Supplemental methods

Reagents: Recombinant murine CXCL12, CXCL13, and IL-2 were purchased from Peprotech, Inc (Rocky Hill, NJ). IL-2 antibody (JES6-1) was purchased from Bio X Cell (West Lebanon, NH). Epoxy Dynabeads (DYNAL®, Dynabeads®) were purchased from Thermo Fisher Scientific (Waltham, MA). Thymidine, [Methyl-3H] was purchased from PerkinElmer (Waltham, MA).

Mice: Mice homozygous for the $Rag1^{tm1Mom}$ mutation (RAG knock out) on C57BL/6J back ground were purchased from The Jackson Laboratory (Bar Harbor, ME) (85).

Migration assay: Negative selected splenic B cells were suspended in RPMI at $5x10^6$ cells/mL, and 100µl was placed in the upper chamber of a 24-transwell plate with a 5µm filter. Chambers were placed into wells containing media containing no chemokine (control), recombinant mouse CXCL12 (100ng/mL) or CXCL13 (100ng/mL). Migration was permitted for 3 hours, and cells in the lower chamber were collected, stained with Live-dead staining, CD45, CD19. Cells were counted for 60 seconds on high speed on a Beckman Coulter FC500 flow cytometer. B cells were doublet-discriminated and then gated on the live-$CD45^+CD19^+$. Absolute counts of B cells were calculated using counting beads (CountBright absolute counting beads; Invitrogen, Thermo Fisher Scientific, Waltham, MA) following the manufacturer's recommendations.

In vivo regulatory T cells induction: $p110\delta^{WT/WT}$ and $p110\delta^{D910A/D910A}$ mice were injected intra-peritoneally with IL-2:IL-2 antibody complex (1ug: 5ug) daily for five days as previously described (86). Spleens were harvested on day six for further isolation.

Murine T cell purification: For regulatory T cells, spleen lymphocytes were first negatively selected for $CD4^+$ T Cells using EasySep™ Mouse $CD4^+$ T Cell Isolation Kits according to the manufacturers' instructions (StemCell Technologies, Vancouver, BC). $CD25^-$ and $CD25^+$ cells were further sorted using FACSAria (BD Biosciences, Franklin Lakes, NJ). For $CD3^+$, $CD4^+$ and $CD8^+$ T cells, spleen lymphocytes from $p110\delta^{WT/WT}$ and $p110\delta^{D910A/D910A}$ mice were first negatively selected for pan T cells ($CD3^+$) using EasySep™ Mouse T Cell Isolation Kits (StemCell Technologies, Vancouver, BC), doublet-discriminated, live-$CD3^+CD4^+$ or live-$CD3^+CD8^+$ T cells were further sorted using FACSAria (BD Biosciences, Franklin Lakes, NJ).

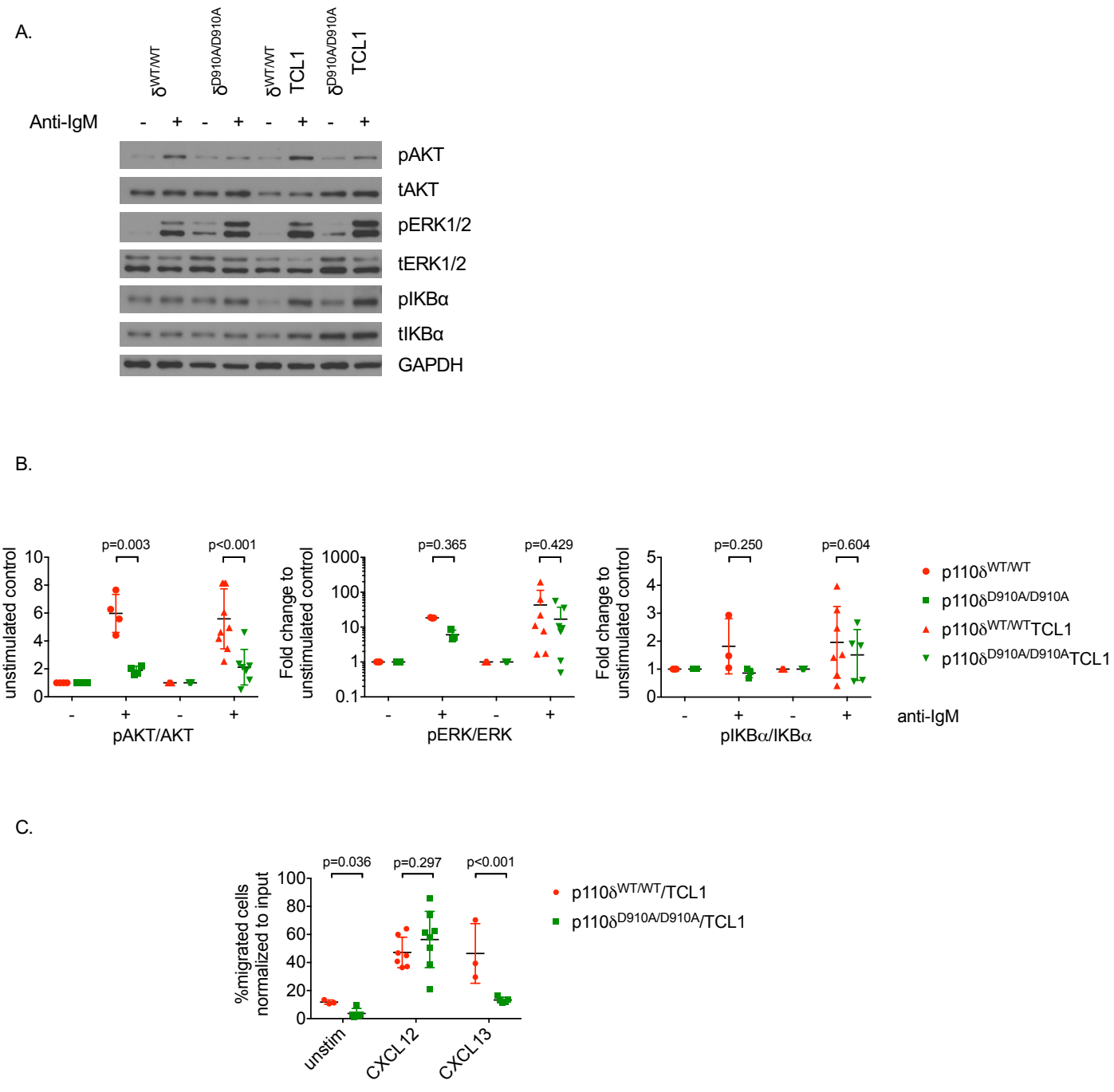
Regulatory T cells suppression assays: FACS sorted $CD4^+CD25^+$ T cells were cultured with $CD4^+CD25^-$ T cells in the presence of Epoxy Dynabeads (DYNAL®, Dynabeads®) coated with anti-CD3 and anti-CD28 mAbs as previously described (one bead per cell) (87). After 3 days, the incorporation of Tritiated

