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# Structural basis for the antiviral activity of BST-2/tetherin and its viral antagonism

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The interferon-inducible host restriction factor bone marrow stromal antigen 2 (BST-2/tetherin) blocks the release of HIV-1 and other enveloped viruses. In turn, these viruses have evolved specific antagonists to counteract this host antiviral molecule, such as the HIV-1 protein Vpu. BST-2 is a type II transmembrane protein with an unusual topology consisting of an N-terminal cytoplasmic tail (CT) followed by a single transmembrane (TM) domain, a coiled-coil extracellular (EC) domain, and a glycosylphosphatidylinositol (GPI) anchor at the C terminus. We and others showed that BST-2 restricts enveloped virus release by bridging the host and virion membranes with its two opposing membrane anchors and that deletion of either one completely abrogates antiviral activity. The EC domain also shows conserved structural properties that are required for antiviral function. It contains several destabilizing amino acids that confer the molecule with conformational flexibility to sustain the protein's function as a virion tether, and three conserved cysteine residues that mediate homodimerization of BST-2, as well as acting as a molecular ruler that separates the membrane anchors. Conversely, the efficient release of virions is promoted by the HIV-1 Vpu protein and other viral antagonists. Our group and others provided evidence from mutational analyses indicating that Vpu antagonism of BST-2-mediated viral restriction requires a highly specific interaction of their mutual TM domains. This interpretation is further supported and expanded by the findings of the latest structural modeling studies showing that critical amino acids in a conserved helical face of these TM domains are required for Vpu–BST-2 interaction and antagonism. In this review, we summarize the current advances in our understanding of the structural basis for BST-2 antiviral function as well as BST-2-specific viral antagonism.

**Keywords: HIV-1, Vpu, BST-2, transmembrane, restriction factor, antagonist, interaction**

## INTRODUCTION

As a result of exposure to viral pathogens over millions of years, humans and other mammals evolved intrinsic immunity proteins that provide resistance to infection by directly interfering with different stages of the viral life cycle. These so-called host restriction factors are normally induced by interferon-α (IFN-α) during induction of the innate immune response by viral infection. A case in point is HIV-1, an extensively studied pathogen for which four major restriction factors have been identified: the apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3) family of cytidine deaminases (Sheehy et al., 2002); the α-isoform of the tripartite motif-containing protein 5 (TRIM5α; Stremlau et al., 2004); the bone marrow stromal antigen 2 (Neil et al., 2008; Van Damme et al., 2008; BST-2, also known as tetherin or CD317, referred to hereafter as BST-2), which is the subject of this review article; and, more recently, SAMHD1 (Hrecka et al., 2011; Laguette et al., 2011). HIV-1, in turn, evolved countermeasures to overcome the antiviral activity of their host restriction factors, mainly by acquiring a series of *trans*-acting viral accessory proteins, including Vif and Vpu. Vif blocks the above-described APOBEC3 proteins that mediate extensive deamination of cytosines in single-stranded viral DNA, thus halting HIV replication. Vpu is another viral antagonist of the transmembrane BST-2 protein that blocks the release of enveloped viruses by physically binding the budding viral particles to the membrane of infected cells. Likewise, in HIV-2 and related simian immunodeficiency viruses, Vpx acts as an antagonist of SAMHD1 that blocks HIV-1 replication in dendritic and myeloid cells. It should be noted that HIV-1 is not susceptible to human TRIM5α antiviral action (Stremlau et al., 2004). In this review, we focus on current advances in structure-based analyses of BST-2 and viral antagonists.

## BST-2: MOLECULAR CHARACTERISTICS

BST-2 is an interferon-induced type II membrane glycoprotein of unusual topology (Ishikawa et al., 1995; Kupzig et al., 2003), which efficiently blocks the release of diverse mammalian enveloped viruses by directly tethering viral particles to the membranes of infected cells. Viruses restricted by BST-2 are found among diverse families, including filoviruses, arenaviruses, paramyxoviruses (Jouvenet et al., 2009; Kaletsky et al., 2009; Sakuma et al., 2009a; Radoshitzky et al., 2010), gamma-herpesviruses (Mansouri et al., 2009; Pardieu et al., 2010), rhabdoviruses (Weidner et al., 2010), and a wide array of retroviruses from several mammal host species (Arnaud et al., 2010; Dietrich et al., 2011; Xu et al., 2011).

