Figure S1.

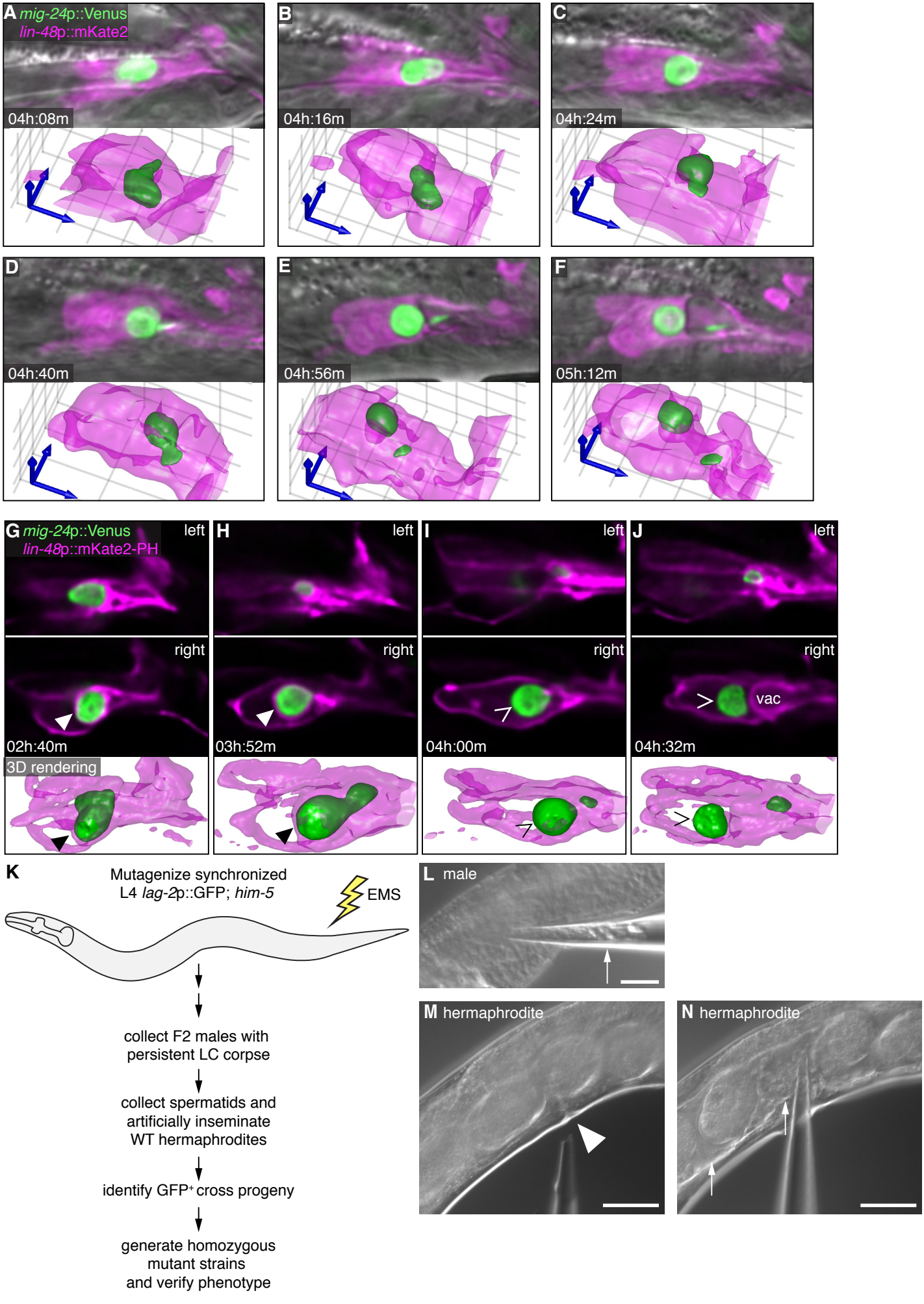
A mig-24p::Venus
lin-48p::mKate2
04h:08m
B
04h:16m
C
04h:24m
D
04h:40m
E
04h:56m
F
05h:12m
G mig-24p::Venus
lin-48p::mKate2-PH
left
right
02h:40m
3D rendering
H
03h:52m
I
04h:00m
J
vac
04h:32m
K
Mutagenize synchronized
L4 lag-2p::GFP; him-5
EMS
collect F2 males with
persistent LC corpse
collect spermatids and
artificially inseminate
WT hermaphrodites
identify GFP+ cross progeny
generate homozygous
mutant strains
and verify phenotype
L male
M hermaphrodite
N hermaphrodite

Figure S1. Competitive phagocytosis is dynamic and subsequent genetic screen for linker cell degradation mutants. Related to Figures 1, 2 and 4.

(**A-F**) Additional movie frames of animal shown in Figure 1, demonstrating back-and-forth tugging events leading to linker cell splitting. Scale bar, 10 µm.

