SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Mice. B6.*Tlr9*$^{-/-}$ mice (Hemmi et al., 2000) were obtained from Dr. Iwasaki, Yale University. To generate B6$^{vic1I/LnJ}$ congenic mice, we crossed C57BL/6J females to I/LnJ males to produce F_1 females. F_1 females were then backcrossed to C57BL/6J males and the resulting N_2 females were genotyped with *D17Mit46* and *D17Mit89* flanking the I/LnJ locus containing the *vic1* gene. Females inheriting the I/LnJ *vic1* locus were backcrossed to C57BL/6J males to produce N_3 offspring. At each generation, only those offspring receiving the I/LnJ *vic1* locus were selected for the next round of backcrossing. Two tenth-generation carriers of the I/LnJ allele of the *vic1* locus were intercrossed, and offspring homozygous for both I/LnJ *vic1* alleles were selected to continue the line. B6$^{vic1I/LnJ}$ congenic mice were crossed to B6.*Tlr7*$^{-/-}$ and the heterozygous offspring were intercrossed to produce B6$^{vic1I/LnJ}$. *Tlr7*$^{-/-}$ mice. C57BL/10ScN.*Tlr4*$^{Lps-d}$ mice were purchased from The Jackson Laboratory. B6.*Myd88*$^{-/-}$ and C57BL/10ScN.*Tlr4*$^{Lps-d}$ mice were backcrossed for 12 generations to C3H/HeN mice and intercrossed to produce C3H/HeN. *Tlr4*$^{Lps-d}$, and C3H/HeN. *Myd88*$^{-/-}$*Tlr4*$^{Lps-d}$mice. The studies described in this paper have been reviewed and approved by the Animal Care and Use Committee at the University of Chicago.

Immunofluorescence. MMTV was detected using the mouse monoclonal anti-p27CAGag antibody (Ab) (Purdy et al., 2003), followed by donkey anti-mouse Abs coupled to AlexaFlour 568 (Invitrogen). MuLV was detected with the rat monoclonal anti-p30CA Ab (ATCC CRL-1912) followed by rabbit anti-rat Abs coupled to AlexaFluor 594 (Invitrogen). Early endosomes were detected using goat anti-mouse early endosome antigen 1 (EEA1) Abs (Santa Cruz Biotechnology) followed by donkey anti-goat Abs coupled to AlexaFlour 488 (Invitrogen). Late endosomes were detected using goat anti-mouse Rab7 Abs (Santa Cruz Biotechnology) followed by donkey anti-goat

Abs coupled to AlexaFlour 488. Cells were viewed with the 100X or 63X objective lens of a Nikon A1 Confocal Microscope and captured with NIS-Elements software (Nikon Inc).

Flow Cytometry. Mononuclear peripheral blood lymphocytes were stained with FITC- or PE-coupled monoclonal Abs against the Vß14, Vß6, and Vß2 T-cell receptor (TCR) chains (BD Biosciences). Anti-CD4 Abs coupled to APC (Invitrogen) were used in the second dimension. Leukocytes were recovered from heparinized blood samples by centrifugation through a Ficoll/Hypaque cushion. Stained lymphocytes were analyzed using a FACSCanto™ (Becton Dickinson) flow cytometer and the FACSDiVa^{TM} software program (Becton Dickinson).

PCR and RT-PCR. The HP transgene is a genetically engineered MMTV provirus in which the 3' half (including the 3'LTR, and the *env* and *sag* genes) is derived from the exogenous MMTV(C3H) virus, and the 5' half (including the 5'LTR, and the *gag* and *pol* genes) is derived from the endogenous *Mtv1* locus (Shackleford and Varmus, 1988) (Figure S1A). To identify the HP transgene, high-molecular-weight DNA isolated from spleens or tails of HP-transgenic and non-transgenic I/LnJ mice was amplified using the forward *Mtv1* LTR-specific primer A (5'GACTAATAGAACATATTAAGG3') and the reverse *Mtv1 gag*-specific primer B (5'CCTTCTCTTTTCTCTTACCC3') (Figure S1A). To identify newly integrated HP viruses, DNA was amplified using the MMTV(C3H) LTR-specific forward primer C (5'GGACTAATAGAACATTATTC3') and the reverse primer B (Figure S1A).

