FIGURE S1

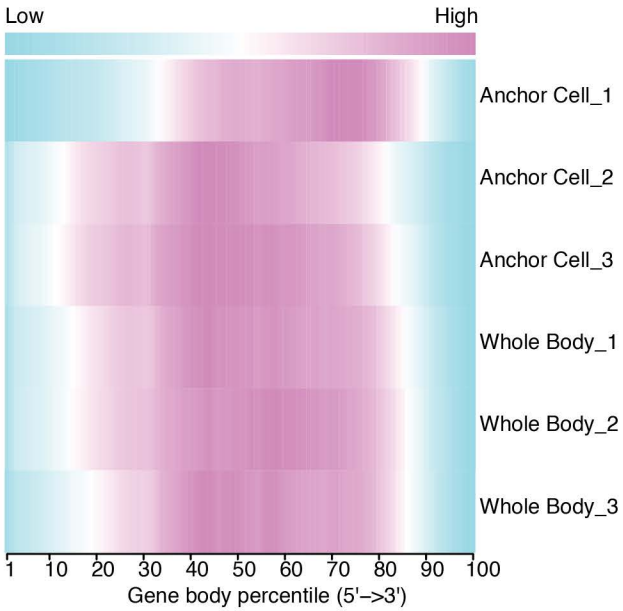

Fig. S1. Gene body coverage analysis of single-cell RNA sequencing.
Heatmap of read coverage profiles over gene body to evaluate uniformity of 5' to 3' coverage (pink = high coverage, blue = low coverage).

