Supporting Information

Karp and Greenwald 10.1073/pnas.1222377110

SI Materials and Methods

A complete list of strains used in this study is found in Table S1. Strains are organized by the figure in which they appear. Information regarding all transgenes used in this study is found in Table S2. Transgenes are organized alphabetically.

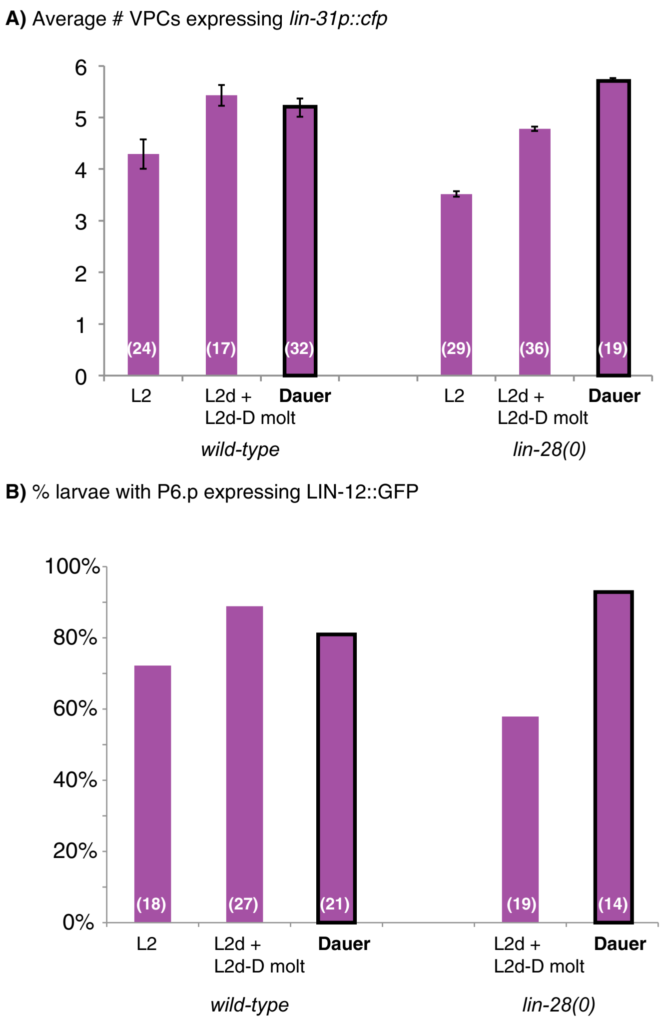

Fig. S1. Vulval precursor cell (VPC) identity markers are expressed in dauer larvae. *lin-31p::cfp* and LIN-12::GFP are expressed in multipotent VPCs during larval (L)2 stage and before induction in the L3 stage (1–3). Their expression in dauer larvae indicates that specification marker expression is not absent because of a general repression of transgenes in dauer or loss of VPC identity. (*A*) *lin-31p::cfp* expression remains high in VPCs of dauer larvae. Average number of VPCs expressing *lin-31p::cfp* ± SEM. Larvae were grown at 25 °C. (*B*) LIN-12::GFP remains expressed in VPCs in dauer larvae. Percentage of larvae in which P6.p expressed LIN-12::GFP at 25 °C. We note that in continuous development, LIN-12 is down-regulated in P6.p and its descendants after induction (3, 4), so the reduced expression in *lin-28* larvae before dauer may include animals in which down-regulation occurred because of precocious induction. In the bars, number of larvae scored is indicated in parentheses, and the dauer stage is highlighted with a black outline.

1. Myers TR, Greenwald I (2005) lin-35 Rb acts in the major hypodermis to oppose ras-mediated vulval induction in C. elegans. *Dev Cell* 8(1):117–123.
2. Tan PB, Lackner MR, Kim SK (1998) MAP kinase signaling specificity mediated by the LIN-1 Ets/LIN-31 WH transcription factor complex during C. elegans vulval induction. *Cell* 93(4): 569–580.
3. Levitan D, Greenwald I (1998) LIN-12 protein expression and localization during vulval development in C. elegans. *Development* 125(16):3101–3109.
4. Shaye DD, Greenwald I (2002) Endocytosis-mediated downregulation of LIN-12/Notch upon Ras activation in Caenorhabditis elegans. *Nature* 420(6916):686–690.

