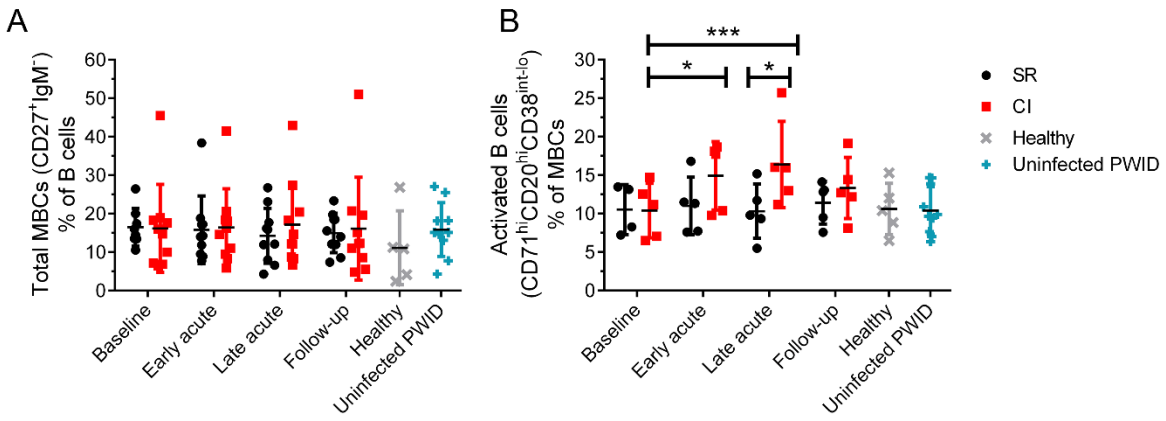
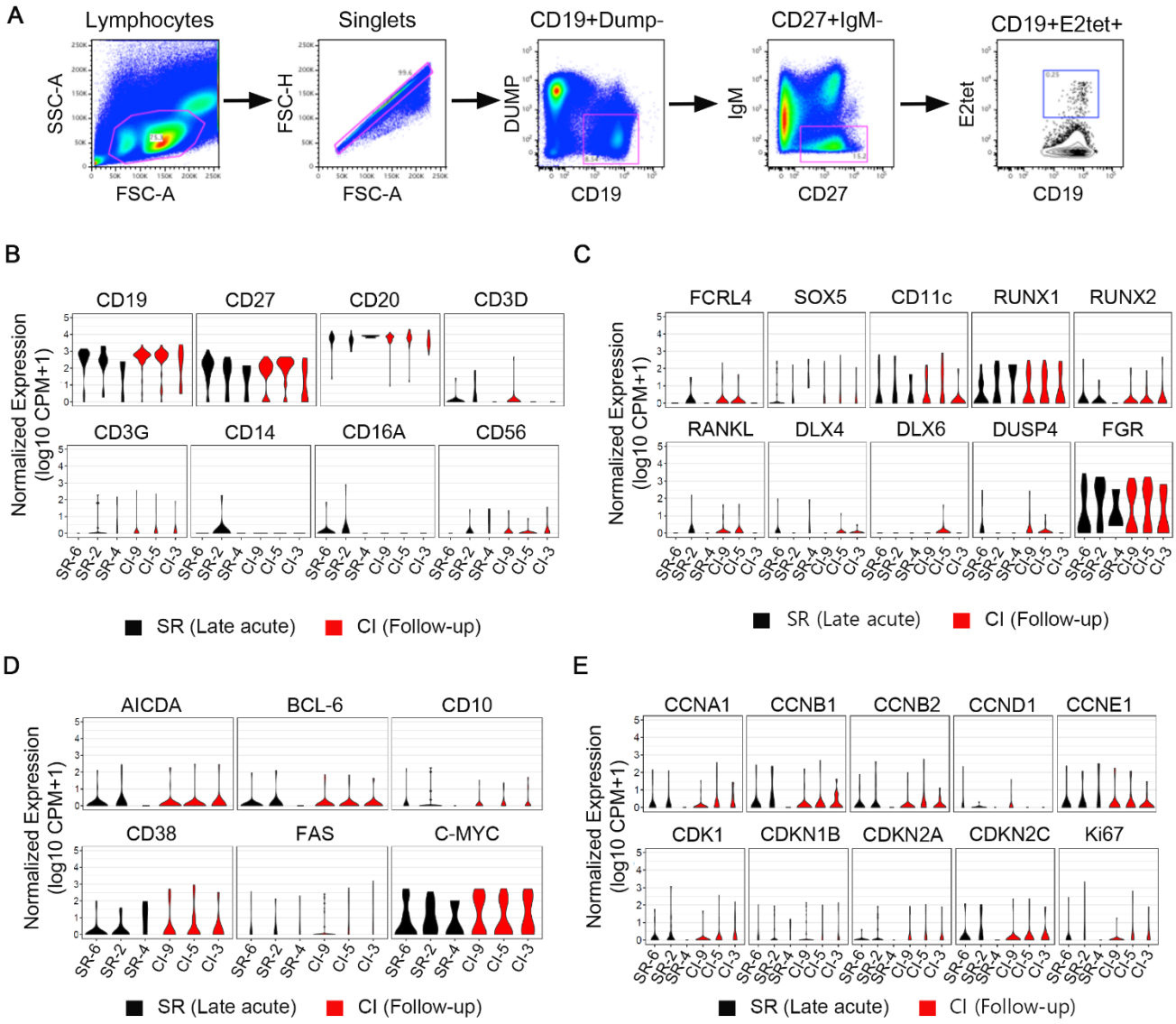
SUPPLEMENTAL FIGURES AND TABLES

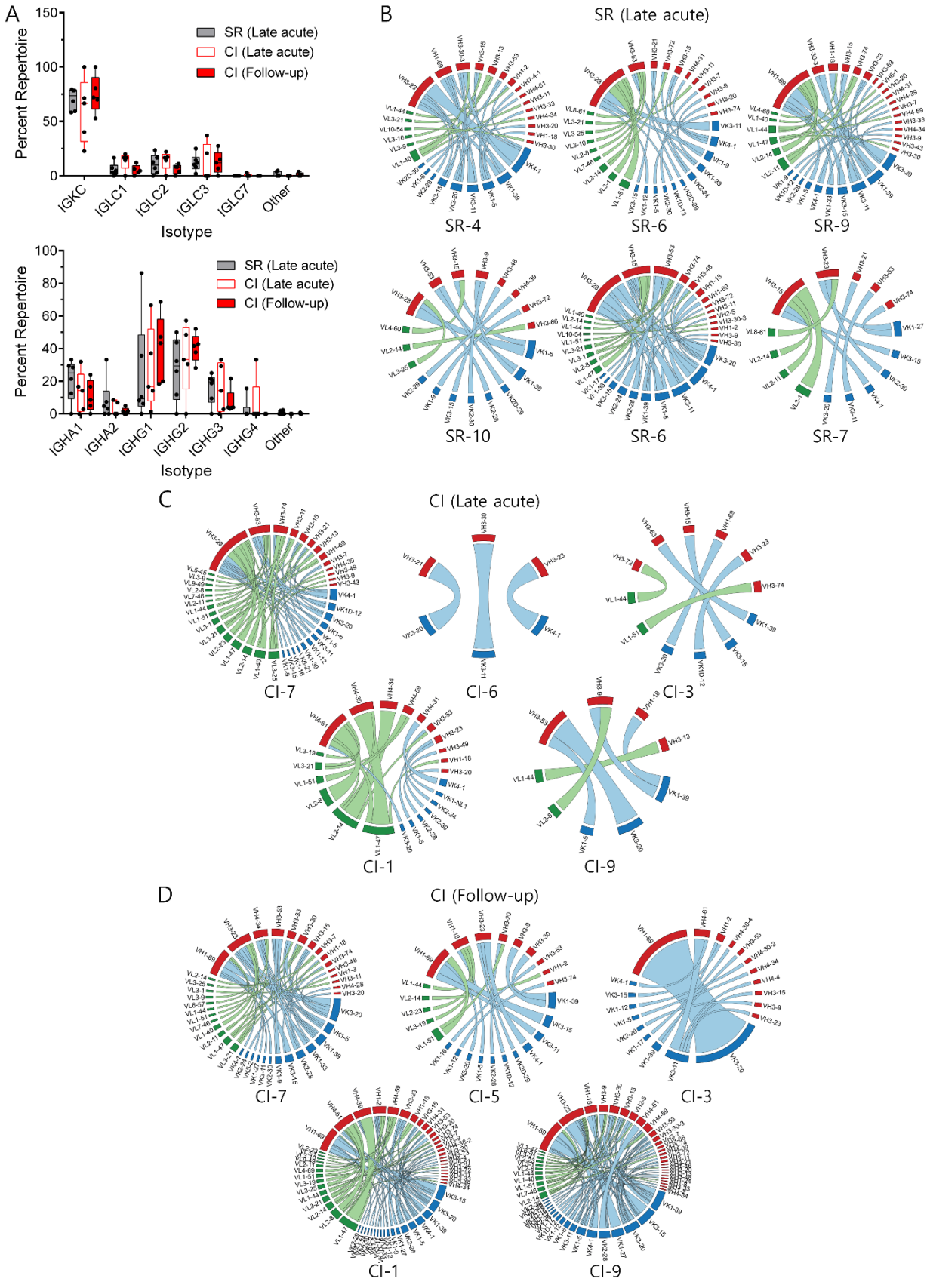


Supplemental Figure 1. Total, activated B cells increase during acute infection in chronically-infected subjects but not in resolvers

(**A**) Frequency of bulk MBCs ($CD27^{+}IgM^{-}$) for resolvers (n=10, black) and chronically-infected subjets (n=10, red) at different time points: baseline, > 8 weeks before EDI; early acute, 8 ± 2 weeks after EDI; late acute, 20 ± 4 weeks after EDI; follow-up, > 51 weeks after EDI. (**B**) Frequency of bulk-activated MBCs ($CD71^{+}CD20^{hi}CD38^{int-lo}$). Controls are healthy donors (n=10, gray) and PWID without HCV infection (n=10, teal). Results are expressed as the mean of subjects for each group and error bars represent SD. Two-way repeated measure ANOVA with Tukey post-hoc test, *p< 0.05; **p< 0.01; ***p< 0.001.

