# Supplemental Figure 1

| | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 99 |
|---|---|---|---|---|---|---|---|---|---|---|
| $HIV_{NL4-3}$ | PQITLWQRPL | VTIKIGGQLK | EALLDTGADD | TVLEEMNLPG | RWKPKMIGGI | GGFIKVRQYD | QILIEICGHK | AIGTVLVGPT | PVNIIGRNLL | TQIGCTLNF |
| $HIV_{SQV-5\mu M}$ | .........I | .......... | .......... | ......D... | .......V.. | ...V...... | ..P....... | ..C....... | ...V.....M | ......... |
| $HIV_{APV-5\mu M}$ | .........F | .......... | .......... | .......... | .....I...V | .......... | .......... | .......... | ....V..... | ......... |
| $HIV_{LPV-5\mu M}$ | .........F | .......... | .......... | .I........ | .....IA... | .......... | .......... | V......... | ...V...... | ......... |
| $HIV_{IDV-5\mu M}$ | .........F | .......... | ...I...... | .......... | .....I.... | ...V...... | ..P....... | V.S....... | .T........ | ......... |
| $HIV_{NFV-5\mu M}$ | .........F | .........T | .........N | .......... | .....I.... | .......... | .......... | V..S...... | .......... | ......... |
| $HIV_{ATV-5\mu M}$ | .......... | .......... | ..I....... | ...Q...... | ..I..I...L | A......... | ..P....... | V......... | .T........ | ......... |
| $HIV_{TPV-15\mu M}$ | .........I | .......... | .......... | ..I..I.... | .....I.... | ...VR..... | .VP....... | V.S....... | .T.......M | ..L...... |
| $HIV_{DRV^{R}P20}$ | .........I | ....V....R | ...I...... | .I...I.... | .....L.... | .......... | ..P....... | .......... | .A......M. | ......... |
| $HIV_{DRV^{R}P30}$ | .........I | ....V....R | ...I...... | .I...I.... | .....L.... | .......... | ..P......Q | .......... | .A.V....M. | ......... |
| $HIV_{DRV^{R}P51}$ | .........I | ....V....R | ...I...... | .IF..I.... | .....L.... | ...M...... | ..P......Q | .......... | .I.V....M. | ......... |
| $HIV_{F16}$ | .........F | I.V.....QM | .......... | .IV.AI.... | .....IV... | ...M..K... | .VPV...... | ..TA...... | ...V....VM | ......... |
| $HIV_{F39}$ | .........V | I.VRV....T | .......... | .IF..I.... | K....L.... | ...L..K..E | ..P....E.T | IMS....... | ...V....VM | ..L...... |
| $HIV_{V42}$ | .........V | .VV.V....M | .......... | .IF....... | ..T..IV... | ...M.....E | NVP...Y.K. | ILS...I... | .A......VM | ......... |
| $HIV_{T45}$ | .........F | IPV.V...PT | .......... | .IF.GI.... | .......... | ...V...... | .VP......T | I.S.....A. | ...V....VM | ..L...... |
| $HIV_{T48}$ | .........I | ..V.V...V. | ...I...... | .IF....... | ..E..L.... | ...L.....E | ..P....... | V.S....... | .A.V...... | ......... |
| $HIV_{F71}$ | .........I | IKV......V | .......... | .IF.GID... | .....LV... | ...M..KE.. | ..PV.V.... | V.S....... | ...V....MM | ......... |

**Figure S1. Amino acid sequence of the PI-resistant HIV-1 variants.** Amino acid substitutions identified in protease-encoding region of laboratory-selected FDA-approved HIV variants ($HIV_{SQV-5\mu M}$, $HIV_{APV-5\mu M}$, $HIV_{LPV-5\mu M}$, $HIV_{IDV-5\mu M}$, $HIV_{NFV-5\mu M}$, $HIV_{ATV-5\mu M}$, and $HIV_{TPV-15\mu M}$, and $HIV_{DRV^{R}}$s: $HIV_{DRV^{R}P20}$, $HIV_{DRV^{R}P30}$, and $HIV_{DRV^{R}P51}$), and recombinant clinical HIV isolates ($_{rCL}HIV_{F16}$, $_{rCL}HIV_{F39}$, $_{rCL}HIV_{V42}$, $_{rCL}HIV_{T45}$, $_{rCL}HIV_{T48}$, and $_{rCL}HIV_{F71}$) compared to the wild-type $HIV_{NL4-3}$ are illustrated. Identity with the sequence at individual amino acid positions is indicated by a dot.

