## SUPPLEMENTARY INFORMATION

## Supplementary information S1 (table) | Evidence for class V myosin-dependent organelle transport in vivo

Organelle– type V myosin	Proposed function	Colocalization with endogenous myosin	Colocalization with GFP-tagged myosin	Organelle and myosin colocalization during transport	Transport speed (nm s <sup>-1</sup> )	Methods used to show defects in organelle movement and/or distribution in cells lacking class V myosin	Wild-type class V myosin complements the defects	Correlate organelle speed with mechanochemical properties of myosin V	Organelle receptor identified	Transport complex reconstituted in vitro	Regulatory mechanisms of motor:receptor complex identified
Secretory vesicles- yeast Myo2	Vectorial transport of the secretory vesicles that drives the growth of the bud	Yes	No	Yes (IF)	~2500	KO, DN	Yes	Yes	Ypt31–Ypt32 and Sec4	No	PtdIns4P levels, GEF for Sec4
Late Golgi–yeast Myo2	Transport of late Golgi elements into the bud to facilitate inheritance of this compartment	Yes	Yes	No	~220	КО	Yes	No	Ypt11 and Ret2	No	No
Vacuole–yeast Myo2	Transport of portions of the vacuole into the bud to facilitate inheritance of this compartment	Yes	No	Yes (IF)	~150	KO, DN	Yes	No	Vac17 and Vac8	No	Cell cycle regulated synthesis, degradation and phosphorylation of Vac17
Peroxisomes-yeast Myo2	Transport of peroxisomes into the bud to facilitate inheritance of this compartment	Yes	No	No	~450	КО	Yes	No	Inp2	No	Cell cycle regulated synthesis and degradation and phosphorylation of Inp2, degradation of Inp2 controlled by positional cues
mRNAs-yeast Myo4	Transport of mRNAs to the bud tip	Yes	No	No	~300	КО	Yes	No	She3, She2	Yes	No
Pigment granules– D. melanogaster myosin V	Transport of pigment granules within photoreceptor cells in response to light to create a functional pupil (sensory adaptation)	Yes	No	Yes (IF)	ND	КО	Yes	No	Lightoid (RAB-like)	No	Elevated Ca <sup>2+</sup>
Secretory vesicles– D. melanogaster myosin V	Transport of secretory vesicles that support the growth of the sensory membrane at the rhabdomere	Yes	No	Yes (IF)	ND	КО	No	No	RAB11 and RIP11	No	No
Melanosome– mouse myosin Va	Capture and local movement of melanosomes in the cell periphery	Yes	Yes	Yes	~150	KO, RNAi, DN	Yes	No	RAB27A-melanophilin	Yes	GEF and GAP for RAB27A, cargo-dependent unfolding and activation of myosin Va
Melanosome– frog myosin V	Capture and local movement of melanosomes in the central cytoplasm	Yes	No	No	~50	DN	No	No	No, but most likely similar to mouse myosin Va	No	Phosphorylation of myosin V's GTD
CV membranes- D. discoideum MyoJ	Cortical capture of CV membranes and translocation of CV tubules following water discharge	Yes	Yes	Yes	~500	KO, DN	Yes	Yes	No	No	No
Recycling endosome- mouse myosin Vb	Activity-dependent transport of recycling endosomes onto the dendritic spines of hippocampal neurons to support functional and structural spine plasticity	Yes	Yes	Yes	ND	DN, RNAi, chemical genetic mutant	Yes	No	RAB11A, FIP2	No	Ca <sup>2+</sup> -dependent unfolding and activatio of myosin Vb, GEF and GAP for RAB11
SER-mouse myosin Va	Transport of SER tubules into the dendritic spines of cerebellar Purkinje neurons to support synaptic plasticity	Yes	Yes	Yes	400	КО	Yes	Yes	No	No	No

CV, contractile vacuole; D. discoideum, Dictyostelium discoideum; D. melanogaster, Drosophila melanogaster; DN, dominant-negative construct in wild-type background; FIP2, RAB11 family-interacting protein 2; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GFP, green fluorescent protein; GTD, globular tail domain; IF, immunofluorescence; Inp2, inheritance of peroxisomes 2; KO, knockout; ND, not determined; PtdIns4P, phosphatidylinositol-4-phosphate; RIP11, RAB11-interacting protein; RNAi, RNA interference; SER, smooth endoplasmic reticulum; She, Swi5-dependent HO expression; Vac, vacuole-related.