IFN-g Induces Histone 3 Lysine 27 Trimethylation in a Small Subset of Promoters to Stably Silence Gene Expression in Human Macrophages

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Graphical Abstract

Highlights
- IFN-γ stably represses select anti-inflammatory genes in human macrophages
- Gene silencing is associated with H3K27me3 at promoters
- IFN-γ induces recruitment of EZH2
- Silenced genes are refractory to induction by anti-inflammatory factors

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In Brief
Qiao et al. demonstrate that IFN-γ stably represses expression of select anti-inflammatory genes in human macrophages by inducing recruitment of EZH2 and deposition of negative histone mark H3K27me3. These results identify a mechanism by which IFN-γ regulates gene expression and provide insights into how macrophage activation is sustained.

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IFN-γ Induces Histone 3 Lysine 27 Trimethylation in a Small Subset of Promoters to Stably Silence Gene Expression in Human Macrophages

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SUMMARY

The mechanisms by which IFN-γ activates expression of interferon-stimulated genes that have inflammatory and host defense functions are well understood. In contrast, little is known about how IFN-γ represses gene expression. By using transcriptomic and epigenomic analysis, we found that stable repression of a small group of genes by IFN-γ is associated with recruitment of the histone methyltransferase EZH2 and deposition of the negative mark histone 3 lysine 27 trimethylation (H3K27me3) at their promoters. Reppressed genes included MERTK, PPARG, and RANK, which have anti-inflammatory functions and promote osteoclast differentiation. Gene repression and H3K27me3 persisted after IFN-γ signaling was terminated, and these silenced genes were no longer responsive to glucocorticoids, IL-4, and M-CSF. These results identify cytokine-induced H3K27 trimethylation as a mechanism that stabilizes gene silencing in macrophages. IFN-γ-induced macrophage activation is thus reinforced by a chromatin-based mechanism that blocks anti-inflammatory and opposing pathways.

INTRODUCTION

Interferon-gamma (IFN-γ) is a key activator of macrophages that induces expression of chemokines and antigen-presenting molecules and primes macrophages for enhanced microbial killing and increased inflammatory cytokine production in response to infectious or inflammatory challenges (Hu and Ivashkiv, 2009). Mechanisms by which IFN-γ activates the Jak-STAT pathway to induce expression of interferon-stimulated genes (ISGs) via binding of STAT1 to promoters and enhancers are well understood (Stark and Darnell, 2012). Since the early days of transcriptional profiling, it has been apparent that IFN-γ also represses gene expression (Ramana et al., 2001; Stark and Darnell, 2012). In contrast to gene activation, little is known about the role of repression in macrophage biology, and about repressive mechanisms. To date, mechanistic explanations of suppression of gene expression by IFN-γ mostly have been limited to inhibition of upstream signaling pathways required to induce gene expression (Hu and Ivashkiv, 2009), and mechanisms underlying direct repression of target genes by IFN-γ have not been elucidated.

Gene repression is mediated by multi-subunit corepressor complexes, negative histone marks, and methylated CpG DNA motifs (Glass and Saijo, 2010). Corepressor complexes are recruited to genes by DNA-binding repressors and contain subunits with enzymatic activity that suppress gene expression by erasing positive histone marks (histone deacetylases, HDACs; histone demethylases), depositing negative histone marks such as H3K9me3 and H3K27me3, and remodeling nucleosomes to close chromatin at regulatory elements. Many histone marks and remodeling of nucleosomes are regulated in a dynamic manner by chromatin regulators with opposing enzymatic activities. Thus, although chromatin-mediated mechanisms can stabilize gene expression to confer “short-term transcriptional memory” (Monticelli and Natoli, 2013), chromatin marks can be erased within minutes and are thus reversible by environmental stimuli. However, the two negative histone marks H3K9me3 and H3K27me3 can be long-lived and stably silence gene expression (Chen and Dent, 2014; Margueron and Reinberg, 2011). H3K9me3 is deposited in areas of heterochromatin that are stably silenced in differentiated cells and maintains repression of retro-elements. H3K27me3 is deposited by Polycomb Repressive Complex (PRC) 2, which is comprised of the histone methyltransferase EZH2 (and in some cell types EZH1), SUZ12, Eed, and the DNA-interacting protein JARID2. PRC2 has been extensively studied in early development, X chromosome inactivation, stem cell pluripotency and differentiation, and cell transformation and is generally considered to promote proliferation and function as an oncogene. Little is known about
the role of PRC2 in terminally differentiated non-proliferating cells and in cells of the immune system. In this study, we investigated whether IFN-\(\gamma\) can suppress gene expression by chromatin-mediated mechanisms, and whether repression of anti-inflammatory genes contributes to IFN-\(\gamma\)-mediated macrophage activation. To maximize physiological relevance for human inflammatory conditions, we used primary human monocytes and macrophages that play a key role in human inflammatory diseases. We found that IFN-\(\gamma\) stably silences a small group of genes including MERTK, PPARG, and RANK; gene silencing is stabilized by maintenance of H3K27me3 on gene promoters. IFN-\(\gamma\) treatment made these genes refractory to activation by the inducers glucocorticoids, IL-4, and M-CSF, respectively. These results identify a mechanism of stable gene repression by IFN-\(\gamma\). As PPARG and MERTK have anti-inflammatory functions (Rothlin et al., 2015; Straus and Glass, 2007) and RANK is essential for differentiation down a non-inflammatory pathway toward osteoclasts (Guerrini and Takayanagi, 2014), these findings suggest that one component of macrophage activation by IFN-\(\gamma\) is epigenetic silencing of select anti-inflammatory pathways.

