# Supplementary material

Supplementary table S1: Table of intercorrelated lipoproteins and lipids and their cluster representatives

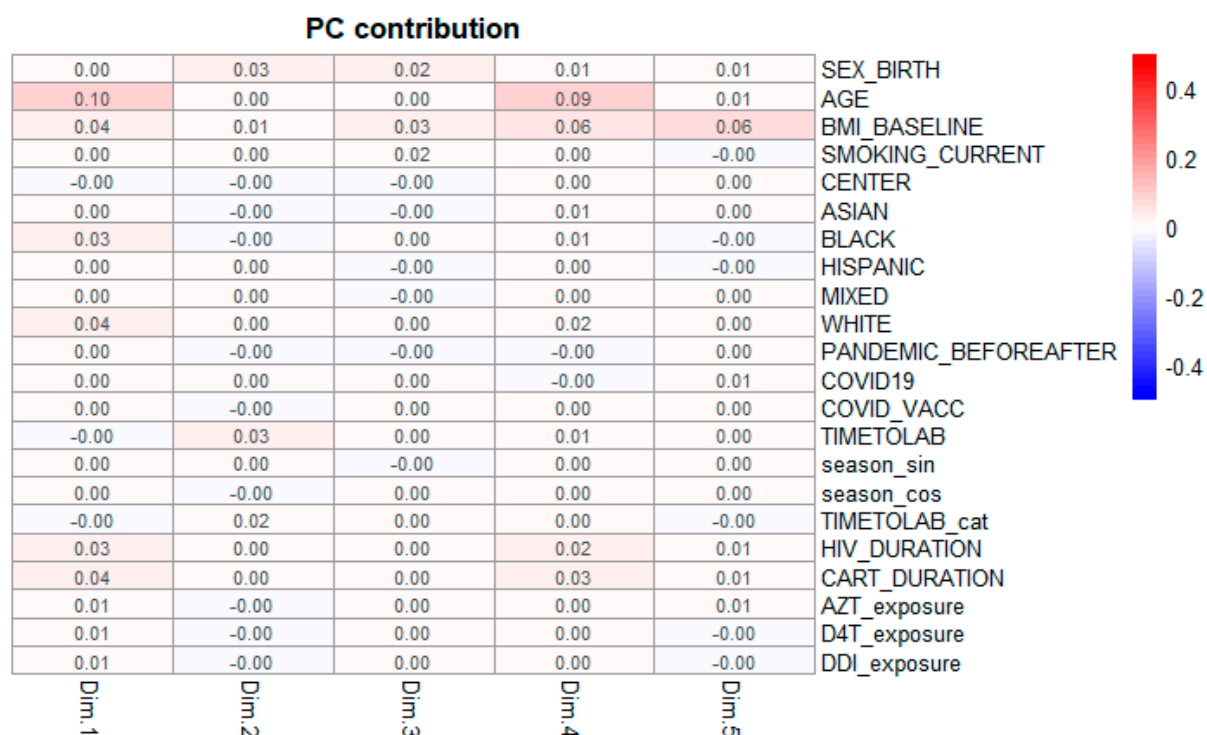| Cluster no	Cluster representative	Lipoproteins in cluster
1	M_LDL_C	ApoB, L_LDL_P, LDL_CE, LDL_L, LDL_P, LDL_PL, M_LDL_CE, M_LDL_L, M_LDL_P, M_LDL_PL, M_VLDL_C, M_VLDL_CE, non_HDL_C, S_LDL_C, S_LDL_CE, S_LDL_L, S_LDL_P, S_LDL_PL, M_LDL_C
2	XS_VLDL_C	IDL_P, Remnant_C, XS_VLDL_CE, XS_VLDL_FC, XS_VLDL_L, XS_VLDL_P, XS_VLDL_PL, XS_VLDL_C
3	L_VLDL_C	L_VLDL_CE, M_VLDL_FC, M_VLDL_L, M_VLDL_P, M_VLDL_PL, S_VLDL_C, S_VLDL_CE, S_VLDL_FC, S_VLDL_L, S_VLDL_P, S_VLDL_PL, VLDL_C, VLDL_CE, VLDL_FC, VLDL_L, VLDL_P, VLDL_PL, XL_VLDL_C, XL_VLDL_CE, L_VLDL_C
4	LDL_TG	HDL_TG, IDL_TG, L_LDL_TG, M_HDL_TG, M_LDL_TG, MUFA, SFA, Total_FA, XS_VLDL_TG, LDL_TG
5	Total_PL	LA, Omega_6, PUFA, Total_L, Total_PL
6	IDL_C	Clinical_LDL_C, IDL_CE, IDL_FC, IDL_L, IDL_PL, L_LDL_C, L_LDL_CE, L_LDL_FC, L_LDL_L, L_LDL_PL, LDL_FC, M_LDL_FC, Total_C, Total_CE, Total_FC, IDL_C
7	HDL_C	ApoA1, HDL_CE, HDL_FC, HDL_L, HDL_P, HDL_PL, M_HDL_C, M_HDL_CE, M_HDL_FC, M_HDL_L, M_HDL_P, Total_P, HDL_C
8	L_HDL_C	HDL_size, L_HDL_CE, L_HDL_FC, L_HDL_L, L_HDL_P, L_HDL_PL, L_HDL_C
9	VLDL_TG	L_VLDL_FC, L_VLDL_L, L_VLDL_P, L_VLDL_PL, L_VLDL_TG, M_VLDL_TG, S_HDL_TG, S_LDL_TG, S_VLDL_TG, Total_TG, VLDL_size, XL_VLDL_FC, XL_VLDL_L, XL_VLDL_P, XL_VLDL_PL, XL_VLDL_TG, XXL_VLDL_C, XXL_VLDL_CE, XXL_VLDL_FC, XXL_VLDL_L, XXL_VLDL_P, XXL_VLDL_PL, VLDL_TG
10	XL_HDL_C	XL_HDL_CE, XL_HDL_L, XL_HDL_P, XL_HDL_PL, XL_HDL_C
11	S_HDL_C	S_HDL_FC, S_HDL_L, S_HDL_P, S_HDL_PL, S_HDL_C
12	Omega_3	Omega_3

