SAMHD1-dependent retroviral control and escape in mice

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Supplementary Information

Supplementary Figure Legends

Supplementary Figure 1. Generation of SAMHD1-deficient mice

A. *Samhd1* knockout strategy. The *Samhd1* gene is located on mouse chromosome 2 and comprises 16 exons. The region encompassing exons 1 to 3 is shown here. Step 1: We constructed the targeting vector pACYC-AGSKO5-3 by cloning the fragment between the SacII and BshT1 (=AgeI) restriction sites. We introduced (i) a loxP site into the SpeI restriction site present between exons 1 and 2 and (ii) a second loxP site together with a neomycin cassette flanked by FRT sites into the BamHI restriction site between exons 2 and 3. Gray shading indicates that these restriction sites were mutated during loxP insertion. The vector backbone contains diphtheria toxin A as a negative selection marker and was linearized with AscI before transfection into ES cells. We used two types of ES cells that have a mixed S6/B6 or pure B6 genetic background. Following selection with G418, two successfully targeted clones were selected by long-range PCR and Southern blot: clone 1F8 (mixed S6/B6) and clone 5D6 (pure B6). These clones were used to generate chimeric founder animals that successfully transmitted the targeted allele to their offspring. Step 2: Animals with one targeted *Samhd1* allele were bred with a transgenic line expressing Cre-recombinase under the ubiquitous PGK promoter, deleting exon 2 in all tissues and generating a *ko(neo)* (knockout-with-neomycin-cassette) allele. *Samhd1 +/ko(neo)* mice were intercrossed to generate homozygous knockouts. Splicing of exon 1 to exon 3 generates a frameshift bringing a stop codon in exon 3 into frame. Step 3: *Samhd1 +/ko(neo)* mice were bred to a line expressing Flp recombinase under the ubiquitous human beta actin promoter to delete the neomycin cassette. We used brother-sister-mating of the

resulting *Samhd1 +/ko* animals to produce homozygous knockout animals. *Samhd1 ko(neo)* and *ko* alleles are denoted as "-" in this manuscript and the wild-type allele as "+".

B. BM-DCs from littermate *Samhd1* +/+, +/- or -/- animals (derived from ES clone 5D6, neomycin cassette present) were stimulated overnight with 1000 U/ml IFN-A/D or left untreated. We determined *SAMHD1* mRNA levels in bulk cultures by RT Q-PCR using primers within exon 2 (left) or outside exon 2 (right). Average relative expression levels from three measurements compared to *GAPDH* mRNA are shown and error bars represent the standard deviation.

C. BM-DCs from (B) and MEFs from Fig. 1E were tested for SAMHD1 expression by Western blot using a polyclonal mSAMHD1 anti-serum. A non-specific band is marked with an asterisk and serves as a loading control.

D. (top) Mouse SAMHD1 has two annotated splice isoforms that use different terminal exons indicated in blue and orange. Isoform 1 (ENSMUST00000057725; CCDS16973.2) and isoform 2 (ENSMUST00000088523; CCDS50783.1) encode 658 and 651 amino acid proteins, respectively. Upon deletion of exon 2, splicing of exon 1 to exon 3 generates a frameshift bringing a stop codon in exon 3 into frame (highlighted in red). This affects both isoforms. mRNA expression of SAMHD1 isoforms was analyzed by RT-PCR using RNA from MEFs of the indicated *Samhd1* genotypes (Fig. 1E). As a negative PCR control, water was used instead of cDNA. Exons are shown as boxes and primers as horizontal arrows; primer names are in italics. The positions of start and stop codons and of the SAM and HD domains are indicated. Drawn to scale. The expected sizes of PCR products were: PCR-A, 504 bp from wild-type cDNA (both isoforms), 437 bp from exon 2 deleted knockout cDNAs; PCR-B1, 1642 bp from wild-type isoform

1 cDNA, no product from isoform 2 and exon 2 deleted cDNAs; PCR-B2, 1621 bp from wild-type isoform 2 cDNA, no product from isoform 1 and exon 2 deleted cDNAs. (bottom) PCR products were loaded on an agarose gel and visualized with SYBRsafe (Invitrogen).

E. MEFs from Fig. 1E were tested for SAMHD1 expression and localization by immunofluorescence using the polyclonal mSAMHD1 anti-serum or pre-immune serum as a control (bottom). Nuclei were visualized with DAPI staining.

F. *Samhd1* +/- animals were crossed and the genotype of the offspring was determined by PCR. Numbers of *Samhd1* +/+, +/- or -/- animals born to heterozygous breeders are shown for the pure B6 and mixed S6/B6 background, with the neomycin cassette (neo) present or not as indicated. These data were compiled into Fig. 1A.

Supplementary Figure 2. Inflammatory responses induced by *E. coli* DNA in SAMHD1-depleted BM-DCs

A. Wild-type B6 BM-DC cultures were infected with lentiviral vectors expressing shRNA targeting SAMHD1 or control scrambled shRNA (scr). Transduced cells were selected with puromycin and *SAMHD1* mRNA levels were determined by RT Q-PCR.

B. Wild-type B6 BM-DC cultures were depleted of SAMHD1 as in (A) and then transfected or not with *E. coli* DNA. The indicated mRNAs were assessed by RT Q-PCR.

(A-B) Relative expression levels compared to *GAPDH* mRNA from three (A) or two (B) measurements are shown and error bars represent the standard deviation (A) or range (B).

Supplementary Figure 3. IFN production is not detectable after infection with HIV-1-GFP

BM-DCs of the indicated *Samhd1* genotypes were infected with VSV-G pseudotyped HIV-1-GFP (pRRLsin.eGFP / pCMVΔ8.2) using different multiplicities of infection (MOI). 48 hours after infection, supernatants were transferred to the NIH3T3-ISRE-GFP reporter cell line stably expressing GFP under the control of an IFN inducible promoter. The fraction of GFP-expressing reporter cells was determined by flow cytometry after 24 hours. IFN-A/D served as a standard at the indicated concentrations. Averages from three DC cultures per group from independent mice are shown and error bars represent the standard deviation. The IFN-A/D standard was done in duplicate and error bars represent the range. Cells and mice were on the B6 background (5D6).

