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Batf3 Deficiency Reveals a Critical Role for CD8α⁺ Dendritic Cells in Cytotoxic T Cell Immunity

Kai Hildner[1,2], **Brian T. Edelson**[1], **Whitney E. Purtha**[3], **Mark Diamond**[1], **Hirokazu Matsushita**[1], **Masako Kohyama**[1,2], **Boris Calderon**[1], **Barbara Schraml**[1], **Emil R. Unanue**[1], **Michael S. Diamond**[3], **Robert D. Schreiber**[1], **Theresa L. Murphy**[1], and **Kenneth M. Murphy**[1,2,*]

[1]Department of Pathology and Immunology, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110, USA

[2]Howard Hughes Medical Institute, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110, USA

[3]Departments of Medicine, Molecular Microbiology, Pathology & Immunology, 660 S. Euclid Ave., St. Louis, MO, 63110, USA

Abstract

While in vitro observations suggest that cross-presentation of antigens is mediated primarily by CD8α$^+$ dendritic cells, in vivo analysis has been hampered by the lack of systems that selectively eliminate this cell lineage. Here we show that deletion of the transcription factor *Batf3* ablated development of CD8α$^+$ dendritic cells, allowing us to examine their role in immunity in vivo. Dendritic cells from *Batf3*$^{-/-}$ mice were defective in cross-presentation and *Batf3*$^{-/-}$ mice lacked virus-specific CD8$^+$ T cell responses to West Nile virus. Importantly, rejection of highly immunogenic syngeneic tumors was impaired in *Batf3*$^{-/-}$ mice. These results suggest an important role for CD8α$^+$ dendritic cells and cross-presentation in responses to viruses and in tumor rejection.

During antigen 'cross-presentation' (1), antigens generated in one cell are presented by MHC class I molecules of a second cell. It remains unclear whether all antigen presenting cells (APCs) use cross-presentation and whether this pathway plays a role in immune responses in vivo (2). Dendritic cells (DCs) are a heterogeneous group of APCs with two major subsets, plasmacytoid dendritic cells (pDCs) and conventional CD11c$^+$ dendritic cells (cDCs) (3). Subsets of cDCs include CD8α$^+$, CD4$^+$, and CD8α$^-$CD4$^-$ populations that may exert distinct functions in immune responses. Evidence has suggested that CD8α$^+$ cDCs are important for cross-presentation during infections, but is based on ex vivo analysis (4-6) or in vitro antigen loading (7). Evidence both for and against a role for cross-presentation in responses against tumors has been reported (8-10).

Attempts have been made to study the in vivo role of dendritic cells by selective depletion. Diphtheria toxin treatment can deplete all CD11c^{hi} cells in one transgenic mouse model (11), but affects splenic macrophages and activated CD8$^+$ T cells (12). Gene targeting of transcription factors (e.g., *Irf2*, *Irf4*, *Irf8*, *Stat3* and *Id2*) has caused broad defects in several DC subsets, T cells and macrophages (13). To identify genes regulating DC development, we performed global gene expression analysis across many tissues and immune cells (fig S1A). *Batf3* (*p21SNFT*) (14) was highly expressed in cDCs, with low to absent expression in other

*To whom correspondence should be addressed. E-mail murphy@pathology.wustl.edu.

immune cells and non-immune tissues. Thus, we generated *Batf3*$^{-/-}$ mice lacking expression of the Batf3 protein (fig. S1B-D).

In spleens of *Batf3*$^{-/-}$ mice we found a selective loss of CD8α^{+} cDCs, without abnormalities in other hematopoietic cell types or architecture (Fig. 1, fig. S2-S11). CD8α^{+} cDC coexpress DEC205, CD24, and low levels of CD11b (3,15). *Batf3*$^{-/-}$ mice lacked splenic CD11c^{hi} CD8α^{+} DEC205^{+} cells (Fig. 1A), showed a loss of CD11c^{hi} CD11b^{dull} cells and CD11c^{hi} CD8α^{+} CD24^{+} cells (Fig. 1B), but had normal populations of CD4^{+} and CD8α^{-}CD4^{-} cDC subsets (Fig. 1B). Lymph nodes and thymi of *Batf3*$^{-/-}$ mice lacked CD8α^{+} DCs but had normal distributions of CD8α^{-} CD11c^{+} cells (Fig. 1C). DEC205^{int} and DEC205^{hi} DCs were present in lymph nodes draining the skin of *Batf3*$^{-/-}$ mice (Fig. 1C), and showed normal migration from skin to lymph node after topical application of fluorescein-5-isothiocyanate (fig. S3A). *Batf3*$^{-/-}$ mice had normal development of pDCs (CD11c^{int} CD11b^{-} B220^{+}) (fig. S3B), interstitial DCs of pancreatic islets (CD11c^{+} CD8α^{-}) (fig. S3C, D), monocytes, neutrophils (fig. S3E) and SIGN-R1^{+} and MOMA-1^{+} marginal zone macrophages (Fig. 2A). CD8α^{+} cDCs developed normally in heterozygous *Batf3*$^{+/-}$ mice (fig. S4A), and were absent in *Rag2*$^{-/-}$ *Batf3*$^{-/-}$ mice (fig. S4B).