Supplementary figure S1: Principal component analysis of untargeted metabolomics. Two samples had were identified as outliers (SD>4) and removed from further analysis.
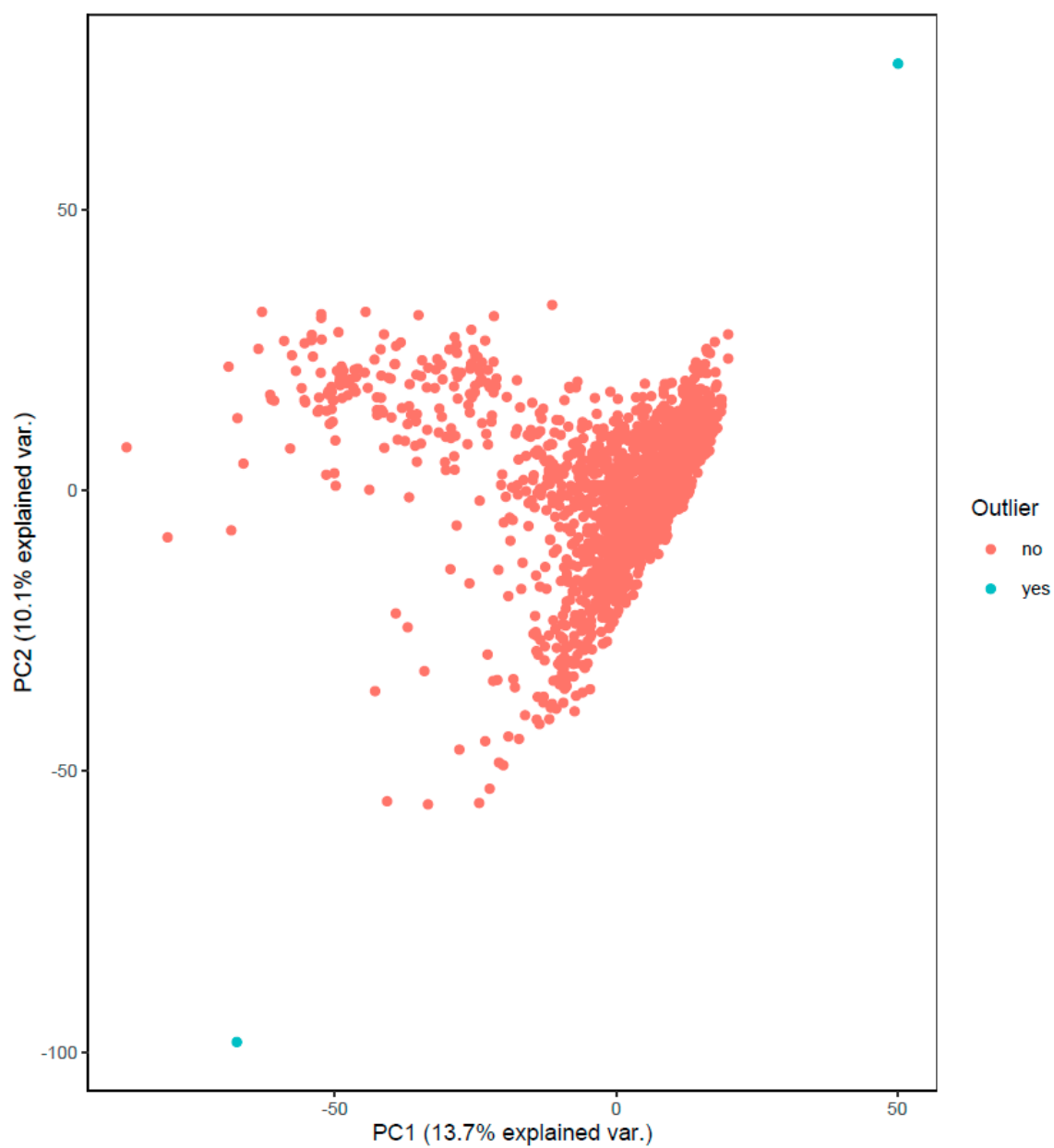
Supplementary figure S2: Contributions of the different principal components (PC) to explain differences in metabolite levels in the 2000HIV cohort. Only PC1 and PC2 had an influence of >5% on metabolite levels.
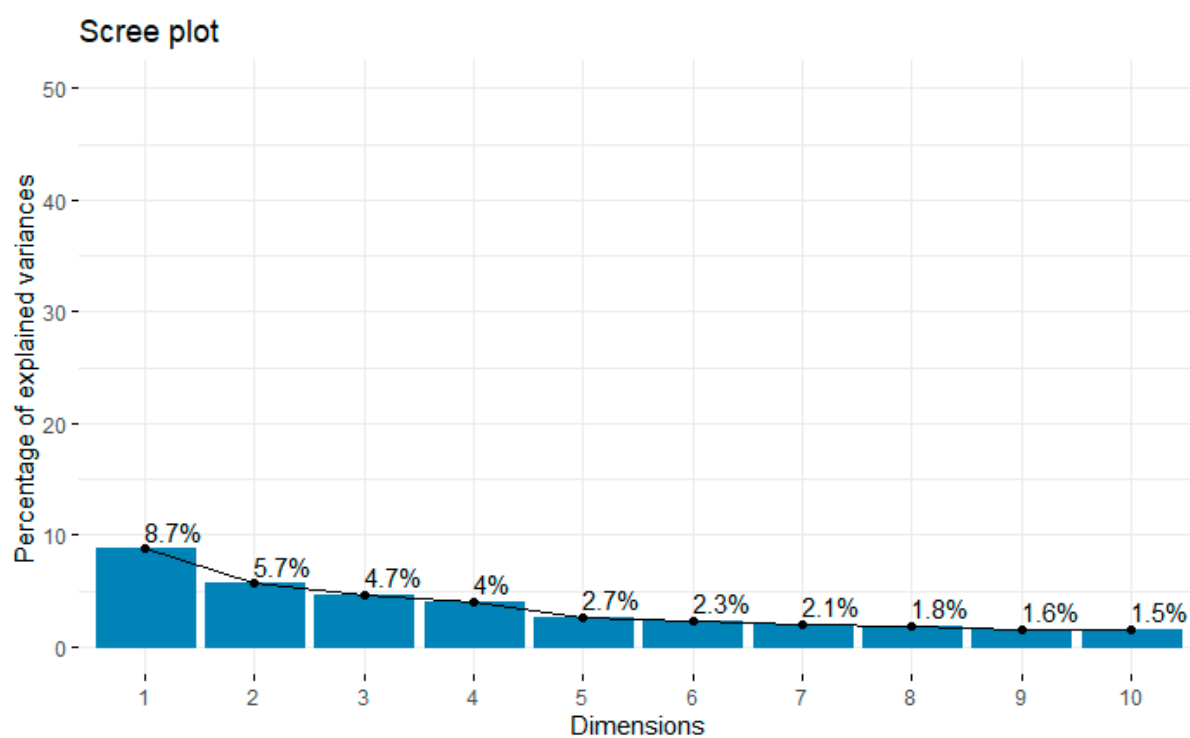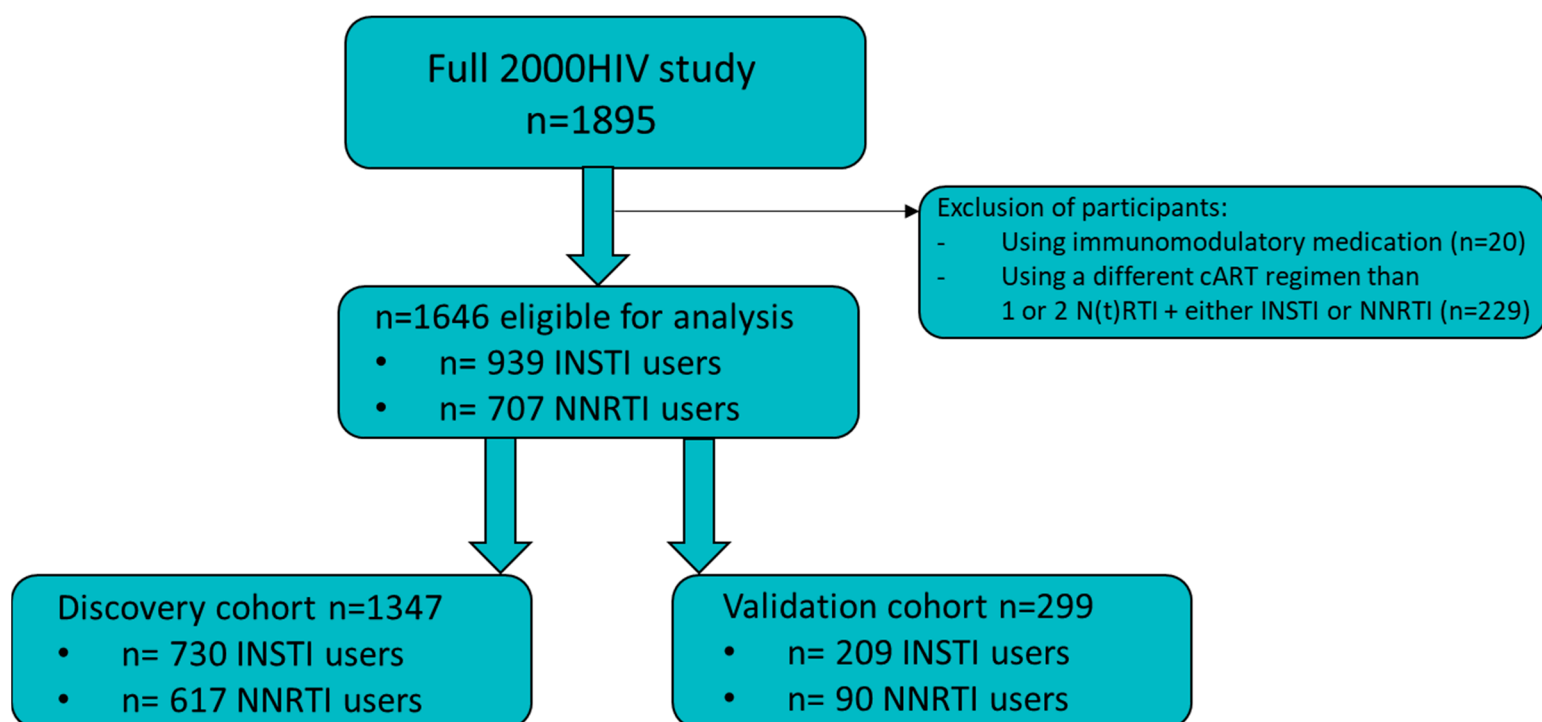
Supplementary figure S3: Principal component analysis of metabolite levels. Only age appears to have confounding influence on metabolite levels.

