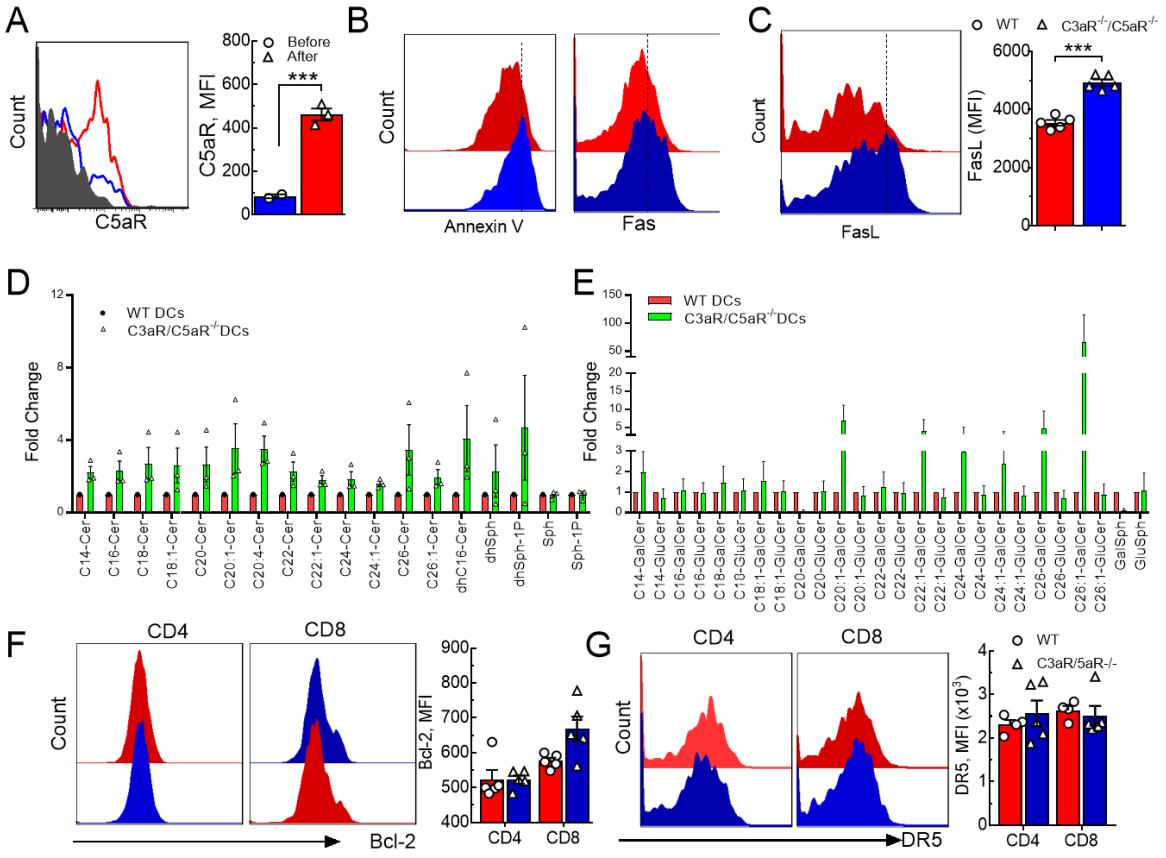


731

732 **Fig. S1. (A-E) Deficiency of C3aR/C5aR increases ceramide-triggered mitophagy in recipient DCs after HCT. (A)** WT BALB/c
733 mice were lethally irradiated at 700 cGy (*n=3*). Representative histograms for the C5aR expressed on MHCII^{+}CD11c^{+} DCs 24hr after
734 TBI. **(B and C)** WT and C3aR$^{-/-}$/C5aR$^{-/-}$ mice (*n=5*) were lethally irradiated at 700 cGy. Representative histograms or summary bar
735 graph for the expression of annexin V and Fas **(B)** are shown. (**D** and **E**) Irradiated BM derived-DCs were matured with 20 ng/mL
736 LPS. The relative ratios of amounts of sphingosine ceramide (**D**) and glucosyl/galactosyl ceramide (**E**) in DCs differentiated from
737 bone marrow of WT and C3aR$^{-/-}$/C5aR$^{-/-}$ mice (*n=3*) are shown. (**F** and **G**) Lethally irradiated WT or C3aR$^{-/-}$/C5aR$^{-/-}$ recipients (*n=5*)
738 were transplanted with CFSE-labelled T-cells. The splenic cells were analyzed 4d after cell transfer. Representative flow cytometry
739 histogram and summary bar graphs for the expression of FasL of recipient DCs (**C**); and bcl-2 (**F**) and DR-5 (**G**) of donor H2^{b+} CD4^{+}
740 or CD8^{+} Tc are shown. Unpaired two-tail *t*-test was used to compare between groups. Data are representative of two independent
741 experiments and presented as mean ± S.E.M., and *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

