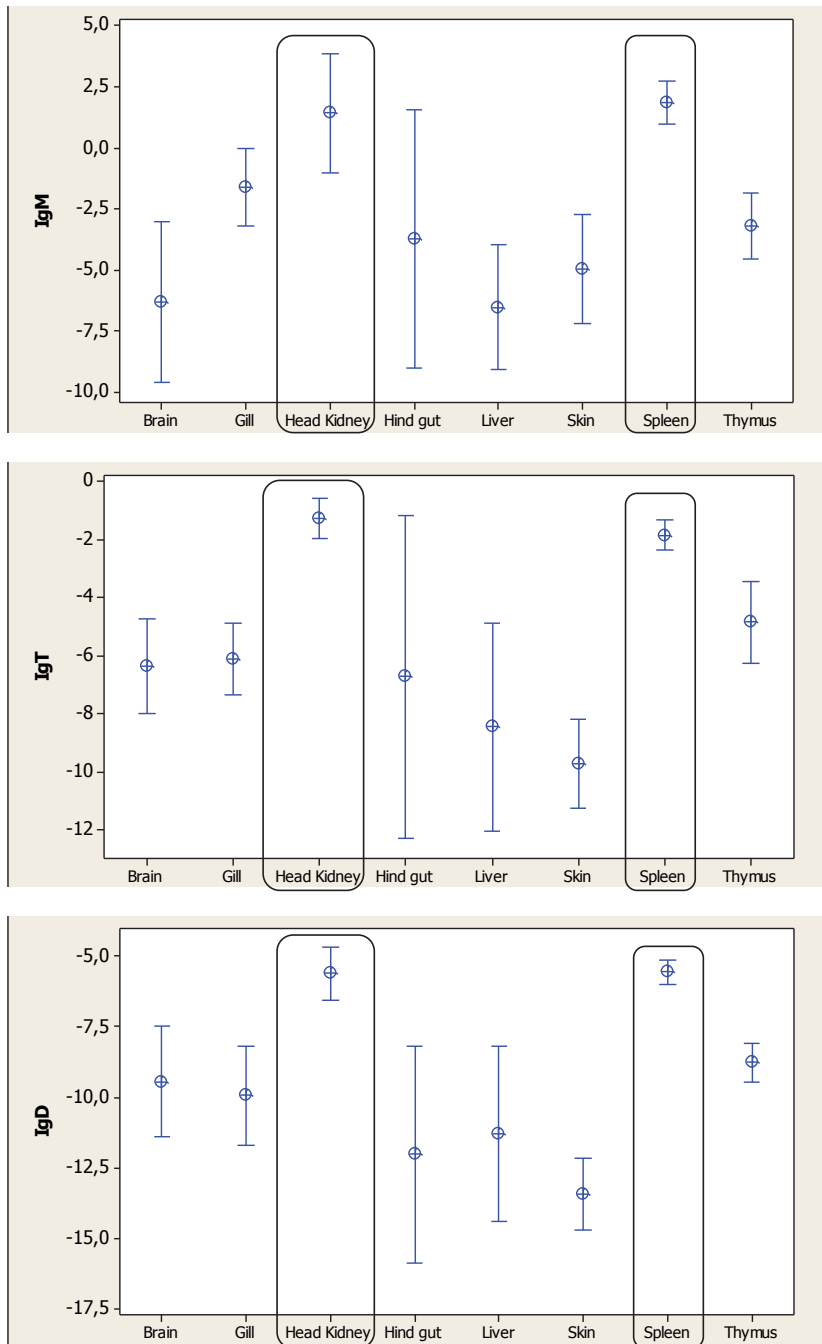


Appendix



Appendix 1. Interval plot of IgM, IgT, and IgD expression in different tissues. 95% confidence interval for the mean (based on $-\Delta\text{Ct}$ values normalized against EF1A) is shown. Tissues with highest expression (spleen and head kindey) are boxed. Note also individual variation.

