Exon trapping
Exon trapping was carried out as previously described[1]. Briefly, we amplified STAT1 genomic DNA (NC_000002) from nucleotides 15844 to 16416 with the following primers: Forward: 5′cgcCTCGAG(Xho-I)gcctaaagtctttggaagttgc3′, Reverse: 5′cgcGGATCC(BamH-I) tgttttgacagggtccattc3'. PCR products and empty pSPL3 plasmids were digested with *Xho*I and *Bam*H1 (New England Biolabs). Plasmids were dephosphorylated and 50ng of purified plasmid was ligated with 250 ng of purified PCR products, with T4 ligase. The ligation products were then used to transform HB101 competent cells (Promega). Plasmids were used to transfect HEK293T and COS7 cells with the Lipofectamine kit (Invitrogen). Cells were harvested 24 hours after transfection, and total RNA extraction, cDNA synthesis and PCR were conducted according to the kit manufacturer's instructions. For analyses of splicing, the amplification mixture included the SD6 and SA2 primers from pSPL3 plasmids.

Electrophoretic mobility shift assay (EMSA)
EMSA was carried out as previously described[2]. Briefly, we incubated 10 µg of nuclear extract with ^{32}P ATP (α-dATP)-labeled GAS (from the FCGR1 promoter) or ISRE (from the ISG15 promoter) probes and subjected the products to electrophoresis in a polyacrylamide gel. Binding activities were quantified with ImageQuant (Amersham Bioscience).

Flow cytometry
EBV-B cells (1 million per experimental point) were incubated for 1 hour in RPMI supplemented with 1% fetal calf serum (1 million cells per ml), and were then activated with 10^5 IU/ml IFN-γ or IFN-α for 30 minutes at 37°C. Activation was stopped by adding 1 ml of cold 1× PBS. The cells were then incubated with 4% PFA for 10 min at room temperature, washed in 1× PBS and incubated with 100% methanol at 4°C for 10 min. The cells were washed twice and incubated with 1× PBS + 1% FCS + 0.1% saponin at 4°C for 10 minutes. They were then washed and incubated for 1 h at 4°C with antibodies against tyrosine-701-phosphorylated-STAT1 (612132, BD Transduction Laboratories) or STAT1 (610116, BD Transduction Laboratories), or with the corresponding isotypic antibodies. The cells were then washed and incubated with Alexa G488 (Molecular Probes, Invitrogen)-labeled secondary antibodies for 20 min at 4°C, washed three times, and the signals were analyzed with a FACS Calibur machine, using CELLQuestTM software (Becton Dickinson).

Viral assays
Viral assays were performed as previously described [3]. Briefly, SV40-fibroblasts were either left untreated or treated with 10^5 IU/ml IFN-α for 18h. The cells were then infected by incubation with vesicular stomatitis virus (VSV) at a multiplicity of infection of 10 for 30 minutes. The remaining inoculum was rinsed away with PBS and DMEM supplemented with 10% FBS was added. Samples were collected at the indicated times and the 50% tissue culture infectious dose (TCID50) was determined on Vero E6 cells.

REFERENCES

1.Chapgier A, Kong XF, Boisson-Dupuis S, et al. A partial form of recessive STAT1 deficiency in humans. *J Clin Invest*. 2009;119:1502–1514.

2.Kong XF, Vogt G, Chapgier A, et al. A novel form of cell type-specific partial IFNgammaR1 deficiency caused by a germ line mutation of the IFNGR1 initiation codon. Hum Mol Genet;19:434-444. 3.Dupuis S, Jouanguy E, Al-Hajjar S, et al. Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. *Nat Genet*. 2003;33:388–391.

Figure S1
(A). Schematic representation of pcDNA3.1 *STAT1* wild-type (WT) anddeletionofexon8 (Δex8) which causes a frame-shift and leads to a premature stop codon in exon 9. Blue arrows show the location of primers to quantify the transcription of transfected cells (B) Western Blotting analysis of protein extracts from 293T primers to quantify the transcription of transfected cells. (B). Western Blotting analysis of protein extracts from 293T or U3C cells transfected with 5μg of mock (M), wild-type (WT), Δex8 plasmids together with EBV-B cells from WT/WT, P1, 1928insA/1928insA subjects. Upper panel, Anti-STAT1 (H95, recognizing N terminal of 69-169aa of STAT1); Lower panel, Anti-GAPDH. (C). Q-PCR analysis of vector STAT1 mRNA transcription level in 293T (upper panel) and U3C (lower panel) cells using specific primers (shown in A) and SYBR green. The results are normalized with respect to the levels of GAPDH mRNA.

