Supplementary Materials

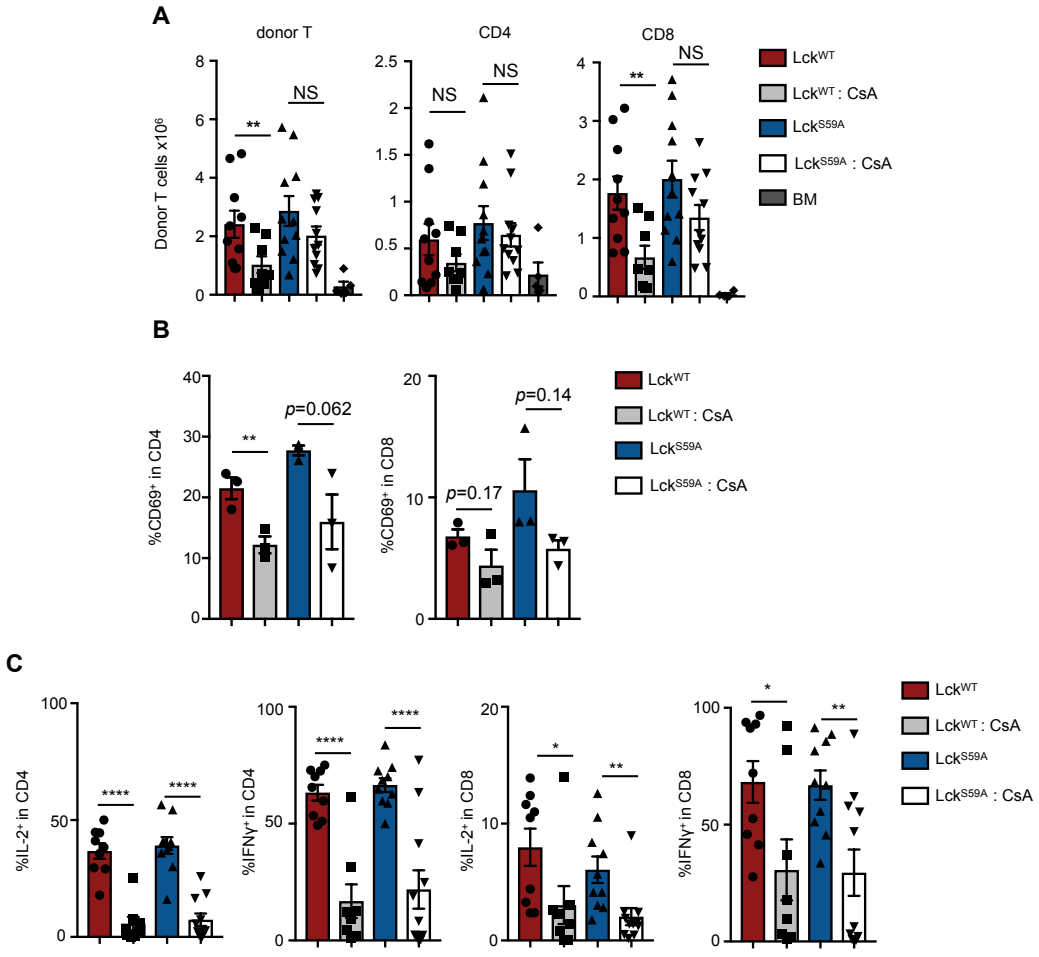

Figure S1. Lck^{S59A} T cell expansion in spleen in aGVHD is insensitive to CsA. (**A**) Splenocytes were collected from mice receiving B6 WT (n=10), WT + CsA (n=8), Lck^{S59A} (n=11), or Lck^{S59A} + CsA (n=11) BM plus lymph node cells or control WT BM alone (n=5) at day 7. (**B**) Freshly-isolated splenocytes were stained and analyzed by flow cytometry and cells gated on CD4$^+$ and CD8$^+$ T cells. The graphs show the mean ± SEM of each group (n=3). (**C**) Splenocytes from day 7 recipients were restimulated for 3 hr and stained for the indicated

cytokines. The graphs show the mean ± SEM of n=8-10/group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$, NS= not significant, Student's t-test.

