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Batf3 expression by CD8 T cells critically regulates the development of memory populations

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Abstract

The basic leucine zipper transcription factor ATF-like 3 (BATF3) is required for the development of conventional type 1 dendritic cells that are essential for cross-presentation and CD8 T cell mediated immunity against intracellular pathogens and tumors. However, whether *Batf3* intrinsically regulates CD8 T cell responses is not well studied. Here, we report a novel role for cell intrinsic *Batf3* expression in regulating the establishment of circulating and resident memory T cells after foodborne *Listeria monocytogenes* infection of mice. Consistent with other studies, *Batf3* expression by CD8 T cells was dispensable for the primary response. However, *Batf3*$^{-/-}$ T cells underwent increased apoptosis during contraction to contribute to a substantially reduced memory populations. *Batf3*$^{-/-}$ memory cells had an impaired ability to mount a robust recall response but remained functional. These findings reveal a novel cell intrinsic role of *Batf3* in regulating CD8 T cell memory development.

Introduction

The basic leucine zipper transcription factor ATF-like 3 (BATF3) is a member of the AP-1 transcription factor family and required for the development of conventional type 1 dendritic cells (cDC1) (1–3). cDC1 are essential for cross-presentation of exogenous antigens to prime CD8 T cells *in vivo* and *Batf3*$^{-/-}$ mice that lack cDC1 have defective CD8 T cell responses to intracellular pathogens and tumors (1). *Batf3*-dependent cDC1 may also affect the CD8 T cell response through other functions such as providing a critical source of IL-12 during *Toxoplasma gondii* infection and transporting bacteria to the splenic T cell zone to establish a productive infection during systemic *Listeria monocytogenes* (*Lm*) infection (4, 5). Moreover, cDC1-mediated cross-priming has also been shown to promote skin CD8 tissue-resident memory T (T_{RM}) cell development by inducing committed T_{RM} cell precursors without affecting their differentiation (6).

Batf3 gene expression is low in naïve CD4 and CD8 T cells (1). However, CD4 T cells upregulate *Batf3* gene expression during *in vitro* T_H1, T_H2, T_H17, and T_H9 cell differentiation but not Treg cell differentiation (1, 7–9). While *Batf3*$^{-/-}$ CD4 T cells show

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Disclosures

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normal T_H1, T_H2 and T_H17 cell differentiation, *Batf3* induces T_H9 cell differentiation by binding to the *Il9* promoter through a BATF3/IRF4 complex (1, 8, 9). *Batf3* inhibits Treg differentiation by repressing *Foxp3* expression and *Batf3*$^{-/-}$ mice have increased Treg cells (7, 10). *Batf3* is also associated with lymphomagenesis in B and T cell lymphomas. Knockdown of *Batf3* expression in lymphoma cell lines resulted in reduced proliferation and enhanced apoptosis that was associated with BATF3 binding to the *Myc* promoter (11). Much less is known about the intrinsic role of *Batf3* in CD8 T cell responses to pathogens. *Batf3* expression by CD8 T cells does not appear to regulate the primary CD8 T cell response as adoptive transfer of *Batf3*$^{-/-}$ CD8 T cells into *Rag2*$^{-/-}$ mice led to a normal anti-viral CD8 T cell response 7 days after infection (1). However, whether *Batf3* intrinsically regulates other phases of the CD8 T cell response has not been explored.

Consistent with previous studies, *Batf3* expression by CD8 T cells was dispensable for the primary response to foodborne *Lm* infection. However, this study revealed the novel observation that *Batf3* expression by CD8 T cells critically regulated memory T cell development. *Batf3*$^{-/-}$ CD8 T cells were largely absent from the circulation and secondary lymphoid tissues during memory homeostasis. The remaining *Batf3*$^{-/-}$ CD8 T cells isolated during memory homeostasis possessed a predominantly terminally differentiated phenotype. Moreover, *Batf3*$^{-/-}$ CD8 T cells were also unable to establish a robust T_{RM} cell population in the intestinal epithelium. *Batf3* expression by CD8 T cells played an important role in promoting the survival of CD8 T cells as *Batf3*$^{-/-}$ CD8 T cells had increased apoptosis during contraction. Furthermore, the intrinsic *Batf3* expression is important for CD8 T cells to mount a robust recall response but is not required for their function. These results reveal a critical and intrinsic role of *Batf3* in regulating CD8 T cell memory development and recall responses.

Materials and Methods

Mice

Female and male B6 mice (C57BL/6J and B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ) were purchased from the Jackson Laboratory and used between 8 and 12 weeks of age. OT-I *Rag1*$^{-/-}$ mice were bred in house. B6.129S(C)-*Batf3*^{tm1kmm}/J (*Batf3*$^{-/-}$) mice were purchased from the Jackson Laboratory. OT-I *Rag1*$^{-/-}$ mice were crossed with B6.129S(C)-*Batf3*^{tm1kmm}/J mice to make *Batf3*$^{+/-}$ OT-I *Rag1*$^{-/-}$ heterozygous. Wild-type (WT) and *Batf3*$^{-/-}$ OT-I *Rag1*$^{-/-}$ mice used in experiments were generated by heterozygous breeding of *Batf3*$^{+/-}$ OT-I *Rag1*$^{-/-}$ mice. All mice were housed under specific-pathogen-free condition. All procedures were carried out in accordance with National Institute of Health guidelines and approved by the Stony Brook University Institutional Animal Care and Use Committee.

Bacteria and foodborne infection

Listeria monocytogenes strain 10403s carrying a mutation in the internalin A protein and expressing a truncated form of ovalbumin ($InlA^M$ r*Lm*-OVA) was used. Prior to infection, mice were deprived of food and water for 6 hours. Foodborne infection was performed by providing ~1 cm^3 piece of bread inoculated with $InlA^M$ r*Lm*-OVA in PBS to individually

housed mice. The doses for primary and recall infection were 2×10^9 and 2×10^{10} cfu, respectively.

Cell sorting

Naïve OT-I cells were obtained by sorting CD8 T cells from the spleen of OT-I *Rag1*$^{-/-}$ mice. To obtain effector OT-I cells, 1×10^4 naïve CD45.1$^+$ OT-I cells were adoptively transferred into CD45.2$^+$ recipient mice. One day later, mice were foodborne infected with 2×10^9 cfu InlA^M r*Lm*-OVA. 6 days post infection (dpi), effector OT-I cells were enriched from the mesenteric lymph nodes (MLN) using positive magnetic selection (Miltenyi Biotec) and sorted on a FACSARIA III cell sorter (BD Biosciences) based on the CD45 congenic marker.

