Title: Live-attenuated Varicella-zoster virus vaccine does not induce HIV-target cell activation

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Supplemental Material

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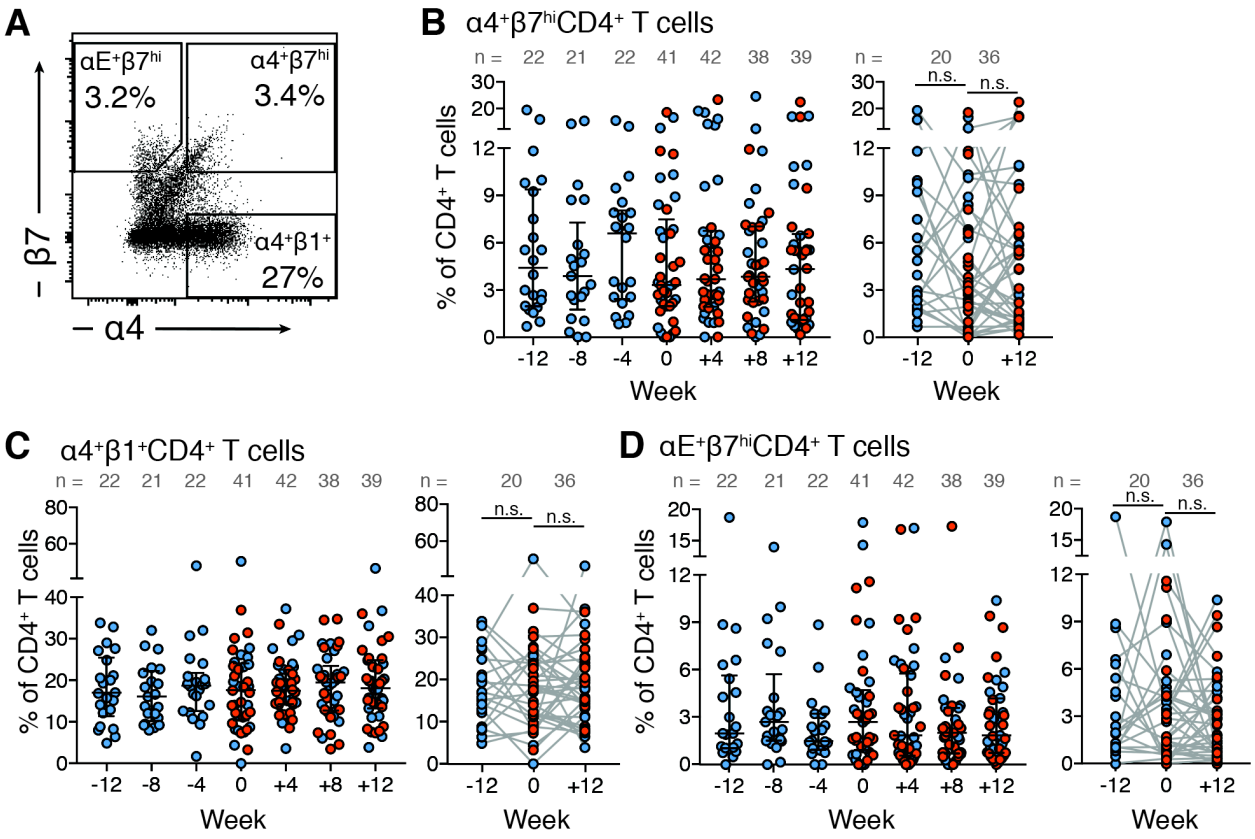

