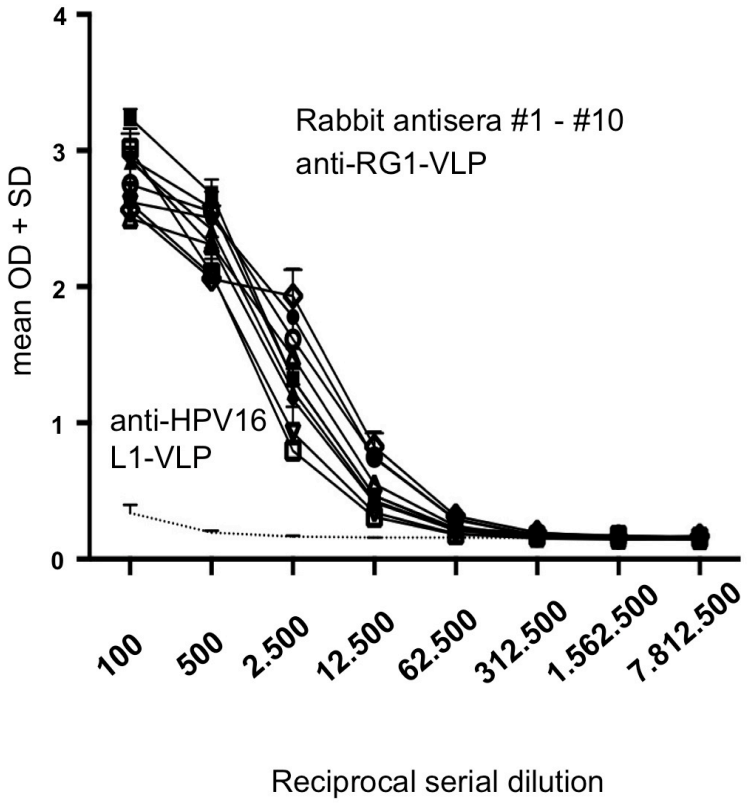
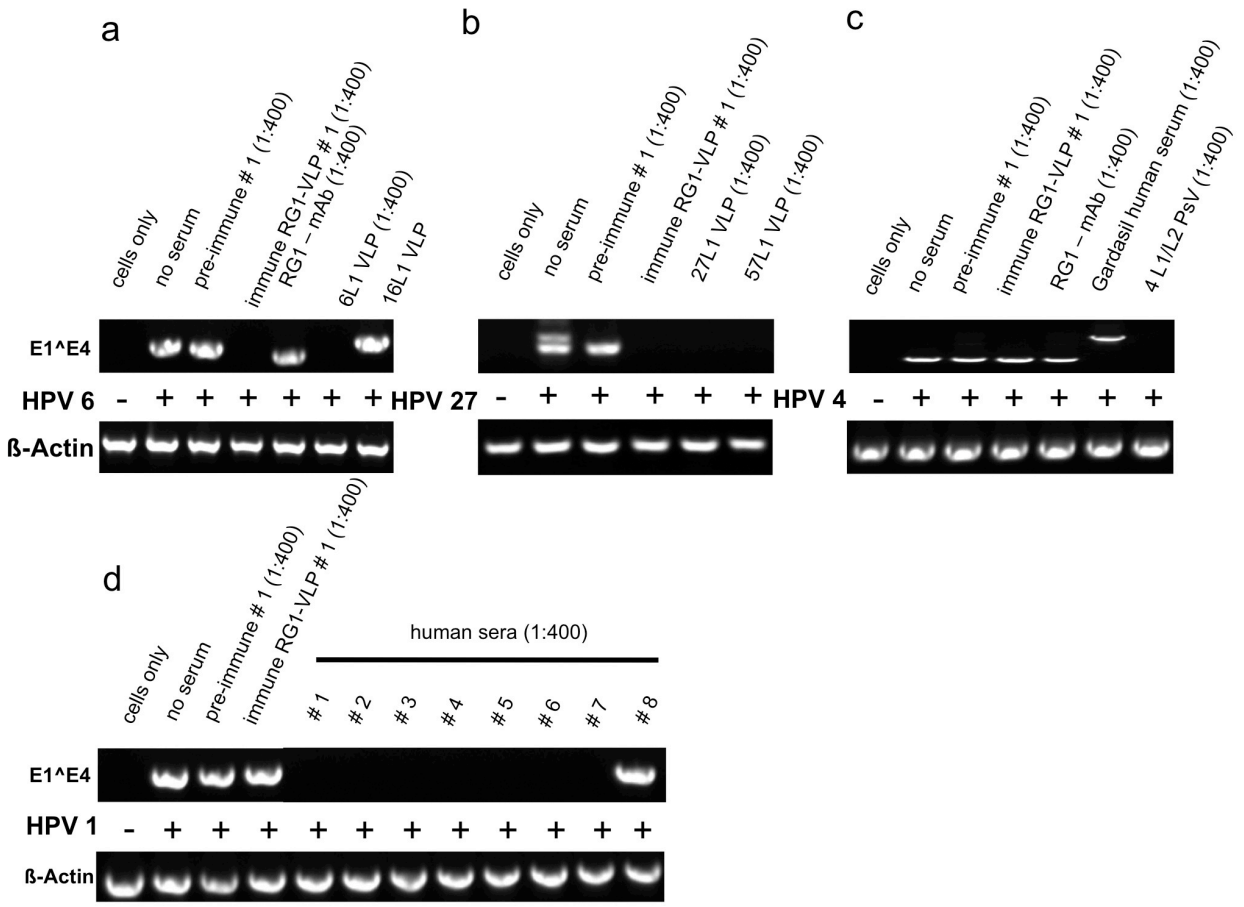
Supplementary Material

