Supplemental Figures

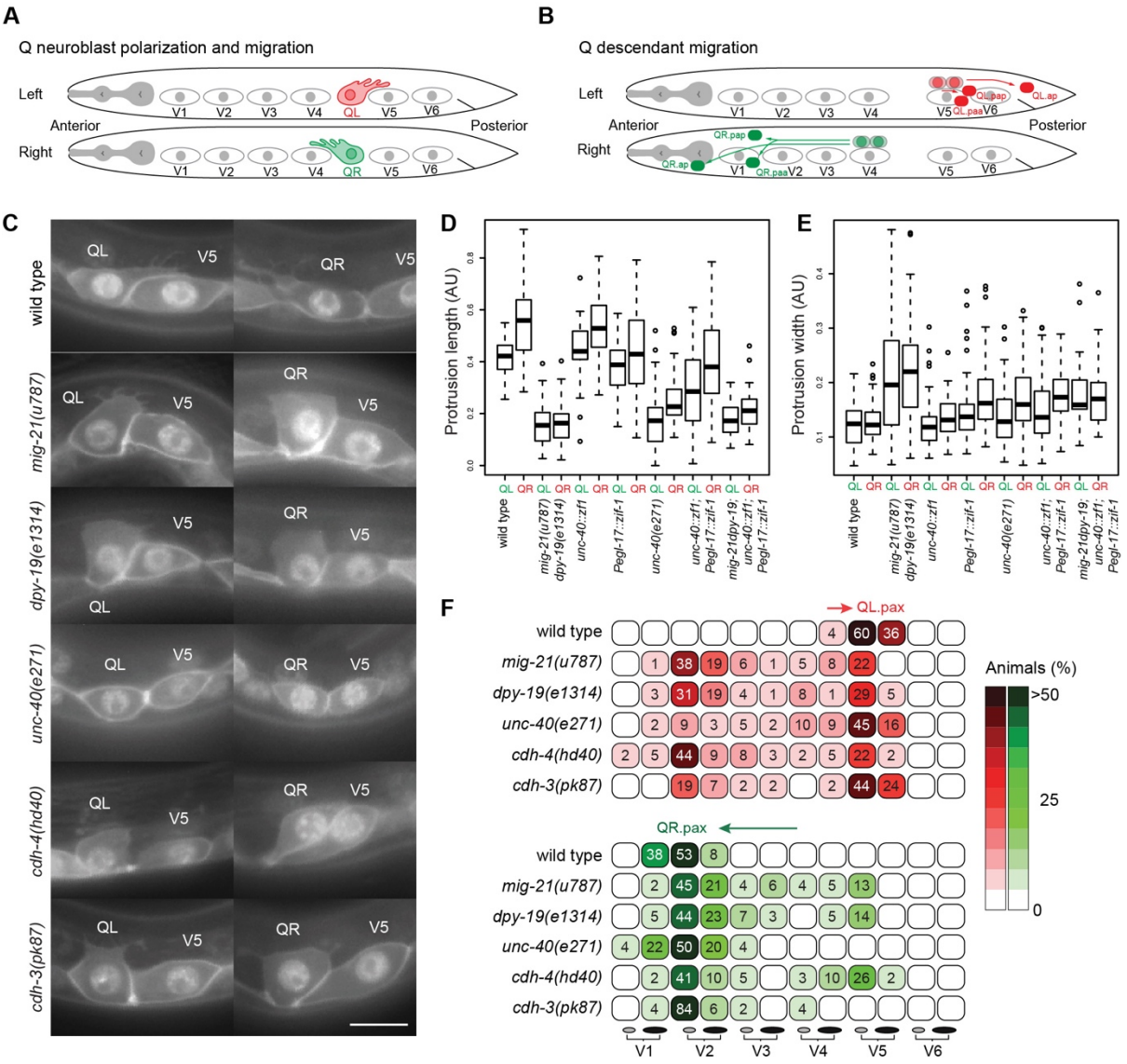
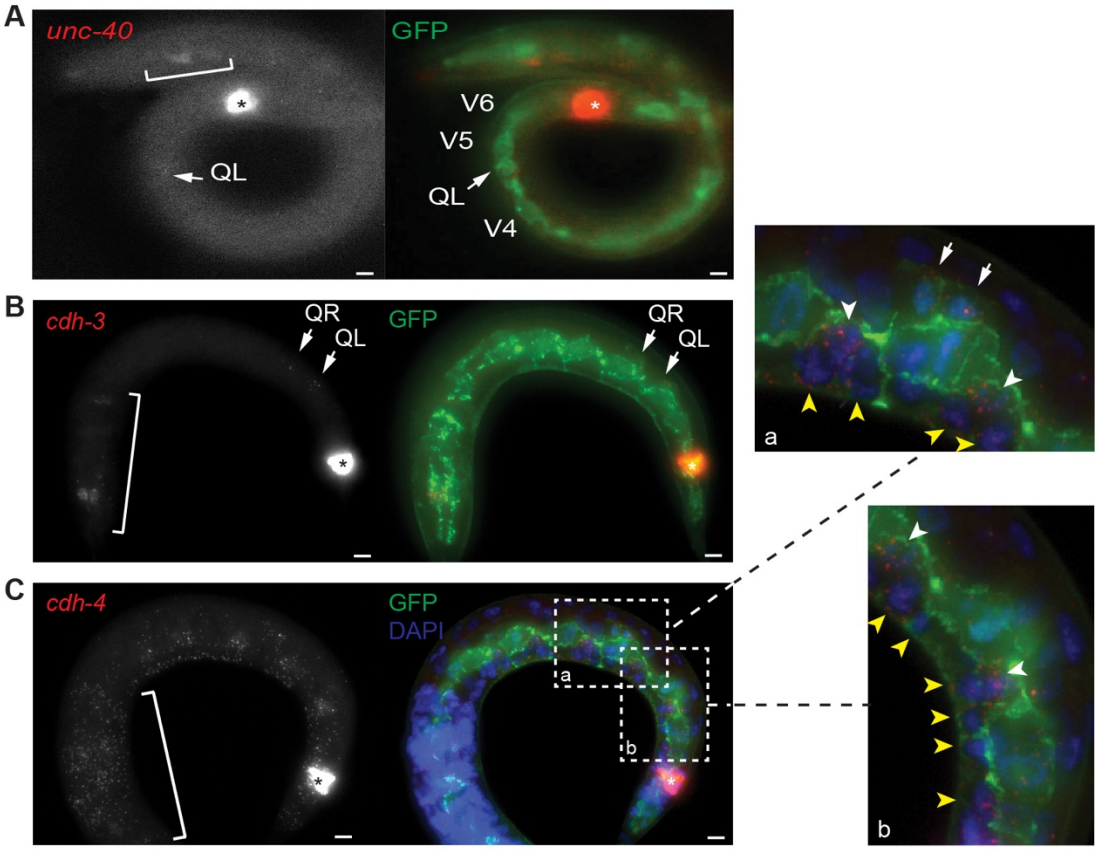


