

Figure S1. Heat maps of KRCV-1-infected crab-eating macaques

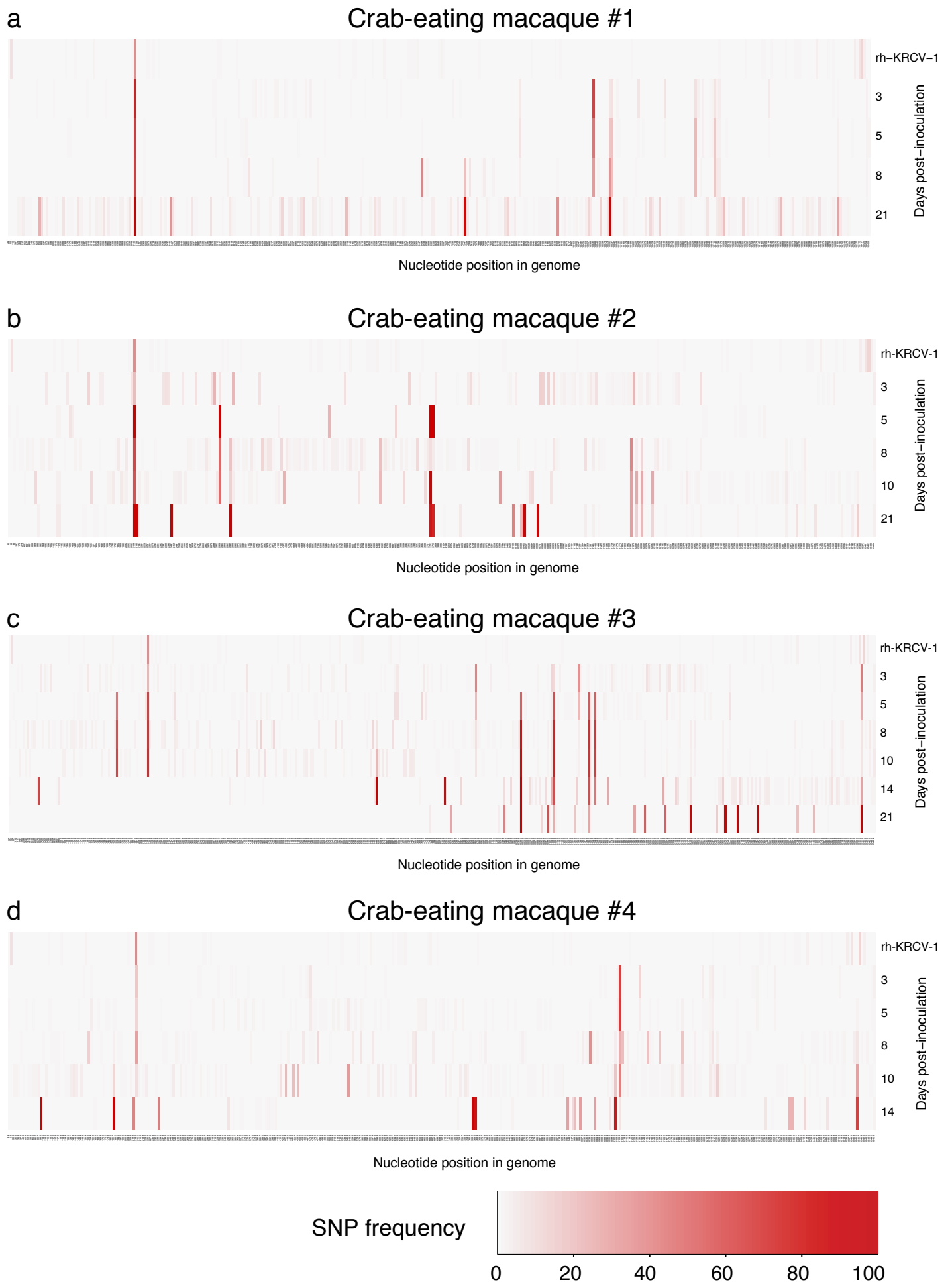


Fig. S1 Heat maps of KRCV-1-infected crab-eating macaques. All SNPs detected in $\geq 1\%$ of sequencing reads for each isolate are depicted in a heatmap. The frequency of the SNP is shown by its color, going from gray (low frequency) to red (100% frequency).

