The long non–coding RNA Morrbid regulates Bim and short–lived myeloid cell lifespan

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Neutrophils, eosinophils and ‘classical’ monocytes collectively account for about 70% of human blood leukocytes and are among the shortest-lived cells in the body1,2. Precise regulation of the lifespan of these myeloid cells is critical to maintain protective immune responses and minimize the deleterious consequences of prolonged inflammation1,2. However, how the lifespan of these cells is strictly controlled remains largely unknown. Here we identify a long non-coding RNA that we termed Morrbid, which tightly controls the survival of neutrophils, eosinophils and classical monocytes in response to pro-survival cytokines in mice. To control the lifespan of these cells, Morrbid regulates the transcription of the neighbouring pro-apoptotic gene, Bcl2l11 (also known as Bim), by promoting the enrichment of the PRC2 complex at the Bcl2l11 promoter to maintain this gene in a poised state. Notably, Morrbid regulates this process in cis, enabling allele-specific control of Bcl2l11 transcription. Thus, in these highly inflammatory cells, changes in Morrbid levels provide a locus-specific regulatory mechanism that allows rapid control of apoptosis in response to extracellular pro-survival signals. As Morrbid is present in humans and dysregulated in individuals with hypereosinophilic syndrome, this long non-coding RNA may represent a potential therapeutic target for inflammatory disorders characterized by aberrant short-lived myeloid cell lifespan.

Neutrophils, eosinophils and ‘classical’ monocytes represent a first line of defense against nearly all pathogens1,2. However, these short-lived myeloid cells also contribute to the development of several inflammatory diseases1,2. Cytokines and metabolites tightly regulate the function and lifespan of these cells, but how these cues are translated into an optimal cellular lifespan is largely unknown. Emerging evidence indicates that certain long non-coding RNAs (lncRNAs) can integrate extracellular inputs with chromatin-modification pathways allowing cells to rapidly adapt to their environment3,4. As such, we investigated whether lncRNAs control the function or lifespan of short-lived myeloid cells in response to extracellular cues. We first analysed multiple RNA sequencing (RNA-seq) datasets for mouse lncRNAs that are preferentially expressed by mature short-lived myeloid cells5,6. We identified an uncharacterized lncRNA (Gm14005) that we termed Morrbid (myeloid RNA regulator of Bim-induced death). Morrbid is conserved across species, contains five exons, is polyadenylated and is localized predominately to the nucleus bound to chromatin (Fig. 1a, b). Morrbid is highly and specifically expressed by mature eosinophils, neutrophils and classical monocytes in both mice and humans (Fig. 1c, d).

To investigate the role of Morrbid in vivo, we deleted the Morrbid locus to generate Morrbid-deficient mice (Extended Data Fig. 1g). Notably, and in accordance with the expression profile of Morrbid, we found that eosinophils, neutrophils and Ly6C+ classic monocytes were markedly reduced in the blood and tissues of these mice (Fig. 1e, f). This defect was highly specific to these three cell types, as well as blood Ly6C+ monocytes (Extended Data Fig. 2a), which are suggested to be progeny of Ly6C+ monocytes3. All other lymphoid and myeloid cell types were unaffected (Extended Data Fig. 1i, 2a). Similarly, knockdown of Morrbid in vivo also led to a specific reduction in the frequency of short-lived myeloid cells in blood and spleen (Extended Data Fig. 2b–e). Finally, as these cells have a critical role in protective immunity and in the development of immunopathology, we found that Morrbid-deficient mice were highly susceptible to bacterial (Listeria monocytogenes) infection (Fig. 1f, g), and protected from eosinophil-driven allergic lung inflammation (Extended Data Fig. 2f–h). Altogether, these results support an important and selective role for Morrbid and potentially DNA elements within its locus in short-lived myeloid cell homeostasis.

Eosinophils, neutrophils and Ly6C+ monocytes originate from common progenitors in the bone marrow (BM)1,8, with extracellular cues driving the developmental programs needed to produce each of these cell types1,8. Using mixed BM chimaeras, we found that Morrbid-deficient BM cells have a significant defect in the generation of short-lived myeloid cells (Extended Data Fig. 3a–e), indicating that Morrbid acts in a cell-intrinsic manner. We next sought to determine whether Morrbid regulates short-lived myeloid cell development. Early progenitors of each of these cell types express low levels of Morrbid and its expression increases throughout development to reach maximal levels in fully mature eosinophils, neutrophils and Ly6C+ monocytes (Extended Data Fig. 3f–h). In accordance with this pattern of expression, the progenitors of each of these cell types were intact in Morrbid-deficient mice (Fig. 2a, Extended Data Fig. 3g–h). These results suggest that Morrbid regulates the frequency of mature eosinophils, neutrophils and monocytes, but not their progenitors.

Mature populations of myeloid cells are controlled by several mechanisms, including homeostatic proliferation, trafficking, and cell death. We found no defects in homeostatic proliferation in Morrbid-deficient mice (Extended Data Fig. 4a). Mature short-lived myeloid cells are

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Figure 1 | IncRNA Morrbid is a critical regulator of eosinophils, neutrophils and Ly6C<sup>hi</sup> monocytes. a, Human neutrophil and mouse granulocyte normalized RNA-seq and ChIP-seq tracks at the Morrbid locus. b, Single molecule Morrbid RNA fluorescence in situ hybridization (FISH). c, d, qPCR expression of mouse (n = 3; representative of 3 independent experiments) and human Morrbid in indicated cell types and tissues (n = 7) (d). e, Wild-type and Morrbid-deficient flow cytometry plots and absolute counts (n = 3–5; representative of 7 independent experiments). f, g, L. monocytogenes infection of wild-type and Morrbid-deficient mice. f, Survival and weight loss (n = 9, representative of 3 independent experiments). g, Colony-forming units (CFUs) per g from indicated organs (n = 5; representative of 3 independent experiments). Error bars show s.e.m. *P < 0.05, **P < 0.01, and ***P < 0.001 (two-sided t-test, e, f (right); Mantel–Cox test, f (left)).

Figure 2 | Morrbid controls eosinophil, neutrophil and Ly6C<sup>hi</sup> monocyte lifespan. a, Schematic of short-lived myeloid cell development and absolute numbers of the indicated cell types in BM from wild-type and Morrbid-deficient mice (n = 3–5; representative of 3 independent experiments). b, Frequency of Casp<sup>3</sup> (Z-VAD-FMK<sup>+</sup>) cultured BM cells (n = 3 mice; representative of 2 independent experiments). c, Half-life of BrdU pulse-labelled neutrophils in blood in vivo (n = 4 mice; representative of 3 independent experiments). d, Bcl2l11 qPCR expression in indicated cell types sorted from BM (n = 3; representative of 2 independent experiments). e, Bcl2l11 protein expression assessed by flow cytometry in indicated BM cell types. Left, representative histograms. Right, mean fluorescence intensity (MFI) quantification (n = 3–5 mice, representative of 3 independent experiments). Error bars show s.e.m. *P < 0.05, **P < 0.01 and ***P < 0.001 (two-sided t-test).
substantially reduced in the BM of Morrbid-deficient mice and there was a near absence of in vitro BM-differentiated eosinophils (Fig. 2a, Extended Data Fig. 4b, c), suggesting that Morrbid controls a dominant process independent of cell trafficking. Notably, Morrbid-deficient eosinophils, neutrophils and Ly6C hi monocytes were all highly prone to apoptosis in BM cultured ex vivo (Fig. 2b, Extended Data Fig. 4d). Furthermore, we observed significantly increased apoptosis in vitro in BM-derived eosinophils (Extended Data Fig. 4e), and in vivo during L. monocytogenes infection in the absence of Morrbid (Extended Data Fig. 4f). Given the close relationship between apoptosis and cellular lifespan, we hypothesized that Morrbid is a regulator of short-lived myeloid cell half-life. Using BrdU to label circulating neutrophils and determine their decay rate, we observed an ∼2-fold decrease in the half-life of these cells (Fig. 2c, Extended Data Fig. 4g). These results indicate that Morrbid regulates short-lived myeloid cell lifespan through control of apoptosis.