Figure S2. U3C cells were transientlyy transfected with a mock pplasmid (M),or with a STAT1 plasmid of the following genotype: wild-type (WT), K201N, E320Q, P696S, L706S
Cells were stimulated with 10^5 IU/ml IFN-α or IFN-γ or left unstimulated for 30 minutes, 48 hours after transfection. Tyr-701-phosphorylated STAT1 and STAT1 levels were measured by western blotting.

Figure S3. U3C cells were transiently transfected with a mock plasmid (M), or a STAT1 plasmid of one of the following genotypes: wild-type (WT), K201N, F77A
A pulse-chase experiment was carried out 48 hours after transfection. Cells were stimulated with 10^5 IU/ml IFN-γ for 30 minutes, and staurosporine was added and the cells were incubated for the time indicated. Tyr-701-phosphorylated STAT1 and STAT1 levels were determined by western blotting.

Figure S4. STAT1 translocation in response to stimulation with 10^3 IU/ml IFN-γ and IFN-α.SV-40 fibroblasts from WT/WT, P1, P696S/P696S, and 1928insA/1928insA subjects were stimulated with 10^5 IU/ml IFN-γ or IFN-α for 30 minutes
An antibody against STAT1 and secondary antibody conjugated with FITC were used to stain the STAT1 molecule.

Figure S5. The K201N STAT1 allele has normal DNA-binding activity after IFN-γ stimulation
U3C cells were transiently transfected with various amounts of WT or K201N expression plasmids. The cells were stimulated with 10^3 IU/ml IFN-γ for 15 minutes, 48 hours after transfection. Nuclear extracts were subjected to EMSA (upper panel) and STAT1 levels were assessed by western blotting (lower panel).

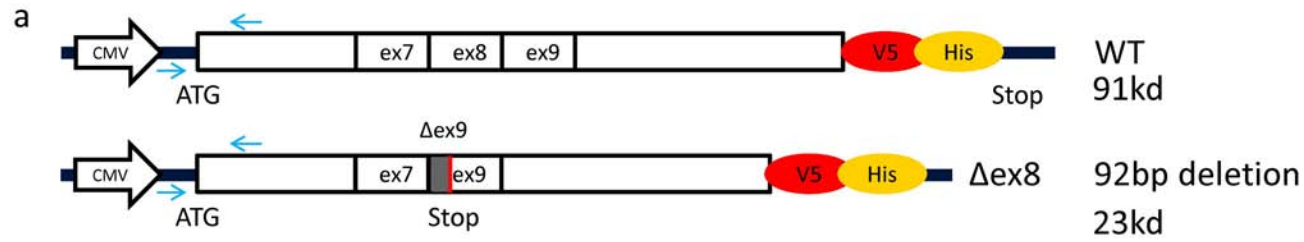
Figure S6. No dominant-negative effect by Δex8onthe GAF DNA bindingactivity of wild type STAT1
U3C cells were transiently co-transfected with various amounts of WT and Δex8 expression plasmids. The cells were stimulated or not with 10^3 IU/ml IFN-γ for 15 minutes, 48 hours after transfection. Nuclear extracts were subjected to EMSA.

Figure S7. The EBV B cells of P1 display normal IFIT1 (A) and SOCS3 (B) mRNA induction after stimulation with 10^3 IU/ml IFN-α. IFIT1 induction is impaired in the STAT1$^{-/-}$ cells, but these cells display normal SOCS3 induction
The results are representative of 3 independent experiments.

Figure S8. Upon stimulation with 100 ng/ml IL-27, the EBV-B cells of P1 displayed impaired CXCL10 induction at 1 hour, as shown byy comparison with healthyy controls, P696S/P696S, and −/− cells displayed a significant and sustained impairment of induction
One red asterisk indicates that P<0.05, three red asterisks indicates that P<0.005. The results are representative of 3 independent experiments

Figure S9. Upon stimulation with 20ng/ml IFN-λ, the EBV-B cells of P1 displayed normal ISG15 induction, as shown by comparison with healthy controls, whereas P696S/P696S and −/− cells displayed a more severe impairment of induction
Three red asterisks indicate that P<0.005. The results are representative of 3 independent experiments.

