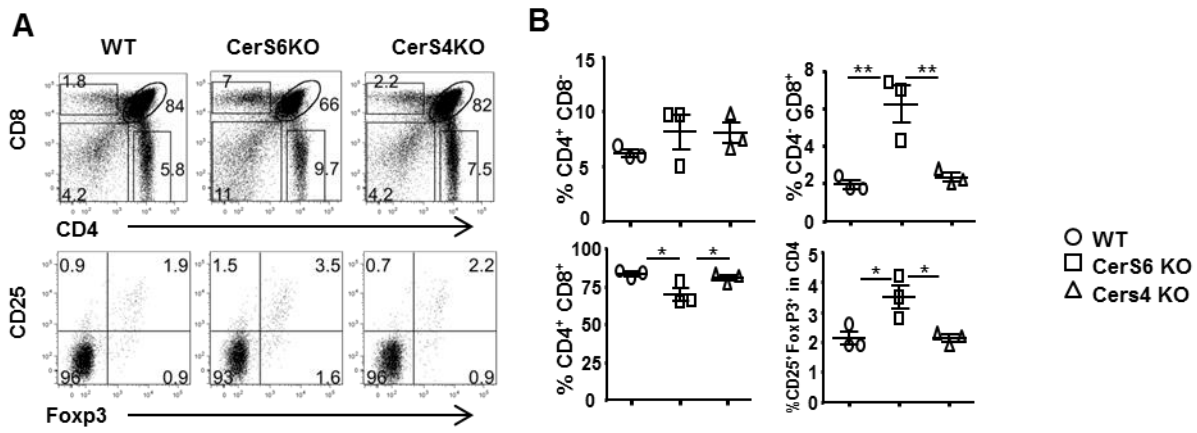
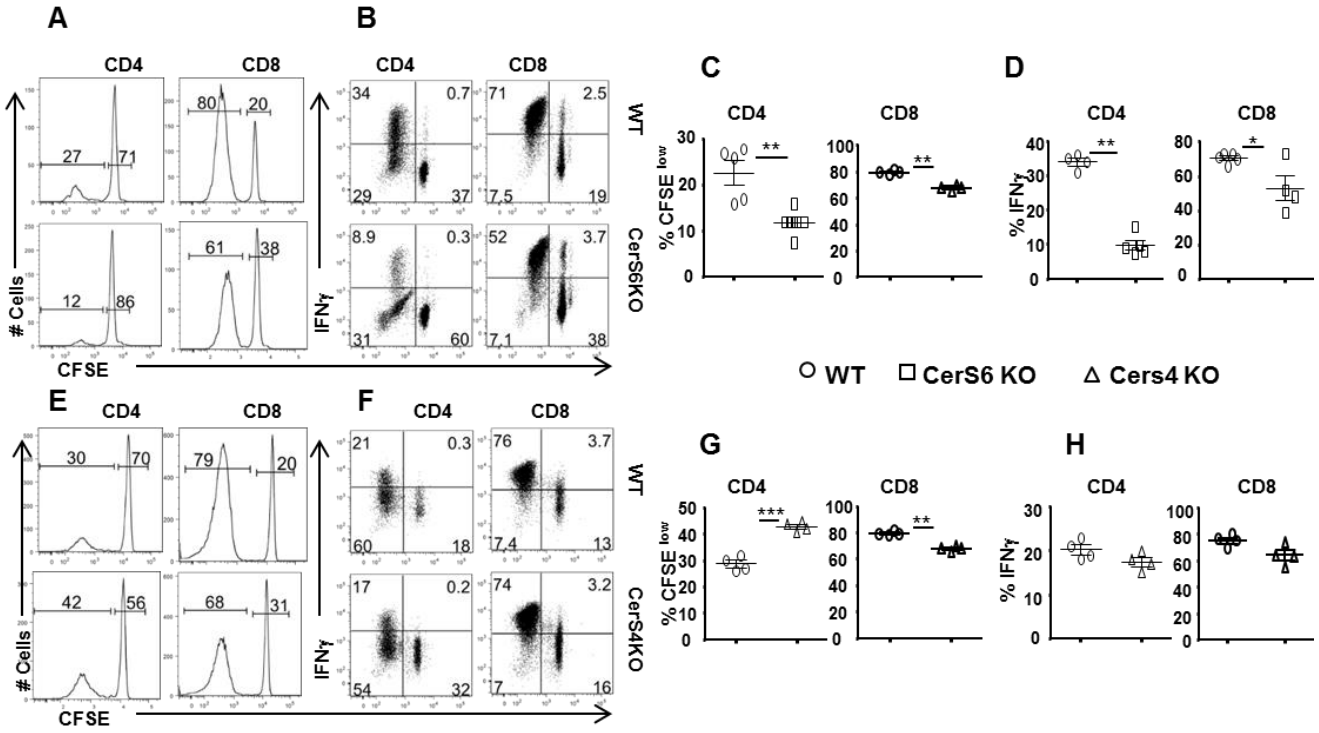


