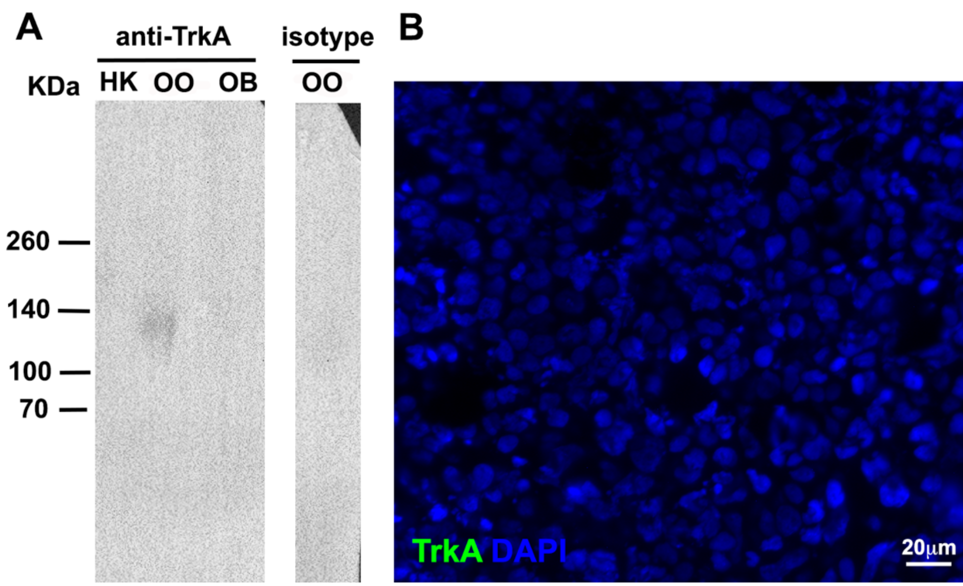
1 **Supplementary Information**

2 Supplementary Figure 1: Detection of TrkA in trout OO but not OB or head-kidney (HK) tissues.
3 (**A**) Detection of TrkA in trout OO but not HK or OB lysates by immunoblotting. Immunoblots
4 detecting TrkA showed a ~130 KDa band in OO lysates. Isotype control consisted of blots
5 incubated with normal rabbit IgG. Immunoblot is representative of two independent experiments.
6 (**B**) Immunofluorescence staining of control rainbow trout HK cryosection stained with anti-TrkA
7 antibody (FITC, green) confirming absence of $TrkA^+$ cells in this tissue. Cell nuclei were stained
8 with DAPI DNA stain (blue). Scale bar: 20 μm.



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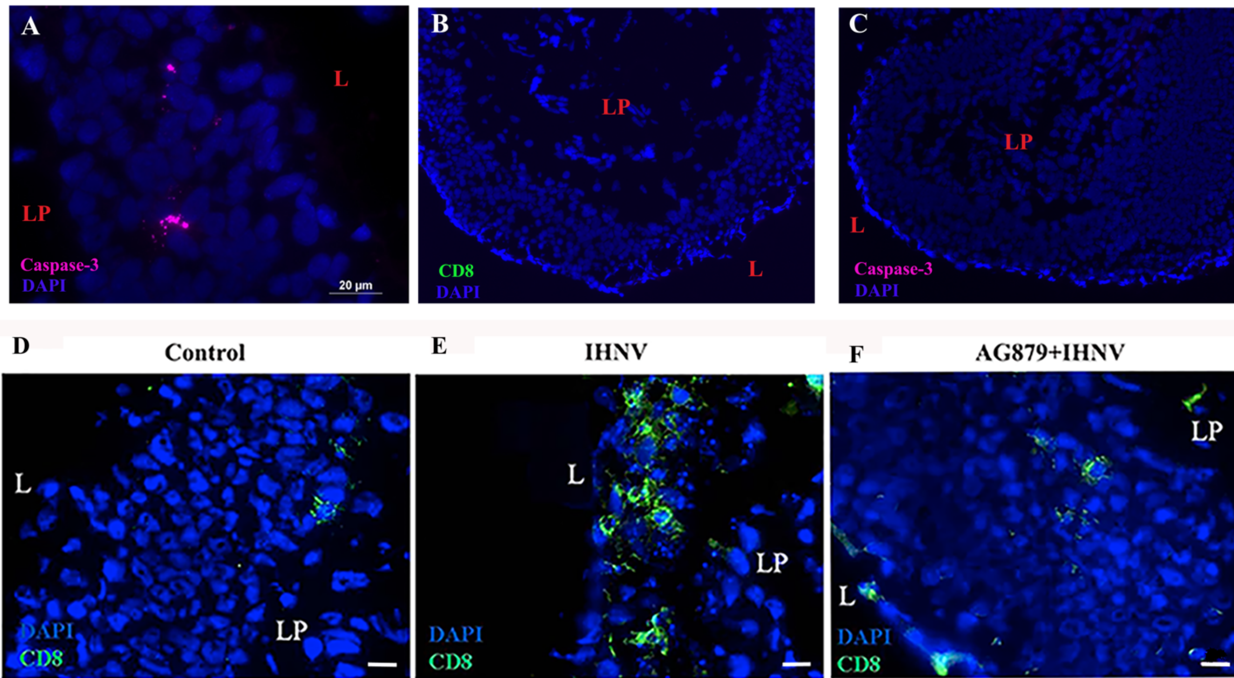
www.pnas.org/cgi/doi/10.1073/pnas.1900083116

18 Supplementary Figure 2: (**A**) Rainbow trout (N=3) received 30 µl of PBS containing 50 µg of poly
19 (I:C) (Sigma) in each nare. Fish were sampled 4 h later and OO frozen sections stained with anti-
20 caspase 3 antibody (magenta). Cell nuclei are stained with DAPI (blue). Scale bar: 20 µm. (**B**) *In*
21 *vivo* inhibition of caspase 3 by treatment with Z-DEV-FMK inhibits IHNV-induced caspase 3
22 staining in trout OO. Representative trout OO cryosection stained with anti-caspase 3 antibody
23 (magenta). Cell nuclei are stained with DAPI (blue). Scale bar: 50 µm. (**C**) *In vivo* blocking of
24 TrkA by treatment with AG879 inhibits IHNV-induced caspase 3 staining in trout OO.
25 Representative trout OO cryosection stained with anti-caspase 3 antibody (magenta). Cell nuclei
26 are stained with DAPI (blue). Scale bar: 50 µm. (**D-F**) Immunofluorescence staining of a control
27 (**D**) IHNV only (**E**) and AG879+IHNV (**F**) trout OO cryosection stained with anti-CD8α (FITC,
28 green). Scale bar, 10 µm. Cell nuclei were stained with DAPI DNA stain. L, lumen; LP, lamina
29 propria.



