Supplemental Information

CDK8 Kinase Phosphorylates Transcription Factor STAT1 to Selectively Regulate the Interferon Response

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Figure S1. Analysis of Flavopiridol Effects on IFN- and Stress-Induced S727 Phosphorylation of STAT1 in Fibroblasts (A, B and C), of Flavopiridol Effects on STAT1 Promoter Recruitment (D), and of the Effects of Transcription Inhibition on S727 Phosphorylation of STAT1 (E)

(A) Flavopiridol inhibits IFN-γ- and IFN-β-induced S727 phosphorylation of STAT1 in fibroblasts. Mouse fibroblasts were stimulated for 40 min with IFN-γ or IFN-β after pretreatment or control treatment for 15 min with flavopiridol (FP) (500 nM). Cell extracts were analyzed by Western blotting using antibodies to phosphorylated S727 of STAT1 (pS727-STAT1), phosphorylated
Y701 of STAT1 (pY701-STAT1) and STAT1 C-terminal antibody (not recognizing the STAT1β isoform).

(B) Flavopiridol inhibits IFN-γ- but not stress-induced S727 phosphorylation of STAT1 in fibroblasts. Mouse fibroblasts were stimulated for 40 min with the stress inducer anisomycin (Aniso) or IFN-γ after pretreatment or control treatment for 15 min with flavopiridol (FP). Cell extracts were analyzed as in (A).

(C) Mouse fibroblasts were stimulated for 40 min with IFN-γ or left untreated followed 15 min-pretreatment with flavopiridol (FP) at indicated concentrations. Cell extracts were analyzed as in (A).

(D) STAT1 promoter recruitment is not impaired by flavopiridol. MEFs were stimulated for 30 min with IFN-γ after pretreatment or control treatment for 30 min with flavopiridol (FP). Chromatin recruitment of STAT1 was analyzed by ChIP using STAT1 antibody or unspecific IgG. A representative gel with PCR-amplified Irf1 promoter using immunoprecipitated DNA as template is shown. Total chromatin DNA was amplified for input control.

(E) Ongoing transcription is not required for IFN-γ-induced S727 phosphorylation of STAT1. MEFs were stimulated for 40 min with IFN-γ after pretreatment or control treatment for 15 min with actinomycin D (ActD). Cell extracts were analyzed by Western blotting using antibodies to phosphorylated S727 of STAT1 (pS727-STAT1), phosphorylated Y701 of STAT1 (pY701-STAT1) and total STAT1.
Figure S2. Effects of Cdk7, Cdk8 and Cdk9 Silencing and of Combined Cdk8 and Cdk9 Silencing on STAT1 S727 Phosphorylation (A, B, D and E), and Silver-Stained Gels of Purified Kinase Complexes Used for In Vitro Kinase Assays (C)

(A) Quantitative analysis of effects of Cdk7, Cdk8 and Cdk9 silencing on IFN-γ-induced STAT1 S727 phosphorylation. MEFs were treated for 48 h with siRNA to Cdk7, Cdk8, Cdk9 or control siRNA (Ctrl) and subsequently stimulated for 40 min with IFN-γ. Cell extracts of three experiments were analyzed by Western blotting using antibodies to phosphorylated S727 of STAT1 (pS727-STAT1), phosphorylated Y701 of STAT1 (pY701-STAT1) and total STAT1. The Western blot signal was quantified using the Odyssey Imager (LI-COR Biosciences), and the pS727 signals were normalized to total STAT1 signals. Values of the normalized pS727 signals
for the individual Cdk siRNA relative to control siRNA (Ctrl) are depicted. Error bars represent standard deviations (SDs) (n = 3).

(B) Combined silencing of Cdk8 and Cdk9 does not result in more efficient inhibition of IFN-γ-induced STAT1 S727 phosphorylation than silencing of Cdk8 alone. MEFs were treated for 48 h with siRNA to Cdk8, Cdk9, both Cdk8 + Cdk9 (Cdk8/9), or control siRNA (Ctrl) and subsequently stimulated for 40 min with IFN-γ or left unstimulated. Cell extracts were analyzed by Western blotting using antibodies to phosphorylated S727 of STAT1 (pS727-STAT1), phosphorylated Y701 of STAT1 (pY701-STAT1) and total STAT1. Silencing was confirmed by Western blotting of the same extracts using antibodies to CDK8 and CDK9. Equal loading was controlled by antibodies to ERK1/ERK2 (pan ERK).

(C) Silver stained gels of purified CDK8 module, TFIIH, and P-TEFb used for kinase assays.

(D) Stress-induced STAT1 S727 phosphorylation is not impaired in fibroblasts silenced for Cdk8. Fibroblasts silenced for the expression of Cdk8 or control-silenced cells were treated for 30 min with anisomycin (Aniso) or IFN-γ or left untreated. Cell extracts were analyzed using antibodies to phosphorylated S727 of STAT1 (pS727-STAT1), phosphorylated Y701 of STAT1 (pY701-STAT1) and total STAT1. Note that anisomycin does not cause Y701 phosphorylation.
Figure S3. Recruitment of S727-Phosphorylated STAT1 to Gbp2 (A, B) and Tap1 (C, D) Promoters and to Chromatin (F), STAT1-Dependent Recruitment of CDK8 to TSS and Gene Body of Irf1 (E) and Effect of Missing TAD on Recruitment of MED1 and RNAPII (G)

(A–D) Recruitment of STAT1 precedes accumulation of S727-phosphorylated STAT1 at the Gbp2 and Tap1 promoters. Mouse fibroblasts were treated for indicated times with IFN-γ or left untreated. Association of STAT1 and S727-phosphorylated STAT1 with the Gbp2 (A) and Tap1 (C) promoters was examined by ChIP using respective antibodies or unspecific IgG. A representative gel with PCR-amplified Gbp2 or Tap1 promoters using immunoprecipitated DNA as template is shown. Total chromatin DNA was amplified for input control. Quantitative analysis (using ImageJ) of data shown in (A) and (C) is depicted in (B) and (D), respectively: the pS727 signal was normalized to input DNA after subtracting the signals of IgG.

(E) IFN-γ induces STAT1-dependent CDK8 recruitment to the Irf1 transcription start site (TSS) but not to the gene body. WT and STAT1−/− MEFs (right panel) were treated for indicated times
with IFN-γ, and CDK8 recruitment to the TSS or the gene body of the *Irf1* gene was determined by ChIP. CDK8 increased after IFN-γ treatment at the start site but not at the gene body in WT cells. No increase of CDK8 was observed in STAT1−/− cells throughout the gene. CDK8 recruitment was determined by ChIP using CDK8 antibodies or unspecific IgG. Immunoprecipitated DNA was analyzed by qPCR for the *Irf1* TSS and gene body (8 kb downstream of TSS), carried out in triplicates. Signals were normalized to input DNA. Error bars represent standard deviations (SDs) (n = 3).

(F) Accumulation of S727-phosphorylated STAT1 on chromatin reaches a plateau at approximately 60 min of IFN-γ treatment. MEFs were treated for indicated times with IFN-γ and processed as for ChIP analysis. The chromatin fractions were analyzed by Western blotting using antibodies to phosphorylated S727 of STAT1 (pS727-STAT1), phosphorylated Y701 of STAT1 (pY701-STAT1) and total STAT1 (left panel), and quantitated using Image Studio (Odyssey system from LI-COR) by normalizing the pS727-STAT1 signal to the STAT1 signal (right panel).

(G) MED1 and RNAPII are less efficiently recruited by STAT1beta than by STAT1alpha. MEFs derived from mice expressing solely STAT1alpha or STAT1beta were treated for indicated times with IFN-γ. Association of MED1 (left panel) and RNAPII (right panel) with the *Irf1* TSS was examined by ChIP. Error bars represent SDs (n = 3).
Figure S4. Measurement of mRNA Stability (A), Total RNA Fractions from Analysis of Newly Transcribed mRNA (B) and Amounts of Primary Transcripts (C)

(A) *Irf1, Gbp2* and *Tap1* mRNA stability in STAT1 WT and S727A cells. STAT1 WT and S727A MEFs were treated with IFN-γ for 4 h and actinomycin D (act D) was added for the indicated times. RNA was isolated, and the amounts of *Irf1, Gbp2* and *Tap1* mRNA were determined by qRT-PCR. Relative values to *Hprt* are shown. Error bars represent standard deviations (SDs) (n = 3).
(B) 4sU was added to the cell culture medium simultaneously with IFN-γ, or 60 min and 210 min after IFN-γ stimulation, or without IFN-γ treatment. The labeling was performed in WT and S727A MEFs for 30 min followed by RNA isolation and separation to collect the 4sU-labeled RNA fractions and total RNA fractions. Total RNA representing accumulated RNA at 0, 30 min, 90 min and 240 min of IFN-γ treatment is shown for Irf1, Tap1, Gbp2, Irf8 and Isg15. mRNA was quantitated by qRT-PCR. ActB was used for normalization. Error bars represent SDs (n = 3).

(C) S727A mutation affects levels of primary transcripts. STAT1 WT and S727A MEFs were treated with IFN-γ for 4 h or left untreated. RNA was isolated, and the amounts of primary transcripts of Tap1 and Gbp2 genes were determined by qRT-PCR for intronic amplicons. Relative values to ActB are shown. Error bars represent standard deviations (SDs) (n = 3).
Figure S5. Schematic Representation of the \textit{Irf1}, \textit{Gbp2} and \textit{Tap1} Gene Loci (A) and Positions of ChIP Amplicons and Relative Abundance of S2-Phosphorylated RNAPII at the TSS and the Gene Body of \textit{Irf1} and \textit{Tap1} (C)

(A) Positions of the regulatory GAS and ISRE elements are marked. Note that GAS and ISRE in the Tap1 gene are downstream of the transcription start site. "Stop" marks the end of \textit{Irf1} (GenBank NM_001159393.1) and \textit{Tap1} (GenBank NM_013683.2) transcripts. Amplicons for ChIP analyses are depicted as TSS (transcription start site), GAS (GAS elements) and gene body with the approximate position relative to TSS.

(B) Ratios of S2-phosphorylated RNAPII to total RNAPII at TSS and the gene body of the \textit{Irf1} and \textit{Tap1} genes. WT MEFs treated for indicated times with IFN-\gamma were examined by ChIP using antibodies to RNAPII and pS2-RNAPII. Immunoprecipitated DNA was analyzed by qPCR for the both TSS and the gene body of \textit{Irf1} and \textit{Tap1}, normalized to input DNA, and the ratios S2-phosphorylated RNAPII to total RNAPII were calculated. Error bars represent SDs (n = 3).
Figure S6. Control Experiments Showing Silencing Efficiency for *Cdk8* and *CycC* (A and D), Fold Differences in Microarray (C) and no Effects of the Transfection Procedure (B and E)

(A) Silencing efficiency of *Cdk8* by siRNA in microarray experiments. Efficiency of silencing of *Cdk8* in STAT1 WT (left panel) or STAT1 S727A (right panel) cells stimulated with IFN-γ or left unstimulated was assessed with measurements of *Cdk8* mRNA levels using qRT-PCR. Relative values to *Hprt* are shown. Error bars represent standard deviations (SDs) of biological triplicates (n = 3).
(B) siRNA transfection procedure has no effect on Cdk8 expression. STAT1 WT and STAT1 S727A cells were left untreated (no transf.), treated with transfection reagent alone (transf. reagent), control siRNA (siCtrl), siRNA for Cdk8 (siCdk8), or siRNA for CycC (siCycC). Cdk8 mRNA levels were determined by qRT-PCR. Relative values to Hprt are shown. Error bars represent standard deviations (SDs) (n = 3).

(C) Differences in expression of Irf1, Tap1, Gbp2 and Irf8 observed in the microarray experiment shown in Figure 7A and 7B. Differences in IFN-γ-induced expression levels of the indicated genes between siCdk8- and siCtrl-treated WT and S727A cells are depicted. The values are antilog numbers extracted from Table S2.

(D) Silencing efficiency of CycC by siRNA in experiments showing similar effect of CycC and CDK8 on expression of IFN-γ-induced genes. Efficiency of silencing of CycC in STAT1 WT (left panel) or STAT1 S727A (right panel) cells stimulated with IFN-γ or left unstimulated was controlled by measurements of CycC mRNA levels using qRT-PCR. Relative values to Hprt are shown. Error bars represent standard deviations (SDs) (n = 3).

