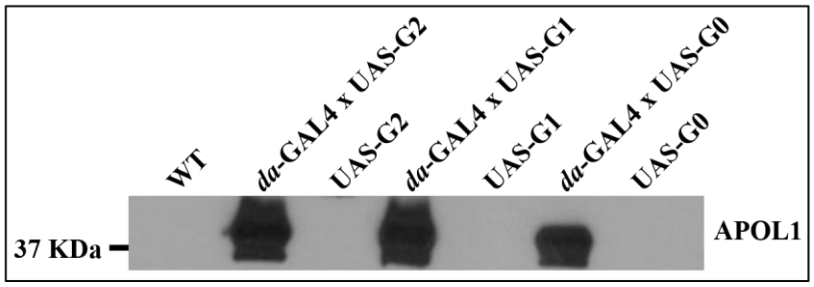
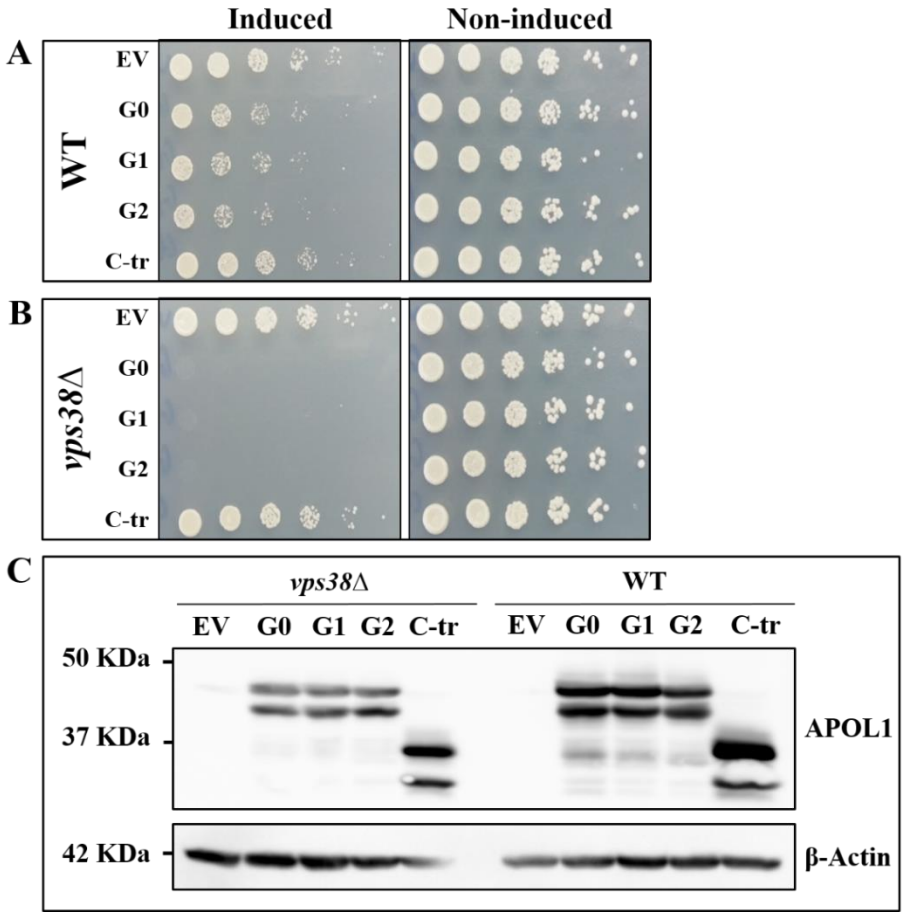
SUPPLEMENTAL INFORMATION

Figure S1.



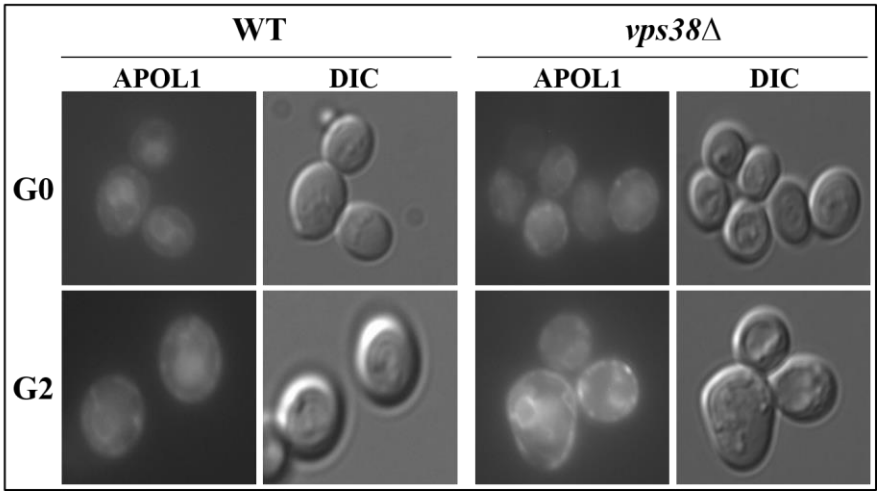
Supplemental Figure 1. Western analysis of *D. melanogaster* larvae hemolymph, expressing APOL1 G0, G1, G2 gene products under *da*-GAL4. APOL1 is expressed in the hemolymph of larvae. Hemolymph was extracted from individual larvae and pooled for each transgenic strain. Equal total protein (50 μg) per lane was loaded.

