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Supplemental information

Divergent fates of antigen-specific CD8$^+$ T cell clones in mice with acute leukemia

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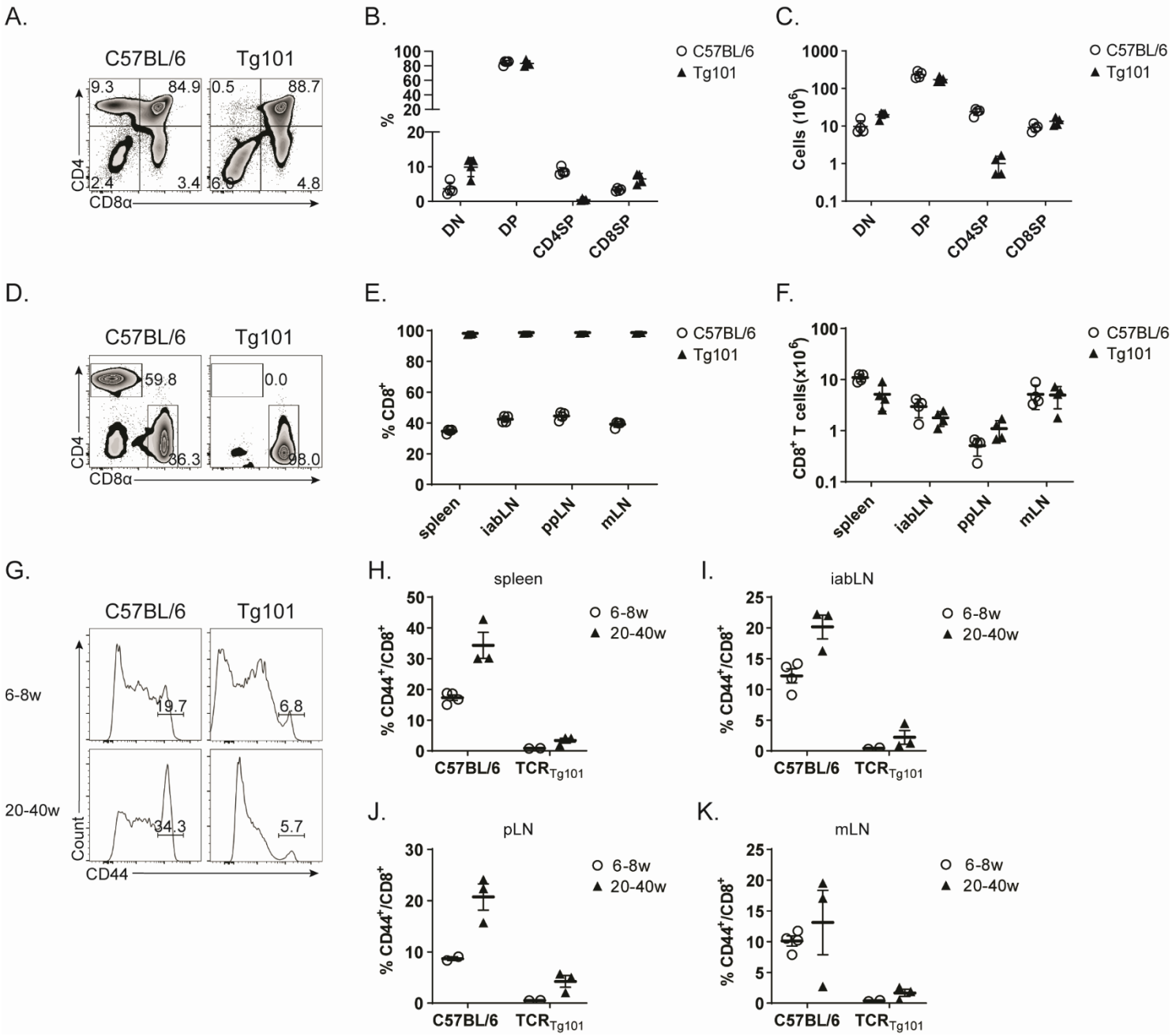
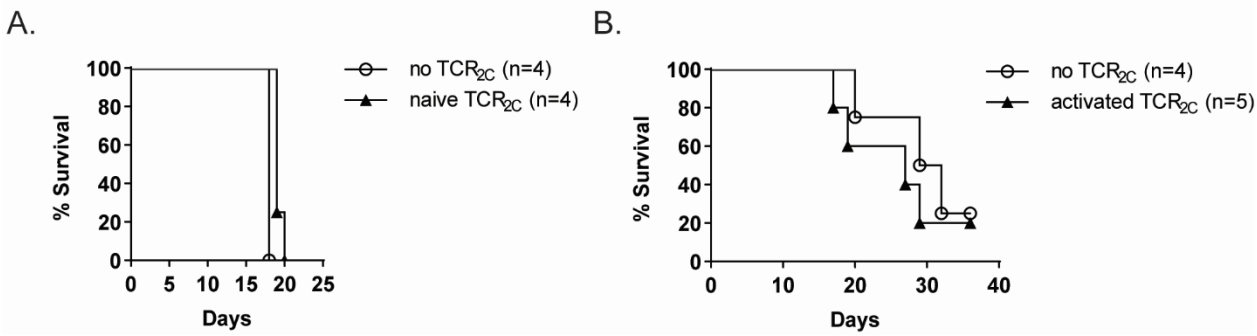
Supplementary Figure 1



