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Paradoxical roles of BST2/Tetherin in promoting IFN-I response and viral infection

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Abstract

Bone marrow stromal antigen-2 (BST2) is a transmembrane protein that prevents virus release from infected cells. It was also reported that BST2 inhibits type-I-interferon (IFN-I) production by plasmacytoid DC (pDC). To determine BST2 impact on antiviral responses in vivo we generated $BST2^{-/-}$ mice. Following infection with a murine retrovirus, $BST2^{-/-}$ mice had slightly elevated viral loads; however, infection with other enveloped viruses revealed unexpected roles of BST2. $BST2^{-/-}$ mice showed reduced IFN-I production by pDC. Moreover, $BST2^{-/-}$ mice had lower viral titers in lungs following intranasal infection with VSV-OVA and influenza B, and increased numbers of virus-specific CD8+T cells in the lungs, suggesting that BST2 may facilitate entry and/or replication of enveloped viruses and modulate priming of CD8+T cells. These findings suggest complex roles of BST2 beyond retroviral control in vivo, possibly reflecting the involvement of BST2 in endocytosis and intracellular trafficking of viruses, viral nucleic acids and antigens.

Keywords

BST2; tetherin; virus; interferon; plasmacytoid dendritic cell

Introduction

Bone marrow stromal antigen 2 (BST2, also known as tetherin, CD317, HM1.24) is a type II membrane protein that was first identified on plasma cells and stromal cells (1, 2). More recently, it has been shown that BST2 is expressed by plasmacytoid dendritic cells (pDC) and is induced on most cell types following exposure to type I and II interferons (IFN). (3). BST2 is a rather unique protein with atypical membrane topology (4). It consists of an N-terminal transmembrane domain, extracellular coiled-coiled domain and a C-terminal glycosylphosphatidylinositol (GPI) anchor. BST2 is found on the cell surface, with the GPI anchor inserted in cholesterol-rich lipid microdomains; and also in the transgolgi network following internalization via clathrin-dependent endocytosis (5, 6). These localizations and the biased expression of BST2 in plasma cells, which secrete immunoglobulins, and pDC, which produce IFN-I, have suggested that it might be involved in the trafficking of secreted proteins.

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Cross-linking BST2 on mouse pDC with a specific antibody reduces the IFN-α responses to CpGA suggesting that it may function as a modulator of IFN-I production (3). Furthermore, it was recently discovered that human BST2 interacts with a receptor expressed on pDC called immunoglobulin-like transcript (ILT7) receptor. ILT7 triggers a FcRγ-dependent signaling pathway that leads to the reduction of IFN-I and proinflammatory cytokine secretion by pDC in response to Toll like receptor (TLR) 9 and 7 ligands (7). In addition to the role of BST2 in regulating the secretion of IFN-I, there is a growing literature illustrating its function in limiting viral spread and infection. BST2 has been shown to be an essential molecule that inhibits the egress, release and spread of retroviruses such as human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) as well as other enveloped viruses (8–15); specifically, BST2 tethers budding viruses at the cell surface by having the transmembrane domain inserted in the cell membrane and the GPI anchor inserted into the virions during the process of budding. However, the role of BST2 in vivo has remained unclear.

In this study, we investigated the impact of BST2 on viral infections in vivo using newly generated BST2$^{-/-}$ mice. Following infection with Moloney murine leukemia virus (Mo-MLV), we observed an expected modest increase in retroviral infection in BST2$^{-/-}$ mice. However, infection with enveloped viruses revealed unexpected roles of BST2 in viral infections. BST2$^{-/-}$ pDC and mice secreted moderately less IFN-I than their wildtype (WT) counterparts in response to enveloped viruses in vitro and in vivo. Furthermore, BST2$^{-/-}$ mice had lower viral titers in the lung following intranasal infection with vesicular stomatitis virus expressing ovalbumin (VSV-OVA) or influenza B (FluB) at early timepoints. Finally, we observed that BST2$^{-/-}$ mice had increased numbers of virus-specific CD8 T cells in the lungs after VSV-OVA infection. These findings suggest complex roles of BST2 in viral infections in vivo, which involve IFN-I production, replication of enveloped viruses and induction of virus-specific CD8 T cells.

