SUPPORTING INFORMATION – TABLES

Table S1. Conditions used for the virus stock preparation.

Virus	Incubation time and temperature of virus stock plates	Plates used for virus stocks
HRPV-1	3 days at 37°C	Semiconfluent
HRPV-2	3 days at 34°C	Confluent
HRPV-3	5 days at 37°C	Confluent
HRPV-6	4 days at 37°C	Confluent
HHPV-1	4 days at 37°C	Semiconfluent
His2	3 days at 37°C	Confluent
HGPV-1	4 days at 37°C	Confluent

Table S2. Composition of the salt waters.

Compound	Artificial salt water					
	30%	23%	18%	15%	12%	9%
NaCl	4.109 M	3.150 M	2.465 M	2.054 M	1.644 M	1.233 M
$MgCl_2 \cdot 6H_2O$	148 mM	113 mM	89 mM	74 mM	59 mM	44 mM
$MgSO_4 \cdot 7H_2O$	142 mM	109 mM	85 mM	71 mM	57 mM	43 mM
KCl	94 mM	72 mM	56 mM	47 mM	38 mM	28 mM
$CaCl_2$	5 mM	3.8 mM	3.0 mM	2.5 mM	2.0 mM	1.5 mM
Tris-HCl (pH 7.2)	80 mM	61 mM	48 mM	40 mM	32 mM	24 mM

Table S3. N-terminal sequences.

Virus	Sample	Protein[a]	Determined N-terminal sequence[b]
His2	Untreated virions	VP27 (63 amino acids)	MNYNLKVGAI... (1...)
		VP28 (273 amino acids)	AGTLYVGTSE... (41...)
		VP29 (523 amino acids)	XHDCDTTDALV... (38...)
		VP32 (62 amino acids)	XNPIDELMAV... (2...)
	Virions treated with proteinase K at high salinity	VP28 (273 amino acids)	GANVFG... (247...)
HRPV-3	Virions treated with proteinase K at high salinity	VP2 (544 amino acids)	GDLGGAG... (508...)
HGPV-1	Untreated virions	VP3 (70 amino acids)	MDIDLN... (4...)
	Virions treated with proteinase K at low salinity	VP2 (135 amino acids)	AGNSVT... (45...)
HRPV-6	Untreated virions	VP4 (133 amino acids)	ASSYRNS... (2...)
	Virions treated with proteinase K at high salinity	VP5 (537 amino acids)	IAPLVGYA... (1...)

[a] Number of the amino acids of the protein based on the genome sequence (AF191797, JN882265, JN882267, JN882266) is given in parentheses.

[b] Number of the first amino acid of the N-terminal sequence is given in parentheses.

SUPPORTING INFORMATION – FIGURES

A

Virus	3% UA (infectivity%)	1% AM (infectivity%)
HRPV-1	<1	35
HRPV-2	<1	89
HRPV-3	8.7	9.3
HRPV-6	<1	100
HGPV-1	18	70
HHPV-1	<1	18
His2	<1	2.5

B

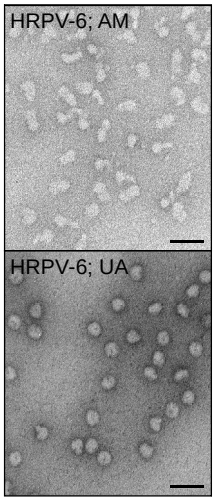

Figure S1. Virion infectivity in negative-stains.
A. Virus particles were treated with 3% uranyl acetate (UA) for 1 min or 1% ammonium molybdate (AM) for 2 min and the infectivity was determined.
B. Negative-stain electron micrographs of the '1× purified' HRPV-6 virions using AM or UA. Bars, 100 nm.

