Polycomb Repressive Complex 2 Confers BRG1 Dependency on the *CIITA* Locus

Mohamed Abou El Hassan,* Tao Yu,* Lan Song,* and Rod Bremner*,†,‡

CIITA (or MHC2TA) coordinates constitutive and IFN-γ–induced expression of MHC class II genes. IFN-γ responsiveness of *CIITA* requires BRG1 (SMARCA4), the ATPase engine of the chromatin remodeling SWI/SNF complex (also called BAF). SWI/SNF is defective in many human cancers, providing a mechanism to explain IFN-γ resistance. BRG1 dependency is mediated through remote elements. Short *CIITA* reporters lacking these elements respond to IFN-γ, even in BRG1-deficient cells, suggesting that BRG1 counters a remote repressive influence. The nature of this distal repressor is unknown, but it would represent a valuable therapeutic target to reactivate IFN-γ responsiveness in cancer. In this article, we show that the polycomb repressive complex 2 (PRC2) components EZH2 and SUZ12, as well as the associated histone mark H3K27me3, are codetected at interenhancer regions across the *CIITA* locus. IFN-γ caused a BRG1-dependent reduction in H3K27me3, associated with nucleosome displacement. SUZ12 knockdown restored IFN-γ responsiveness in BRG1-null cells, and it mimicked the ability of BRG1 to induce active histone modifications (H3K27ac, H3K4me) at the −50-kb enhancer. Thus, PRC2 confers BRG1 dependency on the *CIITA* locus. Our data suggest that, in addition to its known roles in promoting stemness and proliferation, PRC2 may inhibit immune surveillance, and it could be targeted to reactivate CIITA expression in SWI/SNF deficient cancers. ***The Journal of Immunology*****, 2015, 194: 5007–5013.**

CIITA (or MHC2TA) is a transcriptional coactivator that is essential for both constitutive and IFN-γ–induced MHC class II gene expression (1, 2). Inactivating mutations in *CIITA* cause bare lymphocyte syndrome, a lethal immune disorder, and the gene is associated with various other disorders, including cancer (reviewed in Refs. 3, 4). *CIITA* is constitutively expressed in APCs where MHC class I expression is also constitutive, and it can be induced by IFN-γ in other cell types. Four alternative promoters (pI–pIV) were identified, with pIII and pIV being the major constitutive and IFN-γ–responsive promoters, respectively (reviewed in Refs. 5, 6).

Previously, we showed that the chromatin-remodeling enzyme BRG1 is essential for IFN-γ induction of *CIITA* (7). BRG1 is the ATPase that drives activity of the multisubunit SWI/SNF (or BAF) complex. IFN-γ stimulates phosphorylation, dimerization, and nuclear translocation of STAT1, and STAT1 cooperates with IRF1 to induce *CIITA* (8–10). BRG1 is dispensable for IFN-γ–induced STAT1 recruitment to the *IRF1* promoter and for *IRF1* induction, but it is essential for subsequent recruitment of STAT1 and IRF1 proteins to *CIITA* pIV (11). SWI/SNF is also required for chromatin access and induction at several other IFN-γ genes, activation of numerous IFN-α targets by the STAT1-STAT3-IRF9 trimer, and for STAT3-mediated upregulation of IL-6 gene targets, indicating a broad role for this chromatin-remodeling enzyme in cytokine signaling (11–16).

SWI/SNF is defective in ∼20% of human cancers (17), providing a mechanism through which tumors cells could avoid IFN-γ–induced *CIITA* expression and, thus, MHC class II–mediated activation of Th cells. The use of artificial vectors to reconstitute tumors with CIITA enhances immunogenicity (18); thus, there is considerable interest in deducing how to reactivate endogenous expression. Whether this is feasible in SWI/SNF-deficient cancer cells is unknown.

Most initial studies on *CIITA* regulation focused on promoter-proximal mechanisms. We pinpointed a series of five enhancers distributed across 110 kb upstream and downstream of pIV that are essential for IFN-γ responsiveness (19). Chromosome conformation capture analyses revealed that these remote enhancers form a three-dimensional (3D) structure through DNA loops. A basal 3D structure forms independently of BRG1 by an unknown mechanism, and the only event known to be triggered following the addition of BRG1 is methylation of histone H3 lysine 4 (H3K4), which marks poised or active enhancers (19). This modification occurs at an enhancer 50 kb upstream of pIV, which is also where BRG1 is targeted. The recruitment of BRG1, and presumably the methylation that poises the −50-kb enhancer, are critical for all subsequent events following IFN-γ treatment, including factor binding to all five remote enhancers and pIV, epigenetic remodeling, tightening of existing loops, formation of new 3D contacts, RNA polymerase II recruitment, and transcription (19). We observed similar 3D structures at other IFN-γ targets (20). Moreover, several of the remote elements used to regulate human pIV are also enhancers for alternate *CIITA* promoters and are conserved in rodents (21).

Although the endogenous *CIITA* locus and a 200-kb reporter are both BRG1 dependent, shorter reporters with as much as 8 kb of

*Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario M5G1X5, Canada; †Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada; and ‡Department of Ophthalmology and Vision Science, University of Toronto, Toronto, Ontario M5T 3A9, Canada

Received for publication January 5, 2015. Accepted for publication March 12, 2015.

This work was supported by funds from the Canadian Cancer Society Research Institute and the Krembil Foundation.

Address correspondence and reprint requests to Dr. Rod Bremner, Lunenfeld Tanenbaum Research Institute, 600 University Avenue, Room 840, Toronto, ON M5G 1X5, Canada. E-mail address: bremner@lunenfeld.ca

The online version of this article contains supplemental material.

Abbreviations used in this article: Ad-BRG1, adenovirus expressing a GFP-BRG1 fusion protein; Ad-GFP, adenovirus expressing GFP; ChIP, chromatin immunoprecipitation; 3D, three dimensional; H3K4, histone H3 lysine 4; H3K27, histone H3 lysine 27; H3K27ac, H3K27 acetylation; H3K27me2, H3K27 dimethylation; PRC2, polycomb repressive complex 2; siCtrl, control siRNA; siRNA, small interfering RNA; siSUZ12, siRNA against SUZ12; TSS, transcription start site; WB, Western blot.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1403247

sequence upstream of pIV respond to IFN-γ in the absence of BRG1 (19). We hypothesized that BRG1 might counter the effects of a repressor that targets remote sites, thus explaining why short reporters, lacking these distal inhibitory elements, are BRG1 independent. If such a repressor could be identified, it would explain the mechanism underlying BRG1 dependency at *CIITA* and provide an opportunity to reactivate expression in SWI/SNF-deficient cancer cells. However, whether such a repressor exists is unclear. In this study, we identify the repressor and show that its removal mimics the epigenetic and transcriptional effects of adding BRG1.

Materials and Methods

Cell culture and adenoviruses

Human cervical carcinoma HeLa and adrenal carcinoma SW-13 cells were grown as described (11) and treated with IFN-γ (Invitrogen) at 0.1 mg/ml. SW-13 cells were transduced with adenovirus expressing GFP (Ad-GFP) or adenovirus expressing a GFP-BRG1 fusion protein (Ad-BRG1), as described (11), with the final steady-state of BRG1 equivalent to that in HeLa cells, as judged by Western blot (WB) analysis.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) was carried out as described previously (19). A list of Abs is provided in Table I.

Real-time PCR

For ChIP analysis, amplification was performed on an Applied Biosystems 7900 HT thermal cycler using previously described primers (19). Amplicons were detected using SYBR Green, as described previously (19). For RNA analysis, cDNA was generated using Superscript II Reverse Transcriptase (Invitrogen; cat no. 18064-014), as described previously (19). cDNA was amplified for 35 cycles using the following primer pairs: CIITA sense (5′-GGGACTCAATGCACTGACAT-3′) and antisense (5′-CCATTAGCTGTTTCCCTGCT-3′) and IRF1 sense (5′-TGAAGAACATGGATGCCACCT-3′) and antisense (5′-GTGCACAGGGAATGGCCT-3′).

Western blotting

WBs were performed as described previously (22). A full list of Abs is provided in Table I. WBs were quantified using Odyssey imagers (LI-COR).

