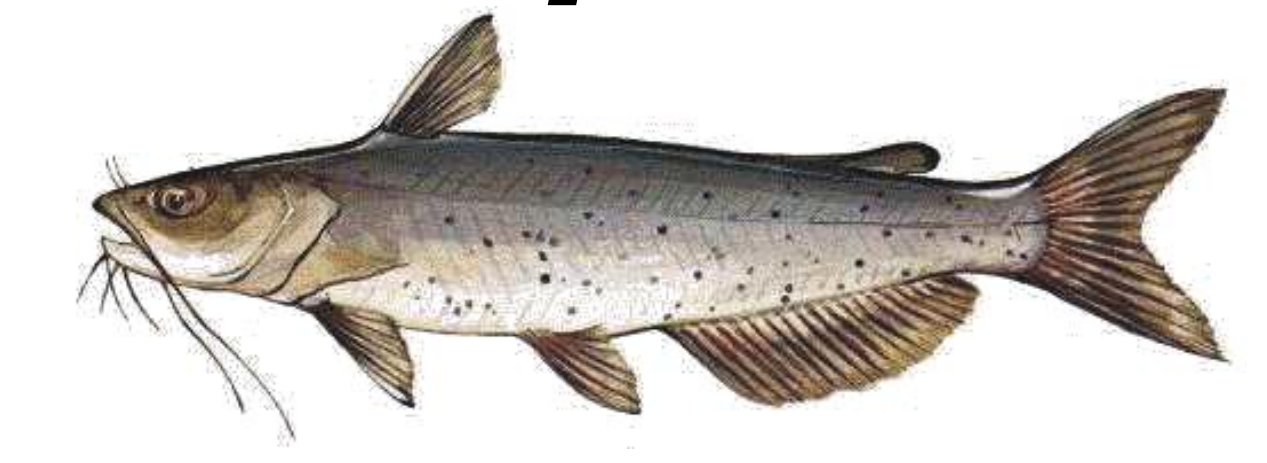
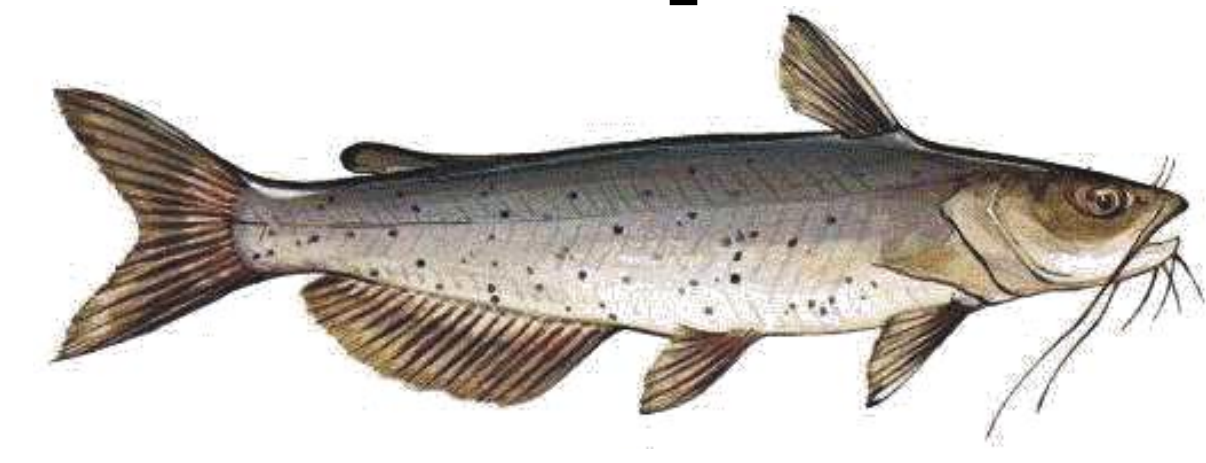




# Development and characterization of monoclonal antibodies to channel catfish, *Ictalurus punctatus*

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## Abstract

Currently, there are only few mAb reagents available for the channel catfish. These include antibodies specific to immunoglobulin heavy and light chains (Ig $\mu$  and IgL F and G isotypes), CD45, a thrombocyte marker (4-20), a neutrophil marker (51A), and a marker cytotoxic cells (CC41). Thus, the highest priority reagents for the catfish research community are mAbs that recognize specific receptors on T and B cell subsets. As part of the US Veterinary Reagent Network, we have developed and partially characterized mAbs to the newly identified catfish IgL isotypes Ig $\sigma$  and Ig $\lambda$ , and to the TCR co-receptor molecule CD4; our goal is to prove specificity by flow cytometry, Western blot, and immunoprecipitation with peptide sequencing. At the present four anti-Ig $\sigma$  and one anti-Ig $\lambda$  mAbs are being characterized. Notably, their use in cell distribution studies using magnetic-activated cell sorting (MACS) combined with flow cytometry and RT-PCR analyses demonstrate that while all four IgL isotypes are expressed in catfish IgM $^+$  B cells, IgD $^+$ IgM $^+$  B cells preferentially express Ig $\sigma$  chains. As for CD4, preliminary experiments indicate that anti-CD4 #75 mAb reacts with catfish clonal TS.32.17 T cells that express CD4 message, but not with other catfish T cell lines, which do not express CD4, or with catfish B cell and macrophage cell lines. Collectively these mAbs represent new and valuable resources for the catfish research community and complement the available mAbs that are used to identify catfish cell populations.

