

Table S1. Number of IL-2, IL-4, IL-21, and/or INF- γ Positive Cells per Million CD4 T Cells by Previous Exposure to HPV.

Measurement at Study Timepoint	HPV-16			HPV-18		
	ELISA-Negative and DNA-Negative	ELISA-Positive or DNA-Positive	All Subjects	ELISA-Negative and DNA-Negative	ELISA-Positive or DNA-Positive	All Subjects
Day 0						
N	33	11	44	34	10	44
Min, Max	0, 291	0, 1888	0, 1888	0, 416	0, 2329	0, 2329
Geometric Mean (GSD)	59 (1)	94 (3)	66 (2)	33 (2)	76 (6)	40 (3)
Day 67						
N	33	11	44	34	10	44
Min, Max	108, 2746	113, 3866	108, 3866	53, 3211	296, 3371	53, 3371
Geometric Mean (GSD)	672 (2)	873 (3)	718 (2)	588 (2)	1137(2)	683 (2)
95% CI of Geometric Mean [1]	498, 908	416,1832	544, 947	436, 793	608, 2126	521, 897
P-Value[2]	0.0197	0.0314	0.0003	< 0.0001	0.0002	< 0.0001
Responders (%) ³	30 (91%)	9 (82%)	39 (89%)	26 (77%)	9 (90%)	35 (80%)
95% CI for Responders (%) ⁴	76%, 98%	48%, 97%	75%, 96%	59%, 89%	56%, 100%	65%, 90%
Day 187						
N	29	8	37[5]	30	8	38
Min, Max	233, 3718	140, 4557	140, 4557	173, 2690	234, 2126	173, 2690
Geometric Mean (GSD)	972 (2)	1021 (3)	983 (2)	841(2)	972(3)	867 (2)
95% CI of Geometric Mean[1]	751, 1259	366, 2845	751, 1286	662, 1069	455, 2077	690, 1089
P-Value[2]	0.0002	0.0459	< 0.0001	< 0.0001	0.0008	< 0.0001
Responders (%) ³	28 (97%)	6 (75%)	34 (92%)	27 (90%)	6 (75%)	33 (87%)
95% CI for Responders (%) ⁴	82%, 100%	35%, 97%	78%, 98%	73%, 98%	35%, 97%	72%, 96%

Note: N = Number of subjects in the prospective cohort. GSD=Geometric Standard Deviation; CI=Confidence Interval.

[1]CI is estimated using a two-sided Normal Approximation Method.

[2]Based on a regression analysis with baseline in the model using a 0.05% significance level testing that the slope equals one.

[3]A responder was defined as subjects whose value is greater than baseline value and with >180 HPV-16+ or >296 HPV-18+ CD4 T cells per million of total CD4 T cells.

[4]CI is estimated using a two-sided Exact (Clopper-Pearson) Method .

[5]HPV-16 test in one of the samples was not qualified for data analysis.

Table S2. Baseline and Post-Vaccination ELISA Results in Prospective Cohort Stratified by HPV Exposure Status.

	Antibody Levels by ELISA (IU/mL)					
	HPV-16			HPV-18		
	ELISA-Negative and DNA-Negative	ELISA-Positive or DNA-Positive	All Subjects	ELISA-Negative and DNA-Negative	ELISA-Positive or DNA-Positive	All Subjects
Day 0 (pre dose 1)						
N	36	11	47	37	10	47
Min, Max	0.0, 1.3	0.1, 354.0	0.0, 354.0	0.0, 0.9	1.6, 12.8	0.0, 12.8
Geometric Mean (GSD)	0.2 (2.8)	9.80 (9.0)	0.4 (9.2)	0.2 (2.8)	3.9 (2.2)	0.3 (5.2)