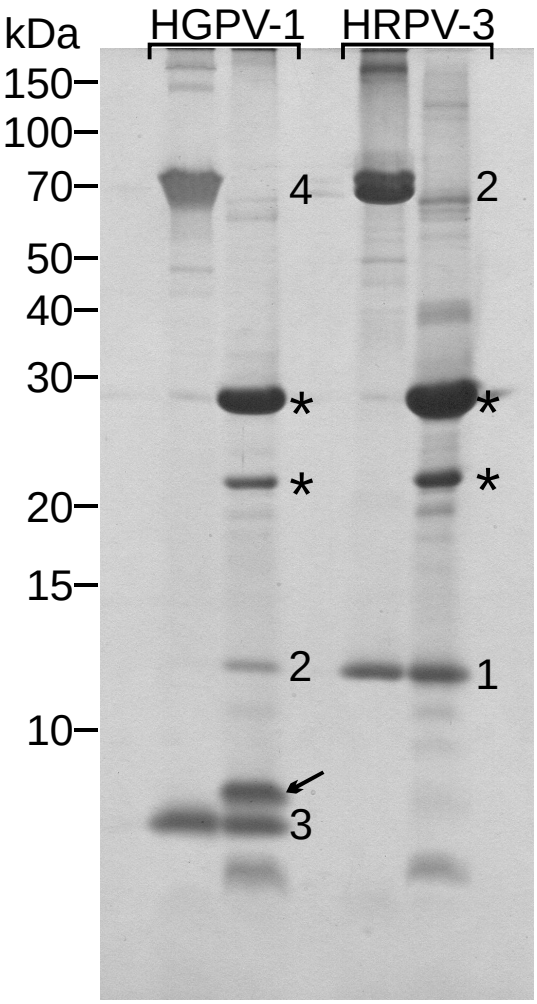

Figure S2. Protein analysis of HGPV-1 and HRPV-3.
The first lanes show the untreated '2× purified' virions and the second lanes the virions treated with proteinase K at low salinity at 37°C. Numbers on the gel indicate the virion protein (VP) number and numbers on the left the molecular mass markers. Asterisks indicate the proteinase K bands. The arrow indicates the digestion product of HGPV-1 VP2 identified by N-terminal sequencing (see Table S3). The intact VP2 protein is indicated based on the molecular mass.

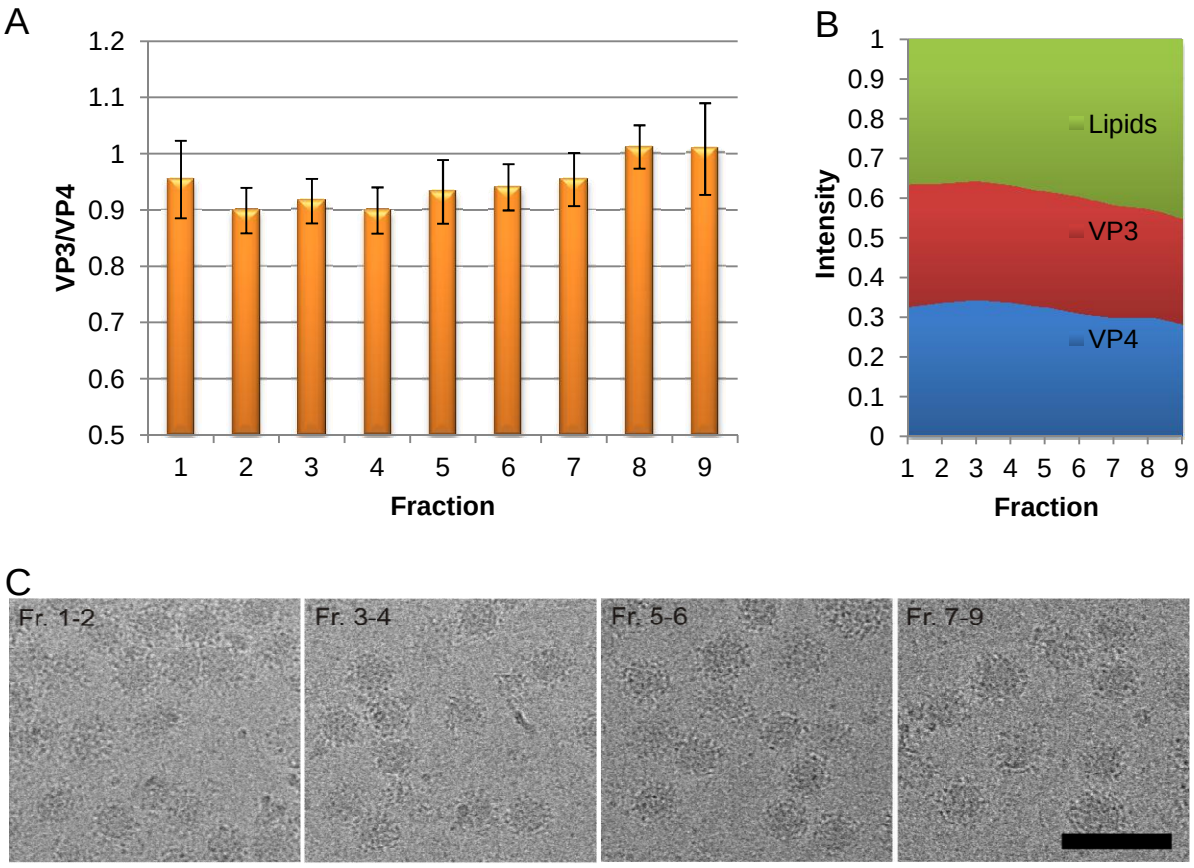

Figure S3. Analyses of the HRPV-1 virus zone after rate zonal centrifugation. Fractions 1 to 9 correspond to those in Fig. 4B.
A. Ratio of VP3 and VP4 band intensities and their strandard deviations.
B. Ratio of VP3, VP4, and lipid band intensities.
C. Cryo-EM analysis of the fractions combined as indicated. Scale bar, 100 nm.

