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Supporting Information

for

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**Four primordial immunoglobulin light chain isotypes, including λ and κ ,
identified in the most primitive living jawed vertebrates**

Michael F. Criscitiello and Martin F. Flajnik

CGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCCGGGCTGCAGGAATTC
>LDR
 GGCACGAGTACACTCGCTGAAGGCAGGCAGTTCTCTGACAGAAATGATGCG
 A R V H S L K A G S S L T E M M R

 GATCTTGTGCTTTTCTCTGTTCTCTGGCACTGTGGCTGGGCTGTGGACAGTG
 I L C F L C S L A L W L G C G Q C
>FR1
 TCAGACTCTGACTCAGCCTCCAGACCTGACTGTGATGCCGGGAAACGCCGC
 Q T L T Q P P D L T V M P G N A A
>CDR1 >FR2
 CAACTCAGCTGTAACATTGGAAGTGTGATCGCTATTACGTTGCCTGGTA
 T L S C N I G T V D R Y Y V A W Y
>CDR2
 CAAGCAGACCCAGGATCAGTTCCTCAGTGGATCCTGTACTACTATCACTC
 K Q T P G S V P Q W I L Y Y Y H S
>FR3
 TCTCAGTGCCCCGACCTATGGGTCTGGGTTTAGCAGCGACCGTTTTACATC
 L S A P T Y G S G F S S D R F T S

 GACCGTCAACAGCGGCCACAACATTTACAACCTTAATCATCAAAAACGTGAA
 T V N S G H N I Y N L I I K N V K
>CDR3
 GGTCGATGATGCCGCCGCTCTACTGTGGAAAGTGGGTTTCTCAGCTAG
 V D D A A V Y Y C G K W V S S A S
>FR4 >CONSTANT
 TACTAGGGTGTGGGCAAGGGACCAAGCTCTTTGTTGCAGCTCAGCAACT
 T R V F G Q G T K L F V A A Q Q L

 CCCTGACCCTTCAGTAAACCTGCTTGGGCCTTCAGCTGAGGAGATGTCCAG
 P D P S V N L L G P S A E E M S S

 TAAAGGAGCTGGTACATTAGTTTGTCTGGTCAGCAAAGTGTCCATGGGCTT
 K G A G T L V C L V S K L S M G F

 TGCTGCAGTCAGCTGGACAGTGGATGGGAGTCTGACGAGCAGTGAGGTCCA
 A A V S W T V D G S L T S S E V Q

 AACCAGCTTGGTGTCCCAGCGACCCGACAACAGCTTCAGTCTCAGCAGCTA
 T S L V S R D P D N S F S L S S Y

 CCTGACTGTCCCTGGCAGGGATTGGAGCAGTGGGAAGGTGTACTCCTGCAC
 L T V P G R D W S S G K V Y S C T

 AGTGCAACAAGGGACAGCTTCAAAAACAACCGCAACGATCTCACAGTCAG
 V Q Q G T A S K T T A T I S Q S R

 CTGTTAAATAAGTTTTCATTTGTGTCTGCTGTCTAACCTTAGCCAGAAAATG
 C *

 TCACTTTTCTTCCACCCCTTACCATTACCCACCCTCTTCTCTGGAATCC

 GCTGCACTTTGG

Figure S1. Clone 61.8. Leader, frameworks, CDRs and constant regions are marked.

