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The MYCL and MXD1 transcription factors regulate the fitness of murine dendritic cells

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We previously found that MYCL is required by a Batf3-dependent classical dendritic cell subset (cDC1) for optimal C8B T cell priming, but the underlying mechanism has remained unclear. The MAX-binding proteins encompass a family of transcription factors with overlapping DNA-binding specificities, conferred by a C-terminal basic helix-loop-helix domain, which mediates heterodimerization. Thus, regulation of transcription by these factors is dependent on divergent N-terminal domains. The MYC family, including MYCL, has actions that are reciprocal to the MXD family, which is mediated through the recruitment of higher-order activator and repressor complexes, respectively. As potent proto-oncogenes, models of MYC family function have served to largely derive from their activity at supraphysiological levels in tumor cell lines. MYC and MYCN have been studied extensively, but empirical analysis of MYCL function had been limited due to highly restricted, lineage-specific expression in vivo. Here we observed that MYC is expressed in immature cDC1s but repressed on maturation, concomitant with Mxd1 induction in mature cDCs. We hypothesized that MYCL and Mxd1 regulate a shared, but reciprocal, transcriptional program during cDC1 maturation. In agreement, immature cDC1s in Mycl−/− deficient mice exhibited reduced expression of genes that regulate core biosynthetic processes. Mature cDC1s from Mxd1−/− mice exhibited impaired ability to inhibit the transcriptional signature otherwise supported by MYCL. The present study reveals LMYC and Mxd1 as regulators of a transcriptional program that is modulated during the maturation of Batf3-dependent cDCs.

Myc, Mycn, and Myel compose a highly conserved family of proto-oncogenes that support elevated transcription in transformed cells (1–5). All members of the MYC family are structural partners of MAX, which shares a basic helix-loop-helix (bHLH) domain and confers DNA-binding specificity to the heterodimer (6, 7). The MYC-MAX dimer in turn activates transcription via transcriptional activation domains intrinsic to MYC (8). Analysis of mice deficient in Myc and Mycn revealed that these factors have nonredundant, essential roles in the regulation of embryogenesis (9, 10). However, developmental defects associated with Mycn deficiency can be rescued by expression of the Myc coding sequence from the endogenous Myc locus (11). Notwithstanding, redundancy is context-dependent, and Myc expression is insufficient to rescue all the cell-intrinsic functions of Mycn, such as during myogenesis and lymphocyte proliferation (11, 12). Despite significant overlap in the functions of MYC, MYCN, and MYCL, distinct enhancer elements at their respective genomic loci enforce a requirement for both Myc and Mycn. This paradox has since been extended to a number of developmental pathways, including hematopoiesis. Sustained production of lymphocytes, for example, requires early expression of Mycn by hematopoietic stem cells and subsequent transition to Myc after restriction to lymphoid lineages (13–15).

Unlike Myc and Mycn, Myel is dispensable for development into adulthood (16, 17). However, Myel is known to retain transcriptional activity and to serve as a functional proto-oncogene in numerous cancer cell lines (3, 18, 19). Following a precise analysis of transcription factors expressed by dendritic cells (DCs), the first hematopoietic lineage that requires normal Myel expression for its function was identified. DCs, including plasmacytoid DCs (pDCs) and both subsets of classical DCs (cDCs), develop normally in Myel−/− deficient mice, but these mice exhibit an impaired capacity to prime CD8 T cells in response to bacterial and viral infections (20). This effect has been attributed specifically to the Batf3-dependent cDC subset, called cDC1, thus providing a model for studying MYCL in a primary cell lineage.

Terminal differentiation of diverse cellular lineages is associated with reduced expression of MYC, coincident with reductions in the rate of growth and proliferation (21). MYC-supported transcription can also be repressed directly by bHLH domain-containing repressors, such as the Msd family of genes. Like the MYC proteins, MXD proteins dimerize with MAX. As repressors of transcription, dimerization and DNA binding impose reciprocal actions at MYC-regulated loci (22). Surveys of the major hematopoietic lineages have revealed that Msd1 is expressed primarily by granulocytes, innate lymphoid cells, and mature DCs (23, 24). In addition, recent studies have revealed suppression of Myel and induction of Msd1 expression during the transition of cDCs from the immature state to the mature state (25, 26).