This loss of CD8α^{+} cDCs could result from a cell-autonomous hematopoietic defect or a cell-extrinsic requirement for *Batf3*. To distinguish these possibilities, we generated chimeras in which CD45.2^{+} *Batf3*$^{+/+}$ or CD45.2^{+} *Batf3*$^{-/-}$ bone marrow (BM) was transplanted into lethally irradiated CD45.1^{+}CD45.2^{+} recipients (Fig. 2B). Upon reconstitution (fig. S5A), we found CD8α^{+} cDCs developed only from *Batf3*$^{+/+}$ donor BM cell (Fig. 2B), indicating a cell-intrinsic hematopoietic defect in *Batf3*$^{-/-}$ mice.

Treatment of mice with fms-like tyrosine kinase 3 (flt3) ligand-Fc (FL-Fc) expanded CD8α^{+} cDCs, CD8α^{-} cDCs and pDCs in *Batf3*$^{+/+}$ mice, but failed to expand CD8α^{+} cDC in *Batf3*$^{-/-}$ mice (Fig. 2C). In vitro culture of BM with FL generates cell populations corresponding to pDCs (CD11c^{+}CD45RA^{+}) and cDCs (CD11c^{+}CD45RA^{-}) (3,16) (Fig. 2D). These in vitro-derived cDCs do not express CD8α or CD4, but contain a CD24^{+}Sirp-α$^{lo\text{-}int}$ population corresponding to CD8α^{+} cDC (16). *Batf3*$^{+/+}$ or *Batf3*$^{-/-}$ BM cells treated with FL produced similar ratios of pDCs and cDCs (Fig. 2D and fig. S5B). However, *Batf3*$^{-/-}$ BM generated far fewer CD24^{+} Sirp-α^{-} cells compared to *Batf3*$^{+/+}$ BM (Fig. 2D), corresponding to loss of CD8α^{+} cDCs. Finally, DCs generated from *Batf3*$^{-/-}$ BM were selectively deficient in TLR3-induced IL-12 production (fig. S5C), a specific feature of CD8α^{+} cDCs (16). Similarly, CD11c^{+} cDCs from the spleens of *Batf3*$^{-/-}$ mice were selectively deficient in TLR3-induced IL-12 production but had normal responses to TLR4 and TLR9 ligands (fig. S6A).

We next tested whether APCs from *Batf3*$^{-/-}$ mice could prime CD4^{+} and CD8^{+} T cell responses. Similar proliferative responses of OT-II transgenic CD4^{+} T cells (17) occurred with soluble ovalbumin presented by *Batf3*$^{+/+}$ and *Batf3*$^{-/-}$ cDCs (fig. S6B). However, *Batf3*$^{-/-}$ cDCs were defective in an assay for cross-presentation of cellular antigen to CD8^{+} T cells (2,18) (Fig. 3A). OT-I T cells proliferated in response to *Batf3*$^{+/+}$ cDCs cocultured with ovalbumin-loaded cells, but failed to proliferate in response to *Batf3*$^{-/-}$ cDCs in this assay.