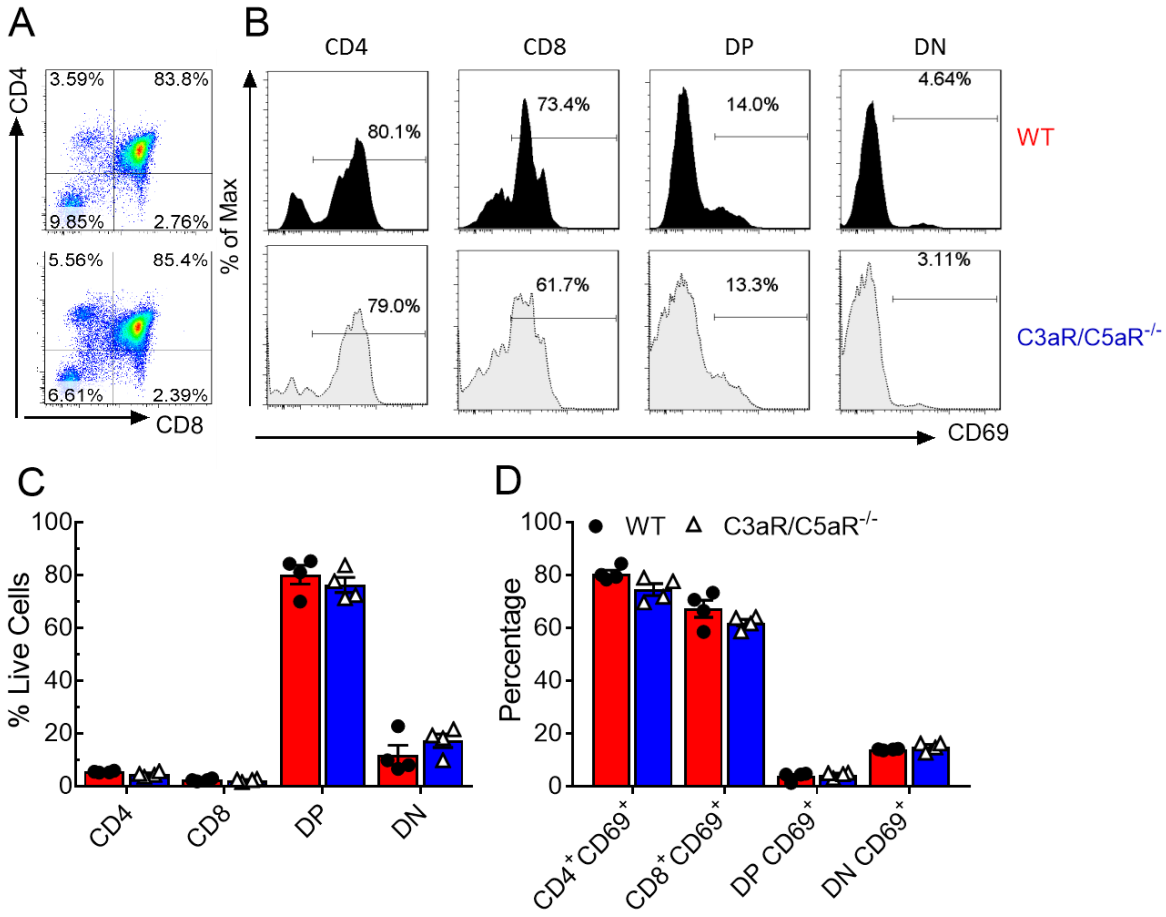


Fig. S2. C3aR/C5aR deficiency does not affect T-cells development and maturation in thymus. WT or C3aR$^{-/-}$/C5aR$^{-/-}$ BALB/c mice (*n=4*) were euthanized and thymus were isolated and analyzed by flow cytometry. Flow color contour plots present the frequencies of CD4^{+}, CD8^{+}, CD4^{+}CD8^{+} (DP), CD4^{-}CD8^{-} (DN). Flow histograms illustrate the fluorescence intensity of CD69^{+}CD4^{+}, CD69^{+}CD8^{+}, DP and DN cells in the thymus. The result is representative of two independent experiments. Unpaired two-tail *t*-test was used to compare between groups.