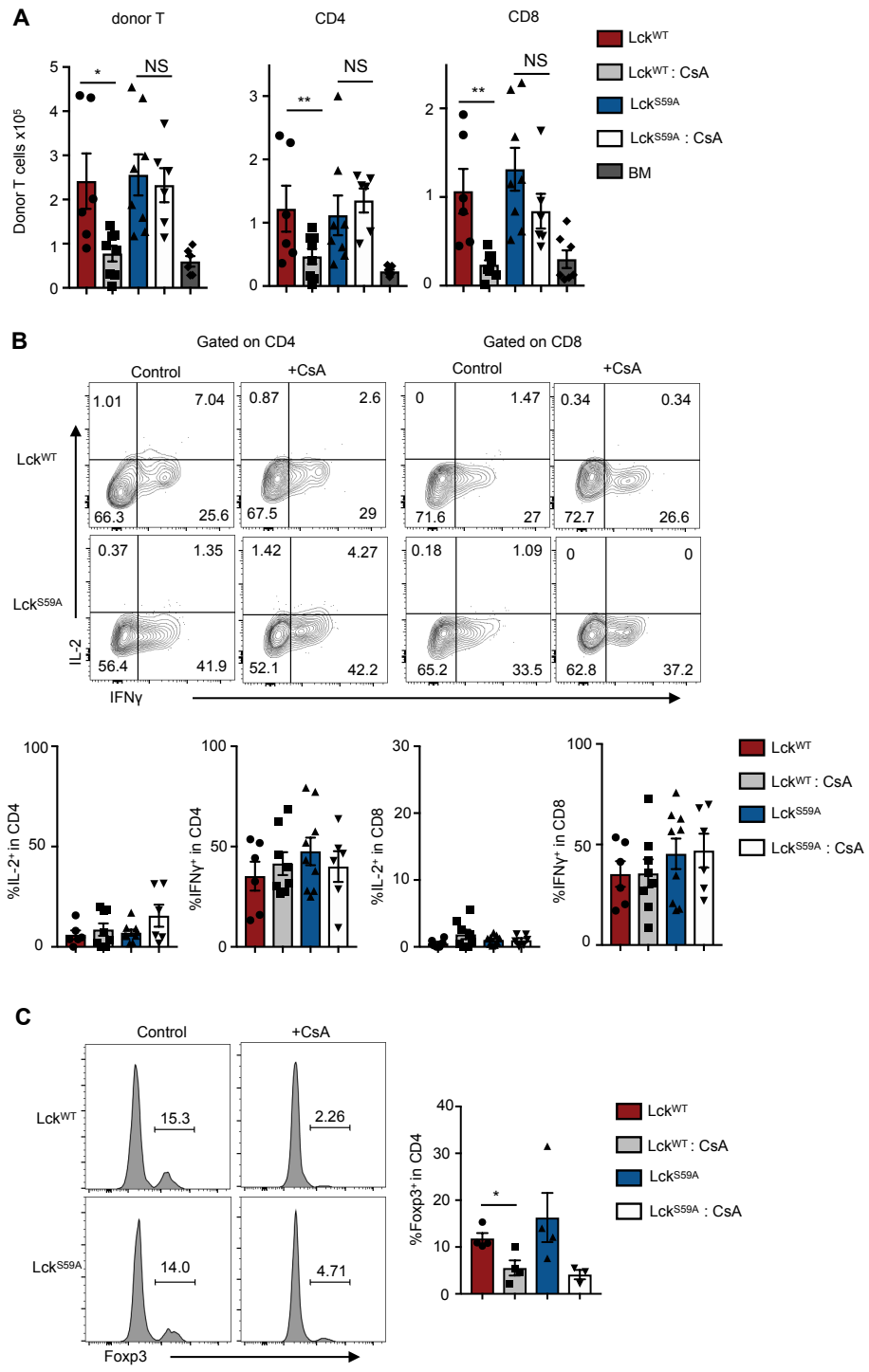

Figure S2. Infiltration of Lck^{S59A} T cells into the liver in aGVHD is not affected by CsA. (**A**) Liver-infiltrating cells were collected from recipient mice (n=6-9/group) at day 30. (**B**) Isolated liver-infiltrating cells from day 30 recipient mice were restimulated for 3 hr and stained for the

indicated cytokines. Representative contour plots of cytokine expression gated on $CD4^+$ and $CD8^+$ T cells is shown. The graph show the mean ± SEM of n=6-9/group from two independent experiment. (**C**) The proportions of $Foxp3^+$ liver-infiltrating $CD4^+$ T cells as detected by flow cytometry. The graphs show the mean ± SEM of n=3-4/group. *$P < 0.05$, **$P < 0.01$, Student's t-test.

