

Supplementary Materials for

**Interferon-Induced SCYL2 Limits Release of HIV-1 by Triggering  
PP2A-Mediated Dephosphorylation of the Viral Protein Vpu**

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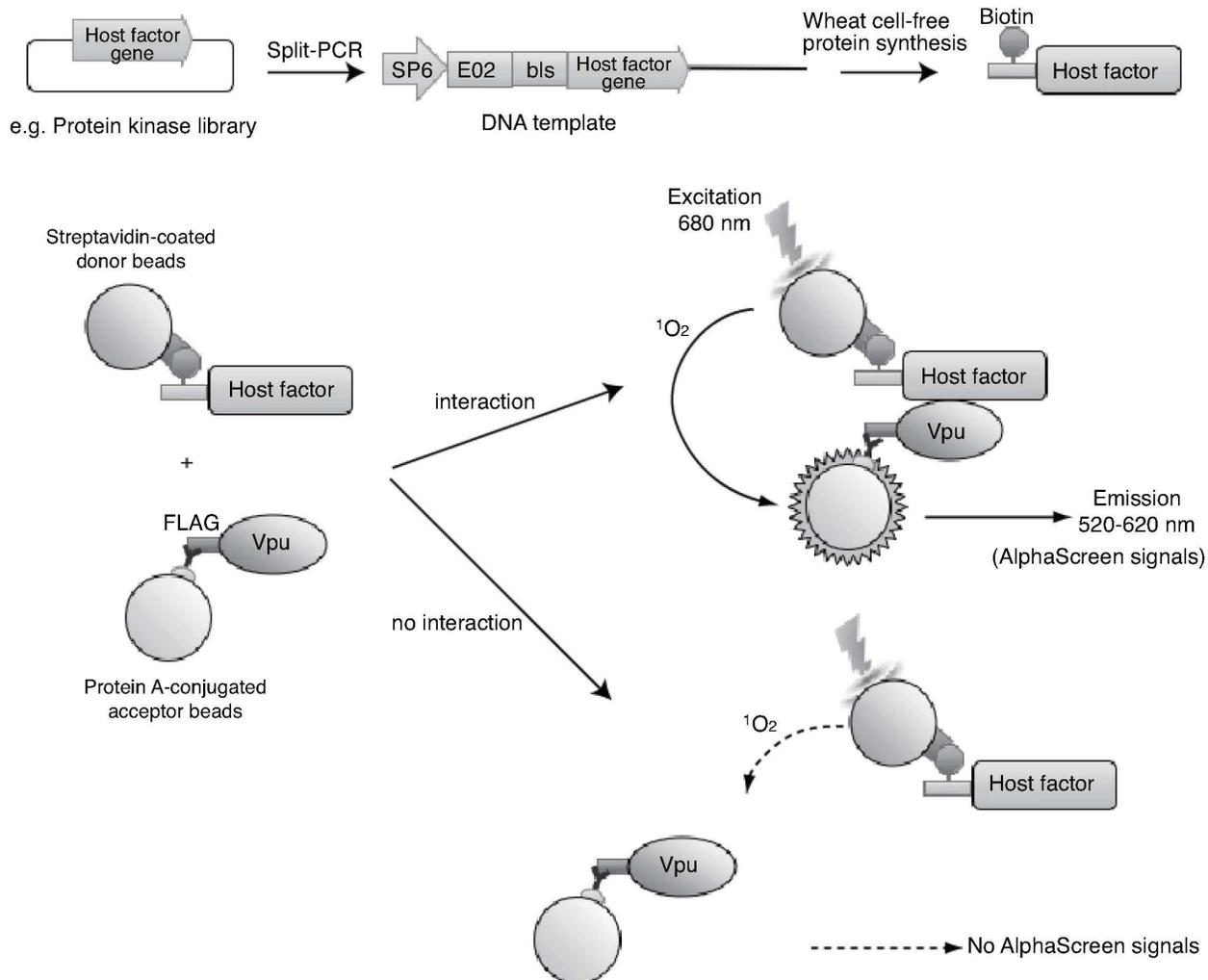
