# Supplementary Tables and Figures

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## Supplementary tables

Table S1: X-ray crystallography data collection and refinement statistics.

	SFV GII RBD <sup>D</sup> (native) data	<b>RBD<sup>D</sup> (derivative) data</b>	SFV GII RBD <sup>G</sup> (native) data
	(PBD 8AEZ)		(PBD 8AIC)
Data collection			
Wavelength	0.9786	1.907	0.9786
Space group	P3 <sub>2</sub> 21	P3121	P61
Cell dimensions			
<i>a, b, c</i> (Å)	99.5, 99.5, 120.6	99.6, 99.6, 120.9	123.6, 123.6, 191.6
a, b, g (°)	90, 90, 120	90, 90, 120	90, 90, 120
Resolution range (Å)	49.73 - 2.574 (2.666 - 2.574) ª	46.06 - 3.171 (3.284 - 3.171)	46.73 - 2.8 (2.9 - 2.8)
Total reflections	453419 (44048)	238580 (24149)	875971 (87278)
Unique reflections	22363 (2191)	12192 (1165)	40619 (4041)
Completeness (%)	99.91 (99.50)	99.60 (96.75)	99.58 (99.14)
Redundancy	20.3 (20.1)	19.6 (20.1)	21.6 (21.6)
R <sub>merge</sub>	0.2051 (0.9362)	0.1776 (0.9898)	0.199 (1.705)
R <sub>pim</sub>	0.04701 (0.2117)	0.04098 (0.2256)	0.04364 (0.3719)
I/s (/)	10.10 (1.85)	20.30 (5.19)	13.35 (1.45)
CC <sub>1/2</sub>	0.986 (0.846)	0.998 (0.917)	0.997 (0.689)
Refinement			
No. reflections	22355 (2190)	/	40615 (4040)
No. of reflections for <i>R</i> <sub>free</sub> <sup>b</sup>	1052 (110)	/	2111 (208)
Rwork/Rfree	0.213/0.253	/	0.194/0.229
No. non-hydrogen atoms	2945	/	6086
Macromolecules	2711	/	5448
Ligands	203	/	531
Water	93	/	321
Mean B value (Ų)			
Protein and sugar	73.88	/	65.44
Ligand/Ion	94.16	/	114.49
Water	60.68	/	55.18
R.m.s. deviations			
Bond lengths (Å)	0.012	/	0.004
Bond angles (°)	1.62	/	0.77
Ramachandran	95.99/0.31	/	95.69/0.00
favored/outliers (%)			

<sup>a</sup> Values in parentheses are for highest-resolution shell.
 <sup>b</sup> The free set represents a random 5% of reflections not included in refinement

Domain	Helical <sup>†</sup> (%)	β-strands (%)	Other <sup>‡</sup> (%)
RBD	30	14	56
Lower	45	17	38
Upper	20	12	68

† -  $\alpha\text{-}$  and 310 helices

‡ - B, S, T, X

The secondary structure content was calculated using 2StrucCompare webserver <sup>1</sup> at https://2struccompare.cryst.bbk.ac.uk/index.php.

### Table S3: Intramolecular interactions within GII RBD

The intramolecular interactions were analyzed by ProteinTools program <u>https://proteintools.uni-bayreuth.de</u><sup>2</sup>.

Van der V	Van der Waals contacts in the SFV RBD									
Cluster #	<sup>•</sup> # Area (Å <sup>2</sup> ) # of residues Location (dom									
1	153	4	Lower							
2	1002	22	Lower							
3	2543	51	Lower + upper							
4	82	2								
5	77	2	Upper							
6	86	2								

Polar co	ontacts in the SFV RBD	
Lower s	ubdomain	
Cluster	Donor - Acceptor	Distance (Å)
1	HIS225-ND1 GLN222-OE1	3.3
2	ARG226-NH1 GLU337-OE1	3.1
2	ARG226-NH2 GLU337-OE2	2.4
3	HIS234-ND1 TYR323-OH	3.1
4	THR242-OG1 GLN492-OE1	3.4
5	HIS314-ND1 THR313-OG1	2.9
6	TYR327-OH ASP320-OD2	2.5
7	ASN331-ND2 ASN336-OD1	3.4
	LYS342-NZ GLU339-OE1	3.3
8	ARG343-NE GLU339-OE2	2.8
	ARG343-NH2 GLU339-OE2	3.2
0	ASN351-ND2 GLU502-OE1	2.5
9	TYR551-OH GLU502-OE2	2.5
10	LYS352-NZ GLU495-OE2	3.2
10	TYR497-OH GLU495-OE1	3.1
11	ASN368-ND2 ASN373-OD1	3.0

Upper subdomain							
Cluster	Donor - Acceptor	Distance (Å)					
10	GLN244-NE2 GLN491-OE1	2.6					
12	GLN491-NE2 SER488-OG	3.0					
13	TYR267-OH ASP468-OD2	2.9					
1.4	THR288-OG1 GLU442-OE1	2.9					
14	THR288-OG1 GLU442-OE2	3.4					