A recent study characterizing a feline BST-2 ortholog reported the protein's strong activity against FIV particle release *in vitro* (Dietrich et al., 2011). BST-2 comprises a short, 21-amino-acid cytoplasmic N-terminal tail (CT), followed by an α-helical transmembrane (TM) domain, an extracellular domain (EC) that is predominantly helical and contains an extended parallel coiled-coil, and a C-terminal glycosylphosphatidylinositol (GPI) component that acts as a second anchor linking the protein back to the cell membrane (Kupzig et al., 2003; **Figure 1A**). This double-anchor topology is extremely unusual and is only shared by an isoform of the prion protein (Moore et al., 1999).

Accumulating evidence supports the view that the structural features of BST-2 are key to its antiviral activity, as discussed in detail in the following sections. In agreement with a direct tethering mechanism, a requirement for both the TM and GPI anchors has been found for BST-2's antiviral activity (Neil et al., 2008; Iwabu et al., 2009; Perez-Caballero et al., 2009). Additionally, the EC of BST-2 contains a series of important residues that are conserved throughout the protein's mammalian orthologs, and these residues are essential to the inhibition of viral release (Van Damme et al., 2008; Andrew et al., 2009; Sakuma et al., 2009b). Whereas the stability of BST-2 is maintained by disulfide-links (Hinz et al., 2010; Schubert et al., 2010), the EC forms an extended coiled-coil domain that contains several conserved destabilizing amino acid residues, providing the conformational flexibility necessary for the molecule to sustain its role as a physical tether, as described later. Salient BST-2 structural motifs important for antiviral function are summarized in **Table 1**.

Based on the identification of these structural features critical for BST-2's antiviral activity, Perez-Caballero et al. (2009) through domain replacement experiments, were able to show that BST-2's configuration rather than its primary sequence is critical for antiviral activity. In an elegant demonstration, the authors generated a completely artificial BST-2-like protein made of structurally similar domains from three unrelated heterologous proteins (the TM from the transferrin receptor, the coiled-coil from dystrophia myotonica protein kinase, and the GPI anchor from the urokinase plasminogen activator receptor). Despite its lack of sequence homology with native BST-2, this artificial protein reproduced the latter's antiviral activity as it was able to inhibit the release of HIV-1 and Ebola virus-like-particles.

## BOTH TM AND GPI ANCHOR ARE IMPORTANT FOR THE RESTRICTION OF VIRUS RELEASE

The TM (amino acid positions 22–43) of BST-2 is a short single-pass α-helix that anchors the molecule to the plasma membrane, while the GPI anchor is located at the C-terminal region of the protein (Kupzig et al., 2003). These two membrane anchors in part determine the antiviral function of BST-2. This unusual topology suggests a model that BST-2 directly tethers budding virions to the membrane of infected cells. Indeed, unequivocal support for this model has come from immunoelectron microscopy studies demonstrating that BST-2 is associated with virions and located between the viral and cell membranes as well as between tethered virions (Neil et al., 2008; Fitzpatrick et al., 2010; Hammonds et al., 2010).

