**SUPPLEMENTARY INFORMATION**

## I. Supplementary Figure Legends

**Figure S1. Venus::UNC-6 expression prior to and during AC invasion.**

**(a-c)** Nomarski images overlayed with Venus::UNC-6 (ΔSP) expression shown in yellow (left), and Venus::UNC-6 (ΔSP) fluorescence alone (right). Venus::UNC-6 (ΔSP) lacks a signal sequence and was thus retained in the cells in which *unc-6* is expressed. Venus::UNC-6 was retained within the neurons of the ventral nerve cord (VNC, small bracket) under the AC (arrow) prior to invasion at the P6.p 1- and 2-cell stages (large brackets, **a** and **b**), and during the time of invasion at constant levels (**c**). Scale bar in (**a**) is 5μm in this and all subsequent supplementary figures.

**Figure S2. Venus::UNC-6 expression and localization is not dependent on the vulval cells.** Nomarski image (left), and corresponding fluorescence image (right) of an animal expressing Venus::UNC-6 at the early L3 stage after laser-mediated ablation of the vulval cells in the early L2 larval stages. Venus::UNC-6 was expressed normally in the VNC and localized to the basement membrane (arrowhead) under the AC (arrow) as in wild-type animals ($n$ = 24/24 animals). Inset highlights the basement membrane localization of Venus::UNC-6 (arrowhead) under the AC.

**Figure S3. AC-specific expression of UNC-40::GFP.**

(**a**) Nomarski image overlayed with UNC-40::GFP fluorescence (green). The *unc-40::GFP* construct is driven by the AC-specific regulatory element of the *cdh-3* gene. (**b**) The

corresponding fluorescence image of UNC-40::GFP fluorescence alone. The *cdh-3* AC-specific promoter drives expression solely in the AC (arrow), and not in neighboring cells, including vulval cells (brackets). The asterisk marks autofluorescence from the gut granules.

**Figure S4. The Rac orthologs GFP::MIG-2 and GFP::CED-10 are polarized along the invasive cell membrane of the AC. (a-e)** Nomarski images (left), corresponding fluorescence images (right). Time is post-hatching at 20ºC. (**a**) GFP::MIG-2 expression driven by its endogenous promoter was first expressed in the AC during the late L2 (9.5 hours before invasion) and was not polarized (16/16 animals). (**b**) MIG-2 was first polarized at the L2 molt (arrowhead; 7/11 animals). (**c-e**). Polarized MIG-2 was maintained along the invasive cell membrane from the early L3 stage through the time of invasion (20/20 animals for each stage). (**f**) Projected confocal z-stack showing uterine and vulval expression of GFP::CED-10 driven by its endogenous promoter (left) and the corresponding spectral representation of the fluorescence intensity (right). CED-10 was expressed in all uterine and vulval cells (bracket outlines the 1º VPCs), and was polarized along the basal (invasive) membrane of the AC (arrow) but not in neighboring uterine cells (arrowheads).

**Figure S5. PAR-3::GFP and apical AJM-1::GFP are localized normally in *unc-6* mutants.** (**a**, **b**) Nomarski images (left), corresponding fluorescence images (right). (**a**) PAR-3::GFP was localized to apical and lateral membranes of wild-type ACs prior to invasion (arrowheads), and this polarity was not altered in *unc-6* mutants (**b,** arrowheads; *n* = 20/20 animals). (**c**) In wild-type animals AJM-1::GFP (green) localized to nascent junctions (arrowhead) in the apical region of the AC (expressing *zmp-1>mCherry* in magenta). (**d**) In

*unc-6* mutants apical AJM-1::GFP junctions were formed and positioned normally compared to wild-type animals ($n \geq 60$ wild-type and *unc-6* animals examined).

**Figure S6. Pan-uterine expression of mCherry:: PLCδ$^{PH}$ reveals a unique PI(4,5)$P_2$ rich invasive membrane domain in the AC.** Fluorescence (left) overlaid on corresponding Normarski image (right). (**a**) Expression of the PI(4,5)$P_2$ sensor mCherry::PLCδ$^{PH}$ in the AC revealed strong polarization at the basal (invasive) cell membrane (arrowhead). (**b**) In contrast, a section lateral to the AC through the neighboring ventral and dorsal uterine cells (VU/DU) expressing PLCδ$^{PH}$::mCherry, revealed that adjacent uterine cells did not have PI(4,5)$P_2$ concentrated in basal membranes (arrowheads).

**Figure S7. GFP::MIG-2 is localized normally in the AC of *fos-1(ar105)* animals.** Nomarski images (left), corresponding fluorescence images (right). In (**a**) wild-type animals and (**b**) *fos-1(ar105)* mutants, MIG-2::GFP was strongly polarized to the invasive cell membrane of the AC (arrowhead; $n = 20/20$ *fos-1*a mutants examined). (**c**) In *unc-6* animals, MIG-2::GFP polarity was perturbed.

**Video S1. Hemicentin::GFP deposition under the AC in wild-type animals.** Hemicentin::GFP was localized at low levels along gonadal and ventral epidermal basement membranes, but was deposited at high levels (green, orange arrows) under the AC's invasive cell membrane (expressing *cdh-3*>mCherry::moeABD in magenta) during AC invasion. Little hemicentin::GFP was deposited along apical or lateral domains of the AC (note small deposit at white arrowhead).

**Video S2. Hemicentin::GFP deposition is perturbed *unc-6* mutants.**

In *unc-6* mutants there was a dramatic decrease in hemicentin deposited under the AC (expressing *cdh-3*>mCherry::moeABD in magenta) at the site of basement membrane contact (green, orange arrows), while apical and lateral accumulations of hemicentin increased (white arrowheads).

