

Comprehensive validation of T- and B-cell deficiency in *rag1*-null zebrafish: Implication for the robust innate defense mechanisms of teleosts

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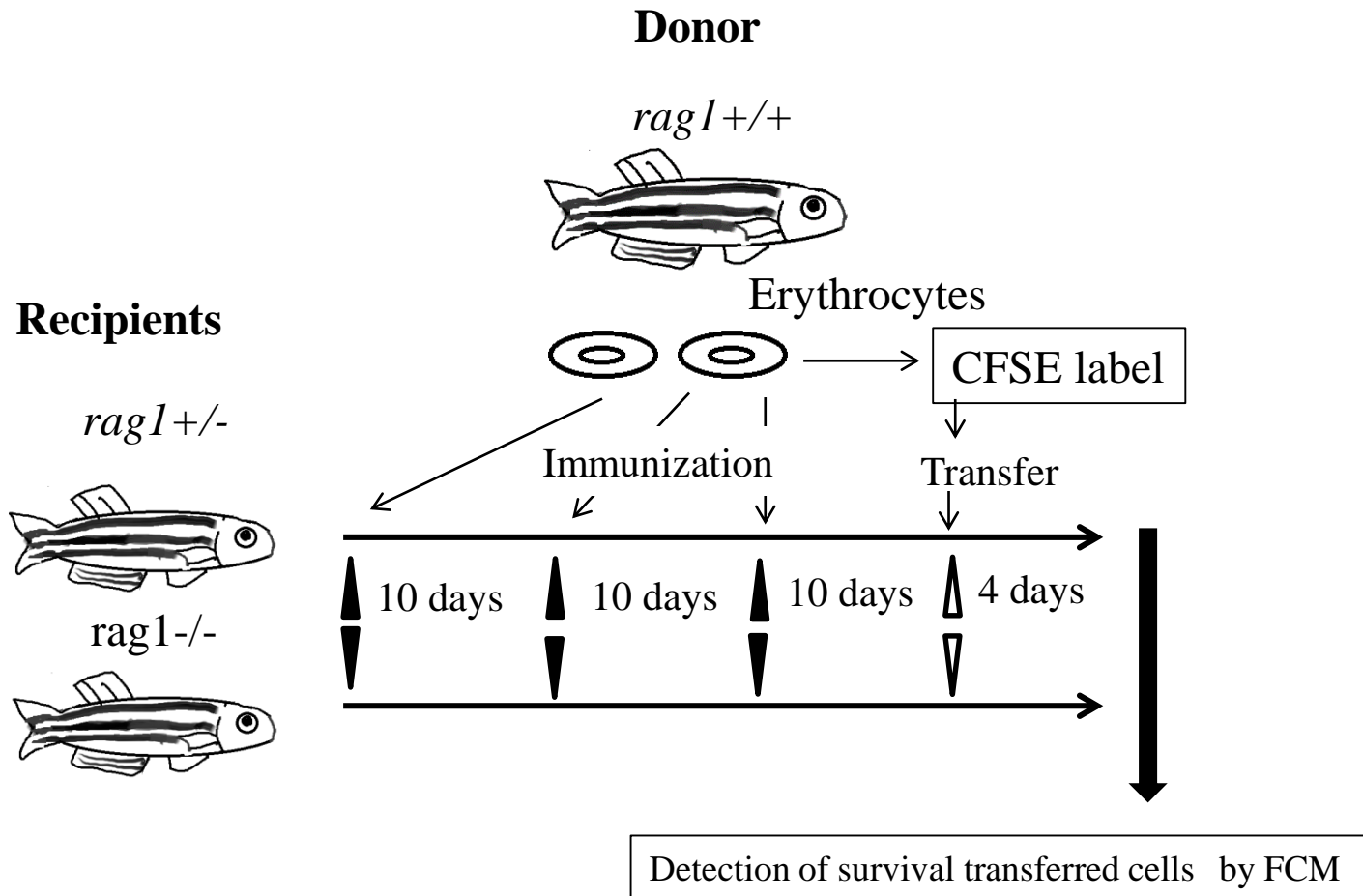


Fig. S1. Experimental scheme for the rejection of in vivo allogeneic erythrocytes in zebrafish. Black arrowheads indicate immunization with 1.0×10^6 allogeneic erythrocytes. Open arrowheads indicated transplantation with 1.0×10^7 5(6)-carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE)-labeled allogeneic erythrocytes. Arrow indicates the collection of erythrocytes from the recipient and detection of CFSE-labeled erythrocytes by flow cytometry. Three pairs of donors and recipients were tested in each strain of fish.