<u>atagcctcacatagtgacagctctggaac</u> ATG ACTCCTCTACTCTTCTTCGCCTTCCTCCTC	61
M T P L L F F A F L L	11
CTCTCTCATCTGCCAGGCTCTCTCTGTAGGGTGACCACCGTGGGTGACCTGGCGGTCTCTG	121
<u>L S H L P G</u> S L C R V T T V G D L A V L	31
GAAGGTCCGGTCGGTGATGATCCCCTGCCATTACGGGCCGAATACGCCAGTTATGTGAAG	181
E G R S V M I P C H Y G P Q Y A S Y V K	51
TACTGGTGCCATGGCAGTGTAAGGACCTCTGTACCAGTCTGGTTTCGCAGCGATGCCCCA	241
Y W C H G S V K D L C T S L V R S D A P	71
CGGGGGCAGGCTGCACCTGGAGAAGACAAGGTGGCCATGTTTGTATGACCCGGTACACGAG	301
R G Q A A P G E D K V A M F D D P V Q Q	91
GTGTTACAGTAACAATGACAGAGCTGCAGAAGGAGACTCTGGATGGTACTGGTGTGGT	361
V F T V T M T E L Q K E D S G W Y W C G	111
GTGGAGGTGGGAGGAGTCTGGAGTGCTGATGTACAGCCTCCCTTACATCAATGTTATC	421
V E V G G V W S A D V T A S L H I N V I	131
CAAGGTATGTCTGTGGTGAACAGCATGGTGAGCGGAGAGGAAGGGACCAGCGTTACTGTC	481
Q G M S V V N S M V S G E E G T S V T V	151
CAGTGTCTTACAGCCAGGGATACAGGCAGCATGAGAAGCGTTGGTGTCTAGTGGTGAC	541
Q C L Y S Q G Y R Q H E K R W C R S G D	171
TGGAGCTCCTGTCTAGTGACAGATGGTGAAGGGCGGTATGAAGACCAGGCAGTAGAGATC	601
W S S C L V T D G E G R Y E D Q A V E I	191
AGAGATGACCTGACCAAGGCTTTCACTGTACCCTGAAGGGACTGGCCCGGAGAGATACA	661
R D D L T K A F T V T L K G L A R R D T	211
<u>GGTGGTACTGGTGTGCTGCAGGACAACAACAAGTGGTGTCTACATCCTGGTCACTCCT</u>	721
G W Y W C A A G Q Q Q V A V Y I L V T P	231
CCATCCACAACAGCACCTGCTCCTACAGTGACGTCCCACCAGAAGAAAGCCCCAGTCT	781
P S T T A P A P T V T S P P E E S P Q S	251
GTTCTGTGTCTCCGTCTGTGTCTCCGTCTGTGTCTCCGTCTGTGTCTCCTTCCAGA	841
V P V S P S V S P S V S P S V S P L P R	271
CACGTAGCTAAAGGAGCAGACCACCACAGGCCACTATGGGAGTTTCTCTGATGGTGTGT	901
H V A K G A D H H R P L W E F P L M V C	291
GGAGTTCTGTTTCATCTTGTATGGTTCTGGTGTCTGCCATGGAAGATACTGGACCAATAC	961
G V L F I L M V L V L L P W K I L D Q Y	311
<u>AACAAAAACACAGGACAAGACAGGCAGAGCTGGAAGCCAGACTCAGT</u> <u>GAC</u> CCCCCAGGT	1021
N K T H R T R Q A E L E A R L S D P P G	331
GACGACTGGCAGAACACCTCTGTCTGTCTTCTCAACTCCGCTCACAGAAGGTGTACGGT	1081
D D W Q N T S V V F L N S A S Q K V Y G	351
TTC TGA cccgcgccctccagtcacctctgacctctaacgtccaccctctatctctgg	1141
F *	352
ccacctttcatcagagctgggcgccactaaatattccatccatccaacgatatttttcagt	1201
accatggtgatgatccatcattaccacaaatggtattgtgatcattacctgacatgta	1261
aataatgttaccatgtttactgaaatcagtggtatataatctactgcaactatttgttt	1321
gtattacatatttacatttctatttaaaaaaaaaaacatttt	1361

