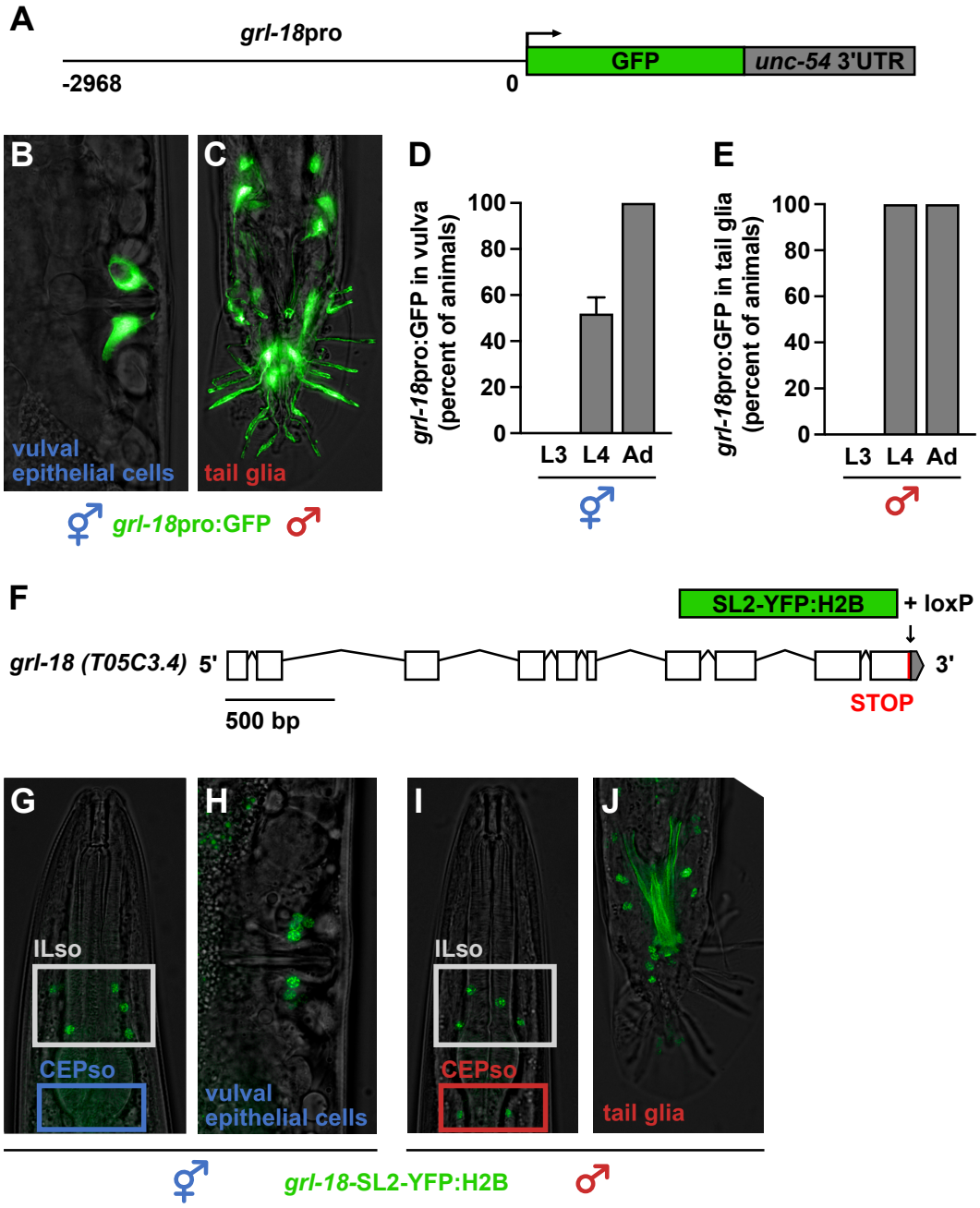

Figure S1. Transgenic and endogenous *grl-18* reporters show sex-specific expression. Related to Figure 1.

(**A**) Schematic of transcriptional reporter transgene *grl-18*pro:GFP, containing 2968 bp upstream of the translational start site. (**B-C**) Expression of *grl-18*pro:GFP in 1-day adult (B) hermaphrodite vulval epithelial cells and (C) male tail glia, including ray structural and hook

socket glia. (**D-E**) Fraction of hermaphrodites (n=48) or males (n=50) at the indicated ages showing *grl-18*pro:GFP expression in (D) vulval cells and (E) tail glia. The same individuals were followed and scored as in Figure 1E and 1F. Error bars, SEM. (**F**) Schematic of *grl-18* endogenous transcriptional reporter. White boxes, exons; lines, introns; gray, 3' untranslated region (UTR). An insertion encoding the splice leader 2 (SL2) sequence followed by a YFP-tagged histone H2B (YFP:H2B) was inserted immediately after the stop codon. A loxP site and short linker sequence remain after removal of a selection cassette. (**G-J**) Expression of *grl-18* endogenous reporter in 1-day adult hermaphrodite (G) ILso and (H) vulva nuclei and in 1-day adult male (I) ILso, CEPso, and (J) tail glia nuclei. Due to relatively dim expression, autofluorescence is visible in spicules of the male tail.

