

Supplemental Table S1. Clinical scoring used in the present study

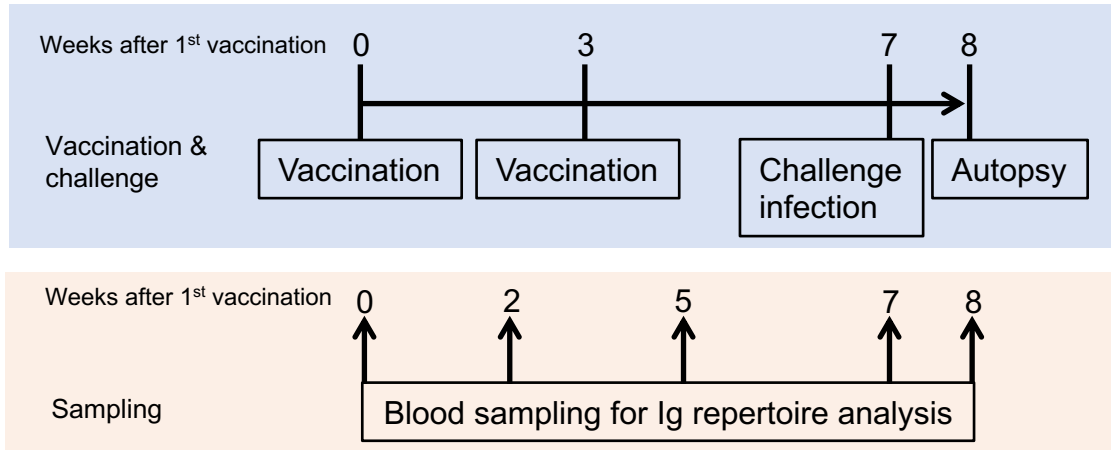
Parameter	Degree of parameter	Possible score
Fever	Normal (< 39°C)	0
	Elevated temperature (39 - 40°C)	3
	High temperature (> 40°C)	5
Posture	Piloerection of body hair	1
	Decreased activity, decreasing normal behavior/Occasionally lying down, huddled, active when people in room	2
	Huddled on camera, active when people in room/Lying down, getting up when approached, using cage for support	3
	Huddled when people in room, shaking, toes and hands clenched/Lying down, not getting up when approached or prompted	5
Respiration	Increased or decreased; mild cough and clear nasal discharge	3
	Labored breathing through mouth; severe cough and severe nasal discharge	5
Appetite	Slightly decreased	1
	Decreased	2
	Severely decreased	5
Skin	Flushed appearance	2
	Visible rash	2
	Bleeding	5

Animals were monitored every day during the study to be clinically scored. Animals would be scheduled to be euthanized if their clinical scores reached 15 (humane endpoint).

Supplemental Table S2. Identification numbers of cynomolgus macaques used in the present study

Inactivation for WPV	Vaccines	Animal ID		
		WPV	SV	Saline/control
Formalin and β -propiolactone	H1N1	H1W1	H1S1	H1C1 H1C2 H1C3
		H1W2	H1S2	
		H1W3	H1S3	
β -propiolactone	H1N1	H1W4	H1S4	
		H1W5	H1S5	
		H1W6	H1S6	
β -propiolactone	B-Victoria	BW1	BS1	BC1
		BW2	BS2	BC2
		BW3	BS3	BC3

Schedule of the present study



Supplemental Figure S1. Schedule of the study

Cynomolgus macaques were subcutaneously immunized with the whole virus particle vaccine or with the split vaccine twice with a 3-week interval between injections. Four weeks after the 2nd vaccination, the challenge viruses were inoculated into the nostrils of the macaques.

Blood samples for Ig gene repertoire analysis were collected 5 times as indicated.