Since the initial report that described Myel expression in the hematopoietic system (20), more precise surface markers have

dendritic cells | transcription factors | maturation | MYC | cancer

Models that use genetic deficiency to infer gene function can be confounded by compensatory actions of coexpressed paralogs. For the MAX-binding proteins, such as MYC, compensation can occur during embryogenesis and hematopoiesis. The present study defines the roles of MYCL and Mxd1 in Batf3-dependent dendritic cells. Our results support the prevailing model of antagonism between MYC and MXD family members. We show that MYCL and Mxd1 have reciprocal actions that converge on a shared transcriptional program of biosynthesis during dendritic cell maturation. More broadly, we identify a physiological setting in which compensation is insufficient to rescue transcriptional deficiencies in Myel−/− and Msd1−/− mice.

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Data deposition: Data have been deposited at the National Center for Biotechnology Information (NCBI) (BioProject: PRJNA593609), and can be downloaded from the Gene Expression Omnibus (GEO) (accession no. GSE141492).

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been defined to distinguish immature and mature cDC1 across tissues and species (25, 27). The present study extends the analysis of Mycl mice and reports that expression of Myel is restricted to immature subsets of DCs. Among cDCs, the immature cDC1 subset expresses the highest level of Myel across all the tissues examined. Therefore, we set out to examine the impact of Myel deficiency on immature splenic cDC1s at steady state and during inflammation. We also asked whether MXD1 acts to suppress the transcriptional program supported by MYCL during inflammatory maturation. The results demonstrate that cDC1 cells from Mxd1−/−mice have an impaired capacity to suppress the same core biosynthetic processes that are otherwise supported by Myel. Therefore, we conclude that Myel and Mxd1 cooperate to regulate the fitness of cDC1s in vivo through the regulation of a shared transcriptional program of core biosynthetic processes.

**Results**

***MycL Is Highly Expressed in cDC1s and Is Repressed on Homeostatic Maturation in Vivo.*** We recently reported that GFP expression driven from the Myel locus in Myclgfp/+ mice is restricted to DCs, and that Myc is not coexpressed with Myel (20). Myclgfp/+ mice do not express a functional Myel transcript, and thus served as an in vivo model to demonstrate that MYCL is required for optimal CD8 T cell priming in vivo (20). Recent whole-transcriptome analyses of DCs during homeostatic and inflammatory maturation found suppression of Mycl, suggesting that its expression is most likely limited to immature cDCs (25, 26). Thus, we examined Mycl-GFP expression in Myclgfp/+ mice, using CCR7 expression as a canonical marker of maturation status of DCs in peripheral lymphoid organs (28). In the skin-draining lymph nodes (LNs), we show that mature CD11c−MHCII+CCR7− cDCs are GFP−, while immature CD11c+MHCII+CCR7+ cDCs are GFP+.

![Flow cytometry results](image.png)

**Fig. 1.** Mycl expression is restricted to immature cDC1s in lymphoid organs. (A and B) Flow cytometry of CD24+ cDC1s and CD172a+ cDC2s (A) and expression of Mycl-GFP and CCR7 (B) in skin-draining lymph nodes of Myclgfp/+ mice. (C) Heatmap of Pearson correlation coefficients for expressed genes differentially expressed among DC subsets from expression microarray analysis published by the ImmGen Consortium (23, 24). Modules characteristic of immature and mature phenotypes defined by hierarchical clustering and highlighted in red and blue, respectively. (D and E) Definition of immature/resident and mature/migratory CD24+ cDC1s and CD172a+ cDC2s based on CD11c and MHCII expression (D) and histograms of Mycl-GFP and CCR7 median fluorescence intensity (E) for immature and mature subsets of cDC1s and in skin-draining lymph nodes of Myclgfp/+ mice analyzed by flow cytometry. (F) For respective populations defined in D, histograms of Mycl-GFP median fluorescence intensity were analyzed by flow cytometry. Expression of Mycl-GFP in splenic B cells served as a negative control for GFP. Flow cytometry results are representative of three to five independent experiments each with two or three mice.
confirming that Mycl expression is suppressed on maturation in this tissue (Fig. 1A and B).

