# Supporting Information

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### SI Materials and Methods

**Reagents and Antibodies.** Recombinant IFNγ (R&D Systems); D-galactosamine hydrochloride (D-Gal) (Sigma); peptidoglycan (PGN) (Sigma); TNFα and IL-1β (R&D Systems); mouse monoclonal antibodies against Flag (Sigma), HA (Origene), and β-actin (Sigma); rabbit monoclonal antibodies against JAK1, JAK2 (Abcam), pan phosphotyrosine, IKKβ, phosphoserine177-IKKβ (Cell Signaling), phosphotyrosine701-STAT1, phosphoserine727-STAT1, and STAT1 (Santa Cruz) were purchased from the indicated companies. Rabbit and mouse anti-SNX8 sera were raised against a recombinant human SNX8 protein.

**Expression Screens.** Approximately 10,000 individual human cDNA expression clones were obtained from Origene, Inc. The clones (0.1 μg) were individually transfected into 293 cells ($1 \times 10^5$) together with the IRF1 promoter reporter plasmid (0.1 μg) by calcium phosphate precipitation method. Twenty hours after transfection, cells were left untreated or treated with IFNγ (100 ng/mL) for 12 h, and then dual-specific luciferase assays were performed.

**Constructs.** Mammalian expression plasmids for HA- or Flag-tagged SNX8 and its mutants were constructed by standard molecular biology techniques.

**Transfection and Reporter Assays.** The 293 cells were seeded in 24-well dishes and transfected the following day by standard calcium phosphate precipitation method. To normalize for transfection efficiency, pRL-TK (*Renilla* luciferase) reporter plasmid was added to each transfection. Luciferase assays were performed using a dual specific luciferase assay kit (Promega). Firefly luciferase activities were normalized on the basis of *Renilla* luciferase activities. All reporter assays were repeated at least three times. Data shown were average values ± SD from one representative experiment.

**Real-Time PCR.** Total RNA was isolated from cells using TRIzol reagent (TAKARA), reverse transcribed, and subjected to real-time PCR analysis to measure mRNA levels of tested genes. Data shown are the relative abundance of the indicated mRNA normalized to that of *GAPDH*. Gene-specific primer sequences were as follows:

*GAPDH:* GACAAGCTTCCCGTTCTCAG (forward) and GAGTCAACGGATTTGGTGGT (reverse); *SNX8*: GGTAAGCAGGAAGAGAACGACG (forward) and CATCAGGCTGTACTTGTGCAGG (reverse); *GBP1*: TAGCAGACTTCTGTTCCTACATCT (forward) and CCACTGCTGATGGCATTGACGT (reverse); *STAT1*: ATGGCAGTCTGGCGGCTGAATT (forward) and CCAAACCAGGCTGGCACAATTG (reverse); *CXCL9*: CTGTTCCTGCATCAGCACCAAC (forward) and TGAACCATTCTTCAGTGTAGCA (reverse); *CXCL10*: GGTGAGAAGAGATGTCTGAATCC (forward) and GTCCATCCTTGGAAGCACTGCA (reverse); *IRF1*: GAGGAGGTGAAAGACCAGAGCA (forward) and TAGCATCTCGGCTGGACTTCGA (reverse); *SOCS1*: TTCGCCCTTAGCGTGAAGATGG (forward) and TAGTGCTCCAGCAGCTCGAAGA (reverse); *Gapdh*: ACGGCCGCATCTTCTTGTGCA (forward) and ACGGCCAAATCCGTTCACACC (reverse); *Gbp1*: AGATGCCCACAGAAACCCTCCA (forward) and AAGGCATCTCGCTTGGCTACCA (reverse); *Stat1*: GCCTCTCATTGTCACCGAAGAAC (forward) and TGGCTGACGTTGGAGATCACCA (reverse); *Socs1*: AGTCGCCAACGGAACTGCTTCT (forward) and GTAGTGCTCCAGCAGCTCGAAA (reverse); *Irf1*: TCCAAGTCCAGCCGAGACACTA (forward) and ACTGCTGTGGTCATCAGGTAGG (reverse); *Cxcl9*: CCTAGTGATAAGGAATGCACGATG (forward) and CTAGGCAGGTTTGATCTCCGTTC (reverse); *Cxcl10*: ATCATCCCTGCGAGCCTATCCT (forward) and GACCTTTTTTGGCTAAACGCTTTC (reverse); *Cxcl11*: CCGAGTAACGGCTGCGACAAAG (forward) and CCTGCATTATGAGGCGAGCTTG (reverse); *Tnf*: GGTGATCGGTCCCCAAAGGGATGA (forward) and TGGTTTGCTACGACGTGGGCT (reverse); *Il6*: TCTGCAAGAGACTTCCATCCAGTTGC (forward) and AGCCTCCGACTTGTGAAGTGGT (reverse); and *Il1β*: AAAGCCTCGTGCTGTCGGACC (forward) and CAGGGTGGGTGTGCCGTCTT (reverse).