nurse shark sigma AQQLPDPVSNLLGPSAEEMSSKAGTLVCLVSKLSMGFAAVSWTV--DGLTSSSE-VQTSLVSRDPDNFSLSVSLTVPGRDWSSGKVYSCVTVQQ-GTASKIT-ATISQSRG----

horn shark sigma AQQLPDPVSKLLGPSAEVSTKAGTLVCLVNLKLSMGFAAVSWAV--DGSPTSSE-VQTSAVSRNTDNTFSLSSSLTVPGTDWSSGKVYSCVTVQQGTASKIT-ATVTSQSVG----

dogfish shark sigma AKQLPEPVSVKLLGPSAEEMSSKAGTLVCLVSKLSMGFAAVSWTV--DGSPTSSE-VQTSVTSVQNTDNTFSLSSSLTVPG-----

carp L2 DAAAAAPBLNLLRPSA-REBELSSKLLTLVCLINHMSGAFADVRWLIV--NGNSVTEG-VFTGSAEEQPKKFKMSSSLTIESESEWDKDAQLTCEATAASKTNRKS---IKKSECSG----

fugu T2 --SSLPPPVLTVPPPS-RAELQSNKATLVCLV-RLSAPFAEIVSWLLI--GDTSVSSG-ISTSTPVQQAQDTFQISSLSILTSWDQAQVYTKVYVGSQTAEKS---IKKSECEE----

zebrafish T2 DAAAAAPPVLTLLRPSREBELSSKVTLLCLINHMSVASADVRWLIV--NGNSVTEG-VFTGSAEQQDPHKFKSSSLTIQRESEWDKRELTCEATVASKTSRAS---IRKSECSG----

X laevis sigma --DKFPEPALLVFPYTTDDNESKDSSTLTCHEISKLEVLVNVNWLII--DGTTVQDG-VITSNPVRESDNTEFSSMSSVLTLSKDKINKDRLYSCMIKQEGSSAFIS-KGVKLSQC----

nurse shark NS5 --DDRKPSVLLLPSSPEQIDT-GSATLCLVSRKPKGLVQVLSVSV--DKETDSG-VITRAVSPDSQVRLSSSLTVPATDWWKGRSRYSCSVKHSGLTSPLL-NTISSSDCOL----

horn shark I SSEDKRPVLLLPSSSEEDS-GMATLCLVSRKPKGFVRLVRLV--DDKETDSG-VITGTVSTDSQSYSLSSSLRVPATAWNKGSYSYTCVVDHESLSSPLL-KTISSTACSG----

skate I SSESRKPSLLLPSSPEETGT-GSATLCLVSRKPKGLVALRWAV--DGVETESG-VITGAVSPDAEQYRLSSSLRVPAAAWGKGTYSYCSVAHSSLGSPLR-HTVSSSSCAN----

nurse shark NS4 --DRSQPKLTLLPSPDQVTKGATLVCLANBFYDDELQVQWKK--DCAVISDG-VQTSNYLRASDSTYSVSSSLTLSGSDWESNARFSCALTHVTLSLSSPLS-KSISRSECA----

horn shark III --REKSQPLTLLPSSPEEYKAKGATLVCLADHFYDDEVGVWKK--DCAAISAG-VQTSNYLRASDSTYSVSSSLTLSGSDWESNARFSCALTHVTLSLSSPLS-KSISRSECV----

sturgeon GSPTAPSSVLLLPSSKLELDKSGATLVCLVNFYDVPDIKWLIV--DGVAQSSG-VLTSMTKQK-DGKYSASSSLTLKAVWNSKTYTCTVVKHETLSSPLS-ESIKRSECTLLDA

fugu T1 --SGVVRPRLTVLPSSPEELQ-QGSATLVCLASGGSPSQWKLWV--CGSSTIT-PTSLSLEVLGSDGRFSWSSLTNLPADQWKKVDSVTCEASLSGQSAVTQ--TLDPHSCSV----

trout T1 --SNSAPRLTVLPSSSEELSSITTTATLCLANKGFPDWTIRWV--DGPSSQ--KQGTSSRVLEKDGLYSWSSSLTLTLEWTKAGEVTCBAQN-SQTSVTK--TLRRADG----

carp L1a --TATRPALTVLPPSRDELQ-QKATVLCVASKGFPDVKLWV--DGSRRS-GVNLSPSQLOKDGLYSWSSSLTLESEWSRATTVSCDATHPSHNAVTN--SLNTKQCNDQ----