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<http://www.umass.edu/vetimm/>

## Catfish Immune reagents

Monoclonal antibodies (mAb) to catfish immune molecules are needed to:

- identify leukocyte cell populations, e.g. B, T and NK cells, neutrophils, macrophages, etc.
- evaluate changes during disease and after vaccinations

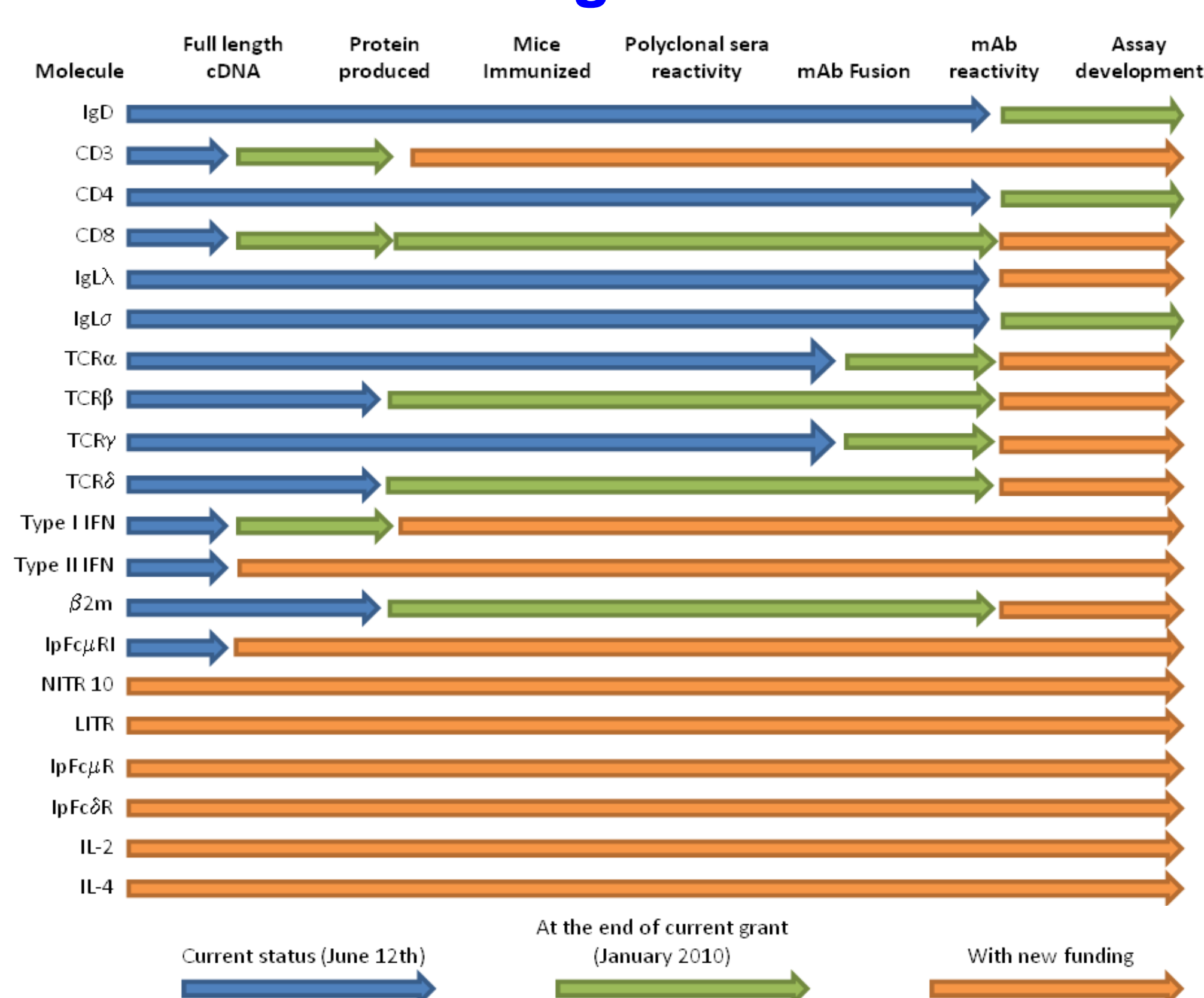
Only a small number of anti-catfish mAb have been fully characterized.

Most anti-catfish mAb are available through specific research laboratories.

## AVAILABLE CATFISH REAGENTS

ANTIBODIES	SOURCE
anti-Ig $\mu$ chain (2 mAbs)	M. Wilson - UMMC
anti-Ig $\delta$ chain (2 mAbs)	M. Wilson - UMMC
anti-IgL F chain	M. Wilson - UMMC
anti-IgL G chain	M. Wilson - UMMC
anti-MHC class II $\alpha$ and $\beta$	T. McConnell - E. Carolina
anti-LFA1	M. Wilson - UMMC
anti-cytotoxic cell marker, CC41	M. Wilson - UMMC
anti-thrombocyte (putative CD41/C61)	M. Wilson - UMMC
anti-CD45 (2 mAbs)	M. Wilson - UMMC
anti-neutrophil (51A)	C. Rice - Clemson
anti-NCCRP1	Harlan Bioproducts

