Supplement

Inhibiting the coregulator CoREST impairs Foxp3+ Treg function and promotes antitumor immunity

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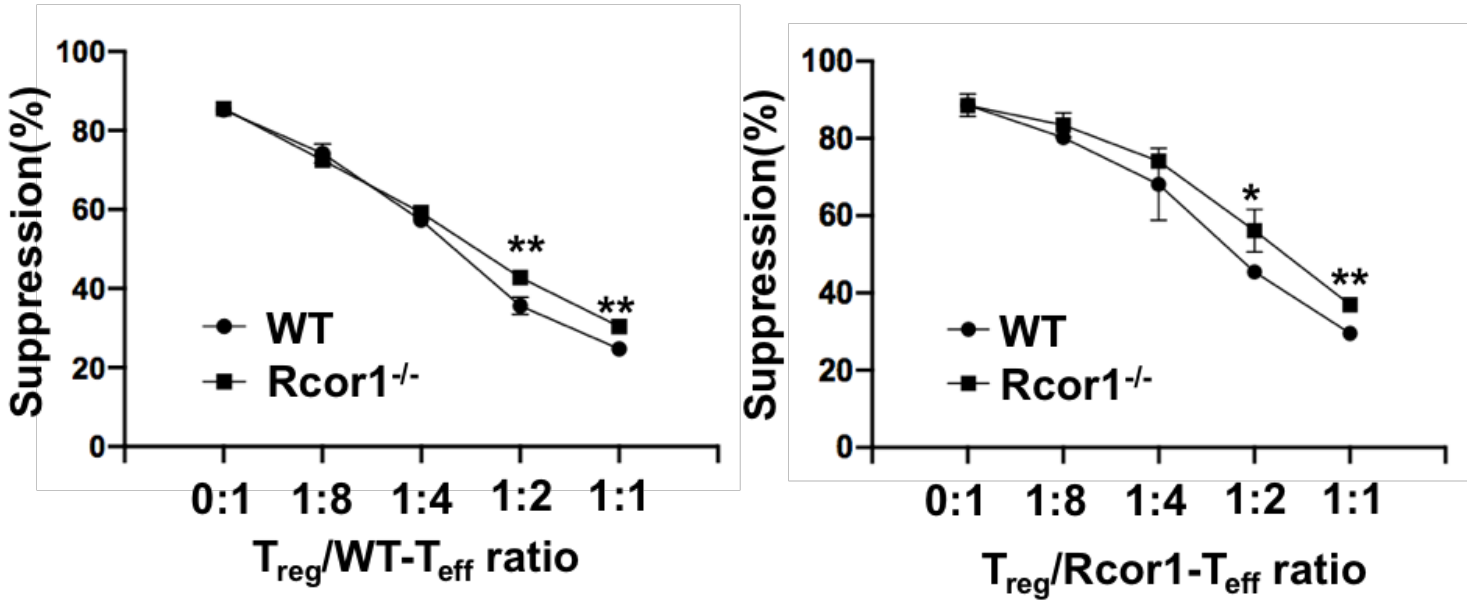
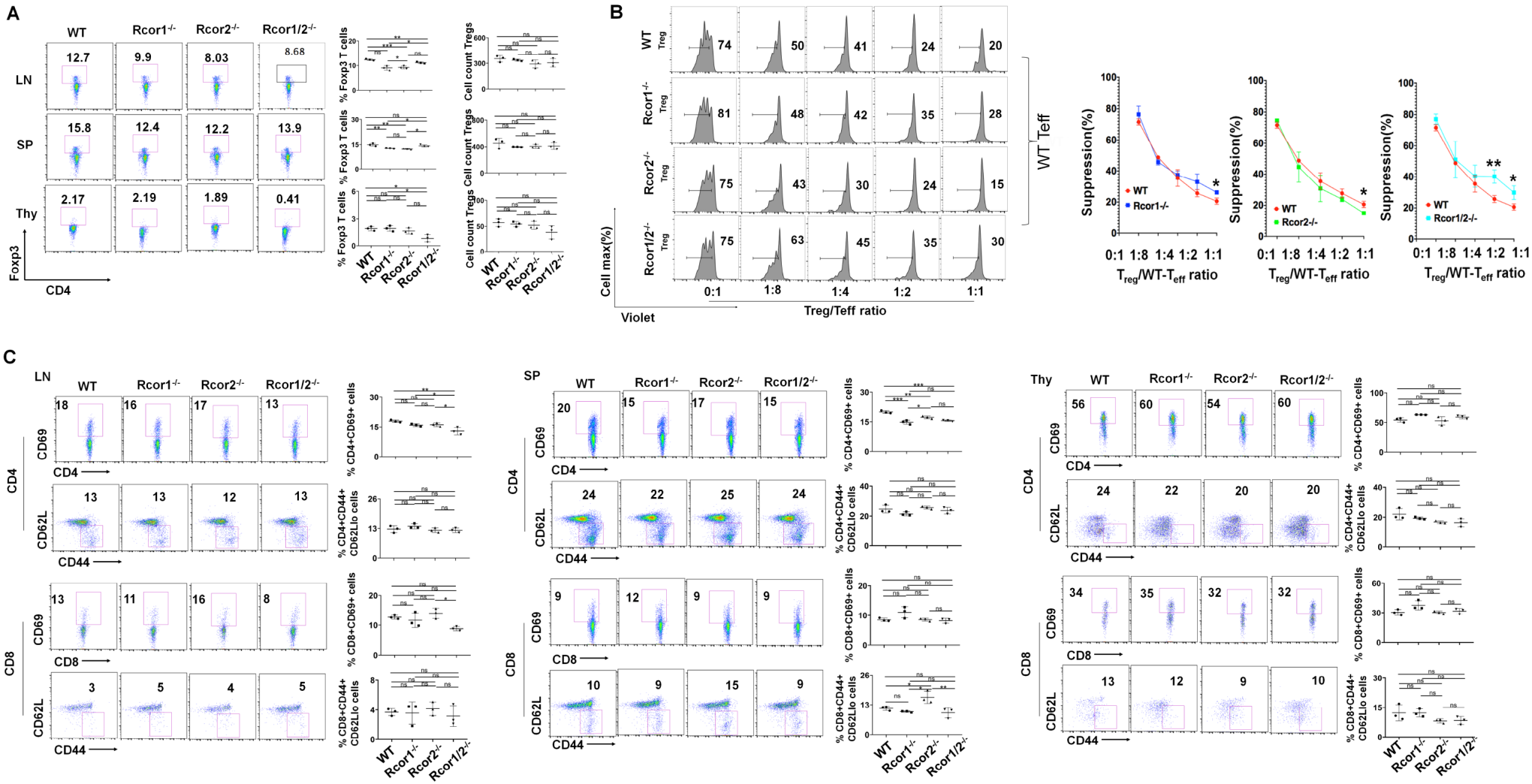
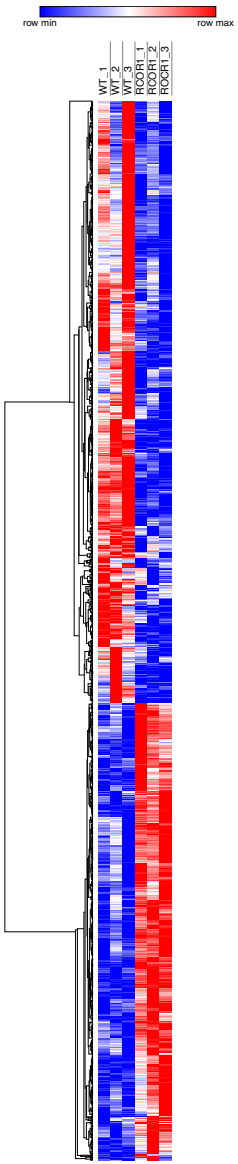


