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Interaction of human immunodeficiency virus gp120 with the voltage-gated potassium channel BEC1

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ABSTRACT

Retrovirus replication critically depends on a dynamic interplay between retroviral and host proteins. We report on the binding of the surface subunit (glycoprotein 120 (gp120)) of the human immunodeficiency virus type 1 (HIV-1) envelope protein (Env) to the cytoplasmic C-terminus of the voltage-gated potassium channel BEC1 (brain-specific ether-a-go-go-like channel 1), an interaction that can result in the repression of BEC's activity and the inhibition of HIV-1 particle-release. BEC1 protein was found to be expressed in T cells and macrophages, the major target cells of HIV-1. Thus, gp120/BEC1 interaction may be involved in HIV-1 life cycle and/or pathogenesis.

Structured summary:
MINT-7968695: *BEC1* (uniprotkb:Q9ULD8) *physically interacts* (MI:0915) with *gp160* (uniprotkb:P04578) by *anti bait coimmunoprecipitation* (MI:0006)
MINT-7968714: *BEC1* (uniprotkb:Q9ULD8) *physically interacts* (MI:0915) with *gp160* (uniprotkb:P04578) by *anti tag coimmunoprecipitation* (MI:0007)
MINT-7968675: *BEC1* (uniprotkb:Q9ULD8) *physically interacts* (MI:0915) with *gp160* (uniprotkb:P04578) by *pull down* (MI:0096)

1. Introduction

Retroviruses interact with multiple host factors [1,2]. One example of virus/host cell protein interaction is that of the human immunodeficiency virus type 1 (HIV-1) envelope proteins (Env) subunit glycoprotein 120 (gp120) with host cell CD4 molecules and co-receptors [3]. HIV-1 Env is synthesized as a gp160 poly-protein precursor that is subsequently processed by a host cell protease to generate the gp120 surface (SU) and gp41 transmembrane (TM) subunits.

The voltage-gated potassium (K^+) channel BEC1 has originally been described as brain-specific eag (ether-a-go-go)-like channel 1, due to its predominant expression in human telencephalon [4]. Voltage-gated K^+ channels constitute an evolutionary related multigene superfamily, which can be classified into the *Shaker* and *eag* families. Most K^+ channels are homotetrameric, with each subunit containing a voltage sensor and contributing to a central pore through which K^+ ions can permeate [5]. Voltage-gated K^+ channels play an essential role in controlling cellular excitability by allowing K^+ to flow out of a cell, thereby restoring the transmembrane potential to a negative value after depolarization [5].

2. Material and methods

2.1. Cells and plasmids

293T and COS-1 cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS). Jurkat and A3.01 cells were cultured in RPMI 1640 with 10% FBS. Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats by gradient centrifugation using Ficoll-Paque™ Plus (GE Healthcare). Monocyte-derived macrophages were generated from PBMC by selective adherence and subsequent culturing in RPMI 1640 with 20% FBS and 5% human AB serum for 6 days. Plasmid constructs and cloning strategies are described in Supplementary data.

Abbreviations: HIV, human immunodeficiency virus; gp120, glycoprotein 120; Env, envelope proteins; hERG, potassium voltage-gated channel subfamily H member 2, voltage-gated potassium channel subunit Kv11.1

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2.2. GST pull-down assays and immunoprecipitations

GST pull-down assays and immunoprecipitation were carried out as described previously [6]. For co-immunoprecipitations, 293T cells (plated in two 10 cm diameter dishes per experimental condition) were transfected with pSG5-gp120-HA or pSG5-gp41-HA (8 μg/dish) using Nanofectin I (PAA). In the experiments shown in Fig. 2D, cells were co-transfected (8 μg plasmid/dish) with pSG5-gp120-HA and either pSG5-BEC1-Flag or pSG5-BEC1(1-861)-Flag. Cells were harvested 48 h after transfection and immunoprecipitations were performed with anti-BEC1 serum or pre-immune serum. Western blots were carried out as described elsewhere [7]. Membranes were developed with anti-BEC1 serum at a dilution of 1:1000, a rat monoclonal anti-HA antibody (3F10, Roche) diluted 1:100, or anti-Flag antibody diluted 1:500.

