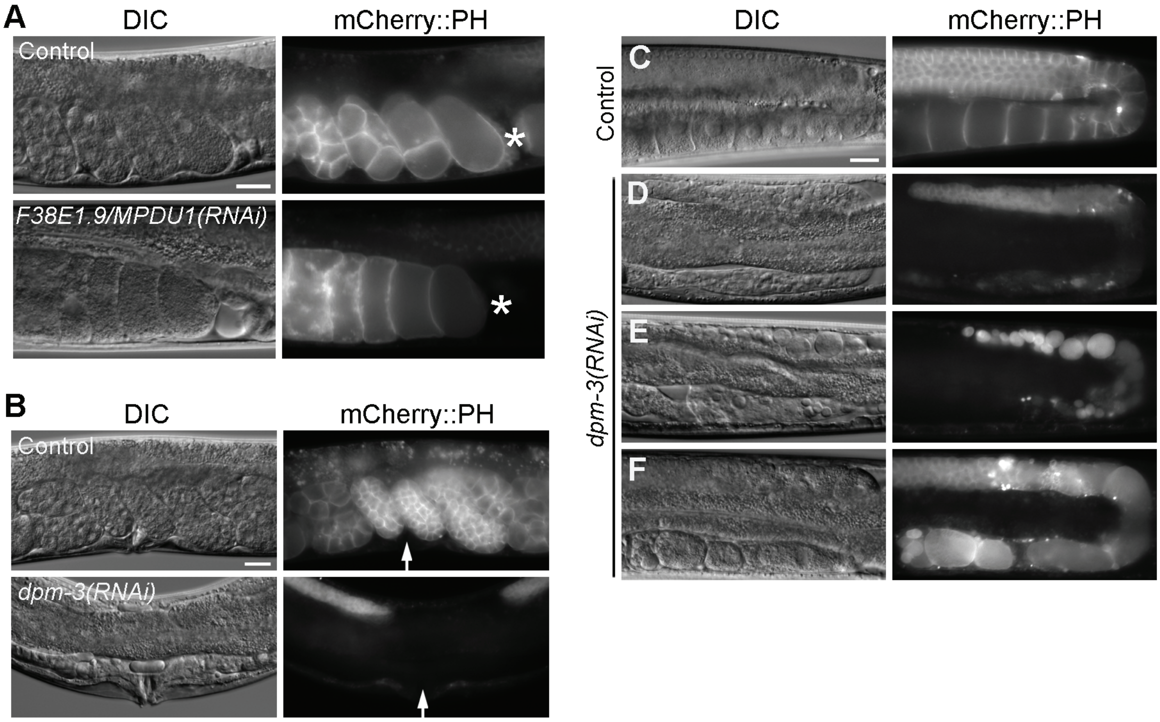
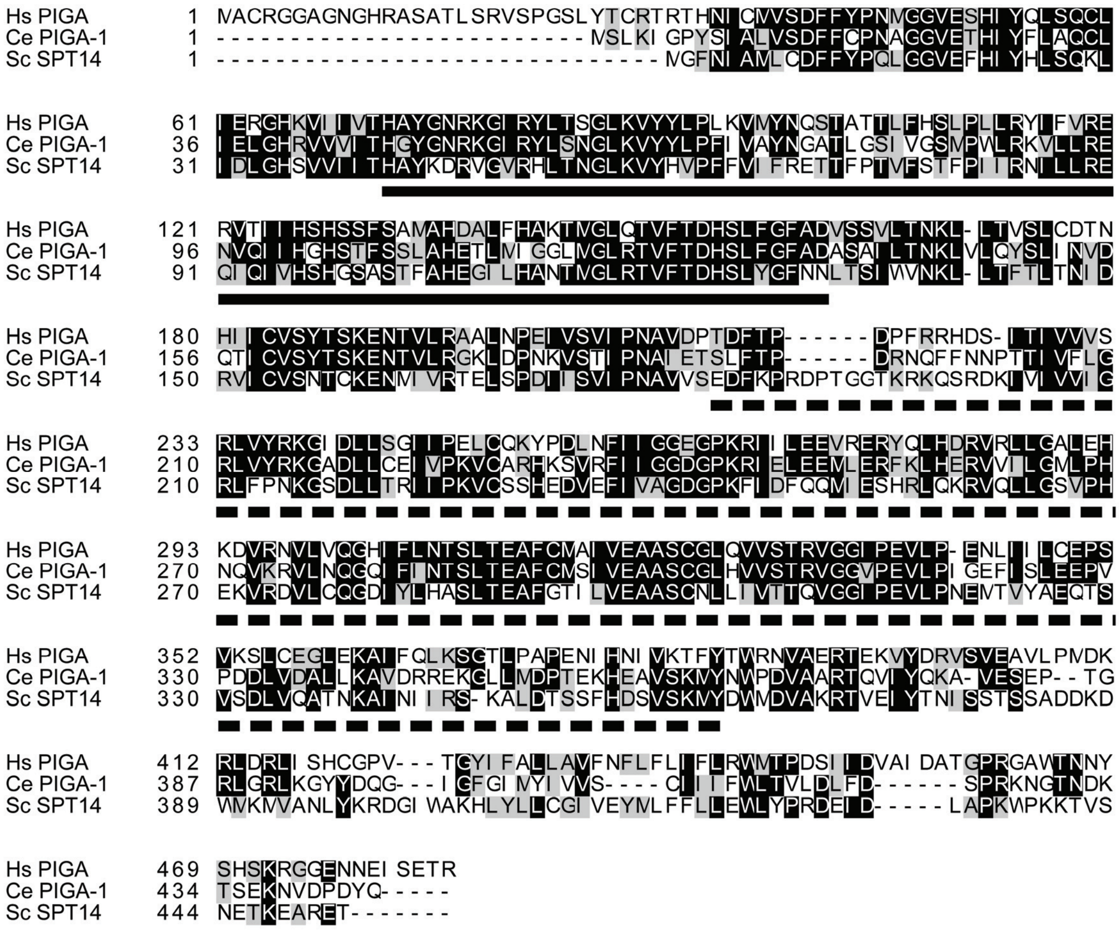
Supplementary Figure 1



