S1 Table. SFV Env proteins produced for the study

| Name | Description ${ }^{\text {a }}$ | Expression <br> level |
| :--- | :--- | :--- | :--- | :--- | :--- |
| ${ }^{\text {Im }}$ |  |  |


| ${ }^{\text {GII }} \mathrm{DN7}$ | N346>A | Normal | Panel C | Fig. 3 |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| ${ }^{\text {GII }} \Delta N$ 7' | N373>A | Reduced | Panel C | Fig. 3 |  |
| ${ }^{\text {GII }} \mathrm{\Delta N} 9$ | N404>A | Reduced | Panel C | Fig. 3 |  |
| ${ }^{\text {GIII }} \mathrm{DN} 10$ | N411>A | Reduced | Panel C | Fig. 3 |  |
| ${ }^{\text {GII }}$-N9N10 | N404>A + N411>A | Insufficient | Panel C | - | Aggregates |
| ${ }^{\text {GII }}$ swap407 | 407RYNVNET413>KDIKSEI | Reduced | Panel D | Fig. 3 |  |
| ${ }^{\text {III }} \triangle$ RBDj | DF396-G480 | Normal | Panel E | Fig. 4 |  |
| ${ }^{\text {GII }}$ swapRBDj | GII-F396-G480>GI-F396-A482 | Reduced | Panel E | Fig. 7 |  |
| ${ }^{\text {GII }} \mathrm{LL} 2$ | -K278-Y293 | Reduced | Panel F | Fig. 4 |  |
| ${ }^{\text {GII }} \Delta \mathrm{L} 3$ | $\Delta \mathrm{V} 410-\mathrm{R} 433$ | Reduced | Panel F | Fig. 4 |  |
| ${ }^{\text {GII }} \mathrm{LL} 4$ | $\Delta \mathrm{E} 442$-P458 | Normal | Panel F | Fig. 4 |  |
| ${ }^{\text {GII }} 263{ }^{\text {glyc }}$ | D263N | Normal | Panel G | Fig. 4 |  |
| ${ }^{\text {GII }} 426{ }^{\text {glyc }}$ | H428> ${ }^{\text {T }}$ | Normal | Panel I | Fig. 4 |  |
| ${ }^{\text {GII }} 450{ }^{\text {glyc }}$ | D450>N | Normal | Panell | Fig. 4 |  |
| ${ }^{\text {GII }}$ A438-A443 | 438-REGKKE-443>AAGAAA | Insufficient |  |  |  |
| ${ }^{\text {GIII }} 459{ }^{\text {glyc }}$ | E459>N | Normal | Panel I | Fig. 4 |  |
| ${ }^{\text {III }} 485^{\text {glyc }}$ | E485>N | Normal | Panel I | Fig. |  |
| ${ }^{\text {GII }} 364{ }^{\text {glyc }}$ | K364>N + G366>T | Reduced | Panell |  | No nAb blocking and no cell binding |
| ${ }^{\text {GII }} 351{ }^{\text {glyc }}$ | L353T | Reduced | Panel H | Fig. 5, <br> Supplementary Fig. 5 | Aggregates; experiments repeated with SEC purified immunoadhesin |
| ${ }^{\text {GII }} 350{ }^{\text {glyc }}$ | G350>N + K352>S | Reduced | Panel I | Fig. 5 | Aggregates, moderate |
| ${ }^{\text {GIII }} 349_{+\mathrm{E}}$ | E inserted afterT348 | Reduced | Panel I | Fig. 5 |  |
| ${ }^{\text {GII }}$ swap333 | GII-L333-S345>CI-EQNERFLLNKLN | Reduced | Panel D | Fig. 5, Supplementary Fig. 6 |  |
| ${ }^{\text {GII }}$ swap345 | GII-S345-N351>GI-NNLTELTS | Normal | Panel D | Fig. 5, Supplementary <br> Fig. 6 |  |
| ${ }^{\text {GII }} \mathrm{\Delta T348-L353}$ | $\Delta \mathrm{T} 348$-L353 + GG | Undetectable |  |  |  |
| ${ }^{\text {GII }}$ swap349 | GII-I349-N355>Cl-SGTSVLKK | Insufficient |  |  |  |
| ${ }^{\text {GIIIE502A }}$ | E502>A | Reduced | Panel D | Fig. 5 |  |


| ${ }^{\text {GIILL505N}}$ | L505>N | Reduced | Panel D | Fig. 5 |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Tagged proteins | Proteins fused to a Strep-tag |  |  |  |  |
| ${ }^{\text {MLV }} \mathrm{SU}$ | MLV SU, strain FB29 | Not applicable | A | Fig. 2 | Three bands are visible in non-reduced conditions that correspond to oligomers formed by the free cysteine thiol group [52]. |
| GII-K74 SU(m) | WT SU produced in mammalian cells | Not applicable | nd | Supplementary Fig. 2 | Purified by size exclusion chromatography [14] |
| GII-K74 SU (i) | WT SU produced in insect cells | Not applicable | nd | Supplementary Fig. 2 | Purified by size exclusion chromatography [14] |
| GII-K74 Ecto(i) | Env 91-907, produced in insect cells | Not applicable | nd | Supplementary <br> Fig. 2 | Purified by size exclusion chromatography [14] |
| ${ }^{\text {GIIK}}$ K342A/R343A | K342>A+R343>A into GII-Ecto(i) | Normal | nd | Fig. 5 | Purified by size exclusion chromatography [14] |
| ${ }^{\text {GIIR }}$ R356A/R369A | R356>A+R369>A into GII-Ecto(i) | Normal | nd | Fig. 5 | Purified by size exclusion chromatography [14] |

${ }^{\text {a }}$ aa positions indicated are those of each protein; for GII-K74, some differ from the CI-PFV based numbering (Supplementary Fig. 1). The symbols $>$ and $\Delta$ designate aa substitutions and deletions, respectively.
${ }^{\mathrm{b}}$ The level of expression was assessed on crude supernatants of transfected cells and categorized as undetected, insufficient to perform the experiments, decreased, or normal relative to the WT counterpart.