2.3. FluxOr™ thallium detection assay

COS-1 cells were plated in 10 cm dishes and co-transfected with either pSG5-BEC1 or pSG5-BEC1(1-861)-Flag and pSG5-gp120-HA or pSG5-gp41-HA employing 8 μg of each plasmid and Nanofectin I (PAA). Two days later, transfected cells were plated at 2×10^4 cells/well in 96-well plates. BEC1 conductance was measured with the FluxOr™ thallium detection kit according to the manufacturer's protocol (Invitrogen). Following addition of the stimulus buffer the fluorescence signal was measured every 30 s on a MR580 Microelisa Autoreader (Dynatech) at 490 nm excitation wavelength.

2.4. HIV-1 particle release assay

293T cells were plated in 10 cm dishes and transfected with plasmids pSG5-BEC1 or pSG5-BEC1(1-861)-Flag (8 μg/dish) using Nanofectin I. On the following day, cells were again transfected (Lipofectamin, Invitrogen) with 4 μg of each of the DNA plasmids pTN7-Stopp and pENV035x. pTN7-Stopp carries the *Renilla luciferase* reporter gene instead of the *nef* gene and lacks a functional *env* gene, thus set to produce only a single-round of infection [8]. pENV035x was a kind gift of Christian Jassoy, University of Leipzig, and expresses a CCR5-tropic HIV-1 *env* gene. Co-transfection of the two plasmids results in the release of pseudo-typed HIV-1 particles. Virus-containing supernatants were harvested 48 h after transfection, filtered through 0.45 μm cellulose–acetate filters (Schleicher and Schuell), and stored in 1 ml aliquots at −80 °C. To monitor transfection efficiency of pTN7-Stopp in 293T cells, Renilla luciferase activity was measured with a luciferase kit (Promega) in 293T cell lysates 48 h after transfection. HIV-1 particles in the supernatants were quantitated by a standard p24-ELISA-kit (Bio-Rad). Additionally, Renilla luciferase activity was measured in PM1 cells infected with HIV-1 pseudo-typed particle containing supernatants (details upon request).

3. Results

3.1. Physical interaction of HIV-1 gp120 with the C-terminus of BEC1

To study the binding of gp120 to BEC1 (Fig. 1) in vitro, the C-terminal 224 amino acids of BEC1 (860–1083), and gp120 and gp41 were analyzed by GST pull-down assays (Fig. 2A). gp120 but not gp41 bound to BEC1 (860–1083). GST pull-down assays with two fragments of the BEC1 C-terminus further narrowed down the gp120 interacting region to the C-terminal 111 amino acids (973–1083) of BEC1 (Fig. 2B). BEC1 protein (~117 kDa) was specifically recognized by our rabbit polyclonal anti-BEC1-antiserum

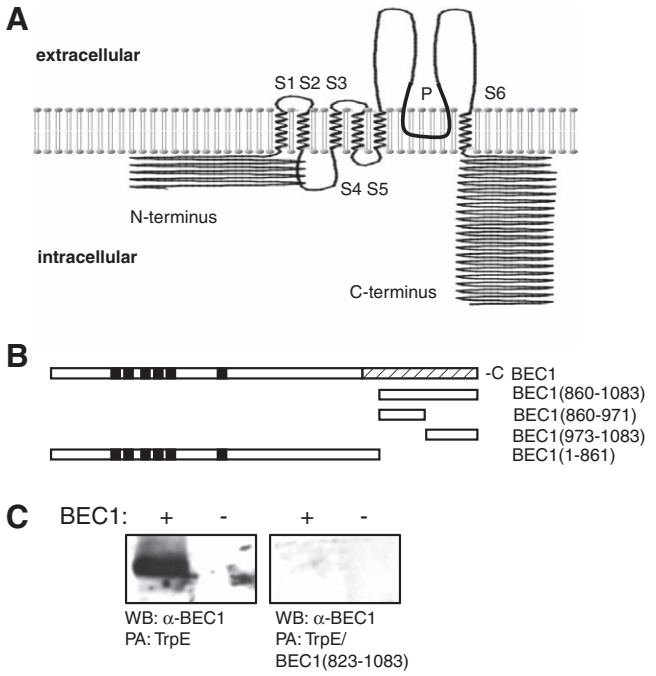

Fig. 1. (A) Transmembrane structure of a BEC1 subunit. Membrane-spanning domains, S1–S6; pore region, P. (B) BEC1 constructs used in this study. Transmembrane regions: black boxes. C-terminal sequence used for the generation of the polyclonal rabbit BEC1-antiserum: grey box. (C) Western blot (WB) analysis of protein lysates from COS-1 cells transfected with full-length BEC1 (+) or empty vector (−) for 48 h. Western blots were developed with BEC1-antiserum (α-BEC1) either pre-adsorbed (PA) with TrpE alone or with TrpE–BEC1 (823–1083) fusion protein.