FIGURE S2

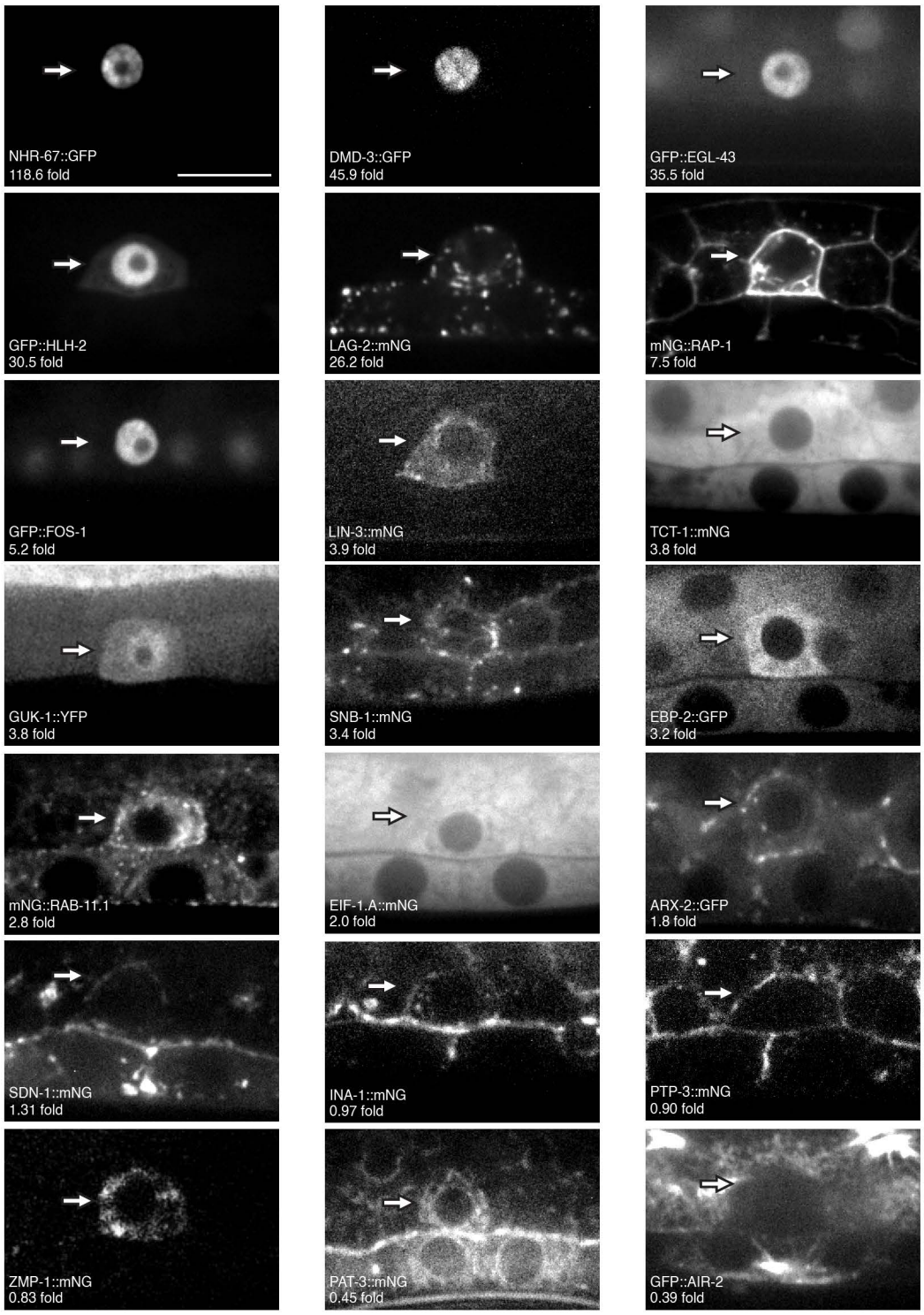
NHR-67::GFP
118.6 fold
DMD-3::GFP
45.9 fold
GFP::EGL-43
35.5 fold
GFP::HLH-2
30.5 fold
LAG-2::mNG
26.2 fold
mNG::RAP-1
7.5 fold
GFP::FOS-1
5.2 fold
LIN-3::mNG
3.9 fold
TCT-1::mNG
3.8 fold
GUK-1::YFP
3.8 fold
SNB-1::mNG
3.4 fold
EBP-2::GFP
3.2 fold
mNG::RAB-11.1
2.8 fold
EIF-1.A::mNG
2.0 fold
ARX-2::GFP
1.8 fold
SDN-1::mNG
1.31 fold
INA-1::mNG
0.97 fold
PTP-3::mNG
0.90 fold
ZMP-1::mNG
0.83 fold
PAT-3::mNG
0.45 fold
GFP::AIR-2
0.39 fold

Fig. S2. Levels of fluorescently tagged proteins of select AC transcriptome genes at the P6.p 2-cell AC.

AC (arrows) protein expression of endogenous reporters generated through genome editing, except GUK-1::YFP which was generated as a translational reporter. Fold change of the AC compared to whole-body transcriptomes are shown (bottom left of each image). DMD-3, RAP-1, TCT-1, SNB-1, EBP-2, RAB-11.1, and AIR-2 are also shown in Figure 1, but included here for completeness. Scale bar: 5 μm.

