Supporting Information

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SI Methods

Clinical Specimens. Samples were collected at Pointe Noire hospitals with verbal informed consent from patients before the time of their death. All specimens ($n = 24$) were received along with names and ages of patients at the Centre International de Recherches Médicales de Franceville (CIRMF), Franceville, Gabon, from October until November 2, 2010, i.e., 10 d before the first supplementary immunization activity in response to the outbreak (1). After initial investigation samples were transferred to the University of Bonn without further opening of sample containers. Control sera ($n = 12$) were collected in neighboring Gabon between May and June 2011, several weeks after an oral polio vaccine (OPV) campaign in Gabon between January and April 2011. Additional control sera ($n = 34$) were obtained from German medical students and from German outpatients ($n = 17$) sent to the National Reference Center for Poliomyelitis and Enteroviruses (NRC PE) at the Robert Koch Institute for confirmation of anti-polio virus immunity.

RT-PCR. RNA was purified from all specimens using the Qiagen Viral RNA kit (Qiagen) and tested at CIRMF for enterovirus RNA by real time RT-PCR targeting the viral 5′-UTR (2). Confirmatory testing was done at the Institute of Virology, using the 5′-UTR real-time (2) and additional (hemi)nested RT-PCR assays targeting the viral 5′-UTR, VP2, and VP1 genomic regions (3–5). The full genome was determined by long-range PCR and Sanger sequencing. Genome termini were determined using a RACE strategy (Roche). Further PCR protocols are available upon request.

Exclusion of Alternative Outbreak-Associated Etiologies. Testing for viruses other than poliovirus type 1 (PV1) included PCR or RT-PCR for cytomegalovirus, human herpesvirus type 6, herpes simplex virus type 1 and 2, varicella–zoster virus, rotavirus serogroup A, norovirus genogroups 1 and 2, sapovirus, astrovirus, human parechovirus, and influenza viruses A and B. Broad-range RT-PCR was used for the genera *Enterovirus*, *Flavivirus*, *Alphavirus*, *Phlebovirus*, *Mastadenovirus*, *Alphacoronavirus*, and *Betacoronavirus* and the family *Paramyxoviridae* (2–20).

Cell Culture. Human rhabdomyosarcoma (RD) cells were used for virus isolation, according to World Health Organization (WHO) guidelines. Neutralization assays in RD, human epidermoid carcinoma 2 (HEp-2) Cincinatti, and Vero cells were done including an in-house reference serum calibrated against the International Standard Serum with known neutralizing activity in each test to control reproducibility of results (21). Calibrated reference antibody sera against PV1-3 obtained from the National Institute for Biological Standardization and Control (NIBSC) (Hertfordshire, UK; NIBSC code 82/585) were used additionally to control results. Briefly, heat-inactivated sera (30 min at 56 °C) were diluted twofold (starting dilutions varied according to the available serum volumes for each laboratory; see Tables S2–S7) and incubated with 100 $TCID_{50}$ of challenge virus at 37 °C for 1–3 h. After adding RD, HEp-2C, or Vero cells, microtiter plates were incubated at 37 °C with 5% (vol/vol) CO_2 for 2 d (Vero cells) or 5 d (RD and HEp-2C cells). Virus-induced cytopathogenic effect (CPE) was evaluated by direct microscopy. Endpoint titers were defined as reciprocals of the highest serum dilutions inhibiting CPE in 50% of replicate wells. The titers of seed virus were redetermined from control wells receiving no neutralizing serum in each assay (back titration) in agreement with WHO protocols (22).