(**G-J**) Linker cell splitting correlates with loss of mKate2-PH, an open phagosome marker. Scale bar, 10 µm. (**G-H**) Arrowhead, mKate2-PH plasma membrane marker around linker cell prior to cell splitting (**I-J**) Caret, mKate2-PH plasma membrane marker absent from phagosome after cell splitting.

(**K**) Genetic screen scheme.

(**L-N**) Artificial insemination procedure. (**L**) Needle filled with buffer plunged into a 24h mutant adult male gonadal cavity. Spermatids automatically flow into the needle (arrow). (**M**) Needle aligned with a wild type hermaphrodite vulva (arrowhead). (**N**) Needle inserted into the vulva and spermatids released. Released spermatids fill the hermaphrodite gonadal cavity (arrows).

Figure S2.

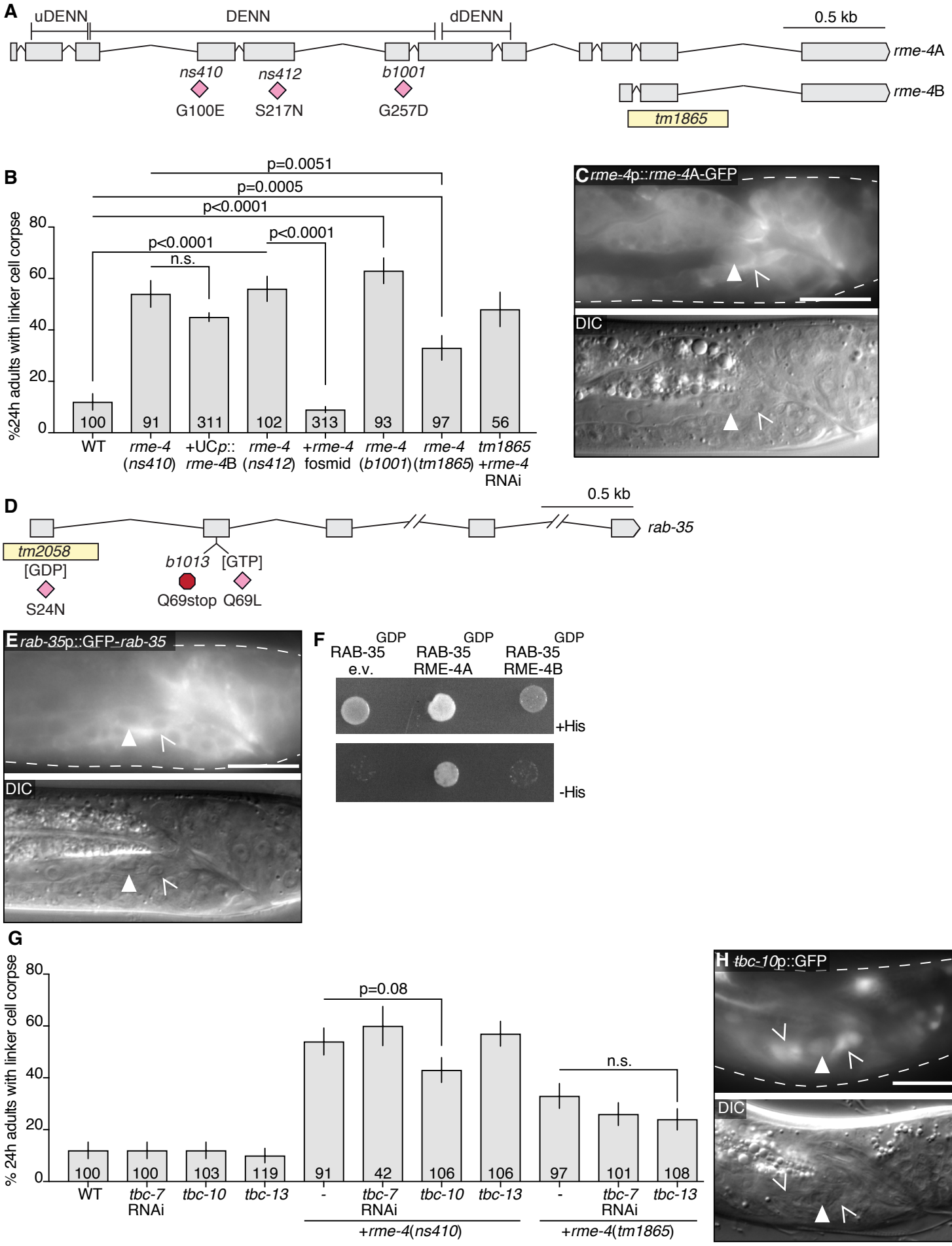
A
uDENN
DENN
dDENN
0.5 kb
rme-4A
rme-4B
ns410
G100E
ns412
S217N
b1001
G257D
tm1865
B
p=0.0051
p=0.0005
p<0.0001
p<0.0001
p<0.0001
n.s.
%24h adults with linker cell corpse
0
20
40
60
80
100
91
311
102
313
93
97
56
WT
rme-4 (ns410)
+UCp:: rme-4B
rme-4 (ns412)
+rme-4 fosmid
rme-4 (b1001)
rme-4 (tm1865)
tm1865 +rme-4 RNAi
C rme-4p::rme-4A-GFP
DIC
D
0.5 kb
rab-35
tm2058
[GDP]
S24N
b1013
[GTP]
Q69stop
Q69L
E rab-35p::GFP-rab-35
DIC
F
GDP
RAB-35 e.v.
GDP
RAB-35 RME-4A
GDP
RAB-35 RME-4B
+His
-His
G
% 24h adults with linker cell corpse
0
20
40
60
80
p=0.08
n.s.
100
100
103
119
91
42
106
106
97
101
108
WT
tbc-7 RNAi
tbc-10
tbc-13
-
tbc-7 RNAi
tbc-10
tbc-13
-
tbc-7 RNAi
tbc-13
+rme-4(ns410)
+rme-4(tm1865)
H tbc-10p::GFP
DIC