Some lncRNAs regulate the expression of neighbouring genes10–13. The pro-apoptotic gene Bcl2l11 (Bim) is located ∼150-kb downstream of Morrbid (Extended Data Fig. 1a). Bcl2l11 has been shown to be an important regulator of myeloid homeostasis14,15. Thus, we reasoned that Morrbid regulates short-lived myeloid cell lifespan through its control of Bcl2l11 expression. Indeed, the protein and mRNA levels of Bcl2l11 were markedly elevated in eosinophils, neutrophils and Ly6C hi monocytes from Morrbid-deficient mice (Fig. 2d, e, Extended Data Fig. 4h–k). In concordance with the pattern of Morrbid expression, Bcl2l11 was maximally elevated in the mature state of each of these cell lineages in Morrbid-deficient mice (Extended Data Fig. 4l), and was not dysregulated in other myeloid and lymphoid cell populations (Extended Data Fig. 4m). Importantly, key myeloid lineage transcription factors and other genes neighbouring Morrbid were largely unaffected in the absence of Morrbid (Extended Data Fig. 5a–c). These results suggest that Morrbid represses Bcl2l11 expression in short-lived myeloid cells.

To specifically address the role of Morrbid RNA in the regulation of Bcl2l11 expression, we first established an in vitro eosinophil culture system in which we could study the function of Morrbid RNA in the absence of genetic disruptions8 (Extended Data Fig. 6a). Using this system, we found that short hairpin RNA (shRNA)-mediated knockdown of Morrbid RNA results in a significant elevation in BCL2L11, which was accompanied by a substantial decrease in eosinophil survival (Figure 3a–c, Extended Data Fig. 6b–d). We observed similar results using transfection of locked nucleic acids (LNAs) as an independent knockdown technique (Extended Data Fig. 6e). We next sought to corroborate these results in a different cell type within the myeloid cell lineage. Interestingly, we found that lipopolysaccharide (LPS)-stimulated BM-derived macrophages (BMDMs) highly upregulated Morrbid (Extended Data Fig. 6f). Notably, LNA knockdown of Morrbid, deletion of the Morrbid promoter, or deletion of its locus in LPS-stimulated BMDMs resulted in a marked increase in Bcl2l11 expression and apoptosis (Extended Data Fig. 6f–l). Altogether, these results indicate that Morrbid RNA is a critical regulator of Bcl2l11 expression and short-lived myeloid cell survival.

Pro-survival cytokines can potentiate the lifespan of immune cells. One well-described mechanism of this control is through the repression of Bcl2l11 (refs 15, 16). We hypothesized that cytokines from the common β-chain receptor family (IL-3, IL-5 and GM-CSF), which are known to promote the survival of eosinophils, neutrophils and Ly6C hi monocytes, regulate Bcl2l11 expression through the induction of Morrbid. To test this hypothesis, we first withdrew cytokines from cultured BM-derived eosinophils and observed a loss of Bcl2l11 expression and an increase in Bcl2l11 levels (Fig. 3d). Subsequent addition of IL-5, IL-3 or GM-CSF induced Morrbid expression, which was accompanied by Bcl2l11 repression (Fig. 3d). Similarly, ex-vivo β-chain cytokine stimulation, but not G-CSF stimulation, significantly induced Morrbid and a corresponding repression of Bcl2l11 in neutrophils and Ly6C hi monocytes (Fig. 3e, Extended Data Fig. 6m). Importantly, Morrbid-deficient neutrophils were unable to inhibit Bcl2l11 expression upon addition of β-chain cytokines (Fig. 3f). These results suggest that β-chain cytokines repress Bcl2l11 expression in short-lived myeloid cells in a Morrbid-dependent manner.

Dysregulated immune cell survival is central to many human haematological and inflammatory diseases. Hypereosinophilic syndrome (HES) is a group of disorders characterized by eosinophilia and a wide range of clinical manifestations17. Several HES subtypes have been associated with increased production or responsiveness to IL-5 (ref. 17). We therefore reasoned that eosinophils from individuals with HES would overexpress Morrbid, and that this overexpression would positively correlate with IL-5 levels. We screened patients with varied subtypes of HES (Fig. 3g), and found that eosinophils from these patients expressed significantly higher levels of Morrbid than of
healthy controls (Fig. 3h). Additionally, we observed that MORRBID expression in eosinophils was positively correlated with plasma IL-5 levels (Fig. 3i). These results suggest a potential role for MORRBID in HES and other inflammatory diseases characterized by high levels of β-chain cytokines and altered short-lived myeloid cell lifespan.

Genes that require both tight regulation and the ability to be rapidly activated frequently have activating (H3K4me3) and repressive (H3K27me3) histone marks in their promoters, termed bivalent promoters18. The Bcl2l11 gene has previously been described as having a bivalent promoter, which allows this pro-apoptotic gene to be maintained in a poised state19. A number of lncRNAs have been shown to repress expression by promoting the enrichment of polycomb repressive complex 2 (PRC2) at target genes, which in turn catalyzes the deposition of H3K27me3 (refs 20, 21). We therefore hypothesized that MORRBID represses Bcl2l11 expression and prevents short-lived myeloid cell apoptosis by promoting PRC2 enrichment and H3K27me3 deposition at the bivalent promoter of Bcl2l11.

To test this hypothesis, we first performed chromatin immunoprecipitation followed by quantitative PCR (ChIP–qPCR) for total polymerase II (Pol II), H3K27me3 and the PRC2 subunit EZH2 in neutrophils from wild-type and Morrbid-deficient mice. In line with the elevated levels of Bcl2l11 in Morrbid-deficient cells, we found that Pol II occupancy was significantly increased (Extended Data Fig. 7a), and the levels of H3K27me3 and EZH2 were markedly reduced at the promoter of Bcl2l11 in the absence of Morrbid (Fig. 4a, b). We next asked whether the induction of Morrbid expression promotes the accumulation of PRC2 at the Bcl2l11 promoter. Using the BMDM system in which Morrbid is induced upon LPS stimulation, we found that Morrbid levels and EZH2 occupancy at the Bcl2l11 promoter concurrently increase in a Morrbid-dependent manner (Extended Data Fig. 7b). Finally, using ChIP–seq and ATAC–seq (assay for transposase-accessible chromatin using sequencing), we did not detect changes in the activating histone marks H3K4me1 and H3K4me3, and only a modest increase in chromatin accessibility at the Bcl2l11 promoter in the absence of Morrbid (Extended Data Fig. 7c–f). Altogether, these results indicate that Morrbid represses Bcl2l11 expression in short-lived myeloid cells by promoting the deposition of H3K27me3 at the bivalent promoter of Bcl2l11.

IncRNAs have been suggested to promote the recruitment of PRC2 to target genes through direct IncRNA–PRC2 interactions or indirect mechanisms1, 2, 24. To understand further the mechanism by which Morrbid promotes PRC2 enrichment at the Bcl2l11 promoter, we first examined whether Morrbid RNA associates with PRC2. Using a recently generated EZH2 photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR–CLIP) dataset22, we found that Morrbid associates with EZH2 (Extended Data Fig. 8a). To further support this association, we performed RNA immunoprecipitation against EZH2 in myeloid cells and found that Morrbid significantly co-immunoprecipitates with this PRC2 subunit (Extended Data Fig. 8a). To further support this association, we performed RNA immunoprecipitation against EZH2 in myeloid cells and found that Morrbid significantly co-immunoprecipitates with this PRC2 subunit (Extended Data Fig. 8a). To further support this association, we performed RNA immunoprecipitation against EZH2 in myeloid cells and found that Morrbid significantly co-immunoprecipitates with this PRC2 subunit (Extended Data Fig. 8a).
looming. Using chromosome conformation capture (3C), we indeed observed a long-distance association between Bcl2l11 and the Morrbid locus in short-lived myeloid cells (Fig. 4d, Extended Data Fig. 8d). Altogether, these results suggest a model in which the Morrbid proximity to Bcl2l11, mediated through DNA looping, enables Morrbid RNA to promote PRC2 enrichment within the Bcl2l11 promoter through direct Morrbid–PRC2 interactions and potentially through additional indirect mechanisms.

Our findings suggest an important role for PRC2 in Morrbid-dependent repression of Bcl2l11. Yet, whether short-lived myeloid cell survival depends on PRC2-mediated transcriptional repression of Bcl2l11 is not known. We cultured eosinophils in the presence of a specific inhibitor of EZH2, GSK126. We observed a dose-dependent increase in Bcl2l11 and eosinophil apoptosis upon PRC2 inhibition (Fig. 4e, f, Extended Data Fig. 8e, f). Importantly, Bcl2l11-deficient eosinophils were resistant to cell death following abrogation of PRC2 activity (Fig. 4f, Extended Data Fig. 8e, f). Altogether, these results demonstrate that PRC2 regulates short-lived myeloid cell survival specifically through repression of Bcl2l11 expression, further supporting a critical role for Morrbid in the regulation of the lifespan of these highly inflammatory cells.

