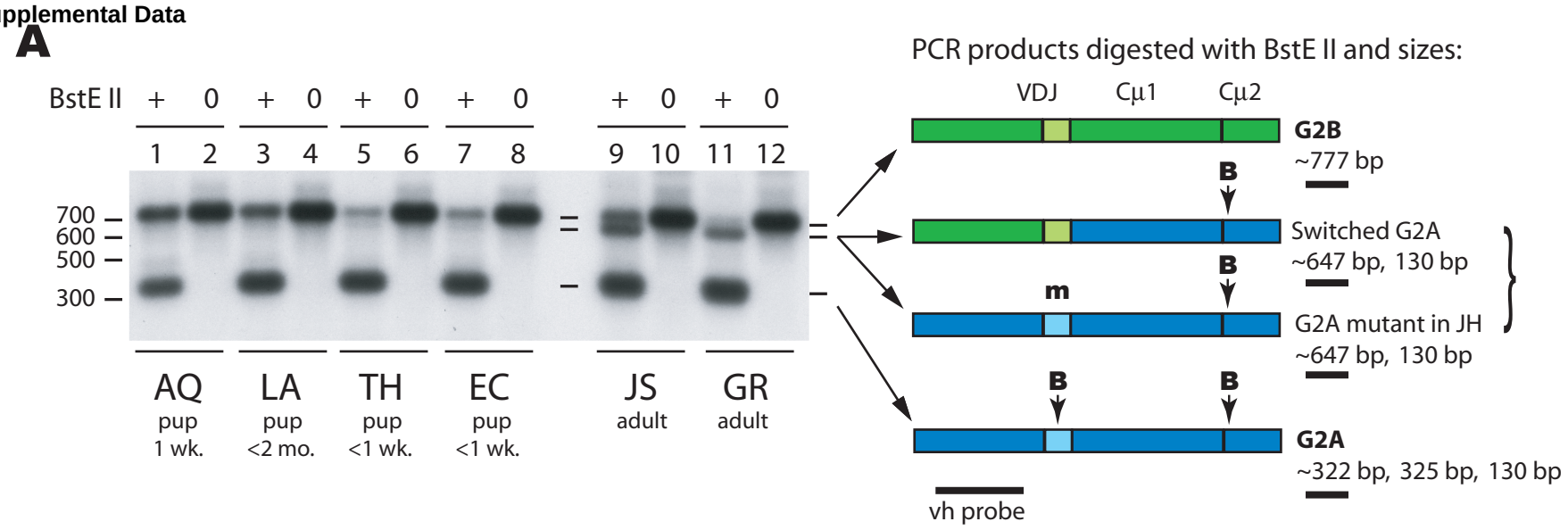
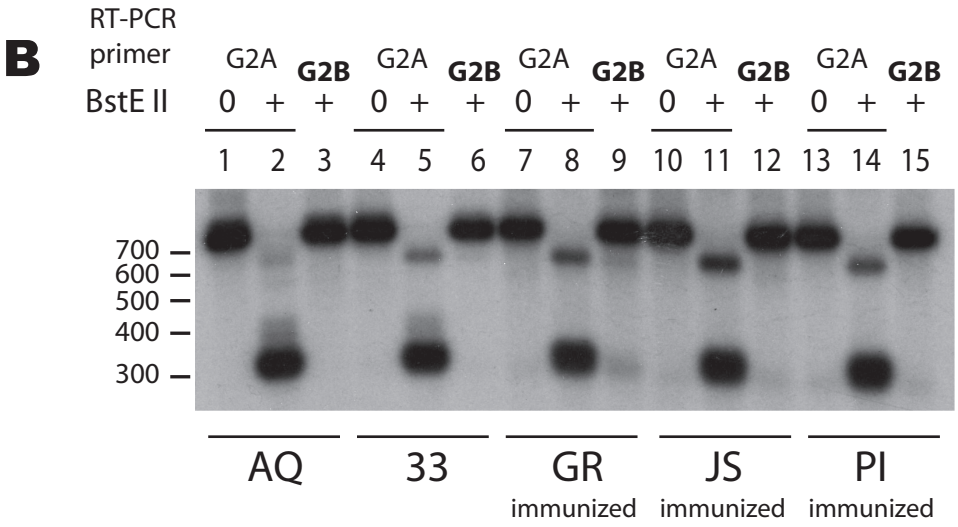
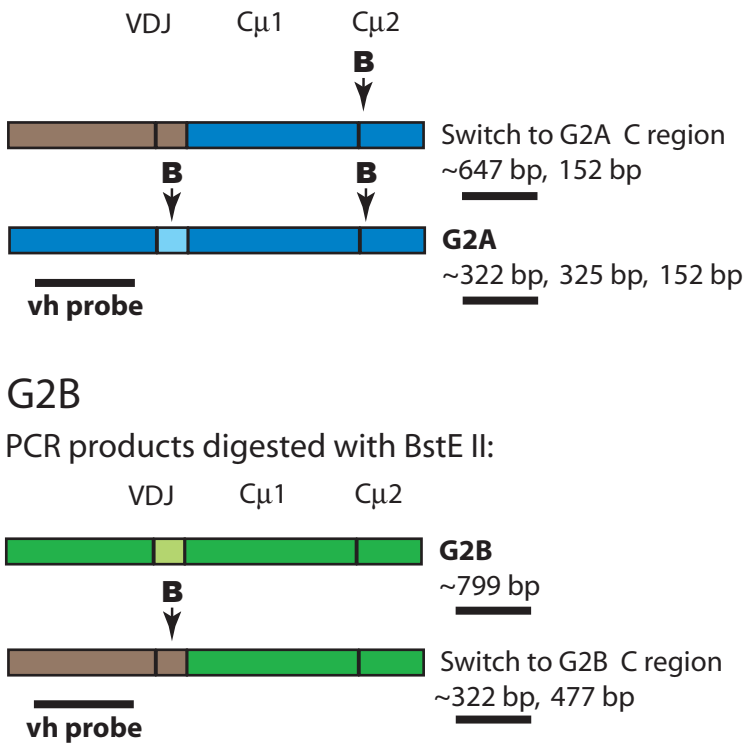
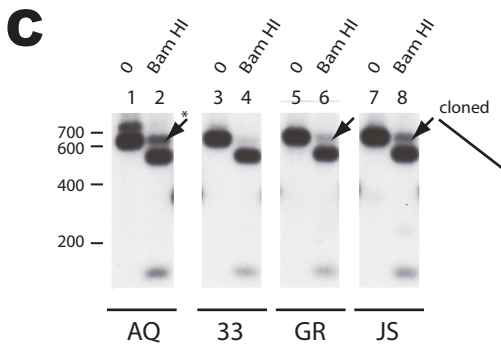
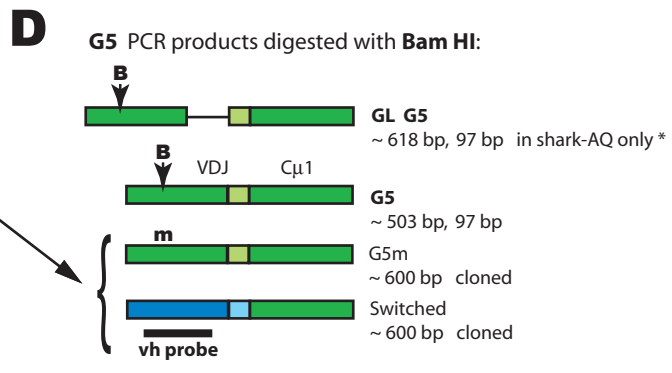










E

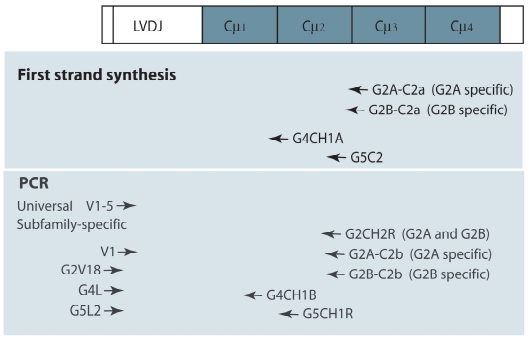
AQ	GR	JS	
156	96	235	total number clones analyzed
5	36	33	BamHI negative sequences
2	24	17	mutation at BamHI
0	10(10)	10(7)	switched: G2 or G4 VDJ to G5 C (unique clones)
3	2	6	in vitro hybrids

Figure S1. Switch frequency in individual sharks.

Panel A. Switched Ig in shark pups. **Left**. RNA was isolated from non-immunized pups AQ, LA, TH, and EC and immunized sharks JS (HEL) and GR (Ebola virus). First strand cDNA was primed with a universal CH2 primer (CH2-3') and PCR was performed with G2A/B-specific reverse primer (G2CH2R) and universal forward primer (V1-5) for 30 cycles. The PCR products were incubated with (+) and without BstEII (0) and electrophoresed.The filter was incubated with a probe detecting only the VH, and the lane between 8 and 9 shows marks for the hybridizing PCR fragments sized ~777 bp, ~647 bp, and ~322 bp. **Right**. Predicted PCR products and results of their cleavage with BstEII. Non switched G2B (green) contains no BstEII sites whereas non switched G2A (blue) contains one in JH segment and one in Cμ2 (arrows). Digestion with BstEII generates a ~322 bp G2A band and ~777 bp G2B band detected by vh probe (as underlined). The ~647 bp band found in adults but not in pups indicates few G2 sequences are switched (or mutated) in very young animals.

