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Supplemental Information

HCV Broadly Neutralizing Antibodies Use a CDRH3 Disulfide Motif to Recognize an E2 Glycoprotein Site that Can Be Targeted for Vaccine Design

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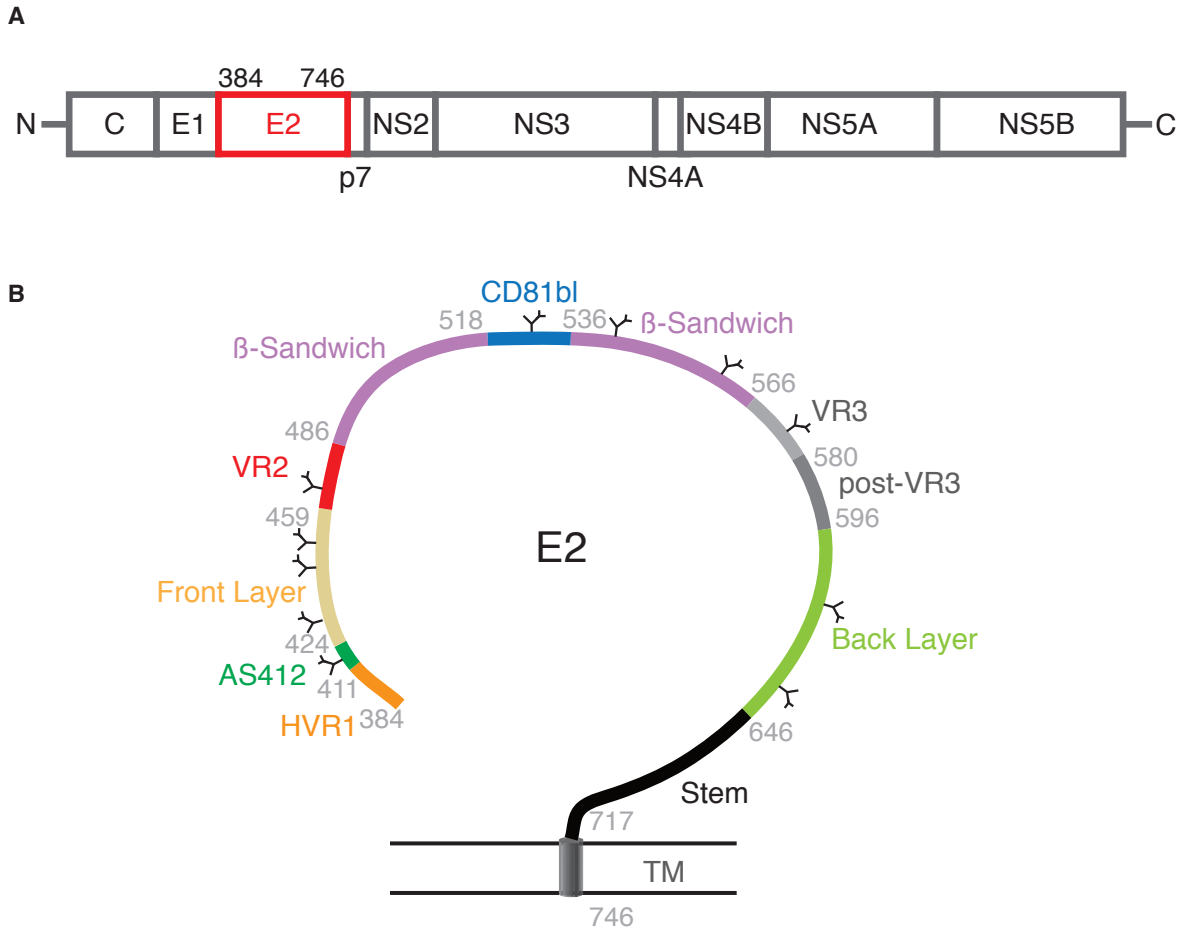

Figure S1. The schematic representation of HCV polyprotein (A) and HCV E2 (B). E2 regions are colored by structural components: HVR1 (orange), AS412 (dark green), front layer (yellow), VR2 (red), ß-sandwich (violet), CD81bl (blue), VR3 (light grey), post-VR3 (dark grey), back layer (green), and stem (black). Related to introduction and Figure 2.

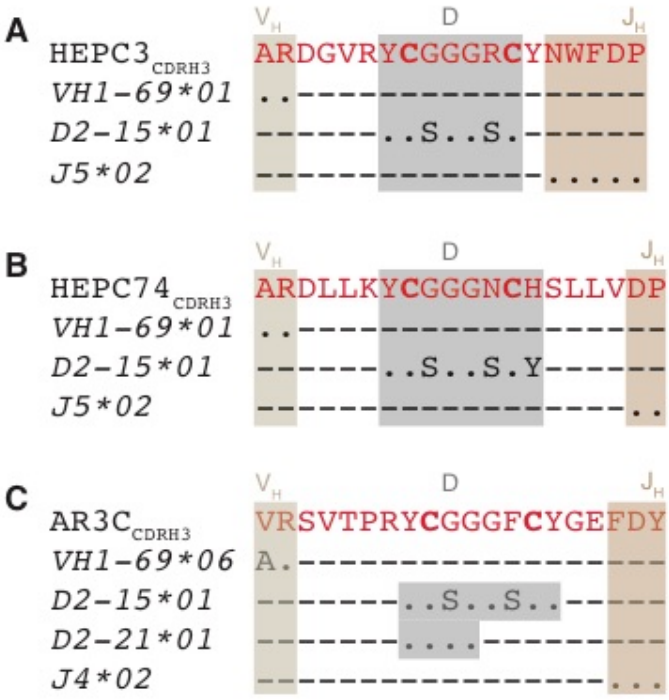

Figure S2. Sequence alignments of HEPC3 (A), HEPC74 (B), or AR3C (C) CDRH3 sequences with potential germline precursor genes. Dots indicated identical amino acids and dashes indicate regions encoded by other gene segments or N-nucleotide additions. Two cysteines in CDRH3 are highlighted in bold. The two most likely D-gene segments for AR3C are indicated. Related to Figure 1.

