Supporting Information

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SI Materials and Methods

Quantitative RT-PCR Measurement. RT-PCR analysis was performed as previously described (1). Briefly, mRNA was extracted from livers stored in 1 mL of TRIzol (Invitrogen) according to the manufacturer's instructions. After homogenization with a tissue lyser (Qiagen), 200 μL of chloroform was added, and samples were vortexed for at least 15 s. Then samples were centrifuged at 14,480 × *g* at 4 °C for 10 min, then supernatant was transferred into a new tube, and RNA was precipitated with 2-isopropanol for 20 min at room temperature. After washing with 75% ethanol, pallets were air-dried and resuspended in DEPC-treated water (Ambion). Gene expression of GAPDH, Irf7, and Usp18 was performed with kits from Applied Biosystems. For analysis, the expression levels of all target genes were normalized to GAPDH expression (ΔCt). Gene expression values were then calculated based on the ΔΔCt method, using the mean of three naïve mice as a standard to which all other samples were compared. Relative quantities (RQ) were determined by using the equation $RQ = 2^{-\Delta\Delta Ct}$.

Gradient Centrifugation. Detailed gradient centrifugation procedures (2) were as follows. 100% Percoll (Percoll) was 9× Percoll reagent (GE Healthcare) and 10× PBS. Livers were homogenized, centrifuged (421 × *g* for 5 min), and resuspended in 40% Percoll (4× Percoll + 6× RPMI medium 1640) in 50-mL Falcon tubes. Slowly, 70% Percoll (7× Percoll + 3× RPMI medium 1640) was underlaid with a Pasteur pipette (VWR). After centrifugation at 859 × *g* for 20 min without brake, the interface was harvested carefully. After one wash with HBSS, the lymphocyte population was resuspended in PBS containing 5 mM EDTA and 1% FCS.

T-Cell Proliferation. First, 96-well plates were coated overnight with anti-CD3 (3 μg/mL; BD Pharmingen) antibody at 4 °C. Negatively sorted T cells were labeled with 2.5 μM 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) in PBS for 10 min at 37 °C. After two washes (Iscove's modified Dulbecco's media/10% FCS), cells were resuspended in Iscove's modified Dulbecco's media/10% FCS containing 2 μg/mL anti-CD28 antibody at a frequency of 2×10^6 per mL. Then, 2×10^5 T cells were coincubated with 10^5 purified NK cells on the precoated plate. IFN-α4 (PBL) was added before coincubation at an activity of 10^4 U/mL After 72 h, the number of $CD8^+$ and $CD4^+$ T cells was analyzed.

Flow Cytometry (FCM) Analysis. Tetramer was provided by the National Institutes of Health Tetramer Core Facility at Emory University. Tetramer, surface, and intracellular FCM staining were performed as described previously (1). Briefly, single suspended splenocytes or liver or peripheral blood lymphocytes were stained with allophycocyanin-labeled gp33 MHCI tetramers ($gp33/H\text{-}2D^b$) for 15 min at 37 °C, followed by staining with anti–CD8-PerCP (BD Biosciences) for 30 min at 4 °C. For determination of their activation status, lymphocytes were stained with anti–PD-1 and anti–IL-7Rα (both BD Pharmingen) for 30 min at 4 °C. For intracellular cytokine staining, single suspended splenocytes or liver cells were incubated with the lymphocytic choriomeningitis virus (LCMV)-specific peptides gp33 and np396. After 1 h, brefeldin A (eBioscience) was added, followed by additional 5-h incubation at 37 °C. After surface staining of CD8 (eBioscience), cells were fixed with 2% formalin and permeabilized with PBS containing 1% FCS and 0.1% saponin and stained with anti–IFN-γ (eBioscience) for 30 min at 4 °C. Anti-NK1.1, anti-CD4, anti-CD3e, anti-2B4, anti-NKG2D, anti-Thy1.1, and anti-CD45.1 antibodies were from eBioscience. NKG2D–human IgG (hIgG) was used to indicate NKG2D ligand expression (3) on T cells and was stained with anti-hIgG Cy3 (Dianova).