## II. Supplementary Figures and Tables

### Figure S1

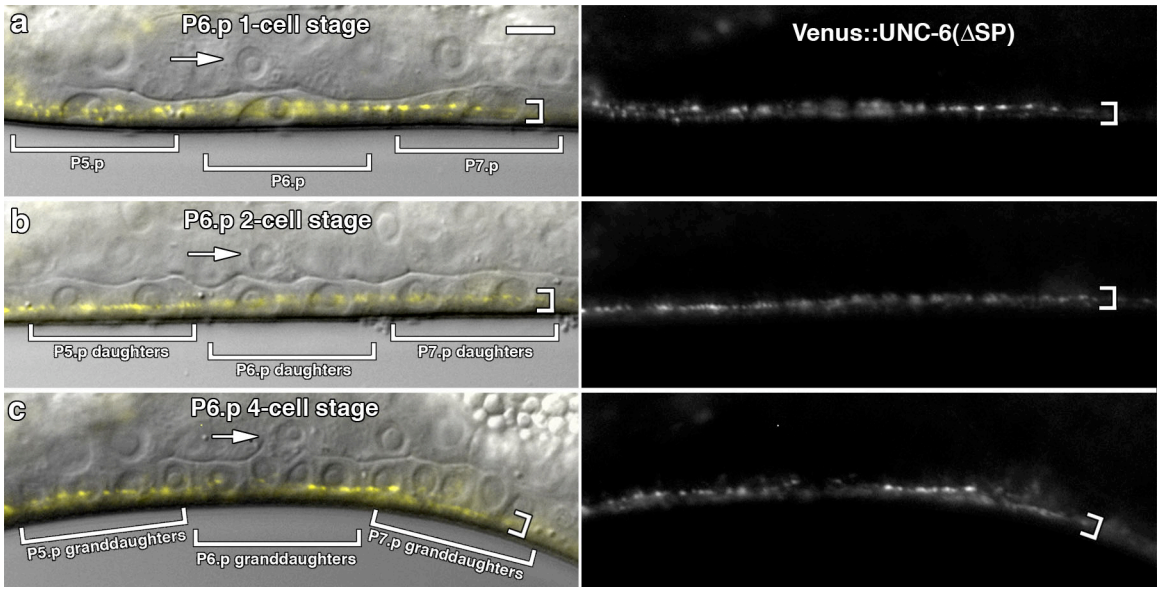

**Figure S2**

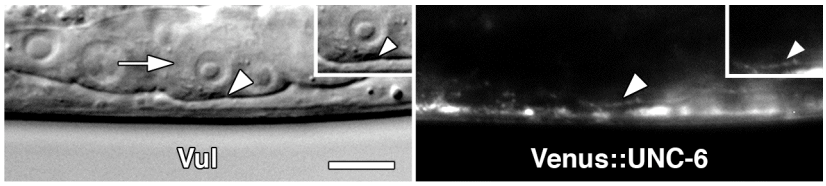

**Figure S3**

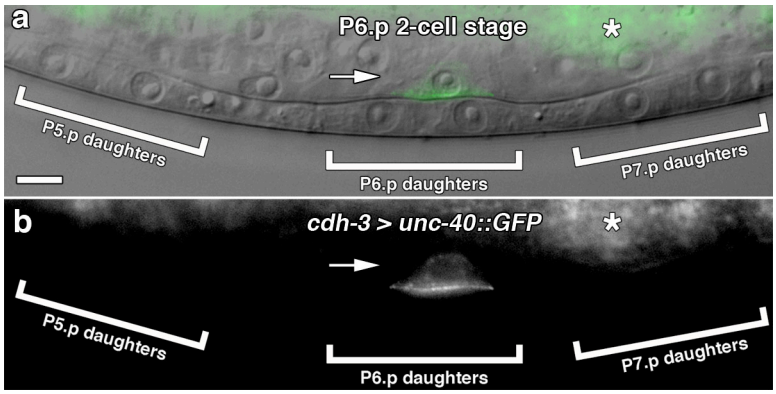
a
P6.p 2-cell stage
*
P5.p daughters
P6.p daughters
P7.p daughters
b
cdh-3 > unc-40::GFP
*
P5.p daughters
P6.p daughters
P7.p daughters

**Figure S4**

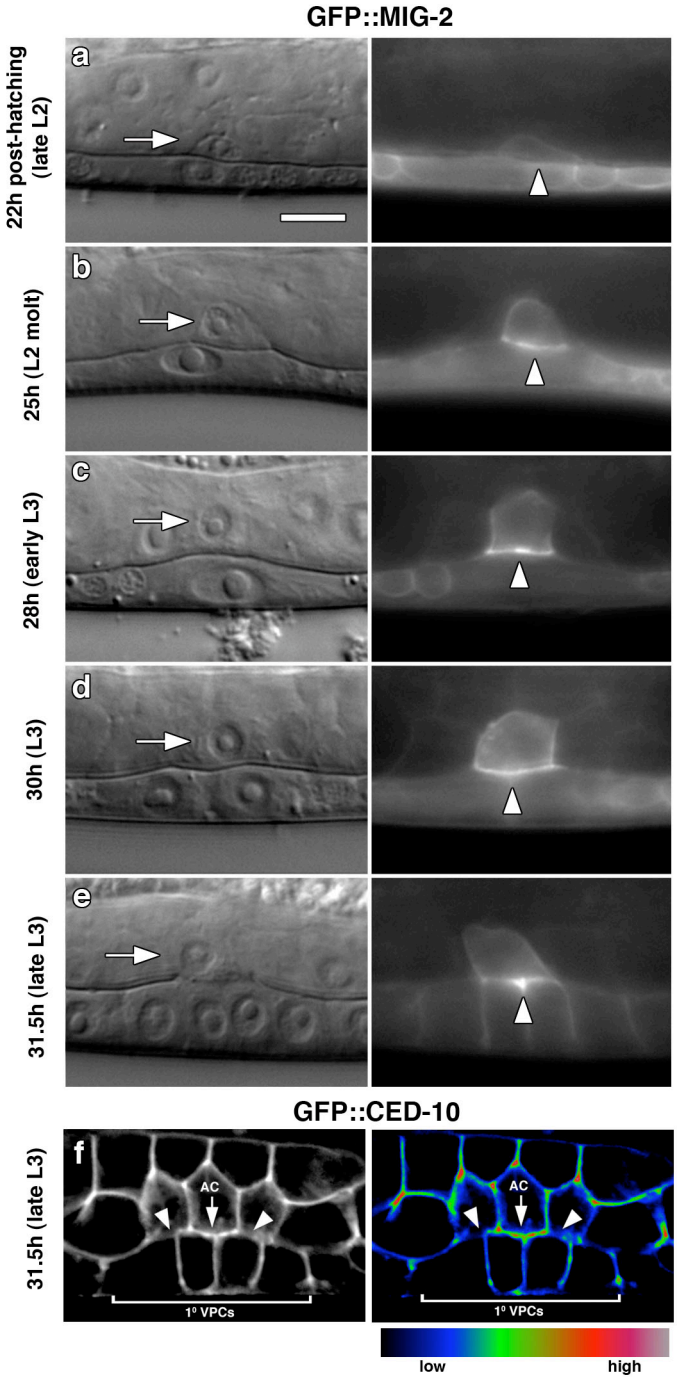
GFP::MIG-2
22h post-hatching (late L2)
25h (L2 molt)
28h (early L3)
30h (L3)
31.5h (late L3)
a
b
c
d
e
GFP::CED-10
31.5h (late L3)
f
AC
1° VPCs
AC
1° VPCs
low
high