RNA interference

Small interfering RNAs (siRNAs) were obtained from QIAGEN (cat. no. SI03246817 and SI04294017). SW-13 cells were transfected at a density of 2 million cells/10-cm dishes and with 50 nM siRNA for 3–4 d using DharmaFECT 1 (Thermo Scientific), according to the manufacturer's instructions. Cells were subcultured at a density of 2 million cells/10-cm dishes and subjected to a second cycle of transfection.

Results

Polycomb repressive complex 2 marks intraenhancer domains at CIITA

To identify potential repressors that block IFN-γ signaling at *CIITA*, we assessed epigenetic signatures across ∼150 kb of the locus in HeLa cells. H3K9me3, a heterochromatin mark, was absent with the exception of low levels 6.4 kb downstream of the transcription start site (TSS) (Fig. 1). Previously, we reported an H3K79me3 mark at the +59-kb enhancer, and its maintenance in BRG1-deficient cells raised the possibility that it might confer BRG1 dependency (19). H3K79me3 is functionally linked to gene activation, but it is sometimes associated with repressed loci (23). In the basal state, H3K79me3 was confined to the +59-kb site (Fig. 1). Although IFN-γ did not change H3K79me3 levels at +59 kb, it induced time-dependent de novo H3K79 methylation at around pIV. DOT1L is the sole H3K79 methyltransferase (reviewed in Ref. 24); although there was little DOT1L at the *CIITA* locus in the basal state, IFN-γ induced recruitment to pIV, explaining the change in H3K79me3, but DOT1L levels also were induced at remote sites, including −50, −16, −8, +40, and +59 kb. Conceivably, DOT1L detection at these sites is an indirect effect of interenhancer DNA looping that we reported previously (19, 20), or DOT1L methylates distal, but not proximal, nucleosomes. Importantly, the positive correlation between *CIITA* induction and the increase in DOT1L recruitment and H3K79me3 levels at the promoter and the majority of the discovered remote sites suggest that this mark is linked to activation, rather than repression, of *CIITA*.

Polycomb repressive complex 2 (PRC2) methylates histone H3 lysine 27 (H3K27) and silences gene expression (25). Other investigators reported that PRC2 binds and regulates *CIITA* pIV (26–28), but it is unknown whether it also acts at remote sites and whether it explains BRG1 dependency. H3K27 dimethylation (H3K27me2) is absent at the *CIITA* locus (19), which we confirmed in this study and extended to additional remote sites (Fig. 2). However, we found an undulating pattern of H3K27me3 across *CIITA* (Fig. 2). This signature was elevated at intraenhancer regions and reduced at remote *CIITA* enhancers located at −50, −16, −8, +40, and +59 kb relative to the *CIITA* TSS (19), which is noteworthy because BRG1 and other SWI/SNF subunits associate constitutively with these elements in HeLa cells (19, 29). Bulk H3 analysis suggested that reduced H3K27me levels at the −16-kb and +40-kb enhancers were due, in part, to nucleosome displacement; however, at other enhancers, H3 levels were unaffected

Table I. Abs

Target	Application	Supplier	Catalog No.
H3	WB, ChIP	Abcam	ab1791
H3K4Me1	ChIP	Abcam	ab8895
H3K4Me2	ChIP	Millipore	07-030
H3K9Me3	ChIP	Millipore	07-442
H3K27Me2	ChIP	Millipore	07-452
H3K27Me3	WB, ChIP	Millipore	07-449
H3K27Ac	WB, ChIP	Abcam	ab4729
H3K79Me3	WB, ChIP	Abcam	ab2621
SUZ12	WB, ChIP	Abcam	ab12073
EZH2	WB	BD	612666
EZH2 (ENX-1)(H-80)	ChIP	Santa Cruz Biotechnology	sc-25383
DOT1L	ChIP	Abcam	ab57827
STAT1	WB	Millipore	06-501
p-STAT1	WB	Cell Signaling	91715
IRF1 (c-20)	WB	Santa Cruz Biotechnology	sc-497
Tubulin	WB	Sigma	T-9026
Goat anti-rabbit	WB	Odyssey	926-32211
Goat anti-mouse	WB	Odyssey	926-32220

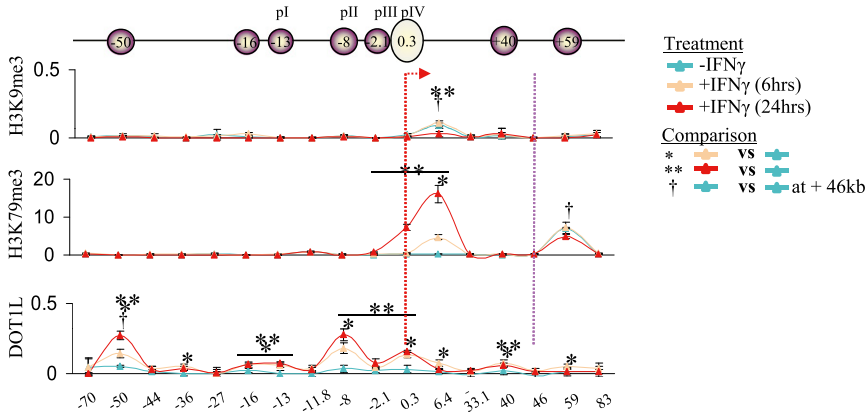


FIGURE 1. Epigenetic regulators and histone marks at the *CIITA* locus in HeLa cells. ChIP-quantitative PCR analyses at *CIITA* of the indicated factors and chromatin modifications in HeLa cells 6 or 24 h after IFN-γ treatment. Data are presented as percentage input, and values are mean ($n \geq 3$) ± SD. To define DNA sites with significant basal levels of the studied factors and histone marks, ANOVA was followed by the two-sided Dunnett test, taking the +46-kb site as the negative control (purple dotted line). The diagram above the graphs summarizes proximal and remote regulatory elements. Green circles indicate remote regulatory elements, and the gray oval represents pIV. Alternative promoters (pI–III) are indicated above the diagram. The red dotted line indicates the TSS at pIV. The distance between the depicted sites and the TSS (in kb) is indicated below. *,**,†≥2-fold significant differences ($p < 0.05$) relative to controls (key). The p values were calculated by ANOVA, followed by the Fisher test.

(Fig. 2, Supplemental Fig. 1). The PRC2 subunits EZH2 and SUZ12 confer enzyme activity and complex stability, respectively (30–32), and recruitment of these proteins across *CIITA*, in general, matched the H3K7me3 epigenetic mark across the CIITA locus, with the exception of the −8-kb site (Fig. 2). These data raise the notion that BRG1 may shield *CIITA* remote enhancers against silencing by PRC2.

Next, we examined whether IFN-γ treatment affects the PRC2 signature. Robust *CIITA* induction is detected 6 h after IFN-γ treatment (7, 11); at this time, we observed ~4-fold less H3K27me3 at *CIITA* pIV and the −8-kb enhancer and ~2-fold less H3K27me3 at the −2-kb, −13-kb, and −70-kb regions (see "*" in Fig. 2 for significant effects of IFN-γ). EZH2 and SUZ12 also were reduced at pIV; although modest depletion of the former at −13 and −8 kb reached significance, that of the latter did not, likely due to slight differences in Ab accessibility (Table I) and/or technical variability (Fig. 2). We did not detect the H3K27me3 demethylase JMJD3 at the *CIITA* locus in untreated or IFN-γ–treated cells, and although we did not examine UTX, H3 ChIP analysis suggested that nucleosome depletion explained the IFN-γ–induced reduction in H3K27me3 at most *CIITA* regions (see "*" in Fig. 2 and Supplemental Fig. 1A for significant effects of IFN-γ). The IFN–γ-mediated reduction in H3K27me3 levels was paralleled by increases in H3K27 acetylation (H3K27ac), an active mark, at the promoter and remote enhancers; comparison with H3 ChIP data indicated that the remaining nucleosomes were actively acetylated (see "*" and "**" in Fig. 2 and Supplemental Fig. 1A for significant effects of IFN-γ). These data fit our prior findings that IFN-γ induces recruitment of the histone acetyl transferases CBP and p300 to *CIITA* (11, 19). Thus, in the pres-

