marr

Stacks

Supplemental Information: Sex biases in ILC1 phenotypes do not alter immune control of cytomegalovirus

Remi Marrocco[1*], Meghan Nguyen[1], Andrea Balcan[1], Kaia Robinson[1] & Chris A. Benedict[1*]

Collaborators: Susan Kovats + 2 other reviewers

[1] Center for Vaccine Innovation and Center for Autoimmunity and Inflammation, La Jolla Institute for Immunology, La Jolla, CA, USA

Recommended Citation

Marrocco, R., M. Nguyen, A. Balcan, K. Robinson, and C. A. Benedict. 2026. Sex biases in ILC1 phenotypes do not alter immune control of cytomegalovirus. Stacks Journal: 26004. https://doi.org/10.60102/stacks-26004.

Accepted by 3 of 3 reviewers

Conflicts of Interest

The authors declare no conflicts of interest.

Publishing History

Submitted	August 20 2025
Accepted	February 9 2026
Published	March 31 2026

Corresponding Author

Remi Marrocco
rmarrocco@lji.org

Chris A. Benedict
benedict@lji.org

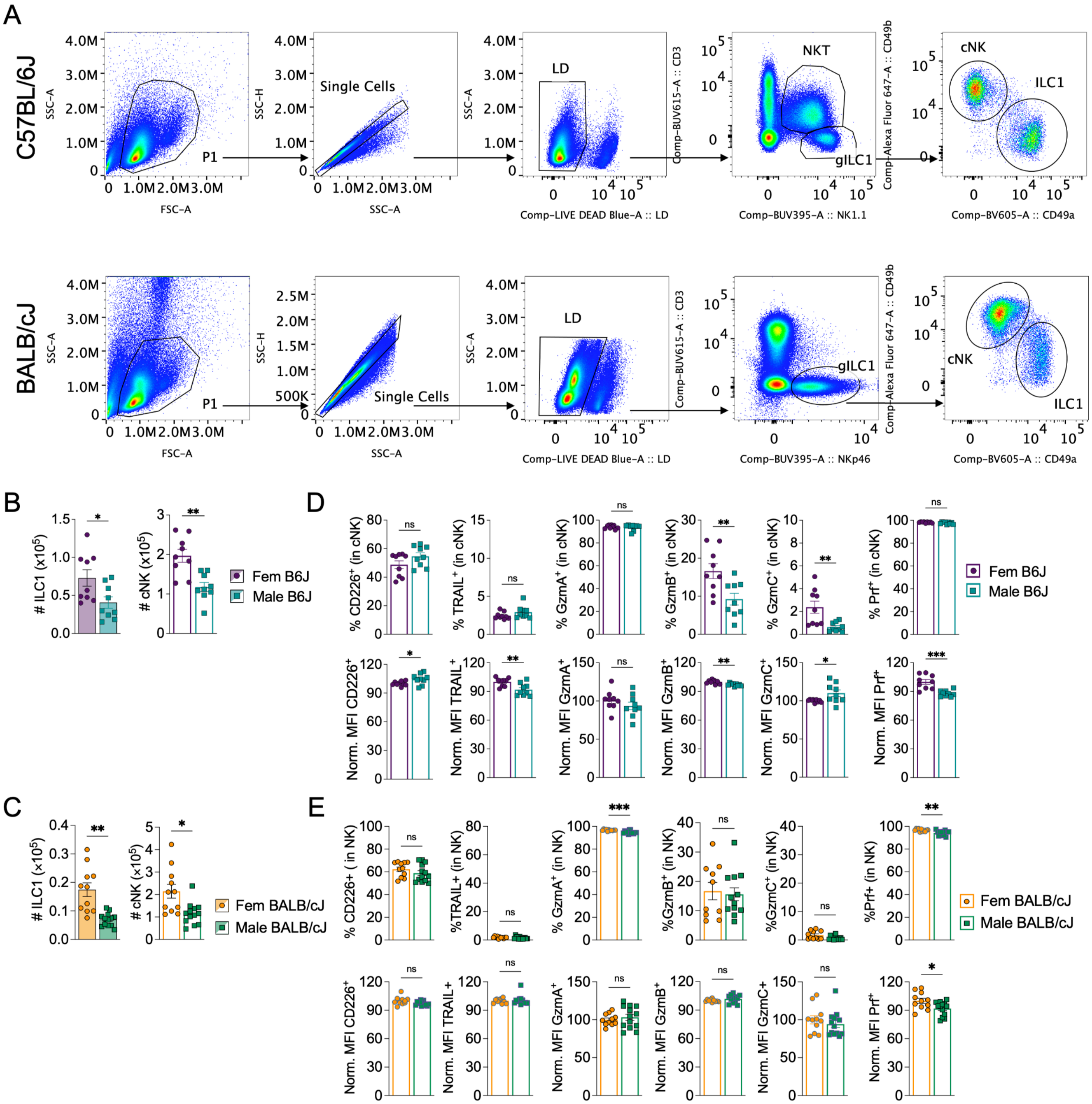

Figure S1. Sex-bias only slightly impact cNK phenotype. Male and female adult mice were euthanized and perfused with PBS before harvesting the liver for cytometry analysis *ex vivo*. (A) Gating strategy used to discriminate gILC1, and then ILC1 and cNK in B6J and BALB/cJ mice. (B,D) Analysis performed on B6J mice. Representative of 2 experiments (C-E) Analysis performed on BALB/cJ mice. Pool of 2 independent experiments. Data were analyzed by unpaired t-test. Normality and homoscedasticity were assessed. Welch’s t-test was performed when samples showed heteroscedasticity. Mann-Whitney was performed when normality tests were negative. (ns: non-significant, *p<0.05, **p<0.01, ***p<0.001).