Day 67 (day 7 post dose 2)						
N	34	11	45	35	10	45
Min, Max	7.4, 2650.0	10.4, 4320.0	7.4, 4320.0	1.7, 788.2	21.5, 823.5	1.7, 823.5
Geometric Mean (GSD)	165.0 (3.6)	499.0 (6.7)	216.3(4.5)	39.2 (4.1)	165.5(3.4)	54.0 (4.4)
95% CI of Geometric Mean [1]	105.9, 257.0	138.2, 1801.5	137.5, 340.1	24.2, 63.7	68.3, 400.7	34.6, 84.5
P-Value [2]	0.0004	0.0926	< 0.0001	0.0007	0.4052	< 0.0001
Responders (%) [3]	34 (100.0%)	11 (100.0%)	45 (100.0%)	35 (100.0%)	10 (100.0%)	45 (100.0%)
95% CI for Responders (%) [4]	89.7%, 100.0%	71.5%, 100.0%	92.1%, 100.0%	90.0%, 100.0%	69.2%, 100.0%	92.1%, 100.0%
Day 187 (day 7 post dose 3)						
N	32	9	41	32	9	41
Min, Max	20.1, 780.0	75.8, 2840.0	20.1, 2840.0	2.5, 947.1	23.7, 444.7	2.5, 947.1
Geometric Mean (GSD)	207.8 (2.3)	442.0 (3.4)	245.3 (2.6)	74.3 (3.8)	120.8 (2.4)	82.7 (3.5)
95% CI of Geometric Mean [1]	154.8, 279.0	172.1, 1135.5	181.2,331.8	46.0, 119.9	61.9, 235.8	55.8, 122.6
P-Value [2]	< 0.0001	0.0091	< 0.0001	0.0014	0.1439	< 0.0001
Responders (%) [3]	32 (100.0%)	9 (100.0%)	41 (100.0%)	32 (100.0%)	9 (100.0%)	41 (100.0%)
95% CI for Responders (%) [4]	89.1%, 100.0%	66.4%, 100.0%	91.4%, 100.0%	89.1%, 100.0%	66.4%, 100.0%	91.4%, 100.0%
Day 365						
N	31	9	40	32	8	40
Min, Max	20.5, 604.0	24.0, 1400.0	20.5, 1400.0	2.4, 399.4	13.8, 374.1	2.4, 399.4
Geometric Mean (GSD)	110.8 (2.2)	243.3 (3.7)	132.3 (2.6)	27.5 (3.5)	78.8 (2.8)	34.0 (3.6)
95% CI of Geometric Mean [1]	83.5, 147.1	88.8, 666.7	97.3, 179.	17.5, 43.2	33.8, 183.6	22.7, 51.0
P-Value [2]	< 0.0001	0.0148	< 0.0001	0.0001	0.1631	< 0.0001
Responders (%) [3]	31 (100.0%)	9 (100.0%)	40 (100.0%)	32 (100.0%)	8 (100.0%)	40 (100.0%)
95% CI for Responders (%) [4]	88.8%, 100.0%	66.4%, 100.0%	91.19%, 100.0%	89.1%, 100.0%	63.1%, 100.0%	91.2%, 100.0%
Day 545						
N	29	9	38	30	8	38
Min, Max	11.2, 409.0	14.4, 779.0	11.2, 779.0	0.0, 201.8	11.4, 327.7	0.0, 327.7
Geometric Mean (GSD)	65.0 (2.3)	172.5 (3.9)	81.6 (2.8)	13.7 (5.0)	50.9 (2.7)	18.1 (4.9)
95% CI of Geometric Mean [1]	47.7, 88.5	60.5, 492.2	58.2, 115.2	7.5 , 25.0	22.3, 116.3	10.8, 30.4
P-Value [2]	< 0.0001	0.0183	< 0.0001	0.0078	0.2333	< 0.0001
Responders (%) [3]	29 (100.0%)	9 (100.0%)	38 (100.0%)	29 (96.7%)	8 (100.0%)	37 (97.4%)
95% CI for Responders (%) [4]	88.1%, 100.0%	66.4%, 100.0%	90.8%, 100.0%	82.8%, 99.9%	63.0%, 100.0%	86.2%, 99.9%
Day 730						
N	26	9	35	28	7	35
Min, Max	17.3, 370.0	12.1, 606.0	12.1, 606.0	0.8, 155.9	8.1, 68.8	0.8, 155.9
Geometric Mean (GSD)	50.7 (2.3)	142.7 (4.2)	66.2 (3.0)	10.9 (3.5)	32.2 (2.2)	13.5 (3.5)
95% CI of Geometric Mean [1]	36.4, 70.6	47.5, 428.7	45.5, 96.2	6.7, 17.7	15.6, 66.4	8.8, 20.7