Supplementary Figure 1. RNAi of genes involved in the synthesis of both GPI-anchor and N-linked/O-linked oligosaccharides resulted in severe germline phenotypes. (A) Knockdown of the *F38E1.9/MPDU1* resulted in the oocyte abnormality similar to the phenotype shown in Figure 3E. Asterisks indicate the spermatheca. (B) In *dpm-3* RNAi-treated worms, no eggs were found in the uteri. Arrows indicate the position of the vulva. (C-F) Abnormalities of germline cells in *dpm-3* RNAi-treated worms with no eggs (B). Wild-type gonad with normal oocytes in control RNAi-treated worms (C). Gonad with no matured oocytes (D, 42%, *n*=24), with disrupted germ cell proliferation and germline syncytium (E, 33%, *n*=24) and with irregularly shaped oocytes (F, 25%, *n*=24) in *dpm-3* RNAi-treated worms. Bars, 20 μm.

Supplementary Figure 2



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Hs PIGA       1  MACRGGAGNGHRASATLSRVSPGSLYTCRTRTHNICMVSDFFYPNMGGVESHIYQLSQCL
Ce PIGA-1     1  -------------------------MSLKIGPYSIALVSDFFCPNAGGVETHIYFLAQCL
Sc SPT14      1  ------------------------------MGFNIAMLCDFFYPQLGGVEFHIYHLSQKL

Hs PIGA      61  IERGHKVIIVTHAYGNRKGIRYLTSGLKVYYLPLKVMYNQSTATTLFHSLPLLRYIFVRE
Ce PIGA-1    36  IELGHRVVVITHGYGNRKGIRYLSNGLKVYYLPFIVAYNGATLGSIVGSMPWLRKVLLRE
Sc SPT14     31  IDLGHSVVIITHAYKDRVGVRHLTNGLKVYHVPFFVIFRETTFPTVFSTFPIIRNILLRE

Hs PIGA     121  RVTIIHSHSSFSAMAHDALFHAKTMGLQTVFTDHSLFGFADVSSVLTNKL-LTVSLCDTN
Ce PIGA-1    96  NVQIIHGHSTFSSLAHETLMIGGLMGLRTVFTDHSLFGFADASAILTNKLVLQYSLINVD
Sc SPT14     91  QIQIVHSHGSASTFAHEGILHANTMGLRTVFTDHSLYGFNNLTSIWVNKL-LTFTLTNID

Hs PIGA     180  HIICVSYTSKENTVLRAALNPEIVSVIPNAVDPTDFTP------DPFRRHDS-ITIVVVS
Ce PIGA-1   156  QTICVSYTSKENTVLRGKLDPNKVSTIPNAIETSLFTP------DRNQFFNNPTTIVFLG
Sc SPT14    150  RVICVSNTCKENMIVRTELSPDIISVIPNAVVSEDFKPRDPTGGTKRKQSRDKIVIVVIG