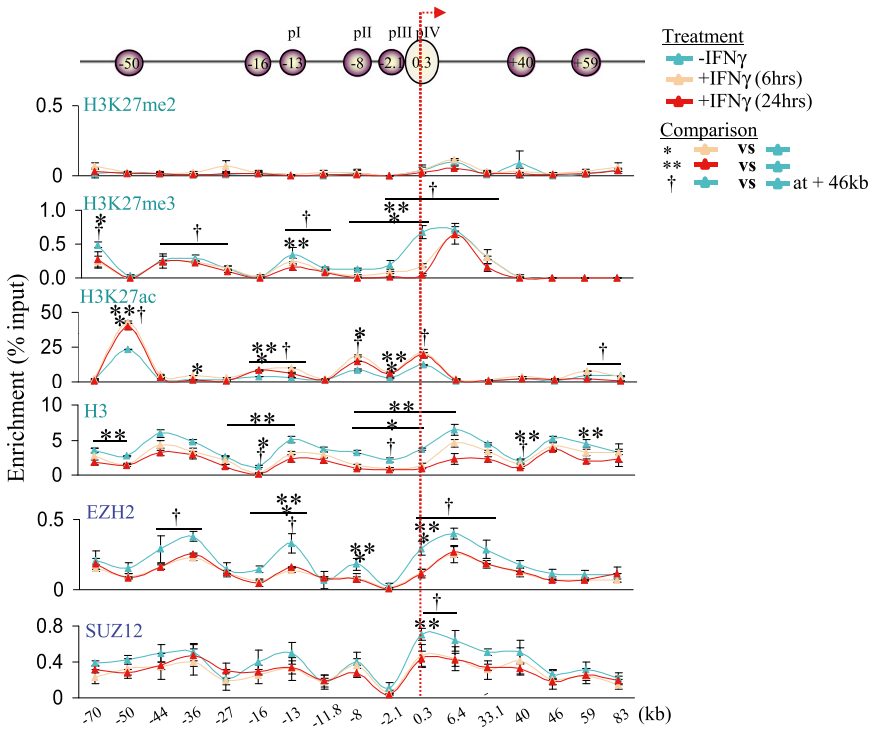


FIGURE 2. PRC2 marks the *CIITA* locus. ChIP-quantitative PCR analyses at *CIITA* of the indicated factors and chromatin modifications in HeLa cells 6 or 24 h after IFN-γ treatment. Data are presented as percentage input and values are mean ($n \geq 3$) ± S.D. Schematic representation as in Fig. 1. *,**,†≥2-fold significant differences ($p < 0.05$) relative to controls (key). The p values were calculated as in Fig. 1.

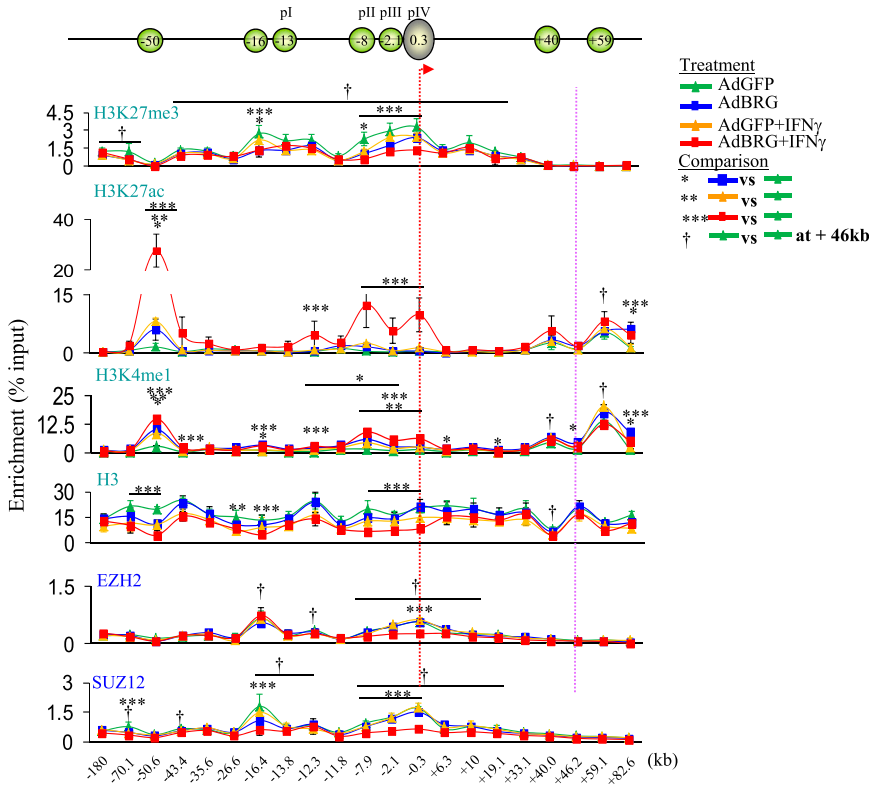


FIGURE 3. BRG1 poises the *CIITA* locus for IFN-γ induction. ChIP-quantitative PCR analyses at *CIITA* of the indicated factors and chromatin modifications in SW-13 cells transduced with Ad-GFP or Ad-BRG1 for 24 h and then left untreated or treated with IFN-γ for 6 h. Data are presented as percentage input, and values are mean ($n \geq 3$) ± S.D. Schematic representation as in Fig. 1. *,**,***,†≥2-fold significant differences ($p < 0.05$) relative to controls (key). The p values were calculated as in Fig. 1.

ence of BRG1, IFN-γ induces an epigenetic switch in which PRC2 and H3K27me3 are replaced with histone acetyl transferases and H3K27ac at *CIITA*.

BRG1 alters positive and negative chromatin marks to poise CIITA *for IFN-γ induction*

Next, we examined these findings in another cell type and assessed whether BRG1 affects H3K27 modification at *CIITA*. For this, we turned to BRG1-deficient SW-13 adrenal carcinoma cells, which were transduced with Ad-GFP or Ad-BRG1; physiological levels of the latter protein rescue IFN-γ stimulated gene responsiveness at multiple BRG1-dependent genes (11).

The H3K27me3 and PRC2 patterns across *CIITA* in GFP-expressing control SW-13 cells were remarkably similar to those in HeLa cells (compare Fig. 3 and Fig. 2). Thus, both lines had undulating distribution of H3K27me3 domains, with troughs at the −50-kb and +40/+59-kb enhancers, peaks at pIV and intra-enhancer regions, and recruitment of EZH2 and SUZ12 at most regions of elevated K27 trimethylation ("†" in Fig. 3 marks significant differences relative to control ChIP at +46 kb). The H3K27me3 trough at +40 kb in both lines correlated with nucleosome displacement (H3 ChIP, Figs. 2, 3). Subtle differences included an overall higher level of H3K27me3 across the locus in SW-13 cells, particularly at the −16-kb and −8-kb enhancers, which could explain the lower basal *CIITA* transcript levels in SW-13 cells versus HeLa cells (19).

