Supplemental Experimental Procedures

Mice

All mice were between 4 and 6 weeks of age at the start of the experiment. All of the animal work was approved by the Fourth Military Medical University Institutional Animal Care and Use Committee and handled in strict accordance with good animal practice as defined by the relevant national animal welfare bodies.

Flow cytometry and cell sorting.

Cells from thymus, lymph node, spleen, blood and liver were mechanically disrupted and the red blood cells were removed with red cell lysis buffer (Tiangen) for 10 min on ice. In vitro-cultured cells were collected and washed with PBS + 1% BSA prior to antibody labeling. Antibodies to the following antigens were used: PerCp-CD3ε (Catalog number 553067, BD), PE-CD19 (557399, BD), FITC-CD4 (553052, BD), PE-CD8 (553033, BD), PE-TCRβ (109208, Biolegend), PE-Cy7-TCRγδ (118124, Biolegend), APC-NK1.1 (17-5941, Biolegend), PE-PLZF (145804, Biolegend), FITC-Bcl11b (ab123449, Abcam), FITC-Nkp46 (137605, Biolegend). Cells were incubated with antibody for 30 minutes at 4° C before being washed. Data acquisition was performed using a FACS Calibur (BD Biosciences) with dead cells exclusion based on their scatter profile or PI inclusion. Analysis was performed using FlowJo (Tree Star) software. Sorting was performed using FACS Aria (BD Biosciences).

Real-time quantitative PCR

Total RNA obtained from thymocytes was extracted using Trizol reagent (Invitrogen). cDNA was synthesized from 1 μg of total RNA using the RNA reverse transcriptase kit (Takara). Real-time quantitative PCR was performed using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems), and the TAKARA SYBR Premix Ex Taq was used for amplification according to the manufacturer's instructions. cDNA input was standardized and PCR was performed for 40 cycles..

Western blot analyses

Freshly isolated thymocytes were immediately homogenized in protein lysis buffer. 30 μg of protein was isolated on 10% SDS-PAGE, transferred onto a PVDF membrane, and blotted using a goat-anti-mouse Bcl11b antibody (R&D Systems) or rabbit-anti-mouse PLZF antibody (Abcam). After several washes, the membranes were incubated with HRP-conjugated anti-goat or anti-rabbit IgG (R&D Systems) and then visualized using ECL reagents (Amersham Bioscience).

OP9-DL1 stromal feeder cell culture

OP9-DL1 stromal feeder cells were cultured in alpha-MEM (Sigma) with 20% FCS, 1% penicillin/streptomycin, and 2 mM L-glutamine (Life Technologies). Cells were passaged every 2 days by trypsinization (Invitrogen). A monolayer (70%-80% confluent) of OP9-DL1 cells was prepared 24 hours prior to co-culture.

Hematoxylin-eosin staining and Immunofluorescence Staining

Liver cancer tissues or melanoma tumor sections were prepared for paraffin sectioning, then stained with anti-NK cell monoclonal antibody (PK136) followed by Dylight 594-conjugated anti-mouse IgG.

Mouse IgG was used as the primary antibody as a control. Sections were examined by light microscopy.

Supplementary Figures

FigureS1.

