

# Interferon-inducible factor 16 is a novel modulator of glucocorticoid action

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**ABSTRACT** Previously, we used cDNA expression profiling to identify genes associated with glucocorticoid (Gc) sensitivity. We now identify which of these directly influence Gc action. Interferon-inducible protein 16 (IFI16), bone morphogenetic protein receptor type II (BMPRII), and regulator of G-protein signaling 14 (RGS14) increased Gc transactivation, whereas sialyltransferase 4B (SIAT4B) had a negative effect. Amyloid  $\beta$  (A4) precursor-protein binding, family B, member 1 (APBB1/Fe65) and neural cell expressed developmentally down-regulated 9 (NEDD9) were without effect. Only IFI16 potentiated Gc repression of NF- $\kappa$ B. In addition, IFI16 affected basal expression, and Gc induction of endogenous target genes. IFI16 did not affect glucocorticoid receptor (GR) expression, ligand-dependent repression of GR expression, or the ligand-dependent induction of GR phosphorylation on Ser-211 or Ser-203. Coimmunoprecipitation revealed an interaction, suggesting that IFI16 modulation of GR function is mediated by protein crosstalk. Transfection analysis with GR mutants showed that the ligand-binding domain of GR binds IFI16 and is the target domain for IFI16 regulation. Analysis of human lung sections identified colocalization of GR and IFI16, suggesting a physiologically relevant interaction. We demonstrate that IFI16 is a novel modulator of GR function and show the importance of analyzing variation in Gc sensitivity in humans, using appropriate technology, to drive discovery.—Berry, A., Matthews, L. Jangani, M., Plumb, J., Farrow, S., Buchan, N., Wilson, P. A., Singh, D., Ray, D., W., Donn, R. P. Interferon-inducible factor 16 is a novel modulator of glucocorticoid action. *FASEB J.* 24, 1700–1713 (2010). [www.fasebj.org](http://www.fasebj.org)

**Key Words:** IFI16 • steroid sensitivity • nuclear receptor • inflammation

GLUCOCORTICOIDS (GCS) EXERT DIVERSE effects on virtually all cell types and tissues. Changes in tissue sensitivity to Gcs can result in pathological states (1, 2). Hypersensitivity to Gcs may result in metabolic diseases such as visceral obesity-related insulin resistance and ischemic heart disease (3). Resistance or insensitivity to Gcs occurs within inflamed tissues and insensitivity to the therapeutic properties of exogenous Gcs arises (4).

This limits the use of Gcs in the management of diseases such as rheumatoid arthritis, inflammatory bowel disease, and asthma. Furthermore, certain pathological conditions such as small cell lung cancer and chronic obstructive pulmonary disease (COPD) are profoundly steroid-resistant (5, 6). The response to Gcs is known to vary greatly among individuals within a population (7–10). The glucocorticoid receptor (GR) is a ubiquitous intracellular receptor that mediates the actions of Gcs and polymorphism of the GR contributes, in part, to variations in Gc sensitivity (10, 11). Understanding the genetic factors that influence Gc sensitivity is crucial for elucidating the mechanisms responsible for resistance and also for the development of more specific treatment regimens.

We have previously used microarray technology for transcriptome profiling of lymphocytes from a healthy cohort and identified a panel of discriminatory genes that showed small but significant changes in expression between Gc-sensitive and Gc-resistant groups (7). The aim of the current study was to identify novel genes capable of influencing Gc sensitivity by determining the effects of the genes, identified by microarray, in model cell systems on GR function. Of the 20 discriminating genes originally reported, a proportion were predicted simply to be acting as markers and only some to directly influence Gc action.

In an attempt to prioritize genes for further *in vitro* evaluation a combination of standard literature searches and systems biology informatics was used. Data-mining informatics allows “hypothesis-free” interactions to be

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identified (12). Predicting the functional effects of our genes of interest from such database mining, combined with searching for potential interactions with a GR signaling pathway, may provide an efficient screening process before individual gene expression studies *in vitro*, using simple reporter gene validation. Finally, we sought interactions between selected gene products and the GR itself to provide mechanistic insights.

## MATERIALS AND METHODS

### Plasmids

TAT3-luciferase (Luc), which contains 3 copies of the glucocorticoid response element (GRE) from the tyrosine amino transferase plasmid, was a kind gift from Professor Keith Yamamoto (University of California, San Francisco, CA, USA) and Dr. Jorge Iniguez-Lluhi (University of Michigan Medical School, Ann Arbor, MI, USA). The NRE-Luc reporter construct contains 5 copies of an NF- $\kappa$ B response element (Stratagene, La Jolla, CA, USA).

The human wild-type GR $\alpha$  in pcDNA3 vector has been described previously (13). Two deletion constructs, GR  $\Delta$ AF1, which lacks aa 77-262, and GR N500, which lacks aa 500-777, were prepared from the human GR $\alpha$  plasmid by restriction enzyme digestion.

The SRC2 expression vector, in a pcDNA3 vector backbone, was a kind gift from Dr. Julie Stimmel (GlaxoSmithKline, Research Triangle Park, NC, USA). Full-length bone morphogenetic protein receptor type II (BMPRII), amyloid  $\beta$  (A4) precursor-protein binding, family B, member 1 (APBB1/Fe65), interferon-inducible protein 16 (IFI16), sialyltransferase 4B (SIAT4B), and regulator of G-protein signaling 14 (RGS14) cDNAs cloned into the CMV-SPORT6 vector using *NotI/SalI* restriction sites were obtained from Geneservice (Cambridge, UK). Empty vector control was obtained by cleaving the IFI16 insert out of CMV-SPORT6 using *NotI/SalI* and religating the vector. Neural cell expressed developmentally down-regulated 9 (NEDD9) cDNA was obtained from the German Resource Center for Genome Research (<http://www.imagenes-bio.de>). *Renilla* (sea pansy) luciferase plasmid was used to correct for transfection efficiency (Promega, Southampton, UK). The control TAT3 $\Delta$ GRE plasmid was generated by cleaving the 3 GREs from the TAT3-Luc vector backbone with *SalI/XhoI* restriction enzymes.

### Antibodies

The following antibodies were used: anti-GR (mouse, clone 41) from BD Biosciences (San Jose, CA, USA); anti-phospho (Ser-211)-GR from Cell Signaling Technology Inc. (Danvers, MA, USA); anti-phospho (Ser-203)-GR and anti-phospho (Ser-226)-GR from Abcam Inc. (Cambridge, MA, USA); anti-GR (rabbit, H-300) and anti-IFI16 (rabbit and mouse) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA); anti-tubulin from Sigma-Aldrich (St. Louis, MO, USA); anti-human CD3 (clone PS1) from Vision Biosystems, Newcastle, UK); anti-human CD20 (clone L26) from Vector Laboratories, Peterborough, UK); and horseradish peroxidase-conjugated anti-mouse and anti-rabbit from GE Healthcare (Little Chalfont, UK). Fluorophore-conjugated (Alexa Fluor 546, 568, and 488) anti-mouse and anti-rabbit antibodies were from Invitrogen (Paisley, UK).

### Bioinformatics

To complete the bioinformatics analysis, two knowledge base resources were queried: the Ingenuity Knowledge Base (12)

and an interaction repository, which is based on cpath (14) and includes interactions curated by GeneGo (<http://www.genego.com>) and Ingenuity. Networks retrieved from the latter were visualized using Cytoscape (15). Both resources were queried using established network searching algorithms to infer how the derived differential expression data may interact with established Gc pathways. Based on these analyses, it was possible to direct the described confirmatory experiments detailed in the Results section.

### Cell culture

Human epithelial carcinoma (HeLa) and human embryonic kidney (HEK) cells were obtained from the European Collection of Cell Cultures (Salisbury, UK) and maintained in DMEM supplemented with GlutaMAX I and 10% FCS (Invitrogen) in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C.

### Reporter gene assay

Cells were cotransfected with 2  $\mu$ g of firefly luciferase (TAT3-Luc, NRE-Luc) and 0.5  $\mu$ g of *Renilla* luciferase reporter together using FuGENE 6 (3  $\mu$ l/ $\mu$ g of DNA; Roche Diagnostics, Indianapolis, IN, USA). For some experiments, cells were also transfected with 0.6 or 1.2  $\mu$ g of coactivator or an empty expression vector control or 1  $\mu$ g of wild-type human GR $\alpha$  (GR $\alpha$ ), GR  $\Delta$ AF1, or GR N500 expression plasmids. After 24 h, cells were transferred to medium containing charcoal dextran-stripped serum, treated as specified in the Results section before lysis, and then assayed for luciferase activity following the manufacturer's instructions (Promega) (16). To control for transfection efficiency, cells were taken from a single transfected pool and divided into the different treatment conditions. All firefly luciferase readings were normalized to *Renilla* luciferase.

### Small interfering RNA (siRNA) transfection

HeLa cells were transfected with 10 nM IFI16 siRNA (catalog no. 4392420, siRNA ID s7138; Ambion, Austin, TX, USA) or 10 nM lamin siRNA (4390771, siRNA ID s82222; Ambion) using Lipofectamine RNAiMax (Invitrogen) in accordance with the manufacturer's instructions. Forty-eight hours later, cells were treated as specified in the Results section and processed accordingly.

### Immunoblot analysis

Cells were treated as specified in the Results section and lysed in RIPA buffer (50 mM Tris-Cl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, and 1 mM EDTA) containing protease (Calbiochem, San Diego, CA, USA) and phosphatase inhibitors (Sigma-Aldrich Corp.). Lysates were electrophoresed on SDS-acrylamide gels and transferred to 0.2- $\mu$ m nitrocellulose membranes (Bio-Rad Laboratories, Hertfordshire, UK) overnight at 4°C. Membranes were blocked for 6 h (0.15 M NaCl, 1% dried milk, and 0.1% Tween 20) and incubated with primary antibodies (diluted in blocking buffer) overnight at 4°C. After three 10-min washes (88 mM Tris, pH 7.8; 0.25% dried milk; and 0.1% Tween 20), membranes were incubated with a species-specific horseradish peroxidase-conjugated secondary antibody (diluted in wash buffer) for 1 h at room temperature and washed a further 3 times, each for 10 min. Immunoreactive proteins were visualized using enhanced chemiluminescence (ECL Advance, GE

Healthcare). Expression levels were quantified using ImageJ software (<http://rsb.info.nih.gov/ij/>).

### Quantitative RT (qRT)-PCR

After siRNA and dexamethasone (Dex) treatment, total RNA was prepared from HeLa cells using an RNeasy mini kit with DNase I digestion (Qiagen, Valencia, CA, USA), and cDNA was synthesized using a SuperScript III Platinum Two-Step qRT-PCR kit with SYBR Green (Invitrogen).

Seven Gc-regulated genes were selected from our previous microarray expression studies. qRT-PCR primer sequences are available on request. Expression levels were calculated using the comparative  $C_t$  method, normalizing to the glyceraldehyde-3-phosphate dehydrogenase control.

