Supplemental Material for

A Distinct Form of Fat Fibrosis is Linked to Insulin Resistance in People with HIV

Diana L. Alba, Alaa Abdellatif, Moon K. Choi, Stephen Brown Mayfield, Thuy An T. Pham, David Berrios, Antonio E. Rodriguez, Marin Ewing, Tony Figueroa, Judy Gonzalez-Vargas, Ningyan Zhang, Zhiqiang An, Dawei Bu, Steven G. Deeks, Philipp E. Scherer, Peter W. Hunt, Suneil K. Koliwad

Corresponding authors: Diana L. Alba diana.alba@ucsf.edu

Suneil K. Koliwad: Suneil.koliwad@ucsf.edu

The PDF file includes Supplemental Methods, Supplemental Figures 1-7, and Supplemental Tables 1-11. Other supplemental material for this manuscript includes the Supporting Data Values file.

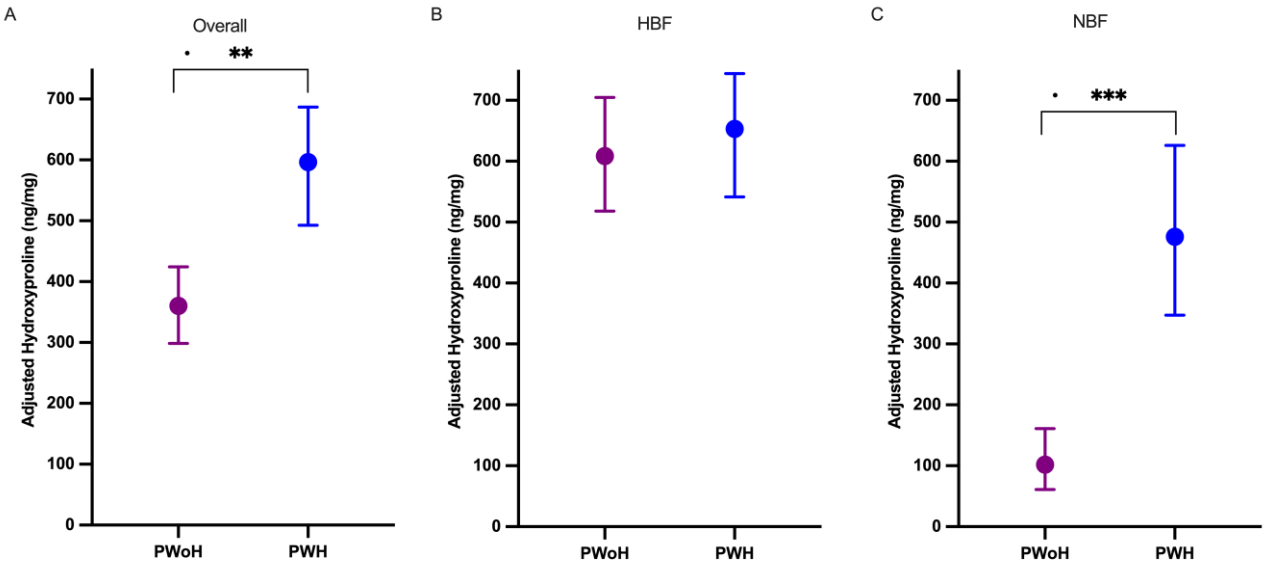
Supplemental Methods

Body composition and anthropometric measurements. Participants' height and weight were measured using a standard stadiometer and scale, with BMI (kg/m2) calculated from two averaged measurements. Body composition was estimated by DXA using a Hologic Horizon/A scanner (3-minute whole-body scan, <0.1 mGy) per manufacturer protocol. This device accurately measures participants up to 450 lbs. and employs high-performance and “offset” scanning techniques on those whose bodies are wider than the table. Standard DXA readouts include total percentage body fat (%BF), fat mass, lean mass, total body mass and VAT volume. A single technologist analyzed all DXA measurements using Hologic Apex software (13.6.0.4:3) following International Society for Clinical Densitometry guidelines. VAT was measured in a 5-cm-wide region across the abdominal region above the iliac crest, coincident with the fourth lumbar vertebrae, matching the region used for computed tomography (CT)-based VAT analysis (89), and approached validated against CT (r^2: 0.949-0.959) (90).

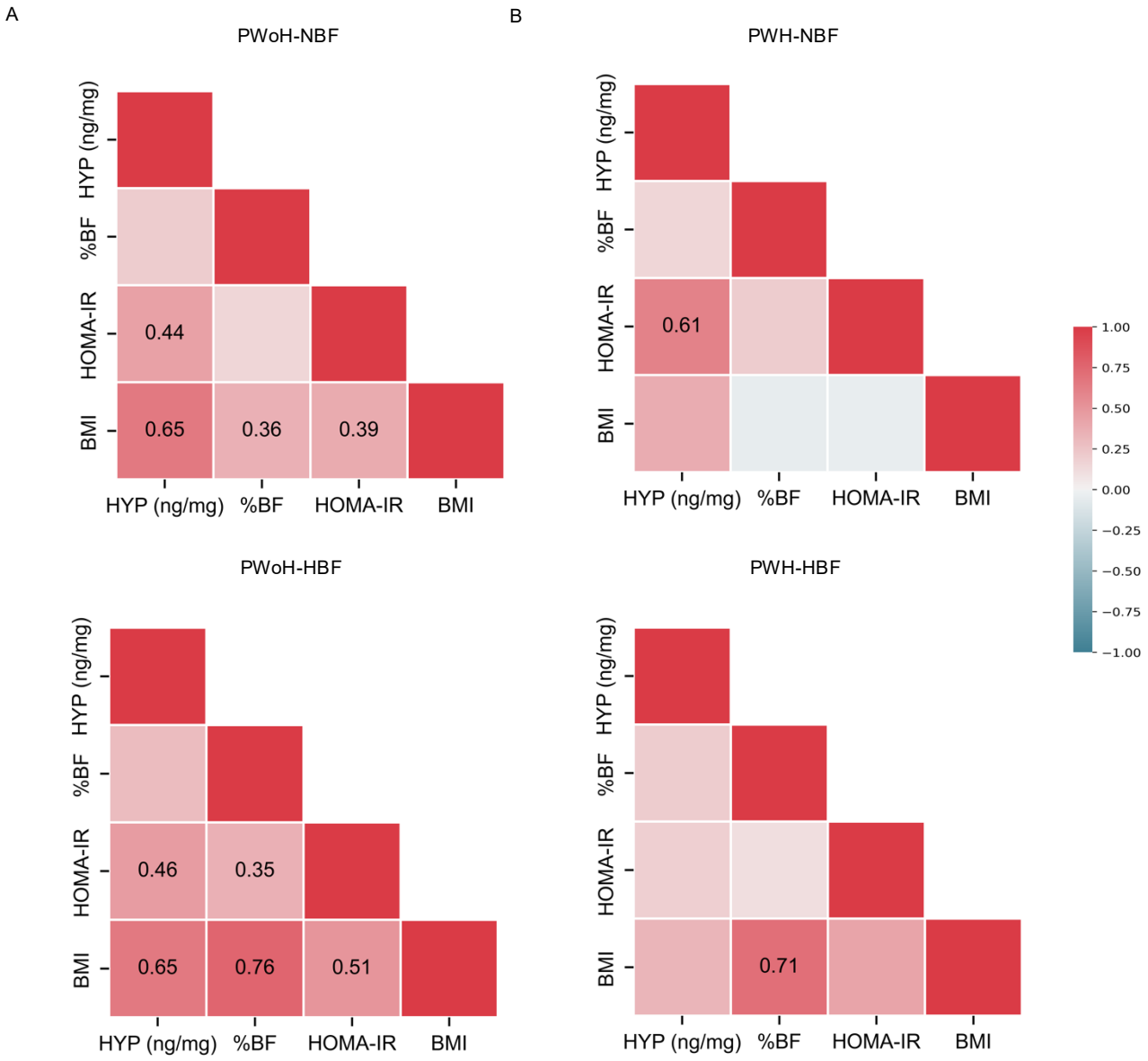
AT sample collection. SAT samples were collected from most participants after a 10-hour fast via aspiration biopsy under local anesthesia using 2% lidocaine. A 2.1 mm blunt-sided, ported liposuction catheter (Tulip CellFriendly™ GEMS Miller Harvester, Tulip Medical Products, San Diego, CA) was used to collect tissue from approximately 5 cm lateral to the umbilicus. In some participants, SAT was instead collected intraoperatively during bariatric surgery. After removal of visible connective tissue, blood, and clots, samples were washed with Krebs-Ringer bicarbonate buffer containing 1% BSA and immediately frozen in liquid nitrogen.