Figure S1. Polarization and migration of the Q neuroblasts and their descendants. (**A**) Schematic overview of the initial polarization and migration of the QL and QR neuroblasts. QL forms a lamellipodium-like protrusion towards the posterior and QR towards the anterior. After stable protrusion formation, the QL and QR cell bodies migrate to positions dorsal to V5 and V4 respectively. (**B**) After migration, QL and QR undergo an identical pattern of division, generating three neurons and two cells that undergo apoptosis. On the left side, the QL.p descendants remain close to the position of QL division, while the QL.a descendant QL.ap migrates to a position in the tail. On the right side, both the QR.a and QR.p descendants migrate anteriorly. (**C**) Representative images of Q neuroblast polarization in wild type and polarity mutants (1-2 hours after hatching). Anterior is left and dorsal is up. Scale bar is 10 μm. Quantification of protrusion length (**D**) and protrusion width (**E**) in wild type and polarity mutants (1-2 hours after hatching). (**F**) Final position of the Q. descendants Q.paa and Q.pap (Q.pax) relative to the seam cells. Arrows indicate direction of migration. Numbers and color coding represent percentages, n > 45. See Table S1 for statistical analysis of Q.pax position.



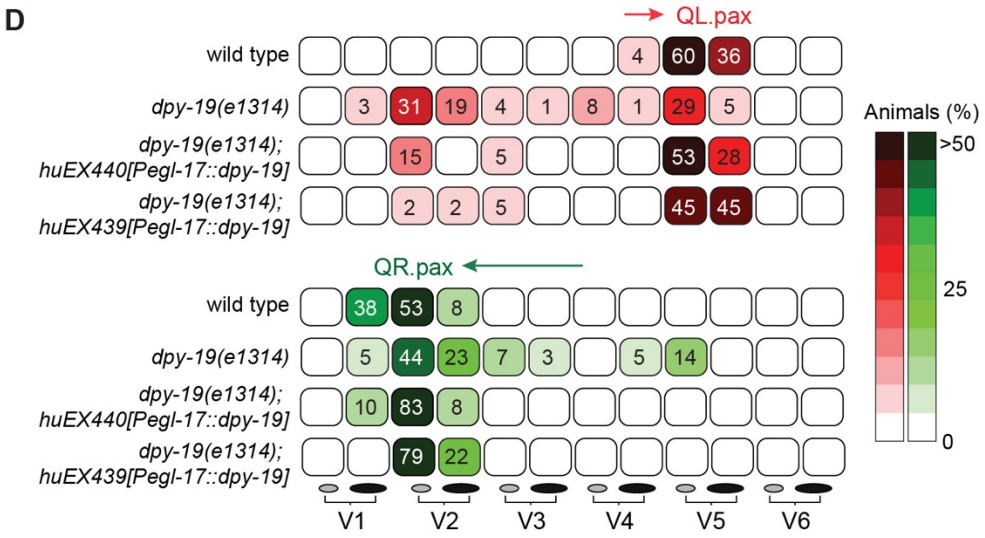


Figure S2. Expression patterns of *unc-40*, *cdh-3* and *cdh-4*. (A, B, C) smFISH at 1-2 hours after hatching. The Q neuroblasts and seam (V) cells are labeled with GFP (heIs63). Arrows indicate the Q neuroblasts, brackets expression in the head region and asterisk expression of the co-injection marker. (**C a**, **b**) Higher magnification images showing expression in the Q neuroblasts (white arrows), juvenile ventral cord neurons (yellow arrowheads) and P cells (white arrowheads). Anterior is left and dorsal is up. Scale bars are 5 μm. (**D**) Final position of the Q. descendants Q.paa and Q.pap (Q.pax) relative to the seam cells. Arrows indicate direction of migration. Numbers and color coding represent percentages, n > 40. See Table S1 for statistical analysis of Q.pax position.