Panel B. Frequency of switching to G2A alone or to G2B alone.**Left**. RNA was isolated from non-immunized neonate AQ (lane 1-3), and adult 33 (4-6) and from immunized sharks GR (lane 7-9), JS (lane 10-12), and PI (lane 13-15). The organ source was spleen in all cases except shark-33 epigonal organ. First strand cDNA was primed with G2A or G2B-specific oligonucleotide (G2A-C2a, G2B-C2a) and PCR was performed respectively with G2A or G2B-specific reverse primers (G2A-C2b, G2B-C2b) and universal forward primers (V1-5) for 35 cycles. The PCR products were standardized for uniform signal intensity to vh probe and these adjusted amounts were incubated with (+) and without BstEII (0) and electrophoresed. The G2B PCR products were also incubated without BstEII, but are not shown. The filter was hybridized with a probe detecting only the VH. **Right**. Predicted PCR products and results of their cleavage with BstEII. Non-switched G2B (green) contains no BstEII sites whereas non switched G2A (blue) contains one in JH segment (lighter shade) and one in Cμ2. Digestion with BstEII generates a ~322 bp band with G2A and ~777 bp G2B detected by vh probe in non switched sequences. Left. The ~647 bp band in lanes 5, 8, 11, and 14 indicates the absence of the BstEII site in the JH of G2A, either a mutation or non-G2A VDJ (tan) that is from G2B, G3, or G4 (see Figure 3, lack of BstEII sites). Right. A ~322 bp band appearing in lanes 3, 6, 9, 12 or 15 would have indicated a non-G2B VDJ that is from G2A, G1 or G5 (see Figure 3, presence of BstEII sites). The BstEII fragment detectable by the probe is underlined.

Panel C-E. Switching to G5 constant region. **C.** RNA was isolated from neonate AQ (lane 1, 2), adults 33 (3, 4), GR (5, 6), and JS (7, 8). First strand cDNA was primed with G5C region primer (G5C2) and PCR was performed universal forward primers (V1-5) and G5 C region primer (G5CH1R) for 40 cycles. The PCR products were incubated with and without Bam HI for one hour and electrophoresed. The filter was hybridized with a probe detecting only the VH. Arrows in lanes 4, 6 and 8 indicate sequences not cleaved by Bam HI, the ~600 bp fragments that isolated and cloned. **D.** G5 PCR products digested with Bam HI. Digestion of non-rearranged (GL G5) transcript in shark-AQ is depicted at the top. Rearranged sequences were cleaved into ~503 bp and 97 bp fragments. G5 with mutated Bam HI sites or non-G5 VDJ (switched) did not cleave and these were isolated (arrows) by gel extraction and cloned into pGEM. **E.** Tabulation of results for switch to G5 C region from three individuals. After BamHI digestion the 600 bp resistant fragments were cloned and analyzed. Of the portion that was negative for the BamHI site on re-screening, some sequences proved to be mutants and others non-G5 (switched) VDJ. In the neonate AQ the 600 bp fragment that remained after Bam HI digestion proved to be almost entirely non-rearranged. Accession numbers of switched sequences from JS are (GQ359852, GQ359856, GQ359858, GQ282627) and from GR are (GQ359826-GQ359831, GQ359848-GQ359851).



I. G4A VDJ to G5 C region

Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G4A	TGTGCAAGAGAC		ACTACTTTGATAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	TGACACCGTCTTCC				
G4G5-39	TGTGCC	CGGGGGGCAACTGGGGGAGC	CTTTGAAAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	V1-5/G5 C	GQ359830
104	TGTGCC	CGGGGGGCAACTGGGGGAGC	CTTTGAAAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
G4G5-33	TGTGCA	CAAGTATCTAATGAGTACCCCCGAACAAGTATTTCGGGA	TTTGATAACTGGGGACAAGGAACCATGGTGACTGTAGCTTACG	CAACACCGTCTTCT	G4A-G5	GR	V1-5/G5 C	GQ359829
G4G5-3*	TGTACAAGA	CGTTCGCACGAGTATTCAGTTCGGCTTGATACTAAGTGGGACCCCCTT	TTTGATAACTGGGGTGAAGGAACCATGGTCACTGTGAGCTTAG	CAACACCGTCTTCT	G4A-G5	GR	V1-5/G5 C	GQ359827
G4G5-66	TGTGTA	CTAGAGACGACTGGGGGAGTTCCGGGGG	ACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	V1-5/G5 C	GQ359831
G4G5-17*	TGTCAAAG	TCACCGATACTACAGTAGGCAGGGGACGCTCCGAGACACT	TGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	V1-5/G5 C	GQ359826
G4G5-4	TGTGCAAG	GGCGCCATACAGTAGGTACAGACTGGGCC	ACTTTGATAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	V1-5/G5 C	GQ359828
118*	TGTGTAACA	CGGTACTACAACCGTGGCTGGGGCCCTCGATCA	TTTGATAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
38	TGTGC	TCGGACCGTATGGCCCC	ATAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
108	TGTGCAT	CATCTAGCCACAGGGGT	TTCGATAACTGGGGAAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
119	TGTGCAAG	GCAGAAAGGTAGACTCACTGGGGGACCTCCG	GATAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
111	TGTACAAGAG	GCCGACTACCCCAATCACTGGGTGTT	TTTGATAACTGGGGACAAGGAACCATGGTGACTGTCGCTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
105	TGTG	TCTCCGATACGCGATATCCACGACTGGCGCCTGG	CTACTTTGATATTTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
37	TGTGCAA	TCTCGGTGACTACAGTCACGCACCTGGGCTGCT	ACTACTTTGATAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
84	TGTACAAG	CCGCCATCCCTTCCGGGGGTGG	TTTGATAACTGGGGACAAGGAACCGTGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
86	TGTGCAAGA	TCGGTACAGCGCCTCAGGTATACCGCCA	ATAATTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
61*	TGTGTAAGGGA	GGGTACTACATACTTGGGCATATATT	ATAACTGGGGAGCAGGAACAATGGTGACTGTTGCTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	JQ272821
53	TGTACAAG	GCGCGGGACATCTTTCACCCGGG	ACTACTTTGATCTCTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4A-G5	GR	G4L/G5 C	
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	CAACACCGTCTTCT				

II. G4CG VDJ to G5 C region

Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G4CG	TGTGCAAGAGAC		ACGAATTCGCTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	TGACACCGTCTTCC				
G4G5-81	TGTGCAA	TATCTAGCCACAGGGGT	TTCGATAACTGGGGAAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4CG-G5	GR	V1-5/G5 C	GQ359850
151	TGTGCAA	CAGCTAGCCACAGGGGT	TTCGATGTCTGGGGAAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4CG-G5	GR	G4L/G5 C	
G4G5-88	TGTGCA	GTAAGAATCGGACGCCCG	TTCGCTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4CG-G5	GR	V1-5/G5 C	GQ359851
G4G5-76	TGTACA	GTAAAGATCGGACGGAGG	TTCGCTGACTGGGGACAAGGAACCATGGTGACTGTGACCGAGG	CAACACCGTCTTCT	G4CG-G5	GR	V1-5/G5 C	GQ359849
G4G5-46	TGTGCAAGAGAC	CGAGTCGGTACGATGCCAACCTGGGGGGCCGAGAGAGCG	GAATTCGCTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4CG-G5	GR	V1-5/G5 C	GQ359848
114	TGTGCA	TTAGTCTGGTCAGTGGGTGGATATACGGTAC	AATTCGCCTCCTGGGGACAAGGAACCATGGTGACTGTTCGTTTAG	CAACACCGTCTTCT	G4CG-G5	GR	G4L/G5 C	
146	TGTGCA	GAAAGCCGCAGGGGTGG	ATTCGCTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4CG-G5	GR	G4L/G5 C	
150	TGTG	TCGTCAAGCTCGGACGGAGT	TTCGCTGACTGGGGACAAGGAACCATGGTGACAGTGACTTTAG	CAACACCGTCTTCT	G4CG-G5	GR	G4L/G5 C	
16	TGTGCAAGA	CTCATAGAGTCGGTGGCCCGGAGGGAGGG	ATTCGGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4CG-G5	GR	G4L/G5 C	
98	TGTGCAAGA	ATGTCTACAGTGTGGGCTTACACGG	ACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4CG-G5	GR	G4L/G5 C	
36	TGTACAA	AGACGGGGAGCCCGCCAAAG	TACTTTCCTTACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	CAACACCGTCTTCT	G4CG-G5	GR	G4L/G5 C	
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	CAACACCGTCTTCT				