We examined responses of *Batf3*$^{-/-}$ mice to West Nile virus (WNV) (19,20). *Batf3*$^{-/-}$ mice showed normal WNV-specific antibody responses (Fig. 3B), memory B cells (fig. S6C) and CD4^{+} T cell responses (fig. S6D) but had a dramatic reduction in WNV-specific CD8^{+} T cell responses (Fig. 3C) and in vivo CTL killing of WNV peptide-loaded target cells (fig. S7A, B). *Batf3*$^{-/-}$ mice lacked WNV-specific memory CD8^{+} T cells and had impaired formation of CD8^{+} CD44^{hi} CD62L^{low} cells (fig. S7). Adoptive transfer of *Batf3*$^{-/-}$ CD8^{+} T cells into *Rag2*$^{-/-}$ mice generated normal WNV-specific CD8^{+} T cell response (Fig. 3D), but adoptive transfer of *Batf3*$^{+/+}$ CD8^{+} T cells into *Batf3*$^{-/-}$ *Rag2*$^{-/-}$ mice generated an impaired WNV-

specific $CD8^+$ T cell response (fig. S7C). This shows that impaired WNV-specific CTL responses in *Batf3*$^{-/-}$ mice results from a defect of DCs rather $CD8^+$ T cells.

We challenged *Batf3*$^{+/+}$ and *Batf3*$^{-/-}$ mice with syngeneic fibrosarcomas that normally are rapidly rejected in a $CD4^+$ and $CD8^+$ T cell-dependent manner (21,22) (fig. S8A). Two independent fibrosarcomas were rapidly rejected by *Batf3*$^{+/+}$ mice, but grew progressively in *Rag2*$^{-/-}$ mice and *Batf3*$^{-/-}$ mice (Fig. 4A,fig. S8B and C). Moreover, *Batf3*$^{-/-}$ mice failed to develop tumor-specific CTLs (Fig. 4B). Tumor-infiltrating $CD8^+$ T cells, but not $CD4^+$ T cells, were significantly reduced in *Batf3*$^{-/-}$ mice (Fig. 4C). The failure of *Batf3*$^{-/-}$ mice to reject these tumors was not due to defective NK cell development or function (fig. S2B and S9A-C). We considered whether *Batf3*$^{-/-}$ T cells have an intrinsic dysfunction, since overexpression studies had suggested *Batf3* might affect IL-2 transcription (14). While *Batf3* overexpression reduces IL-2 reporter activity in Jurkat T cells (fig. S10B), *Batf3*$^{-/-}$ $CD4^+$ T cells showed normal IL-2 production (fig. S10D) and normal T_H1, T_H2 and T_H17 differentiation (fig. S10C-E, S11B). Finally, *Batf3*$^{-/-}$ $CD8^+$ T cells showed normal allospecific effector responses (fig. S11A) and cytokine production (fig. S11B).

Other DC subsets may cross-present, although less efficiently than $CD8\alpha^+$ DCs (23-26), suggesting there may be residual cross-presentation capacity in *Batf3*$^{-/-}$ mice. We therefore challenged mice using reduced tumor-cell numbers which might allow effective responses in the setting of reduced cross-presentation (fig. S8). While 10^4 and 10^5 tumor cells grew in all *Rag2*$^{-/-}$ mice, some *Batf3*$^{-/-}$ mice controlled this lower tumor burden (fig. S8D, 8E) and developed tumor-specific CTL response (fig. S8F). Whereas adoptive transfer of wild type DCs led to partial control of tumor growth in *Batf3*$^{-/-}$ mice, transfer of *Batf3*$^{-/-}$ DCs did not (fig. S12).

Subsets of cDCs have recently been described with functional similarities to $CD8\alpha^+$ cDCs. Migratory $Langerin^+$ dermal and lung DC subsets express $DEC205^+$ and $CD103^+$, and like $CD8\alpha^+$ cDCs, are $CD11b^{lo/-}$ (27,28). $CD8\alpha^+$ cDC and migratory $CD103^+$ DC populations share the distinctive properties of TLR3 responsiveness (27) and capacity for cross-presentation (26), further supporting the idea that these $CD103^+$ subsets may be related. In spleen, CD103 is co-expressed with CD8α on cDCs (fig. S13A) (29) and is selectively expressed by the 'CD8α equivalent' $CD24^+Sirp\text{-}\alpha^{lo\text{-}int}$ cDC subset derived from FL-treated *Baft3*$^{+/+}$ BM (fig. S13C), but is not expressed by *Batf3*$^{-/-}$ splenic cDCs (fig. S13B) or FL-treated *Batf3*$^{-/-}$ BM cells. This suggests that CD103 expression may require *Batf3*. In agreement, *Batf3*$^{-/-}$ mice showed reduced CD103 expression on $DEC205^+CD8\alpha^-CD11b^{lo/-}$ dermal DCs in skin draining lymph nodes (fig. S14).

