SUPPLEMENTAL INFORMATION

Supplemental Methods

Mass spectrometry

Sample preparation - Established methods of in-gel digestion were used for processing of IEF gel[1]. In brief, slices were washed with 25 mM ammonium bicarbonate (ABC)/50 % acetonitrile for removal of the stain agent. Supernatant was removed and gel pieces covered in100% acetonitrile until gel pieces were dehydrated. Acetonitrile was removed and gel pieces were vacuum dried. Next, the gel pieces were rehydrated and reduced in 10 mM dithiothreitol (DTT) (Sigma), for 45 min at 56°C. Supernatant was removed and gel pieces alkylated with 55mM iodoacetamide (IAA) (Sigma) in the dark for 30 minutes. At the end of the alkylation step, the gel pieces were washed 25 mM ABC, then dehydrated with neat acetonitrile and vacuum dried. Gel pieces were rehydrated in sequencing grade trypsin (12.5 ng/μl prepared in 25 mM ammonium bicarbonate) (Promega) at 4°C for 45 minutes, after which excess trypsin solution was removed and 25 mM ABC was placed on the top of the rehydrated gel pieces to ensure submersion in solution. After an overnight incubation at 37°C, digestion was stopped by the addition of .1% formic acid (FA). Samples were spun down and the water extract containing the peptides collected. Remaining peptides were extracted by shaking the gel pieces with .1% FA for 15 minutes at room temperature. This step was repeated one more time and extracts were combined. Peptide solutions were concentrated down to 10 μl by vacuum centrifugation. LCMS analysis was performed using an Eksigent NanoLC Ultra 2D Plus

HPLC system coupled to a Thermo Scientific LTQ Orbitrap Velos (Waltham, MA) via an Advance CaptiveSpray Ionization source (Michrom BioResources, Auburn, CA).

Database Searches - The raw files generated on Orbitrap LTQ Velos mass spectrometer were converted to [mgf] format using Mascot Daemon software version 2.2.2 (Matrix Science) with prior to their submission to the search engine ProteinPilot ™ (release 4.5, revision 1656, AB Sciex) that uses the Paragon algorithm[2]. The initial search of peptides mapping to CSF-associated IgG-VH clusters were performed against the VHref-CSF database appended with the UniProtKB database of human isoform sequences of common contaminants of human origin (release 31-Oct-2012), and of peptides exclusively mapping to PBMC-derived sequences the initial search was performed against the VHref-PB database appended with UniProtKB. The search databases contained 41,473 (VHref-CSF plus UniProtKB) and 489,419 (VHref-PB plus UniProtKB) total entries. Thus, our approach permitted only detection of OCB peptides that were represented either in our VHref databases containing over 450,000 IgG-VH transcripts and/or could be matched to UniProtKB. Next generation immune repertoire sequencing used to generate our IgG-VH reference databases is far-more comprehensive than Sanger-sequencing based approaches, and has a greater chance of capturing the true diversity of IgG-VH transcripts present in CSF samples, and a significant portion of IgG-VH diversity in PB. Nonetheless, it is likely that an undeterminable number of tryptic OCB peptides present after the in-gel digestion, were eliminated from further analysis if their respective IgG-VH transcript was not found in VHref-CSF or VHref-PB. All

peptides were blasted against the IMGT IGHV germline database[3] using IgBLAST (http://www.ncbi.nlm.nih.gov/igblast/).

Confirmatory searches were then performed against a combination of all three datasets (VHref-CSF plus VHref-PB plus UniProtKB). OCB peptides from a certain patient's CSF mapping to only the same patient's IgG-VH were identified as "patient-specific" (Tables S2 and S3). Patient non-specific OCB peptides mapped to IgG-VH transcripts of >1 patient or could not be assigned to the same patient's IgG-VH (Table S4) and were not analyzed further. Mass spectra of all "patient-specific" peptides were manually inspected to assess the quality of the match and importantly, to establish the extent of peptide sequence coverage by the detected product ions. Only OCB peptide sequences for which full tandem mass-spectrometry evidence was seen were utilized in further analysis.