## Supplemental Figure 2

**a.** $\mathrm{HIV}_{P30}^{22wkDRV}$

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CCT CAA ATC ACT CTT TGG CAA CGA CCC ATC GTC ACA ATA AAG GTA GGG GGG CAA CTA AGG GAA GCT CTA ATA GAT ACA GGA   81
Pro Gln Ile Thr Leu Trp Gln Arg Pro Ile Val Thr Ile Lys Val Gly Gly Gln Leu Arg Glu Ala Leu Ile Asp Thr Gly   27

GCA GAT GAT ACA ATA TTT GAA GAA ATA AAT TTG CCA GGA AGA TGG AAA CCA AAA TTG ATA GGG GGA ATT GGA GGT TTT ATG  162
Ala Asp Asp Thr Ile Phe Glu Glu Ile Asn Leu Pro Gly Arg Trp Lys Pro Lys Leu Ile Gly Gly Ile Gly Gly Phe Met   54

AAA GTA AGA CAG TAT GAT CAG ATA CCC ATA GAA ATC TGT GGG CAT CAA GCT ATA GGT ACA GTA TTA GTA GGA CCT ACA CCT  243
Lys Val Arg Gln Tyr Asp Gln Ile Pro Ile Glu Ile Cys Gly His Gln Ala Ile Gly Thr Val Leu Val Gly Pro Thr Pro   81

ATC AAC GTA ATT GGA AGA AAT ATG TTG ACT CAG ATT GGC TGC ACC TTA AAT TTC  297
Ile Asn Val Ile Gly Arg Asn Met Leu Thr Gln Ile Gly Cys Thr Leu Asn Phe   99
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**b.** $\mathrm{HIV}_{P30}^{45wk003-15}$

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CCT CAA ATC ACT CTT TGG CAA CGA CCC ATC GTC CCA ATA AAG GTA GGG GGG CAA CTA AGG GAA GCT CTA ATA GAT ACA GGA   81
Pro Gln Ile Thr Leu Trp Gln Arg Pro Ile Val Pro Ile Lys Val Gly Gly Gln Leu Arg Glu Ala Leu Ile Asp Thr Gly   27

GCA GAT GAT ACA ACA TTA AAA GAA ATA AAT TTG CCA GGA AGA TGG AAA CCA AAA TTG ATA GGG GGA ATT GGA GGT TTT ATC  162
Ala Asp Asp Thr Thr Leu Lys Glu Ile Asn Leu Pro Gly Arg Trp Lys Pro Lys Leu Ile Gly Gly Ile Gly Gly Phe Ile   54

AAA GTA AGA CAG TAT GAT CAG ATA CCC ATA GAA ATC TGT GGG CAT CAA GCT ATA GGT ACA GTA TTA GTA GGA CCT ACA CCT  243
Lys Val Arg Gln Tyr Asp Gln Ile Pro Ile Glu Ile Cys Gly His Gln Ala Ile Gly Thr Val Leu Val Gly Pro Thr Pro   81

ATC AAC GTA ATT GGA AGA AAT ATG TTG ACT AAG ATT GGC TGC ACC TTA AAT TTC  297
Ile Asn Val Ile Gly Arg Asn Met Leu Thr Lys Ile Gly Cys Thr Leu Asn Phe   99
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**c.** $\mathrm{HIV}_{P30}^{45wk001-15}$

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CCT CAA ATC ACT CTT TGG CAA CGA CCC ATC GTC ACA ATA AAG GTA GGG GGG CAA CTA AGG GAA GCT CTA ATA GAT ACA GGA   81
Pro Gln Ile Thr Leu Trp Gln Arg Pro Ile Val Thr Ile Lys Val Gly Gly Gln Leu Arg Glu Ala Leu Ile Asp Thr Gly   27

GCA GAT GAT ACA GTA TTA GAA GAA ATA AAT TTG CCA GGA AGA TGG AAA CCA AAA TTG ATA GGG GGA ATT GGA GGT TTT ATC  162
Ala Asp Asp Thr Val Leu Glu Glu Ile Asn Leu Pro Gly Arg Trp Lys Pro Lys Leu Ile Gly Gly Ile Gly Gly Phe Ile   54

AAA GTA AGA CAG TAT GAT CAG ATA CCC ATA GAA ATC TGT GGG CAT CAA GTT ATA GGT ACA GTA TTA GTA GGA CCT ACA CCT  243
Lys Val Arg Gln Tyr Asp Gln Ile Pro Ile Glu Ile Cys Gly His Gln Val Ile Gly Thr Val Leu Val Gly Pro Thr Pro   81

GTC AAC GTA ATT GGA AGA AAT ATG TTG ACT CAG ATT GGC TGC ACC TTA AAT TTC  297
Val Asn Val Ile Gly Arg Asn Met Leu Thr Gln Ile Gly Cys Thr Leu Asn Phe   99
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**Figure S2. Nucleotide and amino acid sequences of HIV variants selected with DRV, GRL-003-15, and GRL-001-15.** Nucleotide and amino acid sequences of the protease-encoding region of HIV variants obtained from selection of $\mathrm{HIV}_{DRV}{}^{R}{}_{P30}$ in the presence of DRV (a), GRL-003-15 (b), or GRL-001-15 (c) are shown. Note that only the major clones obtained on weeks 22, 45, and 45 with DRV ($\mathrm{HIV}_{P30}^{22wkDRV}$), GRL-003-15 ($\mathrm{HIV}_{P30}^{45wk003-15}$), and GRL-001-15 ($\mathrm{HIV}_{P30}^{45wk001-15}$), respectively, are shown.