**Coimmunoprecipitation and Immunoblot Analysis.** The 293 cells or THP1 cells were lysed in 1 mL Nonidet P-40 lysis buffer (20 mM Tris·HCl, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 10 μg/mL aprotinin, 10 μg/mL leupetin, and 1 mM phenylmethylsufonyl fluoride). For each immunoprecipitation, a 0.4-mL aliquot of lysate was incubated with 0.5–2 μg of the indicated antibody or control IgG and 25 μL of a 1:1 slurry of Protein-G Sepharose (GE Healthcare) for at least 2 h. The Sepharose beads were washed three times with 1 mL of lysis buffer containing 500 mM NaCl. The precipitates were fractionated on SDS/PAGE, and immunoblot analysis was performed following standard methods.

**RNAi.** Double-stranded oligonucleotides corresponding to the target sequences were cloned into the pSuper-Retro RNAi plasmid (Oligoengine). The following sequences were targeted for human SNX8 cDNA: no. 1 (5′-CGGCAGATCTTCTCATATT-3′) and no. 2 (5′-CGGCAGATCTTCTCATATT-3′).

**RNAi-Transduced THP1 Cells.** The 293 cells were transfected with two packaging plasmids, pGag-Pol (10 μg) and pVSV-G (3 μg) and control or SNX8-RNAi retroviral plasmid (10 μg) by calcium phosphate precipitation. Cells were washed 12 h after transfection and new medium without antibiotics was added for an additional 24 h. The recombinant virus-containing medium was filtered and used to infect THP1 cells in the presence of polybrene (4 μg/mL). The infected THP1 cells were selected with puromycin (0.5 μg/mL) for 2 wk before additional experiments were performed.

**CRISPR-Cas9 Knockout.** Double-stranded oligonucleotides corresponding to the target sequences were cloned into the lenti-CRISPR-V2 vector and cotransfected packaging plasmids into 293 cells. Lentiviral particles were collected and used to transduce HeLa cells. The infected HeLa cells were selected with puromycin (1 μg/mL) for 2 wk before additional experiments were performed. The following sequences were targeted for human SNX8 cDNA: no. 1 (5′-GGGCAGGCACCATACGGTAG-3′) and no. 2 (5′-GACCTGCTGCACGATGGCCT-3′); human JAK1 cDNA: no. 1 (5′-GCTGCCTCGAAGAAGGCCTG -3′) and no. 2 (5′-GAGGTACACGATGTGTTTGT-3′); and human IKKβ cDNA: no. 1 (5′-GGCTGCCCGAGATGTCCCTG -3′) and no. 2 (5′-GCCCCCGGAACCGAGAGCGG-3′).

**RNA-Seq.** Cells were left untreated or treated with IFNγ for 6 h then RNAs were extracted using standard methods and cDNA libraries were prepared using the Illumina TruSeq RNA Sample Preparation Kit. Sequencing was carried out by Illumina HiSEq. 4000. Following sequencing, BAM files were converted to merged, demultiplexed FASTQ files and mapped to the University of California Santa Cruz (hg19) human transcriptome.

Fragments per kilobase of transcript per million mapped reads (FPKM) scores were computed in our analysis. The cutoff for significant changes of mRNA levels was set for twofold.

**ELISA.** BMDMs and BMDCs were stimulated with IFNγ for 18 h. The culture media were collected for measurement of CXCL9 and CXCL10 by ELISA.