Supplemental References

1. Jimenez CR, Huang L, Qiu Y, Burlingame AL. In-gel digestion of proteins for MALDI-MS fingerprint mapping. Curr Protoc Protein Sci. 2001;Chapter 16:Unit 16 14
2. Shilov IV, Seymour SL, Patel AA et al. The Paragon Algorithm, a next generation search engine that uses sequence temperature values and feature probabilities to identify peptides from tandem mass spectra. Mol Cell Proteomics. 2007;6:1638-1655
3. Lefranc MP, Giudicelli V, Ginestoux C et al. IMGT, the international ImMunoGeneTics information system. Nucleic Acids Res. 2009;37:D1006-1012

Supplemental Tables

ID	Age	Sex	Duration (months)	# Attacks	EDSS	Most recent MRI (months)	Gd
MS-1	29	F	11	3	1.	7	+
MS-2	37	F	24	1	2.	1	-
MS-3	39	F	>3	>1	2.	7	-
MS-4	39	F	24	2	1.5	2	-
MS-5	22	M	9	2	1.5	.25	+

Table S1. Additional clinical information of patients studied. *Duration* is disease duration in months, "*# Attacks*" refers to number of clinical MS attacks since diagnosis, *EDSS* is the Expanded Disability Scoring Scale at the time of lumbar puncture, "*Most recent MRI*" is time in months since last brain or spinal cord MRI, "*Gd*" indicates whether the most recent MRI displayed pathological contrast enhancement.

CSF	Total peptides	Not patient-specific	Patient-specific	Tandem Mass-Spec confirmed
MS-1	29	13	16	7
MS-2	106	42	64	20
MS-3	129	40	89	38
MS-4	10	5	5	3
MS-5	111	35	76	12
Sum	**385**	**135**	**250**	**80**

Table S2. OCB peptides identified by Mass-Spectrometry. *CSF*, ID of patient from which CSF OCB peptides were obtained; *Total peptides*, all peptides identified by mass-spectrometry; *Not patient-specific*, OCB peptides mapping to >1 patient in VHref-CSF; *Patient-specific*, OCB peptides uniquely mapping to the same patient's IgG-VH sequences in VHref-CSF or VHref-PB (see Methods); *Tandem Mass-Spec confirmed*, fully sequence confirmed OCB peptides per patient used for mapping to IgG-VH clusters. Refer to Tables S3 and S4 for further details on confirmed OCB peptides.