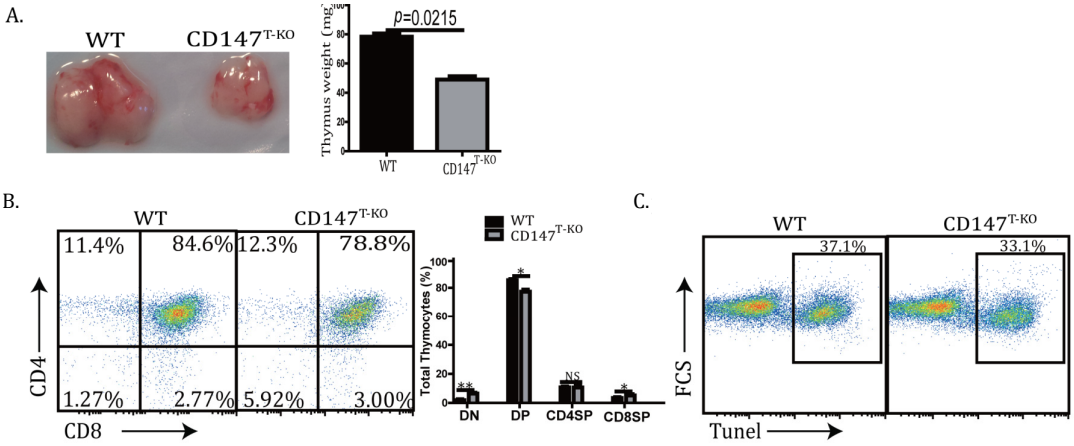

Figure S1. Thymocytes development in CD147$^{T\text{-}KO}$ mice. A. The weight of the thymus between the WT and CD147$^{T\text{-}KO}$ mice were compared and total thymic weight calculated. B. Analysis of the CD4 and CD8 thymocyte population using flow cytometry. C. TUNEL staining was performed to detect the amount of apoptosis in the DP cell population. * $p < 0.05$; ** $p < 0.01$; NS, no significance. Data are representative of 6 experiments.

FigureS2.

