Using the zebrafish lateral line to characterize the HOPS/CORVET complex in axon maintenance

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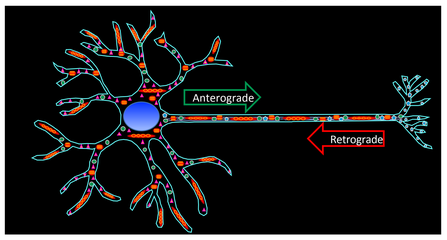
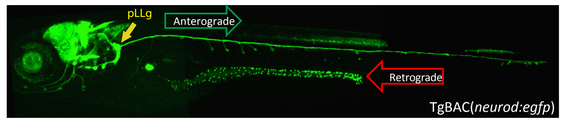
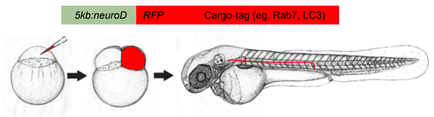
Background

Axonal Transport of cargos in neurons



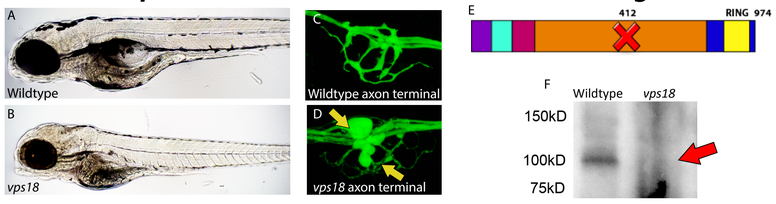
Using the zebrafish lateral line to model axon transport





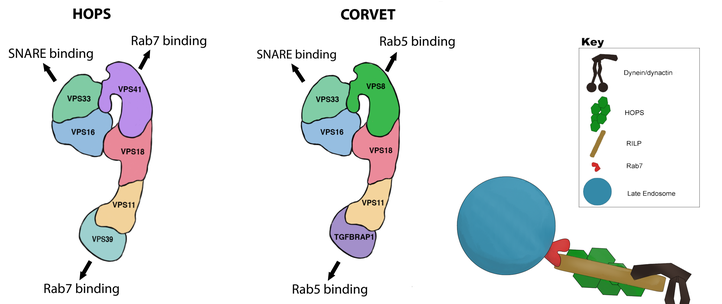
By doing one-cell stage injections, a single axon is expressing. With a single axon, transport of cargos can be quantified and analyzed.

vps18 mutants have axon terminal swellings



(A,B) Wildtype and *vps18* mutant zebrafish larvae at 4 days post-fertilization (dpf). (C,D) HIgh magnification view of a posterior lateral line (pLL) axon terminal at 4dpf shows axon terminal swellings in the *vps18* mutant (arrows). (E) Schematic of vps18 protein showing early stop site in the middle of exon 4 found in the *vps18* mutant strain. (F) Western blot analysis of Vps18 protein from 4dpf larvae demonstration loss of the 100kD band in the mutant strain.

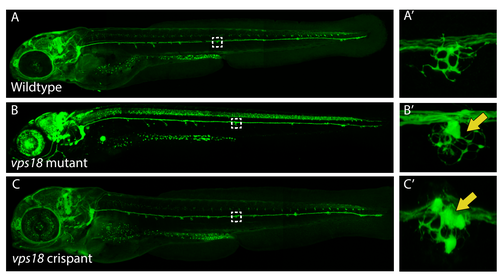
The HOPS/CORVET complexes



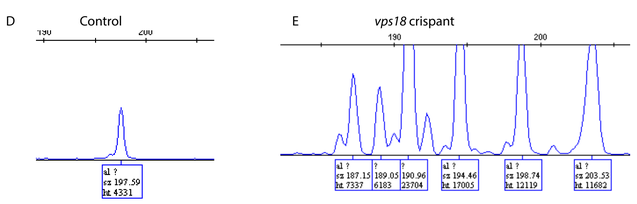
The HOPS and CORVET complexes have been implicated in vesicle fusion in yeast through their specific Rab binding domains. In mammalian cells, HOPS has been shown to interact with the dynein-dynactin binding protein RILP. The exact role HOPS is playin in axonal health, e.g. vesicle fusion and/or mediation of retrograde transport, is unclear

