Supplementary Materials for

Loss-of-function variants in *SAT1* cause X-linked Childhood-onset Systemic Lupus Erythematosus

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Materials and Methods

Whole exome sequencing and bioinformatics analysis

Genomic DNA was extracted from peripheral blood and processed using the Illumina TruSeq kit (Illumina, San Diego, CA, USA). Exome capture was performed using the TruSeq Exome Enrichment Kit, which targets ~62 Mb of the protein-coding and regulatory sequence of 20,794 genes in the human genome according to the manufacture's manual. The enriched libraries were size-checked and quantitated using the Agilent 2100 BioAnalyzer, and then subjected to 2x100 bp paired-end massively parallel sequencing using the Illumina HiSeq2000 sequencing system (Illumina, San Diego, CA, USA). Sequence reads were trimmed using Trimmomatic v0.36[1] and aligned to the GRCh37 human reference sequence using BWA-MEM v0.7.15[2]. Duplicate reads were marked and removed using Picard v2.9.0 (https://broadinstitute.github.io/picard/). Final alignments were generated after local indel realignment and base quality score recalibration using the Genome Analysis Toolkit (GATK) v3.7[3]. Variant calling and coverage analyses were carried out using GATK v3.7 and Samtools v1.3.1[4]. All detected variants were annotated using ANNOVAR v2017Jul16[5]. Only variants in protein-coding region and splice site variants were considered for further analysis. Variants with a minor allele frequency higher than 1% in the general population or any subpopulation in the Genome Aggregation Database (gnomAD) and 1000 Genomes Project were excluded. Variants were prioritized based on the transmission pattern (X-linked, autosomal recessive and de novo) and the predicted functional impact.

SAT1 Sanger sequencing

To confirm the two rare variants in the two whole-exome sequenced families and to explore additional rare SAT1 variants among SLE patients (a total of 562 SLE patients enriched in multiplex male lupus and pediatric patients), we used primer sets to PCR the Amplicon #1 (837bp) and Amplicon # 2 (1129bp) that covers 5'UTR to exon 1-3 region and exon 4-6 to 3'UTR region of *SAT1*, respectively (online supplementary figure 1D). Subsequently, the two resulting PCR products were resolved in an 1.5% agarose gel to purify the PCR products

using Zymoclean Gel DNA Recover Kit (Zymo Research) for standard Sanger sequencing (GENEWIZ). Primer sequences are shown in online supplementary table 11.

In silico functional studies

SIFT (Sorting Intolerant From Tolerant; http://sift.bii.a-star.edu.sg/), PolyPhen-2 (Polymorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph2/), LRT (Likelihood Ration Test Query; http:// http://www.genetics.wustl.edu/), PROVEAN (Protein Variation Effect Analyzer; http://provean.jcvi.org/), Meta-SVM (meta-analytic support vector machine; https://omictools.co/metasvm-tool/), Mutation Taster (http://www.mutationtaster.org/), Mutation Assessor (http://mutationassessor.org/) and FATHMM (Functional Analysis through Hidden Markov Models; http://fathmm.biocompute.org.uk/) were used to predict the functional effects that the *SAT1* missense variant (p.Asp40Tyr) may have on protein function. To evaluate the potential pathogenicity of the splice site variation, Human Splice Finder V3.1 (http://www.umd.be/HSF3/), an in-silico splice site prediction program, was used to analyze whether the variants (p.Asp40Tyr and rs900361022) could cause changes to the splice sites. We used the gene name of *SAT1* and mutants as input for the analysis. p.Asp40Tyr was predicted to result in a "Broken WT Donor site" and rs900361022 was predicted to result in a "Broken Branch Point site", which mean that the variants likely affect splicing.

Minigene assay

This assay is based on a modified protocol described[6]. Briefly, genomic DNA fragment containing either the Tyr40 or Asp40 SAT1 variant encompassing all of intron 1, exon 2, intron 2, exon 3 and intron, was amplified and cloned into the XhoI and BamHI site of the exon trapping vector pET01 (Boca scientific).

After Sanger sequencing to confirm the purified DNA construct contains the correct content and orientation, either the wild-type or the mutant mini-gene construct was transfected into the 293T and HeLa cell lines using FuGene 6 transfection reagent (Promega) following manufacturer's instructions.

Total RNA from exon trapping construct-transfected 293T and HeLa cells were reverse-transcribed and PCR-amplified. The resulting RT-PCR products from WT and mutant minigenes were visualized by gel electrophoresis, and their sequence composition was determined by Sanger sequencing (GENEWIZ). Primer sequences are shown in online supplementary table 14.

Structural model.

The molecular structure of human SSAT1 was retrieved from the Protein Data Bank (https://www.rcsb.org/) code PDB 2B5G5[7]. The transcripts 1, 2 and 3 of *SAT1* caused by p.Asp40Tyr were then submitted to the SWISS-MODEL server (https://swissmodel.expasy.org/) for model building.

Mice

The *Sat1*$^{p.Glu92Leufs*6}$ mouse was generated by CRISPR/Cas9-based gene manipulation in C57/BL6J (B6; Jackson Laboratory) mice at the MUSC Transgenic and Genome Editing Core and was backcrossed with C57BL/6J for at least two generations. Mice were maintained in specific-pathogen free conditions. Mouse experiments were performed according to governmental and institutional guidelines for animal welfare.

CRISPR/Cas9-mediated genome editing

To determine the functional significance of p.Glu92Leufs*6 mutation, we used CRISPR/Cas9-mediated genome editing to make a mouse model mirroring this TT duplication at the same position of mouse *Sat1*. Furthermore, to mimic the human mutant allele as closely as possible, we introduced a c.276A>G substitution in its mouse ortholog to yield a K93R substitution in the truncated protein, thus matching exactly the –LRTSS C-terminus of the mutant human *SAT1*. To protect against potential re-cutting of the already edited target sequence, two silent blocking mutations were introduced, i.e., c.255T>C and c.258C>G. The guide RNA (gRNA; tacagcaacttgccaatccangg) and single-stranded

oligonucleotide donor (ssODN;tcttctttgcatctcatgggatactctgattacaggacatagcattgttgggttcgccatgtactattttacctatgacccatggatCggGaagttgctgtatcttTTgaGgacttcttcgtgatgagtgattacagaggtacagttgactttgggattggaggtagtttaaggagaaatcagagctctttggcct) were designed and validated at the Genome Engineering and iPSC Center (GEiC), Washington University, St. Louis, MO and synthesized at Integrated Technologies, Inc (IDT); for design strategy and sequences, see online supplementary figure 4A. To make functional gRNA, corresponding crRNA was annealed to tracrRNA (IDT) by combining 5 µl of crRNA (1 µg/µl) with 10 µl of tracrRNA (1 µg/µl) in a PCR tube for incubation in a thermocycler (95 °C for 5 min followed by ramp down to 25 °C at 5 °C/min) as described[8]; the resulting ctRNA duplexes (gRNA) were stored at -70 °C until use. Delivery of CRISPR reagents into single-cell C57BL/6J mouse embryos was performed by pronuclear injection of single-cell embryos essentially as described[9]. Prior to microinjection (MI), annealed MS1088.Sat.sp9 ctRNA (12.5 ng/µl) was combined and complexed with Alt-R™ s.P. Cas9 nuclease (IDT) at a concentration of 25 ng/µl in MI buffer (10 mM Tris-HCl, pH 7.4; 0.25 mM EDTA) at room temperature for 10 min. Subsequently, the concentrations were adjusted by 5-fold dilution with MI buffer on ice and the addition of ssODN (50 ng/µl) (MS108.Sat1.InsTT/AtoG.anti.PTO); accordingly, the final concentrations in the MI cocktail were 2.5 ng/µl (sgRNA), 5 ng/µl (Cas9), and 10 ng/µl (ssODN). Successful targeting and faithful editing were achieved as determined by polymerase chain reaction and sequence analysis of DNA extracted from tail biopsies of pups derived from injected embryos.