RESULTS

IFN-\(\gamma\) Regulates H3K27 Modification to Repress Gene Expression

Modification of H3K27 plays a key role in regulation of gene expression; acetylation (H3K27Ac) is associated with gene expression, and trimethylation (H3K27me3) is associated with gene silencing (Chen and Dent, 2014). To test whether IFN-\(\gamma\) could repress gene expression by decreasing H3K27Ac or increasing H3K27me3, we performed chromatin immunoprecipitation sequencing (ChIP-seq) experiments to measure regulation of these marks genome-wide in primary human macrophages, and correlated changes in H3K27 marks with changes in gene expression that were measured by RNA sequencing (RNA-seq). We analyzed ChIP-seq data using ChiPseeker and HOMER (Escoubet-Lozach et al., 2011; Giannopoulou and Elemento, 2011) to detect H3K27Ac- and H3K27me3-enriched regions (peaks) (Figure S1). A small subset of 3,000 loci exhibited both H3K27Ac and H3K27me3 marks (termed bivalent loci) that were enriched in promoters (Figures 1A and S1B, bottom; Figure S1C). As expected (Chen and Dent, 2014), genes with promoter-associated H3K27Ac, but not with H3K27me3, showed the highest...
expression; genes with bivalent promoters were expressed at intermediate levels, and genes with promoter-associated H3K27me3, but not H3K27Ac, were minimally expressed (Figure 1B). These results corroborate previous findings in other cell types (Chen and Dent, 2014) and support the validity of our ChIP-seq data and approach in primary human macrophages.

Next, we investigated the relationship between IFN-γ-induced H3K27 modification and IFN-γ-mediated gene expression. In accord with our recent report (Qiao et al., 2013), stimulation with IFN-γ for 24h resulted in a minimal (4%) change in the total number of H3K27Ac peaks (Figure S1A), but in pervasive changes in H3K27Ac read density: H3K27Ac levels changed in 49% of peaks, and approximately 60% of these peaks underwent more than two fold changes in H3K27Ac read density (Figure 1C). In sharp contrast, only 6% of H3K27me3 peaks were modulated by IFN-γ; H3K27me3 read density was increased by >2-fold at only 1.2% and decreased by >2-fold at 0.1% of peaks (Figure 1C). Thus, regulation of H3K27me3 by IFN-γ was focused on a relatively small number of gene loci. IFN-γ-mediated regulation of H3K27me3 marks at gene promoters correlated highly significantly with gene expression (Figure 1D). These results show a genome-wide correlation of IFN-γ-mediated modulation of H3K27me3 at promoters and gene expression, and suggest changes in H3K27me3 may regulate the IFN-γ response in human macrophages.

**IFN-γ Induces H3K27me3 at Promoters of Select Anti-Inflammatory Genes**

IFN-γ was previously shown to regulate histone acetylation (Qiao et al., 2013; Stark and Darnell, 2012) via STAT-associated histone acetyltransferases, and thus we focused on the finding of H3K27me3 regulation. To evaluate the functional significance of H3K27me3 modulation in the IFN-γ response, we analyzed the functional characteristics of the genes regulated by this mark. This analysis identified a small group of 20 genes whose promoters demonstrated a greater than 2-fold increase H3K27me3 after IFN-γ treatment. 15 of these genes were expressed at baseline, and expression of 13 out of these 15 genes decreased after IFN-γ stimulation (Figure 2A). Another small group of 20 genes showed >2-fold decreased H3K27me3 at their promoters and increased expression after IFN-γ stimulation (Table S1). Modulation of H3K27me3 in more distal regulatory elements was less clearly associated with gene expression and thus we focused on promoters and their immediately downstream genes. Strikingly, the genes whose downregulation by IFN-γ was associated with increased promoter H3K27me3 included genes with clear anti-inflammatory functions: MERTK (mediates effecrosis) (Rothlin et al., 2015), PPARG (suppresses inflammatory gene expression) (Straus and Glass, 2007; Vats et al., 2006), and TNFRSF11A (hereafter termed RANK; the key mediator of differentiation down the alternative osteoclast differentiation pathway that is associated with suppressed inflammatory responses) (Guerrini and Takayanagi, 2014; Mochizuki et al., 2006). We focused on regulation of MERTK, PPARG, and RANK.