FIGURE S3

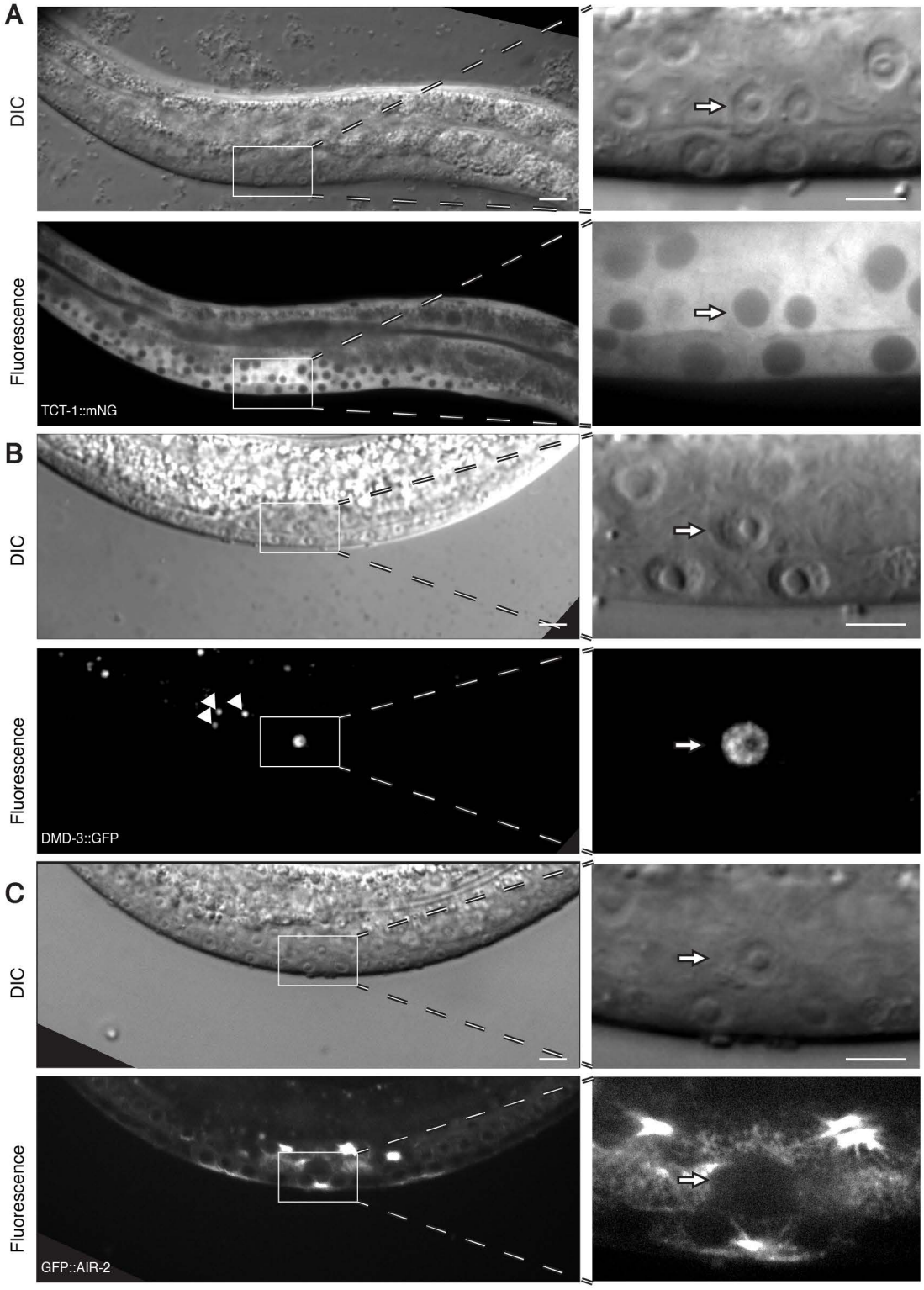

Fig. S3. Levels and cellular distribution of endogenously tagged proteins of select AC enriched and underenriched genes.

(A-C) Endogenous proteins in ACs (arrows) at the P6.p 2-cell stage when the AC transcriptome was generated. AC enrichment was based on comparison to WB gene expression at the same developmental stage. A central body view (left, 400x magnification) showing where the AC is located (box) is compared to a magnified view of the AC region (1000X magnification, right). Arrowheads indicate autofluorescent gut granules. Endogenous tagging of translational proteins generated from the highly enriched *tct-1* and *dmd-3* genes (A,B) and the underenriched *air-2* (C) gene. Scale bars: Left, 10 μm. Right, 5 μm.