This study describes a transcription factor that controls development of $CD8\alpha^+$ cDCs. *Batf3*$^{-/-}$ mice exhibit impaired antigen cross-presentation, impaired CTL responses against viral infection, and impaired responses to tumor challenge. These results suggest an important role for in vivo cross-presentation in CTL responses and provide support for therapeutic approaches that utilize $CD8\alpha^+$ cDCs for the induction of effective immune responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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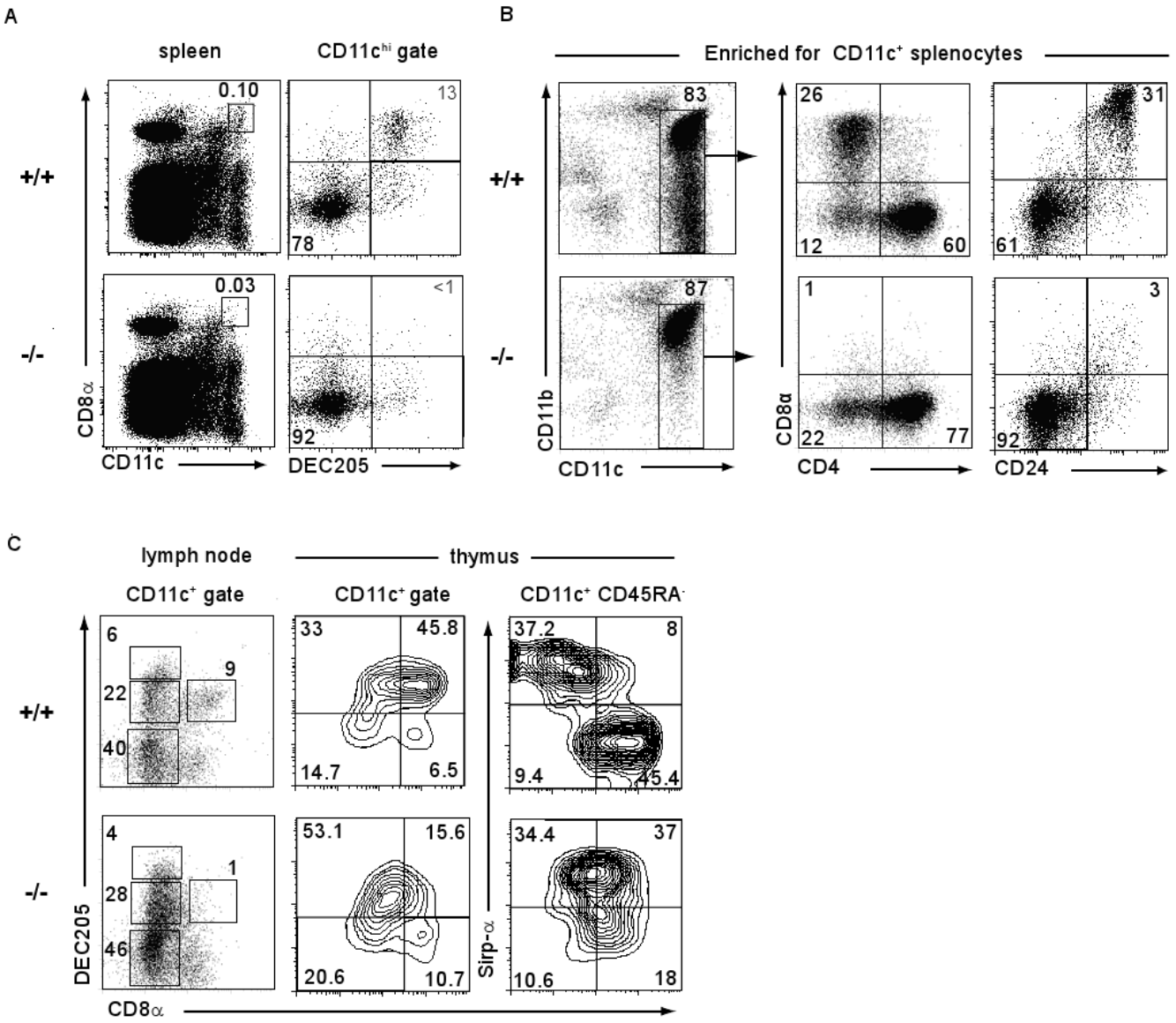