Figure S1: **Effect of CerS6 (Ceramide synthase 6) on T-cell development**. Thymus was obtained from un-manipulated age and sex-matched WT, CerS4 KO (Ceramide synthase 4 KO) or CerS6 KO (Ceramide synthase 6 KO) C57BL/6 (B6) background. Thymocytes were individually processed and counted before FACS staining for expression of CD4, CD8, CD25 and Foxp3. (A) CD4 and CD8 expression is shown on gated live thymocytes, and CD25 and Foxp3 expression on $CD4^{+}CD8^{-}$ cells (A). The mean ± SD of each cell subset is shown from 3 mice in each group. The data is one representative of 3 independent experiments. Significance was determined by using ANOVA test. Asterisks indicate statistical significance *p<.05.



Figures S2: Role of CerS6 (Ceramide synthase 6) in T-cell allogeneic response. Purified T-cells from WT, CerS4KO (Ceramide synthase 4 KO) or CerS6KO (Ceramide synthase 6 KO) mice on B6 background were labeled with CFSE and were co-cultured with T-depleted allogenic APCs (1:3 ratio) for 5 days. T-cell proliferation and IFNγ production is shown in one representation sample in each group (A, B, E and F), or mean ± SD of 3-4 samples per group (C, D, G and H). Data represents one of 2 independent experiments. Significance determined by Student's *t* test. Asterisks indicate statistical significance *p<.05. ** p<.01; *** p<.001.

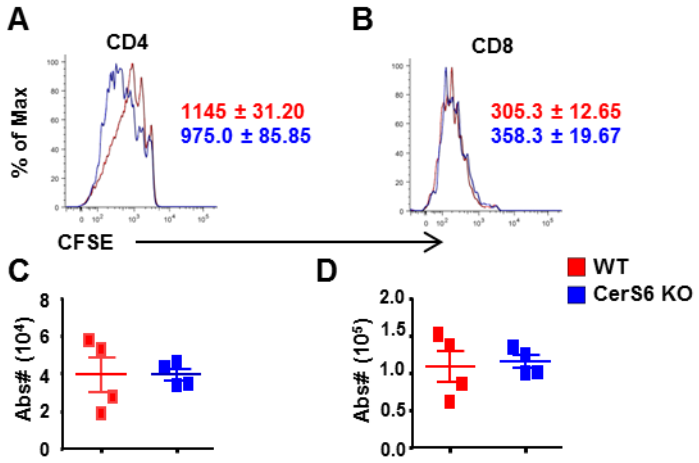


Figure S3: Effect of CerS6 (Ceramide synthase 6) on T-cell syngeneic response. Purified T cells from WT and CerS6KO (Ceramide synthase 6 KO) mice on B6 background (CD45.2) were labeled with CFSE and intravenously injected into lethally irradiated B6 mice (CD45.1) at 2×10^6/mouse. Five days after cell transfer, spleens were collected from recipient mice and subjected to cell counting and FACS staining. (A) Mean fluorescence intensity of CFSE labelled donor-derived ($H2K^{b+}CD45.2^+$) $CD4^+$ and $CD8^+$ cells. (B) The absolute numbers of donor derived $CD4^+$ (C) and $CD8^+$ (D) cells. Data shown here is from one of the two independent experiments. Data represents mean ± SEM (for panel A and B).

