Supporting Information

Liberatore and Bieniasz 10.1073/pnas.1113694108

SI Materials and Methods

Generation of Tetherin-Deficient Mice. A targeting construct was generated by amplifying the relevant regions of the *tetherin* gene from C57BL/6J (B6) genomic DNA and then inserting them into the vector pL451 (1), which contains a *Neo* cassette flanked by 2 *FRT* sites and one *loxP* site. An additional *loxP* site was inserted into the first exon of the *tetherin* (*Bst2*) gene, immediately 5′ to the translation start site (Fig. S1*A*). The targeting construct was electroporated into B6-Albino ES cells, and correct targeting was confirmed by Southern blot analysis of cell clones. Targeted ES cells were then injected into B6 blastocysts, and the resulting chimeric animals were screened for germ-line transmission of the targeted allele. Animals were genotyped with PCR assays and the following primers: RL92, 5′-AGCCACTGTTAAGTCGAGTCCCA-3′; RL100, 5′-GCCTGGTCTACAGAGTGACTTTTA-3′; and RL131, 5′-GTATAGGAACTTCATCAGTCAGGTA-3′ (Fig. S1*A*). Founder mice were crossed to FLP1-expressing transgenic mice [B6.Cg-Tg(ACTFLPe)9205Dym/J; The Jackson Laboratory] to remove the *Neo* cassette, leaving two *loxP* sites and one remaining *FRT* site and creating the tetherin conditional knockout (CKO) allele ($Bst2^{tm1Bsz}$; Fig. S1*A*). Mice bearing this allele were then crossed to animals expressing Cre recombinase under the control of the *Zp3* promoter [C57BL/6-Tg(Zp3-cre)93Knw/J; The Jackson Laboratory) to delete the bulk of the first exon of *tetherin* in the germ line. Mice heterozygous for the deleted allele were intercrossed to generate WT (+/+), heterozygous (+/−), and homozygous knockout (−/−) mice.

Viruses. Moloney murine leukemia virus (Mo-MLV) was produced by transfecting NIH/3T3 cells with the infectious clone pNCS (2). Viral stocks were titered on NIH/3T3 cells by focal immunoassay. LP-BM5 virus was harvested from chronically infected SC-1 cells obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD: SC-1/MuLV LP-BM5 from Herbert Morse and Janet Hartley (catalog no. 1215). The LP-BM5 stock was generated by infecting, cocultivating chronically infected and fresh SC-1 cells (CRL-1404; ATCC), and harvesting the supernatant 6 d later. The stock was titered on SC-1 cells by focal immunoassay.

Cells and in Vitro Virus Replication Assays. Cell lines stably expressing murine tetherin were generated by inserting a murine tetherin cDNA into the retroviral vector pLHCX (Clontech), followed by retroviral transduction of NIH/3T3 cells. Single-cell clones were derived, and surface levels of tetherin were measured by flow cytometry. Clones expressing various levels of tetherin were selected. Murine embryonic fibroblasts (MEFs) were generated from embryos derived from interheterozygous matings according to standard protocols (3). For in vitro replication assays, NIH/3T3 cells or MEFs were infected with virus in the presence or absence of 100 U/mL IFNα, and aliquots of supernatant were removed daily and titered on NIH/3T3 cells or SC-1 cells by focal immunoassay.

Focal Immunoassay for Quantitation of Mo-MLV and LP-BM5. Focal immunoassays were performed by infecting cells with serially diluted virus in the presence of 4 μg/mL polybrene. At 48 h after infection, cells were fixed in 4% paraformaldehyde, and infected cells were detected with an antibody to the viral Gag protein (anti-p12, CRL-1890; ATCC) followed by an HRP-conjugated secondary antibody. The signal was developed with DAB substrate (Vector Laboratories), and foci of infection were counted at an appropriate dilution and expressed as focus-forming units/milliliter (FFU/mL).

Mouse Husbandry, Treatments, and Infections. Mice were housed in a pathogen-free facility and maintained in accordance with Institutional Animal Care and Use Committee guidelines. WT C57BL/6J mice were obtained from The Jackson Laboratory. IFN (α, β, and ω) receptor 1 (IFNAR1)-deficient mice ($Ifnar1^{tm1Agt}$) (4) were a gift from Ralph Steinman (The Rockefeller University, New York, NY) and were maintained as homozygous knockouts. For IFNα treatments, mice were given i.p. injections of 0.5×10^4 U of recombinant mouse $IFN\alpha_1$ (PBL InterferonSource). For poly(I:C) treatments, mice were given i.p. injections of 50 μg of poly(I:C) (Sigma).

