

SUPPORTING INFORMATION

Supplementary Tables

Table S1. List of strains.

Table S2. Measurements of distances between AC and VPCs. Raw data, in microns.

Supplementary Figures

Figure S1. (A) Tracking AC positioning and VPC movement relative to cellular reference landmarks. To study temporal changes in positioning of AC and VPCs during the L2 and L3 stages, we inferred their positions with respect to cellular landmarks, i.e. body wall muscle nuclei in the vicinity of AC and studied VPCs (Sulston and Horvitz, 1977), as previously described (Sternberg and Horvitz 1986). The example DIC image depicts an early L2 larva (wild type, N2 strain) shortly after the birth of the AC. Body wall muscles (BWM, red) on right and left side, AC (blue) and VPCs (green) can be visualized by going through different focal planes. Relative distances between AC, VPC and BWM were drawn to infer movements of AC and VPCs. **(B) VPC and AC movements in individuals with intact VPCs.** Movement was assessed with respect to body wall muscle nuclei in *lon-1(e185)* individuals (Fig. S1A); cell positions were observed at multiple timepoints to establish movement patterns. We selected animals (N=6) with the AC initially positioned between P5.p and P6.p at the mid-late L2 stage and then observed temporal changes in AC-VPC alignment until the mid-L3 stage. In all individuals examined, one or more VPCs showed movement towards the AC; in contrast, the AC did not show any significant movement. Dash: no movement. Black arrow: VPC moved slightly towards the AC. Red arrow: VPC moved under the AC. nd: not determined. **(C) Movement of isolated P8.p towards the AC.** To observe the movement of an isolated VPC, we ablated all VPCs except P8.p at the early L2 stage (wild-type, N2 strain) and

assessed movement with respect to body wall muscle nuclei. Individuals (N=12) were followed from mid-L2 until the 4-cell stage (late L3 stage) to infer cell lineages/fates. In all cases, P8.p moved towards the AC while the AC did not show any significant movement.* P8.p movement was initially inhibited by cell debris of P7.p but reached the AC at 4-cell stage. ** P8.p initiated movement towards the AC but stopped at the debris of P7.p. **(D) Migration of isolated P5.p and P8.p towards the AC.** Example of temporal progression of P5.p (black) and P8.p (grey) nuclear positions after P(3,4,-,6,7,-) ablations at the L1 lethargus. P5.p and P8.p started migrating at the L2 lethargus stage, and P5.p aligned fully with the AC during the L3 stage.

Fig S2. Cell fate patterns of VPCs in mutant contexts. (A) *lin-3(n378)*; (B) *lin-3(e1417)*; (C) *bar-1(ga80)*; (D) *cwn-1(ok546)*; *egl-20(n585)*. Each line represents the vulval pattern of a single individual, and individuals are ordered from highest to lowest index (I) of vulval induction. Vulval cell fate patterns of P3.p to P8.p were, whenever feasible, separately inferred for their anterior and posterior daughters (Pn.pa and Pn.pp). Black lines separate individuals with complete vulval induction (I=3), partial induction ($0 < I < 3$) and no induction (I=0). Colour coding of vulval cell fates (1°: blue, 2°: red) and non-vulval cell fates (3°: yellow, 4°: grey). Induced vulval cells that could not be clearly assigned to either a 1° or 2° fate are coded in purple; missing cells are coded in white. Vulval fate patterns were determined in early to mid L4 individuals using Nomarski microscopy, anaesthetized with sodium azide. We counted the Pn.p progeny and determined their fates as previously described (Sternberg and Horvitz 1986; Braendle and Félix 2008). The presented mutant cell fate patterns were obtained from a previously published data set, which reported levels of induction but not cell fate patterns as shown here (Braendle and Félix 2008).

Figure S3. AC-VPC positioning at the early L3 stage in EGF/Ras/MAPK mutants. The data shown in Figure 5A are plotted here as histograms of AC positioning relative to P(5-7).p. **(A) *lin-3(mf75)*, n=34; (B) *let-23(sy1)*, n=27;**

(C) *let-23(sy1); lin-3(n378)*, n=24; (D) *lin-3(mfls55)*, n=20; (E) *lin-15(e1763)*, n=57; (F) *lin-3(e1417); lin-15(e1763)*, n=60; (G) *let-60(n1046)*, n=58; (H) *lin-3(e1417) let-60(n1046)*, n=62.