**Figure S5**

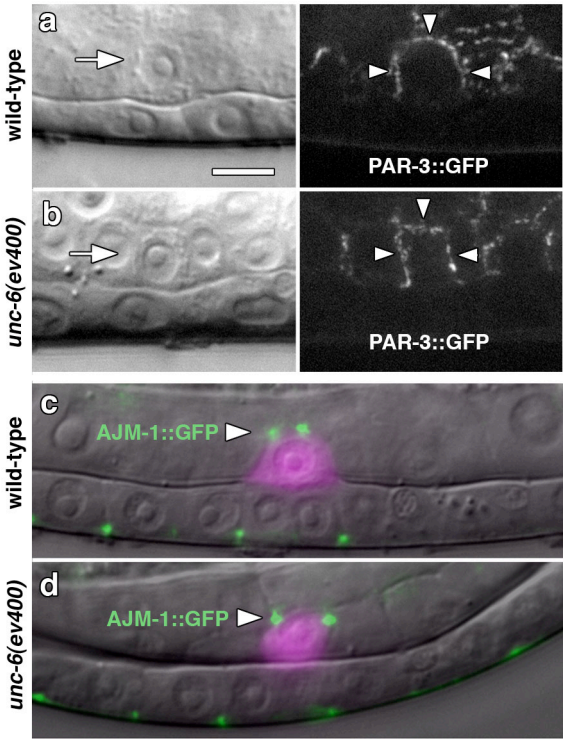
a
wild-type
PAR-3::GFP
b
unc-6(ev400)
PAR-3::GFP
c
wild-type
AJM-1::GFP
d
unc-6(ev400)
AJM-1::GFP

