Supplemental Figures and Figure Legends:

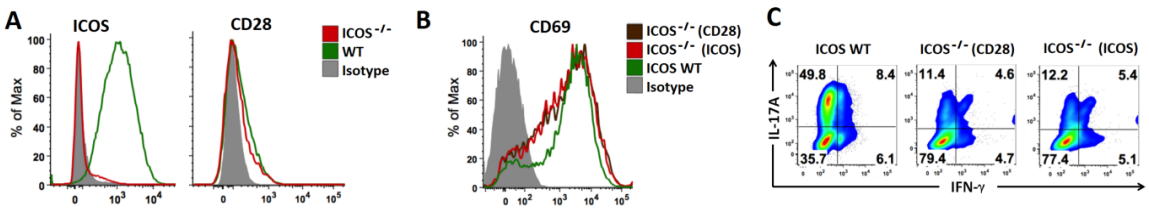

Figure S1. ICOS deficient Th17 cells become activated *in vitro*, yet secrete less IL-17A. A-B, ICOS-/-Th17 cells do not express ICOS, yet express CD28 and become activated. Activation status on WT and ICOS$^{-/-}$Th17 cells was revealed by their high CD69 expression. Representative flow cytometry analysis of (A) ICOS and CD28 expression on ICOS-/- and wild-type (WT) CD4^{+} T cells on day 0 and (B) CD69 on WT Th17 cells and ICOS-/- Th17 cells expanded with either ICOS or CD28 agonist on day 8 of culture. C, genetic ablation of ICOS diminishes Th17 function. (C) Representative flow analysis of IL-17A by IFN-γ secretion by WT Th17 cells and ICOS-/- Th17 cells expanded with either ICOS or CD28 agonist on day 8 of culture.

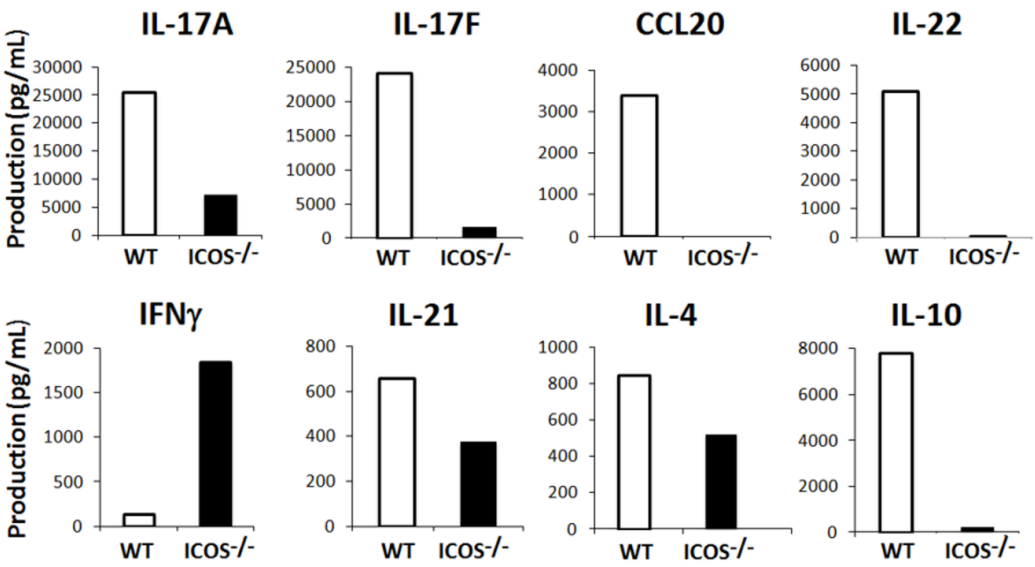

Figure S2. Th17 cells deficient in ICOS produce less IL-17A, IL17F, CCL20, IL-22, IL-10, IL-21, IL-4 but more IFNγ. Representative ELISA analysis of cytokine production on day 3 of peptide activated TRP-1 ICOS-/- and WT Th17 cultures. The experiment was performed twice with similar results.