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35 Supplementary Figure 3: IHNV activates sensory neurons in the OO *in vitro*. (**A**) Representative
36 dot plots of control (left) and IHNV (right) trout OO extracted cells stained with anti-pERK
37 antibody showing the mean percentage of positive cells from the neuronal cell gate. (**B**)
38 Quantification of flow cytometry data in (A) indicating a significant increase in the percentage of
39 $pERK^+$ cells 15 min after adding IHNV (multiplicity of infection 1:3) *in vitro*. Results are
40 representative of three independent experiments (N = 5). $^*p < 0.05$. (**C**) Immunoblots of control
41 and IHNV treated trout OO and OB (15 min intranasal in vivo treatment) showing the increased
42 expression of pERK in IHNV-treated compared to control tissues. Negative control consisted in
43 IHNV treated tissue lysates probed with rabbit IgG isotype control as primary antibody. (**D**)
44 Quantification of immunoblot images shown in C). Data are shown as the relative expression of
45 pERK using Image Lab analysis software.

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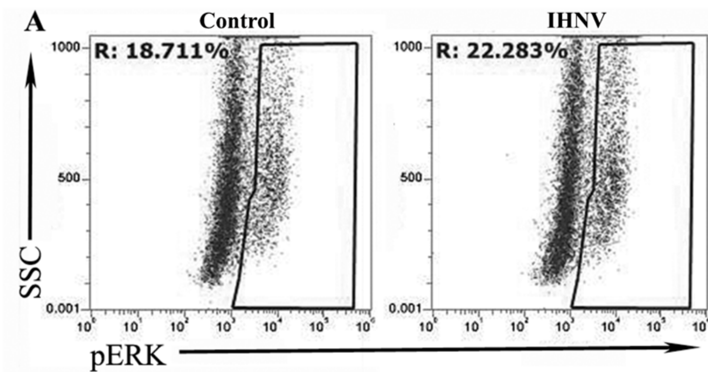

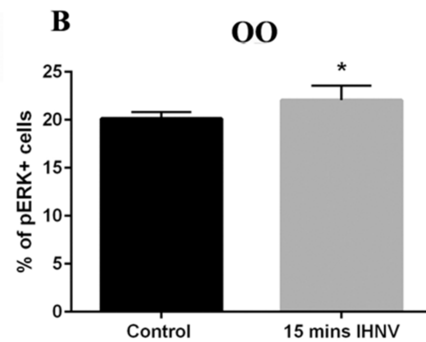

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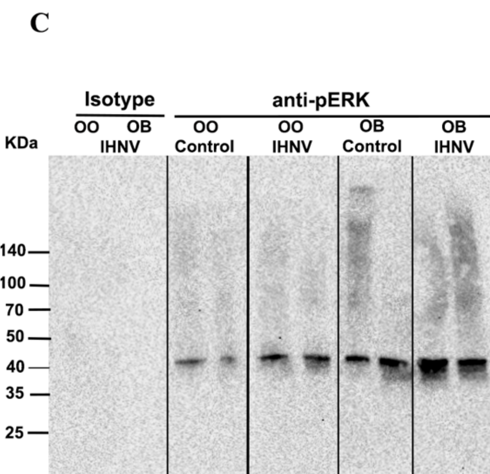

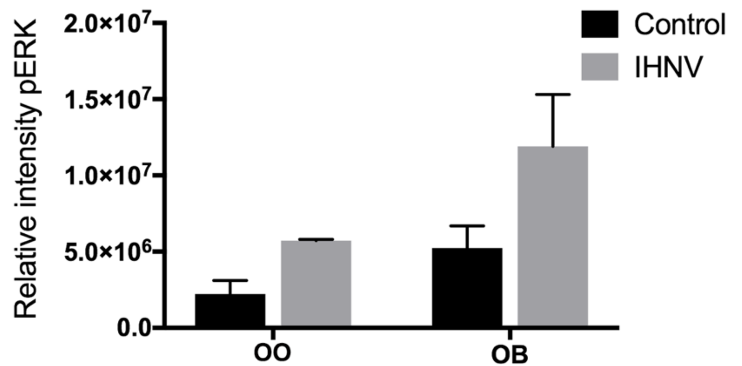

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50 Supplementary Figure 4: Nasal delivery of IHNV does not result in presence of virus in the OB
51 and does not alter blood brain barrier integrity 15 min after delivery. (**A**) Immunofluorescence
52 staining with anti-IHNV Abs (Cy3, red) showing no IHNV staining in the OO of control rainbow
53 trout. (**B**) Immunofluorescence staining with anti-IHNV Abs (Cy3, red) showing the presence of
54 IHNV (white arrows) in the OO of IHNV treated rainbow trout 15 min after nasal delivery. (**C**)
55 Immunofluorescence staining with anti-IHNV Abs (Cy3, red) showing no detection of IHNV at
56 OB of control rainbow trout. (**D**) Immunofluorescence staining with anti-IHNV Abs (Cy3, red)
57 showing the absence of IHNV in the OB of IHNV treated rainbow trout 15 min after nasal delivery.
58 (**E**) Ct values for IHNV N protein gene expression by RT-qPCR of total viral vaccine (positive
59 control), virus-free supernatant, OB of PBS-treated trout and OB of IHNV treated rainbow trout.
60 N.D: not detected. IHNV positive control consisted of Scale bar, 20 μm. (**F-I**) Differential
61 interference contrast (DIC) images merged with FITC (green) fluorescence image of trout OB
62 cryosections following intravenous injection of FITC- conjugated dextran. No changes in the BBB
63 integrity of PBS-treated fish (**F & H**) or IHNV-treated fish (**G & I**) were observed as demonstrated
64 by the absence of FITC leakage into the OB. Scale bar: 100 μm.