(E) The procedure of siRNA transfection does not alter sensitivity of cells to VSV infection. STAT1 WT cells were treated with control siRNA (siCtrl) or were left untreated. After 24 h of incubation with IFN-γ at indicated concentrations cells were infected with VSV (MOI = 0.1), and the cell survival was monitored as in (Figure 7F).
Table S1. IFN-γ-Induced Genes Significantly (p < 0.05) Downregulated at Least 2-fold by the STAT1 S727A Mutation

| Genbank AC   | Gene symbol         | WT-g/WT-0 | SA-g/WT-g |
|--------------|---------------------|-----------|-----------|
| NM_001034859 | Gm4841              | 9.8       | -3.0      |
| NM_001033767 | Gm4951              | 8.7       | -1.1      |
| NM_010260    | Gbp2                | 8.1       | -1.8      |
| NM_018734    | Gbp3                | 7.0       | -1.3      |
| NM_194336    | Mpa2l               | 6.8       | -2.7      |
| NM_001101475 | F830016B08Rik       | 6.5       | -2.0      |
| NM_145545    | Gbp6                | 6.3       | -1.0      |
| NM_001039647 | Gbp11               | 6.3       | -2.9      |
| NM_001168294 | Serpina3f          | 6.2       | -1.1      |
| NM_010724    | Psmb8               | 6.1       | -1.6      |
| NM_013585    | Psmb9               | 5.7       | -2.1      |
| NM_001135115 | Gm12250             | 5.2       | -1.1      |
| NM_030738    | Vmn1r65             | 5.2       | -1.6      |
| NM_001037713 | Xaf1                | 4.9       | -1.1      |
| NM_194336    | Mpa2l               | 4.8       | -2.3      |
| NM_013683    | Tap1                | 4.8       | -2.2      |
| NM_001256005 | Gbp4                | 4.6       | -3.2      |
| NM_008320    | Irf8                | 4.6       | -2.1      |
| NM_009277    | Trim21              | 4.2       | -1.4      |
| NM_009896    | Socs1               | 3.6       | -1.0      |
| NM_001045540 | Gm12185             | 3.5       | -1.2      |
| NM_011530    | Tap2                | 3.2       | -2.2      |
| NM_177820    | Apol10b             | 2.9       | -1.9      |
| NM_020557    | Cmpk2               | 2.9       | -2.1      |
| NM_021443    | Cci8                | 2.5       | -2.0      |
| NM_181545    | Slfn8               | 2.5       | -1.8      |
| NM_020557    | Cmpk2               | 2.5       | -1.8      |
| NM_023835    | Trim12a             | 2.5       | -1.5      |
| NM_001199940 | Serpina3i           | 2.3       | -1.2      |
| NM_001081083 | Armc3               | 2.2       | -1.6      |
| NM_023835    | Trim12a             | 2.1       | -1.4      |
| ENSMUST00000114230 | Psmb9 | 1.9 | -1.0 |
| NM_199146    | Trim30d             | 1.9       | -2.4      |
| NM_001037713 | Xaf1                | 1.9       | -1.3      |
| XM_136331    | Gm4955              | 1.9       | -1.3      |
| NM_001146007 | 9230105E10Rik       | 1.7       | -1.6      |
| NM_181542    | Slfn10-ps           | 1.7       | -1.3      |
| NM_001146007 | 9230105E10Rik       | 1.7       | -1.3      |
| NM_001013828 | Iligp1b             | 1.6       | -1.0      |
| XM_136331    | Gm4955              | 1.6       | -1.4      |
| Genbank ID     | Gene Symbol | SA    | WT    |
|---------------|-------------|-------|-------|
| NM_011333     | Ccl2        | 1.6   | -1.6  |
| NM_029419     | Apol7a      | 1.6   | -1.8  |
| NM_013819     | H2-M3       | 1.6   | -1.5  |
| NM_001146007  | 9230105E10Rik | 1.5  | -1.3  |
| NM_183087     | Fam189a1    | 1.5   | -3.3  |
| NM_001039646  | Gbp10       | 1.4   | -1.3  |
| NM_178745     | Tmem229b    | 1.4   | -1.6  |
| NM_007987     | Fas         | 1.4   | -1.7  |
| NM_025829     | Eif4e3      | 1.4   | -2.3  |
| NM_013654     | Ccl7        | 1.4   | -2.3  |
| NM_001190466  | Dact1       | 1.3   | -3.6  |
| NM_053109     | Clec2d      | 1.3   | -1.1  |
| NR_033577     | Gm8221      | 1.3   | -1.3  |
| NM_010171     | F3          | 1.3   | -2.1  |
| NM_175291     | Dock10      | 1.3   | -2.1  |
| NM_001190466  | Dact1       | 1.3   | -3.2  |
| NM_010555     | Ii1r2       | 1.1   | -1.3  |
| NM_033541     | Oas1c       | 1.1   | -1.9  |
| NM_001083925  | Oas1b       | 1.1   | -1.1  |
| NM_008362     | Ii1r1       | 1.0   | -1.4  |
| NM_029419     | Apol7a      | 1.0   | -1.0  |

Genes are ordered according to their induction (log 2-transformed) by IFN-γ in WT cells (column: WT-g/WT-0). Fold difference (log 2) between S727A (SA) and WT cells is shown in the column "SA-g/WT-g". Genes that appear more than once have different probe set IDs for each listing. Data are extracted from the Table S4.
Table S2. IFN-γ-Induced Genes Significantly (p < 0.05) Upregulated at Least 2-fold by the STAT1 S727A Mutation

| Genbank AC | Gene symbol | WT-g/WT-0 | SA-g/WT-g |
|------------|-------------|-----------|-----------|
| NM_021274  | Cxcl10      | 6.2       | 2.6       |
| NM_029000  | Gvin1       | 6.0       | 4.3       |
| NM_009251  | Serpina3g   | 5.3       | 1.2       |
| NM_197986  | Tmem140     | 4.4       | 1.1       |
| NM_001045481 | Ifi203 | 4.2       | 2.6       |
| NM_028967  | Batf2       | 4.1       | 1.7       |
| NM_008329  | Ifi204      | 4.0       | 2.8       |
| NM_015783  | Isg15       | 4.0       | 1.3       |
| NM_015783  | Isg15       | 3.9       | 1.5       |
| NM_001033450 | Mnda | 3.7       | 2.3       |
| NM_001004174 | AA467197   | 3.6       | 4.0       |
| BC010546   | Ifi204      | 3.5       | 2.6       |
| AK019325   | Gm9706      | 3.4       | 1.3       |
| NM_029000  | Gvin1       | 3.3       | 3.9       |
| NM_007609  | Casp4       | 3.1       | 3.5       |
| XM_001000891 | Gm1966 | 3.0       | 4.2       |
| NM_007609  | Casp4       | 2.9       | 3.5       |
| NM_001045481 | Ifi203 | 2.9       | 2.5       |
| NM_001204910 | A607873 | 2.8       | 2.8       |
| NM_001139519 | Zbp1 | 2.7       | 1.6       |
| XM_003084464 | Gm16340 | 2.5       | 2.8       |
| NM_178005  | Lrtrm2      | 2.3       | 1.0       |
| NM_033601  | Bcl3        | 2.1       | 2.3       |
| NM_178446  | Rbm47       | 2.1       | 1.9       |
| NM_172393  | Aim1        | 2.0       | 3.6       |
| NM_199015  | D14Ertd668e | 1.9       | 5.1       |
| NM_023137  | Ubd         | 1.9       | 1.3       |
| ENSMUST00000104958 | Psme2 | 1.8       | 2.3       |
| AK087205   | 9530082P21Rik | 1.7   | 1.1       |
| NM_175475  | Cyp26b1     | 1.7       | 1.9       |
| AK156907   | Cxcl10      | 1.5       | 2.1       |
| NM_011019  | Osmr        | 1.4       | 1.1       |
| NM_013498  | Crem        | 1.4       | 2.2       |
| NM_008381  | Inhb        | 1.4       | 1.9       |
| NM_009344  | Phlda1      | 1.3       | 1.9       |
| NM_013498  | Crem        | 1.3       | 2.0       |
| NM_178446  | Rbm47       | 1.2       | 1.8       |
| NM_010392  | H2-Q2       | 1.2       | 1.0       |
| NM_010942  | Nsg1        | 1.2       | 2.1       |
| NM_178005  | Lrtrm2      | 1.2       | 1.0       |
| NM_172875  | Adc         | 1.2       | 1.5       |
| NM_026637  | Ggct        | 1.1       | 1.5       |
| Gene ID     | Gene Symbol | WT-g/WT-0 | SA-g/WT-g |
|------------|-------------|-----------|-----------|
| NM_009256  | Serpinb9    | 1.1       | 2.1       |
| NM_011580  | Thbs1       | 1.1       | 1.5       |
| NM_009780  | C4b         | 1.0       | 1.9       |
| NM_001033207 | Nlr5       | 1.0       | 1.2       |
| NM_008599  | Cxcl9       | 1.0       | 2.2       |
| NM_207648  | H2-Q6       | 1.0       | 1.1       |

Genes are ordered according to their induction (log 2-transformed) by IFN-γ in WT cells (column: WT-g/WT-0). Fold difference (log 2) between S727A (SA) and WT cells is shown in the column "SA-g/WT-g". Genes that appear more than once have different probe set IDs for each listing. Data are extracted from the Table S4.
Table S3. IFN-γ-Induced Genes Not Significantly Affected by the STAT1 S727A Mutation

| Genbank AC   | Gene symbol | WT-g/WT-0 |
|--------------|-------------|-----------|
| NM_001145164 | Tgtp2       | 9.5       |
| NM_018738    | Igtp        | 8.6       |
| NM_001146275 | Igtp1       | 7.7       |
| NM_019440    | Irgm2       | 7.5       |
| NM_001033207 | Nlrc5       | 6.8       |
| NM_011854    | Oas1l2      | 6.7       |
| NM_008330    | Ifi47       | 6.1       |
| NM_008326    | Irgm1       | 5.8       |
| NM_011579    | Tgtp1       | 5.7       |
| NM_021893    | Cd274       | 5.7       |
| NM_001145164 | Tgtp2       | 5.5       |
| NM_001039530 | Parp14      | 5.4       |
| NM_008326    | Irgm1       | 5.2       |
| ENSMUST00000073997 | BC023105 | 5.2       |
| NM_001168294 | Serpina3f   | 5.0       |
| NM_023386    | Rtp4        | 5.0       |
| NM_008331    | Ifit1       | 4.7       |
| NM_001146275 | Igtp1       | 4.5       |
| NM_008390    | Ifit1       | 4.3       |
| NM_030253    | Parp9       | 4.2       |
| NM_010501    | Ifit3       | 4.1       |
| NM_001013371 | Dtx3l       | 4.1       |
| NM_001163576 | Parp10      | 3.8       |
| AK034303     | 9330175E14Rik | 3.8       |
| NM_001005858 | 1830012O16Rik | 3.7       |
| NM_001168660 | Apol9b      | 3.5       |
| NM_001199940 | Serpina3i   | 3.5       |
| NM_009283    | Stat1       | 3.3       |
| NM_011909    | Usp18       | 3.3       |
| NM_019963    | Stat2       | 3.3       |
| NM_001163621 | Apol6       | 3.2       |
| NM_029219    | Rnf19b      | 3.1       |
| NM_173786    | Apol9a      | 3.1       |
| NM_173786    | Apol9a      | 3.1       |
| NM_013606    | Mx2         | 3.0       |
| NM_001114679 | 9930111J21Rik1 | 3.0       |
| NM_001159417 | Ifr9        | 3.0       |
| NM_016850    | Ifr7        | 2.9       |
| NM_030684    | Trim34a     | 2.9       |
| NR_033332    | Gm12216     | 2.8       |
| AK135804     | Gm10839     | 2.7       |
| NM_023279    | Tubb3       | 2.7       |
| NM_027835    | Ifih1       | 2.7       |
| Gene ID       | Gene Symbol | Expression |
|--------------|-------------|------------|
| NM_021394    | Zbp1        | 2.7        |
| NM_172729    | Nod1        | 2.6        |
| NM_008358    | Il15rA      | 2.6        |
| NM_010156    | Samd9l      | 2.6        |
| NM_023738    | Uba7        | 2.6        |
| NM_013640    | Psmb10      | 2.5        |
| NM_001005858 | Il830012O16Rik | 2.5     |
| NM_145391    | Tappbpl     | 2.5        |
| NM_010156    | Samd9l      | 2.4        |
| NM_010821    | Mpeg1       | 2.4        |
| ENSMUST00000030584 | Rnf19b   | 2.4        |
| NM_029084    | Slamf8      | 2.4        |
| AK090152     | Dtx3l       | 2.3        |
| NM_025992    | Herc6       | 2.2        |
| NM_025992    | Herc6       | 2.2        |
| NM_198095    | Bst2        | 2.2        |
| NM_172893    | Parp12      | 2.1        |
| NM_001013371 | Dtx3l       | 2.1        |
| NM_010681    | Lama4       | 2.1        |
| ENSMUST000000102642 | Ube2l6 | 2.1        |
| NM_153564    | Gbp5        | 2.0        |
| NM_009396    | Tnfaip2     | 2.0        |
| NM_145227    | Oas2        | 2.0        |
| NM_172689    | Ddx58       | 2.0        |
| NM_172689    | Ddx58       | 2.0        |
| NM_009396    | Tnfaip2     | 2.0        |
| NM_175449    | Fam26f      | 1.9        |
| ENSMUST00000093902 | Rnf213 | 1.9        |
| NM_008223    | Serpind1    | 1.9        |
| NM_199252    | Unc93a      | 1.8        |
| AK017289     | 5430410E06Rik | 1.8    |
| NM_001141949 | Nmi         | 1.8        |
| NM_027320    | Il15        | 1.8        |
| NM_001025313 | Tapbp       | 1.8        |
| NM_009808    | Casp12      | 1.7        |
| NM_011486    | Stat3       | 1.7        |
| NM_001159393 | Irf1        | 1.7        |
| NM_011832    | Insrr       | 1.6        |
| NM_146125    | IltpkA      | 1.6        |
| NM_008357    | Il15        | 1.6        |
| NR_045032    | Gdap10      | 1.6        |
| NM_019949    | Ube2l6      | 1.6        |
| NM_001005748 | Phactr1     | 1.6        |
| NM_001005748 | Phactr1     | 1.6        |
| NM_198004    | 5133401N09Rik | 1.6    |
| NM_001177351 | AW112010    | 1.5        |
| Gene ID     | Description | Fold Change |
|------------|-------------|-------------|
| NM_213659  | Stat3       | 1.5         |
| NM_126166  | Tlr3        | 1.5         |
| NM_145211  | Oas1a       | 1.5         |
| NM_009546  | Trim25      | 1.5         |
| NM_027383  | Ilf1h1      | 1.5         |
| ENSMUST00000170226 | Plec | 1.5         |
| NM_183162  | BC006779    | 1.5         |
| ENSMUST00000112869 | Prune2 | 1.5         |
| NM_001005748 | Phactr1 | 1.4         |
| NM_011163  | Eif2ak2     | 1.4         |
| NM_010531  | I18bp       | 1.4         |
| NM_018851  | Samhd1      | 1.4         |
| NM_194346  | Rnf31       | 1.4         |
| NM_194346  | Rnf31       | 1.3         |
| NM_026913  | Mitd1       | 1.3         |
| NM_183426  | Sbno2       | 1.3         |
| NM_001033196 | Znfx1    | 1.3         |
| NM_011190  | Psme2       | 1.3         |
| NM_001139520 | Samhd1 | 1.3         |
| NM_009895  | Cish        | 1.3         |
| NM_028261  | Tmem173     | 1.3         |
| NM_001025208 | LOC547349 | 1.3         |
| NM_010398  | H2-T23      | 1.3         |
| NM_008230  | Hdc         | 1.3         |
| NM_011710  | Wars        | 1.3         |
| NM_011190  | Psme2       | 1.3         |
| NM_007707  | Socs3       | 1.3         |
| NM_001014996 | Cenpj  | 1.3         |
| NM_009834  | Ccrn4l      | 1.3         |
| NM_008744  | Ntn1        | 1.3         |
| ENSMUST000000100186 | LOC100503847 | 1.2       |
| NM_001199967 | Gm11127 | 1.2         |
| NM_001243760 | Ciita    | 1.2         |
| NM_001038587 | Adar    | 1.2         |
| NM_028035  | Snx10       | 1.2         |
| ENSMUST00000054384 | Trim56 | 1.2         |
| NM_001141981 | Rbm43   | 1.2         |
| NM_001164314 | Wars    | 1.2         |
| NM_028807  | 1200009i06Rik | 1.2      |
| NM_028035  | Snx10       | 1.2         |
| NM_030743  | Rnf114      | 1.1         |
| NM_201373  | Trim56      | 1.1         |
| NM_030711  | Erap1       | 1.1         |
| NM_001243916 | Trim34b   | 1.1         |
| NM_172767  | Vwa5a       | 1.1         |
| NM_172767  | Vwa5a       | 1.1         |
| Gene ID         | Gene Symbol or Name  | Induction |
|----------------|----------------------|-----------|
| NM_001001892   | H2-K1                | 1.1       |
| NM_145153      | Oas1f                | 1.1       |
| NM_011189      | Psme1                | 1.1       |
| NM_011390      | Slc12a7              | 1.1       |
| NM_010392      | H2-Q2                | 1.1       |
| NM_144830      | Tmem106a             | 1.1       |
| NM_011150      | Lgals3bp             | 1.1       |
| NM_001033136   | Fam82a2              | 1.0       |
| NM_181402      | Parp11               | 1.0       |
| NM_001081032   | Gm8909               | 1.0       |
| NM_010393      | H2-Q5                | 1.0       |
| NR_038025      | 4933412E12Rik        | 1.0       |