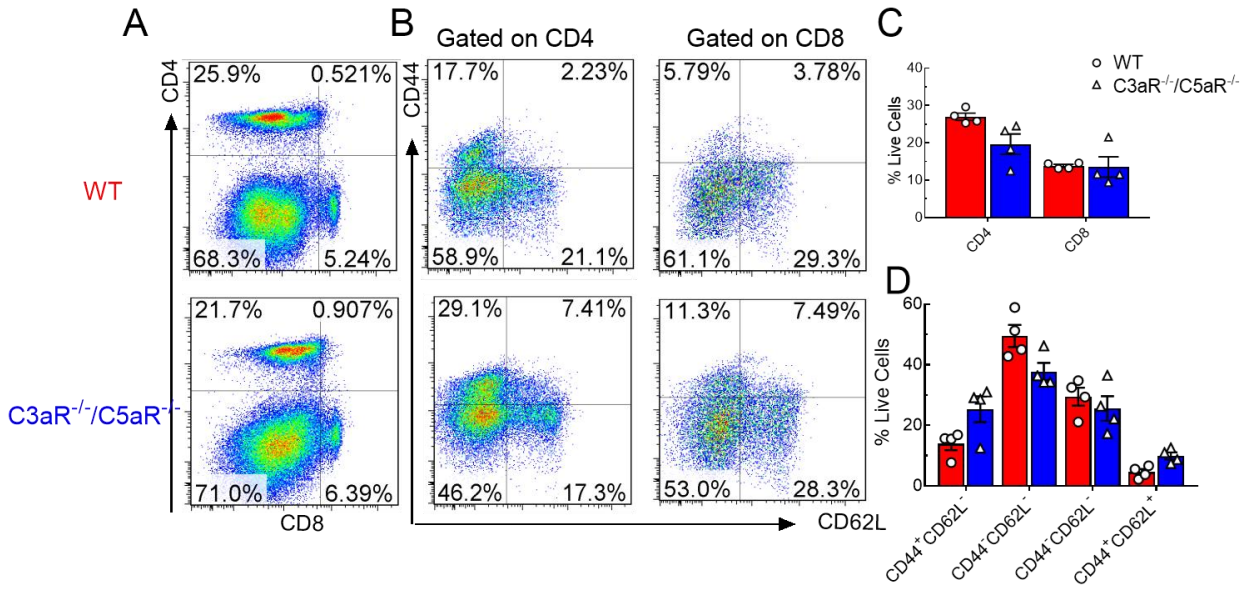


Fig. S3. C3aR/C5aR deficiency does not affect mature T-cells in spleen. WT or C3aR$^{-/-}$/C5aR$^{-/-}$ BALB/c mice (n=4) were euthanized and spleen were isolated and analyzed by flow cytometry. Flow color contour plots present the frequencies and of CD4^{+} or CD8^{+} in the spleen (A); memory effector phenotype CD44^{+}CD62L^{+} gated on splenic CD4^{+} or CD8^{+} T-cells (B), the bar graphs summarize the frequencies of CD4^{+} or CD8^{+} (C) and memory/effector phenotype of splenic lymphocytes (D). The result is representative of two independent experiments. Unpaired two-tail *t*-test was used to compare between groups.

