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## Supplemental information

# *Wnt5* controls splenic myelopoiesis and neutrophil functional ambivalency during DSS-induced colitis

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## Figure S1. Related to Figure 1

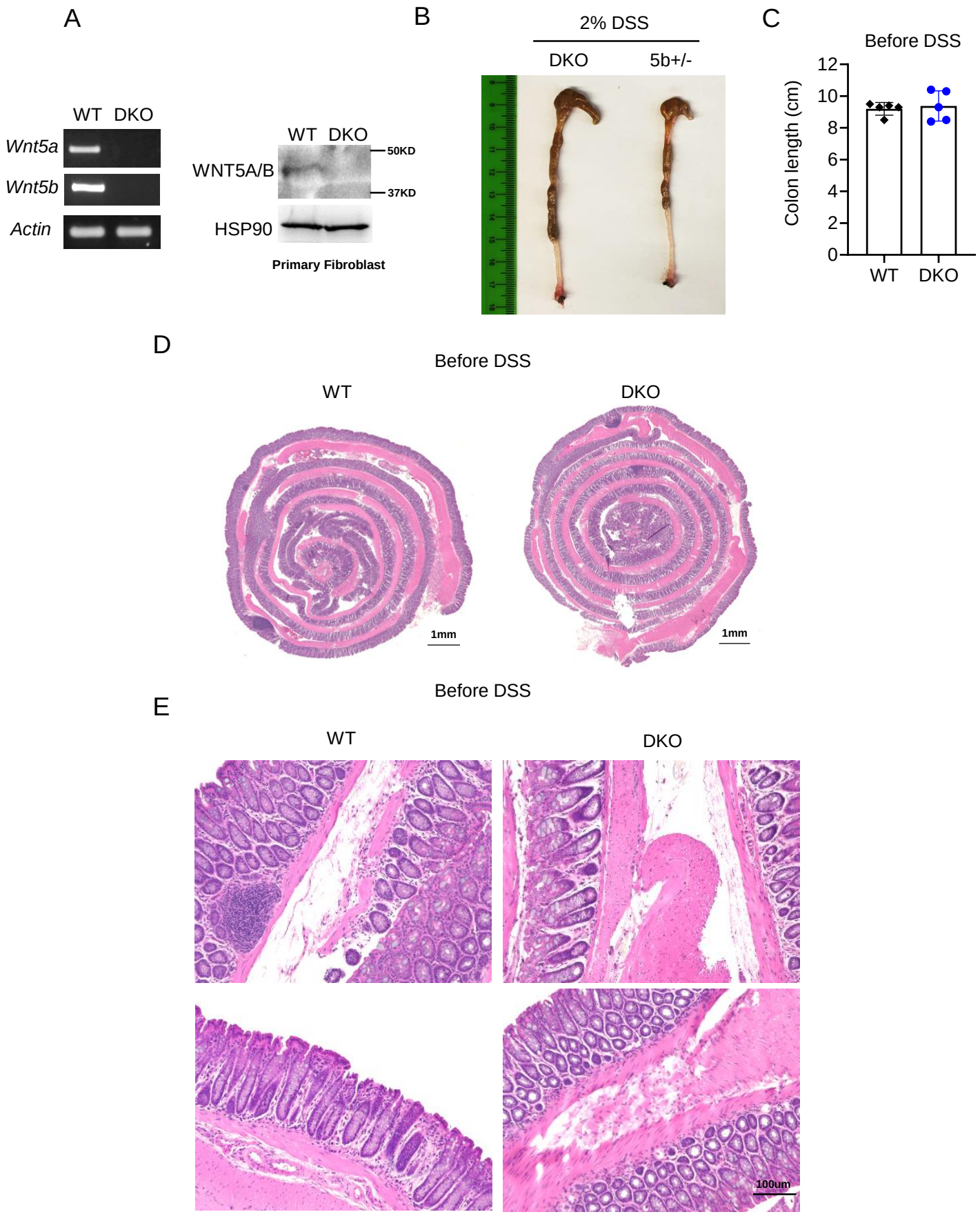

**Figure S1. Loss of WNT5 protects mice from DSS-induced colitis. Related to Figure 1.**

(**A**) RT-PCR and Western blot validation of *Wnt5a* and *Wnt5b* knockout efficiency. (**B**) Representative colons from DKO mice and control mice after 7-day DSS treatment are shown. (**C**-**E**) Colon length and H&E sections of colons from the DKO mice and control mice collected before DSS treatment are shown. Data in (**C**) are presented as means±sem. Each datum point represents one mouse. Each independent experiment consists of at least three technical replicates.