1. Supplementary Data



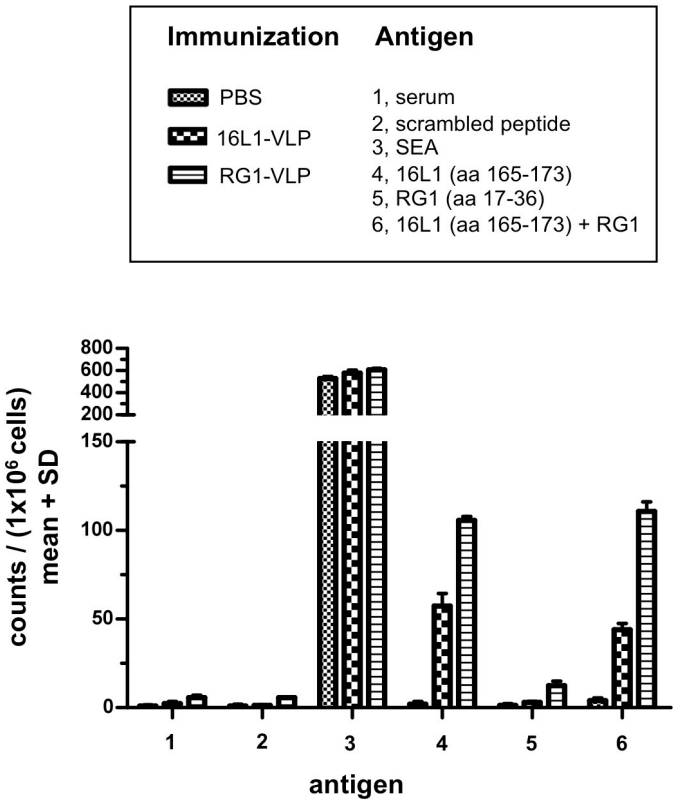
HPV16 L2 peptide ELISA

Ten NZW rabbits were immunized with either 50µg RG1-VLP, week 0, 4, 6 and 8 (n=6); or 20µg of RG1-VLP, week 0, 3, 6 (n=4) using Alum-MPL adjuvant. Antisera drawn two weeks after the last boost were serially diluted 5-fold and tested in triplicates by ELISA using bacterially expressed HPV16 L2-protein (aa 11-200) as antigen. End-point dilution revealed robust ELISA titers of 2,500-12,000 in all animals vaccinated with RG1-VLP, regardless of immunization scheme. In contrast, a serum from an HPV16 L1-VLP vaccinated animal showed no reactivity against L2. Data are shown as mean OD+SD.