III. G1 VDJ to G5 C region

Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G1	TGTGCAAAAAGC		GCTATCTTGATCACTGGGGACAAGGGACCATGGTGACCGTGACTGCAG	TGACAACATCTTCT				
G1G5-3	TGTGTAA	ACCCTATTCGGGGTAGGGTGGATCG	TCTTGATCACTGGGGCGAAGGGACCATGGTGACCGTTGCTGCAG	CAACACCGTCTTCT	G1-G5	JS	V1/G5 C	GQ282628
G1G5-7	TGTGCAA	AATGTAGGCTAAGTGGGGCTGAG	TATCTTGCGCACTGGGGACATGGGACCAAGGTGACCGTGACTGCAG	CAACACCGTCTTCT	G1-G5	JS	V1/G5 C	GQ359847
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	CAACACCGTCTTCT				

IV. G2A VDJ to G5 C region

Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G2A	TGTGCAAGAGAC		ACTATTTTGATTACTGGGGACAAGGGACCATGGTGACCGTGACTTTAG	TGACACCGTCTTCC				
G2G5-F27	TGTGCAAGAGAC	TCGTATGCTGACGGTAG	TTTTGATTACTGGGGACAAGGGACCATGGTGACCGTGACTTTAG	CAACACCGTCTTCT	G2A-G5	JS	V1-5/G5 C	GQ359852
G2G5-B9	TGTGCAAGAGAC	TCGTATGCTGACGGTAG	TTTTGATTACTGGGGACAAGGGACCATGGTGACCGTGACTTTAG	CAACACCGTCTTCT	G2A-G5	JS	V1-5/G5 C	GQ359856
G2G5-C6	TGTGCAAGAGAC	TCGTATGCTGACGGTAG	TTTTGATTACTGGGGACAAGGGACCATGGTGACCGTGACTTTAG	CAACACCGTCTTCT	G2A-G5	JS	V1-5/G5 C	GQ359858
G2G5-34	TGTGCAAGAGA	GAGGCCAGTAGGGCGGTGGTATTT	TTTTGATTACTGGGGACGCGGGACCATGGTAACCGTGACTTTAG	CAACACCGTCTTCT	G2A-G5	PI	V1-5/G5 C	GQ282627
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	CAACACCGTCTTCT				

V. G2B VDJ to G5 C region

Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G2B	TGTGCAAGAGAC		ACTATTTTGGTTACTGGGGACAAGGGACCATGGTGACAGTGACTTTAG	TGACACCGTCTTCC				
G2G5-E11	TGTGCAAGAGAC	GCCTACAGTGGTGGGGATAGCCGGCCA	TTTGGTTACTGGGGACAAGGGACCATGGTGACAGTGACTTTAG	CAACACCGTCTTCT	G2B-G5	PI	V1-5/G5 C	GQ359832
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	CAACACCGTCTTCT				

VI. G1 VDJ to G2A C region

Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G1	TGTGCAAAAAGC		GCTATCTTGATCACTGGGGACAAGGGACCATGGTGACCGTGACTGCAG	TGACAACATCTTCT				
G1G2-1	TGTACAA	GACAGCTAGCCTGGCTTGGCCGGGCT	TATCTTGATCACTGGGGACAAGGGACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G1-G2A	PI	V1/G2A C	GQ282630
G2A	TGTGCAAGAGAC		ACTATTTTGATTACTGGGGACAAGGGACCATGGTGACCGTGACTTTAG	TGACACCGTCTTCC				

VII. G1 VDJ to G2B C region

Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G1	TGTGCAAAAAGC		GCTATCTTGATCACTGGGGACAAGGGACCATGGTGACCGTGACTGCAG	TGACAACATCTTCT				
G1G2-19	TGTGCAAAA	CTCCGCACGGTTGGACCACCCCTGGATTGGAC	TCTTGATCACTGGGGACAAGGGACCATGGTGACCGTTCTGCAG	TGACACCGTCTTCC	G1-G2B	PI	V1/G2B C	
G1G2-16	TGTACGGAAA	CACATGGGGGCGTGCGCTGGGCTTA	TGATCACTGGGGACAAGGGACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G1-G2B	PI	V1/G2B C	
G2B	TGTGCAAGAGAC		ACTATTTTGGTTACTGGGGACAAGGGACCATGGTGACAGTGACTTTAG	TGACACCGTCTTCC				

VIII. G5 VDJ to G2A C region

Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	CAACACCGTCTTCT				
G5G2-13	TGTGCAAC	CGCATACTACAGCGGGCCCATACCG	TCCGGGAACTGGGGACAAGGAACCATGGTGTTCGTGACTACAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359839
G5G2-16	TGTGCAAC	GCCTCCCGTGGGCGGGGGCAGGGACGCCGTAAC	TTACTGGGGACAAGGAACCATGGTGACCGTGACGACAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359840
G5G2-17	TGTGCAAC	TCTCGTCTGGGGACTACTCCTATATG	ACTACTCCGGCCTGTGGGGACAAGGAACCATGGTGACCGTGACCACAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359841
G5G2-31	TGTGCAACA	CGAATAAGTGGCGGGGCGTACTTGACGGGA	TCCGGTTGCTGGGGAGGAGGAACCATGGTGACCGTGACTACAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359842
G5G2-33	TGTGGGCGAG	TGGGGGTATACTTGGAGGG	CGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359843
G5G2-9	TGTGCCACA	CATGAACGGTACTTCAGTGAGACATTCGTGGCCCTGGCGAAAC	ACTACTCCGGCGACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359844
G2A	TGTGCAAGAGAC		ACTATTTTGATTACTGGGGACAAGGGACCATGGTGACCGTGACTTTAG	TGACACCGTCTTCC				

IX. G5 VDJ to G2B C region

Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	CAACACCGTCTTCT				
G5G2-21	TGTGCAAC	GGCGGCTTCAGAGGGTTCCTGGACGG	CCGTTTACTGGGGAGCAGGAACCATGGTGACCGTGACTACAG	TGACACCGTCTTCC	G5-G2B	GR	G5L2/G2 C	GQ359846
G2B	TGTGCAAGAGAC		ACTATTTTGGTTACTGGGGACAAGGGACCATGGTGACAGTGACTTTAG	TGACACCGTCTTCC				

X. G5 VDJ to G4CG C region

Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	CAACACCGTCTTCT				
G5G4-69n	TGTGCAAC	TTTTCGACCGTAAACGCTTGGAG	CCGCTTACTGGGGAGGAGGAACCAAGGTGACCGTGACCACAG	TGACACCGTCTTCC	G5-G4CG	GR	G5L2/G4 C	
G5G4-38n	GGTGTAAAAGAT	GTGGGTGGGCGGTTTTCACAAAG	ACTGGGGACACCGAACCATGGTGACCGTGACGTCAG	TGACACCGTCTTCC	G5-G4CG	GR	G5L2/G4 C	JQ272819
G5G4-142n	GGTGTAAAAGAT	GTTGGTGGGCGGTTTTCACAAAG	ACTGGGGACACCGAACCATGGTGACCGTGACGTCAG	TGACACCGTCTTCC	G5-G4CG	GR	G5L2/G4 C	JQ272820
G4CG	TGTGCAAGAGAC		ACGAATTCGCTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	TGACACCGTCTTCC				

XI. G5 VDJ to G4A C region

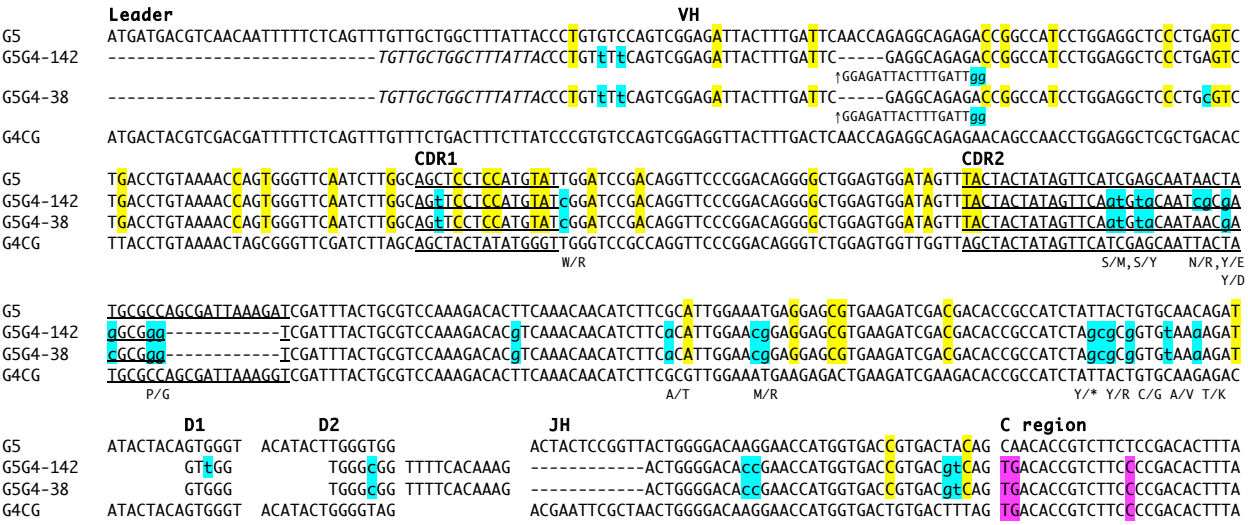
Clone	VH flank		JH	CH	Switch	shark	primer	Accession number
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	CAACACCGTCTTCT				
G5G4137n	GGTGCAAC	CCACGATCCCGTTTACTCAGCCTATACTTGCCCGT	CTAGGTTTACTGGGGAAAAGGAACCATGGTGACCGTGACCACAG	ins;spliced to	G4A C	GR	G5L2/G4 C	
G5G4-39	TGTGCAACAGA	CGGGCGCAACCGCT	ACTACTCCGCTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	TGACACCGTCTTCC	G5-G4A	GR	G5L2/G4 C	
G5G4-68	TGTGCAACA	CTGGGAACGTGGA	ACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	TGACACCGTCTTCC	G5-G4A	GR	G5L2/G4 C	
G5G4-81	TGTGGAAC	TCGGATCAACGCGAATTACTTGGG	CGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG	TGACACCGTCTTCC	G5-G4A	GR	G5L2/G4 C	
G4A	TGTGCAAGAGAC		ACTACTTTGATAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	TGACACCGTCTTCC				

Figure S2. CDR3 and J/C junctions of switched VDJ.

