Supplement

Donor T-cell isolation

Splenocytes and lymph nodes were used to purify donor T-cells using biotin-labeled anti-CD19 (1D3), CD45R (RA3-6B2), CD11b (M1/70), CD11c (N418), CD49b (DX5), NK1.1 (PK136), TCR γδ (GL3) and TER-119 (TER-119) (StemCell Technologies) followed by streptavidin RapidSpheres depletion with EasySep magnet (StemCell Technologies).

Pulmonary function tests

Pulmonary function tests were performed as described previously[1]. Briefly, Nembutal anesthetized mice were intubated and ventilated using the Flexivent system (Scireq). Pulmonary resistance, elastance and compliance were recorded and analyzed using the Flexivent software version 5.1.

Histopathology and immunostaining

Lung, liver and skin were embedded in Optimal Cutting Temperature (OCT) compound, snap frozen in liquid nitrogen, and stored in -80°C. Lungs were inflated by 75% OCT prior to harvest. For trichrome staining 8 µm cryosections were fixed overnight in Bouin's solution and stained with the Masson's trichrome staining kit (Sigma HT15). Collagen deposition was quantified as a ratio of blue area to total area by ImageJ. For immunostaining, acetone fixed 8µm cryosections were stained with indicated markers. For macrophages staining, frozen sections of lung or skin were stained with anti CD68-ef660 (FA-11; Thermo Fischer Scientific). For immunoglobulin deposition, sections of

lung were stained with goat anti-mouse Ig (55401; BD). Confocal images were acquired on an Olympus FluoView500 Confocal Laser Scanning Microscope.