(Fig. 1C). We then studied BEC1/gp120 interaction in eukaryotic cells by co-immunoprecipitation utilizing the BEC1-antiserum. Initial experiments had revealed that 293T cells constitutively express BEC1; however, BEC1 protein was only observed in immunoprecipitated samples but not in total protein lysates, indicating that the levels in 293T cells were low. gp120 but not gp41 was detected in BEC1-immunoprecipitates from 293T cell transfected to produce gp120 or gp41 (Fig. 2C). Furthermore, Bec1 failed to interact with Epstein-Barr-Virus LMP1; with the Env transmembrane domains of HERV-K, HTLV-1 and HIV-1; and with HERV-K Gag. These data demonstrate that ectopic gp120 can associate with endogenously expressed BEC1 in 293T cells. To confirm that HIV-1 gp120 interacts with the C-terminus of BEC1 in living cells, Flag-tagged full-length BEC1 or Flag-tagged C-terminally truncated BEC1 (1-861) were co-expressed with gp120 in 293T cells and subsequently immunoprecipitated with an anti-Flag antibody. gp120 was only co-immunoprecipitated with full-length BEC1 (Fig. 2D). Thus, gp120 binds to the C-terminus of BEC1 in vitro and in living cells.

3.2. HIV-1 gp120 suppresses BEC-1 activity

We next asked whether the electrophysiological properties of BEC1 might be affected by gp120. We addressed this question by employing a Tl^+ detection kit that takes advantage of the well-described permeability of K^+ channels to Tl^+ ions [9]. When added to the culture medium simultaneously with a stimulus to open K^+ channels, Tl^+ flows through K^+ channels down its concentration gradient into cells. By loading cells with a Tl^+-sensitive indicator dye that shows a strong increase in fluorescence upon Tl^+ binding, Tl^+-induced fluorescence can be used as a surrogate indicator for K^+ channel activity. In non-transfected COS-1 control cells only a