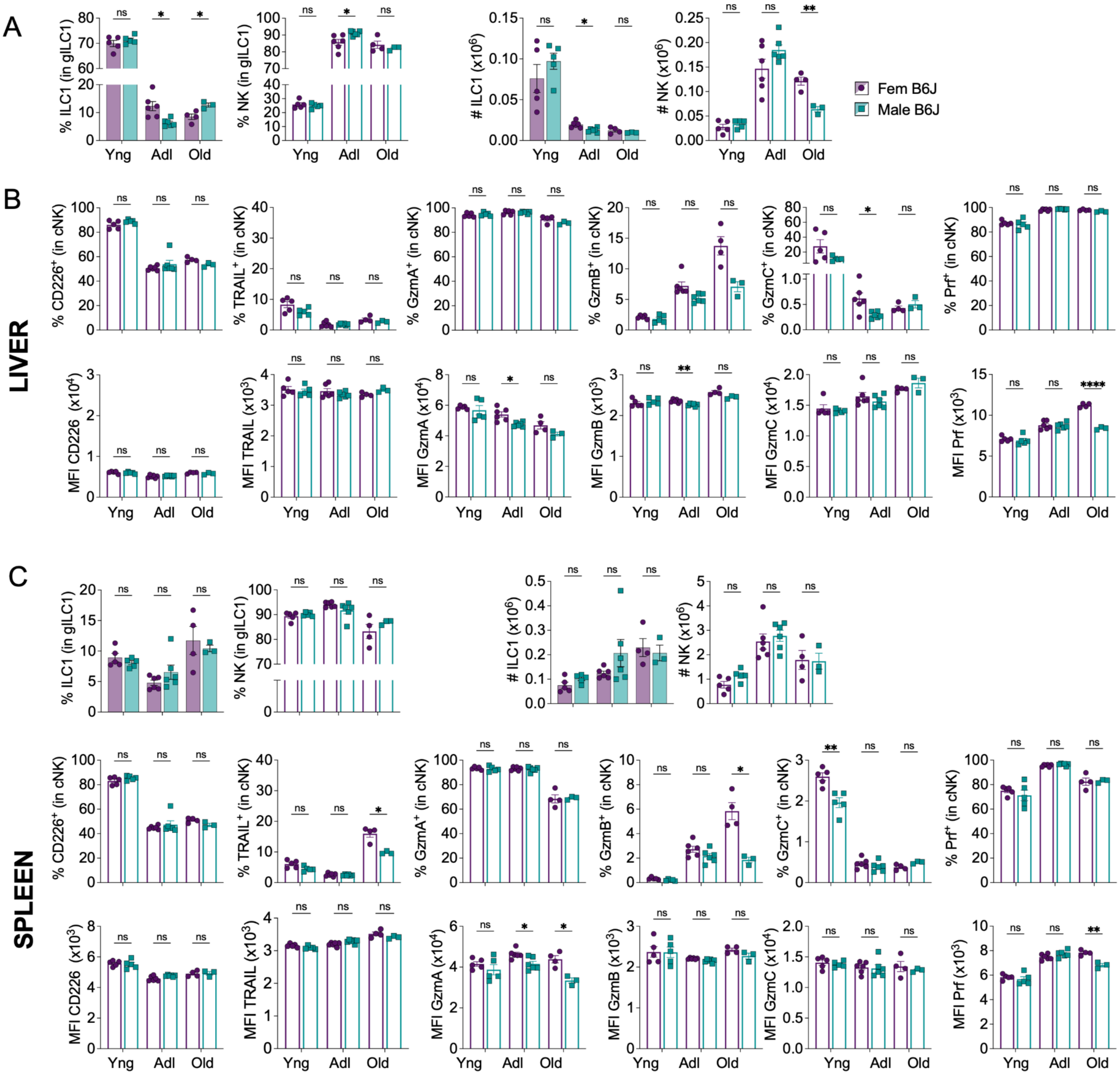

Figure S2. Sex-bias in ILC1 peaks in adulthood but weakly impacts cNK. Young (3w), adult (12w), and old (14m) male and female B6J mice were euthanized and perfused with PBS before harvesting the liver for cytometry analysis *ex vivo*. (A) Percentage and absolute number of ILC1 and cNK. (B) cNK phenotyping. Yng: young; Adl: adult. Data were analyzed by multiple unpaired t-tests, with Holm-Šídák correction. (ns: non-significant, *$p<0.05$, **$p<0.01$, ****$p<0.0001$).