Next, we assessed the effect of BRG1 reconstitution on basal epigenetic marks at the *CIITA* locus (see "*" in Fig. 3 for sig-

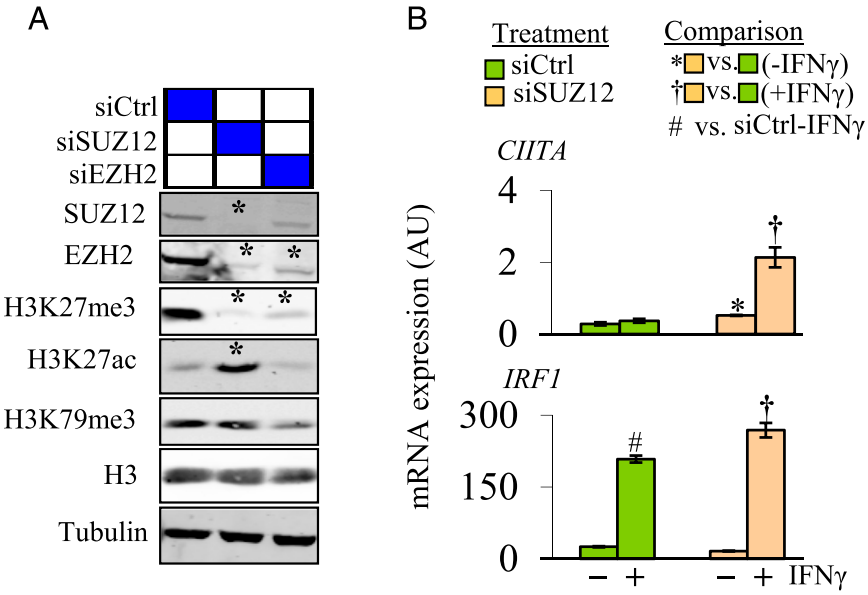


FIGURE 4. PRC2 confers BRG1 dependency on *CIITA*. (**A**) Representative WBs of the indicated factors and histone modifications. Analyses were performed using lysates of SW-13 cells after two cycles of transfection with siCtrl, siSUZ12, or EZH2 siRNA (siEZH2). siRNA treatments are indicated in blue. For quantification of protein levels see Supplemental Fig. 3B. Asterisks indicate significant changes compared with siCtrl group ($p < 0.05$). (**B**) Real-time PCR analysis of the indicated genes in SW-13 cells treated as in (A). Treatments are indicated in blue. Gene expression was normalized to tubulin and presented in arbitrary units (AU). Values are mean ($n \geq 3$) ± SD. *,†,#Significant changes ($p < 0.05$), according to the indicated comparison (key). The p values were calculated by ANOVA, followed by the Fisher test.

nificant effects of BRG1). Confirming prior data (19), we found that BRG1 induced methylation of H3K4, a marker of poised enhancers (23, 33), at the −50-kb enhancer; ~3.7-fold induction in H3K4me1 was observed at this site, and a more modest elevation was detected at and around the −8-kb enhancer (Fig. 3). BRG1 also induced the active H3K27ac mark ~3.8-fold at −50 kb. There was also a modest reduction in H3K27me3 at the −16-kb and −8-kb enhancers, but PRC2 component levels were unaffected (Fig. 3). These effects on H3K4 and H3K27 were not due to a global reduction in PRC2 subunits, bulk H3, or H3K27me3 or to a global increase in H3K27ac (Supplemental Fig. 2). After normalizing for H3 levels at each site, the BRG1-dependent increases in basal H3K27ac and H3K4me1 were still evident at the −50-kb and −8-kb enhancers, whereas small reductions in H3K27me3 at −16 and −8 kb were not ("*" in Supplemental Fig. 1B). Because BRG1 is recruited directly to the −50-kb site (19), we envisage that its main function is to poise this enhancer by facilitating the subsequent recruitment of histone acetylases and methylases and that modest displacement of H3K27me3 nucleosomes at other sites may be a secondary consequence of these effects. Overall, BRG1 enhances positive H3K4me1 and H3K27ac marks and modestly antagonizes H3K27me3 at remote *CIITA* sites, consistent with its indispensable role in poising this locus for IFN-γ responsiveness.

Next, we examined the effect of IFN-γ with or without BRG1. IFN-γ had no effect on H3K27me3, EZH2, or SUZ12 levels in control BRG1-deficient SW-13 cells, but it significantly reduced H3K27 methylation and PRC2 levels at pIV and the −8-kb and −16-kb enhancers in BRG1-reconstituted cells (Fig. 3, compare "**" with "***"). BRG1 did not affect activation of STAT1 or IRF1 (Supplemental Fig. 2). We observed BRG1-dependent IFN-γ induction of H3K27ac (absolute levels or normalized to H3) most prominently at −50 kb, but also at other enhancers and pIV, again consistent with the role of the −50-kb enhancer in recruiting BRG1. This was also the case for H3K4me1, although only after normalizing for H3 (Fig. 3, Supplemental Fig. 1B). The levels of H3K27me3 before or after IFN-γ were similar in BRG1-reconstituted cells, suggesting that basal levels are the prime target of regulation (Fig. 3, Supplemental Fig. 1B). Bulk nucleosome (i.e., H3) ChIP analysis suggested that, similar to HeLa cells (Fig. 2), IFN-γ–driven reduction in H3K27me3 in BRG1-reconstituted SW-13 cells was mainly linked to nucleosome displacement (Fig. 3, Supplemental Fig. 1B), which was not due to a global reduction in bulk H3 (Supplemental Fig. 2). Altogether, these data extend the notion that BRG1 is critical for all IFN-γ–induced events at multiple sites across the *CIITA* locus (19).

PRC2 confers BRG1 dependency on CIITA

The above data suggest that PRC2 could be the repressor that blocks IFN-γ induction of *CIITA* in SWI/SNF-deficient cells. To assess this issue, we depleted SUZ12, which is essential for PRC2 stability (32). SW-13 cells were transfected with control siRNA (siCtrl) or siRNA against SUZ12 (siSUZ12) for two cycles to obtain efficient knockdown (Fig. 4A, Supplemental Fig. 3). As expected, siSUZ12 also reduced EZH2 levels, and the Ab used to detect the latter protein was validated using siRNA against EZH2 (Fig. 4A, Supplemental Fig. 3). This reduction in PRC2 levels was mirrored by a drop in total H3K27me3 and a concomitant induction of H3K27ac, without any effect on bulk H3 or other chromatin modifications, like H3K79me3 (Fig. 4A, Supplemental Fig. 3).

Next, we analyzed gene induction; strikingly, siSUZ12 rescued IFN-γ responsiveness of *CIITA* in BRG1-deficient cells (Fig. 4B). As expected, siSUZ12 had a marginal effect on IRF1, a BRG1-independent IFN-γ stimulated gene (7, 11). Restoration of *CIITA* induction was not an off-target effect, because a distinct siSUZ12 also rescued IFN-γ responsiveness (Supplemental Fig. 4A, 4B) and did not alter levels or activation of STATs or IRF1 (Supplemental Fig. 4C, 4D).

Next, we assessed whether PRC2 loss caused direct epigenetic changes at the *CIITA* locus. Indeed, siSUZ12 dramatically reduced H3K27me3 levels across the *CIITA* locus (Fig. 5). Moreover, siSUZ12 recapitulated other BRG1-dependent epigenetic changes, including induction of H3K4 methylation and H3K27ac at the −50-kb enhancer and modest induction of H3K4me1 at other sites, including the −8-kb and −16-kb enhancers (compare Fig. 3 and Fig. 5). Thus, PRC2 loss mimics the epigenetic effects of BRG1. These data link BRG1 and PRC2 as antagonistic regulators of IFN-γ responsiveness at the *CIITA* locus.

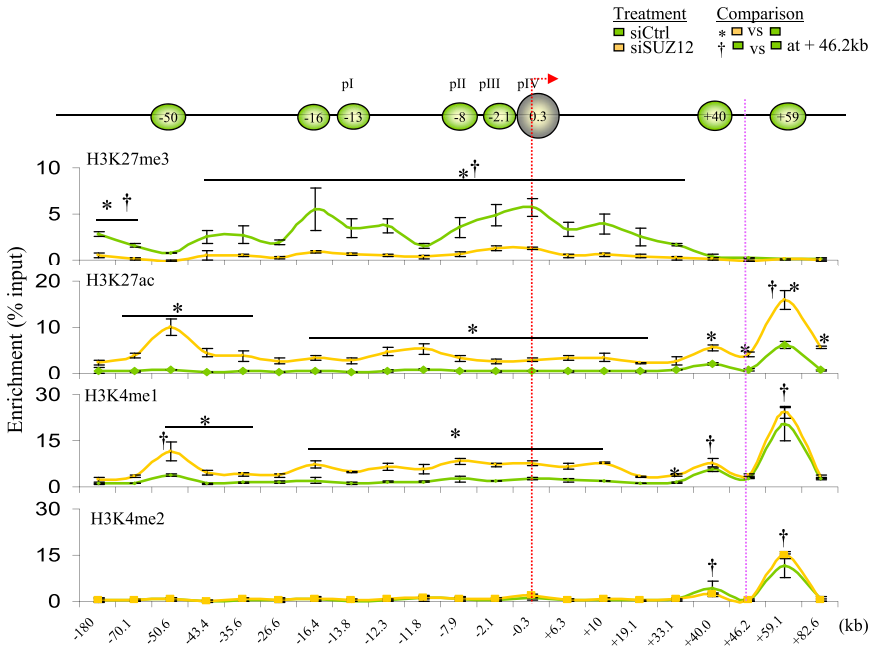


FIGURE 5. SUZ12 knockdown mimics the epigenetic effects of BRG1 at *CIITA*. ChIP-quantitative PCR analyses at *CIITA* of the indicated epigenetic modifications in SW-13 cells transfected with siCtrl or siSUZ12. Data are presented as percentage of the input DNA, and values are mean ($n > 3$) ± SD. Schematic representation as in Fig. 1. *,**,†Sites where the ChIP signal is changed significantly ($p < 0.05$) by ≥2 fold, according to the indicated comparisons (key). The p values were calculated as in Fig. 1.