Figure S2. RAB-35, RME-4, and TBC-10 reagents and interactions. Related to Figure 2.

(**A**) *rme-4* gene locus. Pink diamonds, locations of point mutations. Yellow box, deletion allele *tm1865*.

(**B**) Histogram details as in Figure 2b.

(**C**) *rme-4* translational reporter expression. Arrowhead, linker cell. Caret, U.lp or U.rp cell. Top: GFP. Bottom: DIC. Scale bar, 10 µm.

(**D**) *rab-35* gene locus. Yellow box, deletion allele *tm2058*. Pink diamonds, point mutations. Red octagon, early stop allele *b1013.*

(**E**) *rab-35* translational reporter expression. Arrowhead, linker cell. Caret, U.lp or U.rp cell. Top: GFP. Bottom: DIC. Scale bar, 10 µm.

(**F**) Yeast two-hybrid assay with LexA-RAB-35[S24N] (GDP) as bait, and Gal4-AD (GAD) empty vector, GAD-RME-4A, or GAD-RME-4B as prey. Top: histidine present. Bottom: histidine absent. Growth on -His plates, physical interaction.

(**G**) Histogram details as in (**B**).

(**H**) *tbc-10* transcriptional reporter. Arrowhead, linker cell. Caret, U.l/rp cells. Top: GFP. Bottom: DIC. Scale bar, 10 µm.

Figure S3.