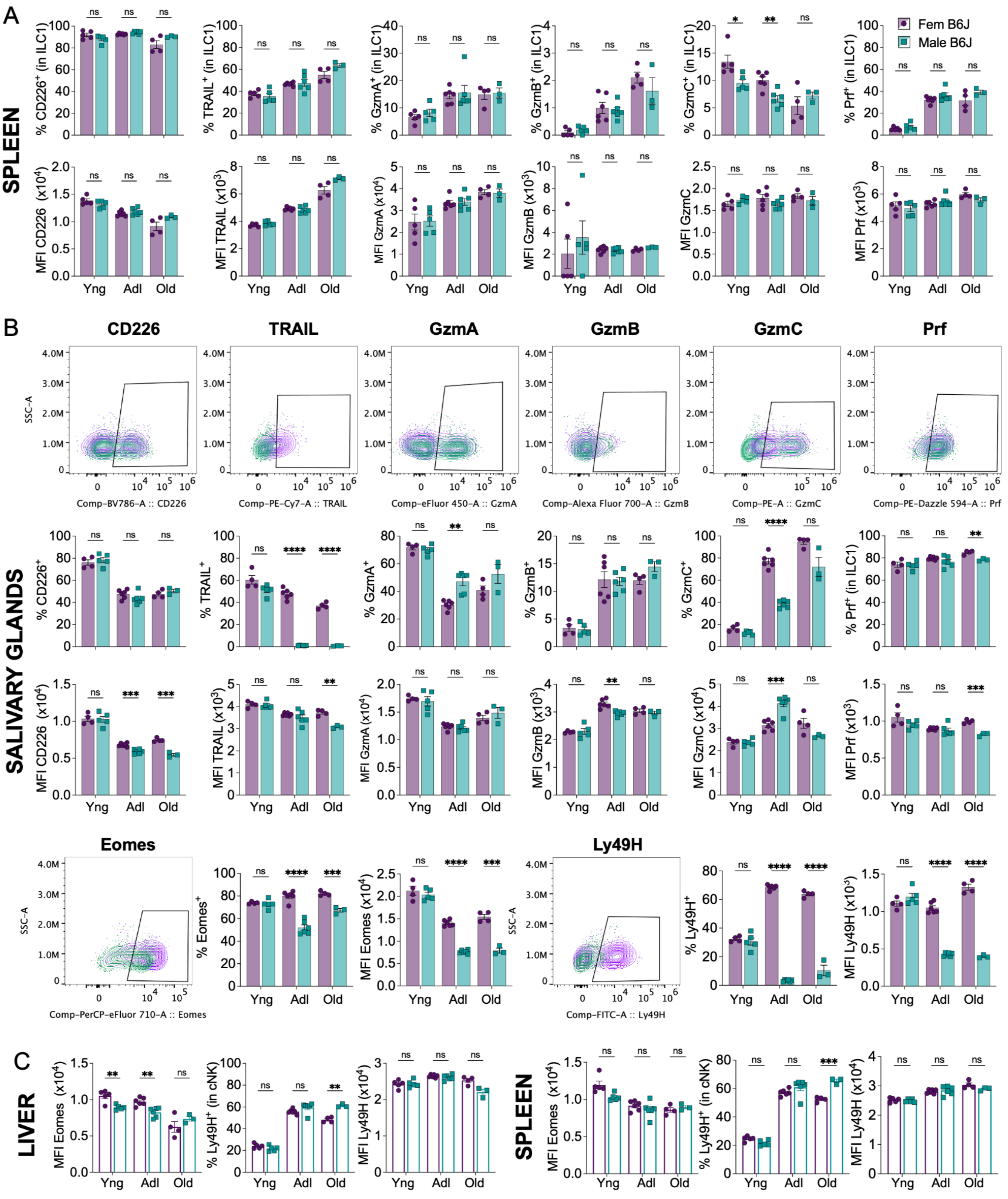
A
SPLEEN
% CD226+ (in ILC1)
% TRAIL+ (in ILC1)
% GzmA+ (in ILC1)
% GzmB+ (in ILC1)
% GzmC+ (in ILC1)
% Prf+ (in ILC1)
Fem B6J
Male B6J
MFI CD226 (x10^4)
MFI TRAIL (x10^3)
MFI GzmA (x10^4)
MFI GzmB (x10^3)
MFI GzmC
MFI Prf (x10^3)
Yng
Adl
Old
ns
*
**
B
CD226
TRAIL
GzmA
GzmB
GzmC
Prf
SSC-A
Comp-BV786-A :: CD226
Comp-PE-Cy7-A :: TRAIL
Comp-eFluor 450-A :: GzmA
Comp-Alexa Fluor 700-A :: GzmB
Comp-PE-A :: GzmC
Comp-PE-Dazzle 594-A :: Prf
SALIVARY GLANDS
% CD226+
% TRAIL+
% GzmA+
% GzmB+
% GzmC+
% Prf+ (in ILC1)
MFI CD226 (x10^4)
MFI TRAIL (x10^3)
MFI GzmA (x10^4)
MFI GzmB (x10^3)
MFI GzmC (x10^4)
MFI Prf (x10^3)
***
****
Eomes
Comp-PerCP-eFluor 710-A :: Eomes
% Eomes+
MFI Eomes (x10^4)
Ly49H
Comp-FITC-A :: Ly49H
% Ly49H+
MFI Ly49H (x10^3)
C
LIVER
MFI Eomes (x10^4)
% Ly49H+ (in cNK)
MFI Ly49H (x10^4)
SPLEEN
MFI Eomes (x10^4)
% Ly49H+ (in cNK)
MFI Ly49H (x10^4)