Top, RT-PCR was performed to raise switched Ig sequences from several adults (GR, JS, PI). The first strand primers are listed, with the approximate positions below the H chain transcript. For PCR, the forward primer was in the leader or VH FR1 and the reverse in the C region. Only the CDR3 and J/C splice are shown below; some sequences were entered into GenBank, as indicated.

Bottom, I-XI, sequences are compared to the IgH subfamily (identities highlighted in yellow in VDJ and C in pink). Mutations are marked in blue; red indicates non-productive sequences. Sequences shown as sharing CDR3 differ in mutations throughout VDJ. Sets I-XI: [I] G4A VDJ-G5 C region. First strand primed with G5-specific G5C2. (A) PCR with universal V1-5 and specific G5CH1R. Fragments of ~600 bp were digested with BamHI; non-digested fragments were cloned into pGEM and analyzed. (B) Direct PCR was done with combination G4L and G5CH1R. [II] G4CG VDJ-G5 C region. See legend to I. [III] G1 VDJ to G5 C region. Direct PCR primer V-1 and G5CH1R. [IV] G2A VDJ to G5 C region. See legened to I. [V] G2B VDJ to G5 C region. See legend to I. [VI] G1 VDJ to G2A C region. First strand primed with G2A-C2a. Direct PCR primers V-1 and G2A-C2b [VII] G1 VDJ to G2B C region. First strand primed with G2B-C2a. Direct PCR with primers V-1 and G2B-C2b. [VIII] G5 VDJ to G2A C region. First strand primed with G2A-C2a. Direct PCR with G5L2 and G2A-C2b, expected size ~840 bp. [IX] G5 VDJ to G2B C region. First strand primed with G2B-C2a. Direct PCR with G5L2 and G2B-C2b, expected size about 840 bp. [X] G5 VDJ to G4CG C region. First strand primed with G4CH1A. Direct PCR with G5L2 and G4CH1B, expected size ~552 bp. [XI] G5 VDJ to G4A C region.

A. Non-productive G5 VDJ with G4 C region



B. Five related cDNAs from phage library, G2B VDJ with G2A C region

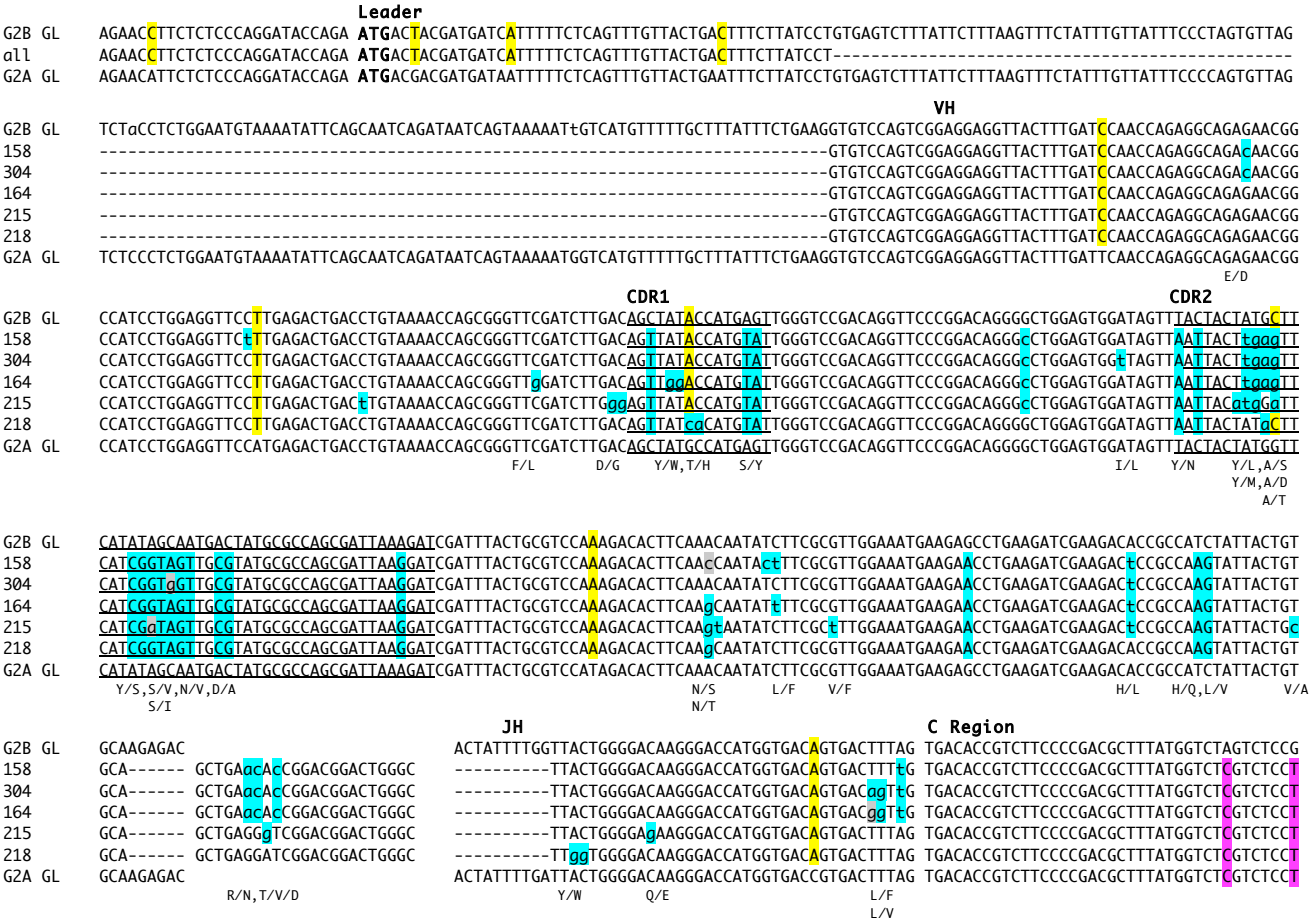
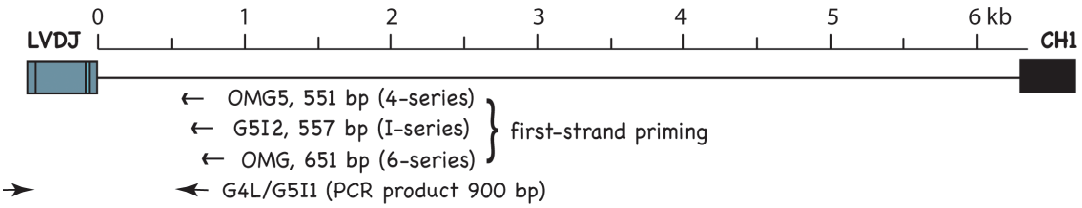


Fig. S3. Clonally related, switched Ig sequences.

A. Non-productive G5 VDJ switched to G4CG C region. G5 VDJ switched to G4 C region were amplified from shark-GR cDNA with subfamily-specific primers directed to the G5 leader (G5L2) and G4 C region (G4CH1B). Two out-of-frame VDJ were related, sharing a common CDR3; clones G4G5-142 and -38 shared a deletion (dashes) and duplication (arrow and sequence) in FR1, a deletion in CDR2 (gapped with dashes) and multiple mutations. The VDJ are out-of-frame, but mutations that would have otherwise lead to amino acid substitutions are indicated from CDR1 to FR3. Asterisk in Y/* indicates mutation tyrosine to stop codon. Accession numbers JQ272819-20.

B. Five switched sequences isolated from the shark-GR epigonal organ cDNA library. Five cDNAs sharing one CDR3 are compared with reference GL sequences from G2A and G2B. See Fig. 2B legend. Mutant nucleotides common to all five cDNAs are in upper case; when there is more than one change at a position, the second is marked in gray. Mutations leading to amino acid substitutions are indicated beneath codons.