Quantitative PCR

RNA was isolated using RNeasy Plus Micro Kit (QIAGEN) and cDNA was generated using iScript Advanced cDNA Synthesis Kit (Bio-Rad). Real-time RT-PCR was performed on a Bio-Rad CFX96™ using primers purchased from Bio-Rad (qMmuCID0020840 for *Batf3* and qMmuCID0022816 for *Hmbs*).

Adoptive co-transfers

WT and *Batf3*$^{-/-}$ OT-I cells bearing different CD45 congenic markers (2×10^3 each) were intravenously co-transferred into naïve congenic mice one day prior to foodborne infection. Congenic allele use was based on mice availability but always performed to distinguish donors from each other and the recipient.

Flow cytometric analysis and reagents

To isolate cells for flow cytometric analysis, spleen and MLN were mashed through 70 μm cell strainers. Small intestine intraepithelial lymphocytes (IEL) were isolated as previously described (12, 13). For functional analysis, IEL were mixed with equal number of congenically different naïve splenocytes and subsequently stimulated with or without 1 μg/ml of SIINFEKL peptide at 37℃ for 5 hours in the presence of brefeldin A followed by intracellular staining of IFNγ and TNFα. Granzyme B staining was performed directly *ex vivo*. Reagents for flow cytometric analysis are listed in Supplemental Table 1. Data were acquired on a LSRFortessa (BD Biosciences) and analyzed with FlowJo software (Tree Star).

Statistical Analysis

Statistical analyses were performed in Prism (GraphPad Software) using unpaired *t* test for Figure 1 and paired *t* test for all other figures. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$.

Results and Discussion

Batf3 expression by CD8 T cells is dispensable for the primary response but critically regulates memory development

It is well established that *Batf3*-dependent cDC1 play a crucial role in mounting anti-viral and anti-tumor CD8 T cell response by cross-presenting exogenous antigens to naïve CD8 T cells. However, only cursory examinations of *Batf3* intrinsic effects on the CD8 T cell response have been evaluated. As such, effector OT-I cells isolated from the MLN of mice at 6 days post infection (dpi) with a mouse adapted *Listeria monocytogenes* expressing ovalbumin ($InlA^M$ r*Lm*-OVA) were evaluated for *Batf3* gene expression. Effector CD8 T cells rapidly upregulated *Batf3* gene expression (Fig. 1), suggesting that cell intrinsic *Batf3* expression may play a role in the CD8 T cell response to infection.

To determine whether cell intrinsic *Batf3* expression regulates the CD8 T cell response, wild-type (WT) and $Batf3^{-/-}$ OT-I cells bearing different congenic markers generated from $Batf3^{+/-}$ OT-I $Rag1^{-/-}$ heterozygous breeders were co-transferred into congenically distinct B6 mice prior to foodborne infection with $InlA^M$ r*Lm*-OVA. Donor OT-I cells were gated based on congenic markers and analyzed at different time points after infection (Fig. 2A and Supplemental Fig. 1). A comparable ratio of WT and $Batf3^{-/-}$ OT-I cells was observed in the blood at 6 dpi (Fig. 2B). At 9 dpi, the peak of the primary CD8 T cell response, the ratio remained comparable in the blood and spleen, while it was slightly skewed towards $Batf3^{-/-}$ OT-I cells in the MLN (Fig. 2B), suggesting that *Batf3* does not play a critical role in CD8 T cell proliferation during the effector phase. Thus, *Batf3* deficiency in CD8 T cells had little impact on the primary T cell response. At 15 dpi, the ratio of WT and $Batf3^{-/-}$ OT-I cells started to skew towards WT OT-I cells (Fig. 2B). Strikingly, at 30 dpi, the ratio was severely skewed towards WT OT-I cells as $Batf3^{-/-}$ OT-I cells were almost undetectable in the blood, spleen and MLN (Fig. 2B). Thus, CD8 T cell intrinsic *Batf3* expression critically regulates the establishment of circulating memory cells.

The memory potential of CD8 T cells was further evaluated by assessing the compositions of short-lived effector cells (SLEC; $CD127^-$ $KLRG\text{-}1^+$), double positive effector cells (DPEC; $CD127^+$ $KLRG\text{-}1^+$) and memory precursor effector cells (MPEC; $CD127^+$ $KLRG\text{-}1^-$). SLEC rapidly undergo apoptosis during T cell contraction while MPEC have the greatest capacity to form long-lived memory cells (14). Some DPEC can lose KLRG-1 expression and also differentiate into long-live memory cells (15). At 9 dpi, WT and $Batf3^{-/-}$ OT-I cells had similar compositions of SLEC, DPEC and MPEC in the blood, spleen and MLN (Fig. 2C and Supplemental Fig. 2). Only subtle differences were observed. Overall, these data suggest that *Batf3* expression by CD8 T cells was largely dispensable for the primary response and the differentiation of effector T cells. At 15 dpi, although the ratio started to skew towards WT OT-I cells, the composition of SLEC, DPEC and MPEC remained largely comparable between WT and $Batf3^{-/-}$ OT-I cells with the exception that $Batf3^{-/-}$ OT-I cells that had slightly less DPEC in the blood (Fig. 2C and Supplemental Fig. 2). However, by 30 dpi, $Batf3^{-/-}$ OT-I cells had a significantly higher proportion of SLEC phenotype cells in the blood and spleen and a significantly lower proportion of MPEC phenotype cells in the blood, spleen and MLN (Fig. 2C and Supplemental Fig. 2). $Batf3^{-/-}$

OT-I cells also had a significantly lower proportion of DPEC in the blood (Fig. 2C). The heavy skew towards terminally differentiated SLEC but away from the memory potential of MPEC and DPEC phenotypes between 15 and 30 dpi reflects the fate of *Batf3*$^{-/-}$ OT-I cells at 30 dpi. Thus, cell intrinsic *Batf3* expression may regulate the MPEC lineage to promote memory cell development during contraction.