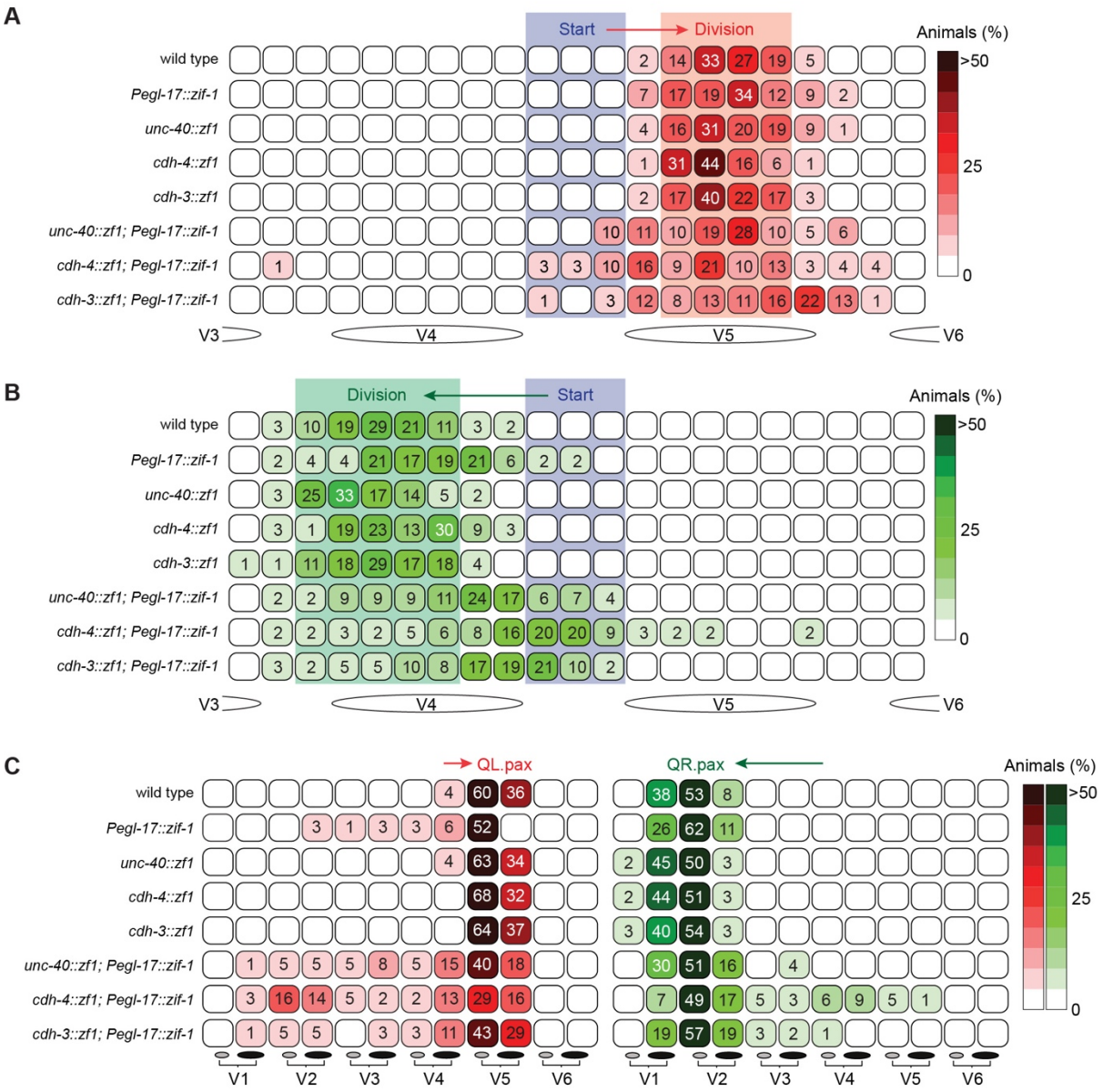


Figure S3. Q lineage specific depletion of UNC-40, CDH-4 and CDH-3. Quantification of QL (**A**) and QR (**B**) division position in animals in which *unc-40*, *cdh-4* and *cdh-3* are endogenously tagged with the *zf-1* sequence, and in which ZIF-1 is specifically expressed in the Q neuroblast lineage (*Pegl-17::zif-1*). Blue area represents the starting point of the migration and the red and green boxes the point where QL and QR divide in wild type animals. Numbers and color coding represent percentages, n > 45. (**C**) Final position of the Q. descendants Q.paa and Q.pap (Q.pax) relative to the seam cells. Arrows indicate direction of migration. Numbers and color coding represent percentages, n > 25. See Table S1 for statistical analysis of Q division position and Q.pax position.

