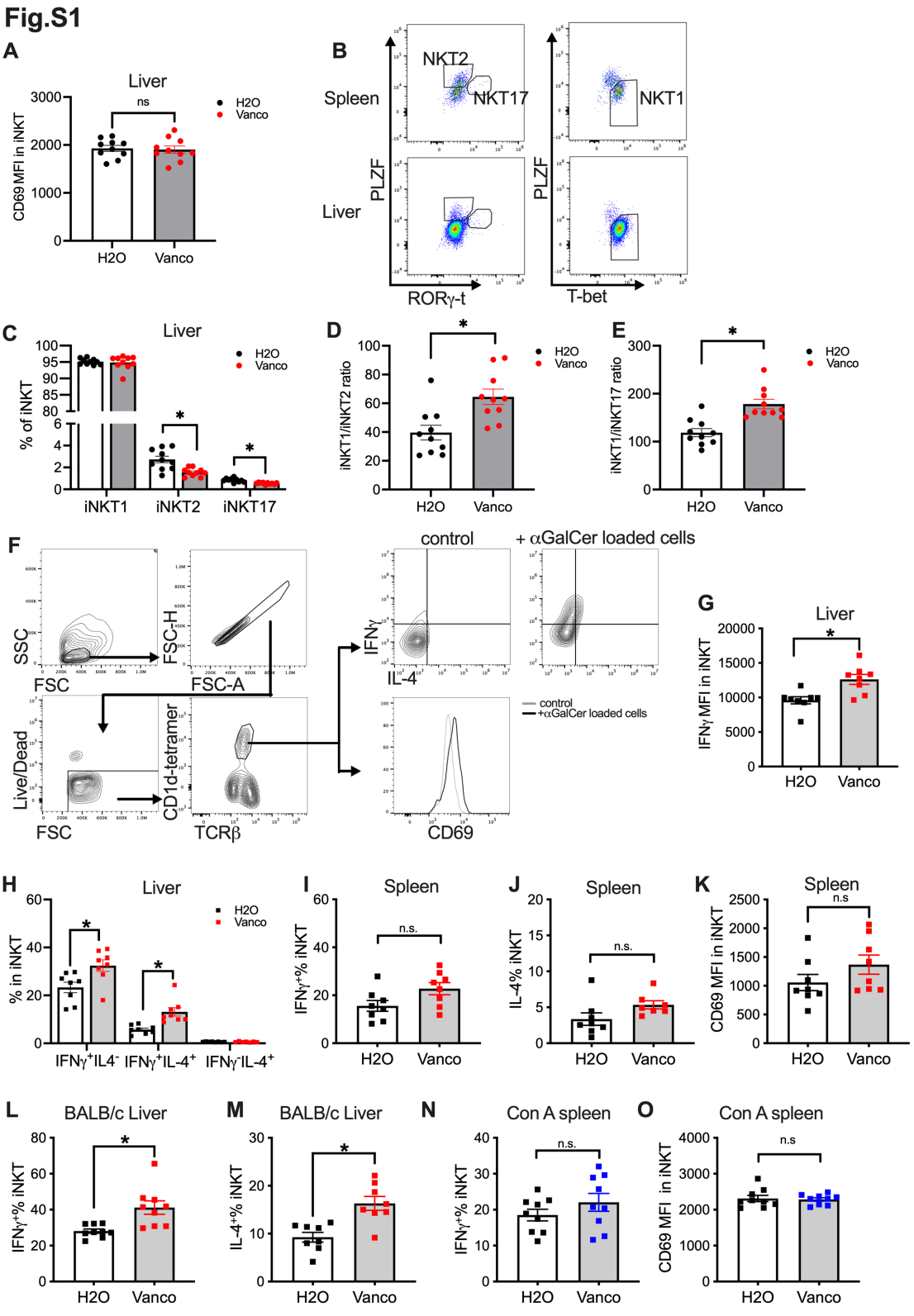
Supplementary figure and legend



Supplementary Figure 1, related to Figure 1.

(**A**) C57BL/6 mice were kept with vancomycin or H2O for 3 weeks. Liver iNKT CD69 MFI was measured by flow cytometry. Results are presented as mean+/- SEM from two independent experiment, n=10, student t-test.

