The long noncoding RNA *Morrbid* regulates CD8 T cells in response to viral infection

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The transcriptional programs that regulate CD8 T-cell differentiation and function in the context of viral infections or tumor immune surveillance have been extensively studied; yet how long noncoding RNAs (lncRNAs) and the loci that transcribe them contribute to the regulation of CD8 T cells during viral infections remains largely unexplored. Here, we report that transcription of the lncRNA *Morrbid* is specifically induced by T-cell receptor (TCR) and type I IFN stimulation during the early stages of acute and chronic lymphocytic choriomeningitis virus (LCMV) infection. In response to type I IFN, the *Morrbid* RNA and its locus control CD8 T cell expansion, survival, and effector function by regulating the expression of the proapoptotic factor, *Bcl2l1*, and by modulating the strength of the PI3K-AKT signaling pathway. Thus, our results demonstrate that inflammatory cue-responsive lncRNA loci represent fundamental mechanisms by which CD8 T cells are regulated in response to pathogens and potentially cancer.

CD8 T cells | lncRNAs | viral infection

Long noncoding RNAs (lncRNAs) are a recently described class of RNA defined as RNAs that do not code for protein that are greater than 200 bp in length (1, 2). lncRNAs are typically transcribed by Polymerase II (PolII), capped, spliced, and polyadenylated (3). In recent years, lncRNAs have been shown to play critical roles in regulating gene expression at multiple levels from the epigenetic control of chromatin accessibility, to splicing and posttranscriptional regulation of gene expression (3). Furthermore, recent findings indicate that either the lncRNA or transcription of lncRNA genes can regulate 3D architecture of the genome and gene expression regulation (4, 5). While the roles of lncRNAs in immune cell development and protective immunity have started to be elucidated (6), the function of these enigmatic molecules or transcription of these loci during adaptive immune responses against viral infections remains poorly understood. Addressing this gap in knowledge may reveal therapeutic avenues to boost protective immune responses against both pathogens and cancer.

CD8 T cells play a key role in adaptive immunity and contribute to host defense and antitumor responses. Upon encountering antigen and initial activation, CD8 T cells undergo proliferative expansion and differentiate into effector CD8 T cells (7). The great majority of these effector CD8 T cells will die through apoptosis during the contraction phase following antigen clearance, but a subset of antigen-specific memory precursors survives and differentiates into long-lived memory T cells (8). Memory CD8 T cells have the capacity for long-term host

protection as they can undergo robust recall responses upon secondary antigen challenge (9). Due to their significance in protective immunity and tumor immunosurveillance, substantial efforts have been devoted to understanding the cellular, molecular, and transcriptional processes that regulate CD8 T-cell differentiation and function. However, how lncRNAs or the loci from which they are transcribed regulate CD8 T-cell differentiation or function during viral responses remains largely unexplored.

Here, we describe a critical role for the locus of the recently described lncRNA *Morrbid* in CD8 T-cell responses during lymphocytic choriomeningitis virus (LCMV) infection. While the

Significance

CD8 T cells are immune cells that protect against viral infections and help fight tumors. Not surprisingly, the regulatory mechanisms that control CD8 T cells have been extensively studied. However, whether a new class of RNA molecules, termed long noncoding RNAs (lncRNAs), regulate CD8 T cells during viral infections remains largely unexplored. Here, we show that in response to potent antiviral cytokines (type I IFNs), the lncRNA *Morrbid* and the genomic region from where it is produced tightly regulate CD8 T-cell survival and function. Thus, we now provide evidence that a lncRNA locus and the RNA it produces can cooperate to control immune responses mediated by CD8 T cells. Moreover, these results may represent a therapeutic avenue to combat viral infections and tumors.

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Morbid lncRNA was originally identified as a critical regulator of myeloid cells under homeostatic conditions (10), we now show that transcription of *Morbid* is induced in CD8 T cells following viral infection in response to T-cell receptor (TCR) and type I IFN stimulation. Furthermore, we show that the *Morbid* locus and its RNA are important in the negative regulation of CD8 T-cell expansion and effector function. These results demonstrate that *Morbid*, and likely other extracellular cue-responsive lncRNA loci, is critical in finely tuning the protective and pathogenic potential of cytotoxic CD8 T cells.

Results

***Morbid* Is Induced in CD8 T Cells During Viral Infection and in Response to TCR and Type I IFN Stimulation.** Following a primary infection, naive CD8 T cells are activated by antigen-presenting cells, clonally expand, and differentiate into short-lived effector and long-lived memory cell populations (8). To provide protective immunity and limit immunopathology, proliferation and the life span of antigen-specific CD8 T cells are tightly controlled (8). As we previously demonstrated that the lncRNA *Morbid* strictly controls the life span of myeloid cells at homeostasis, we hypothesized that this lncRNA or its locus might regulate the life span of other immune cells under nonhomeostatic conditions, such as CD8 T cells following viral infection. To address this hypothesis, we utilized LCMV Armstrong, a well-characterized model of acute viral infection (11). At homeostasis, *Morbid* was lowly expressed by CD8 T cells, in both the thymus and in the periphery (Fig. 1A). However, following infection with LCMV Armstrong (Arm), *Morbid* expression was induced by approximately sevenfold in gp33-tetramer specific CD8 T cells at day 6 postinfection, and returned to near baseline following this time point (GSE41867; Fig. 1B). Similar kinetics of induction were observed following infection with LCMV clone 13 (Cl13), a viral strain that establishes persistent infection (Fig. 1C).

To better understand transcription across the *Morbid* locus in CD8 T cells during infection, we utilized a previously published total-RNA transcriptomics dataset of LCMV-specific CD8 T cells following LCMV Armstrong infection (GSE88987). Several regions of the *Morbid* locus are transcribed during the effector phase of these cells at day 8 postinfection, including nonexonic regions. Additionally, when examining sequencing reads that align across exons, it became clear that CD8 T cells likely express a second isoform of *Morbid* (Fig. 1D). Using 5'- and 3'-rapid amplification of cDNA ends (RACE) in stimulated CD8 T cells, we confirmed the expression of both a short isoform that includes exons 1 and 2 (isoform 1), and a longer second isoform that includes exons 1, 3, and 5 (isoform 2; Fig. 1E). We also confirmed the expression of both isoforms in stimulated CD8 T cells by qPCR using primers spanning exons 1–2 to quantify the short transcript (isoform 1), and primers spanning exons 3–5 to quantify the longer transcript (isoform 2) (SI Appendix, Fig. S1A and C–E). Neutrophils were used as a positive control and were shown to express both isoform 1 and isoform 2, although they more highly expressed isoform 1 than isoform 2 (SI Appendix, Fig. S1B).