NK Cell Cytotoxicity Assay. A total of 2×10^6 splenocytes from LCMV-infected (2 d after infection) and naïve mice were incubated for 5 h at 37 °C in 96-well plates with YAC-1 target cells that had been previously loaded with 400 μCi/mL ^{51}Cr (Perkin-Elmer). Then, 50 μL of the culture supernatant was counted from each well by using a Wallac Wizard counter (Perkin-Elmer). Maximal release was induced by adding 100 μL of 1 M HCl to target cells. Specific lysis (given in percentage) was calculated as follows: (cpm sample release − cpm spontaneous release)/(cpm maximal release − cpm spontaneous release) × 100.

1. Lang PA, et al. (2009) Hematopoietic cell-derived interferon controls viral replication and virus-induced disease. *Blood* 113:1045–1052.
2. Lang PA, et al. (2010) Tissue macrophages suppress viral replication and prevent severe immunopathology in an interferon-I-dependent manner in mice. *Hepatology* 52(1): 25–32.
3. Cerwenka A, et al. (2000) Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* 12:721–727.

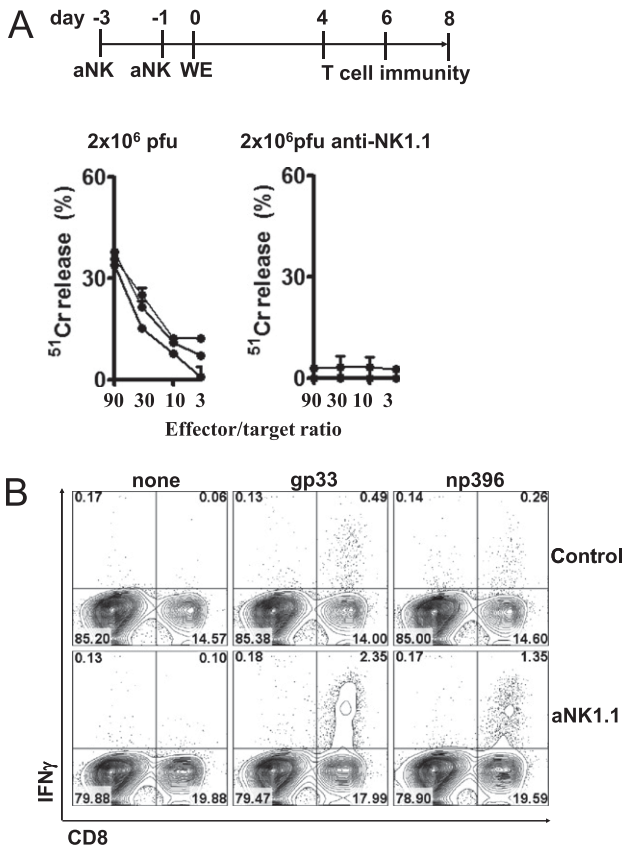

Fig. S1. Anti-NK1.1 treatment depletes NK cells and improves CD8$^+$ T-cell immunity. (*A*) C57BL/6 mice were treated with anti-NK1.1 on days −3 and −1. After depletion, animals were infected with 2×10^6 pfu of LCMV strain WE (LCMV WE). Splenocytes were incubated with ^{51}Cr-labeled YAC-1 cells in different effector/target ratios, and radioactivity was analyzed in the supernatant ($n = 3$; mean ± SEM of duplicates; one of two experiments is shown). (*B*) Representative flow cytometry analysis of splenocytes of NK cell-depleted and control animals restimulated with the virus-specific epitopes gp33 and np396.