## Catfish Progress & Plans



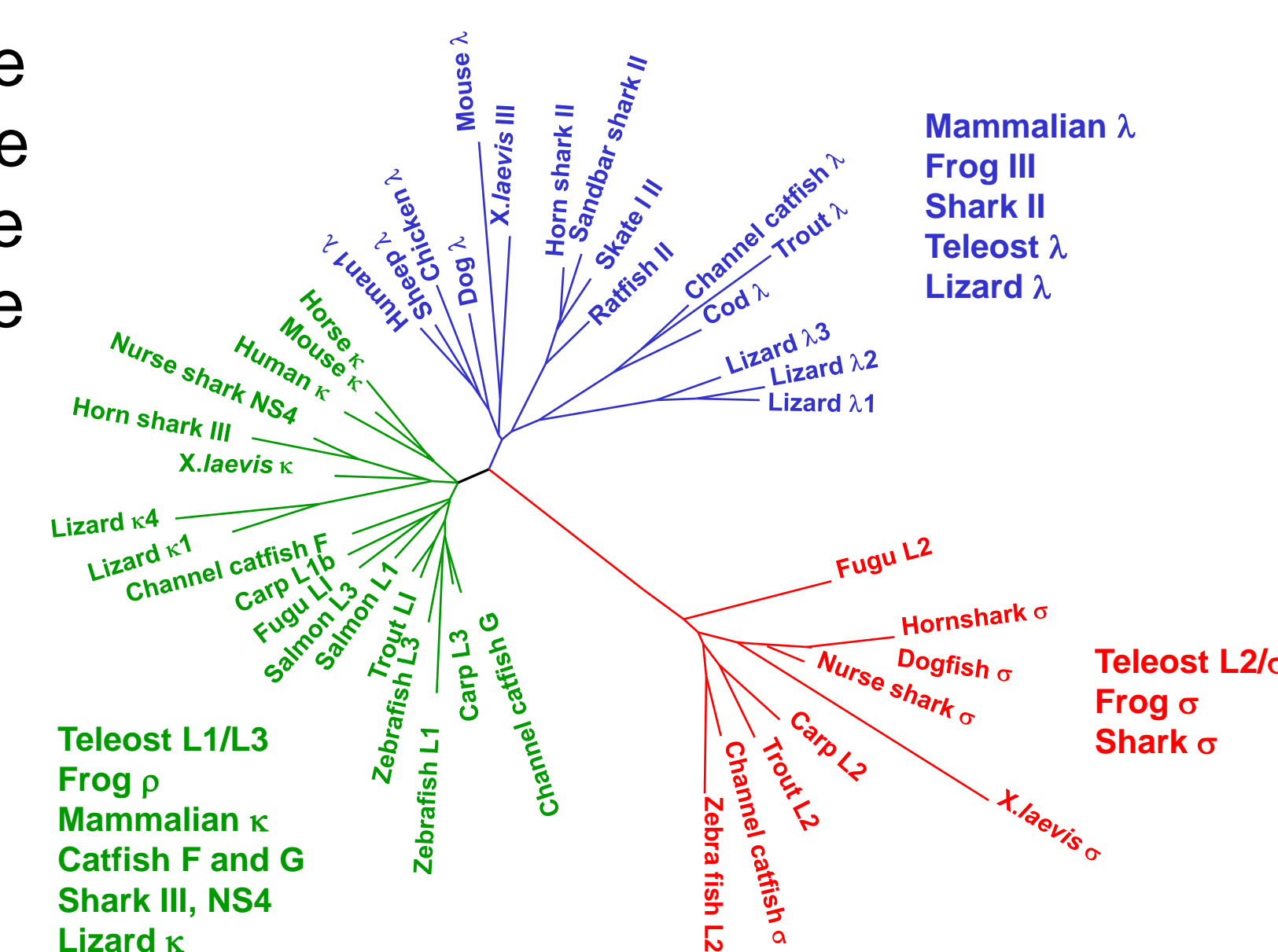
## Methods

- Recombinant (r) proteins are produced in *E. coli* and/or COS cells and purified using Ni $^{2+}$  resin.
- *E. coli* proteins are re-folded using stepwise dialysis.
- Balb/c mice are immunized with r protein for hybridoma production.
- Hybridoma supernatants are first screened in ELISA, then by flow cytometry and Western blot against the r protein.
- Irrelevant r proteins are used as control to test for anti-tag specific antibodies.
- Hybridoma supernatants are also tested on catfish lymphocyte populations and serum (if relevant).

## Teleost IgL chains

Teleost IgL chains can be classified into four isotypes:

- Type I  $\kappa$  type
- Type II  $\sigma$  type
- Type III  $\kappa$  type
- Type IV  $\lambda$  type

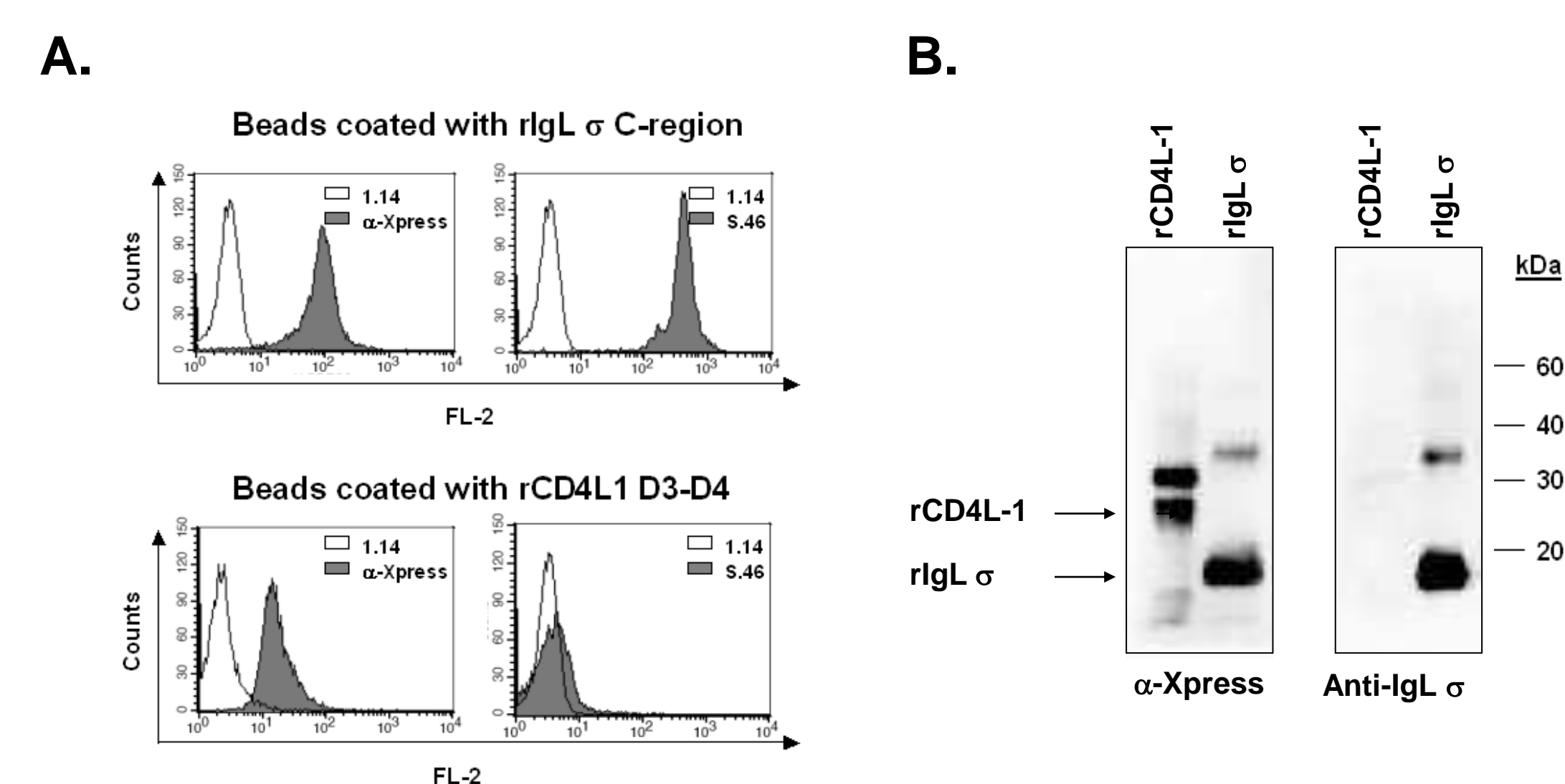


Classifications are based on:

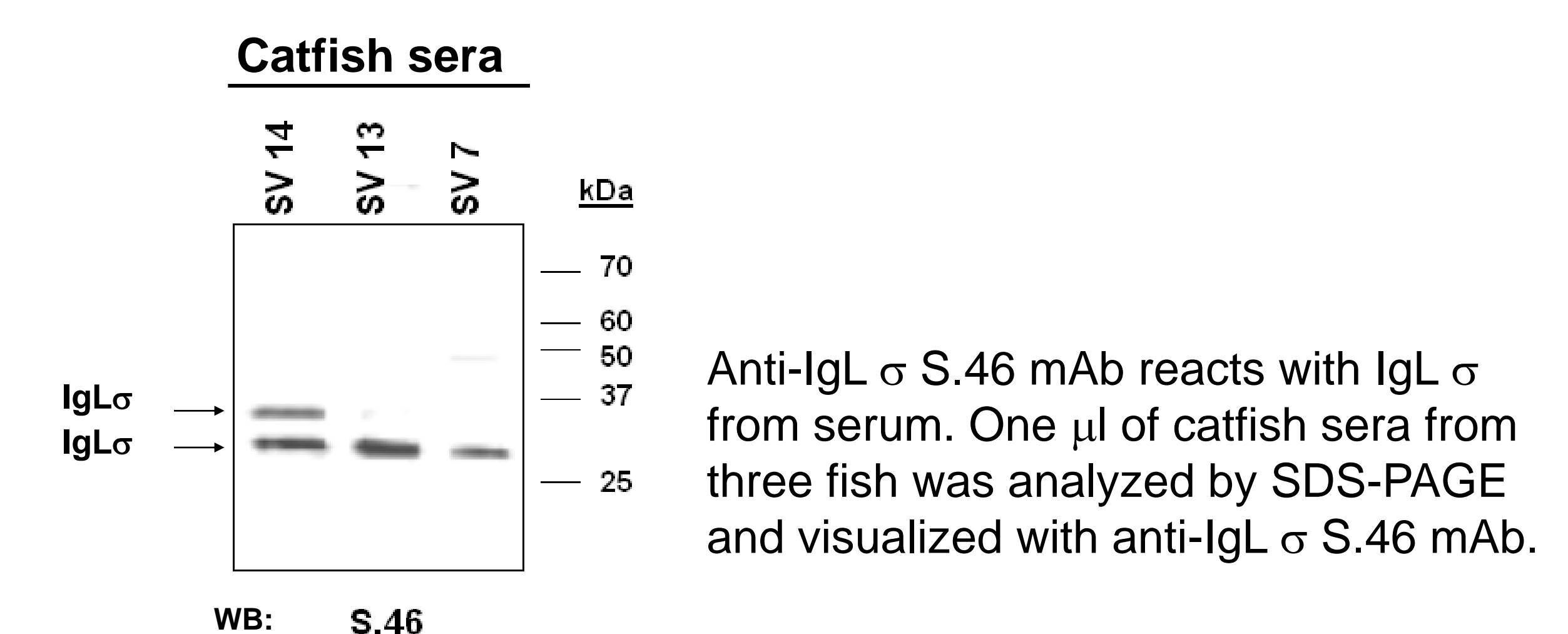
- CDR lengths
- VL homology
- Recombination Signal Sequence (RSS) orientation

## Anti-IgL $\sigma$ mAbs

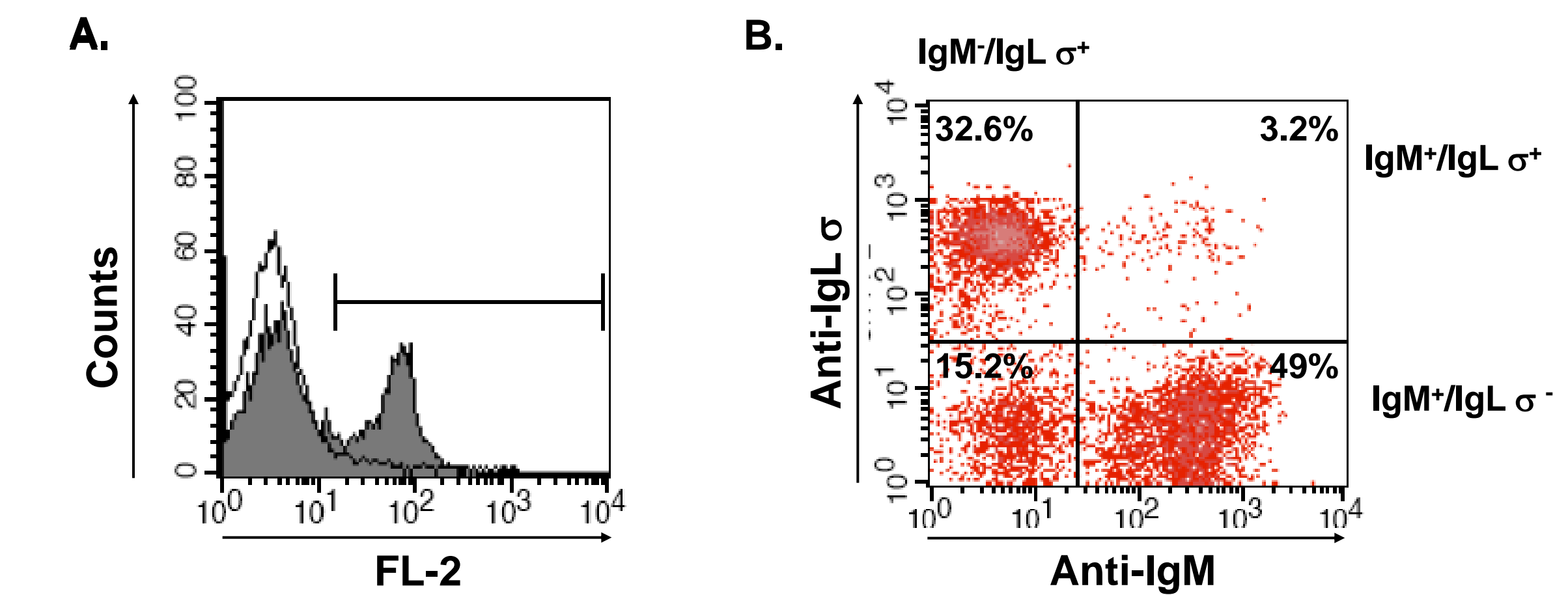
Four mAbs were characterized. Three recognize denatured IgL  $\sigma$  (S.4, S.15, S.46). One reacts with the native form of IgL  $\sigma$  (S.11).