thymidine ([^{3}H]TdR) (PerkinElmer, Waltham, MA) was measured after 12 hours incubation on TopCount® NXT™ (Packard, Packard Bioscience Co, Meriden, CT).

RAG knockout T cell adoptive transfer assay: FACS sorted $CD3^+$, $CD4^+$ and $CD8^+$ T cells ($1x10^6$) from $p110\delta^{WT/WT}$ and $p110\delta^{D910A/D910A}$ were injected in to RAG knockout mice through retro-orbital injections. One week later, TCL1 leukemia cells ($1x10^5$) from a predetermined donor were injected through a lateral tail vein.

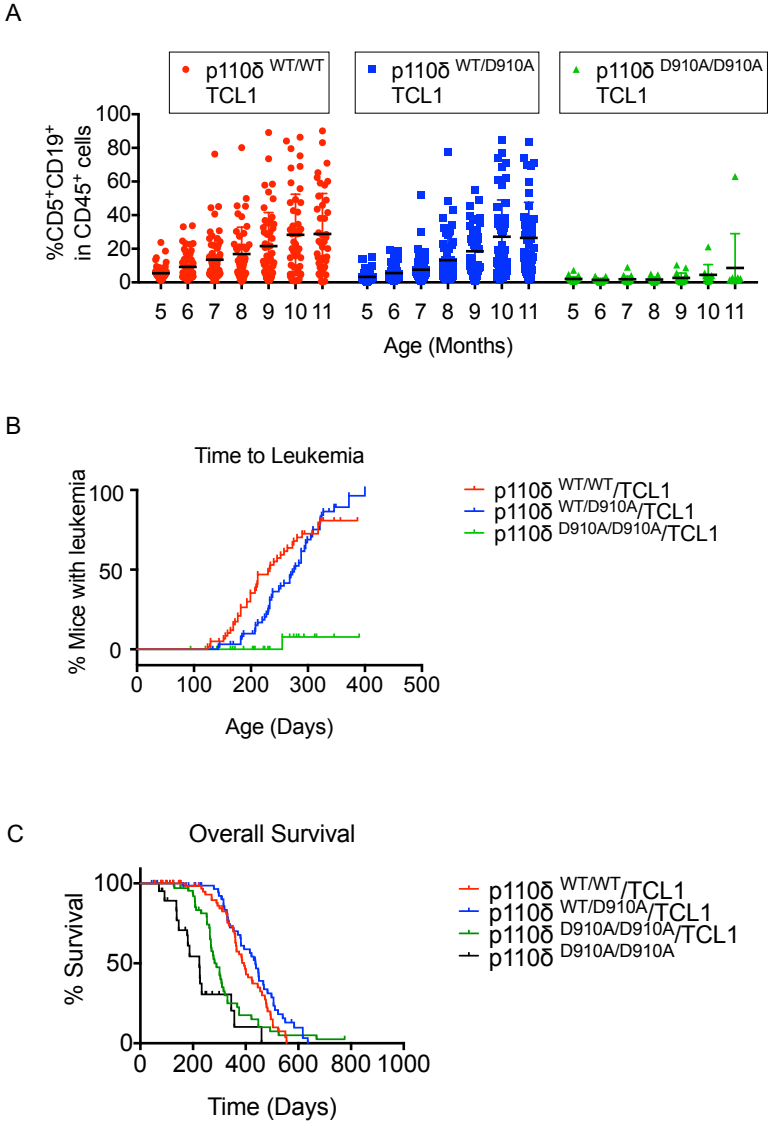
Regulatory T cells adoptive transfer study: $p110\delta^{WT/WT}$ and $p110\delta^{D910A/D910A}$ were intra-peritoneally injected with IL-2:IL-2Ab complex as previously described (86) prior spleen harvesting. $CD4^+CD25^+$ T cells ($1x10^5$) from $p110\delta^{WT/WT}$ and $p110\delta^{D910A/D910A}$ mice were engrafted into $p110\delta^{D910A/D910A}$ mice through retro-orbital injections. Three days later, TCL1 leukemia cells ($1x10^5$) from a predetermined donor were injected through a lateral tail vein.