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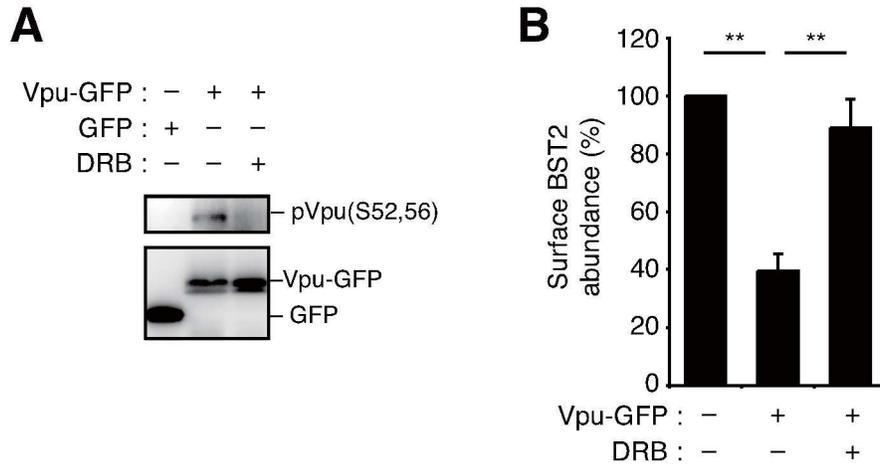
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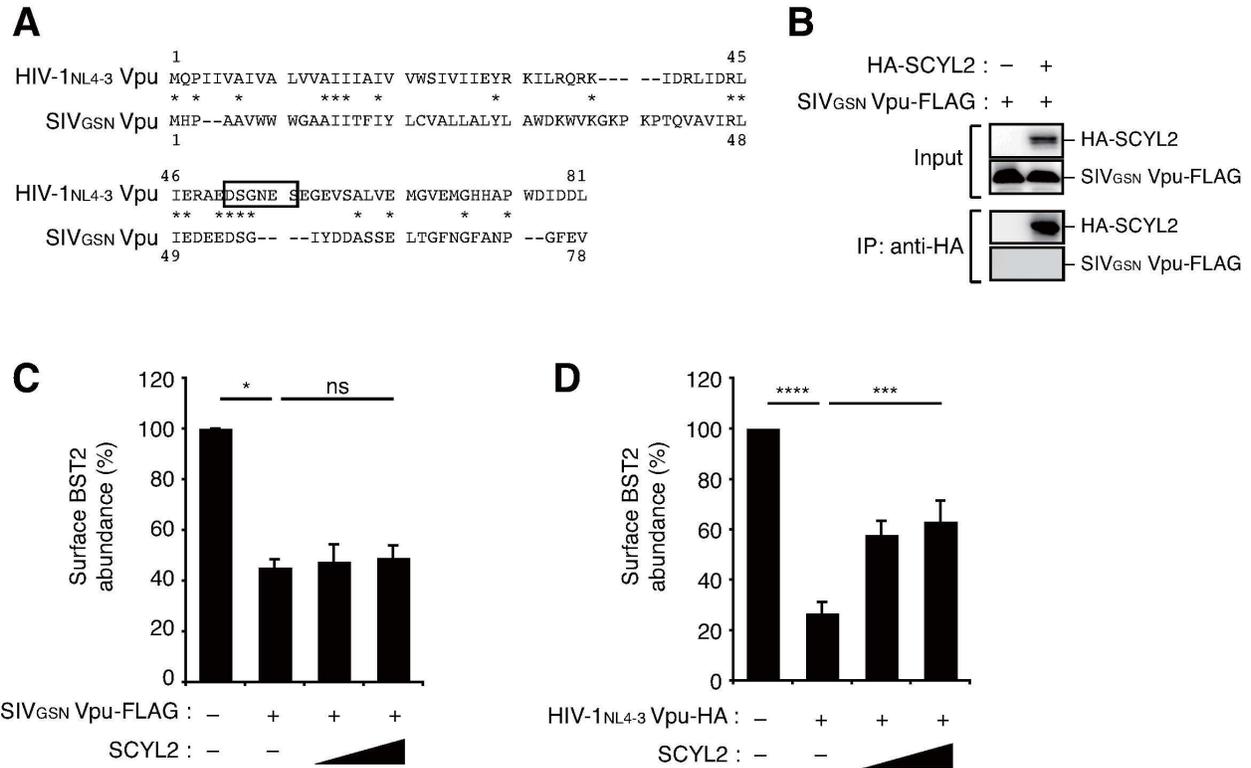
- Fig. S1. Schematic representation of the initial screening method.