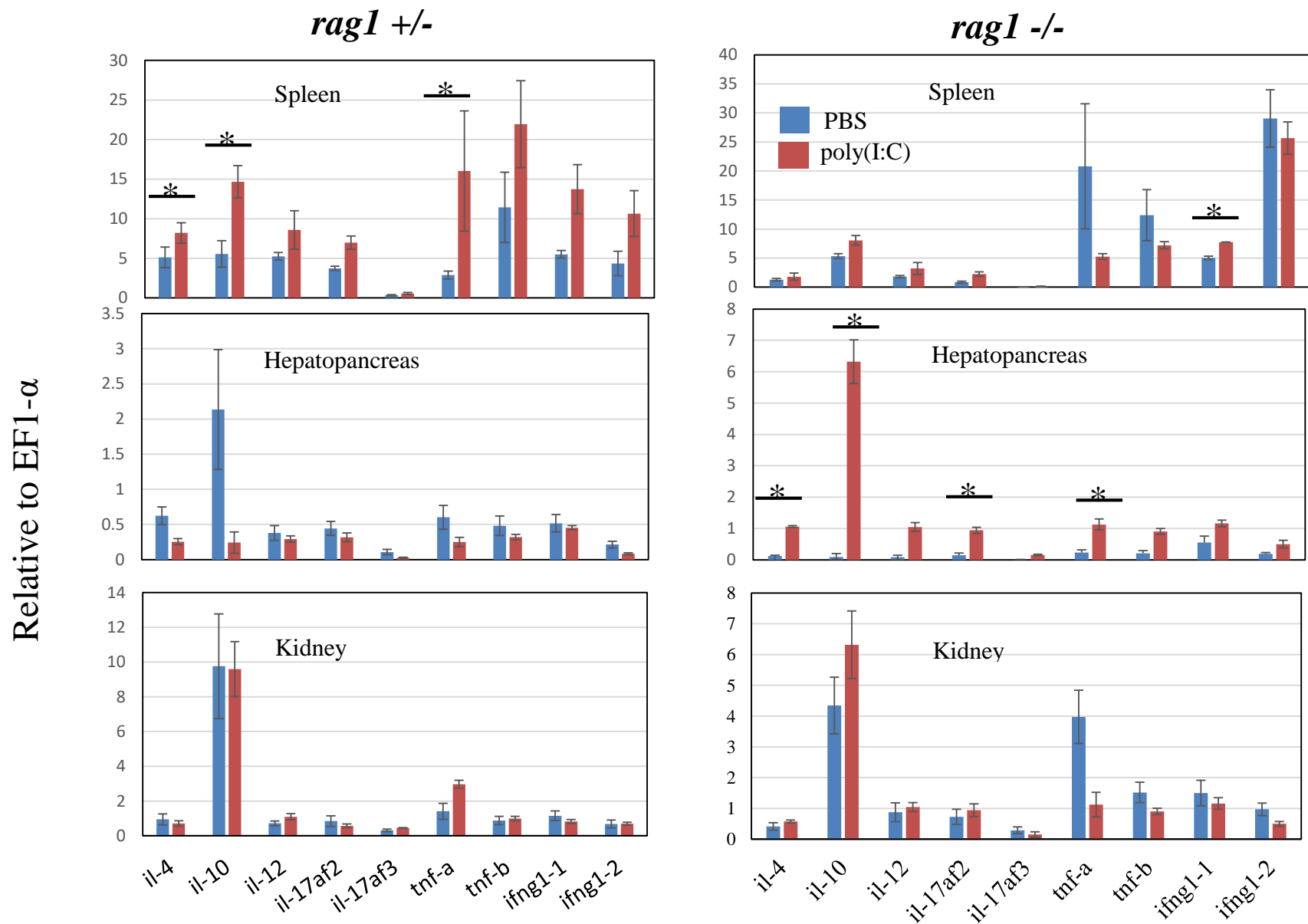


Fig. S2. Expression of cytokines in the spleen, hepatopancreas and kidney from *ragl*^{+/-} and *ragl*^{-/-} fish that were injected with PBS (■) and poly(I:C) (■). Data from the three individual experiments are shown as the mean of relative value to EF1- α . Statistical comparisons between stimulated and control fish were made using an unpaired *t*-test. Asterisks indicate significant differences at $P < 0.05$.

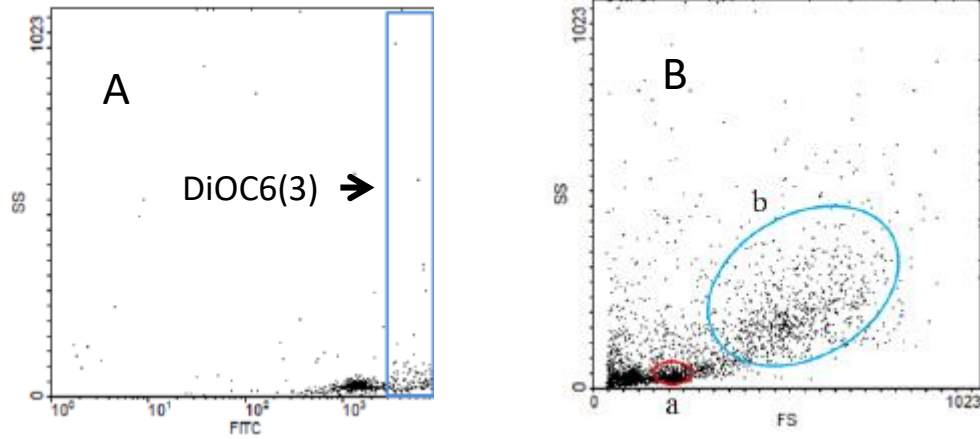


Fig. S3. Representative scatter plots of kidney leukocytes from wildtype zebrafish in flow cytometry analysis. The blue rectangle gate indicates DiOC6(3)-positive cells (A). Forward scatter (FS) and side scatter (SS) analysis: Lymphocyte (a) and macrophage/neutrophil (b) gate (B).

