

## Supplementary materials:

### Studies on the use of flagellin as an immunostimulant and vaccine adjuvant in fish aquaculture

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**Figure S1** A multiple alignment of recombinant *Y. ruckeri* flagellin (YRF) and its muteins YRF-N, YRF-C and YRF-NC.

**Table S1** Primers used for real-time PCR analysis of gene expression

**Table S2** Fold change of transcript expression of immune-relevant genes in the spleen, liver, gills and skin of fish ip injected with YRF.

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YRF      MGA VINTNSLSLLTQNNLNK SQGSLGTAIERLSSGLRINS AKDDAAGQAIANRFTSNING 60
YRF-NC  MGA VINTNSLSLLTQNNLNK SQGSLGTAIERLSSGLRINS AKDDAAGQAIANRFTSNING 60
YRF-N   MGA VINTNSLSLLTQNNLNK SQGSLGTAIERLSSGLRINS AKDDAAGQAIANRFTSNING 60
YRF-C   ----- 0

YRF      LTQAARNANDGISLSQTAEGALGEINNNLQRVRDLTVQAQNS SNSASDIDSIQSEVNQRM 120
YRF-NC  LTQAARNANDGISLSQTAEGALGEINNNLQRVRDLTVQAQNS SNSASDIDSIQSEVNQRM 120
YRF-N   LTQAARNANDGISLSQTAEGALGEINNNLQRVRDLTVQAQNS SNSASDIDSIQSEVNQRM 120
YRF-C   ----- 0

YRF      EEINRVTKQTFDNGIKVLDNR TAANA EYAFQVGSQDAQKINIEIGSSAGWNLATAGAGGT 180
YRF-NC  EEINRVTKQTFDNGIKVL SGGGSGGGGSGGGGS ----- 148
YRF-N   EEINRVTKQTFDNGIKVLDNR TAANA EYAFQVGSQDAQKINIEIGSSAGWNLATAGAGGT 180
YRF-C   ----- MCDNR TAANA EYAFQVGSQDAQKINIEIGSSAGWNLATAGAGGT 44
          :

YRF      SSDVVNDSTQISKAKETVVQ T LSGKTEAQINTALTKFTT DVKAATDAAGVVTAKGALTTA 240
YRF-NC  ----- 148
YRF-N   SSDVVNDSTQISKAKETVVQ T LSGKTEAQINTALTKFTT DVKAATDAAGVVTAKGALTTA 240
YRF-C   SSDVVNDSTQISKAKETVVQ T LSGKTEAQINTALTKFTT DVKAATDAAGVVTAKGALTTA 104

YRF      LGLKADADLGTAVSSAAF GTDLSVDQIAGVKSGVYSAAINGANYATAKTEAEVSAAQAGA 300
YRF-NC  ----- 148
YRF-N   LGLKADADLGTAVSSAAF GTDLSVDQIAGVKSGVYSAAINGANYATAKTEAEVSAAQAGA 300
YRF-C   LGLKADADLGTAVSSAAF GTDLSVDQIAGVKSGVYSAAINGANYATAKTEAEVSAAQAGA 164

YRF      KTAGAMVNGNFRSVEAKGFDV LKGNVTGGATGTATGTTPLADIDAALKAVDSQRSSLGAS 360
YRF-NC  ----- VEA KGFV LKGNVTGGATGTATGTTPLADIDAALKAVDSQRSSLGAS 201
YRF-N   KTAGAMVNGNFRS----- 327
YRF-C   KTAGAMVNGNFRSVEAKGFDV LKGNVTGGATGTATGTTPLADIDAALKAVDSQRSSLGAS 224

YRF      QNRFESTITNLNNTVNNLTSARSRIQDADYSTEVS NMSRAQILQQAGTSVLAQANQVPQT 420
YRF-NC  QNRFESTITNLNNTVNNLTSARSRIQDADYSTEVS NMSRAQILQQAGTSVLAQANQVPQT 261
YRF-N   ----- 327
YRF-C   QNRFESTITNLNNTVNNLTSARSRIQDADYSTEVS NMSRAQILQQAGTSVLAQANQVPQT 284

YRF      VL SLLR ASSA HHHHHHHHHH 440
YRF-NC  VL SLLR ASSA HHHHHHHHHH 281
YRF-N   ----- ASSA HHHHHHHHHH 327
YRF-C   VL SLLR ASSA HHHHHHHHHH 304

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**Figure S1 A multiple alignment of recombinant *Y. ruckeri* flagellin (YRF) and its mutants YRF-N, YRF-C and YRF-NC.** The aa sequences derived from the expression vector are highlighted in green. A GS linker (SGGGSGGGGSGGGGS) inserted in YRF-NC is highlighted in red.

