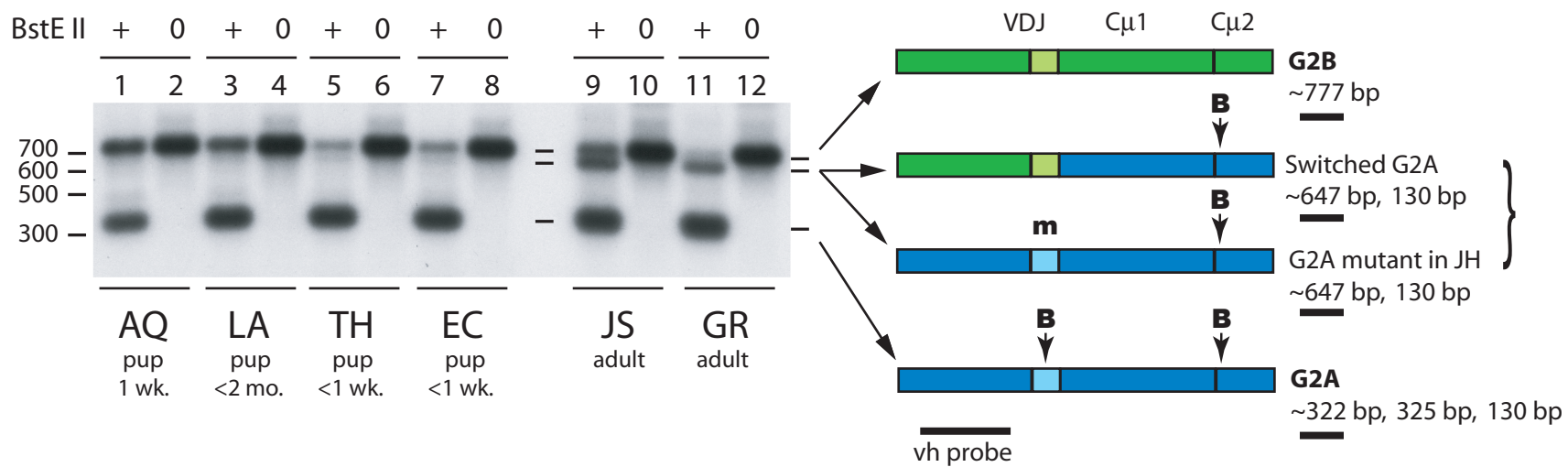
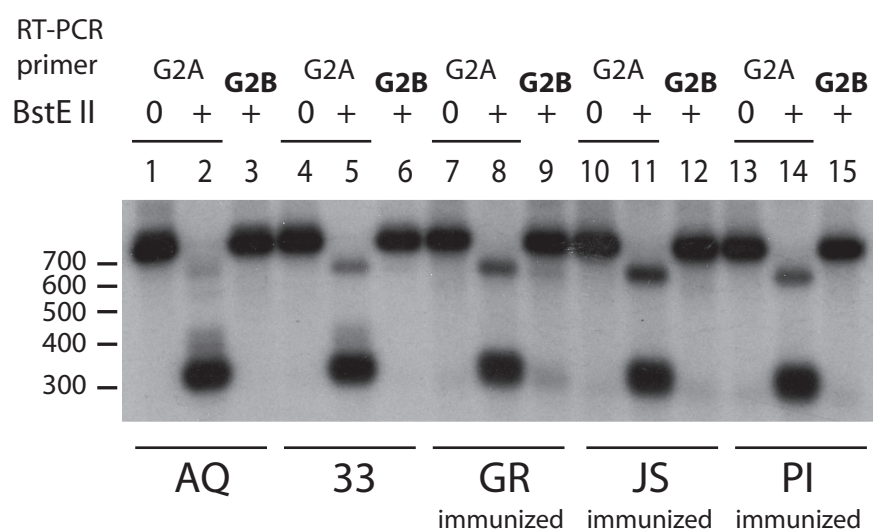
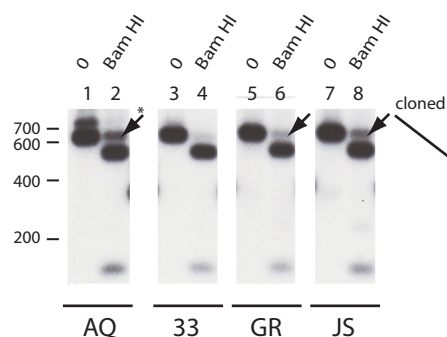
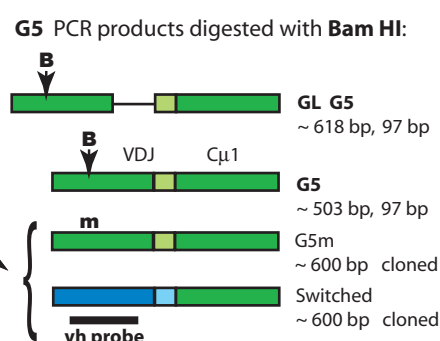


**A****B****C****D****E**

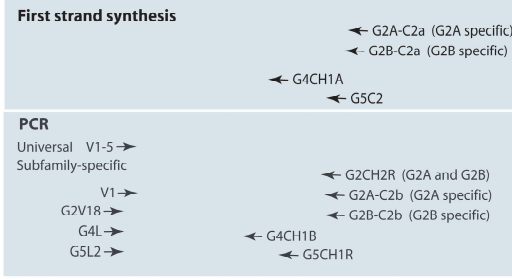
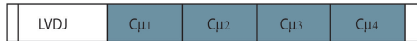
AQ	GR	JS	
156	96	235	total number clones analyzed
5	36	33	BamHI negative sequences
2	24	17	mutation at BamHI
0	10 (10)	10 (7)	switched: G2 or G4 VDJ to G5 C (unique clones)
3	2	6	in vitro hybrids

**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 C $\mu$ 2 (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 C $\mu$ 2. 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	ACTACTTTGATACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	TGACACCGTCTTCC				
G4G5-39	TGTGCC	CGGGGGGCAACTGGGGGAGC	CTTTGAAA	G4A-G5	GR	V1-5/G5 C	GQ359830
104	TGTGCC	CGGGGGGCAACTGGGGGAGC	CTTTGAAA	G4A-G5	GR	G4L/G5 C	
G4G5-33	TGTGCA	CAAGTATCTAATGAGTACCCCGAACAAGTATTTCCGGA	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	V1-5/G5 C	GQ359829
G4G5-3*	TGTACAAAGA	CGTTCGCACGAGTATTCAGTTCCGGCTTGATACTAAGTGGGACCCCTT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	V1-5/G5 C	GQ359827
G4G5-66	TGTGTA	CTAGAGACGACTGGGGAGTTCCGGGG	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	V1-5/G5 C	GQ359831
G4G5-17*	TGTCAAAG	TCACCGATACTACAGTAGGACGGGGACGCTCCGAGACACT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	V1-5/G5 C	GQ359826
G4G5-4	TGTGCAAG	GGCGCCATACAGTAGGTACAGACTGGGG	ACTTTGATACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	V1-5/G5 C	GQ359828
118*	TGTGTAACA	CGGTACTACAACCGTGGCTGGGGCCCTCGATCA	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	
38	TGTGC	TCGGACCGTATGGCCCC	ATACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	
108	TGTGCAT	CATCTAGCCACAGGGGT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	
119	TGTGCAAG	GCAGAAAGGTAGACTCACTGGGGGACCTCCG	GATACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	
111	TGTACAAGAG	GCCGACTACCCCAATCACTGGGTGTT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	
105	TGTG	TCTCCGATACGCGATATCCACGACTGGCGCTGG	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	
37	TGTGCAA	TCTCGTGACTACAGTACCGACCTGGGTGCT	ACTACTTTGATACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	
84	TGTCAAAG	CCGCCATCCCTCCGGGGGTGG	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	
86	TGTGCAAGA	TCGGTACAGCGCTCAGGTATACCCGCA	ATAA	G4A-G5	GR	G4L/G5 C	
61*	TGTGTAAGGGA	GGGTACTACATACTTGGGCATATATT	ATACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	JQ272821
53	TGTCAAAG	GCGGGGGACATCTTTACCCCGGG	ACTACTTTGATACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4A-G5	GR	G4L/G5 C	

**II. G4CG VDJ to G5 C region**

Clone	VH flank	JH	CH	Switch	shark	primer	Accession number
G4CG	TGTGCAAGAGAC	ACGAATTCGCTA	TGACACCGTCTTCC				
G4G5-81	TGTGCAA	TATCTAGCCACAGGGGT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4CG-G5	GR	V1-5/G5 C	GQ359850
151	TGTGCAA	CAGCTAGCCACAGGGGT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4CG-G5	GR	G4L/G5 C	
G4G5-88	TGTGCA	GTAAGAATCGGACGCCG	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4CG-G5	GR	V1-5/G5 C	GQ359851
G4G5-76	TGTACA	GTAAGAATCGGACGGAGG	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4CG-G5	GR	V1-5/G5 C	GQ359849
G4G5-46	TGTGCAAGAGAC	CGAGTCGGTACGATGCCAACCTGGGGGCCGAGAGAGCC	GAAATTCGCTA	G4CG-G5	GR	V1-5/G5 C	GQ359848
114	TGTGCA	TTAGCTGGTCAGTGGGTGGATATACGGTAC	AATTCGCTC	G4CG-G5	GR	G4L/G5 C	
146	TGTGCA	GAAAGCCGACGGGGTGG	ATTTCGCTA	G4CG-G5	GR	G4L/G5 C	
150	TGTG	TCGTCAAGCTCGGACGGAGT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4CG-G5	GR	G4L/G5 C	
16	TGTGCAAGA	CTCATAGAGTCGGTGGCCGGAGGGAGGG	ATTTCGCTA	G4CG-G5	GR	G4L/G5 C	
98	TGTGCAAGA	ATGTCTACAGTGTGGGCTTACACGG	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4CG-G5	GR	G4L/G5 C	
36	TGTCAA	AGACGGGGAGCCGCCAAAG	TACTTTCTTACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4CG-G5	GR	G4L/G5 C	
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G4CG-G5	GR	G4L/G5 C	

