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2021 L Street NW, Suite 900,  
Washington, DC 20036  
Phone: 202-776-0544 | Fax 202-776-0545  
editorial@hematology.org

## CXXC1-dependent IRF4 activity promotes myeloma cell fitness and sustains lenalidomide resistance

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Pierangela Sabbattini (Hugh & Josseline Langmuir Centre for Myeloma Research, Centre for Haematology, Imperial College London, United Kingdom) Nikolaos Trasanidis (Hugh & Josseline Langmuir Centre for Myeloma Research, Centre for Haematology, Imperial College London, United Kingdom) Jason Taslim (Hugh & Josseline Langmuir Centre for Myeloma Research, Centre for Haematology, Imperial College London, United Kingdom) Harry Whitwell (Department of Metabolism, Digestion and Reproduction, Imperial College London, United Kingdom) Yiming Huang (Hugh & Josseline Langmuir Centre for Myeloma Research, Centre for Haematology, Imperial College London, United Kingdom) Han Leng Ng (Hugh & Josseline Langmuir Centre for Myeloma Research, Centre for Haematology, Imperial College London, United Kingdom) Aristeidis Chaidos (Hugh & Josseline Langmuir Centre for Myeloma Research, Centre for Haematology, Imperial College London, United Kingdom) Katrina Fordwor-Hepburn (Hugh & Josseline Langmuir Centre for Myeloma Research, Centre for Haematology, Imperial College London, United Kingdom) Qizhen Jia (Hugh & Josseline Langmuir Centre for Myeloma Research, Centre for Haematology, Imperial College London, United Kingdom) Valentina Caputo (School of Applied Science, London South Bank University, United Kingdom) Alexia Katsarou (Department of Haematology, Hammersmith Hospital, Imperial College Healthcare National Health Service Trust, United Kingdom) Maria Atta (Centre for Haematology, Department of Immunology and Inflammation, Imperial College London, London, United Kingdom, United Kingdom) Marco Bua (Imperial College Healthcare NHS Foundation Trust,, United Kingdom) Jose Ros-Soto (Hammersmith Hospital, Imperial College Healthcare NHS Trust, United Kingdom) Irene Roberts (Oxford University, United Kingdom) Niklas Feldhahn (Imperial College London, United Kingdom) Nicholas Crump (Imperial College London, United Kingdom) Anastasios Karadimitris (Hugh & Josseline Langmuir Centre for Myeloma Research, Centre for Haematology, Imperial College London, United Kingdom)

### Abstract:

Regulation of oncogenic transcriptional programs in multiple myeloma requires the interplay of histone modifications, their writers and readers with lineage-affiliated transcription factors. The transcription factors IKZF1/3, IRF4 and MYC form an aberrant, myeloma-specific regulatory loop that drives myelomagenesis and resistance to immunomodulatory drugs (IMiDs) such as lenalidomide. Chromatin-based mechanisms that regulate these processes remain incompletely understood. Here we investigate the role of CXXC1, a core component of the H3K4 methyltransferase complex COMPASS, in the activity of the IKZF1/3-IRF4-MYC regulatory loop. We find that clinically, high CXXC1 expression is associated with high-risk proliferative, adverse prognosis disease. Consistent with this, CXXC1 is a myeloma dependency and it regulates cellular fitness programs including cell cycle, MYC targets and DNA damage response. High CXXC1 expression in primary myeloma cells is associated with higher chromatin accessibility, while acute depletion of degron-tagged CXXC1 further validates its role in regulating myeloma cell fitness programs and high-risk transcriptional signatures. CXXC1 interacts with and extensively co-binds to chromatin with IRF4 and IKZF3. Notably, in both lenalidomide-sensitive and -resistant myeloma cells, CXXC1 depletion results in loss of IRF4 and IKZF3 chromatin binding and in parallel it 'breaks' the IRF4 transcriptional self-regulatory loop. Thus, CXXC1 and COMPASS emerge as novel therapeutic targets in IMiD-sensitive and -resistant myeloma by regulating the activity of IRF4 and essential myeloma cell fitness programs.

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**Agreement to Share Publication-Related Data and Data Sharing Statement:** The data supporting this study are available upon reasonable request. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD073121. Raw and processed RNA-seq and ChIP-seq data generated in this study have been deposited to the GEO database under the accession codes GSE318161 and GSE318058. Additional MM patient RNA-seq data were obtained from the MMRF CoMMpass study database (<https://research.themmr.org>), primary MM ATAC-seq data from GSE153381, and primary MM DNA methylation data from the BLUEPRINT Epigenome Project repository ([https://ftp.ebi.ac.uk/pub/databases/blueprint/data/homo\\_sapiens/GRCh38/bone\\_marrow](https://ftp.ebi.ac.uk/pub/databases/blueprint/data/homo_sapiens/GRCh38/bone_marrow)). MAF ChIPseq data were retrieved from GSE165060. Histone mark ChIP-seq data were obtained from the ENCODE Project (accession ENCBS241TLI), and corresponding epigenomic chromatin state (ChromHMM) maps were generated as previously described<sup>45</sup>.

**Clinical trial registration information (if any):**

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Pierangela Sabbattini<sup>1</sup>, Nikolaos Trasanidis<sup>1</sup>, Jason Taslim<sup>1</sup>, Harry Whitwell<sup>2</sup>, Yiming Huang<sup>1</sup>,  
Valentina Caputo<sup>3</sup>, Han Leng Ng<sup>1</sup>, Aristeidis Chaidos<sup>1,4</sup>, Katrina Fordwor-Hepburn<sup>1</sup>, Qizhen  
Jia<sup>1</sup>, Alexia Katsarou<sup>1,4</sup>, Maria Atta<sup>4</sup>, Marco Bua<sup>4</sup>, Jose Ros-Soto<sup>4</sup>, Irene AG Roberts<sup>5</sup>, Niklas  
Feldhahn<sup>1</sup>, Nicholas T Crump<sup>1</sup>, Anastasios Karadimitris<sup>1,4</sup>

<sup>1</sup>Hugh & Josseline Langmuir Centre for Myeloma Research, Centre for Haematology,  
Department of Immunology and Inflammation, Imperial College London, London, United  
Kingdom,

<sup>2</sup>Department of Metabolism, Digestion and Reproduction, Imperial College London, London,  
United Kingdom,

<sup>3</sup>School of Applied and Health Science, London South Bank University, London, United  
Kingdom

<sup>4</sup>Department of Haematology, Hammersmith Hospital, Imperial College Healthcare National  
Health Service Trust, London, United Kingdom

<sup>5</sup>MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine,  
Department of Paediatrics, University of Oxford, Oxford, United Kingdom

## Short title

Role of CXXC1 in Lenalidomide sensitive and resistant myeloma

## Data sharing

Accession codes for the data generated in this study are PXD073121 for proteomics,  
GSE318161 and GSE318058 for genomics

## Correspondence

Anastasios Karadimitris  
Hugh & Josseline Langmuir Centre for Myeloma Research  
Centre for Haematology  
Department of Immunology and Inflammation  
Imperial College London  
Du Cane Road, London W12 0NN, UK  
e-mail: [a.karadimitris@imperial.ac.uk](mailto:a.karadimitris@imperial.ac.uk)

## Abstract

Regulation of oncogenic transcriptional programs in multiple myeloma requires the interplay of histone modifications, their writers and readers with lineage-affiliated transcription factors. The transcription factors IKZF1/3, IRF4 and MYC form an aberrant, myeloma-specific regulatory loop that drives myelomagenesis and resistance to immunomodulatory drugs (IMiDs) such as lenalidomide. Chromatin-based mechanisms that regulate these processes remain incompletely understood. Here we investigate the role of CXXC1, a core component of the H3K4 methyltransferase complex COMPASS, in the activity of the IKZF1/3-IRF4-MYC regulatory loop. We find that clinically, high *CXXC1* expression is associated with high-risk proliferative, adverse prognosis disease. Consistent with this, *CXXC1* is a myeloma dependency and it regulates cellular fitness programs including cell cycle, MYC targets and DNA damage response. High *CXXC1* expression in primary myeloma cells is associated with higher chromatin accessibility, while acute depletion of degron-tagged *CXXC1* further validates its role in regulating myeloma cell fitness programs and high-risk transcriptional signatures. *CXXC1* interacts with and extensively co-binds to chromatin with IRF4 and IKZF3. Notably, in both lenalidomide-sensitive and -resistant myeloma cells, *CXXC1* depletion results in loss of IRF4 and IKZF3 chromatin binding and in parallel it 'breaks' the *IRF4* transcriptional self-regulatory loop. Thus, *CXXC1* and COMPASS emerge as novel therapeutic targets in IMiD-sensitive and -resistant myeloma by regulating the activity of IRF4 and essential myeloma cell fitness programs.

## Key points

High *CXXC1* expression marks high risk, proliferative myeloma

*CXXC1* sustains myeloma fitness transcriptional programs, IRF4 chromatin binding and IMiD resistance

## Introduction

Multiple myeloma (MM) remains a mostly incurable cancer of the bone marrow plasma cells (PC). Myeloma initiation often involves translocation of oncogenes (e.g. *MAF*, *MMSET*, or *CCND1*) giving rise to the asymptomatic condition monoclonal gammopathy of undetermined significance (MGUS)<sup>1-3</sup>. Progression to MM requires secondary genetic events<sup>1-3</sup>, alongside the co-opting of lineage-defining transcription factors (TFs) such as IRF4<sup>4-6</sup>. IRF4 establishes an aberrant feedback loop with MYC, leading to the enhanced expression of both transcription factors<sup>5</sup>. IRF4 constitutes the highest dependency amongst TFs<sup>7,8</sup>, thus remaining a high priority target in MM therapeutics.

IKZF1 and IKZF3 are also PC lineage-defining TFs and major myeloma dependencies. Critically, they underpin the therapeutic effect of immunomodulatory drugs (IMiDs) such as Lenalidomide (Len). Len, by interacting with the cereblon (CRBN) component of the CRL4<sup>CRBN</sup> E3 ubiquitin, promotes proteasomal degradation of IKZF1/IKZF3<sup>9,10</sup>, with consequent reduced expression of their transcriptional target *IRF4* and disruption of the IRF4-MYC feedback loop. IMiD and Len resistance are often a result of loss of *CRBN* expression<sup>11,12</sup>, failure to degrade IKZF1/IKZF3 and preservation of the IRF4-MYC feedback loop (reviewed in<sup>13,14</sup>). Len resistance can also be sustained by TFs such as ETV4<sup>15</sup> and BATF<sup>16</sup>, even when IKZF1/3 are efficiently degraded by Len.