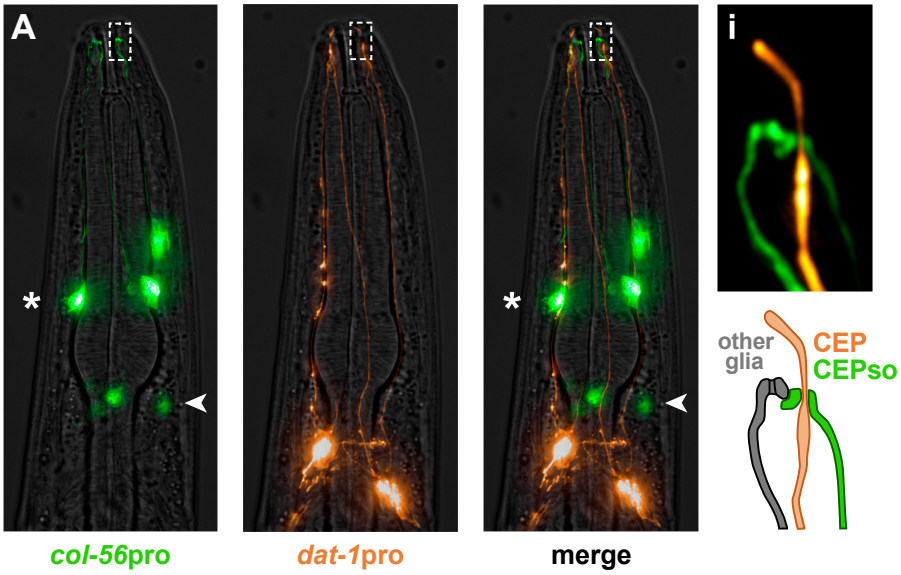

Figure S2. Identification of a novel marker for CEPso glia. Related to Figure 2.

(**A**) *col-56* was identified as a novel marker for CEPso glia (arrowhead), which were identified by their cell body position posterior to the first pharyngeal bulb and by their endings that form a channel wrapping the CEP cilium (inset, **i**). *col-56* is also expressed in OLso glia (asterisk). Green, *col-56*pro:GFP in CEPso and OLso glia; orange, *dat-1*pro:mApple in CEP neurons.