We confirmed the IFN-γ-induced H3K27me3 accumulation at MERTK, PPARG, and RANK promoters by using ChIP-qPCR (Figure 2B); conversely, and as specificity control, IFN-γ induced depletion of H3K27me3 at the promoters of SOCS1 and ETV7 genes that were robustly induced by IFN-γ (Figure 2B). As a negative control, H3K27me3 was not detected at the TNF promoter regulated by distinct mechanisms (Ramirez-Carrozzi et al., 2006). Repression of MERTK, PPARG, and RANK expression was confirmed by qPCR (Figure 2C), as was repression of additional genes (Figure S2A). Furthermore, IFN-γ treatment resulted in decreased occupancy of RNA polymerase II (Pol II) at these gene promoters (Figure 2D). Gene tracks showing H3K27Ac and H3K27me3 peaks at the MERTK, PPARG, and RANK promoters are shown in Figure 2E. IFN-γ treatment resulted in H3K27me3 accumulation at all three promoters, without any loss of H3K27Ac at MERTK or PPARG promoters. The SOCS1 and ETV7 genes that were induced by IFN-γ (Figure 2F) exhibited the converse pattern of decreased H3K27me3 after IFN-γ treatment (Figure 2E).

Next, we investigated the kinetics of IFN-γ-induced gene downregulation to address whether genes are first induced and then silenced or whether they are always targeted for silencing. Analysis of MERTK, PPARG, and RANK mRNA after 1, 3, 6, 24, and 48 hr of IFN-γ stimulation revealed no evidence for early gene induction and instead showed downregulation that was first observed at 3 hr and increased progressively over time (Figure 2C). Downregulation of PPARG mRNA occurred with slower kinetics than MERTK or RANK mRNA. Because stability of mRNA transcripts could obscure decreases in transcription at early time points, we also measured primary unspliced transcripts that more directly reflect transcription. Strikingly, MERTK and RANK primary transcripts were substantially decreased after only 1 hr of IFN-γ treatment, whereas PPARG primary transcripts were downregulated with slower kinetics (Figure S2B), in accord with slower kinetics of mRNA downregulation. Examination of all 13 genes that were downregulated concurrent with H3K27me3 accumulation (Figure 2A) revealed that 11/13 genes were progressively downregulated over time, while PLTP and SPRED1 were transiently induced and subsequently downregulated (Figure S2C). We were surprised by the rapid decrease in transcription and investigated the kinetics of H3K27me3 accumulation. After IFN-γ stimulation, increased H3K27me3 was detected at 6 and 24 hr at the RANK promoter, but not until 24 hr at the MERTK and PPARG promoters (Figure S2D). These results suggest that a distinct mechanism suppresses gene expression at early time points, while deposition of H3K27me3 at later time points could serve to stabilize repression or to make genes refractory to inducing stimuli.

**IFN-γ Regulates EZH2 Recruitment to Select Gene Promoters**

The regulation of H3K27me3 by cytokines has not been previously appreciated and could be mediated by changes in EZH2 that deposit this mark or the demethylases JmjD3 and ultra-thorax (UTX) that erase it (Chen and Dent, 2014; Margueron and Reinberg, 2011). We did not detect changes in expression of mRNAs encoding these proteins and thus examined EZH2 recruitment to gene promoters. Strikingly, IFN-γ induced recruitment of EZH2 to MERTK, PPARG and RANK promoters, while decreasing EZH2 occupancy at SOCS1 and ETV7 promoters (Figure 3A). EZH2-containing PRC2 can cooperatively function
Figure 2. IFN-γ Induces H3K27me3 at Promoters of Select Anti-inflammatory Genes Whose Expression Is Downregulated

(A) Heatmap of gene expression (left) and H3K27me3 read density (right) of 13 genes whose promoters showed a >2-fold increase in H3K27me3 associated with a decrease in gene expression.

(B) Confirmation of H3K27me3 regulation using ChIP-qPCR with primers for the TSS region of the indicated genes.

