Supporting Information

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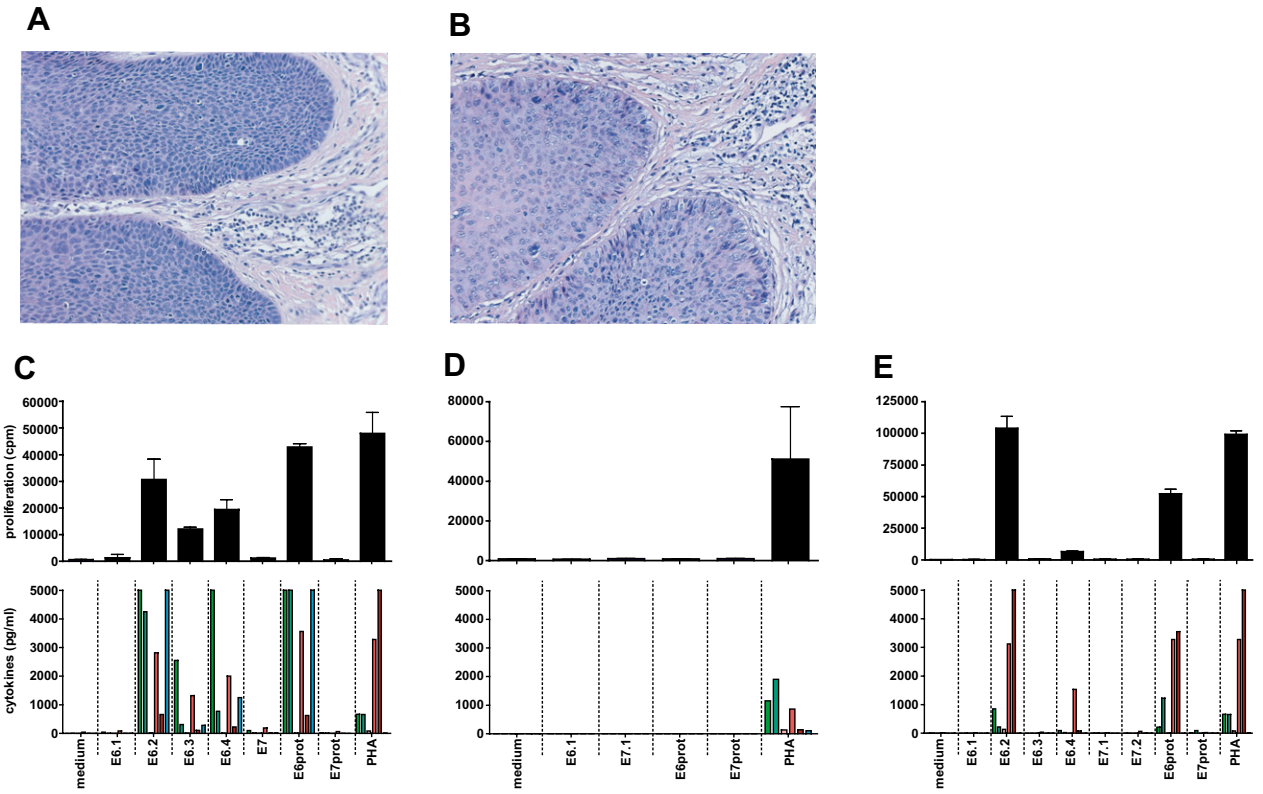

Fig. S1. HPV16-specific T cells cultured from biopsies. Typical example of H&E staining of a vulvar intraepithelial neoplasia (VIN) lesion before vaccination (*A*) and at 3-mo follow-up (*B*), showing T cells infiltrated into the stroma beneath the atypical epithelial cells of the lesion. For the same patient, biopsies were taken from (*C*) the last vaccine injection site 2 wk after vaccination, (*D*) the VIN lesion before the vaccinations, and (*E*) the VIN lesion 3 mo after the last vaccination. T cells from these biopsies were cultured and tested for HPV16 specificity in a proliferation assay (*Upper*) as well as for the secretion of cytokines in the supernatants taken after 2 d of proliferation (*Lower*). From left to right, the cytokines analyzed are IFNγ, TNFα, IL-10, IL-5, IL-4, and IL-2.