(**B-E**) C57BL/6 mice were kept with vancomycin or H2O for 3 weeks. iNKT subsets were measured by flow cytometry. (**B**)Gating strategy for iNKT subsets. Liver iiNKT subset composition (**C**), iNKT1/iNKT2 ratio (**D**) and iNKT1/iNKT17 ratio (**E**) were measured. Results are presented as mean+/- SEM from two independent experiment, n=10, $p<0.05$, two-way ANOVA for (**C**), student t-test for (**D,E**).

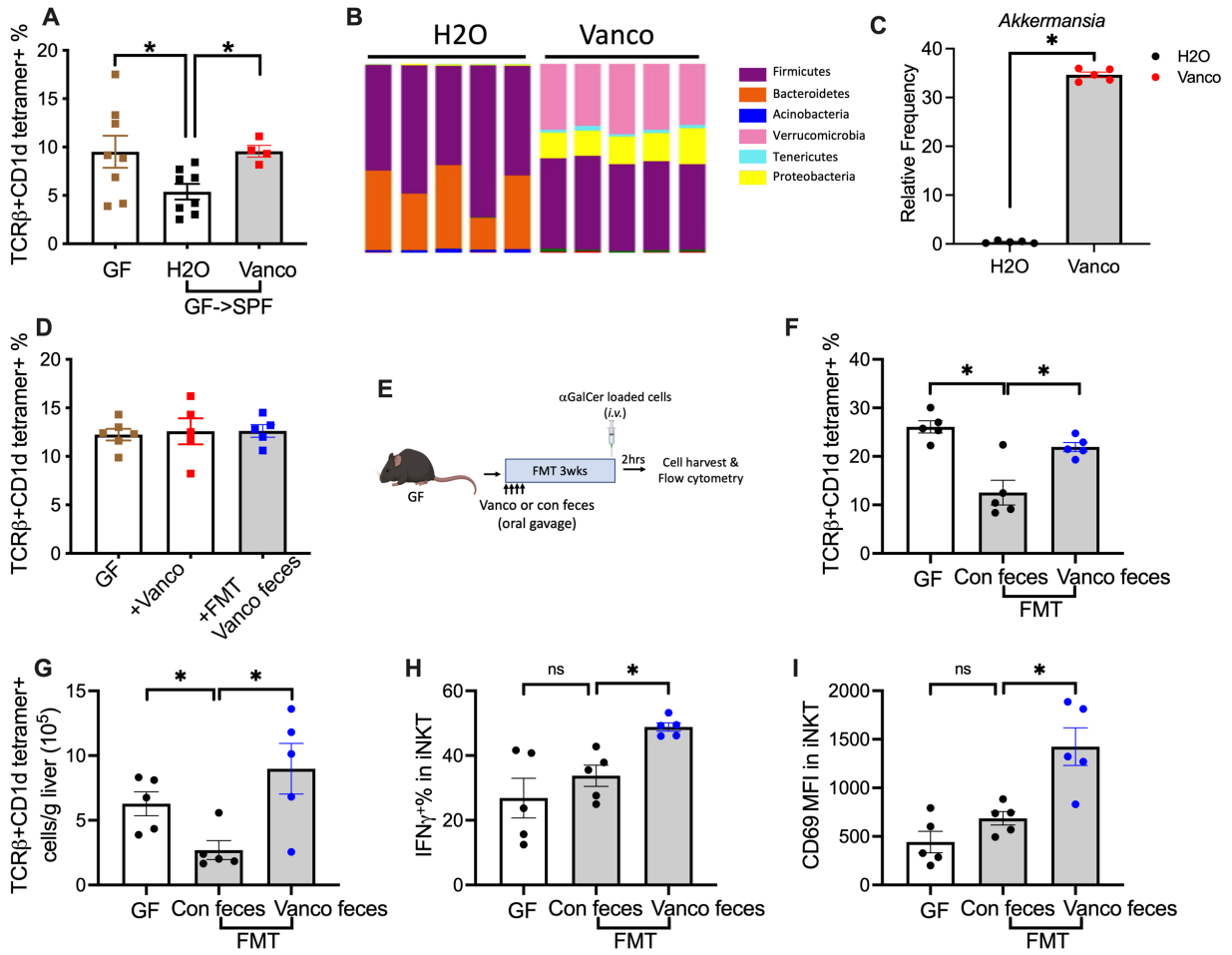
(**F**)Gating strategy for cytokine producing iNKT cells.