Batf3 expression by CD8 T cells promotes T_{RM} cell development in the intestinal epithelium

Batf3-dependent cDC1-mediated cross-priming has been shown to promote skin CD8 T_{RM} cell development (6). Foodborne *Lm* infection drives robust CD103$^+$ T_{RM} cell development in the intestine (16). As such, the role of cell intrinsic *Batf3* expression by CD8 T cells in CD103$^+$ T_{RM} cell development in the intestinal epithelium was evaluated. WT and *Batf3*$^{-/-}$ OT-I cells had comparable expression of gut-homing receptors integrin $\alpha_4\beta_7$ and chemokine receptor CCR9 in the blood at 6 dpi (Fig. 3A), suggesting that *Batf3* expression by CD8 T cells did not regulate gut-homing receptor expression or intestinal migration capacity. At 9 dpi, the ratio of WT and *Batf3*$^{-/-}$ OT-I cells was comparable in the intestinal epithelium (IEL compartment) (Fig. 3B). Both WT and *Batf3*$^{-/-}$ OT-I cells comprised similar compositions of effector subsets (Fig. 3C). Although *Batf3*$^{-/-}$ OT-I cells had a slight disadvantage in upregulating CD69 and CD103 expression, a similar number of WT and *Batf3*$^{-/-}$ OT-I cells had differentiated into CD103$^+$ T_{RM} cell precursors (Fig. 3D), suggesting that *Batf3* expression by CD8 T cells does not regulate the generation of intestinal CD103$^+$ T_{RM} cell precursors. A similar phenomenon was observed at 15 dpi (Fig. 3B–3D). However, by 30 dpi, *Batf3*$^{-/-}$ OT-I cells were significantly reduced in the IEL compartment (Fig. 3B), suggesting *Batf3* deficiency in CD8 T cells impairs T_{RM} cell development. Although *Batf3*$^{-/-}$ OT-I cells had a slightly increased ability to differentiate into MPEC and CD103$^+$ T_{RM} cells (Fig. 3C and 3D), the number of CD103$^+$ T_{RM} cells in the IEL compartment at 30 dpi was significantly lower among *Batf3*$^{-/-}$ OT-I cells (Fig. 3D). Overall, these data suggest that *Batf3* expression by CD8 T cells promotes CD103$^+$ T_{RM} cell development in the intestinal epithelium without affecting the expression of gut-homing receptors or the generation of CD103$^+$ T_{RM} cell precursors. It should also be noted that T_{RM} cells appeared less impacted by cell intrinsic *Batf3* deficiency compared to their circulating counterparts, suggesting that distinct environmental signals present in the gut overcomes the defect of cell intrinsic *Batf3* deficiency on CD8 T cells.

Batf3 expression by CD8 T cells promotes CD8 T cell survival

Batf3 expression drives lymphomagenesis by promoting proliferation and survival (11). *Batf3*$^{-/-}$ OT-I cell numbers were normal during the effector phase suggesting that any potential impact on effector cell proliferation would be minimal. However, *Batf3*$^{-/-}$ OT-I cells decreased drastically between 15 and 30 dpi (Fig. 2B), suggesting an important role for *Batf3* in regulating CD8 T cell survival during contraction. As such, apoptosis of WT and *Batf3*$^{-/-}$ OT-I cells was evaluated. Compared to WT OT-I cells, *Batf3*$^{-/-}$ OT-I cells had a significant increase in early apoptotic cell (Apotracker$^+$ Live/dead$^-$) populations at 9, 15 and 30 dpi, and the increase enlarged over time (Fig. 4). An increase in late apoptotic cells (Apotracker$^+$ Live/dead$^+$) also emerged in *Batf3*$^{-/-}$ OT-I cells by 30 dpi (Fig. 4). These data suggest that *Batf3* expression regulates memory CD8 T cell development in part by

promoting CD8 T cell survival. The survival of effector CD8 T cells during contraction is dependent on IL-7 and IL-15, which upregulates the anti-apoptotic molecule Bcl-2 to overcome the effect of TGFβ (14). As Bcl2 family members are known targets of AP-1 transcription factors (17), *Batf3* may regulate memory development by promoting the anti-apoptotic machinery. The closely related molecule, BATF, can regulate Bcl-2 expression and survival of effector CD8 T cells (18). A defect in homeostatic division during memory maintenance may also contribute to loss of *Batf3*$^{-/-}$ memory T cells. This is particularly relevant for T_{RM} cells as *Batf3*$^{-/-}$ IEL are reduced but still detectable at 30 dpi. Memory CD8 T cells undergo IL-7 and IL-15 dependent homeostatic division (19). However, homeostatic turnover is slower in intestinal T_{RM} cells (20), which may contribute to the reduced impact of *Batf3* deficiency on T_{RM} cell populations.

Batf3 expression by CD8 T cells is important for recall but dispensable for function.

To determine whether cell intrinsic *Batf3* expression impacts recall to a secondary challenge, mice outlined in Fig. 2A were challenged at 30 dpi with a secondary infection and donor OT-I cells were analyzed at 6 days post recall infection (dpr). We were unable to detect a clear *Batf3*$^{-/-}$ OT-I cell population in the spleen and MLN (data not shown); thus, the analysis focused on donor cells from the blood and IEL compartment. While *Batf3*$^{-/-}$ OT-I cells were hardly detectable in the blood at memory and 6 dpr, their numbers increased after recall (Fig. 5A an B), suggesting that they are able to mount a recall response. However, while WT OT-I cells increased more than 20-fold after recall infection, *Batf3*$^{-/-}$ OT-I cells increased less than 10-fold (Fig. 5B), suggesting that *Batf3* deficiency in CD8 T cells impaired their ability to mount a robust recall response. Furthermore, WT and *Batf3*$^{-/-}$ OT-I cells had different compositions of effector subsets at 6 dpr with *Batf3*$^{-/-}$ OT-I cells having less SLEC and more DPEC (Supplemental Fig. 3A). The analysis in the IEL compartment revealed that the ratio of WT and *Batf3*$^{-/-}$ OT-I cells was severely skewed towards WT OT-I cells (about 9:1) (Fig. 5C), which was more striking than that at memory (about 2:1) (Fig. 3B). These data suggest that *Batf3*-deficient OT-I cells are less competitive than their *Batf3*-sufficient counterparts at secondary expansion to challenge infection. The impaired ability of *Batf3*$^{-/-}$ OT-I cells to mount a robust recall response in the IEL was consistent with the lack of an emergent SLEC and CD69^{-} population at 6 dpr (Supplemental Fig. 3B and 3C). Whether this defect is due to a lack of *in situ* proliferation and differentiation or a lack of newly recalled intestinal immigrants remains to be determined.

