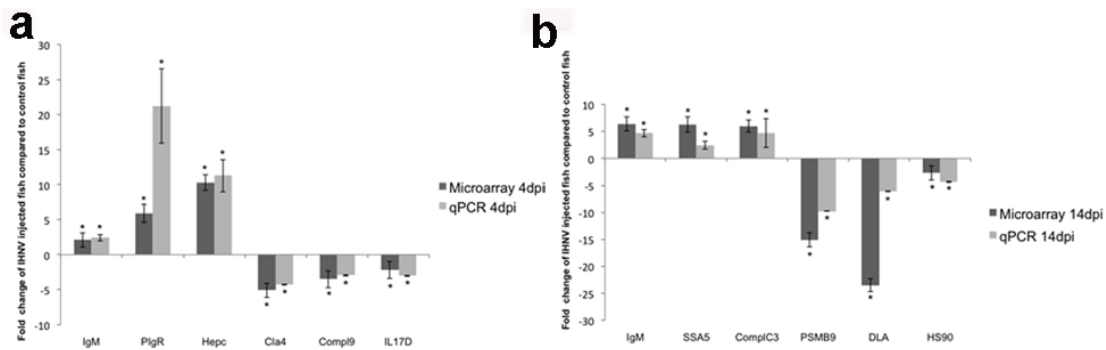
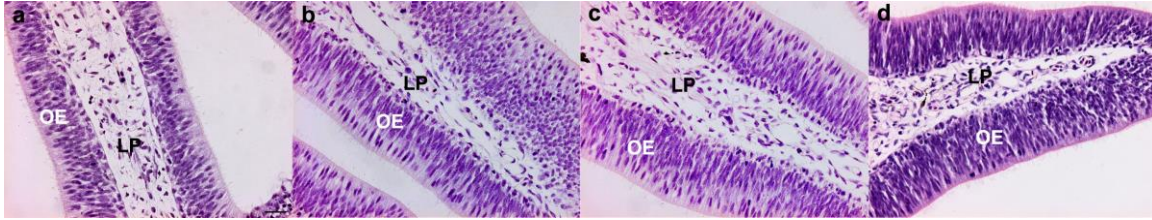


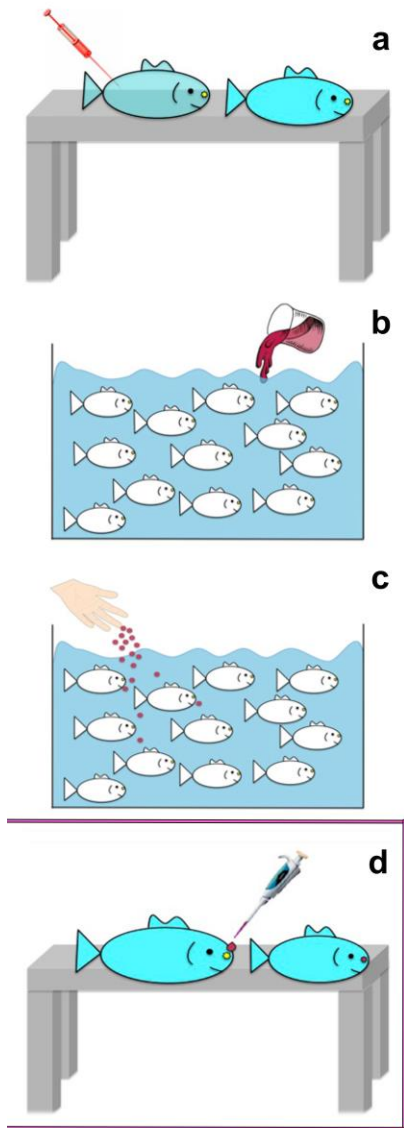
Supplementary Figure 1: The lamina propria of the rainbow trout olfactory epithelium does not harbor IgT or IgM B cells. Representative rainbow trout olfactory organ cryosection where the lamina propria (LP) is negatively stained with anti-trout IgM and anti-trout IgT antibodies. OE: olfactory epithelium.



Supplementary Figure 2: Confirmation of microarray studies by quantitative real-time polymerase chain reaction (qRT-PCR) of mRNAs of six selected genes in the olfactory organ of rainbow trout. (a) 4 dpi (b) 14 dpi nasal vaccination with IHNV vaccine. Supplementary Bars represent mean \pm SEM (N=4) and asterisks indicate significant ($p < 0.05$) differences. The genes chosen for the 4 dpi time point were: IgM, pIgR, Heparin-binding EGF-like motif containing protein 2 (Hepc), Claudin 4 (Cla4), Complement c9 (Compl9), IL-17D. The genes selected for the 14 dpi time point were: IgM, serum amyloid A 5 (SSA5), Complement C3 (complC3), PSMB9, DLA class I, Heat shock protein 90 (Hs90).