(C) Confirmation of gene expression by qPCR.

(D) Regulation of RNA polymerase II occupancy by ChIP-qPCR. In (B) and (D) IFN-γ-induced differences were statistically significant (p < 0.05, one-tailed t test), except for HBB, for pooled data from independent experiments.

(E) Read density for H3K27me3 and H3K27Ac at the indicated genes in control or IFN-γ-treated macrophages. Gene tracks were visualized using the UCSC genome browser.

(F) Confirmation of gene expression by qPCR. Data are representative of two (A and E) or at least three (B–D and F) experiments.
with PRC1 to silence gene expression; EZH2-deposited H3K27me3 recruits PRC1, which adds another repressive histone mark H2A-K119Ub to further stabilize and reinforce gene repression (Schwartz and Pirrotta, 2013). Consistent with this model, H2AK119-Ub was increased at promoters of genes with increased EZH2 and H3K27me3 and decreased at genes that exhibited lower EZH2 and H3K27me3 after IFN-γ stimulation (Figure 3B). To test the causal role of EZH2 in IFN-γ-mediated gene repression, we knocked down EZH2 expression using antisense locked nucleic acids (LNAs) (Figure 3C). Substantial knockdown of EZH2 (approximately 80% under IFN-γ-stimulated conditions) did not reverse IFN-γ-mediated suppression of RANK (Figure 3D, bar 5 versus bar 6) or of PPARG or MERTK (Figure S3A). These results are consistent with distinct and redundant IFN-γ-activated suppressive mechanisms, possibly related to the early phase suppression described above (Figures S2B–S2D). We then considered the possibility that, instead of inducing suppression, the role of EZH2 could be to stabilize the repressed state and make genes with high H3K27me3 resistant to induction by M2-related cytokines. Supportive of this notion, EZH2 knockdown resulted in increased RANK induction by M-CSF under IFN-γ-stimulated conditions (Figure 3D, bar 7 versus bar 8). However, similar induction of PPARG under IFN-γ-repressed conditions was not observed, and induction of MERTK was not consistently observed in all donors when EZH2 was knocked down (Figure S3A). We obtained similar results using the EZH inhibitor UNC1999 (Figure S3B), although in this case UNC1999 treatment resulted in increased induction by IL-10 of the IFN-γ-silenced gene HSTST1 but did not broadly reverse gene suppression (Figure S3C; data not shown). It is possible that these gene-specific results could be related to incomplete knockdown or inhibition of EZH2, the function of the distinct early-acting suppressive pathway, or stabilization of repression by H2A-K119-Ub (Figure 3B). Collectively, the results suggest that there are likely other players and several repressive pathways that are partially redundant that work together to suppress gene expression.

**IFN-γ Stably Silences Gene Expression**

Macrophage phenotypes are generally considered to be plastic and reversible in response to environmental cues (Gosselin et al., 2016).
and an important question is whether IFN-γ induces stable changes in macrophage gene expression that are resistant to changes in the environment. We therefore tested the stability of IFN-γ-induced H3K27me3 and gene repression in human macrophages, and whether genes silenced by this mechanism became refractory to induction by cytokines that oppose IFN-γ functionally. First, we found that repression of MERTK, PPARG and RANK and the concomitant increase in promoter H3K27me3 was maintained for at least 4 days after addition of one dose of IFN-γ (Figures 4A and 4B), even though canonical IFN-γ-Jak-STAT1 signaling mostly resolves after 2 days (Hu et al., 2005). To further address the question of whether IFN-γ-induced chromatin changes persist after the IFN-γ signal is terminated, we washed out IFN-γ after one day of stimulation. IFN-γ-induced H3K27me3 at MERTK, PPARG and RANK promoters persisted without any detectable decrease for at least 3 days after wash out of IFN-γ (Figure 4C; data not shown). This stability of H3K27me3 was likely explained, at least in part, by maintenance of EZH2 occupancy (Figure S3D). We then tested whether IFN-γ-mediated silencing made genes refractory to activation by their major inducers. MERTK, PPARG, and RANK were activated by the glucocorticoid dexamethasone (dex), IL-4, and M-CSF, respectively (Figure 4D, bar 3 versus bar 1). IFN-γ-induced repression of these genes was not reversed by dex, IL-4, or M-CSF (Figure 4D, bar 4 versus bar 3), which was in accord with maintained high levels of H3K27me3 at these gene promoters (Figure 4E). Similar results were obtained when IFN-γ was washed out for 2 days prior to challenge with dex, IL-4, or M-CSF (Figure 4F). Collectively, these results show that IFN-γ induces stable H3K27me3 chromatin marks that persist well after IFN-γ signaling, which meets one definition of an epigenetic mechanism (Monticelli and Natoli, 2013), and that genes silenced by this stable mechanism are refractory to induction by opposing cytokines.

