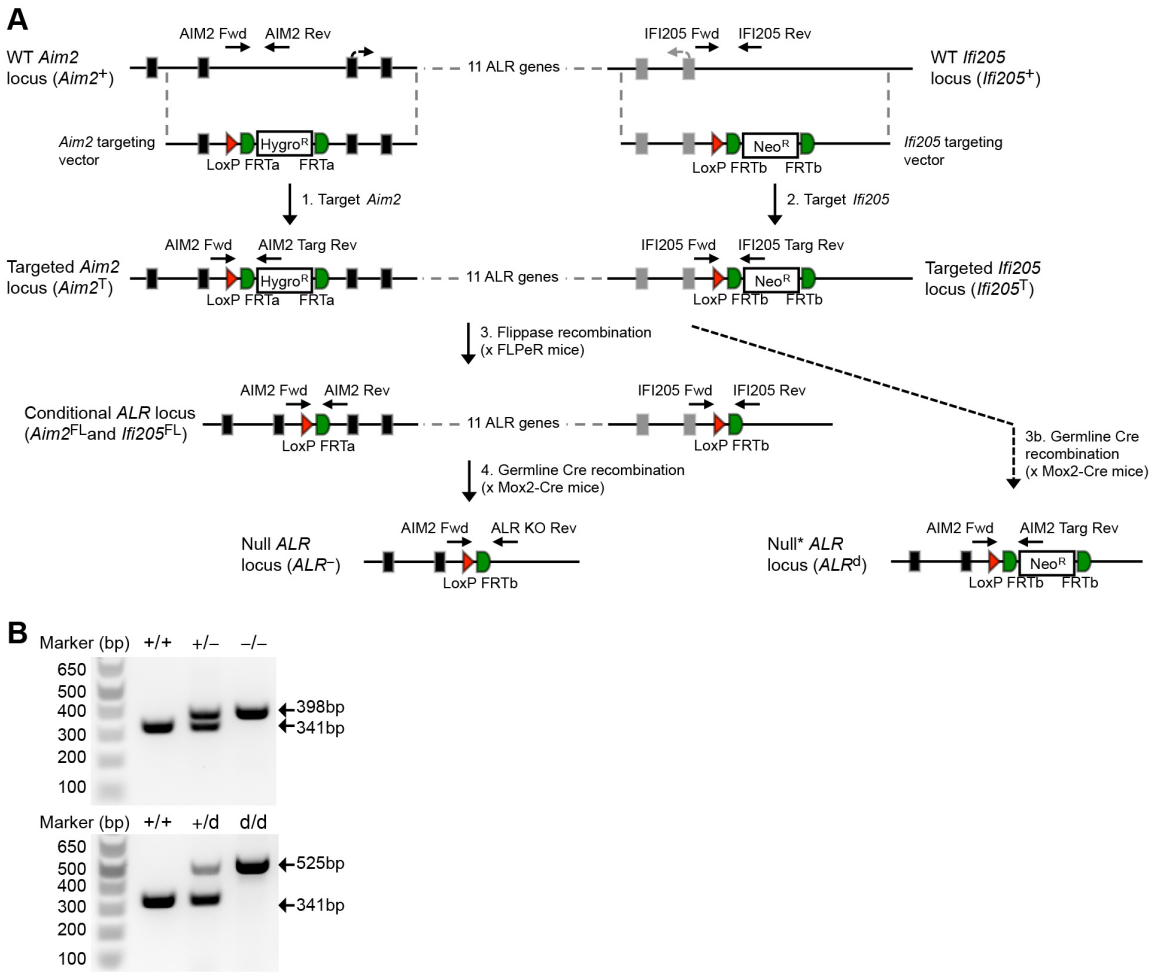
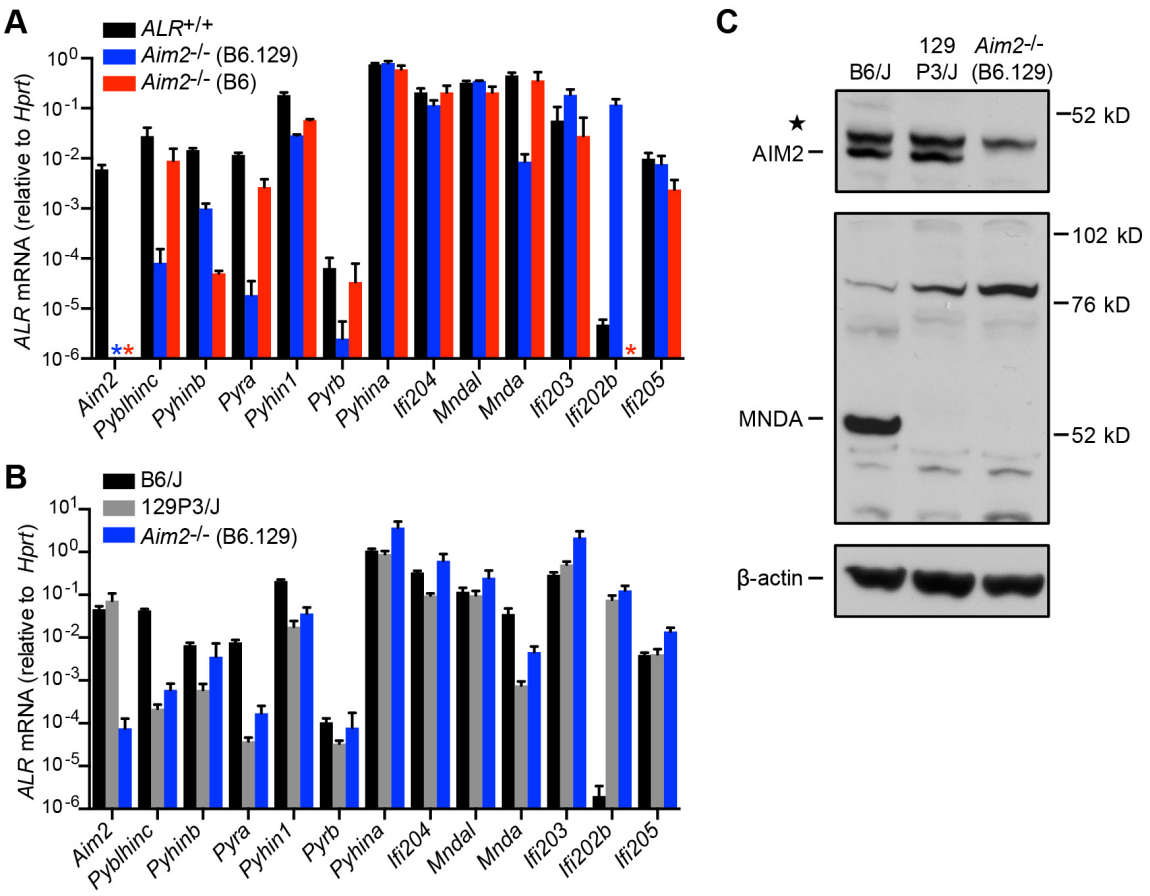