For in vivo Mo-MLV infections, animals were infected by i.p. injection with 0.05 mL of a 9.5×10^6 FFU/mL virus stock at 24–36 h after birth. For in vivo LP-BM5 infections, adult mice (6–10 wk old) were infected by i.p. injection with 0.2 mL of 1.68×10^4 FFU/mL LP-BM5 stock. Infected cells were quantitated by using infectious center assays, which were performed by making single-cell suspensions of spleen, thymus, and bone marrow tissues and plating serially diluted cells (10–10^6 cells per well) on NIH/3T3 cell monolayers. The following day, the medium was changed; thereafter, cells were fixed and stained for viral Gag protein 48 h after infection. The number of infected cells in each cell suspension was inferred from the number of Gag^+ foci and expressed as infected cells per 10^6 cells. Plasma was separated from whole blood by centrifugation (1,500 × *g* for 15 min) in the presence of heparin. Plasma viremia was measured by serial dilution and focal immunoassay on NIH/3T3 cells (Mo-MLV assays) or SC-1 cells (LP-BM5 assays).

Flow Cytometry. Adherent cultured cells (NIH/3T3 and MEFs) were harvested in PBS plus 5 mM EDTA, washed in FACS buffer (PBS plus 2% BSA), and stained with antibodies. Alternatively, single-cell suspensions were made from spleen, thymus, and bone marrow tissues, resuspended in red blood cell lysis buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, and 0.05 mM EDTA), washed in FACS buffer, and stained with antibodies. Dead cells were excluded by DAPI staining. All data were acquired on an LSR II flow cytometer (Becton Dickinson), and data were analyzed with FlowJo software (Tree Star). The following antibodies were used: FITC anti–Siglec-H (Hycult Biotech); phycoerythrin (PE) anti–PDCA-1/tetherin (Miltenyi Biotec); and allophycocyanin (APC)-Cy7 anti-CD11b, FITC anti-CD3, APC anti-CD11c, PerCP-Cy5.5 anti-CD4, V500 anti-CD8, PE anti–c-Kit, PE-Cy-7 anti–Sca-1, FITC anti-CD34, Alexa Fluor 700 anti-B220, purified anti-CD16/CD32, PE Rat IgG2b isotype control (clone R35-38), and APC mouse lineage antibody mixture (BD Pharmingen).

RT-PCR. Total RNA was extracted from spleens with TRIzol Reagent (Invitrogen), and first-strand cDNA was synthesized with the SuperScriptIII First-Strand Synthesis System (Invitrogen). Primers for PCR assays were as follows: RL117, 5′-AGCTCACCCGCACCCAGGACA-3′ and RL157, 5′-GAGCAGGAACAGTGACACTTTGA-3′ for *tetherin*; and RL172, 5′-ACAAAATGGTGAAGGTCGGTGTGA-3′ and RL173, 5′-GATGACCCTTTTGGCTCCACCCT-3′ for *Gapdh*.

1. Liu P, Jenkins NA, Copeland NG (2003) A highly efficient recombineering-based method for generating conditional knockout mutations. *Genome Res* 13:476–484.
2. Yueh A, Goff SP (2003) Phosphorylated serine residues and an arginine-rich domain of the Moloney murine leukemia virus p12 protein are required for early events of viral infection. *J Virol* 77:1820–1829.
3. Nagy A, Gertsenstein M, Vintersten K, Behringer R (2003) *Manipulating the Mouse Embryo* (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY), pp 371–373.
4. Müller U, et al. (1994) Functional role of type I and type II interferons in antiviral defense. *Science* 264:1918–1921.