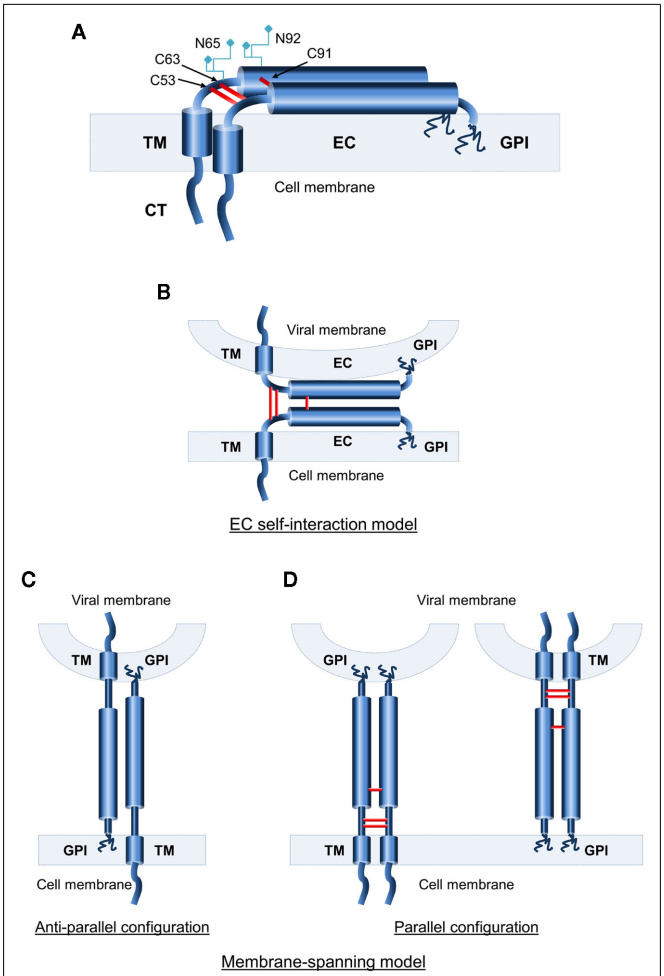


**FIGURE 1 | Topological characteristics of human BST-2. (A)** Schematic representation of the domain structure of BST-2, a type II transmembrane (TM) protein. BST-2 features a short cytoplasmic N-terminus followed by an α-helical single-pass TM domain and an extended coiled-coil extracellular domain that is linked back to the plasma membrane by a C-terminal GPI anchor. *N*-glycosylation sites and cysteine residues for disulfide-bond formation in the extracellular domain (EC) are noted. **(B–D)** Topological models of BST-2's functional state. **(B)** The EC self-interaction model, in which individual BST-2 monomers are anchored at both ends to the same membrane, with interaction between the ECs of cell-bound and virion-bound monomers. **(C)** Membrane-spanning anti-parallel model. Monomers are anchored in both membranes with opposing orientations. **(D)** Membrane-spanning parallel model. Monomers are anchored in both membranes with the same orientation.

As shown in **Table 1**, two structural elements are absolutely required for BST-2-mediated restriction of viral release; (1) the presence of both the TM and the GPI anchor (Neil et al., 2008; Van Damme et al., 2008; Iwabu et al., 2009; Perez-Caballero et al., 2009); and (2) homodimer formation through EC disulfide-bond interactions (Andrew et al., 2009; Perez-Caballero et al., 2009). The latter is discussed in greater detail in a later section of this review. These two elements form the basis of the two proposed topological models of BST-2. In the "EC self-interaction model (**Figure 1B**)," individual BST-2 monomers are anchored at

**Table 1 | Salient structural features of human BST-2.**

| Domain | Structural motif | Function | Necessary for antiviral action? | Reference |
|---|---|---|---|---|
| CT (1–21) | $YxY_{6–8}$ | Clathrin-dependent internalization | No | Masuyama et al. (2009), Rollason et al. (2007) |
| | $DDIWK_{14–18}$ | Nef recognition sequence | No | Yang et al. (2010a), Sauter et al. (2009), Lim et al. (2010) |
| | K18 | Putative ubiquitination site by K5 | No | Mansouri et al. (2009), Pardieu et al. (2010) |
| TM (22–43) | Alpha-helix (22–43) | Membrane anchor | Yes | Neil et al. (2008), Perez-Caballero et al. (2009), Iwabu et al. (2009) |
| | I34, L37, L41 | Vpu recognition face | No | Iwabu et al. (2009), Gupta et al. (2009a), Rong et al. (2009), McNatt et al. (2009), Kobayashi et al. (2011), Skasko et al. (2011b) |
| EC (44–160) | N65, N92 | N-linked glycosylation | No | Sakuma et al. (2009a), Andrew et al. (2009), Ohtomo et al. (1999) |
| | C53, C63, C91 | Putative disulfide-bond formation | Yes | Perez-Caballero et al. (2009), Andrew et al. (2009), Hinz et al. (2010) |
| | Coiled-coil (68–138) | Molecular ruler | Yes | Hinz et al. (2010), Yang et al. (2010a), Swiecki et al. (2011), Schubert et al. (2010) |
| | C91, V95, L98, L102, E105, V113, L116, I120, L123, L127, V134, L137 | Destabilizing residues at core heptad positions | Yes | |
| GPI anchor | GPI signal peptide | Membrane anchor | Yes | Kupzig et al. (2003), Perez-Caballero et al. (2009), Iwabu et al. (2009) |

both ends to the same membrane (cellular or viral), and interaction between the EC domains of cell-bound and virion-bound monomers is required for the restriction of virus release. The alternative is the "membrane-spanning model (**Figures 1C,D**)," in which both BST-2 end tails (TM and GPI anchor) are anchored in different membranes (i.e., cellular and viral). Theoretically, the BST-2 monomers in this model can be arranged in either an anti-parallel (**Figure 1C**) or parallel (**Figure 1D**) configuration.

The first approach to resolve the topology of BST-2 involves cleavage of the GPI anchor by treatment with the hydrolytic enzyme phosphatidyl inositol-specific phospholipase C (Pi–PLC). However, the enzymatic treatment does not effectively release restricted virions from the cell membrane (Fitzpatrick et al., 2010), supporting either a membrane-spanning anti-parallel configuration (**Figure 1C**) or the EC self-interaction model (**Figure 1B**), in which monomers would be able to remain attached to the respective membrane by the TM domain even after cleavage of the GPI anchor.

