1 **Supplementary Information**

2 Cubam receptor-mediated endocytosis in the hindgut-derived pseudoplacenta of a viviparous teleost

3 *Xenotoca eiseni*

4

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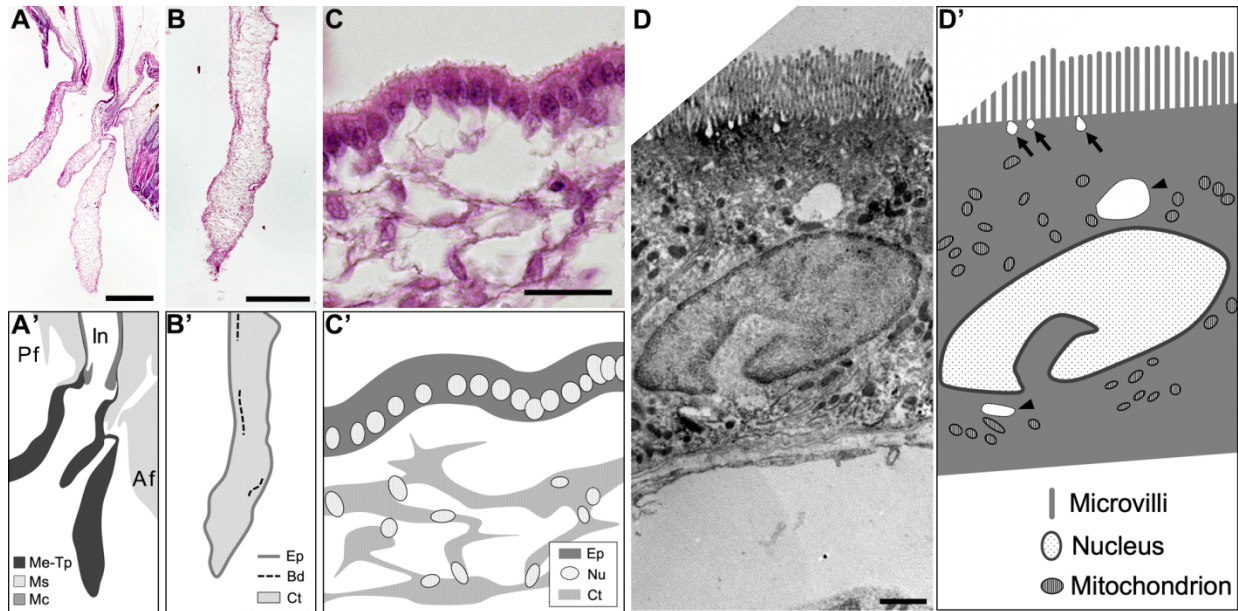
11 **This PDF file includes:**

12   Figures S1 to S6

13   Tables S1 to S7

14

15 **Other supplementary materials for this manuscript include the following:**

16   Dataset S1

17   **Additional Figures**

19   **Figure S1. Tissue structures of the trophotaenia in *Xenotoca eiseni* embryos.**

20   **A-A'**. Hematoxylin and eosin (HE)-stained section (A) and corresponding illustration (A') of the tissue

21   structure around the anus at the border of the hindgut and trophotaenia of the embryo at the fourth

22   gestational week. Trophotaeniae are sequential to the mesenchymal layer of the intestine. In, intestine; Pf,

23   pelvic fin; Af, anal fin; Me-Tp, mesenchyme-to-trophotaenia; Ms, muscle layer; Mc, mucosal layer. Scale

24   bar: 500 µm. **B-B'**. HE-stained section (B) and corresponding illustration (B') of the internal structure of the

25   trophotaenia. Ep, epithelial layer cells; Bd, blood vessel; Ct, connective tissue. Scale bar: 500 µm. **C-C'**.