FIGURE S4

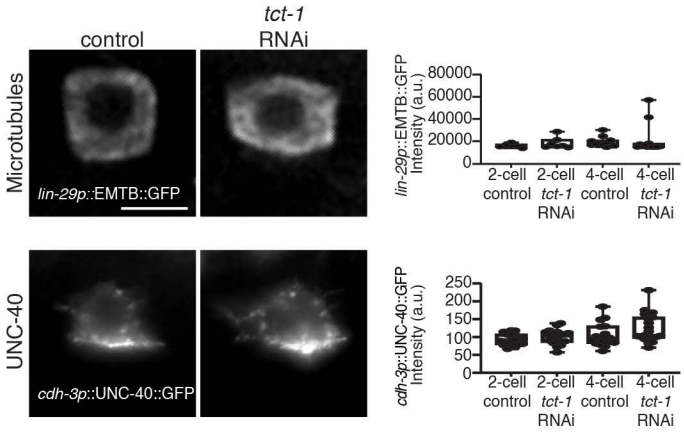

Fig. S4. Loss of TCT-1 does not alter translation of AC-promoter driven transgenes. AC promoters driving transgenes expressing proteins marking microtubules (*lin-29p*::EMTB::GFP) and UNC-40 (*cdh-3p*::UNC-40::GFP) in control empty vector RNAi and after *tct-1* RNAi treatment. Graphs show quantification of mean fluorescence intensity (right, boxplot n≥ 10 each, unpaired two-tailed t-tests). Scale bar: 5 μm.

FIGURE S5

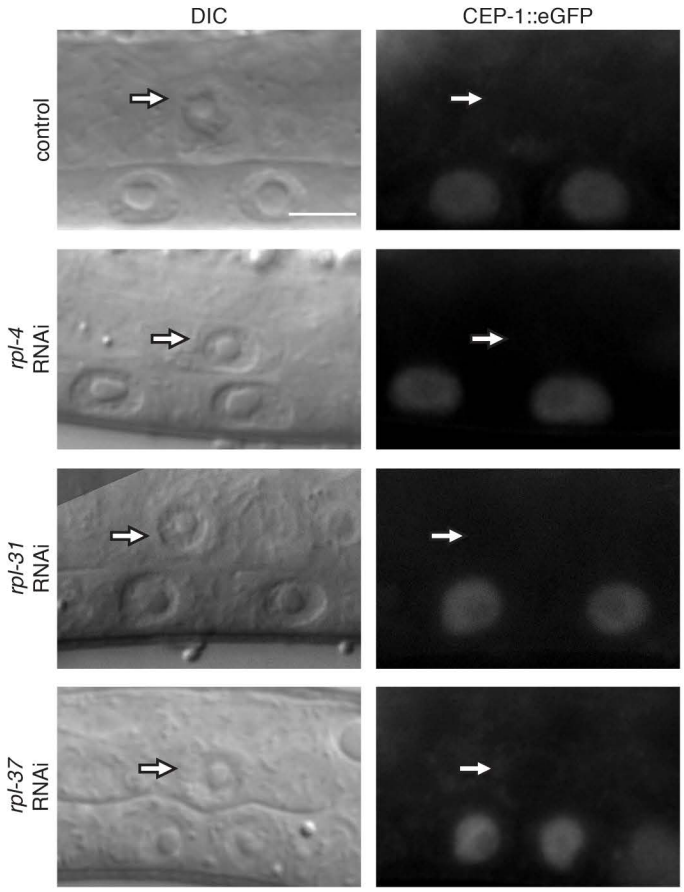

Fig. S5. CEP-1 (p53) is not stabilized/activated in the AC after RNAi mediated reduction of large ribosomal subunit proteins.

Examples of ACs (left, DIC; right, CEP-1::eGFP (p53); arrow) at the P6.p 2-cell stage after treatment with control RNAi and after RNAi mediated knockdown of *rpl-4, rpl-31,* and *rpl-37*. CEP-1::GFP was not detected in the AC after any treatment (n = 10 for each). Scale bars: 5 µm.