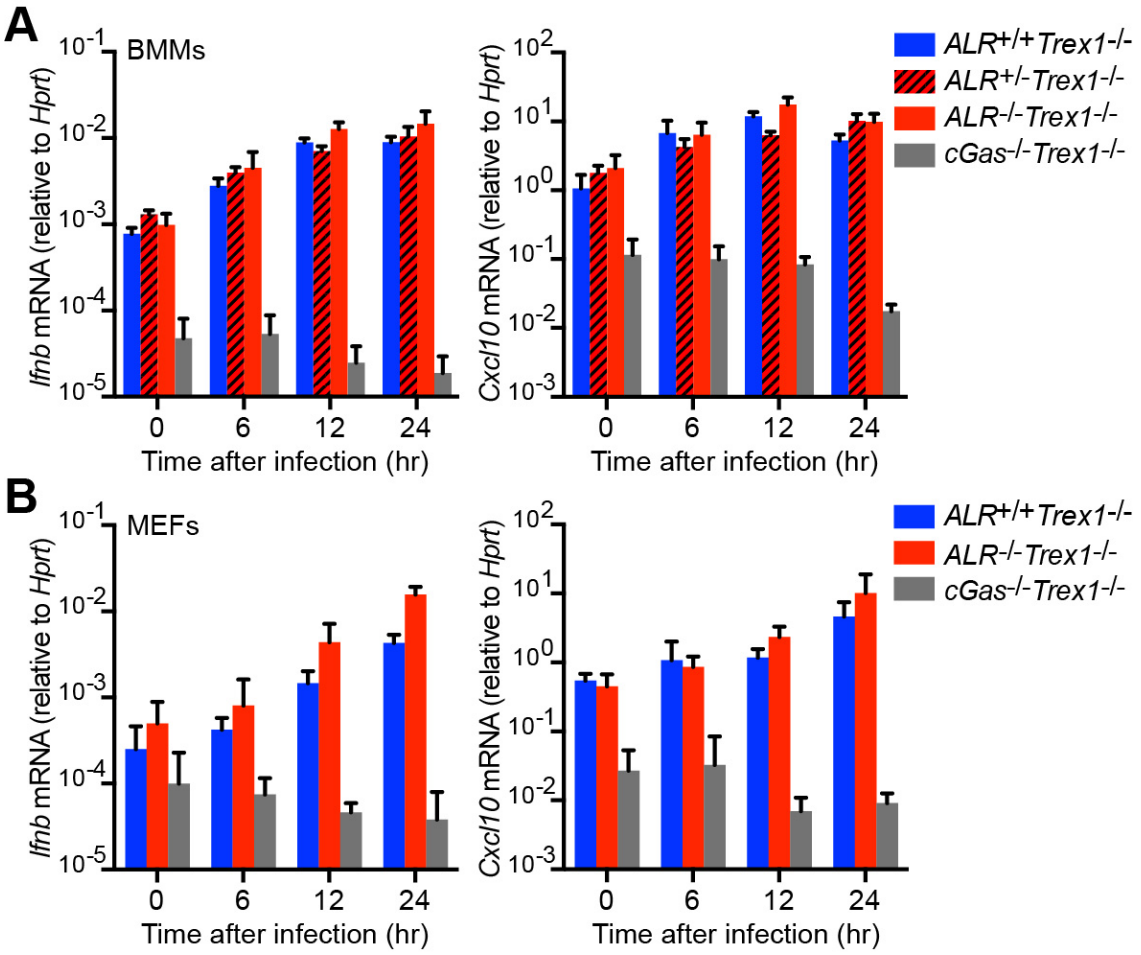


