Table S1. Fly stocks used in this study. (BDSC:Bloomington Drosophila Stock Center, VDRC:Vienna Drosophila RNAi Center)

Stock name	Source [reference]	Stock ID
$Atg6^{1}$	EH. Baechrecke [1]	
UAS-Atg6-myc	EH. Baechrecke [1]	
UAS-GFP-2xFYVE	M. Gonzalez-Gaitan [2]	
tubRab4-YFP	S. Eaton [3]	
tubRab11-YFP	S. Eaton [3]	
tubRab5-CFP, tubRab7-YFP	T. Klein [4]	
UAS-Lamp1-GFP	H. Krämer [5]	
tubLamp1-GFP	H. Krämer [5]	
CathD-mCherry	G. Juhasz [6]	
ptcGal4,UAS-myc,UAS-mCherry-Atg8	G. Juhasz [7]	
MsGal4	BDSC	8860
MsGal4;UAS-Dcr-2	BDSC	25706
UAS-Atg6-RNAi	BDSC	28060
UAS-UVRAG-RNAi	BDSC	34368
UAS-Atg14-RNAi	VDRC	108559
UAS-Vps34-RNAi	VDRC	100296
UAS-Vps15-RNAi	BDSC	34092
UAS-Atg8a-RNAi	BDSC	28989
UAS-N	BDSC	26820
UAS-Hrp-CD2	BDSC	8763
UAS-Clc-GFP	BDSC	7107
UAS-FLP	BDSC	4539
FRT82B,ubiGFP,Minute($RpS3^{Plac92}$)	BDSC	5627
FRT82B,ubiGFP	BDSC	32655
FRT82B	BDSC	2035
hsFLP	BDSC	8862
UAS-Dcr-2;enGal4,UAS-EGFP	BDSC	25752
enGal4	BDSC	30564
enGal4,RFP	BDSC	30557
UAS-Dcr-2;enGal4,UAS-myrRFP,NRE-EGFP	BDSC	30730

Table S2. Antibodies used in this study. (DSHB:Developmental Studies Hybridoma Bank, IHC: immunohistochemistry, IEM: immunoelectron microscopy, TA:traffic assay)

Antibody	Application and dilution	Source
mouse anti-Flamingo	IHC: 1:50	DSHB: Flamingo #74
mouse anti-Armadillo	IHC: 1:100	DSHB: N2 7A1 ARMADILLO
mouse anti-Fasciclin III	IHC: 1:100	DSHB: 7G10 anti-Fasciclin III
rat anti-Cadherin	IHC: 1:100	DSHB: DCAD2
mouse anti-Discs large	IHC: 1:100	DSHB: 4F3 anti-discs large
mouse anti-β-integrin	IHC: 1:80	DSHB: CF.6G11
rabbit anti-GFP	IHC: 1:800, IEM: 1:100	Abcam: ab290-50
mouse anti-Notch extracellular domain	IHC: 1:100 TA: 1:25	DSHB: C458.2H
mouse anti-Notch intracellular domain	IHC: 1:100	DSHB: C17.9C6
mouse anti-Delta extracellular domain	IHC: 1:100	DSHB: C594.9B
mouse anti-Wingless	IHC: 1:100	DSHB: 4D4
rabbit anti-p62	IHC: 1:2000	G.Juhasz [8]
rat anti-mCherry	IHC: 1:300	G. Juhasz [7]
rabbit anti-Cleaved Caspase-3	IHC: 1:300	Cell Signaling Technology®: #9661
Sheep anti-Digoxigenin-AP conjugate	TUNEL: 1:4000	Roche: 11093274910
DyLight®594 horse anti-Mouse	IHC: 1:500	Vector: Dl-2594
DyLight®594 goat anti-Rabbit	IHC: 1:500	Vector: Dl-1594
Alexa Fluor®488 goat anti-Mouse	IHC: 1:500	Invitrogen: A11001
Alexa Fluor®488 goat anti-Rabbit	IHC: 1:500	Invitrogen: A11008
Alexa Fluor®568 goat anti-Rat	IHC: 1:500	Invitrogen: A11077
18nm gold-conjugated goat anti-Rabbit	IEM: 1:100	Jackson ImmunoResearch: 111-215-144