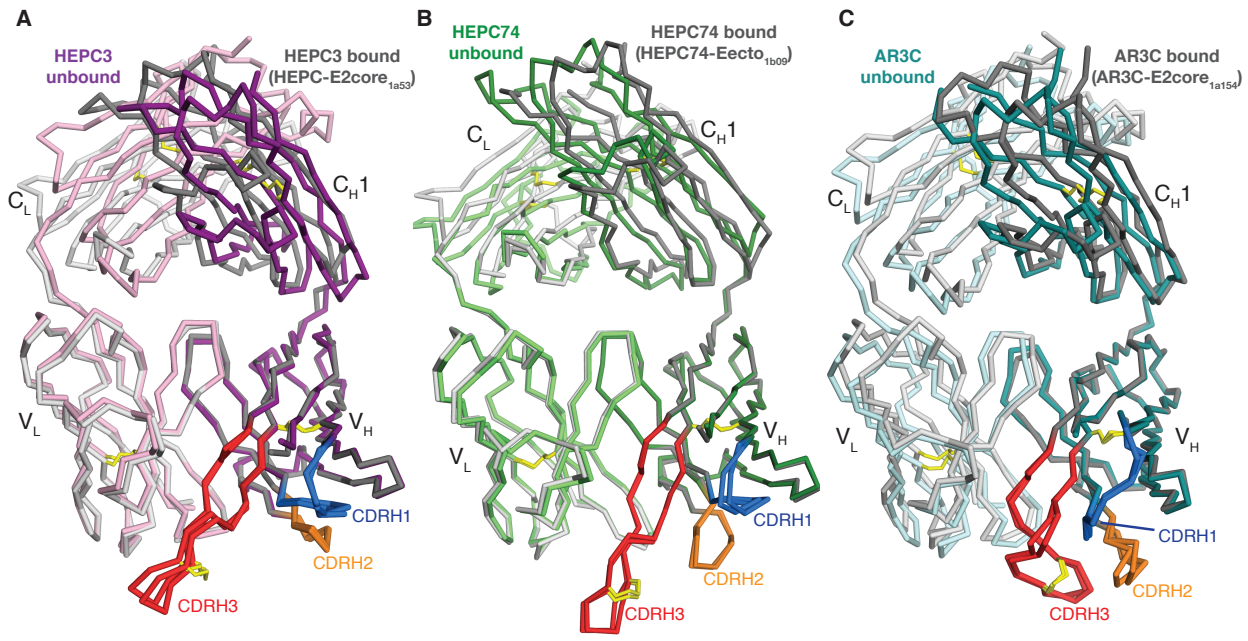

Figure S3. Comparison of HEPC3, HEPC74, and AR3C structures in unliganded and bound states. Superposition of unliganded (colored) and bound (grey) Fab structures of HEPC3 (A), HEPC74 (B), or AR3C (C) (PDB 4MWF for the AR3C-bound Fab structure). The Fabs were superimposed on their V_H domains. Protein backbones are shown as ribbons and CDR loops are blue (CDRH1), orange (CDRH2), and red (CDRH3). Disulfide bonds are shown as yellow sticks. Related to Figure 3, 4.

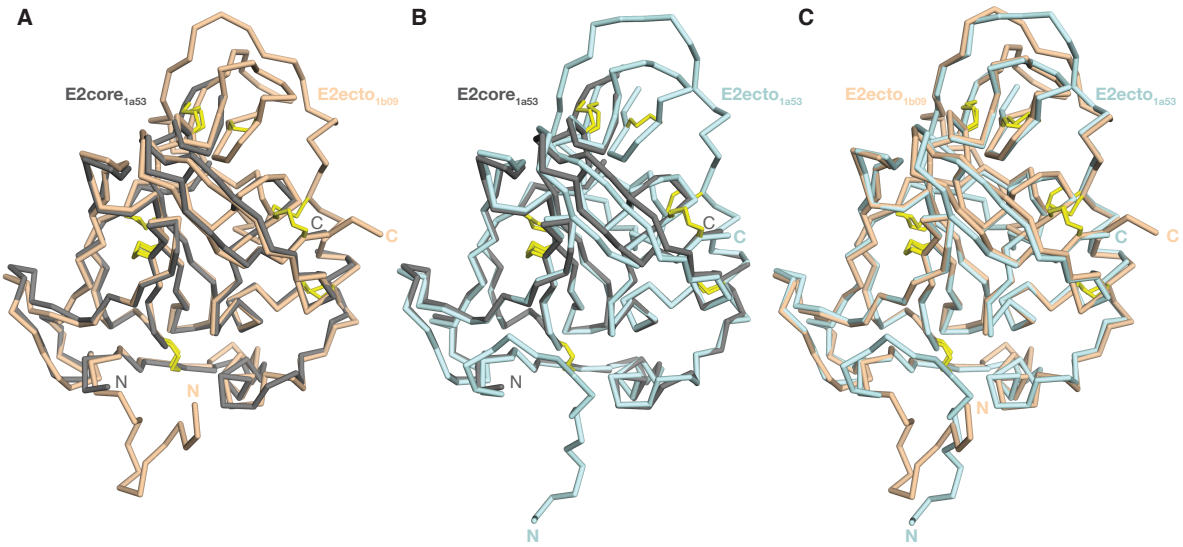

Figure S4. Comparison of E2core and E2ecto structures. Superposition of E2core$_{1a53}$ (grey) and E2ecto$_{1b09}$ (wheat) (A); E2core$_{1a53}$ (grey) and E2ecto$_{1a53}$ (light blue) (B); and E2ecto$_{1b09}$ (wheat) and E2ecto$_{1a53}$ (light blue) (C) structures. Rmsds calculated for superposition of 168 Cα atoms between the E2core and E2ecto structures (A-B) and 189 Cα atoms between the E2ecto structures (C). The HVR1, AS412, and VR2 regions were omitted from the rmsd calculations. Protein backbones are shown as ribbons and disulfide bonds are shown as yellow sticks. Related to Figure 5.

