Supplemental figure legends

Supplemental Figure 1. Flagellin-mediated LSK cell proliferation is dependent on flagellin receptors. Data shown is a duplicate study of Figure 2B-D. C57BL/6 WT and N4T5-DKO mice were treated with flagellin as in Figure 1. Whole bone marrow cells from these mice were analyzed for LSK cells proliferation by flow cytometry. LSK cell% in BM (A), LSK cell number (B) and BrdU incorporation (C) in WT versus N4T5-DKO mice; Statistics were analyzed by unpaired t-test, error bars depict SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P \leq 0.001$; ns, not significant.

Supplemental Figure 2. Flagellin-induced LSK cell proliferation is independent of liver TLR5. Albumin-Cre-TLR5-floxed mice (labeled as Alb-Cre-TLR5$^{fl/fl}$) and its sibling control (TLR5$^{fl/fl}$) were treated as in Figure 1. Fifteen hours following injection, the mice were labeled with 10 mg BrdU for 1 hour, and then sacrificed. Whole bone marrow cells from these mice were analyzed for LSK cells proliferation by flow cytometry. Representative flow cytometry charts comparing LSK proliferation (A); LSK cell% in BM (B), LSK cell number (C) and BrdU incorporation (D) in control versus albumin-Cre-TLR5-floxed mice are shown. Statistics were analyzed by unpaired t-test, error bars depict SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P \leq 0.001$.

Supplemental Figure 3. **Flagellin activates TLR5 on bone marrow-derived cells to induce LSK proliferation.** WT and TLR5 KO Bone marrow chimeric mice were treated with flagellin as in Figure 1. Whole bone marrow cells from these mice were analyzed for LSK cells proliferation by flow cytometry for LSK cell% in BM (A) and LSK cell number (B). Statistics were analyzed by unpaired t-test, error bars depict SEM. ***, $P \leq 0.001$; ns, not significant.

Supplemental Figure 4. Flagellin activates its receptors to induce LSK cell proliferation ex vivo. Whole bone marrow cells from C57BL/6 WT or N4T5-DKO mice were incubated in complete RPMI with or without 50 ng/mL of flagellin for 15 hours. The cells were then labeled with 10 μM BrdU for 1 hour and analyzed by flow cytometry. LSK cell% in BM (A), LSK cell number (B) and BrdU incorporation (C) in PBS-treated versus flagellin-treated BM cells are shown. Statistics were analyzed by unpaired t-test, error bars depict SEM. ***, $P \leq 0.001$.

Supplemental Figure 5. **Flagellin indirectly activates LSK proliferation in bone marrow.** WT and N4T5-DKO bone marrow chimeric mice were treated with flagellin or PBS as in Fig 1. Whole bone marrow cells from these mice were analyzed for LSK cell% in BM (A) and LSK cell number (B) by flow cytometry. Statistics were analyzed by unpaired t-test, error bars depict SEM. **, $P < 0.01$; ***, $P \leq 0.001$.

Supplemental Figure 6. Mouse bone marrow neutrophils express TLR5. Flow cytometry analysis of mouse bone marrow cells, showing the expression of TLR5 on neutrophils ($CD11b^+$ $Ly\text{-}6G^+$) from WT B6 mice but not TLR5 KO B6 mice.

Supplemental Figure 7. Flagellin treatment does not affect LT-HSC proliferation. Bone marrow cells from C57BL/6 mice were treated with flagellin for 15 hours and analyzed by flow cytometry using antibodies including SLAM code CD48 and CD150 to determine LT-HSC proliferation. Representative flow cytometry charts for LT-HSC proliferation (A), LT-HSC cell

number (B) and BrdU incorporation (C) are shown. Statistics were analyzed by unpaired t-test, error bars depict SEM. ***, $P \leq 0.001$; ns, not significant.

Supplemental Figure 8. Flagellin-activated whole bone marrow cells do not show an increased repopulation ability. Mouse whole bone marrow cell repopulation assay were performed as described in methods. $2x10^5$ whole bone marrow cells (competitor cells) from $CD45.2^+$ WT B6 mice and $1x10^5$ or $1x10^6$ of whole bone marrow cells from PBS or flagellin-treated $CD45.1^+$ WT B6 mice (test donor cells) were co-transferred to recipient mice. Recipient mice were housed under sterile conditions with sterile food and sterile water containing 2 mg/mL of neomycin. After 4 weeks, whole blood of these mice was analyzed for peripheral repopulation. Repopulation Units (RU) for test bone marrow cells (A) and RU calculated based on myeloid and lymphoid cell repopulation (B) are shown. Statistics were analyzed by unpaired t-test, error bars depict SEM. *, $P < 0.05$; ns, not significant.

Supplemental Figure 9. Flagellin increases production of neutrophils in blood. C57BL/6 mice were treated with PBS or PBS containing 20 μg Flagellin for 15 hours. 200 μl of whole blood cells was analyzed by flow cytometry for neutrophils using antibodies to CD45, CD11b and Ly-6G.