Autoimmune mouse model

For apoptotic thymocyte-induced autoimmune model, thymocytes from 10-week-old WT mice were collected, and single-cell suspensions were exposed to 2 mJ/cm2/s of radiation in a UVP lamp (CL-1000s, UK) followed by 2 h of incubation at 37 °C in RPMI 1640 plus 1% BSA (Gibco). Flow cytometry revealed that > 95% cells were apoptotic, as determined by annexin V and 7-amino-actinomycin D (7-AAD) staining (Biolegend). WT or *Sat1*$^{p.Glu92Leufs*6}$

variant mice were immunized with apoptotic thymocytes (10^7 per mouse) through four weekly tail vein injections.

High-throughput colorimetric in vitro assay of SSAT1

The assay is based on the protocol of Lin et al[10]. Briefly, liver homogenates of 5-week-old WT and KI male mice in 25mM tris-HCL and 0.5 mM EDTA, PH 7.5 (Sigma-Aldrich) were prepared using an ultrasonic homogenizer, adjusted to 25 μg/ml protein using a bicinchoninic acid (BCA) protein assay as the substrate. Substrate solution contained 50 mM Tris-HCl, 1 mM EDTA, 0.5 mM acetyl-CoA and variable concentrations of spermine/spermidine, pH7.5; enzyme solution contained 50 mM Tris-HCl, 1 mM EDTA and variable concentrations of enzyme, pH7.5; Ellman's reagent contained 50 mM Tris-HCl, 1 mM EDTA, 2 mM DTNB, pH7.5. Each reaction contained 25 μl enzyme solution mixed with 25 μl substrate solution in a 96-well microplate, reactions times were varied at 30°C and stopped by heating at 85°C for 5 min, cooled down to 4°C for 5 min, followed by addition of 50 μl Ellman's reagent, and kept at room temperature for 30 min. The released CoA was measured at OD412 nm with a BioTek plate reader.

RNA isolation and qPCR

Total RNA was isolated from spleen single cells by Trizol reagent (Invitrogen) according to the standard protocols. cDNA was prepared by reverse transcription-PCR (RT-PCR) with High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Thermo Fisher) following manufacture's protocols. SYBR Green PCR Master Mix (Thermo Fisher) was used for quantitative PCR (qPCR) and each group was detected in triplicate. Primer sequences are shown in online supplementary table 11.

Western Blot

Briefly, the harvested cells were washed twice with cold PBS, resuspended in RIPA buffer (Thermo Fisher) on ice for 30 min in the presence of complete Mini Protease Inhibitor tablet

(Roche), and then centrifuged (13,000g for 10 min at 4 °C). Protein concentration was estimated using the BCA Kit (Thermo Fisher) following the manufacturer's instructions. Each sample was separated on 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Thermo Fisher) and transferred to a polyvinylidene fluoride membrane (PVDF) (Thermo Fisher). After the membrane was blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) (Thermo Fisher), the membrane was incubated for with various primary antibodies specific for SSAT1 (61586, Cell Signaling Technology), LC3, p62, LAMP1 or β-actin (3700, Cell Signaling Technology) overnight at 4°C, respectively. After washing in TBST, the membranes were incubated for 1 h at room temperature with poly (HRP)-conjugated anti-rabbit or anti-mouse IgG (Thermo Fisher). The reaction products were visualized with a Super Signal West Pico PLUS Chemiluminescent Substrate (Thermo Fisher) and a Luminoimage analyzer (Fluor Chem R system). Protein expression was quantified by densitometric analysis with ImageJ software (NIH, USA).

Flow cytometric analysis

Single-cell suspensions in MACS buffer (PBS, 1%FBS, 5mM EDTA) (eBioscience) were prepared before staining. Primary cells from mouse spleen or bone marrow mononuclear cells were first blocked with anti-mouse FC blocker (BD Bioscience) in phosphate buffered saline/0.5% bovine serum albumin/5mM EDTA for 10 minutes at 4°C, were incubated with various specific monoclonal antibodies. Cells were washed and re-suspended to the proper density with MACS buffer for LSRFortessa X-20 (BD Bioscience) analysis.

For surface staining, cells were isolated and analyzed by staining for 15 min at room temperature in PBS supplemented with 0.5% (v/v) human serum using the following fluorophore-conjugated antibodies in online supplementary table 10.

For intracellular transcription factor and cytokine staining, cells were fixed with Foxp3 Fix/Perm Buffer (eBioscience) washed with Perm Buffer (eBioscience) and stained with intracellular antibodies or stimulated by phorbol 12-myristate 13-acetate (PMA, Sigma) and

ionomycin (Sigma) and fixed with Intracellular (IC) Fixation Buffer (eBioscience), washed with Perm Buffer, and stained with antibodies in online supplementary table 10, respectively. For detection of cellular apoptosis, FITC Annexin V Apoptosis Detection Kit with 7-90HD (Biolegend) and CellTrace Kit (Invitrogen) were used, following the manufacturer's instructions. Cell acquisition was performed on LSR Fortessa X-20 (BD Bioscience), and data was analyzed using FlowJo software (Tree Star).

For detection of ANA+ autoreactive B cells, they were performed similarly as described previously[11,12]. Briefly, nuclei from HeLa cells were isolated with the Nuclei EZ Lysis Kit (Sigma, St Louis, Mo), fragmented by vertexing with 0.5-mm cell disruption glass beads (Scientific Industries, Bohemia, NY), and biotinylated with EZ-Link-Sulfo-NHS-LC-biotin (Thermo Scientific, Waltham, Mass). Next, staining with 10 μl of biotinylated nuclear extract was done in 1.5% nonfat dry milk (Thermo Fisher) in HBSS (Gibco) or in permeabilization buffer for intracellular staining (eBioscience). The cells were spun down, resuspended, and stained for 15 minutes on ice with various specific monoclonal antibodies (BV421-conjugated streptavidin for biotinylated nuclear extract, Biolegend). The percent - age of allophycocyanin-positive cells in the streptavidin control was subtracted for each B-cell population to obtain the percentage of ANA+ autoreactive B cells.

Detection and scoring of antinuclear autoantibodies (ANA)

ANA was tested by immunofluorescence using human epithelial (Hep-2) cells on 12-well slides (Diasorin Inc). Mice sera were diluted 1:5 and one sample from a mouse with a previously determined high ANA score and one with a low ANA score were used on the same slide to confirm the sensitivity and specificity of the test during each scoring. ANA scores were evaluated by two experienced Rheumatology specialists, unaware of the autoimmune phenotypes.