**III. G1 VDJ to G5 C region**

Clone	VH flank	JH	CH	Switch	shark	primer	Accession number
G1	TGTGCAAAAGC	GCTATCTTGAT	TGACAAATCTTCT				
G1G5-3	TGTGAA	ACCCTATTCCGGGTAGGGTGGATCG	TCTTGAT	G1-G5	JS	V1/G5 C	GQ282628
G1G5-7	TGTGCAA	AATGTAGGCTAAGTGGGGCTGAG	TATCTTCCG	G1-G5	JS	V1/G5 C	GQ359847
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG				

**IV. G2A VDJ to G5 C region**

Clone	VH flank	JH	CH	Switch	shark	primer	Accession number
G2A	TGTGCAAGAGAC	ACTATTTTGATTACTGGGGACAAGGGACCATGGTGACTGTGACTTTAG	TGACACCGTCTTCC				
G2G5-F27	TGTGCAAGAGAC	TCGTATGCTGACGGTAT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G2A-G5	JS	V1-5/G5 C	GQ359852
G2G5-B9	TGTGCAAGAGAC	TCGTATGCTGACGGTAT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G2A-G5	JS	V1-5/G5 C	GQ359856
G2G5-C6	TGTGCAAGAGAC	TCGTATGCTGACGGTAT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G2A-G5	JS	V1-5/G5 C	GQ359858
G2G5-34	TGTGCAAGAGA	GAGGCCAGTAGGGCGGTGGTATTT	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G2A-G5	PI	V1-5/G5 C	GQ282627
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG				

**V. G2B VDJ to G5 C region**

Clone	VH flank	JH	CH	Switch	shark	primer	Accession number
G2B	TGTGCAAGAGAC	ACTATTTGGTTACTGGGGACAAGGGACCATGGTGACTGTGACTTTAG	TGACACCGTCTTCC				
G2G5-F11	TGTGCAAGAGAC	GCCTACAGTGGTGGGGATAGCCGGCCA	TTTGTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG	G2B-G5	PI	V1-5/G5 C	GQ359832
G5	TGTGCAACAGAT		ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG				

VI. G1 VDJ to G2A C region		JH	CH	Switch	shark	primer	Accession number
Clone	VH flank						
G1	TGTGCAAAAAGC	GCTATCTTGATCTACTGGGGACAAGGGACCATGGTGACCGTGACTGCAG	TGACAACATCTTCT				
G1G2-1	TGTACAA GACAGCTAGCCTGGCTTGCCGGGCT	TATCTTGATCTACTGGGGACAAGGGACCATGGTGACCGTGACTGCAG	TGACAACCTCTTCC	G1-G2A	PI	V1/G2A C	GQ282630
G2A	TGTGCAAGAGAC	ACTATTTTGATTACTGGGGACAAGGGACCATGGTGACCGTGACTTTCAG	TGACAACCTCTTCC				
VII. G1 VDJ to G2B C region		JH	CH	Switch	shark	primer	Accession number
Clone	VH flank						
G1	TGTGCAAAAAGC	GCTATCTTGATCTACTGGGGACAAGGGACCATGGTGACCGTGACTGCAG	TGACAACATCTTCT				
G1G2-19	TGTGCAAAA CTCCGCACGGTTGGACCACCCCTGGATTGGAC	TCTTGATCTACTGGGGACAAGGGACCATGGTGACCGTTCTGCAG	TGACAACCTCTTCC	G1-G2B	PI	V1/G2B C	
G1G2-16	TGTACGGAAA CATATGGGGCGTGCTGGGCTTA	TGATCTACTGGGGACAAGGGACCATGGTGACCGTGACTGCAG	TGACAACCTCTTCC	G1-G2B	PI	V1/G2B C	
G2B	TGTGCAAGAGAC	ACTATTTTGATTACTGGGGACAAGGGACCATGGTGACAGTGACTTTCAG	TGACAACCTCTTCC				
VIII. G5 VDJ to G2A C region		JH	CH	Switch	shark	primer	Accession number
Clone	VH flank						
G5	TGTGCAACAGAT	ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	CAACACCGTCTTCT				
G5G2-13	TGTGCAAC CGCATACTACAGGGGCCCATACCG	TCCGGAACTGGGGACAAGGAACCATGGTGCTGTGACTACAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359839
G5G2-16	TGTGCAAC GCCTCCCCTGGCGGGGGCAGGGACGCCGTAAAC	TTACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359840
G5G2-17	TGTGCAAC TCTCGTCTGGGGACTACTCCTATATG	ACTACTCCGGCTGTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359841
G5G2-31	TGTGCAACA CGAATAAGTGGCGGGCGTACTTTGACGGGA	TCCGGTTGCTGGGGAGGAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359842
G5G2-33	TGTGCGCGAG TGGGGGTACTTTGGAGGG	CGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359843
G5G2-9	TGTGCAACA CATGAACGGTACTTCACTGAGACATCTTGCCCTGGCGAAAC	ACTACTCCGGCGACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G2A	GR	G5L2/G2 C	GQ359844
G2A	TGTGCAAGAGAC	ACTATTTTGATTACTGGGGACAAGGGACCATGGTGACCGTGACTTTCAG	TGACACCGTCTTCC				
IX. G5 VDJ to G2B C region		JH	CH	Switch	shark	primer	Accession number
Clone	VH flank						
G5	TGTGCAACAGAT	ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	CAACACCGTCTTCT				
G5G2-21	TGTGCAAC GCGCGCTTACAGAGGTTCTCTGGACGG	CCGTTTACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G2B	GR	G5L2/G2 C	GQ359846
G2B	TGTGCAAGAGAC	ACTATTTTGATTACTGGGGACAAGGGACCATGGTGACAGTGACTTTCAG	TGACACCGTCTTCC				
X. G5 VDJ to G4CG C region		JH	CH	Switch	shark	primer	Accession number
Clone	VH flank						
G5	TGTGCAACAGAT	ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	CAACACCGTCTTCT				
G5G4-69	TGTGCAAC TTTTCGACCGTAAACGCTTTGGAG	CCCGTTACTGGGGAGGAGGAACCAAGGTGACCGTGACCACAG	TGACACCGTCTTCC	G5-G4CG	GR	G5L2/G4 C	
G5G4-38	GGTGTAAAGAT GTGGGTGGGCGGTTTTCACAAAG	ACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G4CG	GR	G5L2/G4 C	JQ272819
G5G4-142	GGTGTAAAGAT GTTGGTGGGCGGTTTTCACAAAG	ACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G4CG	GR	G5L2/G4 C	JQ272820
G4CG	TGTGCAAGAGAC	ACGAATTCGTAACCTGGGGACAAGGAACCATGGTGACTGTGACTTTCAG	TGACACCGTCTTCC				
XI. G5 VDJ to G4A C region		JH	CH	Switch	shark	primer	Accession number
Clone	VH flank						
G5	TGTGCAACAGAT	ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	CAACACCGTCTTCT				
G5G4137	GGTGTGCAAC CCACGATCCCGTTTACTAGCCTATACTTGCCCGT	CTAGTTTACTGGGGAAAAGGAACCATGGTGACCGTGACCACAG	ins:spliced to	G4A C	GR	G5L2/G4 C	
G5G4-39	TGTGCAACAGA CCGGCGCAACCGCT	ACTACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G4A	GR	G5L2/G4 C	
G5G4-68	TGTGCAACA CTGGGAACGTGGA	ACTCCGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G4A	GR	G5L2/G4 C	
G5G4-81	TGTGCAAC TCGGATCAACGCAATTACTTTGGG	CGGTTACTGGGGACAAGGAACCATGGTGACCGTGACTGCAG	TGACACCGTCTTCC	G5-G4A	GR	G5L2/G4 C	
G4A	TGTGCAAGAGAC	ACTACTTTGTAACCTGGGGACAAGGAACCATGGTGACTGTGACTTTCAG	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 legend 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