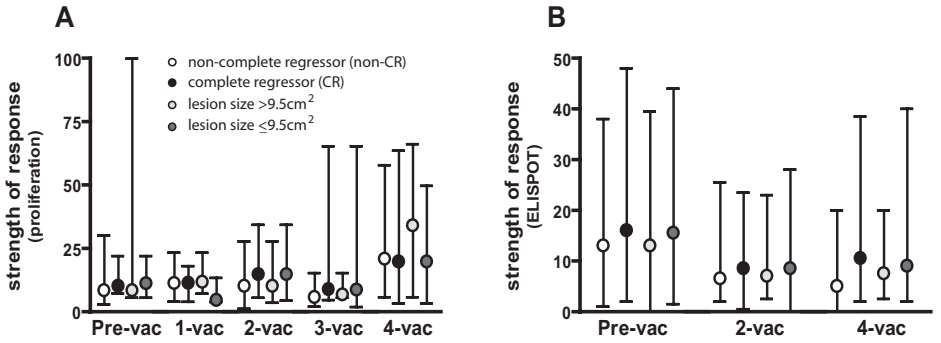

Fig. S2. No differences were seen in memory response mix (MRM)- or influenza virus M1-specific T-cell responses. (*A*) The strength of the T-cell response (depicted as median stimulation index + interquartile range as determined by lymphocyte stimulation test (LST) against the MRM is shown in patients grouped according to the clinical outcome [non-complete regressor (non-CR) versus CR] or lesion size [large (>9.5 cm^2) vs. small (≤9.5 cm^2). (*B*) The strength of the T-cell response [in median specific spots per 10^5 peripheral blood mononuclear cells (PBMC) + interquartile range] against Influenza virus M1 as determined by IFNγ-ELISPOT is shown for patients grouped by clinical response or lesion size.

Table S1. Percentages of IFNγ-producing CD4⁺ T cells in response to 22-mer peptide pools of HPV16 after 10-d prestimulation

Patient ID	E6.1		E6.2		E6.3		E6.4		E6 protein		E7.1		E7.2		E7 protein	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	–	–	–	**0.22**	–	**0.05**	–	**0.38**	–	**0.15**	–	*0.01*	–	**0.05**	–	*0.01*
2	–	*0.03*	–	**0.38**	–	**0.13**	–	*0.03*	–	*0.04*	–	*0.03*	–	**0.21**	–	*0.08*
3	–	**0.38**	–	**0.62**	–	**0.18**	–	**0.68**	–	**0.17**	–	*0.06*	–	**0.72**	–	*0.09*
6		**0.16**		**0.61**		**0.36**		**1.47**		**1.14**		**0.12**		**0.61**		**0.08**
7		**0.06**		**0.08**		**0.08**		**0.71**		**0.23**		–		**0.25**		*0.01*
8	–	**0.07**	–	**0.36**	–	**0.24**	–	**0.35**	–	**0.26**	–	–	–	**0.15**	–	**0.07**
9	–	**0.29**	–	**2.60**	–	**1.59**	–	**0.54**	–	**0.74**	–	*0.01*	–	**0.73**	–	**0.18**
10	–	–	–	**0.63**	–	**0.08**	–	**0.06**	–	*0.01*	–	–	–	**0.16**	–	*0.02*
11	–	**0.29**	–	**1.89**	–	**1.06**	–	**1.04**	–	**0.86**	–	*0.05*	–	**1.22**	–	**0.46**
12	NT															
13		**0.07**		**0.18**		**0.68**		**0.13**		*0.03*		–		**0.30**		*0.04*
16		**0.13**		**0.63**		**1.69**		**0.29**		*0.05*		–		**2.30**		–
18	-	*0.03*	**0.38**	**0.14**	–	**0.12**	–	**0.34**	–	*0.01*	–	**0.12**	–	**0.38**	–	**0.23**
22	**0.22**	**0.52**	**0.41**	**0.78**	**0.59**	**1.82**	–	**0.35**	–	*0.06*	–	**0.11**	**0.13**	**0.05**	–	**0.22**
23	**0.06**	*0.01*	**0.07**	**0.35**	–	**0.10**	**0.07**	**3.28**	–	**0.08**	–	*0.04*	–	*0.07*	–	*0.16*
26	NE															
27		**0.10**		**0.50**		**1.81**		**5.91**		**0.29**		**0.77**		**0.41**		**0.91**
28	**0.54**	**0.39**	**0.19**	**2.03**	–	**0.19**	–	**0.50**	–	**0.06**	–	–	–	**0.48**	–	*0.01*
29	–	–	–	**0.71**	–	**0.35**	–	**0.20**	–	*0.04*	–	*0.01*	–	**0.13**	–	*0.01*
30	–	–	–	–	–	*0.01*	–	-	–	–	–	**0.02**	–	**0.15**	–	**0.02**

