CycaCh25h\_MT602517 AGGACCACTGTGGCTATGACCTGCCATGGGCGACACACAGAC**TGGTGCCTTTTGGTCTGT** 660

CycaCh25h\_MT602518 AGGACCACTGTGGCTATGACCTGCCATGGGCGACACACAGAC**TGGTGCCTTTTGGTCTGT** 659

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

CycaCh25h\_MT602517 ATGGAGGAGCTCCGCACCATGATGTCCACCATCAGAAGTTCAAGTCCAACTATGCT**CCAT** 720

CycaCh25h\_MT602518 ACGGAGGAGCTCCGCACCATGATGTCCACCATCAGAAGTTCAAGTCCAACTATGCT**CCAT** 719

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CycaCh25h\_MT602517 **ACTTCACTCACTGGGACA**AGCTCTTTGGGACACTGCACTCAGAATGAACTG- 771

CycaCh25h\_MT602518 **ACTTCACTCACTGGGACA**AGCTCTTTGGGACACTGCACTCTGAATGAACTGA 771

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**Supplementary Figure S1.** Localisation of the primers used in the qPCR in the common carp (Cyca) *ch25h\_b* genes.

Onts\_XM\_024409452 CCCTAACCTGCTGGGCTGCCACCCGCTCACCAAGATGTTCTTCTTCACCCTGAAC**ATCTG** 1020

Onts\_XM\_024425406 CCCTAACCTGCTGGGCTGCCACCCGCTCACCAAGATGTTCTTCTTCACCCTGAAC**ATCTG** 738

Onmy\_XM\_021611166 CCCTAACCTGCTGGGCTGCCACCCGCTCACCAAGATGTTCTTCTTCACCCTGAAC**ATCTG** 621

Onts\_XM\_024386464 CCCTCTGCTGCTGGGCTGCCACCCCCTGACAGAGATGCTCTTCTATGTACTGAAT**ATCTG** 623

Onmy\_XM\_021564786 CCCTCTGCTGCTGGGCTGCCACCCCCTGACAGAGATGCTCTTCTATGTACTGAAT**ATCTG** 620

Onts\_XM\_024387282 CCCTCTGCTGCTGGGCTGCCACCCCCTGACAGAGATGCTCTTCTATGTTCTGAAT**ATCTG** 639

Onmy\_XM\_021575763 CCCTCTGCTGCTGGGCTGCCACCCCCTGACAGAGATGCTCTTCTATATTCTAAAT**ATCTG** 610

Onts\_XM\_024387283 CCCTCTGCTGCTGGGCTGCCACCCCCTGACAGAGATGCTCTTCTATGTTCTGAAT**ATCTG** 631

Onmy\_XM\_021575762 CCCTCTGCTGCTGGGCTGCCACCCCCTGACAGAGATGCTCTTCTATGTTCTGAAT**ATCTG** 627

\*\*\*\* \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* \*\* \*\* \*\*\*\*\* \*\*\*\*\*\* \*\* \*\* \*\*\*\*\*