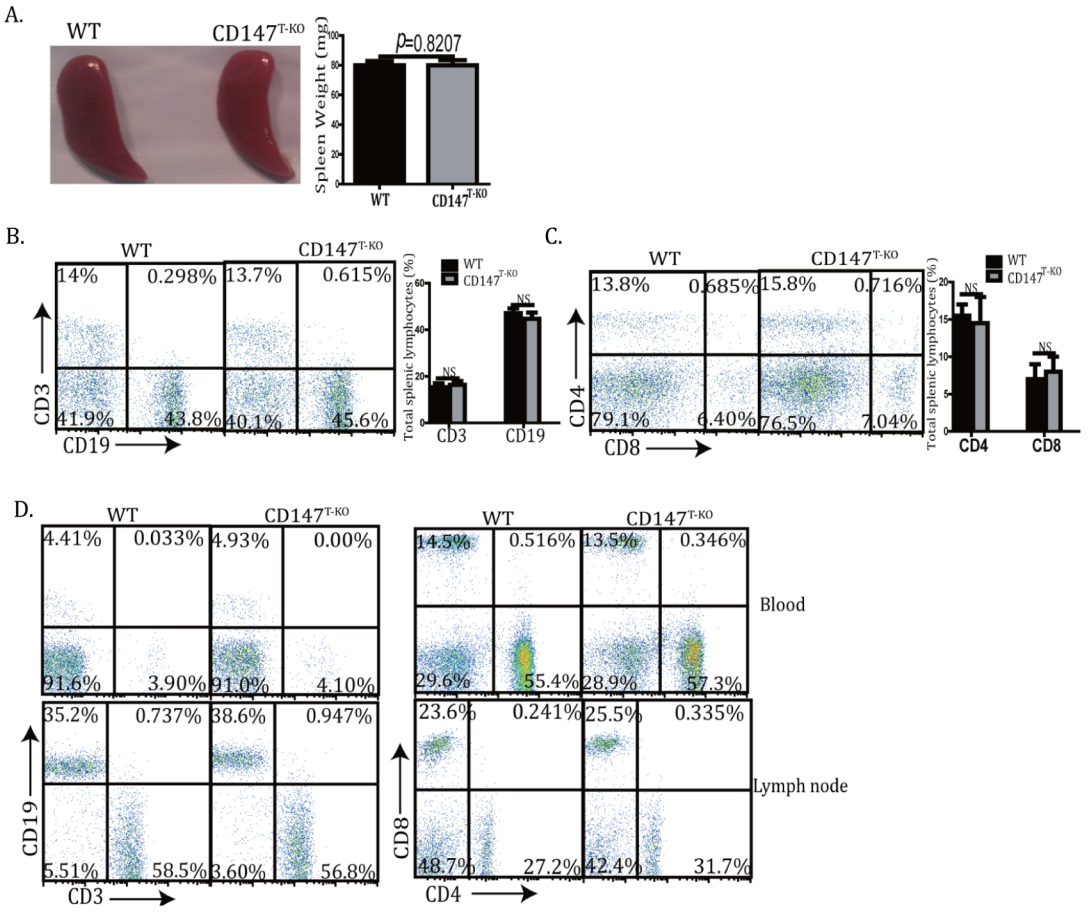

Figure S2. The phenotypes of peripheral lymphoid tissues were compared between the WT and CD147$^{T\text{-}KO}$ mice. A. Spleens were isolated from WT and CD147$^{T\text{-}KO}$ mice and their weights were compared. B. Analysis of CD3+ T cells and CD19+ B cells in splenocytes using flow cytometry and the total percentage of splenocytes were analyzed in both the WT and CD147$^{T\text{-}KO}$ populations. C. Analysis of CD4+ T cells and CD8+ T cells in splenocytes using flow cytometry. D. Analysis of CD3+, CD19+, CD4+, and CD8+ T cells in the blood and lymph node. * $p < 0.05$; ** $p < 0.01$; NS, no significance. Data are representative of 6 experiments.

FigureS3.

