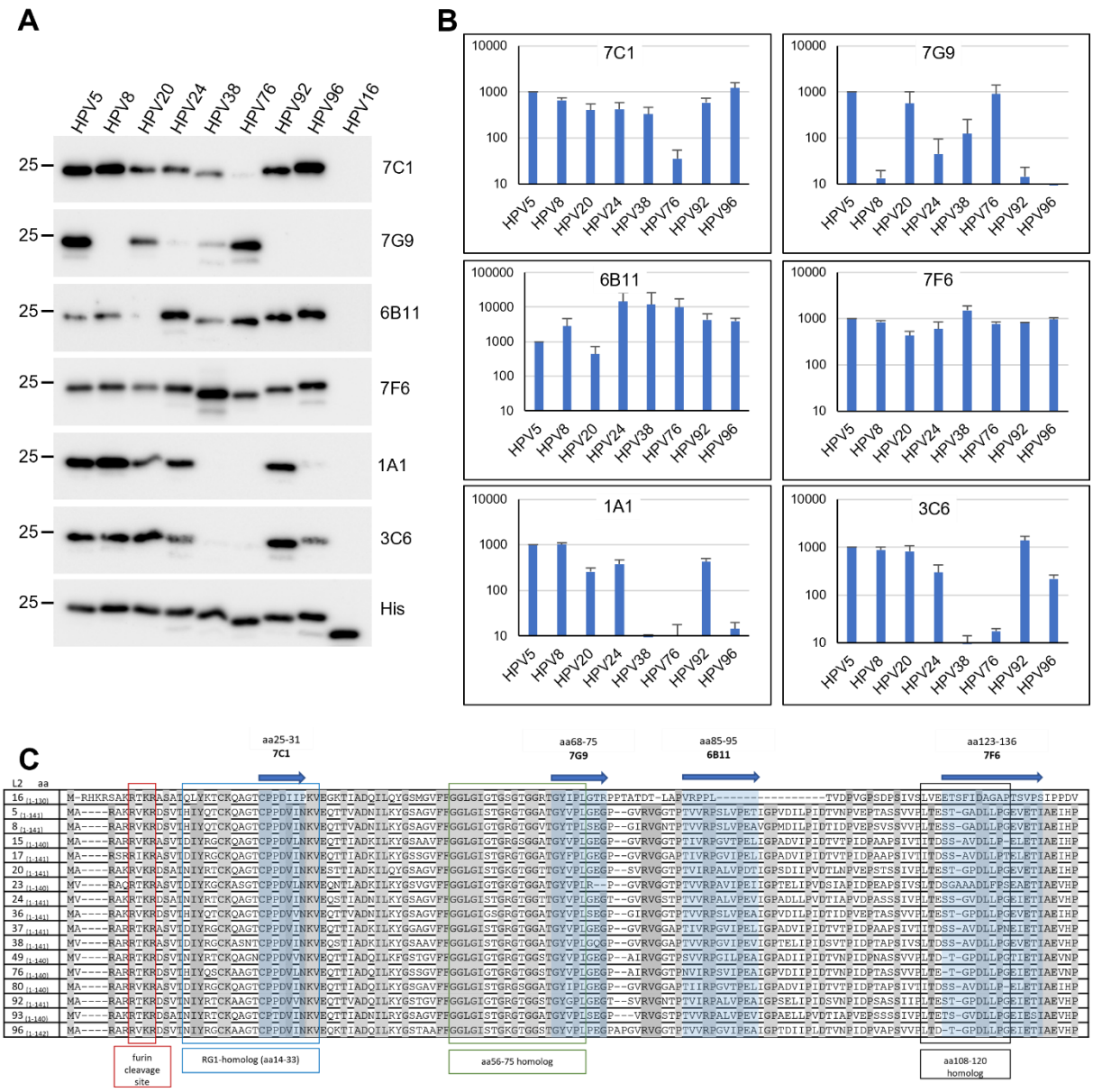
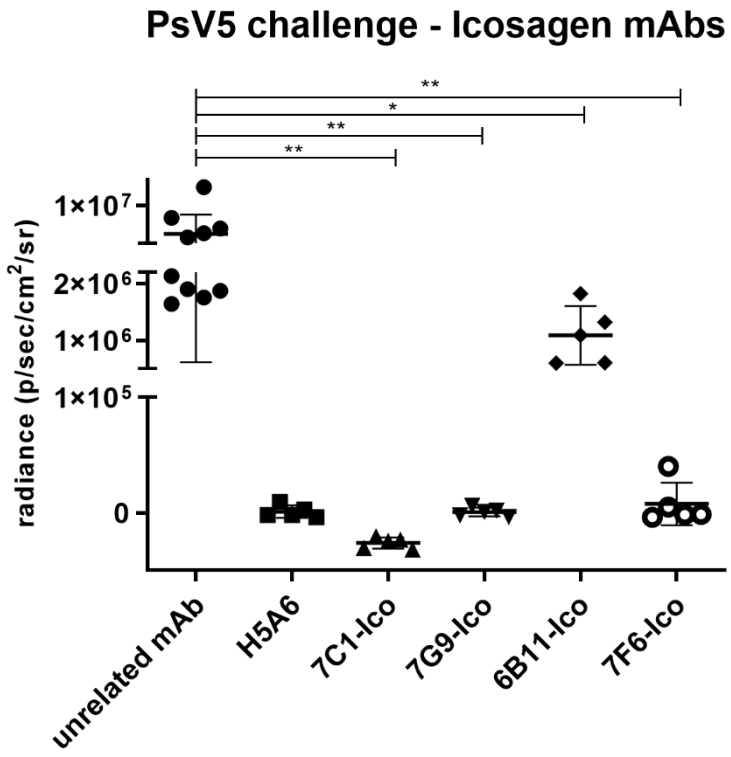
Supplements