PC contribution

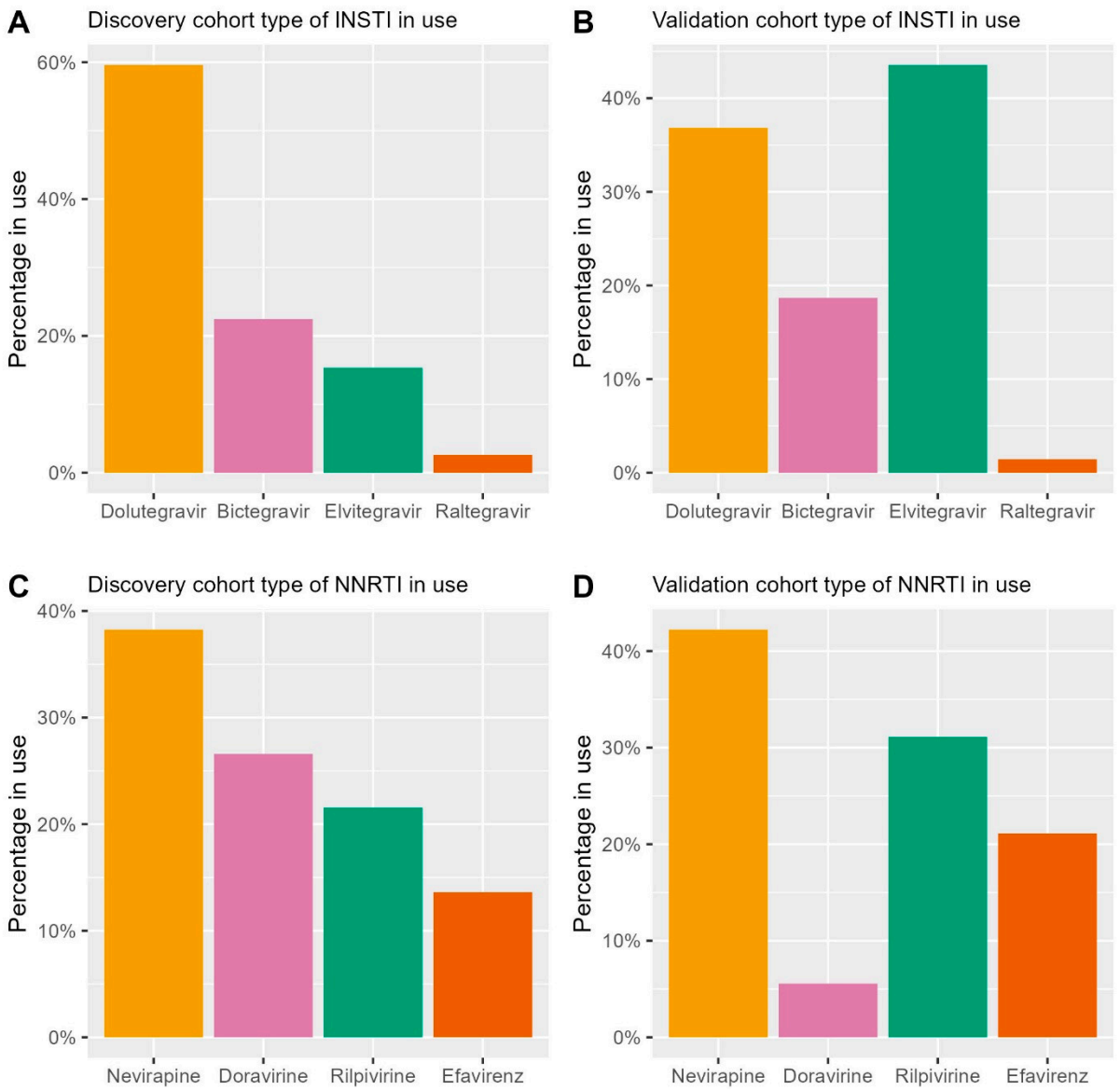| Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	
0.00	0.03	0.02	0.01	0.01	SEX_BIRTH
0.10	0.00	0.00	0.09	0.01	AGE
0.04	0.01	0.03	0.06	0.06	BMI_BASELINE
0.00	0.00	0.02	0.00	-0.00	SMOKING_CURRENT
-0.00	-0.00	-0.00	0.00	0.00	CENTER
0.00	-0.00	-0.00	0.01	0.00	ASIAN
0.03	-0.00	0.00	0.01	-0.00	BLACK
0.00	0.00	-0.00	0.00	-0.00	HISPANIC
0.00	0.00	-0.00	0.00	0.00	MIXED
0.04	0.00	0.00	0.02	0.00	WHITE
0.00	-0.00	-0.00	-0.00	0.00	PANDEMIC_BEFOREAFTER
0.00	0.00	0.00	-0.00	0.01	COVID19
0.00	-0.00	0.00	0.00	0.00	COVID_VACC
-0.00	0.03	0.00	0.01	0.00	TIMETOLAB
0.00	0.00	-0.00	0.00	0.00	season_sin
0.00	-0.00	0.00	0.00	0.00	season_cos
-0.00	0.02	0.00	0.00	-0.00	TIMETOLAB_cat
0.03	0.00	0.00	0.02	0.01	HIV_DURATION
0.04	0.00	0.00	0.03	0.01	CART_DURATION
0.01	-0.00	0.00	0.00	0.01	AZT_exposure
0.01	-0.00	0.00	0.00	-0.00	D4T_exposure
0.01	-0.00	0.00	0.00	-0.00	DDI_exposure

Color scale: 0.4 / 0.2 / 0 / -0.2 / -0.4

Explanation of variables: SEX_BIRTH: sex at birth; CENTER: site where participant participated in the study; MIXED: of mixed ethnicity; PANDEMIC_BEFOREAFTER: participant participated before or after the onset of the covid-19 pandemic in the Netherlands (march 2020); COVID19: did participant have COVID-19 before participation; COVID_VACC: was participant vaccinated for COVID-19 before participation; season_cos/season_sin: seasonality influence of metabolite levels; TIMETOLAB_cat: categorical varible of time between sample draw and sample processing (< vs > 24hours);

Supplementary figure S4: Participant selection for analysis from 2000HIV study. For the current analysis we considered all participants in the 2000HIV study and selected only participants not using immunomodulatory medication and those using one or two N(t)RTI's in combination with either one INSTI or one NNRTI. Previously, all participants were already divided into a discovery and validation cohort based on their site of participation.