Fig. 1. *Batf3*$^{-/-}$ mice selectively lack the CD8α^{+} DC subset

(A) Splenocytes from *Batf3*$^{+/+}$ (+/+) or *Batf3*$^{-/-}$ (-/-) mice were stained for CD11c, CD8α and DEC205. Left panels are gated on live cells. Numbers indicate the percentage of splenocytes within the CD11c^{hi}CD8α^{+} gate. Right panels are gated on CD11c^{hi} cells. (B) Splenocytes were depleted of B220^{+} B cells and Thy1.2^{+} T cells and positively selected for CD11c expression by antibody coated magnetic beads (MACS). Cells were then stained for CD11c, CD11b, and either CD8α and CD4 or CD8α and CD24, and analyzed by FACS. Numbers represent the percentage of cells within the indicated gates. (C) Lymph node cells pooled from cervical, axillary and inguinal lymph nodes and depleted of Thy1.2^{+} T cells, or light density cells of the thymus were stained for CD11c, CD45RA CD8α, DEC205 or Sirp-α. Plots are gated on the indicated populations.

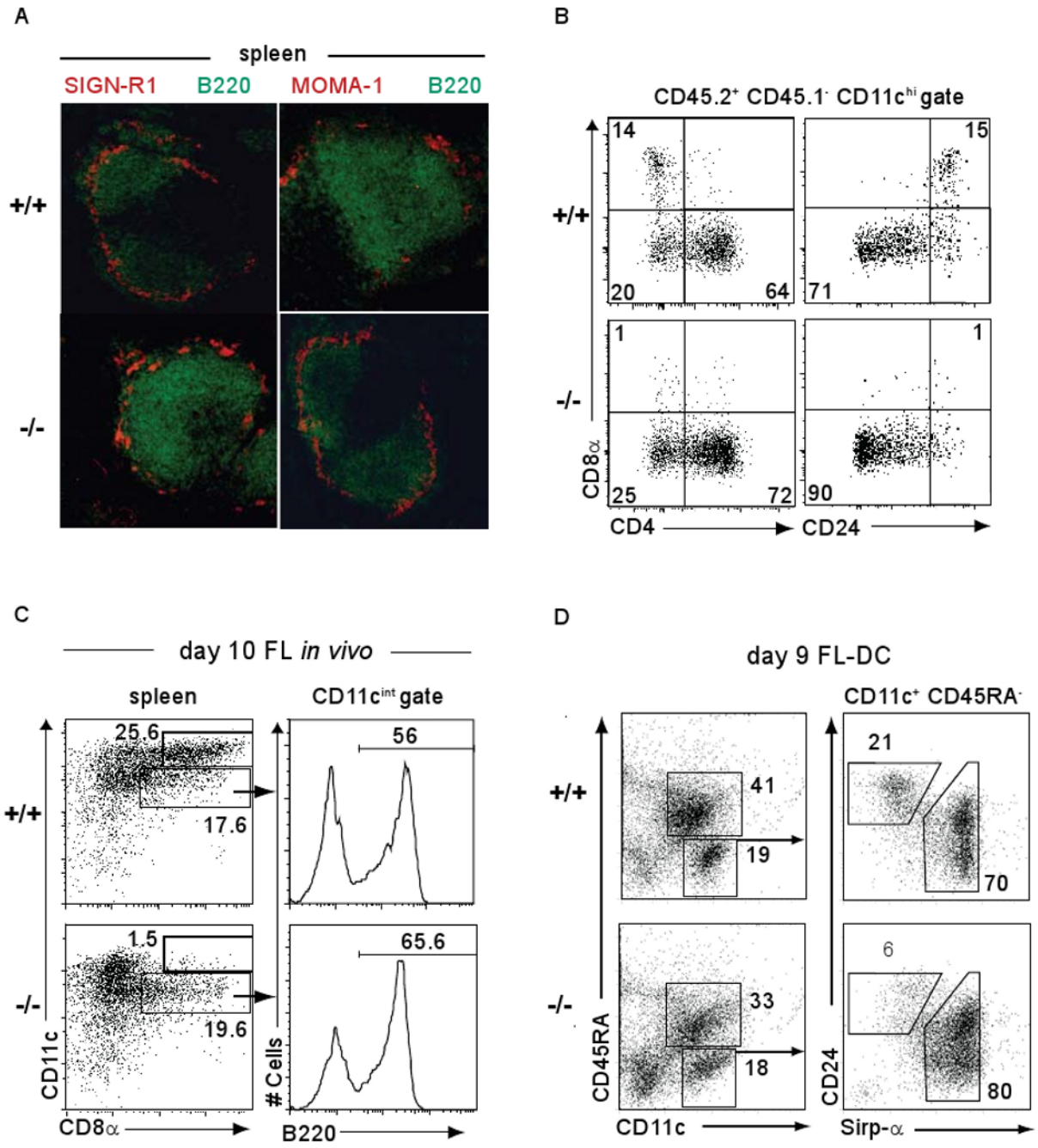

Fig. 2. Functional loss of CD8α$^+$ cDCs in Batf3$^{-/-}$ mice is cell-intrinsic to the hematopoietic system