Materials and Methods

Mice and infections

All animal studies were approved by the Washington University Animal Studies Committee. BST2$^{-/-}$ mice were generated with 129/SvJ ES cells and maintained on a 129/SvJ background. Wildtype and BST2$^{-/-}$ 129/SvJ mice were bred in house and used between 6–12 wks of age. Salivary gland stocks of murine cytomegalovirus (MCMV) Smith strain (2×10^4 pfu) were administered i.p. Mice were infected with different doses of VSV-OVA i.v. or i.n., specified in text and/or figure legends. Influenza B/Yamagata strain/88 (2×10^5 pfu) was administered i.n. Herpes simplex 1 (HSV-1) KOS strain was injected i.v. at 1×10^7 pfu per mouse. Supernatant from Mo-MLV infected cells was injected i.p.

Viral titers

VSV-OVA, MCMV and FluB titers were determined as previously described (16, 17). Mo-MLV titers were determined by qPCR.

Cell culture and preparation

Primary cells were grown in complete RPMI: RPMI 1640 with 10% fetal calf serum, 1% glutamax, 1% nonessential amino acids, 1% sodium pyruvate and 1% kanamycin sulfate (Gibco-Invitrogen). Single cell suspensions were prepared as previously described (16, 18). Whole blood was collected by cardiac puncture and bone marrow cells were harvested from tibias and femurs. For in vitro stimulations and infections, primary cells were cultured with viruses or TLR ligands and supernatants were analyzed by ELISA.

Antibodies, flow cytometry and cytokine analysis

The following reagents were from BD Biosciences, eBioscience or Biolegend: fluorochrome labeled anti-Siglec-H (551), anti-B220 (RA3-6B2), anti-CD11c (HL3), anti-CD8α (53–6.7), anti-CD4 (GK1.5), anti-CD19 (1D3) and Streptavidin. Anti-BST2 (clone 927, rat IgG2b) antibody (3) was purified from ascites and biotinylated using the Fluororeporter Minibiotin Kit (Invitrogen). Virus-specific CD8 T cells in mice infected with VSV-OVA were detected with H-2K^b $OVA_{257–264}$ peptide tetramers (Beckman Coulter). Dead cells were excluded with propidium iodide. All flow cytometry was conducted on a dual laser FACSCalibur flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star, Inc.). B220$^+$Siglec-H$^+$ pDC were sorted from bone marrow on a FACSAria II high speed cell sorter (BD Biosciences). Purities were always greater than 98%. Supernatants, serum samples and tissue samples were collected at various timepoints p.i. and IFN-α levels were determined by ELISA (PBL Interferon Source).

Statistical analysis

The statistical significance of differences in mean values was analyzed with unpaired, two-tailed Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

Results and Discussion

Generation and characterization of BST2$^{-/-}$ mice

To investigate the impact of BST2 on antiviral responses in vivo we generated mice lacking BST2 on a pure 129/SvJ background. The targeting construct contained a neomycin cassette replacing exons 1–4. BST2 expression or lack thereof was confirmed in WT and BST2$^{-/-}$ mice by DNA blot, PCR and antibody staining before and after systemic infection with HSV-1 (Supplemental Figure 1A and 1B; data not shown). BST2$^{-/-}$ mice are viable and resemble their WT counterparts in terms of size and weight. Furthermore, BST2$^{-/-}$ mice have no apparent defects in lymphocyte development in spleen, bone marrow or thymus (Supplemental Figure 1C and data not shown). These data indicate that BST2$^{-/-}$ mice can be used to study the functions of this molecule in vivo.