Supplementary Figure 4. Retroviral transduction of SAMHD1 deficient cells.

A. Cells freshly isolated from whole spleens were transduced with the indicated retroviral vectors. After overnight incubation, GFP and CD11c expression was analyzed by flow cytometry. The number of GFP^+CD11c^+ cells per spleen was calculated. Averages from three different spleens per group are shown and error bars represent the standard deviation.

B. Fresh bone marrow from mice of the indicated *Samhd1* genotypes was transduced by spin-infection with undiluted 293T supernatants containing the indicated retroviral vectors or medium alone as a negative control (mock). Supernatant containing the pCSGW / p8.91 based HIV-1-GFP vector was also diluted 1:10 prior to infection (right). Cells were cultured with GM-CSF for 6 days and GFP expression was determined by flow cytometry.

C. Immortalized MEFs of the indicated *Samhd1* genotypes were infected with different GFP-expressing retroviral vectors. Two days later, transduction was analyzed by flow cytometry. Each histogramm corresponds to one MEF cell line from an individual embryo; numbers in square brackets refer to the cell line identifier (right).

(A-C) Cells and mice were on the B6 background (5D6).

Supplementary Figure 5. HIV-1-GFP transduction of BM-DCs.

Representative FACS plots corresponding to Fig. 6B are shown.

Supplementary Figure 6. FACS analysis of *in vivo* transduced splenocytes

Fresh splenocytes were stained for surface expression of NK1.1, CD3, CD11b and Gr-1 (stain I, top) or B220, CD11c, MHCII and F4/80 (stain II, bottom). Cells staining positive with DAPI were excluded from the analysis. Autofluorescent cells were included for the analysis of $F4/80^{+}$ macrophages (bottom), but were otherwise excluded.

Supplementary Figure 7. *In vivo* infection with HIV-1-GFP (pCSGW / p8.91).

C57BL/6 wild type and SAMHD1-deficient (5D6) animals were injected intravenously with HIV-1-GFP (pCSGW / p8.91, 10^8 293T infectious units). Five days later, transduction of splenocytes was analyzed by flow cytometry. GFP expression in total spleen cells and in the indicated splenocyte subsets was analyzed after pre-gating on DAPI negative cells. T, T-cells; NK, NK-cells; Mono, inflammatory monocytes; Neut, neutrophils; B, B-cells; DC, dendritic cells; pDC, plasmacytoid dendritic cells. The differences between the samples from *Samhd1*$^{+/+}$ and *Samhd1*$^{-/-}$ mice are not statistically significant.

Supplementary Figure 8. Alignment of human and mouse SAMHD1

Human and mouse SAMHD1 protein sequences were downloaded from ensemble.org (hSAMHD1: ENSP00000262878; mSAMHD1 isoform 1: ENSMUSP00000059717, isoform 2: ENSMUSP00000085880) and aligned with DNASTAR MegAlign software using the Clustal W method. The sequence of the human protein is shown in white on black background. Identical amino acids in mouse SAMHD1 are highlighted in the same way, while non-identical residues are shown in black on white background. The CDK1 phosphorylation site T_{592}PQK in human SAMHD1 is marked by a red line. A similar site matching the CDK1 consensus sequence S/T-P-X-K/R is present in mouse SAMHD1 isoform 1.

Supplementary Figure 9. Mouse SAMHD1 escapes Vpx-mediated degradation

A. PMA-differentiated human THP1 cells were treated with medium only or with virus-like particles (VLPs) that do or do not contain Vpx for 24 hours. hSAMHD1 and actin protein levels were assessed by Western blot using antibodies against hSAMHD1 and actin.

B. Mouse BM-DMs or BM-DCs were treated with the same VLPs as in (A) for 24 hours. mSAMHD1 protein levels were assessed by Western blot using a polyclonal mSAMHD1 anti serum. The membrane was re-probed with an α-actin antibody as a loading control.

Supplementary Experimental Procedures

Generation of SAMHD1-deficient mice

Targeting vector

The mouse 129 strain PAC library RPCI-21 (Osoegawa et al, 2000) was screened with a radioactive probe YC1 (chr2:156954584-156955773; using oligonucleotides YC-1F and YC-2R) to identify a clone (PAC 567N18) containing the target region of *Samhd1*. A BshTI (=AgeI) + SacII digest was performed to clone the fragment chr2:156951255-156960865, which contains exons 1-3 of Samhd1, into a pUC19 vector (Yanisch-Perron et al, 1985) modified to contain AscI, SacII, BshTI, XhoI and NotI sites to create plasmid pYC-AGS15. In order to introduce a loxP site into the SpeI site (chr2:156956377-156956382) distal to exon 2 (chr2:156955586-156955652), a BamHI-SacII (chr2:156954488-156960865) subclone of pYC-AGS15 was generated in a pBluescript-II-SK (Stratagene) vector and the loxP site created in the SpeI site using 5'-phosphorylated primers YC-loxPF and YC-loxPR. The resulting insertions were verified by sequencing and the modified BamHI-SacII fragment was transferred back into pYC-AGS15 to generate pYC-AGS15-lox211.

To provide a positive selection marker and to introduce a loxP site into the BamHI site (chr2:156954488-156954493) proximal to exon 2, the neo-loxP cassette from pL451 (Liu et al, 2003) (modified by replacing the KpnI-EcoRI polylinker with a BglII site) was excised by BamHI + BglII digestion and ligated into the BamHI site to create plasmid pYC-AGS15-lox211-neo4. The orientation of the insert was verified by sequencing.

A negative selection marker (DTA) was then inserted at the Samhd1-vector junction by transferring the NotI-SalI fragment from pPGKdta (Soriano, 1997) into

the NotI-XhoI sites in the polylinker to generate pYC-AGS15-lox211-neo4-DTA3. This high copy number plasmid proved to be unstable when grown in large volume culture and the Samhd1-DTA insert was excised by AscI-NotI digestion and transferred to a low copy vector derived from pACYC177 (Chang & Cohen, 1978) by replacing the BstEII-BamHI fragment (b3279-3320, accession X06402) with an AscI-NotI linker to generate pACYC-AGSKO5-3. High quality DNA was prepared using a Qiagen Maxiprep kit following the manufacturers instructions. AscI digestion was used to generate linearized DNA for electroporation into ES cells.