Genes are ordered according to their induction (log 2-transformed) by IFN-γ in WT cells (column: WT-g/WT-0). Genes that appear more than once have different probe set IDs for each listing. Data are extracted from the Table S4.
Table S4. Microarray Evaluation Showing the Effect of S727A Mutation and Cdk8 Silencing on Expression of IFN-γ-Induced Genes

| Probe set ID | Genbank AC | Gene symbol | Gene name | WT-siCtrl-g/WT-siCtrl-0 | SA-siCtrl-g/SA-siCtrl-0 | SA-siCdk8-0/SA-siCtrl-0 | WT-siCdk8-0/WT-siCtrl-0 | SA-siCdk8-0/SA-siCtrl-0 | p-value: WT-siCtrl-g/WT-siCtrl-0 | p-value: SA-siCtrl-g/WT-siCtrl-0 |
|--------------|------------|-------------|-----------|-------------------------|------------------------|-------------------------|------------------------|------------------------|-----------------------------|-----------------------------|
| A_55_P1981461 | AK017289   | 5430410E06Rik | RIKEN cDNA 5430410E06 gene | 1.8 | 1.7 | 0.8 | 0.0 | -0.1 | 0.0 | 0.3 | 0.00 | 0.00 |
| A_66_P101942  | AK019325   | Gm9706      | predicted gene 9706 | 3.4 | 3.9 | 1.3 | -0.2 | -0.1 | -0.1 | -0.4 | 0.00 | 0.01 |
| A_55_P2276224 | AK034303   | 9330175E14Rik | RIKEN cDNA 9330175E14 gene | 3.8 | 2.7 | -0.3 | 0.0 | -0.2 | -0.1 | -0.4 | 0.00 | 0.45 |
| A_55_P2470474 | AK087205   | 9530082P21Rik | RIKEN cDNA 9530082P21 gene | 1.7 | 2.0 | 1.1 | -0.2 | 0.0 | -0.3 | -0.3 | 0.00 | 0.00 |
| A_66_P106060  | AK090152   | Dtx3l       | deltex 3-like (Drosophila) | 2.3 | 1.8 | 0.4 | -0.2 | -0.1 | 0.0 | -0.6 | 0.00 | 0.52 |
| A_55_P2079535 | AK135804   | Gm10839     | predicted gene 10839 | 2.7 | 3.0 | 0.3 | 0.3 | -0.1 | 0.1 | 0.2 | 0.00 | 0.48 |
| A_55_P2016459 | AK156907   | Cxcl10      | chemokine (C-X-C motif) ligand 10 | 1.5 | 3.2 | 2.1 | -0.2 | -0.8 | 0.0 | 0.1 | 0.00 | 0.00 |
| A_55_P2066578 | BC010546   | Ifi204      | interferon activated gene 204 | 3.5 | 2.7 | 2.6 | 0.1 | 0.0 | 0.9 | 0.0 | 0.00 | 0.00 |
| A_66_P124724  | ENSMUST0000030584 | Rnf19b | ring finger protein 19B | 2.4 | 1.9 | 0.2 | 0.0 | -0.4 | 0.1 | 0.1 | 0.00 | 0.48 |
| A_55_P2358679 | ENSMUST0000054384 | Trim56 | tripartite motif-containing 56 | 1.2 | 1.1 | 0.2 | -0.2 | -0.2 | 0.0 | -0.2 | 0.00 | 0.28 |
| A_52_P480044  | ENSMUST0000073997 | BC023105 | cDNA sequence BC023105 | 5.2 | 4.7 | -0.6 | -1.1 | -0.9 | -0.1 | 0.2 | 0.00 | 0.08 |
| A_51_P159503  | ENSMUST0000093902 | Rnf213 | ring finger protein 213 | 1.9 | 1.8 | 0.1 | -0.4 | -0.1 | 0.1 | 0.1 | 0.00 | 0.88 |
| A_55_P2035003 | ENSMUST00000100186 | LOC100503847 | hypothetical LOC100503847 | 1.2 | 1.3 | -0.3 | 0.0 | 0.4 | -0.1 | 0.0 | 0.02 | 0.61 |
| A_55_P2141943 | ENSMUST00000102642 | Ube2l6 | ubiquitin-conjugating enzyme E2L 6 | 2.1 | 2.4 | -0.8 | -0.3 | -0.1 | -0.5 | -0.1 | 0.00 | 0.08 |
| A_55_P2025612 | ENSMUST00000104958 | Psme2 | proteasome (prosome, macropain) 28 subunit, beta | 1.8 | 0.6 | 2.3 | -0.1 | 0.1 | 0.0 | -0.1 | 0.00 | 0.00 |
| A_55_P2042096 | ENSMUST00000112869 | Prune2 | prune homolog 2 | 1.5 | 2.2 | 0.8 | -0.7 | -0.5 | -0.1 | 0.2 | 0.01 | 0.06 |
| Acc   | Gene ID          | Gene Name          | Description                                                                 | 1.9 | 0.8 | -1.0 | -0.4 | -0.1 | 0.1 | -0.2 | 0.00 | 0.00 |
|-------|------------------|--------------------|------------------------------------------------------------------------------|-----|-----|-------|-------|-------|-----|-------|------|------|
| A_55_P2038983 | ENSMUST00000114230 | Psm9              | proteasome (prosome, macropain) subunit, beta type 9 (large multifunctional peptidase 2) | 1.9 | 0.8 | -1.0 | -0.4 | -0.1 | 0.1 | -0.2 | 0.00 | 0.00 |
| A_55_P2006983 | ENSMUST00000170226 | Plec              | plectin                                                                     | 1.5 | 1.4 | 0.0  | 0.0  | 0.1  | 0.2 | 0.2  | 0.0  | 0.98 |
| A_55_P2049647 | NM_001001892     | H2-K1             | histocompatibility 2, K1, K region                                          | 1.1 | 0.6 | 0.9  | -0.2 | -0.2 | 0.1 | 0.0  | 0.00 | 0.00 |
| A_55_P2137049 | NM_001004174     | AA467197          | expressed sequence AA467197                                                | 3.6 | 3.8 | 4.0  | -1.1 | -0.5 | 0.3 | 0.5  | 0.00 | 0.00 |
| A_52_P605517  | NM_001005748     | Phactr1           | phosphatase and actin regulator 1                                           | 1.6 | 0.3 | -0.4 | -0.1 | -0.3 | -0.1 | -0.9 | 0.00 | 0.29 |
| A_51_P182572  | NM_001005748     | Phactr1           | phosphatase and actin regulator 1                                           | 1.6 | 0.3 | -0.5 | -0.3 | -0.3 | -0.2 | -0.9 | 0.00 | 0.17 |
| A_55_P2068233 | NM_001005748     | Phactr1           | phosphatase and actin regulator 1                                           | 1.4 | 0.4 | -0.4 | -0.1 | -0.3 | -0.2 | -0.8 | 0.00 | 0.36 |
| A_66_P117933  | NM_001005858     | I830012O16Rik     | RIKEN cDNA I830012O16 gene                                                  | 3.7 | 3.5 | 3.6  | -0.8 | -0.5 | -0.6 | -0.7 | 0.00 | 0.36 |
| A_55_P1972872 | NM_001005858     | I830012O16Rik     | RIKEN cDNA I830012O16 gene                                                  | 2.5 | 2.8 | 0.4  | -1.0 | -0.3 | -0.5 | -0.2 | 0.01 | 0.72 |
| A_55_P2174541 | NM_001013371     | Dtx3l             | deltax 3-like (Drosophila)                                                  | 4.1 | 3.6 | 0.6  | -0.2 | -0.3 | 0.4  | 0.1  | 0.00 | 0.05 |
| A_55_P2059154 | NM_001013371     | Dtx3l             | deltax 3-like (Drosophila)                                                  | 2.1 | 2.4 | 0.5  | -0.2 | -0.2 | 0.0  | 0.2  | 0.00 | 0.06 |
| A_55_P2112787 | NM_001013828     | Ilgplb            | interferon inducible GTPase 1B                                               | 1.6 | 0.8 | -1.0 | -0.7 | -0.5 | -0.1 | 0.1  | 0.00 | 0.01 |
| A_51_P174434  | NM_001014996     | Cenpj             | centromere protein J                                                         | 1.3 | 1.2 | -0.3 | -0.2 | 0.0  | 0.1  | 0.4  | 0.00 | 0.37 |
| A_55_P1966660 | NM_001025208     | LOC547349         | similar to MHC class I antigen precursor                                     | 1.3 | 0.9 | 0.8  | -0.3 | -0.4 | -0.4 | -0.3 | 0.00 | 0.00 |
| A_55_P2149763 | NM_001025313     | Tappb             | TAP binding protein                                                          | 1.8 | 1.0 | 0.9  | 0.0  | 0.0  | 0.2  | 0.0  | 0.00 | 0.00 |
| A_51_P233027  | NM_001033136     | Fam82a2           | family with sequence similarity 82, member A2                               | 1.0 | 0.7 | -0.2 | 0.0  | 0.0  | 0.3  | 0.1  | 0.01 | 0.72 |
| A_55_P2076757 | NM_001033196     | Znfx1             | zinc finger, NFX1-type containing 1                                          | 1.3 | 1.4 | -0.4 | -0.1 | -0.1 | 0.0  | -0.1 | 0.00 | 0.11 |
| A_66_P113043  | NM_001033207     | Nlrc5             | NLR family, CARD domain containing 5                                        | 6.8 | 5.3 | 0.1  | -0.3 | -0.4 | 0.2  | 0.0  | 0.00 | 0.88 |
| Gene ID | Annotation | Description | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------|------------|-------------|---|---|---|---|---|---|---|---|---|---|---|---|
| A_55_P2016034 | NM_001033307 | Nlrc5 | 1.0 | 1.9 | 1.2 | 0.0 | 0.0 | 0.2 | -0.3 | 0.00 | 0.00 |
| A_55_P1962918 | NM_001033450 | Mnda | predicted gene 4951 | 8.7 | 7.3 | -1.1 | -1.1 | -1.3 | -0.2 | -0.3 | 0.00 | 0.00 |
| A_55_P2015405 | NM_001034859 | Gm4841 | predicted gene 4841 | 9.8 | 6.8 | -3.0 | -1.2 | -2.0 | -0.1 | 0.0 | 0.00 | 0.00 |
| A_55_P1968838 | NM_001037713 | Xaf1 | predicted gene 1 | 4.9 | 5.1 | -1.1 | -0.3 | -0.2 | 0.0 | -0.2 | 0.00 | 0.01 |
| A_55_P1969078 | NM_001038587 | Adar | predicted gene 203 | 1.2 | 1.4 | -0.1 | -0.2 | -0.3 | -0.1 | 0.0 | 0.01 | 0.91 |
| A_51_P514712 | NM_001039530 | Parp14 | predicted gene 203 | 5.4 | 4.7 | 0.2 | -0.5 | -0.5 | 0.0 | 0.0 | 0.00 | 0.68 |
| A_55_P1978521 | NM_001039646 | Gbp10 | predicted gene 4951 | 2.2 | 7.3 | -1.1 | -1.1 | -1.3 | -0.2 | -0.3 | 0.00 | 0.00 |
| A_55_P2045813 | NM_001039647 | Gbp11 | predicted gene 4951 | 6.3 | 2.7 | -2.9 | -0.3 | -0.2 | 0.0 | -0.6 | 0.00 | 0.00 |
| A_55_P1959064 | NM_0010398202 | Ili203 | predicted gene 4951 | 6.3 | 2.7 | -2.9 | -0.3 | -0.2 | 0.0 | -0.6 | 0.00 | 0.00 |
| A_52_P494730 | NM_001045540 | Gm12185 | predicted gene 4951 | 3.5 | 1.4 | -1.2 | -1.0 | -0.2 | 0.1 | 0.0 | 0.00 | 0.00 |
| A_55_P1956160 | NM_001081032 | Gm8909 | predicted gene 4951 | 1.0 | 0.8 | 0.6 | -0.2 | -0.3 | -0.3 | 0.03 | 0.08 |
| A_55_P2008016 | NM_001081083 | Arm3 | predicted gene 4951 | 2.2 | 0.7 | -1.6 | 0.1 | 0.0 | 0.1 | -0.1 | 0.00 | 0.00 |
| A_55_P1959064 | NM_001083925 | Oas1 | predicted gene 4951 | 1.1 | 0.1 | -1.1 | -0.1 | 0.0 | 0.0 | -0.1 | 0.00 | 0.00 |
| A_66_P125389 | NM_001011475 | F830016B08Rik | predicted gene 4951 | 6.5 | 4.5 | -2.0 | -1.2 | -1.5 | -0.2 | -0.2 | 0.00 | 0.00 |
| A_55_P2015405 | NM_0011114679 | 9930111J21Rik1 | predicted gene 4951 | 3.0 | 1.2 | -0.3 | -0.6 | -0.2 | 0.1 | 0.0 | 0.00 | 0.37 |
| A_55_P2057986 | NM_0011315115 | Gm12250 | predicted gene 4951 | 5.2 | 4.1 | -1.1 | -0.5 | -0.6 | -0.1 | -0.1 | 0.00 | 0.01 |
| A_55_P1994042 | NM_001139519 | Zbp1 | predicted gene 4951 | 2.7 | 4.2 | 1.6 | 0.1 | -0.4 | 0.0 | 0.1 | 0.00 | 0.00 |
| A_55_P2019699 | NM_001139520 | Samhd1 | SAM domain and HD domain, 1 | 1.3 | 1.5 | 0.5 | -0.4 | -0.3 | -0.1 | -0.1 | 0.00 | 0.16 |
| A_55_P2034705 | NM_001141949 | Nmi | N-myc (and STAT) interactor | 1.8 | 1.5 | 0.3 | -0.1 | -0.1 | 0.2 | 0.0 | 0.00 | 0.23 |
| A_55_P2017491 | NM_001141981 | Rbm43 | RNA binding motif protein 43 | 1.2 | 0.7 | -0.1 | 0.1 | 0.2 | 0.2 | 0.0 | 0.00 | 0.79 |
| A_55_P2062246 | NM_001145164 | Tgtp2 | T-cell specific GTPase 2 | 9.5 | 9.2 | -0.6 | -0.8 | -0.8 | 1.1 | 0.0 | 0.00 | 0.07 |
| A_55_P1989225 | NM_001145164 | Tgtp2 | T-cell specific GTPase 2 | 5.5 | 4.9 | -0.6 | -1.3 | -0.5 | -0.1 | 0.0 | 0.00 | 0.06 |
| A_55_P2039061 | NM_001146007 | 9230105E10Rik | RIKEN cDNA 9230105E10 gene | 1.7 | 0.8 | -1.6 | 0.1 | 0.2 | 0.1 | -0.1 | 0.00 | 0.00 |
| A_55_P2163857 | NM_001146007 | 9230105E10Rik | RIKEN cDNA 9230105E10 gene | 1.7 | 0.8 | -1.3 | -0.2 | 0.1 | 0.1 | 0.1 | 0.00 | 0.00 |
| A_55_P2064652 | NM_001146007 | 9230105E10Rik | RIKEN cDNA 9230105E10 gene | 1.5 | 0.5 | -1.3 | 0.5 | 0.1 | 0.0 | -0.2 | 0.01 | 0.00 |
| A_55_P1990633 | NM_001146275 | Iigp1 | interferon inducible GTPase 1 | 7.7 | 7.3 | -0.7 | -1.3 | -1.1 | 0.8 | 0.2 | 0.00 | 0.57 |
| A_55_P2410304 | NM_001146275 | Iigp1 | interferon inducible GTPase 1 | 4.5 | 4.0 | -0.7 | -1.2 | -1.2 | -0.2 | -0.4 | 0.00 | 0.06 |
| A_55_P2000067 | NM_001159393 | Irf1 | interferon regulatory factor 1 | 1.7 | 1.7 | -0.1 | -0.3 | -0.3 | -0.1 | 0.1 | 0.00 | 0.87 |
| A_55_P2114938 | NM_001159417 | Irf9 | interferon regulatory factor 9 | 3.0 | 2.1 | -0.1 | -0.3 | -0.4 | -0.2 | -0.5 | 0.00 | 0.81 |
| A_55_P2130970 | NM_001163576 | Parp10 | poly (ADP-ribose) polymerase family, member 10 | 3.8 | 2.9 | 0.0 | -0.2 | -0.2 | 0.7 | 0.1 | 0.00 | 0.99 |
| A_66_P104314 | NM_001168294 | Serpina3f | serine (or cysteine) peptidase inhibitor, clade A, member 3F | 6.2 | 4.7 | -1.1 | -1.1 | -1.5 | -0.1 | -0.3 | 0.00 | 0.01 |
| A_55_P2104975 | NM_001168294 | Serpina3f | serine (or cysteine) peptidase inhibitor, clade A, member 3F | 5.0 | 3.1 | -0.5 | -1.2 | -1.3 | -0.2 | -0.7 | 0.00 | 0.16 |
| A_66_P110633 | NM_001168660 | Apol9b | apolipoprotein L 9b | 3.5 | 4.1 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.00 | 0.05 |
| A_52_P1020860 | NM_001177351 | AW112010 | expressed sequence AW112010 | 1.5 | 1.2 | -0.3 | 0.1 | 0.0 | 0.4 | 0.2 | 0.04 | 0.66 |
