Hyenne et al., http://www.jcb.org/cgi/content/full/jcb.201504136/DC1

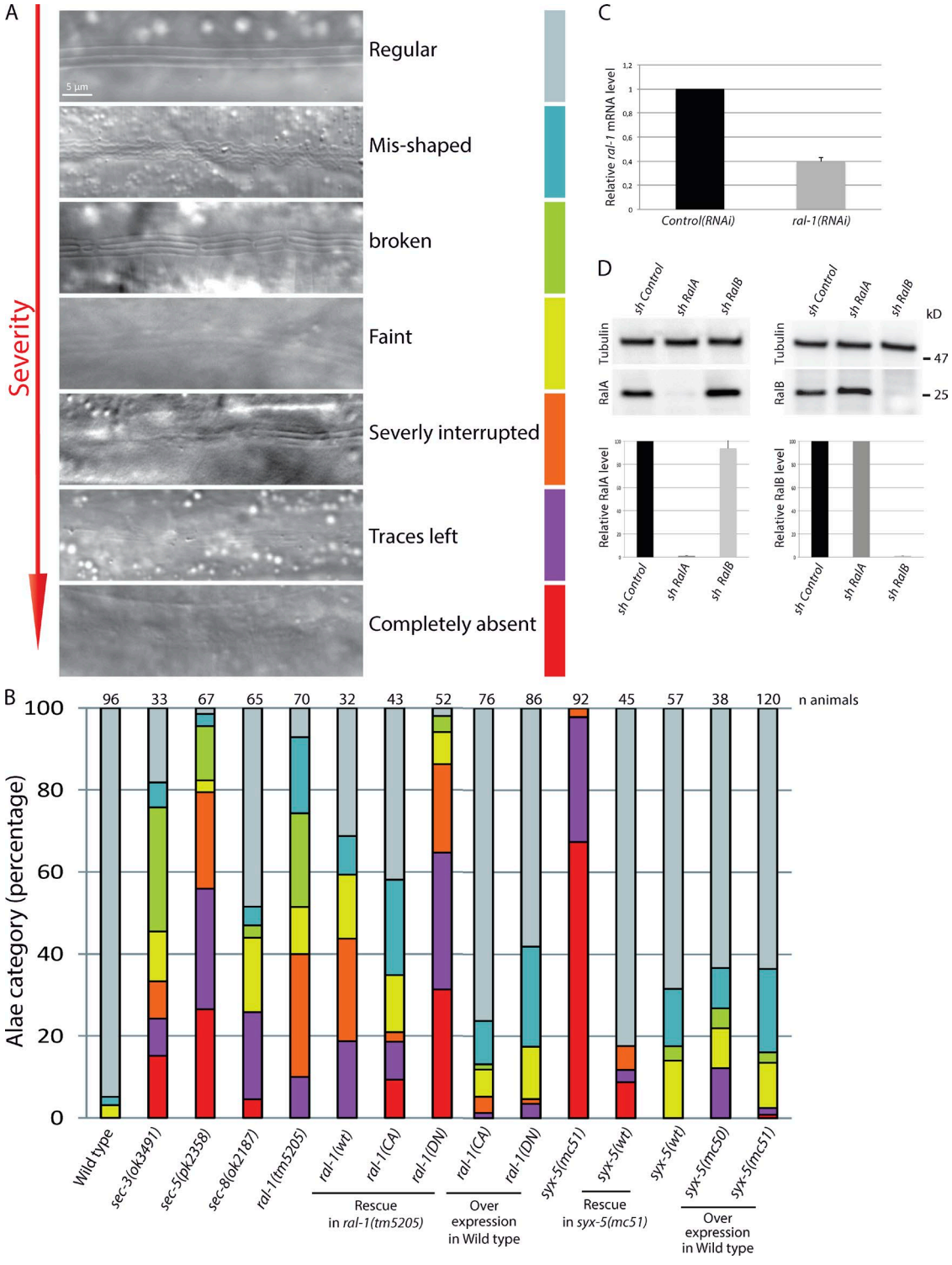

Figure S1. **Classification of alae defects.** (A) Examples of the different categories of alae defects. (B) Percentage of each category of alae defect in the different genotypes. The number of animals analyzed in each genotype is provided above the graph. (C) Quantitative RT-PCR experiments establishing that *ral-1(RNAi)* decreases *ral-1* mRNA levels by 60% compared with *control(RNAi)* (mean of two independent experiments; quantitative RT-PCR performed in triplicate). (D) Western blots of RalA and RalB in 4T1 mice mammary tumor cells stably expressing sh control, sh RalA or sh RalB. Quantification over cellular tubulin, normalized to control (mean of two experiments) reveals a strong efficiency (>98% reduction compared with control). Errors bars, SD.

