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Supplementary Information for

A Missense Variant in *SLC39A8* Confers Risk for Crohn's Disease by Disrupting Manganese Homeostasis and Intestinal Barrier Integrity

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This PDF file includes:

Figures S1 to S6

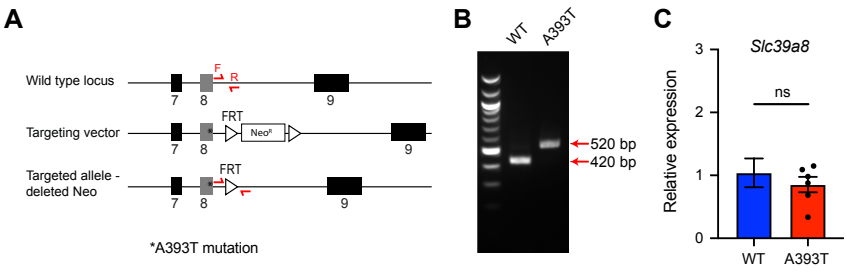

Fig. S1. Generation of Slc39a8 A391T knock-in mouse.
(**A**) Strategy for generating *Slc39a8* A391T knock-in mice. A393T missense variant (corresponding to human A391T) was introduced into the *Slc39a8* locus by gene targeting. (**B**) PCR analysis of genomic DNA isolated from WT and A391T mice. (**C**) Quantitative analysis of Slc39a8 expression in the colonic epithelial cells isolated from WT and A391T mice (n=4-6 per genotype). The values were normalized to Gapdh expression. Similar results were obtained in two independent experiments. A two-tailed unpaired t-test was used for statistical analysis. Data represent mean ± SEM.

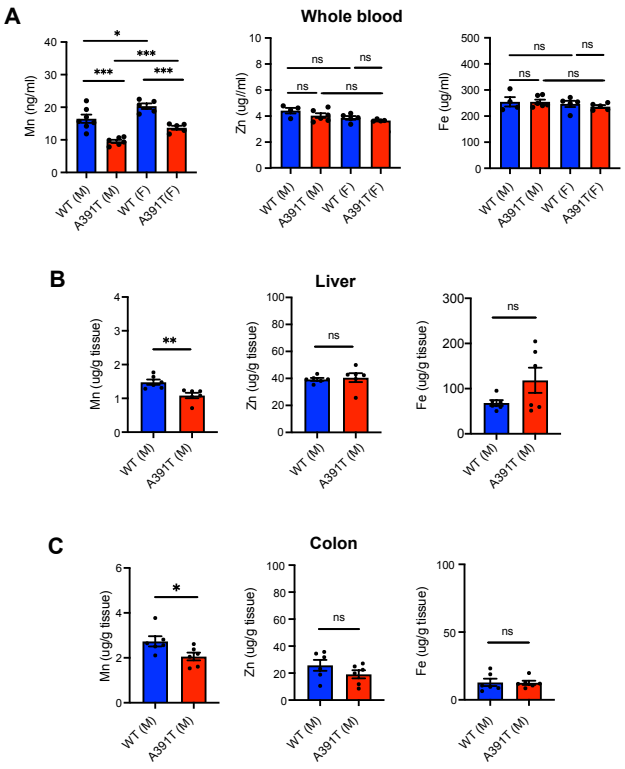

Fig. S2. Slc39A8 A391T male mice show severe Mn deficiency in the blood and tissues.

(**A, B, C**) Trace element levels (Mn, Zn, Fe) in whole blood (A), liver (**B**) and colon (**C**) of WT and *Slc39a8* A391T mice. Trace element measurement was performed by ICP-MS (n=4-6 mice per genotype). Similar results were obtained in at least two independent experiments. *p<0.05, **<0.01, ***<0.001 using one-way ANOVA with Tukey's multiple comparison test (**A**) and a two-tailed unpaired t-test (**B, C**). All data represent mean±SEM.

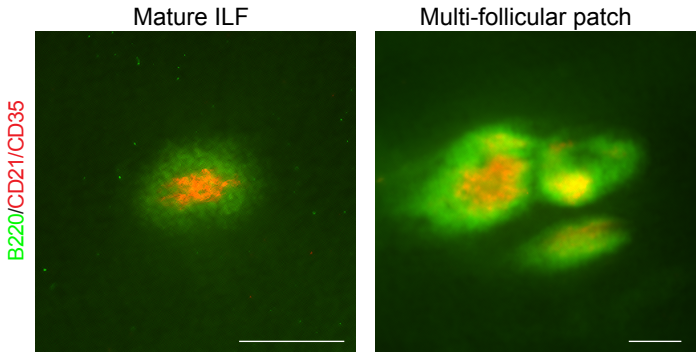

Fig. S3. Whole-mount immunostaining of lymphoid tissues. Representative image of mature lymphoid follicle (mILF) and multifollicular patch. Colon tissues were stained with B220 (green) and CD21/CD35 (red) antibodies. Scale bar, 500 μm.