Table S3. Statistics

Figure	Group	N	Mean	SD	P-value	Test and post hoc
Fig. 2a-e GFP-FYVE	control	30	8.2510	3.0501	P<0.0001	Kruskal-Wallis test (ctrl-Atg6: P<0.0001, ctrl-UVRAG: P<0.0001, ctrl-Atg14: P=1.0000, Atg6-UVRAG: P=1.0000, Atg6-Atg14: P<0.0001, UVRAG-Atg14: P<0.0001
	Atg6 RNAi	30	0.6510	1.0100		
	UVRAG RNAi	30	0.7843	0.9453		
	Atg14 RNAi	30	8.5140	4.2216		
Fig. 2f-h Atg6,Clc	control	30	7.5703	3.4941	P=0.1761	Mann-Whitney test
	Atg6 RNAi	30	6.2923	6.7276		
Fig. 2i,j Atg6,Rab4	control	30	9.6607	2.9445	P<0.0001	Wilcoxon's signed ranks test
	Atg6 RNAi	30	29.9700	5.9617		
Fig. 2k,l Atg6,Rab5	control	30	9.1597	4.4263	P<0.0001	Wilcoxon's signed ranks test
	Atg6 RNAi	30	22.5687	7.5319		
Fig. 2m,n Atg6,Rab7	control	30	7.4573	3.6079	P<0.0001	Wilcoxon's signed ranks test
	Atg6 RNAi	30	20.3950	6.5058		
Fig. 2o,p Atg6,Rab11	control	30	0.6627	0.8570	P=0.1494	Wilcoxon's signed ranks test
	Atg6 RNAi	30	1.8077	2.4607		
Fig. 2q,r Atg6,Lamp1	control	30	0.9523	1.0936	P<0.0001	Wilcoxon's signed ranks test
	Atg6 RNAi	30	13.6137	7.4301		
Fig. 2s,t Atg6,CathD	control	30	3.1570	1.7227	P<0.0001	Wilcoxon's signed ranks test
	Atg6 RNAi	30	15.3590	7.6345		
Fig. 2u,v UVRAG,Lamp1	control	30	0.5913	0.5869	P<0.0001	Wilcoxon's signed ranks test
	UVRAG RNAi	30	6.8510	4.3742		
Fig. 2w,x Atg14,Lamp1	control	30	1.0047	0.6866	P=0.4662	Wilcoxon's signed ranks test
	Atg14 RNAi	30	0.8627	0.7680		
Fig. 5e,f Atg6,p62	control	30	0.5380	0.1205	P<0.0001	Wilcoxon's signed ranks test
	Atg6 RNAi	30	5.2780	0.2916		
Fig. 5g,h Atg14,p62	control	30	0.7723	0.1216	P<0.0001	Wilcoxon's signed ranks test
	Atg14 RNAi	30	3.3040	0.2311		
Fig. 5i,j UVRAG,p62	control	30	0.8763	0.1612	P=0.9658	Wilcoxon's signed ranks test
	UVRAG RNAi	30	0.9503	0.2771		
Fig. 6a,b Atg6 null,NECD	control	25	1.9696	1.1696	P<0.0001	Wilcoxon's signed ranks test
	Atg6 null mutant	25	16.3632	3.3465		
Fig. 6c,d Atg6,NECD	control	20	0.6615	1.0481	P=0.0001	Wilcoxon's signed ranks test
	Atg6 RNAi	20	14.7090	4.8574		
Fig. 6e,f UVRAG,NECD	control	20	1.0630	1.2620	P=0.0001	Wilcoxon's signed ranks test
	UVRAG RNAi	20	12.6330	5.4172		
Fig. 6g,h Atg14,NECD	control	20	3.4385	3.2858	P=0.0966	Wilcoxon's signed ranks test
	Atg14 RNAi	20	2.2240	1.9584		
Fig. S1a,b Atg6,NICD	control	20	0.5050	0.8977	P=0.0001	Wilcoxon's signed ranks test
	Atg6 RNAi	20	17.2225	4.4057		
Fig. S1c,d Atg6,Delta	control	20	0.6775	1.0024	P=0.0002	Wilcoxon's signed ranks test
	Atg6 RNAi	20	5.6810	4.4954		

Table S4. Genotypes of the animals examined in this study.