### Immunofluorescence

Cells were treated as specified and then were fixed with 4% paraformaldehyde for 30 min at 4°C and permeabilized (0.02% Triton X-100 in PBS) for 30 min at room temperature. Fixed cells were blocked (1% FCS in PBS) for 4 h at room temperature with agitation and then in primary antibody (diluted in blocking buffer) overnight at 4°C. After three 10-min washes in PBS, cells were incubated in secondary antibody (diluted in PBS) for 2 h. After three further 10-min washes, coverslips were mounted using Vectashield hard-set mounting compound containing the nuclear DAPI stain (Vector Laboratories). Images were acquired on a Delta Vision RT (Applied Precision, Issa-

quah, WA, USA) restoration microscope using an  $\times 60/1.42$  Plan Apo objective and the Sedat filter set (Chroma 89000; Chroma Technology Corp., Rockingham, VT, USA). The images were collected using a CoolSNAP HQ (Photometrics, Tucson, AZ, USA) camera with a Z optical spacing of 0.5  $\mu\text{m}$ . Raw images were deconvolved using Softworx software (Applied Precision, Inc., Issaquah, WA, USA), and maximum intensity projections of these deconvolved images were processed using ImageJ.

### Immunoprecipitation

Cell extracts (500  $\mu\text{g}$  of protein) were precleared with protein A/G-coated Sepharose beads. These beads were pelleted by centrifugation (1800  $g$ ) and discarded. The supernatant was incubated with either 5  $\mu\text{g}$  of primary antibody or equivalent IgG from nonimmunized animals and protein A/G-coated Sepharose beads overnight at 4°C. After incubation, samples were centrifuged at 1800  $g$  to pellet the protein A/G-coated Sepharose beads. Supernatants were discarded, the pellets were washed 3 times in ice-cold PBS and boiled for 5 min in reducing loading buffer, and the beads were removed before electrophoresis.

### Lung immunohistochemistry

Lung tissue was obtained from 2 patients undergoing surgery for lung cancer. One patient had a diagnosis of COPD with a forced expired volume in 1 s (FEV1) of 62% predicted, an FEV1/forced vital capacity ratio of 0.68, and

TABLE 1. Summary of the discriminate panel of Gc sensitivity genes

Description	Ingenuity ID	Ingenuity Pathways Analysis	Experimentally tested
Regulator of G-protein signaling 14	RGS14	✓	✓
Sialyltransferase 4B ( $\beta$ -galactosidase $\alpha$ -2,3-sialyltransferase)	SIAT4B		✓
Bone morphogenetic protein receptor, type II (serine/threonine kinase)	BMPRII	✓	✓
ATPase, class I, type 8B, member 2	ATP8B2		
Propionyl coenzyme A carboxylase, $\alpha$ polypeptide	PCCA	✓	
Elongation of very long chain fatty acids (FEN1/Elo2,SUR4/Elo3, yeast)-like 1	ELOVL1	✓	
Acidic nuclear phosphoprotein 32 family member E	ANP32E		
Metallophosphoesterase	MPPE1		
Amyloid $\beta$ (A4) precursor protein-binding, family B, member 1 (Fe65)	APBB1	✓	✓
SMT3 suppressor of mif2 3 homologue 4	SUMO4	✓	
ATPase, class I type 8A member 1	ATP8A1		
Down-regulator of transcription 1, TBP-binding (negative cofactor 2)	DR1	✓	
X-prolyl aminopeptidase (aminopeptidase P)-like	XPNPEP1	✓	
Cadherin, EGF LAG seven-pass G-type receptor 1	CELSR1		
Neural cell expressed developmentally down-regulated 9	NEDD9	✓	✓
Phosphatidylinositol glycan, class B	PIGB	✓	
Interferon, $\gamma$ -inducible protein 16	IFI16	✓	✓
RAB3 GTPase-activating protein	RAB3GAP1	✓	
Heterogenous nuclear ribonucleoprotein A1	HNRPA1	✓	

Microarray technology used for transcriptome profiling of lymphocytes from a healthy cohort identified a panel of discriminatory genes that showed small but significant changes in expression between Gc-sensitive and Gc-resistant groups. In some cases, the genes have been renamed relative to the original publication to reflect the current annotation within the Affymetrix database, *i.e.*, HEF1 = NEDD9, MGC5350 = nuclear phospho protein, Fe65 = APBB1, AL031133 = SMT3, and AL390738 = HNRPA1. Also, the gene now denoted as MPPE1 was independently identified twice in the original study (hypothetical protein FLJ11585 and gb:BC002877.1/DEF = homo sapiens) (7). Thirteen discriminatory genes were identified as interacting directly or indirectly with the canonical Gc signaling pathway by Ingenuity Pathways Analysis (<http://www.ingenuity.com>). Six of these were selected for further study after literature searches identified potential interactions with Gc signaling. RGS14, BMPRII, APBB1, NEDD9, and IFI16 were predicted to alter GR function by bioinformatic analysis, and SIAT4B was not.

a 180-pack/yr smoking history. The other patient had normal lung function and no smoking history. After informed consent and ethical approval were received, lung tissues (as far distal to tumor as possible) were fixed in formalin and paraffin-embedded, and 4- $\mu$ m sections were cut and mounted onto polysine-coated glass slides. Sections were dewaxed and rehydrated, and epitope was retrieved (10 mM trisodium citrate buffer, pH 6.0, for 20 min).

Sections were blocked (1.5% normal goat serum and 0.5% Triton X-100) before incubation with rabbit polyclonal anti-IFI16 (Santa Cruz Biotechnology, Inc.) overnight at 4°C. After detection with Alexa Fluor 488-conjugated goat anti-rabbit IgG (Invitrogen), sections were washed and incubated with either anti-human CD3, anti-human GR, or anti-human CD20 overnight at 4°C. Sections were labeled with Alexa Fluor 568-conjugated goat anti-mouse IgG (Invitrogen) and then were washed and counterstained with DAPI (Invitrogen). Digital micrographs were obtained using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan) equipped with a QImaging digital camera (QImaging, Surrey, BC, Canada) and ImagePro Plus 5.1 software (MediaCybernetics, Marlow, UK).

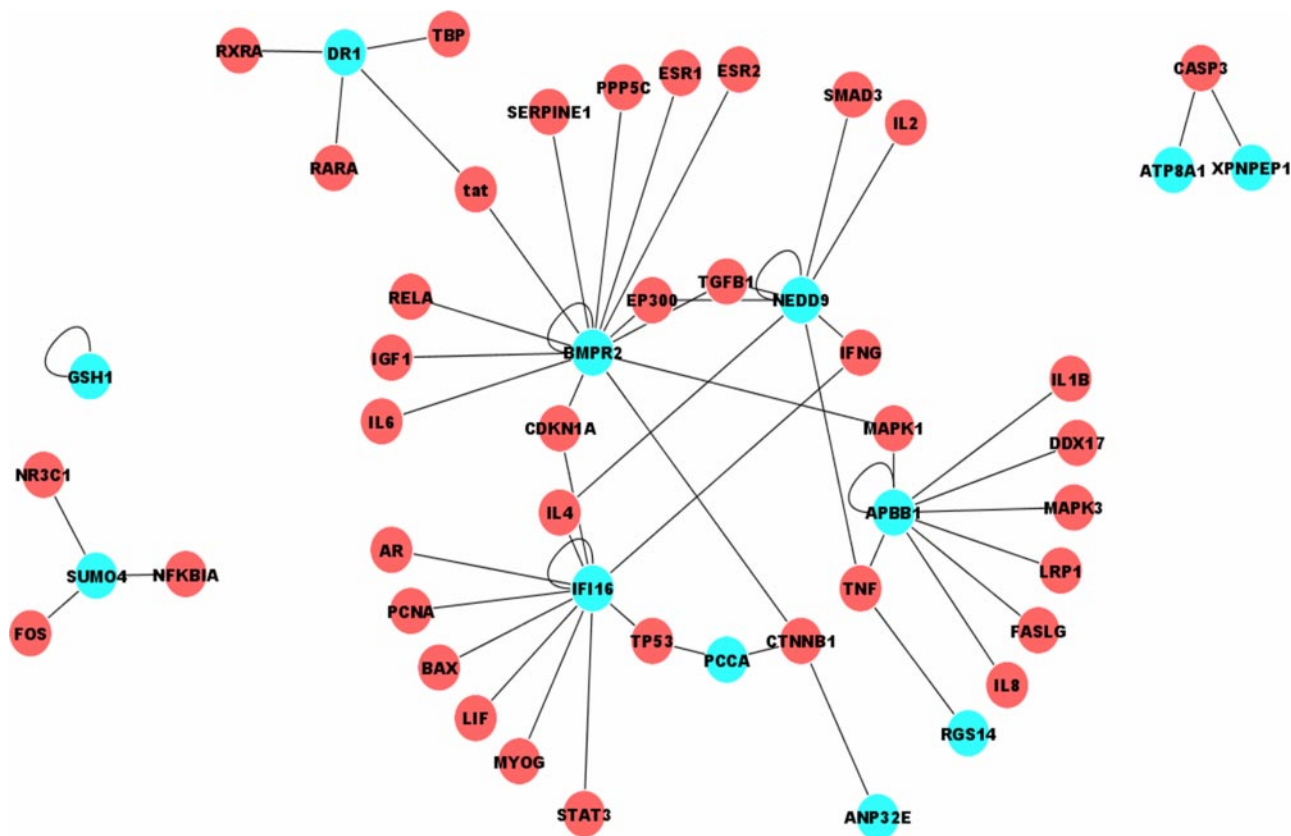
### Statistical analysis

Data were analyzed in multiple samples after  $n = 3$  determinations using the SPSS software package (SPSS Inc., Chicago, IL, USA) and, where appropriate, are expressed as means  $\pm$  sd. For reporter gene assays, means were compared by 1-way ANOVA followed by Dunnett's *post hoc* test. For qRT-PCR, data were analyzed by the Mann-Whitney test, and  $P < 0.05$  was considered statistically significant.