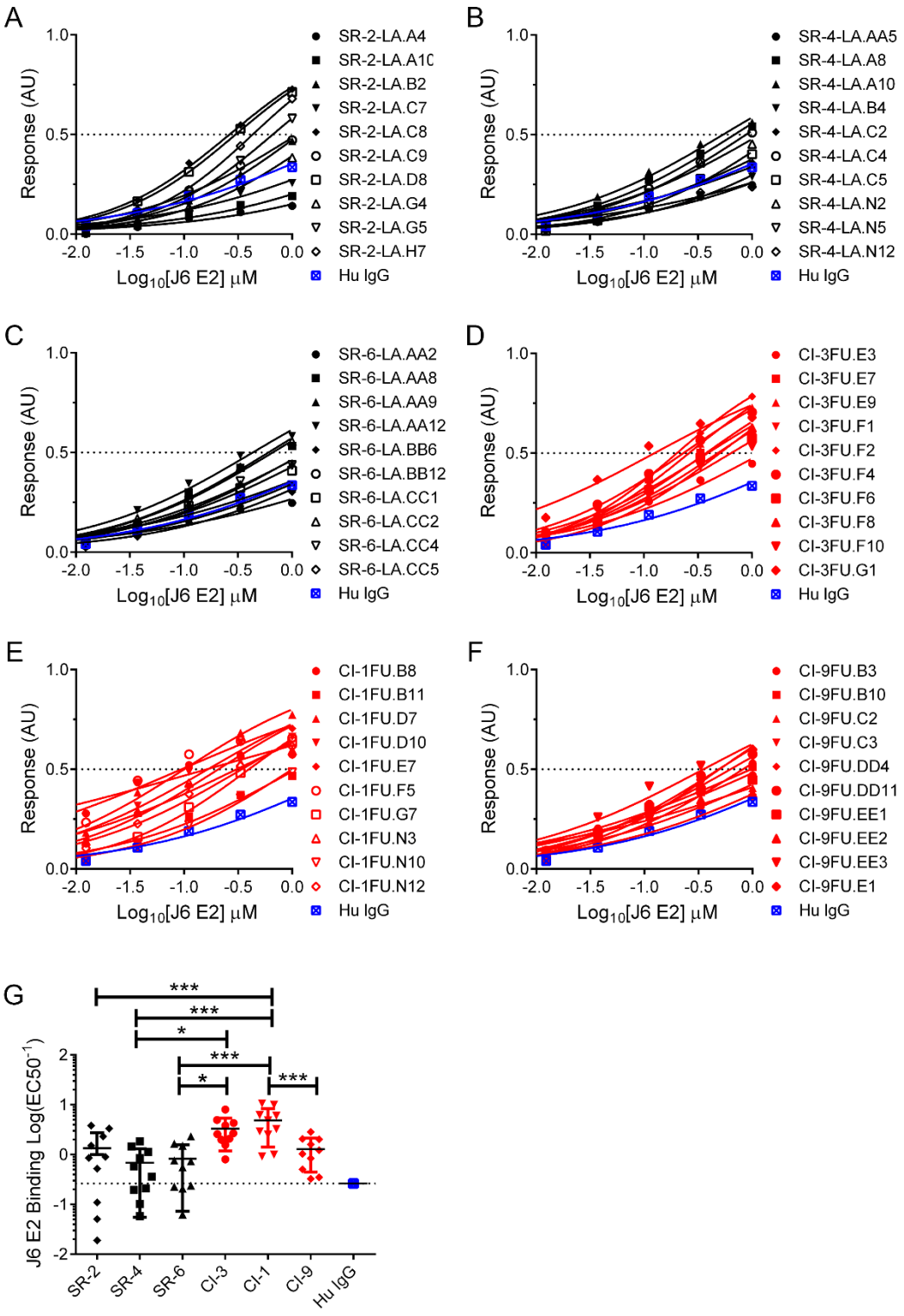


Supplemental Figure 2. E2-specific MBC from resolvers and chronically-infected subjects express low levels of genes associated with B cell exhaustion, proliferation, and germinal center. (**A**) Gating strategy for sorting single E2-specific MBCs (live, dump (CD3CD14CD16)$^-$, CD19+CD27+IgM$^-$E2-tet+). (**B-G**) Violin plots showing differentially expressed genes (DEGs) between E2-specific MBCs of resolvers (black, n=3) and chronically-infected subjects (red, n=3) for MBC markers (**B**), B-cell exhaustion (**C**), GC markers (**D**), and proliferation (**E**). Data are shown as counts per million (CPM) in a $\log_{10}$ scale. For all analyses, number of single cells from 47 spontaneous resolvers and 95 CIs. Statistical significance was determined based on adjusted p-value < 0.05 and fold-change greater than 1.5.


A
Percent Repertoire
SR (Late acute)
CI (Late acute)
CI (Follow-up)
IGKC
IGLC1
IGLC2
IGLC3
IGLC7
Other
Isotype
IGHA1
IGHA2
IGHG1
IGHG2
IGHG3
IGHG4
B
SR (Late acute)
SR-4
SR-6
SR-9
SR-10
SR-6
SR-7
C
CI (Late acute)
CI-7
CI-6
CI-3
CI-1
CI-9
D
CI (Follow-up)
CI-7
CI-5
CI-3
CI-1
CI-9

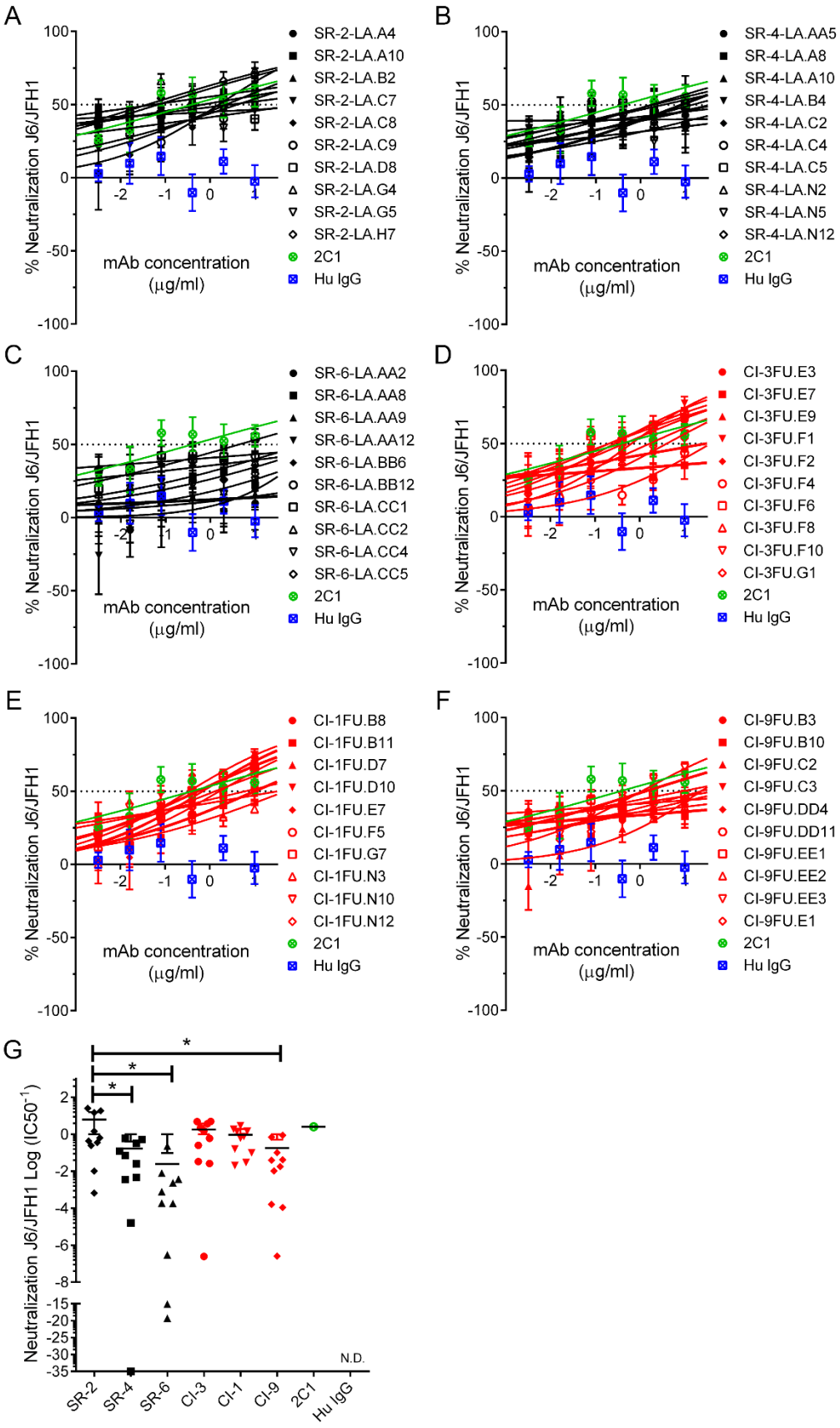
Supplemental Figure 3. BCR repertoire of E2-specific MBCs from resolvers and chronically-infected subjects have similar Ig isotypes but differ in V gene usage.