All restriction enzymes with the exception of BshTI (Fermentas) were purchased from New England Biolabs. Genome positions are based on the NCBI37/mm9 build.

ES cell work

Gene targeting of ES cells was performed using standard techniques (Robertson, 1987). Two ES cell lines were used: (i) PRX-B6N #1, a C57BL/6N derived ES cell line obtained from Primogenix Inc., and (ii) a hybrid (129S6, C57BL/6J) ES cell line (S6/B6 Clone 2 produced at Cancer Research UK London Research Institute - Clare Hall). 10^7 cells were electroporated in DMEM with 20 μg of linearized plasmids using voltage and capacitance settings of 240 V and 500 μF, respectively. ES cells were plated onto 5 six well plates containing a layer of fibroblast feeder cells and were grown in ES cell culture media (DMEM, high glucose, with sodium pyruvate containing 15% (v/v) FCS Fetal Calf Serum, 2 mM Glutamine, 100x nonessential amino acids, 10^{-4} M β-mercaptoethanol; 1000 IU/ml leukocyte inhibitory factor (LIF), 50 U/ml Pen/Strep). After 24 hours, the media was substituted with media containing geneticin sulphate (G418) to a final concentration of 300 μg/ml. Selection was maintained for 7 – 8 days after which G418-resistant

colonies were isolated and expanded for freezing and DNA analysis by long-range PCR and Southern blot to identify homologous recombinants.

Chimera generation and breeding

To generate chimeric male founders, a targeted ES clone (5D6) derived from the PRX-B6N #1 cell line was injected into albino C57BL/6N (Tyrc-Brd) host blastocysts. The targeted ES clone (1F8) derived from the S6/B6 ES cell line was injected into C57BL/6J blastocyst. Chimeras from the PRX-B6N #1 (5D6) cell line were crossed to C57BL/6N (Tyrc-Brd) mice. Chimeras from the S6B6 cell line (1F8) were crossed to C57BL/6J mice. In both cases the appearance of black offspring indicated germ line transmission. This was confirmed by PCR using DNA extracted from ear biopsies and oligonucleotides YC-BamHIF and Neo 3' sense.

Crosses and genotyping

Crosses to transgenic lines expressing Cre and FLP recombinase (both C57BL/6 genetic background) are described in Fig. S1A. The *Samhd1* WT allele was identified by PCR using oligonucleotides A1494 and A485, and the *Samhd1 ko(neo)* and *ko* alleles using oligonucleotides A465-A487 and A465-A485, respectively. A detailed laboratory protocol is available upon request.

Cells

BM-DCs were obtained from fresh bone marrow using GM-CSF as described (Robinson et al, 2009). BM-DMs were grown using the same protocol, except that M-CSF (Peprotech, 20 ng/ml) or 20% L929 supernatant was used instead of GM-CSF. If not indicated otherwise, BM-DCs and BM-DMs were used for further experiments on day 6. Spleen cells were prepared by digestion with 1 mg/ml collagenase IV (Worthington, cat. nb. LS004188) and 0.2 mg/ml DNase I (Roche, cat. nb.

11284932001), followed by red blood cell lysis (Sigma, cat. nb. R7757). For primary MEFs, we crossed *Samhd1* +/- and *Samhd1* -/- animals and obtained cells from embryos at day 13.5 using standard techniques. The genotype of these cells was determined by PCR. MEFs were immortalized as described (Schulz et al, 2010).

NIH3T3 cells and MEFs were grown in DMEM medium. THP1 cells, RAW264.7 cells, BM-DCs, BMDMs and spleen cells were grown in RPMI 1640 medium. All media contained 10% FCS, 2 mM glutamine, 100 units/ml penicillin and 100 mg/ml streptomycin. 50 mM 2-mercaptoethanol was additionally added to RPMI medium.

RT Q-PCR

RNA extraction from cells, reverse transcription and quantitative PCR have been described (Schulz et al, 2010). Mouse tissue samples were lysed in RLT-buffer (Qiagen, RNeasy mini kit) using glass beads (Sigma, G8772) and a FastPrep F120 instrument (Thermo; 6 m/s for 30 seconds or 2x30 seconds depending on tissue type). RNA was then extracted using the RNeasy mini kit (Qiagen, including Qiashredder and DNase treatment). Predeveloped Taqman assay reagents containing primers and fluorescent probe for murine *GAPDH*, *SAMHD1*, *IFIT1*, *IFIT2*, *IFI-44*, *IFNβ*, *IL-6* and *TNFα* (all from Applied Biosystems) were used for quantitative PCR. Alternatively, we used SYBR Green Q-PCR mix from Invitrogen (cat. nb. P/N 56717) to detect the following transcripts: murine *SAMHD1* (primers from Qiagen, cat. nb. QT00150241; one of the primers is located in exon 2); murine *IL-1β* (primers from (Nolte et al, 2007)); MusD (primers from (Karimi et al, 2011)); IAP (primers from (Oakes et al, 2010)); Mu-ERV-L (primers from (Macfarlan et al, 2011)); and m-poly-MLV (primers from (Yoshinobu et al, 2009)).

Antibodies, Western blot, immunofluorescence and FACS

Rabbits were immunized with recombinant, full-length and N-terminally 3xFLAG-tagged mSAMHD1 produced in and purified from insect cells using baculovirus technology. The resulting antiserum was used 1:100,000 in Western blot. α-hSAMHD1 and α-actin antibodies were from Abcam (cat. nb. 67820, 1:1,000) and Santa Cruz Biotechnology (cat. nb. sc-47778, 1:10,000). Secondary HRP-coupled antibodies were from Southern Biotech. For immunofluorescence, MEFs were grown in LabTekII chambers. Cells were fixed with 2% paraformaldehyde in PBS, permeabilized with 0.2% Triton X-100 in PBS and blocked with 5% FCS / 0.2% Triton X-100 in PBS for 90 min. The SAMHD1 anti-serum and control pre-immune serum were used 1:500 in blocking solution overnight at 4°C. Secondary goat-α-rabbit-Alexa633 antibody (Invitrogen A21072) was used 1:500 and DNA was stained with DAPI (1 μg/ml). Images were acquired using a Zeiss Axiovert S100 inverted microscope with an Orca-ER camera (Hamamatsu).