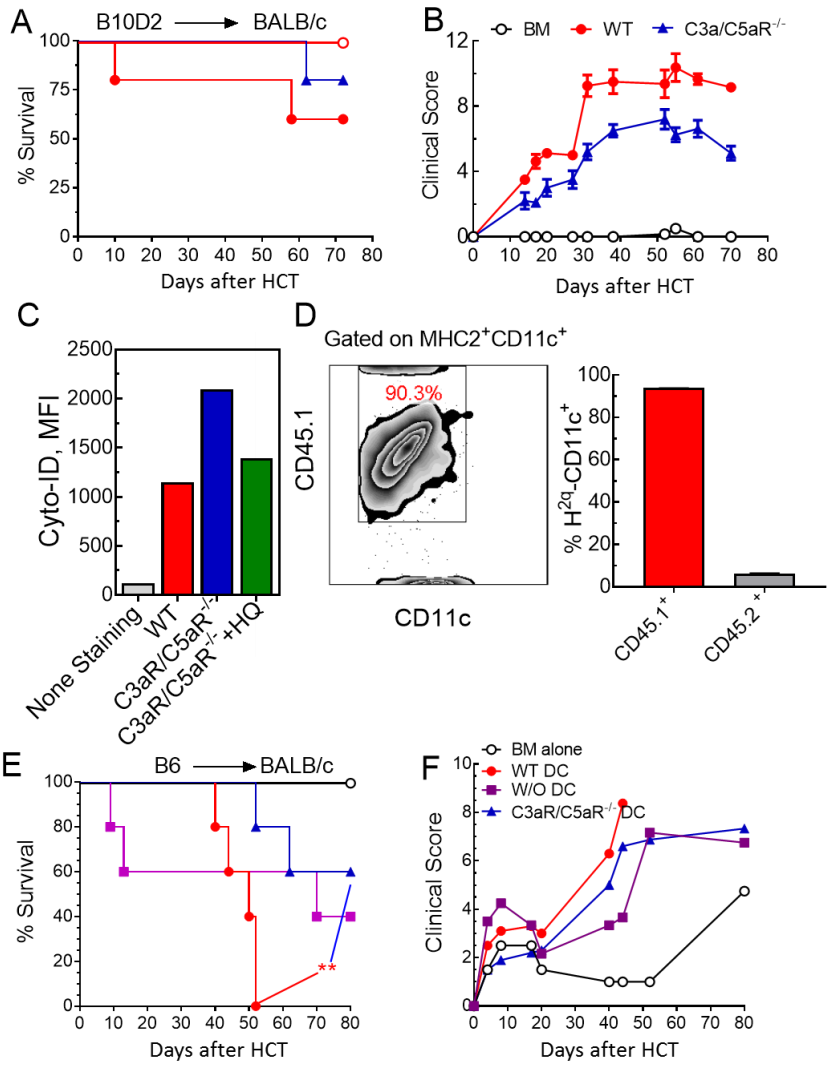


Fig. S4. (A-E) Deficiency C3aR$^{-/-}$/C5aR$^{-/-}$ in recipient increases mitophagy in host DCs and reduces GVHD development. (**A-B**) Lethally irradiated BALB/c mice (*n=6-7*) were transplanted with BM alone (5×10^6/mouse) or plus splenocytes (5×10^6/mouse) from B10.D2 donor mice. The survival (**A**) and GVHD clinical score (**B**) were monitored throughout experiment (**C**) Irradiated BM derived-DCs were stimulated with 20 ng/mL LPS and are stained with Cyto-ID green. The summary bar for mean of fluorescence of cyto-ID expression in WT, C3aR$^{-/-}$/C5aR$^{-/-}$ and HQ-treated C3aR$^{-/-}$/C5aR$^{-/-}$ DCs are shown. (**D**) Lethally irradiated B6 recipients were transplanted with BM alone (3×10^6/mouse) or plus purified T-cells (1×10^6/mouse) from FVB donors. Recipients also received 2x10^6 WT or C3aR$^{-/-}$/C5aR$^{-/-}$ B6 BM-DCs. The representative zebra color and the summary bar for percentage of co-transplanted DCs (CD45.1$^+$CD11c$^+$) in transplanted recipient peripheral blood are shown. (**E**) Lethally irradiated BALB/c recipients (*n=5*) were transplanted with BM alone (3×10^6/mouse) or plus purified T-cells (1×10^6/mouse) from B6 donors. Recipients also received 2x10^6 WT or C3aR$^{-/-}$/C5aR$^{-/-}$ BALB/c BM-DCs. The survival (**E**) and clinical score (**F**) were monitored throughout the experiment. Wilcoxon rank-sum test (A,E) and nonparametric Mann-Whitne U test (B,F) were used to compare between groups. Data are representative of two independent experiments and presented as mean ± S.E.M., and **p < 0.05, **p < 0.01, ***p < 0.001.*