Supplemental Figure 1



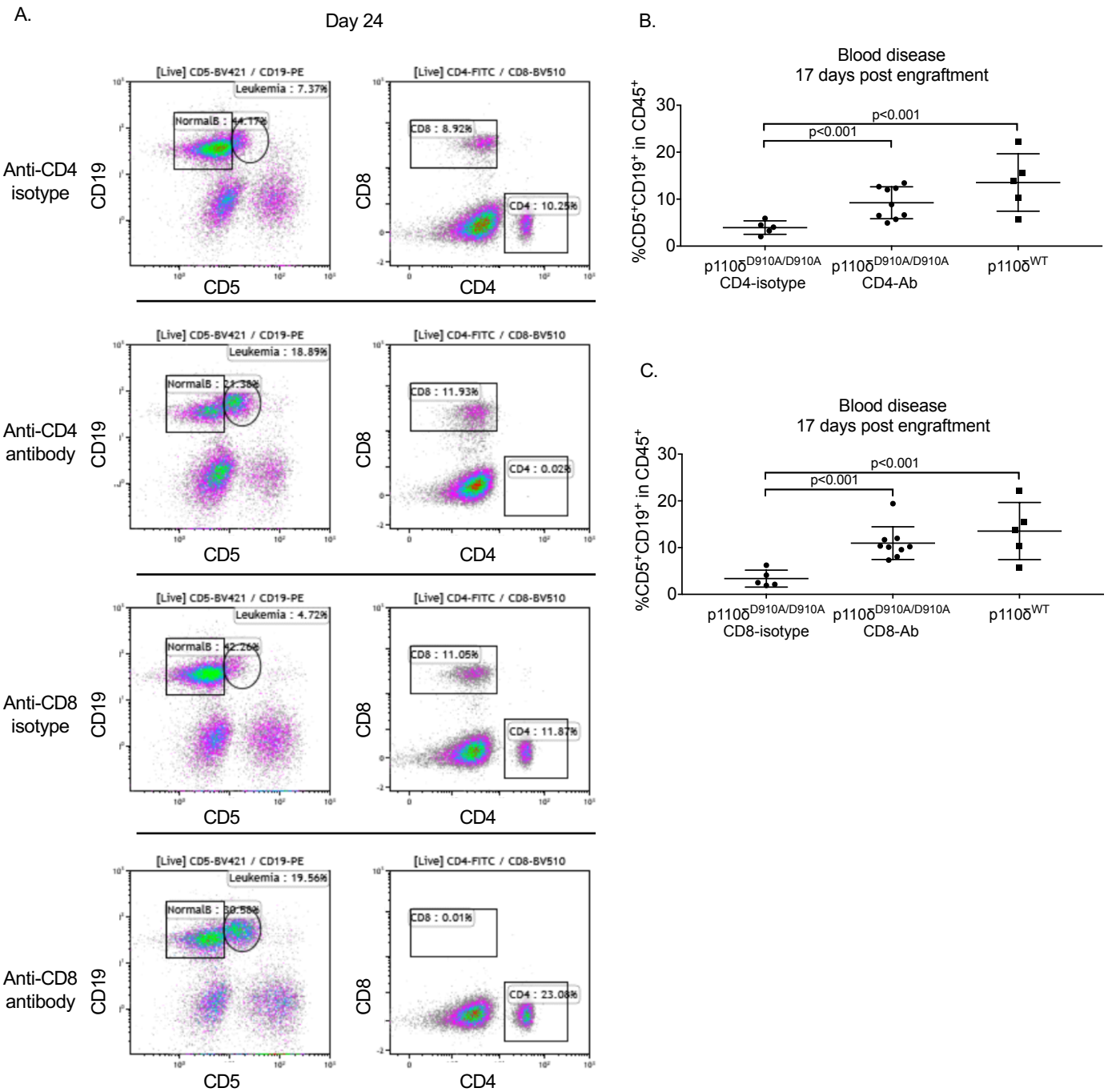
Supplemental Figure 1 (related to Figure 1): (A) B cells were purified from spleens of 3- to 5-month old p110δ$^{WT/WT}$, p110δ$^{D910A/D910A}$, p110δ$^{WT/WT}$/TCL1 and p110δ$^{D910A/D910A}$/TCL1 mice and stimulated with anti-IgM (10 μg/ml) for 15 minutes. Cell lysates were immunoblotted for pATK^{S473}, total AKT, pERK1/2$^{Thr202/Tyr204}$, total ERK, pIKBα$^{Ser32/36}$, total IKBα, and GAPDH. The blots are representative of four independent experiments. (B) Densitometry analysis was conducted using ImageJ analysis software (National Institutes of Health). All data were normalized to unstimulated control. Stimulated samples from 4-9 mice from each genotype were included for statistical analysis. Analysis of variance (ANOVA) methods were used to compare condition means; data were log-transformed to stabilize variance. (C) B cells were purified from spleens of 3- to 5-month old p110δ$^{WT/WT}$/TCL1 and p110δ$^{D910A/D910A}$/TCL1 mice and migrated towards CXCL12 and CXCL13 for 3 hours. Migrated Live-CD45^{+}CD19^{+} cells were counted and normalized to total input. Estimated differences between two groups using ANOVA based on the log-transformed data. Bars represent mean ± SD