Figure S4. AC-VPC positioning at the early L3 stage in Wnt pathway mutants. The data shown in Figure 5B are plotted here as histograms of AC positioning relative to P(5-7).p. (A) *cwn-1(ok546)*, n=27; (B) *egl-20(n585)*, n=26; (C) *lin-44(n1792)*, n=28; (D) *mom-2* RNAi, n=24; (E) *mom-2* RNAi at 15°C, n=13; (F) *lin-44(n1792)* treated with *mom-2* RNAi, n=15; (G) *cwn-1(ok546); egl-20(n585)*, n=12; (H) *cwn-1(ok546); egl-20(n585) cwn-2(ok895)*, n=14; (I) *lin-44(n1792); cwn-1(ok546); cwn-2(ok895)*, n=12; (J) *lin-17(n671)*, n=20; (K) *lin-18(e620)*, n=26; (L) *mom-5(zu193)*, n=18; (M) *mig-1(nu225)*, n=22; (N) *cfz-2(ok1201)*, n=18; (O) *bar-1(mu63)*, n=30; (P) *bar-1(ga80)*, n=30; (G) *pry-1(mu38)*, n=15; (R) *mab-5(e1239)*, n=25 and (S) *lin-39(n1760)*, n=17.

Figure S5. AC-VPC positioning at the early L3 stage in double mutants of the EGF and Wnt pathways. The data shown in Figure 5C are plotted here as histograms of AC position relative to P(5-7).p. (A) *lin-3(n378); bar-1(mu63)*, n= 25; (B) *lin-3(n378); bar-1(ga80)*, n= 29; (C) *let-23(sy1); bar-1(ga80)*, n= 17; and (D) *pry-1(mu38); lin-3(n378)*, n= 28.

Figure S6. Early VPC-gonad positioning in Wnt pathway mutants. (A) To test for early mispositioning of P6.p in the late L1 stage, we assessed its position relative to the centre of the gonad primordium as illustrated in the cartoon. (B) Box-Plots of gonad-P6.p alignment at the L1 lethargus: wild type N2, n=14; *bar-1(ga80)*, n=15, (Brown-Forsythe test, $F_{1,27} = 0.49$, $P=0.49$; Mood's Median Test, $Z=-0.055$, $P=0.58$); *lin-39(n1760)*, n=13, (Brown-Forsythe test, $F_{1,29} = 1.02$, $P=0.32$; Mood's Median Test, $Z=5.10$, $P<0.0001$); *cwn-1(ok546); egl-20(n585)*, n=11, (Brown-Forsythe test, $F_{1,29} = 20.78$, $P<0.0001$; Mood's Median Test, $Z=-1.01$, $P=0.31$); *lin-44(n1792); cwn-*

1(ok546); *cwn-2(ok895)*, n=11, (Brown-Forsythe test, $F_{1,23} = 7.16$, $P=0.01$; Mood's Median Test, $Z=-0.022$, $P=0.82$).

Figure S7. AC-VPC positioning after Pn.p-specific RNAi. (A) Box-Plots of AC-P6.p alignment in control animals and upon *lin-12* (n=18) or *apx-1* (n=18) RNAi knock-down in Pn.p cells. **(B)** Histogram of AC position relative to P(5-7).p after *lin-12* RNAi knock-down in Pn.p cells. *lin-12* RNAi treatment did not affect variance of AC-P6.p alignment (Brown-Forsythe test, $F_{1,30} = 0.04$, $P=0.85$) or median positioning of the AC relative to P6.p (Mood's Median Test, $Z=0.72$, $P=0.47$). **(C)**. Histogram of AC position relative to P(5-7).p after *apx-1* RNAi knock-down in Pn.p cells. *apx-1* RNAi treatment did not affect variance of AC-P6.p alignment (Brown-Forsythe test, $F_{1,37} = 0.84$, $P=0.37$) or median positioning of the AC relative to P6.p (Mood's Median Test, $Z=0.90$, $P=0.37$).

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