PBMC were prestimulated for 10 d with the complete peptide set covering HPV16 E6 or E7. Then the cells were subjected to autologous monocytes loaded with indicated peptide pools or proteins and incubated with Brefeldin A for the intracellular cytokine (i.e., IFNγ) staining. E6.1–E6.4, E7.1, and E7.2 are the peptide pools tested; positive T-cell responses are in boldface type. The numbers indicated in italic type reflect the frequency of detected interferon-gamma producing T cells that was too close to the background to consider this to be a positive T-cell response. Pre, prevaccination; Post, postvaccination (2 wk after last vaccination); NE, not evaluable; NT, not tested because of limitation in PBMC availability; –, no detectable T-cell response.

Table S2. Percentages of IFNγ-producing CD8⁺ T cells in response to 10-mer peptide pools of HPV16 after 10-d prestimulation

Patient ID	E6											E7
	1–19	11–29	21–39	31–49	41–59	51–69	91–109	101–119	111–129	131–149	141–158	41–59
1				**–/0.27**				**–/1.42**				
2	**–0.37**	**–/0.20**										
3						**–/0.14**			**–/0.04**			
6				**–/0.10**				**–/0.08**				
7						**–/0.11**						
8		**–/0.19**		**–/0.82**		**–//0.80**		**–/0.20**			**–/0.11**	**–/4.88**
9					**–/0.10**	**–/0.67**						
10						**–/0.06**						
11		**–/0.10**										
12	NT											
13						**–/0.16**	**–/0.08**					
16						**–/0.06**	**–/0.02**					
18												**–/0.05**
22		**-/0.08**				**-/0.46**						
23				**–/0.06**			**–/0.03**			**–/0.85**		
26	NE											
27												
28												
29	**–/0.45**		**–/0.30**				**–/0.21**					
30												

As in Table S1, PBMC were prestimulated for 10 d with the complete peptide set covering HPV16 E6 or E7. The cells then were subjected to autologous monocytes loaded with indicated peptide pools and incubated with Brefeldin A for the intracellular cytokine (i.e., IFNγ) staining. T-cell response is shown only when a positive T-cell response was detected both before and after vaccination. Ranges (e.g., 1–19) indicate amino acid sequences of HPV16 E6 covered by the 10 pooled peptides. NE, not evaluable; NT, not tested because of limitations in PBMC availability; –, no detectable T-cell response.

Table S3. Strength of the cytokines response by HPV16-specific proliferative T cells in patients with large (>9.5 cm^2) and small (≤9.5 cm^2) lesions at study entry