Phylogenetic Analysis. Because poliovirus 1 (PV1) fulfilled the necessary population genetic criteria of both the hosts (a connected population exchanging the virus) and the virus (one species comprising genetically closely related viruses that originate from a common ancestor), coalescent-based analysis was performed. Complete VP1 gene sequences were aligned with all wild PV1 (WPV1) sequences available in GenBank. Sequences with unknown isolation dates and reference sequences that were >98% identical to any other sequence were excluded from further analysis. Based on published data on WPV1 molecular clock analyses, only strains detected during the last 20 y were included, to avoid saturation of branches (23). This dataset was analyzed under an SRD06 substitution model optimized for coding sequences with a relaxed uncorrelated lognormal clock and constant population growth assumptions. Phylogenetic analysis was done with Bayesian evolutionary analysis by sampling trees (BEAST) 1.4.7 (24) using 50,000,000 generations sampled every 1,000 steps, resulting in 50,000 trees of which 25% were discarded as burn-in. Identical tree topology was yielded in confirmatory analyses with MrBayes Version 3.1 (25) using a GTR+G+I nucleotide substitution model and default chain and run parameters over 2,000,000 generations.

Statistics. All statistics were calculated using SPSS V22 (IBM).

Ethics. This study was part of an outbreak control operation coordinated by the Republic of Congo Ministry of Health and the WHO Central Africa Regional Office. The study was additionally approved by the CIRMF scientific review board. Sera from German vaccinees were obtained during poliovirus neutralization exercises during medical courses and from outpatients sent for PV routine diagnostics.

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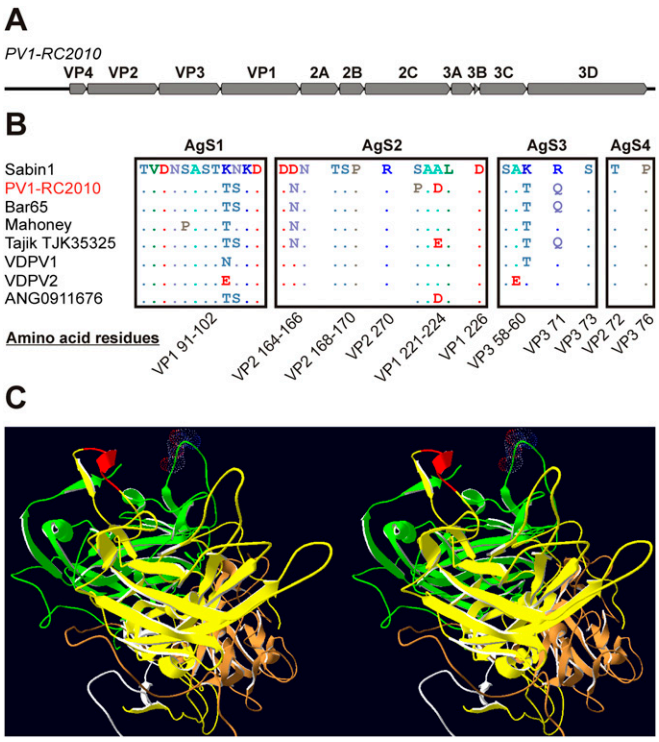

Fig. S1. Genome organization of *PV1-RC2010*, PV1 antigenic sites (AgS), and PV1 structural protein folding (stereo image). (*A*) Representation of the genome organization of *PV1-RC2010* including 5′- and 3′-UTRs. (*B*) PV1 AgS1–4 annotated according to refs. 1 and 2 with amino acid residues indicated below. GenBank accession numbers, where applicable: CHAT, AJ416942; Sabin-1, V01150; Mahoney, V01149; VDPV1, AF462418; Bar65, AY278553; PV1-RC2010, JF838278; Tajik TJK35325, KC880372; and Angola 2009 VP1, JF313102. Amino acid identities with Sabin-1 are indicated by dots; deviations are spelled out. (*C*) The AgS2 domain 2a (VP1 residues 221–226) is highlighted as a red loop with a small alpha-helix part. The opposing domain 2b (VP2 residues 168–170) is shown as a loop surrounded by dots (van der Waals radii, shown for clarity of presentation only). All proteins are shown as C trace and ribbons without side chains; therefore neither specifically depicts PV1 Sabin nor *PV1-RC2010* strains.