We extended these observations across several other cDC subsets in various tissues. We identified a strong positive correlation between Mycl expression and immature DC markers, such as Kit, Wdfy4, Clec9a, Nana, and Sna22 (Fig. 1C) (23–25, 29–31). Inversely, a strong negative correlation exists between the expression of Mycl and canonical markers of mature cDCs, such as Ccr7, Idol, CD40, Msd1, and Cd274. To establish whether this pattern of Mycl expression is conserved across cDCs in peripheral lymphoid organs in vivo, we analyzed GFP expression in Myclgfp/gfp mice. The results demonstrate that Mycl expression is restricted to immature cDCs, as defined by CD11chIMHCIIint cells, in the skin-draining LNs, spleen, mesenteric LNs, mediastinal LNs, and lung (Fig. 1D–F).

**MYCL Regulates cDC1 Cell Size and Supports Transcription Broadly.** Although MYCL is required for optimal CD8 T cell priming by cDCs (20), the transcriptional mechanisms for this action of MYCL are not established. A hallmark of MYC deficiency, from insects to mammals, is a reduction in cell size (32–34). Therefore, we asked whether cDCs in MYCL-deficient mice would exhibit a similar phenotype. By flow cytometry, steady-state splenic cDCs in Mycl−/− mice exhibited significantly reduced forward scatter area (FSC-A) compared with Mycl+/+ mice, an indication of reduced cell size (Fig. 2A–C). Although CD172a+ splenic cDC2 cell size is also affected by Mycl deficiency (Fig. 2B and C), a role for MYCL in the regulation of cDC2 function has not been established. In addition, Mycl expression is highest in immature cDC1s in all the tissues examined (Fig. 1E and F). Therefore, to define the mechanism by which MYCL supports transcription, we focused our analysis on splenic CD24+ cDC1 cells.

To define the transcriptional footprint of MYCL, we used RNA spike-in controls to normalize signal intensity to RNA content (35). This allowed for an examination of the effect of Mycl deficiency on cDC1 transcriptional fitness with more precision than in our initial studies (20). We hypothesized that MYCL, like MYC, might support transcription broadly as a transcriptional activator. This model would predict a uniform reduction in mRNA levels for genes that are supported transcriptionally by MYCL. Normalization of expression microarray signal intensity to RNA spike-in concentration identified a uniform and significant reduction in transcription in splenic cDC1s from Myclgfp/gfp mice relative to Mycl+/+ mice (Fig. 2D). To rule out an effect of GFP expression, an independent experiment demonstrated the same effect when cDC1s were compared between Myclgfp/gfp and Mycl+/+ mice (Fig. 2E).

**MYCL Supports Core Biosynthetic Processes in cDC1s at Homeostasis and during Inflammation.** Since MYC is a positive regulator of transcription with broad activity, MYC deficiency in primary cells is characterized by a directional, downward shift in mRNA levels (35). We used gene set enrichment analysis (GSEA) to determine

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**Fig. 2.** Mycl expression supports cDC1 fitness with respect to cell size and global transcription in vivo. (A) Representative flow cytometry analysis of splenic CD24+ cDC1s and CD172a+ cDC2s from Mycl+/+, Mycl−/−, and Myclgfp/gfp mice. Populations (Right) are pre gated on CD11c and MHCII expression (Left). (B) Relative cell size of splenic CD24+ (Top) and CD172a+ (Bottom) cDCs as measured by median fluorescence intensity of the FSC-A. *P < 0.05, Student’s t test between the indicated populations in Myclgfp/gfp and Mycl+/+ mice. Data were pooled and normalized from three independent experiments (n = 11 to 25). (C) Representative 2D histograms (Left) of cell size for cDC subsets defined in A, measured as a function of FSC-A and side scatter area (SSC-A) for the indicated genotypes. Representative histogram (Right) of FSC-A from which the data in B are derived. Colors correspond to the indicated genotypes (Left). (D and E) Violin plots of microarray expression levels of genes with significant signal to noise ratios between Myclgfp/gfp and Mycl+/+ splenic cDC1s (n = 5 per genotype) mice and Myclgfp/gfp and Mycl+/+ (n = 4 to 5 per genotype), respectively. Results of Welch’s two-sample t test are reported as P values or indicated as *P < 0.05, **P < 0.01, or ***P < 0.005.