Finally, we found that Morrbid-heterozygous mice largely recapitulated the phenotype of mice lacking both alleles of Morrbid (Extended Data Fig. 8g). In light of this dominant heterozygous phenotype and the observed Morrbid-Bcl2l11 DNA loop, we hypothesized that Morrbid functions in cis to repress Bcl2l11. As such, we expected that deletion of Bcl2l11 on the same chromosome as that of the Morrbid-deficient allele will normalize Bcl2l11 expression in short-lived myeloid cells and rescue their numbers, but that deletion of Bcl2l11 on the opposite chromosome would not (Fig. 4g). We therefore generated all permutations of Morrbid and Bcl2l11 double-heterozygous mice (Extended Data Fig. 9). Notably, deletion of Bcl2l11 in cis, but not in trans, of the Morrbid-deficient allele normalized Bcl2l11 expression (Fig. 4h) and rescued short-lived myeloid cell numbers (Fig. 4i, j, Extended Data Fig. 10a, b). Other cell types were largely unaltered in these genetic backgrounds (Extended Data Fig. 10b–d). This complete rescue in cis double-heterozygous mice indicates that Morrbid acts in an allele-specific manner to regulate Bcl2l11 expression and short-lived myeloid cell lifespan.

Here we show that Morrbid integrates extracellular signals to control the lifespan of eosinophils, neutrophils and monocytes through allele-specific suppression of Bcl2l11 expression (Extended Data Fig. 10e). As this lncRNA is present in humans and dysregulated in patients with HES, a better understanding of how Morrbid RNA and potentially DNA elements within its locus regulate Bcl2l11 may provide new therapeutic approaches for several human inflammatory diseases. Finally, our results demonstrate that lncRNAs can function as highly cell-type specific local effectors of extracellular cues to control immunological processes that require rapid and strict regulation.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Information ATAC–seq and ChIP–seq data have been deposited in the Gene Expression Omnibus under accession numbers GSE58073. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.W. (adam.williams@jax.org), R.A.F. (richard.flavell@yale.edu) or J.H.-M. (jhenao@mai.inesp.uc.pt).

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METHODS

Mice. All mice were bred and maintained under pathogen-free conditions at an American Association for the Accreditation of Laboratory Animal Care accredited animal facility at the University of Pennsylvania or Yale University. Mice were housed in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals under an animal study proposal approved by an institutional Animal Care and Use Committee. Male and female mice between 4 and 12 weeks of age were used for all experiments. Littermate controls were used whenever possible.

C57BL/6 (wild type) and B6-SJL-Ptprc<Pepe>/Boy (B6-SJL) mice were purchased from The Jackson Laboratory. We generated Morrbid-deficient mice and the in cis and in trans double heterozygous mice (Morrbid<−/−>, Bel2l11−/−) by using the CRISPR/Cas9 system as previously described26. In brief, to generate Morrbid-deficient mice, single guide RNAs (sgRNAs) were designed against regions flanking the first and last exon of the Morrbid locus (Extended Data Fig. 1g). Cas9-mediated double-stranded DNA breaks resolved by non-homologous end joining (NHEJ) ablated the intervening sequences containing Morrbid in C57BL/6N one-cell embryos. The resulting founder mice were Morrbid<−/−>, which were then bred to wild-type C57BL/6N and then intercrossed to obtain homozygous Morrbid<−/−> mice. One Morrbid-deficient line was generated. To control for potential off-target effects, mice were crossed for at least 5 generations to wild-type mice and then intercrossed to obtain homozygous Morrbid<−/−> mice. Littermate controls were used when possible throughout all experiments.

To generate the in cis and in trans double heterozygous mice (Morrbid<−/−>, Bel2l11−/−) mice, we first obtained mouse one-cell embryos from a mating between Morrbid<−/−> female and wild-type male mice. As such, the resulting one-cell embryos were heterozygous for Morrbid (Morrbid<−/+>). We then micro-injected sgRNAs designed against intronic sequences flanking the second exon of Bel2l11 (which contains the trahalase start site codon), into Morrbid<−/−>-one-cell embryos (Extended Data Fig. 9). Cas9-mediated double-stranded DNA breaks resolved by NHEJ ablated the intervening sequences containing the second exon of Bel2l11 in Morrbid<−/−> (C57BL/6N) one-cell embryos, generating founder mice that were heterozygous for both Bel2l11 and Morrbid (Bel2l11<−/−; Morrbid<−/+>). Founder heterozygous mice were then bred to wild-type C57BL/6N to interrogate for the segregation of the Morrbid-deficient and Bel2l11-deficient alleles (Extended Data Fig. 9). Pups that segregated such alleles were named in trans and pups that did not segregate were labelled in cis. One line of in cis and in trans double heterozygous mice (Bel2l11<−/−; Morrbid<−/+>) lines were generated. To control for potential off-target effects, mice were crossed for at least 5 generations to wild-type (C57BL/6N) mice (for in cis) and to Morrbid<−/−> mice (for in trans) to maintain homozygosity.

To determine genetic rescue, samples from mice containing different permutations of Morrbid and Bel2l11 alleles (Fig. 4g–j) were analysed in a blinded manner by a single investigator not involved in the breeding or coding of these samples. Flow cytometry staining, analysis and cell sorting. Cells were isolated from the indicated tissues (blood, spleen, bone marrow, peritoneal exudate, adipose tissue). Red blood cells were lysed with ACK. Single-cell suspensions were stained with CD16/32 and with indicated fluorochrome-conjugated antibodies. If run live, cells were stained with 7-AAD (7-aminoactinomycin D) to exclude non-viable cells. Otherwise, before fixation, Live/Dead Fixable Violet Cell Stain Kit (Invitrogen) was used to exclude non-viable cells. Active caspase staining using Z-VAD-FMK (CaspGLOW, ebiosciences) was performed according to the manufacturer’s specifications. Apoptosis staining by annexin V+ (Annexin V Apoptosis Detection kit) was performed according to the manufacturer’s recommendations. BrdU staining was performed using BrdU Staining Kit (eBioscience) according to the manufacturer’s recommendations. For BCL2L11 staining, cells were fixed for 15 min in 2% formaldehyde solution, and permeabilized with flow cytometry buffer supplemented with 0.1% Triton X-100. All flow cytometry analysis and cell-sorting procedures were done at the University of Pennsylvania Flow Cytometry and Cell Sorting Facility using BD LSRII cell analysers and a BD FACsAria II sorter running FACSDiva software (BD Biosciences). FlowJo software (version 10 TreeStar) was used for data analysis and graphic rendering. All fluorochrome-conjugated antibodies used are listed in Supplementary Table 2.

Western blotting. 1 × 10⁶ wild-type and Morrbid-deficient neutrophils sorted from mouse bone marrow were assayed for BCL2L11 protein expression by western blotting (Bim C3435 rabbit monoclonal antibody; Cell Signaling), as previously described. ChIP-qPCR. 2 × 10⁶ wild-type and Morrbid-deficient neutrophils sorted from mouse bone marrow were cross-linked in a 1% formaldehyde solution for 5 min at room temperature while rotating. Crosslinking was stopped by adding glycine (0.2 M in 1 × PBS (phosphate buffered saline)) and incubating on ice for 2 min. Samples were spun at 2500g for 5 min at 4°C and washed 4 times with 1 × PBS. The pellets were flash frozen and stored at −80°C. Cells were lysed, and nuclei were isolated and sonicated for 8 min using a Covaris S220 (105 Watts, 2% duty cycle, 200 cycles per burst) to obtain approximately 200–500 bp chromatin fragments. Chromatin fragments were pre-cleared with protein G magnetic beads (New England BioLabs) and incubated with pre-bound anti-H3K27me3 (Qiagen), anti-EZH2 (ebiosciences), or mouse IgG1 (Santa Cruz Biotechnology) antibody-protein G magnetic beads overnight at 4°C. Beads were washed once in low-salt buffer (20 mM Tris, pH 8.1, 1 mM EDTA, 50 mM NaCl, 1% Triton X-100, 0.1% SDS), twice in high-salt buffer (20 mM Tris, pH 8.1, 2 mM EDTA, 500 mM NaCl, 1% Triton X-100, 0.1% SDS), once in LiCl buffer (10 mM Tris, pH 8.1, 1 mM EDTA, 0.25 mM LiCl, 1% NP-40, and 100 mM LiCl) for 30 min at 56°C, washed and then eluted overnight at 65°C. Washed beads were eluted twice with 100 μl of elution buffer (1% SDS, 0.1 M NaHCO3) and de-crosslinked (0.1 mg ml⁻¹ RNase, 0.3 mM NaCl and 0.3 mg ml⁻¹ Proteinase K) overnight at 65°C. The DNA samples were purified with Qiaquick PCR columns (Qiagen). qPCR was carried out on a ViiA7 Real-Time PCR System (ThermoFisher) using the SYBR Green detection system and indicated primers. Expression values of target loci were directly normalized to the indicated positive control loci, such as MxyD1 for H3K27me3 and EZH2 ChiP analysis, and Actb for Pol II ChiP analysis. ChiP–qPCR primer sequences are listed in Supplementary Table 1.