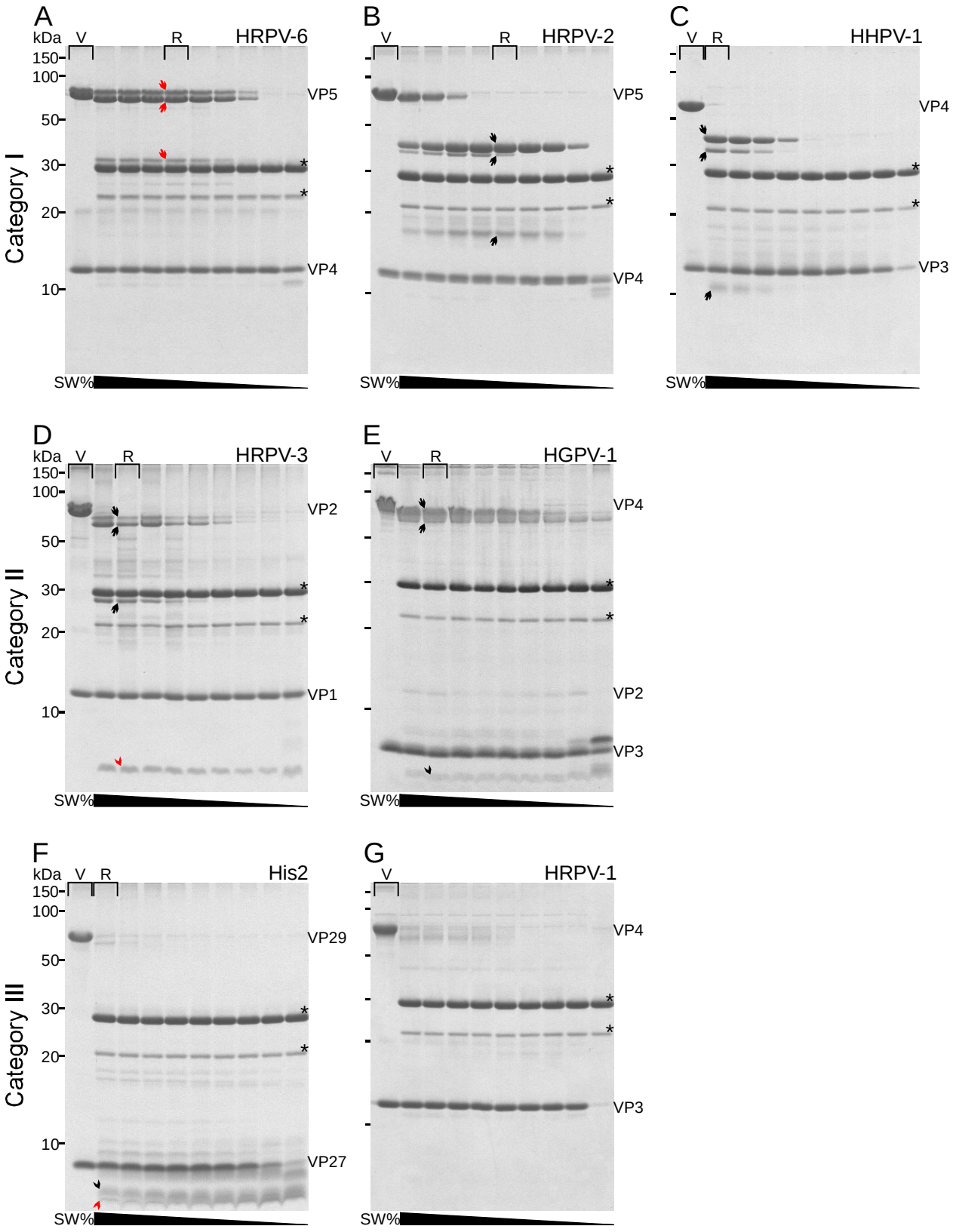

Figure S4. Protease sensitivity of the virions. The virions were treated with proteinase K at different salinities at 37°C and analysed by tricine-SDS-PAGE and Coomassie-blue staining. The untreated ('V') and treated viruses in the decreasing SW concentration are shown from left to right. 'R' indicates the treatments analysed by rate zonal centrifugation. The arrows indicate soluble and arrow heads membrane associated fragments of the spike proteins. The red arrows and arrow heads indicate the fragments identified by N-terminal sequencing. The membrane-associated C-terminal domain of the VP4 spike protein of HRPV-1 is not observed with the virus amount used here but has been verified in the previous study (48). The positions of the molecular mass markers are shown next to the gels and the molecular masses are indicated next to the left-most gels. The asterisks indicate the proteinase K bands. The HGPV-1 gel was partially destained to show the VP4 protein.