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[Diagram and figures representing flow cytometry analysis and gene expression analysis are included, showing the effects of Mycl deficiency on cDC1 cell size and transcription.]
the direction of the effect of Mycl deficiency on the transcription of genes grouped by functional annotations with Gene Ontology (GO) terms (36–39). Signal-to-noise ratios for all expressed genes were calculated, with positive values corresponding to reduced expression in cDC1s from Mycl1gfp/gfp mice relative to cDC1s from Mycl1+/+ or Myc+/+ mice. When compared with a random normal distribution of signal-to-noise ratios, the top 1,000 genes, ranked on the basis of statistical significance, were more highly expressed in Mycl1+/+ and Mycl1+/+ cDC1s (Fig. 1 A and B). These results demonstrate that LMYC functions to activate transcription in cDC1s at steady state.

Analysis of the functional consequences of Mycl deficiency on transcription revealed a consistent enrichment of GO terms associated with core biosynthetic processes (Fig. 3 C–E). These results support a model in which LMYC enhances the expression of genes that regulate core biological processes. MYC family members amplify transcription at loci in which transcription has already been initiated by increasing the rate of RNA polymerase elongation (40–42). Consistent with this model, Mycl deficiency did not result in the absolute inhibition of transcription from target loci. Rather, cDC1s from mice with at least one copy of Mycl exhibited broad amplification of gene expression (Fig. 3 A–C). Genes that were not amplified by MYCL were grouped by GSEA into poorly supported functional gene sets as a result of lower signal-to-noise ratios (Fig. 3 B–D).

The cDC1 lineage can provide critical signals required for the innate immune responses in some contexts (43). Thus, we next asked whether MYCL regulates the transcriptional fitness of cDC1s after acute activation. The synthetic analog to pathogen-associated double-stranded RNA, poly(I:C), is sufficient to activate innate immune cells in vivo and to induce inflammatory maturation of DCs (25, 38, 44). We activated splenic cDC1s in vivo using poly(I:C) and analyzed their transcriptional response in Mycl+/+ or Mycl+/+ mice after 5 h. After activation, pairwise comparisons of the normalized enrichment scores of enriched gene sets revealed shared transcriptional support by MYCL at steady state and after activation. This is illustrated in Fig. 4A, where the gene sets with the highest enrichment scores are associated with higher expression of constituent genes in Mycl+/+ splenic cDC1s. At the gene level, nearly one-half of the genes that contribute to the core enrichment signature are unique to steady-state or inflammatory conditions (Fig. 4B). Therefore, we asked whether LMYC controls discrete biological processes at steady state and activation. We generated an adjacency matrix of the core enrichment genes from steady-state and activating conditions (Fig. 4B and SI Appendix, Tables S1 and S3). In turn, the matrix was visualized as a network of significantly enriched gene sets and their corresponding core enrichment genes, which were clustered using a prefuse force-directed layout algorithm in
Fig. 4. The transcriptional program of biosynthesis supported by MYCL is independent of activation status. Results from GSEA of expression microarray data from two independent experiments, in which splenic cDC1s were isolated from Mycl\textsuperscript{gfp/gfp} (n = 4 to 5) and Mycl\textsuperscript{+/+} (n = 5) mice under homeostatic conditions or activation with poly I:C. (A) Scatterplot of normalized enrichment scores of all biological process GO gene sets for the indicated experiments. Each gene set is represented as a colored dot whose color and size are scaled to their respective FDR. (B) Venn diagram of genes classified as part of the core enrichment signature by GSEA at homeostasis and under activation with poly I:C. (C–F) Network analysis of genes that contribute to the core enrichment signature for a given gene set. Individual genes are represented as nodes, which are colored based on their membership to the core-enrichment signatures defined in B. Edges are lines that connect individual genes to the functional gene sets, represented as black nodes, to which they are annotated by GO. Clusters were generated by a prefuse force-directed algorithm in Cytoscape, in which nodes that are connected by one or more edges are positioned closer together and disconnected nodes are drawn farther apart. Gene node color corresponds to whether they are shared or unique to a given experimental condition, as illustrated in B. The clusters of genes are centered around gene set nodes associated broadly with respiration (C), regulation of chromatin and transcription (D), protein processing (E), and translation (F). (G and H) Heat maps of core enrichment genes expressed by splenic cDC1s at homeostasis (G) or after poly I:C activation (H). Genes are grouped based on their inclusion in the indicated functional gene set, which are derived from GSEA results in SI Appendix, Table S3. Normalized expression is scaled by row z-score.
MXD1 Regulates the Repression of MYCL-Supported Transcription in Mature cDCs. We also found that Mxd1 expression changes during cDC maturation across various tissues, being induced rather than suppressed (Fig. 1C), in agreement with a recent in vivo study (25). Given the established role of MXD1 as a transcriptional repressor that can antagonize MYC-supported transcription (46, 47), we set out to test whether the suppressive activity of MXD1 in mature cDCs overlaps with the support of transcription by MYC in immature cDCs. GSEA results identified elevated expression of genes with promoter-proximal MYC:MAX and E2F1 DNA-binding motifs (Fig. 5B and C). In addition, a model for reciprocal action of MXD1 and MYCL in activated cDCs is supported by a large subset of genes with concordance between MXD1-mediated suppression and MYCL-mediated support (Fig. 5D).