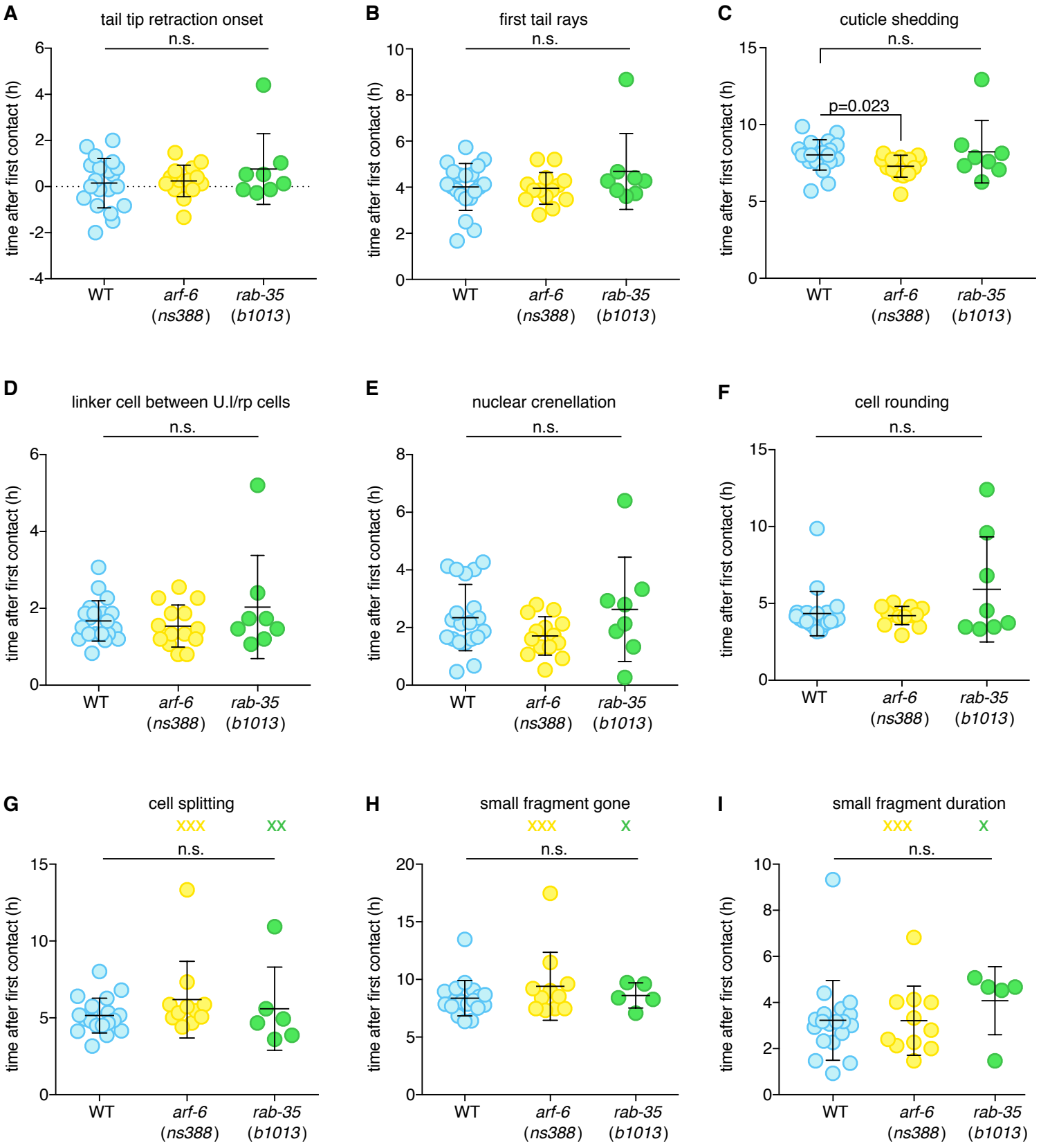

Figure S3.Characterization of stereotypical events during linker cell clearance in rab-35 and arf-6 mutants. Related to Figures 1, 2, and 4.

(A-I) Strains contain linker cell reporter [*mig-24*p::Venus], U.l/rp cell reporter [*lin-48*p::mKate2], *him-5*(*e1490*). Dots, individual events in single animals in hours with respect to first contact. X, event did not occur; not factored into statistical analysis. Bars, mean ± std. Statistical significance, Student's t-test.

Figure S4.