Results

1. Our *vps18* mutant replicates a CRISPR KO loss of function

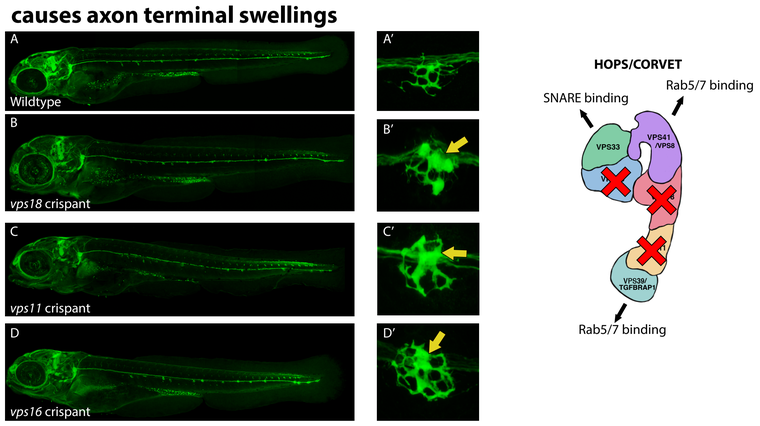


(A) Wildtype 4dpf larva with the *Tg-BAC(neurod:egfp)* transgene with (A') axon terminal of pLL (boxed in A). (B) *vps18* mutant with axon terminal swellings (arrow). (C) *vps18* crispants phenocopy the *vps18* mutant. (D,E) Genotyping of a control (D) and a representitive *vps18* crispant (E). Multiple peaks indicate effective generation of indels at the target site.

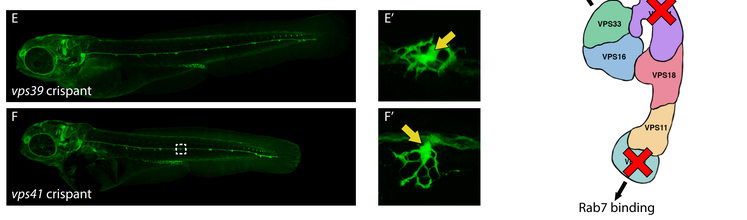


2. G0 Crispant screen reveals that loss of HOPS and not CORVET causes axon terminal swellings

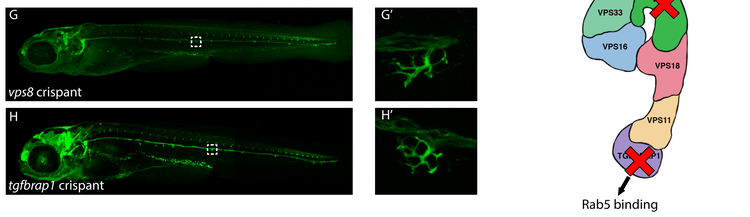
Loss of shared HOPS/CORVET components causes axon terminal swellings



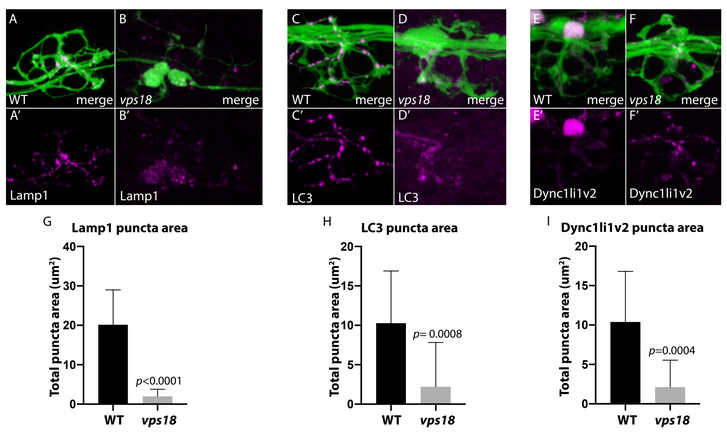
Loss of HOPS-specific components causes axon terminal swellings