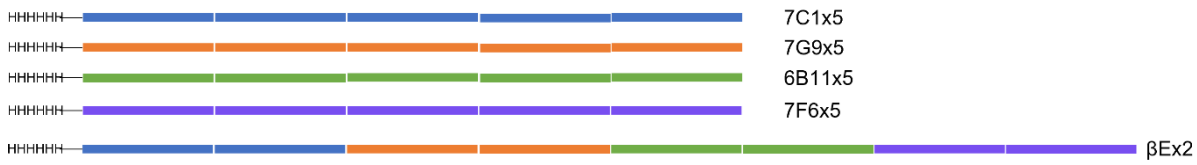
Supplemental Figure 1: mAb western blot characterization.

(A) Western Blot reactivity of mAbs to L2 fragments aa10-142 (or aa141 for HPV76; aa143 for HPV96 and aa13-130 for HPV16) of genus ßHPV and hr HPV16. MAbs 7C1, 6B11 and 7F6 showed very broad reactivity, clone 7G9 detected 5/8, clone 1A1 also 5/8 and 3C6 6/8 tested ßHPV, but none of the mAbs bound to L2 of the more distantly related genus αHPV16. Incubation with anti-6xHis antibody confirmed comparable loading of L2 fragments. (B) Quantification of western blot signals obtained with the 6 mAbs from three technical replicates adjusted to the relative expression levels detected with anti-6xHis antibody. The signals on HPV5 L2 were arbitrarily set to 1000 on a logarithmic scale. (C) Alignment of L2 N-termini of diverse βHPV and mucosal hr type HPV16. Indicated in grey are conserved aa. Boxed

in red is the presumed furin cleavage site; in blue the HPV16 RG1-homolog; in green the homolog to aa56-75 and in black the homolog to aa108-120. Novel L2 cross-neutralization epitopes are marked in blue shadows and arrows. MAbs 7C1, 7G9, 6B11 and 7F6 are derived from immunizations with ßHPV L2 N-terminal proteins, and mAbs 1A1 and 3C6 from immunizations with chimeric HPV16L1 presenting the epitope 6B11middle on the VLP surface.