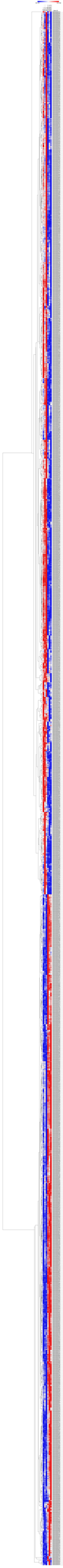
Supplemental Figure 1: Treg suppression assay data relevant to Fig 1G. Left panel involves WT T-eff cells and right panel involves Rcor1-/- T-eff cells. In both cases, Rcor1-/- Tregs had impaired suppressive activity compared to WT Treg cells. Assays were performed in triplicate using cells from 3 individual mice and repeated at least 3 times; *p<0.05, **p<0.01 vs. WT control.

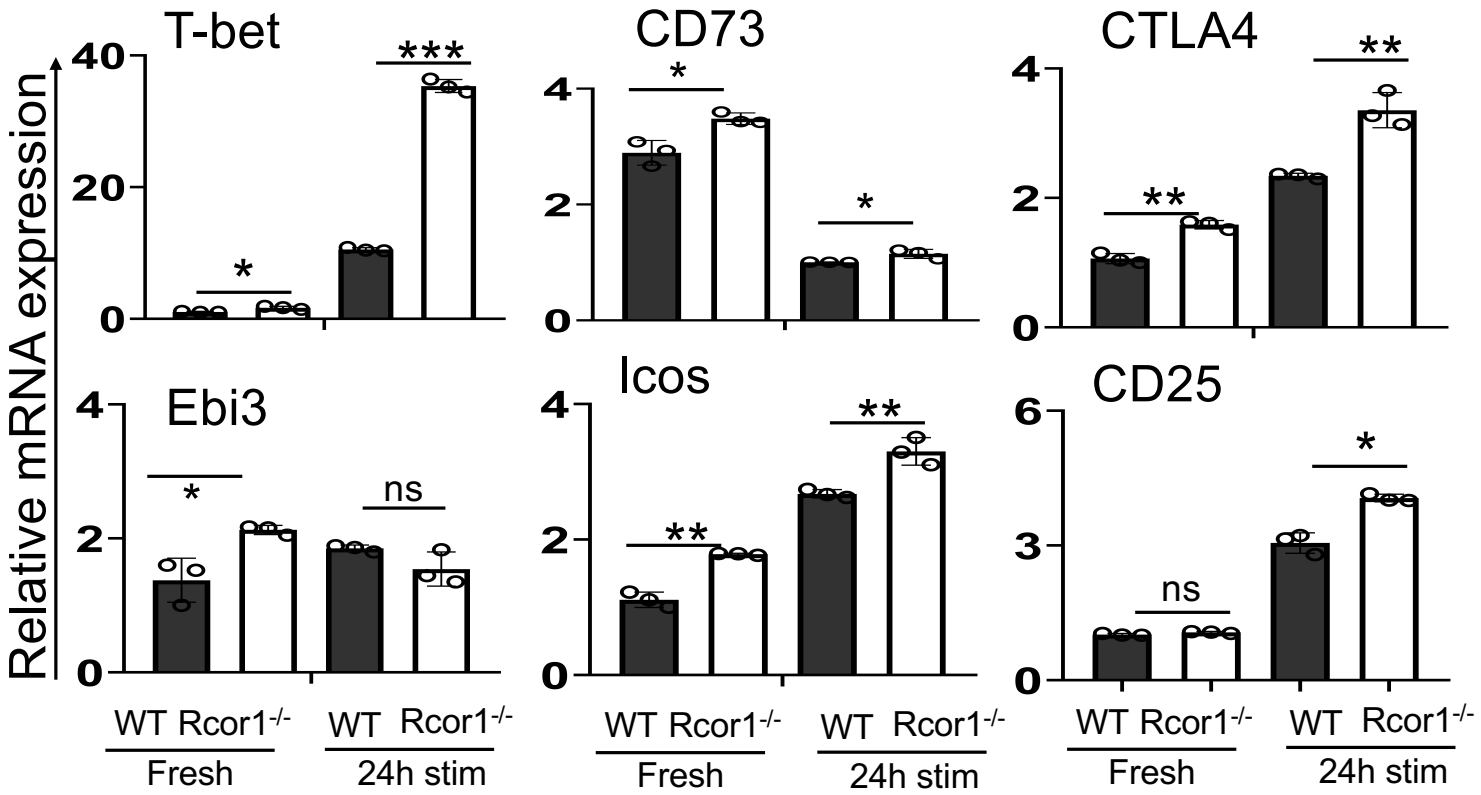


Supplemental Figure 2: Effects of conditional deletion of Rcor1, Rcor2 or Rcor1/2 on Treg function. (**A**) Percentages of CD4+Foxp3+ Tregs in lymph nodes, spleens and thymii of WT, Rcor1-/-, Rcor2-/- and dual Rcor1/2-/- mice, shown as representative plots (left) and with statistical analyses (right). (**B**) Treg suppression assay using Tregs from lymph nodes and spleens of WT, Rcor1-/-, Rcor2-/- and dual Rcor1/2-/- mice, with representative data (left) and cumulative data (right); the percentage of proliferating cells are shown in each panel. (**C**) T cell activation makers in CD4 and CD8 T cells of WT, Rcor1-/-, Rcor2-/- and dual Rcor1/2-/- mice were analyzed as % of gated cells; data shown as representative plots (left) and with statistical analyses (right). Data are shown as mean ± SD, 3 mice/group. Statistical analyses were performed with one-way ANOVA followed by Tukey's post-hoc test; *p<0.05, **p<0.01 or ns (not significant) vs. WT.