# Figure S2. Related to Figure 2

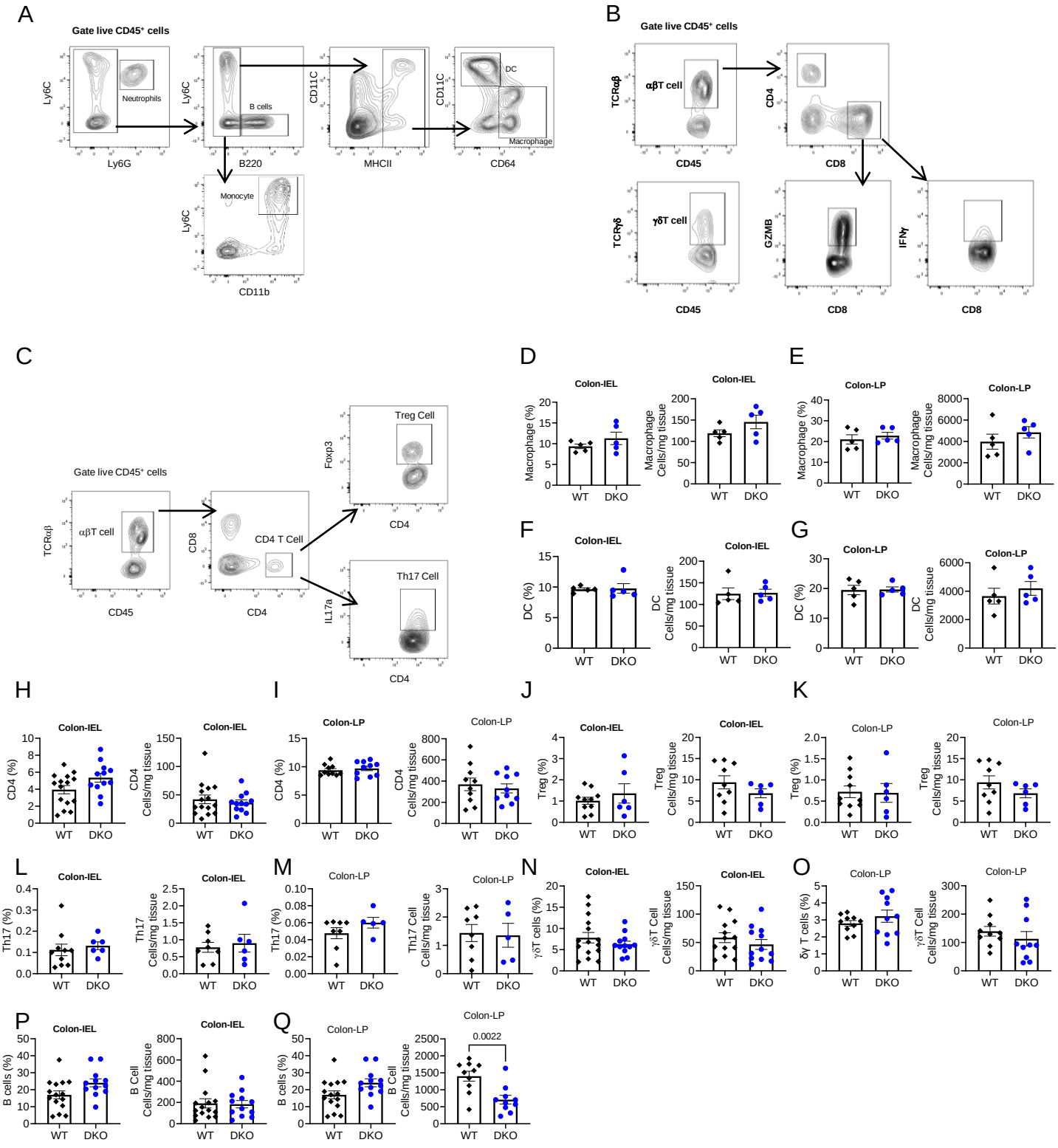

**Figure S2. Immune cell compositions in colitic colons of *Wnt5* DKO mice and control mice. Related to Figure 2.**

(**A**-**C**) Gating strategies for flow cytometry analysis of colon immune cells. (**D**-**Q**) Mice were treated as in Fig. 1, and percentage (in $CD45^+$) and absolute number of various immune cell populations were determined by flow cytometry. Data are shown as means±SEM with P value (two-tailed Student's *t*-test). Each datum point represents one mouse. Each independent experiment consists of at least three technical replicates.