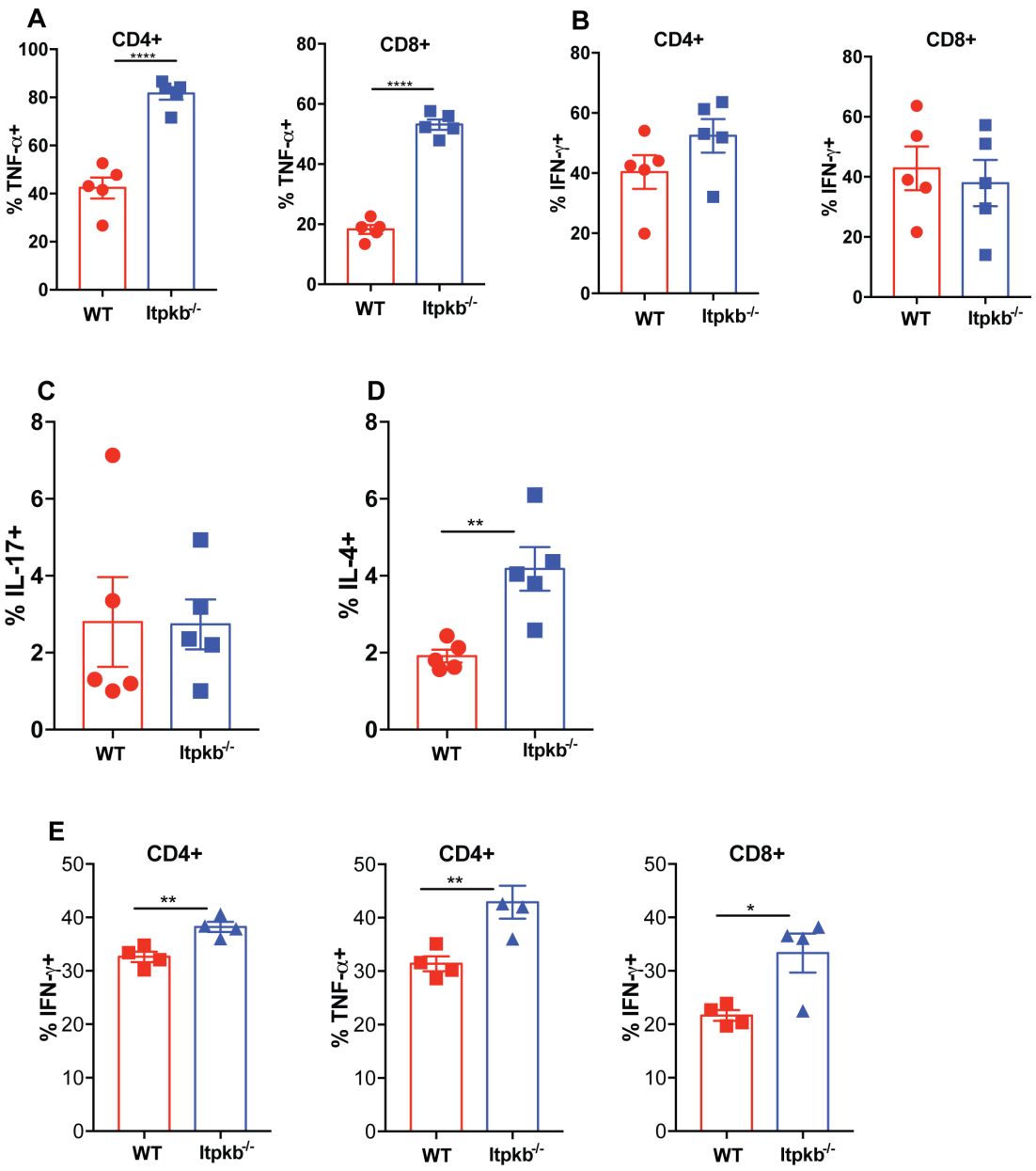
Flow Cytometry

Following flow antibodies: anti–H-2Kb (clone AF6-88.5), anti-CD4 (clone RM4-5/GK1.5), anti-CD8 (clone 53-6.7), anti–CD16/CD32 (clone 93), anti-CD62L (clone MEL-14), anti-CD44 (clone IM7), anti–IFN-γ (clone XMG1.2), anti–TNF-α (clone MP6-XT22), anti-CD45.1 (clone A20), anti-CD45.2 (clone 104), anti-CXCR5 (clone SPRCL5), anti-PD-1 (clone J43), anti-CD19 (clone 1D3), anti-GL7 (clone GL-7,), anti-Fas (clone J02), anti-Foxp3 (clone FJK-16s), IL-22 (IL22JOP), TCR Vα2 (B20.1) and fixable viability dye (catalog 65-0865) were purchased from Thermo Fischer Scientific and Biolegend. Active caspase-8 staining was determined using CaspGLOW™ Fluorescein Active Caspase-8 Staining Kit (Thermo Fischer Scientific). Tfh cells were defined as PD-1 and CXCR5 high CD4 T-cells. Cells were analyzed on BD LSRFortessa.

Statistical analysis

GraphPad Prism 6 was used to conduct the statistical analysis. Data were analyzed by Student's t-tests. Error bars indicated means +/- standard error of the mean (SEM). Survival studies are represented by Kaplan Meier survival curves, with statistical comparisons determined using log rank statistics. Significance: *P<0.05; **P<0.01; ***P< 0.001; ****P<0.0001.

