**I36T↑T Mutation in South African Subtype C (C-SA) HIV-1 Protease Significantly Alters Protease-Drug Interactions**

Sibusiso B. Maseko, Eden Padayachee, Thavendran Govender, Yasien Sayed, Gert Kruger, Glenn E.M. Maguire and Johnson Lin

**Supplementary material**



**Figure S1** A ribbon presentation of Wild type C-SA HIV protease (A) and I36T↑T mutant (B).

The Figure shows the positions of the tryptophan within the HIV-1 C-SA protease. Shown in yellow are the aspartic residues (Asp 25.25’). Insertion mutations are shown in pink. The red spheres are amino acid mutations. Trytophan 3 residues are shown in blue. Figure was created using UCSF Chimera version 1.



**Figure S2** Inhibition (*K*i) of the protease activities of wild type (A) and I36T↑T (B) by APV.

The reaction mix contained an increasing substrate concentration (0-250 μM); protease enzyme (50 nM), protease inhibitor (0-10 nM) in a 96-well plate with a total volume of 100 µl. (n=3).

**Table S1** Stern Volmer quenching constants (*K*sv) at different temperatures for both wild type (WT) and mutant (36T↑T).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Temp. | 293 K | 298 K | 303 K | 310 K |  | 293 K | 298 K | 303 K | 310 K |
| APV | Ksv (nM­-1) | ATV | Ksv (nM-1) |
| WT | 0.0436±0.0042 | 0.0867±0.002 | 0.08724±0.0091 | 0.0885±0.0426 | WT | 0.1040±0.0066 | 0.1140±0.0021 | 0.1420±0.0037 | 0.1720±0.0311 |
| I36T↑T | 0.0217±0.0050 | 0.0805±0.0018 | 0.08514±0.0055 | 0.0861±0.0199 | I36T↑T | 0.0454±0.0074 | 0.0704±0.0227 | 0.1400±0.0354 | 0.1820±0.0021 |
|  |
| DRV | 293 K | 298 K | 303 K | 310 K | IDV | 293 K | 298 K | 303 K | 310 K |
| WT | 0.0287±0.0075 | 0.0822±0.0094 | 0.0813±0.0028 | 0.1490±0.0060 | WT | 0.0067±0.0237 | 00.0949±0.087 | 0.0933±0.0154 | 0.1090±0.0220 |
| I36T↑T | 0.0484±0.0038 | 0.0508±0.0028 | 0.0648±0.0036 | 0.0683±0.0033 | I36T↑T | 0.0450±0.0107 | 0.0747±0.0021 | 0.0605±0.0166 | 0.0883±0.0164 |
|  |
| LPV | 293 K | 298 K | 303 K | 310 K | RTV | 293 K | 298 K | 303 K | 310 K |
| WT | 0.0384±0.0161 | 0.0572±0.0121 | 0.1490±0.0216 | 0.1700±0.0158 | WT | 0.0805±0.0086 | 0.0828±0.001 | 0.1072±0.0015 | 0.1730±0.0364 |
| I36T↑T | 0.0208±0.0282 | 0.0287±0.0086 | 0.1390±0.0272 | 0.1730±0.0147 | I36T↑T | 0.0356±0.0085 | 0.041±0.001 | 0.0795±0.0200 | 0.1030±0.0021 |
|  |
| TPV | 293 | 298 | 303 | 310 | SQV | 293 K | 298 K | 303 K | 310 K |
| WT | 0.0384±0.01 | 00.0484±0.01 | 0.1072±0.0015 | 0.1730±0.0364 | WT | 0.0408±0.0017 | 0.0686±0.0042 | 0.0669±0.0120 | 0.0819±0.0011 |
| I36T↑T | 0.0156±0.0085 | 0.0366±0.0004 | 0.0795±0.0200 | 0.1030±0.0021 | I36T↑T | 0.0106±0.0033 | 00.0217±0.005 | 0.0711±0.0033 | 0.0933±0.0018 |
| NFV | 293 | 298 | 303 | 310 | NFV | 293K | 298K | 303K | 310K |
|  I36T↑T | 0.0484±0.0038 | 0.0561±0.002 | 0.0648±0.0036 | 0.0683±0.0033 | WT | 0.0450±0.0107 | 0.0759±0.002 | 0.0605±0.0166 | 0.0883±0.0164 |