| A_51_P175567 | NM_001190466 | Dact1 | dapper homolog 1, | 1.3 | 1.6 | -3.6 | -0.5 | 0.0 | 0.2 | 0.3 | 0.00 | 0.00 |
| Gene Symbol | Gene Name | Description | Effect Size | Interferon | Histidine | Clade D, Member 1 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 | Peptidase A, Member 31 |
|------------|-----------|-------------|-------------|------------|-----------|------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| A_55_P2177899 | NM_001190466 | Dact1 | antagonist of beta-catenin (xenopus) | 1.3 | 1.3 | -3.2 | -0.4 | -0.1 | 0.3 | 0.3 | 0.00 | 0.00 |
| A_55_P1966774 | NM_001199940 | Serpina3i | serine (or cysteine) peptidase inhibitor, clade A, member 31 | 3.5 | 1.4 | -1.0 | -0.9 | -1.0 | -0.8 | -1.4 | 0.01 | 0.37 |
| A_55_P2142232 | NM_001199940 | Serpina3i | serine (or cysteine) peptidase inhibitor, clade A, member 31 | 2.3 | 1.5 | -1.2 | -0.8 | -0.7 | -0.4 | 0.0 | 0.00 | 0.00 |
| A_55_P2125049 | NM_001199967 | Gm11127 | predicted gene 11127 | 1.2 | 0.7 | 0.9 | -0.3 | -0.3 | -0.2 | -0.1 | 0.00 | 0.00 |
| A_55_P2081105 | NM_001204910 | Al607873 | expressed sequence Al607873 | 2.8 | 3.7 | 2.8 | 0.8 | 0.2 | 0.5 | 0.6 | 0.00 | 0.00 |
| A_55_P2179074 | NM_001243760 | Ciita | class II transactivator | 1.2 | 1.3 | 0.1 | -0.2 | -0.3 | -0.2 | 0.0 | 0.02 | 0.90 |
| A_55_P1973229 | NM_001243916 | Trim34b | tripartite motif-containing 34B | 1.1 | 0.7 | -0.3 | -0.2 | 0.1 | 0.2 | -0.2 | 0.00 | 0.31 |
| A_55_P2103837 | NM_001256005 | Gbp4 | guanylate binding protein 4 | 4.6 | 1.4 | -3.2 | -0.6 | -0.6 | 0.0 | -0.1 | 0.00 | 0.00 |
| A_55_P2091461 | NM_007609 | Casp4 | caspase 4, apoptosis-related cysteine peptidase | 3.1 | 2.0 | 3.5 | 0.3 | -0.1 | 0.4 | 0.2 | 0.00 | 0.00 |
| A_55_P1984168 | NM_007609 | Casp4 | caspase 4, apoptosis-related cysteine peptidase | 2.9 | 2.0 | 3.5 | 0.3 | 0.0 | 0.3 | 0.3 | 0.00 | 0.00 |
| A_51_P474459 | NM_007707 | Soc53 | suppressor of cytokine signaling 3 | 1.3 | 1.4 | 0.6 | -0.2 | -0.4 | 0.0 | -0.2 | 0.00 | 0.01 |
| A_55_P2091676 | NM_007987 | Fas | Fas (TNF receptor superfamily member 6) | 1.4 | 1.8 | -1.7 | 0.3 | -0.8 | 0.3 | -0.6 | 0.01 | 0.00 |
| A_51_P468140 | NM_008223 | Serpind1 | serine (or cysteine) peptidase inhibitor, clade D, member 1 | 1.9 | 1.8 | 0.0 | -0.5 | -0.1 | 0.0 | 0.0 | 0.00 | 0.95 |
| A_51_P254656 | NM_008230 | Hdc | histidine decarboxylase | 1.3 | 0.8 | -0.5 | -0.3 | 0.2 | 0.0 | 0.2 | 0.00 | 0.03 |
| A_52_P354823 | NM_008320 | Irf8 | interferon regulatory factor 8 | 4.6 | 2.7 | -2.1 | -0.9 | -1.3 | -0.1 | 0.0 | 0.00 | 0.00 |
| A_55_P1981479 | NM_008326 | Irgm1 | immunity-related GTPase family M member 1 | 5.8 | 4.5 | -0.4 | -0.4 | -0.2 | 0.3 | -0.1 | 0.00 | 0.13 |
| A_51_P262171 | NM_008326 | Irgm1 | immunity-related | 5.2 | 4.3 | -0.1 | -0.3 | -0.3 | 0.2 | -0.2 | 0.00 | 0.70 |
| Gene ID    | Accession  | Description                                      | IFI204 | IL47  | IFI15 | IFI15RA  | NTR1  | CXCL9  | SERPINA3G  | TRIM21 | STAT1  | PHILDA1 | TNAIP2  | TNAIP2  |
|------------|------------|--------------------------------------------------|--------|-------|-------|---------|-------|--------|------------|--------|--------|---------|--------|--------|
| A_55_P1975560 | NM_008329  | Ifi204 interferon activated gene 204             | 4.0    | 3.1   | 2.8   | 0.4     | 0.1   | 1.0    | 0.2        | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| A_55_P1998416 | NM_008330  | Ifi47 interferon gamma inducible protein 47      | 6.1    | 5.9   | -0.3  | 0.0     | 0.0   | 0.6    | 0.2        | 0.0    | 0.0    | 0.68    | 0.0    | 0.68    |
| A_51_P327751 | NM_008331  | Ifit1 interferon-induced protein with tetraicosopeptide repeats 1 | 4.7    | 4.4   | 0.5   | -0.5    | -0.6  | -0.3   | -0.5       | 0.0    | 0.47   | 0.0     | 0.0    | 0.47    |
| A_52_P15461 | NM_008357  | Ifi15 interleukin 15                              | 1.6    | 1.1   | -0.4  | 0.0     | 0.4   | -0.1   | 0.2        | 0.0    | 0.1    | 0.1     | 0.0    | 0.1    |
| A_55_P2041738 | NM_008358  | Ifi15ra interleukin 15 receptor, alpha chain     | 2.6    | 2.4   | -0.2  | -0.7    | -0.7  | -0.2   | -0.3       | 0.0    | 0.59   | 0.0     | 0.0    | 0.59    |
| A_51_P271503 | NM_008362  | Il1r1 interleukin 1 receptor, type I             | 1.0    | 0.7   | -1.4  | 0.2     | -0.7  | 0.3    | -0.2       | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| A_55_P2096422 | NM_008381  | Inhbb inhibin beta-B                              | 1.4    | -0.2  | 1.9   | 0.0     | 0.3   | 0.2    | 0.3        | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| A_55_P2000062 | NM_008390  | Irf1 interferon regulatory factor 1               | 4.3    | 3.8   | 0.0   | -0.1    | -0.1  | 0.1    | 0.1        | 0.0    | 0.95   | 0.0     | 0.0    | 0.95    |
| A_51_P461665 | NM_008599  | Cxcl9 chemokine (C-X-C motif) ligand 9            | 1.0    | 3.1   | 2.2   | 0.3     | -0.7  | -0.1   | 0.1        | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| A_55_P2162935 | NM_008744  | Ntn1 netrin 1                                     | 1.3    | 0.6   | 0.0   | -0.1    | -0.2  | 0.0    | 0.2        | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| A_51_P326191 | NM_009251  | Serpina3g serine (or cysteine) peptidase inhibitor, clade A, member 3G | 5.3    | 4.0   | 1.2   | -0.9    | -1.1  | -0.5   | -1.1       | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| A_55_P2134246 | NM_009256  | Serpinb9 serine (or cysteine) peptidase inhibitor, clade B, member 9 | 1.1    | 0.6   | 2.1   | -0.2    | -0.2  | -0.2   | -0.2       | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| A_55_P1962344 | NM_009277  | Trim21 tripartite motif-containing 21             | 4.2    | 3.8   | -1.4  | -0.3    | -0.2  | 0.3    | 0.1        | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| A_55_P1955906 | NM_009283  | Stat1 signal transducer and activator of transcription 1 | 3.3    | 2.9   | -0.4  | -0.5    | -0.4  | 0.0    | 0.2        | 0.0    | 0.0    | 0.33    | 0.0    | 0.33    |
| A_51_P195958 | NM_009344  | Phlda1 pleckstrin homology-like domain, family A, member 1 | 1.3    | 0.1   | 1.9   | -0.4    | -0.1  | -0.1   | 0.1        | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| A_55_P2098697 | NM_009396  | Tnfaip2 tumor necrosis factor, alpha-induced protein 2 | 2.0    | 0.7   | -0.6  | 0.1     | -0.2  | 0.3    | 0.2        | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| A_51_P364485 | NM_009396  | Tnfaip2 tumor necrosis factor, alpha-              | 2.0    | 0.7   | -0.6  | 0.1     | -0.2  | 0.3    | 0.2        | 0.0    | 0.0    | 0.0     | 0.0    | 0.0     |
| Accession | Gene ID | Gene Symbol | Gene Description | Fold Change | Fold Change | Fold Change | Fold Change | Fold Change | Fold Change | p-value | p-value |
|-----------|---------|-------------|------------------|-------------|-------------|-------------|-------------|-------------|-------------|---------|---------|
| A_55_P2098071 | NM_009546 | Trim25 | trimpartite motif-containing 25 | 1.5 | 1.4 | -0.7 | -0.2 | -0.3 | -0.4 | 0.00 | 0.01 |
| A_55_P2078633 | NM_009780 | C4b | complement component 4B (Childo blood group) | 1.0 | 0.9 | 1.9 | 0.0 | 0.0 | 0.4 | -0.2 | 0.04 | 0.00 |
| A_55_P1983853 | NM_009808 | Casp12 | caspase 12 | 1.7 | 1.2 | 0.6 | 0.0 | -0.1 | 0.2 | -0.3 | 0.00 | 0.00 |
| A_55_P1968723 | NM_009834 | Ccrn4l | CCR4 carbon catabolite repression 4-like (S. cerevisiae) | 1.3 | 1.2 | 0.8 | 0.1 | 0.0 | 0.1 | 0.4 | 0.00 | 0.01 |
| A_51_P470715 | NM_009895 | Cish | cytokine inducible SH2-containing protein | 1.3 | 1.4 | 0.5 | -0.1 | -0.2 | 0.0 | -0.2 | 0.00 | 0.01 |
| A_51_P279606 | NM_009896 | Socs1 | suppressor of cytokine signaling 1 | 3.6 | 2.8 | -1.0 | -0.2 | -0.2 | -0.1 | -0.2 | 0.00 | 0.00 |
| A_66_P121787 | NM_010156 | Samd9l | sterile alpha motif domain containing 9-like | 2.6 | 2.7 | 0.1 | 0.0 | -0.1 | 0.2 | 0.0 | 0.00 | 0.85 |
| A_55_P2151601 | NM_010156 | Samd9l | sterile alpha motif domain containing 9-like | 2.4 | 2.6 | 0.2 | 0.1 | 0.0 | 0.1 | 0.0 | 0.00 | 0.56 |
| A_65_P08971 | NM_010171 | F3 | coagulation factor III | 1.3 | 1.0 | -2.1 | -0.2 | -0.1 | 0.2 | 0.0 | 0.01 | 0.00 |
| A_51_P203955 | NM_010260 | Gbp2 | guanylate binding protein 2 | 8.1 | 8.0 | -1.8 | -0.8 | -1.1 | -0.2 | -0.2 | 0.00 | 0.00 |
| A_51_P425048 | NM_010392 | H2-Q2 | histocompatibility 2, Q region locus 2 | 1.2 | 0.6 | 1.0 | -0.3 | -0.1 | -0.1 | -0.1 | 0.00 | 0.00 |
| A_51_P219789 | NM_010392 | H2-Q2 | histocompatibility 2, Q region locus 2 | 1.1 | 0.6 | 0.9 | -0.2 | 0.0 | 0.0 | 0.0 | 0.00 | 0.00 |
| A_51_P400752 | NM_010392 | H2-Q5 | histocompatibility 2, Q region locus 5 | 1.0 | 0.6 | 0.8 | -0.3 | -0.1 | 0.0 | 0.0 | 0.00 | 0.00 |
| A_51_P237754 | NM_010398 | H2-T23 | histocompatibility 2, T region locus 23 | 1.3 | 0.7 | 0.8 | -0.3 | -0.3 | -0.2 | -0.1 | 0.00 | 0.00 |
| A_51_P359570 | NM_010501 | Ilf3 | interferon-induced protein with tetratricopeptide repeats 3 | 4.1 | 2.8 | 0.9 | -0.6 | -0.5 | -0.2 | -0.3 | 0.00 | 0.07 |
| A_52_P577384 | NM_010531 | Il18bp | interleukin 18 binding protein | 1.4 | 0.9 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.00 | 0.41 |
| A_51_P470079 | NM_010555 | Il1r2 | interleukin 1 receptor, type II | 1.1 | 0.1 | -1.3 | 0.8 | 0.1 | 0.4 | -0.1 | 0.00 | 0.00 |
| Gene Name     | Accession   | Description                                                                 | A_51_P212420 | A_51_P345367 | A_51_P390538 | A_52_P467726 | A_51_P319460 | A_51_P359636 | A_52_P559919 | A_51_P101196 | A_55_P1996862 | A_55_P2025611 | A_51_P288737 | A_51_P421734 | A_55_P1991219 | A_55_P2017645 |
|--------------|-------------|-----------------------------------------------------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Lama4        | NM_010681   | laminin, alpha 4                                                            | 2.1          | 6.1          | 2.4          | 1.2          | 1.4          | 1.1          | 1.4          | 1.1          | 1.3          | 1.3          | 1.6          | 1.2          | 1.7          | 3.2          |
| Psmb8        | NM_010724   | proteasome (prosome, macropain) subunit, beta type 8 (large multifunctional peptidase 7) | 1.2          | 5.2          | 2.7          | 0.3          | 1.1          | 1.1          | 1.4          | 1.0          | 1.0          | 0.9          | 2.7          | 0.3          | 0.7          | 3.3          |
| Mpeg1        | NM_010821   | macrophage expressed gene 1                                                 | 0.4          | -1.6         | -0.4         | -0.2         | -0.1         | -0.1         | 0.0          | 0.5          | 0.0          | 0.0          | 0.4          | 0.0          | 0.7          | 3.3          |
| Nsg1         | NM_010942   | neuron specific gene family member 1                                        | 0.6          | -0.2         | 0.0          | 0.1          | 0.3          | 0.1          | 0.0          | 2.1          | 0.1          | 0.0          | 0.1          | 0.3          | 0.7          | 3.3          |
| Osmr         | NM_011019   | oncostatin M receptor                                                        | 0.0          | -0.3         | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          |
| Lgals3bp     | NM_011150   | lectin, galactoside-binding, soluble, 3 binding protein                     | 0.4          | 0.7          | 0.4          | 0.6          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          |
| Eil2ak2      | NM_011163   | eukaryotic translation initiation factor 2-alpha kinase 2                   | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          |