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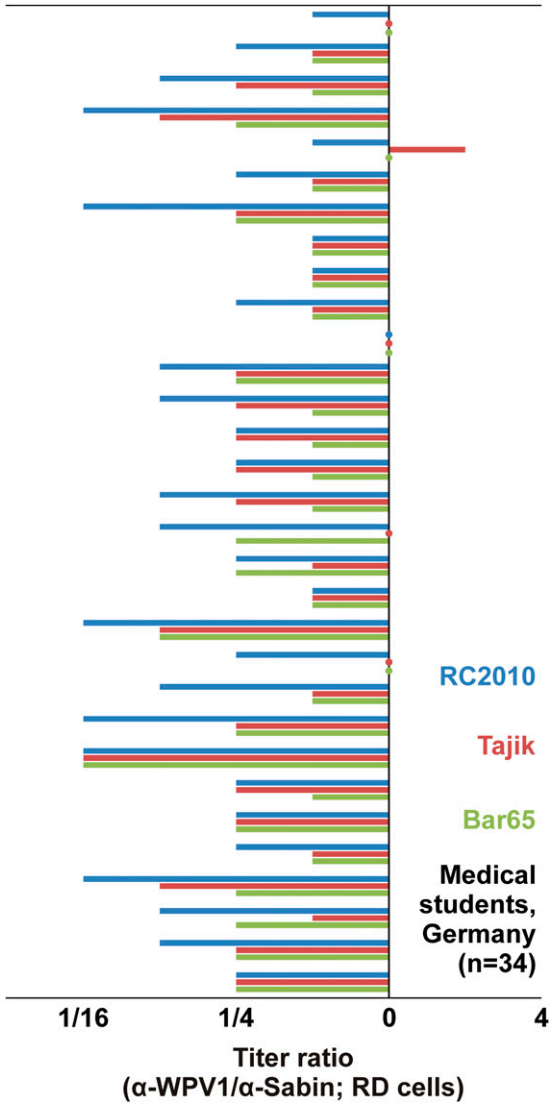

Fig. S2. Deviations between serum neutralization of WPV1 strains *PV1-RC2010*, Bar65, and Tajik compared with Sabin-1. Relative reciprocal neutralization titers determined on RD cells against each WPV1 divided by those against Sabin-1 ("α-Sabin"). Bars pointing left indicate an excess of antibodies against Sabin-1 and vice versa. Null deviations are indicated by circles color-coded by each WPV1.

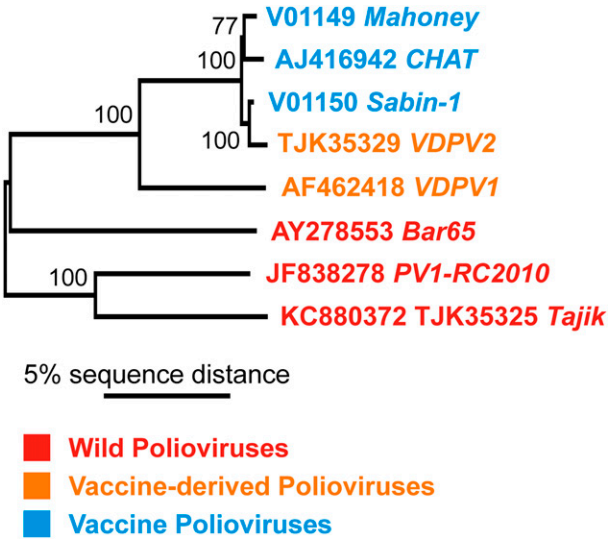

Fig. S3. Phylogenetic relationships between PV1 used for comparative neutralization tests. The neighbor-joining phylogeny was calculated using Molecular Evolutionary Genetics Analysis Version 5 (1) with a percentage nucleotide distance substitution model and a complete deletion option. Full genomes were used for all viruses identified by strain names and GenBank accession numbers, where applicable. Values at nodes indicate statistical support from 1,000 bootstrap replicates.