For FACS analysis, cells were resuspended in ice cold FACS buffer (PBS containing 2 mM EDTA, 0.02% sodium azide and 1% FCS) and, if required, stained using the following antibodies, alone or in different combinations: α-CD11c-PerCP-Cy5.5 (1:200; Biolegend cat. nb. 117328); α-B220-PE (1:400; BD Biosciences cat. nb. 553090); α-MHCII-eFluo.780 (1:200; E-Bioscience cat. nb. 47-5321-82); α-F4/80 (1:300; Biolegend cat. nb. 122610); α-CD11b-PerCP-Cy5.5 (1:300; BD Biosciences cat. nb. 550993); α-Gr1-PE (1:400; BioLegend cat. nb. 108408); α-NK1.1-Pe-Cy7 (1:200; E-Bioscience cat. nb. 25-5941-81); α-CD3 APC (1:200; BD Biosciences cat. nb. 553066); α-CD45-eFluo.780 (1:200; E-Bioscience cat. nb. 47-0451-80). Monoclonal antibody 83A25 was a kind gift from Stefan Bauer with

permission from Leonard Evans. PE-α-rat was from Jackson ImmunoResearch (1:200). DAPI (1 μg/ml) was added to the samples immediately prior to data acquisition on Beckmann Coulter CyAn or BD Biosciences LSR II or LSRFortessa cell analysers. Data from at least 2×10^4 and 10^6 cells were collected in *in vitro* and *in vivo* experiments, respectively.

IFN measurement

We employed four different approaches to detect IFN in serum or cell culture supernatant samples:

(i) ELISA for mouse IFNα (Schulz et al, 2010)

(ii) LL171 bioassay (Rehwinkel et al, 2010)

(iii) *IFIT1* mRNA induction in NIH3T3 cells. 50,000 NIH3T3 cells were seeded in 96-wells, incubated for 24 hours, and then exposed for 3 hours to 25 μl sample (for example, serum). *IFIT1* transcript levels were quantified by RT Q-PCR. IFN-A/D (kind gift from I. Kerr) served as a standard.

(iv) NIH3T3-ISRE-GFP bioassay. To generate an IFN reporter cell line, NIH3T3 cells were transduced with a lentivirus derived from pGreenFire-ISRE (System Biosciences), which expresses GFP in response to IFN. A stably transduced clone was selected and expanded. 25,000 NIH3T3-ISRE-GFP cells were seeded in 96-wells, incubated for 24 hours, and then exposed for 24 hours to 50 μl sample. The fraction of GFP-expressing reporter cells was then determined by flow cytometry and compared to an IFN standard.

Cell stimulation

25,000 MEFs were seeded in 24-well plates and, after 7 hours, transfected with Neo^{1-99} IVT-RNA (Rehwinkel et al, 2010), poly dA:dT (Sigma cat. nb. P0833) or *E. coli* DNA (USB, cat. nb. 14380) using lipofectamine 2000 (Invitrogen). After 24 hours, culture supernatants were analyzed for the presence of IFN using the LL171 bioassay.

BM-DMs were seeded in 96-well plates (400,000 cells per well), incubated over night, transfected with IVT-RNA or c-di-GMP (Biolog; cat. nb. C 057) using lipofectamine 2000, and culture supernatants were collected 6 hours after transfection. IFNα was detected by ELISA.

Depletion of SAMHD1 using shRNAs

BM-DCs were depleted of SAMHD1 using lentiviral shRNA delivery (target sequence CCCTCTCCTTATCAGAATCAT) as described previously (Robinson et al, 2009). On day 6, cells were transfected with water ("mock") or *E. coli* DNA using lipofectamine 2000, and RNA was extracted after 24 hours for RT Q-PCR analysis.

Viruses

EMCV was a kind gift from I. Kerr. Mo-MLV was produced as described before (Liberatore & Bieniasz, 2011). Ecotropic retroviral vectors expressing GFP (pFB-GFP and pMSCV-GFP) were made in Phoenix cells as described earlier (Sancho et al, 2009). VSV-G pseudotyped retroviral vectors that express GFP were produced in HEK293T cells transfected with three plasmids: the retroviral backbones pCNCG (MLV based) (Hatziioannou et al, 2003), pCSGW (HIV based) (Bainbridge et al, 2001) and pLL3.7 (Rubinson et al, 2003) were used in conjunction with p8.91

(lacking accessory genes) and pVSV-G; the HIV-1-based backbone pRRLsin.eGFP was used with pCMVΔ8.2 (encoding Vif, Vpr, Vpu and Nef accessory proteins) or pCMVΔ8.2 V148I and pVSV-G. The plasmids pRRLsin.eGFP, pCMVΔ8.2 and pCMVΔ8.2 V148I were a kind gift from Florence Margottin-Goguet. HEK293T supernatants were filtered (0.45 μm) and, if required, concentrated by centrifugation. Titers of concentrated stocks were determined by infection of HEK293T cells with a dilution series of virus stocks, followed by FACS analysis of GFP expression. A detailed laboratory protocol is available upon request. The pCSGW / p8.91 and pRRLsin.eGFP / pCMVΔ8.2 based lentiviral vectors are referred to as HIV-1-GFP in this manuscript.

***In vitro* infection models**

MEFs were seeded in 6-well plates and transduced in the presence of 8 μg/ml polybrene (Sigma) by adding different dilutions of supernatants collected from 293T cells producing retroviral vectors or by adding concentrated and titrated virus stocks. In the latter case, multiplicities of infection (MOIs) were calculated based on 293T infectious units and are indicated in the figures. After 3 hours, the cell supernatant was replaced with fresh medium and cells were incubated for 24-48 hours. GFP expression was analyzed by flow cytometry.