**DISCUSSION**

In contrast to gene induction in IFN-γ-mediated macrophage activation, the mechanisms and functions of gene repression in IFN-γ responses are poorly understood. In this study, we identified IFN-γ-induced recruitment of EZH2 and associated deposition of the negative histone mark H3K27me3 as a mechanism by which IFN-γ silences gene expression in macrophages. Gene silencing associated with EZH2-H3K27me3 was stable, resistant to challenge by opposing cytokines, and abrogated the expression of a small group of genes with anti-inflammatory functions. Our findings support a model in which IFN-γ engages chromatin-based epigenetic mechanisms to silence select anti-inflammatory pathways in macrophages to achieve and stabilize an activated state.

Previous work analyzing gene repression by cytokines implicated competition between opposing STATs or recruitment of the Sin3a corepressor complex (Hu and Ivashkiv, 2009; Icardi et al., 2012). We identify a distinct mechanism whereby IFN-γ induces recruitment of a different repressor complex containing the histone methylase EZH2. EZH2 has been previously described to bind to and silence expression of the IFN-γ-inducible gene CIITA in uveal melanoma cells, where knockdown of EZH2 resulted in increased IFN-γ inducibility of CIITA (Holling et al., 2007); induction of EZH2 occupancy and H3K27 deposition by IFN-γ has not been previously described. The highly selective recruitment of EZH2 to a small group of gene promoters suggests a specific mechanism that will be investigated in future work.

A kinetic analysis revealed that IFN-γ rapidly suppresses gene transcription, prior to deposition of H3K27me3. This indicates that IFN-γ engages additional mechanisms to induce the initial suppression of the group of target genes where it increases levels of H3K27me3 at a later time point. One possible mechanism is decreased histone acetylation, but it is likely that there are additional suppressive mechanisms at play. These additional mechanisms are sufficient to induce gene suppression, as knockdown or inhibition of EZH2 did not prevent IFN-γ-mediated gene suppression. Our results suggest that instead of inducing gene suppression, IFN-γ-mediated increases in H3K27me3 work together with other repressive mechanisms to stabilize gene silencing over time, and to make genes refractory to induction by opposing M2 cytokines. Such cooperative functions of mechanisms to silence gene expression is in accord with the literature (Schwartz and Pirrotta, 2013).

The functional significance of IFN-γ-mediated gene repression for IFN-γ-induced macrophage activation has not been clarified. Our results suggest that one component of IFN-γ-mediated macrophage activation is H3K27me3-associated stable silencing of genes, such as MERTK and PPARG, that could otherwise promote deactivation of macrophages by opposing or anti-inflammatory environmental factors. Refractoriness of MERTK and PPARG to induction by glucocorticoids and IL-4 compromises the ability of these factors to deactivate macrophages in the face of IFN-γ stimulation. One important function of IFN-γ is to suppress osteoclast differentiation and limit bone resorption in inflammatory settings (Takayanagi et al., 2000). RANK is a key nondendritic receptor required for...
osteoclastogenesis and silencing of RANK to make cells refractory to its ligand RANKL will limit bone erosion (Ji et al., 2009; Lorenzo et al., 2008). Thus, our findings suggest an important role for IFN-γ in stably diverting myeloid precursors from the osteoclast differentiation pathway, which limits bone damage and simultaneously enables cells to polarize in an M1 direction important for host defense.

Our work highlights the importance of gene repression in IFN-γ-mediated macrophage activation. IFN-γ silencing of a small number of anti-inflammatory genes was stabilized via H3K27me3 deposition at their promoters; this can make genes resistant to induction by environmental cues. Future work is needed to identify additional mechanisms of gene repression by IFN-γ and how they relate to macrophage functions important in inflammation and host defense.

EXPERIMENTAL PROCEDURES

Cell Culture
CD14+ human monocytes were purified from peripheral blood mononuclear cells (PBMCs) by positive selection using anti-CD14 magnetic beads (Miltenyi Biotec 130-050-201) using a protocol approved by the Hospital for Special Surgery Institutional Review Board. Monocytes were cultured in RPMI 1640 medium (Invitrogen, cat # 11875119) supplemented with 10% fetal bovine serum (FBS) (HyClone Fisher, cat # SH30031.02) and 10 ng/ml M-CSF (Peprotech, cat # 300-25).