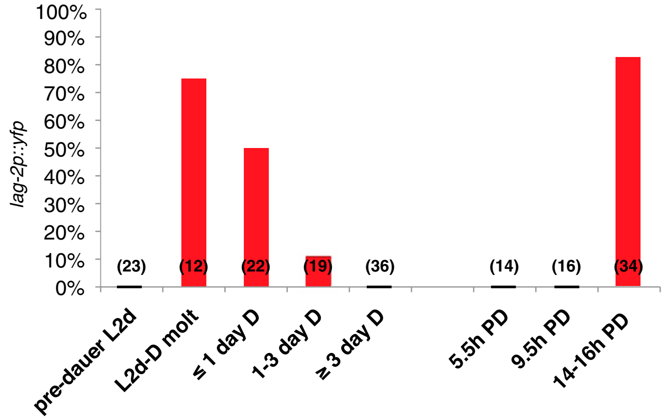

Fig. S2. *lag-2p::yfp* expression during the dauer life history at 20 °C. Otherwise wild-type *arIs131[lag-2p::yfp]* larvae underwent the dauer life history as a result of addition of dauer pheromone to the culture medium (predauer and dauer larval stages). Dauer larvae isolated from crowded and starved plates did not display *lag-2* expression in P6.p during dauer (Fig. 1), but expression resumed after ~14 h postdauer, the time at which cell divisions had resumed. Numbers in parentheses are the number of larvae scored. PD, postdauer.