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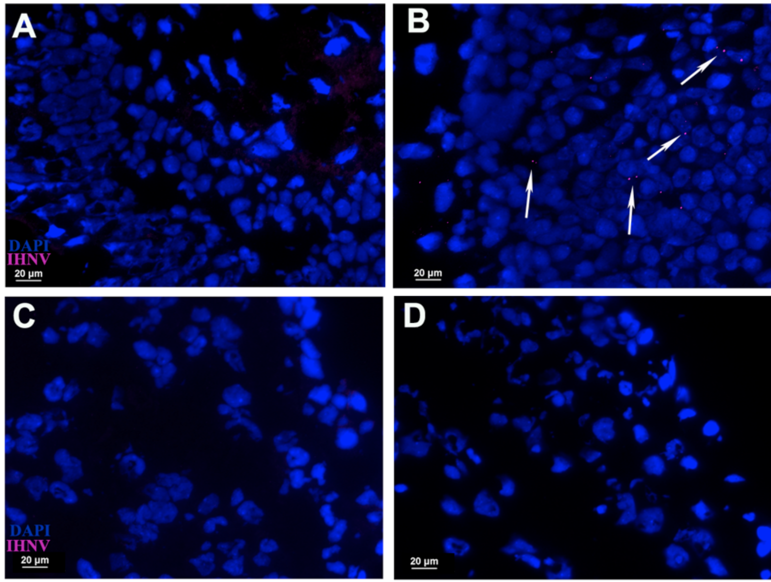

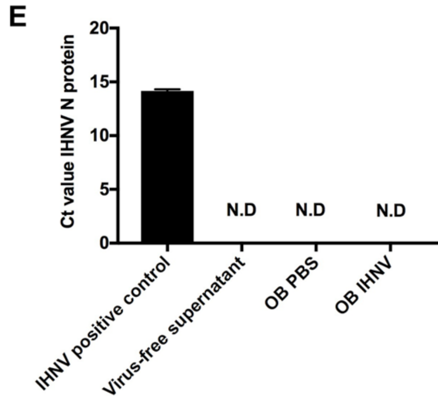

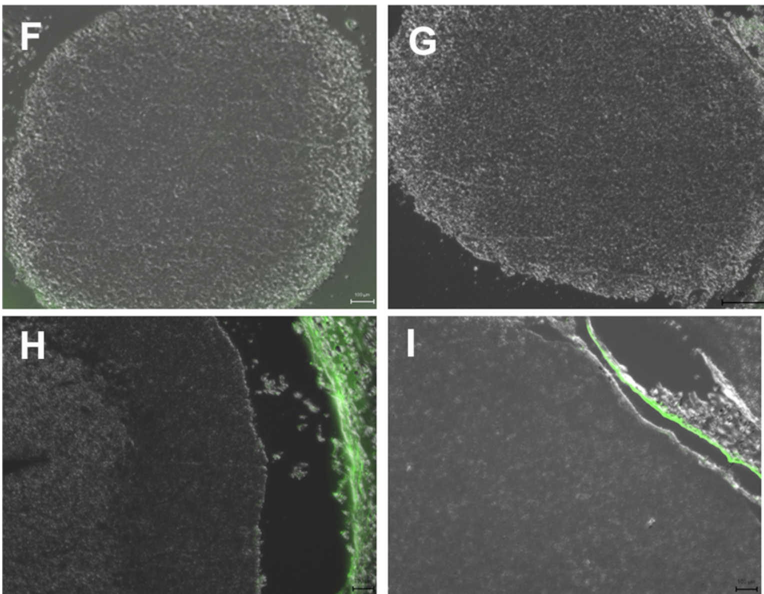

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69 Supplementary Figure 5: Leukocyte recruitment occurs as early as 15 min after IHNV delivery as
70 visualized by enlargement lamina propria (LP) of the olfactory lamellae of IHNV-treated
71 compared to control fish. (**A**) Immunofluorescence staining of control (left) and IHNV-treated
72 (right) rainbow trout OO stained with anti-trout TrkA (FITC, green) showing our image analysis
73 strategy and the enlargement in the apical and medial regions of the LP in the IHNV-treated fish.
74 Cell nuclei were stained with DAPI DNA stain (blue). Results are representative of two different
75 experiments (N = 3). Scale bar, 20 µm. (**B**) The width of LP at the apical (100 µm from the lamellar
76 tip, red line) and lateral (250 µm from the lamellar tip, yellow line) regions of the olfactory lamella
77 were measured by image analysis of 10 individual lamellae from three different fish per treatment.
78 The mean distance ± SE is shown. (**C**) Representative hematoxylin-eosin stain of adult rainbow
79 trout olfactory organ showing that leukocyte recruitment occurs as early as 15 min after IHNV
80 delivery in the olfactory lamellae of IHNV-treated (middle and right) compared to control fish
81 (left). L, lumen; LP, lamina propria. Scale bar: 50 µm.

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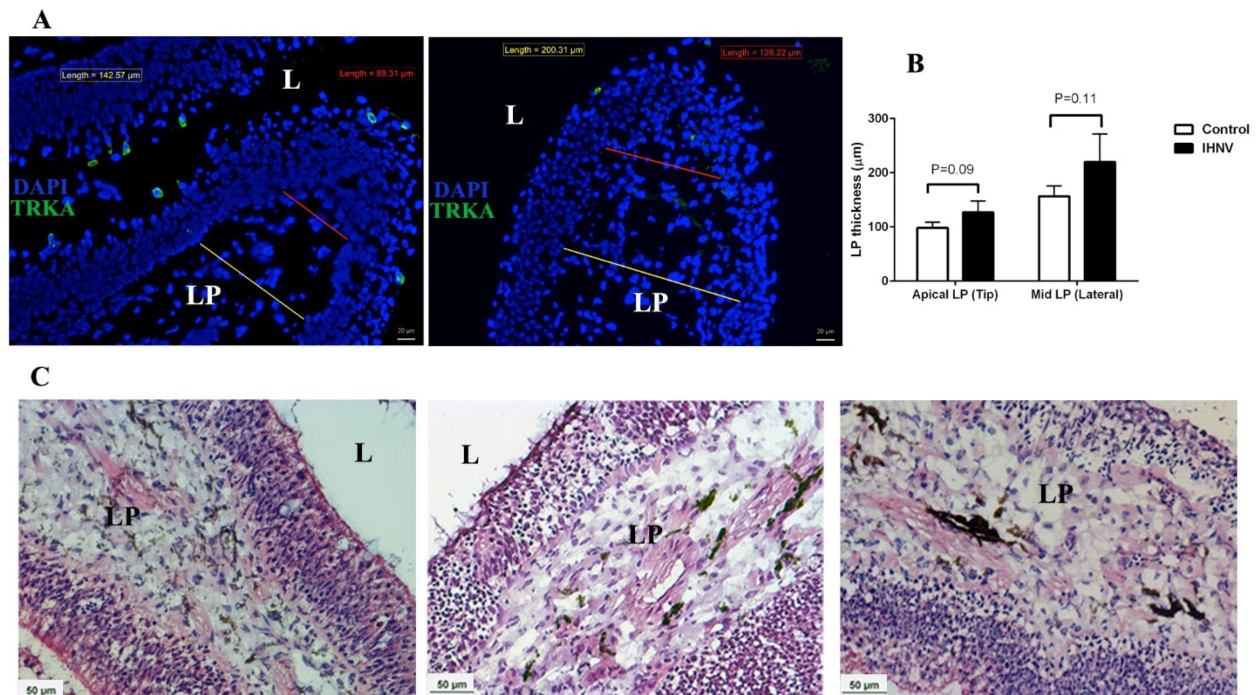
83 