ChiP and ChiP-Seq
ChiP was performed as previously described (Qiao et al., 2013). For regular ChiP assays, immunoprecipitated DNA was analyzed by quantitative real-time PCR and normalized relative to input DNA amount. For ChiP-seq experiments, more than 100 million nonclonal mapped tags were obtained for each condition in each experiment and analyzed using ChIPseeker (Giannopoulou and Elemento, 2011). Peak calling was normalized to input DNA sequencing data with p < 10^-15, fold induction > 2, with FDR < 0.025 for all datasets. Two biological replicates were performed, and scatter plots and Pearson correlation coefficients between peak heights in biological replicates are shown in the Supplemental Experimental Procedures.

RNA-Seq
Sequencing libraries were prepared using TruSeq RNA Library Preparation Kits (Illumina), and more than 40 million non-clonal reads were obtained for each sample. TopHat was used for alignment and Cufflinks was used for gene differential expression analysis.

Western Blotting
Cyttoplasmic, nuclear, or whole-cell extracts were fractionated on 7.5%–10% polyacrylamide gels by SDS-PAGE, transferred to polyvinylidene fluoride membranes (Millipore), and incubated with specific antibodies; enhanced chemiluminescence (Amersham) was used for detection.

Statistical Analysis
Statistical analyses were performed using a one-tailed t test or one-way ANOVA as indicated.

ACKNOWLEDGMENTS
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REFERENCES


Supplemental Information

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Supplemental Inventory

1. Supplemental Figures and Tables
   - Figure S1, related to Figure 1
   - Figure S2, related to Figure 2
   - Figure S3, related to Figure 3
   - Table S1
   - Table S2
   - Table S3

2. Supplemental Experimental Procedures

3. Supplemental References
**Figure S1. Related to Figure 1**

### A

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### B

- **Active**
  - K27Ac
  - K27me3
  - GAPDH
  - ACTB

- **Silenced**
  - K27Ac
  - K27me3
  - SOX1
  - HOX

- **Bivalent**
  - K27Ac
  - K27me3
  - SOCS3
  - IL15RA

### C

- H3K27Ac unique peaks
  - 11
  - 23
  - 35
  - 2

- H3K27me3 unique peaks
  - 36
  - 34
  - 2

- Bivalent peaks
  - Promoter
  - Downstream
  - Exons
  - Introns
  - Distal
  - Intergenic

### D

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<td>PCP pathway</td>
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<tr>
<td>Wnt/β-catenin Signaling</td>
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<tr>
<td>eNOS Signaling</td>
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<tr>
<td>p70S6K Signaling</td>
<td>10^{-4.99}</td>
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<tr>
<td>Nitric Oxide Signaling in the Cardiovascular System</td>
<td>10^{-4.19}</td>
</tr>
<tr>
<td>Virus Entry via Endocytic Pathways</td>
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**K27Ac-K27me3- gene canonical pathways**

- EIF2 Signaling
- Protein Ubiquitination Pathway
- Regulation of eif4 and p70S6K Signaling
- mTOR Signaling
- Superpathway of Inositol Phosphate Compounds
- PI3K/AKT Signaling
- 3-phosphoinositide Biosynthesis
- JAK/Stat Signaling
- ERK/MAPK Signaling
- Assembly of RNA Polymerase II Complex

**K27Ac-K27me3+ gene canonical pathways**

- Axonal Guidance Signaling
- Human Embryonic Stem Cell Pluripotency
- Regulation of the Epithelial-Mesenchymal Transition Pathway
- Hepatic Fibrosis / Hepatic Stellate Cell Activation
- GABA Receptor Signaling
- Neuropathic Pain Signaling in Dorsal Horn Neurons
- Bladder Cancer Signaling
- Wnt/Ca+ pathway
- Adipogenesis pathway
- PCP pathway
- Wnt/β-catenin Signaling
- eNOS Signaling
- p70S6K Signaling
- Nitric Oxide Signaling in the Cardiovascular System
- Virus Entry via Endocytic Pathways
Figure S2. Related to Figure 2

**A** IFNγ-repressed genes with increased K27me3

- PID1
- MTSS1
- PHLDA
- MAPK13

**B** Primary transcript

- MERTK
- PPARG
- RANK

**C** RNA-seq

<table>
<thead>
<tr>
<th>Gene</th>
<th>IFN-γ -</th>
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<th>IFN-γ 24h</th>
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**D** H3K27me3 ChIP

