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ORIGINAL ARTICLE

A novel anti-viral role for STAT3 in IFN-α signalling responses

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Abstract The cytokine, Interferon (IFN)-α, induces a wide spectrum of anti-viral mediators, via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. STAT1 and STAT2 are well characterised to upregulate IFN-stimulated gene (ISG) expression; but even though STAT3 is also activated by IFN-α, its role in anti-viral ISG induction is unclear. Several viruses, including Hepatitis C and Mumps, reduce cellular STAT3 protein levels, via the promotion of ubiquitin-mediated proteasomal degradation. This viral immune evasion mechanism suggests an undiscovered anti-viral role for STAT3 in IFN-α signalling. To investigate STAT3's functional involvement in this Type I IFN pathway, we first analysed its effect upon the replication of two viruses, Influenza and Vaccinia. Viral plaque assays, using Wild Type (WT) and STAT3-/- Murine Embryonic Fibroblasts (MEFs), revealed that STAT3 is required for the inhibition of Influenza and Vaccinia replication. Furthermore, STAT3 shRNA knockdown also enhanced Influenza replication and hindered induction of several, well characterised, anti-viral ISGs: PKR, OAS2, MxB and ISG15; while STAT3 expression had no effect upon induction of a separate ISG group: Viperin, IFI27, CXCL10 and CCL5. These discoveries reveal, for the first time, an anti-viral role for STAT3 in the IFN-α pathway and characterise a requirement for STAT3 in the expression of specific ISGs. These findings also identify STAT3 as a therapeutic target against viral infection and highlight it as an essential pathway component for endogenous and therapeutic IFN-α responsiveness.

Keywords
Janus kinase/signal transducer of activator of transcription (JAK/STAT) · Interferon-alpha (IFN-α) · STAT3 · IFN-stimulated gene (ISG) · Vaccinia virus · Influenza virus · Anti-viral

Abbreviations

AA	Amino acid
ANOVA	Analysis of variance
BCL6	B-cell lymphoma 6
DC	Dendritic cell
EIF2A	Eukaryotic translation initiation factor 2A
EMCV	Encephalomyocarditis virus
EMSA	Electrophoretic mobility shift assay
GAS	Gamma-activated sequence
HCV	Hepatitis C virus
HIF-1α	Hypoxia-inducible factor 1 alpha
HIV	Human immunodeficiency virus
IAV	Influenza A virus
IFN	Interferon
IFN-α	Interferon alpha

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IFN-αR	IFN alpha receptor
IL	Interleukin
IRF	IFN regulatory factor
ISG	IFN-Stimulated Gene
ISGF3	IFN-stimulated gene factor 3
ISRE	IFN-stimulated response element
JAK	Janus kinase
MDCK	Madin–Darby canine kidney
MEF	Murine embryonic fibroblast
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MxA/MxB	Myxovirus resistance gene A/B
NK	Natural killer
OAS2	2′-5′-Oligoadenylate synthetase 2
PKR	Protein kinase R
PRR	Pathogen recognition receptor
qRT-PCR	Quantitative real time polymerase chain reaction
RIG-I	Retinoic acid-inducible gene I
RK13	Rabbit kidney 13
RPS15	Ribosomal protein S15
SH2	Src Homology 2
shRNA	Short hairpin RNA
SIE	Sis-inducible element
SOCS	Suppressor of cytokine signalling
STAT	Signal transducer and activator of transcription
TNF-α	Tumour necrosis factor alpha
TGF-β	Transforming growth factor beta
Tyk2	Tyrosine kinase 2
VACV	Vaccinia virus
VSV	Vesicular stomatitis virus
WT	Wild type

Introduction

Type I IFNs (IFN-α, -β, -ω, -κ and -ε) are the first line of defence against viral infection and act through autocrine and paracrine pathways to induce an intracellular, anti-viral state [31]. Pathogen recognition receptor (PRR) detection of viral components, including RNA, DNA, protein and glycoprotein, stimulates Type I IFN synthesis. Once secreted by the virally infected cell, Type I IFNs activate the IFN-αR (composed of IFN-αR1 and IFN-αR2 chains), which initiates signalling via the JAK/STAT pathway. Intracellular JAK1 and Tyrosine kinase (Tyk)2 phosphorylate receptor tyrosine residues, which provide docking sites for cytoplasmic STAT proteins [42]. STATs are a family of seven mammalian transcription factor proteins: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6. STATs promote induction of a wide range of genes, encoding proteins with diverse activities, including cell growth, differentiation and immune defence. Receptor bound STATs are themselves phosphorylated by JAKs and subsequently dissociate from the receptor to form homo- and hetero-dimers which translocate into the nucleus. STAT1 and STAT2 are well-established mediators of the anti-viral response, which bind IFN regulatory factor (IRF)9, thus forming the IFN-stimulated gene factor (ISGF)3 complex ([14]; [44]). Once ISGF3 translocates to the nucleus, it binds to the IFN-stimulated response element (ISRE) promoter site, which induces expression of several hundred ISGs, many of which are characterised as anti-viral, including protein kinase R (PKR), myxovirus resistance genes (Mx) and 2′-5′-oligoadenylate synthetase (OAS)2 [7, 11, 24, 28]. These genes use a spectrum of anti-viral processes to effectively eliminate infection, including apoptosis, suppression of cell growth and direct inhibition of virus transcription and translation [46]. ISGs are also involved in propagating innate and adaptive immunity via processes such as MHC class I synthesis and induction, which enhances CD8 cytotoxic T cell activity [16]; activation of NK cells, which selectively kills virally infected cells [43]; maturation of DCs, which enhances viral antigen presentation [34] and stimulation of B cell responses [35].

While the role of STAT1 and STAT2 in IFN-α signalling is extensively documented, a role for STAT3 is uncertain. IFN-α phosphorylates STAT3 and promotes the formation of STAT3:STAT3 and STAT1:STAT3 dimers [5]. The role of STAT3 has been historically limited to the transcription of oncogenes including c-Fos [52] and HIF-1α [40]; pro-inflammatory genes, such as TGF-β [32], IL-6 [50] and TNF-α [9]; cell-cycle genes, including p21 [4] and Bcl-xL [6] and anti-apoptotic genes, including Bcl-2 [10] and Survivin [22]. While STAT3's role in cell proliferation and oncogenesis is clear, its contribution to anti-viral activity remains elusive. Recent studies have shown that STAT3 activity is targeted by Hepatitis C and Mumps viruses [47, 48]. Since both viruses actively deplete STAT3 expression, we hypothesised that this immune evasion strategy reveals an, as-of-yet, undiscovered anti-viral role for STAT3.

