Supplementary materials

Maternal and child immune profiles are associated with neurometabolite measures of early-life neuroinflammation in children who are HIV-exposed and uninfected: a South African birth cohort

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1 Supplementary Table 1. Studies reporting peripheral blood immune marker alterations in mothers living with HIV and/or children who are HIV-exposed and uninfected

Legend: Children (a); Pregnant women (b)

Controls are either defined as (a) children born to mothers who are not living with HIV; or (b) pregnant women not living with HIV

Cases are either defined as (a) children born to mothers who are living with HIV; or (b) pregnant women living with HIV

Study, year		Country	Technique	N controls	N cases	Timepoint / Age	Reported differences in serum marker levels between groups
(1)	Sachdeva *et al.,* 2008	United States	Biochip Array	15	35	Pregnancy, gestational age 12–15 weeks	**TNFα** was higher in women living with HIV compared to women without HIV.
(2)	Richardson *et al*., 2011	United States	ELISA & CLIA	18	20	Pregnancy, 2nd and 3rd trimesters	**IFN-γ**, **IL1**, **IL4**, **IL8**, **IL10**, and **TNFα** were higher in women living with HIV compared to women without HIV. There were no significant changes in plasma cytokines and other biomarkers from early to late pregnancy.
(3)	López *et al*., 2016	Spain	ELISA	36	36	1st trimester	In both trimesters, **CD14** was higher in women living with HIV compared to women without HIV.
						3rd trimester	
(4)	Maharaj *et al.,* 2017	South Africa	CBA	50	45	Pregnancy, gestational age 35–36 weeks	**IL-2**, **IL-6**, and **TNF-α** were lower in women living with HIV compared to women without HIV.
(5)	Prendergast *et al*., 2017	Zimbabwe	ELISA	197	194	6 weeks	No group differences in **IL-6** levels were detected at any timepoint.
						6 months	
(6)	Evans *et al*., 2017	Zimbabwe	ELISA	97	223	6 weeks	**C-reactive protein** was significantly higher in HEU infants compared to their HU peers.
(7)	Miyamoto *et al*., 2017	Brazil	Luminex & ELISA	20	19	Birth	No group differences were detected.
				19	19	12 months	A slight decay in **CD14** levels was observed from 12 months to 6–12 years in HEU children. At 6–12 years, **IL-4** was higher in HEU compared to HU children.
				18	20	6–12 years	
(8)	Dirajlal-Fargo *et al.*, 2019	Brazil	ELISA	88	86	Delivery	**IL-6** and **CD14** were higher in women living with HIV compared to women without HIV.
				88	86	Birth	**IL-6** and **CD14** were higher in HEU compared to HU infants.
						6 months	**IL-6** remained significantly higher in HEU infants.
(9)	Ray *et al*., 2019	Kenya	Luminex	43	44	Delivery	**IFN-γ**, **IL-1β**, **IL-6**, **IL-10**, **IL-12p70**, **IL-17A**, **IL-17E**, **IL-17F**, **IL-21**, **IL-22**, **IL-23**, and **TNFα** were significantly lower in mothers with HIV compared to those without HIV.

				43		44		Birth	No difference was detected for any cytokine measured in cord blood between HEU and HU neonates.
(10)	Shafiq *et al*., 2021	India	Luminex	149		69		Pregnancy, gestational age 28–30 weeks	Higher **IL-1β** levels during pregnancy were associated with preterm birth in women living with and without HIV. Higher **CD14** levels during pregnancy were associated with growth deficits at birth in mothers living with HIV.
(11)	Sevenoaks *et al*., 2021	South Africa	Luminex & ELISA	190		77		Pregnancy, gestational age ≈26 weeks	**GM-CSF** and **MMP-9** were lower in mothers living with HIV compared to mothers without HIV. **IL-1β** and **IL-4** were also lower in mothers living with HIV prior to correction for multiple comparisons. In mothers living with HIV, and prior to correction for multiple comparisons, **IFN-γ**, **IL-10**, **IL-12p70** and **IL-7** were associated with lower composite scores for language in HEU children at 24–28 months, and **TNF-α** was associated with lower cognitive scores.
				159		63		6–10 weeks	**IFN-γ** and **IL-1β** were lower in HEU compared to HU infants. **IL-12p70** and **IL-4** were also lower in HEU infants prior to correction for multiple comparisons. In HEU infants, **GM-CSF**, **IFN-γ**, **IL-10**, **IL-12p70**, **IL-1β**, **IL-2**, **IL-4**, **IL-6**, and **NGAL** were associated with motor development at 24–28 months. Prior to correction for multiple comparisons, **MMP-9** was associated with motor and language outcomes, and **IL-1β** was associated with language outcomes.
				190		77		24–28 months	**IFN-γ**, **IL-1β**, **IL-2** and **IL-4** were lower in HEU compared to HU children. In HEU children, and prior to correction for multiple comparisons, **IL-10** was associated with lower cognitive scores.
(12)	Akoto *et al*., 2021	South Africa	Luminex	68		56		Pregnancy, 1st trimester	Detection of **IL-1β**, **IFN-β** and **IFN-λ2/3** was lower in women living with HIV compared to those without HIV. Detection of **IP-10**, **IL-2**, **IL-5**, **IL-6**, **IL-9**, **IL-10**, and **IL-17A** was higher in women living with HIV compared to those without HIV.
								2nd trimester	Detection of **IL-1β**, **IFN-β** and **IFN-λ2/3** was lower in women living with HIV compared to those without HIV. Detection of **IFN-λ1**, **IP-10**, **IL-2**, **IL-5**, **IL-10**, **IL-12p70**, and **IL-17A** was higher in women living with HIV compared to those without HIV.
								3rd trimester	Detection of **IFN-β** and **IFN-λ2/3** was lower in women living with HIV compared to those without HIV. Detection of **IFN-λ1**, **IP-10**, and **IL-5** was higher in women living with HIV compared to those without HIV.
(13)	Schnittman *et al*., 2021	Uganda	ELISA	—	—	759		Pre-pregnancy, 1st, 2nd, and 3rd trimester pregnancy, and postpartum	**IL-6** declined by 29% in the 1st trimester but increased toward pre-pregnancy baseline by the 3rd trimester. **CD14** declined by 17%–18% in the 1st and 2nd trimesters. **CD27** and **CD163** declined in the 1st trimester. **IP-10** declined by 30%–40% during pregnancy.
(14)	Vyas *et al*., 2021	India	Luminex	150		70		Pregnancy, gestational age 13–34 weeks	In the second trimester, **CD14**, **TNFα**, **IL-6**, and **IL-17a** were higher, and **CD163** lower, in pregnant women living with HIV compared to women without HIV. In the third trimester, **CD14** and **IL-6** were higher in women living with HIV compared to women without HIV.

(15)	Shiau *et al*., 2023	United States	EIA	76	🚺	188	🚺	Pregnancy, gestational age 13–27 weeks	**IL-6**, **CD14**, and **CD163** were higher in pregnant people living with HIV compared to those without HIV. Among people living with HIV, **CD14** and **CD163** were higher in those with perinatally acquired HIV versus non-perinatally acquired HIV.
(16)	Bebell *et al*., 2024	Uganda	Luminex	142	🚺	147	🚺	Delivery	Partial Least Squares Discriminant Analysis identified top markers distinguishing cytokine profiles, which included higher **IL-5** in pregnant women living with HIV, higher **IL-8** and **MIP-1α** in women without HIV, and higher **RANTES** and **E-selectin** in umbilical cord plasma from HU newborns.
(17)	Ray *et al.*, 2024	Kenya	Luminex	58	👶	59	👶	From birth to 54 weeks of age	There were no significant interaction effects between maternal HIV status and time for any of the cytokines measured, indicating that cytokine trajectories did not differ between HEU and HU children.
(18)	Hindle *et al*., 2024	Canada	Luminex	22	🚺	144	🚺	Pregnancy, 2nd and 3rd trimesters	In both trimesters, **AGP** was higher and **IFN-β** lower in pregnant people living with HIV compared to those without HIV. In the second trimester, **HMGB1**, **IFN-γ**, and **IFN-α** were lower in pregnant people living with HIV compared to those without HIV.
(19)	Yin *et al*., 2024	United States	Luminex	18	🚺	46	🚺	Within 2 days prior to delivery	**IL-1β**, **IL-21**, **TNF-α**, **CCL5**, **CXCL9**, **sCD27**, **sCD40L**, and **sCD163** were higher in pregnant women living with HIV compared to those without HIV, whereas **APRIL** was lower. **CXCL9** and **CXCL10** were significantly higher in pregnant women living with HIV who were not virally suppressed compared to those who were virally suppressed.
				50	👶	46	👶	Birth	**IL-1β**, **IL-6**, **TNF-α**, **IL-10**, **IL-1RA**, **IL-21**, **IL-22**, **CCL4**, **CXCL9**, **sCD14**, **sCD27**, **sCD40L**, **sCD163**, and **APRIL** were significantly higher in HEU newborns compared to HU.
				50	👶	46	👶	6 months	**IL-1β**, **TNF-α**, **IL-21**, **CCL4**, **sCD14**, **sCD40L**, and **APRIL** were significantly higher in HEU children compared to HU.

CLIA: Chemiluminescence Immunoassay; **ELISA**: Enzyme-Linked Immunosorbent Assay; **CBA**: Cytometric Bead Array; **EIA**: Enzyme Immunoassay; **HEU**: HIV-exposed uninfected; **HU**: HIV-unexposed.

References

1. Sachdeva N, Oshima K, Cotter A, Ashman M, Davila L, Okazaki T, *et al*. Analysis of Immunological Markers Associated with Pregnancy and HIV-1 Infection: Relevance in Perinatal Transmission in HIV-1-Infected Pregnant Women with Low Plasma Viral Load. *Am J Reprod Immunol* (2008) 60(3):264–73

2. Richardson K, Weinberg A. Dynamics of Regulatory T-Cells during Pregnancy: Effect of HIV Infection and Correlations with Other Immune Parameters. *PLoS One* (2011) 6(11):e28172

3. López M, Figueras F, Coll O, Goncé A, Hernández S, Loncá M, *et al*. Inflammatory Markers Related to Microbial Translocation Among HIV-Infected Pregnant Women: A Risk Factor of Preterm Delivery. *J Infect Dis* (2016) 213(3):343–50

4. Maharaj NR, Phulukdaree A, Nagiah S, Ramkaran P, Tiloke C, Chuturgoon AA. Pro-Inflammatory Cytokine Levels in HIV Infected and Uninfected Pregnant Women with and without Preeclampsia. *PLoS One* (2017) 12(1):e0170063

5. Prendergast AJ, Chasekwa B, Rukobo S, Govha M, Mutasa K, Ntozini R, *et al*. Intestinal Damage and Inflammatory Biomarkers in Human Immunodeficiency Virus (HIV)–Exposed and HIV-Infected Zimbabwean Infants. *J Infect Dis* (2017) 216(6):651–61

6. Evans C, Chasekwa B, Rukobo S, Govha M, Mutasa K, Ntozini R, *et al*. Cytomegalovirus Acquisition and Inflammation in Human Immunodeficiency Virus–Exposed Uninfected Zimbabwean Infants. *J Infect Dis* (2016) 215(5):698.

7. Miyamoto M, Gouvêa AFTB, Ono E, Succi RCM, Pahwa S, de Moraes-Pinto MI. Immune development in HIV-exposed uninfected children born to HIV-infected women. *Rev Inst Med Trop Sao Paulo* (2017) 59:e30

8. Dirajlal-Fargo S, Mussi-Pinhata MM, Weinberg A, Yu Q, Cohen R, Harris DR, *et al*. HIV-exposed-uninfected infants have increased inflammation and monocyte activation. *AIDS* (2019) 33(5):845–53

9. Ray JE, Dobbs KR, Ogolla SO, Daud II, Vulule J, Sumba PO, *et al*. Reduced Transplacental Transfer of Antimalarial Antibodies in Kenyan HIV-Exposed Uninfected Infants. *Open Forum Infect Dis* (2019) 6(6)

10. Shafiq M, Mathad JS, Naik S, Alexander M, Yadana S, Araújo-Pereira M, *et al*. Association of Maternal Inflammation During Pregnancy With Birth Outcomes and Infant Growth Among Women With or Without HIV in India. *JAMA Netw Open* (2021) 4(12):e2140584–e2140584

11. Sevenoaks T, Wedderburn CJ, Donald KA, Barnett W, Zar HJ, Stein DJ, *et al*. Association of maternal and infant inflammation with neurodevelopment in HIV-exposed uninfected children in a South African birth cohort. *Brain Behav Immun* (2021) 91:65–73

12. Akoto C, Norris SA, Hemelaar J. Maternal HIV infection is associated with distinct systemic cytokine profiles throughout pregnancy in South African women. *Sci Rep* (2021) 11(1):1–15

13. Schnittman SR, Byakwaga H, Boum Y, Kabakyenga J, Matthews LT, Burdo TH, *et al*. Changes in Immune Activation During Pregnancy and the Postpartum Period in Treated HIV Infection. *Open Forum Infect Dis* (2021) 8(6)

14. Vyas P, Mathad JS, Leu CS, Naik S, Alexander M, Araújo-Pereira M, *et al*. Impact of HIV status on systemic inflammation during pregnancy. *AIDS* (2021) 35(14):2259–68

15. Shiau S, Jacobson DL, Huo Y, Kacanek D, Yee LM, Williams DB, *et al*. Unique Profile of Inflammation and Immune Activation in Pregnant People With HIV in the United States. *J Infect Dis* (2023) 227(5):720–30

16. Bebell LM, Ngonzi J, Butler A, Kumbakumba E, Adong J, Loos C, *et al*. Distinct cytokine profiles in late pregnancy in Ugandan people with HIV. *Sci Rep* (2024) 14(1):1–12

17. Ray JE, Dobbs KR, Ogolla SO, Daud II, Midem D, Omenda MM, *et al*. Clinical and immunological outcomes of HIV-exposed uninfected and HIV-unexposed uninfected children in the first 24 months of life in Western Kenya. *BMC Infect Dis* (2024) 24(1):1–13.