Onts\_XM\_024409452 **GCTGTCTGTGGAGGA**CCACTCAGGTTATGACCTGCCCTGGGCCCCTCACAGACTGGTACC 1080

Onts\_XM\_024425406 **GCTGTCTGTGGAGGA**CCACTCAGGTTATGACCTGCCCTGGGCCCCTCACAGACTGGTACC 798

Onmy\_XM\_021611166 **GCTGTCTGTGGAGGA**CCACTCAGGTTATGACCTGCCCTGGGCCCCTCACAGACTGGTACC 681

Onts\_XM\_024386464 **GCT**T**TCTGTGGAGGA**CCACTGTGGCTATGACCTGCCCTGGTCCACGCATAGACTGGTGCC 683

Onmy\_XM\_021564786 **GCT**T**TCTGTGGAGGA**CCACTGTGGCTATGACCTGCCCTGGTCCACGCATAGACTGGTGCC 680

Onts\_XM\_024387282 **GCT**T**TCTGTGGAGGA**CCACTCTGGCTATGACCTGCCCTGGTCCACGCATAGACTGGTGCC 699

Onmy\_XM\_021575763 **GCT**T**TCTGTGGAGGA**CCACTCTGGCTATGACCTGCCCTGGTCCACGCATAGACTGGTGCC 670

Onts\_XM\_024387283 **GCT**T**TCTGTGGAGGA**CCACTCTGGCTATGACCTGCCCTGGTCCACGCATAGACTGGTGCC 691

Onmy\_XM\_021575762 **GCT**T**TCTGTGGAGGA**CCACTCTGGCTATGACCTGCCCTGGTCCACACATAGACTGGTGCC 687

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Onts\_XM\_024409452 CTTTGGGCTCTATGGCGGATCTCCYCACCACGACCTCCACCATCTCAAGTTCATGGTCAA 1140

Onts\_XM\_024425406 CTTTGGGCTCTATGGCGGATCTCCGCACCACGACCTCCACCATCTCAAGTTCATGGTCAA 858

Onmy\_XM\_021611166 CTTTGGGCTCTATGGCGGATCTCCACACCACGACCTCCACCATCTCAAGTTCATGGTCAA 741

Onts\_XM\_024386464 CTTTGGGCTCTACGGTGGAGCTCCGCACCATGACCTGCACCACCTGAAGTTCAAATCCAA 743

Onmy\_XM\_021564786 CTTTGGACTCTACGGTGGAGCTCCGCACCATGACATGCACCACCTGAAGTTCAAATCCAA 740

Onts\_XM\_024387282 CTTTGGGCTCTACGGTGGAGCTCCGCACCACGACCTGCACCATCTGAAGTTCAAGTCCAA 759

Onmy\_XM\_021575763 CTTTGGGCTCTACGGTGGAGCTCCGCACCACGACCTGCACCATCTGAAGTTCAAGTCCAA 730

Onts\_XM\_024387283 CTTTGGGCTCTACGGTGGAGCTCTGCACCACGACCTGCACCATCTGAAGTTCAAGTCCAA 751

Onmy\_XM\_021575762 CTTTGGGCTCTACGGTGGAGCTCCGCACCACGACCTGCACCATCTGAAGTTCAAGTCCAA 747

\*\*\*\*\*\* \*\*\*\*\* \*\* \*\*\* \*\*\* \*\*\*\*\* \*\*\* \* \*\*\*\*\* \*\* \*\*\*\*\*\*\* \*\*\*

Onts\_XM\_024409452 CTACGCACCCT**ACTTCACACACTGGGACAG**GCTGTTCGGCTCGCTGCTGCATACAGACAA 1200

Onts\_XM\_024425406 CTACGCACCCT**ACTTCACACACTGGGACAG**GCTGTTCGGCTCGCTGCTGCATACAGACAA 918

Onmy\_XM\_021611166 CTACGCACCCT**ACTTCACACACTGGGACAG**GCTGTTTGGCTCGCTGCTGCATACAGACAA 801

Onts\_XM\_024386464 CTACGCTCCGT**ACTTCACACACTGGGACAG**GCTTTTTGGGACCTTGCACAAGAGCTCTGA 803

Onmy\_XM\_021564786 CTACGCTCCGT**ACTTCACACACTGGGACAG**GCTTTTTGGGACCTTGCACAAGAGCTCTGA 800

Onts\_XM\_024387282 CTATGCTCCGT**ACTTCACACACTGGGACAG**GGTTTTTGGGACATTGCACAAGCATTCAGA 819

Onmy\_XM\_021575763 CTATGCTCCGT**ACTTCACACACTGGGACAG**GGTTTTTGGGACATTGCACAAGCATTCAGA 790

Onts\_XM\_024387283 CTATGCTCCGT**ACTTCACACACTGGGACAG**GGTTTTTGGGACATTGCACAAGCATTCAGA 811

Onmy\_XM\_021575762 CTATGCTCCGT**ACTTCACACACTGGGACAG**GGTTTTTGGGACATTGCACAAGCATTCAGA 807

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**Supplementary Figure S2.** Localisation of the primers used in the qPCR in the rainbow trout (Onmy) and Chinook salmon (Onst) *ch25h\_b* genes. The point mutation G/T in reverse primers was not present in the TSA and WGS sequences (GenBank ID: CDQ94151, GBTD01125112, GBTD01125114, CDQ61409, GBTD01125112) used for primer design.