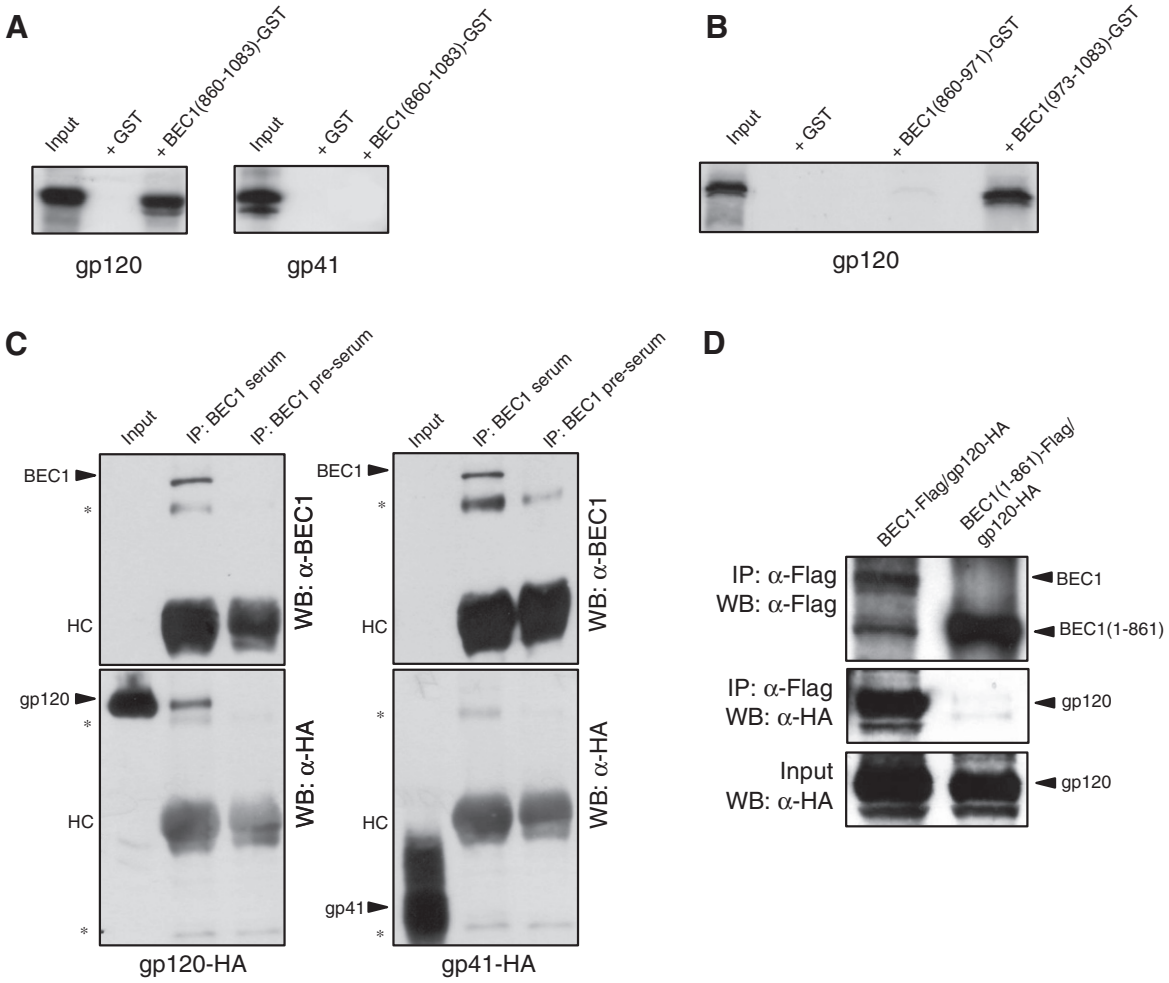

Fig. 2. (A) Binding of ^{35}S-labeled gp120 or gp41 (Input) to either GST alone or BEC1 (860–1083) in GST pull-down assays. (B) Binding of gp120–BEC (973–1083). (C) 293T cells producing BEC1 were transfected with either HA-tagged HIV-1 gp120 (gp120-HA) or gp41 (gp41-HA) for 48 h. Expression of the transfected proteins was confirmed in total cellular protein lysates (Input). Endogenously expressed BEC1 was immunoprecipitated with BEC1 serum (IP: BEC1 serum) or the corresponding pre-immune serum (IP: BEC1 pre-serum) as control. Western blots (WB) were developed with either BEC1-antiserum (α-BEC1) or an anti-HA antibody (α-HA). BEC1, gp120, and gp41 proteins are marked by arrows. Asterisks indicate unspecific background bands. HC, immunoglobulin heavy chains. (D) Interaction of gp120 with the C-terminus of BEC1 in living cells. 293T cells were co-transfected with gp120-HA and either Flag-tagged full-length BEC1 (BEC1-Flag) or a Flag-tagged C-terminally truncated BEC1 construct (BEC1(1-861)-Flag)) for 48 h. Immunoprecipitations (IP) were performed with an anti-Flag antibody (α-Flag) and Western blots (WB) were developed with either α-Flag or α-HA antibody. Expression of gp120 in transfected cells was confirmed in total cellular protein lysates (Input).

small increase in fluorescence could be observed with the Tl^+ assay, probably resulting from constitutively expressed Tl^+ permeable channels or transporters in these cells (Fig. 3). In contrast, transfection of COS-1 cells with BEC1 led to a strong increase of the fluorescence signal, indicating that the cells expressed functionally intact BEC1. As a positive control, we also employed COS-1 cells transduced with the well-characterized human voltage-gated K^+ channel potassium voltage-gated channel subfamily H member 2 (hERG) [10]. hERG like BEC1 acted as a Tl^+ channel in the Tl^+ assay (Fig. 3). Strikingly, co-expression of BEC1 and gp120 resulted in a significant suppression of BEC1 activity ($P = 0.00035$), comparable to the level of non-transfected control cells (Fig. 3, upper panel). Using increasing amounts of gp120 plasmid DNA (1–8 μg), this suppressive effect was found to be dose-dependent (data not shown). In contrast, BEC1 conductance was unaffected in COS-1 cells co-expressing BEC1 and gp41. The C-terminally truncated BEC1 (1-861) mutant that lacks the gp120-interacting domain induced a robust fluorescence increase in the Tl^+ assay, with a maximum fluorescence signal only about 25% lower than that of full-length BEC1. Importantly, in contrast to full-length BEC1, the gp120 binding-deficient BEC1 (1-861) was not suppressed by gp120 (Fig. 3, lower panel). Combined, these data strongly suggest that gp120 can suppress BEC1 conductance through the physical interaction with its cytoplasmic C-terminus.

