Supplemental Material

Supplemental Materials and Methods

Assessment of T cell viability

Cryopreserved PBMCs isolated from HIV negative individuals were thawed and rested overnight as described above. The viability of T cells was repeatedly assessed by using trypan blue staining. Briefly, 10 µl of cell suspension was mixed with 10 µl of 0.4 % trypan blue and loaded onto a cell counting slide. Afterwards, the number of live cells was determined using an automatic cell counter EVE. To determine whether living cells undergo early apoptosis cells were stained repeatedly with Annexin V. Cells were washed with staining buffer (PBS supplemented with 2% FCS) and stained extracellularly for 20 min with fluorescently conjugated antibody: anti-CD3-APC-Cy7 (clone UCHT1; Biolegend). Afterwards, cells were washed with Annexin V Binding Buffer (Biolegend) and stained with anti-AnnexinV-FITC (Biolegend) antibody for 15 min. Subsequently, 400 µl of Annexin V Binding Buffer were added to the cells. Data were collected at FACS Celesta (BD) and analyzed with FlowJo Software version 10.0.7 (TreeStar).

FCS concentration test

Cryopreserved PBMCs isolated from HIV negative individuals were thawed, rested overnight and on the next day CD8 T cells were isolated using CD8 isolation beads (Miltenyi). Cells were then stimulated with anti-CD3/CD28 beads (Gibco, Life Technologies) at a cell-to-bead ratio of 1:1 in the presence of IL-2 (30 U/ml) (eBioscience) and DMSO or DTG for 3 days. Cells were cultured in R10 media supplemented with 10%, 30% or 50% FCS in humidified atmosphere (5% CO_2) at 37 °C. Afterwards, the metabolic assay was performed as described above.