Discussion

The requirement for BRG1 at IFN targets was discovered over a decade ago (7, 13, 14), yet the reason for this dependency has remained unclear. We demonstrate that BRG1 antagonizes the repressive effects of PRC2 at *CIITA*. The repressive H3K9me3 mark was largely absent at the locus, and H3K79me3 and DOT1L recruitment correlated with *CIITA* activation rather than repression. However, we detected an undulating pattern of PRC2 subunits and H3K27me3 across *CIITA*, which was interrupted at remote BRG1-dependent enhancers. Extended H3K27 methylation also was observed at loci encoding developmental transcription factors in embryonic stem cells (34). The presence of these repressive marks at remote sites explains our prior observations that distal regions are essential to confer BRG1 dependency on pIV (19).

BRG1 dramatically enhanced the basal levels of H3K4me1 and H3K27ac, especially at the −50-kb enhancer where it is directly recruited when introduced into BRG1-deficient cells (19). BRG1 recruitment had no effect on PRC2 recruitment and only modest effects on H3K27me3 levels through nucleosome depletion. Strikingly, however, PRC2 depletion mimicked the effect that BRG1 reconstitution had on H3K4me1 and H3K27ac marks. These data raise the intriguing model that BRG1 and PRC2 may not battle each other directly, but instead that BRG1 promotes, whereas PRC2 antagonizes, the recruitment of histone acetyl and methyl transferases that poise the locus for responsiveness. CBP and p300 are recruited to *CIITA* (11), but the identity of the H3 methyl transferase is unknown, and the mechanism by which BRG1 and PRC2 influence recruitment of these enzymes also requires additional analysis. BRG1 can have ATPase-independent effects (35, 36), but *CIITA* induction requires enzyme activity (7), suggesting that nucleosome movement is required for transferase recruitment.

CIITA induces expression of MHC class II and the invariant chain, which is required to present peptides to the receptor. MHC class II–peptide complexes activate the helper arm of the adaptive immune response, which has several critical roles in immunity, including activation of killer T cells. *CIITA* is often downregulated and/or unresponsive to IFN-γ in cancer cells (7, 26, 28, 37–45), or in some cases its activity is redirected through translocation and fusion to other regulators (46). Re-expression of *CIITA* in various cancers improves immunogenicity and can lead to both tumor clearance and sustained antitumor immunological memory (reviewed in Ref. 18). Therefore, it is of some note that PRC2 is overexpressed in multiple cancers, and SWI/SNF subunits are defective in ∼20% of human cancers (17). Based on our observations, targeting PRC2 would be predicted to overcome defective *CIITA* regulation in both of these scenarios, potentially reactivating immune sensitivity in a large spectrum of cancer cells. Indeed, in a parallel study, we showed that PRC2 represses IFN-γ targets in multiple cancer cell types. Unexpectedly, this work also revealed that PRC2 represses many other cytokine pathways (M. Abou El Hassan, K. Huang, M.B.K. Eswara, M. Zhao, L. Song, T. Yu, Y. Liu, J.C. Liu, S. McCurdy, A. Ma, J. Wither, J. Jin, E. Zacksenhaus, J.L. Wrana, and R. Bremner, submitted for publication). Thus, our discovery that PRC2 confers BRG1 dependency on CIITA has relevance for immune control well beyond the master regulator of MHC class II expression.

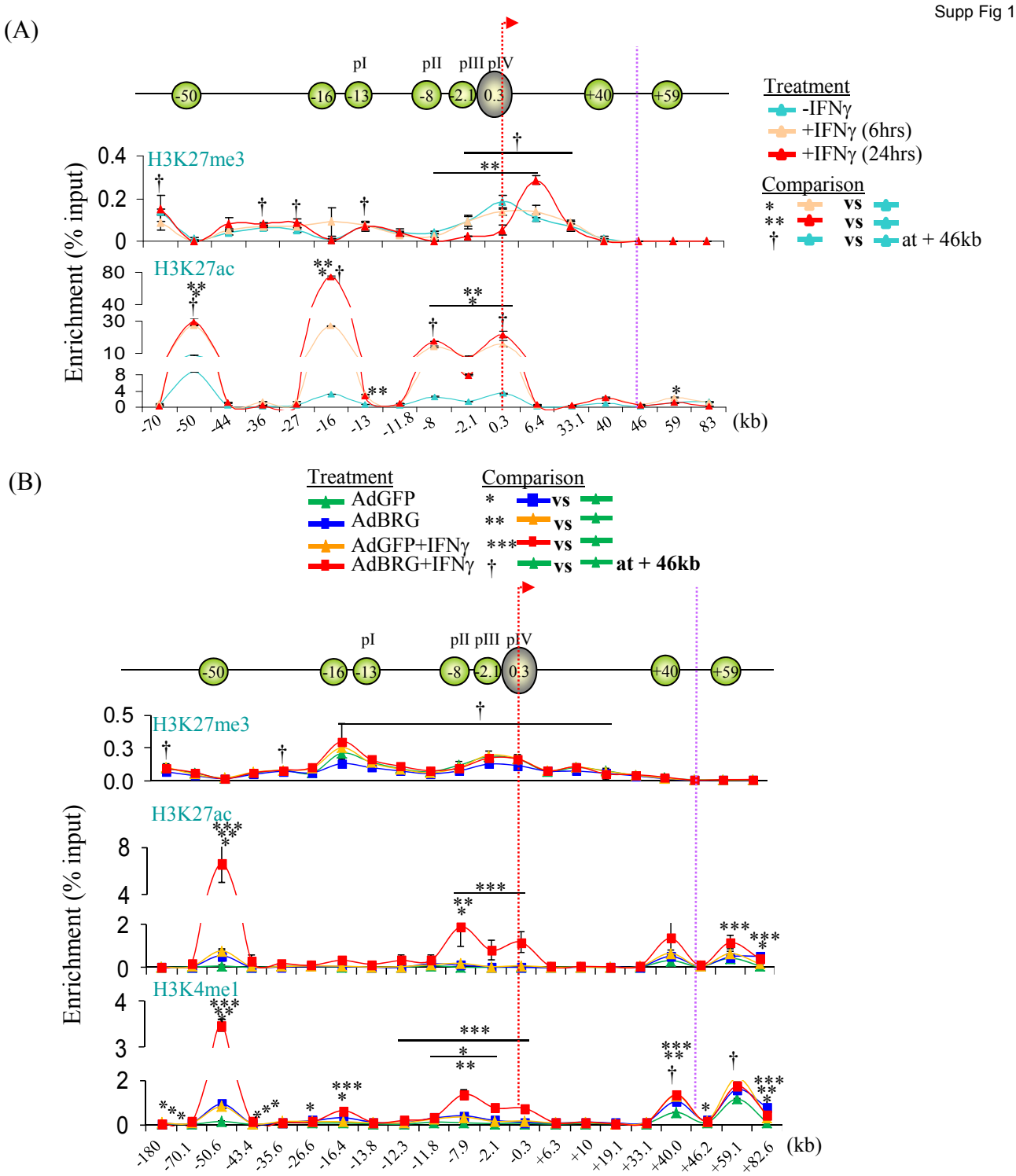
Disclosures

The authors have no financial conflicts of interest.