Appendix 2A. Nucleotide and deduced amino acid sequences of Salsal pIgR (GQ892057). The start and stop codons are in red font. The predicted regions shown are: Leader (L) (underlined), domain 1 (shaded light gray), domain 2 (white font in dark gray background), transmembrane (highlighted red). Each potential *O*-glycosylation site is shown in green. Translated nucleotides are in upper case, and 5' and 3' untranslated regions are in lower case letters. The starting position of each exon is double underlined.

actgtgagcagacacactgatgcggttgaccagctcaccatctacatattactttga 60
 catgctgtacacgtctatgtgactaaggagagagtagactaacattgggtattgacacaat 120
 tagaacaataagtagacac**ATG**GTTTCTCTTTATCTTCTCATCCTTGCTTTGTCCATGGA 180
 M V S L Y L L I L V F V H G 14
 CCATCAGGTTGCAACAGCCTGTGGACTGTGACAAAAGTACTGCAAAGAGTGGCGGAGCC 240
P S G C N S L W T V T K S T A K S G G A 34
 ATCACGATCCCCTGCCACTACCACCGCATGTTGAGAGACCATGCCAAATACTGGTGTAAAG 300
I T I P C H Y H R M F R D H A K Y W C K 54
 GGCGAACCTGGCCCTTATGTACTGTGATGGCAAGCACCGACCCAAGCGGGAACAGAGGA 360
G R T W P L C T V M A S T D P R R N R G 74
 GGCATGTCAATCACAGACATCCCAGAGGAGCTGGTCTTCACTGTGACCATGAAGAACCTA 420
G M S I T D I P E E L V F T V T M K N L 94
 CAGGAGACTGACACCAACAGGTACTGGTGTGCTCTGAAAGTGGGTGGGATAGGCAAGCCA 480
Q E T D T N R Y W C A L K V G G I G K P 114
 GATGTCAAAGTATCCGTGGACCTCACAGTCACCCAAGGCTCTCCTGATCTGTCTAGTGGTT 540
D V K V S V D L T V T Q G S P D L S V V 134
 GATGAGCTGGTATCTGGTGAGGAGGGGGGCGAGTGTCACTGTACAGTGTCTCTACAGTGAC 600
D E L V S G E E G G S V S V Q C L Y S D 154
 ACACTCAGAGGCAAAGAGAAGAAGTGGTGCAGGAGTGGGGACCGGCATTCTGTCTAGACA 660
T L R G K E K K W C R S G D R H S C Q T 174
 CAGACAGATACTAGCCAGAATGCATCTGTTGTGATCAGTGATGGCAAGAGGGGAGTGTTC 720
Q T D T S Q N A S V V I S D G K R G V F 194
 AATGTGACAATGAAACAAC TAGAGAAGAAAGATGCAGGCTGGTATTGGTGCTCTGTTGGA 780
N V T M K Q L E K K D A G W Y W C S V G 214
 GACCTACAGGCTGCAGTTCATATCAACGTCACTCAGAGATCCACAGCACACAGGAACACT 840
D L Q A A V H I N V T Q R S T A H R N T 234
 GCAGAAAGCAGTGACTACTCCAACGCTGCCCATGTTACTCCTCGCATTTCTCGACATTTGCA 900
A E A V T T P T L P M L H S A F S T F A 254
 GACTCCACAGCTACCTCTACCTCAAAGACAAACCCTCCGCCACAGTAGACTCTGCTCTG 960
D S T A T S T S K T N P S A T V D S A L 274
 ACAACATCCTCATATGGCTCTGCAACTCCCTATCCATCTGTCTCAGTTCATACATCACA 1020
T T S S Y G S A T P Y P S V S V P Y I T 294
 ATCCATTCTTCCACACTGACTTCTTCACTAACACAGTACAACCTACATGCTGTCTCTTCT 1080
I H S S T L T S S L T T V Q P T C C P S 314
 ACTGAATCTACAGCTACTTACAAGAAAATCAGCAACCTGCCCTGGCATGCTCTTATTCTC 1140
T E S T A T Y K K I S N L P W H A L I L 334
 ATTTGTTGGCCATGGTGTGTTGGTTATTGTTGTTATGGCTGCAGTTAACATCTATAGA 1200
I V L A M V L L V I V V M A A V N 354
 TATTCAGGAATAATATCAGACCTGTGGAAGGAGAGATGACTGAGCTGGTGATAAATCAA 1260
Y S R N N I R P V E G E M T E L V I N Q 374
 GATCAATAAatcaattaaaagagcctaaaaataaattgaaatcataaaaattactgtaa 1320
D Q * 376
 gtttgctttaatatcaccaaaataatcacatgtacagtaaattggattgtagcaataaacac 1380
 Aattagctagagaacagagaaaaaa 1406