Fresh bone marrow, fresh spleen cells and differentiated BM-DCs and BM-DMs were all transduced by a single round of spin-infection in the presence of 4 μg/ml polybrene. Retroviral vectors were added to cells in 6-well or 24-well dishes, and plates were then spun at room temperature for 90 minutes at 2500 rpm. Supernatants were replaced with fresh medium after centrifugation. GFP expression was analyzed by flow cytometry after 24 hours (fresh spleen cells and differentiated

BM-DCs and BM-DMs) or after 6 days of GM-CSF culture (fresh bone marrow). Fresh bone marrow and fresh spleen cells were transduced using different dilutions of supernatants collected from 293T cells producing retroviral vectors. BM-DCs and BM-DMs were transduced with concentrated stocks of these vectors and MOIs were calculated based on 293T infectious units and are indicated in the figures.

***In vivo* infection models**

EMCV

Mice were infected with 10^6 plaque-forming units of EMCV in PBS by intraperitoneal injection. Serum was collected from animals sacrificed 24 hours after infection and IFNα was detected by ELISA.

Mo-MLV

Samhd1 +/- and *Samhd1* -/- animals were crossed and neonates were infected with Mo-MLV (Liberatore & Bieniasz, 2011) 1 day after birth (intraperitoneal injection of $2x10^5$ focus forming units in PBS). After 14 days, animals were sacrificed. DNA extracted from tail samples was used to determine the genotype by PCR. Virus titers in serum samples were determined using the following protocol. 8000 NIH3T3 cells were seeded in 96-well plates and infected with serum dilutions the next day in the presence of 4 μg/ml polybrene. After 48 hours, the cells were fixed with ice-cold acetone : methanol (50:50) for 5 hours at 4°C, washed 3 x with PBS, and stained for 2 hours with S/N from 1912 hybridoma cells (Chesebro et al, 1983) diluted 1:20 in PBS / 1% FCS. Cells were then washed 3 x with PBS, the secondary antibody was added for 1 hour (goat-α-mouse β-galactosidase conjugate; Southern Biotech cat. nb. 1010-06; 1:400 in PBS / 1% FCS), and cells were washed 3 x with PBS. Substrate solution (PBS containing 1 mg/ml X-Gal; 5 mM $K_3Fe(CN)_6$; 5 mM $K_4Fe(CN)6•3H_2O$; 2 mM

$MgCl_2$) was added and the number of blue foci (indicating the presence of Mo-MLV capsid protein and thus infection of the cell) was determined microscopically after over-night incubation at 37°C.

HIV-1-GFP

Mice were infected with 5×10^7 to 10^8 infectious units HIV-1-GFP by intravenous injection. 5 to 6 days post infection, animals were sacrificed, spleens collected and digested and splenocytes were analyzed by flow cytometry (Rowe et al, 2006).

Virus-like particles

VSV-G pseudotyped virus-like particles were produced by transfecting HEK293T cells with pSIV3+ (Vpx sufficient) or pSIV4+ (Vpx deficient)(Negre et al, 2000) and pVSV-G. HEK293T supernatants were filtered (0.45 μm). $5x10^5$ THP1 cells were seeded in 24-well plates and treated with 30 ng/ml PMA for 24 hours. VLPs were added to PMA-treated THP-1 cells or BM-DCs and BMDMs, and cells were spin-infected as above in the presence of 4 μg/ml polybrene. After 24 hours, cell lysates were prepared for Western blot analysis.

dNTP measurement

Extracts were prepared from BM-DCs and BM-DMs following a published protocol (Wilson et al, 2011). The single nucleotide incorporation (primer extension) assay was performed as described in the literature (Diamond et al, 2004) using extract from 1 million cells per reaction. A Typhoon 9400 phosphoimager (Amersham) was used to scan dried polyacrylamide gels. The fluorescence-based assay was carried out as published (Wilson et al, 2011) on a light cycler (ABI 7500, Applied Biosciences)

using extract from 1 million cells per reaction. Normalized fluorescence units were calculated by subtracting background values (H_2O only) from sample values after 20 cycles. A dTTP dilution series was used as a standard to determine the number of dTTP molecules per BM-DC sample. These values were converted into intracellular dTTP concentrations based on the cell volume of BM-DCs. This was determined to be 2244 μm^3 by measuring the average diameter of 100 cells by confocal microcopy and is based on the assumption that the cells are spherical.

Oligonucleotides

oligonucleotide	**sequence 5' -> 3'**
YC-1F	ACATGGTTCACTACAGTAGGTTC
YC-2R	TACAGTACAGGCTAACACAAGTC
YC-loxPF	CTAGATAACTTCGTATAATGTATGCTATACGAAGTTAT
YC-loxPR	CTAG ATAACTTCGTATAGCATACATTATACGAAGTTAT
YC-BamHIF	GACTTGTGTTAGCCTGTACTGTA
IL-1b F	CAACCAACAAGTGATATTCTCCATG
IL-1b R	GATCCACACTCTCCAGCTGCA
Neo 3' sense	GCTCCCGATTCGCAGCGCATC
A1494	TTTGGTTCTAGGCACTCTTCTG
A485	TCTCCTTATGGATGGTTGTAAGCC
A465	TCTGCATGGATTTCAGGATAGC
A487	ATCAGTCAGGTACATAATATAACTTCGT
MusD F	GTGCTAACCCAACGCTGGTTC
MusD R	CTCTGGCCTGAAACAACTCCTG
IAP-Forward	ATAATCTGCGCATGAGCCAAGG
IAP-Reverse	AGGAAGAACACCACAGACCAGA
MuERV-L F	ATCTCCTGGCACCTGGTATG
MuERV-L R	AGAAGAAGGCATTTGCCAGA
polyMLV F	CCGCCAGGTCCTCAATATAG
mpolyMLV R	CGTCCCAGGTTGTATAGAGG
XhoI-kozak-mSamhd1-F1	actcgagGCCGCCatgcagagcgcacccttggagcag
mAGS5-R 411	GATGATTCTGATAAGGAGAGGGTG
mSamhd1-F exon 2	TTTGGATGAGGATCGTCTGG
EcoRI-mSamhd1-isof1-R	agaattcTTAAAATTTTAGACATGTTTTTACT
EcoRI-mSamhd1-isof2-R	agaattcTTACAGCTGGTTGTGAGCCG