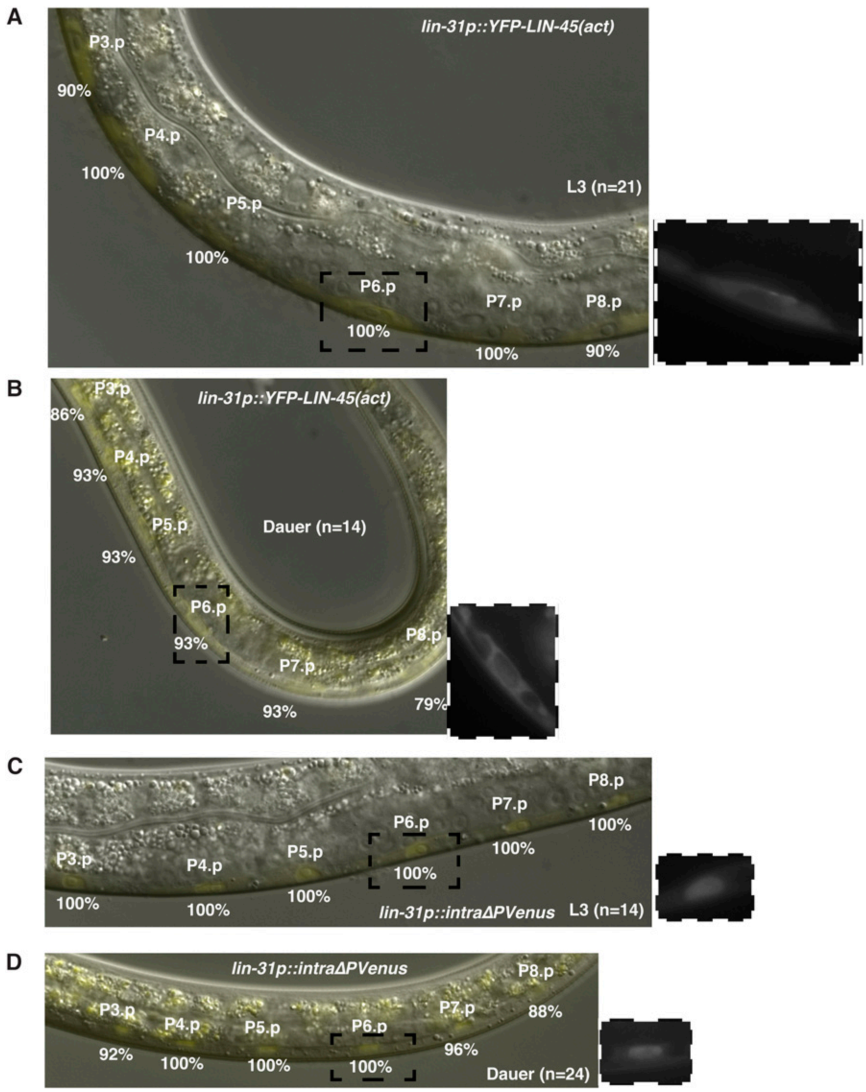

Fig. S3. LIN-45(act) and LIN-12(intraΔP) are stable in dauer larvae. (*A–D*) Percentage of larvae in which each VPC expresses the indicated reporter out of the total larvae examined. A merged image of YFP and differential interference contrast (DIC) channels is shown. Note that dauer larvae display a high degree of background autofluorescence because of the increased fat storage in the intestine and hypodermis. A larger image of the YFP channel alone is shown for a representative VPC (P6.p) for each picture (boxes). (*A*) *arEx1626[lin-31p::YFP-LIN-45(AAED)]* in L3 using a 63× objective and a 1,000-ms exposure time. YFP-LIN-45 is cytoplasmic. (*B*) *arEx1626[lin-31p::YFP-LIN-45(AAED)]* in dauer larvae using a 63× objective and a 1,000-ms exposure time. YFP-LIN-45 is cytoplasmic. (*C*) *arEx1719[lin-31p::LIN-12(intraΔP)-Venus]* in L3 using a 63× objective and a 800-ms exposure time. YFP is nuclear. (*D*) *arEx1719[lin-31p::LIN-12(intraΔP)-Venus]* in L3 using a 63× objective and a 800-ms exposure time. YFP is nuclear. The brightness and contrast were adjusted identically for all of the boxed insets; the merged images show the unadjusted photographs.

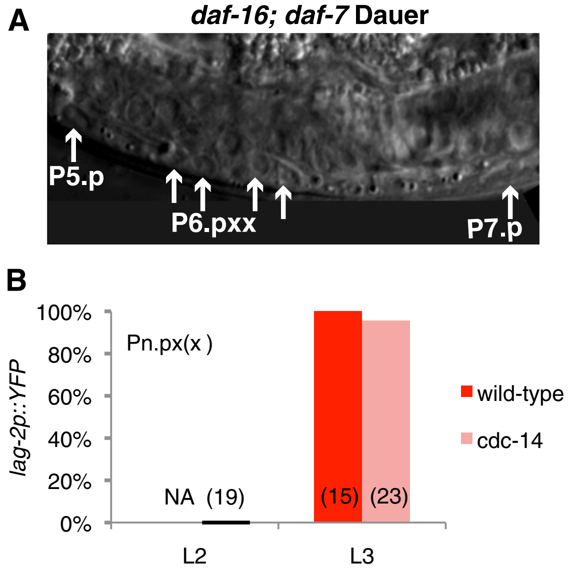

Fig. S4. Precocious cell division does not cause precocious specification. VPCs in *daf-16(0); daf-7* dauer larvae often divide, whereas VPC divisions are not observed in either wild-type or *daf-7* dauer larvae (1, 2) (Fig. 5). These divisions could occur either as a result of specification or could be the cause of specification in dauer larvae. Our data indicates that the former is the case: first, after normal vulval induction in continuous or postdauer L3 stages VPC divisions occur synchronously (1, 3), whereas divisions occur asynchronously in *daf-16(0)* dauer larvae (*A*). Second, forced progression through the cell cycle does not result in the expression of a specification marker (*B*). Finally, precocious specification markers expression is observed even in VPCs that have not yet divided (compare numbers in Fig. 5 *A* and *B*). (*A*) The VPC divisions in *daf-16(0); daf-7* dauer larvae are not coordinated, in contrast to the approximately synchronous vulval lineages observed in the L3 stage during continuous development. In this dauer larva, P6.p has divided twice, whereas P5.p and P7.p have not divided. DIC photograph taken with a 63× objective. (*B*) Loss of *cdc-14* activity results in cell division during the L2 stage (4). These cells do not undergo precocious specification because the daughters of P6.p never display *lag-2p::yfp* expression during the L2 stage but always display *lag-2p::yfp* expression during the L3 stage.

1. Euling S, Ambros V (1996) Reversal of cell fate determination in Caenorhabditis elegans vulval development. *Development* 122(8):2507–2515.
2. Braendle C, Félix M-A (2008) Plasticity and errors of a robust developmental system in different environments. *Dev Cell* 15(5):714–724.
3. Sulston JE, Horvitz HR (1977) Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. *Dev Biol* 56(1):110–156.
4. Saito RM, Perreault A, Peach B, Satterlee JS, van den Heuvel S (2004) The CDC-14 phosphatase controls developmental cell-cycle arrest in C. elegans. *Nat Cell Biol* 6(8):777–783.