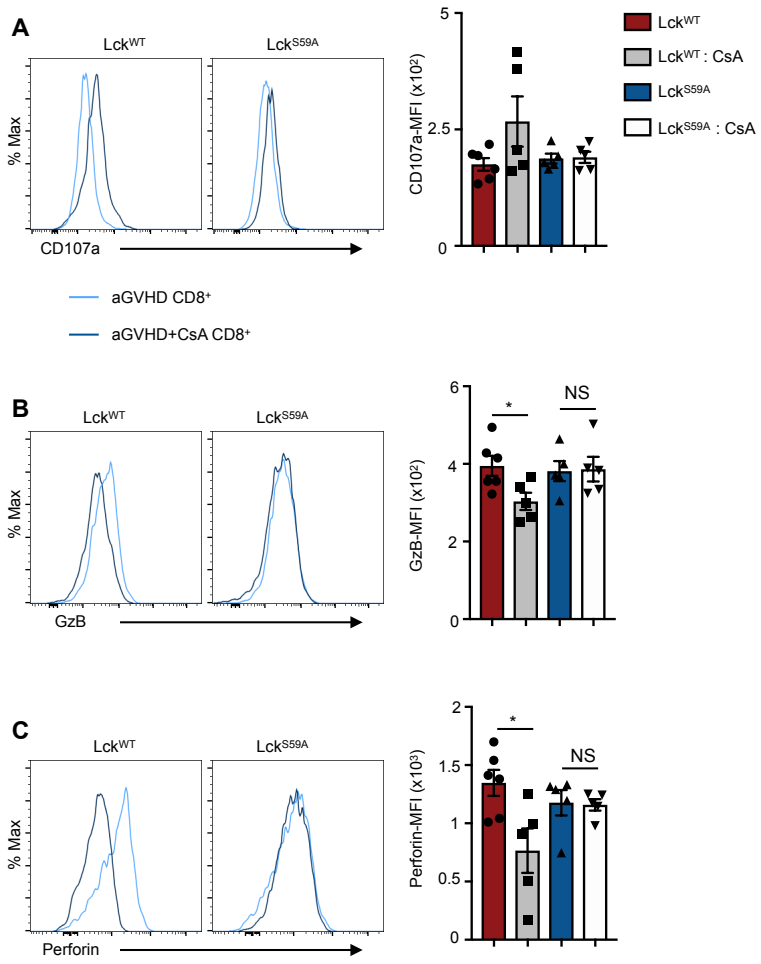

Figure S3. Perforin expression in CD8$^+$ T cells from spleen. (**A-C**) Freshly-isolated splenocytes from mice receiving B6 WT BM plus lymph node cells (n=5-6) were stained for CD107a (**A**), granzyme B (**B**), and perforin (**C**) and the results with CD8$^+$ T cells are shown. The graphs show the mean ± SEM. *$P < 0.05$, Student's t-test.