Hs PIGA     233  RLVYRKGIDLLSGIIPELCQKYPDLNFIIGGEGPKRIILEEVRERYQLHDRVRLLGALEH
Ce PIGA-1   210  RLVYRKGADLLCEIVPKVCARHKSVRFIIGGDGPKRIELEEMLERFKLHERVVILGMLPH
Sc SPT14    210  RLFPNKGSDLLTRIIPKVCSSHEDVEFIVAGDGPKFIDFQQMIESHRLQKRVQLLGSVPH

Hs PIGA     293  KDVRNVLVQGHIFLNTSLTEAFCMAIVEAASCGLQVVSTRVGGIPEVLP-ENLIILCEPS
Ce PIGA-1   270  NQVKRVLNQGQIFINTSLTEAFCMSIVEAASCGLHVVSTRVGGVPEVLPIGEFISLEEPV
Sc SPT14    270  EKVRDVLCQGDIYLHASLTEAFGTILVEAASCNLLIVTTQVGGIPEVLPNEMTVYAEQTS

Hs PIGA     352  VKSLCEGLEKAIFQLKSGTLPAPENIHNIVKTFYTWRNVAERTEKVYDRVSVEAVLPMDK
Ce PIGA-1   330  PDDLVDALLKAVDRREKGLLMDPTEKHEAVSKMYNWPDVAARTQVIYQKA-VESEP--TG
Sc SPT14    330  VSDLVQATNKAINIIRS-KALDTSSFHDSVSKMYDWMDVAKRTVEIYTNISSTSSADDKD

Hs PIGA     412  RLDRLISHCGPV---TGYIFALLAVFNFLFLIFLRWMTPDSIIDVAIDATGPRGAWTNNY
Ce PIGA-1   387  RLGRLKGYYDQG---IGFGIMYIVVS----CIIIFWLTVLDLFD------SPRKNGTNDK
Sc SPT14    389  WMKMVANLYKRDGIWAKHLYLLCGIVEYMLFFLLEWLYPRDEID-----LAPKWPKKTVS