As LCMV Arm is known to be cleared by day 8 postinfection (11), the kinetics of *Morbid* expression suggests that its transcription is induced downstream of CD8 T-cell activation. As such, we first stimulated sorted splenic CD8 T cells with varying doses of α CD3 in combination with α CD28 or phorbol 12-myristate 13-acetate (PMA)/ionomycin (I), a pharmacological surrogate for strong TCR signaling. Interestingly, we found that both isoforms of *Morbid* were only induced at maximal non-physiologic doses of α CD3 or PMA/I stimulation (Fig. 1F and SI Appendix, Fig. S1C), highlighting that additional signals are likely important for induction of *Morbid* transcription in vivo.

T cells are activated in the context of several signals including the TCR, surface costimulatory receptors such as CD28, and the inflammatory cytokine milieu (12). As such, we provided TCR

Fig. 1. *Morbid* is induced in CD8 T cells during viral infection and in response to TCR and type I IFN stimulation. (A) *Morbid* transcript expression was assessed by qPCR in sorted double-negative (DN), double-positive (DP), single-positive (sp) CD4, and sp CD8 T-cell thymocytes, as well as splenic CD4 and CD8 T cells. Sorted neutrophils were used as positive control ($n = 3$ biological replicates; these data are representative of two independent experiments). (B and C) *Morbid* expression in gp33-tetramer-specific CD8 T cells by microarray after (B) LCMV Armstrong (Arm) and (C) LCMV clone 13 (Cl13) infection represented relative to uninfected (GSE41867; $n = 3$ –4 biological replicates). (D, Top) Schematic of the *Morbid* locus and its predicted exons. (D, Bottom) Day 8 post-LCMV P14 CD8 T-cell total RNA-seq reads that map to the *Morbid* locus (GSE88987). Lines indicate reads spanning two locations. (E, Top) 5'- and (Bottom) 3'-rapid amplification of cDNA ends (RACE) of the *Morbid* locus from CD8 T cells stimulated with α CD3/ α CD28/IFN- β . The arrows indicate gene-specific primers. (F) qPCR of *Morbid* transcript expression in sorted splenic CD8 T cells from naive WT spleens stimulated with the indicated doses of plate-bound α CD3 and 1 μ g/mL soluble α CD28, or PMA/I for 4 h ($n = 3$ biological replicates). (G and H) qPCR of (G) *Morbid* isoform 1 and (H) isoform 2 in negatively selected splenic CD8 T cells stimulated for indicated time with 1 μ g/mL plate-bound α CD3, 1 μ g/mL soluble α CD28, and 20 ng/mL IFN- α or IFN- β . Expression is represented as fold change relative to 0 h ($n = 3$ biological replicates; these data are representative of three independent experiments). (I and J) qPCR of (I) *Morbid* isoform 1 and (J) isoform 2 transcript in negatively selected splenic CD8 T cells unstimulated or stimulated for 48 h with IFN- α or IFN- β ($n = 3$ biological replicates; these data are representative of three independent experiments). (K) *Morbid* expression in WT or *Ifnar1*^{-/-} P14 CD8 T cells by microarray after acute LCMV infection represented relative to uninfected (GSE57355; $n = 3$ biological replicates). Error bars show SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (unpaired two-sided t test, B, C, F–H, and K).

and CD28 costimulation in the context of a panel of different cytokines known to be important for CD8 T-cell activation during viral infection. Interestingly, using a lower dose of α CD3/ α CD28 that alone did not induce *Morbid* expression (Fig. 1F), we consistently observed a strong induction of both isoforms of

this lncRNA with the addition of either IFN- α or IFN- β 48 h following stimulation (Fig. 1 *G* and *H* and *SI Appendix, Fig. S1 D and E*), but not other cytokines known to play important roles early during LCMV infection such as IL-7 or IL-2 (8). Importantly, IFN- α and IFN- β alone were unable to similarly induce the transcription of *Morbid*, demonstrating that its induction likely requires multiple signals (Fig. 1 *I* and *J*). Consistent with the importance of type I IFN for *Morbid* induction in vitro, *Morbid* expression is reduced by threefold following LCMV infection in *Ifnar1*-deficient LCMV-specific CD8 T cells relative to their LCMV-specific wild-type (WT) counterparts in vivo (GSE57355; Fig. 1*K*). These results suggest that, following viral infection in vivo, transcription of *Morbid* is induced in part through type I IFN signaling.

Morbid Is a Negative Regulator of CD8 T Cell Numbers Following Viral Infection. In accordance with the low expression of both isoforms of *Morbid* at homeostasis in the thymus and periphery, mice deficient for the *Morbid* locus did not demonstrate defects in circulating peripheral CD8 T-cell numbers (Fig. 2*A*). Additionally, we did not observe gross developmental defects in the thymus, but we did note an increase in thymic cellularity and an increase in mature CD24^{lo}TCR β ^{hi} CD8 T cells in the absence of *Morbid* (*SI Appendix, Fig. S1 F and G*). We next interrogated

whether the *Morbid* locus impacts CD8 T-cell function following LCMV Arm infection. First, we independently transferred 20×10^6 WT or *Morbid*^{-/-} splenocytes into congenic (CD45.1⁺) WT hosts, infected these mice with LCMV Arm, and assessed CD8 T-cell numbers over the course of infection in blood. Although the same number of WT and *Morbid*^{-/-} CD8 T cells were initially transferred before infection, the *Morbid*^{-/-} bulk CD8 T-cell population expanded to greater numbers, and maintained increased numbers following the acute phase of this infection (Fig. 2*B*). To control for any differences in the TCR repertoires between WT and *Morbid*^{-/-} CD8 T cell populations, as well as to assess the antigen-specific response to LCMV, we generated *Morbid*^{-/-} P14 mice, which express a transgenic TCR specific for the gp33 epitope of LCMV (13). We subsequently adoptively transferred a 1:1 mix of congenic P14 CD8 T cells from WT (CD45.1⁺CD45.2⁺) and *Morbid*-deficient (CD45.2⁺) mice into WT (CD45.1⁺) hosts and assessed CD8 T-cell frequency and number in blood over time following LCMV Arm infection (Fig. 2*C*). We observed a greater number and frequency of *Morbid*-deficient CD8 T cells compared with WT CD8 T cells at nearly all time points analyzed (Fig. 2*D* and *E*). As *Morbid* was also induced following infection with LCMV clone 13 (Cl13) (Fig. 1*C*), we next used the P14 cotransfer model to assess the role of *Morbid* in CD8 T cells during chronic viral infection.