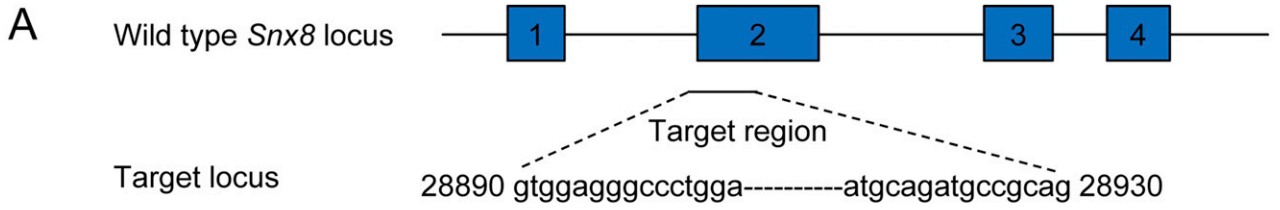
**Flow Cytometry.** The spleen, thymus, and peripheral lymph nodes were obtained from *Snx8*$^{+/+}$ and *Snx8*$^{-/-}$ mice, and single-cell suspensions were prepared. After depletion of red blood cells by ammonium chloride, cells were subject to staining with the indicated antibodies for 30 min followed by flow analysis. The antibodies used in this study were CD4-PerCP (1:200, 553052; BD), CD8-PB (1:200, 558207; BD), CD3-FITC (1:500, 561801; BD), B220-APC (1:200, 553092; BD) and CD25-FITC (1:500, 553072; BD).

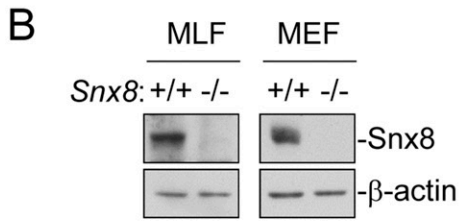
***Listeria monocytogenes* Infection in Mice.** Mice were infected with *L. monocytogenes* i.p. The viability of the infected mice was monitored for 12 d. The mouse sera were collected at 6 h after infection to measure cytokine production by ELISA. All animal experiments were performed in accordance with the Wuhan University Medical Research Institute animal care and use committee guidelines.

**Mouse PGN Injection.** Mice were injected intraperitoneally with PGN (15 μg/g body weight) plus D-galactosamine (1 mg/g body weight). The mouse sera were collected at 3 h after injection to measure cytokine production by ELISA.

**Cell Fractionation Assays.** HeLa cells ($4 \times 10^7$) left untreated or treated with IFNγ for the indicated times were washed with PBS and lysed by douncing 20 times in 2 mL homogenization buffer [10 mM Tris·HCl (pH 7.4), 2 mM MgCl2, 10 mM KCl, and 250 mM sucrose]. The homogenate was centrifuged at 500 × *g* for 10 min, and the pellet was saved as crude nuclei. The supernatant was saved as cytosol.

**Statistical Analysis.** Student's *t* test was used for statistical analysis with Microsoft Excel and GraphPad Prism Software. For the mouse survival study, Kaplan–Meier survival curves were generated and analyzed by log-rank test; $P < 0.05$ was considered significant.

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| Genotypes | +/+ | +/- | -/- |
|---|---|---|---|
| Total | 86 | 179 | 94 |
| % | 23 | 50 | 27 |