# Figure S3. Related to Figure 3.

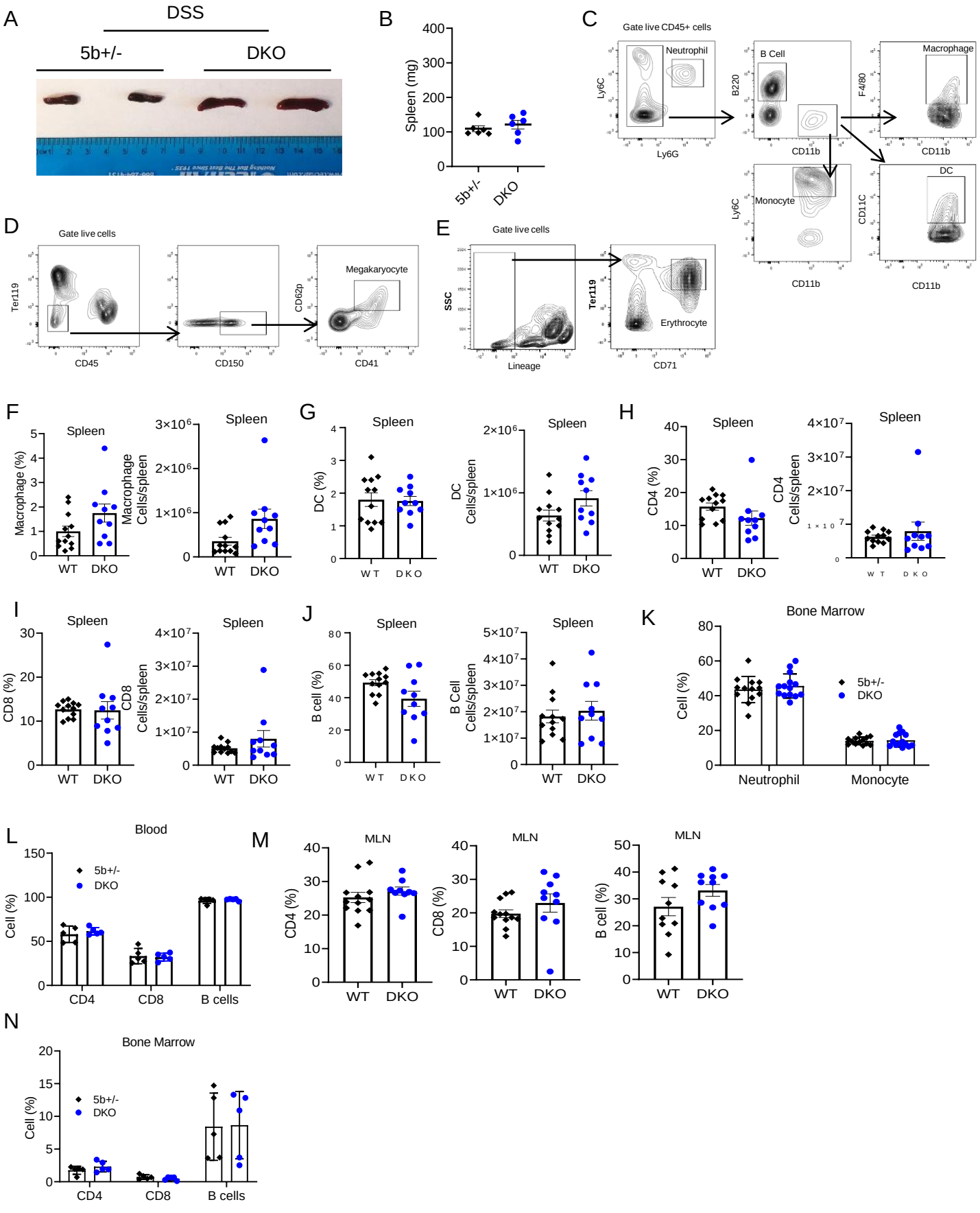

**Figure S3. Colitic *Wnt5* DKO mice develop splenomegaly. Related to Figure 3.**

(**A**) Representative images of spleens from the DKO mice and control mice on Day 7 of DSS treatment. (**B**) Spleen weight of *Wnt5* DKO mice and control mice without DSS treatment. (**C**-**E**) Flow cytometry gating strategies for the analysis in spleens. The same gating strategy was used for flow cytometry analysis of blood and MLN cells. (**F**-**N**) Mice were treated as in Figure 1, and the percentage (in $CD45^+$) and the absolute number of immune cells were determined by flow cytometry. Results in (**B**, **F**-**N**) are presented as means±sem with P value (two-tailed Student's *t*-test). Each datum point represents one mouse. Each independent experiment consists of at least three technical replicates.