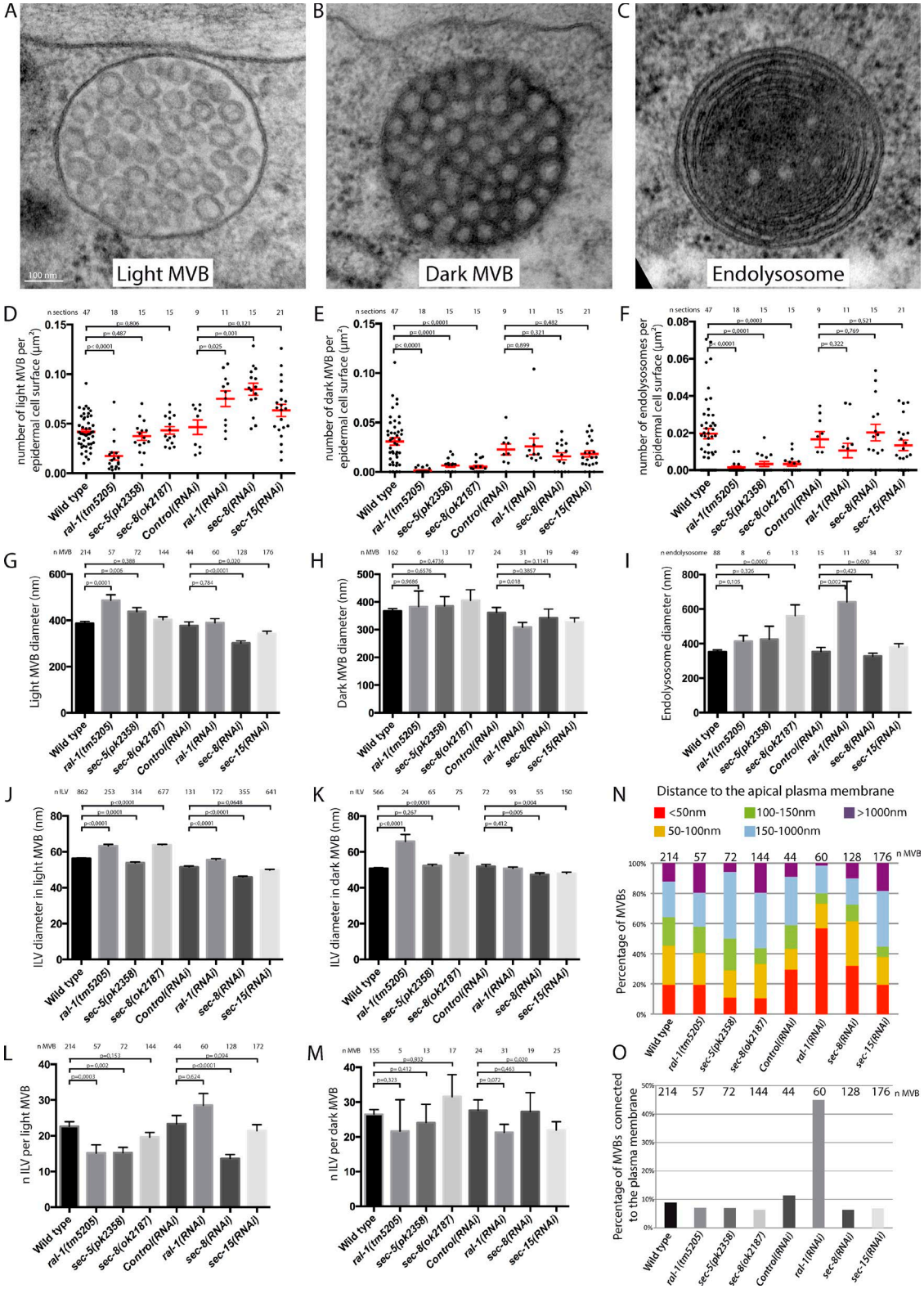

Figure S2. **Systematic and quantitative analysis of MVBs and endolysosomes by electron microscopy.** (A–C) Examples of the different types of MVBs and endolysosomes. (D–M) Graphics representing the density (D–F), diameter (G–I), ILV diameter (J and K), and number of ILVs per MVB surface (L and M) in the case of light MVBs (D, G, J, and L), dark MVBs (E, H, K, and M), and endolysosomes (F and I) in WT, RNAi, and mutant animals. P values are indicated above the graphs (Mann-Whitney test). Numbers above the graphs indicate the numbers of sections in D–F; MVBs in G, H, and L–O; endolysosomes in I; and ILVs in J and K. (N) Distance between MVBs and the apical plasma membrane. (O) Percentage of MVBs connected to the apical plasma membrane. Error bars, SD.