To identify HP RNA secreted in the milk, five μg of RNA isolated from the milk-filled stomachs of newborn pups were subjected to RT-PCR using SMARTScribe Reverse Transcriptase (Clontech Laboratories Inc.). Amplification of HP viral RNA was accomplished using a non-specific MMTV LTR forward primer

(5'TTCGGAGAACTCGACCTTCC3') and a reverse MMTV(C3H) LTR-specific primer (5'GAAGATCTTAATGTTCTATTAGTCCAGCCACTG3') (Golovkina et al., 1998).

Analysis of endogenous MuLVs. Genomic DNA isolated from mouse spleens was digested with *EcoRI* (New England BioLabs) and subjected to Southern blot analysis using a probe specific for ecotropic (Chattopadhyay et al., 1980) or xenotropic/polytropic MuLV (Chattopadhyay et al., 1982).

RNA isolated from infected and uninfected cells or from mouse spleens was subjected to northern blot analysis using a probe specific for ecotropic (Chattopadhyay et al., 1980) or xenotropic/polytropic MuLV (Chattopadhyay et al., 1982).

Assay for IL-6. 1.5 x 10^6 splenocytes were incubated with MMTV virions (isolated from ~100µl of MMTV-containing milk) in 300µl of Click's medium (Irvine Scientific) supplemented with 5% FCS in 48-well plates for 14h. IL-6 was measured by an ELISA kit according to the manufacturer's protocol (BD Biosciences). ODs obtained in cell supernatants exposed to heat-inactivated (85^{o}C for 15 min) virus were subtracted.

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. I/LnJ-HP transgenic mice secrete infectious virions.

A) Diagram of the hybrid provirus (HP) transgene and newly integrated provirus. Arrows represent primers used to distinguish transgene from newly integrated provirus. Long terminal repeats (LTRs) are homogenized after one round of viral replication as a result of reverse transcription.

B) RNA isolated from the milk of indicated mice was subjected to MMTV-specific RT-PCR. Lane 1: RNA from C3H/HeN-HP milk. Lanes 2 - 3: RNA from I/LnJ-HP milk. Lane 4: DNA from the spleen of an I/LnJ-HP mouse. Lane 5: RNA from I/LnJ non-transgenic milk. Lane 6: water. Lanes 7 - 9: RT(-) samples corresponding to samples in lanes 1 - 3, respectively. M: 1 kb plus marker (Invitrogen). HP: Hybrid provirus specific fragment.

C) DNA isolated from spleens of I/LnJ-HP transgenic mice was subjected to PCR specific for either the HP transgene or newly integrated HP provirus (Figure 1A). Lanes 1, 2 and 8: non-transgenic I/LnJ mice fostered by I/LnJ-HP females (these mice produce uninfected virus, Figure 1D). Lanes 3 - 7, 9 and 10: I/LnJ-HP mice. Lanes 11 and 12: DNA from tails of #9 and #10 HP transgenic I/LnJ mice. Lane 13; no DNA.

Figure S2. Endogenous MuLVs inherited by I/LnJ mice.

A) DNA isolated from spleens of various mice was digested with *EcoRI* and subjected to southern blot analysis using an LTR probe specific for ecotropic (right) or xeno/polytropic (left) viruses.

B) (Top) Ten µg of RNA isolated from SC-1 cells (1), SC-1 cells co-cultured with splenocytes of aged Ab-producing I/LnJ mice (2), or from I/LnJ and BALB/cJ spleens (taken from 2-month-old mice) (3, 4) were subjected to northern blot analysis using polytropic/xenotropic (Chattopadhyay et al., 1982) or ecotropic (Chattopadhyay et al.,

1980) probes. (Bottom) The same RNA samples were stained with ethidium bromide prior to transfer to verify their integrity. 28S and 18S: ribosomal RNA subunits.
C) Exogenous virus produced by an endogenous provirus up-regulated in aged I/LnJ mice was injected into 6-8 week-old I/LnJ mice. Serum samples were tested for reactivity against MuLV virion proteins by ELISA. Anti-mouse IgG2a secondary Abs coupled to AP were used at the second step. none: uninfected I/LnJ mice. Error bars represent the standard error of the mean (SEM), three to five mice were used per group in a single experiment.

Figure S3. Live and UV-irradiated retrovirus localizes to early endosomes and stimulates Ab responses only in virus-resistant animals.