carp L1b --TATRPALTVLPPSRDELQ-QKATVLCVASKGFPDVKLWV--DGSRRS-GVNLSPSQLOKDGLYSWSSSLTLESEWSRATTVSCDATHPSHNAVTK--TLNTQCNDQ----

carp L3 TGPVAVKPSVSLLPSSLOISG-DSALLCLLSSYSPQCAQVSWRI--DGSSEVTEG--VQTSAESERDGRYSRSSLVSLSKARWEAGERFVCRVTHDGAQETS-FLK-SSEC----

zebrafish T1 -----PQVSVLPSSAEELSSKRLATLCLVANKGFPDWRVWVWVLPDGSSSSGQESSAGLLEKDGLYSWSSSLTLEQOWMESASVSCBATRSQGPALTAGDTVRRQOCSE----

zebrafish T3 TGSVPKPSVSLGSSSLOISG-DSALLCLLSSYSPQKAVSWRI--DGSSELEEG--FTLSAESQDGRYSCTSVLKLKREWEKREPYACRVTHAGGDEIP----FPK-----

catfish G --RLTQPSVTVLPSSSEELQ-QEAVTLVCLVAYKGFPSDWRVLSWV--DGSSEWSS-GESHSTAVLQADGLYSWSSSLTLEHPQW-RNKVVTCBAASKDNQPPVVS--TVNTECC----

catfish F TGPTVKPSVSLLPSSLOISE-GSALLCLLSSYSPQCALVSWTV--DGSSEVKG--VLTSAEERKDEYTHSSSLTLSKALWEKGEFVCKVSHDNVDHPVT-FRK--SCEV----

salmon T1 --SNSAPRLTVLPSSSEELSSITTTATLCLANKGFPDWTMSWV--DGTSSK--KQEAEPGVLEKDGLYSWSSSLTLEQWTKAGEVTCBAQQISQTPVTK--TLRRADCGS----

salmon T3 --SDVRPRLTVLPSSVELQ-QKATLCLANKGFPDVKLWV--DGSSSSWEVTEGSPFLEKDGHEYSWSSLTLPVDQWKKVGSVVCBATQGSQSPLE--TLRRDQCSG----

X laevis rho --RNDKAPAVFIKRPDQVKE-GNETAVCLINNFPRDLTVTKV--DQDVSDDQKSTDFMKSDDSTYSQSSMLTLTKDK-DKADKFKLEKVKHK--TAQIT-QSFSKSGS----

human kappa --RTVAAPVSVFIPPSDEQLKS-GNASVCLLNFFPREAKVQWV--DNALQSNQKQSVTEQDSKDDSTYSLSLTLTKADYEKHKVYACVETHQGLSSSPVT-KSFNRGEC----

mouse kappa --RADAAPVSVIFIPPSSEQLTS-GGASVCFLLNFFPKDINVKWKI--DGSERQNGVLNWTDDQSKDDSTYSMSSSLTLTKDEYERHNSYTCBATHTKSTSTPIV-KSFNRNEC----

ratfish II --NPMAP-SVTVLPSSPDQITAKSKATLVCLVDFHPGAVFVWV--DGSARSQ-VETSAIKQVEDNTEFSSVSLTLPASEWESHALYSCLVTHETRADPLK-TTIAIRSTV----

nurse shark NS3 --NPRAP-SLSLLPPSPVQITEKSKATLVCLVSGNPGAAEIQWIV--DGNVRNG-VETSRIQEQDNTFSSMSSVLTLSASEWNSHELYSQVVKHETLNPLO-KSITKSSCV----

horn shark II SSEDKRPVLLLPSSSEEDS-GMATLCLVSRKPKGFVRLVRLV--DDKETDSG-VITGTVSTDSQSYSLSSSLRVPATAWNKGSYSYTCVVDHESLSSPLL-KTISSTACSG----

sandbar shark II --SPRSP-TVSVLPSSDQITAKNMTLVCLVSGFVPGAAEIEWIV--DGSVRNG-VETSRIQEQADNTFSSVSLTLSASDWNSELHYSQVVKHETQANPLQ-TSISRSSCM----

skate II --SPRSP-TVSVLPSSMGEVTAESTATLVCLVSGNPGVVDIKWIV--DGSARSQ-AATSRVQEKDNEFSASSSLTLPAAWNSHDLYSQVVKHETQATPIK-ANLARSSCL----