## RESULTS

### Identification of Gc signaling pathway interacting genes: bioinformatics

The discriminatory genes identified by microarray analysis (Table 1) were used to generate a “hypothetical network” of genes (Fig. 1). The gene products were not found to interact directly with each other or with steroid metabolic pathways; however, 12 genes (Fig. 1, blue) were identified as interacting with the GR itself (NR3C1), with GR target genes (IL-6 and leukemia inhibitory factor), or with genes known to regulate GR function (RelA and p53). Based on the above analysis and further interrogation of the literature, it was possible to highlight the strongest candidates for further analysis: IFI16 is known to bind p53 (17), which in turn is capable of binding the GR to affect its activity. IFI16 also binds to the androgen receptor to up-regulate both its expression and activity (18). BMPRII is the ligand-binding partner of the signaling bone morphogenetic protein receptor type I. Bone morphogenetic proteins are members of the TGF- $\beta$  superfamily, which act on brain and bone and in development (19). We have previously identified BMPRII as a modulator of GR function (7). RGS14 is expressed in both brain and



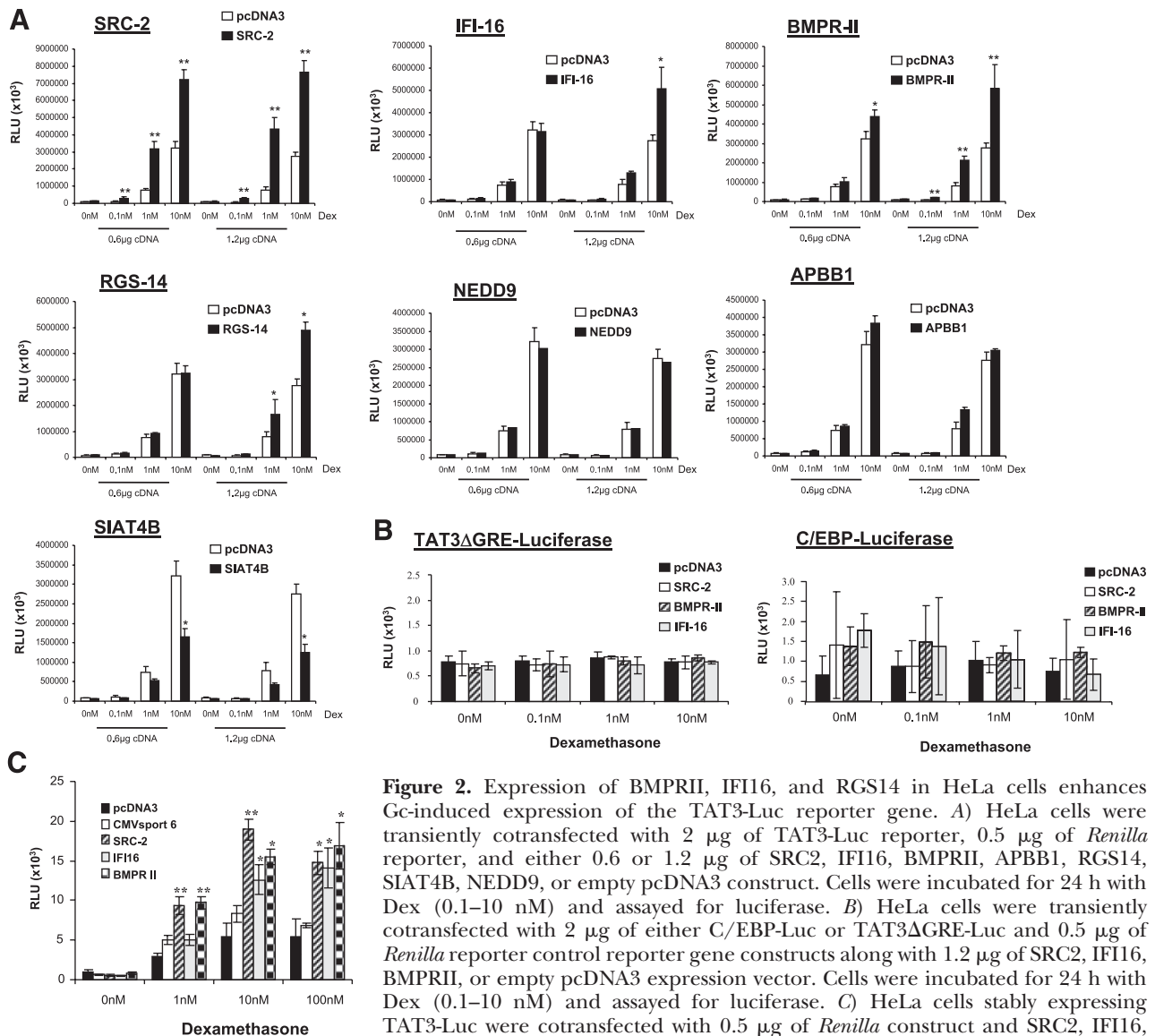
**Figure 1.** Bioinformatic analysis of 20 candidate Gc sensitivity modulating genes. Network analysis describing interactions between genes/gene products from the steroid-sensitive gene list with each other and with other well-characterized genes defined by canonical Gc metabolic and GR signaling pathways. Blue represents genes/gene products from the steroid-sensitive gene list and red denotes genes represented on canonical Gc pathways. Connecting lines represent interactions.

spleen (20, 21) and down-regulates signaling by heterotrimeric G proteins. Neural cell expressed developmentally down-regulated 9 (NEDD9) is subject to tyrosine phosphorylation in response to ligation of  $\beta 1$  integrin (22, 23). The adapter protein APBB1 (also known as Fe65), can interact directly with estrogen receptor  $\alpha$  (24) and itself is phosphorylated by serum and glucocorticoid-induced kinase 1 (SGK1) (25). Because SIAT4B was not predicted to interact with GR in this analysis, it was selected for further analysis as a control gene.

### IFI16, BMPRII, and RGS14 affect Gc transactivation

The ability of the selected genes to modulate GR transactivation was tested in HeLa cells transfected with the Dex-responsive luciferase reporter, TAT3-

Luc. In line with our previous work, SRC2 and BMPRII both potentiated GR transactivation, and, in addition, IFI16, and RGS14 also effectively enhanced Dex-induced GR transactivation (Fig. 2A). In contrast, expression of SIAT4B significantly impaired GR transactivation (Fig. 2A). Expression of NEDD9 or APBB1 had no effect on GR transactivation. Cells transfected with increasing amounts of IFI16, BMPRII, or RGS14 showed a greater enhancement of Dex response, whereas increasing the amount of transfected pcDNA3 or SRC2 was without effect (Fig. 2A). Notably, neither a reporter gene with deleted GRE elements nor an unrelated CCAAT/enhancer-binding protein (C/EBP)-responsive reporter was regulated by IFI16, BMPRII, or SRC2 (Fig. 2B). After stable integration of the TAT3-Luc reporter cassette into HeLa cells, overexpression of SRC2, BMPRII, or IFI16 still enhanced GR transactivation (Fig. 2C).



**Figure 2.** Expression of BMPRII, IFI16, and RGS14 in HeLa cells enhances Gc-induced expression of the TAT3-Luc reporter gene. **A)** HeLa cells were transiently cotransfected with 2  $\mu$ g of TAT3-Luc reporter, 0.5  $\mu$ g of *Renilla* reporter, and either 0.6 or 1.2  $\mu$ g of SRC2, IFI16, BMPRII, APBB1, RGS14, SIAT4B, NEDD9, or empty pcDNA3 construct. Cells were incubated for 24 h with Dex (0.1–10 nM) and assayed for luciferase. **B)** HeLa cells were transiently cotransfected with 2  $\mu$ g of either C/EBP-Luc or TAT3 $\Delta$ GRE-Luc and 0.5  $\mu$ g of *Renilla* reporter control reporter gene constructs along with 1.2  $\mu$ g of SRC2, BMPRII, or empty pcDNA3 expression vector. Cells were incubated for 24 h with Dex (0.1–10 nM) and assayed for luciferase. **C)** HeLa cells stably expressing TAT3-Luc were cotransfected with 0.5  $\mu$ g of *Renilla* construct and SRC2, IFI16, BMPRII, or empty pcDNA3/CMV SPORT6 construct. Twenty-four hours after transfection, cells were incubated with Dex (0.1–10 nM) for 16 h before harvest and luciferase analysis. Graphs depict means  $\pm$  SD of triplicate wells, representative of 3 independent experiments. \* $P$  = <0.05, \*\* $P$  = <0.01 vs. empty vector control. RLU, relative light units.

## IFI16 enhances Gc transrepression of an NF- $\kappa$ B reporter

In HeLa cells, 0.5 ng/ml TNF- $\alpha$  maximally activated an NF- $\kappa$ B-Luc reporter gene, and higher concentrations progressively impaired the Gc inhibitory response, mimicking the acquired Gc resistance seen in inflammation loci. (Fig. 3). None of the comodulator genes had a significant effect. At 5 ng/ml TNF- $\alpha$ , only IFI16 was able to restore Gc repression of NF- $\kappa$ B activity. Under control conditions, Dex repressed 0.5 ng/ml TNF- $\alpha$  driven reporter activity by 46%, which was reduced to 30% repression when the reporter was driven by 5 ng/ml TNF- $\alpha$ . IFI16 overexpression restored repression (48% repression with 0.5 ng/ml TNF- $\alpha$  compared with 49% repression with 5 ng/ml TNF- $\alpha$ ) (Fig. 3). Notably, GR expression was not affected by treatment with TNF- $\alpha$  (data not shown).

## IFI16 alters Gc-regulated gene expression via GR-independent and GR-dependent actions

To explore the role of IFI16 in regulating endogenous GR target genes siRNA specific to IFI16 was used, with lamin siRNA as a control (Fig. 4A). From our previous microarray expression studies, 7 Gc-regulated genes were selected for analysis. IFI16 knockdown altered the baseline expression of 6 of 7 well-characterized GR target genes; with only metallothionein 1X (MT1X) showing no alteration in expression. FK506 binding protein 5 (FKBP5) expression was augmented by loss of IFI16, and, in contrast, aspartate  $\beta$ -hydroxylase (ASPH), IL-6 signal transducer (gp130 oncostatin M receptor) (IL6ST), myosin 1B (MYO1B),

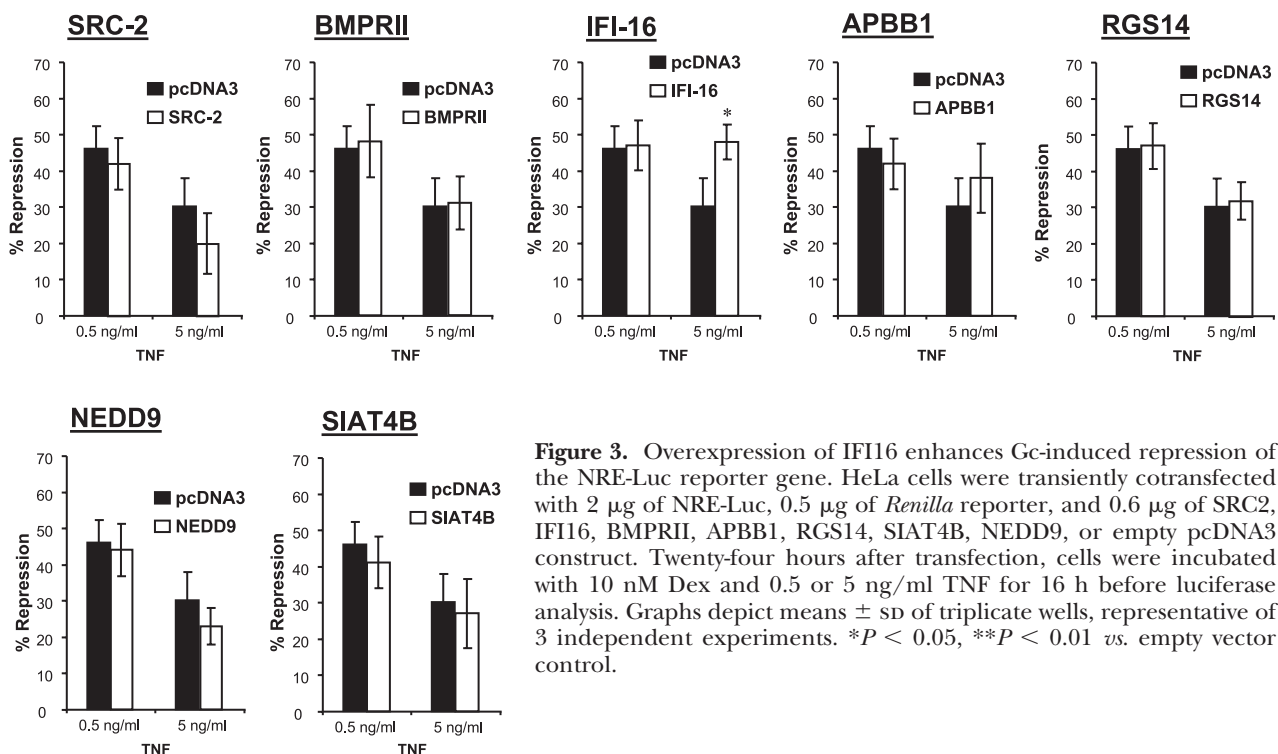
glutamate ammonia ligase (glutamine synthase) (GLUL), and TSC22 domain family 3 (GILZ, TSC22D3) were suppressed by IFI16 knockdown (Fig. 4B).