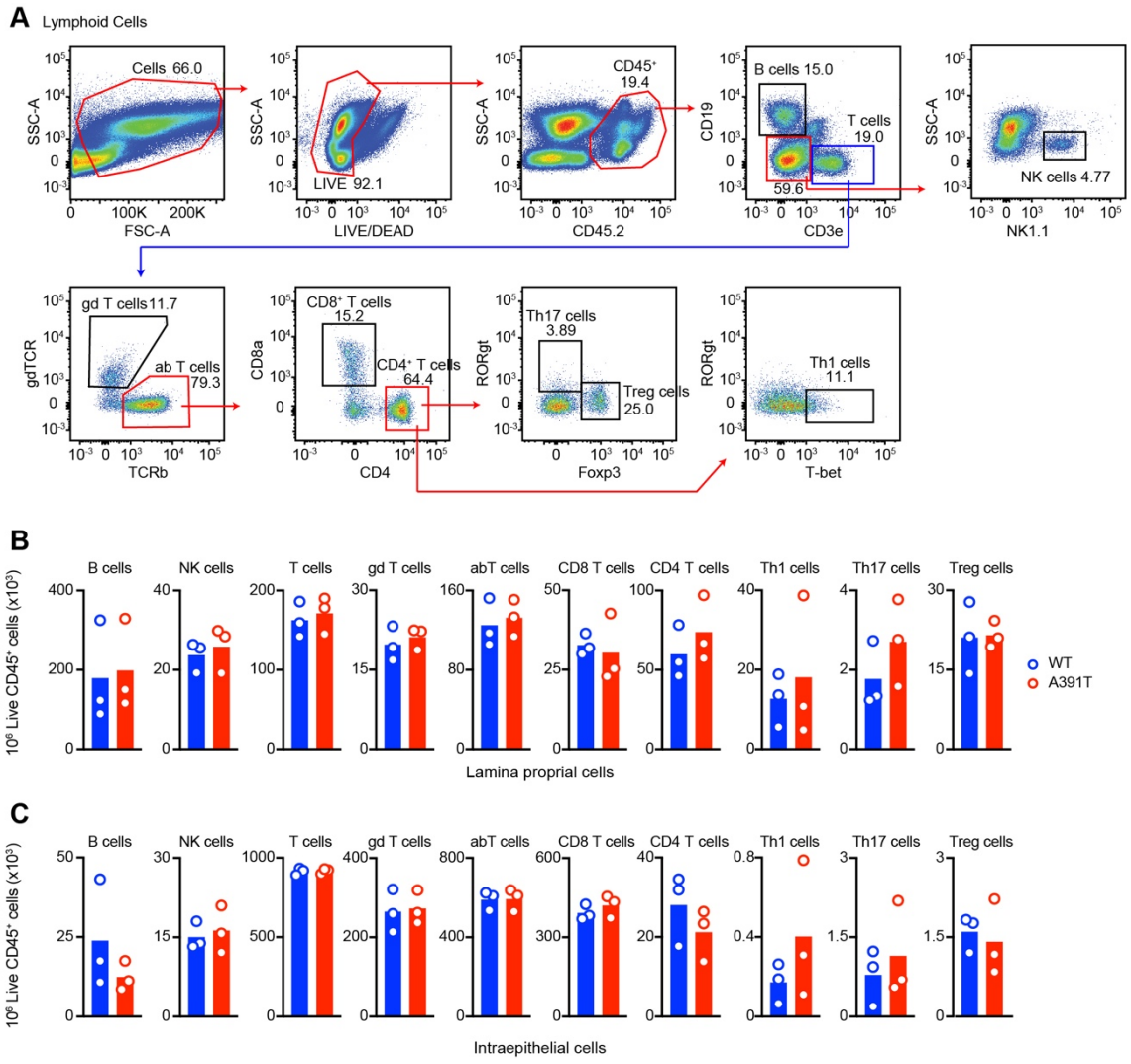

Fig. S4. Immunophenotyping of colonic lymphoid cells.