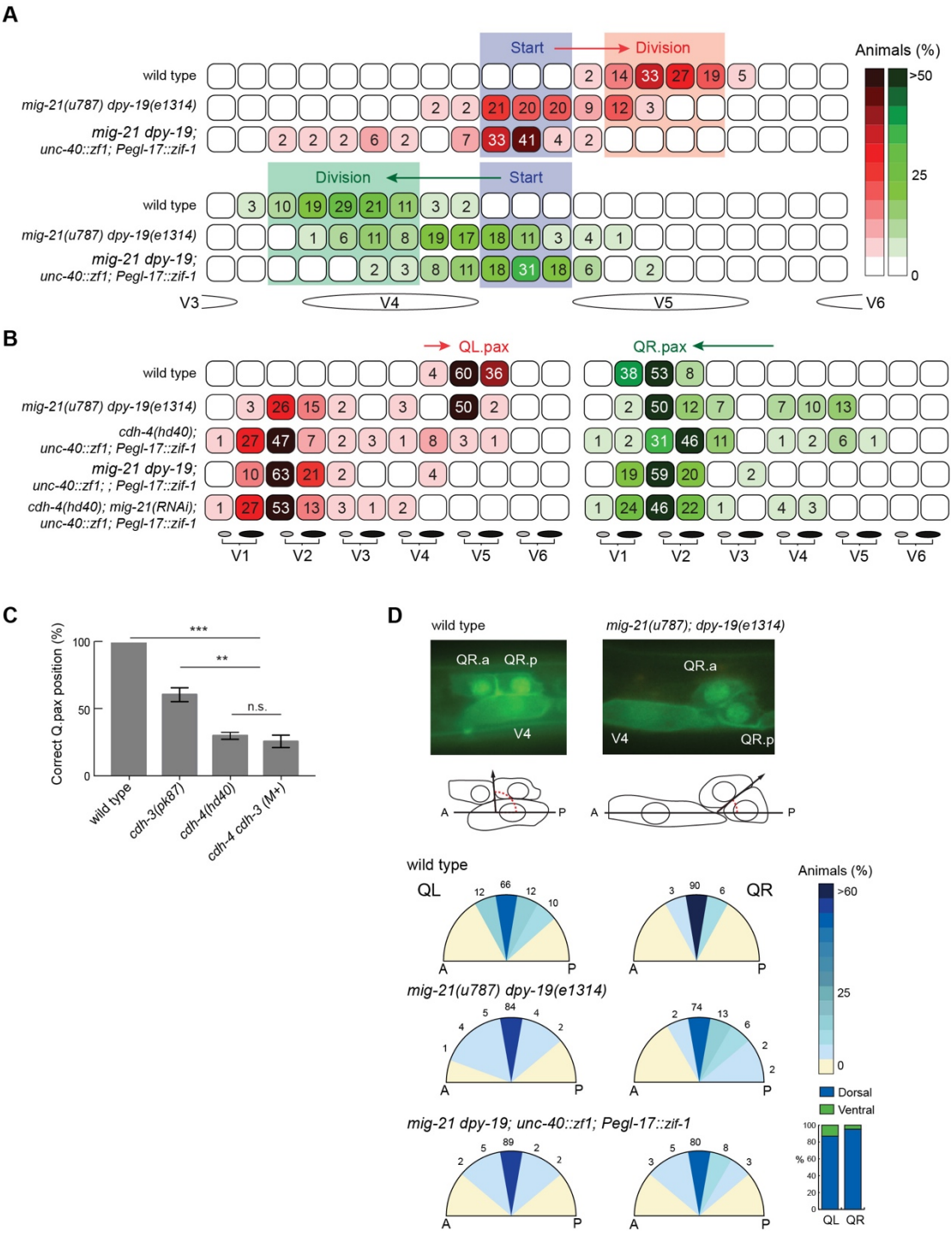


Figure S4. Polarization and migration of the Q neuroblasts and their descendants in the combined absence of polarity pathways. (**A**) Quantification of QL and QR division position in *mig-21(u787) dpy-19(e1314)* double mutants combined with Q lineage specific depletion of UNC-40 (*unc-40::zf-1; Pegl-17::zif-1*). Blue area represents the starting point of the migration and the red and green boxes the point where QL and QR divide in wild type animals. Numbers and color coding represent percentages, n > 50. (**B**) Final position of the Q. descendants Q.paa and Q.pap (Q.pax) relative to the seam cells. *mig-21* was knocked down by feeding RNAi. Arrows indicate direction of migration. Numbers and color coding represent percentages, n > 50. See Table S1 for statistical analysis of Q division position and Q.pax position. (**C**) Quantification of Q.pax position in *cdh-3(pk87)* and *cdh-4(hd40)* single and

maternally rescued (M+) double mutants. Numbers represent mean ± SD from three independent experiments (each n > 50). **p < 0.003, ***p < 0.0004, n.s., not significant (Student's t-test). (**D**) Quantification of the division plane of QL and QR. In wild type, a line through the point of contact between Q.a and Q.p is at a 90^{o} angle with the anteroposterior axis. In *mig-21(u787) dpy-19(e1314)* double mutants, the orientation of the Q division is more variable. Numbers and color coding represent percentages, n > 50. When UNC-40 is depleted (*unc-40::zf-1; Pegl-17::zif-1*) in *mig-21 dpy-19* double mutants, the Q neuroblasts occasionally divide ventral to the seam cells (bar diagram).