Figure S1. Integrin expression on cervical CD4 T cells. (**A**) Representative flow cytometry plot for the identification of cervical $\alpha4^+\beta7^{hi}$, $\alpha4^+\beta1^+$ and $\alpha E^+\beta7^{hi}$ $CD4^+$T cells. Cells were pre-gated on lymphocytes, singlets, viability by LIVE/DEAD staining, $CD3^+$ and $CD4^+$ cells. Expression of (**B**) $\alpha4^+\beta7^{hi}$, (**C**) $\alpha4^+\beta1^+$, (**D**) $\alpha E^+\beta7^{hi}$ on cervical $CD4^+$ T cells prior to vaccination (weeks -12 to 0) and post-vaccination (weeks +4 to +12). Graphs at the left show median with interquartile ranges (IQR) and graphs at the right the paired comparison between weeks -12 and 0 and between weeks 0 and +12 for each of the graphs. Individuals in the immediate and delayed groups are shown in red and blue, respectively, and were grouped according to time from vaccination for this analysis. Time points were compared to week 0 using Wilcoxon signed rank-test. No significant change was observed between any of the time-points (unadjusted for multiple comparisons). *n.s.* not significant.

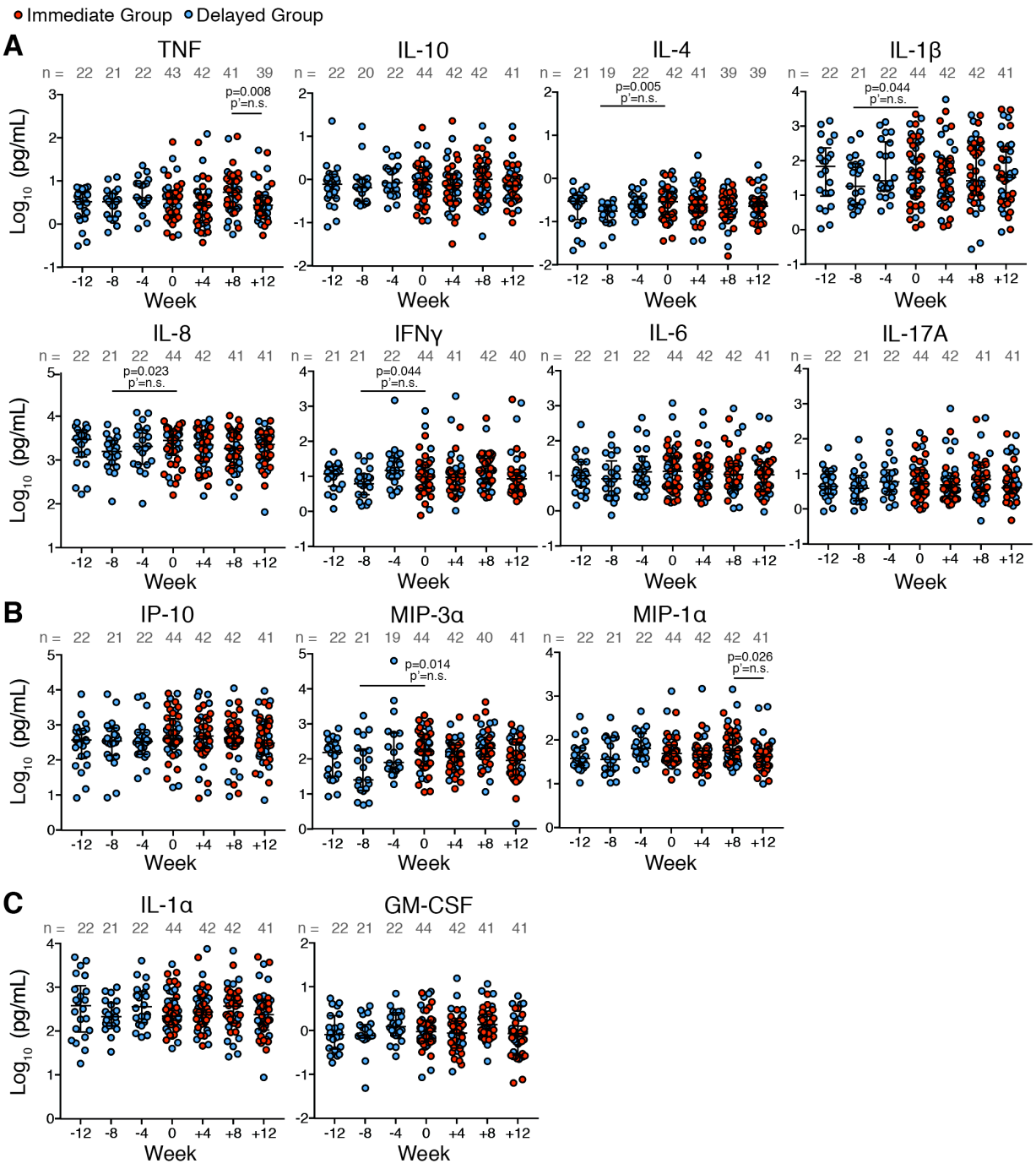

Figure S2. Cytokine concentrations in cervico-vaginal secretions (CVS). Concentration of individual cytokines composing (**A**) PC1 (**B**) PC2 and (**C**) PC3. Graphs show median with interquartile ranges (IQR). Time points were compared to week 0 using Wilcoxon signed rank-test. *p* and *p'* show p values unadjusted and adjusted for multiple comparisons, respectively. *n.s.* not significant.

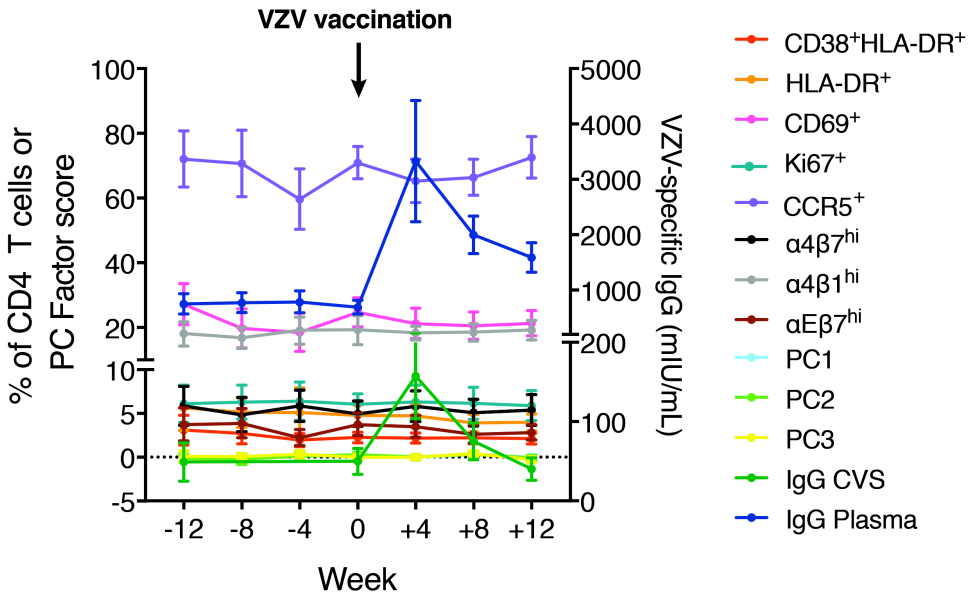

Figure S3. IA and VZV-specific IgG levels prior to and post-VZV vaccination. Graph shows mean and 95% confidence interval for the frequency of cervical CD4 T cells expressing HLA-DR, CD69, Ki67, CCR5, $\alpha4\beta7^{hi}$, $\alpha4\beta1^{+}$, $\alpha E\beta7^{hi}$, or co-expressing CD38 and HLA-DR, PC1, PC2 and PC3 factor scores for cervico-vaginal secretion (CVS) as well as the concentration of VZV-specific IgG (mIU/mL) measured in plasma and in CVS prior to vaccination (weeks -12 to 0) and post-vaccination (weeks +4 to +12).

