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Supplementary Information for

Enhanced susceptibility to chemically-induced colitis caused by excessive endosomal TLR signaling in LRBA-deficient mice

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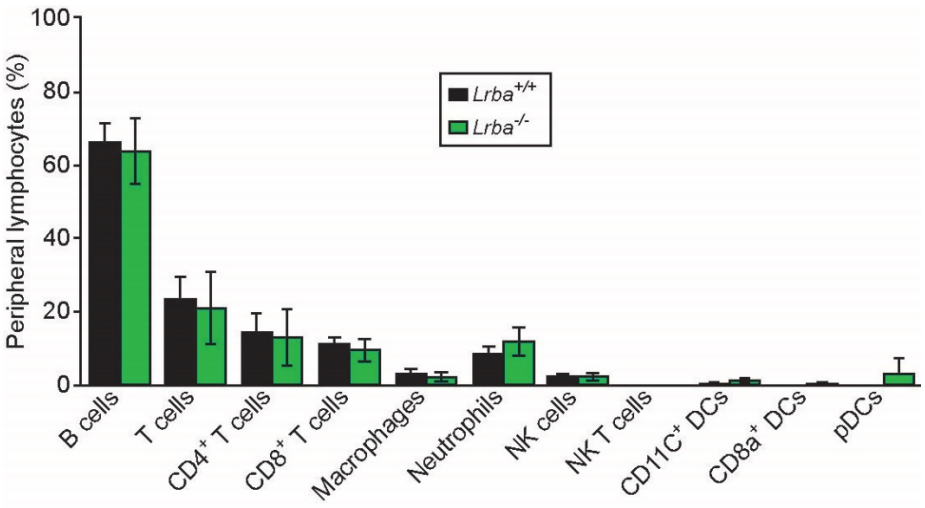

Fig. S1. Immune cell populations in the blood of *Lrba*$^{-/-}$ mice. (A) Percentage of each cell type in the blood (n=5 mice per genotype). Data are representative of 3 independent experiments (mean ± s.d.).

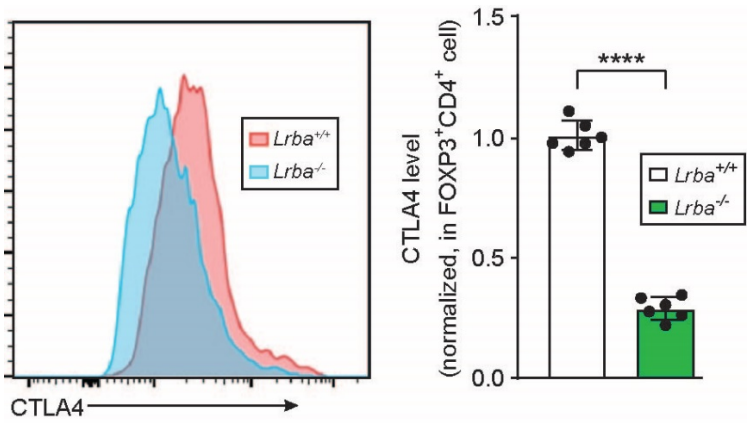

Fig. S2. Reduced CTLA4 protein expression in FoxP3$^+$CD4$^+$ cells from *Lrba*$^{-/-}$ spleen. *Left*, Flow cytometry of splenocytes from *Lrba*$^{+/+}$ and *Lrba*$^{-/-}$ mice, assessing expression of CTLA4 in CD3$^+$CD4$^+$FoxP3$^+$ cell population. *Right*, Quantification of CTLA4 protein expression in splenic CD3$^+$CD4$^+$FoxP3$^+$ cells (mean fluorescence intensity normalized to wild-type). Each symbol represents an individual mouse. **** $P < 0.0001$ (two-tailed Student's *t*-test). Data are representative of 3 independent experiments (mean ± s.d.).