(A) Frozen sections from *Batf3*$^{+/+}$ (+/+) or *Batf3*$^{-/-}$ (-/-) mice were stained for B220 (green) and SIGN-R1 (red) expression (left panels) or for B220 (green) and MOMA-1 (red) (right panels). (B) Irradiated F1(B6.SJL/129SvEv) mice (CD45.1$^+$ CD45.2$^+$) were reconstituted with 2 ×10^7 bone marrow cells from *Batf3*$^{+/+}$ (+/+) or *Batf3*$^{-/-}$ (-/-) CD45.1$^-$CD45.2$^+$ mice. After 10 weeks, donor cells (CD45.1$^-$ CD45.2$^+$) were analyzed for CD11c, CD8α, CD4 and CD24 expression. Shown are plots for CD8α and CD4 (left panels) or CD8α and CD24 (right panels) gated on CD11c^{hi} donor-derived cells. Numbers represent the percentage of cells within the indicated gates. (C) *Batf3*$^{+/+}$ (+/+) or *Batf3*$^{-/-}$ (-/-) mice were treated i.p. with 10μg FL-Fc. After 10 days, splenocytes were enriched for CD11c$^+$ by MACS and stained for CD11c, CD8α and

B220. Plots are gated on live cells (left) or $CD11c^{int}CD8\alpha^{+}$ cells (right). Numbers represent the percentage of cells within the indicated gates. (D) *Batf3*$^{+/+}$ (+/+) or *Batf3*$^{-/-}$ (-/-) BM cells were cultured in FL (20 ng/ml) for 9 days, and non-adherent cells analyzed for CD11c, CD45RA, CD24 and Sirp-α expression. Plots are gated on live cells (left) or $CD11c^{+}$ $CD45RA^{-}$ cells (right).