(**G-K**) αGalCer-loaded EL4 cells were injected *i.v.* into C57BL/6 mice kept on vancomycin or H2O for 3 weeks. Liver iNKT cells IFNγ MFI (**G**) and IFNγ or IL-4 positive frequencies (**H**) were measured. IFNγ% (**I**), IL-4% (**J**) and CD69 MFI (**K**) of spleen iNKT cells were determined. Results are presented as mean+/- SEM of two independent experiments, n=8, student t-test.

(**L, M**) BALB/c mice were kept on vancomycin or H2O for 3 weeks. αGalCer-loaded A20 cells plus brefeldin A were injected *i.v.*. Two hours later, liver mononuclear cells were isolated and liver iNKT cell IFNγ% (**L**) and IL-4% (**M**) were measured. Results are presented as mean+/- SEM of two independent experiments, n=8, $p<0.05$, student t-test.

(**N, O**) 50ug ConA was injected *i.v.* in C57BL/6 mice kept on vancomycin or H2O for 3 weeks. Spleen iNKT cell IFNγ% (**N**) and CD69 MFI (**O**) were measured. Results are presented as mean+/- SEM from two independent experiments, n=9, $p<0.05$, student t-test.

Fig.S2



Supplementary Figure 2, related to Figure 2.

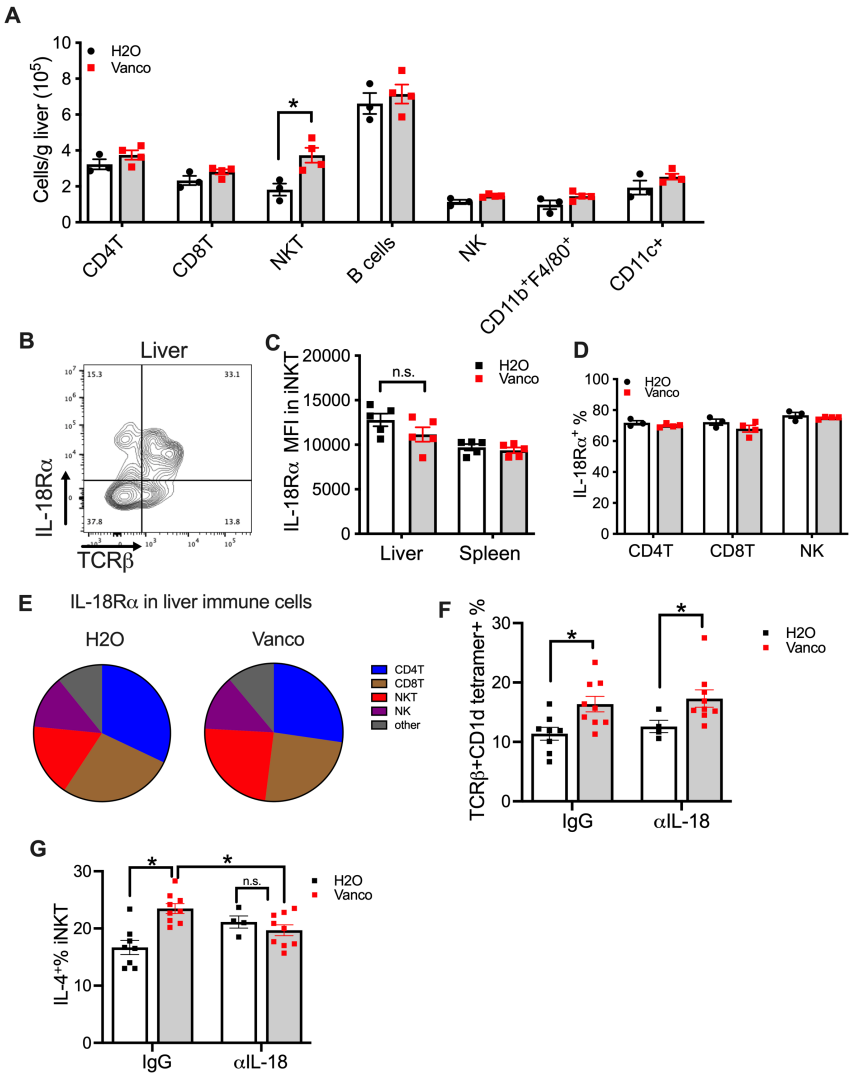
(**A**) BALB/c germ free mice were transferred to SPF facility to recolonize gut microbiome. Then the conventionalized mice were kept on vancomycin for H2O for 3 weeks. Frequency of liver iNKT cells were measured. Results are presented as mean+/- SEM from two independent experiments, n=8 for GF, 8 for GF->SPF H2O, 4 for GF->SPF Vanco, $p<0.05$, one-way ANOVA.