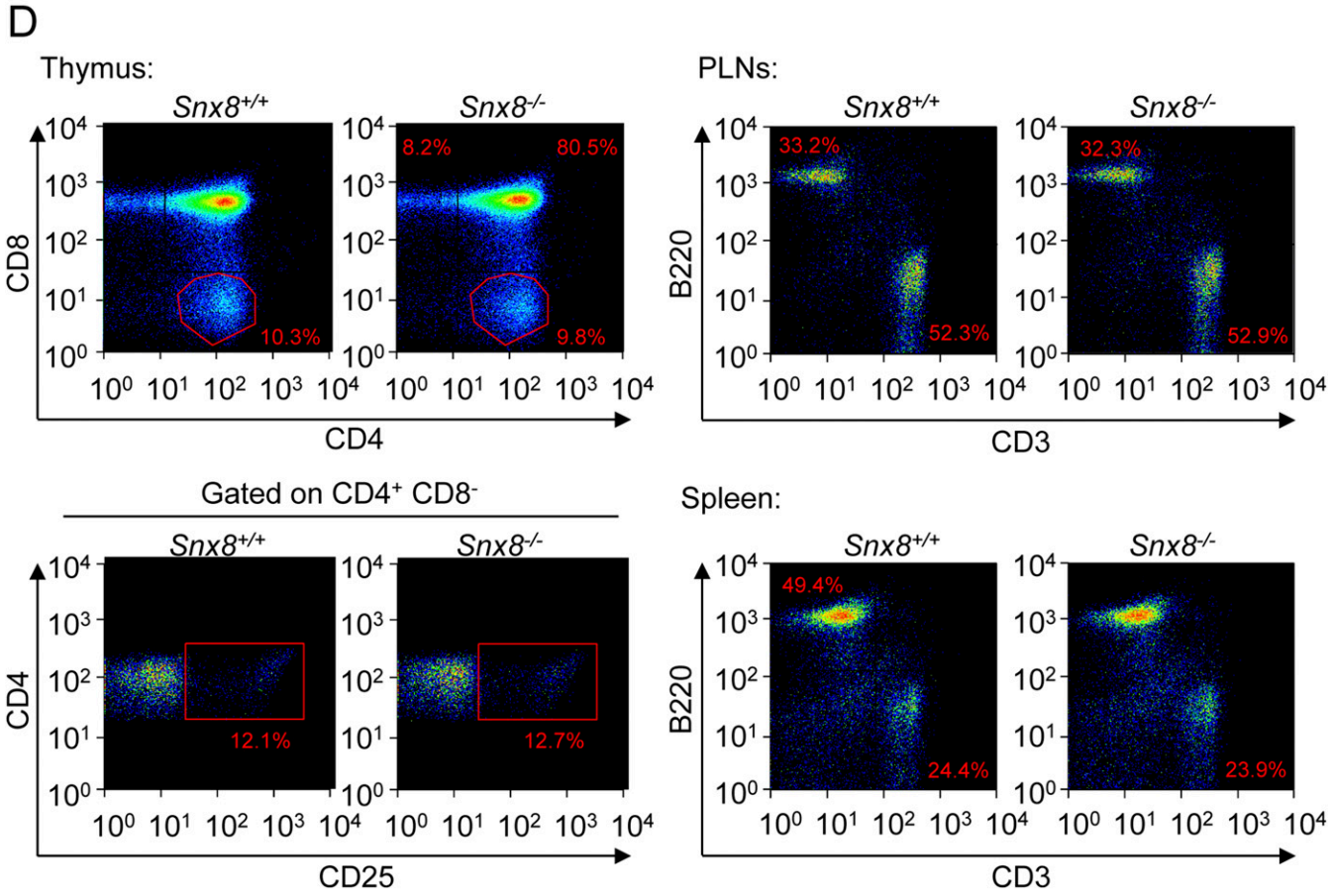



**Fig. S1.** Generation and analysis of SNX8-deficient mice. (*A*) Schematic representation of wild-type *Snx8* locus shows its exons (boxes) and introns (lines). The *Snx8* locus was disrupted by the indicated targeting vector. Ten nucleotides were deleted in the second exons of *Snx8*, resulting in the loss of SNX8 expression. (*B*) Immunoblot analysis of SNX8 protein levels in MLFs and MEFs of $Snx8^{+/+}$ and $Snx8^{-/-}$ mice. (*C*) Genotypes of the offspring from the breeding of *Snx8* heterozygous mice. (*D*) Cells from thymus, spleen, and peripheral lymph nodes were analyzed by FACS after staining with the indicated antibodies.