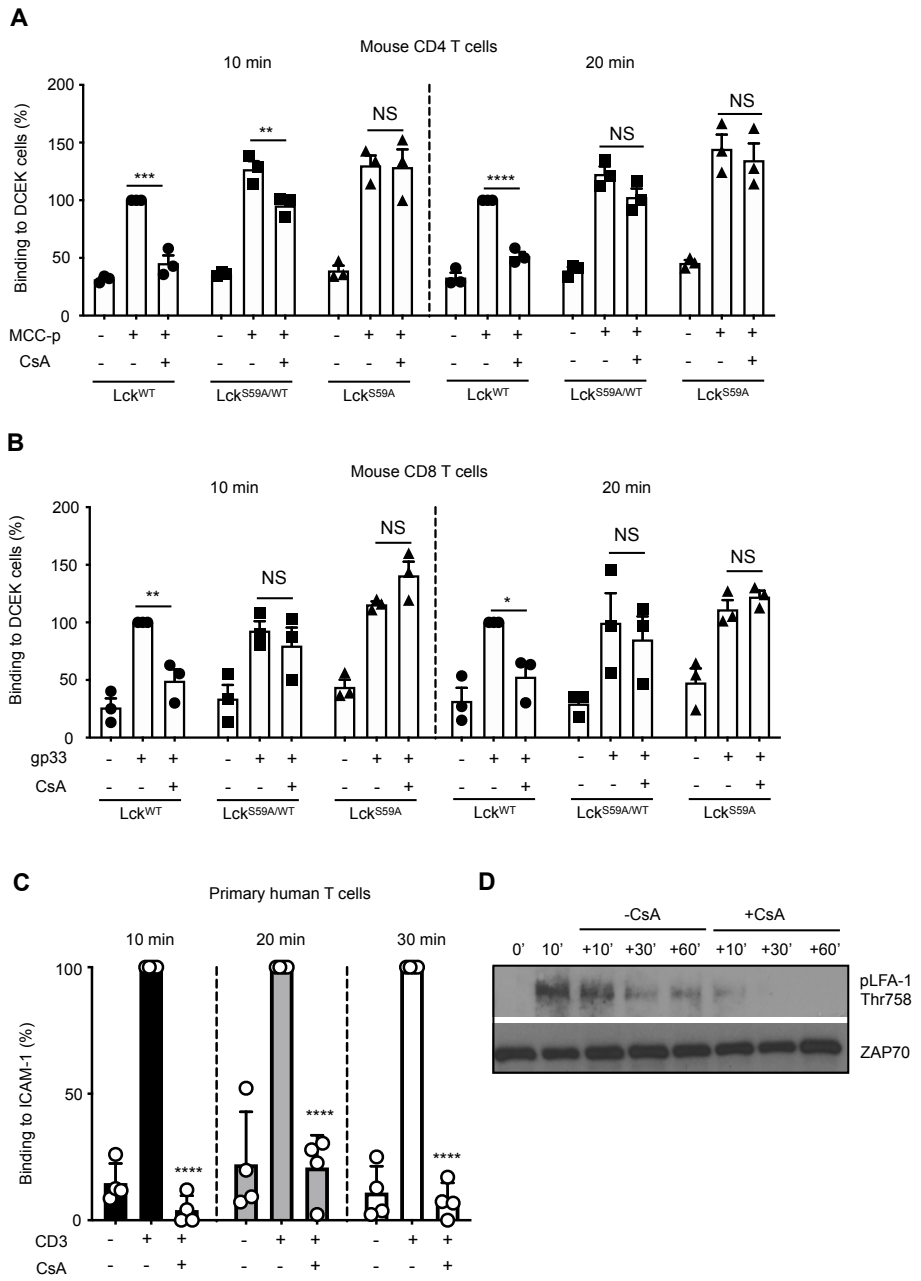

Figure S4. CsA rapidly reverses existing antigen-induced LFA-1/ICAM1 adhesion. (**A** and **B**) AND or P14 T cells were cultured for 30 min on antigen-pulsed DCEK or DCEK-D^b cells, respectively, to allow adhesion to occur. At that time CsA was added to the cultures. The number of AND (**A**) and P14 (**B**) T cells bound to APCs were counted by trypan blue exclusion and light microscopy at the indicated times. Results are presented relative to the number of activated WT

T cells bound at each time, set as 100%. The graphs show the mean ± SEM of three or four independent experiments. (**C**) Human primary T cells were stimulated with anti-CD3 cross-linked with anti-mouse IgG for 30 min and then incubated in ICAM1-coated plates. After 30 min, CsA was added to the cultures and the number of bound cells was quantitated at the indicated times. Results are presented as in (**A)** and (**B**). The graphs show the mean ± SEM of four independent experiments. (**D**) Human primary T cells were stimulated with soluble anti-CD3 cross-linked with anti-mouse-IgG. Ten min after stimulation CsA was added and the cells lysed at the indicated times. Lysates were immunoblotted with anti-phosphorylated $CD18^{T758}$. $*P < 0.05$, $**P < 0.01$, $***P < 0.0001$, $****P < 0.00001$, NS= not significant, 1-way ANOVA with Tukey's multiple-comparison post hoc test.