X laevis III --GDVKAPSVSIFIPPSVEELATK-KATVCLSLDFPRGATVWV--DCKDQTDV-QVSSGLSKQSDNLYMESLSSLTADQWLRHETYSQVSHQ--GKEII-QTLKRSECV----

chicken lambda GQPKVAPITLFPSSKEELNEATKATLVCLINDFVSPVVDWV--DGS--TRS-GETAPQRQSNQYMASSVLSASDWSSEHETVTCRVTHN--GTSIT-KTLKRSEC----

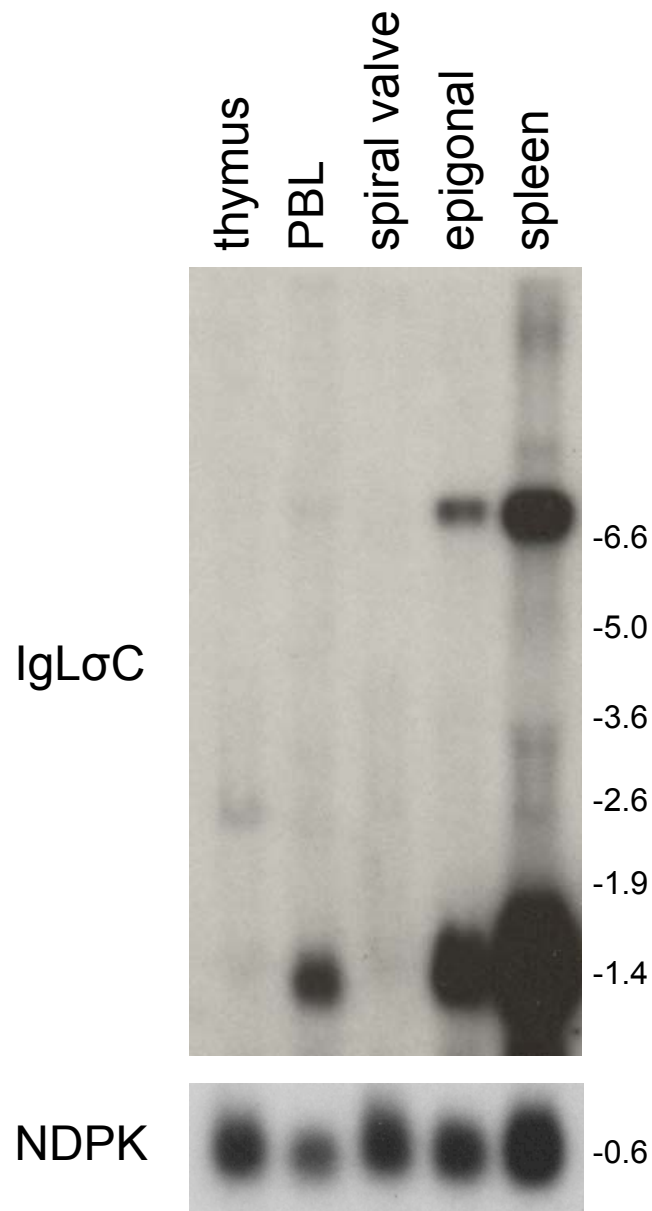
mouse lambda --QPKSTPRLTVFPSSSEELKEN-KATLVCLISNFSVSGVTVAWKA--NGTPITQGVDSNPTKEGN-KFMASSELHLSLSDQWRSHNSFTICQVTHE--GDIVE-KSLSPAEC----

human lambda --PKANPRLTVFPSSSEELQAN-KATLVCLISDFVPGAVVWKA--DGSVPKAC-VETTKPKSQSNKYAASSVLSLTPQWKSRSYSQVTHE--GSTVE-KTVAPTECS----