Figure S2. Heat maps of SHFV-infected crab-eating macaques

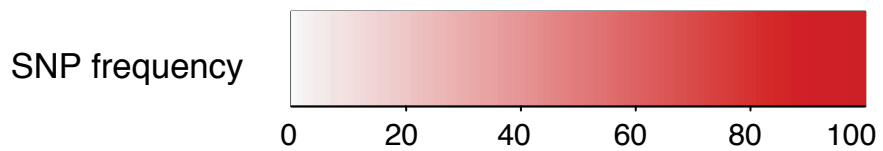
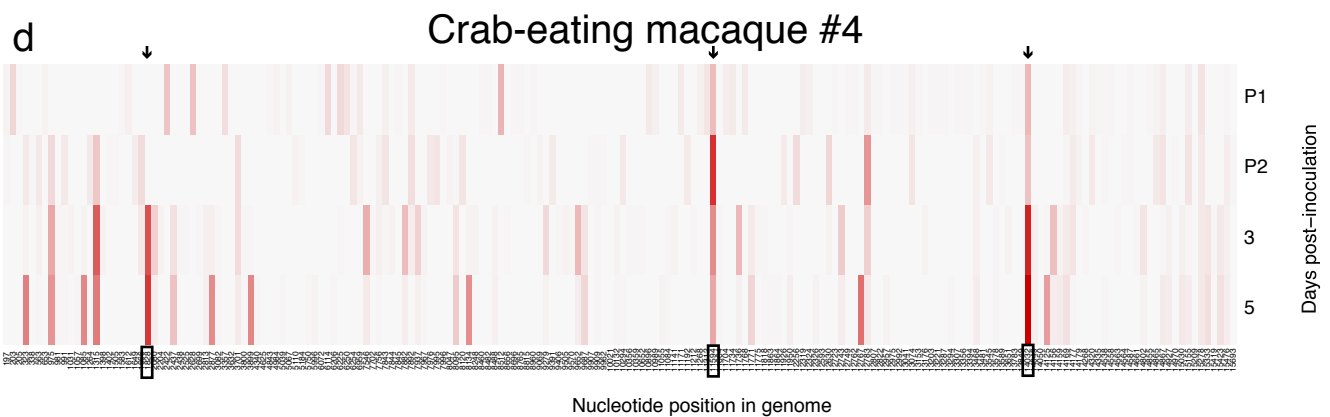