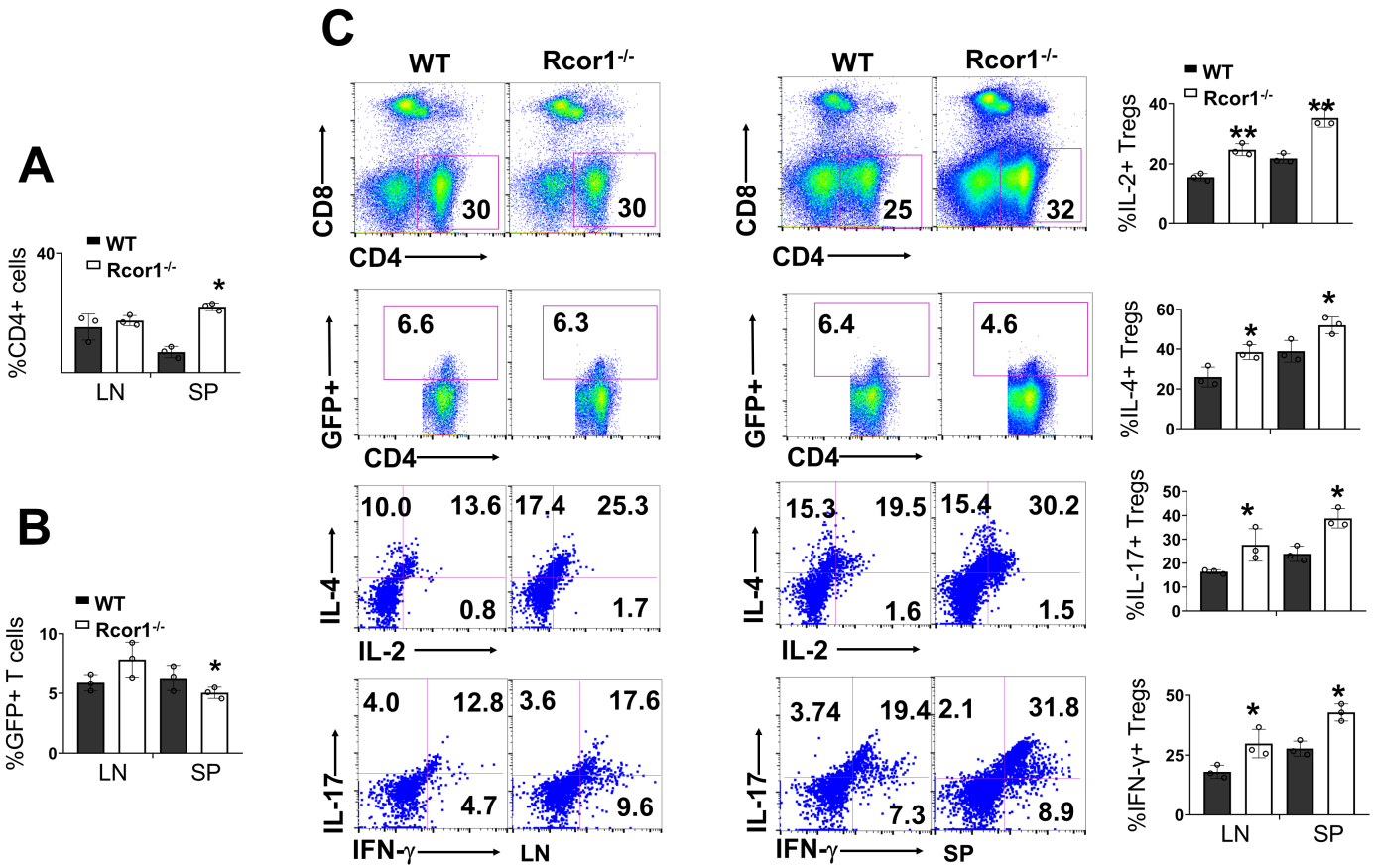


Supplemental Figure 3. Overview of hierarchical clustering map for differential gene expression by WT vs. Rcor1-/- Treg cells. This panel is available as a separate pdf supplementary file (Cluster.pdf) that can be more readily magnified to examine specific areas of interest and has a single gene view of the row hierarchical clustering (metric: One minus Pearson correlation, linking method: average).





Supplemental Figure 4: qPCR results of gene expression in WT vs. Rcor1-/- Tregs that were freshly isolated or cultured under activating conditions for 24 h (1:1 ratio of CD3/CD28 mAb- coated beads); data are shown as mean ± SD, 3 mice/group, Student's t test for unpaired data, *p<0.05, **p<0.01, ***p<0.001 for the indicated comparisons.