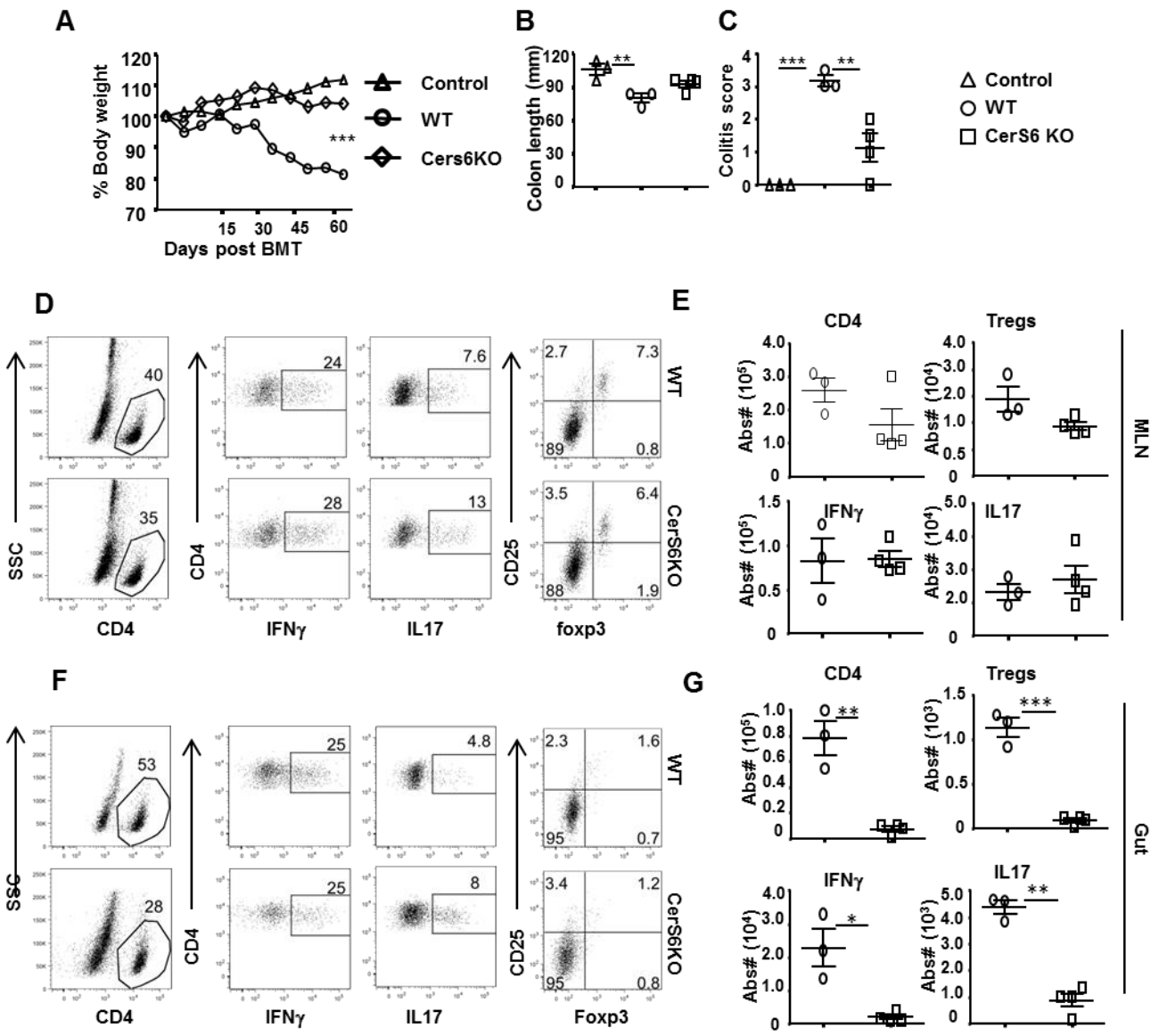
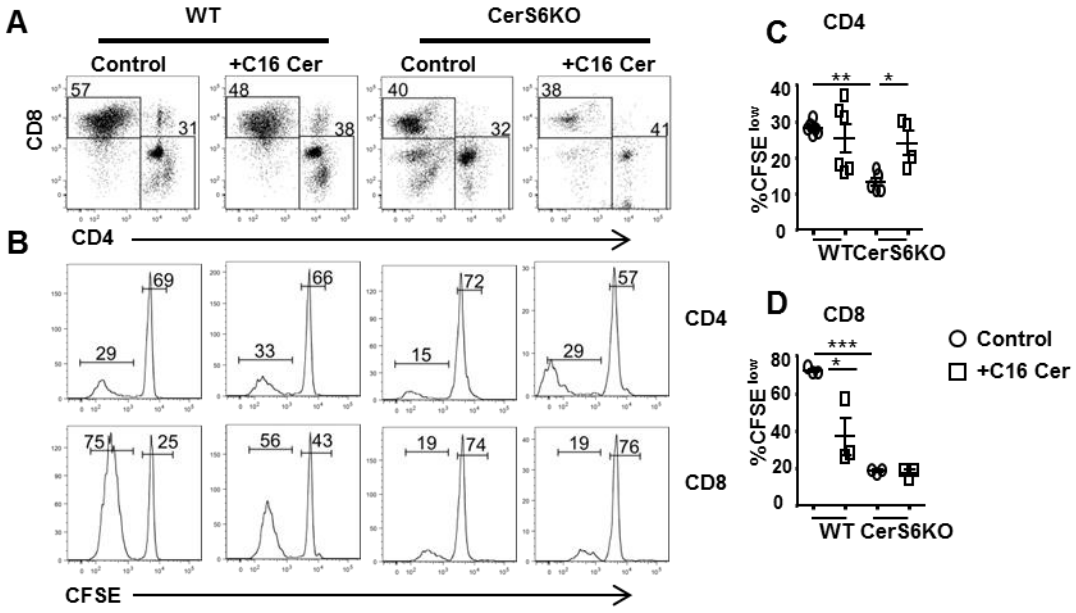