Collagen content measurement. The collagen content of SAT was measured by determining tissue HYP levels using the Quick Zyme Sensitive Tissue Hydroxyproline Assay (QuickZyme Biosciences, the Netherlands) according to the manufacturer’s instructions. Briefly, SAT samples were acid hydrolyzed using 6M HCl at 95°C for 20 hours. The HYP content was then assessed by spectrophotometry at 570nm.

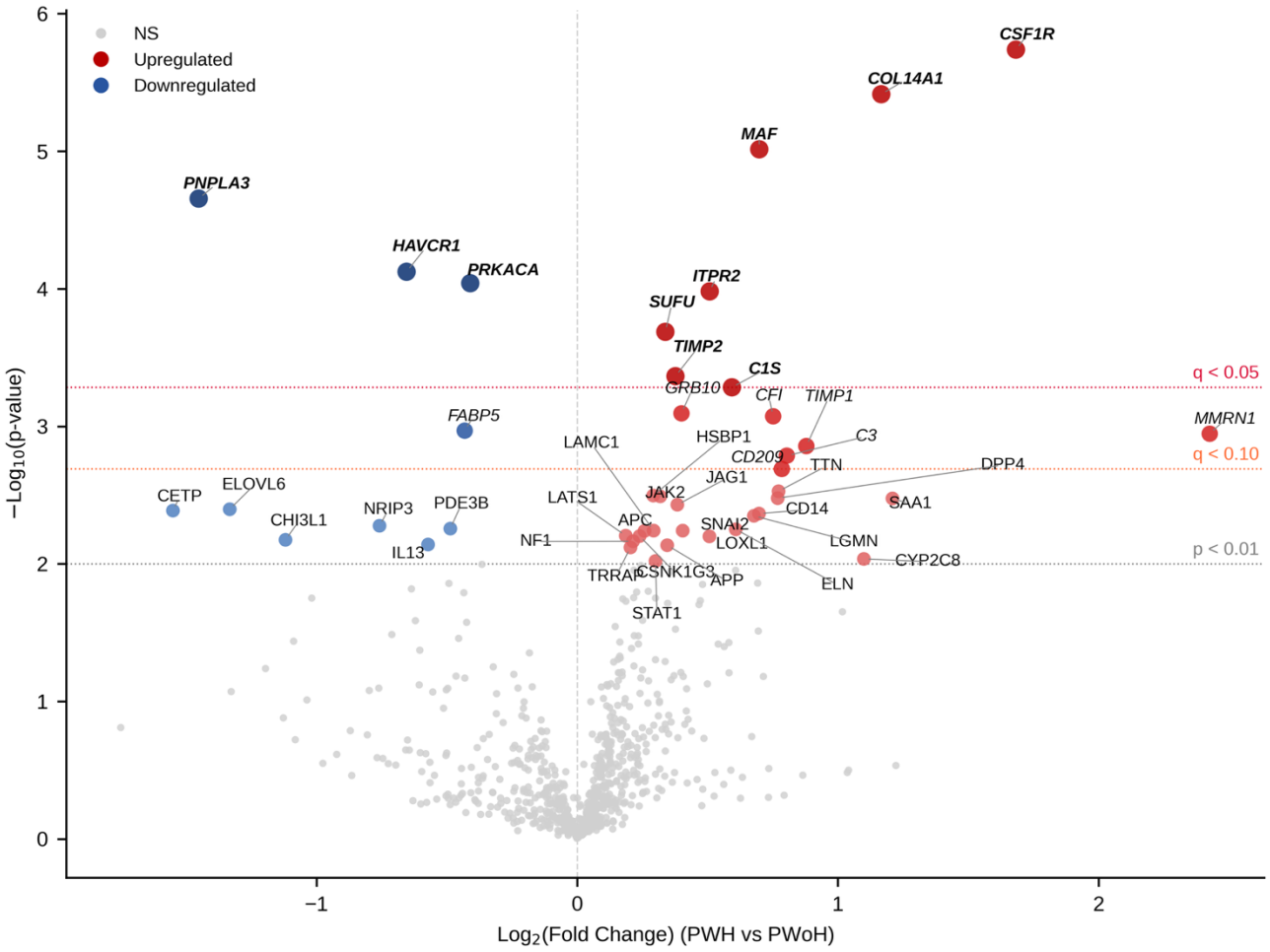
ELISA measurements of circulating ETP. Plasma samples were freshly prepared from blood samples collected after a 10 hour fast and preserved at –80°C until analysis. To quantitatively determine the levels of circulating ETP*,* 96-well plates (Corning Costar) were coated with rabbit monoclonal anti-endotrophin antibodies prepared in house at 2 μg/mL concentration. Plasma samples were titrated at a series of dilutions in 1X PBS, then added to an anti-endotrophin coated plate. A high affinity specific anti-endotrophin antibody (ETN-1Rb) was utilized as primary, with an anti-rabbit Fab2-HRP antibody (111-035-006**,** Jackson ImmunoResearch) used as a secondary antibody to amplify the ETP signal, at dilutions suggested by the manufacturer. The coating antibody and the primary detection antibody (ETN-1Rb) were both generated in-house in the laboratory of N. Zhang and Z. An (Texas Therapeutics Institute, Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX). A purified recombinant ETP protein was titrated across a series of concentrations (0–50 ng/mL) to establish a standard curve for calculating plasma ETP levels.



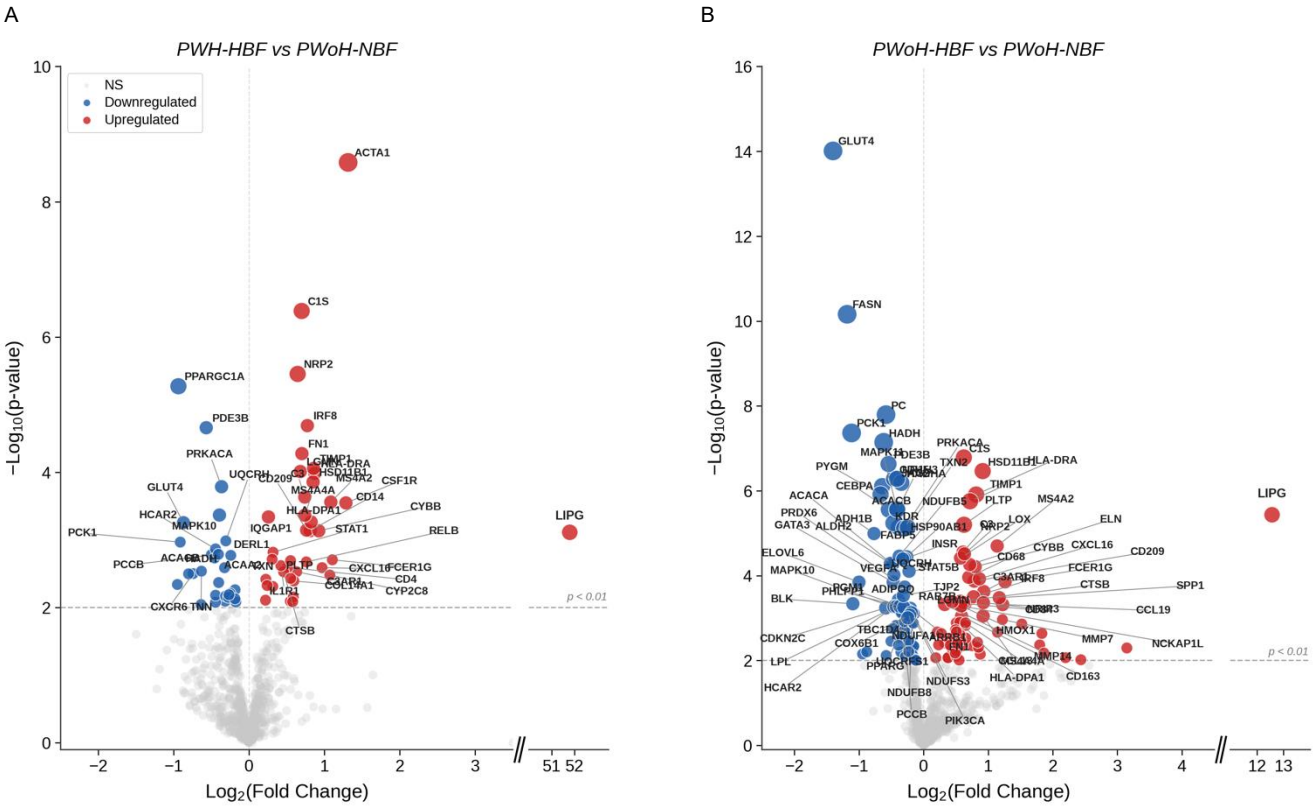
Supplemental Figure 1. Shown are model-derived adjusted estimates of SAT hydroxyproline (HYP) levels in people with HIV (PWH) and people without HIV (PWoH). HYP levels were estimated using multivariable linear regression models with HIV status as the primary exposure, adjusting for sex, age, and race/ethnicity, and analyses were stratified by adiposity category as defined in the Methods. **(A)** In the full cohort, PWH exhibited higher adjus ted SAT HYP levels compared with PWoH (PWoH n = 74, PWH n = 46). **(B)** Among participants with high adiposity (%BF ≥ 25% in males and ≥ 35% in females), adjusted SAT HYP levels did not differ between PWH and PWoH (PWoH n = 43, PWH n = 26). **(C)** Among participants with normal adiposity (% BF < 25% in males and < 35% in females), adjusted SAT HYP levels were significantly higher in PWH compared with PWoH (PWoH n = 31, PWH n = 20). Adjusted values shown are derived from the fitted regression models; error bars represent 95% confidence intervals. P-values correspond to the regression coefficient for HIV status within each model and were calculated from the t-statistic using appropriate degrees of freedom. **p < 0.01; ***p < 0.001.