26   Enlarged image of the HE-stained section (C) and corresponding illustration (C') of the epithelial layer of the

27   trophotaenia. Ep, epithelial layer; Nu, nucleus; Ct, connective tissue. Scale bar: 20 µm. **D-D'**. Electron

28   microscopy (D) and corresponding illustration (D') of an epithelial layer cell of the trophotaenia. Microvilli

29   are distributed on the apical surface of the cells. Arrows indicate endocytic vesicles during the invagination

30   phase. Arrowheads indicate intracellular vesicles after endocytosis. Scale bar: 1 µm.
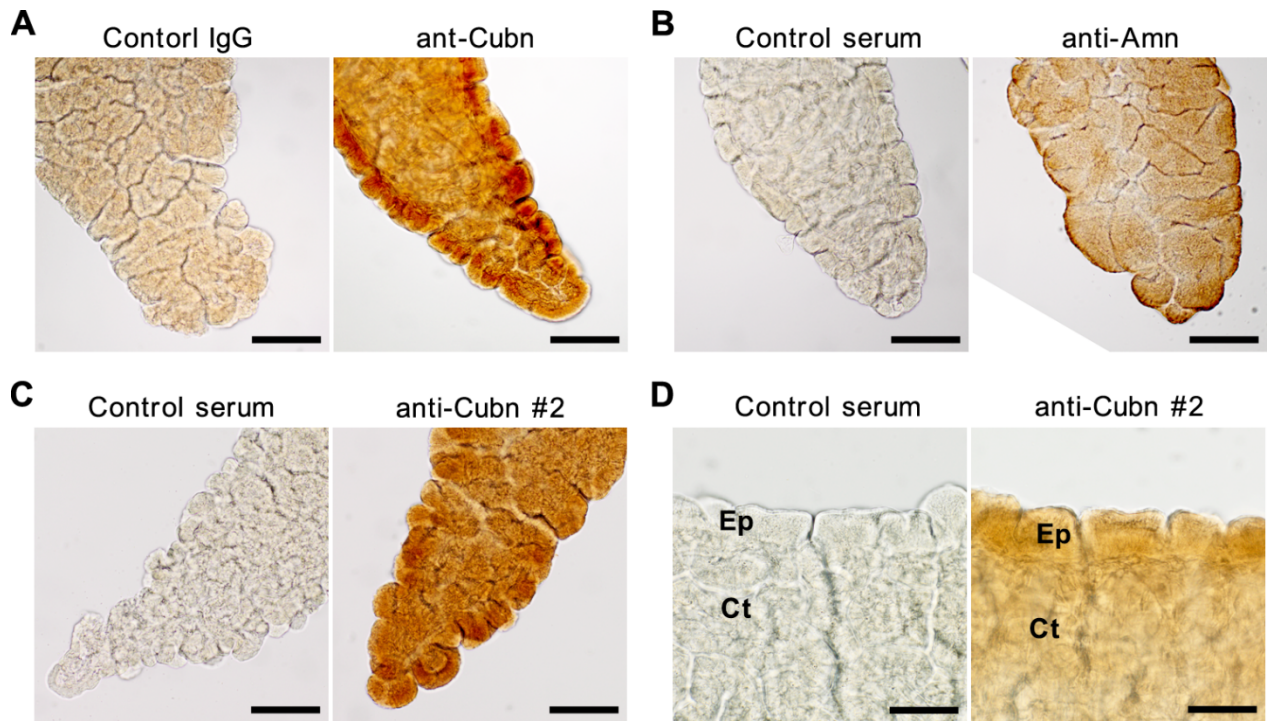
Figure S2. Immunohistochemistry of Cubn or Amn in the trophotaenia.

A-B. Immunohistochemistry using the Cubn antibody (A) or Amn antiserum (B) in the apical terminal of the trophotaenia. Scale bar: 100 µm. **C-D**. Immunohistochemistry using the Cubn antiserum in the apical terminal (C) or the epithelial layer (enlarged image) (D) of the trophotaenia. Ep, epithelium layer; Ct, connective tissue. Scale bar: 100 µm (C) or 50 µm (D).