Hs PIGA     469  SHSKRGGENNEISETR
Ce PIGA-1   434  TSEKNVDPDYQ-----
Sc SPT14    444  NETKEARET-------
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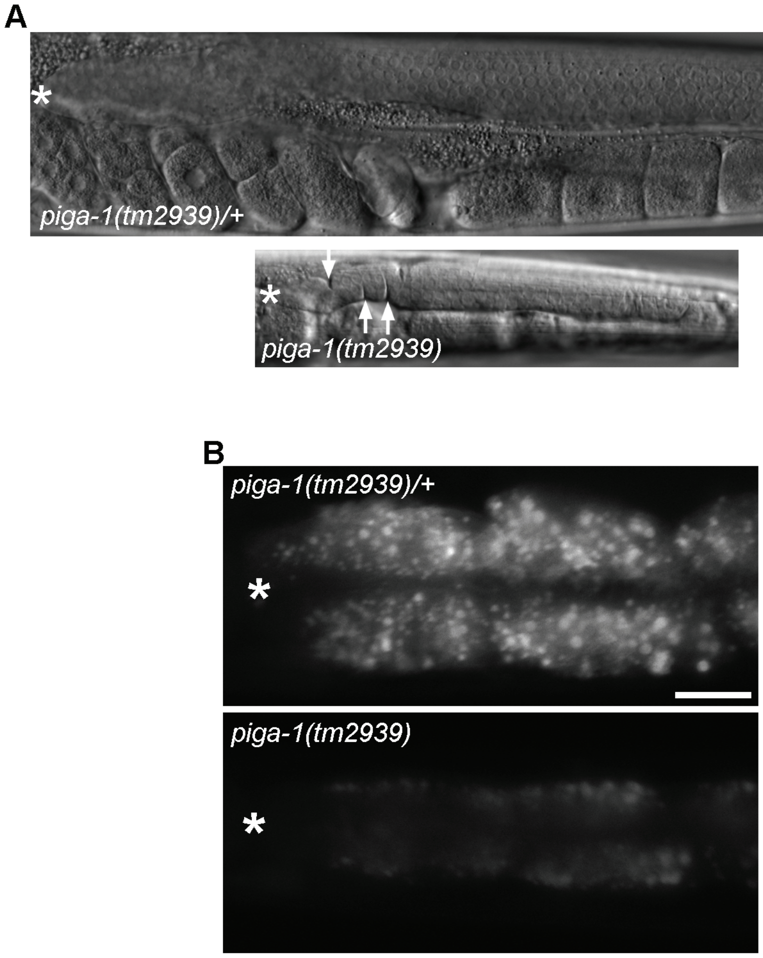
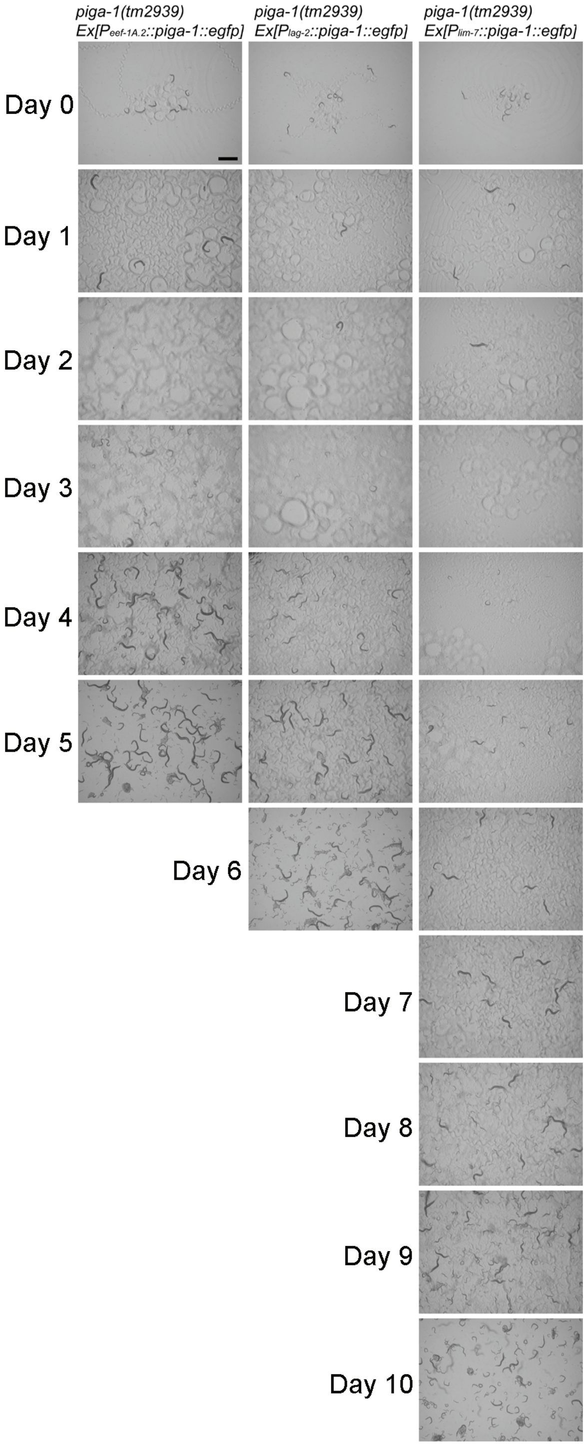
Supplementary Figure 2. ClustalW alignment of the predicted amino acid sequences of *H. sapiens* PIGA (Hs PIGA), *C. elegans* PIGA-1 (Ce PIGA-1) and *S. cerevisiae* SPT14 (Sc SPT14). Identical amino acids are shaded in black and similar amino acids are shaded in gray. The PIGA domain is underlined with a solid line, and the glycosyltransferase group 1 domain is shown as a dashed line.