FKBP5, MT1X, GLUL, and GILZ were up-regulated and ASPH, IL6ST, and MYO1B were down-regulated in response to treatment with Dex, which is in accordance with previous findings (7, 26, 27) (Fig. 4C). FKBP5 and MT1X were dramatically less responsive to Gc in the absence of IFI16, whereas GILZ and GLUL became more Gc responsive. Loss of IFI16 prevented Dex inhibition of ASPH and indeed increased the transcript abundance. The Gc effects on IL6ST and MYO1B were not significantly affected by the IFI16 expression level (Fig. 4C). Most interesting, increased baseline expression of FKBP5 correlated well with the impaired induction observed with Gc. Conversely, the reduced baseline of GLUL and GILZ gave rise to a much larger induction in the presence of Gc.

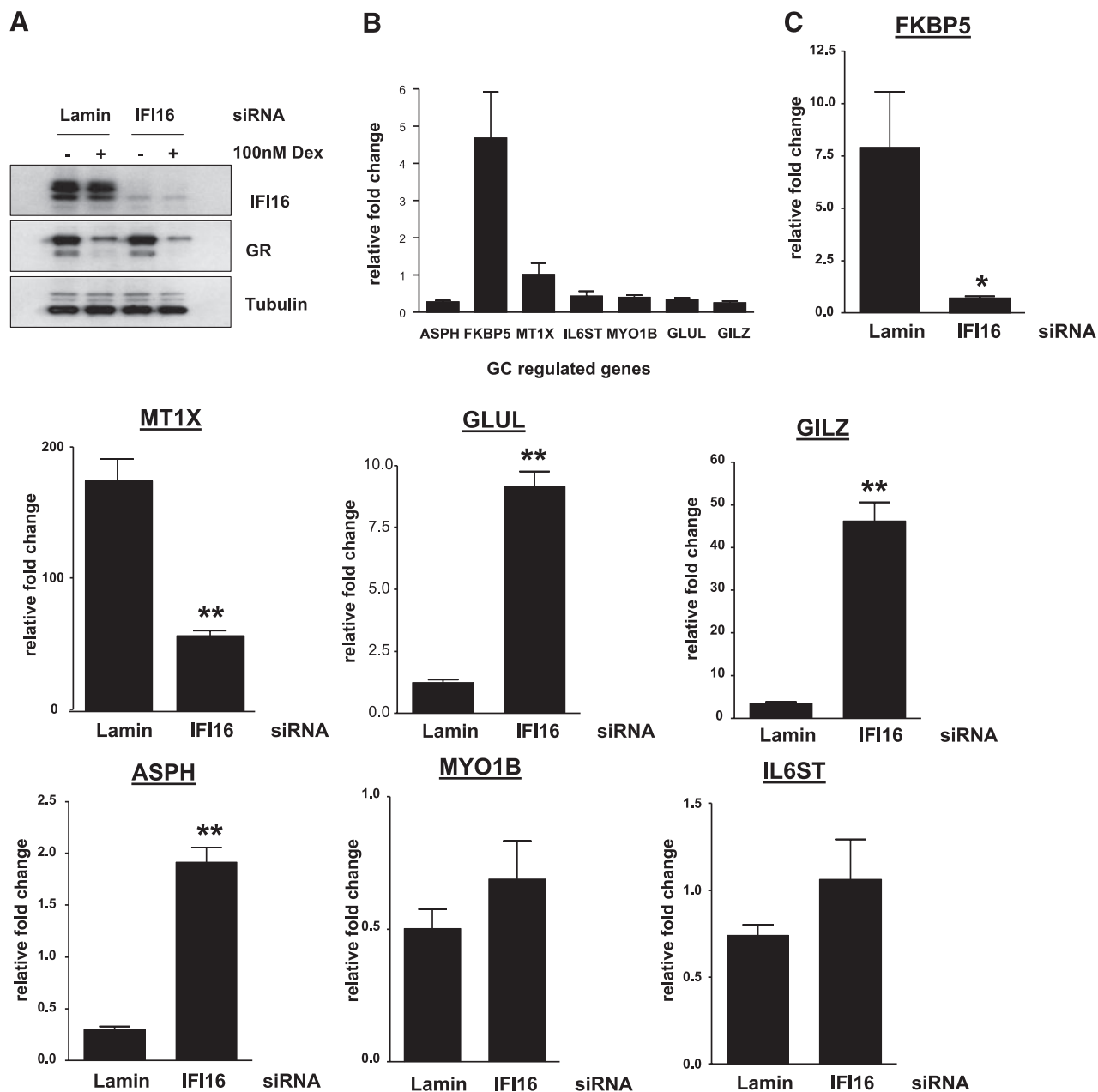
## IFI16, BMPRII, SIAT4B, and RGS14 do not affect GR expression or phosphorylation

GR expression powerfully regulates target cell Gc sensitivity, and so the expression and regulation of GR protein were measured. Dex treatment for 24 h caused a 60% reduction in GR expression. A similar reduction was observed in cells overexpressing IFI16, BMPRII, SIAT4B, and RGS14, suggesting that Gc modulatory effects are not mediated through altered GR expression or stability (Fig. 5A).

Treatment with Dex also induces rapid phosphorylation of GR on multiple residues. Cells incubated with 100 nM Dex for 30 min showed a rapid increase in GR phosphory-



**Figure 3.** Overexpression of IFI16 enhances Gc-induced repression of the NRE-Luc reporter gene. HeLa cells were transiently cotransfected with 2  $\mu$ g of NRE-Luc, 0.5  $\mu$ g of *Renilla* reporter, and 0.6  $\mu$ g of SRC2, IFI16, BMPRII, APBB1, RGS14, SIAT4B, NEDD9, or empty pcDNA3 construct. Twenty-four hours after transfection, cells were incubated with 10 nM Dex and 0.5 or 5 ng/ml TNF for 16 h before luciferase analysis. Graphs depict means  $\pm$  SD of triplicate wells, representative of 3 independent experiments. \* $P$  < 0.05, \*\* $P$  < 0.01 vs. empty vector control.



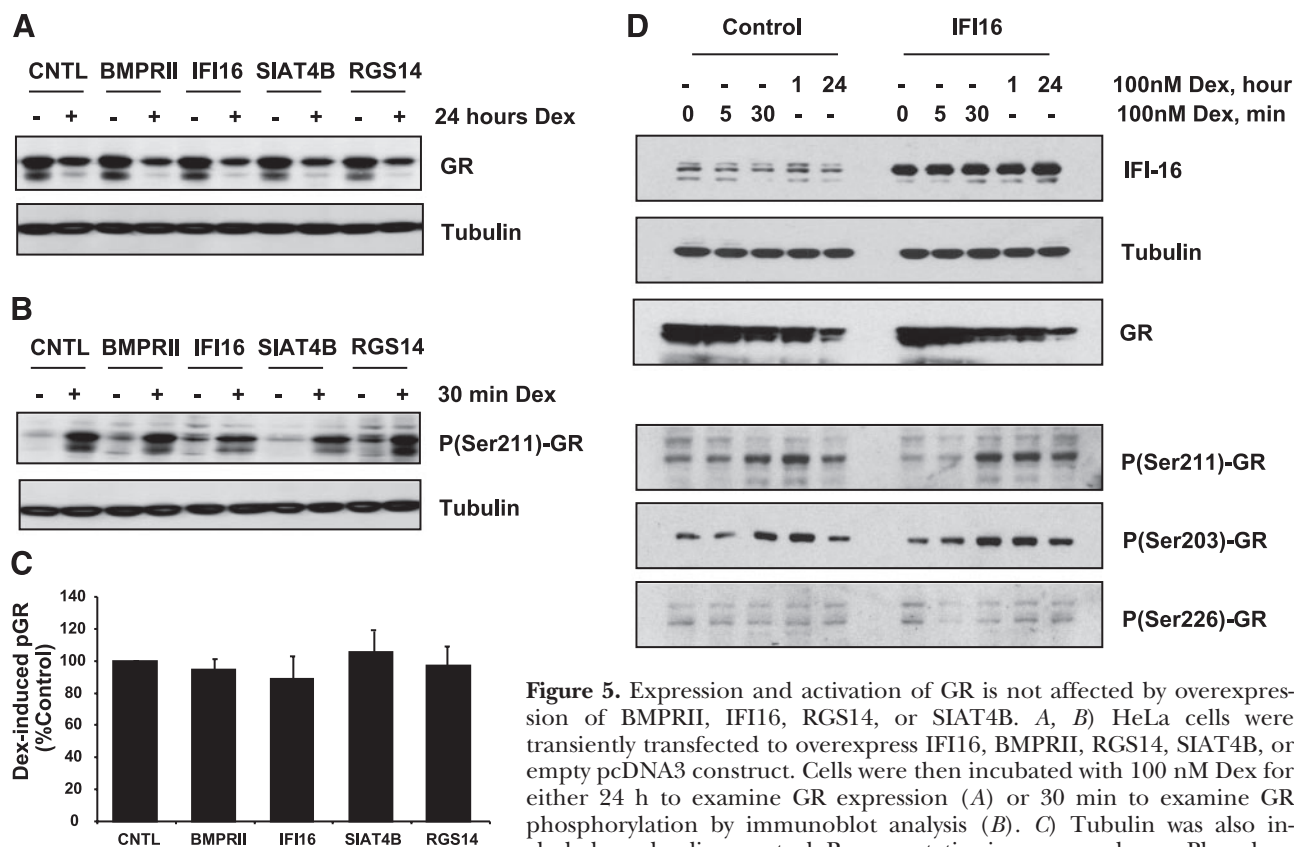
**Figure 4.** IFI16 modulates GR regulation of endogenous genes. *A*) HeLa cells transfected with 10 nM IFI16 or lamin siRNA for 48 h were lysed and immunoblotted for IFI16, GR, or tubulin. Representative images are shown. *B, C*) HeLa cells were transfected with 10 nM IFI16 or lamin siRNA, treated with vehicle or 100 nM Dex for 4 h, and then lysed and RNA-processed. Effect on basal (*B*) and Gc-regulated (*C*) expression of 7 GC-regulated genes (ASPH, FKBP5, MT1X, IL6ST, MYO1B, GLUL, and GILZ) was analyzed by qRT-PCR. Graphs depict means  $\pm$  SD of triplicate wells, representative of 3 independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$  vs. lamin siRNA control.

lation at serine 211. The phosphorylation status of GR was unaffected by overexpression of IFI16, BMPRII, SIAT4B, or RGS14 (Fig. 5*B, C*). More detailed time course analysis in cells overexpressing IFI16 demonstrates no effect of IFI16 in modulating Dex-induced phosphorylation of GR on either Ser-211 or Ser-203 (Fig. 5*D*). Phosphorylation of GR at Ser-226 was not induced by addition of Dex in these cells.