Table S1. **Genes identified in the RNAi screen for alae defects**

Gene	Clone	Category
arf-3	F57H12.1	Small GTPase
ral-1	Y53G8AR	Small GTPase
rab-1	C39F7.4	Small GTPase
rab-2	F53F10.4	Small GTPase
rab-7	W03C9.3	Small GTPase
rab-8	D1037.4	Small GTPase
rab-11	F53G12.1	Small GTPase
rab-27	Y87G2A.4	Small GTPase
rab-35	Y47D3A.25	Small GTPase
rab-39	D2013.1	Small GTPase
sec-8	Y106G6H.7	Exocyst
sec-10	C33H5.9	Exocyst
sec-15	C28G1.3	Exocyst
exoc-8	Y105E8B.2	Exocyst
cdc-42	R07G3.1	Cytoskeleton
gei-4	W07B3.2	Cytoskeleton
byn-1	F57B9.5	Cytoskeleton
pes-7	F09C3.1	Cytoskeleton
evl-20	F22B5.1	Cytoskeleton
arx-2	K07C5.1	Cytoskeleton
ani-1	Y49E10.19	Cytoskeleton
Y19D2B.1	Y19D2B.1	Cytoskeleton
flna-1	C23F12.1	Cytoskeleton
goa-1	C26C6.2	Cytoskeleton
hgrs-1	C07G1	pre-ESCRT
tsg-101	C09G12	ESCRT
vps-28	Y87G2A	ESCRT
vps-37	CD4.4	ESCRT
vps-25	W02A11.2	ESCRT
vps-22	C27F2.5	ESCRT
vps-36	F17C11.8	ESCRT
vps-20	Y65B4A.3	ESCRT
vps-32	C56C10.3	ESCRT
vps-4	Y34D9A.10	ESCRT disassembly
tfg-1	Y63D3A.5	ER-Golgi
syx-5	F55A11.2	ER-Golgi
sft-4	C54H2.5	ER-Golgi
emc-2	Y57G7A.10	ER-Golgi
NA	ZK686.3	ER-Golgi
npp-20	Y77E11A.13a	ER-Golgi
soap-1	C13F10.4	Secretion
lpr-5	W04G3.2	Secretion
ify-1	C27A2.3	Cell division
ect-2	T19E10.1	Cell division
paa-1	F48E8.5	Cell division
R08D7.1	R08D7.1	Cell division
cdc-48.3	K04G2.3	Cell division
cki-1	T05A6.1	Cell division
xpo-2	Y48G1A_54.b	Cell division
bub-1	R06C7.8	Cell division
cdk-9	H25P06.2a	Cell division
plk-1	C14B9.4	Cell division
cdk-1	T05G5.3	Cell division
cdk-4	F18H3.5a	Cell division
spd-1	Y34D9A.4	Cell division
let-92	F38H4.9	Other
nekl-2	ZC581.1	Other
vhp-1	F08B1.1	Other
vha-9	ZK970.4	Other
ccdc-55	C16C10.6	Other
NA	T22H9.1	Other

Table S1. **Genes identified in the RNAi screen for alae defects** *(Continued)*

Gene	Clone	Category
chp-1	Y110A7A.13	Other
dcp-66	C26C6.5	Other
cdt-2	T01C3.1	Other
NA	F25H9.6	Unknown
NA	Y6B3B.9	Unknown
smgl-1	F20G4.1	Unknown
F52C6.13	F52C6.13	Unknown
D1043.1	D1043.1	Unknown
C14C10.4	C14C10.4	Unknown
srh-49	C10G11.4	Unknown
NA	Y41D4B.11	Unknown
NA	C23H3.5	Unknown

Table S2. **Quantification of alae defects**

Animals	Percentage of alae defects	SE	No. of animals observed	No. of experiments	Mann-Whitney[a]
Adult animals					
WT	4.25	2.02	96	6	NA
sec-3(ok3491)	86.1	7.12	33	3	0.0238
sec-5(pk2358)	97.05	1.71	67	4	0.0095
sec-8(ok2187)	50.85	3.27	65	4	0.0139
exoc-7(ok2006)	25.26	3.11	22	3	0.025
exoc-8(ok2523)	42.91	2.13	34	3	0.025
ral-1(tm5205)	93.86	2.24	70	4	0.0095
Rescued *ral-1(tm5205) pdpy-7::ral-1(WT)*, 1 ng	68.75	0.95	32	2	0.1333
Rescued *ral-1(tm5205) pdpy-7::ral-1(CA)*, 1 ng	59.86	8.43	43	4	0.0286
Rescued *ral-1(tm5205) pdpy-7::ral-1(DN)*, 1 ng	98.24	1.75	52	3	0.4
Overexpression of RAL-1(CA), 10 ng	24.23	6.79	76	4	0.0325
Overexpression of RAL-1(DN), 10 ng	39.97	4.88	86	5	0.0043
L1 animals					
syx-5(mc51)[b]	100	0	92	4	0.0095
Rescued *syx-5(mc51)* by *syx-5(wt)*[c]	11.24	1.72	35	3	0.0571
Adult animals					
Overexpression of SYX-5(WT)	29.39	8.66	57	4	0.0667
Overexpression of SYX-5(mc50)	32.13	8.17	38	3	0.0476
Overexpression of SYX-5(mc51)	38.76	8.2	120	5	0.0043