Supplemental Table S3. Number of sequence reads in total Ig genes and each Ig class

Vaccine	Monkey	Ig class	Weeks after 1 st vaccination ¹					Total read
			0	2	5	7	8	
Saline	H1C1	Total Ig	105969 ²	132352	20915	30401	34294	323931
		IgA	38323	53676	6973	12301	11287	122560
		IgG	34519	40454	2525	8694	3363	89555
		IgM	33127	38222	11417	9406	19644	111816
	H1C2	Total Ig	77508	152548	17729	53834	31682	333301
		IgA	44585	57667	8262	25414	12039	147967
		IgG	3523	54601	3347	13308	7001	81780
		IgM	29400	40280	6120	15112	12642	103554
	H1C3	Total Ig	114250	134684	19861	40186	30573	339554
		IgA	56088	55630	8095	18267	10202	148282
		IgG	30870	41266	1276	9342	7071	89825
		IgM	27292	37788	10490	12577	13300	101447
SV	H1S1	Total Ig	64003	111577	15358	55742	44104	290784
		IgA	23912	38432	5459	20358	16162	104323
		IgG	20170	34520	4409	13147	10908	83154
		IgM	19921	38625	5490	22237	17034	103307
	H1S2	Total Ig	95461	143130	22970	32526	26607	320694
		IgA	37438	54379	7332	14168	11508	124825
		IgG	25763	40947	6914	5218	1303	80145
		IgM	32260	47804	8724	13140	13796	115724
	H1S3	Total Ig	54206	123090	26239	31847	11243	246625
		IgA	10360	57978	10463	14161	3722	96684
		IgG	7337	25180	5542	2327	791	41177
		IgM	36509	39932	10234	15359	6730	108764
H1W1	Total Ig	45236	119018	34755	57944	44794	301747	
	IgA	8328	52033	11783	21314	19486	112944	
	IgG	11593	31488	12284	18055	10334	83754	
	IgM	25315	35497	10688	18575	14974	105049	
H1W2	Total Ig	33083	138234	25451	19575	18144	234487	
	IgA	7186	57664	10058	8834	7539	91281	
	IgG	3751	35272	5594	267	1955	46839	
	IgM	22146	45298	9799	10474	8650	96367	
H1W3	Total Ig	31564	132350	33692	31982	30621	260209	
	IgA	3410	53497	10456	15189	12732	95284	
	IgG	3795	38695	14923	5008	7377	69798	
	IgM	24359	40158	8313	11785	10512	95127	

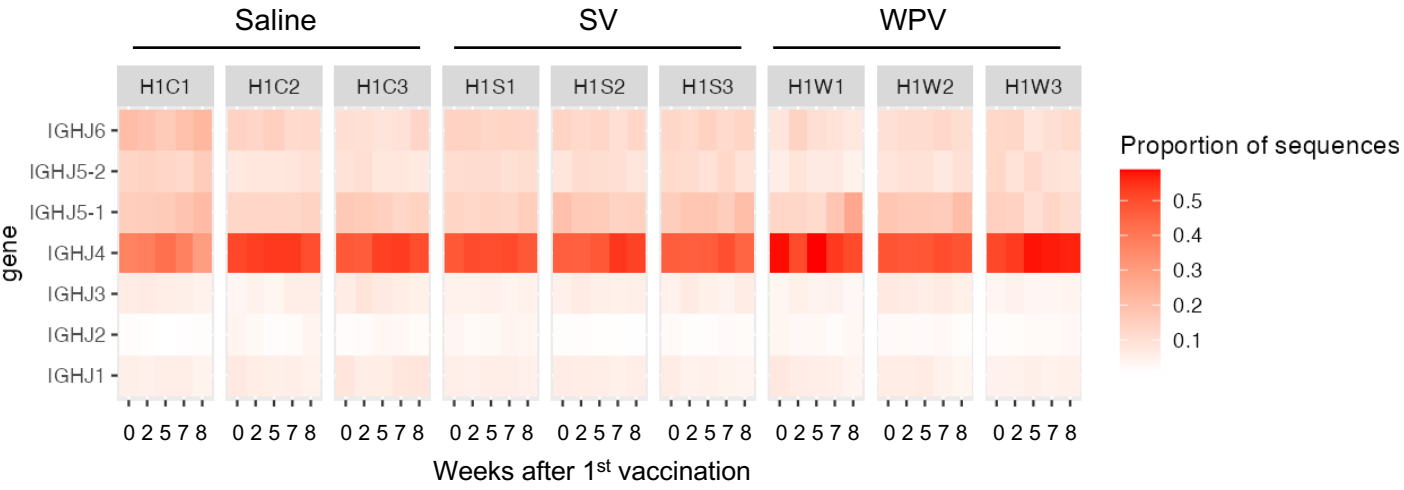
¹ The number of sequence reads in each sample collected at the indicated weeks before and after the 1st vaccination.