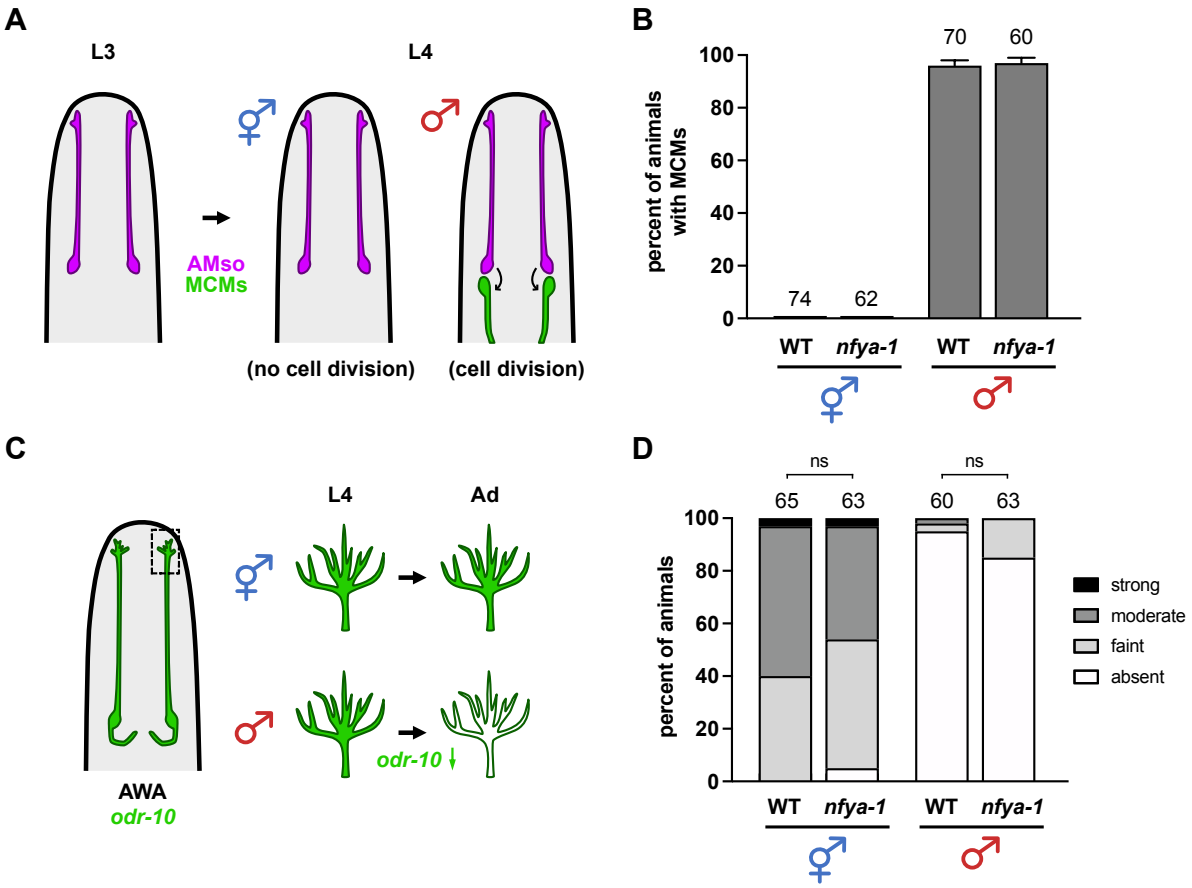

Figure S3. *nfya-1* does not affect other male-specific phenotypes in glia and neurons. Related to Figure 3.

(**A**) Schematic showing amphid socket glia (AMso, magenta) divide to generate MCM interneurons (green) at the L4 stage only in males[S1]. (**B**) Fraction of 1-day adult wild-type or *nfya-1(ok1174)* mutant animals of each sex with MCM interneurons, as visualized by *pdf-1*pro:RFP. Sample sizes are indicated above the bars. Error bars, SEM. (**C**) Schematic showing that expression of the ODR-10 chemoreceptor (ODR-10:GFP) in AWA neuronal cilia is reduced or absent in adult males compared to adult hermaphrodites[S2]. (**D**) Fraction of 1-day adult wild-type or *nfya-1(ok1174)* mutant animals of each sex with ODR-10:GFP expression at the indicated levels. Expression levels were binned as 0, absent; 1, faint; 2, moderate; and 3, strong,

and the Mann-Whitney test was used as previously described[S2-S4]; ns = no statistically significant difference ($p > 0.05$).

