

A

	L17P	I41T	D53Y	
Human	-----MPTAA--APIISSVQKLVLYETRAR--YFLVGSNNAETKYRVLKIDR--TEPKDLVVIDDR--HVYTQQEVRELLGRLD	*	*	71
<i>Dros mel</i>	MNNDN--PNIIVFNPLISSIQKVLYETRARLY--LVGSNNRETRFRLLTIDRLAHNR--LSI--EENANEFNSLEIR-----RF--			71
Human	LGNRTKMGQKGS SGLFRAVSAFVGVCFVRFLEGGYIVLITKRRK--MADIGGHAHYKVEDTNNMIYIPND--SVRVTHPDEA			148
<i>Dros mel</i>	VAS---LS--GSP---KVTISAYGVLGFVRFLEGGYLLLVTKR--KCCAHHGRELHYVYTIKIPYVRY--SNVYISORPPHPHED			141
Human	RYLRIFQNVDLSSNFYFSYSYDLSHSLOQYNLTVDLRMPLEMLKSEMTQNRQESF--DIFEDEGITIQG-GSG---V--FGICS			222
<i>Dros mel</i>	RYKRMFQNIIDLRSNFYFSYSYDLTRTLQYNESAPRY--VGA--KVDL--DRDEPLPD--W--NT--I--TSNVDKAHERVDYAFRS			213
Human	EPYMK--YVWNGELLDI IKSTVHRDWLLYTIHGFVCGOSKLLIYGRPVYVTLIARRSSKFAGTRFLKRGANCFEGDVANEVET			301
<i>Dros mel</i>	DS--RKRFEVWVAYLLOPMEGIMLKDWLLEVTHTGFVSQSCISIFGREVNVCLVARRSSRFAGTRFLKRGANFQGDVANEVET			292
Human	EQILCDAS--VMSFTAGSYSSYVQVRGSPVLYWSODISTMMPKPPITLDDQADPFAHVAALHFDQ--MFORFGSPITIIILNLVK			379
<i>Dros mel</i>	EQIVSDCQRICAFI-----QMRGSI PSHWSQDISKMWPKPQIQLDIDCPVAQTPSLHFERLLEH--YCAPLIMLNLVK			363
Human	EREKRKHERRILSEELVAAVTYLNOFLPPEHTIVYIPWDMAKYTKSKLC--NVLDRLNVI--AESVVKKTC--FFVNRPDSYC			455
<i>Dros mel</i>	KRERRKHESIIISKELIYSTIRYLNQFLPPEHRMKHLEHEDMAR--QSRLSGGNVMEQL--AHAEASIVQMTGMEF--K--A-A			435
Human	SILRPDEKWNELGGCVIPTGRLQGTGILRTNCVDCLDRTNTAQEFMVGKCALAYQLYSLGL--DK--PNLOFDIDAVRLFEELY	*		534
<i>Dros mel</i>	G-----SE-----P--G--LQGTGIVRTNCVDCLDRTNSAQEFAIGKCALGHQLERLGFV--KSAKLEFSDCVTMLLENLY			498
Human	EDHGDITSLQYGGSQLVHRVKTYRKTAPW--TQHSKDIMQTLSTRYYSNAFSDADRODSINLFLGVFHP--T--EGKPHLWEL			610
<i>Dros mel</i>	EEHGDITLALQYGGSQLVHRIRKTYRKTAPWGSQGS--DVMQTLSTRYYSNTFSDTEKQHSINLFLGLIYKPSLTIKOGPP--I--WEL			576
Human	PTDFY--LHHKNTMRLLETR---RSYIYVWWTPEVTKH--L--P--LPYDEVICA--VNLKKLIVKK--F--HKYE--EEIDIH--NEF			677
<i>Dros mel</i>	QTD--YDMH--NAF--V--P--RADS KATIDW---V--RHKVRACLPYS---CADSN--K--L--VKELFRVHSSGLEMIDAYSN--Y			638
Human	FRPYELSSFDDTFCCLAM--TSS--A--RDEMP--KTVGIDPSPFTVRKPDDETGKSVLGNKNSNREE--AVL--QRKTAASAPPPPS			750
<i>Dros mel</i>	HQSFKWDIASE--H--LAFEISQLALR--YMPTRFT--NF--SPFQ--RQI--QT--S---RKA--RONPSMTGQSSTG--SMN---S			700
Human	EEAVSSSSE--DDSGTDR EE--EGSVSQR--STPVKMTD--AGDSAKV--TENVQVQ--MKELYG--INLSDGLSEEDFSIYSRFV			822
<i>Dros mel</i>	N--S--SSSSEGDDSSD--EELSASF AEKEANQTESTEPA--AATLAT--GL--PSMEEIYGCTIN--PP--SKQSMAVYKQYV			769
Human	QLGQ--SQ--HK--QDKNSQ--OPCSFCSDGVIKLTPISAFSODNIY--EVQPPRVDRKS--TELEQA--HIQA--S--QGIMQP			889
<i>Dros mel</i>	QMCKLSSGGARPAQTAVAQORDQELAKIMRG--ITLRPLSDYGTDS--YLSVRPVPVPRKSLT--IY--AEYCRTRSTFNAV--P			843
Human	-LGKE--DSSMYREYIRNRYL			907
<i>Dros mel</i>	KL--EEFDV--LY--QYVQ--K--L			858