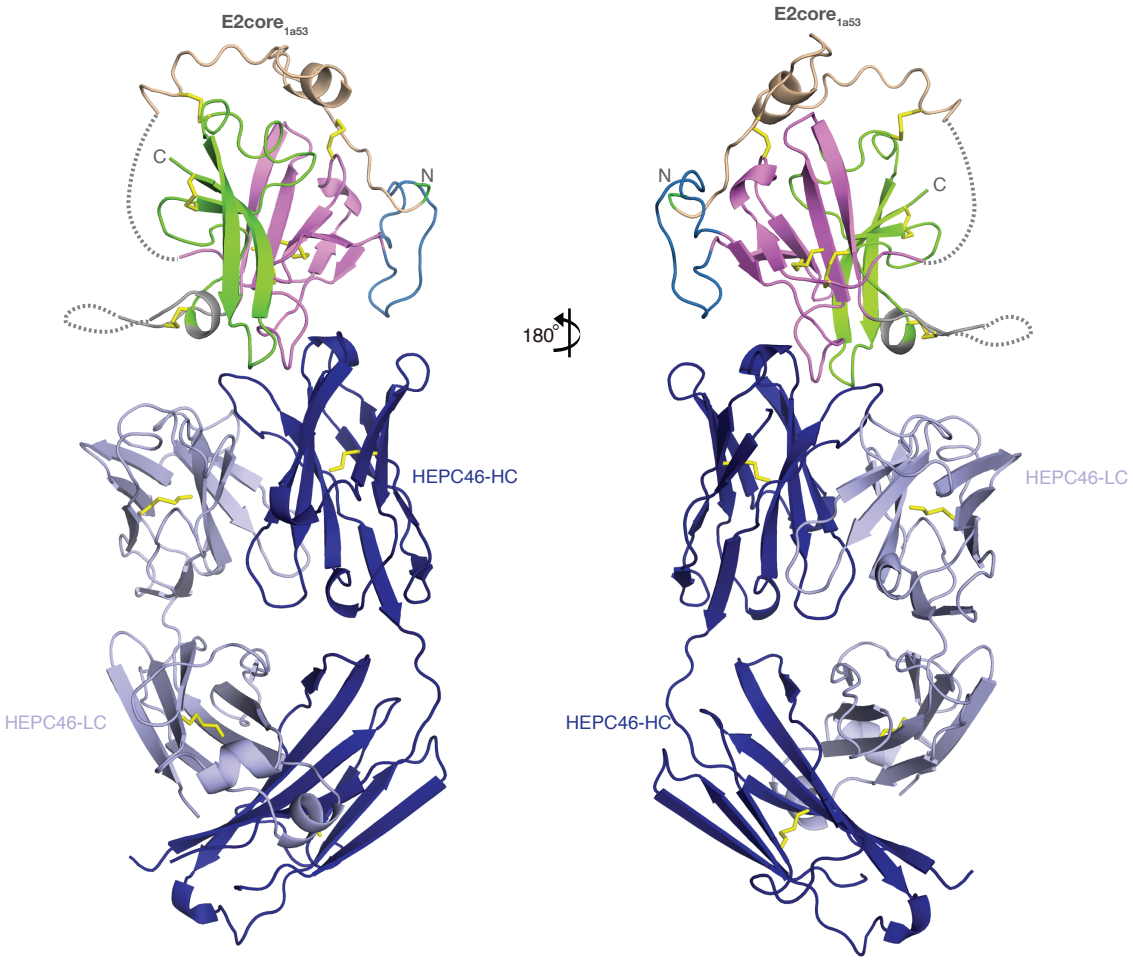

Figure S5. The structure of E2core$_{1a53}$ in complex with HEPC46. Cartoon representation of the crystal structure of HEPC3-E2core$_{1a53}$-HEPC46 complex (HEPC3 Fab not shown for clarity). E2core is colored according to structural elements: Front layer (yellow), β-sandwich (violet), CD81bl (blue), VR3 (light grey), post-VR3 (dark grey), and back layer (green). HEPC46-HC is dark blue and HEPC46-LC is light blue. Disulfide bonds are shown as yellow sticks; dashed lines indicate disordered regions. Related to Figure 2.

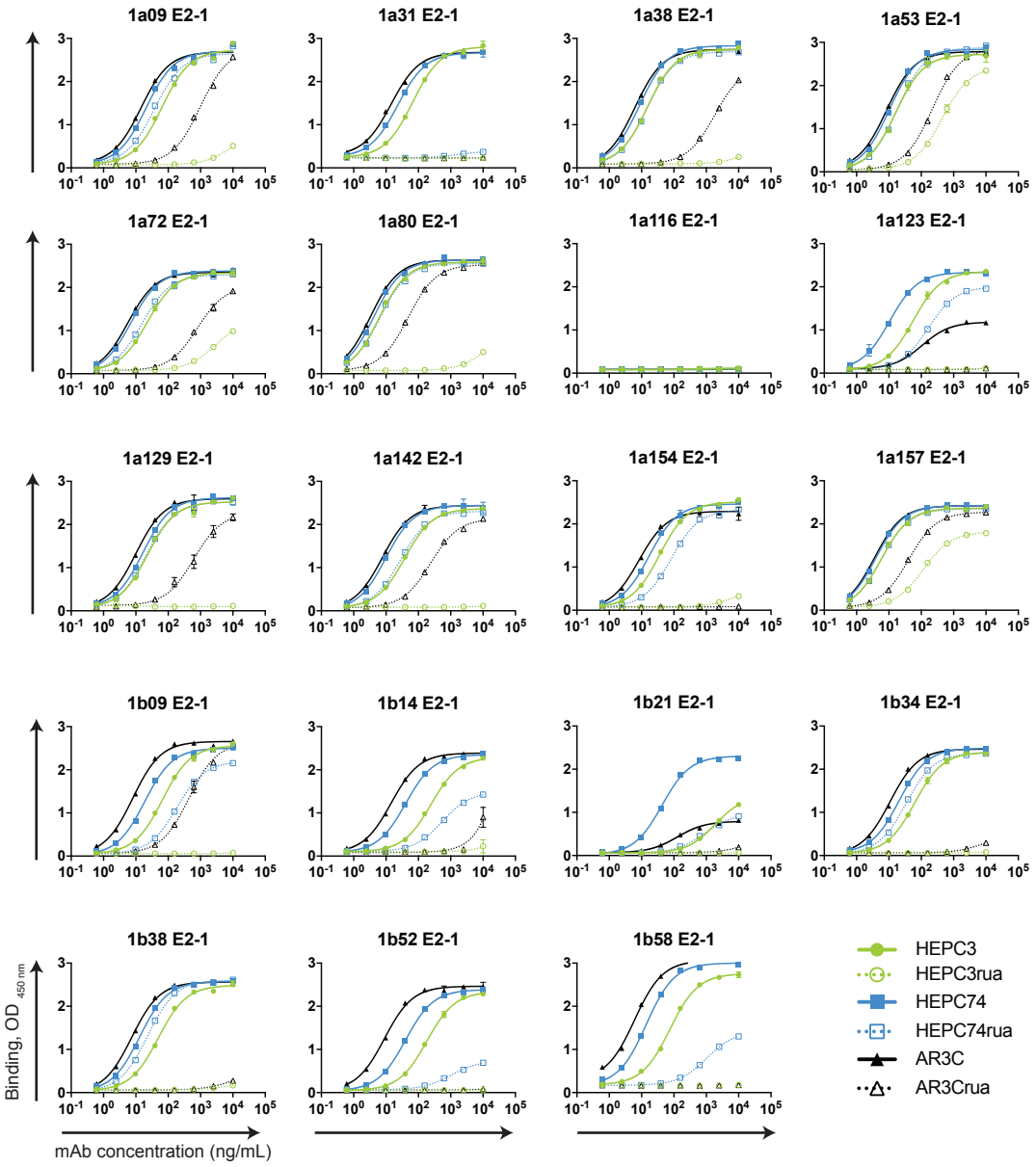

Figure S6. Binding of HEPC3, HEPC74, AR3C and their germline precursors to a panel of 19 genotype 1 E2ecto proteins. Values shown are means ± s.d. of duplicates. One experiment representative of two independent experiments is shown. Related to Figure 7.