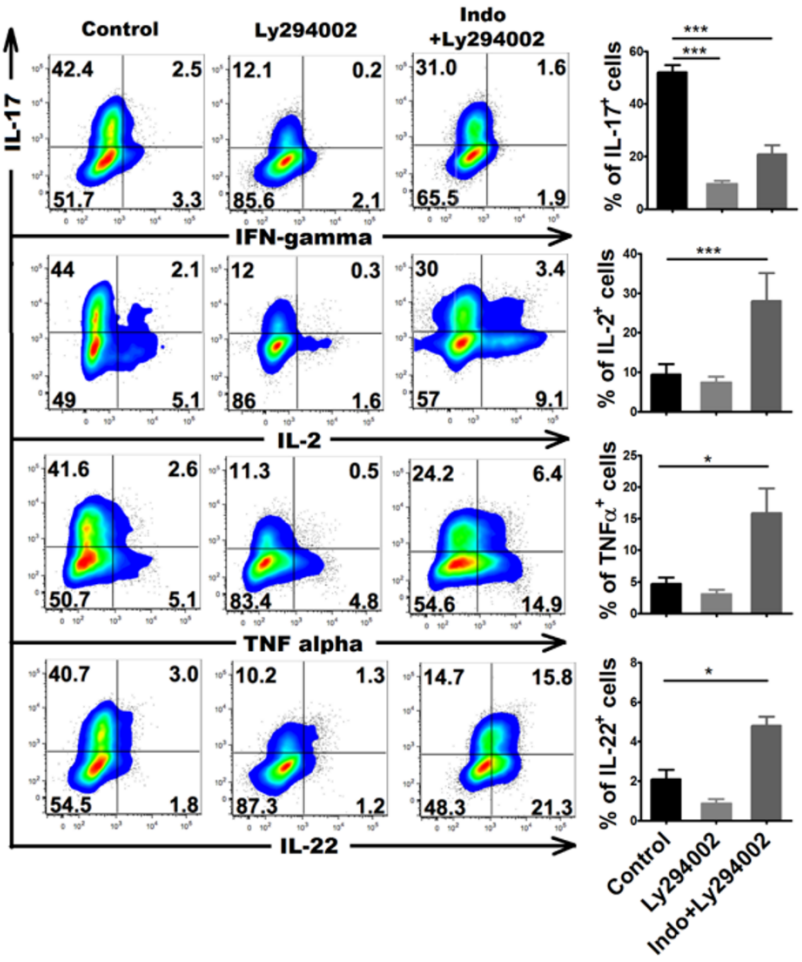

Figure S3. Pharmacologic inhibition of pan PI3 Kinases activity and Wnt/β-catenin signaling pathway alter the cytokine profile of ICOS stimulated Th17 cells. Inhibition of pan PI3 Kinases activity suppress IL-17A and IFN-γ production, but concomitant inhibition of β-catenin increase IL-2, TNF-α, IL-22 secretion by ICOS activated Th17 cells. Representative FACS plot and quantification of cytokine production in CD4+ cells polarized towards a Th17 phenotype, co-stimulated with ICOS agonist for 8 days (control) and expanded in the presence of total PI3K inhibitor (Ly294002, 10**μ**M) and specific β-catenin inhibitor – Indomethacin (Indo, 60**μ**M). Data represent mean ± SEM of at least three independent experiments. *,$P < 0.05$; ***,$P < 0.001$.