#### GR and IFI16 colocalize to the same cellular compartments in HeLa cells

Endogenous (Fig. 6*A*) and overexpressed (Fig. 6*B*) IFI16 had a heterogeneous intracellular distribution

with some cells demonstrating predominantly nuclear expression and others mainly cytoplasmic expression. It was evident that in cells expressing predominantly nuclear IFI16, GR tended to localize within the nucleus also, even in the absence of Dex (Fig. 6*A, B*, indicated by arrows). Treatment with Dex increased nuclear GR, which was also accompanied by increased nuclear IFI16 (Fig. 6*C*). Thus, there was clear overlap of GR and IFI16 subcellular localization, both in the absence of ligand and after addition of Dex (Fig. 6*A–C*). Although subcellular localization of either protein does not appear to be exclusively



**Figure 5.** Expression and activation of GR is not affected by overexpression of BMPRII, IFI16, RGS14, or SIAT4B. *A, B*) HeLa cells were transiently transfected to overexpress IFI16, BMPRII, RGS14, SIAT4B, or empty pcDNA3 construct. Cells were then incubated with 100 nM Dex for either 24 h to examine GR expression (*A*) or 30 min to examine GR phosphorylation by immunoblot analysis (*B*). *C*) Tubulin was also included as a loading control. Representative images are shown. Phosphorylated GR was analyzed using ImageJ, and densitometry from 3 independent experiments was expressed as a ratio of phospho (P)-GR/tubulin. *D*) HeLa cells transfected to overexpress IFI16 or empty pcDNA3 construct were incubated with 100 nM Dex for up to 24 h and immunoblotted for GR, P-Ser-203-, P-Ser-211-, and P-Ser-226-GR. Immunoblots for IFI16 and tubulin are included as controls (CTRL). Representative images are shown.

dependent on the other, localization to the same subcellular compartment does allow for potential interaction.

### GR interacts with a specific IFI16 isoform

HeLa cells transfected with the IFI16 expression vector were treated with vehicle or Dex for 1 h and then lysed, and cell extracts were subjected to immunoprecipitation with two different antibodies (mouse and rabbit) to both IFI16 or GR and then to immunoblotting for IFI16 and GR. Although evidence for a direct interaction between GR and IFI16 was observed in samples immunoprecipitated with both GR antibodies, no GR was immunoprecipitated with either IFI16 antibody (Fig. 6D). The GR-IFI16 interaction was unaffected by the presence of ligand (Fig. 6D). Strikingly, both GR antibodies immunoprecipitated the B isoform of IFI16 (Fig. 6E). Neither the GR nor IFI16 was precipitated by incubation with normal rabbit or normal mouse IgG.

### The ligand-binding domain (LBD) of GR is required for the potentiation of Gc signaling by IFI16

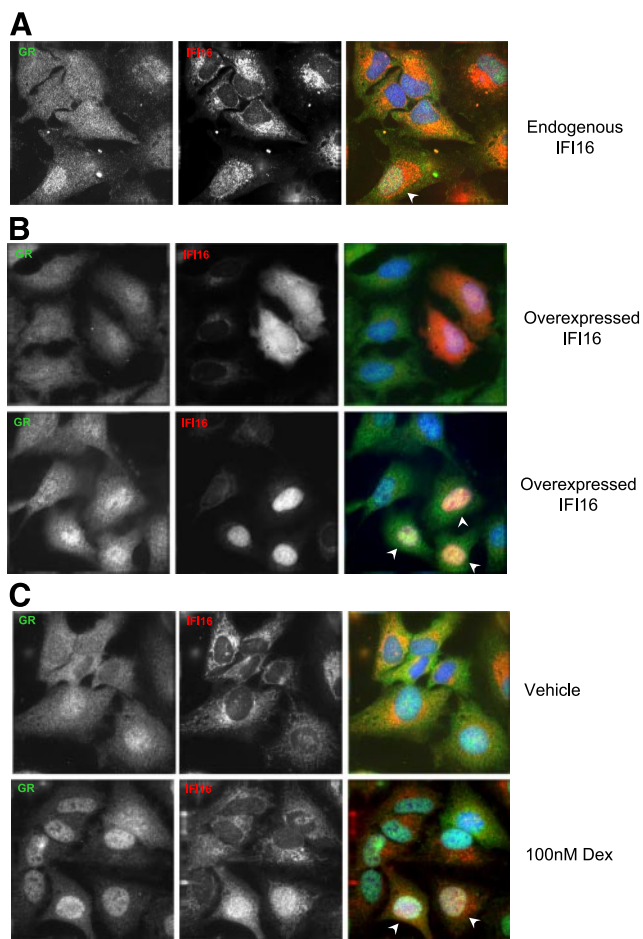
To assess which domain of GR functionally interacts with IFI16, two deletant GR constructs were used in

transfection studies with the GR-deficient HEK293 cell line: GR $\Delta$ AF1, which lacks the AF1 transactivation domain and has reduced transactivation potential, and GR N500, which lacks the LBD and is constitutively active. IFI16 still potentiated the Dex-induced transactivation in cells transfected with GR  $\Delta$ AF1 (Fig. 7A) but not with GR N500 (Fig. 7B). This result suggests that although the interaction between GR and IFI16 does not require ligand, the LBD is the domain required for interaction.

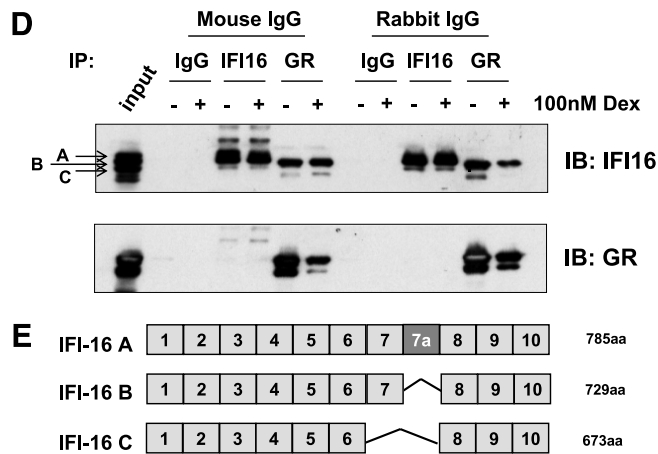
### The LBD of GR is required for interaction of GR and IFI16

To determine which domain of GR is required for physical interaction with IFI16, full-length GR, GR  $\Delta$ AF1, and GR N500 constructs fused to a myc tag were transfected into the GR-deficient HEK293 cell line. GR was isolated using a myc antibody, and the precipitate was immunoblotted for IFI16. Figure 7C demonstrates that IFI16 binding to GR requires intact LBD. IFI16 was not precipitated by incubation with normal mouse IgG. Immunoblotting with a GR antibody raised against the AF1 domain was used to determine the presence of this domain in the expressed deletants (Fig. 7C).





immunoprecipitated with mouse or rabbit IgG were included as species-specific immunoglobulin controls. *E*) Schematic diagram depicting the exons encoding the three IFI16 isoforms.



**Figure 6.** IFI16 and GR subcellular localization. *A, B*) HeLa cells expressing endogenous IFI16 (*A*) or transfected with 1.2  $\mu$ g of IFI16 expression vector (*B*) were fixed and double-labeled with antibodies specific to GR (green) and IFI16 (red), and nuclei were counterstained with DAPI (blue). *C*) Arrowheads identify cells in which IFI16 and GR are localized to the nucleus. HeLa cells expressing endogenous IFI16 were incubated with vehicle or 100 nM Dex for 2 h. Cells were fixed and then were labeled with antibodies specific to GR (green) and IFI16 (red), and nuclei were counterstained with DAPI (blue). Arrowhead indicates Dex-treated cells in which GR and IFI16 localize to the nucleus. *D*) HeLa cells transfected with IFI16 expression vector (IFI16) were treated with vehicle or Dex for 1 h and then lysed, and cell extracts were immunoprecipitated (IP) with two different antibodies (rabbit and mouse) for either IFI16 or GR. Precipitates were immunoblotted (IB) for IFI16 and GR. Samples immunoprecipitated with mouse or rabbit IgG were included as species-specific immunoglobulin controls. *E*) Schematic diagram depicting the exons encoding the three IFI16 isoforms.

