Appendix

LET-381/FoxF and its target UNC-30/Pitx2 specify and maintain the molecular identity of *C. elegans* mesodermal glia that regulate motor behavior

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log2 Fold Change

Appendix Figure S1. Analysis of cis-regulatory regions of GLR glia-expressed genes.

(A) – (E) Cis-regulatory dissection analysis for the genes lgc-55 (A), egl-6 (B), gly-18 (C), hlh-1 (F) and inx-18 (E).

(F) A stable transgenic strain expressing nuclear YFP under the GLR glia-specific *nep-2prom7* was used to isolate GLR glia for the transcriptome analysis. Anterior is left, dorsal is up and scale bar is 10 µm.

(G) Volcano plot show significantly enriched and depleted genes (p-value >0.05) among all GLR glia expressed genes (>50 reads). *let-381* and *unc-30*, the two transcription factor genes we found to have important roles in GLR glia development, are among the most highly enriched genes.



Appendix Figure S2. *let-381/FoxF* is required for GLR glia fate specification.

(A) *let-381::gfp* is expressed in the coelomocytes (red dashed box). Asterisks denote non-specific gut granule fluorescence. Nomarski image on the right panel.

(B) Number of GLR glia expressing *gly-18prom::gfp*, *hlh-1::gfp* and *unc-30::gfp* expression in wild type and homozygous *let-381(gk302)* mutants. Expression of all genes tested is absent from GLR cells in *let-381* homozygous null mutants.

(C), (D) Animals transgenic (2 independent lines) for the fosmid genomic clone WRM069bF08 carrying wild-type *let-381* have (B) 6 GLR glia and (C) no extra head muscle cells.

Data information: Anterior is left, dorsal is up and scale bars are 10µm for all animal images. Un-paired t-test used for statistical analysis in (B) and (C).

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Appendix Figure S3. Postembryonic *let-381* knockdown does not affect GLR fate specification.

(A) Animals carying the GLR glia-specific *let-381* RNAi arrays do not have extra head muscle cells.

(B) In addition, *let-381* RNAi does not cause anteriorly displaced Nerve Ring. Yellow dashed box outlines the Nerve Ring (green) on the left image and arrow on the right image shows a head muscle arm extending into the Nerve Ring. As evidenced, the Nerve Ring is located in its normal position between the two pharyngeal bulbs (grey asterisks). Data information: Anterior is left, dorsal is up and scale bars are 10µm for all animal images.

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K-NAA dependent LET-381::AID degradation

Appendix Figure S4. LET-381::GFP::AID is rapidly degraded in the presence of the

K-NAA auxin analog.

Temporal analysis of *let-381::gfp::aid* degradation in presence of the auxin analog K-NAA.

let-381::gfp expression is lost in GLR glia within 2 hours of exposure to K-NAA in both L1

and L4 stage animals.

Data information: n=60 for each time point. Error bars show standard error of the mean.



[WRM0617aE02 (unc-30 fosmid) + unc-122prom::rfp + rab-3prom1::2xnls::rfp]

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Appendix Figure S5. UNC-30 acts cell autonomously to control GLR glia gene expression and morphology.

(A) Fluorescence images (top) and schematic representation (bottom) of endogenous unc-30::gfp expression in wild-type animals. unc-30::gfp is expressed in the MS-lineage derived GLR glia (vellow dashed boxes), and in the ASG and AVJ head neurons, DD and VD Ventral Nerve Cord neurons and PVP neurons of the preanal ganglion, all deriving from the neuroectodermal-like lineage of the blast cell AB. We performed mosaic analysis to determine whether *unc-30* acts cell autonomously to control GLR glia gene expression and morphology. To this end we used unstable extrachromosomal arrays carrying a) wild type copies of the unc-30 locus (unc-30 fosmid WRM0617aE02) that can rescue the unc-EV3A) 30(e191) phenotype (Fig. and b) *rfp* based panneuronal (rab-3prom1::2xnls::tagrfp) and coelomocyte (unc-122prom::rfp) transgenes to follow the array.