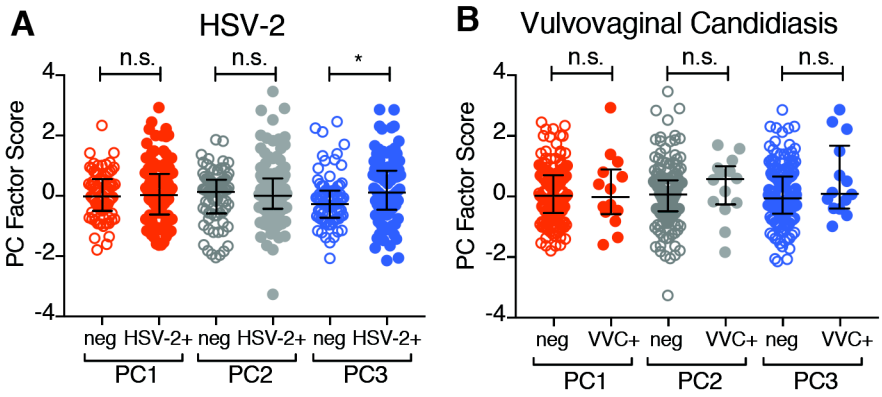

Figure S4. Impact of HSV-2 and candidiasis on genital IA. Cumulative PC factor scores for (**A**) HSV-2+ (n=143) and HSV-2 neg (n=91) records and for (**B**) vulvovaginal candidiasis + (VVC+, n=14) and candidiasis – (neg, n=220) Graphs show median with interquartile ranges (IQR). *$p < 0.05$ as determined by a two-tailed Mann-Whitney test and adjusted for multiple comparisons using step-down procedure. *n.s.* not significant.