Figure S4: Impact of CerS6 (Ceramide Synthase 6) on T-cell induced colitis. Naïve 1x10^6 CD4$^+$ T-cells from either WT or CerS6KO mice were intravenously injected into lymphopenic *Rag1*$^{-/-}$ hosts to induce colitis. The control mice were injected with PBS only. Body weight, colon length and colitis score are shown (A-C). Expression of IFNγ, IL17, CD25 and Foxp3 is shown on gated CD4$^+$ cells (D and F). Absolute numbers of CD4$^+$ IFNγ, IL17 and Tregs are shown at mean ± SD in mesenteric lymph nodes and colon from 3-4 mice per group (E and G). Data shown here are one of two independent experiments. Body weight loss was compared using a nonparametric Mann-Whitney *U* test. To compare colon length and colitis score, significance was determined by using ANOVA test; and for cytokine levels a 2-tailed Student *t* test was performed. Asterisks indicate statistical significance *p<.05; ** p<.01; *** p<.001.



Figures S5: Role of C_{16}-ceramide in T-cell proliferation in vitro. CFSE-labelled T cells were stimulated with allogenic APCs for 5 days in the presence or absence of C_{16}-ceramide (C_{16}-cer). On day 5, cells were analyzed for T-cell proliferation on one representative mouse (A and B), or mean ± SD of different mice (C and D). Date represent one of two independent experiments (C). Significance was determined by using ANOVA test. Asterisks indicate statistical significance *p<.05.