² The numbers of reads in the total Ig heavy chain genes are the sums of the read numbers of IgA, IgG, and IgM.

Supplemental Figure S2. IGVH family usage frequency

The Ig heavy chain gene sequences shown in Supplemental Table S3 were compared with germline IgVH gene segments, and the percentages of IgVH gene usage in the total Ig gene reads were calculated. The IGHV4S7 gene fragment was the most frequently identified (up to approximately 40%). Gray squares mean no reads in the indicated IgVH genes in the samples.

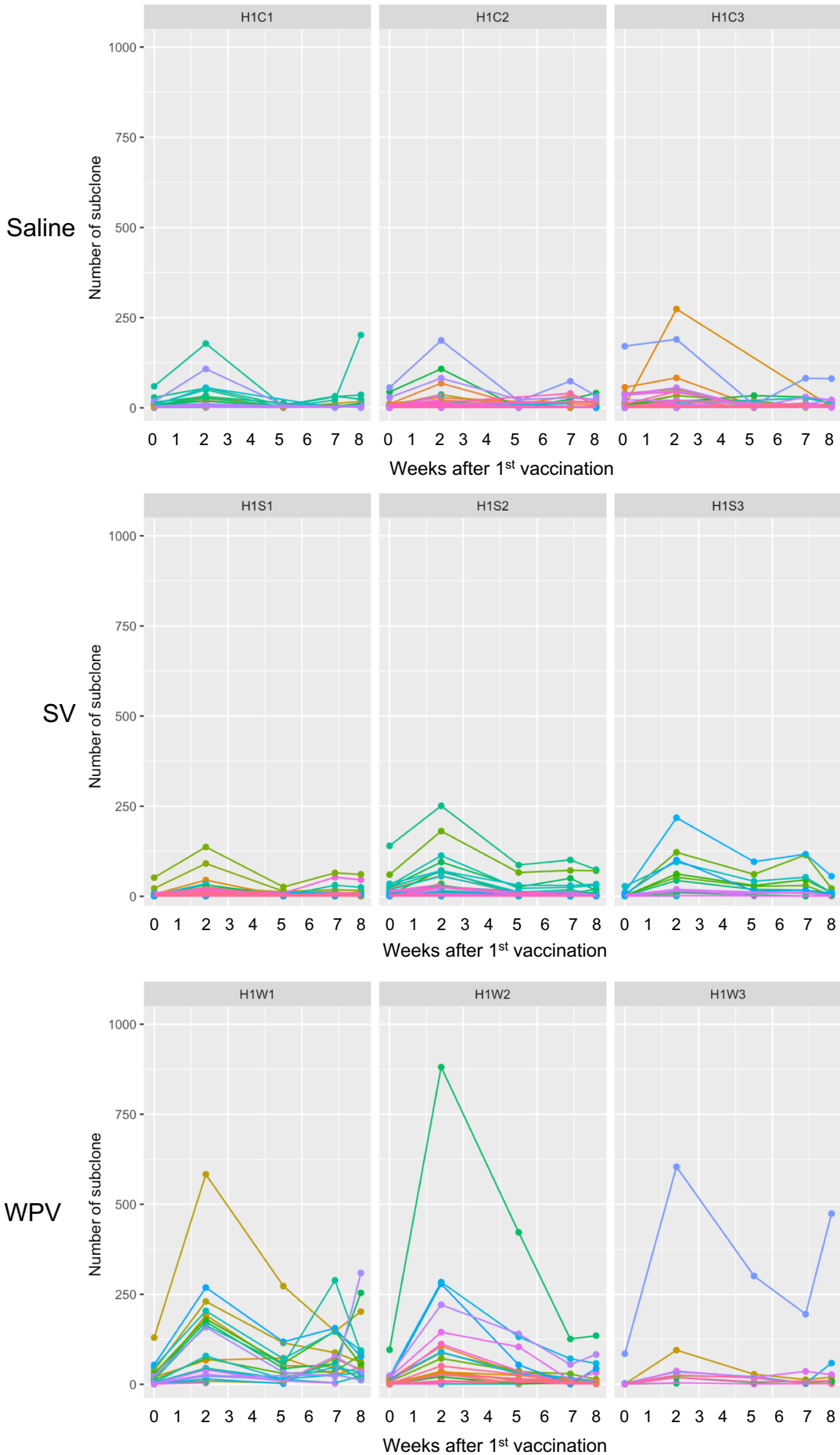
Supplemental Figure S3. IGJH Family usage frequency