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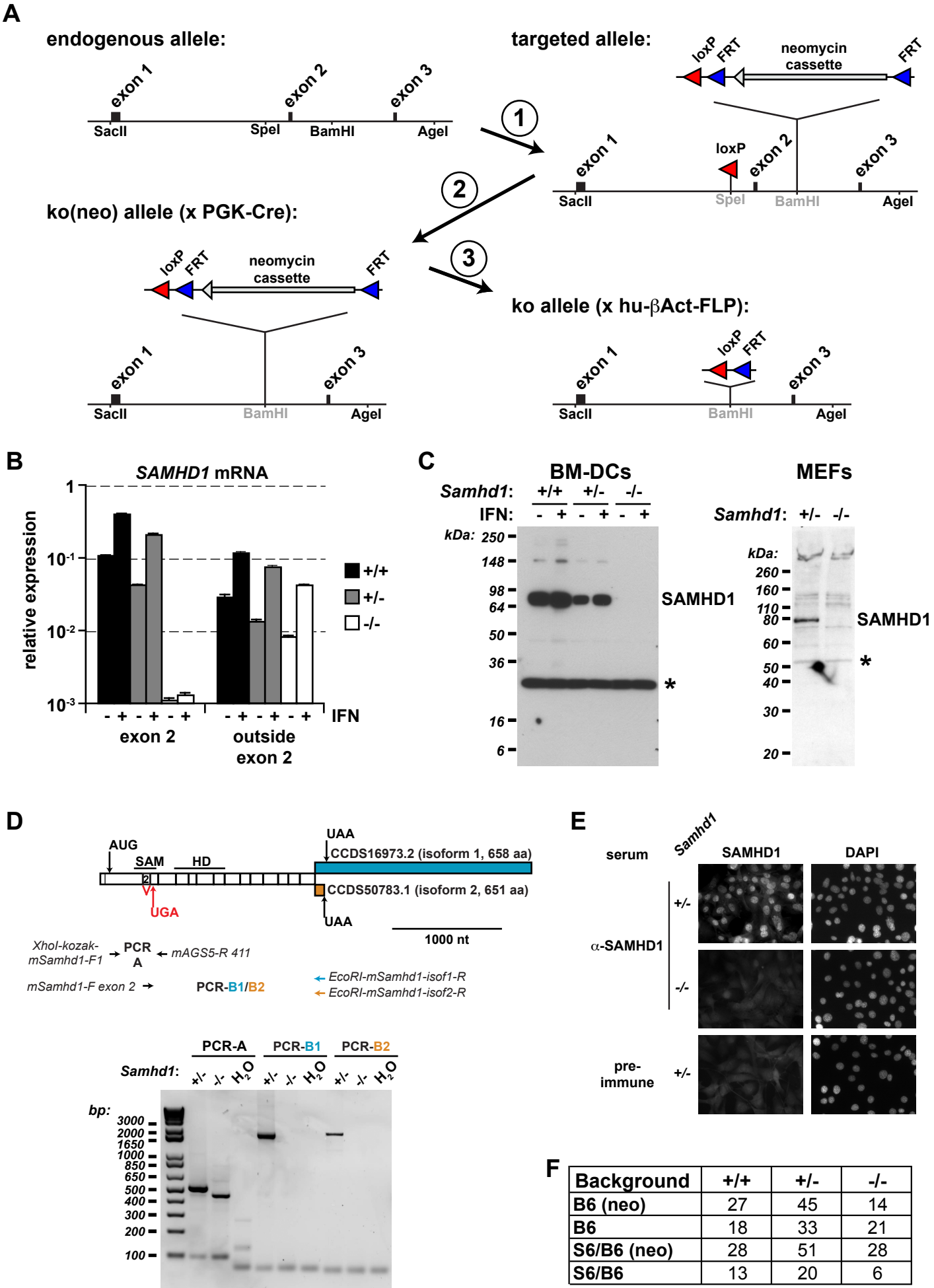
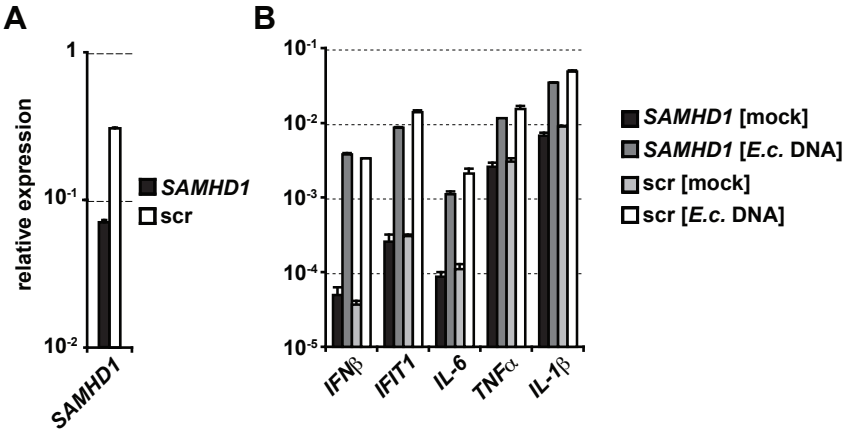
Supplementary Figure 1



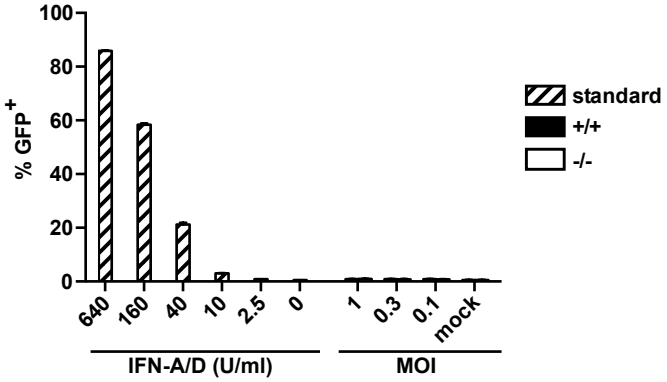
F

Background	+/+	+/-	-/-
B6 (neo)	27	45	14
B6	18	33	21
S6/B6 (neo)	28	51	28
S6/B6	13	20	6