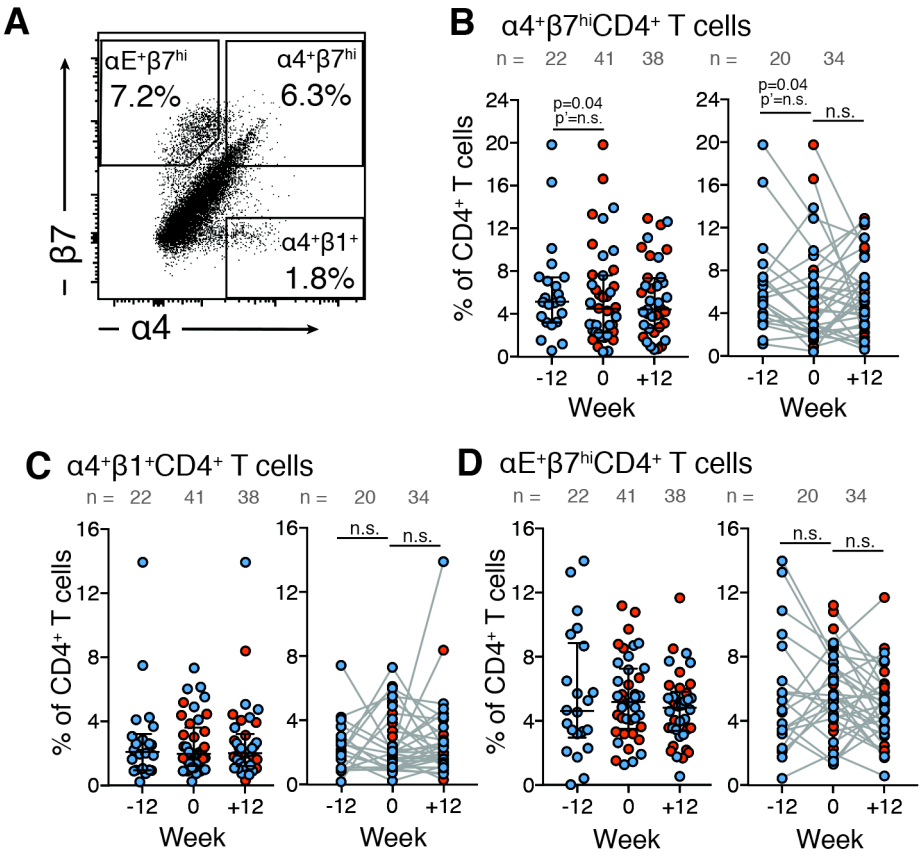

Figure S5. Integrin expression on rectal CD4 T cells. (**A**) Representative flow cytometry plot for the identification of cervical $\alpha4^+\beta7^{hi}$, $\alpha4^+\beta1^+$ and $\alpha E^+\beta7^{hi}$ $CD4^+$T cells. Cells were pre-gated on lymphocytes, singlets, viability by LIVE/DEAD staining, $CD3^+$ and $CD4^+$ cells. Expression of (**B**) $\alpha4^+\beta7^{hi}$, (**C**) $\alpha4^+\beta1^+$, (**D**) $\alpha E^+\beta7^{hi}$ on rectal $CD4^+$ T cells prior to vaccination (weeks -12 and 0) and post-vaccination (week +12). Graphs at the left show median with interquartile ranges (IQR) and graphs at the right the paired comparison between weeks -12 and 0 and between weeks 0 and +12 for each of the graphs. Individuals in the immediate and delayed groups are shown in red and blue, respectively, and were grouped according to time from vaccination for this analysis. Time points were compared to week 0 using Friedman Test, followed by Wilcoxon signed rank-test. *p* and *p'* show p values unadjusted and adjusted for multiple comparisons using step-down procedure, respectively. *n.s.* not significant.