### IFI16 is expressed in lymphoid follicles in inflammatory lung disease and is coexpressed with GR

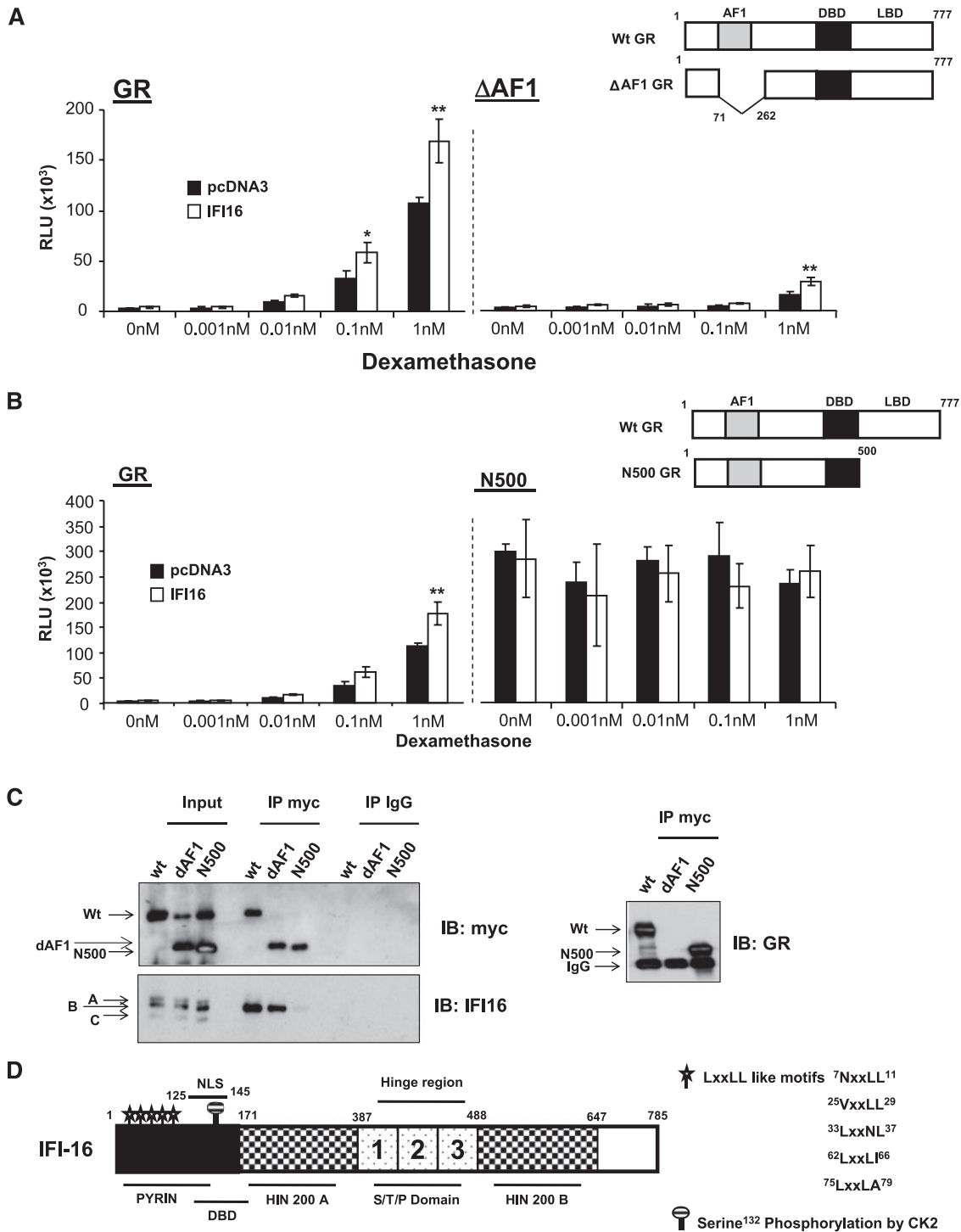
To explore the possible physiological relevance of IFI16 to GR action, expression was sought in human lung tissue. As Gcs are frequently used to treat patients with pulmonary inflammation (28), IFI16 expression was analyzed in lung tissue from a patient with COPD. COPD is characterized by the presence of lymphoid follicles within inflamed lung tissue (29, 30). Expression of IFI16 was found in the airway epithelium and lymphoid follicles with mainly nuclear localization. There was clear coexpression of IFI16 with the T lymphocyte marker CD3 (Fig. 8A) and with the B lymphocyte marker CD20 (Fig. 8B), suggesting a potential for GR-IFI16 interaction within immune cells of inflamed lung tissue.

Colocalization of IFI16 with GR within the inflammatory cell infiltrate was evident in lung sections from a patient with a diagnosis of COPD (Fig. 9A). The number of inflammatory cells seen in the non-COPD lung is substantially less; however, IFI16/GR colocalization can be clearly seen (Fig. 9B).

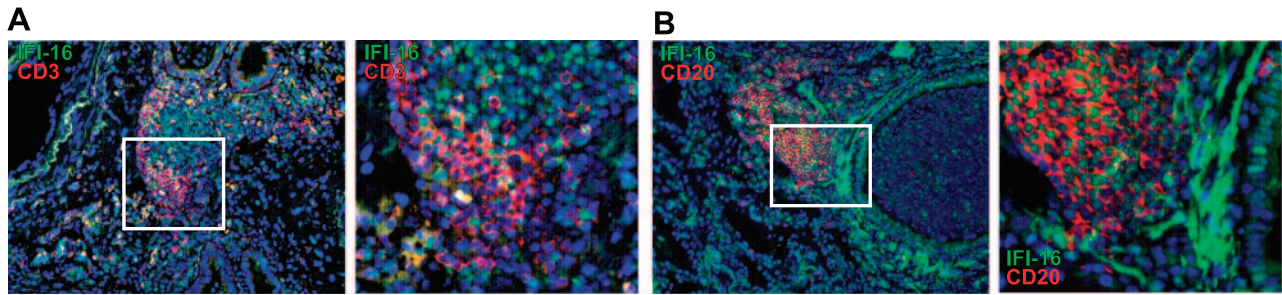
### DISCUSSION

A systems biology approach was used to prioritize our discriminatory panel of genes for functional analysis. For 5 of these 6 candidate genes (IFI16, BMPRII, Fe65, NEDD9, and RGS14), literature searches suggested a role in influencing Gc action, further supporting the bioinformatic pathway analysis. A functional effect on Gc signaling was confirmed *in vitro* for BMPRII, as documented previously (7), IFI16, and RGS14. However, neither APBB1/Fe65 nor NEDD9 was found to affect transactivation or transrepression. SIAT4B was included in our *in vitro* functional screen as a representative gene not predicted by the bioinformatics to affect Gc action. However, SIAT4B has been shown to alter expression and activity of sialyltransferase in inflammation (31), and therefore was of interest. Despite the informatic software not predicting this gene to interact functionally with GR, we found that SIAT4B significantly inhibited Gc transactivation in HeLa cells, indicating the necessity for experimental verification of bioinformatically identified "hits."

GR expression directly affects Gc sensitivity (32). However, immunoblot analysis found no effect of the genes on GR expression levels under basal or post-Gc conditions. Phosphorylation at serine 211 occurs rapidly on ligand binding



**Figure 7.** IFI16 interacts with the GR in an LBD-dependent manner. *A, B*) HEK293 cells were transiently transfected with 2  $\mu$ g of TAT3-Luc, 0.5  $\mu$ g of *Renilla*, 1.2  $\mu$ g of IFI16 expression vector, and 1  $\mu$ g of either full-length GR $\alpha$  or GR  $\Delta$ AF-1, which lacks the AF-1 domain (*A*), or GR N500, which lacks the LBD (*B*). After transfection cells were incubated with increasing concentrations of Dex (0.001–1 nM) for 16 h before analysis. Graphs depict means  $\pm$  SD of triplicate wells, representative of 3 independent experiments. \* $P$  < 0.05, \*\* $P$  < 0.01. RLU, relative light units. *C*) HEK293 cells were transiently transfected with 2  $\mu$ g of full-length GR $\alpha$ , GR  $\Delta$ AF-1, or GR N500 myc fusion proteins and then lysed, and cell extracts were immunoprecipitated (IP) with an antibody raised against myc. Precipitates were immunoblotted (IB) for myc, IFI16, and GR. Samples immunoprecipitated with mouse IgG were included as controls. wt, wild-type. *D*) Schematic representation of the putative domain structure of IFI16 protein. The N-terminal domain of IFI16 (solid black) contains several regions important for IFI16 activity, including the DNA-binding domain (DBD), nuclear localization signal (NLS), pyrin/dapin/PAAD domain (pyrin domain), and site of phosphorylation by casein kinase 2 (CK2). Checkered regions indicate duplicate HIN 200 domains A and B. These are separated by a serine-threonine-proline (S/T/P)-rich spacer region (hinge region). IFI16 isoforms arise due to alternative RNA splicing in exons encoding the S/T/P domain. Numbers identify amino acid residues. LxxLL-like motifs, serine phosphorylation, and nuclear localization signal are also shown.



**Figure 8.** Expression of IFI16 in human lung. Representative photomicrographs of IFI16 expression in areas of T- and B-lymphocyte aggregates within human lung tissue. Detection of IFI16 (green) within immune cells of a parenchymal tertiary lymphoid follicle from a patient with COPD. IFI16 was expressed in CD3<sup>+</sup> T cells (A) and CD20<sup>+</sup> B cells (B). Left panels are low-magnification views; right panels are expanded views.

and enhances the interaction of the GR with the DRIP/TRAP coactivator complex and so is important for function (33–35). GR Ser-211 phosphorylation was unaltered by expression of the candidate genes. Furthermore, in cells overexpressing IFI16 no effect on GR phosphorylation at Ser-203 or Ser-226 was seen.

Only one gene, IFI16, a member of the HIN 200 family of proteins that can modulate cell growth, differentiation, and antiviral responses (36–38), significantly altered both GR transactivation and transrepression. Its ability to modulate GR transrepression is of particular interest, with relevance to acquired changes in Gc sensitivity seen in inflammation, an effect predominantly mediated by repression of NF- $\kappa$ B activity (39, 40).