Fig. 2. *Morbid* is a negative regulator of CD8 T-cell numbers following viral infection. (A) Number of splenic CD4 and CD8 T cells from WT and *Morbid*-deficient mice assessed by flow cytometry ($n = 5$ mice per group; these data are representative of three independent experiments). (B) Number of donor CD8 T cells in blood over time in mice that received 10×10^6 WT or *Morbid*-deficient splenocytes followed by LCMV Arm infection ($n = 5$ mice per group). (C) Schematic of P14 adoptive transfer experiments. (D–G) Number and frequency of donor P14 cells assessed by flow cytometry in blood over time in mice that received a 1:1 mix of 250×10^6 WT (CD45.1⁺, CD45.2⁺) and 250×10^6 *Morbid*-deficient (CD45.2⁺) P14 CD8 T cells followed by (D and E) LCMV Arm or (F and G) LCMV Cl13 infection ($n = 5$ mice per group; these data are representative of three independent experiments). (H) Frequency of donor P14 cells assessed by flow cytometry in spleens of mice that received a 1:1 mix of 0.5×10^6 WT and *Morbid*-deficient P14 CD8 T cells followed by LCMV Cl13 infection ($n = 5$ mice per group; these data are representative of two independent experiments). (I and J) Frequency of BrdU incorporation as measured by flow cytometry in cotransferred *Morbid*-deficient and WT P14 CD8 T cells in spleen at (I) day 4 and (J) day 5 following LCMV Cl13 infection. BrdU was injected 12 h before takedown ($n = 5$ mice per group). (K) Frequency and (L) representative flow cytometry plots of Annexin V and propidium iodide staining of WT and *Morbid*-deficient P14 CD8 T cells stimulated with $1 \mu\text{g/mL}$ plate-bound αCD3 and $1 \mu\text{g/mL}$ soluble αCD28 for 48 h ($n = 3$ mice per group; these data are representative of two independent experiments). Error bars show SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (unpaired two-sided t test, A, B, and K; paired two-sided t test, D–J).

Following LCMV Cl13 infection, we observed nearly identical results, with a greater number and frequency of *Morbid*-deficient CD8 T cells relative to that of WT at all time points analyzed (Fig. 2 *F* and *G*). KLRG1, together with IL-7R α , have been used as markers to identify effector CD8⁺ T-cell subsets (8). KLRG1⁺IL-7R α ^{lo} are well-characterized short-lived effector CD8⁺ T cells that have a limited potential to become memory cells (8). In contrast, effector CD8⁺ T cells that do not express KLRG1 (KLRG1^{lo}IL-7R α ^{hi}) have been referred to as memory precursor effector T cells, which display increased survival during the contraction phase, and retain the capacity to differentiate into multiple memory cell lineages (8). Interestingly, we observed minimal differences in the expansion of KLRG1^{lo}IL-7R α ^{hi} population, and short-lived effector CD8⁺ T cells (KLRG1^{hi}IL-7R α ^{lo}) both following acute and chronic infection (*SI Appendix*, Fig. S1 *H–K*). Additionally, we did not observe a significant difference in the expression of programmed cell death 1 (PD-1), a negative regulator of T-cell activation, in chronic infection (*SI Appendix*, Fig. S1*L*). Taking all this together, our results demonstrate that the *Morbid* locus negatively regulates CD8 T-cell numbers following viral infection, even when these cells are exposed to the same antigen and cytokine environment.

CD8 T-cell numbers following infection can be influenced by proliferation, cell death, and trafficking. We observed an increase in the frequency and numbers of *Morbid*^{−/−} CD8 T cells following LCMV infection in all of the tissues that we examined, including blood, spleen, liver, and lung, suggesting that trafficking was likely not responsible for the increase in circulating CD8 T cells. Cotransferred congenically marked *Morbid*^{−/−} P14 CD8 T cells did not demonstrate a competitive advantage over their WT counterparts in vivo until ~5 d post-LCMV infection (Fig. 2*H*), a time point after which responding CD8 T cells have completely diluted cytoplasmic dyes such as CFSE (14). To examine T-cell proliferation at these later stages, we pulsed the mice with the thymidine analog bromodeoxyuridine (BrdU) and assessed its incorporation, as well as Ki67, a surrogate of cell cycle entry, 12 h following BrdU injection. BrdU incorporation and Ki67 frequency were similar between cotransferred WT and *Morbid*^{−/−} P14 CD8 T cells at day 4 and 5 postinfection (Fig. 2 *I* and *J*), suggesting that the competitive advantage of *Morbid*^{−/−} CD8 T cells is not likely secondary to increased proliferation. To further analyze T-cell proliferation, we stimulated CFSE-labeled CD8 T cells from WT and *Morbid*^{−/−} spleens with α CD3/ α CD28 and assessed the number of cells per division after 3 d. Similar to what we observed in vivo, *Morbid*-deficient CD8 T-cell proliferation was similar to that of WT CD8 T cells (*SI Appendix*, Fig. S1*M*). However, when we stimulated these cells in vitro with α CD3/ α CD28 for 48 h and stained with Annexin V and propidium iodide to examine activation-induced cell death, we noted that, relative to that of WT, a greater percentage of *Morbid*-deficient CD8 T cells remained alive (Fig. 2 *K* and *L* and *SI Appendix*, Fig. S1*N*). These data suggest that, following stimulation, *Morbid*-deficient CD8 T cells have a survival advantage over WT CD8 T cells.

***Morbid* Controls CD8 T Cell Effector Function and Naive Cell Homeostasis in a Cell-Intrinsic Manner.** In addition to expanding their numbers, CD8 T cells must also develop appropriate effector functions, such as cytokine production and degranulation, to mount efficient antipathogen responses (15). Thus, we asked whether *Morbid* impacts CD8 T-cell effector function. To establish the baseline effector profile of *Morbid*^{−/−} relative to WT P14 CD8 T cells, we activated these cells in vitro under conditions that induce *Morbid* transcription (Fig. 1 *G* and *H*) and compared cytokine production and degranulation by flow cytometry. We observed a significant increase in the frequency of IFN- γ -producing *Morbid*-deficient P14 CD8 T cells compared with that of WT P14 CD8 T cells (Fig. 3 *A* and *B*). In addition, *Morbid*-

deficient CD8 T cells also showed a significant increase in the cellular quantity of IFN- γ , TNF- α , and Granzyme B (GzMB), as assessed by mean fluorescent intensity (MFI) (Fig. 3 *C–E*), a phenomenon that was recapitulated in non-TCR transgenic TCR *Morbid*^{−/−} CD8 T cells (*SI Appendix*, Fig. S2 *A–C*). These results indicated that, in addition to cellular expansion, the *Morbid* locus also restrains IFN- γ production and other effector functions following stimulation of CD8 T cells.