FIGURE S6

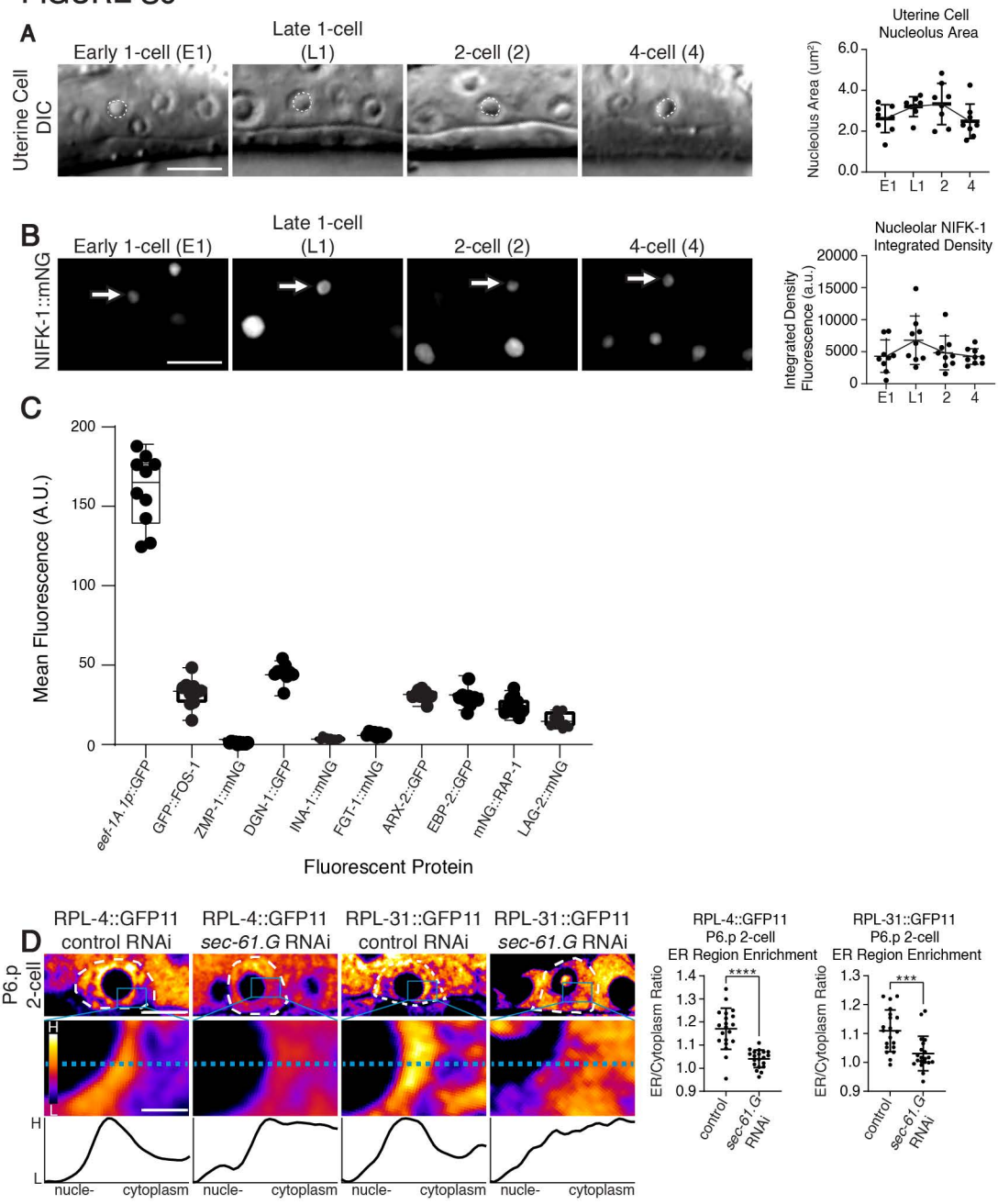

Fig. S6. Uterine cell nucleolus size, AC NIKF-1::mNG expression, *eef-1a.1* promoter expression, *sec-61.G* RNAi and ribosome localization.