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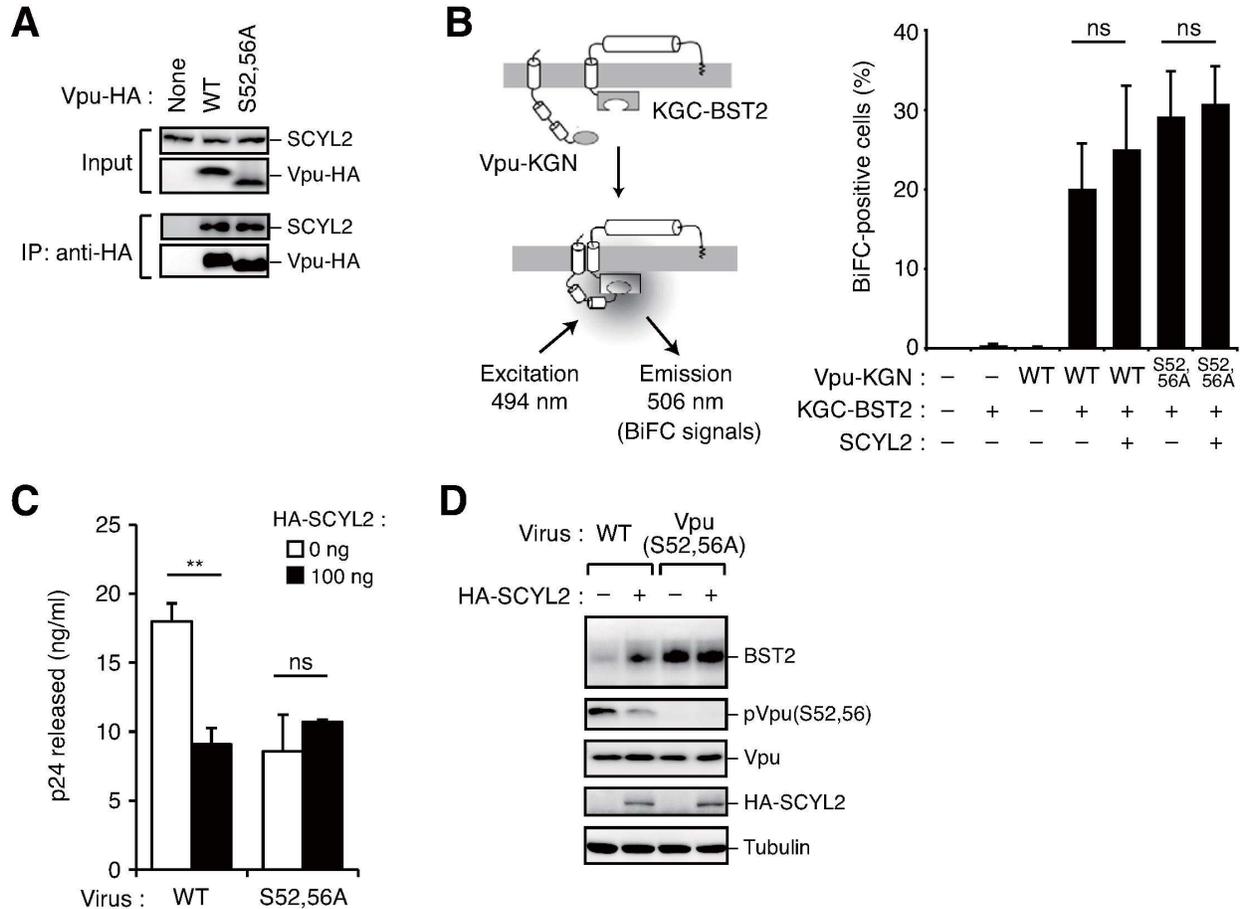
**Fig. S1.** Schematic representation of the initial screening method. DNA templates for transcription were constructed by the host factor (protein kinase) library and split-primer PCR technique, by which the biotin ligation site (bls) was fused onto the N-terminal-coding regions of all genes for protein biotinylation. Biotinylated host factors were incubated with FLAG-tagged Vpu protein for 1 hour at 37°C. Subsequently, protein A-conjugated acceptor beads with anti-FLAG antibody and streptavidin-coated donor beads were added and allowed to bind to the tagged proteins. Upon laser excitation, donor beads converted ambient oxygen to singlet oxygen. In cases in which both proteins are within 200 nm of each other, singlet oxygen is transferred across the gap to activate acceptor beads and subsequently emit light at 520 to 620 nm (AlphaScreen signals). If the proteins do not interact, no AlphaScreen signal is produced because the singlet oxygen fails to transfer from the donor beads to the acceptor beads because of the increased distance (> 200 nm).