Figure S2. C domain alignment. Hyphens denote gaps. Common names of organisms and original IgL nomenclature are highlighted based on our proposed four isotype scheme; σ = pink, σ' = yellow, κ = green and λ = blue (consistent throughout rest of figures). Accession numbers of sequences used are zebrafish NITR (NM001005577), skate I (L25568), horn shark I (X15315), nurse shark NS5 (xxx), chicken λ (M24403), human λ (AAA59013), mouse λ (AA053422), *X. laevis* TIII (L76575), ratfish II (L25549), skate II (L25566), horn shark II (L25560), nurse shark (xxx), sandbar shark II (M81314), *X. laevis* ρ (XELIGLVAA), horn shark III (L25561), nurse shark NS4 (GSU15144), mouse κ (MUSIGKACN), human κ (S46371), catfish F (U25705), salmon L3 (AF406956), fugu L1 (AB126061), carp L1b (AB073332), zebrafish L3 (AB246193), catfish G (L25533), sturgeon (CAB44624), trout L1 (X65260), salmon L1 (AF273012), zebrafish L1 (AF246185), carp L1a (AB073328), carp L3 (AB073335), trout L2 (AAB41310), carp L2 (AB103558), fugu L2 (DQ471453), zebrafish L2 (AF246183), catfish σ (CK403931), *X. laevis* σ (S78544), *X. tropicalis* σ (scaffold 289), dogfish σ (CX662707), skate σ (CV222129), nurse shark σ (xxx) and horn shark σ (xxx). Solid lines at bottom mark predicted β -strands.