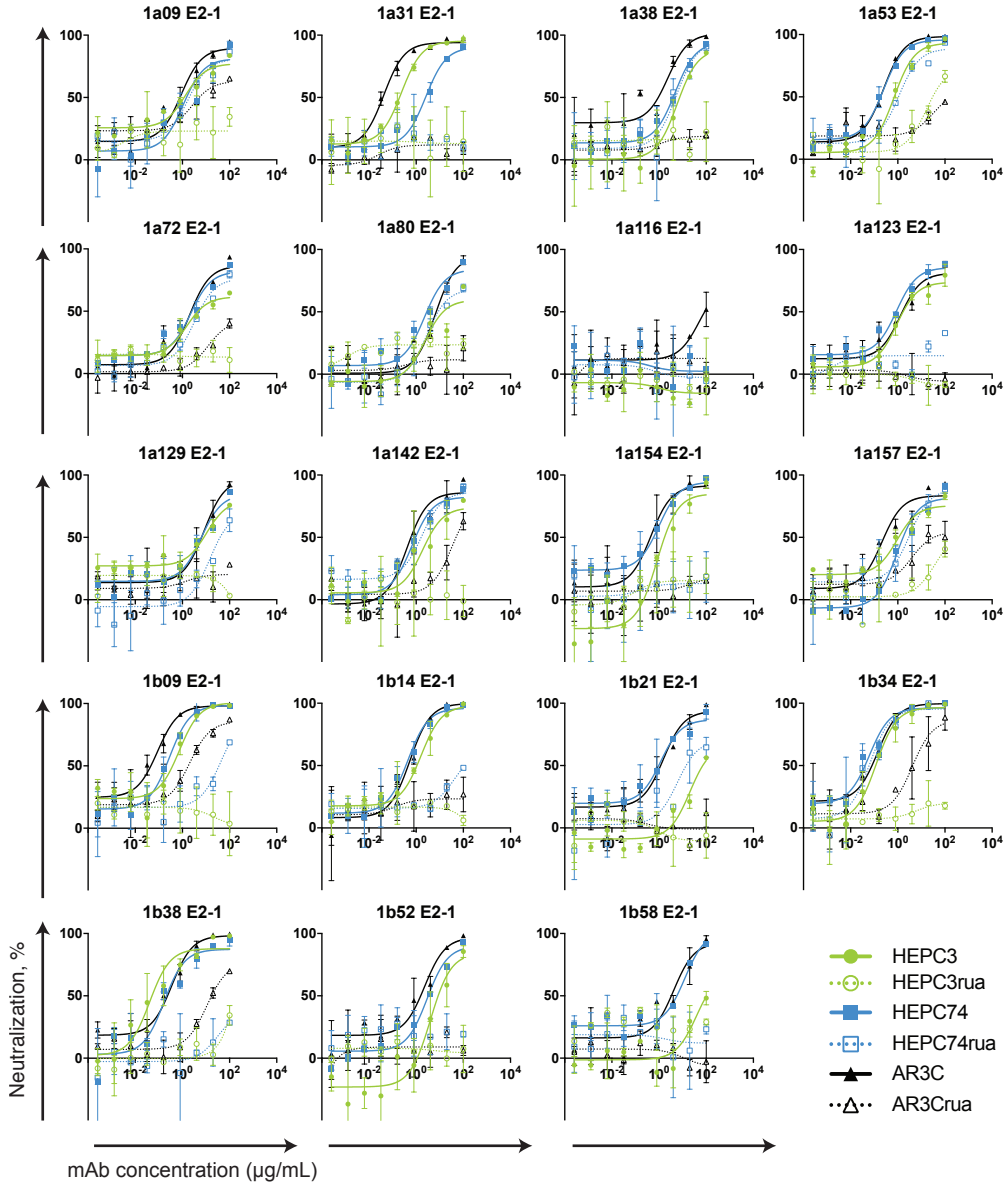

Figure S7. Neutralization capacity of HEPC3, HEPC74, AR3C and their germline precursors against a panel of genotype 1 HCVpp. Values shown are means ± s.d. of duplicates. Related to Figure 7.

Table S1 Data collection and refinement statistics for unliganded Fab structures.
Related to Figure 1 and Figure S3.

	HEPC3	HEPC74	AR3C	HEPC46
Data collection				
Wavelength (Å)	1.19	0.98	1.00	0.98
Resolution range (Å)	38.32 - 2.04 (2.11 - 2.04)	33.55 - 1.36 (1.41 - 1.36)	54.28 - 2.90 (3.00 - 2.90)	32.28 - 1.41 (1.46 - 1.41)
Space group	$P22_12_1$	P1	I222	$P2_12_12_1$
Cell, Å (a, b, c)	98.91, 108.69 166.25	55.13, 63.59, 73.76	76.67, 146.87, 175.25	54.64, 71.30, 108.59
Angles (α, β, γ)	90, 90, 90	80, 74, 84	90, 90, 90	90, 90, 90
Total reflections	502,575 (49,122)	1,209,301 (119,316)	129,013 (13,533)	402,422 (40,082)
Unique reflections	114,386 (11,299)	190,820 (18,843)	22,311 (2,207)	81,622 (8,076)
Multiplicity	4.4 (4.3)	6.3 (6.3)	5.8 (6.1)	4.9 (5.0)
Completeness (%)	99.9 (99.8)	93.7 (92.4)	99.5 (99.5)	99.0 (99.7)
I/σI	9.17 (1.50)	11.54 (1.41)	8.03 (1.12)	11.10 (1.71)
Wilson B-factor ($Å^2$)	32.35	17.62	75.33	16.43
R_{merge} (%)	10.16 (95.94)	6.77 (120.80)	16.67 (170.3)	6.40 (81.06)
R_{pim} (%)	5.35 (51.08)	2.83 (51.48)	7.55 (74.81)	3.07 (39.19)
$CC_{1/2}$	99.7 (55.7)	99.9 (48.7)	98.9 (52.8)	99.8 (66.6)
Refinement statistics				
R_{work}	18.13	16.75	22.14	17.46
R_{free}	21.60	19.08	25.45	20.08
Number of atoms	10,921	7,996	3,290	3,821
Protein	9,889	6,592	3,290	3,224
Ligands	30	-	-	-
Solvent	1,002	1,404	-	597
Protein residues	1,305	873	436	432
R.m.s deviations				
Bond lengths (Å)	0.011	0.009	0.011	0.009
Bond angles (°)	1.36	1.06	1.53	1.05
Clashscore	3.94	4.06	11.09	5.03
Average B-factor	36.94	26.6	78.15	24.95

Numbers in parentheses correspond to the highest resolution shell

Table S2 Data collection and refinement statistics for bNAb Fab-E2 complexes.
Related to Figure 2 and Figure 5.

