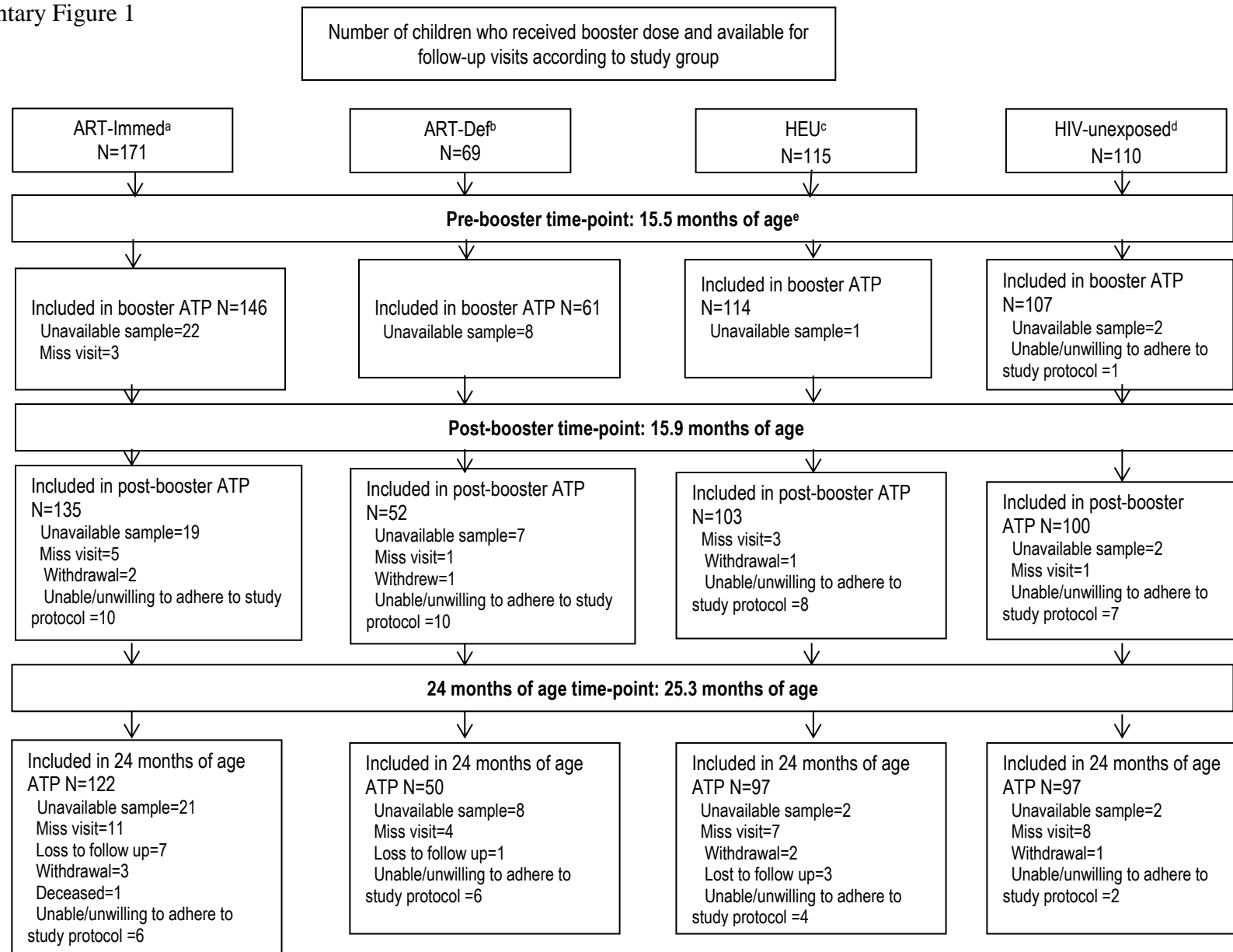


Supplementary Figure 1



Supplementary Figure 1. Reasons for exclusion from analyses at the different study time-points.