The second approach is to evaluate the gap between the cellular and viral membranes in electron microscopy studies. If the BST-2 monomers are positioned parallel to the cellular and viral membranes (EC self-interaction model; **Figure 1B**), virions would be tethered very close to the membrane, less than 3–5 nm, as described in (Hinz et al., 2010). However, imaging studies show larger distances between virions and cells (Neil et al., 2008; Perez-Caballero et al., 2009; Hammonds et al., 2010), thus supporting a membrane-spanning model (**Figures 1C,D**).

The third approach to this problem has been the systematic determination of BST-2 function in mutational analyses. We have previously shown that the anchoring of BST-2 through both its N-terminal and C-terminal regions is required for antiviral activity (Iwabu et al., 2009). Briefly, mutagenesis studies using GPI-anchor-deleted and CD4 signal peptide chimeric versions of BST-2, in which the protein is linked to the cell membrane only through one of its ends, showed that removal of either end abrogated the antiviral effect of BST-2 on virus production. Therefore, we concluded that membrane binding through both the TM and GPI anchor of BST-2 is critical for its antiviral activity, supporting the model of the membrane-spanning parallel configuration (**Figure 1D**). Further evidence for this parallel-dimer model comes from the analysis of residual BST-2 found in virions released through proteolytic treatment with subtilisin (Perez-Caballero et al., 2009).

Finally and more importantly, four different groups have combined high-resolution crystallography (1.6–2.8Å), and small-angle X-ray scattering-based modeling to determine the structures of the entire human and murine BST-2 EC, and have shown that BST-2 forms parallel coiled-coil arrangements (Hinz et al., 2010; Schubert et al., 2010; Yang et al., 2010a; Swiecki et al., 2011). Taken together, these observations suggest that the antiviral state of BST-2 present at the cell membrane corresponds to the membrane-spanning parallel configuration model as shown in **Figure 1D**.

## THE EC MEDIATES HOMODIMERIZATION

The BST-2 EC (amino acid positions 44–160) is predominantly an α-helical coiled-coil structure that contains a series of residues highly conserved among mammalian orthologs: two asparagines that are N-linked glycosylation sites (N65, N92), and three cysteines (C53, C63, C91) responsible for intermolecular disulfide-bonds that result in homodimerization (**Figure 1A**; Ohtomo et al., 1999; Andrew et al., 2009). Disulfide linkage through these cysteine residues is critical for the restriction of HIV production

(**Table 1**). Mutational analyses demonstrate that partial disulfide-bond formation through at least one such cysteine residue is necessary for the retention of antiviral activity, whereas mutations at all three positions result in the total loss of antiviral function even though expression of the protein at the cell membrane remains unaltered (Andrew et al., 2009; Perez-Caballero et al., 2009; Hinz et al., 2010), although this is not the case for filovirus or arenavirus (Lassa virus) particles (Perez-Caballero et al., 2009; Sakuma et al., 2009a).

Several conserved amino acids within the EC domain, which are also thought to stabilize the dimers through weak coiled-coil domain interactions, include two interhelical salt bridges (E105–K106, and E133–R138) and one interhelical hydrogen bond (N141), and contribute to stabilize the EC domain interface (Hinz et al., 2010). Glycosylation of residues N65 and N92 was shown to contribute to anterograde transport and correct protein folding, but mutations in these positions had no effect on BST-2 antiviral activity (**Table 1**; Andrew et al., 2009; Sakuma et al., 2009a). In summary, all evidence thus far suggests that BST-2 EC contains a dimeric coiled-coil that is stabilized by C53–C53, C63–C63, and C91–C91 disulfide-bonds, with the conservation of at least one of these, along with weak interactions within the coiled-coil domain, and is required for dimer stability and the antiviral activity of BST-2.

## THE BST-2 EC EXHIBITS CONFORMATIONAL FLEXIBILITY

The most recent structural studies provide valuable clues to the biological function of the EC while at the same time reconciling the topological models of BST-2 dimer configuration with available electron microscopy data, as outlined above. Resolution of the crystal structure of human BST-2 EC (Hinz et al., 2010; Schubert et al., 2010; Yang et al., 2010a) together with small-angle X-ray scattering data suggest an elongated extracellular domain forming a long rod-like structure and a greatly extended EC separating the two membrane anchors, acting as a molecular ruler with a predicted distance of 170Å (**Table 1**). This distance would correspond to the predicted separation between membrane-tethered virions and the plasma membrane of the host cells, or between tethered viral particles, and is in agreement with the separation determined in published electron micrographic studies. This finding seems to be consistent with the aforementioned membrane-spanning model (**Figure 1D**).