B

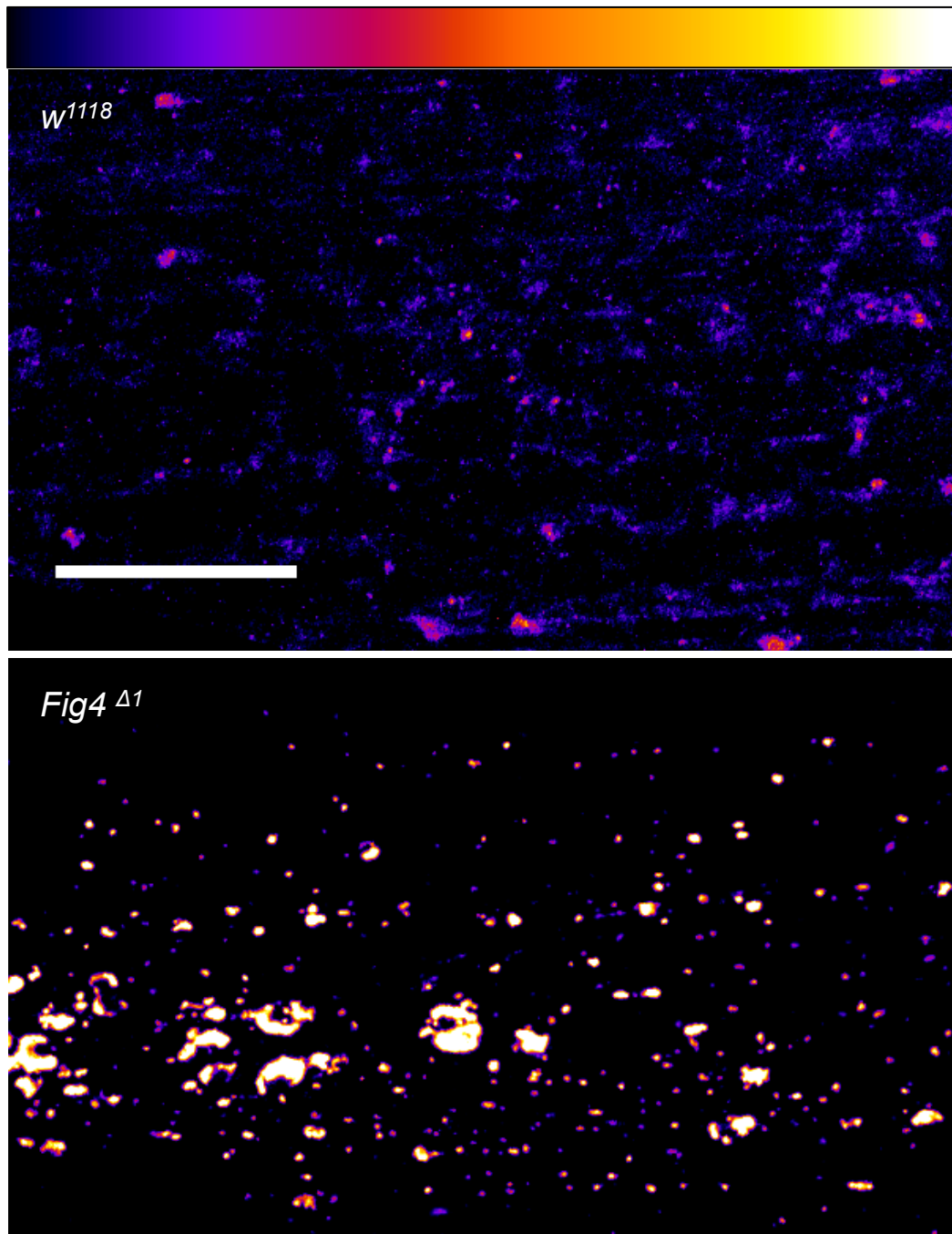
	L17P	I41T	D53Y	
Mouse	ISSVQKLVLYETRAR [*] YFLVGSNHAETKYRVLKIDRTEPKDLVVIDDRHVVY [*] QQEVRELLGRLDLG [*] NR	*	*	75
Human	ISSVQKLVLYETRAR [*] YFLVGSNNAETKYRVLKIDRTEPKDLVVIDDRHVVY [*] QQEVRELLGRLDLG [*] NR	*	*	75
<i>Dros mel</i>	ISSIQKVVLYETRARLYLVGSNNRETRFRLLTIDRLAHNRLSIEENANEFNSLEIRRFVASLSGS--			78
<i>C. elegans</i>	PCRLRKITVYETKSRFYIIGCDSTGSTRYVNLKIDRIDPKALITGEPYDYTR [*] EILEL [*] LATISDGSS			68