A Discovery cohort type of INSTI in use

B Validation cohort type of INSTI in use

C Discovery cohort type of NNRTI in use

**D** Validation cohort type of NNRTI in use

Supplementary figure S6: Principal component (PC) analysis of untargeted plasma metabolites of the types of INSTI and NNRTI in the discovery cohort. X-axis shows PC1, Y-axis shows PC2.
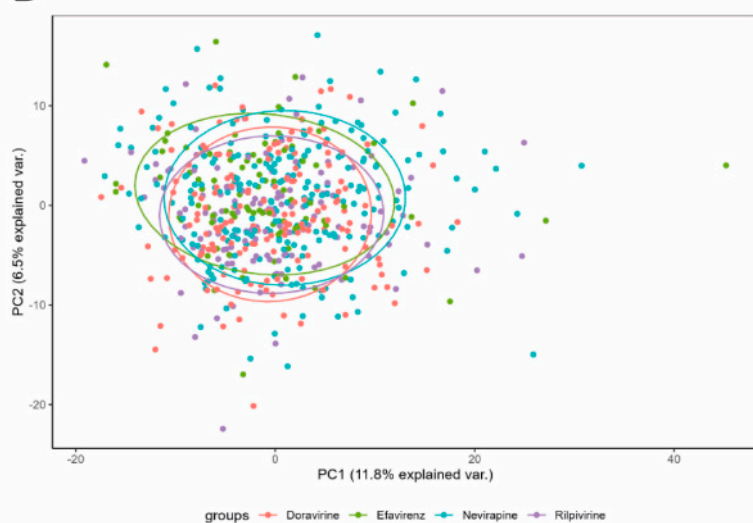
A: All types of INSTI showed overlap in PC analysis, showing homogeneity between untargeted plasma metabolomics between the different INSTI types.

B: All types of NNRTI showed overlap in PC analysis, showing homogeneity between untargeted plasma metabolomics between the different NNRTI types.
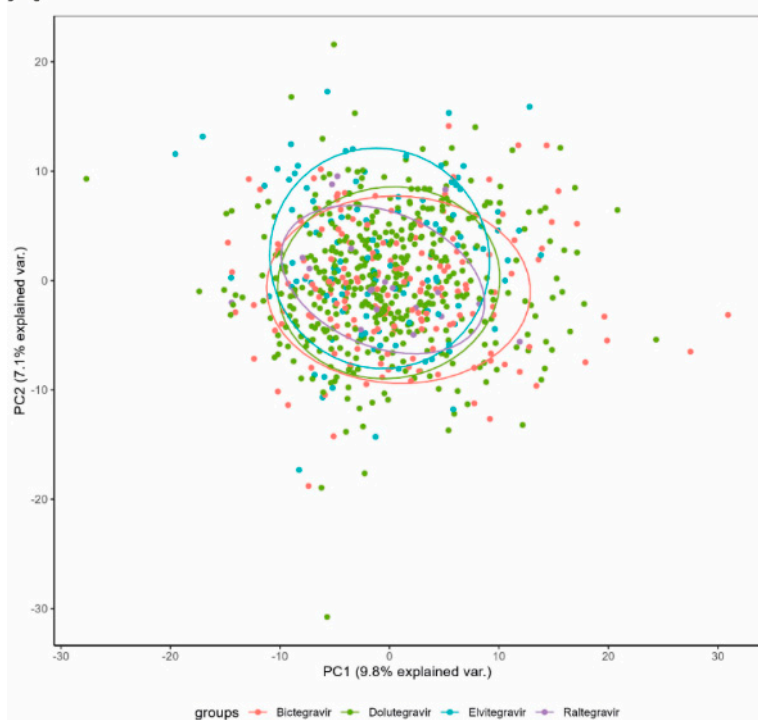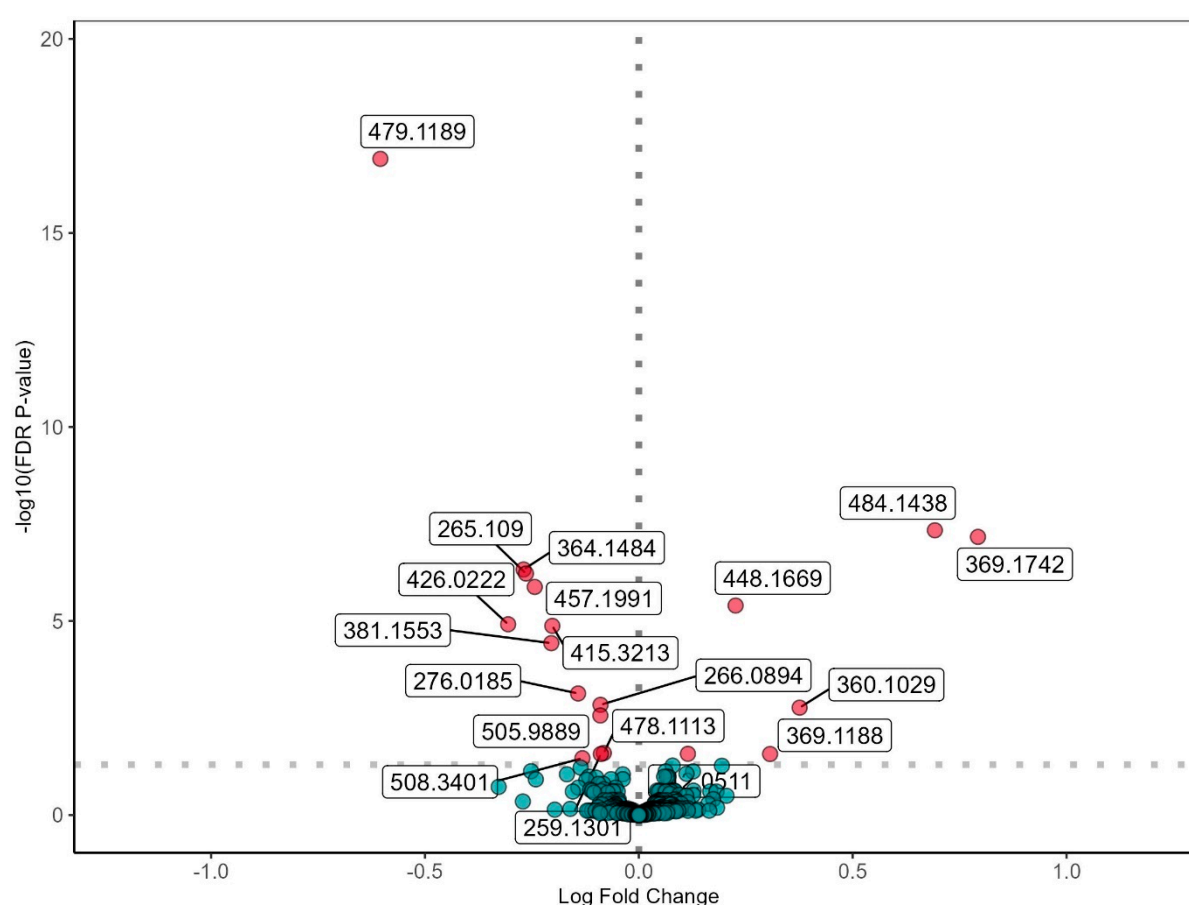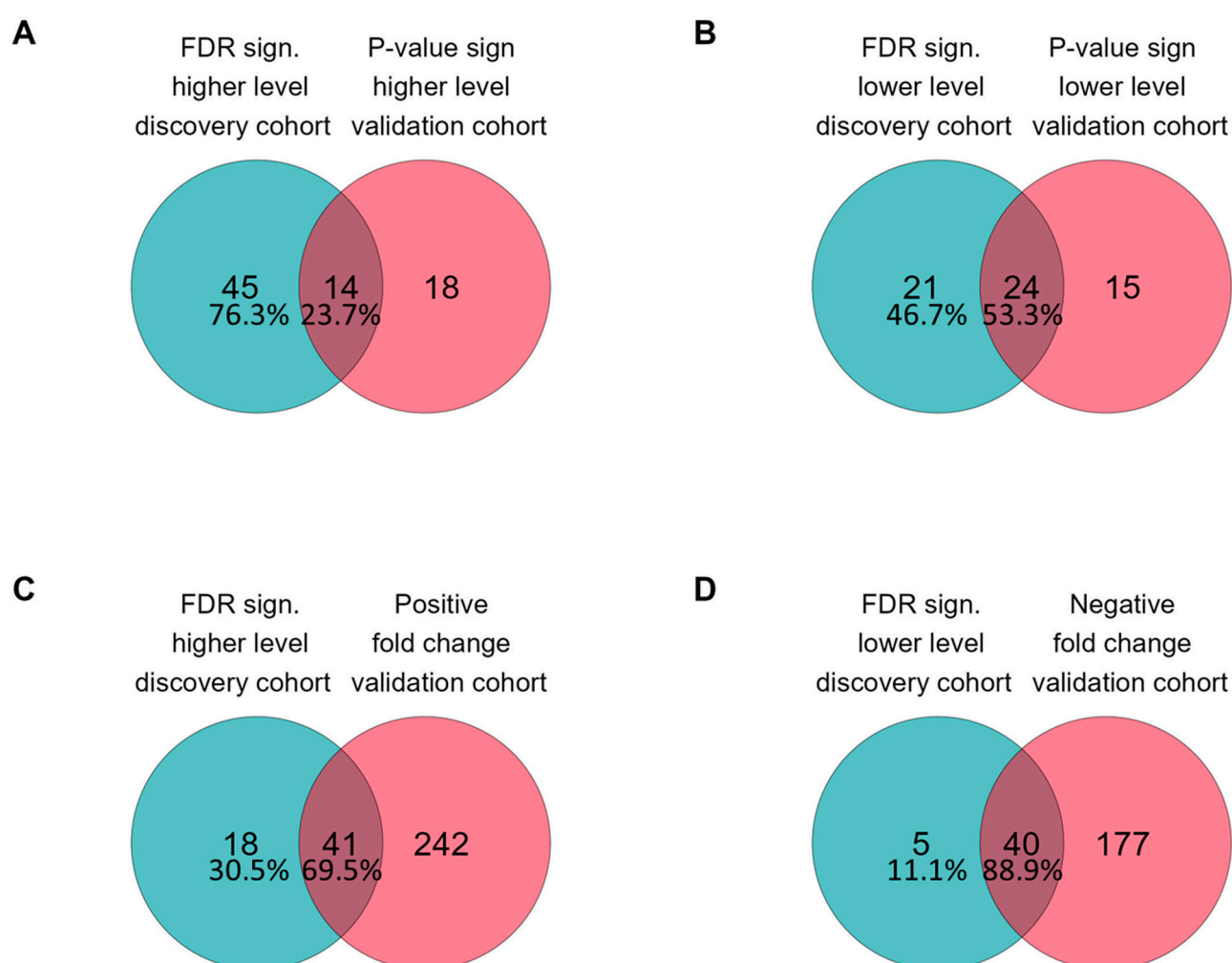
Supplementary figure S7: Differentially expressed untargeted metabolites in INSTI users compared to NNRTI users in the validation cohort (INSTI n = 207; NNRTI n = 89). Differential expression analysis using a linear model with sex at birth and age as covariates on 500 metabolites known to be present in serum. Y-axis shows the p-value through -log10(p-value), x-axis shows the log fold change. Horizontal dotted line represents border of significance (p-value<0.05), vertical dotted line represents border between higher levels (right) and lower levels (left) of metabolites in the INSTI group. Significantly differentially expressed metabolites are shown in red. Numbers specify the significant differentially expressed ion m/z ratio. In the validation cohort cohort, 32 metabolites were significantly higher and 39 metabolites were significantly lower in the INSTI group. In addition, 283 metabolites had a positive log fold change and 217 metabolites had a negative log fold change.