**Table S1 Primers used for real-time PCR analysis of gene expression**

Gene	Forward (5' to 3')	Reverse (5' to 3')	Acc. No.
<b>House-keeping gene</b>			
<b>EF-1<math>\alpha</math></b>	CAAGGATATCCGTCGTGGCA	ACAGCGAAACGACCAAGAGG	AF498320
<b>IL-1 Family</b>			
<b>IL-1<math>\beta</math>1</b>	CCTGGAGCATCATGGCGTG	GCTGGAGAGTGCTGTGGAAGAACATATAG	AJ278242
<b>IL-1<math>\beta</math>2</b>	GAGCGCAGTGGAAGTGTGG	AGACAGGTTCAAATGCACTTTATGGT	AJ245925
<b>IL-1<math>\beta</math>3</b>	CTGAAGGCCGTCACAATCCA	CTGGTCCTTACAGCGCTCCAA	AM181685
<b>nIL-1Fm</b>	CCCATTCTCGTGACACCAG	CTGGACGACCTGGAGAGTGACT	AJ555869
<b>IL-18</b>	GAGCAATGCAAAGCAGATGATTG	CATGTTTTGAGCAGCCAATGTAGTC	AJ556990
<b>TNF family</b>			
<b>TNF<math>\alpha</math>1</b>	TGTGTGGGGTCTCTTAATAGCAGGTC	CCTCAATTCATCCTGCATCGTTGA	AJ277604
<b>TNF<math>\alpha</math>2</b>	CTGTGTGGCGTTCTCTTAATAGCAGCTT	CATTCGTCCTGCATCGTTGC	AJ401377
<b>TNF<math>\alpha</math>3</b>	GCTGCACTCTTCTTTACCAAGAAACAAG	CCACTGAGGACTTGTATCACCATAGGT	HE798544
<b>IL-6 family</b>			
<b>IL-6</b>	GGGAGAAAATGATCAAGATGCTCGT	GCAGACATGCCTCCTTGTGG	DQ866150
<b>IL-11</b>	CTCTCGTGCTATTGGCCCA	TCTCGAATGCATGTTCTTCAATAGAT	AJ535687
<b>M17</b>	GTGGACCTCTTAAAAACATACAAGCTCAG	GGATGGTGGCTGTAAGTCTGTCTG	FM866399
<b>CNTF</b>	GCACTTATCTTCTGGAGCTATATAGGGAGA	AACTCCATCAACCTCCTCATTGC	FM866401
<b>IL-12 family</b>			
<b>P19</b>	ACCTAAGAGCAGATTCAATGCCTTG	TCTTCCCAGCTTTCACCTTCTG	KP410548
<b>P28A</b>	GCAGCTGCTCAGGAGATATAAGGAGG	TCTCTCAGGTATGCTGGGTTTTGG	HG794528
<b>P28B</b>	GCAGCTGCTCATGAGATATAAAGAGGA	GCTGCTCTCTGTTCCACCTTATCCAC	HG794529
<b>P35A1</b>	GGAACACCACATTCAGTGAGAGTGC	CGTCTGCAACTTGTGAGGAAGGAT	HE798148
<b>P35A2</b>	GGAACACCACATTCAGTGAGAGTGA	CAACCTGTGAGGAAGACACCCA	HG917950
<b>P35B1</b>	TGCCAAACGCCAAGCTTTATTTTG	GCTGTTGAGTGCTTTTGGTCTTTGG	HG917951
<b>P40B1</b>	CCCTTCTACATCCGAGAAATAGTGAAAC	GTTGGTTTTCACTATAAACACCTTTTCTT	HE798149
<b>P40B2</b>	CCGTTCTACATACGAGAAATAGTGGAGA	TCAGAGTCACAGCTTTCCTGG	HG917952
<b>P40C</b>	TTAAAGACAACGGAAAGGAGGAGC	CCTCCCCTAACCACATTTTTCC	AJ548830
<b>EBI3</b>	ACATCGCCACCTACAGTATGAAAGG	GGGTCCGGCTTACAATGT	AJ620467
<b>Chemokines</b>			
<b>CXCL_F1a</b>	TCATCTACATGATACAAGGG A	TCAAGTGCTAGGGGTGGTATG	DQ191448
<b>CXCL_F1b</b>	CGTCATCTGCATGATACAAGGGAGAAC	TCCGGATTTCAAGTGTACAATGAG	NM_001124562
<b>CXCL_F1c</b>	GCTGCCTTTGCCCAAAGTG	CCTGCATTCTTTGGTTGATC	HE578132
<b>CXCL_F2</b>	TCTGTGAGCCAACGATGTTTGTG	GCACTCCATTCTTAAGGCTAACAATGAA	BX859166
<b>CXCL_F4</b>	TGCCTACTCCTTACAGTGGGTA	TCCGCTGGTTTTCTTCCA	HE578134
<b>CXCL_F5</b>	TGGGAGTTTGTATACAGCTCAAC	CTTCTTCCAACGGGGTTTCAAGG	HE578133
<b>CXCL8</b>	AGAGACACTGAGATCATTGCCAC	CCCTCTTCACTTGTGTTGGC	AJ310565
<b>CXCL11_L1</b>	TCTGCTCCTGGCCAATGTTGAAG	TCCCTTCTCCGTTCTTCAAGT	AJ417078
<b>CXCL12a</b>	GACATACGTTTCTTTCGCACA	GCTTCTTCACTTGTGAGGGCA	HE578135
<b>CXCL12b</b>	CATGGATGTACCTCCCTCTACG	CTTCTTCACTTGTGTTAATGGCA	HE578136
<b>CXCL13</b>	CAGGTTTCAACAAGTTGGAGA	TCCCTTGTGTTACTTTGTAAC	HE578137
<b>CXCL14</b>	TCCCTACAAGCTGAAGCCTAC	TGCTTATCCTTCCAGATCCG	HE578138
<b>IFN<math>\gamma</math></b>			
<b>IFN<math>\gamma</math>1</b>	CAAACCTGAAAGTCCACTATAAGATCTCCA	TCCTGAATTTCCCTTGCATATTT	AJ616215
<b>IFN<math>\gamma</math>2</b>	CAAACCTGAAAGTCCACTATAAGATCTCCA	GGTCCAGCCTCTCCCTCAC	FM864345

