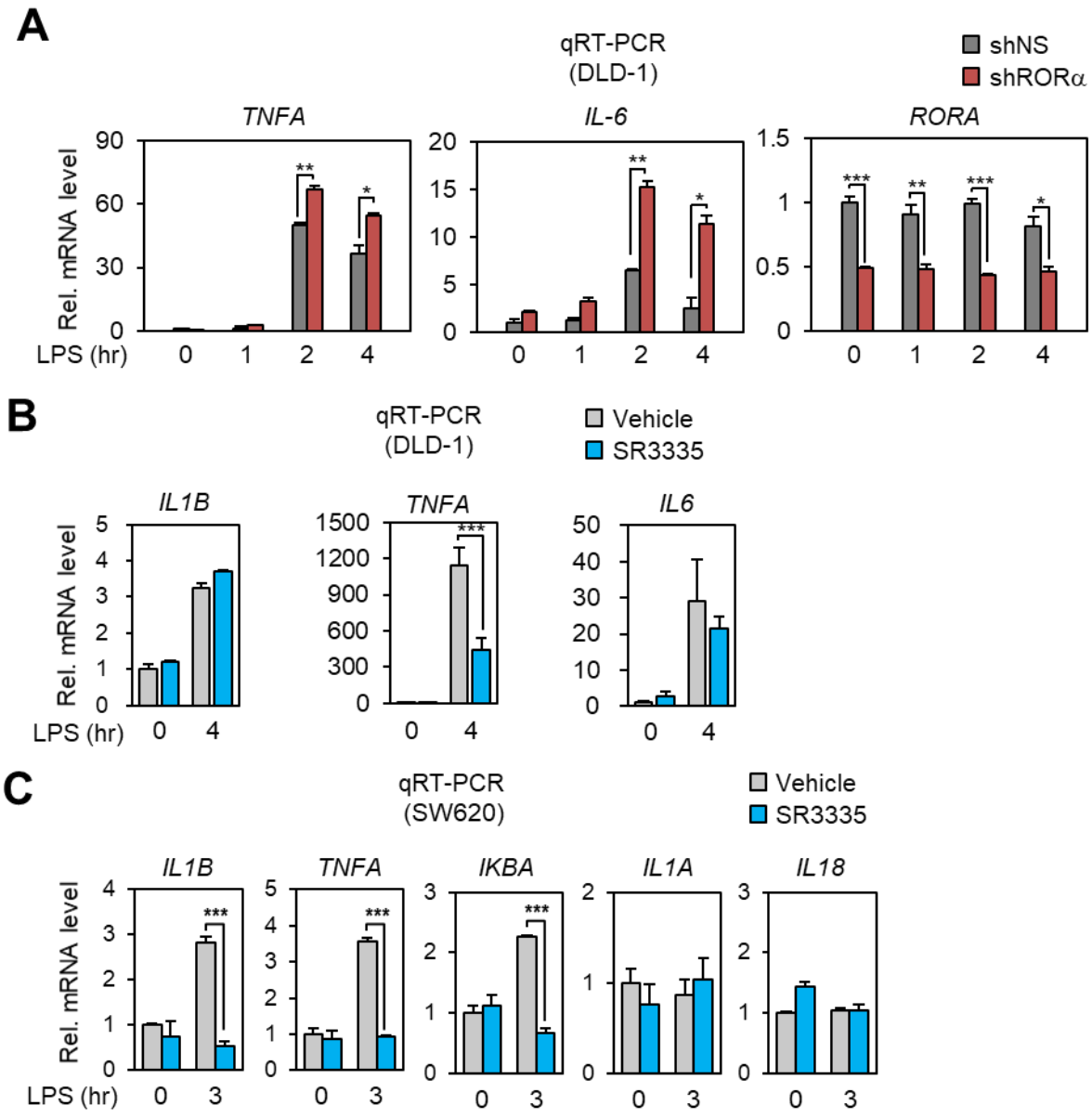

Fig. S7. RORα suppresses transcription of NF-κB target genes in colorectal cancer cell lines after LPS treatment. (**A**) qRT-PCR analysis of inflammatory genes in DLD-1 cells after LPS (1 μg/ml) treatment for the indicated times. (**B** and **C**) Colorectal cancer cell lines including DLD-1 and SW620 were treated with LPS (1 μg/ml) for the indicated times. Either SR3335 (5 μM) or DMSO was treated with LPS at the same points. **P*<0.05, ***P*<0.01, ****P*<0.001. Statistical analysis was performed using unpaired *t*-test. (**A, B** and **C**; mean ± SEM) Data are from three independent experiments.