# Figure S4. Related to Figure 4

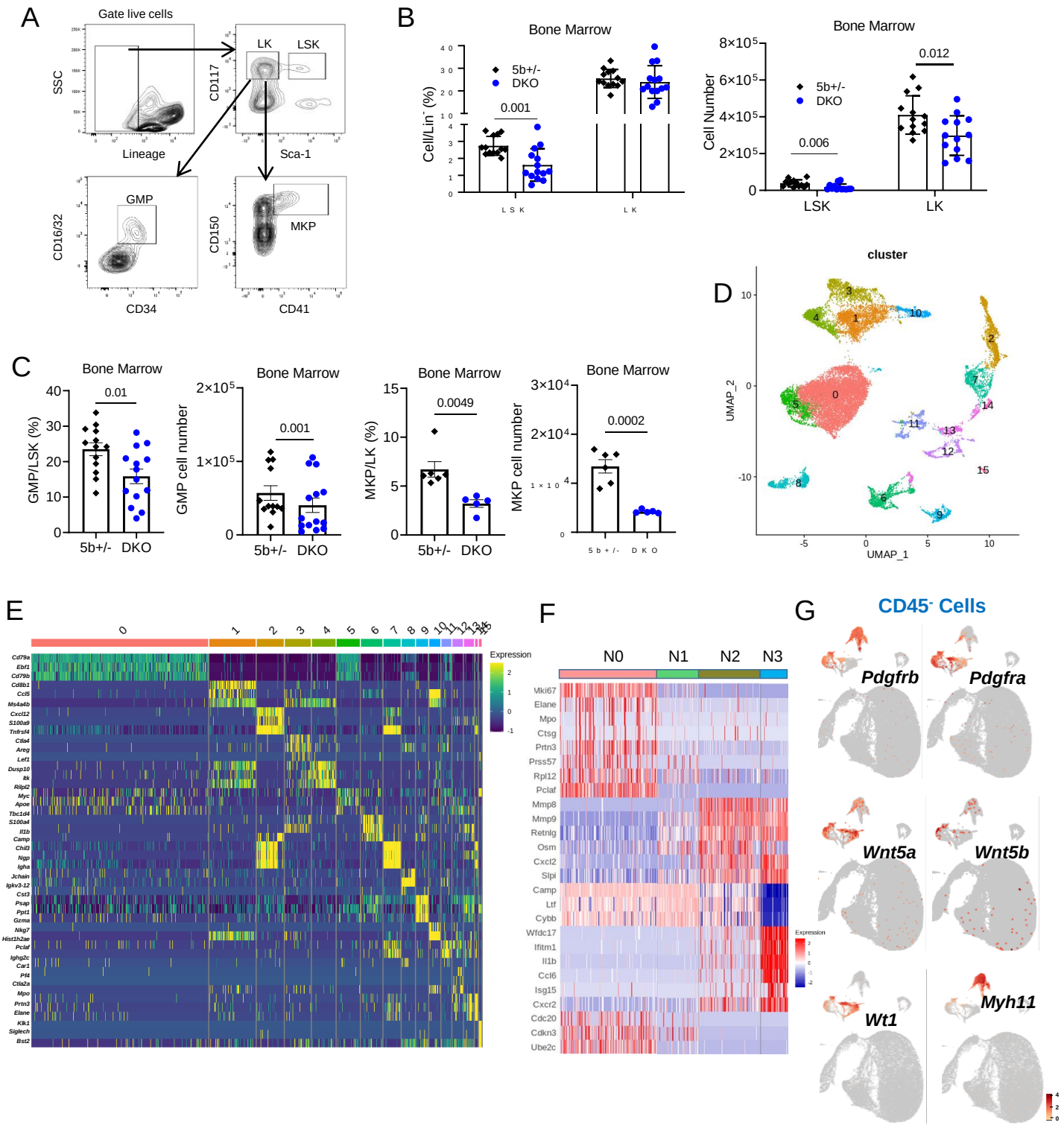

**Figure S4. *Wnt5* DKO mice enhance splenic extramedullary hematopoiesis upon colitis induction. Related to Figure 4.**

(**A**) Gating strategies for flow cytometry analysis of hematopoietic cell stem/progenitor populations during hematopoiesis in the bone marrow and spleen are shown. (**B**-**G**) Mice were treated as in Figure 1 and the percentage (in Lineage-) and the absolute number of LK and LSK (**B**), percentage (in LK) and absolute number of GMP and MKP (**C**) in the bone marrow were determined by flow cytometry. **(D)** UMAP plot of all of the splenic cells (21,745 in total) from DSS-treated WT mice and DKO mice that were analyzed by scRNA-seq and passed quality control. **(E)** The major DEGs among the clusters identified in **(D)**. **(F)** The expression of neutrophil development marker genes in the subclusters. **(G)** WT mice and *Wnt5* DKO mice were treated as in Figure 1. Spleen $CD45^-$ cells were collected and subjected to single-cell RNA sequencing. Expression of *Pdgfrα, Pdgfrβ, Wnt5a, Wnt5b, Wt1, and Myh11* are shown. Results in (**B**-**C**) are shown as means±sem with P values (two-tailed Student's *t*-test). Each datum point represents one mouse. Each independent experiment consists of at least three technical replicates.