(A) Timeline of nucleolus area (DIC images, dotted circles) of ventral uterine cells from the early P6.p 1-cell through the P6.p 4-cell stage. Graph shows quantification of nucleolus area over developmental time (n ≥ 10 ACs per timepoint each, mean ± SD, one-way ANOVA with *post hoc* Tukey's test).

(B) The timeline of endogenous NIFK-1 (NIFK-1::mNG) expression in the AC (arrow). NIFK-1 shows a spike in levels at the P6.p late 1-cell stage. Graph shows quantification of total fluorescence levels (right) over developmental time (n ≥ 10 ACs per timepoint, mean ± SD, one-way ANOVA with *post hoc* Tukey's test). Quantification is from one experiment in which several animals at each timepoint were processed in parallel.

(C) Fluorescence intensity of *eef-1a.1p*::GFP at the P6.p 2-cell stage is higher than all endogenously-tagged pro-invasive proteins (n ≥ 8 ACs per strain, boxplot).

(D) Spectral fluorescence-intensity map displaying the minimum and maximum pixel value range of the acquired data of RPL-31::GFP11 and RPL-4::GFP11 in the AC (dashed outlines) after control empty vector RNAi or *sec-61.G* RNAi treatment. Blue box depicts area containing the linescan. Linescan (blue dashed line, magnified bottom panels) shows peak levels of RPL- 31::GFP11 and RPL-4::GFP11 surrounding the nucleus in control RNAi, whereas RPL-31::GFP11 and RPL-4::GFP11 are more uniform in the cytosol after *sec-61.G* RNAi. Graphs show quantification of ER region to cytoplasm ratio in P6.p 2-cell ACs expressing RPL-4::GFP11 or RPL-31::GFP11 in control empty vector RNAi and after *sec-61.G* RNAi treatment (boxplots, n ≥ 20 each, ***p<0.001, ****p<0.0001 , unpaired two-tailed t-test). Scale bars: 1 μm inset, 5 μm all others.

Table S1. Anchor cell (AC) transcriptome compared with mid L3 larval stage whole body (WB) gene expression.

Three replicates of AC and WB gene expression profiles are shown. Genes are ordered based upon log2 fold change values (the log2 transformed ratio of the average of the 3 AC replicates normalized reads over the average of the 3 WB replicates normalized reads). Log2 fold change of 1 corresponds to a fold change of 2. Genes listed were considered to be part of the AC transcriptome if they had at least 10 reads in one of the three AC replicates.

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Table S2. AC transcripts with significantly enriched expression versus mid L3 larval stage WB expression.

Three replicates of AC and WB gene expression profiles are shown. Genes are ordered based upon log2 fold change values (the log2 transformed ratio of the average of the 3 AC replicates normalized reads over the average of the 3 WB replicates normalized reads). Log2 fold change of 1 corresponds to a fold change of 2. A list of 1,502 AC genes with log2 fold change ≥ 1 compared with WB gene expression and an adjusted p-value < 0.1.

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Table S3. Analysis of transgenic and genome edited reporters of genes present in the AC transcriptome.

Complete list of genes previously annotated and newly identified as having AC expression based on visual analysis of transgenic or endogenously tagged strains. Log2 fold change is shown for each gene (the log2 transformed ratio of the average of the 3 AC replicates normalized reads over the average of the 3 WB replicates normalized reads). Log2 fold change of 1 corresponds to a fold change of 2. Sources are listed by PubMed reference number (PMID).