(**B,C**) Feces from C57BL/6 mice kept on vancomycin or H2O were analyzed by 16S rRNA sequencing. Fecal bacterial composition at phylum level (**B**) and relative abondance of *Akkermansia muciniphila* (**C**) are shown.

(**D**) BALB/c germ free mice were kept on vancomycin or FMT with vanco feces. Frequency of liver iNKT cells were measured. Results are presented as mean+/- SEM from one experiment, n=6 for GF, 5 for vanco, 5 for vanco feces, one-way ANOVA.

(**E-I**) C57BL/6 ferm free mice received FMT of control or vanco feces. Three weeks later mice were given *i.v.* injection of αGalCer-loaded EL4 cells with brefeldin A. Two hours later mice were euthanized, and liver mononuclear cells were isolated for flow cytometry analysis. Liver iNKT absolute numbers (**F**), frequencies(**G**), IFNγ% (**H**) and CD69MFI (**I**) were measured. Results are presented as mean+/- SEM from one experiment, n=5, $p<0.05$, one-way ANOVA.

Fig.S3



Supplementary Figure 3, related to Figure 3.

(**A**) Frequency of different immune cells in the liver of mice kept on vancomycin or H2O. Results are presented as mean+/- SEM from one experiment, n=4, $p<0.05$, two-way ANOVA.

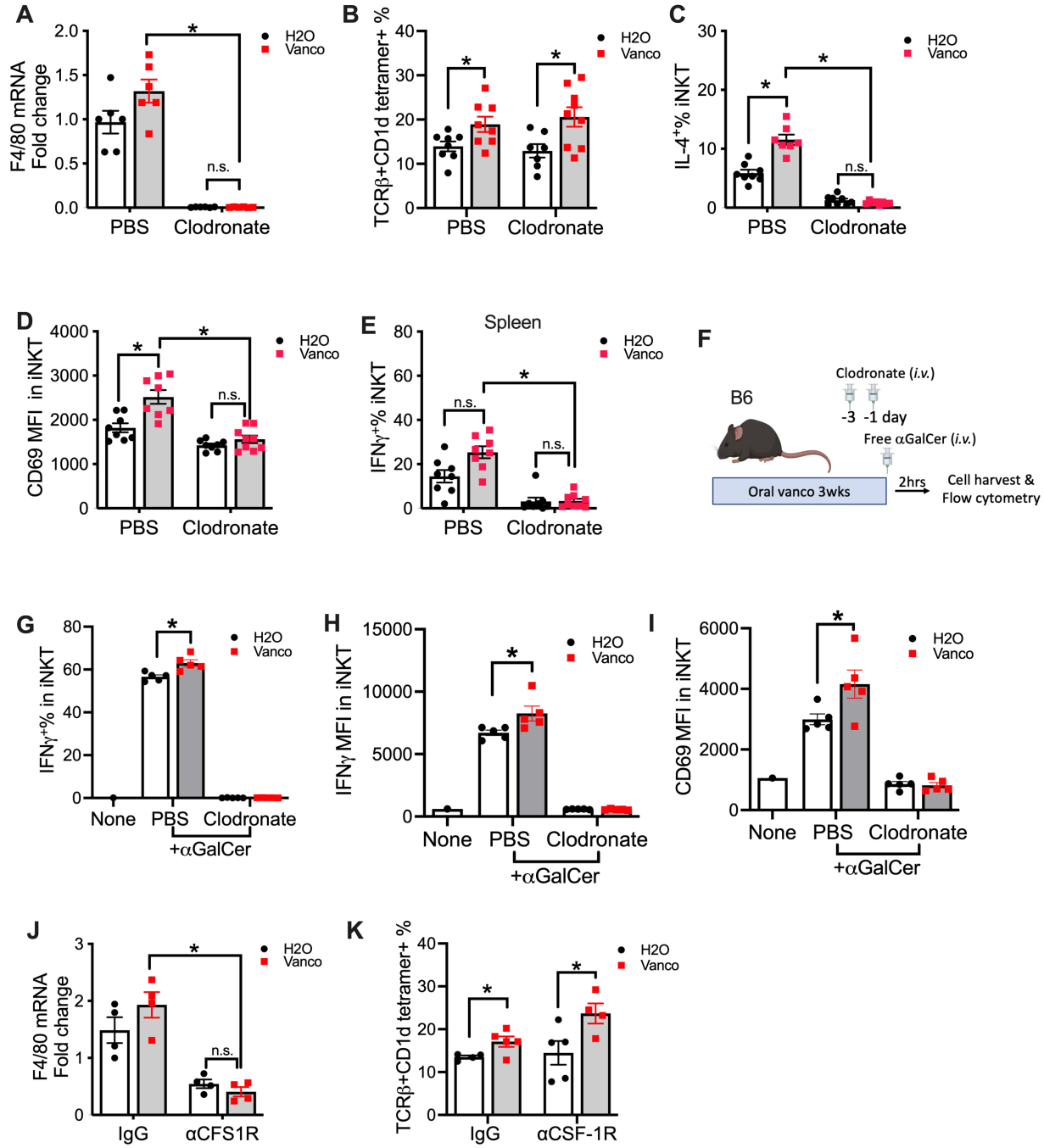
(**B**) Representative plot of IL-18Ra staining of liver iNKT cells.