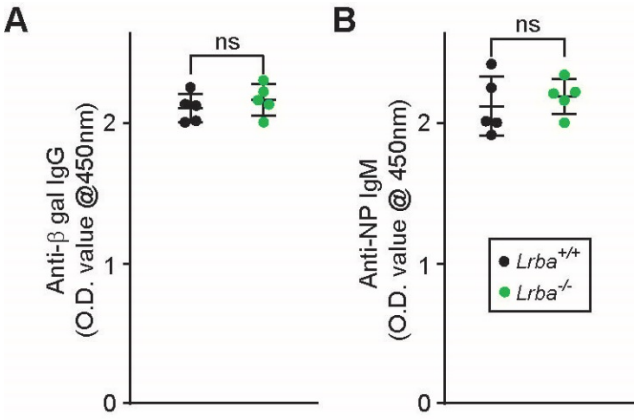

Fig. S3. Normal antibody responses to immunization in *Lrba*$^{-/-}$ mice. (A) Quantification of β-gal-specific IgG in the blood 13 days after immunization with recombinant Semliki forest virus (rSFV)-encoded β-galactosidase. (B) Quantification of NP-specific IgM in the blood 6 days after immunization with NP-Ficoll. Each symbol represents an individual mouse. Data are representative of 3 independent experiments (mean ± s.d.).

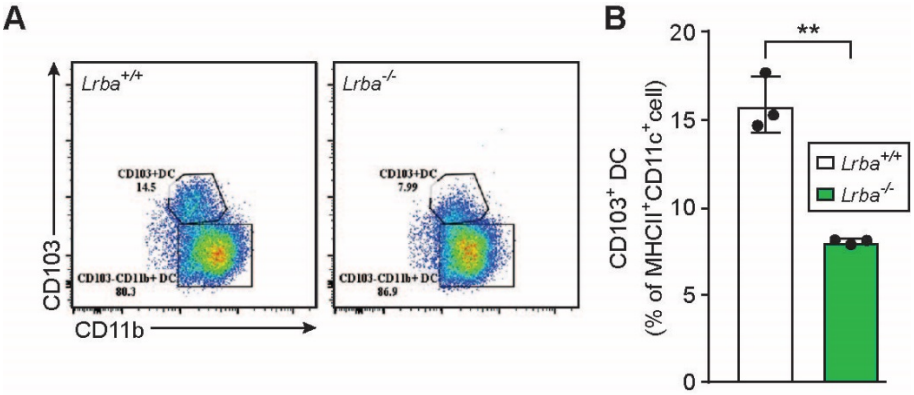

Fig. S4. Reduced CD103$^+$ dendritic cells in colon lamina propria of *Lrba*$^{-/-}$ mice. (A) Flow cytometry of colon lamina propria cells from *Lrba*$^{+/+}$ and *Lrba*$^{-/-}$ mice, assessing expression of CD11b and CD103 in MHC II$^+$CD11c$^+$ cell population. Numbers next to boxed regions represent percent cells in each. (B) Quantification of percentage of CD103$^+$ cells among MHC II$^+$CD11c$^+$ dendritic cells. Each symbol represents an individual mouse. ** $P < 0.01$ (two-tailed Student's *t*-test). Data are representative of 3 independent experiments (mean ± s.d.).

