Supplemental information.

Supplemental Materials & Methods.

Flow Cytometry. To stain for T cell activation and regulatory T-cells, single cell suspensions from the spleen, skin-draining lymph nodes, or mesenteric lymph nodes were surface stained, fixed overnight at 4^{o}C using FoxP3 Fixation/Permabilization buffer (eBioscience), permeabilized using Foxp3 Permeabilization buffer (eBioscience) and then subjected to intracellular staining. Surface markers were identified with anti-TCRβ (H57-597) conjugated to peridinin chlorophyll-a protein-cyanin 5.5 (PerCP-Cy5.5) (BD Pharmingen), anti-CD4 (GK1.5) conjugated to allophycocyanin (APC), anti-CD44 (IM7) conjugated to phycoerythrin (PE) (eBioscience), anti-CD62L (MEL-14) conjugated to phycoerythrin protein-cyanin 7 (PE-Cy7) (eBioscience). After staining intracellularly with anti-Foxp3 (FJK-16s) conjugated to eFlour450 (eBioscience), FoxP3 expression among $CD4^{+}TCR\beta^{+}$ cells was analyzed by FACS. To assess the composition of cell types in the blood, blood samples were stained with CD45 (30-F11) conjugated to PerCP-Cy5.5 (BD Biosciences) and anti-B220 (RA3-6B2; BD Biosciences), CD3e (145-2C11; BD Pharmingen), or Ly6G (RB6-8C5; Biolegend) conjugated to PE and $CD45^{+}$ cells were analyzed. To characterize the maturity of the B-cells, blood samples were stained with anti-B220 (RA3-6B2) conjugated to PerCP-Cy5.5 (Biolegend), anti-IgM (RMM-1) conjugated to APC (Biolegend), anti-IgD (11-26) conjugated to eFlour450 (eBioscience), anti-CD93 (AA4.1) conjugated to PE-Cy7 (Biolegend), and anti-CD23 (B3B4) conjugated to PE (BD Pharmingen); $B220^{+}$ cells were analyzed by FACS.

TEWL Measurements. Trans epidermal water loss (TEWL) was measured on the midline back skin between the shoulder blades at postnatal day 9 (P9) using VapoMeter (Delfin Technology) according to manufacturer's instructions, the instrument was allowed to equilibrate between each measurement. The average of two measurements from each mouse is shown for each data point.

Supplemental Figures

Figure S1. (Related to Fig 1)

Trans-epidermal water loss (TEWL) confirms the similar levels of barrier defect in GF and CR RBPjCKO mice (CONV-R wild-type, n=7; CONV-R RBPjCKO, n=6; GF wild-type, n=4; GF RBPjCKO, n=4). *, $p<0.05$.

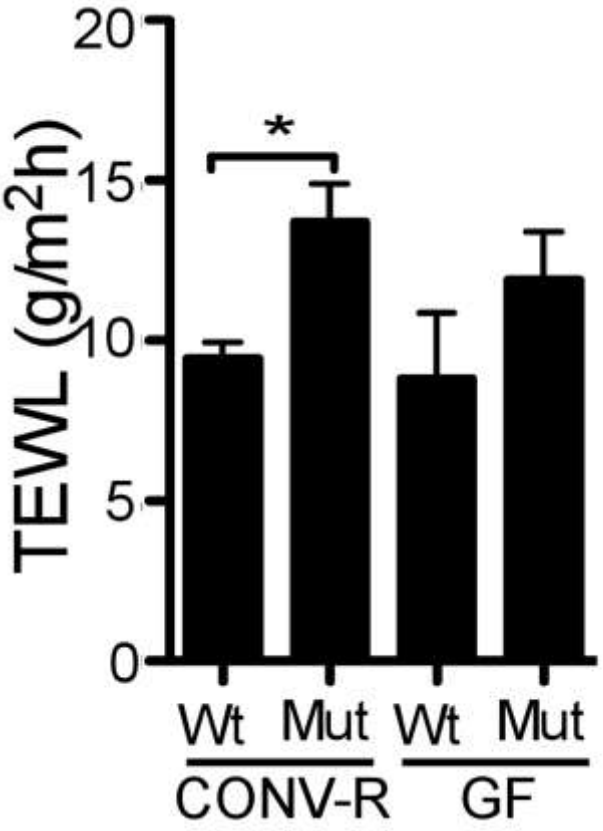

Figure S2. (Related to Fig 2)

FACS gated on CD4+ cells from (A) the skin-draining lymph node (LN) (CONV-R wild-type, n=3; CONV-R RBPjCKO, n=6; GF wild-type n=3; GF RBPjCKO, n=6) and (B) mesenteric LN (CONV-R wild-type, n=2; CONV-R RBPjCKO, n=5; GF wild-type n=2; GF RBPjCKO, n=5) detecting effector ($CD44^{hi}$ $CD62L^{-}$ cells) and naïve ($CD44^{lo}$ $CD62L^{+}$ cells).