ATAC–seq preparation, sequencing, and analysis. 50,000 wild-type and knock-out cells, in triplicate, were spun at 500g for 5 min at 4°C, washed once with 50 μl of cold 1× PBS and centrifuged in the same conditions. Cells were resuspended in 50 μl of ice-cold lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% IGEPA, CA-630). Cells were immediately spun at 500g for 10 min at 4°C. Lysates were pipetted away from the pellet, which was then resuspended in 50 μl of the transposition reaction mix (25 μl 2× TD buffer, 2.5 μl Tn5 Transposase (Illumina), 22.5 μl nuclease-free water) and then incubated at 37°C for 30 min. DNA purification was performed using a Qiagen MiniElute kit and eluted in 12 μl of Elution buffer (10 mM Tris pH 8.0). To amplify library fragments, 6 μl of the eluted DNA was mixed with NEBNext High-Fidelity 2× PCR Master Mix, 25 μl of customized Nextera PCR primers 1 and 2 (Supplementary Table 1), 100x SYBR Green1 and used in PCR as follows: 72°C for 5 min; 98°C for 30s; and thermocycling 4 times at 98°C for 10s: 63°C for 30s; 72°C for 1 min. 5 μl of the 5 cycles PCR amplified DNA was used in a qPCR reaction to estimate the additional number of amplification cycles. Libraries were amplified for a total of 10–11 cycles and were then purified using a Qiagen PCR Cleanup kit and eluted in 30 μl of Elution buffer. The libraries were quantified using qPCR and bioanalyzer data, and then normalized and pooled to 2 nm. Each 2 nm pool was then denatured with a 0.1 N NaOH solution in equal parts then further diluted to form a 20 pM denatured pool. This pool was then further diluted down to 1.8 pM for sequencing using the NextSeq500 machine on V2 chemistry and sequenced on a 1× 75 bp Illumina NextSeq flow cell.

ATAC sequencing cells was done on Illumina NextSeq at a sequencing depth of 40–60 million reads per sample. Libraries were prepared in triplicates. Raw reads were deposited under GEO:GSE85073. 2× 75 bp paired-end reads were mapped to the mouse mm9 genome using ‘bwa’ algorithm with ‘mem’ option. Only reads that uniquely mapped to the genome were used in subsequent analysis. Duplicate reads were eliminated to avoid potential PCR amplification artifacts and to eliminate the high numbers of duplicate reads. Nux library sequences are listed in Supplementary Table 1. Normalized ChIP–seq data were deposited under GSE85073. 2× 75 bp paired-end reads were mapped to the mouse mm9 genome using ‘bwa’ algorithm with ‘mem’ option. Only reads that uniquely mapped to the genome were used in subsequent analysis. Duplicate reads were eliminated to avoid potential PCR amplification artifacts and to eliminate the high numbers of duplicate reads. Nux library sequences are listed in Supplementary Table 1. Normalized ChIP–seq data were deposited under GSE85073. Normalized ChIP–seq data were deposited under GSE85073.
and washed out with 100% ethanol. Dried DNA pellets were resuspended in 30 μl of 10 mM Tris HCL, pH 8.0, and DNA concentrations were quantified using Qubit. Starting with 10 ng of DNA, ChiP–seq libraries were prepared using the KAPA Hyper Prep Kit (Kapa Biosystems, Inc.) with 10 cycles of PCR. The libraries were quantified using qPCR and bioanalyzer data then normalized and pooled to 2 nM. Each 2 nM pool was then denatured with a 0.1 N NaOH solution in equal parts then further diluted to form a 20 pM denatured pool. This pool was then further diluted down to 1.8 pM for sequencing using the NextSeq500 machine on V2 chemistry and sequenced 157 × 75 bp Illumina NextSeq flow cell.

ChiP sequencing was done on an Illumina NextSeq at a sequencing depth of ~30–40 million reads per sample. Raw reads were deposited under GSE85073. 75 bp single-end reads were mapped to the mouse mm9 genome using ‘bowtie2’ algorithm. Duplicate reads were eliminated to avoid potential PCR amplification artifacts and only reads that uniquely mapped to the genome were used in subsequence analysis. Depicted tracks were normalized to control IgG input sample. ChiP–seq-enriched regions (peaks) in each sample was identified using MACS2 using the below settings:

MACS2-2.1.0.20140616/bin/mac2 callpeak -c < ChiP tag file> - g < control tag file> -f BED-gm- mm-nomodel –extsize=250 –hgsq-broad - n < output peak file>

RIP–qPCR. 107 immortalized BMDMs were collected by trypsinization and resuspended in 2 ml PBS, 2 ml nuclear isolation buffer (1.28 M sucrose; 40 mM Tris-HCl, pH 7.5; 20 mM MgCl2; 4% Triton X-100), and 6 ml water on ice for 20 min (with frequent mixing). Nuclei were pelleted by centrifugation at 2,500 g for 15 min. Nuclear pellets were resuspended in 1 ml RNA immunoprecipitation (RIP) buffer (150 mM KCl, 25 mM Tris, pH 7.4, 5 mM EDTA, 0.5 mM DTT, 0.5% NP40; 100 μl 1 SUPERaseIn, Amion; complete EDTA-free protease inhibitor, Sigma). Resuspended nuclei were split into two fractions of 500 μl each (for mock and immunoprecipitation) and were mechanically sheared using a dounce homogenizer. Nuclear membranes and debris were pelleted by centrifugation at 15,800 g for 10 min. Antibody to EZH2 (Cell Signaling 4905S; 1:30) or normal rabbit IgG (mock immunoprecipitation, SantaCruz; 10 μg) were added to supernatant and incubated for 2 hours at 4 °C with gentle rotation. 25 μl of protein G beads (New England BioLabs S1430S) were added and incubated for 1 hour at 4 °C with gentle rotation. Beads were pelleted by magnetic field, the supernatant was removed, and beads were resuspended in 500 μl RIP buffer and repeated for a total of three RIP buffer washes, followed by one wash in PBS. Beads were resuspended in 1 ml of Trizol. Co-precipitated RNAs were isolated, reverse-transcribed to cDNA, and assayed by qPCR for the Hprt and Morbid–isoform1. Primer sequences are listed in Supplementary Table I.

PAR–CLIP analysis. EZH2 PAR–CLIP dataset (GSE49435) was analysed as previously described22. Adapter sequences were removed from total reads and those longer than 17 bp were kept. The Fastx toolkit was used to remove duplicate sequences, and the resulting reads were mapped using BOWTIE allowing for two mismatches. The four independent replications were pooled and analysed using PARalyzer, requiring at least two T tags for two mismatches. The four independent replicates were pooled and analysed

Chromosome conformation capture (3C). 1 × 107 wild-type bone marrow derived mouse eosinophils were fixed with 1% formaldehyde for 10 minutes at room temperature, and crosslinks were reversed by proteinase K digestion (300 μg) for 1 hour at 37 °C. Culture was readjusted to 2 ml at 2.5 × 106 cells per ml in PBS or PBS alone every 24 hours. Mice were killed 12 hours after the last challenge. Bronchoalveolar lavage was collected in two 1 ml lavages of PBS. Cellular lung infiltrates were collected after 1 hour digestion in RPMI supplemented with 5% FCS, 1 mg ml−1 collagenase D (Roche) and 10 μg ml−1 DNase I (Invitrogen) at 37 °C. Homogenates were passed through a cell strainer and infiltrates separated with a 27.5%, Optiprep gradient (Axis-Shield) by centrifugation at 1,750 g for 30 min at 4 °C, and CD45.2+ cells reseeded on 60 mm gelatinized plates.

Bone marrow chimaeras. Congenic C57BL/6 (wild-type) bone marrow expressing CD45.1 and CD45.2 and Morbid-deficient bone marrow expression CD45.2 was mixed in a 1:1 ratio and injected into C57BL/6 hosts irradiated twice with 5 Gy 3 hours apart that express CD45.1 (B6.SJL–Ptprc Peplf1/BoyJ). Mice were analysed between 4–9 weeks after injection.

Bone–derived macrophage cultures. Bone marrow was isolated and cultured as previously described23. Briefly, unfragmented bone marrow cell cultures were pelleted with 100 ng ml−1 stem cell factor (SCF) and 100 ng ml−1 FLT3-ligand (FLT3-L). At day 4, the media was replaced with media containing 10 ng ml−1 interleukin (IL-3). Mature bone–marrow-derived eosinophils were analysed between day 10–14.