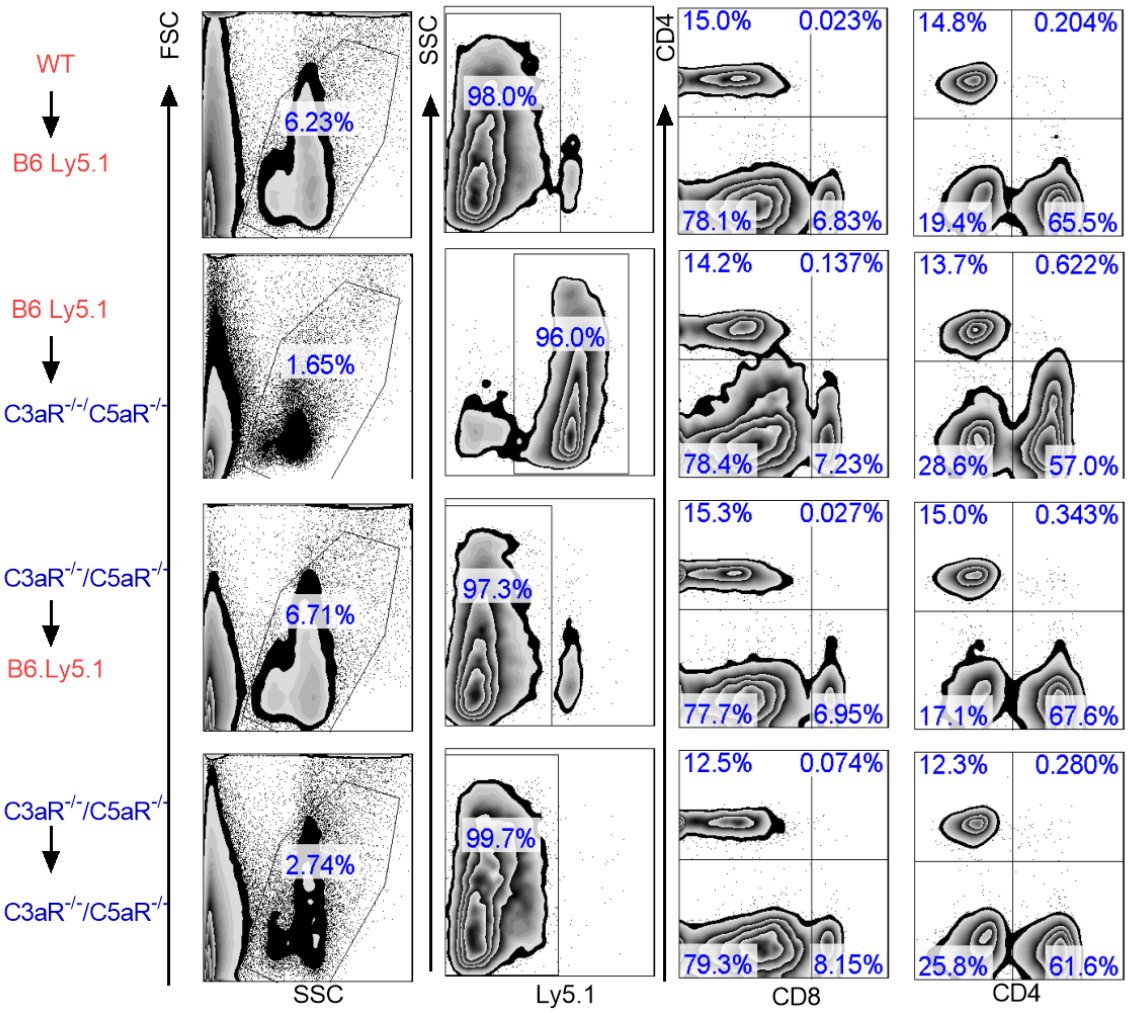


Fig. S5. Generation of BM chimeras. Lethally irradiated WT or C3aR$^{-/-}$/C5aR$^{-/-}$ B6 mice (*n=10*) were reconstituted with TCD-BM from WT or C3aR/C5aR donor. In order to distinguish donor vs. host, B6 Ly5.1 congnic mice were used either as donor or recipients when possible. After three months, the peripheral blood was collected for the analysis of donor cell reconstitution reflecting by expression of Ly5.1, CD4 and CD8. The representative flow cytometric zebra blots are shown.