Supplemental Figures

Fig. S1

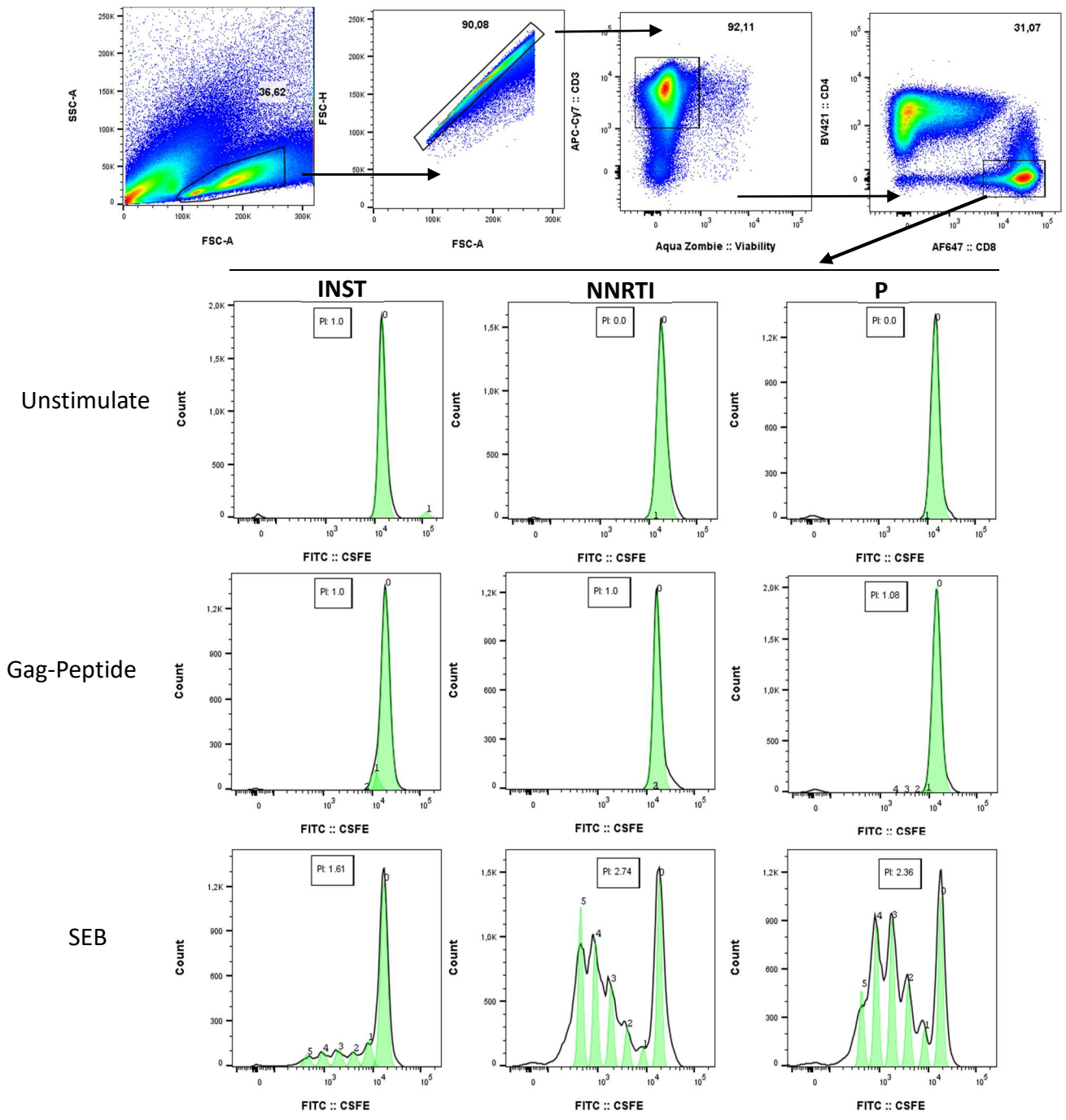

Figure S1. Representative gating strategy for the assessment of CD8 T cell proliferation from HIV-positive individuals treated with different ART regimen *ex vivo*. Based on size and granularity, lymphocytes were discriminated using forward and side scatter. Subsequently, lymphocytes were further divided into living CD3 and CD8 T cells. The Proliferation Index was calculated through the FlowJo's Proliferation platform (Version 10) and division was characterized by the reduction in the signal intensity of CSFE.