| Bright field | DAPI | Alexa488 | Merge |

Control IgG
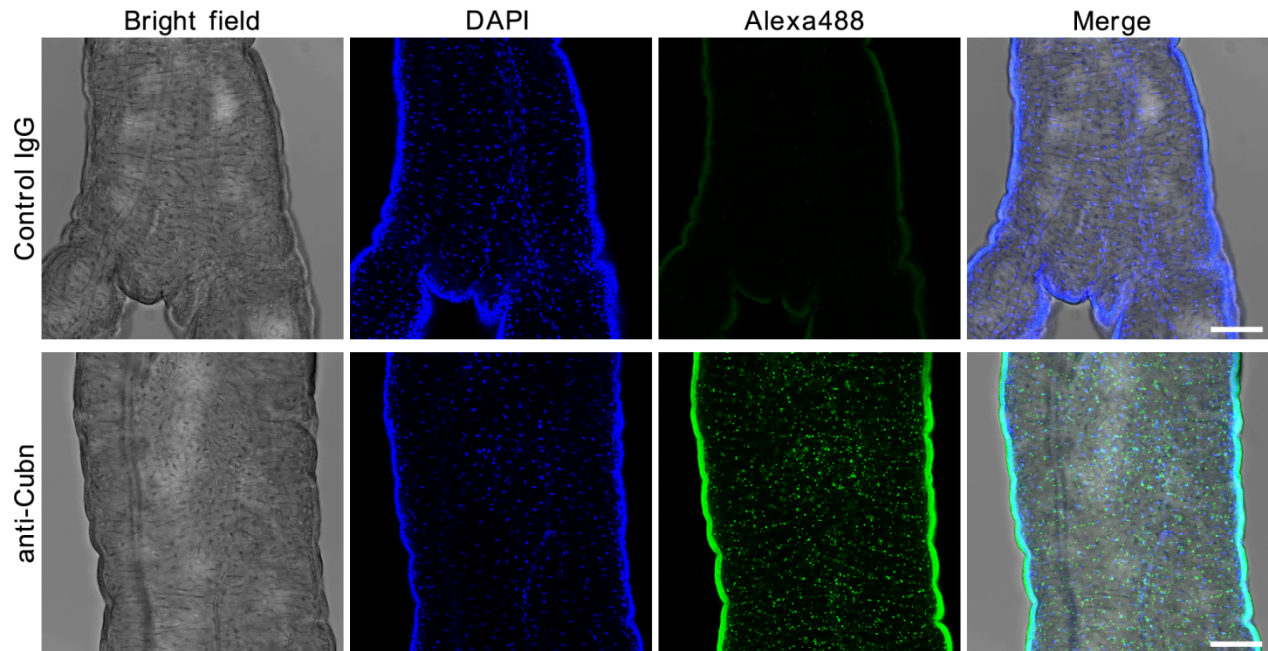
anti-Cubn

Figure S3. Fluorescent immunohistochemistry using Cubn antibody in the trophotaenia.

Confocal microscopy analysis of the trophotaenia of *Xenotoca eiseni* embryos at the fourth week of gestation. Anti-Cubn signals are observed in the epithelial layer cells of the trophotaenia. DAPI, 4′,6-diamidino-2-phenylindole. Scale bar: 20 μm.

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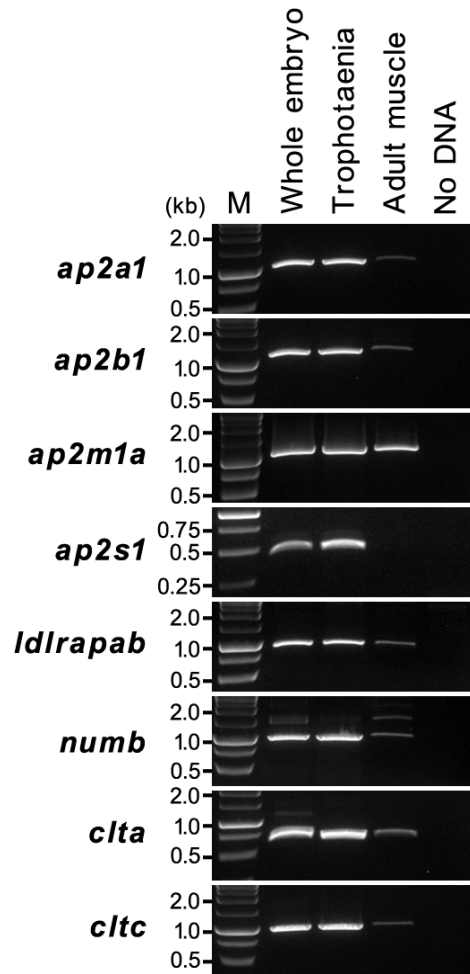
Figure S4. Gene expression analysis of the endocytic adaptor and vesicle coating proteins in the

respective tissues.

M, marker.