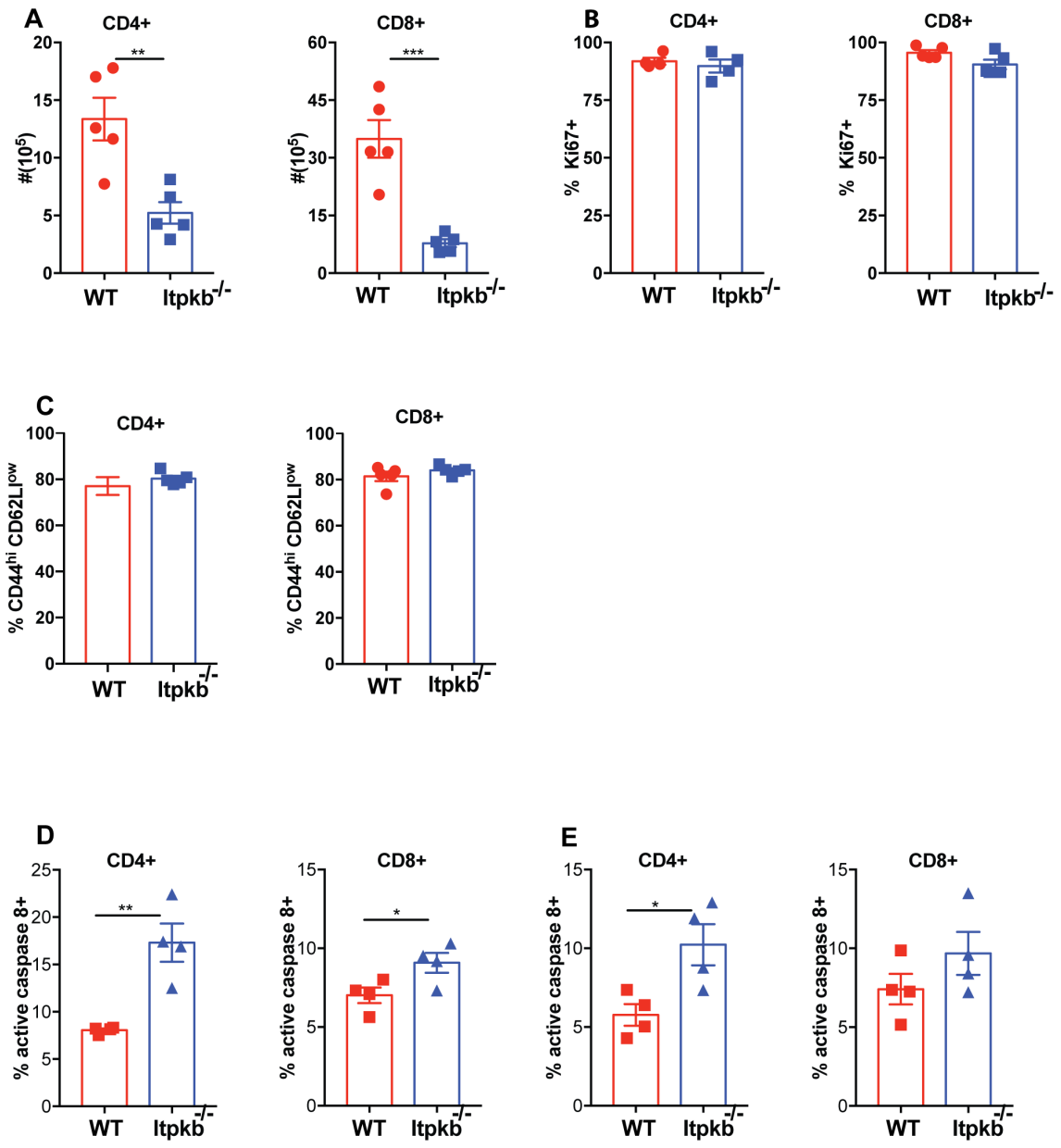
1. Du J, Paz K, Flynn R, et al. Pirfenidone ameliorates murine chronic GVHD through inhibition of macrophage infiltration and TGF-beta production. *Blood*. 2017;129(18):2570-2580.



Supplemental Figure 1. A higher frequency of Itpkb$^{-/-}$ vs. congenic WT T-cells expressed TNF-α and IL-4 with comparable IFNγ and IL-17 expression

(A-D) Lethally irradiated BALB/c recipients were infused with B6 BM. Cohorts were co-infused B6 CD45.1 WT and CD45.2 Itpkb$^{-/-}$ purified T-cells (0.75 x 10^6 each). Percentages of (A) TNF-α, (B) IFN-γ on donor T cells, C) IL-17 D) IL-4 on donor CD4+ T cells in spleens of recipients on day 6 post-transplant is shown. (E) The frequency of