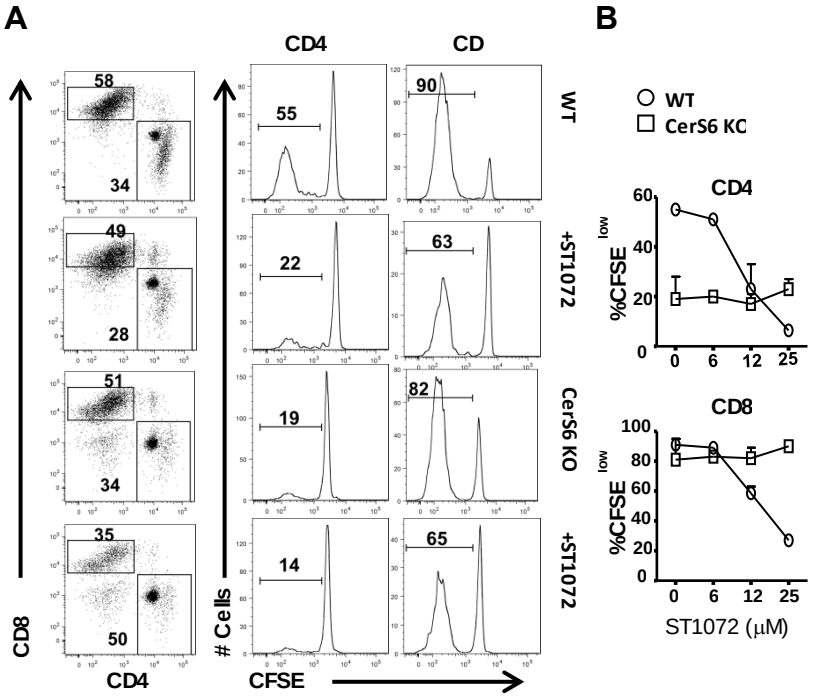
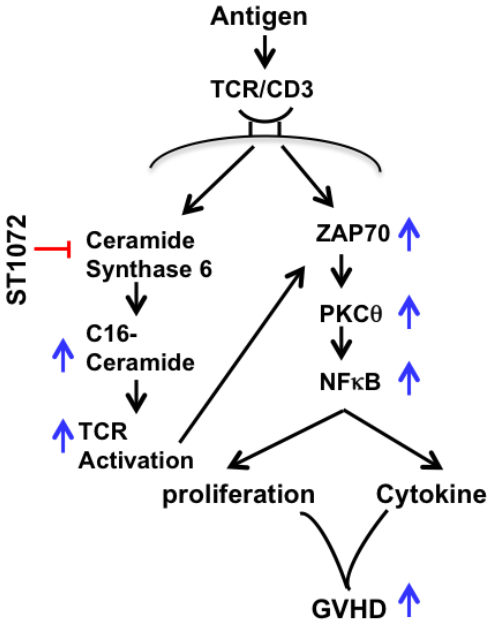


Figure S6: Impact of CerS6 (Ceramide synthase 6) inhibition on T-cell allogeneic responses. CFSE-labelled T cells from WT or CerS6KO mice were stimulated with allogeneic APCs in the presence or absence of different concentration of CerS6 inhibitor ST1072 ranging from 6μM to 25 μM for 5 days. Cells were subjected to FACS staining and analyzed for T-cell proliferation. (A) CFSE dilution is shown on gated $CD4^+$ or $CD8^+$ cells. (B) CFSE diluted cells with different concentrations of ST1072 are shown on gated CD4 and CD8 cells.



Figures S7: CerS6 regulates T-cell activation, proliferation and GVHD induction through the generation of C16-ceramide. TCR ligation up-regulates CerS6 that results in *de novo* generation of C16-ceramide, which in turn promotes activation of ZAP70 and co-localization of PKCθ with TCR. Subsequently, allogeneic T-cell activation, proliferation, migration, and cytokine production lead to GVHD development. Inhibition of CerS6 using ST1072 reverses the positive effect of CerS6 on T cells and thus alleviate GVHD.