Bone–derived macrophage cultures. Bone marrow cells were isolated and cultured in media containing recombinant mouse M-CSF (10 ng ml−1) for 7–8 days. On day 7–8, cells were re-plated for use in experimental assays. Bone–derived macrophage cultures were stimulated with LPS (250 ng ml−1) for the indicated periods of time.

ChIP–qPCR. Briefly, 40 μl Immortalized bone–marrow-derived macrophages were fixed with 40 μl of 1% glutaraldehyde for 10 min at room temperature. Crosslinking was quenched with 0.125 M glycine for 5 min. Cells were rinsed with PBS, pelleted for 4 min at 2,000g, snap-frozen in liquid nitrogen, and stored at −80 °C. Cell pellets were thawed at room temperature and resuspended in 800 μl of lysis buffer (50 mM Tris-HCl, pH 7.0, 10 mM EDTA, 1% SDS, 1 mM PMSF, complete protease inhibitor (Roche), 0.1 μM −1 Supersase In (Life Technologies)). Cell suspension was sonicated using a Covaris S220 machine (Covaris; 100 W, duty factor 20%, 200 cycles per burst) for 60 minutes until DNA was in the size range of 100–500 bp. After centrifugation for 5 min at 16100 g at 4 °C, the supernatant was aliquoted, snap-frozen in liquid nitrogen, and stored at −80 °C. 1 ml of chromatin was diluted in 2 ml hybridization buffer (750 mM NaCl, 1% SDS, 50 mM Tris HCl, pH 7.0, 1 mM EDTA, 15% formamide) and input RNA and DNA aliquots were removed. 100 μl of probes (Supplementary Table I) and 10 μl DNA elution buffer (50 mM NaHCO3, 1% SDS, 200 mM NaCl, 100 μg ml−1 RNase A, 100 μg ml−1 RNase H) incubated for 30 min at 37 °C. DNA elutions were combined and treated with 15 μl (20 mg ml−1) Proteinase K for 45 min at 50 °C. DNA was purified using phenol: chloroform:isoamyl and assayed by qPCR using the indicated primer sequences (Supplementary Table I).

shRNA generation and transduction. shRNAs of indicated sequences (Supplementary Table I) were cloned into pGreen shRNA cloning and expression vectors. Equal volume of RNA was reverse-transcribed and assayed by qPCR using Hprt and Morbid–isoform1 primer sets (Supplementary Table I). DNA was eluted from remaining bead fraction twice using 150 μl DNA elution buffer (50 mM NaHCO3, 1% SDS, 200 mM NaCl, 100 μg ml−1 RNase A, 100 μg ml−1 RNase H) incubated for 30 min at 37 °C. DNA elutions were combined and treated with 15 μl (20 mg ml−1) Proteinase K for 45 min at 50 °C. DNA was purified using phenol: chloroform:isoamyl and assayed by qPCR using the indicated primer sequences (Supplementary Table I).

For transduced BM-derived eosinophils, cultured BM cells on day 3 of previously described culture conditions were mixed 1:1 with indicated lentivirus and performed pseudo-typed lentivirus was generated as previously described, and 293T cells were transfected with a packaging plasmid, envelop plasmid, and the generated shRNA vector plasmid using Lipofectamine 2000. Virus was collected 14–16h and 48h after transfection, combined, 0.4 μM filtered, and stored at −80 °C. For generation of in vivo BM chimaeras, virus was concentrated 6 times by ultracentrifugation using an Optiprep gradient (Axis-Shield).

For transduced in vivo BM chimaeras, BM cells were cultured at 2.5 × 106 cells per ml in mL−3 (10 ng ml−1), mL−6 (5 ng ml−1) and mSCF (100 ng ml−1) overnight at 37 °C. Culture was readjusted to 2 ml at 2.5 × 106 cells per ml in a 6-well
plate, and spinfected for 2 h at 260g at 25 °C with 5yg ml−1 polybrene. Cells were incubated overnight at 37 °C. On the day before transfer, recipient hosts were irradiated twice with 5 Gy 3 hours apart. Mice were analysed between 4 and 5 weeks following transfer.

**Locked nucleic acid knockdown.** Bone marrow-derived macrophages (BMDMs) were transfected with pooled *Morribid* or scrambled locked nucleic acid (LNA) antisense oligonucleotides of equivalent total concentrations using Lipofectamine 2000. *Morribid* LNA pools contained *Morribid* LNA 1-4 sequences at a total of 50 or 100nm oligonucleotide (Supplementary Table 1). After 24h, the transfection media was replaced. The BMDMs were incubated for an additional 24h and subsequently stimulated with LPS (250ng ml−1) for 8–12h.

Eosinophils were derived from mouse BM as previously described. On day 12 of culture, 1×102 to 2×106 eosinophils were transfected with 50nm of *Morribid* LNA 3 or scrambled LNA (Supplementary Table 1) using TransIT-oligo according to manufacturer’s protocol. RNA was extracted 48h after transfection.

**Morribid promoter deletion.** Guide RNAs (gRNAs) targeting the 5’ and 3’ flanking regions of the *Morribid* promoter were cloned into Cas9 vectors pSPCas9(BB)-2A-GFP(PX458) (Addgene plasmid 48138) and pSPCas9(BB)-2A-mCherry (a gift from the Stitzel lab, JAX-GM) respectively. gRNA sequences are listed in Supplementary Table 1. The cloned Cas9 plasmids were then transfected into RAW 264.7, a macrophage cell line using Lipofectamine 2000, according to manufacturer’s protocol. Forty–eight hours post transfection the double positive cells expressing GFP and mcherry, and the double negative cells lacking GFP and mcherry were sorted. The bulk sorted cells were grown in a complete media containing 20% FBS, assayed for deletion by PCR, as well as for *Morribid* and Bcl2111 transcript expression by qPCR.

**Ex vivo cytokine stimulation.** BM–derived eosinophils, or neutrophils or Ly6Chi monocytes sorted from mouse BM, were rested for 4–6 hours at 37 °C in complete media. Cells were subsequently stimulated with IL–3 (10 ng ml−1; Biologend), IL–5 (10 ng ml−1; Biologend), GM-CSF (10 ng ml−1; Biologend), or G-CSF (10 ng ml−1; Biologend) for 4–6h. RNA was collected at each time-point using TRIzol (Life Technologies).

**GSK126 treatment.** Wild-type and Bcl2111−/− BM–derived eosinophils were generated as previously described6. On day 8 of culture, the previously described IL–5 media was supplemented with the indicated concentrations of the EZH2–specific inhibitor GSK126 (Toronto Research Chemicals). Media was exchanged for fresh IL–5 GSK126 containing media every other day. Cells were assayed for numbers and cell death by flow cytometry every day for 6 days following GSK126 treatment.

**RNA extraction, cDNA synthesis and quantitative RT–PCR.** Total RNA was extracted from TRIzol (Life Technologies) according to the manufacturer’s instructions. Gycogen (ThermoFisher Scientific) was used as a carrier. Isolated RNA was quantified by spectrophotometry, and RNA concentrations were normalized. cDNA was synthesized using SuperScript II Reverse Transcriptase (ThermoFisher Scientific) according to the manufacturer’s instructions. Resulting cDNA was analysed by SYBR Green (KAPA SYBR Fast, KAPABiosystems) or Taqman-based (KAPA Probe Fast, KAPABiosystems) using indicated primers. Primer sequences are listed in Supplementary Table 1. All reactions were performed in duplicate using the MEGAclear kit (Life Technologies). RNA was quantified using spectrophotometry and serial dilutions of *Morribid* RNA of calculated copy number were spiked into *Morribid*-deficient RNA isolated from *Morribid*-deficient mouse spleen. Samples were reverse transcribed in parallel with wild–type–sorted neutrophil RNA and B-cell RNA isolated from known cell number using gene–specific *Morribid* primers, and the *Morribid* standard curve and wild-type neutrophils and B cells were assayed using qPCR with *Morribid*-exon1–1 and other known cytoplasmic and nuclear transcripts. Primer sequences are listed in Supplementary Table 1.