	TYR456-OH GLU442-OE2	3.2
	ARG297-NH1 ASP402-OD2	2.9
15	ARG297-NH2 ASP402-OD1	2.6
15	ARG297-NH2 ASP402-OD2	2.9
	SER397-OG ASP402-OD2	2.8
	ARG372-NH1 ASP378-OD2	2.8
16	ARG372-NH2 ASP378-OD1	2.9
16	ARG382-NH1 ASP378-OD1	2.8
	SER375-OG ASP378-OD2	2.9
17	TRP399-NE1 ASP254-OD1	2.7
18	THR406-OG1 ASN409-OD1	3.4
19	ARG407-NE GLU400-OE2	3.1
20	ARG433-NH2 – GLU464-OE2	2.7
21	TRP435-NE1 ASN462-OD1	3.0
22	ARG436-NH2 – ASP254-OD1	2.6
23	THR452-OG1 ASP450-OD1	2.9
24	SER461-OG GLU439-OE2	2.5
25	GLN482-NE2 SER473-OG	3.1
	ARG537-NE GLU384-OE1	3.0
	ARG537-NH1 TYR269-OH	3.4
26	ARG537-NH2 GLU384-OE2	2.8
20	ARG537-NH2 ASP274-OD2	2.8
	TYR269-OH ASP274-OD1	2.6
	TYR275-OH GLU384-OE2	2.6

## Supplementary figures



Figure S1: The fold of the FV RBD is maintained by hydrophobic and polar interactions

**Figure S1 legend:** The FV RBD core is formed by the hydrophobic residues grouped in 6 clusters - 2 in the lower subdomain (clusters #1 and #2), 3 in the upper subdomain (clusters #4, #5, #6), and the largest hydrophobic cluster (BSA=2451 Å<sup>2</sup> with 51 participating residues; cluster #3 shown in green) running in the direction of the longer axis of the RBD and containing residues from both domains. There are 24 networks of residues whose side chains contribute to 43 hydrogen bonds, with 21 charged residues forming 9 salt bridges. The area of the hydrophobic interfaces in the lower subdomain is about 6 times larger than in the upper subdomain, while the hydrogen bonds and salt bridges are more prevalent in the upper subdomain. The full list of intramolecular interactions and relevant details are given in Table S3. The intramolecular interactions were analyzed by ProteinTools program <a href="https://proteintools.uni-bayreuth.de">https://proteintools.uni-bayreuth.de</a> <sup>2</sup>.



Figure S2: Mobile loops decorate the apex of the RBD

**Figure S2 legend: A)** The upper subdomains loops are designated as follows: loop 1 (L1, residues 253-270, connecting  $\beta 2$  and  $\eta 1$ ) in blue, loop 2 (L2, residues 276-281, connecting  $\eta 1$  and  $\beta 3$ ) in orange, loop 3 (L3, residues 414-436, connecting  $\alpha 5$  and  $\beta 9$ ) in green, and loop 4 (L4, residues 446-453, connecting  $\beta 10$  and  $\beta 11$ ) in dark purple. **B)** The RBD structure is shown in 'tube' presentation to illustrate the mobility, with the more flexible regions shown as thicker tubes. Coloring scheme corresponds to the C<sub> $\alpha$ </sub> atomic B-factors (low to high B factors shown in blue to orange spectrum). The images were generated in Pymol <sup>3</sup>.



### Figure S3: Comparison of glycosylated vs deglycosylated RBD structures

**Figure S3 legend: A)** Schematic representation of SFV Env and the 17 predicted N-glycosylation sites, labeled as <u>N1</u> to <u>N15</u><sup>4</sup>. The sites that are 100% conserved in all FV Envs (Asn<sup>141</sup> (<u>N3</u>), Asn<sup>390</sup> (<u>N8</u>), Asn<sup>781</sup> (<u>N13</u>), Asn<sup>807</sup> (<u>N14</u>), Asn<sup>832</sup> (<u>N15</u>)) are indicated with red underscored numbers. The two furin sites are represented by scissors. LP, SU and TM are the abbreviations for the leader peptide, surface subunit and transmembrane subunit, respectively. The sugar residues, N-acetyl glucosamine (NAG) and mannose (MAN) that could be resolved in RBD<sup>D</sup> or RBD<sup>G</sup> are shown.

The N-linked oligosaccharide core is shown in the grey inlet, with the cleavage sites indicated for the EndoD and EndoH glycosidases. A fraction of proteins expressed in insect cells contains an  $\alpha$ 1-6 fucose bound to the first NAG, rendering the sugar sensitive to cleavage by EndoD, but resistant to EndoH. Thus, both EndoD and EndoH were used for deglycosylation of the recombinant RBD. The figure was created in Biorender.com.

**B)** Superposition of the RBD<sup>G</sup> (purple) and RBD<sup>D</sup> (grey) structures done in Pymol <sup>3</sup>. For clarity reasons, only polypeptide chains are displayed. The boxed region on the right structure is magnified in panel C)

**C)** The polypeptide chain (in wire model) and sugars <u>N7'</u>, <u>N8</u> and <u>N9</u> (shown as sticks) are displayed to illustrate absence of local changes induced by the sugars in RBD<sup>G</sup>. Only molecule A is shown.