<sup>a</sup>ART-Immed: HIV-infected children with CD4+%  $\geq 25\%$  at enrolment who were initiated on antiretroviral therapy (ART) at enrolment;

<sup>b</sup>ART-Def: HIV-infected children with CD4+  $\geq 25\%$  at enrolment randomized to deferred ART; <sup>c</sup>HEU: HIV-uninfected children born to HIV-infected mothers; <sup>d</sup>HIV-unexposed: HIV-uninfected children born to HIV-uninfected mothers; <sup>e</sup>Pre-booster: 15.5 months of age, all children received a booster dose of DTP and no booster dose of hepatitis-B vaccine was administered.

Explanatory notes: ATP: analysis time-point; Unavailable sample: sample unavailable for further testing; Lost to follow-up: due to relocation or the children stopped the study without reason; Withdrew: parent or guardian withdrew informed consent; Missed visit: children missed a schedule visit; Unable/unwilling to adhere to study protocol.

Number of participants who left the study (excluded, missed visit, lost to follow-up, withdrawals, deceased) are represented as cumulative pre- and post-vaccination.

Supplementary Table 1: Comparison of children included in analysis versus those not included in analysis.

Variable	All children included		Children excluded from the current analysis		p-value	
Number	465		29			
Male, n (%)	218 (47)		19 (65)		0.051	
Black race, n (%)	420 (90)		29 (100)		0.079	
Coloured race, n (%)	45 (10)		0 (0)			
Soweto study center, n (%)	312 (67)		25 (86)		0.032	
Stellenbosch study center, n (%)	153 (33)		4 (14)			
Mean time in months between last dose in primary series and booster dose ( $\pm$ SD)	12.0 (0.6)		12.9 (2.1)		<0.001	
Mean age in months ( $\pm$ SD)	15.5 (0.5)		15.3 (0.4)		0.148	
Pre-booster <sup>a</sup>						
Post-booster <sup>b</sup>	15.9 (0.5)		15.8 (0.5)		0.454	
24 months of age	25.3 (0.6)		25.4 (0.6)		0.388	
	ART-Immed.	ART-Def	ART-Immed.	ART-Def	p-value <sup>c</sup>	p-value <sup>d</sup>
Total initiated ART between first and last dose of primary series n/N (%)	146/146 (100)	9/61 (15)	25/25 (100)	2/8 (25)	NE <sup>e</sup>	0.539
Total on ART between last dose of primary series and booster dose n/N (%)	146/146 (100)	52/61 (85)	25/25 (100)	7/8 (88)	NE	0.962
Time to ART initiation relative to dose 1 of primary series, median months (range)	ND	4 (3 to 8) n=59	ND	3 (2 to 4) n=7	ND	0.183

<sup>a</sup>Blood draw was undertaken just prior to and on the same day of DTP booster vaccination;

<sup>b</sup>Children who received booster dose within protocol-defined window periods and available immunogenicity data within 1-2 weeks after booster; <sup>c</sup>p-value ART-Immed. all children included compared to ART-Immed. children excluded from the current analysis; <sup>d</sup>p-value ART-Def all infants included compared to ART-Def Infants excluded from the current analysis; <sup>e</sup>NE: not evaluable; <sup>f</sup>ND: not done.

Supplementary Table 2. Geometric mean concentrations and proportion of children in the two ART Immed groups with sero-protective antibody titers and sero-conversion rates at pre-booster, post-booster and at 2 years of age

