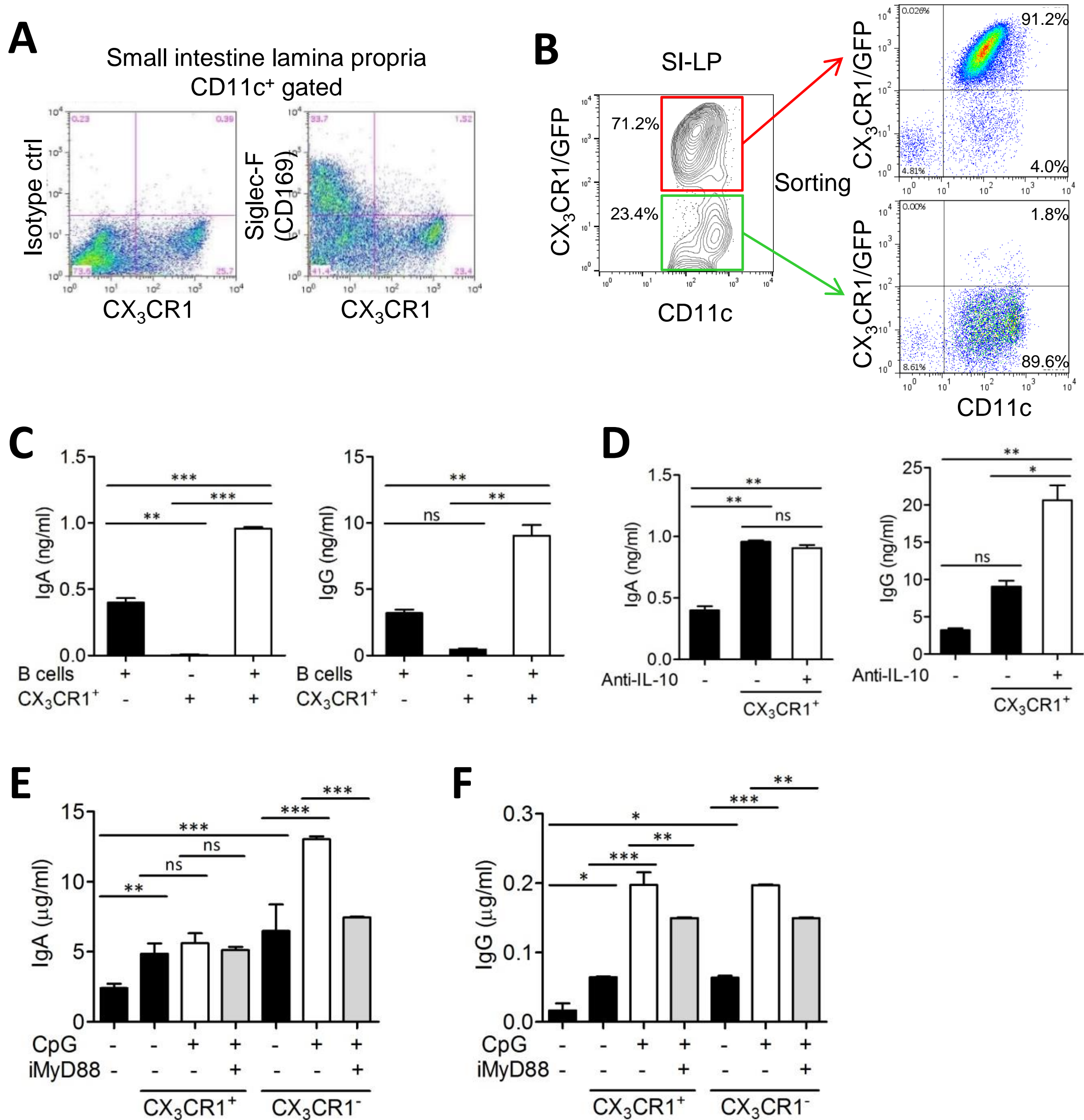


**FIGURE S1**. Phenotype and sorting purity of intestinal $CX_3CR1^+CD11c^+$ macrophages.

(**A**) $CD11c^+$ cells isolated from small intestinal lamina propria of $CX_3CR1^{(GFP/+)}$ mice were analyzed for expression of Siglec F/CD169 in the intestinal $CX_3CR1^+CD11c^+$ macrophages. (**B**) $CD11c^+$ cells were isolated from lamina propria of small intestine of $CX_3CR1^{(GFP/+)}$ mice and sorted to $CX_3CR1^+$ and $CX_3CR1^-$ subsets. Purity of sorted subsets from intestinal $CD11c^+$ cells were about 90%. (**C**) Total IgA and IgG level from culture supernatants of $CD11c^+CX_3CR1^+$ macrophages co-cultured with B cells or each cell alone in presence of anti-IgM (10 μg/ml). (**D**-**F**) The level of total IgA and IgG were determined from culture supernatants of B cells co-cultured with $CD11c^+CX_3CR1^+$ macrophages (**D**) in presence of 10 μg/ml anti-mouse IL-10 or (**E** and **F**) CpG (1 μM) and MyD88 inhibitor (100 Mm, IMG2005; Novus Biologicals, Littleton, CO). Data are representative of three independent experiments. Graphs show mean ± SEM. ns; not significant, *p < 0.05, **p < 0.01 and ***p < 0.001 using one-way ANOVA.