3.3. Inhibition of HIV-1 particle release by BEC-1

Interaction of HIV-1 Vpu with the human acid-sensitive background K^+ channel TASK-1 inhibits HIV-1 particle release [11]. We thus wanted to analyze whether the interaction of gp120 and BEC1 might similarly influence the release of HIV-1 particles. 293T cells were transfected with either full-length BEC1 or the truncated BEC1 (1-861) plasmids. Twenty-four hours later cells were again transfected with an Env-defective HIV-1 proviral construct (pTN7-stopp) carrying a *Renilla luciferase* reporter gene, and a plasmid expressing a CCR5-tropic HIV-1 *env* gene. Supernatants were harvested 48 h later and their virus content was quantitated by p24 ELISA. As a further quantitation, PM1 cells were infected with virus present in the supernatants for 48 h and subsequently assayed for Renilla luciferase activity (Fig. 4A). Enzyme activity in the 293T cells was lower in BEC1-transfected cells than in non-transfected control cells, suggesting that BEC1 inhibits HIV-1 particle release (data not shown). Full-length BEC1 led to a twofold reduction of HIV-1 particle release compared to BEC1

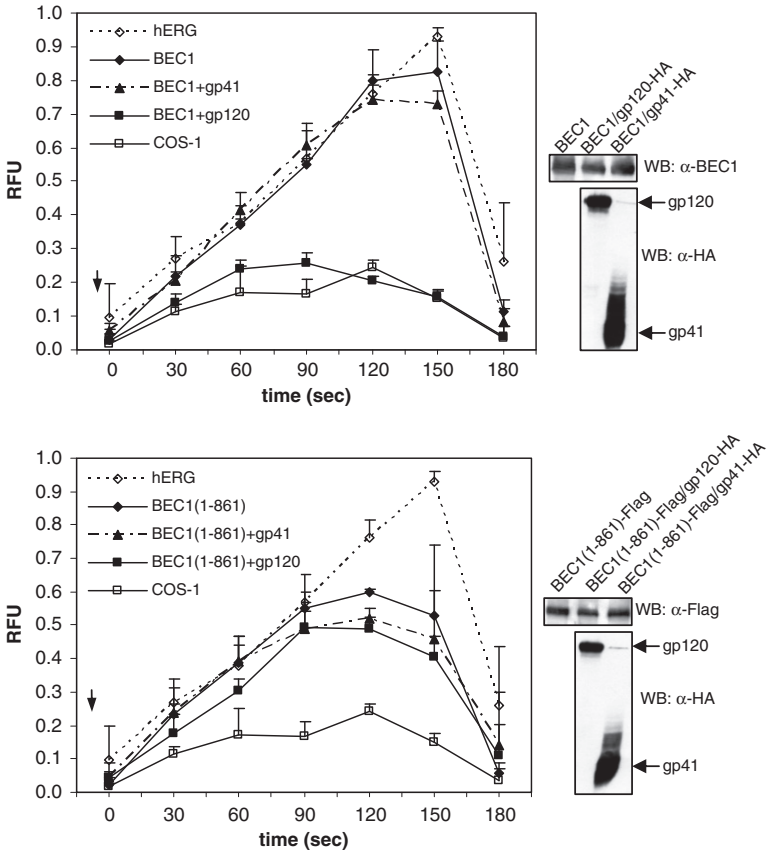

Fig. 3. COS-1 cells were transfected with either full-length BEC1 (upper panel) or a Flag-tagged C-terminally truncated BEC1 construct (BEC1 (**1**-861), lower panel)) and HA-tagged gp120 or gp41 as indicated. As a positive control, cells were transduced with the human voltage-gated K^+ channel hERG. Untransfected cells (COS-1) served as negative control. Diagrams show fluorescence kinetics, expressed in relative fluorescence units (RFU), and represent the mean plus standard deviation of three independent experiments carried out in triplicates. Uniform expression of transfected proteins was confirmed by Western blots.