Besides co-option of TFs, histone modifiers are also required to establish the epigenetic states that drive oncogenic gene regulation<sup>17</sup>. An example of this are bromodomain inhibitors that prevent the H3K27 acetyl-transferases CBP/p300 from binding to the *IRF4* super-enhancer, leading to *IRF4* transcriptional downregulation<sup>18</sup>, and trigger a profound anti-myeloma effect with clinical trials now investigating a CBP/p300 inhibitor-IMiD combination ([NCT04068597](#)). However, little is known about the role of H3K4me3 methyltransferases and associated complexes in MM. We previously showed that, relative to normal PCs, CXXC1, a chromatin binding factor and COMPASS H3K4me3 methyltransferase complex component, is predicted to acquire a large number of additional binding sites on the chromatin of myeloma PCs of all genetic subgroups, especially in those with *MAF* translocation<sup>7</sup>. In its physiological role, CXXC1 directly binds unmethylated CpG islands and guides H3K4me3 broad domain deposition mostly at active promoters catalysed by its partners SETD1A or SETD1B<sup>19-23</sup>. CXXC1 has several developmental roles including in hematopoiesis<sup>24</sup> and T cell development<sup>24,25</sup>, while in the B cell lineage it is required for germinal centre (GC) formation and activation of the relevant transcriptional programs<sup>26</sup>. By promoting the BCL6-mediated repression of *Irf4*, CXXC1 inhibits premature exit of B cells from the GC and thus limits the formation of memory B cells and differentiation to plasmablasts<sup>26</sup>.

Here we address the hypothesis that Len-sensitive and -resistant myeloma cells repurpose CXXC1 to regulate their transcriptional state in cooperation with lineage-defining and oncogenic TFs and histone marks.

## Methods

### Cell Lines, degrons and Len-resistant cultures

The MM.1S and H929 myeloma cell lines were used to generate CXXC1-degron clones. Len-resistant cell cultures (LenR) were then isolated by culturing degron-tagged clones in increasing concentration of Len (up to 64  $\mu$ M) for around 12 weeks and then maintained in 64  $\mu$ M Len. Control cell cultures were grown in parallel in DMSO.

Cell cycle, apoptosis, shRNA, ChIP-seq and RNA-seq were performed and analyzed as previously described<sup>6,27</sup>.

Accession codes for the data generated are: PXD073121 for proteomics; GSE318161 and GSE318058 for genomics.

Detailed methods are described in Supplemental Methods.

## Results

### CXXC1 regulates essential transcriptional programs in myeloma

Amongst >1000 CCLE cancer cell lines, CXXC1 expression is highest in myeloma cells (**Suppl Fig 1A**), supporting a relatively selective role in MM compared to other cancers.

We previously showed<sup>7</sup> that shRNA-mediated depletion of CXXC1 in myeloma cell lines with distinct myeloma initiating events (*MAF*-translocated MM1.S and *NSD2*-translocated H929) causes cell death. We extended this analysis and found that shRNA-mediated depletion of *SETD1A* (**Suppl Figure 1B&C**) is also toxic to myeloma cells (**Suppl Figure 1D**), suggesting an essential role of both these COMPASS components in the biology of MM.

Since in our previous work CXXC1 binding was predicted to be higher in *MAF*-translocated myeloma cells<sup>7</sup>, we performed transcriptome analysis in MM1.S cells. We found that shRNA-mediated CXXC1 depletion resulted in downregulation and upregulation of 1281 and 1035 genes, respectively, while upon *SETD1A* depletion, 1958 genes were downregulated and 1661 were upregulated (**Figure 1A & Table S1**). Gene set enrichment analysis (GSEA) of 472 genes downregulated by both CXXC1 and *SETD1A* depletion identified cell cycle genes (E2F targets, G2-M checkpoint), MYC targets, stress response functions (DNA damage, unfolded protein response) and mTORC1 signalling as the most enriched (**Figure 1B & Suppl Figure 1E**). Upregulated enriched pathways included interferon response and TNF $\alpha$  signalling-related inflammatory response (**Figure 1B**).

Consistent with these findings, cells with CXXC1 and *SETD1A* knock-down showed increased DNA damage, as assessed by H2AXS139ph staining (**Figure 1C&D**), rapid induction of cell cycle arrest in *SETD1A*-depleted (**Figure 1E&F**) and apoptosis in both *SETD1A*- and CXXC1-depleted myeloma cells (**Figure 1G&H**).

Similar to MM1.S cells, shRNA-mediated depletion of CXXC1 and *SETD1A* in H929 myeloma cells was associated with induction of apoptosis and cell cycle arrest (**Suppl Fig 1F&G**); transcriptome and GSEA analyses of differentially expressed genes showed enrichment of

downregulated genes for cell cycle, MYC targets and mTORC1 signalling pathways (**Suppl Figure 1H&I**).

We conclude that *CXXC1* and *SETD1A* are myeloma dependencies and promote cellular fitness and oncogenic programs in myeloma PCs.

### **Impact of *CXXC1* expression on disease risk, biology, outcome and chromatin accessibility**

We sought to explore the significance of *CXXC1* expression in a clinical context using patient datasets from the CoMMpass study (NCT01454297).

Genes with positive correlation with *CXXC1* expression ( $r > 0.3$ ; **Figure 2A & Table S1**), were significantly enriched for E2F targets, DNA repair, G2-M checkpoint and MYC targets (**Figure 2B**), mirroring pathways enriched upon depletion of *CXXC1*. Supporting a role of *CXXC1* in myeloma cell proliferation, its expression modestly but significantly correlated with the CoMMpass PC proliferation index<sup>28</sup> and UAMS70 gene high-risk transcriptional signature<sup>29</sup>, which is enriched in proliferative genes (**Figure 2C**). Moreover, expression of *CXXC1* positively and significantly correlated with high-risk genetic features including chr1q21 gain/amplification and *RB1* loss while it negatively correlated with hyperdiploidy and MYC amplification (**Figure 2D**).

We then evaluated the prognostic relevance of *CXXC1* expression (**Suppl Figure 2A, Suppl Table S2.C**). In a univariate Cox model, high *CXXC1* expression was associated with shorter overall survival (OS) (hazard ratio (HR)=1.37;  $p=8 \times 10^{-4}$ ; **Figure 2E**) but had no impact on progression free survival (PFS; HR=1.12,  $p=0.13$ ; **Suppl Figure 2B**). Association with OS became more pronounced in a multivariate model (adjusted HR=1.65,  $p=1 \times 10^{-5}$ , **Figure 2E**) after accounting for established clinical and cytogenetic risk factors<sup>30</sup>. Of note, in the multivariate model, *CXXC1* expression was also significantly associated with shorter PFS (HR=1.23,  $p=0.02$ , **Suppl Figure 2B**).

Upon cross-validation, the multivariate model including *CXXC1* achieved strong discrimination, with a mean of time-dependent area under the curve (AUC) of 0.737 and a C-index of 0.715, both substantially higher than those of the univariate model (AUC: 0.554; C-index: 0.554) (**Figure 2F**). For progression-free survival (PFS), predictive performance was generally lower (AUC: 0.687 vs 0.540; C-index: 0.643 vs 0.527; **Suppl Figure 2C**). Survival analysis of the independent Arkansas dataset<sup>48</sup> confirmed that higher expression of *CXXC1* is associated with worse survival (**Suppl Figure 2D**).

Of note, in the IMiD-treated subgroup of the CoMMpass cohort, higher *CXXC1* expression remained significantly associated with OS and PFS in both univariate and multivariable analyses (**Suppl Table S2.D, Suppl Figure 2E-H**), with larger effect sizes than those observed in the full cohort. The univariate association with PFS, which was not significant in the full cohort, became significant in the IMiD-treated subgroup, supporting a stronger prognostic relevance of *CXXC1* in this clinically relevant treatment context.

Further analysis of another three independent gene expression datasets showed that progression from MGUS to MM is associated with significantly higher *CXXC1* expression (**Suppl Figure 2I**).

Therefore, high *CXXC1* expression is associated with progression from MGUS to MM and proliferative, high risk, adverse prognosis myeloma and is a strong, independent predictor of OS, including in IMiD-treated patients.

To explore how *CXXC1* expression impacts chromatin states, we compared the transcriptomes and chromatin accessibility of primary myeloma cells with high and low *CXXC1* expression (n=5 patients each), using normal PC (NPC) as reference, from our previously described cohort<sup>7</sup> (**Figure 2G & Table S3**). Comparison of transcriptomes (**Table S3**) showed that, in line with the findings of *CXXC1* knockdown (KD), genes and pathways promoting cell cycle were highly upregulated and enriched in *CXXC1*<sup>high</sup> cells (**Suppl Figure 2J&K**). *CXXC1*<sup>high</sup> myeloma PCs collectively had a higher number of myeloma-specific accessible peaks, i.e., not present in normal PC, than *CXXC1*<sup>low</sup> cells (~39,000 vs 31,000 peaks respectively, **Figure 2H**), suggesting higher *CXXC1* expression is associated with a more open chromatin. When accessibility was overlapped with *CXXC1* binding peaks in MM1.S myeloma cells (see below), the overlap was >5 fold greater in *CXXC1*<sup>high</sup> than in *CXXC1*<sup>low</sup> myeloma PC (odds ratio: 6.16, p-value< 2.2x10<sup>-16</sup>), mostly distributed at intronic and intergenic areas (**Figure 2H**). These *CXXC1*-binding accessible peaks showed a significant enrichment for IRF motifs (including IRF4) in *CXXC1*<sup>high</sup> but not *CXXC1*<sup>low</sup> myeloma PC, suggesting co-binding with IRF4 (**Figure 2I**). These findings are consistent with *CXXC1* dose-dependent effects on chromatin accessibility in primary myeloma cells and suggest the ability of *CXXC1* to regulate transcription of oncogenic programs through co-operation with IRF4.

#### **Chromatin proteomics reveal *CXXC1* interaction with the PC lineage-defining TFs**

To obtain unbiased insights into the on-chromatin protein partnerships of *CXXC1*, we performed immunoprecipitation (IP) of endogenous *CXXC1* and *SETD1A* in MM1.S nuclear extracts followed by Liquid Chromatography-Mass Spectrometry (LC-MS).

We identified 1086 and 1044 proteins enriched (FDR <0.05) in *CXXC1* and *SETD1A* IPs respectively (**Figure 3A, Suppl Figure 3A and Suppl Table S4**).

As expected, among the *CXXC1* and *SETD1A* interactors, we identified several COMPASS components (**Figure 3A&E and Suppl Figure 3A**). Key TFs including IRF4, IKZF1 and IKZF3 and the RUNX family RUNX1 and RUNX3, but not MAF, also showed interaction with *CXXC1*. GO analysis revealed significant enrichment of proteins associated with chromatin functions (**Figure 3B**).