Supplementary table S2: Overlapping significant differentially expressed metabolites in INSTI compared to NNRTI users with metabolite annotations in discovery and validation cohort with FDR/P-values and log fold changes. Metabolites with identical ionMz share colors.

| ionMz	name	logfc discovery	fdr discovery	logfc validation	pvalue validation	ionMz	name	logfc discovery	fdr discovery	logfc validation	pvalue validation
457.1362	12-Hydroxynevirapine glucuronide	-1,170858737	7,77581E-72	-1,294790654	6,34292E-23	224.0785	Aciclovir	0,09748719	0,001052967	0,127764519	0,023955153
457.1362	2-Hydroxynevirapine glucuronide	-1,170858737	7,77581E-72	-1,294790654	6,34292E-23	478.1113	Petunidin 3-glucoside	-0,039701752	0,001990301	-0,081900186	0,000805794
457.1362	3-Hydroxynevirapine glucuronide	-1,170858737	7,77581E-72	-1,294790654	6,34292E-23	426.0222	ADP	-0,090240251	0,002206316	-0,305559259	2,18393E-07
457.1362	8-Hydroxynevirapine glucuronide	-1,170858737	7,77581E-72	-1,294790654	6,34292E-23	102.0555	Dimethylglycine	-0,051815557	0,003356823	-0,072853297	0,034513634
479.1189	3'-O-Methylepicatechin 7-O-glucuronide	-0,554835062	4,3495E-63	-0,60426381	4,92754E-20	102.0555	gamma-Aminobutyric acid	-0,051815557	0,003356823	-0,072853297	0,034513634
479.1189	4'-O-Methyl-(-)-epicatechin 3'-O-glucuronide	-0,554835062	4,3495E-63	-0,60426381	4,92754E-20	102.0555	L-alpha-Aminobutyric acid	-0,051815557	0,003356823	-0,072853297	0,034513634
369.1742	Androsterone sulfate	0,743880297	1,60521E-38	0,792810872	5,42996E-10	102.0555	D-alpha-Aminobutyric acid	-0,051815557	0,003356823	-0,072853297	0,034513634
369.1742	5a-Dihydrotestosterone sulfate	0,743880297	1,60521E-38	0,792810872	5,42996E-10	102.0555	2-Aminoisobutyric acid	-0,051815557	0,003356823	-0,072853297	0,034513634
369.1742	Epiandrosterone sulfate	0,743880297	1,60521E-38	0,792810872	5,42996E-10	102.0555	(S)-beta-Aminoisobutyric acid	-0,051815557	0,003356823	-0,072853297	0,034513634
448.1669	Glisoxepide	0,211828559	1,07168E-30	0,226176781	6,38694E-08	102.0555	3-Aminoisobutanoic acid	-0,051815557	0,003356823	-0,072853297	0,034513634
251.1081	Cimetidine	-0,134399869	3,45922E-24	-0,116438234	0,006122943	96.9592	Sulfate	0,031318802	0,005163462	0,062582775	0,00391384
364.1484	Propericiazine	-0,218339292	4,21302E-20	-0,269504059	4,69445E-09	203.0827	L-Tryptophan	0,048379387	0,005332126	0,063383446	0,031678514
484.1438	Ketotifen-N-glucuronide	0,274942201	3,0648E-19	0,692051409	2,75413E-10	203.0827	D-Tryptophan	0,048379387	0,005332126	0,063383446	0,031678514
265.109	Nevirapine	-0,180694241	3,77874E-17	-0,263818024	7,17281E-09	203.0827	3-Hydroxymethylantipyrine	0,048379387	0,005332126	0,063383446	0,031678514
457.1991	2-Hydroxy-desipramine glucuronide	-0,184537655	4,13818E-17	-0,243330316	1,85236E-08	203.0827	Ethotoin	0,048379387	0,005332126	0,063383446	0,031678514
112.0511	Creatinine	0,109513658	2,75081E-12	0,114711351	0,000897632	203.0827	Nirvanol	0,048379387	0,005332126	0,063383446	0,031678514
167.0212	Uric acid	0,145224431	2,96648E-12	0,126667806	0,003866144	203.0827	4-Hydroxyantipyrine	0,048379387	0,005332126	0,063383446	0,031678514
381.1553	Ibuprofen glucuronide	-0,149986012	2,78661E-11	-0,204637832	8,17831E-07	203.0827	S-nirvanol	0,048379387	0,005332126	0,063383446	0,031678514
462.0555	Chondroitin sulfate	-0,075592181	1,47488E-09	-0,064914894	0,008556359	195.0654	Homoveratric acid	-0,099877343	0,005392802	-0,154365506	0,030736962
315.0511	Isorhamnetin	-0,03467816	1,66986E-09	-0,037406184	0,005122368	195.0654	3-(3-Hydroxyphenyl)-2-methyllactic acid	-0,099877343	0,005392802	-0,154365506	0,030736962
383.1532	16alpha-Hydroxy DHEA 3-sulfate	-0,252454815	9,07659E-09	-0,251970071	0,003558627	195.0654	3-(3,4-Dihydroxyphenyl)-2-methylpropionic acid	-0,099877343	0,005392802	-0,154365506	0,030736962
266.0894	Adenosine	-0,045440073	2,24795E-05	-0,089556921	3,75408E-05	195.0654	Dihydroferulic acid	-0,099877343	0,005392802	-0,154365506	0,030736962
266.0894	Deoxyguanosine	-0,045440073	2,24795E-05	-0,089556921	3,75408E-05	195.0654	3-(3-hydroxy-4-methoxyphenyl)propanoic acid	-0,099877343	0,005392802	-0,154365506	0,030736962
266.0894	Vidarabine	-0,045440073	2,24795E-05	-0,089556921	3,75408E-05	276.0185	DOPA sulfate	-0,0590246	0,005392802	-0,141979154	1,76683E-05
266.0894	Zidovudine	-0,045440073	2,24795E-05	-0,089556921	3,75408E-05	415.3213	24,25-Dihydroxyvitamin D	-0,054332113	0,01042519	-0,202276475	2,66684E-07
360.1029	5-Hydroxyomeprazole	0,157370187	3,20968E-05	0,375888271	4,78265E-05	415.3213	25,26-dihydroxyvitamin D	-0,054332113	0,01042519	-0,202276475	2,66684E-07
360.1029	Omeprazole sulfone	0,157370187	3,20968E-05	0,375888271	4,78265E-05	415.3213	Calcitriol	-0,054332113	0,01042519	-0,202276475	2,66684E-07
360.1029	3-Hydroxyomeprazole	0,157370187	3,20968E-05	0,375888271	4,78265E-05	415.3213	3beta-Hydroxy-5-cholestenoic acid	-0,054332113	0,01042519	-0,202276475	2,66684E-07
427.2251	Irbesartan	0,154868448	4,20224E-05	0,194148894	0,002362308	415.3213	Paricalcitol	-0,054332113	0,01042519	-0,202276475	2,66684E-07
128.0349	Pyroglutamic acid	-0,07251005	0,000140932	-0,089274319	0,025839989	259.1301	gamma-Glutamylisoleucine	-0,034999121	0,010792483	-0,088837457	0,001015475
128.0349	dimethadione	-0,07251005	0,000140932	-0,089274319	0,025839989	259.1301	gamma-Glutamylleucine	-0,034999121	0,010792483	-0,088837457	0,001015475
367.1588	Dehydroepiandrosterone sulfate	0,195192514	0,000228188	0,205008978	0,045121886	182.0463	4-Pyridoxic acid	-0,080798755	0,011311974	-0,108927018	0,023094951
367.1588	Testosterone sulfate	0,195192514	0,000228188	0,205008978	0,045121886	195.0521	1,3-Dimethyluric acid	-0,120179041	0,019570213	-0,240823458	0,008913258
146.0456	L-Glutamic acid	-0,083316051	0,00024846	-0,136087126	0,002754033	195.0521	3,7-Dimethyluric acid	-0,120179041	0,019570213	-0,240823458	0,008913258
146.0456	O-Acetylserine	-0,083316051	0,00024846	-0,136087126	0,002754033	195.0521	1,9-Dimethyluric acid	-0,120179041	0,019570213	-0,240823458	0,008913258
146.0456	D-Glutamic acid	-0,083316051	0,00024846	-0,136087126	0,002754033	195.0521	7,9-Dimethyluric acid	-0,120179041	0,019570213	-0,240823458	0,008913258
146.0456	DL-Glutamate	-0,083316051	0,00024846	-0,136087126	0,002754033	195.0521	1,7-Dimethyluric acid	-0,120179041	0,019570213	-0,240823458	0,008913258
183.139	Undecylenic acid	0,07609794	0,000359433	0,111445782	0,004655494	508.3401	LysoPE(20:0/0:0)	-0,051859038	0,027230985	-0,131899344	0,001370067
164.072	L-Phenylalanine	0,037448861	0,000725874	0,059139569	0,006770527	508.3401	LysoPC(17:0/0:0)	-0,051859038	0,027230985	-0,131899344	0,001370067
350.0279	Meloxicam	-0,082092378	0,000843835	-0,122548288	0,009334358	116.0502	Indole	0,034202513	0,035415296	0,060391926	0,0284867

Supplementary figure S8: Comparison of untargeted metabolites found in the discovery and validation cohort. Circles indicate number of metabolites found in certain category. Overlaying parts of the circles indicate number of metabolites found to overlap between the two categories. A: Overlap between FDR significant higher levels of metabolites in discovery cohort and p-value significant higher levels of metabolites in validation cohort. B: Overlap between FDR significant lower levels of metabolites in discovery cohort and p-value significant lower levels of metabolites in validation cohort. C: Overlap between FDR significant higher levels of metabolites in discovery cohort and metabolites with a positive fold change in the validation cohort. D: Overlap between FDR significant lower levels of metabolites in discovery cohort and metabolites with a negative fold change in the validation cohort.