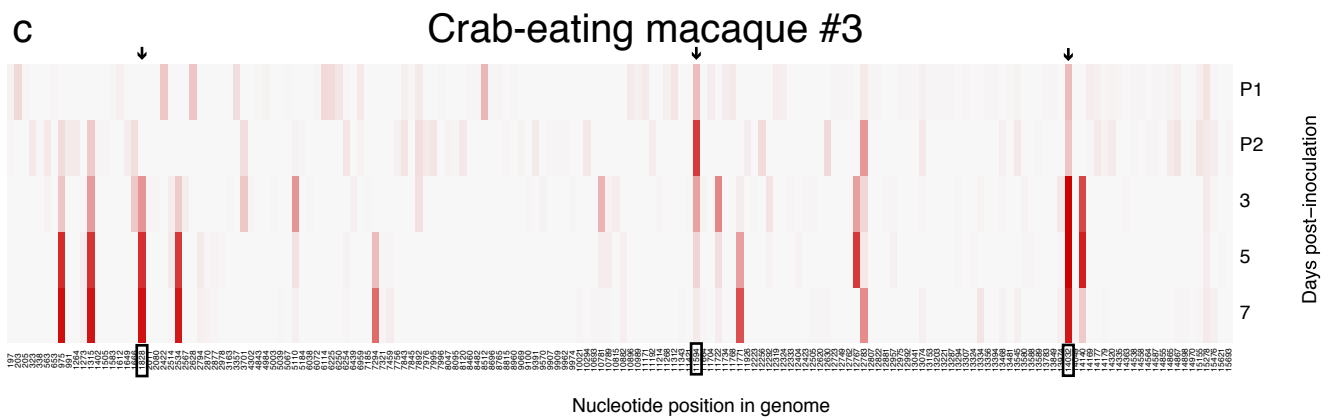
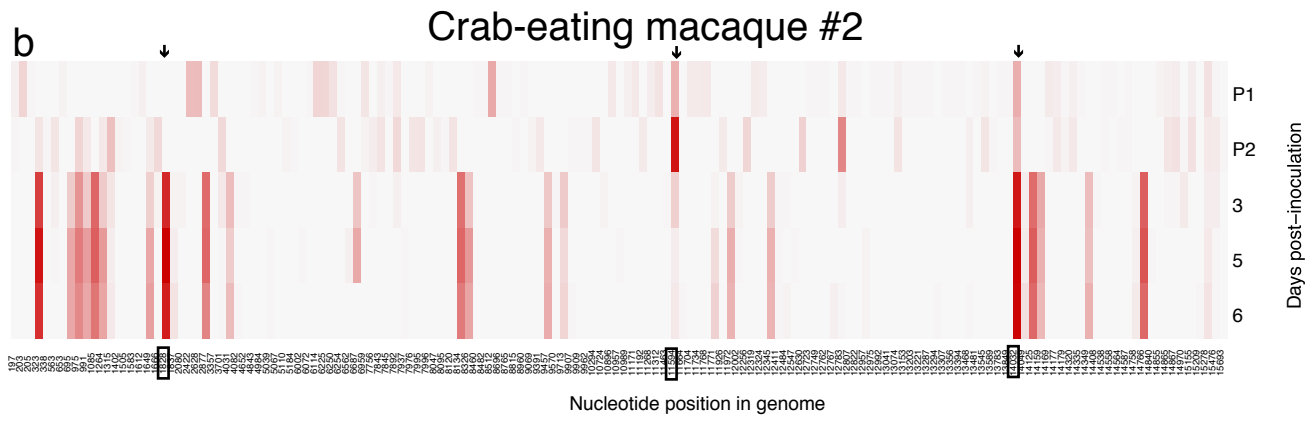
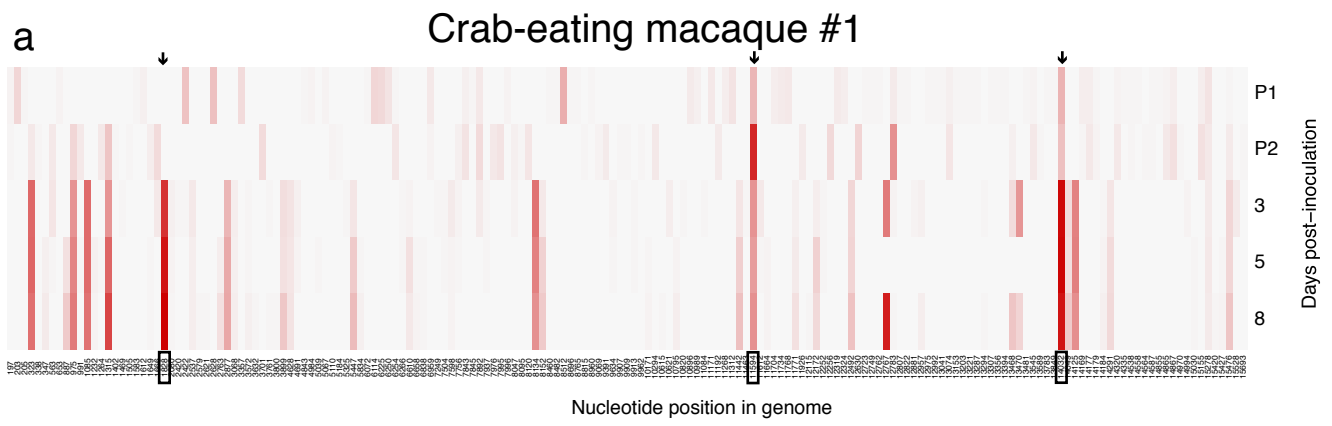