	HEPC74-E2ecto$_{1b09}$	**HEPC3-E2ecto$_{1b09}$**	**HEPC3-E2ecto$_{1a53}$-HEPC46**	**HEPC3-E2core$_{1a53}$-HEPC46**
Data collection				
Wavelength (Å)	0.98	1.00	1.00	0.98
Resolution range (Å)	43.76 - 1.99 (2.06 - 1.99)	69.48 - 2.90 (3.00 - 2.90)	78.27 - 2.80 (2.90 - 2.80)	76.97 - 3.10 (3.21 - 3.10)
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
Cell, Å (a, b, c)	66.31, 69.77, 168.58	65.81, 75.79, 174.00	86.44, 76.64, 118.26	75.40, 105.59, 337.28
Angles (α, β, γ)	90, 90, 90	90, 90, 90	90, 106, 90	90, 90, 90
Total reflections	317,277 (24,655)	104,643 (10,272)	114,902 (10,691)	524,186 (44,182)
Unique reflections	53,843 (4,890)	19,947 (1,968)	36,828 (3,637)	49,943 (4,864)
Multiplicity	5.9 (5.0)	5.2 (5.2)	3.1 (2.9)	10.5 (9.1)
Completeness (%)	98.6 (90.9)	98.6 (100.0)	99.7 (99.1)	99.9 (99.7)
I/σI	7.32 (1.36)	6.50 (3.40)	6.81 (1.35)	12.72 (2.31)
Wilson B-factor (Å^2)	36.03	37.76	64.73	77.54
R_{merge} (%)	11.15 (86.45)	17.81 (29.37)	10.75 (74.46)	13.84 (88.36)
R_{pim} (%)	4.96 (47.49)	8.87 (14.03)	7.25 (51.38)	4.44 (30.41)
$CC_{1/2}$	95.9 (29.7)	96.9 (93.2)	98.3 (79.6)	99.7 (78.0)
Refinement statistics				
R_{work}	18.62	20.98	19.88	22.4
R_{free}	22.98	27.53	27.10	29.0
Number of atoms	5,089	5,589	8,500	15,747
Protein	4,588	5,202	8,333	15,435
Ligands	98	183	167	312
Solvent	403	204	-	-
Protein residues	603	693	1,099	2,044
R.m.s deviations				
Bond lengths (Å)	0.008	0.007	0.010	0.008
Bond angles (°)	1.29	1.25	1.49	1.35
Clashscore	4.02	8.56	16.39	15.53
Average B-factor	50.9	32.04	75.1	76.9

Numbers in parentheses correspond to the highest resolution shell

Table S3. HEPC3 and AR3C interfaces with E2cores. Shared E2core contact residues and hydrogen bonds are shaded grey.
Data for the AR3C-E2core1a154 complex was derived from PDB 4MWF. Related to Figure 2.

List of HEPC3 heavy chain (chain H)-E2core$_{1a53}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Heavy chain	Bond	BSA (Å^2)	E2core$_{1a53}$	Bond	BSA (Å^2)
H:GLN 1		68	C:LEU 427		49
H:VAL 2	H	17	C:ASN 428		25
H:GLY 27		15	C:CYS 429	H	35
H:THR 28	H	101	C:ASN 430		22
H:LEU 29		78	C:ASP 431	HS	37
H:ASN 30	H	60	C:SER 432		12
H:SER 31		4	C:LEU 433		36
H:TYR 32		5	C:ASN 434	H	134
H:GLU 33		11	C:THR 435	H	60
H:THR 52		14	C:GLY 436		36
H:PRO 53		45	C:TRP 437		13
H:ILE 54		47	C:LEU 438		42
H:PHE 55		5	C:ALA 439		52
H:GLU 73	HS	19	C:GLY 440		11
H:SER 76		1	C:PHE 442		30
H:ARG 94	HS	35	C:TYR 443		89
H:VAL 97		59	C:HIS 444		5
H:ARG 98	H	96	C:HIS 445		3
H:TYR 99		36	C:LYS 446	HS	134
H:CYS 100	H	78	C:PHE 447	H	45
H:GLY 100A		14	C:ASP 448	H	63
H:GLY 100B		50	C:TRP 529		42
H:GLY 100C		59	C:GLU 531		22
H:CYS 100E		32			
H:ASP 101	H	7			
H:PRO 102		4			
Total		959			1000

Hydrogen bonds

##	Heavy chain	Distance (Å)	E2core$_{1a53}$
1	H:CYS 100[O]	3.1	C:CYS 429[N]
2	H:ARG 98[O]	2.8	C:ASP 431[N]
3	H:ASP 101[OD1]	3.2	C:ASN 434[ND2]
4	H:GLU 73[OE2]	2.9	C:LYS 446[NZ]
5	H:THR 28[O]	2.7	C:ASP 448[N]
6	H:CYS 100[N]	2.9	C:CYS 429[O]
7	H:ARG 98[NE]	2.8	C:ASP 431[O]
8	H:ARG 94[NH1]	3.9	C:ASP 431[OD2]
9	H:VAL 2[N]	3.0	C:ASN 434[O]
10	H:ARG 94[NH1]	2.3	C:ASN 434[OD1]
11	H:THR 28[N]	3.0	C:THR 435[O]
12	H:ASN 30[N]	2.8	C:LYS 446[O]
13	H:ASN 30[ND2]	3.3	C:PHE 447[O]

Salt bridges

##	Heavy chain	Distance (Å)	E2core$_{1a53}$
1	H:GLU 73[OE1]	2.8	C:LYS 446[NZ]
2	H:GLU 73[OE2]	2.9	C:LYS 446[NZ]
3	H:ARG 94[NH1]	3.9	C:ASP 431[OD2]

List of AR3C heavy chain (chain H)-E2core$_{1a154}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Heavy chain	Bond	BSA (Å^2)	E2core$_{1a154}$	Bond	BSA (Å^2)
H:ASP 30		40	C:ILE 422		40
H:ASN 31		7	C:THR 425		2
H:TYR 32	H	33	C:LEU 427	H	74
H:VAL 52		3	C:ASN 428		11
H:LEU 53		60	C:CYS 429	H	35
H:PHE 54		97	C:ASN 430		14
H:THR 56		15	C:GLU 431	H	5
H:VAL 96		11	C:SER 432	H	18
H:THR 97	H	48	C:LEU 433		48
H:PRO 98		62	C:GLY 436		23
H:ARG 99	H	87	C:LEU 438		45
H:TYR 100		30	C:ALA 439		28
H:CYS 100A	H	80	C:LEU 441		22
H:GLY 100B		5	C:PHE 442		117
H:GLY 100C	H	59	C:TYR 443	H	112
H:GLY 100D		28	C:GLN 444		2
H:CYS 100F		6	C:LYS 446		12
			C:TRP 529		78
			C:ALA 531		9
			C:TYR 613		9
Total		671	**Total**		704