	Leader	VH			
G5	ATGATGACGTCACAAATTTTCTCAGTTTGTGCTGGCTTTATTACCTTGTGTCCAGTCGGAGATTACTTTGATTCACCCAGAGGCGAGACCGGCCATCTCTGGAGGCTCCTGAGTC				
G5G4-142	-----TGTGCTGGCTTTATTACCTTGTETECAGTCGGAGATTACTTTGATTC-----GAGGCAGAGACCGGCCATCTCTGGAGGCTCCTGAGTC				
G5G4-38	-----TGTGCTGGCTTTATTACCTTGTETECAGTCGGAGATTACTTTGATTC-----GAGGCAGAGACCGGCCATCTCTGGAGGCTCCTGAGTC				
G4CG	ATGACTACGTCGACGATTTTCTCAGTTTGTGCTGACTTTCTTATCCCGTGTCCAGTCGGAGGTTACTTTGACTCAACCAGAGGCGAGAAACAGCCAACTGGAGGCTCGTGACAC				
G5	TGACCTGTA AAAACAGTGGGTTCAATCTTGGAGCTCTCCATGTAATTGGATCCGACAGGTTCCCGGACAGGGCTGGAGTGGATAGTTTACTACTATAGTTTCATCGAGCAATAACTA	CDR1	CDR2		
G5G4-142	TGACCTGTA AAAACAGTGGGTTCAATCTTGGAGCTCTCCATGTAATTGGATCCGACAGGTTCCCGGACAGGGCTGGAGTGGATAGTTTACTACTATAGTTTCATCGAGCAATAACTA				
G5G4-38	TGACCTGTA AAAACAGTGGGTTCAATCTTGGAGCTCTCCATGTAATTGGATCCGACAGGTTCCCGGACAGGGCTGGAGTGGATAGTTTACTACTATAGTTTCATCGAGCAATAACTA				
G4CG	TTACCTGTA AAAACAGCGGGTTCATCTTAGCAGCTACTATATGGTGGGTCGCCAGGTTCCCGGACAGGGCTGGAGTGGTGGTTAGCTACTATAGTTTCATCGAGCAATAACTA				
		W/R	S/M, S/Y N/R, Y/E Y/D		
G5	TGCGCCAGCGATTAAAGATCGATTTACTGCGTCAAAGACACTTCAAACAACATCTTCCGATTGGAAATGAAGGAGCGTGAAGATCGAGACACCGCCATCTATTACTGTGCAACAGAT				
G5G4-142	gCGGad-----TCGATTTACTGCGTCAAAGACACTTCAAACAACATCTTCCGATTGGAAATGAAGGAGCGTGAAGATCGAGACACCGCCATCTAGCGCGGTGAAAGAT				
G5G4-38	gCGGad-----TCGATTTACTGCGTCAAAGACACTTCAAACAACATCTTCCGATTGGAAATGAAGGAGCGTGAAGATCGAGACACCGCCATCTAGCGCGGTGAAAGAT				
G4CG	TGCGCCAGCGATTAAAGGTCGATTTACTGCGTCAAAGACACTTCAAACAACATCTTCCGATTGGAAATGAAGGAGCTGAAGATCGAAGACACCGCCATCTATTACTGTGCAAGAGAC				
	P/G	A/T M/R	Y/* Y/R C/G A/V T/K		
G5	ATACTACAGTGGGT ACATACTGGGGTGG ACTACTCCGGTACTGGGGACAAGGAACCATGGTGACCGTACTACAG CAACACCGTCTTCCGACACTTTA	D1	D2	JH	C region
G5G4-142	GTGGG TGGGGG TTTTCACAAAG -----ACTGGGACACCGAACCATGGTGACCGTACTACAG TGACACCGTCTTCCGACACTTTA				
G5G4-38	GTGGG TGGGGG TTTTCACAAAG -----ACTGGGACACCGAACCATGGTGACCGTACTACAG TGACACCGTCTTCCGACACTTTA				
G4CG	ATACTACAGTGGGT ACATACTGGGGTGG ACGAATTCGCTAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG TGACACCGTCTTCCGACACTTTA				

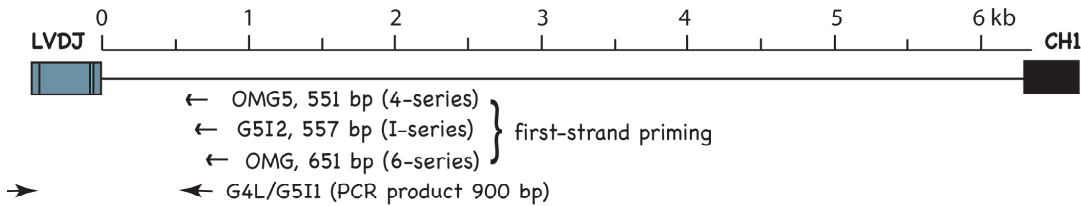
## B. Five related cDNAs from phage library, G2B VDJ with G2A C region

	Leader	VH	
G2B GL	AGAACCCTCTCCAGGATACCAGA ATGACTACGATGATCAATTTTCTCAGTTTGTACTGACTTTCTTATCTCTGTGAGTCTTTATCTTTAAGTTTCTATTTGTTATTTCCCTAGTGTTAG		
a11	AGAACCCTCTCTCCAGGATACCAGA ATGACTACGATGATCAATTTTCTCAGTTTGTACTGACTTTCTTATCTCT-----		
G2A GL	AGAACCCTCTCTCCAGGATACCAGA ATGACTACGATGATCAATTTTCTCAGTTTGTACTGAAATTTCTATCTCTGTGAGTCTTTATCTTTAAGTTTCTATTTGTTATTTCCCTAGTGTTAG		
G2B GL	TCTCCTCTGGAATGTA AAAATATTAGCAATCAGATAATCAGTAAAAATGTCATGTTTTGCTTTATTTCTGAAGGTGCCAGTGGAGGTTACTTTGATTC AACCCAGAGGCGAGAACCG		
158	-----GTGCCAGTCGGAGGAGTTACTTTGATTC AACCCAGAGGCGAGAACCG		
304	-----GTGCCAGTCGGAGGAGTTACTTTGATTC AACCCAGAGGCGAGAACCG		
164	-----GTGCCAGTCGGAGGAGTTACTTTGATTC AACCCAGAGGCGAGAACCG		
215	-----GTGCCAGTCGGAGGAGTTACTTTGATTC AACCCAGAGGCGAGAACCG		
218	-----GTGCCAGTCGGAGGAGTTACTTTGATTC AACCCAGAGGCGAGAACCG		
G2A GL	TCTCCTCTGGAATGTA AAAATATTAGCAATCAGATAATCAGTAAAAATGTCATGTTTTGCTTTATTTCTGAAGGTGCCAGTGGAGGTTACTTTGATTC AACCCAGAGGCGAGAACCG		
			E/D
G2B GL	CCATCCTGGAGGTTCTTGAGACTGACCTGTA AAAACACAGCGGGTTCGATCTTGACAGCTATACCATGAGTTGGTCCGACAGGTTCCCGGACAGGGCTGGAGTGGATAGTTTACTACTATGCTTT	CDR1	CDR2
158	CCATCCTGGAGGTTCTTGAGACTGACCTGTA AAAACACAGCGGGTTCGATCTTGACAGCTATACCATGAGTTGGTCCGACAGGTTCCCGGACAGGGCTGGAGTGGATAGTTTACTACTATGCTTT		
304	CCATCCTGGAGGTTCTTGAGACTGACCTGTA AAAACACAGCGGGTTCGATCTTGACAGCTATACCATGAGTTGGTCCGACAGGTTCCCGGACAGGGCTGGAGTGGATAGTTTACTACTATGCTTT		
164	CCATCCTGGAGGTTCTTGAGACTGACCTGTA AAAACACAGCGGGTTCGATCTTGACAGCTATACCATGAGTTGGTCCGACAGGTTCCCGGACAGGGCTGGAGTGGATAGTTTACTACTATGCTTT		
215	CCATCCTGGAGGTTCTTGAGACTGACCTGTA AAAACACAGCGGGTTCGATCTTGACAGCTATACCATGAGTTGGTCCGACAGGTTCCCGGACAGGGCTGGAGTGGATAGTTTACTACTATGCTTT		
218	CCATCCTGGAGGTTCTTGAGACTGACCTGTA AAAACACAGCGGGTTCGATCTTGACAGCTATACCATGAGTTGGTCCGACAGGTTCCCGGACAGGGCTGGAGTGGATAGTTTACTACTATGCTTT		
G2A GL	CCATCCTGGAGGTTCTCATGAGACTGACCTGTA AAAACACAGCGGGTTCGATCTTGACAGCTATGCCATGAGTTGGTCCGACAGGTTCCCGGACAGGGCTGGAGTGGATAGTTTACTACTATGCTTT		
	F/L D/G Y/W, T/H S/Y	I/L Y/N Y/L, A/S Y/M, A/D A/T	
G2B GL	CATATAGCAATGACTATGCGCCAGCGATTAAAGATCGATTTACTGCGTCAAAGACACTTCAAACAATATCTTCCGTTGAAATGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGT		
158	CATCGGTAGTTGCTATGCGCCAGCGATTAAAGATCGATTTACTGCGTCAAAGACACTTCAAACAATATCTTCCGTTGAAATGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGT		
304	CATCGGTAGTTGCTATGCGCCAGCGATTAAAGATCGATTTACTGCGTCAAAGACACTTCAAACAATATCTTCCGTTGAAATGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGT		
164	CATCGGTAGTTGCTATGCGCCAGCGATTAAAGATCGATTTACTGCGTCAAAGACACTTCAAACAATATCTTCCGTTGAAATGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGT		
215	CATCGGTAGTTGCTATGCGCCAGCGATTAAAGATCGATTTACTGCGTCAAAGACACTTCAAACAATATCTTCCGTTGAAATGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGT		
218	CATCGGTAGTTGCTATGCGCCAGCGATTAAAGATCGATTTACTGCGTCAAAGACACTTCAAACAATATCTTCCGTTGAAATGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGT		
G2A GL	CATATAGCAATGACTATGCGCCAGCGATTAAAGATCGATTTACTGCGTCAAAGACACTTCAAACAATATCTTCCGTTGAAATGAAGAGCCTGAAGATCGAAGACACCGCCATCTATTACTGT		
	Y/S, S/Y, N/V, D/A S/I N/S N/T L/F V/F H/L H/Q, L/V V/A		
G2B GL	GCAAGAGAC ACTATTTGTACTGGGGACAAGGACCATGGTGACAGTGACTTTAG TGACACCGTCTTCCCGACGCTTTATGGTCTAGTCTCCG	JH	C Region
158	GCA-----GCTGaacACCGGACGACTGGG -----TTACTGGGGACAAGGACCATGGTGACAGTGACTTTAG TGACACCGTCTTCCCGACGCTTTATGGTCTGTCTCT		
304	GCA-----GCTGaacACCGGACGACTGGG -----TTACTGGGGACAAGGACCATGGTGACAGTGACTTTAG TGACACCGTCTTCCCGACGCTTTATGGTCTGTCTCT		
164	GCA-----GCTGaacACCGGACGACTGGG -----TTACTGGGGACAAGGACCATGGTGACAGTGACTTTAG TGACACCGTCTTCCCGACGCTTTATGGTCTGTCTCT		
215	GCA-----GCTGAGGTCGGACGACTGGG -----TTACTGGGGACAAGGACCATGGTGACAGTGACTTTAG TGACACCGTCTTCCCGACGCTTTATGGTCTGTCTCT		
218	GCA-----GCTGAGGTCGGACGACTGGG -----TTACTGGGGACAAGGACCATGGTGACAGTGACTTTAG TGACACCGTCTTCCCGACGCTTTATGGTCTGTCTCT		
G2A GL	GCAAGAGAC ACTATTTGATTAAGGACAAGGACCATGGTGACAGTGACTTTAG TGACACCGTCTTCCCGACGCTTTATGGTCTGTCTCT		
	R/N, T/V/D Y/W Q/E L/F L/V		