Supplementary Figure 3: Histological studies of i.m vaccinated trout olfactory organ. Trout that receive an i.m injection of live attenuated IHNV vaccine have similar olfactory organ to that of control fish. a) Control, b) i.m vaccinated 4 dpi c) i.m vaccinated 14 dpi d) i.m vaccinated 28 dpi. Results are representative of one experiment N=5. LP: lamina propria, OE: olfactory epithelium. Scale bar: 100 μ m.



Supplementary Figure 4: Cartoon of different fish vaccination methods. Injection, immersion and oral delivery (a-c) methods have been used in fish farming over the past thirty years. Pink box indicates the new proposed nasal vaccination method (d).

Supplementary Table 1: Primers used in this study

Gene	Primer name	Primer Sequence (5'-3')	Application
MX1	MXF	GATGCTGCACCTCAAGTCCTACTA	qPCR
	MXR	CGGATCACCATGGGAATCTGA	qPCR
IFN γ	IFNgF	GCTGTTCAACGGAAAACCTGTTT	qPCR
	IFNgR	TCACTGTCCTCAAACGTG	qPCR
IL2	IL2F	CATGTCCAGATTCAGTCTTCTATACACC	qPCR
	IL2R	GAAGTGTCCGTTGTGCTGTTCTC	qPCR
TNF α	TNFaF	GGGGACAAACTGTGGACTGA	qPCR
	TNFaR	GAAGTTCTTGCCCTGCTCTG	qPCR
IL6	IL6F	ACTCCCCTCTGTCACACACC	qPCR
	IL6R	GGCAGACAGGTCTCCACTA	qPCR
IL8	IL8F	AGAATGTCAGCCAGCCTTGT	qPCR
	IL8R	TCTCAGACTCATCCCCTCAGT	qPCR
IgD	IgDF	CAGGAGGAAAGTTCGGCATCA	qPCR
	IgDR	CCTCAAGGAGCTCTGGTTTGGA	qPCR
IgT	IgTF	CAGACAACAGCACCTCACCTA	qPCR
	IgTR	GAGTCAATAAGAAGACACAACGA	qPCR
IgM	IgMF	AAGAAAGCCTACAAGAGGGAGA	qPCR
	IgMR	CGTCAACAAGCCAAGCCACTA	qPCR
TCR α	TCRaF	CAGCTTGAAGTCAAGAAATAC	qPCR
	TCRaR	TATCAGCACGTTGAAAACGAT	qPCR
TCR β	TCRbF	CTCCGCTAAGGAGTGTGAAGATAG	qPCR
	TCRbR	CAGGCCATAGAAGGTACTCTTAGC	qPCR
pIgR	pIgRF	GTACAGCAGGTGTTACAGTAAC	qPCR
	pIgRR	CCACAGACAGACCTTGATAAC	qPCR
Hepcidin	HepF	GCTGTTCCTTTCTCCGAGGTGC	qPCR
	HepR	GTGACAGCAGTTGCAGCACCA	qPCR
Claudin 4	Cla4F	GCTGGACTGGAGATCCTTGG	qPCR
	Cla4R	CCACACAAGACATCCAGAGCCC	qPCR
Complement c9	Compl9F	GGAAGTGCCTGTGTCTCTGTCTG	qPCR
	Compl9R	CCTGAGGAACACTCTCCACG	qPCR
IL17D	IL17DF	CCTGAGTGC GTTCCACCACAC	qPCR
	IL17DR	GTCGTGGGAGATCCTGTATGC	qPCR
SSA5	SSA5F	GCTCAAGGTGCTAAAGACATGTG	qPCR
	SSA5R	GAGTCTCATGTCTCGACC	qPCR

Complement C3	ComplC3F ComplC3R	CCTCACAACAAGAGTGCACATC CCAAGTGGGCAAACCTCATCTCC	qPCR qPCR
PSMB9	PSMB9F PSMB9R	CCTCAAGTTCGTTTCAGCTGC GGTACTCTGCATCAACAAACC	qPCR qPCR
Heat shock 90	HS90F HS90R	CCACCCTATTGTTGAGACTCTG CCTGTAGATGCGATTGCGTGG	qPCR qPCR
DLA class I	DLAF DLAR	CCTGCATTGAGTGGCTGAAG CTGCCAGAACACCATGACTCC	qPCR qPCR
IL1 β	IL1bF IL1bR	ACATTGCCAACCTCATCATCG TTGAGCAGGTCCTTGTCCTTG	qPCR qPCR
IHNV	IHNVgF IHNVgR	CGCTATGCACAAAGGCTCCAT ATTTTCGTGAAGCTGGTAGCGC	qPCR qPCR
EF-1a	EF-1aF EF-1aR	CAACGATATCCGTCGTGGCA ACAGCGAAACGACCAAGAGG	qPCR qPCR