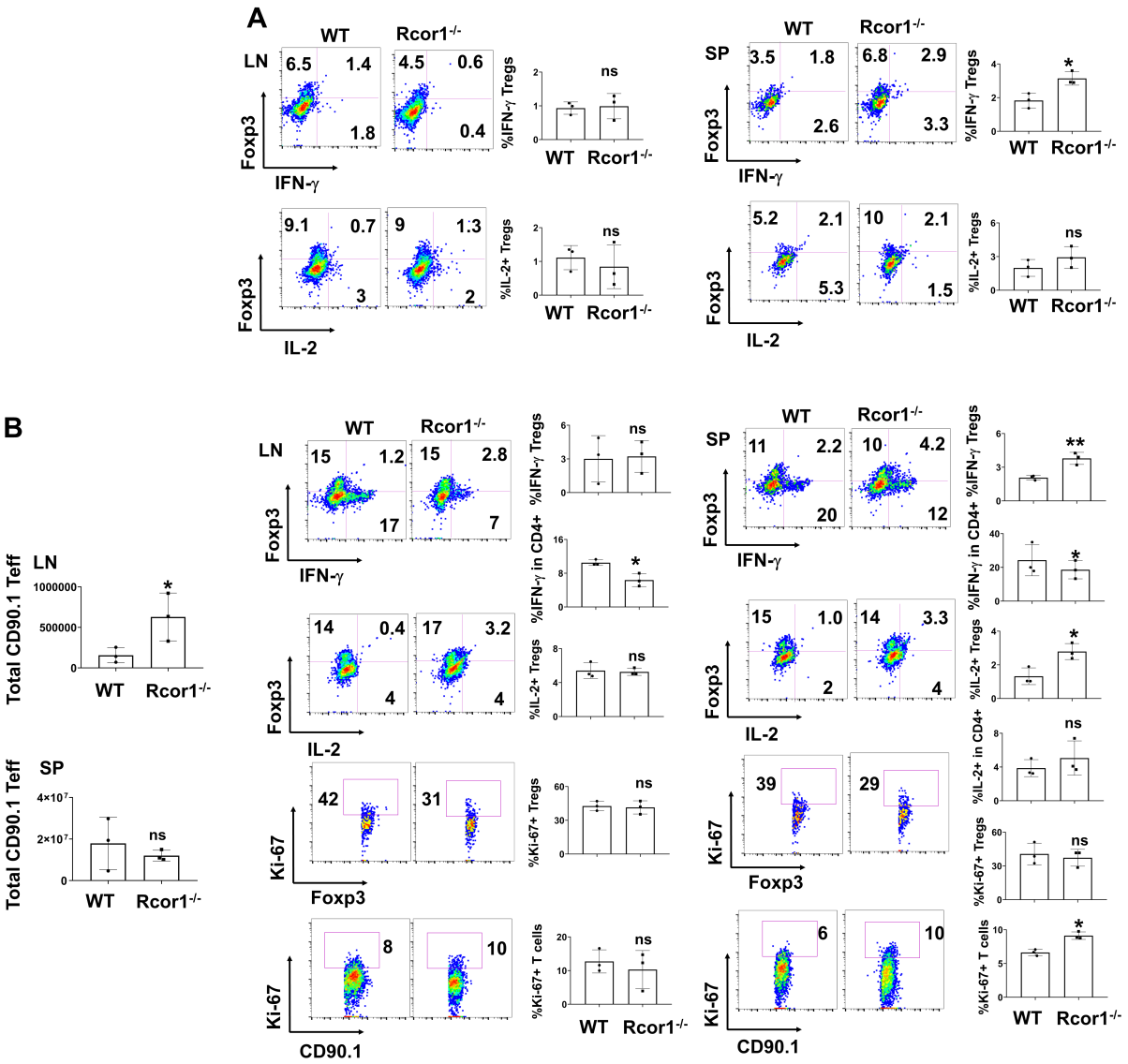


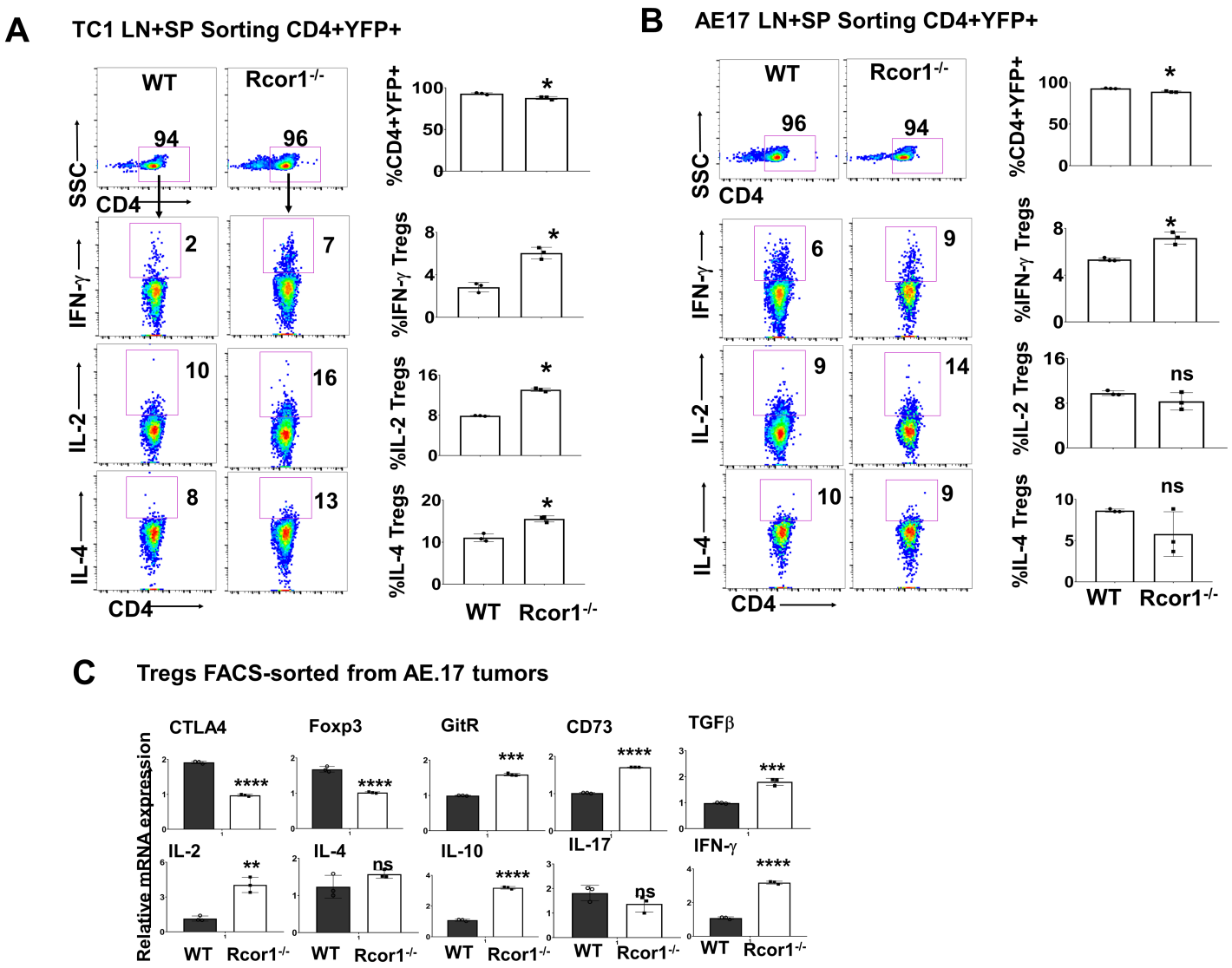
Supplemental Figure 5: Tregs with conditional deletion of Rcor1-/- had increased production of proinflammatory cytokines. Cells from lymph nodes or spleen were stimulated with PMA/ionomycin in the presence of Golgi-stop for 4 h. The results of gating on (**A**) CD4+ T cells and (**B**) GFP+ Tregs are shown. (**C**) Analysis the percentage of IL-2+, IL-4+, IL-17+ and IFN-γ+ Tregs; numbers in quadrants indicate the percentage of each cell population. Data are shown as mean ± SD, 3 mice/group at 8-12 weeks of age, using Student's t-test for unpaired data, *p<0.05, **p<0.01 compared with WT.

Supplemental Figure 6: Rcor1 deletion impaired Treg function *in vivo*.

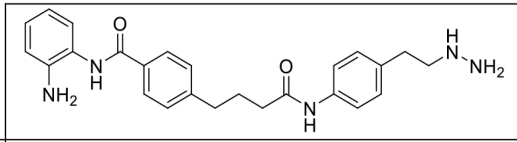
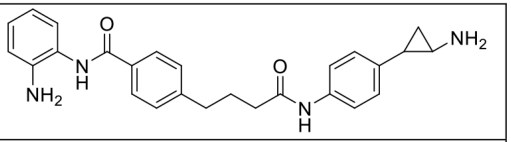
(**A**) WT or Rcor1-/- Tregs ($0.5x10^6$) and co-transferred T-effector cells ($1x10^6$) were injected into Rag1-/- mice (n=3/group), with analysis at 7 d of %IL-2+ Tregs and %IFN-γ+ Tregs in lymph nodes (LN) and spleen (SP).