WT and A391T male mice were used (n=3 per genotype). (**A**) Gating strategy of lymphoid cell populations. Gates used for descendent analyses are shown in red or blue and directed by the same color arrows. Representative plots were shown from colonic lamina proprial cells. (**B**) Relative amount of the lymphoid cell population per 10^6 live CD45+ lamina proprial cells. (**C**) Relative amount of the lymphoid cell populations per 10^6 live CD45+ intraepithelial cells. In (**B**) and (**C**), each dot represents a sample from one mouse and each bar represents an average of replicate samples. Similar results were obtained in two independent experiments.

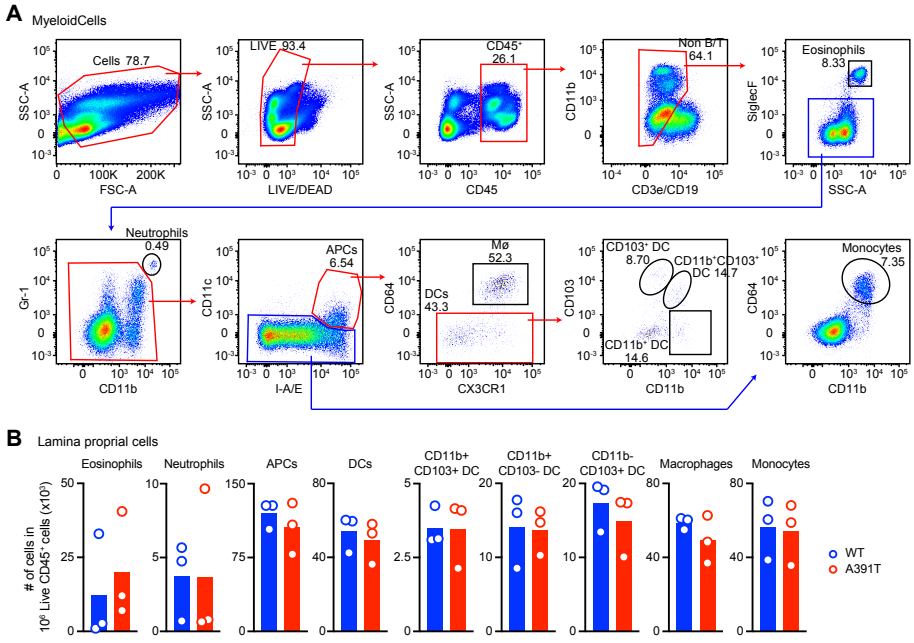

Figure S5. Immunophenotyping of colonic myeloid cells.
WT and A391T male mice were used (n=3 per genotype). (**A**) Gating strategy of myeloid cell populations. Gates used for descendent analyses are shown in red or blue and directed by the same color arrows. Representative plots were shown from colon lamina proprial cells. (**B**) Relative amount of the myeloid cell populations per 10^6 live CD45+ lamina proprial cells. Each dot represents a sample from one mouse and each bar represents an average of replicate samples. Similar results were obtained in two independent experiments.

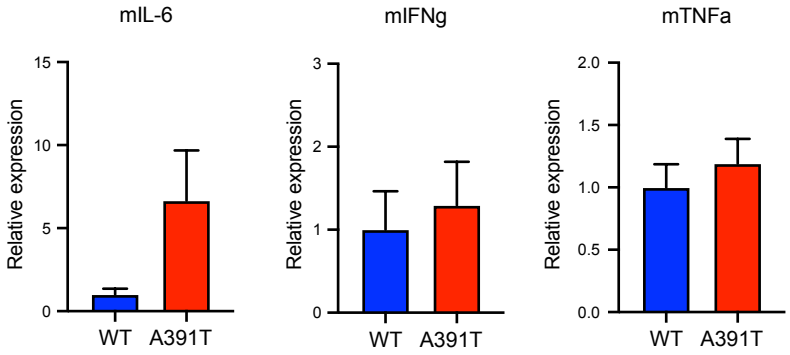

Figure S6. Proinflammatory cytokine levels in Slc39a8 WT and Slc39a8 A391T mice following DSS treatment.
qRT-PCR analysis of proinflammatory cytokine levels in the distal colon of Slc39a8 WT and Slc39a8 A391T mice on day 10 (n=6 per genotype). Data are representative of two independent experiments. All data represent mean ± SEM.