Supplementary Figure 1. T cell phenotyping of Tg101 TCR transgenic mice. Related to Figure 1. (**A-C**) Thymocyte development in *Rag2*$^{-/-}$ Tg101 mice**.** CD4 and CD8 expression on live thymocytes from 6-8 week-old C57BL/6 and *Rag2*$^{-/-}$ Tg101 mice were analyzed by flow cytometry. Representative FACS plots are shown in (**A**). Quantified data are shown in (**B and C**) as mean ± SD. DN – double negative; DP – double positive; SP - single positive. (**D-F**) Frequencies and absolute numbers of CD4^{+} and CD8^{+} T cells in SLO of C57BL/6 and *Rag2*$^{-/-}$ Tg101mice (gated on CD3^{+} cells). Representative FACS plots are shown in (**D**) and quantified results are shown in

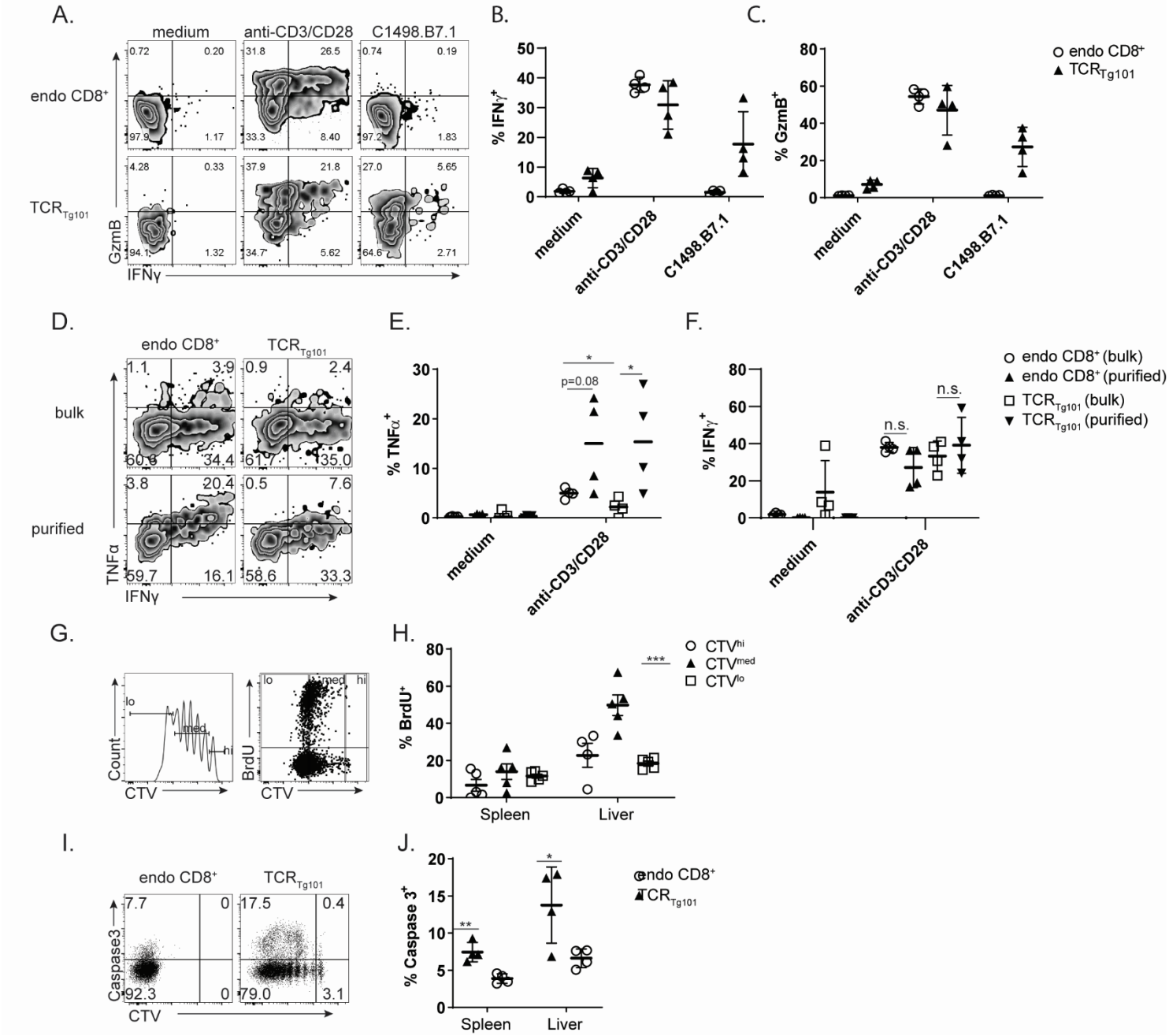
(**E and F**). Sp - spleen; iabLN - pooled inguinal, axillary and brachial lymph lodes; pLN – periaortic lymph node; mLN - mesenteric lymph lode. (**G-K**) Analysis of CD44 expression on $CD8^+$ T cells from C57BL/6 and *Rag2*$^{-/-}$ Tg101 mice. Representative FACS plots depicting the frequency of CD44-expressing splenic $CD8^+$ T cells from younger (6-8 weeks) and older (20-40 weeks) mice are shown in (**G**). Summary plots of different SLO are shown in (**H-K**) as mean ± SD. Data are representative of at least 2 experiments with 2-4 mice/group.

Supplementary Figure 2



Supplementary Figure 2. Adoptive transfer of naïve or activated TCR_{2C} does not impact survival of mice with leukemia. Related to Figure 2. (**A and B**) Survival of C57BL/6 mice challenged i.v. with C1498.SIY cells (10^6) and transferred or not with naive (**A**) or *in vitro* activated (**B**) TCR_{2C} ($4x10^6$) 3-5 days later.