**Figure S6**

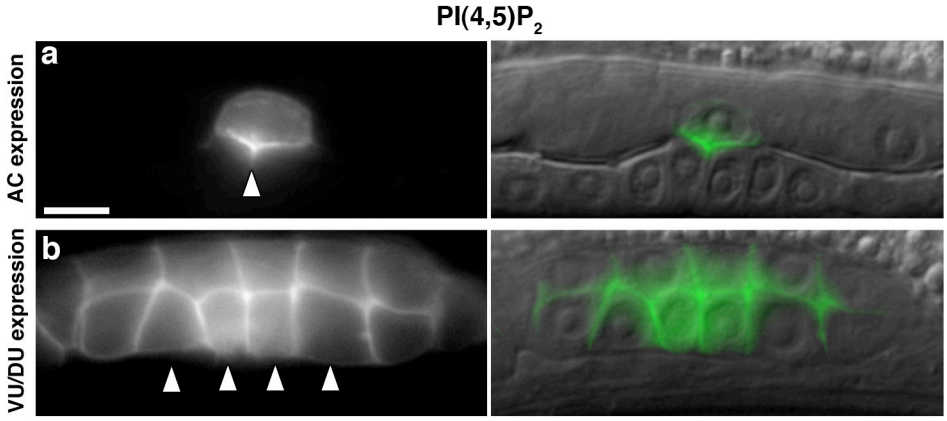

**Figure S7**

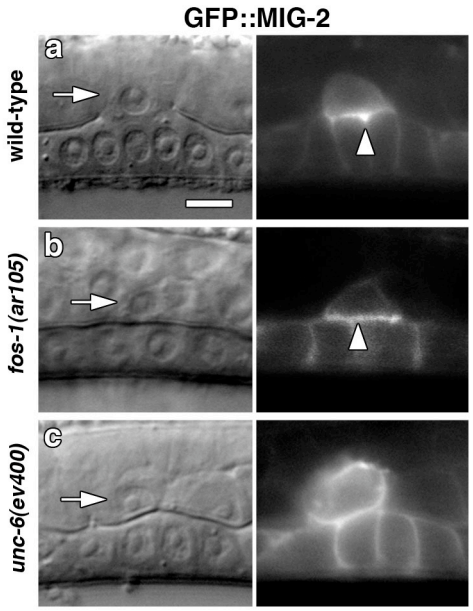

**Table S1. AC invasion in mutant strains with roles in cell motility and axon outgrowth**

| *C. elegans* gene | Encoded Product | Invasion at P6.p 4-cell stage (number of ACs invaded/number observed)[a] | Invasion at P6.p 8-cell stage (number of ACs invaded/number observed) |
|---|---|---|---|
| *arf-1.2(ok796)* | ADP-ribosylation factor 1 homolog | 10/10 | 10/10 |
| *arf-1.2(ok1322)* | ADP-ribosylation factor 1 homolog | 9/9 | 4/4 |
| *bar-1 (ga80)* | Armadillo/beta-Catenin/plakoglobin | 11/11 | 9/9 |
| *C25F6.4(ok874)* | protein tyrosine kinase homolog that is also homologous to human RS1 | 15/15 | 16/16 |
| *cam-1(gm122)* | receptor tyrosine kinase of the immunoglobulin superfamily that is orthologous to human ROR1 | 7/9 | 28/35 |
| *cam-2(gm124)* | uncloned locus that affects migration of canal associated neurons | 6/6 | 5/5 |
| *cdh-4(ok1323)* | member of the cadherin superfamily | 10/10 | 8/8 |
| *cdh-5(hc181)* | member of the cadherin superfamily | 10/10 | 7/7 |
| *cdh-7(ok428)* | contains a cadherin domain | 10/10 | not observed |
| *ced-2(e1752)* | Src homology (SH) 2 and 3-containing adaptor protein | 10/10 | 11/11 |
| *ced-5(n1812)* | homolog of the human protein DOCK180 | 10/10 | 13/13 |
| *ced-12(k149)* | Regulator of Rac1, required for phagocytosis and cell migration | 11/11 | 12/12 |
| *ceh-10(ct78)* | Paired-like class of homeodomain proteins | 10/10 | 8/8 |
| *ces-1(n1414)* | C2H2-type zinc finger transcription factor that is a member of the Snail family of proteins | 11/11 | 11/11 |
| *ces-1(n703)* | C2H2-type zinc finger transcription factor that is a member of the Snail family of proteins | 16/16 | 14/14 |
| *cle-1(cg120)* | collagen protein with endostatin domain | 9/9 | 13/13 |
| *crb-1(ok931)* | homolog of Drosophila CRUMBS | 9/9 | 9/9 |
| *daf-1(m40)* | TGF-beta type I receptor homolog | 8/9 | 6/6 |
| *daf-4(e1364)* | type II transforming growth factor-beta (TGF-b) receptors | 11/11 | 14/14 |
| *daf-7(e1372)* | member of the transforming growth factor beta (TGFbeta) superfamily | 12/12 | 14/14 |
| *dgn-2(ok209)* | dystroglycan | 27/27 | 20/20 |
| *dbl-1(wk70)* | member of the transforming growth factor beta (TGFbeta) superfamily | 21/21 | 6/6 |
| *dpy-19(e1295)* | novel transmembrane protein | 7/7 | 7/7 |
| *efn-4(bx80)* | member of the ephrin family of ligands | 10/10 | 17/17 |
| *egl-15(MT3324)* | FGF-like receptor tyrosine kinase | 13/13 | 7/7 |
| *egl-17(e1313)* | fibroblast growth factor-like protein | 13/13 | 8/8 |
| *evl-20(ar103)* | a functional ortholog of human ADP-ribosylation factor-like protein 2 | not observed | 4/4 |
| *F11D5.3(ok574)* | a putative tyrosine kinase homologous to human RS1 | 11/11 | 22/22 |
| *flt-1(ok722)* | a putative homolog of flectin, an extracellular matrix protein | 10/10 | 8/8 |
| *gon-1(e1254)/eDf18* | a functional metalloprotease that defines a new sub-family of secreted proteases known as MPT (metalloprotease with thrombospondin type 1 repeats) | 8/8 | 11/11 |
| *gpn-1(ok377)* | glypican, a heparan sulfate proteoglycan anchored to the cell membrane by a GPI linkage | 13/13 | 14/14 |
| *hlh-8(nr2061)* | helix-loop-helix protein required for normal muscle development | 10/10 | 18/18 |
| *lad-1(ok1244)/sax-7* | ortholog of human L1CAM | 10/10 | 13/13 |
| *let-756(S2613)* | fibroblast growth factor (FGF)-like ligand | 9/11 | 21/21 |
| *lon-2(e678)* | member of the glypican family of heparan sulfate proteoglycans | 13/13 | 19/19 |
| *mab-20 (ev574)* | semaphorin | 9/10 | 13/13 |
| *mab-20(bx24)* | semaphorin | 10/10 | 10/10 |
| *max-1(ju39)* | conserved PH/MyTH4/FERM domain-containing protein | 15/15 | 10/10 |
| *mig-1(e1787)* | Frizzled-like receptor | 10/10 | 13/13 |
| *mig-2(gm103)* | Rho family of GTP-binding proteins, similar to Rac | 12/15 | 36/37 |
| *mig-6(e1931)* | uncloned locus involved in cell migration | 9/10 | 15/15 |
| *mig-14(ga62)* | homologous to drosophila Wntless | 10/10 | 15/15 |
| *mig-15(rh326)* | Nck-interacting kinase (NIK) | 8/9 | 10/10 |