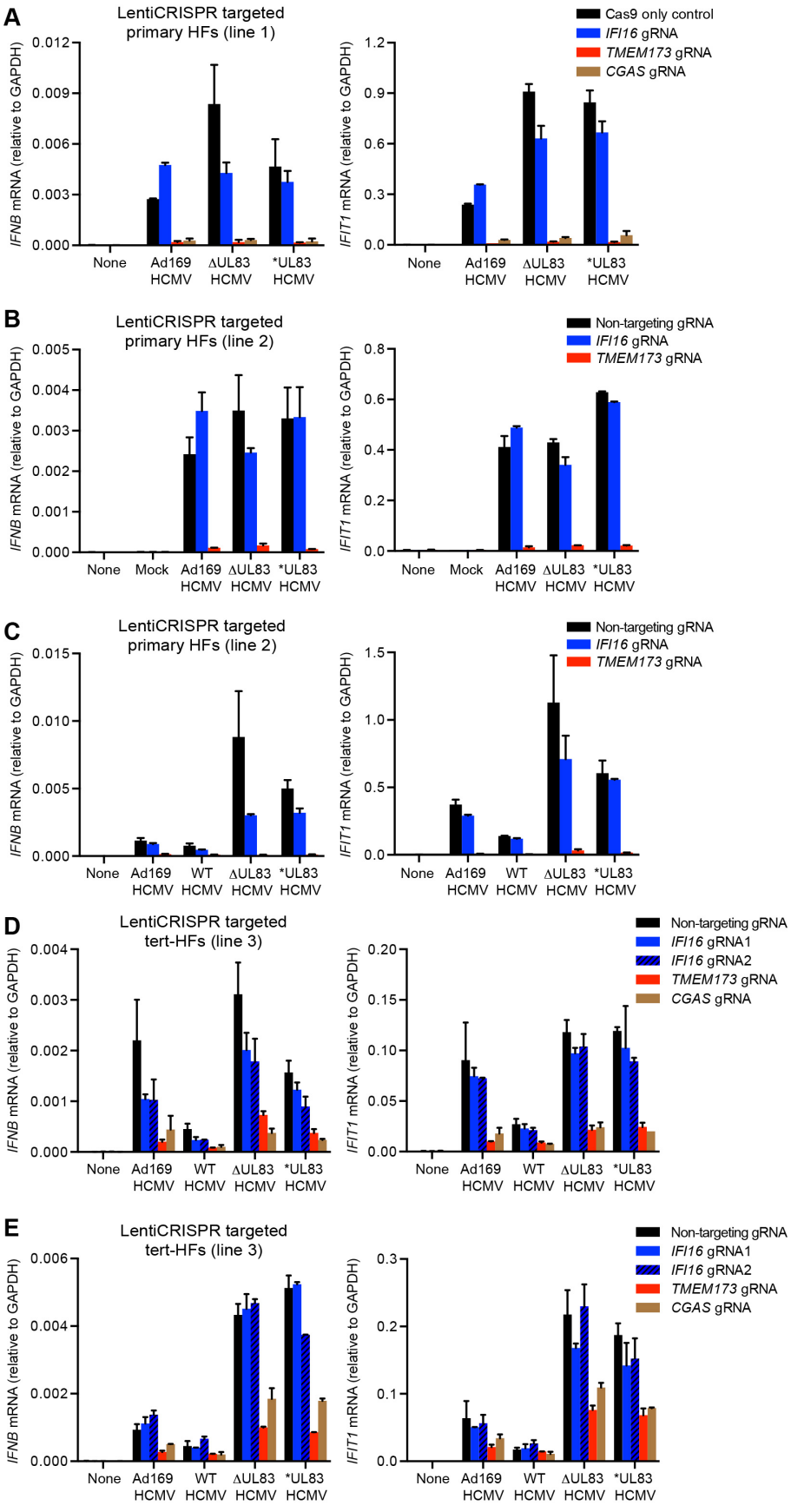
Supplemental Figure 1, related to Figure 1. ALR locus targeting schematic. (A) Schematic of the wild type and targeted *ALR* gene locus. Exons are indicated with solid black or gray squares and primers used for genotyping PCR are indicated with arrows. The targeted ALR^{T} locus contains LoxP and hygromycin and neomycin resistance cassettes upstream of the first coding exons of *Aim2* and *Ifi205*, respectively. The ALR^{FL} mice were generated by crossing the ALR^{T} mice to FLPeR mice (Farley et al., 2000) to remove the FRT-flanked hygromycin and neomycin resistance cassettes. ALR-deficient ($ALR^{-/-}$) mice were generated by crossing ALR^{FL} mice to Mox2-Cre mice (Tallquist and Soriano, 2000) for germline excision of the entire LoxP-flanked ALR locus. ALR-deficient ($ALR^{d/d}$) mice that retain the neomycin resistance cassette were generated by directly crossing ALR^{T} mice to Mox2-Cre mice. **(B)** Genotyping PCR to distinguish $ALR^{+/+}$, $ALR^{+/-}$, $ALR^{-/-}$, $ALR^{+/d}$, and $ALR^{d/d}$ mice using primers described in Supplemental Table 1 (ALR Fwd, AIM2 Rev, and ALR KO Rev (top panel) or AIM2 TARG Rev (bottom panel).