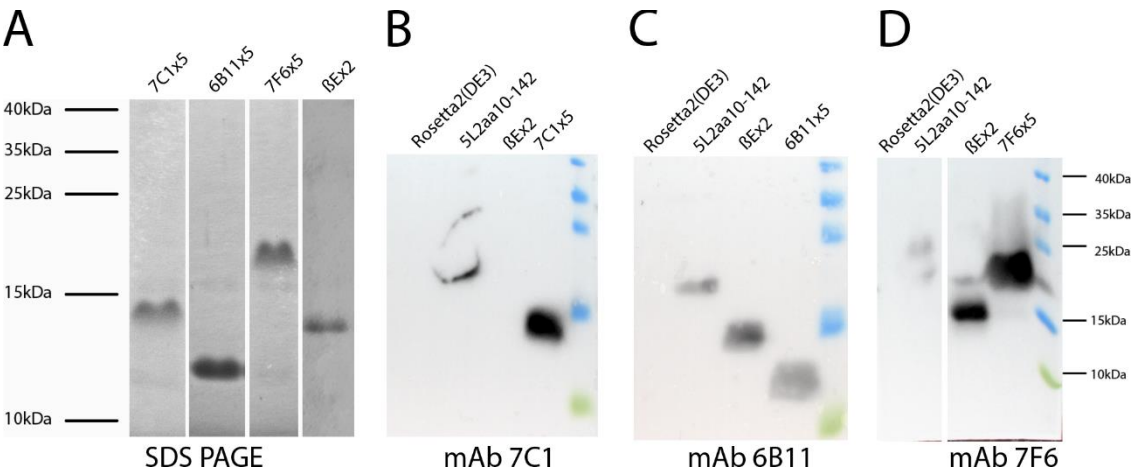


Supplemental Figure 2: In vivo protective vaccine efficacy against experimental HPV5 PsV challenge. Balb/c mice were passively immunized with unrelated mAb, the recombinantly expressed beta L2 mAbs (Icosagen) or HPV5 L1-derived mAb H5.A6 (for all 50µg). Mice were vaginally challenged one day later and infection assessed after three days using the IVIS bioluminescence imager. Luciferase activity was measured as p/s/cm2/sr (average radiance) and quantified after background subtraction (measurement at an uninfected site of the same mouse) using Living Image Software (PerkinElmer). All mAbs show significant differences to the unrelated mAb group. Reported are p-values of unpaired, two-tailed t-test, Welch's corrected if groups with unequal sizes were analyzed; * p≤ 0.05; ** p≤ 0.005; **** p< 0.0001; p>0.05 were considered not significant (n.s.).



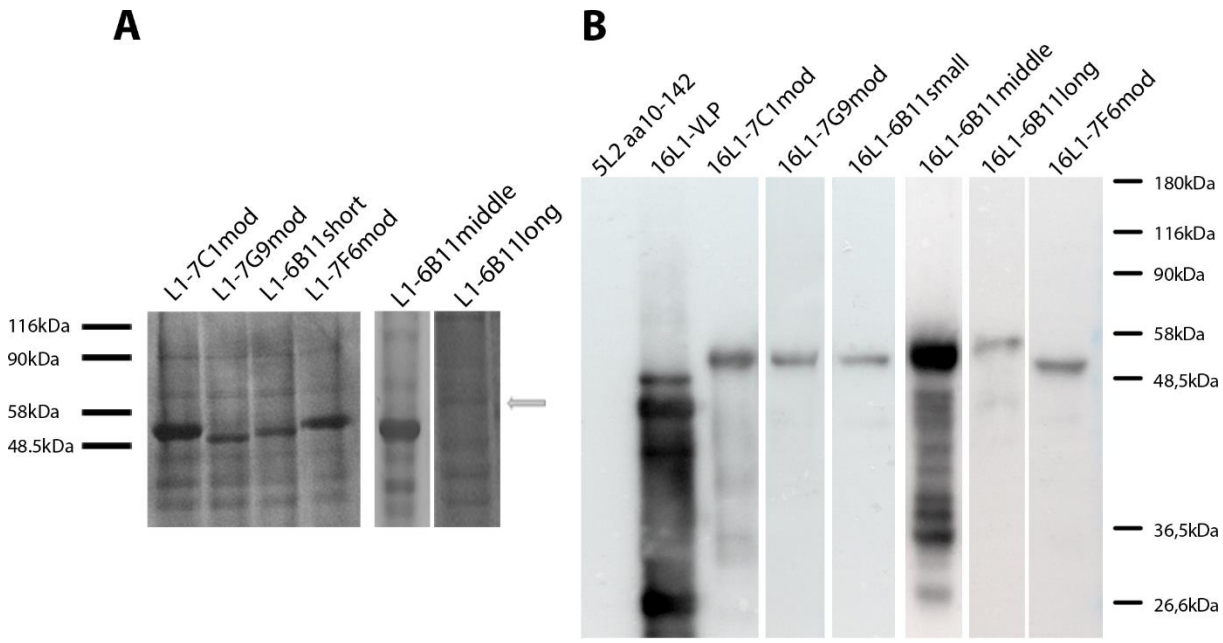
Supplemental Figure 3: Schematic design of ßHPV L2 epitope polymers.

Pentameric repeats of the respective HPV5 L2 epitopes were expressed with an N-terminal His tag (7C1 aa24-31 in blue; 7G9 aa68-75 in orange, 6B11 aa 85-95 in green and 7F6 aa124-136 in violet). An additional polymer consisted of tandem repeats of all four novel epitopes arranged in their N- to C-terminal order (beta epitope x2, βEx2).