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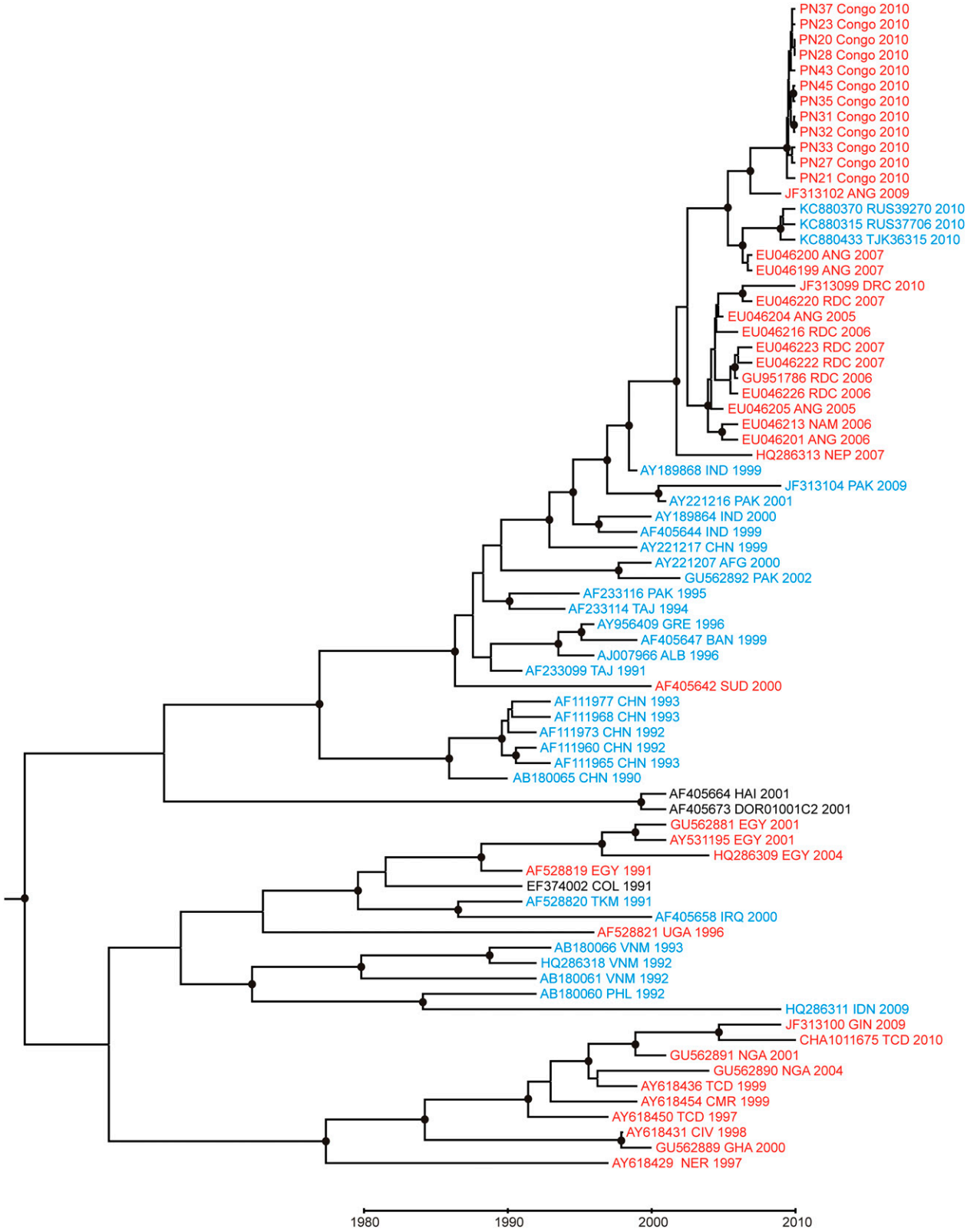

Fig. S4. WPV1 complete VP1 gene phylogeny including the RC2010 viruses and PV1 reference strains. The phylogenetic reconstruction was created with BEAST Version 1.4.7 (1) for the complete VP1 genome region of 76 PV1 strains isolated from 1990 to 2010. (Scale bar: 1980–2010; indicates evolutionary timing in years.) Filled circles at tree nodes indicate posterior probabilities above 0.95. African viruses are in red, Asian in blue, and others in black. Viruses from the RC2010 outbreak are given with patient codes.