Fig. S2

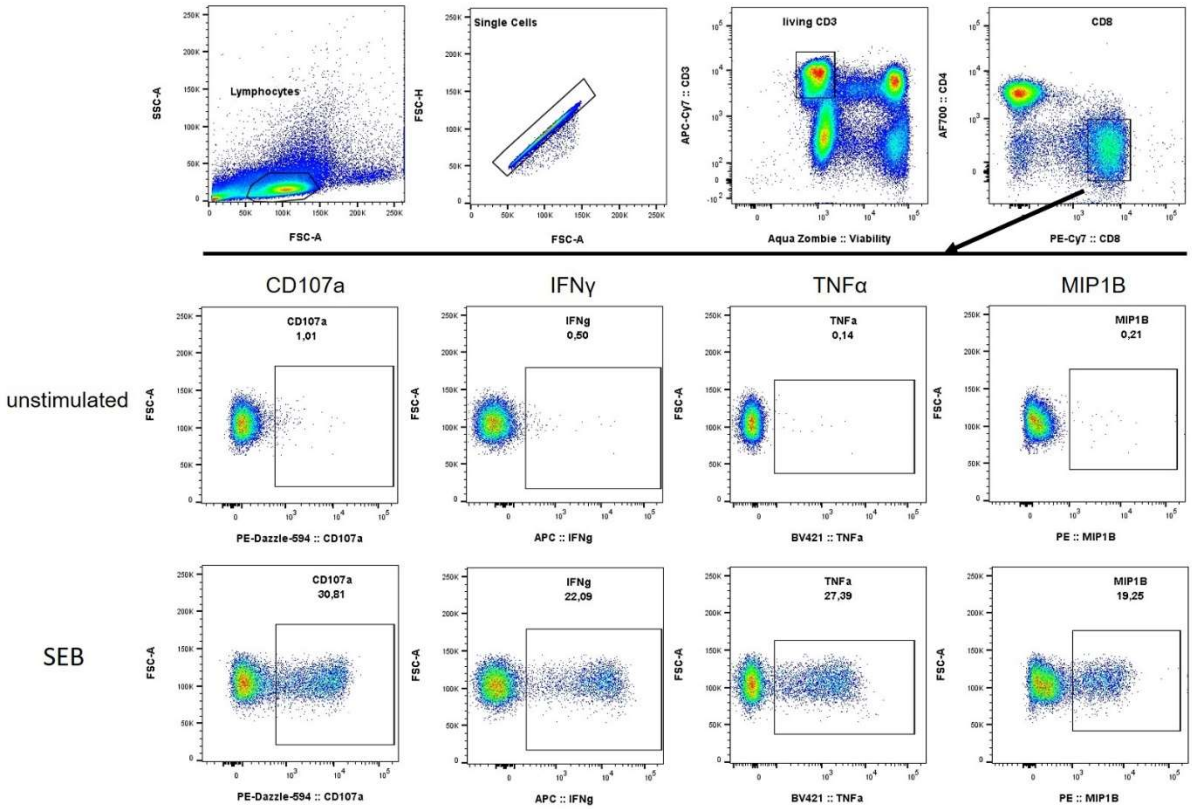

Figure S2. Representative gating strategy for the assessment of CD8 T cell polyfunction from HIV-positive individuals treated with different ART regimen *ex vivo* (here shown for an individual who received an NNRTI-containing ART regimen*).* Based on size and granularity, lymphocytes were discriminated using forward and side scatter. Subsequently, lymphocytes were further divided into living CD3 and CD8 T cells and gates for the individual cytokines (CD107a, IFNγ, TNFα and MIP1β) were chosen on the unstimulated control for every sample separately. The frequency of responding cells was calculated by subtracting negative control from the stimulated sample. Afterwards, Boolean gating allowed to analyze polyfunctionality via SPICE Data Analysis.