In vitro RT-PCR neutralization assays using native mucosal lr HPV6, or cutaneous HPV27, HPV4, or HPV1 virions

Type-specific neutralizing anti-L1 VLP antisera (HPV6, 27) or anti L1-L2 PsV antiserum (HPV4), were used as respective controls. Sera of 8 pre-pubertal girls (#1-#8) were used as controls in the HPV1 assay, as a type-specific neutralizing serum was not available.



INF-γ ELISPOT assay detecting cytotoxic T-cell responses to vaccination with RG1-VLP

Female C57/BL6 mice (3 x 3) were vaccinated with 2μg of HPV16 L1-VLP, RG1-VLP, or PBS, respectively. Isolated splenocytes were stimulated with serum (1), scrambled peptide (2), SEA (3), HPV16 L1 peptide (aa 165-173) (4), HPV16 L2 RG1 peptide (aa 17-36) (5), or HPV16 L1 plus RG1 peptides combined (6). Spots representing IFN-γ producing cells were counted and results expressed as mean number/10^6 cells + SD of triplicate cultures.

	Neutralizing titer (over time)					
	# 1			# 2		
Week (Immunizations)	**10** (4)	**52** (4)	**54** (5)	**10** (4)	**52** (4)	**54** (5)
Pseudovirions						
HPV 16	**100,000**	**1,000**	**100,000**	**100,000**	**1,000**	**100,000**
HPV 18	**1,000**	**50**	**1,000**	**1,000**	**100**	**1,000**
HPV 31	**10,000**	**100**	**1,000**	**1,000**	**100**	**10,000**
HPV 45	**1,000**	<25	**1,000**	**100**	**100**	**1,000**
HPV 52	**100**	<25	**1,000**	**50**	<25	**50**
HPV 58	**1,000**	<25	**10,000**	**1,000**	**100**	**1,000**
HPV 6	**100**	<25	**1000**	**50**	<25	**100**
HPV 5	**100**	<25	**1,000**	**50**	<25	**100**

RG1-VLP induced cross-neutralizing antibody titers over time (12 months)

Two NZW rabbits (#1 and #2) were kept for additional 10 months following the 4th immunization with RG1-VLP in Alum-MPL. Sera were drawn before and two weeks after a 5th injection at week 52 with 50μg of antigen plus Alum-MPL, and tested by PBNA for indicated types. Serum titers are shown and compared to sera obtained at week 10 as indicated.

2, Supplementary Methods

Enzyme-linked Immunosorbent Assays (ELISA)

L2-specific serum titers were determined using His-tagged HPV16 L2 aa 11-200 polypeptide as ELISA antigen (Schellenbacher *et al.*, 2009).

Pseudovirion-based Neutralization Assay (PBNA)

Production of PsV and PsV-based neutralization assays (PBNA) were carried out as described (http://home.ccr.cancer.gov/lco/protocols.asp) with minor modifications (Schellenbacher *et al.*, 2009).

Pseudovirion (PsV) Plasmids

Packaging plasmids for HPV16, 18, 45, 5, 6 and BPV were provided by J. Schiller, NIH, Bethesda (plasmid maps and references: http://home.ccr.cancer.gov/Lco/plasmids.asp); HPV31, 52 and 58 by T. Kanda, NIID, Tokyo (Kondo *et al.*, 2008), HPV11 (Mossadegh *et al.*, 2004) by M. Müller, DKFZ, Heidelberg.