A) Live/UV-irradiated MMTV or MuLV were incubated with NMuMG or XC cells, respectively, for 90 minutes at 4^0C, and then shifted to 37^0C for 15 minutes. Cells were stained with Abs against the early endosomal marker EEA1 (green) and respective viral Gag proteins (red). Top: Maximum intensity 3D reconstruction of z-stacks. Middle panels: boxed areas of enlarged single 0.2μM thick optical slices. Asterisks indicate colocalization of viral Gag and EE1; corresponding images with orthogonal views (XZ projection) are shown below.
B-C) Virus-resistant I/LnJ and virus-susceptible BALB/cJ and C3H/HeN mice were injected with UV-irradiated MMTV (A) or MuLV (B) (the same virus isolate used in Figures 3B and C). Serum samples collected within 6 weeks post-injection were tested for reactivity against MMTV or MuLV virion proteins by ELISA. All sera were used at 5 x 10^{-3} dilution. Anti-mouse Abs coupled to AP were used at the second step. Error bars represent the standard error of the mean (SEM), three to five mice were used per group in a single experiment.

Figure S4. Retrovirus triggers production of a pro-inflammatory cytokine in a MyD88-dependent TLR9-independent manner.

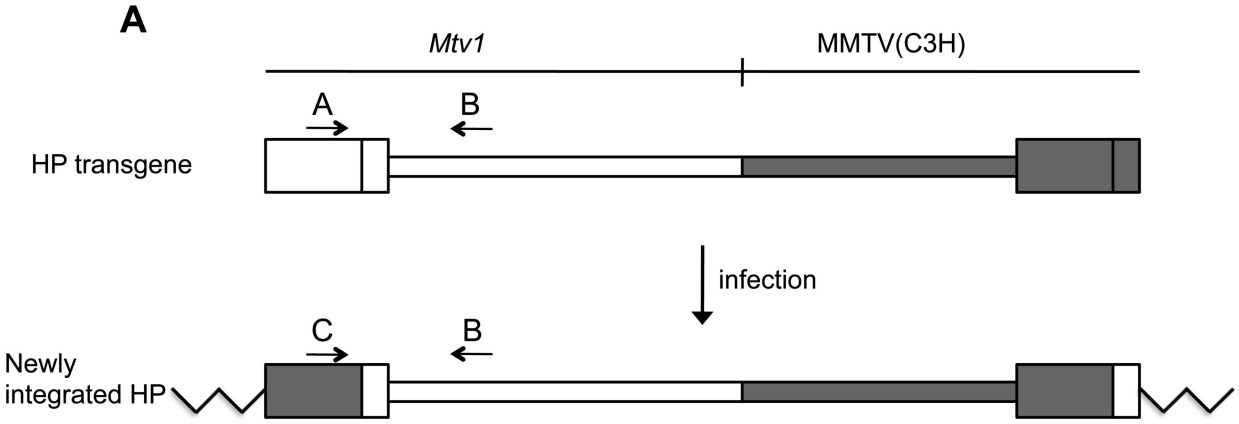
A) To overcome the host's anti-virus immune response MMTV subverts TLR4 signaling in macrophages and dendritic cells to induce IL-6-dependent production of the immunosuppressive cytokine IL-10 (Jude et al., 2003). To look at MyD88-mediated anti-virus response that is unmasked by the subversion pathway, we compared IL-6 production by virus-exposed splenocytes from TLR4-deficient (*Tlr4*$^{Lps\text{-}d}$) and *Tlr4*$^{Lps\text{-}d}$ *Myd88*$^{-/-}$ C3H/HeN mice. Backgrounds readings obtained with 85^{0}C-heated virus were subtracted.

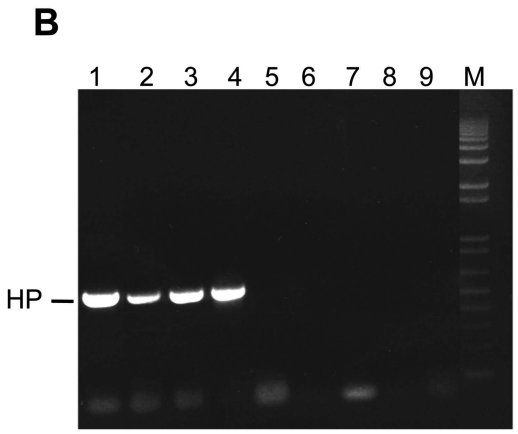
B) wt and *Tlr9*$^{-/-}$ B6 mice were injected with MuLV and analyzed 10 weeks post-infection for production of anti-viral Abs by western blot and presence of infectious virus by XC-PFU test. Anti-mouse IgG2c-specific Abs coupled to HRP were used at the second step. B6: sera from uninfected B6 mice. Numbers correspond to individual mice within a single experiment. a-p30Gag, monoclonal Ab against p30Gag; 2^0 Ab, anti-mouse-HRP Abs. gp70Env, product of the *env* gene; p65Gag and p30Gag, two differentially processed forms of Gag.