Fig. S3

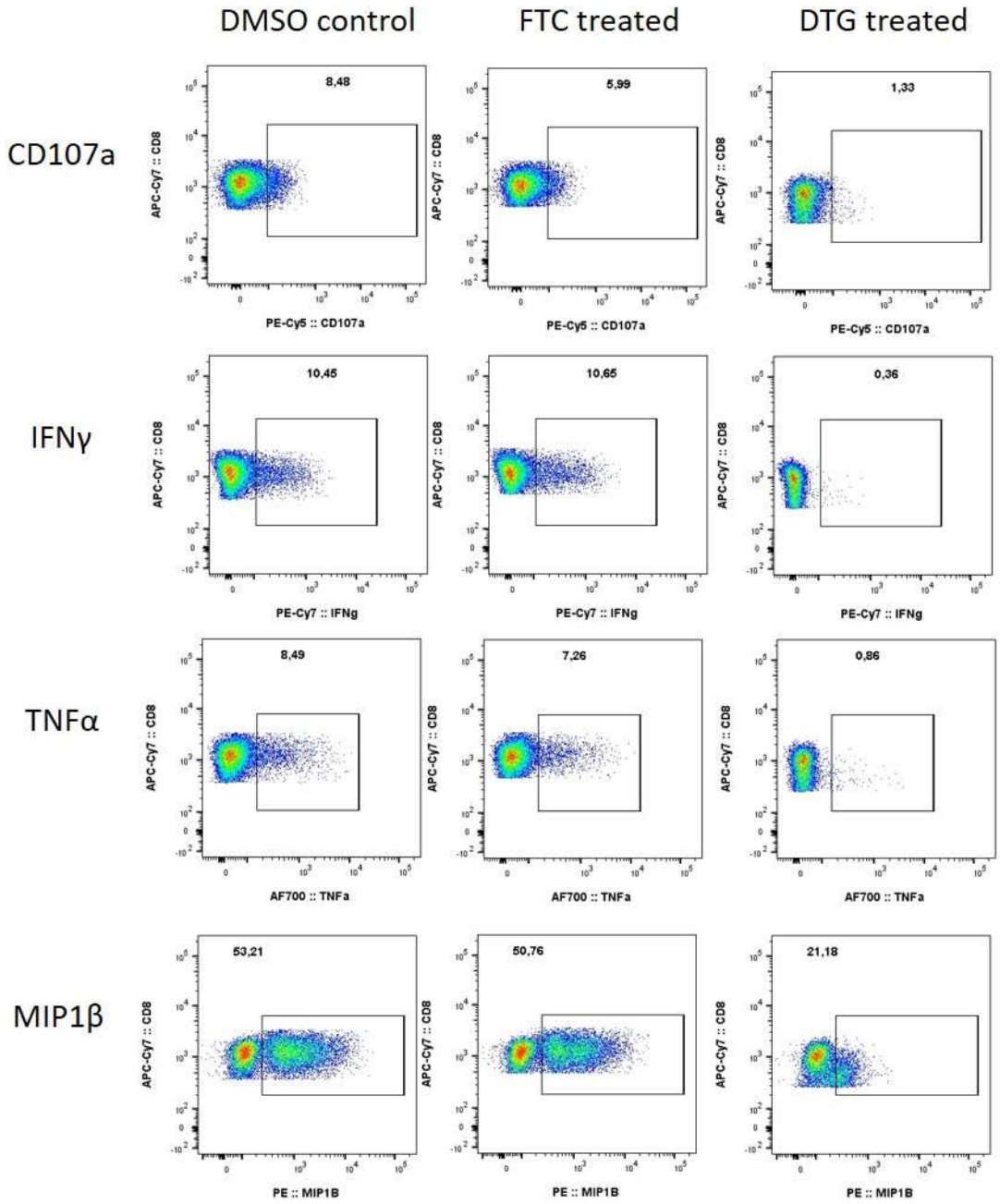

Figure S3. Representative gating strategy for the assessment of the T cell cytokine expression profile. Cryopreserved PBMCs isolated from HIV negative individuals and treated with different ART regiments (here shown: DMSO control, emtricitabine and dolutegravir) at described bio-active plasma concentrations for 3 days. Afterwards, cells were stimulated with 5 µg/ml SEB for 6h and stained with fluorochrome-conjugated antibodies. Based on size and granularity, lymphocytes were discriminated using forward and side scatter. Subsequently, lymphocytes were further divided into living CD3 and CD8 T cells and gates for the individual cytokines (CD107a, IFNγ, TNFα and MIP1β) were chosen on the unstimulated control for every HIV-negative donor separately. The frequency of responding cells was calculated by subtracting negative control from the stimulated sample.