Figure S1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ex8
ex9
V5
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ATG
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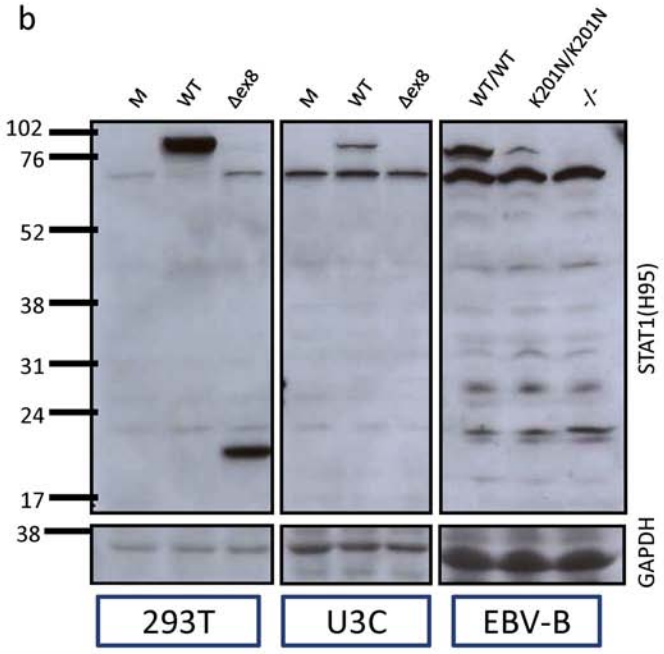
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M
WT
Δex8
M
WT
Δex8
WT/WT
K201N/K201N
-/-
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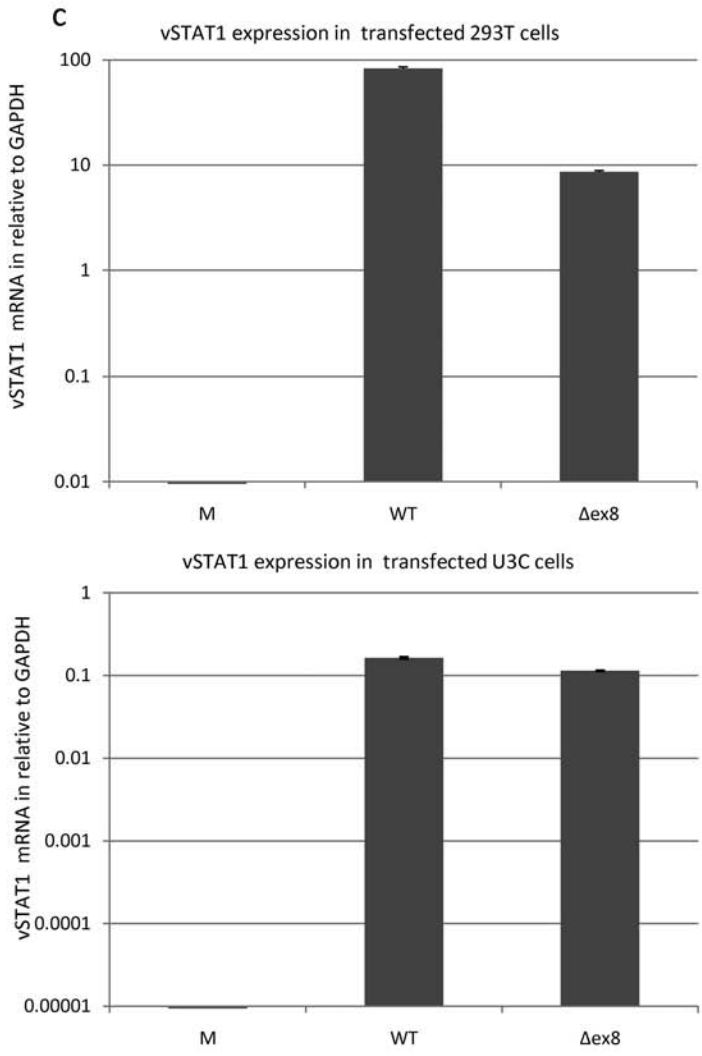
17
38
STAT1(H95)
GAPDH
293T
U3C
EBV-B