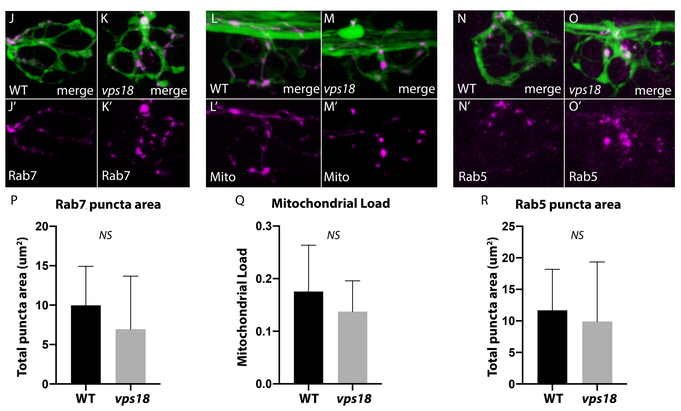
Loss of CORVET-specific components does not cause axon terminal swellings



3. Loss of Vps18 leads to a decrease of some vesicular cargos, retrograde motor component in axon terminals



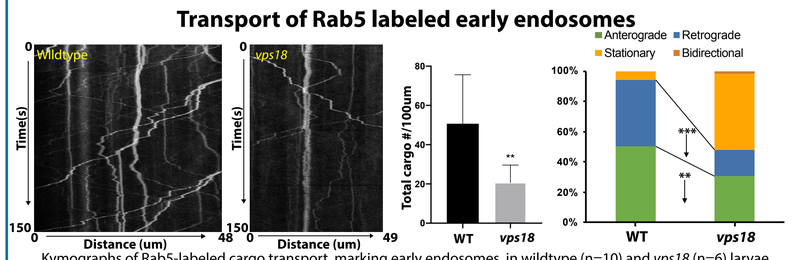
(A-F) Representative live images of 4dpf WT and *vps18* larvae expressing Lamp1 (late endosome/lysosome), LC3 (autophagosome), or dync1li1V2 (dynein) tagged with RFP in a single axon terminal. (G-I) Graphs of total area of all puncta in axon terminals show significant decrease in *vps18* mutants compared to wildtype.



(J-O) Representative live images of 4dpf WT and *vps18* larvae expressubg Rab7 (late endosomes), Mitochondrial targeting sequence, or Rab5 (early endosomes) tagged with RFP. (P,R) Graphs of total area of all puncta in axon terminals show no significant difference in area between *vps18* mutants and wildtype larvae and no significant difference in Mitochondrial load (Q), a measure of total mitochondrial area/expressing axon area

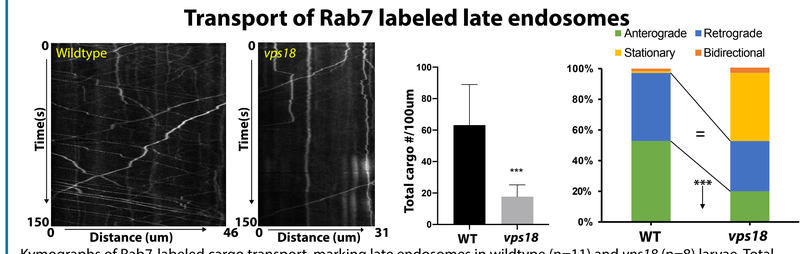
4. Axonal transport of vesicular cargos is disrupted in *vps18* mutants