Numerous factors have been identified as sufficient to induce maturation, but none has been shown to be absolutely required for maturation at steady state or during immune responses in vivo (26). The precise description of transcriptional changes that occur during cDC1 maturation in vivo can now be used to identify putative regulators of this process. Therefore, we conducted preliminary analyses that can be used to inform future work on the role of MXD1 in cDC1 function. Using curated gene sets related to cDC1 maturation (24), we performed GSEA to investigate whether MXD1 activity correlated with maturation gene sets. In activated cDCs from Mxd1+/− mice, there was significant enrichment and elevated expression of genes known to be

Fig. 5. Broad transcriptional support of biosynthesis is suppressed by MXD1 during inflammatory maturation. Results of GSEA of expression microarray data of splenic cDCs isolated from Mxd1+/− and Mxd1−/− after activation with poly I:C (n = 3). (A) Violin plot of expression levels for genes with signal-to-noise ratio >1. P values reported for Welch two-sample t tests. (B and C) Rank-ordered genes, normalized enrichment scores, and FDR for gene sets defined as having known cis-regulatory elements for the transcription factors MYC:MAX (B) and E2F1 (C) within 4 kb of their transcription start sites. (D) Scatterplot of signal-to-noise ratios of genes enriched in activated cDCs from experiments described here and in Fig. 4. (E–G) Rank-ordered gene sets as described in B and C for published gene sets related to cDC1 maturation (25). (H and I) Heat maps of gene expression for gene sets of cDC1 maturation markers (H) and core biosynthetic processes (I), which are derived from GSEA results in SI Appendix, Tables S4 to S6. Expression is scaled by row z-score.
repressed on maturation (Fig. 5E). Functionally, these gene sets corresponded to cell cycle control and DNA replication (Fig. 5F and G). Finally, GO-based GSEA revealed that MXD1 deficiency on global transcription as a function of cDC1 maturation state. Genetic analyses have demonstrated that Myc and Mycn are necessary for the development of hematopoietic and nonhematopoietic lineages during embryogenesis and adulthood (9, 10, 48, 49). However, models of transcriptional regulation by MYC family members are based largely on empirical studies of MYC function. All MYC family members have the ability to function as proto-oncogenes (2, 3, 5, 18, 19, 50–52). Mediated by interactions with MAX, their functions can be redundant when amplified to supraphysiological levels in transformed cells (11, 53). Analysis of transformed cell lines has identified that a core transcriptional program is conserved across MYC family members, but MYC-, MYCN-, and MYCL-specific signatures have also been reported (11, 54). For example, MYCN is required during neurogenesis, and MYCN-amplified cancers are enriched with a gene signature associated with neuronal function (10, 16). Likewise, both MYC and MYCN are necessary for hematopoiesis and immune responses, and thus MYC- and MYCN-amplified cancers are enriched with a gene signature associated with cytokines and immune responses (13, 48).