In co-IP/western blot assays, IP with anti-*SETD1A* and -IRF4 antibodies immunoprecipitated *CXXC1* (**Figure 3C and Suppl Figure 3B**) and, reciprocally, IP against *CXXC1* pulled down IRF4 and IKZF3/IKZF1 (**Figure 3D and Suppl Figure 3B**), further validating their individual interactions with *CXXC1*.

Among the several chromatin complexes with important transcriptional roles enriched by *CXXC1* and *SETD1A* IP were the Mediator complex<sup>31</sup>, the chromatin remodellers INO80<sup>32</sup> and SWI/SNF<sup>49</sup> and the NuA4<sup>33</sup> and Sin3<sup>34</sup> chromatin regulator complexes. *CXXC1* but not *SETD1A* IPs were enriched for the cleavage and polyadenylation factor group of proteins that are known to be involved in mRNA processing and cleavage<sup>35</sup> (**Figure 3E**). Co-IP/WB assays confirmed the interaction of *CXXC1* with MED1 (**Suppl Figure 3C**) and the cleavage and polyadenylation factor group proteins CPSF1-3 (**Suppl Figure 3D**).

## Chromatin co-binding of CXXC1 with lineage-affiliated TFs and MAF

Using ChIP-seq in MM1.S cells, we mapped the genome-wide binding profiles of CXXC1 and SETD1A and integrated them with binding of MED1, RNA Polymerase II, EZH2 and several histone marks. We identified 45,535 CXXC1 binding sites which mostly overlap with transcriptionally active chromatin and with limited overlap with repressive chromatin (**Figure 4A**). SETD1A ChIP-seq detected a smaller number of peaks mainly overlapping with the ~10% most robust CXXC1 peaks, possibly reflecting the ability of CXXC1 but not of SETD1A to directly bind DNA, and/or differences in the Ab affinities or epitope accessibility (**Figure 4A**). ChromHMM annotation showed that CXXC1 binds to active enhancers and promoters (**Figure 4B**). Moreover, we identified 615 and 773 CXXC1-bound neo-promoters and neo-enhancers respectively (i.e., not active in mature B and normal PCs, **Figure 4C**), suggesting that CXXC1 participates in conferring myeloma-specific regulatory potential in malignant PC.

Integration of CXXC1 ChIP-seq with the transcriptome of CXXC1 knock-down cells by BETA<sup>36</sup>, identified 686 and 553 genes that are predicted to be directly bound and activated or repressed, respectively, by CXXC1 (**Figure 4D & Suppl Table S5**). GSEA confirmed that CXXC1-activated genes are more highly and significantly enriched than CXXC1-repressed genes in cell cycle, E2F1, MYC targets and DNA repair pathways (**Figure 4E**).

Similarly, SETD1A is predicted to activate 743 genes enriched for MYC and E2F targets, cell cycle, mTORC1 signalling, unfolded protein response and DNA Repair (**Suppl Figure 4A&B; Table S5**).

CXXC1 footprints in newly accessible chromatin are particularly pronounced in MAF-translocated myeloma<sup>7</sup>. Overlaying of MAF and CXXC1 ChIP-seq peaks showed considerable overlap, with 25% of CXXC1 binding sites overlapping with MAF binding, corresponding to 65% of all MAF binding sites (**Figure 4F&G**). We identified 34 genes (24 activated, 10 repressed) and regulatory regions that are co-occupied and co-regulated by MAF and CXXC1; (**Suppl Figure 4C & Suppl Table S5**). Amongst these was the neo-enhancer of the cell-migration *CCR1* gene which we previously showed is activated by oncogenic MAF<sup>6</sup> (**Suppl Figure 4C&D**). We found co-binding of CXXC1, IRF4 and MAF at accessibility peaks that were identified as significant for *CCR1* transcriptional regulation by Peaks-to-Genes (P2G) analysis of primary myeloma cells subjected to paired ATAC-seq and RNA-seq analyses<sup>7</sup> (**Suppl Figure 4D**).

Comparison of the IRF4, IKZF1/3 and CXXC1 cistromes found that nearly all CXXC1 binding sites were co-occupied by IRF4, while 70% and 40% were co-occupied by IKZF1 and IKZF3, respectively. A higher fraction of all IKZF3 than IKZF1 peaks (40% vs 25% of all) co-bound with CXXC1, while >50% of IRF4 binding sites were co-bound by CXXC1 (**Figure 4F&G**). The IRF4, IKZF1/3 and CXXC1 co-bound regions showed higher chromatin accessibility in primary myeloma PC expressing higher CXXC1 levels (**Figure 4F**).

Using TOPmentation<sup>37</sup>, we extended these studies to myeloma PC from two patients (PT0511 with t(11;14) and UPN108 with hyperdiploid MM; **Figure 4H & Suppl Figure 4E**). Among the CXXC1 BETA targets, we found that CXXC1 occupied 69% of CXXC1 BETA regions and 85% of BETA genes in PT0511, and 49% regions and 80% genes in UPN108, with variable IKZF3 and IRF4 co-binding and presence of active chromatin marks (H3K27ac, H3K4me3).

GSEA of BETA activated genes bound by CXXC1 in the patient myeloma PC showed significant enrichment for E2F-, MYC-targets and DNA repair (**Suppl Figure 4F**).

### **Degron-mediated depletion of CXXC1 reveals dual chromatin function of CXXC1**

For inducible degradation of CXXC1 and functional analysis upon acute loss of CXXC1, we generated two FKBP12<sup>F36V</sup>- and HA-tagged CXXC1-degion clones (M1&M2) in MM1.S cells<sup>38</sup> (**Figure 5A**). Degron-tagged endogenous CXXC1 was expressed at a level similar to the endogenous native protein of the parental cells, while treatment with the degion inducer dTAG-V1<sup>38</sup> resulted in loss of total CXXC1 as early as 4hrs (**Figure 5B and Suppl Figure 5A**). In competition assays, while co-cultured parental MM1.S cells survived after dTAG-V1 treatment, M1&M2 cells were selectively depleted (**Figure 5C&D**).

Transcriptome analysis of M1 and M2 cells showed hundreds of genes up- and down-regulated at 24 hours, and >1,000 up- and down-regulated genes at 60 hours post CXXC1 depletion (**Figure 5E & Table S6**). GSEA of downregulated genes in CXXC1-depleted cells highlighted enrichment for cell cycle, MYC-dependent transcription, DNA repair, mTORC1 signalling and unfolded protein response (**Figure 5F**). CXXC1 depletion resulted in cell cycle arrest of both M1 and M2 cells (**Suppl Figure 5B**).

Chromatin analysis of M1 revealed ~45,000 CXXC1 binding peaks, mostly distributed between promoters and enhancers (**Figure 5G**). While significant loss of CXXC1 binding to chromatin was observed upon dTAG-V1-induced CXXC1 depletion, this was not complete at 60hrs, even with nearly full loss of total CXXC1 protein detection, suggesting that chromatin-bound CXXC1 may not be as susceptible to degion-induced depletion. To further explore this, we carried out ChIP-seq with anti-HA Ab and identified only 4,441 significant CXXC1 peaks, suggesting possible lower affinity or accessibility of the HA epitope for the antibody (**Suppl Figure 5C**). Nearly all of these peaks were also identified by the anti-CXXC1 Ab and were severely depleted upon dTAG-V1 treatment. Notably, these anti-HA peaks showed extensive SETD1A co-binding and corresponded to the strongest CXXC1 Ab peaks and unlike peaks identified by the CXXC1 Ab, lacked IRF4 and IKZF1/3 co-binding, were highly enriched in unmethylated CpG islands (**Suppl Figure 5C**) and were devoid of IRF4 motifs (**Suppl Figure 5D**).

Therefore, in addition to its reported function at unmethylated CpG islands<sup>19-21</sup>, CXXC1 also serves to regulate gene transcription in myeloma cells through co-binding with TFs.

Chromatin profiling of H3K4me3, the histone mark deposited by COMPASS<sup>40</sup>, notably showed no significant differences between DMSO control and dTAG-V1-treated myeloma cells (**Suppl Figure 5E**) except for broad domains (BDs) (>4 kb) linked to transcriptional activation of lineage identity genes<sup>23</sup> (**Figure 5H&I**). Indeed, BD loss was found at PC lineage-defining genes such as *IRF4*, *PRDM1*, *XBP1* but less so at *IKZF1/3* (**Figure 5J**). There were only limited changes in H3K27ac distribution upon CXXC1 depletion (**Suppl Figure 5F**).

### **CXXC1-dependent IRF4 transcription and chromatin binding**

To explore the impact of CXXC1 in Len resistance, we generated Len-resistant derivatives (LenR) from clones M1 and M2 (Len IC50 565 and 587  $\mu$ M respectively) and compared them

with their Len-sensitive (LenS) counterparts, showing sensitive range<sup>15,16,41</sup> (Len IC50 0.049 and 0.017  $\mu$ M; **Figure 6A**). As expected with Len treatment, expression of IKZF3 and of its feed-forward loop partners IRF4 and MYC decreased; by contrast, expression remained robust for all three TFs in LenR cells (**Suppl Figure 6A&B**). Despite these differences, dTag-V1 treatment resulted in death of LenS and LenR degons at a similar rate, suggesting that myeloma cells were dependent on CXXC1 for survival regardless of Len sensitivity (**Figure 6B&C**).

Mechanistically, CXXC1 depletion in LenS M1 cells reduced expression of MYC and IRF4, but not IKZF3. In LenR M1, Len-resistance was consistent with loss of expression of CRBN resulting in sustained expression of IKZF3, while CXXC1 depletion decreased expression of MYC but not IRF4 or IKZF3 (**Figure 6D**). mRNA expression broadly mirrored this pattern (**Suppl Figure 6C**).

Crucially, ChIP-seq analysis showed that overall binding of IRF4 to chromatin in CXXC1-depleted cells was significantly impaired in both LenS and LenR cells (**Figure 6E**). Thus, IRF4 binding to chromatin is CXXC1-dependent, even when IRF4 expression is not altered upon CXXC1 depletion.

CXXC1 depletion moderately but significantly reduced the genome-wide binding of IKZF3 in LenR but not in Len S cells (**Figure 6E**).

In CXXC1-depleted and -replete LenS and LenR M2 cells, IKZF3 and MYC protein expression mirrored what was observed in M1 cells, although changes at the mRNA level were less apparent (**Figure 6G** and **Suppl Figure 6D**). However, *IRF4* mRNA and protein levels decreased in both LenS and LenR M2 cells (**Figure 6G** and **Suppl Figure 6D**). At the chromatin level, this was reflected by significant reduction of both IRF4 and IKZF3 binding in CXXC1-depleted LenS and LenR cells, showing CXXC1-dependence of IRF4 and IKZF3 chromatin binding in two distinct Len-resistant derivatives (**Figure 6H**).