Figure S3. Sex-bias strongly impacts gILC1 in salivary glands. Young (3w), adult (12w), and old (14m) male and female B6J mice were euthanized and perfused with PBS before harvesting liver, spleen, and salivary glands for cytometry analysis *ex vivo*. (A) ILC1 phenotype in the spleen. (B) gILC1 phenotyping in the salivary glands (C) Eomes and Ly49H expression in liver-cNK and spleen-cNK. . Yng: young; Adl: adult. Data were analyzed by multiple unpaired t-tests, with Holm-Šídák correction. (ns: non-significant, *p<0.05, **p<0.01, ****p<0.0001).

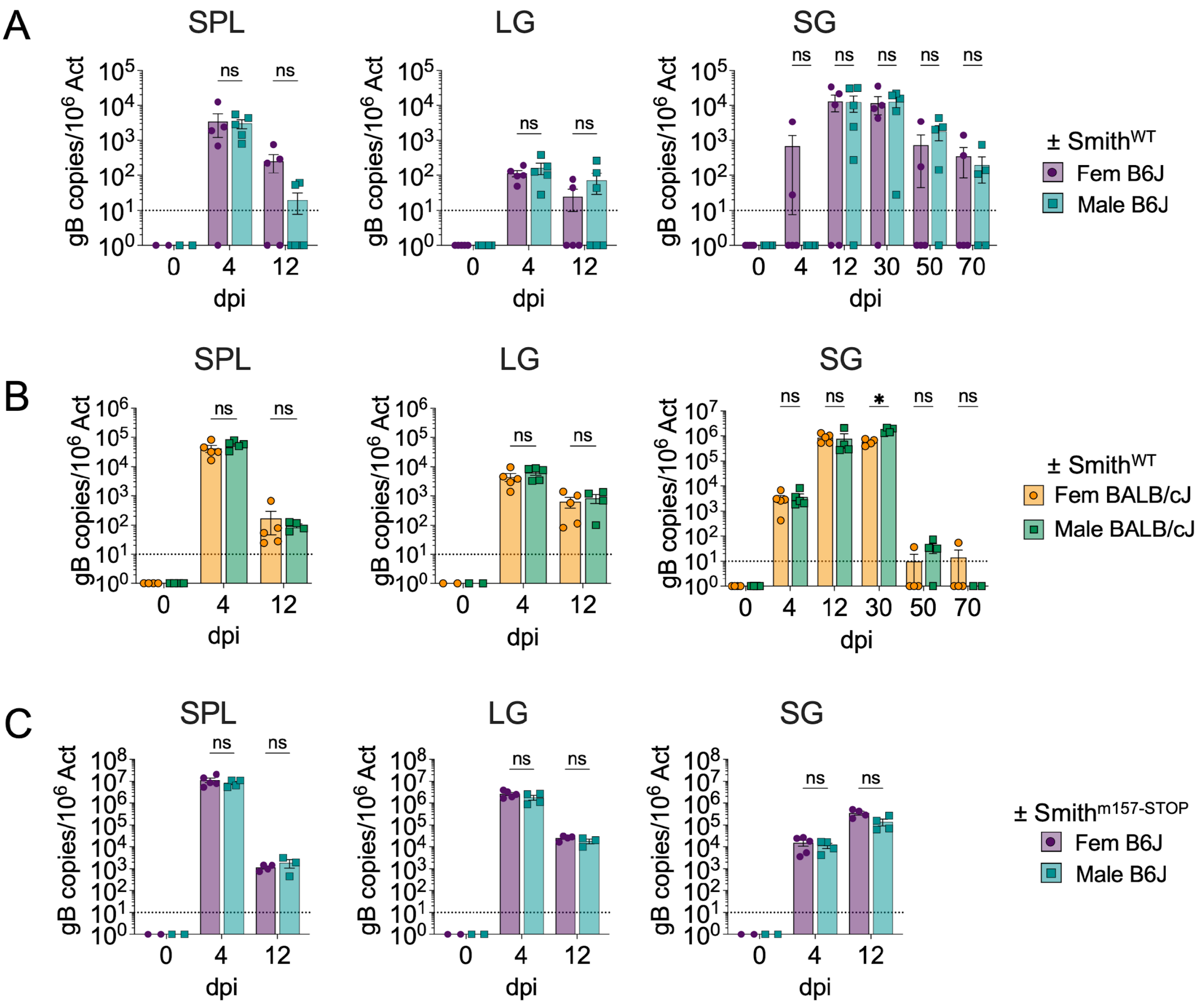

Figure S4. Males and females show similar ability to control MCMV infection. Male and female adult mice were infected with various strains of virus and euthanized 4- and 12-days post infection (dpi). In each case, mice were infected through intraperitoneal injection of 106pfu of *in