Antibody	Threshold	Time-point	ART/12m <sup>a</sup> N=70	ART/24m <sup>b</sup> N=76	p-value
TT	GMC	Pre-Booster	0.08 (0.06-0.09)	0.08 (0.07-0.10)	0.40
		Post-booster	7.3 (6.1-8.9)	6.0 (4.9-7.4)	0.24
		Fold-change <sup>c</sup>	100.5 (76.0-133.1)	78.1 (58.0-105.0)	0.45
		24 months	0.74 (0.60-0.92)	0.82 (0.68-0.98)	0.32
		Fold-change <sup>d</sup>	8.7 (6.7-11.3)	11.3 (9.3-13.7)	0.15
	≥0.01	Pre-booster: N/n; % (95% CI)	69/70; 99 (92-100)	76/76; 100 (95-100)	0.30
		Post-booster: N/n; % (95% CI)	63/63; 100 (94-100)	72/72; 100 (95-100)	NE <sup>e</sup>
		24 months: N/n; % (95% CI)	56/56; 100 (94-100)	66/66; 100 (95-100)	NE
	≥0.1	Pre-booster: N/n; % (95% CI)	36/70; 51 (40-63)	36/76; 47 (37-58)	0.38
		Post-booster: N/n; % (95% CI)	63/63; 100 (94-100)	72/72; 100 (95-100)	NE
		24 months: N/n; % (95% CI)	56/56; 100 (94-100)	66/66; 100 (95-100)	NE
	≥1	Pre-booster: N/n; % (95% CI)	0/70; 0	1/76; 1 (0.2-7)	0.34
		Post-booster: N/n; % (95% CI)	61/63; 97 (89-99)	69/72; 96 (88-99)	0.97
		24 months: N/n; % (95% CI)	18/56; 32 (21-45)	26/66; 39 (28-51)	0.96
	DT	GMC	Pre-Booster	0.10 (0.9-0.12)	0.10 (0.08-0.11)
Post-booster			3.2 (2.9-3.7)	2.8 (2.3-3.2)	0.23
Fold-change			33.1 (26.6-41.4)	28.1 (21.8-36.3)	0.71
24 months			0.45 (0.38-0.54)	0.47 (0.39-0.55)	0.91
Fold-change			4.2 (3.3-5.3)	5.0 (4.1-6.1)	0.45
≥0.01		Pre-booster: N/n; % (95% CI)	70/70; 100 (95-100)	76/76; 100 (95-100)	NE
		Post-booster: N/n; % (95% CI)	63/63; 100 (94-100)	72/72; 100 (95-100)	NE

		24 months: N/n; % (95% CI)	56/56; 100 (94-100)	66/66; 100 (95-100)	NE
	≥0.1	Pre-booster: N/n; % (95% CI)	40/70; 57 (46-68)	39/76; 51 (40-62)	0.84
		Post-booster: N/n; % (95% CI)	63/63; 100 (94-100)	72/72; 100 (95-100)	NE
		24 months: N/n; % (95% CI)	56/56; 100 (94-100)	65/66; 98.5 (92-100)	0.36
	≥1	Pre-booster: N/n; % (95% CI)	0/70; 0	0/76; 0	NE
		Post-booster: N/n; % (95% CI)	61/63; 97 (89-99)	64/72; 89 (80-94)	0.12
		24 months: N/n; % (95% CI)	7/56; 13 (6-24)	9/66; 14 (7-24)	0.75
HBsAg	GMC	15-18 months	721.5 (573.6-907.5)	816.4 (647.0-1030.1)	0.74
		24 months	211.8 (174.4-257.1)	271.1 (220.6-33.2)	0.67
		Fold-change	0.33 (0.24-0.45)	0.34 (0.25-0.46)	0.75
	≥10	15-18 months: N/n; % (95% CI)	70/70; 100 (95-100)	76/76; 100 (95-100)	NE
		24 months: N/n; % (95% CI)	56/56; 100 (94-100)	66/66; 100 (94-100)	NE
	≥100	15-18 months: N/n; % (95% CI)	68/70; 97 (90-99)	74/76; 97 (91-99)	0.86
		24 months: N/n; % (95% CI)	53/56; 95 (85-98)	59/66; 89 (80-95)	0.34
	FHA	GMC	Pre-Booster	36.1 (30.4-42.8)	32.2 (27.6-37.5)
Post-booster			437.1 (354.3-539.4)	416.5 (346.9-500.0)	0.99
Fold-change			12.8 (9.5-17.3)	13.2 (10.3-17.0)	0.55
24 months			208.3 (159.7-271.8)	190.4 (157.9-229.5)	0.74
Fold-change			5.5 (4.1-7.5)	6.1 (4.8-7.7)	0.74
≥4 fold		Post-booster: N/n; % (95% CI)	56/63; 90 (80-96)	60/69; 87 (77-93)	0.68
PT	GMC	Pre-booster	30.5 (26.2-35.6)	33.0 (28.6-37.5)	0.04
		Post-booster	953.3 (741.9-1224.9)	949.9 (749.4-1203.9)	0.46
		Fold-change	31.0 (22.6-42.5)	29.3 (22.4-38.2)	0.94