Pat ID	OCB Peptide	Mutated?	Cluster Compartment	IPG BAND	Multi band?	IGHV	H-CDR 3	IGHJ
MS-1	L**A**CTASGF**R**	y	C	F	n	3-48	CVRDQGGYYGNFFLDYW	4
MS-1	G**T**GG**GW**YY**R**	y	C	F	n	3-23	CARGTGGGWYYRIHFDYW	4
MS-1	**V**EDSGVYYCA**GR**	y	C	D	n	3-48	CAGRRYLESSGPPEFPW	5
MS-1	**VMGFYEDSGYR**	CDR3	B	F	n	4-4	CARVMGFYEDSGYRYFTGLNDYW	4
MS-1	**SIF**DGGTTD**F**AAPV**R**	y	C	D	n	3-15	CTTDEGGNSGYYFEYW	4
MS-1	V**A**MS**I**DTSR	y	C	F	n	4-59/61	CARSGPGGEPVYYFDPW	5
MS-1	L**T**SVTAADTAK	y	C	F	n	4-59/61	CARSGPGGEPVYYFDPW	5
MS-2	**AFFNPFDY**WGQGALVTVSSASTK	CDR3	C	J	n	4-39	CARHPDSSDNTGRAFFNPFDYW	4
MS-2	**YFAWSAGK**	CDR3	B,C	D	n	4-39	CVAVRYFAWSAGKLFDYW	4
MS-2	ASDTAMYYCAR*	n	C	H	n	5-51	CARRIAPLRGTYSMFAFDIW	3
MS-2	VTISV**VP**SK	y	C	C	n	4-59/61	CAKRAAYCDGKRCSRAFDYW	4
MS-2	**FDQ**WGQGTLVTVSSASTK	CDR3	C	J	n	4-39	CAGDGSGSYYTRFDQW	4
MS-2	**GLLWLDGDYR**	CDR3	C	D	y	3-30/33rn	CTRGLLWLDGDYRDYW	4
MS-2	AEDTA**I**YYC**T**R	y	C	D	y	3-30/33rn	CTRGLLWLDGDYRDYW	4
MS-2	**Y**TL**N**LQM**D**SLR	y	C	J	y	3-30/33rn	CTRGLLWLDGDYRDYW	4
MS-2	FTF**T**SYGMHWVR	y	C	J	y	3-30/33rn	CTRGLLWLDGDYRDYW	4
MS-2	**L**TIS**M**DTS**R**	y	C	D	n	4-30-4/31	CARRASPHHYDGSGEDYW	4
MS-2	VTISV**E**TSK	y	C	J	n	4-30-4/31	CGRDGRGRELLPFPGFDSW	4
MS-2	**ELLPFPGFDS**WGQGTLVTVSSASTK	CDR3	C	J	n	4-30-4/31	CGRDGRGRELLPFPGFDSW	4
MS-2	**GRELLPFPGFDS**WGQGTLVTVSSASTK	CDR3	C	J	n	4-30-4/31	CGRDGRGRELLPFPGFDSW	4
MS-2	GLEWVS**T**IYR	y	C	J	y	3-53/66	CAGGQTGYCTGGNSQRCYGMDVW	6
MS-2	**D**G**A**T**S**YAD**T**VK	y	C	A	y	3-53/66	CAGGQTGYCTGGNSQRCYGMDVW	6
MS-2	VTISVDTS**N**NHFSLK	y	C	J	n	4-59/61	CARGSDILTGYSLGGWFDPW	5
MS-2	AEDT**G**VYYCA**K**	y	C	C	n	3-53/66	CAKVKGRQLVHYYYGMDVW	6
MS-2	V**A**IS**A**DA**AP**K	y	C	J	n	4-39	CARGLPRLLSPHSDW	4
MS-2	S**T**STA**N**L**H**WSSLK	y	C	J	n	5-a	CARPQRSSSSTFDHW	4
MS-2	LSSVTAADTAVY**H**CAR	y	C	J	n	4-30-4/31	CARVDRTAGYYFDNW	4
MS-3	V**IL**SVDTSK	y	C	K	y	4-39	CARHELVQTPATSKFGPW	5
MS-3	GLEWIGSV**Y**K	y	C	J	y	4-39	CAGHELLHTPTTSKFGPW	5
MS-3	**HEFLQTPTTSK**	CDR3	C	J	y	4-39	CARHEFLQTPTTSKFGPW	5
MS-3	**HELLQTPTTSK**	CDR3	C	J	y	4-39	CVRHELLQTPTTSKFGPW	5
MS-3	**HELVQTPATSK**	CDR3	C	D	y	4-39	CARHELVQTPATSKFGPW	5
MS-3	**HELVQTPTTSK**	CDR3	C	A	y	4-39	RARHELVQTPTTSKFGPW	5