Here we show that inhibition of both Vaccinia virus (VACV) and Influenza A virus (IAV) replication is dependent on STAT3. A sub-group of ISGs (PKR, OAS2, MxB and ISG15), all with different anti-viral functions, is dependent on STAT3 for its induction. However, a separate group of ISGs (Viperin, IFI27, CXCL10 and CCL5) are unaffected by STAT3 knockdown. Together, these results reveal an essential, gene-specific anti-viral role for STAT3 in IFN-α signalling, which may be instrumental in developing new therapeutics that target viral infection.

Materials and methods

Cell culture

WT and STAT3−/− MEFs, Huh7 cells and A549 cells were grown in DMEM, supplemented with 10% FCS, 250U/ml penicillin and 250 μg/ml streptomycin. Cells were treated with 1000 IU/ml human IFN-α2A (Roche) or 1000 IU/ml murine IFN-α (Bio-techne), as appropriate.

Immunoblotting

To prepare whole cell lysates, cells were harvested in RIPA lysis buffer, supplemented with protease and phosphatase inhibitors (1 mM Sodium orthovanadate (Na_3VO_4), 1 mM Phenylmethylsulfonylfluoride (PMSF), 1 mM leupeptin), 25U/ml benzonase and 1 mM DTT. Following 30 min incubation at 4 °C, SDS loading buffer was added and samples were boiled at 95 °C for 10 min. Equal quantities of whole cell lysates were resolved by electrophoresis on a denaturing SDS-polyacrylamide gel according to the method of Laemmli [33] and transferred to a PVDF membrane. Following Immunoblotting using anti-STAT1 (Cell signalling), anti-STAT2 (Santa Cruz), anti-phospho-STAT1 (Cell Signalling), anti-STAT3 (Santa Cruz) and anti-β-actin antibodies (Sigma), the membrane was developed using enhanced chemiluminescent horse radish peroxidase (HRP) substrate (BioRad) and analysed using the BioRad Imaging system.

shRNA

Huh7 or A549 cells were transfected in 6 well plates with 3 μg of either scrambled or STAT3-specific shRNA (InvivoGen), using Lipofectamine 2000 transfection reagent (Life Technologies). Huh7 experiments: after 48 h transfection, cells were incubated in fresh media for 4 h, prior to IFN-α stimulation. A549 cell experiments: After 24 h transfection, cells were infected with IAV for 24 h.

qRT-PCR

RNA was isolated from cells using the TRI Reagent (Sigma) method. qRT-PCR was carried out as described previously [12]. Gene amplifications were normalised to Ribosomal protein S 15 (RPS15) (human) or 18S (murine). Primers used in the study are listed in Table 1 (human) and Table 2 (murine).

Viral infection and plaque assays

VACVΔE3L (formerly called vP1080) was engineered by in vivo recombination to delete the E3L gene and

Table 1 Human primers used in study

Gene	Forward sequence	Reverse sequence
PKR	TCTCAGCAGATACATCAGAGATA	AGTATACTTTGTTTCTTTCATGT
OAS	GAAGCCCTACGAAGAATGTCAGA	TCGGAGTTGCCTCTTAAGACTGT
MxB	AAGCAGTATCGAGGCAAGGA	TCGTGCTCTGAACAGTTTGG
ISG15	TCCTGCTGGTGGTGGACAA	TTGTTATTCCTCACCAGGATGCT
IFI27	GGCAGCCTTGTGGCTACTCT	CCCAGGATGAACTTGGTCAATC
Viperin	CGTGGAAGAGGACATGACGGAAC	CCGCTCTACCAATCCAGCTTC
CCL5	CAAGGAGCGGGTGGGGTAGGA	ATCCCCCAAACTGGCTGTCCCG
CxCL10	GGAAGCACTGCATCGATTTTG	CAGAATCGAAGGCCATCAAGA
STAT3	GAGAAGGACATCAGCGGTAAGAC	GCTCTCTGGCCGACAATACTTT
RPS15	CGGACCAAAGCGATCTCTTC	CGCACTGTACAGCTGCATCA

Table 2 Murine primers used in study

Gene	Forward sequence	Reverse sequence
MxB	GTCGCCTATTCACCAGGCTC	AGCATAACCTTTTGCGAAATTCT
ISG15	TGAGAGCAAGCAGCCAGAAG	ACGGACACCAGGAAATCGTT
Viperin	ATCGCTTCAACGTGGACGAA	GGAAAACCTTCCAGCGCAC
CxCL10	CTGAATCCGGAATCTAAGACCA	GAGGCTCTCTGCTGTCCATC
STAT3	AGATCATGGATGCGACCAACA	CCGTTATTTCCAAACTGCATCA
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
GAPDH	GATGCCCCCATGTTTGTGAT	GGTCATGAGCCCTTCCACAAT