Supplementary Figure 3



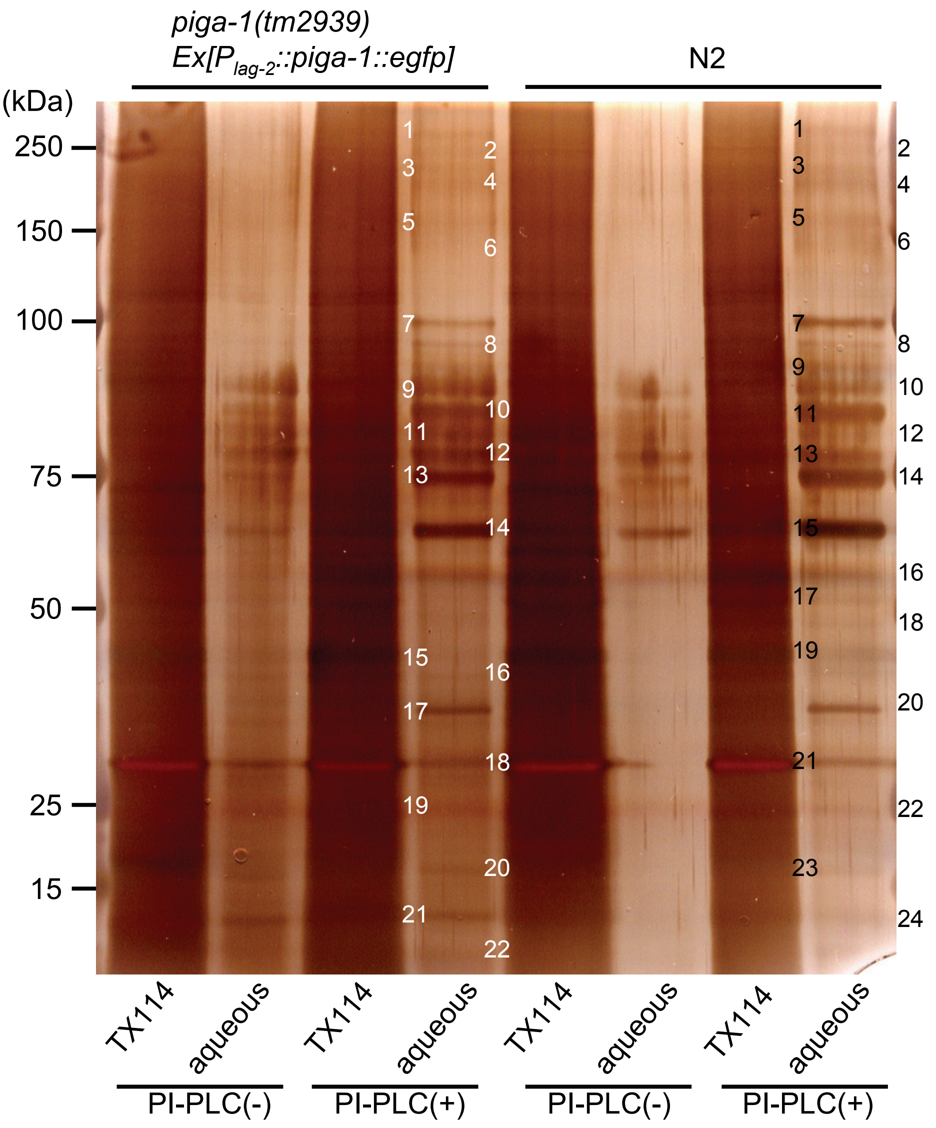
Supplementary Figure 3. Abnormalities of somatic tissues in the *piga-1(tm2939)* worm. (A) Gonads of *piga-1(tm2939)/+* and *piga-1(tm2939)* worms. Gonad size was decreased in *piga-1(tm2939)* worms. Some animals showed wrinkled gonadal arms (arrows). Asterisks indicate DTCs. (B) Intestines of *piga-1(tm2939)/+* and *piga-1(tm2939)* worms. Autofluorescent granules in the intestinal cells were visualized using a DAPI filter under UV illumination. Autofluorescence of the granules was decreased in the intestine of *piga-1(tm2939)* worms. Asterisks indicate the position of the pharyngeal-intestinal valve. Bars, 50 μm (A) and 20 μm (B).

Supplementary Figure 4



Supplementary Figure 4. Growth of the *piga-1(tm2939)* rescue lines on NGM plate. Ten rescued L4 worms were placed on a fresh NGM plate seeded with OP50 and maintained at 20° C. Microscopic images of worms on the plates were acquired with an OLYMPUS SZX12 microscope (OLYMPUS) equipped with a digital camera (Nikon COOLPIX 990, Nikon) every 24 h until bacteria on the plate were depleted. The $P_{lim\text{-}7}$*::piga-1::egfp* rescue line showed slow growth and proliferation compared with the $P_{eef\text{-}1A.2}$*::piga-1::egfp* rescue line and the $P_{lag\text{-}2}$*::piga-1::egfp* rescue line. It took 10 days for the $P_{lim\text{-}7}$*::piga-1::egfp* rescue line to eat up all the OP50 bacteria on the plate, while it took 5 and 6 days for the $P_{eef\text{-}1A.2}$*::piga-1::egfp* rescue line and $P_{lag\text{-}2}$*::piga-1::egfp* rescue line, respectively. Bar, 1 mm (measured with an eyepiece micrometer).

