**Supplementary material**



**Figure S1:** Gene ontology (GO) of immunoprecipitated proteins using α-EhVps35 antibody, were carried out using the GeneOntology database (http://geneontology.org/). GO analysis allows the association of a given gene list with specific functional annotations, which are further divided into functional clusters listed according to an enrichment P-value. The 10 most significant enriched GO terms in biological process, molecular function and cellular component branches are presented. All the adjusted statistically significant P-values of the terms were negative 10-base log normalized.

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| Table S1. Proteins of vesicular trafficking.  |
| Protein | **Access number** |
| AP-1 complex subunit mu 2 | EHI\_089880 |
| AP-2 complex subunit mu | EHI\_124560 |
| Vacuolar protein sorting-associated protein | EHI\_012580 |
| Rab5 | EHI\_026420 |
| Rab11D | EHI\_056100 |
| Rab2C | EHI\_067850 |
| Rab7B | EHI\_081330 |
| RabC1 | EHI\_153690 |
| RabD2 | EHI\_164900 |
| Rab11B | EHI\_107250 |
| Vacuolar sorting protein 26 | EHI\_008470/EHI\_062490 |
| Vacuolar sorting protein 35 | EHI\_002990 |

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| Table S2. Proteins associated with motility \*. |
| Protein | **Access number** |
| Beta-hexosaminidase subunit beta | EHI\_007330 |
| Pyruvate phosphate dikinase | EHI\_009530 |
| Cysteine proteinase 2 | EHI\_033710 |
| RabX31 | EHI\_040310 |
| V-type ATPase, A subunit | EHI\_043010 |
| Actin-related protein 2/3 complex subunit | EHI\_045000 |
| Heat shock protein 70 | EHI\_052860 |
| RAP1 | EHI\_058090 |
| RACC | EHI\_070730 |
| Protein disulfide isomerase | EHI\_071590 |
| Steroid 5-alpha reductase | EHI\_076870 |
| Rab7B | EHI\_081330 |
| Small ribosomal subunit protein uS2 | EHI\_081410 |
| Ubiquitin | EHI\_083270 |
| 4-alpha-glucanotransferase | EHI\_084680 |
| DEAD/DEAH box helicase | EHI\_093900 |
| Filamin 2 | EHI\_104630 |
| Rab11B | EHI\_107250 |
| Myosin II heavy chain | EHI\_110180 |
| Grainin | EHI\_120360 |
| 60S Ribosomal protein L9 | EHI\_126140 |
| Peptidase-CP-C6 | EHI\_127030 |
| RACG | EHI\_129750 |
| Enolase 1 | EHI\_130700 |
| Protein disulfide-isomerase | EHI\_133970 |
| Calreticulin | EHI\_136160 |
| Dipeptidyl-peptidase  | EHI\_136440 |
| RabC3 | EHI\_143650 |
| Gal/GalNAc lectin light subunit | EHI\_148790 |
| Alcohol dehydrogenase | EHI\_150490 |
| RabC1 | EHI\_153690 |
| 3-ketoacyl-CoA synthase  | EHI\_158240 |
| 90 kDa Heat shock protein | EHI\_163480 |
| Grainin 1 | EHI\_167300 |
| Grainin 2 | EHI\_167310 |
| Cysteine proteinase 5 | EHI\_168240 |
| RabX11 | EHI\_177520 |
| V-type ATPase, B subunit | EHI\_189850 |
| Sulfate adenylyltransferase | EHI\_197160 |
| 70 kDa Heat shock protein | EHI\_199590 |
| \* Markiewicz et al., 2011. |

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| Table S3. Proteins associated with phagocytosis \*\*. |