B. Insertion (I58, I41)

```
        CDR3       JH                                                  1                  ↓
G4CG                ACGAATTCGCTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG GTAAGAACCTTGAATGTTTT-------CAGCAACCGTTATATTTGTTTTAACTGCATATT 53
I58     CCCCCATACTGCGACT TTCGCTAACTGGGGACATGGAACCATGGTGACTGTGACTTCGG GTAAGAACCTTGAATGTTTTGCGATCTTAACAACCGCGATCTCTATTTTCGTTATTTCGT 60
I41     CCCCCATACTGCGAGG TTCGCTAACTGGGGACATGGAACCATGGTGACAGTTAGTGAGG GTAAGAACCTTGAATGTTTTGTGATCTTAACAACCGCGATCTCTATGGTCGTTATTTCGT 60
G5                  ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG GTAAGAAAATCAAACAT-------TCTTAGCAACCGGTATCTTTATTTTAATTATTTATT 53
```

C. “Deletion”-insertion (I68, 6-42)

```
        CDR3       JH                                                  1                                                  ↓
G4CG                ACGAATTCGCTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTGCATATTAATTTGAGTAATTCATGATTCAAA 77
I68     GGGAAGCCGGGGTGAG TTCGCTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG GTAAGAGCCTTGCCTGTTTTCACTAACCGTTATATTTCATTGGA----------AGGGGGGTAATTCATGAGTTCAA 67
6-42    GGGAAGCCGGGGTGAG TTCGCTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG GTAAGAGCCTTGAATGTTTTCAGCAACCGTTATATTTCATTGGA----------AGGGGGGTAACACATGGTTTCCA 67
G5                  ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAATTATTTATTGATGTGAGTAATTCATGAGTTCAA 77
```

D. “Deletion” (4-5, I3-8, I3-52, I3-96)

(I)

```
        1                                                   ↓
G4A     GTAAGAACCTTGAATGTTTTCAGCAACCGTTATA------TTTGTTTTAACTGTATATTAATTTG // CCTTTTCTGCAAAGTAATTATAAAATCCATTTACGCCTCAGCAAATAAGTAAACTGCTGG 179
4-5     GTAAGAACCTTGACGGTTTTCAGCAA-CGAGATAAGGTGGTATACTGATATCGTATAT-------    ------------------------CCCTCGTGACCGTCTCAGCAACTGAATGCAGTGCTGC  94
G5      GTAAGAAAATCAAACATTCTTAGCAACCGGTATC------TTTATTTTAATTATTTATTGATGTG // ATTTTTCTGCAAAGTGATTAGAACCCTCGTTTAAGTCTCAGCAAATGAATGAAGTGCTGC 179
```

(II)

```
                                                                                CDR3                                                JH
G4A     TGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGTGCAAGAGAC                                                          ACTACTTTGATAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG
4-5     TGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGTCAAAG---- TCACCGATACTACAGCAGACCGGGGACGCTCCGAGACACT --------------TGGGGACAAGGAACCATGGTGACTGTGTCTTTAG
I3-8    TGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGTCACGG---- TCACCGATACTACAGTAGACTGGGGACGCTCCGAGACACT --------------TGGGGGCAAGGAACCATGGTGACTGTGACTTTAG
I3-52   TGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGTCAAAG---- TCACCGATACTACAGTAGGCAGGGGACGCTCCGAGACACT --------------TGGGGACAAGGAACCATGGTGACTGTGACTTTAG
I3-96   TGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGTCAAAG---- TCACCGATACTACAGTAGGCAGGGGACGCTCCGAGACACT --------------TGGGGACAAGGAACCATGGTGACTGTGACTTTAG

        1                   (duplication)                                   ↓
G4A     GTAAGAACCTTGAATGTTTTC---------------AGCAACCGTTATA-------TTTGTTTTAACTGTATATTAATTTGAG // AAAGTAATTATAAAATCCATTTACGCCTCAGCAAATAAGTAAACTGCTGG 179
4-5     GTAAGAACCTTGACGGTTTTC---------------AGCA-ACGAGATA-AGGTGGTATACTGATATCGTATAT---------    -------------CCCTCGTGACCGTCTCAGCAACTGAATGCAGTGCTGC  94
I3-8    GTAAGAACCTTGAATGTTTTCACCTTGAAACCTTTCAGCA-CCGTTATA-GGGTTCTATAGTATTATCGTATAT---------    -------------CCCTCGT----------CCACACAGGGACTATGCTGC  99
I3-52   GTAAGAACTTTGAATGTTTTC---------------AGCA-GCGTTATACAGGTGGTATAGGGATATCGTATAT---------    -------------CCCTCGTTTAAGTCTCAGCAACTGCATGAAGTGCTGC  95
I3-96   GTAAGAACTTTGAATGTTAGC---------------AACACCGTGGCAAACGGTGGTATAGGGATATCGTATAT---------    -------------CCCTCGTTTAATTCTCAGCAATTGCAGCTAGTGCTGC  96
G5      GTAAGAAAATCAAACATTCTT---------------AGCAACCGGTATC-------TTTATTTTAATTATTTATTGATGTGAG // AAAGTGATTAGAACCCTCGTTTAAGTCTCAGCAAATGAATGAAGTGCTGC 179
```

E. “Deletion” (4-7)

```
        1                                                   ↓
G4A     GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTGTATATTAATTTGAGTA // GTGAGAGATTCAGTTATTCGGCGTTTCTCTCATTTTTATCAG-ATGGAAAGACAGTTTG 236
4-7     GTAAGAATCTTGACCTCTTTCAGCAACCGTTACGGCGGTTTTAACTGTATATTAATTTGA---    ----------------CTCGGTATTTCACTCTTTTTTTCCCGCAGCAGCATACTGCGTG 103
G5      GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAATTATTTATTGATGTGAGTA // CTGAGGGATTCAGCTGCTCGGTATTTCACTCTTTTTTTCCCACATGGGCATACTGCGTG 237
```

F. “Deletion”-insertion (4-70)

```
        1                                                   ↓
G4A     GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTGTATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCTGCGCTGTGTTTAGTTTGGTTCCAATTGC 120
4-70    GTAAGAACCTCTAATGTTTTCAGCAAACGTTAGGTTTGCTTTAACTGT-TATTGATGTGAGTAACTCTTAGGTGGGTGGTAGGGC--------------------------TAGAATTCA  94
G5      GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAATTATTTATTGATGTGAGTAATTCATGAGTTCAATGAAACTCAAATTCTGTGCTGAGTTTAGTTTGGTTAGAATTGA 120
```

G. “Deletion”-insertion (I7)

```
        1                                                   ↓
G4A     GTAATTCATGATTCAAATGAAACTCGAATTCTGCGCTGTGTTTAGTTTGGTTCCAATTGCCTT // CAAACATTAAGAATTTAAAAGTGTTTAAAA-------AAA----AACAAAATATGCTAAAAT 384
I7      GTTCCTCATGATTCAAACGACACTCCCATTCTGCGCTGTGTTTAGTTTGGTTCCAACTGCC--    ----ACGTAACCTTTTAAAGATATTCGAAATACTGCCAAATTATTGACAAAATATGCTAAAAT 179
G5      GTAATTCATGAGTTCAATGAAACTCAAATTCTGTGCTGAGTTTAGTTTGGTTAGAATTGATTT // GAAACGTTAAGAATTTAAAGATATTCGAAATACTGCCAAATTATTGACAAAATATGCTAAAAT 399
```

H. “Deletion”-insertion (I16)

```
        1   ↓
G4A     GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTGTATATTAATTTGA // CTTTTCTGCAAAGTAATTATAAAATCCATTTACGCCTCAGCAAATAAGTAAACTGCTGGT 180
I16     GTAAGAACCG-------------------------------------------------    ---------AAAGCTATTAGAGGCCTCGTTTAAGTCTCAGCAAATGAATGAAGTGCCCTT  61
G5      GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAATTATTTATTGATGTGA // TTTTTCTGCAAAGTGATTAGAACCCTCGTTTAAGTCTCAGCAAATGAATGAAGTGCTGCT 180
```

I. “Deletion”-insertion (I29)

```
        1                   ↓
G4A     GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAA // AGATGGAAAGACAGTTTGGCTGGTTTAATTAAAG----AAAAATGTCTGAAACAGTATGACGTTTCAACAATGATTAA 292
I29     GTAAGAACCTTGAATGTTTTTAGCAACCGTTATATT--------    --------GACTGGGGAGTCTGGGGTCATAGATACACCGAAAACCTCTTAGA-ATTATGATGAATAAACATTGTTTTA 105
G5      GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAA // ACATGGGCATACTGCGTGTCTGGTTTCATTGAAACACCGAAAACCTCTTAGA-ATTATGATGAATAAACATTGTTTAA 296
```

J. “Deletion”, homology? (I36)

```
        181                                                                                                homology
G4A     GAGAGATTCAGTTATTCGGCGTTTCTCTCATTTTTATC-AGATGGAAAGACAGTTTGGCT // ----------AAAAACAAAATATGCTAAAATATTATTGTCAATTTCTTCAAT 405
I36     GAGAGATTCAGTTATTCGGCGTTTCTCTCAT----------------------------    ----------AACTACAAGCTATGCTAGAATATCATTACTAACTCCTTGGAT 253
G5      GAGGGATTCAGCTGCTCGGTATTTCACTCTTTTTTTCCCACATGGGCATACTGCGTGTCT // TACTGCCAAATATTGACAAAATATGCTAAAATATCATTACTAATTCCTTGAAT 420
```