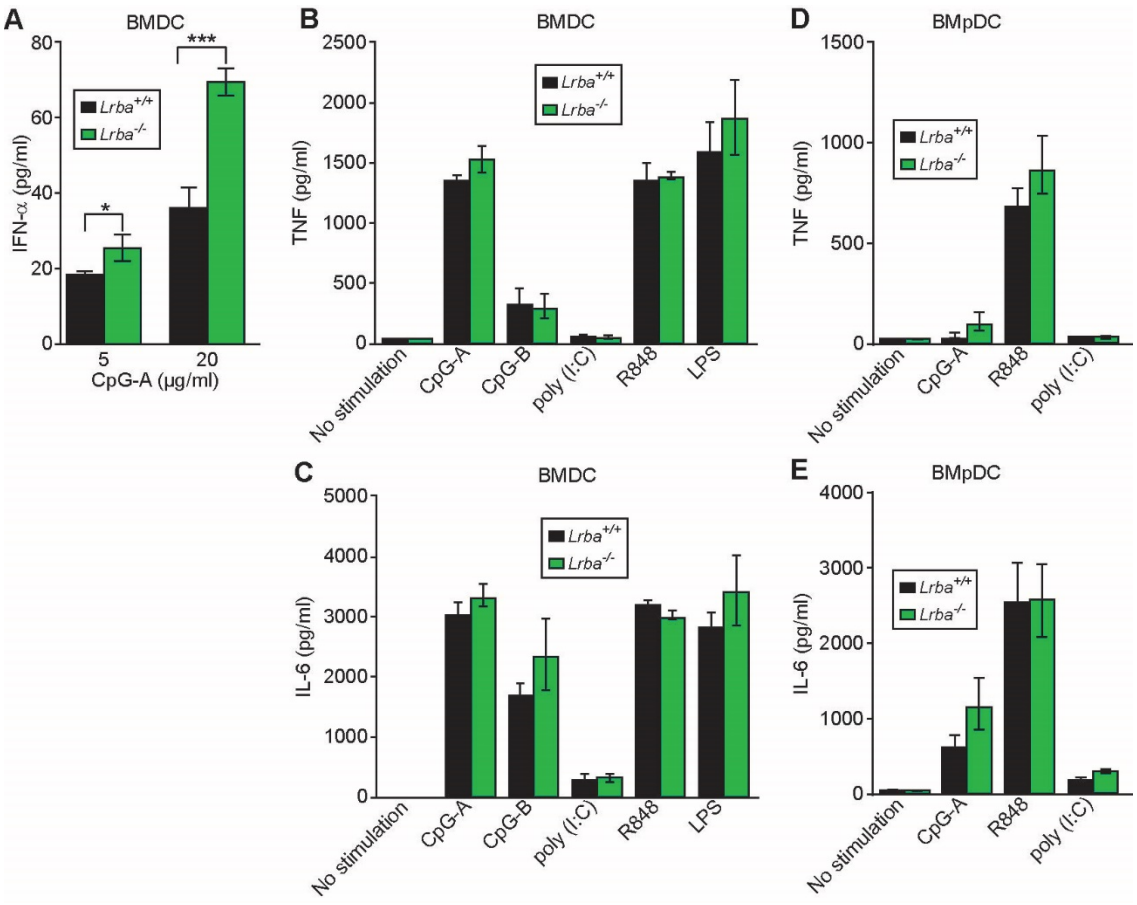

Fig. S5. Normal TNF and IL-6 responses to endosomal TLR stimulation in *Lrba*$^{-/-}$ dendritic cells. *In vitro* differentiated BMDC or BMpDC were stimulated with TLR ligands for 16 h. (A) Concentration of IFN-α in the culture supernatant of BMDC after stimulation with CpG-A (n=3 independent cultures of each genotype from separate mice). (B-E) Concentration of (B,D) TNF and (C,E) IL-6 in the supernatant of (B,C) BMDC or (D,E) BMpDC after stimulation with the indicated ligands (n=3 independent cultures of each genotype from separate mice). * $P < 0.05$; *** $P < 0.001$ (two-tailed Student's *t*-test). Data are representative of 4 independent experiments (mean ± s.d.).