# Figure S5. Related to Figure 5

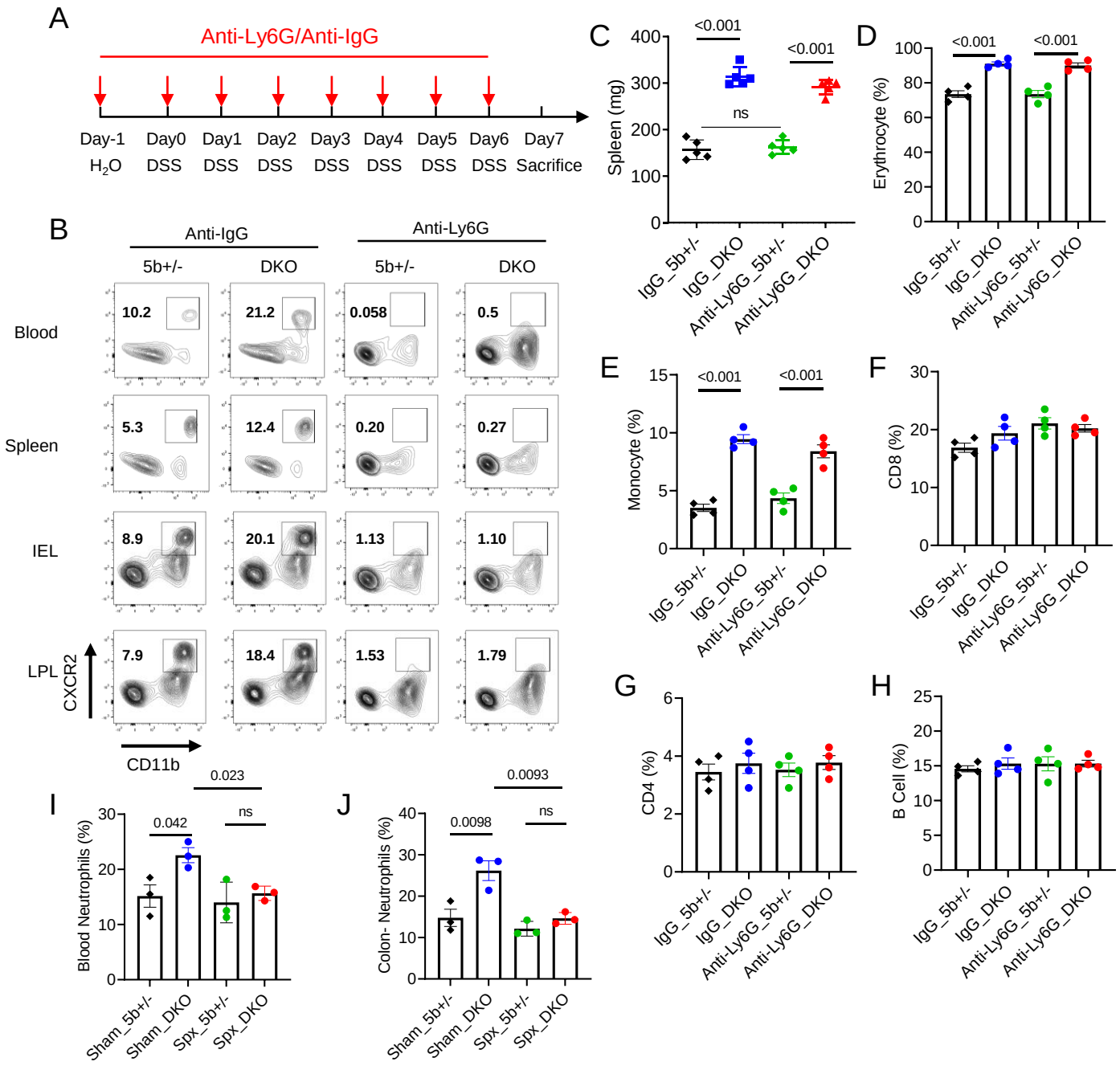

**Figure S5. Colitic phenotypes of *Wnt5* DKO mice depend on neutrophils and spleens. Related to Figure 5.**

**(A)** Schematic representation of the procedure used to deplete neutrophils in the *Wnt5* DKO mice and control mice subjected to DSS-induced colitis. (**B-H**) *Wnt5* DKO mice and the control mice were treated with DSS and subjected to neutrophil depletion as in **A**. Neutrophil depletion efficiency in various tissues is shown in **(B),** and spleen weight is shown in **(C)**. The frequencies of erythrocytes (in $CD45^-$) and monocytes (in $CD45^+$) in the spleen are shown in **(D)** and **(E)**. The frequencies of $CD8^+$ T cells, $CD4^+$ T cells, and B cells (in $CD45^+$) in the colon are shown in **(F-H)**. **(I-J)** *Wnt5* DKO mice and the control mice were subjected to splenectomy before being treated with DSS**.** The frequencies of neutrophils (in $CD45^+$) in the blood and colon are shown in **(I)** and **(J)**. Data in (**C-J**) are presented as means±sem with P values (two-tailed two-way ANOVA). Each datum point represents one mouse. Each independent experiment consists of at least three technical replicates.