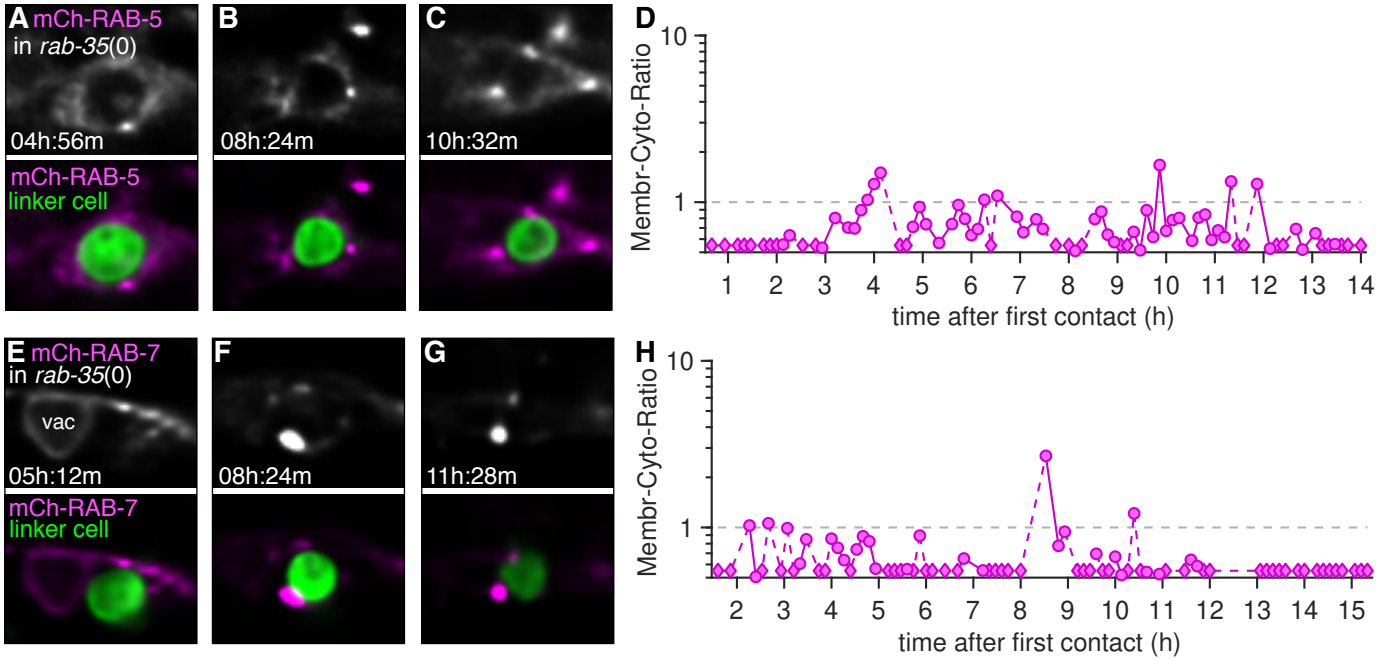

Figure S4. RAB-5 and RAB-7 do not sustained around the phagosome membrane in the absence of RAB-35. Related to Figure 3.
(**A-C**) Localization of mCh-RAB-5 (top) within the U.l/rp cells examined during linker cell (YFP) death and degradation in a *rab-35*(*b1013*) animal. Bottom: linker cell, green; mCh-RAB-5, magenta. Scale bar, 5 µm. (**D**) Ratio of average fluorescence intensity on phagosome membrane over the cytoplasm in hours after the first contact of animal shown in adjacent images. Logarithmic scale, where 1 indicates equal amount of cytoplasmic and phagosome membrane bound fluorescence. Any data point with a ratio below 0.5 indicated with a diamond and dotted line. (**E-G**) Imaging details as in (**A**), except that mCh-RAB-7 is imaged in a *rab-35*(*b1013*) animal (top). Bottom: linker cell, green; mCh-RAB-7, magenta. (**H**) Same details as in (**D**).

Figure S5.

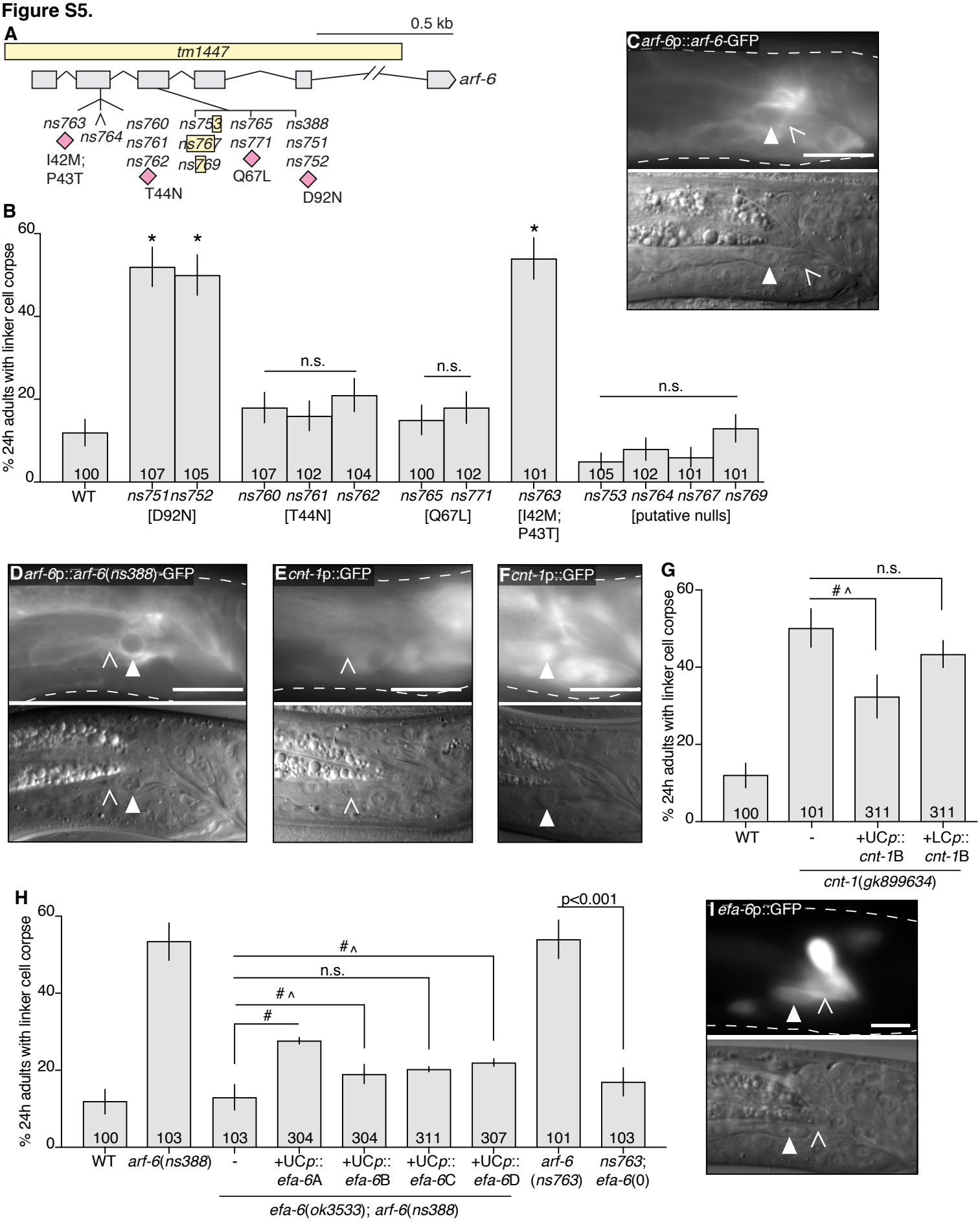
A
0.5 kb
tm1447
arf-6
ns763
ns764
I42M;
P43T
ns760
ns761
ns762
T44N
ns753
ns767
ns769
ns765
ns771
Q67L
ns388
ns751
ns752
D92N
B
% 24h adults with linker cell corpse
n.s.
WT
ns751 ns752
[D92N]
ns760 ns761 ns762
[T44N]
ns765 ns771
[Q67L]
ns763
[I42M; P43T]
ns753 ns764 ns767 ns769
[putative nulls]
C arf-6p::arf-6-GFP
D arf-6p::arf-6(ns388)-GFP
E cnt-1p::GFP
F cnt-1p::GFP
G
% 24h adults with linker cell corpse
WT
+UCp:: cnt-1B
+LCp:: cnt-1B
cnt-1(gk899634)
H
% 24h adults with linker cell corpse
p<0.001
WT
arf-6(ns388)
+UCp:: efa-6A
+UCp:: efa-6B
+UCp:: efa-6C
+UCp:: efa-6D
arf-6 (ns763)
ns763; efa-6(0)
efa-6(ok3533); arf-6(ns388)
I efa-6p::GFP