## Figure S4: Comparison of the SFV RBD fold with that of the RBD of Orthoretroviruses

**Figure S4 legend:** Structures of RBDs from gammaretroviruses and of SU from HIV (lentivirus) are shown to illustrate a lack of structural homology between the RBDs from 3 different genera of retroviruses.





**Figure S5:** The buried surface area (BSA) for each sugar residue was calculated as a percent of the total surface area ( $Å^2$ ) in ePISA <sup>10</sup> and plotted. Sugars that establish hydrogen bonds with the amino acids are indicated with letter 'h'. Sugars 6 and 7 are colored in grey for RBD<sup>D</sup> because they were not resolved in the structure.

## Figure S6: Sequence conservation of FV Env

		LP <sup>1-126</sup>									
		1	10	20 🔥	N1 30	40	50	60	70	80	90
Simian	Gorilla GII Gorilla GI PFV Chimp CI GMonkey Macaque Marmoset Orangutan Bovine Equine Feline	MAP PMSL MAP PMSL MAP PMTL MAP PMTL MAP PMTL MAP PMTL MAP PMTL MAP PMTL MEQEH VMTL	QQWIIWNKM QQWIIWNKM QQWIIWNKM QQWIIWNKM QQWIIWKKM TEWIIWNKK QQWIWDRM QQWIQWRYN PEWMQWRYN KEWMEWNAH	HQAHQALQN HQAHQALQN NKAHEALQN NKAHEALQN SQAHQALEN RNMTIMTSNI QKANEALKS LETTNLLQM QNVNKIREA KQLQKLQSTI	TLVTEEQK STLVTEEQK TTVTEQQK STLVTEQQK STTVTDQQK SSLTEEQK TTLEQK TTLTEQK TTLTEQK TTLTEQK TAVSEEEK NPKME (PDV PEL	EQTILEIQNED EQTILEIQNED EQTILEIQNEE EQTILEIQNEE EQTILEIQNEE QQVIIDIQE QQVIIDIQE EHLILEIQNEE SVCLPDFDPPG QTDLPVYNPDM HVDIPEDIPLV	VIPTRMDRV VVPTRMDRV VVPTRNDKF VRPTRKDKI VVPTRMDRV VVPTRMDKL FIKPTIQQRL IIPTKVDRV DEEVS.LRIRC FKPLP.LCLRI PEKVP.LKMRM	KYFLYTCCAT KYFLYTCCAT RYLLYTCCAT KYLAYACCAT SYTCYLACAT KYLLYTCCAT KYLLYTCCAT KYWIYLCCAT KYWYLLCAT RYRCYTLCAT	SSRVLAWMILA SSRVLAWMILA SSRVLAWMILA SSRVLAWMLLV SSRVLCWIVLV STRVLCWIVLV TTRIMIWILFC TTRILAWLFLF STRIMAWIVFI SPRVMAWLLFV STRIMFWILFF	CILFIIIIVS CILFIIIVS CLLFIVVVS CVLLIVVVS CVLLIVVFS CVLLIVVFIS CVLLVVFIS CVLIVVFIS LTVLSILIS CVLSVMIA LLCFSIVTS	LISRIQWNKD CFITLSRIQWNKD CFUTISRIQWNKD CFUTISRIQWNRD CFUTVARIQWNRD CFUTNARIQWNRD CFTTAARIQWNRD CFTTAARIQWNAA CFITIARIQWNQD VLIAVFRLQWKGA VIVTVFRMQWKAA CFIISILRYQWKEA
				I P <sup>1-126</sup>	SU127-570						
		100	<≻N2	120	¥ 130		150	160	170	<sup>180</sup> <b>♦N4</b>	190
-	Gorilla GII Gorilla GI PFV Chimp CI GMonkey Macaque Marmoset <u>Orangutan</u> Bovine Equine Feline	IQVLGPVID IQVLGPVID IQVLGPVID IQVLGPVID IQVLGPVID IAVFGPVID IITPGPVID IQVYGPVID IESPCPILV IDVPGPVLF ITHPGPVLS	WINTQRAVY WINTRAV WINTRAV WINTRAV WINTQRAVY WINTRAV WINTRAV WINTRAV WINTRAV WINTRAV WINTRAV WINTRAV WINTRAV WINTRAV WINTRAV WINTRAV WINTRAV WINTRAV WINTRAV WINTAV WI	QPL.QLRRI QPL.QLRRI QPL.QTRRI QPL.QTRRI QQL.KAARI QQL.KAARI PAQQRARRI KPL.LMKRI QPT.TPPHY VPP.VGPHY MGG.NTSSS	RALRAQHP RALRAQHP RSLRMQHP RSLRMQHP RSIRVEHA RSIRVEHA RDL.IKVT RSIRTHYP SRLRRAIHL RLRRAVHL SRRRDIQY	VPKYVEVNMTS VPKYVEVNMTS VPKYVEVNMTS VPKYIEVNMTS HISYISINMSS TETYVEVNMTS