(1-861) (Fig. 4B). This reduction was significant ($P = 0.0097$) and consistently observed in both HIV-1 particle detection assays (p24 ELISA and luciferase assay). To confirm that this reduction depended upon binding of BEC1 and gp120, BEC1 or BEC1 (1-861) transfected cells were subsequently transfected with pTN7-stopp only (no HIV-1 *env* plasmid). Under these conditions, p24 levels did not differ in the supernatants of BEC1 and BEC1 (1-861) transfected cells (data not shown). Together, these results indicate that overexpression of BEC1 inhibits HIV-1 particle release from 293T cells and that this requires the C-terminus of BEC1.

3.4. Expression of BEC1 in HIV-1 target cells

BEC1 mRNA expression was originally reported to be restricted to human brain [4]. Nevertheless, a subsequent RT-PCR based study detected *BEC1* mRNA also in various hematopoietic cells [12]. Immunoprecipitations identified BEC1 protein in freshly isolated human PBMC and cultured monocyte-derived human macrophages, as well as in the human T cell lines Jurkat and A3.01 (Fig. 4C). BEC1 was also observed in COS-1, Gos 3, HeLa, LN405, HaCaT, human fibroblasts, and EBV-transformed B lymphoblastoid cell lines (data not shown). In sum, our results show that BEC1 is expressed in target cells of HIV-1.

4. Discussion

We have documented here that the voltage-gated K^+ channel BEC1 expressed in HIV target cells can physically and functionally interact with HIV-1 gp120 in the cytoplasm. A potentially important functional consequence is the marked suppression of BEC1 conductance in cells co-expressing BEC1 and gp120. Inhibition of K^+ channels by HIV-1-encoded proteins, either by direct interaction or through indirect mechanisms, has been recognized before: The HIV-1 accessory protein Vpu was shown to physically interact with the acid-sensitive human K^+ channel TASK-1, leading to the suppression of TASK-1 current [11]. Expression of the HIV-1 accessory protein Nef in CEM lymphocytes inhibited the activity of a Ca^{2+}-dependent K^+ channel [13]. HIV-1 Nef also inactivated a large-conductance K^+ channel in U251 human glial cells [14]. HIV-1 gp160 decreased the current of the voltage-gated K^+ channel Kv1.3 in Jurkat E6.1 cells, probably by increasing the channel's phosphorylation [15]. Finally, cardiac myocytes showed a delay in repolarization associated with a reduction in outward K^+ currents [16]. Together, it therefore seems possible that modulation of K^+ channel activity and the associated changes in the transmembrane electric potential may be of relevance for HIV-1 life cycle and pathogenesis. How does the BEC1/gp120 interaction fit into this scenario? Notably, HIV-infection of human PBMC caused an increase of intracellular K^+ levels that correlated with HIV-1-induced cytopathic effects [17]. Since BEC1 transports K^+ out of the cell, BEC1 inhibition is likely to cause increased intracellular K^+ concentration.

In T lymphocytes, the major target cells of HIV-1, functional K^+ channels are required to maintain the negative resting membrane potential as an important driving force for Ca^{2+} influx, which in turn is crucial in the antigen-driven activation of T lymphocytes [18]. Indeed, high affinity antagonists of K^+ channels are known to depolarize human T cells (render the resting membrane potential less negative), thereby inhibiting Ca^{2+}-dependent T cell activation [19,20]. Along this line, inhibition of a Ca^{2+}-dependent K^+ channel