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Table S1. Results in laboratory-confirmed fatal poliomyelitis cases

Patient		RT-PCR			
Code	Age, y	Throat swab	Rectal swab*	Cerebrospinal fluid	Virus characterization
PN20	21	–	+	N/A	VP1
PN21	14	+	+	–	VP1
PN23	18	+	+	N/A	VP1
PN27	12	–	+	N/A	VP1
PN28	23	–	+	N/A	VP1
PN30	18	N/A	–	+	—
PN31	22	–	+	–	VP1
PN32	18	N/A	+	N/A	VP1
PN33	20	+	+	N/A	VP1
PN35	20	–	+	N/A	VP1
PN37	24	+	+	–	VP1
PN43	20	N/A	+	N/A	VP1
PN45	20	N/A	+	N/A	Full genome, isolate PV1 RC2010

N/A, not available; —, negative.

*Second rectal swab samples for further laboratory confirmation could not be confirmed for the reason of death.

Table S2. Neutralizing antibody titers: Laboratory-confirmed poliomyelitis cases

Patient		Neutralization titer					
		Reciprocal, laboratory 1, Vero cells	Reciprocal, laboratory 2, RD cells		Reciprocal, laboratory 1, Vero cells		
Code	Age, y	PV1, PV2, PV3 Sabin		PV1 Mahoney	PV1 Sabin; repeated testing for comparison with RC2010	PV1 RC2010	Virus characterization
PN20	21	5, 80, 160	320, 160, 40	20	10	80	VP1
PN21	14	640, 640, 80	160, 1,280, 320	N/A*	640	40	VP1
PN23	18	2,560, 640, 80	160, 2,560, N/A*	N/A*	160	80	VP1
PN27	12	320, 320, 160	5,120, 640, 160	320	5,120	320	VP1
PN28	23	640, 640, 160	1,280, 640, 320	640	1,280	640	VP1
PN30	18	5,120, 1,280, 80	160, 40, N/A*	N/A*	640	80	—
PN31	22	160, 640, 80	2,560, 2,560, 320	N/A*	2,560	320	VP1
PN32	18	80, 320, 20	N/A*	N/A*	80	40	VP1
PN33	20	1,280, 640, 160	N/A*	80	160	80	VP1
PN35	20	160, 10, 10	2,560, 320, 320	N/A*	80	10	VP1
PN37	24	640, 320, <5*	N/A*	640	640	160	VP1
PN43	20	320, 320, 160	2,560, 640, 1,280	160	5,120	160	VP1
PN45	20	80, 640, 640	640, 640, 320	320	80	320	Full genome, Isolate PV1 RC2010

*These titers could not be finally determined due to exhaustion of serum samples.

Table S3. Neutralizing antibody titers: Epidemiologically linked poliomyelitis cases without laboratory confirmation

Patient		Neutralization titer				
		Reciprocal, laboratory 1, Vero cells	Reciprocal, laboratory 2, RD cells	Reciprocal, laboratory 1, Vero cells		
Code	Age, y	PV1, PV2, PV3 Sabin		PV1 Mahoney	PV1 Sabin; repeated testing for comparison with RC2010	PV1 RC2010
PK	23	80, 640, 160	5,120, 1,280, 320	N/A*	80	40
PN6	19	2,560, 1,280, 10	320, N/A*, N/A*	N/A*	1,920	240
PN8	26	5, N/A*, N/A*	N/A*	10	10	80
PN9	21	1,280, 320, 80	N/A*	1,280	1,280	320
PN10	17	160, 20, N/A*	80, 160, 40	160	160	80
PN11	11	640, 320, 320	1,280, 320, 40	N/A*	640	160
PN12	13	320, 160, 10	160, 640, 1,280	2,560	320	160
PN17	26	320, 2,560, 160	2,560, 1,280, 640	640	640	2,560
PN18	21	160, 2,560, N/A*	N/A*	160	160	80
PN19	17	640, 640, 320	320, N/A*, 160	2,560	1,280	640
PN41	22	640, 640, 80	320, 320, N/A*	N/A*	640	160

*These titers could not be finally determined due to exhaustion of serum samples.