**Copy number analysis.** *Morribid* cDNA was cloned into reference plasmid (pC DNA3.1) containing a T7 promoter. The plasmid was linearized and *Morribid* RNA was in vitro transcribed using the MEGASHortscript T7 kit (Life Technologies), according to the manufacturer’s recommendations, and purified using the MEGAclear kit (Life Technologies). RNA was quantified using spectrophotometry and serial dilutions of *Morribid* RNA of calculated copy number were spiked into *Morribid*-deficient RNA isolated from *Morribid*-deficient mouse spleen. Samples were reverse transcribed in parallel with wild–type–sorted neutrophil RNA and B-cell RNA isolated from known cell number using gene–specific *Morribid* primers, and the *Morribid* standard curve and wild-type neutrophils and B cells were assayed using qPCR with *Morribid*-exon1–1 primer sets (Supplementary Table 1).

**Bromodeoxyuridine incorporation assay.** Cohorts of mice were given a total of 4g mg bromodeoxyuridine (BrDU; Sigma Aldrich) in 2 separate intraperitoneal (i.p.) injections 3 h apart and monitored over the subsequent 5 days, unless otherwise indicated. For analysis cells were stained according to manufacturer protocol (BrDU Staining Kit; eBioScience; anti–BrDU, Biologend). A one–phase exponential curve was fitted from the peak labelling frequency to 36h after peak labelling within each genetic background, and the half–life was determined from this curve.

**Human samples. Human subject cohort 1.** Study subjects were recruited and consented in accordance with the University of Pennsylvania Institutional Review Board. Peripheral blood was separated by Ficoll–Paque density gradient centrifugation, and the mononuclear cell layer and erythrocyte/granulocyte pellet were isolated and stained for fluorescence–associated cell sorting as previously described (Life Technologies). RNA was quantified using spectrophotometry and serial dilutions of *Morribid* RNA of calculated copy number were spiked into *Morribid*-deficient RNA isolated from *Morribid*-deficient mouse spleen. Samples were reverse transcribed in parallel with wild–type–sorted neutrophil RNA and B-cell RNA isolated from known cell number using gene–specific *Morribid* primers, and the *Morribid* standard curve and wild-type neutrophils and B cells were assayed using qPCR with *Morribid*-exon1–1 primer sets (Supplementary Table 1).

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or to provide controls for in vitro research (NCT00090662). All participants gave written informed consent. Eosinophils were purified from peripheral blood by negative selection and frozen at −80°C in TRIzol (Life Technologies). Purity was >97% as assessed by cytospin. RNA was purified according to the manufacturer’s instructions. Expression analysis by qPCR was performed in a blinded manner by an individual not involved in sample collection or coding of these of these samples. Plasma IL-5 levels were measured by suspension array in multiplex (Millipore). The minimum detectable concentration was 0.1 pg ml$^{-1}$.

**Cell lines.** RAW 264.7 cells were obtained from ATCC and were not authenticated, but were tested for mycoplasma contamination biannually. Immortalized C57/B6 macrophages were obtained as a generous gift from I. Brodsky. These cells were not authenticated, but were tested for mycoplasma contamination biannually.

**Statistics.** Samples sizes were estimated based on our preliminary phenotyping of Morrbid-deficient mice. Preliminary cell number analysis of eosinophils, neutrophils, and Ly6C$^+$ monocytes suggested that there were very large differences between wild-type and Morrbid-deficient samples, which would allow statistical interpretation with relatively small numbers and no statistical methods were used to predetermine sample size. No animals were excluded from analysis. All experimental and control mice and human samples were run in parallel to control for experimental variability. The experiments were not randomized. Experiments corresponding to Fig. 3g–i and Fig. 4g–j were performed and analysed in a single-blinded manner. All other experiments were not blinded to allocation during experiments and outcome assessment. Correlation was determined by calculating the Spearman correlation coefficient. Half-life was estimated by calculating the one-phase exponential decay constant from the peak of labelling frequency to 36 h after peak labelling. *P* values were calculated using a two-way *t*-test, Mann–Whitney *U*-test, one-way ANOVA with Tukey post-hoc analysis, Kaplan–Meier Mantel–Cox test, and false discovery rate (FDR) as indicated. FDR was calculated using trimmed mean of M-values (TMM)-normalized read counts and the DiffBind R package as described in Extended Data Fig. 7c, d. All error bars indicate mean plus and minus the standard error of mean (s.e.m.).
Extended Data Figure 1 | See next page for caption.
Extended Data Figure 1 | *Morrbid* transcript expression, localization, and conservation across species. **a**, Left: mouse, human and cow *Morrbid* transcripts. Human neutrophil, mouse granulocyte and cow peripheral blood RNA-seq data are represented as read density around the *Morrbid* transcript of each species. Right: the *Morrbid* loci and surrounding genomic regions of the indicated species were aligned with mVista and visualized using the rankVista display generated with mouse as the reference sequence. Green highlights annotated mouse exonic regions and corresponding regions in other indicated species. **b**, Quantification of *Morrbid* FISH spots per indicated cell population. Cells were stained with *Morrbid* RNA probes conjugated to 2 different fluorophores, and spots colocalizing in both fluorescent channels were quantified. **c**, Cytoplasmic and nuclear subcellular RNA fractionation of LPS-stimulated BMDMs with qPCR of indicated target transcripts (n = 3 macrophages generated from independent mice). **d**, Cytoplasmic, nuclear and chromatin subcellular RNA fractionation of LPS-stimulated immortalized BMDMs with qPCR of indicated target transcripts (average of 4 independent experiments). **e**, Mature eosinophil transcriptome sorted in descending order of log(RPKM) gene expression, with annotated select reported eosinophil-associated genes. **f**, Average number of *Morrbid* RNA copies per cell in sorted neutrophils and B cells. Left: standard curve generated using *in vitro* transcribed *Morrbid* RNA spiked into *Morrbid*-deficient RNA isolated from spleen. Right: calculated per cell *Morrbid* RNA copies (n = 3 replicates from independent mice). **g**, Representation of CRISPR–Cas9 targeting of the *Morrbid* locus with indicated guide RNA (gRNA) sequences and genotyping primer sets. Target gRNA sequences are bolded. **h**, Cells isolated from the blood of wild-type mice. Representative flow cytometry plots demonstrating the gating strategy for neutrophils (CD45^+^CD11b^+^LY6G^-^), T cells (CD45^-^LY6G^-^CD3^+^), B cells (CD45^-^LY6G^-^CD3^-^CD19^+^), eosinophils (CD45^-^CD3^-^CD19^-^LY6G^-^SiglecF^-^SSC^-^), Ly6C^-^ monocytes (CD45^-^CD3^-^CD19^-^LY6G^-^SSC^-^SiglecF^-^Ly6C^-^CSF-1R^-^), NK cells (CD45^-^CD3^-^CD19^-^LY6G^-^SSC^-^SiglecF^-^CSF-1R^-^NK1.1^-^). **i**, Total cell numbers of the indicated cell populations isolated from the spleen of wild-type and *Morrbid*-deficient mice (n = 3–5 mice per group, results representative of 8 independent experiments). Error bars show s.e.m. *P < 0.05, **P < 0.01, and ***P < 0.001 (two-sided t-test, c, f, i; one-way ANOVA with Tukey post-hoc analysis, d).
Extended Data Figure 2 | Myeloid cell populations in tissue following Morrbid deletion, and blood and spleen following Morrbid knockdown in vivo. a, Representative flow cytometry plots and absolute counts of the indicated cell populations in wild-type and Morrbid-deficient mice (n = 3–5 mice per group, representative of 3–7 independent experiments). b, shRNA knockdown of Morrbid RNA relative to control vector in BM-transduced with the indicated GFP vector, sorted on GFP, differentiated into eosinophils and assessed by qPCR (each dot represents eosinophils generated from independent mice). c, Schematic of control and Morrbid shRNA1 BM chimaera generation. d, e, Frequency of indicated cell populations within total GFP+ transduced cells from blood (d) and spleen (e) (n = 3–4 mice per transduction group). f–h, Wild-type and Morrbid-deficient mice challenged with papain or PBS. f, Absolute numbers of indicated cell populations in lung tissue and broncholalveolar lavage (BAL). g, qPCR expression in lung tissue. h, Representative haematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) stain lung histology at 40× magnification (n = 3–4 mice per group; representative of two independent experiments). Error bars show s.e.m. *P < 0.05, **P < 0.01, and ***P < 0.001 (two-sided t-test, a, b, d, e; Mann-Whitney U-test, f, g).
Extended Data Figure 3 | See next page for caption.
Extended Data Figure 3 | Morbid regulation of mature neutrophils, eosinophils and Ly6C<sup>hi</sup> monocytes is cell intrinsic. a–e, Morbid-deficient competitive BM chimaera generation. a, Schematic of mixed BM chimaera generation. Congenically labelled wild-type CD45.1<sup>+</sup>CD45.2<sup>+</sup> and Morbid-deficient CD45.2<sup>+</sup> BM cells were mixed 1:1 and injected into an irradiated CD45.1<sup>+</sup> host. b, Ratio of mixed congenically labelled wild-type CD45.1<sup>+</sup>CD45.2<sup>+</sup> and Morbid-deficient CD45.2<sup>+</sup> BM cells before injection into an irradiated CD45.1<sup>+</sup> host. c, d, Ratio of Morbid-deficient to wild-type short-lived myeloid and control immune cells in blood (c) and representative flow cytometry plots of these cell populations (d). e, Morbid-deficient to wild type ratio of additional immune cell populations (n = 4–8 mice per group; pooled from two independent experiments). f, Schematic of myeloid differentiation and Morbid qPCR expression in the indicated sorted progenitor and mature cells (n = 3–5 mice per group; representative of 3 independent experiments). g, Cells isolated from the BM of wild-type mice. Representative flow cytometry plots demonstrating the gating strategy for common myeloid progenitor (CMP): lineage (Sca1, CD11b, GR-1, CD3, Ter-119, CD19, B220, NK1.1), IL7Ra<sup>−</sup> C-kit<sup>−</sup>CD34<sup>−</sup>CD16/32<sup>lo/int</sup>; granulocyte/monocyte progenitor (GMP): lineage IL7Ra<sup>−</sup> C-kit<sup>−</sup>CD34<sup>−</sup>CD16/32<sup>hi</sup>; monocyte/dendritic cell progenitor (MDP): lineage IL7Ra<sup>−</sup> C-kit<sup>−</sup>CD115<sup>−</sup>CD135<sup>+</sup> ; eosinophil progenitor (EosP): lineage IL7Ra<sup>−</sup> C-kit<sup>−</sup>CD34<sup>−</sup>CD16/32<sup>hi</sup>IL-5Ra<sup>+</sup>. h, Cells isolated from the BM of wild-type mice. Representative flow cytometry plots demonstrating the gating strategy for eosinophils: dump<sup>−</sup> (dump: CD3, NKp46, Ter119, CD19, Ly6G, Sca1), CSF-1R<sup>−</sup> C-kit<sup>−/lo</sup>SiglecF<sup>+</sup> SSC<sup>hi</sup>; monocytes: dump<sup>−</sup> CSF-1R<sup>+</sup> C-kit<sup>−</sup> MHCII<sup>−</sup> Ly6Chi; common monocyte progenitor (cMoP): dump<sup>−</sup> CSF-1R<sup>−</sup> C-kit<sup>−</sup> Ly6Ch<sup>hi</sup>CD11b<sup>lo</sup>. Flow cytometry count beads are visualized and gated by forward and side scatter area (g, h). Error bars show s.e.m. *P < 0.05, **P < 0.01, and ***P < 0.001 (one-way AVONA with Tukey post-hoc test analysis).
Extended Data Figure 4 | See next page for caption.
Extended Data Figure 4 | Morbid regulates neutrophil, eosinophil and Ly6C^hi monocyte lifespan through cell-intrinsic regulation of Bcl2l11.