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
G4CG      ACGAATTCGCTA ACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG GTAAGAACCTTGAATGTTTT-----CAGCAACCGTTATATTTGTTTAACTGCATATT 53
I58       CCCCCATACTGCGACT TTCGCTA ACTGGGGACA TGGAAACCATGGTGACTGTGACTTTAG GTAAGAACCTTGAATGTTTTGCGGATCTTAA CAACCGCGATCTGTATTTT CGTTATTTTCGT 60
I41       CCCCCATACTGCGAGG TTCGCTA ACTGGGGACA TGGAAACCATGGTGACTGTGACTTTAG GTAAGAACCTTGAATGTTTTGTGATCTTAA CAACCGCGATCTGTATTTT CGTTATTTTCGT 60
G5        ACTACTCCGGTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG GTAAGAAAATCAACAT-----TCTT AGCAACCGGTATCTTTATTTTAAATTTATTATT 53
  
```

## C. "Deletion"-insertion (I68, 6-42)

```

CDR3      JH
G4CG      ACGAATTCGCTA ACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG GTAAGAACCTTGAATGTTTT CAGCAACCGTTATATTTGTTTAACTGCATATTAATTTGAGTAATTCATGATTCAA 77
I68       GGGAAAGCCGGGTGAG TTCGCTA ACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG GTAAGAACCTTGAATGTTTTCACTAACCGTTATATTT CATTGGA-----AGGGGGTAATTCATGATTCAA 67
6-42     GGGAAAGCCGGGTGAG TTCGCTA ACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG GTAAGAACCTTGAATGTTTT CAGCAACCGTTATATTT CATTGGA-----AGGGGGTAACATGATTTC 67
G5        ACTACTCCGGTACTGGGGACAAGGAACCATGGTGACCGTGACTACAG GTAAGAAAATCAACATTTCTAGCAACCGGTATCTTTATTTTAAATTTATTGATGTGAGTAAATTCATGATTCAA 77
  
```

## D. "Deletion" (4-5, I3-8, I3-52, I3-96)

(I)

```

1
G4A      GTAAGAACCTTGAATGTTTTAGCAACCGTTATA-----TTTGTTTAACTGTATATTAATTTG // CCTTTTCTGCAAAGTAAATATAAAATCCATTTACGCCCTCAGCAAAATAAGTAAACTGCTGG 179
4-5     GTAAGAACCTTGAACGGTTTTAGCAA-CGAGATAAAGGTGGTATACTGATTCGTATAT----- CCTTCGTGACCGTCTCAGCAACTGAATGAGTGTCTG 94
G5      GTAAGAAAATCAACATCTTAGCAACCGGTATC-----TTTATTTAATTTATTATTGATGTG // ATTTTCTGCAAAGTATTAGAAACCTTCGTTAAAGTCTCAGCAAAATGAATGAAGTGTCTG 179
  
```

(II)

```

CDR3      JH
G4A      TGAAGAGCCTGAAGATCGAAGACACCCCATCTATTACTGTGCAAGAGAC ACTACTTTGATAACTGGGGACAAGGAACCATGGTGACTGTGACTTTAG
4-5     TGAAGAGCCTGAAGATCGAAGACACCCCATCTATTACTGTCAAAG---- TCACCGATACTACAGCAGACCGGGGACGCTCCGAGACACT -----TGGGGACAAGGAACCATGGTGACTGTGCTTTAG
I3-8    TGAAGAGCCTGAAGATCGAAGACACCCCATCTATTACTGTCAAAG---- TCACCGATACTACAGTAGACTGGGGACGCTCCGAGACACT -----TGGGGACAAGGAACCATGGTGACTGTGACTTTAG
I3-52   TGAAGAGCCTGAAGATCGAAGACACCCCATCTATTACTGTCAAAG---- TCACCGATACTACAGTAGGGCAGGGGACGCTCCGAGACACT -----TGGGGACAAGGAACCATGGTGACTGTGACTTTAG
I3-96   TGAAGAGCCTGAAGATCGAAGACACCCCATCTATTACTGTCAAAG---- TCACCGATACTACAGTAGGGCAGGGGACGCTCCGAGACACT -----TGGGGACAAGGAACCATGGTGACTGTGACTTTAG
  
```

(duplication)

```

1
G4A      GTAAGAACCTTGAATGTTTT-----AGCAACCGTTATA-----TTTGTTTAACTGTATATTAATTTGAG // AAAGTAATTATAAAATCCATTTACGCCCTCAGCAAAATAAGTAAACTGCTGG 179
4-5     GTAAGAACCTTGAACGGTTTT-----AGCA-ACGAGATA-AGTGGTATACTGATATCGTATAT----- -----CCCTCGTGACCGTCTCAGCAACTGAATGAGTGTCTG 94
I3-8    GTAAGAACCTTGAATGTTTTCACTTGAACCTTTTCAGCA-CCGTATA-GGTCTATAGTATTATCGTATAT----- -----CCCTCGT-----CCACACAGGACTATGCTG 99
I3-52   GTAAGAACCTTGAATGTTTT-----AGCA-GCGTTATACAGGTGGTATAGGGATATCGTATAT----- -----CCCTCGTTAAAGTCTCAGCAACTGCATGAAGTGTCTG 95
I3-96   GTAAGAACCTTGAATGTTAGC-----AACACCGTGGCAAACCGTGGTATAGGGATATCGTATAT----- -----CCCTCGTTAAATCTCAGCAACTGCAGTGTGCTG 96
G5      GTAAGAAAATCAACATCTT-----AGCAACCGGTATC-----TTTATTTAATTTATTATTGATGTGAG // AAAGTATTAGAACCTTCGTTAAAGTCTCAGCAAAATGAATGAAGTGTCTG 179
  
```

## E. "Deletion" (4-7)

```

1
G4A      GTAAGAACCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGTATATTAATTTGAGTA // GTGAGAGATTCACTATTTCGCCGTTTCTCTCATTTTATCAG-ATGGAAAGACAGTTTG 236
4-7     GTAAGAACTTGAACCTTTAGCAACCGTTACGGCGGTTTTAACTGTATATTAATTTGAGTA--- -----CTCGGTATTTCACTCTTTTTTCCCGCAGCAGGATACTGCGTG 103
G5      GTAAGAAAATCAACATCTTAGCAACCGGTATCTTTATTTAATTTATTATTGATGTGAGTA // CTGAGGATTACAGCTGTCGTTATTTCACTCTTTTTTCCCATGGGACTATGCGTG 237
  
```

## F. "Deletion"-insertion (4-70)

```

1
G4A      GTAAGAACCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGTATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATCTGCCGCTGTGTTAGTTGGTTCCAATTGC 120
4-70    GTAAGAACCTTGAATGTTTTAGCAAACCGTTAGGTTTCTTAACTGTATATTTGAGTAACTCTTAGGTTGGTGGTAGGGC-----TAGAATTCA 94
G5      GTAAGAAAATCAACATCTTAGCAACCGGTATCTTTATTTAATTTATTATTGATGTGAGTA // GTGAGTAATTCATGAGTTCAATGAAACTCAAATCTGTGCTGAGTTAGTTGGTTGAGTAATTGA 120
  
```

## G. "Deletion"-insertion (I7)