Figure S2.



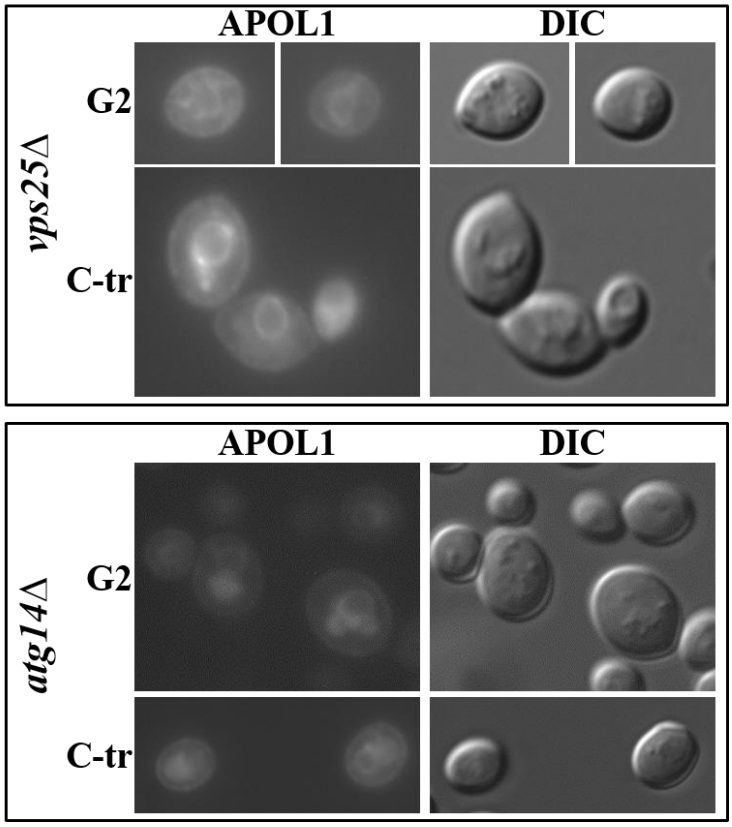
Supplemental Figure 2. Hypersensitivity of the *vps38Δ* to ApoL1. Plasmids containing the human *APOL1* variants, under the yeast GAL1 promoter, were transformed into WT and *vps38Δ* strains. Drop titration growth assay results are shown for (**A**) WT and (**B**) *vps38*Δ. Each row represents serial fivefold dilutions (left to right) of a suspension of cells transformed with the indicated plasmid, spotted on plates containing glucose (right panel – non-induced conditions) or galactose (left panel - induced conditions). (**C**) Western analysis of APOL1 expression. APOL1 was induced for 3 hours on synthetic minimal media containing essential amino acids and galactose. β-actin serves as a control for protein loading. EV- empty vector, C-tr- C-truncated *APOL1*.

Figure S3.



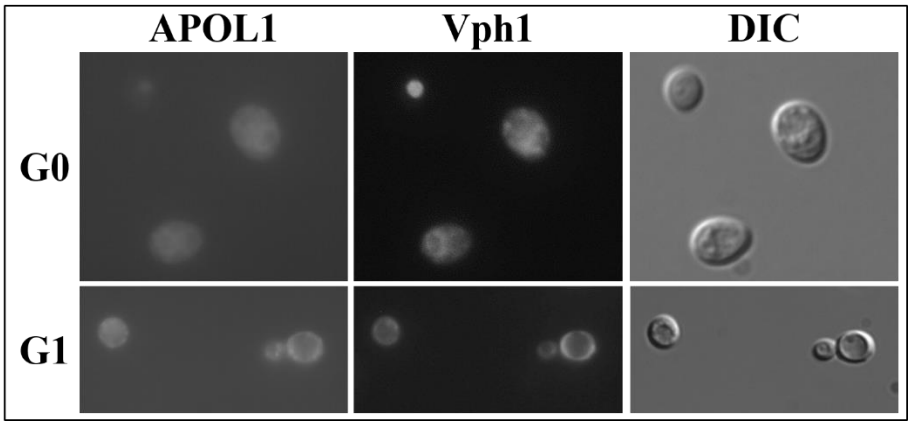
Supplemental Figure 3. Lack of APOL1 vacuolar localization in the *vps38*Δ strain. *APOL1* variants were tagged with mCherry at their C-terminus and expressed in WT and *vps38*Δ strains. APOL1 expression was induced for 3 hours on synthetic minimal media containing essential amino acids and galactose. DIC- differential interference contrast.