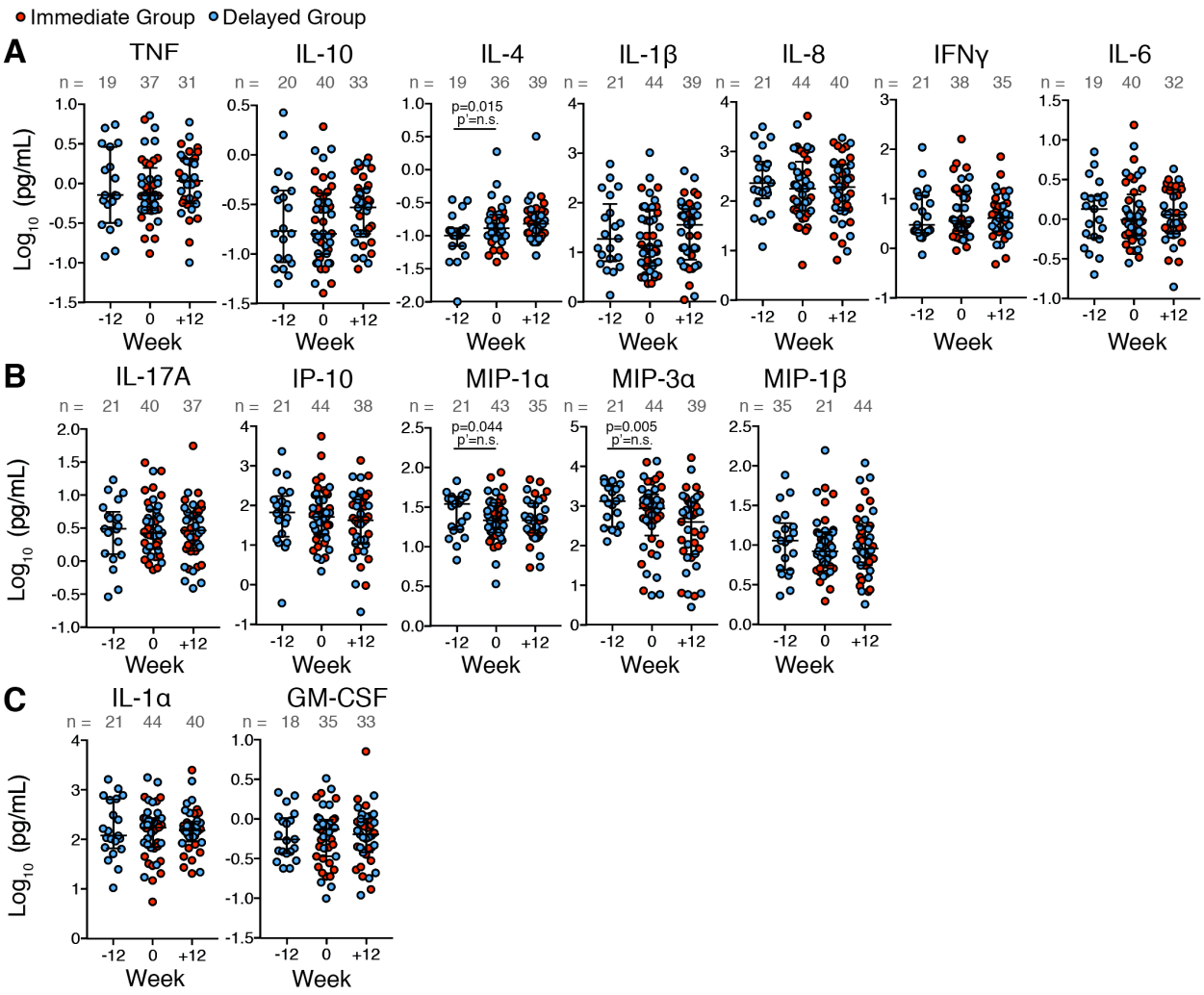

Figure S6. Cytokine concentrations in rectal secretions (RS). Concentration of individual cytokines composing (**A**) PC1 (**B**) PC2 and (**C**) PC3. Graphs show median with interquartile ranges (IQR). Time points were compared to week 0 using Wilcoxon signed rank-test. *p* and *p'* show p values unadjusted and adjusted for multiple comparisons, respectively. *n.s.* not significant.

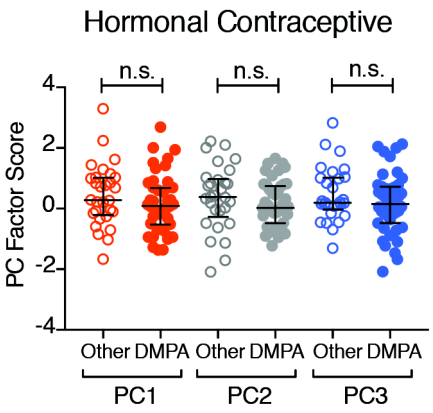

Figure S7. Impact of DMPA use on rectal IA. Cumulative PC factor scores for records of DMPA users (n=43) and for records of women using other contraceptives (n=29). Graph shows median with interquartile ranges (IQR). Unpaired comparison using two-tailed Mann-Whitney test. No significant change was observed between any of the time-points (unadjusted for multiple comparisons). *n.s.* not significant.

