Figure legends (Supplemental figures)

<u>Figure S1.</u> Amino acid sequence and genomic structure of *pst-1*

(A) Amino acid sequence alignment of *C. elegans* PST-1b (Ce_PST-1b/1-440) with human PAPST1 (Hs_PAPST1/1-432) and *Drosophila* SLALOM (Dm_SLALOM/1-465). Khaki lines and the red line show the nine predicted transmembrane domains and the missing region, respectively, predicted in *pst-1*(tm3364). (B) Genomic structure of *pst-1* (*M03F8.2*) and nearby genes. Note the 2 kb scale bar (lower right). (C) The *pst-1* gene intron-exon structures and reporter constructs. The 5'-upstream regions contain the promoters included in the reporter constructs ($P_{pst\text{-}1a}$*::egfp* and $P_{pst\text{-}1bc}$*::egfp*). The reporter constructs end at the ATG transcription initiation sequences. The full-length constructs (*pst-1ab(fl)::egf*p and *pst-1c(fl)::egfp*) end at the TAA signal for the addition of a polyA tail. Note the 1 kb scale bar (lower right).

<u>Figure S2</u>. Amino acid sequence and genomic structure of *pst-2*

(A) Amino acid sequence alignment of *C. elegans* PST-2a (Ce_PST-2a/1-364) with human PAPST2 (Hs_PAPST2/1-401) and *Drosophila* PAPST2 (Dm_PAPST2/1-396). Khaki lines and the red line show the 10 predicted transmembrane domains and the missing region, respectively, predicted in *pst-2*(tm3316). (B) Genomic structure of *pst-2* (*F54E7.1*) and nearby genes. Note the 1 kb scale bar (lower right). (C) The *pst-2* intron-exon gene structure and reporter constructs. The 5'-upstream regions contain the promoters included in the reporter constructs ($P_{pst\text{-}2}$*::egfp* and *pst-2b(fl)::egfp*). The reporter constructs end at the ATG transcription initiation sequences. The full-length construct (*pst-2a(fl)::egfp*) ends at the TAG stop codon. Note the 0.5 kb scale bar (lower right).

<u>Figure S3</u>. Defects in seam cell maturation and DTC migration in *pst-1*(*tm3364*) mutant animals.

(A-D) Animals were either wild-type or heterozygous for a *pst-1* deletion; to visualize seam cells, both expressed AJM-1::GFP. (A) In wild-type animals, the seam cells (arrows) were aligned on each side during larval development. (C) They fused to each other and formed two parallel syncytia in the late-L4 to adult stage. (B) In *pst-1*(*tm3364*) homozygotes, the seam cells were often discontinuous in the L3 to L4 stage. (D) They remained unfused and formed abnormal doughnut-shaped structures in the late- L4 to adult stage (arrowheads). (B and D, bracket) Some seam cells were lost or fused to the hypodermis. GFP signals from vulval cells are indicated with a "V". Note that the *pst-1*(*tm3364*) young adult has increased intestinal auto-fluorescence. (E) A representative DIC image of a wild-type hermaphrodite gonad. DTC that reached the dorsal side migrated along the dorsal body wall muscle and formed a U-shaped gonadal arm. (F) A representative DIC image of a *pst-1*(*tm3364*) gonad. The DTC that reached the dorsal side migrated toward the ventral side and formed a P-shaped gonadal arm. (G) Quantification of the DTC migration defects.

Figure S4. Subcellular localization of PST-1 and PST-2

Confocal images of (A) anti-GFP antibody staining and (A') anti-DsRed antibody staining in the epidermis of a transgenic worm carrying $P_{dpy\text{-}7}$*::pst-1b::egfp* and $P_{dpy\text{-}7}$*::aman-2::mCherry* transgene constructs. The epidermis is enclosed in the dashed lines. Confocal images of (B) anti-GFP antibody staining and (B') anti-DsRed antibody staining in the intestine of a transgenic worm carrying *pst-2(fl)::egfp* and $P_{aman\text{-}2}$*::aman-2::mCherry* transgene constructs. The intestine is enclosed in the dashed lines. (A") Merged A and A’ images; (B") Merged B and B’ images. The area within the box in A" and B" were enlarged for A"' and B"'. Scale bar = 20 mm. (C) Quantitative comparison of the Golgi localization of PST-1 and PST-2. The percentages of co-localization with PST proteins and Golgi marker are shown.