Supplemental Figure 2



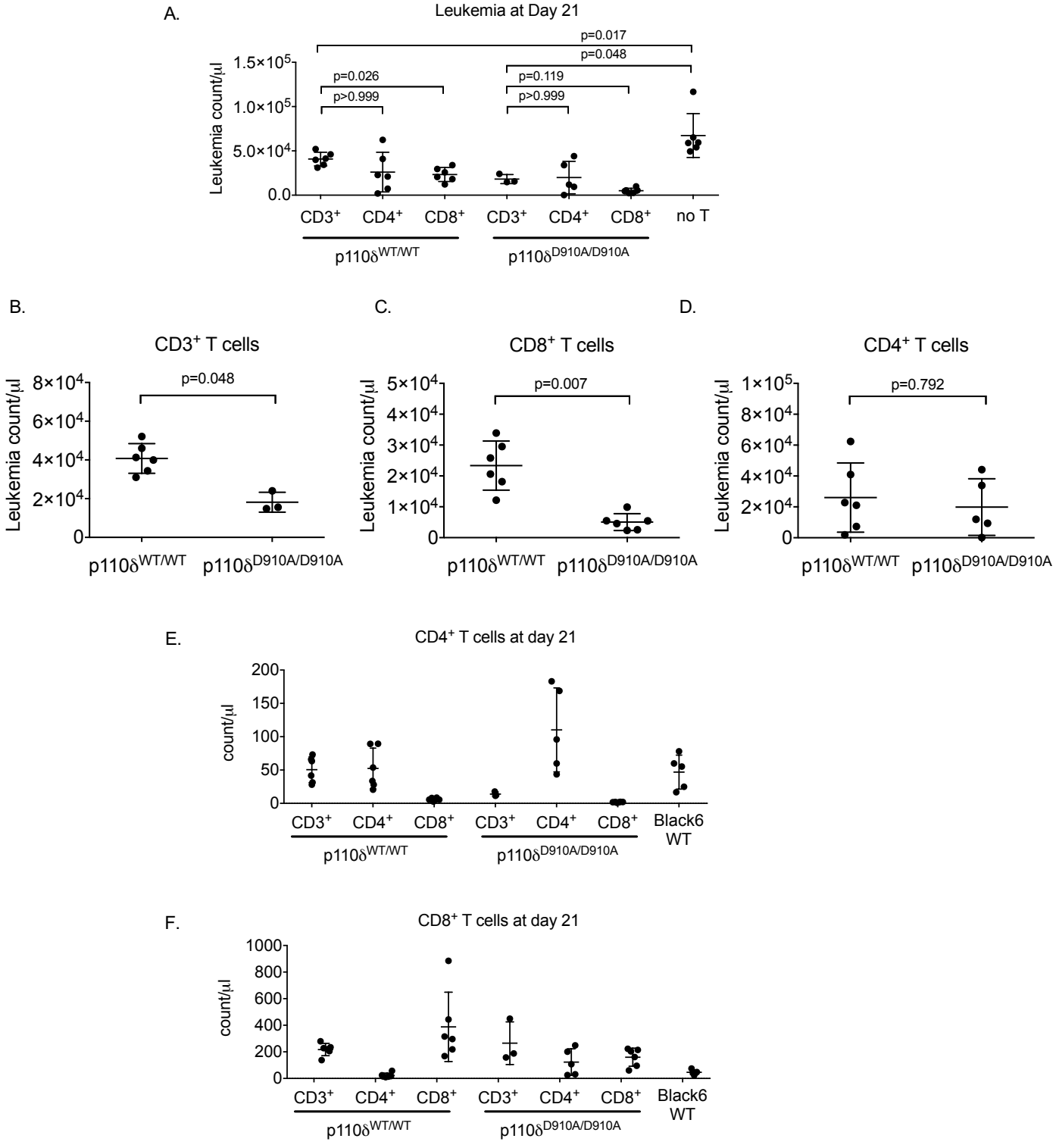
Supplemental Figure 2 (related to Figure 2):
(A) Mice of p110δ $^{WT/WT}$/TCL1 (n=43-65) , p110δ $^{WT/D910A}$/TCL1 (n=45-59) and p110δ $^{D910A/D910A}$/TCL1 (n=9-39) were monitored monthly for leukemia development through immunophenotyping of $CD5^+CD19^+$ cells in the peripheral blood by flow cytometry. Bars represent mean ± SD. (B) Time to leukemia (leukemia is determined by having ≥10% $CD5^+CD19^+$ of total $CD45^+$ cells in blood) of p110δ$^{WT/WT}$/TCL1 (n=61), p110δ$^{WT/D910A}$/TCL1 (n=65), p110δ$^{D910A/D910A}$/TCL1 (n=44) mice. (*P<0.001 comparing p110δ$^{D910A/D910A}$/TCL1 with p110δ$^{WT/WT}$/TCL1) Kaplan-Meier method and the log-rank test were used to evaluated differences between curves. (C) Overall survival of p110δ$^{WT/WT}$/TCL1 (n=88), p110δ$^{WT/D910A}$/TCL1 (n=130), p110δ$^{D910A/D910A}$/TCL1 mice (n=79). (*p<0.001 comparing p110δ$^{D910A/D910A}$/TCL1 with p110δ$^{WT/WT}$/TCL1). Kaplan-Meier method and the log-rank test were used to evaluated differences between curves.