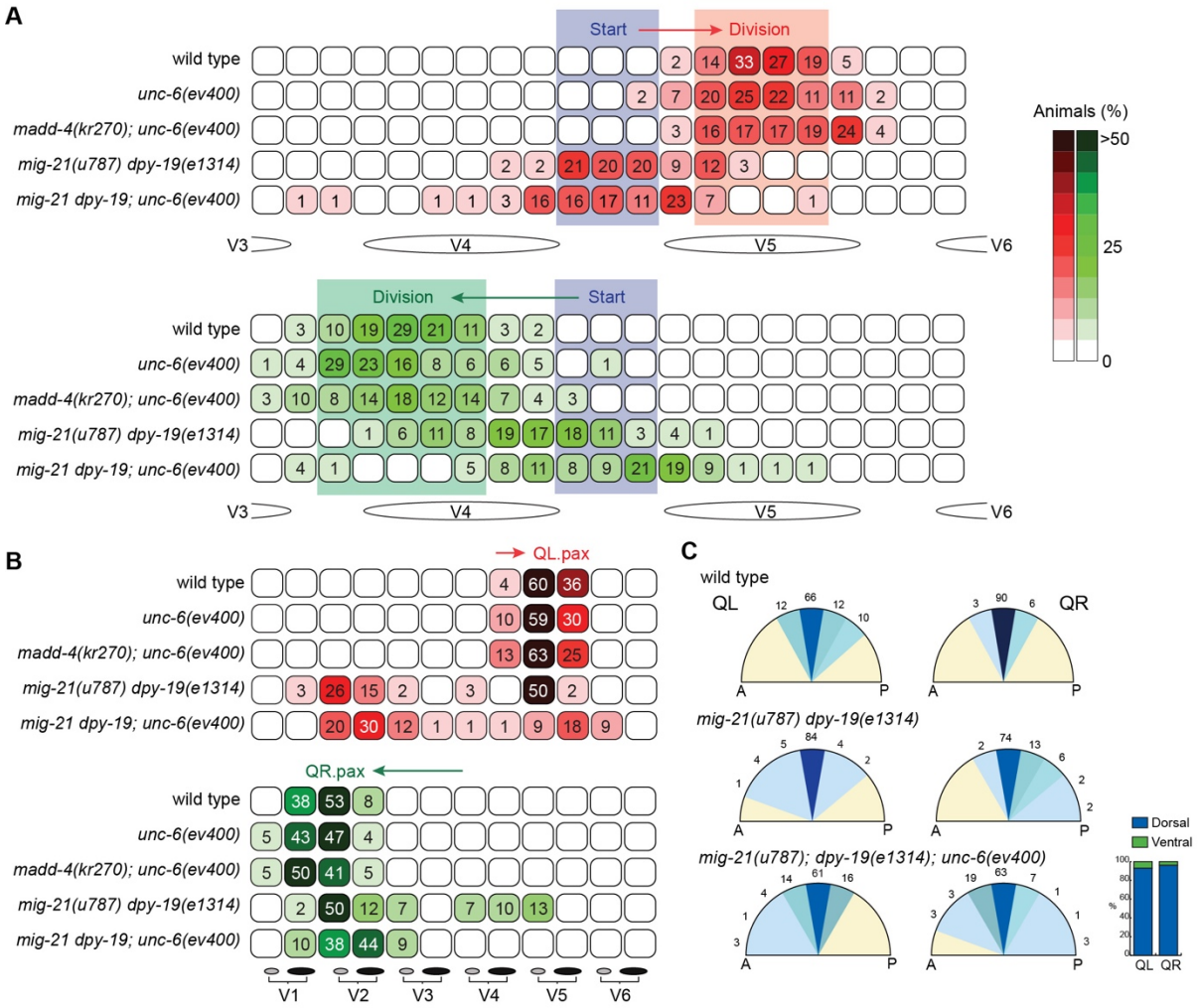


Figure S5. Polarization and migration of the Q neuroblasts and their descendants in the absence of the UNC-40 ligands UNC-6 and MADD-4. (**A**) Quantification of QL and QR division position. Blue area represents the starting point of the migration and the red and green boxes the point where QL and QR divide in wild type animals. Numbers and color coding represent percentages, n > 33. (**B**) Final position of the Q. descendants Q.paa and Q.pap (Q.pax) relative to the seam cells. Arrows indicate direction of migration. Numbers and color coding represent percentages, n > 47. See Table S1 for statistical analysis of Q division position and Q.pax position. (**C**) Quantification of the division plane of QL and QR. In wild type, a line through the point of contact between Q.a and Q.p is at a 90° angle with the anteroposterior axis. In *mig-21(u787) dpy-19(e1314)* double mutants and *mig-21 dpy-19*; *unc-6(ev400)* triple mutants, the orientation of the Q division is more variable. Numbers and color coding represent percentages, n > 50. In *mig-21 dpy-19*; *unc-6* triple mutants, the Q neuroblasts occasionally divide ventral to the seam cells (bar diagram).

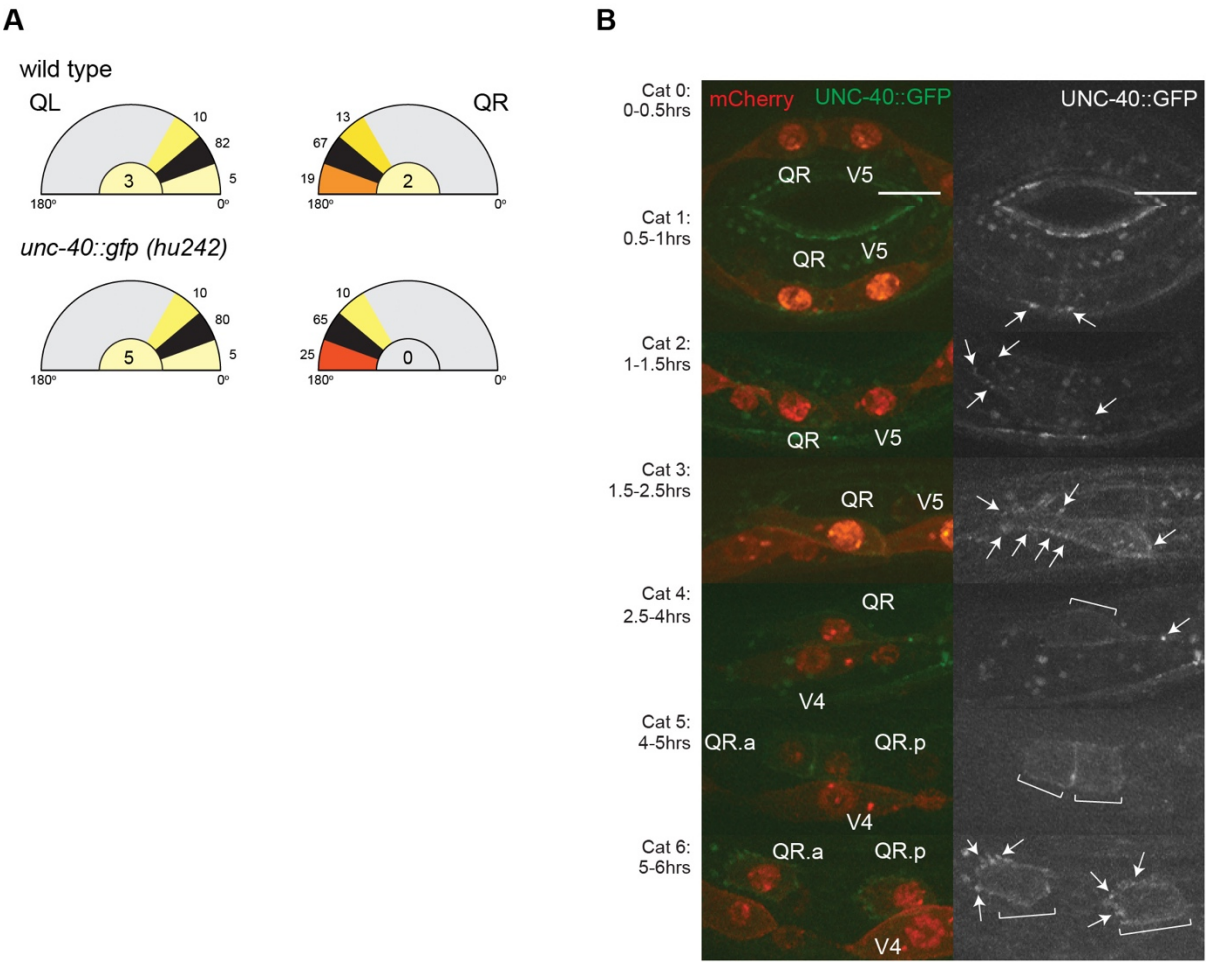


Figure S6. Endogenous UNC-40::GFP expression. (**A**) Quantification of protrusion formation and protrusion direction in wild type and *unc-40::gfp(hu242)* at 1-2 hours after hatching. Numbers and color coding represent percentages, n > 20. The percentage of cells that fail to form a major protrusion is indicated in the center of the polar graphs. See Table S1 for statistical analysis of protrusion formation and direction. (**B**) Representative images of UNC-40::GFP localization during polarization, migration and division (indicated by category and time after hatching). The plasma membrane and nucleus of the Q neuroblasts and seam (V) cells are labeled with mCherry (*huIs166*). UNC-40::GFP punctae are indicated by arrows. Brackets indicate diffuse UNC-40::GFP localization at the plasma membrane. Anterior is left and dorsal up. Scale bar is 10 μm.

