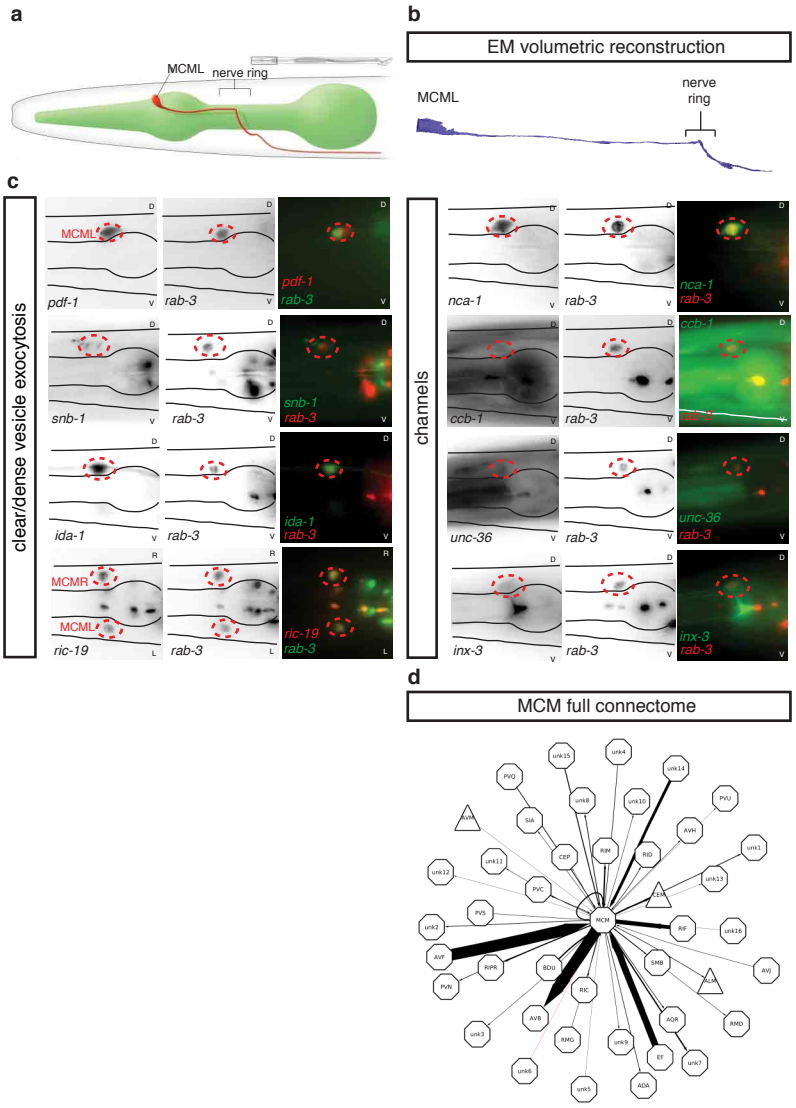
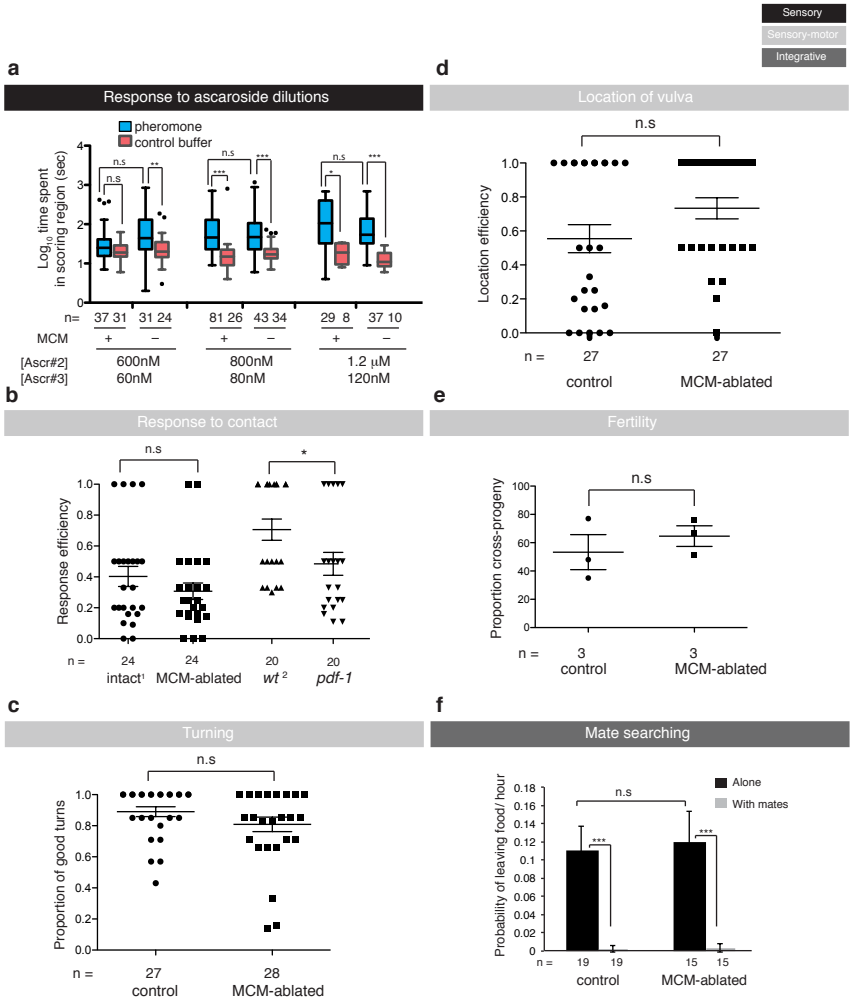
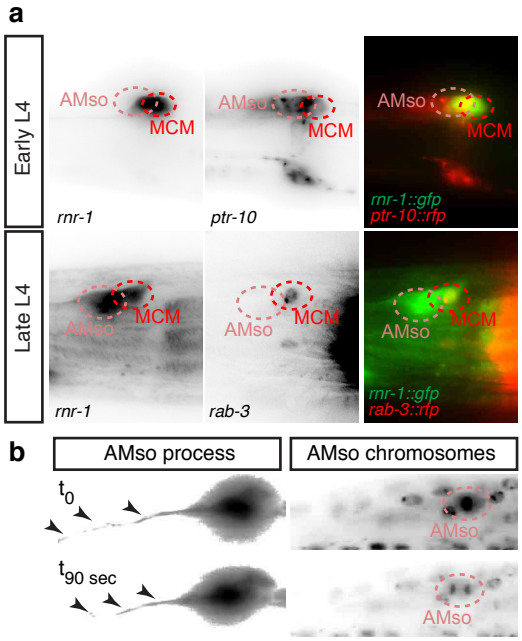
Extended Data Figure 1