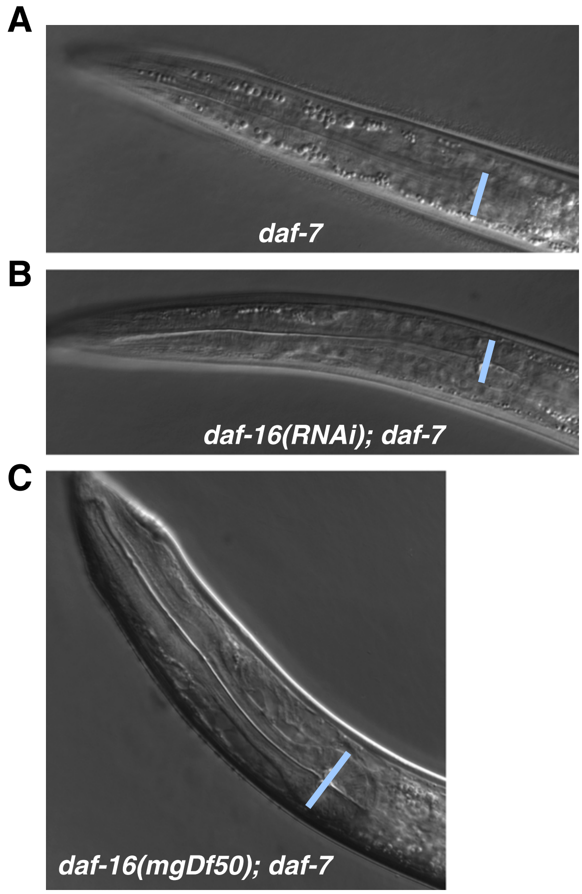

Fig. S5. *daf-16(RNAi); daf-7* dauer larvae do not display the morphological defects observed in *daf-16(0); daf-7* dauer larvae. *daf-16(RNAi); daf-7(e1372)* dauer larvae have a constricted pharynx, similar to wild-type or *daf-7(e1372)* dauer larvae. By contrast, *daf-16(0); daf-7(e1372)* dauer larvae do not have a normally constricted pharynx. Bars indicate the width of the pharynx. DIC photographs were taken with a 63× objective.

Table S1. List of strains

Fig.	Strain	Genotype
1 *C–F*	GS5572	*lin-28(n719); arIs131*
1 *C* and *D*	GS5745	*lin-28(n719); arIs98*
1 *C* and *D*	GS6056	*lin-28(n719); arIs116*
1 *C* and *D*	GS5605	*lin-28(n719)); arIs107*
1 *G* and *H*	GS4892	*arIs131*
1 *G* and *H*	GS4815	*arIs98*
1 *G* and *H*	GS5231	*arIs116*
1 *G* and *H*	GS6107	*arIs107*
2*B*, *Left*	GS4892	*arIs131*
2*B*, *Right*	GS5528	*arIs131; lin-1(n304)*
2*C*, *Left*	GS5801	*arEx1120*
2*C*, *Right*	GS5851	*arEx1213*
3*A*, *Upper Left*	GS4892	*arIs131*
3*A*, *Upper Right*	GS6065	*arIs131; arEx1287*
3*A*, *Lower Left*	GS5620	*daf-7(e1372) arIs131*
3*A*, *Lower Right*	GS6385	*daf-7(e1372) arIs131; arEx1287*
3*B*, *Upper Left*	GS5231	*arIs116*
3*B*, *Upper Right*	GS5236	*arIs116; arEx1080*
3*B*, *Lower Left*	GS6106	*daf-7(e1372); arIs116*
3*B*, *Lower Right*	GS6637	*daf-7(e1372); arIs116; arEx1080*
4*B*	GS5620	*daf-7(e1372) arIs131*
4*B*	GS5933	*daf-2(e1370) arIs131*
4*B*	GS5989	*daf-5(m512); daf-2(e1370) arIs131*
4*C*	GS5934	*arIs131; daf-9(dh6); dhEx24[daf-9+, sur-5::GFP]**
4*D*	GS6072	*arIs131; daf-12(rh273)*
5*A*	GS6656	*daf-7(e1372); arIs98*
5*A*	GS6657	*daf-16(mgDf50); daf-7(e1372); arIs98*
5*A*	GS5620	*daf-7(e1372) arIs131*
5*A*	GS5997	*daf-16(mgDf50); daf-7(e1372) arIs131*
5*A*	GS6106	*daf-7(e1372); arIs116*
5*A*	GS6163	*daf-16(mgDf50); daf-7(e1372); arIs116*
5*A*	GS6148	*daf-7(e1372); arIs107*
5*A*	GS6048	*daf-16(mgDf50); daf-7(e1372); arIs107*
5*B*, *Upper*	GS6106	*daf-7(e1372); arIs116*
5*B*, *Upper*	GS5620	*daf-7(e1372) arIs131*
5*B*, *Lower*	GS6163	*daf-16(mgDf50); daf-7(e1372); arIs116*
5*C*, *Left*	GS5620	*daf-7(e1372) arIs131*
5*C*, *Right*	GS6810	*daf-7(e1372) arIs131; rde-1(ne300)V; arEx1720*
5*D*	GS5620	*daf-7(e1372) arIs131*
5*E*	GS5997	*daf-16(mgDf50); daf-7(e1372) arIs131*
S1*A*	GS3858	*dpy-20(e1282); arEx574*
S1*A*	GS6039	*lin-28(n719); arEx574*
S1*B*	GS6473	*pha-1(e2123); arEx1575*
S1*B*	GS6699	*lin-28(n719); pha-1(e2123); arEx1575*
S2	GS4892	*arIs131*
S3 *A* and *B*	GS6572	*pha-1(e2123); arEx1626*
S3 *C* and *D*	GS6790	*pha-1(e2123); arEx1719*
S4*A*	GS5997	*daf-16(mgDf50); daf-7(e1372) arIs131*
S4*B*	GS4892	*arIs131*
S4*B*	GS6073	*cdc-14(he141); arIs131*
S5 *A* and *B*	GS5620	*daf-7(e1372) arIs131*
S5*C*	GS5997	*daf-16(mgDf50); daf-7(e1372) arIs131*