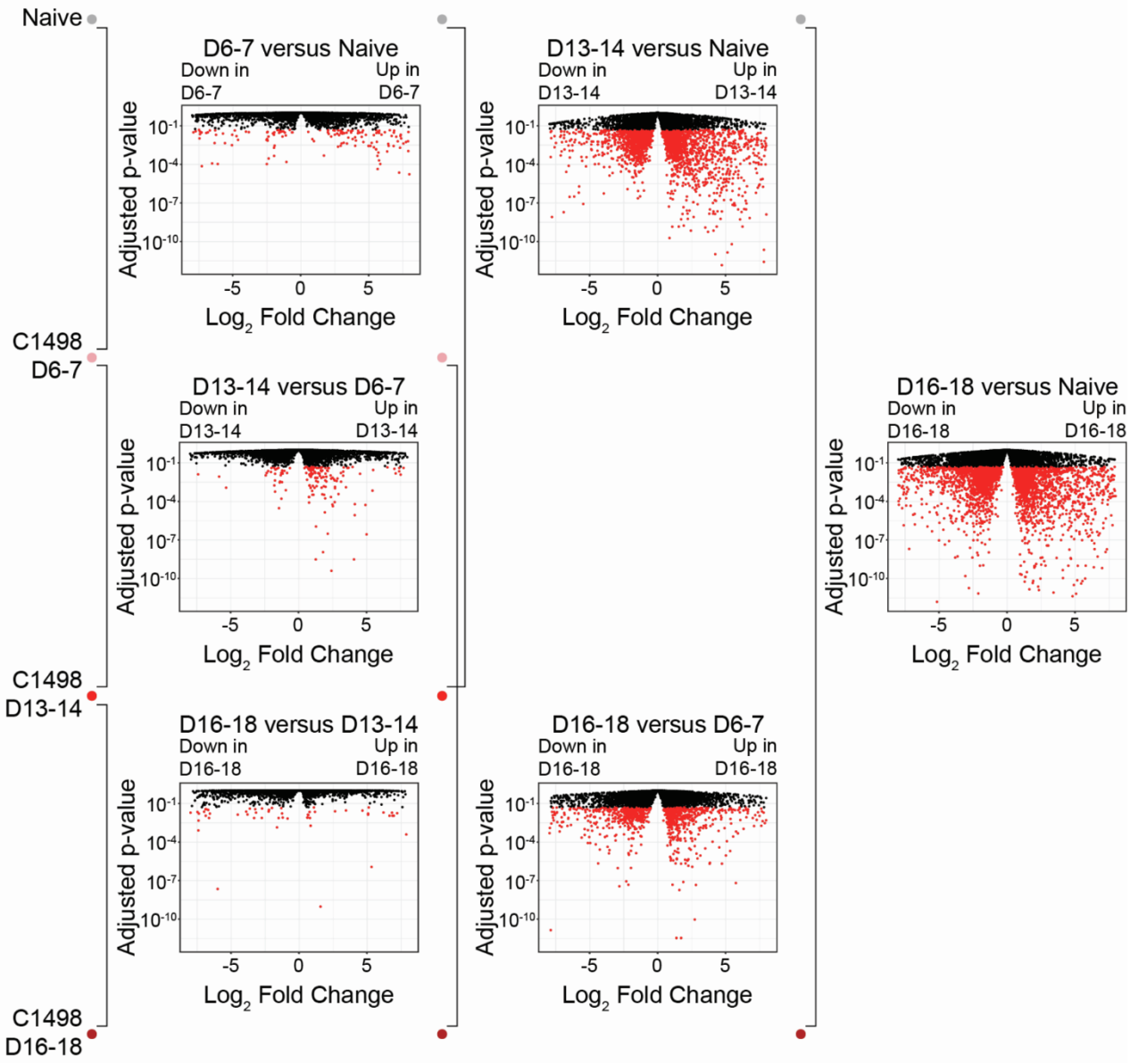
Supplementary Figure 3



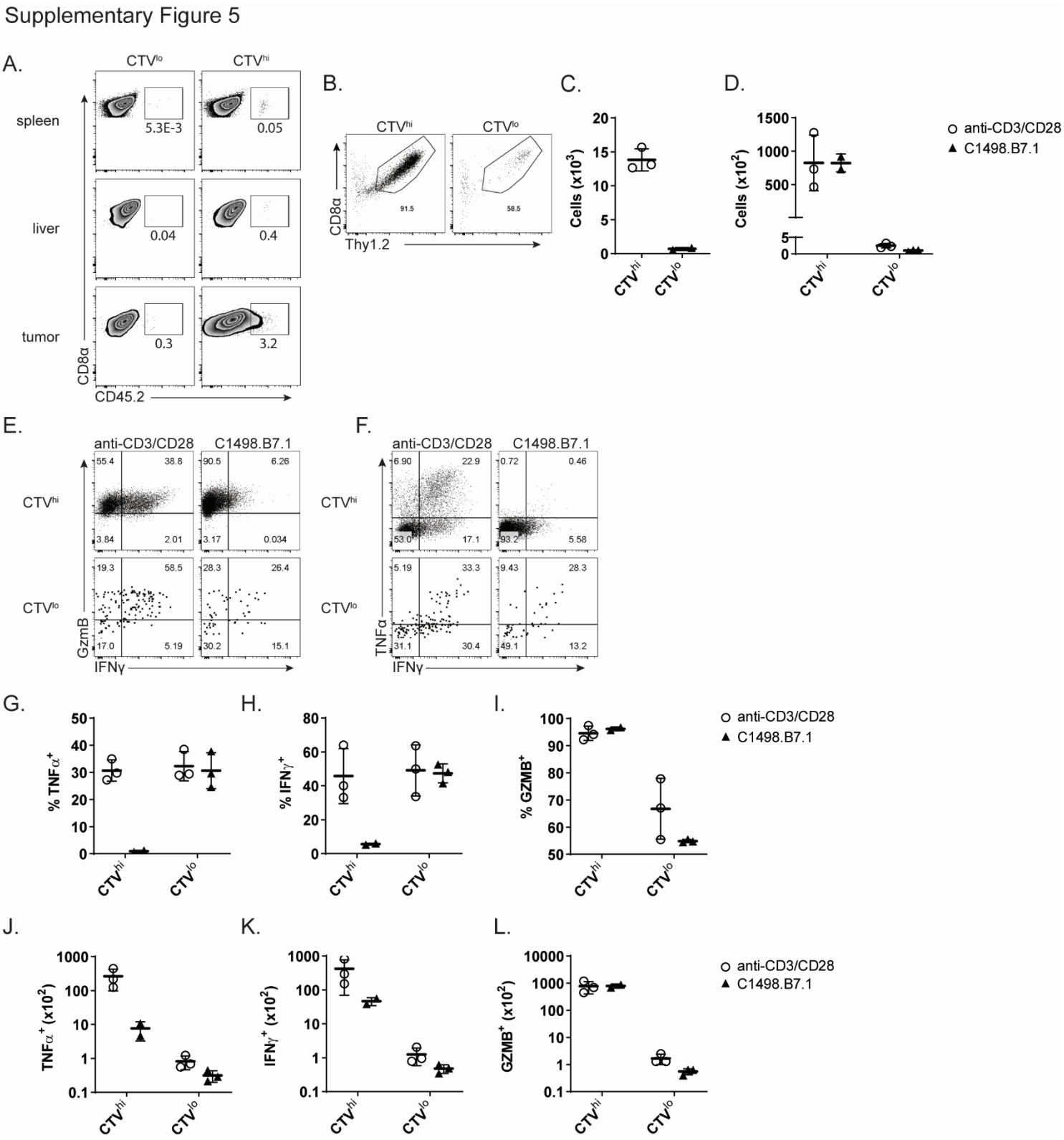
Supplementary Figure 3. TCR_{Tg101} effector cytokine, proliferation and apoptosis profiles. Related to Figure 2. (**A-C**) Mononuclear cells isolated from livers of mice 18 days after C1498 cell inoculation (19 days following TCR_{Tg101} (CD45.1.2) adoptive transfer) were restimulated with anti-CD3 and anti-CD28 antibodies or irradiated C1498.B7.1 cells with IL-2 (50 U/ml), IL-7 (5 ng/ml), and IL-12 (20 ng/ml) overnight. Cytokine production was analyzed by flow cytometry. Gating was performed on $TCR\beta^+CD8^+CD45.1$ cells (endogenous $CD8^+$ T cells) or $TCR\beta^+CD8^+CD45.1.2$ cells (TCR_{Tg101}). (**A**) Representative FACS plots showing IFNγ and Granzyme B (GzmB)