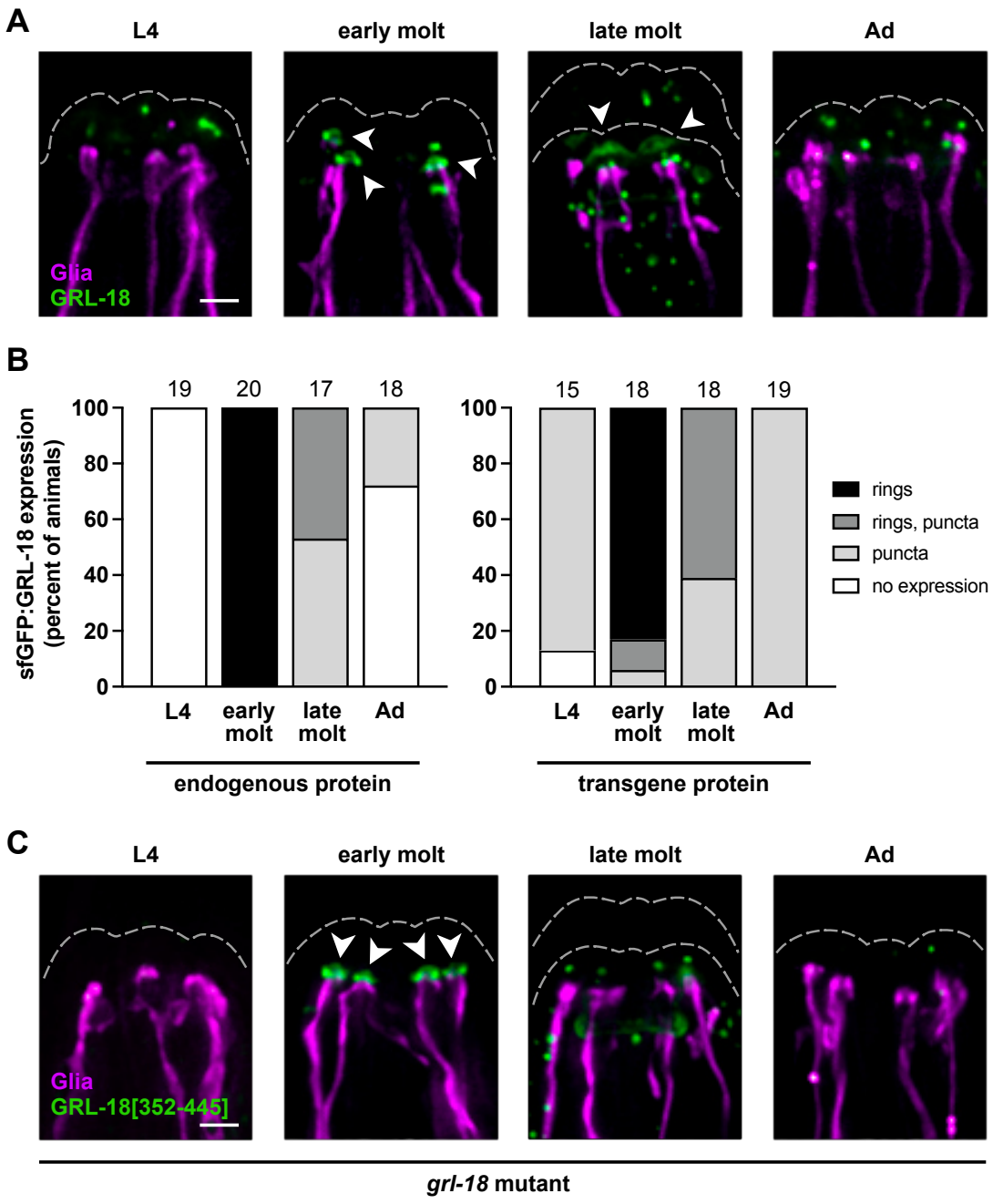

Figure S4. sfGFP-tagged GRL-18 localizes to transient rings near glial endings. Related to Figure 4.

(**A**) Consistent with results obtained using an endogenous insertion, sfGFP-GRL-18 expressed from a low-copy transgene localizes to rings at the nose tip in males during the L4 to adult (Ad)

cuticle molt; sfGFP tag is inserted at the N-terminus of full-length GRL-18 protein (see Figure 4F). (**B**) The fraction of males at the indicated stages that show no expression or localization to rings, diffuse puncta, or both is similar between strains with an endogenous insertion (left) or low-copy transgene (right). Sample sizes are indicated above the bars. (**C**) Localization of sfGFP-Grl domain [352-445] transgene at nose tip of *grl-18(hmn341)* mutant males through the L4 to Ad transition. *hmn341* deletes the sequence encoding the Grl domain (see Figure S5) and therefore lack full-length GRL-18. Dashed lines, outline of cuticle. Arrowheads, sfGFP-GRL-18 rings. All scale bars, 2 μm.