| | | | |
|---|---|---|---|
| *mig-17(k113)* | secreted metalloprotease that is a member of the ADAM (A Disintegrin And Metalloprotease) protein family | 10/10 | 13/13 |
| *mom-2(or42)* | member of the Wnt family of secreted signaling glycoproteins | 6/6 | 20/20 |
| *mom-5(or57)* | Frizzled-like receptor | 2/5 | 7/8 |
| *pkc-3(RNAi)* | Serine/threonine protein kinase, atypical Protien Kinase C | 15/15 | no data |
| *pld-1(ok986)* | phospholipase D1 | 10/10 | 2/2 |
| *plx-1(nc37)* | plexin ortholog, semaphorin receptor | 10/10 | 10/10 |
| *ptp-3(op147)* | receptor-like tyrosine phosphatase | 10/10 | 15/15 |
| *qid-7(mu533)* | uncloned locus that affects Q neuroblast polarization and migration | 11/11 | 10/10 |
| *qid-8(mu342)* | uncloned locus that affects Q neuroblast polarization and migration | 10/10 | 13/15 |
| *rac-2(ok326)* | Rho family GTPase that is one of three C. elegans Rac-related proteins | 10/10 | 7/7 |
| *rig-4(ok1160)* | Immunoglobulin C-2 Type/fibronectin type III domains | 10/10 | 5/5 |
| *sax-3(ky123)* | homologous to Drosophila roundabout | 11/11 | 10/10 |
| *sdn-1(ok449)* | a type I transmembrane heparan sulfate proteoglycan, syndecan | 13/13 | 5/5 |
| *slt-1(eh15)* | homolog of Drosophila slit | 16/16 | 11/11 |
| *slt-1(ok255)* | homolog of Drosophila slit | 80/81 | 59/59 |
| *sma-6(wk7)* | serine/threonine protein kinase that is orthologous to type I TGF-beta receptors | 10/10 | 15/15 |
| *smp-1 (ev715)* | semaphorin | 10/11 | 12/12 |
| *smp-2 (ev709)* | semaphorin | 10/10 | 10/10 |
| *syg-1(ky652)* | a novel transmembrane protein | 9/9 | 12/12 |
| *syg-2(ky671)* | transmembrane protein, immunoglobulin superfamily | 10/10 | 4/4 |
| *tag-150(gk261)* | Guanine nucleotide exchange factor for Rho and Rac GTPases | 10/10 | 11/11 |
| *unc-3(e151)* | a protein with homology to immunoglobulin (Ig) domain-containing transcription factors | 10/10 | 10/10 |
| *unc-5(e53)* | a netrin receptor required for dorsal cell and axon migration | 10/10 | 22/22 |
| *unc-6(ev400)* | netrin ortholog, secreted guidance molecule that regulates pioneer axons and mesodermal cells | 4/20 | 14/18 |
| *unc-14(e57)* | an activity required for both axonogenesis and sex myoblast migration | 8/10 | 11/11 |
| *unc-18(e81)* | an ortholog of Saccharomyces cervisiae SEC1 | 10/11 | 12/12 |
| *unc-30(e191)* | Pitx homeodomain transcription factor family member | 10/10 | 10/10 |
| *unc-33(mn407)* | CRMP/TOAD/Ulip/DRP homologue | 10/10 | 10/11 |
| *unc-34(e315)* | EVH1 domain-containing protein that is the sole C. elegans Enabled/VASP homolog | 6/10 | 12/12 |
| *unc-34(e566)* | EVH1 domain-containing protein that is the sole C. elegans Enabled/VASP homolog | 5/8 | 5/5 |
| *unc-39(e257)* | homeodomain transcription factor that belongs to the Six4/5 family | 10/10 | 11/11 |
| *unc-40(e271)* | A netrin receptor required for guiding dorsal and ventral cell and axon migrations | 6/21 | 13/18 |
| *unc-44(e1197)* | ankyrin-like protein | 10/10 | 10/10 |
| *unc-44(e362)* | ankyrin-like protein | 10/10 | 2/2 |
| *unc-51 (e369)* | serine/threonine kinase involved in autophagy | 9/10 | 13/13 |
| *unc-51(e1189)* | serine/threonine kinase involved in autophagy | 10/12 | 10/10 |
| *unc-53(e404)* | orthologous to human NAV1, NAV2/RAINB1, and NAV3 | 11/11 | 7/7 |
| *unc-53(n152)* | orthologous to human NAV1, NAV2/RAINB1, and NAV3 | 10/10 | 13/13 |
| *unc-60(e723)* | orthologs of actin depolymerizing factor/cofilin | 15/15 | 16/16 |
| *unc-60(su158)* | orthologs of actin depolymerizing factor/cofilin | 37/39 | 30/30 |
| *unc-71 (e541)* | ADAM, a disintegrin and metalloprotease-containing transmembrane protein | 10/10 | 10/10 |
| *unc-73 (e936)* | guanine nucleotide exchange factor (GNEF) similar to the trio protein | not observed | 39/39 |
| *unc-73 (gm40)* | guanine nucleotide exchange factor (GNEF) similar to the trio protein | 6/11 | 22/22 |
| *unc-76(e911)* | coiled-coil protein that belongs to the FEZ (fasciculation and elongation protein; zygin/zeta-1) family of proteins | 9/11 | 10/10 |
| *unc-78(gk27)* | homolog of actin-interacting protein 1 | 26/26 | 27/27 |
| *unc-97(su10)* | LIM domain-containing protein of the PINCH family | 10/10 | 14/14 |
| *unc-115(e2225)* | Actin-binding LIM Zn-finger protein Limatin involved in axon guidance | 10/10 | 15/15 |
| *unc-115(ky275)* | Actin-binding LIM Zn-finger protein Limatin involved in axon guidance | 67/67 | 68/68 |
| *unc-115(mn481)* | Actin-binding LIM Zn-finger protein Limatin | 8/11 | 24/24 |

| | | | |
|---|---|---|---|
| *unc-129 (ev554)* | member of the transforming growth factor beta (TGFbeta) superfamily | 5/5 | 11/11 |
| *vab-1(e2)* | ephrin receptor | 10/10 | 10/10 |
| *vab-8(e1017)* | novel protein containing an atypical kinesin-like motor domain | 10/10 | 10/10 |
| *vab-9(e1744)* | claudin homolog | 8/9 | 13/13 |
| *ver-1(ok859)* | Fibroblast/platelet-derived growth factor receptor and related receptor tyrosine kinases | 10/10 | 4/4 |
| *ver-2(ok897)* | Fibroblast/platelet-derived growth factor receptor and related receptor tyrosine kinases | 10/10 | 8/8 |
| *ver-3(gk227)* | Fibroblast/platelet-derived growth factor receptor and related receptor tyrosine kinases | 11/11 | 13/13 |
| *ver-4(ok1079)* | Fibroblast/platelet-derived growth factor receptor and related receptor tyrosine kinases | 10/10 | 5/5 |
| *zig-1(ok784)* | secreted 2-immunoglobulin-domain protein | 9/9 | 5/5 |
| *zig-2(ok696)* | secreted 2-immunoglobulin-domain protein | 9/9 | 11/11 |
| *zig-3(gk33)* | secreted 2-immunoglobulin-domain protein | 10/10 | 4/4 |
| *zig-4(gk4)* | secreted 2-immunoglobulin-domain protein | 10/10 | 4/4 |
| *zig-5(ok1065)* | secreted 2-immunoglobulin-domain protein | 11/11 | 10/10 |
| *zig-6(ok723)* | secreted 2-immunoglobulin-domain protein | 10/10 | 3/3 |
| *zig-8(ok561)* | secreted 2-immunoglobulin-domain protein | 9/9 | 4/4 |