ERYVEVNMTS AQKPVEVNMTS AQKPVQVNFTS ADRAVNINITH HKLPVEVNISG	IPQGVFYQPHP IPQGVFYQPHP IPQGVYYEPHP IPQGVYYEPHP IPQGVLYVPHP IPQGVILLPHP IPQGVILLPHP IPQGVILEPHP IPQGVFIEPFP IPQGVFIEPFP	EPIIHTERVL EPIIHTERVL EPIVVKERVL EPIVVTERVL EPIILKERVL EPIILKERIL KPIIQKNRVL KPIISKERVL KPIISKERVL KPIIDKERVL KPIFHKERTL	GLSQVLMINSE GLSQVLMINSE GLSQVLMINSE GLSQVLMINSE GLSQVLMINSE GLSQVLMINSE GLSQULLINSE GLSQVLMINSE GISQIVMIDSG GLSQVILIDSP	NVANSANISQI NVANSANISQI NIANNANITQI NIANNANITQI NIANVANISQI SIASIFNIKQI TVANSANITQI SIASIFNIKQI TUTQKINIEGI SIAQSMNIDI TITQGHIK	TKALLTEMVNEE TKALLTEMINEE VKKLLAEVVNEE TKVLLADMINEE TKVLLADMINEE HKSILTEIIQE AKVLLADMVNEE AKSLLIKTINEE (MKHLLVDMINEE QKAYLVSTINEE
X-ra	Gorilla GII Ay structure	0.0 2	1.0	RBD <sup>218-552</sup> 000000000	-	β1 2 4 0		L1	ηι 270	L2 β3 280 → TT	η2 222
- Feli	Gorilla GII Gorilla GI PFV Chimp CI GMonkey Macaque Marmoset Orangutan Bovine Equine Feline ne AF model	MQGISDVMI MQSLSDVMI MQSLSDVMI MQSLSDVMI LQDLSNQMI MNDLANQMI MNDLANQMI MRSLQDITL LQGLADVMI LISLQDVVL MVALSNVVL MEQLQKTVL	 DFEIPLGDP DFEIPLGDP DFEIPLGDP DFEIPLGDP DFEIPLGDP DFEIPLGDP MFDLFIGMP DFEIPLGDP PFELPVCDP PFDLPIKDP	RD QE QY I HRI RD QD QY I HRI RD QK QY QHI KT QHEY I QS I RD QD QY I HRI ST QD QY I HKI ST QD QY I HKI QC QC QC QC α1	CYQEFAHC CYQEFAHC CYQEFAHC CYQEFAHC CYQEFAHC CYQEFAHC CFQEFAHC CFQEFAHC CYQHFGHC CYQHFGHC CYQUFAHC CYQUFAHC CYQUFAHC	YLVKYKT. PQP           YLVKYKT. PQP           YLVKYKT. PQP           YLVKYKT. PQP           YLVKYKT. PGP           YLVKYKT. PKS           YLVKYKT. TKG           YLVKYKT. TKG           YLVKYKD. PKG           YLVKYKD. PKG           YVHIPG. CKE           YIVWOPG. RRV           YUIAFNG. NKV	P NE G LIAD OC NP NE G LIAD OC NP SE E LIAD OC NP KE G LIAD OC NP SE STVIAD OC NP SE KLIVD OC NP TSE LIQD OC NP TSE LIQD OC NP SO DLIOD OC SP SO DLIOD OC 32	PLPGLA.DVS PLPGYH.AGV PLPCYH.AGL PLPCYH.AGL PLPCYH.AGL PLPGNH.PTV PLPGGE.GL. PLPGVH.IPS PLNNSWLTAL PLPDHPYRPL PLPPRFGNNL	FYPYQAIWDYY FYPYQAIWDYY TYNRQSIWDYY SYKPQSIWDYY YYKYOPIWDYY QYAHQNIWDYY IYKYQAIWDYY IYKYQAIWDYY IYKYQAIWDYY IYKYQAIWDYY GYMQQNAYDIY KYRNHTIWKYY Jlacoc Jlacoc	AKIENIRPANI IKVEITRPANI IKVESIRPANI IKVESIRPANI IKVEITRPANI IKVEITRPANI LKIQNIRPQGI VPFEQIRPEGI LEISNIRPENI IPFQMMRPENI NQPPPRQLSLI LQPPRYGLQN IPLPFKVSSNI TT	$\begin{array}{c} \textbf{ND} \\ \textbf{TTSSK} \textbf{LYGKA} \\ \textbf{TTSSK} \textbf{YGKA} \\ \textbf{TTSXA} \textbf{YGA} \\ \textbf{TTSXS} \textbf{YQCA} \\ \textbf{SSQA} \textbf{YQCA} \\ \textbf{SSQA} \textbf{YYCQA} \\ \textbf{TSSKS} \textbf{YYGTA} \\ \textbf{TSKS} \textbf{YYGTA} \\ \textbf{TSAT} \textbf{YFGQA} \\ \textbf{TSAT} \textbf{YFQA} \\ \textbf{TSAT} \textbf{YFQA} \\ \textbf{TSAT} \textbf{YGA} \\ \textbf{TSAT} \textbf{TGAA } \textbf{TAAA} \textbf{TGAA } \textbf{TAAAA} \textbf{TGAA } TAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA$