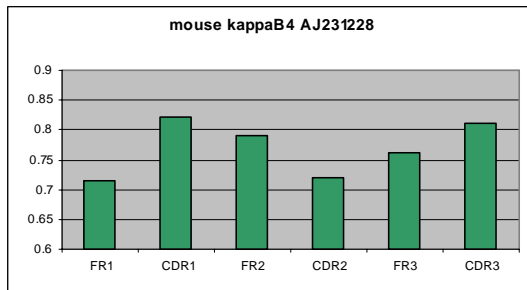
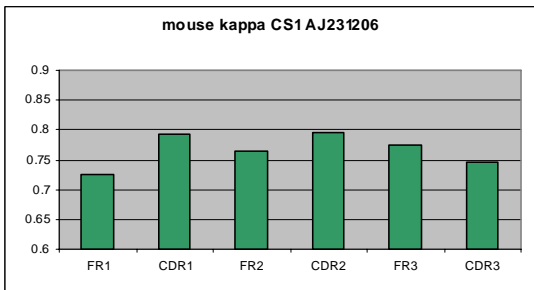
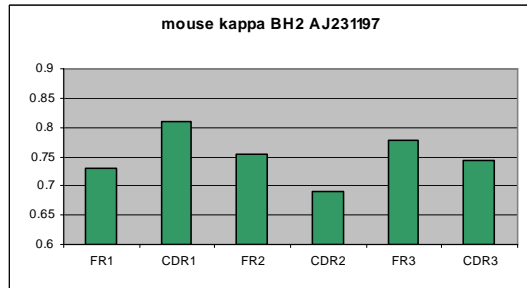
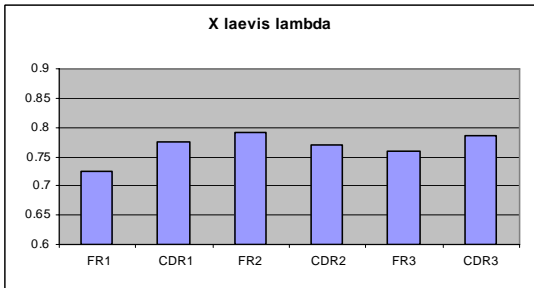
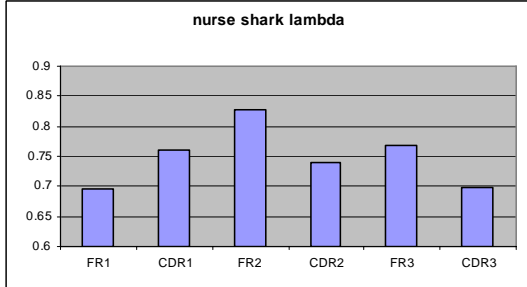
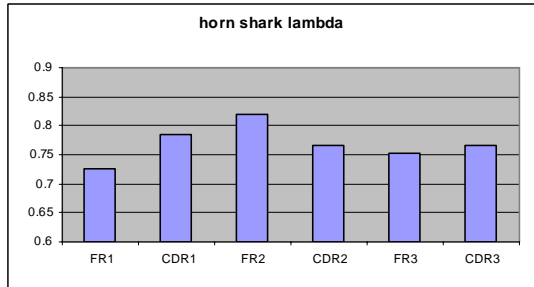
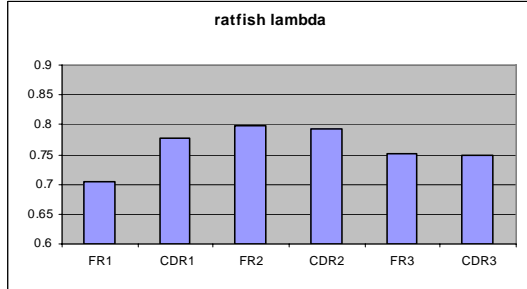
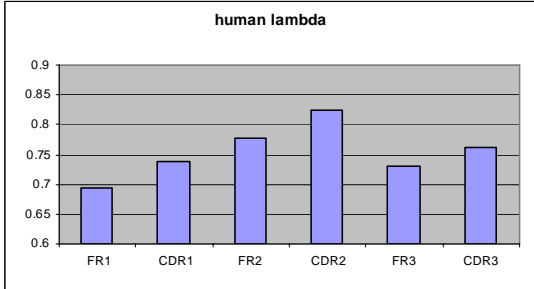
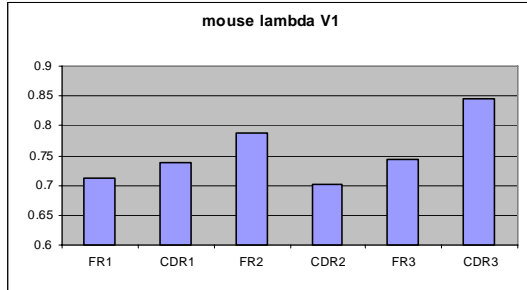
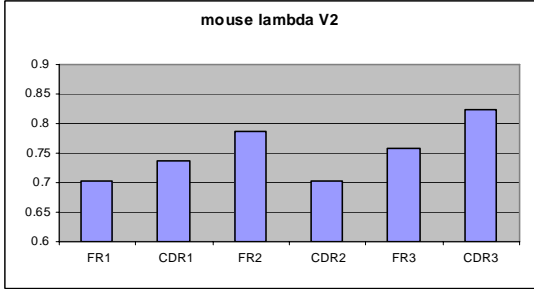
Figure S3 Expression Analysis.

Northern blot of shark RNA probed with IgL σ C and nucleotide diphosphate kinase (NDPK) as a loading control. Size markers shown to the right in kilobases.