# Figure S6. Related to Figure 6

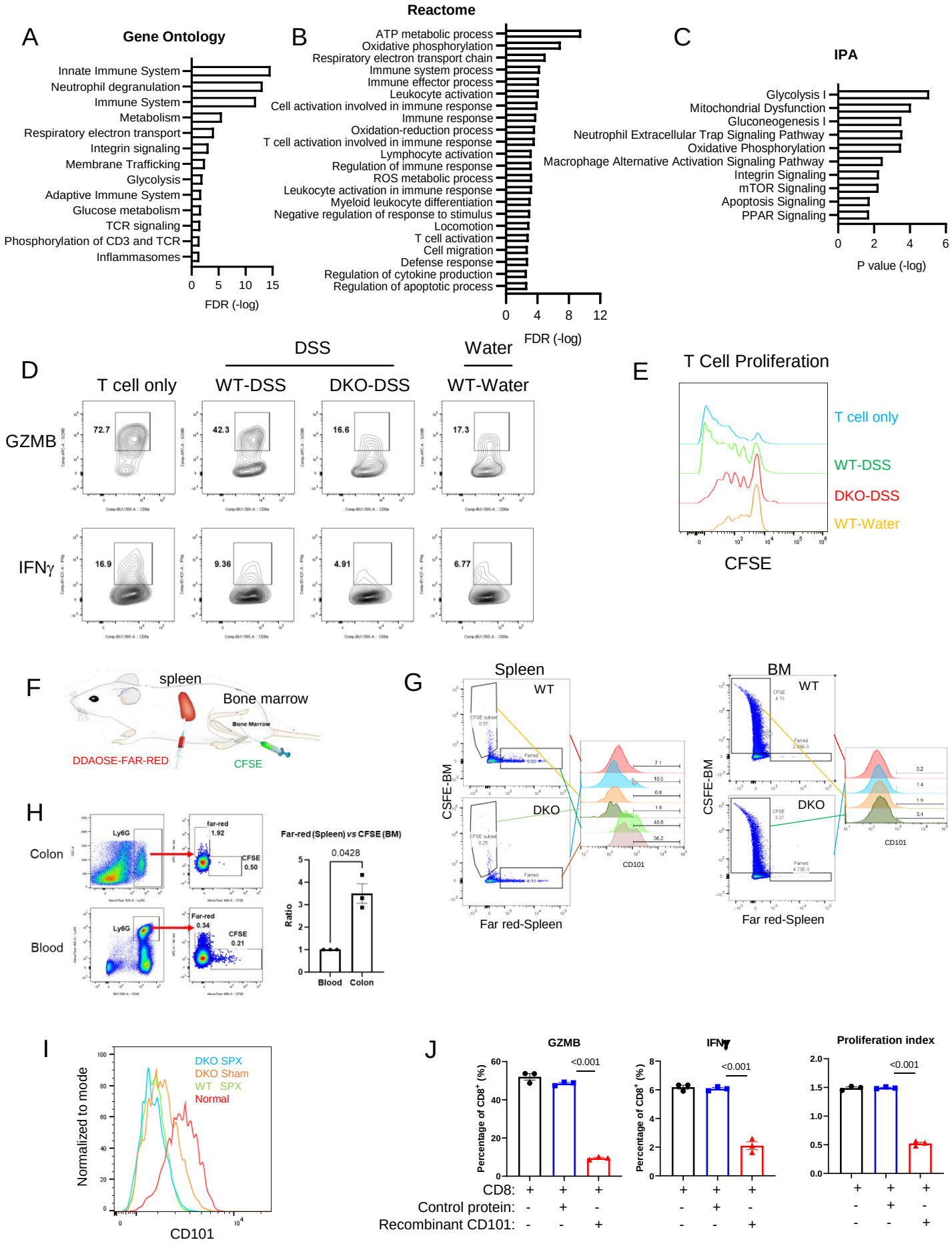

**Figure S6. Colitic *Wnt5* DKO mice produce CD101-high hyper-immunosuppressive neutrophils from the spleen. Related to Figure 6.**

**(A-C)** Gene Ontology, Reactome, and IPA pathway analysis of common DEGs identified from both transcriptomic and proteomic analyses of blood neutrophils. (**D**-**E**) Representative flow cytometry gating and charts for Fig. 6C-E. (**F**). Schematic representation of the cell tracing experiment. (**G**) Cell tracing experiment was performed as in Fig.6J. CD101 expression in dye-labeled BM and spleen cells is shown. (**H**) Cell tracing experiment was performed as in Fig.6J. The infiltration of dye-labeled BM and spleen neutrophils was shown in the inflamed colon. (**I**) WT mice and DKO mice were subjected to splenectomy and sham surgery, and CD101 expression in blood neutrophils was determined by flow cytometry. (**J**) Splenic $CD8^+$ T cells were activated by anti-CD3/CD28 in the presence or absence of the CD101 recombinant protein. GZMB, IFNγ, and T cell expansion were determined by flow cytometry. Figures in (**H**) and (**J**) are presented as means±sem with P values (two-tailed Student's *t*-test). Each independent experiment consists of at least three technical replicates.