Thus far, these data demonstrated that *Batf3* expression by CD8 T cells critically regulated memory development and the robustness of the recall response. However, whether it regulates T cell function is unknown. The functional analysis of WT and *Batf3*$^{-/-}$ OT-I cells was performed at 6 dpr. *Batf3*$^{-/-}$ OT-I cells had a subtle but significant increase in granzyme B in the blood but not IEL (Fig. 5D and 5E). Additionally, *Batf3*$^{-/-}$ OT-I cells produced similar IFNγ, but more cells were IFNγ and TNFα double producers (Fig. 5F). Collectively, these data suggest that *Batf3* deficiency in CD8 T cells did not result in functional impairment during recall.

The impact of *Batf3* deficiency on the CD8 T cell response has been largely attributed to a lack of cDC1. By assessing the impact of *Batf3* deficiency on the CD8 T cell response in

mice with a normal compartment of DC, we uncovered a novel role for CD8 T cell intrinsic *Batf3* expression in the establishment of robust circulating and resident memory T cells. In summary, intrinsic *Batf3* expression by CD8 T cells was dispensable for the primary CD8 T cell response but required for the development of circulating memory T cells. In non-lymphoid tissues, cell intrinsic *Batf3* expression was required for optimal T_{RM} cell development. In the absence of *Batf3*, CD8 T cells underwent more apoptosis and failed to survive into memory, though other mechanisms may also contribute to the reduced memory population. Moreover, cell intrinsic *Batf3* expression is dispensable for their function but is required for CD8 T cells to mount a robust recall response. Altogether, this study uncovered a novel CD8 T cell-intrinsic role of *Batf3* expression in regulating memory development and recall response in part by promoting T cell survival during contraction that may be exploited for rationale vaccine design.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations:

BATF3	basic leucine zipper transcription factor ATF-like 3
cDC1	conventional type 1 dendritic cells
DPEC	double positive effector cells
dpi	days post infection
dpr	days post recall
IEL	intraepithelial lymphocytes
Lm	Listeria monocytogenes
MLN	mesenteric lymph nodes
MPEC	memory precursor effector cells
SLEC	short-lived effector cells
T_{RM}	resident memory T
WT	wild-type

References

1. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, Calderon B, Schraml BU, Unanue ER, Diamond MS, Schreiber RD, Murphy TL, and Murphy KM. 2008 Batf3 deficiency

reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. Science 322: 1097–1100. [PubMed: 19008445]

2. Edelson BT, Kc W, Juang R, Kohyama M, Benoit LA, Klekotka PA, Moon C, Albring JC, Ise W, Michael DG, Bhattacharya D, Stappenbeck TS, Holtzman MJ, Sung SS, Murphy TL, Hildner K, and Murphy KM. 2010 Peripheral CD103+ dendritic cells form a unified subset developmentally related to CD8alpha+ conventional dendritic cells. J Exp Med 207: 823–836. [PubMed: 20351058]
3. Murphy TL, Tussiwand R, and Murphy KM. 2013 Specificity through cooperation: BATF-IRF interactions control immune-regulatory networks. Nat Rev Immunol 13: 499–509. [PubMed: 23787991]
4. Mashayekhi M, Sandau MM, Dunay IR, Frickel EM, Khan A, Goldszmid RS, Sher A, Ploegh HL, Murphy TL, Sibley LD, and Murphy KM. 2011 CD8alpha(+) dendritic cells are the critical source of interleukin-12 that controls acute infection by Toxoplasma gondii tachyzoites. Immunity 35: 249–259. [PubMed: 21867928]
5. Edelson BT, Bradstreet TR, Hildner K, Carrero JA, Frederick KE, Kc W, Belizaire R, Aoshi T, Schreiber RD, Miller MJ, Murphy TL, Unanue ER, and Murphy KM. 2011 CD8alpha(+) dendritic cells are an obligate cellular entry point for productive infection by Listeria monocytogenes. Immunity 35: 236–248. [PubMed: 21867927]
6. Iborra S, Martinez-Lopez M, Khouili SC, Enamorado M, Cueto FJ, Conde-Garrosa R, Del Fresno C, and Sancho D. 2016 Optimal Generation of Tissue-Resident but Not Circulating Memory T Cells during Viral Infection Requires Crosspriming by DNGR-1+ Dendritic Cells. Immunity 45: 847–860. [PubMed: 27692611]
7. Lee W, Kim HS, Hwang SS, and Lee GR. 2017 The transcription factor Batf3 inhibits the differentiation of regulatory T cells in the periphery. Exp Mol Med 49: e393. [PubMed: 29147008]
8. Lee WH, Jang SW, Kim HS, Kim SH, Heo JI, Kim GE, and Lee GR. 2019 BATF3 is sufficient for the induction of Il9 expression and can compensate for BATF during Th9 cell differentiation. Exp Mol Med 51: 143.
9. Tsuda M, Hamade H, Thomas LS, Salumbides BC, Potdar AA, Wong MH, Nunnelee JS, Stamps JT, Neutzsky-Wulff AV, Barrett RJ, Wang Y, Tang J, Funari VA, Targan SR, and Michelsen KS. 2019 A role for BATF3 in TH9 differentiation and T-cell-driven mucosal pathologies. Mucosal Immunol 12: 644–655. [PubMed: 30617301]
10. Zhang X, Xiao X, Lan P, Li J, Dou Y, Chen W, Ishii N, Chen S, Xia B, Chen K, Taparowsky E, and Li XC. 2018 OX40 Costimulation Inhibits Foxp3 Expression and Treg Induction via BATF3-Dependent and Independent Mechanisms. Cell Rep 24: 607–618. [PubMed: 30021159]
11. Lollies A, Hartmann S, Schneider M, Bracht T, Weiss AL, Arnolds J, Klein-Hitpass L, Sitek B, Hansmann ML, Kuppers R, and Weniger MA. 2018 An oncogenic axis of STAT-mediated BATF3 upregulation causing MYC activity in classical Hodgkin lymphoma and anaplastic large cell lymphoma. Leukemia 32: 92–101. [PubMed: 28659618]
12. Sheridan BS, and Lefrancois L. 2012 Isolation of mouse lymphocytes from small intestine tissues. Curr Protoc Immunol Chapter 3: Unit3 19.
13. Qiu Z, and Sheridan BS. 2018 Isolating Lymphocytes from the Mouse Small Intestinal Immune System. J Vis Exp.
14. Qiu Z, Khairallah C, and Sheridan BS. 2018 Listeria Monocytogenes: A Model Pathogen Continues to Refine Our Knowledge of the CD8 T Cell Response. Pathogens 7.
15. Herndler-Brandstetter D, Ishigame H, Shinnakasu R, Plajer V, Stecher C, Zhao J, Lietzenmayer M, Kroehling L, Takumi A, Kometani K, Inoue T, Kluger Y, Kaech SM, Kurosaki T, Okada T, and Flavell RA. 2018 KLRG1(+) Effector CD8(+) T Cells Lose KLRG1, Differentiate into All Memory T Cell Lineages, and Convey Enhanced Protective Immunity. Immunity.
16. Sheridan BS, Pham QM, Lee YT, Cauley LS, Puddington L, and Lefrancois L. 2014 Oral infection drives a distinct population of intestinal resident memory CD8(+) T cells with enhanced protective function. Immunity 40: 747–757. [PubMed: 24792910]
17. Eferl R, and Wagner EF. 2003 AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer 3: 859–868. [PubMed: 14668816]
18. Kuroda S, Yamazaki M, Abe M, Sakimura K, Takayanagi H, and Iwai Y. 2011 Basic leucine zipper transcription factor, ATF-like (BATF) regulates epigenetically and energetically effector CD8 T-