Supplemental Figure 10. MPP3 cells from naïve or flagellin-treated mice showed equal ability to differentiate into myeloid cells and produce neutrophils in blood. Two thousand MPP3 cells were FACS-sorted from whole bone marrow cells of naïve or flagellin-treated $CD45.2^+$ mice and transferred to irradiated $CD45.1^+$ C57BL/6 mice along with $4x10^5$ whole bone

marrow cells from CD45.1$^+$ mice. Whole blood cells of recipient mice were analyzed at indicated time points by flow cytometry using antibody to CD45.1, CD11b and Ly6G to show peripheral blood cells differentiated from MPP3. Number of MPP3-originated myeloid cells (CD45.1$^-$CD11b$^+$) (A) and MPP3-originated neutrophils (CD45.1$^-$CD11b$^+$Ly6G$^+$) (B) per mL of blood are shown. Statistics were analyzed by unpaired t-test, error bars depict SEM. ns, not significant.

Supplemental Figure 11. Number of Donor MPP3 cells from flagellin-treated mice correlates to MPP3-originated neutrophil production in recipient mice. Different numbers of MPP3 cells (1000, 2000 and 4000) were FACS-sorted from whole bone marrow cells of flagellin-treated CD45.2$^+$ B6 mice and transferred to irradiated CD45.1$^+$ B6 mice along with 4x10^5 whole bone marrow cells from CD45.1$^+$ B6 mice. Whole blood cells of recipient mice were analyzed at 14 days post cell transfer by flow cytometry using antibodies to CD45.1, CD11b and Ly6G to show peripheral blood cell differentiated from MPP3. Average number of MPP3-originated neutrophils (CD45.1$^-$CD11b$^+$Ly6G$^+$) per mL of blood are shown, N=4.

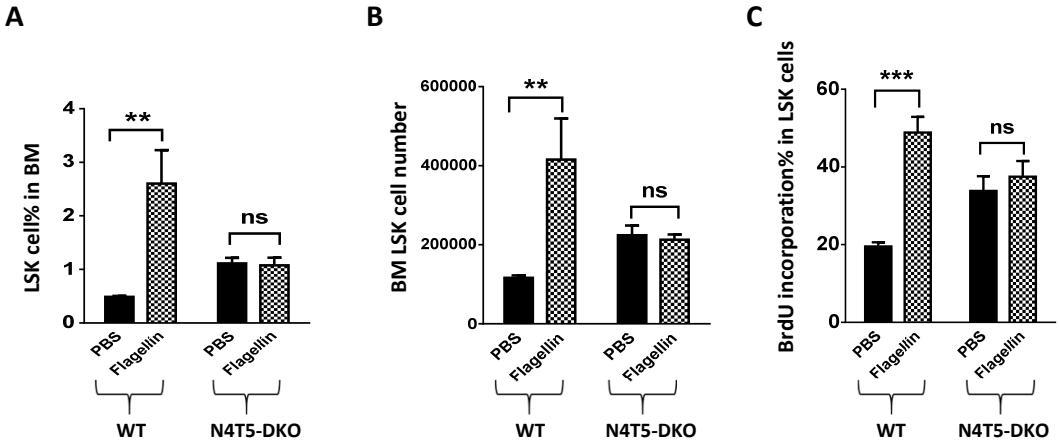
Supplemental Figure 12. Flagellin-induced MPP3 cells significantly increase Swiss Webster mice survival after bone marrow transplantation. Five thousand MPP3 cells were FACS-sorted from whole bone marrow cells from flagellin-treated mice and transferred to irradiated mice along with 1x10^5 whole bone marrow cells from the same species. In parallel, an additional group of mice was irradiated and given whole bone marrow cells only. After bone marrow transplantation, mice were housed under sterile conditions and survival was observed for 30 days. Both donor and recipients were Swiss-Webster mice.

Supplemental Figure 13. Mouse bone marrow MPP3 cells do not express TLR5. Flow cytometry analysis of mouse bone marrow cells, showed no expression of TLR5 on MPP3 cells ($CD34^{+}Flt3^{-}CD48^{+}$ $CD150^{-}$ LSK cells) from mice.

Supplemental Figure 14. Flagellin treatment induced LSK cells proliferation is independent of G-CSF. Whole bone marrow cells of C57BL/6 mice were incubated in complete RPMI in the presence of 50 ng/mL of flagellin, and with or without 4 μg/mL of anti-G-CSF for 15 hours. Cells were pelleted and analyzed by flow cytometry to determine LSK cell% in BM (A) and LSK cell number (B). Statistics were analyzed by unpaired t-test, error bars depict SEM. ***, $P \leq 0.001$.

Supplemental Figure 15. **Flagellin treatment induced LSK cells proliferation is independent of IL-22.** Eight-week-old female IL-22 KO mice were treated with PBS only or with PBS containing 20 μg of flagellin by i.p. injection. Fifteen hours following injection, the mice were labeled with 10 mg BrdU for 1 hour, and then sacrificed. Whole bone marrow cells were prepared and analyzed by flow cytometry. Representative flow cytometry plots (A), LSK cell% in BM (B), LSK cell number (C) and BrdU incorporation (D) are shown. Statistics were analyzed by unpaired t-test, error bars depict SEM. **, $P < 0.01$; ***, $P \leq 0.001$.