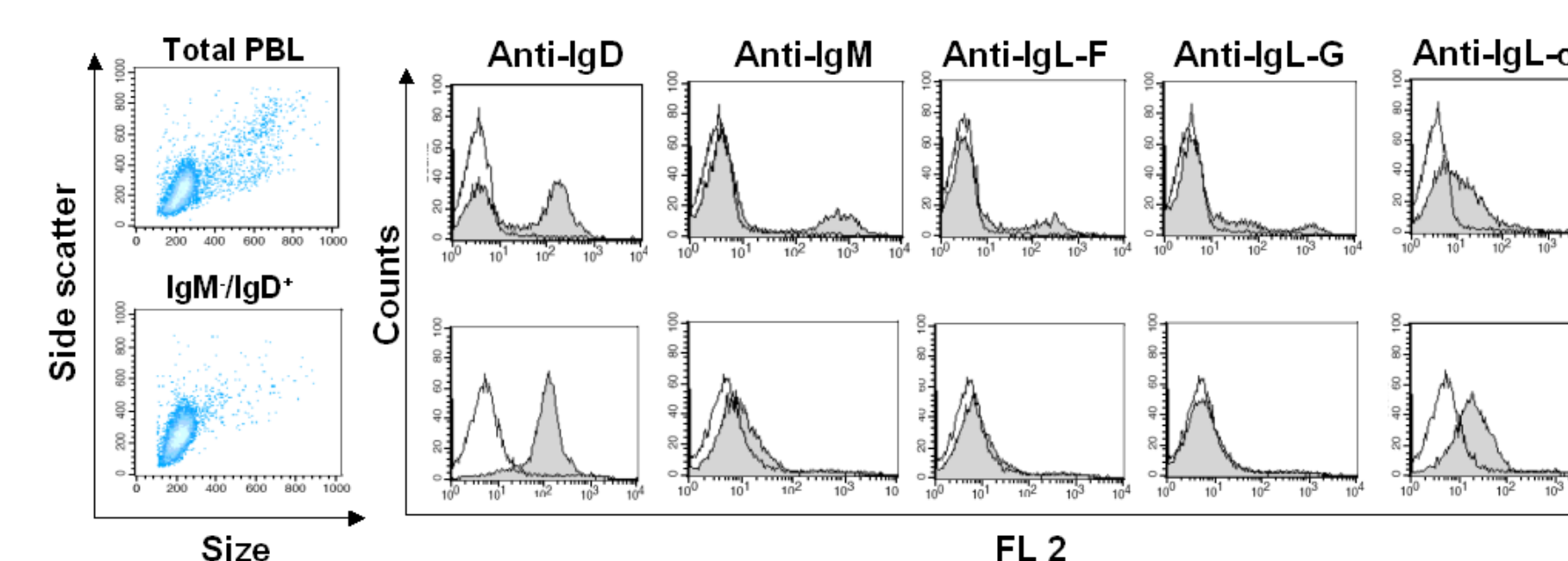
**Anti-IgL  $\sigma$  S.46 mAb reacts with the denatured IgL  $\sigma$  protein.**  
**A.** Latex beads were coated with rIgL  $\sigma$  or rCD4 protein (as irrelevant tagged protein control), incubated with anti-IgL  $\sigma$  S.46 or anti-Xpress tag mAbs, and analyzed by flow cytometry. Anti-trout IgM 1.14 mAb was used as isotype control.  
**B.** rIgL  $\sigma$  and rCD4 proteins were analyzed by SDS-PAGE and visualized by Western blot using anti-IgL  $\sigma$  S.46 mAb.



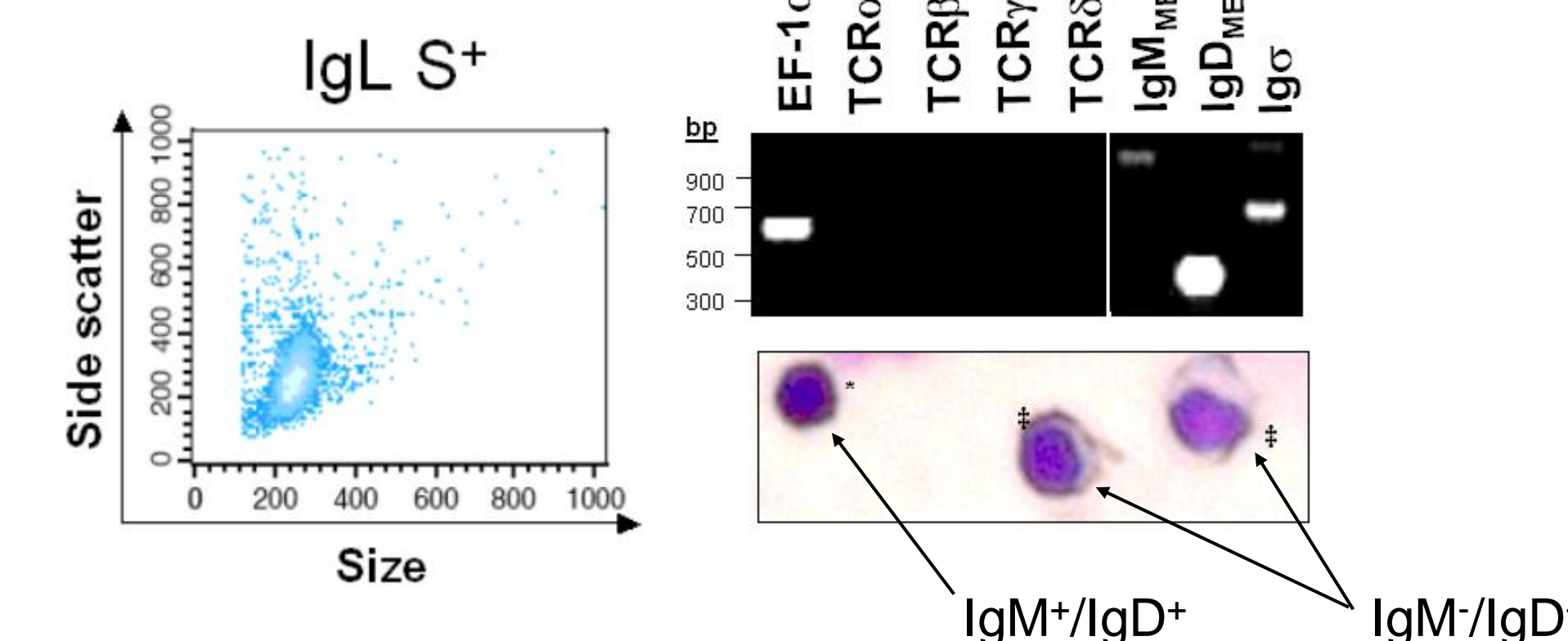
## IgL $\sigma$ preferentially associates with IgD H chains