c
vSTAT1 expression in transfected 293T cells
vSTAT1 mRNA in relative to GAPDH
100
10
1
0.1
0.01
M
WT
Δex8
vSTAT1 expression in transfected U3C cells
vSTAT1 mRNA in relative to GAPDH
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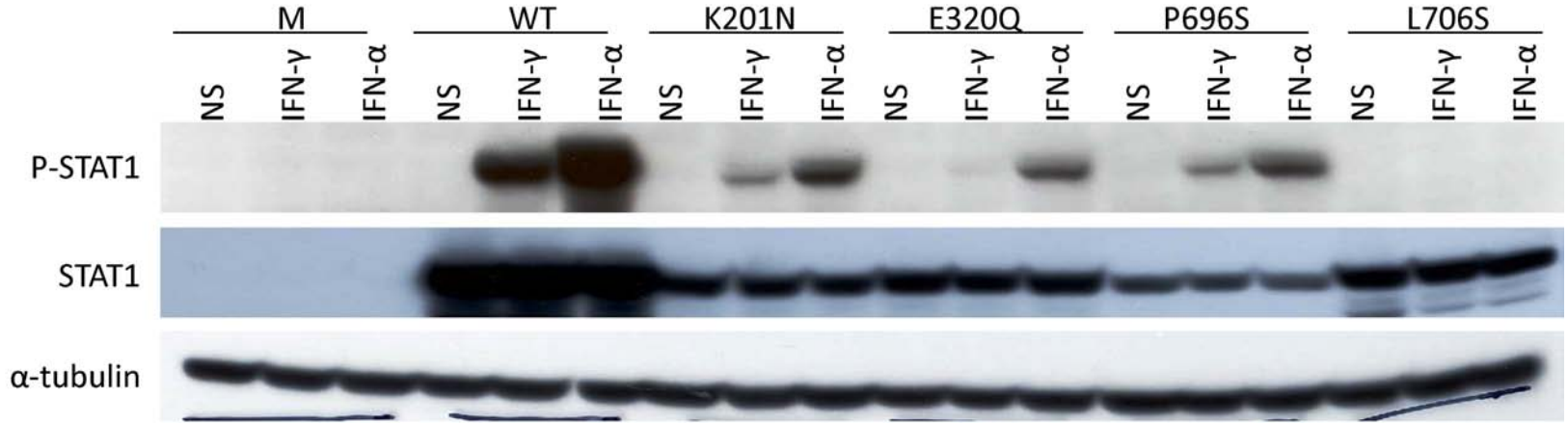
0.1
0.01
0.001
0.0001
0.00001
M
WT
Δex8

Figure S2



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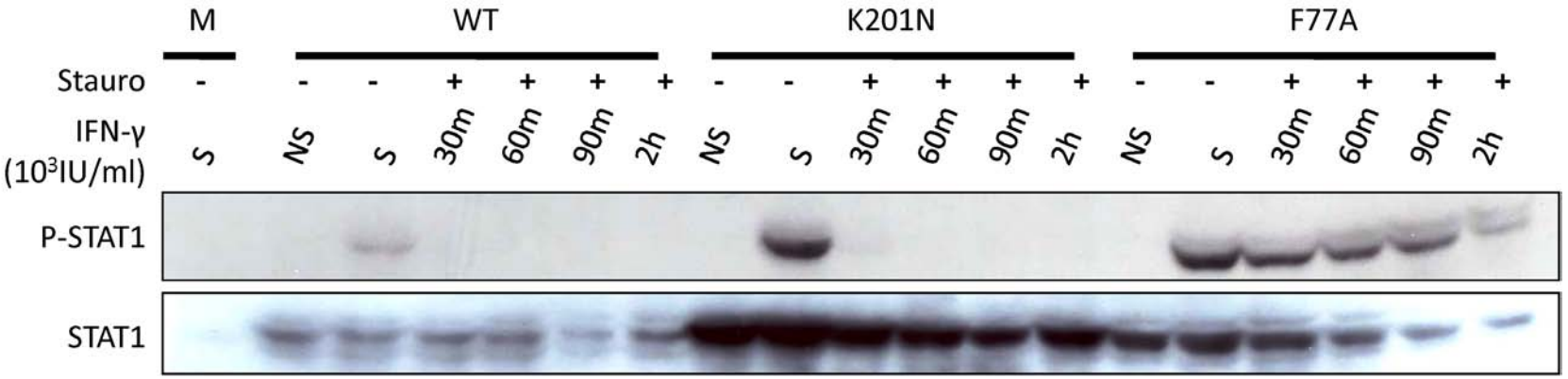