Supplemental Figure S1: Protein alignment of Human and *Drosophila* FIG4.

(A) *Drosophila melanogaster* (*Dros mel*) FIG4 (CG17840) is 59% similar and 42% identical to *Homo sapiens* (Human) FIG4. Asterisks show disease causing missense mutations in the protein interaction domain (PID) that are characterized in this study (see Figure 1). The SAC phosphatase domain is highly conserved, including the conserved CX₅R(S/T) motif (underlined). This study analyzes the effect of the catalytic cysteine to serine (asterisk, C450S in *Drosophila*), known to inactivate the phosphatase. Alignment performed using http://www.bioinformatics.org/sms2/pairwise_align_protein.html (Stothard P (2000) The Sequence Manipulation Suite: JavaScript programs for analyzing and formatting protein and DNA sequences. Biotechniques 28:1102-1104). (B) Multiple sequence alignment (ClustalW) of FIG4 protein-interaction domain showing conservation of disease-associated residues from multiple species. Light blue is identity; dark blue similarity.

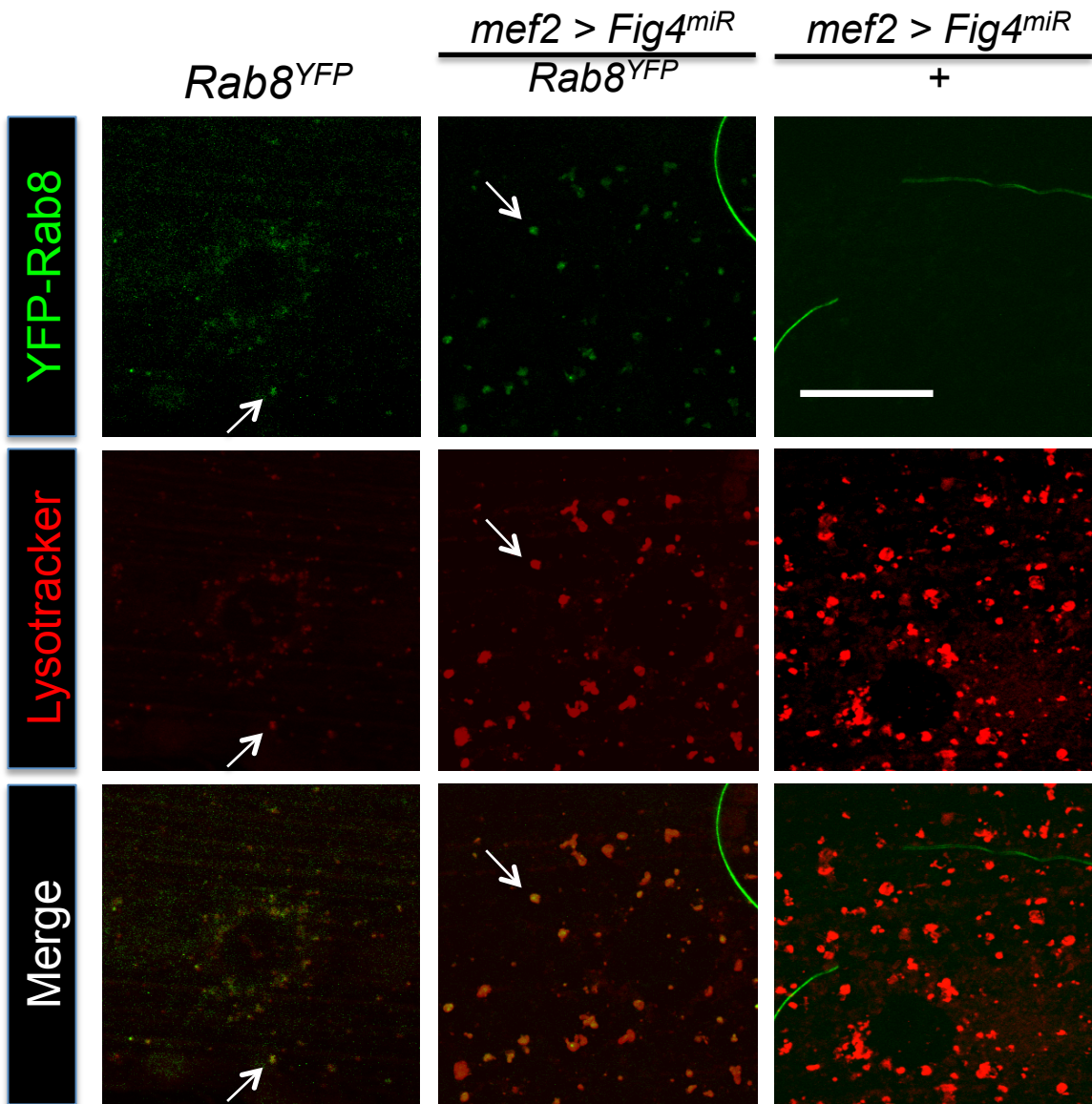
Min

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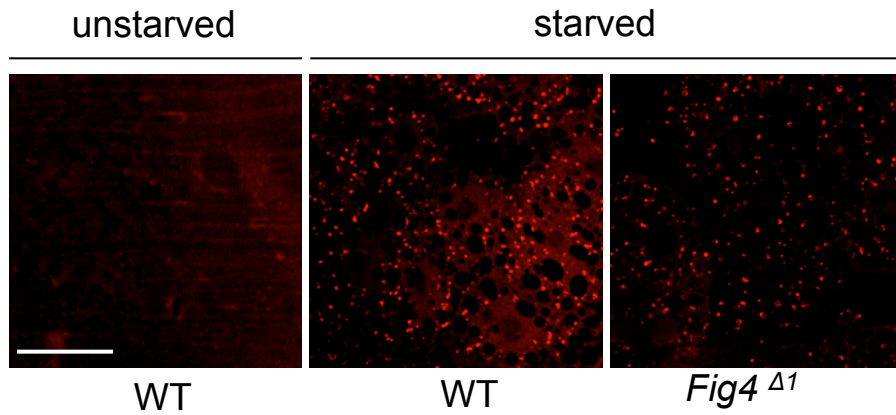
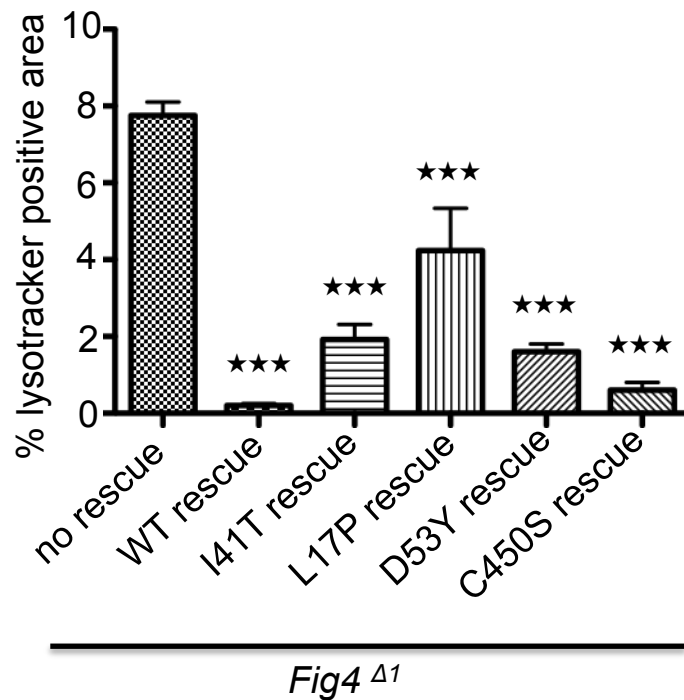
Supplemental Figure S2

Heat-map showing that with confocal settings that detect lysosomes with Lysotracker staining in wild-type muscles, the Lysotracker staining becomes saturated in *Fig4* animals. Scale bar is 20 μ m.