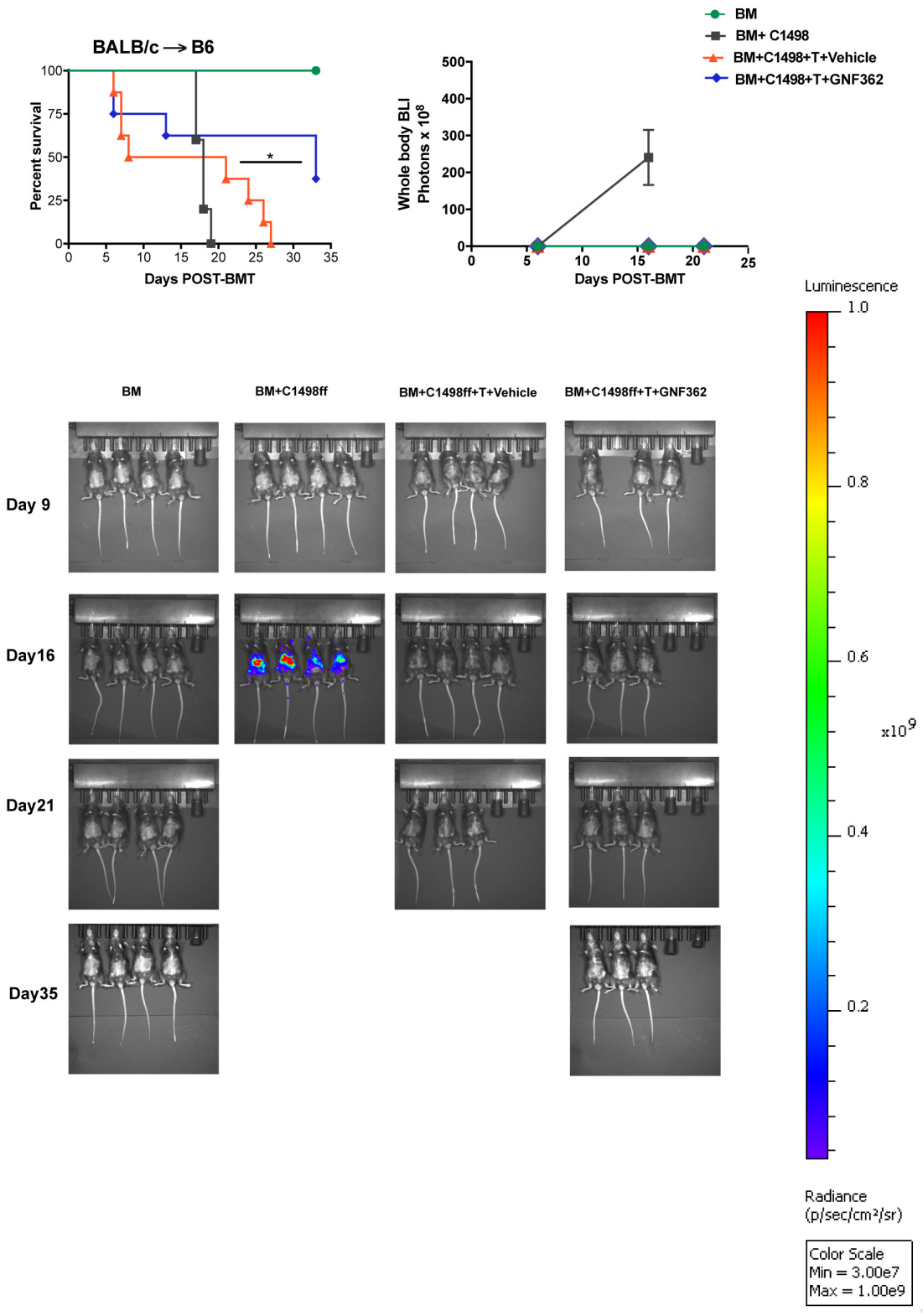
IFN-γ or TNF-α expressing CD4+ or CD8+ T-cells, as indicated, that were isolated from the large intestines of recipients on day 14 post-transplant is shown. n=4-5. Data are presented as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, and ****$P < 0.0001$.



Supplemental Figure 2. Intact proliferative capacity of Itpkb$^{-/-}$ T-cells vs. congenic WT T-cells in aGVHD mice

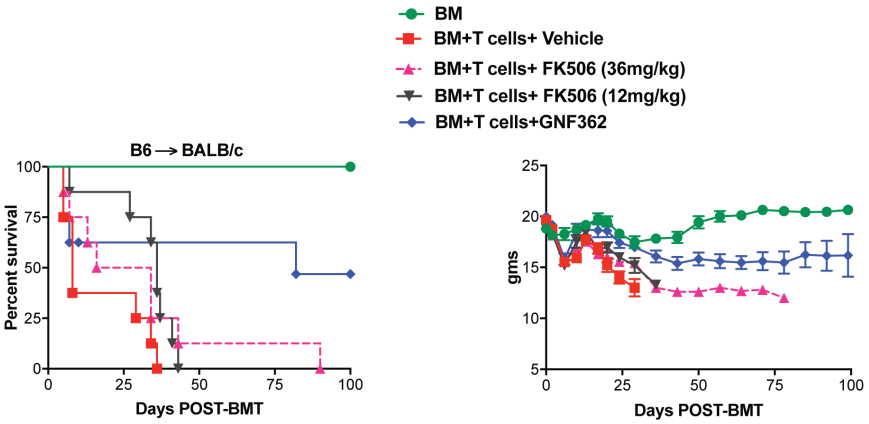
(A-B) Lethally irradiated BALB/c recipients were infused with B6 BM and cohorts co-infused with B6 CD45.1 WT and CD45.2 Itpkb$^{-/-}$ purified T cells (0.75 x 10^6 each). (A) Absolute numbers of WT (CD45.1) and Itpkb$^{-/-}$ (CD45.2) donor T-cells and percentage of (B) Ki-67 and (C) CD44^{hi} CD62L^{lo} expressing donor T-cells from the spleen of day 6