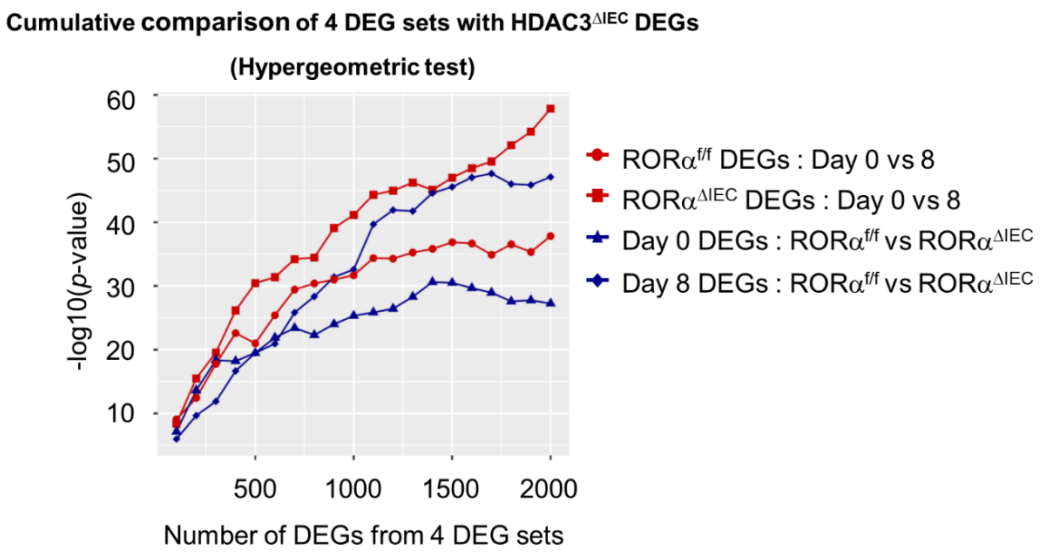

Fig. S8. RORα and HDAC3 share common target genes under DSS-induced injury. "RORα$^{f/f}$ DEGs" represents the DEGs identified in IECs from RORα$^{f/f}$ mice by comparing day 0 with day 8 after DSS treatment. Cumulative comparison of 4 DEG sets (RORα$^{f/f}$ DEGs, RORα$^{\Delta IEC}$ DEGs, Day 0 DEGs, and Day 8 DEGs) with the HDAC3$^{\Delta IEC}$ DEGs. "HDAC3$^{\Delta IEC}$ DEGs" are identified by comparing the gene expression profiles between HDAC3$^{f/f}$ IECs and HDAC3$^{\Delta IEC}$ IECs. The 4 DEG sets are constructed by comparing gene expression of every possible combination of factors including day of treatment and genotype. Thus, each line indicates overlaps of a DEG set with HDAC3$^{\Delta IEC}$ DEGs. Hypergeometric test shows that RORα deletion along with DSS-injury has the highest degree of overlaps with HDAC3$^{\Delta IEC}$ DEGs.