**Supplementary Table S1.** GenBank accession numbers for the genes and proteins encoded by the cholesterol 25 hydroxylase paralogues of common carp, rainbow trout and Chinook salmon. Shaded in grey are the entries for homologues of the zebrafish *ch25h\_b* gene, which expression was measured. EST - expressed sequence tag, TSA - transcriptome shotgun assembly, WGS - whole genome shotgun.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene in *Danio rerio* (GenBank ID)** | **Host** | **Nucleotide**  **GenBank ID** | **Protein**  **GenBank ID** | **EST/TSA/WGS**  **GenBank ID** | **Locus** |
| *ch25h\_a* (MF095413) | Common carp | XM\_019097130 | XP\_018952675 |  | LOC109082232 |
|  |  | XM\_019091588 | XP\_018947133 |  | LOC109075736 |
|  | Rainbow trout | XM\_021621937 | XP\_021477612 |  | LOC110536255 |
|  | Chinook salmon | XM\_024442579 | XP\_024298347 |  | LOC112265362 |
|  |  | XM\_024438376 | XP\_024294144 |  | LOC112262684 |
|  |  | XM\_024442578 | XP\_024298346 |  | LOC112265362 |
|  |  | XM\_024438375 | XP\_024294143 |  | LOC112262684 |
| *ch25h\_b* (MF095414) | Common carp | XM\_019113012/MT602518 | XP\_018968557 | JZ503992 | LOC109099495 |
|  |  | XM\_019113007/MT602517 | XP\_018968552 |  | LOC109099486 |
|  | Rainbow trout | XM\_021611166 | XP\_021466841 | CDQ94151 | LOC110529015 |
|  |  | XM\_021575763 | XP\_021431438 | GBTD01125112 | LOC110498991 |
|  |  | XM\_021564786 | XP\_021420461 | GBTD01125114/ CDQ61409 | LOC110491381 |
|  |  | XM\_021575762 | XP\_021431437 | GBTD01125112 | LOC110498990 |
|  | Chinook | XM\_024425406 | XP\_024281174 |  | LOC112253430 |
|  | salmon | XM\_024409452 | XP\_024265220 |  | LOC112241335 |
|  |  | XM\_024386464 | XP\_024242232 |  | LOC112223449 |
|  |  | XM\_024387283 | XP\_024243051 |  | LOC112223991 |
|  |  | XM\_024387282 | XP\_024243050 |  | LOC112223989 |
| *ch25h\_c1* | Rainbow trout | XM\_021571258 | XP\_021426933 |  | LOC110495809 |
| (MF095415) | Chinook salmon | XM\_024379494 | XP\_024235262 |  | LOC112218585 |
| *ch25h\_c2* | Common carp | XM\_019122127 | XP\_018977672 |  | LOC109109020 |
| (MF095416) | Chinook salmon | XM\_024380417 | XP\_024236185 |  | LOC112219235 |
| *ch25h\_d* (MF095417) | Common carp | XM\_019073746 | XP\_018929291 |  | LOC109056552 |
|  |  | XM\_019071129 | XP\_018926674 |  | LOC109053824 |
|  |  | XM\_019080025 | XP\_018935570 |  | LOC109062966 |
|  | Rainbow trout | XM\_021601299 | XP\_021456974 |  | LOC110522829 |
|  |  | XM\_021590696 | XP\_021446371 |  | LOC110509673 |
|  | Chinook salmon | XM\_024396823 | XP\_024252591 |  | LOC112230551 |