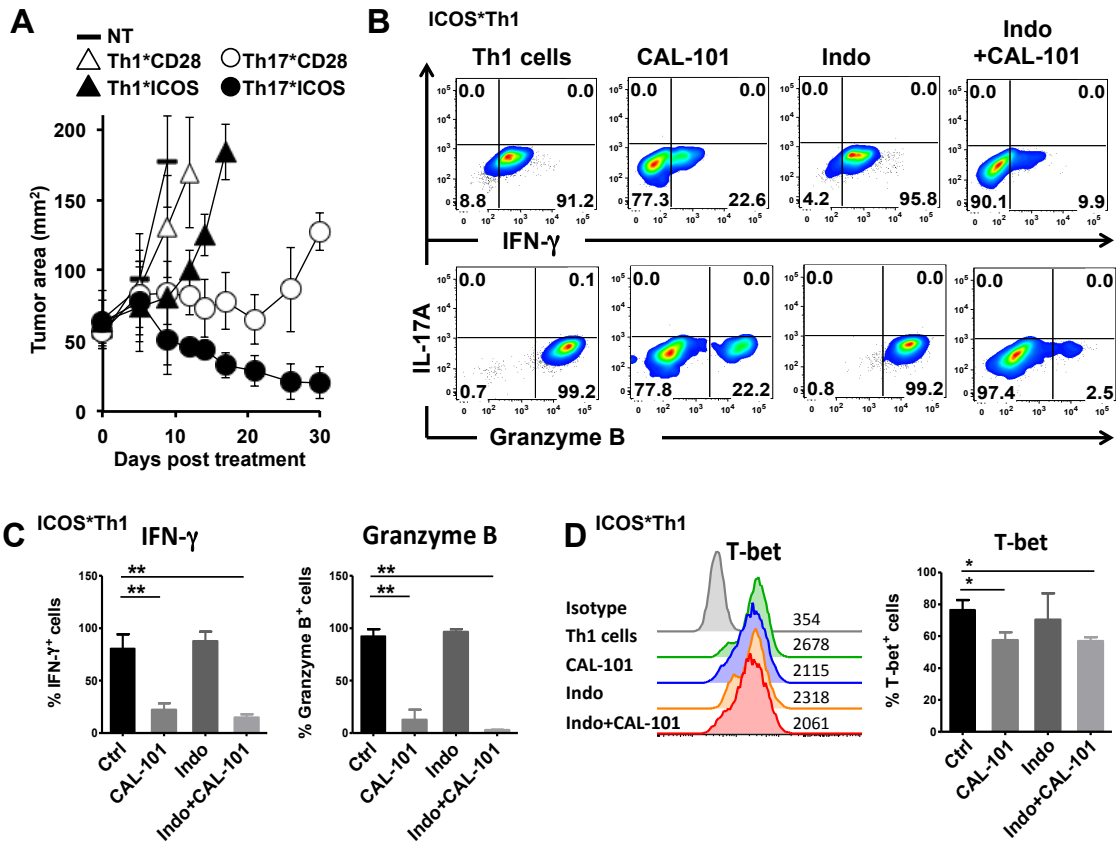

Figure S4. PI3K/p110δ and β-catenin co-inhibition reduces IFN-γ, granzyme B and T-bet in ICOS-stimulated Th1 cells. A. Th1 cells stimulated with ICOS are significantly more effective at slowing tumor growth than Th1 cells stimulated with CD28 ($P<0.05$). Th17 cells stimulated with ICOS are significantly more effective at regressing tumor than those stimulated with CD28 ($P<0.01$). Th17 cells stimulated with ICOS are significantly more effective at slowing tumor growth than Th1 cells stimulated with ICOS ($P<0.001$). **B**. Inhibition of p110δ and/or β-catenin activity suppresses IL-IFN-γ and Granzyme by flow cytometry. **C**. Representative quantification of cytokine production in TRP-1 $CD4^+$ T cells polarized towards a Th1 phenotype, co-stimulated with ICOS agonist for 8 days (control) and expanded in the presence of p110δ inhibitor (CAL-101, 10μM) and specific β-catenin inhibitor – Indomethacin (Indo, 60μM). **D**. Inhibition of p110δ and/or β-catenin activity suppresses T-bet by flow cytometry and represented by quantification in $CD4^+$ T cells polarized towards a Th1 phenotype, co-stimulated with ICOS agonist for 8 days (control) and expanded in the presence of p110δ inhibitor (CAL-101, 10μM) and specific β-catenin inhibitor–Indomethacin (Indo, 60μM). Data represent mean ± SEM of at least three independent experiments. *,$P < 0.05$; **,$P < 0.01$.