**Table S1. Continued.**

Gene	Forward (5' to 3')	Reverse (5' to 3')	Acc. No.
<b>IL-17 family</b>			
<b>IL-17A/F1a</b>	CAAACGTACACTTTTTGATGGTGCTG	GGGACTCATCATAGGTGGTGTGGT	KJ921977
<b>IL-17A/F1b</b>	AGGACCACAACACAGCAACAGA	CATGTAGGGATTGAGCCTTCAACC	KJ921978
<b>IL-17A/F2a</b>	CACCCTGGACCTGGAAAAGCAC	GGCCACAGACAGGAAGGAGG	AJ580842
<b>IL-17A/F2b</b>	CGCACCTTCTCCACTCAAG	AGTCAATTCTTCTGGCTTTGTGGG	KJ921979
<b>IL-17A/F3</b>	GCAACGTTTCACTCTGAAGAGACAG	CTGGGGAAGTAGACAAAGGGCATC	KJ921980
<b>IL-17N</b>	ACCCACAGCACAGCAGAGACAGA	GATGTCATTGTGGCAAACGACTAAC	KJ921981
<b>IL-17C1</b>	CTGGCGGTACAGCATCGATA	GAGTTATATCCATAATCTTCGATTCCGGC	FM955455
<b>IL-17C2</b>	CTGGCGGTACAGCATCGATA	CAGAGTTATATGCATGATGTTGGGC	FM955456
<b>IL-17D</b>	GAAGAAATCCTCGAGCAGATGTTTG	GGGTCGTGGGAGATCCTGTATG	AJ580843
<b><math>\gamma</math>-chain cytokines</b>			
<b>IL-2</b>	TGATGTAGAGGATAGTTGCATTGTTGC	GAAGTGTCCGTTGTGCTGTTCTC	AM422779
<b>IL-4/13A</b>	ACCACCACAAAGTGCAAGGAGTTCT	CACCTGGTCTTGGCTCTTACAAC	FN820501
<b>IL-4/13B1</b>	GAGATTCATCTACTGCAGAGGATCATGA	GCAGTTGGAAGGGTGAAGCTTATTGTA	HG794522
<b>IL-4/13B2</b>	GAGACTCATCTATTGCGTATGATCATCG	TGCAGTTGGTTGGATGAAACTTATTGTA	HG794523
<b>IL-15</b>	TGGAATTGCTTCATAATATTGAGCTGCC	TGGTAGTTATCTGTGACCGACATGTCCTC	AJ628345
<b>IL-21</b>	AAAGTTATCAAAAACCTCAACAACCGAA	CCAGTCTACTGATGGCCTTTGAAG	FM883702
<b>Anti-inflammatory cytokines</b>			
<b>IL-10A</b>	GGATTCTACACCACTTGAAGAGCCC	GTCGTTGTTGTTCTGTGTTCTGTTGT	AB118099
<b>IL-10B</b>	GGGATTCTAGACCACATCAAGAGTCC	GATGGGAGATTTAAAGTTGTGTGTTCC	FR691804
<b>TGF<math>\beta</math>1A</b>	CTCACATTTTACTGATGTCACCTTCTGT	GGACAACGCTCCACCTTGTG	OMY7836
<b>TGF<math>\beta</math>1B</b>	CATGTCCATCCCCAGAACT	GGACAACGTTCCACCTTGTGTT	FN822750
<b>Other cytokines</b>			
<b>IL-20</b>	CAAGAACCTGAGGCAATGTCACTG	TCTCCTATAGCCTTACTGCTGCCG	FN386780