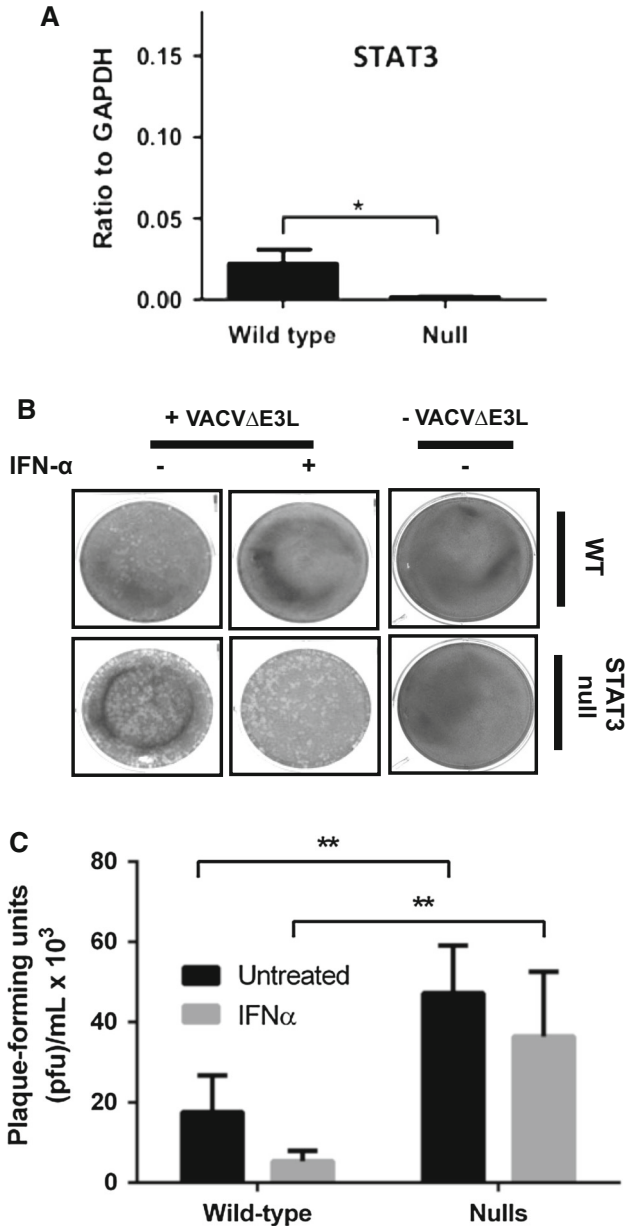

Fig. 1 STAT3 is required for anti-viral activity against Vaccinia virus. **a** STAT3 mRNA levels were confirmed in MEFs derived from WT and STAT3−/− ("null") mice by qRT-PCR, before they were infected with vaccinia virus (strain VACVΔE3L) and **b** treated with IFN-α for 0 and 24 h, after which viral plaque assays using RK13 cells were carried out and **c** quantified. The data shown in Fig. 1C are the mean plaque-forming units/mL × 10^3 obtained from 4 replicates carried out over 3 independent experiments

replace it with a lacZ gene at this locus [3]. MEFs were infected with VACVΔE3L at an MOI of 0.1. After 1 h at 37 °C, inoculum was aspirated, replaced with 2 ml 2.5% FCS DMEM, treated with IFN-α and harvested after 24 h. Cells were washed with PBS, subjected to three freeze–thaw cycles and sonicated. Viral yields were quantified by plaque titration on rabbit kidney (RK)13 cells. RK13 cells were infected with serial dilutions of VACVΔE3L samples from MEFs. After 1.5 h, inoculum was replaced with 1.5% carboxycellulose/MEM (2.5% FCS) overlay for 48 h, before cells were stained with 0.1% crystal violet for 1 h, dried and plaques counted.

MEFs or STAT3 shRNA-transfected A549 cells were infected with IAV A/Puerto Rico/8/1934 (PR8) strain at an MOI of 0.3 and 0.01, respectively. After 1 h at 37 °C, inoculum was aspirated, cells were washed with PBS and overlayed with serum free DMEM with 0.3% BSA (Fraction V, GIBCO), plus TPCK trypsin (Worthington BioChem) and incubated at 37 °C for 24, 48 or 72 h, at which points supernatants were harvested. Supernatants were titrated by plaque assay on MDCK cells and plaque assay plates were incubated at 37 °C for 3 days before staining with crystal violet.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.01 for Windows. Groups were compared by paired or unpaired student's *t* test for parametric samples with Gaussian distributions, and two-way ANOVA with Bonferroni's multiple comparison post-test analysis, as appropriate $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

Results

STAT3 is required for anti-viral activity against Vaccinia virus

To determine if STAT3 has a functional anti-viral role, we initially examined VACV replication following IFN-α treatment of WT and STAT3-/- MEFs. VACV is a large dsDNA poxvirus, well known to elicit a robust Type I IFN response [41]. As the VACV-derived E3 protein is a potent inhibitor of the IFN response [23, 38], we used a VACV deletion mutant lacking its expression: VACV-ΔE3L. After confirming the presence and absence of STAT3 mRNA in WT and STAT3-/- MEFs, respectively (Fig. 1a), we infected them with VACVΔE3L, before treating with IFN-α for 24 h, following which viral titres were quantified by plaque assay titration on RK13 cells. We found that viral plaque formation was considerably increased in RK13 cells incubated with inoculum from STAT3−/− MEFs compared with WT, demonstrating, for the first time, that STAT3 suppresses viral activity (Fig. 1b, c). Furthermore, while treatment with the anti-

