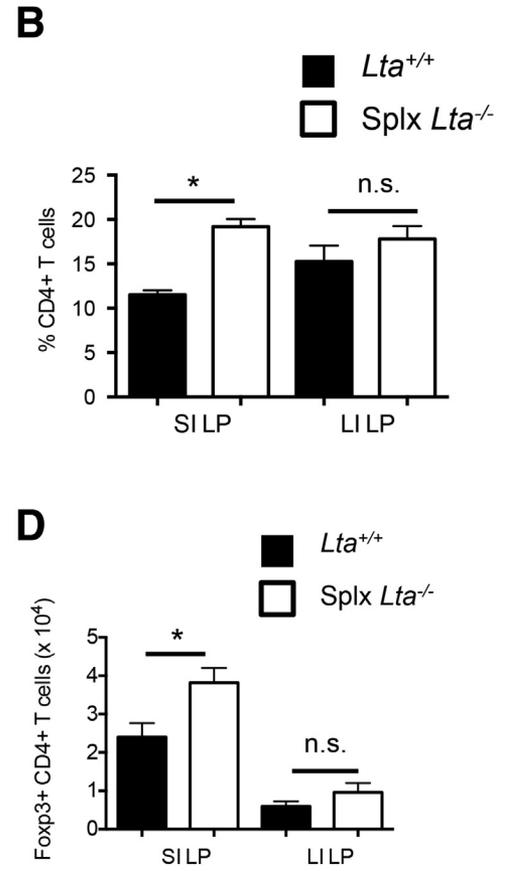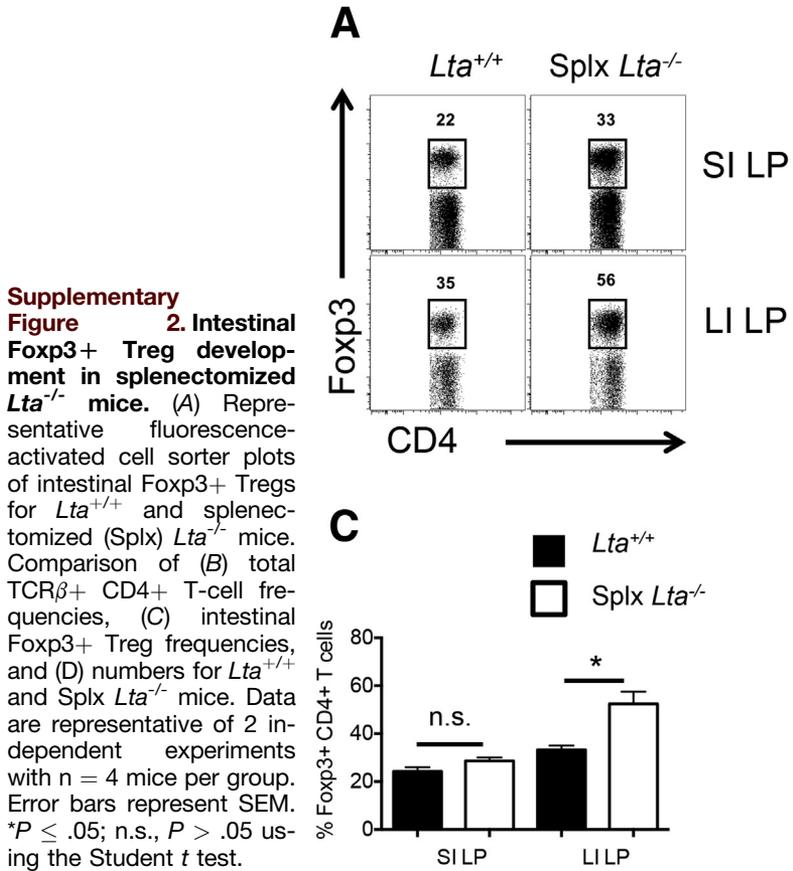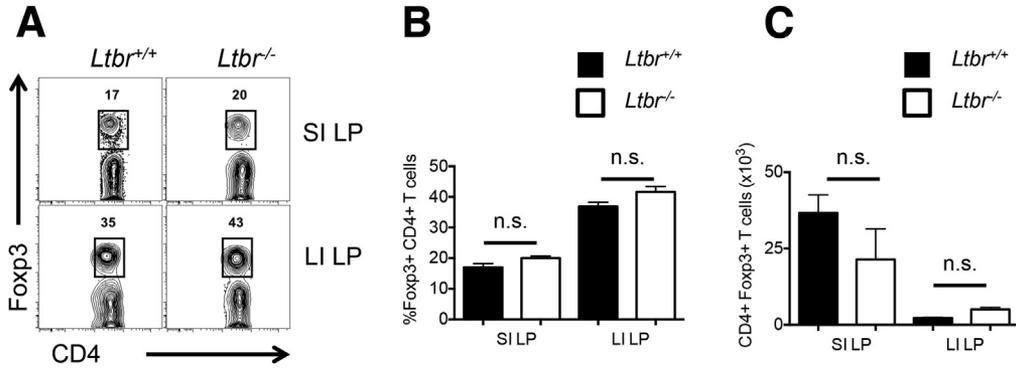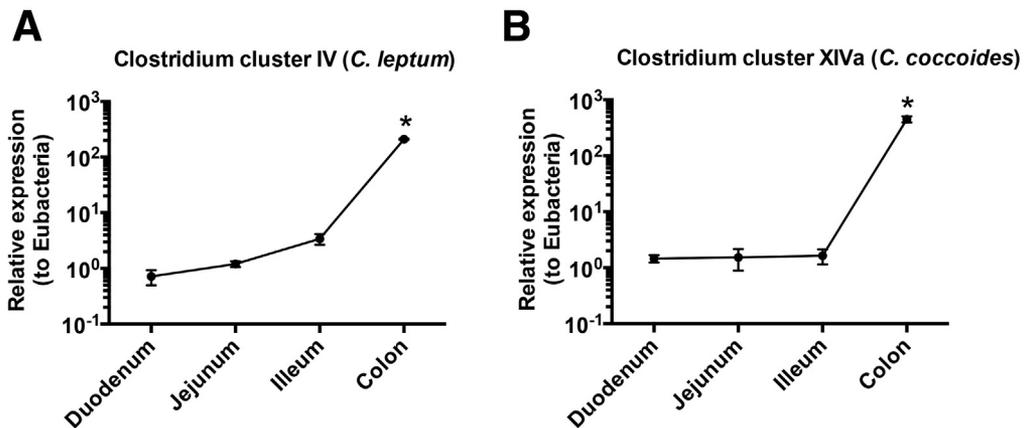**Supplementary Figure 1. CD4+ T cells accumulate in the intestinal LP independent of CCR7.** Comparison of TCRβ+ CD4+ cell frequencies in the SI and LI LP between *Ccr7*$^{+/+}$ and *Ccr7*$^{-/-}$ mice. Data are representative of 2 independent experiments with n = 4 mice per group. Error bars represent SEM. *$P \leq$ .05; NS, $P >$ .05 using the Student *t* test.

Supplementary Figure 2. Intestinal Foxp3+ Treg development in splenectomized *Lta*$^{-/-}$ mice. (*A*) Representative fluorescence-activated cell sorter plots of intestinal Foxp3+ Tregs for *Lta*$^{+/+}$ and splenectomized (Splx) *Lta*$^{-/-}$ mice. Comparison of (*B*) total TCRβ+ CD4+ T-cell frequencies, (*C*) intestinal Foxp3+ Treg frequencies, and (D) numbers for *Lta*$^{+/+}$ and Splx *Lta*$^{-/-}$ mice. Data are representative of 2 independent experiments with n = 4 mice per group. Error bars represent SEM. *$P \leq$ .05; n.s., $P >$ .05 using the Student *t* test.