Figure	Genotype
Fig. 1 Control	+/+; tubGal4/+
Fig. 1 Atg6 RNAi	UAS-Dcr-2/+; tubGal4/UAS-Atg6-RNAi
Fig. 1 UVRAG RNAi	UAS-Dcr-2/+; tubGal4/UAS-UVRAG-RNAi
Fig. 1 Atg14 RNAi	UAS-Atg14-RNAi /+; tubGal4/+
Fig. 2a	MsGal4/+; UAS-Dcr-2/UAS-GFP-2xFYVE; +/+
Fig. 2b	MsGal4/+; UAS-Dcr-2/UAS-GFP-2xFYVE; UAS-Atg6-RNAi/+
Fig. 2c	MsGal4/+; UAS-Atg14-RNAi/UAS-GFP-2xFYVE,UAS-Dcr-2; +/+
Fig. 2d	MsGal4/+;UAS-GFP-2xFYVE/+; UAS-UVRAG-RNAi/+; +/+
Fig. 2f	MsGal4/+; UAS-Dcr-2/UAS-Clc-GFP;+/+
Fig. 2g	MsGal4/+; UAS-Dcr-2/UAS-Clc-GFP; UAS-Atg6-RNAi/+
Fig. 2i	enGal4,RFP/UAS-Dcr-2; UAS-Atg6-RNAi/tubRab4-YFP
Fig. 2k	enGal4,RFP/UAS-Dcr-2; UAS-Atg6-RNAi/tubRab5-CFP
Fig. 2m	enGal4,RFP/UAS-Dcr-2; UAS-Atg6-RNAi/tubRab7-YFP
Fig. 2o	enGal4,RFP/UAS-Dcr-2; UAS-Atg6-RNAi/tubRab11-YFP
Fig. 2q	enGal4,RFP/tubLamp1-GFP; UAS-Atg6-RNAi/UAS-Dcr-2
Fig. 2s	UAS-Dcr-2/+; enGal4,UAS-EGFP/CathDmCherry; UAS-Atg6-RNAi/+
Fig. 2u	enGal4,RFP/tubLamp1-GFP; UAS-UVRAG-RNAi/UAS-Dcr-2
Fig. 2w	enGal4,RFP/tubLamp1-GFP,UAS-Atg14-RNAi; UAS-Dcr-2/+
Fig. 3a	MsGal4/+; UAS-Dcr-2/+; +/+
Fig. 3b	MsGal4/+; UAS-Dcr-2/+; UAS-Atg6-RNAi/+
Fig. 3c	MsGal4/+; UAS-FLP/+; FRT82bAtg6^{1}/FRT82b,ubiGFP,Minute(RpS3^{Plac92})
Fig. 3d	MsGal4/+; UAS-Dcr-2/+; UAS-Atg8a-RNAi/+
Fig. 3e	MsGal4/+; UAS-Dcr-2/+; UAS-UVRAG-RNAi/+
Fig. 3f	MsGal4/+; UAS-Dcr-2/UAS-Atg14-RNAi; +/+
Fig. 4a,b	MsGal4/+; UAS-Dcr-2/UAS-Lamp1-GFP; UAS-Atg6-RNAi/+
Fig. 4c	MsGal4/+; UAS-Dcr-2/+; +/+
Fig. 4d-f	MsGal4/+; UAS-Dcr-2/+; UAS-Atg6-RNAi/+
Fig. 4g	MsGal4/+; UAS-Dcr-2/UAS-HRP-CD2; +/+
Fig. 4h	MsGal4/+; UAS-Dcr-2/UAS-Hrp-CD2; UAS-Atg6-RNAi/+
Fig. 5a	ptc-Gal4, UAS-mCherry-Atg8a, UAS-Myc/+; +/+
Fig. 5b	ptc-Gal4, UAS-mCherry-Atg8a, UAS-Myc/+; UAS-Atg6-RNAi /+
Fig. 5c	ptc-Gal4, UAS-mCherry-Atg8a, UAS-Myc/ UAS-Atg14-RNAi; +/+
Fig. 5d	ptc-Gal4, UAS-mCherry-Atg8a, UAS-Myc/+; UAS-UVRAG-RNAi /+
Fig. 5e	UAS-Dcr-2/+; enGal4,UAS-EGFP/+; UAS-Atg6-RNAi/+
Fig. 5g	UAS-Dcr-2/+;enGal4,UAS-EGFP/UAS-Atg14-RNAi;+/+
Fig. 5i	UAS-Dcr-2/+; enGal4,UAS-EGFP/+; UAS-UVRAG-RNAi/+
Fig. 6a	hsFLP/+; +/+; FRT82bAtg61/FRT82b,ubiGFP
Fig. 6c	UAS-Dcr-2/+; enGal4,UAS-EGFP/+;UAS-Atg6-RNAi/+
Fig. 6e	UAS-Dcr-2/+; enGal4,UAS-EGFP/+; UAS-UVRAG-RNAi/+
Fig. 6g	UAS-Dcr-2/+; enGal4,UAS-EGFP/UAS-Atg14-RNAi; +/+
Fig. 6i	enGal4/UAS-Lamp1-GFP; UAS-Atg6-RNAi/UAS-Dcr-2
Fig. 7a	UAS-Dcr-2/+; enGal4, UAS-myr-mRFP,NRE-EGFP/+; UAS-Atg6-RNAi/+