# Figure S7. Related to Figure 7

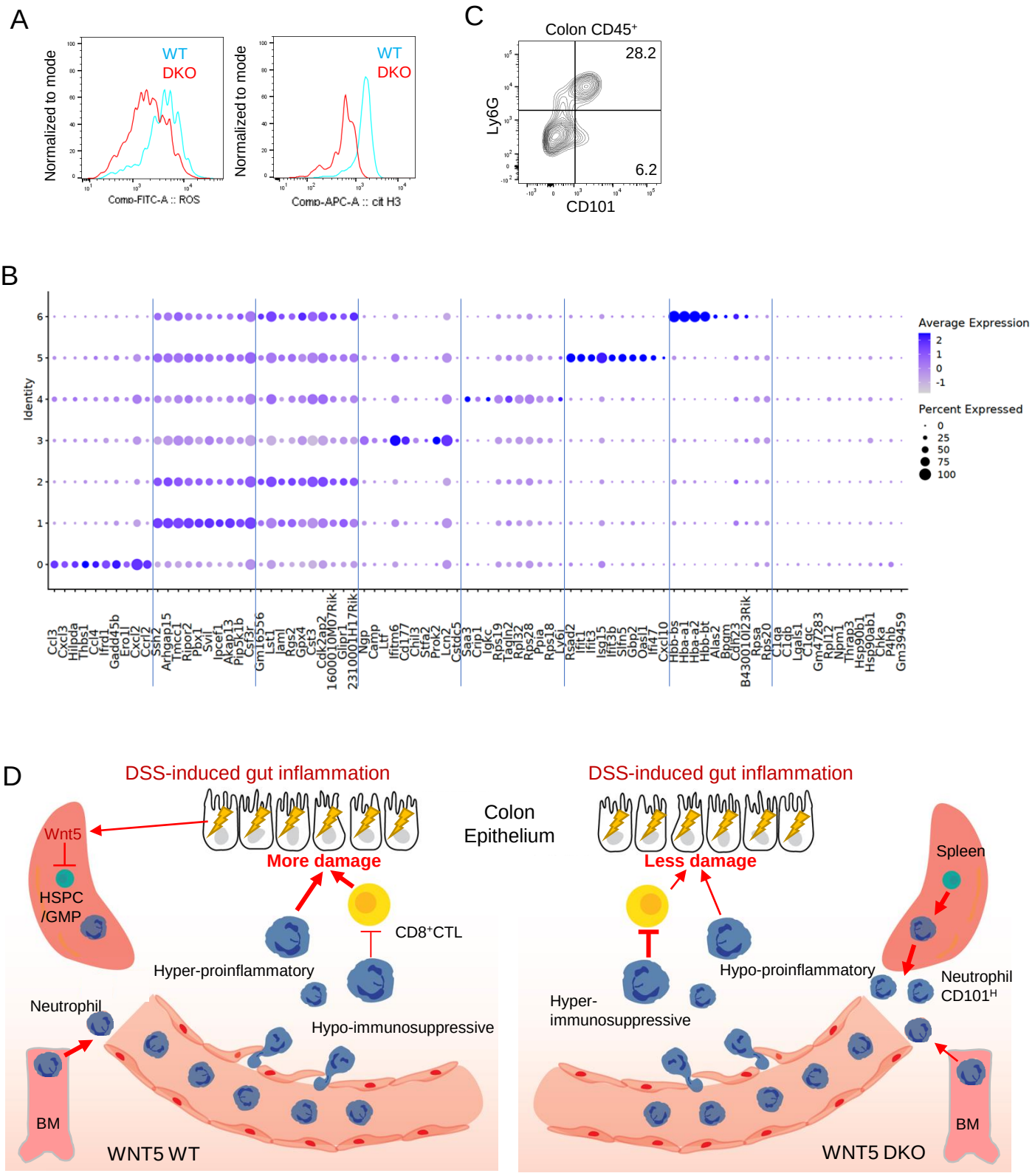

**Figure S7. Transcriptomic profiles of blood neutrophils inform the functions of colon neutrophils. Related to Figure 7.**

(**A**) Representative flow cytometry charts of ROS and citrullinated histone determination for Fig. 7B. (**B**) Signature genes of the subclusters shown in Fig. 7B. (**C**) CD101 expression in $Ly6G^+$ and $Ly6G^-$ cells of DKO-DSS colon $CD45^+$ cells were determined by flow cytometry. (**D**) Graphic summary. The loss of WNT5 proteins in DSS-treated mice leads to splenomegaly, EMH, and generation of neutrophils in spleens with heightened suppressive activity toward cytotoxic $CD8^+$ T cells and reduced pro-inflammatory activities. These splenic neutrophils help to protect mice from DSS-induced colitis. Each independent experiment consists of at least three technical replicates.