Figure S4.



Supplemental Figure 4. APOL1 vacuolar localization is defective in endocytic mutant (*vps25*Δ), but not in the autophagic mutant (*atg14*Δ). *APOL1* variants were tagged with mCherry at their N-terminus sequence (after the signal peptide, see Concise methods) and expressed in the indicated strains. APOL1 expression was induced for 3 hours on synthetic minimal media containing essential amino acids and galactose. C-tr- C-truncated APOL1; DIC- differential interference contrast.

Figure S5.



Supplemental Figure 5. Defective transport of Vph1 in yeast expressing G0 and G1 *APOL1* variants. Plasmids containing the human APOL1 variants, under the yeast GAL1 promoter, were transformed into *S. cerevisiae* expressing GFP-Vph1. In the WT strain, as well in the C-tr APOL1, Vph1-GFP localized normally to the vacuole, whereas APOL1 expression (G0, G1) inhibited Vph1 trafficking to the vacuole, which was missorted to multiple punctate compartments. DIC-differential interference contrast.

Supplemental Table S1: APOL1 variants increase lethality of *vps38Δ* strain.

APOL1 variant comparison	variant 1: 8h/0h ratio	variant 2: 8h/0h ratio	p value for difference†
G0–EV	0.28	0.83	<0.0001
G1–G0	0.22	0.28	0.0070
G2–G0	0.20	0.28	0.0001
G2–G1	0.20	0.22	0.1663
C-trunc–EV	0.78	0.83	0.4149

APOL1 variant	WT: 8h/0h ratio	*vps38Δ*: 8h/0h ratio	p value for difference
EV	0.86	0.83	0.5441
G0	0.71	0.28	<0.0001
G1	0.55	0.22	<0.0001
G2	0.45	0.20	<0.0001
C-trunc	0.82	0.78	0.4466

Survival assays were conducted as described in Methods, with measurement of surviving cells determined as colony forming units (CFU) expressed as ratios at 0 hours and 8 hours of induction for each experimental condition. Each pair of experiments was conducted in parallel for all conditions 3 times, and p values for differences determined as described in Methods. Top table show ratios of surviving cells at 8 versus 0 hours for each experimental condition in the comparison pair and the corresponding p value for the difference. The bottom table shows the survival ratios for each APOL1 experimental condition in the WT (non-*vps38Δ*) versus *vps38Δ* strain, and the corresponding p value for the comparison. EV- empty vector, C-tr- C-terminus truncated APOL1.

Supplemental Table S2: *S. cerevisiae* deleted strains that enhanced *APOL1* toxicity.

Golgi/ endosome to endosome trafficking	Endosome to Golgi trafficking	Endosome maturation-MVB	Endosome to vacuole trafficking	Golgi to vacuole trafficking	Endosome /vacuole acidification
CORVET	**RETROMER**	**ESCRT 0**	***HOPS***	*apm3*Δ	*vma1*Δ
*vps3*Δ	*vps5*Δ	*vps27*Δ	*vps39*Δ		*vma21*Δ
*vps8*Δ	*vps17*Δ		*vps41*Δ		
*vps16*Δ	*vps26*Δ		*vps16*Δ		
*vps18*Δ	*vps29*Δ		*vps18*Δ		
*vps33*Δ	*vps35*Δ		*vps33*Δ		
*pep5*Δ		**ESCRT I**	*vam3*Δ		
*pep7*Δ		*vps37*Δ	*vam7*Δ		
*pep12*Δ		*vps28*Δ			
*vps13*Δ		**ESCRT II**			
*ypt6*Δ		*vps25*Δ			
		*vps36*Δ			
		ESCRT III			
		*vps2*Δ			
		*vps20*Δ			
		*vps24*Δ			

CORVET- class C core vacuole/endosome tethering factor, **ESCRT**- endosomal sorting complex required for transport, **HOPS**- homotypic fusion and vacuolar protein sorting. **RETROMER**- retrograde endosomal to Golgi trafficking. PI(3)P class III (VPS34, VPS30, VPS15 and VPS38) interacts with the endosomal sorting complex ESCRT-I machinery, CORVET and the late endosomal/lysosomal HOPS.