Initial examination of the Mycgfp/+ mutant mouse model demonstrated that GFP+ hematopoietic cells in vivo were restricted to the DC lineage, including cDC1, cDC2, and pDC subsets (20). More recent whole-transcriptome datasets suggest that Myel expression is regulated differentially between tissues and among DC subsets, however (Fig. 1C) (23–26). We found that expression of Myel is an order of magnitude higher in cDC1s than in cDC2s (Fig. 1E and F). Therefore, in the present study we focused on the role of LMYC in the transcriptional fitness specifically of cDC1s, which correspond to a single developmental lineage of Batf3- and Irf8-dependent cDCs present in lymphoid and nonlymphoid tissues (55, 56). Our analysis revealed that maturation is associated with suppression of Myel expression across tissues (Fig. 1B–E).

Immature cDC1s are present in all major lymphoid organs and are the predominant population among DC1s in the spleen. Therefore, we focused on splenic cDC1s at steady state and during acute inflammation to determine the impact of Myel deficiency on the cDC1 fitness. Examination of cDC1 cell size and mRNA content revealed that both were reduced in Myel−/− mice (Fig. 2A–E). This phenomenon is also associated with MYC deficiency in cancer and activated lymphocytes, suggesting that the core function of MYCL in cDC1 cells is conserved with other MYC family members (32, 34).

Detailed expression microarray analysis using GSEA revealed that MYCL supports transcription broadly in cDC1s at steady state (Fig. 3). We found that MYCL regulates the transcription of genes associated with core biosynthetic processes, such as nucleic acid and peptide biosynthesis (Fig. 3C–E). Independent of activation status, MYCL activated transcription uniformly, manifested as a uniform reduction in mRNA signal intensity in cDC1s from Mycl−/− (Figs. 3A and B and 4) (44). A major fraction of MYCL-supported genes was unique to cDC1s isolated from steady-state and activating conditions (Fig. 4B); however, GSEA revealed that the broader functions of these genes converged on the regulation of the biosynthetic processes (Fig. 4A and C–F). The mechanism by which MYCL-regulated gene expression is sensitive to activation status was not examined here, but in vivo analysis revealed that cDC1 maturation coincides with suppression of Myel expression (Fig. 1C–F). In other contexts, such as lymphocyte activation and MYC amplification, MYC is known to function as an analog regulator of gene expression, where the level of MYC protein expression positively correlates with transcription and binding to dose-dependent enhancer elements (34, 42, 57). Given the ability of poly(IC) to induce cDC1 maturation in vivo, it is possible that MYCL levels vary between activating and steady-state conditions (25). Additional work is needed to identify the DNA-binding sites of MYCL in cDC1s in immature and activated cDC1s. To inform future investigations, the evidence presented here supports a role for MYCL in the support of transcription of genes that regulate core biosynthetic processes and thus the fitness of cDC1s.

Proteins structurally similar to MYC, such as MXD1, can dimerize with MAX and represses transcription at MYC-regulated loci (22, 46, 58). Generation of an MXD1-deficient mouse model demonstrated a role for MXD proteins in granulocyte cell cycle exit, and Mad1 expression is associated with terminal differentiation of a number of cellular lineages (59–63). cDC1 maturation is marked by the induction of Mad1 and concomitant repression of Myel (Fig. 1C) (23–26). Induction of cDC1 maturation in vivo with poly(IC) revealed broad inhibition of transcription by MXD1, demonstrated by significantly enhanced mRNA signal intensities in cDC1s from Mad1−/− mice (Fig. 5A). The repressive activity of MXD1 correlated significantly with genes enriched with promoter-adjacent MYC/MAX and E2F DNA-binding motifs (Fig. 5B and C). Qualitatively, a large fraction of genes repressed by MXD1 are otherwise supported by MYCL in immature cDC1s (Fig. 5D).