Histology and Immunohistochemistry of kidney lesions

For mice, half of the kidney was isolated and fixed in histological 10% formalin solution fixative (Sigma-Aldrich) and embedded in paraffin. Three micrometer sections of the kidney tissue were cut, and images observed for various morphologic lesions after hematoxylin-eosin (H&E) staining were collected on an Olympus BX51 microscope.

Renal or spleen tissues were embedded in Tissue-Tec OCT medium, frozen in liquid nitrogen, and stored at -80℃ until sectioning. Renal slides were stained with goat anti-mouse IgG (1:150 dilutions; Abcam, Cambridge, UK) or goat anti-mouse complement C3 (1:150 dilutions; Abcam, Cambridge, UK). Images were acquired on Zeiss 880, processed, and analyzed using Zen (Carl Zeiss) software and fluorescence intensity was quantified using ImageJ software.

Measurement of autoantibodies, albumin, creatinine, and cytokines

Serum levels of IgG anti-double stranded DNA (IgG anti-dsDNA; Alpha Diagnostic, Catalog 5110) and IL-17A (RayBiotech, Catalog ELM-IL17-1) were measured using commercial ELISA kit, according to the manufacturer's protocol. Serum BUN (StressMarq Biosciences, Catalog SKT-213-192) and creatinine (Abcam, Catalog ab65340) were measured according to the manufacturer's protocol. Urine mouse albumin was detected using the Mouse Albumin ELISA kit (Bethyl Laboratories, Catalogue E99-134) according to the manufacturer's instructions.

Neutrophil isolation

Bone marrow neutrophils were isolated using a modified protocol of a Percoll-based density gradient as described before[13]. Briefly, femurs and tibias were harvested from mice of different groups. Bone marrow was rinsed with a syringe filled with medium and collected in a tube, and single-cell suspensions were generated. Centrifuge the bone marrow suspension at 250 × g for 5 min at room temperature. Layer each dilution of Percoll (52%, 64%,72%) onto the one beneath in a sterile 15 mL conical tube. Start with 2 ml of the 72% Percoll dilution, followed by 2 ml of the 64% dilution and then 2 ml of the 52% dilution, 2 mL cell

suspensions on the top using a 2 mL serological pipette. Centrifuge the gradient at 2000g for 30 min at room temperature. Next, carefully remove the first (top) and second cell layers (between the top and 52% and between 52% and 64% layers) and then remove the third (neutrophil) layer and transfer the cells to a clean 15 mL conical tube. Repeat this procedure with a clean transfer pipette until the third layer is entirely transferred, or the layers begin to mix. The neutrophils are ready to use in the following experiments.

NET formation

BM isolated neutrophils were seeded onto µ-Dish coated with poly-l-lysine (Sigma-Aldrich). Then neutrophils were incubated in RPMI-1640 supplemented with L-glutamine and 10% FBS. In experiments with either no stimulation or 100 nM PMA (Sigma-Aldrich), incubation was for 24 hours at 37°C. Cells were fixed with 4% Paraformaldehyde (PFA) and then blocked with 10% Bovine serum albumin (BSA); cells were not explicitly permeabilized. DNA was stained with Hoechst 33342 (Invitrogen). Protein staining was with rabbit polyclonal antibodies to neutrophil elastase (Abcam), signals were detected on Zeiss 880 confocal microscope (Carl Zeiss AG). At least 15 random fields were selected from each of the three independent experiments.

Phagocytosis assays

The phagocytosis assay was performed similarly as described in Linyu et al[12]. BM-isolated neutrophils were cocultured with AC for 30 or 60 minutes at 37°C. Slides were then washed 3 times with sterile phosphate-buffered saline (PBS, Gibco) to remove any undigested AC and fixed using 4% paraformaldehyde prior to confocal microscopy. Neutrophils were labeled by CD11b (Bioscience).

Autophagic flux assay

For autophagic flux assay, bone marrow-isolated neutrophils were stained by adding the Autophagy Tandem Sensor RFP-GFP-LC3B Kit (Invitrogen) to the cells in RPMI-1640

supplemented with L-glutamine and 10% FBS and cultured at 37°C ≥16h. Then neutrophils were stimulated by PMA for 4 hours. Slides were then washed three times with sterile phosphate-buffered saline (PBS, Gibco). Finally, slides were mounted with Hoechst 33342 and image the cells using the appropriate instrument filter sets for GFP and RFP detection on Zeiss 880 confocal microscope (Carl Zeiss AG). At least 15 random fields were selected from each of three independent experiments and were subjected to quantitative analyses of the mean fluorescence intensity (MFI) of puncta positive for GFP and RFP.

Quantitative real-time PCR of mitochondrial and chromosomal genes

The assay is based on the previous reports of Lood C et al[14]. Briefly, bone marrow-isolated neutrophils (5×10^5 cells/ml), stimulated with PMA (Sigma), were cultured for 4 h. After addition of micrococcal nuclease (NEB) for 30 min at 37 °C, cell-free supernatants were incubated with anti-8-OHdG antibodies coupled to protein G beads (Santa Cruz) at 4 °C overnight. After centrifugation and extensive washing, non-8-OHdG DNA (supernatant) as well as 8-OHdG+ DNA (bound to antibody-bead complex), were treated with proteinase K (Thermo Scientific) for 4 h at 56 °C. 8-OHdG+ DNA and non-8-OHdG DNA were isolated by phenol chloroform extraction as described previously[14], and were mixed with SYBR green master mix (Thermo Fisher) and primers (50 nM) for 16S (Forward, 5'-GGATAACAGCGCAATCCTA-3'; Reverse, 5'-GATTGCTCCGGTCTGAACTC-3') or 18S (Forward, 5'-GTAACCCGTTGAACCCCATT-3'; Reverse, 5'-CCATCCAATCGGTAGTAGCG-3') at final volume of 20 ul, respectively. Activation of enzyme at 95°C for 15 min was followed by 40 cycles with 95°C for 15 s and 60°C for 60 s.

Participants and sample collection

A total of 26 treatment-naïve, newly diagnosed SLE patients and 20 HCs were included in this study.

All SLE patients met at least 4 of the 11 American College of Rheumatology (ACR) criteria for the classification of SLE[15]. SLE activity was evaluated according to SLE Disease Activity

Index (SLEDAI) scores. Blood samples were collected from patients with SLE and from HCs, and cells were removed from plasma by centrifugation for 5 minutes at 2000 rpm at 20℃ within 10 minutes. Following centrifugation, the supernatant plasma was harvested and stored at -80℃. Information about clinical symptoms, and physical examination were registered in a database when serum sampling was performed. This study was approved by the Ethical Committee of the First Affiliated Hospital of Nanjing Medical University, and all donors provided written informed consent.