Given the increased numbers and augmented effector profile of *Morbid*^{−/−} CD8 T cells following stimulation, we next investigated whether this hyperactivity impacts the capacity of *Morbid*^{−/−} CD8 T cell to maintain a naive phenotype at homeostasis in vivo. Loss of negative regulators of T-cell activation such as tuberous sclerosis component 2 (Tsc1), a negative regulator of mammalian target of rapamycin (mTOR), can result in expansion of memory-like CD44^{hi} CD8 T cells (16). In *Morbid*-deficient P14 mice, we observed a minor but significant decrease in naive (CD62L^{hi}CD44^{lo}) CD8 T cells with a concordant increase in the central memory (CM) (CD62L^{hi}CD44^{hi}) compartment (Fig. 3 *F* and *G*). In nontransgenic *Morbid*-deficient mice, we also observed a decrease in naive CD8 T cells, but with an increase in effector memory (EM) (CD62L^{lo}CD44^{hi}) CD8 T cells (*SI Appendix*, Fig. S2 *D* and *E*). Altogether, these results demonstrate that *Morbid* is important for maintaining the homeostasis of naive CD8 T cells. As this expansion of memory-phenotype CD8 T cells in *Morbid*-deficient mice potentially contributed to the rapid responsiveness and hyperactivity exhibited by these cells, we sorted naive (CD62L^{hi}CD44^{lo}) WT and *Morbid*^{−/−} CD8 T cells and stimulated these cells in vitro. Stimulated naive *Morbid*^{−/−} CD8 T cells produced more IFN- γ , TNF- α , and GzMB than their stimulated naive WT counterparts (Fig. 3 *H* and *I* and *SI Appendix*, Fig. S2*F*), demonstrating that *Morbid*^{−/−} hyperresponsiveness stems in part from the naive CD8 compartment.

As short-lived myeloid cells are absent in *Morbid*-deficient animals (10), we questioned whether the hyperactivity of *Morbid*-deficient CD8 T cells occurs in a cell-intrinsic or -extrinsic manner. Although our adoptive transfer studies suggest that the phenotype is cell-intrinsic, we sought to determine whether development in the absence of a full myeloid compartment contributes to *Morbid*-deficient CD8 T-cell hyperactivity. First, we transferred WT splenocytes into WT and *Morbid*^{−/−} hosts to assess the cell-extrinsic impact of host *Morbid* deficiency on CD8 T-cell homeostasis. After 8 wk, we did not observe any difference in IFN- γ production or the memory phenotype of WT cells exposed to either a WT or *Morbid*-deficient environment (*SI Appendix*, Fig. S2*G*). To more rigorously address this question, we generated competitive bone marrow chimeras using sorted LSK cells from WT P14 and *Morbid*-deficient P14 bone marrow mixed 1:1 and transferred into irradiated WT hosts. After allowing the bone marrow to reconstitute for 9 wk, we observed a greater frequency of IFN- γ ⁺ *Morbid*-deficient CD8 T cells in response to PMA/I stimulation compared with that of WT CD8 T cells, suggesting that increased production of IFN- γ with *Morbid* deficiency occurs in a cell-intrinsic manner in the absence of the *Morbid* locus (Fig. 3*J*). In addition, we observed a similar loss of naive and expansion of central memory CD8 T cells in the *Morbid*-deficient P14 populations compared with WT P14 CD8 T cells in the same recipient hosts (Fig. 3*J*), a phenomenon that was recapitulated when reconstituting mice with non-P14 LSK cells (*SI Appendix*, Fig. S2*H*).

We next aimed to establish the functionality of these cells in the context of viral infection in vivo. Thus, we adoptively transferred WT P14 CD8 T cells and *Morbid*^{−/−} P14 CD8 T cells into WT hosts and assessed viral titers in the spleens at days 5 and 6 (LCMV-Arm) and in serum at day 8, 15, 21, and 28 (LCMV-Cl13) postinfection. At these doses of virus and number of adoptively transferred P14 CD8 T cells, *Morbid* deficiency does

not significantly impact primary Arm or Cl13 LCMV viral kinetics (*SI Appendix, Fig. S2 I and J*). Altogether, our findings suggest that the *Morbid* locus and potentially its RNA molecule functions in a cell-intrinsic manner to restrain effector memory phenotype CD8 T cells at steady state, and to control effector cytokine production following CD8 T-cell activation. Moreover, our data indicate that these alterations are largely independent of the defects in the myeloid compartment of *Morbid*-deficient mice.

The *Morbid* Locus Is Required for the Up-Regulation of *Bcl2l1* Expression in CD8 T Cells Following TCR Stimulation. As previously discussed, *Bcl2l1*, the key downstream target of *Morbid* in short-lived myeloid cells, is required for appropriate contraction of CD8 T cells following viral clearance (17–19). CD8 T cells deficient for *Bcl2l1* fail to contract at later stages of infection (17–19), which closely mirrors the kinetics of *Morbid*-deficient CD8 T cells in the later stages of acute and chronic LCMV infection (Fig. 2 D–G). Moreover, similar alterations in the memory compartment present in *Morbid*^{−/−} mice are also observed in the absence of *Bcl2l1* (19). However, no published studies have demonstrated that *Bcl2l1* regulates CD8 T-cell expansion or effector function. We first aimed to determine whether

Morbid RNA and/or its locus regulate *Bcl2l1* expression. At homeostasis, *Bcl2l1* expression was not altered in naive *Morbid*-deficient CD8 T cells at the transcript or protein levels (Fig. 4 A and B). However, in contrast to short-lived myeloid cells, *Bcl2l1* expression was decreased in *Morbid*^{-/-} CD8 T cells following stimulation in vitro, in central memory and effector memory CD8 T cells, as well as at day 8 post-LCMV Arm infection in vivo (Fig. 4 C–E and [SI Appendix, Fig. S3 A and B](#)). It is important to highlight that *BCL2L1* protein expression was decreased in *Morbid*-deficient CD8 T cells under stimulation conditions and time points at which we did not previously observe significant *Morbid* RNA expression (Fig. 4F and [SI Appendix, Fig. S3 A and B](#)), suggesting that the locus, not the RNA, might be primarily responsible for controlling *Bcl2l1* expression under these conditions. Thus, to better understand the contribution of the *Morbid* locus and its RNA to *Bcl2l1* expression in CD8 T cells, we independently overexpressed both isoforms of *Morbid* in WT or *Morbid*^{-/-} CD8 T cells and determine its contribution to the regulation of *Bcl2l1* expression ([SI Appendix, Fig. S3C](#)). In WT CD8 T cells, we did not observe an impact of either isoform of *Morbid* RNA on *Bcl2l1* expression relative to empty vector (Fig. 4G). Interestingly, we observed a small but significant increase in *Bcl2l1* expression

upon overexpression of *Morbid* isoform 2 in *Morbid*^{-/-} CD8 T cells (*SI Appendix*, Fig. S3D), suggesting that through either direct or indirect mechanisms *Morbid* isoform 2 RNA has the capacity to contribute to the promotion of *Bcl2l1* expression.