Hydrogen bonds

##	Heavy chain	Distance (Å)	E2core$_{1a154}$
1	H:ARG 99[O]	3.5	C:GLU 431[N]
2	H:ARG 99[O]	3.1	C:SER 432[N]
3	H:CYS 100A[O]	3.2	C:CYS 429[N]
4	H:TYR 32[N]	3.4	C:TYR 443[OH]
5	H:THR 97[N]	3.5	C:TYR 443[OH]
6	H:ARG 99[NH1]	3.6	C:SER 432[O]
7	H:ARG 99[NH1]	3.5	C:SER 432[OG]
8	H:CYS 100A[N]	2.7	C:CYS 429[O]
9	H:GLY 100C[N]	3.6	C:LEU 427[O]

No salt bridges observed

List of HEPC3 light chain (chain L)-E2core$_{1a53}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Light chain	Bond	BSA (Å^2)	E2core$_{1a53}$	Bond	BSA (Å^2)
L:TYR 49		7	C:ASN 430		8
L:SER 56		5	C:SER 432		5
Total		12	**Total**		13

List of AR3C light chain (chain L)-E2core$_{1a154}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Light chain	Bond	BSA (Å^2)	E2core$_{1a154}$	Bond	BSA (Å^2)
L:SER 31		9	C:ASN 430		1
L:ASN 32		23	C:GLU 431		2
L:TYR 49		22	C:LEU 433		115
L:GLY 50		14	C:LYS 446		23
L:THR 53		1			
L:THR 56		21			
L:TYR 91		11			
Total		101	**Total**		141

HEPC3 heavy chain (H) and light chain (L) residues interacting with E2core$_{1a53}$ glycans (chain C) (interactive partners are grouped together)

Ab chain	Bond	BSA (Å^2)	E2core$_{1a53}$	Bond	BSA (Å^2)
L:TYR 49		27	C:NAG4301		56
L:ASN 53		13			
L:SER 56		16			
H:TYR 99		41	C:NAG4301		44
H:SER 74		12	C:BMA6233		17
H:THR 75		4			
Total		114	**Total**		117

AR3C heavy chain (H) and light chain (L) residues interacting with E2core$_{1a154}$ glycans (chain C) (interactive partners are grouped together)

Ab chain	Bond	BSA (Å^2)	E2core$_{1a154}$	Bond	BSA (Å^2)
L:SER 30		10	C:NAG 4301	H	101
L:TYR 92	H	42			
L:ARG 93		28			
H:TYR 100		34	C:NAG 4301		44
L:SER 30	H	20	C:NAG 4302	H	53
L:TYR 92	H	35			
L:ARG 93		2			
L:GLN 27		3	C:MAN 4304		3
Total		174	**Total**		201

* Type of putative interation: H - hydrogen bond, S - salt bridge. BSA: buried surface area, Å.
The AR3C-E2core$_{154}$ interface was previously reported to be dominated by V_H domain (86% of the total BSA) and particularly CDRH3 loop (44% of the BSA) (Kong et al., 2013)

Table S4: HEPC3 and HEPC74 interfaces with E2 ectodomains.

Shared E2ecto contact residues, hydrogen bonds, and salt bridges are shaded grey. Related to Figure 2.

List of HEPC3 heavy chain (chain H)-E2ecto$_{1b09}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Heavy chain	Bond	BSA (Å^2)	E2ecto$_{1b09}$	Bond	BSA (Å^2)
H:GLN 1	H	47	C:ILE 414		16
H:GLY 26		1	C:ASN 417	H	19
H:GLY 27	H	14	C:GLY 418		26
H:THR 28	H	73	C:SER 419	H	27
H:LEU 29		76	C:TRP 420	H	5
H:ASN 30	H	60	C:LEU 427		52
H:SER 31		5	C:ASN 428		10
H:GLU 33	H	10	C:CYS 429	H	34
H:THR 52		9	C:ASN 430		19
H:PRO 53	H	47	C:ASP 431	HS	40
H:ILE 54		36	C:HIS 434	HS	90
H:PHE 55		5	C:THR 435	H	33
H:GLU 56		2	C:GLY 436	H	30
H:GLU 73	S	16	C:PHE 437		1
H:ARG 94		24	C:LEU 438		48
H:GLY 96		3	C:ALA 439		42
H:VAL 97		70	C:ALA 440		15
H:ARG 98	HS	83	C:LEU 441		1
H:TYR 99		31	C:PHE 442		37
H:CYS 100	H	77	C:TYR 443	H	98
H:GLY 100A		9	C:HIS 445		2
H:GLY 100B		53	C:LYS 446	HS	133
H:GLY 100C	H	86	C:PHE 447	H	27
H:ARG 100D	H	66	C:ASN 448	H	65
H:CYS 100E		47	C:ALA 449		1
H:ASP 101	HS	26	C:TRP 529		26
H:PRO 102		5	C:GLU 531		29
Total		980	**Total**		926

Hydrogen bonds

##	Heavy chain	Distance (Å)	E2ecto$_{1b09}$
1	H:GLY 100C[O]	2.9	C:TRP 420[N]
2	H:CYS 100[O]	3.1	C:CYS 429[N]
3	H:ARG 98[O]	3.0	C:ASP 431[N]
4	H:ASP 101[OD2]	2.9	C:HIS 434[NE2]
5	H:GLU 33[OE1]	3.9	C:TYR 443[OH]
6	H:PRO 53[O]	2.3	C:LYS 446[NZ]
7	H:THR 28[O]	2.8	C:ASN 448[N]
8	H:GLY 27[O]	2.9	C:ASN 448[ND2]
9	H:ARG 100D[NH:	2.7	C:ASN 417[O]
10	H:ARG 100D[NH	3.6	C:SER 419[OG]
11	H:CYS 100[N]	2.9	C:CYS 429[O]
12	H:ARG 98[NH1]	3.1	C:ASP 431[OD2]
13	H:GLN 1[N]	3.9	C:HIS 434[O]
14	H:THR 28[OG1]	3.1	C:THR 435[O]
15	H:THR 28[OG1]	3.7	C:GLY 436[O]
16	H:ASN 30[N]	2.9	C:LYS 446[O]
17	H:ASN 30[ND2]	3.4	C:PHE 447[O]