(**A**) Antibody isotype of heavy (top) and light (bottom) chains of total isolated E2-specific MBCs from resolvers at late acute (n=6, gray bars) and chronically-infected subjects at late acute (n = 5, red border, white bars) and follow-up (n=5, red bars). Each dot represents one individual subject. Results are shown as means of subjects from each group and error bars represent SD. (**B-D**) Circos diagrams showing relative frequencies of V gene pairs from heavy and light chains of E2-specific MBCs from individual resolvers at late acute (n=6, **B**), chronically-infected subjects at late acute (n=5, **C**), and follow-up (n=5, **D**). The width of each ribbon indicates the frequency of the VH-VK/VL pairing. Arc length corresponds to V gene frequency.


A
Response (AU)
Log10[J6 E2] μM
SR-2-LA.A4
SR-2-LA.A10
SR-2-LA.B2
SR-2-LA.C7
SR-2-LA.C8
SR-2-LA.C9
SR-2-LA.D8
SR-2-LA.G4
SR-2-LA.G5
SR-2-LA.H7
Hu IgG
B
SR-4-LA.AA5
SR-4-LA.A8
SR-4-LA.A10
SR-4-LA.B4
SR-4-LA.C2
SR-4-LA.C4
SR-4-LA.C5
SR-4-LA.N2
SR-4-LA.N5
SR-4-LA.N12
C
SR-6-LA.AA2
SR-6-LA.AA8
SR-6-LA.AA9
SR-6-LA.AA12
SR-6-LA.BB6
SR-6-LA.BB12
SR-6-LA.CC1
SR-6-LA.CC2
SR-6-LA.CC4
SR-6-LA.CC5
D
CI-3FU.E3
CI-3FU.E7
CI-3FU.E9
CI-3FU.F1
CI-3FU.F2
CI-3FU.F4
CI-3FU.F6
CI-3FU.F8
CI-3FU.F10
CI-3FU.G1
E
CI-1FU.B8
CI-1FU.B11
CI-1FU.D7
CI-1FU.D10
CI-1FU.E7
CI-1FU.F5
CI-1FU.G7
CI-1FU.N3
CI-1FU.N10
CI-1FU.N12
F
CI-9FU.B3
CI-9FU.B10
CI-9FU.C2
CI-9FU.C3
CI-9FU.DD4
CI-9FU.DD11
CI-9FU.EE1
CI-9FU.EE2
CI-9FU.EE3
CI-9FU.E1
G
J6 E2 Binding Log(EC50-1)
SR-2
SR-4
SR-6
CI-3
CI-1
CI-9
Hu IgG

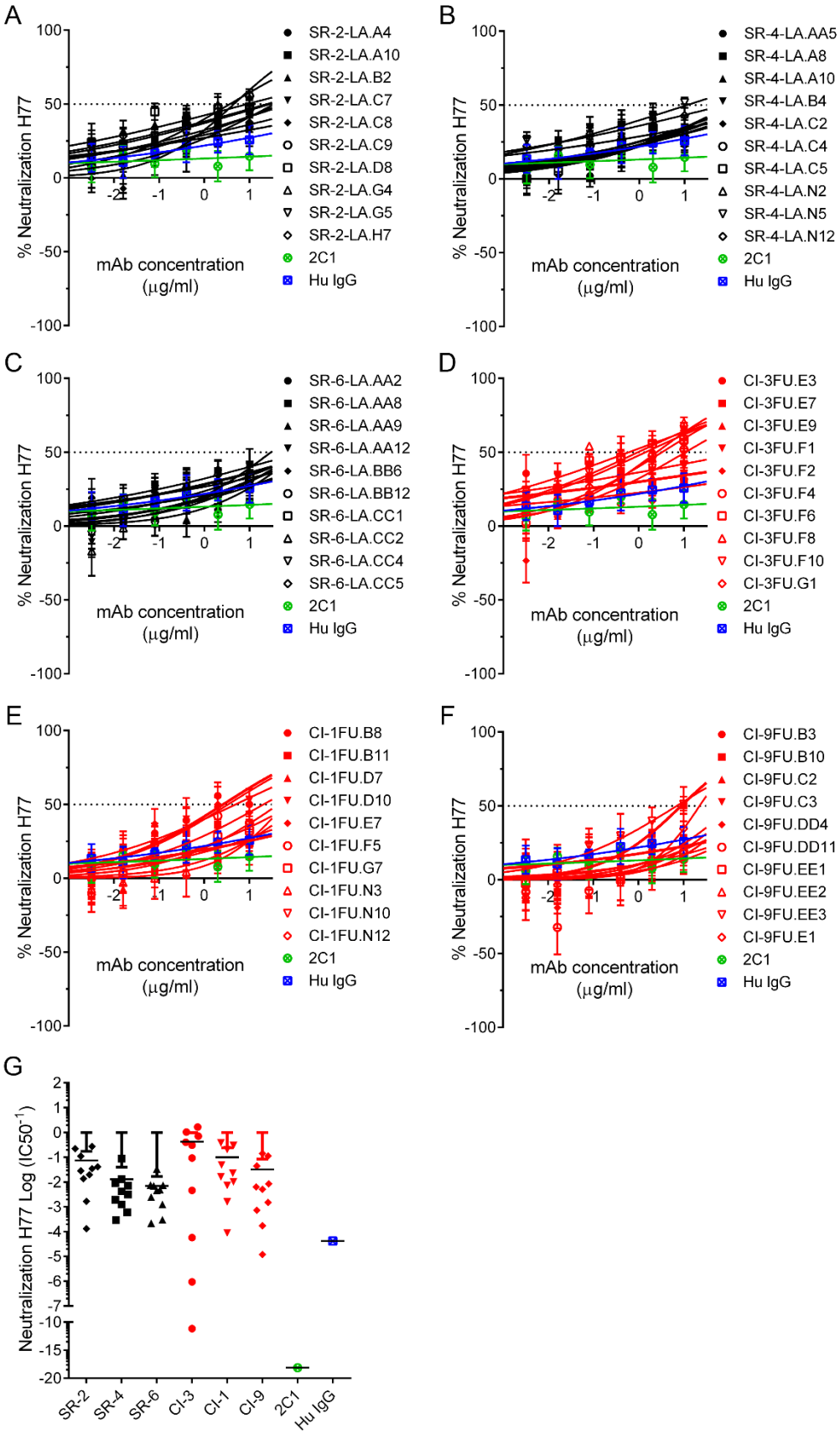
Supplemental Figure 4. MAbs from chronically-infected subjects bind to J6 E2 more efficiently than mAbs from resolvers.

(**A–F**) Binding curves from individual mAbs derived from 3 resolvers (**A–C**, 10 from each subject) and 3 chronically-infected subjects (**D–F**, 10 from each subject). Each symbol shows data from a single mAb; curves are 4PL dose-response nonlinear regression curves fitted to response intensities as a function of increasing J6 E2 molar concentrations. Binding curve of isotype human IgG antibody is represented in blue. (**G**) Combined data from **A-F** pooled for each subject, represented as mean ± SD. One-way ANOVA with Tukey post-hoc test, *p< 0.05; ***p< 0.001.


A
% Neutralization J6/JFH1
mAb concentration (μg/ml)
SR-2-LA.A4
SR-2-LA.A10
SR-2-LA.B2
SR-2-LA.C7
SR-2-LA.C8
SR-2-LA.C9
SR-2-LA.D8
SR-2-LA.G4
SR-2-LA.G5
SR-2-LA.H7
2C1
Hu IgG
B
SR-4-LA.AA5
SR-4-LA.A8
SR-4-LA.A10
SR-4-LA.B4
SR-4-LA.C2
SR-4-LA.C4
SR-4-LA.C5
SR-4-LA.N2
SR-4-LA.N5
SR-4-LA.N12
C
SR-6-LA.AA2
SR-6-LA.AA8
SR-6-LA.AA9
SR-6-LA.AA12
SR-6-LA.BB6
SR-6-LA.BB12
SR-6-LA.CC1
SR-6-LA.CC2
SR-6-LA.CC4
SR-6-LA.CC5
D
CI-3FU.E3
CI-3FU.E7
CI-3FU.E9
CI-3FU.F1
CI-3FU.F2
CI-3FU.F4
CI-3FU.F6
CI-3FU.F8
CI-3FU.F10
CI-3FU.G1
E
CI-1FU.B8
CI-1FU.B11
CI-1FU.D7
CI-1FU.D10
CI-1FU.E7
CI-1FU.F5
CI-1FU.G7
CI-1FU.N3
CI-1FU.N10
CI-1FU.N12
F
CI-9FU.B3
CI-9FU.B10
CI-9FU.C2
CI-9FU.C3
CI-9FU.DD4
CI-9FU.DD11
CI-9FU.EE1
CI-9FU.EE2
CI-9FU.EE3
CI-9FU.E1
G
Neutralization J6/JFH1 Log (IC50-1)
N.D.
SR-2
SR-4
SR-6
CI-3
CI-1
CI-9
2C1
Hu IgG