Appendix 2B. Nucleotide and deduced amino acid sequences of Salsal pIgRL (HM452379). Each potential *O*-glycosylation site, located in the connecting region, is highlighted green. Description of predicted regions and color codes are as in Appendix 2A.

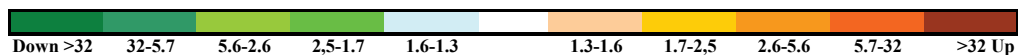
ctgacacacaccagtgaccattcaaacataaaagctaccagagaatttctctctcatcta 62
 atc**ATG**GCTCCATATCTCCTCAGTGTCTGCACATCCTTTTCTTCTCACTGGACTGTCA 122
M A P Y L L S V L H I L F F L T G L S 19
 GGTATTCACGGTGTGTCCACAGTGAGTCATGTGTCTGTAAGTGAAGGAGGCTCCATCACC 182
G I H G V S T V S H V S V S E G G S I T 39
 ATCCCATGTCTCTATCATCATGGCTCTGAAAAACATGTGAAATACTGGTGTAGTGGATAT 242
I P C L Y H H G S E K H V K Y W C S G Y 59
 TTTTTCATTTTGTCTACTCTAATACGTACTGACTCCCAGAGTGCATCTAATTGGTTG 302
F F H F C S T L I R T D S Q S A S N W L 79
 TCCATCGCTGATAATGTTACTACAAGAGTCTTCACTGTGACCATGAAGAATCTGCAACTA 362
S I A D N V T T R V F T V T M K N L Q L 99
 GGGGTCTCTGGATATTTCTGGGGTGTGTGGAGAAAGGTGACACCACACATCTATATCTG 422
G V S G Y F W G A V E K G D T T H L Y L 119
 TCAGTTCCACAGGTACTGCAGGACTCTATGTGGACCAACAACATGTGACTGGAGTTGAA 482
S V S T G T A G L Y V D Q Q H V T G V E 139
 GGACAGAGTGTCACTGTCAATTGTAACATATAGTAACTCTGGAGGTTTCTGCTGGTGCAGG 542
G Q S V T V N C N Y S N S G G F C W C R 159
 CTGGGTGGCTCTTGCATGGAGAGGAGTGTGGGAATTTAGATGGAGCATCAGTAGAAATA 602
L G G S C M E R S V G N L D G A S V E I 179
 AAGCGGATCTTGCCAATGGAAAAAAGTCATGATCGTAACAATGAGTCAACTAAAAATT 662
K R I L A N G K K V M I V T M S Q L K I 199
 AAGAACACTGGCTGGTACTGGTGTGCAGTTGGAGATCTACAGATACCTGTTTCATATCACT 722
K N T G W Y W C A V G D L Q I P V H I T 219
 GTTAGTCAACCAACCACAACACAGAGTAAACCACCACATCACAAACAGAACAATGGGGGA 782
V S Q P T T T Q S N T T I T N Q N N G G 239
 ACTGACGAAAGCGACAGGAAGGATCCAGAGTTTGTCTGGTAGTCTGGTCACTTCTCTG 842
T D E A K R Q E R I Q S L L V V L V I P L 259
 AGCCTGTGGTGGTGTGTGATAGTTGGTACCTTGGTCACTTGAAGATATTCAGAAAACAT 902
S L L V V L I V G T L V T I K I F R K H 279
 AAGGACAAGAAGGCAAAGGACCAACCACCAACACCCACAGTCTGCTGACTCTGAG 962
K D K K A K D Q P P N T P V Q S A D S E 299
 CAGAACATTACCTACAGCACTGTGAGTACACCAGAAGAACAGCACAACAGGACCCATTA 1022
Q N I T Y S T V S H T R R T A Q Q D P L 319
 CCTGATGATGCAGTGACATACAGCACCGTGGTACCAAGAACAAGACCCAACCAATGCA 1082
P D D A V T Y S T V V T K N K T Q P N A 339
 GCAAACAGATGTGGTCTACAGCACAGTGGCCGCACACCAAGA**TAG**caggaggcaaca 1142
A K P D V V Y S T V A A H Q R * 354
 gaacagactgattgaagtccctccaaattaaataggccttgttccattttactcccta 1202
 gctcttacctctttgggggttactggttactaatgcctgtctattacagtcttggttcttt 1262
 tccattgttcaggctctgctggcttaattatgccagtgattgtgtattctagacaaaggc 1322
 agattgagtgaatTTTTGATTTTCTGAAGTTAATGTACACACTGATGTGTATTTCAT 1382
 gcttcataatctgaataaaactactttaagaactt 1417