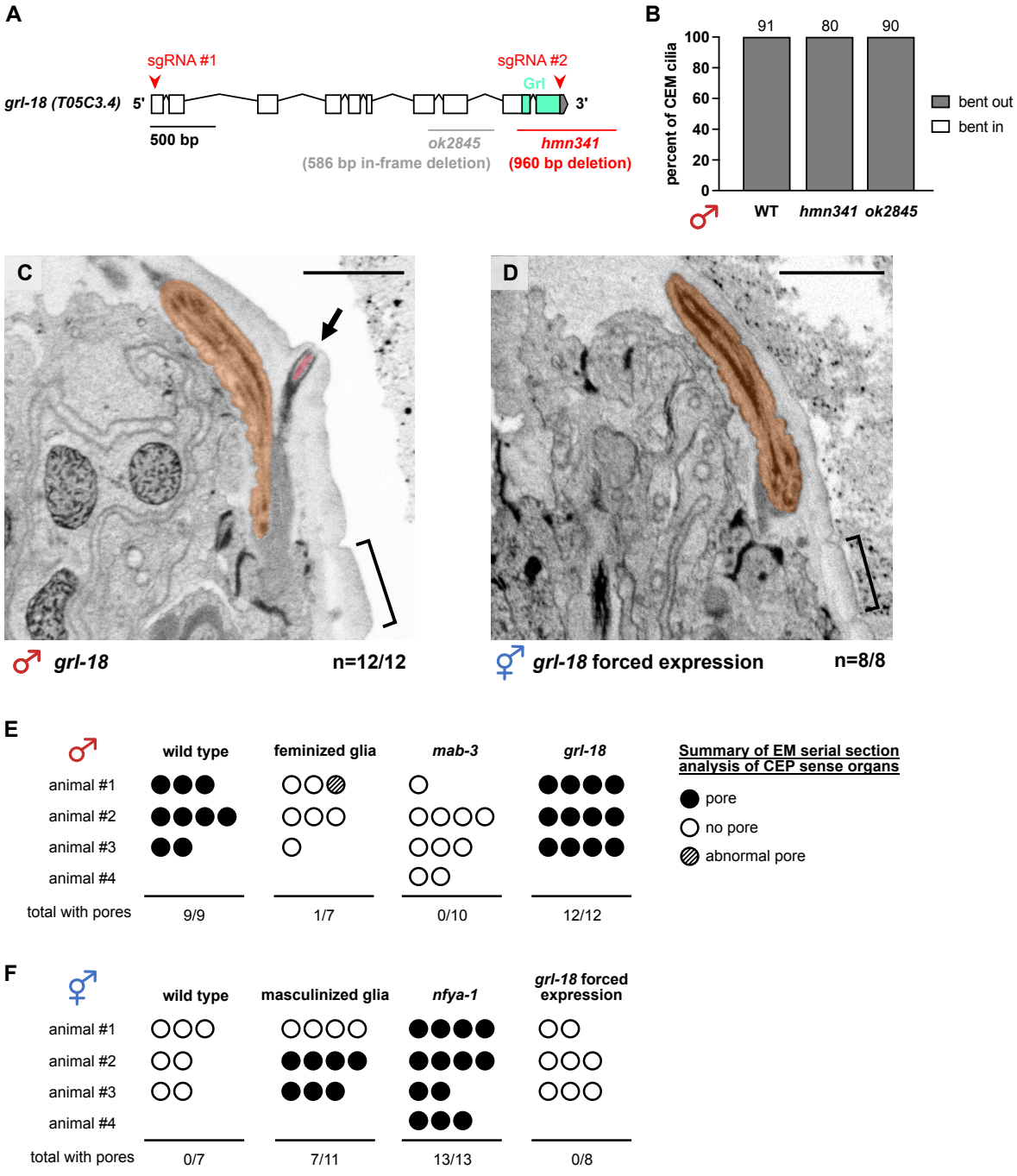

Figure S5. Male-specific gene expression in CEPso glia causes cuticle pore formation. Related to Figure 5.

(**A**) Schematic of the *grl-18* gene showing existing allele *ok2845* and a novel allele *hmn341*. *ok2845* is an in-frame deletion that is predicted to spare the Grl domain. *hmn341* deletes the

sequence encoding the Grl domain. White boxes, exons; lines, introns; gray, 3' UTR. Red arrowheads indicate sites targeted in the dual-sgRNA CRISPR/Cas9 approach used to generate the *hmn341* deletion. (**B**) Quantification of CEM cilia trajectories in *grl-18* mutants. Sample sizes are indicated above the bars. (**C-D**) Electron micrographs of longitudinal sections through single CEP sense organs in adult (C) *grl-18(hmn341)* mutant male and (D) hermaphrodite with forced expression of *grl-18* in socket glia. While cuticle pores are present in *grl-18(hmn341),* subtle changes are sometimes observed including a more gradual boundary between the pore and the surrounding cuticle and more anterior positioning of the pore relative to the first annulus (compare C with Figure 5E, 5I, and 5J). First annulus, bracket; arrow, cuticle pore. All scale bars, 1 μm. The fraction of CEP sense organs exhibiting the represented phenotype out of the total number scored is shown. (**E, F**) Summary of observations made for each CEP sense organ scored in EM serial sections of adult (E) males and (F) hermaphrodites (n=3-4 animals per genotype, numbered #1-#4). Each animal has four CEP sense organs, but due to missing or damaged sections, not all four were scored in every animal. Filled circle, cuticle pore; open circle, no pore; striped circle, abnormal pore. In the sense organ with an abnormal pore, the CEM ending appears to break through the cuticle adjacent to the CEP nubbin, reminiscent of a phenotype previously described for CEP in *cat-6* mutants[S5].

	Genotype	Age	*grl-18*$^{+}$ CEPso glia (percent of animals)	n
♂	I. DM Domain Transcription Factors			
	wild type males	1d	100	67
	mab-23(gk664)	1d	98[a]	58
	dmd-3(ok1327)	1d	100	68
	dmd-5(ok1394)	1d	98[a]	42
	dmd-6(gk287)	1d	100	61
	dmd-7(ok2276)	1d	100	69
	dmd-8(ok1294)	1d	100	41
	dmd-9(ok1438)	1d	100	68
	dmd-10(gk1125)	1d	97[a]	71
	[a] phenotype is partially penetrant; at least one CEPso fails to express marker			

Table S1. Other DM domain transcription factors are not required for sex-specific CEPso glial gene expression. Related to Table 1.