Time point	IFNγ (pg/mL)			TNFα (pg/mL)			IL-10 (pg/mL)			IL-5 (pg/mL)		
	>9.5 cm^2	≤9.5 cm^2	*P* value	>9.5 cm^2	≤9.5 cm^2	*P* value	>9.5 cm^2	≤9.5 cm^2	*P* value	>9.5 cm^2	≤9.5 cm^2	*P* value
Pre	0.2	2.3	0.31	0.0	0.0	0.82	0.0	0.0	0.74	0.0	0.0	0.42
1-vac	534	1982	0.0003	8.2	38	0.01	6.3	61.6	<0.0001	53	309	<0.0001
2-vac	162	623	<0.0001	4.7	7.8	0.03	9.2	25	0.001	20	60	0.0004
3-vac	92	610	<0.0001	4.2	8.0	<0.01	2.2	23	<0.0001	12	112	<0.0001
4-vac	174	734	<0.0001	6.8	11	0.01	9.8	32	<0.0001	59	205	0.0003
	IFNγ/TNFα ratio			IFNγ/IL-10 ratio			IFNγ/IL-5 ratio			Proliferation (stimulation index)		
	>9.5 cm^2	≤9.5 cm^2	*P* value	>9.5 cm^2	≤9.5 cm^2	*P* value	>9.5 cm^2	≤9.5 cm^2	*P* value	>9.5 cm^2	≤9.5 cm^2	*P* value
Pre	0.0	0.0	0.54	0.0	0.0	0.52	0.0	0.0	0.20	0.8	0.9	0.57
1-vac	41	29	0.15	17	22	0.44	7.5	5.6	0.59	3.8	8.4	0.18
2-vac	30	39	0.07	9.2	24	0.007	7.4	8.3	0.86	3.3	6.4	0.02
3-vac	18	31	0.007	6.0	20	0.009	5.4	3.8	0.62	2.6	8.8	<0.0001
4-vac	20	31	0.002	13	28	0.001	2.6	3.7	0.02	3.5	12.2	0.004

The proliferation assay (LST) was performed with the blood samples at different time points: before vaccination (pre) and after one (1-vac), two (2-vac), three (3-vac), and four (4-vac) vaccinations. Supernatants of the LST, harvested at day 6, were subjected to cytometric bead array to measure the cytokine production. The strength of cytokine response per patient group is given as the median cytokine production in response to all six peptide pools for all patients in that group. *P* values from Mann–Whitney test; $P < 0.05$ is considered significant.

Table S4. Strength of the cytokine response by HPV16-specific proliferative T cells in non-CR and CR patients

Time point	IFNγ (pg/mL)			TNFα (pg/mL)			IL-10 (pg/mL)			IL-5 (pg/mL)		
	Non-CR	CR	*P* value	Non-CR	CR	*P* value	Non-CR	CR	*P* value	Non-CR	CR	*P* value
Pre	0.0	2.8	0.23	0.0	0.0	0.58	0.0	0.0	0.47	0.0	0.1	0.07
1-vac	373	1982	<0.0001	6.7	51	0.0001	3.9	75	<0.0001	40	451	<0.0001
2-vac	217	502	0.04	5.0	6.9	0.12	9.4	23	0.01	24	49	0.01
3-vac	127	402	0.003	4.0	9.7	0.0011	2.2	16	0.0002	17	89	0.0002
4-vac	205	734	<0.0001	5.4	12	<0.0001	9.3	34	<0.0001	53	178	0.0003
	IFNγ/TNFα ratio			IFNγ/IL-10 ratio			IFNγ/IL-5 ratio			Proliferation (stimulation index)		
	Non-CR	CR	*P* value	Non-CR	CR	*P* value	Non-CR	CR	*P* value	Non-CR	CR	*P* value
Pre	0.0	0.0	0.54	0.0	0.0	0.31	0.0	0.0	0.15	0.9	0.9	0.38
1-vac	49	23	0.52	22	20	0.89	7.6	5.2	0.87	6.8	4.2	0.99
2-vac	35	33	0.95	13	22	0.09	9.4	6.1	0.38	4.3	6.4	0.08
3-vac	25	24	0.56	7	16	0.03	4.7	3.9	0.96	3.5	8.2	0.03
4-vac	23	26	0.07	20	18	0.09	3.2	3.5	0.18	4.1	11.8	0.003

The proliferation assay (LST) was performed with the blood samples at different time points: before vaccination (pre) and after one (1-vac), two (2-vac), three (3-vac), and four (4-vac) vaccinations. Supernatants of the LST, harvested at day 6, were subjected to cytometric bead array to measure the cytokine production. The strength of cytokine response per patient group is given as the median cytokine production to all six peptide pools for all patients in that group. *P* values from Mann–Whitney test; $P < 0.05$ is considered significant).