**Table S1. Vpu Constructs Used in This Study, Related to Figures 1–4, 6, and 7**
The sequences, modifications, and uses of the Vpu-based constructs in this study. The phosphorylation sites on Vpu are indicated in red in the first construct. TMDs are in cyan. Epitope tags are in green. The TEV protease site is in orange. Oppositely-charged coiled-coil sequences are in magenta. GpA is glycophorin A, VSVG is vesicular stomatitis virus glycoprotein. Epitope tags used are HA (AYPYDVPDYA) and His6 (HHHHHH).

| Name | Vector | Sequence Information | Uses in paper |
|---|---|---|---|
| Vpu | pCDNA5/FRT/TO | MVPIIVAIVALVVAIIIAIVVWSIVIIEYRKIL RQRKIDRLIDRLIERAEDSGNESEGEVSALV EMGVEMGHHAPWDIDDLENLYFQGAYPY DVPDYA | Fig. 1A, 1B, 2A, 2D, 4D, 4E, S1B, S1C, S1D, S1E, S2A, S2B, S2C, S5C, S5D |
| Vpu-SN | pCDNA5/FRT/TO | S52N/S56N mutations in cytosolic domain | Fig. 1B, 1C, 2A, 2D, 4D, S1B, S1C, S1D, S1F, S1G, S2A, S5C |
| Vpu-M1 | pCDNA5/FRT/TO | I17F/V21F/V25L mutations in the TMD of Vpu. | Fig. 1A, 4D, S1E, S5C, S5D |
| Vpu-M2 | pCDNA5/FRT/TO | GpA(G13L) TMD in place of Vpu TMD. | Fig. S1C, S1F |
| Vpu-M3 | pCDNA5/FRT/TO | VSVG TMD in place of Vpu TMD. | Fig. S1C, S1F |
| Vpu-SN-M1 | pCDNA5/FRT/TO | Vpu-M1 construct containing SN mutation. | Fig. 1C, S5C |
| Vpu-SN-M2 | pCDNA5/FRT/TO | Vpu-M2 construct containing SN mutation. | Fig. S1C, S1F |
| Vpu-SN-M3 | pCDNA5/FRT/TO | Vpu-M3 construct containing SN mutation. | Fig. S1C, S1F |
| untagged Vpu | pCDNA3.1 | Vpu without any tags | Fig. 7A, 7B, 7C |
| untagged Vpu-SN | pCDNA3.1 | Vpu containing SN mutation without any tags | Used in experiments not shown |
| untagged Vpu-M1 | pCDNA3.1 | Vpu-M1 construct without any tags | Fig. 7A, 7C |
| rec-Vpu | pRSETA | MVPIIVAIVALVVAIIIAIVVWSIVIIEYRKIL RQRKIDRLIDRLIERAEDSGNESEGEVSALV EMGVEMGHHAPWDIDDLAYPYDVPDYAG SENLYFQGAHHHHHHST | Fig. 3B, 3C, 3D, 4A, 4B, 4C, 6A, 6C, S3C, S3D, S4B-S4F, S5A, S6A, S7A, S7B, S7C |
| rec-Vpu-SN | pRSETA | S52N/S56N mutations in cytosolic domain of rec-Vpu | Fig. 3D, S4B |
| rec-Vpu-M1 | pRSETA | I17F/V21F/V25L mutations in TMD of rec-Vpu. | Fig. S4B, S7 |
| rec-Vpu-cyto | pRSETA | MRGSHHHHHHEYRKILRQRKIDRLIDRLIE RAEDSGNESEGEVSALVEMGVEMGHHAP WDIDDL | Fig. 3F, S3A |
| rec-Vpu-SN-cyto | pRSETA | S52N/S56N mutations in rec-Vpu-cyto | Fig. S3A |
| His-FLAG-Vpu | pRSETA | MRGSHHHHHHGMASMTGGQQMGRDLYD DDDKDRWGSMVPIIVAIVALVVAIIIAIVV WSIVIIEYRKILRQRKIDRLIDRLIERAEDSG NESEGEVSALVEMGVEMGHHAPWDIDDL | Fig. S3B |
| Coil-Vpu | pET-Duet (with Coil-CD4) | MGSSHHHHHHSQDPGSENLYFQG*LEIRAAF LRQRNTALRTEVAELEQEVQRLENEVSQYETR YGPL*GGGSEYRKILRQRKIDRLIDRLIERAE DSGNESEGEVSALVEMGVEMGHHAPWDI DDLGYPYDVPDYA | Fig. 3F |