Percent of 1-day adult (1d) males expressing the transcriptional reporter *grl-18*pro:mApple in CEPso glia for mutants in DM domain transcription factors other than *mab-3* (Table 1), except the lethal mutant *dmd-4*. Expression in ILso glia and male tail glia were unaffected.

Oligonucleotide Name	**Sequence**	**Notes**
col-56pro_FW	TGCATGCCTGCAGGGAGGAGCATGGCGAACAAGATGTTG	Forward primer for *col-56* promoter with overhangs and SbfI site
col-56pro_RV	TGCATGGGCGCGCCGATTTTATAGAATTTGGAGAATTGTCAAAAC	Reverse primer for *col-56* promoter with overhangs and AscI site
fem-3_cloning_F	ATCAGGCGCGCCATGGAGGTGGATCCGGGTTCAGATG	Forward primer to amplify *fem-3* cDNA from *Prab-3:fem-3:mCherry:unc-54 3'UTR*, a gift from Douglas Portman, with overhangs and AscI site
fem-3_cloning_R	TCAAGCGGCCGCTCATCGTTTCCTGGAGCAATCAGTAGC	Reverse primer to amplify *fem-3* cDNA from *Prab-3:fem-3:mCherry:unc-54 3'UTR*, a gift from Douglas Portman, with overhangs and NotI site
tra-2_FW	GCATGGGCGCGCCATGGAATTCTCAATCAAACGATC	Forward primer to amplify *tra-2* intracellular fragment from *Prab-3:tra-2:mCherry:unc-54 3'UTR*, a gift from Douglas Portman, with overhangs and AscI site
tra-2_RV	AGCGAGCGGCCGCTTAAACCTCTGGGTCTGATAGGTCGC	Reverse primer to amplify *tra-2* intracellular fragment from *Prab-3:tra-2:mCherry:unc-54 3'UTR*, a gift from Douglas Portman, with overhangs and NotI site
pkd-2pro_FW	TGCATGCCTGCAGGCGCTCACCTGTATACTGTAGATC	Forward primer for *pkd-2* promoter with overhangs and SbfI site
pkd-2pro_RV	ACCGGTGGCGCGCCTGAAGACGGCTCGCTGAAACAG	Reverse primer for *pkd-2* promoter with overhangs and AscI site
grl-18amp_FW2	TCGACTGGCGCGCCATGGCAAAATACTTATTTTTCTACTTATCACTTGTTTTATACTTTCATGAAACGACAGCTATATTTTTCCCTCAACTTGGATTCGGATCCATGAACTGCCAATGCC	Forward primer for *grl-18* gDNA with overhangs and SbfI site; removes first intron and introduces a T87A silent mutation to generate a BamHI site for sfGFP insertion at N-terminus
grl-18amp_RV	TAGCGAGCGGCCGCTTCACCCGGGAGGTTGAAAGAAAACGAAGCATGTCAACGCC	Reverse primer for *grl-18* gDNA with overhangs and NotI site

grl-18(31-351)-amp_RV	TAGCGAGCGGCCGCTCAATCTCGGTCAAATGGGAACTTTGGAG	Reverse primer for *grl-18* gDNA(31-351) fragment lacking the Grl domain with overhangs and NotI site
grl-18(352-445)-fPCR_FW	TACAAAGGATCCGAAAACGTAAACAAATGGACGAGTAAACGG	Forward primer for *grl-18* gDNA(352-445) fragment containing the Grl domain only with overhangs and BamHI site
nfya-1-amp_FW	AAAACTGGCGCGCCATGAATGGAGCGTCGAGGGGCG	Forward primer for *nfya-1* cDNA with overhangs and AscI site
nfya-1-amp_RV	TAGCGAGCGGCCGCTTAGAGATTCGTGAAACTTTGTCCATC	Reverse primer for *nfya-1* cDNA with overhangs and NotI site
nfya-1pro_FW	TGCATGCCTGCAGGCAGTATAGATACAATTCGGAAAATCAAC	Forward primer for *nfya-1* promoter with overhangs and SbfI site
nfya-1pro_RV	ATTCATGGCGCGCCTTTGTCTTGTTTTTTGATGATTGGATTTACCTG	Reverse primer for *nfya-1* promoter with overhangs and AscI site
grl-18-sgRNA #1	AGTTTACCGAATCCAAGTTG	CRISPR guide target for 5' end of *grl-18* to generate null allele
grl-18-sgRNA #2	GAGTATCAAAGTTTGAATCA	CRISPR guide target for 3' end of *grl-18* to insert SL2-YFP:H2B sequence and to generate null allele
ss-oligo_ unc-58(e665)	ATTTTGTGGTATAAAATAGCCGAGTTAGGAAACAAATTTTTCTTTCAGGTTTCTCAGTAGTGACCA TGTGCGTGGATCTTGCGTCCACACATCTCAAGGCGTACTT	*unc-58(e665)* repair oligo to introduce the gain-of-function allele; AF-JA-76 from Arribere et al., 2014[S6]
mam-5pro_FW	GCATGCCTGCAGGGCACACAAGGGTTTCAGATAATATC	Forward primer for *mam-5* promoter with overhangs and SbfI site
mam-5pro2_RV	GAGCATGGCGCGCCACCCAAGTAGCTGGTATGAGAG	Reverse primer for *mam-5* promoter with overhangs and AscI site