Supplemental Table 1: Primers used for plasmid construction

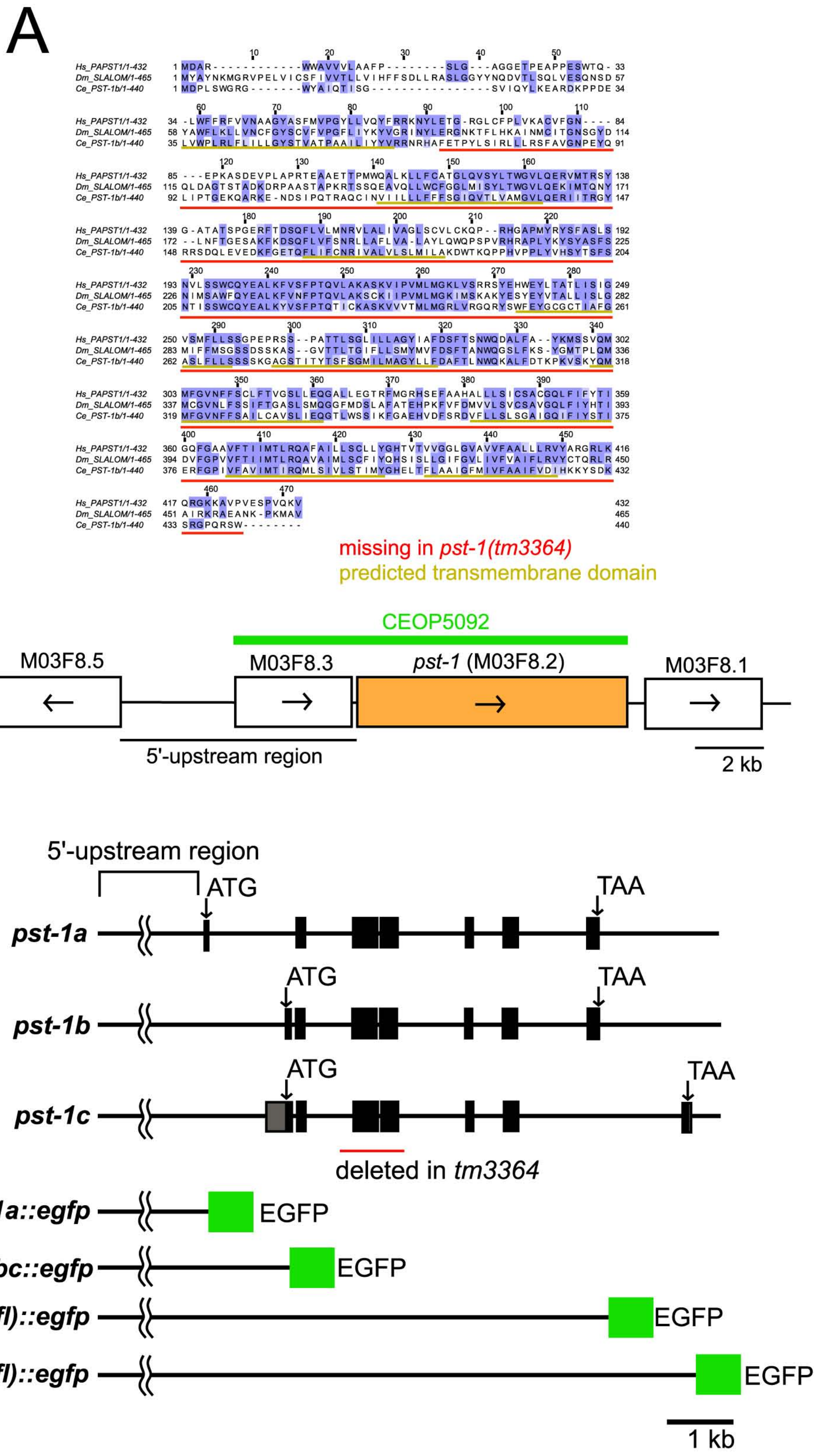
egfp: enhanced green fluorescent protein;