K. “Deletion”-insertion (I69)

```
        1                   ↓
G4CG    GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTGCATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCTGCGCTGTGTTTAGTTTGGTTCCAATTGC 120
I69     GTAAGAACCTTGAACGTCCTCAGCAATCGTCC------------------------------------------------------------TCTGTGCTGAGTATCTTTTGGTTAGAATTGA 63
G5      GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAATTATTTATTGATGTGAGTAATTCATGAGTTCAATGAAACTCAAATTCTGTGCTGAGTTTAGTTTGGTTAGAATTGA 120
```

L. “Deletion”-insertion (I117)

```
        1                                                                                  ↓
G4CG    GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTGCATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTC // TCATTT-TTATCAGATGGAAAGACAGTTTGGCT 239
I117    GTAAGA--TTGAATGTTTTCAGCAACCGTTAT-TTTGTTTTAACTGCATATTAATTTGAGTAATTCATGATTCGAGTGAATCTCGA----    ----GCACCCCCACATGGGCATACTGCGTGTCT 113
G5      GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAATTATTTATTGATGTGAGTAATTCATGAGTTCAATGAAACTCAAATTC // TCTTTTTTTCCCACATGGGCATACTGCGTGTCT 240
```

M. Insertion (I148)

```
        1                                                                                  ↓
G4A     GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGT-TTTAACTGTATATTAATTTGAGTAATTCATGATTCA---------AATGAAACTCGAATTCTGCGCTGTGTTTAGTTTGG 110
I148    GTAAGAACCTTGAATGTTAAGAGCAACCGTTATCTTTGCCTTTAACTATATATTAATTTGAGTAATTCATGAGTCAGGGACCCCGAAAGAGGAGCAAGCTCTGTACTGAGTTTAGTTTGG 120
G5      GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTAT-TTTAATTATTTATTGATGTGAGTAATTCATGAGTTC---------AATGAAACTCAAATTCTGTGCTGAGTTTAGTTTGG 110
```

N. “Duplication”-insertion (I53)

(I)

```
        1
G4A     GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTGTATA-TTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCTGCGCTGTGTTTAGTTTGGTTCCAATTG 119
I53     GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTGACTAATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCTGCGCTGTGTTTAGTTTGGTTCCAATTG 120
G5      GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAATTATTTA-TTGATGTGAGTAATTCATGAGTTCAATGAAACTCAAATTCTGTGCTGAGTTTAGTTTGGTTAGAATTG 119

G4A     CCTTTTCTGCAAAGTAATTATAAAATCCATTTACGCCTCAGCAAATAAGTAAACTGCTGGTGAGAGATTCAGTTATTCGGCGTTTCTCTCATTTTTATC-AGATGGAAAGACAGTTTGGC 238
I53     CCTTTTCTGCAAAGTAATTATAAAATCCATTTACGCCTCAGCAAATAAGTAAACTTACGGTGAGAGATTCAGTTATTCGGCGTTTCTCTCATTTTTATC-AGATGGAAAAAACAGTTTGGC 239
G5      ATTTTTCTGCAAAGTGATTAGAACCCTCGTTTAAGTCTCAGCAAATGAATGAAGTGCTGCTGAGGGATTCAGCTGCTCGGTATTTCACTCTTTTTTTCCCACATGGGCATACTGCGTGTC 239
                                                  ⇑see G5 at 169 bp (below)
                                                                                     ↓
G4A     TGGTTTAATTAAAG----AAAAATGCCTGAAACAGTATGACGTTTCAACAATGATTAATGCCAGTGACACCCAATATCTCCGAGTAT 322  AAGTAAACTGCTGGTGAGAGATTCAGTTATTCGGCG 201
I53     TGGTTTAATTAAAG----AAAAATGTCTGAAACAGTATGACGTTTAAACAATGATTAATGCCAGTGACACCCAATAGGCACTGC--- 319  ---TGAAGTGCTACTGAGGCTTTCAGCTGCTCGGTA 352
G5      TGGTTTCATTGAAACACCGAAAACCTCTTAGA-ATTATGATGAATAAACATTGTTTAATATCTGTGACACCCCATTTCTGCGAGTTT 326  GAATGAAGTGCTGCTGAGGGATTCAGCTGCTCGGTA 201
```

(II) **Switch junction:**

```
                                                                                     ↓
G4A     ATGACGTTTCAACAATGATTAATGCCAGTGACACCCAATATCTCCGAGTATTAGTATCGACAACAAACATTAAGAATTTAA 351
I53     ATGACGTTTAAACAATGATTAATGCCAGTGACACCCAATAGGCACTGCTGAAGTGCTACTGAGGCTTTCAGCTGCTCGGTA 352
G5      TTTTTCTGCAAAGTGATTAGAACCCTCGTTTAAGTCTCAGCAAATGAATGAAGTGCTGCTGAGGGATTCAGCTGCTCGGTA 201
```

O. “Duplication”-insertion (I22)

(I)

```
        1
G4A     GTA-AGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTG-------------TATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCTGCGCTGTGTTTAGTTTGGTTCC 114
I22     GTAGAGACCCTTGAATGTTTTCAGCAATTGTTATATTTGTTTTAACTGCTCCGCGAAACTGTATATTATTTTGAGTAATTCATAGCAATAATGAAACTCGAATTCTGCGCTGTGTTTAGTTTGGTTCC 128
G5      GTA-AGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAATTA-------------TTTATTGATGTGAGTAATTCATGAGTTCAATGAAACTCAAATTCTGTGCTGAGTTTAGTTTGGTTAG 114
                                                          ⇑see G5 at 28 bp (below)
                                                                                     ↓
G4A     AATTGCCTTTTCTGCAAAGTAATTATAAAATCCATTTACGCCTCAGCAAATAAGTAAACTGCTGGTGAGAGATTCAGTTATTCGGCG 201  --TATATTAA-TTTGAGTAATTCATGATTCAAA  77
I22     AACTGCCTTTTCTGCAAAGTAATTATAAAATCCATTCACGCCTCC-CAAATAGACATACTGCTGGTTAGAGATTCAGTTATCGC--- 211  ----TATTGAAAGTGAGTAATTGTTGAGTTCAC 240
G5      AATTGATTTTTCTGCAAAGTGATTAGAACCCTCGTTTAAGTCTCAGCAAATGAATGAAGTGCTGCTGAGGGATTCAGCTGCTCGGTA 201  --TTTATTGA-TGTGAGTAATTCATGAGTTCAA  77
```

(II) **Switch junction:**

```
                                                                                     ↓
G4A     AATTGCCTTTTCTGCAAAGTAATTATAAAATCCATTTACGCCTCAGCAAATAAGTAAACTGCTGGTGAGAGATTCAGTTATTCGGCGGCGTTTCTCTCATTTTTATCAGATGG 224
I22     AACTGCCTTTTCTGCAAAGTAATTATAAAATCCATTCACGCCTCCC-AAATAGACATACTGCTGGTTAGAGATTCAGTTATCGCTATTGAAAGTGAGTAATTGTTGAGTTCAC 240
G5                                      GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAATTATTTATTGA-TGTGAGTAATTCATGAGTTCAA  77
```

P. Homology (I167)

```
        1                                                                                  ↓   or   ↓
G4A     GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTGTATATTAATTTGAGTAATTCATGATTCAAATGAAACTCG-AATTCTGCGCTG // ATATCTCCGAGTATTAGTATCGACAACAAACA 339
I167    GTAAGAACCTTGAATGTTTTCAGCAACCCTTATATTTGTTTTAACTGTATATTACTTTGAGTAATTCATGATTCAAATGAAACTCGTATTTCTGCGA--    ---------AGTTTTAGGATAGACAAGGAACG 120
G5      GTAAGAAAATCAAACATTCTTAGCAACCGGTATCTTTATTTTAATTATTTATTGATGTGAGTAATTCATGAGTTCAATGAAACTCA-AATTCTGTGCTG // ATTTCTGCGAGTTTTAGGATAGACAAGAAACG 343
```

Switch area shaded

```
G4A     GTAAGAACCTTGAATGTTTTCAGCAACCGTTATATTTGTTTTAACTGTATATTAATTTGAGTAATTCATGATTCAAATGAAACTCG--AATTCTGCGCTGTGTTTAGTTTGGTTCCAATTG 119
I167    GTAAGAACCTTGAATGTTTTCAGCAACCCTTATATTTGTTTTAACTGTATATTACTTTGAGTAATTCATGATTCAAATGAAACTCGT-ATTTCTGCGAAGTTTTAGGATAGACAAGGAACG 120
G5      ATAAACATTGTTTAATAGGTTTCATTGAAACACCGAAAACCTCTTAGAATTATGATGAATAAACATTGTTTAATATCTGTGACACCCCATTTCTGCG-AGTTTTAGGATAGACAAGAAACG 343
```

Figure S4. Switch junctions.
Top, diagram of primers used (separately) for first strand cDNA and their locations along the J-C intron (position 1 is first position after JH). Spleen (OMG5, G5I2) and epigonal organ (OMG) RNA from shark-GR were used. PCR primer pair G4L/G5I1 amplify fragments of about ~900 bp and G4 VDJ joined to G5 intron were obtained. Only the switch junction is shown for most of the sequences.