MS-3	**N**G**IA**YYNPSL**T**SR	y	C	J	y	4-39	CARHELVQTPATSKFGPW	5
MS-3	SG**IA**F**T**NPSL**T**SR	y	C	J	y	4-39	CARHELVQRPAISKFGPW	5
MS-3	SG**IA**Y**H**NPSL**T**SR	y	C	J	y	4-39	CARHEAVQTPTTSKFGPW	5
MS-3	SG**IA**YYNPSL**TT**R	y	C	B	y	4-39	GARHELLQTPSTSKFGPW	5
MS-3	SG**IV**YYNPSL**T**SR	y	C	J	y	4-39	CARHELVQTPATSKFGPW	5
MS-3	SG**VA**YYNPSL**TT**R	y	C	J	y	4-39	CVRHELLQTPTTSKFGPW	5
MS-3	S**VIA**YYNPSL**TG**R	y	C	J	y	4-39	CARHELVQTPTTSKFGPW	5
MS-3	L**N**SV**A**AADTA**F**YYCAR	y	C	J	y	4-39	CARHELVQAPTTSKFGPW	5
MS-3	L**N**SV**A**AADTA**I**YYCAR	y	C	J	y	4-39	CARHELLQTPTTSKFGPW	5
MS-3	**YGP**WGQGTLVTVSSASTK	y	C	J	y	4-39	CARHELVQTPATSKYGPW	5
MS-3	L**T**SVTAADTAVYYC**V**R	y	C	J	n	4-30-4/31	CVRSKYDILTGYYDKGHAFHIW	3
MS-3	AEDTAVYYC**V**K	y	C	J	n	3-23	CVKDQGDYIWGTYPSTFDYW	4
MS-3	GLEWIGSI**HSG**GSTYYNP**VV**K	y	C	J	n	4-39	CASPRAIGATGPFDYW	4
MS-3	SVTAADTA**M**Y**F**CAR	y	C	J	n	4-59/61	CARTYGSGTYSRGYYYGMDVW	6
MS-3	**E**DGSE**S**YYVDSVK	y	C	J	n	3-7	CARRGTTTPRGRTPDSW	4
MS-3	**TLDS**WGQGTLVTVSSASTK	CDR3	C	J	n	3-7	CTRRGATTPRGRTLDSW	4
MS-3	GLEWIG**NMQ**Y**R**	y	C	J	n	4-39	CASSRSWKGHFQHW	1
MS-3	NTLYLQM**T**SLR	y	C	K	n	3-30/33rn	CARKPDRGYNLDDYGEYKPTSPFDYW	4
MS-3	**SAMDV**WGQGTTVTVSSASTK	CDR3	C	J	n	4-59	CARVRSAMDVW	6
MS-3	SVTAADTAVYYC**R**	y	C	B	n	4-34	CRWGGDMDVW	6
MS-3	**SHFAD**WGQGTLVSVSSPSTK	CDR3	C	J	n	4-39	CASSTGTTLRRSHFADW	4
MS-3	LSSVTAADT**GI**YY**CASSTGTTLR**	CDR3	C	J	n	4-39	CASSTGTTLRRSHFADW	4
MS-3	**DHQWLVLGR**	CDR3	C	J	n	3-7	CVRDHQWLVLGRRCDSW	5
MS-3	GL**Q**WVANIK	y	C	J	n	3-7	CARVLTVRGVSSQGFDSW	4
MS-3	**GVSSQGFDS**WGQGTLVSVSSASTK	CDR3	C	J	n	3-7	CARVLTVRGVSSQGFDSW	4
MS-3	LSCAASGF**I**F**R**	y	C	K	n	3-15	CTTVKLGWRSSYYDTAFDYW	4
MS-3	GLEWVANI**NPE**G**N**EK	y	C	K	n	3-7	CAKDWTDAGAVTNVFDYW	4
MS-3	AEDTAVYYCA**NR**	y	C	B	n	3-23	CANRYLVDSSGSYRDDPFDIW	3
MS-3	**DAPDCGGDCYSLPTVR**	CDR3	C	K	n	3-7	CARDAPDCGGDCYSLPTVRFDYW	4
MS-3	**DYNPSVDS**SGSTYYNPSLK	y	C	K	n	4-30-4/31	CARDYDYGDKWFDPW	5
MS-3	AEDT**G**VYYCAR	y	C	J	n	3-7	CARDYGSATYYASYYHGMGVW	6
MS-3	VTISVDT**A**K	y	C	K	n	4-39	CARHIALPARAMYYHDTIGTPGPLDFW	5
MS-4	AEDTAVY**F**CAR	y	C	D	n	3-53/66	CARDVFDAWCDHRFDFW	4
MS-4	NTL**F**LQMNSLR	y	C	D	n	3-53/66	CARDVFDAWCDHRFDFW	4
MS-4	LSCAAS**N**F**R**	y	C	D	n	3-23	CAKSDDYDFHNIDSW	4