Figure S2. Heat maps of SHFV-infected crab-eating macaques

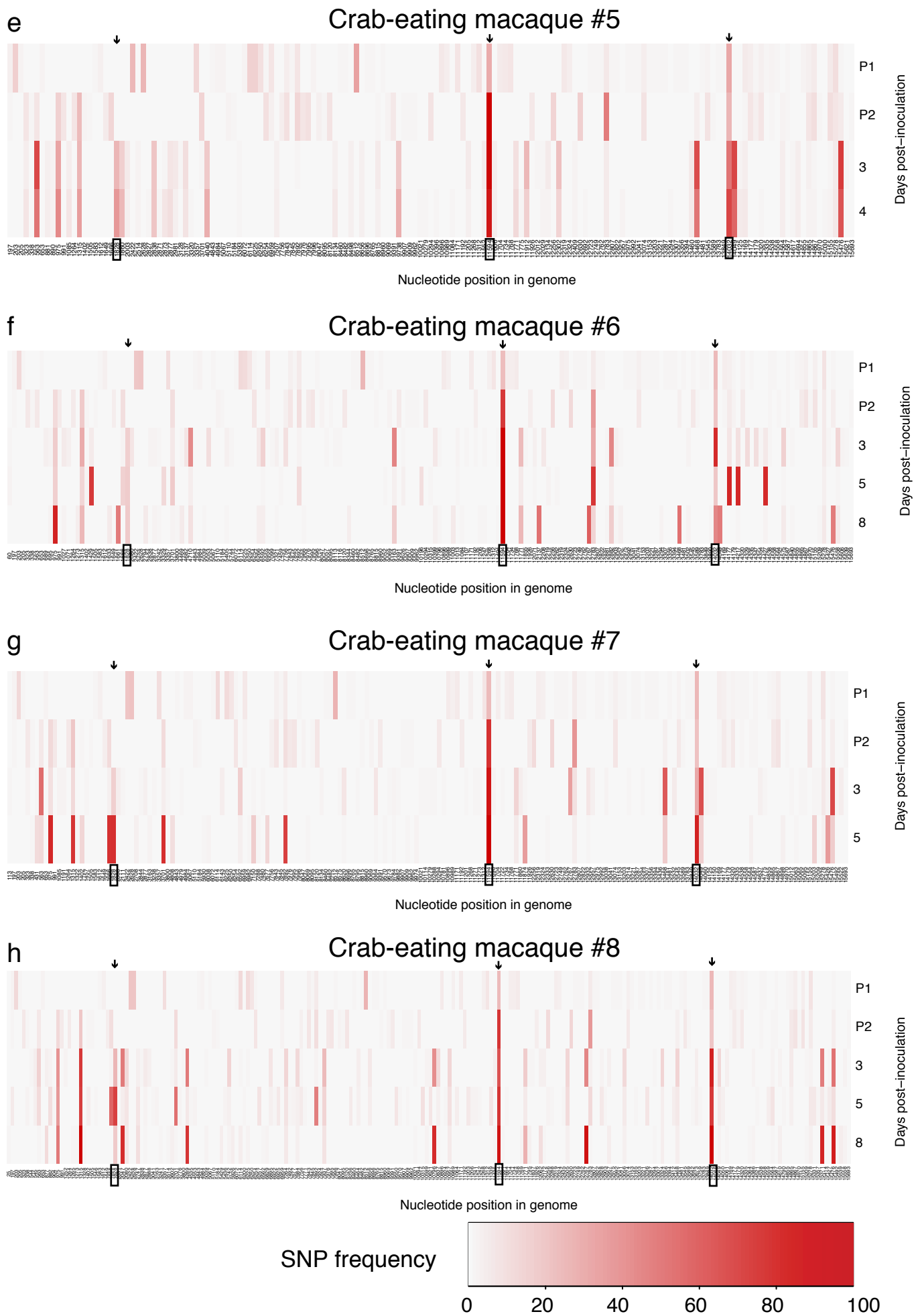


Fig. S2 Heat maps of SHFV-infected crab-eating macaques. All SNPs detected in $\geq 1\%$ of sequencing reads for each isolate are depicted in a heatmap. The frequency of the SNP is shown by its color, going from gray (low frequency) to red (100% frequency). Arrows above the plots and boxes around the polymorphic site identity indicate the 3 SNPs whose trajectories were plotted in Fig. 6 d,e and f.