		24 months	255.3 (212.6-306.6)	253.9 (214.5-300.6)	0.98
		Fold-change	8.3 (6.8-10.2)	8.0 (6.4-10.0)	0.83
	≥4 fold	Post-booster: N/n; % (95% CI)	59/63; 95 (87-98)	67/69; 97 (90-99)	0.52

Footnote: Geometric mean concentration (95% confidence interval); Percentage of participants with concentration above threshold (95% confidence interval); Units for antibody to TT, DT, PT and FHA are IU/ml; Units for HBsAg are mIU/ml. <sup>a</sup>ART/12m: HIV-infected children with CD4+ ≥25% at enrolment who were initiated on ART at enrolment and in whom ART was interrupted at 12-months of age; <sup>b</sup>ART/24m: HIV-infected children with CD4+ ≥25% at enrolment who were initiated on ART at enrolment and in whom ART was interrupted at 24-months of age; <sup>d</sup>Fold-change: Fold-change in GMC between pre-booster and post-booster; <sup>e</sup>Fold-change: Fold-change in GMC between post-booster and 24 months of age; <sup>c</sup>NE: not evaluable.

#### Explanatory notes:

p-values were either calculated by linear or logistic regression and adjusted for age, gender, race, study center, pre-booster antibody concentration (for post-booster and 24 months of age time-points) and CD4+ cell percentage in HIV-infected children; or by Pearson's chi-square test for values in italics. Similar results were obtained by univariate analysis.

Supplementary Table 3. Geometric mean concentrations at pre-booster, post-booster and at 24 months of age

Antibody	Threshold	Time-point	HIV-unexposed <sup>a</sup> N=107	ART-Immed <sup>b</sup> N=146	p-value <sup>c</sup>	ART-Def <sup>d</sup> N=61	p-value <sup>e</sup>	p-value <sup>f</sup>	HEU <sup>g</sup> N=114	p-value <sup>h</sup>
TT	GMC	Pre-Booster	0.13 (0.12-0.15)	0.08 (0.07-0.09)	<0.001	0.11 (0.08-0.15)	0.09	0.07	0.13 (0.12-0.15)	0.47
		Post-booster	17.9 (15.8-20.4)	6.6 (5.7-7.6)	<0.001	4.4 (3.2-6.1)	<0.001	0.01	15.4 (13.3-17.9)	0.72
		Fold-change <sup>i</sup>	135.6 (114.1-161.0)	87.9 (71.7-107.7)	<0.001	39.8 (23.0-68.8)	<0.001	0.001	116.6 (97.5-139.3)	0.72
		24 months	1.7 (1.5-1.9)	0.79 (0.69-0.90)	<0.001	0.23 (0.15-0.35)	<0.001	<0.001	1.3 (1.1-1.5)	0.01
		Fold-change <sup>j</sup>	-10.1 (8.7-11.8)	-9.7 (8.0-11.7)	0.74	-21.3 (13.8-32.7)	<0.001	0.002	-11.9 (9.8-14.5)	0.08
DT	GMC	Pre-Booster	0.08 (0.07-0.09)	0.10 (0.09-0.11)	0.22	0.12 (0.10-0.15)	0.05	0.05	0.07 (0.05-0.09)	0.07
		Post-booster	3.9 (3.5-4.3)	3.0 (2.7-3.3)	0.15	2.3 (1.8-3.0)	0.003	0.04	4.6 (4.1-5.2)	0.002
		Fold-change <sup>i</sup>	49.6 (41.4-59.4)	30.4 (25.7-36.0)	0.08	17.9 (12.2-26.2)	0.001	0.001	68.4 (48.9-95.8)	0.004
		24 months	0.68 (0.60-0.77)	0.46 (0.41-0.52)	<0.001	0.20 (0.15-0.27)	<0.001	<0.001	0.61 (0.53-0.70)	0.37
		Fold-change <sup>j</sup>	-5.5 (4.8-6.4)	-6.8 (5.8-8.0)	0.008	-11.4 (8.3-15.7)	0.001	0.008	-7.7 (6.5-9.1)	0.004
FHA	GMC	Pre-booster	24.6 (20.9-28.9)	34.0 (30.3-38.1)	0.001	31.9 (26.9-38.0)	0.09	0.23	34.5 (30.1-39.6)	0.01
		Post-booster	467.6 (395.8-552.4)	426.0 (371.6-488.3)	0.71	402.2 (324.9-497.9)	0.78	0.50	806.2 (703.2-924.2)	<0.001
		Fold-change <sup>i</sup>	18.7 (14.8-23.6)	13.0 (10.8-15.8)	0.22	12.3 (9.1-16.6)	0.36	>0.99	23.1 (19.0-28.1)	0.01
		24 months	170.3	198.4	0.26	103.9 (70.3-153.7)	0.02	0.001	140.9	0.14