post-transplant recipients are shown. (D, E) The percentage of active caspase-8 in donor T-cells isolated from D) large intestines and E) small intestines of transplanted recipients on post-transplant day 14 is shown. n=4-5. Data are presented as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.



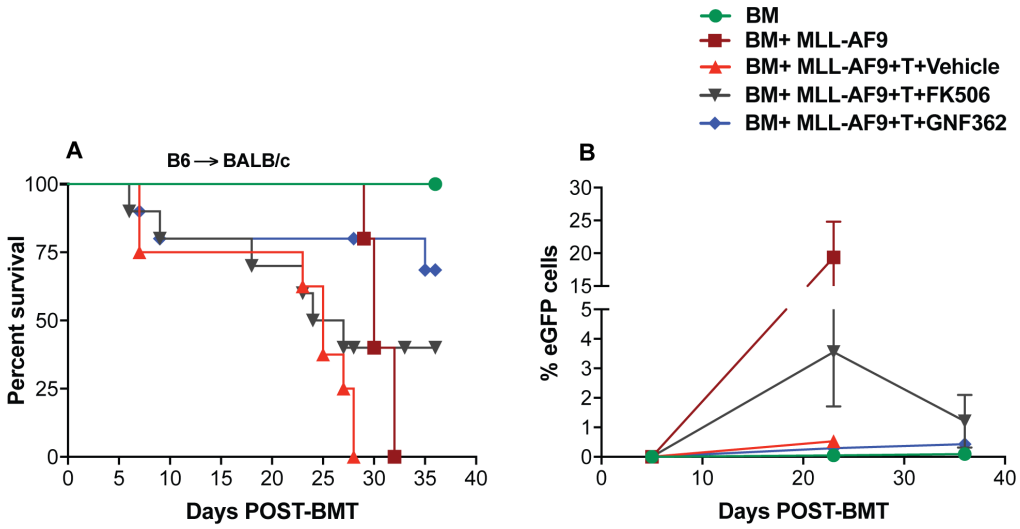
Supplemental Figure 3. GNF362 treatment spared the GVL effect against acute myeloid leukemia.

Survival and BLI of lethally irradiated B6 recipients infused with BALB/c BM ± C1498 ff leukemia cells ($3x10^4$) ± BALB/c purified T cells ($2.5x10^6$) ± GNF362. Tumor burden was quantified using BLI at the indicated time points after BMT. One experiment was performed. n=5 mice/BM group; 5 mice/BM+C1498ff leukemia group; 8 mice/BM+ C1498ff +T group. Data are shown as the mean ± SEM. $*P < 0.05$.