a, Flow cytometric analysis of percentage of BrdU incorporation in the indicated wild-type and Morbid-deficient immune cell populations from blood. Mice were analysed 24 h after one dose of 2 mg BrdU (n = 3 mice per group). b, Representative flow cytometry plots and absolute counts of mature eosinophils (live, CD45^hi SSC^hi CD11b^hi Siglec F^-) of BM-derived eosinophil culture on day 12 in wild-type and Morbid-deficient mice (n = 3 mice per group, results representative of 3 independent experiments). c, Morbid expression of developing wild-type BM-derived eosinophils at indicated time points of in vitro culture (n = 3 mice per group). d, Percentage of annexin V^+ wild-type and Morbid-deficient BM cell populations at indicated time points of ex vivo culture (n = 3 mice per group; data are representative of 3 independent experiments). e, Percentage of annexin V^+ eosinophils (gated on annexin-V^+ CD45^hi SSC^hi CD11b^hi SiglecF^-) of BM-derived eosinophil culture on day 12 in wild-type and Morbid-deficient mice (n = 3 mice per group, results representative of 3 independent experiments). f, Percentage of annexin V^+ wild-type and Morbid-deficient neutrophils and Ly6C^hi monocytes 4 days after L. monocytogenes infection (n = 3 mice per group, representative of 2 independent experiments). g, Flow cytometric analysis of percentage and absolute number of blood neutrophils from wild-type or Morbid-deficient mice that were pulsed two times with 2 mg BrdU 3 h apart and monitored over 5 days (n = 4 mice per group; data are representative of three independent experiments). h, Western blot analysis of BCL2L11 protein expression in wild-type and Morbid-deficient sorted BM neutrophils. I, BCL2L11 protein expression measured by flow cytometry in blood neutrophils from wild-type, Morbid-deficient and Bcl2l11-deficient mice (n = 1–4 mice per group). j, k, BCL2L11 protein expression in mixed BM chimaera model. Quantification of mean fluorescence intensity (MFI) of BCL2L11 protein expression in indicated cell populations from blood (j) and BM (k) (n = 4–8 mice per group, results representative of two independent experiments). I, BCL2L11 protein expression in the indicated progenitors and mature cell types from wild-type and Morbid-deficient mice. ‘n/a’ indicates that too few cells were present for MFI quantification (n = 3–5 mice per group, results representative of 3 independent experiments). m, BCL2L11 expression measured in the indicated cell populations from wild-type and Morbid-deficient mice (n = 3, results representative of two independent experiments). Error bars show s.e.m. *P < 0.05, **P < 0.01, and ***P < 0.001 (two-sided t-test).
Extended Data Figure 5 | **Morrbid** specifically controls Bcl2111 expression. a, Schematic representation of genes surrounding the **Morrbid** locus. b, c. Expression of indicated transcripts assessed by qPCR in neutrophils (b) and Ly6C<sup>hi</sup> (c) monocytes sorted from wild-type and **Morrbid**-deficient mice. ND (not detected) indicates expression was below the limit of detection (n = 3 mice per group, representative of 2 independent experiments). Error bars show s.e.m. *P < 0.05, **P < 0.01, and ***P < 0.001 (two-sided t-test).
Extended Data Figure 6 | See next page for caption.
Extended Data Figure 6 | Knockdown of *Morrbid* leads to *Bcl2l11* upregulation and cell death.  