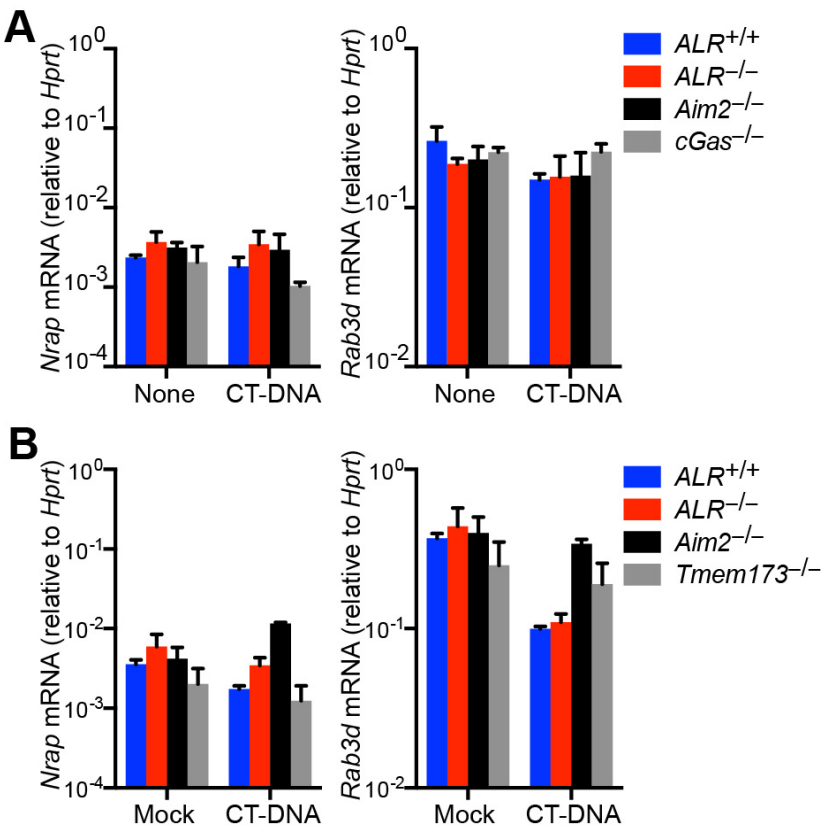
Supplemental Figure 2, related to Figure 1. ALR gene expression is variable in B6 compared to 129 mouse strains. (A) Quantification by RT-PCR of ALR gene transcripts in bone marrow-derived macrophages (BMMs) from $ALR^{+/+}$ mice compared to $Aim2^{-/-}$ mice generated in B6 ($Aim2^{-/-}$ (B6)) (Jones et al., 2010) or 129 ES cells ($Aim2^{-/-}$ (B6.129)) (Rathinam et al., 2010). The $Aim2^{-/-}$ mice generated in 129 ES cells were backcrossed to B6, but retain the 129-derived ALR locus linked to *Aim2* (* = not detected). **(B)** Quantification by RT-PCR of ALR gene transcripts in BMMs from B6/J, 129P3/J, or $Aim2^{-/-}$ (B6.129) mice. **(C)** AIM2 and MNDA protein expression evaluated in IFNβ-primed BMMs cultured from mice of the indicated genotype. Error bars represent mean ± SD. Data represent one experiment.