Transport of Rab5 labeled early endosomes



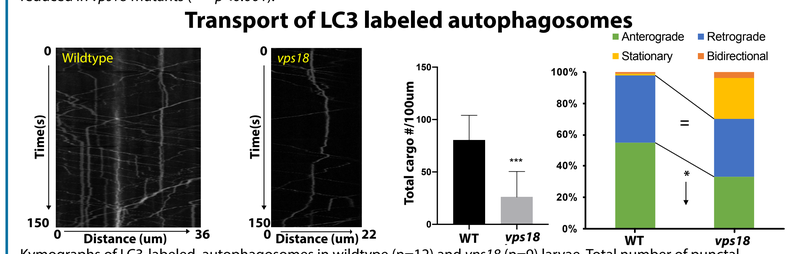
Kymographs of Rab5-labeled cargo transport, marking early endosomes, in wildtype (n=10) and *vps18* (n=6) larvae. Total number of punctal cargo (Wilcoxson Rank Sum) and the proportion of those cargos moving (Wilcoxson Rank Sum) are reduced in *vps18* mutants (***-*p*<0.001, **- *p*<0.01).

Transport of Rab7 labeled late endosomes



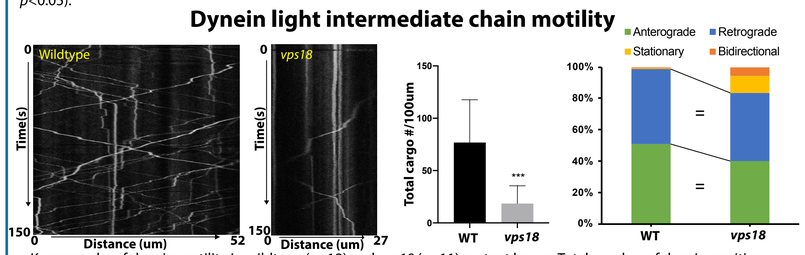
Kymographs of Rab7-labeled cargo transport, marking late endosomes in wildtype (n=11) and *vps18* (n=8) larvae. Total number of punctal cargo (Wilcoxson Rank Sum) and the proportion of those cargos moving (Wilcoxson Rank Sum) are reduced in *vps18* mutants (***-*p*<0.001).

Transport of LC3 labeled autophagosomes



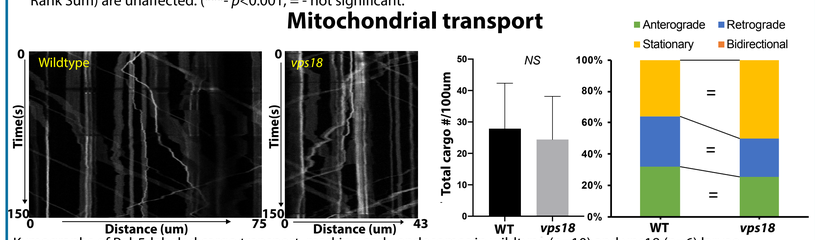
Kymographs of LC3-labeled autophagosomes in wildtype (n=12) and *vps18* (n=9) larvae. Total number of punctal cargo (ANOVA) and the proportion of those cargos moving (ANOVA) are reduced in *vps18* mutants (***-*p*<0.001, *-*p*<0.05).

Dynein light intermediate chain motility



Kymographs of dynein motility in wildtype (n=12) and *vps18* (n=11) mutant larvae. Total number of dynein-positive puncta (Wilcoxson Rank Sum) is reduced in *vps18* mutants but the proportion moving in either direction (Wilcoxson Rank Sum) are unaffected. (***- *p*<0.001, = - not significant.

Mitochondrial transport



Kymographs of Rab5-labeled cargo transport, marking early endosomes in wildtype (n=10) and vps18 (n=6) larvae. Total number of punctal cargo (Wilcoxson Rank Sum) and the proportion of those cargos moving (Wilcoxson Rank Sum) are reduced in vps18 mutants (***-*p*<0.001, **- *p*<0.01).

Conclusions

- VPS18 loss of function leads to axon terminal swellings
- Loss of the HOPS complex is responsible for the phenotype
- Transport and localization of cargos in endolysosomal pathway and retrograde motor is disrupted in *vps18* mutants

Future Directions

- Determine if Vps18 interaction with RILP underlies cargo transport defects
- Determine what is accumulating in these axon terminals

Acknowledgements

We thank the NICHD zebrafish facility for zebrafish husbandry and our funding from the National Institute of Child Health & Human Development.