Supplementary Table 2: List of selected mRNAs, grouped according to functional classes (shown in bold), found to be up- and down-regulated by nasal vaccination with IHNV (4 dpi) in the trout olfactory organ. The selection was based on manual assignment of function and genes with greatest fold differences in expression are presented, the genes that are down-regulated are denoted by (-) value. In this table the genes within each group are ordered by mean fold-change expression level following infection. The genes shown were significant at $p < 0.05$ following unpaired t-test ($p < 0.05$) with Benjamini-Hochberg False Discovery Rate (FDR) correction and greater than 2-fold change. ¹Indicates the unique code for the feature on the microarray, ²Accession number of the cDNA sequence. ³Mean of fold-change (\pm SEM) for genes increased/decreased in expression following IHNV infection. ⁴Identity of the probe target as determined by BLASTX and BLASTN searches.

Probe name ¹	ACC ²	FC \pm SEM ³	Identity ⁴
NALT			
TC162079	BT125321	50 \pm 1.2	C-C motif chemokine 19
TC167667	BT045725	2.9 \pm 1.4	L-selectin
TC157883	BT072605	2.6 \pm 1.1	Vascular cell adhesion protein 1
TC150169	AM713180	6.1 \pm 1.2	Integrin alpha-M
TC171435	NM_001173753	-4.1 \pm 1.0	Carbohydrate sulfotransferase 1
IMM58	P22362	-2.1 \pm 1.1	C-C motif chemokine 28
Innate immunity			
TC152594	XM_678994	73.2 \pm 1.5	Interferon-induced very large GTPase 1
IMM1052	NM_001124355	22.5 \pm 1.2	Interleukin-1 receptor
IMM406	NM_001171871	16.2 \pm 1.3	Tumor necrosis factor receptor
TC169834	NM_001146166	16.1 \pm 1.4	Suppressor of cytokine signaling 1
IMM486	NM_001124480	15.2 \pm 1.1	Cathelicidin 1
TC155613	AY160981	11.8 \pm 1.2	Interleukin-8
TC132324	NM_001124412	11.2 \pm 1.0	Toll-like receptor 13
IMM831	AF281354	10.3 \pm 1.1	Hepcidin
IMM592	NM_001124578	9.1 \pm 1.2	Toll-like receptor 3
TC138478	NM_001165216	8.7 \pm 1.4	Proteasome subunit beta type-8
IMM1040	BT047745	7.9 \pm 1.1	Interleukin-10 receptor subunit beta
TC132590	NM_001124258	7.6 \pm 1.2	Proteasome subunit beta type-9
TC155771	NM_001139852	6.8 \pm 1.0	Interferon regulatory factor 6
TC155762	EF165029	6 \pm 1.4	Interleukin-10

IMM313	NM_001124421	5.3±1.2	MyD88
CUST_135_PI420312184	NM_001160506	5.2±1.3	TRIM25
TC159551	NM_001173710	4.2±1.0	Interleukin-6 receptor subunit alpha
TC171674	BT026755	4.1±1.4	Interferon regulatory factor 8
TC155105	Q5M7X9	3.4±1.2	Mitochondrial ubiquitin ligase activator of nfkb 1-A
TC132478	NM_001124392	3±1.1	Interleukin-12 subunit beta
IMM459	NM_001124623	2.7±1.0	Interleukin-7 receptor subunit alpha
IMM417	NM_001140256	2.2±1.1	TGF-beta receptor type-2
CUST_118_PI420312184	NM_001124438	2.1±1.0	Interferon regulatory factor 2
TC150027	NM_001124581	-4.6±1.3	Macrophage migration inhibitory factor
TC137604	NM_001124399	-2.18±1.2	Interleukin-17D
IMM589	NM_001146167	-2±1.0	Suppressor of cytokine signaling 2
Acute response			
TC165091	NM_001124436	29.3±1.2	Serum amyloid A
TC169258	NM_001146565	19.7±1.4	Serum amyloid A-5
TC134895	O19063	6.3±1.0	Serum amyloid P-component
Adaptive immunity			
IMM992	AY263797	23.2±1.5	CD83
TC157426	BT058951	17±1.3	High affinity immunoglobulin gamma Fc receptor I
TC147178	BT072250	10.7±1.1	B-cell receptor CD22
TC159660	BT047230	9.8±1.2	Regulator of G-protein signaling 1
TC132567	FR688119	9±1.0	Class I histocompatibility antigen F10
TC162887	BT074080	7.4±1.2	Beta-2-microglobulin
TC132611	AY523659	7.3±1.1	BOLA class I histocompatibility antigen
TC145984	DQ831751	7±1.2	Ig heavy chain Mem5
TC139133	BT046889	7±1.0	High affinity immunoglobulin epsilon receptor
TC155919	FJ940682	5.9±1.3	Polymeric immunoglobulin receptor
TC144208	BT060117	5.7±1.4	Low affinity immunoglobulin gamma
TC144852	AF273017	5.4±1.2	Ig kappa chain V
TC132398	NM_001124500	4.3±1.0	Cytotoxic and regulatory T-cell molecule
TC135926	Q8WML4	3.4±1.3	Mucin-1
TC170503	BT072728	2.9±1.2	B-cell lymphoma 3 protein
TC152757	Q9Y6R7	2.8±1.3	IgGFc-binding protein
TC162586	P98089	2.8±1.0	Intestinal mucin-like protein
IMM398	NM_001165113	2.2±1.1	T-cell surface glycoprotein CD3 zeta
IMM249	X65261	2.1±1.0	Ig mu
TC163654	EF467298	-3.8±1.2	T-cell receptor alpha
Viral induced			
IMM536	NM_001124332	52.6±1.3	VHSV-induced protein-3
IMM538	NM_001124334	52.4±1.3	VHSV-induced protein-5
CUST_22_PI420312184	NM_001129984	22±1.2	VHSV-induced protein-8
IMM1057	NM_001124285	21.8±1.0	VIG-2
CUST_20_PI420312184	NM_001124333	18.2±1.1	VHSV-induced protein-4
CUST_15_PI420312184	AF483535	9.4±1.2	VHSV-induced C-lectin
Oxidative stress			