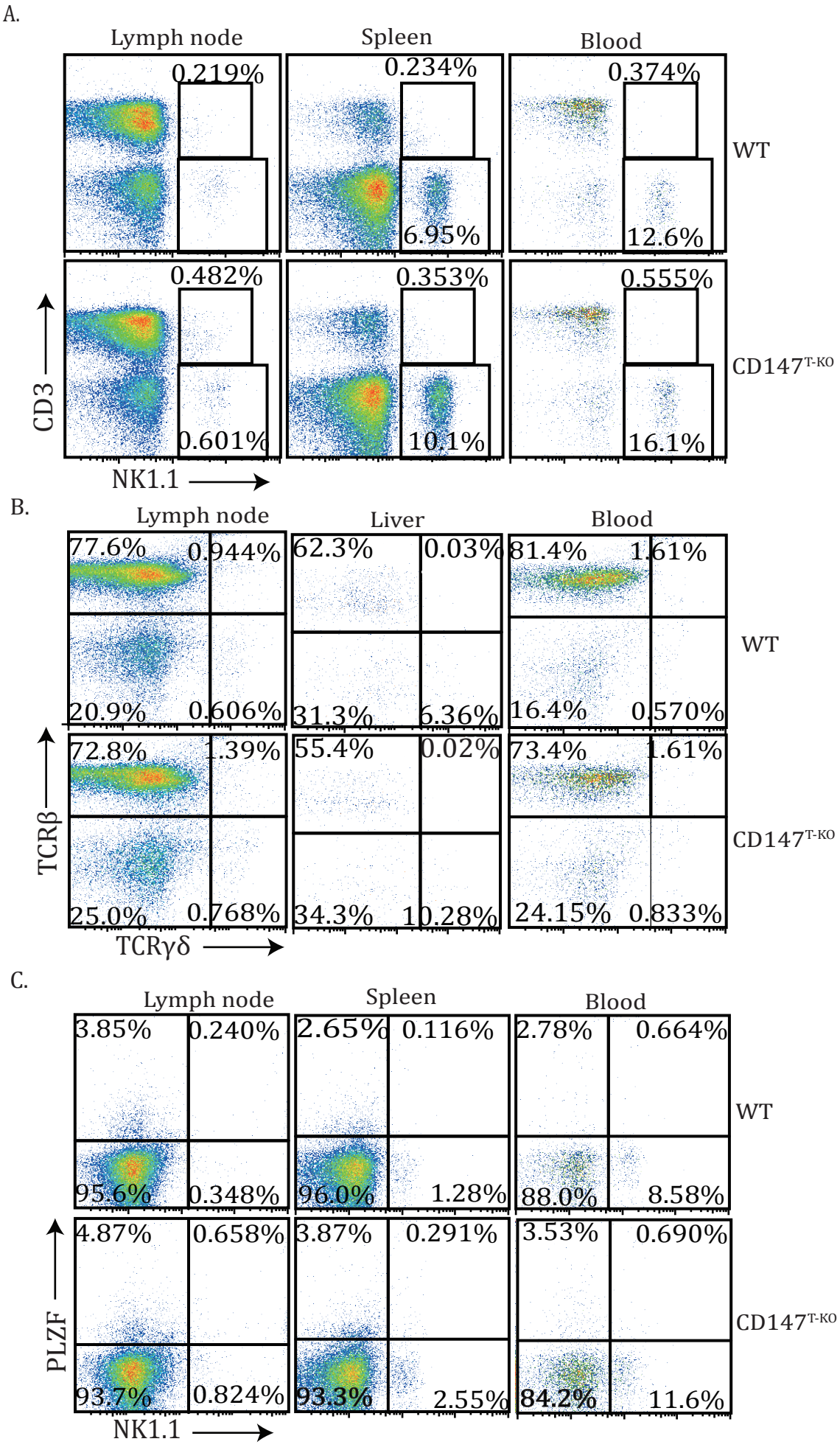

Figure S3. Detection of NK, NKT, TCR β /TCR γ δ , and PLZF+ cells in the lymph node, spleen and blood. A. Analysis of NK and NKT cells from both WT and CD147$^{T\text{-}KO}$ mice using flow cytometry. B. Analysis of TCR β and TCR γ δ T cells from WT and CD147$^{T\text{-}KO}$ mice using flow cytometry. C. Analysis of PLZF+ cells from WT and CD147$^{T\text{-}KO}$ mice using flow cytometry. * $p < 0.05$; ** $p < 0.01$; NS, no significance. Data are representative of 6 experiments.

FigureS4.