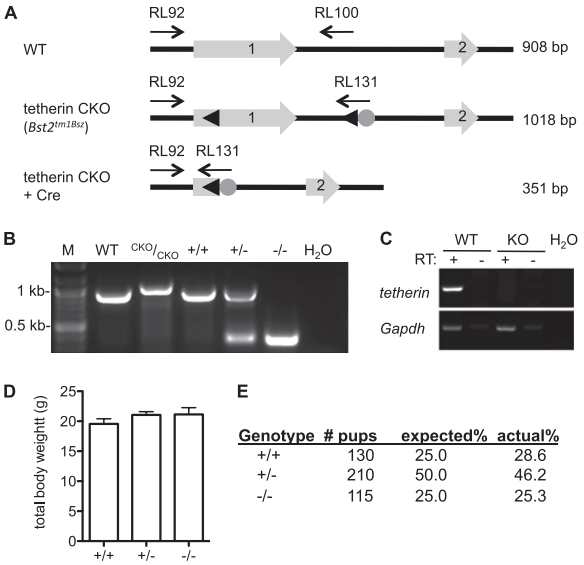

Fig. S1. Generation of a tetherin CKO mouse. (*A*) Schematic of the WT *tetherin* (*Bst2*) locus, the *tetherin CKO* (*Bst2*^{tm1Bsz}) allele, or the *tetherin CKO* allele after Cre recombinase expression (*tetherin CKO* + Cre). Arrows indicate the primers used for genotyping. Black triangles indicate *loxP* sites, and gray circles indicate *FRT* sites. The expected size of the PCR product generated by each primer pair is indicated to the right of each diagram. (*B*) PCR assays performed on genomic DNA extracted from animals with the indicated genotypes by using primers RL92, RL100, and RL131 (as in *A*). (*C*) RT-PCR, with (+) or without (–) reverse transcriptase (RT), was performed on total RNA isolated from spleens of animals of the indicated genotypes by using primers specific for *Gapdh* or the deleted portion of *tetherin*. (*D*) Total body weight of +/+ (n = 5), +/– (n = 4), and –/– (n = 6) mice. (*E*) Numbers and percentages of +/+, +/–, and –/– pups born after matings where both parents were +/–.