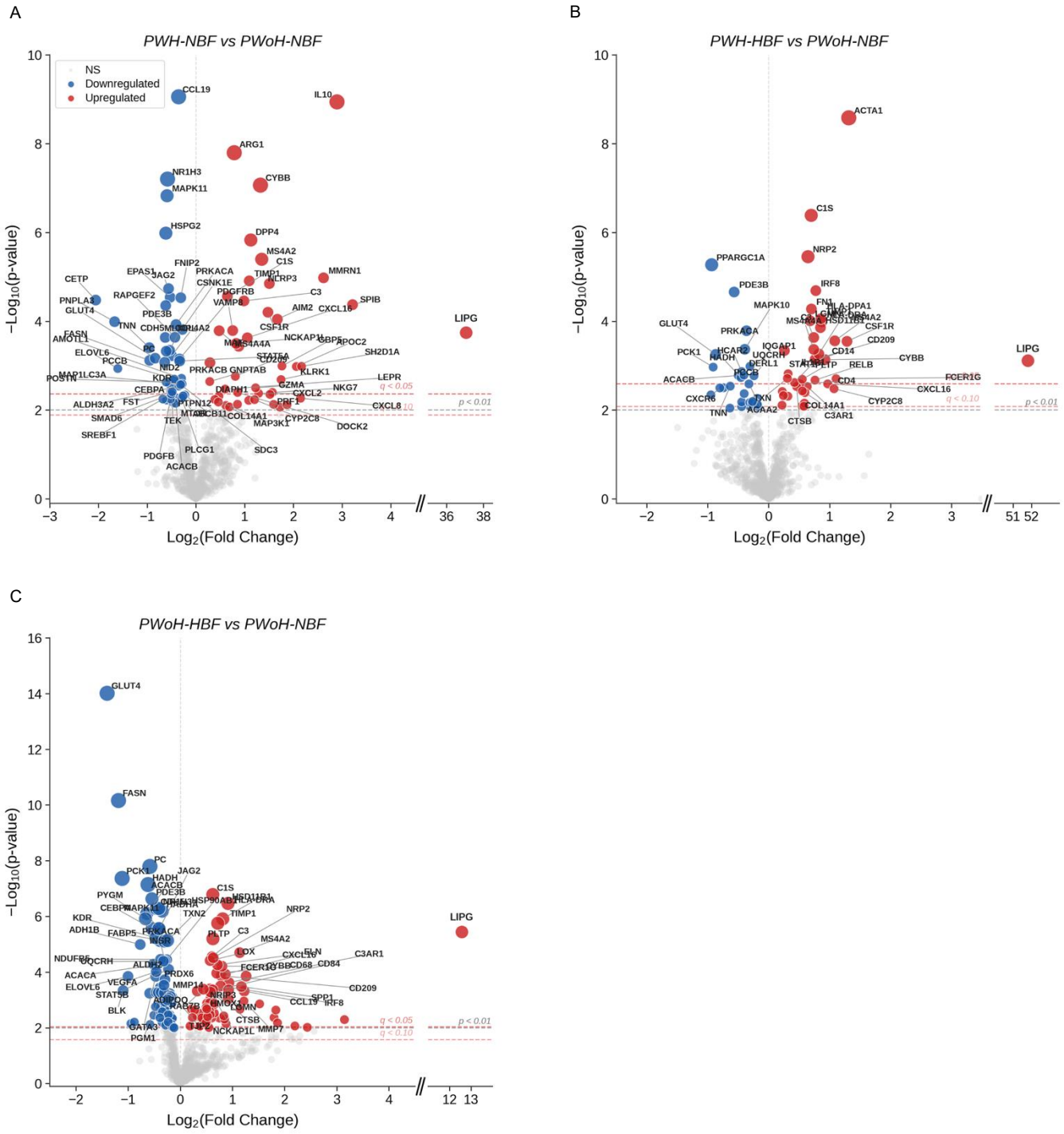
Supplemental Figure 2. Partial Spearman correlation analyses were performed to evaluate relationships among subcutaneous adipose tissue hydroxyproline (HYP), insulin resistance (HOMA-IR), BMI and whole-body percent fat (%BF) in PWoH and PWH, further grouped by normal adiposity and high adiposity as previously defined. **(A)** Correlation matrices for PWoH with normal adiposity (NBF, n = 30) and high adiposity (HBF, n = 43). **(B)** Correlation matrices for PWH with normal adiposity (NBF, n = 20) and high adiposity (HBF, n = 26). Spearman's rank-order correlation coefficients were calculated for each pair of variables while adjusting for age, sex, race and legacy d-drugs. Heatmaps display partial Spearman correlation coefficients (rho) only for associations reaching statistical significance ($p < 0.05$).



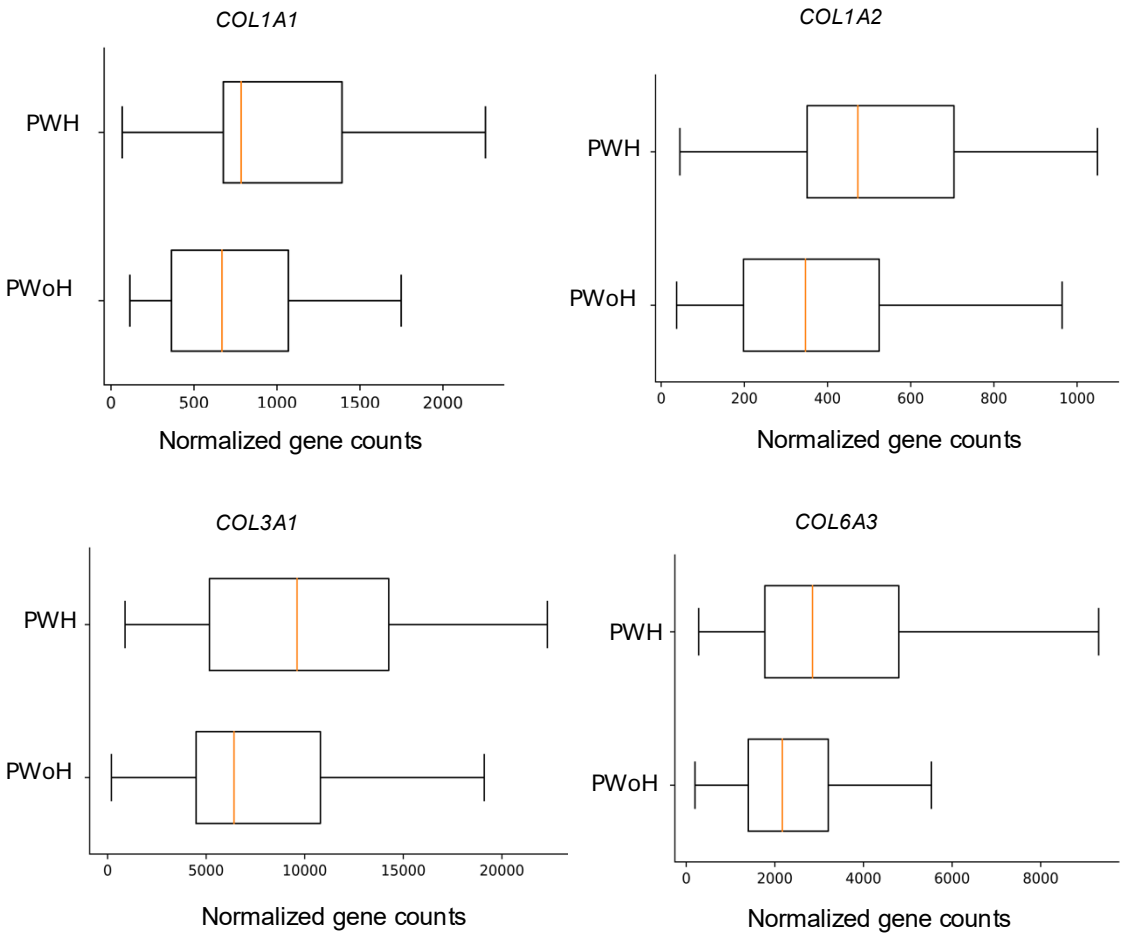
Supplemental Figure 3. Volcano plot of differential gene expression in SAT between PWH and PWoH, with FDR-adjusted thresholds. Each point represents a single gene. Red points indicate upregulated genes and blue points indicate downregulated genes in PWH relative to PWoH; gray points denote genes that did not reach nominal significance. Horizontal dotted lines indicate thresholds for nominal $p < 0.01$ (gray), $q < 0.10$ (orange), and $q < 0.05$ (red). Differential expression was assessed using a negative binomial model adjusting for age, sex, race, batch, and whole-body percent fat. FDR correction was performed using the Benjamini-Hochberg method.