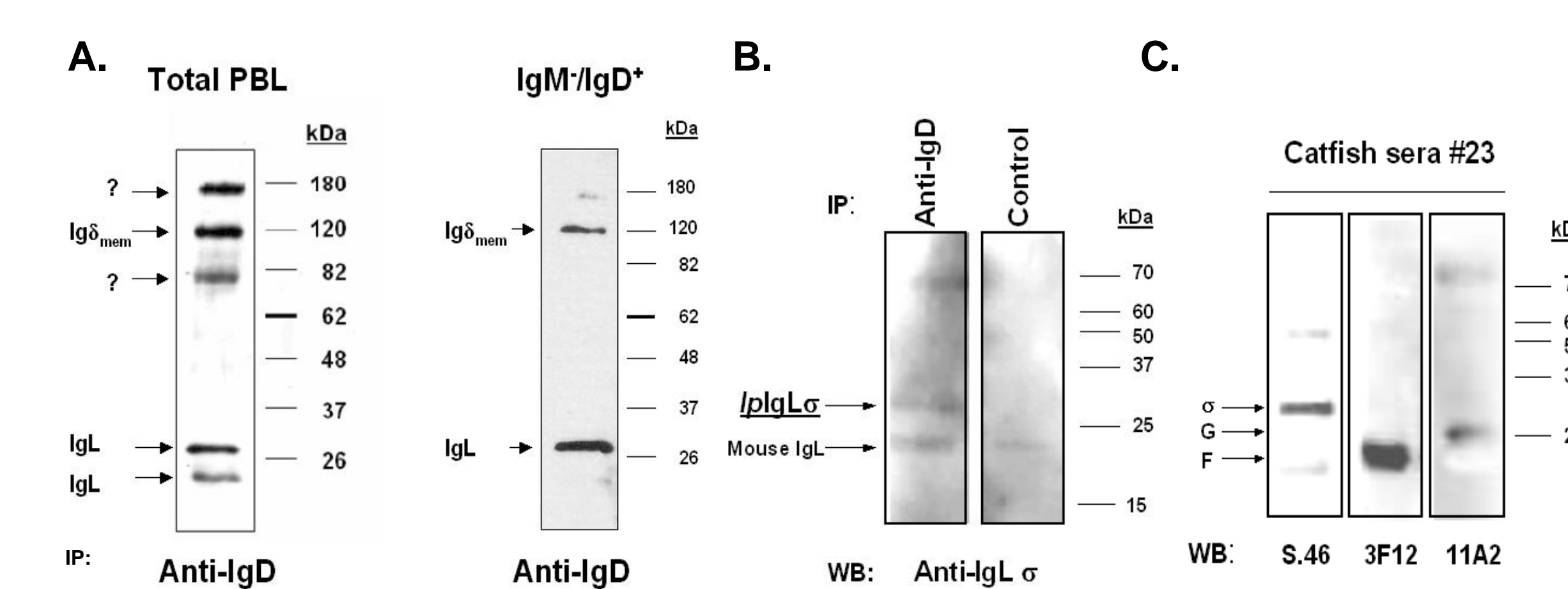
**Anti-IgL  $\sigma$  S.11 mAb stains an IgM $^+$  subpopulation in catfish PBL.**  
**A.** Histogram shows 35% cells stained positive for IgL  $\sigma$ . Cells stained with S.11 mAb are in gray; isotype control staining is in white.  
**B.** Double staining of catfish PBL using anti-IgM 9E1 and anti-IgL  $\sigma$  S.11 mAbs. Percent staining cells are shown in each quadrant. Flow cytometry was performed using freshly isolated PBL.



IgD, IgM, and IgL expression in PBL from a catfish with a large population of IgM/IgD $^+$  B cells. Freshly isolated PBL were selected by magnetically activated cell sorting (MACS) from the same fish using anti-IgD 7D11 mAb. Aliquots from the unsorted (top) and sorted (bottom) populations were stained with anti-IgD, anti-IgM, anti-IgF, anti-IgG and anti-IgL  $\sigma$  mAbs. Most IgM/IgD $^+$  B cells stained with anti-IgL  $\sigma$ . Isotype controls are shown in white.



PBL were sorted with anti-IgL  $\sigma$  mAb and the resulting cells were shown to be mostly IgM/IgD $^+$  B cells. Total RNA isolated from these cells was used in RT-PCR with primers specific for IgM, IgD, IgL  $\sigma$ , TCR chains, and EF1- $\alpha$  as a control. All PCR products were verified by sequencing.