Bottom, alignments with G4 and G5. Positions shared with G4 highlighted in yellow, shared with G5 in pink, mutations in blue. The intron positions are numbered in all sequences. Arrow indicates where the G4A sequence identity ceases; hotspot motifs RGYW/WRCY are underlined in its vicinity or that of G5. Sequences were entered into Genbank, Accession numbers JQ272797-JQ272818.

B. Two sequences, I58 and I41, that share CDR3 are compared to G4 and G5. B. I58, I41. Two sequences share CDR3 (as indicated), some mutations (in blue) and switch point at position 20.
C. I68, 6-42. Two sequences share CDR3 (as indicated), some mutations (in blue), and switch point at position 41. Slashes indicate reference sequence not shown, portion deleted in cDNA clone.
D. 4-5, I3-8, I3-52, I3-96. I. The switch junction of 4-5 is shown, position 52 (arrow). II. The I3-series was obtained using first strand primer G5I4 at 1202 bp in the G5 J-C intron and PCR with G4L and G5I3 (at 1098 bp) to generate fragments of ~1498 bp. Three sequences shared CDR3 and switch point with 4-5. The duplication in I3-8 is marked.
E. 4-7. Switch point at position 60.
F. 4-70. Switch point marked by 1 bp deletion after position 48.
G. I7. Switch point at position 62, followed by large “deletion” and 3 bp insertion.
H. I16. Switch point at position 9, followed by “deletion” and 1 bp insertion before joining with G5 sequence.
I. I29. Switch point at position 36, followed by large “deletion. The 9 bp insertion is most likely a duplication of the adjacent GTCTGGGGT.
J. I36. Switch point at position 211, followed by large deletion. An alternative interpretation is the joining occurred through homology between G4A and G5 (underlined); the deletion and mutations would have occurred after switching.
K. I69. Switch point at position 30, “deletion” with 2 bp insertion.
L. Switch point at position 87, followed by large “deletion” and 5 bp insertion.
M. Switch point at position 76, followed by 9 bp insertion and extensive downstream changes.
N. I53. I. Switch point in G4A sequence is at position 311 but is taken up at position 169 in G5, generating a “duplication”, as if unequal crossingover had taken place. II. The actual I53 sequence is aligned with the disparate regions of G4A and G5 to show insertion of 8 bp at the switch point before the sequence continues as G5.
O. I22. I. Switch point in G4A sequences is at position 195 but is taken up at position 28 in G5 generating a “duplication”. II. I22 is aligned with the disparate regions of G4A and G5 to show insertion of 3 bp CGC before I22 continues as G5. P. I167.
P. I167. The switch junction of I167 includes 9 bp of sequence that is shared between G4A (86-95) and G5 (311-319) that may be involved in homology pairing.

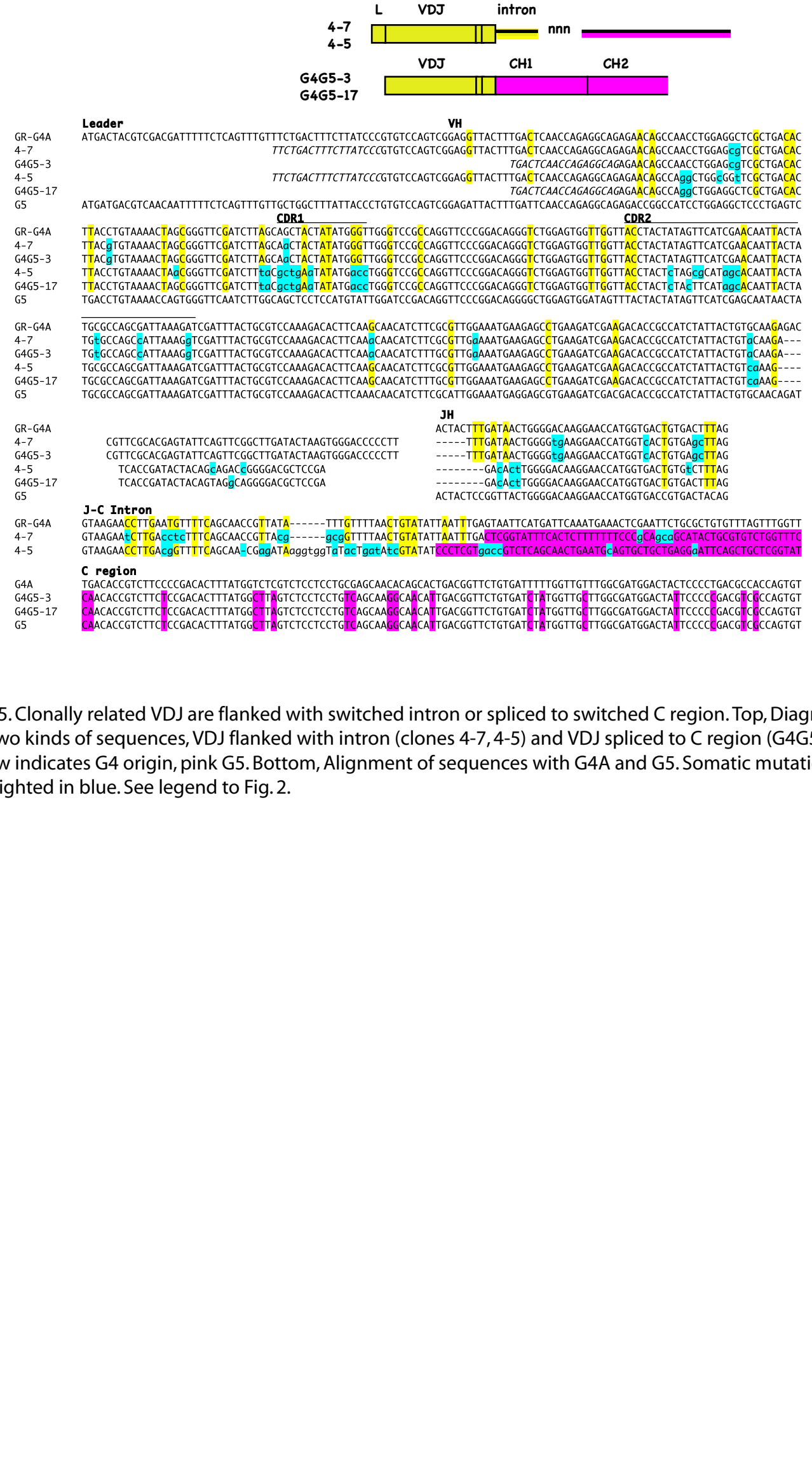
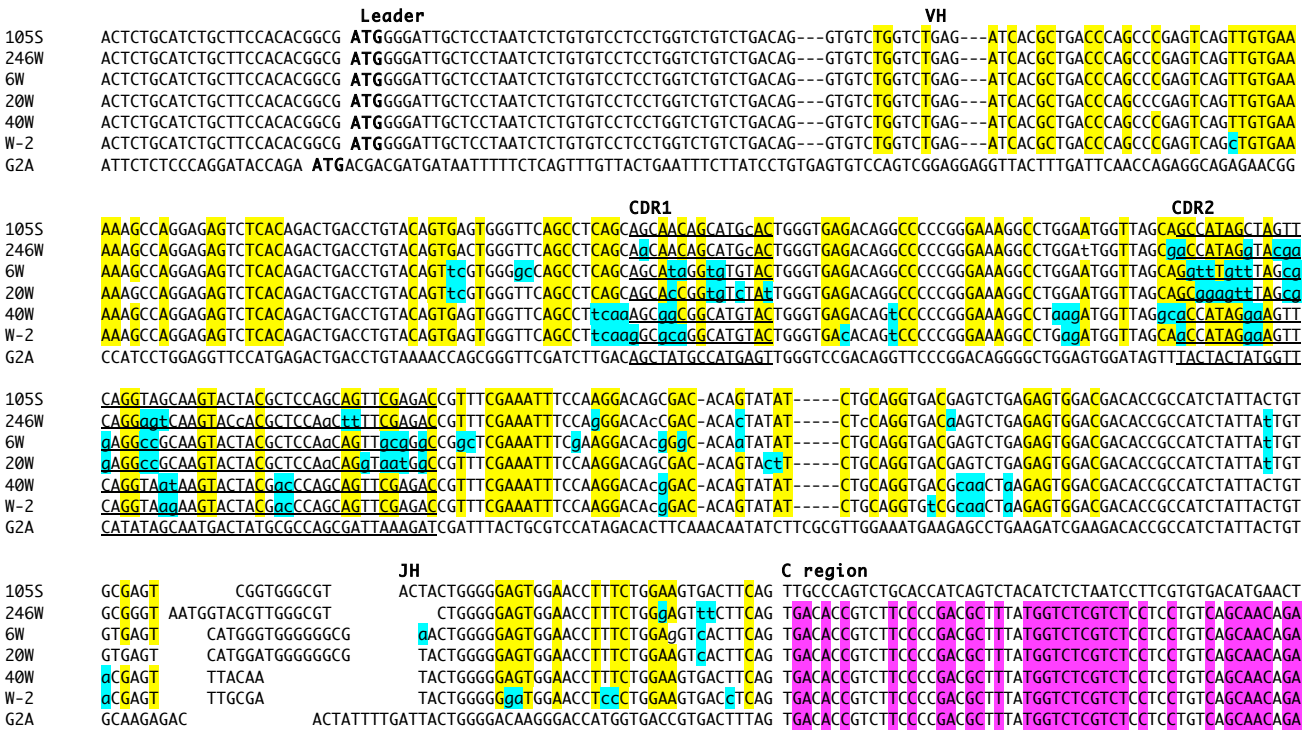