BST2-deficiency reduces IFN-I secretion by pDC

It has been reported that BST2 inhibits pDC responses to TLR9/7 ligands in vitro (3, 7). Therefore, we hypothesized that BST2$^{-/-}$ pDC would have more robust IFN-I responses to TLR ligands than pDC from WT mice. To test whether BST2 expression altered IFN-I secretion by pDC, we sorted B220$^+$Siglec-H$^+$ pDC from the bone marrow of WT and BST2$^{-/-}$ mice and stimulated them with viruses or synthetic TLR9/7 ligands then measured IFN-α in supernatants. To our surprise, BST2$^{-/-}$ pDC secreted less IFN-I than WT pDC under most conditions (Figure 1A). Similar findings were obtained when total splenocytes were incubated with viruses or CpGA (Figure 1B). We next evaluated IFN-I responses by pDC in vivo. Mice were infected i.p. or i.v. with MCMV (Figure 1C) or VSVOVA (Figure 1D), respectively and serum IFN-α was measured. We have previously reported that MCMV and VSV-OVA infections induce IFN-α production by pDC in vivo at early timepoints p.i. (16). Infection with either virus resulted in modest reductions of serum IFN-α in BST2$^{-/-}$ mice compared to WT mice. Taken together, these data suggest that BST2$^{-/-}$ mice have reduced IFN-I responses by pDC and perhaps other cell types to viruses, which might promote viral replication in vivo.

BST2$^{-/-}$ mice have decreased viral burden in lungs following respiratory infection with VSV-OVA or Influenza B

Given the reported role of BST2 in controlling retroviral egress, we infected WT and BST2$^{-/-}$ mice i.p. with Mo-MLV and measured viral burden in the spleen by qPCR on day 11. We found that BST2$^{-/-}$ mice had slightly elevated levels of Mo-MLV compared to WT mice, which did not reach statistical significance (Figure 2A). We next infected mice systemically with other enveloped viruses and determined viral burden in various organs by plaque assay. Viral titers in WT and BST2$^{-/-}$ mice infected with MCMV i.p. were similar in spleens and salivary glands on days 3 and 14 p.i., respectively (Figure 2B). It has been reported that BST2 inhibits the release of VSV from infected cells (19, 20) so we next infected mice i.v. with VSV-OVA. Infection of WT and BST2$^{-/-}$ mice with different doses of VSV-OVA revealed that both groups of mice were able to control viral replication in spleens and liver to a similar degree (Figure 2C and data not shown). These data suggest that during certain systemic infections, BST2 deficiency does not strongly impact viral replication or spread.

We next asked whether a local infection would be controlled in the absence of BST2. To this end, we infected mice intranasally (i.n.) with VSV-OVA and measured viral titers in the lungs at different timepoints p.i. Interestingly, BST2$^{-/-}$ mice had reduced viral titers at 24 h p.i. compared to WT mice; however, on day 3 p.i., both groups of mice had similar viral loads and on day 5 p.i. viral burden in WT and BST2$^{-/-}$ mice was below detection limit as determined by plaque assay (Figure 3A and data not shown). Consistent with viral titers, evaluation of IFN-I in the lung tissue of VSV-infected mice revealed that BST2$^{-/-}$ mice also had significantly lower levels of IFN-α compared to their WT counterparts (Figure 3B).

The difference in viral titers and IFN-I levels we observed in the lungs after VSV-OVA infection compelled us to look at another respiratory virus infection with FluB. It has been shown that BST2 does not restrict Influenza infection but does prevent the release of influenza virus-like particles into supernatants of MDCK-infected cells (21). On day 3 p.i. the lungs of FluB-infected BST2$^{-/-}$ mice contained less virus than WT mice; however, on day 6 p.i. both groups had comparable levels of virus (Figure 3C and data not shown). Taken together these data suggest that BST2 may promote the entry or early replication of some viruses in vivo during local infections, at least in the lung.