In parental M1 cells, depletion of CXXC1 was also associated with significant chromatin-wide reduction in IKZF1 and MYC binding (**Suppl Figure 6E**).

We tested the transcriptional effects of CXXC1 depletion in LenS and LenR M1/M2 cells by BETA-integrating the corresponding transcriptome changes with the CXXC1 ChIP-seq profile. GSEA of BETA genes in LenS and LenR M1 and M2 cells confirmed higher enrichment of E2F1- and MYC-targets, mTORC1 signalling and unfolded response amongst the downregulated genes and interferon signatures among the upregulated (**Figure 6F&I** & **Suppl Table S7**). Downregulation of several of these CXXC1-activated BETA genes in CXXC1-depleted M1/M2 cells was also validated by reverse transcriptase-qPCR (**Suppl Figure 6F**). Analysis of the *IRF4* promoter and super-enhancer in LenS & LenR M1 and M2 cells showed that, upon CXXC1 reduction, IRF4 binding was markedly diminished, whereas IKZF3 binding was not or only mildly affected (**Figure 6J**). Accessibility peaks that were identified as significant for IRF4 transcriptional regulation by P2G analysis of primary myeloma cells were extensively co-bound by CXXC1, IRF4 and IKZF3 (**Figure 6J**).

These findings are consistent with a feed-forward transcriptional loop whereby IRF4 sustains its own transcription through CXXC1-dependent co-binding.

To confirm this, we performed bidirectional CXXC1-IRF4 ChIP-re-ChIP at three sites of the IRF4 super-enhancer. This showed simultaneous binding of both factors at the IRF4 super-enhancer, thus further supporting CXXC1-IRF4 interaction and co-binding (**Suppl Figure 6G**).

Amongst several other genes relevant to myeloma cell fitness that are predicted to be regulated by CXXC1 marked by loss of IRF4 and/or IKZF3 upon CXXC1 depletion (**Suppl Figure 6H&I**) was *APOBEC3B*, a protein that underpins genomic instability and high-risk myeloma in the context of MAF-translocated and other forms of MM<sup>42,43</sup>.

Together, these findings demonstrate a CXXC1-dependent activity of IRF4 and IKZF3 and co-regulation of transcriptional programs required for Len response and resistance.

To investigate the role of CXXC1 in Len resistance in a different genetic background, we generated LenR CXXC1-degron-tagged H929 cells (clone H1-LenR; Len IC50 384  $\mu$ M) (**Suppl Figure 7A&B**) by growth in Len. Parental LenS H929 cells (IC50 0.57  $\mu$ M) responded to Len with reduction of MYC and IKZF3 but not IRF4 expression, while H1-LenR cells retained IRF4 and MYC but not IKZF3 expression (**Suppl Figure 7C**). CXXC1 depletion resulted in loss of H1-LenR cells in co-culture with H929 cells (**Suppl Figure 7D**). Transcriptome analysis of H1-LenR upon CXXC1-depletion confirmed that CXXC1 regulates myeloma fitness programs such cell cycle and MYC targets (**Suppl Figure 7E&F & Suppl Table S7**). Consistently, cell cycle in CXXC1-depleted cells was arrested with significant reduction of G2/M cells, coinciding with increase of subG0/1 apoptotic cells (**Suppl Figure 7G**). Notably, H1-LenR cells preserved CRBN expression despite exposure to Len and responded with reduced expression of MYC but not of IRF4 upon CXXC1 depletion (**Suppl Figure 7H**). ChIP-seq in H1-LenR cells showed extensive chromatin co-binding of CXXC1 and IRF4. Induction of CXXC1 depletion resulted in significant loss of IRF4 chromatin binding parallel to the loss of CXXC1 binding (**Suppl Figure 7I**). BETA integration of the CXXC1 cistrome of H1-LenR myeloma cells upon CXXC1 depletion with the corresponding transcriptomes, confirmed that CXXC1 activates cell cycle, E2F, MYC targets and mTORC1 signalling genes (**Suppl Figure 7J**).

Together, these findings corroborate in different genetic backgrounds the CXXC1-dependent IRF4 chromatin binding and regulation of myelomagenic transcriptional programs in Len-resistant cells, independent of cereblon expression status.

## Discussion

We identified CXXC1 as a key enabler of the IRF4-dependent oncogenic gene regulatory networks that control myeloma cell proliferation and fitness. Importantly for myeloma treatment, our data showed that targeting CXXC1 can overcome IMiD resistance by directly dysregulating IRF4, providing a promising strategy for relapsed/refractory disease.

High CXXC1 expression marks high-risk genetic features, proliferative transcriptional signatures and adversely impacts patient survival, thus supporting its importance in the biology of high-risk myeloma. Mechanistically, these effects of CXXC1 are linked to its ability to directly regulate expression of genes involved in essential cellular functions in myeloma PC and to instruct chromatin binding of IRF4 and IKZF3.

Changes in H3K4me3 upon CXXC1 depletion affect mostly H3K4me3 BDs at PC lineage-defining genes and not in total H3K4me3 chromatin modification, consistent with previous reports for COMPASS<sup>23</sup>. Similarly, in GC B cells, CXXC1 deletion does not impact overall H3K4me3 chromatin levels except for loss of BDs at genes related to B lineage and mitotic cell cycle<sup>26</sup>. These limited H3K4me3 changes in the absence of CXXC1 likely reflect redundancy between the COMPASS and the KMT2A/B histone methyl-transferase activities<sup>23</sup>.

Beyond the previously reported recruitment of the glucocorticoid receptor by SETD1A to chromatin in response to dexamethasone<sup>44</sup>, little is known on the interaction of CXXC1 and COMPASS with TFs. Our co-IP-MS approach revealed interaction of CXXC1 with the PC lineage-defining TFs IKZF3 and IRF4. These interactions were corroborated at the chromatin level by finding extensive co-binding of all three factors and by demonstrating that CXXC1 depletion disrupts co-binding of IRF4 and IKZF1/IKZF3 and dysregulates cell proliferation and fitness transcriptional programs. These functions are not limited to CXXC1 binding to CpG islands and the ensuing deposition of H3K4me3. Indeed, the combinatorial analysis of CXXC1, IKZF3, IRF4 and histone marks allowed the delineation of two patterns of CXXC1-dependent chromatin regulation. The majority of the CXXC1-bound regions are co-bound by these DNA binding factors, whereas a second set, which includes only ~10% of CXXC1 binding sites, is characterised by high level of SETD1A co-occupancy and exclusion of IRF4 and IKZF3. This latter pattern is enriched in chromatin regions of unmethylated CpG islands and is consistent with the role of CXXC1 and COMPASS in protecting CpG islands from repressive DNA methylation<sup>19,20</sup>. Similar patterns of CXXC1 chromatin binding were previously reported in early B cell progenitors showing a segregated pattern of CXXC1 chromatin binding whereby B lineage-defining transcription factors co-bind with CXXC1 outside the unmethylated CpG islands<sup>39</sup>.

Given the extensive CXXC1-IKZF1/IKZF3-IRF4 interplay and the critical role of these TFs in the regulation of IMiD response, we extended our investigation into the role of CXXC1 in the context of Len resistance, a clinical outcome that eventually affects most IMiD-treated patients<sup>13,14</sup>. CXXC1 depletion resulted in significant loss of IKZF3 binding to chromatin despite preserved expression of the latter in LenR cells. Importantly, for IRF4, CXXC1 depletion provided evidence of two levels of CXXC1-IRF4 interaction. One that entails CXXC1-dependent co-binding of IRF4 with CXXC1 on chromatin irrespective of changes in IRF4 expression; and another, in which CXXC1, by co-binding with IRF4 to the *IRF4* super-enhancer and promoter, directly regulates *IRF4* transcription and thus indirectly decreases IRF4 binding to chromatin. This is consistent with a CXXC1-dependent, self-regulation of *IRF4* expression in a feed-forward loop.

Despite the distinct CXXC1-IRF4-IKZF3 interplay in LenR vs LenR myeloma cells, CXXC1 depletion results in transcriptional downregulation of genes regulating myeloma cell fitness and IMiD resistance, including those for cell cycle, MYC-signaling, DNA repair and proteostasis.

Further supporting the notion that CXXC1 regulates transcriptional programs that overlap with those activated by IMiDs, genes directly repressed by CXXC1 in both LenS and LenR myeloma cells are enriched in IFN pathway response<sup>15,16</sup>. We also note the ability of CXXC1

to directly regulate expression of *APOBEC3B*, responsible for mutational signatures that underpin genomic instability and high-risk myeloma<sup>42,43</sup>.

In conclusion, CXXC1 is required for IRF4 and IKZF3 activity and together regulate genes critical for cell fitness in IMiD-resistant as well as -sensitive myeloma cells. Since direct or indirect targeting of IRF4 remains one of the highest priorities in MM therapeutics, our findings showing that IRF4 activity is CXXC1-dependent provides a strong rationale for therapeutic targeting of CXXC1 itself and the COMPASS complex more broadly in the context of IMiD-resistant as well as -sensitive myeloma. Supported by the observation that, while CXXC1 promotes the proliferative GC B cell reaction, it is dispensable for normal PC differentiation<sup>26</sup>, such an approach could follow the principles of therapeutic targeting of the oncogenic KMT2A methyltransferase complex in acute myeloid leukaemia by Menin inhibitors<sup>45</sup> and would complement emerging epigenetic and transcriptional approaches of IRF4 therapeutics that target CBP/p300 histone acetyl-transferases<sup>18,46</sup> and the SWI/SNF family of chromatin remodelling complexes<sup>47</sup>.