The authors of those studies also described the presence of irregularities in the 90-Å coiled-coil motif. The irregularities arise from the introduction of destabilizing residues (see **Table 1**) that are arranged regularly in core heptad positions, i.e., amino acid residues located at the center of the α–helix. The destabilizing residues loosen regular coiled-coil packing increasing the pitch and radius of the α-helix, accounting for the low stability of BST-2's coiled-coil under reducing conditions in vitro. These positions are conserved throughout all available BST-2 sequences, and their mutations result in loss of the antiviral function of BST-2 (Hinz et al., 2010). Yet, despite this intrinsic instability, the disulfide-bonds are still able to be formed, restabilizing the EC domains in a dimeric form. These findings suggest that conformational flexibility allows adaptation to the dynamic events of virion budding, while disulfide-bond-mediated dimerization prevents major separation of the coiled-coils. Together, these two properties result in a dynamic structure that permits dimer dissociation and restabilization during the process of virion trapping (Hinz et al., 2010; Swiecki et al., 2011). A high-resolution crystal structure of the full-length mouse BST-2 EC confirmed the presence of an elongated EC characteristically unstable due to the insertion of destabilizing residues (Swiecki et al., 2011). In that study, structural and biophysical analyses of murine and human BST-2 EC domains revealed that an unstable coiled-coil motif is evolutionarily conserved. This evidence provides further support for the aforementioned model of conformational flexibility.

## THE GPI ANCHOR MEDIATES SURFACE LOCALIZATION AND THE CT IS CRITICAL FOR BST-2 TRAFFICKING

BST-2 localizes both to the plasma membrane and internal compartments, particularly the trans-Golgi network (TGN) and recycling endosomes (Kupzig et al., 2003; Rollason et al., 2007; Dube et al., 2009; Masuyama et al., 2009; Habermann et al., 2010). At the cell surface, BST-2 localizes into cholesterol-enriched lipid rafts, due to its GPI anchor. This localization is implicated in the promotion of clathrin-mediated endocytosis (Rollason et al., 2007; Masuyama et al., 2009) and, importantly, it allows BST-2 to directly interfere with the virion-release process, as lipid rafts are the preferential site of budding of several enveloped viruses (Aloia et al., 1993; Panchal et al., 2003; Waheed and Freed, 2009). This also positions BST-2 at the virological synapse (VS; Casartelli et al., 2010; Jolly et al., 2010; Pais-Correia et al., 2010), but its potential to restrict cell-to-cell viral spread remains controversial. With respect to internalization and cell trafficking, it was previously shown that rodent BST-2 is internalized from the cell surface in a clathrin-dependent manner (Rollason et al., 2007; Masuyama et al., 2009). Internalization requires a non-canonical dual tyrosine motif at amino acid positions 6 and 8 of the protein's CT ($YxY_{6–8}$; **Table 1**). This motif is highly conserved through all mammalian orthologs and sequentially participates in the interaction of BST-2 with the clathrin adaptors AP-2, which mediates internalization by endocytosis, and AP-1, which retrieves BST-2 to the TGN. The CT domain of BST-2 indirectly interacts with the underlying actin cytoskeleton through a series of adaptor proteins (RICH2, EBP50, ezrin), although additional studies are required to understand the implications of these interactions for BST-2 function (Rollason et al., 2009).

## VIRAL ANTAGONISM OF BST-2

Since BST-2 targets the lipid bilayer of the host cell, viruses cannot evade it simply by escape mutations. Therefore, enveloped viruses had been obliged to evolve trans-acting countermeasures specifically to overcome BST-2 restriction. Among primate lentiviruses, three different viral gene products are known to antagonize BST-2. In most SIV strains, the viral Nef protein antagonizes primate BST-2, while in HIV-1 and HIV-2, the Vpu protein and the Env glycoprotein, respectively, antagonize human BST-2. Other BST-2 antagonists include the Kaposi's sarcoma-associated herpesvirus (KSHV) K5 protein and the Ebola virus glycoprotein (GP). With the exception of Ebola GP, all of these viral proteins downregulate BST-2 at the plasma membrane, thus effectively removing it from viral budding sites.