BST2$^{-/-}$ mice have increased virus-specific CD8 T cells in the lung after intranasal VSV-OVA infection

We next examined virus-specific CD8 T cell responses in WT and BST2$^{-/-}$ mice. Frequencies of MHC class I tetramer^{+} CD8 T cells were determined at different timepoints p.i. by flow cytometry. Analysis of mice infected i.n. with VSVOVA indicated that BST2$^{-/-}$ mice had higher numbers of OVA-specific CD8 T cells in the lungs on day 15 p.i. but not at day 7 p.i. (Figure 4A). Thus, even though BST2$^{-/-}$ mice produced less IFN-I and had lower viral burden than WT mice early on and cleared the virus around the same as WT mice, they appeared to have an increase or accumulation of virus-specific CD8 T cells at later timepoints. In contrast, after i.v. infection with VSV-OVA, the spleens of WT and BST2$^{-/-}$ mice contained similar numbers of CD8 T cells and OVA-specific CD8 T cells on days 7 and 14 p.i. (Figure 4B and data not shown). When higher doses of VSV-OVA were administered i.v. we also observed similar frequencies and numbers of OVA-specific CD8 T cells in spleens of WT and BST2$^{-/-}$ mice (data not shown).

In summary, we have generated BST2$^{-/-}$ mice to study the impact of BST2 on antiviral responses in vivo. Our data indicate that BST2 has a modest impact in limiting Mo-MLV infection in mice. A recent study corroborates our findings, showing that BST2 moderately

inhibits Mo-MLV replication in vivo, unless BST2 expression is upregulated by IFN-I (22). Together, these findings suggest that BST2 inhibits Mo-MLV egress and that Mo-MLV, in contrast to HIV and SIV (8, 9, 15, 23), is inefficient at antagonizing BST2, such that Mo-MLV spreading is slightly higher in BST2$^{-/-}$ mice than WT mice. The impact of BST2 deficiency on MLV spreading is likely to be more evident when mice are infected with MLV strains that induce stronger IFN-I responses and subsequent expression of BST2 (22).

The most surprising finding in our study is that the influence of BST2 on antiviral responses is more complex than previously anticipated. Although earlier studies have shown that BST2 inhibits pDC secretion of IFN-I and proinflammatory cytokines, we found that pDC and splenocytes from BST2$^{-/-}$ mice have reduced IFN-I secretion in response to a purified TLR9 ligand and viruses known to engage TLR9/7 in pDC. In addition, BST2$^{-/-}$ mice produced less IFN-I than their WT counterparts in vivo after systemic viral infections that have been shown to induce IFN-I secretion by pDC through TLR9/7 (16). These results suggest that BST2 promotes pDC secretion of IFN-I while the modulatory effects of BST2 previously reported may be the result of an artificial cross-linking by antibodies.

Because it has been shown that BST2 restricts viral release and we observed lower levels of IFN-I in BST2$^{-/-}$ mice, we expected to see increased viral burden in mice lacking BST2. However, our data indicate that in the lung, replication of VSV-OVA and FluB was initially reduced in BST2$^{-/-}$ mice compared to WT mice. These data imply that BST2$^{-/-}$ mice might have defects in viral entry. Indeed, a recent study has shown that BST2 enhances the entry of human cytomegalovirus (HCMV) by a reverse-tethering mechanism (24). Thus, certain viruses such as HCMV and perhaps VSV and FluB, may exploit BST2 to gain entry into BST2-expressing cells. The fact that we did not observe increased viral burden or spreading during systemic infections in BST2$^{-/-}$ mice suggests that (i) the viruses used in these studies may have mechanisms to antagonize BST2 functions and (ii) that BST2 may play a predominant role in the early phase of infection when viral replication is confined to a mucosal barrier, and becomes less important when the virus has spread beyond the initial site of infection.

Finally, we observed that BST2$^{-/-}$ mice had increased numbers of virusspecific CD8 T cells in the lungs following i.n. infection with VSV-OVA even though the virus was cleared around the same time as WT mice (day 5). These findings suggest that BST2 may inhibit presentation of viral antigens and subsequent CD8 T cell responses. By promoting viral entry in antigen presenting cells, BST2 may accelerate their death, reducing their ability to migrate into the draining lymph nodes and present viral antigens to CD8 T cells. BST2 may also preferentially direct newly synthesized viral proteins towards intracellular viral factories rather than endolysosomal compartments where viral peptides are processed and loaded onto MHC. Thus, the lack of BST2 may result in sustained antigenic stimulation, even in the absence of obvious viral replication.