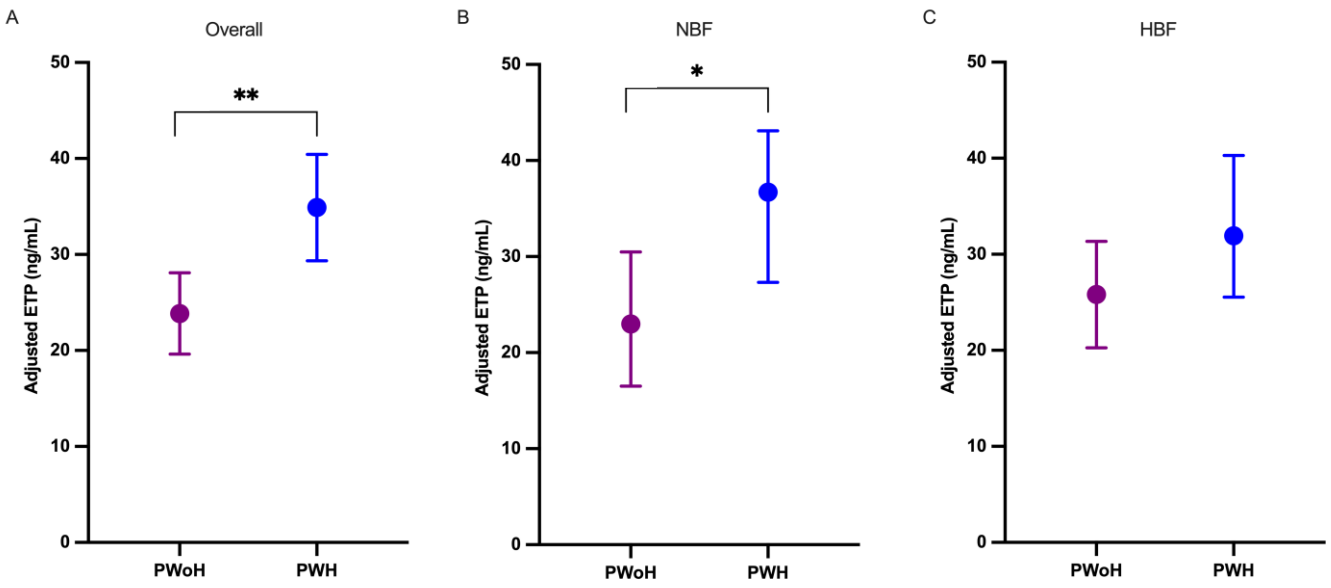
Supplemental Figure 4. Differential gene expression in adipose tissue by HIV and body fat status. Volcano plots showing differential gene expression in subcutaneous adipose tissue. The x-axis shows $\log_2$ fold change, and the y-axis shows $-\log_{10}$ p value. Genes meeting the nominal significance threshold ($p < 0.01$) are highlighted (red, upregulated *relative to PWoH-NBF*; blue, downregulated *relative to PWoH-NBF*) . Dashed line, $p < 0.01$ threshold. A broken x-axis (//) accommodates the LIPG gene. **(A)** PWH-HBF vs PWoH-NBF**. (B)** PWoH-HBF vs PWoH-NBF. Differential expression was analyzed using NanoStringDiff within a covariate-adjusted negative binomial generalized linear modeling framework. Models adjusted for age, sex, race, %BF, and batch.



Supplemental Figure 5. Differential gene expression in adipose tissue by HIV and body fat status. Volcano plots showing differential gene expression in subcutaneous adipose tissue. Red, upregulated ($p < 0.01$); blue, downregulated ($p < 0.01$); gray, NS: non-significant. Dashed gray line, $p < 0.01$ threshold; dashed red and orange lines, $q < 0.05$ and $q < 0.10$ thresholds (Benjamini–Hochberg FDR-adjusted p-values). A broken x-axis (//) accommodates the LIPG outlier. **(A)** PWH-NBF vs PWoH-NBF. **(B)** PWH-HBF vs PWoH-NBF. **(C)** PWoH-HBF vs PWoH-NBF. Differential expression was analyzed using NanoStringDiff within a covariate-adjusted negative binomial generalized linear modeling framework. Models adjusted for age, sex, race, batch, and %BF.



Supplemental Figure 6. Comparison of normalized expression of collagen-encoding transcripts in SAT from PWH and PWoH. NanoString-based quantification of *COL1A1*, *COL1A2*, *COL3A1*, and *COL6A3* expression in SAT samples from PWH and PWoH. Although differences between groups did not reach statistical significance ($p = 0.16$ for *COL1A1*; $p = 0.05$ for *COL1A2*; $p = 0.13$ for *COL3A1*; $p = 0.07$ for *COL6A3*), all transcripts showed a trend toward higher expression in PWH. All boxplot boxes display the interquartile range (IQR), with horizontal lines indicating the median. Statistical comparisons were performed using the Wilcoxon rank-sum test.



Supplemental Figure 7. Model-derived adjusted estimates of SAT endotrophin (ETP) levels by HIV status. ETP levels were estimated using multivariable linear regression models with HIV status as the primary exposure, adjusting for sex, age, race and %BF, and analyses were stratified by adiposity category as defined in the Methods. **(A)** In the full cohort, PWH exhibited higher adjusted SAT ETP levels compared with PWoH (PWoH n = 73, PWH n = 46; $p < 0.004$). **(B)** Among participants with normal adiposity (NBF) adjusted SAT ETP levels were significantly higher in PWH compared with PWoH (PWoH n = 31, PWH n = 20; $p = 0.041$). **(C)** Among participants with high adiposity (HBF), adjusted SAT ETP levels did not differ between PWH and PWoH (PWoH n = 42, PWH n = 26; $p = 0.16$. Adjusted values are derived from the fitted regression models; error bars represent 95% confidence intervals. P-values correspond to the regression coefficient for HIV status within each model. *$p < 0.05$; ***$p < 0.001$.

Supplemental Table 1. Multivariable linear regression examining associations between HIV status and VAT mass as a proportion of total body mass (%VAT), adjusting for sex, race/ethnicity, and age.

Predictor	β	SE	95% CI	P-value
HIV Status (PWH vs PWoH)	0.05	0.06	−0.06, 0.16	0.380
Sex (Male vs Female)	0.02	0.05	−0.08, 0.12	0.696
Age (years)	0.01	0.00	0.01, 0.02	*<0.001*
Race: Black	−0.07	0.07	−0.22, 0.07	0.319
Race: Other	−0.04	0.05	−0.014, 0.06	0.445

Although %VAT differed modestly between people with HIV (PWH) and HIV-negative participants in unadjusted analyses (two-sample t test $p = 0.06$), HIV status was not independently associated with %VAT after adjustment for age, sex, and race/ethnicity. Regression coefficients (β) represent the estimated change in the dependent variable per unit increase in each continuous covariate or relative to the reference category for categorical variables. Reference categories: HIV-negative (HIV status), female (sex), White (race). The "Other" race category includes Asian, Native Hawaiian/Pacific Islander, American Indian, and individuals reporting more than one race. Statistically significant associations ($p < 0.05$) are shown in italics. N = 115.

Supplemental Table 2. Multivariable OLS regression examining the relationship between Hydroxyproline levels (HYP) in subcutaneous adipose tissue (SAT) and HIV status, sex, race, age, and whole-body percent fat (N = 120)

Predictor	β	95% CI	p-value
HIV status (PWH vs PWoH)	*5.05*	*(2.25, 7.84)*	*0.001*
Sex (Male vs Female)	*7.46*	*(4.35, 10.57)*	*<0.001*
Race: Black (vs White)	0.28	(−3.34, 3.90)	0.878
Race: Other (vs White)	1.91	(−0.65, 4.46)	0.142
Age (years)	0.02	(−0.08, 0.12)	0.705
Body fat percentage (%)	*0.65*	*(0.47, 0.83)*	*<0.001*

HYP was Box-Cox transformed (λ = 0.35) to satisfy normality and homoscedasticity assumptions prior to model fitting. β coefficients represent the estimated change in transformed hydroxyproline per unit increase in each continuous predictor or relative to the reference category for categorical variables. Reference categories: HIV-negative (PWoH) for HIV status, female for sex, and White for race. “Other” race includes Asian, Native Hawaiian/Pacific Islander, and more than one race. N = 120. Statistically significant associations ($p < 0.05$) are italicized.