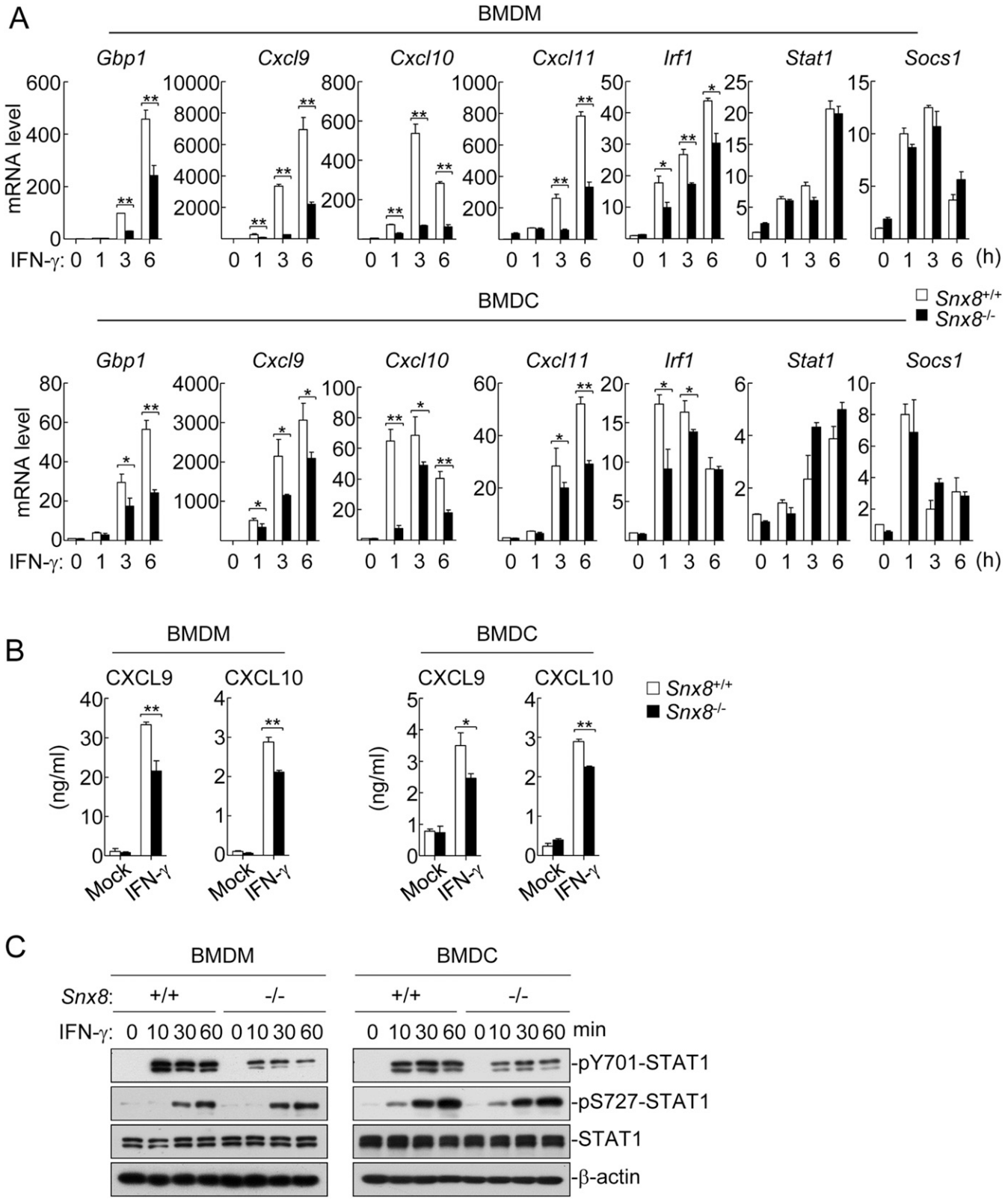
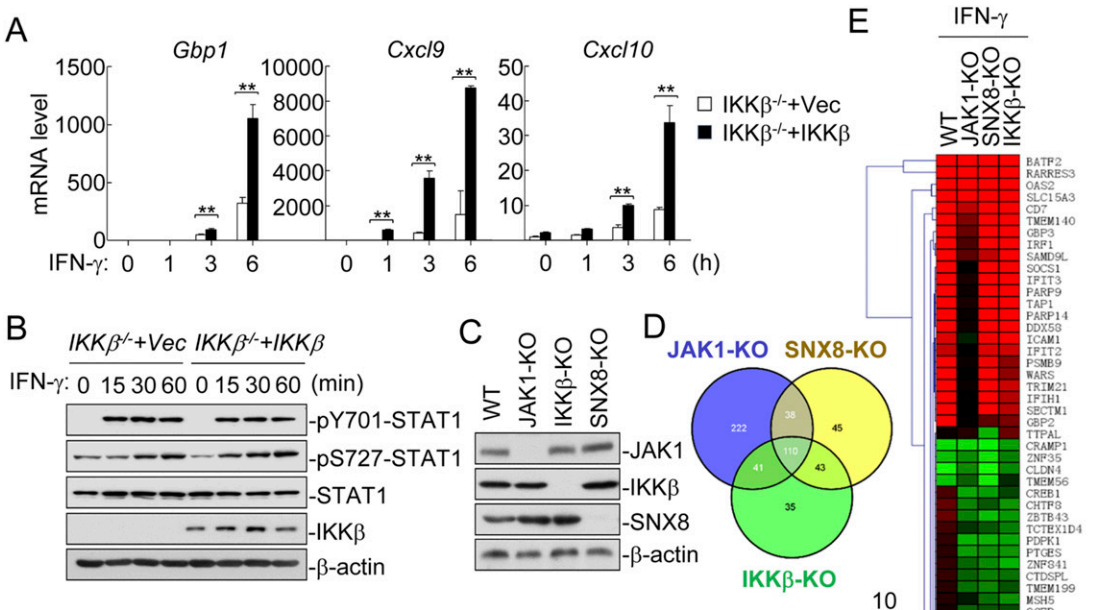