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H.sapiens DAB2 (NP_001334.2)  MSNEVETSATN-GQPDQ--------------QAAPKAPSKKEKKKGPEK 34
X.eiseni  dab2                MSTEVENSVPATADPSSPSTASTTISPTTSPVTTTPKAQLKKEKKKVPEK 50
                              **.***.*..  .:*..                ::*** ****** ***

H.sapiens DAB2 (NP_001334.2)  TDEYLLARFKGDGVKYKAKLIGIDDVPDARGDKMSQDSMMKLKGMAAAGR 84
X.eiseni  dab2                TDEYLLSRFQGDGVRYKAKLIGIDDVSEARGDKMCQDSMMKLKGMAVAAR 100
                              ******:**:****:***********.:******.**********.*.*

H.sapiens DAB2 (NP_001334.2)  SQGQHKQRIWVNISLSGIKIIDEKTGVIEHEHPVNKISFIARDVTDNRAF 134
X.eiseni  dab2                SQGKHKQRIWVNISMSGLKIIDEKSGVIEHEHVVNKISFIARDVTDNRAF 150
                              ***:**********:**:******:****** *****************

H.sapiens DAB2 (NP_001334.2)  GYVCGGEGQHQFFAIKTGQQAEPLVVDLKDLFQVIYNVKKKEEEK-KKIE 183
X.eiseni  dab2                GYVCGAEGQHQFFAIKTAQQAEPLVIDLKDLFQVIFNMRKKEAESSQKAE 200
                              *****.***********.*******:*********:*::*** *. :* *

H.sapiens DAB2 (NP_001334.2)  EASKAVENGSEALMILDDQTNKLKSGVDQMDLFGDMSTPPDLNSPTESKD 233
X.eiseni  dab2                NGSAVVENGG-ALQSTDGES-KAAQPVEQLDLFGDITTPPDIRAP----- 243
                              :.* .****. **    *.:: *  .  *:*:*****::****:.:*

H.sapiens DAB2 (NP_001334.2)  ILLVDLNSEIDTNQNSLRENPFLTNGITSCSLPRPTPQASFLPENAFSAN 283
X.eiseni  dab2                --------------------------------------------------


H.sapiens DAB2 (NP_001334.2)  LNFFPTPNPDPFRDDPFTQPDQSTPSSFDSLKSPDQKKENSSSSSTPLSN 333
X.eiseni  dab2                ----------------------------------------NSGSS------ 248
                                                                      **.**

H.sapiens DAB2 (NP_001334.2)  GPLNGDVDYFGQQFDQISNRTGKQEAQAGPWPFSSSQTQPAVRTQNGVSE 383
X.eiseni  dab2                --------------------------------------------------


H.sapiens DAB2 (NP_001334.2)  REQNGFSVKSSPNPFVGSPPKGLSIQNGVKQDLESSVQSSPHDSIAIIPP 433
X.eiseni  dab2                --------------------------------------------------


H.sapiens DAB2 (NP_001334.2)  PQSTKPGRGRRTAKSSANDLLASDIFAPPVSEPSGQASPTGQPTALQPNP 483
X.eiseni  dab2                ------------------DLFGTDLFVPPVS------SETSPADLFNNTP 274
                                                **:.:*:*.****       * *.. .:: .*

H.sapiens DAB2 (NP_001334.2)  LDLFKTSAPAPVGPLVGLGGVTVTLPQAGPWNTASLVFNQSPSMAPGAMM 533
X.eiseni  dab2                TNTVPSTIPALG--SLQLGPTATSVPAVGMWGTSPAVPAMFP--MPGIVT 320
                               : . :: **      : ** .:.::* .* *.*:. *    *   ** :

H.sapiens DAB2 (NP_001334.2)  GGQPSGFSQPVIFGT--SPAVSGWNQPSPFAASTPPPVPVVWGPSASVAP 581
X.eiseni  dab2                PGLRPNFPQPTAFGVPMQPPVWAPQVVPQFSAAPLSPPHLQWGQPATS-- 368
                               *  ..*.**. **.  .*.* .:  . *:*:. .*  : ** .*:

H.sapiens DAB2 (NP_001334.2)  NAWSTTSPLGNPFQSNIFPAPAVSTQPPSMHSSLLVTPPQPPPRAGPPKD 631
X.eiseni  dab2                ----------NPFQ-------AMGDHGPSRP------PPRPPVKETPPR- 394
                                        ****        *:. : **       **:** :  **:

H.sapiens DAB2 (NP_001334.2)  ISSDAFTALDPLGDKEIKDVKEMFKDFQLRQPPAVPARKGEQTSSGTLS- 680
X.eiseni  dab2                VENSAFTALDPLGDKEKKTGKDMFKNFQIAKLPAIPARKGELMPSSTPPP 444
                              :...************ *  *:***:**: : **:****** .*.* .