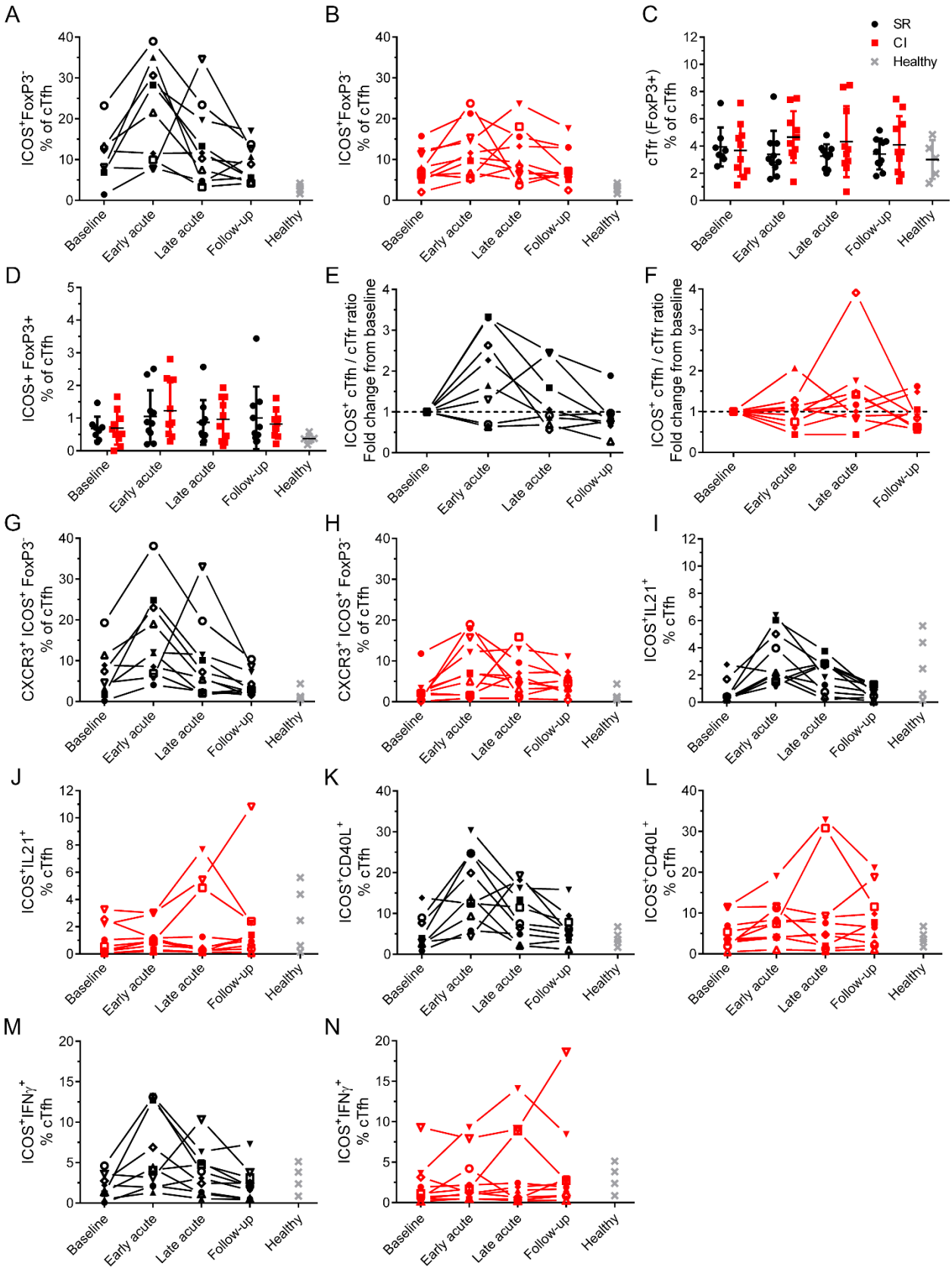
Supplemental Figure 5. MAbs from resolvers and chronically-infected subjects neutralize J6/JFH1 HCVpp with comparable efficiency.

(**A–F**) Neutralization curves against J6-JFH1 HCVpp from individual mAbs derived from 3 resolvers (**A–C**, 10 from each subject) and 3 chronically-infected subjects (**D–F**, 10 from each subject). Each symbol shows data from a single mAb; curves are 4PL dose-response nonlinear regression curves fitted to % neutralization as a function of increasing mAb concentrations. Neutralization curves of 2C1 monoclonal anti-HCV E2 antibody (green); isotype human IgG (blue) were used as control. (**G**) Combined data from **A–F** pooled for each subject, shown as mean ± SD. Results are from three independent experiments. One-way ANOVA with Dunn's multiple comparisons test, *$p < 0.05$.


A
% Neutralization H77
mAb concentration (µg/ml)
SR-2-LA.A4
SR-2-LA.A10
SR-2-LA.B2
SR-2-LA.C7
SR-2-LA.C8
SR-2-LA.C9
SR-2-LA.D8
SR-2-LA.G4
SR-2-LA.G5
SR-2-LA.H7
2C1
Hu IgG
B
SR-4-LA.AA5
SR-4-LA.A8
SR-4-LA.A10
SR-4-LA.B4
SR-4-LA.C2
SR-4-LA.C4
SR-4-LA.C5
SR-4-LA.N2
SR-4-LA.N5
SR-4-LA.N12
C
SR-6-LA.AA2
SR-6-LA.AA8
SR-6-LA.AA9
SR-6-LA.AA12
SR-6-LA.BB6
SR-6-LA.BB12
SR-6-LA.CC1
SR-6-LA.CC2
SR-6-LA.CC4
SR-6-LA.CC5
D
CI-3FU.E3
CI-3FU.E7
CI-3FU.E9
CI-3FU.F1
CI-3FU.F2
CI-3FU.F4
CI-3FU.F6
CI-3FU.F8
CI-3FU.F10
CI-3FU.G1
E
CI-1FU.B8
CI-1FU.B11
CI-1FU.D7
CI-1FU.D10
CI-1FU.E7
CI-1FU.F5
CI-1FU.G7
CI-1FU.N3
CI-1FU.N10
CI-1FU.N12
F
CI-9FU.B3
CI-9FU.B10
CI-9FU.C2
CI-9FU.C3
CI-9FU.DD4
CI-9FU.DD11
CI-9FU.EE1
CI-9FU.EE2
CI-9FU.EE3
CI-9FU.E1
G
Neutralization H77 Log (IC50-1)
SR-2
SR-4
SR-6
CI-3
CI-1
CI-9
2C1
Hu IgG

Supplemental Figure 6. MAbs from resolvers and chronically-infected subjects neutralize H77 HCVpp with comparable efficiency.

(**A-F**) Neutralization curves against H77 HCVpp from individual mAbs derived from 3 resolvers (**A-C**, n=10 for each subject) and 3 chronically-infected subjects (**D–F**, n=10) subjects. Each symbol provides data from a single mAb; curves are 4PL dose-response nonlinear regression curves fitted to % neutralization as a function of increasing mAb concentrations. Neutralization curves of 2C1 monoclonal anti-HCV E2 antibody (green); isotype human IgG (blue) were used as control. (**G**) Combined data from **A–F** pooled for each subject, shown as mean ± SD. Results are from three independent experiments. One-way ANOVA with Dunn's multiple comparisons test, all non-significant (ns, $p > 0.05$).


A
B
C
D
E
F
G
H
I
J
K
L
M
N
SR
CI
Healthy
Baseline
Early acute
Late acute
Follow-up
Healthy
ICOS+FoxP3- % of cTfh
cTfr (FoxP3+) % of cTfh
ICOS+ FoxP3+ % of cTfh
ICOS+ cTfh / cTfr ratio Fold change from baseline
CXCR3+ ICOS+ FoxP3- % of cTfh
ICOS+IL21+ % cTfh
ICOS+CD40L+ % cTfh
ICOS+IFNγ+ % cTfh

Supplemental Figure 7. Resolvers but not chronically-infected subjects have increased activation and function of cTfh cells during EA infection. cTfh analysis for resolvers (**A, E, G, I, K, M**; n=10; black) and chronically-infected subjects (**B, F, H, J, L, N**; n=10; red) for indicated time points (see Figure S1). Each symbol connected with line represent a single subject. (**A-B**) Frequencies of activated cTfh cells (ICOS+) as % total cTfh (CXCR5+PD1+CD45RA-FoxP3-). (**C**) Frequencies of total regulatory cTfh (cTfr, FoxP3+) as frequency of cTfh for both groups. (**D**) Frequencies of activated cTfr (ICOS+ FoxP3+) as frequency of cTfh for both groups. (**E-F**) Ratio of activated cTfh over cTfr ratio cells, shown as fold-change from baseline for indicated time points. (**G-H**) Frequencies of Th1-like activated cTfh cells (CXCR3+ICOS+) as % total cTfh. (**I-J**) Frequencies of IL-21-producing activated cTfh cells after 5 hrs stimulation with PMA and ionomycin. (**K-l**) Frequencies of CD40L-expressing activated cTfh cells after 5 hrs stimulation with PMA and ionomycin. (**M-N**) Frequencies of IFNγ-producing activated cTfh cells after 5 hrs stimulation with PMA and ionomycin. Results are expressed as the mean of subjects for each group and error bars represent SD. Two-way repeated measure ANOVA with Tukey post-hoc test, *$p < 0.05$; **$p < 0.01$.