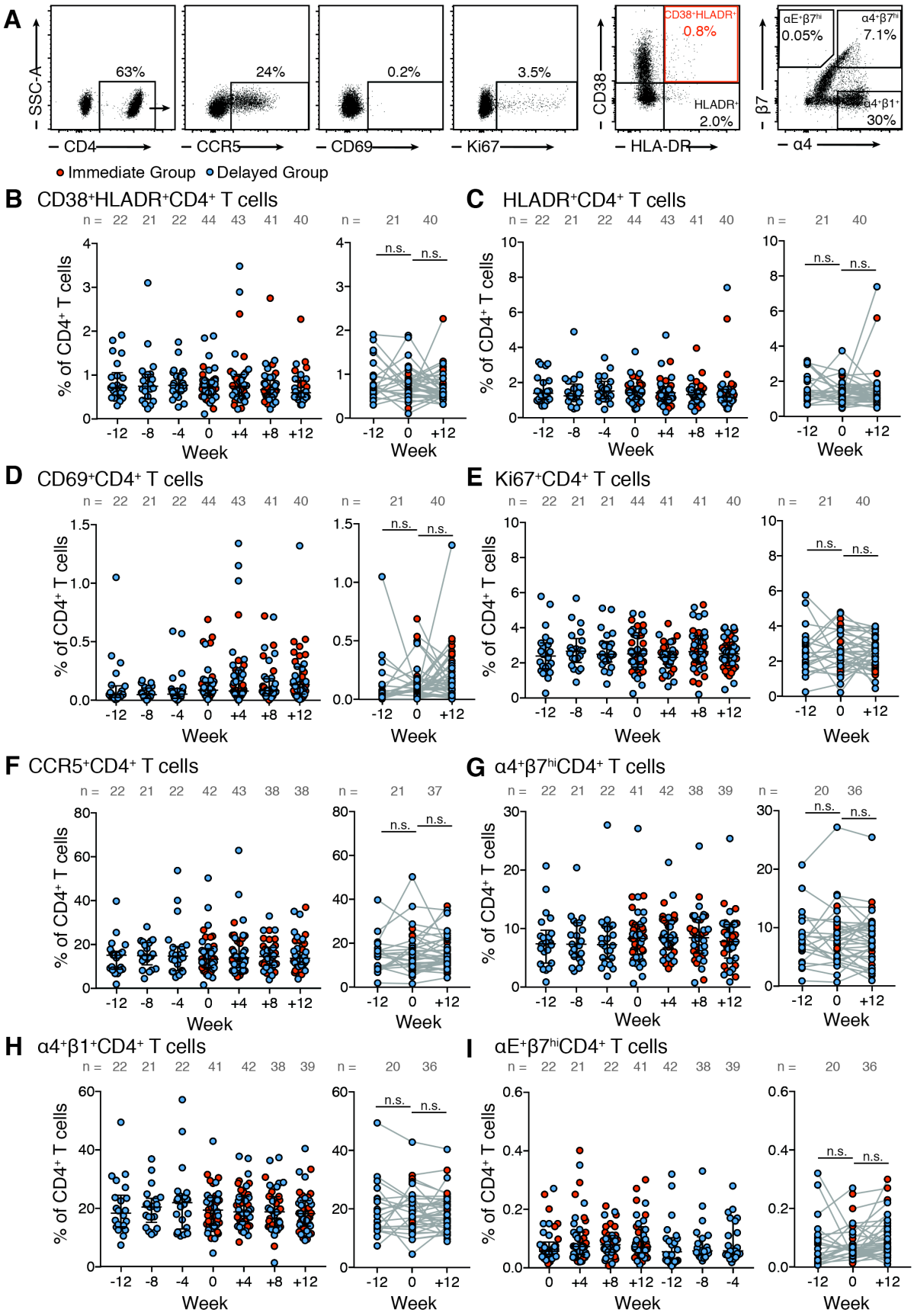
A
SSC-A
63%
CD4
24%
CCR5
0.2%
CD69
3.5%
Ki67
CD38
CD38+HLADR+
0.8%
HLADR+
2.0%
HLA-DR
β7
αE+β7hi
0.05%
α4+β7hi
7.1%
α4+β1+
30%
α4
Immediate Group
Delayed Group
B CD38+HLADR+CD4+ T cells
C HLADR+CD4+ T cells
D CD69+CD4+ T cells
E Ki67+CD4+ T cells
F CCR5+CD4+ T cells
G α4+β7hiCD4+ T cells
H α4+β1+CD4+ T cells
I αE+β7hiCD4+ T cells
% of CD4+ T cells
Week
n.s.

Figure S8. Expression of IA markers on CD4 T cells isolated from blood. (**A**) Representative flow cytometry plots for the identification of blood $CD4^+$ T cells, and $CCR5^+$, $CD69^+$, $Ki67^+$, $CD38^+HLADR^+$, $HLADR^+$, $\alpha4^+\beta7^{hi}$, $\alpha4^+\beta1^+$ and $\alpha E^+\beta7^{hi}$ $CD4^+$T cells. Cells were pre-gated on lymphocytes, singlets, live and $CD3^+$ cells. Expression of (**B**) $CD38^+HLADR^+$, (**C**) $HLADR^+$, (**D**) $CD69^+$, (**E**) $Ki67^+$, (**F**) $CCR5^+$, (**G**) $\alpha4^+\beta7^{hi}$, (**H**) $\alpha4^+\beta1^+$, (**I**) $\alpha E^+\beta7^{hi}$ on circulatory $CD4^+$ T cells prior to vaccination (weeks -12 to 0) and post-vaccination (weeks +4 to +12). Graphs at the left show median with interquartile ranges (IQR) and graphs at the right the paired comparison between weeks -12 and 0 and between weeks 0 and +12 for each of the graphs. Individuals in the immediate and delayed groups are shown in red and blue, respectively, and were grouped according to time from vaccination for this analysis. Time points were compared to week 0 using Wilcoxon signed rank-test. No significant change was observed between any of the time-points (unadjusted for multiple comparisons). *n.s.* not significant.