Fig. S4

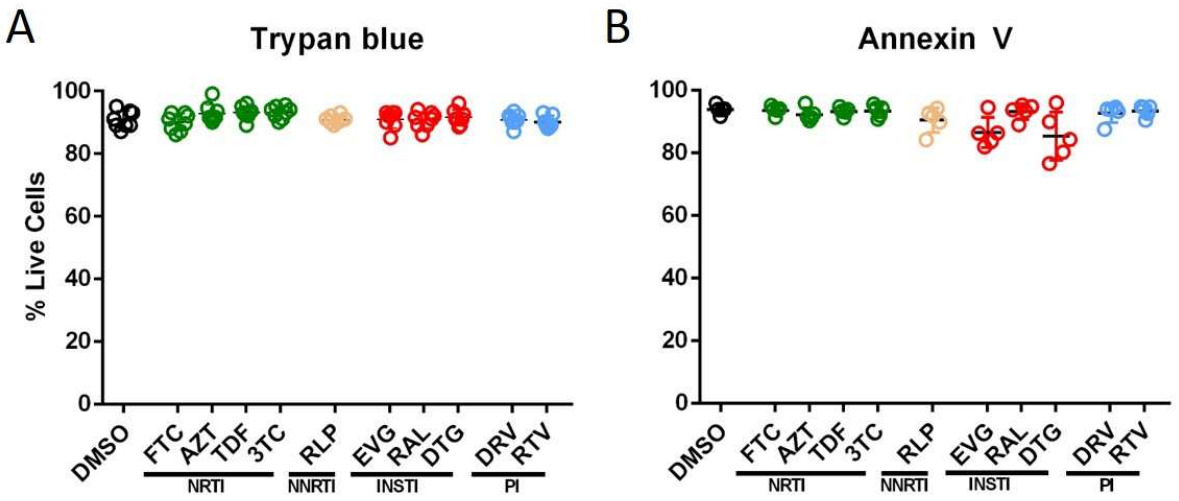

Figure S4. Assessment of cell viability in the presence of different ART regimens for three days. (A) Viability assessed of PBMCs by Trypan blue (n=8) and **(B)** by Annexin V (n=5). Plots show individual values with the mean ± SD.

Fig S5

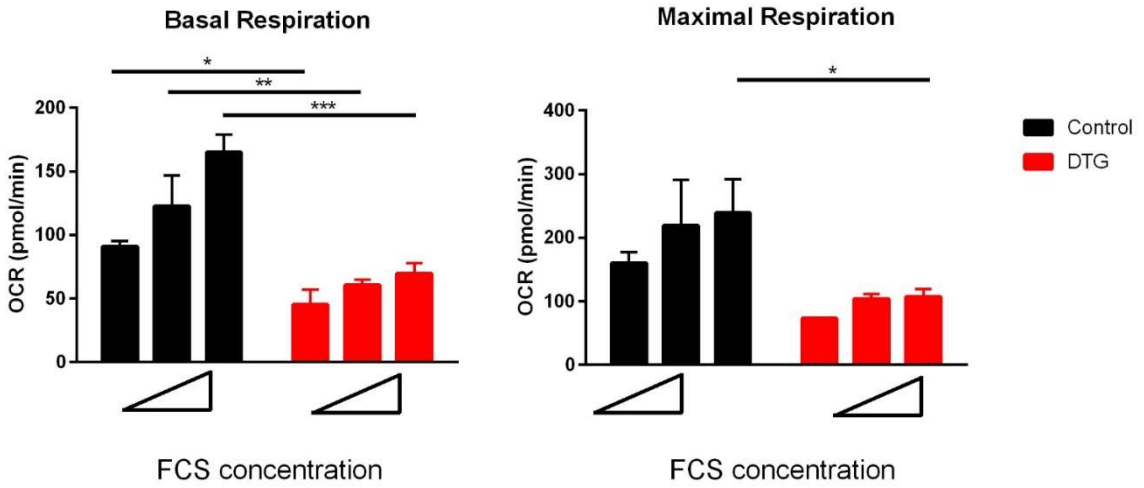

Figure S5. Impact of increased FCS concentration on respiration. Increasing FCS concentration (triangles) 10%, 30% and 50% was corresponding to increased basal and maximal respiration in CD8 T cells from HIV positive and treated donor (n=1), which were treated with 1-fold plasma concentration of DTG. Experiment was carried-out in replicates. Charts show the mean value ± SEM. Statistical significance was assessed by RM one-way ANOVA test with Holm-Sidak´s multiple comparison test. (*P<0.05; **P<0.01; ***P<0.01).

Supplemental Tables

Table S1

A

Individuals Ex-Vivo **Proliferation**

Demographics		Overall	INSTI	NNRTI	PI	P-value
Age						0.1469
	Mean ± std	47.4 ± 12.5	47.1 ± 11.3	42.8 ± 12.4	53.0 ± 13.5	
Gender						
	Male	42	18	13	11	
	Female	4	3	0	1	
CD4 count*						0.1178
	Median	564.1 ± 146.2	604.5 ± 145.8	512.5 ± 119.7	549.3 ± 163.2	
CD8 count*						0.6071
	Median	645.7 ± 210.1	615.9 ± 187.6	663.0 ± 251.7	679.2 ± 210.2	
Days on treatment						0.007
	Mean ± std	709.7 ± 696.5	392.9 ± 147. 9	617.6 ± 361.4	1363.8 ± 1065.0	