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85 Supplementary Figure 6: A low degree of amino acid conservation between IHNV G protein and
86 HSV secreted G protein and generation of recombinant IHNV G protein. (**A**) Amino acid sequence
87 alignment of HSV-2 sG protein (accession number GD_HHV23) and trout IHNV G protein
88 (sequenced obtained from the live attenuated IHNV used in this study) performed in CLUSTALW
89 showing a low degree of amino acid conservation. **(B**) Production of recombinant FLAG-tagged
90 IHNV G protein by mammalian expression system. Immunoblot using anti-FLAG antibody
91 confirmed the presence of the recombinant protein (IHNV G protein) band at expected (~50 KDa)
92 molecular weight.

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A

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HSV-2 sG        ----------MGRLTSGVG--TAALLVVAVGLRVVCAKYALADPSLKMAD
IHNV G          MDTTITTPLILILITCGANSQTVNPDTVSESDQPTWSNPLFTYPEGCTLD
                            :  :*.*..  *.   .*: . : . ::  :: *.     *

HSV-2 sG        PNRFRGKNLPVLDRLTD---------PPGVKRVYHIQPSLEDPFQPPSIP
IHNV G          KLSKVNASQLRCPRIFDDENRGLIAYPTSIRSLAVGNNLGEIHTQGTHIH
                      . .    *: *          *..:: :    :    *   * . *

HSV-2 sG        ITVYYAVLER-------ACRSVLLHAPSEAPQIVRGASDEARKHTYNLTI
IHNV G          KVLYRTICSTGFFGGQTIEKALVEMKLSTKEAGAYDTTTAAALYFPAPRC
                 .:* :: .            ::::    *      . .::  *  :

HSV-2 sG        AWYR---MGDNCAIPITVMEYTECPYN-------KSLGVCPIR-TQPRWS
IHNV G          QWYTDNVQNDLIFYYTTPKSVLRDPYTRDFLDSDFIGGKCTKSPCQTHWS
                 **      .*       *   .   . **.            * *.    *.:**

HSV-2 sG        ------------------------YYDSFSAVSEDNLGFLMHAPAFETAGT
IHNV G          NVVWMGDAGIPACDSSQEIKGHLFVDKISNRVVKATSYGHHPWGLHQACM
                                         : *.:*    .  .:  *. .:. *

HSV-2 sG        YLRLVKINDWTEITQFILEHRARASCKYALPLRIPPAACLTSKAYQQGVT
IHNV G          IEFCGGQWIRTDLGDLISVVYNSGSKILSFPKCEDKTVGMRGNLDDFAYL
                            *:: ::*       .*   ::*       :. : .:  : .

HSV-2 sG        VDSIGMLPR------FIPENQRTVALYSLKIAGWHGPKPPYTSTLLPPEL
IHNV G          DDLVKASESREECLEAHAEIISTNSVTPYLLSKFRSPHPGINDVYAMHKG
                 * :               .*   * :: .  :: ::.*:*  ...    :

HSV-2 sG        SDTTNATQPELVPEDPEDSALLEDPAGT----------------------
IHNV G          SIYHGMCMTVAVDEVSKDRTTYRAHRATSFTKWERPFGDEWEGFHGLHGN
                *    .    .   * * .:* :   .    .*

HSV-2 sG        ----------VSSQIPPNWHIPSIQDVAPHHAPAAPSNPGLIIGALAGST
IHNV G          NTTIIPDLEKYVAQYKTSMMEPMSIKSVPHPSILALYNETDVSGISIRKL
                              :*   ..    *    . .** :  *  *    : *     .


HSV-2 sG        -------------LAVLVIGGIAFWVRRRAQMAPKRLRLPHIRDD--DAP
IHNV G          DSFDLQSLHWSFWPTISTLGGIPFVLLLAVAAYCCWSGRPPTPSAPQSIP
                                :: .:***.* :    .          *   .   . *

HSV-2 sG        PSHQPLFY
IHNV G          IYHLANRS
                  * .
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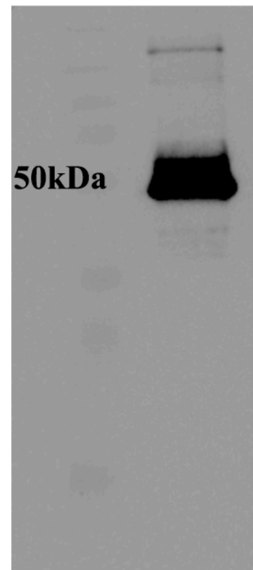
B