MS-5	NSLYLQMN**T**LR	y	B,C	I	y	3-9	CAKEPDSSGWSTGGFDPW	5
MS-5	**V**EDTALYYCAK	y	B,C	G	y	3-9	CAKEPDSSGWSTGGFDPW	5
MS-5	AEDTALYYCAK*	n	B,C	G	y	3-9	CAKEILRSKYDLWSGYYKPFDIW	3
MS-5	NSLYLQM**S**SLR	y	B,C	L	y	3-9	CAKEILRSKYDLWSGYYKPFDIW	3
MS-5	VTITADESTR*	n	B,C	I	n	1-69	CAPGIYIAGAGGKTYFDYW	4
MS-5	NTLYLQMNSLK*	n	B,C	I	n	3-15	CTTDRTVGATRDFGYW	4
MS-5	AEDTAVYYC**S**R	y	B,C	I	n	3-74	CSRDQHNFWTGSPYYMDAW	6
MS-5	QDG**TD**KYYVDSVK	y	C	I	n	3-7	CVGFNPPIDYR	4
MS-5	**LNM**SVD**A**SK	y	C	L	n	4-30-4/31	CARRTSRREGMNWFDPW	5
MS-5	IYTSGSTNYNPSLK*	n	B,C	K	n	4-59/61	CAGRGFWSPYYYYMDAW	6
MS-5	**VG**DTA**I**YYCAR	y	C	A	n	3-7	CARGSAVAGNYW	4
MS-5	NS**V**YLQM**H**SLR	y	C	I	n	3-7	CARNADYDILTGYYRPGNFDFW	4

Table S3: OCB Peptides. Summary of patient-specific and fully sequence-confirmed OCB peptides. Peptides are ordered by patient (*Pat ID*); shown are the peptide AA sequences (*OCB Peptide*), IGHV/IGHJ usage, and *H-CDR3* regions representative of the associated IgG-VH cluster. Peptides marked with a "*" are identical to the respective IGHV germline sequence; AA mutations in comparison to the closest IGHV are in bold letters and shaded in gray. The column "Mutated?" lists whether a peptide is identical to germline ("n", not mutated); different from germline ("y"; yes); or contains >2 aminoacids mapping to the H-CDR region (*CDR3*). Compartmental distribution of IgG-VH clusters is "*Cluster Compartment*"; "C" are CSF-restricted clusters identified in VHref-CSF; "B,C" are clusters identified in VHref-CSF with contributions from blood and CSF; "B" is a cluster in patient MS-1 identified in VHref-PB. IEF gel band from which a peptide was obtained by mass-spectrometry is shown in column "*IEF*". Peptides belonging to IgG-VH clusters detected in >1 band on IEF of the same patient's OCB are labeled *"y"* (yes) in column "*Multi band"*, "*n*" (no) if only detected in a single band. Boxed are peptides belonging to the same IgG-VH cluster. *Shaded in gray* is an OCB peptide that could not be matched to the same patient's VHref-CSF but instead mapped exclusively to this patient's IgG-VH in VHref-PB; this peptide is highly private to patient MS-1 as it is entirely H-CDR derived.

CSF	OCB Peptide	Pts in VHref-CSF	VHref-CSF				
			MS-1	MS-2	MS-3	MS-4	MS-5
MS-1	GTGGGWYYR	1	12				
MS-1	VAMSIDTSR	1	8				
MS-1	SIFDGGTTDFAAPVR	1	3				
MS-1	VEDSGVYYCAGR	1	29				
MS-1	LACTASGFR	1	24				
MS-1	LTSVTAADTAK	1	8				
MS-2	GLEWVSTIYR	1		1			
MS-2	AEDTAIYYCTR	1		1			
MS-2	VTISVVPSK	1		18			
MS-2	GLLWLDGDYR	1		1			
MS-2	FDQWGQGTLVTVSSASTK	1		2			
MS-2	YTLNLQMDSLR	1		1			
MS-2	AFFNPFDYWGQGALVTVSSASTK	1		2			
MS-2	VTISVDTSNNHFSLK	1		6			
MS-2	VAISADAPK	1		4			
MS-2	GRELLPFPGFDSWGQGTLVTVSSASTK	1		1			
MS-2	AEDTGVYYCAK	1		5			
MS-2	DGATSYADTVK	1		2			
MS-2	YFAWSAGK	1		3			
MS-2	LSSVTAADTAVYHCAR	1		3			
MS-2	ASDTAMYYCAR*	1		6			
MS-2	VTISVETSK	1		1			
MS-2	LTISMDTSR	1		5			
MS-2	ELLPFPGFDSWGQGTLVTVSSASTK	1		1			
MS-2	FTFTSYGMHWVR	1		1			
MS-2	STSTANLHWSSLK	1		1			
MS-3	YGPWGQGTLVTVSSASTK	1			1		
MS-3	SHFADWGQGTLVSVSSPSTK	1			1		
MS-3	SVTAADTAVYYCR	1			1		
MS-3	SGIVYYNPSLTSR	1			1		
MS-3	SGIAYYNPSLTTR	1			176		
MS-3	DYNPSVDSSGSTYYNPSLK	1			1		
MS-3	TLDSWGQGTLVTVSSASTK	1			15		
MS-3	LSCAASGFIFR	1			1		