| Protein | **Access number** |
| Calcium-regulated actin-bundling protein C-terminal domain-containing protein | EHI\_004550  |
| Hydroxylamine reductase | EHI\_004600 |
| TNase-like domain-containing protein | EHI\_005150  |
| Beta-hexosaminidase subunit beta  | EHI\_007330  |
| NECAP PHear domain-containing protein | EHI\_007350  |
| Aminopeptidase | EHI\_008380  |
| Calcium-regulated actin-bundling protein C-terminal domain-containing protein | EHI\_010570  |
| Elongation factor 1α | EHI\_011210  |
| 60S ribosomal protein L10a | EHI\_012480  |
| Proton-translocating NAD(P)(+) transhydrogenase | EHI\_014030  |
| Ribosomal protein L15 | EHI\_020300  |
| Uncharacterized protein | EHI\_020330  |
| NADP-dependent alcohol dehydrogenase | EHI\_023110  |
| Calmodulin | EHI\_023500  |
| Cysteine synthase A | EHI\_024230  |
| 14-3-3 protein 1 | EHI\_025360  |
| PPi-type phosphoenolpyruvate carboxykinase 2 | EHI\_030750  |
| GTP-binding protein | EHI\_031410  |
| Polyadenylate-binding protein | EHI\_033250  |
| Cysteine protease 2  | EHI\_033710  |
| Uncharacterized protein | EHI\_035730  |
| Rho guanine nucleotide exchange factor | EHI\_039480  |
| Aminoacyl-histidine dipeptidase | EHI\_042170  |
| 60S ribosomal protein L10 | EHI\_044810  |
| Uncharacterized protein | EHI\_047800  b |
| Lysine-tRNA ligase | EHI\_047810  |
| Purine nucleoside phosphorylase | EHI\_048740  |
| Nitrogen fixation protein NifU | EHI\_049620  |
| phosphoglycerate mutase | EHI\_050940  |
| Serine carboxypeptidase  | EHI\_054530  |
| RAP1 | EHI\_058090  |
| Vacuolar protein sorting 26 (EhVps26) | EHI\_062490  |
| Vacuolar protein sorting 35 (EhVps35) | EHI\_002990  |
| Adenosylhomocysteinase  | EHI\_068250  |
| Inositol-3-phosphate synthase | EHI\_070720  |
| RACC | EHI\_070730  |
| ADP-ribosylation factor 1 | EHI\_073470  |
| Mitogen-activated protein kinase | EHI\_073650  |
| Long-chain-fatty-acid--CoA ligase | EHI\_079300  |
| START domain-containing protein | EHI\_080260  |
| Filopodin | EHI\_080740  |
| Rab7B  | EHI\_081330  |
| Small ribosomal subunit protein uS2 | EHI\_081410  |
| Coronin | EHI\_083590  |
| Seryl-tRNA synthetase | EHI\_092640  |
| DEAD/DEAH box helicase | EHI\_093900  |
| 14-3-3 protein 2 | EHI\_098280  |
| Phosphotransferase | EHI\_098290  |
| Cortexillin | EHI\_104560  |
| Rab11B \* | EHI\_107250  |
| Rab1A \* | EHI\_108610  |
| LIM zinc finger domain containing protein | EHI\_110280  |
| Glutamate synthase beta subunit | EHI\_110520  |
| PCTP-like protein | EHI\_110720  |
| ADF-H domain-containing protein | EHI\_118750  |
| Enhancer binding protein-1 | EHI\_121780  |
| AP-2 complex subunit mu | EHI\_124560  |
| Peptidyl-prolyl cis-trans isomerase | EHI\_125840  |
| RACG | EHI\_129750  |
| Purine nucleoside phosphorylase | EHI\_130960  |
| Protein disulfide-isomerase | EHI\_133970  |
| S-phase kinase-associated protein 1A | EHI\_134960  |