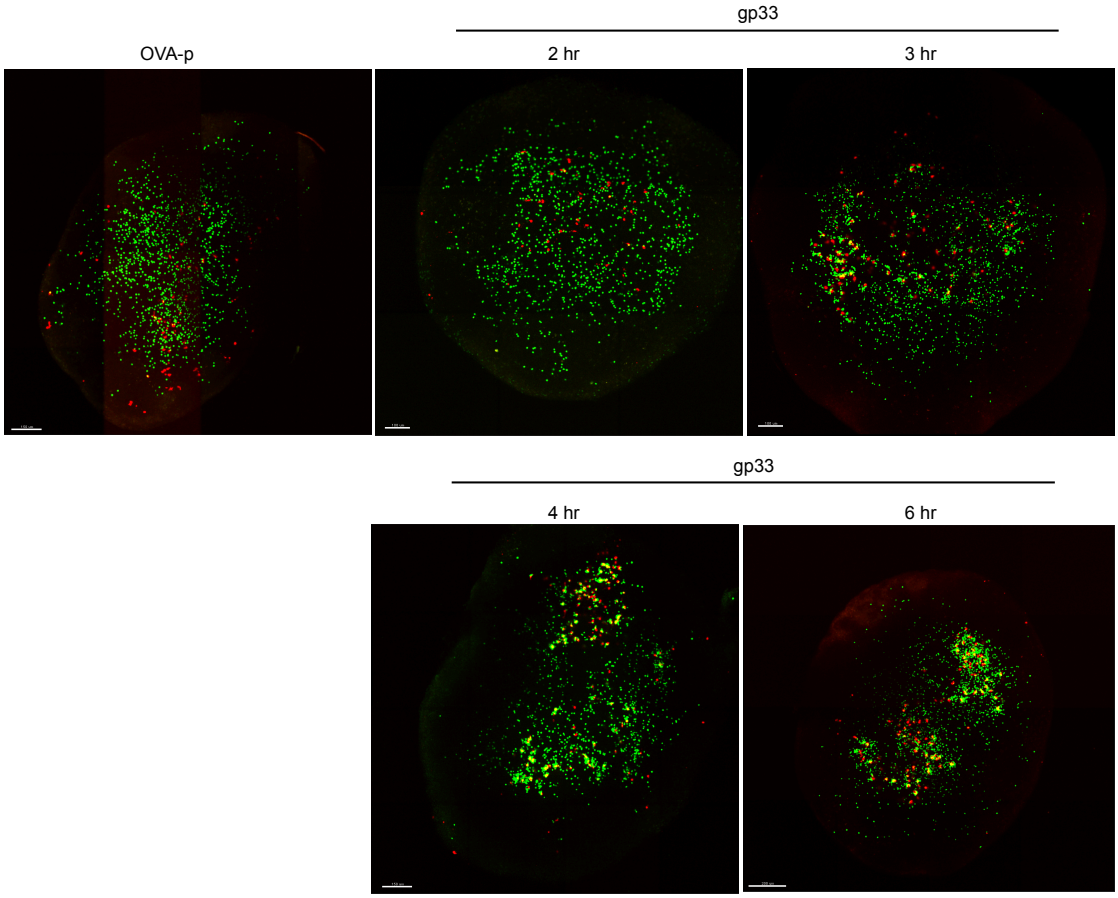

Figure S5. Distribution of injected DCs and P14 T cells. Example images of whole pLN sections showed the distribution of P14 T cells (green) and DCs (red) at the indicated times after T cell transfer. Bar, 100 μm

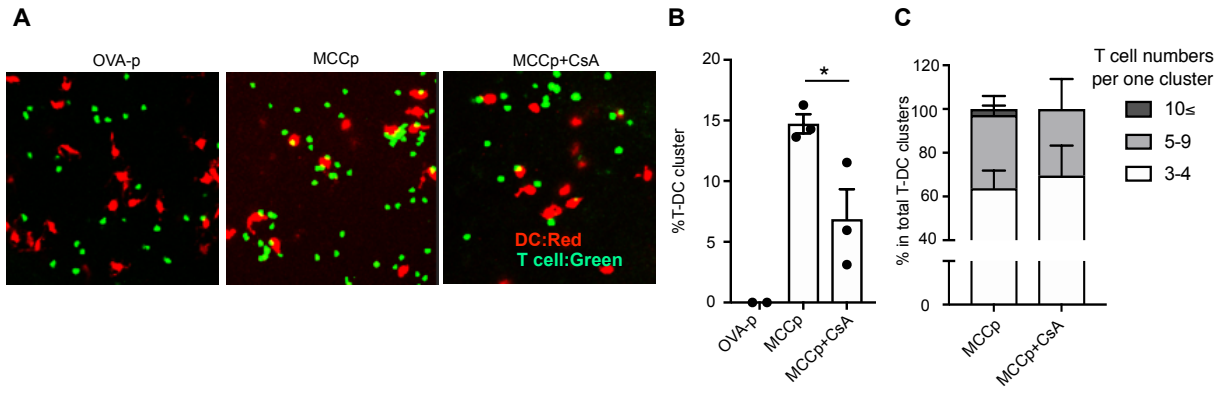

Figure S6. CsA inhibits CD4$^+$ T cell:DC clustering *in vivo*. Mice were injected in the footpad with MCCp or OVA-p pulsed DCs labeled with Deep Red Dye. After 18-20 hr, recipients were injected intravenously with green CMFDA-labeled AND T cells. Mice were injected i.p. with CsA the day before and again at the time of T cell transfer. pLNs were removed at 6 hr and cleared. Representative images are shown in (**A**). The percentage of T:DC clusters (**B**) and the number of T cells per cluster (**C**) were analyzed by Imaris software. The graphs show the mean ± SEM of three independent experiments. *$P < 0.05$, 1-way ANOVA with Tukey's multiple-comparison post hoc test.