- MERTK
- PPARG
- RANK
- SOCS1
Figure S3. Related to Figure 3

A

PPARG

MERTK

% of GAPDH

0.00

0.05

0.10

0.15

LNA  EZH2  Ctrl  E  C

IFNg  +  +  +  +

MCSF  -  -  3  3h

% of GAPDH

PPARG

MERTK

RANK

DMSO  UNC1999

0

2

4

6

8

10

12

14

16

0 h  3 h  24 h

0

5

10

15

20

25

0 h  3 h  24 h

0

2

4

6

8

10

12

14

16

0 h  3 h  24 h

0

0.5

1

1.5

2

2.5

3

0 h  3 h  24 h

C

UNC1999  M-CSF

3 days  IFN-γ

3 days  M-CSF

3 days  IFN-γ

HS3ST1

% of GAPDH

IFN-γ  -  +  +  +  +

EZHi  -  -  +  -  +

IL-10  -  -  -  +  +

D

EZH2 ChIP

RANK

control

% of input

IFN-γ  +  +  +  +

M-CSF  IL-4  IL-10

IFN-γ  +  +  +  +

M-CSF  IL-4  IL-10
**Figure S1, related to Fig. 1.**

(A) Annotation of H3K27Ac and H3K27me3 peaks in resting and IFN-γ-stimulated human primary monocytes. Promoters: ±2kb from TSS; downstream: ±2kb from TES; Distal: 2kb-50kb upstream of TSS; Intergenic: >50kb from TSS.

(B) Examples of the occupancy of H3K27me3 and H3K27Ac. Top: transcriptionally active locus; middle: silenced locus; bottom: bivalent locus.

(C) Composition of each genomic compartment in each group of peaks calculated by genomic annotation to hg19.

(D) Functional analysis of the three groups of genes with differential H3K27 modification and gene expression levels. Genes in each of the 3 groups shown in Figure 1B (promoters H3K27Ac+/me3-, H3K27Ac+/me3+ or H3K27Ac-/me3+) were analyzed using Ingenuity Pathway Analysis to detect canonical pathways that were significantly enriched in each gene group. Genes associated with different patterns of H3K27 modification fell into distinct functional categories, and as expected promoters of genes expressed in other cell types but not in myeloid cells were enriched for only H3K27me3.

**Figure S2, related to Fig. 2.**

(A) Confirmation of transcriptional regulation of selected genes by real time qPCR normalized to GAPDH. Shown is one of three representative experiments.

(B) qPCR analysis of the indicated primary transcripts, normalized relative to GAPDH, during a time course of IFN-γ stimulation.

(C) Heat map of gene expression of 13 genes at the indicated time points after IFN-γ stimulation.

(D) ChIP-qPCR analysis of H3K27me3 during a time course of IFN-γ stimulation.
Figure S3, related to Fig. 3.

(A) Gene expression after EZH2 knockdown. Cells were cultured with IFN-γ for 3 days and then stimulated with IL-4 (50 ng/ml) or M-CSF (100 ng/ml) for 3 hr. PPARγ results are representative of at least three experiments. MERTK results were variable among different donors and the increased expression in bar 3 versus bar 4 was not observed in all donors.

(B) qPCR analysis of the indicated mRNAs, normalized relative to GAPDH. Cells were stimulated with IFN-γ for 3 hrs or 24 hrs after treatment with vehicle control DMSO or 2 μM UNC1999 for 3 days. Inhibition of EZH2 using UNC1999 did not prevent IFN-γ-mediated gene repression.

(C) HS3ST1 expression in monocytes pretreated with EZH inhibitor (UNC1999) or DMSO for 3 days and cultured with IFN-γ for 3 days and then stimulated with IL-10 (50 ng/ml) for 3 hrs. Although UNC1999 did not prevent IFN-γ-mediated repression of HS3ST1 (bar 3 versus bar 2), it did enable induction of HS3ST1 by IL-10 (bar 5 versus bar 4). Data are representative of 3 experiments.

(D) ChIP assays for EZH2 at the RANK promoter in macrophages cultured with IFN-γ for 24 hr. IFN-γ was then removed and 24 hr later cells were stimulated with indicated factors for 3 hrs. EZH2 occupancy was maintained at the RANK promoter relative to negative control (TNF promoter).
Table S1. List of genes induced by IFN-γ with decreased H3K27me3 around promoters

<table>
<thead>
<tr>
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<th>Gene</th>
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<tbody>
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<td>AKAP2</td>
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<tr>
<td>ARNTL2</td>
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<td>ATF3</td>
<td>IL15RA</td>
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<td>CD38</td>
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<tr>
<td>DUSP5</td>
<td>SOCS3</td>
</tr>
<tr>
<td>ETV7</td>
<td>SOCS1</td>
</tr>
<tr>
<td>FAM20A</td>
<td>MUC1</td>
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<td>GUCY1A3</td>
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### Table S2. Primers for mRNA expression