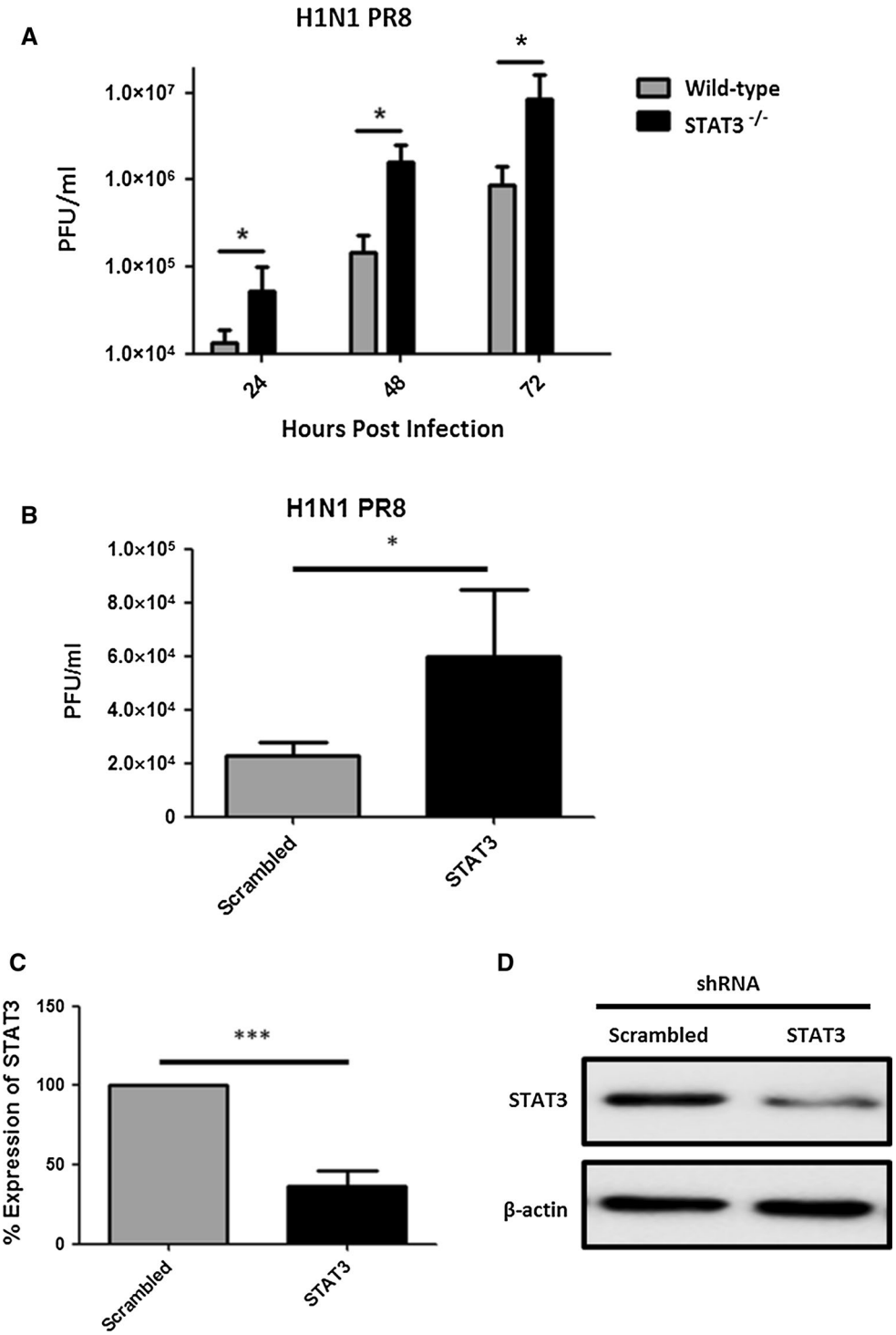

Fig. 2 STAT3 is required for anti-viral activity against Influenza A virus. **a** WT and STAT3−/− MEF cells were infected with PR8 H1N1 virus. After incubation with Influenza, cells were washed 3 times, overlayed with media and incubated at 37 °C. At 24, 48 and 72 h post infection, samples of the supernatant were collected and titrated by plaque assay on MDCK cells ($n = 3$). **b** A549 cells were transfected with scrambled control or STAT3-specific shRNA for 24 h before infection with IAV for 24 h. Supernatants were titrated by plaque assay on MDCK cells. At 48 h post transfection **c** STAT3 mRNA and **d** protein levels were determined by qRT-PCR or immunoblotting, respectively ($n = 3$)

viral cytokine, IFN-α, reduced VACV replication in WT cells (STAT3 expressing cells), IFN-α had virtually no effect upon the formation of viral plaques without STAT3 expression (STAT3−/− cells) (Fig. 1b, c). These results demonstrate a lack of effective IFN-α-driven responses in the absence of STAT3 and thus reveal a new anti-viral role for STAT3 in IFN-α signalling.

STAT3 is required for anti-viral activity against Influenza A virus

While VACV is a DNA virus and IAV an RNA virus, PRR detection of either induces Type I IFN expression that subsequently mediates anti-viral activity through the JAK/STAT pathway [2, 49]. Having discovered that STAT3 is

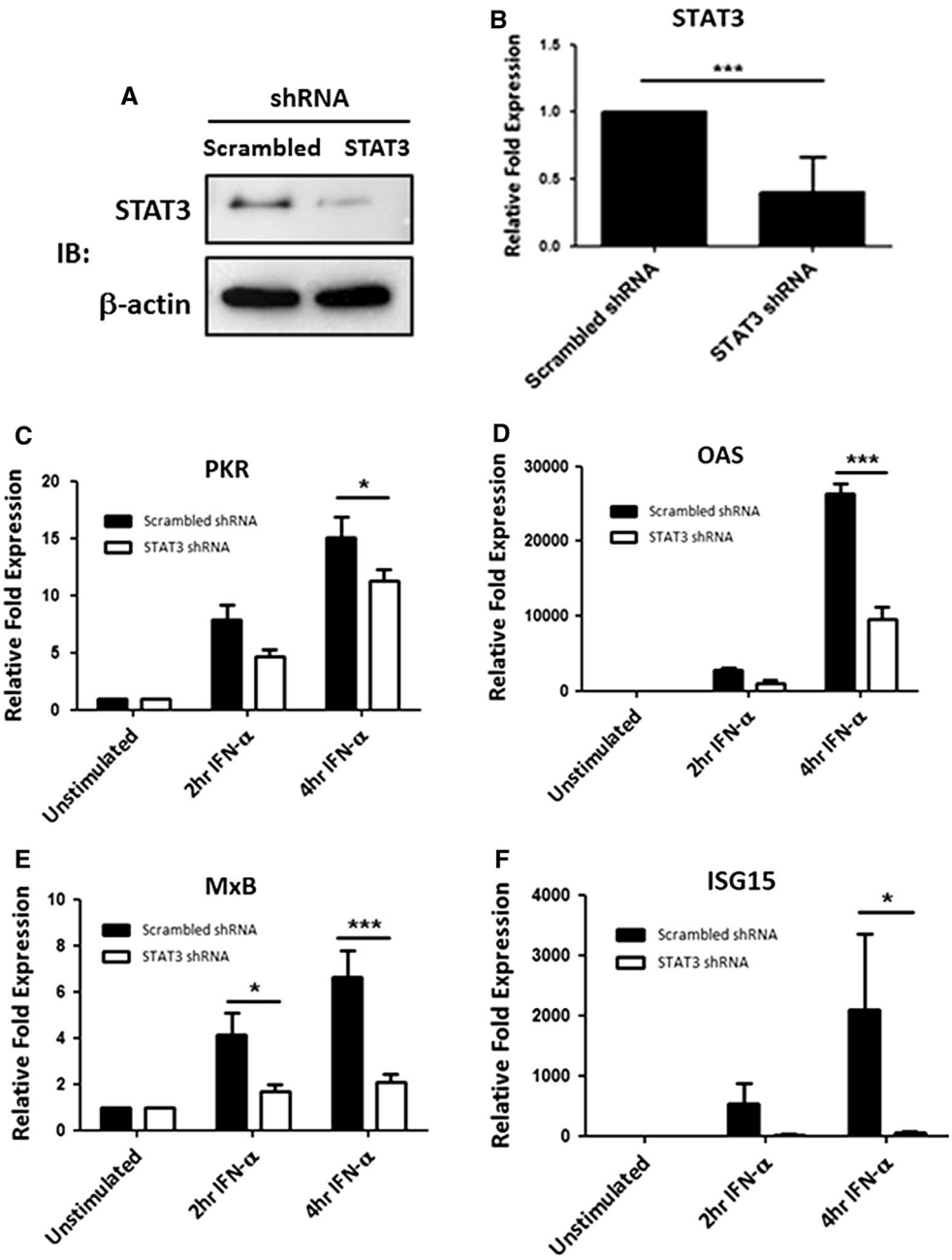

Fig. 3 STAT3 knockdown significantly reduces IFN-α induction of PKR, OAS, MxB and ISG15. Huh7 hepatocytes were transfected with scrambled control or STAT3-specific shRNA for 48 h. After confirming **a** STAT3 protein and **b** mRNA knockdown, cells were stimulated for 2 or 4 h with IFN-α. qRT-PCR was performed to measure **c** PKR, **d** OAS, **e** MxB and **f** ISG15 mRNA induction