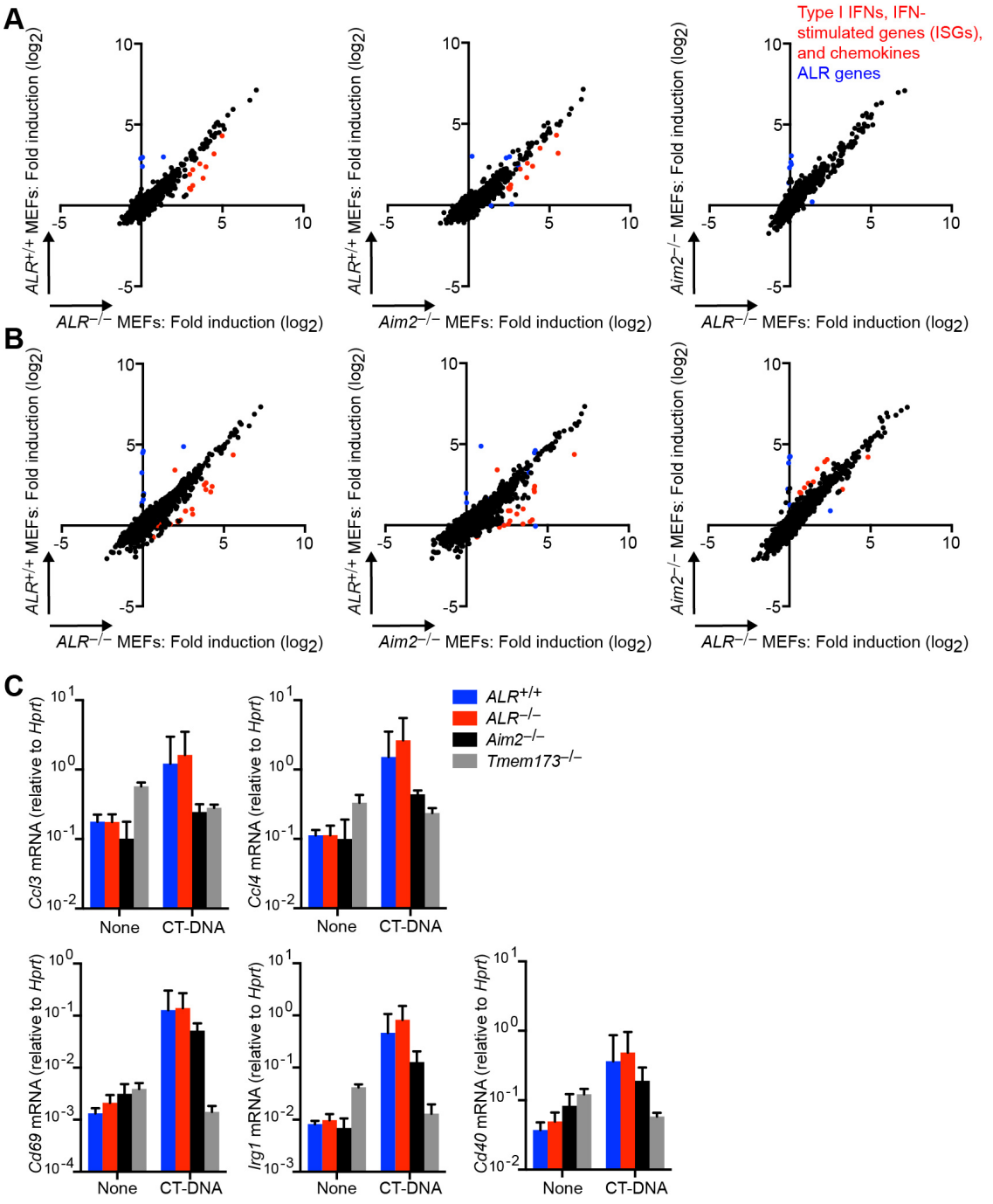
Supplemental Figure 3, related to Figure 3. Time course of the IFN response to lentivirus infection. Quantification of *Ifnb* (left panels) and *Cxcl10* (right panels) mRNA induction by RT-PCR in **(A)** BMMs and **(B)** MEFs from *Trex1*$^{-/-}$, *ALR*$^{-/-}$*Trex1*$^{-/-}$, and *cGas*$^{-/-}$*Trex1*$^{-/-}$ mice infected with a VSVg-pseudotyped, self-inactivating lentivirus at an MOI=1 for 6, 12, or 24h. Error bars represent mean ± SD. Data are representative of two independent experiments.