| Psme1        | NM_011189   | proteasome (prosome, macropain) 28 subunit, alpha                          | 0.2          | -0.1         | -0.1         | -0.2         | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          |
| Psme2        | NM_011190   | proteasome (prosome, macropain) 28 subunit, beta                           | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          |
| Psme2        | NM_011190   | proteasome (prosome, macropain) 28 subunit, beta                           | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          |
| Ccl2         | NM_011333   | chemokine (C-C motif) ligand 2                                              | 0.4          | -0.3         | 0.0          | 0.3          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          |
| Slc12a7      | NM_011390   | solute carrier family 12, member 7                                         | 0.5          | 0.2          | 0.0          | 0.2          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          |
| Stat3        | NM_011486   | signal transducer and activator of transcription 3                         | 0.1          | 0.1          | 0.0          | 0.1          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          |
| Tap2         | NM_011530   | transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)                 | 0.2          | -0.2         | 0.0          | 0.2          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          | 0.0          |
| Accession | NM_011579 | Tgtp1   | T-cell specific GTPase 1 | 5.7 | 5.3 | -0.4 | -1.0 | -0.6 | 0.1 | 0.2 | 0.00 | 0.40 |
|-----------|------------|---------|--------------------------|-----|-----|------|------|------|-----|-----|------|------|
| A_55_P2017636 | NM_011580 | Thbs1  | thrombospondin 1         | 1.1 | 0.0 | 1.5  | 0.5  | -0.7 | 0.8 | -0.7 | 0.01 | 0.00 |
| A_55_P2082902 | NM_011710 | Wars   | tryptophanyl-tRNA synthetase | 1.3 | 1.0 | -0.4 | -0.3 | 0.1  | 0.2 | 0.2  | 0.00 | 0.23 |
| A_55_P1983708 | NM_011832 | Insrr  | insulin receptor-related receptor | 1.6 | 0.8 | -0.6 | -0.1 | 0.0  | -0.1 | -0.1 | 0.00 | 0.02 |
| A_51_P387123 | NM_011854 | Oasl2  | 2'-5' oligoadenylate synthetase-like 2 | 6.7 | 6.3 | -0.1 | -0.4 | -0.2 | -0.2 | -0.2 | 0.00 | 0.93 |
| A_55_P2114953 | NM_011909 | Usp18  | ubiquitin specific peptidase 18 | 3.3 | 3.8 | 0.8  | -0.6 | -0.3 | -0.3 | -0.1 | 0.00 | 0.19 |
| A_51_P423976 | NM_013498 | Crem   | cAMP responsive element modulator | 1.4 | 1.7 | 2.2  | -0.4 | -0.2 | -0.2 | 0.1  | 0.00 | 0.00 |
| A_52_P460957 | NM_013498 | Crem   | cAMP responsive element modulator | 1.3 | 1.6 | 2.0  | -0.3 | -0.1 | -0.3 | 0.0  | 0.00 | 0.00 |
| A_51_P369803 | NM_013585 | Psmb9  | proteasome (prosome, macropain) subunit, beta type 9 (large multifunctional peptidase 2) | 5.7 | 5.1 | -2.1 | -0.3 | -0.1 | 0.1 | 0.1  | 0.00 | 0.00 |
| A_51_P514085 | NM_013606 | Mx2    | myxovirus (influenza virus) resistance 2 | 3.0 | 3.6 | 0.6  | 0.0  | 0.0  | 0.1 | 0.0  | 0.00 | 0.27 |
| A_52_P570266 | NM_013640 | Psmb10 | proteasome (prosome, macropain) subunit, beta type 10 | 2.5 | 2.2 | 0.4  | -0.2 | -0.2 | 0.0 | -0.2 | 0.00 | 0.12 |
| A_51_P436652 | NM_013654 | Ccl7   | chemokine (C-C motif) ligand 7 | 1.4 | 3.2 | -2.3 | 0.8  | -0.2 | 1.3 | 0.6  | 0.00 | 0.00 |
| A_51_P100327 | NM_013683 | Tap1   | transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) | 4.8 | 4.3 | -2.2 | -0.5 | -0.1 | 0.7 | 0.2  | 0.00 | 0.00 |
| A_51_P469968 | NM_013819 | H2-M3  | histocompatibility 2, M region locus 3 | 1.6 | 1.6 | -1.5 | 0.2  | 0.4  | 0.4 | 0.5  | 0.00 | 0.00 |
| A_66_P128537 | NM_015783 | Isg15  | ISG15 ubiquitin-like modifier | 4.0 | 4.4 | 1.3  | -0.2 | 0.0  | -0.2 | -0.5 | 0.00 | 0.01 |
| A_55_P2103698 | NM_015783 | Isg15  | ISG15 ubiquitin-like modifier | 3.9 | 4.7 | 1.5  | -0.1 | -0.1 | -0.2 | -0.1 | 0.00 | 0.00 |
| A_51_P421876 | NM_016850 | Irf7   | interferon regulatory factor 7 | 2.9 | 3.4 | -0.2 | -0.7 | -0.1 | -0.2 | -0.1 | 0.00 | 0.72 |
| A_55_P2472435 | NM_018734 | Gbp3   | guanylate binding protein 3 | 7.0 | 6.7 | -1.3 | -0.6 | -0.6 | -0.1 | -0.2 | 0.00 | 0.00 |
| Accession | Gene ID | Description | Log2 Fold Change | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|-----------|---------|-------------|-----------------|---|---|---|---|---|---|---|---|---|----|
| A_51_P112355 | NM_018738 | Igtp | interferon gamma induced GTPase | 8.6 | 8.8 | -0.5 | -0.4 | -0.5 | 0.6 | 0.2 | 0.00 | 0.13 |
| A_52_P466090 | NM_018851 | Samhd1 | SAM domain and HD domain, 1 | 1.4 | 1.5 | 0.7 | -0.3 | -0.2 | 0.1 | 0.0 | 0.00 | 0.01 |
| A_55_P2137611 | NM_019440 | Irgm2 | immunity-related GTPase family M member 2 | 7.5 | 7.2 | -0.8 | -0.6 | -0.5 | 0.2 | -0.1 | 0.00 | 0.03 |
| A_55_P2031125 | NM_019949 | Ube2l6 | ubiquitin-conjugating enzyme E2L 6 | 1.6 | 1.4 | -0.9 | -0.2 | -0.2 | -0.3 | -0.4 | 0.00 | 0.00 |
| A_55_P2059606 | NM_019963 | Stat2 | signal transducer and activator of transcription 2 | 3.3 | 2.5 | -0.5 | -0.3 | -0.2 | -0.1 | -0.2 | 0.00 | 0.08 |
| A_55_P2158404 | NM_020557 | Cmpk2 | cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial | 2.9 | 0.8 | -2.1 | -0.2 | 0.0 | 0.0 | -0.5 | 0.00 | 0.00 |
| A_52_P186937 | NM_020557 | Cmpk2 | cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial | 2.5 | 0.7 | -1.8 | -0.1 | 0.1 | -0.1 | -0.5 | 0.00 | 0.00 |
| A_55_P2016462 | NM_021274 | Cxcl10 | chemokine (C-X-C motif) ligand 10 | 6.2 | 5.6 | 2.6 | -0.6 | -0.8 | -0.5 | -0.2 | 0.00 | 0.00 |
| A_66_P139683 | NM_021394 | Zbp1 | Z-DNA binding protein 1 | 2.7 | 3.5 | 0.8 | -0.5 | -0.4 | 0.0 | 0.2 | 0.00 | 0.13 |
| A_51_P464703 | NM_021443 | Ccl8 | chemokine (C-C motif) ligand 8 | 2.5 | 2.0 | -2.0 | 0.0 | -0.2 | 0.1 | -0.1 | 0.00 | 0.00 |
| A_51_P248666 | NM_021893 | Cd274 | CD274 antigen | 5.7 | 4.4 | -0.9 | -0.8 | -0.8 | -0.2 | 0.1 | 0.00 | 0.04 |
| A_52_P338066 | NM_023137 | Ubd | ubiquitin D | 1.9 | 3.2 | 1.3 | 0.3 | 0.2 | -0.1 | 0.3 | 0.00 | 0.00 |
| A_55_P2041828 | NM_023279 | Tubb3 | tubulin, beta 3 | 2.7 | 1.3 | -0.6 | -0.4 | 0.4 | 0.0 | 0.5 | 0.00 | 0.05 |
| A_51_P304170 | NM_023386 | Rtp4 | receptor transporter protein 4 | 5.0 | 5.1 | -0.1 | -0.2 | -0.1 | 0.0 | -0.2 | 0.00 | 0.96 |
| A_55_P2026233 | NM_023738 | Uba7 | ubiquitin-like modifier activating enzyme 7 | 2.6 | 1.3 | 0.0 | -0.3 | 0.0 | 0.2 | -0.4 | 0.00 | 0.98 |
| A_52_P267391 | NM_023835 | Trim12a | tripartite motif-containing 12A | 2.5 | 2.5 | -1.5 | 0.0 | 0.1 | -0.1 | 0.1 | 0.00 | 0.00 |
| A_55_P2064659 | NM_023835 | Trim12a | tripartite motif-containing 12A | 2.1 | 1.5 | -1.4 | -0.1 | 0.2 | 0.2 | 0.0 | 0.00 | 0.00 |
| A_51_P187842 | NM_025829 | Eif4e3 | eukaryotic translation initiation factor 4E member 3 | 1.4 | 0.6 | -2.3 | 0.1 | 0.4 | 0.0 | 0.0 | 0.00 | 0.00 |
| A_52_P679860 | NM_025992 | Herc6 | hect domain and | 2.2 | 2.1 | 0.4 | -0.2 | 0.1 | 0.0 | 0.7 | 0.00 | 0.19 |
| Accession | Symbol   | Description                                      | RLD 6 | 2.2 | 2.0 | 0.6 | -0.5 | 0.1 | 0.1 | 0.3 | 0.00 | 0.20 |
|-----------|----------|--------------------------------------------------|-------|-----|-----|-----|------|-----|-----|-----|------|------|
| A_52_P64514 | NM_025992 | Herc6                                            | hect domain and RLD 6 | 1.1 | 1.0 | 1.5 | 0.2  | -0.6 | 0.5 | -0.4 | 0.02 | 0.00 |
| A_52_P641758 | NM_026637 | Ggct                                             | gamma-glutamyl cyclotransferase | 0.6 | 0.5 | -0.4 | 0.02 | 0.00 | 0.20 |
| A_66_P122110 | NM_026913 | Mtd1                                             | MIT, microtubule interacting and transport, domain containing 1 | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_51_P414889 | NM_027320 | Il35                                              | interferon-induced protein 35 | 0.3 | 0.2 | -0.2 | 0.00 | 0.00 | 0.00 |
| A_55_P1978987 | NM_027835 | Ifih1                                            | interferon induced with helicase C domain 1 | 0.3 | 0.2 | -0.2 | 0.00 | 0.00 | 0.00 |
| A_55_P2005783 | NM_027835 | Ifih1                                            | interferon induced with helicase C domain 1 | 0.3 | 0.2 | -0.2 | 0.00 | 0.00 | 0.00 |
| A_51_P120093 | NM_028035 | Snx10                                            | sorting nexin 10 | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_66_P110343 | NM_028035 | Snx10                                            | sorting nexin 10 | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_51_P240801 | NM_028261 | Tmem173                                          | transmembrane protein 173 | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_55_P2022604 | NM_028807 | 1200009I06Rik                                     | RIKEN cDNA 1200009I06 gene | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_51_P165182 | NM_028967 | Botf2                                            | basic leucine zipper transcription factor, ATF-like 2 | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_55_P1996973 | NM_029000 | Gvin1                                            | GTPase, very large interferon inducible 1 | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_52_P535484 | NM_029000 | Gvin1                                            | GTPase, very large interferon inducible 1 | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_51_P444290 | NM_029084 | Slamf8                                           | SLAM family member 8 | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_55_P2068607 | NM_029219 | Rnf19b                                           | ring finger protein 19B | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_55_P1964262 | NM_029419 | Apol7a                                           | apolipoprotein L 7a | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_51_P497724 | NM_029419 | Apol7a                                           | apolipoprotein L 7a | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_55_P2142863 | NM_030253 | Parp9                                            | poly (ADP-ribose) polymerase family, member 9 | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| A_66_P105689 | NM_030684 | Trim34a                                          | tripartite motif- | 0.5 | 0.4 | -0.3 | 0.02 | 0.00 | 0.00 |
| Accession | Gene ID | Gene Description                                      | Fold Change | Log2 FC | p-value | Fold Change | Log2 FC | p-value | Fold Change | Log2 FC | p-value |
|-----------|---------|------------------------------------------------------|-------------|---------|---------|-------------|---------|---------|-------------|---------|---------|
| A_55_P04284 | NM_030711 | *Erap1* endoplasmic reticulum aminopeptidase 1 | 1.1         | 1.0     | -0.7    | 0.2         | 0.4     | 0.5     | 0.5         | 0.00   | 0.00    |
| A_55_P2166049 | NM_030738 | *Vmn1r65* vomeronasal 1 receptor 65 | 5.2         | 3.1     | -1.6    | -0.9        | -1.6    | -0.2    | -0.6        | 0.00   | 0.00    |
| A_55_P1958255 | NM_030743 | *Rnf114* ring finger protein 114 | 1.1         | 1.0     | -0.1    | -0.1        | 0.2     | 0.1     | 0.1         | 0.78   |         |
| A_55_P1998957 | NM_033541 | *Oas1c* 2'-5' oligoadenylate synthetase 1C | 1.1         | 1.0     | -1.9    | 0.2         | 0.5     | 0.3     | 0.2         | 0.00   | 0.00    |
| A_55_P2066116 | NM_033601 | *Bcl3* B-cell leukemia/lymphoma 3 | 2.1         | 1.5     | 2.3     | -0.4        | -0.2    | -0.7    | -0.2        | 0.00   | 0.00    |