required for IFN-α-mediated inhibition of VACV replication (Fig. 1b, c), we subsequently assessed the role of STAT3 in IAV infection, using WT and STAT3−/− MEFs. We found that IAV replicated to significantly higher titres at 24, 48 and 72 h post infection in STAT3−/− MEFs, compared with STAT3-expressing WT MEFs (Fig. 2a). To examine the cell-type specificity of this anti-viral effect, we also infected human alveolar basal epithelial (A549) cells with IAV following shRNA-mediated knockdown of STAT3 and investigated replication of the virus 24 h post infection. Suppression of endogenous STAT3 levels in A549 epithelial cells led to significantly higher titres of IAV (Fig. 2b). Knockdown of STAT3 mRNA and protein in A549 cells was also confirmed by qRT-PCR (Fig. 2c) and immunoblotting (Fig. 2d), respectively. These observations further implicate STAT3 as an essential anti-viral mediator and demonstrate that its activity has immune regulatory implications for both RNA and DNA viral infection.

STAT3 is specifically required for induction of a subset of IFN-α-driven ISGs

Having established a functional role for STAT3 in blocking viral replication, we next assessed the molecular role for

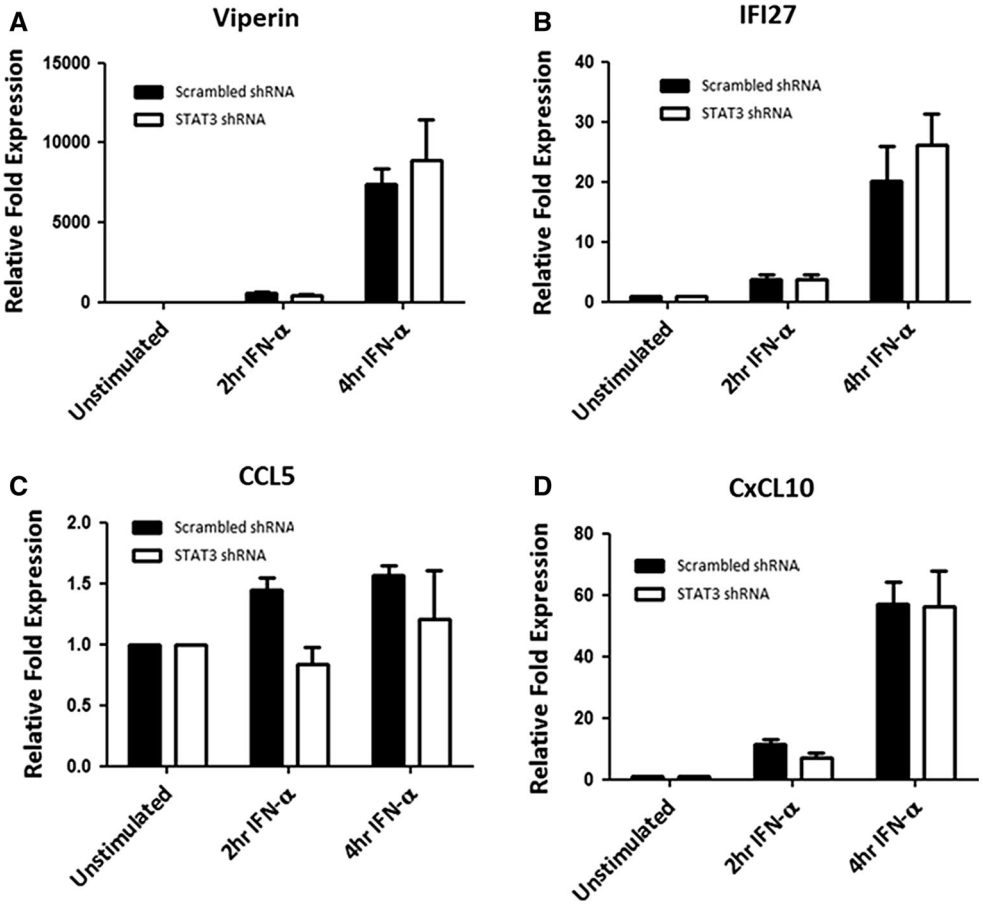

Fig. 4 STAT3 knockdown does not affect IFN-α induction of Viperin, IFI27, CCL5 and CxCL10. Huh7 hepatocytes were transfected with scrambled control or STAT3-specific shRNA for 48 h before stimulation for 2 or 4 h with IFN-α. qRT-PCR was performed to measure **a** Viperin, **b** IFI27, **c** CCL5 and **d** CxCL10 mRNA induction

STAT3 in anti-viral ISG induction, using the IFN-responsive, human hepatocyte cell line, Huh7. Huh7 cells were transfected with either scrambled or STAT3 shRNA for 48 h, before being stimulated for 2 or 4 h with IFN-α. STAT1, 2 and 3 protein levels were also measured in Huh7 cells following STAT3-shRNA knockdown which confirmed that neither STAT1 nor STAT2 protein levels were altered in the absence of STAT3 (Supplementary Fig. 1). Knockdown of STAT3 protein (Fig. 3a) and mRNA (Fig. 3b) was confirmed by immunoblotting and qRT-PCR, respectively. Next, IFN-α-mediated induction of a group of well-defined anti-viral ISGs (PKR, OAS2, MxB, ISG15, Viperin, IFI27, CCL5 and CxCL10) was analysed by qRT-PCR. We found that induction of PKR, OAS2, ISG15 (4 h IFN-α) and MxB (2 and 4 h IFN-α) was significantly reduced upon STAT3 knockdown, compared to the scrambled control (Fig. 3c–f). Of particular significance was the dramatic suppression of ISG15 induction upon STAT3 shRNA-mediated depletion; in fact, this anti-viral gene had virtually no response to IFN-α in the absence of STAT3 (Fig. 3f). In contrast, qRT-PCR analysis showed that IFN-α-induced expression of Viperin, IFI27, CCL5 and CxCL10 (Fig. 4a–d), was unaffected by STAT3 knockdown, compared to scrambled controls. Having observed that STAT3 was required for anti-viral activity against Vaccinia (Fig. 1b, c) and Influenza (Fig. 2a) in MEFs, we next analysed if, as in Huh7 cells, STAT3 was required for specific ISG induction in MEFs. WT and STAT3−/− MEFs were treated with IFN-α for 4 h before ISG15, MxB, Viperin and CxCL10 were measured by qRT-PCR. Interestingly, we found that, as in Huh7 cells (Figs. 3e, f, 4a, d), induction of ISG15 and MxB was reduced in the absence of STAT3, whereby Viperin and CxCL10 induction was not (Fig. 5a–d). Importantly, we also confirmed that STAT3 depletion in the MEF cells had no effect on STAT1 expression nor IFN-α-induced STAT1 phosphorylation (Supplementary Fig. 2). Together, these results reveal that induction of a specific ISG subset relies on STAT3 and indicate an essential role for STAT3 in IFN-α signalling that suppresses replication of both RNA and DNA viruses.

