Hagedorn et al., http://www.jcb.org/cgi/content/full/jcb.201312098/DC1

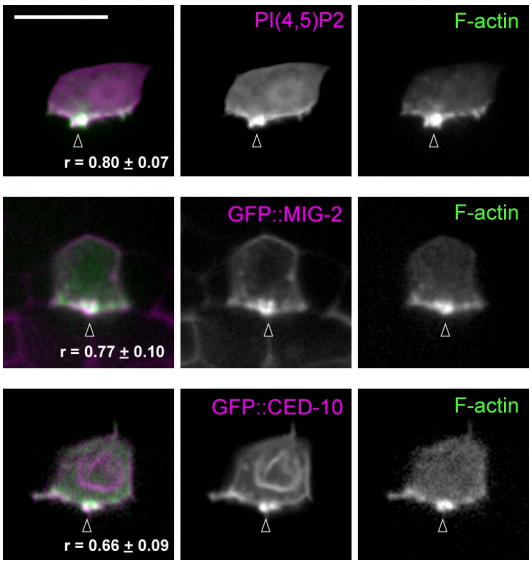


Figure S1. **F-actin and invadopodial membrane components are tightly colocalized in wild-type animals.** Lateral view images show ACs coexpressing the F-actin marker *cdh-3* > mCherry::moeABD (green in overlays, left) with the invadopodial membrane components PI(4,5)P2 (visualized with GFP::$PLC\delta^{PH}$), GFP::MIG-2, and GFP::CED-10 (magenta in overlays). F-actin and all invadopodial membrane components localized tightly together within invadopodia at the invasive cell membrane (arrowheads). Pearson's correlation coefficients (r) for colocalization are shown as mean ± SEM ($n \geq 5$ animals each genotype.

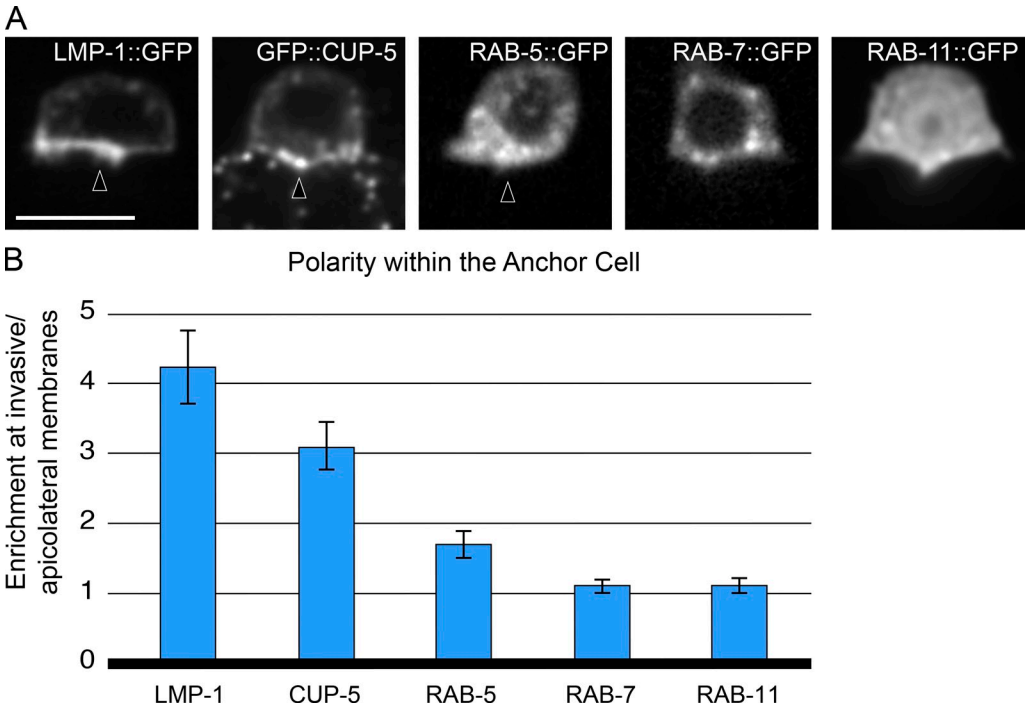


Figure S2. **Polarization of lysosomal but not endosomal proteins during AC invasion.** (A) Early (Rab5; mCherry::RAB-5), late (Rab7; mCherry::RAB-7), and recycling (Rab11; mCherry::RAB-11) endosomal proteins do not enrich at the invasive membrane during AC invasion. However, the lysosomal proteins LMP-1 (mCherry::LMP-1) and CUP-5 (GFP::CUP-5) are highly polarized (three- to fourfold) to the invasive membrane. Lateral-view images; bars, 5 µm. (B) Graphs report the fold enrichment of the endosomal and lysosomal markers at the invasive membrane ($n \geq 5$ animals examined for each genotype, error bars report ± SEM).