Click here to download Table S3

Table S4. Previously tagged genes visually examined for AC expression.
A list of genes that were present at various levels in the AC transcriptome and examined for expression in the AC at the P6.p 2-cell stage. Expression was compared to other uterine and vulval cells using fluorescence microscopy, see Figures S2 and S3. Sources are listed by PubMed reference number (PMID).

Gene	Sequence ID	Source (PMID)	Notes	Fluorophore tagging method
nhr-67	C08F8.8	31806663	nuclear hormone receptor Tailless/TLX	endogenous tag
fos-1	F29G9.4	31806663	basic leucine zipper transcription factor Fos	endogenous tag
hlh-2	M05B5.5	31806663	basic helix-loop-helix transcription factor E/Daughterless	endogenous tag
egl-43	R53.3	31806663	zinc-finger transcription factor EVI1/MEL	endogenous tag
dmd-3	Y43F8C.10	30599092	doublesex	endogenous tag
ina-1	F54G8.3	32585132	alpha integrin	endogenous tag
pat-3	ZK1058.2	32585132	beta integrin	endogenous tag
ptp-3	C09D8.1	32585132	LAR-type receptor phosphotyrosine-phosphatases	endogenous tag
sdn-1	F57C7.3	32585132	syndecan	endogenous tag
lag-2	Y73C8B.4	30799241	Notch receptor ligand	endogenous tag
ebp-2	VW02B12L.3	30080857	homolog of microtubule tip tracking protein EBP-1	endogenous tag
arx-2	K07C5.1	27780040	Arp2/3 subunit	endogenous tag
rap-1	C27B7.8	28829947	small GTPase	endogenous tag
guk-1	T03F1.8	20442418	guanylate kinase	translational reporter
air-2	B0207.4	34014923	Aurora B	endogenous tag
zmp-1	EGAP1.3	30686527	GPI anchored MMP	endogenous tag

Table S5. Focused RNAi screen of AC enriched genes.
A focused RNAi screen of AC enriched genes (versus WB gene expression) encoding transmembrane or secreted proteins, cytoskeleton component and regulators, and transcription factors. AC invasion was scored by assessing the presence of a basement membrane (BM) breach using *lam-1p*::LAM-1::mCherry.

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Table S6. Pathway analysis of AC enriched genes.

Table of AC-enriched annotated terms and protein domains (referred to as pathways in text for simplicity) sorted by p-values identified using Database for Annotation, Visualization and Integrated Discovery (DAVID). Total genes annotated with the term or domain within the AC enriched genes is indicated in the Count column, while the Total genes annotated with Term/Domain lists the total number of genes in the *C. elegans* genome with that term or domain. Fisher Exact p-value was used for gene-enrichment analysis. Resource database used for each term or domain is shown. Notably, cuticular collagens were the most enriched genes in the AC. This is likely for a post-invasion role of the AC in contributing to the apical extracellular matrix of the vulva.

Term/Domains (Pathway)	Count[a]	Total genes annotated with Term/Domain[b]	p-value[c]	Resource Database
Cuticular Collagens	55	147	3.36E-24	GOTERM_MF_DIRECT
Proteasome	17	35	1.09E-09	UP_KEYWORDS
Embryo Development	253	2788	5.13E-05	GOTERM_BP_DIRECT
Protein Heterodimerization	15	61	0.000151	GOTERM_MF_DIRECT
Cytoskeleton	28	176	0.000223	GOTERM_CC_DIRECT
Zinc Finger, C2H2	22	151	0.00372	INTERPRO
Translation Regulation	25	181	0.01	UP_KEYWORDS
GTP binding	26	206	0.0134	GOTERM_MF_DIRECT
Longin-like Domains	5	16	0.0265	INTERPRO
VHS	3	4	0.0297	INTERPRO

[a] The number of AC enriched genes (>2-fold enrichment) belonging to the Term/Domain.

[b] The total number of genes annotated by the resource database that match the Term/Domain.

[c] Fischer Exact 2x2 Tests computed by DAVID (see Methods).