vitro* produced MCMV, diluted in 100µL of PBS. (A) Infection of B6J mice with untouched Smith-MCMV. (B) Infection of BALB/cJ mice with untouched Smith-MCMV. (C) Infection of B6J mice with m157-deficient Smith MCMV. SPL: spleen; LG: lung; SG: salivary gland. Data were analyzed by multiple unpaired t-tests, with Holm-Šídák correction. (ns: non-significant, *p<0.05).

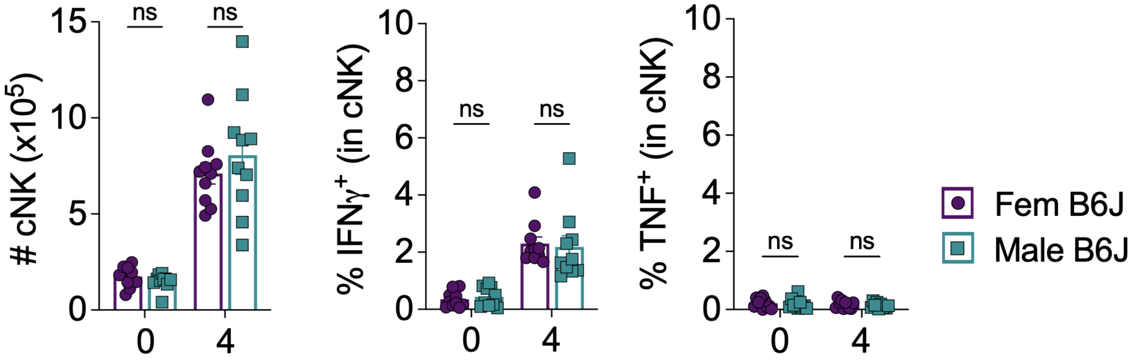

Figure S5. There is no sex-bias in cytokine production by cNK. Male and female B6J adult mice were infected with Smith-MCMV (i.p., 106pfu) and euthanized 4-days post infection (dpi), together with non-infected control mice. Mice were perfused with PBS before harvesting the liver for cytometry analysis following *in vitro* incubation with GolgiPlug. Data were analyzed by multiple unpaired t-tests, with Holm-Šídák correction. (ns: non-significant, *p<0.05, **p<0.01, ***p<0.001).