H.sapiens DAB2 (NP_001334.2)  ------AFASYFNSKVGIPQENADHDDFDANQL-LNKINEPPKPAPRQVS 723
X.eiseni  dab2                ANKESVPFDEYFSNKVGLAQDAADHDDFDINQMSILDGNDAPKQTPVQFT 494
                                  .* .**..**:.*: ******* **: : . *:.** :* *.:

H.sapiens DAB2 (NP_001334.2)  LPVTKS------TDNAFENP-----------------FFKDSFGSSQASV 750
X.eiseni  dab2                APAAAPSFTPDLLDAAFSSAPVPNSSAPTLGQDLSHDMFDQAFGAPDPNP 544
                               *.: .       * **...                 :*.::**:.:..

H.sapiens DAB2 (NP_001334.2)  ASSQPVSS----------EMYRDPFGNPFA 770
X.eiseni  dab2                FGAPPVAMNTVAQTSGSTDAFGDAFGNPFA 574
                               .: **:           : : *.******
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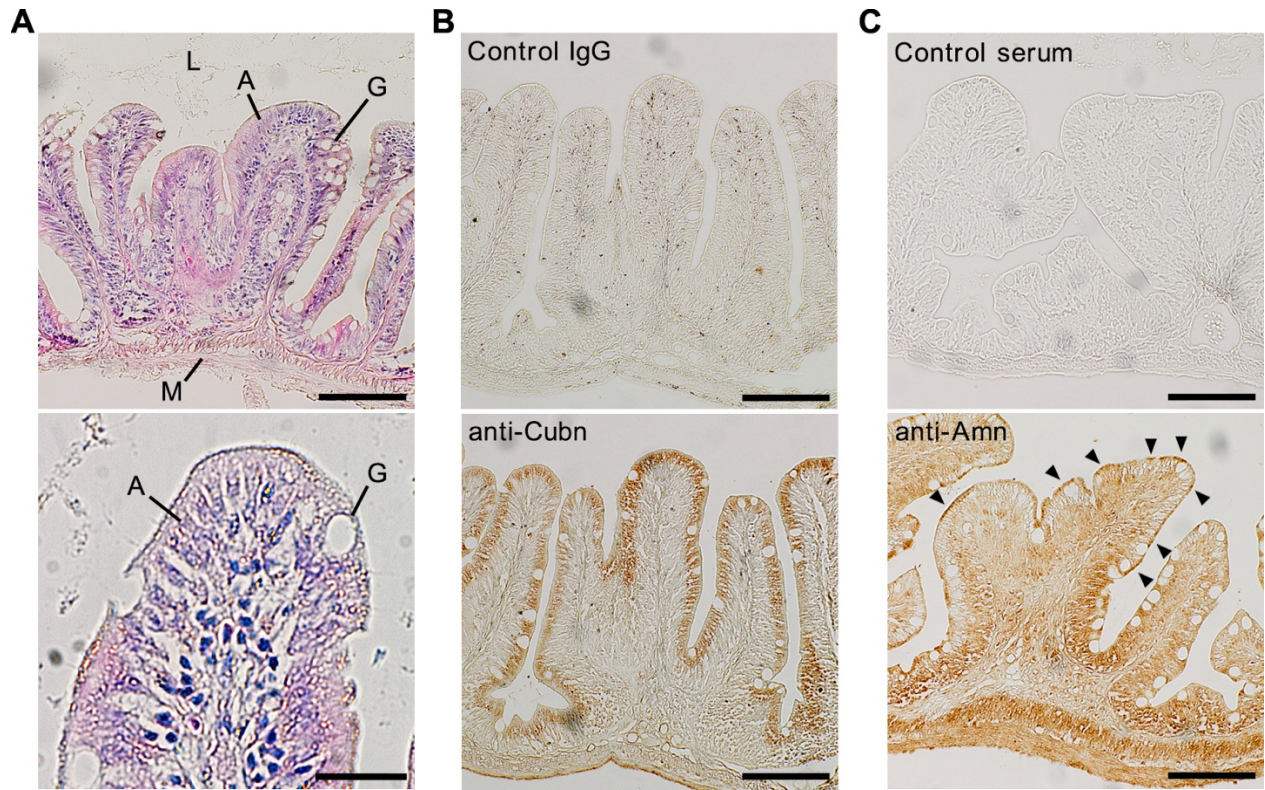
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48 **Figure S5. Amino acid sequence alignment for *Homo sapiens* (*H. sapiens*) DAB2 and *Xenotoca eiseni***

49 **(*X. eiseni*) dab2-like proteins.**

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Figure S6. Immunohistochemistry of Cubn or Amn in the adult intestine of *Xenotoca eiseni*.