Supplemental Figure 1



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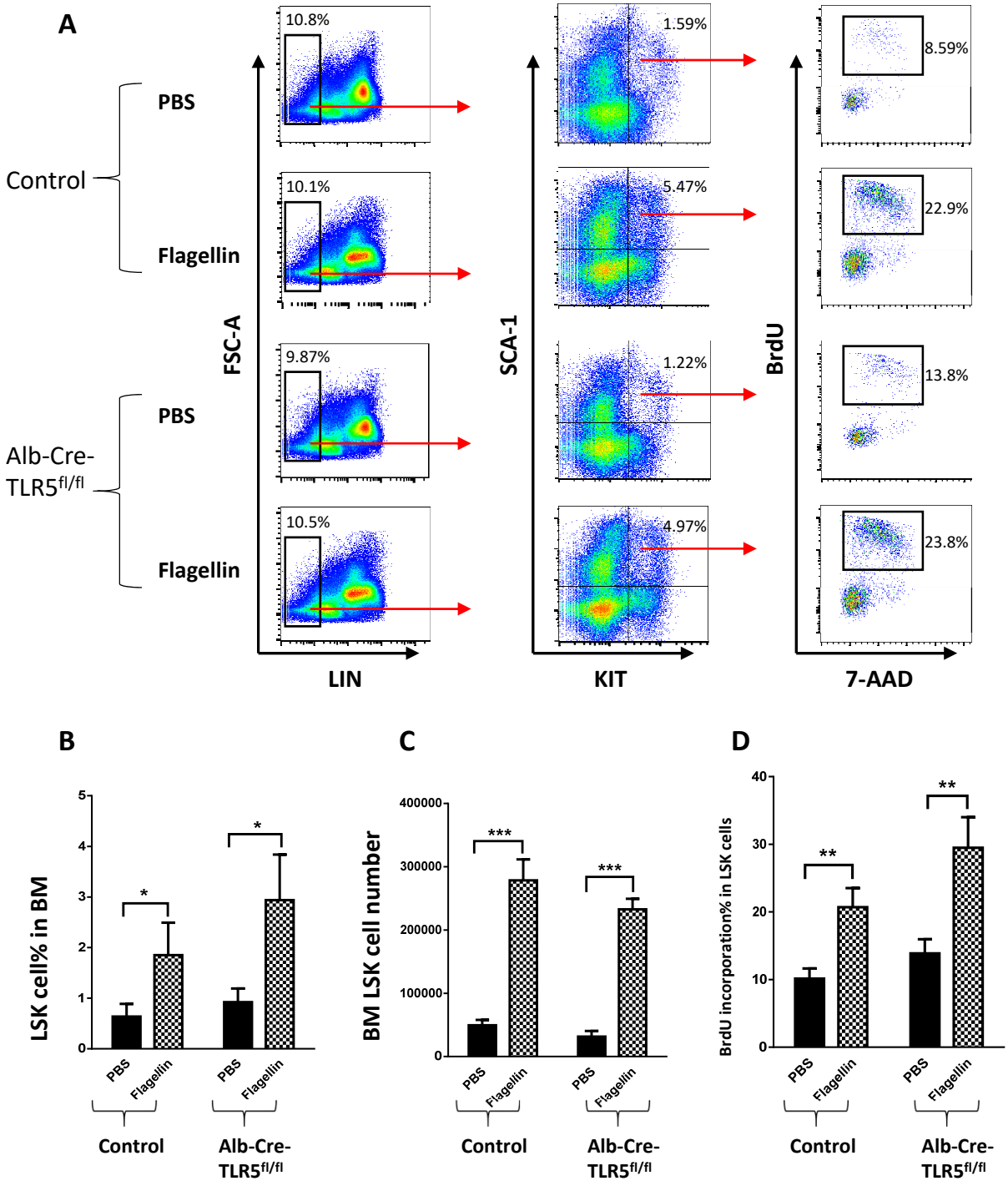

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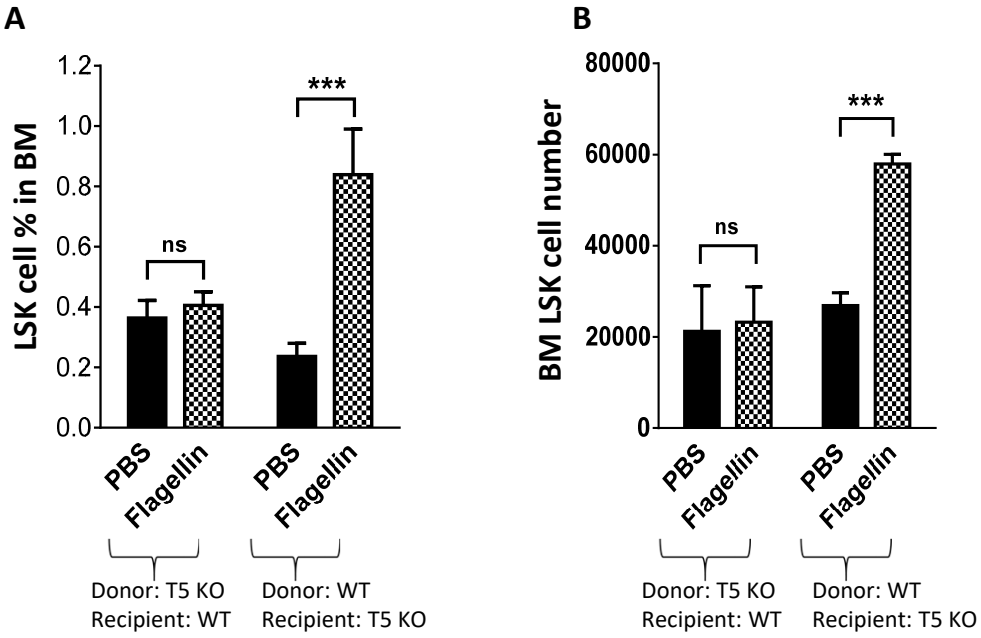