Table S1. Comparison of nucleotide diversity among ORFs for KRCV-1-infected crab-eating macaques

All KRCV-1-infected crab-eating macaques and timepoints were combined for these analyses. π values for each ORF were first compared to every other ORF using a 1-way ANOVA, which showed strong statistical support that diversity was different among ORFs ($F = 10.18$, $P < 0.0001$). Following this result, pairwise comparisons were performed among every possible pair of ORFs using Tukey's multiple comparisons test to correct for multiple comparisons. These analyses were performed for all data using original assemblies as well as for assemblies subsampled to 1000x coverage (the results obtained from subsampled assemblies are shown in parentheses in each cell). The results of this test are shown here. ORFs 2a' and 4' were more diverse than most other ORFs. Blank cell = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

	1a	1b	2a	2a'	2b	3	3'	4	4'	5	5a	6	7
1a													
1b													
2a													
2a'	** (**)	**** (****)	**** (****)										
2b				*** (****)									
3				*** (****)									
3'		* (*)	* (*)			(*)							
4				** (***)									
4'	**** (****)	**** (****)	**** (****)		**** (****)	**** (****)	* (*)	**** (*)					
5				*** (****)			(*)		**** (****)				
5a				*** (***)					**** (****)				
6				**** (****)			* (**)		**** (****)				
7				* (*)					**** (****)				

Table S2. Comparison of nucleotide diversity among ORFs for KRCV-1-infected crab-eating macaque #1

All timepoints were combined for these analyses. π values for each ORF were first compared to every other ORF using a 1-way ANOVA, which showed strong statistical support that diversity was different among ORFs ($F = 11.35$, $P < 0.0001$). Following this result, pairwise comparisons were performed among every possible pair of ORFs using Tukey's multiple comparisons test to correct for multiple comparisons. The results of this test are shown here. These analyses were performed using original assemblies as well as for assemblies subsampled to 1000x coverage (the results obtained from subsampled assemblies are shown in parentheses in each cell). ORFs 2a' and 4' were more diverse than most other ORFs. Blank cell = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

	1a	1b	2a	2a'	2b	3	3'	4'	4	5	5a	6	7
1a													
1b													
2a													
2a'	**** (****)	**** (****)	**** (****)										
2b				**** (****)									
3				**** (****)									
3'				** (**)									
4'	**** (****)	**** (****)	**** (****)		**** (****)	**** (****)	*** (**)						
4				*** (**)				**** (****)					
5				**** (****)				**** (****)					
5a				**** (****)				**** (****)					
6				**** (****)				**** (****)					
7				** (*)				*** (**)					

Table S3. Comparison of nucleotide diversity among ORFs for KRCV-1-infected crab-eating macaque #2

All timepoints were combined for this analysis. π values for each ORF were first compared to every other ORF using a 1-way ANOVA, which showed strong statistical support that diversity was different among ORFs ($F = 3.609$, $P = 0.0004$). Following this result, pairwise comparisons were performed among every possible pair of ORFs using Tukey's multiple comparisons test to correct for multiple comparisons. The results of this test are shown here. These analyses were performed using original assemblies as well as for assemblies subsampled to 1000x coverage (the results obtained from subsampled assemblies are shown in parentheses in each cell). ORF 4' was more diverse than all other ORFs.

Blank cell = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

	1a	1b	2a	2a'	2b	3	3'	4'	4	5	5a	6	7
1a													
1b													
2a													
2a'													
2b													
3													
3'													
4'	** (*)	**** (***)	*** (**)	* (**)	*** (**)	*** (**)	** (*)						
4								*** (**)					
5								*** (**)					
5a								** (**)					
6								*** (***)					
7								** (**)					

Table S4. Comparison of nucleotide diversity among ORFs for KRCV-1-infected crab-eating macaque #3

All timepoints were combined for this analysis. π values for each ORF were first compared to every other ORF using a 1-way ANOVA, which showed strong statistical support that diversity was different among ORFs ($F = 13.42$, $P < 0.0001$). Following this result, pairwise comparisons were performed among every possible pair of ORFs using Tukey's multiple comparisons test to correct for multiple comparisons. The results of this test are shown here. These analyses were performed using original assemblies as well as for assemblies subsampled to 1000x coverage (the results obtained from subsampled assemblies are shown in parentheses in each cell). ORFs 2a', 3' and 4' were more diverse than many other ORFs. Blank cell = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

	1a	1b	2a	2a'	2b	3	3'	4'	4	5	5a	6	7
1a													
1b													
2a													
2a'	*	**	**										
	(*)	(**)	(*)										
2b	****	****	****										
	(****)	(****)											
3				**									
				(**)									
3'	***	****	****		**	****							
	(**)	(****)	(***)			(****)							
4'	*	**	**			**							
	(*)	(**)	(*)			(**)							
4				**			****	*					
				(*)			(****)	(**)					
5				**			****	**					
				(**)			(****)	(**)					
5a				***			****	***					
				(**)			(****)	(**)					
6				**			****	**					
				(**)			(****)	(**)					
7				*			***						
							(**)						