cell differentiation via Sirt1 expression. Proc Natl Acad Sci U S A 108: 14885–14889. [PubMed: 21873234]

19. Tan JT, Ernst B, Kieper WC, LeRoy E, Sprent J, and Surh CD. 2002 Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8+ cells but are not required for memory phenotype CD4+ cells. J Exp Med 195: 1523–1532. [PubMed: 12070280]
20. Schenkel JM, Fraser KA, Casey KA, Beura LK, Pauken KE, Vezys V, and Masopust D. 2016 IL-15-Independent Maintenance of Tissue-Resident and Boosted Effector Memory CD8 T Cells. J Immunol 196: 3920–3926. [PubMed: 27001957]

Key Points

1. *Batf3* expression by CD8 T cells critically regulates memory T cell development.
2. *Batf3*$^{-/-}$ CD8 T cell undergo increased apoptosis during contraction.
3. *Batf3*$^{-/-}$ CD8 T cells have an impaired recall ability but remain functional.

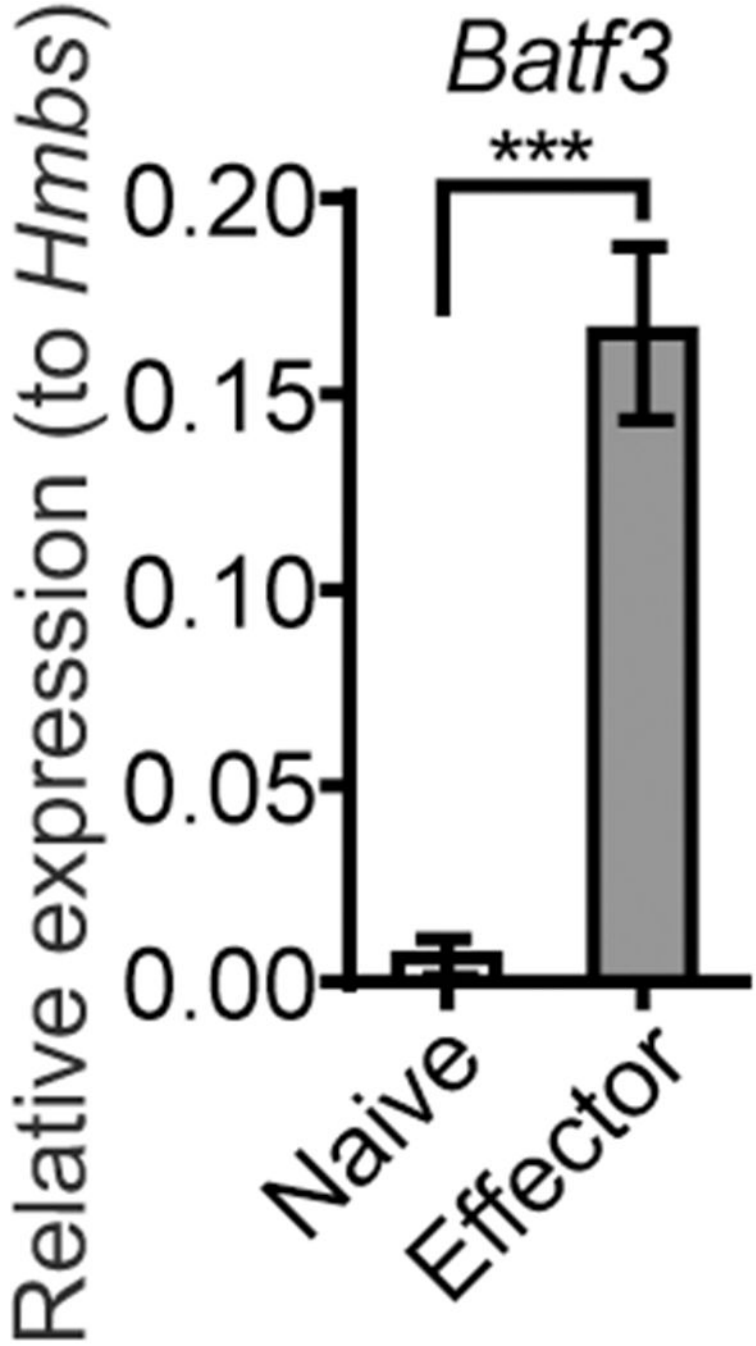

Figure 1.

Effector CD8 T cells upregulate *Batf3* gene expression. OT-I cells sorted from the spleen of naïve OT-I *Rag1*$^{-/-}$ mouse (Naïve) and from the MLN of foodborne infected mice at 6 dpi (Effector) were subjected to RNA extraction and quantitative PCR. Relative expression of *Batf3* mRNA to housekeeping gene *Hmbs* was calculated. The data is representative of 3 independent experiments with n=4 and shown as mean ± SEM.