(B) Rare mosaic animals carrying the extrachromosomal array in the MS-lineage and NOT in the AB-lineage would express RFP only in the coelomocytes and the six MS-derived pharyngeal neurons I3, I4, I6, M1, M4 and M5, easily distinguished by their stereotypic position.

(C) Rare mosaic animals carrying the extrachromosomal array in the AB-lineage and NOT the MS lineage would have broad neuronal expression but no expression in the six MS-derived neurons mentioned above and no expression in coelomocytes.

(D) Fluorescence image of animal carrying the rescuing array in the MS lineage and not the AB lineage. RFP expression is in the MS-derived pharyngeal neurons M4, I4, I6, M1

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and M5. GFP expression in GLR glia (green) shows rescue of the *unc-30(e191)* phenotypes.

(E) Fluorescence image of animal carrying the rescuing array in the AB lineage and not the MS lineage. RFP is broadly expressed in AB-derived neurons. GFP expression in GLR glia (green) does not show rescue of the *unc-30(e191)* phenotypes.

(F) Quantification of the mosaic rescue analysis. Presence of the array in the MS lineage and not the AB lineage is sufficient to rescue the *unc-30(e191)* null mutant phenotype on GLR gene expression and morphology.

Data information: Anterior is left, dorsal is up and scale bars are 10µm for all animal images.

TRANSGENIC REPORTERS MADE BY PCR FUSION			
	primer C	Primer D	Primer D*
<i>gfp/rfp</i> amplicon	agcttgcatgcctgcaggtcg	aagggcccgtacggccgacta	caagaaaaacgccgtcctcg
	Primer A	Primer A*	Primer B: B primers start with CTCTAGAGTCGACCT GCAGGCATGCAAGC (homology with gfp/rfp amplicon), followed by the gene specific sequence listed below
nep-2 prom1	ggacgatgctcttcgcaaag	gctcttcgcaaagtggctcc	atcgggaggcgtccgaccgt
nep-2 prom2	gacgtcagcgcgttcttaac	cagcgcgttcttaaccatgc	atcgggaggcgtccgaccgt
nep-2 prom3	ggacgatgctcttcgcaaag	gctcttcgcaaagtggctcc	gtagatcaaaccgtaatggg
nep-2 prom4	ggacgatgctcttcgcaaag	gctcttcgcaaagtggctcc	cggagtcagaagttatacaa
nep-2 prom5	gactccgcccattccgattc	cccattccgattcccacttg	gtagatcaaaccgtaatggg
nep-2 prom6	cgacctcatcatattttaagtg	cgacctcatcatattttaagtg	gtagatcaaaccgtaatggg
nep-2 prom7	gactccgcccattccgattc	cccattccgattcccacttg	cttaaaatatgatgaggtcg
lgc-55 prom1	ccagcctaacttgctccgtt	ccatgccaatttagcgcatc	ggtctataacaagggtgtcg
lgc-55 prom2	cataggcgtgttttaaagca	gcgctactccaccttgaaga	ggcgtaggctttggcattgg
lgc-55 prom3	ccaatgccaaagcctacgcc	gcctaagcctaagcctaagt	ccaccagtactctttcaatg
lgc-55 prom4	cctggtgggtactataaagc	gggtactataaagcggggca	gtaggcgccaaaacgcttgc
lgc-55 prom5	ctacgtggtagcccaaaaca	gttagtggtactgtgcacaag	cctctcatcttccgagagac
lgc-55 prom6	gcaccacctcaagatttatc	cattggtttcgctcttgtttg	cgccacgaaaaacttgttga
lgc-55 prom7	caacaagtttttcgtggcg	ggacccaaaattctctaccc	ccatttcatttcgacatcta
egl-6 prom1	ccgcaatttttttggtggtg	gaacatttcaccggaatctc	agccgagttaaagtctaaag
egl-6 prom2	cctagtggtgcgttccttcc	ctttccttccaggaatttcg	cagctttctatgttgccagc
egl-6 prom3	cagctgaattgagctaccac	cgttttcttgtcgttttgctc	tgctgaaaagctgtcattgt
egl-6 prom4	cctagtggtgcgttccttcc	ctttccttccaggaatttcg	gaaacctttcggtctcacac
egl-6 prom5	ggtttcaagttagagctact	gagctactactttctattcc	cagctttctatgttgccagc
gly-18 prom1	cttgtcaaacatgctaactg	gagccaactagctgtttctt	gaagtcgcggaagctactta
gly-18 prom2	ctaagtagcttccgcgacttc	ctttacttacctacagtgtcc	cacagttacaaggtgcaccc
gly-18 prom3	ggtgcaccttgtaactgtgt	ctcgtagcttgttgaacgag	ctatactaatgccaatattg
gly-18 prom4	gccactccactatattttcg	ctgtgttcacacaatgactgc	caatactttgacagctgtct
gly-18 prom5	gagacagctgtcaaagtattg	gtatcacttgtgaaaagccc	cttgatacgttttaacacag
gly-18 prom6	gagacagctgtcaaagtattg	gtatcacttgtgaaaagccc	agacatgagagaaattcagg
gly-18 prom7	ctcatgtctaacccttgtgg	cccttgtggaattttgtcaa	cttgatacgttttaacacag
gly-18 prom8	ctcatgtctaacccttgtgg	cccttgtggaattttgtcaa	gaattattggaaaactggga
gly-18 prom9	caattgaagaactccgcgac	caattgaagaactccgcgac	cttgatacgttttaacacag
hlh-1 prom1	ggttataatgagcaccagatg	ggttataatgagcaccagatg	caacgggttaacccagtcta
hlh-1 prom2	ggttataatgagcaccagatg	ggttataatgagcaccagatg	cataaattctcttctcccct
hlh-1 prom3	gaaatgggtcatgggaatag	gggtcatgggaatagtaaag	caacgggttaacccagtcta
inx-18 prom1	ggagaagcttccccagccta	caaccggacagagcagctac	tcgcgaacgtcttcaggcttc
inx-18 prom2	ggaatgcactttctggcctc	ctaaggtttcttgaaccaag	cgtcaactctggaaattttc
inx-18 prom3	ggaatgcactttctggcctc	ctaaggtttcttgaaccaag	ccgtatacacattacatoto
inx-18 prom4	cacatgtaatgtgtatacgg	ggagagcactcatatcatct	cgtcaactctggaaattttc