(**C**,**D**) Mice were kept on vancomycin or H2O for three weeks. IL-18Ra MFI of iNKT cells (**C**) and IL-18Ra positive frequencies of liver $CD4^+$ T, $CD8^+$ T and NK cells (**D**) were measured. Results are presented as mean+/- SEM of one experiment, n=4.

(**E**) The composition of liver IL-18Ra$^+$ immune cells from mice treated with vancomycin or H2O.

(**F, G**) Vancomycin or H2O treated BALB/c mice were given two *i.p.* injections of αIL-18. Then mice were injected with αGalCer-loaded A20 cells. Frequencies of liver iNKT (**F**) and IL-4$^+$ iNKT cells (**G**) were measured. Results are presented as mean+/- SEM from two independent experiments. n=8 for IgG H2O, 9 for IgG vanco, 4 for αIL-18 H2O, 9 for αIL-18 vanco, $p<0.05$, two-way ANOVA.

Fig.S4


A
F4/80 mRNA Fold change
H2O
Vanco
n.s.
PBS
Clodronate
B
TCRβ+CD1d tetramer+ %
C
IL-4+% iNKT
D
CD69 MFI in iNKT
E
Spleen
IFNγ+% iNKT
F
B6
Clodronate (i.v.)
-3 -1 day
Free αGalCer (i.v.)
Oral vanco 3wks
2hrs
Cell harvest & Flow cytometry
G
IFNγ+% in iNKT
None
+αGalCer
H
IFNγ MFI in iNKT
I
CD69 MFI in iNKT
J
IgG
αCFS1R
K
αCSF-1R