Supplemental Table 3. Multivariable OLS regression model restricted to people with HIV (PWH) examining associations between subcutaneous adipose tissue (SAT) hydroxyproline levels antiretroviral treatment characteristics and adiposity (n=46).

Predictor	β	95% CI	p-value
Legacy d-drug exposure	6.01	−11.68, 23.70	0.496
INSTI use	−10.77	−33.49, 11.95	0.344
CD4 T-cell count	0.016	−0.017, 0.048	0.344
%Body fat	0.87	−0.29, 2.03	0.137

β coefficients represent the estimated change in transformed hydroxyproline per unit increase in each continuous predictor or relative to the reference category for categorical variables. Reference categories: no exposure for legacy d-drug use, no current use for INSTI, continuous for CD4+ T-cell count and %BF. Hydroxyproline was Box-Cox transformed ($\lambda = 0.58$) to satisfy normality and homoscedasticity assumptions prior to model fitting. Statistically significant associations ($p < 0.05$) are italicized.

Supplemental Table 4. Sequential Sensitivity analyses for the association of ART exposure with subcutaneous adipose tissue hydroxyproline in people with HIV (n=46).

Predictor	β	95% CI	p-value
Model 1 (Base + Sex)			
INSTI use	−10.31	−33.34, 12.71	0.371
Legacy d-drug exposure	7.25	−11.32, 25.82	0.435
%Body fat	1.04	−0.32, 2.40	0.130
CD4 T-cell count	0.018	−0.017, 0.052	0.305
Sex (Male)	5.75	−17.80, 29.30	0.624
Model 2 (Base + Age)			
INSTI use	−11.33	−34.39, 11.73	0.327
Legacy d-drug exposure	6.97	−11.31, 25.25	0.445
%Body fat	0.88	−0.30, 2.05	0.139
CD4 T-cell count	0.015	−0.018, 0.049	0.352
Age	−0.19	−0.96, 0.58	0.618
Model 3 (Base + Race)			
INSTI use	−8.96	−31.86, 13.93	0.433
Legacy d-drug exposure	5.16	−12.72, 23.04	0.563
%Body fat	0.87	−0.29, 2.04	0.137
CD4 T-cell count	0.019	−0.015, 0.053	0.274
Race: Black	6.65	−11.04, 24.33	0.452
Race: Other	14.19	−5.63, 34.00	0.156

Each model extends the base model (legacy d-drug exposure, INSTI use, CD4+ T-cell count, %BF) with an additional covariate. Hydroxyproline was Box-Cox transformed ($\lambda = 0.58$) prior to model fitting. β coefficients represent the estimated change in transformed hydroxyproline per unit increase in each continuous predictor or relative to the reference category for categorical variables. Reference categories: no exposure for legacy d-drug use, no current use for INSTI, female for sex, White for race. “Other” race includes Asian, Native Hawaiian/Pacific Islander, and more than one race. Statistically significant associations ($p < 0.05$) are italicized

Supplemental Table 5. Demographic and clinical characteristics of the study population stratified by adiposity and HIV status.

	Normal Adiposity, NBF		High Adiposity, HBF	
Parameter	**PWoH (n=31)**	**PWH (n=20)**	**PWoH (n=43)**	**PWH (n=26)**
Age (years)	44 ± 14	52 ± 10	50 ± 12	52 ± 11
Sex at birth (Female/Male)	17/14	3/17	22/21	7/19
Race				
Asian	14	1	15	0
Black	2	6	1	10
White	13	7	20	12
More than one race	1	4	6	1
Other	1	2	1	3
Ethnicity				
Hispanic or Latino	2	4	10	6
Not Hispanic or Latino	29	16	32	20
Unknow	0	0	1	0
BMI (kg/m^2)	23.4 ± 3.1	25.6 ± 3.4	32.7 ± 8.2	32.9 ± 10.1
Body Fat Percentage (%)	25.7 ± 6.0	23.2 ± 3.1	36.0 ± 7.0	34.2 ± 7.0
Fasting glucose (mg/dL)	92 (85, 100)	98 (88, 107)	93 (88,101)	88 (84, 98)
Fasting insulin (mU/L) [A]	5.8 (3.9, 9.5)	9.1 (7.2, 14.4)	14.6 (9.2, 24.4)	11.6 (7.0, 16.7)
HOMA-IR[A]	1.4 (0.8, 2.8)	2.4 (1.7, 3.6)	4.0 (2.0, 6.3)	2.7 (1.5, 4.0)
HbA1c (%)	5.4 ± 0.5	5.6 ± 0.4	5.8 ± 0.7	5.3 ± 0.5
Duration of HIV (years)	-	23.8 ± 9.8	-	20.5 ± 12.9
Duration of ART use (years)	-	11.8 ± 9.0	-	8.6 ± 9.3
CD4$^+$ cell count (cells/mm^3)	-	537.0 (471.8, 774.2)	-	713.5 (605.2, 815.0)
ART regimen				
INSTI, n (%)	-	18 (90%)	-	22 (85%)
NNRTI, n (%)	-	5 (25%)	-	5 (19%)
Protease Inhibitor, n (%)	-	3 (15%)	-	1 (4%)
Exposure to d-drugs, n (%)	-	10 (50%)	-	4 (15%)
Hydroxyproline (ng/mg fat)	72.7 (48.7, 166.5)	448.4 (287.3, 815.9)	389.7 (271.9, 842.5)	654.0 (405.0, 863.1)

Males were classified as NBF (%BF <25%) or HBF (%BF ≥25%); females were classified as NBF (%BF <35%) or HBF (%BF ≥35%). Values are presented as mean ± SD or median (interquartile range). Other category for race includes one individual identifying as American Indian, one as Native Hawaiian or Pacific Islander, and five with unknown or not reported race. Missing data: n = 5 (VAT/total mass), n = 2 (fasting insulin and HOMA-IR). PWH, people with HIV; PWoH, people without HIV; NBF, normal body fat; HBF, high body fat; BMI, body mass index; VAT, visceral adipose tissue; HOMA-IR, homeostatic model assessment for insulin resistance; HbA1c, hemoglobin A1c; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

Supplemental Table 6. Characteristics of Study Participants included in SAT NanoString transcriptional analysis.

Parameter	PWoH (n=38)	PWH (n=37)	p-value
Age (years)	48.5 ± 13.2	52.2 ± 10.8	0.23
Sex at birth			0.07
Female	20	10	
Male	18	27	
Race			*<0.01*
Asian	15	1	
Black	2	15	
White	15	16	
More than one race	6	3	
Other*	0	2	
Ethnicity			0.75
Hispanic or Latino	6	6	
Not Hispanic or Latino	31	31	
Unknown	1	0	
BMI (kg/m²)	29.1 ± 8.0	29.7 ± 7.7	0.47
Body Fat Percentage (%)	32.1 ± 8.1	29.9 ± 7.8	0.18
VAT mass/total mass (%)[A]	0.7 ± 0.3	0.7 ± 0.3	0.48
Fasting glucose (mg/dL)	93.0 (87.5, 98.5)	90.5 (84.8, 99.5)	0.39
Fasting insulin (mU/L)[A]	11.8 (6.2, 19.3)	9.9 (6.8, 16.6)	0.60
HOMA-IR[A]	2.9 (1.4, 4.4)	2.6 (1.4, 3.7)	0.43
Duration of HIV (years)	—	23.1 (15.0, 31.2)	
Duration of ART use (years)	—	5.6 (2.4, 13.1)	
CD4⁺ cell count (cells/mm³)	—	631.0 (521.0, 830.2)	
ART regimen			
INSTI, n (%)	—	32 (89%)	
NNRTI, n (%)	—	6 (17%)	
PI, n (%)	—	3 (8%)	
Exposure to d-drugs, n (%)	-	9 (25%)	-
Hydroxyproline (ng/mg fat)	290.7 (79.6, 757.2)	507.2 (335.8, 877.8)	*0.02*

Values are presented as mean ± SD or median (interquartile range). Differences between the groups were analyzed by Chi-Square test for categorical variables and Wilcoxon rank-sum test for continuous variables. *Other category for race includes one individual identifying as Native Hawaiian or Pacific Islander and one with unknown or not reported race. [A]Missing data: n = 4 (VAT/total mass), n = 2 (fasting insulin, HOMA-IR). PWH, people with HIV; PWoH, people without HIV; BMI, body mass index; VAT, visceral AT; HOMA-IR, homeostatic model assessment for insulin resistance: HbA1c, hemoglobin A1c; ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor. ART regimen categories are not mutually exclusive.