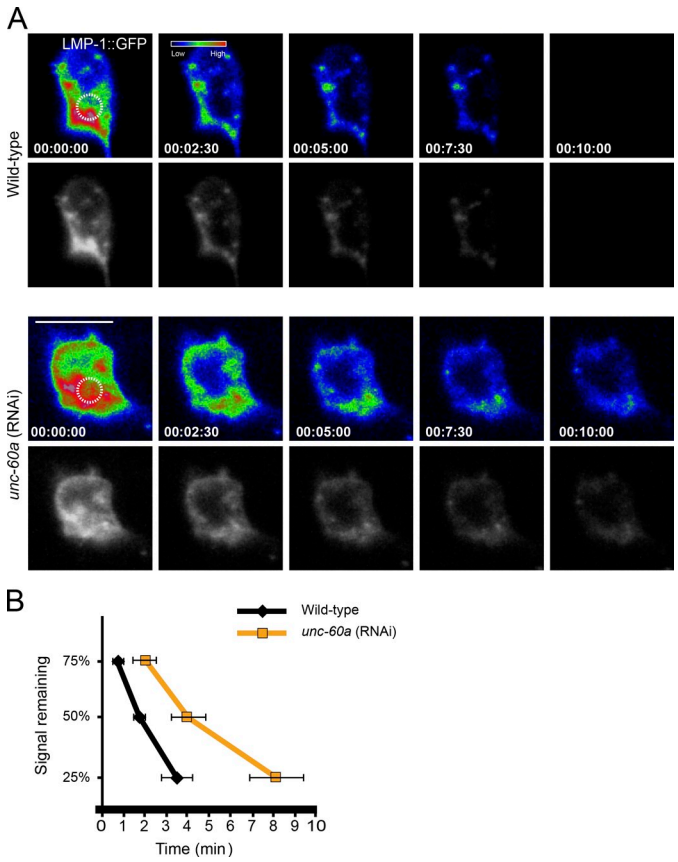
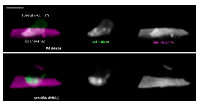
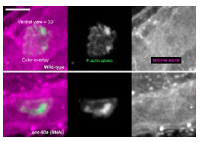


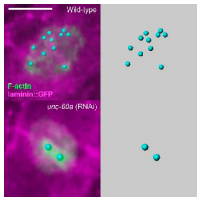
Figure S3. **Invadopodial membrane recycling from the endolysosome is regulated by UNC-60A.** (A) Focused laser photobleaching of an ∼1.0-µm region above the invasive membrane (circle) in a wild-type AC (top row) resulted in over 50% loss of the endolysosome LMP-1::GFP signal throughout the AC within 2.5 min (time-points, minutes). The LMP-1::GFP signal persisted for longer in *unc-60a* (RNAi) animals. The original fluorescent images are shown underneath images depicting a heat map of the fluorescent intensity. (B) Graphs report LMP-1::GFP signal remaining at the nonbleached invasive membrane over time ($n \geq 5$ animals each genotype; $P < 0.05$ for 25, 50, and 75% signaling remaining; Student's *t* test).



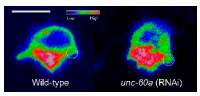
Video 1. **UNC-60A (ADF/cofilin) regulates F-actin dynamics during invadopodia formation.** Lateral-view time-lapses show 3D reconstruction of F-actin in wild-type (top) and *unc-60a* RNAi-treated (bottom) ACs. F-actin is visualized with cdh-3 > mCherry::moeABD (green); the basement membrane is visualized with laminin::GFP (magenta). Images were acquired using a spinning-disk confocal microscope (CSU-10 scan head; Yokogawa Corporation of America) mounted on an upright microscope (AxioImager; Carl Zeiss). 43-min time-lapses are shown with time-points acquired every 60 s. Projections of seven z-sections (step size of 1 µm) are shown. The video plays at 10 frames per second. Bar, 5 µm. This video corresponds to Fig. 2 C.