Together, these data indicate that the *Morbid* locus is required for promoting *Bcl2l1* expression following CD8 T-cell stimulation, and that *Morbid* RNA *in trans* may contribute to the regulation of this proapoptotic gene in these cells. Further

Fig. 4. *Bcl2l1* expression is reduced in the absence of the *Morbid* locus in CD8 T cells following stimulation but does not significantly contribute to *Morbid*-deficient expansion or effector function. (A) qPCR of *Bcl2l1* transcript expression in sorted naive (CD62L^{hi}CD44^{lo}) WT and *Morbid*-deficient CD8 T cells ($n = 3$ mice per group; these data are representative of two independent experiments). (B and C) BCL2L1 protein mean fluorescent intensity (MFI) measured by flow cytometry in (B) unstimulated or (C) 4-h PMA/I-stimulated WT and *Morbid*-deficient mice CD8 T cells ($n = 3$ mice per group; these data are representative of five independent experiments). (D) BCL2L1 protein MFI by flow cytometry in WT or *Morbid*-deficient cotransferred P14 cells 8 d post-LCMV Arm infection ($n = 5$ mice per group; these data are representative of three independent experiments). (E) BCL2L1 protein MFI by flow cytometry in naive (NV) (CD62L^{hi}CD44^{lo}), central memory (CM) (CD62L^{hi}CD44^{hi}), and effector memory (EM) (CD62L^{lo}CD44^{hi}) WT and *Morbid*-deficient CD8 T cells from blood ($n = 5$ mice per group; these data are representative of three independent experiments). (F) BCL2L1 protein MFI by flow cytometry in WT and *Morbid*-deficient CD8 T cells stimulated with αCD3/αCD28 for 24 h ($n = 5$ mice per group; these data are representative of three independent experiments). (G) BCL2L1 protein MFI by flow cytometry in WT CD8 T cells transduced with the empty vector, *Morbid* isoform (iso) 1, or *Morbid* iso 2 ($n = 3$ biological replicates; data pooled from three independent experiments). (H) Representative flow cytometry plot of IFN-γ production in WT, *Morbid*-deficient, and *Bcl2l1*-heterozygous CD8 T cells stimulated for 3 h with PMA/I and IFN-β ($n = 3$ mice per group; these data are representative of four independent experiments). (I) Frequency of donor P14 cells in blood over time in mice that received a 1:1 mix of 250 WT and 250 *Bcl2l1*-deficient P14 CD8 T cells and infected with LCMV Arm. Represented as the ratio of donor *Bcl2l1*-deficient to donor WT ($n = 5$ mice per group; these data are representative of two independent experiments). (J) BCL2L1 protein MFI of CD8 T cells from blood of WT, *Bcl2l1*^{+/-}, and *Morbid*^{-/-}; *Bcl2l1*^{+/-} mice ($n = 4$ –5 mice per group). (K, Left) Representative flow cytometry plots and (Right) frequency of IFN-γ production in CD8 T cells from *Bcl2l1*^{+/-}, and *Morbid*^{-/-}; *Bcl2l1*^{+/-} mice 3 h post-PMA/I and IFN-β stimulation ($n = 2$ mice per group; these data are representative of two independent experiments). (L and M) Frequency of donor P14 cells in blood over time in mice that received a 1:1 mix of 250 *Bcl2l1*^{+/-} and 250 *Morbid*^{-/-}; *Bcl2l1*^{+/-} and infected with LCMV Arm. (L) Ratio of frequencies of the indicated donor populations. (M) Ratio of BCL2L1 protein MFI of the indicated donor populations. ($n = 5$ mice per group; these data are representative of two independent experiments). Error bars show SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (unpaired two-sided t test, A–G, J, and K; paired two-sided t test, I, L, and M).

studies are required to determine the contribution of *Morbid* RNA to *Bcl2l1* regulation *in cis* in this context. Altogether, these results suggest that *Morbid*-deficient alterations in CD8 T-cell contraction during LCMV infection and loss of naive homeostasis are likely secondary to decreased *Bcl2l1* expression due to loss of the *Morbid* locus and potentially *Morbid* RNA.

Since *Bcl2l1* has not been previously associated with alterations in CD8 T-cell expansion or effector function, we next formally tested the impact of *Bcl2l1* on these processes. As the reduction in *Bcl2l1* expression observed in *Morbid*-deficient mice was similar to that observed in *Bcl2l1* heterozygous mice (*SI Appendix, Fig. S3 E and F*), we first stimulated *Morbid*^{-/-}, *Bcl2l1*^{+/-}, and WT CD8 T cells and examined their production of effector molecules. Interestingly and in concordance with previous reports, we found that *Bcl2l1*^{+/-} CD8 T cells have a nearly identical IFN- γ production profile to that of WT cells (Fig. 4H and *SI Appendix, Fig. S3 G and H*). We subsequently cotransferred WT and *Bcl2l1*^{-/-} P14 CD8 T cells into congenic hosts and assayed the number and frequency of these cells following acute LCMV Arm infection. We did not observe differences in expansion between WT and *Bcl2l1*^{-/-} P14 cells during the early phase following LCMV Arm (Fig. 4I). These results, coupled with the pattern of expression of *Morbid* in CD8 T cells *in vitro* and *in vivo*, suggest that the *Morbid* locus and/or its RNA may contribute to the regulation of CD8 T-cell expansion and effector function in a *Bcl2l1*-independent manner.