Table S5. Estimates of π N and π S from assemblies subsampled to 1000x coverage

KRCV-1, Kibale red colobus virus 1; SHFV, Simian hemorrhagic fever virus; π , nucleotide diversity; ORF, open reading frame; sd, standard deviation. To ensure that uneven genome coverage did not influence the signatures of selection we detected, we calculated estimates of π N and π S using assembly files subsampled to 1000x coverage. This subsampling approach was implemented in Popoolation version 1.2.2 using the subsample-pileup.pl script and results in even coverage at every position in the genome. All timepoints and animals are combined for this analysis. The mean π N, and π S values for each ORF are shown \pm the standard deviation. Statistical significance was assessed in R by performing a paired t-test against the null hypothesis that π N = π S. ORFs with π N/ π S > 1 are shown in red. Significance values are as follows. ns = not significant, * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001.

Virus	ORF	π N/ π S	π N \pm standard deviation	π S \pm standard deviation	significance
KRCV-1	1a	0.63	0.00133 \pm 0.000325	0.00210 \pm 0.000822	***
KRCV-1	1b	0.58	0.00134 \pm 0.000383	0.00230 \pm 0.000707	****
KRCV-1	2a'	1.50	0.00299 \pm 0.00147	0.00200 \pm 0.00121	*
KRCV-1	3'	0.56	0.00193 \pm 0.000820	0.00345 \pm 0.00256	**
KRCV-1	4'	0.91	0.00273 \pm 0.00118	0.00301 \pm 0.00226	ns
KRCV-1	2a	2.62	0.00418 \pm 0.00543	0.00159 \pm 0.000991	ns
KRCV-1	2b	0.53	0.00138 \pm 0.000532	0.00259 \pm 0.00173	**
KRCV-1	3	0.67	0.00127 \pm 0.000376	0.00189 \pm 0.000757	***
KRCV-1	4	0.63	0.00145 \pm 0.000463	0.00230 \pm 0.00173	*
KRCV-1	5a	0.68	0.00143 \pm 0.000191	0.00211 \pm 0.000463	***
KRCV-1	5	0.71	0.00143 \pm 0.000363	0.00202 \pm 0.000697	**

KRCV-1	6	0.54	0.00119 ± 0.000312	0.00221 ± 0.000547	****
KRCV-1	7	0.955	0.00191 ± 0.000833	0.00200 ± 0.000610	ns
SHFV	1a	0.57	0.00162 ± 0.000103	0.00283 ± 0.000412	****
SHFV	1b	0.44	0.00132 ± 0.000103	0.00302 ± 0.000516	****
SHFV	2a'	0.63	0.00170 ± 0.000632	0.00271 ± 0.00124	**
SHFV	2b'	0.66	0.00150 ± 0.000329	0.00226 ± 0.000751	****
SHFV	3'	0.68	0.00188 ± 0.00119	0.00276 ± 0.000892	*
SHFV	4'	0.59	0.00183 ± 0.000428	0.00310 ± 0.00140	***
SHFV	2a	0.37	0.00133 ± 0.000204	0.00359 ± 0.00179	****
SHFV	2b	0.58	0.00144 ± 0.000616	0.00247 ± 0.00109	**
SHFV	3	0.67	0.00159 ± 0.000360	0.00238 ± 0.000897	***
SHFV	4	0.68	0.00140 ± 0.000232	0.00207 ± 0.000629	****
SHFV	5a	1.14	0.00526 ± 0.00213	0.00463 ± 0.003631	ns
SHFV	5	0.46	0.00155 ± 0.000278	0.00335 ± 0.00143	****
SHFV	6	0.40	0.00139 ± 0.000200	0.00349 ± 0.000959	****
SHFV	7	0.79	0.00272 ± 0.00134	0.00345 ± 0.00189	ns

Table S6. Comparison of nucleotide diversity among ORFs for SHFV-infected crab-eating macaques