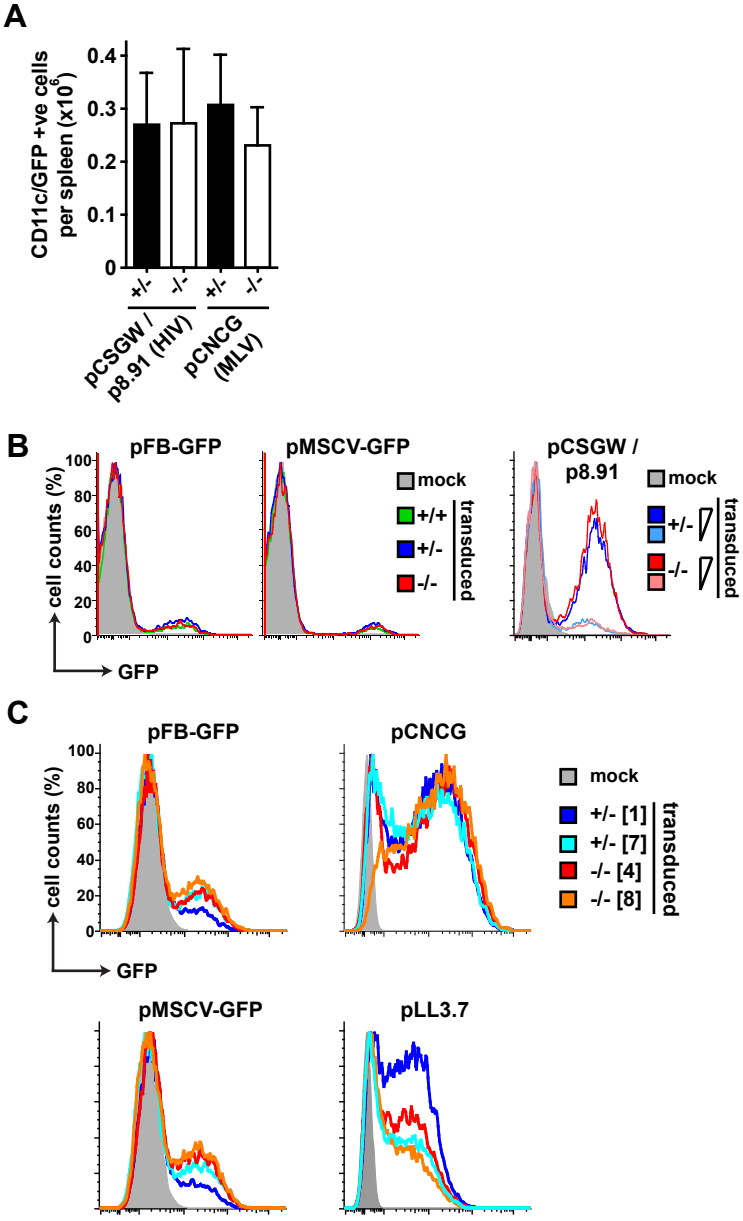
Supplementary Figure 2



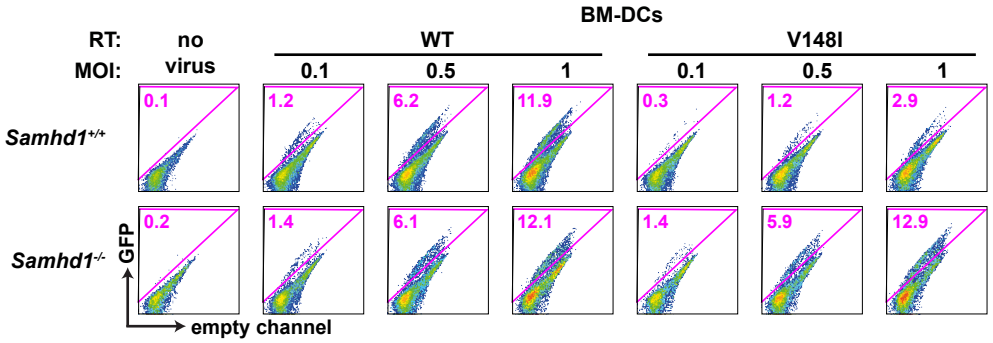
Supplementary Figure 3



Supplementary Figure 4



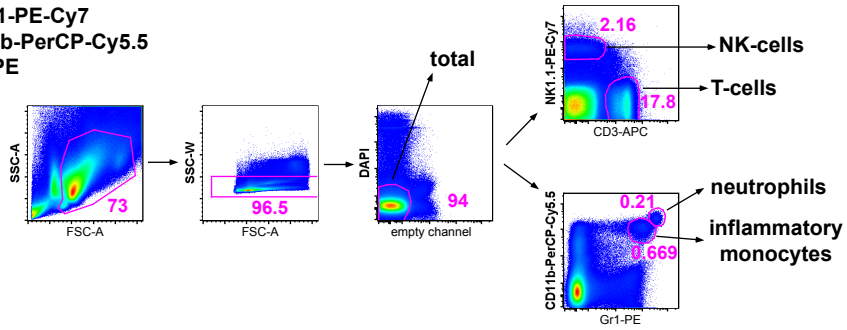
Supplementary Figure 5



Supplementary Figure 6

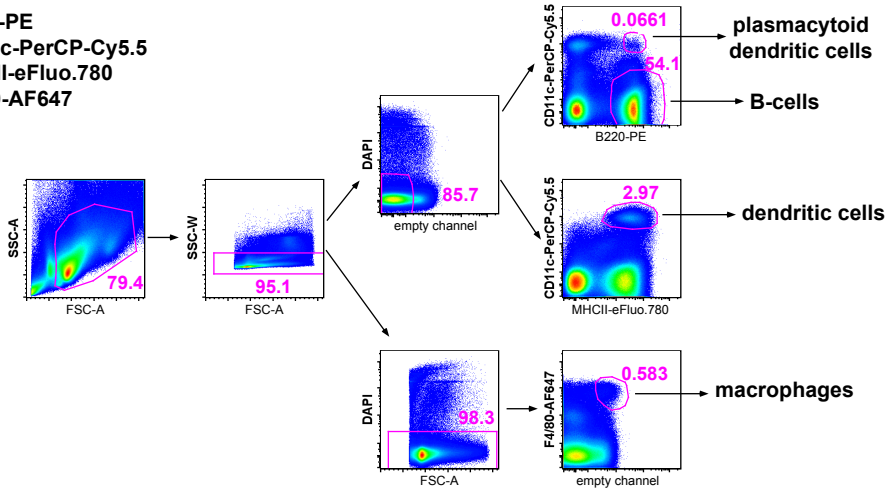