**Fig. S2.** The CKII inhibitor DRB phenocopies the effect of increased SCYL2 abundance. **(A)** DRB inhibits Vpu phosphorylation on Ser<sup>52</sup> and Ser<sup>56</sup>. HeLa cells were transfected with a vector encoding Vpu-GFP. Twenty-four hours later, cells were washed and treated with 100  $\mu$ M DRB for 18 hours. Cells were processed for Western blotting analysis with anti-pVpu and anti-GFP antibodies. Data are representative of 3 experiments. **(B)** DRB inhibits the Vpu-induced reduction in BST2 abundance. The cell-surface abundance of BST2 on cells used in (A) was monitored by flow cytometry. \*\* $P < 0.01$ ,  $n = 3$  experiments.



**Fig. S3.** SCYL2 fails to inhibit SIV<sub>GSN</sub> Vpu-induced counteraction of BST2. **(A)** The sequence alignment of HIV-1<sub>NL4-3</sub> Vpu (Accession No. AF324493) and SIV<sub>GSN</sub> Vpu (Accession No. AF468659). Invariant residues are highlighted with an asterisk. The boxed region shows the  $\beta$ -TrCP-binding consensus motif. **(B)** SCYL2 fails to interact with SIV<sub>GSN</sub> Vpu. HEK 293T cells were transfected with plasmids encoding SIV<sub>GSN</sub> Vpu-FLAG and HA-SCYL2. Cell lysates were immunoprecipitated with an anti-HA antibody and then analyzed by Western blotting with either anti-FLAG or anti-HA antibodies. Data are representative of 3 experiments. **(C)** LLC-MK2 cells (which have endogenous rhesus BST2) were transfected with plasmids encoding SIV<sub>GSN</sub> Vpu (0 or 100 ng) and SCYL2 (0, 500, or 1,000 ng). The cell-surface abundance of endogenous rhesus BST2 was monitored by flow cytometry. ns: not significant, \* $P = 0.0280$ ,  $n = 3$  experiments. **(D)** HeLa cells (which have endogenous BST2) were transfected with plasmids encoding HIV-1<sub>NL4-3</sub> Vpu (0 or 100 ng) and SCYL2 (0, 500, or 1,000 ng). The cell-surface abundance of endogenous human BST2 was monitored by flow cytometry. ns: not significant, \*\*\*\* $P < 0.0001$ , \*\*\* $P = 0.0008$ ,  $n = 3$  experiments.



**Fig. S4.** SCYL2 inhibits Vpu function through a phosphorylation-dependent mechanism. **(A)** Endogenous SCYL2 interacts with both wild-type Vpu and the Vpu(S52,56A) mutant. HEK 293T cells were transfected with plasmids encoding the indicated Vpu-HA proteins. Cell lysates were then immunoprecipitated with anti-HA antibodies and bound proteins were visualized by Western blotting analysis. **(B)** SCYL2 does not interfere with the association between BST2 and either wild-type Vpu or the Vpu S52,56A mutant in live cells. BiFC analysis of HEK 293T cells expressing KGC-BST2 and Vpu-KGN (left panel). The BiFC signals derived from the BST2-Vpu interaction in the presence or absence of SCYL2 were quantified by flow cytometry (right panel). ns: not significant. **(C and D)** SCYL2 inhibits the release of wild-type HIV-1, but not its derivative harboring the Vpu S52,56A mutant. HeLa cells were cotransfected with the indicated molecular clones (100 ng) together with SCYL2 expression plasmid (100 ng). **(C)** Forty-eight hours after transfection, culture supernatants were subjected to p24 ELISA. **(D)** Cell lysates were processed for Western blotting analysis with the indicated antibodies. ns: not significant,  $**P = 0.0088$ ,  $n = 3$  experiments. Data in **(A)** and **(D)** are representative of 3 experiments.