18. Hindle S, Girard S, Cote HCF, Money D, Mann E, Boucoiran I. Circulating levels of inflammatory mediators in pregnant people living with HIV according to antiretroviral therapy regimen. *Front Microbiol* (2023) 8;14:1282291

19. Yin L, Venturi GM, Barfield R, Fischer BM, Kim-Chang JJ, Chan C, *et al*. Maternal immunity shapes biomarkers of germinal center development in HIV-exposed uninfected infants. *Front Immunol* (2024) 15:1443886

2 Supplementary Table 2

2A. Sociodemographic characteristics of the subset of children included in the MRS analysis

	DCHS complete MRS subset (N=83)		
	CHEU (n=36) Median (IQR) or n/N (%)	**CHU (n=47)** Median (IQR) or n/N (%)	**P value**
Child age at scan (in months)	34.00 (2.00)	35.00 (1.00)	0.21
Sex			0.14
Female	11/36 (30.6%)	23/47 (48.9%)	
Male	25/36 (69.4%)	24/47 (51.1%)	
Monthly household income (in ZAR)			0.98
<R1,000	10/36 (27.8%)	14/47 (29.8%)	
R1,000–5,000	23/36 (63.9%)	29/47 (61.7%)	
>R5,000	3/36 (8.3%)	4/47 (8.5%)	
Maternal education			0.83
Primary	3/36 (8.3%)	3/47 (6.4%)	
Some secondary	22/36 (61.1%)	26/47 (55.3%)	
Completed secondary	10/36 (27.8%)	15/47 (31.9%)	
Tertiary	1/36 (2.8%)	3/47 (6.4%)	
Employed mother	9/36 (25.0%)	9/47 (19.1%)	0.71
Maternal relationship status (partnered)	19/36 (52.8%)	17/47 (36.2%)	0.20
Maternal age at delivery (in years)	29.49 (6.15)	24.93 (6.74)	**<0.0001**
Gestational age at delivery (in weeks)	39.00 (2.25)	39.00 (2.00)	0.66
Premature birth (<37 weeks' gestation)	5/36 (13.9%)	6/47 (12.8%)	1.00
Child birthweight (in grams)	3120.00 (547.50)	3210.00 (645.00)	0.26
Exclusive breastfeeding for 5 or more months	7/36 (19.4%)	4/47 (8.5%)	0.26
Exclusive breastfeeding duration (in months)	0.92 (3.10)	1.84 (2.04)	0.07
Nutritional status at 2 years old			
Stunting (height-for-age Z-score < -2)	6/31 (19.4%)	3/43 (7.0%)	0.20
Underweight (weight-for-age Z-score < -2)	1/32 (3.1%)	1/43 (2.3%)	0.90
Wasting (weight-for-length Z-score < -2)	1/32 (3.1%)	1/43 (2.3%)	0.90

Maternal anaemia during pregnancy	11/36 (30.6%)	14/47 (29.8%)	1.00
Maternal smoking during pregnancy	7/36 (19.4%)	17/47 (36.2%)	0.16
Maternal alcohol use during pregnancy	4/34 (11.8%)	11/46 (23.9%)	0.28
Maternal depression during pregnancy	1/28 (3.6%)	11/42 (26.2%)	**0.019**
Maternal hospitalization during pregnancy	3/36 (8.3%)	4/47 (8.5%)	1.00
Maternal HIV diagnosis timepoint			
Before pregnancy	16/36 (44.4%)		
During pregnancy	20/36 (55.6%)		
Maternal lowest CD4 cell count during pregnancy[§]			
≤500 cells/mm^3	13/26 (50.0%)		
>500 cells/mm^3	13/26 (50.0%)		
Highest maternal viral load during pregnancy			
(undetectable) <40 copies/mL	24/29 (82.8%)		
40–1000 copies/mL	2/29 (6.9%)		
>1000 copies/mL	3/29 (10.3%)		
Antiretroviral therapy initiation			
Before pregnancy	16/36 (44.4%)		
During pregnancy	20/36 (55.6%)		
First-line antiretroviral therapy during pregnancy			
Efavirenz + Emtricitabine + Tenofovir (FDC)	33/36 (91.7%)		
Lamivudine + Zidovudine + Nevirapine	2/36 (5.6%)		
Lamivudine + Zidovudine + Efavirenz	1/36 (2.8%)		
Cotrimoxazole prophylaxis	31/32 (96.9%)		
Infant prophylaxis			
Nevirapine monotherapy	28/36 (77.8%)		
Nevirapine + zidovudine	8/36 (22.2%)		

Data are median (IQR) or n/N (%). Percentages calculated out of available data. Continuous data was assessed for normality using Shapiro-Wilk tests. Comparisons between CHEU and CHU were made using Wilcoxon Rank Sum (Mann Whitney U) tests for continuous data, and X^2 tests for categorical data. **DCHS**: Drakenstein Child Health Study; **CHEU**: Children who are HIV-Exposed and Uninfected; **CHU**: Children who are HIV-Unexposed; **ZAR**: South African Rand; **FDC**: Fixed Dose Combination.

Missing data: nutritional conditions at 2 years old (n=5 in the CHEU group, n=4 in the CHU group); maternal alcohol use during pregnancy (n=2 in the CHEU group, n=1 in the HU group); maternal depression during pregnancy (n=8 in the CHEU group, n=5 in the HU group); maternal lower CD4 cell count during pregnancy (n=10); maternal highest viral load during pregnancy (n=7); cotrimoxazole prophylaxis (n=4). §The lowest maternal CD4 cell count within 1 year before birth and 3 months after birth was used to maximise numbers.

2B. Sociodemographic characteristics of the subset of children included in the MRS analysis, compared to all children invited for neuroimaging at age 2–3 years

	DCHS neuroimaging cohort at age 2 years		
	Complete MRS subset (N=83) Median (IQR) or n/N (%)	**Original cohort (N=156)** Median (IQR) or n/N (%)	**p value**
Child age at scan (in months)	34.00 (2.00)	34.00 (2.00)	0.71
Sex			0.95
Male	49/83 (59.0%)	90/156 (57.7%)	
Female	34/83 (41.0%)	66/156 (42.3%)	
Monthly household income (in ZAR)			0.67
<R1,000	24/83 (28.9%)	37/156 (23.7%)	
R1,000–5,000	52/83 (62.7%)	104/156 (66.7%)	
>R5,000	7/83 (8.4%)	15/156 (9.6%)	
Maternal education			0.96
Primary	6/83 (7.2%)	9/156 (5.8%)	
Some secondary	48/83 (57.8%)	92/156 (59.0%)	
Completed secondary	25/83 (30.1%)	46/156 (29.5%)	
Tertiary	4/83 (4.8%)	9/156 (5.8%)	
Employed mother	18/83 (21.7%)	42/156 (26.9%)	0.46
Maternal relationship status (partnered)	36/83 (43.4%)	74/156 (47.4%)	0.64
Maternal age at delivery (in years)	27.09 (7.43)	27.26 (7.56)	0.69
Gestational age at delivery (in weeks)	39.00 (2.00)	39.00 (2.00)	0.99
Premature birth (<37 weeks' gestation)	11/83 (13.3%)	20/156 (12.8%)	1.00
Birthweight (in grams)	3170.00 (540.00)	3180.00 (657.50)	0.87
Exclusive breastfeeding for 5 or more months	11/83 (13.3%)	18/156 (11.5%)	0.86
Exclusive breastfeeding duration (in months)	1.84 (2.42)	1.00 (2.54)	0.31
Nutritional status at 2 years old			
Stunting (height-for-age Z-score < -2)	9/74 (12.2%)	18/138 (13.0%)	0.97
Underweight (weight-for-age Z-score < -2)	2/75 (2.7%)	134/139 (96.4%)	0.89
Wasting (weight-for-length Z-score < -2)	2/75 (2.7%)	5/139 (3.6%)	0.89

Maternal anaemia during pregnancy	25/83 (30.1%)	48/156 (30.8%)	1.00
Maternal smoking during pregnancy	24/83 (28.9%)	41/156 (26.3%)	0.78
Maternal alcohol use during pregnancy	15/80 (18.8%)	25/152 (16.4%)	0.82
Maternal depression during pregnancy	12/70 (17.1%)	30/128 (23.4%)	0.53
Maternal hospitalization during pregnancy	7/83 (8.4%)	10/154 (6.5%)	0.50
Maternal HIV status			0.98
Positive	36/83 (43.4%)	66/156 (42.3%)	
Negative	47/83 (56.6%)	90/156 (57.7%)	

Data are median (IQR) or n/N (%). Percentages calculated out of available data. Continuous data was assessed for normality using Shapiro-Wilk tests. Comparisons between CHEU and CHU were made using Wilcoxon Rank Sum (Mann Whitney U) for continuous data, and X^2 tests for categorical data. **DCHS**: Drakenstein Child Health Study; **CHEU**: Children who are HIV-Exposed and Uninfected; **CHU**: Children who are HIV-Unexposed; **ZAR**: South African Rand; **FDC**: Fixed Dose Combination.

Missing data: nutritional conditions at 2 years old (n=9 in the complete MRS subset, n=18 in the full cohort); maternal hospitalization during pregnancy (n=2 in the full cohort); maternal alcohol use during pregnancy (n=3 in the complete MRS subset, n=4 in the full cohort); maternal depression during pregnancy (n=13 in the complete-case cohort, n=28 in the original cohort).

3 Supplementary Table 3. Maternal, infant, and child serum marker concentrations

3.1 Maternal serum marker concentrations during pregnancy (log-scaled)

Biomarker	Mothers not living with HIV (n=78)	Mothers living with HIV (n=60)	Effect size		95% CI	P-value	BH
GM-CSF	3.76 ± 0.98	3.29 ± 0.89	0.47	0.16	0.79	**0.004**	**0.034**
IFN-γ	2.27 (1.1)	2.29 (1.45)	-0.08	-0.33	0.18	0.54	0.88
IL-1β	0.58 (1.02)	0.54 (1.32)	0.09	-0.18	0.36	0.51	0.88
IL-2	0.77 (1.18)	0.78 (1.58)	0.02	-0.30	0.34	0.93	0.98
IL-5	0.76 ± 0.77	0.82 ± 0.88	-0.06	-0.35	0.22	0.66	0.90
IL-6	0.67 (1.59)	0.50 (1.64)	0.00	-0.45	0.44	0.98	0.98
IL-7	2.31 (0.8)	2.25 (0.92)	-0.01	-0.21	0.19	0.88	0.98
IL-8	1.28 (0.91)	1.26 (0.88)	-0.06	-0.32	0.20	0.67	0.98
TNFα	1.58 ± 0.55	1.71 ± 0.57	-0.14	-0.33	0.06	0.16	0.49
IL-4	3.51 (1.68)	2.99 (2.06)	0.32	-0.13	0.73	0.18	0.66
IL-10	2.18 ± 1.01	2.15 ± 1.08	0.03	-0.32	0.39	0.85	0.90
IL-12p70	1.25 (1.28)	1.24 (1.18)	0.03	-0.23	0.32	0.77	0.98
IL-13	1.55 (1.43)	1.19 (1.64)	0.25	-0.17	0.67	0.26	0.77
CD14	7.46 ± 0.34	7.59 ± 0.38	-0.13	-0.25	0.00	**0.044**	0.20
CD163	6.32 ± 0.48	6.31 ± 0.5	0.02	-0.15	0.19	0.82	0.90
NGAL	5.21 ± 0.51	4.99 ± 0.56	0.22	0.04	0.40	**0.018**	0.11
MMP-9	7.11 ± 0.62	6.64 ± 0.75	0.48	0.24	0.72	**0.0001**	**0.002**
YKL-40	3.51 (0.87)	3.61 (1.03)	-0.12	-0.38	0.13	0.32	0.77

T-Test for normally-distributed data; Wilcoxon Rank-Sum Test (Mann-Whitney U Test) for not normally-distributed data. Data presented as mean ±SD or median (IQR) per each group.