Supplemental Figure S3

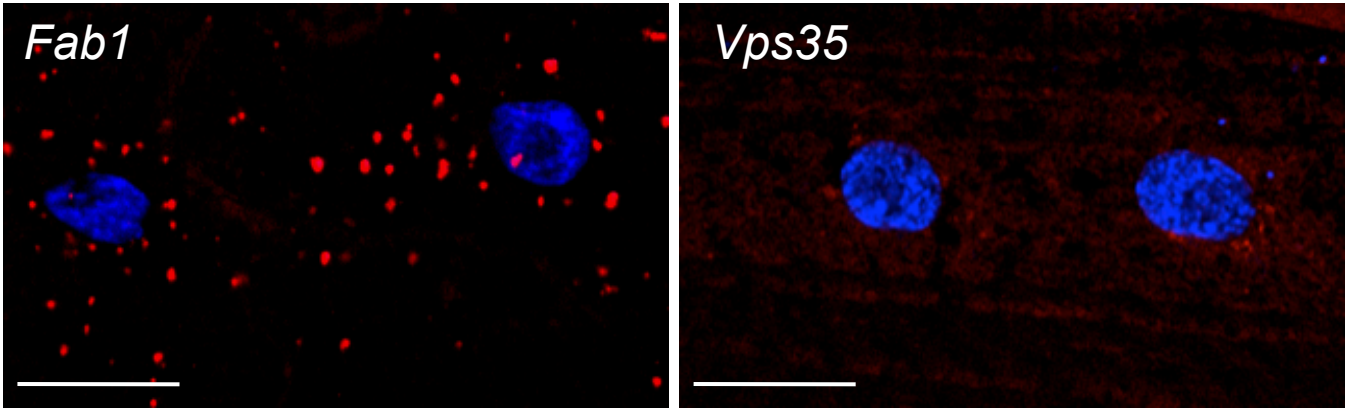
Endogenous Rab8^{YFP} colocalizes with Lysotracker (arrows) in wild-type (left) and *Fig4* knockdown (*Fig4^{miR}*) animals (middle). Note that punctae are much larger with *Fig4* knockdown. Right panels show that using identical confocal settings, there is no crossover of Lysotracker into YFP channel (note trachea autofluorescence). Scalebar is 20 μ m.

A**B****Supplemental Figure S4**

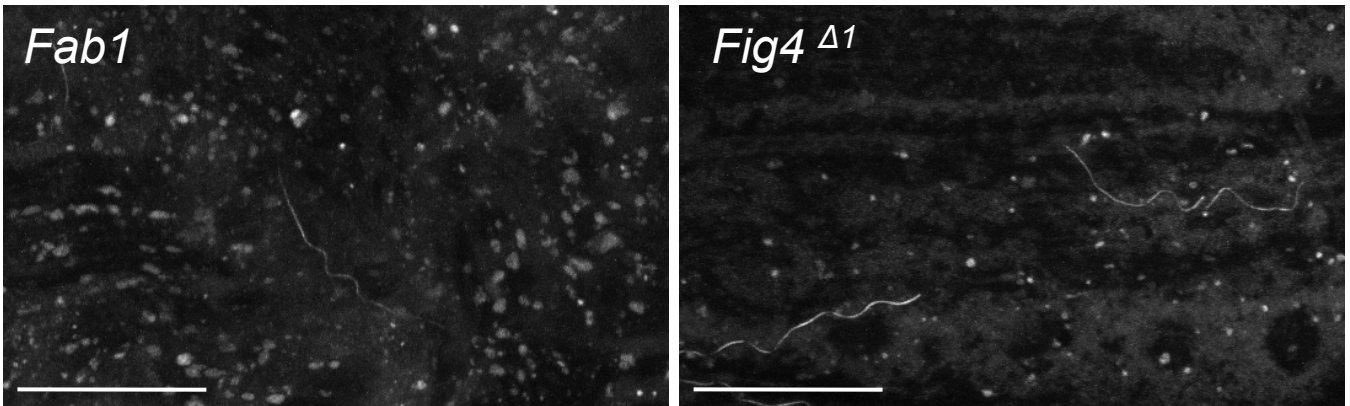
(A) Starvation-induced autophagolysosome formation does not appear to be disrupted in the fat body of *Fig4*^{Δ1} third-instar larvae. Scale bar 70 μ m.