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106 Supplementary Figure 7: The interaction between viral glycoprotein (G protein) and crypt neuron
107 TrkA is necessary for inducing crypt neuron-mediated nasal immune responses in trout. **(A)**
108 Multiple sequence alignment (performed with CLUSTALW http://align.genome.jp/) of rainbow
109 trout (Accession number: XP_021415297.1), mouse (Accession number: NP_001028296.1) and
110 human (Accession number: EAW52902.1) TrkA domain 5 (domain known to interact with cognate
111 ligand) showing conservation of aa at sites previously described to be critical for NGF binding to
112 TrkA. **(B)** Nasal delivery of recombinant IHNV G protein recapitulates IHNV-induced changes in
113 crypt neurons and CD8 T cell immune responses. Immunofluorescence staining of trout olfactory
114 organs 15 min after receiving PBS or 100 ng of recombinant FLAG-tagged IHNV G protein
115 intranasally stained with anti-TrkA (FITC, green), anti-FLAG (Cy3, magenta) and DAPI (blue)
116 showing the co-localization of TrkA and IHNV G protein in the FLAG-tagged IHNV G protein
117 delivered group but not controls. **(C)** Quantification of the mean number of $TrkA^+$ crypt neurons
118 in the OO of control trout and trout that received recombinant FLAG-tagged IHNV G protein IN
119 ($N = 3$). **(D)** Quantification of the mean number of $CD8\alpha^+$ T cells in control and FLAG-tagged
120 IHNV G protein treated rainbow trout OO ($N = 3$) by immunofluorescence microscopy. **(E)** *In*
121 *vivo* antibody blocking of IHNV G protein reverts IHNV-induced changes in crypt neurons and
122 CD8 T cell immune responses. Live attenuated IHNV was incubated with anti-IHNV G protein
123 monoclonal antibody, anti-IHNV N protein monoclonal antibody or not treated for 30 min at RT
124 prior to *in vivo* nasal delivery. Quantification of the mean number of $TrkA^+$ crypt neurons by
125 immunofluorescence microscopy in control, anti-G protein antibody treated + IHNV, anti-N
126 protein antibody treated IHNV and IHNV alone in the OO of rainbow trout ($N = 3$). **(F)**
127 Quantification of the mean number of $CD8\alpha^+$ T cells by immunofluorescence microscopy in the
128 OO of control, anti-G protein antibody treated + IHNV, anti-N protein antibody treated IHNV and
129 IHNV-treated rainbow trout ($N = 3$). Results are representative of two independent experiments
130 ($N = 3$). One-way ANOVA and a Tukey post hoc analysis test were performed to identify
131 statistically significant differences among groups. $*p < 0.05$, $**p < 0.01$.

A

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TrkA trout     TKVQEGGNLTFVCQVTGVPMPVVRWRTNKLLSRYTVQERFWGSTLELELQ
TrkA mouse     DSVEVGDDVFLQCQVEGLALQQADWILTELEGAATVKK--FGDLPSLGLI
TrkA human     ASVDVGDDVLLRCQVEGRGLEQAGWILTELEQSATVMK--SGGLPSLGLT
               .*: *.:: : *** *  :  . *  .:*    ** :   *.  .* *

TrkA trout     LWNMSSEDNLHNLTCEAENRAGPGEEKVTLDIEFPSKIIFLHNAEQQHHW
TrkA mouse     LVNVTSDLNKKNVTCWAENDVGRAEVSVQVSVSFP-ASVHLGLAVEQHHW
TrkA human     LANVTSDLNRKNVTCWAENDVGRAEVSVQVNVSFP-ASVQLHTAVEMHHW
               * *::*: * :*:** *** .* .* .* :.:.**   : *  * : ***

TrkA trout     CFPFKVDGNPEPTIRWLFNGSNLTEGLYTYTQFIPDSDD-GSVKHGCLFL
TrkA mouse     CIPFSVDGQPAPSLRWLFNGSVLNETSFIFTQFLESALTNETMRHGCLRL
TrkA human     CIPFSVDGQPAPSLRWLFNGSVLNETSFIFTEFLEPAAN-ETVRHGCLRL
               *:**.***:* *::******* *.*  : :*:*:  :    :::**** *

TrkA trout     NKPTHLNNGLYTIIVGN
TrkA mouse     NQPTHVNNGNYTLLAAN
TrkA human     NQPTHVNNGNYTLLAAN
               *:***:*** **::..*
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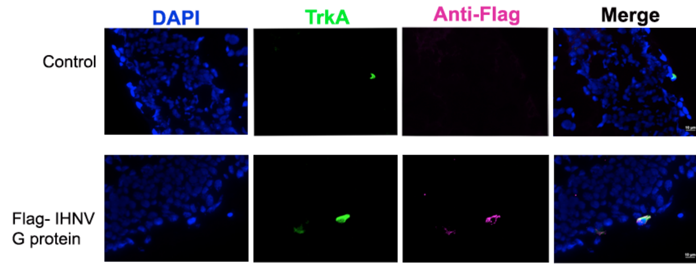

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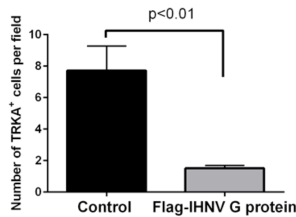

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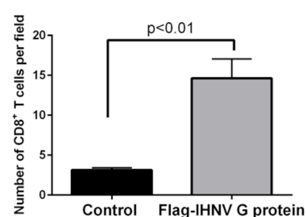

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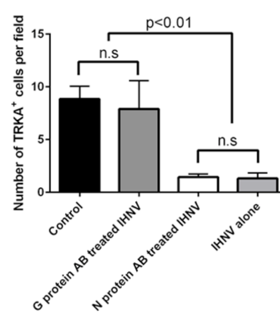

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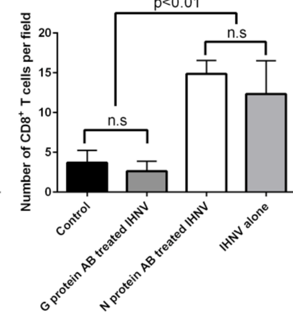

Table S1. List of antibodies

Antibodies	Vendor	Cat#
Trk A anti-rabbit polyclonal IgG	Santa Cruz Biotechnology	Cat# sc-118
Caspase-3 anti-rabbit polyclonal IgG	Abcam	Cat# ab13847
Normal rabbit IgG	Santa Cruz Biotechnology	Cat# sc-3888
Cy3 AffiniPure Goat anti-rabbit IgG (H+L)	Jackson ImmunoResearch	Cat# 111-165-144
Phospho-p44/42 MAPK(ERK1/2) anti-rabbit polyclonal IgG	Cell signaling	Cat# 9101
FITC affiniPure donkey anti-rat IgG	Jackson ImmunoResearch	Cat#712-095-153
FITC donkey anti-rabbit IgG	Jackson ImmunoResearch	Cat# 115-165-003
Rat anti-trout CD8α polyclonal IgG	(1)	

Rabbit anti-trout IgT	(2)	
Mouse anti-trout IgM	(2)(1.14)	
Mouse anti-IHNV mAb	(3)	Pool of anti-IHNV mAbs containing mAbs 1H8, 6A7, and 5AG (2 mg/ml)
Cy3 anti-mouse IgG	Jackson ImmunoResearch	Cat# 715-165-150
Peroxidase AffiniPure Donkey Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch	Cat# 711-035-152
Mouse anti-FLAG M2 IgG	Sigma	Cat# F3165
Monoclonal ANTI-FLAG® M2-Cy3™ antibody produced in mouse	Sigma	Cat# A9594
murine mAb: anti-Infectious hematopoietic necrosis virus G (anti-IHNV G)	EVAg	Cat# 015A-01754
murine mAb: anti-Infectious hematopoietic necrosis virus N (anti-IHNV N)	EVAg	Cat# 015A-01753