Supplemental Table 7. Characteristics of Study Participants included in SAT NanoString transcriptional analysis, stratified by adiposity and HIV status.

	Normal Adiposity, NBF		High Adiposity, HBF	
Parameter	**PWoH (n=18)**	**PWH (n=16)**	**PWoH (n=20)**	**PWH (n=21)**
Age (years)	45.6 ± 13.2	52.3 ± 10.6	50.5 ± 13.1	52.1 ± 11.1
Sex at birth (Female/Male)	9/9	3/13	11/9	7/14
Race				
Asian	7	1	8	0
Black	1	5	1	10
White	7	6	8	10
More than one race	3	3	3	0
Other*	0	1	0	1
Ethnicity				
Hispanic or Latino	2	3	4	3
Not Hispanic or Latino	16	13	15	18
Unknown	0	0	1	0
BMI (kg/m²)	24.1 ± 3.2	25.1 ± 2.4	32.5 ± 8.5	32.9 ± 8.6
Body Fat Percentage (%)	26.1 ± 5.6	23.4 ± 3.3	36.2 ± 6.9	34.5 ± 6.8
Fasting glucose (mg/dL)	95.5 (87.5, 104.5)	98.0 (87.5, 107.0)	92.0 (87.5, 95.0)	88.0 (84.0, 95.0)
Fasting insulin (mU/L) [A]	5.7 (3.7, 13.0)	8.9 (6.8, 11.8)	17.1 (9.3, 25.2)	12.3 (7.2, 17.4)
HOMA-IR [A]	1.4 (0.8, 3.2)	2.2 (1.5, 3.2)	4.0 (2.1, 5.9)	2.8 (1.5, 3.8)
HbA1c (%)	5.6 ± 0.4	5.5 ± 0.4	5.6 ± 0.4	5.3 ± 0.5
Duration of HIV (years)	—	23.6 ± 9.7	—	21.6 ± 13.6
Duration of ART use (years)	—	11.6 ± 9.3	—	8.5 ± 9.9
$CD4^+$ cell count ($cells/mm^3$)	—	536.0 (450.5, 680.0)	—	774.0 (624.0, 864.0)
ART regimen				
INSTI, n (%)	—	15 (100%)	—	17 (81%)
NNRTI, n (%)	—	1 (7%)	—	5 (24%)
Protease Inhibitor, n (%)	—	2 (13%)	—	1 (5%)
Exposure to d-drugs, n (%)	—	7 (47%)	—	2 (10%)
Hydroxyproline (ng/mg fat)	100.6 (55.3, 205.6)	410.4 (268.9, 687.5)	375.6 (271.9, 915.0)	657.8 (423.9, 899.5)

Adiposity classification: males were classified as NBF (%BF <25%) or HBF (%BF ≥25%); females were classified as NBF (%BF <35%) or HBF (%BF ≥35%). Values are presented as mean ± SD or median (interquartile range). *Other category for race includes one individual identifying as Native Hawaiian or Pacific Islander and one with unknown or not reported race. [A] Missing data: n = 4 (VAT/total mass), n = 2 (fasting insulin, HOMA-IR). PWH, people with HIV; PWoH, people without HIV; NBF, normal body fat; HBF, high body fat; BMI, body mass index; VAT, visceral adipose tissue; HOMA-IR, homeostatic model assessment for insulin resistance; HbA1c, hemoglobin A1c; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

Supplemental Table 8. Genes differentially expressed in adipose tissue across groups compared to the reference group (PWoH-NBF).

A. DGE analysis of PWH-NBF group vs control (PWoH-NBF). Total significant genes ($p < 0.01$): 89; Upregulated: 44; Downregulated: 45

Upregulated genes (n = 44)

Gene	logFC	p-value	q-value
IL10	2.8961	1.15e-9	4.36e-7
ARG1	0.7873	1.60e-8	4.05e-6
CYBB	1.3255	8.60e-8	1.31e-5
DPP4	1.1278	1.48e-6	1.40e-4
MS4A2	1.3512	4.02e-6	3.40e-4
MMRN1	2.6200	1.05e-5	7.99e-4
TIMP1	1.0954	1.23e-5	8.49e-4
NLRP3	1.5083	1.42e-5	8.97e-4
C1S	0.6465	2.74e-5	0.0014
C3	0.9872	3.48e-5	0.0015
SPIB	3.2162	4.23e-5	0.0017
CSF1R	1.4787	6.27e-5	0.0023
AIM2	1.6723	8.99e-5	0.0031
VAMP8	0.7558	1.62e-4	0.0046
MAF	0.4776	1.63e-4	0.0046
LIPG	37.0635	1.79e-4	0.0049
CXCL16	1.0558	2.36e-4	0.0058
NCKAP1L	0.8280	3.15e-4	0.0075
MS4A4A	0.8807	3.67e-4	0.0084
PRKACB	0.2885	8.57e-4	0.0151
GBP5	1.7662	0.0010	0.0173
SH2D1A	2.1685	0.0010	0.0173
APOC2	2.0627	0.0010	0.0173
CD209	0.8098	0.0017	0.0275
KLRK1	1.7486	0.0021	0.0301
GNPTAB	0.2843	0.0023	0.0326
GZMA	1.2260	0.0032	0.0406
LEPR	0.5996	0.0034	0.0426
NKG7	1.5701	0.0040	0.0476
DOCK2	0.8559	0.0040	0.0476
CXCL2	1.2861	0.0042	0.0491
PRF1	1.5200	0.0044	0.0503
DIAPH1	0.4731	0.0049	0.0528
CXCL8	2.1480	0.0054	0.0557
MAP3K1	0.3894	0.0058	0.0567
CYP2C8	1.2042	0.0058	0.0567
CXCR4	1.0848	0.0061	0.0576
PELI1	0.4620	0.0066	0.0618
MS4A1	0.8535	0.0074	0.0665
EGR1	1.5963	0.0074	0.0665
PTGS2	1.8701	0.0076	0.0670
COL14A1	0.6150	0.0079	0.0688
IRF8	0.6902	0.0085	0.0734
CCL4	1.7254	0.0086	0.0734

Downregulated genes (n = 45)

Gene	logFC	p-value	q-value
CCL19	-0.3549	8.80e-10	4.36e-7
NR1H3	-0.5822	6.27e-8	1.19e-5
MAPK11	-0.5919	1.49e-7	1.89e-5
HSPG2	-0.6163	1.04e-6	1.13e-4
JAG2	-0.5612	1.84e-5	0.0011
EPAS1	-0.5292	2.89e-5	0.0014
FNIP2	-0.3101	2.95e-5	0.0014
CETP	-2.0569	3.33e-5	0.0015
PDE3B	-0.6200	4.43e-5	0.0017
PNPLA3	-1.6681	1.02e-4	0.0034
PRKACA	-0.4078	1.18e-4	0.0037
CSNK1E	-0.2798	1.53e-4	0.0046
RAPGEF2	-0.4307	2.30e-4	0.0058
CDH5	-0.6293	2.32e-4	0.0058
TNN	-0.9571	3.93e-4	0.0088
MLXIPL	-0.5405	4.51e-4	0.0098
PC	-0.6087	4.72e-4	0.0100
COL4A2	-0.4985	5.98e-4	0.0123
GLUT4	-0.8365	6.79e-4	0.0136
PDGFRB	-0.3369	7.06e-4	0.0138
FASN	-0.9488	7.61e-4	0.0145
STAT5A	-0.3332	7.85e-4	0.0145
NID2	-0.6404	8.37e-4	0.0151
ELOVL6	-1.6034	0.0012	0.0188
AMOTL1	-0.2947	0.0019	0.0299
PCCB	-0.4434	0.0020	0.0301
KDR	-0.4584	0.0020	0.0301
POSTN	-0.6099	0.0023	0.0329
MAP1LC3A	-0.2830	0.0026	0.0355
CEBPA	-0.5357	0.0026	0.0355
SDC3	-0.3137	0.0027	0.0357
ALDH3A2	-0.3526	0.0029	0.0378
TEK	-0.4872	0.0039	0.0476
FST	-0.5163	0.0039	0.0476
SMAD6	-0.5027	0.0043	0.0500
ABCB11	-0.2295	0.0046	0.0511
PTPN12	-0.2613	0.0048	0.0528
MTOR	-0.2671	0.0052	0.0552
ACACB	-0.4198	0.0052	0.0552
PLCG1	-0.2916	0.0054	0.0557
PDGFB	-0.5216	0.0056	0.0566
SREBF1	-0.6769	0.0057	0.0566
COX4I2	-0.6023	0.0059	0.0572
MAP2K1	-0.4588	0.0068	0.0629
PDE2A	-0.4154	0.0070	0.0644

B. DGE analysis of PWH-HBF group vs control (PWoH-NBF). Total significant genes ($p < 0.01$): 68; Upregulated: 39; Downregulated: 29

Upregulated genes (n = 39)