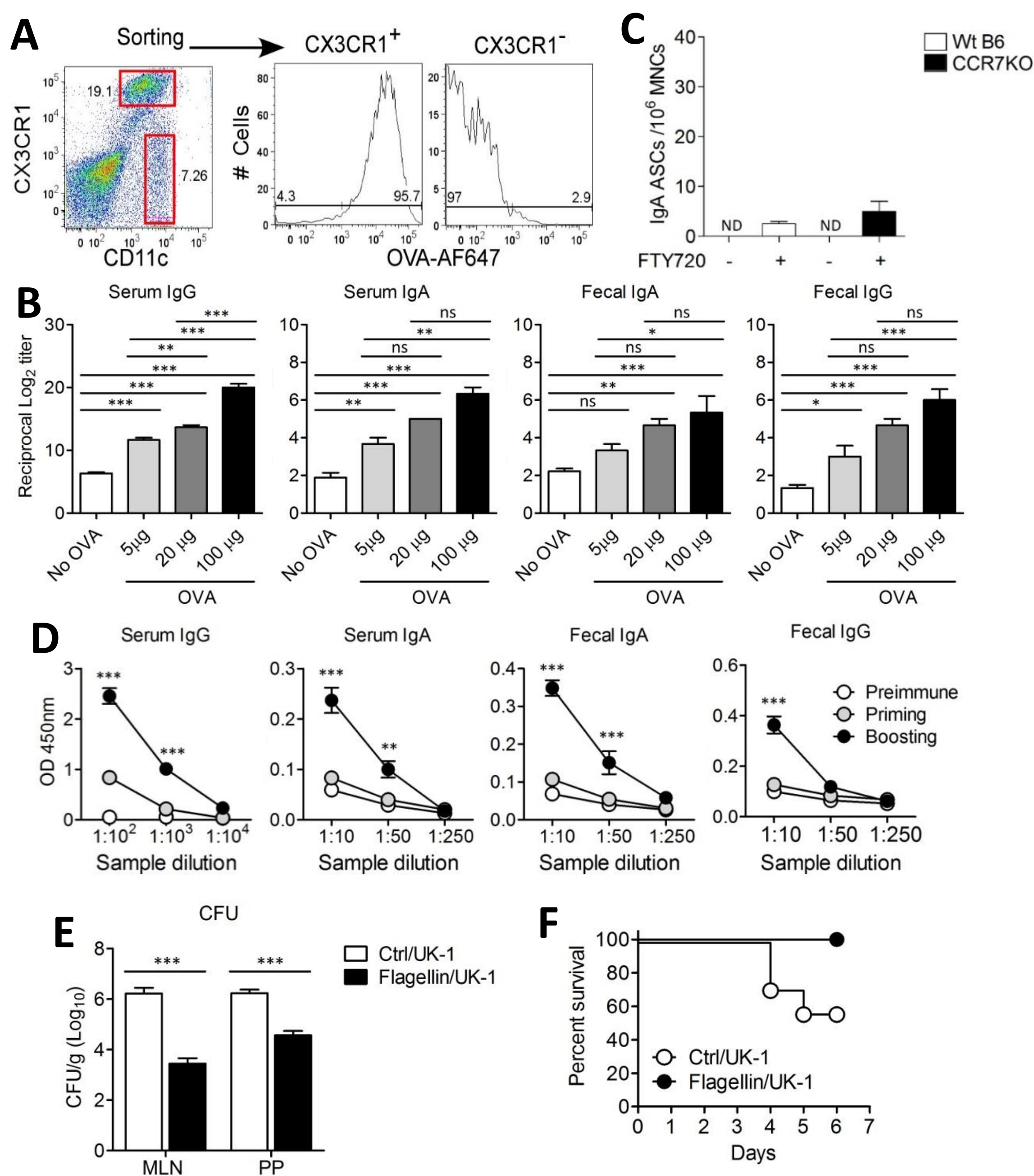


**FIGURE S2.** Preferential uptake of circulatory Ag by intestinal $CX_3CR1^+$ macrophages.
(**A**) $CX_3CR1^{(GFP/+)}$ mice *i.v.* injected with 100 μg OVA-AF647. At 1hr following injection, $CD11c^+$ cells were isolated from small intestinal lamina propria of $CX_3CR1^{(GFP/+)}$ mice and sorted to $CX_3CR1^+$ and $CX_3CR1^-$ subsets. Fluorescence signal from OVA-AF647 was analyzed to investigate the preferential uptake of circulatory antigen by $CD11c^+CX_3CR1^+$ macrophages. (**B**) WT B6 mice were *i.v.* injected with three different doses of OVA and boosted 2 weeks later. Serum and feces were collected 1 week after boosting (n=3). The titers of OVA specific serum IgG, serum IgA, fecal IgA and fecal IgG were analyzed. (**C**) The generation of circulatory Ag specific IgA ASCs was not induced in the MLN. WT B6 and CCR7 KO mice were *i.v.* immunized with 100 μg OVA and boosted 2 weeks later. Mice were treated with FTY720 and then OVA specific IgA ASCs were determined in the MLN. ND, not detected. (D-F) WT mice were *i.v.* injected with 2 μg flagellin isolated from *Salmonella Typhimurium* and boosted 2 weeks later. At 10 days following final boosting, mice were challenged orally with $10^7$ CFU of wild-type invasive *Salmonella* Typhimurium. (D) Serum and feces were collected 1 week after boosting (n=10). The level of flagellin specific serum IgG, serum IgA, fecal IgA and fecal IgG were analyzed. (E) CFU from PP and MLN were determined at day 6 following challenge and (F) survival rates were monitored.