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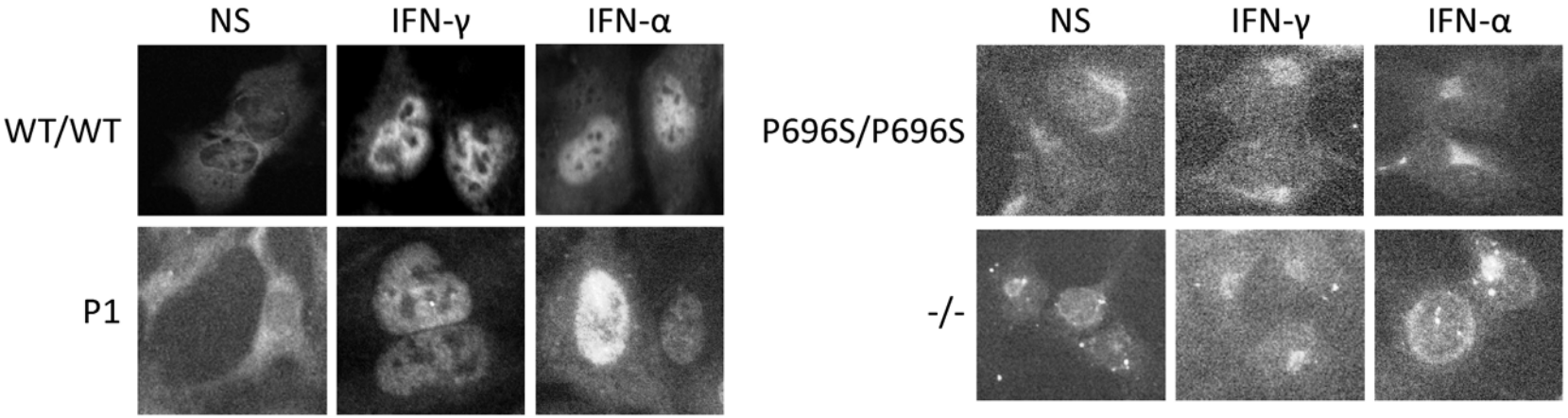

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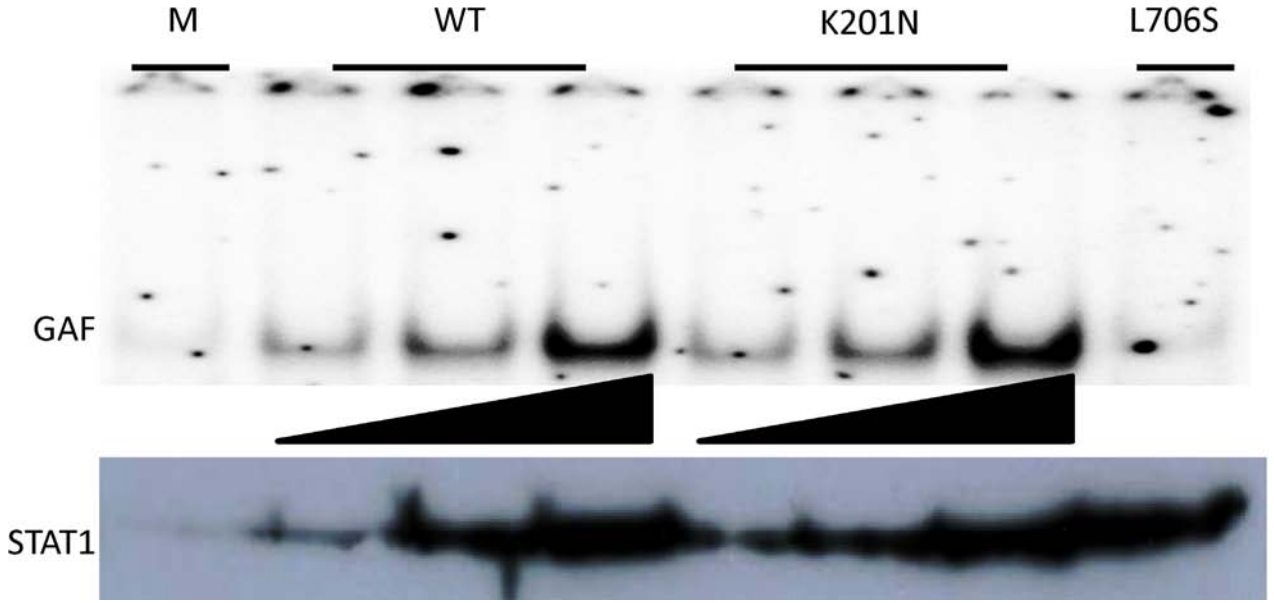