| A_55_P2115442 | NM_053109 | *Clec2d* C-type lectin domain family 2, member d | 1.3         | 2.2     | -1.1    | 0.1         | -0.3    | -0.1    | -0.4        | 0.03   | 0.01    |
| A_52_P85174 | NM_126166 | *Tlr3* toll-like receptor 3 | 1.5         | 1.4     | 0.1     | -0.3        | 0.1     | 0.3     | 0.1         | 0.00   | 0.95    |
| A_55_P2037121 | NM_144830 | *Tmem106a* transmembrane protein 106A | 1.1         | 0.9     | 0.0     | -0.3        | 0.2     | 0.2     | 0.2         | 0.00   | 1.00    |
| A_51_P154842 | NM_145153 | *Oas1f* 2'-5' oligoadenylate synthetase 1F | 1.1         | 1.2     | -0.2    | 0.0         | 0.1     | 0.3     | 0.1         | 0.04   | 0.71    |
| A_55_P1998943 | NM_145211 | *Oas1a* 2'-5' oligoadenylate synthetase 1A | 1.5         | 1.7     | 0.4     | 0.2         | 0.1     | -0.1   | -0.1        | 0.03   | 0.55    |
| A_55_P2019719 | NM_145227 | *Oas2* 2'-5' oligoadenylate synthetase 2 | 2.0         | 1.8     | -0.3    | -0.1        | 0.3     | -0.1   | -0.1        | 0.01   | 0.79    |
| A_51_P505795 | NM_145391 | *Tapbpl* TAP binding protein-like | 2.5         | 1.9     | -0.3    | -0.2        | -0.1    | -0.1   | -0.1        | 0.00   | 0.28    |
| A_51_P463846 | NM_145545 | *Gbp6* guanylate binding protein 6 | 6.3         | 6.3     | -1.0    | -0.8        | -0.5    | 0.0     | -0.1        | 0.00   | 0.03    |
| A_51_P273609 | NM_146125 | *Iltpka* inositol 1,4,5-trisphosphate 3-kinase A | 1.6         | 1.5     | 0.3     | -0.4        | -0.1    | 0.0     | 0.1         | 0.00   | 0.39    |
| A_52_P327664 | NM_153564 | *Gbp5* guanylate binding protein 5 | 2.0         | 1.5     | -0.3    | 0.0         | -0.3    | -0.1   | -0.5        | 0.00   | 0.50    |
| A_55_P2116059 | NM_172393 | *Aim1* absent in melanoma 1 | 2.0         | 0.2     | 3.6     | -0.4        | 0.0     | -0.4   | 0.0         | 0.00   | 0.00    |
| A_52_P385536 | NM_172689 | *Ddx58* DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 | 2.0         | 2.4     | 0.4     | -0.5        | -0.3    | -0.4   | -0.6        | 0.00   | 0.22    |
| A_55_P1965000 | NM_172689 | *Ddx58* DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 | 2.0         | 2.5     | 0.1     | -0.5        | -0.2    | -0.5   | -0.3        | 0.00   | 0.92    |
| A_51_P234113 | NM_172729 | *Nod1* nucleotide-binding | 2.6         | 2.7     | 0.7     | -0.3        | -0.8    | -0.2   | -0.3        | 0.00   | 0.10    |
| Gene ID         | Accession ID | Gene Symbol | Description                                                                 | Log2 Fold Change | Log2 Fold Change | Log2 Fold Change | Log2 Fold Change | Log2 Fold Change | Log2 Fold Change | Log2 Fold Change |
|----------------|--------------|-------------|------------------------------------------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| A_55_P2131088 | NM_172767    | Vwa5a       | von Willebrand factor A domain containing 5A                                | 1.1              | 1.1              | -0.3             | -0.5             | -0.3             | -0.4             | 0.04             | 0.62             |
| A_66_P131754  | NM_172767    | Vwa5a       | von Willebrand factor A domain containing 5A                                | 1.1              | 1.2              | 0.1              | -0.4             | -0.2             | -0.3             | -0.2             | 0.04             | 0.95             |
| A_66_P140742  | NM_172875    | Adc         | arginine decarboxylase                                                       | 1.2              | 0.5              | 1.5              | 0.0              | 0.0              | 0.2              | -0.2             | 0.01             | 0.00             |
| A_51_P214747  | NM_172893    | Parp12      | poly (ADP-ribose) polymerase family, member 12                              | 2.1              | 2.1              | -0.1             | -0.6             | -0.2             | -0.2             | 0.1              | 0.00             | 0.94             |
| A_55_P2107775 | NM_173786    | Apol9a      | apolipoprotein L 9a                                                          | 3.1              | 3.8              | 0.5              | -0.1             | 0.0              | 0.1              | 0.3              | 0.00             | 0.15             |
| A_55_P2043367 | NM_173786    | Apol9a      | apolipoprotein L 9a                                                          | 3.1              | 3.7              | 0.6              | 0.0              | 0.0              | 0.0              | 0.0              | 0.00             | 0.02             |
| A_52_P650028  | NM_175291    | Dock10      | dedicator of cytokinesis 10                                                  | 1.3              | 0.0              | -2.1             | 0.5              | 0.0              | 0.8              | 0.2              | 0.01             | 0.00             |
| A_51_P100852  | NM_175449    | Fam26f      | family with sequence similarity 26, member F                                | 1.9              | 2.1              | 0.3              | -0.2             | 0.0              | 0.0              | -0.2             | 0.00             | 0.58             |
| A_51_P501844  | NM_175475    | Cyp26b1     | cytochrome P450, family 26, subfamily b, polypeptide 1                       | 1.7              | 0.4              | 1.9              | 0.4              | -0.5             | 0.6              | -0.5             | 0.00             | 0.00             |
| A_55_P2006494 | NM_177820    | Apol10b     | apolipoprotein L 10b                                                         | 2.9              | 2.1              | -1.9             | 0.3              | 0.0              | 0.4              | 0.0              | 0.00             | 0.00             |
| A_55_P2084418 | NM_178005    | Lrtrm2      | leucine rich repeat transmembrane neuronal 2                                 | 2.3              | 1.7              | 1.0              | -0.4             | -1.1             | -0.4             | -0.8             | 0.00             | 0.00             |
| A_55_P2084413 | NM_178005    | Lrtrm2      | leucine rich repeat transmembrane neuronal 2                                 | 1.2              | 1.6              | 1.0              | -0.5             | -0.9             | 0.0              | -0.2             | 0.00             | 0.00             |
| A_55_P1959303 | NM_178446    | Rbm47       | RNA binding motif protein 47                                                 | 2.1              | 0.5              | 1.9              | -0.1             | -0.1             | -0.1             | -0.2             | 0.00             | 0.00             |
| A_55_P1959305 | NM_178446    | Rbm47       | RNA binding motif protein 47                                                 | 1.2              | 0.5              | 1.8              | 0.2              | 0.0              | 0.2              | 0.4              | 0.04             | 0.00             |
| A_52_P220783  | NM_178745    | Tmem229b    | transmembrane protein 229B                                                   | 1.4              | 0.8              | -1.6             | -0.1             | -0.1             | 0.1              | -0.1             | 0.00             | 0.00             |
| A_55_P2113256 | NM_181402    | Parp11      | poly (ADP-ribose) polymerase family, member 11                              | 1.0              | 0.6              | 0.0              | -0.1             | 0.0              | 0.3              | 0.0              | 0.01             | 0.00             |
| A_55_P2135200 | NM_181542    | Sifn10-ps   | schlafen 10, pseudogene                                                      | 1.7              | 0.6              | -1.3             | -0.2             | 0.2              | -0.1             | -0.3             | 0.03             | 0.01             |
| GenBank acc | NM acc | Gene | Description | EXP | FPKM | FPKM | FPKM | FPKM | FPKM | FPKM | FPKM |
|-------------|--------|------|-------------|-----|------|------|------|------|------|------|------|
| A_55_P2117656 | NM_181545 | Slfn8 | schlafen 8 | 2.5 | 0.8 | -1.8 | -0.2 | 0.1 | 0.1 | 0.6 | 0.00 | 0.00 |
| A_55_P2026572 | NM_183087 | Fam189a1 | family with sequence similarity 189, member A1 | 1.5 | 0.8 | -3.3 | 0.7 | 0.5 | 0.9 | 0.4 | 0.00 | 0.00 |
| A_55_P1959953 | NM_183162 | BC006779 | cDNA sequence BC006779 | 1.5 | 1.6 | 0.2 | -0.1 | 0.0 | 0.1 | 0.1 | 0.00 | 0.64 |
| A_52_P616392 | NM_183426 | Sbno2 | strawberry notch homolog 2 (Drosophila) | 1.3 | 0.9 | 0.2 | 0.0 | -0.2 | 0.2 | -0.3 | 0.00 | 0.68 |
| A_55_P2052385 | NM_194336 | Mpa2l | macrophage activation 2 like | 6.8 | 4.3 | -2.7 | -0.6 | -0.2 | 0.1 | -0.1 | 0.00 | 0.00 |
| A_55_P2052380 | NM_194336 | Mpa2l | macrophage activation 2 like | 4.8 | 2.3 | -2.3 | -0.9 | -0.6 | -0.4 | -0.4 | 0.00 | 0.00 |
| A_55_P2165074 | NM_194346 | Rnf31 | ring finger protein 31 | 1.4 | 1.1 | 0.0 | 0.1 | 0.0 | 0.2 | 0.0 | 0.00 | 0.95 |
| A_51_P191469 | NM_194346 | Rnf31 | ring finger protein 31 | 1.3 | 1.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.00 | 1.00 |
| A_52_P463977 | NM_197986 | Tmem140 | transmembrane protein 140 | 4.4 | 3.6 | 1.1 | -0.4 | -0.4 | 0.2 | -0.1 | 0.00 | 0.01 |
| A_52_P335609 | NM_198004 | 5133401N09Rik | RIKEN cDNA 5133401N09 gene | 1.6 | 1.5 | -0.9 | -0.3 | -0.4 | -0.1 | -0.2 | 0.00 | 0.00 |
| A_51_P169693 | NM_198095 | Bst2 | bone marrow stromal cell antigen 2 | 2.2 | 1.6 | 0.5 | 0.1 | 0.2 | 0.6 | 0.2 | 0.00 | 0.03 |
| A_55_P2015687 | NM_199015 | D14Ertd668e | DNA segment, Chr 14, ERATO Doi 668, expressed | 1.9 | 3.7 | 5.1 | 0.1 | 0.2 | 0.0 | 0.6 | 0.00 | 0.00 |
| A_52_P199633 | NM_199146 | Trim30d | tripartite motif-containing 30D | 1.9 | 0.2 | -2.4 | -0.1 | 0.2 | 0.1 | -0.2 | 0.00 | 0.00 |
| A_52_P326664 | NM_199252 | Unc93a | unc-93 homolog A (C. elegans) | 1.8 | 1.8 | -0.2 | -0.6 | -0.2 | 0.2 | 0.1 | 0.01 | 0.83 |
| A_55_P2139763 | NM_201373 | Trim56 | tripartite motif-containing 56 | 1.1 | 1.0 | -0.4 | -0.2 | -0.3 | -0.1 | -0.3 | 0.00 | 0.17 |
| A_55_P1978506 | NM_207648 | H2-Q6 | histocompatibility 2, Q region locus 6 | 1.0 | 0.5 | 1.1 | -0.2 | -0.1 | -0.1 | 0.0 | 0.00 | 0.00 |
| A_51_P201480 | NM_213659 | Stat3 | signal transducer and activator of transcription 3 | 1.5 | 0.8 | -0.1 | -0.1 | -0.3 | 0.0 | -0.2 | 0.00 | 0.93 |
| A_55_P2100620 | NR_033332 | Gm12216 | predicted gene 12216 | 2.8 | 2.1 | -0.4 | 0.1 | -0.2 | 0.0 | -0.2 | 0.00 | 0.24 |
| A_52_P69558 | NR_033577 | Gm8221 | apolipoprotein L, 3-like | 1.3 | 0.6 | -1.3 | -0.2 | 0.3 | 0.2 | 0.0 | 0.00 | 0.00 |
| A_55_P2213828 | NR_038025 | 4933412E12Rik | RIKEN cDNA 4933412E12 gene | 1.0 | 1.2 | 0.1 | -0.1 | -0.2 | -0.1 | -0.2 | 0.00 | 0.87 |
| Accession   | Record Name  | Description                      | Fold Difference | Log2 Fold Difference | P-value  |
|-------------|--------------|----------------------------------|-----------------|----------------------|----------|
| A_55_P2243431 | NR_045032    | Gdap10 - ganglioside-induced differentiation-associated-protein 10 | 1.6             | -0.9                 | 0.00     |
| A_52_P431615   | XM_001000891 | Gm1966 - predicted gene 1966       | 3.0             | 2.8                  | 0.40     |
| A_55_P2173210   | XM_003084464 | Gm16340 - predicted gene 16340    | 2.5             | 3.3                  | 0.00     |
| A_55_P1972582   | XM_136331    | Gm4955 - predicted gene 4955       | 1.9             | 0.6                  | 0.00     |
| A_55_P2004007   | XM_136331    | Gm4955 - predicted gene 4955       | 1.6             | 0.2                  | 0.00     |