RBPjCKO animals have a significantly higher percentage of effector and lower percentage of naïve cells than WT in both conditions. FACS gated on CD4+ cells in the skin-draining LN (C) and mesenteric LN (D) shows similar levels of T regulatory (Foxp3+) cells. Percentage of Foxp3+ cells is significantly higher in CONV-R RPBjCKO compared to CONV-R wild-type mice. (CONV-R wild-type, n=3; CONV-R RBPjCKO, n=6; GF wild-type n=3; GF RBPjCKO, n=6). *, p<0.05; **, p<0.01, *** , p<0.001 (Students t-test)

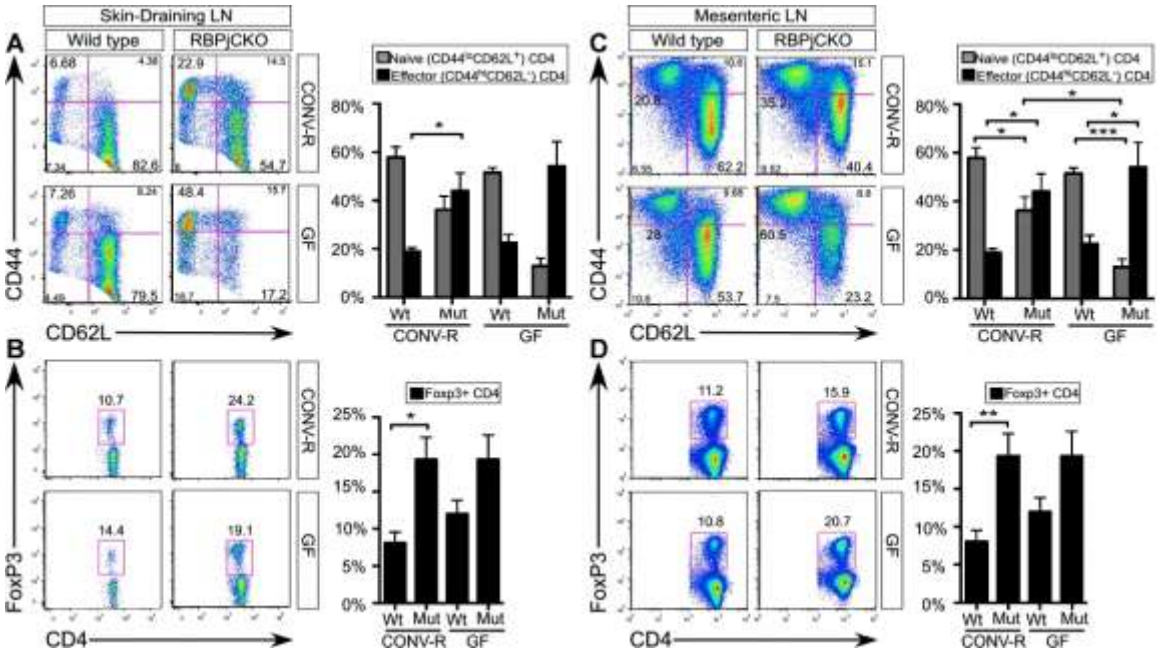

Figure S3 (Related to Fig 4)

(A) Flow cytometry analysis of B220+ cells in blood shows decreased percentage of mature ($CD23^+$ $CD93^-$) B-cells in GF and CONV-R RPBjCKO. On the right, graphic representation of the percentage of mature B cells compiled from 5 independent experiments (CONV-R wild-type, n=5; CONV-R RBPjCKO, n=11; GF wild-type, n=4; GF RBPjCKO, n=11). (B) There is a positive correlation between LDH levels and HCT for both GF and CONV-R RBPjCKO (n=12). (C) In CONV-R RBPjCKO, haptoglobin correlates negatively with HCT while in GF RBPjCKO, haptoglobin correlates positively with HCT (n=12). *, p<0.05; **, p<0.01; ***, p<0.001)