To more thoroughly test whether this lncRNA and/or its locus have *Bcl2l1*-independent roles in CD8 T cells, we generated P14 transgenic mouse strains with equally reduced levels of *Bcl2l1* expression in the presence or absence of *Morbid* (*Morbid*^{-/-}; *Bcl2l1*^{+/-} and *Bcl2l1*^{-/-}; Fig. 4J). Using these strains normalized for *Bcl2l1* expression, we subsequently tested CD8 T-cell effector cytokine production following stimulation, expansion, and contraction upon LCMV infection. Interestingly, following stimulation, *Morbid*^{-/-}; *Bcl2l1*^{+/-} CD8 T cells express significantly more IFN- γ and TNF- α than their *Bcl2l1*^{-/-} counterparts (Fig. 4K and *SI Appendix, Fig. S3 I–L*), further supporting that *Morbid* impacts the effector profile of CD8 T cells in a *Bcl2l1*-independent manner. Next, we cotransferred *Bcl2l1*^{+/-} and *Morbid*^{-/-}; *Bcl2l1*^{+/-} P14 CD8 T cells into naive recipients, infected these mice with LCMV Arm, and assessed the frequency of these populations at days 7, 14, and 21 post-infection. During the expansion phase at day 7, a time point at which this proapoptotic factor does not seem to play a large role, *Morbid*^{-/-}; *Bcl2l1*^{+/-} P14 T cells outcompeted their *Bcl2l1*^{+/-} counterparts (Fig. 4L), with no significant difference in their expression of *Bcl2l1* (Fig. 4M and *SI Appendix, Fig. S3M*). However, during the contraction phase (days 14 and 21), *Morbid*^{-/-}; *Bcl2l1*^{+/-} P14 CD8 T cells outcompeted their *Bcl2l1*^{-/-} counterparts and expressed significantly less *Bcl2l1* (Fig. 4L and M). Due to the key role that *Bcl2l1* plays in the contraction phase of CD8 T-cell responses, these results suggest that the decrease in *Bcl2l1* in *Morbid*^{-/-}; *Bcl2l1*^{+/-} CD8 T cells might be a key contributor to their slower contraction following viral clearance. Altogether, these data strongly support a *Bcl2l1*-independent role for *Morbid* in negatively regulating CD8 T-cell expansion and effector function following acute viral infection.

The *Morbid* Locus and Its RNA Represses AKT Signaling in CD8 T Cells Downstream of Type I IFN. *Morbid* transcription in CD8 T cells in the early stages of acute LCMV infection is largely dependent on type I IFN signaling. Thus, we hypothesized that some of the regulatory roles of *Morbid* that are independent of *Bcl2l1* could be associated with controlling the impact of type I IFN signaling on CD8 T cells. Type I IFNs are well recognized for their potent effects on CD8 T-cell activation, proliferation, differentiation, and survival (20). Although the functional consequences of type I

IFNs can be diverse depending on the relative timing of IFN exposure and TCR stimulation, within the LCMV system type I IFNs have been reported to act directly on CD8 T cells to promote their survival and effector function (21–23). To start to address this hypothesis, we first tested whether *Morbid*-deficient CD8 T cells have altered surface expression of IFN- α /beta receptor 1 (IFNAR1) and found no significant differences in expression between naive WT and *Morbid*^{-/-} CD8 T cells (*SI Appendix, Fig. S4A*). We next asked whether downstream canonical signaling was dysregulated, and tested the phosphorylation of signal transducer and activator 1 (STAT1) and STAT4 in WT and *Morbid*^{-/-} CD8 T cells in response to varying doses of IFN- β in combination with α CD3. *Morbid*^{-/-} CD8 T cells were similarly sensitive to IFN treatment, with a minor reduction in STAT1 and STAT4 phosphorylation (*SI Appendix, Fig. S4 B–D*). Although reduced but imbalanced STAT1 to STAT4 signaling may contribute to *Morbid*^{-/-} hyperactivity, these results suggest that canonical type I IFN signaling is not grossly dysregulated in *Morbid*^{-/-} CD8 T cells.

To take an unbiased approach and identify potential pathways regulated by the *Morbid* RNA or its locus downstream of type I IFN signaling, which could contribute to the regulation of CD8 T-cell function and expansion, we performed RNA sequencing of sorted naive (CD62L^{hi}CD44^{lo}) WT and *Morbid*-deficient splenic CD8 T cells stimulated with α CD3/ α CD28 with and without IFN- β , and asked what pathways were altered. Importantly, within 1 Mb of the *Morbid* locus, apart from *Bcl2l1*, we observed no significant differences in *cis* gene expression between WT and *Morbid*-deficient CD8 T cells under any stimulation condition (Fig. 5A), supporting the notion that *Morbid* could be acting *in trans*. Interestingly, using gene set enrichment analysis (GSEA), we found that several noncanonical type I IFN signaling pathways, including PI3K-AKT and NF- κ B, were significantly dysregulated in the absence of *Morbid* (Fig. 5B and *SI Appendix, Fig. S4E*). AKT is a well-described key signaling molecule downstream of IFN receptor stimulation that promotes autophagy, glucose uptake, and translation of IFN-stimulated genes (ISGs), as well as the proliferation, survival, and effector function of CD8 T cells (24–27). AKT signaling was of particular interest as it was only enriched in *Morbid*^{-/-} CD8 T cells under conditions in which this lncRNA is induced, such as α CD3/ α CD28 and IFN- β , but not with α CD3/ α CD28 alone (Fig. 5B). This dysregulation of AKT targets was also apparent when visualizing individual targets statistically significant by differential expression (Fig. 5C). Therefore, we hypothesized that, in addition to decreased expression of *Bcl2l1*, increased PI3K-AKT activity could contribute to the alterations observed in *Morbid*-deficient CD8 T cells. To test this possibility, we first asked whether phosphorylation of AKT is enhanced following TCR and IFN stimulation in *Morbid*^{-/-} CD8 T cells. Indeed, we observed that under conditions capable of inducing *Morbid* expression such as α CD3/ α CD28 with IFN- β or PMA/I, but not α CD3/ α CD28 alone, there is a more rapid phosphorylation of AKT at S473 in *Morbid*^{-/-} CD8 T cells (Fig. 5D and E). AKT is also a fundamental signaling hub downstream of the TCR and costimulatory receptors (28). Importantly, we did not observe gross alterations in key proximal TCR signaling pathways such as calcium flux and ERK phosphorylation (*SI Appendix, Fig. S4 F–H*), indicating that increased AKT activity in *Morbid*^{-/-} CD8 T cells is restricted to conditions in which CD8 T cells are stimulated with type I IFN and that there is no global dysregulation of TCR signaling in these cells. To determine whether the alteration in *Morbid*-deficient CD8 T-cell numbers following viral infection is partially dependent on dysregulated PI3K-AKT-mTOR signaling, we used rapamycin to target this pathway *in vivo*. WT and *Morbid*-deficient P14 CD8 T cells were cotransferred into WT hosts that were subsequently treated with rapamycin from day -1 before LCMV-Cl13 infection to day 27 postinfection.