Salt bridges

##	Heavy chain	Distance (Å)	E2ecto$_{1b09}$
1	H:ASP 101[OD2]	2.9	C:HIS 434[NE2]
2	H:GLU 73[OE1]	3.8	C:LYS 446[NZ]
3	H:ARG 98[NH1]	3.1	C:ASP 431[OD2]

List of HEPC74 heavy chain (chain H)-E2ecto$_{1b09}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Heavy chain	Bond	BSA (Å^2)	E2ecto$_{1b09}$	Bond	BSA (Å^2)
H:GLY 26		3	C:TRP 420		1
H:GLY 27	H	18	C:HIS 421	H	27
H:THR 28	H	70	C:LEU 427		51
H:TYR 29		138	C:ASN 428		1
H:ILE 30	H	77	C:CYS 429	H	29
H:ASN 31		31	C:ASN 430		16
H:TYR 32	H	16	C:ASP 431	H	35
H:ALA 33		3	C:SER 432		7
H:PRO 52A		29	C:HIS 434	H	114
H:ILE 53		47	C:THR 435	H	58
H:SER 54		7	C:GLY 436		34
H:ASN 55		49	C:PHE 437		13
H:ARG 94	H	24	C:LEU 438		57
H:LEU 96		46	C:ALA 439		38
H:LEU 97		73	C:ALA 440		16
H:LYS 98	H	72	C:PHE 442		80
H:TYR 99		20	C:TYR 443		73
H:CYS 100	H	68	C:HIS 445		54
H:GLY 100A		7	C:LYS 446	H	59
H:GLY 100B	H	45	C:PHE 447		56
H:GLY 100C	H	69	C:ASN 448	H	63
H:ASN 100D		10	C:ALA 449		1
H:CYS 100E		39	C:TRP 529	H	32
H:VAL 100J		11	C:GLU 531	H	45
H:ASP 101	H	13	C:TRP 616		5
			C:PRO 619		6
Total		987	**Total**		972

Hydrogen bonds

##	Heavy chain	Distance (Å)	E2ecto$_{1b09}$
1	H:GLY 27[O]	3.0	C:ASN 448[ND2]
2	H:THR 28[O]	2.9	C:ASN 448[N]
3	H:LYS 98[O]	3.0	C:ASP 431[N]
4	H:GLY 100B[O]	3.3	C:TRP 529[NE1]
5	H:GLY 100C[O]	2.7	C:HIS 421[NE2]
6	H:ASP 101[O]	3.8	C:HIS 434[ND1]
7	H:CYS 100[N]	3.8	C:CYS 429[O]
8	H:ARG 94[NH1]	3.0	C:HIS 434[O]
9	H:TYR 32[OH]	2.8	C:THR 435[O]
10	H:ILE 30[N]	2.9	C:LYS 446[O]
11	H:GLY 100B[N]	2.2	C:GLU 531[OE2]

List of HEPC3 light chain (chain L)-E2ecto$_{1b09}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Light chain	Bond	BSA (Å^2)	E2ecto$_{1b09}$	Bond	BSA (Å^2)
L:TYR 32	H	29	C:GLY 418	H	24
L:TYR 49		15	C:ASN 430		16
L:GLN 55		16	C:SER 432		11
L:SER 56		11	C:HIS 434		15
Total		71	**Total**		65

Hydrogen bonds

##	Light chain	Distance (Å)	E2ecto$_{1b09}$
1	L:TYR 32[OH]	3.4	C:GLY 418[O]

List of HEPC74 light chain (chain L)-E2ecto$_{1b09}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Light chain	Bond	BSA (Å^2)	E2ecto$_{1b09}$	Bond	BSA (Å^2)
L:TYR 49			C:ASN 430		14
L:GLU 55	HS	29	C:SER 432	H	32
L:THR 56	H	48	C:LEU 433		9
			C:HIS 434	HS	33
Total		77	**Total**		88

Hydrogen bonds

##	Light chain	Distance (Å)	E2ecto$_{1b09}$
1	L:GLU 55[OE1]	2.9	C:HIS 434[NE2]
2	L:THR 56[OG1]	3.1	C:SER 432[O]

Salt bridges

##	Heavy chain	Distance (Å)	E2ecto$_{1b09}$
1	L:GLU 55[OE1]	2.9	C:HIS 434[NE2]
2	L:GLU 55[OE2]	3.8	C:HIS 434[NE2]

HEPC3 heavy chain (H) and light chain (L) residues interacting with E2ecto$_{1b09}$ glycans (chain C) (interactive partners are grouped together)

Ab chain	Bond	BSA (Å^2)	E2ecto$_{1b09}$	Bond	BSA (Å^2)
H:ALA 24		1	C:NAG4481	H	153
H:SER 25		13			
H:GLY 26	H	25			
H:GLY 27	H	16			
H:LEU 29		8			
H:ASN 30		16			
H:TYR 32	H	3			
H:SER 76		12			
L:TYR 49	H	28	C:NAG4301	H	84
L:ASN 53		24			
L:LEU 54		9			
L:SER 56		20			
H:GLN 3		1	C:NAG4482	H	52
H:SER 25	H	19			
H:GLY 26		26			
H:TYR 99		38	C:NAG4301		42
L:LEU 54		1	C:NAG4302	H	42
L:SER 56	H	27			
L:GLY 57		7			
H:ARG 100D	H	31	C:NAG4171	H	31
L:SER 60	H	29	C:BMA4303	H	24
L:ASN 30		21	C:NAG4171		19
Total		374	**Total**		447

HEPC74 heavy chain (H) and light chain (L) residues interacting with E2ecto$_{1b09}$ glycans (chain C) (interactive partners are grouped together)

Ab chain	Bond	BSA (Å^2)	E2ecto$_{1b09}$	Bond	BSA (Å^2)
H:GLN 1	H	21	C:NAG4481	H	178
H:THR 24		7			
H:SER 25		17			
H:GLY 26	H	24			
H:GLY 27	H	18			
H:TYR 29		4			
H:ILE 30		26			
H:SER 76		10			
L:TYR 49	H	25	C:NAG4301	H	79
L:LYS 50		10			
L:SER 53		14			
L:LEU 54		6			
L:THR 56		11			
H:LYS 98		2	C:NAG4301		16.99
H:TYR 99		15			
L:LEU 54		10	C:NAG4302		9
Total		219	**Total**		283

* Type of putative interation: H - hydrogen bond, S - salt bridge. BSA: buried surface area, Å^2.

Table S5: Interface between HEPC46 and E2 proteins ($E2ecto_{1a53}$, $E2core_{1a53}$).

Shared E2 contact residues, hydrogen bonds, and salt bridges are shaded grey. Related to Figure 2 and Figure S5.