MS-3	LSSVTAADTGIYYCASSTGTTLR	1			1		
MS-3	SGIAYHNPSLTSR	1			2		
MS-3	GVSSQGFDSWGQGTLVSVSSASTK	1			1		
MS-3	AEDTGVYYCAR	1			1		
MS-3	GLEWVANINPEGNEK	1			1		
MS-3	GLEWIGSIHSGGSTYYNPVVK	1			22		
MS-3	HELVQTPTTSK	1			98		
MS-3	HELLQTPTTSK	1			117		
MS-3	SAMDVWGQGTTVTVSSASTK	1			1		
MS-3	GLEWIGNMQYR	1			21		
MS-3	SGVAYYNPSLTTR	1			27		
MS-3	LNSVAAADTAFYYCAR	1			20		
MS-3	LNSVAAADTAIYYCAR	1			6		
MS-3	GLQWVANIK	1			1		
MS-3	DAPDCGGDCYSLPTVR	1			2		
MS-3	SVIAYYNPSLTGR	1			1		
MS-3	GLEWIGSVYK	1			6		
MS-3	SGIAFYNPSLTSR	1			2		
MS-3	NGIAYYNPSLTSR	1			24		
MS-3	EDGSESYYVDSVK	1			16		
MS-3	VTISVDTAK	1			2		
MS-3	HEFLQTPTTSK	1			1		
MS-3	HELVQTPATSK	1			76		
MS-3	NTLYLQMTSLR	1			10		
MS-3	VILSVDTSK	1			38		
MS-3	DHQWLVLGR	1			2		
MS-3	AEDTAVYYCANR	1			1		
MS-3	AEDTAVYYCVK	1			13		
MS-3	SVTAADTAMYFCAR	1			21		
MS-3	LTSVTAADTAVYYCVR	1			2		
MS-4	AEDTAVYFCAR	1				6	
MS-4	LSCAASNFR	1				11	
MS-4	NTLFLQMNSLR	1				6	
MS-5	NSLYLQMNTLR	1					3
MS-5	AEDTAVYYCSR	1					21
MS-5	NSLYLQMSSLR	1					3
MS-5	VTITADESTR*	1					16

MS-5	VGDTAIYYCAR	1					7
MS-5	AEDTALYYCAK*	1					14
MS-5	LNMSVDASK	1					47
MS-5	IYTSGSTNYNPSLK*	1					15
MS-5	NTLYLQMNSLK*	1					6
MS-5	VEDTALYYCAK	1					2
MS-5	QDGTDKYYVDSVK	1					34
MS-5	NSVYLQMHSLR	1					1

Table S4: "Patient-specific" and fully sequence-confirmed OCB peptides**.** OCB peptides were termed "patient-specific" if they were found in the same patient's CSF IgG proteome and transcriptome (column "Pts in VHref-CSF"). In this table, the first column (*CSF*) denotes the CSF in which the indicated peptide (*OCB Peptide*) was identified using mass-spectrometry. In column "*VHref-CSF*" numbers of individual IgG-VH sequences containing exact sequence matches of the indicated OCB peptides as found in each patient are shown. The same OBC peptides as in Table S3 are represented here, except for peptide VMGFYEDSGYR from patient MS-1 which was private to this patient in VHref-PB.