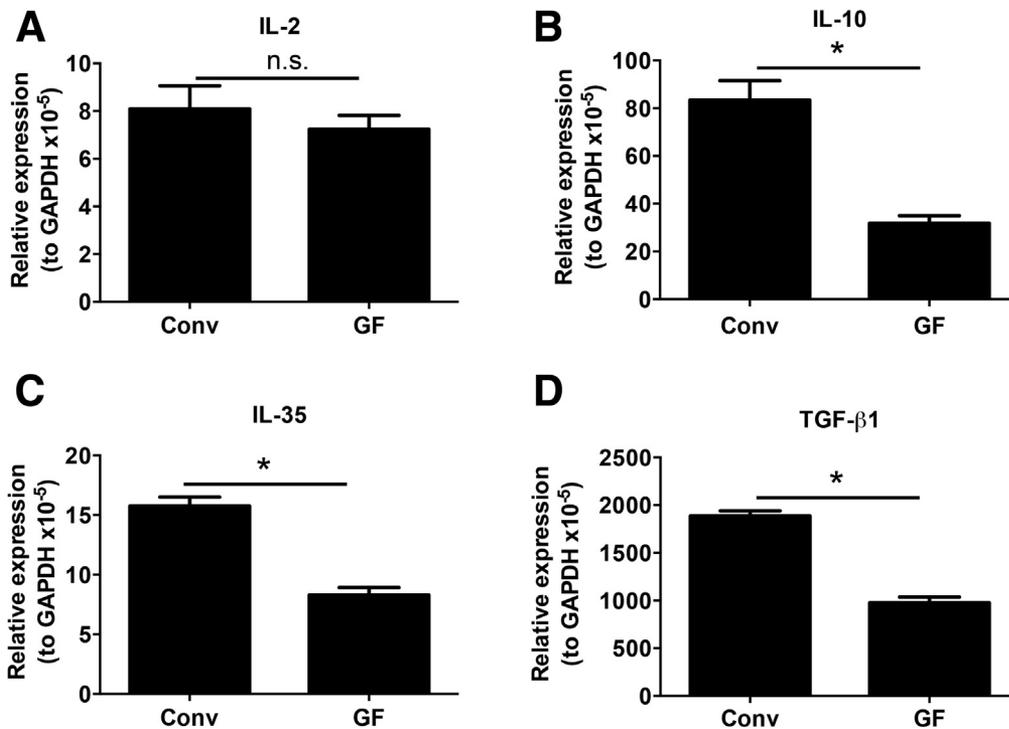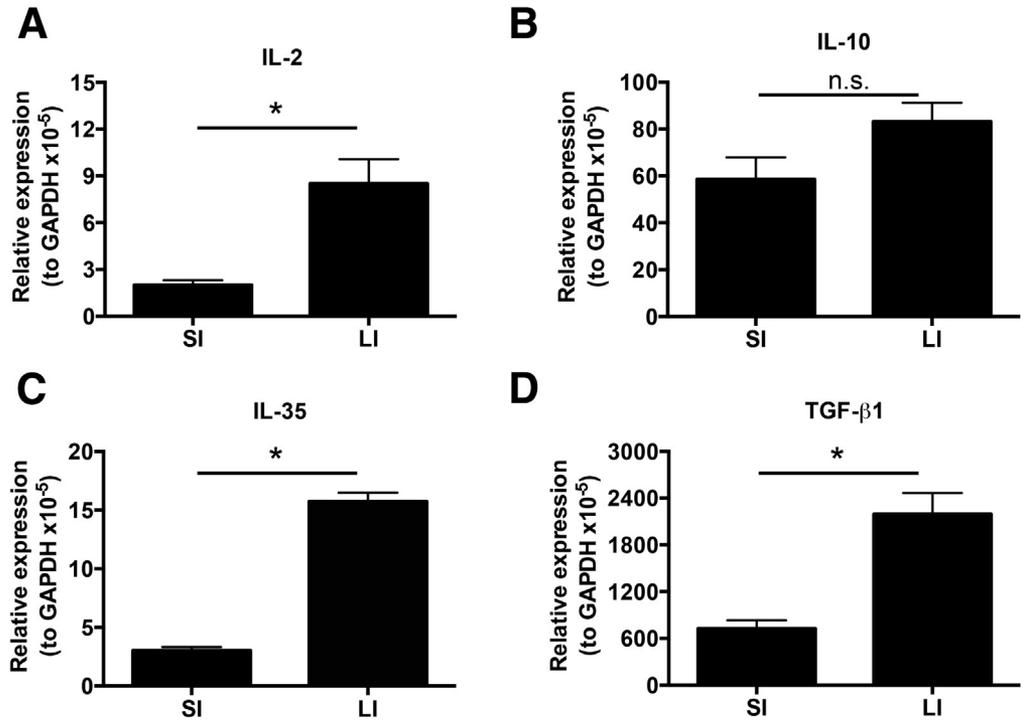
Supplementary Figure 3. Intestinal Foxp3+ Treg development in *Ltbr*^-/- mice. (*A*) Representative fluorescence-activated cell sorter plots of intestinal Foxp3+ Tregs in *Ltbr*^+/+ and *Ltbr*^-/- mice. Comparison of (*B*) intestinal Foxp3+ Treg frequencies and (*C*) numbers in *Ltbr*^+/+ and *Ltbr*^-/- mice. Data are representative of 2 independent experiments with n = 4 mice per group. Error bars represent SEM. n.s., $P > .05$ using the Student t test.

Supplementary Figure 4. Analyses of *Clostridium* clusters IV and XIVa in the SI and LI. DNA was isolated from the upper (duodenum), middle (jejunum), and lower (ileum) small intestine, and colon then was analyzed for 16S ribosomal DNA for Clostridium IV and XIVa groups. Quantitation of bacteria is shown relative to Eubacteria. Data are representative of 2 independent experiments with n = 4 mice per group. Error bars represent SEM. *$P \leq .05$ using the Student t test.

Supplementary Figure 5. Treg-inducing cytokines are expressed preferentially in the LI. (*A*) IL2, (*B*) IL10, (*C*) IL35, and TGF-β1 mRNA expression in SI and LI. Data are representative of 2 independent experiments with n = 3 mice per group. Error bars represent SEM. *$P \leq$ .05; n.s., $P >$ .05 using the Student *t* test. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Supplementary Figure 6. Influence of the microbiota on Treg-inducing cytokine expression in the LI. (*A*) IL2, (*B*) IL10, (*C*) IL35, and TGF-β1 mRNA expression in the LI of WT C57BL/6 mice housed under conventional (Conv) or germ-free (GF) conditions. Data are representative of 2 independent experiments with n = 2 mice per group. Error bars represent SEM. *$P \leq$ .05; n.s., $P >$ .05 using the Student *t* test. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.