Figure S5. *Vic1*-mediated anti-MMTV Ab responses are TLR7-dependent.

A) Genetic distance and predicted locations of *vic1* on chromosome 17. Region between markers *D17Mit46* and *D17Mit143* of I/LnJ chromosome 17 were transferred to the C57BL/6J genetic background to create B6$^{vic1I/LnJ}$ congenic mice.

B) C57BL/6J, C57BL/6J$^{vic1I/LnJ}$, and *Tlr7*$^{-/-}$ C57BL/6J$^{vic1I/LnJ}$ were i.p. injected with MMTV(LA). Serum samples were tested for reactivity against MMTV virion proteins by ELISA 10-12 weeks post-infection. Either polyisotypic or IgG2c-specific secondary Abs coupled to HRP were used. Four mice per each group were tested in a single experiment (three mice for C57BL/6J).


A
Mtv1
MMTV(C3H)
A
B
HP transgene
infection
C
B
Newly
integrated HP


B
1 2 3 4 5 6 7 8 9 M
HP

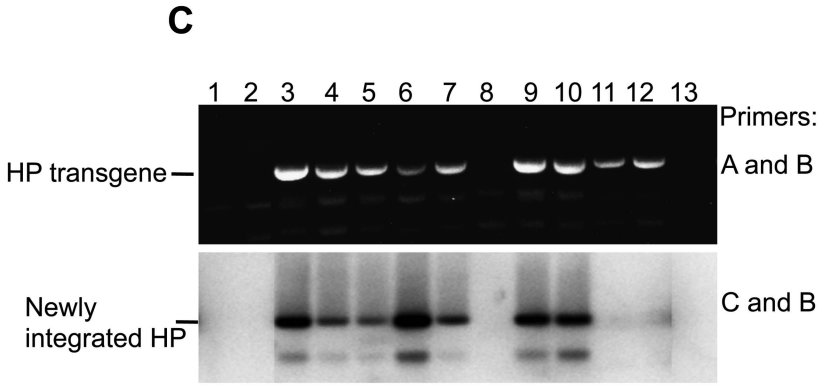
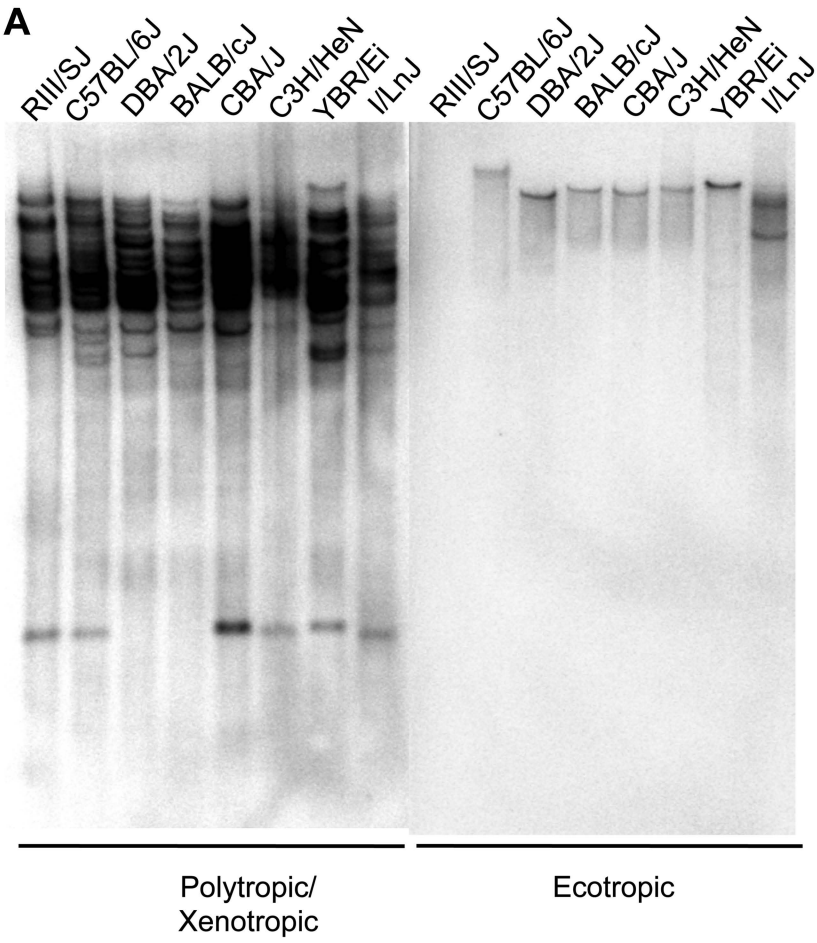