(**B**) WT or Rcor1-/- Tregs ($1x10^6$) were co-transferred with T-effector cells ($0.5x10^6$) injected into Rag1-/- mice (n=3/group) for 4 weeks. The first column has the total number of CD90.1+ cells in LN and SP. Columns 2 and 3 show %IL-2+ Tregs, %IFN-γ+ Tregs and %Ki-67+ Tregs in LN. Columns 4 and 5 show corresponding SP data. Numbers in quadrants indicate % of cell population, and data are shown as mean ± SD, 3 mice/group at 8-12 weeks of age, Student's t-test for unpaired data, *p<0.05, **p<0.01 compared to WT.

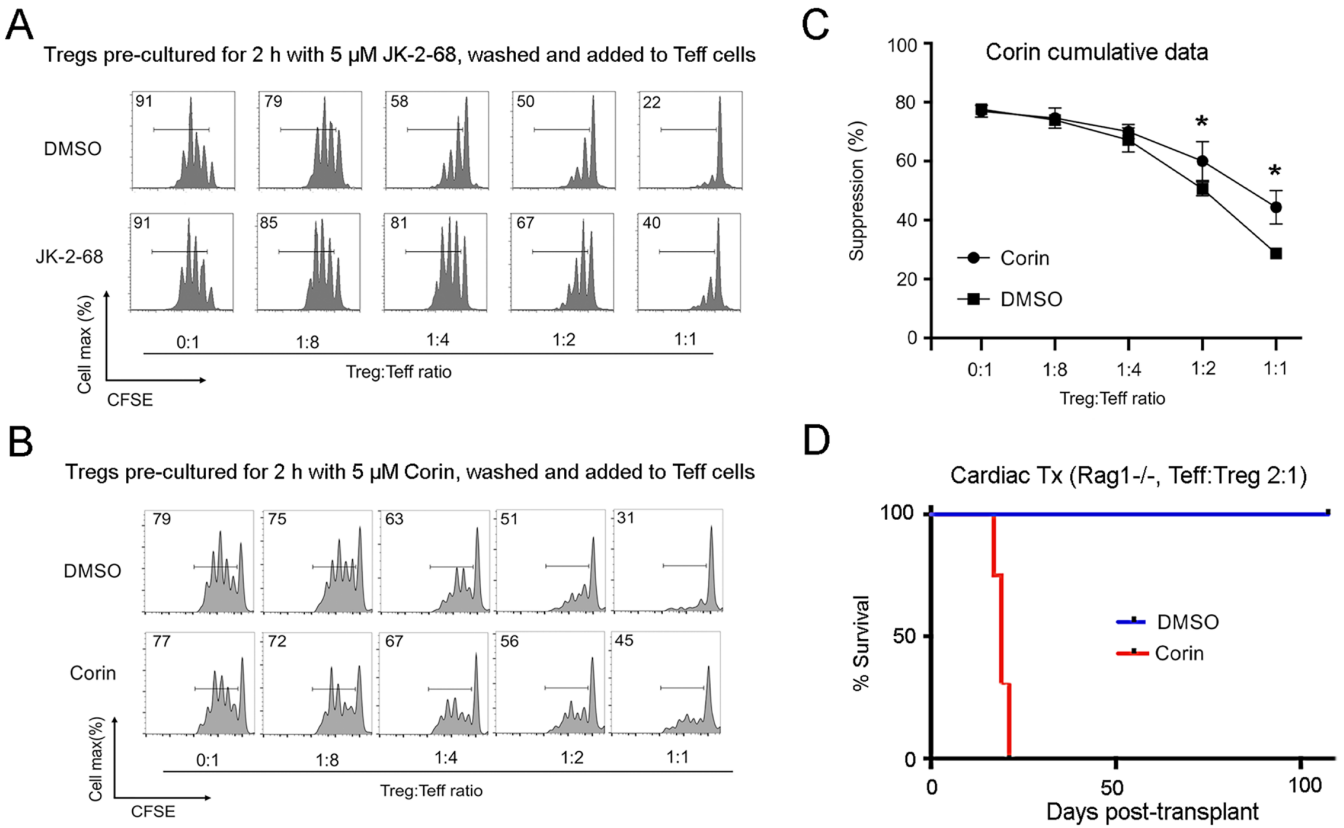




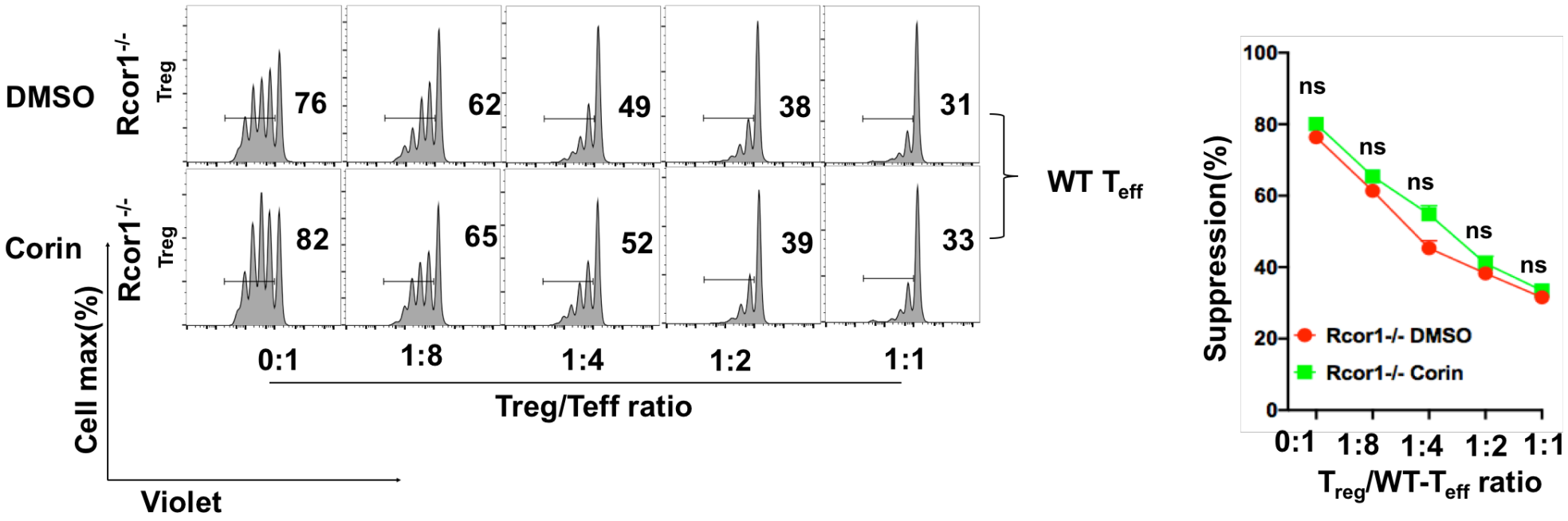
Supplemental Figure 7: Deletion of Rcor1 in Tregs increased production of pro-inflammatory cytokines in draining LN and in tumors. %CD4+ YFP+ cells, %CD4+YFP+ cells, %IL-2+ Tregs, %IL-4+ Treg and IFN-γ Tregs in LN of Rcor1-/- or WT mice (3 mice/group), bearing (**A**) AE17 or (**B**) TC1 tumors. (**C**) qPCR analysis of gene expression of CTLA4, Foxp3, GitR, CD73, TGF-b, IFN-γ, IL-2, IL-4, IL-10 and IL-17 by FACS-sorted tumor-infiltrating Tregs, isolated from AE17 tumors at 14 d. Data are shown as mean ± SD, 3 samples/group. Student's t-test for unpaired data; *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 vs. WT control.