Supplemental Figure 3



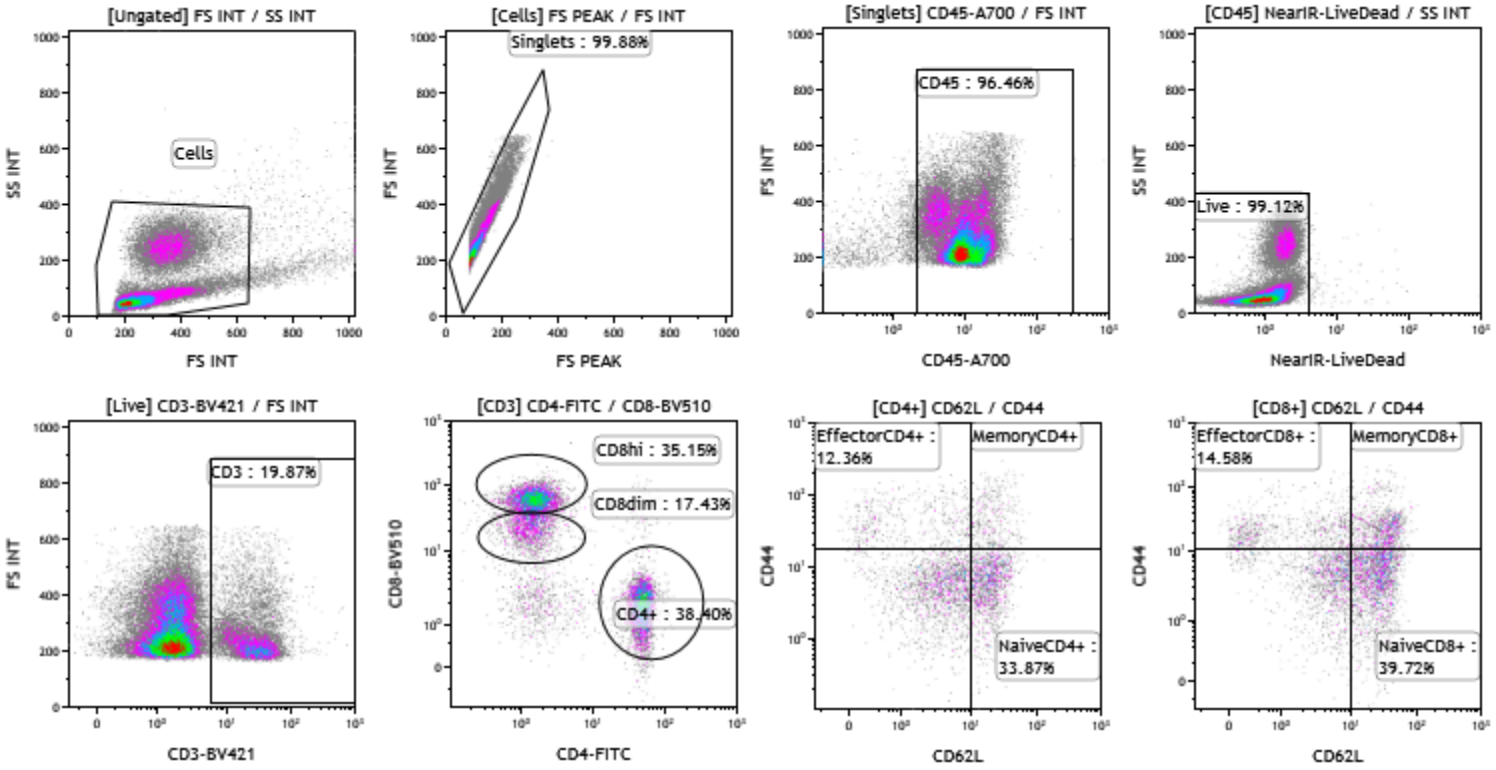
Supplemental Figure 3 (related to Figure 5): p110δ$^{WT/WT}$ and p110δ$^{D910A/D910A}$ mice adoptively transferred with Eμ-TCL1 leukemia cells. p110δ$^{D910A/D910A}$ mice were subjected to CD4 or CD8 depleting antibodies injections once in three days. Isotype controls were included as controls. Blood from each group were stained with near-IR live dead staining, CD45$^+$, CD4$^+$, CD8$^+$, CD5$^+$ and CD19$^+$ fluorescent antibody in the same tube. After staining, red cells were lysed with RBC lysis buffer. (A) Cells were gated on CD45$^+$ and live population and subjected to CD4$^+$/CD8$^+$ and CD5$^+$/CD19$^+$ analysis to confirm successful CD4 or CD8 depletion and assess CD5$^+$CD19$^+$ leukemia burden. Data are representative from treatment groups of p110δ$^{D910A/D910A}$ mice on day 24 post engraftment. (B) And (C) Blood disease of p110δ$^{WT/WT}$ mice and p110δ$^{D910A/D910A}$ mice receiving CD4 or CD8 depleting antibodies and their corresponding isotype. Group differences estimated using a mixed effects models. Bars represent mean ± SD.