*at the time the sample was taken

INSTI		n
DTG	ABC+3TC	11
EVG	FTC+TAF	10
	total:	21
NNRTI		
RLP	FTC+TAF	1
RLP	FTC+TDF	12
	total:	13
PI		
RTV+DRV	FTC+TDF	9
RTV+ATV	FTC+TDF	2
RTV+ATV	FTC+TAF	1
	total:	12

B

Individuals Ex-Vivo **Polyfunctional**

Demographics		Overall	INSTI	NNRTI	PI	P-value
Age						0.0554
	Mean ± std	46.1 ± 13.9	44.9 ± 13.1	41.2 ± 12.5	54.1 ± 13.7	
Gender						
	Male	34	11	13	10	
	Female	2	2	0	0	
CD4 count*						0.219
	Median	615.2 ± 209.0	708.5 ± 296.3	550.9 ± 106.7	577.5 ± 132.1	
CD8 count*						0.7676
	Median	691.3 ± 209.1	671.5 ± 249.1	727.2 ± 179.9	670.5 ± 203.2	
Days on treatment						0.0036
	Mean ± std	915.1 ± 690.9	588.7 ± 280.6	681.7 ± 393.1	1642.9 ± 851.0	

*at the time the sample was taken

INSTI		n
DTG	ABC+3TC	10
EVG	FTC+TAF	3
	total:	13
NNRTI		
RLP	FTC+TAF	3
RLP	FTC+TDF	10
	total:	13
PI		
RTV+DRV	FTC+TDF	9
LPV	FTC+TDF	1
	total:	10

Table S1. List of participants and their regiments in the *ex-vivo* assays. (A) Characteristics of individuals (n=46) used in the *ex vivo* proliferation assay and their individual ART regimens. All participants in this study had two NRTI as a backbone in combination with a third drug from INSTI, NNRTI or PI class listen in the first column. Plus-minus values are means ± SD. **(B)** Characteristics of individuals (n=36) used in the *ex vivo* polyfunctional assay and their individual ART regimens. All participants in this study had two NRTI as a backbone combined with a third drug from INSTI, NNRTI or PI class listen in first column. Plus-minus values are means ± SD. It should be mentioned that there are overlaps of participants (n=34) where PBMCs were used in both assays, but this was not the case for every participant due to limited access to PBMCs.

Table S2

Antiretroviral	Abbreviation	Plasma Concentration (ng/ml)		Chosen Concentration (ng/ml)	Source
		Median	Range		
Emtricitabine	FTC	717	21-1072	1000	Gish et al., 2002
Zidovudine	AZT	1150	710-1850	1500	Bergshoeff et al., 2004
Tenofovir DF	TDF	149	120-193	200	Avihingsanon et al., 2015
Tenofovir	TFV	NA*	NA*	200	
Lamivudine	3TC	2077	1264-2893	1500	Bruno et al., 2001
Rilpivirine	RLP	139	128-168	200	Dickinson et al., 2015
Raltegravir	RAL	448	37-5180	1000	Yilmaz et al., 2009
Dolutegravir	DTG	3908	3571-4245	4000	Elliot et al., 2016
Elvitegravir	EVG	1675	1557-1884	2000	Elliot et al., 2016
Darunavir ethanolate	DRV	3930	1800-12900	5000	Yilmaz et al., 2009
Ritonavir	RTV	746	646-1045	1000	Boffito et al., 2011

*variable plasma concentrations according to the prodrug

Table S2. List of tested ART drugs with previously determined plasma concentration and concentration chosen in the assays

Supplemental References

1. Gish RG, Leung NW, Wright TL, Trinh H, Lang W, Kessler HA, Fang L, Wang LH, Delehanty J, Rigney A, Mondou E, Snow A, Rousseau F. 2002. Dose range study of pharmacokinetics, safety, and preliminary antiviral activity of emtricitabine in adults with hepatitis B virus infection. Antimicrob Agents Chemother 46:1734-40.
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