All SHFV-infected crab-eating macaques and timepoints were combined for this analysis. π values for each ORF were first compared to each other using a 1-way ANOVA, which showed strong statistical support that diversity was different among ORFs ($F = 46.2$, $P < 0.0001$). Following this result, pairwise comparisons were performed among every possible pair of ORFs using Tukey's multiple comparisons test to correct for multiple comparisons. The results of this test are shown here. These analyses were performed for all data using original assemblies as well as for assemblies subsampled to 1000x coverage (the results obtained from subsampled assemblies are shown in parentheses in each cell). ORF 5a is the only ORF exhibiting nucleotide diversity that is statistically significantly higher than every other ORF. Blank cell = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

	1a	1b	2a	2a'	2b	2b'	3	3'	4	4'	5	5a	6	7
1a														
1b														
2a														
2a'		** (**)												
2b	**** (****)	**** (****)	* (**)											
2b'					**** (****)									
3					*** (***)									
3'	** (**)	**** (****)				** (**)	* (**)							
4					*** (***)			** (**)						
4'					** (**)			* (*)						
5	** (**)	**** (****)				** (**)	* (*)		** (*)					
5a	**** (****)	**** (****)	**** (****)	**** (****)	**** (****)	**** (****)	**** (****)	**** (****)	**** (****)	**** (****)	**** (****)			
6					**** (****)			*** (***)		(***)	*** (***)	**** (****)		
7	* (**)	**** (****)				* (*)			* (*)			**** (****)	** (**)	

Table S7: Primer sequences used to amplify KRCV-1 and SHFV

KRCV-1, Kibale red colobus virus 1; SHFV, simian hemorrhagic fever virus. The binding site refers to the start site of the primer with regard to the rh-KRCV-1 and SHFV p1 reference sequences.

Virus	Binding site	Directions	Sequence
KRCV-1	27	forward	TATTGTGCTATCACTTCTTGTGG
KRCV-1	1677	reverse	TTAGGCACAGTCACGTCAGG
KRCV-1	1576	forward	TGATTTCAACTTTGGGTTTGG
KRCV-1	3245	reverse	GGATACAAGGACGCAAGCAT
KRCV-1	3120	forward	ATTTTTGGGGTGCTGTGGTA
KRCV-1	4770	reverse	GTTCCACGAAGCCTGACT
KRCV-1	4676	forward	AAGGGAGCTTACCCACCTA
KRCV-1	6342	reverse	GCCAGAAGCGATTAGGAGTTT
KRCV-1	6223	forward	CATGGCATTGAATGTGTCAAC
KRCV-1	7871	reverse	GGCTCATTGAGAAGGACCAA
KRCV-1	750	forward	GCCTTCAA AATTGGTCATCC
KRCV-1	9426	reverse	AGTGCCGGGAAGGTCTGTAG
KRCV-1	9323	forward	CCATTGTCGCCATCACTAGA
KRCV-1	10987	reverse	AGTGGCCGGAGGTGTAATAA
KRCV-1	10872	forward	GATGCTGGAGGTTTTTCATCG
KRCV-1	12519	reverse	ATATAGCCCAATGGCGTAG

KRCV-1	12396	forward	GCTGCCTTTCACCATGCTAT
KRCV-1	14133	reverse	AGCCCAGTTACGTTGTGGAG
KRCV-1	13807	forward	GGCGCATTCTAGATTCTTTT
KRCV-1	15466	reverse	CATTTGGTTAATTCACCTATGCAC
SHFV	16	forward	GTGAAGCTCCCTGTGCTTTC
SHFV	1724	reverse	CAAGTCAGGGGTCGTTCTTC
SHFV	1573	forward	TGTTTGTAACGCGTCTCACG
SHFV	3268	reverse	GCCACCGAGACAACAAAA
SHFV	3128	forward	CGACAATGATGACGCTGACT
SHFV	4840	reverse	AACTTCAGTGGAGGGAATGC
SHFV	4669	forward	GTTTGTTTTCCGATGGCAAC
SHFV	6374	reverse	TTCTGGCATGATGATGTCGT
SHFV	6226	forward	TGGAAAATGATGGTCGTTTC
SHFV	7931	reverse	TGTGCTGGACATAGAGAACCA
SHFV	7761	forward	TTGAGATGGCTTGTGCTGAG
SHFV	9469	reverse	CGACCCTTGAGAGGAGTCAA
SHFV	9309	forward	ACGCAATTCAGCCCTGTTAC
SHFV	11040	reverse	TGAAGGAGGAGGAGAAGTGAA
SHFV	10877	forward	CCTACAGCCTGTCACTGTAAGGT
SHFV	12587	reverse	GGCTGTAGTGATGTGGGTGA
SHFV	12426	forward	CTGGAACGGGACTGATCCTA
SHFV	14120	reverse	CCGGTGCTGCTGAATGTAAT

