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Supplemental information

A lipid transfer protein ensures

nematode cuticular impermeability

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S1 Figure: Alignments of C. elegans GM2AP-like proteins (related to the 1st paragraph of result section).

Conservation: HsGM2AP C34E7.4 C05E7.2 C05E7.1 <u>Consensus_aa:</u> <u>Consensus_ss:</u>	1 1 1	9 55 5 5 9 5 MQSLMQAPLLIALGLLAAPAQAHLKKPSQLSSFSWDNCDEGKDPAV MTTRRI-VLLCAVAACVSAASSEISVRIERHFPCSASSGPKKDS MTPSFAHRFQSQML-VVFLASSCLFVSISNALENVELGKDLSVTLELIKHQPCKSGKWTKK MITNCL-ILLAILFGFASTQYUNLNLHRHQSCAWRKSSHWNRK M	47 43 60 42
Conservation: HsGM2AP C34E7.4 C05E7.2 C05E7.1 Consensus_aa: Consensus_ss:	48 44 61 43	5 5 5 5 5 5 9 -IRSLTLEPDPIIVPGNVTLS-VMGSTSV-PLSSPLKVDLVLEKEVAGLWIKIPCTD- LLIKFPSYKTAGVQFHEEIGENGHKCFRMSGGKVEVYAPGLNGSKKYYVHLETRIGIHGKPERCVNA -LEFESGNDRRGPKLEPQADK-PNCFTIG-GQVEVLE-DFGGDFSIYLELKNTAKKNTVPEKCVRQ -IEFQGGHSDSGALLKSTEQKGCYAIS-GKVKIHE-DVSDELLIYLSVSTTGDTORPPEVCRNA .lch.ssh.tsbsphshss.b.h.l.lpppbC.p. 00 0	101 110 122 103
Conservation: HsGM2AP C34E7.4 C05E7.2 C05E7.1 Consensus_aa: Consensus_ss:	102 111 123 104	5 999 5 9 55 5 9 9 55 9 95 5 5559 5 YIGSCTFEHFCDVLDMLIPTGEPCPEPLRTYGLPCHCPFKEGTYSLPKSEFVVPDLE-LP -DANGCGGIGSCVHCDICHTMGGSLKNFVQIFQADKPAQCSTTGLSAGSYSDLSLRVCLPTKNELL -PENGCGGFGSCLYCNACETFGDKLGVRAQLLLDGQPISCA-DGLKKGSYDNLKLAFCLPDIDDVI NPDTGCGGVGSCLVCRPCDBLDSLTKILGAQLVVNGKPAGCEPIKKGEYDNVELRFCLPKLAALL hGSCh0.p.Ccshs.hlsbPh.Cshp.GpYs.p.hhlPpb.l. eee hhhhh h eheese eeeee hhhhh	160 175 186 168
Conservation: HsGM2AP C34E7.4 C05E7.2 C05E7.1 Consensus_aa: Consensus_ss:	161 176 187 169	555 SWLTTGNYRIESVLSSSG PFLDQNASRAEQLWDLFVSSRARSGEIPLVIAARLFDRPINNLD SSQGLSKESFLQLIQGDDGNSVRAMGIFATVYVFDSDVSKQMATQIKIEGVYRRTKYSFFKDTQ EWQGISEDALDHILAATTQEGNGPPKLSLFVTVVLFDKDIKPLLVSQRKLESRIRELR-KLGADEP 	178 219 250 233
Conservation: HsGM2AP C34E7.4 C05E7.2 C05E7.1 Consensus_aa: Consensus_ss:	179 220 251 234	5955555 KRLGCIKIAASLKGI 193 AKTLNTILHDSKEGMIGCHWIYATVSQPN 248 LPAEVYWSLPFNQIIKN-QQSYVACHKIYGNLKVNRK 286 VDSQTYWNLPFNQIIKK-QSVFVGCHKLYGTISLSDIQAKKK 274	

S1 Figure: Alignment of GMAP-1 protein sequences of *C. elegans* paralogues to the human GM2AP. Protein sequences were retrieved from WormBase or Uniprot and aligned using PROMALS3D (A). HsGM2AP = *Homo sapiens (Uniprot ID*, P17900), C34E7.4 = *Caenorhabditis elegans* (GMAP-1), C05E7.2 = *C. elegans* (GMAP-3), C05E7.1 = *C. elegans* (GMAP-2). Sequence color representation; red = predicted alpha helices, blue = predicted beta strands; Consensus Structure (ss) symbols: e = beta strand, h = alpha helix. Consensus amino acid symbols: conserved amino acids = bold and uppercase letters; aliphatic (I, V, L): *I*; aromatic (Y, H, W, F): @; hydrophobic (W, F, Y, M, L, I, V, A, C, T, H): *h*; alcohol (S, T): o; polar residues (D, E, H, K, N, Q, R, S, T): p; tiny (A, G, C, S): t; small (A, G, C, S, V, N, D, T, P): s; bulky residues (E, F, I, K, L, M, Q, R, W, Y): b; charged (D, E, K, R, H): c. Bold residues in the consensus sequence represent greater than 80% consensus. Numbers in the first row represent a level of conservation above 4.