Gene	logFC	p-value	q-value
ACTA1	1.3152	2.62e-9	1.99e-6
C1S	0.6989	4.12e-7	1.57e-4
NRP2	0.6456	3.51e-6	8.90e-4
IRF8	0.7741	2.04e-5	0.0028
FN1	0.7019	5.29e-5	0.0057
TIMP1	0.8571	8.75e-5	0.0079
LGMN	0.6810	9.74e-5	0.0079
HLA-DRA	0.8718	1.03e-4	0.0079
HSD11B1	0.8502	1.39e-4	0.0096
C3	0.7400	2.32e-4	0.0136
MS4A2	1.0858	2.76e-4	0.0144
CSF1R	1.2861	2.84e-4	0.0144
MS4A4A	0.7397	4.31e-4	0.0193
IQGAP1	0.2581	4.57e-4	0.0193
HLA-DPA1	0.8248	5.43e-4	0.0211
CD209	0.7645	7.16e-4	0.0242
CD14	0.8125	7.28e-4	0.0242
CYBB	0.9252	7.33e-4	0.0242
LIPG	51.7935	7.67e-4	0.0243
STAT1	0.3191	0.0015	0.0412
TXN	0.3071	0.0019	0.0439
FCER1G	1.1062	0.0020	0.0439
PLTP	0.5514	0.0020	0.0440
RELB	0.7604	0.0021	0.0446
IL1R1	0.4234	0.0024	0.0489
CD4	0.5132	0.0025	0.0489
CXCL16	0.9695	0.0026	0.0489
C3AR1	0.6332	0.0030	0.0524
CTSB	0.4590	0.0030	0.0524
CYP2C8	1.0730	0.0033	0.0547
VAMP8	0.5530	0.0037	0.0600
CTNNB1	0.2236	0.0038	0.0604
COL14A1	0.5948	0.0040	0.0627
LAMC1	0.2411	0.0047	0.0689
GNB4	0.3153	0.0049	0.0699
LIPA	0.5874	0.0070	0.0893
PDGFRB	0.2193	0.0078	0.0947
CD163	0.5491	0.0081	0.0947
NCKAP1L	0.5794	0.0082	0.0947

Downregulated genes (n = 29)

Gene	logFC	p-value	q-value
PPARGC1A	-0.9364	5.34e-6	0.0010
PDE3B	-0.5672	2.19e-5	0.0028
PRKACA	-0.3641	1.63e-4	0.0103
MAPK10	-0.3920	4.29e-4	0.0193
GLUT4	-0.8697	5.56e-4	0.0211
UQCRH	-0.3071	0.0010	0.0314
PCK1	-0.9120	0.0011	0.0315
HCAR2	-0.4439	0.0014	0.0382

PCCB	-0.4057	0.0016	0.0415
ACACB	-0.4999	0.0017	0.0415
DERL1	-0.2416	0.0017	0.0415
HADH	-0.4562	0.0019	0.0439
ACAA2	-0.3243	0.0026	0.0489
TNN	-0.6311	0.0029	0.0524
CXCR6	-0.7411	0.0031	0.0536
FASN	-0.8017	0.0032	0.0536
PGM1	-0.4025	0.0043	0.0649
PNPLA3	-0.9494	0.0046	0.0679
CHUK	-0.1849	0.0055	0.0769
HIKESHI	-0.2500	0.0062	0.0855
UQCRFS1	-0.2659	0.0065	0.0876
PC	-0.4433	0.0066	0.0876
PHLPP1	-0.3096	0.0068	0.0887
SERPINF1	-0.2739	0.0070	0.0893
TXN2	-0.1856	0.0074	0.0925
FABP5	-0.3158	0.0081	0.0947
PPM1A	-0.1751	0.0083	0.0947
FST	-0.4450	0.0084	0.0947
IL13	-0.6363	0.0090	0.1010

C. DGE analysis of PWoH-HBF group vs control (PWoH-NBF). Total significant genes ($p < 0.01$): 148; Upregulated: 70; Downregulated: 78

Upregulated genes (n = 70)

Gene	logFC	p-value	q-value
C1S	0.6236	1.64e-7	2.08e-5
HSD11B1	0.9152	3.44e-7	3.26e-5
HLA-DRA	0.8139	1.22e-6	6.65e-5
TIMP1	0.7189	1.76e-6	8.94e-5
LIPG	12.5659	3.62e-6	1.45e-4
PLTP	0.6263	6.32e-6	2.29e-4
MS4A2	1.1392	2.00e-5	5.85e-4
C3	0.6112	2.79e-5	7.85e-4
NRP2	0.6326	3.07e-5	8.32e-4
LOX	0.5728	3.84e-5	9.34e-4
ELN	0.7150	5.49e-5	0.0012
CYBB	0.7993	6.14e-5	0.0013
CD68	0.6992	1.09e-4	0.0021
CXCL16	0.8650	1.19e-4	0.0022
CD209	0.7840	1.35e-4	0.0024
FCER1G	1.2622	1.40e-4	0.0024
C3AR1	0.9329	2.37e-4	0.0038
IRF8	0.7731	3.15e-4	0.0049
SPP1	1.1718	3.31e-4	0.0050
CTSB	0.5693	4.02e-4	0.0059
RAB7B	0.4428	4.10e-4	0.0059
NRIP3	0.9268	4.33e-4	0.0060
LGMN	0.6207	4.35e-4	0.0060
CD84	0.6423	4.58e-4	0.0061
MMP14	0.3262	4.78e-4	0.0062
CCL19	1.2252	4.80e-4	0.0062
NCKAP1L	0.5801	5.19e-4	0.0064
HMOX1	0.5864	5.20e-4	0.0064

CCL13	0.9219	8.95e-4	0.0092
CD163	0.5885	9.05e-4	0.0092
MMP7	1.2207	0.0011	0.0106
HLA-DPA1	0.5608	0.0013	0.0119
MS4A4A	0.6493	0.0013	0.0119
FN1	0.5062	0.0013	0.0119
SERPINE1	1.5204	0.0014	0.0122
LIPA	0.6483	0.0015	0.0129
S100A4	0.4946	0.0019	0.0158
VAMP8	0.5088	0.0021	0.0172
AP2S1	0.2137	0.0021	0.0172
NLRP3	1.1444	0.0022	0.0173
ATP6V1G2	1.8316	0.0023	0.0180
CTSD	0.2766	0.0023	0.0180
LOXL4	0.5650	0.0025	0.0189
ADCY7	0.5109	0.0028	0.0214
FBP1	0.6532	0.0030	0.0218
ANGPTL4	0.3924	0.0030	0.0219
NCEH1	0.5736	0.0034	0.0238
THBS1	0.8349	0.0037	0.0258
ADAM9	0.3295	0.0039	0.0266
SYK	0.5243	0.0040	0.0271
TXN	0.2331	0.0043	0.0282
ACSM3	1.7979	0.0043	0.0282
LOXL1	0.4136	0.0044	0.0282
CD14	0.6026	0.0045	0.0284
CCN2	0.7503	0.0045	0.0284
CYP27A1	0.5031	0.0047	0.0293
MMP9	0.8497	0.0049	0.0299
C9	3.1466	0.0051	0.0308
FGD2	0.4360	0.0051	0.0308
RELB	0.5090	0.0060	0.0359
CCL4	1.8639	0.0067	0.0383
SCIN	0.4900	0.0067	0.0383
IL12RB2	0.8784	0.0071	0.0400
LEP	0.5090	0.0080	0.0438
AMOTL2	0.3766	0.0086	0.0458
ADH6	2.1973	0.0086	0.0458
CD4	0.3783	0.0086	0.0458
TIMP2	0.1896	0.0086	0.0458
CFHR5	2.4348	0.0096	0.0503
PLCB2	0.5505	0.0098	0.0508

Downregulated genes (n = 78)

Gene	logFC	p-value	q-value
GLUT4	-1.4010	9.77e-15	7.43e-12
FASN	-1.1828	6.92e-11	2.63e-8
PC	-0.5810	1.60e-8	4.07e-6
PCK1	-1.1136	4.33e-8	8.24e-6
HADH	-0.6168	7.19e-8	1.09e-5
PDE3B	-0.5423	2.33e-7	2.53e-5
CDH5	-0.4566	5.20e-7	3.97e-5
NR1H3	-0.4114	5.22e-7	3.97e-5
HADHA	-0.3448	6.49e-7	4.48e-5
ACACB	-0.6410	7.77e-7	4.92e-5