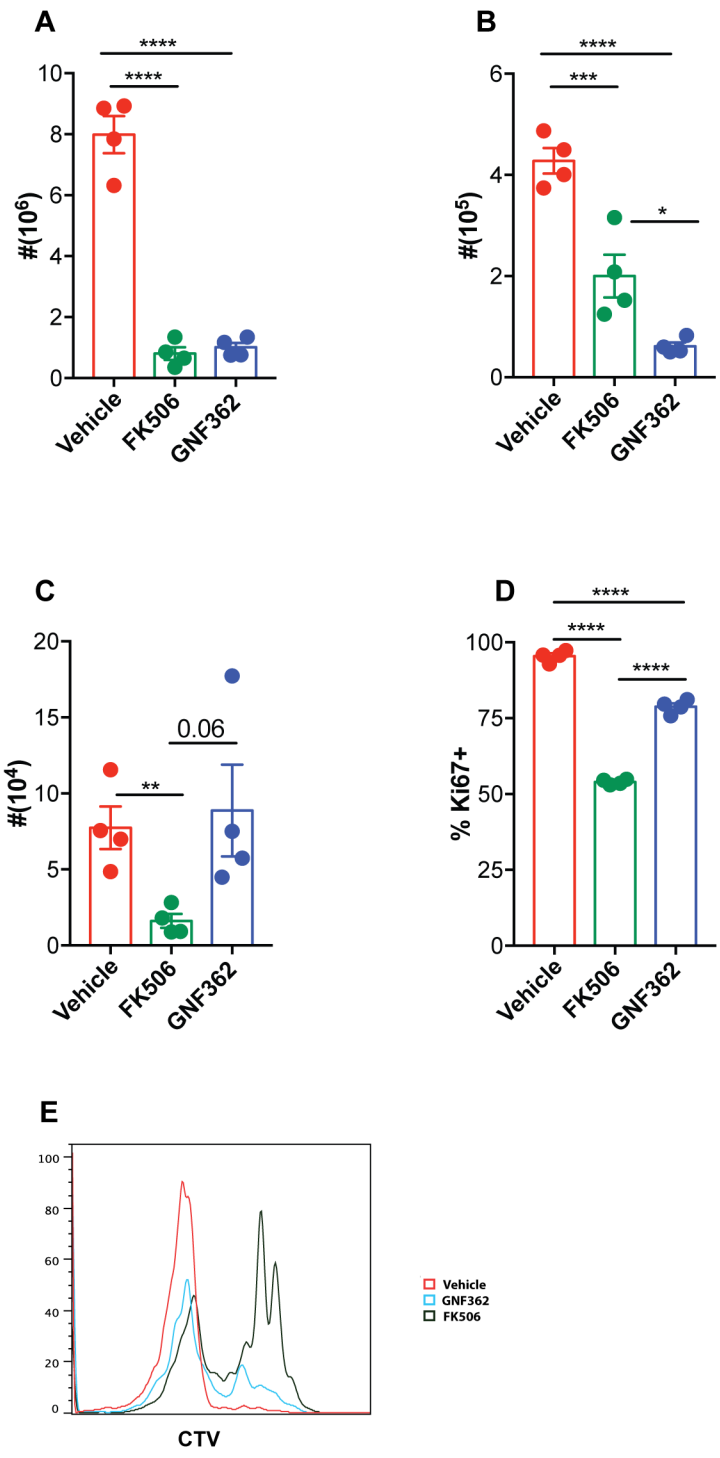
Supplemental Figure 4. Comparative GNF632 and FK506 aGVHD effects

Survival and weight curves are shown for irradiated BALB/c recipients given B6 BM ± purified T cells (1.5 x10^6) and treated from day 3 to 28 post BMT with vehicle, FK506 (36mg/kg or 12mg/kg) or GNF362. n=5 mice/BM group; 8 mice/BM+T group. FK506 (12mg/kg) or GNF632 treated recipients survived longer than vehicle treated recipients ($p<0.05$; $p=0.05$, respectively). There was no significant difference in the survival rate between vehicle vs FK506 (36mg/kg) ($P=0.2$), GNF362 vs FK506 ($P=0.10$) treated recipients. One experiment was performed.