<b>IL-22</b>	GAAGGAACACGGCTGTGCTATTAAC	GATCTAGGCGTGCACACAGAAGTC	AM748538
<b>IL-34</b>	AGGCAGAAGACGTAACATGAAACACA	TCCACCCTCGCCCTCAGCTT	FN820429
<b>SOCS genes</b>			
<b>CISHa1</b>	CATTCTACCTTGATACCTCAGGCTGGT	CCTGCTGCACCTTCTCCTCC	AM903340
<b>CISHa2</b>	TCTTCTACCTTGATACCTCAGGCTGGT	CCTTGCCCTTCTGTACCTTCTTGT	HG003693
<b>CISHb1</b>	GAATGATGGTGAGAGAGGAGATTTGTGT	GACCCCCAGTACCAACCTGAGTT	FR873795
<b>CISHb2</b>	GATCATGGTGAGAGAGGAGGGTCA	GACCCCCAGTACCAACCTGAGTT	HG003694
<b>SOCS1A</b>	GCTAGGAGTGGAGGAGGCTCACA	AGAGGCAGGGGAAAGAGTGCTC	AM748721
<b>SOCS1B</b>	AGCTTCTCATCAGGGACAGCA	GCCTATGGGATGACTCCACGTAGAA	KY387584
<b>SOCS2A1</b>	CAAAAACAAGACGCGTGGGAGAA	CGTCGGAATCTACAACCCGTGACA	AM748722
<b>SOCS2A2</b>	CCACTCGAAATAGGACGATTCATTT	TCATCGGAGTCTACAACCTGCGACT	KY387585
<b>SOCS2B</b>	CTCACCTTCAAGGAGTCAGGCTGGTA	ACATGCCAGTGGCGTGCTCTATAC	FR874096
<b>SOCS3A</b>	CACAGAGAAACCGTTAAAAGGACTATCC	AAGGGGCTGCTGCTCATGAC	AM748723
<b>SOCS3B1</b>	CACATGCACACTACCAACAGGTGCTGT	GGGGCATGTGTGTGCAGCA	KY387586
<b>SOCS3B2</b>	CACGCACACTACCAACAGGTGCTGT	GGGCGTGTGCGTGCAGTG	KY387587
<b>SOCS4</b>	CGTTGTCTCTCTAAAACCTATTTTTAAAAGCACAGAG	AGGTGGAACGCGCTCCTTG	KY387588
<b>SOCS5A1</b>	GTGTTTATAAAAAGACAAAAGGGTCTTGGTCTC	GACGTCGTCCCCAGTAACCAT	KY387589
<b>SOCS5A2</b>	CTGGTTTTAAAAGACAAAAGGGGCTTG	TCTCATTGTCTTTTCTGTCTGTGGTTC	KY387590
<b>SOCS5B1</b>	GCAAGGAATACTGTTTTTTTTAAGCCTCAAG	GACGTATGGCCTTTTTTCTTCTCTCTC	AM903341
<b>SOCS5B2</b>	GAACAACGAAGTTTTTATGCCCAAGT	CAGCTTGCTCATGGGACGTCTG	KY387591
<b>SOCS6A1</b>	GTTGAAAAACATACTGTAGAAAGGGATGACA	TCCTCATGATTGAAGTCGTTGCG	AM903342
<b>SOCS6A2</b>	GTTTTTAACAGAGGATGACAGGGATGACT	CTTCTCCTCGTGATCAAATCGTTACA	KY387592
<b>SOCS6B1</b>	TGCAGATGTTTCATCTAACGAAAGCAAAG	TTGGCAGGGGCGGCTACTCT	KY387593
<b>SOCS6B2</b>	GCAGATGGTAATCTAACGAAGGGATGAGT 70	CCCTCCTTCTCCTTGCCCTTGT	KY387594