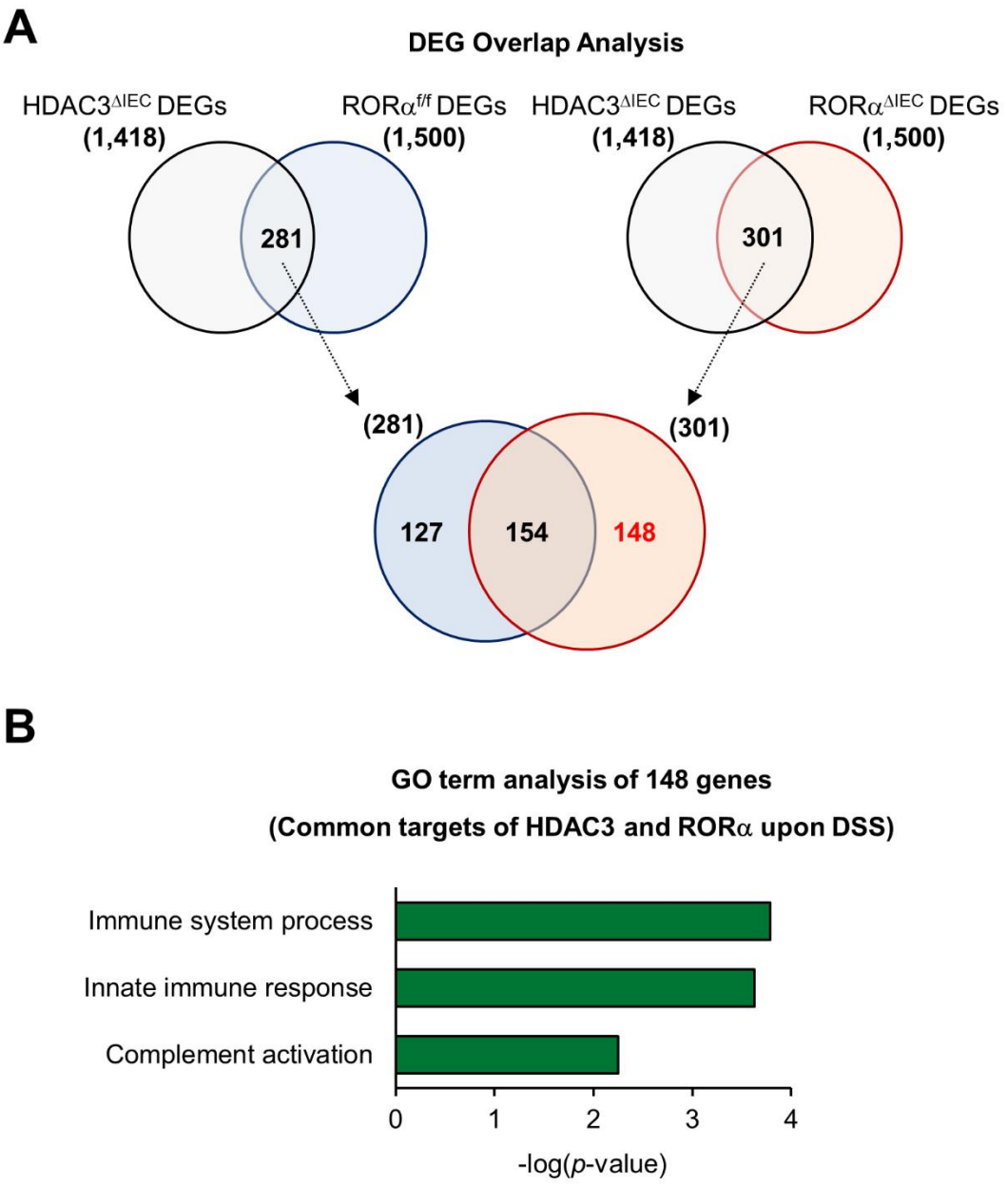

Fig. S9. RORα and HDAC3 share common inflammatory target genes under DSS-induced injury. (**A**) Three different comparison of DEGs shown as Venn diagram. (**B**) GO term analysis for the shared targets of HDAC3 and RORα upon DSS treatment (n= 148).

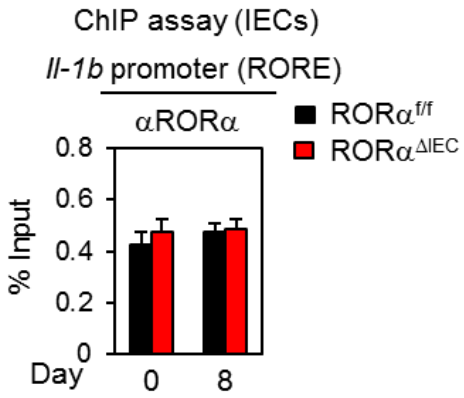

Fig. S10. ChIP assays were performed on the *Il-1b* promoter (ROR-response element) in IECs from RORα$^{f/f}$ and RORα$^{\Delta IEC}$ mice after 8 days of 2 % DSS (n = 5 per group). Promoter occupancy by RORα was analyzed. Statistical analysis was performed using unpaired *t*-test. (mean ± SEM) Data are from three independent experiments.