TC143962	BT059657	5.5±1.3	Heat shock 60
TC144473	BT059713	5.1±1.4	DnaJ homolog subfamily A member 2
TC171997	NM_200044	5±1.3	DnaJ homolog subfamily C member 21
TC137463	XM_002716458	4.7±1.4	DnaJ homolog subfamily B member 11
TC160594	Q58DL1	4.6±1.2	Arginase-2 mitochondrial
TC161098	BT072794	4.2±1.0	Glutathione peroxidase 3
TC161884	BC065970	3.3±1.1	Heat shock 70
IMM220	NM_001124231	2.9±1.3	Heat shock 90 beta
TC146008	U19370	2.2±1.2	Heat shock 30
TC159384	BT045539	2.1±1.0	Glutathione reductase
TC145126	U89945	-6.9±1.4	Heat shock alpha
TC151247	CR700599	-3.4±1.2	Glutathione peroxidase 2

Chemokines

TC162079	BT125321	50±1.5	CCL19
IMM926	AJ417078	43.4±1.2	CXCL10-like
IMM1005	NM_001124489	31.2±1.3	CCL4
TC132837	NM_001124316	22.2±1.2	Virus-induced CXC chemokine
IMM111	NM_001124406	13.6±1.3	CXCR1
TC132449	AF260960	6.9±1.0	IL-8R
TC141029	NM_001160689	4.6±1.2	CCL13
TC154962	NM_001089524	3±1.1	CCR4
TC143794	NM_001124625	3±1.2	CXCR3
TC148292	NM_001140855	-5.7±1.3	CXC9
TC143681	BT056553	-2.9±1.1	CXC14

Complement

TC148206	Q9ES30	17.3±1.2	Complement C1q
TC171606	L24433	10.7±1.3	Complement C3
TC132318	NM_001124385	7.1±1.4	Complement C4
IMM964	NM_001124408	4.8±1.0	Complement C8 gamma
IMM963	NM_001124621	4.5±1.1	Complement C6
TC136854	NM_001124426	-3.5±1.2	Complement C9

Energy related

TC154720	NM_053962	66.5±1.3	L-serine dehydratase/L-threonine deaminase
TC170517	BT059114	30.9±1.1	Glyceraldehyde 3-phosphate dehydrogenase
TC161187	BT043786	4.6±1.2	Fructose-bisphosphate aldolase A
TC143586	NM_001141724	3.2±1.0	Glucose-6-phosphate 1-dehydrogenase

Protein metabolism

TC138833	BT049918	44.3±1.2	Polyubiquitin-C
TC150657	NM_001173966	20.7±1.1	NEDD8-conjugating enzyme
TC138749	Q5GLZ8	14.2±1.4	E3 ubiquitin-protein ligase HERC4
TC152463	NM_001124304	9.5±1.1	Cathepsin B
TC134251	BT075243	9.15±1.5	Ribosomal RNA processing protein 1
TC132725	NM_001124491	8±1.2	Calpain-2 catalytic subunit
TC144585	BT082794	6.5±1.0	Cathepsin L

TC134551	NM_001141697	6.3±1.3	60S ribosomal protein L6
TC135968	BT071960	4.7±1.1	28S ribosomal protein S2
TC133997	BT074236	3.8±1.0	Proteasome subunit alpha
IMM615	BT043515	3.4±1.2	Cathepsin D
TC164056	BT059055	2.9±1.3	Caspase-9
TC146850	FR751081	2.7±1.0	Caspase-3
TC149669	BT047994	-11.9±1.4	40S ribosomal protein S3
TC172176	AJ312339	-3.5 ±1.2	60S ribosomal protein L27a
TC142208	NM_001140206	-2.4±1.0	Cathepsin F