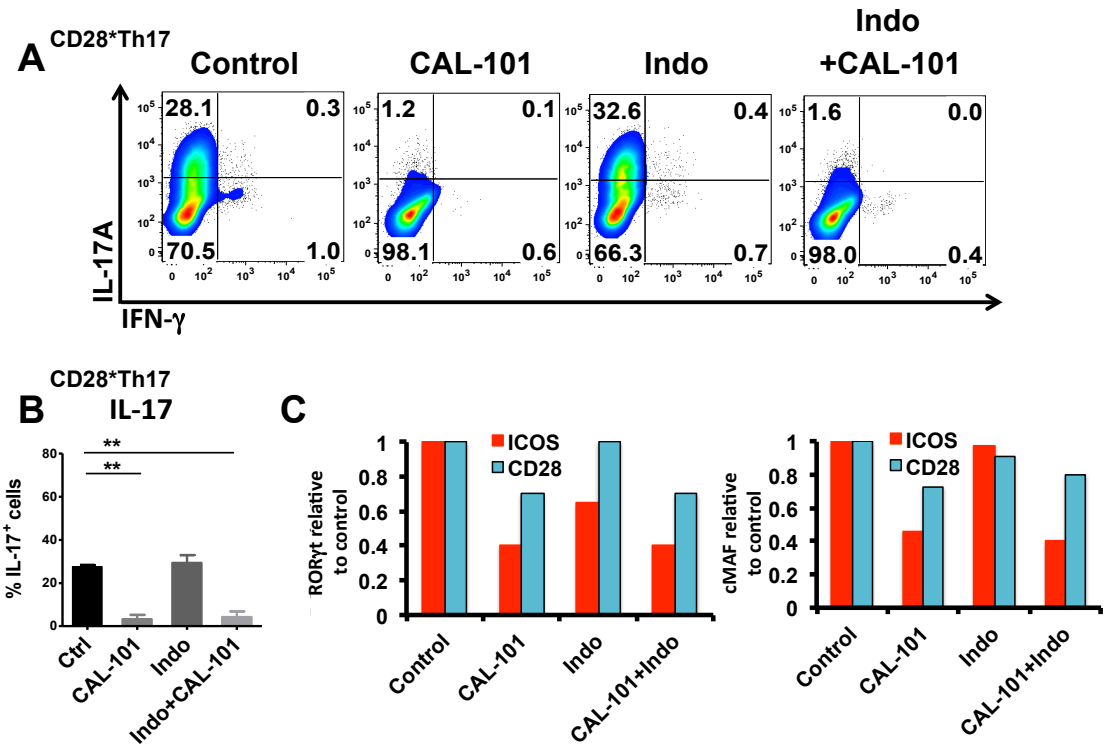

Figure S5. p110δ and β-catenin co-inhibition reduces IL-17A significantly yet slightly decreases RORγt and cMaf in CD28-stimulated Th17 cells. A-B. Inhibition of p110δ and/or β-catenin suppresses IL-I7A by flow cytometry and representative quantification of IL-17 production in $CD4^+$ T cells polarized towards a Th17 phenotype, co-stimulated with CD28 agonist for 8 days (control) and expanded in the presence of p110δ inhibitor (CAL-101, 10**μ**M) and specific β-catenin inhibitor – Indomethacin (Indo, 60**μ**M). Data represent mean ± SEM of at least three independent experiments. *,$P < 0.05$; **,$P < 0.01$. **C**. Drugs suppress Th17 transcription in Th17 cells stimulated with ICOS compared to those stimulated with CD28. Th17 cells stimulated with CD28 relative to those stimulated with ICOS. $CD4^+$ TRP-1 cells polarized towards a Th17 phenotype, co-stimulated with CD28 or ICOS agonist for 8 days (control) and expanded in the presence of p110δ inhibitor (CAL-101, 10**μ**M) and specific β-catenin inhibitor – Indomethacin (Indo, 60**μ**M).

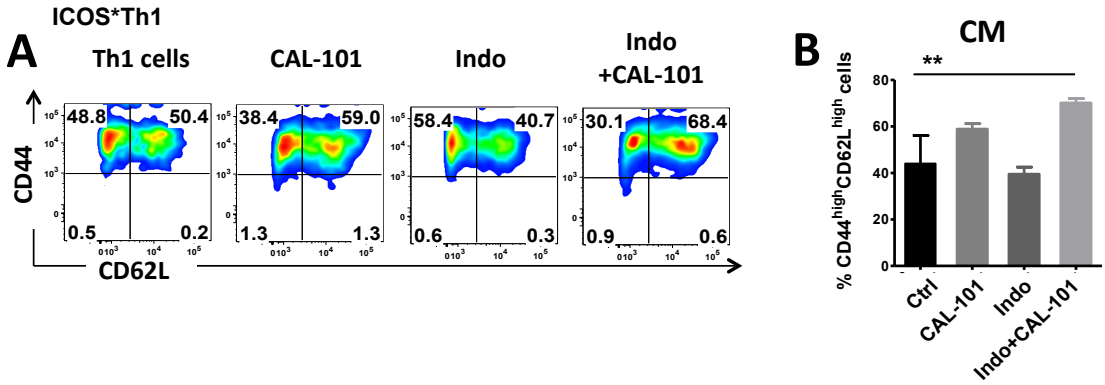

Figure S6. CD62L is elevated in ICOS-stimulated Th1 cells co-inhibited of p110δ and β-catenin, as shown by flow (**A**). **B**. Data represents mean ± SEM of at least three independent experiments. **,$P < 0.01$.