Figure S5. CNT-1/ACAP2 and EFA-6/EFA6 regulate ARF-6. Related to Figures 4 and 6.

(**A**) *arf-6* genomic locus. Yellow box, deletion alleles. Caret, single nucleotide insertion allele *ns764*. Pink diamonds, point mutations.

(**B**) Histogram details as in Figure 2b.

(**C**) *arf-6* translational reporter expression. Arrowhead, linker cell. Caret, U.lp or U.rp cell. Top: GFP; bottom: DIC. Scale bar, 10 µm.

(**D-F**) Expression reporter for *arf-6*(*ns388)*, and *cnt-1*.

(**G**) Histogram as in B. UC*p* = *lin-48*p. LC*p* = *mig-24*p. Error bars represent standard error of the proportion or standard error of the mean. #, significantly different from both *cnt-1*(*gk899634*) and wild type. ^, only two lines out of three show significant rescue.

(**H**) Histogram as in B. UC*p* = *lin-48*p. LC*p* = *mig-24*p. Error bars represent standard error of the proportion or standard error of the mean. #, significantly different from both *efa-6*(*ok3533*); *arf-6*(*ns388*) and *arf-6*(*ns388*). ^, only one line out of three show significant rescue.

(**I**) *efa-6* transcriptional reporter. Annotations as in (**C**).

Figure S6.