Supplementary Figure 5



Supplementary Figure 5. The extracted GPI-anchored proteins were separated by SDS-PAGE and stained with Silver stain MS kit (Wako). The silver-stained gel including samples before PI-PLC treatment (PI-PLC (-)) and after PI-PLC treatment (PI-PLC(+)) is shown. Numbers indicate the positions of possible GPI-anchored protein bands. Black numbers, N2; white numbers, *piga-1(tm2939) Ex[P$_{lag-2}$::piga-1::egfp]*.

Supplementary Table 1

Band	CDS/ Public name	Description	Phenotype	Human homolog
1	Y16B4A.2	serine-type carboxypeptidase activity	n/a	lysosomal protective proteine isoform a precursor
4	Y41D4B.16/ hpo-6	uncharacterized	n/a	isoform 2 of mucin-3A
6	F23B2.11/ pcp-3	prolyl carboxypeptidase like	n/a	thymus-specific serine protease
	Y40D12A.2	serine carboxypeptidases (lysosomal cathepsin A)	Lva, Emb, protein aggregation variant	lysosomal protective protein isoform a precursor
	C29F3.7	positive regulation of growth rate	maternal Ste	n/a
7	F32A5.3	serine carboxypeptidases (lysosomal cathepsin A)	n/a	lysosomal protective protein isoform a precursor
	C31C9.1/ tag-10	apical gut membrane polyprotein	n/a	n/a
	C34H4.3/ tag-244	uncharacterized	n/a	n/a
	F53C11.1	uncharacterized	n/a	n/a
	K08D8.6	uncharacterized	n/a	n/a
8	Y47H9C.1	uncharacterized	n/a	n/a
9	T03G6.3	type I phosphodiesterase/nucleotide pyrophosphatase	n/a	ENPP6
	C53B7.1/ rig-3	neuronal IGCAM	Ste, Let	isoform 5 of neural cell adhesion molecule 1
	F54E2.1	uncharacterized	brood size variant	n/a
	R173.1/ cah-5	carbonic anhydrase	n/a	carbonic anhydrase 7
	F41D9.3/ wrk-1	ephrin receptor-interacting immunoglobulin superfamily protein	n/a	MAM domain-containing GPI-anchored protein 2
10	F35E12.10	uncharacterized	n/a	n/a
	ZK6.10/ dod-19	determination of adult life span	ventral cord patterning variant	n/a
11	ZK6.11a	uncharacterized	ventral cord patterning variant	n/a
	C05D9.3	integrin beta subunit	maternal Ste	isoform β-3A of integrin β-3
	F13B6.1	determination of adult life span	Emb	n/a
12	Y119C1B.9	uncharacterized	n/a	n/a

Supplementary Table 1. The list of possible GPI-anchored proteins identified in N2 by SyproRuby staining (Figure 1C). Ste, sterile; Emb, embryonic lethal; Let, Lethal; Lva, Larval arrest