	590	600	610	620	630	640	650	660	670	680
Gorilla GII Gorilla GI PFV Chimp CI GMonkey Macaque Marmoset Orangutan Bovine Equine Feline	GAVQTLAQI GAVQTLAQI GAVQTLSQI GAVQTLSQI GAVQTLSQI NAVTQIAKI QAVTLSKI QAITTLSKL QAISTLAKI NAITTVAKI	SDINDQNLQQ SDINDENLQQ SDINDENLQQ SDINDENLQQ SDINDERLQQ SDINDERLQQ SDINDERLQQ SDINDERLQQ SDINDERLQQ SDINDENLAA SDINDENLAA SDINDENLAA SDINDQKLAK	CIYLLRDHI GIYLLRDHY GIYLLRDHV GYYLLRDHV GYYLLRDHV GYYLLRDHY GIYLLRDHI GIHLLOHI GIHLLOHI GVHLLRDHY	VTLMEATLED VTLMEATLED ITLMEATLED VTLMEATLED VTLMEAALED VTLMEATLED VTLMEATLED VTLMEATLED VTLMEATLED VTLMEATLED VTLMEATLED VTLMEATLED	ISIMECMFA ISIMECMFA ISVMECMFA ISVMECMFA ITIMECMLA VSIMECMLA VSALGNVVTI ISLMECMLA VSLLGHMTS ISMLEAAHGI IVSLGEGIQ	VQHVHTHINHI VQHVHTHINHI VQHLHTHINHI VQHLHTHINHI QHVHTHINHI QHVHTHINHI QHVHTHINHI QHLHTHINHF QHLHTHINTF QILHTHISTI EHIHNHITSL	RTMLMERRI RTMLMERRI KTMLLERRI KTMLLERRI KTILLMRKI KTMLLMRKI KTMLLERRI KNLLIGNRV RLLLTENRV KLLTENRI	WTYMSSSWLQ WTYMSSSWLQ WTYMSSSWLQ WTYMSSSWLQ WTFIKSSWLQ WTFIRSDWIQ WTFIRSDWIQ WTFINSDWLQ WTFINSDWLQ WSVLENKWIQ WNLIDSTWIQ WRFINDSWIQ	TOTOKSDDEMK TOTOKSDDEMK QOTOKSDDEMK QOTOKSDDEMK QOTOKSDDEMK QOTOKTDDEMK QOTOKTDDEMK QOTOKTDDEMK QOTOQPTDHMK EETKYTDEVMN QOTOADEALMN EETGVSDNIMK	VIKRTARSLVYY VIKRTARSLVYY VIKRIAKSLVYY UIRRTAKSLVYY LIRRTAKSLVYY LIRRTARSLVYY UIRRTARALIYN VIRRTARSLYYY VIRRTARSITYD VIRRTARSITYD VIRRTARSMYR VIRKTARCIPYN
	. 7	00 7	10	720	730	740	750	760	770	. <b>◆</b> N13 <sup>790</sup>
Gorilla GII Gorilla GI PFV Chimp CI GMonkey Macaque Marmoset Orangutan Bovine Equine Feline	KQTYNSLTA KQTYNSLTA KQTYNSLTA KQTYNSPTA TQTSSSTTA TQTSSSPTA EEIDFRPTS EQTSNSPTA QNVKNTSDS IQQINRPDM KQTRNLNTS	TAWEIGIYYE TAWEIGIYYE TAWEIGIYYE TSWEIGIYYE TSWEIGIYYE TSWEIGIYYE TSWEIGIYYE TSWEIQIYYE TMWEIYIYYE TLWELGIYYE TAWEIYIYYE	LIIPRHIYL LIIPRHIYL LVIPKHIYL ITIPKHYYL ITIPKHIYL IVIPKHIYL LIIPRHIYL LIIPRRIWI LIIPKKVWL IIIPTTIYT	NNWQIVNIGH NNWQVVNIGH NNWNVVNIGH NNWQVINIGH NNWQVINIGH NNWQVINGH TNWEVHNIGH NNWQIKNIGH RNWQVANIGH TNWKIQNIGH QNWNIKNIGH	LIKSAGQLTH LIKSAGQLTH LVKSAGQLTH LVESAGHLTI LVESAGHLTI LIESAGHTT LUSAGSLTI LHSAQLTH LTHNSGYLTH LIKNAGHLAH LVRNAGYLSH	VTLSHPYEII VTLSHPYEII VTLAHPYEII VTIAHPYEII IRVKHPYEII VTIQHPYTIV VTIQHPYTIV VTIDHPYEIL VTIHHPYEIV VVELQHPYEIV VELQHPYEIV	NRECSNTLY NRECSNTLY NKECVETIY NKECTYEQY NKECSTYEQY NQECGETKY NRECETKY NQECETKY NQCCELTF NQCCELTF NQCCGTNIY	HLEECRRLDY HLEECRRLDY HLEDCTRQDY HLEDCIREQY HLECIREDY HLEECRRDY HHEECTEQDY HLEQCIKQDY HLEQCIKQDY HLVDCHEQDY ELKGCQELDY HMEECVDQDY	VICDVVKIVQP VICDVVKIVQP VICDVVKIVQP VICDVVEIVQP VICDTVQIVQP VICDIVQIVQP VICDIVQIVQP VICDIVQIVQP LICEQVTEVLP LICEEVMEVEP LICEEVMELPP	CGNSSDSSDCPVW CGNSSDSSDCPVW CGNSSDTSDCPVW CGNSTDTSDCPVW CGNSTTTSDCPVT CGNATELSDCPVT CGNLTG.SDCPVL CGNLTG.SDCPVL CGNLTG.SDCPVL CGNLTG.SDCPVL CGNQTG.SDCPVL CGNGTG.SDCPVL