Lipid metabolism

TC135848	BT072598	12.8±1.4	Perilipin-2
TC141932	XM_001381263	9.6±1.2	Lipid phosphate phosphohydrolase 3
TC147397	Q8K4F2	7.5±1.1	Arachidonate 15-lipoxygenase B
TC159982	XM_001512920	4.4±1.3	Glycerol-3-phosphate acyltransferase 3
TC135251	NM_001173770	2.8±1.0	Lipase member H
TC169585	XM_001089527	2.6±1.2	Elongation of very long chain fatty acids
TC159562	NM_001141203	-19±1.4	Fatty acid-binding protein adipocyte
TC167389	XM_002663239	-4.9±1.2	Long-chain-fatty-acid
TC147270	BT048822	-3.5±1.0	Apolipoprotein A

Cell cycle

TC153816	NM_200653	11.5±1.2	Transcriptional regulator ATRX
TC144283	NM_001126433	11.4±1.3	M-phase phosphoprotein 8
TC170735	FQ231962	10.5±1.1	RNA polymerase-associated protein RTF1
TC141671	BT056522	7.2±1.0	Transcription initiation factor TFIID
IMM988	NM_001124447	6±1.3	CCAAT/enhancer-binding protein beta
TC159950	NM_001173931	4±1.4	Cell division cycle 5-like
TC154625	XM_536753	2.8±1.0	DNA replication factor Cdt1

Cell proliferation/apoptosis

TC155993	NM_001141351	21.2±1.2	Programmed cell death 1 ligand 1
TC151400	NM_001114446	5.4±1.0	Transforming growth factor beta regulator 1
IMM46	O15519	5±1.1	CASP8 and FADD-like apoptosis regulator
TC142441	NM_001140256	4.7±1.0	TGF-beta receptor type-2
TC168626	BT072249	4.1±1.3	Growth arrest-specific protein 6
TC137408	NM_001173565	3.4±1.2	Cell cycle progression protein 1
TC141106	NM_001140346	2.7±1.2	Apoptosis inhibitor 5-B
CUST_32_PI420312184	BT072239	2.5±1.0	Bcl-2-like
TC146370	BT059075	2.8±1.1	Retinoic acid receptor responder protein 3
TC134353	BT046832	-6.9±1.2	TP53-regulated inhibitor of apoptosis 1
TC171862	NM_001141656	-4.4±1.2	CIDE-B

Permeability

TC132896	BT060383	17.9±1.3	Collagenase 3
TC141152	BT072217	12.1±1.0	Fibronectin
TC138422	NM_001165403	8.7±1.2	Claudin D
TC167201	XM_003225626	7±1.1	Protocadherin-7
TC150169	AM713180	6.1±1.3	Integrin alpha-M

TC147436	P23229	5.2±1.0	Integrin alpha-6
TC151231	Q9UGQ3	4.2±1.0	Solute carrier family 2
TC132918	NM_001124435	3.7±1.3	Integrin beta-2
TC138660	HQ206591	3.6±1.1	Organic solute transporter subunit alpha
TC135491	Q6L708	3.5±1.3	Claudin-1
TC172265	BT058818	3.4±1.1	Integrin beta-1
TC171123	XM_002688579	3±1.0	Solute carrier family 35
TC144562	Q5EBI0	2.7±1.2	Solute carrier family 26
TC146276	Q9YH92	2.4±1.3	Claudin-7
TC152062	CR711779	2.4±1.1	Tight junction protein ZO-2
TC168612	Q9EPR4	-7.4±1.1	Solute carrier family 23
TC138901	O14493	-5.1±1.0	Claudin-4
TC148044	P56746	-4.8±1.3	Claudin-15
Neuronal system			
TC144610	NM_001141101	-153±1.4	Somatostatin-1A
TC150931	L37836	-98.3±1.2	Glutamate receptor subunit 2B
TC157273	BT125271	-34.2±1.2	Glucagon family neuropeptides
TC148591	FJ973564	-21.9±1.3	Neuropeptide Y
TC146280	BT043925	-21.7±1.2	Parvalbumin beta 2
TC159186	DQ641434	-17.7±1.0	Neurexin-3-beta
TC140897	NM_001140648	-8.6±1.3	Neuroendocrine protein 7B2
TC168111	GU108576	-5±1.1	Brain-derived neurotrophic factor
TC152088	BT072067	-2.2±1.0	Neural cell adhesion molecule L1-
TC132692	BT073379	-2.1±1.3	Calmodulin
TC158828	CR637442	-2±1.2	Selenoprotein M
Vessel			
TC154308	NM_001032683	59.2±1.4	Guanylate cyclase soluble subunit alpha-1
TC157886	AJ717301	6±1.2	Vascular endothelial growth factor A
TC134243	BT072605	4.3±1.0	Vascular cell adhesion protein 1
TC160858	Q2TA49	2.2±1.2	Vasodilator-stimulated phosphoprotein
TC160119	GU817337	2.1±1.0	Vascular endothelial growth factor receptor
Platelet			
CUST_2_PI420312184	NM_001124316	28.2±1.5	Platelet basic protein
IMM610	BT057627	4.4±1.0	CD63 antigen
TC142442	BT072759	2.3±1.1	Platelet-derived growth factor receptor
TC141971	XM_002710305	2.3±1.0	Beta-type platelet-derived growth factor receptor
Metalloproteinases			
IMM302	NM_001124370	14.6±1.5	Matrix metalloproteinase-9
IMM301	BT072316	5.2±1.2	Matrix metalloproteinase-23
TC148182	BT060428	5±1.0	Metalloproteinase inhibitor 2
IMM306	Q95220	4.6±1.0	Matrix metalloproteinase-14
Globin			
TC150624	NM_001160555	7.2±1.4	Hemoglobin subunit beta-1
TC164941	AF281329	7±1.2	Haptoglobin 1