[a] ACs showing any degree of invasion were scored as invaded and invasion was scored over the entire range of the 4-cell stage, including the L3 molt. In contrast, Table S2 4-cell stage animals were only scored at beginning of the 4-cell stage at the mid-to-late L3 stage, and partial invasions were scored.

**Table S2. Timing and degree of AC invasion into the vulval epithelium**

| Genotype/Treatment | ACs showing full, partial or no invasion | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | P6.p 4-cell stage (mid-to-late L3 stage) | | | | P6.p 8-cell stage (early L4 stage) | | | |
| | % Full Invasion | % Partial Invasion | % No Invasion | *n* = | % Full Invasion | % Partial Invasion | % No Invasion | *n* = |
| wild-type (N2) | 100 | 0 | 0 | >100 | 100 | 0 | 0 | >100 |
| <u>*unc-6*</u>/site of action | | | | | | | | |
| *unc-6(ev400)* | 0 | 26 | 74 | 54 | 52 | 26 | 22[h] | 54 |
| *ghIs8[unc-6>Venus::unc-6]; unc-6(ev400)*[a] | 91 | 4 | 5 | 55 | 100 | 0 | 0 | 53 |
| *unc-6(ev400); ghEx15[glr-1p>Venus::unc-6; tph-1p>GFP]*[b] | 90 | 4 | 6 | 50 | 96 | 4 | 0 | 50 |
| *unc-6(ev400);ghEx13[egl-17>Venus::unc-6]*[c] | 8 | 22 | 70 | 50 | 60 | 16 | 25 | 58 |
| *rde-1(ne219);ghEx11[egl-17>rde1::mRFP]; unc-6(RNAi)*[d] | 100 | 0 | 0 | 51 | 100 | 0 | 0 | 34 |
| *lin-3(n1059)/lin-3(n378) (Vul)*[e] | 21 | 0 | 79 | 52 | 19 | 0 | 81[i] | 52 |
| *lin-3(n1059)/lin-3(n378);unc-6(ev400)* | 0 | 0 | 100 | 50 | 0 | 0 | 100[j] | 50 |
| *laser killed P3.p-P8.p(Vul); unc-6(ev400)* | 0 | 0 | 100 | 20 | 0 | 0 | 100[k] | 20 |
| *kyIs299 [hs>unc-6::HA]*[f] | 50 | 26 | 24 | 50 | nd | nd | nd | nd |
| *N2(mock heat shock)* | 98 | 2 | 0 | 51 | nd | nd | nd | nd |
| Netrin receptors/*unc-40*/site of action | | | | | | | | |
| *unc-40(e271)* | 2 | 31 | 67 | 54 | 58 | 25 | 17[l] | 53 |
| *unc-40(e271); unc-6(ev400)* | 2 | 29 | 69 | 51 | 57 | 21 | 21[m] | 70 |
| *unc-40(e271); qyIs66[cdh-3>unc-40::gfp]* | 96 | 2 | 2 | 54 | 98 | 2 | 0 | 53 |
| *unc-5(e51)* | 100 | 0 | 0 | 58 | 100 | 0 | 0 | 64 |
| Intracellular effectors | | | | | | | | |
| *mig-10(ct41)* | 100 | 0 | 0 | 59 | 100 | 0 | 0 | 81 |
| *ced-10(n1993)* | 98 | 2 | 0 | 61 | 100 | 0 | 0 | 57 |
| *mig-2(mu28)* | 100 | 0 | 0 | 54 | 100 | 0 | 0 | 64 |
| *ced-10(n1993); mig-2(mu28)* | 24 | 10 | 66 | 29 | 69 | 5 | 25 | 55 |
| *unc-34(gm104)* | 64 | 16 | 20 | 50 | 100 | 0 | 0 | 50 |
| *qyIs23(cdh-3>mCherry::PLC$\delta^{PH}$)*[g] | 85 | 13 | 2 | 55 | 100 | 0 | 0 | 50 |
| *qyIs23(cdh-3>mCherry::PLC$\delta^{PH}$); mig-2(mu28)* | | 18 | 12 | 114 | 99 | 1 | 0 | 110 |
| *qyIs23(cdh-3>mCherry::PLC$\delta^{PH}$); unc-34(gm104)* | 41 | 18 | 41 | 51 | 89 | 9 | 2 | 56 |
| *fos-1* pathway interaction | | | | | | | | |
| *unc-40(e271); rrf-3(pk1426)* | 8 | 27 | 65 | 51 | 50 | 22 | 28 | 50 |
| *unc-40(e271); rrf-3(pk1426); fos-1(RNAi)* | 4 | 11 | 85 | 54 | 12 | 12 | 77 | 52 |
| *rrf-3(pk1426); fos-1(RNAi)* | 10 | 8 | 82 | 50 | 16 | 26 | 58 | 50 |

[a] Venus::unc-6 driven by its own promoter. [11]

[b] Ventral nerve cord specific expression of Venus::unc-6 driven by *glr-1* promoter. [11]

[c] 1º VPC specific expression of Venus::unc-6 driven by the *egl-17* promoter. [11]

[d] Targeted RNAi mediated knockdown of *unc-6* in the 1º VPCs. [11]

[e] A similar percentage of ACs have been shown to invade when vulval cells are removed by ablation. [5]

[f] 2h heat shock at 30ºC followed by 4h recovery at 15ºC.