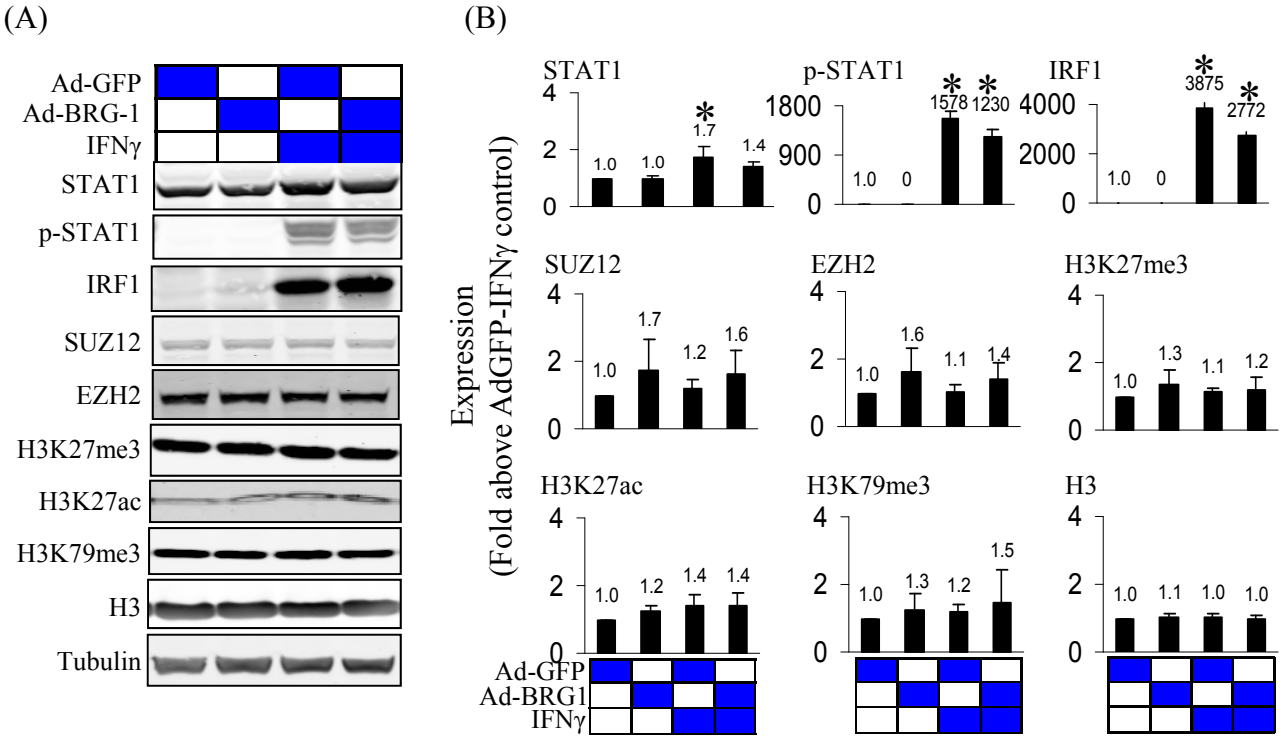
References

1. Steimle, V., L. A. Otten, M. Zufferey, and B. Mach. 1993. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). *Cell* 75: 135–146.
2. Steimle, V., C. A. Siegrist, A. Mottet, B. Lisowska-Grospierre, and B. Mach. 1994. Regulation of MHC class II expression by interferon-gamma mediated by the transactivator gene CIITA. *Science* 265: 106–109.
3. Devaiah, B. N., and D. S. Singer. 2013. CIITA and Its Dual Roles in MHC Gene Transcription. *Front. Immunol.* 4: 476.
4. Holling, T. M., M. C. van Eggermond, M. J. Jager, and P. J. van den Elsen. 2006. Epigenetic silencing of MHC2TA transcription in cancer. *Biochem. Pharmacol.* 72: 1570–1576.
5. LeibundGut-Landmann, S., J. M. Waldburger, M. Krawczyk, L. A. Otten, T. Suter, A. Fontana, H. Acha-Orbea, and W. Reith. 2004. Mini-review: Specificity and expression of CIITA, the master regulator of MHC class II genes. *Eur. J. Immunol.* 34: 1513–1525.
6. Wright, K. L., and J. P. Ting. 2006. Epigenetic regulation of MHC-II and CIITA genes. *Trends Immunol.* 27: 405–412.
7. Pattenden, S. G., R. Klose, E. Karaskov, and R. Bremner. 2002. Interferon-gamma-induced chromatin remodeling at the CIITA locus is BRG1 dependent. *EMBO J.* 21: 1978–1986.
8. Muhlethaler-Mottet, A., W. Di Berardino, L. A. Otten, and B. Mach. 1998. Activation of the MHC class II transactivator CIITA by interferon-gamma requires cooperative interaction between Stat1 and USF-1. *Immunity* 8: 157–166.
9. Piskurich, J. F., M. W. Linhoff, Y. Wang, and J. P. Ting. 1999. Two distinct gamma interferon-inducible promoters of the major histocompatibility complex class II transactivator gene are differentially regulated by STAT1, interferon regulatory factor 1, and transforming growth factor beta. *Mol. Cell. Biol.* 19: 431–440.
10. Xi, H., D. D. Eason, D. Ghosh, S. Dovhey, K. L. Wright, and G. Blanck. 1999. Co-occupancy of the interferon regulatory element of the class II transactivator (CIITA) type IV promoter by interferon regulatory factors 1 and 2. *Oncogene* 18: 5889–5903.
11. Ni, Z., E. Karaskov, T. Yu, S. M. Callaghan, S. Der, D. S. Park, Z. Xu, S. G. Pattenden, and R. Bremner. 2005. Apical role for BRG1 in cytokine-induced promoter assembly. *Proc. Natl. Acad. Sci. USA* 102: 14611–14616.
12. Cui, K., P. Tailor, H. Liu, X. Chen, K. Ozato, and K. Zhao. 2004. The chromatin-remodeling BAF complex mediates cellular antiviral activities by promoter priming. *Mol. Cell. Biol.* 24: 4476–4486.
13. Huang, M., F. Qian, Y. Hu, C. Ang, Z. Li, and Z. Wen. 2002. Chromatin-remodelling factor BRG1 selectively activates a subset of interferon-alpha-inducible genes. *Nat. Cell Biol.* 4: 774–781.
14. Liu, H., H. Kang, R. Liu, X. Chen, and K. Zhao. 2002. Maximal induction of a subset of interferon target genes requires the chromatin-remodeling activity of the BAF complex. *Mol. Cell. Biol.* 22: 6471–6479.
15. Ni, Z., and R. Bremner. 2007. Brahma-related gene 1-dependent STAT3 recruitment at IL-6-inducible genes. *J. Immunol.* 178: 345–351.
16. Yan, Z., K. Cui, D. M. Murray, C. Ling, Y. Xue, A. Gerstein, R. Parsons, K. Zhao, and W. Wang. 2005. PBAF chromatin-remodeling complex requires a novel specificity subunit, BAF200, to regulate expression of selective interferon-responsive genes. *Genes Dev.* 19: 1662–1667.
17. Kadoch, C., D. C. Hargreaves, C. Hodges, L. Elias, L. Ho, J. Ranish, and G. R. Crabtree. 2013. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nat. Genet.* 45: 592–601.
18. Accolla, R. S., L. Lombardo, R. Abdallah, G. Raval, G. Forlani, and G. Tosi. 2014. Boosting the MHC Class II-Restricted Tumor Antigen Presentation to CD4+ T Helper Cells: A Critical Issue for Triggering Protective Immunity and Re-Orienting the Tumor Microenvironment Toward an Anti-Tumor State. *Front. Oncol.* 4: 32.
19. Ni, Z., M. Abou El Hassan, Z. Xu, T. Yu, and R. Bremner. 2008. The chromatin-remodeling enzyme BRG1 coordinates CIITA induction through many interdependent distal enhancers. *Nat. Immunol.* 9: 785–793.
20. Abou El Hassan, M., and R. Bremner. 2009. A rapid simple approach to quantify chromosome conformation capture. *Nucleic Acids Res.* 37: e35.
21. Lohsen, S., P. Majumder, C. D. Scharer, B. G. Barwick, J. W. Austin, W. M. Zinzow-Kramer, and J. M. Boss. 2014. Common distal elements orchestrate CIITA isoform-specific expression in multiple cell types. *Genes Immun.* 15: 543–555.
22. Dorval, K. M., B. P. Bobechko, K. F. Ahmad, and R. Bremner. 2005. Transcriptional activity of the paired-like homeodomain proteins CHX10 and VSX1. *J. Biol. Chem.* 280: 10100–10108.
23. Barski, A., S. Cuddapah, K. Cui, T. Y. Roh, D. E. Schones, Z. Wang, G. Wei, I. Chepelev, and K. Zhao. 2007. High-resolution profiling of histone methylations in the human genome. *Cell* 129: 823–837.
24. Nguyen, A. T., and Y. Zhang. 2011. The diverse functions of Dot1 and H3K79 methylation. *Genes Dev.* 25: 1345–1358.
25. Di Croce, L., and K. Helin. 2013. Transcriptional regulation by Polycomb group proteins. *Nat. Struct. Mol. Biol.* 20: 1147–1155.
26. Holling, T. M., M. W. Bergevoet, L. Wilson, M. C. Van Eggermond, E. Schooten, R. D. Steenbergen, P. J. Snijders, M. J. Jager, and P. J. Van den Elsen. 2007. A role for EZH2 in silencing of IFN-gamma inducible MHC2TA transcription in uveal melanoma. *J. Immunol.* 179: 5317–5325.
27. Mehta, N. T., A. D. Truax, N. H. Boyd, and S. F. Greer. 2011. Early epigenetic events regulate the adaptive immune response gene CIITA. *Epigenetics* 6: 516–525.
28. Truax, A. D., M. Thakkar, and S. F. Greer. 2012. Dysregulated recruitment of the histone methyltransferase EZH2 to the class II transactivator (CIITA) promoter IV in breast cancer cells. *PLoS ONE* 7: e36013. Available at: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0036013.
29. Euskirchen, G. M., R. K. Auerbach, E. Davidov, T. A. Gianoulis, G. Zhong, J. Rozowsky, N. Bhardwaj, M. B. Gerstein, and M. Snyder. 2011. Diverse roles