S2 Figure: Electron microscopy analysis of N2 and gmap-1 (related to Figure 3).



S2 Figure: Electron microscopy analysis of N2 and *gmap-1*. Worms were fixed with glutaraldehyde and formaldehyde in cacodylate buffer prior to osmium staining.

S3 Figure: Lipidomic analysis of the cuticle (related S1 Table).







S3 Figure: (A) Molecular network of lipids extracted from the external layer of the *C. elegans* cuticle and organized by MetGem 1.3.6. Each node represents one lipid identified by LC-MS/MS operated in the positive mode. Clusters are labeled according to the lipids identified in each of them. The relative surface of the node is divided according to the abundance of this lipid in each genotype: green: N2; Red: *gmap-1*; and Blue: *gmap-1* rescued in hypodermis. Two of the most modulated lipids are indicated: PE C18:1/C17:2 and PE C18:1/C18:1 that are increased in the cuticle of *gmap-1* (red) compared to N2 (green) and *gmap-1* rescued in hypodermis (blue). (B) For each lipid family, we identified the number of nodes/lipids regulated >30% in *gmap-1* compared to N2 and *gmap-1* rescued in hypodermis. The fraction of nodes upregulated, downregulated or not modulated is represented for each lipid family.

B)

atg acg acc cga aga atc gta ctg ttg tgt gcc gtt gcc gcg tgc gtc tca gcc gcc tcc Μ T R R I V L L C A V A A C V S A A S teg gag ate tet gte ege ate gaa aga eac tte eea tge tea get agt tea ggt eea aag V Н F P S R I E C S A S P aaa gat tot ttg oto ato aag tto coa toa tao aaa aco goo gga gtt caa tto cao gaa K D S L L I K F P S Y K T A Ğ V Q F H E gaa att gga gag aat gga cac aaa tgc ttc aga atg tcc ggt ggc aaa gtt gag gtt tat E I G E N G H K C F R M S G G K V E V Y gee eca gga eta aat gge tee aag aaa tae tat gtg eat ett gag aca aga ate gga ate G L N G S K K Y Y L E P V T R G cac gga aag cca gaa cga tgc gta aat gct gat gct aat ggg tgt gga gga atc gga tca H G K P E R C V N A D A N G C G G I G S tgt gtt cae tge gae ate tgt eae aet atg gga gga tet ete aag aae ttt gtt eaa ate C V H C D I C H T M G G S L K N F V Q I DICHTMGGSLKNF VQI tte caa gee gat aag eea get caa tge tet ace aeg gga ete teg gea gga tea tae age A O C S T D K T G L S G S 0 A D . A Y gae ttg tee etc ege gtg tge ett eca ace aaa aae gaa ett ete eca tte etc gat eaa D L SLR V C L P T K N E L L P F L D aad gee tet egt gee gag eag ete tgg gat ete tit gie age tee egt get egt tee ggi NASRAEQLWDLF VSSRARS gag ate eea ett gte att gee gee egt ett tte gat aga eea ate aat aat ttg gat gee I P L V I A A R L F D R P I N N L D A aag aca ttg aac act att ctt cac gac tog aag gaa gga atg atc gga tge cac tgg atc T L NTILHDSKEGMI G C H W tac gog acg gtg tee cag eca aac gaa tte tet aga gaa aac etg tac tte caa gog cae Y A T V S Q P N E F S R E N L Y F Q A H cat cae cat cae cat cat cae tga ннннн TGAACTAGTGCCTGCAGTCTCGACA #100 # 75 Culture supernantnat



S4 Figure: (A) Coding region and translated amino acid sequence of GMAP-1. (B) SDS-PAGE analysis of 8X His purified GMAP-1. Elution was performed using increasing concentrations of imidazole including 35 mM (lanes 2 and 3), 100 mM (Lanes 4 and 5) and 500 mM (lanes 6-8). Upper band represents glycosylated species while lower band represents de-glycosylated species.