BH: Benjamini-Hochberg corrected p-value.

3.2 Infant serum marker concentrations at 6 weeks of age (log-scaled)

Biomarker	CHU (n=56)	CHEU (n=41)	Effect size	95% CI		P-value	BH
GM-CSF	2.68 (1.07)	2.35 (1.08)	0.24	-0.11	0.59	0.19	0.86
IFN-γ	1.43 ± 0.97	1.41 ± 1.03	0.02	-0.39	0.44	0.91	0.99
IL-1β	-0.08 ± 1.05	-0.42 ± 1.00	0.34	-0.08	0.76	0.11	0.66
IL-2	0.01 (1.46)	0.10 (1.43)	0.01	-0.41	0.45	0.95	0.97
IL-5	0.41 (0.96)	0.44 (0.89)	-0.12	-0.47	0.20	0.42	0.97
IL-6	0.49 (2.08)	0.35 (1.84)	0.01	-0.55	0.56	0.96	0.97
IL-7	1.68 (0.96)	1.76 (0.82)	-0.09	-0.36	0.16	0.49	0.97
IL-8	1.86 (0.65)	2.01 (1.07)	-0.08	-0.35	0.21	0.56	0.97
TNFα	2.79 ± 0.42	2.87 ± 0.71	-0.08	-0.33	0.17	0.52	0.99
IL-4	2.20 (2.62)	1.93 (2.02)	0.08	-0.47	0.64	0.77	0.97
IL-10	2.46 ± 0.82	2.49 ± 0.65	-0.03	-0.32	0.27	0.86	0.99
IL-12p70	0.60 ± 1.00	0.45 ± 0.85	0.15	-0.22	0.52	0.43	0.99
IL-13	1.24 (1.70)	1.10 (1.50)	0.04	-0.45	0.57	0.88	0.97
CD14	7.38 ± 0.28	7.46 ± 0.26	-0.09	-0.20	0.02	0.11	0.66
CD163	6.41 ± 0.45	6.46 ± 0.50	-0.05	-0.25	0.14	0.60	0.99
NGAL	4.50 (0.53)	4.32 (0.47)	0.19	0.02	0.35	**0.032**	0.51
MMP-9	5.79 (0.51)	5.71 (1.05)	0.07	-0.22	0.35	0.66	0.97
YKL-40	3.43 ± 0.53	3.37 ± 0.46	0.06	-0.14	0.26	0.56	0.99

T-Test for normally-distributed data; Wilcoxon Rank-Sum Test (Mann-Whitney U Test) for not normally-distributed data. Data presented as mean ±SD or median (IQR) per each group.

CHU: Children who are HIV-Unexposed; **CHEU**: Children who are HIV-Exposed and Uninfected; **BH**: Benjamini-Hochberg corrected p-value.

3.3 Child serum marker concentrations at 2 years of age (log-scaled)

Biomarker	CHU (n=65)	CHEU (n=46)	Effect size	95% CI		P-value	BH
GM-CSF	4.62 ± 0.88	4.44 ± 0.85	0.18	-0.15	0.52	0.27	0.63
IFN-γ	2.12 ± 0.68	1.97 ± 0.65	0.15	-0.10	0.41	0.24	0.63
IL-1β	0.71 (1.57)	0.13 (1.13)	0.33	0.04	0.66	**0.031**	0.27
IL-2	0.89 (1.30)	0.63 (0.87)	0.28	-0.01	0.55	0.06	0.27
IL-5	1.06 (0.85)	1.06 (0.71)	0.03	-0.19	0.27	0.75	0.82
IL-6	1.20 (0.83)	0.95 (0.96)	0.17	-0.16	0.46	0.28	0.47
IL-7	2.16 ± 0.54	2.09 ± 0.43	0.07	-0.11	0.26	0.43	0.65
IL-8	2.23 (1.05)	2.23 (1.43)	0.08	-0.31	0.44	0.62	0.75
TNFα	2.49 (0.72)	2.46 (0.64)	-0.03	-0.22	0.18	0.79	0.82
IL-4	3.66 (1.18)	3.28 (1.65)	0.36	-0.01	0.74	0.06	0.27
IL-10	2.82 ± 0.77	2.70 ± 0.53	0.12	-0.13	0.36	0.34	0.63
IL-12p70	1.55 (1.35)	1.26 (1.05)	0.22	0.00	0.44	**0.048**	0.27
IL-13	2.23 ± 1.03	1.96 ± 0.93	0.27	-0.10	0.64	0.15	0.63
CD14	7.71 ± 0.34	7.65 ± 0.34	0.06	-0.07	0.19	0.35	0.63
CD163	6.52 ± 0.42	6.61 ± 0.47	-0.09	-0.26	0.08	0.29	0.63
NGAL	5.21 ± 0.63	5.14 ± 0.62	0.07	-0.17	0.31	0.56	0.72
MMP-9	6.64 ± 0.64	6.69 ± 0.60	-0.05	-0.28	0.19	0.70	0.79
YKL-40	3.27 (0.83)	3.36 (0.92)	-0.07	-0.33	0.16	0.58	0.75

T-Test for normally-distributed data; Wilcoxon Rank-Sum Test (Mann-Whitney U Test) for not normally-distributed data. Data presented as mean ±SD or median (IQR) per each group.

CHU: Children who are HIV-Unexposed; **CHEU**: Children who are HIV-Exposed and Uninfected; **BH**: Benjamini-Hochberg corrected p-value.

4 Supplementary Table 4. Linear Mixed-Effects Models to examine child trajectories in serum marker concentrations from 6 weeks to 2 years of age

	Fixed effects							Random effects		Model fit			
Serum marker	**Baseline** log(levels)	**Effect of time** (p-value)		**Effect of maternal HIV** (p-value)		**Interaction** (p-value)		**Intercept**	**Residual SD**	**AIC**	**BIC**	**LogLik**	**Deviance**
GM-CSF	0.09	-0.01	(0.91)	-0.21	(0.15)	0.01	(0.95)	0.18	0.42	594.96	614.99	-291.48	582.96
IFN-γ	0.10	≈0.00	(0.95)	-0.08	(0.63)	-0.12	(0.42)	0.72	0.69	576.56	596.58	-282.28	564.56
IL-1β	0.17	-0.05	(0.64)	-0.30	(0.06)	0.02	(0.92)	0.67	0.71	573.70	593.72	-280.85	561.70
IL-2	0.08	0.05	(0.64)	-0.17	(0.28)	-0.20	(0.25)	0.53	0.84	590.91	610.94	-289.46	578.91
IL-5	-0.03	0.11	(0.39)	0.06	(0.68)	-0.26	(0.17)	0.28	0.95	597.85	617.88	-292.93	585.85
IL-6	≈0.00	-0.02	(0.90)	≈0.00	(0.99)	0.02	(0.92)	0.39	0.92	599.02	619.05	-293.51	587.02
IL-7	0.02	0.07	(0.52)	≈0.00	(0.99)	-0.20	(0.26)	0.51	0.85	592.65	612.68	-290.33	580.65
IL-8	-0.01	0.02	(0.88)	0.03	(0.83)	-0.07	(0.66)	0.55	0.83	591.83	611.85	-289.91	579.83
TNFα	-0.04	0.02	(0.89)	0.10	(0.53)	-0.05	(0.77)	0.57	0.81	589.90	609.92	-288.95	577.90
IL-4	0.06	-0.04	(0.62)	-0.07	(0.69)	-0.11	(0.44)	0.76	0.65	570.25	590.27	-279.12	558.25
IL-10	0.03	0.05	(0.63)	-0.07	(0.68)	-0.17	(0.28)	0.63	0.76	581.87	601.90	-284.94	569.87
IL-12p70	0.07	0.05	(0.89)	-0.13	(0.26)	-0.16	(0.66)	0.63	0.76	581.21	601.23	-284.60	569.21
IL-13	0.07	0.03	(0.79)	-0.16	(0.29)	-0.13	(0.48)	0.52	0.84	592.29	612.32	-290.15	580.29
CD14	-0.03	0.16	(0.21)	0.07	(0.61)	-0.37	(**0.06**)	0.25	0.95	599.28	619.33	-293.64	587.28
CD163	-0.08	-0.02	(0.88)	0.15	(0.34)	0.08	(0.63)	0.55	0.83	593.15	613.20	-290.57	581.15
NGAL	0.08	-0.07	(0.53)	-0.24	(0.11)	0.16	(0.34)	0.48	0.86	594.53	614.58	-291.26	582.53
MMP-9	≈0.00	-0.05	(0.70)	≈0.00	(0.98)	0.11	(0.56)	0.22	0.97	602.52	622.57	-295.26	590.52
YKL-40	≈0.00	-0.11	(0.27)	0.03	(0.88)	0.25	(0.11)	0.69	0.72	581.36	601.42	-284.68	569.36

SD: Standard Deviation; **AIC**: Akaike Information Criterion; **BIC**: Bayesian Information Criterion; **LogLik**: Log-likelihood.

5 Supplementary Table 5. Associations between serum marker concentrations and child neurometabolite ratios

5.1 Maternal serum markers during pregnancy

5.1.1 Child neurometabolite ratios in the midline parietal grey matter voxel

5.1.1.1 Child glutamate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
40	34	Pro-inflammatory	GM-CSF	-0.23	-0.76 to 0.31	0.27	0.40	0.49				
			IFN-γ	-0.44	-1.03 to 0.16	0.30	0.15	0.35				
			IL-1β	-0.52	-1.14 to 0.09	0.31	0.09	0.26				
			IL-2	-0.35	-0.82 to 0.12	0.23	0.14	0.47				
			IL-5	-0.72	-1.61 to 0.17	0.45	0.11	0.14				
			IL-6	-0.31	-0.74 to 0.12	0.21	0.15	0.19				
			IL-7	-0.78	-1.74 to 0.18	0.48	0.11	0.12				
			IL-8	-0.24	-0.88 to 0.39	0.32	0.45	0.79				
			TNFα	-0.32	-1.30 to 0.66	0.49	0.52	0.76				
		Anti-inflammatory	IL-4	-0.28	-0.66 to 0.10	0.19	0.15	0.16				
			IL-10	-0.59	-1.13 to -0.05	0.27	0.032	0.05				
			IL-12p70	-0.68	-1.35 to 0.00	0.34	0.05	0.07				
			IL-13	**-0.44**	**-0.80 to -0.07**	**0.18**	**0.019**	**0.030**	**-0.41**	**-0.80 to -0.02**	**0.19**	**0.038**
		Monocyte activation	CD14	-0.28	-2.20 to 1.64	0.96	0.77	0.75				
			CD163	-0.05	-1.11 to 1.02	0.53	0.93	0.93				
		Neuroinflammatory	NGAL	0.11	-0.81 to 1.02	0.46	0.82	0.89				
			MMP-9	**-0.85**	**-1.58 to -0.13**	**0.36**	**0.022**	**0.044**	**-0.85**	**-1.57 to -0.12**	**0.36**	**0.023**
			YKL-40	-0.58	-1.23 to 0.07	0.32	0.08	0.17				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.1.1.2 Child myo-inositol ratios