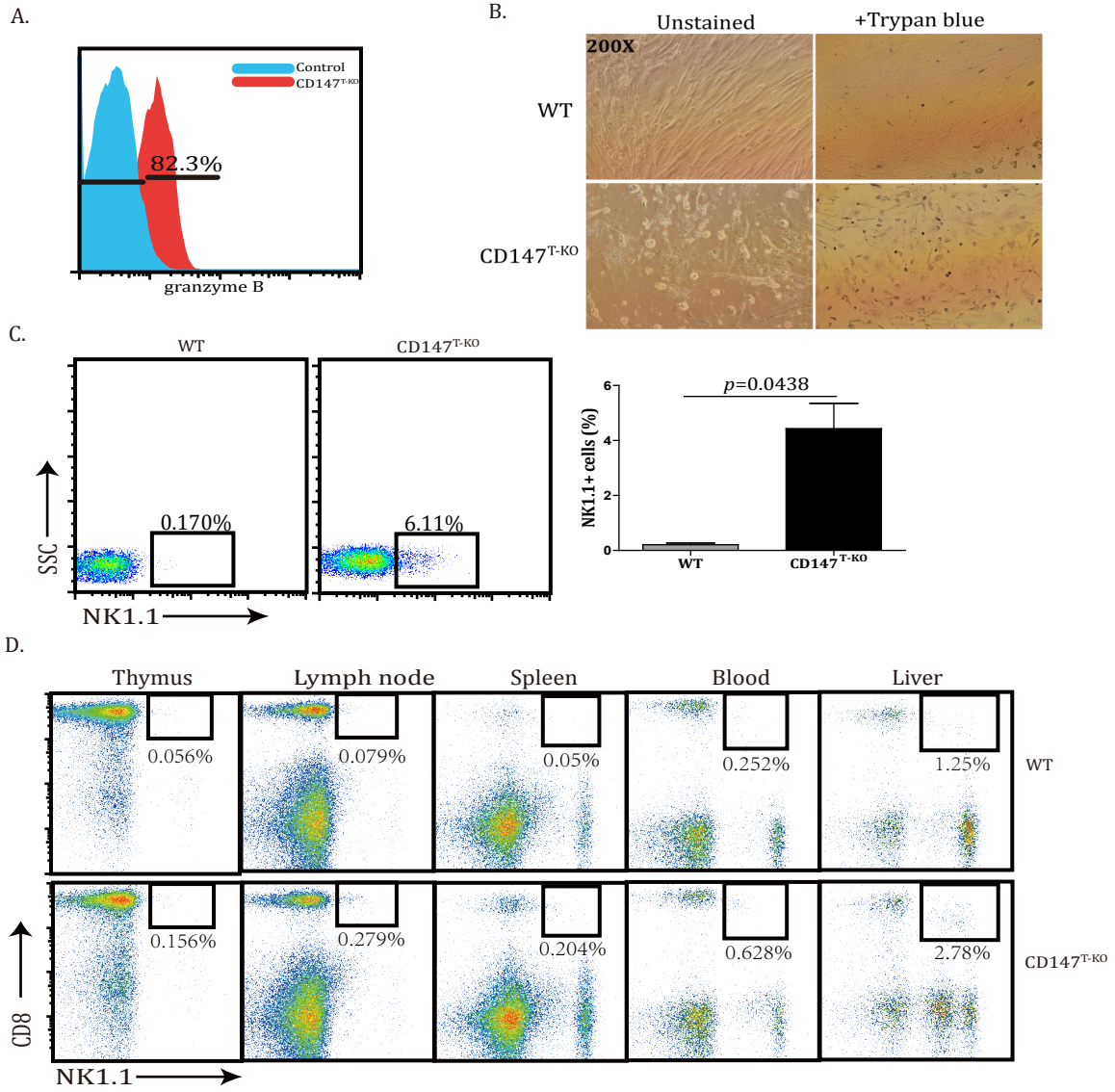

Figure S4. CD147 deletion reprograms peripheral CD8+ T cells into the NK-like lineage. A. The expression of granzyme B in CD147T-KO DN3-converted NK1.1 cells. CD147$^{T\text{-}KO}$ DN3 thymocytes co-cultured with OP9-DL1 feeder cells for 14 days, supplemented with 100 ng/ml IL-2. Flow cytometry analysis the expression of granzyme B in NK1.1+ cells. The control is blank control. B. Killing of OP9-DLI stromal cells by co-cultured DP thymocytes using Trypan Blue staining, magnification at 200X. C. Flow cytometry analysis of co-cultured peripheral CD8+ T cells with NK1.1. D. Analysis of CD8+NK1.1+ cells in the thymus and peripheral organs of WT and CD147$^{T\text{-}KO}$ mice using flow cytometry. All data are representative of 3 experiments.

FigureS5.