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<td>CCATCATCTTTGGCGTTTG</td>
<td>AGCTGTGAGTCTTTCCCT</td>
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<tr>
<td>PPARG</td>
<td>GCTGGCCTCTTTGATGAATA</td>
<td>TTGGGCTCATAAAGTCACC</td>
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<tr>
<td>MERTK</td>
<td>CCACAATTTCTTGGGAAAGA</td>
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</tr>
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<td>SOCS1</td>
<td>GACGCCTGCGGATTCTACT</td>
<td>TGCCATCCAGGTGAAAGC</td>
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<tr>
<td>ETV7</td>
<td>CCAAGGGTGAACCATGACCTAC</td>
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### Table S3. Primers for ChIP assay

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<tr>
<td>HBB</td>
<td>GAGGGCTGAGGGGTGAAGT</td>
<td>TGCTCCTGGGAGTAGATTG</td>
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2. Supplemental Experimental Procedures

Cell Culture Reagents

Monocytes were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, Cat # 11875119) supplemented with 10% heat-inactivated defined FBS (HyClone Fisher, Cat # SH30031.02), penicillin/streptomycin (Invitrogen, Cat # 15070-063), L-glutamine (Invitrogen, Cat # 25030-081), and 10 ng/ml human macrophage colony-stimulating factor (M-CSF; Peprotech, Cat # 300-25) in the presence or absence of 100 U/ml human IFN-γ (Roche, Cat # 11040596001) as indicated. In certain experiments, cells were stimulated with 50 ng/ml IL-4 (Peprotech, Cat # 200-04), 50 ng/ml of M-CSF or 1 μM dexamethasone (Sigma-Aldrich, Cat # D1756) for 3 hours.

Chromatin Immunoprecipitation (ChIP) and ChIP-sequencing (ChIP-seq)

Chromatin Immunoprecipitation was performed according to the protocol that was previously described (Qiao et al., 2013). For regular ChIP assays, immunoprecipitated DNA was analyzed by quantitative real-time PCR and normalized relative to input DNA amount. Anti-RNA polymerase II (Cat # sc-899) was from Santa Cruz Biotechnology; H3K27me3 antibody (Cat # 07-449) from Millipore; EZH2 antibody from Active Motif (Cat # 39875), and anti-H3K27Ac (Cat # ab-4729) from Abcam.

For ChIP-seq experiments, 10 ng of co-immunoprecipitated DNA per sample were ligated with adaptors and 100-300 bp DNA fragments were purified to prepare DNA libraries using Illumina ChIP-seq Sample Prep Kit following the manufacturer’s instructions. ChIP libraries were sequenced (50 bp single end reads) using an Illumina HiSeq 2000 Sequencer at the Weill Cornell Medical College Epigenomic Core Facility.
per manufacturer's recommended protocol. Image capture, analysis and base calling were performed using Illumina's CASAVA 1.7. Sequence tags were mapped to the human reference sequence (GRCh37/hg19) using Bowtie (Langmead and Salzberg, 2012) with default parameters and clonal reads were removed from further analysis. More than 100 million nonclonal mapped tags were obtained for each condition in each experiment for analysis. ChIPseeqer was used for peak detection, annotation, comparison between different lists of peaks and peak clustering. Peak calling was normalized to input DNA sequencing data with \( p < 10^{-15} \), fold induction >2. The peaks were defined with minimum length of 500 bp, minimum distances between peaks as 500 bp and minimum peak height as 30 tag counts, and FDR < 0.025 for all data sets. Two biological replicates were performed in the ChIP-seq experiments, and scatter plots and Pearson correlation coefficients between peak heights in biological replicates are shown immediately below.
**RNA-sequencing (RNA-seq)**

CD14⁺ human monocytes were cultured with MCSF (10 ng/ml) in the presence or absence of human IFN-γ (100 U/ml) for 24 hours before harvest and RNA extraction. RNA library was prepared using the TruSeq RNA Library Preparation Kit (Illumina). High throughput sequencing (50 bp, paired end) was performed at the Weill Cornell Medical College Epigenomic Core Facility. More than 40 million non-clonal reads were obtained for each sample. TopHat (Kim et al., 2013) was used for alignment and Cufflinks (Trapnell et al., 2010) was used for gene differential expression analysis. Two biological replicates were performed in the RNA-seq experiments, and scatter plots and Pearson correlation coefficients between peak heights in biological replicates are shown immediately below.

Western Blotting Reagents

Anti-STAT1 (Cat # sc-346) and p38α (Cat# 9212) were from Santa Cruz Biotechnology. Anti-phospho-STAT1 (Tyr701, Cat # 9171S) was from Cell Signaling.
3. Supplemental References