## References

1. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. *Nat Rev Cancer*. 2012;12(5):335-348.
2. Palumbo A, Anderson K. Multiple myeloma. *N Engl J Med*. 2011;364(11):1046-1060.
3. Smith D, Yong K. Multiple myeloma. *BMJ*. 2013;346:f3863.
4. Klein U, Casola S, Cattoretti G, et al. Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. *Nat Immunol*. 2006;7(7):773-782.
5. Shaffer AL, Emre NC, Lamy L, et al. IRF4 addiction in multiple myeloma. *Nature*. 2008;454(7201):226-231.
6. Katsarou A, Trasanidis N, Ponnusamy K, et al. MAF functions as a pioneer transcription factor that initiates and sustains myelomagenesis. *Blood Adv*. 2023;7(21):6395-6410.
7. Alvarez-Benayas J, Trasanidis N, Katsarou A, et al. Chromatin-based, in cis and in trans regulatory rewiring underpins distinct oncogenic transcriptomes in multiple myeloma. *Nat Commun*. 2021;12(1):5450.
8. de Matos Simoes R, Shirasaki R, Downey-Kopyscinski SL, et al. Genome-scale functional genomics identify genes preferentially essential for multiple myeloma cells compared to other neoplasias. *Nat Cancer*. 2023;4(5):754-773.
9. Kronke J, Udeshi ND, Narla A, et al. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. *Science*. 2014;343(6168):301-305.
10. Lu G, Middleton RE, Sun H, et al. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. *Science*. 2014;343(6168):305-309.
11. Gooding S, Ansari-Pour N, Towfic F, et al. Multiple cereblon genetic changes are associated with acquired resistance to lenalidomide or pomalidomide in multiple myeloma. *Blood*. 2021;137(2):232-237.
12. Zhu YX, Braggio E, Shi CX, et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. *Blood*. 2011;118(18):4771-4779.
13. Bird S, Pawlyn C. IMiD resistance in multiple myeloma: current understanding of the underpinning biology and clinical impact. *Blood*. 2023;142(2):131-140.

14. Teoh PJ, Koh MY, Mitsiades C, Gooding S, Chng WJ. Resistance to immunomodulatory drugs in multiple myeloma: the cereblon pathway and beyond. *Haematologica*. 2025;110(5):1074-1091.
15. Neri P, Barwick BG, Jung D, et al. ETV4-Dependent Transcriptional Plasticity Maintains MYC Expression and Results in IMiD Resistance in Multiple Myeloma. *Blood Cancer Discov*. 2024;5(1):56-73.
16. Welsh SJ, Barwick BG, Meermeier EW, et al. Transcriptional Heterogeneity Overcomes Super-Enhancer Disrupting Drug Combinations in Multiple Myeloma. *Blood Cancer Discov*. 2024;5(1):34-55.
17. Xiong S, Zhou J, Chng WJ. Deciphering the dynamics of histone acetylation and chromatin remodeling in multiple myeloma: a tale beyond the tails. *Blood*. 2025;146(13):1550-1560.
18. Nicosia L, Spencer GJ, Brooks N, et al. Therapeutic targeting of EP300/CBP by bromodomain inhibition in hematologic malignancies. *Cancer Cell*. 2023;41(12):2136-2153 e2113.
19. Clouaire T, Webb S, Skene P, et al. Cfp1 integrates both CpG content and gene activity for accurate H3K4me3 deposition in embryonic stem cells. *Genes Dev*. 2012;26(15):1714-1728.
20. Thomson JP, Skene PJ, Selfridge J, et al. CpG islands influence chromatin structure via the CpG-binding protein Cfp1. *Nature*. 2010;464(7291):1082-1086.
21. Hughes AL, Kelley JR, Klose RJ. Understanding the interplay between CpG island-associated gene promoters and H3K4 methylation. *Biochim Biophys Acta Gene Regul Mech*. 2020;1863(8):194567.
22. Park S, Kim GW, Kwon SH, Lee JS. Broad domains of histone H3 lysine 4 trimethylation in transcriptional regulation and disease. *FEBS J*. 2020;287(14):2891-2902.
23. Sze CC, Ozark PA, Cao K, et al. Coordinated regulation of cellular identity-associated H3K4me3 breadth by the COMPASS family. *Sci Adv*. 2020;6(26):eaaz4764.
24. Cao W, Guo J, Wen X, et al. CXXC finger protein 1 is critical for T-cell intrathymic development through regulating H3K4 trimethylation. *Nat Commun*. 2016;7:11687.
25. Kiuchi M, Onodera A, Kokubo K, et al. The Cxxc1 subunit of the Trithorax complex directs epigenetic licensing of CD4+ T cell differentiation. *J Exp Med*. 2021;218(4).
26. Zhao Y, Gong S, Yang Y, et al. CFP1 promotes germinal center affinity maturation and restrains memory B cell differentiation through H3K4me3 modulation. *Nat Commun*. 2025;16(1):8013.
27. Trasanidis N, Katsarou A, Ponnusamy K, et al. Systems medicine dissection of chr1q-amp reveals a novel PBX1-FOXO1 axis for targeted therapy in multiple myeloma. *Blood*. 2022;139(13):1939-1953.
28. Skerget S, Penaherrera D, Chari A, et al. Comprehensive molecular profiling of multiple myeloma identifies refined copy number and expression subtypes. *Nat Genet*. 2024;56(9):1878-1889.
29. Shaughnessy JD, Jr., Zhan F, Burington BE, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. *Blood*. 2007;109(6):2276-2284.
30. Avet-Loiseau H, Davies FE, Samur MK, et al. International Myeloma Society/International Myeloma Working Group Consensus Recommendations on the Definition of High-Risk Multiple Myeloma. *J Clin Oncol*. 2025;43(24):2739-2751.

31. Richter WF, Nayak S, Iwasa J, Taatjes DJ. The Mediator complex as a master regulator of transcription by RNA polymerase II. *Nat Rev Mol Cell Biol.* 2022;23(11):732-749.
32. Bowman GD. Mechanistic insights into INO80-type chromatin remodelers. *Curr Opin Struct Biol.* 2025;92:103030.
33. Xue Y, Wong J, Moreno GT, Young MK, Cote J, Wang W. NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. *Mol Cell.* 1998;2(6):851-861.
34. Adams GE, Chandru A, Cowley SM. Co-repressor, co-activator and general transcription factor: the many faces of the Sin3 histone deacetylase (HDAC) complex. *Biochem J.* 2018;475(24):3921-3932.
35. Liu L, Manley JL. Modulation of diverse biological processes by CPSF, the master regulator of mRNA 3' ends. *RNA.* 2024;30(9):1122-1140.
36. Wang S, Sun H, Ma J, et al. Target analysis by integration of transcriptome and ChIP-seq data with BETA. *Nat Protoc.* 2013;8(12):2502-2515.
37. Crump NT, Smith AL, Godfrey L, et al. MLL-AF4 cooperates with PAF1 and FACT to drive high-density enhancer interactions in leukemia. *Nat Commun.* 2023;14(1):5208.
38. Nabet B, Roberts JM, Buckley DL, et al. The dTAG system for immediate and target-specific protein degradation. *Nat Chem Biol.* 2018;14(5):431-441.
39. Bai W, Yang Y, Zhao Y, et al. CFP1-mediated H3K4me3 broad domains control early B cell lineage fate determination. *Nucleic Acids Res.* 2026;54(4).
40. Shilatifard A. The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. *Annu Rev Biochem.* 2012;81:65-95.
41. Chrisochoidou Y, Scarpino A, Morales S, et al. Evaluating the impact of CRBN mutations on response to immunomodulatory drugs and novel cereblon E3 ligase modulators in myeloma. *Blood.* 2025;145(22):2630-2644.
42. Grasedieck S, Panahi A, Jarvis MC, et al. Redefining high risk multiple myeloma with an APOBEC/Inflammation-based classifier. *Leukemia.* 2024;38(5):1172-1177.
43. Walker BA, Wardell CP, Murison A, et al. APOBEC family mutational signatures are associated with poor prognosis translocations in multiple myeloma. *Nat Commun.* 2015;6:6997.
44. Greulich F, Wierer M, Mechtidou A, Gonzalez-Garcia O, Uhlenhaut NH. The glucocorticoid receptor recruits the COMPASS complex to regulate inflammatory transcription at macrophage enhancers. *Cell Rep.* 2021;34(6):108742.
45. Thakur RK, Wang ES. The promise of menin inhibitors: from approval to triplet regimens. *Hematology Am Soc Hematol Educ Program.* 2025;2025(1):599-606.
46. Lenoir WF, McKeown MR, Giorgetti G, et al. Catalytic Inhibition of p300 Preferentially Targets IRF4 Oncogenic Activity and Tumor Growth in Multiple Myeloma. *Cancer Res.* 2025.
47. Bolonsky A, Ceribelli M, Scheich S, et al. IRF4 requires ARID1A to establish plasma cell identity in multiple myeloma. *Cancer Cell.* 2024;42(7):1185-1201 e1114.
48. Zhan, F. et al. The molecular classification of multiple myeloma. *Blood* 108, 2020–2028 (2006).
49. Brownlee PM, et al. The SWI/SNF chromatin remodelling complex: Its role in maintaining genome stability and preventing tumourigenesis. *DNA Repair (Amst)* 32:127-133. (2015).

## Contributions

PS designed and performed experiments, analysed data, wrote manuscript, NT performed computational analysis, wrote manuscript, JT performed experiments, HW performed MS and data analysis, YH performed data analysis, HN performed data analysis, KFH and QJ performed experiments, AC, VC, AK, MA, MB and JRS provided patient samples, IAG co-wrote manuscript, NF performed data analysis, NC supervised research and performed data interpretation, AK designed and supervised the overall research, performed data analysis and wrote the manuscript.

All authors read and approved the final draft of the manuscript.

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## Declaration of Interests

NT is currently employed by GSK. The authors declare no other relevant competing interests.

## Figure Legends

### Figure 1. CXXC1 regulates essential transcriptional programs in myeloma

**A.** Heatmaps of differentially expressed genes identified by RNA-seq (n=2, biological replicates per condition) upon shRNA-mediated depletion of CXXC1 and SETD1A in MM1.S cells for three days. Right heatmap shows DEG shared by CXXC1 and SETD1A-depleted cells (Log2F change > |1|; padj < 0.05)

**B.** GSEA of shared up and downregulated genes from A.

**C.** Immunofluorescence assay to assess DNA damage response after staining for DAPI and with anti-H2AXS139ph Ab of MM1.S cells three days after their treatment with CXXC1- and SETD1A- targeting shRNA or scramble control. Scale bar, 5µm.

**D.** Cumulative data of C (n=3-4; mean ± SEM is shown, t test against scrb control).

**E.** Flow-cytometric analysis of cell cycle of MM1.S cells (day 4) after propidium iodide staining using the indicated shRNAs and scramble controls.

**F.** Cumulative data of E (n=3, mean ± SEM is shown, one way-Anova).

**G.** Flow-cytometric analysis of apoptosis of MM1.S cells after propidium iodide and Annexin V staining (day 4) using the indicated shRNAs and scramble controls.

**H.** Cumulative data of G showing Annexin V+PI+ cells (n=3, mean ± SEM is shown, t test against scrb control).

\*p<0.05, \*\*p<0.01, \*\*\*\*p<0.0001

### Figure 2. CXXC1 expression correlates with chromatin accessibility and high risk, adverse prognosis proliferative myeloma

**A.** Correlation of *CXXC1* expression with other genes expressed in primary myeloma cells. Data from the MMRF CoMMpass (n= 789 patients with myeloma plasma cell RNA-seq).