Further studies will need to determine whether MYCL and MXD1 execute their reciprocal actions on the same loci. With respect to the independent actions of MXD1 during cDC1 maturation, genes that are normally repressed in mature cDC1s had elevated expression in Mad1−/− mice (Fig. 5E–H). Therefore, MXD1 broadly represses the transcription of genes that regulate biosynthesis as a function of cDC1 maturation. It is widely accepted that DC maturation correlates with the capacity of DCs to effectively regulate immune responses (28). The present study reveals mechanisms by which MYCL and MXD1 regulate cellular fitness in a primary cell lineage. We demonstrate that MYCL and MXD1 have overlapping but reciprocal actions that regulate biological processes associated with cDC1 maturation. This contributes to the growing body of evidence supporting the evolutionary conservation of structure and function of MAX-binding transcription factors across species and cell types (46, 64).

Materials and Methods

Mice. The generation of Mycgfp/mice has been described previously, and the strain used in this study, B6.129S6(Q-Myc11.1Kmm/J), is available publicly from The Jackson Laboratory (20). Mice were maintained in a pathogen-free animal facility and experiments conducted in accordance with institutional guidelines and protocols established by the Animal Studies Committee at Washington University in St. Louis. The generation of Mycl−/− mice has been described previously (73). Mice were provided by R. Eisenman, Fred Hutchinson Cancer Research Center, and maintained as described above at Washington University in St. Louis. Mice aged 8 to 12 wk were used for all experiments.

Antibodies and Flow Cytometry. Cells were prepared for staining and analysis at 4 °C in PBS with 0.5% BSA and 2 mM EDTA (MACS buffer). Antibodies used in this study were manufactured by BD Biosciences, Tonbo Biosciences, BioLegend, and Thermo Fisher Scientific. Anti-CD16/32 was used as an Fc-block (2.4G2). The following antibodies were used for staining and depletion: CD11c (N418), MHCII (M5/114/15/2), CD24 (M1/69), CD172a (P84), CD45 (103000), CD40 (1F4), and concomitant repression of Myel and MXD1 broadly represses the transcription of genes that regulate biosynthesis as a function of cDC1 maturation. It is widely accepted that DC maturation correlates with the capacity of DCs to effectively regulate immune responses (28). The present study reveals mechanisms by which MYCL and MXD1 regulate cellular fitness in a primary cell lineage. We demonstrate that MYCL and MXD1 have overlapping but reciprocal actions that regulate biological processes associated with cDC1 maturation. This contributes to the growing body of evidence supporting the evolutionary conservation of structure and function of MAX-binding transcription factors across species and cell types (46, 64).
dividing FSC-A of each sample by the mean FSC-A within the same experiment and genotype, followed by –log2 transformation. Welch’s t test was performed on the normalized sample mean and variance.

Cell Activation, Isolation, and Preparation. Splenocytes, skin-draining lymph nodes, mesenteric lymph nodes, mediastinal lymph nodes, lungs, and spleens were dissociated mechanically and enzymatically with 250 μg/mL collagenase B (Roche) and 30 U/mL DNase I (Sigma-Aldrich) with gentle agitation at 37 °C for 45 min in up to 5 mL of lsoceve’s modified Dulbecco’s medium. Red blood cells were lysed with hypotonic buffer containing NH4Cl and KHC03. Before analysis, cells suspensions were filtered through 70-μm mesh. For microarray experiments, maturation was induced by activation with i.p. injection of anti-CD3e, CD19, NK1.1, TER-119, and Ly6G and depletion by negative selection with MojoSort streptavidin microbeads (Biolegend). Lineage-positive cells were also excluded by staining with Qdot 605 streptavidin conjugate (Thermo Fisher Scientific). Cells were sorted into microcentrifuge tubes containing MACS buffer at 4 °C.

Expression Microarray Analysis. Cells were lysed in cell lysis buffer provided with the RNeasy Micro Kit (Qiagen). The volume of lysis buffer used for each sample was normalized to cell number as estimated by FACS. An equal volume of internal RNA Controls Consortium (ERCC) RNA spike-in mix diluted in lysis buffer (Thermo Fisher Scientific) was added to each sample for downstream normalization of expression microarray signal intensities (79). Total RNA was isolated and genomic DNA digested as described in the manufacturer’s protocol. RNA was amplified with the GeneChip WT Pico Kit (Applied Biosystems) in accordance with the manufacturer’s protocol and hybridized to GeneChip Mouse Gene 1.0 ST microarrays (Affymetrix). Data were processed by robust microarray average (RMA) summarization.

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