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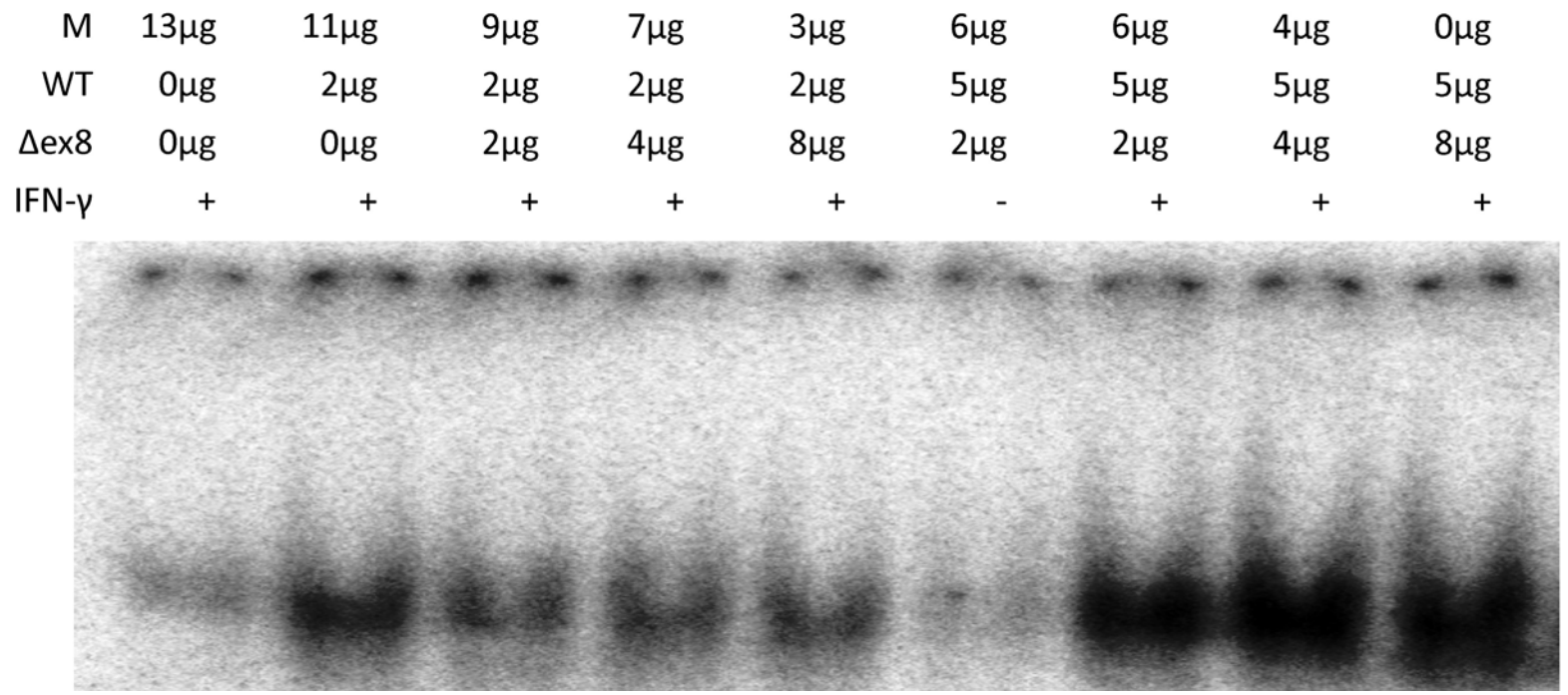
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WT 0μg 2μg 2μg 2μg 2μg 5μg 5μg 5μg 5μg
Δex8 0μg 0μg 2μg 4μg 8μg 2μg 2μg 4μg 8μg
IFN-γ + + + + + - + + +