Construction		Primer sequences (5'- -3')
$P_{pst\text{-}1a}$*::egfp*	Forward	5’- ACTGTTTCGTGGCAAGATCA –3’
	Reverse	5’- CATGATTGCTCTGAATACCTGG –3’
$P_{pst\text{-}1bc}$*::egfp*	Forward	Same as $P_{pst\text{-}1a}$*::egfp* Forward
	Reverse	5’- CATTAACCCGTTCGTGATGTCTC –3’
pst-1ab(fl)::egfp	Forward	Same as $P_{pst\text{-}1a}$*::egfp* Forward
	Reverse	5’- CCAACTCCTCTGTGGTCCTC –3’
pst-1c(fl)::egfp	Forward	Same as $P_{pst\text{-}1a}$*::egfp* Forward
	Reverse	5’- ATTTGAGACGAAACGGTTCAG –3’
$P_{pst\text{-}2a}$*::egfp*	Forward	5’- TTGACTCCTTTATTTGCCTGAAA –3’
	Reverse	5’- CATTTGAAAGAGTGGGTGGAAAG –3’
$P_{pst\text{-}2b}$*::egfp*	Forward	Same as $P_{pst\text{-}2a}$*::egfp* Forward
	Reverse	5’- CATTGTTGCTACTGTGAAAAAGG –3’
pst-2a(fl)::egfp	Forward	Same as $P_{pst\text{-}2a}$*::egfp* Forward
	Reverse	5’- AACCGTCATTGGATCCTTCC –3’
$P_{unc\text{-}119}$*::egfp*	Forward	5’- TGCAATTGTTTTGTGCCAAG –3’
	Reverse	5’- CTCTGCCTTCATATATGCTGTTG –3’
$P_{rgef\text{-}1}$*::egfp*	Forward	5’- TGTATGATTCCTCGAATGTATTGAA –3’
	Reverse	5’- GCTCATCGTCGTCGTCGT –3’
$P_{dpy\text{-}7}$*::pst-1b::egfp*	Forward	5’- TGGCGGCCGCGATCCATTGAGCTGGGGT –3’
	Reverse	5’- TGGCGGCCGCACCAACTCCTCTGTGGTCCTC –3’
$P_{dpy\text{-}7}$*::pst-2a::egfp*	Forward	5’- TGGCGGCCGCACTGCAGCTCAAATACATTCTG –3’
	Reverse	5’- TGGCGGCCGCAAACCGTCATTGGATCCTTCC –3’
$P_{eft\text{-}4}$*::hPAPST1::Venus*	Forward	5’- TGGGCGGCCGCATGGACGCCAGATGGTGGGC –3’
	Reverse	5’- TGGGCGGCCGCAAACCTTCTGCACAGGAGAC –3’
$P_{eft\text{-}4}$*::hPAPST2::Venus*	Forward	5’- TGGGCGGCCGCATGGACTTGACACAGCAAGC –3’
	Reverse	5’- TGGGCGGCCGCATACAGTCTGTGCCAGCGTC –3’

Supplemental Table 1 (continued) : Primers used for plasmid construction

Construction		Primer sequences (5'- -3')
YEp352GAP-II-pst-1a-HA	Forward	5’- AAAAAGCAGGCTTCGCCGCCACCATGGATCGGTCAATCATGCCGATTG -3’
	Reverse	5’- AGAAAGCTGGGTACCAACTCCTCTGTGGTCC -3’
YEp352GAP-II-pst-2-HA	Forward	5’- AAAAAGCAGGCTTCGCCGCCACCATGACTGCAGCTCAAATACATTCTGATTGT -3’
	Reverse	5’- AGAAAGCTGGGTAAACCGTCATTGGATCCTTCCTAG -3’
attB adaptive primers	Forward	5’- GGGGACAAGTTTGTACAAAAAAGCAGGCT -3’
	Reverse	5’- GGGGACCACTTTGTACAAGAAAGCTGGGT -3’