Fig. S5. Clonally related VDJ are flanked with switched intron or spliced to switched C region. Top, Diagram depicts the two kinds of sequences, VDJ flanked with intron (clones 4-7, 4-5) and VDJ spliced to C region (G4G5-3, -17). Yellow indicates G4 origin, pink G5. Bottom, Alignment of sequences with G4A and G5. Somatic mutations are highlighted in blue. See legend to Fig. 2.

A. IgW VDJ and IgM C region



B. IgM VDJ and IgW C region

```
G4CG      ----------------------------------------------------------------------------------------------------------------------------------
c57       CGGCCCAAGAGACCAAGAATCAGAGCAGAGTTACACTCGAGTGTGTAATCACTGGATTCTACCCAGACCTCATACAGGTCTCCTGGGAGAAGGATAGCAGCCTGATCTCCTCCAATACC
3 1-7     CGGCCCAAGAGACCAAGAATCAGAGCAGAGTTACACTCGAGTGTGTAATCACTGGATTCTACCCAGACCTCATACAGGTCTCCTGGGAGAAGGATAGCAGCCTGATCTCCTCCAATACC

G4CG      ----------------------------------------------------------------------------------------------------------------------------------
c57       CGTGCTGGCCTCACTGCTCTGGAGCAGACGGGGACATTCAGTGTCaGGCACTACCTGACTGTGAGCACAGAGGAGTGGAGGAAAGGTTCAGTCTTCACCTGTGTAGTGTCTCATCCACC
3 1-7     CGTGCTGGCCTCACTGCTCTGGAGCAGACGGGGACATTCAGTGTCaGGCACTACCTGACTGTGAGCACAGAGGAGTGGAGGAAAGGTTCAGTCTTCACCTGTGTAGTGTCTCATCCACC
                                                                  CH3
G4CG      ----------------------------------------------------------GTTTCGGAGTGCCGTGATTACACAGCTAAAATACTACCGCCACCAGTTGAACAGGTCTTACTGG
c57       ATCCAACTTTACCAGCAGTAAGGAGGTGAAGAATGTTCAAGATGAATGTTTGGACACTGGAATCTCAGTCACCTTAAGCAAGCCTCCATTTGCAGAGATCTGGAGGAACAGAACAGCAA
3 1-7     ATCCAACTTTACCAGCAGTAAGGAGGTGAAGAATGTTCAAGATGAATGTTTGGACACTGGAATCTCAGTCACCTTAAGCAAGCCTCCATTTGCAGAGATCTGGAGGAACAGAACAGCAA

G4CG      AGGCGACGGTGACGTTAACCTGCGTTGTGTACAATCTTCATTTTGAAGCCAACTTCACCTGGATACACGACAAGAAGACTCTGAAATCAGAGATTGTTGACCAGTCCGGAGAGCATTCC
c57       CCATTCTGTGTGAGGTACTTTACCGTGATTTAGAGGGAGTCAGGGTAACCTGGAAAGTGGATGGAATCATGAGACAAGATGGAGTGAAGACCCAGGGTCCCAAGAAAAGTGGACATAAA
3 1-7     CCATTCTGTGTGAGGTACTTTACCGTGATTTAGAGGGAGTCAGGGTAACCTGGAAAGTGGATGGAATCATGAGACAAGATGGAGTGAAGACCCAGGGTCCCAAGAAAAGTGGACATAAA

G4CG      GAAGGTGTGATCAGCAAGTTAGATATTTCTACTGAAGCCTGGCTGAGTGGGGATAAATTTGAATGCGTGGTGAACAATCAGTATTTACCGACTCCACTGAGAAACTCCATCCACAAAGA
c57       GAGATCATCTTCAGTAGACTGACAGTCCCTGCTGCAGAGTGGGAGAGTGGGGTCGAGTATATGTGTTTGGTGGAGGACAAAAATTTGCCAACACCAGAGAAGAGGTTCATCAGGAAATC
3 1-7     GAGATCATCTTCAGTAGACTGACAGTCCCTGCTGCAGAGTGGGAGAGTGGGGTCGAGTATATGTGTTTGGTGGAGGACAAAAATTTGCCAACACCAGAGAAGAGGTTCATCAGGAAATC
                CH4
G4CG      GAAATTGGTAAATCCGCTGGAACCATCGGTGTCCGTCCTCCTGCCAGCGACAGAAGAAGTCTCCGCACAGAGGTTTGTTTCGCTCACCTGTTTAGTGAGAGGTTTCAGGCCCCGAGAGA
c57       CCAAGATGATATTAAGACTCACCCTCAAGTCTACCTCCTGCCCCCTTCACCGGATGAGATGGAGACTGCTCATACTGCGACCCTGGTTTGCCTGGTCACTGGATTCTCTCCCGTAGATA
3 1-7     CCAAGATGATATTAAGACTCACCCTCAAGTCTACCTCCTGCCCCCTTCACCGGATGAGATGGAGACTGCTCATACTGCGACCCTGGTTTGCCTGGTCACTGGATTCTCTCCCGTAGATA

G4CG      TCTTTGTCAAGTGGACAACCAACGACAAGCCGGTTAATCCCACTAACTATAAGAACACCGAGGTGACGGCAGAGAGCGATAACACCTCCTTCTTCTTGTACAGCCTATTGTCCATTGCT
c57       TCGATCTGGCTTGGATGGCCAATGATACCCTTCTGAAGACAGGGTTCATTCA---TCAGCCAGTGACTGAGGATGGCAGAGGAGGCTGGAACTCAGG---CAGTCGCCTGACGGTCTCT
3 1-7     TCGATCTGGCTTGGATGGCCAATGATACCCTTCTGAAGACAGGGTTCATTCA---TCAGCCAGTGACTGAGGATGGCAGAGGAGGCTGGAACTCAGG---CAGTCGCCTGACGGTCTCT
                                                                                                                  Tailpiece
G4CG      GCGGAGGAGTGGGCCAGCGGTGCTTCTTACTCCTGTGTGGTGGGACATGAAGCGATTCCTTTGAAGATCATCAACAGAACCGTGGATAAATCCAGCGGTAAACCGAGTTTTGTGAATAT
c57       ACAGAGGAGTGGGACAGCGGCACCACATACTCCTGTGTGGTGGGACATGAGTCAGTCACAACAAATGTATTCAGAAGCATCAATAAATATCACAGCAAACCCAACCTGGTTAATGTCTC
3 1-7     ACAGAGGAGTGGGACAGCGGCACCACATACTCCTGTGTGGTGGGACATGAGTCAGTCACAACAAATGTATTCAGAAGCATCAATAAATATCACAGCAAACCCAACCTGGTTAATGTCTC

G4CG      TTCACTTGCACTGATGGACACCATTAATTCATGCCAA
c57       ACTTGTTTTGACTGAAAGTTTTAACTCCTGTTCA---
3 1-7     ACTTGTTTTGACTGAAAGTTTTAACTCCTGTTCA---
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Figure S6. A. IgW VDJ and IgM C region. Five cDNA sequences isolated from shark-GR epigonal library (Table 2) are compared with an IgW H chain cDNA (top, 105S; accession number AY609244) and IgM G2A H chain (bottom). Identities in VDJ highlighted in yellow, those in C region in pink; presumed mutations are in lower case and marked in blue. All cDNA were full-length and identical in C region sequence to the secreted form of G2A. Accession numbers JQ272792-96.

B. IgM VDJ and IgW C region. Clone c57 from shark-GR cDNA library is aligned with the IgM G4CG H chain sequence in the VH. Mutations are in lower case and highlighted in blue. Identities between G4CG and c57 JH highlighted in yellow. The C region of c57 (1961 bp, Accession number JN802117) is almost identical to that of clone 3 1-7 (AY524280), an IgW H chain sequence with a partial JH (as shown). The C region has been identified as belonging to the G5 subfamily of Igω genes (unpublished results). There are two gapping mismatches between 3 1-7 and c57 (shaded in 3 1-7), but the sequence of c57 has been confirmed and can be read through to its end. The C region is labeled CH1-4 according to the G4CG H chain sequence. The IgW sequences have two additional domains.