				Linear regression with robust standard errors								
Participants		**Serum markers**		**Unadjusted analysis**					**Adjusted analysis***			
n CHU	**n CHEU**	**Marker type**	**Marker name**	**β**	**95% CI**	**SE**	**P-value**	**BH**	**β**	**95% CI**	**SE**	**P-value**
40	**34**	**Pro-inflammatory**	**GM-CSF**	0.03	-0.45 to 0.51	0.24	0.91	0.99				
			IFN-γ	0.04	-0.59 to 0.67	0.32	0.90	0.97				
			IL-1β	0.38	-0.08 to 0.84	0.23	0.10	0.63				
			IL-2	0.28	-0.11 to 0.68	0.20	0.15	0.72				
			IL-5	**0.79**	**0.25 to 1.33**	**0.27**	**0.005**	**0.047**	**0.79**	**0.24 to 1.34**	**0.27**	**0.005**
			IL-6	0.22	-0.18 to 0.62	0.20	0.27	0.45				
			IL-7	0.36	-0.49 to 1.22	0.43	0.40	0.67				
			IL-8	0.27	-0.13 to 0.66	0.20	0.18	0.65				
			TNFα	0.51	-0.33 to 1.34	0.42	0.23	0.47				
		Anti-inflammatory	**IL-4**	0.19	-0.14 to 0.53	0.17	0.25	0.54				
			IL-10	0.28	-0.29 to 0.86	0.29	0.33	0.41				
			IL-12p70	0.38	-0.19 to 0.95	0.29	0.19	0.57				
			IL-13	0.11	-0.29 to 0.50	0.20	0.59	0.91				
		Monocyte activation	**CD14**	-0.37	-1.61 to 0.86	0.62	0.55	0.59				
			CD163	-0.16	-1.24 to 0.92	0.54	0.77	0.75				
		Neuroinflammatory	**NGAL**	0.16	-0.89 to 1.21	0.53	0.77	0.77				
			MMP-9	-0.03	-0.76 to 0.69	0.36	0.93	0.98				
			YKL-40	0.18	-0.71 to 1.07	0.45	0.69	0.66				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.1.1.3 Child N-acetyl-aspartate ratios

				Linear regression with robust standard errors								
Participants		**Serum markers**		**Unadjusted analysis**					**Adjusted analysis***			
n CHU	**n CHEU**	**Marker type**	**Marker name**	**β**	**95% CI**	**SE**	**P-value**	**BH**	**β**	**95% CI**	**SE**	**P-value**
40	**34**	**Pro-inflammatory**	**GM-CSF**	-0.16	-0.67 to 0.34	0.25	0.52	0.65				
			IFN-γ	-0.15	-0.70 to 0.40	0.28	0.59	0.81				
			IL-1β	-0.41	-0.93 to 0.12	0.26	0.13	0.29				
			IL-2	-0.22	-0.62 to 0.17	0.20	0.26	0.60				
			IL-5	-0.72	-1.39 to -0.05	0.34	**0.035**	0.11				
			IL-6	-0.24	-0.58 to 0.11	0.17	0.18	0.35				
			IL-7	-0.60	-1.34 to 0.15	0.37	0.11	0.17				
			IL-8	-0.09	-0.76 to 0.57	0.33	0.78	0.98				
			TNFα	-0.15	-0.84 to 0.54	0.35	0.66	0.96				
		Anti-inflammatory	**IL-4**	-0.23	-0.60 to 0.15	0.19	0.23	0.24				
			IL-10	-0.47	-0.90 to -0.03	0.22	**0.036**	0.10				
			IL-12p70	-0.66	-1.16 to -0.16	0.25	**0.011**	0.07				
			IL-13	-0.25	-0.56 to 0.06	0.15	0.11	0.22				
		Monocyte activation	**CD14**	-0.25	-2.28 to 1.79	1.02	0.81	0.77				
			CD163	-0.42	-1.44 to 0.60	0.51	0.42	0.47				
		Neuroinflammatory	**NGAL**	-0.32	-1.18 to 0.54	0.43	0.46	0.72				
			MMP-9	**-1.01**	**-1.74 to -0.28**	**0.37**	**0.008**	**0.013**	**-1.01**	**-1.74 to -0.27**	**0.37**	**0.008**
			YKL-40	-0.40	-1.08 to 0.27	0.34	0.23	0.55				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.1.2 Child neurometabolite ratios in the left parietal white matter voxel

5.1.2.1 Child glutamate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
40	34	Pro-inflammatory	GM-CSF	0.06	-0.55 to 0.67	0.31	0.84	0.90				
			IFN-γ	0.05	-0.57 to 0.67	0.31	0.86	0.86				
			IL-1β	-0.15	-0.77 to 0.48	0.31	0.64	0.86				
			IL-2	-0.05	-0.54 to 0.44	0.25	0.84	0.91				
			IL-5	-0.33	-1.19 to 0.54	0.43	0.45	0.54				
			IL-6	0.08	-0.34 to 0.50	0.21	0.72	0.82				
			IL-7	-0.09	-1.07 to 0.89	0.49	0.86	0.96				
			IL-8	0.01	-0.50 to 0.53	0.26	0.96	0.95				
			TNFα	-0.35	-1.26 to 0.55	0.46	0.44	0.56				
		Anti-inflammatory	IL-4	-0.03	-0.39 to 0.34	0.18	0.89	0.94				
			IL-10	-0.08	-0.64 to 0.48	0.28	0.78	0.95				
			IL-12p70	-0.03	-0.75 to 0.69	0.36	0.93	1.00				
			IL-13	-0.11	-0.56 to 0.35	0.23	0.64	0.75				
		Monocyte activation	CD14	-0.27	-2.55 to 2.00	1.14	0.81	0.82				
			CD163	0.32	-0.85 to 1.49	0.59	0.59	0.85				
		Neuroinflammatory	NGAL	0.01	-1.01 to 1.04	0.52	0.98	0.98				
			MMP-9	-0.09	-0.83 to 0.65	0.37	0.81	0.88				
			YKL-40	-0.15	-0.74 to 0.43	0.29	0.60	0.74				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.1.2.2 Child myo-inositol ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
40	34	Pro-inflammatory	GM-CSF	-0.10	-0.59 to 0.40	0.25	0.70	0.68				
			IFN-γ	-0.03	-0.60 to 0.55	0.29	0.92	0.92				
			IL-1β	0.00	-0.53 to 0.53	0.27	0.99	0.99				
			IL-2	-0.04	-0.46 to 0.38	0.21	0.85	0.85				
			IL-5	0.49	-0.06 to 1.05	0.28	0.08	0.40				
			IL-6	0.18	-0.33 to 0.69	0.25	0.48	0.40				
			IL-7	0.00	-0.92 to 0.92	0.46	1.00	1.00				
			IL-8	0.22	-0.27 to 0.71	0.25	0.37	0.90				
			TNFα	0.31	-0.52 to 1.14	0.42	0.46	0.95				
		Anti-inflammatory	IL-4	0.03	-0.33 to 0.38	0.18	0.89	0.91				
			IL-10	0.21	-0.34 to 0.76	0.27	0.45	0.71				
			IL-12p70	0.23	-0.26 to 0.73	0.25	0.35	0.77				
			IL-13	-0.02	-0.41 to 0.37	0.20	0.91	0.90				
		Monocyte activation	CD14	0.58	-0.81 to 1.96	0.69	0.41	0.43				
			CD163	0.48	-0.80 to 1.76	0.64	0.46	0.41				
		Neuroinflammatory	NGAL	0.36	-0.49 to 1.21	0.42	0.40	0.49				
			MMP-9	0.37	-0.40 to 1.14	0.38	0.34	0.55				
			YKL-40	0.40	-0.54 to 1.34	0.47	0.40	0.26				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.1.3 Child neurometabolite ratios in the right parietal white matter voxel

5.1.3.1 Child glutamate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
40	34	Pro-inflammatory	GM-CSF	-0.09	-0.55 to 0.38	0.24	0.72	0.96				
			IFN-γ	0.03	-0.56 to 0.63	0.30	0.91	0.90				
			IL-1β	-0.15	-0.68 to 0.38	0.27	0.57	0.77				
			IL-2	-0.07	-0.49 to 0.35	0.21	0.74	0.88				
			IL-5	-0.17	-0.92 to 0.58	0.37	0.65	0.80				
			IL-6	0.07	-0.36 to 0.50	0.22	0.75	0.70				
			IL-7	-0.35	-1.20 to 0.49	0.42	0.40	0.56				
			IL-8	0.07	-0.46 to 0.59	0.26	0.80	0.77				
			TNFα	0.18	-0.68 to 1.05	0.43	0.68	0.68				
		Anti-inflammatory	IL-4	-0.01	-0.41 to 0.40	0.20	0.98	0.98				
			IL-10	-0.42	-0.92 to 0.07	0.25	0.09	0.12				
			IL-12p70	-0.31	-0.80 to 0.19	0.25	0.22	0.44				
			IL-13	-0.03	-0.49 to 0.43	0.23	0.90	0.88				
		Monocyte activation	CD14	-0.29	-1.88 to 1.30	0.80	0.72	0.67				
			CD163	0.34	-0.95 to 1.64	0.65	0.60	0.95				
		Neuroinflammatory	NGAL	-0.44	-1.33 to 0.45	0.45	0.33	0.64				
			MMP-9	-0.06	-0.84 to 0.72	0.39	0.89	0.87				
			YKL-40	**-0.75**	**-1.37 to -0.14**	**0.31**	**0.018**	**0.032**	**-0.90**	**-1.47 to -0.33**	**0.29**	**0.002**

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.1.3.2 Child myo-inositol ratios

				Linear regression with robust standard errors								
Participants		**Serum markers**		**Unadjusted analysis**					**Adjusted analysis***			
n CHU	**n CHEU**	**Marker type**	**Marker name**	**β**	**95% CI**	**SE**	**P-value**	**BH**	**β**	**95% CI**	**SE**	**P-value**
40	**34**	**Pro-inflammatory**	**GM-CSF**	-0.08	-0.51 to 0.34	0.21	0.70	0.72				
			IFN-γ	-0.12	-0.60 to 0.36	0.24	0.62	0.65				
			IL-1β	0.03	-0.42 to 0.48	0.23	0.91	0.92				
			IL-2	0.00	-0.42 to 0.42	0.21	0.99	0.99				
			IL-5	0.38	-0.17 to 0.93	0.28	0.18	0.41				
			IL-6	0.38	0.09 to 0.68	0.15	**0.012**	0.09				
			IL-7	0.03	-0.83 to 0.88	0.43	0.95	0.94				
			IL-8	**0.62**	**0.11 to 1.14**	**0.26**	**0.018**	**0.009**	**0.64**	**0.10 to 1.17**	**0.27**	**0.020**
			TNFα	0.73	-0.16 to 1.62	0.45	0.11	0.25				
		Anti-inflammatory	**IL-4**	0.12	-0.16 to 0.40	0.14	0.39	0.66				
			IL-10	0.20	-0.28 to 0.68	0.24	0.41	0.70				
			IL-12p70	0.05	-0.45 to 0.55	0.25	0.83	0.84				
			IL-13	0.11	-0.24 to 0.47	0.18	0.52	0.68				
		Monocyte activation	**CD14**	-0.06	-1.59 to 1.47	0.77	0.94	0.93				
			CD163	0.44	-0.53 to 1.40	0.48	0.37	0.59				
		Neuroinflammatory	**NGAL**	0.75	0.01 to 1.49	0.37	**0.047**	0.09				
			MMP-9	0.54	-0.12 to 1.19	0.33	0.11	0.17				
			YKL-40	0.22	-0.46 to 0.89	0.34	0.52	0.64				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.1.3.3 Child N-acetyl-aspartate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
40	34	Pro-inflammatory	GM-CSF	-0.22	-0.63 to 0.19	0.21	0.29	0.47				
			IFN-γ	0.08	-0.55 to 0.72	0.32	0.80	0.97				
			IL-1β	-0.15	-0.70 to 0.41	0.28	0.60	0.58				
			IL-2	-0.04	-0.49 to 0.41	0.23	0.84	0.83				
			IL-5	-0.38	-1.01 to 0.24	0.31	0.23	0.74				
			IL-6	-0.33	-0.71 to 0.06	0.19	0.10	0.10				
			IL-7	-0.13	-0.91 to 0.65	0.39	0.74	0.83				
			IL-8	-0.21	-0.92 to 0.50	0.35	0.56	0.77				
			TNFα	-0.30	-0.99 to 0.40	0.35	0.40	0.94				
		Anti-inflammatory	IL-4	-0.23	-0.54 to 0.09	0.16	0.16	0.23				
			IL-10	-0.20	-0.70 to 0.29	0.25	0.41	0.47				
			IL-12p70	-0.33	-0.84 to 0.18	0.26	0.20	0.42				
			IL-13	-0.19	-0.55 to 0.16	0.18	0.28	0.33				
		Monocyte activation	CD14	-1.11	-2.76 to 0.54	0.83	0.18	0.19				
			CD163	-0.52	-1.92 to 0.87	0.70	0.46	0.37				
		Neuroinflammatory	NGAL	-0.47	-1.36 to 0.43	0.45	0.30	0.37				
			MMP-9	-0.70	-1.55 to 0.15	0.43	0.11	0.13				
			YKL-40	-0.20	-0.97 to 0.57	0.39	0.60	0.71				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.2 Infant serum markers at 6 weeks of age