[g] *qyIs23(cdh-3> mCherry::PLC$\delta^{PH}$)* binds and sequesters $PI(4,5)P_2$ in the AC, thus reducing its levels there.

[h-m] The number of AC's that detached from the basement membrane at the L4 stage was as follows (number of detached/number that failed to invade): h = 2/12; i =11/42; j = 47/50; k = 18/20 l = 0/9; m= 2/15.

nd = not determined

**Table S3. Polarized marker localization in wild-type and mutant ACs**

| Genotype | Marker | Classification of marker localization patterns in wild-type and various mutant ACs | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P6.p 1-cell stage | | | | P6.p 2-cell stage | | | | P6.p 4-cell stage | | | |
| | | Polarized (%±SE) | Apicolateral Accumulation (%±SE) | No Polarity (%±SE) | N= | Polarized (%±SE) | Apicolateral Accumulation (%±SE) | No Polarity (%±SE) | N= | Polarized (%±SE) | Apicolateral Accumulation (%±SE) | No Polarity (%±SE) | N= |
| wild-type | mCherry::PLC$\delta^{PH}$ | 85±8 | 15±8 | 0 | 20 | 86±8 | 14±8 | 0 | 21 | 95±5 | 0 | 5±5 | 20 |
| wild-type | GFP::MIG-2 | 86±7 | 13±7 | 0 | 22 | 100 | 0 | 0 | 22 | 100 | 0 | 0 | 23 |
| wild-type | mCherry::moeABD | 100 | 0 | 0 | 18 | 100 | 0 | 0 | 21 | 95±5 | 5±5 | 0 | 21 |
| wild-type | UNC-40::GFP | 90±7 | 5±5 | 5±5 | 20 | 100 | 0 | 0 | 21 | 90±6 | 10±6 | 0 | 20 |
| *unc-6(ev400)* | mCherry::PLC$\delta^{PH}$ | 22±10 | 38±12 | 38±12 | 18 | 15±9 | 53±12 | 32±11 | 19 | 0 | 69±12 | 31±12 | 16 |
| *unc-6(ev400)* | mCherry::moeABD | 29±11 | 53±12 | 18±10 | 17 | 29±11 | 71±11 | 0 | 17 | 18±10 | 76±11 | 6±6 | 17 |
| *unc-6(ev400)* | UNC-40::GFP | 21±10 | 32±11 | 47±12 | 19 | 5±5 | 42±12 | 53±12 | 20 | 0 | 25±10 | 75±10 | 20 |
| *unc-6(ev400)* | GFP::MIG-2 | 33±11 | 38±11 | 29±10 | 21 | 14±8 | 43±11 | 43±11 | 21 | 25±10 | 40±11 | 35±11 | 20 |
| Vul[a] | mCherry::PLC$\delta^{PH}$ | 84±9 | 16±9 | 0 | 19 | 90±7 | 10±7 | 0 | 20 | 73±12 | 27±12 | 0 | 15 |
| Vul[a] | UNC-40::GFP | 94±6 | 6±6 | 0 | 17 | 86±8 | 14±8 | 0 | 21 | 94±6 | 6±6 | 0 | 17 |
| Vul[a] | GFP::MIG-2 | 95±5 | 5±5 | 0 | 20 | 87±7 | 13±7 | 0 | 23 | 76±10 | 24±10 | 0 | 21 |
| Vul[a] | mCherry::moeABD | 88±9 | 6±6 | 6±6 | 16 | 86±8 | 14±7 | 0 | 21 | 90±7 | 10±6 | 0 | 20 |
| *hs> unc-6::HA*[b] | UNC-40::GFP | nd | nd | nd | nd | nd | nd | nd | nd | 33±13 | 33±13 | 33±13 | 15 |
| wild-type (mock)[c] | UNC-40::GFP | nd | nd | nd | nd | nd | nd | nd | nd | 93±7 | 7±7 | 0 | 15 |

[a] Vul (vulvaless) animals were of the genotype *lin-3(n1059)/lin-3(n378)*.
[b] Heat shock directed expression of *unc-6::HA* from the integrated transgene *kyIs299*, 2h heat shock at 30ºC followed by 4h recovery at 15ºC.
[c] wild-type animals expressing UNC-40::GFP in the AC were subjected to an identical experimental regimen to confirm that heat shock alone does not cause defects in UNC-40::GFP localization.