A
LentiCRISPR targeted primary HFs (line 1)
Cas9 only control
IFI16 gRNA
TMEM173 gRNA
CGAS gRNA
IFNB mRNA (relative to GAPDH)
IFIT1 mRNA (relative to GAPDH)
None
Ad169 HCMV
ΔUL83 HCMV
*UL83 HCMV
B
LentiCRISPR targeted primary HFs (line 2)
Non-targeting gRNA
IFI16 gRNA
TMEM173 gRNA
Mock
C
LentiCRISPR targeted primary HFs (line 2)
WT HCMV
D
LentiCRISPR targeted tert-HFs (line 3)
IFI16 gRNA1
IFI16 gRNA2
E
LentiCRISPR targeted tert-HFs (line 3)

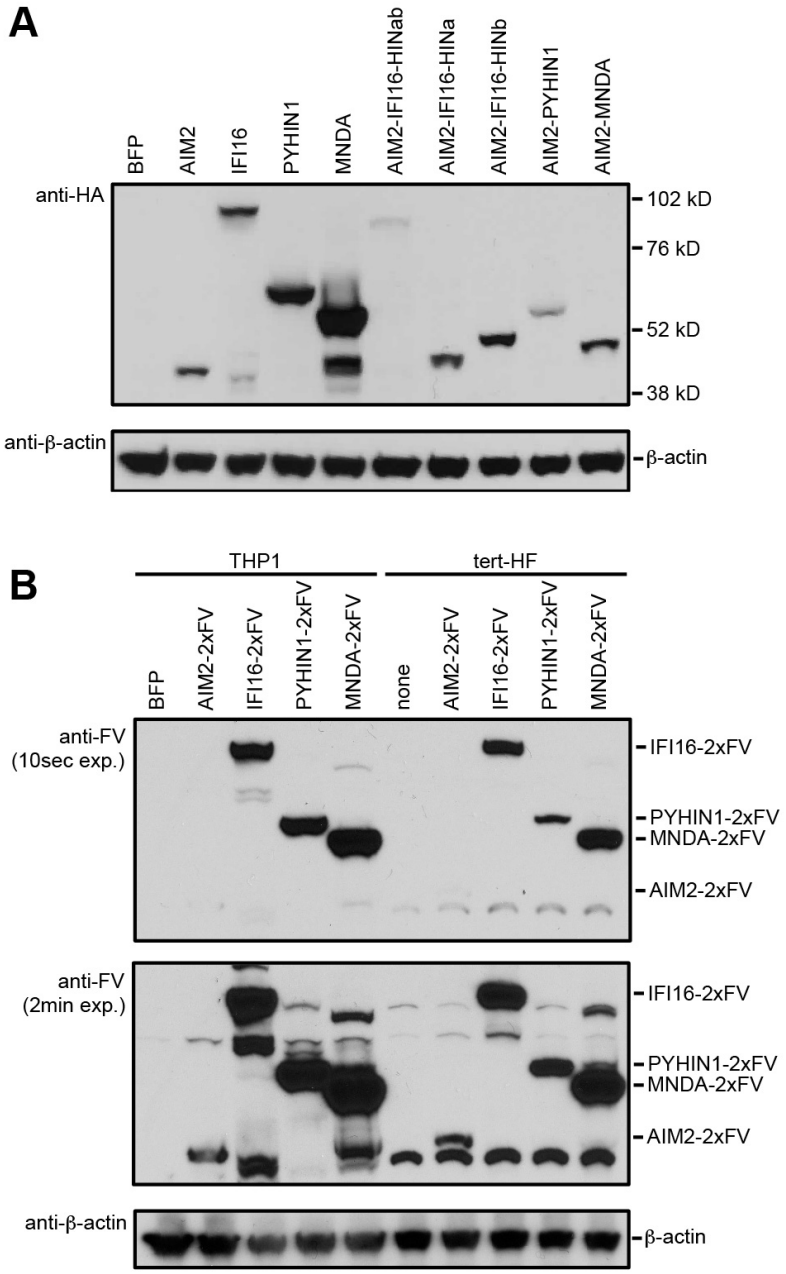
Supplemental Figure 4, related to Figure 5. Data from individual HCMV infection experiments. Quantification of *IFNB* (left panels) and *IFIT1* (right panels) mRNA induction by RT-PCR in targeted HFs, described in Figure 5A, infected with the indicated HCMV viral strains (MOI = 3) for 6h. Panels A-E each represent data from an individual experiment. Error bars represent mean ± SD. Three independently generated LentiCRISPR-targeted primary HF or telomerase-immortalized HF (tert-HF) cell lines were used in panels A, B/C, or D/E as indicated.



Supplemental Figure 5, related to Figure 6. Validation of microarray data. Evaluation of *Nrap* and *Rab3d* gene expression by quantitative RT-PCR in bone marrow-derived macrophages (BMMs) cultured from mice of the indicated genotype transfected with 5μg calf-thymus DNA (CT-DNA) for 4h (**A**) or 8h (**B**). Error bars represent mean ± SD. Data in each panel are representative of one experiment.



Supplemental Figure 6, related to Figure 6. Microarray analysis of the transcriptional response to transfected DNA in fibroblasts. (**A-B**) Microarray analysis of gene expression changes in *ALR*$^{+/+}$, *ALR*$^{-/-}$, and *Aim2*$^{-/-}$ MEFs transfected with 5μg calf-thymus DNA (CT-DNA) for 4h (**A**) or 8h (**B**) plotted as fold induction relative to mock-treated cells (treated with lipofectamine alone). Selected type I IFNs, cytokines, and IFN-stimulated genes are highlighted in red, and ALR genes are highlighted in blue. Data are representative of one experiment. (**C**) Quantification by RT-PCR of gene expression in *ALR*$^{+/+}$, *ALR*$^{-/-}$, *Aim2*$^{-/-}$ and *Tmem173*$^{-/-}$ MEFs cultured from mice of the indicated genotype transfected with 1μg calf-thymus DNA (CT-DNA) for 8h. Error bars represent mean ± SD. Data are representative of one experiment.



Supplemental Figure 7, related to Figure 7. Expression of ALR constructs in transduced cells.
Evaluation by Western blot of (**A**) ALR-HA protein expression in AIM2-deficient THP1 cells reconstituted with lentiviral constructs expressing HA-tagged wild type ALRs (left lanes) or chimeric ALRs (right lanes), and (**B**) ALR-2xFV protein expression in THP1 cells (left lanes) or tert-HFs (right lanes) reconstituted with lentiviral constructs expressing ALR-2xFV proteins. Two different exposure (exp.) times are shown for the anti-FV blot; note that AIM2-2xFV is expressed at lower levels than the other ALR-2xFVs. Data in each panel are representative of one experiment.

Supplemental Table 1, related to Figure 1. Genotyping primers for ALR-deficient mice. Primers for genotyping PCR to distinguish ALR^{+}, ALR^{-}, ALR^{d}, $Aim2^{FL}$, $Aim2^{T}$, $Ifi205^{FL}$, and $Ifi205^{T}$ alleles.

Supplemental Table 2, related to Figure 6. Microarray analysis of the transcriptional response to transfected DNA in bone marrow-derived macrophages. List of probes with ≥2-fold change in expression comparing $ALR^{+/+}$, $ALR^{-/-}$, and $AIM2^{-/-}$ macrophages treated with lipofectamine alone (mock) or transfected with 5μg calf thymus DNA (CT-DNA) for 4 or 8 hours.