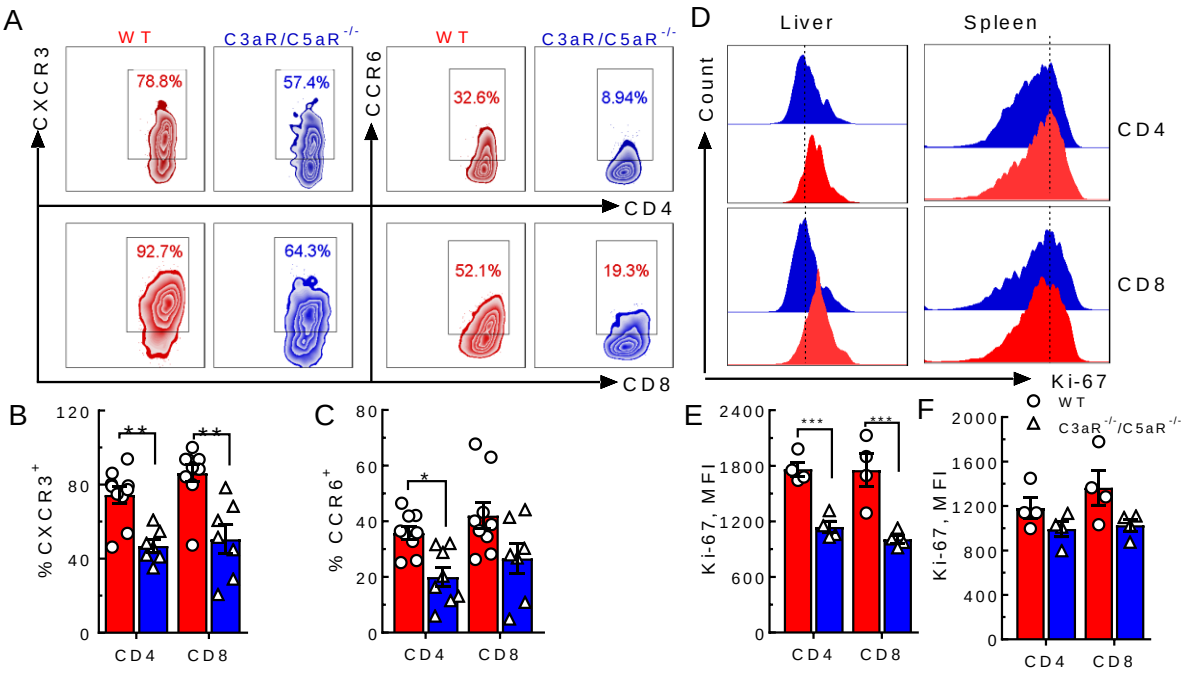
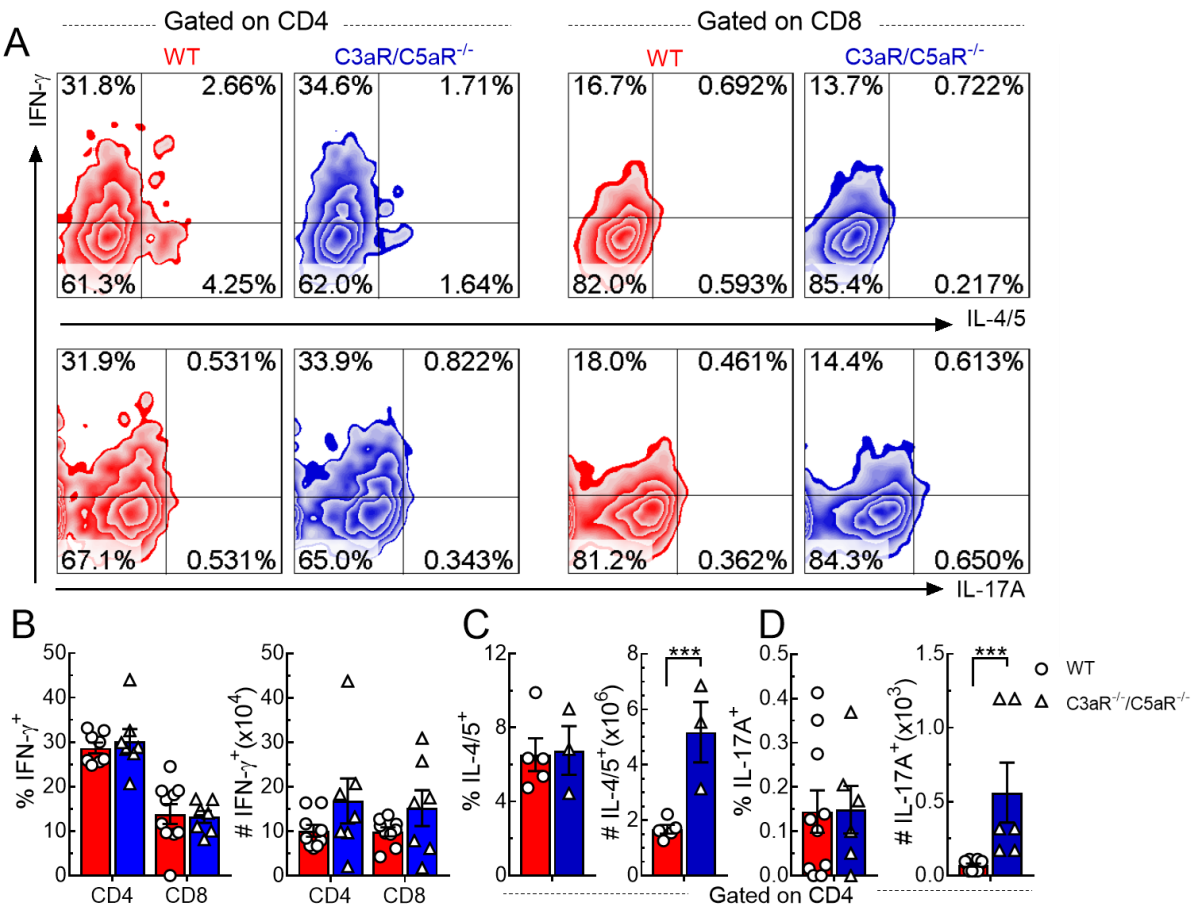


Fig. S6. Host C3aR/C5aR deficiency decreases the migration of donor T-cells toward target organs. HCT was performed as in **Fig. 4**. Recipient spleen and liver cells were isolated 14 post-transplant and stained for CD4, CD8, $H2^b$, CXCR3, and CCR6. Representative zebra plots of $CXCR3^+$ and $CCR6^+$ lymphocytes in the spleens (**A**). Graphs summarize frequencies of $CXCR3^+$ (**B**)/$CCR6^+$ (**C**) donor lymphocytes (*n=8*). Representative histogram and summary graphs for MFI of ki-67 on $H2^{b+}CD4^+/CD8^+$ in the liver (**D**, **E**) and spleen (**D**, **F**) (n=4). The result is combined of two independent experiments. Unpaired two-tail *t*-test was used to compare between groups. Data are presented as mean ± S.E.M. and *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