PYGM	-0.6661	1.21e-6	6.65e-5
MAPK11	-0.4086	2.73e-6	1.23e-4
CEBPA	-0.5381	2.77e-6	1.23e-4
JAG2	-0.4067	2.90e-6	1.23e-4
KDR	-0.4664	5.78e-6	2.20e-4
FABP5	-0.3853	7.31e-6	2.43e-4
TXN2	-0.2448	7.34e-6	2.43e-4
PRKACA	-0.3003	7.69e-6	2.43e-4
ADH1B	-0.7668	1.02e-5	3.10e-4
NDUFB5	-0.3771	3.49e-5	9.12e-4
HSP90AB1	-0.2589	3.60e-5	9.12e-4
INSR	-0.2935	4.00e-5	9.34e-4
UQCRH	-0.3219	4.06e-5	9.34e-4
ALDH2	-0.5134	5.14e-5	0.0011
ACACA	-0.4791	5.47e-5	0.0012
STAT5B	-0.2243	7.87e-5	0.0016
VEGFA	-0.4594	9.63e-5	0.0019
ELOVL6	-0.9998	1.43e-4	0.0024
GATA3	-0.4702	1.43e-4	0.0024
PRDX6	-0.2926	1.86e-4	0.0031
TJP2	-0.3128	2.95e-4	0.0047
ADIPOQ	-0.3874	3.69e-4	0.0055
BLK	-1.0948	4.59e-4	0.0061
PGM1	-0.3668	5.28e-4	0.0064
HCAR2	-0.4585	5.37e-4	0.0064
LPL	-0.4208	5.38e-4	0.0064
PHLPP1	-0.3058	5.56e-4	0.0065
CDKN2C	-0.5854	5.77e-4	0.0066
ARRB1	-0.2037	5.90e-4	0.0067
NDUFA1	-0.2396	6.70e-4	0.0074
MAPK10	-0.3277	6.70e-4	0.0074
PCCB	-0.3310	7.23e-4	0.0078
PIK3CA	-0.1894	7.47e-4	0.0080
NDUFS3	-0.1619	7.85e-4	0.0083
TBC1D4	-0.2145	8.54e-4	0.0089
UQCRFS1	-0.2440	9.87e-4	0.0099
COX6B1	-0.2515	0.0010	0.0103
PPARG	-0.2958	0.0012	0.0117
NDUFB8	-0.1792	0.0013	0.0119
SMAD4	-0.1573	0.0013	0.0120
AMOTL1	-0.2485	0.0013	0.0120
FLT4	-0.4274	0.0015	0.0129
FABP4	-0.3652	0.0015	0.0129
IRS1	-0.4752	0.0018	0.0148
MMUT	-0.2952	0.0019	0.0158
CAT	-0.2750	0.0022	0.0176
NOTCH4	-0.3443	0.0027	0.0202
COX4I2	-0.3738	0.0029	0.0214
EPAS1	-0.2324	0.0032	0.0235
ANAPC1	-0.2002	0.0033	0.0237
PLCG1	-0.2298	0.0034	0.0240
C5	-0.5046	0.0035	0.0245
COX6A1	-0.1978	0.0040	0.0271
SLC25A10	-0.3887	0.0044	0.0282
CSNK1E	-0.1994	0.0044	0.0282
DEPDC5	-0.1524	0.0046	0.0286

CUL1	-0.1475	0.0048	0.0298
COX7C	-0.2631	0.0059	0.0351
STAT5A	-0.2292	0.0062	0.0364
SCD	-0.8793	0.0062	0.0365
MLXIPL	-0.3500	0.0063	0.0367
CETP	-0.9444	0.0070	0.0399
GSK3B	-0.1709	0.0074	0.0412
DLL1	-0.5786	0.0076	0.0423
PDHA1	-0.2799	0.0080	0.0439
SMARCC2	-0.1469	0.0093	0.0492
NDUFC1	-0.1354	0.0098	0.0509
SUGT1	-0.1185	0.0099	0.0509

Supplemental Table 9. Results from a multivariable linear regression examining the relationship between Endotrophin (ETP) plasma levels and HIV status, sex, race, age, and whole-body percent fat (%BF).

Predictor	β	95% CI	P value
HIV status (PWH vs PWoH)	0.96	(0.28, 1.64)	*0.006*
Sex (male vs female)	0.00	(−0.76, 0.76)	1.000
Age (years)	0.02	(−0.003, 0.046)	0.079
Body fat (%)	0.04	(−0.003, 0.085)	0.068
Race (Black vs White)	−0.35	(−1.23, 0.53)	0.432
Race (Other vs White)	−0.45	(−1.08, 0.17)	0.152

Multivariate OLS regression of plasma endogenous thrombin potential (ETP) on HIV status, adjusted for sex, age, percent body fat, and race. ETP was Box-Cox transformed ($\lambda = 0.29$) to satisfy normality and homoscedasticity assumptions prior to model fitting. β coefficients represent the estimated change in transformed ETP per unit increase in each continuous predictor or relative to the reference category for categorical variables. Reference categories: HIV-negative (PWoH) for HIV status, female for sex, and White for race. "Other" race includes Asian, Native Hawaiian/Pacific Islander, and more than one race. N = 119. Statistically significant associations ($p < 0.05$) are italicized.

Supplemental Table 10. Multivariable linear regression models restricted to people with HIV (PWH) examining associations between plasma endotrophin (ETP) levels, antiretroviral treatment characteristics, adiposity and demographic factors.

Predictor	Model 1		Model 2		Model 3		Model 4	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Legacy d-drug exposure (yes vs no)	0.18 (-0.27, 0.62)	0.44	0.24 (-0.23, 0.71)	0.31	0.08 (-0.36, 0.53)	0.70	0.19 (-0.27, 0.65)	0.41
Current INSTI use (yes vs no)	-0.01 (-0.58, 0.55)	0.96	0.00 (-0.57, 0.57)	0.99	0.04 (-0.52, 0.59)	0.89	0.02 (-0.56, 0.61)	0.93
CD4+ T-cell count	0.0003 (-0.0003, 0.0009)	0.37	0.0003 (-0.0003, 0.0009)	0.27	0.0003 (-0.0003, 0.0008)	0.35	0.0003 (-0.0003, 0.0009)	0.33
%BF	0.01 (-0.02, 0.04)	0.39	0.02 (-0.01, 0.06)	0.21	0.01 (-0.02, 0.04)	0.38	0.01 (-0.02, 0.04)	0.50
Sex (Male vs Female)	—	—	0.30 (-0.29, 0.89)	0.31	—	—	—	—
Age (years)	—	—	—	—	0.02 (-0.00, 0.04)	0.06	—	—
Race: Black (vs White)	—	—	—	—	—	—	-0.10 (-0.53, 0.34)	0.66
Race: Other (vs White)	—	—	—	—	—	—	-0.41 (-1.24, 0.42)	0.32

Model 1 (base): legacy d-drug exposure, current INSTI use, CD4+ T-cell count, and %BF. Model 2: Model 1 + sex. Model 3: Model 1 + age. Model 4: Model 1 + race. ETP was Box-Cox transformed ($\lambda = 0.09$) prior to model fitting. β coefficients represent the estimated change in transformed ETP per unit increase in each continuous predictor or relative to the reference category for categorical variables. Reference categories: no legacy d-drug exposure, no INSTI use, female for sex, White for race. “Other” race includes Asian, Native Hawaiian/Pacific Islander, and more than one race. — indicates variable not included in that model. Statistically significant associations ($p < 0.05$) are italicized.

Supplemental Table 11. Adiposity-stratified multivariable linear regression models examining associations between HIV status and plasma endotrophin (ETP) levels among individuals with normal body fat (NBF) and high body fat (HBF).

Predictor	NBF (n = 51)		HBF (n = 68)	
	β (95% CI)	p-value	β (95% CI)	p-value
HIV status	0.98 (0.03, 2.00)	*0.037*	0.63 (−0.31, 1.56)	0.186
Sex (Male)	0.48 (−0.97, 1.93)	0.513	0.66 (−0.60, 1.91)	0.299
Age	0.03 (−0.01, 0.06)	0.120	0.02 (−0.01, 0.05)	0.238
Race: Black	−0.97 (−2.28, 0.34)	0.143	0.18 (−1.01, 1.36)	0.768
Race: Other	−0.45 (−1.40, 0.50)	0.344	−0.56 (−1.42, 0.29)	0.192
%BF	0.05 (−0.09, 0.18)	0.463	*0.13 (0.04, 0.21)*	*0.005*

Multivariate OLS regression of Box-Cox–transformed plasma (ETP) on HIV status, stratified by body fat group and adjusted for sex, age, %BF, and race. ETP was Box-Cox transformed ($\lambda = 0.27$ for NBF, $\lambda = 0.30$ for HBF) to satisfy normality and homoscedasticity assumptions prior to model fitting. β coefficients represent the estimated change in transformed ETP per unit increase in each continuous predictor or relative to the reference category for categorical variables. Reference categories: HIV-negative (PWoH) for HIV status, female for sex, and White for race. "Other" race includes Asian, Native Hawaiian/Pacific Islander, and more than one race. Statistically significant associations ($p < 0.05$) are italicized.