Sample preparation and LC/MS analysis

Aliquots of 100μl of serum and 100μl of internal standard were transferred to a LoBind microcentrifuge tube (Eppendorf) and vortexed for 30 s. For protein precipitation, 400μl and 1% formic acid methanol (Sigma) was added to precipitate the protein, vortexed at 2000 rpm for 3 minutes, centrifuged at 4°C for 5 minutes (12000 rpm), and the supernatant was transferred to a new tube. The supernatant was mixed with 300μl of 0.1% formic acid in Acetonitrile (Merck). The mixture was incubated at room temperature for 1 h. LC-MS/MS analysis were then performed on a SCIEX Exion LCTM 20 AD system connected a mass spectrometer AB Sciex 6500 QTRAP (AB SCIEX, Foster City, CA, USA).

For spermine, chromatographic separation was performed on an Acquity UPLC r HSS T3 column (1.8 μm; 2.1 mm~100 mm) (Waters, USA). The gradient employed was 0~2 min, 3% B; 2~4 min, 3~60% B; 4~6 min, 60~97% B; 6~8 min, 97% B; 8~8.1 min, 97~3% B, with the mobile phases being 0.5% formic acid in water (Solvent A) and 0.1% formic acid in Acetonitrile (Solvent B). The flow rate was 0.3 mL/min, the injection volume was 5 μL and the column temperature was set at 40 ℃.

For the other polyamines (except spermine), chromatographic separation was performed on an Acquity UPLC BEH Amide column (1.7 μm; 2.1 mm~100 mm) (Waters, USA). The gradient employed was 0~2 min, 95% B; 2~10 min, 95~60% B; 10~14 min, 60% B; 14~14.01 min, 60~95% B; 14.01~16 min, 95% B, with the mobile phases being 100 mM

ammonium acetate (Solvent A) and acetonitrile (Solvent B). The flow rate was 0.3 mL/min, the injection volume was 2.5 μl and the column temperature was set at 40 °C.
The triple quadrupole operated in the positive electrospray ionization mode (ESI+). Ion source settings were set as followed: ion source voltage floating of 4500 V, ion source temperature of 450°C, curtain gas of 25 psi, nebulizer gas and auxiliary gas of 50 psi. Multiple reaction monitoring (MRM) is used for scanning. Quantitative determination was performed using the multiple reaction monitoring (MRM) mode, and the transitions for each compound are detailed in online supplementary table 9.

Plasma cell-free DNA concentration

The cell-free DNA levels of plasma samples were measured by Quant-iT™ PicoGreen® dsDNA Reagent and Kits (Invitrogen) according to the manufacturer's instructions, with slight modifications. Briefly, 3 μl of patient's plasma was added into each micro-well containing 100 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5) followed by addition of 100 μl of PicoGreen working solution. Reaction mixture was dark incubated for 5 min and fluorescence was measured (at 485 nm excitation, 535 nm emission wavelengths) using a fluorescence microplate reader (Perkin Elmer Wallac 1420). Each sample was performed in duplicate. The absolute concentration of the cell-free DNA was calculated by using a low-range standard curve, which consisted of five different dilutions of genomic DNA (Applied Biosystems, cat #4312660, #360486; 250, 1000, 2500, 5000 and 10,000 pg/μl).

Statistical analysis.

GraphPad Prism 9 software (GraphPad Software, San Diego, CA) was used to assess statistical significance by applying two-tailed unpaired t-tests and two-tailed Mann–Whitney tests as described in the figure legends. Correlations between polyamine metabolites and SLE disease activities were evaluated using partial correlation. *P* values < 0.05 were considered statistically significant.

Supplementary figure 1

Genomic map, exon structure, exome sequencing coverage and Sanger sequencing approach of *SAT1*.

(A) Genomic map, exon structure of *SAT1*. Below the ideogram of the human X chromosome a genomic region of *SAT1* located at Xp22.11 is shown. This figure was adapted from the UCSC Genome Browser.

(B) Exon structure of different transcripts of *SAT1* and their mean expression levels across all tissues. Transcript expression was calculated from GTEx v7 RNA-Seq data. *Denotes the Ensemble canonical transcript of *SAT1* and RefSeq transcript NM_002970 of *SAT1* from NCBI annotations.

(**C**) Sequencing depth (per base) at *SAT1* and locations of known loss-of-function (LOF) *SAT1* variants are depicted. Most known LOFs located in coding regions not present in the highly expressed transcripts in the gnomAD population database, except p.Asp99_Tyr100insTer variant that has a minor allele frequency of 0.0005%, 1/183495 individuals who is an African-American. Each exon of *SAT1* is well sequenced in gnomAD with an average 50X sequencing depth. This figure was adapted from the gnomAD browser.

(D) PCR amplification of *SAT1* for Sanger sequencing. The 2-kb coding region of *SAT1* was amplified by two PCR reactions (PCR products P1and P2) for bidirectional sequencing.

Supplementary figure 2

Evolutionary conservation and computational prediction for functional impact of p.Asp40Tyr and p.Glu92Leufs*6.

(A) Alignments of multiple vertebrate species at p.Asp40Tyr and p.Glu92Leufs*6. This figure was adapted from the UCSC Genome Browser.

(B) Assessment of the functional impact of p.Asp40Tyr. The missense variant p.Asp40Tyr is predicted to be deleterious using Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen-2), Functional Analysis through Hidden Markov Models (FATHMM), LRT, Mutation Taster, Mutation Assessor, PROVEAN, and MetaSVM.

Supplementary figure 3

Sanger sequencing chromatograms depicting the aberrant transcripts produced by variant c. 118G>T (p.Asp40Tyr) in family #1

(A) A single wild-type Asp40 containing transcript, which consists of pET01 exon V1, exon 2, exon 3 and pET01 exon V2, is spliced as expected. The three isoforms of variant Tyr40 containing transcripts include Transcript 1 that shows complete skipping of exon 2, Transcript 2 that shows normally splicing with p.Asp40Tyr containing, and Transcript 3 that shows intron 2 retained, includes pET01 exon V1, exon 2, intron 2, exon 3, and pET01 exon V2. c.118G>T (p.Asp40Tyr) variant is indicated by a red line. Vector exons, V1 and V2, are depicted as black boxes, intron 2 of SAT1 is in orange, and exons 2 and 3 of SAT1 are in gray. TAG stop codon is underlined (star symbol).

(B) Sanger sequencing-verified transcripts 1, 2 and 3 represent the skipped exon 2, normally sliced, and the intron 2 retained transcript from Tyr40-containing transfected construct indicated by red dashed lines. Tyr40 variant is indicated by a red line. Vector exons, V1 and V2, are depicted as black boxes, intron 2 of *SAT1* is in orange, and exons 2 and 3 of *SAT1* are in gray. Vector exon-specific primers are indicated by black arrows.

(C) Schematic representation of protein models of WT and p.Asp40Tyr *SAT1* transcripts. N^1-acetyltransferase domain of SSAT1 protein is shown in blue; Red star symbol depicts the termination code (TAG); Missense and insertion mutations are shown in the schematics in orange.

Supplementary figure 4

CRISPR-Cas9 mediated generation of a *Sat1*$^{p.Glu92Leufs*6}$ KI C57BL/6J mouse model.

(A) The design strategy, and sequences of guide RNA and ssODN.

(B) Chromosome locations and genomic positions of the gene editing target and two predicted potential off-target sites that were not detected in the founders.