Fig. 7b	UAS-Dcr-2/+; enGal4, UAS-myr-mRFP,NRE-EGFP/+; UAS-N/+
Fig. 7c	enGa4,UAS-myr-mRFP,NRE-EGFP/UAS-Dcr-2;UAS-Atg6-RNAi/UAS-N
Fig. 7d	UAS-Dcr-2/+;enGal4,UAS-myr-mRFP,NRE-EGFP/+; UAS-UVRAG-RNAi/+
Fig. 7e	UAS-Dcr-2/+; enGal4, UAS-myr-mRFP,NRE-EGFP/UAS-Atg14-RNAi; +/+
Fig. 7f	UAS-Dcr-2/+; enGal4, UAS-myr-mRFP,NRE-EGFP/+; +/+
Fig. 8a	UAS-Dcr-2/+; enGal4,UAS-EGFP/+; UAS-Atg6-RNAi/+
Fig. 8b	UAS-Dcr-2/+; enGal4,UAS-EGFP/+; UAS-UVRAG-RNAi/+
Fig. 8c	UAS-Dcr-2/+;enGal4,UAS-EGFP/UAS-Atg14-RNAi;+/+
Fig. 9a,f	MsGal4/+; UAS-Dcr-2/+; +/+
Fig. 9b-d	MsGal4/+; UAS-Dcr-2/+; UAS-Atg6-RNAi/+
Fig. 9e	MsGal4/+; UAS-Dcr-2/+; UAS-Vps15-RNAi/+
Fig. 9g,h	MsGal4/+; UAS-Dcr-2/+; UAS-UVRAG-RNAi/+
Fig. 9i	MsGal4/+; UAS-Dcr-2/UAS-Vps34-RNAi; +/+
Fig. 9j	MsGal4/+;UAS-Dcr-2/UAS-Atg14-RNAi;+/+
Fig. 9k	MsGal4/y; UAS-FLP/+; FRT82b,ry^{506}/FRT82b,ubiGFP,Minute($RpS3^{Plac92}$)
Fig. 9l	MsGal4/y; UAS-FLP/+; FRT82b$Atg6^{1}$/FRT82b,ubiGFP,Minute($RpS3^{Plac92}$)
Fig. 9m	MsGal4/y; UAS-FLP/UAS-Atg6-myc; FRT82b$Atg6^{1}$/FRT82b,ubiGFP,Minute($RpS3^{Plac92}$)
Fig. 10a-h	UAS-Dcr-2/+; enGal4,UAS-EGFP/+; UAS-Atg6-RNAi/+
Fig. 11a,c,e,g	UAS-Dcr-2/+;enGal4,UAS-EGFP/UAS-Atg14-RNAi;+/+
Fig. 11b,d,f,h	UAS-Dcr-2/+; enGal4,UAS-EGFP/+; UAS-UVRAG-RNAi/+
Fig. S1a,c	UAS-Dcr-2/+; enGal4,UAS-EGFP/+; UAS-Atg6-RNAi/+
Fig. S3a,e	MsGal4/+; UAS-Dcr-2/+; +/+
Fig. S3b,f	MsGal4/+;UAS-Dcr-2/UAS-Atg14-RNAi;+/+
Fig. S3c,g	MsGal4/+; UAS-Dcr-2/+; UAS-Atg6-RNAi/+
Fig. S3d,h	MsGal4/+; UAS-Dcr-2/+; UAS-UVRAG-RNAi/+

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