Supplemental Tables

Table S1. Statistical analysis of Q polarization.
Click here to Download Table S1

Table S2. Statistical analysis of Q position of division
Click here to Download Table S2

Table S3. Statistical analysis of Q.pax position
Click here to Download Table S3

Table S4. Transgenic mosaic analysis to determine the site of action of *dpy-19*

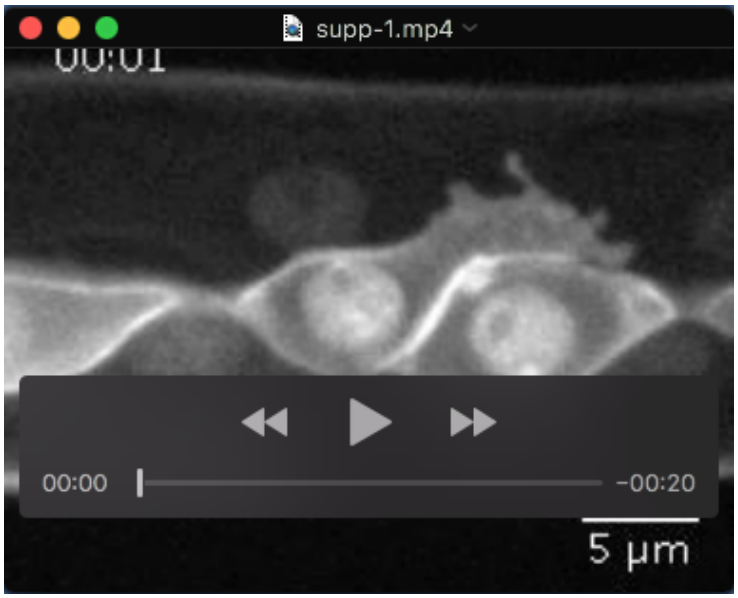
Genotype	**Body shape**	**Percentage Q.pax migration defect***
dpy-19(e1314); huEx439[Pegl-17::dpy-19]		
Transgenic	Dpy	35.3 ± 5.0**
Transgenic	WT	21.3 ± 7.0
Non-transgenic siblings	Dpy	59.0 ± 5.6
dpy-19(e1314); huEx440[Pegl-17::dpy-19]		
Transgenic	Dpy	20.3 ± 4.0**
Transgenic	WT	7.90 ± 0.1
Non-transgenic siblings	Dpy	66.9 ± 8.2

Expression of wild type *dpy-19* in the Q neuroblast lineage of *dpy-19(e1314)* mutants rescues Q.pax migration (Fig. S2). However, in some of the transgenic animals, the Dpy phenotype is rescued as well. This indicates that despite the specificity of the *egl-17* promoter for the Q cell lineage, these transgenes may display a low level of *dpy-19* expression in the hypodermis. To circumvent this problem, we made use of the fact that extra-chromosomal transgenes are frequently lost during somatic cell divisions, leading to mosaic expression of the transgene. Using this approach, we found that transgenic mosaics that remain Dpy still show significant rescue of Q.pax migration, supporting our conclusion that *dpy-19* functions cell autonomously in the Q cell lineage. * mean ± SD. **t-test siblings versus Dpy: p=0.0056 and 0.0034 for *huEx439* and *huEx440*, respectively.

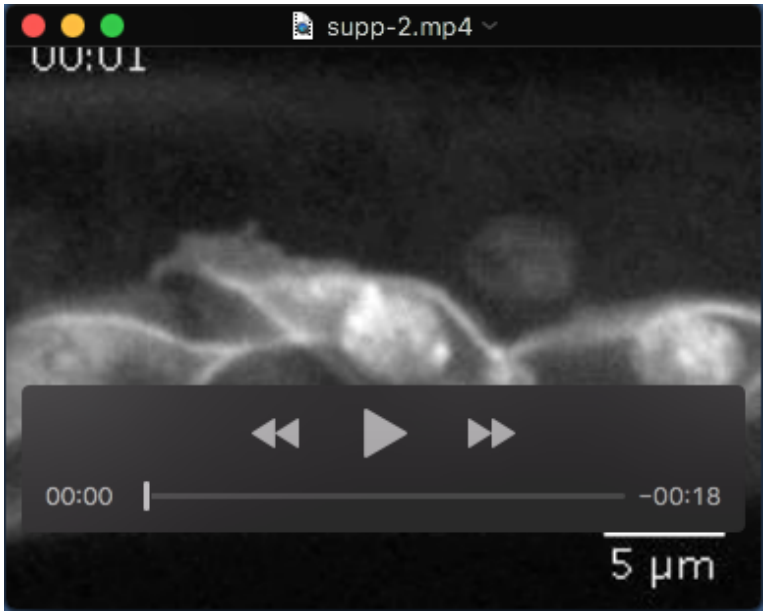
Table S5. Primers and oligonucleotides

	Sequence
***dpy-19* expression construct**	
dpy-19 FW	GGGGACAAGTTTGTACAAAAAAGCAGGCTATGGCAAAGAAACCCAAGAATTC
dpy-19 RV	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGATTTTCAAAACAATATAATTCCT
Guide RNAs zf1 tagging	
unc-40	CCTCCTATTGCGAGATGTCTTGATGGATAAGTAATAATAATCGTTTAAGAGCTATGCTGG
cdh-4	CCTCCTATTGCGAGATGTCTTGGTGAATTAAAGAAATACTGAGTTTAAGAGCTATGCTGG
cdh-3	CCTCCTATTGCGAGATGTCTTGTAGAGACGGGCACATTAATAGTTTAAGAGCTATGCTGG
ssODN zf1 repair templates	
unc-40	ACAACAATTACAGCACAATTTGCATTTTGAGACGAGTATGGATAAGACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGATAATAATAATCTAGTATTTTTTTGATAATTTGAATTATTAATTTTG
cdh-4	TTTGATGATATAGACGAAGAAGTCAACATTCACATCTCTACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGATAATAACATTCAGTATTTCTTTAATTCACAATTTCTCAA
cdh-3	CTGCACCATCTTACCGTAGAGACGGGCACATTAATATAGCTTACTTAACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGATAGAGATATTTTATTTTCATCATTTTCGATTCATTTTTACGGGTT
SEC cassette cloning	
unc-40 sgRNA	GTAATACGACTCACTATAGGATGGATAAGTAATAATAATCGTTTTAGAGCTAGAAATAGC
dpy-19 sgRNA	GTAATACGACTCACTATAGGAAATAGTTTATAATGCTAATGTTTTAGAGCTAGAAATAGC
reverse sgRNA primer	AAAAGCACCGACTCGGTGC
HA 1 *dpy-19* FW	ACGTTGTAAAACGACGGCCAGTCGCCGGCAACAGTTGAATGTCAAGGGAGAA
HA 1 *dpy-19* RV	CATCGATGCTCCTGAGGCTCCCGATGCTCCGATTTTCAAAACAATATAATTTCTATTAGC
HA 2 *dpy-19* FW	CGTGATTACAAGGATGACGATGACAAGAGATGATTTTTTTCTATTTTGTTTTTAAATTTA
HA 1 *dpy-19* RV	GGAAACAGCTATGACCATGTTATCGATTTCTTTAGGCGGAAACCTCAGAC
HA 1 *unc-40* FW	ACGTTGTAAAACGACGGCCAGTCGCCGGCACGGTGGACGGCAAGTTCCAGTTGGAAGAGC
HA 1 *unc-40* RV	CATCGATGCTCCTGAGGCTCCCGATGCTCCCTTATCCATACTCGTCTCAAAATGCAAATT
HA 2 *unc-40* FW	CGTGATTACAAGGATGACGATGACAAGAGATAATAATAATCTGGTATTTTTTTGATAATT
HA 1 *unc-40* RV	GGAAACAGCTATGACCATGTTATCGATTTCGGATGTTAATCTGACAACTCTCATCTCC