Plasmids for HPV38 (Faust *et al.*, 2010) and HPV3, 32, 33, 68, 76 (Faust *et al.*, 2013) were provided by J. Dillner, Karolinska Institutet, Stockholm; HPV18, 45, 31, 33, 52, 58, 35, 39, 51, 56, 59, 73, 68, 26, 53, 66, 34, 69, 70 by R. Roden, JHU, Baltimore (manuscript in preparation, Kwak et al.); HPV4 PsV were generated by codon modification of NCBI reference sequence NC001457.1 as described by Buck et al. (http://home.ccr.cancer.gov/lco/codonmodification.htm). ”As-different-as-possible” altered L1 and L2 sequences were synthesized (Invivogen) and cloned into expression vector pVITRO1-neo-mcs. For PsV plasmids of HPV5, 6, 11, 16, 18, 31, 45, 52, 58 and CRPV) one may be referred to Schellenbacher et al (Schellenbacher *et al.*, 2009).

Target Plasmids: pYSEAP (encoding for secreted-alkaline phosphatase) and pCLucf (encoding firefly luciferase) were generously provided by J. Schiller and C. Buck, NIH, Bethesda.

Enzyme-Linked Immuno Spot (ELISPOT) assays

C57BL/6 mice (3 groups of 3) were primed (day 0) and boosted (day 10) s.c. with 2µg HPV16 L1-VLP, RG1-VLP, or PBS control. Spleens and sera were isolated day 20. Splenic single-cell suspensions were obtained by mechanical dissociation and 10^6 cells/well added onto 96-well plates with nitrocellulose base (Millipore), pre-coated with interferon-gamma (IFN-γ) capture antibody. Cells were incubated for 24 hours in the presence of serum (1), 5µM scrambled control peptide (YVRFQRLEHHHHHH) (2), 2 µg/ml S. aureus enterotoxin A (SEA) (Sigma) (3), 5µM HPV16L1 peptide aa 165-173 (AGVDNRECI, a described CTL epitope (Ohlschlager *et al.*, 2003)) (4), 5µM RG1 peptide (QLYKTCKQAGTCPPDIIPKV) (5), or 2,5µM HPV16L1 plus 2,5µM RG1 peptides combined (6) (Severn Biotech). Following addition of biotinylated anti-IFN-γ mAb and avidin-HRP, spots representing IFN-γ producing cells of duplicate cultures were counted (mean + SD).

RT-PCR Primer Pairs

outside: upstream (UO), downstream (DO); inside: upstream (UI), downstream (DI)