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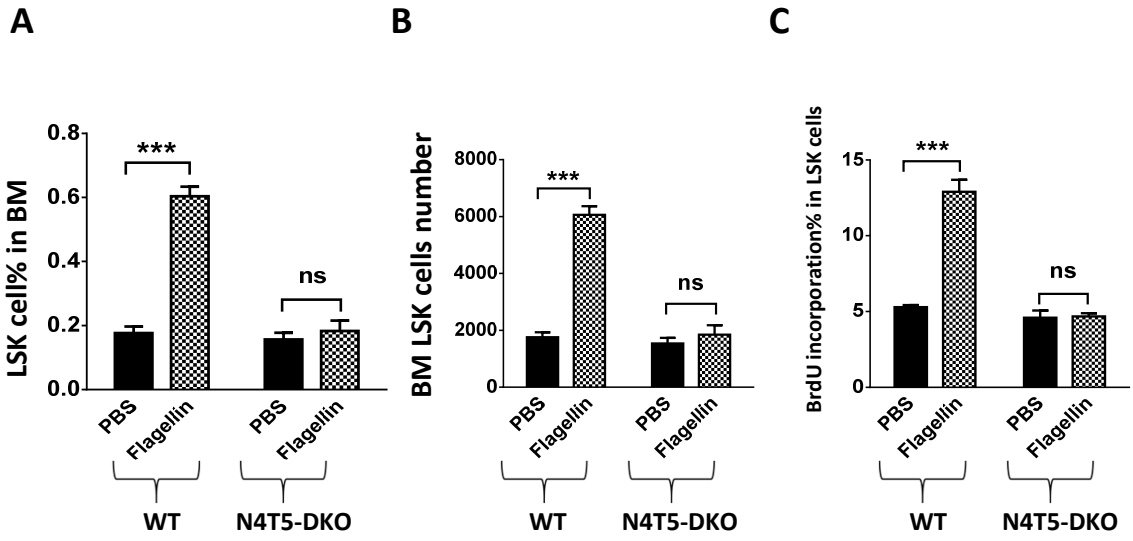
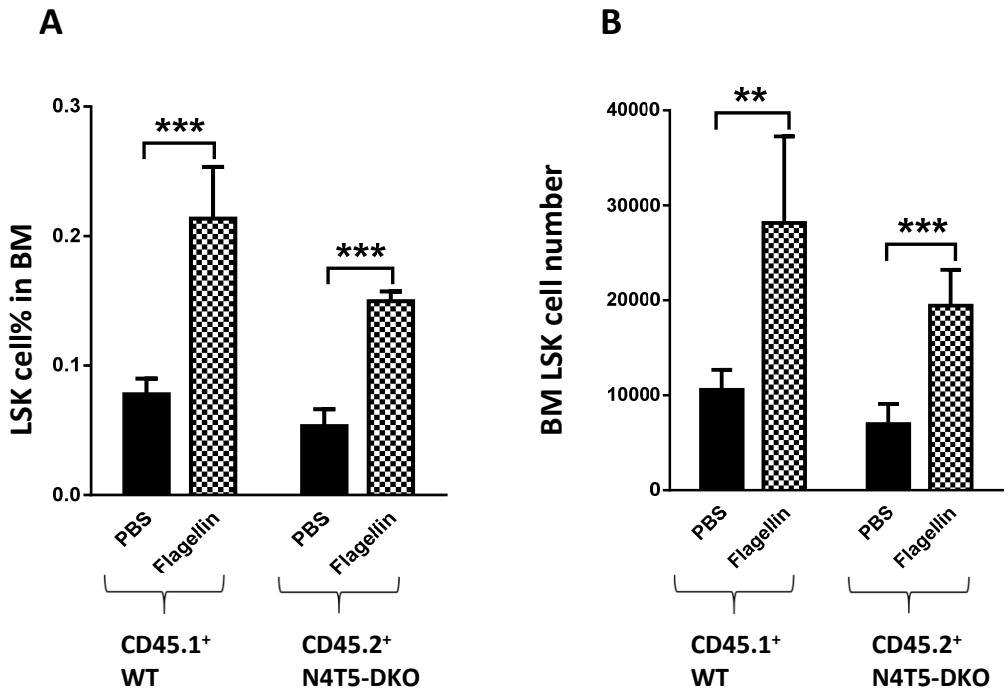