(C) Breeding schemes of wild type (WT) and knock in (KI) male littermates, as well as heterozygous (HET) and KI female littermates for experiments. Blue box denotes male WT and KI littermates; Red box denotes female WT, HET, and KI mice.

(D) Sanger sequencing data of founders and genotype results of WT, HET and KI mice.

(E) Diagrammatic timeline representation of either spontaneous or apoptotic cell-induced lupus-like disease development in WT or *Sat1*$^{p.Glu92Leufs*6}$ KI littermates. WT, wild type; KI, knock in. AC, Apoptotic cells.

Supplementary figure 5

Effect of p.Glu92Leufs*6 variant on expression of *Sat1* and SSAT1 and activity of SSAT1 activity.

(A and B) Relative expression levels of *Sat1* mRNA by RT-qPCR (**A**) and SSAT1 protein (**B**) by Western blot using splenocytes from 10-week-old male WT and KI littermates with or without DENSpem (a polyamine analog) stimulation. Data are mean ± SD. Mann-Whiney U test.

(C) Detection of the SSAT1 activity in liver cell homogenates from 10-week-old male WT and KI littermates by colorimetric assay. N1, N11-diethylnorspermine (DENSpm), 10µM for 24 hours; WT, wild type; KI, knock in; M, marker. Data are mean ± SD. Mann-Whiney U test.

Supplementary figure 6

Phenotype of *Sat1*$^{p.Glu92Leufs*6}$ male littermates.

(A and B) Representative image of spleens (**A**), body weights, and changes in body weights (**B**) at different ages of male WT and KI littermates. Open circle: WT; closed circle: KI.

(C) Representative image of serum ANA staining of Hep2 cells and ANA Scores from 5 to 52-week-old male WT and KI littermates. ANA Scores were blindly evaluated by two Rheumatology specialists. Bar: 50μm.

(D) Serum IL-17A levels in sera of 5 to 52-week-old male WT and KI littermates.

(E) Relative expression levels of type I interferon stimulated genes (ISGs) in splenocytes of 5- to 52-week-old WT and KI male littermates. Black: 5-wk-old; Purple: 10-wk-old; Green: PBS-treated 20-wk-old; Red: AC-treated 20-wk-old groups. Blue: 52-week-old. PBS, phosphate buffer saline; AC, apoptotic cell. Data are mean ± SD. Mann-Whiney U test.

Supplementary figure 7

Phenotype comparison among female WT, HET and KI mice injected with either PBS or apoptotic cells.

(A and B) Representative image of spleen and spleen index (**A**), and body weight change (**B**) in 20-week-old WT, heterozygote (HET) and KI female mice. Open circle, WT; Semi-solid circle, HET; Closed circle: KI; black: PBS treated 20-wk-old; Red: AC treated 20-wk-old groups. * Compared with AC-HET group. Data are mean ± SD. Mann-Whiney U test.

(C to E) Proteinuria (**C**), representative image of hematoxylin-eosin staining of kidney section (**D**), calculated type I IFN scores (**E**) in 20-week-old female WT, HET, and KI mice. Bar: 50μm.

(F) Pearson correlation of IFN scores with levels of proteinuria, serum anti-dsDNA and blood urea nitrogen (BUN) in splenocytes of 20-week-old KI mice treated with PBS or AC.

(G) Relative expression levels of ISGs in splenocytes of 20-week-old WT* and KI female mice. ISGs, IFN-simulated genes. Green: PBS-treated 20-wk-old; Red: AC-treated 20-wk-old groups. * Denotes WT female mice were chosen from other cages of similar dates of birth with the KI female mice.

(H) Levels of IgG, anti-dsDNA, and BUN in serum samples from 20-week-old WT and KI female mice. Data are mean ± SD. Mann-Whiney U test.

Supplementary figure 8

Genotypic and sex differences in responses to AC treatment between *Sat1*$^{p.Glu92Leufs*6}$ male and female mice.

(A) Representative image of hematoxylin-eosin-stained kidney sections in 20-week-old male and female WT and KI mice treated with either PBS or apoptotic cells. Bar: 50μm. (**B to G**) Levels of proteinuria, IFN-Score (**B**), serum levels of BUN, IgG anti-dsDNA and IL-17A (**C**) in spleen cells from 20-week-old WT and KI male and female mice. Frequencies of plasmacytoid dendritic cells (pDC), macrophage cells (**D**), proportions of Foxp3-related subsets, including $CD4^+Foxp3^+$ T, $CD4^+Foxp3^+CD25^+$ Treg, $CD4^+Foxp3^+CXCR5^{++}PD\text{-}1^{++}$ Tfr cells, $CD4^+Foxp3^-CXCR5^-PD\text{-}1^{++}$ Tph, $CD4^+Foxp3^-CXCR5^{++}PD\text{-}1^{++}$ Tfh cells, and Tfh/Tfr ratio (**E**), ANA^+ autoreactive B cell subsets, and $CD19^+CD11C^+CD21^+$ Age-associated B cells (**F**), $CD19^+CD23^{low}CD21^{high}$ marginal zone (MZ), $CD19^+CD23^{hi}CD21^{int}$ Follicular, $CD19^+CD95^{hi}GL7^{hi}$ Germinal center (GC), $CD19^+CD23^{hi}CD21^{int}$ plasmablast B cells, plasma cells (**G**), in splenocytes of 20-week-old male and female mice treated with PBS or AC. PBS, phosphate buffer saline; AC, apoptotic cells; Blue: male littermates; Red: female mice; Circle, WT; Solid dot, KI. Data are mean ± SD. *P*-values in green for sex differences of the same genotypes; *P*-values in black for genotypic differences. Mann-Whiney U test.

Supplemental figure 9

Perturbed development of Foxp3-related T cell subsets in naïve 5- to 10-week-old male KI mice.

(A and B) Representative gating strategy and proportion of T cell subsets in thymocytes from 5 or 10-wk-old male WT and KI littermates, including $CD4^+CD25^+$ T cells and $CD4^+CD25^+Foxp3^+$ T cells. Mann-Whiney U test (left). Unpaired *t*-test (right). Data presented as mean ± SD.

(C) Gating strategy and percentage of splenic $CD4^+$ T cell subsets including $CD4^+Foxp3^+$ cells, $CD4^+CD25^+Foxp3^+$ T regulatory cells (Treg), $CD4^+$ $Foxp3^+CXCR5^+PD1^+$ T follicular regulatory cells (Tfr), and $CXCR5^-PD\text{-}1^+$ T peripheral helper (Tph) cells, $CXCR5^+PD\text{-}1^+$ T follicular helper cells (Tfh) from $CD4^+Foxp3^-$ cells, and Tfh/Tfr ratios in 5- to 20-week-old KI and WT male mice. Open circle, WT mice; closed circle, KI mice. Mann-Whiney U test. Data presented as mean ± SD.

Supplementary figure 10

Expression of SAT1 mainly enriched in Thio-induced peritoneal neutrophils of C57/BL6J mice and human neutrophils.

These figures were adapted from ImmGen (**A**) and Human Protein Atlas (**B**) database.

Supplementary table 1. Variants identified by whole exome sequencing in two multiplex families.