C
1 2 3 4 5 6 7 8 9 10 11 12 13
Primers:
HP transgene
A and B
Newly
integrated HP
C and B

Figure S1


A
RIII/SJ
C57BL/6J
DBA/2J
BALB/cJ
CBA/J
C3H/HeN
YBR/Ei
I/LnJ
RIII/SJ
C57BL/6J
DBA/2J
BALB/cJ
CBA/J
C3H/HeN
YBR/Ei
I/LnJ
Polytropic/
Xenotropic
Ecotropic

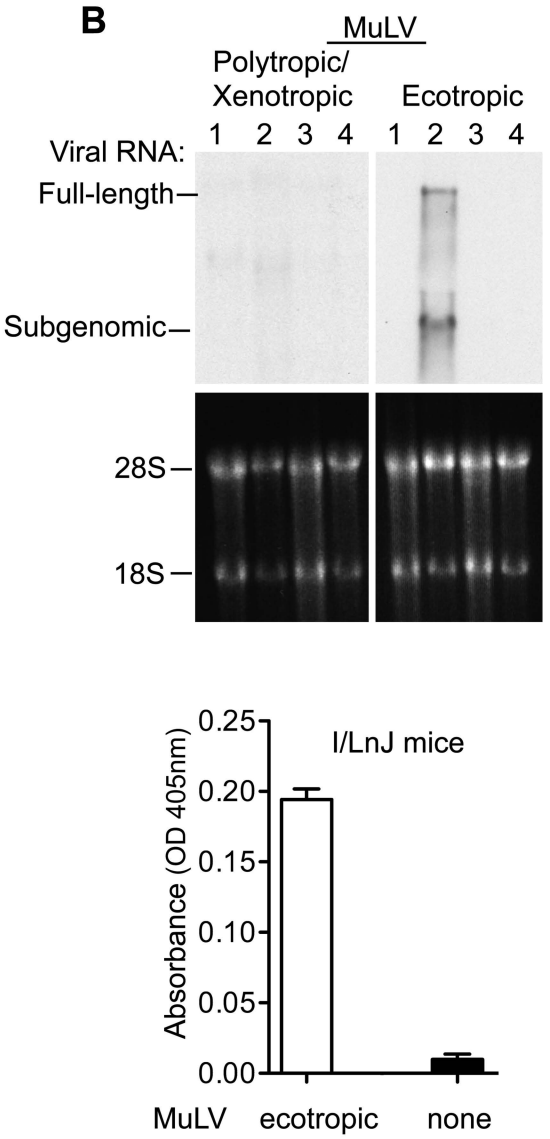
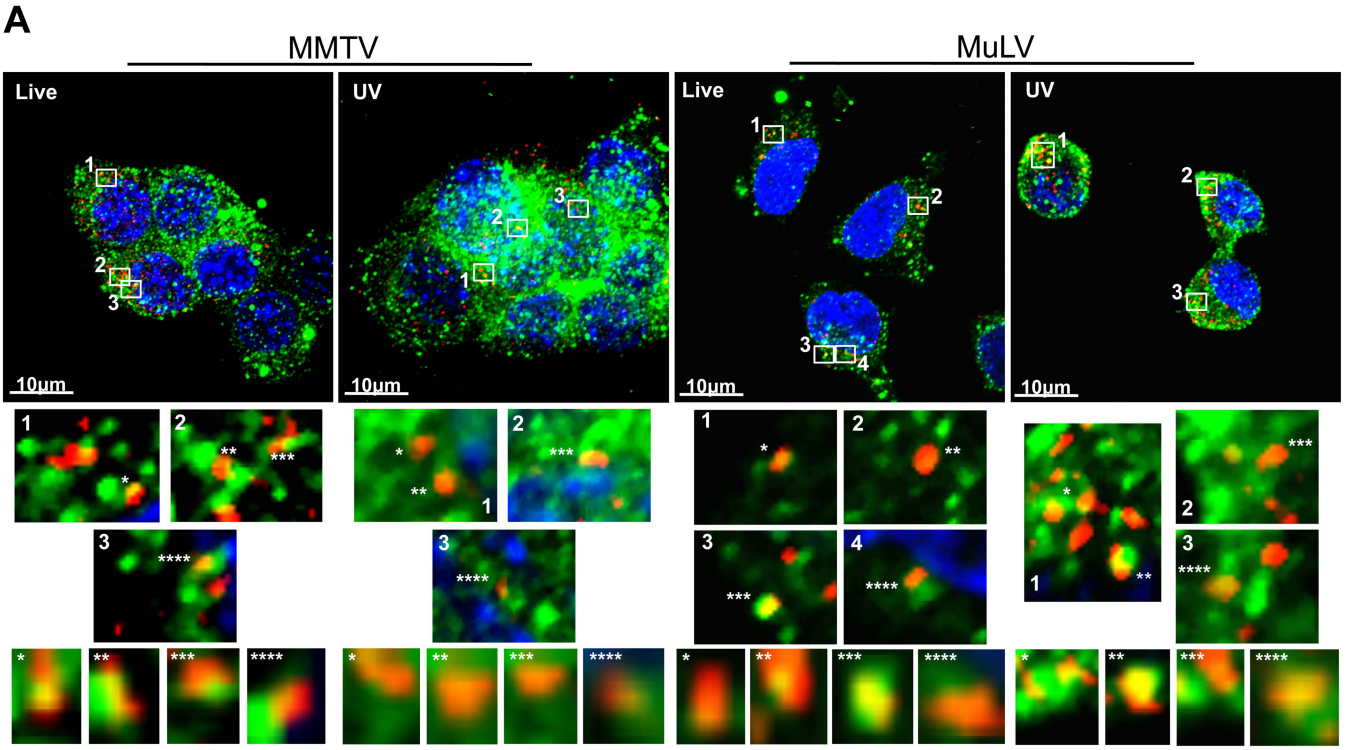
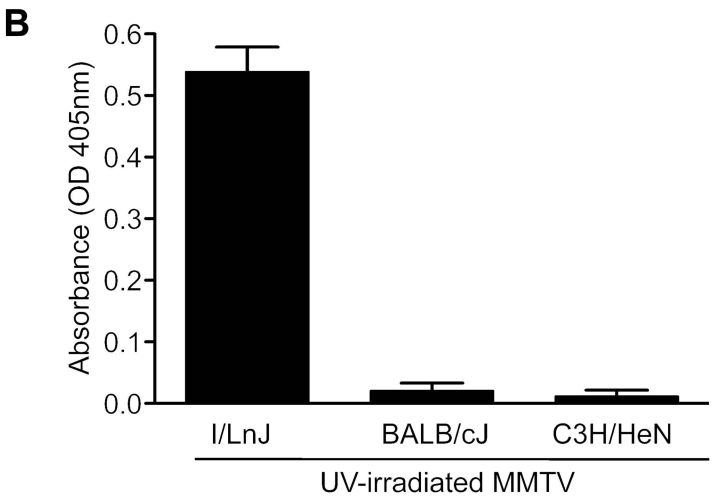

B
MuLV
Polytropic/
Xenotropic
Ecotropic
1 2 3 4 1 2 3 4
Viral RNA:
Full-length
Subgenomic
28S
18S
Absorbance (OD 405nm)
0.25
0.20
0.15
0.10
0.05
0.00
I/LnJ mice
MuLV ecotropic none

Figure S2


A
MMTV
MuLV
Live
UV
Live
UV
10µm
10µm
10µm
10µm


B
Absorbance (OD 405nm)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
I/LnJ
BALB/cJ
C3H/HeN
UV-irradiated MMTV