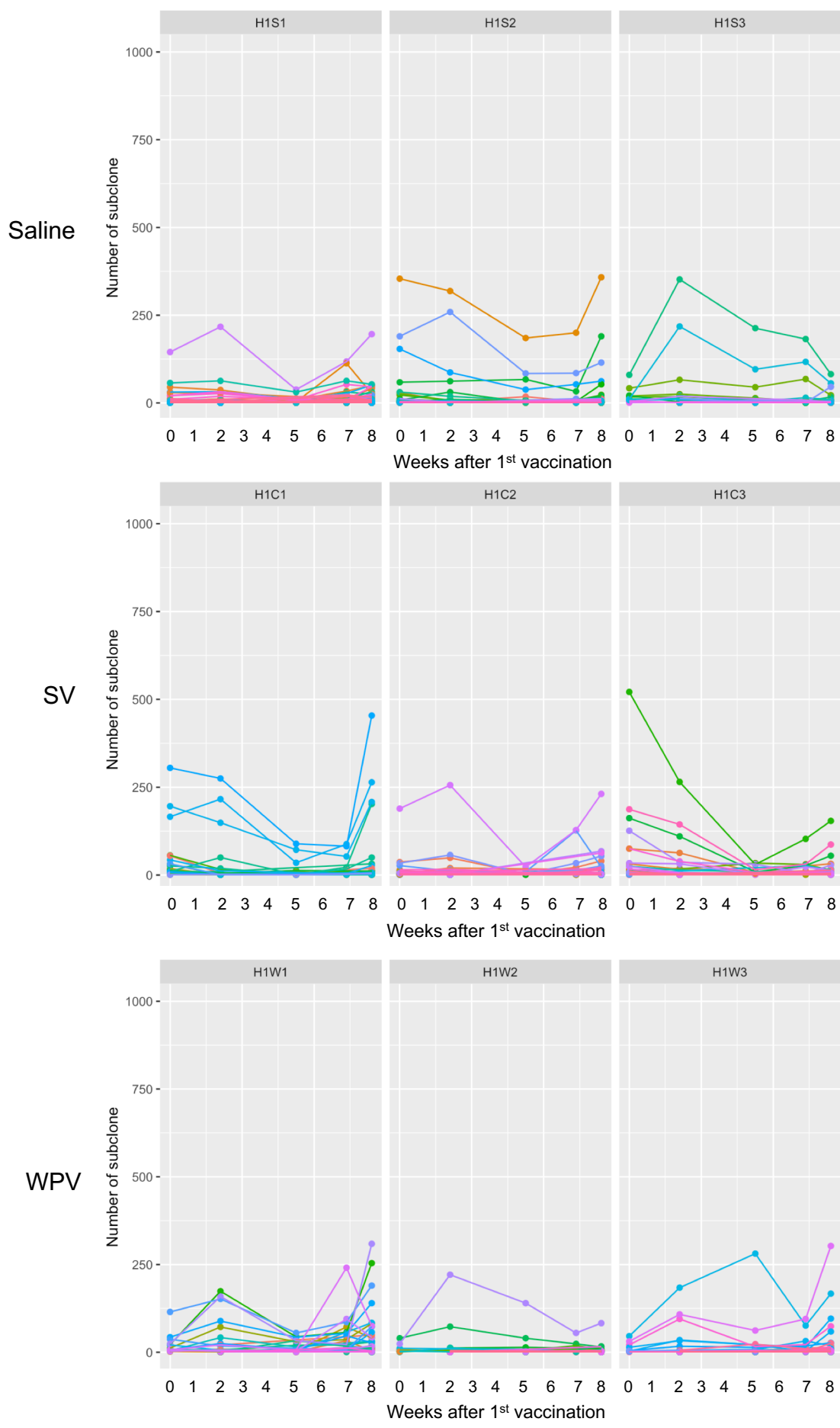
Supplemental Figure S3. IGJH family usage frequency