Supplemental Figure 4



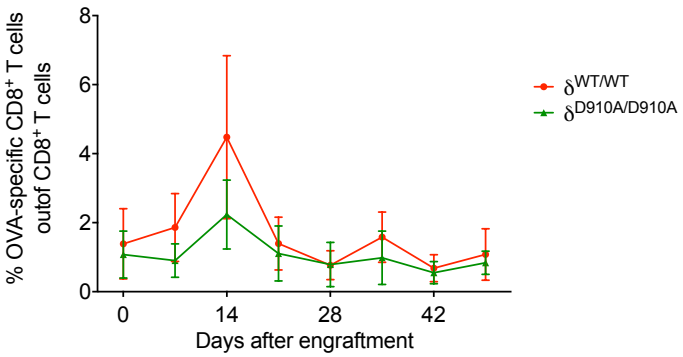
Supplemental Figure 4 (related to Figure 5): Recombination activating gene 1 knock-out ($RAG^{-/-}$) mice were reconstituted with $CD3^+$, $CD4^+$ and $CD8^+$ T cells from $p110\delta^{WT/WT}$ and $p110\delta^{D910A/D910A}$ mice followed by TCL1 leukemia engraftment. (A) Blood disease (total $CD5^+CD19^+$ cells counts) of all groups of $RAG^{-/-}$ mice. (B)-(D) Blood disease of $RAG^{-/-}$ mice from (A) were graphed to demonstrate the difference of $CD3^+$, $CD4^+$ and $CD8^+$ T cells between $p110\delta^{WT/WT}$ and $p110\delta^{D910A/D910A}$ mice. Blood $CD4^+$ T cell count (E) and $CD8^+$ T cell count (F) were confirmed in $RAG^{-/-}$ mice reconstituted with $CD3^+$, $CD4^+$ and $CD8^+$ T cells from $p110\delta^{WT/WT}$ and $p110\delta^{D910A/D910A}$ mice. The nonparametric Wilcoxon rank sum test as used to compare groups. P-values were adjusted for multiple comparisons using Holm's procedure. Bars represent mean ± SD.

Supplemental Figure 5



Supplemental Figure 5 (related to Figure 6): p110δ$^{WT/WT}$ and p110δ$^{D910A/D910A}$ mice after adoptively transferred with 2 × 10^{7} secondary Eμ-TCL1 leukemia cells. Blood were stained with near-IR live dead staining, CD45^{+}, CD4^{+}, CD8^{+}, CD5^{+} and CD19^{+} fluorescent antibody in the same tube. After staining, red cells were lysed with RBC lysis buffer and washed with PBS. Cells were gated on CD45^{+}, live population, CD3^{+} and subjected to CD4^{+}/CD8^{+} subsets for further analysis. . Data is representative flow cytometry analysis showing the gating strategy.

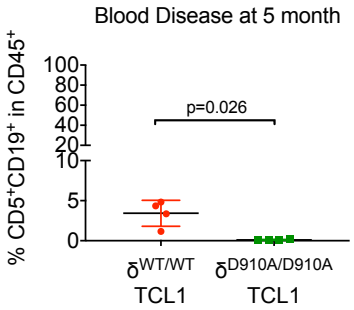
Supplemental Figure 6



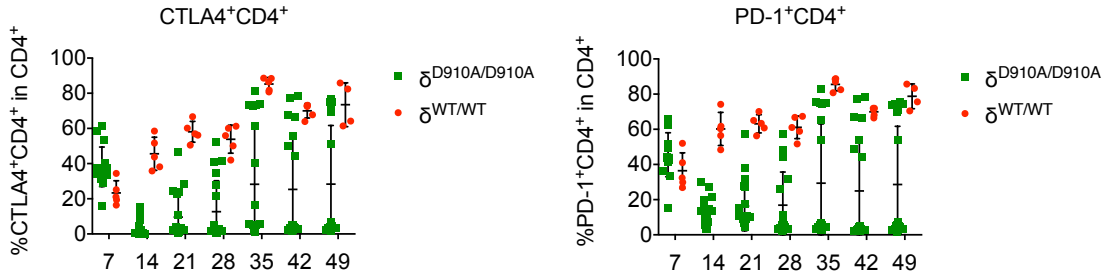
Supplemental Figure 6 (related to Figure 6): Repeat of p110$\delta^{WT/WT}$ (n=11) and p110$\delta^{D910A/D910A}$ (n=10) mice adoptively transferred with OVA-TCL1 cells as seen in Figure 5 (L) using a different OVA-TCL1 donor cell. OVA-specific $CD8^+$ T cells were examined weekly using fluorescent tagged OVA-tetramer by flow cytometry. Data represent mean ± SD.

Supplemental Figure 7

A.