Table S7. AC transcriptome enriched translational regulators.

Genes that encode translational regulators and are enriched within the AC transcriptome versus WB gene expression. Genes were identified using Database for Annotation, Visualization and Integrated Discovery (DAVID) and WormBase ParaSite Biomart was used to identify human orthologs (Methods).

Gene	Sequence ID	Human Ortholog	Ensembl Stable ID
asd-1	R74.5	RBFOX1	ENSG00000078328
eif-1	T27F7.3	EIF1B	ENSG00000114784
ife-2	R04A9.4	EIF4E2	ENSG00000135930
ife-2	R04A9.4	EIF4E3	ENSG00000163412
ife-4	C05D9.5	EIF4E2	ENSG00000135930
mrps-11	W04D2.5	MRPS11	ENSG00000181991
rpl-5	F54C9.5	RPL5	ENSG00000122406
rpl-17	Y48G8AL.8	RPL17	ENSG00000265681
rpl-18	Y45F10D.12	RPL18	ENSG00000063177
rpl-19	C09D4.5	RPL19	ENSG00000108298
rpl-21	C14B9.7	RPL21	ENSG00000122026
rpl-25.2	F52B5.6	RPL23A	ENSG00000198242
rpl-29	B0513.3	RPL29	ENSG00000162244
rpl-30	Y106G6H.3	RPL30	ENSG00000156482
rpl-31	W09C5.6	RPL31	ENSG00000071082
rpl-33	F10E7.7	RPL35A	ENSG00000182899
rpl-37	C54C6.1	RPL37	ENSG00000274242
rpl-38	C06B8.8	RPL38	ENSG00000172809
rpl-39	C26F1.9	RPL39	ENSG00000355315
rps-10	D1007.6	RPS10	ENSG00000124614
rps-22	F53A3.3	RPS15A	ENSG00000134419
snr-1	Y116A8C.42	SNRPD3	ENSG00000100028
snr-4	C52E4.3	SNRPD2	ENSG00000125743
snr-5	ZK652.1	SNRPF	ENSG00000139343
snr-6	Y49E10.15	SNRPE	ENSG00000182004
snrp-27	R05D11.7	SNRNP27	ENSG00000124380

Table S8. Genetic mutants examined for AC invasion defects.

A list of genetic mutants examined for AC invasion defects. AC invasion was scored by DIC microscopy and assessing the presence or break in the phase dense line under the AC, which corresponds to the BM.

	Identifier	Genotype	% ACs with incomplete invasion[a]	p-value[b]	*n*[c]
1	N2	Wild-type	0%	n/a	50
2	RB925	*ire-1(ok799)* II	25%	0.001	20
3	MC817	*ddx-52(gc51)* I	16%	0.01	25
4	FK312	*sma-5(n678)* X	0%	ns	25
5	NK2694	*qy110(rpl-31::GFP11)* I, *bmd15(eft-3p::GFP1-10::unc54 3'UTR-[let858 terminator]-myo-2p::mCherry-3xHA tbb-2 3'UTR')* I	0%	n/a	41
6	NK2902	*qyIs463(lin-29p::ZIF-1::SL2::mCh)*, *rpl-31(qy189[rpl-31::ZF1::GFP11])* I, *bmd15(eef-1A.1p::GFP1-10::unc-54 3'UTR; myo-2p::mCherry tbb-2 3'UTR)* I; *zif-1(gk117)* III	45%	<0.0001	40

[a] All animals were scored for invasion at the VPC P6.p 4-cell stage (see Figure 1A).

[b] Fischer Exact 2x2 Tests were used. Genetic mutants compared to N2 for statistical analysis. NK2902 was compared to NK2694 for statistical analysis.

[c] Number of animals observed for each condition.

Table S9. Strains used in study.

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Table S10. Oligonucleotide sequences used in genome edited strains and RNAi constructs.

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