**S1 Table. Sequences containing known cis-acting elements left undisturbed in synonymously mutated viruses**

|  |  |  |
| --- | --- | --- |
| **Site name** | **Position1** | **Reference** |
| **Splice sites2** |  | Reviewed in[21, 23] |
| D1 | 744 |  |
| A1 | 4911 |  |
| D2 | 4963 |  |
| D3 | 5464 |  |
| A2 | 5388 |  |
| A3 | 5775 |  |
| A4c | 5935 |  |
| A4a | 5952 |  |
| A4b | 5958 |  |
| A5 | 5974 |  |
| D4 | 6045 |  |
| A7 | 8373 |  |
| **Splicing regulators** |  |  |
| ESS2b3 | 4988-5007 | [32] |
| ESSV | 5422-5436 | [45] |
| ESS2 | 5846-5855 | [46] |
| ESS3 | 8450-8465 | [47] |
| ESS2P | 5779-5785 | [25] |
| ESE2b3 | 5008-5032 | [32] |
| ESE vpr3 | 5437-5460 | [38] |
| ESE tat3 | 5807-5837 | [48] |
| (GAA)3 | 8418-8428 | [49] |
| ESE2 | 5838-5845 | [50] |
| ESEM1 | 4933-4939 | [28] |
| ESEM2 | 4956-4962 | [28] |
| ESE vif | 4918-4927 | [29] |
| ISS | 8331-8353 | [51] |
| SD2 G4 | 4968-4971 | [29] |
| **Other3** |  | Reviewed in [2, 21] |
| TAR, Poly A, Psi. | 1-811 |  |
| GagPol frameshift | 2085-2136 |  |
| RRE | 7705-8059 |  |
| 3’PPT | 9553-9586 |  |
| cPPT | 4781-4799 |  |
| CTS | 4883-4898 |  |

1 Coordinates are given for positions in the HIV-1NHG reporter virus (S3 Data, S4 Data) and reflect sequences that were not changed in the mutant proviruses rather than precisely demarcating the locations of the named elements.

2Splice donors were maintained in the synonymously mutated viruses by leaving the 3 and 6 nucleotides positioned 5’ and 3’ to the splice site intact. Splice acceptors were maintained by leaving the PPT, branch point as well as 3 nucleotides and 1 nucleotide positioned 5’ and 3’ to the splice site intact. Cnonical splice sites are indicated. Cryptic splice sites, used at extremely low frequency (D1a, A1a and D2b) [37, 52] were not considered in the mutagenesis strategy.

3Splicing regulatory signals not yet reported at the time of mutant design were altered by synonymous mutagenesis