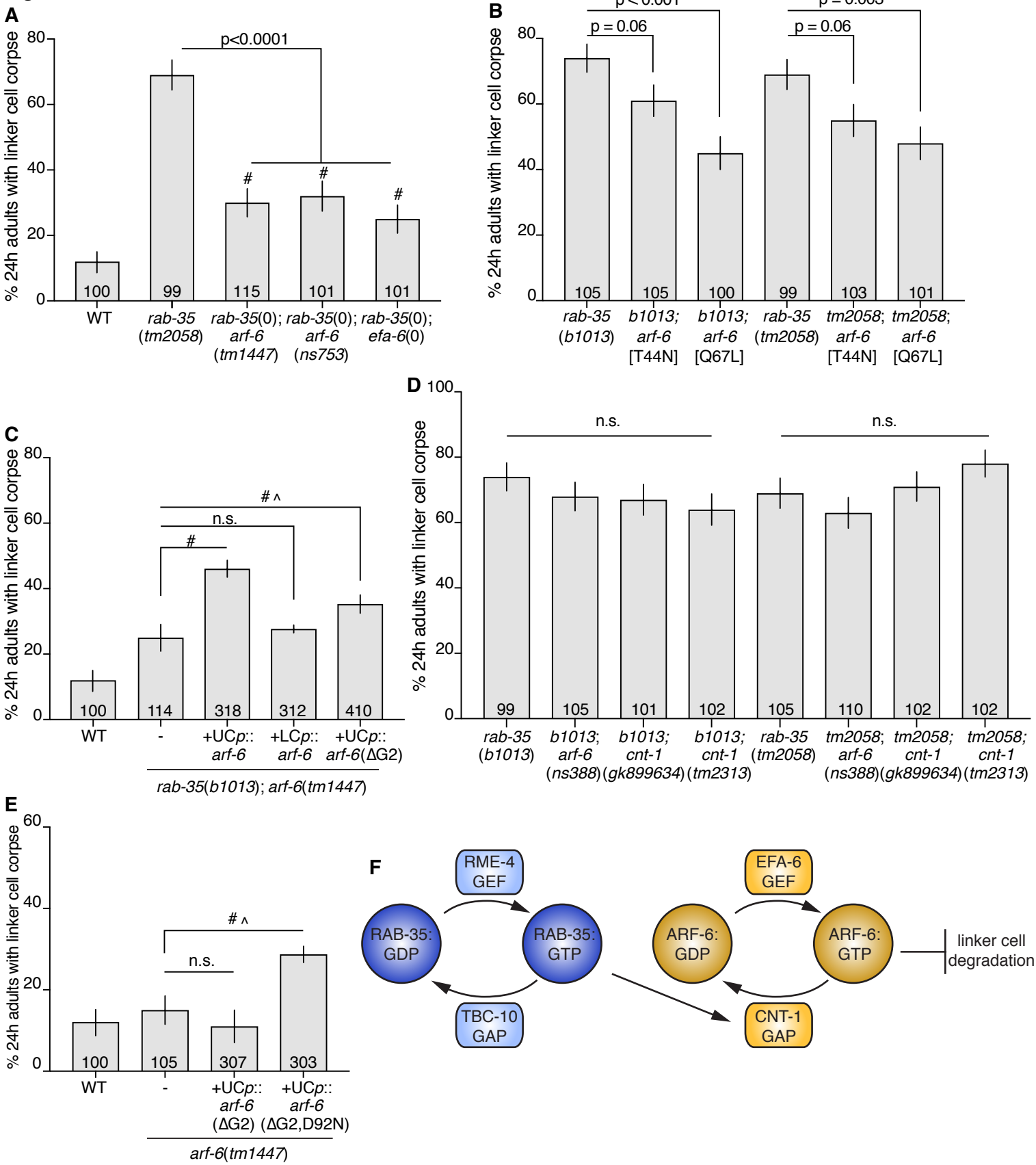

Figure S6. RAB-35 and ARF-6 are in the same genetic pathway. Related to Figure 7.
(**A-E**) Histogram details as in Figure 2B. ΔG2 = deletion of glycine2, necessary for myristoylation. #, (**A**) significantly different from single mutants, (**C**) significantly different from both *rab-35*(*b1013*); *arf-6*(*tm1447*) and *rab-35*(*b1013*), or (**E**) significantly different from both *arf-6*(*tm1447*) and *arf-6*(*ns388*). ^, only 2/4 lines showed significant rescue. (**F**) RAB-35:GTP promotes linker cell degradation by interacting with the ARF-6 GAP CNT-1. CNT-1 is required for turning ARF-6 off and removing it from the membrane. ARF-6:GTP inhibits linker cell degradation.
GAP, GTPase activating protein. GEF, guanine nucleotide exchange factor.

Table S1. Reporter identity does not affect linker cell degradation (mean ± SE of proportion). Related to Figures 1-5, 7.

Genotype	% linker cell corpses in 24h adults	N
qIs56[LCp::GFP]	12 ± 3.2	100
nsIs65[LCp::Venus]	11 ± 3.1	100
nsIs60[LCp::Venus]	5 ± 2.1	103
arf-6(*ns388*); *nsIs65*	64 ± 4.8	101
rab-35(*b1013*); *nsIs65*	79 ± 4.0	102
nsIs589[UCp::mKate2]; *nsIs65*	5 ± 5.0	101
arf-6(*ns388*); *nsIs589; nsIs65*	57 ± 6.6	56
rab-35(*b1013*); *nsIs589; nsIs65*	54 ± 8.4	35
nsIs653[UCp::mKate2-PH]; *nsIs65*	1 ± 1.0	104
nsIs60; nsIs622[UCp::mCh-RAB-5]	0	100
nsIs592[UCp::mCh-RAB-7]; *nsIs65*	8 ± 2.8	91
nsIs586[UCp::CTNS-1-mKate2]; *nsIs65*	3 ± 1.7	101
nsIs650[LCp::mKate2]	4 ± 2.0	101
nsIs595[UCp::YFP-RAB-35]; *nsIs650*	2 ± 1.4	101
rab-35(*b1013*); *nsIs595; nsIs650*	1 ± 1.0	100
arf-6(*tm1447*); *nsIs595; nsIs650*	3 ± 1.7	101
nsIs625[UCp::ARF-6-YFP]*; nsIs650*	10 ± 3.9	59
arf-6(*tm1447*); *nsIs625; nsIs650*	4 ± 2.6	55
rab-35(*b1013*)*; nsIs625; nsIs650*	51 ± 6.9	53
arf-6(*tm1447*); *rab-35*(*b1013*)*; nsIs625; nsIs650*	43 ± 5.2	90
nsIs636[UCp::ARF-6 (D92N)-YFP]; *nsIs650*	56 ± 4.9	101
rab-35(*b1013*)*; nsIs713*[UCp::YFP-RAB-35]; *nsIs65*	0	53

UCp = *lin-48*p; LCp = *mig-24*p, except for *qIs56*, which is *lag-2*p. All animals carried a *him-5*(*e1490*) mutation in addition to the transgenes listed above.

Table S2. Canonical apoptotic engulfment genes play a minor role in linker cell corpse removal (mean ± SE of proportion). Related to Figure S1.