**Table S1** Partition and distribution coefficient of DRV, GRL-003-15, GRL-001-15, GRL-121-13, and GRL-142-13

| | Concentration (μM) | | | | | |
|---|---|---|---|---|---|---|
| Compounds | 1-Octanol | Water | 1-Octanol | TBS | Log $P_{octanol/partition}$[*] | Log $D_{octanol/TBS}$[*] |
| DRV | 0.8 | 9.4 | 0.6 | 9.3 | -1.08 | -1.48 |
| GRL-003-15 | 1.6 | 9.4 | 1.5 | 9.4 | -0.76 | -1.11 |
| GRL-001-15 | 1.5 | 8.6 | 1.1 | 8.4 | -0.76 | -1.19 |
| GRL-121-13 | 1.1 | 9.5 | 0.8 | 9.9 | -0.92 | -1.37 |
| GRL-142-13 | 1.9 | 9.0 | 1.7 | 8.8 | -0.67 | -1.01 |

[*] The partition (log *P*) and distribution (log *D*) coefficients of GRL-003-15, GRL-001-15, GRL-121-13, and GRL-142-13 were determined. DRV was used as a control. 1-Octanol, an organic alcohol, and water were used for log *P* determination, while tris buffered saline (TBS) (pH 7.4) and octanol were utilized for the log *D* assay. Prior to the retrieval of actual values, a standard curve was generated as a reference. The drug concentrations for each compartment (octanol, water, and TBS) were measured at an absorbance of 230 using a light spectrophotometer. Log *P* and log *D* values were calculated according to the formulas given in Materials and Methods.

**Table S2** Antiviral effects of GRL-003-15, GRL-001-15, and GRL-121-13 against selected PI-resistant HIV-1 variants and recombinant clinical HIV-1 isolates compared to that of DRV

| HIV strains | Fold change against DRV* | | | |
|---|---|---|---|---|
| | DRV | GRL-003-15 | GRL-001-15 | GRL-121-13 |
| $HIV_{NL4-3}$ | 1 | 0.016 | 0.020 | 0.10 |
| $HIV_{SQV\text{-}5\mu M}$ | 1 | 0.00011 | 0.013 | 0.00052 |
| $HIV_{APV\text{-}5\mu M}$ | 1 | 0.000021 | 0.0000070 | 0.00069 |
| $HIV_{LPV\text{-}5\mu M}$ | 1 | 0.00000043 | 0.000012 | 0.0000064 |
| $HIV_{IDV\text{-}5\mu M}$ | 1 | 0.000017 | 0.0000091 | 0.00025 |
| $HIV_{NFV\text{-}5\mu M}$ | 1 | 0.00017 | 0.000038 | 0.0047 |
| $HIV_{ATV\text{-}5\mu M}$ | 1 | 0.000035 | 0.000083 | 0.0019 |
| $HIV_{TPV\text{-}15\mu M}$ | 1 | 0.000017 | 0.0020 | 0.0030 |
| $HIV_{DRV^{R}P20}$ | 1 | 0.027 | 0.0046 | 0.053 |
| $HIV_{DRV^{R}P30}$ | 1 | 0.015 | 0.00079 | 0.11 |
| $HIV_{DRV^{R}P51}$ | 1 | 0.014 | 0.013 | 0.037 |
| $_{rCL}HIV_{F16}$ | 1 | 0.00000025 | 0.00000018 | 0.00080 |
| $_{rCL}HIV_{F39}$ | 1 | 0.011 | 0.011 | 0.013 |
| $_{rCL}HIV_{V42}$ | 1 | 0.011 | 0.012 | 0.043 |
| $_{rCL}HIV_{T45}$ | 1 | 0.019 | 0.019 | 0.017 |
| $_{rCL}HIV_{T48}$ | 1 | 0.000010 | 0.0011 | 0.0019 |
| $_{rCL}HIV_{F71}$ | 1 | 0.012 | 0.012 | 0.051 |

*The values indicate fold change of $EC_{50}$ values of each HIV variant against those of DRV.