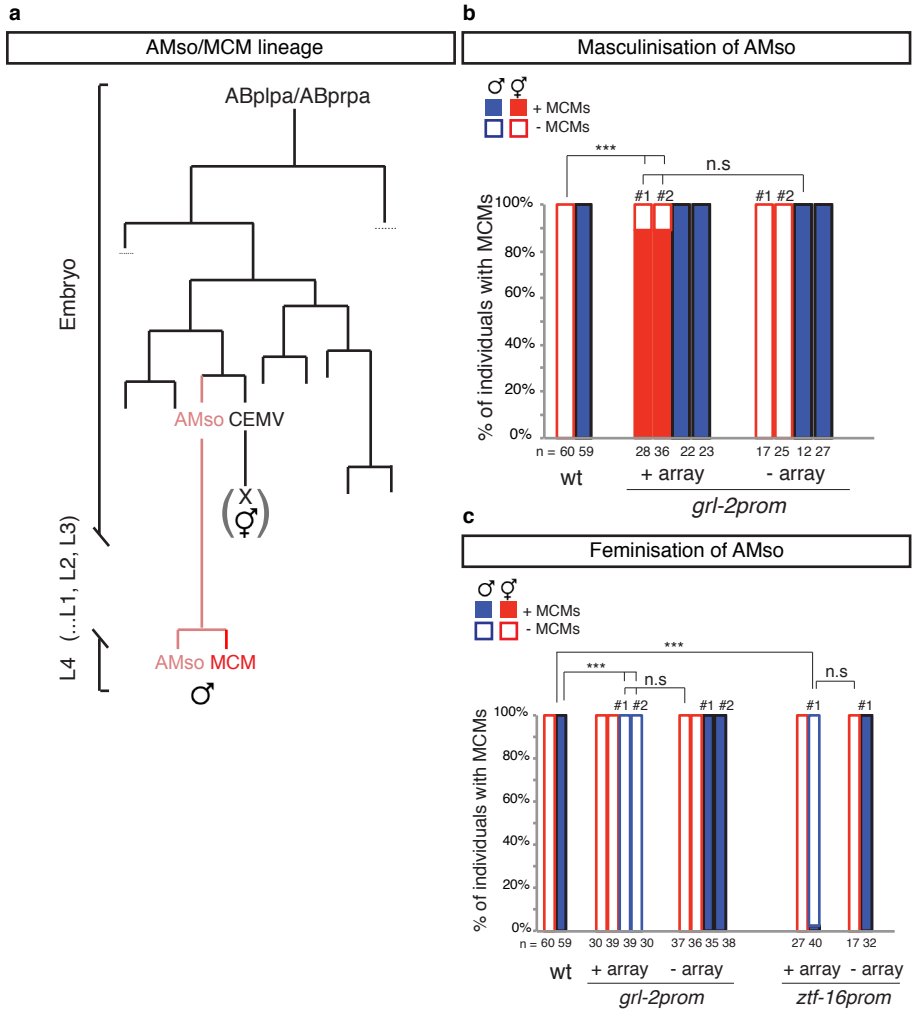
Extended Data Figure 2



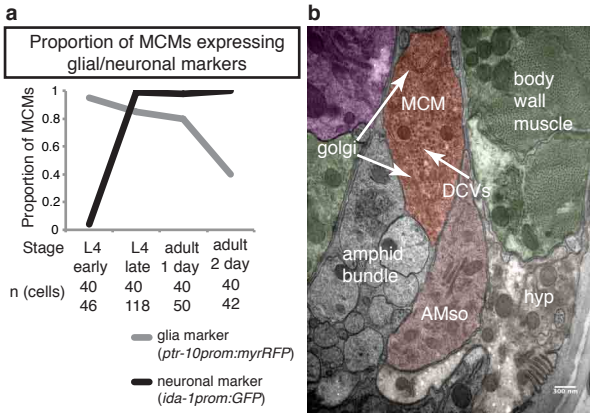
Extended Data Figure 3



Extended Data Figure 4



Extended Data Figure 5



Extended Data Table 1: Reporter transgenes for neuronal markers tested for MCM expression