Supplemental Figure 5

Donor cells: 50% CD45.1^{+} WT BM / 50% CD45.2^{+} N4T5-DKO BM

Recipient: CD45.2^{+} N4T5-DKO mice



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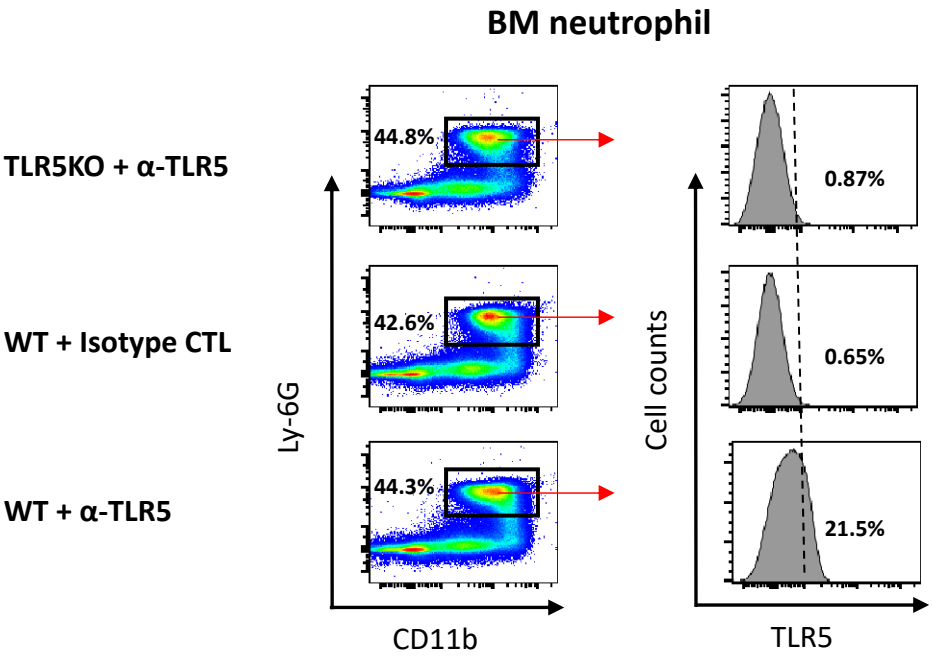

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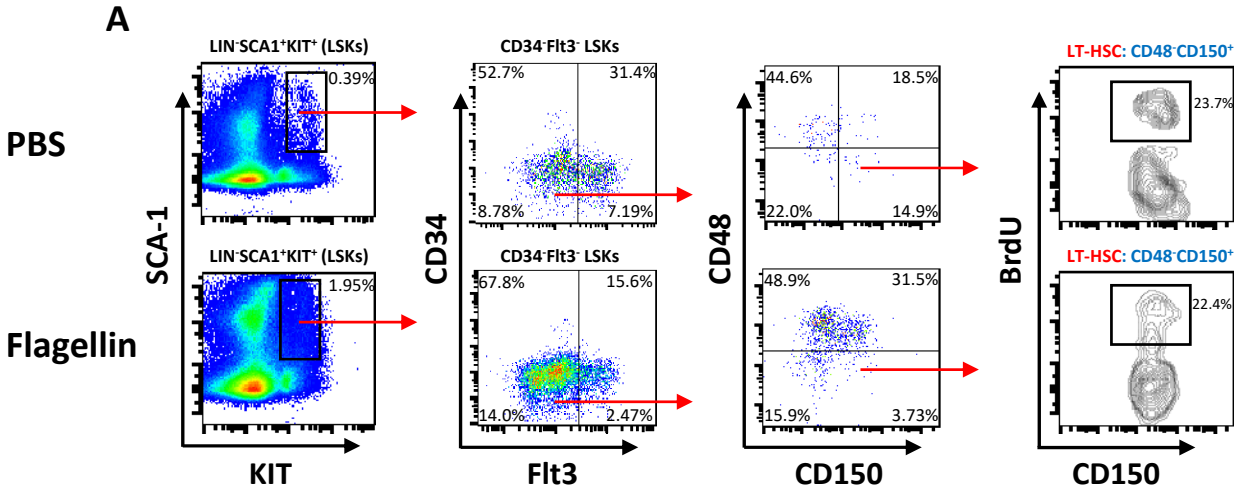
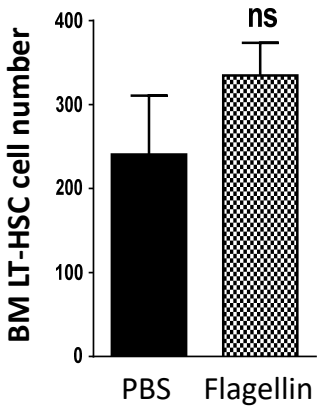
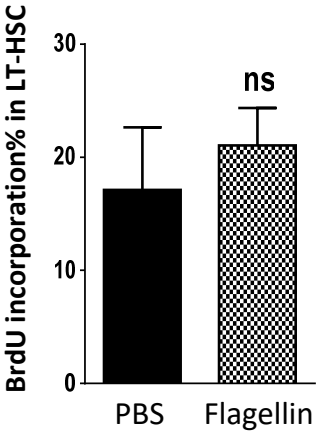
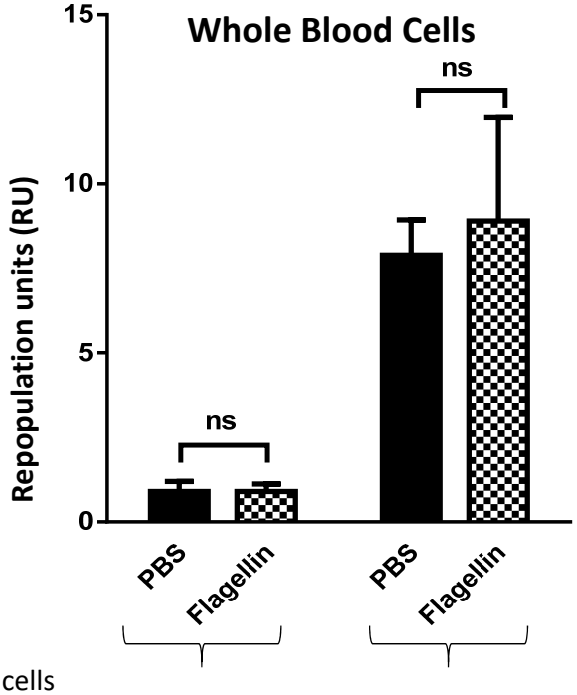