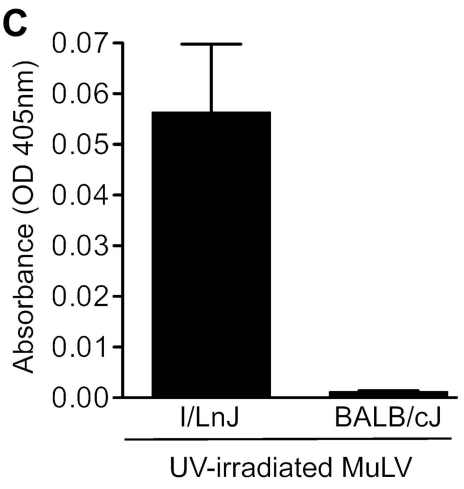
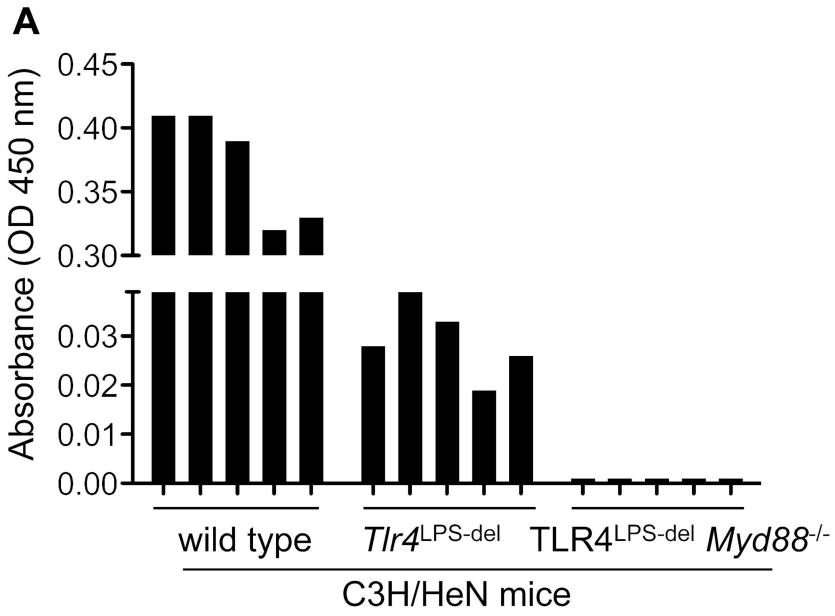

C
Absorbance (OD 405nm)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
I/LnJ
BALB/cJ
UV-irradiated MuLV

Figure S3


A
Absorbance (OD 450 nm)
0.45
0.40
0.35
0.30
0.03
0.02
0.01
0.00
wild type
Tlr4LPS-del
TLR4LPS-del
Myd88-/-
C3H/HeN mice

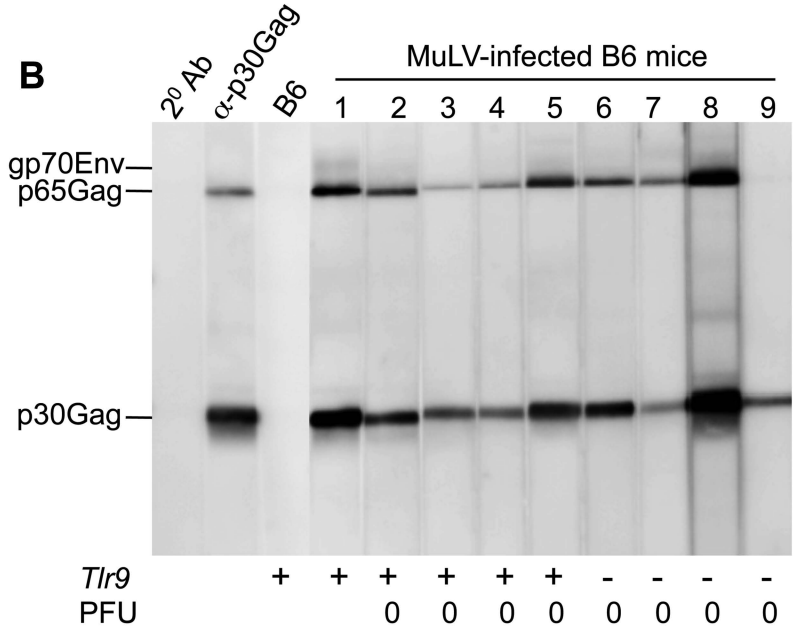

B
2° Ab
α-p30Gag
B6
MuLV-infected B6 mice
1 2 3 4 5 6 7 8 9
gp70Env
p65Gag
p30Gag
Tlr9 + + + + + + - - - -
PFU 0 0 0 0 0 0 0 0