Figure S1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Ce_PST-1b/1-440 1 MDPLSWGRG-------WYAIQTISG----------------SVIQYLKEARDKPPDE 34
60 70 80 90 100 110
Hs_PAPST1/1-432 34 -LWFFRFVVNAAGYASFMVPGYLLVQYFRRKNYLETG-RGLCFPLVKACVFGN---- 84
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Ce_PST-1b/1-440 35 LVWPLRLFLILLGYSTVATPAAILIYYVRRNRHAFETPYLSIRLLLRSFAVGNPEYQ 91
120 130 140 150 160
Hs_PAPST1/1-432 85 ---EPKASDEVPLAPRTEAAETTPMWQALKLLFCATGLQVSYLTWGVLQERVMTRSY 138
Dm_SLALOM/1-465 115 QLDAGTSTADKDRPAASTAPKRTSSQEAVQLLWCFGGLMISYLTWGVLQEKIMTQNY 171
Ce_PST-1b/1-440 92 LIPTGEKQARKE-NDSIPQTRAQCINVIILLLFFFSGIQVTLVAMGVLQERIITRGY 147
180 190 200 210 220
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Dm_SLALOM/1-465 172 --LNFTGESAKFKDSQFLVFSNRLLAFLVA-LAYLQWQPSPVRHRAPLYKYSYASFS 225
Ce_PST-1b/1-440 148 RRSDQLEVEDKFGETQFLIFCNRIVALVLSLMILAKDWTKQPPHVPPLYVHSYTSFS 204
230 240 250 260 270 280
Hs_PAPST1/1-432 193 NVLSSWCQYEALKFVSFPTQVLAKASKVIPVMLMGKLVSRRSYEHWEYLTATLISIG 249
Dm_SLALOM/1-465 226 NIMSAWFQYEALKFVNFPTQVLAKSCKIIPVMLMGKIMSKAKYESYEYVTALLISLG 282
Ce_PST-1b/1-440 205 NTISSWCQYEALKYVSFPTQTICKASKVVVTMLMGRLVRGQRYSWFEYGCGCTIAFG 261
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Hs_PAPST1/1-432 250 VSMFLLSSGPEPRSS--PATTLSGLILLAGYIAFDSFTSNWQDALFA--YKMSSVQM 302
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Ce_PST-1b/1-440 262 ASLFLLSSSSKGAGSTITYTSFSGMILMAGYLLFDAFTLNWQKALFDTKPKVSKYQM 318
350 360 370 380 390
Hs_PAPST1/1-432 303 MFGVNFFSCLFTVGSLLEQGALLEGTRFMGRHSEFAAHALLLSICSACGQLFIFYTI 359
Dm_SLALOM/1-465 337 MCGVNLFSSIFTGASLSMQGGFMDSLAFATEHPKFVFDMVVLSVCSAVGQLFIYHTI 393
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460 470
Hs_PAPST1/1-432 417 QRGKKAVPVESPVQKV 432
Dm_SLALOM/1-465 451 AIRKRAEANK-PKMAV 465
Ce_PST-1b/1-440 433 SRGPQRSW-------- 440
missing in pst-1(tm3364)
predicted transmembrane domain
B
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M03F8.5
M03F8.3
pst-1 (M03F8.2)
M03F8.1
5'-upstream region
2 kb
C
5'-upstream region
ATG
TAA
pst-1a
ATG
TAA
pst-1b
ATG
TAA
pst-1c
deleted in tm3364
Ppst-1a::egfp
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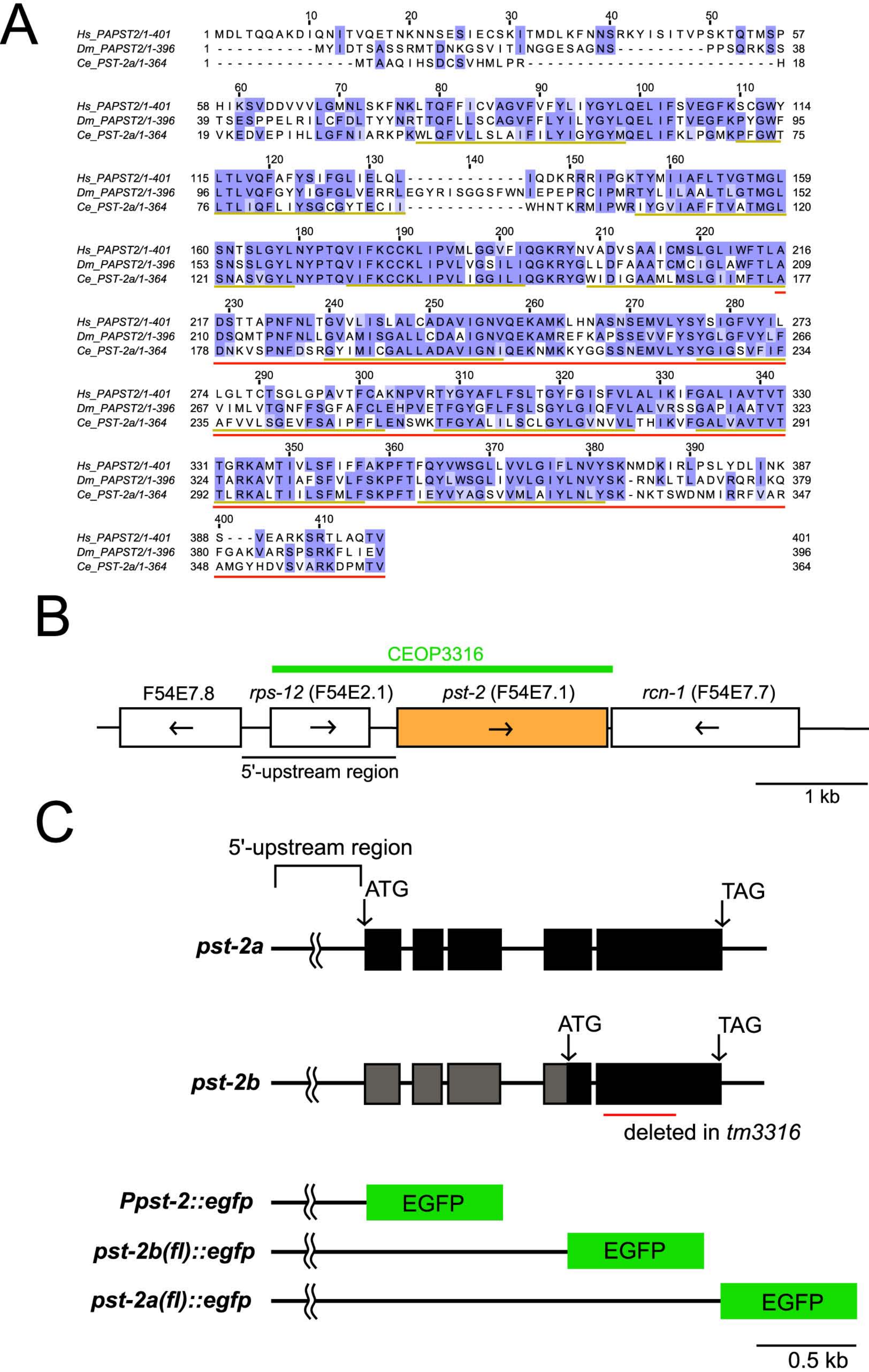
EGFP
pst-1ab(fl)::egfp
EGFP
pst-1c(fl)::egfp
EGFP
1 kb