Table S2. Oligonucleotide information. Related to STAR Methods.

Allele	Sequence	Notes
hmn289	GATGGGAAGCGTGTC[C>T]GTGACCCACACTGTG	R90C mutation
hmn304	CGGGAACGTGCGTCT[G>A]GTTTGCTGTGCCGTA	W831STOP mutation
hmn305	AAACGTGCGGAATTT[G>A]TATGGAGAACATTTT	C216Y mutation
hmn316	CCGCCAAACTGCAAG[g>a]ttagggagttttaca	splice site mutation
hmn317	CAAGCTCCGGCAAAG[C>T]GACCAATTCCGCCTA	R21STOP mutation
hmn318	AACAAGCTGCCGAG[T>G]GTCGAATTTGCCGGA	C144G mutation
hmn319	CCAGCTCCGGTTTCT[C>T]AATCACAACCACAGA	Q146STOP mutation
hmn340	TTCTTTCAACCTTGA[->atggctgtctcatcctactttcacctagttaactgcttgtcttaaaatctatg cttctctttagtatctaaaattttcctagaagcttacaagtatataaatggtctcttctcaataaaggttgtatatttattca tcttattgaatctgccatttcctcgtttttgcgagtttatatacttccaatttttctttctattgtattttcaacttctaatttaa ttcagggaaactgctaccggtATGAGTAAAGGAGAAGAACTTTTCACTGGAGTT GTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATT TTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTT ACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGgta agtttaaacatatatatactaactaaccctgattatttaaattttcagCCAACACTTGTCACTACTTT CGGTTATGGTCTTCAATGCTTCGCGAGATACCCAGATCATATGAAA CGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGG AAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTaag tttaaacagttcggtactaactaaccatacatatttaaattttcaggtGCTGAAGTCAAGTTTGAAG GTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAA AGAAGATGGAAACATTCTCGGACACAAATTGGAATACAACTATAAC TCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATC AAAGTTgtaagtttaaacatgattttactaactaactaatctgatttaaattttcagAACTTCAAAATT AGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATC AACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAA CCATTACCTGTCCTATCAATCTGCCCTTTCGAAAGATCCCAACGAAA AGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCtGGGATT ACACATGGCATGGATGAACTATACGAATTCCCACCAAAGCCATCTG CCAAGGGAGCCAAGAAGGCCGCCAAGACCGTTACGAAGCCAAAGG ACGGAAAGAAGAGACGTCATGCCCGTAAGGAATCATACTCCGTCTA CATCTACCGTGTCCTCAAGCAAGTTCATCCAGACACTGGAGTTTCCT CCAAAGCCATGTCTATCATGAACTCTTTTGTCAACGATGTCTTCGAG CGTATTGCTGCTGAAGCATCCCGTCTTGCTCACTACAACAAGCGTTC CACAATCTCATCCCGCGAAATTCAGACCGCTGTCCGTCTGATCCTTC CAGGAGAGCTTGCCAAGCACGCCGTGTCTGAGGGAACCAAGGCCG TTACCAAGTACACTTCCAGCAAGTAGTGAgcggccgcaaggtaagtttaaaataact tcgtatagcatacattatacgaagttattttcagggtggcagcggaggtaccggcggtagtggaggcacg]ttca aactttgatac	insertion of SL2-YFP:H2B + loxP + linker sequence immediately after the stop codon of the endogenous *grl-18* locus
hmn341	GAAAAGATGAAACATC[AACTCCAAAGTTCCCATTTGACCGAGATG AAAACGTAAACAAATGGACGAGTAAACGGAAAATTCGAGAAAAAG AAGAAATTAATTCAAAATGTAATAATCCGATTCTAAAGGATCTTAT GGAAATGgtaatgagtcactggccaacagattccaagttaatatttttttttagAAAATGACAACG TCTCCTTCAATATCGAAACAAATGATTTATTCAGCAGCAACCGAAA TGTGGATGGGAAGGAATGTGAATGTGATATGTTCGAAACATTCATT TTCATATGTCGTTGTGACTTCTCCAATTTTTTGTGAACACAGGAAAA AGGCGTTGACATGCTTCGTTTTCTTTCAACCTTGAttcaaactttgatactcttttttt attgttagggtgctttttgataataaattattatttattccatgttatactaaaatgtatgactgaaaatctacttttttctgctt gaactcagcggatatgaaacctttttcaaattttgttgtaaaattctgaaaaaacacaaaatttgcaacaaaatggcta cggggatttttgaaaaagtttgtttgaacccagatattagttttaaaaaattggaaaatctactaattttgcatataaatg gtcggttttagtcaatctcatctcaagctgtgcactgtgctagaaatgtgcaaaaaaaagcaaaaattagacgaaa attgaaaaatgttagacaaaaaagttttttaattcagaaaaaattttaactattttaatttttctttctgttctgccaatatgt ttcctctagagtcctgctaatcgttttgatttttttgaaaatttctattaaaaatttcacagttattttagtcatttagggcta aaaacccacatacgctacttcaaagtattcgattcataattctatttttatttatttgtcaaaactacacaagcagctttc aattagtc>-]gaaaattctagtcatc	deletion spanning Grl domain of the endogenous *grl-18* locus
syb6299	ATATTTTTCCCTCAA[CTT>TTG]GGATTCG[gtaaactttaattgtaactccctggaaag tccatgaattaaaattcagGT>GATCCATGAGCAAAGGAGAAGAACTTTTCACT GGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCA CAAATTTTCTGTCCGTGGAGAGGGTGAAGGTGATGCTACAAACGGA AAACTCACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCC GTGGCCAACACTTGTCACTACTCTGACCTATGGTGTTCAATGCTTTT CCCGTTATCCGGATCACATGAAACGGCATGACTTTTTCAAGAGTGC	removal of the first intron, insertion of sfGFP, and synonymous mutations to the endogenous *grl-18* locus

	CATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGAT GACGGGACCTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATA CCCTTGTTAATCGTATCGAGTTAAAGGGTATTGATTTTAAAGAAGAT GGAAACATTCTTGGACACAAACTCGAGTACAACTTTAACTCACACA ATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTA ACTTCAAAATTCGCCACAACGTTGAAGATGGTTCCGTTCAACTAGC AGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTT TACCAGACAACCATTACCTGTCGACACAATCTGTCCTTTCGAAAGA TCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACT GCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAAGGA]TCC ATGAACTGCCAATGCCAAAATTCGTGT[AG>TC]CTCTCCACCGGCAC AA	

Table S3. Alleles generated in this study. Related to STAR Methods.

Upper case, exons; lower case; introns.

Supplemental References:

S1. Sammut, M., Cook, S.J., Nguyen, K.C.Q., Felton, T., Hall, D.H., Emmons, S.W., Poole, R.J., and Barrios, A. (2015). Glia-derived neurons are required for sex-specific learning in C. elegans. Nature *526*, 385–390. 10.1038/nature15700.

S2. Ryan, D.A., Miller, R.M., Lee, K., Neal, S.J., Fagan, K.A., Sengupta, P., and Portman, D.S. (2014). Sex, Age, and Hunger Regulate Behavioral Prioritization through Dynamic Modulation of Chemoreceptor Expression. Current Biology *24*, 2509–2517. 10.1016/j.cub.2014.09.032.

S3. Lawson, H., Vuong, E., Miller, R.M., Kiontke, K., Fitch, D.H., and Portman, D.S. (2019). The Makorin lep-2 and the lncRNA lep-5 regulate lin-28 to schedule sexual maturation of the C. elegans nervous system. eLife *8*, e43660. 10.7554/eLife.43660.

S4. Wexler, L.R., Miller, R.M., and Portman, D.S. (2020). C. elegans Males Integrate Food Signals and Biological Sex to Modulate State-Dependent Chemosensation and Behavioral Prioritization. Current Biology *30*, 2695-2706.e4. 10.1016/j.cub.2020.05.006.

S5. Perkins, L.A., Hedgecock, E.M., Thomson, J.N., and Culotti, J.G. (1986). Mutant sensory cilia in the nematode Caenorhabditis elegans. Developmental Biology *117*, 456–487. 10.1016/0012-1606(86)90314-3.

S6. Arribere, J.A., Bell, R.T., Fu, B.X.H., Artiles, K.L., Hartman, P.S., and Fire, A.Z. (2014). Efficient Marker-Free Recovery of Custom Genetic Modifications with CRISPR/Cas9 in *Caenorhabditis elegans*. Genetics *198*, 837–846. 10.1534/genetics.114.169730.