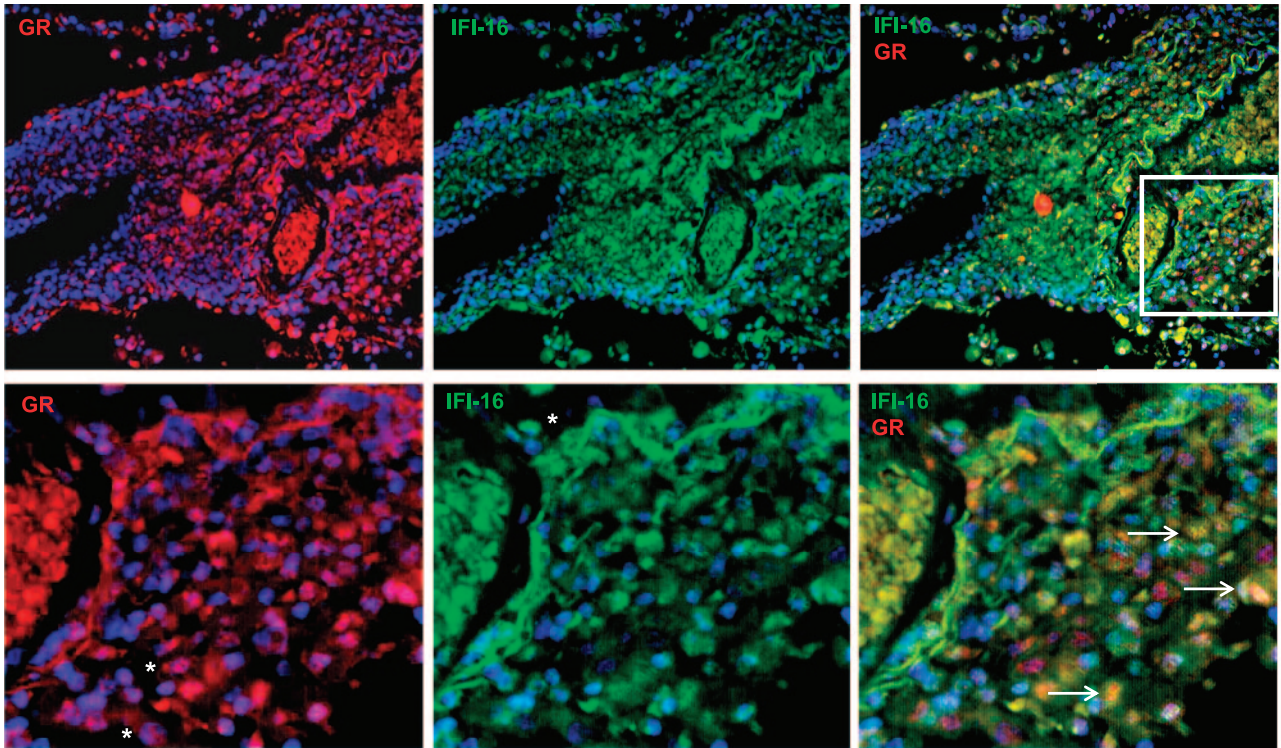
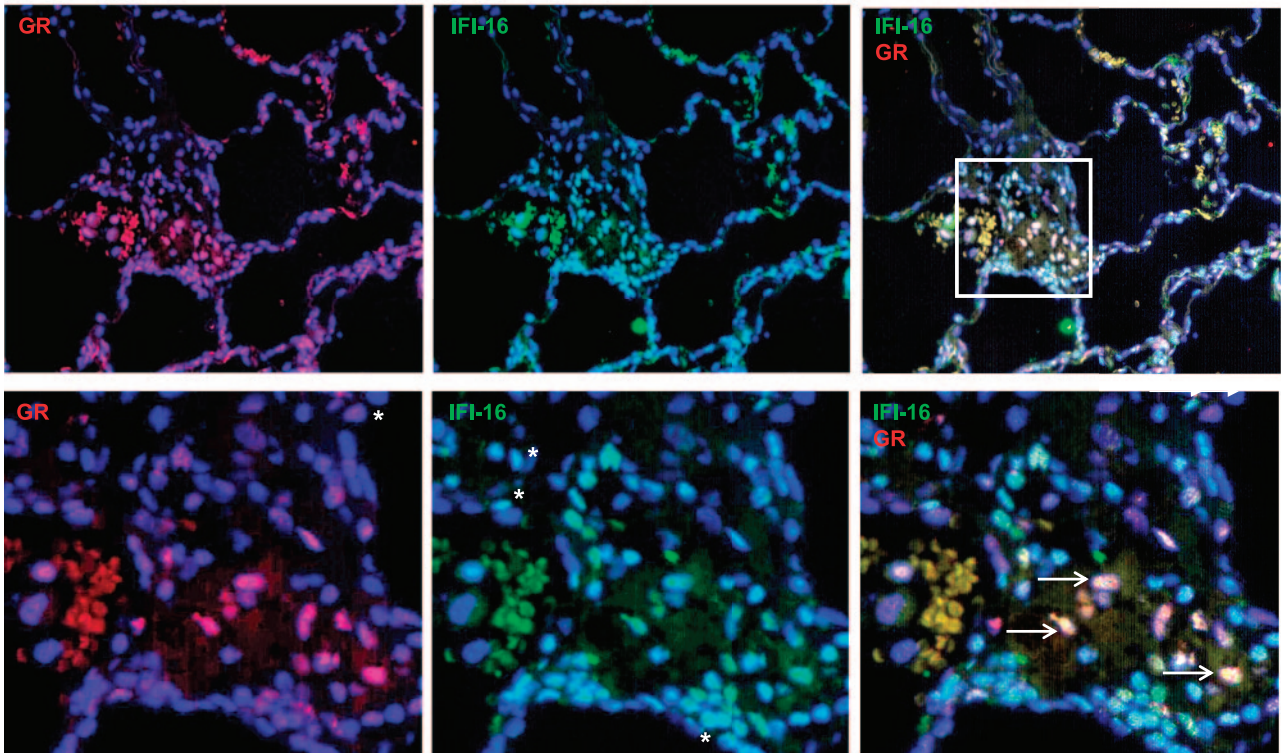
To support our reporter gene studies, we looked at the effect of IFI16 on endogenous gene expression. The panel selected comprised known Gc-regulated genes. We show that IFI16 affects basal expression of most of these genes, supporting a GR-independent effect of IFI16 on their expression. Furthermore, we show that IFI16 directly modulates the effect of Dex on the 7 genes studied. Therefore, there is evidence for both direct regulation of GR target gene expression by independently acting IFI16 and, in addition, an effect of IFI16 on GR transregulation. MT1X is of particular interest as its expression is unaffected by alteration in IFI16 expression, but loss of IFI16 dramatically impairs Gc induction. Therefore, the IFI16 effect is dependent on GR transactivation. Taken together, the effects of IFI16 on GR function suggest widespread changes to the network of GR-regulated genes.

The gene regulation results show a functional effect of IFI16 on GR function, and coimmunofluorescence studies show that IFI16 and GR are colocalized in both cytoplasmic and nuclear compartments. Coimmunoprecipitation experiments reveal IFI16 interactions with the GR, both in the presence and absence of GR ligand. The interaction appears quite specific with the B isoform of IFI16 being preferentially pulled down by GR. In our cells there appear to be up to four different IFI16 isoforms expressed, with very similar molecular mass. Their identity was confirmed using IFI16 siRNA studies, and multiple isoforms, arising from differential splicing, have been reported previously, although func-

tional differences have not been described (41). It is relevant that the IFI16 B isoform has been reported to be selectively up-regulated in the inflammatory disease systemic lupus erythematosus (42). It is possible that isoform differences discovered here may partly reflect specificity of the antibodies used, although two different IFI16 antibodies were tested for completeness, and both gave similar results. However, these results further support a direct effect of IFI16 on GR function, as suggested by the MT1X gene expression study. Such a direct interaction may also explain why loss of IFI16 causes a different Gc response for ASPH, FKBP5, GLUL, and GILZ.

To further dissect out the mechanism of IFI16 action on the GR domain, deletant GR constructs were examined. These studies showed that the N-terminal transactivation domain (AF1) was unaltered by loss of IFI16, but that the C-terminal transactivation domain (AF2) was selectively potentiated by IFI16. AF2 function is difficult to dissect more precisely, as disruption to the C-terminal domain also abolishes ligand-binding function, but it is known that agonist ligands induce a conformational change to the GR C terminus to open up an interaction surface for coactivator  $\alpha$  helices with the consensus LxxLL motif (43). However, although IFI16 contains a series of LxxLL motifs in its N-terminal pyrin domain (Fig. 7D), it is unlikely that these are required for GR binding. First, the GR-IFI16 interaction was independent of GR ligand binding, required to reveal the LxxLL interaction cleft, and second, the interaction was specific to the IFI16 B isoform, which differs from the nonbinding A and C isoforms only at exon 7 and not in the N-terminal pyrin domain. The mode of action of IFI16 on GR is interesting, but the ligand independence of the interaction and the colocalization of the two proteins in both cytoplasm and nucleus suggests that whereas direct effects on assembly and function of the GR occur at its target genes, additional effects are also likely, as, for example, the cytoplasmic crosstalk between GR and protein kinase A (44, 45).

Notably, we were able to show expression of IFI16 in human COPD lung lymphoid follicles, a relevant site for Gc action, suggesting that it may be playing a role to modulate Gc sensitivity *in vivo*. IFI16 has been documented previously to be highly expressed in B and T

**A****B**

**Figure 9.** Colocalization of IFI16 and GR in human lung. Representative photomicrographs of IFI16 and GR $\alpha$ . Lung sections from a patient with COPD (*A*) and a nonsmoking individual with normal lung function (*B*) were subject to immunofluorescence with GR and IFI16 antibodies. All sections were counterstained with DAPI (blue). Arrows indicate inflammatory cells coexpressing GR and IFI16. Asterisk indicates single expression of GR or IFI16. Top rows are low-magnification views; bottom rows are expanded views.

cells, whereas within healthy lung its expression is restricted to epithelial cells (46), and GR expression in the lung is similarly found in epithelium and in lymphocytes (47).

The IFI family can also affect inflammation directly through a conserved DAPIN domain (commonly found in proteins involved in regulatory pathways that promote NF- $\kappa$ B activation) (48), and indeed the murine homologue of IFI16 binds and inhibits NF- $\kappa$ B function (49–51).

In this study we report a bioinformatic approach to discover genes capable of altering GR function. Of the six genes initially identified only four were found to have functional effects in our *in vitro* model systems. It is possible the others had effects that were too subtle to be detected or that were GR target gene-specific, but experimental validation of two-thirds of the proposed genes supports the validity of such an approach. In addition, SIAT4B, not predicted by bioinformatic search to interact with GR, was found to influence GR transactivation *in vitro*.

The functional importance of IFI16 for GR-mediated anti-inflammatory action now needs to be fully explored. Understanding the mechanism of this interaction may lead to new insights for modulating target tissue response to Gc and so improve the management of patients with inflammatory diseases. EJ

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## REFERENCES

- Kino, T., and Chrousos, G. P. (2001) Glucocorticoid and mineralocorticoid resistance/hypersensitivity syndromes. *J. Endocrinol.* **169**, 437–445
- Franchimont, D., Louis, E., Dupont, P., Vrindts-Gevaert, Y., Dewe, W., Chrousos, G., Geenen, V., and Belaiche, J. (1999) Decreased corticoid sensitivity in quiescent Crohn's disease: an *in vivo* study using whole blood cell cultures. *Dig. Dis. Sci.* **44**, 1208–1215
- Van Rossum, E. F., and Lamberts, S. W. (2004) Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition. *Recent Prog. Horm. Res.* **59**, 333–357
- Szatmary, Z., Garabedian, M. J., and Vilcek, J. (2004) Inhibition of glucocorticoid receptor-mediated transcriptional activation by p38 mitogen-activated protein (MAP) kinase. *J. Biol. Chem.* **279**, 43708–43715
- Waters, C. E., Stevens, A., White, A., and Ray, D. W. (2004) Analysis of co-factor function in a glucocorticoid-resistant small cell carcinoma cell line. *J. Endocrinol.* **183**, 375–383
- Adcock, I. M., and Ito, K. (2005) Glucocorticoid pathways in chronic obstructive pulmonary disease therapy. *Proc. Am. Thorac. Soc.* **2**, 313–319
- Donn, R., Berry, A., Stevens, A., Farrow, S., Betts, J., Stevens, R., Clayton, C., Wang, J., Warnock, L., Worthington, J., Scott, L., Graham, S., and Ray, D. (2007) Use of gene expression profiling to identify a novel glucocorticoid sensitivity determining gene, BMPRII. *FASEB J.* **21**, 402–414
- Huizenga, N. A., Koper, J. W., de Lange, P., Pols, H. A., Stolk, R. P., Grobbee, D. E., de Jong, F. H., and Lamberts, S. W. (1998) Interperson variability but intraperson stability of baseline plasma cortisol concentrations, and its relation to feedback sensitivity of the hypothalamo-pituitary-adrenal axis to a low dose of dexamethasone in elderly individuals. *J. Clin. Endocrinol. Metab.* **83**, 47–54
- Chriguer, R. S., Elias, L. L., da Silva, I. M., Jr., Vieira, J. G., Moreira, A. C., and de Castro, M. (2005) Glucocorticoid sensitivity in young healthy individuals: *in vitro* and *in vivo* studies. *J. Clin. Endocrinol. Metab.* **90**, 5978–5984
- Stevens, A., Ray, D. W., Zeggini, E., John, S., Richards, H. L., Griffiths, C. E., and Donn, R. (2004) Glucocorticoid sensitivity is determined by a specific glucocorticoid receptor haplotype. *J. Clin. Endocrinol. Metab.* **89**, 892–897
- Van Rossum, E. F., Voorhoeve, P. G., te Velde, S. J., Koper, J. W., Delemarre-van de Waal HA, Kemper, H. C., and Lamberts, S. W. (2004) The ER22/23EK polymorphism in the glucocorticoid receptor gene is associated with a beneficial body composition and muscle strength in young adults. *J. Clin. Endocrinol. Metab.* **89**, 4004–4009
- Mayburd, A. L., Martlinez, A., Sackett, D., Liu, H., Shih, J., Tauler, J., Avis, I., and Mulshine, J. L. (2006) Ingenuity network-assisted transcription profiling: identification of a new pharmacologic mechanism for MK886. *Clin. Cancer Res.* **12**, 1820–1827
- Stevens, A., Garside, H., Berry, A., Waters, C., White, A., and Ray, D. (2003) Dissociation of steroid receptor coactivator 1 and nuclear receptor corepressor recruitment to the human glucocorticoid receptor by modification of the ligand-receptor interface: the role of tyrosine 735. *Mol. Endocrinol.* **17**, 845–859
- Cerami, E. G., Bader, G. D., Gross, B. E., and Sander, C. (2006) cPath: open source software for collecting, storing, and querying biological pathways. *BMC Bioinformatics* **7**, 497
- Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498–2504
- Kudrin, A., Scott, M., Martin, S., Chung, C. W., Donn, R., McMaster, A., Ellison, S., Ray, D., Ray, K., and Binks, M. (2006) Human macrophage migration inhibitory factor: a proven immunomodulatory cytokine? *J. Biol. Chem.* **281**, 29641–29651
- Gugliesi, F., Mondini, M., Ravera, R., Robotti, A., de Andrea, M., Gribaudo, G., Gariglio, M., and Landolfo, S. (2005) Up-regulation of the interferon-inducible IFI16 gene by oxidative stress triggers p53 transcriptional activity in endothelial cells. *J. Leukoc. Biol.* **77**, 820–829
- Alimirah, F., Chen, J., Xin, H., and Choubey, D. (2006) Androgen receptor auto-regulates its expression by a negative feedback loop through upregulation of IFI16 protein. *FEBS Lett.* **580**, 1659–1664
- Lane, K. B., Machado, R. D., Pauculo, M. W., Thomson, J. R., Phillips, J. A., III, Loyd, J. E., Nichols, W. C., and Trembath, R. C. (2000) Heterozygous germline mutations in BMPR2, encoding a TGF- $\beta$  receptor, cause familial primary pulmonary hypertension. The International PPH Consortium. *Nat. Genet.* **26**, 81–84
- Kimple, R. J., Kimple, M. E., Betts, L., Sondek, J., and Siderovski, D. P. (2002) Structural determinants for GoLoco-induced inhibition of nucleotide release by G $\alpha$  subunits. *Nature* **416**, 878–881
- Sierra, D. A., Gilbert, D. J., Householder, D., Grishin, N. V., Yu, K., Ukidwe, P., Barker, S. A., He, W., Wensel, T. G., Otero, G., Brown, G., Copeland, N. G., Jenkins, N. A., and Wilkie, T. M. (2002) Evolution of the regulators of G-protein signaling multigene family in mouse and human. *Genomics* **79**, 177–185