Discussion

STAT3 is well known to have essential roles in cell survival, differentiation and proliferation. It is often constitutively active in a wide range of cancers, including

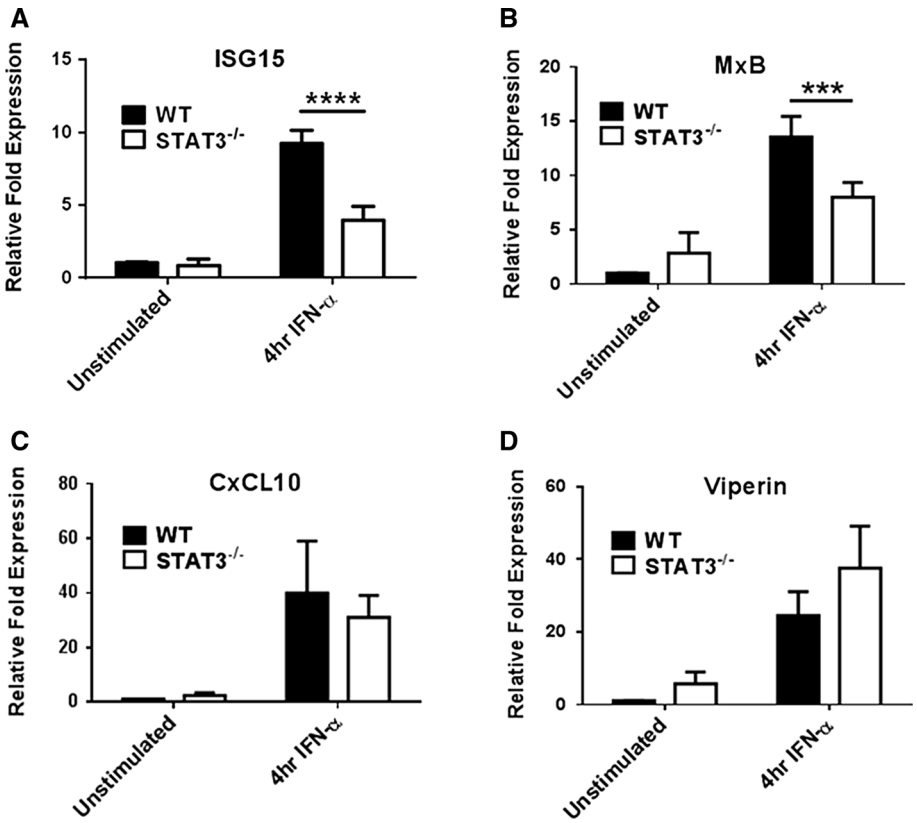

Fig. 5 STAT3-null MEFs display significantly decreased IFN-α induction of ISG15 and MxB, but not CXCL10 or Viperin; MEFs derived from WT and STAT3−/− mice MEFs were stimulated for 4 h with murine IFN-α. qRT-PCR was performed to measure **a** ISG15, **b** MxB, **c** CXCL10 and **d** Viperin mRNA induction ($n = 3$)

leukaemia [20], lymphoma [39], multiple myeloma [25]; along with carcinomas of the breast [18], head and neck [37], ovary [30] and prostate [36]. While this proliferative function for STAT3 is extensively defined, its effect on anti-viral activity remains undecided. Here, we identify a new anti-viral role for STAT3 in IFN-α signalling, by demonstrating that STAT3 is required to block replication of both VACV and IAV and is essential for the optimum induction of a specific subset of anti-viral ISGs.

STAT1 and STAT2 are classically linked to anti-viral responses [29], with viruses such as Respiratory syncytial virus [15], Simian virus 5 (strain W3A) [1], Sendai virus [19] and HCV [47], targeting them for degradation to avoid the host immune response. Our study reveals STAT3 as another essential anti-viral mediator, thus explaining why it is also targeted for degradation by HCV and Mumps [47, 48]. Here, we have shown that loss of STAT3 specifically inhibits a unique group of ISGs (PKR, OAS2, ISG15 and MxB), well characterised to combat viral infection through a broad spectrum of mechanisms, including inhibition of viral mRNA translation (PKR) [17], ISGylation (ISG15) [13], viral RNA degradation (OAS) [7] and reduced replication and DNA integration (MxB) [21].

Although STAT3 is known to be activated by IFN-α [5], its anti-viral role in IFN-α signalling has remained poorly defined. The primary immunodeficiency disease, Hyper-immunoglobulin(Ig)-E recurrent-infection syndrome (or Jobs), is caused by STAT3 mutations, that affect its DNA-binding domain and SH2 domain [8, 27]. While "Jobs" is commonly linked to bacterial skin disease and pneumonia, patients also have decreased ability to control Varicella zoster and Epstein-Barr viruses [45], further supporting STAT3's role as an essential anti-viral mediator.

Other groups have previously explored STAT3's anti-viral role [26, 51], however, we are first to investigate these effects in human hepatocytes and specifically against VACV and IAV. Ho et al., suggested that STAT3 might sequester STAT1 in the THP-1 monocytic cell line, thus reducing IRF-1, CXCL-9 and CXCL-10 induction [26]. Due to their robust IFN-α responsiveness, we used the human Huh7 hepatoma cell line during our ISG analysis, which may explain the differences observed by Ho et al., in THP-1 cells and thus reveal cell type variation in STAT3's anti-viral activity. While Wang et al., found that knocking out STAT3 in MEFs suppressed titres of Encephalomyocarditis virus (EMCV) and vesicular stomatitis virus (VSV) [51], we found it enhanced VACV and IAV replication, possibly identifying a viral specificity for STAT3's activity.

In summary, our results demonstrate a previously undiscovered anti-viral function for STAT3 in IFN-α signalling, which is crucial for clearance of both VACV and IAV. These results reveal an anti-viral gene-specific role for STAT3, which is instrumental to our overall

understanding of innate immunity against viruses. We anticipate these exciting findings to have wider implications against other viruses and be fundamental in the development of new immune regulatory therapeutics.