807

808 **Fig. S7. Recipient C3aR/C5aR is required for Th1 differentiation while suppressing Treg**
809 **generation after HCT.** HCT was performed as described in Fig. 5 A and B. On day 14 after
810 transplantation, recipient spleen cells were stained with $H2^b$, live/death, CD4, CD8 for surface
811 and intracellularly with IFN-γ, IL-4/5, IL-17A, and Foxp3. The flow zebra plots of IFN-γ^+, IL-$4/5^+$,
812 IL-17^+ donor cells from liver (**A**) are displayed. Bar graphs summarize the percentage and
813 absolute number of $H2^{b+}$IFN-γ^+ (**B**) (*n=8*), $H2^{b+}$IL-$4/5^+$ (**C**) (*n=3-4*), and $H2^{b+}$IL-$17A^+$ (**D**) (*n=8*)
814 cells gated on CD4. Data were combined from two independent experiments. Unpaired two-tail
815 *t*-test was used to compare between groups**.** Results are presented as mean ± S.E.M. and *$p <$
816 *0.05, **$p < 0.01$, ***$p < 0.001$.*

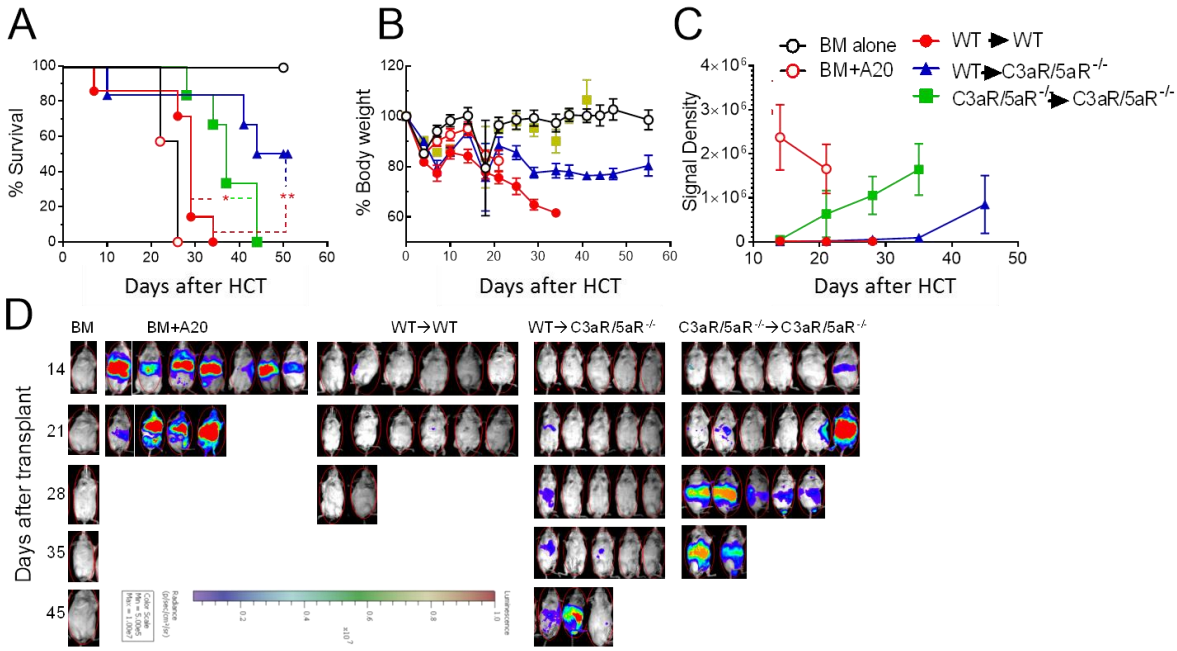


Fig. S8. Recipient C3aR/C5aR is essential for optimal GVL activity. Lethally WT or C3aR$^{-/-}$/C5aR$^{-/-}$ BALB/c recipients (n=7-10) were transplanted with TCD-BM (5×10^6/mouse) with or without purified T cells from WT or C3aR$^{-/-}$/C5aR$^{-/-}$ B6 donors (1.0×10^6/mouse). In addition, recipients received 2×10^3 A20-luc at the time of transplant. Recipients were monitored for survival (**A**), GVHD related body weight loss (**B**), and tumor growth determined by whole-body BLI (**C, D**). The log-rank (Mantel-Cox) test (A) and nonparametric Mann-Whitney U test (B, C) were used to compare between groups. Data are presented as mean SEM; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