Figure S2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Ce_PST-2a/1-364 1 --------------MTAAQIHSDCSVHMLPR-------------------------H 18
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Dm_PAPST2/1-396 39 TSESPPELRILCFDLTYYNRTTQFLLSCAGVFFLYILYGYLQELIFTVEGFKPYGWF 95
Ce_PST-2a/1-364 19 VKEDVEPIHLLGFNIARKPKWLQFVLLSLAIFILYIGYGYMQELIFKLPGMKPFGWT 75
120 130 140 150 160
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Dm_PAPST2/1-396 153 SNSSLGYLNYPTQVIFKCCKLIPVLVGSILIQGKRYGLLDFAAATCMCIGLAWFTLA 209
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230 240 250 260 270 280
Hs_PAPST2/1-401 217 DSTTAPNFNLTGVVLISLALCADAVIGNVQEKAMKLHNASNSEMVLYSYSIGFVYIL 273
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Ce_PST-2a/1-364 178 DNKVSPNFDSRGYIMICGALLADAVIGNIQEKNMKKYGGSSNEMVLYSYGIGSVFIF 234
290 300 310 320 330 340
Hs_PAPST2/1-401 274 LGLTCTSGLGPAVTFCAKNPVRTYGYAFLFSLTGYFGISFVLALIKIFGALIAVTVT 330
Dm_PAPST2/1-396 267 VIMLVTGNFFSGFAFCLEHPVETFGYGFLFSLSGYLGIQFVLALVRSSGAPIAATVT 323
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350 360 370 380 390
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400 410
Hs_PAPST2/1-401 388 S---VEARKSRTLAQTV 401
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Ce_PST-2a/1-364 348 AMGYHDVSVARKDPMTV 364
B
CEOP3316
F54E7.8
rps-12 (F54E2.1)
pst-2 (F54E7.1)
rcn-1 (F54E7.7)
←
→
→
←
5'-upstream region
1 kb
C
5'-upstream region
ATG
TAG
pst-2a
ATG
TAG
pst-2b
deleted in *tm3316*
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pst-2b(fl)::egfp
EGFP
pst-2a(fl)::egfp
EGFP
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Figure S3