Supplemental Table 1: Subject demographics, disease parameters, and immune analyses

Subject	Group	Sex	Age at EDI	Time between last negative and first positive (days)	Time point	Time from EDI (days)	HCV Genotype	HCV RNA	Viral load	AST (U/L)	ALT (U/L)	Anti-E2 and anti-NS3 ELISA (plasma)	Anti-HBs and anti-Rubella (plasma)	HCVpp neutralization (plasma)	Flow cytometry	Flow cytometry activated B cells	Single cell sort and RNA-seq	Single cell sort and Repertoire analysis	mAbs cloning
SR-1	HCV Resolver	M	39	92 (Ab*)	Baseline	-218	3	-	ND	20	17	X	X	X	X	X			
					Early acute	55		+	105	22	41	X	X	X	X	X			
					Late Acute	129		+	31	40	83	X	X	X	X	X			
					Follow-up	305		-	0	16	11	X	X	X	X	X			
SR-2	HCV Resolver	F	21	87 (RNA)	Baseline	-140	1a	-	ND	27	16	X	X	X	X				
					Early acute	44		+	17,095,395	32	23	X	X	X	X				
					Late Acute	141		+	349,856	292	117	X	X	X	X		X	X	X
					Follow-up	497		-	0	26	18	X	X	X	X				
SR-3	HCV Resolver	F	32	84 (Ab/RNA)	Baseline	-224	3a	-	ND	27	26	X	X	X	X	X			
					Early acute	42		+	4,356,748	807	618	X	X	X	X	X			
					Late Acute	134		-	0	29	27	X	X	X	X	X			
					Follow-up	197		-	0	28	19	X	X	X	X	X			
SR-4	HCV Resolver	M	49	97 (RNA)	Baseline	-138	2	-	ND	29	10	X	X	X	X				
					Early acute	79		+	61	17	38	X	X	X	X				
					Late Acute	179		-	0	14	13	X	X	X	X		X	X	X
					Follow-up	436		-	0	21	11	X	X	X	X				
SR-5	HCV Resolver	M	28	103 (Ab/RNA)	Baseline	-51	3a	-	ND	29	12	X	X	X	X	X			
					Early acute	52		+	1,615	805	1183	X	X	X	X	X			
					Late Acute	135		-	210	43	24	X	X	X	X	X			
					Follow-up	402		-	0	27	13	X	X	X	X	X			
SR-6	HCV Resolver	M	31	93 (RNA)	Baseline	-46	3a	-	ND	23	13	X	X	X	X				
					Early acute	47		+	5,238,000	150	236	X	X	X	X				
					Late Acute	151		+	14	30	34	X	X	X	X		X	X	X
					Follow-up	612		-	0	20	13	X	X	X	X				
SR-7	HCV Resolver	F	19	84 (Ab)	Baseline	-42	1	-	ND	44	33	X	X	X	X	X			
					Early acute	55		+	380	380	692	X	X	X	X	X			
					Late Acute	139		-	0	54	52	X	X	X	X	X		X	
					Follow-up	248		-	0	27	20	X	X	X	X	X			

SR-8	HCV Resolver	M	37	90 (RNA)	Baseline	-234	1a	-	ND	59	33	X	X	X	X				
					Early acute	68		+	1,152,815	28	28	X	X	X	X				
					Late Acute	180		-	0	24	24	X	X	X	X				
					Follow-up	446		-	0	23	11	X	X	X	X				
SR-9	HCV Resolver	F	21	N/A	Baseline	No Baseline**	2					N/A	N/A	N/A	X				
					Early acute	70		+	12,468	54	86	X	X	X	X				
					Late Acute	158		-	0	35	47	X	X	X	X			X	
					Follow-up	384		-	0	64	80	X	X	X	X				
SR-10	HCV Resolver	F	21	N/A	Baseline	No Baseline**	1					N/A	N/A	N/A	N/A	N/A			
					Early acute	75		+	24,690	27	70	X	X	X	X	X			
					Late Acute	131		-	0	26	21	X	X	X	X	X		X	
					Follow-up	389		-	0	20	13	X	X	X	X	X			
CI-1	Chronic HCV	M	21	92 (Ab/RNA)	Baseline	-46	1a	-	ND	31	31	X	X	X	X				
					Early acute	58		+	2,181,570	284	566	X	X	X	X				
					Late Acute	177		+	114,720	116	216	X	X	X	X			X	
					Follow-up	428		+	116,815	167	275	X	X	X	X		X	X	X
CI-2	Chronic HCV	M	48	70 (RNA)	Baseline	-147	1a	-	ND	33	18	X	X	X	X				
					Early acute	89		+	11,487,369	684	497	X	X	X	X				
					Late Acute	195		+	3,854,523	369	170	X	X	X	X				
					Follow-up	641		+	14,950,132	44	27	X	X	X	X				
CI-3	Chronic HCV	M	29	91 (Ab)	Baseline	-45	1a	-	ND	31	19	X	X	X	X				
					Early acute	89		+	636,978	289	607	X	X	X	X				
					Late Acute	180		+	55,733	16	10	X	X	X	X			X	
					Follow-up	554		+	404,980	65	97	X	X	X	X		X	X	X
CI-4	Chronic HCV	M	35	70 (RNA)	Baseline	-111	3a	-	ND	28	20	X	X	X	X	X			
					Early acute	55		+	216,852	595	773	X	X	X	X	X			
					Late Acute	135		+	8,143,140	ND	ND	X	X	X	X	X			
					Follow-up	273		+	191,290	145	301	X	X	X	X	X			
CI-5	Chronic HCV	M	55	87 (RNA)	Baseline	-43	1a	-	ND	22	18	X	X	X	X	X			
					Early acute	44		+	2,957,999	25	24	X	X	X	X	X			
					Late Acute	136		+	251	81	367	X	X	X	X	X			
					Follow-up	476		+	93,209	95	202	X	X	X	X	X		X	
CI-6	Chronic HCV	F	27	80 (RNA)	Baseline	-229	1a	-	ND	25	16	X	X	X	X	X			
					Early acute	68		+	3,192	175	744	X	X	X	X	X			
					Late Acute	141		-	109	28	36	X	X	X	X	X		X	

					Follow-up	383		+	2,991,514	282	638	X	X	X	X	X			
CI-7	Chronic HCV	F	40	123 (RNA)	Baseline	-61	1a	-	ND	11	11	X	X	X	X	X			
					Early acute	62		+	> 100 000 000	23	27	X	X	X	X	X			
					Late Acute	124		+	12,852,282	403	429	X	X	X	X	X		X	
					Follow-up	327		+	5,724,912	48	42	X	X	X	X	X		X	
CI-8	Chronic HCV	F	40	81 (RNA)	Baseline	-131	1a	-	ND	29	10	X	X	X	X	X			
					Early acute	58		+	1,919,086	107	186	X	X	X	X	X			
					Late Acute	140		+	3,444,866	115	153	X	X	X	X	X			
					Follow-up	484		+	694,224	71	124	X	X	X	X	X			
CI-9	Chronic HCV	M	34	101 (RNA)	Baseline	-50	1a	-	ND	16	15	X	X	X	X				
					Early acute	51		+	1,727,451	71	129	X	X	X	X				
					Late Acute	155		+	430,185	85	96	X	X	X	X			X	
					Follow-up	392		+	325,022	33	39	X	X	X	X		X	X	X
CI-10	Chronic HCV	M	33	145 (Ab/RNA)	Baseline	-268	3	-	ND	15	10	X	X	X	X				
					Early acute	91		+	29	33	32	X	X	X	X				
					Late Acute	187		+	73	23	22	X	X	X	X				
					Follow-up	360		+	666,827	56	115	X	X	X	X				

* Type of test that was positive first (Ab: Antibody or RNA)

** No baseline sample available for research (only clinical data of HCV negative test)

X: Sample utilized for experiment

N/A: No baseline blood sample available for these subjects

ND: Not done

Supplemental Table 2: Number and plate designation of single, E2-specific MBC sequenced for each subject.

Subject	Plate ID													Total
	P1S1	P2S23[a]	P3S2	P4S2	P6S3	P7S4	P8S3	P9S3	P10S3	P11S4	P12S3	P13S3	P14S4	
CI-7_FU	0	0	0	0	0	0	0	0	0	94	0	0	0	94
CI-5_FU	0	0	0	0	33	0	0	0	0	0	0	0	0	33
CI-3_FU	17	0	0	0	0	0	0	0	0	0	0	0	0	17
CI-1_FU	0	0	0	0	0	0	87	0	0	0	0	0	0	87
CI-9_FU	0	0	58	94	0	0	0	0	0	0	0	0	0	152
CI-7_LA	0	0	0	0	0	0	0	94	0	0	0	0	0	94
CI-6_LA	0	0	0	0	0	0	0	0	11	0	0	0	0	11
CI-5_LA	0	0	0	0	0	0	0	0	5	0	0	0	0	5
CI-3_LA	0	0	0	0	0	0	0	0	0	0	8	0	0	8
CI-1_LA	0	0	0	0	0	0	0	0	0	0	45	0	0	45
CI-9_LA	0	0	0	0	0	0	0	0	0	0	0	10	0	10
SR-4_LA	0	24	0	0	15	0	0	0	0	0	0	0	0	39
SR-9_LA	0	0	0	0	0	54	0	0	0	0	0	0	0	54
SR-2_LA	21	0	0	0	0	0	0	0	0	0	0	0	0	21
SR-10_LA	0	0	0	0	0	0	0	0	0	0	0	0	22	22
SR-6_LA	0	70	36	0	0	0	0	0	0	0	0	0	0	106
SR-7_LA	0	0	0	0	0	0	0	0	0	0	0	0	27	27

[a]P2S23 – used modified HMC protocol

Supplemental Table 3: BCR repertoire and functional characteristics of mAbs isolated from resolvers at late acute.