5.2.1 Child neurometabolite ratios in the midline parietal grey matter voxel

5.2.1.1 Child glutamate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
29	23	Pro-inflammatory	GM-CSF	-0.31	-0.92 to 0.31	0.31	0.32	0.35				
			IFN-γ	-0.36	-0.93 to 0.22	0.29	0.22	0.52				
			IL-1β	-0.31	-1.00 to 0.38	0.34	0.38	0.89				
			IL-2	-0.32	-1.11 to 0.47	0.39	0.42	0.95				
			IL-5	-0.25	-1.40 to 0.90	0.57	0.66	0.99				
			IL-6	-0.19	-0.72 to 0.33	0.26	0.47	0.98				
			IL-7	-0.64	-1.57 to 0.30	0.47	0.18	0.25				
			IL-8	-0.57	-1.74 to 0.60	0.58	0.33	0.30				
			TNFα	0.18	-1.05 to 1.41	0.61	0.77	0.82				
		Anti-inflammatory	IL-4	-0.29	-0.77 to 0.18	0.24	0.22	0.42				
			IL-10	-0.22	-0.89 to 0.46	0.34	0.52	0.92				
			IL-12p70	-0.52	-1.28 to 0.23	0.38	0.17	0.44				
			IL-13	-0.25	-0.66 to 0.17	0.21	0.24	0.85				
		Monocyte activation	CD14	0.01	-1.92 to 1.94	0.96	0.99	0.99				
			CD163	0.84	-0.74 to 2.42	0.79	0.29	0.26				
		Neuroinflammatory	NGAL	1.28	-0.38 to 2.93	0.82	0.13	0.13				
			MMP-9	-0.37	-0.99 to 0.24	0.31	0.23	0.60				
			YKL-40	0.20	-1.20 to 1.61	0.70	0.77	0.80				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.2.1.2 Child myo-inositol ratios

				Linear regression with robust standard errors								
Participants		**Serum markers**		**Unadjusted analysis**					**Adjusted analysis***			
n CHU	**n CHEU**	**Marker type**	**Marker name**	**β**	**95% CI**	**SE**	**P-value**	**BH**	**β**	**95% CI**	**SE**	**P-value**
29	**23**	**Pro-inflammatory**	**GM-CSF**	-0.20	-0.93 to 0.53	0.36	0.58	0.87				
			IFN-γ	0.01	-0.61 to 0.64	0.31	0.96	0.96				
			IL-1β	0.30	-0.17 to 0.76	0.23	0.21	0.48				
			IL-2	-0.12	-0.71 to 0.47	0.29	0.68	0.92				
			IL-5	0.03	-0.74 to 0.81	0.38	0.93	0.93				
			IL-6	-0.02	-0.45 to 0.41	0.21	0.94	0.98				
			IL-7	-0.43	-1.21 to 0.35	0.39	0.28	0.54				
			IL-8	0.29	-0.29 to 0.87	0.29	0.31	0.83				
			TNFα	-0.05	-1.11 to 1.02	0.53	0.93	0.94				
		Anti-inflammatory	**IL-4**	0.01	-0.51 to 0.54	0.26	0.96	0.97				
			IL-10	-0.25	-0.92 to 0.42	0.33	0.46	0.94				
			IL-12p70	-0.22	-1.05 to 0.62	0.41	0.60	0.91				
			IL-13	-0.24	-0.68 to 0.21	0.22	0.29	0.42				
		Monocyte activation	**CD14**	-0.71	-3.85 to 2.43	1.56	0.65	0.76				
			CD163	-0.85	-1.81 to 0.11	0.48	0.08	0.25				
		Neuroinflammatory	**NGAL**	-0.67	-1.65 to 0.31	0.49	0.18	0.26				
			MMP-9	0.38	-0.07 to 0.83	0.22	0.10	0.34				
			YKL-40	-0.27	-2.18 to 1.65	0.95	0.78	0.87				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.2.1.3 Child N-acetyl-aspartate ratios

				Linear regression with robust standard errors								
Participants		**Serum markers**		**Unadjusted analysis**					**Adjusted analysis***			
n CHU	**n CHEU**	**Marker type**	**Marker name**	**β**	**95% CI**	**SE**	**P-value**	**BH**	**β**	**95% CI**	**SE**	**P-value**
29	**23**	**Pro-inflammatory**	**GM-CSF**	0.11	-0.52 to 0.75	0.31	0.72	0.95				
			IFN-γ	0.18	-0.38 to 0.73	0.28	0.52	0.50				
			IL-1β	0.05	-0.56 to 0.65	0.30	0.88	0.90				
			IL-2	0.15	-0.52 to 0.82	0.33	0.65	0.86				
			IL-5	0.31	-0.60 to 1.22	0.45	0.49	0.77				
			IL-6	-0.12	-0.58 to 0.34	0.23	0.59	0.84				
			IL-7	0.21	-0.74 to 1.16	0.47	0.65	0.58				
			IL-8	-0.48	-1.62 to 0.66	0.57	0.40	0.43				
			TNFα	0.00	-1.24 to 1.23	0.61	1.00	1.00				
		Anti-inflammatory	**IL-4**	-0.09	-0.59 to 0.41	0.25	0.72	0.84				
			IL-10	0.32	-0.25 to 0.90	0.28	0.26	0.39				
			IL-12p70	0.18	-0.51 to 0.88	0.35	0.60	0.78				
			IL-13	-0.10	-0.49 to 0.29	0.19	0.62	1.00				
		Monocyte activation	**CD14**	1.63	0.05 to 3.20	0.78	**0.043**	0.18				
			CD163	1.10	-0.28 to 2.48	0.69	0.12	0.08				
		Neuroinflammatory	**NGAL**	1.14	0.03 to 2.26	0.55	**0.044**	0.12				
			MMP-9	-0.08	-0.77 to 0.60	0.34	0.81	0.99				
			YKL-40	0.22	-1.33 to 1.76	0.77	0.78	0.73				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.2.2 Child neurometabolite ratios in the left parietal white matter voxel

5.2.2.1 Child glutamate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
29	23	Pro-inflammatory	GM-CSF	-0.68	-1.28 to -0.09	0.30	**0.026**	0.09				
			IFN-γ	-0.62	-1.35 to 0.10	0.36	0.09	0.17				
			IL-1β	**-0.86**	**-1.37 to -0.35**	**0.25**	**0.001**	**0.015**	**-0.76**	**-1.34 to -0.18**	**0.29**	**0.011**
			IL-2	-0.60	-1.55 to 0.35	0.47	0.21	0.25				
			IL-5	-0.81	-1.63 to 0.01	0.41	0.05	0.13				
			IL-6	-0.36	-1.03 to 0.31	0.33	0.29	0.41				
			IL-7	-0.86	-1.92 to 0.21	0.53	0.11	0.20				
			IL-8	-0.61	-1.57 to 0.34	0.47	0.20	0.43				
			TNFα	-0.06	-1.22 to 1.11	0.58	0.92	0.98				
		Anti-inflammatory	IL-4	-0.46	-0.94 to 0.03	0.24	0.06	0.18				
			IL-10	-0.55	-1.60 to 0.50	0.52	0.29	0.56				
			IL-12p70	-0.69	-1.58 to 0.19	0.44	0.12	0.19				
			IL-13	-0.37	-0.81 to 0.06	0.22	0.09	0.31				
		Monocyte activation	CD14	1.48	-0.44 to 3.41	0.96	0.13	0.38				
			CD163	0.66	-0.95 to 2.27	0.80	0.41	0.62				
		Neuroinflammatory	NGAL	-0.08	-1.91 to 1.75	0.91	0.93	0.97				
			MMP-9	-0.62	-1.43 to 0.19	0.40	0.13	0.25				
			YKL-40	-0.43	-1.47 to 0.62	0.52	0.42	0.61				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.2.2.2 Child myo-inositol ratios

				Linear regression with robust standard errors								
Participants		**Serum markers**		**Unadjusted analysis**					**Adjusted analysis***			
n CHU	**n CHEU**	**Marker type**	**Marker name**	**β**	**95% CI**	**SE**	**P-value**	**BH**	**β**	**95% CI**	**SE**	**P-value**
29	**23**	**Pro-inflammatory**	**GM-CSF**	-0.19	-0.76 to 0.38	0.28	0.50	0.69				
			IFN-γ	-0.17	-0.75 to 0.41	0.29	0.56	0.59				
			IL-1β	-0.12	-0.74 to 0.50	0.31	0.70	0.74				
			IL-2	-0.42	-0.91 to 0.07	0.24	0.09	0.13				
			IL-5	-0.15	-0.89 to 0.58	0.37	0.68	0.79				
			IL-6	-0.03	-0.44 to 0.39	0.21	0.90	0.98				
			IL-7	-0.58	-1.25 to 0.09	0.33	0.09	0.13				
			IL-8	-0.01	-0.61 to 0.58	0.29	0.96	0.96				
			TNFα	-0.36	-1.19 to 0.47	0.41	0.39	0.72				
		Anti-inflammatory	**IL-4**	-0.04	-0.40 to 0.33	0.18	0.84	0.83				
			IL-10	-0.30	-1.24 to 0.63	0.46	0.51	0.70				
			IL-12p70	-0.32	-0.89 to 0.24	0.28	0.25	0.26				
			IL-13	-0.18	-0.62 to 0.26	0.22	0.41	0.32				
		Monocyte activation	**CD14**	0.41	-1.49 to 2.30	0.94	0.67	0.86				
			CD163	-0.93	-1.81 to -0.05	0.44	**0.038**	0.11				
		Neuroinflammatory	**NGAL**	-0.88	-1.76 to 0.00	0.44	**0.049**	0.19				
			MMP-9	-0.17	-0.70 to 0.36	0.26	0.53	0.92				
			YKL-40	0.11	-2.25 to 2.47	1.17	0.93	0.99				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.2.3 Child neurometabolite ratios in the right parietal white matter voxel

5.2.3.1 Child glutamate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
29	23	Pro-inflammatory	GM-CSF	-0.38	-0.89 to 0.13	0.25	0.14	0.29				
			IFN-γ	-0.51	-1.16 to 0.13	0.32	0.12	0.13				
			IL-1β	-0.41	-0.95 to 0.13	0.27	0.14	0.53				
			IL-2	-0.27	-0.93 to 0.40	0.33	0.42	0.53				
			IL-5	-0.48	-1.30 to 0.34	0.41	0.25	0.33				
			IL-6	-0.32	-0.66 to 0.03	0.17	0.07	0.18				
			IL-7	-0.71	-1.54 to 0.12	0.41	0.09	0.15				
			IL-8	0.08	-0.99 to 1.15	0.53	0.88	0.84				
			TNFα	0.00	-1.50 to 1.49	0.74	0.99	0.99				
		Anti-inflammatory	IL-4	-0.34	-0.81 to 0.12	0.23	0.14	0.19				
			IL-10	-0.19	-1.21 to 0.83	0.51	0.71	0.68				
			IL-12p70	-0.49	-1.24 to 0.25	0.37	0.19	0.23				
			IL-13	-0.22	-0.65 to 0.20	0.21	0.29	0.48				
		Monocyte activation	CD14	1.62	-0.60 to 3.83	1.10	0.15	0.24				
			CD163	0.40	-0.84 to 1.64	0.62	0.52	0.67				
		Neuroinflammatory	NGAL	-0.36	-1.68 to 0.96	0.66	0.59	0.73				
			MMP-9	-0.61	-1.60 to 0.38	0.49	0.22	0.14				
			YKL-40	-0.27	-1.73 to 1.20	0.73	0.71	0.67				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.2.3.2 Child myo-inositol ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
29	23	Pro-inflammatory	GM-CSF	-0.19	-0.78 to 0.39	-0.78	0.39	0.29				
			IFN-γ	-0.23	-0.92 to 0.46	-0.92	0.46	0.34				
			IL-1β	-0.04	-0.49 to 0.41	-0.49	0.41	0.22				
			IL-2	-0.21	-0.79 to 0.37	-0.79	0.37	0.29				
			IL-5	-0.10	-0.69 to 0.49	-0.69	0.49	0.29				
			IL-6	-0.20	-0.56 to 0.16	-0.56	0.16	0.18				
			IL-7	-0.43	-1.33 to 0.46	-1.33	0.46	0.45				
			IL-8	-0.02	-0.88 to 0.83	-0.88	0.83	0.42				
			TNFα	-0.18	-1.28 to 0.93	-1.28	0.93	0.55				
		Anti-inflammatory	IL-4	-0.18	-0.47 to 0.10	-0.47	0.10	0.14				
			IL-10	-0.33	-1.15 to 0.49	-1.15	0.49	0.41				
			IL-12p70	-0.26	-0.93 to 0.40	-0.93	0.40	0.33				
			IL-13	-0.27	-0.67 to 0.13	-0.67	0.13	0.20				
		Monocyte activation	CD14	0.64	-1.62 to 2.91	-1.62	2.91	1.13				
			CD163	-0.56	-1.77 to 0.64	-1.77	0.64	0.60				
		Neuroinflammatory	NGAL	-0.49	-1.51 to 0.54	-1.51	0.54	0.51				
			MMP-9	-0.04	-0.79 to 0.71	-0.79	0.71	0.37				
			YKL-40	-0.13	-2.41 to 2.14	-2.41	2.14	1.13				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.2.3.3 Child N-acetyl-aspartate ratios