Supplementary Table 2

Band	CDS/ Public name	Description	Phenotype	Human homolog
1	F57F4.4	positive regulation of growth rate	Ste, Emb, Sck, Let	stabilin-2
	F57F4.3*/ gfi-1	positive regulation of growth rate	locomotion variant	mucin-5AC (fragment)
6	Y41D4B.16*/ hpo-6	uncharacterized	n/a	isoform 2 of mucin-3A
	F23B2.11*/ pcp-3	prolyl carboxypeptidase like	n/a	thymus-specific serine protease
9	K11H12.4*	uncharacterized	n/a	n/a
	ZK896.5	uncharacterized	n/a	n/a
9	F19C7.2	serine-type peptidase activity	n/a	thymus-specific serine protease
10	C31C9.1a*/ tag-10	apical gut membrane polyprotein	n/a	n/a
	K08D8.6*	uncharacterized	n/a	n/a
	F56F10.1	serine-type peptidase activity	n/a	thymus-specific serine protease
11	C34H4.3*/ tag-244	uncharacterized	n/a	n/a
	T16D1.2/ pho-4	acid phosphatase activity	n/a	lysosomal acid phosphatase
	T03G6.3*	type I phosphodiesterase/nucleotide pyrophosphatase	n/a	ENPP6
	W05E10.4a*/ tre-3	alpha,alpha-trehalase activity	n/a	isoform 1 of trehalase
	W05E10.4b*/ tre-3	alpha,alpha-trehalase activity	n/a	isoform 1 of trehalase
12	F54E2.1*	uncharacterized	brood size variant	n/a
	C34B7.1	a divergent MYST acetyltransferase	n/a	n/a
13	C34H4.1*	integral to membrane	n/a	n/a
	C05D9.3*	integrin beta subunit	maternal Ste	isoform b-3A of integrin b-3
	F44E2.4	predicted receptor-like serine/threonine kinase	protein aggregation variant	low density lipoprotein receptor
14	ZK6.10*/ dod-19	determination of adult life span	ventral cord patterning variant	n/a
	F35E12.10*	uncharacterized	n/a	n/a
16	F44D12.2	transmembrane receptor protein S/T kinase activity	n/a	n/a
17	ZK6.11a*	uncharacterized	ventral cord patterning variant	n/a
18	F15G9.5	uncharacterized	n/a	n/a
	F01G10.6	uncharacterized	n/a	n/a
	F32A5.3	serine carboxypeptidases (lysosomal cathepsin A)	n/a	lysosomal protective protein isoform a precursor
19	F54E4.3*	uncharacterized	n/a	n/a
	R173.1 */ cah-5	carbonic anhydrase	n/a	carbonic anhydrase 7
20	C44B7.5*	dopamine beta-monooxygenase activity	n/a	n/a
	F27E5.4*/ phg-1	embryonic development ending in birth or egg hatching, mitosis	Slo, mitosis variant	growth arrest-specific protein 1
21	D1054.10	uncharacterized	n/a	n/a
22	K12B6.9	uncharacterized	n/a	n/a
23	K11H12.7	uncharacterized	n/a	n/a
	Y40D12A.2*	serine carboxypeptidases (lysosomal cathepsin A)	Lva, Emb, protein aggregation variant	lysosomal protective protein isoform a precursor
24	C15H9.9*	uncharacterized	n/a	n/a

Supplementary Table 2. The list of possible GPI-anchored proteins identified in N2 by silver staining (Suppementary Figure 5). Ste, sterile; Emb, embryonic lethal; Let, Lethal; Sck, unhealthy; Slo, Slow growth rate; Lva, Larval arrest. Proteins listed both in Supplementary Table 2 and 3 are indicated with asterisks.

Supplementary Table 3

Band	CDS/ Public name	Descripttion	Phenotype	Human homolog
2	F15E11.4	predicted receptor	n/a	n/a
	C12D12.1a	uncharacterized	n/a	mucin-2
3	F57F4.3* gfi-1	positive regulation of growth rate	locomotion variant	mucin-5AC (fragment)
	Y16B4A.2	serine-type carboxypeptidase activity	n/a	lysosomal protective proteine isoform a precursor
6	Y41D4B.16*	uncharacterized	n/a	isoform 2 of mucin-3A
7	W05E10.4a*/ tre-3	alpha,alpha-trehalase activity	n/a	isoform 1 of trehalase
	W05E10.4b*/ tre-3	alpha,alpha-trehalase activity	n/a	isoform 1 of trehalase
8	Y37E11AR.6/ vab-2	ephrin, ligand for ephrin receptor tyrosine kinase	head notched, head morphology variant, aldicarb hypersensitive, oraganism morphology variant	ephrin-B2
	F35E12.8a	uncharacterized	n/a	n/a
	F23B2.11*/ pcp-3	prolyl carboxypeptidase like	n/a	thymus-specific serine protease
9	ZK896.4	uncharacterized	n/a	n/a
	C31C9.1a* / tag-10	apical gut membrane polyprotein	n/a	n/a
	K08D8.6*	uncharacterized	n/a	n/a
	F58H7.1	uncharacterized	n/a	collagen, type XI, α 2
10	T03G6.3*	type I phosphodiesterase/nucleotide pyrophosphatase	n/a	ENPP6
	F54E2.1*	uncharacterized	brood size variant	n/a
	C34H4.3*/ tag-244	uncharacterized	n/a	n/a
11	K11H12.4*	uncharacterized	n/a	n/a
12	C05D9.3*	integrin beta subunit	maternal Ste	isoform β-3A of integrin β-3
	C34H4.1*	unnamed protein	n/a	n/a
	F35E12.10*	uncharacterized	n/a	n/a
13	ZK6.10*/ dod-19	determination of adult life span	ventral cord patterning variant	n/a
14	ZK6.11a*	uncharacterized	ventral cord patterning variant	n/a
16	R173.1*/ cah-5	carbonic anhydrase	n/a	carbonic anhydrase 7
	Y40D12A.2*	serine carboxypeptidases (lysosomal cathepsin A)	Lva, Emb, protein aggregation variant	lysosomal protective protein isoform a precursor
17	C44B7.5*	dopamine beta-monooxygenase activity	n/a	n/a
19	F54E4.3*	uncharacterized	n/a	n/a
21	C15H9.9*	uncharacterized	n/a	n/a
	F27E5.4*/ phg-1	embryonic development ending in birth or egg hatching, mitosis	Slo, mitosis variant	growth arrest-specific protein 1
22	C02B10.3	a predicted secreted protein containing EGF-like repeats	n/a	cDNA FLJ51576, highly similar to Jagged-1