P-Value [2]	< 0.0001	0.0356	< 0.0001	0.0010	0.0009	< 0.0001
Responders (%) [3]	26 (100.0%)	9 (100.0%)	35 (100.0%)	27 (96.4%)	7 (100.0%)	34 (97.1%)
95% CI for Responders (%) [4]	86.8%, 100.0%	66.4%, 100.0%	90.0 %, 100.0%	81.7%, 99.9%	59.0 %, 100.0%	85.1%, 99.9%

Note: N = Number of subjects in the prospective cohort. GSD=Geometric Standard Deviation; CI=Confidence Interval.[1] CI is estimated using a two-sided Normal Approximation Method [2] Based on a regression analysis with baseline in the model using a 0.05% significance level testing that the slope equals one. [3] A responder was defined as seropositive by the laboratory. The denominator for percentages is based on the total number of subjects (n) with a result at the visit being summarized. [4] CI is estimated using a two-sided Exact (Clopper-Pearson) Method.

Table S3. Percentage of CFSE^{low} CD4 T cells (%) by Previous Exposure to HPV.

	HPV-16			HPV-18		
	ELISA-Negative and DNA-Negative	ELISA-Positive or DNA-Positive	All Subjects	ELISA-Negative and DNA-Negative	ELISA-Positive or DNA-Positive	All Subjects
Day 0						
N	35	10	45	36	9	45
Min, Max	0.00, 1.11	0.00, 0.54	0.00, 1.11	0.00, 1.24	0.00, 1.18	0.00, 1.24
Geometric Mean (GSD)	0.00 (4.09)	0.00 (3.73)	0.00 (3.97)	0.00 (8.69)	0.00 (18.23)	0.00 (10.86)
Day 365						
N	31	9	40	32	8	40
Min, Max	0.00, 14.73	0.00, 5.57	0.00, 14.73	0.00, 12.33	0.00, 4.43	0.00, 12.33
Geometric Mean (GSD)	0.85 (5.22)	0.32 (5.59)	0.68 (5.46)	0.59 (5.43)	0.44 (12.59)	0.56 (6.39)
95% CI of Geometric Mean[1]	0.47, 1.57	0.08, 1.19	0.38, 1.18	0.32, 1.09	0.05, 3.64	0.31, 1.01
P-Value [2]	0.0250	0.0216	0.0011	0.0002	0.0079	< 0.0001
Responders (%) [3]	25 (80.6%)	6 (66.7%)	31 (77.5%)	30 (93.8%)	5 (62.5%)	35 (87.5%)
95% CI for Responders (%) [4]	62.5%, 92.6%	29.9 %, 92.5 %	61.6%, 89.2%	79.2%, 99.2 %	24.5%, 91.5%	73.2%, 95.8%
Day 545						
N	28	9	37	29	8	37
Min, Max	0.00, 16.15	0.04, 7.61	0.00, 16.15	0.02, 16.65	0.00, 7.77	0.00, 16.65
Geometric Mean (GSD)	1.126 (6.14)	0.57 (5.42)	0.95 (5.97)	0.75 (6.30)	0.51 (15.23)	0.69 (7.58)
95% CI of Geometric Mean[1]	0.56, 2.28	0.156, 2.09	0.53, 1.73	0.37 1.50	0.052, 4.95	0.35, 1.35
P-Value [2]	0.1435	0.4711	0.0920	0.0018	0.3280	0.0002
Responders (%) [3]	25 (89.3%)	7 (77.8%)	32 (86.5%)	28 (96.6%)	4 (50.0%)	32 (86.5%)
95% CI for Responders (%) [4]	71.8%, 97.7%	40.0%, 97.2%	71.2 %, 95.5%	82.2%, 99.9%	15.7%, 84.3%	71.2 %, 95.5%
Day 730						
N	26	9	35	28	7	35
Min, Max	0.08, 9.48	0.10, 8.73	0.08, 9.48	0.06, 4.77	0.16, 10.48	0.06, 10.48
Geometric Mean (GSD)	1.39 (3.22)	0.65 (4.76)	1.14 (3.67)	0.77 (3.28)	1.97 (3.77)	0.93 (3.51)
95% CI of Geometric Mean[1]	0.87 2.23	0.20, 2.15	0.73, 1.79	0.48, 1.21	0.58, 6.73	0.60, 1.43
P-Value [2]	0.0010	0.1949	0.0006	< 0.0001	0.0241	< 0.0001
Responders (%) [3]	24 (92.3%)	7 (77.8%)	31 (88.6%)	26 (92.9%)	4 (57.1%)	30 (85.7%)
95% CI for Responders (%) [4]	74.9%, 99.1%	40.0%, 97.2%	73.3%, 96.8 %	76.5 %, 99.1%	18.4%, 90.1%	69.7%, 95.2%