		
Compound ID	**JK-2-68 (compound 1)**	**JKD-1-51 (Corin)**
isol. LSD1 ($k_{inact}/K_{i(inact)}$)	0.55 min^{-1} μM^{-1}	1.70 min^{-1} μM^{-1}
CoREST-LSD1 (IC_{50})	1.8 ± 0.3 μM	0.33 ± 0.05 μM
isol. HDAC1 (IC_{50})	0.158 ± 0.003 μM	0.147 ± 0.007 μM
CoREST-HDAC1 (IC_{50})	0.230 ± 0.014 μM	0.206 ± 0.035 μM

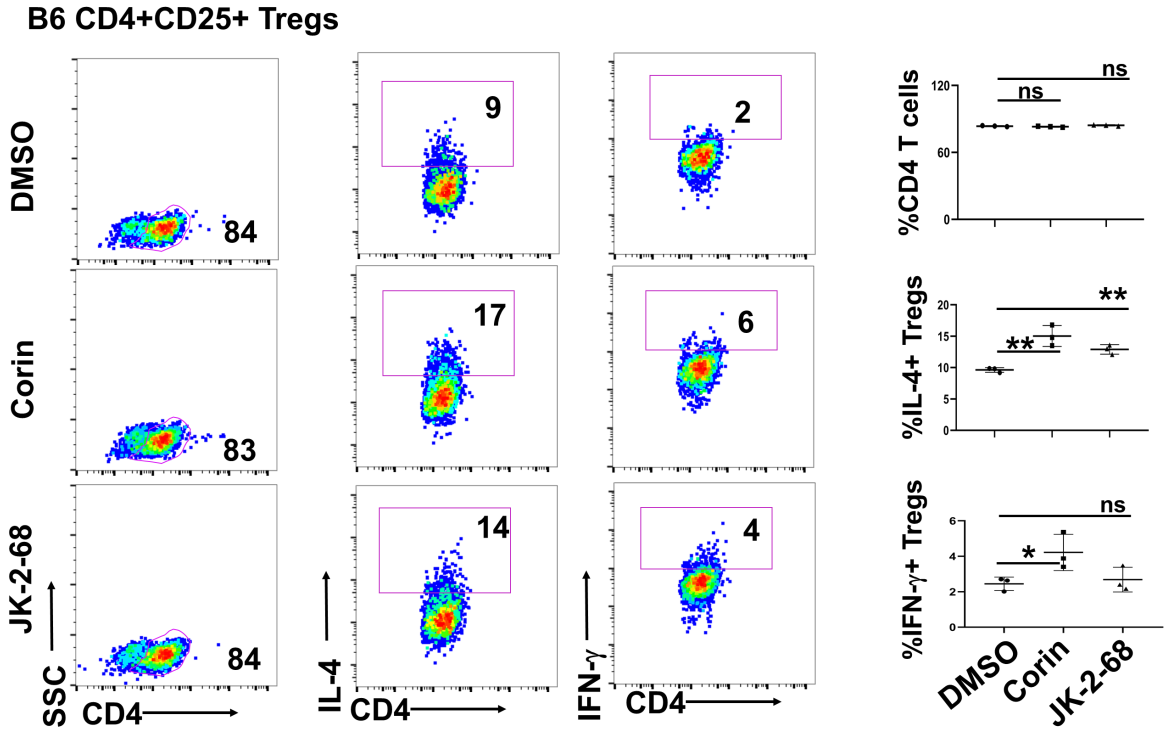
Supplemental Figure 8. Inhibitory properties for JK-2-68 and JKD-1-51 against isolated CoREST complex subunits (isol.) and CoREST ternary complex-bound LSD1 and HDAC1, as reported (33).



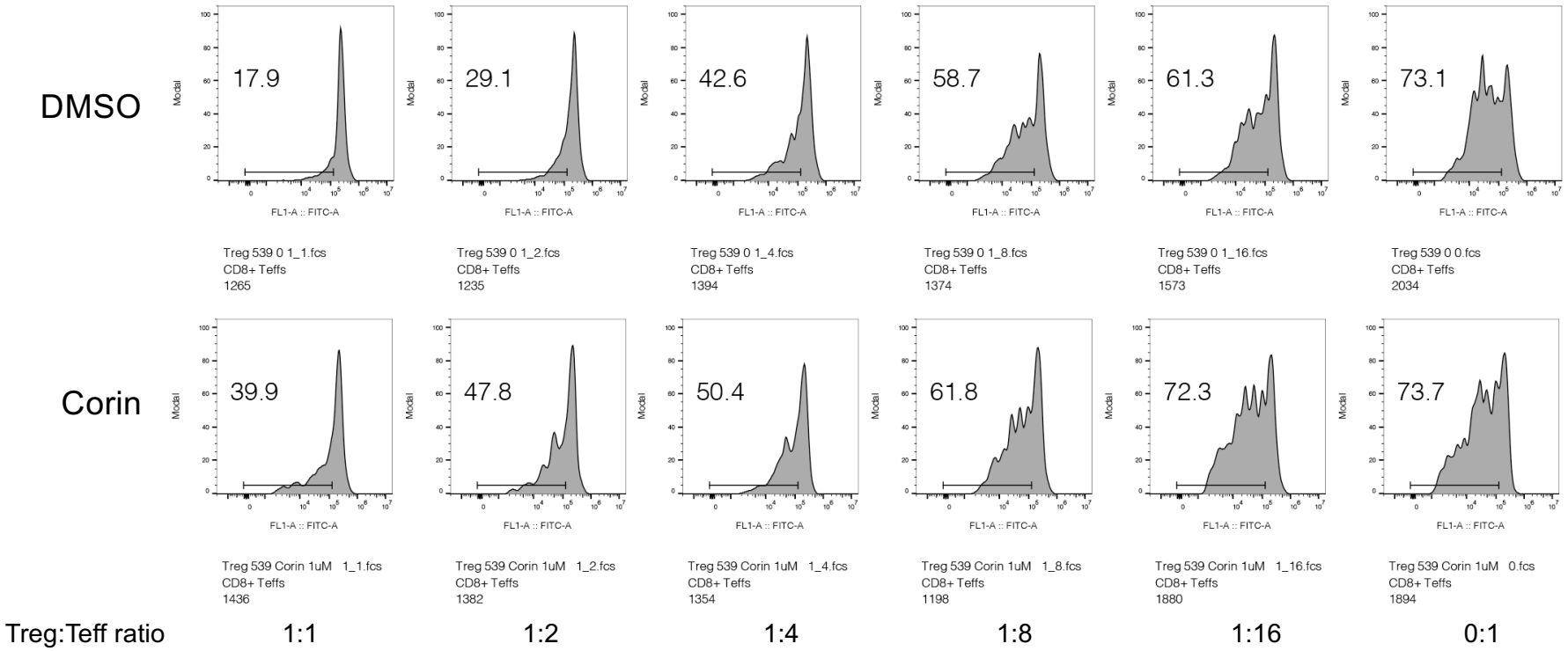
Supplemental Figure 9: CoREST complex inhibitors impair Treg suppressive function. Treg suppression assay using Tregs from lymph nodes and spleens of WT and Rcor1-/- mice, treated with JK-2-68 or corin (5 µM) for 2 h, washed and cultured for 72 h. Representative data for JK-2-68 shown in panel (A) and for corin in panel (B); the percentage of proliferating WT CD4 T cells is shown for each ratio of Treg:Teff cells. Statistics for corin data are shown in panel (C); mean ± SD, 3 mice/group; student's t-test for unpaired data; *$p<0.05$, **$p<0.01$ or ns (not significant) vs. WT. Panel (D) shows how corin (10 mg/kg/d, 14 d) impaired cardiac allograft survival ($p<0.01$) in the adoptive transfer model (BALB/c->C57BL/6 Rag1-/- mice, see text).