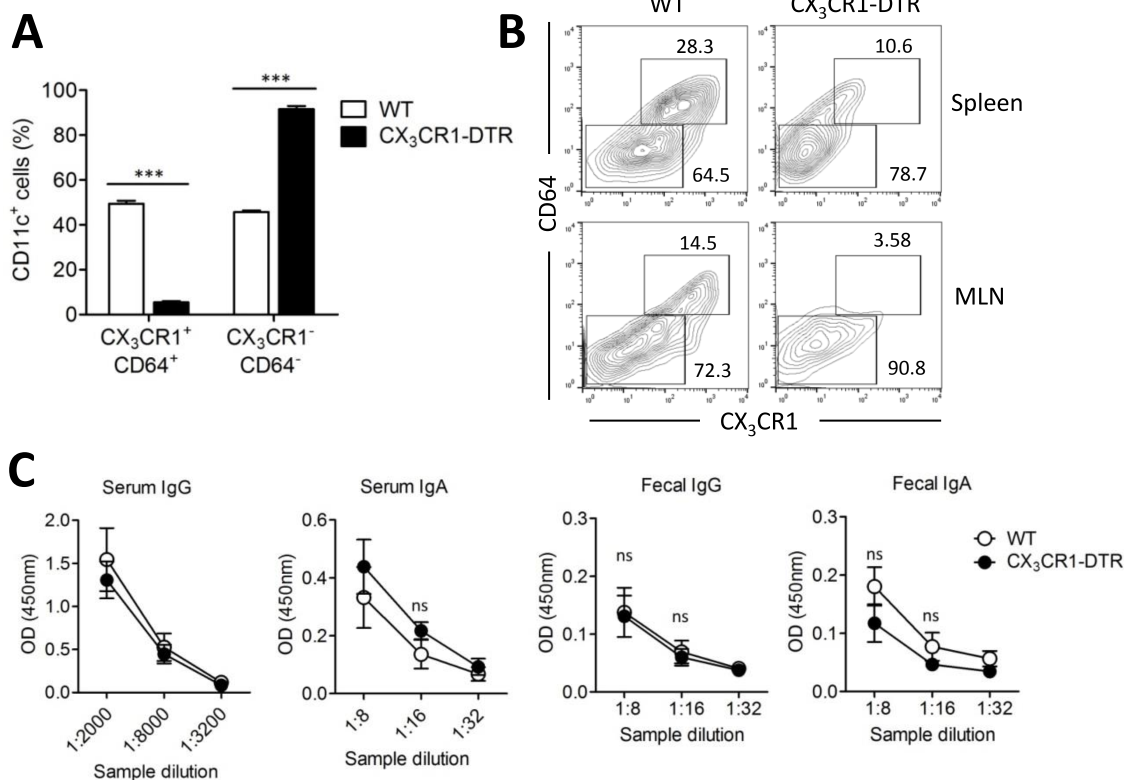


**FIGURE S3.** Generation of antigen specific IgA antibodies in the gut upon delivery of antigen by oral gavage partially reduced in absence of $CX_3CR1^+$ macrophages.

For depletion of $CX_3CR1^+$ macrophages, mice were *i.p.* injected with 200 ng diphtheria toxin before 1 day and after 2 days of OVA immunization. (**A-B**) To confirm the depletion of $CD11c^+CX_3CR1^+$ macrophages, $CD11c^+$ cells were isolated from the small intestinal lamina propria, spleen, and MLN. $CD64^+CX_3CR1^+$ cells were analyzed from the $CD11c^+$ population. (**A**) The percentages of $CD64^+CX_3CR1^+$ population in the lamina propria among $CD11c^+$ cells were summarized. (**B**) Depletion of $CD64^+CX_3CR1^+$ cells in the spleen and MLN. (**C-F**) WT B6 and $CX_3CR1$-DTR mice were treated with 10 mg OVA with 10 μg cholera toxin and boosted 2 weeks later. Serum and feces were collected 1 week after 2nd immunization (n=5). (**C**) OVA specific serum IgG, (**D**) serum IgA, (**E**) fecal IgG and (**F**) fecal IgA were analyzed. Graphs show mean ± SEM. ns; not significant, using Student's *t*-test.