1. HPV1

UO: 5′ -GATCTGGGCAAAGCAACTC-3′

DO: 5′ -GCTTCCTGTGCCTATTGCGAG-3′

UI: 5′ -GCACTACCTATCCTCTCGG-3′

DI: 5′ -CGATTGACCGTCCTCGCGG-3′

2. HPV2

UO: 5′ -CTCTTGTCAGGAACTCTGTACG-3′

DO: 5′ -GGGTGGTAACTACCTGCTG-3′

UI: 5′ -CCCACCCGCCCAGTGCCAC-3′

DI: 5′ -CAGAACCGTCCGGCTGGTGG-3′

3. HPV4

UO: 5′ -GCGTCTCTTTGGTCAGTGCTATC-3′

DO: 5′ -GAGTCGGTGGTTCCATTTAG-3′

UI: 5′ -GTGAGCAATCTCCAACCCAGTTC-3′

DI: 5′ -CATTGTATGCTGCTGAGCTCG-3′

4. HPV6

UO: 5′ -CGGGACAGTAACACACAAGTAGAG-3′

DO: 5′ -TGCAACAGCTTCTGTTGGGAACAC-3′

UI: 5′ -GTTCCTGCAGCTATTTGTACAGG-3′

DI: 5′ -ACATAGTGTGTCCCATCTGCG-3′

5. HPV26

UO: 5′ -GGTCACTACTCGTGTCC-3′

DO: 5′ -GGAAGACGTGTCCTTGG-3′

UI: 5′ -GTGATGGCCTCCACTTG-3′

DI: 5′ -GCCATCAGTGTGCTGC-3′

6. HPV27

UO: 5′ -CACTGGTGTACCACACTGTCAC-3′

DO: 5′ -GCCTACGGGGTGGTAACAAC-3′

UI: 5′ -CACGTGGAGGACACCCTGTC-3′

DI: 5′ -GTGCGGACCAGAAGACATAAG-3′

7. HPV40

UO: 5′ -GGCGTGCGTGTTCTGTCT-3′

DO: 5′ -GGGCACATTACATATAGTGT-3′

UI: 5′ -TGCCCACAGTAGTGGTGAT-3′

DI: 5′ -CCCCAACTGTGCAGCTACA-3′

3, L2 RG1 Epitope AA Sequence Homology

L2 (RG1) homology motifs (RG1*: HPV16 L2 aa 17-36)	PV	Homology with RG1 (%)	Neutralization by antiserum to RG1-VLP (in vitro / in vivo)
QLYKTCKQAGTCPPDIIPKV*	**HPV 16**	100	**in vivo**
QLYKTCKQAGTCPPDVIPKV	**HPV 73**	95	**in vivo**
QLYKTCKQSGTCPPDIIPKV	**HPV 34**	95	**in vivo**
DLYRTCKQAGTCPPDVIPKV	**BPV 1**	85	**in vitro**
DLYRTCKQAGTCPPDIIPR	**HPV 2**	85	**in vitro**
DLYRTCKQAGTCPPDIIPRL	**HPV 27**	85	**in vitro**
DLYRTCKQAGTCPPDIIPRV	**HPV 57**	85	
QLYRTCKAAGTCPPDVIPKV	**HPV 35**	85	**in vivo**
QLYQTCKAAGTCPPDVIPKV	**HPV 67**	85	
QLYRTCKAAGTCPPDVIPKV	**HPV 3**	85	**in vitro**
ELYKTCKAAGTCPPDVIPKV	**HPV 77**	85	
QLYRTCKAAGTCPPDVIPKV	**HPV 28**	85	
ELYKTCKVAGTCPPDVIPKV	**HPV 29**	85	
QLYSTCKAAGTCPPDVIPKV	**HPV 82**	85	
QLYQTCKAAGTCPSDIIPKV	**HPV 44**	85	**in vivo**
QLYQTCKAAGTCPSDIIPKV	**HPV 55**	85	
DLYKTCKQSGTCPPDVVPKV	**HPV 18**	80	**in vivo**
QLYRTCKASGTCPPDVIPKV	**HPV 117**	80	
QLYRTCKASGTCPPDVIPKV	**HPV 94**	80	
QLYRTCKASGTCPPDVIPKV	**HPV 10**	80	
ELYKTCKQSGTCPPDVINKV	**HPV 68**	80	
DLYRTCKAAGTCPPDVIPKV	**HPV 102**	80	
QLYQTCKASGTCPPDVIPKV	**HPV 52**	80	**in vivo**
QLYQTCKASGTCPPDVIPKV	**HPV 58**	80	**in vivo**
DLYKTCKQAGTCPSDVINKV	**HPV 59**	80	**in vivo**
DLYKTCKAAGTCPPDVIPKI	**HPV 69**	80	
DLYKTCKAAGTCPPDVIPKI	**HPV 26**	80	**in vivo**
QLYQTCKASGTCPPDVIPKV	**HPV 42**	80	
QLYQTCKASGTCPPDVIPKV	**HPV 13**	80	