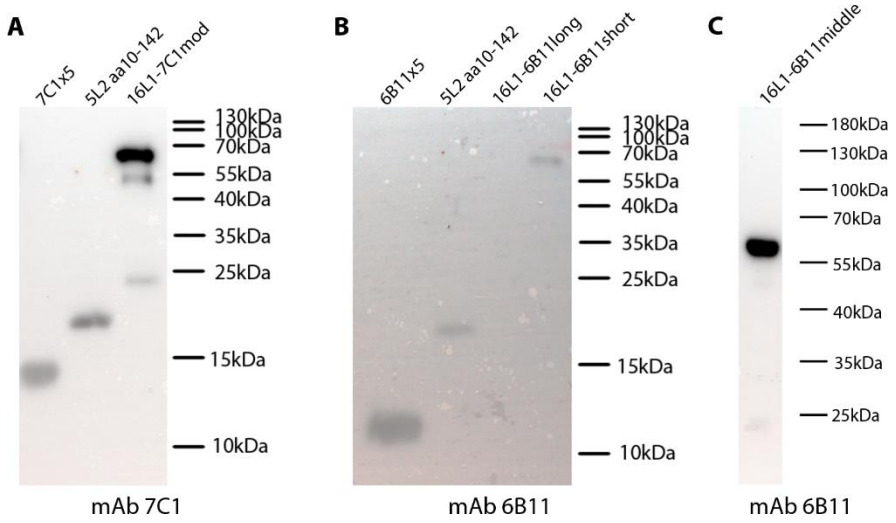
Supplemental figure 4: ßHPV L2 epitope polymers.

(A) SDS PAGE/Coomassie staining of epitope polymers 7C1x5, 6B11x5 and 7F6x5 expressed in Rosetta2 (DE3) cells (7G9x5 failed to be expressed – not shown). All polymers migrated slower than their calculated size (Serial Cloner 2.6): 7,89kDa (7C1x5), 9,59kDa (6B11x5), 10,18kDa (7F6x5) and 11,87kDa (βEx2, a polymer of each single epitope in tandem including 7G9). Western Blot reactivity of polymers (B) 7C1x5 and βEx2 with mAb 7C1, (C) 6B11x5 and βEx2 with mAb 6B11 and (D) 7F6x5 and βEx2 with mAb 7F6. HPV5L2 aa10-141, or aa122-136, or Rosetta 2 (DE3) cells were used as positive and negative controls, respectively.



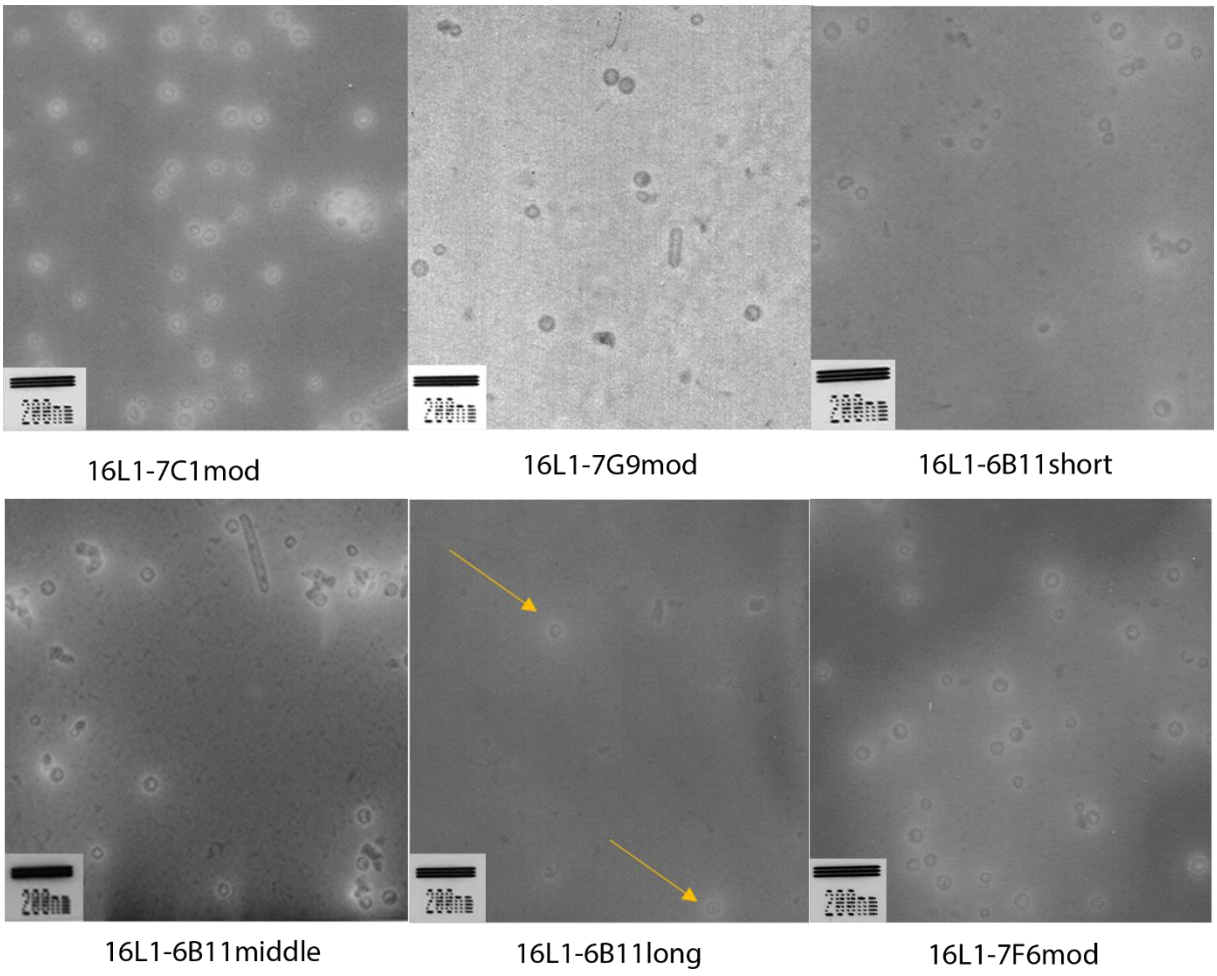
Supplemental figure 5: Chimeric fusion protein expression.