Genotype	Mammalian homolog	% linker cell corpses in 24h adults	N
WT		12 ± 3.2	100
ced-1(*e1735*)	CD91	14 ± 3.4	103
ced-6(*n2095*)[a]	Gulp	9 ± 2.9	100
ced-7(*n1892*)	ABCA	7 ± 2.3	120
ced-2(*e1752*) [a]	CrkII	20 ± 4.0*	102
ced-5(*n1812*) [a]	Dock180	12 ± 2.9	125
ced-12(*k149*)	Elmo	27 ± 4.5*	96
ced-10(*n1993*)	Rac1	26 ± 4.5*	97
ced-1; ced-5		21 ± 3.2	162
ced-7; ced-10		27 ± 4.5*	98
nuc-1(*e1392*)	DNaseII	31 ± 4.5**	104
cep-1(*gk138*)	p53	12 ± 3.2	103
ced-8(*n1891*)	XK	7 ± 2.5	104
dyn-1(*ky51*)	dynamin	22 ± 4.1	103
cdc-42(*gk388*)[b]	Cdc42	17 ± 7.8	23
ttr-52(*tm2003*)	transthyretin-like	7 ± 2.5	101
ttr-52(*tm2078*)	transthyretin-like	4 ± 1.9	102
psr-1(*ok714*)	phosphatidylserine receptor	7 ± 2.5	103
psr-1(*tm469*)	phosphatidylserine receptor	4 ± 2.6	56
piki-1(*ok2346*)	PI3K	13 ± 3.4	94
unc-108(*n3263*)	Rab2	5 ± 2.3	88
sand-1(*or552*)	Mon1	38 ± 4.9***	97
sand-1(*ok1963*)	Mon1	58 ± 4.8***	104
rab-7(*ok511*)[b]	Rab7	79 ± 9.5***	19
rme-1(*b1045*)[c]	EHD4	19 ± 6.1	42
scav-1(*ok2598*)	Scarb1	12 ± 5.1	41
scav-2(*ok877*)	CD36	4 ± 2.7	54
scav-3(*ok1286*)	CD36	3 ± 2.1	64
scav-5(*ok1606*)	Scarb1	5 ± 2.9	56
cdh-3(*gk178860*)	cadherin	13 ± 4.5	56
cdh-3(*pk87*)	cadherin	3 ± 2.8	36

[a]*nsIs1*[*lag-2*p::GFP, *rol-6*(+)]; [b]Strain maintained with mIn1 balancer; [c]*nsIs65*[*mig-24*p::Venus]; *him-8*; *: $p < 0.05$, Fisher's exact test; **: $p < 0.005$, Fisher's exact test; ***: $p < 0.0001$, Fisher's exact test. All animals carried a *him-5*(*e1490*) mutation and *qIs56*[*lag-2*p::GFP] linker cell marker, unless otherwise noted.

Table S3. Other putative ARF-6 GAPs have no linker cell defect (mean ± SE of proportion). Related to Figure 6.

Genotype	% linker cell corpses in 24h adults	N	homolog
WT	12 ± 3.2	100	
F23H11.4(*gk585084*)	8 ± 4.3	39	ARAP1-3/ ArfGAP
F23H11.4(*gk165637*)	13 ± 3.4	96	ARAP1-3/ ArfGAP
git-1(*ok1848*)	10 ± 3.9	59	GIT1
git-1(*gk392605*)	15 ± 5.6	41	GIT1
gap-2(*ok1001*)	10 ± 4.2	52	SynGAP/RasGAP
cnt-2(*gm377*)	12 ± 3.2	102	ArfGAP

All animals carried a *him-5*(*e1490*) mutation and *qIs56*[*lag-2*p::GFP] linker cell marker.

Table S4. Mutations in the canonical apoptotic engulfment genes *ced-1* and *ced-5* do not enhance mutations in *rab-35* (mean ± SE of proportion). Related to Figure 2.

Genotype	% linker cell corpses in 24h adults	N
WT	12 ± 3.2	100
ced-1(*e1735*)	14 ± 3.4	103
ced-5(*n1812*)[a]	12 ± 2.9	125
rab-35(*b1013*)	74 ± 4.2	105
rab-35(*tm2058*)	69 ± 4.6	99
ced-1; *rab-35*(*b1013*)	77 ± 4.2	101
ced-5; *rab-35*(*b1013*)	63 ± 4.8	101
ced-1; *ced-5*; *rab-35*(*b1013*)	62 ± 4.8	103
ced-1; *rab-35*(*tm2058*)	56 ± 4.9	102
ced-5; *rab-35*(*tm2058*)	58 ± 4.9	102
ced-1; *ced-5*; *rab-35*(*tm2058*)	63 ± 4.7	104

[a] *nsIs1*[*lag-2*p::GFP, *rol-6*(+)].All animals carried a *him-5*(*e1490*) mutation and *qIs56*[*lag-2*p::GFP] linker cell marker, unless otherwise noted.