mAb	E2 Binding [EC50-1]	H77 Neutralization [IC50-1]	J6/JFH1 Neutralization [IC50-1]	VH Gene	JH Segment	VL Gene	JL Segment	CDR3H	CDR3L	CDR3H Length (aa)	SHM	H77 Rank[a]	J6/JFH1 Rank[a]	Sum of Ranks[b]
SR-2LA.G4	0.521028	0.041234	25.3815	IGHV1-69*10	IGHJ5*02	IGKV3-20*01	IGKJ1*01	ASKMGDGYNLR GPPWFDP	QQYGSSPET	18	6	18	1	19
SR-2LA.C8	3.812341	0.221344	0.639028	IGHV3-74*01,IGHV3-74*03	IGHJ4*02	IGLV2-11*01	IGLJ2*01,IGLJ3*01	ARAGHRSYAPFEY	CSYAGSYTP	13	6	9	17	26
SR-2LA.A10	0.051277	0.034904	1.460955	IGHV1-18*01	IGHJ3*02	IGLV1-47*02	IGLJ3*02	VRAGAIAYVVDAFDI	AAWDDSLSAWV	15	9	19	11	30
SR-2LA.C9	0.903971	0.271534	0.24686	IGHV3-30-3*01	IGHJ4*02	IGKV3-11*01	IGKJ2*01	ARDDAPGAIGYYFDY	QHRSNWPFMYT	15	4	8	26	34
SR-2LA.C7	0.109905	0.028405	0.436721	IGHV3-30-3*01	IGHJ5*02	IGKV1-39*01,IGKV1D-39*01	IGKJ3*01	ARPQAQGSGYTGSDWDTRKNWFDP	QQSYSTPPF	24	6	21	22	43
SR-2LA.A4	0.01902	0.019757	0.356173	IGHV6-1*01	IGHJ6*02	IGLV1-44*01	IGLJ2*01,IGLJ3*01	AGGRHYASGYYYGMDV	AAWDDSLNGPVV	16	4	23	23	46
SR-6LA.CC2	1.655092	0.03379	0.008033	IGHV3-53*01	IGHJ4*02,IGHJ5*02	IGKV2-24*01	IGKJ4*01	ARVGGLQGYFDSSGYG	MQATQFLT	16	39	20	43	63
SR-4LA.A8	1.460982	0.008838	0.123662	IGHV3-30-3*01	IGHJ5*02	IGLV1-40*01	IGLJ2*01,IGLJ3*01	ARPQAQGSGYTGSDWDTRKNWFDP	QSYDSTLSRDVV	24	14	28	29	57
SR-4LA.C5	0.581953	0.007092	0.321035	IGHV1-69*01,IGHV1-69*12,IGHV1-69D*01	IGHJ4*02	IGKV1-39*01,IGKV1D-39*01	IGKJ1*01	ARGRSYYYDSTASFGY	QQSYSHPGT	16	30	33	24	57
SR-2LA.B2	0.85841	0.00168	18.70727	IGHV4-59*08	IGHJ5*02	IGLV1-47*02	IGLJ3*02	ARHILKSPYNWLDP	AAWDDSLSAWV	14	3	44	2	46
SR-2LA.D8	3.343006	0.111446	0.000658	IGHV4-34*01	IGHJ6*02	IGKV1-33*01,IGKV1D-33*01	IGKJ5*01	ARGFMSSDDFLSDYFYYHYYSLDV	QQYDNLPIT	24	7	13	49	62
SR-2LA.G5	1.514734	0.01375	0.010333	IGHV1-2*02	IGHJ4*02,IGHJ5*02	IGKV1-39*01,IGKV1D-39*01	IGKJ1*01	ARVEVYYGXGMVG	QQSYSPLWT	13	13	25	42	67
SR-6LA.CC1	0.5571947	0.004898	0.228916	IGHV3-15*01	IGHJ4*02	IGLV1-47*01	IGLJ3*02	TTGPFYRGWELPLGDEY	AAWDDSLSGWV	17	11	37	27	64
SR-4LA.C2	0.36238	0.001254	0.600123	IGHV3-23*01,IGHV3-23*04,IGHV3-23D*01	IGHJ4*02	IGKV3-11*01	IGKJ4*01	AKDLHEWLVAGNFDS	QERDNRRPLT	15	31	47	20	67
SR-4LA.N5	0.195469	0.087996	0.000016	IGHV3-23*01,IGHV3-23D*01	IGHJ4*02	IGKV1-39*01,IGKV1D-39*01	IGKJ1*01	ARDLVGDMRGGADYFDC	QQSYSTPT	17	16	15	54	69
SR-4LA.N12	0.058247	0.013098	0.003575	IGHV3-53*01	IGHJ4*02	IGKV1-6*01	IGKJ1*01	TRVQYDYGGNLDF	LHDYNYPWT	13	17	26	46	72
SR-6LA.AA12	2.310509	0.00755	0.003738	IGHV3-53*02	IGHJ4*02,IGHJ5*02	IGKV4-1*01	IGKJ1*01	AAGSTAITLR	QEYYSTLWT	10	7	30	45	75
SR-4LA.A10	1.861293	0.004221	0.071456	IGHV3-23*01,IGHV3-23D*01	IGHJ3*01	IGKV4-1*01	IGKJ1*01	ARDGDDSRPPDAFGV	QQYYSTPST	15	26	40	32	72
SR-4LA.N2	0.845745	0.000603	0.529863	IGHV3-23*01,IGHV3-23D*01	IGHJ4*02	IGKV3-15*01	IGKJ4*01	AKGVDYYDSSGYRVYYFDY	QQYNNWPPLT	19	4	50	21	71

SR-2LA.H7	2.323291	0.000132	14.69457	IGHV1-69*10	IGHJ6*02	IGLV2-14*01	IGLJ3*02	ARDFPPLRMATTDYYGMDV	SSYTSTYTRV	19	8	55	3	58
SR-4LA.AA5	0.102871	0.003166	0.025257	IGHV4-61*01	IGHJ5*02	IGKV3-20*01	IGKJ4*01	ARGPYSSFHS	QQYGGSPLVT	10	27	41	38	79
SR-6LA.CC4	0.7316723	0.007358	0.000796	IGHV3-23*04	IGHJ6*02	IGKV2-28*01,IGKV2D-28*01	IGKJ2*01	AKGIYDYALDV	MQGLQTPYT	11	13	31	48	79
SR-6LA.BB6	0.2084555	0.007052	0.000181	IGHV3-11*04	IGHJ4*02	IGLV1-51*01	IGLJ2*01,IGLJ3*01	ATLSNWNYFHY	GTWDNSLSAVI	11	11	34	51	85
SR-4LA.B4	0.210361	0.000291	0.004639	IGHV4-34*01,IGHV4-34*12	IGHJ3*02	IGLV3-9*01	IGLJ1*01	ARKKARMTMTREVYAFDM	QVWDTSSFYV	18	32	52	44	96
SR-6LA.AA8	1.510681	0.004516	5.44267E-20	IGHV3-48*02	IGHJ5*02	IGLV2-8*01	IGLJ3*02	ARDWAYAFDL	SSYAGPMNWV	10	22	39	59	98
SR-6LA.AA2	0.06287165	0.002469	7.53918E-16	IGHV3-15*01	IGHJ4*02	IGLV1-47*01	IGLJ2*01,IGLJ3*01	TRGYCSGGSCSN	AAWDDSLSGRV	12	1	42	58	100
SR-6LA.CC5	0.2276717	0.000217	0.002383	IGHV3-15*01	IGHJ3*02	IGLV2-8*01	IGLJ1*01	ATDKPACGGDCYHALDI	SSYAGGNNFV	17	9	53	47	100
SR-6LA.BB12	0.2391654	0.000302	0.000189	IGHV3-23*01,IGHV3-23D*01	IGHJ4*02	IGKV3-11*01	IGKJ5*01	TKDEGRGGGLSPFDS	QQRINWPIT	15	15	51	50	101
SR-4LA.C4	1.138883	0.001932	1.00173E-35	IGHV3-23*01,IGHV3-23*04,IGHV3-23D*01	IGHJ6*02	IGLV1-44*01	IGLJ2*01,IGLJ3*01,IGLJ3*02	AKSIVTYYYYSSGYYSPGYYGMDV	AAWDDSLTGYVV	24	22	43	60	103
SR-6LA.AA9	0.762652	0.001252	2.98885E-07	IGHV3-15*01	IGHJ3*02	IGLV3-1*01	IGLJ1*01	TTDRYGAFDI	QAWDSSTVV	10	0	48	55	103

[a]Ranks were determined from pooled mAbs from both groups (regardless of infection outcome).

[b]Sum of Ranks for Neutralization of H77 and J6/JFH1 HCVpp.

29 **Supplemental Table 4: BCR repertoire and functional characteristics of mAbs isolated from chronically-infected individuals at follow-up.**