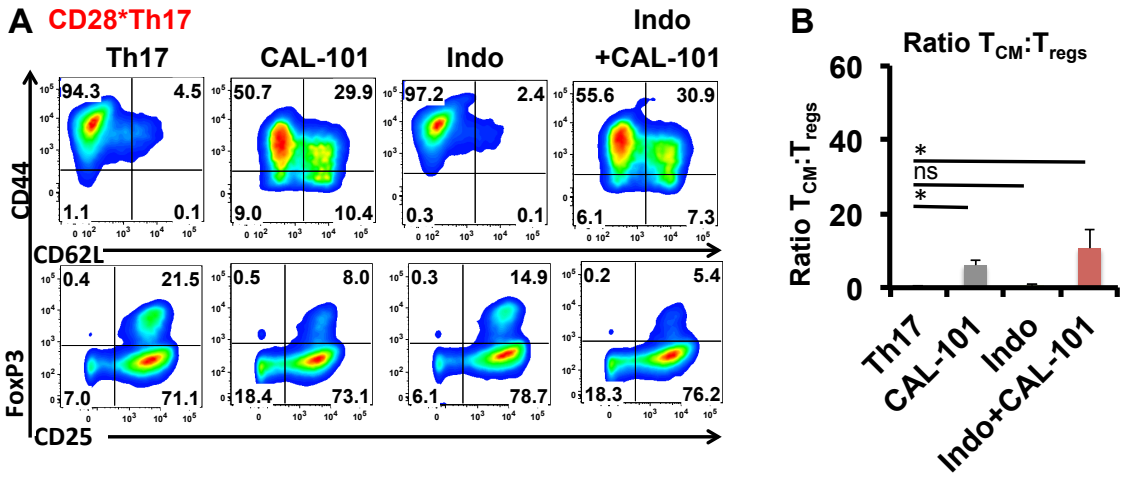

Figure S7. CD62L is elevated while FoxP3 is decreased in CD28-stimulated Th17 cells co-inhibited of p110δ and β-catenin, as shown by flow (**A**). **B**. Relative CD62L+ to FoxP3 Th17 cells represented as mean ± SEM of three independent experiments. *,P < 0.05.

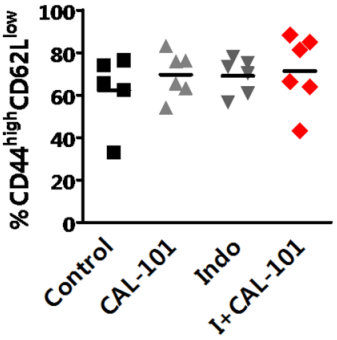

Figure S8. Th17 cells stimulated with ICOS and primed with Indo and CAL-101 acquire an effector memory phenotype *in vivo*. Quantification of donor Vβ14+ tumor-specific $CD44^{high}CD62L^{low}$ effector memory cells in the spleen 14 days post ACT of ICOS activated Th17 cells primed with the inhibitors or untreated, as indicated.

