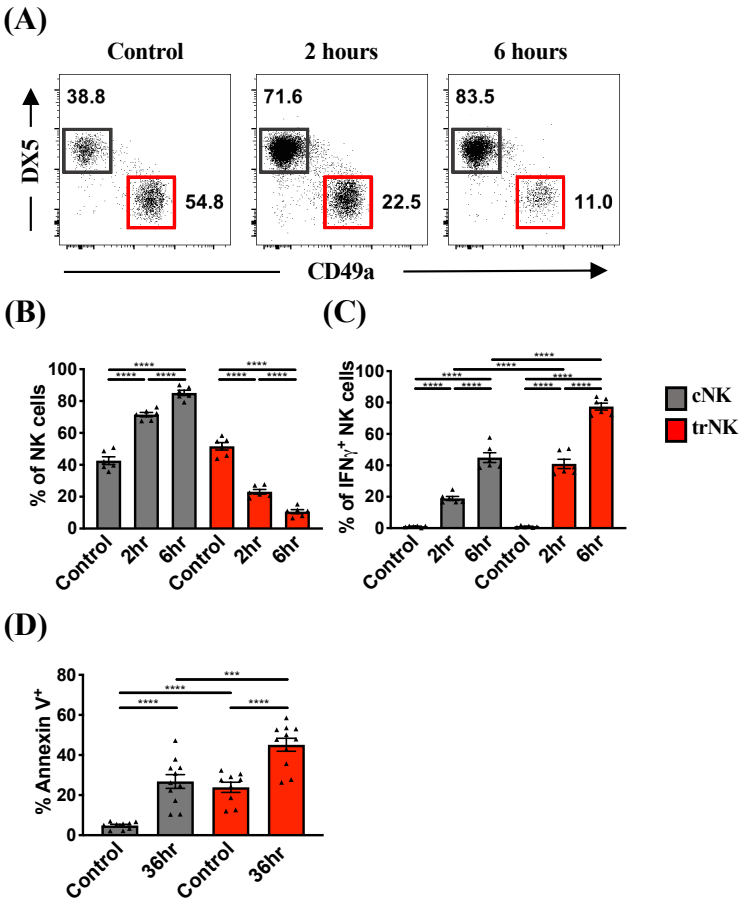
Cell Reports, Volume *32*

Supplemental Information

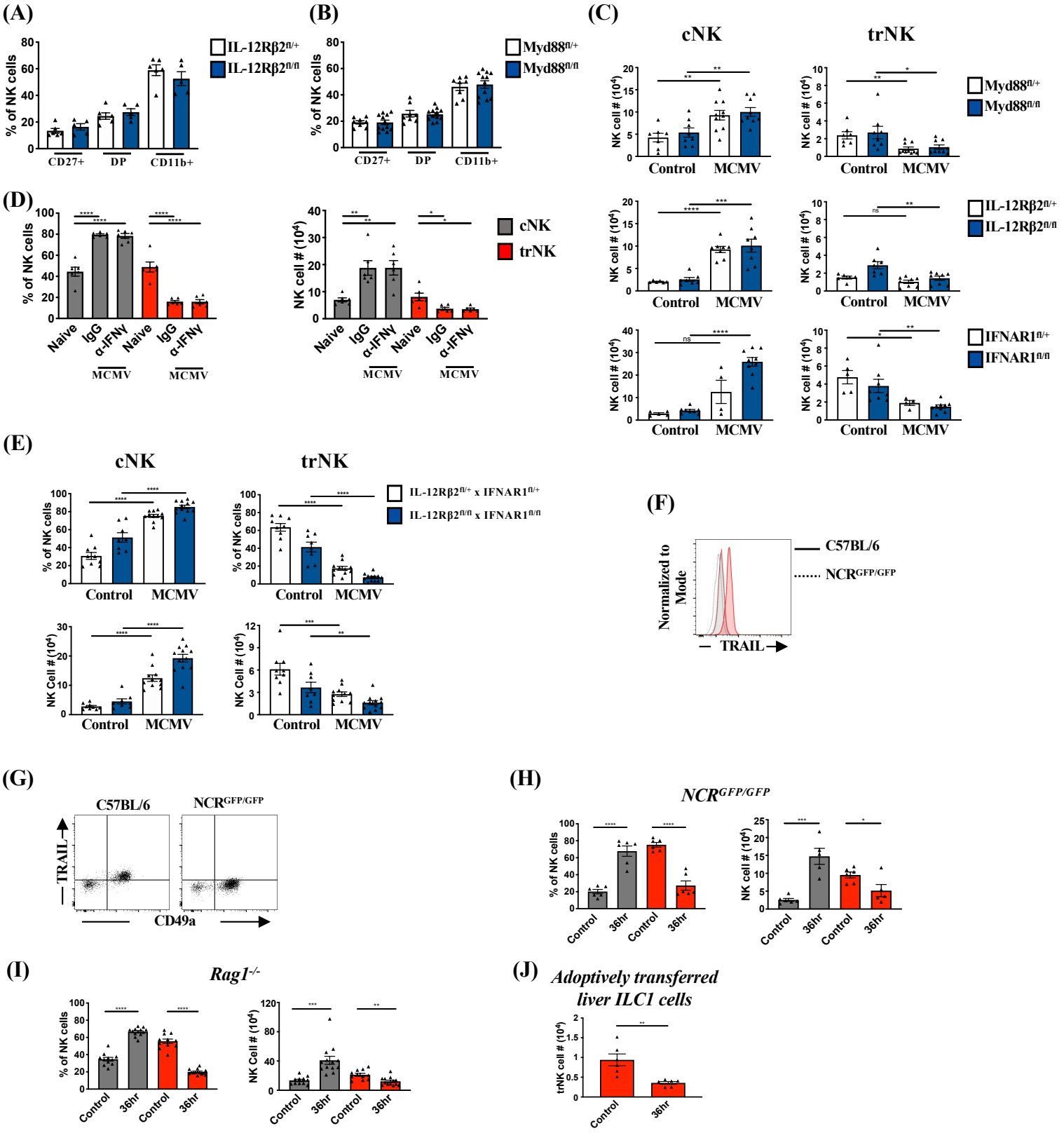
Inflammation-Induced Lactate Leads to Rapid Loss of Hepatic Tissue-Resident NK Cells