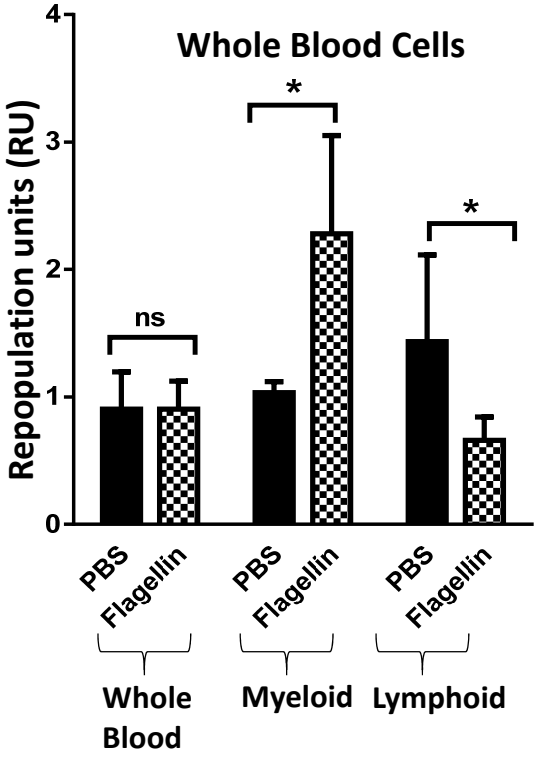
Supplemental Figure 8

A



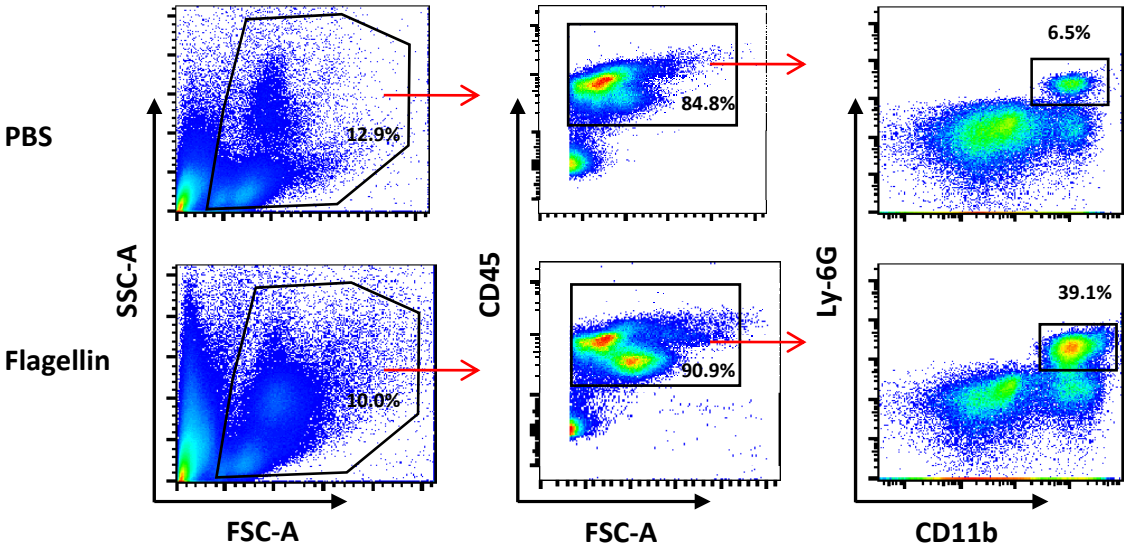
Test donor cells (CD45.1$^+$):	$1x10^5$	$1x10^6$
Competitor cells (CD45.2$^+$):	$2x10^5$	$2x10^5$

B



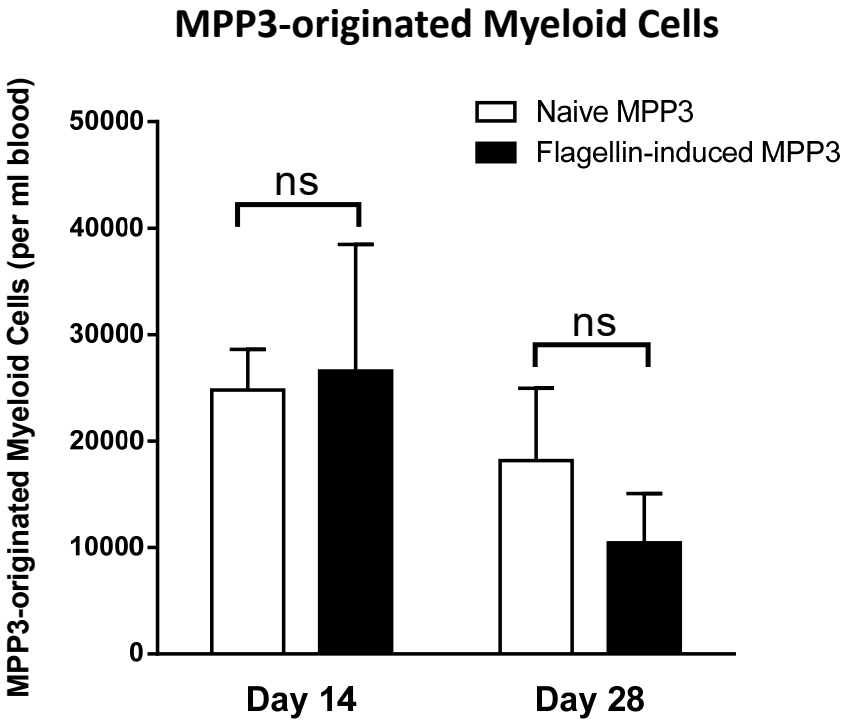
Test donor cells (CD45.1$^+$):	$1x10^5$
Competitor cells (CD45.2$^+$):	$2x10^5$