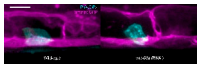
Video 2. **UNC-60A (ADF/cofilin) regulates F-actin dynamics during invadopodia formation.** Ventral-view time-lapses show 3D reconstruction of F-actin in wild-type (top) and *unc-60a* RNAi-treated (bottom) ACs. F-actin is visualized with cdh-3 > mCherry::moeABD (green); the basement membrane is visualized with laminin::GFP (magenta). Images were acquired using a spinning-disk confocal microscope (CSU-10 scan head; Yokogawa Corporation of America) mounted on an upright microscope (AxioImager; Carl Zeiss). 60-min time-lapses are shown with time-points acquired every 15 s. Projections of eight z-sections (step size of 0.5 µm) are shown. The video plays at 10 frames per second. Bar, 5 µm. This video corresponds to Fig. 2 C.



Video 3. **Spot-tracking analysis of AC invadopodia lifetimes.** Ventral-view time-lapses show a wild-type (top) and *unc-60a* RNAi-treated animal (bottom) just before the normal time of basement membrane breaching in wild-type animals. F-actin (*mCherry::moeABD*) is shown in green and laminin (*laminin::GFP*) is shown in magenta; spots (cyan) are overlaid on fluorescence (left) and shown alone (right). Time points were acquired every 15 s and the video plays at a rate of 10 frames per second. Bar, 5 µm. This video corresponds to Fig. 2 D.



Video 4. **FLIP analysis reveals dynamic recycling of invadopodial membrane through the endolysosomal compartment.** Lateral-view time-lapses show spectral representation of fluorescence intensity of LMP-1::GFP in wild-type (left) and *unc-60a* RNAi-treated (right) ACs. Small 1-µm regions of the invasive membrane (circles) were continuously photobleached using 100% laser power and 20 iterations. The first time-point shown is immediately before the start of photobleaching. This analysis was preformed using a confocal microscope (LSM 510; Carl Zeiss) equipped with a 100× objective. 10-min time-lapses are shown with time-points acquired every 30 s. The video plays at 10 frames per second. Bar, 5 µm. This video corresponds to Fig. 5 A.



Video 5. **The invadopodial membrane component PI(4,5)P_2 localizes to static intracellular membrane vesicles in the absence of *unc-60a* (ADF/cofilin).** Lateral-view time-lapses show 3D reconstruction of PI(4,5)P_2 in wild-type (left) and *unc-60a* RNAi-treated (right) ACs. PI(4,5)P_2 is visualized with cdh-3 > mCherry::PLCδ^{PH} (cyan); the basement membrane is visualized with laminin::GFP (magenta). Images were acquired using a spinning-disc confocal microscope (CSU-10 scan head; Yokogawa Corporation of America) mounted on an upright microscope (AxioImager; Carl Zeiss). 40-min time-lapses are shown with time points acquired every 60 s. Projections of seven z-sections (step size of 1 µm) are shown. The video plays at 10 frames per second. Bar, 5 µm. This video corresponds to Fig. 5 B.

Table S1. **Extrachromosomal array and integrated stain generation**

Strain designation[a]	PCR fusion created	Injected concentration	Co-injection marker
qyEx237	*unc-60 > GFP::unc-60a*	0.1 ng/µl	unc-119+
qyEx236, qyIs224	*cdh-3 > GFP::Cbrunc-60*	0.1 ng/µl	unc-119+
qyEx235, qyIs222, qyIs223	*cdh-3 > GFP::unc-60a*	0.1 ng/µl	unc-119+
qyEx282	*cdh-3 > Dendra2::act-1*	0.1 ng/µl	unc-119+
qyEx411	*zmp-1 > lmp-1::mCherry*	0.1 ng/µl	unc-119+
qyEx403	*cdh-3 > GFP::cup-5*	0.1 ng/µl	unc-119+
qyIs211	*cdh-3 > lmp-1::GFP*	0.1 ng/µl	unc-119+
qyIs205	*cdh-3 > mCherry::rab-11*	0.1 ng/µl	unc-119+
qyIs252	*cdh-3 > mCherry::rab-7*	0.1 ng/µl	unc-119+
qyIs256	*cdh-3 > mCherry::rab-5*	0.1 ng/µl	unc-119+

[a]*Ex* signifies an extrachromosomal transgenic line, whereas *Is* denotes stably integrated lines.