**Table S1. Continued.**

Gene	Forward (5' to 3')	Reverse (5' to 3')	Acc. No.
<b>SOCS genes</b>			
<b>SOCS7A1</b>	GGACCAGGACACAGGGAGCAAAC	GAAGGCAGGACCGAAGGCCAC	AM903343
<b>SOCS7A2</b>	CAGACTTGGACACAGGGAGCAAACA	GAAGGCAGGACCGAAGGCCAA	KY387595
<b>SOCS7B1</b>	CGCTGCCCAAACCACTGATCA	CCAGTTACGTCTGAGACTCCGCCTCTT	KY387596
<b>SOCS7B2</b>	CGCTGCCCAAACCACTGATCA	CAGTTACGTCTGAGACTCGGCCACTTG	KY387597
<b>APPs</b>			
<b>SAA</b>	GGTGAAGCTGCTCAAGGTGCTAAAG	GCCATTACTGATGACTGTTGCTGC	AM422447
<b>SAP1</b>	GCTGTTATGGTGACCTTCAAGATCTCTC	GCGTTTGTACAACAACAAATCATTGTC	X99385
<b>SAP2</b>	GGTTGTTATGCTGAACATCAAGATCTCTC	CCACCCTTTGATTGCATACACAGATT	EZ763346
<b>AMPs</b>			
<b>CATH1</b>	ACCAGCTCCAAGTCAAGACTTTGAA	TGTCGGAATCTTCTGCTGCAA	AY594646
<b>CATH2</b>	ACATGGAGGCAGAAGTTCAGAAGA	GAGCCAAACCCAGGACGAGA	AY542963
<b>Hepcidin</b>	GCTGTTCCCTTCTCCGAGGTGC	GTGACAGCAGTTGCAGCACCA	CA369786
<b><math>\beta</math>-defensin 1</b>	GGTTTTCTATTGCTTAATGTTGTGG	GACACACAGTTAAGTCATGG	AM282655
<b><math>\beta</math>-defensin 2</b>	GCTGACAGCAGTGCAAGCTGATGACAC	GCAAAGCACAGCATCTTAATCTGC	FM212656
<b><math>\beta</math>-defensin 3</b>	GCTTGTGGAATACAAGAGTCATCTGC	GCATACATTGGCCATGTACATCC	FM212657
<b><math>\beta</math>-defensin 4</b>	GCAACTCTTCTAAAGAACAGT	CGTGGGCGACACAGCATACAAATCC	FM212658
<b>LEAP2A</b>	GGTTCCTGGTGTCTGCTGCT	AGTGGCCACCCCTGCAAAT	AY362186
<b>LEAP2B</b>	TGCACGGTCAAACCCACAGCA	AGGAATGAACTGCCCCACTGA	AY362187
<b>Complement genes</b>			
<b>Bf-1</b>	ACAATTGTATCGAAATGGCTGTTACTG	CCACCGGAGTCACCTTTGC	AF089861
<b>Bf-2</b>	GGCGAATTGTATTGAATTGGCTACTA	CCACCGGAGTCACCTTTGC	AF089860
<b>C1r</b>	CCTCCCTGAGTGCCACATAATTG	TTGTCTCTCCATGTTCTATCTGCTGC	AJ519930
<b>C1 inhibitor</b>	AACAGTTGGGTTGCAAAGCAGAC	GGAACATCGCCACCTGTGC	AJ519929
<b>C3-3</b>	TTGGCTGTGGATTGAGGAGAGTCT	GGGTTCCAGACACACAGATTCCATG	U61753
<b>C3-4</b>	GGATGACCGATTGCAAAGTTTGG	GGGTTCCAGACACACAGATTCCATG	AF271080
<b>C4</b>	ACAGACGACCTGGACAAGCTAAAGA	CATCCCCTGAACACAATGTTGAAAC	AJ544262
<b>C5</b>	GAGGAAGATCAGAGAGGGCATCAC	CTTTGACGTATTTGTCCACCTGTCC	AF349001
<b>C6</b>	CATCTCCATTTTCAAAGGCAAAATG	GAGCACACCTTCTGATGCACA	AJ622903
<b>C7-1</b>	CTTCCATACCAATGCCAGCCTTC	ATCCGACCAATCACAATCACTGTC	AJ566190
<b>C7-2</b>	AAATGCCTTATGAGTGCACGTCTTC	TTGTCCGTCAACAAGTCTCCCA	AJ622902
<b>C8<math>\alpha</math></b>	GAGATGTATGGCAGCTCCAGTTTGA	CAAATATGGGCAGGGTCTCAAATC	NM_001124624
<b>C8<math>\beta</math></b>	GGCTGTTCTCAAAGGCACCA	GCCTCCGCTGCTAGGTGC	NM_001124607
<b>C8<math>\gamma</math></b>	AGCACTACCACACGCCTTAACC	GGTGAGCTGTCCCTGTTTCTG	AJ622904
<b>C9</b>	GCGCTGGTGGATAAGGTGATAACTC	GAATGGTTCTGGCTCACTGCT	NM_001124426
<b>CR1 like</b>	CCTACTCCACATGCAAAGATGGTG	CCTATAACACAACAAAACCAAGTCCACC	NM_001124404
<b>C3aR</b>	ATTTAAGAAAGGAAAGCTCAAAGATGG	GTCAGCTATGGAGAAGGGTATGGA	AJ616902
<b>C5aR</b>	CCATACGCCTGAAATCTAAGTCACTC	ACCCAGCAGGAAGACCAAGCC	NM_001124467
<b>Arginase genes</b>			
<b>Arginase 1a</b>	CAGAGGTGGATCGCCTTGAATA	GCAGACAGCATCCCTGTCTGACA	KX998965
<b>Arginase 1b</b>	AGGTGGATCGCCTTGAATCG	GCAGACAGCAGCCCTGTCTGACT	KX998966
<b>Arginase 2a</b>	TCCAGAGAGTCATGGAAGTCACTTTCC	CCATCACTGACAACAACCCTGTGTT	KX998967
<b>Arginase 2b</b>	TCCAGAGAGTCATGGAAGTCTCTTTCCG	CATCACCGACAACAACCCTGTGTT	KX998968
<b>TLR5</b>			
<b>TLR5S</b>	GCGCATCACTTCAGGGGGAT	GCATTTCACTTGCAGGTAGA	AB062504
<b>TLR5M</b>	GCGCTCATACTTCAGGGGGAT	GCATTTCACTTGCAGGTATT	AB091105

**Table S2. Fold change of transcript expression of immune-relevant genes in the spleen, liver, gills and skin of fish ip injected with YRF.** The fold change was calculated as the average expression level of YRF injected fish divided by that of time matched controls (N=7). Asterisks indicate significant differences (One-way-ANOVA with Bonferroni correction) between YRF injected fish and controls, as follows; \* =  $p < 0.05$ , \*\* =  $p < 0.01$  and \*\*\* =  $p < 0.001$ .