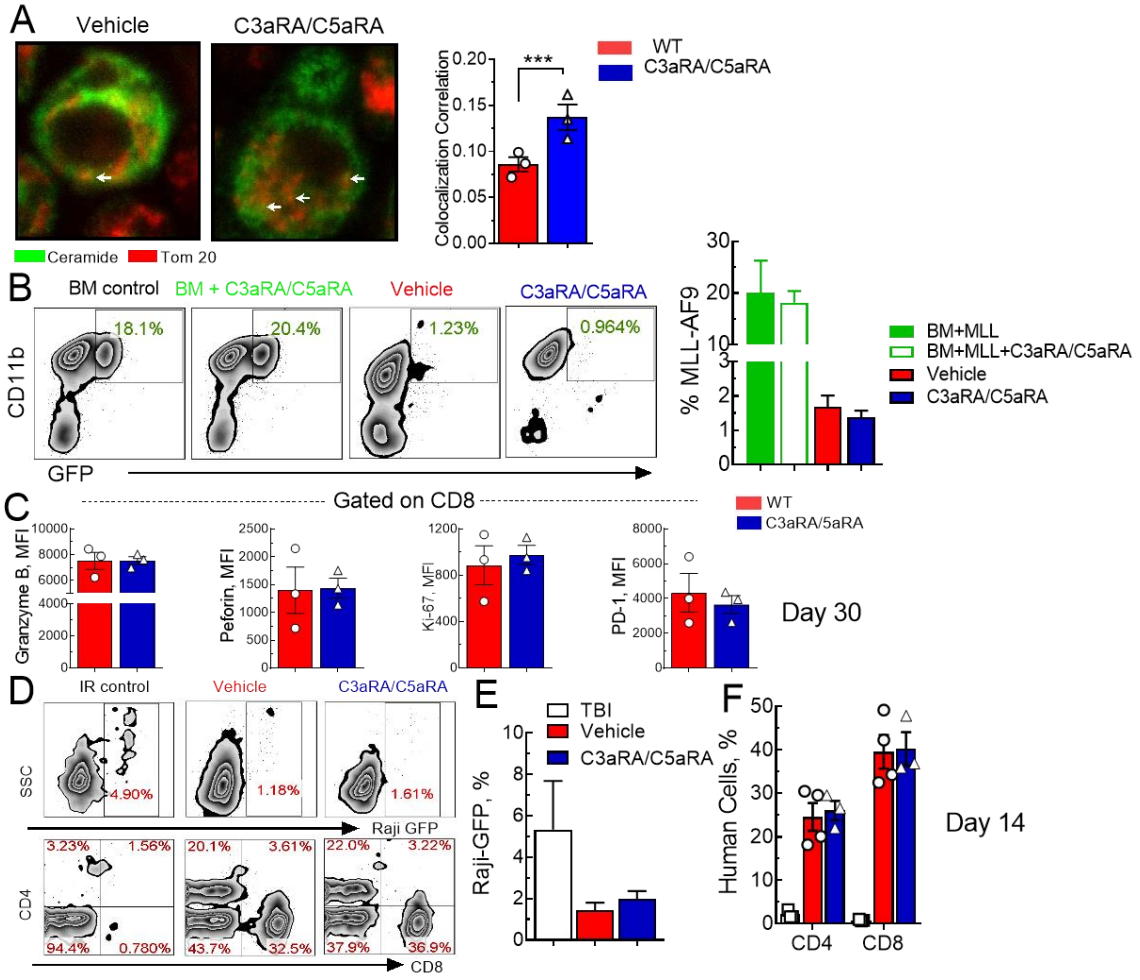


Fig.S9. ***C3aRA/C5aRA induced mitophagy in DCs. (A)*** Irradiated BM derived-DCs were cultured with or without C3aRA (2 mg/mL) and C5aRA (1mg/mL) and stimulated with 20 ng/mL LPS following by dual staining with ceramide antibody and autophagosomal marker Tom 20 and visualized using confocal microscopy. White arrows indicate colocalization. Data were combined from two independent experiments (*n=3*) and are presented as mean ± S.D. and ****p < 0.001.* (**B-C**) HCT *(n=8)* was performed and C3RA/C5aRA was administered as described above. At the time of HCT recipients also received MLL-AF9 GFP tumor cells. The tumor growths were indicated by the frequencies of $GFP^+/CD11b^+$ double positive cells. The representative flow zebra plots of MLL-AF9 GFP tumor cells and the summary bar graphs for the frequency of MLL-AF9 GFP are shown (**B**). On day 30 post-transplant, peripheral blood cells were stained for H^{2b}, CD4, CD8 and intracellular staining for granzyme B (grzm B), perforin, and CD107a. The bar columns for the mean of fluorescence of grzm B, perforin, Ki-67 and PD-1 are shown (**C**) (*n=3*). (**D-F**) NSG-A2 mice (*n=5-7*) were irradiated at the dose of 280 cGy, treated with a combination of C3RA/C5aRA and transfused with HLA-A2 negative human PBMCs (15×0^6 i.v.). $eGFP^+$ Raji cells ($1x10^6$/mouse) were injected i.v. on day 0. Tumor burden was determined by measuring GFP^+ cells in peripheral blood of transplant recipients. The engraftment was determined by monitoring frequencies of human donor $CD4^+$ and $CD8^+$cells in peripheral blood. The representative flow zebra plots and summary bar graph of frequencies of MLL-AF9 GFP tumor cells (**D-E**) and $CD4^+$ or $CD8^+$ (**D-F**) are shown (*n=4-5*). Unpaired two-tail *t*-test was used to compare between groups**.** ****p < 0.001*.