Fig. 5. The *Morbid* locus and its RNA negatively regulate AKT signaling in CD8 T cells, and *Morbid*-deficient effector hyperactivity is type I IFN dependent. (A–C) RNAseq analysis of sorted naive (CD62L^{hi}CD44^{lo}) WT and *Morbid*-deficient CD8 T cells stimulated with α CD3/ α CD28 with or without IFN- β for 6 h. (A) Expression of genes within 1 Mb of the *Morbid* locus. (B) GSEA of hallmark PI3K-AKT-mTOR gene set in WT and *Morbid*-deficient CD8 T cells under the indicated stimulation conditions. α CD3/ α CD28 (NES 0.88, FDR 0.59) and α CD3/ α CD28/IFN- β (NES -1.51, FDR 0.06). (C) Heatmap of genes significantly differentially expressed with FDR < 0.05 following stimulation α CD3/ α CD28/IFN- β that are also identified within the hallmark PI3K-AKT-mTOR pathway. Reads were normalized using DESeq2 ($n = 3$ –4 biological replicates per genotype per condition). (D) Western blot of pS473 AKT in WT and *Morbid*-deficient CD8 T cells at the indicated time points following stimulation with α CD3/ α CD28 with or without IFN- β (these data are representative of two independent experiments). (E) Phospho-flow analysis as measured by gMFI of pS473 AKT in CD8 T cells from WT and *Morbid*-deficient mice stimulated with PMA/I for 15 min ($n = 3$ mice per group; these data are representative of two independent experiments). (F) Frequency of donor P14 CD8 T cells in blood at the indicated time points in mice that received a 1:1 mix of 250 WT and 250 *Morbid*^{-/-} P14 CD8 T cells and were infected with LCMV CI13. Mice were treated daily with rapamycin or PBS i.p. from day -1 before LCMV-CI13 infection to day 27 postinfection ($n = 3$ –5 mice per group; these data are representative of two independent experiments). (G) Phospho-flow analysis as measured by gMFI of pS473 AKT in *Morbid*-deficient CD8 T cells that were transduced with empty, *Morbid* isoform 1, or *Morbid* isoform 2 expressing vectors. Cells were plated in triplicate, and then stimulated with α CD3/ α CD28/IFN- β for 15 min ($n = 3$ replicate per group). (H, Left) Frequency and (Right) MFI of IFN- γ production in WT, *Morbid*^{-/-}, and *Morbid*^{-/-}; *Ifnar1*^{fl/fl}; *Cd4-Cre* CD8 T cells stimulated with α CD3/ α CD28/IFN- β for 24 h ($n = 4$ mice per group; data pooled from three independent experiments). (I and J) Flow cytometry analysis of cotransferred *Morbid*^{-/-} and *Morbid*^{-/-}; *Ifnar1*^{fl/fl}; *Cd4-Cre* P14 CD8 T cells following LCMV Arm or CI13 infection. (I) Frequency of the cotransfer donor P14 cells represented as the ratio of *Morbid*^{-/-} to *Morbid*^{-/-}; *Ifnar1*^{fl/fl}; *Cd4-Cre* at the indicated time points following infection. (J) BCL2L11 protein MFI in each donor P14 population 21 d post-CI13 infection ($n = 5$ mice per group; these data are representative of two independent experiments). Error bars show SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (DESeq2 FDR, A and C; GSEA FDR, B; unpaired two-sided t test, E, G, and H; paired two-sided t test, F, I, and J).

Interestingly, *Morbid*-deficient P14 CD8 T cells were more sensitive to rapamycin treatment compared with WT P14 CD8 T cells at all of the time points analyzed (Fig. 5F). These results indicate that the competitive expansion and delayed contraction of *Morbid*-deficient CD8 T cells is in part PI3K-AKT-mTOR dependent. Taken together, these results suggest that, in response

to type I IFN, the *Morbid* locus plays a critical role in controlling the strength of PI3K-AKT signaling.

We next assessed the contribution of *Morbid* RNA to the regulation of AKT signaling downstream of the type I IFN receptor. To do so, we overexpressed *Morbid* RNA in WT or *Morbid* locus-deficient CD8 T cells. Strikingly, WT and

Morbid-deficient CD8 T cells overexpressing either isoform of *Morbid* demonstrated a dramatic reduction in AKT phosphorylation following stimulation with α CD3/ α CD28 and IFN- β relative to their control counterparts (Fig. 5G and *SI Appendix, Fig. S4f*), effectively rescuing the aberrant increase in AKT phosphorylation observed in the absence of this lncRNA. These results suggest that *Morbid* RNA plays a critical role in downregulating the PI3K–AKT pathway downstream of type I IFN signaling in CD8 T cells.

As type I IFN induces *Morbid* expression and this lncRNA and its locus contribute to the regulation of noncanonical type I IFN signaling through the PI3K–AKT pathway in CD8 T cells, we next aimed to formally test whether the alterations that we observed in *Morbid*^{−/−} CD8 T cells could be explained by enhanced type I IFN-dependent signaling. To address this possibility, we first generated *Morbid*-deficient mice that lack the type I IFN signaling receptor IFNAR1 (*Morbid*^{−/−}; *Ifnar1*^{fl/fl}; *Cd4-Cre*) and determined whether the absence of type I IFN signaling could rescue the hyperactive effector function of *Morbid*-deficient CD8 T cells. Indeed, following in vitro stimulation with α CD3/ α CD28 and IFN- β , we find that *Morbid*^{−/−}; *Ifnar1*^{fl/fl}; *Cd4-Cre* CD8 T cells display an effector profile similar to that of WT CD8 T cells (Fig. 5H and *SI Appendix, Fig. S4f*), demonstrating that *Morbid* regulates CD8 T-cell effector function in a type I IFN-dependent manner. Of note, absence of the type I IFN receptor did not rescue decreased BCL2L11 expression in the absence of *Morbid* (*SI Appendix, Fig. S4k*). These data suggest that the *Morbid* locus and its RNA function downstream of type I IFN signaling as negative autoregulators of the stimulatory effects of type I IFN on CD8 T cells.

As *Morbid*-deficient CD8 T-cell AKT signaling and cytokine effector profile are increased in a type I IFN-dependent manner, we hypothesized that the increased expansion and decreased contraction of *Morbid*^{−/−} cells in vivo is also type I IFN dependent. To test this hypothesis, we performed cotransfer experiments in which we could directly compare the kinetics of *Morbid*^{−/−} and *Morbid*^{−/−}; *Ifnar1*^{fl/fl}; *Cd4-Cre* P14 CD8 T cells upon acute and chronic LCMV infection. Interestingly, while we did not observe significant differences in expansion in the context of either acute or chronic LCMV infection, we noted a clear reduction in *Morbid*^{−/−}; *Ifnar1*^{fl/fl}; *Cd4-Cre* P14 CD8 T cells 21 d following chronic LCMV Cl13 infection (Fig. 5I). As we did not observe a difference in BCL2L11 expression at any of the time points that we examined, including 21 d post-Cl13 infection (Fig. 5J), these results suggest that the impact of *Morbid* on *Bcl2l11* is independent of type I IFN signaling. More importantly, restoring the kinetics of contraction of *Morbid*-deficient CD8 T cells by ablating the type I IFN receptor suggests that regulation of PI3K–AKT signaling strength downstream of this receptor by *Morbid* is critical for tuning CD8 T-cell numbers during chronic viral infections. Moreover, it highlights the possibility that a lncRNA and its locus could have independent roles in CD8 T cells during viral infection and that these roles have different degrees of relevance and are highly contextualized.