Supplemental Table 3, related to Figure 6. Microarray analysis of the transcriptional response to transfected DNA in fibroblasts. List of probes with ≥2-fold change in expression comparing $ALR^{+/+}$, $ALR^{-/-}$, and $AIM2^{-/-}$ fibroblasts treated with lipofectamine alone (mock) or transfected with 5μg calf thymus DNA (CT-DNA) for 4 or 8 hours.

Supplemental Experimental Procedures

Mice

To generate ALR-deficient mice, we performed consecutive gene targeting in Itl IC1 C57BL/6NTac-derived embryonic stem (ES) cells to introduce LoxP sites flanking the ALR locus. One LoxP site was integrated upstream of the first coding exon of *Aim2* together with a FRTa-flanked hygromycin resistance cassette. Correctly targeted clones were then used to introduce a LoxP site and a FRTb-flanked neomycin resistance cassette upstream of the first coding exon of *Ifi205* at the other end of the locus (**Supplemental Figure 1A**; note that *Aim2* and *Ifi205* face each other and that the two LoxP sites are in the same orientation, outside the coding exons). We tested the resulting ES cells for cis-targeting of both loci by transfecting these cells with a Cre recombinase expression vector. PCR analysis using primers that bind outside the two LoxP sites revealed deletion of the entire ALR locus in 2 of 6 clones, thus demonstrating both cis-targeting in these clones and the feasibility of Cre-mediated recombination over this large genomic distance (not shown). One of these clones was injected into blastocysts to make chimeric mice, and we confirmed germline transmission of both LoxP sites in the offspring of these chimeras (not shown). These *ALR*-targeted ($ALR^{T/+}$) mice were crossed to FLPeR mice (Farley et al., 2000) to remove the FRT-flanked hygromycin and neomycin cassettes, crossed to Mox2-Cre mice (Tallquist and Soriano, 2000) for germline excision of the entire ALR locus in all tissues, and intercrossed to generate ALR-deficient ($ALR^{-/-}$) mice (**Supplemental Figure 1A**, bottom left panel). We also bred $ALR^{T/+}$ mice directly to Mox2-Cre mice and intercrossed their progeny to generate ALR-deficient ($ALR^{d/d}$) mice that retain the FRTb-flanked neomycin cassette (**Supplemental Figure 1A**, bottom right panel). Deletion of the ALR locus was confirmed by sequencing PCR products using primers flanking the ALR locus (not shown). We observed no phenotypic differences between $ALR^{-/-}$ and $ALR^{d/d}$ mice. In figures throughout this manuscript, we simply denote "$ALR^{-/-}$ mice" to indicate that $ALR^{-/-}$ or $ALR^{d/d}$ mice were used. Mice were genotyped using primers listed in **Supplemental Table 1**.

All mouse studies were carried out in a specific-pathogen-free facility at the University of Washington. Sentinel mice (Crl:CD1[ICR]; Charles River, Wilmington, MA) were tested quarterly and were negative for endo- and ectoparasites, MNV, mouse hepatitis virus, mouse parvovirus, and rotavirus and annually for Mycoplasma pulmonis, pneumonia virus of mice, reovirus 3, Sendai virus, and Theiler murine encephalomyelitis virus.

Cell lines and tissue culture

Bone marrow-derived macrophages (BMMs) and mouse embryonic fibroblasts (MEFs) were generated and cultured as described (Lau et al., 2015). 293T cells, primary human fibroblasts (HF) and telomerase-immortalized HF (tert-HF; kindly provided by Denise Galloway, Fred Hutchinson Cancer Research Center) were grown in DMEM supplemented with 10% fetal calf serum, L-glutamine, penicillin/streptomycin, sodium pyruvate, and HEPES. THP1 cells were cultured in RPMI supplemented as above. THP1 cells were differentiated by culturing in 100nM PMA for 24 hours and then culturing for an additional 24 hours in fresh medium (without PMA) prior to treatment.

IL-1β ELISA

BMMs were seeded at a density of $4x10^5$ cells per well in a 24-well plate, primed with 200ng/ml LPS for 4h, and transfected with 1μg calf thymus DNA (CT-DNA) using Lipofectamine 2000 (Life Technologies). The supernatant was harvested 6 hours later and IL-1β secretion was quantified using a mouse IL-1β ELISA kit (Becton Dickinson) and an iMark Microplate Reader (Bio-Rad Laboratories).

Quantification of cell death

PMA-differentiated THP1 cells, HFs, and BMMs were seeded at a density of $8x10^4$, $2x10^4$, and $15x10^4$ cells per well, respectively, in a 24-well plate. Cells were transfected with 2μg calf thymus DNA (CT-DNA) or ISD 100-mer oligonucleotides (Stetson and Medzhitov, 2006) using Lipofectamine 2000 (Life Technologies). Cell death was assayed with a 2-color IncuCyte Zoom in-incubator imaging system (Essen Biosciences, Ann Arbor, MI, USA) and analyzed as described (Orozco et al., 2014). SytoxGreen and SytoGreen (25nM, Life Technologies) were used to quantify the frequency of dead cells.