			(151.2-191.8)	(169.7-232.0)					(120.1-165.4)	
		Fold-change <sup>j</sup>	-2.6 (2.1-3.2)	-2.1 (1.7-2.6)	0.40	-3.6 (2.3-5.6)	0.04	0.03	-5.8 (4.8-7.0)	<0.001
PT	GMC	Pre-booster	38.3 (33.9-43.3)	31.8 (28.7-35.2)	0.004	27.2 (21.6-34.2)	0.004	0.38	39.5 (35.3-44.3)	0.64
		Post-booster	2168.8 (1856.7-2533.3)	951.5 (802.7-1127.8)	<0.001	767.6 (594.8-990.7)	<0.001	0.19	3138.2 (2650.6-3715.5)	<0.001
		Fold-change <sup>i</sup>	56.1 (45.6-69.0)	30.1 (24.6-36.8)	0.01	27.9 (18.3-42.5)	0.04	0.89	79.7 (65.7-96.7)	0.001
		24 months	268.4 (228.3-315.5)	254.6 (225.3-287.7)	0.54	67.2 (47.9-94.3)	<0.001	<0.001	348.6 (288.6-421.2)	0.09
		Fold-change <sup>j</sup>	-7.7 (6.1-9.7)	-4.0 (3.3-4.9)	0.001	-12.0 (8.6-16.7)	0.01	<0.001	-9.6 (7.6-12.2)	0.06
HBsAg	GMC	5 months <sup>k</sup>	2521.0 (2249.8-2824.9)	924.9 (778.3-1099.1)	<0.001	536.6 (373.0-772.0)	<0.001	0.02	2019.3 (1740.3-2343.0)	0.04
		15-18 months	1198.5 (1033.9-1389.4)	769.4 (654.4-904.7)	0.001	700.2 (587.3-834.7)	0.002	0.55	880.1 (719.2-1077.0)	0.02
		Fold-change <sup>l</sup>	-2.1 (1.8-2.5)	-1.2 (0.9-1.5)	<0.001	0.8 (0.50-1.3)	<0.001	0.13	-2.3 (1.8-3.0)	0.78
		24 months	396.1 (332.5-471.8)	242.0 (209.9-279.1)	0.001	54.8 (32.2-93.0)	<0.001	<0.001	311.9 (264.8-367.4)	0.11
		Fold-change <sup>m</sup>	-3.0 (2.5-3.7)	-3.0 (2.4-3.7)	0.94	-13.3 (7.6-23.2)	<0.001	<0.001	-3.0 (2.3-4.0)	0.97

Footnote: Geometric mean concentration (95% confidence interval); Units for geometric mean concentration (GMC) are IU/ml for TT, DT, FHA and PT; mIU/ml for HBsAg. <sup>a</sup>HIV-unexposed: HIV-uninfected children born to HIV-uninfected mothers; <sup>b</sup>ART-Immed: HIV-infected children with CD4+  $\geq$ 25% at enrolment who were initiated on ART at enrolment; <sup>c</sup>P value: HIV-unexposed vs. ART-Immed;