Note: N = Number of subjects in the prospective cohort. GSD=Geometric Standard Deviation; CI=Confidence Interval. [1] CI is estimated using a two-sided Normal Approximation Method. [2] Based on a regression analysis with baseline in the model using a 0.05% significance level testing that the slope equals one. [3] A responder was defined as having a result two times above the baseline value. The denominator for percentages is based on the total number of subjects (n) with a result at the visit being summarized. [4] CI is estimated using a two-sided Exact (Clopper-Pearson) Method.

Table S4: Comparison of Elapsed Time in Days from Last Vaccination at Enrollment in Retrospective Cohorts.

Statistics	Retrospective With 2 Prior HPV Vaccinations (N=74)	Retrospective With 3 Prior HPV Vaccinations (N=77)	Difference 3 Prior versus 2 Prior
Mean (SD)	748 (619)	1581 (222)	833 (461)
Median	471 (16 months)	1608(54 months)	
Min, Max	181, 2419	1100, 1986	
95% CI of Mean			684,980 [1]
P-Value			<0.0001 [2]

Note: N=subjects with samples can be tested. [1] 95% CI was calculated using a two-sided test using Normal Approximation Method. [2] P-value is based on a two-sample t-test using a 2-sided 0.05% significance level.

Table S5: Antibody Levels in Retrospective Cohorts.

	ELISA (IU/mL)			Pseudovirion Neutralizing Assay (1/dilution)		
	Retro 2 (N=74)	Retro 3 (N=77)	Difference 3 Prior - 2 Prior	Retro 2 (N=74)	Retro 3 (N=77)	Difference 3 Prior - 2 Prior
HPV-16						
Min, Max	3.4, 794.0	0.1, 1280.0	N/A	100.0, 51200.0	0.0, 25600.0	N/A
Geometric Mean (GSD)	52.5 (3.7)	65.9 (3.8)	1.3 (3.7) [6]	1289.9 (4.2)	1814.8 (3.8)	1.4 (4.0) [6]
95% CI of Geometric Mean [1]	38.9, 70.9	48.7, 89.1	0.8, 1.9[6]	922.6, 1803.3	1340., 2456.5	0.9, 2.2[6]
P-Value [2]			0.2885			0.1335
Seropositive (%) [3]	74 (100.0%)	76 (98.7%)	-1.3% [7]	74 (100.0%)	75 (97.4%)	-2.6% [7]
95% CI for Seropositive (%) [4]	95.1 %, 100.0%	93.0%, 100.0%	-3.8 %, 1.2 % [7]	95.1 %, 100.0 %	90.9 %, 99.7%	-6.2%, 1.0% [7]
P-Value [5]			0.3253			0.1628
HPV-18						
Min, Max	0.8, 190.6	0.3, 183.5	N/A	0.0, 6400.0	0.0, 12800.0	N/A
Geometric Mean (GSD)	9.7 (3.0)	15.5 (3.4)	1.6 (3.2) [6]	228.0 (3.3)	487.6 (4.4)	2.1 (3.9) [6]
95% CI of Geometric Mean [1]	7.5, 12.5	11.7, 20.4	1., 2.3[6]	172.6, 301.2	347.9, 683.3	1.4, 3.3[6]
P-Value [2]			0.0142			0.0007
Seropositive (%) [3]	69 (93.2%)	73 (94.8%)	1.6% [7]	68 (91.9%)	73 (94.8%)	2.9% [7]
95% CI for Seropositive (%) [4]	84.9%, 97.8%	87.2%, 98.6%	-6.0%, 9.1% [7]	83.2%, 97.0%	87.2%, 98.6%	-5.0%, 10.9% [7]
P-Value [5]			0.6853			0.4717