protein/function	gene	array	MCM expression
Neuronal GTPase	*rab-3*	*otIs291 (rab-3prom:: NLS YFP+rol-6) and otIs356(rab-3prom::rfp)* [1]	+ 62/62
Neuronal GEF	*rgef-1*	*evIs111 (rGEFprom:: GFP)* [2]	+
Neuropeptide	*pdf-1*	*myEx696 (pdf-1prom::RFP+unc-122::GFP)* [3]	+
Phogrin/Exocytosis	*ida-1*	*inIs179(ida-1prom::GFP)* [4]	+ 59/59
Neuropeptide exocytosis	*ric-19*	*otEx6173 (ric-19prom6::NLS-TagRFP)* (gift from Hobert lab) [5]	+
Syntaxin	*unc-64*	*otEx4553 (fosmid-based transcriptional reporter)* (gift from Hobert lab) [5]	+
Synaptobrevin	*snb-1*	*otEx5422 (fosmid-based translational reporter)* (gift from Hobert lab)	+
Synaptotagmin	*snt-1*	*otEx5924(fosmid-based transcriptional reporter)* (gift from Hobert lab) [5]	+
Na^{2+} channel subunit	*nca-1*	*hpEx528 (nca-1prom::GFP)* (gift from Zhen lab)	+
Ca^{2+} channel subunit	*ccb-1*	*sEX10836(T28F2.5prom::GFP+pCeh361)* [6]	+
Ca^{2+} channel subunit	*unc-36*	*sEx12299(C50C3.9prom::GFP+pCeh361)* [6]	+
Innexin	*inx-3*	*zwEx103 [inx-3prom::GFP +lin-15(+)]* [7]	+
VGLUT	*eat-4*	*otIs388 (fosmid-based reporetr)* [8]	–
Choline transporter	*cho-1*	*otIs323 (fosmid-based reporter)* [9]	–
VGAT	*unc-47*	*otIs348[unc-47prom(300bp)::mCHOPTI::unc54-3'UTR), pha-1(+)]* [9]	–
Intraflagellar transport (marker for ciliated neurons)	*osm-6*	*mnIs17(osm-6prom::osm-6:GFP)* [10]	–
Fatty acid hydroxylase (marker for the XXX cell)	*daf-9*	*dhIs59(daf-9prom::GFP)* [11]	–
Octopamine synthesis	*tbh-1*	*nIs107[tbh-1prom::GFP+lin-15(+)]* [12]	–
Neuropeptide	*flp-21*	*nyIs80 (flp-21prom::GFP)* [13]	–
Nematocin receptor	*ntr-1*	*lstEx33 [ntr-1prom::gfp]* [14]	–
Ca^{2+} channel subunit	*cca-1*	*sEx14060(C54D2.5aprom::GFP+pCeh361)* [6]	–
K^{+} channel subunit	*unc-103*	*rgEx[unc-103prom::YFP + pBx1]* [15]	–
Innexin	*inx-12*	*zwEx112 [inx-12prom::GFP +lin-15(+)]* [7]	–
Innexin	*inx-13*	*zwEx113 [inx-13prom::GFP +lin-15(+)]* [7]	–
Innexin	*inx-5*	*zwEx105 [inx-5prom::GFP +lin-15(+)]* [7]	–
Innexin	*inx-12*	*zwEx112 [inx-12prom::GFP +lin-15(+)]* [7]	–

Extended Data Table 2: MCM connectivity

type	presynaptic cell	postsynaptic cell	number of synapses	number of EM sections
chemical	AVF	MCM	16	61
chemical	AVJ	MCM	1	2
chemical	AVM	MCM	1	1
chemical	BDU	MCM	3	7
chemical	CEM	MCM	2	5
chemical	CEP	MCM	1	1
chemical	EF	MCM	6	35
chemical	MCM	ADA	1	3
chemical	MCM	ALM	2	3
chemical	MCM	AQR	2	3
chemical	MCM	AVB	29	61
chemical	MCM	AVF	1	2
chemical	MCM	AVH	1	2
chemical	MCM	BDU	1	3
electrical	MCM	CEM	1	1
chemical	MCM	CEM	5	8
chemical	MCM	CEP	1	1
chemical	MCM	EF	2	3
chemical	MCM	MCM	2	6
chemical	MCM	PVC	1	2
chemical	MCM	PVN	3	3
chemical	MCM	PVU	1	1
chemical	MCM	RIC	2	3
chemical	MCM	RID	1	3
chemical	MCM	RIF	11	23
chemical	MCM	RIM	3	7
chemical	MCM	RIPR	3	6
chemical	MCM	RMD	1	2
chemical	MCM	SIA	1	2
chemical	MCM	SMB	2	3
chemical	MCM	unk1[1]	2	4
chemical	MCM	unk10[1]	1	2
chemical	MCM	unk11[1]	1	2
chemical	MCM	unk12[1]	1	1
chemical	MCM	unk13[1]	1	1
chemical	MCM	unk2[1]	1	3
chemical	MCM	unk3[1]	1	2