<sup>d</sup>ART-Def: HIV-infected children with CD4+  $\geq 25\%$  at enrolment randomized to deferred ART; <sup>e</sup>*P* value: HIV-unexposed vs. ART-Def; <sup>f</sup>*P* value: ART-Immed vs. ART-Def; <sup>g</sup>HEU: HIV-uninfected children born to HIV-infected mothers; <sup>h</sup>*P* value: HIV-unexposed vs. HEU; <sup>i</sup>Fold-change: Fold-change in GMC between pre-booster and post-booster; <sup>j</sup>Fold-change: Fold-change in GMC between post-booster and 24 months of age; <sup>k</sup>5 months: results of primary vaccination and have been published in Simani et al 2014 [1]; <sup>l</sup>Fold-change: Fold-change in GMC between 5 months and 15-18 months of age; <sup>m</sup>Fold-change: Fold-change in GMC between 15-18 months and 24 months of age.

#### Explanatory notes:

Geometric mean concentration: All p-values were calculated by linear regression and adjusted for age, gender, race, study center, pre-booster antibody concentration (for post-booster and 24 months time-point) and CD4+ cell percentage in HIV-infected children.

Supplementary Table 4. Percentage of children with sero-protective antibody titers at pre-booster, post-booster and at 24 months of age

Antibody	Threshold	Time-point	HIV-unexposed <sup>a</sup> N=107	ART-Immed <sup>b</sup> N=146	p-value <sup>c</sup>	ART-Def <sup>d</sup> N=61	p-value <sup>e</sup>	p-value <sup>f</sup>	HEU <sup>g</sup> N=114	p-value <sup>h</sup>
TT	≥0.01	Pre-booster: n/N; % (95% CI)	107/107; 100 (97-100)	145/146; 99 (96-100)	0.39	61/61; 100 (94-100)	NE	0.52	114/114; 100 (97-100)	NE <sup>i</sup>
		Post-booster: n/N; % (95% CI)	100/100; 100 (96-100)	135/135; 100 (97-100)	NE	52/52; 100 (93-100)	NE	NE	103/103; 100 (96-100)	NE
		24 months: n/N; % (95% CI)	97/97; 100 (96-100)	122/122; 100 (97-100)	NE	48/50; 96 (87-99)	0.05	0.03	97/97; 100 (96-100)	NE
	≥0.1	Pre-booster: n/N; % (95% CI)	85/107; 79 (71-86)	72/146; 49 (41-57)	<0.001	26/61; 43 (31-55)	<0.001	0.38	90/114; 79 (71-85)	0.63
		Post-booster: n/N; % (95% CI)	100/100; 100 (96-100)	135/135; 100 (97-100)	NE	52/52; 100 (93-100)	NE	NE	103/103; 100 (96-100)	NE
		24 months: n/N; % (95% CI)	97/97; 100 (96-100)	122/122; 100 (97-100)	NE	36/50; 72 (58-83)	<0.001	<0.001	95/97; 98 (93-99)	0.16
	≥1	Pre-booster: n/N; % (95% CI)	0/107; 0	1/146; 0.7 (0.1-4)	0.39	1/61; 2 (0.3-9)	0.18	0.47	1/114; 0.9 (0.2-5)	0.33

		Post-booster: n/N; % (95% CI)	100/100; 100 (96-100)	130/135; 96 (92-98)	0.05	44/52; 85 (73-92)	<0.001	0.01	103/103; 100 (96-100)	NE
		24 months: n/N; % (95% CI)	74/97; 76 (67-84)	44/122; 36 (28-45)	<0.001	7/50; 14 (7-26)	<0.001	0.01	64/97; 66 (56-75)	0.05
DT	≥0.01	Pre-booster: n/N; % (95% CI)	107/107; 100 (97-100)	146/146; 100 (97-100)	NE	61/61; 100 (94-100)	NE	NE	105/114; 92. (86-96)	0.003
		Post-booster: n/N; % (95% CI)	100/100; 100 (96-100)	135/135; 100 (97-100)	NE	52/52; 100 (93-100)	NE	NE	103/103; 100 (96-100)	NE
		24 months: n/N; % (95% CI)	97/97; 100 (96-100)	122/122; 100 (97-100)	NE	50/50; 100 (93-100)	NE	NE	97/97; 100 (96-100)	NE
	≥0.1	Pre-booster: n/N; % (95% CI)	41/107; 38 (30-48)	79/146; 54 (46-62)	0.001	35/61; 57 (45-69)	0.02	0.95	61/114; 54 (44-62)	0.03
		Post-booster: n/N; % (95% CI)	100/100; 100 (96-100)	135/135; 100 (97-100)	NE	52/52; 100 (93-100)	NE	NE	103/103; 100 (96-100)	NE
		24 months: n/N; % (95% CI)	96/97; 99 (94-100)	121/122; 99 (96-100)	0.76	40/50; 80 (67-89)	0.02	0.004	95/97; 98 (93-99)	0.49
	≥1	Pre-booster: n/N; % (95% CI)	0/107; 0	0/146; 0	NE	0/61; 0	NE	NE	0/114; 0	NE