production by endogenous $CD8^+$ T cells or TCR_{Tg101}. Quantitative data are shown in (**B and C**) as mean ± SD. (**D-F**) $CD8^+$ T cells (endogenous and TCR^{Tg101}) were purified or not from Ficoll-enriched mononuclear cells from livers of mice 18 days after C1498 cell inoculation. Bulk mononuclear cells or purified $CD8^+$ T cells were restimulated with anti-CD3 and anti-CD28 antibodies plus IL-2 and IL-12 overnight, and cytokine production was analyzed by flow cytometry. Gating was performed on $TCR\beta^+CD8^+CD45.1$ cells (endogenous $CD8^+$ T cells) or $TCR\beta^+CD8^+CD45.1.2$ cells (TCR_{Tg101}). (**D**) Representative FACS plots showing TNFα and IFNγ production by endogenous $CD8^+$ T cells or TCR_{Tg101} from bulk or purified $CD8^+$ T cell populations. Quantitative data are shown in (**E and F**) as mean ± SD. * $p < 0.05$; n.s. – not significant (**G and H**) BrdU incorporation by CTV-labeled TCR_{Tg101} in livers of leukemia-bearing mice 17 days after C1498 cell challenge. (**G**) Representative FACS plots showing CTV^{hi}, CTV^{med}, and CTV^{low} TCR_{Tg101} populations (left) and BrdU incorporation by CTV dilution of TCR_{Tg101} (right). Gating was performed on $TCR\beta^+CD8^+CD45.2$ cells. (**H**) Quantified data from (**G**) as mean ± SD. (**I** and **J**) Activated caspase 3 in endogenous $CD8^+$ T cells or TCR_{Tg101} from spleens or livers of leukemia-bearing mice 17 days following C1498 cell challenge. (**I**) Representative FACS plots showing activated caspase 3 expression in endogenous $CD8^+$ T cells and TCR_{Tg101} in livers of leukemia-bearing animals. (**J**) Quantified data shown as mean ± SD. Data are pooled from 2 experiments with 2-3 mice/group. * $p < 0.05$; ** $p < 0.01$. *** $p < 0.001$. n.s. – not significant.