and interactions of the SWI/SNF chromatin remodeling complex revealed using global approaches. *PLoS Genet.* 7: e1002008. Available at: http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1002008.
30. Cao, R., L. Wang, H. Wang, L. Xia, H. Erdjument-Bromage, P. Tempst, R. S. Jones, and Y. Zhang. 2002. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* 298: 1039–1043.
31. Müller, J., C. M. Hart, N. J. Francis, M. L. Vargas, A. Sengupta, B. Wild, E. L. Miller, M. B. O'Connor, R. E. Kingston, and J. A. Simon. 2002. Histone methyltransferase activity of a *Drosophila* Polycomb group repressor complex. *Cell* 111: 197–208.
32. Pasini, D., A. P. Bracken, M. R. Jensen, E. Lazzerini Denchi, and K. Helin. 2004. Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. *EMBO J.* 23: 4061–4071.
33. Heintzman, N. D., R. K. Stuart, G. Hon, Y. Fu, C. W. Ching, R. D. Hawkins, L. O. Barrera, S. Van Calcar, C. Qu, K. A. Ching, et al. 2007. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat. Genet.* 39: 311–318.
34. Lee, T. I., R. G. Jenner, L. A. Boyer, M. G. Guenther, S. S. Levine, R. M. Kumar, B. Chevalier, S. E. Johnstone, M. F. Cole, K. Isono, et al. 2006. Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* 125: 301–313.
35. Chaiyachati, B. H., A. Jani, Y. Wan, H. Huang, R. Flavell, and T. Chi. 2013. BRG1-mediated immune tolerance: facilitation of Treg activation and partial independence of chromatin remodelling. *EMBO J.* 32: 395–408.
36. Jani, A., M. Wan, J. Zhang, K. Cui, J. Wu, P. Preston-Hurlburt, R. Khatri, K. Zhao, and T. Chi. 2008. A novel genetic strategy reveals unexpected roles of the Swi-Snf-like chromatin-remodeling BAF complex in thymocyte development. *J. Exp. Med.* 205: 2813–2825.
37. Croce, M., A. De Ambrosis, M. V. Corrias, V. Pistoia, M. Occhino, R. Meazza, J. Giron-Michel, B. Azzarone, R. S. Accolla, and S. Ferrini. 2003. Different levels of control prevent interferon-gamma-inducible HLA-class II expression in human neuroblastoma cells. *Oncogene* 22: 7848–7857.
38. Londhe, P., B. Zhu, J. Abraham, C. Keller, and J. Davie. 2012. CIITA is silenced by epigenetic mechanisms that prevent the recruitment of transactivating factors in rhabdomyosarcoma cells. *Int. J. Cancer* 131: E437–E448.
39. Meissner, M., T. L. Whiteside, P. van Kuik-Romein, E. M. Valesky, P. J. van den Elsen, R. Kaufmann, and B. Seliger. 2008. Loss of interferon-gamma inducibility of the MHC class II antigen processing pathway in head and neck cancer: evidence for post-transcriptional as well as epigenetic regulation. *Br. J. Dermatol.* 158: 930–940.
40. Radosevich, M., Z. Song, J. C. Gorga, B. Ksander, and S. J. Ono. 2004. Epigenetic silencing of the CIITA gene and posttranscriptional regulation of class II MHC genes in ocular melanoma cells. *Invest. Ophthalmol. Vis. Sci.* 45: 3185–3195.
41. Radosevich, M., M. Jager, and S. J. Ono. 2007. Inhibition of MHC class II gene expression in uveal melanoma cells is due to methylation of the CIITA gene or an upstream activator. *Exp. Mol. Pathol.* 82: 68–76.
42. Satoh, A., M. Toyota, H. Ikeda, Y. Morimoto, K. Akino, H. Mita, H. Suzuki, Y. Sasaki, T. Kanaseki, Y. Takamura, et al. 2004. Epigenetic inactivation of class II transactivator (CIITA) is associated with the absence of interferon-gamma-induced HLA-DR expression in colorectal and gastric cancer cells. *Oncogene* 23: 8876–8886.
43. Shi, B., A. Vinyals, P. Alia, C. Broceño, F. Chen, M. Adrover, C. Gelpi, J. E. Price, and A. Fabra. 2006. Differential expression of MHC class II molecules in highly metastatic breast cancer cells is mediated by the regulation of the CIITA transcription Implication of CIITA in tumor and metastasis development. *Int. J. Biochem. Cell Biol.* 38: 544–562.
44. van der Stoep, N., P. Biesta, E. Quinten, and P. J. van den Elsen. 2002. Lack of IFN-gamma-mediated induction of the class II transactivator (CIITA) through promoter methylation is predominantly found in developmental tumor cell lines. *Int. J. Cancer* 97: 501–507.
45. Yazawa, T., H. Kamma, M. Fujiwara, M. Matsui, H. Horiguchi, H. Satoh, M. Fujimoto, K. Yokoyama, and T. Ogata. 1999. Lack of class II transactivator causes severe deficiency of HLA-DR expression in small cell lung cancer. *J. Pathol.* 187: 191–199.
46. Steidl, C., S. P. Shah, B. W. Woolcock, L. Rui, M. Kawahara, P. Farinha, N. A. Johnson, Y. Zhao, A. Telenius, S. B. Neriah, et al. 2011. MHC class II transactivator CIITA is a recurrent gene fusion partner in lymphoid cancers. *Nature* 471: 377–381.