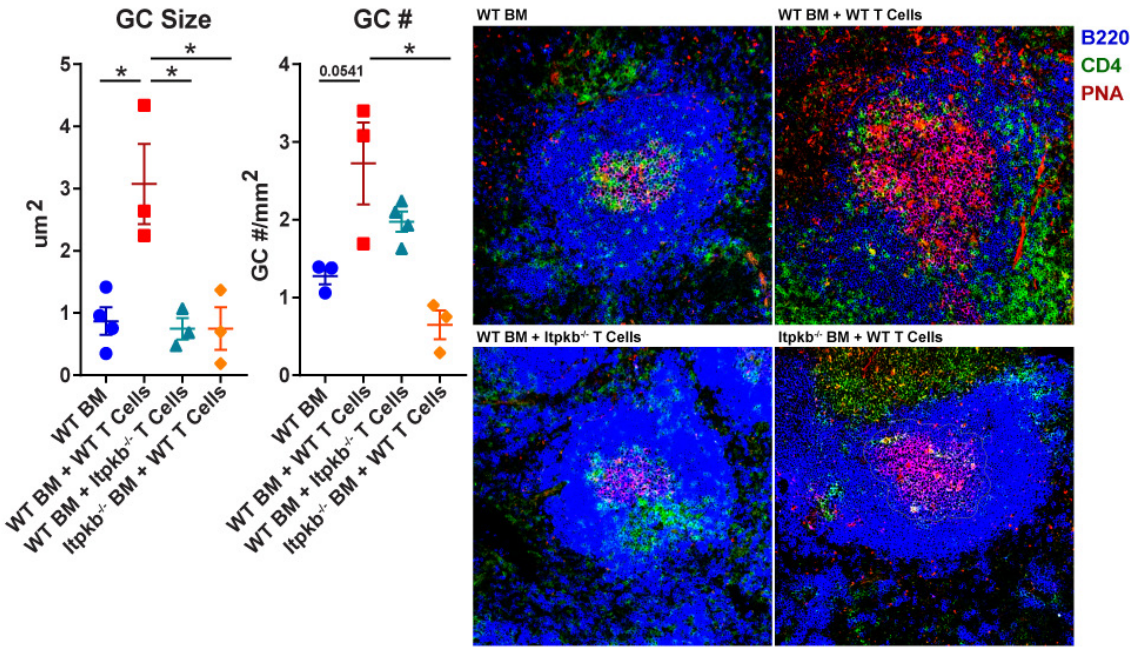
Supplemental Figure 5. FK506 effects on aGVHD and GVL

A) Survival of lethally irradiated BALB/c recipients infused with B6 BM ± MLL-AF9 eGFP+ leukemia cells (10^4) and with or without supplemental purified T-cells ($1.5x10^6$). BM+ MLL-AF9+T recipients were treated with either vehicle, FK506 (12mg/kg) or GNF362 from days 0-28. B) Line graphs show the changing frequency of eGFP+ cells (tumor cells) in the peripheral blood at the indicated time points. n=5 mice/BM group; 5 mice/BM+ MLL-AF9 group; 8-10 mice/BM+ MLL-AF9 GFP+T group. GNF362 treated recipients were significantly survived longer than vehicle ($P < 0.01$). There was a statistical trend toward improved survival in the GNF362 as compared to FK506 group (P=0.07). One experiment was performed. Data are shown as the mean ± SEM.



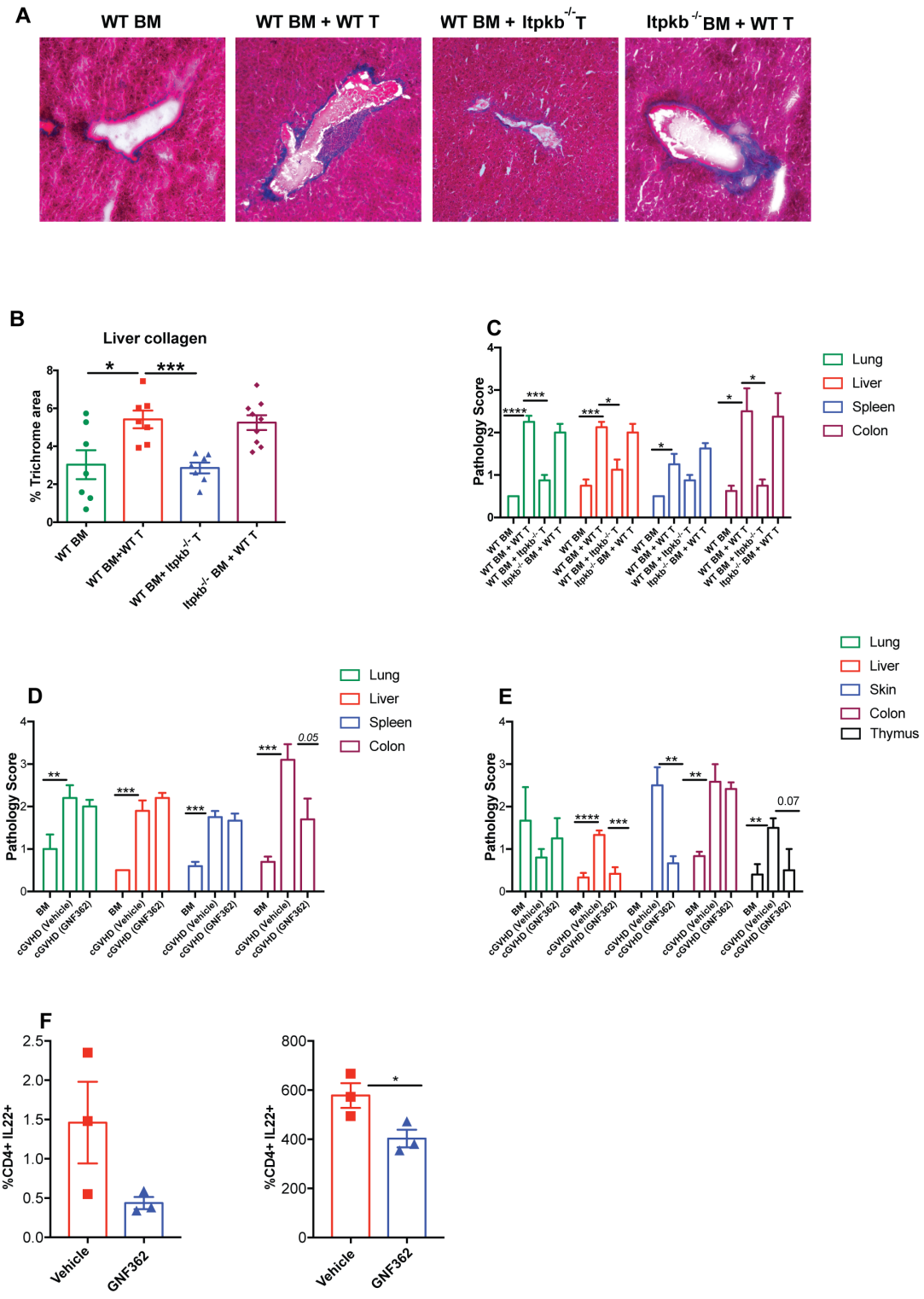
Supplemental Figure 6. GNF362 selectively eliminated antigen-activated TCR Tg T cells without affecting bystander T cell proliferation