```

1
G4A      GTAATTCATGATTCAAATGAAACTCGAATTCCTGCTGTGTTAGTTGGTTCCAATTGCGTT // CAAACATTAAGAAATTTAAAAGTGTTTAAAA-----AAA-----AACAAAATATGCTAAAAAT 384
I7      GTCTCCATGATTCAAACGACTCCATCTGCTGCTGTTAGTTGGTTCCAATGCGC-----ACGTAACCTTTAAAAGATTTGAAATACTGCGCAAAATTTGACAAAATATGCTAAAAAT 179
G5      GTAATTCATGATTCAAATGAAACTCAAATCTGTGCTGAGTTAGTTGGTTAGAATTGATTT // GAAACGTTAAGAAATTTAAAAGATTTGAAATACTGCGCAAAATTTGACAAAATATGCTAAAAAT 399
  
```

## H. "Deletion"-insertion (I16)

```

1
G4A      GTAAGAACCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGTATATTAATTTGAGTA // CTTTCTGCAAAGTAAATATAAAATCCATTTACGCCCTCAGCAAAATAAGTAAACTGCTGGT 180
I16     GTAAGAACCG-----AAAGCTATTAGAGGCCCTGTTAAAGTCTCAGCAAAATGAATGAAGTGTCTG 61
G5      GTAAGAAAATCAACATCTTAGCAACCGGTATCTTTATTTAATTTATTATTGATGTGAGTA // TTTTCTGCAAAGTATTAGAACCTTCGTTAAAGTCTCAGCAAAATGAATGAAGTGTCTG 180
  
```

## I. "Deletion"-insertion (I29)

```

1
G4A      GTAAGAACCTTGAATGTTTTAGCAACCGTTATATTTGTTTAA // AGATGAAAGACAGTTTGGCTGGTTAATTTAAAG----AAAAATGTCTGAAACAGTATGACGTTTCAACAATGATTTAA 292
I29     GTAAGAACCTTGAATGTTTTAGCAACCGTTATATTT-----GACTGGGGAGTCTGGGTCATAGATACACCGAAAACCTCTTAGA-ATTATGATGAATAAACATGTTTTA 105
G5      GTAAGAAAATCAACATCTTAGCAACCGGTATCTTTATTTTAA // ACATGGGCATACTGCGTCTGTTTATTGAAACCCGAAAACCTCTTAGA-ATTATGATGAATAAACATGTTTTAA 296
  
```

### J. "Deletion", homology? (I36)

```
181
G4A GAGAGATTGATTATTCGGCGTTTCCTCATTTTTATC-AGATGGAAGACAGTTTGGCT // -----AAAAACAAAATATGCTAAAATATTATTGTCAATTTCTTCAAT 405
I36 GAGAGATTGATTATTCGGCGTTTCCTCAT-----AACTACAAGCTATGCTAATAATCATTACTAACTCCTTGGAT 253
G5 GAGGGATTGAGTCTCGGTATTTCACTCTTTTTTCCACATGGGCATACTCGCTGTCT // TACTGCCAAATATTGACAAAATATGCTAAAATATTACTTAATTCCTTGAAT 420
```

### K. "Deletion"-insertion (I69)

```
1
G4CG GTAAGAACCCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGCATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCGCGCTGTGTTAGTTGGTTCCAATTGC 120
I69 GTAAGAACCCTTGAACCTCTCAGCAATCTGTC-----TCTCTGCTGAGTATCTTTTGGTTAGAATTGA 63
G5 GTAAGAAAATCAAACATCTTAGCAACCGGTATCTTTATTTAATTTATTTATGATGTGAGTAATTCATGAGTTCAATGAAACTCAAATCTGCTGCTAGTTAGTTGGTTAGAATTGA 120
```

### L. "Deletion"-insertion (I117)

```
1
G4CG GTAAGAACCCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGCATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTC // TCATTTT-TTATCAGATGGAAGACAGTTTGGCT 239
I117 GTAAGAA--TTGAATGTTTTAGCAACCGTTATTTTGTAACTGCATATTAATTTGAGTAATTCATGATTCAGTGAACTCTCA----GCACCCCAAGATGGCATACTGCGTCT 113
G5 GTAAGAAAATCAAACATCTTAGCAACCGGTATCTTTATTTAATTTATTTATGATGTGAGTAATTCATGAGTTCAATGAAACTCAAATTC // TCTTTTTTCCCAATGGCATACTGCGTCT 240
```

### M. Insertion (I148)

```
1
G4A GTAAGAACCCTTGAATGTTTTAGCAACCGTTATATTTGT-TTTAACTGTATATTAATTTGAGTAATTCATGATTC-----AATGAAACTCGAATTCGCGCTGTGTTAGTTGG 110
I148 GTAAGAACCCTTGAATGTTAAGAGCAACCGTTATCTTTGCCTTTAACTATATATTAATTTGAGTAATTCATGATTCAGGGACCCCGAAAGGAGCAAGCTCTGACTGAGTTAGTTGG 120
G5 GTAAGAAAATCAAACATCTTAGCAACCGGTATCTTTAT-TTTAATTTATTTATGATGTGAGTAATTCATGATTC-----AATGAAACTCAAATTCGCTGCTAGTTAGTTGG 110
```

### N. "Duplication"-insertion (I53)

```
(I) 1
G4A GTAAGAACCCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGTATA-TTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCGCGCTGTGTTAGTTGGTTCCAATTG 119
I53 GTAAGAACCCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGACTAATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCGCGCTGTGTTAGTTGGTTCCAATTG 120
G5 GTAAGAAAATCAAACATCTTAGCAACCGGTATCTTTATTTAATTTATTTA-TTATGATGTGAGTAATTCATGAGTTCAATGAAACTCAAATTCGCTGCTGAGTTAGTTGGTTAGAATTG 119

G4A CCTTTTCTGCAAAGTAATTAATAATCCATTACGCTCAGCAAAATAGTAACTGCTGGTGAGAGATTCAGTTATTCGGCGTTCTCTCATTTTTATC-AGATGGAAGACAGTTTGGC 238
I53 CCTTTTCTGCAAAGTAATTAATAATCCATTACGCTCAGCAAAATAGTAACTGCTGGTGAGAGATTCAGTTATTCGGCGTTCTCTCATTTTTATC-AGATGGAAGAACAGTTTGGC 239
G5 ATTTTTCTGCAAAGTGATTAGAACCCCTGTTTAAAGTCTCAGCAAAATGAATGAAGTGTCTGAGGGATTGAGTTTCACTCTTTTTTCCACATGGCATACTGCGTGTCT 239
↑see G5 at 169 bp (below)

G4A TGGTTTAATTAAG----AAAAATGCTGAAACAGTATGAGTTTCAACAATGATTAATGCCAGTGACACCAATATCTCCGAGTAT 322 AAGTAACTGCTGGTGAGAGATTCAGTTATTCGGCG 201
I53 TGGTTTAATTAAG----AAAAATGCTGAAACAGTATGAGTTTCAACAATGATTAATGCCAGTGACACCAATAGGCACTGG---319 ---TGAAGTGTCTGAGGCTTTCAGCTGCTCGGTA 352
G5 TGGTTTCATTGAAACACCGAAACCTCTTAGA-ATTATGATGAATAAACATTGTTAATATCTGTGACACCCATTTCTGCGAGTTT 326 GAATGAATGCTCTGAGGGATTCAGCTGCTCGGTA 201
```

### (II) Switch junction:

```
↓
G4A ATGACGTTTCAACAATGATTAATGCCAGTGACACCAATATCTCCGAGTATTAGTATCGACAACAACATTAAGAATTTAA 351
I53 ATGACGTTTAAACAATGATTAATGCCAGTGACACCAATAGGCACTGCTGAAGTGCTACTGAGGCTTTCAGCTCTCGGTA 352
G5 TTTTCTGCAAAGTATTAGAACCCCTGTTTAAAGTCTCAGCAAAATGAATGAAGTGTCTGAGGGATTCAGCTGCTCGGTA 201
```

### O. "Duplication"-insertion (I22)

```
(I) 1
G4A GTA-AGAACCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTG-----TATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCGCGCTGTGTTAGTTGGTTCC 114
I22 GTAAGAACCTTGAATGTTTTAGCAACCTGTTATATTTGTTTAACTGCTCCCGCAAACTGATATTTATTTGAGTAATTCATAGCAATTAATGAAACTCGAATTCGCGCTGTGTTAGTTGGTTCC 128
G5 GTA-AGAAAATCAAACATCTTAGCAACCGGTATCTTTATTTAATTTA-----TTTATGATGTAGTAATTCATGAGTTCAATGAAACTCAAATTCGCTGCTGAGTTAGTTGGTTAG 114
↑see G5 at 28 bp (below)
```

```
G4A AATTGCTTTTCTGCAAAGTAATTAATAATCCATTACGCTCAGCAAAATAGTAACTGCTGGTGAGAGATTCAGTTATTCGGCG 201 --TATATTA-TTTGAGTAATTCATGATTCAAA 77
I22 AACTGCTTTTCTGCAAAGTAATTAATAATCCATTACGCTCCTCAAATAGACATACTGCTGGTTAGAGATTCAGTTATTCGG---211 ----TATTGAAAGTGAAGTAATTTGTTGAGTTTCA 240
G5 AATTGATTTTCTGCAAAGTATTAGAACCCCTGTTTAAAGTCTCAGCAAAATGAATGAAGTGTCTGAGGGATTCAGCTGCTCGGTA 201 --TTTATTA-TG-TGAGTAATTCATGATTCAAA 77
```

### (II) Switch junction:

```
↓
G4A AATTGCTTTTCTGCAAAGTAATTAATAATCCATTACGCTCAGCAAAATAGTAACTGCTGGTGAGAGATTCAGTTATTCGGCGGCTTTCTCTCATTTTTATCAGATGG 224
I22 AACTGCTTTTCTGCAAAGTAATTAATAATCCATTACGCTCCTCAAATAGACATACTGCTGGTTAGAGATTCAGTTATTCGGTATTGAAAGTGAAGTAATTTGTTGAGTTTCA 240
G5 GTAAGAAAATCAAACATCTTAGCAACCGGTATCTTTATTTAATTTATTTATTTGAG-TGAGTAATTCATGATTCAAA 77
```

### P. Homology (I167)

```
1
G4A GTAAGAACCCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGTATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCGCGCTGTGTTAGTTGGTTCCAATTG 119
I167 GTAAGAACCCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGTATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCGCGCTGTGTTAGTTGGTTCCAATTG 120
G5 GTAAGAAAATCAAACATCTTAGCAACCGGTATCTTTATTTAATTTATTTATGATGTGAGTAATTCATGAGTTCAATGAAACTCAAATTCGCTGCTGAGTTAGTTGGTTAGAATTGA 343
```

### Switch area shaded

```
G4A GTAAGAACCCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGTATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCGCGCTGTGTTAGTTGGTTCCAATTG 119
I167 GTAAGAACCCTTGAATGTTTTAGCAACCGTTATATTTGTTTAACTGTATATTAATTTGAGTAATTCATGATTCAAATGAAACTCGAATTCGCGCTGTGTTAGTTGGTTCCAATTG 120
G5 ATAACATTTGTTAATAGTTTTCATTGAAACACCGAAACCTCTTAGAATATGATGAATAAACATTGTTAATATCTGTGACACCCATTTCTGCG-AGTTTAGATAGACAAAGAAC 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, G512) and epigonal organ (OMG) RNA from shark-GR were used. PCR primer pair G4L/G511 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.

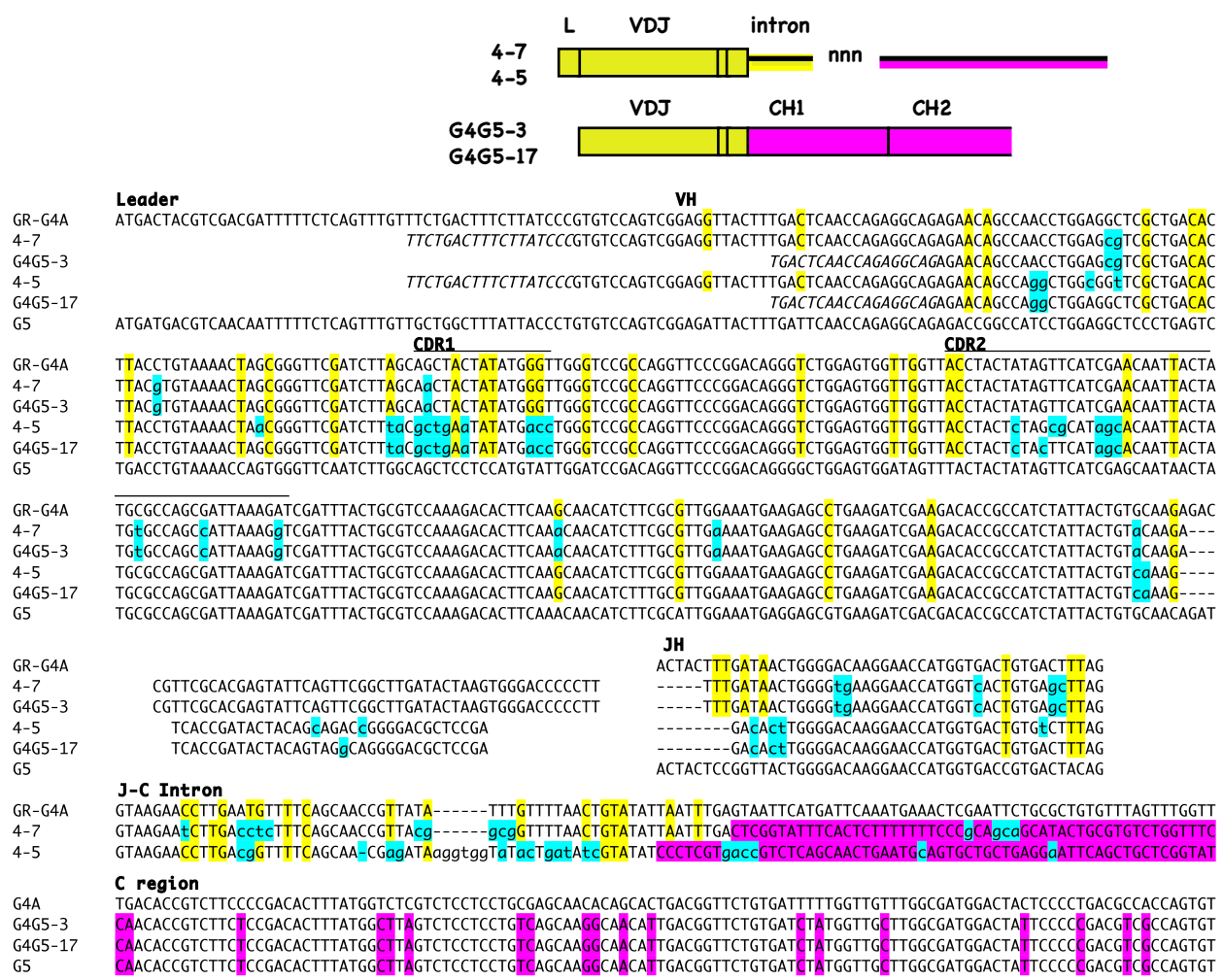


Fig. S5. Clonally related VDJ are flanked with switched intron or spliced to switched C region. Top, Diagram depicts the two kinds of sequences, VDJ flanked with intron (clones 4-7, 4-5) and VDJ spliced to C region (G4G5-3, -17). Yellow indicates G4 origin, pink G5. Bottom, Alignment of sequences with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



## A. IgW VDJ and IgM C region

**Leader** **VH**

105S ACTCTGCATCTGCTTCCACACGGCG **ATG**GGGATTGCTCTAATCTCTGTGTCTCTCTGGTCTGTCTGACAG---GTGCTGGTCTGAG---ATCACGCTGACCCAGCCGAGTCAGTTGTGAA  
 246W ACTCTGCATCTGCTTCCACACGGCG **ATG**GGGATTGCTCTAATCTCTGTGTCTCTCTGGTCTGTCTGACAG---GTGCTGGTCTGAG---ATCACGCTGACCCAGCCGAGTCAGTTGTGAA  
 6W ACTCTGCATCTGCTTCCACACGGCG **ATG**GGGATTGCTCTAATCTCTGTGTCTCTCTGGTCTGTCTGACAG---GTGCTGGTCTGAG---ATCACGCTGACCCAGCCGAGTCAGTTGTGAA  
 20W ACTCTGCATCTGCTTCCACACGGCG **ATG**GGGATTGCTCTAATCTCTGTGTCTCTCTGGTCTGTCTGACAG---GTGCTGGTCTGAG---ATCACGCTGACCCAGCCGAGTCAGTTGTGAA  
 40W ACTCTGCATCTGCTTCCACACGGCG **ATG**GGGATTGCTCTAATCTCTGTGTCTCTCTGGTCTGTCTGACAG---GTGCTGGTCTGAG---ATCACGCTGACCCAGCCGAGTCAGTTGTGAA  
 W-2 ACTCTGCATCTGCTTCCACACGGCG **ATG**GGGATTGCTCTAATCTCTGTGTCTCTCTGGTCTGTCTGACAG---GTGCTGGTCTGAG---ATCACGCTGACCCAGCCGAGTCAGTTGTGAA  
 G2A ATTCTCTCCAGGATACCAGA **ATG**ACGACGATGATAATTTTCTCAGTTTGTACTGAATTTCTTATCTGTGAGTTCAGTCCAGTCCGAGGAGGTTACTTTGATTCAACCAGAGGCAGAGAACGG

**CDR1** **CDR2**

105S AAAGCCAGGAGAGTCTCACAGACTGACCTGTACAGTGGTTCAGCCTCAGCAGCAACAGCATGcAcTGGGTGAGACAGGCCCCCGGAAAGGCTGGAATGGTTAGCAGCCATAGCTAGTT  
 246W AAAGCCAGGAGAGTCTCACAGACTGACCTGTACAGTGGTTCAGCCTCAGCAGCAACAGCATGcAcTGGGTGAGACAGGCCCCCGGAAAGGCTGGAATGGTTAGCAGCCATAGCTAGTT  
 6W AAAGCCAGGAGAGTCTCACAGACTGACCTGTACAGTGGTTCAGCCTCAGCAGCAACAGCATGcAcTGGGTGAGACAGGCCCCCGGAAAGGCTGGAATGGTTAGCAGCCATAGCTAGTT  
 20W AAAGCCAGGAGAGTCTCACAGACTGACCTGTACAGTGGTTCAGCCTCAGCAGCAACAGCATGcAcTGGGTGAGACAGGCCCCCGGAAAGGCTGGAATGGTTAGCAGCCATAGCTAGTT  
 40W AAAGCCAGGAGAGTCTCACAGACTGACCTGTACAGTGGTTCAGCCTCAGCAGCAACAGCATGcAcTGGGTGAGACAGGCCCCCGGAAAGGCTGGAATGGTTAGCAGCCATAGCTAGTT  
 W-2 AAAGCCAGGAGAGTCTCACAGACTGACCTGTACAGTGGTTCAGCCTCAGCAGCAACAGCATGcAcTGGGTGAGACAGGCCCCCGGAAAGGCTGGAATGGTTAGCAGCCATAGCTAGTT  
 G2A CCATCTGAGAGTCCATGAGACTGACCTGTAAAACAGCGGGTTCGATCTTGACAGCTATGCGCATGAGTTGGTCCGACAGTTCGCGGACAGGGCTGGAGTGGATAGTTTACTACTATGTT