		Post-booster: n/N; % (95% CI)	99/100; 99 (95-100)	125/135; 93 (87-96)	0.08	44/52; 85 (73-92)	0.02	0.14	100/103; 97 (92-99)	0.45
		24 months: n/N; % (95% CI)	31/97; 32 (24-42)	16/122; 13 (8-20)	0.001	1/50; 2 (0.3-1.0)	0.003	0.03	26/97; 27 (19-36)	0.40
FHA	≥4 fold increase <sup>j</sup>	Post-booster: n/N; % (95% CI)	91/100; 91 (84-95)	116/131; 89 (82-93)	0.75	45/50; 90 (79-96)	0.99	0.69	100/103; 97 (92-99)	0.06
PT	≥4 fold increase	Post-booster: n/N; % (95% CI)	99/100; 99 (95-100)	126/131; 96 (91-98)	0.31	45/50; 90 (79-96)	0.10	0.19	102/103; 99 (95-100)	0.88
HepB	≥10	15-18 months: n/N; % (95% CI)	107/107; 100 (97-100)	146/146; 100 (97-100)	NE	61/61; 100 (94-100)	NE	NE	114/144; 100 (97-100)	NE
		24 months: n/N; % (95% CI)	97/97; 100 (96-100)	122/122; 100 (97-100)	NE	36/50; 72 (58-83)	<0.001	<0.001	97/97; 100 (96-100)	NE
	≥100	15-18 months: n/N; % (95% CI)	107/107; 100 (97-100)	142/146; 97 (93-99)	0.08	61/61; 100 (94-100)	NE	0.19	107/114; 94 (88-97)	0.01
		24 months: n/N; % (95% CI)	93/97; 96 (90-98)	112/122; 92 (86-96)	0.28	25/50; 50 (37-63)	<0.001	<0.001	89/97; 92 (85-96)	0.17

Footnote: Percentage of participants with concentration above threshold (95% confidence interval) Units are IU/ml for TT, DT, FHA

and PT; mIU/ml for HBsAg. <sup>a</sup>HIV-unexposed: HIV-uninfected children born to HIV-uninfected mothers; <sup>b</sup>ART-Immed: HIV-infected

children with CD4+  $\geq 25\%$  at enrolment who were initiated on ART at enrolment; <sup>c</sup>p-value: HIV-unexposed vs. ART-Immed; <sup>d</sup>ART-Def: HIV-infected children with CD4+  $\geq 25\%$  at enrolment randomized to deferred ART; <sup>e</sup>p-value: HIV-unexposed vs. ART-Def; <sup>f</sup>p-value: ART-Immed vs. ART-Def; <sup>g</sup>HEU: HIV-uninfected children born to HIV-infected mothers; <sup>h</sup>p-value: HIV-unexposed vs. HEU; <sup>i</sup>NE: not evaluable; <sup>j</sup> $\geq 4$  fold increase: sero-conversion rate, children showing 4 fold increase in antibody concentration from pre-booster to post-booster dose.

Explanatory notes: Percentage participants: p-values were either calculated by logistic regression and adjusted for age, gender, race, study center, pre-booster antibody concentration (for post-booster and 24 months time-point) and CD4+ cell percentage in HIV-infected children; or by Pearson's chi-square test for values in italics.