**Table S1** List of the specific primers used for cloning and qPCR analysis. Sequence accession numbers, primer sequences and amplicon sizes are shown.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **GenBank Acc. No.** | **Sense primer 5’-3’ (Tm)** | **Antisense primer 5’-3’ (Tm)** | **Amplicon (bp)** |
| **Cloning** |  |  |  |  |
| *pept1a* (*slc15a1a*) | NC\_007120.7& | CTTTCACACACACACTCTCT (52 °C) | AACAGACCCCTGTATCATCAT (53 °C) | 2478 |
| **RT-PCR** |  |  |  |  |
| *actb* | NM\_131031.2 | CGTGACATCAAGGAGAAGCT (54 °C) | ATCCACATCTGCTGGAAGGT (55 °C) | 443 |
| *pept1a* (*slc15a1a*) | NC\_007120.7& | AGAACCGGCTGAGATGTAT (57 °C) | AAATACTGAGGAATCTGGAG (53 °C) | 351 |
| **qPCR** |  |  |  |  |
| *28S* | EF417169.1 | GGTCTAAGTCCTTCTGATGG (55 °C) | GGCTGCATTCCCAAACAAC (55 °C) | 112 |
| *pept1a* (*slc15a1a*) | NC\_007120.7& | AGAACCGGCTGAGATGTAT (57 °C) | GAAGGCTGAAGGCTGGACT (56 °C) | 132 |
| *pept1b (slc15a1b)* | NM\_198064.1 | TGTGACCATCTCTGCTGGAG (56°C) | CCGCGTGCACATTATCAGAC (56°C) | 206 |

GC content, end stability, self/cross-dimer formation, and melting temperature of the oligonucleotides were analyzed by using the software AmplifX version 1.7.0 (<https://inp.univ-amu.fr/en/amplifx-manage-test-and-design-your-primers-for-pcr>). Amplification products from the primer pairs were sequenced and identified by alignment with the reference mRNAs. &From GRCz11 (RefSeq Acc. No. GCF\_000002035.6; GenBank Acc. No. GCA\_000002035.4) Genome Assembly, Chr 9 (NC\_007120.7): 1,136,369-1,163,151. Tm, melting temperature.