Here all types of alae defects are combined. However, their severity can vary (see Fig. S1 for details).
[a]The Mann-Whitney test was performed with WT animals, except for the rescue experiments in which it was performed with mutant animals.
[b]*syx-5(mc50)* and *syx-5(mc51)* show the same phenotype: 68% of animals have invisible alae, and 32% have visible alae with major defects.
[c]For *syx-5(mc51)* rescue, alae defects are rescued, although alae were not always continuous. Rescued animals could reach adulthood (with alae) but failed to give rise to progeny.

Table S3. **Strains used in this study**

Strain	Genotype
N2	*Bristol*
NL2099	*rrf-3(pk1426)*
VC2836	*+/szT1 [lon-2(e678)] I; sec-3(ok3491)/szT1 X*
DV2689	*sec-5(pk2358)/mIn1 [dpy-10(e128) mIs14] II*
VC2648	*sec-8(ok2187) I/hT2 [bli-4(e937) let-?(q782)qIs48] (I;III)*
FT1411	*ral-1(tm5205)/qC1III*
RB1630	*exoc-7(ok2006) I*
RB1928	*exoc-8(ok2523)I*
ML2339	*syx-5(mc50)/+*
ML2340	*syx-5(mc51)/+*
ML2376	*syx-5(mc51)/oxTi711[pelt-3::H2B::tomato]*
ML1783	*mcEX586[psec-8::sec-8::gfp; pvha-5::vha-5::mrfp; pttx-3::gfp]*
ML2311	*mcEX858[psec-15::sec-15::gfp; pvha-5::vha-5::rfp; pttx-3::gfp]*
ML2309	*mcEX856[pral-1::yfp::ral-1; pvha-5::vha-5::rfp; pmyo-2::mCherry]*
ML2040	*mcEX702[pdpy-7::gfp::ral-1(CA); pvha-5::vha-5::rfp; pmyo-2::mCherry]*
ML2199	*mcEX806[pdpy-7::gfp::ral-1(DN); pvha-5::vha-5::rfp; pmyo-2::mCherry]*
ML2033	*mcEX695[pdpy-7::gfp::syx-5; pvha-5::vha-5::rfp; pmyo-2::mCherry]*
ML2034	*mcEX696[pdpy-7::gfp::ral-1(CA); pmyo-2::mCherry]*
ML2037	*mcEX699[pdpy-7::gfp::ral-1(DN); pmyo-2::mCherry]*
ML2400	*ral-1(tm5205)/qC1 mcEX905[pral-1::yfp::ral-1(wt); pmyo-2::gfp]*
ML2313	*ral-1(tm5205)/qC1 mcEX(860)[pdpy-7::gfp::ral-1(CA); pttx-3::gfp]*
ML2314	*ral-1(tm5205) mcEX(861)[pdpy-7::gfp::ral-1(DN); pttx-3::gfp]*
ML2369	*mcEX893[pdpy-7::apex::ral-1(DN); pmyo-2::mCherry]*
ML2324	*mcEX871[pdpy-7::gfp::syx-5; pmyo-2::mCherry]*
ML2325	*mcEX872[pdpy-7::gfp::syx-5(mc50); pmyo-2::mCherry]*
ML2331	*mcEX878[pdpy-7::gfp::syx-5(mc51); pmyo-2::mCherry]*
ML2158	*mcEX775[pdpy-7::gfp::syx-5; pdpy-7::mCherry::ral-1(CA);pmyo-2::mCherry]*
ML2153	*mcEX770[pdpy-7::gfp::syx-5; pdpy-7::mCherry:ral-1(DN);pmyo-2::mCherry]*
ML2399	*syx-5(mc51)/oxTi711[pelt-3::H2B::tomato] mcEX904[psyx-5::gfp::syx-5; pmyo-2::mCherry]*
ML2375	*ral-1(tm5205)/qC1; mcSi56[pCbr-unc-119::Cbr-unc-119(+)::pvha-5::vha-5[E830q]::mRFP1]II; unc-119(ed3)IIIss*