22. Law, S. F., Estojak, J., Wang, B., Mysliwiec, T., Kruh, G., and Golemis, E. A. (1996) Human enhancer of filamentation 1, a novel p130cas-like docking protein, associates with focal adhesion kinase and induces pseudohyphal growth in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **16**, 3327–3337
23. Manie, S. N., Beck, A. R., Astier, A., Law, S. F., Canty, T., Hirai, H., Druker, B. J., Avraham, H., Haghayeghi, N., Sattler, M., Salgia, R., Griffin, J. D., Golemis, E. A., and Freedman, A. S. (1997) Involvement of p130<sup>Cas</sup> and p105<sup>HEF1</sup>, a novel Cas-like docking protein, in a cytoskeleton-dependent signaling pathway initiated by ligation of integrin or antigen receptor on human B cells. *J. Biol. Chem.* **272**, 4230–4236
24. Bao, J., Cao, C., Zhang, X., Jiang, F., Nicosia, S. V., and Bai, W. (2007) Suppression of  $\beta$ -amyloid precursor protein signaling into the nucleus by estrogens mediated through complex formation between the estrogen receptor and Fe65. *Mol. Cell. Biol.* **27**, 1321–1333
25. Lee, E. J., Chun, J., Hyun, S., Ahn, H. R., Jeong, J. M., Hong, S. K., Hong, J. T., Chang, I. K., Jeon, H. Y., Han, Y. S., Auh, C. K., Park, J. I., and Kang, S. S. (2008) Regulation Fe65 localization to the nucleus by SGK1 phosphorylation of its Ser566 residue. *BMB Rep.* **41**, 41–47
26. Vermeer, H., Hendriks-Stegeman, B. I., van der Burg, B., van Buul-Offers, S. C., and Jansen, M. (2003) Glucocorticoid-induced increase in lymphocytic FKBP51 messenger ribonucleic acid expression: a potential marker for glucocorticoid sensitivity, potency, and bioavailability. *J. Clin. Endocrinol. Metab.* **88**, 277–284
27. Fan, B. J., Wang, D. Y., Tham, C. C., Lam, D. S., and Pang, C. P. (2008) Gene expression profiles of human trabecular meshwork cells induced by triamcinolone and dexamethasone. *Invest. Ophthalmol. Vis. Sci.* **49**, 1886–1897
28. Slatore, C. G., Bryson, C. L., and Au, D. H. (2009) The association of inhaled corticosteroid use with serum glucose concentration in a large cohort. *Am. J. Med.* **122**, 472–478
29. Hogg, J. C., Chu, F., Utokaparch, S., Woods, R., Elliott, W. M., Buzatu, L., Cherniack, R. M., Rogers, R. M., Sciruba, F. C., Coxson, H. O., and Pare, P. D. (2004) The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N. Engl. J. Med.* **350**, 2645–2653
30. Van der Strate, B. W., Postma, D. S., Brandsma, C. A., Melgert, B. N., Luinge, M. A., Geerlings, M., Hylkema, M. N., van den Berg, A., Timens, W., Kerstjens, H. A. (2006) Cigarette smoke-induced emphysema: a role for the B cell? *Am. J. Respir. Crit. Care Med.* **173**, 751–758
31. Yasukawa, Z., Sato, C., and Kitajima, K. (2005) Inflammation-dependent changes in  $\alpha$ 2,3-,  $\alpha$ 2,6-, and  $\alpha$ 2,8-sialic acid glycotopes on serum glycoproteins in mice. *Glycobiology* **15**, 827–837
32. Szapary, D., Huang, Y., and Simons, S. S., Jr. (1999) Opposing effects of corepressor and coactivators in determining the dose-response curve of agonists, and residual agonist activity of antagonists, for glucocorticoid receptor-regulated gene expression. *Mol. Endocrinol.* **13**, 2108–2121
33. Almlof, T., Wright, A. P., and Gustafsson, J. A. (1995) Role of acidic and phosphorylated residues in gene activation by the glucocorticoid receptor. *J. Biol. Chem.* **270**, 17535–17540
34. Ismaili, N., and Garabedian, M. J. (2004) Modulation of glucocorticoid receptor function via phosphorylation. *Ann. N. Y. Acad. Sci.* **1024**, 86–101
35. Ismaili, N., Blind, R., and Garabedian, M. J. (2005) Stabilization of the unliganded glucocorticoid receptor by TSG101. *J. Biol. Chem.* **280**, 11120–11126
36. Alimirah, F., Chen, J., Davis, F. J., and Choubey, D. (2007) IFI16 in human prostate cancer. *Mol. Cancer Res.* **5**, 251–259
37. Trapani, J. A., Dawson, M., Apostolidis, V. A., and Browne, K. A. (1994) Genomic organization of IFI16, an interferon-inducible gene whose expression is associated with human myeloid cell differentiation: correlation of predicted protein domains with exon organization. *Immunogenetics* **40**, 415–424
38. Johnstone, R. W., and Trapani, J. A. (1999) Transcription and growth regulatory functions of the HIN-200 family of proteins. *Mol. Cell. Biol.* **19**, 5833–5838
39. Adcock, I. M., and Caramori, G. (2001) Cross-talk between pro-inflammatory transcription factors and glucocorticoids. *Immunol. Cell Biol.* **79**, 376–384
40. Reichardt, H. M., Tuckermann, J. P., Gottlicher, M., Vujic, M., Weih, F., Angel, P., Herrlich, P., and Schutz, G. (2001) Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor. *EMBO J.* **20**, 7168–7173
41. Johnstone, R. W., Kershaw, M. H., and Trapani, J. A. (1998) Isotypic variants of the interferon-inducible transcriptional repressor IFI 16 arise through differential mRNA splicing. *Biochemistry* **37**, 11924–11931
42. Kimkong, I., Avihingsanon, Y., and Hirankarn, N. (2009) Expression profile of HIN200 in leukocytes and renal biopsy of SLE patients by real-time RT-PCR. *Lupus* **18**, 1066–1072
43. Bledsoe, R. K., Montana, V. G., Stanley, T. B., Delves, C. J., Apolito, C. J., McKee, D. D., Consler, T. G., Parks, D. J., Stewart, E. L., Willson, T. M., Lambert, M. H., Moore, J. T., Pearce, K. H., and Xu, H. E. (2002) Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell* **110**, 93–105
44. Doucas, V., Shi, Y., Miyamoto, S., West, A., Verma, I., Evans, R. M. (2000) Cytoplasmic catalytic subunit of protein kinase A mediates cross-repression by NF- $\kappa$ B and the glucocorticoid receptor. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 11893–11898
45. Rangarajan, P. N., Umesono, K., and Evans, R. M. (1992) Modulation of glucocorticoid receptor function by protein kinase A. *Mol. Endocrinol.* **6**, 1451–1457
46. Gariglio, M., Azzimonti, B., Pagano, M., Palestro, G., de, A. M., Valente, G., Voglino, G., Navino, L., Landolfo, S. (2002) Immunohistochemical expression analysis of the human interferon-inducible gene IFI16, a member of the HIN200 family, not restricted to hematopoietic cells. *J. Interferon Cytokine Res.* **22**, 815–821
47. Adcock, I. M., Gilbey, T., Gelder, C. M., Chung, K. F., and Barnes, P. J. (1996) Glucocorticoid receptor localization in normal and asthmatic lung. *Am. J. Respir. Crit. Care Med.* **154**, 771–782
48. Fiorentino, L., Stehlik, C., Oliveira, V., Ariza, M. E., Godzik, A., and Reed, J. C. (2002) A novel PAAD-containing protein that modulates NF- $\kappa$ B induction by cytokines tumor necrosis factor- $\alpha$  and interleukin-1 $\beta$ . *J. Biol. Chem.* **277**, 35333–35340
49. Min, W., Ghosh, S., and Lengyel, P. (1996) The interferon-inducible p202 protein as a modulator of transcription: inhibition of NF- $\kappa$ B, c-Fos, and c-Jun activities. *Mol. Cell. Biol.* **16**, 359–368
50. The International FMF Consortium (1997) Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell* **90**, 797–807
51. Ma, X. Y., Wang, H., Ding, B., Zhong, H., Ghosh, S., Lengyel, P. (2003) The interferon-inducible p202a protein modulates NF- $\kappa$ B activity by inhibiting the binding to DNA of p50/p65 heterodimers and p65 homodimers while enhancing the binding of p50 homodimers. *J. Biol. Chem.* **278**, 23008–23019

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