**B.** GSEA of positively correlated genes ( $r>0.3$ ; red coloured in A).

**C.** Scatterplots showing correlation of *CXXC1* expression with transcriptome-based proliferation index (top) and the gene expression signature-based UAMS-70 Proliferation Score (bottom), as retrieved from MMRF CoMMpass.

**D.** Correlation of *CXXC1* expression with different myeloma genetic subgroups as retrieved from MMRF CoMMpass.

**E.** Kaplan–Meier curves showing OS probability over a 10-year time scale across *CXXC1* expression quantiles in univariate (top) and multivariate (middle) Cox proportional hazards models. The multivariate model adjusts for sex, age, ISS stage, LDH, and high-risk cytogenetic alterations (NSD2 and MAF translocations, 1q21 gain, 17p13 and 1p22 deletions). Wald p-values and hazard ratios (HRs) with 95% confidence intervals are shown. *CXXC1* mRNA expression range for each quantile is shown at the bottom.

**F.** Cross-validated performance of OS prediction models including *CXXC1*. Time-dependent area under the curve (AUC; top graph) and the concordance index (C-index; bottom graph) with 95% confidence intervals across folds are shown for both univariate with multivariate models.

**G.** mRNA expression of *CXXC1* in *CXXC1* low and high primary myeloma cells (n=5 each) and normal PC (n=3).

**H.** Stepwise identification of myeloma-specific (i.e. not present in normal plasma cells) chromatin accessible sites in *CXXC1*-high and -low myeloma plasma cells and fraction of myeloma-specific ATAC-seq peaks bound by *CXXC1* in *CXXC1* high and low cells (Fisher's exact test). In addition, promoter vs enhancer distribution of *CXXC1*-bound, myeloma-specific accessible sites is shown. MM and NPC data derived from reference 7.

**I.** Motif analysis and enrichment of *CXXC1*-bound ATAC-seq peaks in *CXXC1*-high and -low myeloma cells.

### **Figure 3. Chromatin protein interactome reveals novel interactions of *CXXC1* with IRF4 and IKZF3**

**A.** Volcano plot of proteins differentially and significantly enriched after immunoprecipitation with *CXXC1* and in relation to IgG control upon MS analysis (FDR<0.05). Proteins of interest are highlighted.

**B.** Ontology enrichment over-representation analysis of proteins that are significantly enriched upon *CXXC1* immunoprecipitation.

**C.** *CXXC1* interaction with indicated proteins. Cell lysates were first immunoprecipitated with anti-*CXXC1*, -SETD1A, -IRF4 or IgG control Abs and detected by western blot with anti-*CXXC1* Ab.

**D.** IRF4, IKZF3 and IKZF1 interaction with *CXXC1*. Cell lysates were first immunoprecipitated with anti-*CXXC1* or IgG control Abs and detected by western blot with anti-IRF4, -IKZF3 or -IKZF1 Abs.

**E.** Heatmap of normalised values of proteins (see Methods) identified by MS as enriched after immunoprecipitation with anti-*CXXC1*, -SETD1A or IgG control Abs. Protein members of the Mediator, INO80, Sin3, Nu4A, mRNA cleavage and COMPASS complexes are shown. For normalisation of protein abundance values, the coefficient of variation (SD/mean) of proteins present in all four replicates samples was calculated. Among these proteins,

proteins with coefficient of variation <60% were classified as background, the sum of their intensity was calculated, and all protein spectra were divided by this value and then Log2 transformed.

#### **Figure 4. Chromatin co-binding of CXXC1 with lineage-affiliated TF and oncogenic MAF**

**A.** Heatmap of chromatin binding of CXXC1 and SETD1A and other indicated chromatin factors and histone modifications as assessed by ChIP-seq in MM1.S myeloma cell line. Top metagene graphs illustrate binding of chromatin factors and histone enrichment in regions bound solely by CXXC1 or SETD1A or by both.

**B.** Heatmap of indicated epigenomic states of chromatin regions bound solely by CXXC1 or SETD1A or by both.

**C.** Heatmap of CXXC1 chromatin binding to promoter and enhancer regions that are *de novo* activated in myeloma cells and predominantly inactive across different B-cell development states (Blueprint chromHMM states, as per reference 6).

**D.** Schematic of the principle of BETA analysis and number of genes predicted to be directly regulated, activated or repressed by CXXC1 by BETA analysis.

**E.** GSEA of genes predicted to be activated or repressed by CXXC1 by BETA.

**F.** Heatmap of the indicated transcription and chromatin factors co-binding with CXXC1 centred on CXXC1. Heatmaps of ATAC-seq from patient myeloma plasma cells with high and low expression of CXXC1 are shown.

**G.** Top: Fraction of CXXC1 ChIP-seq peaks showing co-binding with indicated TF (dark blue). Bottom: Fraction of each transcription and chromatin factor binding peaks co-bound by CXXC1 (dark blue). Overall number of peaks for each factor is also shown.

**H.** Venn diagrams showing binding of CXXC1 in patient myeloma PC at chromatin regions bound by CXXC1 in MM1.S cells (left) and are predicted to directly regulate genes (right) in MM1.S cells. The primary genetic event for patient PT0511 is t(11;14) and for patient UPN108 is hyperdiploidy.

#### **Figure 5. Probing the role of CXXC1 on chromatin activity upon its degron-dependent depletion in myeloma cells**

**A.** Schematic of the degron system for CXXC1 protein depletion.

**B.** Western blot using anti-CXXC1 Ab of parental MM1.S cells and of M1 and M2 degron-tagged myeloma cells with dTag-V1-induced CXXC1 depletion at different time points. Vinculin is shown as loading control.

**C&D.** Flow-cytometric analysis (left) and cumulative data (right) of cell viability over 12 days of a 1:1 co-culture of parental MM1.S cells and mNeon-Green-expressing M1 and M2 cells upon treatment with DMSO control or dTag-V1 (n=3 independent experiments).

**E&F.** Volcano plot and shared differentially expressed genes in M1&M2 cells before and at 24 and 60hrs post-degron induction (E). GSEA at 60hrs (F).

**G.** Heatmap showing CXXC1, chromatin binding in parental MM1.S cells and M1 degron-tagged cells 60hrs after DMSO or dTag-V1 treatment. Bar on the right shows promoters and enhancers as assessed by chromHMM. Corresponding metagene profiles are shown on top of heatmaps.

**H.** Heatmap of H3K4me3 broad domains (BDs) (>4kb) with and without degron induction and CXXC1 depletion at 60hrs.

**I.** Comparison of normalised ChIP-seq signal of H3K4me3 broad domains with and without degron induction as shown in J.

J. IGV browser snapshots of examples of H3K4me3 broad domains at regulatory areas of plasma cell lineage affiliated transcription factors. H3K27ac and CXXC1 binding are also shown.

# **Figure 6. CXXC1-dependent IRF4 transcription and binding to chromatin and regulation of lenalidomide resistance**

**A.** IC50 estimation of LenS and LenR M1 and M2 myeloma cells. Cells were exposed to varying concentrations of Lenalidomide for 5 days and viability was assessed by CellGlo assay.

**B&C.** Viability of LenS and LenR M1 and M2 myeloma cells over time as assessed by flow cytometry upon treatment with DMSO or dTAGV1.

**D.** Expression of MYC, IRF4, IKZF3, CRBN and CXXC1 in M1 LenS and LenR myeloma cells upon treatment with DMSO or dTag-V1 for 60 hrs as assessed by Western blot. Duplicate assays are shown.

**E.** Heatmaps of IRF4 and IKZF3 chromatin binding upon treatment with DMSO or dTAGV1 for 60hrs of M1 LenS and R cells. Heatmap of CXXC1 is from Fig 4F. Metagene profiles are also shown on top of heatmaps. Significant differences in factor binding are shown at the bottom of heatmaps. \*\*\*,  $p < 0.0001$

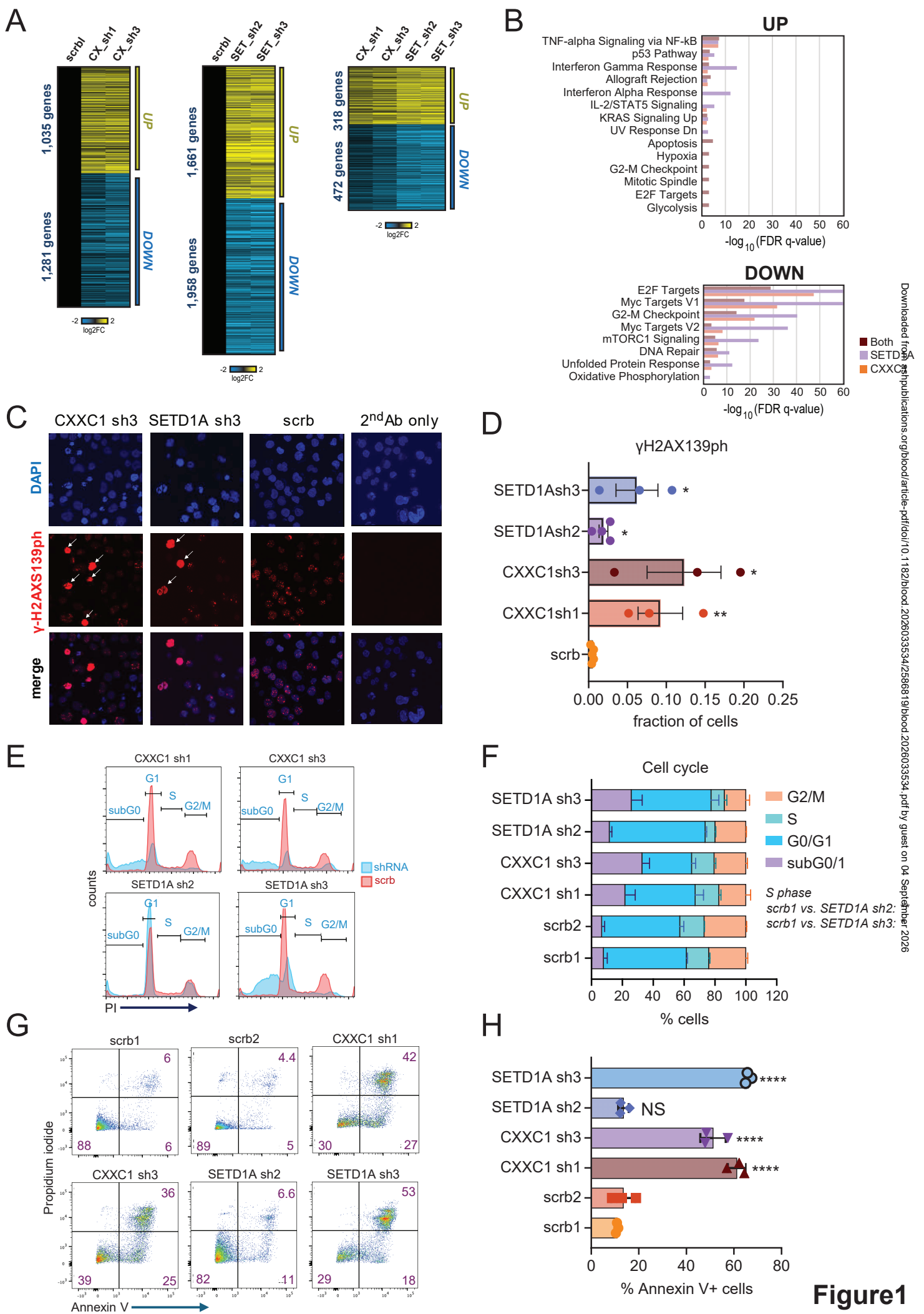
**F.** GSEA of genes identified by BETA as bound by CXXC1 (ChIP-seq) and differentially expressed (RNA-seq) upon CXXC1 depletion in M1 cells.