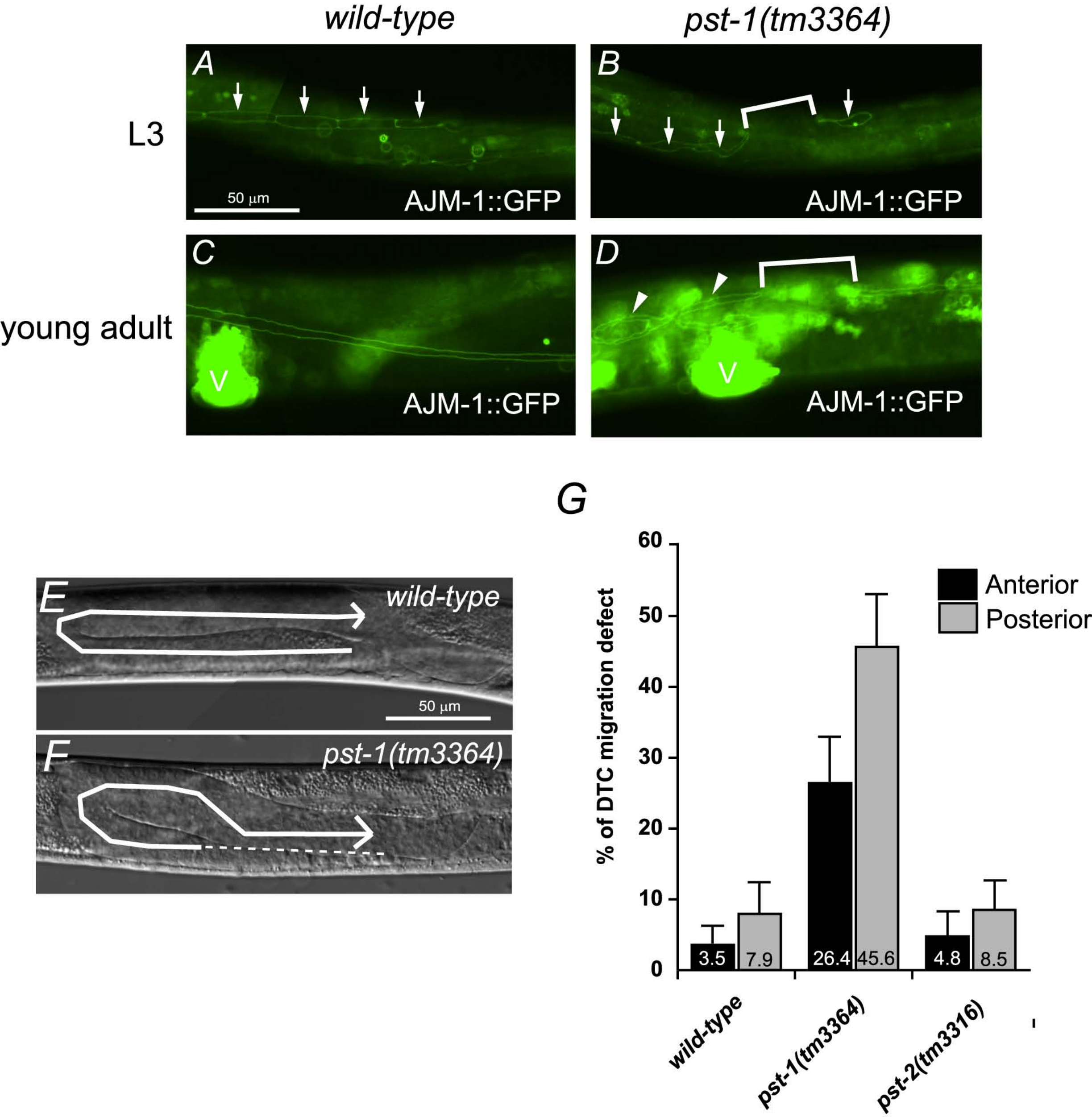
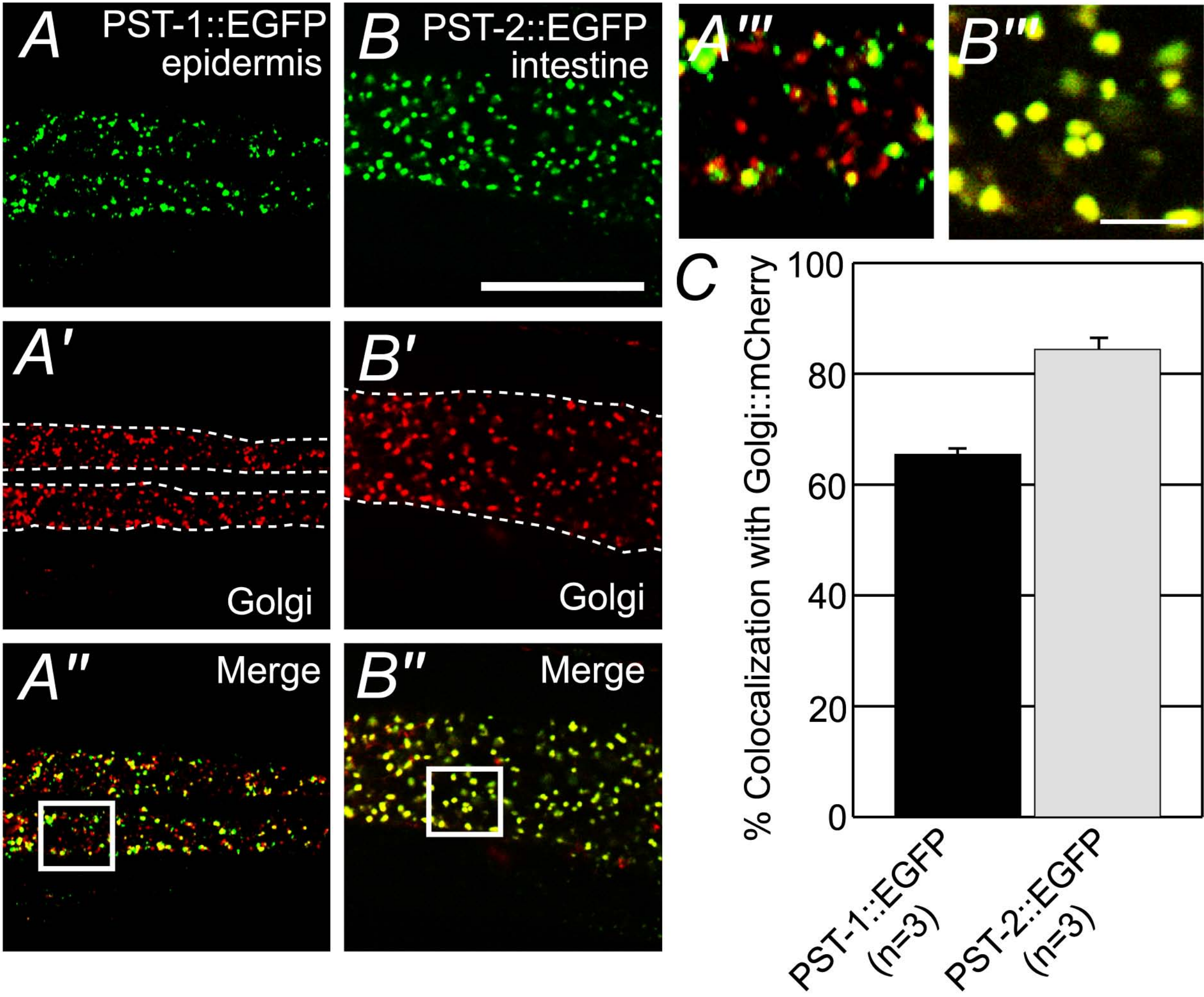

wild-type
pst-1(tm3364)
L3
young adult
A
B
C
D
50 µm
AJM-1::GFP
AJM-1::GFP
AJM-1::GFP
AJM-1::GFP
V
V
E
wild-type
50 µm
F
pst-1(tm3364)
G
% of DTC migration defect
0
10
20
30
40
50
60
Anterior
Posterior
3.5
7.9
26.4
45.6
4.8
8.5
wild-type
pst-1(tm3364)
pst-2(tm3316)

Figure S4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epidermis
B
PST-2::EGFP
intestine
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B'''
A'
Golgi
B'
Golgi
A''
Merge
B''
Merge
C
% Colocalization with Golgi::mCherry
0
20
40
60
80
100
PST-1::EGFP (n=3)
PST-2::EGFP (n=3)