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**Supplementary Figure S3.** Phylogenetic relation of common carp, rainbow trout and Chinook salmon *ch25h* genes in comparison to human CH25H and zebrafish *ch25h a*, *b*, *c1*, *c2* and *d* genes. A maximum likelihood phylogenetic analysis was performed with PhyML on the coding nucleotide sequence of each gene. The primers used for gene expression were able to amplify homologues of the *danio rerio* *ch25h\_b* gene from common carp (Cyca) marked in blue, rainbow trout (Onmy) marked in red and chinnok salmon (Onts) marked in green. Human *CH25H* gene is marked in violet. The phylogenetic tree was rendered with TreeDyn. The phylogenetic analysis was performed using tools available at www.phylogeny.fr. The branch length is proportional to the number of substitutions per site. The branch supporting values are indicated in brown.

HosaCH25H\_NP\_003947 MSCHNCSDPQVLCSSGQLFLQPLWDHLRSWEALLQSPFFPVIFSITTYVGFCLPFVVLDI 60

Cyca\_MT602517 ----------------MFGLQYIWDSILQYEAMLRSPYFPVLFSITVYLSFCLPFVILDV 44

Cyca\_MT602518 ----------------MFGLQYIWDSILQYEAVLRSPYFPVFFSITVYLSFCLPFVALDA 44

Onmy\_XP\_021466841 -------MEPSLESSSPFLLQTLWDKIRAQEDFLRSPLFPVLFSMTLYLSCCLPYLCLDT 53

Onts\_XP\_024281174 -------MEPSLESSSPFLLQTLWDKIRAQEDFLRSPLFPVLFSMTLYLSCCLPYLCLDT 53

Onts\_XP\_024265220 -------MEPSLESSSPFLLQTLWDKIRAQEDFLRSPLFPVLFSMTLYLSCCLPYLCLDT 53

Onmy\_XP\_021420461 -----------------MLLQSLWDLILGYNAWLMSPFFPVLFSLSVYLAFCLPFVVLDL 43

Onts\_XP\_024242232 -----------------MLLQSLWDLILGYNAWLMSPFFPVLFSLSVYLAFCLPFVVLDL 43

Onts\_XP\_024243051 -----------------MLPQSLWDFILGYNAWLRSPFFPVLFSLSIYLTFCLPFVVLDL 43

Onmy\_XP\_021431438 -----------------MLLQSLWDFILGYHAWLRSPFFPVLFSLSVYLTFCLPFVVLDL 43

Onmy\_XP\_021431437 -----------------MLPQSLWDFILGYHAWLRSPFFPVLFSLSVYLTFCLPFVVLDL 43

Onts\_XP\_024243050 -----------------MLLQSLWDFILGHHVWLRSPFFPVLFSLSVYLTFCLPFVVLDL 43

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HosaCH25H\_NP\_003947 LCSWVPALRRYKIHPDFSPSAQQLLPCLGQTLYQHVMFVFPVTLLHWARSPALLPHEAPE 120