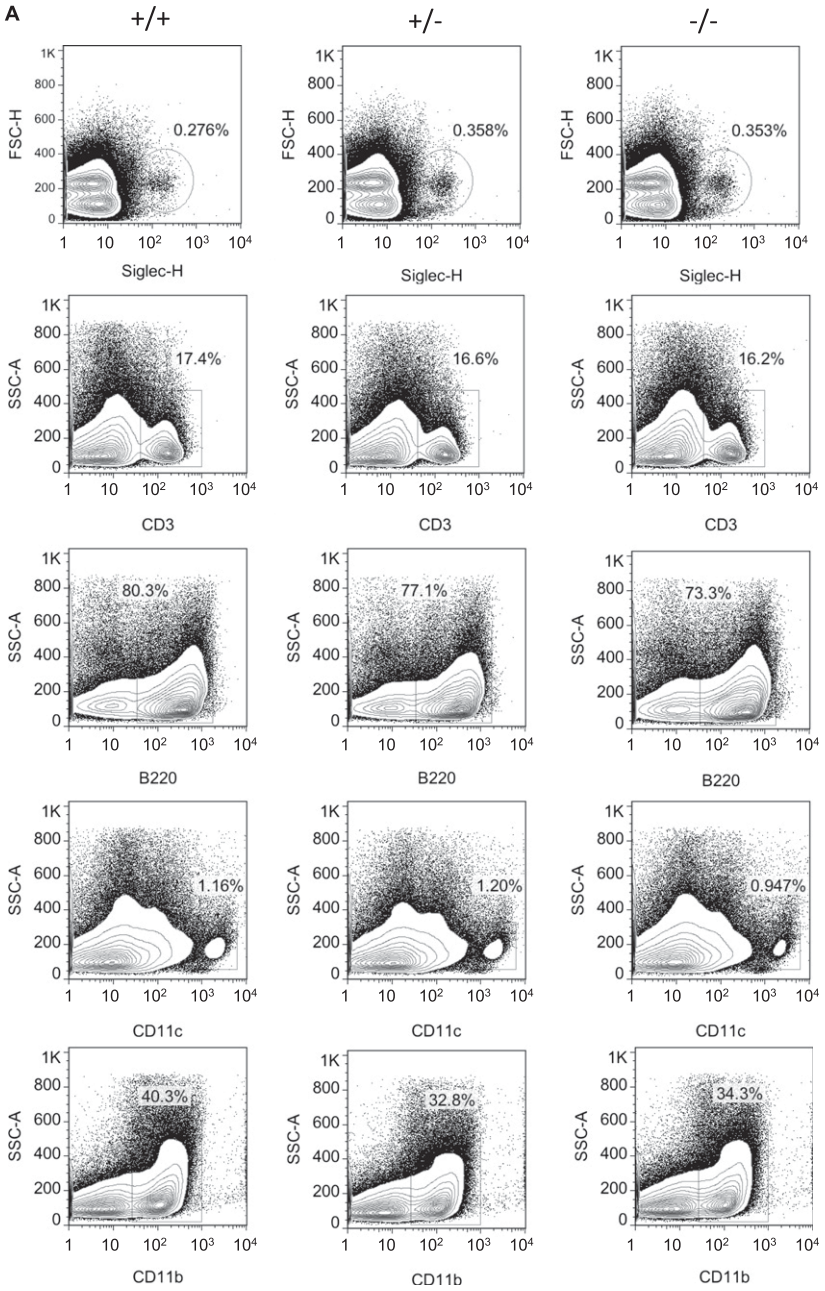

Fig. S2. (Continued)