Supplemental Figure S1

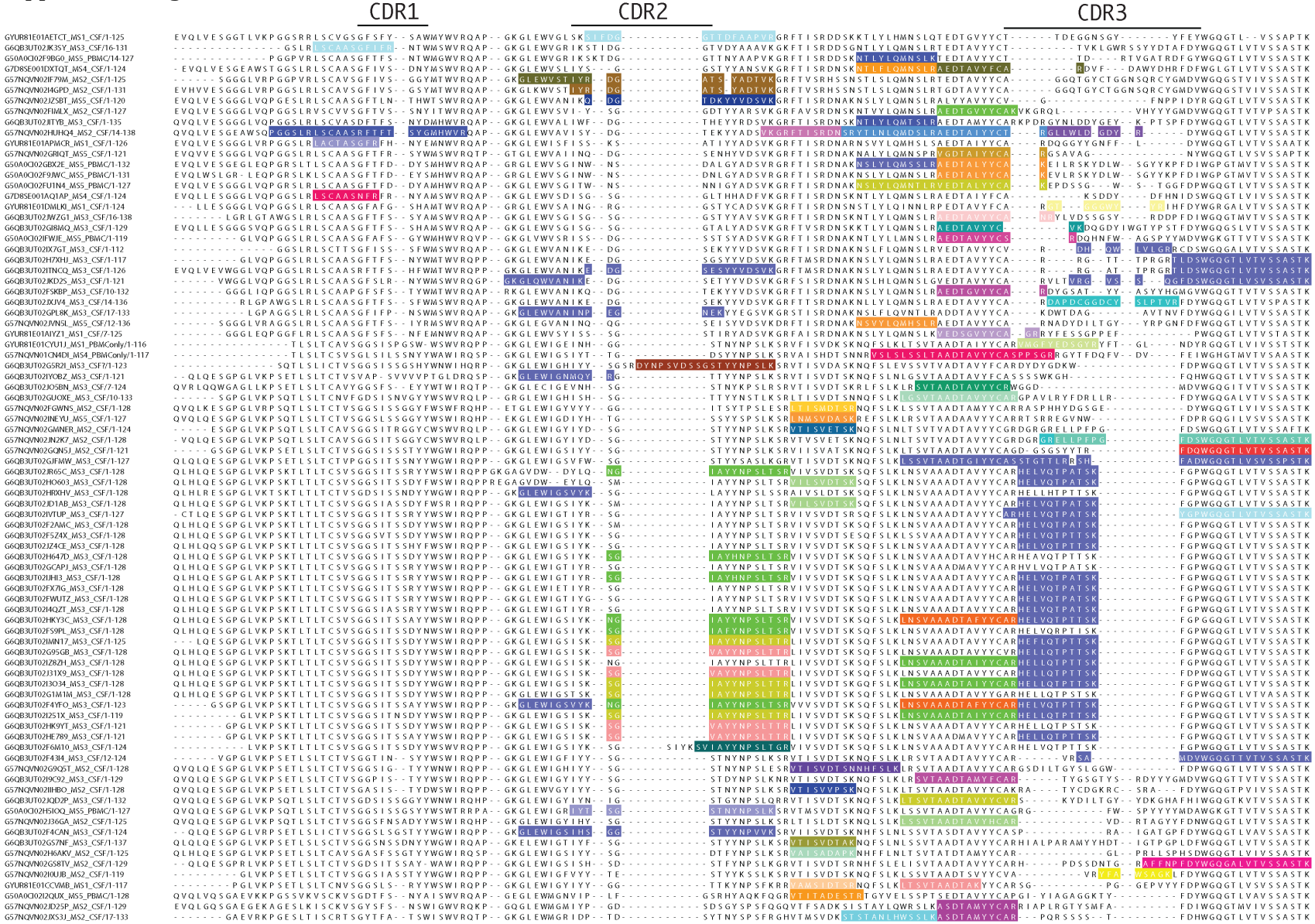

Figure S1: OCB peptides displayed on representative IgG-VH sequences. OCB peptides are indicated in different colors; identical or very similar colors do not necessarily indicate identical OCB peptides. For additional information refer to Table S3.