Cyca\_MT602517 LSPRVALIRRYKIQQKTSVSWTMMWSCLALSLYNHAMYIFPLSVLHWYWRPVSYPAMAPG 104

Cyca\_MT602518 LSSRVSWIRRYKIQQKTSVSWKMMWSCLALSLYNHAVYIFPLSVLHWYWRPVSYPVMAPG 104

Onmy\_XP\_021466841 LSSRVALVHRYKIQSQSRVTWAMAWSCLATSLHTHAVFIFPLSVLHWYWRPVVLPAQAPG 113

Onts\_XP\_024281174 LSSRVALVHRYKIQSQSRVTWAMAWSCLATSLHTHAVFIFPLSVLHWYWRPVVLPAQAPG 113

Onts\_XP\_024265220 LSSRVALVHRYKIQSQSRVTWAMAWSCXATSLHTHAVFIFPLSVLHWYWRPVVLPAXAPG 113

Onmy\_XP\_021420461 LSPRLAWIRTFKIQQKSHVSWTMMWSCLAHSLYNHVVFLFPLTVLHWYWRPASFIAEAPG 103

Onts\_XP\_024242232 LSPRLAWIRTFKIQQKSHVSWTMMWSCLAHSFYNHVVFLFPLTVLHWYWRPASFIAEAPG 103

Onts\_XP\_024243051 LSPRLAWIRTFKIQQKSHVSWTMMWSCLAHSLYNHVVFIFPLTVLHWFWRPATFMPEAPG 103

Onmy\_XP\_021431438 LSPRLAWIRTFKIQQKSHVSWTMMWSCLAHSLYNHVVFLFPLTVLHWFWRPATFMPEAPG 103

Onmy\_XP\_021431437 LSPRLAWIRTFKIQQKSHVSWTMMWSCLAHSLYNHVVFLFPLTVLHWFWRPATFMPEAPG 103

Onts\_XP\_024243050 LSPRLAWIRTFKIQQKSHVSWTMMWSCLAHSLYNHVVFLFPLTVLHWFWRPATFMPEAPG 103

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HosaCH25H\_NP\_003947 LLLLLHHILFCLLLFDMEFFVW**HLLHH**KVPWLYRTF**HKVHH**QNSSSFALATQYMSVWELF 180

Cyca\_MT602517 LLRVIWDLAACLLLFDFQYFVW**HLLHH**KVPWLYRTF**HKVHH**KYTSTFALATEYSGAWEIL 164

Cyca\_MT602518 LLRVIWDLAACLLLFDFQYFVW**HLLHH**KVPWLYRTF**HKVHH**KYTSTFALATEYSGAWETL 164

Onmy\_XP\_021466841 SLRVAWDVLACLLLFDLQYFVW**HVLHH**KVPWLYRTF**HKVHH**RYTATFALTTEHSGIWETL 173

Onts\_XP\_024281174 SLRVAWDVLACLLLFDLQYFVW**HVLHH**KVPWLYRTF**HKVHH**RYTATFALTTEHSGIWETL 173

Onts\_XP\_024265220 SLRVAWDVLACLLLFDLQYFVW**HVLHH**KVPWLYRTF**HKVHH**RYTATFALTTEHSGIWETL 173

Onmy\_XP\_021420461 TLRLIWDVVACLLLFDFQSFIW**HMLHH**KVPWLYRTF**HKVHH**MHTTTFALTTEYSGAWETL 163

Onts\_XP\_024242232 TLRLIWDVVACLLLFDFQSFIW**HMLHH**KVPWLYRTF**HKVHH**MHTTTFALTTEYSGAWETL 163

Onts\_XP\_024243051 TLRLIWDVVACLLLFDFQYFIW**HLLHH**KVPWLYRTF**HKVHH**KHTSTFALTTEYSGAWETL 163

Onmy\_XP\_021431438 TLRLIWDVVACLLLFDFQYFIW**HLLHH**KVPWLYRTF**HKVHH**KHTSTFALTTEYSGAWETL 163

Onmy\_XP\_021431437 TLRLIWDVVACLLLFDFQYFIW**HLLHH**KVPWLYRTF**HKVHH**KHTSTFALTTEYSGAWETL 163

Onts\_XP\_024243050 TLRLIWDVVACLLLFDFQYFIW**HLLHH**KVPWLYRTF**HKVHH**KHTSTFALTTEYSGAWETL 163

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HosaCH25H\_NP\_003947 SLGFFDMMNVTLLGCHPLTTLTFHVVNIWLSVEDHSGYNFPWSTHRLVPFGWYGGVVH**HD** 240