SHFV	13975	forward	GCGACTCCGCTCCTTAACTA
SHFV	15655	reverse	AATTACCCATATGAACCATATAATCA

Table S8. vRNA copies/ml blood and estimated cDNA input copy number used for sequencing each sample.

For each sample sequenced in this study, the vRNA copies/ml blood (as plotted in Fig. 3a and 6a) are reported along side the estimated number of cDNA molecules that went into each PCR and were sequenced. The estimated cDNA copy number input reflects the number of cDNA molecules that were used as input for every individual amplicon PCR. These values are calculated based on the vRNA copies present after vRNA extraction (as quantified by qPCR, see Methods), the number of μl used for reverse transcription, and the μl of cDNA used in each PCR. The volume of cDNA used for each PCR was varied for each sample so as to add the maximum number of cDNA molecules for sequencing. NA indicates that the qPCR was unable to be performed successfully for that sample.

Virus	Animal #	Day	vRNA copies/ml blood	cDNA input copy number
KRCV-1	1	3	1.27×10^7	1.57×10^5
KRCV-1	1	5	1.34×10^8	1.68×10^6
KRCV-1	1	8	8.03×10^7	1.00×10^6
KRCV-1	1	10	3.26×10^6	4.07×10^4
KRCV-1	1	14	NA	NA
KRCV-1	1	21	3.37×10^4	8.43×10^2

KRCV-1	2	3	1.00×10^6	1.34×10^4
KRCV-1	2	5	1.29×10^5	1.61×10^3
KRCV-1	2	8	1.22×10^6	1.53×10^4
KRCV-1	2	10	1.72×10^5	2.16×10^3
KRCV-1	2	21	2.35×10^4	8.82×10^2
KRCV-1	3	3	5.32×10^5	1.33×10^4
KRCV-1	3	5	6.54×10^5	1.64×10^4
KRCV-1	3	8	2.11×10^5	5.26×10^3
KRCV-1	3	10	9.52×10^5	2.38×10^4
KRCV-1	3	14	7.36×10^4	1.83×10^3
KRCV-1	3	21	1.66×10^4	6.22×10^2
KRCV-1	4	3	1.03×10^7	1.28×10^5
KRCV-1	4	5	1.07×10^7	1.33×10^5
KRCV-1	4	8	7.65×10^6	9.45×10^4
KRCV-1	4	14	2.67×10^4	6.66×10^4
SHFV	5	3	6.72×10^8	2.35×10^5
SHFV	5	4	3.74×10^{10}	1.31×10^7
SHFV	6	3	1.90×10^9	6.65×10^5
SHFV	6	5	4.94×10^7	1.73×10^4
SHFV	6	8	1.84×10^9	6.44×10^5
SHFV	7	3	2.42×10^{10}	8.47×10^6
SHFV	7	5	6.53×10^8	2.29×10^5

SHFV	8	3	3.34×10^9	1.17×10^6
SHFV	8	5	9.06×10^7	3.17×10^4
SHFV	8	8	1.17×10^{10}	4.10×10^6
SHFV	1	3	7.97×10^8	2.79×10^5
SHFV	1	5	2.24×10^{10}	7.84×10^6
SHFV	1	6	1.18×10^{10}	4.13×10^6
SHFV	2	3	5.66×10^7	1.98×10^4
SHFV	2	5	1.59×10^9	5.57×10^5
SHFV	2	6	1.50×10^9	5.25×10^5
SHFV	3	3	2.91×10^7	1.02×10^4
SHFV	3	5	6.77×10^8	2.37×10^5
SHFV	3	7	2.08×10^9	7.28×10^5
SHFV	4	3	8.82×10^8	3.09×10^5
SHFV	4	5	3.14×10^{10}	1.10×10^7
SHFV	4	6	2.67×10^{10}	9.35×10^6