Table S4. Neutralizing antibody titers: African healthy controls

Control		Neutralization titer		
		Reciprocal, laboratory 1, Vero cells		
Code	Age, y	PV1 Sabin	PV1 RC2010	PV1 Mahoney
GO	3	80	20	N/A*
JCE	43	20	20	160
GM	43	20	<10*	80
AO	56	80	40	N/A*
AE	48	320	80	N/A*
AN	29	10	10	20
CM	46	160	160	N/A*
EM	29	10	<10*	N/A*
CO	55	80	40	N/A*
MA	N/A	40	10	N/A*
EE	40	40	40	N/A*
ME	38	160	80	N/A*

*These titers could not be finally determined due to exhaustion of serum samples.

Table S5. Neutralizing antibody titers: German healthy controls (Medical students)

Controls		Neutralization titer						
		Reciprocal, laboratory 1, Vero cells			Reciprocal, laboratory 2, RD cells		Reciprocal, laboratory 2, Hep2 cells	
Code	Age, y	PV1 Sabin	PV1 RC2010	PV1 Mahoney	PV1 Sabin	PV1 RC2010	PV1 Sabin	PV1 RC2010
10/14246	26	N/A	N/A	N/A	256	32	512	32
10/18599	27	N/A	N/A	N/A	384	64	512	64
10/18620	23	N/A	N/A	N/A	>512	64	>512	64
10/18627	25	N/A	N/A	N/A	>512	64	>512	64
10/18628	21	N/A	N/A	N/A	128	32	384	64
10/18629	27	N/A	N/A	N/A	512	64	256	64
10/19066	25	N/A	N/A	N/A	>512	64	>512	64
10/20787	24	N/A	N/A	N/A	32	<4*	48	<4*
10/19071	22	80	20	80	192	32	128	96
10/20787	24	20	<5*	5	32	<4*	48	<4*
11/14082	22	20	10	N/A*	192	64	128	128
11/19504	22	80	10	80	128	16	192	24
11/19694	21	80	10	80	256	24	256	96
11/19697	35	40	40	N/A*	384	128	384	384
11/20102	24	80	80	N/A*	>512	384	512	512
11/20203	23	20	<5*	N/A*	256	12	128	48
11/20204	22	5	5	N/A*	192	8	192	64
11/20210	24	20	<5*	N/A*	192	24	128	64
11/20238	22	80	20	N/A*	>512	128	>512	512
11/20247	22	160	20	N/A*	>512	32	>512	128
11/20249	25	20	5	N/A*	256	48	384	64
11/20250	23	10	5	N/A*	192	16	128	32
11/20253	22	40	5	N/A*	192	32	128	48
11/20357	22	>640	40	N/A*	>512	192	>512	48
11/20359	23	80	20	N/A*	>512	96	384	96
11/20496	24	5	<5*	N/A*	96	8	64	16
11/20690	25	5	<5*	N/A*	64	16	48	48
11/20804	22	40	5	N/A*	512	16	384	32
11/20920	23	20	5	N/A*	384	48	256	64
11/20934	23	20	5	N/A*	256	24	256	64
11/21026	28	20	10	N/A*	192	16	256	64
AFT	35	10	<5*	N/A*	32	<4*	32	<4*
RMZ	27	10	<5*	N/A*	16	<4*	32	<4*
SB	30	20	<5*	N/A*	128	<4*	96	<4*

N/A, not available.

*These titers could not be finally determined due to exhaustion of serum samples.

Table S6. Neutralizing antibody titers: German outpatients

Control		Neutralization titer	
		Reciprocal, laboratory 2, Hep2 cells	
Code	Age, y	PV1 Sabin	PV1 RC2010
13-46	55	16	<4*
13-53	35	384	64
13-77	57	12	<4*
13-105	17	512	192
13-116	11	128	96
13-124	8	96	24
13-125	55	512	8
13-126	13	256	256
13-127	24	48	8
13-136	26	96	96
13-137	64	16	<4*
12-645	21	96	16
12-759	N/A	24	4
12-760	N/A	48	16
12-834	21	32	4
12-838	15	64	8
12-848	19	128	128

N/A, not available.