mAb	E2 Binding [EC50-1]	H77 Neutralization [IC50-1]	J6/JFH1 Neutralization [IC50-1]	VH Gene	JH Segment	VL Gene	JL Segment	CDR3H	CDR3L	CDR3H Length (aa)	SHM	H77 Rank[a]	J6/JFH 1 Rank[a]	Sum of Ranks[b]
CI-3FU.F8	2.67502	1.664572	4.881347	IGHV1-69*01,IGHV1-69D*01	IGHJ5*02	IGKV3-20*01	IGKJ1*01	VRCNLGSLFGGSCHGWLDP	QQYGTSPQT	19	21	1	4	5
CI-3FU.F6	2.081772	1.04535	4.870797	IGHV1-69*01,IGHV1-69D*01	IGHJ5*01,IGHJ5*02	IGKV3-20*01	IGKJ1*01	VRCNLGSLFGGSCYGWFDS	HQYGSSPHT	19	28	2	5	7
CI-3FU.F1	1.992488	0.712754	3.680959	IGHV1-69*01,IGHV1-69D*01	IGHJ5*02	IGKV3-20*01	IGKJ4*01	TGGRGSIVGGRFLGWFDP	QQYGSSPQT	18	23	3	6	9
CI-3FU.F2	4.96966	0.402486	2.248968	IGHV1-69*01,IGHV1-69D*01	IGHJ5*01,IGHJ5*02	IGKV3-20*01	IGKJ1*01	VRCNLGSLFGGSCYGWFDS	HQYGSSPHT	19	28	4	8	12
CI-1FU.B8	6.215929	0.382664	1.873191	IGHV4-61*01	IGHJ4*02,IGHJ5*02	IGKV3-15*01	IGKJ4*01	ARGSLPGIAVL	QQYNNWAPLT	11	22	5	9	14
CI-1FU.D10	6.055358	0.296519	2.942823	IGHV3-20*01	IGHJ6*02	IGKV2-28*01,IGKV2D-28*01	IGKJ2*01	ARDDWGTTVTAYYKGMDV	MQALQTPHT	18	12	7	7	14
CI-1FU.E7	4.835731	0.203206	1.442409	IGHV3-20*01	IGHJ6*02	IGKV2-28*01,IGKV2D-28*01	IGKJ5*01	ARGDVPLGYSNYRSYIGMDV	MQALQSPLT	20	10	10	13	23
CI-3FU.E9	3.843892	0.093069	1.541538	IGHV1-2*02	IGHJ5*02	IGKV3-15*01	IGKJ1*01	ANVLIIGYCSGGRCYSGWFDP	HQYDNWPRT	21	21	14	10	24
CI-9FU.EE3	2.853002	0.141953	0.882754	IGHV1-69*02	IGHJ6*02	IGLV7-46*01	IGLJ1*01	ARATVVVGEYFGMDV	LLSYSGARGPTSV	15	20	11	14	25
CI-1FU.G7	2.522985	0.010283	1.445812	IGHV4-39*01	IGHJ4*02	IGKV3-20*01	IGKJ3*01	ARHVPVWGLGHYHDPRYYFDY	QQYGESPGT	21	19	27	12	39
CI-1FU.F5	10.634	0.048929	0.609119	IGHV4-39*01	IGHJ4*02	IGKV1-39*01,IGKV1D-39*01	IGKJ2*01	ARTSDLKVAVAVD	QQSYSIPYT	13	20	16	18	34
CI-3FU.F4	4.328592	0.307083	0.026211	IGHV1-69*01,IGHV1-69D*01	IGHJ4*02	IGKV3-20*01	IGKJ2*01	ARGINLATFGFDY	QHFRSPYT	13	29	6	37	43
CI-9FU.E1	2.030321	0.044476	0.039923	IGHV3-9*01	IGHJ4*02	IGKV1-39*01,IGKV1D-39*01	IGKJ3*01	VKDKGRLEEVPGTLDY	QQSYSALLFT	16	11	17	34	51
CI-1FU.N12	2.935452	0.001598	0.763207	IGHV3-23*01,IGHV3-23D*01	IGHJ4*02	IGKV3-20*01	IGKJ2*01,IGKJ2*02	AGAGRYYGSGTYGVFNPFDY	QQYGSSPST	20	9	45	15	60
CI-1FU.D7	10.01723	0.00735	0.162034	IGHV1-69*06	IGHJ3*02	IGKV1-39*01,IGKV1D-39*01	IGKJ1*01	ARDSCSGGNCYPHDAFDI	QLSYSTLWT	18	17	32	28	60
CI-3FU.F10	1.56784	0.004598	0.602612	IGHV1-69*01,IGHV1-69D*01	IGHJ4*02	IGKV3-20*01	IGKJ5*01	ARAQGSLVQWLPTY	HQYGSSPIT	14	16	38	19	57
CI-1FU.N10	1.000649	0.022768	0.029883	IGHV1-2*02	IGHJ3*01,IGHJ3*02	IGKV3-20*01	IGKJ2*01,IGKJ2*02	ARAGTYYGDPFDF	QHYGSSTGFT	13	21	22	36	58
CI-9FU.C1	0.50706	0.008481	0.041897	IGHV1-69*01,IGHV1-69*12,IGHV1-69D*01	IGHJ4*02	IGKV4-1*01	IGKJ1*01	ARGRSYYYDSTASFGY	QQYYTTPLT	16	14	29	33	62
CI-9FU.B10	1.228562	0.112891	2.56179E-07	IGHV3-23*01 F, or	IGHJ4*02F	IGKV1-27*01	IGKJ1*01	AKGPRWDIVVAPAAQALDY	QKYNNAQWT	19	10	12	56	68

				IGHV3-23D*01 F										
CI-1FU.N3	3.279807	0.016314	0.020474	IGHV1-69*06	IGHJ3*02	IGKV1-39*01,IGKV1D-39*01	IGKJ1*01	ARDSCSGGNCYPHDAFDI	QLSYSTLWT	18	12	24	39	63
CI-9FU.B3	1.035848	0.000012	0.69992	IGHV3-30*18,IGHV3-30-5*01	IGHJ3*02	IGKV4-1*01	IGKJ3*01	AKDGTKCSGENCYSYGFDI	QQYYTTPLT	19	18	58	16	74
CI-9FU.EE1	0.83882	0.000172	0.101189	IGHV3-23*01,IGHV3-23D*01	IGHJ4*02	IGKV1-39*01,IGKV1D-39*01	IGKJ3*01	AKVRSGYYYDFDY	QQSYSTPS	13	10	54	30	84
CI-1FU.B11	0.923015	0.000089	0.098959	IGHV1-2*02	IGHJ5*02	IGKV1-12*01,IGKV1-12*02,IGKV1D-12*01	IGKJ3*01	ARVALYCSALSCSRRYNWFDP	QLPNRFPFT	21	31	56	31	87
CI-3FU.G1	8.030452	0.000001	0.250458	IGHV4-61*01	IGHJ4*02,IGHJ5*02	IGKV3-11*01	IGKJ2*01,IGKJ2*02	ARSSSAWYILL	QQRSEWPPVT	11	32	59	25	84
CI-9FU.DD11	2.058918	0.005107	0.017965	IGHV4-34*01	IGHJ4*02	IGKV1-5*01	IGKJ4*01	ARGAYSLSGPSLIPAGTPSFDY	QQCNSYPIT	22	28	36	40	76
CI-9FU.DD4	1.604307	0.006321	0.000163	IGHV3-66*02	IGHJ2*01	IGLV2-14*01	IGLJ1*01	ARDPPPLWDSSGYRGWYFDL	SSYTSSSTPYV	10	10	35	52	87
CI-9FU.C3	0.327655	0.000725	0.01038	IGHV1-69*01,IGHV1-69D*01	IGHJ4*02	IGKV1-6*01,IGKV1-6*02	IGKJ2*01,IGKJ2*02,IGKJ2*03	ARGRSYYYDSTASFGY	LQDYNFPS	16	10	49	41	90
CI-9FU.EE2	0.349718	0.001497	0.000111	IGHV3-53*01	IGHJ5*02	IGKV2-28*01,IGKV2D-28*01	IGKJ4*01	ARNRHCNGDSCYGA	MQDLQIPLT	14	11	46	53	99
CI-3FU.E3	0.806485	6.94835E-12	0.033393	IGHV3-9*01	IGHJ6*02	IGKV2-28*01,IGKV2D-28*01	IGKJ1*01	TKDRGVEAVLTGFPSGMDV	MQALQTPRT	19	7	60	35	95
CI-3FU.E7	2.578755	0.000057	2.4772E-07	IGHV3-53*01	IGHJ4*02	IGKV1-39*01,IGKV1D-39*01	IGKJ4*01	AKAAGWYGSGYFDY	QQSHRTPLT	14	14	57	57	114

[a]Ranks were determined from pooled mAbs from both groups (regardless of infection outcome).

[b]Sum of Ranks for Neutralization of H77 and J6/JFH1 HCVpp.

Supplemental Table 5: Flow cytometry antibodies

	Panel #1 Identification of E2-specific memory B cells (MBCs)			
Antigen	**Fluorophore**	**Clone**	**Catalog#**	**Provider**
CD3	APC-H7	SK7	641415	BD Biosciences
CD14	V500	M5E2	561391	BD Biosciences
CD16	V500	3G8	561393	BD Biosciences
CD19	BUV496	SJ25C1	612938	BD Biosciences
CD20	BV785	2H7	302355	BioLegend
CD27	APC-R700	M-T271	565116	BD Biosciences
CD38	BUV395	HB7	563811	BD Biosciences
CD56	BV510	NCAM16.2	563041	BD Biosciences
CD71	APC	CY1G4	334107	BioLegend
IgM	BB515	G20-127	564622	BD Biosciences
	Panel #2 E2-specific MBCs sorting			
CD3	APC-Cy7	UCHT1	300425	Biolegend
CD14	APC-Cy7	M5E2	301819	Biolegend
CD16	APC-Cy7	3G8	302017	Biolegend
CD19	BV421	HIB19	302233	Biolegend
CD27	APC-R700	M-T271	565116	BD Biosciences
IgM	BB515	G20-127	564622	BD Biosciences
	Panel #3 cTfh phenotyping			
CD3	BUV496	UCHT1	612940	BD Biosciences
CD4	BV605	RPA-T4	562658	BD Biosciences
CD8	BUV395	RPA-T8	563796	BD Biosciences
CD45RA	A700	HI100	560673	BD Biosciences
CD185 (CXCR5)	PerCP-Cy5.5	RF8B2	562781	BD Biosciences
CD278 (PD1)	BV421	EH12.1	562516	BD Biosciences
CD279 (ICOS)	PE	DX29	557802	BD Biosciences
CD 183 (CXCR3)	APC	1C6/CXCR3	561732	BD Biosciences
FoxP3	PE-Cy7	PCH101	12-4776-42	eBioscience
	Panel #4 cTfh stimulation and ICS			
CD3	BUV496	UCHT1	612940	BD Biosciences
CD4	BV605	RPA-T4	562658	BD Biosciences
CD8	BUV395	RPA-T8	563796	BD Biosciences
CD45RA	A700	HI100	560673	BD Biosciences
CD154 (CD40L)	APC-Cy7	TRAP1	563588	BD Biosciences
CD185 (CXCR5)	PerCP-Cy5.5	RF8B2	562781	BD Biosciences
CD278 (PD1)	BV421	EH12.1	562516	BD Biosciences
CD279 (ICOS)	PE	DX29	557802	BD Biosciences
IFNγ	PE-Cy7	B27	557643	BD Biosciences
IL-21	e660	eBio3A3-N2	50-7219-42	eBioscience