Garvin Dodard, Angela Tata, Timothy K. Erick, Diego Jaime, S.M. Shahjahan Miah, Linda Quatrini, Bertrand Escalière, Sophie Ugolini, Eric Vivier, and Laurent Brossay



Supplementary Figure 1

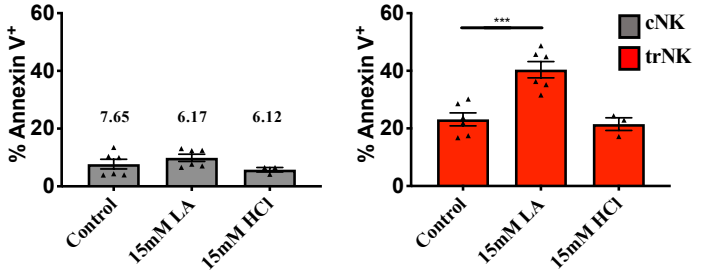
Liver trNK cells serve as early responders to stimuli within the liver microenvironment prior to cell death. **Related to Figures 1 and 2.** (A) Representative staining of liver cNK (grey) and trNK (red) cell populations at 2 and 6 hours post α-GalCer stimulation. (B) Frequency of liver cNK and trNK cells at 2 and 6 hours post α-GalCer stimulation (n = 6). (C) Frequency of IFNγ^+ liver cNK and trNK cells at 2 and 6 hours post α-GalCer stimulation (n = 6). (D) Frequency of Annexin V$^+$ liver cNK and trNK cell populations at 36 hours post α-GalCer stimulation (n = 9-11). (Data are representative of (A) or pooled from 2-3 experiments (B-D), error bars indicate S.E.M.)