Supplemental Figure 9



Supplemental Figure 10

A



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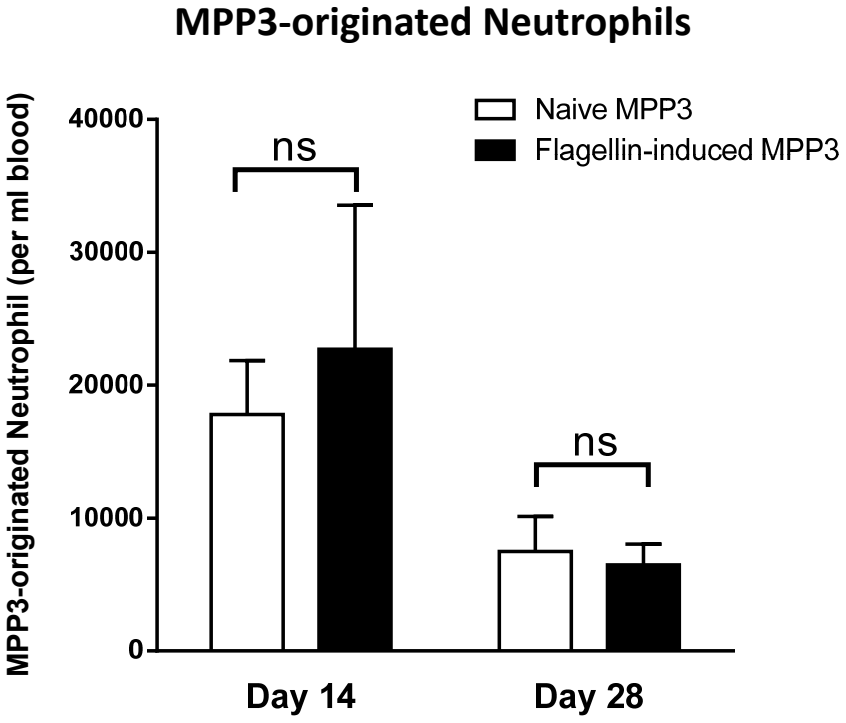

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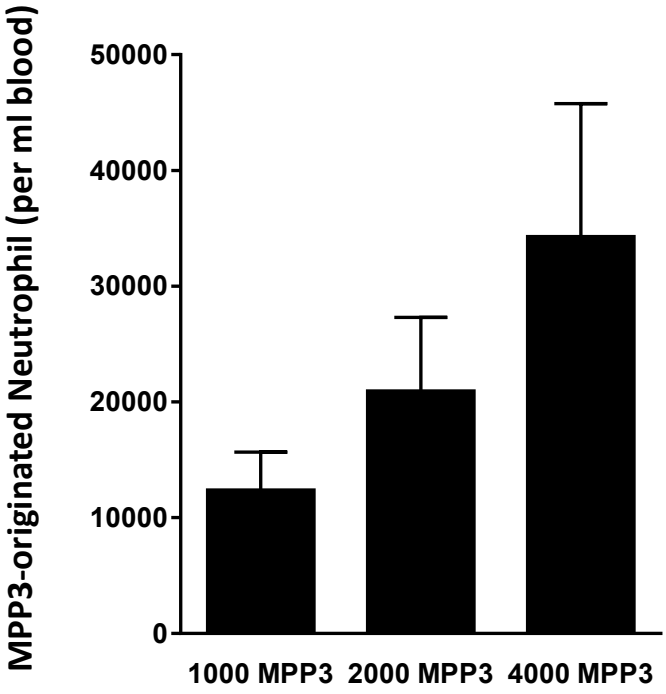