Western blot analysis and antibodies

Cells were harvested in lysis buffer (20mM HEPES pH 7.4, 150mM NaCl, 10% glycerol, 1% Triton-X 100, 1mM EDTA, 1mM DTT) supplemented with Complete protease inhibitor cocktail (Roche), incubated on ice for 15 minutes, and cleared of insoluble material by centrifugation. Cleared lysates were separated using a 4-12% Bis-Tris SDS-PAGE gel (Life Technologies) and transferred to Immobilon-P PVDF membrane (Millipore). Membranes were probed with the following primary antibodies: anti-mouse AIM2 (Cell Signaling, #13095), anti-mouse IFI204/MNDA (Novus Biologicals, NBP2-27153), anti-human AIM2 (Biolegend, clone 3B10, #652801), anti-human IFI16 (Abcam, #ab55328), anti-human STING (Abcam, ab92605), anti-human cGAS (Sigma, #HPA031700), anti-HA (Cell Signaling, clone 6E2, #2367), and anti-FV (Pierce, #PA1-026A, anti-FKBP12). Membranes were then probed with goat anti-mouse-HRP or goat anti-rabbit-HRP (Jackson ImmunoResearch) and developed by ECL chemiluminescence (Pierce). In some experiments, mouse and human cells were primed with 100U/ml mIFNb (R&D Systems) for 6h or 10U/ml hIFNb (R&D Systems) for 5h, respectively.

CRISPR/Cas9 gene targeting

The sequences of the guide RNA (gRNA) target sites are as follows, with the protospacer adjacent motif (PAM) sequence underlined: non-targeting control gRNA: 5'-(G)ACGGAGGCTAAGCGTCGCAA (Sanjana et al., 2014), where the (G) denotes a nucleotide added to enable robust transcription off the U6 promoter; *AIM2* gRNA: GGAGATGTTTCATGCTACAGTGG; *IFI16* gRNA1: GAATGAAGTCTTCCGAGTGAAGG; *IFI16* gRNA2: GGAATATGATAGTCTCCTAGAGG; *cGAS* gRNA: GGCGCCCCTGGCATTCCGTGCGG; *TMEM173* gRNA: GGTGCCTGATAACCTGAGTATGG.

Generation of chimeric ALR-HIN domain constructs

Chimeric ALR-HIN domain constructs were created by cloning the first two coding exons of *AIM2* upstream of the first exon encoding the HIN domains for *IFI16*, *PYHIN1*, and *MNDA* followed by a C-terminal HA tag (IFI16-HINab: coding exons 4-10, IFI16-HINa: coding exons 4-5; IFI16-HINb: coding exons 8-10; PYHIN1-HIN: coding exons 4-7; MNDA-HIN: coding exons 4-6). Full-length wild type ALR constructs followed by a C-terminal HA tag were also cloned. Full-length AIM2 localizes to the cytosol, while IFI16, PYHIN1, and MNDA localize to the nucleus due to NLS signals between the Pyrin and HIN domains (Hornung et al., 2009; Li et al., 2012); the chimeric ALR-HIN domain proteins do not contain NLS signals and should localize to the cytosol. Synonymous mutations were introduced into the *AIM2* gRNA target site and protospacer adjacent motif (PAM) in the third coding exon of *AIM2* to avoid LentiCRISPR-mediated gene disruption of AIM2-HA. Full-length wild type ALR and chimeric ALR-HIN domain constructs were cloned into a pRRL lentiviral vector for SFFV promoter-driven ALR-HA-T2A-blasticidin resistance expression. AIM2-deficient THP1 cells (generated using CRISPR/Cas9) were transduced with lentivirus and selected with 10μg/ml blasticidin (Thermo Fisher Scientific) for three days.

Microarray analysis

BMMs and MEFs were seeded at a density of $7.5x10^5$ and $1x10^5$ per well, respectively, in a 12-well plate. Cells were transfected with 5μg calf thymus DNA (CT-DNA) per well using Lipofectamine 2000 (Life Technologies) for 4 or 8 hours. Cells were harvested with RNA Stat60 (TelTest) and RNA was isolated per manufacturer's instructions. To improve RNA quality, isolated RNA was then purified using the RNeasy kit (Qiagen) per manufacturers instructions. Hybridization to Illumina MouseWG-6 v2.0 Expression BeadChip microarrays was performed by the W.M. Keck Foundation Yale Center for Genomic Analysis. Probes with a low signal across all samples (the sum of signals from all nine macrophage or fibroblast samples was less than 1000) were excluded from analysis. Probes with ≥2-fold change in expression are listed in **Supplemental Tables 2 and 3**.

THP1 cells stably expressing IFI16, PYHIN1, or MNDA-2xFV fusion proteins were differentiated with PMA and seeded at a density of $3x10^5$ cells per well in a 12-well plate. Cells were treated with vehicle or 30nM AP1 (Clontech, also called 'B/B Homodimerizer') for 4 or 12 hours. RNA was purified as described above. Hybridization to Illumina HumanHT-12 v4 Expression BeadChip microarrays was performed by the W.M. Keck Foundation Yale Center for Genomic Analysis. Probes with a low signal across all samples (the sum of signals from all 12 samples was less than 1500) were excluded from analysis.

Supplemental References

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