Figure S4

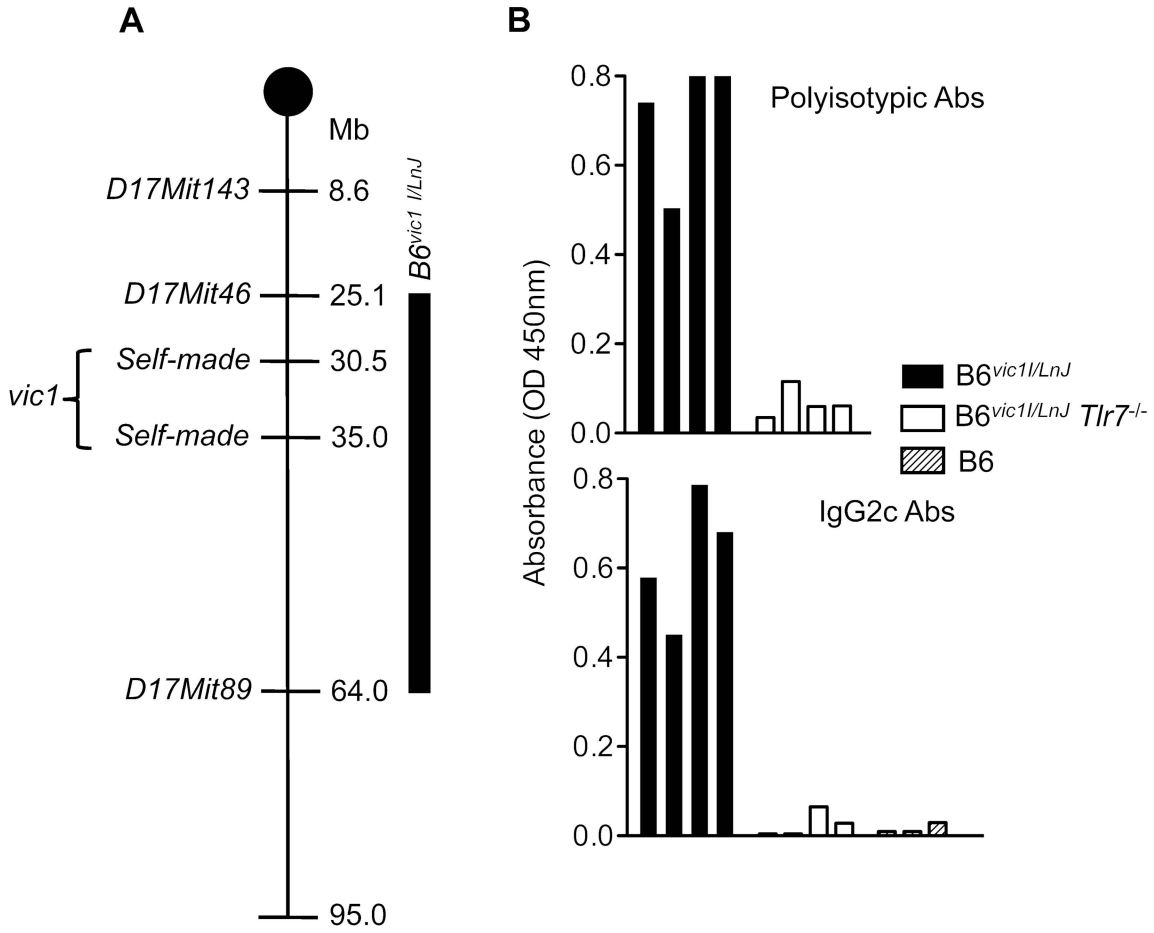

A
Mb
D17Mit143
8.6
D17Mit46
25.1
Self-made
30.5
vic1
Self-made
35.0
D17Mit89
64.0
95.0
B6vic1 I/LnJ
B
Polyisotypic Abs
IgG2c Abs
Absorbance (OD 450nm)
0.8
0.6
0.4
0.2
0.0
B6vic1I/LnJ
B6vic1I/LnJ Tlr7-/-
B6

Figure S5