electrical	MCM	unk6[1]	1	1
chemical	MCM	unk7[1]	3	7
chemical	MCM	unk8[1]	4	4
chemical	MCM	unk9[1]	1	2
chemical	PVC	MCM	1	3
chemical	PVQ	MCM	1	4
chemical	PVS	MCM	1	2
chemical	RIC	MCM	1	1
chemical	RID	MCM	1	1
chemical	RIF	MCM	10	20
chemical	RIM	MCM	3	3
chemical	RMG	MCM	1	2
chemical	SMB	MCM	2	3
chemical	unk14[1]	MCM	3	15
chemical	unk15[1]	MCM	1	5
chemical	unk16[1]	MCM	1	1
chemical	unk4[1]	MCM	2	3
chemical	unk5[1]	MCM	1	1

[1]unk refers to neurons whose identity has not been unambiguously confirmed in the EM serial sections

Extended Data Table 3: Cell ablations of candidate MCM progenitors

cell ablated	stage	animals with loss of MCM/total
AMso	L3	6/7
AMso	L4	0/3
H0 seam cell	L1	1/16
H1 seam cell	L1	0/2
H0+H1	L1	0/1
Hyp1 (lateral nucleus)	L1	0/4
H0+Hyp1(lateral nucleus)	L1	0/1

Extended Data Table 4: Mosaic analysis of sex-transformation arrays, scoring the presence of MCMs

sex reversal	array	array in AMso and other cells[1]	array in other cells[1] only
Masculinised hermaphrodites	*oleEx18 (grl-2prom::fem-3:SL2:mCherry)*	89% (n=28)	0% (n=22)
Feminised males	*oleEx19 (grl-2prom::tra-2IC:SL2:mCherry)*	0% (n=39)	100% (n=7)
Feminised males	*oleEx22 (ztf-16prom::tra-2IC:SL2:mCherry)*	2.5% (n=40)	87.5%(n=16)

[1]Expression of *oleEx18 (grl-2prom::fem-3:SL2:mCherry)* in the head was observed in AMso, excretory duct and pore cells and sometimes in the pharynx and/or hypodermis. Expression of *oleEx19 (grl-2prom::tra-2IC:SL2:mCherry)* in the head was observed in AMso, excretory duct and pore cells and sometimes in the hypodermis. Expression of *oleEx22 (ztf-16prom::tra-2IC:SL2:mCherry)* in the head was observed in AMso and sometimes in the amphid sheath and/or a neuron in the nerve ring.

Table 5: Reporter transgenes for glial/AMso markers

protein/function	gene	array	glial subtype expression	AMso expression
Patched (PTCHD3) related receptor	*ptr-10* [1]	*nsIs108[ptr-10prom::myristyl-Rfp]*	All glia	+
Hedgehog-like/Ground-related	*grl-2* [2]	*sEx12852[T16G1.8prom::GFP+pCeh361]*	AMso and PHso	+
Inositol trisphospate receptor	*itr-1* {Gower:2001ix, Heiman:2009bj}	*jwEx51(itr1promB::GFP+rol-6)* and *nsEX1153 [F16F9.3prom::mCherry+itr-1prom::CFP+rol-6(su1006)]*	AMso	+
C2H2 zinc-finger transcription factor	*ztf-16* [3]	*oleEx22[ztf-16enhancer::tra-2IC::SL2cherry+elt-2::GFP]*	AMso, AMsh, PHso	+
Basic helix-loop-helix transcription factor	*hlh-17* [1]	*leEx1713[hlh-17prom::GFP+unc-119(+)]*	CEPsh	–
Caspr (Neurexin superfamily)	*itx-1* [4]	*otEx[W03D8.6prom::GFP+rol-6]*	OL and IL glia	–

+ indicates presence; – indicates absence. At least 15 animals were examined for each transgene. AMso, amphid socket; PHso, phasmid socket; AMsh, amphid sheath; PHsh, phasmid sheath; CEPsh, cephalic sensilla sheath; OLL, outer labial; IL, inner labial