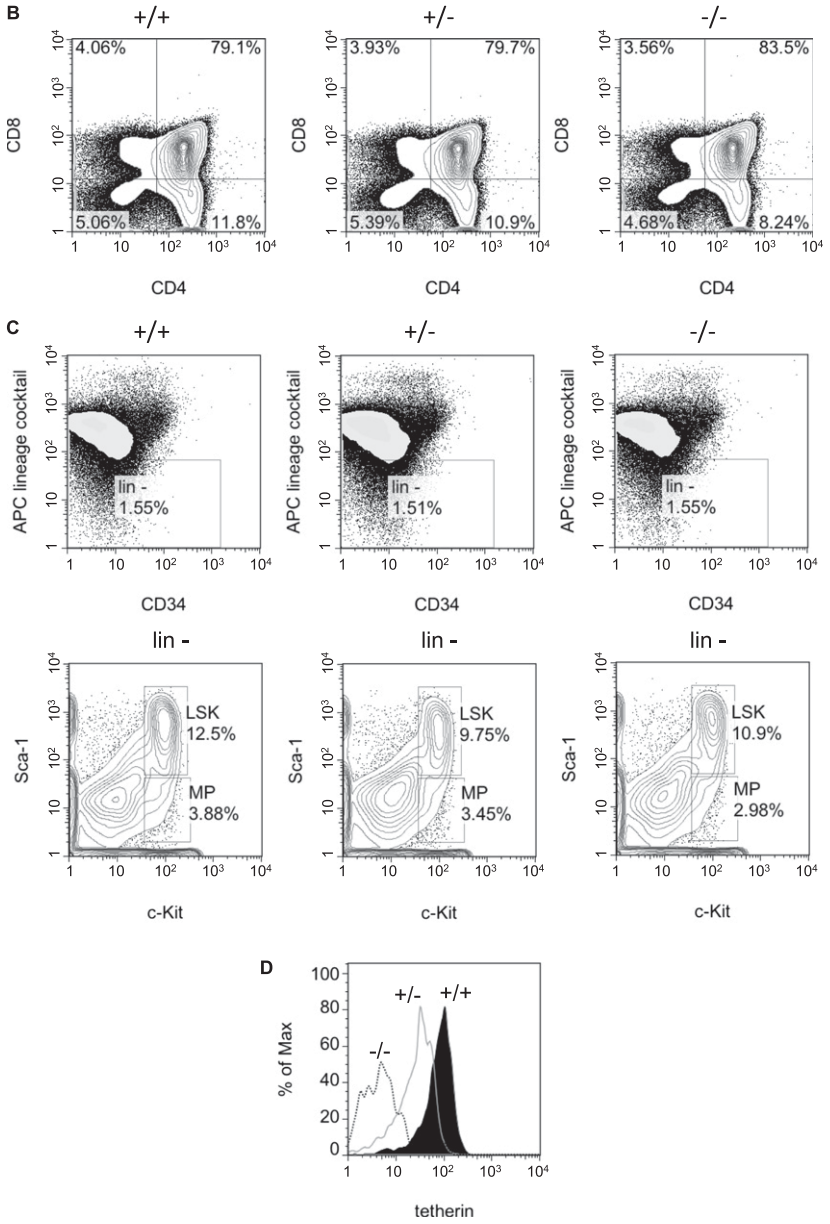

Fig. S2. Tetherin is not required for normal immune system development. (*A*–*C*) Total splenocytes (*A*), thymocytes (*B*), and bone marrow cells (*C*) from +/+, +/–, and –/– 4-wk-old mice were stained with the indicated antibodies and analyzed by flow cytometry. (*C*) Cells not reacting with the antibodies in the lineage mixture (anti-CD3e, anti-CD11b, anti-CD45R/B220, anti–Ly-76, and anti–Ly-6G/C) were selected by gating (lin–) and further interrogated for c-Kit and Sca-1 expression to identify hematopoietic progenitor (lin^- $Sca\text{-}1^+$ $c\text{-}Kit^+$; LSK) cells (enriched in hematopoietic stem cells) and erythromyeloid progenitor (MP) cells. (*D*) Tetherin expression on $Siglec\text{-}H^+$ splenocytes from +/+, +/–, and –/– mice.

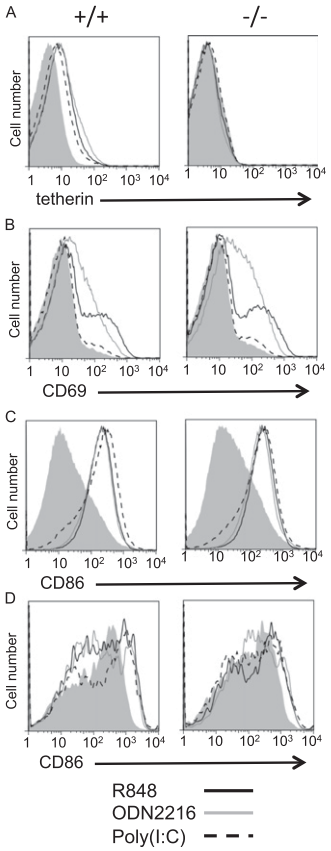

Fig. S3. Tetherin is not required for activation with Toll-like receptor (TLR) agonists. Total splenocytes were treated with agonists of TLR7 (R848), TLR9 (ODN2216), or TLR3 [poly(I:C)] for 12 h and analyzed by FACS. Untreated samples are indicated by shaded histograms, and the samples subjected to the various treatments (R848, solid black line; ODN2216, solid gray line; and poly(I:C), dashed black line) are indicated by unshaded histograms. Tetherin expression was assessed on total splenocytes (*A*), and expression of the indicated activation markers was assessed on $CD3^+$ (*B*), $B220^+$ (*C*), and $CD11c^+$ (*D*) cells.

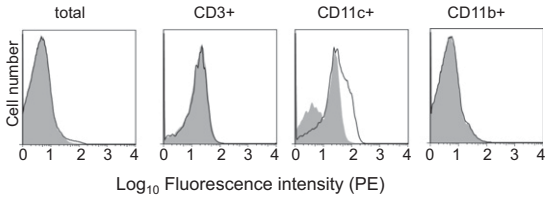

Fig. S4. Tetherin expression on bone marrow cells. Flow cytometric analysis of tetherin expression on bone marrow-derived cells from 3-wk-old WT mice, stained with PE Rat IgG2b isotype control antibody (shaded histograms) or PE anti-tetherin antibody (unshaded histograms).

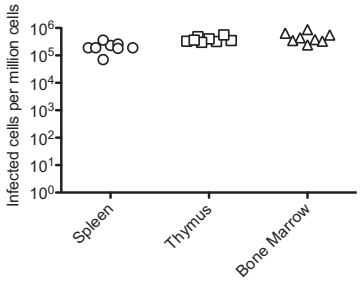

Fig. S5. High levels of Mo-MLV replication in mouse lymphoid tissues at 12 d after infection. Numbers of infected cells were inferred by using infectious center assays of splenocytes, thymocytes, and bone marrow cells harvested at 12 d after infection from WT mice infected with Mo-MLV.

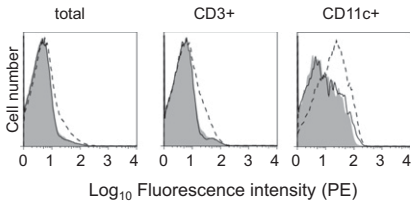

Fig. S6. Tetherin up-regulation during LP-BM5 infection. Flow cytometric analysis of tetherin expression on splenocytes from WT mice is shown. Cells from uninfected, age-matched animals were stained with PE Rat IgG2b isotype control antibody (shaded histograms) or PE anti-tetherin antibody (unshaded histograms, solid line). Cells from LP-BM5–infected mice (14 wk after infection) were stained with PE anti-tetherin antibody (unshaded histograms, dashed line).