A. Hematoxylin and eosin-stained section of the hindgut of an adult female. The lower panel is an

enlarged image of an intestinal villus. A, absorbent epithelium; G, goblet cell; L, intestinal lumen; M,

muscularis mucosae. Scale bar: 100 μm (upper) and 20 μm (lower). **B**. Immunohistochemistry using the

Cubn antibody or control IgG. Scale bar: 100 μm. **C**. Immunohistochemistry using the Amn antiserum or

control serum. Arrowheads indicate signal in the apical surface of the absorbent epithelium. Scale bar:

100 μm.

Additional Tables

		cubn	*amn*	*lrp1aa*	*lrp2a*
Third week	#1	1421	745	6	12
	#2	768	966	4	6
	#3	1414	1269	10	10
	Average	**1201**	**993**	**7**	**9**
Fourth week	#1	1528	1139	14	12
	#2	1464	818	10	7
	#3	1250	1032	8	12
	Average	**1414**	**996**	**11**	**10**

Table S1. The transcripts per million (TPM) values for endocytic receptor genes in the RNA-seq analysis.

		ap2a1	*ap2b1*	*ap2m1a*	*ap2s1*	*ldlrapab*	*numb*
	#1	75	91	383	365	7	19
	#2	60	54	346	318	6	15
Third week	#3	73	98	360	368	7	19
	Average	**69**	**81**	**363**	**350**	**7**	**18**
	#1	78	84	349	359	8	17
	#2	89	83	374	370	8	15
Fourth week	#3	82	85	385	371	6	13
	Average	**83**	**84**	**369**	**367**	**7**	**15**

61 **Table S2. The TPM values for endocytic adaptor genes in the RNA-seq analysis.**

		clta	*cltb*	*cltc*	*cav2*	*cav3*	*flot1b*	*flot2a*
	#1	851	67	337	1	0	229	91
	#2	680	76	209	0	0	196	50
Third week	#3	744	73	308	2	0	221	89
	Average	**758**	**72**	**285**	**1**	**0**	**215**	**77**
	#1	664	97	328	4	0	228	228
	#2	739	127	366	2	0	238	238
Fourth week	#3	817	104	343	3	0	234	234
	Average	**740**	**109**	**346**	**3**	**0**	**233**	**233**

62   **Table S3. The TPM values for vesicle coating protein genes in the RNA-seq analysis.**

		ctsa	*ctsb*	*ctsc*	*ctsh*	*ctsk*	*ctsla*	*ctsl.1*	*ctso*	*ctss2.1*	*ctsz*
	#1	862	474	1867	52	11	831	12749	3	2144	66
	#2	691	234	1113	31	17	736	8412	5	1656	63
Third week	#3	939	429	1907	52	14	939	11227	7	2042	63
	Average	**831**	**379**	**1629**	**45**	**14**	**835**	**10796**	**5**	**1947**	**64**
	#1	751	2686	2273	47	24	960	17795	6	2494	530
	#2	749	2559	2320	55	24	809	18334	7	2500	551
Fourth week	#3	720	1944	1884	48	18	924	15906	8	2342	523
	Average	**740**	**2396**	**2159**	**50**	**22**	**898**	**17345**	**7**	**2445**	**535**