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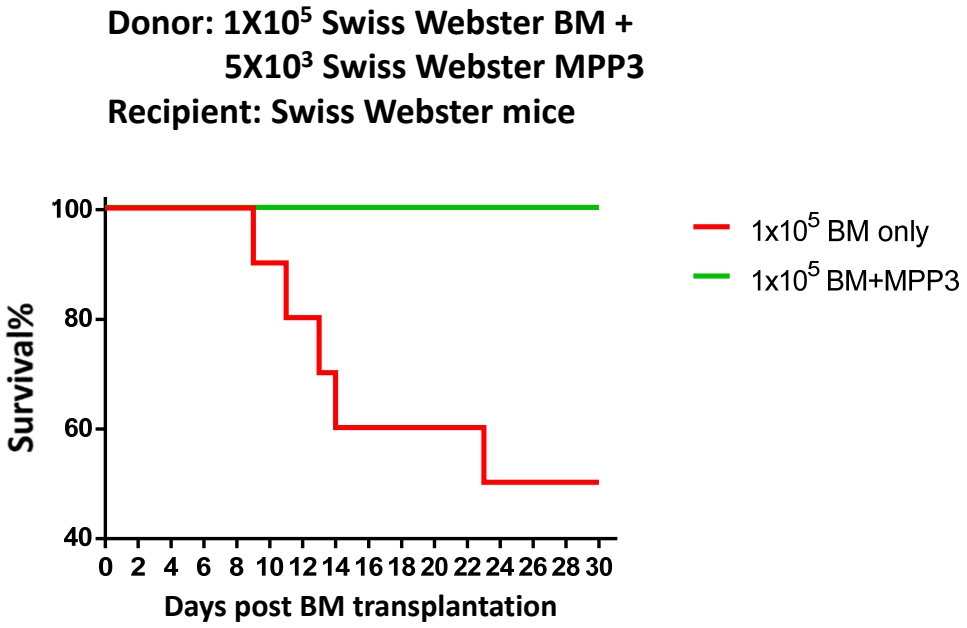

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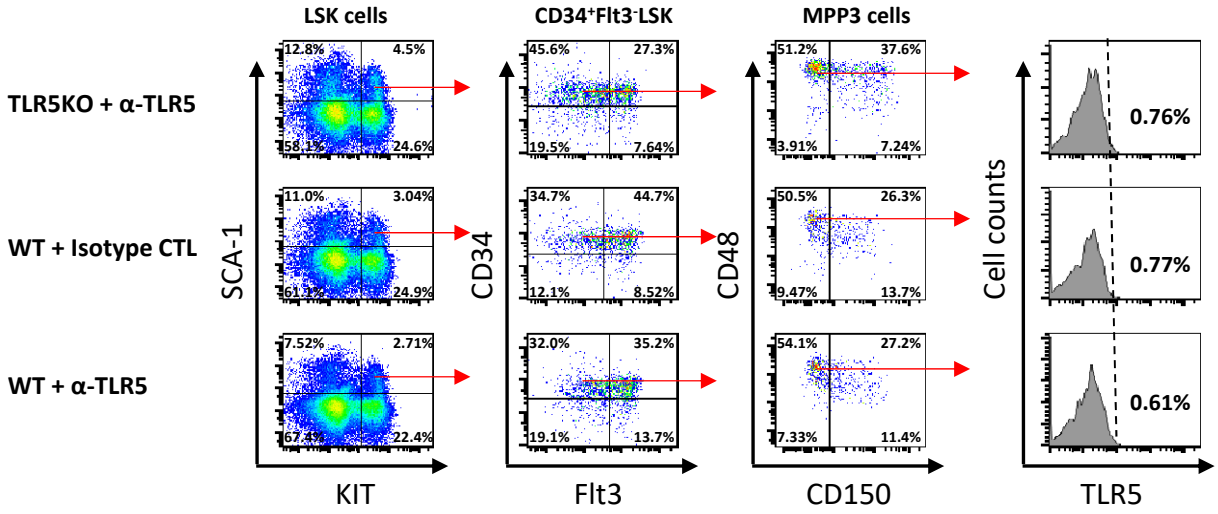

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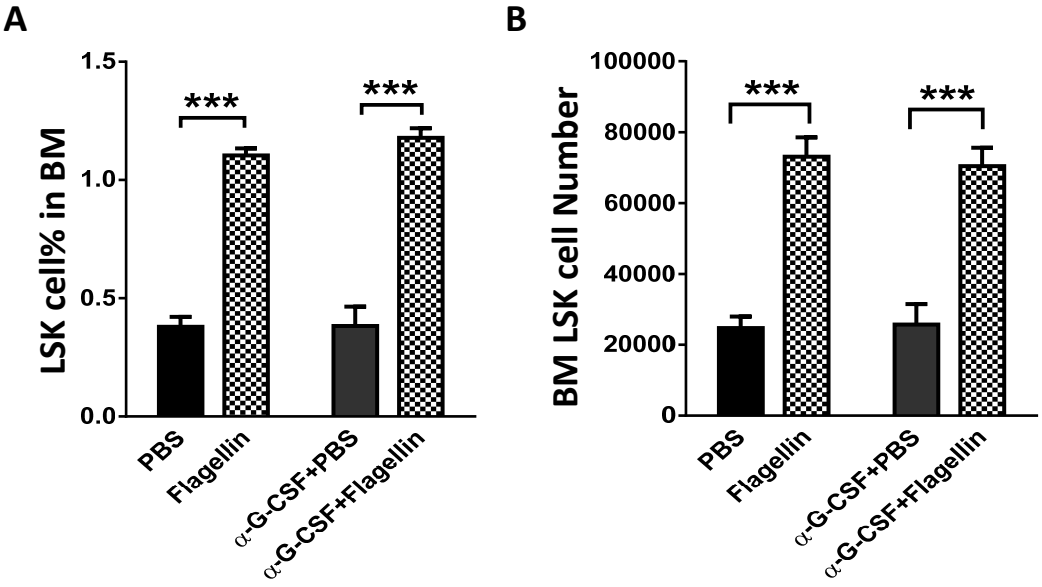

Supplemental Figure 15