Discussion

In this study, we show that transcription of the lncRNA *Morbid* is specifically induced by type I IFN in CD8 T cells in vitro and during the early stages of acute and chronic LCMV infection in vivo. Moreover, this lncRNA locus and/or its RNA regulate CD8 T-cell expansion, contraction, and acquisition of effector functions in the context of viral infection. We find that the *Morbid* locus promotes the expression of the proapoptotic factor BCL2L11, which likely contributes to *Morbid* regulation of CD8 T-cell contraction. However, alterations in CD8 T-cell expansion and effector functions are regulated, at least partially, through the capacity of *Morbid* RNA to modulate signaling strength through the type I IFN receptor. Thus, our results demonstrate

that inflammatory cue-responsive lncRNA loci, through either *cis*-regulatory elements, RNA transcription, or the RNA molecule itself, provide a key regulatory layer to tune the functions of CD8 T cells during antipathogen responses.

BCL2L11 plays a pivotal role in the contraction of CD8 T-cell numbers during viral infection, yet it does not seem to control CD8 T-cell expansion or acquisition of effector functions in the early stages of LCMV infection. In accordance with BCL2L11's role in contraction, *Morbid*-deficient mice express less BCL2L11 following stimulation and have a striking impairment in CD8 T-cell contraction at later stages of LCMV infection. As *Bcl2l11* expression is decreased in CD8 T cells under conditions in which *Morbid* is not expressed, such as upon stimulation with low doses of anti-CD3 alone or during the contraction phase of LCMV infection, and *Morbid* RNA overexpression *in trans* seems to have a modest impact in BCL2L11 expression in this cell type, these results suggest that DNA regulatory elements within the *Morbid* locus or transcription across the locus are likely key in controlling the expression of this proapoptotic factor *in cis*. This *Morbid*–*Bcl2l11* relationship is in stark contrast to that in short-lived myeloid cells, where prosurvival cytokines promote *Morbid* expression, which in turn represses *Bcl2l11* transcription *in cis* to control the life span of these cells. As the CRISPR–Cas9–edited *Morbid*-deficient mice were backcrossed more than six times to WT mice, there is no predicted homology between the CRISPR guide RNAs and the *Bcl2l11* sequence, and littermate controls were used whenever possible, it is unlikely that CRISPR–Cas9 off-target effects in the *Bcl2l11* gene contribute to our observed phenotype. It will be critical for future work to determine how DNA elements within the *Morbid* locus, transcription across the locus, or the RNA itself contribute to the regulation of *Bcl2l11* transcription in CD8 T cells at different stages of infection, and what impact this regulatory circuit has on CD8 T-cell homeostasis and function.

The timing of *Morbid* transcription during LCMV infection, and the presence of CD8 T-cell alterations that could not be explained by *Bcl2l11* dysregulation, suggest that *Morbid* functions through additional mechanisms in this cell type. Our studies indicate that, in response to type I IFN and TCR stimulation, the signaling strength of the PI3K–AKT pathway is increased in *Morbid*-deficient CD8 T cells. While the PI3K–AKT pathway was not the only pathway that was altered at the transcriptional level under these conditions, our *Morbid* RNA overexpression studies strongly support the notion that this lncRNA contributes to the down-regulation of the PI3K–AKT pathway signaling strength. Interestingly, lncRNAs have previously been shown to regulate the PI3K–AKT signaling pathway by directly interacting with PIP3 to promote AKT activation, or by serving as sponges for microRNAs that target a negative regulator of this pathway, PTEN (29–32). The work presented here establishes *Morbid* as an important regulator of this fundamental signaling pathway in CD8 T cells, and implies that this *Morbid*–AKT axis is likely important in other contexts and cell types in which this lncRNA is expressed. Furthermore, as the AKT pathway controls multiple fundamental processes in CD8 T cells during viral infection or tumor immune surveillance, it will be important to determine the exact molecular mechanism by which *Morbid* regulates this pathway, as it may provide insights into how to manipulate AKT signaling to improve antipathogen and anticancer immunity.

The current study of the *Morbid* lncRNA and its locus in CD8 T cells highlights the exciting possibilities that individual lncRNAs may have more than one function, and that the biological significance of each of those functions is dependent on the cellular context in which the lncRNA is expressed and the isoforms being expressed at any given time. For example, our results suggest that *Morbid* may control key signaling pathways in addition to regulating *Bcl2l11* expression. Of note, in short-lived myeloid cells,

Morbid regulation of *Bcl2l1* and survival was striking and dominant; thus, in this context, additional functions of *Morbid* were not thoroughly assessed. As such, it will be important to formally determine whether this lncRNA controls additional signaling pathways such as the PI3K–AKT pathway in these innate myeloid cells. Alternatively, it is possible that different cell types express different isoforms of *Morbid*, which could have alternative subcellular localizations, binding partners, and therefore functions. Although both isoforms of *Morbid* are expressed in short-lived myeloid cells and stimulated CD8 T cells, these isoforms are enriched in the cytoplasm of CD8 T cells. These data differ from the chromatin-enriched predominance in myeloid cells. This dichotomy demonstrates that further work is required to address the function of each *Morbid* isoform, and whether these functions vary depending on the cellular context in which they are transcribed.

In summary, our results provide evidence that lncRNA loci, and potentially the RNA that they transcribe, have the capacity to regulate CD8 T-cell function and kinetics during LCMV infection. Moreover, this work suggests that specific lncRNA may have multiple functions depending on the cellular context in which they are expressed.

Materials and Methods

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. Mice were housed in accordance with the procedures outlined in *Guide for the Care and Use of Laboratory Animals* (33) under an animal study proposal approved by an institutional animal care and use committee. *Morbid*-deficient mice were generated as previously described (10, 34). Samples sizes were estimated based on our preliminary phenotyping of *Morbid*-deficient CD8 T cells following viral infection. No animals were excluded from analysis. All experimental and control mice were run in parallel to control for experimental variability and were not randomized. *P* values were calculated using unpaired two-sided *t* test, paired two-sided *t* test, one-way ANOVA with Tukey post hoc analysis, and false-discovery rate (FDR) as indicated. FDR was calculated using DESeq2 or GSEA algorithms. All error bars indicate mean $\pm$ SEM.

For fully detailed materials and procedures, please refer to *SI Appendix, Materials and Methods*.

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