**Table S4. Primer sequences and templates used for PCR fusions**

| Primer Sequence | Primer Type | Amplicon | Template |
|---|---|---|---|
| 5' TAA TgT gAg TTA gCT CAC TCA TTA gg 3' | forward | *cdh-3/zmp-1>* promoters | *cdh-3>*, pPD107.94/mk62-63; *zmp-1>*, pPD107.94/mk50-51 |
| 5' AAC gAT ggA TAC gCT AAC AAC TTg g 3' | forward nested | *cdh-3/zmp-1>* promoters | *cdh-3>*, pPD107.94/mk62-63; *zmp-1>*, pPD107.94/mk50-51 |
| 5' TTT CTg AgC TCg gTA CCC TCC AAg 3' | reverse | *cdh-3/zmp-1>* promoters | *cdh-3>*, pPD107.94/mk62-63; *zmp-1>*, pPD107.94/mk50-51 |
| 5' TAg gCT TTT CCg TAT AgC ATC CTC 3' | forward | *fos-1a>* promoter | cosmid F29G9 |
| 5' gCC CAA CTC TAg TCA TTT CTA gC 3' | forward nested | *fos-1a>* promoter | cosmid F29G9 |
| 5' TCC ACT CTC TTA TAT AgC AgA ggT gC 3' | reverse | *fos-1a>* promoter | cosmid F29G9 |
| 5' CTT ggA ggg TAC CgA gCT Cag AAA ggT ACC Atg AgT AAA ggA gAA g 3' | *cdh-3/zmp-1* extension, forward | *GFP::unc-34* | *punc-86>GFP::unc-34* |
| 5' Cgg gAA gCT AgA gTA AgT AgT TCg CC 3' | reverse | *GFP::unc-34* | *punc-86>GFP::unc-34* |
| 5' CTC TCA Agg ATC TTA CCg CTg TTg 3' | reverse nested | *GFP::unc-34* | *punc-86>GFP::unc-34* |
| 5' CTT ggA ggg TAC CgA gCT Cag AAA ATg ATT TTg CgA CAT TTC gg 3' | *cdh-3/zmp-1* extension, forward | *unc-40::GFP* | *punc-86>unc-40::GFP* |
| 5' gTg CCA CCT gAC gTC TAA g 3' | reverse | *unc-40::GFP* | *punc-86>unc-40::GFP* |
| 5' gTA Cgg CCg ACT AgT Agg AAA CAg T 3' | reverse nested | *unc-40::GFP* | *punc-86>unc-40::GFP* |
| 5' CTT ggA ggg TAC CgA gCT CAg AAA ATg gTC TCA AAG ggT gAA g 3' | *cdh-3/zmp-1* extension, forward | *mCherry::moeABD* | pJWZ6 |
| 5' CAg gAA ACA gCT ATg ACC ATg 3' | reverse | *mCherry::moeABD* | pJWZ6 |
| 5' gCC gCT CTA gAA TCA TCg TTC 3' | reverse nested | *mCherry::moeABD* | pJWZ6 |
| 5'CTT ggA ggg TAC CgA gCT CAg AAA ATg gCT CAA ACA AAg CCg ATT gCC 3' | *cdh-3/zmp-1* extension, forward | *mCherry::PLC$\delta^{PH}$* | pAA173 |
| 5' TTC gAg CgA Agg TCg CTT TTT ggT C 3' | reverse | *mCherry::PLC$\delta^{PH}$* | pAA173 |
| 5' TTg AAA TCg AgT TgC AAg CgC gCT CC 3' | reverse nested | *mCherry::PLC$\delta^{PH}$* | pAA173 |
| 5'CTT ggA ggg TAC CgA gCT Cag AAA Atg gCT CAA ACA AAg CCg ATT gCC 3' | *cdh-3/zmp-1* extension, forward | *mCherry* | pAA64 |
| 5' TTC gAg CgA Agg TCg CTT TTT ggT C 3' | reverse | *mCherry* | pAA64 |
| 5' TTg AAA TCg AgT TgC AAg CgC gCT CC 3' | reverse nested | *mCherry* | pAA64 |
| 5' gCA CCT CTg CTA TAT AAg AgA gTg gAA Tgg CTC AAA CAA AgC CgA TTg CC 3' | *fos-1a* extension, forward | *mCherry::PLC$\delta^{PH}$* | pAA173 |
| 5' TTC gAg CgA Agg TCg CTT TTT ggT C 3' | reverse | *mCherry::PLC$\delta^{PH}$* | pAA173 |
| 5' TTg AAA TCg AgT TgC AAg CgC gCT CC 3' | reverse nested | *mCherry::PLC$\delta^{PH}$* | pAA173 |
| 5' TgT AAA ACg ACg gCC AgT 3' | forward | *unc-6>* promoter | *pVns-unc-6* |
| 5' TgT AAA ACg ACg gCC AgT 3' | forward nested | *unc-6>* promoter | *pVns-unc-6* |
| 5' gTT CTT CTC CTT TAC TgT TTg TgT gAA Agg gTg Taa AgT ggA3' | reverse | *unc-6>* promoter | *pVns-unc-6* |
| 5' TCC ACT TTA CAC CCT TTC ACA CAA ACA TgA gTA AAg gAg AAg gAg AAg AACTTT TCA CTg g 3' | *unc-6* promoter extension, forward | *venus::unc-6(ΔSP)* | *pVns-unc-6* |
| 5' CAg gAA ACA gCT ATg ACC ATg 3' | reverse | *venus::unc-6(ΔSP)* | *pVns-unc-6* |
| 5' ATg ACC ATg ATT ACg CCA AgC gC 3' | reverse nested | *venus::unc-6(ΔSP)* | *pVns-unc-6* |

**Table S5. Extrachromosomal array and integrated strain generation**

| ***Ex*** **Designation** | ***Is*** **Designation** | **PCR Fusion created** | **Injected Concentration** | **Co-Injection Marker(s)** |
|---|---|---|---|---|
| *qyEx27* | *qyIs23, qyIs24, qyIs25* | *cdh-3>mCherry::PLCδ$^{PH}$* | 0.01ng/µl | *unc-119* |
| *qyEx39* | *qyIs50* | *cdh-3>mCherry::moeABD* | 2ng/µl | *unc-119* |
| *qyEx40* | *qyIs66, qyIs67* | *cdh-3>unc-40::GFP* | 2ng/µl | *unc-119 + myo-2>YFP* |
| *qyEx30* | *qyIs37* | *zmp-1>unc-40::GFP* | 2ng/µl | *unc-119* |
| *qyEx42* | *qyIs61* | *cdh-3>unc-34::GFP* | 1ng/µl | *unc-119* |
| *qyEx60* | not integrated | *fos-1a>mCherry::PLCδ$^{PH}$* | 0.25ng/µl | *pha-1* |
| *qyEx68* | *qyIs7* | *zmp-1>mCherry* | 1.0ng/µl | *unc-119* |
| *qyEx3* | *qyIs17* | pGK41(*lam-1::GFP*) | 10ng/µl | *unc-119* |
| *qyEx19* | *qyIs27* | pPR80(*GFP::ced-10*) | 75ng/µl | *unc-119* |
| *qyEx78* | not integrated | *Venus::unc-6(ΔSP)* | 15ng/µl | *unc-119* |