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137 **Table S2. List of chemicals and reagents**

Chemicals	**Vendor**	**Cat#**
Ampicillin	Sigma Aldrich	Cat# 69-52-3
L-Serine	Acros Organics	Cat# 132660250
Ethyl 3-aminobenzoate methanesulfonate salt (MS-222)	Sigma Aldrich	Cat# 886-86-2
Gallamine triethiodide	Sigma Aldrich	Cat# G8134-25G
RNAlater	Ambion	Cat# AM7021
Paraformaldehyde	Sigma Aldrich	Cat# 30525-89-4
ABsolute Blue RTq-PCR SYBR Green ROX Mix	Thermo Scientific	Cat# AB4162B
2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI)	Sigma Aldrich	Cat# 28718-90-3

TRIzol™ Reagent	Thermo Scientific	Cat# 15596018
Tyrphostin AG879	Sigma Aldrich	Cat# 148741-30-4
Tyrphostin AG879	Cayman Chemical	Cat# *10793*
SuperScript™ III First-Strand Synthesis System	Thermo Scientific	Cat# 18080051
Fluorescein isothiocyanate–dextran	Sigma	Cat# FD10S
DMEM high glucose	Gibco	Cat# 11995040
Fetal bovine serum	Hyclone	Cat# SH30071.03
Penicillin-Streptomycin (10,000 U/mL)	Gibco	Cat# 15140122
StartingBlock™ T20 (TBS) Blocking Buffer	ThermoFisher	Cat# 37543
Metronidazole	Sigma-Aldrich	Cat# M3761
PfuUltra II Fusion HS DNA Polymerase	Agilent	Cat# 600670
Anti-FLAG M2 Magnetic Beads	Sigma	Cat# M8823
3X-FLAG peptide	Sigma	Cat# F4799
Recombinant IHNV glycoprotein	This paper	N/A
Gallamine triethiodide	Sigma Aldrich	Cat# G8134-25G
Potassium chloride	Acros Organics	Cat# 196770010
Agar	VWR	Cat# J637

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139 **Table S3. List of primer sequences.**

flag-ihnv, primers AATAGGTACCGCCATGGATTACAAGGATGACGACGATAAGGACACCACGATCACCACTCCGCTC (forward) and AATACTCGAGCTAGTGGAGTGATTGAAGGTCGAATGAG (reverse)
Trout ef-1a, primers CAACGATATCCGTCGTGGCA (forward) and ACAGCGAAACGACCAAGAGG (reverse)
Trout ck10, primers GGCCAGATGGTGATGGACTGTG (forward) and GGTAGTGAAGACCACAGCGCTG (reverse)
Trout Ifng, primers GCTGTTCAACGGAAAACCTGTTT (forward) and TCACTGTCCTCAAACGTG (reverse)
Trout c-fos, primers CGTCCTTCATCCCTACTGTTACC (forward) and TGTTCCATTTTGCCTCTGC (reverse)
Trout tnfα, primers GGGGACAAACTGTGGACTGA (forward) and GAAGTTCTTGCCCTGCTCTG (reverse)
Trout ptgs2b, primers *CGTCCTTACAGAGGCTAGTGTGC* (forward) *GGTCCCTTCTTTCAGAAGTACTG* (reverse)
Trout tcra, primers GCTGTTCAACGGAAAACCTGTTT (forward) and TATCAGCACGTTGAAAACGAT (reverse)
Trout tcrb, primers CTCCGCTAAGGAGTGTGAAGATAG (forward) and CAGGCCATAGAAGGTACTCTTAGC (reverse)
Trout tcrg, primers CACCCTGCTATGTCTGGCTA (forward) and CCATTCATGCTCCACAGAAC (reverse)

Trout CCR7, primers TTCACTGATTACCCCACAGACAATA (forward) and AAGCAGATGAGGGAGTAAAAGGTG (reverse)
Trout IL10, primers CTGCTGGACGAAGGGATTCTAC (forward) and GGCCTTTATCCTGCATCTTCTC (reverse)
Trout granzyme A, primers GGCCCACTGTACTGACATCAA (forward) and ACGCGACCTTTCACCTTACG (reverse)
Trout perforin, primers TCCTGCGGGTATTACAGCTATC (forward) and CACTGCTGTCTTGAATTCTCGG (reverse)
ZF rps11, primers CCCAGAGAAGCTATTGATGGC (forward) and CCCATGCTTCAGGGATGTGA (reverse)
SVCV (N protein), primers ATCAGGCCGATTATCCTTCCA (forward) and AGATAAGCATTCACATGCTGTAT (reverse)
ZF ccl19-like, primers *GCCCACGTGATGCTGTAATA* (forward) and ACAGCGTCTCTCGATGAACC (reverse)
IHNV (N proteins), primers GGTCGCCGAACTTCTGGAA (forward) and GTAGGGCGCAGGTGAAGAGG (reverse)

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141 **SI Methods:**

142 **Histology, transmission electron microscopy and immunofluorescence microscopy**

143 For transmission electron microscopy (TEM), the OO (N = 3) of rainbow trout that had received
144 live attenuated IHNV IN 15 min prior to sampling were fixed overnight at 4 °C in 2.5 % (v/v)
145 glutaraldehyde in PBS, then transferred to 1 % osmium tetroxide (w/v) in PBS for 2 h at 4 °C.
146 After washing in PBS (3 times, 10 min), samples were dehydrated in a graded series of ethanol
147 (10–100 %) through changes of propylene oxide. Samples were then embedded in Epon resin,
148 sectioned and stained with uranyl acetate and lead citrate before being examined in a PHILIPS
149 TECNAI 12 transmission-electron microscope. Additionally, semithin sections were stained with
150 toluidine blue. Quantification of crypt neuron morphology in semithin sections was performed by
151 two independent investigators by counting 10-20 crypt neurons per OO (N=3 per treatment) and
152 scoring them as healthy or apoptotic/dead.