(B) *Drosophila* FIG4 containing I41T, L17P, and D53Y missense mutations partially rescue the *Fig4*^{Δ1} LysoTracker phenotype. The C450S phosphatase-inactive mutation rescues the LysoTracker phenotype almost as well as wild-type FIG4. n= 30 hemisegments. Data are mean \pm s.e.m, ****P*<0.005 (pairwise t-test compared with no rescue).

A



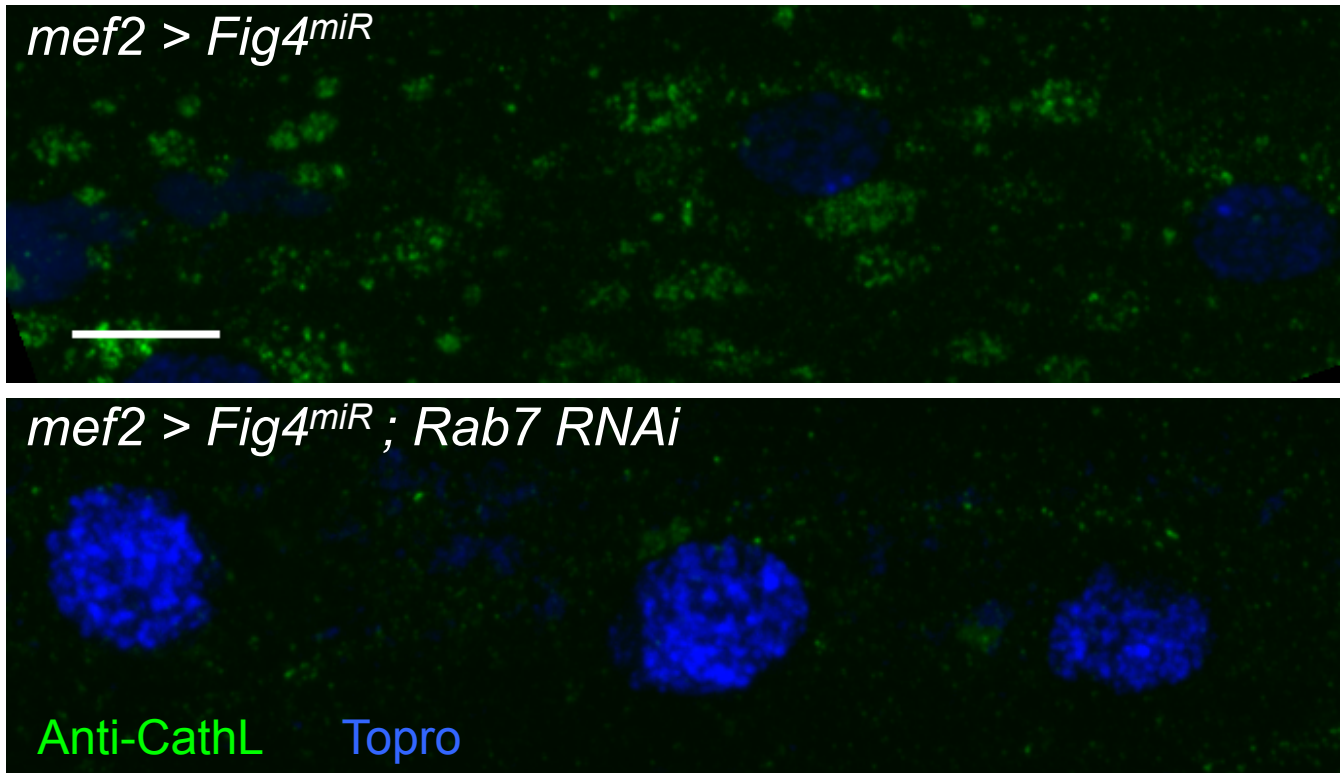
B



Supplemental Figure S5

(A) Lyotracker staining of late third-instar larvae demonstrating that lysosomes are markedly enlarged compared with control animals (*Vps35*, which is not significantly different than wild-type, is shown—same panel as in Figure 6 for comparison). Note *Fab1* late third-instar larvae are significantly smaller than wild-type animals and arrest development at this stage. Scale bar is 20 μ M

(B) Lyotracker staining of early, size-matched third-instar larvae show that the lysosomal expansion phenotype of *Fab1* null animals is at least as severe as that of *Fig4* animals at that stage. Scale bar is 20 μ M



Supplemental Figure S6

Rab7 knockdown suppresses Lysosome expansion (labeled with Cathepsin L antibody) observed with *Fig4* knockdown. Scale bar is 10 μ m.