BST2 is localized in clathrin-rich lipid rafts as well as in the transgolgi network following internalization (4–6). VSV and influenza can gain entry into cells via clathrin-mediated endocytosis (25). IFN-I production by pDC is also dependent on the localization of TLR9/7 and viral nucleic acids in specialized endosomal compartments. Priming of CD8 T cells depends on efficient antigen processing and MHC class I loading in specialized intracellular compartments. A recent study found that targeting antigen to cells via BST2 resulted in protective T cell-mediated immunity suggesting a role for BST2 in antigen presentation (26). Thus, we envision that perturbation of lipid rafts and the transgolgi network in the absence of BST2 may affect endocytosis and vesicular trafficking of viruses or viral nucleic acids and antigen presentation machinery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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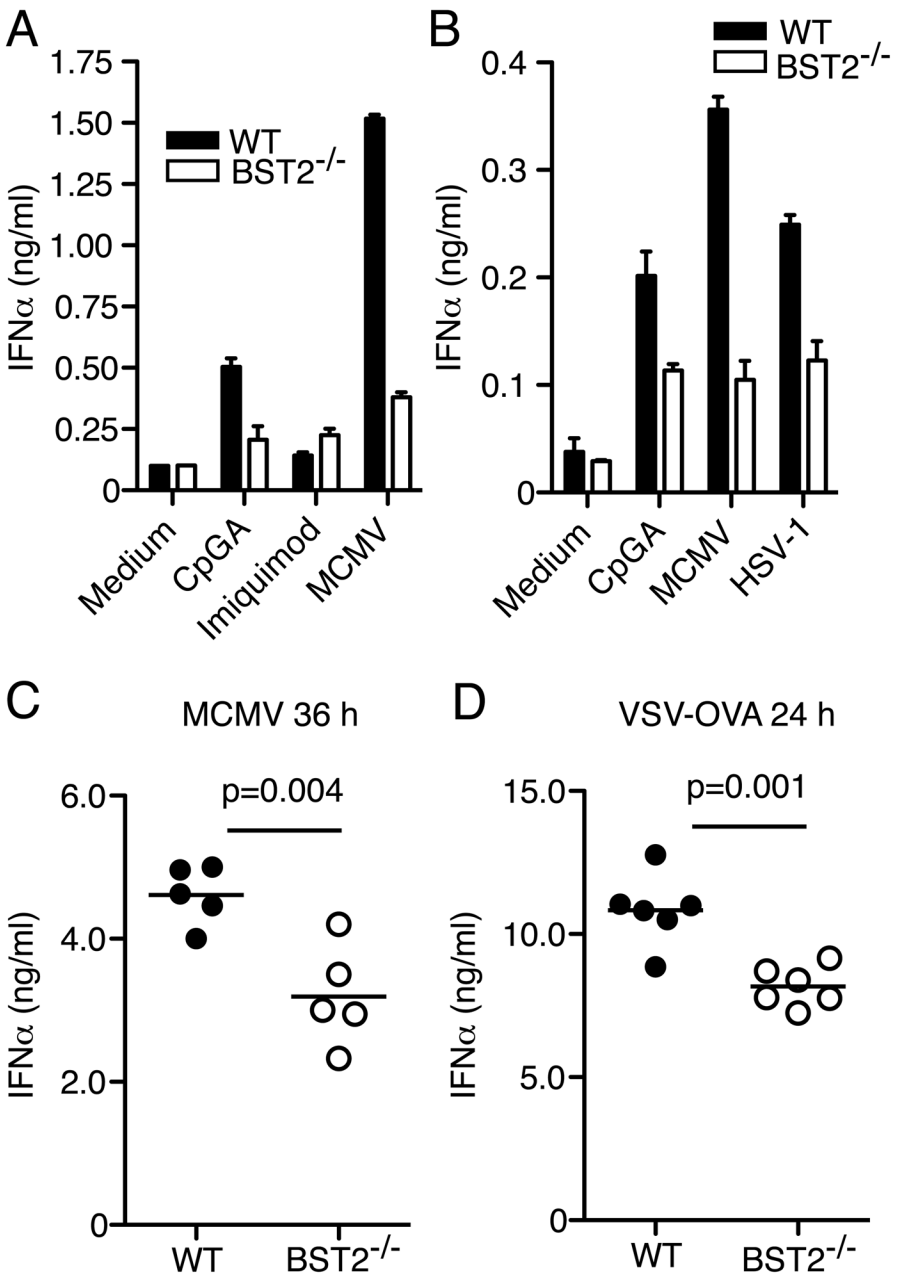