A

FDR sign. higher level discovery cohort   P-value sign higher level validation cohort

45
76.3%   14
23.7%   18

B

FDR sign. lower level discovery cohort   P-value sign lower level validation cohort

21
46.7%   24
53.3%   15

C

FDR sign. higher level discovery cohort   Positive fold change validation cohort

18
30.5%   41
69.5%   242

D

FDR sign. lower level discovery cohort   Negative fold change validation cohort

5
11.1%   40
88.9%   177

Supplementary figure S9: Up- and down-regulation of metabolic pathways in INSTI users compared to NNRTI users in the validation cohort (INSTI n = 207; NNRTI n = 89) using MetaboAnalyst software. Y-axis shows significance through -10log(P-value), with the horizontal line the threshold for significance (p<0.05). X-axis indicates normalized enrichment score (NES) with horizontal line represents border between upregulated (right side) and downregulated (left side). Red dots indicate significant pathways. In the validation cohort nine pathways were significantly downregulated, three pathways were upregulated in INSTI users compared to NNRTI users. In addition, the three pathways that were significantly differentially regulated in the discovery cohort and had same directionality in the validation cohort without reaching the threshold of significance were annotated.
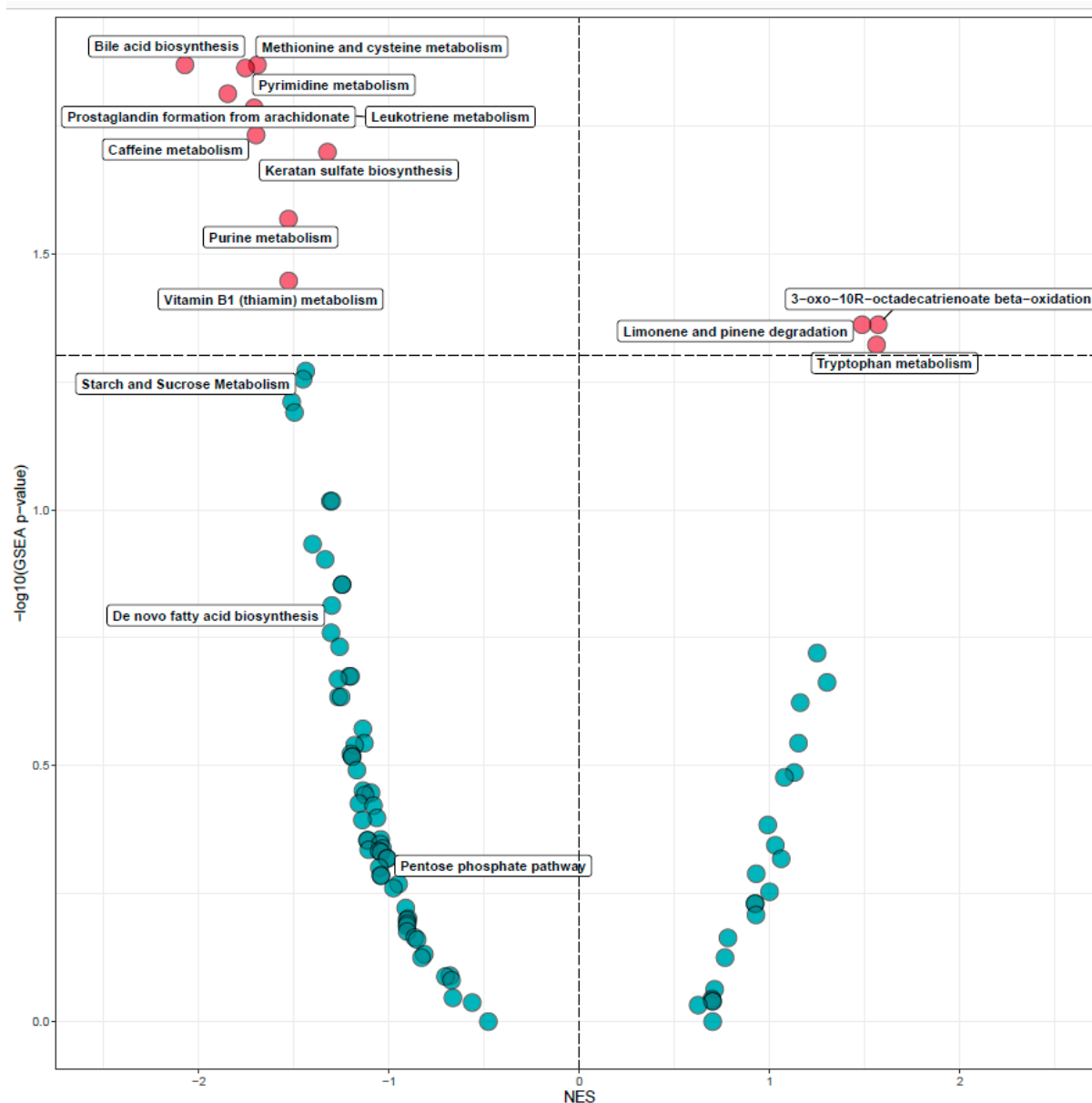
Supplementary figure S10: Nonhierarchical clustering of 132 intercorrelated lipoproteins and lipids through hierarchical Ward-linkage clustering based on Spearman correlation coefficients. Clusters share the same color, all clusters were positively correlated (r>0.75, p<0.05).
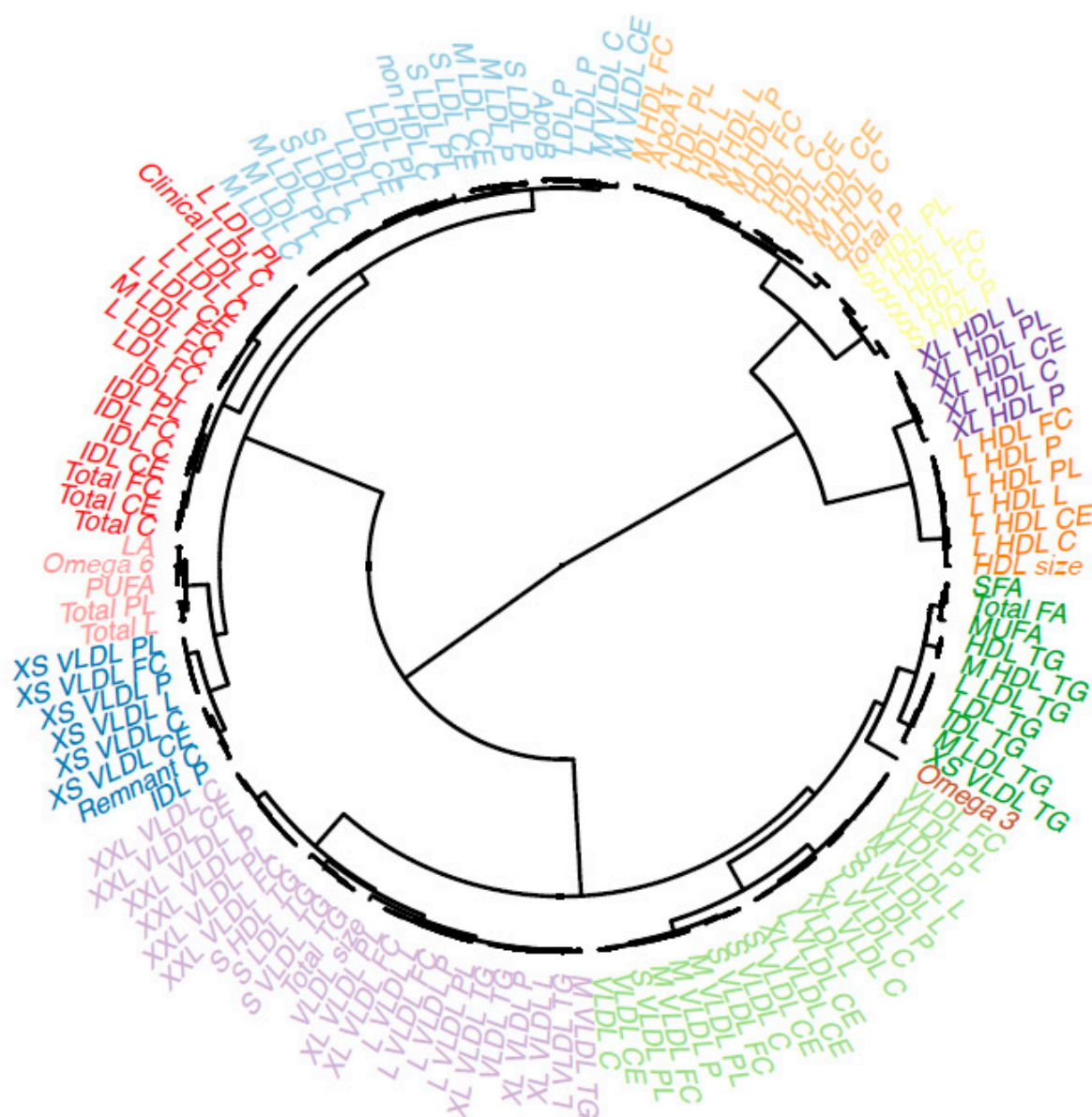
<u>Supplementary figure S11:</u> Principal component (PC) analysis of lipoproteins and lipids of the types of INSTI and NNRTI in the discovery cohort. X-axis shows PC1, Y-axis shows PC2.
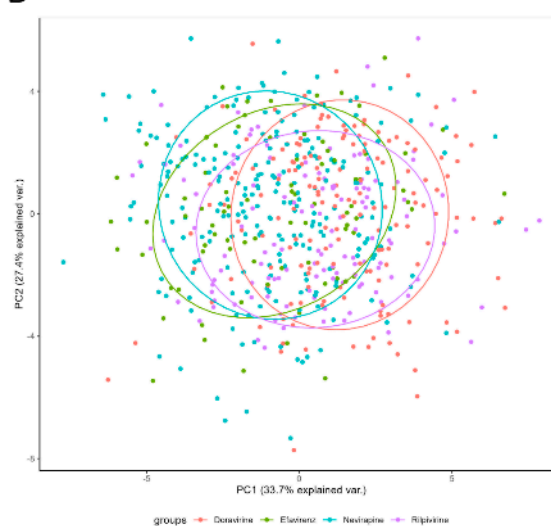
A: All types of INSTI showed considerable overlap in PC analysis, showing homogeneity between lipoproteins and lipids between the different INSTI types.