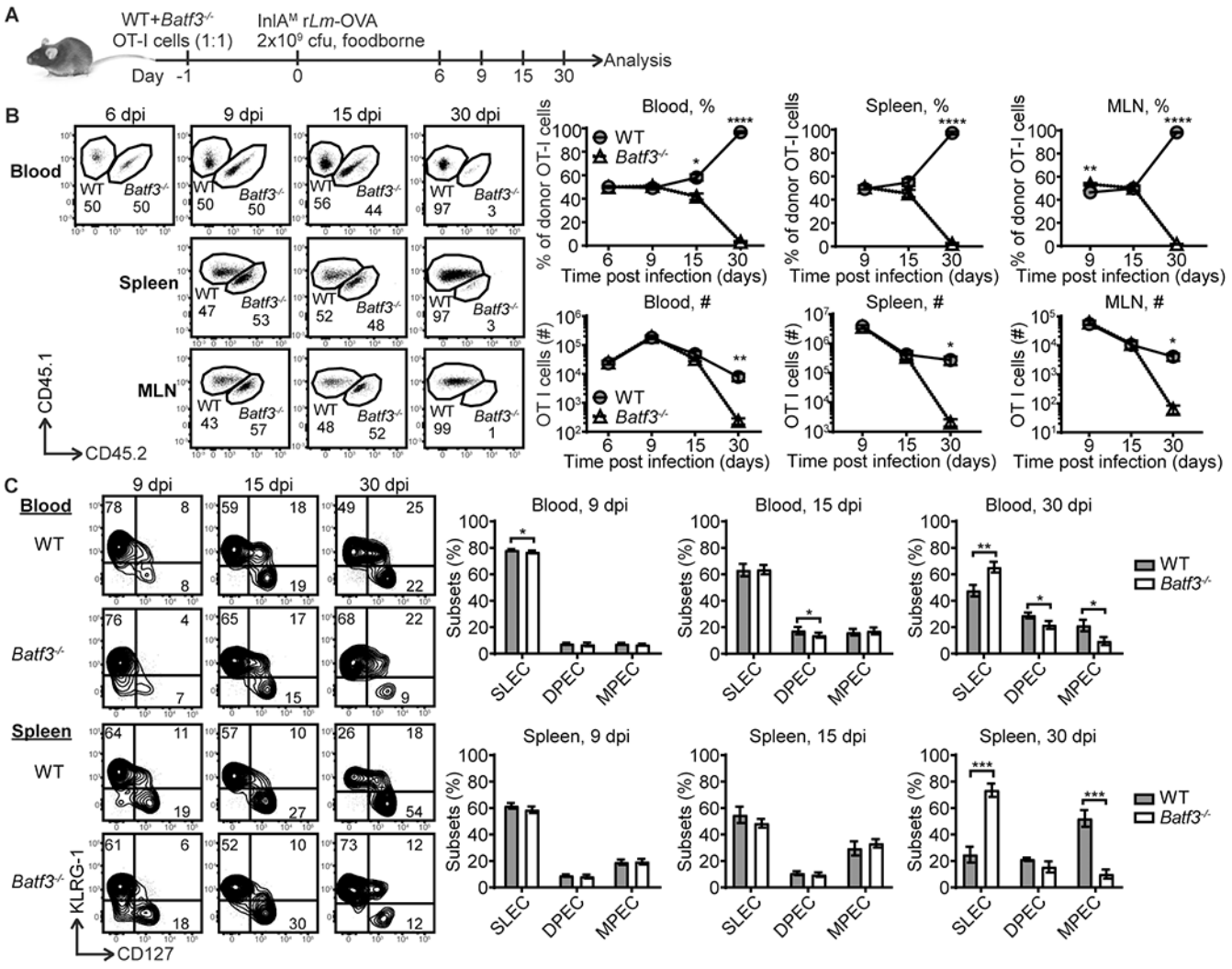

Figure 2.

Batf3 expression by CD8 T cells is dispensable for the primary response but critically regulates memory development. (**A**) Experimental setup. WT and *Batf3*$^{-/-}$ OT-I cells (1:1, 2×10^3 cells each) bearing different congenic markers were intravenously co-transferred into naïve congenic mice one day prior to foodborne infection with InlA^M r*Lm*-OVA followed by analysis at different time points. (**B**) The kinetics of WT and *Batf3*$^{-/-}$ OT-I cells in the blood, spleen and MLN. (**C**) The kinetics of CD127 and KLRG-1 expression by WT and *Batf3*$^{-/-}$ OT-I cells in the blood and spleen. The data are pooled from 3 independent experiments for 6 and 9 dpi with n=36 for 6 dpi and n=12 for 9 dpi and 2 independent experiments for 15 and 30 dpi with n=7. The data are shown as mean ± SEM.