				Linear regression with robust standard errors								
Participants		**Serum markers**		**Unadjusted analysis**					**Adjusted analysis***			
n CHU	**n CHEU**	**Marker type**	**Marker name**	**β**	**95% CI**	**SE**	**P-value**	**BH**	**β**	**95% CI**	**SE**	**P-value**
29	**23**	**Pro-inflammatory**	**GM-CSF**	-0.24	-0.76 to 0.27	0.26	0.35	0.80				
			IFN-γ	-0.07	-0.62 to 0.49	0.28	0.80	0.78				
			IL-1β	-0.15	-0.72 to 0.42	0.28	0.59	0.53				
			IL-2	-0.13	-0.76 to 0.51	0.32	0.69	0.63				
			IL-5	-0.14	-0.89 to 0.60	0.37	0.70	0.85				
			IL-6	-0.22	-0.62 to 0.18	0.20	0.27	0.28				
			IL-7	0.02	-0.89 to 0.94	0.45	0.96	0.95				
			IL-8	-0.04	-1.30 to 1.21	0.62	0.94	0.90				
			TNFα	0.82	-0.25 to 1.89	0.53	0.13	0.08				
		Anti-inflammatory	**IL-4**	-0.20	-0.56 to 0.17	0.18	0.29	0.56				
			IL-10	0.04	-0.96 to 1.05	0.50	0.93	0.90				
			IL-12p70	-0.16	-0.77 to 0.45	0.30	0.60	0.75				
			IL-13	-0.20	-0.52 to 0.11	0.16	0.21	0.45				
		Monocyte activation	**CD14**	-0.02	-1.98 to 1.94	0.98	0.98	0.98				
			CD163	1.20	-0.17 to 2.57	0.68	0.08	**0.047**				
		Neuroinflammatory	**NGAL**	0.41	-0.79 to 1.62	0.60	0.49	0.56				
			MMP-9	-0.11	-0.68 to 0.45	0.28	0.69	0.90				
			YKL-40	0.33	-1.09 to 1.75	0.71	0.64	0.57				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.3 Child serum markers at 2 years of age

5.3.1 Child neurometabolite ratios in the midline parietal grey matter voxel

5.3.1.1 Child glutamate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
35	28	Pro-inflammatory	GM-CSF	-0.17	-0.86 to 0.51	0.34	0.61	0.98				
			IFN-γ	-0.01	-0.90 to 0.89	0.45	0.99	0.99				
			IL-1β	-0.23	-0.98 to 0.53	0.38	0.55	0.99				
			IL-2	-0.76	-1.56 to 0.03	0.40	0.06	0.16				
			IL-5	0.63	-0.40 to 1.65	0.51	0.23	0.43				
			IL-6	0.10	-0.62 to 0.83	0.36	0.77	0.89				
			IL-7	-0.21	-1.36 to 0.94	0.57	0.72	0.99				
			IL-8	0.25	-0.28 to 0.79	0.27	0.35	0.82				
			TNFα	-0.43	-1.39 to 0.53	0.48	0.37	0.56				
		Anti-inflammatory	IL-4	0.01	-0.56 to 0.57	0.28	0.98	1.00				
			IL-10	-0.69	-1.62 to 0.23	0.46	0.14	0.25				
			IL-12p70	-0.68	-1.49 to 0.14	0.41	0.10	0.24				
			IL-13	0.07	-0.56 to 0.70	0.32	0.82	0.93				
		Monocyte activation	CD14	1.13	-0.17 to 2.43	0.65	0.09	0.28				
			CD163	0.52	-0.74 to 1.78	0.63	0.41	0.61				
		Neuroinflammatory	**NGAL**	**1.00**	**0.12 to 1.88**	**0.44**	**0.027**	**0.039**	**1.00**	**0.07 to 1.94**	**0.47**	**0.036**
			MMP-9	0.74	-0.10 to 1.58	0.42	0.08	0.12				
			YKL-40	0.39	-0.51 to 1.29	0.45	0.38	0.58				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.3.1.2 Child myo-inositol ratios

				Linear regression with robust standard errors								
Participants		**Serum markers**		**Unadjusted analysis**					**Adjusted analysis***			
n CHU	**n CHEU**	**Marker type**	**Marker name**	**β**	**95% CI**	**SE**	**P-value**	**BH**	**β**	**95% CI**	**SE**	**P-value**
35	**28**	**Pro-inflammatory**	**GM-CSF**	0.03	-0.51 to 0.57	0.27	0.90	0.92				
			IFN-γ	-0.01	-0.90 to 0.88	0.44	0.98	0.98				
			IL-1β	0.17	-0.49 to 0.83	0.33	0.61	0.98				
			IL-2	0.32	-0.39 to 1.03	0.35	0.37	0.91				
			IL-5	-0.11	-1.43 to 1.20	0.66	0.86	0.82				
			IL-6	-0.05	-0.50 to 0.39	0.22	0.81	0.88				
			IL-7	-0.06	-1.24 to 1.13	0.59	0.92	0.97				
			IL-8	0.31	-0.18 to 0.80	0.25	0.21	0.70				
			TNFα	0.03	-0.69 to 0.76	0.36	0.93	0.99				
		Anti-inflammatory	**IL-4**	0.17	-0.25 to 0.59	0.21	0.41	0.94				
			IL-10	0.16	-0.83 to 1.16	0.50	0.74	0.77				
			IL-12p70	0.24	-0.53 to 1.01	0.39	0.53	0.73				
			IL-13	-0.41	-0.93 to 0.12	0.26	0.13	0.31				
		Monocyte activation	**CD14**	-0.52	-1.72 to 0.67	0.60	0.39	0.51				
			CD163	-0.81	-1.86 to 0.24	0.52	0.13	0.30				
		Neuroinflammatory	**NGAL**	-0.41	-1.16 to 0.33	0.37	0.27	0.71				
			MMP-9	**1.31**	**0.24 to 2.38**	**0.54**	**0.017**	**0.012**	**1.29**	**0.12 to 2.45**	**0.58**	**0.031**
			YKL-40	0.46	-0.56 to 1.47	0.51	0.37	0.70				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.3.1.3 Child N-acetyl-aspartate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
35	28	Pro-inflammatory	GM-CSF	-0.02	-0.57 to 0.53	0.28	0.94	0.94				
			IFN-γ	0.08	-0.79 to 0.94	0.43	0.86	0.85				
			IL-1β	-0.26	-0.86 to 0.34	0.30	0.39	0.53				
			IL-2	-0.63	-1.28 to 0.02	0.33	0.06	0.24				
			IL-5	-0.23	-0.93 to 0.47	0.35	0.51	0.80				
			IL-6	-0.16	-0.64 to 0.31	0.24	0.50	0.85				
			IL-7	-0.15	-1.20 to 0.89	0.52	0.77	0.96				
			IL-8	-0.18	-0.64 to 0.29	0.23	0.44	0.67				
			TNFα	-0.25	-1.14 to 0.63	0.44	0.57	0.66				
		Anti-inflammatory	IL-4	-0.27	-0.69 to 0.16	0.21	0.21	0.30				
			IL-10	-0.68	-1.50 to 0.13	0.41	0.10	0.24				
			IL-12p70	-0.65	-1.36 to 0.07	0.36	0.07	0.15				
			IL-13	-0.15	-0.60 to 0.31	0.23	0.52	0.72				
		Monocyte activation	CD14	0.78	-0.57 to 2.13	0.68	0.25	0.55				
			CD163	0.36	-0.68 to 1.41	0.52	0.49	0.97				
		Neuroinflammatory	NGAL	0.80	-0.13 to 1.73	0.47	0.09	0.09				
			MMP-9	0.59	-0.29 to 1.47	0.44	0.19	0.24				
			YKL-40	-0.21	-1.02 to 0.59	0.40	0.60	0.81				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.3.2 Child neurometabolite ratios in the left parietal white matter voxel

5.3.2.1 Child glutamate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
35	28	Pro-inflammatory	GM-CSF	-0.12	-0.84 to 0.60	0.36	0.74	0.97				
			IFN-γ	0.14	-0.63 to 0.91	0.38	0.72	0.94				
			IL-1β	-0.15	-0.79 to 0.49	0.32	0.64	0.83				
			IL-2	-0.41	-1.23 to 0.40	0.41	0.32	0.79				
			IL-5	0.99	0.08 to 1.90	0.45	0.033	0.06				
			IL-6	0.09	-0.63 to 0.81	0.36	0.81	0.87				
			IL-7	0.14	-0.96 to 1.24	0.55	0.80	0.98				
			IL-8	0.31	-0.21 to 0.83	0.26	0.24	0.55				
			TNFα	-0.10	-0.77 to 0.57	0.33	0.76	0.87				
		Anti-inflammatory	IL-4	0.09	-0.36 to 0.54	0.22	0.70	0.89				
			IL-10	0.09	-1.11 to 1.29	0.60	0.88	0.92				
			IL-12p70	-0.24	-1.23 to 0.75	0.49	0.63	0.96				
			IL-13	0.18	-0.31 to 0.67	0.25	0.47	0.98				
		Monocyte activation	CD14	0.41	-1.20 to 2.01	0.80	0.62	0.59				
			CD163	0.30	-0.88 to 1.48	0.59	0.61	0.70				
		Neuroinflammatory	NGAL	0.47	-0.36 to 1.29	0.41	0.26	0.39				
			MMP-9	-0.27	-1.26 to 0.72	0.49	0.59	0.68				
			YKL-40	0.37	-0.44 to 1.18	0.40	0.37	0.42				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.3.2.2 Child myo-inositol ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
35	28	Pro-inflammatory	GM-CSF	0.09	-0.47 to 0.65	0.28	0.75	0.95				
			IFN-γ	-0.36	-1.32 to 0.60	0.48	0.46	0.35				
			IL-1β	-0.09	-0.75 to 0.57	0.33	0.78	0.78				
			IL-2	0.12	-0.58 to 0.83	0.35	0.73	0.74				
			IL-5	-0.05	-0.87 to 0.77	0.41	0.90	0.91				
			IL-6	-0.14	-0.65 to 0.37	0.26	0.59	0.75				
			IL-7	-0.88	-2.14 to 0.39	0.63	0.17	0.13				
			IL-8	0.06	-0.41 to 0.53	0.23	0.79	0.82				
			TNFα	-0.53	-1.40 to 0.33	0.43	0.22	0.34				
		Anti-inflammatory	IL-4	-0.07	-0.55 to 0.41	0.24	0.76	0.75				
			IL-10	0.11	-0.73 to 0.95	0.42	0.79	0.96				
			IL-12p70	0.01	-0.71 to 0.74	0.36	0.97	0.97				
			IL-13	-0.42	-0.98 to 0.15	0.28	0.15	0.17				
		Monocyte activation	CD14	0.10	-1.26 to 1.46	0.68	0.88	0.94				
			CD163	-0.60	-1.56 to 0.36	0.48	0.22	0.52				
		Neuroinflammatory	NGAL	0.17	-0.67 to 1.01	0.42	0.69	0.79				
			MMP-9	0.79	-0.06 to 1.64	0.42	0.07	0.20				
			YKL-40	0.72	-0.21 to 1.64	0.46	0.13	0.21				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.3.3 Child neurometabolite ratios in the right parietal white matter voxel

5.3.3.1 Child glutamate ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
35	28	Pro-inflammatory	GM-CSF	-0.39	-0.91 to 0.14	0.26	0.14	0.26				
			IFN-γ	0.08	-0.76 to 0.93	0.42	0.84	0.82				
			IL-1β	-0.23	-0.89 to 0.43	0.33	0.49	0.61				
			IL-2	-0.24	-0.87 to 0.40	0.32	0.46	0.65				
			IL-5	0.56	-0.33 to 1.46	0.45	0.21	0.58				
			IL-6	0.05	-0.53 to 0.64	0.29	0.85	0.94				
			IL-7	-0.09	-1.38 to 1.20	0.64	0.89	0.87				
			IL-8	0.27	-0.25 to 0.79	0.26	0.30	0.50				
			TNFα	-0.15	-1.04 to 0.74	0.45	0.73	0.67				
		Anti-inflammatory	IL-4	-0.03	-0.59 to 0.53	0.28	0.93	0.91				
			IL-10	-0.44	-1.23 to 0.35	0.39	0.27	0.56				
			IL-12p70	-0.36	-1.01 to 0.28	0.32	0.26	0.61				
			IL-13	0.16	-0.42 to 0.74	0.29	0.59	0.76				
		Monocyte activation	CD14	0.46	-1.15 to 2.08	0.81	0.57	0.58				
			CD163	0.49	-0.50 to 1.47	0.49	0.33	0.75				
		Neuroinflammatory	NGAL	0.63	-0.20 to 1.46	0.42	0.13	0.18				
			MMP-9	0.16	-0.66 to 0.98	0.41	0.70	0.87				
			YKL-40	-0.11	-0.87 to 0.65	0.38	0.77	0.79				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.3.3.2 Child myo-inositol ratios