Table S2. **Primer sequences and templates used for PCR fusions**

Primer sequence	Primer type	Amplicon	Template
5′-TAATGTGAGTTAGCTCACTCATTAGG-3′	Forward	*cdh-3* > promoter	pPD107.94/mk62-63
5′-AACGATGGATACGCTAACAACTTGG-3′	Forward, nested	*cdh-3* > promoter	pPD107.94/mk62-63
5′-TTTCTGAGCTCGGTACCCTCCAAG-3′	Reverse	*cdh-3* > promoter	pPD107.94/mk62-63
5′-AGTATTGCCAGAAAATCCCGTTGC-3′	Forward	*unc-60* > promoter	Fosmid WRM0613bD01
5′-CTCTTAATGTGAGCCTAGTGCTCG-3′	Forward, nested	*unc-60* > promoter	Fosmid WRM0613bD01
5′-TCACGTGTGAGATCACAGTTGCCG-3′	Forward, nested 2	*unc-60* > promoter	Fosmid WRM0613bD01
5′-GAAAAGTTCTTCTCCTTTACTCATACTCTA GAAAACAGGCACACATAG-3′	*gfp* extension, reverse	*unc-60* > promoter	Fosmid WRM0613bD01
5′-ATGAGTAAAGGAGAAGAACTTTTC-3′	Forward	*GFP*	pPD95_81
5′-TTTGTATAGTTCATCCATGCCATG-3′	Reverse	*GFP*	pPD95_81
5′-CATGGCATGGATGAACTATACAAATCCGGT GTCATGGTCGACCCAGAT-3′	*gfp* extension, forward	*unc-60a*	Fosmid WRM0613bD01
5′-TCATGGTCGTGGAATTACACGGCC-3′	Reverse	*unc-60a*	Fosmid WRM0613bD01
5′-TTAGGCTTAGGCCTGGGCTTAGCC-3′	Reverse, nested	*unc-60a*	Fosmid WRM0613bD01
5′-ATAGGCTCAAGCTTAGGCTTAGGC-3′	Reverse, nested 2	*unc-60a*	Fosmid WRM0613bD01
5′-ATTACACATGGCATGGATGAACTAATGGTGAGT GACTAGTTTTTTGCT-3′	*gfp* extension, forward	*Cbrunc-60*	AF16 genomic DNA
5′-AGGATCCGAACATGGTCAAAACGG-3′	Reverse	*Cbrunc-60*	AF16 genomic DNA
5′-AATATGTGGGATTGCTTTAAAACG-3′	Reverse, nested	*Cbrunc-60*	AF16 genomic DNA
5′-ATGTGTGACGACGAGGTTGC-3′	Forward	*act-1*	*C. elegans* genomic
5′-ACTTCCCTTCCTGTTCAAAG-3′	Reverse	*act-1*	*C. elegans* genomic
5′-CGGAAGTTATCATACAACCG-3′	Reverse, nested	*act-1*	*C. elegans* genomic
5′-ATGAACCTTATTAAGGAAGA-3′	Forward	*Dendra2*	*Dendra2 plasmid*
5′-CCATGCTTGACTTGGTAGAG-3′	Reverse	*Dendra2*	*Dendra2 plasmid*
5′-AAGGGCCCTTCGCCATTTACATCGGCTC-3′	Forward	*cup-5*	pHD736
5′-AAGAGCTCGTACGGCCGACTAGTAGG-3′	Reverse	*cup-5*	pHD736
5′-AATTAATTAAGACGCTGGCATATCCTTGT-3′	Reverse	*lmp-1*	*C. elegans* genomic
5′-AAACCGGTATGTTGAAATCGTTTGTCATC-3′	Forward	*lmp-1*	*C. elegans* genomic
5′-AAAAGCGGTTAGCTCCTTCGGTCC-3′	Reverse	*rab-5*	pHD281
5′-TCTGTGACTGGTGAGTACTCAACC-3′	Reverse, nested	*rab-5*	pHD281
5′-AAAAGCGGTTAGCTCCTTCGGTCC-3′	Reverse	*rab-7*	pHD251
5′-TCTGTGACTGGTGAGTACTCAACC-3′	Reverse, nested	*rab-7*	pHD251
5′-CTTGGAGGGTACCGAGCTCAGAAAATGGTCTCAAA GGGTGAAGA-3′	*cdh-3*, extension, forward	*rab-11*	pJWZ529
5′-CAGGAAACAGCTATGACCATG-3′	Reverse	*rab-11*	pJWZ529
5′-CATTCACAGGACAAAGAGAGGG-3′	Reverse, nested	*rab-11*	pJWZ529