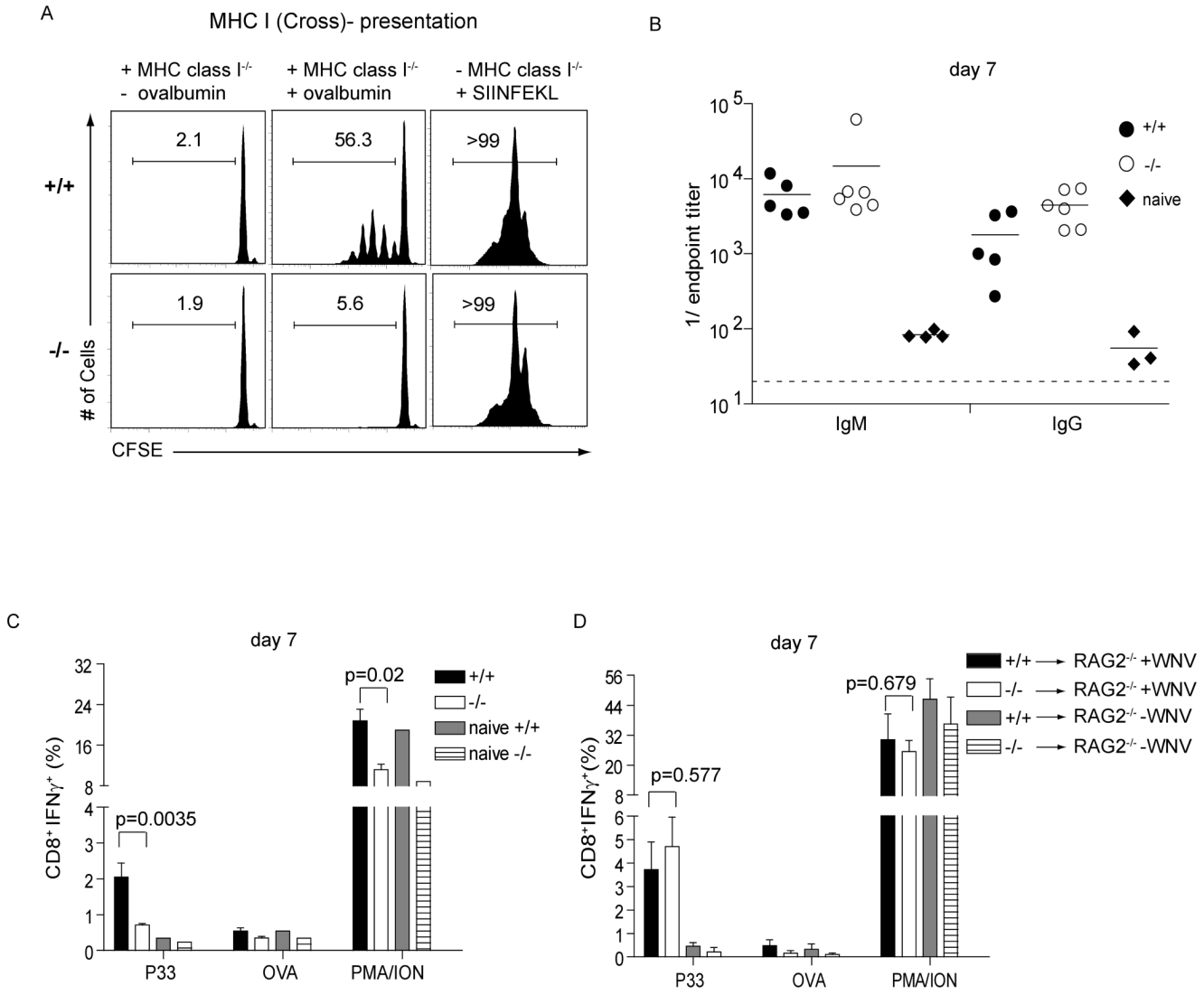

Fig. 3. Lack of cross-presentation and antiviral CTL responses in *Batf3*$^{-/-}$ mice

(A) *Batf3*$^{+/+}$ (+/+) or *Batf3*$^{-/-}$ (-/-) splenocytes were depleted of B220^{+} B cells and Thy1.2^{+} T cells and enriched for CD11c by MACS and cultured with irradiated MHC-class I$^{-/-}$ splenocytes as indicated that were either untreated (-ovalbumin), pulsed with 10 mg/ml soluble ovalbumin (+ovalbumin), or cultured with 1 μM SIINFEKL peptide. CFSE-labeled CD45.1^{+} OT-I T cells were cultured with these cells and proliferation determined by FACS after 60 hours. Single-color histograms of CD8^{+}CD45.1^{+} OT-I T cells show the percentage of cells in the indicated gates. (B) *Batf3*$^{+/+}$ (+/+) or *Batf3*$^{-/-}$ (-/-) mice were infected with 100 PFU of WNV. On day 7, isotype-specific anti-WNV E protein titers were measured. (C) *Batf3*$^{+/+}$ (+/+) or *Batf3*$^{-/-}$ (-/-) mice were infected with 100 PFU of WNV, or left uninfected. After 7 days, splenocytes were stimulated in vitro with the WNV-specific NS4B peptide (P33), OVA peptide, or PMA/ionomycin as described. CD8^{+} T cells were analyzed for expression of intracellular IFN-γ. Data shown are the mean ± SEM (n=9-10). (D) *Batf3*$^{+/+}$ (+/+) or *Batf3*$^{-/-}$ (-/-) CD8^{+} T cells were transferred i.v. into *Rag2*$^{-/-}$ recipients. After 24h, mice were infected with 100 PFU of WNV (+ WNV) or left uninfected (-WNV). After 7 days, splenocytes were harvested and analyzed as described in (C). Data shown are the mean ± SEM (n=6). Three independently performed experiments yielded similar results.