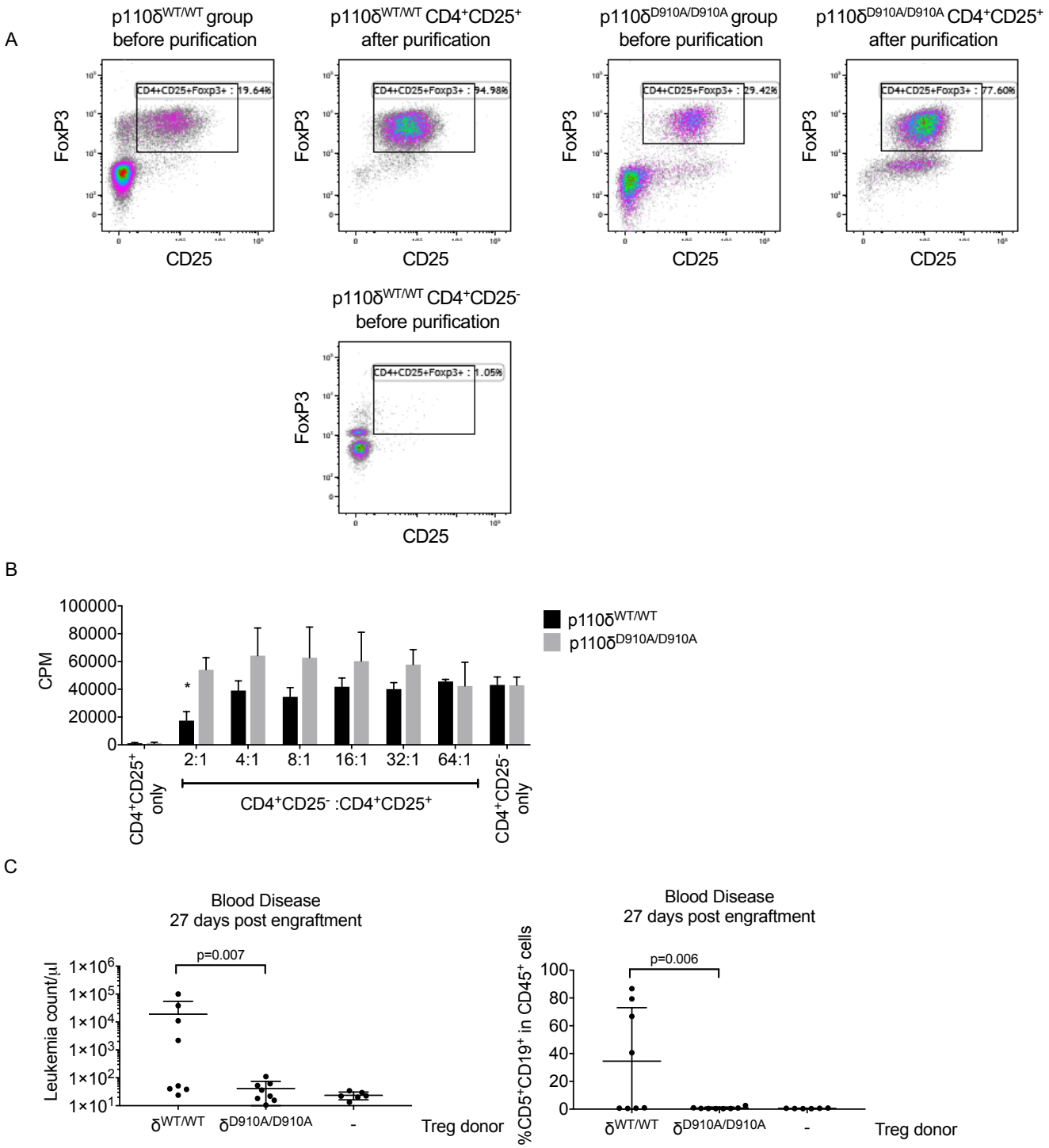


B.



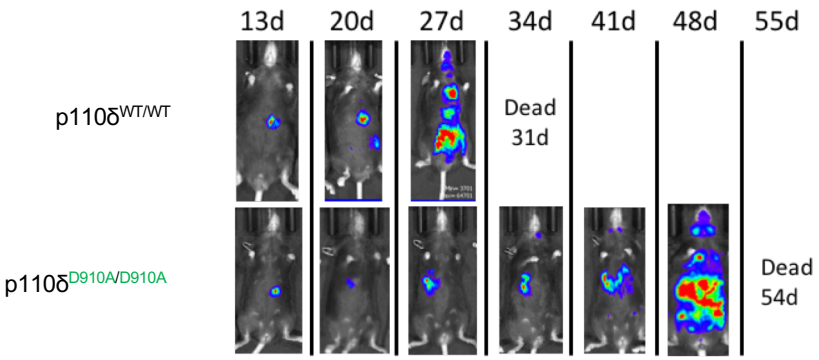
Supplemental Figure 7 (related to Figure 7): (A) Blood from 5-month old p110δ$^{WT/WT}$/TCL1 and p110δ$^{D910A/D910A}$/TCL1 mice was examined for leukemia burden by flow cytometry. %CD5^{+}CD19^{+} in CD45^{+} were compared between groups using a two-sample t-test assuming unequal variances. (B) Blood from p110δ$^{WT/WT}$ and p110δ$^{D910A/D910A}$ mice adoptively transfer with 2e7 TCL1 leukemia cells were examined weekly post adoptive transfer for immune checkpoint CTLA-4, PD-1 expression on CD4^{+} cells by flow cytometry. Bars represent mean ± SD.