(A) SDS PAGE Coomassie staining of HPV16L1-Beta L2 epitope fusion proteins. Prominent bands of indicated HPV16L1-Beta HPV L2 epitope fusion proteins migrated around 50-60kDa. HPV16L1-6B11long showed the least efficient expression (arrow). Additional bands are considered impurities or degradation products. (B) Western Blot reactivity of fusion proteins with Camvir-1. Main fusion protein bands ran at around 50-60kDa. HPV5 L2 protein (aa10-142) was used as a negative control, while HPV16 wild-type L1 protein running at around 50kDa was used as positive control. Faster migrating bands were considered degradation products.



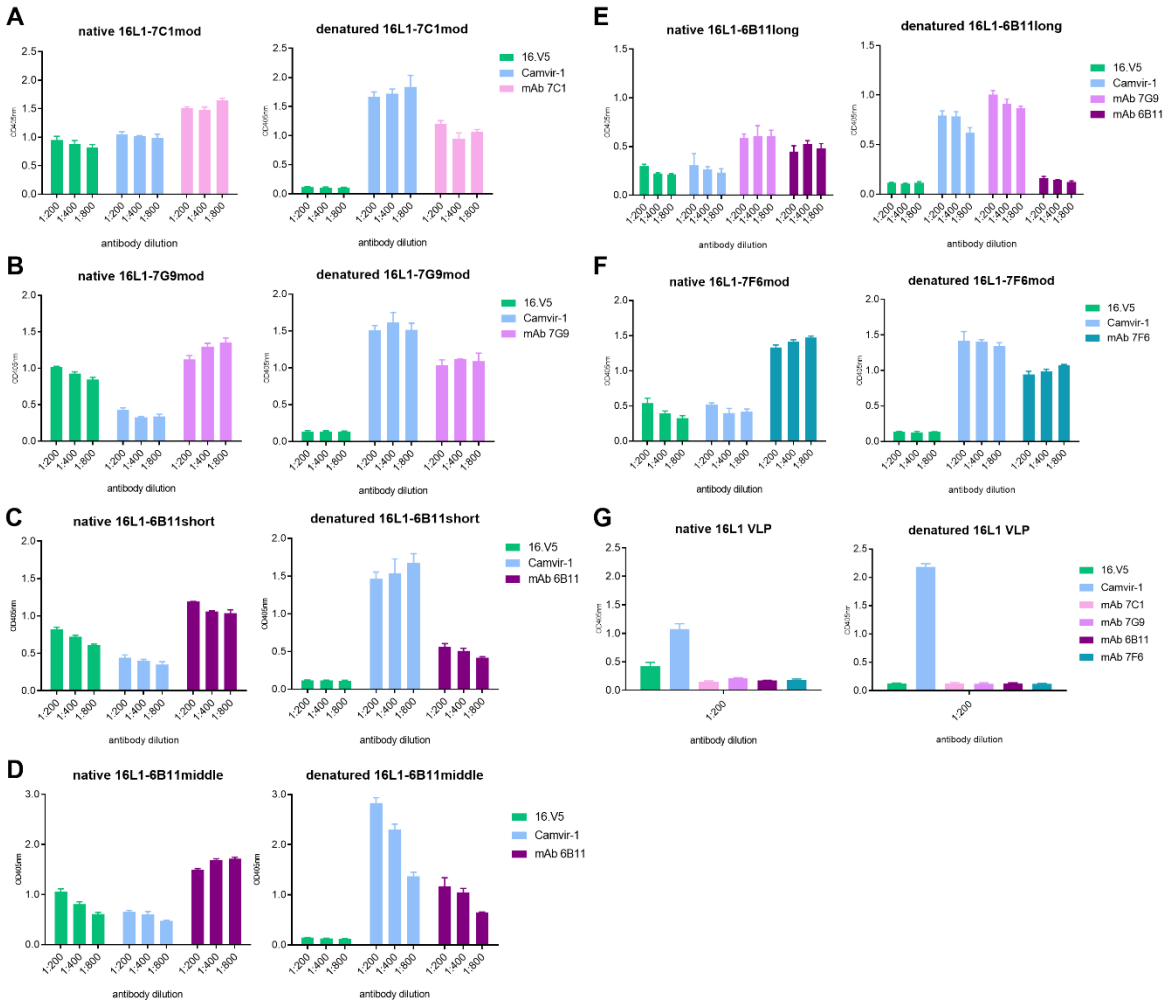
Supplemental figure 6: Western Blot reactivity of indicated fusion proteins with their respective ßHPV L2 mAb.