Tissue Time	Spleen		Liver		Gills		Skin	
	6h	24h	6h	24h	6h	24h	6h	24h
<b>IL-1 Family</b>								
<b>IL-1β1</b>	138.1***	2.3	166.2***	10.1***	47.7***	1.9	468.9***	3.5
<b>IL-1β2</b>	3702.6***	4.5*	186.4***	2.7	259.7***	3.3	166.4***	3.1
<b>IL-1β3</b>	1.3	0.6	1.8	0.8	3.6***	0.7	4.1***	1.1
<b>nIL-1Fm</b>	5.7***	1.1	4.3***	2.0	2.1	1.0	2.6*	1.2
<b>IL-18</b>	2.7***	1.2	1.5	4.9***	1.2	1.2	1.2	1.5
<b>TNF family</b>								
<b>TNFα1</b>	12.8***	0.9	12.6***	1.0	10.4***	0.7	24.3***	1.0
<b>TNFα2</b>	24.4***	2.9	272.5***	13.8	20.8***	1.4	206.6****	3.1
<b>TNFα3</b>	1.6	0.4	73.7***	1.5	2.4	1.7	47.7*	0.5
<b>IL-6 family</b>								
<b>IL-6</b>	999.6***	6.2**	463.5***	7.8**	68.0***	3.4	240.1***	7.7***
<b>IL-11</b>	92.8***	9.7***	1766.1***	22.3***	34.0***	4.3*	112.8***	9.7***
<b>M17</b>	14.7***	6.0***	6.3***	2.2	7.3***	5.4***	4.3**	3.1
<b>CNTF</b>	0.5	0.9	0.8	0.6	1.0	0.6	1.0	0.6
<b>IL-12 family</b>								
<b>P19</b>	10.0***	2.4	1391.6****	64.2***	4.0	0.4	68.7***	1.4
<b>P35A1</b>	1.1	0.9	30.9***	2.4*	1.4	1.2	40.7***	3.9
<b>P35A2</b>	51.9***	2.1	68.3***	0.2	20.8***	1.6	270.7***	1.1
<b>P35B1</b>	2.4	0.3	9.0	0.1	1.6	0.8	13.9	0.9
<b>P28A</b>	2.3	0.9	ND	ND	13.4	1.5	7.5	0.4
<b>P28B</b>	5.9	2.0	ND	ND	ND	ND	ND	ND
<b>P40B1</b>	ND*	ND	ND*	ND	2.1	0.8	3.9	0.8
<b>P40B2</b>	3.1	2.4	2.0	4.4	1.6	0.8	2.0	1.3
<b>P40C</b>	1.2	0.5	2.4	1.3	2.1	0.7	1.5	0.8
<b>EBI3</b>	5.0**	0.3	1.3	0.6	1.2	1.0	0.7	0.7
<b>Chemokines</b>								
<b>CXCL F1a</b>	22.2	103.0	1.5	1.0	0.6	0.7	1.6	0.8
<b>CXCL F1b</b>	0.8	12.2	0.7	2.7	1.5	2.6	1.2	1.6
<b>CXCL F1c</b>	1.1	0.4	0.8	1.2	1.1	0.2***	0.7	0.4
<b>CXCL F2</b>	1.1	0.3	2.0	0.7	1.1	0.5	0.7	0.6
<b>CXCL F4</b>	22.9***	8.0**	38.9***	32.3***	43.4***	10.7***	47.6***	18.9***
<b>CXCL F5</b>	7.4***	2.2	6.5***	1.1	1.6	1.2	4.6***	1.9
<b>CXCL8</b>	344.1***	14.0***	82.9***	13.3***	11.2***	1.5	120.8***	3.0
<b>CXCL11 L1</b>	1.2	0.5	1.2	1.4	0.7	0.4	1.6	0.3
<b>CXCL12a</b>	0.9	0.4	1.8	0.4	0.8	1.6	0.9	1.5
<b>CXCL12b</b>	0.8	0.4	0.7	1.2	0.8	1.7	0.5	0.8
<b>CXCL13</b>	2.7	15.7***	0.7	99.7***	0.8	19.0***	0.5	12.6**
<b>CXCL14</b>	1.6	0.7	0.8	0.6	1.0	1.5	0.9	1.8
<b>IFNγ</b>								
<b>IFNγ1</b>	1.3	0.7	1.0	3.8	0.3	0.9	0.2	0.4
<b>IFNγ2</b>	1.0	1.9	1.3	1.3	0.4	0.8	0.5	0.5
<b>IL-17 family</b>								
<b>IL-17A/F1a</b>	364.5***	233.0***	17.7***	29.3***	0.5	0.3	0.5	0.9
<b>IL-17A/F1b</b>	ND	17.5	ND	ND	1.2	0.6	0.3	0.6
<b>IL-17A/F2a</b>	0.6	0.7	1.9	11.4***	0.4	0.5	0.2	0.4
<b>IL-17A/F2b</b>	0.8	1.1	2.0	2.2	0.9	1.0	0.5	0.9
<b>IL-17A/F3</b>	0.6	1.2	1.6	0.6	0.7	1.1	0.4	0.7
<b>IL-17N</b>	1.9	1.6	1.1	1.2	2.2	1.0	0.9	0.8
<b>IL-17C1</b>	1.9	4.1	4967.4***	718.4***	7.4***	0.5	21.0***	0.8
<b>IL-17C2</b>	3.6	4.1	130.5***	11.7***	3.4	0.6	7.8***	4.2
<b>IL-17D</b>	0.9	0.4	0.4	0.6	1.0	0.8	0.6	0.4