Table 1S: Study power to detect a true difference in the expression of the cellular markers analyzed in the trial.

	Power	Ability to detect	CD38 - HLADR	HLADR	CD69	Ki67	CCR5	α4β7^{hi}	α4β1	αEβ7^{hi}
Cervical CD4 T cells	**80%**	True difference >	**1.35**	**2.34**	**9.53**	**2.68**	**11.44**	**2.55**	**8.60**	**2.88**
		Fold change (from wk 0) >	0.60	0.49	0.39	0.44	0.16	0.51	0.45	0.77
	95%	True difference >	**1.78**	**3.04**	**12.29**	**3.45**	**14.75**	**3.28**	**11.08**	**3.69**
		Fold change (from wk 0) >	0.79	0.64	0.50	0.57	0.21	0.66	0.58	0.99
Rectal CD4 T cells	**80%**	True difference >	**0.59**	**1.01**	**7.91**	**1.53**	**10.86**	**1.62**	**1.33**	**1.58**
		Fold change (from wk 0) >	0.39	0.40	0.30	0.41	0.19	0.29	0.53	0.29
	95%	True difference >	**0.76**	**1.31**	**10.20**	**1.97**	**13.94**	**2.08**	**1.71**	**2.03**
		Fold change (from wk 0) >	0.50	0.52	0.38	0.53	0.25	0.37	0.68	0.37
Blood CD4 T cells	**80%**	True difference >	**0.21**	**0.37**	**0.09**	**0.65**	**4.68**	**1.98**	**5.29**	**0.03**
		Fold change (from wk 0) >	0.27	0.26	0.65	0.25	0.31	0.23	0.26	0.43
	95%	True difference >	**0.27**	**0.48**	**0.12**	**0.84**	**5.80**	**2.54**	**6.80**	**0.04**
		Fold change (from wk 0) >	0.35	0.33	0.84	0.32	0.38	0.29	0.33	0.54

Calculations based on the respective standard deviations of the differences in the frequencies between visits prior to vaccination for each of the markers (intraindividual), the sample size, α=0.05, β=0.80 and 0.95. Numeric-fold change was calculated as the ratio of the true mean difference able to be detected between the final value (week 12) and the initial value (week 0) over the initial value (week 0).The calculations were assisted by the user-friendly algorithm: http://hedwig.mgh.harvard.edu/sample_size/js/js_crossover_quant.html

Table 2S: Study power to detect a true difference in the principal component (PC) factor scores analyzed in the trial.

	Power	Ability to detect	PC1	PC2	PC3
Cervico-vaginal Secretion	**80%**	True difference >	**0.45**	**0.62**	**0.55**
		Fold change (from wk 0) >	2.12	2.41	1.91
	95%	True difference >	**0.58**	**0.80**	**0.72**
		Fold change (from wk 0) >	2.73	3.11	2.47
Rectal Secretion	**80%**	True difference >	**0.94**	**0.59**	**1.05**
		Fold change (from wk 0) >	4.48	2.70	4.39
	95%	True difference >	**1.22**	**0.77**	**1.36**
		Fold change (from wk 0) >	5.81	3.50	5.68

Calculations based on the respective standard deviations of the differences in the PC factor scores between visits prior to vaccination (intraindividual), the sample size, α=0.05, β=0.80 and 0.95. Numeric-fold change was calculated as the ratio of the true mean difference able to be detected between the final value (week 12) and the initial value (week 0) over the initial value (week 0). The calculations were assisted by the user-friendly algorithm:
http://hedwig.mgh.harvard.edu/sample_size/js/js_crossover_quant.html