Supplemental Figure 8



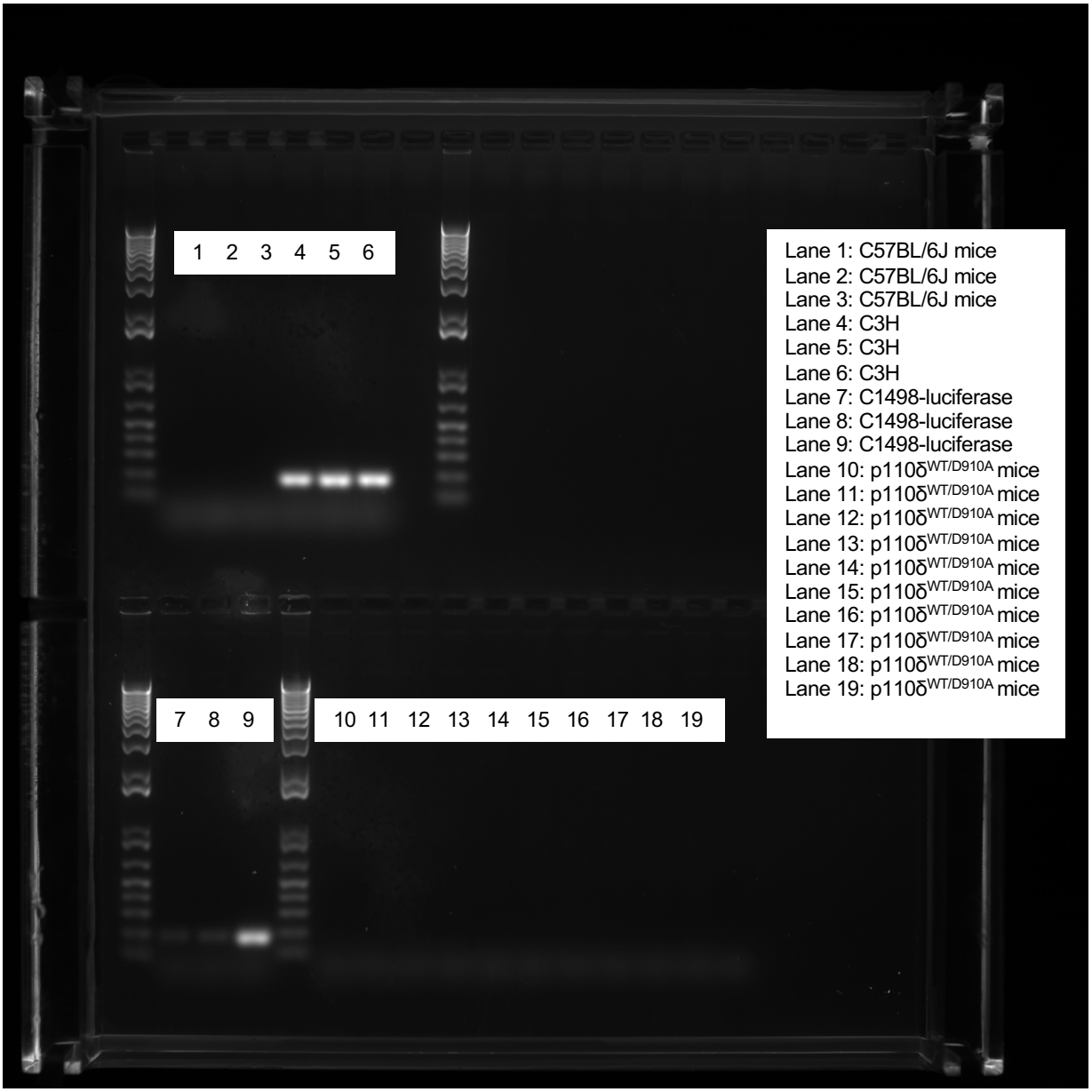
Supplemental Figure 8 (related to Figure 7): (A) The purities of Tregs from flow-sorted $CD4^+CD25^-$ and $CD4^+CD25^+$ were confirm with intranuclear FoxP3 staining. (B) Cocultures of $p110\delta^{WT/WT}$ (n=3) or $p110\delta^{D910A/D910A}$ (n=3) $CD4^+CD25^+$ T cells mixed at ratios from 1:2 to 1:32 relative to $p110\delta^{WT/WT}$ $CD4^+CD25^-$ T cells in the presence of anti-CD3/CD28-coated beads; proliferation was measured after 3 days. Two-sample t-tests using unequal variances was used to compare group means. (*p=0.013, compared with $p110\delta^{WT/WT}$ $CD4^+CD25^+$ only condition) Bars represent mean ± SD. (C) Blood disease total $CD5^+CD19^+$ cells count and % $CD5^+CD19^+$ in $CD45^+$ of $p110\delta^{D910A/D910A}$ mice receiving $p110\delta^{WT/WT}$ or $p110\delta^{D910A/D910A}$, or no $CD4^+CD25^+$ Tregs, followed by TCL1 leukemia transfer. Analysis of variance (ANOVA) methods were used to compare condition means; data were log-transformed to stabilize variance. Bars represent mean ± SD.

Supplemental Figure 9



Supplemental Figure 9 (related to Figure 8): p110δ$^{WT/WT}$ (n=10) and p110δ$^{D910A/D910A}$ (n=10) mice were engrafted with C1498-luciferse cells through tail vein injection as seen in Figure 7. Images of leukemia burden were taken by IVIS-imager.

Supplemental Figure 10



Supplemental Figure 10 (related to Figure 8): p110δ$^{WT/D910A}$ breeders were confirmed to be C57BL/6J. Primers for exon 8 of Nnt gene were custom designed according to previous report. C57BL/6J and C3H purchase from Jackson laboratory were included as control. C1498-luciferase cell line and p110δ$^{WT/D910A}$ mice breeders were tested for Nnt positivity. Nnt gene is present in C57BL/6N and all other strain of mice except C57BL/6J. C1498-luciferase cell line is reported to be C57BL/6N were confirmed.