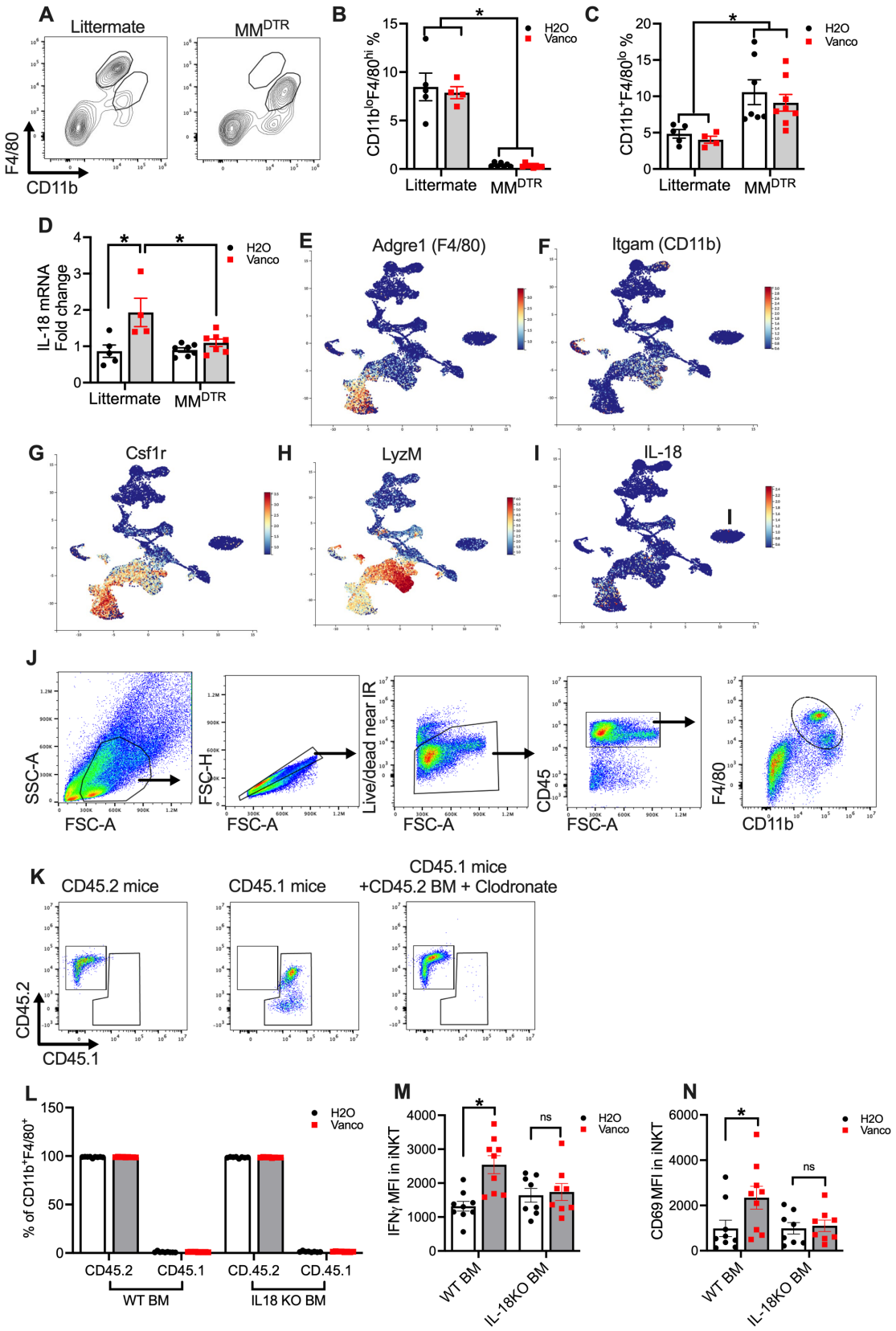
Supplementary Figure 4, related to Figure 4.

(**A-E**) Vancomycin or H2O treated C57BL/6 mice were given two doses of clodronate injections to deplete macrophages. Then mice were *i.v.* injected with αGalCer-loaded EL4 cells with brefeldin A. Two hours later mice were euthanized for analysis. (**A**) Liver F4/80 mRNA was measured by RT-PCR. **(B)** Frequency of iNKT cells in the liver; (**C**) IL-4$^+$ iNKT cells in the liver; (**D**)CD69MFI on iNKT cells in the liver; (**E**) frequencies of IFNγ$^+$ iNKT cells in spleen were measured by flow cytometry. Results are presented as mean+/- SEM of two independent experiments. $p<0.05$, two-way ANOVA.