Note: N=Numbers of subjects with samples can be tested. GSD=Geometric Standard Deviation; CI=Confidence Interval. [1] Confidence Interval (CI) is estimated using a two-sided Normal Approximation Method. [2] P-value is based on a two-sample t-test using a 2-sided 0.05% significance level. [3] A responder was defined as seropositive by the laboratory. The denominator for percentages is based on the total number of subjects (n) with a result at the visit being summarized. [4] CI is estimated using a two-sided Exact (Clopper-Pearson) Method. [5] P-value is based on a two-sample Chi-square test using a 2-sided 0.05% significance level. [6] Difference is the relative difference. A value of one means the response after two doses is the same as the response after three doses. [7] Difference is the absolute difference.

Table S6: Number of HPV + Memory B cells Per Million of Total-IgG secreting B cells by Previous Exposure to HPV.

Memory B Cells (Number of HPV specific memory B cells/per million of total IgG secreting cells)	
HPV-16	HPV-18

	ELISA- Negative and DNA-Negative	ELISA- Positive or DNA-Positive	All Subjects	ELISA- Negative and DNA-Negative	ELISA- Positive or DNA-Positive	All Subjects
Day 0						
N	35	11	46	36	10	46
Min, Max	0, 538	0, 1276	0, 1276	0, 658	0, 906	0, 906
Geometric Mean (GSD)	21 (4)	56 (9)	27 (6)	24 (4)	65 (6)	30 (4)
Day 365						
N	31	9	40	32	8	40
Min, Max	0, 2574	382, 3430	0, 3430	0, 3931	0, 1974	0, 3931
Geometric Mean (GSD)	447 (8)	1068 (2)	544(6)	209 (6)	274 (6)	221(6)
95% CI of Geometric Mean[1]	217, 921	631,1806	306, 965	108, 405	63, 1199	124, 393
P-Value [2]	0.0010	< 0.0001	< 0.0001	0.0006	0.0780	< 0.0001
Responders (%) [3]	20 (64.5%)	7 (77.8%)	27 (67.5%)	13 (40.6%)	3 (37.5%)	16 (40.0%)
95% CI for Responders (%) [4]	45.37%, 80.77%	39.99%, 97.19%	50.87%, 81.43%	23.70%, 59.36%	8.52%, 75.51%	24.86%, 56.67%
Day 545						
N	29	9	38	30	8	38
Min, Max	0, 2684	145, 3228	0, 3228	0, 1629	96, 1630	0, 1630
Geometric Mean (GSD)	610 (3)	562 (3)	599 (3)	238 (4)	339 (3)	256(4)
95% CI of Geometric Mean[1]	397, 938	242, 1305	416, 862	142, 399	131, 878	165, 397
P-Value [2]	< 0.0001	0.0021	< 0.0001	< 0.0001	0.0132	< 0.0001
Responders (%) [3]	19 (65.5%)	4 (44.4%)	23 (60.5%)	9 (30.0%)	3 (37.5%)	12 (31.6%)
95% CI for Responders (%) [4]	45.7%, 82.1%	13.7%, 78.8 %	43.4%, 76.0%	14.7%, 49.4%	8.5%, 75.5%	17.5%, 48.7%
Day 730						
N	26	9	35	28	7	35
Min, Max	0, 1374	48, 2564	0, 2564	0, 1022	24, 1185	0, 1185
Geometric Mean (GSD)	299.7 (5.18)	491.9 (3.24)	340.5 (4.65)	168.2 (4.54)	267.3 (4.06)	184.5 (4.41)
95% CI of Geometric Mean[1]	154.3, 582.4	199.3, 1213.8	200.8, 577.2	93.5, 302.5	73.2, 976.4	110.9, 307.2
P-Value [2]	0.0004	0.0034	< 0.0001	0.0003	0.0404	< 0.0001
Responders (%) [3]	13 (50.0%)	3 (33.3%)	16 (45.7%)	7 (25.0%)	2 (28.6%)	9 (25.7%)
95% CI for Responders (%) [4]	29.9 %, 70.1%	7.5%, 70.1%	28.8%, 63.4%	10.7%, 44.9%	3.7%, 71.0%	12.5%, 43.3%