### HIV-1 Vpu

Just as the study of HIV-1 Vif led to the discovery of APOBEC3 as a host restriction factor (Sheehy et al., 2002), BST-2 was identified by searching for the host restriction factor antagonized by the accessory viral protein Vpu. This 16-kDa type I transmembrane viral protein is a BST-2 antagonist and as such promotes the release of HIV-1 virions (Cohen et al., 1988; Strebel et al., 1988; Malim and Emerman, 2008). Importantly, Vpu can directly mediate the removal of BST-2 away from its site of action on the cell surface, although the mechanisms remain hotly debated (Van Damme et al., 2008; Iwabu et al., 2009, 2010; Ruiz et al., 2010; Lau et al., 2011). Thus far, it appears that Vpu recruits cellular proteins to remove BST-2 from the surface (**Figure 2A**). As we and others have shown, BST-2 downregulation by Vpu involves a beta-transducin repeat-containing protein (β-TrCP)-dependent mechanism (Douglas et al., 2009; Iwabu et al., 2009; Mangeat et al., 2009; Mitchell et al., 2009; Dubé et al., 2010; Tokarev et al., 2011); however, this only partially explains the underlying mechanism, since mutations in the β-TrCP-binding motif of Vpu do not entirely abrogate its antagonism of BST-2 (Schubert and Strebel, 1994; Van Damme et al., 2008; Iwabu et al., 2009).

Whereas several reports suggest that BST-2 downregulation in the presence of Vpu is accomplished at least in part through proteasomal degradation (Goffinet et al., 2009; Gupta et al., 2009a; Mangeat et al., 2009), evidence obtained by our group and others supports a model of BST-2 downregulation through lysosomal degradation (Douglas et al., 2009; Iwabu et al., 2009; Mitchell et al., 2009; Janvier et al., 2011). It is proposed that Vpu causes the retention of BST-2 within endosomes by blocking its recycling after endocytosis (Mitchell et al., 2009; Dubé et al., 2010; Lau et al., 2011). Alternatively, it is hypothesized that Vpu inhibits the membrane transport of BST-2 by causing its intracellular sequestration within the TGN (Dubé et al., 2010; Andrew et al., 2011; Lau et al., 2011). We and others suggested that Vpu directly internalizes BST-2 from the cell surface through TM interactions leading to lysosomes (Iwabu et al., 2009, 2010; Janvier et al., 2011; Skasko et al., 2011a). An additional level of complexity in the BST-2 downregulation mechanism stems from a report that in certain cell lines (CEMx174, H9), Vpu overexpression results in the enhancement of virion production, but without effectively reducing the surface levels of BST-2 (Miyagi et al., 2009). Thus, it is not yet clear how Vpu affects the internalization, recycling, or membrane transport, of BST-2.

Regardless of the mechanisms of Vpu-induced BST-2 downregulation, the ability of Vpu to bind to BST-2 is crucial for the antagonism of BST-2-mediated restriction (**Figure 2A**), as evidenced by data showing that the anti-BST-2 activity of Vpu is abrogated by mutations that disrupt TM-TM interaction. (Gupta et al., 2009a; Iwabu et al., 2009; McNatt et al., 2009; Rong et al., 2009; Skasko et al., 2011a). This interaction is highly specific since single point mutations in either BST-2 (I34, L37, L41; **Table 1**; Kobayashi et al., 2011) or Vpu (A14, A18, and W22; Vigan and Neil, 2010) render BST-2 resistant to Vpu antagonism. Their structural analyses showed that these residues form both hydrophobic faces of the helices, and therefore presumably contribute to their interacting surfaces. Recently, the aforementioned residues have been shown by NMR spectroscopy to interact directly in a membrane-embedded TM–TM interface (Skasko et al., 2011b).

Importantly, a high degree of species-specificity characterizes this interaction. Even though all primate BST-2 proteins are able to block HIV-1 virion-release, non-human BST-2 proteins are mostly insensitive to Vpu antagonism (Goffinet et al., 2009; Gupta et al., 2009a; Jia et al., 2009; Zhang et al., 2009). Analyses of codon-specific positive selection in the primate lineage showed that a mutation of residue T45 in human BST-2 is sufficient to reduce its sensitivity to Vpu (Gupta et al., 2009a). Likewise, the transfer of amino acid positions 30–45 of the human BST-2 TM domain into rhesus BST-2 was sufficient to render it Vpu-sensitive, while a single I48T mutation in rhesus BST-2 conferred partial Vpu sensitivity (Yoshida et al., 2011). These results suggest that this specificity of HIV-1 Vpu for BST-2 depends on conserved amino acids in the latter's TM domain (as described above) that are divergent between the human protein and its simian counterparts.