153 The conjugated antibodies used for immunostaining are listed in the **Table S1.** Trout OO and OB
154 were snap frozen in OCT and 5- µm-thick cryosections were fixed in 4% paraformaldehyde for 3
155 min, blocked for 15 min in T20 StartingBlock blocking solution (ThermoScientific) and labeled
156 with rat anti-trout CD8α (1:50 dilution in PBT, PBS containing 0.01% Triton X-100 and 0.1%
157 BSA), anti- pERK (1:50 dilution in PBT), anti-human TrkA (1:100 dilution in PBT) and rabbit
158 anti-mouse caspase 3 (1:100 dilution in PBT) antibody for immunostaining. Nuclei were stained
159 with DAPI. Samples were observed under a Nikon Ti or Zeiss confocal microscopes. To test the

160 permeability of BBB 15 min after nasal viral delivery, 50 µl of FITC-conjugated 10 kDa dextran
161 particles in PBS were injected i.v into 10 g rainbow trout (N = 6) 1 h before sampling. Trout then
162 received IHNV or PBS IN and 15 min later, trout heads were snap frozen, embedded in OCT and
163 cryosections were examined for fluorescence microscopy.

164 **Electrophysiological recordings**

165 Rainbow trout were anesthetized in a solution of MS222 at 0.1 g/l, and then immobilized with an
166 intra-muscular injection of gallamine triethiodide 3 mg/kg of body weight, in 0.9% saline. Fish
167 were then secured in a V-shape Plexiglas stand partially inundated, whereby gills could be
168 continuously irrigated with aeriated anesthetic solution of MS222 at 0.05 g/l. The olfactory rosette
169 was surgically exposed and borosilicate electrodes, filled with a solution of 3 M KCL in 0.4% agar
170 and connected to solid state electrodes with Ag/AgCl pellets, were placed between olfactory
171 lamellae (signal electrode) and external skin (reference electrode). The olfactory epithelium was
172 continuously irrigated with tap charcoal filtered water and the stimulus was released directly into
173 the nose through a borosilicate tube. The olfactory responses generated after release of the stimuli
174 for 4 s were filtered and amplified by a NeuroLog DC filter and pre-amplifier integrated by an
175 Axon Digidata 1550B, and stored on a PC running Axioscope 10.6 software.

176 *Dose response experiments.* Stimuli were serially diluted from a 1:100 to 1:1000 000 from a stock
177 solution, and applied to the nose to measure amplitude of the olfactory responses. These responses
178 were blank subtracted (i.e. the response to tap charcoal filtered water, negative control) and
179 normalized to those of $_{L}$-serine at 10^{-5} M. Normalized responses to filtered water had an average
180 value of 0.2 ± 0.03. The IHNV stock consisted of live attenuated IHNV at 2 x 10^{8} PFU and culture
181 medium stock was the supernatant of the vaccine after being centrifuged twice at 45,000 G for 60
182 min at 5 °C. The supernatants were confirmed to be virus-free by RT-qPCR using IHNV N protein
183 specific primers. Since the limit of the olfactory detection of IHNV is at a dilution of 1:100 000,
184 we estimate that any remaining virus in the supernatant will be at a lower concentration than 1:100
185 000 and will not activate specific IHNV olfactory receptors.

186 *Cross-adaptation experiments.* We identified dilutions of IHNV and medium that evoked the same
187 EOG amplitude (called the 'unadapted' response). Then the olfactory rosette was continually
188 exposed to IHNV solution at the concentration of the unadapted response at least 1 min, and the

189 response to a sample at double concentration of unadapted response IHNV was recorded (called
190 the self-adapted control, SAC). After that, the response to a mixture IHNV and medium, both at
191 same concentration that unadapted response, was recorded (Mix). Both measures, Mix and SAC,
192 were then calculated as a percentage of the unadapted response. After adaptation, the olfactory
193 rosette was flushed with charcoal filtered water for 20 min, and the process repeated using medium
194 as the adapting solution and IHNV or the mixture IHNV and medium as stimuli. Half of the fish
195 were adapted first to IHNV and the other half first to control.

196 *Inhibition curves.* Responses to 1:1000 to IHNV or medium were recorded (both showed similar
197 amplitude in their olfactory responses). Then the olfactory rosette was continuously exposed to
198 increasing concentrations of AG879 from 10^{-9} M to 10^{-5} M and, under each adapting concentration
199 of the drug, it was measured the olfactory responses to 1:1000 of IHNV or medium. Responses
200 were calculated as ratio between 1:1000 odorant after adaptation to drug solution and 1:1000
201 odorant before adapted to drug. All graphs were produced with Sigma plot 11.0 and EC50
202 concentrations were calculated using the Pharmacology module of the same program.

203 **Western blot, cell isolation and flow cytometry**

204 The conjugated antibodies used for western blot and Flow cytometry are listed in the **Table S1.**
205 Olfactory Organ (OO), head kidney (HK), and olfactory bulb (OB) were extracted and prepared
206 for Western blotting as explained elsewhere (4). Briefly, tissues were placed in 200 µl of RIPA
207 buffer and frozen at -80°C until use. Samples were sonicated (10s intervals, 5 times), centrifuged
208 (10,000 rpm, 15 min, 4°C), and the supernatants collected. The total protein content of the
209 supernatants was quantified using the Pierce 660nm Protein Assay (Thermo Scientific). After all
210 samples were adjusted to the same concentration, 15 µl of each sample were mixed with 15 µl of
211 Laemmli buffer under non-reducing conditions. Samples were boiled for 3 min at 97 °C and
212 resolved on 4–15% SDS-PAGE gels. Gels were run for 50 min at 120 V and transferred onto
213 PVDF membranes. Membranes were blocked in PBS-T (PBS containing 0.05% Tween-20- and
214 5% non-fat milk overnight at 4 °C. Membranes were incubated with anti-TrkA (1:1000) or isotype
215 control for 90 min, washed three times in PBS-T and then incubated for 60 min with HRP-anti-
216 rabbit IgG (1:7500). Isotype controls consisted of samples incubated with rabbit IgG as primary
217 antibody. Detection was performed using ECL Western Blotting Substrate. Immunoblots were