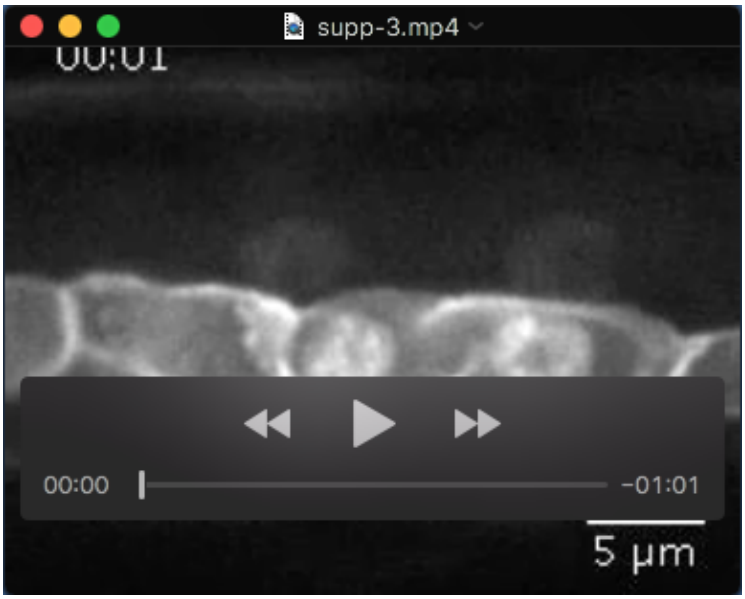
Supplemental Movies



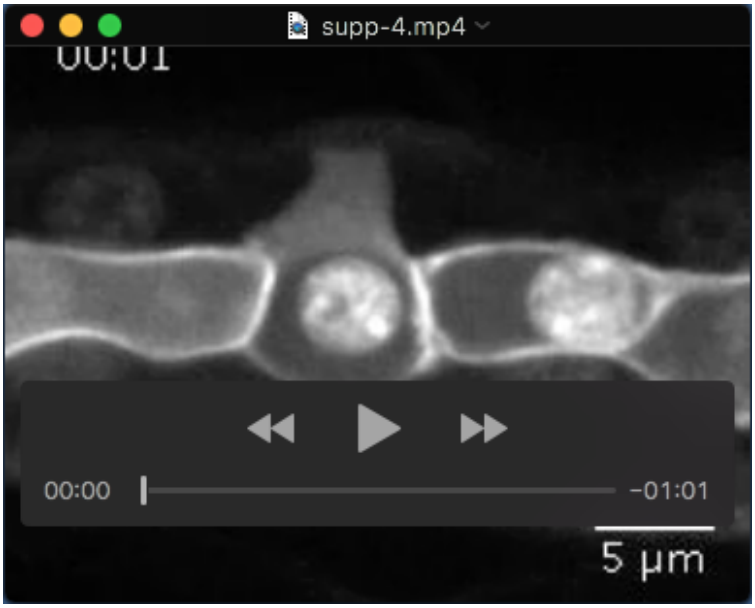
Movie 1. Live time-lapse confocal imaging of wild type animals expressing plasma membrane and nuclear localized GFP in QL and the seam cells (transgene *heIs63*). Anterior is left. Images were acquired every minute for a total duration of 1.5 hours. Movies are played at 5 frames per second.



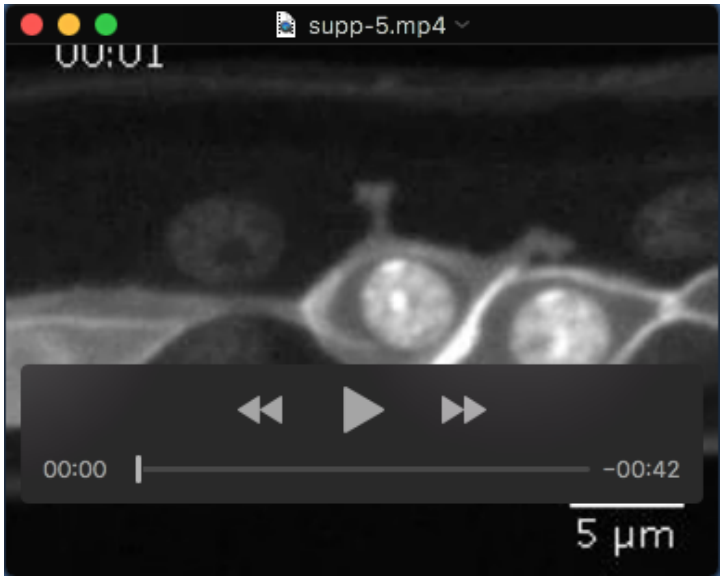
Movie 2. Live time-lapse confocal imaging of wild type animals expressing plasma membrane and nuclear localized GFP in QR and the seam cells (transgene *heIs63*). Anterior is left. Images were acquired every minute for a total duration of 1.5 hours. Movies are played at 5 frames per second.