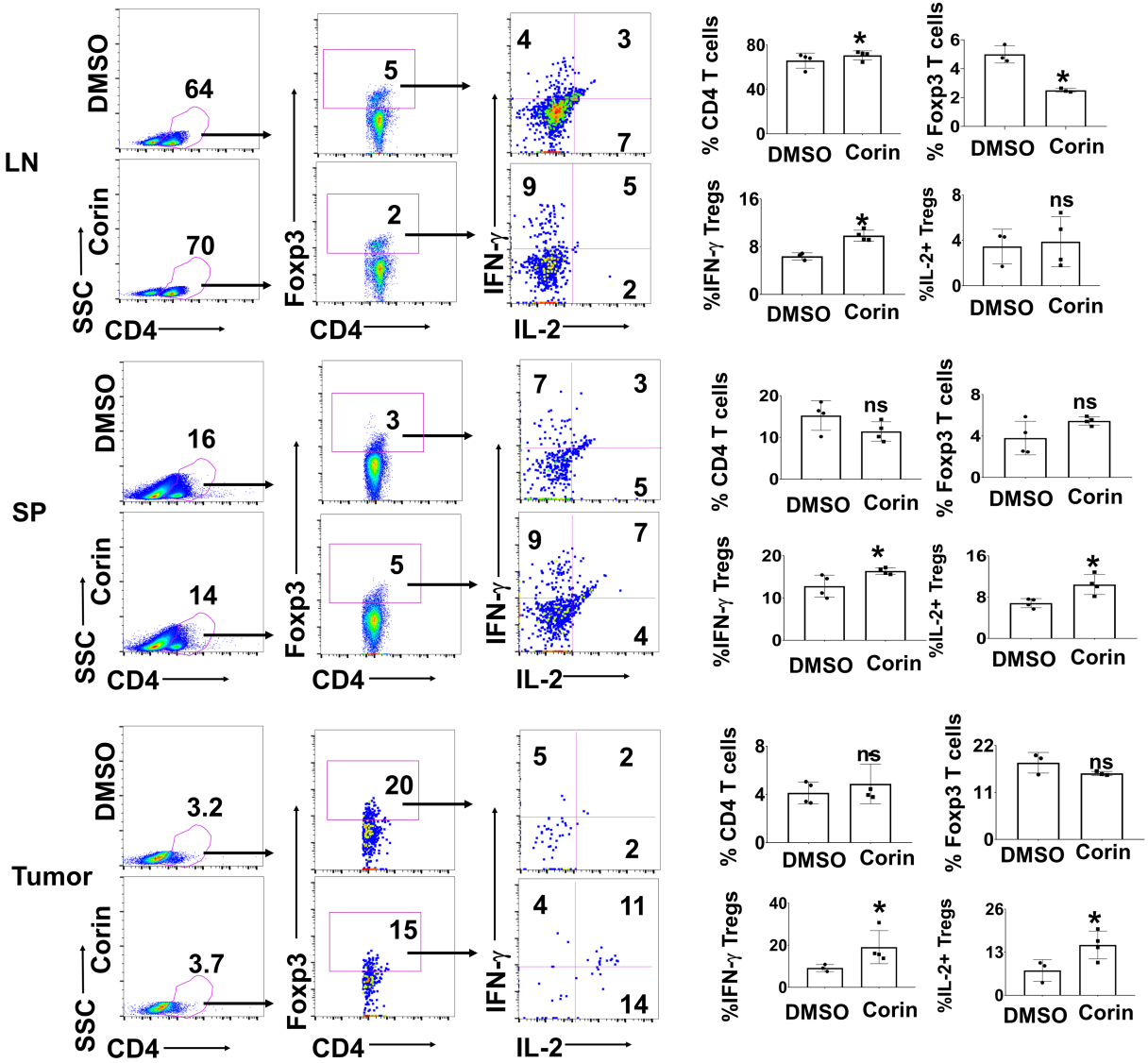
Supplemental Figure 10: Corin had no effect on the suppressive function of Rcor1-/- Tregs. Treg suppression assay using Tregs from lymph nodes and spleens of WT and Rcor1-/- mice, treated with Corin (5 μM) for 2 h, washed and cultured for 72 h. Representative data shown at left, along with the percentage of proliferating WT CD4 T cells in each panel, and statistics at right. Data are shown as mean ± SD, 3 mice/group. Student's t-test for unpaired data; *p<0.05, **p<0.01 or ns (not significant) vs. WT.