 -44 ctttcacacacacactctctcacacacacgctcctgctgccaac

 1 ATGCCAGACTCAAAGATGGACGAGACGAAGAAGAAGAAGAAAAAG

1 **M P D S K M D E T K K K K K KK**

 46 ACGGCTGAGTGCTGTGGATATCCCATCAGCATCTTCTTCATTGTG

16 **T A E C C G Y P I S I F F I V**

 <--------------------------------I-

 91 GTCAATGAGTTCTGTGAGAGATTCTCCTATTATGGGATGCGCGCT

31 **V N E F C E R F S Y Y G M R A**

 ------------------------------->

 136 GTGCTGGTGCTGTATTTCCGCTATTTTCTGCTGTGGGACGATGAT

46 **V L V L Y F R Y F L L W D D D**

 181 CTGGCAACCTCCATCTACCATGCGTTCGTGGCGCTCTGCTACCTG

61 **L A T S I Y H A F V A L C Y L**

 <--------------------------------II---------

 226 ACGCCCATCCTGGGGGCCATCATCGCCGACTCCCGGCTCGGCAAG

76 **T P I L G A I I A D S R L G K**

 ---------------------->

 271 TTCAAGACCATCATATACCTGTCTATAGTGTACGCAGTGGGGCAG

91 **F K T I I Y L S I V Y A V G Q**

 <-------------------------------

 316 GTGGTCATGGCCGTCAGCACTATTCATGACATCACTGACGCTAAC

106 **V V M A V S T I H D I T D A N**

 III------------------------------->

 361 AGAGACGGCACACCGGACAACTTCACCTTACACATTGCTCTCTCT

121 **R D G T P D N F T L H I A L S**

 <-------------------------

 406 ATGCTGGGTTTGGTCCTCATAGCTCTGGGCACCGGAGGAATTAAA

136 **M L G L V L I A L G T G G I K**

 -------IV------------------------------->

 451 CCGTGTGTTGCAGCGTTTGGTGGAGATCAGTTTCAGGAGCATCAG

151 **P C V A A F G G D Q F Q E H Q**

 496 AGTCGGCAGCTCAACACTTTTTTCTCAGTGTTTTATTTGTGCATC

166 **S R Q L N T F F S V F Y L C I**

 <-------------------------------

 541 AACGCTGGAAGTCTGCTTTCCACACTCATCACGCCTGTGCTCAGA

181 **N A G S L L S T L I T P V L R**

 V------------------------------>

 586 GCTCAGGAGTGTGGCATCCACACGCAGCAGCAGTGCTATCCGCTT

196 **A Q E C G I H T Q Q Q C Y P L**

 631 GCTTTCGGGGTCCCGGCAGCTCTCATGGTGGTGTCTCTGGTGGTG

211 **A F G V P A A L M V V S L V V**

 <--------------------------------VI---------

 676 TTTATTGCGGGCAGTGGCATGTACACCAAAACTGCTCCAGAGGGA

226 **F I A G S G M Y T K T A P E G**

 ---------------------->

 721 AACATTATGGGCTCTGTGTGTAAATGCATATGGTTTGCTCTGAAT

241 **N I M G S V C K C I W F A L N**

 766 AACCGTTTCAGACACCGAAGCGATATTTATCCAAAGAGGGAGCAC

256 **N R F R H R S D I Y P K R E H**

 811 TGGATGGACTGGGCGGAGGAGAAATATGATAAACTCCTCATTGCG

271 **W M D W A E E K Y D K L L I A**

 <----------

 856 CAGATAAAGATGGTGCTGAAGGTGTTGTTCCTCTACATCCCCCTG

286 **Q I K M V L K V L F L Y I P L**

 ---------------------VII---------------------

 901 CCCATGTTTTGGACCCTGTTTGACCAGAAGGGCTCCCGCTGGACT

301 **P M F W T L F D Q K G S R W T**

 ---------->

 946 CTACAAGCCACCACCATGACCGGAGACTTTGGAGGGTTCGTCCTG

316 **L Q A T T M T G D F G G F V L**

 991 CAGCCAGACCAGATGCAGACGGTGAACCCCATCCTCATCTTGACC

331 **Q P D Q M Q T V N P I L I L T**

 <----------------------------

1036 CTGGTGCCCATCATGGACAGAATTGTTTTCCCTCTCATAAAAAAG

346 **L V P I M D R I V F P L I K K**

 ---VIII------------------------------>

1081 TGTGGCCTCAATTTCAGCCCTTTGAAGAGAATGACGGTCGGCATG

361 **C G L N F S P L K R M T V G M**

 <-------------

1126 TTGTTCGCTGCCACAGCGTTTATTGCTGCTGCTCTGGTGCAGATG

376 **L F A A T A F I A A A L V Q M**

 -----------IX------------------------>

1171 GAGGTTGATAAAACCTTGCCGAATTTCCCATCATCCTCTGAGAGC

391 **E V D K T L P N F P S S S E S**

1216 CAGCTGAAGGTGGTGAATATGCACAGCGAGTCTCTCATAGTGACT

406 **Q L K V V N M H S E S L I V T**

1261 GTGCCGTCCCAAGAGCCTCTACTGATCGGCTCATTTGAGAGCAGT

421 **V P S Q E P L L I G S F E S S**

1306 CCAGATTACATTACGTTTGGCCAGCAGGACATCAGGTTAGCCTTT

436 **P D Y I T F G Q Q D I R L A F**

1351 TACACAACTCCTGCGATCAATAAAGATTTGAGTTTAATCAAAGGC

451 **Y T T P A I N K D L S L I K G**

1396 AGCCGTCAGACCCTGATCATCCCCTCAGAACCGGCTGAGATGTAT

466 **S R Q T L I I P S E P A E M Y**

1441 CTGAAAGAAGACATCAAGTCTAAACCAAAGGAAGGGAAGAATGCT

481 **L K E D I K S K P K E G K N A**

1486 GTCAGGTTTGTAAACGGCTGGACTGCATATCTGAACATCACTAAC

496 **V R F V N G W T A Y L N I T NN**

1531 CTGGAGTCCAGCCTTCAGCCTTCAGAAACGTCAAACTACACGCTG