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References

1. Andrejeva J, Young DF, Goodbourn S, Randall RE (2002) Degradation of STAT1 and STAT2 by the V proteins of simian virus 5 and human parainfluenza virus type 2, respectively: consequences for virus replication in the presence of alpha/beta and gamma interferons. J Virol 76(5):2159–2167
2. Baum A, Garcia-Sastre A (2011) Differential recognition of viral RNA by RIG-I. Virulence 2(2):166–169
3. Beattie E, Denzler KL, Tartaglia J, Perkus ME, Paoletti E, Jacobs BL (1995) Reversal of the interferon-sensitive phenotype of a vaccinia virus lacking E3L by expression of the reovirus S4 gene. J Virol 69(1):499–505
4. Bellido T, O'Brien CA, Roberson PK, Manolagas SC (1998) Transcriptional activation of the p21(WAF1, CIP1, SDI1) gene by interleukin-6 type cytokines. A prerequisite for their pro-differentiating and anti-apoptotic effects on human osteoblastic cells. J Biol Chem 273(33):21137–21144
5. Caraglia M, Marra M, Pelaia G, Maselli R, Caputi M, Marsico SA, Abbruzzese A (2005) Alpha-interferon and its effects on signal transduction pathways. J Cell Physiol 202(2):323–335. doi:10.1002/jcp.20137
6. Catlett-Falcone R, Landowski TH, Oshiro MM, Turkson J, Levitzki A, Savino R, Ciliberto G, Moscinski L, Fernandez-Luna JL, Nunez G, Dalton WS, Jove R (1999) Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity 10(1):105–115
7. Chakrabarti A, Jha BK, Silverman RH (2011) New insights into the role of RNase L in innate immunity. J Interf Cytokine Res Off J Int Soc Interferon Cytokine Res 31(1):49–57
8. Chandesris MO, Melki I, Natividad A, Puel A, Fieschi C, Yun L, Thumerelle C, Oksenhendler E, Boutboul D, Thomas C, Hoarau C, Lebranchu Y, Stephan JL, Cazorla C, Aladjidi N, Micheau M, Tron F, Baruchel A, Barlogis V, Palenzuela G, Mathey C, Dominique S, Body G, Munzer M, Fouyssac F, Jaussaud R, Bader-Meunier B, Mahlaoui N, Blanche S, Debre M, Le Bourgeois M, Gandemer V, Lambert N, Grandin V, Ndaga S, Jacques C, Harre C, Forveille M, Alyanakian MA, Durandy A, Bodemer C, Suarez F, Hermine O, Lortholary O, Casanova JL, Fischer A, Picard C (2012) Autosomal dominant STAT3 deficiency and hyper-IgE syndrome: molecular, cellular, and clinical features from a French national survey. Medicine (Baltimore) 91(4):e1–e19. doi:10.1097/MD.0b013e31825f95b9
9. Chappell VL, Le LX, LaGrone L, Mileski WJ (2000) Stat proteins play a role in tumor necrosis factor alpha gene expression. Shock 14(3):400–402
10. Choi HJ, Han JS (2012) Overexpression of phospholipase D enhances Bcl-2 expression by activating STAT3 through independent activation of ERK and p38MAPK in HeLa cells. Biochim Biophys Acta 1823(6):1082–1091. doi:10.1016/j.bbamcr.2012.03.015
11. Clemens MJ, Elia A (1997) The double-stranded RNA-dependent protein kinase PKR: structure and function. J Interferon Cytokine Res 17(9):503–524
12. Collins AS, Ahmed S, Napoletano S, Schroeder M, Johnston JA, Hegarty JE, O'Farrelly C, Stevenson NJ (2014) Hepatitis C virus (HCV)-induced suppressor of cytokine signaling (SOCS) 3 regulates proinflammatory TNF-alpha responses. J Leukoc Biol 96(2):255–263. doi:10.1189/jlb.2A1211-608RRRR
13. Dai J, Pan W, Wang P (2011) ISG15 facilitates cellular antiviral response to dengue and west nile virus infection in vitro. Virol J 8:468. doi:10.1186/1743-422x-8-468
14. Darnell JE Jr, Kerr IM, Stark GR (1994) Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264(5164):1415–1421
15. Elliott J, Lynch O, Suessmuth Y, Qian P, Boyd C, Burrows J, Buick R, Stevenson N, Touzelet O, Gadina M, Power U, Johnston J (2007) Respiratory syncytial virus NS1 protein degrades STAT2 by using the Elongin-Cullin E3 ligase. J Virol 81(7):3428–3436 (**JVI.02303-06**)
16. Epperson DE, Arnold D, Spies T, Cresswell P, Pober JS, Johnson DR (1992) Cytokines increase transporter in antigen processing-1 expression more rapidly than HLA class I expression in endothelial cells. J Immunol 149(10):3297–3301
17. Galabru J, Hovanessian A (1987) Autophosphorylation of the protein kinase dependent on double-stranded RNA. J Biol Chem 262(32):15538–15544
18. Garcia R, Yu CL, Hudnall A, Catlett R, Nelson KL, Smithgall T, Fujita DJ, Ethier SP, Jove R (1997) Constitutive activation of Stat3 in fibroblasts transformed by diverse oncoproteins and in breast carcinoma cells. Cell Growth Differ 8(12):1267–1276
19. Garcin D, Marq JB, Strahle L, le Mercier P, Kolakofsky D (2002) All four Sendai Virus C proteins bind Stat1, but only the larger forms also induce its mono-ubiquitination and degradation. Virology 295(2):256–265
20. Gouilleux-Gruart V, Debierre-Grockiego F, Gouilleux F, Capiod JC, Claisse JF, Delobel J, Prin L (1997) Activated Stat related transcription factors in acute leukemia. Leuk Lymphoma 28(1–2):83–88
21. Goujon C, Moncorge O, Bauby H, Doyle T, Ward CC, Schaller T, Hue S, Barclay WS, Schulz R, Malim MH (2013) Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection. Nature 502(7472):559–562
22. Gritsko T, Williams A, Turkson J, Kaneko S, Bowman T, Huang M, Nam S, Eweis I, Diaz N, Sullivan D, Yoder S, Enkemann S, Eschrich S, Lee JH, Beam CA, Cheng J, Minton S, Muro-Cacho CA, Jove R (2006) Persistent activation of stat3 signaling induces survivin gene expression and confers resistance to apoptosis in human breast cancer cells. Clin Cancer Res 12(1):11–19
23. Guerra S, Caceres A, Knobeloch KP, Horak I, Esteban M (2008) Vaccinia virus E3 protein prevents the antiviral action of ISG15. PLoS Pathog 4(7):e1000096. doi:10.1371/journal.ppat.1000096
24. Haller O, Staeheli P, Schwemmle M, Kochs G (2015) Mx GTPases: dynamin-like antiviral machines of innate immunity. Trends Microbiol 23(3):154–163. doi:10.1016/j.tim.2014.12.003
25. Hilbert DM, Kopf M, Mock BA, Kohler G, Rudikoff S (1995) Interleukin 6 is essential for in vivo development of B lineage neoplasms. J Exp Med 182(1):243–248
26. Ho HH, Ivashkiv LB (2006) Role of STAT3 in type I interferon responses. Negative regulation of STAT1-dependent inflammatory gene activation. J Biol Chem 281(20):14111–14118
27. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, Freeman AF, Demidowich A, Davis J, Turner ML, Anderson VL, Darnell DN, Welch PA, Kuhns DB, Frucht DM, Malech HL, Gallin JI, Kobayashi SD, Whitney AR, Voyich JM, Musser JM, Woellner C, Schaffer AA, Puck JM, Grimbacher B (2007)