(A-E) Lethally irradiated (CB6) F1 recipients were infused with B6 BM and a mixture of B6 TEα GFP+, Thy1.1+ 2C, and GFPneg, Thy1.2+ TCR Vα+ OT-1 TCR-Tg T cells (0.5 x 10^6 each). Recipients were treated daily with vehicle or FK506 (12mg/kg) or GNF362 from days 0-3. On day 4 post BMT, spleens were harvested. Absolute numbers of A) TEα or B) 2C T-cells are shown; (C) The OT-1:2C TCR Tg T cell ratios are shown. (D, E) Proliferation of OT-1+ CD8+ T cells, denoted by Ki67 expression, on day 4 post-BMT is shown. n=4 per group. Data are presented as the mean ± SEM. One experiment was performed. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.



Supplemental Figure 7. Donor T-cell Itpkb expression influences GC size in the cGVHD/BO model

Conditioned B10.BR mice were transplanted with B6 WT or Itpkb$^{-/-}$ BM with or without WT or Itpkb$^{-/-}$ donor T-cells (70 x 10^3). Splenic GC size and numbers and representative GC immunofluorescence staining images are shown. Three to four mice from each group were analyzed. Data are shown as the mean ± SEM. *$P < 0.05$.



Supplemental Figure 8. Precluding donor T-cell Itpkb activity reduced cGVHD in the BO model

(A-C). Lethally irradiated B10.BR recipient mice that were transplanted with B6 T-cell depleted BM ± T-cells. Some cohorts received B6 BM from WT or Itpkb$^{-/-}$ donors with or without WT or Itpkb$^{-/-}$ donor T-cells (70 x 10^3) (A) Liver collagen deposition staining, (B) quantification and (C) Histopathology tissue scores. n=4-5 mice/group. (D) Histopathology scores of lethally irradiated B10.BR recipients that were transplanted with B6 WT BM ± B6 WT T-cells ± GNF362 from days 28-56 post-transplant. n=4-5 mice/group. (E-F). Lethally irradiated BALB/c mice were transplanted with B10.D2 BM ± purified T-cells. GNF362 or vehicle treatment started on day 21. (E) Histopathology tissue scores; and F) T-cells isolated from skins of cGVHD recipients on day 49 post-BMT were analyzed to determine the frequency and levels (MFI) of IL-22 in CD4+ T-cells. n=3-6 /group. Data are shown as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.