*Larvae scored were GFP-minus; thus, they had lost the extrachromosomal array.

Table S2. List of transgenes

Transgene	Description	Type	Injection marker(s)	Source
arEx1080	*lin-31p::LIN-12(intraΔP)*	Complex	*myo-3::mCherry*	1
arEx1120	*lag-2(min)p::yfp*	Simple	*pha-1+, ceh-22::GFP*	2
arEx1213	*Lag-2(minΔVPCrep)p::yfp*	Simple	*pha-1+, ceh-22::GFP*	2
arEx1287	*lin-31p::LIN-45(AAED)*	Complex	*myo-3::mCherry*	3
arEx1575	*LIN-12::GFP*	Complex	*pha-1+, ceh-22::GFP*	This work*
arEx1626	*lin-31p::YFP-LIN-45(AAED)*	Complex	*pha-1+, ceh-22::GFP*	3
arEx1719	*lin-31p::LIN-12(intraΔP)-Venus*	Complex	*pha-1+*	Ryan Underwood and I.G. (Columbia University, New York)
arEx1720	*lin-31p::RDE-1(+)*	Simple	*myo-3::mCherry*	This work*
arEx574	*lin-31p::cfp*	Simple	*dpy-20+, ceh-22::GFP*	4
arIs107	*mir-61p::yfp*	Simple	*pha-1+, ttx-3::GFP*	5
arIs116	*lst-5p::yfp*	Simple	*pha-1+, ceh-22::GFP*	1 and 6
arIs131	*lag-2p::yfp*	Simple	*pha-1+, ceh-22::GFP*	1 and 2
arIs98	*apx-1p::yfp*	Simple	*dpy-20+, ceh-22::GFP*	1

*a*rEx1575* was made by injecting GS6014 *pha-1(e2123)* hermaphrodites with linearized plasmids: 4 ng/μL pLIN-12::GFP (7), 1 ng/μL pCW2.1 (ceh-22::GFP), and 1 ng/μL pBX (pha-1+), along with 50 ng/μL cut N2 genomic DNA. *arEx1720* was made by injecting GS6409 daf-7(e1372) arIs131; rde-1(ne300) hermaphrodites with circular plasmids: 60 ng/μL p887 lin-31p::RDE-1 (J. Li and I.G., Columbia University, New York, NY), 20 ng of p716 myo-3::mCherry, and 20 ng of pBluescript.

1. Li J, Greenwald I (2010) LIN-14 inhibition of LIN-12 contributes to precision and timing of C. elegans vulval fate patterning. *Curr Biol* 20(20):1875–1879.
2. Zhang X, Greenwald I (2011) Spatial regulation of lag-2 transcription during vulval precursor cell fate patterning in Caenorhabditis elegans. *Genetics* 188(4):847–858.
3. de la Cova C, Greenwald I (2012) SEL-10/Fbw7-dependent negative feedback regulation of LIN-45/Braf signaling in C. elegans via a conserved phosphodegron. *Genes Dev* 26(22): 2524–2535.
4. Myers TR, Greenwald I (2005) lin-35 Rb acts in the major hypodermis to oppose ras-mediated vulval induction in C. elegans. *Dev Cell* 8(1):117–123.
5. Yoo AS, Greenwald I (2005) LIN-12/Notch activation leads to microRNA-mediated down-regulation of Vav in C. elegans. *Science* 310(5752):1330–1333.
6. Choi MS (2009) Genes that act in specification of the vulval secondary fate in Caenorhabditis elegans.
7. Levitan D, Greenwald I (1998) LIN-12 protein expression and localization during vulval development in C. elegans. *Development* 125(16):3101–3109.