**Fig. S2.** SNX8 is essential for IFNγ-triggered signaling in murine cells. (*A*) Effects of SNX8 deficiency on IFNγ-induced transcription of the indicated genes in BMDMs and BMDCs. The indicated cells were untreated or treated with IFNγ (100 ng/mL) for the indicated times before qPCR experiments were performed (**$P < 0.01$, *$P < 0.05$). (*B*) Effects of SNX8 deficiency on IFNγ-induced secretion of CXCL9 and CXCL10 in BMDMs and BMDCs. The cells were untreated or treated with IFNγ (100 ng/mL) for 18 h. The culture media were collected for quantization of the indicated chemokines by ELISA (**$P < 0.01$, *$P < 0.05$). (*C*) Effects of SNX8 deficiency on IFNγ-induced phosphorylation of STAT1 in BMDMs and BMDCs. The cells were untreated or treated with IFNγ (100 ng/mL) for the indicated times, and immunoblots were performed with the indicated antibodies.

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| Gene symbol | WT-IFNγ | JAK1-KO-IFNγ | SNX8-KO-IFNγ | IKKβ-KO-IFNγ |
|---|---|---|---|---|
| AC005606.1 | 7.476 | 0.617 | 3.097 | 0.276 |
| CHTF8 | 5.174 | 0.954 | 1.301 | 0.823 |
| CREB1 | 4.496 | 0.679 | 0.742 | 1.433 |
| CTD | 6.224 | 2.247 | 1.262 | 0.705 |
| CTDSPL | 3.842 | 1.222 | 1.007 | 1.093 |
| CXCL10 | 27.59 | 4.590 | 8.036 | 2.333 |
| CXCL11 | 9.322 | 2.276 | 2.731 | 3.744 |
| FAM111A | 2.471 | 1.023 | 0.594 | 0.447 |
| GBP1 | 6.728 | 1.006 | 2.653 | 2.868 |
| GBP2 | 14.89 | 2.279 | 5.068 | 5.961 |
| MYC | 26.58 | 0.932 | 1.341 | 1.588 |
| PDPK1 | 3.658 | 0.574 | 0.588 | 0.683 |
| PTGES | 3.506 | 0.760 | 0.883 | 0.818 |
| SLC7A2 | 3.002 | 0.632 | 0.641 | 0.639 |
| TCTEX1D4 | 4.220 | 1.558 | 1.301 | 0.989 |
| TMEM199 | 3.350 | 1.114 | 0.947 | 1.331 |
| ZNF841 | 3.670 | 1.183 | 0.907 | 0.567 |
| ZBTB43 | 5.388 | 0.974 | 0.496 | 0.780 |
| ACSL5 | 5.519 | 1.305 | 5.926 | 5.73 |
| BATF2 | 333.6 | 56.52 | 349.16 | 285.3 |
| CASP1 | 4.414 | 0.783 | 4.396 | 3.48 |
| CD7 | 51.66 | 8.09 | 61.33 | 49.48 |
| IFIT2 | 8.313 | 2.063 | 9.113 | 8.66 |
| IFIT3 | 15.48 | 2.945 | 15.72 | 12.8 |
| ICAM1 | 9.08 | 1.746 | 9.714 | 10.6 |
| PARP9 | 15.66 | 2.59 | 15.28 | 13.11 |
| STAT1 | 5.637 | 1.611 | 5.248 | 4.91 |
| SOCS1 | 14.42 | 2.061 | 17.68 | 13.76 |
| TAP1 | 15.11 | 2.944 | 14.19 | 10.6 |
| TMEM140 | 53.41 | 6.148 | 49.92 | 32.54 |