**G.** Expression of MYC, IRF4, IKZF3, CRBN and CXXC1 in M2 LenS and LenR myeloma cells upon treatment with DMSO or dTAGV1 for 60hrs as assessed by Western blot. Duplicate assays are shown.

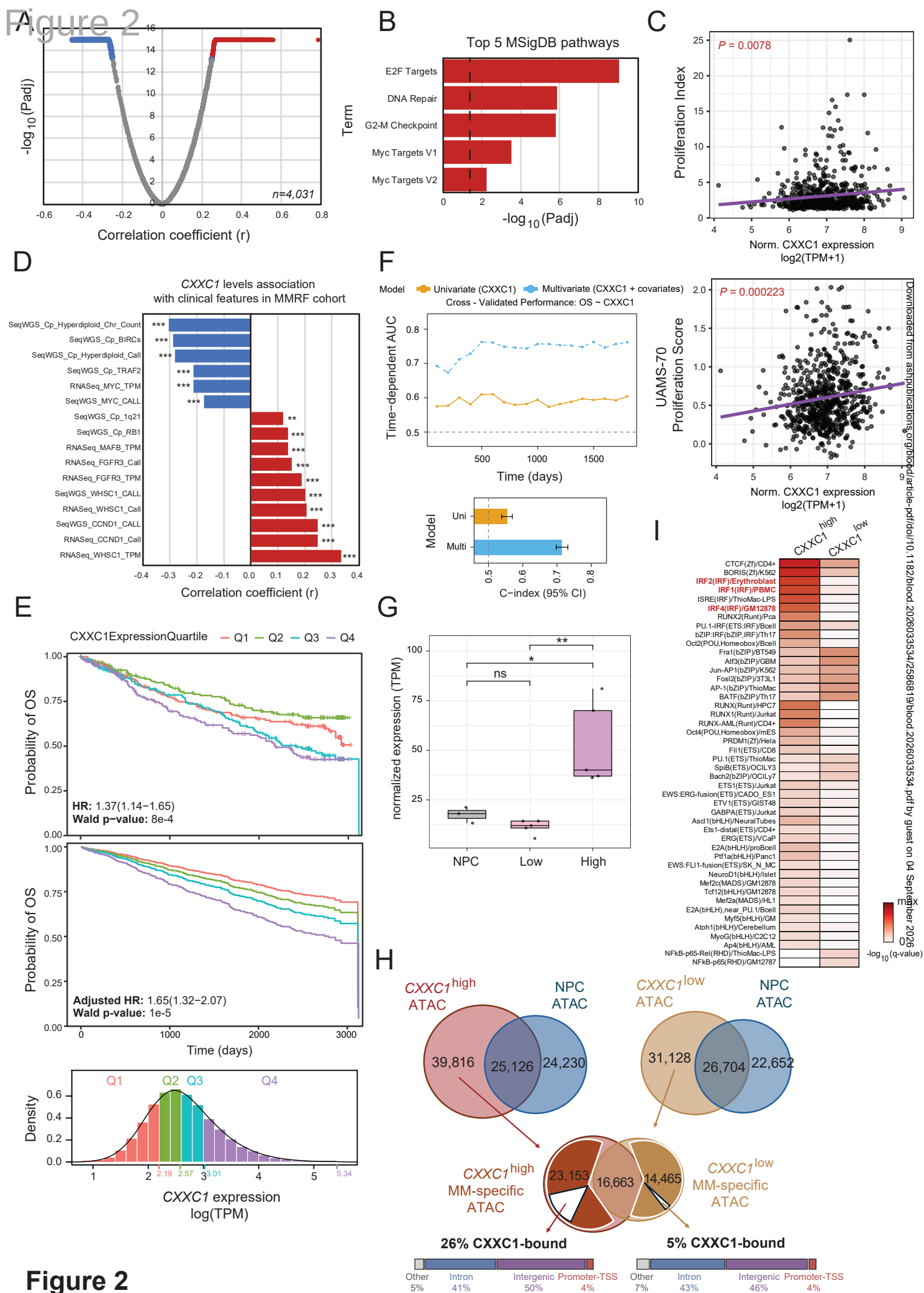
**H.** Heatmaps of IRF4 and IKZF3 chromatin binding upon treatment with DMSO or dTAGV1 for 60hrs of M2 cells. Heatmap of CXXC1 is from Fig 4F. Metagene profiles are also shown on top of heatmaps. Significant differences in factor binding are shown at the bottom of heatmaps. \*\*\*,  $p < 0.0001$

**I.** GSEA of genes identified by BETA as bound by CXXC1 (ChIP-seq) and differentially expressed (RNA-seq) upon CXXC1 depletion in M2 cells.

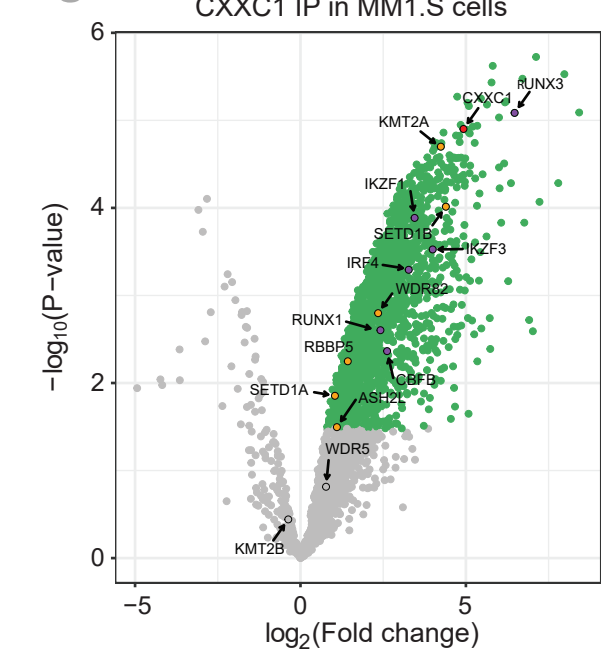
**J.** IGV browser snapshot of *IRF4* and its enhancer showing CXXC1, IRF4 and IKZF3 binding and corresponding changes upon CXXC1 depletion for 60hrs. H3K4me3 and H3K27ac enrichment and ChromHMM states of chromatin are also shown. Significant peak-to-gene (P2G) linkage associations ( $p < 0.05$ ,  $\rho > 0.5$ ) identified in primary MM ATAC-RNA-seq samples are shown at the bottom.



**Figure1**

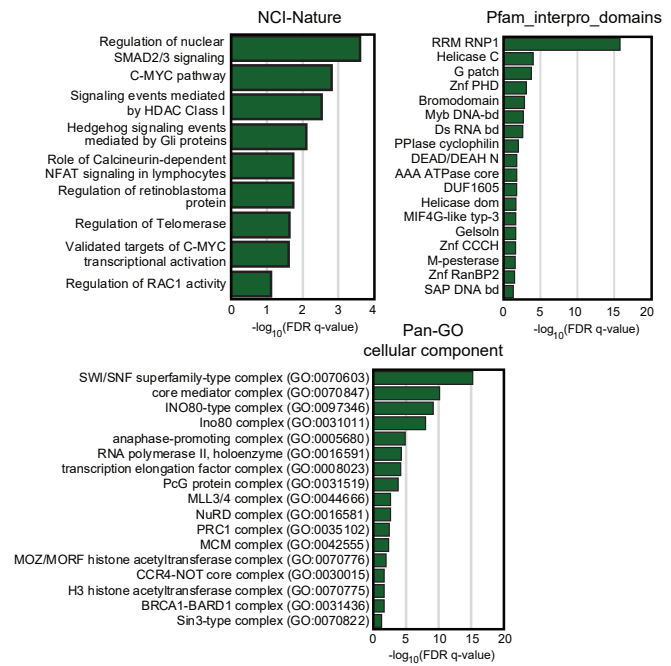


**Figure 3**



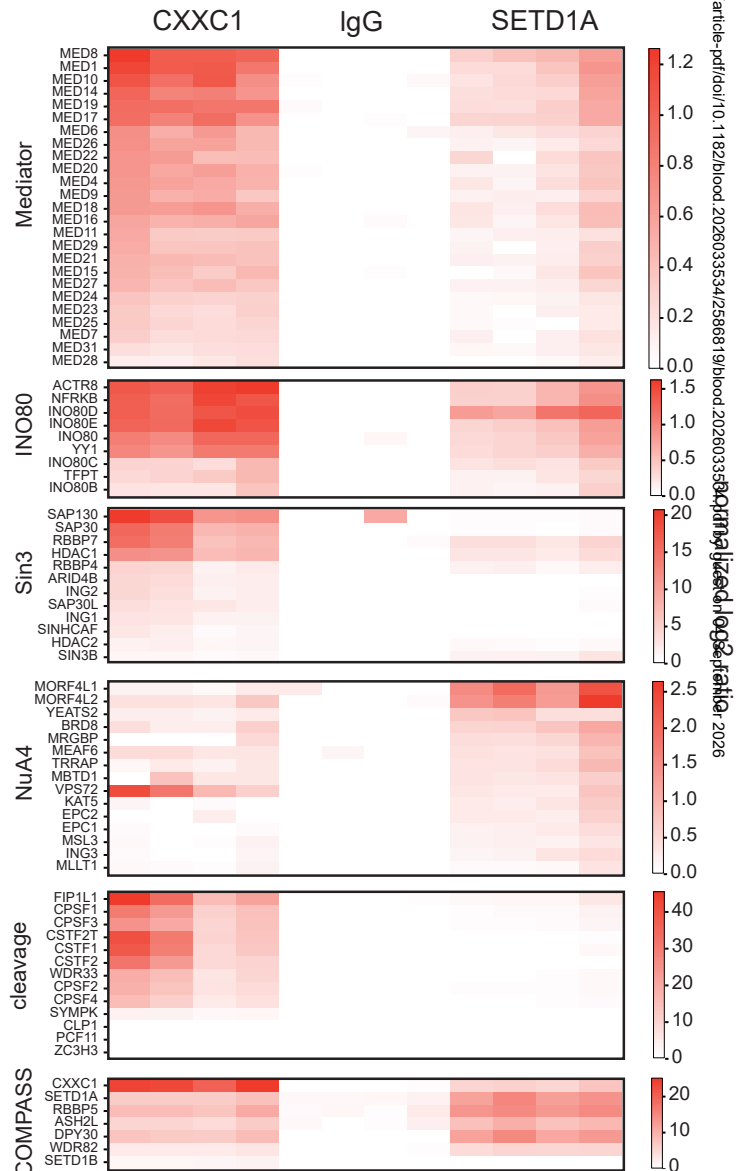
- group
- bait
  - COMPASS (not significant)
  - COMPASS (FDR < 0.05)
  - proteomic data (not significant)
  - proteomic data (FDR < 0.05)
  - TFs (FDR < 0.05)