© Marrocco et al. (2026), *Stacks Journal,* DOI 10.60102/stacks-26004

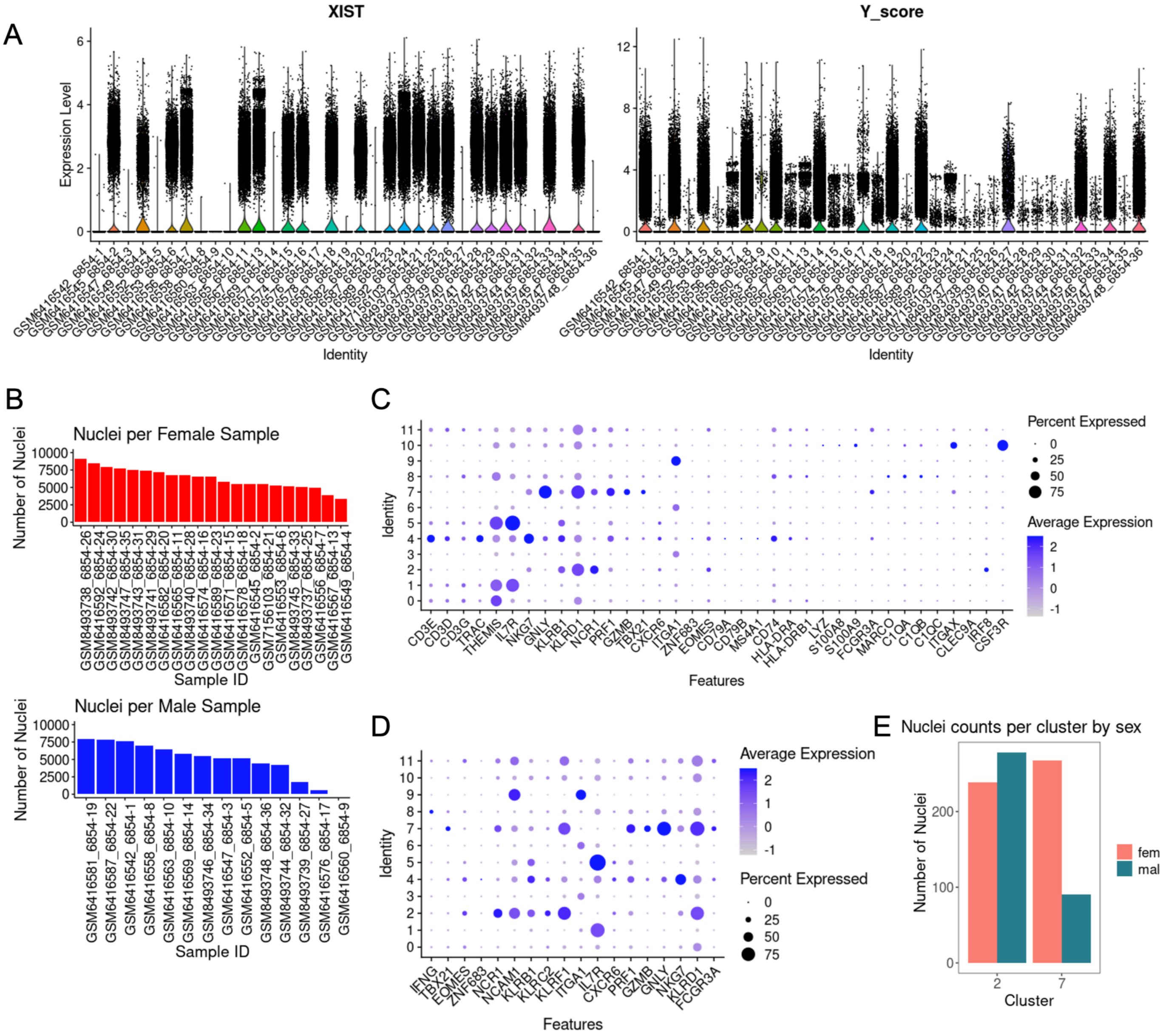

Figure S6. Analysis of a public snRNA-seq dataset on healthy human liver (GSE210077). (A) XIST-score and Y-score violin plot among all 34 samples. (B) nuclei count per sample after QC. (C) Immune genes expression among all 12 immune clusters. (D) expression of the 18 genes used for calculation of the gILC1-signature score. (E) Count of cluster 2 and cluster 7 cells among male and female groups.