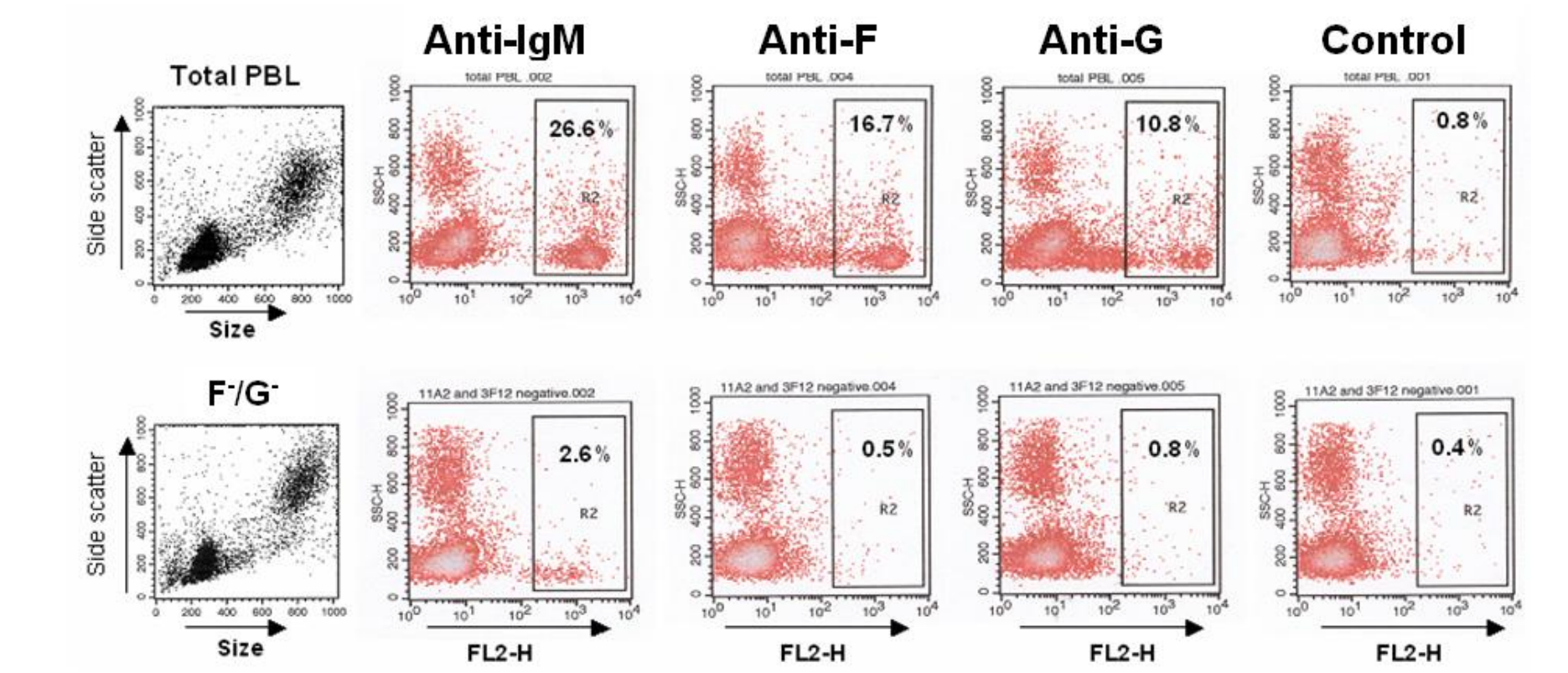


## Catfish IgD preferentially associates with IgL $\sigma$ chains on IgM/IgD $^+$ B cells.

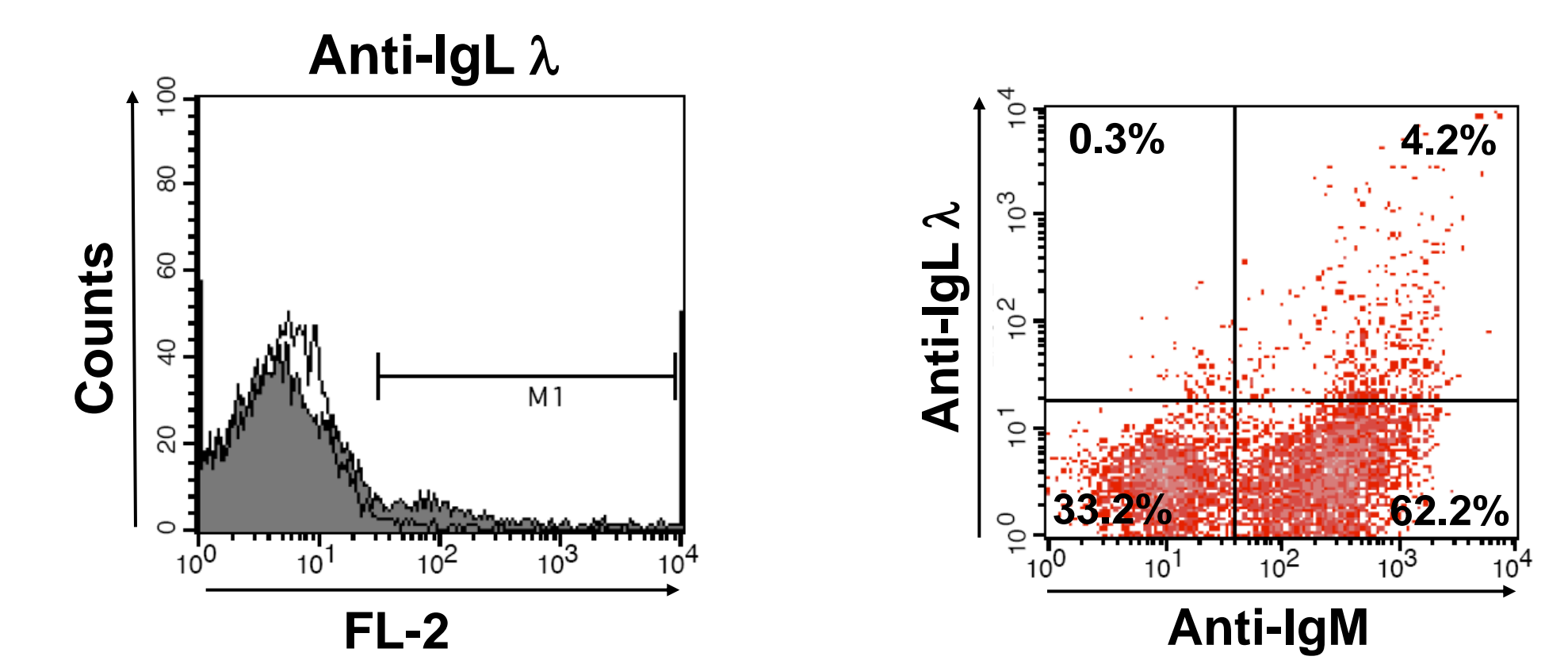
**A.** Immunoprecipitations of biotinylated PBL and IgM/IgD $^+$  B cells using anti-IgD 7D11 mAb. Labeled arrows indicate the appropriate sized Ig $\delta$  H and IgL chains. Membrane Ig $\delta$ , secreted Ig $\delta$ , associated IgL bands and unknown (?), will be verified by sequencing.  
**B.** Total catfish PBL were immunoselected with anti-IgD mAb, proteins were analyzed by SDS-PAGE and visualized using anti-IgL  $\sigma$  as primary mAb, followed by goat anti-mouse IgG2b-HRP secondary mAb. Isotype matched controls were used for immunoprecipitations and Western blots.  
**C.** 0.5  $\mu$ l of catfish sera were analyzed by SDS-PAGE and visualized with anti-IgL  $\sigma$  S.15, anti-IgF 3F12, and anti-IgG 11A2 mAbs. Reactive bands of the appropriate size for catfish IgL  $\sigma$ , IgL F and IgL G as were observed.