TC161955	BT059981	6.8±1.0	Hemoglobin subunit alpha-4
TC162076	NM_001124388	2.6±1.1	Neuroglobin-2
Olfactory			
TC168421	BT072283	9±1.5	Olfactomedin-4
TC142095	BT059075	4.4±1.2	Retinoic acid receptor responder protein 3
TC132610	NM_001124239	3.7±1.0	Olfactory receptor 2AT4
TC154582	BT057899	3.6±1.1	Olfactory receptor 1E3
TC156356	HM133622	3.1±1.0	Olfactory receptor family C

Supplementary Table 3: List of selected mRNAs, grouped according to functional classes (shown in bold), found to be up- and down-regulated 14 dpi nasal vaccination with live attenuated IHNV in the trout olfactory organ. The selection was based on manual assignment of function and genes with greatest fold differences in expression are presented, the genes that are down-regulated are denoted by (-) value. In this table the genes within each group are ordered by mean fold-change expression level following infection. The genes shown were significant at $p < 0.05$ following unpaired t-test ($p < 0.05$) with Benjamini-Hochberg False Discovery Rate (FDR) correction and greater than 2-fold change. ¹Indicates the unique code for the feature on the microarray, ²Accession number of the cDNA sequence. ³Mean of fold-change (\pm SEM) for genes increased/decreased in expression following IHNV infection. ⁴Identity of the probe target as determined by BLASTX and BLASTN searches.

Probe name ¹	ACC ²	FC \pm SEM ³	Identity ⁴
Innate immunity			
TC151419	BT072737	2.6.2 \pm 1.0	Inhibitor of nuclear factor kappa-B kinase
Acute response			
TC169258	NM_001146565	6.3 \pm 1.4	Serum amyloid A-5
IMM1088	NM_001124436	5.3 \pm 1.1	Serum amyloid A-3
Adaptive immunity			
TC152757	Q9Y6R7	8.7 \pm 1.5	IgGFc-binding protein
TC132403	AY870263	6.4 \pm 1.3	Ig mu chain
TC166854	U69990	3.5 \pm 1.2	Ig kappa chain V-I
TC145661	DQ831794	3.5 \pm 1.0	Ig heavy chain Mem5
TC147427	BT047697	2.7 \pm 1.0	Ig kappa chain V-III
TC164241	GQ217535	2.6 \pm 1.2	Immunoglobulin lambda-like
TC132611	AY523659	7.3 \pm 1.4	BOLA class I histocompatibility antigen
TC145984	DQ831751	7 \pm 1.2	Ig heavy chain Mem5
TC165339	BT060181	2.3 \pm 1.3	Ig kappa-b4
TC142338	AF115528	-366 \pm 1.4	Major histocompatibility complex class I
TC132537	AF287486	-264.6 \pm 1.5	Class I histocompatibility antigen F10
TC144852	AF273017	-43.3 \pm 1.2	Proteasome subunit beta type-8
TC169094	AF296373	-23.5 \pm 1.2	DLA class I histocompatibility antigen A9/A9
TC132590	NM_001124258	-15.1 \pm 1.3	Proteasome subunit beta type-9
TC164135	XM_001920312	-2.9 \pm 1.2	Vascular cell adhesion protein 1
Viral induced			