Table S2. Continued.

Tissue Time	Spleen		Liver		Gills		Skin	
	6h	24h	6h	24h	6h	24h	6h	24h
<b><math>\gamma</math>-chain cytokines</b>								
IL-2	1.1	0.7	1.2	1.1	1.0	0.6	1.0	1.1
IL-4/13A	0.9	1.1	0.7	4.6**	0.9	1.5	1.1	0.8
IL-4/13B1	0.1	3.5	0.4	13.1***	0.3	2.2	0.7	0.9
IL-4/13B2	1.2	1.6	0.8	1.1	1.2	1.0	1.2	0.6
IL-15	1.0	0.6	2.7***	8.4***	2.4*	0.7	1.2	1.0
IL-21	1.6	0.9	1.7	1.4	1.5	0.8	0.9	0.9
<b>Anti-inflammatory cytokines</b>								
IL-10A	4.3	7.9	5.5	0.4	0.6	1.8	1.7	1.4
IL-10B	0.9	1.1	5.9	0.7	1.5	1.0	1.4	1.4
TGFB1A	1.6	1.4	1.6	3.3***	1.1	1.4	0.9	1.2
TGFB1B	1.7	1.3	22.8***	0.4	2.0	1.4	1.6	0.8
<b>Other cytokines</b>								
IL-20	6.5	1.2	2.0	2.1	1.0	1.8	1.0	1.1
IL-22	8.9**	3.3	112.1***	12.5***	6.5*	0.6	68.6***	1.6
IL-34	2.9***	1.0	18.7***	0.7	1.7	1.1	3.4***	1.5
<b>SOCS genes</b>								
CISHa1	1.4	3.3	8.0***	0.2***	2.4	0.8	4.4**	1.0
CISHa2	5.4*	2.2	19.6***	0.2**	6.6**	1.0	8.3***	0.9
CISHb1	1.9	2.5	1.4	0.8	1.2	0.6	1.2	0.8
CISHb2	2.8	2.1	0.9	1.0	1.4	1.0	1.8	1.0
SOCS1A	3.6**	1.7	22.2***	1.4	3.0*	1.2	3.1**	1.3
SOCS1B	1.2	1.4	0.5	2.8	0.8	1.2	0.9	1.3
SOCS2A1	2.4	3.7	1.1	0.3	1.3	1.8	1.0	1.3
SOCS2A2	0.6	0.5	3.3	0.2	1.4	1.2	0.7	0.8
SOCS2B	1.0	0.2	2.5	0.4	1.2	0.3	1.1	0.3
SOCS3A	11.0***	6.0**	38.6***	3.8	5.7***	2.4	3.6*	2.6
SOCS3B1	1.5	1.5	1.7	3.8	1.0	2.5	2.2	3.4
SOCS3B2	7.0*	6.2	2.6	16.2**	2.5	4.7	2.5	6.9
SOCS4	1.3	0.3	0.3	0.5	1.8	1.7	0.8	1.3
SOCS5A1	1.2	1.5	0.1	2.3	1.2	1.8	0.8	2.4
SOCS5A2	0.6	1.3	0.1	0.9	0.9	1.6	1.0	1.6
SOCS5B1	1.0	1.0	0.4	1.1	1.0	1.4	1.4	1.3
SOCS5B2	1.4	1.1	0.7	1.4	2.8	1.7	2.8	2.4
SOCS6A1	0.7	1.0	0.4*	1.3	0.7	1.3	0.6	1.1
SOCS6A2	1.0	1.1	0.4***	1.3	1.2	1.0	0.6	1.0
SOCS6B1	1.0	1.0	0.4	0.8	1.2	1.4	0.5	0.9
SOCS6B2	0.8	1.1	0.3**	1.5	1.0	1.3	0.9	1.1
SOCS7A1	1.4	1.0	0.6	1.2	1.7	1.3	1.6	1.3
SOCS7A2	0.9	0.6	1.5	0.9	1.6	1.0	0.9	1.2
SOCS7B1	3.0	2.0	1.5	1.6	1.8	2.5	0.7	1.4
SOCS7B2	1.6	1.4	2.2***	1.7*	1.1	1.4	0.7	1.2
<b>APPs</b>								
SAA	31.1***	15.9***	225.8***	5450.6***	2.8	26.2***	8.7**	111.8***
SAP1	3.9*	3.2	1.4	0.6	0.4	3.5	0.6	0.8
SAP2	1.8	1.8	0.9	1.7	1.1	0.9	0.9	2.1