(A) HPV16 L1-7C1mod reacted with mAb 7C1, while (B, C) HPV16L1-6B11short and HPV16L1-6B11middle, but not HPV16L1-6B11long, reacted with mAb 6B11. Pentameric versions of the respective ßHPV L2 epitope (7C1x5 or 6B11x5), and HPV5L2 aa10-142 were used as controls. Lower MW bands were considered degradational products. Blot A and B were transferred from a 15% and blot C from a 10% polyacrylamide gel.



Supplemental Figure 7: VLP assembly analysis by TEM.

Optiprep gradient-purified preparations of indicated fusion proteins were negatively stained and visualized at 30,000-fold magnification. All six fusion proteins assembled into VLP of ~55nm. The size bars represent 200nm.



Supplemental Figure 8: Analysis of chimeric VLP by ELISA.

Purified VLP preparation of (A) HPV16L1-7C1mod, (B) HPV16L1-7G9mod, (C) HPV16L1-6B11short, (D) HPV16L1-6B11middle, (E) HPV16L1-6B11long, (F) HPV16L1-7F6mod or (G) HPV16 wt L1-VLP were probed under native and denatured conditions using HPV16-specific mAb H16.V5, mAb Camvir-1 and the indicated Beta L2 mAbs. For all chimeric VLP, V5 signal was stronger under native than denaturing conditions, with the opposite binding pattern with Camvir.1, thus pointing towards a higher ordered structure. Nevertheless, since the Camvir-1 recognizes a linear epitope there is still some reactivity seen under native conditions indicative of the presence of unassembled particles, which was more pronounced with 16L1-7C1mod, 16L1-7F6 and 16L1-6B11long. Furthermore, all chimeric VLP reacted with their respective L2 mAb. MAb 6B11 appears to prefer its epitope presentation under native conditions, in contrast to the other beta mAbs that bound equally well under both conditions. Serial dilutions of mAbs were added in triplicates and OD +/- standard deviation reported.

mAb	derived from sequential immunization with
7C1	N-term L2 of HPV8-38-5-20
7G9	N-term L2 of HPV8-38-5-76-20
6B11	N-term L2 of HPV5-8-20-76-5-38
7F6	N-term L2 of HPV8-5-38-76-8-20
1A1	16L1-6B11middle
3C6	16L1-6B11middle

Supplemental table 1: Sequential immunization schemata for ßHPV mAb generation.

overlapping peptides

5L2	7C1	7G9	6B11	7F6
aa10-50	+	-	-	-
aa36-80	-	+	-	-
aa73-110	-	-	+	-
aa100-142	-	-	-	+

5L2	7C1	7G9
aa10-24	-	-
aa17-31	+	-
aa24-38	++	-
aa31-45	-	-
aa38-52	-	-
aa45-59	-	-
aa52-66	-	-
aa59-73	-	-
aa66-80	-	+

5L2	6B11	7F6
aa66-80	-	-
aa73-87	-	-
aa80-94	-	-
aa87-101	-	-
aa94-108	-	-
aa101-115	-	-
aa108-122	-	-
aa115-129	-	-
aa122-136	-	++
aa129-142	-	-

5L2	6B11
aa73-87	-
aa76-90	-
aa79-93	+
aa82-96	+++
aa85-99	+++
aa88-102	-
aa91-105	-
aa94-108	-
aa97-110	-