TCTCAGTGCCCCGACCTATGGGTCTGGGTTTAGCAGCGACCGTTTTACATC
 L S A P T Y G S G F S S D R F T S
 GACCGTCAACAGCGGCCACAACATTTACAACCTTAATCATCAAAAACGTGAA
 T V N S G H N I Y N L I I K N V K
 GGTGATGATGCCGCGTCTATTACTGTGGAAAGTGGGTTTCCTCAGCTAG
 V D D A A V Y Y C G K W V S S A S
 12
 TACTTACGTGT CACAGTGACAGAAGGTAAAACAAAACTGACATCAGGAAA
 T Y V S
 TGTACACTCTATATCTTTGCATTGGATCAAATATTCTTATTGTACGTTTCA
 TAAGTTACTTTAATGTTGTCCTTCTGTGCAGTGTTTAATAATTGTTGTTGT
 TGCATAATTGATAACATCCTGACTGATTGCCCACTGTTGGGTGTTGATGAC
 ATTTTAATGTCAGTGTGTGGATGTTGTTATGAGGCTATTCCTTACTGTCCC
 CCACCAGCTGGGACACTGGGAACACACGATCAGTGCCGGCACTGGACCATC
 TGAAGGTGTTTTGTGTCCAATTGCAGGATCCTGGATAACTGTATGGACTGA
 23
 ACAGTAGAAACATTA CATTGTGTGGGTGTTTGGGCAAGGGACCAAGCTCTT
 W V F G Q G T K L F

Figure S4. RSS orientation of a nurse shark IgL σ locus. Heptamer and nonamers are highlighted in blue, conserved cysteine of FR3 and GxG of FR4 are highlighted in grey.