Table S2. Continued.

Tissue Time	Spleen		Liver		Gills		Skin	
	6h	24h	6h	24h	6h	24h	6h	24h
<b>AMPs</b>								
<b>CATH1</b>	39.0***	5.7	5.2	45.0***	4.3	10.4	29.0***	60.6***
<b>CATH2</b>	69.0***	26.6***	745.0***	14687.1****	19.9***	137.5***	10.7***	128.5***
<b>Hepcidin</b>	278.6***	75.4***	11.9***	235.2***	65.4***	309.5***	66.0***	1891.5***
<b>β-defensin 1</b>	ND	0.0	ND	ND	1.8	0.3	0.9	1.2
<b>β-defensin 2</b>	3.1	5.4	1.5	0.8	0.8	0.7	0.3	1.2
<b>β-defensin 3</b>	4.4	1.3	0.9	1.8	7.6	3.3	3.0	1.3
<b>β-defensin 4</b>	0.9	3.1**	0.5	2.7*	0.9	1.5	0.7	1.1
<b>LEAP2A</b>	0.6	1.3	2.6	0.04***	0.5	0.8	1.3	1.1
<b>LEAP2B</b>	0.8	0.2	1.2	0.05***	1.3	0.6	0.7	0.6
<b>Complement genes</b>								
<b>Bf-1</b>	13.6***	4.3	1.6	1.8	0.8	1.3	0.7	0.8
<b>Bf-2</b>	1.9	1.9	1.7	1.4	1.4	0.9	0.2	0.5
<b>C1r</b>	4.0**	1.0	1.1	0.7	1.4	1.3	0.8	0.8
<b>C1 inhibitor</b>	2.8	1.1	1.0	0.7	0.8	1.2	0.8	1.3
<b>C4</b>	0.8	0.9	1.2	0.9	1.2	1.9	0.6	3.2
<b>C5</b>	7.8	1.4	1.3	1.3	1.1	0.7	0.2	1.2
<b>C6</b>	1.3	1.3	1.1	1.4	1.5	2.2	1.7	6.6***
<b>C7-1</b>	23.1***	19.5***	25.5***	23.2***	11.1***	12.4***	8.7***	12.5***
<b>C7-2</b>	1.2	0.7	0.9	0.8	0.9	1.2	1.3	0.7
<b>C8α</b>	5.4	1.5	0.9	0.8	1.3	0.3	0.6	0.7
<b>C8β</b>	1.9	1.0	1.3	0.6	1.2	0.9	0.7	0.8
<b>C8γ</b>	0.9	0.5	1.2	0.6	1.2	1.4	1.1	0.8
<b>C9</b>	1.0	1.0	1.5	1.8	0.6	0.9	0.6	0.5
<b>C3-3</b>	4.0	3.7*	1.0	1.3	0.6	2.0	0.4	1.4
<b>C3-4</b>	6.2*	2.0	1.1	0.5	1.8	0.9	0.9	0.6
<b>C3aR</b>	1.8	1.7	1.5	1.5	2.5	1.1	1.9	1.6
<b>C5aR</b>	4.6**	2.5	2.2	7.0***	2.9	5.3**	3.6	2.9
<b>CR1 like</b>	3.8***	3.1***	1.7	4.3***	2.7***	1.5	2.6***	2.2**
<b>Arginase genes</b>								
<b>Arginase 1a</b>	31.2***	11.5***	0.8	0.4	1.2	0.9	3.3	2.0
<b>Arginase 1b</b>	2.2	1.3	0.8	0.7	0.5	1.0	3.2	1.0
<b>Arginase 2a</b>	8.2***	3.1**	6.0***	6.5***	1.5	1.5	4.5***	3.5
<b>Arginase 2b</b>	16.3***	5.0**	15.8***	18.9***	3.6	2.6	5.8	3.6
<b>TLRS</b>								
<b>TLR5S</b>	25.2***	1.0	254.5****	23.4***	59.4***		274.4****	476.6***
<b>TLR5M</b>	1.0	0.2	2.6	0.0	0.5	0.9	0.7	1.6

Note

ND, not determined due to low expression levels.

ND\*, detectable in YRF injected fish but not detectable in time-matched controls.