5L2	1A1	3C6
aa79-93	+++	+++
aa82-96	+++	+++
aa85-99	+	-
aa82-93		

truncated peptides

5L2		7C1
aa24-27	TCPP	-
aa24-28	TCPPD	-
aa24-29	TCPPDV	-
aa24-30	TCPPDVI	-
aa24-31	TCPPDVIN	+++
aa24-32	TCPPDVINK	+++
aa24-33	TCPPDVINKV	+++
aa24-34	TCPPDVINKVE	+++
aa25-34	CPPDVINKVE	+++
aa26-34	PPDVINKVE	+
aa27-34	PDVINKVE	-
aa25-31		

5L2		7G9
aa66-71	ATGYVP	-
aa66-72	ATGYVPL	-
aa66-73	ATGYVPLG	-
aa66-74	ATGYVPLGE	+
aa66-75	ATGYVPLGEG	+++
aa66-76	ATGYVPLGEGP	+++
aa66-77	ATGYVPLGEGPG	+++
aa66-78	ATGYVPLGEGPGV	+++
aa66-79	ATGYVPLGEGPGVR	+++
aa66-80	ATGYVPLGEGPGVRV	+++
aa67-80	TGYVPLGEGPGVRV	+++
aa68-80	GYVPLGEGPGVRV	+++
aa69-80	YVPLGEGPGVRV	++
aa70-80	VPLGEGPGVRV	-
aa71-80	PLGEGPGVRV	-
aa68-75		

5L2		6B11	1A1	3C6
aa91-96	LVPETI	-	-	-
aa90-96	SLVPETI	-	-	-
aa89-96	PSLVPETI	-	-	-
aa88-96	RPSLVPETI	-	-	-
aa87-96	VRPSLVPETI	-	-	-
aa86-96	VVRPSLVPETI	-	-	-
aa85-96	TVVRPSLVPETI	+++	+	-
aa85-95	TVVRPSLVPET	+++	+	-
aa85-94	TVVRPSLVPE	-	-	-
aa85-93	TVVRPSLVP	-	-	-
aa85-92	TVVRPSLV	-	-	-
aa85-91	TVVRPSL	-	-	-
aa85-90	TVVRPS	-	-	-
aa85-95				

5L2		7F6
aa122-129	ESTGADLL	-
aa122-130	ESTGADLLP	-
aa122-131	ESTGADLLPG	-
aa122-132	ESTGADLLPGE	-
aa122-133	ESTGADLLPGEV	-
aa122-134	ESTGADLLPGEVE	-
aa122-135	ESTGADLLPGEVET	-
aa122-136	ESTGADLLPGEVETI	+++
aa123-136	STGADLLPGEVETI	+++
aa124-136	TGADLLPGEVETI	++
aa125-136	GADLLPGEVETI	+
aa126-136	ADLLPGEVETI	-
aa127-136	DLLPGEVETI	-
aa128-136	LLPGEVETI	-
aa129-136	LPGEVETI	-
aa123-136		

Supplemental table 2: Overlapping and truncated peptide screen to map the epitopes of ßHPV L2 mAbs. +++ strong, ++ medium and + low binding.

		Icosagen			
		7C1	7G9	6B11	7F6
LoVoT-PBNA	HPV 5	3200	12800	50	12800
	HPV8	12800	-	100	12800
	HPV 20	12800	12800	6400	12800
	HPV 24	6400	800	3200	12800
	HPV 38	6400	12800	200	12800
	HPV 76	-	6400	100	-
	HPV 92	6400	-	200	1600
	HPV 96	12800	-	100	200
L2-PBNA	MmuPV-1	≥100	-	-	-

Supplemental table 3: Recombinantly expressed mAbs (Icosagen AS, Estonia), all in $1^{mg}/_{ml}$ concentration, were tested by fc- and L2-PBNA against a panel of 8 βHPV and Mus musculus PV (MmuPV-1). The cross-neutralization profiles were similar to the parental mAbs (table 1), with the exception that the hybridoma-produced mAb 7F6 did not cross-neutralize HPV96, but the recombinant 7F6 did at low levels. Neutralization titers are indicated for reciprocal serum dilutions resulting in 50% reduction in reporter signal compared to an unrelated mAb or PsV only. Titers of <50 were considered non-neutralizing and indicated as (-).