Supplementary Figure 2

Liver trNK cell disappearance is independent of IL-1, IL-12, IL-18, Type I, Type II IFN, TRAIL signaling, and other lymphocyte populations. Related to Figure 3. (A) Frequency of indicated NK cell maturation markers from IL-12Rβ2$^{fl/fl}$ and IL-12Rβ2$^{fl/het}$ controls (n= 5-6). (B) Frequency of indicated NK cell maturation markers from MyD88$^{fl/fl}$ and MyD88$^{fl/het}$ controls (n= 8-12). (C) Number of liver cNK and trNK cells from MyD88$^{fl/fl}$ and MyD88$^{fl/het}$, IFNAR1$^{fl/fl}$ and IFNAR1$^{fl/het}$, IL-12Rβ2$^{fl/fl}$ and IL-12Rβ2$^{fl/het}$ controls 36 hours post-MCMV infection (n = 4-10). (D) Frequency and number of liver cNK and trNK cell populations of mice treated with IFNγ blocking antibody or isotype control 36 hours post-MCMV infection (n = 6). (E) Frequency and number of liver NK cells from IL-12Rβ2$^{fl/fl}$ x IFNAR1$^{fl/fl}$ and IL-12Rβ2$^{fl/het}$ x IFNAR1$^{fl/het}$ controls 36 hours post-MCMV infection (n= 8-12). (F) TRAIL expression on liver cNK (grey) and trNK (red) in C57BL/6 and NCR$^{GFP/GFP}$ mice. (G) Representative staining of TRAIL expression on liver cNK (CD49a^{-}) and trNK (CD49a^{+}) cell populations in C57BL/6 and NCR$^{GFP/GFP}$ mice. (H) Frequency and number of liver cNK and trNK cell populations 36 hours post-MCMV infection of NCR$^{gfp/gfp}$ mice (n = 6). (I) Frequency and number of hepatic cNK and trNK cells 36 hours post-MCMV infection in *Rag1*$^{-/-}$ mice (n = 11-13). (J) Absolute cell number of adoptively transferred hepatic trNK cells 36 hours post-MCMV infection in *Rag2*$^{-/-}$*IL-2Rγ*$^{-/-}$ mice (n = 6). (Data are representative of (F-G) or pooled from 2-3 experiments (A-E,H-J), error bars indicate S.E.M.)

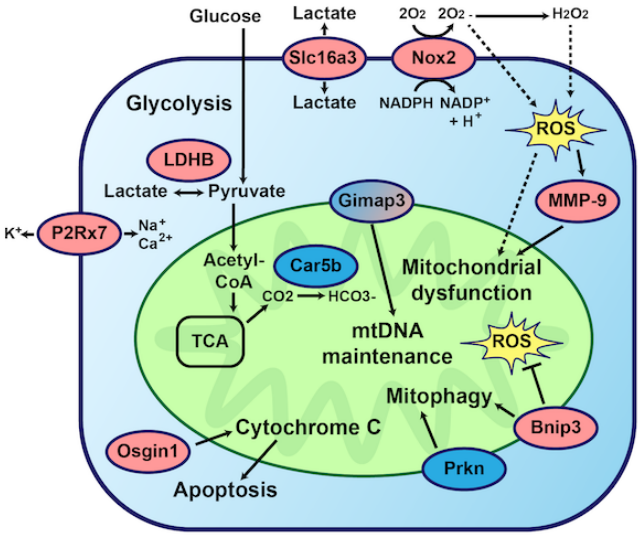
(A)



(B)

	Naïve[a]	44hr MCMV[b]
ROS Production		
Nox2	*	4.13
Mitochondria		
mmp9	13.35	6.19
Osgin1	4.12	5.21
Car5b	-15.57	-1.55
Gimap3	2.06	-5.88
Bnip3	*	4.02
Prkn	*	-7.14
Cellular Apoptosis		
P2Rx7	5.54	6.18
Lactate Metabolism		
Ldhb	*	8.47
Slc16a3	*	4.00

(C)



Supplementary Figure 3

Genes differentially expressed between liver trNK cell and cNK cells by four functional categories. Related to Figure 4. (A) Frequency of Annexin V$^+$ liver cNK (grey) and trNK (red) cells following 4 hours incubation in control media (pH7.65), 15mM lactic acid (pH 6.17), and 15mM HCl (pH 6.12) (n = 6). (B) Table of genes differentially expressed between liver NK cell populations. Higher transcript expression in cNK cells (blue). Higher transcript expression in trNK cells (red). a – Robinette *et al*. Transcriptional programs define molecular characteristics of innate lymphoid cell classes and subsets. *Nature immunology* 16:306-317. b – Quatrini *et al*. Endogenous glucocorticoids control host resistance to viral infection through the tissue-specific regulation of PD-1 expression on NK cells. *Nature immunology* 19:954-962. * - expression levels below a 2-fold difference were not included in analysis. (C) Model of indicated genes and their roles in functional pathways. (Data are representative of (B-C) or pooled from 1-2 experiments (A), error bars indicate S.E.M.)