| 40S ribosomal protein S17 | EHI\_135060  |
| Dipeptidyl-peptidase | EHI\_136440  |
| RabC3 | EHI\_143650  |
| Rho GDP exchange inhibitor | EHI\_147570  |
| RabC1 | EHI\_153690  |
| 4Fe-4S ferredoxin-type domain-containing protein | EHI\_154450  |
| Thioredoxin reductase | EHI\_155440  |
| Proteasome subunit Alpha | EHI\_163650  |
| Actinin-like protein | EHI\_164430  |
| Alcohol dehydrogenase | EHI\_166490  |
| Rab GDP dissociation inhibitor alpha | EHI\_167060 |
| Cysteine protease 5  | EHI\_168240  |
| Aspartyl-tRNA synthetase | EHI\_175050  |
| Profilin | EHI\_176140  |
| RabX11 | EHI\_177520  |
| Dolichyl-diphosphooligosaccharide-protein glycotransferase | EHI\_183010  |
| Eukaryotic translation initiation factor 5A | EHI\_186480  |
| Actin-binding protein | EHI\_186840  |
| Phosphoglycerate kinase | EHI\_188180  |
| Calcium-regulated actin-bundling protein C-terminal domain-containing protein | EHI\_189930  |
| Molybdenum cofactor sulfurase putative | EHI\_194600  |
| Heat shock protein 90 | EHI\_196940  |
| 40S ribosomal protein S10 | EHI\_197030  |
| Sulfate adenylyltransferase | EHI\_197160  |
| Ras family GTPase | EHI\_198330  |
| Polyadenylate-binding protein | EHI\_198750  |
| PRA1 family protein | EHI\_199660  |
| Signal recognition particle receptor alpha subunit | EHI\_200840  |
| \*\* Okada et al., 2005; Watanabe et al., 2023. |

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| Table S4. Proteins associated with secretion \*\*\*. |
| Protein | **Access number** |
| TNase-like domain-containing protein | EHI\_005150 |
| 40S ribosomal protein S4 | EHI\_008210 |
| Tyrosine-tRNA ligase | EHI\_009240 |
| Pyruvate phosphate dikinase | EHI\_009530 |
| Tubulin alpha chain | EHI\_010530 |
| Elongation factor 1-alpha  | EHI\_011210 |
| Proteasome endopeptidase complex | EHI\_011870 |
| Ribosomal protein S14 | EHI\_012360 |
| 60S Ribosomal protein L10a-2 | EHI\_012480 |
| 40S Ribosomal protein S6 | EHI\_013890 |
| NAD(P) transhydrogenase subunit alpha | EHI\_014030 |
| 40S Ribosomal protein S2 | EHI\_020280 |
| Calmodulin | EHI\_023500 |
| 60S Ribosomal protein L12 | EHI\_030710 |
| PPi-type phosphoenolpyruvate carboxykinase 2 | EHI\_030750 |
| GTP-binding protein | EHI\_031410 |
| Cysteine proteinase 2 | EHI\_033710 |
| Ribosomal protein L18a | EHI\_035600 |
| V-type ATPase, A subunit | EHI\_043010 |
| Malic enzyme | EHI\_044970 |
| Actin-related protein 2/3 complex subunit | EHI\_045000 |
| Uncharacterized protein | EHI\_047800 |
| Lysine-tRNA ligase | EHI\_047810 |
| Guanine nucleotide-binding protein subunit beta 2-like 1 | EHI\_050550 |
| Pyruvate:ferredoxin oxidoreductase | EHI\_051060 |
| Elongation factor 1-alpha  | EHI\_052400 |
| Heat shock protein 70 | EHI\_052860 |
| RAP1 | EHI\_058090 |
| Galactose-inhibitable lectin | EHI\_058330 |
| EF-hand calcium-binding domain containing protein | EHI\_060740 |
| EhRab2C | EHI\_067850 |
| 60S Ribosomal protein L5 | EHI\_068660 |