		HPV5 L2 peptide			
		aa24-31	aa66-80	aa85-95	aa124-136
antisera to	7C1x5	12.800	n.d.	n.d.	n.d.
	6B11x5	n.d.	n.d.	800	n.d.
	7F6x5	n.d.	n.d.	n.d.	-
	βEx2	-	-	200	3.200

Supplemental table 4: HPV5 L2 peptide ELISA. Serial dilutions of pooled mouse immune sera raised against the indicated L2 epitope multimers were tested in triplicates against indicated biotinylated HPV5 L2 peptides. Serum titers are reported for OD values greater than the mean OD of pre-immune sera plus 3 standard deviations (SD). Titers <200 were considered non-reactive and indicated as (-); n.d. not determined.

	antiserum			
HPV	a7C1x5	a6B11x5	a7F6x5	aβEx2
5	50	-	-	-
8	50	50	-	-
20	100	50	50	-
24	200	100	>200	>200
38	50	50	50	-
76	-	50	-	50
92	50	-	100	50
96	50	50	50	-

Supplemental table 5: Cross-neutralizing titers induced by vaccination with ßHPV L2 epitope polymers. Serial dilutions of pooled mouse sera were tested for cross-neutralization against eight ßHPV types by FC-PBNA. Neutralization titers are indicated for reciprocal serum dilutions resulting in 50% reduction in reporter signal compared to an unrelated mAb or PsV only. Titers of <50 were considered non-neutralizing and indicated as (-).

	5L2 sequence insertions		length (incl. linker)	aa sequence
	nt #	aa #	aa	
7C1mod	70-102	24-34	19	GSGS TCPPDVINKVE GSGS
7G9mod	178-216	60-72	21	GSGS GRGTGGATGYVPL GSGS
6B11short	250-294	84-98	23	GSGS PTVVRPSLVPETIGP GSGS
6B11middle	235-294	79-98	28	GSGS RVGGTPTVVRPSLVPETIGP GSGS
6B11long	196-294	66-98	41	GSGS ATGYVPLGEGPGVRVGGTPTVVRPSLVPETIGP GSGS
7F6mod	379-414	127-138	20	GSGS DLLPGEVETIAE GSGS

Supplemental table 6: HPV5 L2 amino acid (aa) sequences used for chimeric VLP design. Indicated are nucleotide (nt) and aa positions of HPV5 L2 that include the identified epitopes and surrounding conserved L2 aa. Epitopes were flanked by GSGS-linkers and inserted into HPV16 L1 (between aa position 136/137).

NZW	16L1-VLP	cognate HPV5 L2 peptide	
a16L1-7C1mod	12.800	aa24-34	200
a16L1-7G9mod	3.200	aa36-80	200
a16L1-6B11short	3.200	aa85-96	12.800
a16L1-6B11long	3.200	aa73-110	200
a16L1-7F6mod	12.800	aa127-136	3.200
a16L1-7C1mod[#]	204.800	aa24-34	200

Supplemental table 7: HPV16 L1-VLP and HPV5 L2 ELISA results. Serial dilutions of NZW rabbit sera raised against indicated chimeric fusion proteins/VLP were tested in triplicates against HPV16 L1-VLP or cognate HPV5 L2 peptides. Serum titers are reported for OD values greater than the mean OD of pre-immune sera plus 3 standard deviations (SD). [#] Freund's adjuvanted.

	7C1 epitope (5L2aa 25-31)	7G9 epitope (5L2 aa68-75)	6B11 epitope (5L2 aa85-95)	7F6 epitope (5L2 aa123-136)
HPV5	CPPDVIN	GYVPLGEG	TVVRPSLVPET	STGADLLPGEVETI
HPV8	CPPDVIN	GY**T**PL**S**EG	TVVRPSLVPE**A**	S**S**GADLLPGEVETI
HPV20	CPPDVIN	GYVPLGEG	TV**I**RP**A**LVP**D**T	STG**P**DLLPGEVETI
HPV38	CPPDVIN	GYVPLG**Q**G	TVVRP**GVI**PE**V**	S**SAV**DLLPGEVETI
HPV76	CPPDV**L**N	GYVPLGEG	**N**V**I**RPS**VI**PE**A**	**-**TG**P**DLLPGE**I**ETI
HPV24*	CPPDVIN	GYVPLGEG	TVVRP**A**LVPE**V**	S**S**G**V**DLLPGE**I**ETI
HPV92*	CPPDV**V**N	GY**G**PLGEG	TV**I**RP**A**LVPE**A**	STG**P**DLLPGE**I**ETI
HPV96*	CPPDVIN	GYVPL**P**EG	TVVRP**GVI**PE**A**	**-**TG**P**DLLPGE**I**ETI

Supplemental table 8: L2 alignment of novel epitopes. Marked in blue are differences in aa to HPV5.

Types marked with (*) were not used for mAb generation.