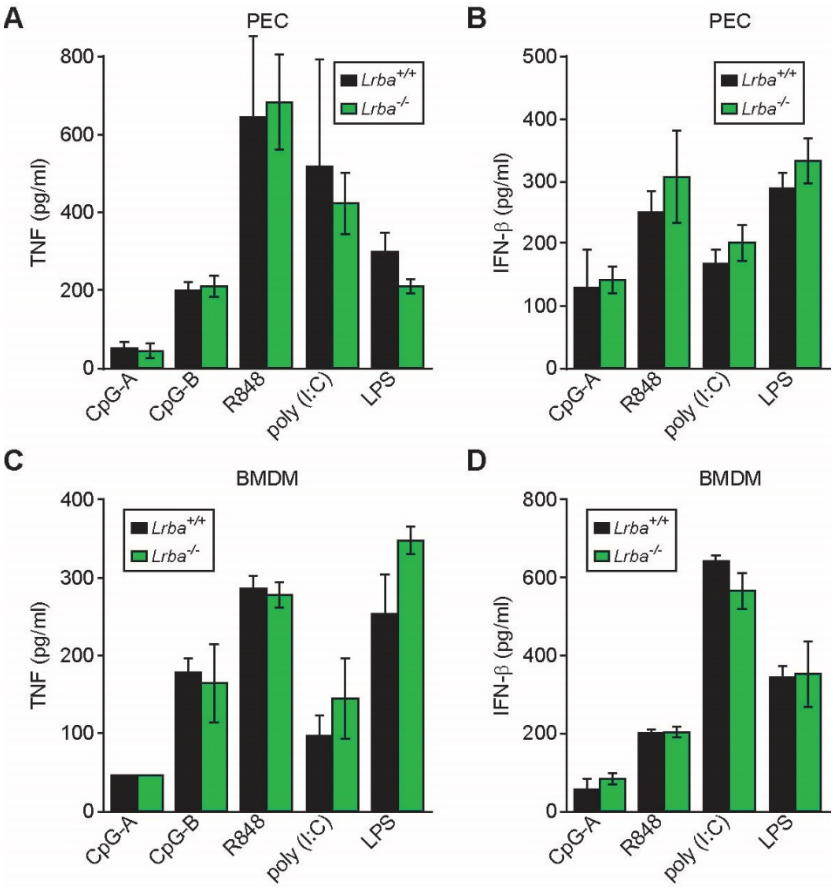

Fig. S6. Normal TNF and IFN-β production by macrophages after TLR stimulation. Concentration of (A,C) TNF and (B,D) IFN-β in the culture supernatant of peritoneal macrophages (PEC, peritoneal exudate cells; n=3 mice per genotype) (A,B) or bone marrow-derived macrophages (n=4 independent cultures of each genotype from separate mice) (C,D) after stimulation with the indicated ligands. Data are representative of 3 independent experiments (mean ± s.d.).