Figure 1. IFN-I secretion in WT and BST2$^{-/-}$ mice

(A) pDC were sorted from the bone marrow of WT and BST2$^{-/-}$ mice and stimulated with MCMV or TLR9/7 ligands. IFN-α was measured in supernatants 24 h p.i. (B) Splenocytes were incubated with viruses or CpGA and levels of IFN-α in supernatants were measured 20 h later. (C, D) Mice were infected i.p. with MCMV (C) or i.v. with VSV-OVA (1×10^6 pfu) (D) and IFN-α was measured in the serum at 36 or 24 h p.i., respectively. (A–D) Data are representative (A, B) or from (C, D) two independent experiments. Statistical significance is indicated by *P* values. Bar graphs represent means ± SEM.

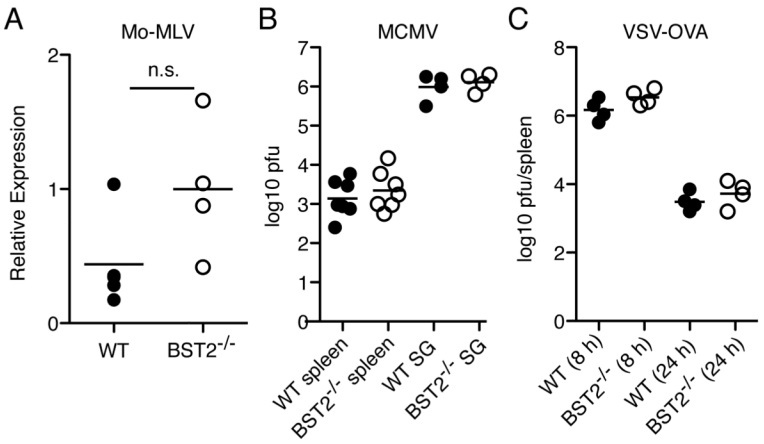

Figure 2. Viral titers in WT and BST2$^{-/-}$ mice after systemic infection

WT and BST2$^{-/-}$ mice were infected systemically with viruses and viral burden in organs were determined by qPCR or plaque assay at different timepoints p.i. (A) Mo-MLV levels in spleens on day 11 p.i. (B) MCMV replication in spleens and salivary glands (SG) on days 3 and 14 p.i., respectively. (C) VSV-OVA replication in spleens 8 and 24 h p.i. after i.v. infection (1×10^6 pfu). Data are from two independent experiments. Statistical significance is indicated by *P* values. Not significant, n.s.