The Ig heavy chain gene sequences shown in Supplemental Table S3 were compared with germline IgJH gene segments, and the percentages of IgJH gene usage in the total Ig gene reads were calculated. The IGHJ4 gene fragment was the most frequently identified (up to approximately 60%).

A Clones increasing 2 weeks after the 1st vaccination (week 0 to week 2)



B Clones increasing 1 week after the challenge infection (week 7 to week 8)



Supplemental Figure S4. Number of subclones in B-lymphocyte clones responding to the 1st vaccination and challenge infection in individual macaques

Peripheral blood cells of cynomolgus macaques were collected at the indicated weeks after the 1st vaccination. The Ig heavy genes were amplified and sequenced. After the differentiation of clones by CDR3 sequences, subclones were identified by the differences in amino acids. Each line indicates the number of subclones in individual clones at the indicated weeks. The numbers of subclones in each clone that increased (A) from week 0 to week 2 and (B) from week 7 to week 8 in individual macaques are shown.

Supplemental Table S4. Average subclone numbers in each B-lymphocyte clone with an increase of more than 2-fold from week 0 to week 2

Vaccine	Monkey	Average subclone numbers in each B-lymphocyte clone ¹							
		Weeks after 1 st vaccination					Average subclone number ratio week 2/ week 0	Average ratio ³	SD
		0	2	5	7	8			
Saline	H1C1	6.3	21.8	3.9	8.9	25.0	3.4	3.0	0.71
	H1C2	2.9	9.8	4.8	7.9	8.2	3.3		
	H1C3	5.1	11.0	6.7	15.2	12.5	2.2		
SV	H1S1	2.4	8.2	2.5	5.7	5.5	3.4	5.8	4.6
	H1S2	4.8	14.0	7.0	8.8	8.3	2.9		
	H1S3	4.3	47.9	28.6	34.8	11.9	11.1		
WPV	H1W1	19.9	118.7 ²	51.5	78.1	82.0	6.0	8.3	2.2
	H1W2	10.8	110.1 ²	54.1	22.0	21.6	10.2		
	H1W3	8.9	79.0 ²	46.7	37.3	68.0	8.9		

¹ Ig clones were determined as described in Table 1. Ig subclones were differentiated by the Ig sequences with different amino acid sequences within clones with identical V and J genes and more than 90% similarity. The subclone numbers were determined as the number of different sequences in each clone.

² The average numbers of subclones in the B-lymphocytes of macaques vaccinated with WPV at week 2 were significantly higher than those of subclones in the B-lymphocytes of macaques vaccinated with WPV at week 0 ($P = 0.01$, Student's *t*-test).

³ A significant difference was detected in the average subclone number ratio of week 2/week 0 between the saline and WPV groups ($P = 0.014$, Student's *t*-test).