Appendix 3A. Nucleotide and deduced amino acid sequences of Salsal CLM1 (NM_001140948). Three putative ITIM motifs (positions 302-307, 324-329, 344-349) are shaded light blue. Description of predicted regions and color codes are as in Appendix 2A. No potential *O*-glycosylation residues were found.

agggggaccacaaacaaagtttccatttccataacctttaagtcatagatttcagataa	60
agattgagtttaagcagaaaag ATG ACTACTAAGATTGGATCAATCTCATCATCTTATGT	120
M T T K I W I N L I I L C	13
TTACTAACAGCCTCATCCCTCTCCGGACCCTCAGAGGTGAAGAGTGCAGTTGGTGAACG	180
L L T A S S L S G P S E V K S A V G E T	33
GTCCACATCTCCTGCCAGTATCACCAGTTCAACAGGGACAAGGTCAAATTCTGGTGCAGG	240
V H I S C Q Y H Q F N R D K V K F W C R	53
GGTTATCACTGGTACTTCTGCACAGTTATCATTTCGATCTGATCATCCTAAATATCTGACC	300
G Y H W Y F F C T V I I R S D H P K Y L T	73
AGTGATGTTACAGATATTGGATGATAAAAACITGGTTATTACAGTCAGTATGAAAGGA	360
S D V Q I L D D K N L G L F T V S M K G	93
GCAACAGCCGAGGACAGTGGCTGGTACTGGTGTGCAATTGAAAGAGCCAGCAGAACTTTG	420
A T A E D S G W Y W C A I E R A S R T L	113
GCATTTTCGTCTTCAGCTGACTGTCTCTGAGTGGCTTGACCTCGGCTCAAACCAGAAACC	480
A F R L Q L T V S E W L V P R L K P E T	133
ACTGAACAGTACAACGAGACCTCAACTTCCCCAACCAACGTTACCTCATCTTGAGACAACA	540
T E Q Y N E T S T S P T T L P H L E T T	153
ACACGGTCCACACCTTGTATGACTCAAACAACAAGTCTCATATCCGCTACAAGCAATTCA	600
T R S T P C M T Q T T S L I S A T S N S	173
ACAAGTGTGACAGTGTCTTGGATCAAGATGATCTGGTGTGGAAAGTATGGAGAGTACTG	660
T S V T V S L D Q D D L V W K V W R V L	193
CGCTGGATGCTCTTCCTCTTCCTGTGTCTGTTCCTTGTTCCTTTCAGTATAACAATGCCAT	720
R W M L F L F L C L F L V L F S I Q C H	213
CGCTGA t atattgtaccattcctgcccagtgatcgcttacctcaatggtgctacattttc	780
R *	214
Cc <a>aa atc <a>aa aatcgttgagaatattgtttgattaaat <a>aa aaaagtaaaatatttctaa	840
Catgcacatatattaataataagcaataaattgaggtcacaacatgttctgagagatat	900
Taatgtaaaagaaacaataactaaagatgtaggatctttatattgaaaccggtttgctacag	960
Caggaaaataatcctgcagcaacagaaaatgtgaaatccatagattaggggtctaatacat	1020
Ttacttaaattggctgatttccttatatgaactgtaactcagtaaaatctttgaaattgt	1080
Tgcatgtcacatgtatatctttaataaaccacagatt	1120