Cyca\_MT602517 SLGFFAAVNPMLLGVHPMTEMLFHMLNMWLSVEDHCGYDLPWATHRLVPFGLYGGAPH**HD** 224

Cyca\_MT602518 SLGFFAAVNPMLLGVHPMTEMLFHILNMWLSVEDHCGYDLPWATHRLVPFGLYGGAPH**HD** 224

Onmy\_XP\_021466841 SLGLFAAVNPNLLGCHPLTKMFFFTLNIWLSVEDHSGYDLPWAPHRLVPFGLYGGSPH**HD** 233

Onts\_XP\_024281174 SLGLFAAVNPNLLGCHPLTKMFFFTLNIWLSVEDHSGYDLPWAPHRLVPFGLYGGSPH**HD** 233

Onts\_XP\_024265220 SLGLFAAVNPNLLGCHPLTKMFFFTLNIWLSVEDHSGYDLPWAPHRLVPFGLYGGSPH**HD** 233

Onmy\_XP\_021420461 SLGFFSSVNPLLLGCHPLTEMLFYVLNIWLSVEDHCGYDLPWSTHRLVPFGLYGGAPH**HD** 223

Onts\_XP\_024242232 SLGFFSSVNPLLLGCHPLTEMLFYVLNIWLSVEDHCGYDLPWSTHRLVPFGLYGGAPH**HD** 223

Onts\_XP\_024243051 SLGFFAGVNPLLLGCHPLTEMLFYVLNIWLSVEDHSGYDLPWSTHRLVPFGLYGGALH**HD** 223

Onmy\_XP\_021431438 SLGFFAGVNPLLLGCHPLTEMLFYILNIWLSVEDHSGYDLPWSTHRLVPFGLYGGAPH**HD** 223

Onmy\_XP\_021431437 SLGFFAGVNPLLLGCHPLTEMLFYVLNIWLSVEDHSGYDLPWSTHRLVPFGLYGGAPH**HD** 223

Onts\_XP\_024243050 SLGFFAGVNPLLLGCHPLTEMLFYVLNIWLSVEDHSGYDLPWSTHRLVPFGLYGGAPH**HD** 223

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HosaCH25H\_NP\_003947 **LHH**SHFNCNFAPYFTHWDKILGTLRTASVPAR---------------------------- 272