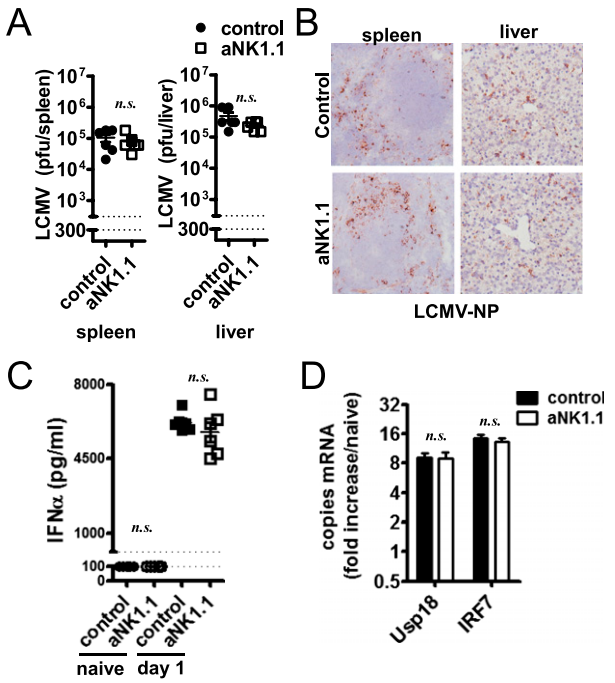

Fig. S2. Normal IFN-I responses trigger similar early virus replication in the presence or absence of NK cells. Control or NK cell-depleted mice were infected with 2×10^6 pfu of LCMV WE. (*A*) Virus titers were obtained from liver and spleen tissue at 4 d postinfection. (*B*) Immunohistochemistry of snap-frozen spleen and liver tissue at 4 d postinfection was stained for LCMV NP. (*C*) IFN-α serum concentration was determined at 24 h after infection ($n = 6$). (*D*) mRNA copies of IRF7 and USP18 relative to naïve animals were analyzed at 24 h after infection with LCMV in WT and NK cell-depleted mice ($n = 6$).

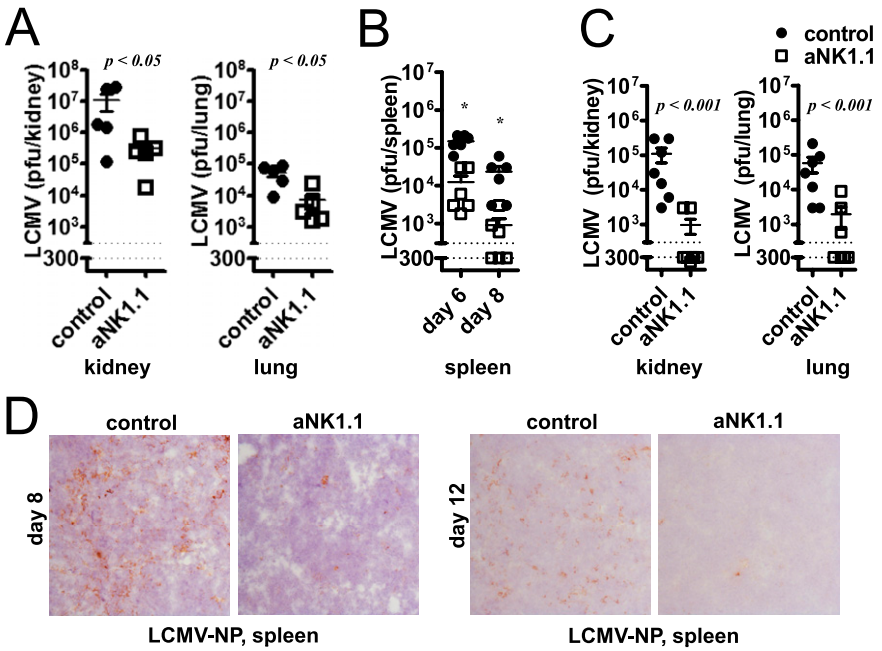

Fig. S3. Anti-NK1.1 treatment improves virus elimination. (*A*) C57BL/6 mice were treated with anti-NK1.1 on days −3 and −1. After depletion, animals were infected with 10^5 pfu of LCMV DOCILE. Virus titers were determined in kidney and lung tissue at 33 d postinfection ($n = 5$). (*B*) Virus titers were determined in the spleen at 6 and 8 d postinfection ($n = 6$) of control and NK cell-depleted mice after infection with 2×10^6 pfu of LCMV WE. (*C*) Virus titers of kidney and lung tissue, after infection with 2×10^6 pfu of LCMV WE, were measured in control and NK cell-depleted animals at 12 d postinfection. (*D*) Immunohistochemistry of LCMV NP in sections from snap-frozen spleen tissue from control and NK cell-depleted mice at 8 and 12 d postinfection.