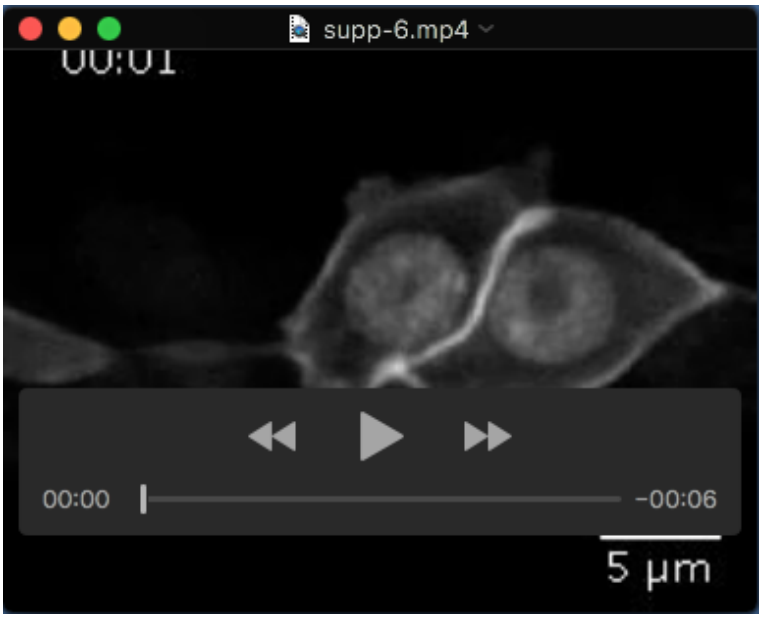
Movie 3. Live time-lapse confocal imaging of *cdh-4(hd40)* animals expressing plasma membrane and nuclear localized GFP in QL and the seam cells (transgene *heIs63*). A nterior is left. Images were acquired every minute for a total duration of 5 hours. Movies are played at 5 frames per second.



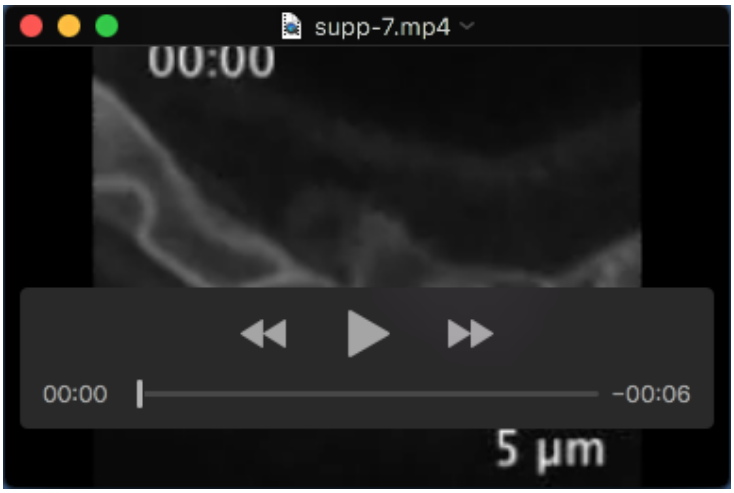
Movie 4. Live time-lapse confocal imaging of *cdh-4(hd40)* animals expressing plasma membrane and nuclear localized GFP in QR and the seam cells (transgene *heIs63*). Anterior is left. Images were acquired every minute for a total duration of 5 hours. Movies are played at 5 frames per second.



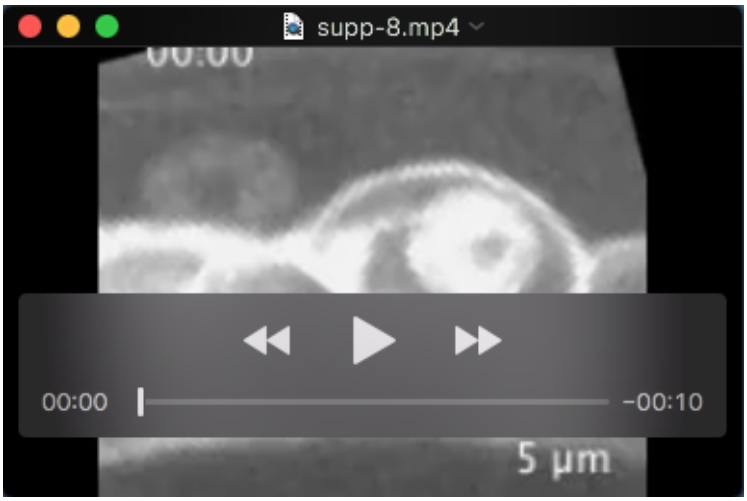
Movie 5. Live time-lapse confocal imaging of *cdh-3(pk87)* animals expressing plasma membrane and nuclear localized GFP in QL and the seam cells (transgene *heIs63*). Anterior is left. Images were acquired every minute for a total duration of 3.5 hours. Movies are played at 5 frame s per second.



Movie 6. Live time-lapse confocal imaging of *unc-40(e271)* animals expressing plasma membrane and nuclear localized GFP in QL and the seam cells (transgene *heIs63*). Anterior is left. Ima ges were acquired every minute for a total duration of 1 hour. Movies are played at 5 frames per second.



Movie 7. Live time-lapse confocal imaging of *unc-40(hu226[unc-40::zf1]); mig-21(u787) dpy-19(e1314); huIs181[Pegl-17::zif-1-SL2-mcherry; Pmyo-2::mcherry]* animals expressing plasma membrane and nuclear localized GFP in QL and the seam cells (transgene *heIs63*). Anterior is left. Images were acquired every 10 minutes for a total duration of 3 hours. Movies are played at 3 frames per second.



Movie 8. Live time-lapse confocal imaging of *unc-40(hu226[unc-40::zf1]); mig-21(u787) dpy-19(e1314); huIs181[Pegl-17::zif-1-SL2-mcherry; Pmyo-2::mcherry]* animals expressing plasma membrane and nuclear localized GFP in QR and the seam cells (transgene *heIs63*). Anterior is left. Images were acquired every 10 minutes for a total duration over 4.5 hours. Movies are played at 3 frames per second.