| MRH domain-containing protein | EHI\_069560 |
| Long-chain-fatty-acid-CoA ligase | EHI\_079300 |
| Filopodin | EHI\_080740 |
| Small ribosomal subunit protein uS2 | EHI\_081410 |
| Coronin | EHI\_083590 |
| 4-alpha-glucanotransferase | EHI\_084680 |
| Serine-tRNA ligase | EHI\_092640 |
| DEAD/DEAH box helicase | EHI\_093900 |
| Glycogen phosphorylase | EHI\_096830 |
| Glycogen debranching enzyme | EHI\_098360 |
| Fructose-1,6-bisphosphate aldolase | EHI\_098570 |
| NAD-dependent epimerase/dehydratase domain-containing protein | EHI\_098800 |
| NAD(FAD)-dependent dehydrogenase | EHI\_099700 |
| Nuclease domain containing protein | EHI\_101240 |
| 60S Ribosomal protein L7a | EHI\_103310 |
| Cortexillin | EHI\_104560 |
| Filamin 2 | EHI\_104630 |
| Rab1A  | EHI\_108610 |
| Phosphoglucomutase | EHI\_110120 |
| Myosin heavy chain | EHI\_110180 |
| Actin-like protein | EHI\_111050 |
| Histone-glutamine methyltransferase  | EHI\_118840 |
| Alcohol dehydrogenase | EHI\_125950 |
| Asparaginyl-tRNA synthetase | EHI\_126920 |
| Ribosomal protein L17 | EHI\_127330 |
| RACG | EHI\_129750 |
| Enolase | EHI\_130700 |
| 40S Ribosomal protein S16 | EHI\_131190 |
| Protein disulfide-isomerase | EHI\_133970 |
| Adenylyl cyclase-associated protein | EHI\_136150 |
| Glycogen phosphorylase | EHI\_138380 |
| RabC3 | EHI\_143650 |
| 40S ribosomal protein S3 | EHI\_148320 |
| Gal/GalNAc lectin light subunit | EHI\_148790 |
| Ribosomal protein S24 | EHI\_148820 |
| 60S acidic ribosomal protein P0 | EHI\_148850 |
| Aldehyde-alcohol dehydrogenase 2 | EHI\_150490 |
| 40S Ribosomal protein S9 | EHI\_152080 |
| Ethanolamine kinase | EHI\_152340 |
| Actin-related protein 2/3 complex subunit 4 | EHI\_152660 |
| Uncharacterized protein | EHI\_160980 |
| Cysteine protease binding protein family 1 | EHI\_164800 |
| AP-3 complex subunit delta | EHI\_164810 |
| Malate dehydrogenase | EHI\_165350 |
| Alcohol dehydrogenase | EHI\_166490 |
| PPi-type phosphoenolpyruvate carboxykinase 1 | EHI\_166920 |
| Cysteine proteinase 5 | EHI\_168240 |
| Aspartyl-tRNA synthetase | EHI\_175050 |
| Profilin 1 | EHI\_176140 |
| Cdc48-like protein | EHI\_176970 |
| 26s proteasome subunit P45 family protein | EHI\_177320 |
| 40S ribosomal protein S25 | EHI\_177470 |
| Leucine-rich repeat containing protein | EHI\_177990 |
| Acetyl-CoA synthetase | EHI\_178960 |
| Actin | EHI\_182900 |
| Glyceraldehyde-3-phosphate dehydrogenase | EHI\_187020 |
| Phosphoglycerate kinase | EHI\_188180 |
| V-type ATPase, B subunit | EHI\_189850 |
| 40S Ribosomal protein S10 | EHI\_197030 |
| Actophorin | EHI\_197480 |
| Alcohol dehydrogenase 3 | EHI\_198760 |
| Actin-related protein 3 | EHI\_198930 |
| PRA1 family protein | EHI\_199660 |
| Clathrin heavy chain | EHI\_201510 |
| Clathrin heavy chain | EHI\_201710 |
| Clathrin heavy chain | EHI\_201940 |
| Vacuolar sorting protein 35 (EhVps35) | EHI\_002990 |
| \*\*\* Sharma et al., 2020 |  |