**B**

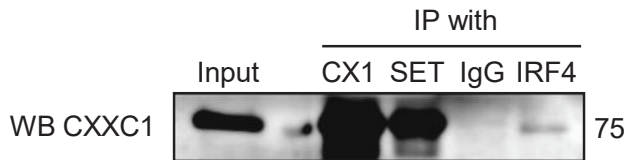


**E**

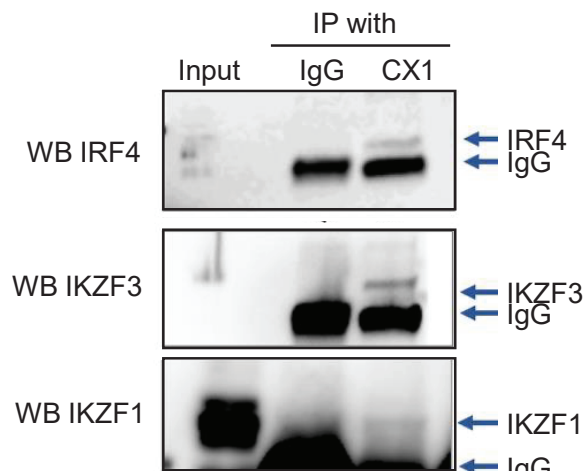
Interactors within complexes (FDR<0.05)



**C**



**D**



**Figure 3**

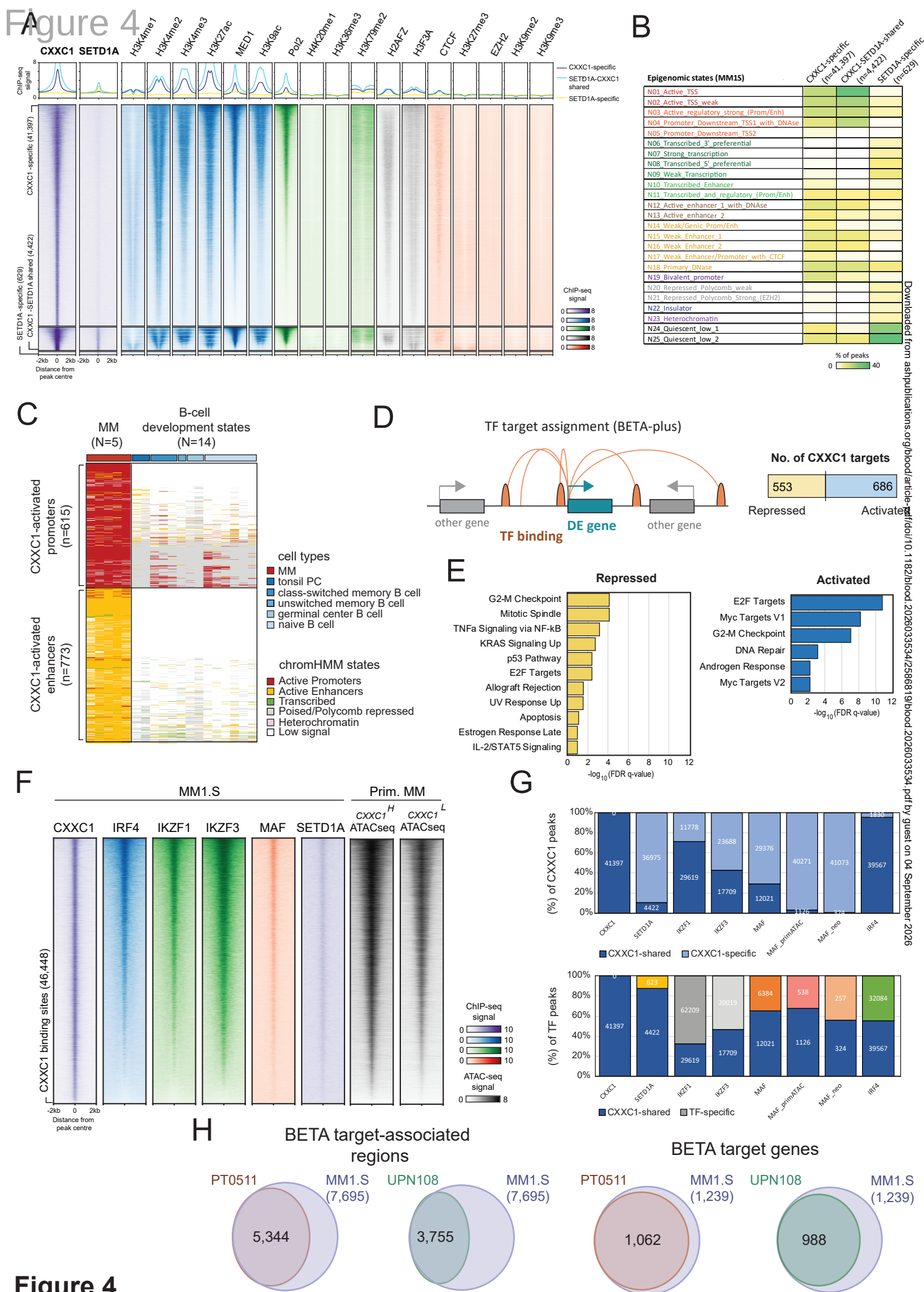


Figure 5

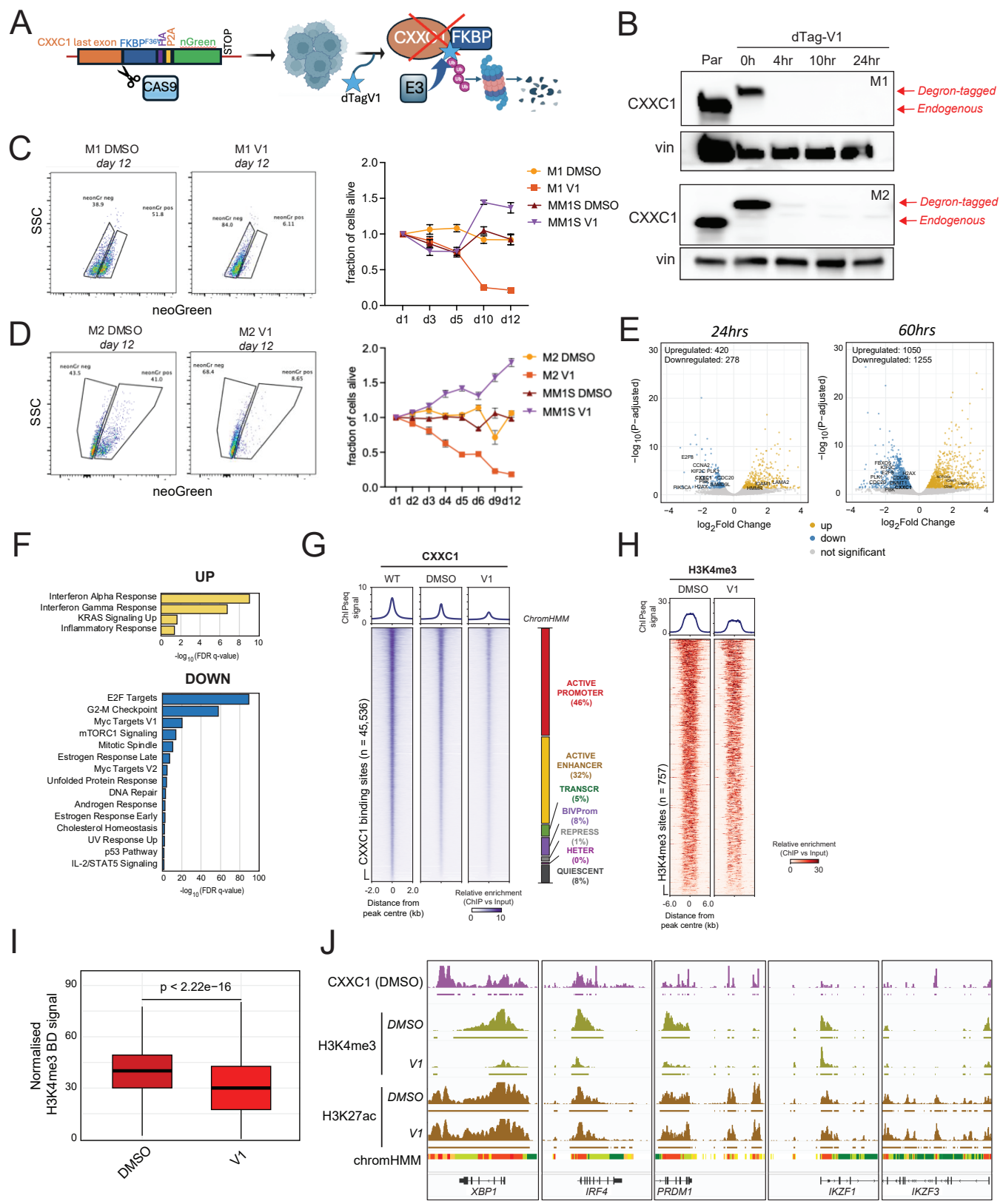


Figure 5

Figure 6

Figure 6