List of HEPC46 heavy chain (chain A)-$E2ecto_{1a53}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Heavy chain	Bond	BSA (Å^2)	$E2ecto_{1a53}$	Bond	BSA (Å^2)
A:ILE 28		67	C:HIS 474		39
A:THR 30		12	C:ILE 496		4
A:SER 31	H	67	C:THR 510		8
A:HIS 32		14	C:VAL 514		17
A:GLY 33		14	C:VAL 538		21
A:TRP 50		29	C:ASN 540		45
A:ILE 51		4	C:ASN 541		8
A:SER 52	H	23	C:THR 542	H	66
A:TYR 53		95	C:ARG 543		29
A:ASN 54		34	C:PRO 544		4
A:TYR 56	H	84	C:PRO 545		58
A:ALA 95		9	C:LEU 546	H	147
A:SER 96		28	C:GLY 547	H	21
A:GLN 97	H	112	C:ASN 548		58
A:ILE 98		65	C:TRP 549		19
			C:PRO 567		17
			C:SER 595	H	3
			C:ARG 596	H	9
			C:VAL 633		0
			C:GLY 634	H	19
			C:VAL 636		24
			C:HIS 638		7
Total		657			622

Hydrogen bonds

##	Heavy chain	Distance (Å)	$E2ecto_{1a53}$
1	A:SER 31[O]	3.3	C:THR 542[N]
2	A:SER 52[OG]	2.9	C:LEU 546[O]
3	A:SER 52[OG]	3.5	C:GLY 547[O]
4	A:TYR 56[OH]	3.8	C:GLY 634[O]
5	A:GLN 97[NE2]	3.9	C:SER 595[O]
6	A:GLN 97[NE2]	3.1	C:ARG 596[O]

List of HEPC46 heavy chain (chain F)-$E2core_{1a53}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Heavy chain	Bond	BSA (Å^2)	$E2core_{1a53}$	Bond	BSA (Å^2)
F:ILE 28		67	C:ILE 496		3
F:THR 30		7	C:THR 510		7
F:SER 31	H	63	C:VAL 514		12
F:HIS 32		24	C:ARG 521		7
F:GLY 33		14	C:VAL 538		24
F:TRP 50		20	C:ASN 540		36
F:ILE 51		4	C:ASN 541		5
F:SER 52	H	16	C:THR 542	H	74
F:TYR 53		94	C:ARG 543		39
F:ASN 54	H	32	C:PRO 544		8
F:TYR 56		65	C:PRO 545	H	77
F:ALA 95		6	C:LEU 546		153
F:SER 96		22	C:GLY 547	H	27
F:GLN 97		89	C:ASN 548		51
F:ILE 98		95	C:TRP 549		22
			C:PRO 567		27
			C:PRO 568		7
			C:CYS 569		4
			C:SER 595		9
			C:ARG 596		14
			C:GLY 634		15
			C:VAL 636		8
Total		617	**Total**		630

Hydrogen bonds

##	Heavy chain	Distance (Å)	$E2core_{1a53}$
1	F:SER 31[O]	3.2	C:THR 542[N]
2	F:SER 31[O]	2.8	C:THR 542[OG1]
3	F:SER 52[OG]	3.8	C:GLY 547[O]
4	F:ASN 54[ND2]	2.7	C:PRO 545[O]

List of HEPC46 light chain (chain B)-$E2ecto_{1a53}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Light chain	Bond	BSA (Å^2)	$E2ecto_{1a53}$	Bond	BSA (Å^2)
B:GLY 29		1	C:GLY 470		1
B:SER 30		37	C:PRO 471		1
B:ASN 31	H	16	C:ILE 472		12
B:TYR 32	H	72	C:SER 473		9
B:ASN 51		1	C:HIS 474	H	29
B:LYS 66		1	C:LEU 546		28
B:TRP 91		28	C:GLU 591		35
B:ASP 93	HS	21	C:ARG 596	HS	52
Total		177	**Total**		168

Hydrogen bonds

##	Light chain	Distance (Å)	$E2ecto_{1a53}$
1	B:ASN 31[OD1]	3.8	C:ARG 596[NH2]
2	B:TYR 32[OH]	3.4	C:HIS 474[N]
3	B:TYR 32[OH]	2.7	C:HIS 474[ND1]
4	B:ASP 93[OD1]	3.5	C:ARG 596[NH2]
5	B:ASP 93[OD2]	3.3	C:ARG 596[NH2]

Salt bridges

##	Light chain	Distance (Å)	$E2ecto_{1a53}$
1	B:ASP 93[OD1]	3.5	C:ARG 596[NH2]
2	B:ASP 93[OD2]	3.3	C:ARG 596[NH2]

List of HEPC46 light chain (chain E)-$E2core_{1a53}$ (chain C) interface residues (the residues on each row are not matched interactive partners)

Light chain	Bond	BSA (Å^2)	$E2core_{1a53}$	Bond	BSA (Å^2)
E:SER 30		15.7337	C:PRO 545		5.86376
E:ASN 31	H	19.9429	C:LEU 546		24.9079
E:TYR 32		43.6506	C:CYS 569		25.165
E:GLN 53		10.3248	C:ALA 570		10.8951
E:TRP 91		31.212	C:ARG 596	HS	87.1744
E:ASP 93	HS	16.9637	C:CYS 597		1.15859
Total		138	**Total**		155

Hydrogen bonds

##	Light chain	Distance (Å)	$E2core_{1a53}$
1	E:ASN 31[OD1]	2.6	C:ARG 596[NH2]
2	E:ASP 93[OD2]	2.5	C:ARG 596[NH2]

Salt bridges

##	Light chain	Distance (Å)	$E2core_{1a53}$
1	E:ASP 93[OD1]	3.1	C:ARG 596[NH2]
2	E:ASP 93[OD2]	2.5	C:ARG 596[NH2]

HEPC46 heavy chain (A) and light chain (B) residues interacting with $E2ecto_{1a53}$ glycans (chain C) (interactive partners are grouped together)

Ab chain	Bond	BSA (Å^2)	$E2ecto_{1a53}$	Bond	BSA (Å^2)
A:GLY 26		8	C:NAG5401		81
A:TYR 27		17			
A:ILE 28		38			
A:SER 31		3			
A:HIS 32		4			
Total		70	**Total**		81

HEPC46 heavy chain (F) and light chain (E) residues interacting with $E2core_{1a53}$ glycans (chain C) (interactive partners are grouped together)

Ab chain	Bond	BSA (Å^2)	$E2core_{1a53}$	Bond	BSA (Å^2)
F:GLY 26		4	C:NAG5401	H	73
F:TYR 27		16			
F:ILE 28	H	37			
Total		57	**Total**		73

* Type of putative interation: H - hydrogen bond, S - salt bridge. BSA: buried surface area, Å^2.