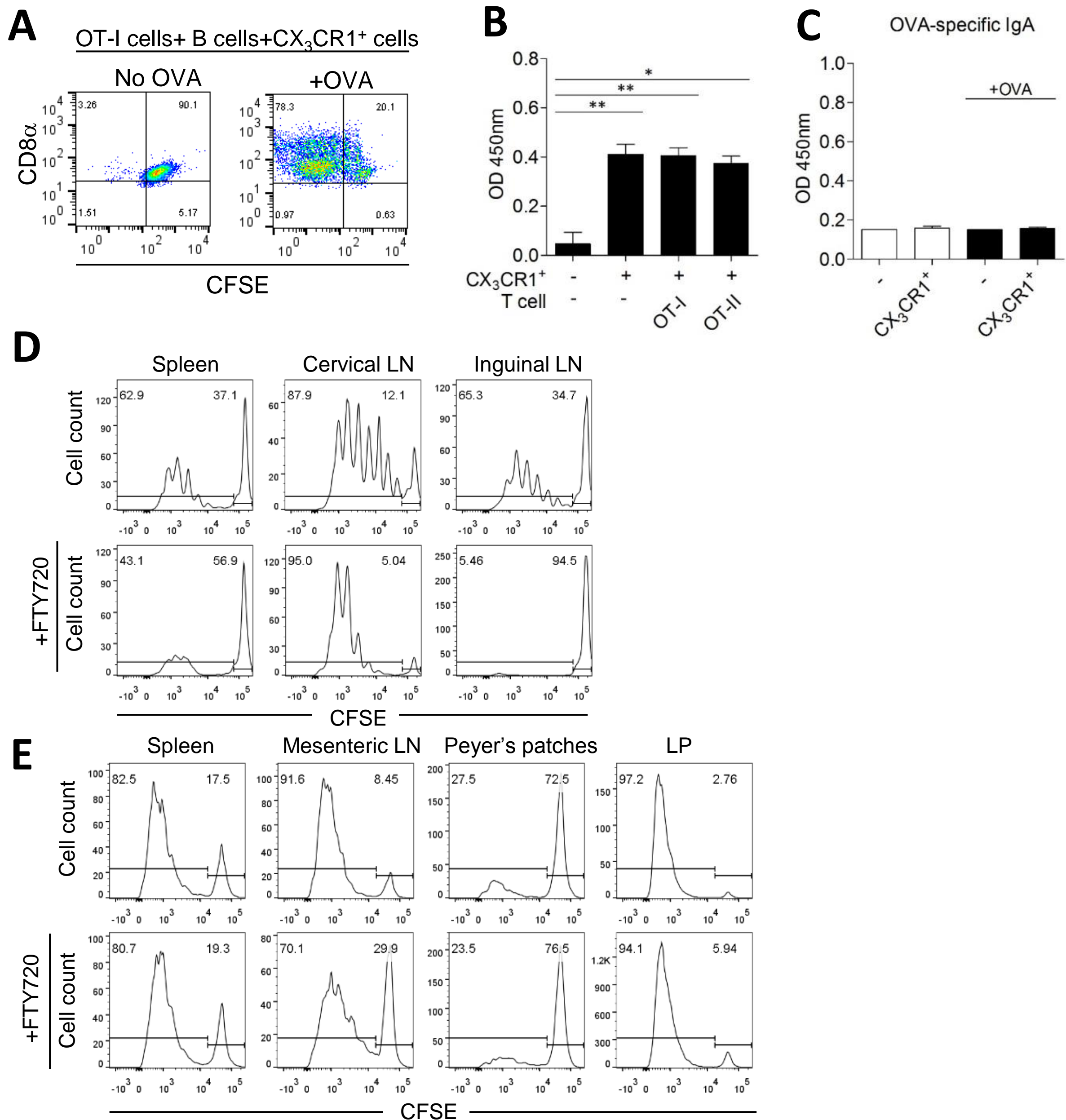


**FIGURE S4.** CD8⁺ T cells could not enhance IgA production without cognate Ag.

(**A**-**C**) CD11c⁺ cells of SI LP from $CX_3CR1^{(GFP/+)}$ mice were isolated and sorted to $CX_3CR1^+$ cells. Splenic B cells stimulated with anti-IgM were co-cultured with OVA pulsed $CD11c^+CX_3CR1^+$ macrophages and OT-I or OT-II cells. (**A**) CFSE dilution of co-cultured OT-I cells were analyzed. (**B**) Total IgA was measured from culture supernatant of B cells co-cultured with $CX_3CR1^+$ macrophages with OT-I or OT-II cells in absence of OVA. (**C**) OVA-specific IgA was measured from culture supernatant of B cells co-cultured with $CX_3CR1^+$ macrophages in the presence of OVA. Graphs show mean ± SEM. *$p < 0.05$ and **$p < 0.01$ using one-way ANOVA with Tukey's post hoc test. (**D**-**E**) OT-I $CD8^+$ T cells labeled with 9 μM CFSE were adoptively transferred to $CD45.1^+$ WT B6 mice. (**D**) Next day these mice were *s.c.* immunized with 20 μg OVA plus 1μg cholera toxin and *i.p.* treated with 20 μg FTY720. At 2 days later, FTY720 was treated again. After 3 days, CFSE dilution of $CD45.2^+CD8\alpha^+$ cells were analyzed from the spleen, cervical LN, and inguinal LN. (**E**) Next day following OT-I cell transfer, OVA was *i.v.* injected and CFSE dilution of $CD45.2^+CD8\alpha^+$ cells were analyzed from the spleen, MLN, Peyer's patches (PP), and small intestine lamina propria (LP) at day 5 following OVA injection.