Figure S1. Wild-type BF520 infection of 293T cells engineered to express either macaque or human CD4/CCR5 receptors. TZM-bl titer of supernatant harvested from cells infected with viruses bearing the BF520 Env (MOI of 0.01). The cells that were infected are shown to the right. “Mix of 293T\textsubscript{hu} and 293T\textsubscript{rhm}” refers to a 3:1 mixture of these cells, respectively. Each data point reports the titer of one viral supernatant sample assayed in triplicate on TZM-bl cells.
Figure S2. Expression of CD4 and CCR5 on 293T<sub>rhm</sub> and 293T<sub>hu</sub> cells. Expression of CD4 (a) and CCR5 (b) on un-transduced 293Ts, 293T<sub>rhm</sub>, and 293T<sub>hu</sub> cells. For each density plot, receptor expression on 10,000 cells taken from one biological sample was measured. Data is representative of multiple independent experiments.
### Supplementary Table 1

#### Fold-change following infection

| Mutation  | Location | 293T<sub>rhm</sub> | 293T<sub>hu</sub> |
|-----------|----------|---------------------|-------------------|
| N656Y     | CHR      | 34.2                | 4.5               |
| N656Q     | CHR      | 32.8                | 4.7               |
| N655I     | CHR      | 31.6                | 8.8               |
| N656G     | CHR      | 27.8                | 1.2               |
| Q653L     | CHR      | 26.8                | 2.8               |
| N656R     | CHR      | 24.2                | 3.3               |
| Q653I     | CHR      | 23.0                | 5.0               |
| K588M     | gp41     | 20.8                | 1.8               |
| Q653N     | CHR      | 20.6                | 3.0               |
| Q653M     | CHR      | 19.8                | 2.3               |
| N554D     | NHR      | 17.3                | 4.4               |
| N656E     | CHR      | 17.2                | 3.4               |
| Q652F     | CHR      | 15.6                | 3.0               |
| Q653E     | CHR      | 15.6                | 1.8               |
| N656H     | CHR      | 15.5                | 3.7               |
| S649F     | CHR      | 15.0                | 6.1               |
| K588F     | gp41     | 14.0                | 1.9               |
| K655F     | CHR      | 13.9                | 2.0               |
| Q653F     | CHR      | 13.5                | 1.1               |
| N656S     | CHR      | 11.4                | 3.1               |
| Q652D     | CHR      | 11.2                | 2.2               |
| Q653Y     | CHR      | 11.1                | 1.0               |
| N656D     | CHR      | 10.8                | 6.3               |
| K655W     | CHR      | 10.7                | 1.6               |
| N656M     | CHR      | 10.5                | 5.2               |
| K655Y     | CHR      | 10.1                | 1.9               |
| R273K     | gp120    | 4.0                 | 10.6              |
| E482K     | gp120    | 7.9                 | 11.7              |

**Table S1.** Fold-change relative to the wild-type residue for all mutants that were enriched greater than 10-fold following either 293T<sub>rhm</sub> or 293T<sub>hu</sub> infection in the DMS.