(**F-I**) Vancomycin or H2O treated C57BL/6 mice were given two doses of clodronate injections to deplete macrophages. Then mice were *i.v.* injected with free αGalCer (0.5ug/mouse) with brefeldin A. Two hours later mice were euthanized for analysis. Liver iNKT cells IFNγ$^+$%(**G**), IFNγ MFI (**H**) and CD69MFI (**I**) were measured by flow cytometry. Results are presented as mean+/- SEM of one experiment. n=5, $p<0.05$, two-way ANOVA.

(**J,K**) Vancomycin or H2O treated BALB/c mice were given two doses of αCSF-1R injections. Then mice were i.v. injected with αGalCer-loaded A20 cells. Two hours later mice were euthanized for analsyis. (**J**) Liver F4/80 mRNA was measured by RT-PCR. (**K**) Frequency of iNKT cells in the liver were measured by flow cytometry. Results are presented as mean+/- SEM from one experiment, n=4 for IgG H2O and IgG Vanco, n=5 for αCSF-1R H2O and αCSF-1R Vanco, $p<0.05$, two-way ANOVA

Fig.S5


A
Littermate
MMDTR
F4/80
CD11b
B
CD11bloF4/80hi %
H2O
Vanco
Littermate
MMDTR
C
CD11b+F4/80lo %
H2O
Vanco
Littermate
MMDTR
D
IL-18 mRNA Fold change
H2O
Vanco
Littermate
MMDTR
E
Adgre1 (F4/80)
F
Itgam (CD11b)
G
Csf1r
H
LyzM
I
IL-18
J
SSC-A
FSC-A
FSC-H
FSC-A
Live/dead near IR
FSC-A
CD45
FSC-A
F4/80
CD11b
K
CD45.2 mice
CD45.1 mice
CD45.1 mice
+CD45.2 BM + Clodronate
CD45.2
CD45.1
L
% of CD11b+F4/80+
H2O
Vanco
CD45.2
CD45.1
CD.45.2
CD.45.1
WT BM
IL18 KO BM
M
IFNγ MFI in iNKT
H2O
Vanco
ns
WT BM
IL-18KO BM
N
CD69 MFI in iNKT
H2O
Vanco
ns
WT BM
IL-18KO BM

78

Supplementary Figure 5, related to Figure 4.

(**A-D**) MM^{DTR} mice or littermates were kept on vancomycin or H2O for 3 weeks. Then the mice were *s.c.* injected with DT 200ng/mouse. One day later, mice received *in vivo* stimulation with αGalCer-loaded EL4 cells. The mice were euthanized two hours later for analysis. (**A**) Representative plots of liver immune cells after DT-treatment are shown. Frequencies of $CD11b^{lo}F4/80^{hi}$ (**B**) and $CD11b^{+}F4/80^{lo}$ macrophages(**C**) in the liver were determined by flow cytometry. (**D**) Liver tissue IL-18 mRNA expression was measured by RT-PCR. Results are presented as mean+/- SEM from two independent experiments, n=5 for littermate H2O, 4 for littermate Vanco, 7 for MM^{DTR} H2O, 8 for MM^{DTR} Vanco, $p<0.05$, two-way ANOVA.

(**E-I**) Gene expression in $CD45^{+}$ mouse liver immune cells were visualized using reported scRNA-Seq dataset at www.livercellatlas.org. The expression of Agre1(F4/80) (**E**), Itgam(CD11b) (**F**), Csf1r(**G**), LyzM (**H**), and IL-18 (**I**) are shown.

(**J-N**) Liver macrophage reconstitution with IL-18 KO or WT macrophages using bone marrow transplantation combined with clodronate treatment. (**J**) Gating strategy for liver $CD11b^{+}F4/80^{+}$ cells. (**K**) Representative plots of CD45.1/45.2 staining of liver $CD11b^{+}F4/80^{+}$ cells from CD45.2 mice, CD45.1 mice or CD45.1 mice received transplantation of CD45.2 bone marrow plus clodronate treatment. (**L**) Confirmation of liver macrophage reconstitution by donor CD45.2 WT or IL-18 KO bone marrow derived macrophages. After *in vivo* stimulation, liver iNKT cells IFNγ MFI (**M**) and CD69 MFI (**N**) were measured by flow cytometry. Results are presented as mean+/- SEM from two independent experiments, n=10, $p<0.05$, two-way ANOVA.