Cyca\_MT602517 **VHH**Q*KFKSN*YAPYFTHWDKLFGTLHSE--------------------------------- 251

Cyca\_MT602518 **VHH**Q*KFKSN*YAPYFTHWDKLFGTLHSE--------------------------------- 251

Onmy\_XP\_021466841 **LHH**LKFMVNYAPYFTHWDRLFGSLLHTDKPDTFDVDVLDASKRCDTASSGVTDVSHAPVY 293

Onts\_XP\_024281174 **LHH**LKFMVNYAPYFTHWDRLFGSLLHTDKPDTFDVDVLDTSKRCDTASSGVTDVSHAPVY 293

Onts\_XP\_024265220 **LHH**LKFMVNYAPYFTHWDRLFGSLLHTDKPDTFDVDVLDXSKRCDTASSGVTDVSHAPVY 293

Onmy\_XP\_021420461 **MHH**L*KFKSN*YAPYFTHWDRLFGTLHKSSD------------------------------- 252

Onts\_XP\_024242232 **LHH**L*KFKSN*YAPYFTHWDRLFGTLHKSSD------------------------------- 252

Onts\_XP\_024243051 **LHH**L*KFKSN*YAPYFTHWDRVFGTLHKHSD------------------------------- 252

Onmy\_XP\_021431438 **LHH**L*KFKSN*YAPYFTHWDRVFGTLHKHSD------------------------------- 252

Onmy\_XP\_021431437 **LHH**L*KFKSN*YAPYFTHWDRVFGTLHKHSD------------------------------- 252

Onts\_XP\_024243050 **LHH**L*KFKSN*YAPYFTHWDRVFGTLHKHSD------------------------------- 252

:\*\* :\* \*:\*\*\*\*\*\*\*\*:::\*:\*

HosaCH25H\_NP\_003947 ----------------- 272

Cyca\_MT602517 ----------------- 251

Cyca\_MT602518 ----------------- 251

Onmy\_XP\_021466841 ETTQSCVKDYEVQFRGK 310

Onts\_XP\_024281174 ETTQSCVKDYEVQFRGK 310

Onts\_XP\_024265220 ETTQSCVKDYEVQFRGK 310

Onmy\_XP\_021420461 ----------------- 252

Onts\_XP\_024242232 ----------------- 252

Onts\_XP\_024243051 ----------------- 252

Onmy\_XP\_021431438 ----------------- 252

Onmy\_XP\_021431437 ----------------- 252

Onts\_XP\_024243050 ----------------- 252

**Supplementary Figure S4.** CLUSTAL Omega (1.2.4) multiple amino acid sequence alignment of cholesterol 25 hydroxylase of human (HosaCH25H GenBank ID: NP\_003947); and cholesterol 25 hydroxylase b of Chinook salmon (ch25h\_b, Onts GenBank ID: Onts\_XP\_024281174; Onts\_XP\_024265220; Onts\_XP\_024242232; Onts\_XP\_024243051; Onts\_XP\_024243050); rainbow trout (Onmy GenBank ID: Onmy\_XP\_021466841; Onmy\_XP\_021420461; Onmy\_XP\_021431438; Onmy\_XP\_021431437) and common carp (Cyca GenBank ID: MT602517; MT602518). The fatty acid hydroxylase superfamily conserved domains are shaded in gray. The histidine cluster motif (HXXHH) characteristic for di-iron-oxo proteins (Fe-O-Fe) such as fatty acid desaturases/hydroxylase enzymes is underlined. This motif is involved in coordination of the reception of electrons in reduction reactions. Putative dilysine motifs (KXKXX, at the C-terminus), thought to signal ER retention, are marked in cursive and underlined. The alignment was performed with the online CLUSTAL Omega tool (https://www.ebi.ac.uk/Tools/msa/clustalo/). Symbols (\* : .) under the alignment indicate levels of similarity of amino acids.



**Supplementary Figure S5.** Effect of the incubation of koi fin 1 (KF-1) cells with 10 µM of 25-hydroxycholesterol (25HC) for 8h prior to infection with CyHV-3 with MOI of 0.001 for 1h, following inoculation in medium with 10 µM of 25HC. Cells were harvested following 96h incubation. Virus content was measured by estimation of TCID50 / mL(A) or virus DNA copy numbers with qPCR (B) in medium and cells. Data presented as a bar indicating mean value for TCID50 /mL or number of virus DNA copies (+SD). \* indicates a statistically significant difference at p<0.05 between control and 25HC pre-treatment. Analysis was performed with two-way ANOVA with subsequent pairwise multiple comparisons using the Holm-Sidak method.

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**Supplementary Figure S6.** Effect of incubation of koi fin cells (KFC) with rainbow trout recombinant IFNa1 (recIFN) on the transcription of mRNA encoding the enzyme involved in the generation of 25-hydroxycholesterol *ch25h\_b* (A), the IFN gene *ifna2* (B) and the IFN stimulated genes mx2 (C) and *vig1* (D). Data presented as a bar indicating mean normalised copy numbers (+SD) from n=3 replicates. \* indicates a statistically significant difference at p<0.05 between control and the recombinant IFN treatment. Analysis was performed with two-way ANOVA with subsequent pairwise multiple comparisons using the Holm-Sidak method.



**Supplementary Figure S7.** Effect of 24h incubation of koi fin cells (KFC) with zoledronic acid on the transcription of mRNA encoding the enzyme involved in the generation of oxysterols *ch25h\_b* (A) *cyp71b* (B), farnesyl diphosphate synthase (*fdps*), a key enzyme in the sterol pathway(C), the IFN gene *ifna2* (D) and the IFN stimulated gene *vig1* (E). Data presented as a bar indicating mean normalised copy numbers (+SD) from n=3 replicates. No statistically significant difference at p<0.05 between control and treated cells was recorded. Analysis was performed with one-way ANOVA with subsequent pairwise multiple comparisons using the Holm-Sidak method.