STAT3 mutations in the hyper-IgE syndrome. N Engl J Med 357(16):1608–1619
28. Honda K, Yanai H, Takaoka A, Taniguchi T (2005) Regulation of the type I IFN induction: a current view. Int Immunol 17(11):1367–1378. doi:10.1093/intimm/dxh318
29. Horvath CM, Stark GR, Kerr IM, Darnell JE (1996) Interactions between STAT and non-STAT proteins in the interferon-stimulated gene factor 3 transcription complex. Mol Cell Biol 16(12):6957–6964
30. Huang M, Page C, Reynolds RK, Lin J (2000) Constitutive activation of stat 3 oncogene product in human ovarian carcinoma cells. Gynecol Oncol 79(1):67–73
31. Isaacs A, Lindenmann J (1957) Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci 147(927):258–267
32. Kinjyo I, Inoue H, Hamano S, Fukuyama S, Yoshimura T, Koga K, Takaki H, Himeno K, Takaesu G, Kobayashi T, Yoshimura A (2006) Loss of SOCS3 in T helper cells resulted in reduced immune responses and hyperproduction of interleukin 10 and transforming growth factor-beta 1. J Exp Med 203(4):1021–1031. doi:10.1084/jem.20052333
33. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259):680–685
34. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF (2001) Type i interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity 14(4):461–470
35. Le Bon A, Thompson C, Kamphuis E, Durand V, Rossmann C, Kalinke U, Tough DF (2006) Cutting edge: enhancement of antibody responses through direct stimulation of B and T cells by type I IFN. J Immunol 176(4):2074–2078
36. Lou W, Ni Z, Dyer K, Tweardy DJ, Gao AC (2000) Interleukin-6 induces prostate cancer cell growth accompanied by activation of stat3 signaling pathway. Prostate 42(3):239–242
37. Mali SB (2015) Review of STAT3 (signal transducers and activators of transcription) in head and neck cancer. Oral Oncol 51(6):565–569. doi:10.1016/j.oraloncology.2015.03.004
38. Myskiw C, Arsenio J, van Bruggen R, Deschambault Y, Cao J (2009) Vaccinia virus E3 suppresses expression of diverse cytokines through inhibition of the PKR, NF-kappaB, and IRF3 pathways. J Virol 83(13):6757–6768
39. Nielsen M, Kaltoft K, Nordahl M, Ropke C, Geisler C, Mustelin T, Dobson P, Svejgaard A, Odum N (1997) Constitutive activation of a slowly migrating isoform of Stat3 in mycosis fungoides: tyrphostin AG490 inhibits Stat3 activation and growth of mycosis fungoides tumor cell lines. Proc Natl Acad Sci USA 94(13):6764–6769
40. Niu G, Briggs J, Deng J, Ma Y, Lee H, Kortylewski M, Kujawski M, Kay H, Cress WD, Jove R, Yu H (2008) Signal transducer and activator of transcription 3 is required for hypoxia-inducible factor-1alpha RNA expression in both tumor cells and tumor-associated myeloid cells. Mol Cancer Res 6(7):1099–1105
41. Perdiguero B, Esteban M (2009) The interferon system and vaccinia virus evasion mechanisms. J Interferon Cytokine Res Off J Int Soc Interferon Cytokine Res 29(9):581–598
42. Qureshi SA, Salditt-Georgieff M, Darnell JE Jr (1995) Tyrosine-phosphorylated Stat1 and Stat2 plus a 48-kDa protein all contact DNA in forming interferon-stimulated-gene factor 3. Proc Natl Acad Sci USA 92(9):3829–3833
43. Salazar-Mather TP, Ishikawa R, Biron CA (1996) NK cell trafficking and cytokine expression in splenic compartments after IFN induction and viral infection. J Immunol 157(7):3054–3064
44. Shuai K (1994) Interferon-activated signal transduction to the nucleus. Curr Opin Cell Biol 6(2):253–259
45. Siegel AM, Heimall J, Freeman AF, Hsu AP, Brittain E, Brenchley JM, Douek DC, Fahle GH, Cohen JI, Holland SM, Milner JD (2011) A critical role for STAT3 transcription factor signaling in the development and maintenance of human T cell memory. Immunity 35(5):806–818
46. Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD (1998) How cells respond to interferons. Annu Rev Biochem 67:227–264. doi:10.1146/annurev.biochem.67.1.227
47. Stevenson NJ, Bourke NM, Ryan EJ, Binder M, Fanning L, Johnston JA, Hegarty JE, Long A, O'Farrelly C (2013) Hepatitis C virus targets the interferon-alpha JAK/STAT pathway by promoting proteasomal degradation in immune cells and hepatocytes. FEBS Lett 587(10):1571–1578
48. Ulane CM, Rodriguez JJ, Parisien JP, Horvath CM (2003) STAT3 ubiquitylation and degradation by mumps virus suppress cytokine and oncogene signaling. J Virol 77(11):6385–6393
49. Unterholzner L (2013) The interferon response to intracellular DNA: why so many receptors? Immunobiology 218(11):1312–1321. doi:10.1016/j.imbio.2013.07.007
50. Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, Bhattacharya R, Gabrilovich D, Heller R, Coppola D, Dalton W, Jove R, Pardoll D, Yu H (2004) Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med 10(1):48–54
51. Wang WB, Levy DE, Lee CK (2011) STAT3 negatively regulates type I IFN-mediated antiviral response. J Immunol 187(5):2578–2585
52. Yang E, Lerner L, Besser D, Darnell JE Jr (2003) Independent and cooperative activation of chromosomal c-fos promoter by STAT3. J Biol Chem 278(18):15794–15799