				Linear regression with robust standard errors								
Participants		Serum markers		Unadjusted analysis					Adjusted analysis*			
n CHU	n CHEU	Marker type	Marker name	β	95% CI	SE	P-value	BH	β	95% CI	SE	P-value
35	28	Pro-inflammatory	GM-CSF	0.10	-0.40 to 0.61	0.25	0.69	0.91				
			IFN-γ	-0.41	-1.14 to 0.32	0.37	0.27	0.28				
			IL-1β	-0.14	-0.72 to 0.45	0.29	0.64	0.67				
			IL-2	0.01	-0.61 to 0.62	0.31	0.98	0.98				
			IL-5	-0.25	-1.21 to 0.72	0.48	0.61	0.78				
			IL-6	-0.01	-0.51 to 0.49	0.25	0.96	0.95				
			IL-7	-0.72	-1.78 to 0.34	0.53	0.18	0.20				
			IL-8	-0.15	-0.71 to 0.40	0.28	0.59	0.58				
			TNFα	-0.62	-1.34 to 0.10	0.36	0.09	0.14				
		Anti-inflammatory	IL-4	-0.08	-0.47 to 0.32	0.20	0.70	0.74				
			IL-10	-0.24	-1.04 to 0.57	0.40	0.56	0.66				
			IL-12p70	-0.08	-0.74 to 0.58	0.33	0.81	0.84				
			IL-13	-0.37	-0.92 to 0.18	0.28	0.19	0.19				
		Monocyte activation	CD14	-0.08	-1.45 to 1.29	0.69	0.91	0.91				
			CD163	-0.53	-1.64 to 0.59	0.56	0.35	0.62				
		Neuroinflammatory	NGAL	0.37	-0.48 to 1.21	0.42	0.39	0.48				
			MMP-9	0.72	-0.32 to 1.75	0.52	0.17	0.33				
			YKL-40	0.72	-0.27 to 1.71	0.49	0.15	0.19				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

5.3.3.3 Child N-acetyl-aspartate ratios

				Linear regression with robust standard errors								
Participants		**Serum markers**		**Unadjusted analysis**					**Adjusted analysis***			
n CHU	**n CHEU**	**Marker type**	**Marker name**	**β**	**95% CI**	**SE**	**P-value**	**BH**	**β**	**95% CI**	**SE**	**P-value**
35	**28**	**Pro-inflammatory**	**GM-CSF**	-0.08	-0.55 to 0.38	0.23	0.72	0.94				
			IFN-γ	0.13	-0.85 to 1.11	0.49	0.79	0.91				
			IL-1β	-0.12	-0.82 to 0.57	0.35	0.72	0.73				
			IL-2	-0.28	-1.05 to 0.49	0.39	0.47	0.49				
			IL-5	-0.05	-0.90 to 0.81	0.43	0.91	0.97				
			IL-6	0.06	-0.49 to 0.61	0.28	0.82	0.79				
			IL-7	0.28	-1.11 to 1.67	0.70	0.69	0.88				
			IL-8	-0.11	-0.60 to 0.38	0.24	0.65	0.86				
			TNFα	0.45	-0.47 to 1.38	0.46	0.33	0.38				
		Anti-inflammatory	**IL-4**	0.03	-0.57 to 0.62	0.30	0.93	0.90				
			IL-10	-0.31	-1.19 to 0.58	0.44	0.49	0.69				
			IL-12p70	-0.33	-1.07 to 0.40	0.37	0.37	0.69				
			IL-13	0.05	-0.72 to 0.81	0.38	0.90	0.84				
		Monocyte activation	**CD14**	-0.81	-2.15 to 0.53	0.67	0.23	0.44				
			CD163	0.13	-0.86 to 1.13	0.50	0.79	0.79				
		Neuroinflammatory	**NGAL**	0.33	-0.54 to 1.21	0.44	0.45	0.40				
			MMP-9	-0.24	-1.09 to 0.61	0.43	0.57	0.70				
			YKL-40	-0.54	-1.28 to 0.20	0.37	0.15	0.46				

CHU: HIV-unexposed children; **CHEU**: HIV-exposed uninfected children; **β**: Effect size; **BH**: Benjamini-Hochberg corrected p-value. *Child age, child sex, and tissue composition.

6 Supplementary Table 6. Sensitivity analyses

				Adjusted analysis[A]			Maternal age at delivery[B]			Maternal depression[C]			Maternal alcohol use[D]		
Timepoint	**Brain region**	**Metabolite ratios**	**Serum marker**	**β**	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**
Pregnancy	**PGM**	Glu	IL-13	-0.41	-0.80 to -0.02	**0.038**	-0.39	-0.75 to -0.05	**0.031**	-0.46	-0.83 to -0.09	**0.016**	-0.50	-0.89 to -0.10	**0.016**
			MMP-9	-0.85	-1.57 to -0.12	**0.023**	-0.81	-1.57 to -0.04	**0.039**	-0.87	-1.63 to -0.11	**0.026**	-0.88	-1.63 to -0.13	**0.023**
		Myo	IL-5	0.79	0.24 to 1.34	**0.005**	0.78	0.25 to 1.32	**0.005**	0.80	0.26 to 1.35	**0.005**	0.78	0.26 to 1.30	**0.004**
		NAA	MMP-9	-1.01	-1.74 to -0.27	**0.008**	-0.99	-1.71 to -0.27	**0.008**	-1.03	-1.77 to -0.29	**0.007**	-1.09	-1.79 to -0.40	**0.003**
	RPWM	Glu	YKL-40	-0.90	-1.47 to -0.33	**0.002**	-0.90	-1.46 to -0.35	**0.002**	-0.91	-1.47 to -0.34	**0.002**	-0.91	-1.52 to -0.31	**0.004**
		Myo	IL-8	0.64	0.10 to 1.17	**0.020**	0.64	0.10 to 1.19	**0.021**	0.64	0.08 to 1.20	**0.025**	0.64	0.10 to 1.18	**0.021**
	All regions	Myo*	IL-5	0.84	0.23 to 1.44	**0.007**	0.81	0.20 to 1.42	**0.010**	0.86	0.24 to 1.47	**0.007**	0.83	0.25 to 1.40	**0.006**
6 weeks	**LPWM**	Glu	IL-1β	-0.76	-1.34 to -0.18	**0.011**	-0.76	-1.32 t -0.21	**0.008**	-0.76	-1.31 t -0.21	**0.008**	-0.76	-1.31 to -0.21	**0.008**
2 years	**PGM**	Glu	NGAL	1.00	0.07 to 1.94	**0.036**	1.06	0.20 to 1.93	**0.017**	1.20	0.27 to 2.14	**0.013**	1.05	0.12 to 1.98	**0.027**
		Myo	MMP-9	1.29	0.12 to 2.45	**0.031**	1.29	0.28 to 2.30	**0.014**	1.26	0.23 to 2.30	**0.018**	1.31	0.29 to 2.34	**0.013**
	L & RPWM	Glu*	NGAL	0.84	0.14 to 1.54	**0.019**	0.85	0.16 to 1.54	**0.016**	0.94	0.24 to 1.63	**0.009**	0.89	0.20 to 1.58	**0.012**

Sensitivity analyses of statistically significant associations between maternal and child immune marker concentrations and child neurometabolite ratios in children who are HIV-exposed and uninfected compared to HIV-unexposed peers. Linear regression models with robust standard errors were constructed including the following covariates:

A. Child age, child sex, and voxel tissue composition (reference)
B. Child age, child sex, and maternal age at delivery (sensitivity analysis B)
C. Child age, child sex, and antenatal maternal depression (sensitivity analysis C) (Note: missing data were handled using the last observation carried forward (LOCF) method)
D. Child age, child sex, and maternal alcohol use during pregnancy (sensitivity analysis D)

MRS: Magnetic Resonance Spectroscopy; **β**: Effect size; **PGM**: Parietal Grey Matter; **LPWM**: Left Parietal White Matter; **RPWM**: Right Parietal White Matter.
*Cross-regional neurometabolite patterns (factor loadings) previously reported in the same cohort, identified with factor analysis.

7 Supplementary Table 7. Mediation analyses

Structural equation modelling estimates for direct, indirect, and total effects of maternal HIV on child neurometabolite ratios mediated by serum marker concentrations

Timepoint	n CHU	n CHEU	Brain region	Neurometabolite ratios	Marker	Effect	Estimate	StdEst	95% CI	SE	z-value	p-value
Pregnancy	**40**	**34**	**PGM**	Glutamate	IL-13	Direct	0.02	0.01	-0.48 to 0.50	0.25	0.08	0.94
						Indirect	-0.02	-0.01	-0.15 to 0.12	0.07	-0.30	0.76
						Total	≈0.00	≈0.00	-0.47 to 0.48	0.25	≈0.00	1.00
					MMP-9	Direct	-0.11	-0.05	-0.61 to 0.40	0.26	-0.41	0.69
						Indirect	0.11	0.05	-0.07 to 0.28	0.09	1.16	0.25
						Total	≈0.00	≈0.00		0.26	≈0.00	1.00
				Myo-inositol	IL-5	Direct	0.09	0.05	-0.38 to 0.51	0.22	0.40	0.69
						Indirect	0.01	0.01	-0.07 to 0.13	0.05	0.26	0.80
						Total	0.10	0.05	-0.35 to 0.53	0.23	0.44	0.66
				N-acetyl-aspartate	MMP-9	Direct	-0.28	-0.14	-0.75 to 0.28	0.27	-1.06	0.29
						Indirect	0.05	0.02	-0.12 to 0.23	0.09	0.56	0.58
						Total	-0.24	-0.12	-0.68 to 0.27	0.24	-0.99	0.33
			RPWM	Glutamate	YKL-40	Direct	0.59	0.29	0.11 to 1.05	0.24	2.48	**0.013**
						Indirect	0.01	≈0.00	-0.07 to 0.09	0.04	0.23	0.82
						Total	0.60	0.29	0.15 to 1.06	0.23	2.54	**0.011**
				Myo-inositol	IL-8	Direct	0.62	0.32	0.22 to 1.04	0.21	2.94	**0.003**
						Indirect	0.00	0.00	-0.05 to 0.10	0.03	0.06	0.95
						Total	0.63	0.32	0.24 to 1.05	0.21	2.99	**0.003**
			All regions	Myo-inositol pattern	IL-5	Direct	0.55	0.24	0.04 to 1.05	0.26	2.12	**0.034**
						Indirect	≈0.00	≈0.00	-0.14 to 0.13	0.07	-0.06	0.95
						Total	0.54	0.23	0.04 to 1.05	0.26	2.10	**0.036**