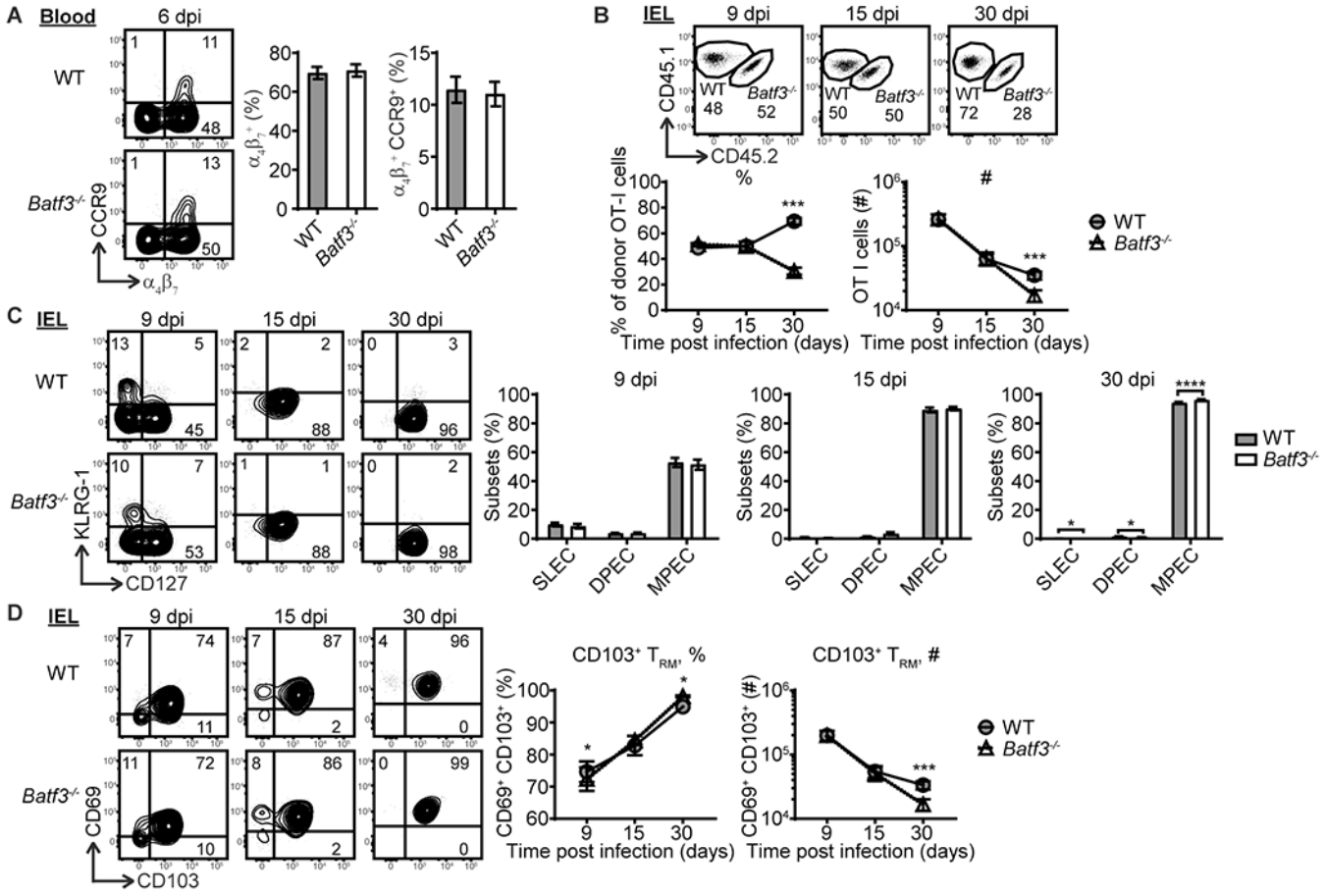

Figure 3.

Batf3 expression by CD8 T cells promotes T_{RM} cell development in the intestinal epithelium. (**A**) Integrin $\alpha_4\beta_7$ and CCR9 expression by WT and *Batf3*$^{-/-}$ OT-I cells in the blood at 6 dpi. (**B**) The kinetics of WT and *Batf3*$^{-/-}$ OT-I cells in the IEL compartment. (**C**) The kinetics of CD127 and KLRG-1 expression by WT and *Batf3*$^{-/-}$ OT-I cells in the IEL compartment. (**D**) The kinetics of CD1103 and CD69 expression by WT and *Batf3*$^{-/-}$ OT-I cells in the IEL compartment. The data in (**A**) are pooled from 3 independent experiments with n=36. The data in (A) are pooled from 3 independent experiments with n=36. The The data in (B-D) are pooled from 3 independent experiments for 9 dpi with n=12 and from 2 independent experiments for 15 and 30 dpi with n=7. The data are shown as mean ± SEM.