IMM857	NM_001124332	9.5±1.2	VHSV-induced protein-3
CUST_19_PI420312184	NM_001124334	5.4±1.1	VHSV-induced protein-5
CUST_20_PI420312184	NM_001124333	4.6±1.0	VHSV-induced protein-4
CUST_23_PI420312184	NM_001124316	4.6±1.3	VHSV-induced protein-7
CUST_25_PI420312184	NM_001124253	4.5±1.1	VIG-1
Oxidative stresses			
TC153634	NM_001141459	3.6±1.3	Microsomal glutathione S-transferase 2
TC172388	NM_001124329	2.6±1.1	Superoxide dismutase
TC148355	BT057727	2.4±1.3	Glutathione peroxidase 7
TC142591	BT043748	-7.4±1.5	Heat shock protein beta-11
TC153065	NM_001141684	-3.5±1.0	Heat shock 71
TC147502	NM_001124748	-2.8±1.3	Heat shock factor 1
TC145126	U89945	-2.7±1.3	Heat shock 90
TC146008	U19370	-2.7±1.3	Heat shock 30
TC145802	BT072794	-2.5±1.2	Glutathione peroxidase 3
Chemokines			
TC148292	NM_001140855	6.5±1.2	CXC9
Complement			
TC132318	NM_001124385	14.4±1.3	Complement C4
TC171606	L24433	6.0±1.1	Complement C3
TC146390	NM_001124265	2.7±1.0	Complement C1q
TC142005	NM_001124427	-2.3±1.1	Complement factor H
Energy related			
TC152317	NM_001173694	4.2±1.3	6-phosphofructokinase
TC134937	BT049461	2.7±1.1	Cytochrome c oxidase subunit 4
TC161187	BT043786	2.4±1.0	Glyceraldehyde 3-phosphate dehydrogenase
TC160550	BT047645	2.3±1.0	Aldose reductase
TC168035	NM_001139709	-3.1±1.3	Fructose-bisphosphate aldolase A
TC141795	NM_001141700	-2.5±1.3	Alpha-enolase
Protein metabolism			
TC170835	BT060180	7.2±1.3	60S ribosomal protein L13a
TC157043	BC155643	3.2±1.1	NEDD8-conjugating enzyme
TC164515	BT048302	2.5±1.2	40S ribosomal protein S2
TC142167	NM_001146570	2.4±1.0	Ubiquitin-conjugating enzyme E2
TC156961	BT081306	2.2±1.2	Cathepsin L1
TC151997	BT072762	2.1±1.0	Cathepsin Z
TC140936	BT082794	2.0±1.0	Cathepsin L
TC153220	BT047689	-2.6±1.3	60S ribosomal protein L35
TC163550	BT046841	-2.4±1.2	26S proteasome complex subunit DSS1
TC132725	NM_001124491	-2.0±1.0	Calpain-2
Lipid metabolism			
TC143031	CR684502	3.9±1.2	Apolipoprotein D

TC149244	XM_001920851	3.5±1.0	Adipocyte enhancer-binding protein 1
TC156121	BC164928	2.9±1.2	Fatty acid-binding protein 10-A
TC140097	NM_213416	2.1±1.3	Elongation of very long chain fatty acids
TC147139	NM_019821	-2.5±1.0	Glycolipid transfer protein
TC167544	AF542091	-2.2±1.1	Low-density lipoprotein receptor
TC164695	FJ842648	-2.0±1.0	Fatty acid synthase

Cell cycle

TC142979	BT048797	4.6±1.2	G2/mitotic-specific cyclin-B1
TC143690	BT072376	3.8±1.1	DNA topoisomerase 2-alpha
TC142811	AM993097	3.6±1.1	ATP-dependent DNA helicase PIF1
TC141671	BT056522	7.2±1.0	Transcription initiation factor TFIID
TC134576	NM_001139779	3.0±1.0	Cell division cycle protein 20
TC161901	Q99741	2.0±1.1	Cell division control protein 6
TC141625	NM_001142720	-2.0±1.0	RNA polymerase II subunit A
TC155293	BT072384	-2.0±1.1	Protein MCM10

Cell proliferation/apoptosis

TC137332	NM_001139999	7.2±1.3	Connective tissue growth factor
TC138015	NM_001141717	5.3±1.2	Serine protease HTRA1
TC158874	FJ966093	3.6±1.2	Transforming growth factor beta-2
TC157377	Q6DC66	3.4±1.2	Bcl-2
TC142408	BX465208	3.3±1.0	Transcription factor Sox-14
TC169661	Q08DY9	3.0±1.3	Caspase-3
TC147580	BT045040	-2.8±1.1	Annexin A13
TC141852	BX936258	-2.2±1.0	TSC22 domain family protein 1
IMM80	BT028551	-2.1±1.1	CD81 antigen

Permeability

TC148044	P56746	3.5±1.2	Claudin-15
TC134765	BT047482	2.7±1.0	Claudin-4
TC145295	HQ206591	2.3±1.3	Organic solute transporter subunit alpha
TC139896	BT072200	2.1±1.0	Solute carrier family 28
TC146632	NM_001140144	-3.2±1.3	Solute carrier family 22