## OTHER BST-2 ANTAGONISTS

Most of the primate lentiviruses that do not encode a Vpu protein instead use Nef to counteract BST-2's antiviral function (Jia et al., 2009; Sauter et al., 2009; Zhang et al., 2009). It should be noted that even though the primate ancestors of HIV-1, SIVcpz, and SIVgor from chimpanzees and gorillas encode Vpu, they also use Nef to antagonize BST-2 (Sauter et al., 2009; Yang et al., 2010b). Analogous to HIV-1 Vpu antagonism of human and chimpanzee, but not other primate BST-2 proteins (Goffinet et al., 2009; McNatt et al., 2009; Hauser et al., 2010), SIV Nef counteracts primate but not human BST-2 orthologs. This selectivity resides in the CT of non-human primate BST-2, which contains a discreet $DDIWK_{14–18}$ sequence (**Table 1**) that is required for the response to SIV Nef but is deleted in the protein's human counterparts (Sauter et al., 2009; Lim et al., 2010; Yang et al., 2010b). Furthermore, antagonism of non-human primate BST-2 is abrogated by mutations in the myristoylation site of SIV Nef (**Figure 2B**; Jia et al., 2009; Zhang et al., 2009). In addition, SIV Nef mutations that impair CD4 and CD28 downregulation also abrogate BST-2 antagonism, suggesting a similar mechanism of interaction (Zhang et al., 2009). By contrast, BST-2 antagonism by some strains of HIV-2 (as well as SIVtan from Tantalus monkeys) is mediated by the Env glycoprotein (**Figure 2C**; Bour and Strebel, 1996; Ritter et al., 1996; Abada et al., 2005; Gupta et al., 2009b). Although the exact determinants of interaction are not well understood, an endocytic motif (GYxxϕ) in the cytoplasmic region of gp41 (Boge et al., 1998) is known to be required to bind to AP-2, triggering BST-2 downregulation (Le Tortorec and Neil, 2009), while extracellular domains of HIV-2 Env apparently bind to the EC of BST-2. It was recently reported that an A100D point mutation of BST-2's EC abrogates the HIV-2 Env-mediated block of BST-2 restriction (Gupta et al., 2009b), supporting a model of interaction between HIV-2 Env and the EC of BST-2.

Other BST-2 antagonists include KSHV K5 protein, which ubiquitinates K18 residue in the CT domain of BST-2 (**Table 1**), leading to reduced surface and intracellular levels of BST-2, presumably through an endolysosomal process (**Figure 2D**; Mansouri et al., 2009; Pardieu et al., 2010). The Ebola virus GP2 appears to use a novel non-sequence-specific mechanism, overcoming