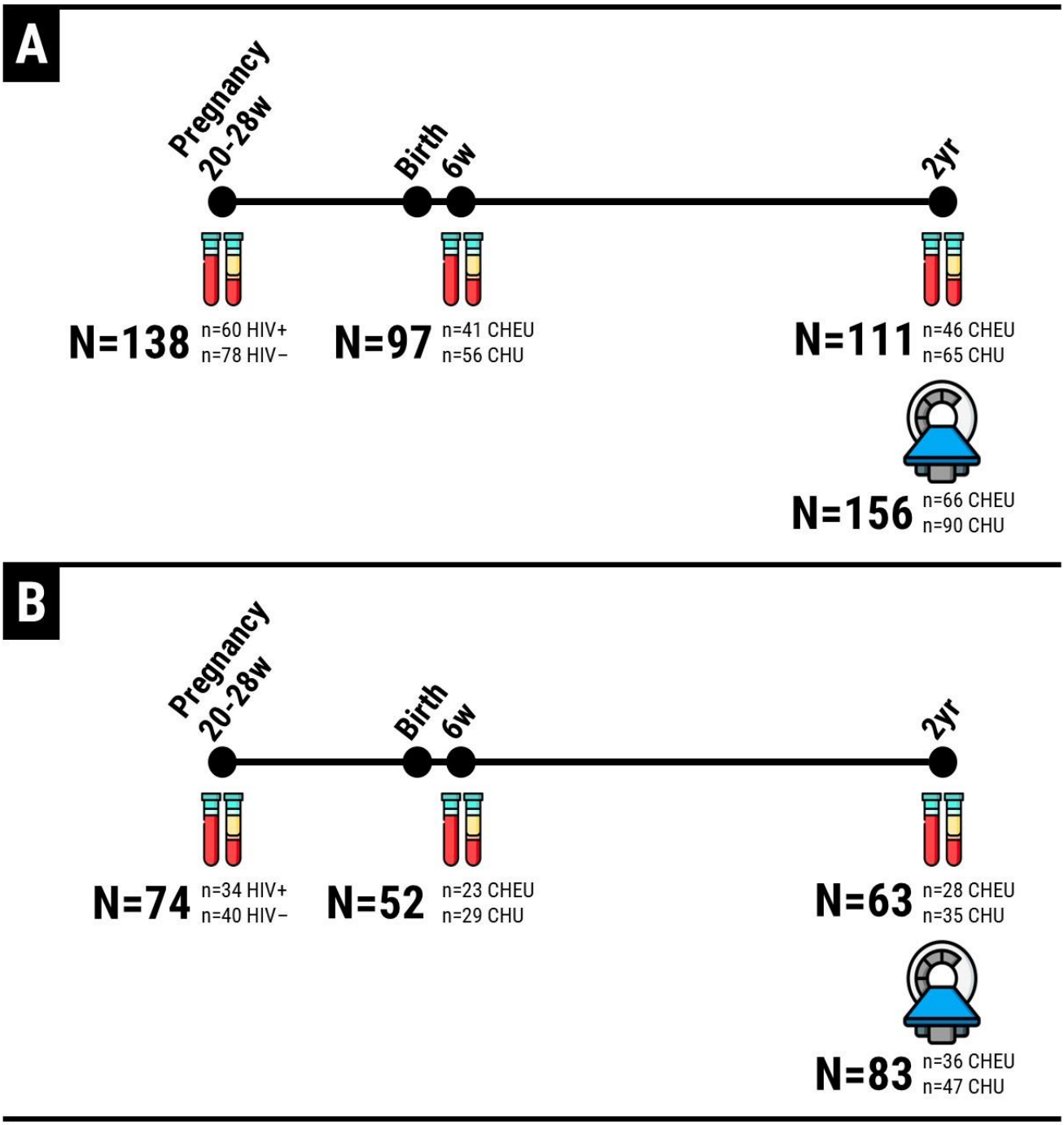
6 weeks	**29**	**23**	**LPWM**	Glutamate	IL-1β	Direct	0.06	0.03	-0.54 to 0.65	0.30	0.21	0.83
						Indirect	≈0.00	≈0.00	-0.34 to 0.23	0.05	0.07	0.95
						Total	0.07	0.03	-0.54 to 0.65	0.30	0.23	0.82
2 years	**35**	**28**	**PGM**	Glutamate	NGAL	Direct	-0.04	-0.02	-0.59 to 0.46	0.27	-0.14	0.89
						Indirect	≈0.00	≈0.00	-0.07 to 0.08	0.04	0.08	0.94
						Total	-0.04	-0.02	-0.57 to 0.48	0.27	-0.13	0.90
				Myo-inositol	MMP-9	Direct	0.03	0.02	-0.45 to 0.51	0.25	0.12	0.90
						Indirect	0.01	≈0.00	-0.06 to 0.08	0.02	0.49	0.62
						Total	0.04	0.02	-0.42 to 0.51	0.24	0.14	0.89
			L & RPWM	Glutamate pattern	NGAL	Direct	0.24	0.14	-0.17 to 0.65	0.21	1.18	0.24
						Indirect	≈0.00	≈0.00	-0.09 to 0.10	0.05	0.15	0.88
						Total	0.25	0.15	-0.16 to 0.64	0.20	1.25	0.21

Predictor: maternal HIV status; **Mediator**: maternal/child serum marker concentrations; **Outcome**: child neurometabolite ratios to total creatine at age 2–3 years.

Direct path: Predictor → Outcome
Indirect path: Predictor → Mediator → Outcome
Total path: Predictor → (Mediator + Outcome)

CHEU: Children who are HIV-Exposed and Uninfected; **CHU**: Children who are HIV-Unexposed; **MRS**: Magnetic Resonance Spectroscopy; **PGM**: Parietal Grey Matter; **LPWM**: Left Parietal White Matter; **RPWM**: Right Parietal White Matter; **Estimate**: Raw unstandardized regression coefficient; **StdEst**: Standardized regression coefficient for interpretability; **SE**: Standard Error.

Supplementary Figure 1. Sample sizes

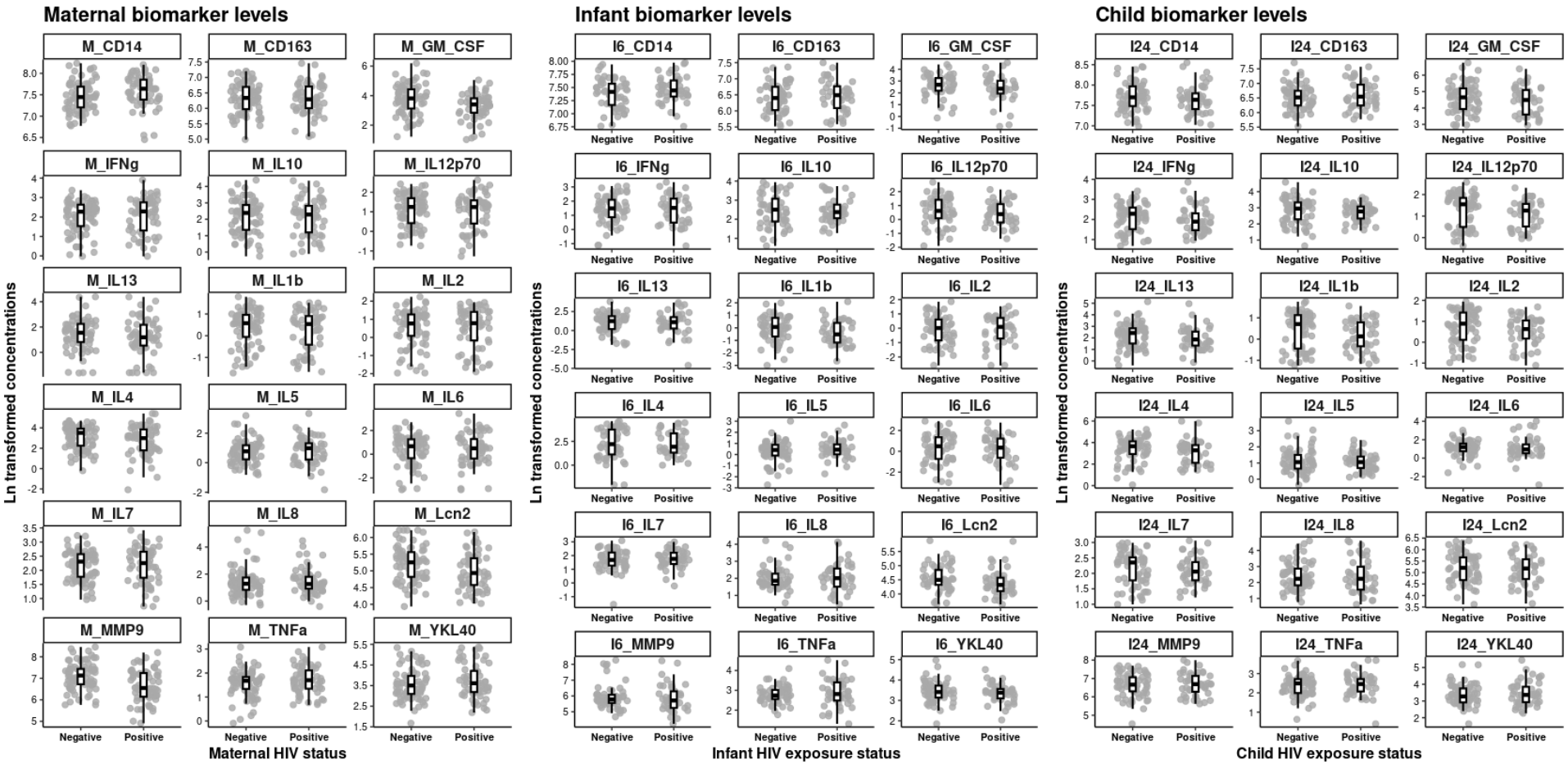


A. Number of participants with serum samples available at each timepoint, out of the entire cohort of children invited for magnetic resonance spectroscopy at 2-3 years of age.

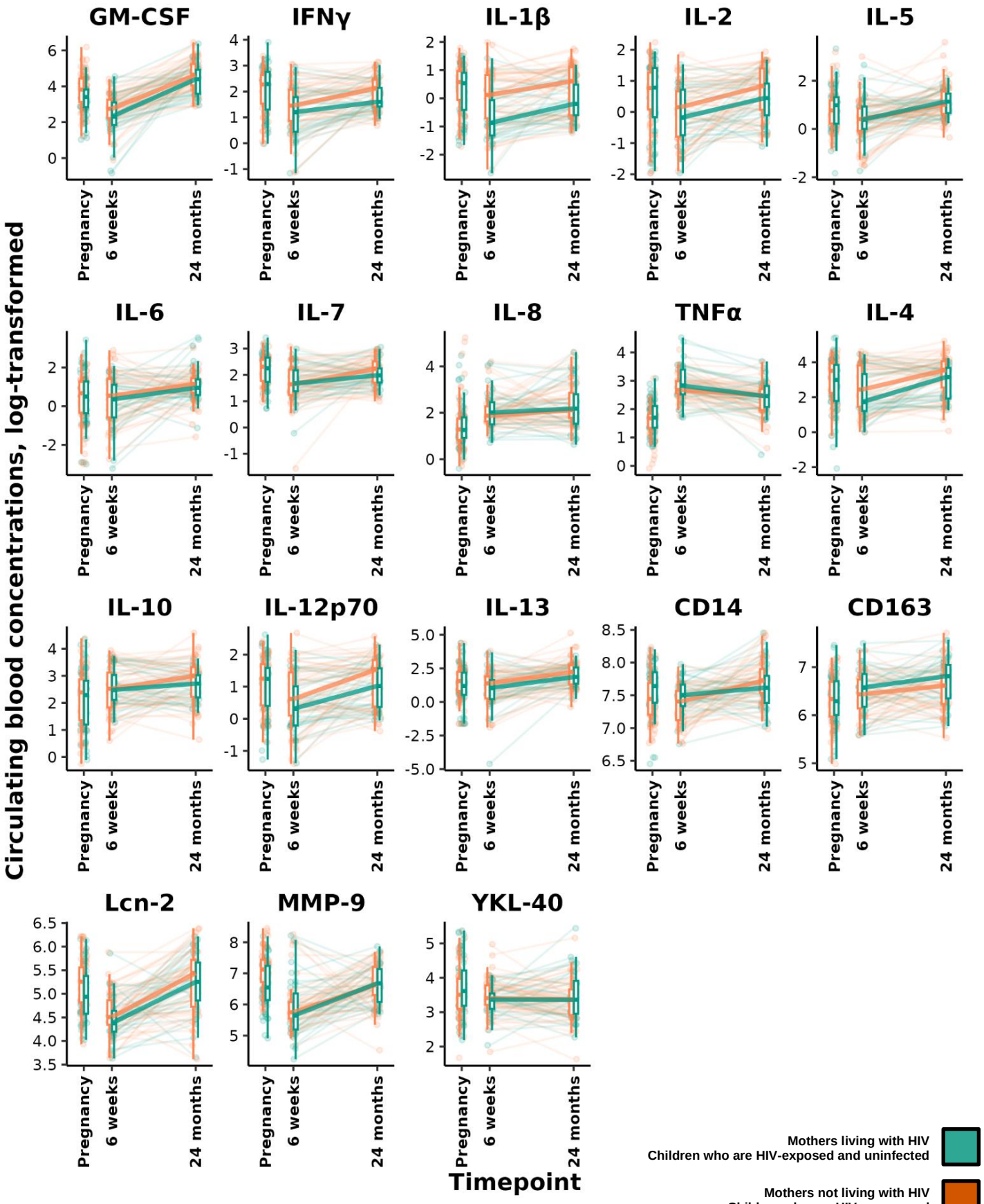
B. Number of participants with serum samples available at each timepoint, out of the entire cohort of children with complete, high-quality magnetic resonance spectroscopy data at 2-3 years of age.

CHEU: Children who are HIV-Exposed and Uninfected; **CHU**: Children who are HIV-Unexposed.

9 Supplementary Figure 2. Maternal, infant, and child serum marker concentrations



Supplementary Figure 3. Trajectories of child serum marker concentrations from 6 weeks to 2 years of age



11 Supplementary Figure 4. Forest plots: Associations between serum marker levels and child neurometabolite ratios in the HIV-exposed group