Supplementary Figure 4



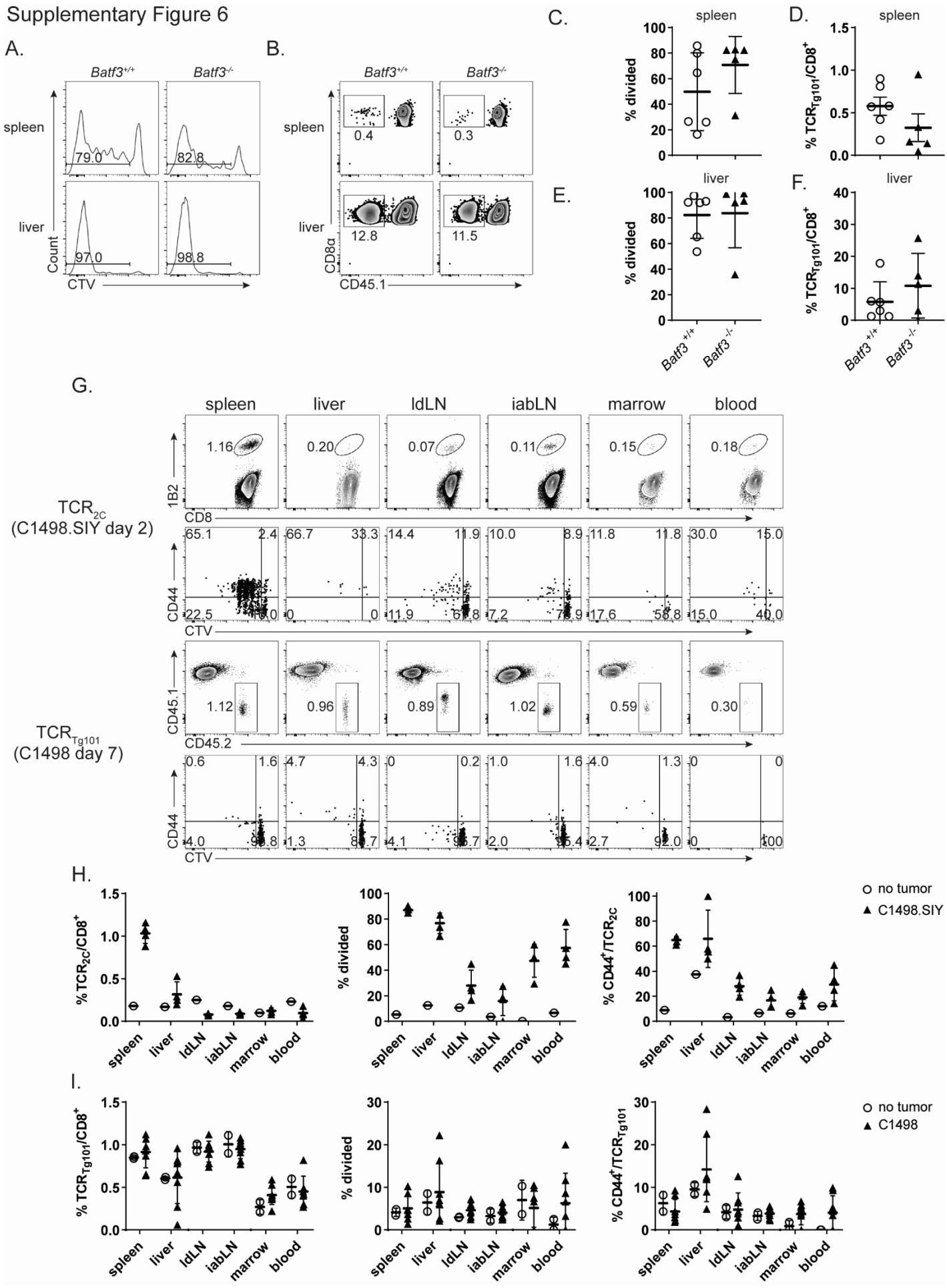
Supplementary Figure 4. Volcano plots showing differentially expressed genes in TCR_{Tg101} at specific time points. Related to Figure 3. Pairwise comparisons of RNA-seq profiles of TCR_{Tg101} from leukemia-bearing mice at early (day 6-7, pink icon), mid (day 13-14, red icon), and late (day 16-18, maroon icon) time points, as well as naïve mice (grey icon). Genes with an adjusted p-value <0.05 (Benjamini-Hochberg) were considered to be differentially expressed and are depicted in red.



Supplementary Figure 5. The dysfunctional TCR_{Tg101} phenotype is not readily reversible. Related to Figure 4. (A) Identification of naïve (CTV^{hi}) or exhausted (CTV^{lo}) TCR_{Tg101} in secondary hosts. Experimental design outlined in Figure 4A. Representative FACS plots showing frequencies of naïve (CTV^{hi}) and exhausted (CTV^{lo}) TCR_{Tg101} (CD45.1.2) in the indicated tissues of secondary B6.SJL mice (CD45.1) with s.c. C1498 tumors. (**B and**