	800	<b>♦</b> N14	820	<sup>830</sup> <b>♦</b> N15	840	850	860	870	880	890
Gorilla GII	AEPVKEPHVQISP	LKNGSYLVLA	SSTDCQIPPY	PSVVTVNETTQ	FGVTFKK	PLVAEE.KTSLE	PQLPHLQLR	LPHLVGII <mark>A</mark> KI	KGIKIEVTSSG	ESIKDQ
Gorilla GI	AEPVKEPHVQISP	LKNGSYLVLA	SSTDCQIPPY	PSVVTVNETTQ	FGVTFKK	PLVAEE.KTSLE	PQLPHLQLR	LPHLVGIIAKI	KGIKIEVTSSG	ESIKDQ
Chimp CI	AFAVKEPFVQVNP	LKNGSYLVLA	SSTDCQIPPY	APSIVIVNETTSC APSIVIVNETTSC	YCLNEKK	PLVAEE.RLSFE	PREPREZER	LPHLVGIIAKI	KGIKIEVISSG	ESIKEQ
GMonkey	AEKVKEPYVOVSA	LKNGSYLVLT	SRTDCSIPAY	PSIVTVNETVK	FGVEFHK	PLYSES. KVSFE	POVPHLKLR	LPHLVGIIANL	ONLEIEVISTO	ESIKDO
Macaque	ALKVKTPYIQVSP	L <mark>KNGSY</mark> LVLS	STKDCSIPAY	/PSVVTVNET <b>VK</b> (	FGVEFHK	PLYAET.KTSYE	PQVPHLKLR	L <mark>PHL</mark> TGII <mark>A</mark> SL	QSLEIEVTSTQ	ENIKDQ
Marmoset	AKTVKPGYVHIES	L R NG SYIYMA	HYQDCGIKPY	PQIVTVNATVKC	LGYEIQP	PLQFEETTSSLT	PQVPSLKLR	LPHLVGIL <mark>A</mark> KL	KNIQIQVTSTW	ESIKDQ
Orangutan	AKAIKSPYTEILP	LKNGSYLVLS	DSTSCNILPY	<b>IPSIVTVNETVE</b>	FGVLFKK	PLTAER.KTDYT	PHIPPLRLR	LPHLLGII <mark>A</mark> KL	KNIKIEVTSTO	ENIKDQ
Bovine	AENIQAPYVYLHP	LKNGSYLLMA	SHTDCSLPPY	PVVVTVNDSLE	YGKPLKR	PLTSHTEIKLFA	PQIPQLRVR	LPHLVGIIAKL	KSLKIKVTSTW	ESIKDQ
Equine	AQKIKDPYVWIYP	LKNGSYLIMS	SHTDCAIPPY	PVLVTVNDTVR	FGTTLKK	PLRTSLETFTFQ	PHIPQLQVR	LPHLVGLIAKI	KGLKIEITSTW	ENIKDQ
Feline	TKPLTDEYLEIEP	LKNGSYLVLS	STTDCGIPAY	PVVITVNDTISC	FDKEFKR	PLKQELKVTKYA	PSVPQLELR	VPRLTSLIAKI	KGIQIEITSSW	ETIKEQ



**Figure S6 legend**: Sequences corresponding to 11 FV Env were aligned in Clustal Omega <sup>5</sup> and the alignment was plotted with ESPript <u>https://espript.ibcp.fr</u><sup>6</sup>, with colors that indicate % identity (white letter, red background 100% identical; red letters, white background >70% identity; black letters, white background, <70% identity). The black, horizontal line separates simian from other FVs.

The secondary structure elements corresponding to the SFV RBD X-ray structure and the AF2 model for the feline FV RBD are plotted above and below the alignment, respectively. The N-linked glycosylation sites are indicated with stars. The already established nomenclature for the N-glycosylation sites (N1 to N15) is applied. The strictly conserved N-glycosylation sites are outlined with a thicker border, and the sites that carry sugars, which could be resolved in our structure, have grey filling. The residues interacting with heparan-sulfate are marked with blue ovals above the alignment. Loops 1-4 are indicated with bars above the alignment and labeled as L1-L4, using the same color code as in Fig. S2. The boundaries for the LP, SU, RBD, TM subunit are shown, as well as for the RBD variable and RBDjoin regions (the numbering corresponds to that of gorilla SFV Env, GII-K74 genotype). To distinguish the TM subunit from the TM domain, which is the region spanning the membrane, the latter is referred to as the TM<sup>anchor</sup>. The two furin sites are indicated with the scissors icon.