Figure S7A

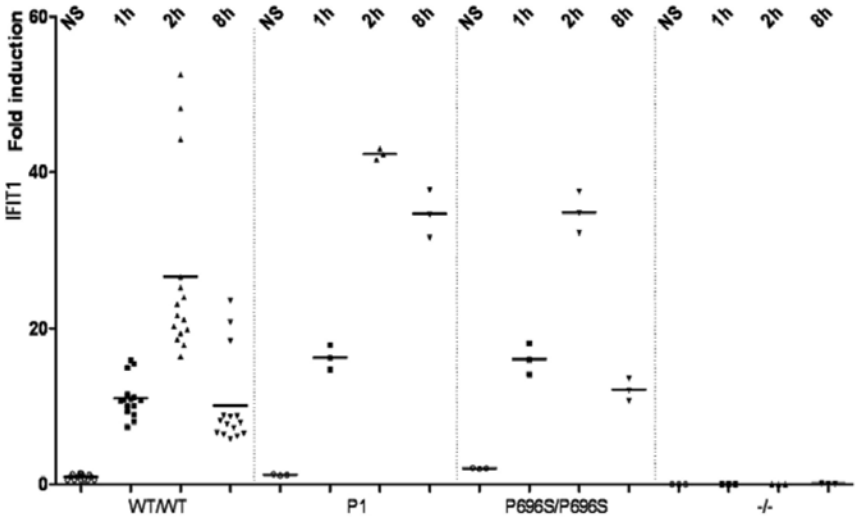


Figure S7B

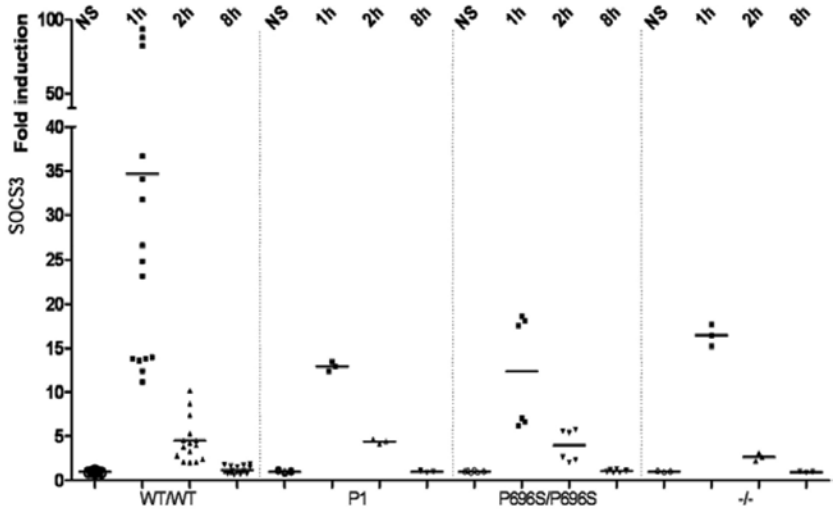


Figure S8

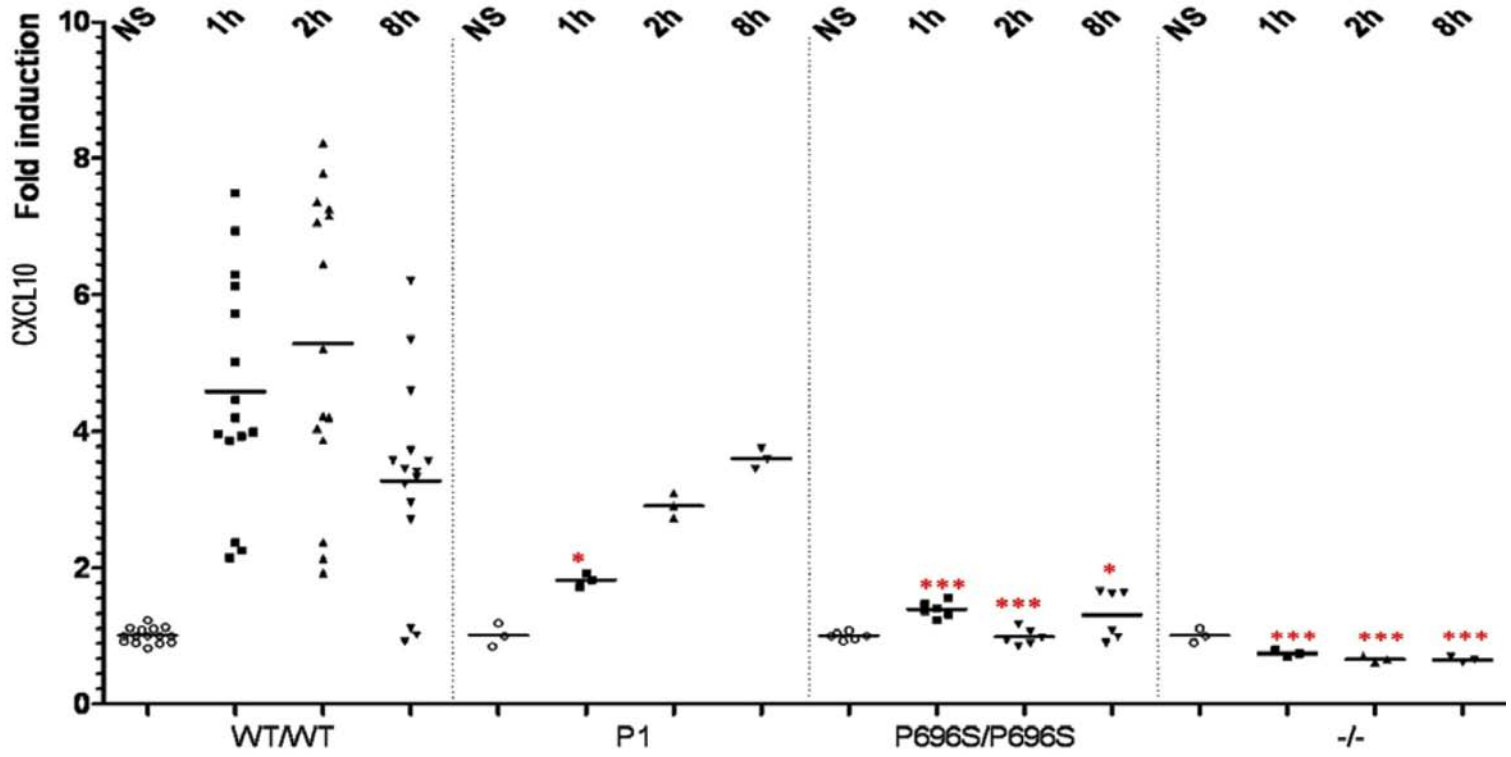
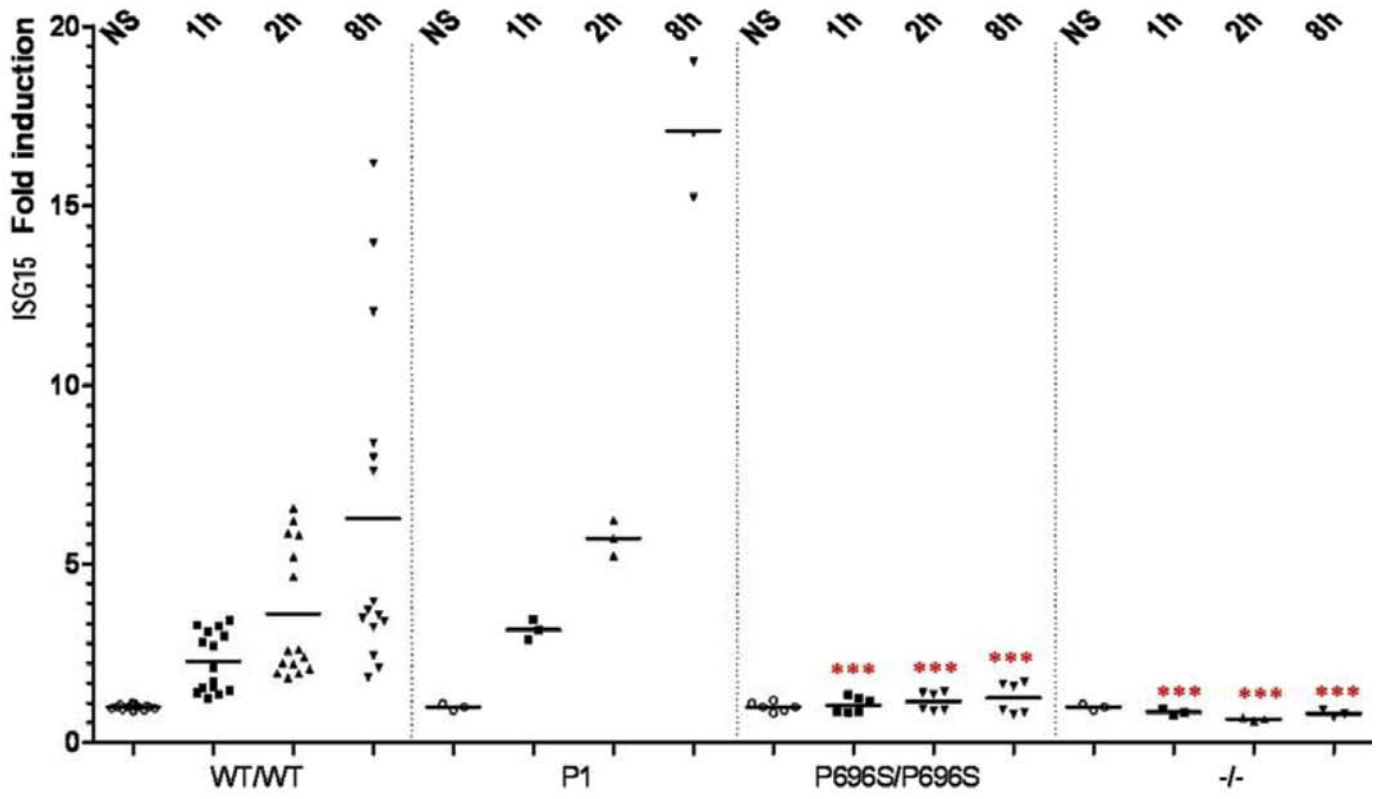

CXCL10 Fold induction
10
8
6
4
2
0
NS
1h
2h
8h
WT/WT
P1
P696S/P696S
-/-
*
***

Figure S9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20
15
10
5
0
NS
1h
2h
8h
***
WT/WT
P1
P696S/P696S
-/-