**Fig. S3.** SNX8 and IKKβ mediate transcription of overlapping genes induced by IFNγ. (*A*) IKKβ is required to activate a subset of IFNγ-stimulated genes. The *IKKβ*$^{-/-}$ cells reconstituted with IKKβ or empty vector were left untreated or treated with IFNγ for the indicated times before qPCR experiments were performed (**$P < 0.05$). (*B*) Effects of IKKβ deficiency on the phosphorylation of STAT1 induced by IFNγ. The *IKKβ*$^{-/-}$ cells reconstituted with IKKβ or empty vector were left untreated or treated with IFNγ for the indicated times before immunoblot analysis was performed. (*C*) Immunoblot analysis of JAK1, IKKβ, and SNX8 protein levels in the wild-type and knockout cells. JAK1-, IKKβ-, and SNX8-deficient HeLa cells were constructed by CRISPR-Cas9 procedures. (*D*) Venn diagram schematic of JAK1-, SNX8-, and IKKβ-mediated genes induced by IFNγ (*E*) Heat map of hierarchical clustering analysis of RNA-sequencing data from IFNγ-induced JAK1-deficient, SNX8-deficient, IKKβ-deficient, or wild-type HeLa cells. Cells were left untreated or treated with IFNγ for 6 h before RNA-sequencing analysis. Fold induction is based on relative level to that in unstimulated wild-type cells. Data were clustered by Euclidean distance algorithm. (*F*) Select list of IFNγ-induced genes. A subset of IFNγ-induced genes was down-regulated in all three groups, whereas a part of IFNγ-induced genes were down-regulated in JAK1-deficient but not SNX8- and IKKβ-deficient cells.

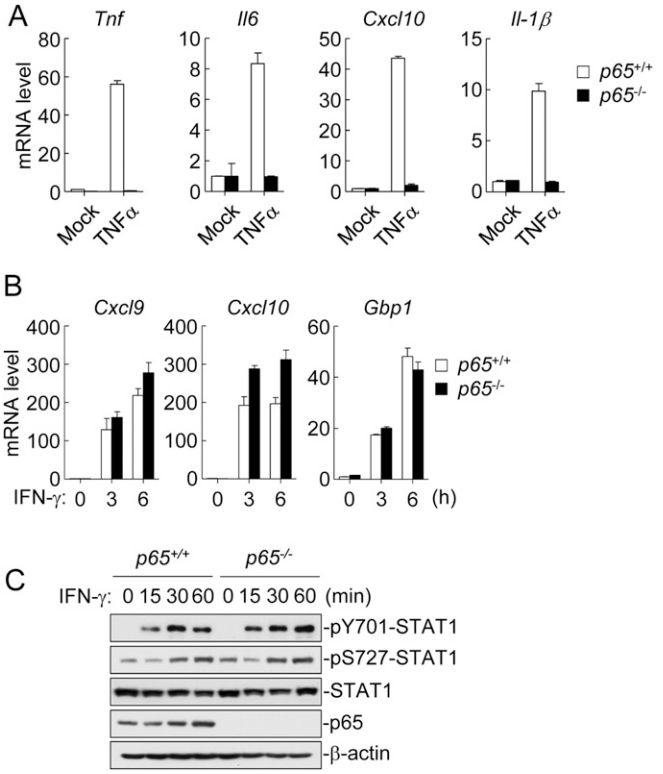


**Fig. S4.** Effects of p65-deficiency on IFNγ-triggered signaling. (*A*) Effects of p65 deficiency on TNFα-induced transcription of downstream genes. MEFs were left untreated or treated with TNFα for 3 h before qPCR experiments were performed. (*B*) Effects of p65 deficiency on IFNγ-induced transcription of downstream genes. MEFs were left untreated or treated with IFNγ for the indicated times before qPCR experiments were performed. (*C*) Effects of p65 deficiency on IFNγ-induced phosphorylation of STAT1. MEFs were left untreated or treated with IFNγ for the indicated times before immunoblot analysis was performed.