Location	Number of variants
Exonic	59,294
Splicing (within 2bp of a splicing junction)	413
5'/3' UTR	71,411
Downstream / upstream (within 1kb)	18,412
Intronic	283,128
Non-coding RNA	63,348
Intergenic	301,530
	797,536

Supplementary table 2. Clinical features of 562 SLE patients enriched in index cases of multiplex pedigrees, male lupus and pediatric SLE patients.

	Multiplex family n=65	**Sporadic pediatric Lupus** n=211	**Sporadic adult Lupus** n=286
Age of onset, mean (range)	20.4 ± 13.3 (5-48)	13.7 ± 3.4 (1-18)	36.2 ± 11.6(19-72)
Male, n (%)	52 (80)	107 (51)	263 (92)
Disease manifestation, n (%)			
Malar rash	47 (68)	108 (51)	94 (33)
Discoid rash	12 (17)	21 (10)	57 (20)
Photosensitivity	34 (49)	68 (32)	112 (39)
Oral ulcer	21 (30)	57 (27)	54 (19)
Arthritis	55 (79)	137 (65)	180 (63)
Serositis	26 (38)	61 (29)	114 (40)
Renal disorder	40 (58)	148 (70)	132 (46)
Proteinuria	38 (55)	106 (50)	126 (44)
Cellular casts	17 (24)	30 (14)	46 (16)
Hematologic disorder	43 (63)	97 (46)	163 (57)
Anemia	17 (24)	25 (12)	23 (8)
Thrombocytopenia	19 (28)	34 (16)	57 (20)
Immunologic disorder	57 (82)	192 (91)	255 (89)
Anti-double stranded DNA	45 (65)	167 (79)	177 (62)
Anti-Smith	11 (16)	38 (18)	77 (27)
Antinuclear antibody	68 (99)	196 (93)	283 (99)
Anti-Cardiolipin	19 (28)	89 (42)	160 (56)

Supplementary table 3. Ethnicity and age of onset characteristics of 562 SLE patients.

Ethnicity	Numbers	Percentage
European-American	245	44%
African-American	125	22%
Hispanic-American	80	14%
Asian-American	68	12%
American Indian	7	1%
Unknown	37	7%

Age of onset (years)	Numbers	Percentage
1-5	5	0.9%
6-10	35	6.2%
11-15	118	21.0%
16-18	97	17.3%
19-72	300	53.4%
Unknown	7	1.2%

Supplementary table 4. Minor Allele Frequencies (MAF) of 12 common variants and 3 additional rare variants of *SAT1* identified from 562 SLE patients in comparison to those from TOPMED and gnomAD database.

SNP	Location	TOPMED		gnomAD		Current Study	
		Allele Number	MAF	Allele Number	MAF	Allele Number	MAF
X-23783180-T-C	5'UTR	-	-	-	-	1	0.18%
X-23783714-T-C	Intron 2	-	-	-	-	1	0.18%
X-23785605-T-C	Intron 5	-	-	-	-	1	0.18%
rs113458958	5'UTR	2159	1.72%	260	1.22%	4	0.71%
rs11550721	5'UTR	19850	15.8%	3841	18.2%	50	8.90%
rs757525953	3'UTR	245	0.20%	18	0.08%	5	0.89%
rs3764885	Intron 1	36609	29.2%	5649	27.1%	370	65.8%
rs770974016	Intron 1	263	0.21%	41	0.19%	2	0.36%
rs1569213511	Intron 1	-	-	5	0.01%	1	0.18%
rs3764884	Intron 1	42802	34.1%	5787	30.3%	351	62.5%
rs191762819	Intron 2	244	0.19%	285	0.16%	1	0.18%
rs140890109	Intron 4	1060	0.84%	367	0.21%	6	1.07%
rs144320087	Intron 5	302	0.24%	51	0.23%	3	0.53%
rs145980431	Exon1	40	0.03%	51	0.03%	1	0.18%
rs139915283	Exon1	40	0.03%	50	0.03%	1	0.18%

Supplementary table 5. No robust evidence for functions in 12 common *SAT1* variants based on HaploReg v4 and Regulome database.

			HaploReg V4					Regulome
Variant	Ref	Alt	No. of Promoter histone marks	No. of Enhancer histone marks	DNase	No. of protein bound by Chip (ENCODE)	No. of regulatory motifs altered	Regulome DB Score
rs113458958	G	A	24	1	53	29	0	4
rs11550721	C	T	24	1	53	29	0	4
rs145980431	G	A	24	0	43	6	4	4
rs139915283	C	A	24	0	43	6	3	3a
rs3764885	A	G	24	0	37	5	5	4
rs3764884	G	C	24	0	10	1	4	4
rs191762819	G	C	24	0	24	2	1	4
rs140890109	T	C	12	7	2	0	2	4
rs144320087	G	A	9	9	2	0	9	5
rs757525953	TTGT	T	NA	NA	NA	NA	NA	4
rs770974016	C	T	NA	NA	NA	NA	NA	2b
rs1569213511	CCCCG	C	NA	NA	NA	NA	NA	3a

Abbreviation: NA denotes not available.

Regulome:

1a~f Likely to affect binding and linked to expression of a gene target

2a~c Likely to affect binding

3a~b Less likely to affect binding

4~6 Minimal binding evidence

Supplementary table 6. Demographic, and clinical characteristics of the treatment naive SLE patients.

Patients	Age	Gender	Clinical manifestations	SLEDAI-2K
#1	31	F	Proteinuria / Rash / Low Complement	6
#2	22	F	Vasculitis / Proteinuria / Low Complement	10
#3	22	F	Pleurisy / Low Complement / Thrombocytopenia	7
#4	32	F	Hematuria / Proteinuria / Pericarditis /Low Complement	12
#5	18	F	Rash / Low Complement / High titers of dsDNA	6
#6	30	F	Hematuria / Proteinuria / Pyuria / Low Complement	14
#7	21	F	Rash / Low Complement / High titer of dsDNA	6
#8	21	F	Hematuria/ Proteinuria / Rash / Alopecia / Pleurisy / Pericarditis / Low Complement / Fever	19
#9	29	F	Rash / Alopecia / Low Complement / High titers of dsDNA / Fever / Leukopenia	10
#10	26	F	Hematuria / Proteinuria / Low Complement	12
#11	36	F	Arthritis / Proteinuria / Alopecia / Low Complement / High titers of dsDNA / Thrombocytopenia	15
#12	30	M	Arthritis / Rash / Low Complement	8
#13	25	F	Proteinuria / Low Complement	6
#14	21	F	Proteinuria / Low Complement	6
#15	18	F	Hematuria / Proteinuria / Low Complement	10

#16	22	F	Proteinuria / Low Complement	6
#17	24	F	Low Complement / High titers of dsDNA	4
#18	24	F	Proteinuria / Low Complement	4
#19	28	F	Proteinuria / High titers of dsDNA / Low Complement	8
#20	33	M	Proteinuria / Rash / Alopecia / Pleurisy / Low Complement / Thrombocytopenia / Leukopenia	14
#21	27	F	Rash / Low Complement / High titers of dsDNA	6
#22	33	F	Proteinuria / Low Complement	6
#23	15	F	Proteinuria / Low Complement / High titers of dsDNA	8
#24	19	F	Rash / Low Complement	4
#25	15	F	Hematuria / Rash / Low Complement	8
#26	23	F	Proteinuria / Low Complement / High titers of dsDNA	8

Supplementary table 7. Gender, ethnicity, and age of 26 SLE patients and 20 healthy controls.