## Anti-IgL $\lambda$ mAbs

One putative anti-IgL  $\lambda$  mAb was obtained, it appears to work in flow cytometry.



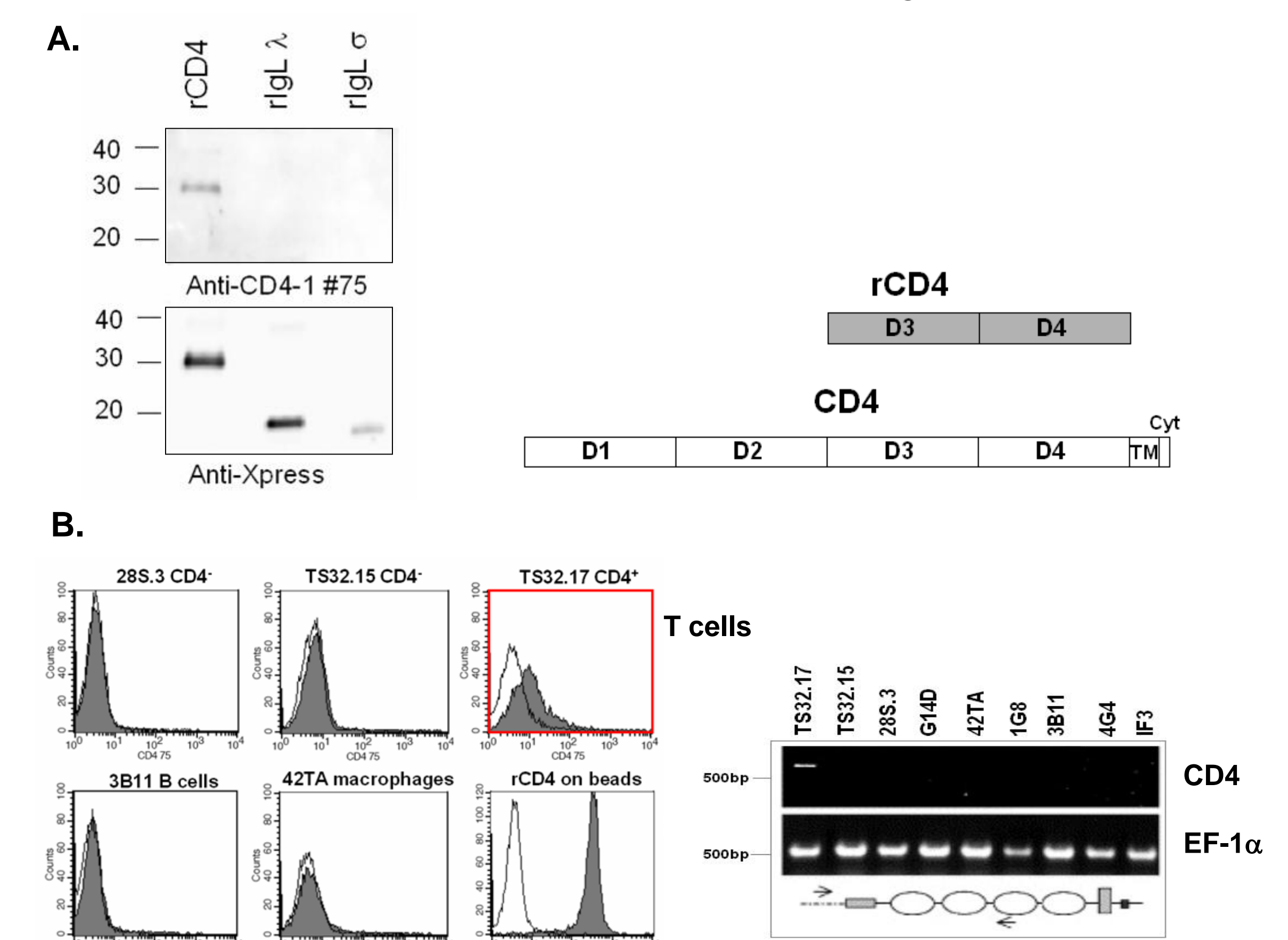
Most IgM $^+$  B cells are either IgL F $^+$  or IgL G $^+$ . However, flow cytometry of IgL F and IgL G MACS depleted cells show a small population of IgM $^+$  cells, which suggests that IgM $^+$  cells can be IgL  $\lambda$  $^+$ .



Putative anti-IgL  $\lambda$  stains an IgM $^+$  subpopulation in catfish PBL

## Anti-CD4

One mAb, #75 was developed for CD4. Preliminary experiments indicate that anti-CD4 mAb reacts with catfish clonal TS.32.17 T cells that express CD4 message. Anti-CD4 does not react with catfish T cell lines, which do not express CD4, or with catfish B and macrophage cell lines.



**Anti-CD4 #75 mAb reacts with native and denatured CD4 protein.**  
**A.** rCD4 protein and IgL  $\sigma$  (as irrelevant tagged protein controls) were analyzed as described for anti-IgL  $\sigma$  S.46. Western blots show that CD4 #75 mAb reacted with rCD4 protein and not the HIS or Xpress tags.  
**B.** Various catfish clonal cell lines were stained with anti-CD4 #75 or an appropriate isotype control, and analyzed by flow cytometry. Only clonal TS32.17 T cells, which express CD4 message stained positive. Latex beads coated with rCD4 protein served as a positive control.

## Conclusions

- Monoclonal Abs to anti-catfish IgL  $\sigma$ , IgL  $\lambda$ , and CD4 were developed from Balb/c mice immunized with *E. coli* produced recombinant protein that was refolded using stepwise dialysis.
- Four anti-catfish catfish IgL  $\sigma$  mAbs were developed and successfully used to characterize catfish IgL  $\sigma$  expressing B cells.
- Characterization of anti-catfish IgL  $\lambda$  and anti-catfish CD4 #75 are ongoing.