QLYQTCKLTGTCPPDVIPKV	**HPV 6**	80	**in vivo**
QLYQTCKATGTCPPDVIPKV	**HPV 11**	80	**in vitro**
QLYQTCKATGTCPPDVIPKV	**HPV 33**	80	**in vivo**
QLYSTCKAAGTCPPDVVNKV	**HPV 51**	80	**in vivo**
HIYQTCKQAGTCPPDVINKV	**HPV 8**	75	
QLYQTCKAAGTCPSDVIPKI	**HPV 31**	75	**in vivo**
DLYRTCKQSGTCPPDVINKV	**HPV 45**	75	**in vivo**
QLYQTCKASGTCPPDVIPKI	**HPV 32**	75	**in vitro**
HIYQTCKQAGTCPPDVINKV	**HPV 5**	75	**in vitro**
HIYQTCKQAGTCPPDVINKV	**HPV 8**	75	
NIYRTCKQAGTCPPDVINKV	**HPV 19**	75	
NIYRTCKQAGTCPPDVINKV	**HPV 14**	75	
NIYRTCKQAGTCPPDVINKV	**HPV 20**	75	
NIYRTCKQAGTCPPDVINKV	**HPV 21**	75	
NIYRTCKQAGTCPPDVINKV	**HPV 24**	75	
QLYKTCKLSGTCPEDVINKV	**HPV 66**	75	
DIYKGCKAAGTCPPDVINKV	**HPV 107**	75	
QLYQTCKAAGTCPPDVVNKV	**HPV 7**	75	
DIYRGCKQAGTCPPDVINKV	**HPV 80**	70	
DIYRGCKQAGTCPPDVINKV	**HPV 17**	70	
NIYRTCKQAGTCPPDVLNKV	**HPV 25**	70	
HIYQTCKQAGTCPPDVVNKV	**HPV 36**	70	
DIYRGCKQAGTCPPDVINKV	**HPV 37**	70	
NIYRTCKQAGTCPTDVINKV	**HPV 93**	70	
NIYRTCKQAGTCPTDVINKV	**HPV 93**	70	
QLYQTCKQSGTCPEDVINKI	**HPV 53**	70	
DLYRTCKQSGTCPPDVVDKV	**HPV 39**	70	
HIYQTCKQAGTCPPDVLNKV	**HPV 12**	70	
HIYQTCKQAGTCPSDVVNKV	**HPV 47**	65	
NIYRTCKQAGNCPPDVVNKV	**HPV 49**	65	
NIYRTCKAAGTCPPDVVNKV	**HPV 92**	65	
NIYRGCKAAGTCPPDVINKV	**HPV 96**	65	
DIYKGCKASGTCPPDVINKV	**HPV 22**	65	
DIYKGCKASGTCPPDVLNKV	**HPV 23**	65	

QLYKTCKLSGTCPEDVVNKI	**HPV 56**	65	**in vivo**
DIYRGCKAAGTCPPDVINKV	**HPV 9**	65	
DIYRGCKQAGTCPPDVLNKV	**HPV 15**	65	
HIYQSCKAAGTCPPDVVNKV	**HPV 75**	60	
HIYQSCKAAGTCPPDVLNKV	**HPV 76**	60	**in vitro**
DIYRGCKASNTCPPDVINKV	**HPV 38**	55	Ø
DIYPSCKISNTCPPDIQNKI	**HPV 1**	50	Ø
NLYAKCQLSGNCLPDVKNKV	**HPV 4**	50	Ø

Faust H, Jelen MM, Poljak M*, et al.* (2013) Serum antibodies to human papillomavirus (HPV) pseudovirions correlate with natural infection for 13 genital HPV types. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*.

Faust H, Knekt P, Forslund O*, et al.* (2010) Validation of multiplexed human papillomavirus serology using pseudovirions bound to heparin-coated beads. *The Journal of general virology* 91:1840-8.

Kondo K, Ochi H, Matsumoto T*, et al.* (2008) Modification of human papillomavirus-like particle vaccine by insertion of the cross-reactive L2-epitopes. *Journal of medical virology* 80:841-6.

Mossadegh N, Gissmann L, Muller M*, et al.* (2004) Codon optimization of the human papillomavirus 11 (HPV 11) L1 gene leads to increased gene expression and formation of virus-like particles in mammalian epithelial cells. *Virology* 326:57-66.

Ohlschlager P, Osen W, Dell K*, et al.* (2003) Human papillomavirus type 16 L1 capsomeres induce L1-specific cytotoxic T lymphocytes and tumor regression in C57BL/6 mice. *Journal of virology* 77:4635-45.

Schellenbacher C, Roden R, Kirnbauer R (2009) Chimeric L1-L2 virus-like particles as potential broad-spectrum human papillomavirus vaccines. *Journal of virology* 83:10085-95.