Note: N = Number of subjects in the prospective cohort. ; GSD=Geometric Standard Deviation; CI=Confidence Interval.

[1] CI is estimated using a two-sided Normal Approximation Method.

[2] Based on a regression analysis with baseline in the model using a 0.05% significance level testing that the slope equals one.

[3] A responder was defined as a subject whose value was greater than the baseline value and greater than the following threshold values: HPV-16: 455; HPV-18: 450.

The denominator for percentages is based on the total number of subjects (n) with a result at the visit being summarized.

[4] CI is estimated using a two-sided Exact (Clopper-Pearson) Method.

Table S7: Number of HPV + Memory B Cells per Million of Total IgG Secreting B cells in Retrospective Cohorts.

	Memory B Cells (Number of HPV specific memory B cells/per million total IgG secreting cells)		
	Retrospective Cohort with 2 prior HPV Vaccinations (N=74)	Retrospective Cohort with 3 prior HPV Vaccinations (N=72)	Difference 3 Prior - 2 Prior
HPV-16			
n	74	72	N/A
Min, Max	0, 2444	0, 2701	N/A
Geometric Mean (GSD)	301 (4)	241 (4)	0.8 (4) [6]
95% CI of Geometric Mean [1]	218, 428	169, 343	0.49, 1.28 [6]

P-Value [2]			0.3369
Seropositive (%) [3]	38 (51.4%)	24 (33.3%)	-18.0% [7]
95% CI for Seropositive (%) [4]	39.4%, 63.15%	22.7%, 45.43%	-33.8%, -2.3%[7]
P-Value [5]			0.0277
HPV-18			
N	74	72	N/A
Min, Max	0, 1726	0, 610	N/A
Geometric Mean (GSD)	90 (5)	74 (4)	0.8 (5) [6]
95% CI of Geometric Mean [1]	62, 129	53, 104	0.5, 1.4[6]
P-Value [2]			0.4545
Seropositive (%) [3]	10 (13.5%)	1 (1.4%)	-12.1% [7]
95% CI for Seropositive (%) [4]	6.7%, 23.5%	0.0%, 7.5 %	-20.4%, -3.9%[7]
P-Value [5]			0.0055

Note: Only subjects who were responders are summarized GSD=Geometric Standard Deviation; CI=Confidence Interval. N/A=Not applicable.

[1] Confidence Interval (CI) is estimated using a two-sided Normal Approximation Method.

[2] P-value is based on a two-sample t-test using a 2-sided 0.05% significance level.

[3] A responder was defined as a subject whose value was greater than the following threshold values: HPV-16: 455; HPV-18: 450. The denominator for percentages is based on the total number of subjects (n) with a result at the visit being summarized.

[4] CI is estimated using a two-sided Exact (Clopper-Pearson) Method.

[5] P-value is based on a two-sample Chi-square test using a 2-sided 0.05% significance level.

[6] Difference is the relative difference. A value of one means the response after two doses is the same as the response after three doses.

[7] Difference is the absolute difference