35 **Supplemental Table 6: Primers used in this study**

Primer Name	Sequence (5' to 3')	Purpose	Reference
L-VH1	ACAGGTGCCCACTCCCAGGTGCAG	Heavy Chain Round 1 PCR	Tiller et al., 2008
L-VH3	AAGGTGTCCAGTGTGARGTGCAG	Heavy Chain Round 1 PCR	Tiller et al., 2008
L-VH4/6	CCCAGATGGGTCCTGTCCCAGGTGCAG	Heavy Chain Round 1 PCR	Tiller et al., 2008
L-VH5	CAAGGAGTCTGTTCCGAGGTGCAG	Heavy Chain Round 1 PCR	Tiller et al., 2008
Cy CH1	GGAAGGTGTGCACGCCGCTGGTC	Heavy Chain Round 1 PCR	Tiller et al., 2008
Cu CH1	GGGAATTCTCACAGGAGACGA	Heavy Chain Round 1 PCR	Tiller et al., 2008
L-Vk1/2	ATGAGGSTCCCYGCTCAGCTGCTGG	Kappa Chain Round 1 PCR	Tiller et al., 2008
L-Vk3	CTCTTCCTCCTGCTACTCTGGCTCCCAG	Kappa Chain Round 1 PCR	Tiller et al., 2008
L-Vk4	ATTTCTCTGTTGCTCTGGATCTCTG	Kappa Chain Round 1 PCR	Tiller et al., 2008
Ck 543	GTTTCTCGTAGTCTGCTTTGCTCA	Kappa Chain Round 1 PCR	Tiller et al., 2008
L VL1	GGTCCTGGGCCCAGTCTGTGCTG	Lambda Chain Round 1 PCR	Tiller et al., 2008
L VL2	GGTCCTGGGCCCAGTCTGCCCTG	Lambda Chain Round 1 PCR	Tiller et al., 2008
L VL3	GCTCTGTGACCTCCTATGAGCTG	Lambda Chain Round 1 PCR	Tiller et al., 2008
L VL4/5	GGTCTCTCTCSCAGCYTGTGCTG	Lambda Chain Round 1 PCR	Tiller et al., 2008
L VL6	GTTCTTGGGCCAATTTTATGCTG	Lambda Chain Round 1 PCR	Tiller et al., 2008
L VL7	GGTCCAATTCYCAGGCTGTGGTG	Lambda Chain Round 1 PCR	Tiller et al., 2008
L VL8	GAGTGGATTCTCAGACTGTGGTG	Lambda Chain Round 1 PCR	Tiller et al., 2008
C lambda	CACCAGTGTGGCCTTGTTGGCTTG	Lambda Chain Round 1 PCR	Tiller et al., 2008
VH3a.S	SARGTGCAGCTGGTGGAG	Heavy Chain Round 2 PCR/Sequencing	Ho et al., 2016
VH3b.S	GAGGTGCAGCTGTTGGAG	Heavy Chain Round 2 PCR/Sequencing	Ho et al., 2016
VH1_5_7.S	CTGCAACCGGTGTACATTCCGAGGTGCAGCTG GTGCAG	Heavy Chain Round 2 PCR/Sequencing	Ho et al., 2016
VH4.S	CTGCAACCGGTGTACATTCCCAGGTGCAGCTG CAGGAG	Heavy Chain Round 2 PCR/Sequencing	Ho et al., 2016
IgG int	GTTCGGGGAAGTAGTCCTTGAC	Heavy Chain Round 2 PCR/Sequencing	Tiller et al., 2008
Pan Vk	ATGACCCAGWCTCCABYCWCCCTG	Kappa Chain Round 2 PCR/Sequencing	Tiller et al., 2008
Ck 494	GTGCTGTCCTTGCTGTCCTGCT	Kappa Chain Round 2 PCR/Sequencing	Tiller et al., 2008
colE.VH1	CTGCAgaattcCGTACATTCCCAGGTGCAGCTGG TGCAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VH3	CTGCAgaattcCGTACATTCTGAGGTGCAGCTGG TGGAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VH3-33	CTGCAgaattcCGTACATTCTCAGGTGCAGCTGG TGGAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VH3-9	CTGCAgaattcCGTACATTCTGAAGTGCAGCTGG TGGAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VH1/5	CTGCAgaattcCGTACATTCCGAGGTGCAGCTGG TGCAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VH4	CTGCAgaattcCGTACATTCCCAGGTGCAGCTGC AGGAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008

colE.VH4-39	CTGCAgaattcCGTACATTCCCAGCTGCAGCTGCAGGAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VH3-23	CTGCAgaattcCGTACATTCTGAGGTGCAGCTGTTGGAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VH1-18	CTGCAgaattcCGTACATTCCCAGGTTCAGCTGGTGCAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VH1-24	CTGCAgaattcCGTACATTCCCAGGTCCAGCTGGTACAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VH4-34	CTGCAgaattcCGTACATTCCCAGGTGCAGCTACAGCAGTG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VH6-1	CTGCAgaattcCGTACATTCCCAGGTACAGCTGCAGCAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
cNheI_JH1_2_4_5	TGCGAAgctagcGGCTGAGGAGACGGTGACCAG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
cNheI_JH3	TGCGAAgctagcGGCTGAAGAGACGGTGACCATTG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
cNheI_JH6	TGCGAAgctagcGGCTGAGGAGACGGTGACCGTG	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.Vk1-9	TTGTGCTGCAgaattcCGTACATTCAGACATCCAGTTGACCCAGTCT	Heavy Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.Vk3-11	TTGTGCTGCAgaattcCGTACATTCAGAAATTGTGTTGACACAGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.Vk4-1	CTGCAgaattcCGTACATTCGGACATCGTGATGACCCAGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.Vk2-24	CTGCAgaattcCGTACATGGGGATATTGTGATGACCCAGAC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.Vk2-30	CTGCAgaattcCGTACATGGGGATGTTGTGATGACTCAGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.Vk2-28	CTGCAgaattcCGTACATGGGGATATTGTGATGACTCAGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.Vk1-5	CTGCAgaattcCGTACATTCTGACATCCAGATGACCCAGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.Vk3-15	CTGCAgaattcCGTACATTCAGAAATAGTGATGACGCAGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.Vk3-20	TTGTGCTGCAgaattcCGTACATTCAGAAATTGTGTTGACGCAGTCT	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.Vk1D-43	CTGCAgaattcCGTACATTGTGCCATCCGGATGACCCAGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
BsiWI_Jk1/4	GCCACcgtacgTTTGATYTCCACCTTGGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
BsiWI_Jk2	GCCACcgtacgTTTGATCTCCAGCTTGGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
BsiWI_Jk3	GCCACcgtacgTTTGATATCCACTTTGGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
BsiWI_Jk5	GCCACcgtacgTTTAATCTCCAGTCGTGTC	Kappa Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VL1	CTGCTAgaattcCTCCTGGGCCCAGTCTGTGCTGACKCAG	Lambda Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VL2	CTGCTAgaattcCTCCTGGGCCCAGTCTGCCCTGACTCAG	Lambda Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VL3	CTGCTAgaattcCTCTGTGACCTCCTATGAGCTGACWCAG	Lambda Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VL4/5	CTGCTAgaattcCTCTCTCTCSCAGCYTGTGCTGACTCA	Lambda Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VL6	CTGCTAgaattcCTCTTGGGCCAATTTTATGCTGACTCAG	Lambda Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
colE.VL7/8	CTGCTAgaattcCTCCAATTCYCAGRCTGTGGTGACYCAG	Lambda Chain Round 2 PCR/Cloning	This study; Tiller et al., 2008
AvrII_Clambda	CTCCTCAcctaggGGYGGGAACAGAGTG	Lambda Chain Round 2 PCR/Cloning/Sequencing	This study; Tiller et al., 2008
Ig colony fwd	GCGCCGTTACAGATCCAAGCTGTGA	Colony PCR Screening	This study
CHIg-IgG1_Rev	CCGTCACCGGTTCGGGGAAGTAGT	Colony PCR Screening	This study
CLIg-hK_Rev	GGAGGGCGTTATCCACCTTCCACT	Colony PCR Screening	This study
CLIg-hL2_Rev	CCACTGTCACGGCTCCCGGGTAGAA	Colony PCR Screening	This study

SUPPLEMENTAL METHODS

sc-RNA-Seq library preparation

RNA was purified from sorted single cells using RNACleanXP Solid Phase Reversible Immobilization (SPRI) beads (Beckman Coulter). Full-length cDNA amplification of single cells was performed using the SMART-Seq v4 Ultra Low-Input RNA protocol. Amplified cDNA was fragmented using Illumina Nextera XT DNA Library Preparation kits and dual-indexed barcodes were added to each sample. Libraries were validated using an Agilent 4200 Tapestation, pooled, and sequenced at 101 SR on an Illumina HiSeq 3000 to an average depth of 1.67 M reads at the Yerkes NHP Genomics Core (http://www.yerkes.emory.edu/nhp_genomics_core/). The number of single cells sorted for each individual subject and their corresponding PCR plate ID is shown in Supplemental Table 2.

We used a modified protocol for plate P2S23 where instead of using the Clontech SMART-Seq v4 kit, we used a version of the SMARter method with Clontech buffers as was previously described (58). The template switching oligos for full-length cDNA synthesis were obtained from Exiqon (Woburn, MA, USA) and the primers for cDNA synthesis from Integrated DNA Technologies (Skokie, IL, USA). The libraries for this plate were also sequenced twice.

Transcriptomic bioinformatics analyses of single cells

The counts were obtained from the STAR ReadsPerGene files. Rstudio was used to perform downstream gene expression analysis (59) using the rocker/rstudio container (60). The scater package v1.12.2 (61) was used to remove low-quality cells. Cells were filtered out if the library size and number of genes were 3 median absolute deviations away and if the percentage of mitochondrial reads was more than 3 absolute deviations. The Ig genes were filtered out. The Seurat package v3.1.0 (62) was used to generate UMAP (63) that showed batch effects between plates. As a result, the subsequent analysis was focused on plates that had cells from both groups. The edgeR library v3.26.8 (64) was used to obtain CPM values. The log2(CPM+1) values were used for showing normalized gene expression using ggplot2 v3.2.1 (65). The differential gene expression analysis was carried out using MAST package v1.10.0 (66) with plate and cell detection rate as cofactors (67).

SUPPLEMENTAL REFERENCES

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