**Table S2. CD4 Constructs Used in This Study, Related to Figures 1–4, 6, and 7**
The sequences, modifications, and uses of the CD4-based constructs in this study. The potential ubiquitination sites (lysines) are indicated (red). TMDs are in cyan. Epitope tags are in green. The TEV protease site is in orange. Oppositely-charged coiled-coil sequences are in magenta. GpA is glycophorin A, VSVG is vesicular stomatitis virus glycoprotein. Epitope tags used are FLAG (DYKDDDDK), 3F4 (KTNMKHMAGAAA), and His6 (HHHHHH).

| Name | Vector | Sequence Information | Uses in paper |
|---|---|---|---|
| CD4 | pCMV | ...STPVQPMALIVLGGVAGLLLFIGLGIFFCV RCRHRRRQAERMSQIKRLLSEKKTCQCPH RFQKTCSPI | Fig. 1A, 1B, 1C, 4D, 7A, 7B, 7C, S1B, S1C, S1D, S1E, S1F, S1G, S5C |
| CD4-M1 | pCMV | GpA TMD in place of the TMD in CD4. | Fig. 1B, 1C, 7A, 7B, 7C, S1B, S1C-S1F, S5C |
| CD4-M2 | pCMV | VSVG TMD in place of the TMD in CD4. | Fig. 1B |
| CD4-M3 | pCMV | Vpu TMD in place of CD4 TMD in CD4. | Fig. S1D, S1G |
| CD4-Δcyto | pCMV | CD4 lacking the cytosolic domain (ends at R425) | Fig. 1C |
| CD4 | SP64 | ...STPVQPMALIVLGGVAGLLLFIGLGIFFCV RCRHRRRQAERMSQIKRLLSEKKTCQCPH RFQKTCSPI | Fig. 1B |
| mCD4 | SP64 | MVQPMALIVLGGVAGLLLFIGLGIFFCVRC RHRRRQAERMSQIKRLLSEKKTCQCPHRF QKTCSPIKTNMKHMAGAAA | Fig. 4A, 4B, 4C, 4E, 6A, 6C, S4B, S4C, S4D, S4E, S4F, S5A, S5D, S6A, S7A, S7B, S7C |
| mCD4-M1 | SP64 | GpA TMD in place of CD4 TMD in the mCD4. | Fig. 4A, 4B, 4C, 4E, 6A, 6C, S4D, S4E, S5A, S7B |
| 3F4-mCD4 | pRSETA | MKTNMKHMAGAAASTPVQPMALIVLGGV AGLLLFI... | Fig. 2C, 2D, S2B, S2C, S3C, S3D |
| glyco-mCD4 | pRSETA | MNKTNMKHMAGAAASTPVQPMALIVLGG VAGLLLFI... | Fig. 2C, 2D |
| mCD4-4KR | pRSETA | Mutation of cytosolic Lys to Arg in 3F4-mCD4. | Fig. S3C |
| His-FLAG-mCD4 | pRSETA | MRGSHHHHHHGMASMTGGQQMGRDLYD DDDKDRWGSSTPVQPMALIVLGGVAGLLL FI... | Fig. 3C, S3B |
| rec-mCD4 | pRSETA | MSTPVQPMALIVLGGVAGLLLFIGLGIFFC VRCRHRRRQAERMSQIKRLLSEKKTCQCP HRFQKTCSPIDYKDDDDKGSENLYFQGAH HHHHHST | Fig. 3B, 3D |
| rec-CD4-cyto | pRSETA | MMRGSHHHHHHCVRCRHRRRQAERMSQI KRLLSEKKTCQCPHRFQKTCSPIDYKDDDD KGS | Fig. 3F |
| Coli-CD4 | pET-Duet (with Coil-Vpu) | M*GLEIEAAFLERENTALETRVAELRQRVQRLR NRVSQNRTRYGPL*GGGSCVRCRHRRRQAER MSQIKRLLSEKKTCQCPHRFQKTCSPIGDY KDDDDK | Fig. 3F |