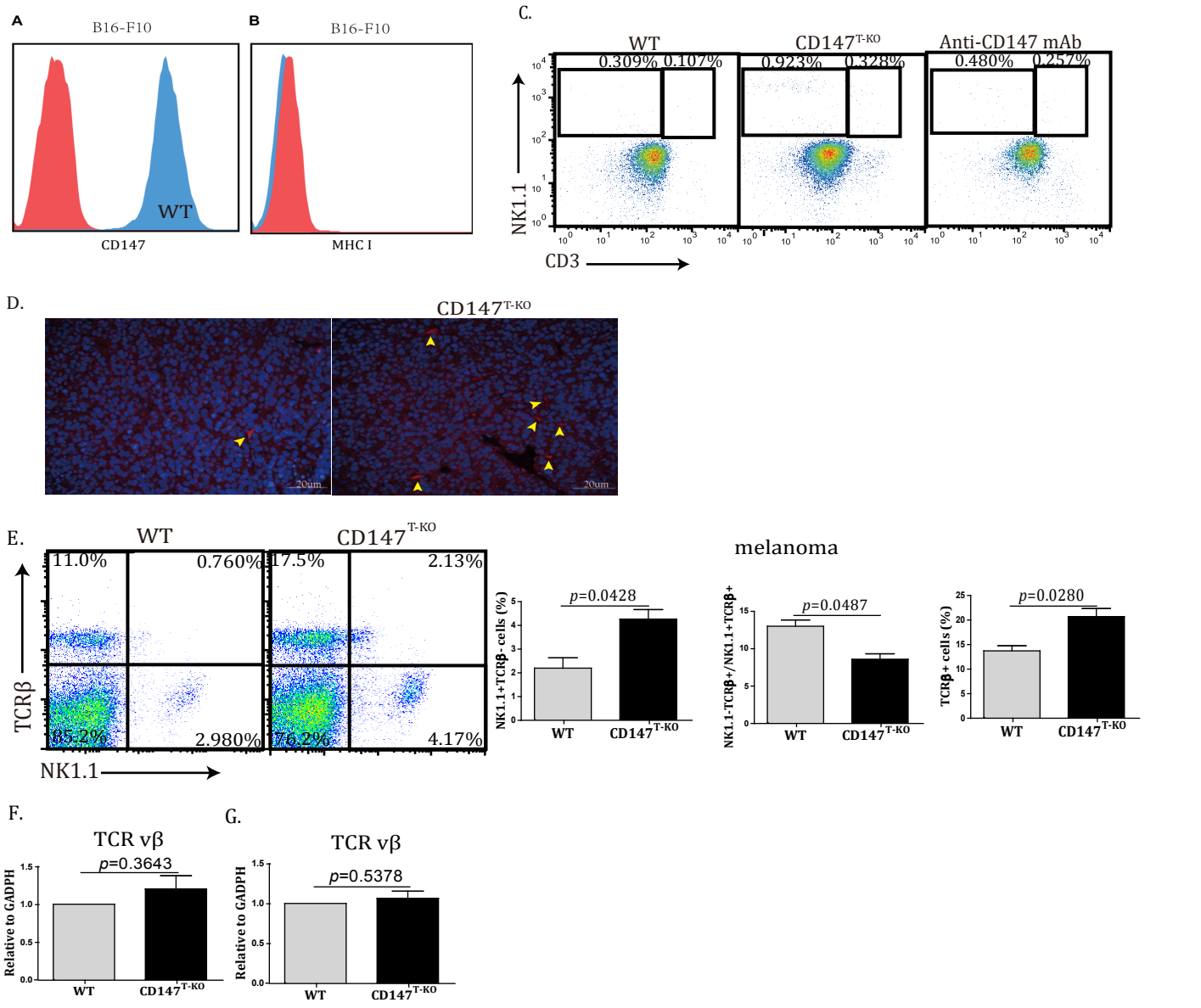

Figure S5. CD147 is an immunomodulatory target for melanoma tumor therapy. A. Flow cytometry analysis of CD147 expression in B16-F10 cells. Data are representative of 3 experiments. B. Flow cytometry analysis of MHCI expression in B16-F10 cells. Data are representative of 3 experiments. C. Flow cytometry analysis of NK (left) and NKT (right) cells in melanoma. Data are representative of 5 experiments. D. Immunofluorescence staining analysis of NK cells in the tumor. Magnification is at 400x. E. Intracellular TCR β expression analyzed by Flow cytometry for melanoma infiltrating NK1.1+ cells. Data are representative of 3 experiments. F. qPCR analysis of TCR v β expression in CD3-NK1.1+ cells sorted from melanoma. Data are representative of 3 experiments. G. qPCR analysis of TCR v β expression in CD3-NK1.1+ cells sorted from spleen without tumor. Data are representative of 3 experiments.