511 **L E S S L Q P S E T S N Y T LL**

1576 GTCTCTCAGGGCATGCGTAAGTTCACGCTAACCAATGGTATTCAG

526 **V S Q G M R K F T L T N G I Q**

1621 TCGTGTGAGTTTTCACGGAAGTTTGGCTTCGGTTCCTCCTACACT

541 **S C E F S R K F G F G S S Y T**

1666 TTCCTGATCCCCAGCGATCTGTTCTCCACTGATTGTGAGTCTATA

556 **F L I P S D L F S T D C E S I**

1711 AAGGAGATTGAAGACATGCAGCCCAACTCGGTGCACATGGCTCTC

571 **K E I E D M Q P N S V H M A L**

 <-------

1756 CAGATTCCTCAGTATTTCCTCATCACTACGGGAGAGGTCATGTTC

586 **Q I P Q Y F L I T T G E V M F**

 -------------------------X-------------------

1801 TCCGTCACCGGTCTGCAGTTCTCATACTCACAGGCTCCCAAAAAC

601 **S V T G L Q F S Y S Q A P K N**

 ------------->

1846 ATGAAGTCGGTGCTGCAGGCCGGCTGGCTGTGCACTAACGCAGTG

616 **M K S V L Q A G W L C T N A V**

 <--------------------------------XI

1891 GGAAACATCATCGTGCTGATCGTGGCGGAGCTGGGGAAACTTCCC

631 **G N I I V L I V A E L G K L P**

 ------------------------------->

1936 AAACAGTGGGCAGAGTATGTGCTGTTTGCGTCGCTGCTAGTAGCT

646 **K Q W A E Y V L F A S L L V A**

 <----------------------------

1981 GTTAGCATCATCTTCTCCATCATGGCGTATTTCTACACCTACATC

661 **V S I I F S I M A Y F Y T Y I**

 --XII-------------------------------->

2026 GACCCAGCGGAGATTGAAGCCGAGATCCTGAAACAGCAAGAGACT

676 **D P A E I E A E I L K Q Q E T**

2071 GATCCAGACAAGAAGAAGAAGAAGGAGACTCTAGAAATGGAGGAA

691 **D P D K K K K K E T L E M E E**

2116 AAGGAGAACGAGCAGGAAATCAAACAAACCAAGATTTAAgacttg

706 **K E N E Q E I K Q T K I \***

2161 aagattgagttacgctcatgctaatatcttccctttacttccgta

2206 tttattaaagcaggtttgaaggactgtgagcagacgtgtgtgtgt

2251 ttatgattgatttgcatctgttgttgatacatttagatgattatt

2296 gatgagagccaattgagtcgacaatcatatgtagcattaaacatt

2341 taatgtgtaaaataaactttgttatggatatggattgtgatgtag

2386 atggactttagtgagtgtttttgtgtgtgttttgacttttttaag

2431 ctaataatcaatggactatgatgatacaggggtctgtt

**Fig. S1** Nucleotide and predicted amino acid sequence of zebrafish *pept1a* (*slc15a1a*) obtained using ORFfinder (<https://www.ncbi.nlm.nih.gov/orffinder/>). Numbers on the left refer to the nucleotide (upper row) and amino acid (lower row) positions. Nucleotides are numbered, starting from the first ATG initiation codon. \* indicates the stop codon. The specific primers used for cloning and PCR analyses (**Table S1**) are indicated in red and green, respectively. In the amino acid sequence, putative transmembrane domains, obtained using the TMHMM v. 2.0 program as implemented in SMART, are indicated and named I to XII. Potential extracellular N-glycosylation sites (white boxes), potential cAMP/cGMP-dependent protein kinase phosphorylation sites at the cytoplasmic surface (light gray boxes) and potential protein kinase C phosphorylation sites at the cytoplasmic surface (dark gray boxes) were obtained using the ScanProsite tool.



**Fig. S2** Current-voltage relationships of transport-associated currents in zebrafish PepT1a, in the presence of 3 mmol/L Gly-Gln in sodium (NaCl) saline buffer (black square) and tetramethylammonium (TMACl) saline buffer (empty circle) at pH 7.6 (see **Methods** for details). Values are means ± SEM from 5 oocytes from one batch each group. The transport-associated current values reported were obtained by subtracting the current recorded in the absence of the substrate to that recorded in its presence.



**Fig. S3** Expression analysis by RT-PCR on *pept1a* (*slc15a1a*) mRNA in different sections of adult zebrafish intestine. **a** RT-PCR assay on cDNA templates from total RNA extracted from whole gut (gut), intestinal bulb (I. bulb), mid intestine (mid) and posterior intestine (posterior); a PCR product of ~350 bp related to *pept1a* (*slc15a1a*) mRNA is present in all intestinal samples; L: 1 Kb Plus DNA ladder (Thermo Fisher Scientific). **b** A graphic representation of the adult zebrafish gut anatomy with its major adjacent tracts.