C) CTV^{hi} (naïve) and CTV^{lo} (exhausted) TCR_{Tg101} were FACS-purified from livers of leukemia-bearing mice17-18 days after i.v C1498 cell challenge, and ($1x10^4$) were cultured with of IL-2 (50 U/ml), IL-7 (5 ng/ml), IL-12 (20 ng/ml) and IL-15 (5 ng/ml) for 5 days, at which point, surviving TCR_{Tg101} were enumerated. (**B**) Representative FACS plots showing live TCR_{Tg101} from CTV^{hi} and CTV^{lo} populations. Quantified data are shown in (**C**). (**D-L**) CTV^{hi} and CTV^{lo} TCR_{Tg101} were FACS-purified from livers of leukemia-bearing mice 17-18 days after i.v C1498 cell challenge, and $2\text{-}3x10^4$ CTV^{hi} or CTV^{lo} TCR_{Tg101} were restimulated with anti-CD3 and anti-CD28 antibodies or irradiated C1498.B7.1 cells in the presence of IL-2 (50 U/ml) and IL-12 (20 ng/ml) for 5 days. Viability and cytokine production by CTV^{hi} and CTV^{lo} TCR_{Tg101} was analyzed by flow cytometry. (**D**) Quantification of live TCR_{Tg101} following 5 days of *in vitro* culture. (**E and F**) Representative FACS plots showing effector cytokine production and GzmB expression among *in vitro* cultured CTV^{hi} and CTV^{lo} TCR_{Tg101} following restimulation with anti-CD3 and anti-CD28 antibodies or with irradiated C1498.B7.1 cells. (**G-I**) Quantified data showing frequencies of IFNγ-, TNFα-, or dual cytokine-producing, viable CTV^{hi} and CTV^{lo} TCR_{Tg101} after restimulation. (**J-L**) Quantified data showing absolute numbers of IFNγ-, TNFα-, or dual cytokine-producing, viable CTV^{hi} and CTV^{lo} TCR_{Tg101} after restimulation. Data are representative of 2 experiments with 2-3 mice/group and shown as mean ± SD.

Supplementary Figure 6



Supplementary Figure 6. TCR_{Tg101} initially encounter antigen in the liver in a cDC1-independent manner. Related to Figures 5 and 6. (**A-F**) CTV-labeled TCR_{Tg101} (CD45.2) were adoptively transferred into *Batf3*$^{+/+}$ (CD45.1) or *Batf3*$^{-/-}$ (CD45.1.2) mice 1 day prior to i.v. challenge with C1498 cells. Fifteen days later, TCR_{Tg101} proliferation and frequencies in spleens and livers of *Batf3*$^{+/+}$ and *Batf3*$^{-/-}$ mice were analyzed by flow cytometry. Representative FACS plots showing CTV dilution (**A**) or frequency (**B**) of TCR_{Tg101} in spleens and livers of leukemia-bearing *Batf3*$^{+/+}$ and *Batf3*$^{-/-}$ mice. (**C-F**) Quantitative data showing percent divided TCR_{Tg101} (**C and E**) and TCR_{Tg101} frequencies (**D and F**) in spleens (**C and D**) and livers (**E and F**) of leukemia-bearing *Batf3*$^{+/+}$ versus *Batf3*$^{-/-}$ mice. Data in **C-E** are pooled from 2 independent experiments with 2-3 mice/group and shown as mean ± SD. (**G-I**) $1x10^6$ CTV-labeled TCR_{2C} or TCR_{Tg101} were adoptively transferred into groups of congenic C57BL/6 mice that received an i.v. challenge with C1498.SIY cells (TCR_{2C}) or C1498 cells (TCR_{Tg101}) the following day, or that received no subsequent leukemia cell challenge. Two (TCR_{2C}) or seven (TCR_{Tg101}) days later, spleens, livers, bone marrow, pooled skin-draining lymph nodes, pooled liver-draining lymph nodes (portal and celiac), and peripheral blood were harvested. Frequencies, proliferation profiles, and CD44 expression among TCR_{2C} or TCR_{Tg101} at the indicated sites were analyzed by flow cytometry. (**G**) Representative FACS plots showing TCR_{2C} (top) or TCR_{Tg101} (bottom) frequency, proliferation (CTV dilution) and CD44 expression at the sites indicated. (**H and I**) Quantitative data from (**G**), including data from leukemia-free control mice. Data in **H** and **I** are pooled of 2 independent experiments with 2-4 mice/group and shown as mean ± SD.