*These titers could not be finally determined due to exhaustion of serum samples.

Table S7. Neutralizing antibody titers: German healthy controls (Medical students)

Control		Neutralization titer					
		Reciprocal, laboratory 3, RD cells					
Code	Age, y	PV1 Sabin*	PV1 RC2010	PV1 Bar65	PV1 Tajik (strain 35325)	VDPV1 (strain 11262)	VDPV2 (strain 35329)
10/14246	26	80	40	80	80	160	160
10/18599	27	160	40	80	80	160	160
10/18620	23	320	40	160	80	320	320
10/18627	25	640	40	160	80	320	320
10/18628	21	40	20	40	80	80	80
10/18629	27	80	20	40	40	160	160
10/19066	25	640	40	160	160	320	320
10/19710	22	20	10	10	10	20	20
10/20787	24	20	10	10	10	10	10
10/19071	22	80	20	40	40	80	80
11/14082	22	40	40	40	40	40	80
11/19507	22	80	<10†	20	20	80	40
11/19694	21	80	<10†	40	20	80	80
11/19697	35	320	80	160	80	320	320
11/20102	24	320	80	160	80	320	320
11/20203	23	80	<10†	40	20	80	40
11/20204	22	80	<10†	20	80	80	<10†
11/20210	24	80	20	20	40	40	40
11/20238	22	320	160	160	160	640	320
11/20247	22	1,280	80	160	160	640	1,280
11/20249	25	80	20	80	80	160	80
11/20250	23	80	<10†	40	40	80	80
11/20253	22	160	<10†	40	40	40	80
11/20357	22	1,280	80	80	80	640	1,280
11/20359	23	320	80	160	80	320	320
11/20496	24	40	<10†	<10†	10	20	40
11/20690	25	40	<10†	20	20	40	20
11/20807	22	160	<10†	40	20	160	160
11/20920	23	320	40	80	160	160	160
11/20934	23	160	20	40	40	80	160
11/21026	28	160	40	40	40	80	80

*Back titration yielded a challenge dose of 300–500 tissue culture infectious dose$_{50}$ (TCID$_{50}$) for Sabin-1; all other viruses were used at 100–150 TCID$_{50}$.

†These titers could not be finally determined due to exhaustion of serum samples.

Table S8. Results of PV1 neutralization assays using three mAbs against AgS2 epitopes located in the VP2 and VP1 proteins

	Virus strains and neutralization test results; duplicate assays											
	Dilution, 1/×											
mAb	16	32	64	128	256	512	1,024	2,048	4,096	8,192	16,384	32,768
	PV1 Mahoney*											
14D2E9	0/0	0/0	0/0	0/0	0/0	+/+	+/+	+/+	+/+	+/+	+/+	+/+
12(237)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	+/+	+/+	+/+	+/+
14(427)	0/0	0/0	0/0	0/0	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
	Escape mutant 1083; site 2a mutant, VP1 S221L†											
14D2E9	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
12(237)	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
14(427)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	+/0	+/+
	Escape mutant 1005; site 2b mutant, VP2 P170S†											
14D2E9	0/0	0/0	0/0	0/0	0/0	0/0	0/0	+/0	+/+	+/+	+/+	+/+
12(237)	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
14(427)	0	0	0	0	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
	PV1-RC2010 outbreak strain											
14D2E9	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
12(237)	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
14(427)	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+

0, no CPE and neutralization of infectivity; +, CPE and no neutralization.

*mAb 14D2E9 binds specifically in site 2a (1) and has been found to correlate well with neutralizing antibody titers against PV1 (2); 237 is a mAb eliciting a VP2 P170S exchange (escape mutant 1005) as published in ref. 3; 427 binds a composite epitope in AgS2.

†*PV1* mutants generated by culture under antibody pressure, as published in ref. 3. Mutant 1083 (designated as "9" in ref. 3) has a mutation in AgS2a, VP1 residue 221 (S→L). Mutant 1005 (designated as "7" in ref. 3) has a mutation in AgS2b, VP2 residue 170 (P→S).

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