Appendix 3C. Nucleotide and deduced amino acid sequences of Salsal CLM8 (NM_001140890). CLM8 has one extracellular domain and the cytoplasmic region is encoded by only four codons. Description of predicted regions and color codes are as in Appendix 2A.

Gene	Day 1	Day 3	Day 5	Day 10	Day 15
trypsin [Oncorhynchus masou]	0.12	1.12	0.64	-2.30	-2.08
alpha amylase [Salmo salar]	1.39	1.57	-0.01	-2.53	-2.93
anionic trypsin [Oncorhynchus keta]	1.01	1.21	0.61	-2.36	-1.95
carboxylic ester hydrolase	1.12	1.55	1.65	-2.07	-2.06
Carboxypeptidase A1 precursor [Salmo salar]	1.13	0.91	0.80	-4.81	-2.96
Carboxypeptidase A2 precursor [Salmo salar]	0.35	1.56	0.58	-2.84	-2.77
Carboxypeptidase B precursor [Salmo salar]	0.64	0.98	0.22	-2.42	-2.11
Chymotrypsin B [Salmo salar]	1.10	1.65	0.80	-4.15	-2.79
Chymotrypsin B [Salmo salar]	1.06	1.48	0.26	-2.16	-2.04
Chymotrypsin-like protease CTRL-1 precursor [Salmo salar]	0.75	1.56	0.97	-3.38	-2.66
Elastase-1	1.40	1.30	0.71	-4.32	-2.80
Endoplasmic reticulum resident protein ERp27 [Salmo salar]	1.05	0.84	1.27	-3.09	-2.91
Guanidinoacetate N-methyltransferase [Salmo salar]	1.20	0.50	0.63	-3.04	-2.59
Guanidinoacetate N-methyltransferase [Salmo salar]	1.51	0.27	0.81	-3.10	-2.43
High choriolytic enzyme 1 precursor [Salmo salar]	1.25	0.73	0.87	-3.20	-2.42
Proproteinase E precursor [Salmo salar]	0.10	1.19	0.74	-4.78	-3.56
Proproteinase E precursor [Salmo salar]	0.37	0.97	0.62	-3.66	-2.84
<i>syncollin</i>	1.01	1.40	0.84	-3.93	-2.84
Trypsin II	1.78	1.48	0.93	-2.46	-1.95
Trypsin II	1.42	1.19	0.44	-2.22	-1.68
Trypsin III	1.28	1.25	0.96	-3.15	-2.47
Trypsin precursor [Salmo salar]	0.54	0.99	0.81	-1.18	-1.77
Trypsin-1 precursor [Salmo salar]	1.14	1.36	0.89	-1.67	-1.49
Trypsin-1 precursor [Salmo salar]	0.33	1.44	1.04	-2.42	-1.92



Appendix 4. Proteases with differential expression in spleen, microarray results. Data are log₂-ER. Fold change (up or down regulation) is indicated by colour scale.