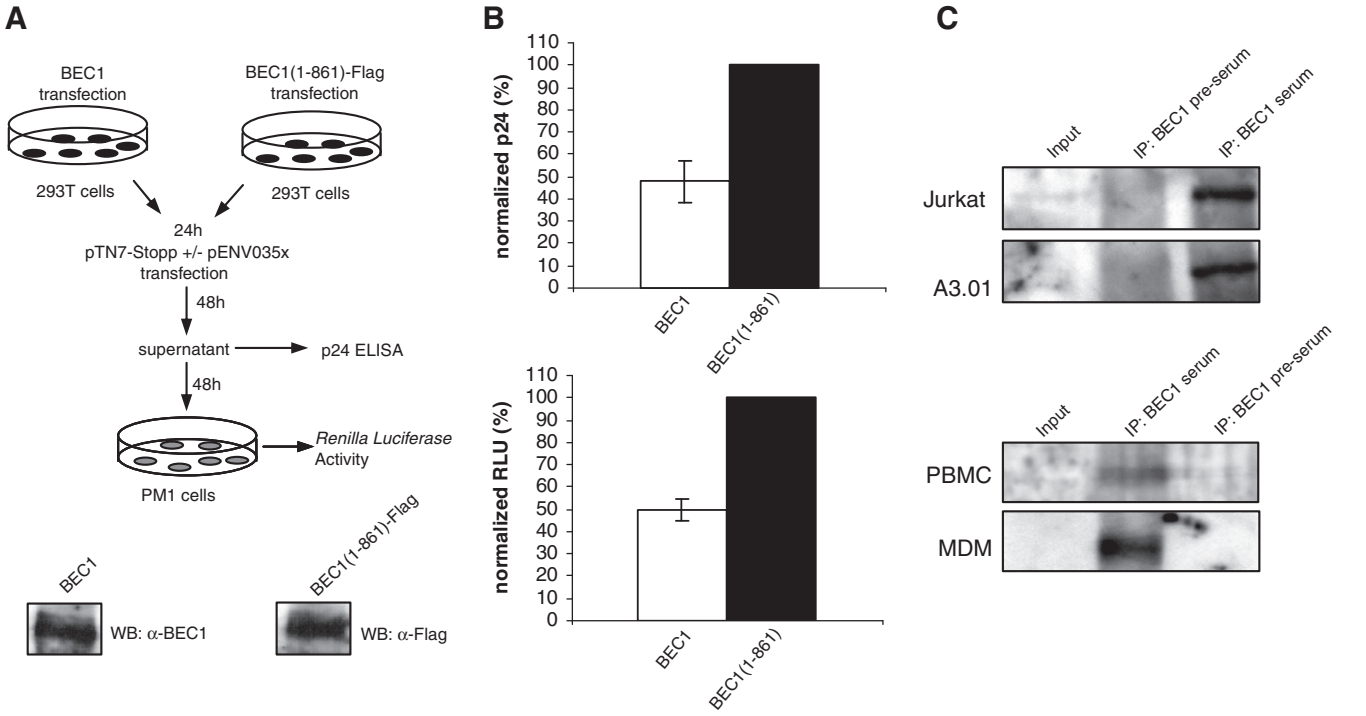

Fig. 4. (A) Flow diagram outlining the quantitation of HIV-1 particle release. (B) p24 levels and Renilla luciferase activity (expressed in relative light units [RLU]) from 293T cells transfected with BEC1 (1-861) were set as 100%. Error bars denote standard deviations from three independent experiments carried out in duplicates. (C) BEC1 immunoprecipitated from the indicated cell lines by BEC1-antiserum. IP: BEC1 pre-serum, protein lysate immunoprecipitated with the corresponding BEC1 pre-immune serum as a control. Western blots were stained with BEC1-antiserum.

by HIV-1 Nef has been found to cause depolarization of CEM lymphocytes [13]. Given the expression of BEC1 in human T cells, it is thus tempting to speculate that suppression of BEC1 conductance by HIV-1 gp120 and the subsequent increase of the intracellular K^+ concentration may lead to depolarization of T cells and the suppression of Ca^{2+}-dependent T cell activation cascades. Moreover, the prominent expression of BEC1 in neurons [4] and the growing evidence that HIV can infect neurons [21,22] raise the possibility that BEC1/gp120 interaction is relevant in this tissue as well. BEC1/gp120 interaction also negatively affected HIV particle release, probably through interference with the binding of gp120 to the HIV-1 Gag protein crucial for particle assembly [23]. However, since BEC1 is a rare protein in all tested cells and gp120 is typically expressed to high levels in HIV-infected cells, the effect of the BEC1/gp120 interaction on particle release is likely to be of minor importance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.07.016.

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