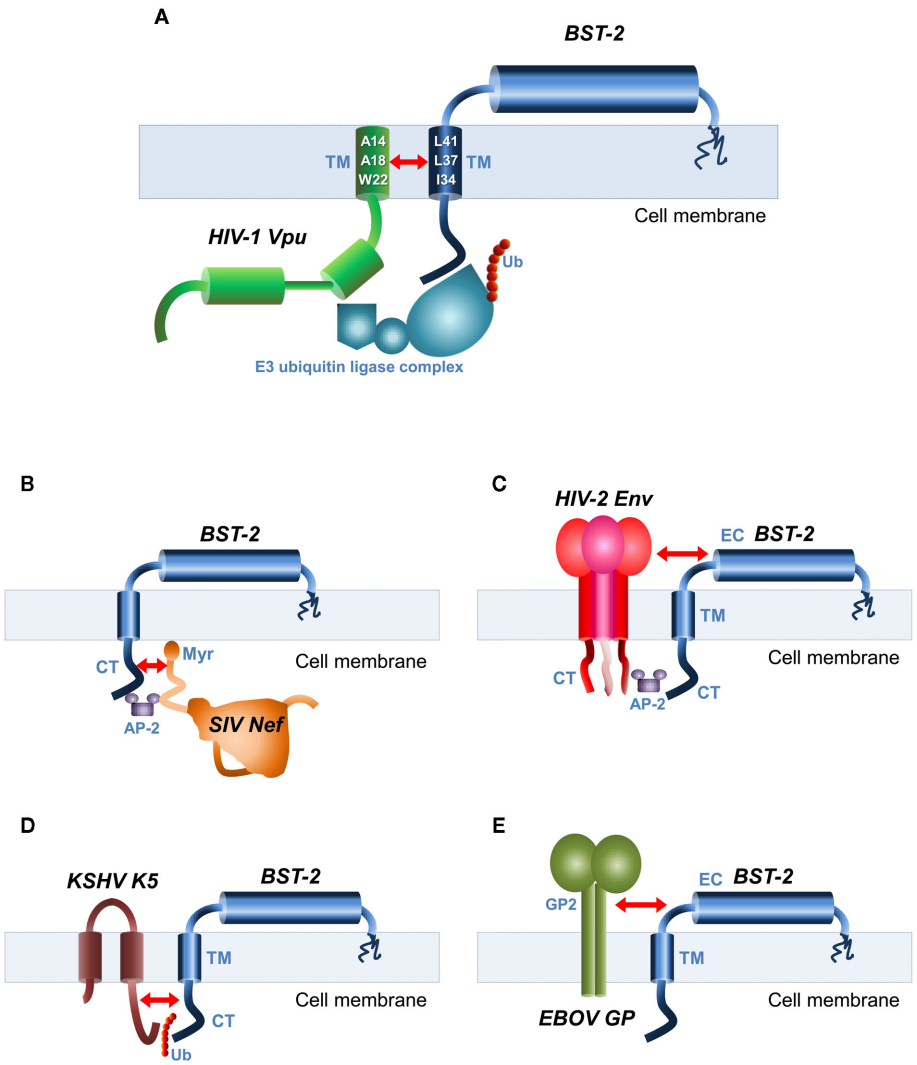


**FIGURE 2 | Viral antagonists of BST-2 and their domains of interaction.** Schematic representation of BST-2 and its known antagonists. The structural domains of interaction are indicated by red arrows. **(A)** HIV-1 Vpu and BST-2 interact through their mutual transmembrane (TM) domains. Key amino acid residues involved in the interaction are depicted in the TM helices. Also shown is the E3 ubiquitin (Ub) ligase complex required for BST-2 internalization. **(B)** SIV Nef recognizes the cytoplasmic (CT) domain of BST-2. The AP-2 clathrin adaptor recruited for BST-2 internalization is also shown. Myr, myristoylation site. **(C)** The envelope glycoprotein (Env) of HIV-2 and SIVtan binds to BST-2 through their mutual ectodomains (EC), and recruitment of AP-2 by the CT domain of Env required for internalization is also shown. **(D)** Kaposi's sarcoma-associated herpesvirus (KSHV) K5 protein that is an ubiquitin ligase ubiquitinates a target lysine motif in the CT domain of BST-2, resulting in its internalization. **(E)** The antagonistic mechanisms of the Ebola virus (EBOV) glycoprotein (GP) are unclear, but require interaction between GP2 subunit of EBOV–GP and BST-2 EC.

BST-2's restriction without significant removal of the protein from the cell surface (**Figure 2E**; Kaletsky et al., 2009; Lopez et al., 2010; Kühl et al., 2011). Influenza virus is suspected of harboring an unidentified viral antagonist against BST-2, since BST-2 expression was unable to block replication-competent influenza virus production but inhibited the release of influenza virus-like-particles (Watanabe et al., 2011).

## CONCLUSION

Considerable progress was made recently in understanding the structure and function of BST-2, as well as the mechanisms by which viral antagonists counteract its activity. Through a combination of biological studies and structural analyses, the functional state of BST-2 is characterized as that of a parallel dimeric coiled-coil that, via its double-membrane anchors, physically binds budding virions to the infected cell. More importantly, current evidence shows that the unusual structural features of BST-2 determine its antiviral function independently of sequence homology. The EC has a prime role acting as a molecular ruler that separates the membrane anchors, in addition to allowing dimerization of BST-2 and providing conformational flexibility to sustain the protein's function as a viral particle tether. Likewise, loss of BST-2's double-membrane anchoring leads to the complete abrogation of the antiviral activity.

Although most of the evidence presented here was obtained from *in vitro* systems, a recent study using BST-2 knockout mice has shown that BST-2 inhibited the replication and release of a murine retrovirus *in vivo*, in a manner completely dependent on IFN-α production. Additionally, BST-2 restricted viral pathogenesis and delayed disease progression, suggesting that it has verifiable antiviral activity not only *in vitro* but also *in vivo*. (Liberatore and Bieniasz, 2011). Another study using rhesus macaques has confirmed the importance of the antagonism of BST-2 antiviral activity by Vpu *in vivo* (Shingai et al., 2011). Further investigation of the antiviral mechanisms exerted by host restriction factors, as well as the evolution of viral countermeasures, will not only advance our understanding of AIDS pathogenesis but also lead to the development of therapeutic alternatives.

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