Supplemental Table S3: Primer list

Name	5'→ 3' sequence
C-ter truncated	CCGAATTCTCACACATCCGTGAGCTTGAC
S342G fw	GATGTGGCCCCTGTAGGCTTCTTTCTTGTG
S342G rev	GCACAAGAAAGAAGCCTACAGGGGCCACATC
I384M fw	GGAGAAGCTAAACATGCTCAACAATAATTAT
I384M rev	ATAATTATTGTTGAGCATGTTTAGCTTCTCC
BamHI-*APOL1* 1 fw	ATGCGGATTCATGAGATTCAAAAGCCACAC
APOL1-mcherry 2 rev	TCCTCGCCCTTGCTCACCATTCCAGCTTCCTCTGCCCTCAC
APOL1-mcherry 3 fw	GTGAGGGCAGAGGAAGCTGGAATGGTGAGCAAGGGCGAGGA
APOL1-mcherry (-stop) 4 rev	ACGTTTTGTTGCACCCTCGCCTTGTACAGCTCGTCCATGC
APOL1-mcherry (-stop) 5 fw	GCATGGACGAGCTGTACAAGGCGAGGGTGCAACAAAACGT
APOL1 6 Xho1 rev	TCAGCTCGAGTCACAGTTCTTGGTCCGCCTG
BamHI-mCherry fw	ACGAGGATCCAATGGTGAGCAAGGGCGAGGA
mCherry-XhoI rev	AGTCCTCGAGTTACTTGTACAGCTCGTCCAT

Supplemental Table S4: Yeast strains that were used in the study

Name	Relevant genotype	Source
WT	BY4741; Mat a; his3Δ1, leu2Δ0, met15Δ0, ura3Δ0	Euroscarf
apm3Δ	BY4741; apm3:: kanMX4	Euroscarf
atg14Δ	BY4741; atg14:: kanMX4	Euroscarf
pep4Δ	BY4741; pep4:: kanMX4	Euroscarf
pep5Δ	BY4741; pep5:: kanMX4	Euroscarf
pep7Δ	BY4741; pep7:: kanMX4	Euroscarf
pep12Δ	BY4741; pep12:: kanMX4	Euroscarf
trk2Δ	BY4741; trk2:: kanMX4	Euroscarf
vam3Δ	BY4741; vam3:: kanMX4	Euroscarf
vam7Δ	BY4741; vam7:: kanMX4	Euroscarf
vma1Δ	BY4741; vma1:: kanMX4	Euroscarf
vma21Δ	BY4741; vma21::kanMX4	Euroscarf
vps2Δ	BY4741; vps2:: kanMX4	Euroscarf
vps3Δ	BY4741; vps3:: kanMX4	Euroscarf
vps5Δ	BY4741; vps5:: kanMX4	Euroscarf
vps8Δ	BY4741; vps8:: kanMX4	Euroscarf
vps13Δ	BY4741; vps13:: kanMX4	Euroscarf
vps15Δ	BY4741; vps15:: kanMX4	Euroscarf
vps16Δ	BY4741; vps16:: kanMX4	Euroscarf
vps17Δ	BY4741; vps17:: kanMX4	Euroscarf
vps18Δ	BY4741; vps18:: kanMX4	Euroscarf
vps20Δ	BY4741; vps20:: kanMX4	Euroscarf
vps24Δ	BY4741; vps24:: kanMX4	Euroscarf
vps25Δ	BY4741; vps25:: kanMX4	Euroscarf
vps26Δ	BY4741; vps26:: kanMX4	Euroscarf
vps27Δ	BY4741; vps27:: kanMX4	Euroscarf
vps28Δ	BY4741; vps28:: kanMX4	Euroscarf
vps29Δ	BY4741; vps29:: kanMX4	Euroscarf
vps30Δ	BY4741; vps30:: kanMX4	Euroscarf
vps33Δ	BY4741; vps33:: kanMX4	Euroscarf
vps34Δ	BY4741; vps34:: kanMX4	Euroscarf
vps35Δ	BY4741; vps35:: kanMX4	Euroscarf
vps36Δ	BY4741; vps36:: kanMX4	Euroscarf
vps37Δ	BY4741; vps37:: kanMX4	Euroscarf
vps38Δ	BY4741; vps38:: kanMX4	Euroscarf
vps39Δ	BY4741; vps39:: kanMX4	Euroscarf
vps41Δ	BY4741; vps41:: kanMX4	Euroscarf
ypt6Δ	BY4741; ypt6:: kanMX4	Euroscarf
VPH1-GFP	BY4741; VPH1-GFP:HIS3	(Spokoini et al., 2012)