Table S1 Primers for real time PCR.

Target genes	Primer sequences (5'→3')
IL-1 β	F: TTCCCCAAGTGCTGCTTATT R: AAGTTAAAACCGCTGGTCA
IL-4	F: AGTCACGCTGCTGATGAAGA R: AACTTGGTCTTGGGCTTTTT
IL-10	F: ATAGGATGTTGCTGGGTTGG R: GTGGATGAAGTCCATTTGTGC
IL-12	F: GCTGAAGGAGTGTTTCCTCAGT R: TGACATCATTTCCCTGTGCTCTC
IL-17AF2	F: CTGCACTGGGCTTCAAAGAT R: TCAATCTGAGGACGGAAAGG
IL-17AF3	F: TCTCTGTCGCCTTGGACATAC R: GTCCTCCTCACCGTCTTTTG
TNF α 1	F: GCGCTTTTCTGAATCCTACG R: TGCCCAGTCTGTCTCCTTCT
TNF α 2	F: AAGCCAAACGAAGAAGGTCA R: AACCCATTTTCAGCGATTGTC
IFN ϕ 1	F: ACGACAGAATCTCTGAACCT R: GTCAGGACTAAAACTTCAC
IFN γ 1-1	F: TTCAGACAACCAGCGCATAAC R: AACCCAATCCTTTGCAAGC
IFN γ 1-2	F: TGCAGAGCTCAGGACGTATG R: CTTTAGCCTGCCGTCTCTTG
EF1 α	F: AACAGCTGATCGTTGGAGTCAA R: TTGATGTATGCGCTGACTTCCT