63   **Table S4. The TPM values for protease genes in the RNA-seq analysis.**

11

		1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
No lysate	#1	68.8	67.8	67.2	65.8	65.5	65.8	65.6	65.2
	#2	66.5	65.9	64.8	62.7	64.0	64.4	63.8	62.7
	#3	66.8	65.4	69.8	63.1	63.3	62.8	61.8	62.6
	Average	**67.4**	**66.3**	**67.3**	**63.8**	**64.3**	**64.3**	**63.8**	**63.5**
Lysate	#1	260.3	345.0	437.7	530.4	579.7	637.3	699.6	694.1
	#2	264.9	351.6	460.3	551.3	617.0	678.4	727.0	748.2
	#3	256.6	339.2	441.8	522.7	587.1	642.9	692.5	720.8
	Average	**260.6**	**345.3**	**446.6**	**534.8**	**594.6**	**652.9**	**706.3**	**721.0**
Lysate + inhibitor	#1	94.0	96.2	98.0	99.6	101.0	106.8	104.4	101.9
	#2	88.1	88.8	91.1	91.5	93.4	94.0	98.0	98.2
	#3	88.3	88.4	89.4	93.1	94.2	96.4	99.4	101.0
	Average	**90.1**	**91.2**	**92.8**	**94.7**	**96.2**	**99.0**	**100.6**	**100.4**

64 **Table S5. The measured fluorescent signal values indicating cathepsin L-dependent proteolysis.**

Gene	Accession #		Primer sequence (5'-3')	Related figures
cubn	LC595284	F	ATGGCTGTTACAGTGCAGCCTTCA	3A
		R	GGCATGTACATACAGGGAATGCTG	
amn	LC595285	F	TGCCCTTTACAAGCAGTGGATTCC	3A
		R	GCGGATTAGCACAACCACAATCAC	
lrp2a	LC595286	F	ATGTGGAGAACACCGCTGCTTCAA	3A
		R	CCACATGGAGCAGTCATCGAAATC	
ap2a1	LC595287	F	GCTGTGTCGAAGGGAGATGGAATG	S4
		R	AGAGCTAGATAGCGCAGATTGGTC	
ap2b1	LC595288	F	GTCATTGCTGCCATGACTGTTGGC	S4
		R	CCACAATAGCCTCCTGAACCACAT	
ap2m1a	LC595289	F	GACGTAATGACGGCCTACTTTGGC	S4
		R	CAGCAGCGGGTTTCATAGATGCCA	
ap2s1	LC595290	F	ATGATCCGCTTCATCCTCATCCAG	S4
		R	CTCTAGTGACTGAAGCATGAGGAG	
ldlrap1b	LC595291	F	GGACGCTTTAAAATCCGCTGGAAG	S4
		R	GCAGCTGAGTCTGTCTCATCTTGG	
numb	LC595296	F	GAATAAGCTACGGCAGAGCTTCCG	S4
		R	TTGGGCATTGGAGCGGAAGAGAAC	
clta	LC595292	F	GGATGATTTTGACATGCTGAACGC	S4
		R	TAACGGACCAGCGGGGACTGCTTT	
cltc	LC595293	F	CTCAGATCCTGCCAATTCGCTTTC	S4
		R	TCTTCTTCCACGCAAACAGACAGC	
ctsl.1	LC595294	F	CAGCATCTCTCTGGAAGATCTCGA	4B
		R	CAGACTAGTGGATAACTTGCTGCG	
actb	LC595295	F	ATGGAAGATGAAATCGCCGCACTG	3A, 4B
		R	GAAGCATTTACGGTGGACGATGGA	

Table S6. List of primers used in the study. F, forward, R, reverse.

Name	Immunogen	Host	Supplier	ID	Related figures
CUBN Polyclonal Antibody (anti-Cubn)	Human CUBN	Rabbit	Invitrogen	PA5-83684	3C, 3E, 3G, S2, S3
Rabbit IgG Isotype Control (Control IgG)	N/A	Rabbit	Invitrogen	02-6102	3C, 3E, 3G, S2, S3
Cubn antiserum (anti-Cubn #2)	*Xenotoca eiseni* cubn	Mouse	N/A	N/A	S2
Amn antiserum (anti-Amn)	*Xenotoca eiseni* amn	Mouse	N/A	N/A	3D, S2
Preimmune serum (Control serum)	N/A	Mouse	N/A	N/A	3D, S2
Goat Anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor Plus 488	Rabbit IgG	Goat	Invitrogen	A32723	3E, S3
Horse Anti-Mouse IgG Antibody (H+L), Peroxidase	Rabbit IgG	Horse	VECTOR	PI-2000	3D, S2
Goat Anti-Rabbit IgG Antibody (H+L), Peroxidase	Mouse IgG	Goat	VECTOR	PI-1000	3C, S2
Goat Anti-Rabbit IgG Antibody (H+L), Biotinylated	Rabbit IgG	Goat	VECTOR	BA-1000	3G

66   **Table S7. List of antibodies used in the study.**

67   N/A, not applicable.

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