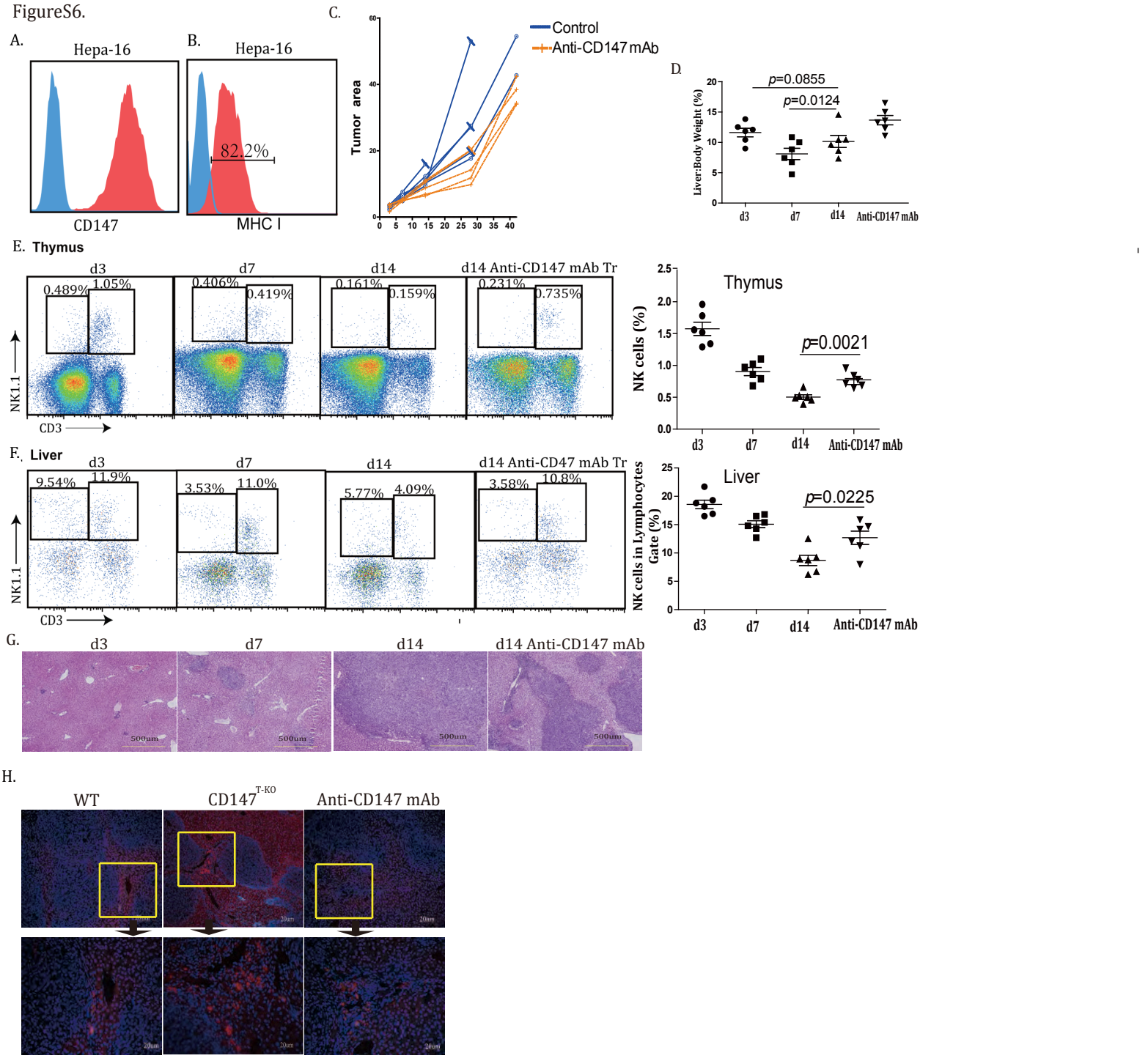

Figure S6. CD147 is an immunomodulatory target for hepatocellular carcinoma therapy. A. Flow cytometry analysis of CD147 expression in Hepa-16 cells. Data are representative of 3 experiments. B. Flow cytometry analysis of CD147 expression in Hepa-16 cells. Data are representative of 3 experiments. C. Highlight area and mice death in the WT and anti-CD147 treatment group. Every line represents a mice. D. Analysis of the ratio of liver to body weight percentages at day 3, 7, 14 of all treatment groups. E. Flow cytometry analysis of NK cells in the thymus after injection of Hepa16 cells. F. Flow cytometry analysis of NK cells in the liver after injection of Hepa16 cells. G. Hematoxylin-eosin staining was used to analyze tumor growth at days 3, 7, and day 14 and in anti-CD147 mAbs treatment mice at day 14. H. Immunofluorescence staining analysis was performed of NK cells in liver tumors at both 200x magnification (above) and 400x magnification (below). All image analysis is shown at 100x magnification.

Tables

Table S1. The absolute number of total thymocytes.

	n	Total number (10^6)
WT	**4**	**141.25 ± 18.75**
$CD147^{T\text{-}KO}$	**4**	**75.75 ± 15.80**
Paired t-test		***p* = 0.0016**

Table S2. The absolute number of DN, DP, SP thymocytes.

	n	DN (10^6)	DP (10^6)	CD4 SP (10^6)	CD8 SP (10^6)
WT	**4**	**7.30 ± 1.85**	**122.75±16.75**	**7.88 ± 1.65**	**3.32 ± 0.65**