B: There were considerable differences between the different types of NNRTI in PC analysis, showing heterogeneity in lipoproteins and lipids between the different NNRTI types.
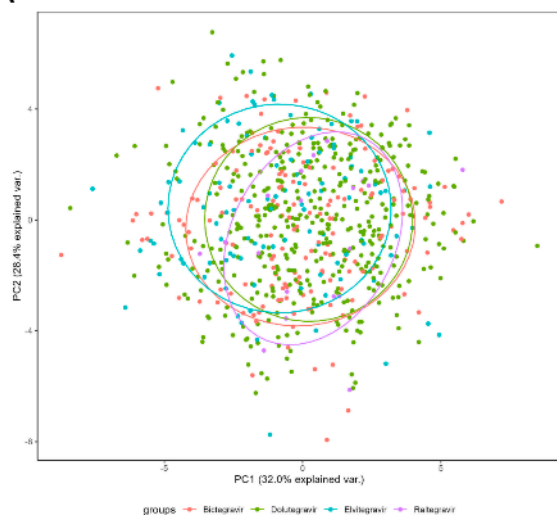
Supplementary table S3: Baseline characteristics of INSTI, NNRTI nevirapine and NNRTI doravirine users in the discovery (A) and validation cohort (B).

Supplementary table S3A:

	Discovery cohort			
	INSTI	**Nevirapine**	**Doravirine**	P-val
	730 patients	**236 patients**	**164 patients**	
Age in years (IQR)	51.0 (41.0 - 58.0)	55.0 (49.8 - 63.0)	50.0 (41.0 - 57.2)	< 0.0001
Sex at birth (male)	619 (84.8%)	202 (85.6%)	141 (86.0%)	0.94
BMI in kg/m2 Median (IQR)	25.0 (22.7 - 27.7)	24.5 (22.2 - 27.3)	24.9 (22.4 - 28.0)	0.20
Ethnicity (white)	543 (74.4%)	192 (81.4%)	115 (70.1%)	0.025
HIV duration in years Median (IQR)	10.8 (5.7 - 16.5)	19.0 (13.1 - 23.3)	11.6 (6.7 - 15.5)	< 0.0001
cART Duration in years Median (IQR)	8.3 (4.7 - 13.4)	16.1 (10.4 - 21.6)	9.5 (5.8 - 13.1)	< 0.0001
Latest CD4 count ($x10^6$ cells/L) Median (IQR)	740.0 (550.5 - 940.0)	673.0 (530.0 - 845.0)	707.5 (553.2 - 941.8)	0.01
CD4 Nadir ($x10^6$ cells/L) Median (IQR)	280.0 (160.0 - 422.5)	230.0 (120.0 - 310.0)	285.0 (164.8 - 413.8)	< 0.0001
Viral Load Zenith (copies/ml) Median (IQR)	97000.0 (36650.5 - 248154.0)	100000.0 (49300.0 - 261000.0)	102347.0 (37075.0 - 300000.0)	0.27
Currently smoking	238 (32.6%)	60 (25.4%)	38 (23.2%)	0.01
Missing	56 (7.7%)	12 (5.1%)	14 (8.5%)	
Packyears Median (IQR)	6.0 (0.0 - 22.0)	11.8 (0.0 - 27.9)	0.3 (0.0 - 15.4)	0.0002
Had Non-AIDS malignancy	28 (3.8%)	18 (7.6%)	6 (3.7%)	0.060
Had previous cardiovascular disease	214 (29.3%)	98 (41.5%)	41 (25.0%)	0.0004
On lipid lowering medication	141 (19.3%)	53 (22.5%)	22 (13.4%)	0.070

Supplementary table S3B:

	Validation cohort			
	INSTI	**Nevirapine**	**Doravirine**	**P-val**
	209 patients	**38 patients**	**5 patients**	
Age in years (IQR)	52.0 (45.0 - 61.0)	54.5 (48.0 - 59.8)	48.0 (37.0 - 54.0)	0.34
Sex at birth (male)	178 (85.2%)	32 (84.2%)	4 (80.0%)	0.76
BMI in kg/m2 Median (IQR)	25.8 (23.0 - 28.4)	25.4 (22.3 - 27.3)	28.2 (24.8 - 28.3)	0.75
Ethnicity (white)	180 (86.1%)	33 (86.8%)	4 (80.0%)	0.74
HIV duration in years Median (IQR)	7.0 (3.5 - 12.7)	15.7 (11.7 - 22.3)	10.1 (3.3 - 10.5)	< 0.0001
cART Duration in years Median (IQR)	6.0 (3.3 - 10.6)	12.9 (9.2 - 21.3)	10.0 (3.3 - 10.4)	< 0.0001
Latest CD4 count (x10^6 cells/L) Median (IQR)	660.0 (480.0 - 810.0)	660.0 (462.5 - 800.0)	700.0 (530.0 - 950.0)	0.73
CD4 Nadir (x10^6 cells/L) Median (IQR)	290.0 (170.0 - 452.5)	280.0 (190.0 - 330.0)	300.0 (130.0 - 420.0)	0.18
Viral Load Zenith (copies/ml) Median (IQR)	156748.0 (39240.8 - 346862.5)	100000.0 (46000.0 - 223214.0)	973984.0 (606326.0 - 3675318.0)	0.005
Currently smoking	59 (28.2%)	15 (39.5%)	1 (20.0%)	0.33
Missing	23 (11.0%)	4 (10.5%)	1 (20.0%)	
Packyears Median (IQR)	6.0 (0.0 - 28.5)	12.5 (0.0 - 36.0)	0.0 (0.0 - 11.0)	0.61
Had Non-AIDS malignancy	12 (5.7%)	2 (5.3%)	0 (0.0%)	1.0
Had previous cardiovascular disease	66 (31.6%)	18 (47.4%)	1 (20.0%)	0.13
On lipid lowering medication	40 (19.1%)	14 (36.8%)	0 (0.0%)	0.029