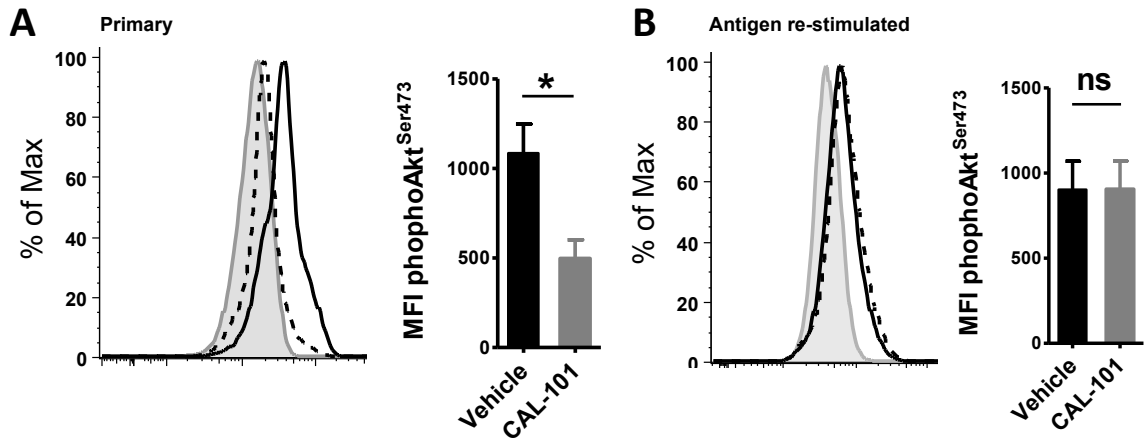

Figure S9. Phosphorylated Akt (serine 473) is impaired in ICOS-stimulated Th17 cells inhibited of p110δ (A) but rebounds post peptide re-stimulation (B), as shown by flow (**A-B**) and graphically represented as mean ± SEM of three independent experiments. *,$P < 0.05$.

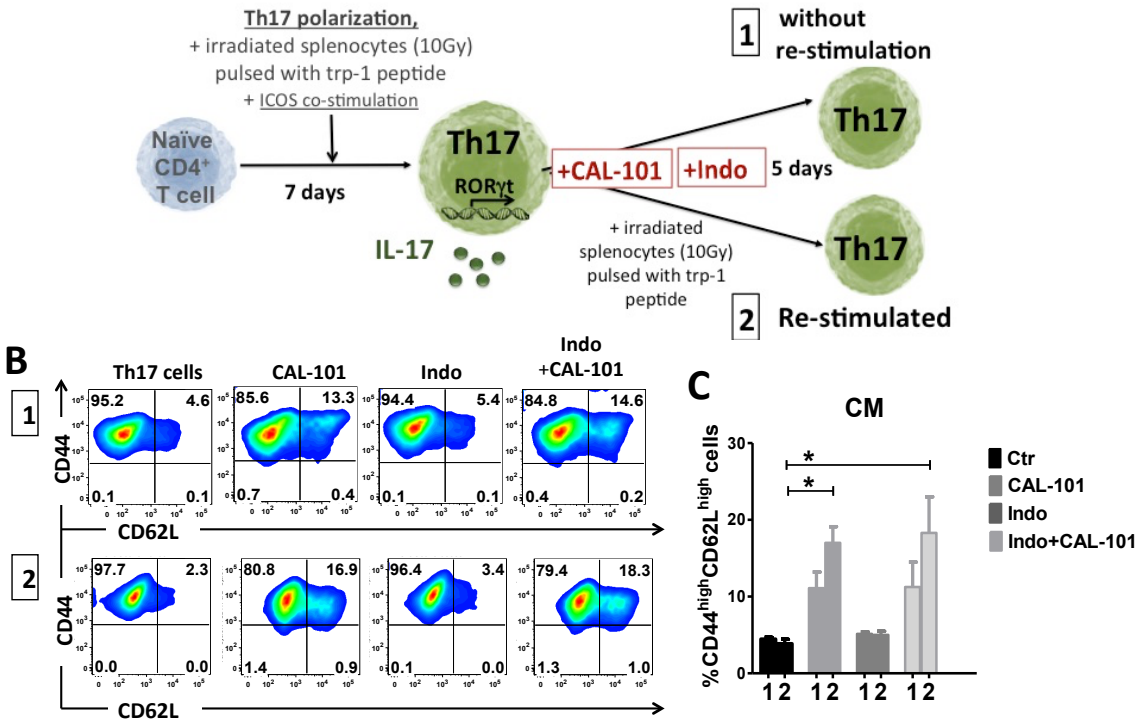

Figure S10. Treating differentiated Th17 cells with p110δ and β-catenin inhibitors rescues the expansion of the few central memory cells in the culture. As in **A**, CAL-101 and/or Indo was added to ICOS-stimulated Th17 cells that were expanded for 7 days, generating of fully differentiated Th17 cells (~95% CD62L-). These drugs were added to one-week cultures either 1) with or 2) without peptide re-stimulation and expanded for an additional 5 days (12 days total). CAL-101 or CAL-101 plus Indo treatment supported the outgrowth of $CD62L^{+}$ cells from this population, regardless of reactivation. As shown by flow (**B**) and graphically (**C**), differentiated Th17 cultures expressed more CD62L when treated with CAL-101 or CAL-101 plus Indo. Represented as mean ± SEM of two independent experiments. *,$P < 0.05$.