**a.** Schematic of shRNA-transduced BM-derived eosinophil system. **b–d.** *In vitro* shRNA BM-derived eosinophil competitive chimaera. **b.** Schematic of transduction of CD45.2+ and CD45.1+ BM cells transduced with GFP scrambled shRNA or GFP *Morrbid*-specific shRNA lentiviral vectors, respectively. GFP+ cells were sorted, mixed 1:1, differentiated into eosinophils, and analysed by flow cytometry. **c.** Representative histogram and MFI quantification of *BCL2L11* expression of mature eosinophils separated by congenic marker. **d.** Percentage of contribution of each congenic BM to the total mature eosinophil pool (*n* = 3 mice per group, each dot represents eosinophils differentiated from the BM of 1 mouse, representative of 2 independent experiments). **e.** *Morrbid* and *Bcl2l11* expression of wild-type BM-derived eosinophils transinfected with *Morrbid*-specific LNA 3 and control LNA (each dot represents the average of 2–3 biological replicates, data pooled from 5 independent experiments). **f.** *Morrbid* and *Bcl2l11* expression of wild-type and *Morrbid*-deficient BM-derived macrophages at the indicated time points following LPS stimulation. Expression is represented as fold change from time 0 (*t*0) (*n* = 3 mice per group, representative of 3 independent experiments). **g–i.** LPS-stimulated BM-derived macrophages transfected with pooled *Morrbid*-specific (LNA 1–4) or scrambled (cntrl LNA) antisense LNAs. **g.** *Morrbid* and *Bcl2l11* qPCR expression; **h.** Annexin V expression; **i.** absolute BM-derived macrophage numbers (*n* = 3 mice per group, representative of 6 independent experiments). **j–l.** *Morrbid* promoter deletion in immortalized BMDMs. **j.** Diagram of *Morrbid* promoter targeting in immortalized BMDMs using CRISPR-Cas9. Immortalized BMDMs were transfected with GFP-expressing Cas9 and Cherry-expressing gRNA vectors of the indicated sequences. **k, l.** GFP+/Cherry+ and GFP−/Cherry− expressing cells were sorted and assayed at the bulk level using PCR for verification of promoter deletion using the indicated primers (*j, k*) and qPCR for *Morrbid* and *Bcl2l11* expression following LPS stimulation for 6 hours (*l*) (*n* = 3 LPS-stimulated cultures, average of 3 independent experiments). **m.** *Morrbid* and *Bcl2l11* transcript expression in wild-type and *Morrbid*-deficient sorted BM-derived neutrophils stimulated with G-CSF for 4 h. Expression is represented as fold change from unstimulated (*n* = 3 mice, representative of 2 independent experiments). Error bars show s.e.m. *P* < 0.05, **P** < 0.01, and ***P*** < 0.001 (two-sided *t*-test).
Extended Data Figure 7 | See next page for caption.
Extended Data Figure 7 | Epigenetic effect of Morrbid deletion on its surrounding genomic region. a, ChIP–qPCR analysis of total Pol II enrichment within the Bcl2l11 promoter and gene body in wild-type and Morrbid-deficient neutrophils. Results are represented as Bcl2l11 enrichment relative to control Actb enrichment within each sample. Each dot represents 1–2 pooled mice. b, ChIP–qPCR analysis of EZH2 enrichment within the Bcl2l11 promoter in wild-type and Morrbid-deficient BMDMs stimulated with LPS for 12 hours. Results are represented as Bcl2l11 enrichment relative to control MyOD1 enrichment within each sample (n = 3, each dot represents BMDMs generated from 1 mouse). c, Relative chromatin accessibility levels at the Bcl2l11, Acox1, Anapc1 and Mertk promoters in Morrbid−/− and wild-type neutrophils as assessed by ATAC–seq. Chromatin accessibility levels were estimated as an average trimmed mean of M-values (TMM)-normalized read count across the replicates. Statistics were obtained by differential open chromatin analysis using the DiffBind R package. The Bcl2l11 promoter is more open in Morrbid−/− neutrophils with a 1.52-fold change with a FDR of < 0.1%. ND (not detected) indicates that no peak was present at the indicated promoter. d, Density plot of log, fold-change distribution for H3K4me1, H3K4me3, H3K27ac and H3K36me3 levels between Morrbid−/− and wild-type neutrophils. Relative fold changes are estimated as the ratio of TMM-normalized read counts within consensus peak regions and were obtained using the DiffBind R package. Positive and negative fold changes indicate higher levels of ChIP binding in Morrbid−/− and wild-type neutrophils, respectively. Dashed green lines show the 5th and 95th percentiles. The green triangles on the x axis mark the change at the Bcl2l11 promoter or gene body between wild-type and Morrbid−/− neutrophils. e, f, ATAC–seq and ChIP–seq for H3K4me1, H3K4me3, H3K27ac and H3K36me3 chromatin modifications were performed on neutrophils sorted from the bone marrow of wild-type and Morrbid-deficient mice. ATAC–seq and ChIP–seq are represented as read density surrounding the Morrbid locus (e) and at the Bcl2l11 locus (f). ATAC–seq tracks are expressed as reads normalized to total reads, and chromatin modification tracks are expressed as reads normalized to input. Error bars show s.e.m. *P < 0.05, **P < 0.01, and ***P < 0.001 (two-sided t-test, a, b; FDR of fold change as described above, c, d).
Extended Data Figure 8 | See next page for caption.
**Extended Data Figure 8 | Morrbid represses Bcl2l11 by maintaining its bivalent promoter in a poised state and phenotype of Morrbid heterozygous mice.**

a, Venn diagram summary of EZH2 PAR–CLIP analysis, with representation of tags and RNA–protein contact sites as determined by PARalyzer mapping to Morrbid. RNA contact sites (RCS) are displayed in red. b, Co-immunoprecipitation of the PRC2 family member EZH2 and Morrbid. Nuclear extracts of immortalized wild-type BMDMs stimulated with LPS for 6–12 h were immunoprecipitated by IgG or anti-EZH2 antibodies. Co-precipitation of indicated RNAs were assayed by qPCR. Data are represented as enrichment over IgG control ($n = 6$ biological replicates pooled from 2 independent experiments, representative of 3 independent experiments). c, Validation of Morrbid RNA pull-down over other RNAs using pools of Morrbid capture probes and LacZ probes ($n = 3$, average of 3 independent experiments). d, Visualized 3C PCR products from bait and indicated reverse primers using template from fixed and ligated BM-derived eosinophil DNA (S1, S2 and S3), BAC control (BAC) or water. The sequence of each reverse primer is listed in Supplementary Table 1. e, f, BM-derived eosinophils from wild-type and Bcl2l11$^{-/-}$ mice treated with EZH2 inhibitor GSK126 over time. Frequency of non-viable (Aqua$^+$) (e) and annexin V (f) staining cells on day 5 following treatment with GSK126 ($n = 3$ independently differentiated eosinophils per dose, results representative of 2 independent experiments). g, Total cell numbers (top) and BCL2L11 protein expression (bottom) of indicated cell populations from the blood of wild-type, Morrbid-heterozygous and Morrbid-deficient mice ($n = 3–5$ mice per group, results representative of 3 independent experiments). Error bars show s.e.m. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ (two-sided $t$-test, c, g; one-way ANOVA with Tukey post-hoc analysis, e, f; Mann–Whitney $U$-test, b).
Extended Data Figure 9 | Generation of \textit{Morrbid-Bcl2l11} double heterozygous mice. Diagram of allele specific CRISPR–Cas9 targeting of \textit{Bcl2l11}. \textit{Bcl2l11} was targeted using indicated gRNA sequences in one-cell embryos from a wild-type by \textit{Morrbid}-deficient breeding. F1 mice with allele-specific \textit{Bcl2l11} deletions in \textit{cis} or in \textit{trans} of the \textit{Morrbid}-deficient allele were bred to a wild-type background to demonstrate linkage or segregation of \textit{Bcl2l11} and \textit{Morrbid} knockout alleles. Second–rightmost lanes of both gels contain \textit{Morrbid}−/−-\textit{Bcl2l11}+/+ DNA, and rightmost lanes contain water, as internal controls.

\textbf{Allele 1: Morrbid} Δ

\textbf{Allele 2: WT}

\textbf{F1 in Cis:} \textit{Bcl2l11} +1Δ x WT

\textbf{F1 in Trans:} \textit{Bcl2l11} +1Δ x WT

\textbf{gRNA1:} 5’ ACTCTAGAATCTTTAACAAAAAGTTTATGAGCTGAAATAGCAAGTTAAAATAAGGCTAG TCCGTTATCAACTTGAAAAAGTGAGCCACCGAGTCGGTGTCTTTTTT 3’

\textbf{gRNA2:} 5’ AAGTTTGTGTCGTGAAATTAGTTTATGAGCTGAAATAGCAAGTTAAAATAAGGCTAG TCCGTTATCAACTTGAAAAAGTGAGCCACCGAGTCGGTGTCTTTTTT 3’

\textbf{\textit{Bcl2l11} primers:} 5’ TCCGATTAGTCCACTCTGC 3’; 5’ TTCCCTTTTACATGCCCTGTTG3’
Extended Data Figure 10 | See next page for caption.
Extended Data Figure 10 | Morrbid regulates Bcl2l11 in an allele-specific manner and working model of the role of Morrbid. a, Diagram of the allele-specific combinations of Morrbid- and Bcl2l11-deficient heterozygous mice studied. b, Representative flow cytometry plots of indicated splenic cell populations in the specified allele-specific deletion genetic backgrounds. Neutrophils (CD45⁺ CD11b⁺ LY6G⁺), monocytes (CD45⁺ CD3⁻ CD19⁻ Ly6G⁻ SScᵐ SiglecF⁻ Ly6Cʰ CSF-1R⁺) and B cells (CD45⁺ Ly6G⁻ CD3⁻ CD19⁺). Wild-type (WT), Morrbid heterozygote (Het), Bcl2l11 heterozygote and Morrbid heterozygote with deletions in trans (Trans), Bcl2l11 heterozygote and Morrbid heterozygote with deletions in cis (Cis). c, d, Absolute counts (c) and BCL2L11 protein expression (d) of indicated splenic cell populations in the specified genetic backgrounds (n = 3–9 mice per genetic background). e, Morrbid integrates extracellular signals to control the lifespan of eosinophils, neutrophils and classical monocytes through the allele-specific regulation of Bcl2l11. Pro-survival cytokines induce Morrbid, which promotes enrichment of the PRC2 complex within the bivalent Bcl2l11 promoter through direct and potentially indirect mechanisms to maintain this gene in a poised state. Tight control of the turnover of these short-lived myeloid cells by Morrbid promotes a balance of host anti-pathogen immunity with host damage from excess inflammation. Error bars show s.e.m. *P < 0.05, **P < 0.01, and ***P < 0.001 (one-way ANOVA with Tukey post-hoc analysis).