105S CAGGTAGCAAGTACTACGCTCCAGAGTTGAGAGCGTTTCGAAATTTCCAAGGACAGCGAC-ACAATATAT----CTGCAGGTGACGAGTCTGAGAGTGGACGACACCCGCATCTATTACTGT  
 246W CAGGaaTCAAGTACcAcTCTCCaAcTTCGAGAGCGTTTCGAAATTTCCAAGGACAGCGAC-ACAATATAT----CTGCAGGTGACAGTCTGAGAGTGGACGACACCCGCATCTATTATGT  
 6W aAGGccGCAAGTACTACGCTCCaAcAGTTTcaGcGcGgcTCGAAATTTCCAAGGACAGCGc-ACAATATAT----CTGCAGGTGACGAGTCTGAGAGTGGACGACACCCGCATCTATTATGT  
 20W aAGGccGCAAGTACTACGCTCCaAcAGTaaTaaGcCGCTTCGAAATTTCCAAGGACAGCGc-ACAATATAT----CTGCAGGTGACGAGTCTGAGAGTGGACGACACCCGCATCTATTATGT  
 40W CAGGTaaTAAAGTACTAcGacCCAGcAGTTTCGAGAGCGTTTCGAAATTTCCAAGGACAGcGAc-ACAATATAT----CTGCAGGTGACGaaCTGAGAGTGGACGACACCCGCATCTATTACTGT  
 W-2 CAGGTaaTAAAGTACTAcGacCCAGcAGTTTCGAGAGCGTTTCGAAATTTCCAAGGACAGcGAc-ACAATATAT----CTGCAGGTGcGaaCTGAGAGTGGACGACACCCGCATCTATTACTGT  
 G2A CATATAGCAATGACTATGCGCCAGCGATTAAAGATCGATTTACTGCGTCCATAGACACTTCAACAATATCTTCGCGTGGAAATGAAGAGCCTGAAGATCGAAGACACCCGCATCTATTACTGT

**JH** **C region**

105S GCGAGT CCGTGGGCGT ACTACTGGGGAGTGAACCTTTCTGGAAAGTCACTCAG TTGCCAGTCTGCACCATCAGTCTACATCTAATCCTTCGTGTGACATGAAC  
 246W GCGGgT AATGTACTCTTGGGCGT CTGGGGAGTGAACCTTTCTGGAGTTCCTCAG TGACACCGTCTTCCCGACGCTTTATGGTCTCGTCTCTCTGTGACGAAACAG  
 6W GTGAGT CATGGGTGGGGGGCG aACTGGGGAGTGAACCTTTCTGGAGTTCCTCAG TGACACCGTCTTCCCGACGCTTTATGGTCTCGTCTCTCTGTGACGAAACAG  
 20W GTGAGT CATGGATGGGGGGCG TACTGGGGAGTGAACCTTTCTGGAAAGTCACTCAG TGACACCGTCTTCCCGACGCTTTATGGTCTCGTCTCTCTGTGACGAAACAG  
 40W aCGAGT TTACAA TACTGGGGAGTGAACCTTTCTGGAAAGTCACTCAG TGACACCGTCTTCCCGACGCTTTATGGTCTCGTCTCTCTGTGACGAAACAG  
 W-2 aCGAGT TTGCGA TACTGGGGGaaTGAACCTccCTGGAAAGTCACTCAG TGACACCGTCTTCCCGACGCTTTATGGTCTCGTCTCTCTGTGACGAAACAG  
 G2A GCAAGAGAC ACTATTTTACTCTGGGCAAGGGACATGGTGAACCTTACTGACTTTAG TGACACCGTCTTCCCGACGCTTTATGGTCTCGTCTCTCTGTGACGAAACAG

## B. IgM VDJ and IgW C region

**Leader** **VH**

G4CG ATGACTACGTGCAGGATTTTTCTCAGTTTGTCTGACTTTCTTATCCCGTGTCCAGTCCGAGGTTACTTTGACTCAACCAGAGGCAGAGAACAGCCAACTGGAGGCTCGCTGACAC  
 c57 ATGACTACGTGCAGGATTTTTCTCAGTTTGTCTGACTTTCTTATCCCGTGTCCAGTCCGAGGTTACTTTGACTCAAGTCCAGAGGCAGAGAACAGCCAAAGTGGAGGCTCGCTGACAC

**CDR1** **CDR2**

G4CG TTACCTGTAATAACTAGCGGGTTCGATCTTAGCAGCTACTATATGGTGGTGGTCCCGCAGGTTCCCGGACAGGGTCTGGAGTGGTGGTTAGCTACTATAGTTTATCAGGCAATTA  
 c57 TTACCTGTAATAACTAGCGGGTTCGATTTTAGCAGTcAcTggaTGGTGGTGGTCCCGCAGGTTCCCGGACAGGGTCTGGAcTGGTTGacTAGTACTATAGTTTcAGgTcTAAgTACTA

G4CG TCGCCAGCGATTAAAGGTCGATTTACTGCGTCCAAAGACACTTCAACAACATCTTCGCGTGGAAATGAAGAGACTGAAGATCGAAGACCCGCATCTATTACTGTGCAAGAGAC  
 c57 TCGCCAGCGATTAAAGGTCGATTTAGGCGTCCAAAGACACTTCAACAATATCTTCGCGTGGAAATGAAGAGACTGAAGTTCGAAGACCCGCATCTATTACTGTGcTAGAGAC

**D1** **D2** **JH** **CH1**

G4CG ATACTACAGTGGGT ACATACTGGGGTAG ACGAATTCGCTAATGGGGCAAGGAACATGGTACTGTGACTTTAG TGACACCGTCTTCCCGACACTTTATGGTCTC  
 c57 TCGCCGGGT GGGTGGGCCC ---AATTcGcgaACTGGGGACAGGAACATGGTACTGTGACTTTAG ATGTCAGATCAAACTTCAGTTTATCTTTCC  
 3 1-7 AAGGCAACCTTTCTGAAAGTGACTTCAAG ATGTCAGATCAAACTTCAGTTTATCTTTCC

G4CG GTCTCTCTCGTGCACACAGCACTGACGGTCTGTGATTTTGGTGTGGTGGGACTACTCCCGACGCCACAGTGTGACCTGGAAGAAAGTGGGAGCCCTTACAG  
 c57 AGTACTTCTGTGACACAAAGCTCAATCAAGATAAGTACGCTCTCTGTCTGGTCAAGGATTTCCAGCCAGGAACATCAACAGACATGGTCCAGCAACAATAAGAGATCAACA  
 3 1-7 AGTACTTCTGTGACACAAAGCTCAATCAAGATAAGTACGCTCTCTGTCTGGTCAAGGATTTCCAGCCAGGAACATCAACAGACATGGTCCAGCAACAATAAGAGATCAACA

G4CG CTGGATTTATGACATACCCATCGGTGAGAAGCAAGAAAGGAACTACACCCTGAGCAGTCACTAGCGGTCATCGAGTCCAGAGGCAGAAATGCCAGAAATACCTGTGAGGTTCCAGAC  
 c57 CTGGATTTCAATAAGATTCAGCAACTTTGGGGCAAAAATATGAAGTACACAAATGAGCAGTGGTGTCTGCTGCTGCTGAGGATTTCCAGCCAGGAACATCAACAGACATGGTCCAGCAACAATAAGAGATCAACA  
 3 1-7 CTGGATTTCAATAAGATTCAGCAACTTTGGGGCAAAAATATGAAGTACACAAATGAGCAGTGTCTGCTGCTGCTGCTGAGGATTTCCAGCCAGGAACATCAACAGACATGGTCCAGCAACAATAAGAGATCAACA

G4CG AGCGGTCCGACAAGAGCACTGGAATGCCAT-----GTTCCACAGATAAAGTCACTCCAATCTTCTCTAACTGTGAGTTCAGTGAAGA  
 c57 TACAAGCAAAATGAAGTGGTGAAGCTGTGATCACAAGCCTTCGCTCCAGCTCAGCCTCAATCTCCAGGTTCTTCTGTTGTTCCATCTGGGAGATGGTCTCAATCAAATAC  
 3 1-7 TACAAGCAAAATGAAGTGGTGAAGCTGTGATCACAAGCCTTCGCTCCAGCTCAGCCTCAATCTCCAGGTTCTTCTGTTGTTCCATCTGGGAGATGGTCTCAATCAAATAC