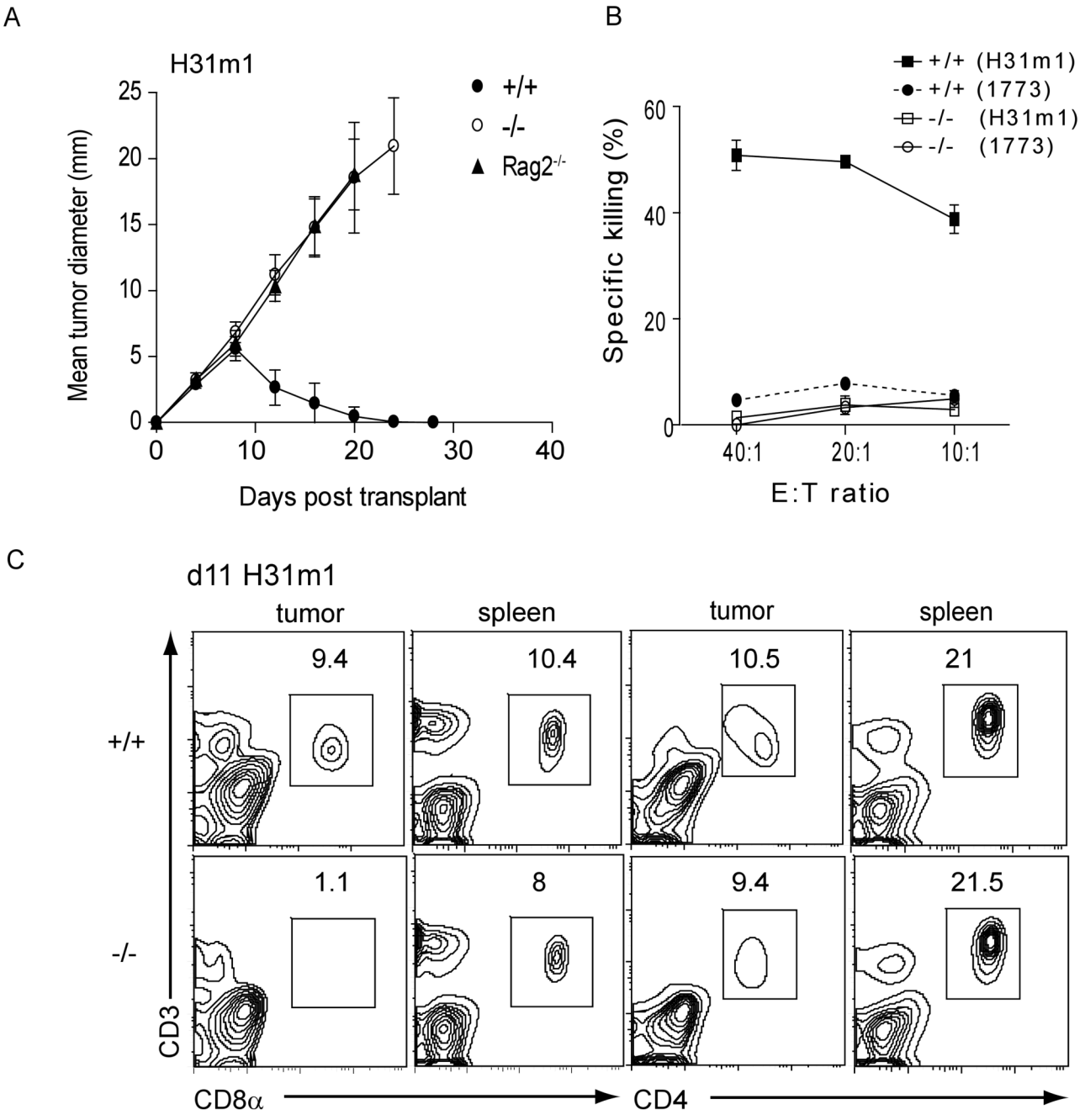

Fig. 4. Lack of tumor rejection in *Batf3*$^{-/-}$ mice

(A) 10^6 H31m1 fibrosarcoma cells were injected subcutaneously into *Batf3*$^{+/+}$ (closed circles), *Batf3*$^{-/-}$ (open circles), or *Rag2*$^{-/-}$ (closed triangles) mice and tumor diameter (± SD) (n=10) was measured. (B) Mice were treated as in (A). After 9 days, splenocytes were harvested and cocultured with IFN-γ pre-treated, irradiated H31m1 tumor cells. After 5 days, a CTL killing assay using ^{51}Cr- labeled H31m1 or 1773 tumor cells as target cells was performed. Shown is specific killing activity as described in the Methods. (C) Tumors and spleens from mice treated as in (A) were removed on day 11 and cells analyzed by FACS. Plots are gated on live CD45.2$^+$ cells and show CD3, CD8α and CD4 expression. Numbers represent the percentage of cells within the indicated gate. Results are representative of at least three mice per group.