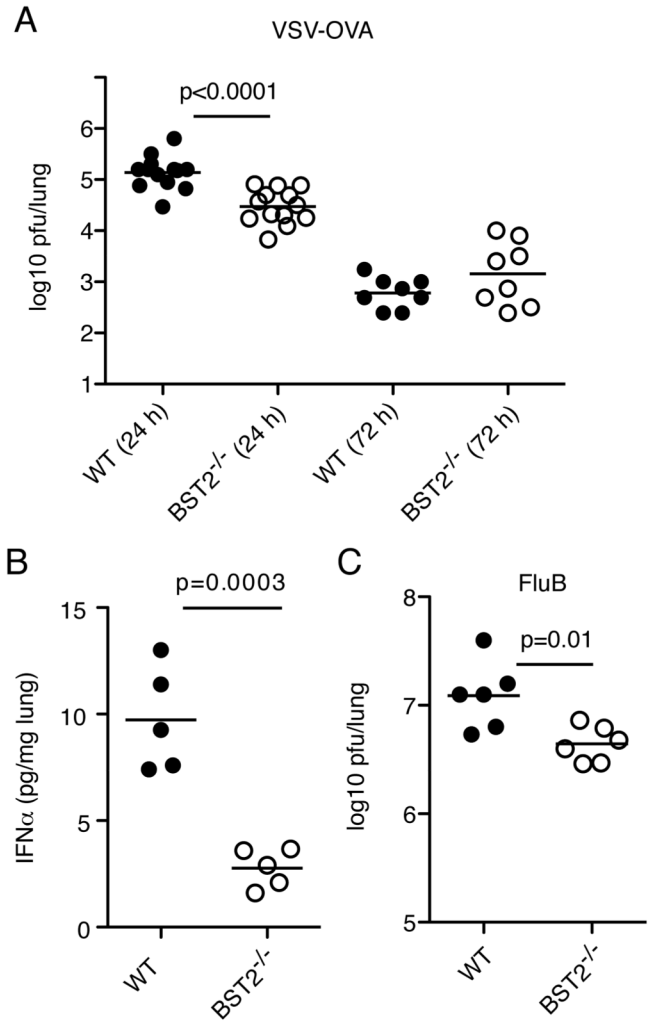

Figure 3. Viral titers and IFN-I secretion in WT and BST2$^{-/-}$ mice after respiratory infection

(A) VSV-OVA replication in the lungs 24 and 72 h p.i. after i.n. infection (5×10^7 pfu). (B) IFN-α levels in lung tissue 18 h after i.n. infection with VSV-OVA (5×10^7 pfu). (C) Viral titers in the lung after i.n. infection with FluB 72 h p.i. Data are from two to four independent experiments. Statistical significance is indicated by *P* values.

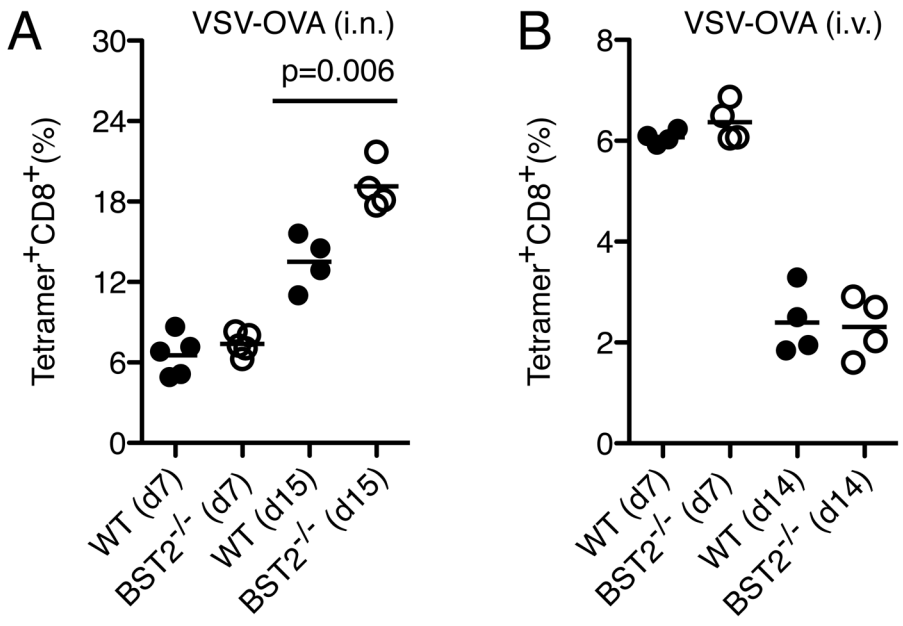

Figure 4. Antiviral CD8 T cell responses in WT and BST2$^{-/-}$ mice

(A) WT and BST2$^{-/-}$ mice were infected with VSV-OVA i.n. (5×10^7 pfu) and frequencies of OVA-specific CD8 T cells were determined in lungs on days 7 and 15 p.i. (B) Mice were infected i.v. with VSV-OVA (3×10^6 pfu) and OVA-specific CD8 T cells were determined in spleens on days 7 and 14 p.i. Data are representative of two independent experiments. Statistical significance is indicated by *P* values.