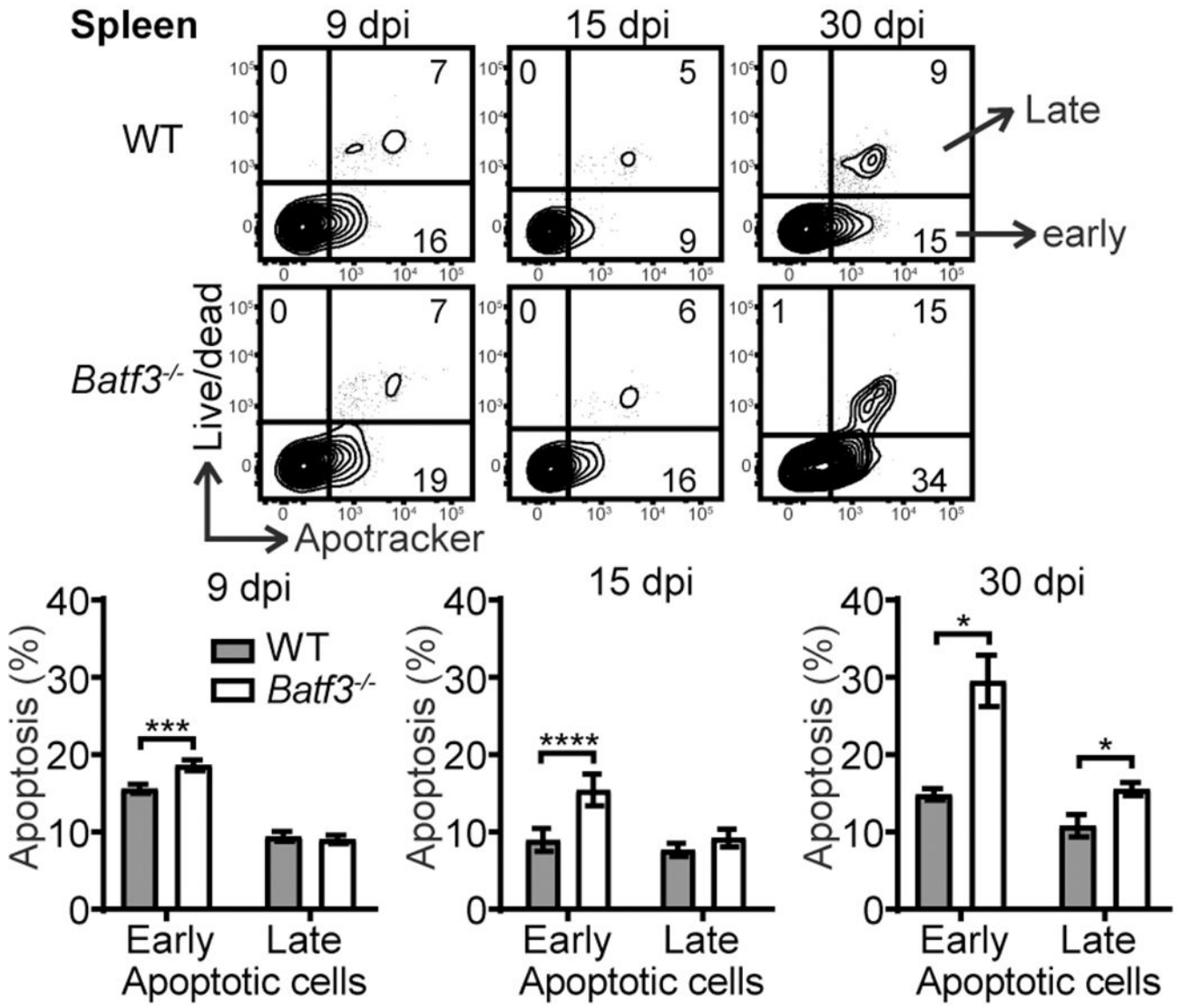

Figure 4.

Batf3-deficient CD8 T cells undergo enhanced apoptosis. The kinetics of apoptosis analysis of WT and *Batf3*$^{-/-}$ OT-I cells in the spleen. Early apoptotic cells are defined as Apotracker^{+} Live/dead^{-} and late apoptotic cells are defined as Apotracker^{+} Live/dead^{+}. The data are pooled from two independent experiments with n=8 for 9 dpi and n=7 for 15 and 30 dpi and shown as mean ± SEM.

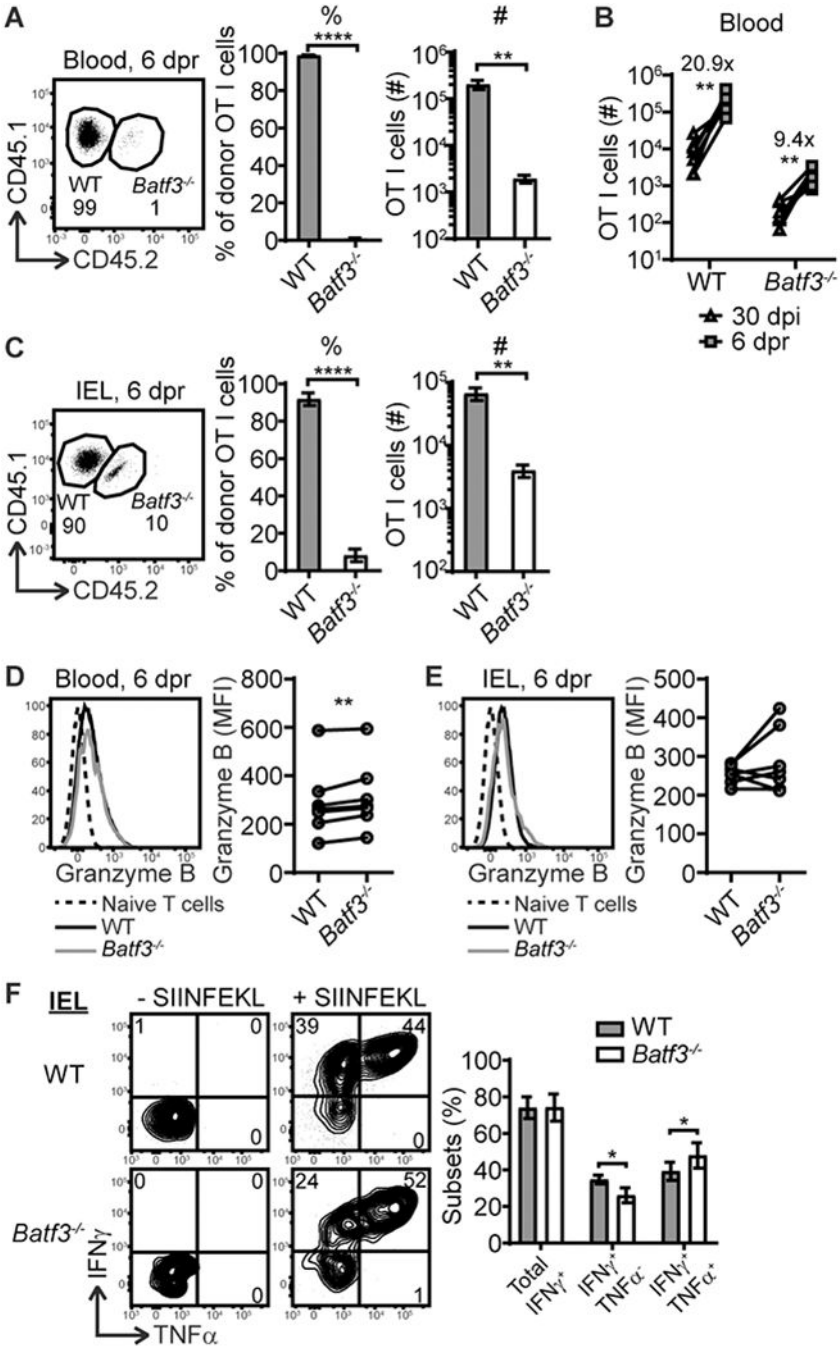

Figure 5.

Batf3 expression by CD8 T cells is important for recall response but dispensable for their function. Mice outlined in Fig. 2A were challenged with recall infection at 30 dpi and the donor OT-I cells were analyzed at 6 dpr. (**A**) The percentage and number of WT and *Batf3*$^{-/-}$ OT-I cells in the blood at 6 dpr. (**B**) The corresponding number of WT and *Batf3*$^{-/-}$ OT-I cells in the blood at 30 dpi and 6 dpr. (**C**) The percentage and number of WT and *Batf3*$^{-/-}$ OT-I cells in the IEL compartment at 6 dpr. (**D**) The granzyme B expression by WT and *Batf3*$^{-/-}$ OT-I cells in the blood at 6 dpr. (**E**) The granzyme B expression by WT and

Batf3$^{-/-}$ OT-I cells in the IEL compartment at 6 dpr. (**F**) The IFNγ and TNFα expression by WT and *Batf3*$^{-/-}$ OT-I cells in the IEL compartment at 6 dpr. The data are pooled from 2 independent experiments with n=7. The data in (A, C, F) are shown as mean ± SEM. In (B, D, E), each dot represents an individual animal.