Supplementary Table 3. The list of possible GPI-anchored proteins identified in *piga-1(tm2939) Ex[*P_{lag-2}*::piga-1::egfp]* by silver staining (Supplementary Figure 5). Ste, sterile; Emb, embryonic lethal; Slo, Slow growth rate; Lva, Larval arrest. Proteins listed both in Supplementary Table 2 and 3 are indicated with asterisks.

Supplementary Table 4

Amplified products		Primer sequences
Y48E1B.2 cDNA	Forward	5’- AAACTCCAAAAACCCCACTACC -3’
	Reverse	5’- TTCCTGTCGTTCCTTCTTCC -3’
T27F7.4 cDNA	Forward	5’- ATGCGCACTACACTTCGTTT -3’
	Reverse	5’- GTACCCATTCCGACACAACAC -3’
C27A12.9 cDNA	Forward	5’- ATGGGATCAATAATTCGAATACTC -3’
	Reverse	5’- GCTTCACTATTCTTGAATTTCTCG -3’
T22C1.3 cDNA	Forward	5’- ATGGCAAAGGACAAGAAATTACA -3’
	Reverse	5’- GTCGTCGCGAGTAAACGAAG -3’
B0511.13 cDNA	Forward	5’- ATGATATGGCTTAAAAATTTGCGA -3’
	Reverse	5’- CTGTTCAGAGCCGGTTGATT -3’
dpm-1 genomic sequence	Forward	5’- TTTCAGATTATTATGACATCCACTCC -3’
	Reverse	5’- CCAAACAAATGCAAACAGATAGA -3’
lag-2 promoter	Forward	5’- CCGGAATTTTGATTGTAGGC -3’
	Reverse	5’- CATTTTCTGAAAAAAGGCAAAT -3’
lim-7 promoter	Forward	5’- GAGGTTGGCTGATCCCTCTC -3’
	Reverse	5’- TTCTGAAAAATGAAAGCTCGATTAAC -3’
sth-1 promoter	Forward	5’- TCTCCCTGATCTGGATTGGA -3’
	Reverse	5’- CATGTTGCTCTAGCACAAAAAGAC -3’
pie-1 promoter	Forward	5’- AATTGAAAGTTTTGTGGGGAAA -3’
	Reverse	5’- CTGGAAAAGAAAATTTGATTTTTAAT -3’
pie-1 3rd intron	Forward	5’- TAGGATCCGTGCTGATCGGATGGAAATT -3’
	Reverse	5’- TAGGATCCGGTCTCCGATTCTGGAAGTAAA -3’
pie-1 3’ sequence	Forward	5’- TAAGATCTTTTTGCCGTATTTTCCATATTTT -3’
	Reverse	5’- TAACATGTCAGGATTTTTCCATCATTTTTCC -3’
piga-1 genomic sequence	Forward	5’- CACGCGGCCGCATGTCGCTAAAAATTGGTCCG -3’
	Reverse	5’- TATGCGGCCGCACTGATAGTCAGGATCAACATTTTTC -3’
wrk-1 genomic sequence	Forward	5’- TATGCGGCCGCATGATGAAATTGGTTCTGCT -3’
	Reverse	5’- TATAGATCTTTAAAATAAAAGATACATTGTGACT -3’
wrk-1 promoter	Forward	5’- TATGCGGCCGCCTCTTTCTCGATGCCCTTCT -3’
	Reverse	5’- TATGCGGCCGCCTGAAAAAAGAAAATGTTGAAAA -3’
egfp	Forward	5’- TATGGATCCATGGTGAGCAAGGGCGAG -3’
	Reverse	5’- TATGGATCCCTTGTACAGCTCGTCCATGC -3’

Supplementary Table 4. The list of primers used in plasmid construction. Restriction sites are underlined.