Genes significantly (p-value < 0.05) induced at least 2-fold by IFN-γ are shown (in alphabetical order). Fold differences (log 2-transformed) and p-values derived from three biological replicates for each condition are depicted. Key: WT = WT MEFs; SA = S727A MEFs; siCtrl = sample treated with control siRNA; siCdk8 = sample treated with Cdk8 siRNA; g = sample stimulated for 4 h with IFN-γ; 0 = sample not stimulated with IFN-γ.
Table S5. Overlap of Genes Similarly Affected by S727A Mutation and Cdk8 Silencing

| Genbank AC | Gene symbol       | Type of change |
|------------|-------------------|----------------|
| NM_001146007 | 9230105E10Rik     | down           |
| NM_029419   | Apol7a            | down           |
| NM_020557   | Cmpk2             | down           |
| NM_020557   | Cmpk2             | down           |
| NM_001190466| Dact1             | down           |
| NM_001190466| Dact1             | down           |
| NM_010171   | F3                | down           |
| NM_001101475| F830016B08Rik     | down           |
| NM_001039646| Gbp10             | down           |
| NM_001039647| Gbp11             | down           |
| NM_010260   | Gbp2              | down           |
| NM_018734   | Gbp3              | down           |
| NM_001256005| Gbp4              | down           |
| NM_145545   | Gbp6              | down           |
| NM_001045540| Gm12185           | down           |
| NM_001135115| Gm12250           | down           |
| NM_001034859| Gm4841            | down           |
| NM_001033767| Gm4951            | down           |
| XM_136331   | Gm4955            | down           |
| XM_136331   | Gm4955            | down           |
| NR_033577   | Gm8221            | down           |
| NM_001013828| Iigp1b            | down           |
| NM_008320   | Irf8              | down           |
| NM_194336   | Mpa2l             | down           |
| NM_194336   | Mpa2l             | down           |
| NM_001083925| Oas1b             | down           |
| NM_010724   | Psmb8             | down           |
| ENSMUST00000114230 | Psmb9 | down |
| NM_013585   | Psmb9             | down           |
| NM_001168294| Serpina3f         | down           |
| NM_001199940| Serpina3i         | down           |
| NM_181542   | Slfn10-ps         | down           |
| NM_181545   | Slfn8             | down           |
| NM_009896   | Socs1             | down           |
| NM_013683   | Tap1              | down           |
| NM_011530   | Tap2              | down           |
| NM_178745   | Tmem229b          | down           |
| NM_023835   | Trim12a           | down           |
| NM_009277   | Trim21            | down           |
| NM_199146   | Trim30d           | down           |
| NM_030738   | Vmn1r65           | down           |
| Gene ID          | Gene Symbol | Change |
|-----------------|-------------|--------|
| NM_001037713    | Xaf1        | down   |
| NM_175475       | Cyp26b1     | up     |
| NM_199015       | D14Ertd68e  | up     |
| NM_26637        | Ggct        | up     |
| XM_003084464    | Gm16340     | up     |
| NM_001045481    | Ili203      | up     |
| NM_008329       | Ili204      | up     |
| BC010546        | Ili204      | up     |
| NM_011019       | Osmr        | up     |
| NM_178446       | Rbm47       | up     |
| NM_011580       | Thbs1       | up     |
| NM_023137       | Ubd         | up     |
| NM_001139519    | Zbp1        | up     |