$CD147^{T\text{-}KO}$	**4**	**7.20 ± 2.25**	**61.23 ± 12.65**	**4.77 ± 1.15**	**2.88 ± 0.45**
Paired t-test		***p* = 0.4247**	***p* = 0.0014**	***p* = 0.0021**	***p* = 0.0548**

.

Table S3. The absolute number of total splenocytes. .

	n	Total number (10^6)
WT	**4**	**81.25 ± 9.30**
$CD147^{T\text{-}KO}$	**4**	**74.75 ± 15.76**
Paired t-test		***p* = 0.2157**

Table S4. The absolute number of T, B, SP splenocytes.

	n	T cell (10^6)	B cell (10^6)	CD4 SP (10^6)	CD8 SP (10^6)
WT	**4**	**22.86 ± 1.45**	**41.44 ± 1.48**	**13.01 ± 0.89**	**4.47 ± 0.98**
$CD147^{T\text{-}KO}$	**4**	**18.41 ± 3.96**	**38.12 ± 3.94**	**11.49 ± 1.65**	**4.51 ± 0.84**
Paired t-test		***p* = 0.0809**	***p* = 0.1214**	***p* = 0.0742**	***p* = 0.9576**

Table S5. The absolute number of $NK1.1^+$ cells in thymus and liver.

	n	Thymus (10^6)	Liver (10^6)
WT	**4**	**0.46 ± 0.09**	**2.46 ± 0.44**
$CD147^{T\text{-}KO}$	**4**	**0.68 ± 0.08**	**4.00 ± 0.52**
Paired t-test		***p* = 0.034**	***p* = 0.015**

Table S6. The absolute number of melanoma tumor infiltrating cells.

	n	CD3-NK1.1+ (10^6)	CD3+NK1.1+ (10^6)
WT	**4**	**1.26 ± 0.57**	**0.49 ± 0.22**
$CD147^{T\text{-}KO}$	**4**	**2.27 ± 0.62**	**0.75 ± 0.38**
Anti-CD147 mAb	**4**	**1.58 ± 0.63**	**0.55 ± 0.48**

Table S7. The morbidity of hepatocellular carcinoma in WT and CD147$^{T\text{-}KO}$ mice.

	Total number	Mice with Tumor-developed	Morbidity
WT	**6**	**6**	**100%**
CD147$^{T\text{-}KO}$	**6**	**3**	**50%**