	SLE (n=26)	Control (n=20)	*P* value
Female, n (%)	24 (92.6)	19 (95.0)	0.7139
Age, mean (range)	24.8 (15-33)	23.3 (14-32)	0.3858
Ancestry, n (%)			
Chinese-Han	26 (100)	20 (100)	1

Supplementary table 8. Correlation between disease activity and plasma polyamine levels in patients with SLE. Correlation coefficients and *P* values were depicted.

	SPD		SPM		N^1PUT		N^1SPD		N^1SPM		L-ORN		L-ARG		SAM	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
SLEDAI	-0.348	0.145	-0.314	0.191	0.416	0.069	-0.008	0.975	-0.384	0.094	-0.022	0.928	0.420	0.065	**0.535**	**0.015**
PU (mg/24h)	-0.146	0.605	-0.117	0.666	0.073	0.788	-0.180	0.505	-0.056	0.836	-0.031	0.909	0.010	0.972	-0.249	0.353
Anti-dsDNA (RU/ml)	**-0.424**	**0.044**	**-0.463**	**0.023**	0.065	0.762	0.096	0.656	-0.243	0.253	0.182	0.395	0.099	0.647	0.398	0.054
Cf DNA (ng/ml)	**-0.575**	**0.013**	-0.391	0.109	0.121	0.621	0.093	0.706	**-0.522**	**0.022**	-0.303	0.208	0.068	0.782	-0.192	0.432
BUN (mmol/L)	0.203	0.390	0.222	0.346	**-0.598**	**0.004**	-0.050	0.829	0.258	0.259	-0.051	0.825	**-0.551**	**0.010**	-0.319	0.159
Cr (μmol/L)	-0.259	0.270	-0.181	0.445	0.166	0.472	-0.022	0.926	-0.180	0.434	0.208	0.367	0.227	0.323	0.423	0.056
HB (g/L)	-0.412	0.080	-0.371	0.117	0.407	0.075	-0.105	0.658	**-0.469**	**0.037**	-0.224	0.344	0.354	0.126	**0.494**	**0.027**
PLT (10^9/L)	-0.252	0.299	-0.066	0.789	0.166	0.485	0.110	0.644	-0.207	0.381	-0.277	0.237	0.149	0.530	0.171	0.471
N (10^9/L)	-0.377	0.069	**-0.662**	**0.000**	-0.056	0.792	0.119	0.572	-0.307	0.135	-0.141	0.501	-0.108	0.608	0.108	0.609
IgG (g/L)	-0.295	0.206	-0.162	0.495	**0.435**	**0.049**	0.016	0.945	-0.371	0.098	-0.226	0.326	0.418	0.059	**0.574**	**0.007**
IgA (g/L)	0.407	0.093	0.319	0.197	-0.176	0.472	0.028	0.910	0.358	0.132	0.171	0.485	-0.119	0.627	-0.252	0.298
IgM (g/L)	0.181	0.445	0.203	0.391	-0.298	0.190	0.317	0.162	0.237	0.300	0.020	0.933	-0.260	0.255	-0.235	0.305
C3 (g/L)	-0.350	0.130	-0.369	0.110	0.317	0.161	-0.011	0.961	-0.343	0.128	-0.159	0.491	0.271	0.235	0.203	0.377
C4 (g/L)	0.280	0.233	0.135	0.569	0.016	0.946	0.153	0.507	0.303	0.182	0.058	0.805	0.044	0.851	**-0.452**	**0.040**
$CD3^+CD4^+$ (%)	0.268	0.299	0.140	0.593	0.132	0.603	-0.077	0.760	0.283	0.255	0.039	0.878	0.108	0.670	-0.454	0.058
$CD3^+CD8^+$ (%)	0.434	0.093	0.190	0.481	0.101	0.701	-0.099	0.707	0.321	0.209	0.072	0.783	0.113	0.667	0.226	0.384
$CD19^+$ (%)	0.108	0.690	0.220	0.413	-0.409	0.103	0.327	0.201	0.166	0.525	0.078	0.765	-0.341	0.180	-0.431	0.085
$CD4^+Foxp3^+$ (%)	**0.475**	**0.022**	**0.597**	**0.003**	-0.222	0.298	-0.307	0.144	**0.451**	**0.027**	0.170	0.426	-0.281	0.183	-0.352	0.092
Tfh (%)	**0.525**	**0.037**	0.333	0.208	-0.151	0.564	0.083	0.752	**0.501**	**0.040**	0.141	0.589	-0.128	0.624	-0.370	0.143
Tph (%)	-0.361	0.170	-0.369	0.145	0.055	0.835	0.420	0.093	-0.189	0.467	0.358	0.158	0.165	0.528	**0.528**	**0.029**

Abbreviations: SLE: systemic lupus erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; PU: Proteinuria; Anti–dsDNA: Anti-double-stranded DNA; Cf DNA: cell-free DNA; BUN: blood urea nitrogen; Cr: Creatinine; HB: Hemoglobin; PLT: Platelet; N: neutrophil; IgG: Immunoglobulin G; IgA: Immunoglobulin A; IgM: Immunoglobulin M; C3: Complement 3; C4: Complement 4; Tfh: T follicular helper; Tph: T Peripheral

Helper; SPD, Spermidine; SPM, Spermine; N^1PUT, N1-acetylputrescine; N^1SPD, N1-acetylspermidine; N^1SPM, N-acetylspermine; L-ORN, L-ornithine; L-ARG, L-arginine; SAM S-adenosyl-L-methionine.

Supplementary table 9. Selected multiple reaction monitoring, collision energy (CE), retention time, and declustering potential (DP) used for each analyte.

Analyte	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Retention Time (min）	DP	CE
Putrescine	89	72.2	10.3	30	16
spermidine	146.1	72.1	12.7	30	20
spermine	203.2	129.2	0.7	30	18
N^1-acetylputrescine	131.1	72	10.0	30	22
N^1-acetylspermidine	188.3	171.1	10.0	30	15.6
N^1-acetylspermine	245.2	100.1	12.4	30	27
L-ornithine	133.1	70.1	10.6	30	22
L-arginine	175.1	70	10.5	30	30
S-adenosyl-L-methionine	399.1	250.1	10.7	30	20

Supplementary table 10. Antibodies used for flow cytometry.