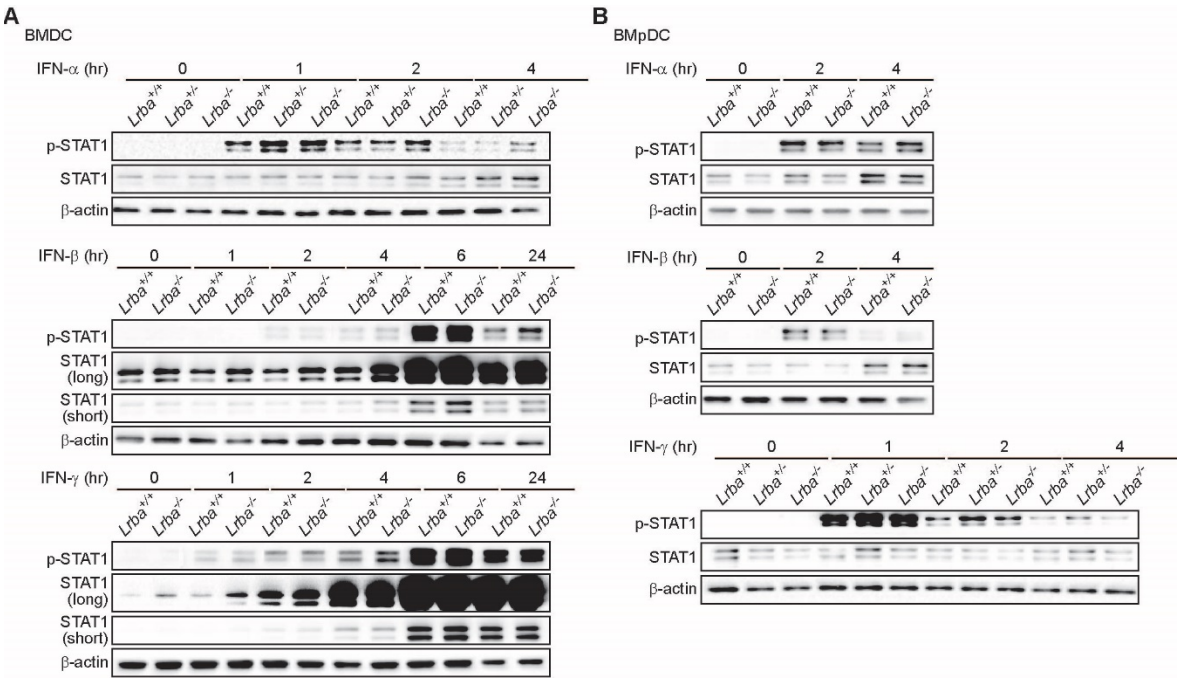

Fig. S7. Normal STAT1 activation in *Lrba*$^{-/-}$ dendritic cells stimulated with type I or type II IFN. Immunoblot analysis of phosphorylated (p) and total STAT1 in *Lrba*$^{+/+}$, *Lrba*$^{+/-}$, and *Lrba*$^{-/-}$ (A) BMDC and (B) BMpDC after stimulation with IFN-α, IFN-β, or IFN-γ. Data are representative of 2 independent experiments.

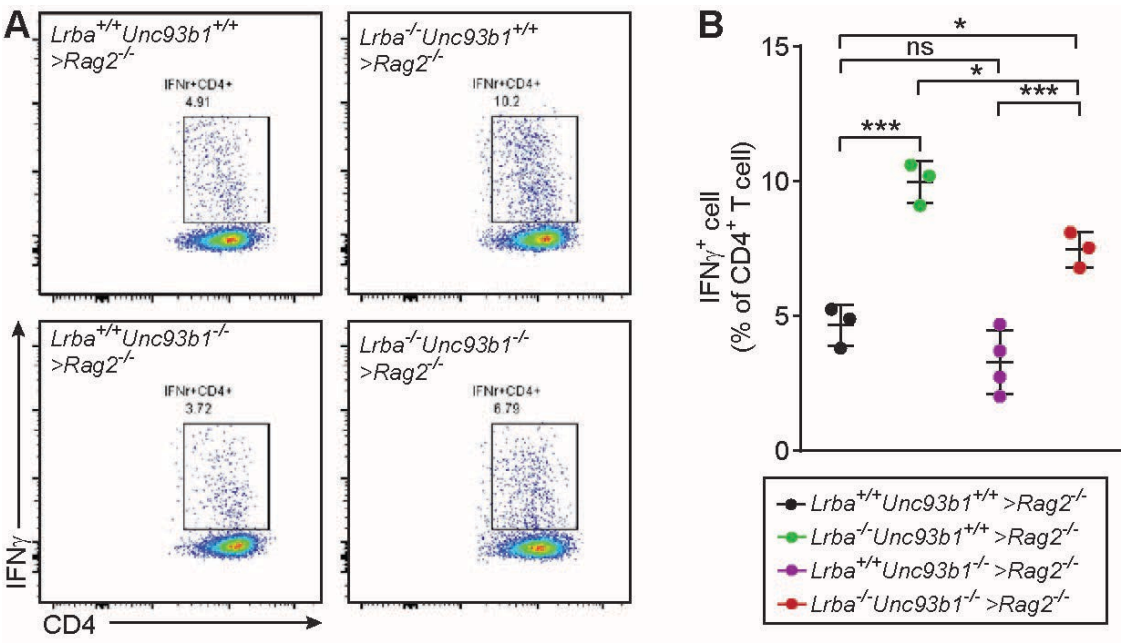

Fig. S8. Knockout of UNC93B1 normalizes frequency of IFN-γ$^+$ CD4$^+$ T cells in *Lrba*$^{-/-}$ spleen. Bone marrow transplantation was performed using mice of the indicated genotypes (donor > recipient). (A) Flow cytometry of splenocytes from bone marrow chimeric mice, assessing expression of IFN-γ by CD4$^+$ T cells. Numbers above boxed regions represent percent cells in each. (B) Quantification of percentage of splenic CD4$^+$ T cells expressing IFN-γ. Each symbol (B) represents an individual mouse. * $P < 0.05$, *** $P < 0.001$ (one-way ANOVA with post hoc Tukey multiple comparisons test). Data are representative of 3 independent experiments (mean ± s.d. in graph).