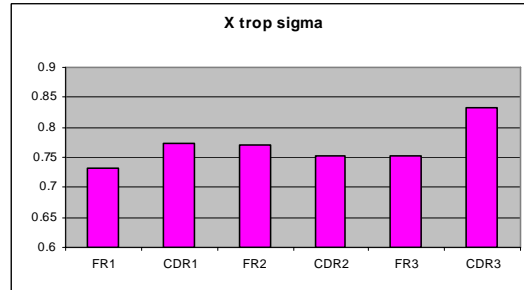
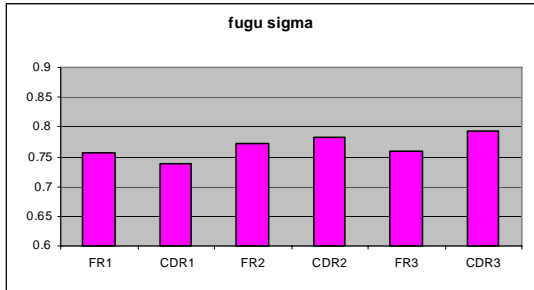
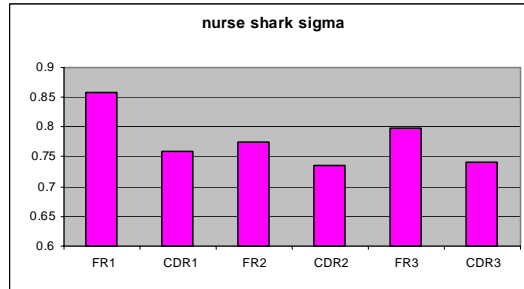
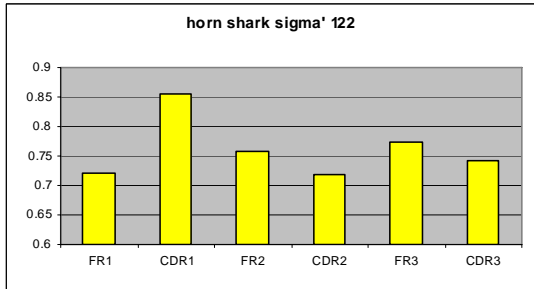
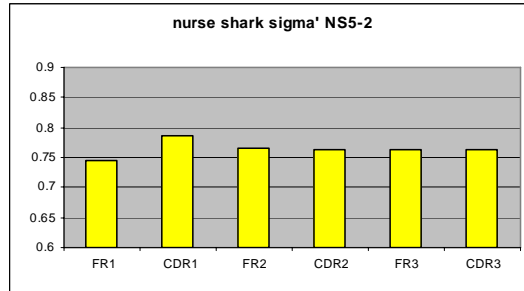
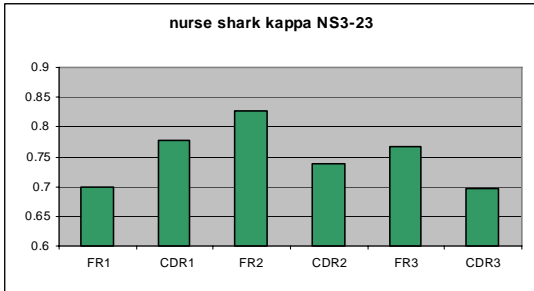
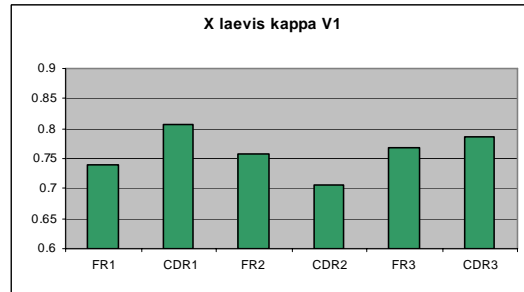
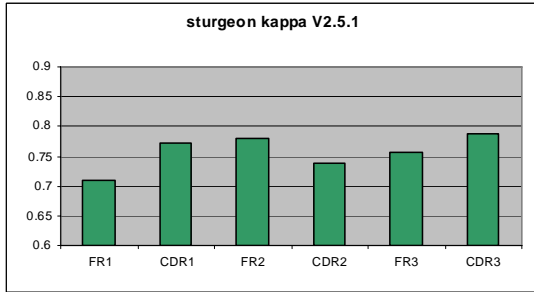
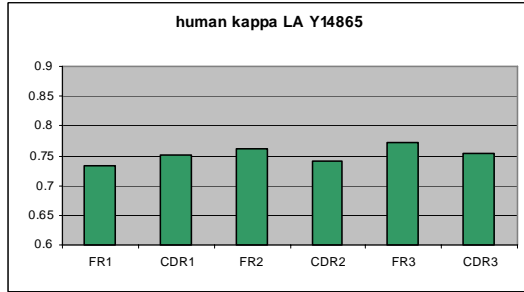
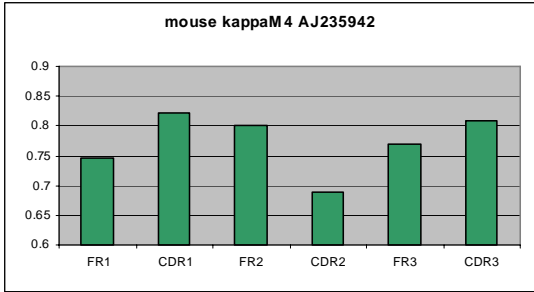


Figure S5. Codon volatility analysis of regions of V domains (preceding two pages). Codon volatility scores (as determined by in Sharp *Mol Biol Evol* 2004) were calculated for all codons and averaged for framework and CDR regions based on alignment in Figure 1. For example, the arginine codon “CGA” is least likely to mutate to another amino acid and thus has the lowest volatility score (0.5), whereas any substitution to the tryptophan and methionine codons will result in a change (volatility score of 1.0).

Figure S6. Positive correlation of CDR1L and CDR3H lengths. Ten murine and human known structures of antibody/ligand complexes (reviewed in Padlan 1994) used to compare length of CDRs. Line of best fit drawn with applet at www.mste.uiuc.edu/users/carvell/PlotPoints

	CDR1L	CDR3H
HyHEL-10	11	5
HyHEL-5	10	7
D1.3	11	8
McPC603	17	11
4/4/2020	16	7
AN02	10	6
B13I2	16	10
BV04-01	16	10
17/9	17	11
NC-41	11	10