IFN-γ-induced genes (in alphabetical order) either down-regulated by both, the S727A mutation and Cdk8 silencing, or up-regulated under the same conditions. Genes that appear more than once have different probe set IDs for each listing. Data are extracted from the Table S4.
Supplemental Experimental Procedures

RNAi-Mediated Silencing
Approx. 2x 10^5 (or 5x 10^5) MEFs were seeded in 3.5 cm (or 6 cm) format in DMEM supplemented with 10% FCS without antibiotics. Next day the medium was replaced with 2.5 ml (or 5 ml) fresh medium and cells were transfected with 50 pmol (or 100 pmol) ON-TARGET plus™ SMART pool siRNA (Dharmacon) using Lipofectamine™ RNAiMAX Reagent in Opti-MEM I (both Invitrogen). After 48 hours cells were used for whole cell extract preparation or RNA isolation.

Immunoblotting
Procedures for whole cell extracts, immunoprecipitation and immunoblotting were as described (Sadzak et al., 2008). Primary antibodies used to detect proteins are described in antibody list (below).

Chromatin Immunoprecipitation
Chromatin Immunoprecipitation assay (ChIP) was performed as in (Hauser et al., 2002) with the following modifications. To pull down the antibodies Protein G Dynabeads (Invitrogen) were used. The amount of immunoprecipitated DNA was quantified in qPCR using Kapa Sybr Fast qPCR Universal Mix (Peqlab) and primers described in the primer list (below). All qPCRs were run on Mastercycler ep realplex² (Eppendorf). Values of immunoprecipitated DNA were shown as % input. Antibodies used in ChIP assay are specified in the antibody list (below).

Purification of Recombinant Proteins
Plasmids encoding STAT1 GST fusion proteins (GST-STAT1-WT aa711-750 and the corresponding S727A mutant) were described (Kovarik et al., 1999). STAT3 and STAT5a GST fusion proteins (GST-STAT3-WT aa715-770 and the corresponding S727A mutant, GST-STAT5a-WT aa704-793 and the corresponding S725A and S779A single mutants and S725A/S779A double mutant) were obtained by cloning the TADs into pGEX-4T1 (Promega). Standard techniques for expression and purification of GST fusion proteins were used. The four protein CDK8 module was expressed and purified according to (Knuesel et al., 2009b). The TFIIH complex was purified from HeLa cells as described in (Knuesel et al., 2009a). P-TEFb was isolated following recombinant expression in insect cells. SF9 cells were co-infected with high-titer virus at a multiplicity of infection ratio of 1:2 (CDK9:Cyc T1) for 48 h at 27°C. P-TEFb was purified from SF9 cell pellets as described (Tahirov et al.).

Kinase Assays
Reactions were performed with 1 μl CDK8 module, 1 μl TFIIH, and 0.75 μl P-TEFb with 500 ng GST-RNAPII CTD and 750 ng of GST-STAT-TAD (STAT1, STAT3, STAT5a and the corresponding mutants) or GST only in kinase buffer (25 mM Tris pH 8.0, 100 mM KCl, 100 μM ATP, 10 mM MgCl₂, and 2 mM DTT) with 2.5 μCi [γ-32P]ATP at 30°C for 60 min. SDS-PAGE was used to separate proteins and the gels were subsequently silver stained, dried at 55°C for 60 min, exposed on a phosphor-imager screen for 18 h, and imaged using a Typhoon 9400 scanner. Quantitation of auto-rad bands was performed using ImageJ.

Microarray Analysis
STAT1 WT and STAT1 S727A MEFs were treated with siRNA as described above and stimulated with IFN-γ for 4 h or left untreated. Total RNA was isolated from cells using TRIzol reagent (Invitrogen) following the manufactures protocol and used for expression analysis using Agilent Whole Mouse Genome Microarrays, 8x60K. Standard protocols for labeling and hybridization were followed. In brief, fluorescent cRNA was generated using Low Input Quick
Amp Labeling Kit (Agilent). The amplified cyanine 3-labeled cRNA samples were then purified using SV Total RNA Isolation System (Promega) and hybridized to microarray slides. Microarray slides were washed and scanned with an Agilent Scanner. Information from probe features was extracted from microarray scan images using the Agilent Feature Extraction software v10.7.3. Further analyses were performed using R and Bioconductor (Gentleman et al., 2004). Arrays were assessed for quality, weighted and quantile normalized. Subsequently the data were log transformed and filtered for low expression. Residual variances were adjusted using an empirical bayes method (Smyth, 2004) to obtain approximately t-distributed differences in gene expression values. P-values were adjusted for multiple testing using the false discovery rate (Reiner et al., 2003). Microarray data have been deposited in Gene Expression Omnibus under the accession number GSE40728 (GEO, http://www.ncbi.nlm.nih.gov/geo/info/linking.html).

Quantitation of Gene Expression by Quantitative RT-PCR (qRT-PCR)
To measure mRNA expression total RNA was reverse transcribed using oligo (dT)18 primer and Mu-MLV reverse transcriptase (Fermentas). cDNA was amplified with GoTaq qPCR Master Mix (Promega) and primers described in the primer list (below). qRT-PCR was performed using Mastercycler ep realplex2 (Eppendorf). To measure the abundance of primary transcript RNA was digested with DNase I (Roche), purified with RNeasy MiniElute Cleanup Kit (Qiagen) and reverse transcribed using random primers (nonamers) and Superscript III Reverse Transcriptase (Invitrogen).

Metabolic labeling of RNA
Metabolic labeling, biotinylation and purification of nascent RNA were performed as described (Dolken et al., 2008). Briefly, 4-thiouridine (4sU) (100 µM) was added to the cell culture medium 30 min prior to IFN-γ stimulation, simultaneously with IFN-γ, or 60 min and 210 min after IFN-γ stimulation. Labeling was stopped after 30 minutes and total cellular RNA was isolated from cells using TRIzol reagent (Invitrogen) following manufacturer’s protocol. RNA was treated with recombinant RNase-free DNase I (Roche) 0.2 U/µl for 30 minutes at 37°C in order to remove genomic DNA contamination. Biotinylation was carried out in biotinylation buffer (10 mM Tris, 1 mM EDTA) and 0.2 mg/mL Biotin-HPDP at a final RNA concentration of 100 ng/µL for 2 h at 25°C with shaking. Unbound biotin was removed with chloroform/isoamylalcohol. RNA was precipitated at 16000g at 4°C with equal volume of isopropanol and 1:10 volume of 5M sodium chloride. Biotinylated RNA was captured using μMACS streptavidin beads and columns (Miltenyi). Nascent RNA was eluted from streptavidin beads by applying twice 100 µl of 100 mM dithiothreitol (DTT) to the column. RNA was recovered from eluates using the RNeasy MinElute Spin columns (Qiagen). RNA was reverse transcribed using SuperScript III (Invitrogen) and oligo-dT(18) primers following manufacturer’s instructions. For reverse transcription reaction 100 ng of nascent RNA or 1 µg of Total RNA was used. ActB was used for normalization. qRT-PCR was performed on Realplex system (Eppendorf) using GoTaq MasterMix (Promega).

mRNA Stability
Approx. 1x 10⁶ MEFs were seeded in 6 cm format were stimulated with IFN-γ for 4 h and actinomycin D (Sigma) was added (5 µg/ml). After 0, 15 30, 45 and 60 min of act D treatment RNA was isolated and processed for qRT-PCR analysis.

Antiviral Assay with Vesicular Stomatitis Virus
MEFs were seeded on 3.5 cm dishes at 30% confluence and 24 h later they were treated with Cyclin C siRNA, control siRNA or left untreated. siRNA transfection was performed using Lipofectamine RNAiMAX (Invitrogen) according the manufacturer protocol and 50 pmol siRNA
per dish. After 48 hours of siRNA transfection cells were seeded on 96 well plates in normal medium and 5 x 10^3 cells per well. Four hours after seeding the medium was replaced with new medium that was supplemented with fresh siRNA and IFN-γ in two-fold serial dilutions starting at 10 units. The medium was again replaced 24 h later with medium without siRNA and without IFN-γ. VSV was then added at the multiplicity of infection (MOI) of 0.1 and the cells were incubated for additional 39 h. The plates were then washed 2x with PBS (Dulbecco’s PBS, PAA) and surviving cells were stained for 1 h at room temperature in the dark with Crystal violet (40 ml 1% crystal violet and 80 ml methanol, 300 ml dH2O). Cells were then washed twice with PBS, air dried and subsequently incubated with 100 µl solubilization buffer (50/50 mixture of 0.1 M NaH2PO4, pH = 4.5 and 50% ethanol) per well. Crystal violet intensity, that was proportional to number of surviving cells, was determined at 595 nm using a microplate reader (BIO-RAD iMark).

List of Antibodies Used for Western Blotting

pS727 STAT1 (Kovarik et al., 1998), dilution 1:1000
pY701 STAT1 (Cell Signalling, Cat. Nr. 9171), dilution 1:1000
C-terminus STAT1 (Kovarik et al., 1998), dilution 1:1000
N-terminus STAT1 (BD Biosciences, Cat. Nr. 610115), dilution 1:1000
pS727 STAT3 (Cell Signaling Cat. Nr. 9134), dilution 1:500
pY705 STAT3, (Cell Signaling, Cat. Nr. 9131), dilution 1:1000
STAT3 (Cell Signaling, Cat. Nr. 9139), dilution 1:1000
pS722 STAT4 (Santa Cruz, Cat. Nr. sc-28296), dilution 1:500
pY694 STAT4 (Cell Signaling, Cat. Nr. 5267), dilution 1:1000
STAT4 (Cell Signaling, Cat. Nr. 2653), dilution 1:1000
pS725/730 STAT5a/b (Abcam, Cat. Nr. ab36153), dilution 1:1000
pY694/699 STAT5a/b (Upstate-Millipore, Cat. Nr. 05-886), dilution 1:1000
STAT5a/b (Santa Cruz, Cat. Nr. sc-835), dilution 1:1000
CDK7 (Santa Cruz, Cat. Nr. sc-529), dilution 1:1000
CDK8 (Santa Cruz, Cat. Nr. sc-1521), dilution 1:500
CDK8 (Santa Cruz, Cat. Nr. sc-5612), dilution 1:300
CDK9 (Santa Cruz, Cat. Nr. sc-484), dilution 1:1000
pan ERK (BD Biosciences, Cat. Nr. 610123), dilution 1:2000

List of Antibodies Used for Chromatin Immunoprecipitation (ChIP)

pS727 STAT1 (Kovarik et al., 1998), 5 µl serum/ChIP
C-terminus STAT1 (Kovarik et al., 1998), 5 µl serum/ChIP
RNAPII (Santa Cruz, Cat. Nr. sc-899), 4 µg/ChIP
pS2 RNAPII (Bethyl, Cat. Nr. A300-654A), 0,7 µg/ChIP
CDK8 (Santa Cruz, Cat. Nr. sc-1521), 5 µg/ChIP
MED1 (also called TRAP220) (Santa Cruz, Cat. Nr. Sc-5334X), 5 µg/ChIP
Control rabbit IgG (Santa Cruz, Cat. Nr. sc-2027), 4 µg/ChIP
Control goat IgG (Santa Cruz, Cat. Nr. sc-2028), 5 µg/ChIP
Pre-immune serum (Kovarik et al., 1998), 1-2 µl serum/ChIP

List of Primers Used for ChIP

Irf1 (GAS): Fwd- GGAGCAGACAGCTGCTTTGACTTT, Rev- CCCACTCGGCCTCATT
Irf1 (TSS): Fwd- TCCCGCTAAGTTTATGATTTC, Rev- TTCCGGTTTGCTTAGACTG
Irf1 (gene body): Fwd- TGGCTCTTGTCTTTGTCTCTC, Rev- CTCTGTGTTGCTGTGCTG
Tap1 (TSS): Fwd- GGTCAGGTCTGAAACTT, Rev- GCTGTTGCTGTTCTGCTCTCC
Tap1 (GAS): Fwd- AGGCCGTGCTAGTGATTTC, Rev- CGTGAGCTGCTCCAGAGTC
Tap1 (gene body): Fwd- TAGTGTTAGAAATCAGCCTTC, Rev- ATCGCTTCAGTTCGTTAC
List of Primers Used for Quantitation of Gene Expression (qRT-PCR)

Irf1: Fwd - CCGAAGACCTATGGAAGCTTCTGT, Rev - GCAATCTCCCTGACCCTCG
Tap1: Fwd - CTGGCAACCAGCTACGGT, Rev - TGAGAAGAGATGTGTTGG
Gbp2: Fwd - TGCTAACCCTGGAACAGG, Rev - GAGCTTGGCAGAGGTTTG
Irf8 (Ouyang et al., 2011): Fwd - GAGCGAAGTTCTGAGATGT, Rev - TGGGCTCCTTCTTGTCTACAT
Isg15 (Wood et al., 2012): Fwd - ATGGCCTGGGACCCTAAG, Rev - TAGGCACACTGGTCCCC
Hprt: Fwd - GGTGTTAATCTACGCTTGT, Rev - ACACCTGTAATTACTGGA
ActB: Fwd - CAACGAGCGGTTCGATG, Rev - GCCACAGGATTCCATCA

List of QuantiTect Primer Assays (Qiagen) for qRT-PCR

Cdk8: Mm_Cdk8_1_SG, Cat. Nr. QT00158697
CycC: Mm_Ccnc_1_SG, Cat. Nr. QT00161420

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