G4CG AATCGAGAGCAAAAAGTTGGCAGGATCTGTCTTCAATCTTGGACTTCAATCAAAGAAATCTCCGTGATTTGGTGAAGATGGAAGAAAGTGAATTCGTTACTTCACTTCT  
 c57 TGCTGCCCTGGGGTGCATACTATCTGATTCTATCTGACAGTGTACAGGTTCTCTGGTTTAAAGCCGAGTCCCAATCTCCCAATCGGGTCCAACTCCCTCCACCAAGGAAAAAG  
 3 1-7 TGCTGCCCTGGGGTGCATACTATCTGATTCTACTGACTGTACAGGTTCTCTGGTTTAAAGCCGAGTCCCAATCTCCCAATCGGGTCCAACTCCCTCCACCAAGGAAAAAG

G4CG CCGTGTGTGAA--GCGAATGGAACCTTCAACGACCACTGACAGTCCCTTCTGTAATGGTTCGACAGAGCACTTACATACATGCCAGTCTGATGAGGGGTCAATCAAG  
 c57 GTGATACATTT--GAAACAGTCTTCTTACTGACTGTACAGGTGGCGGATTTGAAAAAAGGGGATGAATCACTTCCGAGTGTCCCATGAGCCAGCAAGCTTCAAGGACAGGATCAACAT  
 3 1-7 GTGATACATTTTGAACAGTCTTCTTACTGACTGTACAGGTGGCGGATTTGAAAAAAGGGGATGAATCACTTCCGAGTGTCCCATGAGCCAGCAAGCTTCAAGGACAGGATCAACAT

G4CG TCAGAACATCACCAGCCTCAG-----GTTCCACAGATAAAGTCACTCCAATCTTCTCTAACTGTGAGTTCAGTGAAGA  
 c57 GAAGTATCGAGAGGAAATGACTGTCTTCTCCAAAATCTTCAATCAAAGAGCTTTGGATCAACAAGACCCGCAACCTGGTGTGTACAGCAGTGTGTTCTGATTCCAGCCGGTCCGGG  
 3 1-7 GAAGTATCGAGAGGAAATGACTGTCTTCTCCAAAATCTTCAATCAAAGAGCTTTGGATCAACAAGACCCGCAACCTGGTGTGTACAGCAGTGTGTTCTGATTCCAGCCGGTCCGGG

G4CG -----  
 c57 TCACCTGGAAGTAAATGGG--AGACAGAGAAGTAAAGGGTTACAGCTCAGCCACAGAGAGGAAGGACCCAAATACATCTGCTGACGCAACTCCGAACACAGCAGCGAGTGGGA  
 3 1-7 TCACCTGGAAGTAAATGGGAGACAGAGAAGTAAAGGGTTACAGCTCAGCCACAGAGAGGAAGGACCCAAATACATCTGCTGACGCAACTCCGAACACAGCAGCGAGTGGGA

G4CG -----  
 c57 GAGTGGGGTAGAGTTTGTGTTCTGCTCAAAGTGGTCCCTCATCTCACAGTGTCAAAGCGTCAAGGAGTGTAAAAGTTCTGCAAAAAAGTCCGAGGTGAGACTGCTGCCTCCAC  
 3 1-7 GAGTGGGGTAGAGTTTGTGTTCTGCTCAAAGTGGTCCCTCATCTCACAGTGTCAAAGCGTCAAGGAGTGTAAAAGTTCTGCAAAAAAGTCCGAGGTGAGACTGCTGCCTCCAC

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G4CG -----
c57 CGGCCAAGAGACCAAGAATCAGAGCAGAGTTACACTCGAGTGTGTAATCACTGGATTCTACCCAGACCTCATAcAGGTCTCCTGGGAGAAGGATAGCAGCCTGATCTCTCCAATACC
3 1-7 CGGCCAAGAGACCAAGAATCAGAGCAGAGTTACACTCGAGTGTGTAATCACTGGATTCTACCCAGACCTCATAcAGGTCTCCTGGGAGAAGGATAGCAGCCTGATCTCTCCAATACC

G4CG -----
c57 CGTGCTGGCCTCACTGCTCTGGAGCAGACGGGACATTCAAGTGTcaGGCACTACCTGACTGTGAGCACAGAGGAGTGGAGGAAAGGTTCAAGTCTTCACTGTGTAGTGTCTATCCACC
3 1-7 CGTGCTGGCCTCACTGCTCTGGAGCAGACGGGACATTCAAGTGTcaGGCACTACCTGACTGTGAGCACAGAGGAGTGGAGGAAAGGTTCAAGTCTTCACTGTGTAGTGTCTATCCACC
                                     CH3
G4CG -----GTTTCGGAGTGCCGTGATTACACAGCTAAAATACTACCGCCACCAAGTGAACAGGTCTTACTGG
c57 ATCCAACCTTACAGCAGTAAGGAGGTGAAGAATGTTCAAGATGAATGTTTGGACACTGGAATCTCAGTCACCTTAAGCAAGCCTCCATTTGCAGAGATCTGGAGGAACAGAACAGCAA
3 1-7 ATCCAACCTTACAGCAGTAAGGAGGTGAAGAATGTTCAAGATGAATGTTTGGACACTGGAATCTCAGTCACCTTAAGCAAGCCTCCATTTGCAGAGATCTGGAGGAACAGAACAGCAA

G4CG AGGGCAGCGTGACGTTAACTCGCTTGTGTAACAATCTTCAATTTGAAGCCAACCTCACCTGGATACACGACAAGAAGACTCTGAAATCAGAGATTGTTGACCAGTCCGGAGAGCATTCC
c57 CCATTCTGTGTGAGGTACTTTACCGTGATTTAGAGGGAGTCAAGGTAACCTGAAAGTGGATGGAATCATGAGACAAGATGGAGTGAAGACCCAGGGTCCCAAGAAAAGTGGACATAAA
3 1-7 CCATTCTGTGTGAGGTACTTTACCGTGATTTAGAGGGAGTCAAGGTAACCTGAAAGTGGATGGAATCATGAGACAAGATGGAGTGAAGACCCAGGGTCCCAAGAAAAGTGGACATAAA

G4CG GAAGGTGTGATCAGCAAGTTAGATATTTCTACTGAAGCCTGGCTGAGTGGGGATAAATTTGAATGCGTGGTGAACAATCAGTATTTACCGACTCCACTGAGAACTCCATCCACAAGA
c57 GAGATCATCTTCAGTAGACTGACAGTCCCTGCTGCAGAGTGGGAGAGTGGGGTGCAGTATATGTGTTTGGTGGAGGACAAAAATTTGCCAACCCAGAGAGAGGTTTCATCAGGAAATC
3 1-7 GAGATCATCTTCAGTAGACTGACAGTCCCTGCTGCAGAGTGGGAGAGTGGGGTGCAGTATATGTGTTTGGTGGAGGACAAAAATTTGCCAACCCAGAGAGAGGTTTCATCAGGAAATC
                                     CH4
G4CG GAAATTTGTAATCCGCTGGAACCATCGGTGTCGCTCCTCTGCCAGCGACAGAAGAAGTCTCGCACAGAGTGTGTTTCGCTCACCTGTTAGTGAAGGTTTCAGGCCCCGAGAGA
c57 CCAAGATGATATAAAGACTCACCTCAAGTCTACCTCTGCCCTTCCACCGGATGAGATGGAGACTGCTCATACTGCGACCTGGTTTGCCTGGTCACTGGATTCTCTCCCGTAGATA
3 1-7 CCAAGATGATATAAAGACTCACCTCAAGTCTACCTCTGCCCTTCCACCGGATGAGATGGAGACTGCTCATACTGCGACCTGGTTTGCCTGGTCACTGGATTCTCTCCCGTAGATA

G4CG TCTTTGTCAAGTGGACAACCAACGACAAGCCGGTTAATCCCACTAATAAAGAACCAGGTTGACGGCAGAGAGCGATAAACACCTCCTTCTTGTACAGCCTATTGTCCATTGCT
c57 TCGATCTGGCTTGGATGGCCAATGATACCTTCTGAAGACAGGGTTCATTCA---TCAGCCAGTGACTGAGGATGGCAGAGGAGGCTGGAACCTCAGG---CAGTCGCCTGACGGTCTCT
3 1-7 TCGATCTGGCTTGGATGGCCAATGATACCTTCTGAAGACAGGGTTCATTCA---TCAGCCAGTGACTGAGGATGGCAGAGGAGGCTGGAACCTCAGG---CAGTCGCCTGACGGTCTCT
                                     Tailpiece
G4CG GCGGAGGAGTGGCCAGCGGTGCTTCTACTCCTGTGTGGTGGGACATGAAGCGATTCTTTGAAGATCATCAACAGAACCCTGGATAAATCCAGCGGTAACCCGAGTTTTGTGAATAT
c57 ACAGAGGAGTGGGACAGCGCACACATACTCCTGTGTGGTGGGACATGAGTCAGTCACAACAAATGTATTGAGAAAGCATCAATAAATATCACAGCAAAACCCAACTGGTTAATGTCTC
3 1-7 ACAGAGGAGTGGGACAGCGCACACATACTCCTGTGTGGTGGGACATGAGTCAGTCACAACAAATGTATTGAGAAAGCATCAATAAATATCACAGCAAAACCCAACTGGTTAATGTCTC

G4CG TTCACTTGCACTGATGGACACCATTAATTATGCCAA
c57 ACTTGTGTTGACTGAAAGTTTTAACTCCTGTTCA---
3 1-7 ACTTGTGTTGACTGAAAGTTTTAACTCCTGTTCA---

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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 Ig $\omega$  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.