218 scanned using a ChemiDoc Touch Imaging System and band densitometry was analyzed with
219 Image Lab Software.
220 Isolation of trout OO cells was carried out as explained elsewhere (5). Briefly, trout OO were
221 obtained by means of mechanical agitation of both olfactory rosettes in DMEM medium
222 (supplemented with 5% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin) at 4 °C for 30 min.
223 Leukocytes were collected, and the aforementioned procedure was repeated four times. Thereafter,
224 the OO pieces were treated with PBS (containing 0.37 mg/ml EDTA and 0.14 mg/ml dithiothreitol
225 (DTT)) for 30 min followed by enzymatic digestion with collagenase type IV (0.15 mg/ml) for 2
226 h at 20 °C. All cell fractions obtained the OO following the mechanical and enzymatic treatments
227 were pooled, washed with modified DMEM. OB microvessels were extracted then 40 µl of DMEM
228 containing heparin were added to the cavity to collect the remaining blood from the microvessels.
229 This step was repeated twice. Cells were isolated by forcing the tissue through a 100-µm pore
230 nylon cell strainer and washed in DMEM three times. Cell suspensions were counted in a
231 haemacytometer and stained with CD8α, IgM, IgT, pERK or TrkA antibodies as explained
232 elsewhere (3). For pERK and TrkA flow cytometry staining, cells were stained intracellularly by
233 first fixing for 10 min at room temperature in 2% PFA followed washing in Perm/Wash buffer
234 (BD). A total of 30,000 cells were recorded using an Attune NxT flow cytometer. The percentage
235 and total numbers of CD8α$^+$, IgM$^+$ and IgT$^+$ cells were quantified within the lymphocyte gate using
236 their FSC/SSC profile. pERK$^+$ cells were quantified using a neuronal cell gate based on their
237 FSC/SSC profile.
238

239 **Gene expression analysis by real-time quantitative PCR**

240 Total RNA from OO and OB samples were collected and placed in 1 ml TRIzol (**Table S2**) for
241 RNA extraction according to the manufacturer's instructions. cDNA synthesis was performed as
242 explained elsewhere (4). The resultant cDNA was stored at -20 °C. The expression of *ptgs2b, ifng,*
243 *tnfα, ck10* (CCL19-like) and *c-fos* for rainbow trout, zebrafish *ccl19-like*, IHNV N protein and
244 SVCV N protein were measured by RT-qPCR using specific primers (**Table S3**). The RT-qPCR
245 was performed using 3 µl of a diluted cDNA template as described in (6). The relative expression
246 level of the genes was determined using the Pfaffl method (7) as previously described (6).

247 **Recombinant IHNV G protein production**

248 IHNV lacking the predicted C-terminal transmembrane domain (amino acids 1-459) was cloned
249 into pcDNA3.1 with an N-terminal FLAG epitope using 5'-KpnI and 3'-XhoI restriction sites and
250 sequenced confirmed. Recombinant IHNV was purified using the FreeStyle 293 Expression
251 System (Invitrogen). 293-F cells were maintained as 30mL suspension stocks in 293 Expression
252 Medium supplemented with 10% FBS. Cells were grown at 37°C with 8% CO_2 with constant
253 shaking at 125 rpm. For transfection with pcDNA3.1-FLAG:IHNV, cells were scaled to 5x30mL
254 stocks and each transfected using 293-Fectin reagent according to manufacturer's protocol
255 (Invitrogen). Briefly, cells were passaged as ~$1x10^6$/mL in 30 mL suspension stocks and each
256 transfected with 30 μg of pcDNA3.1-FLAG:IHNV plasmid. Cells were then allowed to grow at
257 37°C with 8% CO_2 with constant shaking at 125rpm for 5 days.

258 FLAG:IHNV was purified using affinity chromatography. Cells were harvested and lysed in lysis
259 buffer (50 mM Tris pH=8, 300 mM NaCl, 1mM DTT, protease inhibitors) using 20-30 passes
260 through a 23G needle. Cell lysates were clarified and the supernatant (~10mL) was incubated with
261 1mL of Anti-FLAG M2 Magnetic Bead slurry (Sigma) for 3 h at 4°C and constant rocking. Beads
262 were separated using a magnetic stand and washed 5 times with lysis buffer. FLAG:IHNV was
263 eluted using 3X FLAG Peptide at a concentration of 100 μg/mL in lysis buffer (Sigma). The eluted
264 sample was extensively dialyzed overnight against storage buffer (20 mM Tris pH=8, 150 mM
265 NaCl, 2 mM DTT). The dialyzed FLAG:IHNV was concentrated using a VivaSpin-20
266 concentrator and stored at -80°C until use. Purity and size of the recombinant protein generated
267 was confirmed by western blotting using anti-FLAG antibody (Sigma) (See SI Appendix, Figure
268 S6B). Recombinant protein stocks were diluted in PBS for *in vivo* nasal delivery as explained
269 below.

270 **DNA constructs and generation of transgenic zebrafish larvae**

271 Three kb of the upstream regulatory sequence of the *ora4* gene, which includes its 5'UTR
272 (Ensembl accession number ENSDARG00000078223) was amplified using PfuUltra II Fusion HS
273 DNA Polymerase, the primers 5'-aaggtaccgtgaatgcgtgtgtgtgatgtc-3 and 5'-
274 aaaggatccgctgaagatgctccagagtcc-3, and zebrafish larval genomic DNA as template. The amplicon
275 was digested with *KpnI*/*BamHI*, cloned in the p5E-MCS vector (#228) of the Tol2kit and the
276 *ora4::Gal4VP16* construct were then generated by MultiSite Gateway assemblies using LR

Clonase II Plus according to standard protocols and using Tol2kit vectors described previously (8).

The lines *Tg(ora4::Gal4VP16)^{ums4}* were generated by microinjecting 0.5-1 nl into the yolk sac of one-cell-stage embryos a solution containing 100 ng/µl *ora4::Gal4VP16* construct and 50 ng/µl Tol2 RNA in microinjection buffer (0.5x Tango buffer and 0.05 % phenol red solution) using a pneumatic microinjector.

Cell ablation and live imaging of zebrafish larvae

The lines *Tg(UAS-E1b:nfsb-mCherry)^{c264}* and *Tg(ora4::Gal4VP16)^{ums4}* were crossed. Their offspring were treated at 48 h post-fertilization (hpf) for 24 h with 12 mM metronidazole and kept in dark (9). Images were first obtained at 48 hpf as described below. Thereafter, starting from 72 hpf the prodrug was removed, and larvae imaged once a day up to 120 hpf to confirm the ablation of ora4^{+} crypt neurons.

For live imaging, larvae were anesthetized in tricaine as previously described (10). Images were captured with an epifluorescence MZ16FA stereomicroscope (Leica) equipped with green and red fluorescent filters while animals were kept constantly at 28.5°C.

Viral challenge in zebrafish

The SVCV isolate 56/70 was propagated in EPC cells and titrated in 96-well plates. Thirty 72 hpf zebrafish larvae per group in triplicate were challenged for 24 h at 25°C in disposable Petri dishes by immersion in 10^8 TCID50/fish SVCV. After challenge, the remaining fish in each group were transferred to fresh plates containing egg water and monitored every 12 h over a 6-day period to score mortality (11).

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305

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