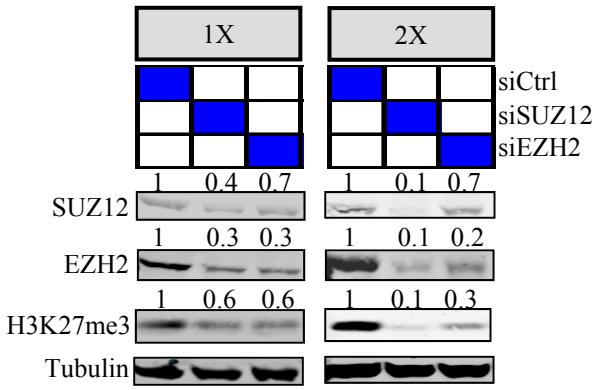


Supp Fig 1. Histone ChIP data from Fig 2 and 3 normalized to H3 ChIP-qPCR analyses at *CIITA* of the indicated factors and chromatin modifications in (**A**) HeLa or (**B**) SW-13 cells 6 or 24 hrs after IFNγ treatment. Marked interactions (*, **, † in **A** and *, **, ***, † in **B**) indicate ≥2-fold significant differences ($P < 0.05$) relative to controls (key). P values were calculated as in Fig 1. Schematic representation also as in Fig 1.

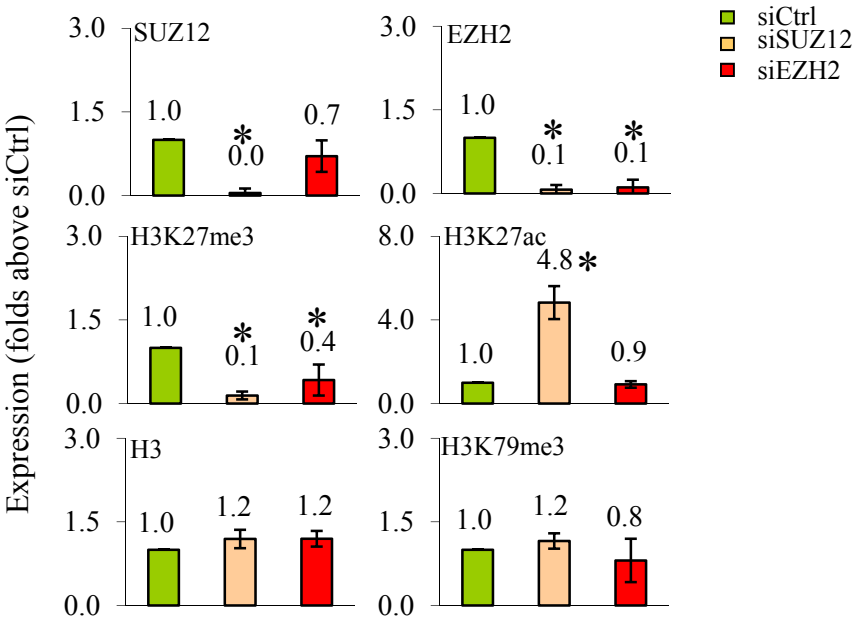


Supp Fig 2. BRG1 does not affect IFNγ-induced STAT1 and IRF1 activation or global PRC2 expression levels or activity. (A) Western blot analyses of the indicated transcription factors, PRC2 subunits or total or modified histone H3 were performed using lysates of SW-13 cells transduced with Ad-GFP or Ad-BRG1 for 24 hrs then left untreated or challenged with IFNγ for 6 hrs. The different treatments are indicated in blue in the table. **(B)** Protein expression was quantified relative to Tubulin and changes in protein levels were expressed as fold above the Ad-GFP minus IFNγ (Ad-GFP-IFNγ) control. Values are mean (n=2-4) +/- S.D. Asterisks indicate a significant increase compared to the Ad-GFP-IFNγ control ($p<0.05$). P values were calculated by ANOVA followed by Fisher test.

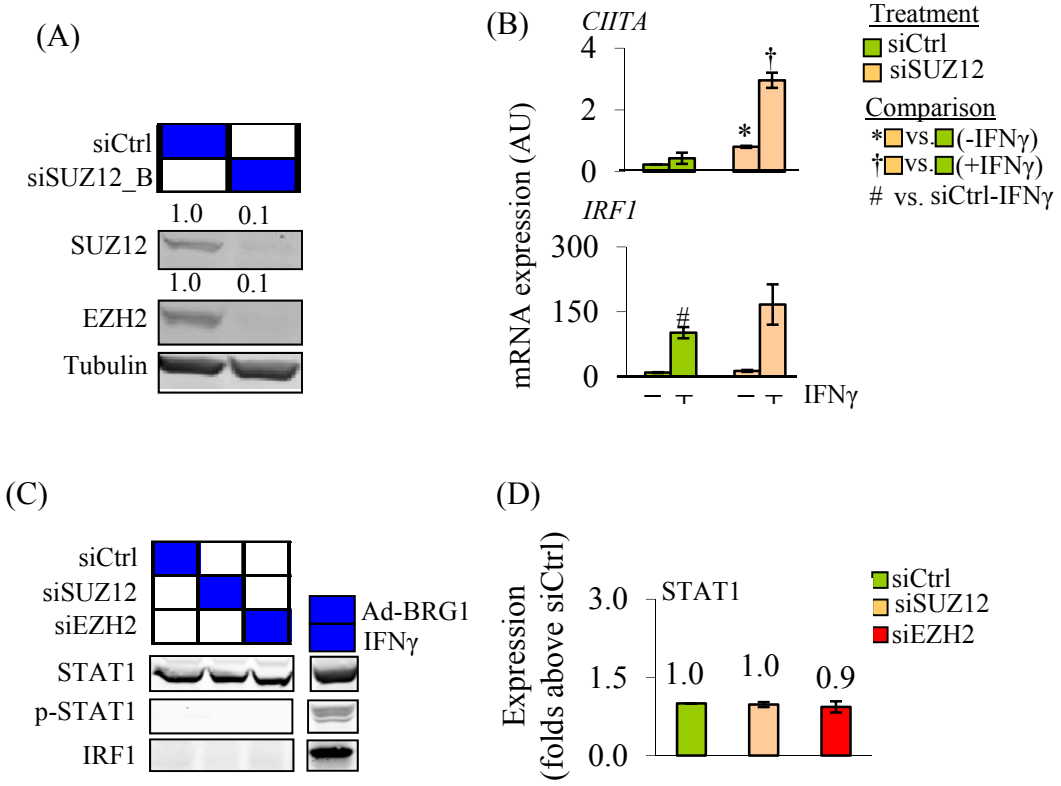
(A)



(B)



Supp Fig 3. SUZ12 Knockdown and its effectson PRC2 and Histones. (A) Representative Western blots of PRC2 subunits and H3K27me3. Analyses were performed using lysates of SW-13 cells after one (1X) or two (2X) cycles of transfection with siCtrl, siSUZ12 or siEZH2 (see Methods). Treatments are indicated in blue. (**B**) Quantification of the blots in **Figure 4A**. Protein levels were quantified relative to Tubulin and changes in expression presented as fold above siCtrl. Values are mean (n=2-4) +/- S.D. Asterisks indicate significant changes compared to siCtrl ($p<0.05$). P values were calculated by ANOVA followed by Fisher test.



Supp Fig 4. Rescue of ISG responsiveness by siSUZ12 is not due to an off target effect or STAT1/IRF1 activation (A) Representative Western blots of PRC2 subunits after knockdown using a second siRNA against SUZ12 (siSUZ12_B). Western blot analyses of SUZ12 and EZH2 was assessed in lysates of SW-13 cells after two cycles of transfection. Tubulin was used as a loading control. Numbers above blots indicate protein expression levels, relative to Tubulin. Changes in gene expression were expressed as fold above siCtrl, which was set to 1. **(B)** Real-time PCR analysis of the indicated genes in SW-13 cells transfected as in **(A)** and left untreated or exposed to IFNγ for 6 hrs. Tables beneath graphs indicate treatments in blue. Gene expression was normalized to Tubulin and presented in arbitrary units (AU). Values are mean ($n \geq 3$) +/- S.D. Marks (#, *, **, †, ‡, and §) indicate significant changes ($P < 0.05$) according to the indicated comparisons (key). P values were calculated by ANOVA followed by Fisher test. (**C**) Western blot analysis of STAT1, p-STAT1, and IRF1 in SW-13 cells transfected with the indicated siRNAs. Lysates of SW-13 cells transduced with AdBRG1 and treated with IFNγ were used as positive control for STAT1 and IRF1 activation. (**D**) STAT1 expression levels expressed as fold above the siCtrl.