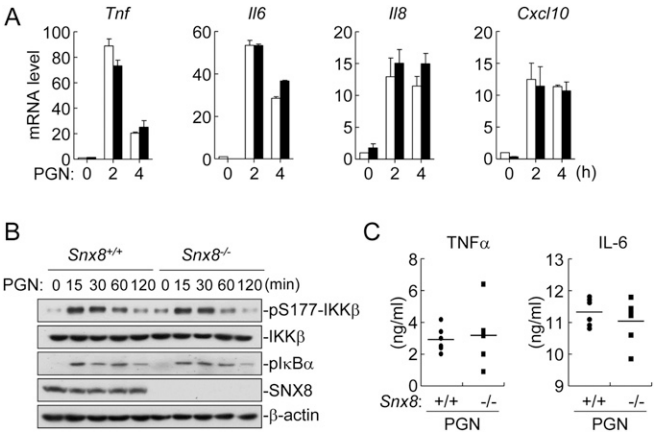


**Fig. S5.** Effects of SNX8 deficiency on PGN-induced signaling. (*A*) Effects of SNX8 deficiency on PGN-induced transcription of downstream genes. BMDCs were left untreated or treated with PGN (50 μg/mL) for the indicated times before qPCR experiments were performed. (*B*) Effects of SNX8 deficiency on PGN-induced phosphorylation of IKKβ and IκBα. BMDCs were left untreated or treated with PGN (50 μg/mL) for the indicated times before immunoblot analysis was performed. (*C*) Effects of SNX8 deficiency on serum levels of TNFα and IL-6 induced by i.p. injection of mice with PGN plus D-gal for 3 h.

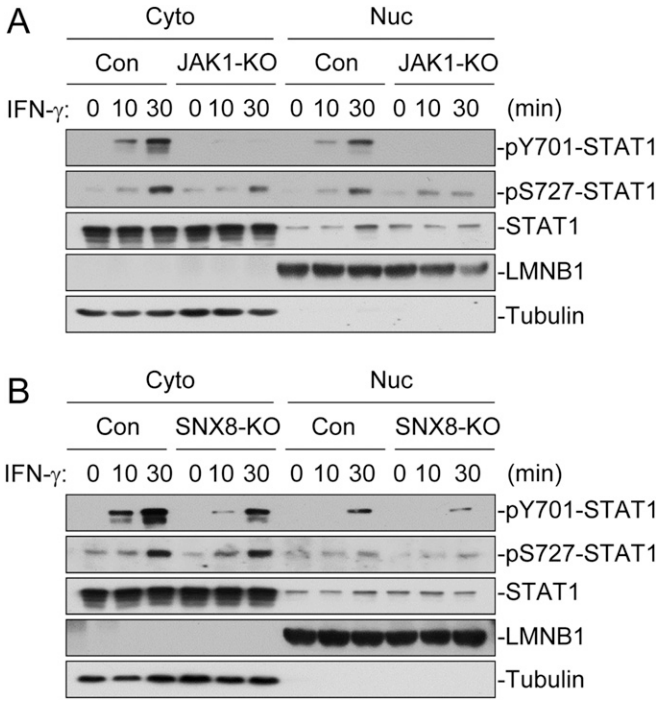


**Fig. S6.** Effects of SNX8 or JAK1 deficiency on the phosphorylation of STAT1 induced by IFNγ. (*A*) Effects of JAK1 deficiency on the phosphorylation of STAT1 induced by IFNγ. HeLa cells were left untreated or treated with IFNγ (100 ng/mL) for the indicated times before cell fractionation assays and immunoblot analysis were performed. (*B*) Effects of SNX8 deficiency on the phosphorylation of STAT1 induced by IFNγ. HeLa cells were left untreated or treated with IFNγ (100 ng/mL) for the indicated times before cell fractionation assays and immunoblot analysis were performed.