Table S1. RT-qPCR Primer Sequences

Primer	Sequences
*Ccl2*_qPCR_F	CTCAGCCAGATGCAGTTAACG
*Ccl2*_qPCR_R	AAACTACAGCTTCTTTGGGACAC
*Ccl3*_qPCR_F	CTTCTCAGCGCCATATGGAG
*Ccl3*_qPCR_R	CTGCCGGTTTCTCTTAGTCAG
*Ccl7*_qPCR_F	CATCCACATGCTGCTATGTC
*Ccl7*_qPCR_R	TTGTCTTGAAGATAACAGCTTCCC
*Cxcl10*_qPCR_F	CAACTGCATCCATATCGATGAC
*Cxcl10*_qPCR_R	TTTCATCGTGGCAATGATCTC
*Gapdh*_qPCR_F	CGACTTCAACAGCAACTCCCACTCTTCC
*Gapdh*_qPCR_R	TGGGTGGTCCAGGGTTTCTTACTCCTT
*Ifit1*_qPCR_F	CGTAGCCTATCGCCAAGATTTA
*Ifit1*_qPCR_R	AGCTTTAGGGCAAGGAGAAC
*Ifit2*_qPCR_F	GTAGATCTGGAAGGGCCTAAATG
*Ifit2*_qPCR_R	CAAGCTGAACCCAGTAGAGAAA
*Ifna*_qPCR_F	GGACTTTGGATTCCCGCAGGAGAAG
*Ifna*_qPCR_R	GCTGCATCAGACAGCCTTGCAGGTC
*Ifnb*_qPCR_F	AACCTCACCTACAGGGCGGACTTCA
*Ifnb*_qPCR_R	TCCCACGTCAATCTTTCCTCTTGCTTT
*Ifng*_qPCR_F	CAGCAACAGCAAGGCGAAA
Ifng_qPCR_R	CTGGACCTGTGGGTTGTTGAC
*Il10*_qPCR_F	AGGACTTTAAGGGTTACTTGGG
*Il10*_qPCR_R	GTCTTCAGCTTCTCACCCAG
*Il17a*_qPCR_F	GCTGGACCACCACATGAA
*Il17a*_qPCR_R	GCATCTTCTCGACCCTGAAA
*Il18*_qPCR_F	TTCCTTTGAGGAAATGGATCCAC
*Il18*_qPCR_R	TGGCAAGCAAGAAAGTGTCC
*Il1b*_qPCR_R	TGCCGTCTTTCATTACACAG
*Il1b*_qPCR_F	CAACCAACAAGTGATATTCTCC
*Il6*_qPCR_F	CTCTGCAAGAGACTTCCATCC
*Il6*_qPCR_R	CGACTTGTGAAGTGGTATAGACAG
*Irf3*_qPCR_F	CTTCATGGAAGGAAGTGGAC
*Irf3*_qPCR_R	TAGGAACAACCTTGACCATCAC
*Irf7*_qPCR_F	TTTCTTCCGAGAACTGGAGGAG
*Irf7*_qPCR_R	ACCAGGATCAGGGTCTTCTC
*Mx2*_qPCR_F	CATCTGTAAATCTCTTCCTCTGCT
*Mx2*_qPCR_R	TTCACTGTCATCTTCTGGTATGTC
*Oas1g*_qPCR_F	TTAGAGTCAAGTTTGAGGTCCA
*Oas1g*_qPCR_R	TGATACTACCATGACCCAGGA
*Rantes*_qPCR_F	TTGCAGTCGTGTTTGTCACTC
*Rantes*_qPCR_R	GCAATGACAGGGAAGCTATACAG
*Rnasel*_qPCR_F	GCTTACAATGTCTCCAGATGAGG
*Rnasel*_qPCR_R	TATAGCGGTTCTCCCAAGTC
*Tnf*_qPCR_F	AGGCTGCCCCGACTACGT
*Tnf*_qPCR_R	GACTTTCTCCTGGTATGAGATAGCAAA
*Trim5*_qPCR_F	CATAGAAATACTACAGGGTGTGGA
*Trim5*_qPCR_R	CTCGGAACTTTCTTTGTTCCC