Marker	Color/Format	Host/Target	Isotype	Clone	Company	Catalog
CD19	PE-Cy7	Rat anti-Mouse	IgG2a κ	6D5	BioLegend	115519
CD23	Brilliant Violet 711	Rat anti-Mouse	IgG2a κ	B3B4	BD Biosciences	563987
CD21	DyLight 350	Mouse anti-Mouse	IgG1 κ	BB6-11C9.6	Novus Biologicals	NBP1-28245UV
CD5	Brilliant Violet 421	Rat anti-Mouse	IgG2a κ	53-7.3	BioLegend	100617
CD11b	FITC	Rat anti-Mouse	IgG2b κ	M1/70	BioLegend	101205
IgM	APC	Rat anti-Mouse	IgG2a κ	RMM-1	BioLegend	406509
CD138	PE	Rat anti-Mouse	IgG2a κ	281-2	BioLegend	142503
CD45R (B220)	Brilliant Violet 650	Rat anti-Mouse	IgG2a κ	RA3-6B2	BD Biosciences	563893
IgG2a	Brilliant Violet 510	Rat anti-Mouse	IgG1 κ	R19-15	BD Biosciences	744531
CD27	APC-Cy7	Armenian Hamster anti-Mouse	IgG	LG.3A10	BioLegend	124225
CD95	APC-R700	Armenian Hamster anti-Mouse	IgG2 λ2	Jo2	BD Biosciences	565130
IgD	Brilliant Violet 786	Rat anti-Mouse	IgG2a κ	11-26c.2a	BD Biosciences	563618
T- and B-Cell Activation Antigen (GL7)	PerCP-Cy5.5	Rat anti-Mouse	IgM κ	GL7	BioLegend	144609
CD3	Brilliant Violet 421	Rat anti-Mouse	IgG2b κ	17A2	BioLegend	100227
CD19	PE-Cy7	Rat anti-Mouse	IgG2a κ	6D5	BioLegend	115519
CD11b	FITC	Rat anti-Mouse	IgG2b κ	M1/70	BioLegend	101205
CD11c	Brilliant Violet 650	Armenian Hamster anti-Mouse	IgG	N418	BioLegend	117339
F4/80	PE	Rat anti-Mouse	IgG2a κ	BM8	BioLegend	123109
Ly-6G	APC	Rat anti-Mouse	IgG2a κ	1A8	BioLegend	127613

Ly-6C	Brilliant Violet 605	Rat anti-Mouse	IgG2c κ	HK1.4	BioLegend	128035
CD56	Brilliant Violet 786	Rat anti-Mouse	IgG2a κ	809220	BD Biosciences	748100
CD45R	APC-Cy7	Rat anti-Mouse	IgG2a κ	RA3-6B2	BioLegend	103223
MHC II	BV 480	Mouse anti-Mouse	IgG2a κ	14-4-4S	BD Biosciences	746291
BST2	Brilliant Violet 711	Mouse anti-Mouse	IgG2b κ	927	BD Biosciences	747604
CD4	PerCP-Cy5.5	Rat anti-Mouse	IgG2b κ	GK1.5	BioLegend	100433
CD25	Brilliant Violet 605	Rat anti-Mouse	IgG1 λ	PC61	BioLegend	102035
FoxP3	PE	Rat anti-Mouse	IgG2b κ	MF-14	BioLegend	126403
CD185	PE-Cy7	Rat anti-Mouse	IgG2b κ	L138D7	BioLegend	145515
PD-1	APC	Rat anti-Mouse	IgG2a κ	29F.1A12	BioLegend	135209
IL-17A	Brilliant Violet 421	Rat anti-Mouse	IgG1 κ	TC11-18H10	BD Biosciences	566286
IL-4	Alexa Fluor 488	Rat anti-Mouse	IgG1 κ	11B11	BioLegend	504111
IFN-gamma	Brilliant Violet 711	Rat anti-Mouse	IgG1 κ	XMG1.2	BioLegend	505835
CD3	Brilliant Violet 510	Rat anti-Mouse	IgG2b κ	17A2	BioLegend	100233
CD8a	Brilliant Violet 650	Rat anti-Mouse	IgG2a κ	53-6.7	BioLegend	100741
Bcl-6	APC-Cy7	Mouse anti-Mouse	IgG1 κ	K112-91	BD Biosciences	563581
CD183	Alexa Fluor 700	Rat anti-Mouse	IgG2a	220803	Novus Biologicals	FAB1685N-100
CCR6	Brilliant Violet 786	Rat anti-Mouse	IgG2a	140706	BD Biosciences	740840

Supplementary table 11. RT-PCR primer sequences for mice.

Gene name	Forward Primer Sequence (5'–3')	Reverse Primer Sequence (5'–3')
Foxp3	CAC CCA GGA AAG ACA GCA ACC	GCA AGA GCT CTT GTC CAT TGA
Mx1	GACCATAGGGGTCTTGACCAA	AGACTTGCTCTTTCTGAAAAGCC
Ifit1	AGCCTGGAGTGTGCTGAGAT	TCTGGATTTAACCGGACAGC
Ifit44	CCAACTGACTGCTCGCAATA	TAGGACCCAGCAGCAGAACT
Prkr	ACCGGCTCATAAGAGGGAAT	GCATCCTAGGGAATGTCCAA
Oas1a	AGAGATGCTTCCAAGGTGCT	CCTTGATGAACTCTCCCCGT
Sat1	TGCGACTGATCAAGGAACTG	TATGTCCTTCAGGGGTCCAG
16S	GGATAACAGCGCAATCCTA	GATTGCTCCGGTCTGAACTC
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
βeta-actin	GACGGCCAGGTCATCACTATTG	AGGAAGGCTGGAAAAGAGCC

Supplementary table 12. Primers for gDNA PCR amplification and direct Sanger sequencing of the human *SAT1* gene core promoter region and coding exons.

	Forward Primer Sequence (5'–3')	Reverse Primer Sequence (5'–3')
5' UTR and Exon 1~3 (Product 1)	GCCTCATTGGATGAGAGGTC	TTCGGCCTGTGTAGTCAGTG
Exon 4~6 and 3' UTR (Product 2)	GCCTGGACTGCACTCACATA	GCCTGGACTGCACTCACATA
Sequencing primer #1 (For Product 1)	ATCACCCCACTCTGGGAAAC	
Sequencing primer #2 (For Product 1)	ATCACCCCACTCTGGGAAAC	
Sequencing primer #3 (For Product 2)	CATTTCAGCCTGCTCTCAAG	
Sequencing primer #4 (For Product 2)	CATTTCAGCCTGCTCTCAAG	

Supplementary table 13. Mouse genotyping probe and Sanger sequencing primer sequences.

	Forward Primer Sequence (5'–3')	Reverse Primer Sequence (5'–3')
Exon 4~6	TAAGGAGGCAGTCCAGTTCC	TCAAACATGCAACAACGTCA
Probe	GACATAGCATTGTTGGGTTCG	TCTGTAATCACTCATCACGAAGAA
Reporter 1 (WT)	CTGTATCTTGAAGACTTC	
Reporter 2 (KI)	TGTATCTTTTGAGGACTTC	

Supplementary table 14. Primers for sequencing and cloning of Minigene.

	Forward Primer Sequence (5'–3')	Reverse Primer Sequence (5'–3')
Human-Asp40Tyr-fragment	GATTCACGTCTTGAGGTCTCG	GTGGTTCTACACAGCCGACA
pET01 primers #1	GGATTCTTCTACACACCC	TCCACCCAGCTCCAGTTG
pET01 primers #2	GCGAAGTGGAGGATCCACAAG	ACCCGGATCCAGTTGTGCCA
XhoL-Asp40Tyr	CTGACTGACTCGAGgattcacgtcttgaggtctcg	
BamHI-Asp40Tyr	TCAGTCAGGGATCCgtggttctacacagccgaca	

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