Neuronal system

TC142036	XM_003201140	7.1±1.3	5-hydroxytryptamine receptor 3A
TC153128	BT079716	5.2±1.2	Myelin and lymphocyte protein
TC152753	XM_002715416	3.0±1.1	Synaptotagmin-10
TC148500	P24727	2.2±1.3	Neurotrophin-4
TC153445	NM_001124694	13.3±1.1	Ependymin-2
TC168099	NM_001199365	-4.4±1.0	Semaphorin-6
TC140939	BT059740	-3.1±1.0	Neuron-specific protein family member 1
TC168111	GU108576	-2.6±1.1	Brain-derived neurotrophic factor
TC141604	NM_001140648	-2.3±1.2	Neuroendocrine protein 7B2

Vessel

TC165769	NM_001140446	8.2±1.2	Angiotensinogen
TC139007	Q95334	7.4±1.1	Glutamyl aminopeptidase

TC163724	NM_001146509	7.0±1.0	Cytokine-like protein 1
Platelet			
IMM855	NM_001124316	3.6±1.3	Platelet basic protein
TC162473	BC165195	2.3±1.2	Coagulation factor VII
TC156035	XM_695441	2.2±1.1	Platelet endothelial aggregation receptor 1
Metalloproteinases			
IMM405	BT060428	7.9±1.3	Metalloproteinase inhibitor 2
TC134956	NM_001140457	6.9±1.2	Matrix metalloproteinase-9
TC162423	BT060428	5±1.1	Metalloproteinase inhibitor 3
TC132896	BT060383	-2.9±1.0	Matrix metalloproteinase-1
Globin			
TC168272	NM_001124352	4.0±1.3	Hemoglobin embryonic subunit alpha
TC152493	NM_001124514	2.6±1.2	Mannose-specific lectin
IMM753	AF279136	4.6±1.0	Haptoglobin
Structural			
TC138527	CU306817	24±1.2	Collagen alpha-1
TC135539	NM_001124353	10±1.2	Keratin type I cytoskeletal 13
TC143643	BT071961	7.0±1.0	Collagen alpha-2
TC133994	XM_002663369	5.2±1.1	Collagen alpha-3
TC156862	NM_001124352	4.9±1.0	Keratin type II cytoskeletal 8
TC155895	AB481206	4.0±1.3	Actin-3
TC155607	Q8WWK9	3.8±1.2	Cytoskeleton-associated protein 2
TC160106	BT059156	3.1±1.3	Tubulin alpha-4A
TC167682	AB076182	2.8±1.2	Myosin heavy chain
TC144889	BT057006	4.9±1.0	Tropomyosin alpha-4
TC165734	DQ680175	-22.5±1.2	Troponin T
TC170243	BT047894	-17.0±1.0	Troponin C
TC140573	XM_001923765	-2.6±1.3	Titin
TC142753	BT057236	-2.0±1.1	Myozenin-2
TC164617	NM_001140492	-2.0±1.10	Tubulin-folding cofactor B

Supplementary Table 4: Statistical analysis of nasal vs i.m injection vaccination trials with the live attenuated IHNV vaccine in rainbow trout. Exact Parameter Estimates, odds ratios, and two-sided p-value for testing the parameter equal to zero.

Parameter	Levels	Estimate	SE	95% CI		p-Value	Odds Est	Odds CI	
Treatment	control	4.1326	1.0251	2.3034	7.8588	<.0001	62.338	10.008	>999.999
Treatment	nasal	1.4109	1.1253	-0.9253	5.3238	0.3687	4.1	0.396	205.156
Challenge	unchalle	0.6982	1.4196	-3.6798	5.0762	1	2.01	0.025	160.168
treatment*challenge	control unchalle	-3.8108	1.5751	-8.563	0.9414	0.1239	0.022	<0.001	2.564
treatment*challenge	nasal unchalle	-1.1819	1.6217	-6.0296	3.6658	1	0.307	0.002	39.086

Supplementary Table 5: Exact Parameter Estimates, odds ratios, and two-sided p-value for testing the parameter equal to zero in the ERM vaccination trial where nasal vaccine delivery was compared to i.p injection vaccination.

Parameter	Levels	Estimate	SE	95% CI		p-Value	Odds Est	Odds CI	
Treatment	control	4.1326	1.0251	2.3034	7.8588	<.0001	62.338	10.008	>999.999
Treatment	nasal	1.4109	1.1253	-0.9253	5.3238	0.3687	4.1	0.396	205.156
Challenge	unchalle	0.6982	1.4196	-3.6798	5.0762	1	2.01	0.025	160.168
treatment*challenge	control unchalle	-3.8108	1.5751	-8.563	0.9414	0.1239	0.022	<0.001	2.564
treatment*challenge	nasal unchalle	-1.1819	1.6217	-6.0296	3.6658	1	0.307	0.002	39.086