The Env sequences used in the alignment were obtained from public databases and with following accession numbers: <u>SFVggo\_huBAK74</u> (GII-K74, genotype II gorilla SFV, GenBank: AFX98090.1), <u>SFVggo\_huBAD468</u> (GI-D468, genotype I gorilla SFV; GenBank: AFX98095.1), <u>SFVpsc\_huHSRV13</u> (CI-PFV, known as Prototype Foamy Virus genotype I Eastern chimpanzee SFV; GenBank: AQM52259.1), <u>SFVcpz</u> (genotype I Western chimpanzee; UniProtKB/Swiss-Prot: Q87041.1), <u>SFVcae\_LK3</u> (Genotype II African green monkey SFV; NCBI Reference: YP\_001956723.2), <u>SFVmcy\_FV21</u> (genotype I macaque SFV; UniProtKB/Swiss-Prot: P23073.3), <u>SFVcja\_FXV</u> (Marmoset FV; GenBank: GU356395.1), <u>SFVppy\_bella</u> (Orangutan SFV; GenBank: CAD67563.1), <u>BFVbta\_BSV11</u> (Bovine\_FV; NCBI Reference: NP\_044930.1), <u>EFVeca\_1</u> (Equine\_FV; GenBank: AAF64415.1), and <u>FFVfca\_FUV7</u> (Feline FV; UniProtKB/Swiss-Prot: O56861.1). Genotypes I and II have been defined for gorilla, chimpanzee, green monkey and macaque FVs <sup>7 8,9</sup>.



#### Figure S7: Functional features of FV EBD mapped onto the structure

**Figure S7: Functional features plotted on the RBD structure.** The conserved 'RBD<sup>cons'</sup> (residues 218-241 and 488-552) and variable regions 'RBD<sup>var'</sup> (residues 242-487) are plotted on the X-ray structure of gorilla SFV RBD and colored in light grey and red, respectively. The glycosylation sites are indicated with the stars on the bottom.



#### Figure S8: AlphaFold2 models of FV RBDs

**Figure S8 legend:** AF2 prediction program <sup>11</sup> was used for *ab initio* modeling of the FV RBDs. The structures are colored according to the perresidue confidence metric called 'predicted local distance difference test' (pLDDT). The pLDDT can have a value between 0 and 100, with the higher model confidence corresponding to the higher pLDDT number. pLDDT > 90 (rendered in blue on the panels) is the high accuracy cut-off, above which the backbone and rotamers are predicted with high confidence; values between 70 and 90 (cyan) correspond to the regions where the backbone conformation is correct; values between 50 and 70 (yellow) have low confidence and are not reliably predicted, and regions with pLDDT below 50 (red) should not be interpreted. Structural superpositions of all AF2 models against each other were carried out using mTM-align server for multiple structural alignments12,13 available at https://yanglab.nankai.edu.cn/mTM-align/. Below each model is a 'template modelling score' (TM-score), which is a length-independent scoring function reflecting the similarity of two structures14. The TM-scores can take values between 0 and 1, with the higher TM-score indicating higher structural similarity. The indicated TM-scores correspond to the pairwise superimposition of each AF2 model onto the X-ray Gorilla GII RBD structure. The accession codes for the AF2 models, which have been deposited in the Model Archive database, are provided in the 'Data Availability' section.



#### Figure S9: FV RBD common core excludes a large portion of the upper subdomain

**Figure S9 legend**: **A)** Superposition of the RBD experimental structure and 11 AF2 models (Fig. S8) yielded a 'common core', model that includes the residues with C $\alpha$  rmsd < 4Å for all pairwise superpositions. The regions containing residues with C $\alpha$  rmsd > 4Å are indicated with dashed lines. The common core regions are highlighted with purple bars on top of the sequence alignment, which is colored using the same scheme as in Fig. S6. The small inlet in the upper left corner represents the entire RBD as a reference for comparison, with the common core colored in purple, and the remaining residues in grey. The structural and sequence alignments were carried out as described in Figs. S8 and S6, respectively.

**B)** Each RBD model is shown as a separate structure to illustrate contrast between the structurally conserved, common core residues, and the upper subdomains, whose loops are predicted to adopt different conformations.



Figure S10: The upper domain loops show poor sequence conservation

**Figure S10 legend: A)** Three SFV RBD protomers fitted in the density maps obtained for PFV Env <sup>15</sup>, as shown in Fig. 4 are rendered by residue conservation. The % identity was calculated in Chimera <sup>16</sup> according to the sequence alignment shown in Fig. S6, and residues were colored with the white to maroon gradient as indicated with the color key. The residues showing less than 30% and more than 90% sequence identity are colored in solid white and maroon, respectively.

**B)** The region outlined with a green dashed-line rectangle in panel A (top view) is magnified, with the two protomers colored in yellow and blue, for clarity. Loops 4 and 2, and loop 3 of the neighboring protomer (L4, L2 and L3\*, respectively) are indicated, along with the disulfide bonds DS5 and DS6, clamping the ends of the loops. On the left panels, residues from L3\*, potentially forming contacts with L4 and L2 are displayed with their side chains as stick models (D419, D427, P429, Y430, S431). On the right panel, the residues from L2 (E280, N281, I282) and L4 (L447, S449, D450), likely to contact L3\*, are shown in stick models.