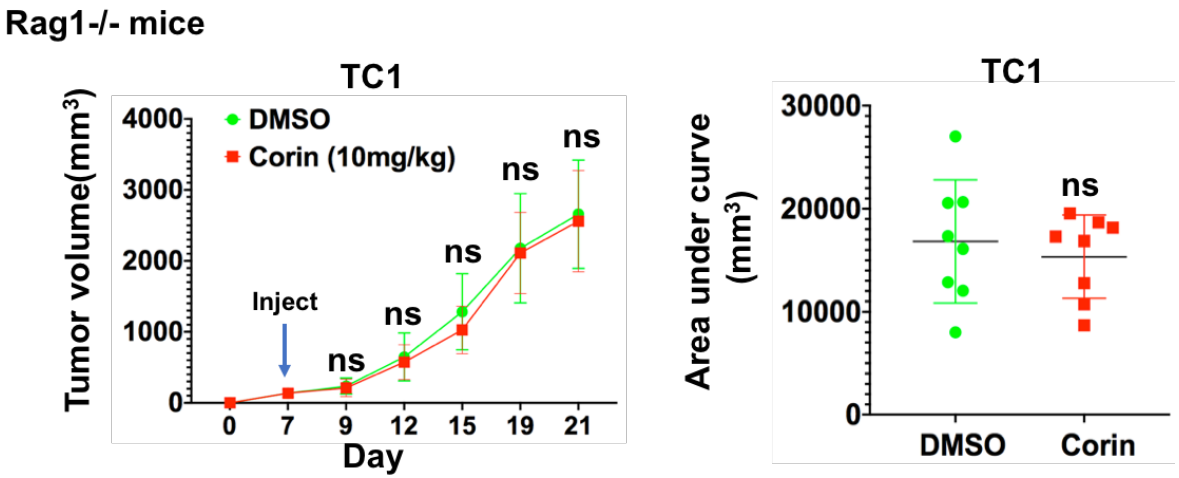
Supplemental Figure 11: CoREST inhibitors increased Treg production of pro-inflammatory cytokines. YFP+ Tregs isolated from C57BL/6 mice were treated with CoREST inhibitor JK-2-68 (5 μM) or Corin (5 μM) for 2 h, cultured with anti-CD3 for 20 h, and stimulated with PMA/ionomycin in the presence of Golgi-stop for 4 h. Percentages of cells gated on CD4+ T cells and %IL-4+ Treg and %IFN-γ+ Tregs are shown. Numbers in quadrants indicate the percentage of cell population, and data are shown as mean ± SD, 3 mice/group at 6-8 weeks of age, Student's t-test for unpaired data, *p<0.05, **p<0.01 compared with DMSO.



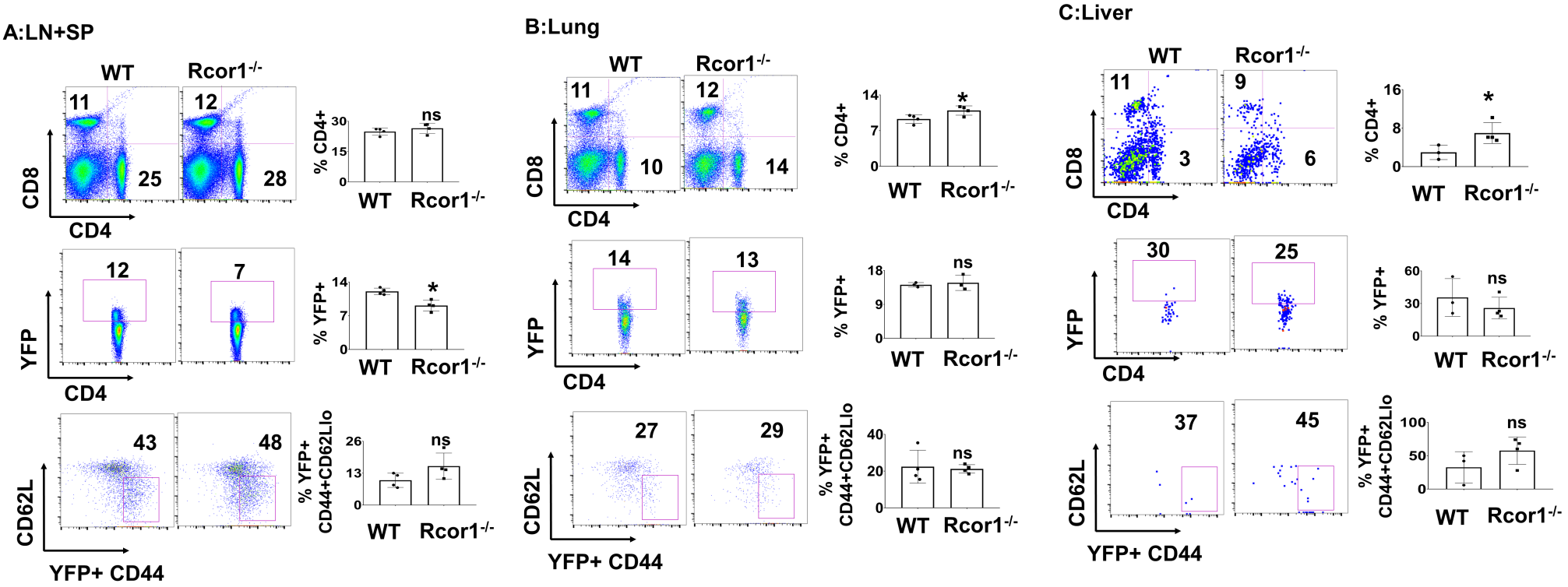
Supplemental Figure 12: Corin decreases the suppressive function of human Tregs. Freshly isolated human peripheral blood Tregs were incubated with Corin (1.0 μM) for 2.5 hours, washed and evaluated for suppression of human CD8+ T cell proliferation; results are representative of 12 separate assays.



Supplemental Figure 13: CoREST inhibitor increased Treg production of pro-inflammatory cytokines in lymphoid tissues and in the tumor environment. C57BL/6 mice bearing TC1 tumors were treated with DMSO or corin (10 mg/kg/day) for 10 d. Panels show % of CD4+Foxp3+, IL-2+ Tregs and IFN-γ+ Tregs in lymph nodes, spleens and tumors from corin and DMSO-treated groups. Data are shown as mean ± SD, 8-10 samples/group. Student's t-test for unpaired data; *p<0.05, **p<0.01 or ns (not significant) vs. control.



Supplemental Figure 14: CoREST inhibitor (Corin) did not affect the growth of tumors in immunodeficient (Rag1-/-) mice. Panels show TC1 tumor volumes and area-under-curve data in Rag1-/- mice treated with corin (10 mg/kg/day) vs. DMSO (n=8/group).



Supplemental Figure 15: Rcor1 deletion in Tregs had no major effects on various non-lymphoid tissues. Cells from lymph nodes, spleen, lung and liver were stained and %CD4+, %CD4+YFP+ and %CD4+YFP+CD44^{hi}CD62L^{lo} determined. Data shown as representative plots (left) and with statistical analyses (right); mean ± SD, 3 mice/group, with Student's t-test for unpaired data; *p<0.05 or ns (not significant) vs. WT.