stain I

DAPI
CD3-APC
NK1.1-PE-Cy7
CD11b-PerCP-Cy5.5
Gr1-PE

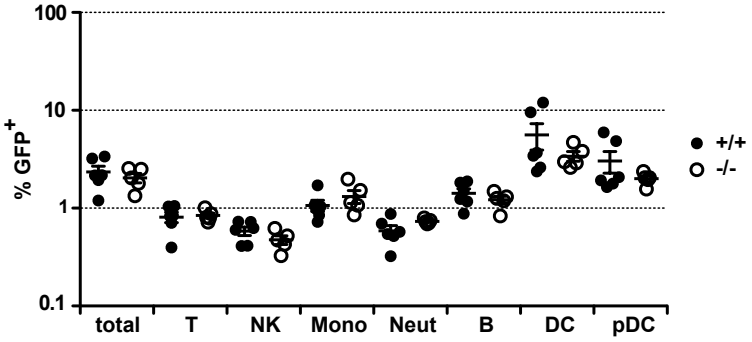


stain II

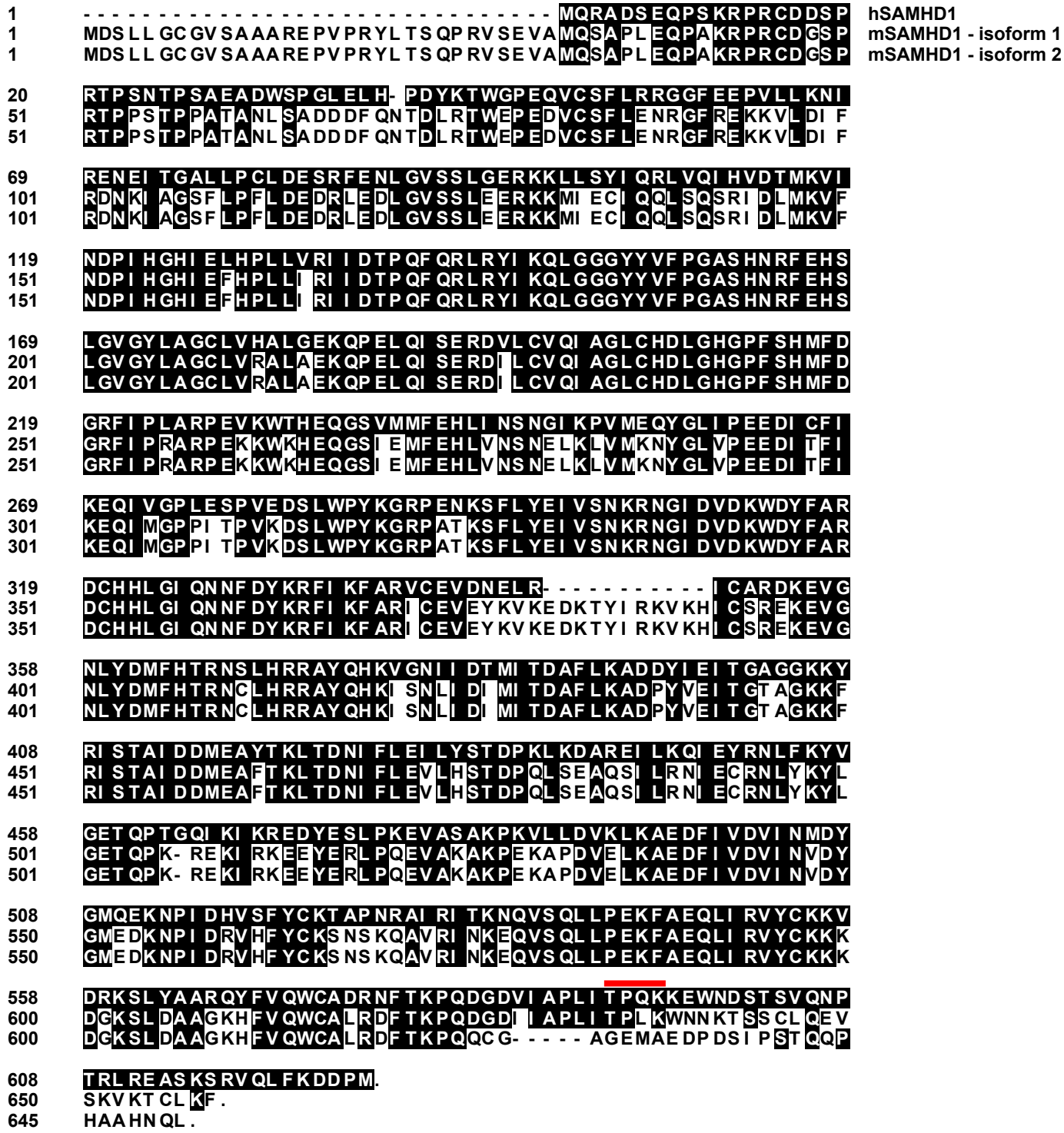
DAPI
B220-PE
CD11c-PerCP-Cy5.5
MHCII-eFluo.780
F4/80-AF647



Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9