**C)** The region outlined with a green dashed-line rectangle in panel A (bottom view) is magnified, showing the L1 loops from 3 protomers, colored in yellow, blue, and grey, with the residues likely to form inter-RBD contacts displayed only for L1, for clarity (A262, D263, V264, S265, F266, Y267).





**Figure S11**: Three batches of FVVs carrying WT or mutated Env were produced. Horizontal lines represent the mean values from tested FVV batches. The dotted lines represent the quantification threshold. Source data are provided as a Source Data file.

**A)** The concentration of FVV particles was quantified by RT-qPCR amplification. Each batch was titrated two times and the mean titers are presented.

**B)** FVVs carrying WT and mutated Env were incubated with HT1080 cells on ice for 1 h before washing and quantification of the remaining vector particles by RT-qPCR. The FVV dose was 1, 10 or 100 FVV particles per cell. Values below the threshold were arbitrarily set at 0.0005. The values obtained for the mutant and WT FVVs were compared using the two-way paired t test.

**C)** FVV infectious titers were quantified on BHK-21 cells. Each batch was titrated two times and the mean titer is plottedblack solid horizontal lines represent the mean values from the three FVV batches. The values obtained for the mutant and WT FVVs were compared using the two-way paired t test.

**D)** The percentage of infectious FVV particles carrying WT,  $\Delta$ L2 and  $\Delta$ L4 Env was calculated as the ratio between the number of infectious particles (panel C) and the amount of vector particles obtained by RT-qPCR (panel A).





**Figure S12 legend**: The size exclusion chromatography (Superdex 200) profiles for the GII RBD expressed in mammalian cells are shown for the WT protein (red line) and the variants (blue and green lines).





**Figure S13 legend**: Flow cytometry gating strategy for the detection of Env binding and HS expression on SFV-susceptible cell lines. Cells were treated with trypsin-EDTA before labelling with Env proteins or anti-HS antibodies.

**A)** Representative example of HT1080 single cell selection: live cells were selected by a gate applied on an FSC-A/SSC-A dot-plot and a single cell gate applied on an SSC-A/SSC-H dot-plot.

**B)** Representative example of Env binding analysis. HT1080 cells were labelled with WT, K342A/R343A (mut1) or R356A/R369A (mut2) ectodomain proteins, StrepMAB-Classic-HRP and anti-HRP-AF488 antibodies. Staining obtained on gated live single cells is presented on the

histogram overlay: MFI is presented on the x-axis and frequency is expressed as the normalized percentage of gated events on the y-axis (%Max). Cells labelled with secondary antibodies only ("control" condition, black curve) were used as a reference. Env-specific staining was quantified by the ratio of MFI from Env treated to untreated cells.

**C)** Representative example of heparan sulfate staining after treatment with heparinase III. HT1080 cells were treated with heparinase III or buffer and stained with the F58-10E4 antibody specific for heparan sulfate (anti-HS) and the F69-3G10 antibody specific for glycans exposed after heparan sulfate removal (anti- $\Delta$ HS). Staining obtained on gated live single cells are presented on the histogram overlay: MFI is presented on the x-axis and frequency is expressed as the normalized percentage of gated events on the y-axis (%Max). Cells labelled with secondary antibodies only ("control" condition, black curve) were used as a reference; HS and  $\Delta$ HS-specific staining was quantified by the ratio of MFI from labelled to control cells.

**D)** HT1080 cells were treated with heparinase III or buffer and stained with antibodies specific for HS (HS) or glycans exposed after heparan sulfate removal ( $\Delta$ HS). Expression levels are calculated as the ratio of MFI from labelled to unlabeled cells (Fig. S13C). Mean values from two independent experiments are shown. Source data are provided as a Source Data file.



#### Figure S14: Effect of mutations on FVV release and infectious titer

**Figure S14 legend**: Five batches of FVVs carrying WT (red), mut1 (blue) and mut2 (green) Env were produced, each represented with a single dot.

A) The concentration of the vector particles was quantified by RT-qPCR of  $\beta$ -galactosidase transgene. Each batch was titrated twice, and mean titers are plotted; solid black, horizontal lines represent mean values from the five FVV batches. The dotted black horizontal line represents the quantification threshold. Source data are provided as a Source Data file.

**B)** FVVs infectious titers were quantified on BHK-21 cells. Each batch was titrated twice, and mean titers are plotted; black solid lines represent mean values from the five FVV batches. The dotted line represents the quantification threshold. The values obtained for the mutant and WT FVVs were compared using the two-way paired t test. Source data are provided as a Source Data file.



Figure S15: Structural basis for RBDjoin region being dispensable for binding to cells



**A)** The regions identified in the bipartite PFV RBD as essential <sup>17</sup> (indicated as RBD1 and RBD2 according to the more recent nomenclature <sup>18</sup>) and non-essential (or RBDjoin <sup>18</sup>) for SFV entry are colored in dark grey and green, respectively, and plotted on the X-ray structure of gorilla SFV RBD. The numbering corresponds to the gorilla GII RBD.

**B)** The AF2 models of the PFV RBD lacking the non-essential RBDjoin region (left panel) and of the whole PFV RBD (right panel) are colored according to the pLDDT values, using the same palette as in Fig. S8.

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