Appendix Table S1. List of primers used in this study.

inx-18 prom5	ggaatgcactttctggcctc	ctaaggtttcttgaaccaag	ctacaaggcggcctactttg
inx-18 prom6	gcacgcaggcagacatgttc	ggcagacatgttcgtacctg	ccgtatacacattacatgtg
inx-18 prom7	ggaatgcactttctggcctc	ctaaggtttcttgaaccaag	cttgaatagggagatggaac
inx-18 prom8	gtcccacattacactttcgc	gtcccacattacactttcgc	ctacaaggcggcctactttg
inx-18 prom9	gtcccacattacactttcgc	gtcccacattacactttcgc	tttattagtatttgatggtc
inx-18	gtaaacataatgcgattgtag	gtaaacataatgcgattgtag	ctacaaggcggcctactttg
prom10			
inx-18	catgtttgctgttttggtgtc	caaacaatatggttcgtagg	ccattgccctggtaaattgt
prom11			
unc-30	cgtctccaactgtgttctgt	tctgttgtcttttacctacg	caaaagctgcaagatcttgg
prom1			
unc-30	ccaagatcttgcagcttttg	gaaagtcttcttggcgacatg	cttgttttggagttacagcc
prom2			
unc-30	cgatagaagtgagcttggtc	ccaaaacaaggtgtctgatc	gaacgctcttttccagttttc
prom3			
unc-30	ctgctgaaaacttagttatg	catatgcagagagaggcttag	cacttgggtgtaacttctca
prom4			
unc-30	tgagaagttacacccaagtg	ggaacacgttgaccgtgaga	cccttatcatcaccagatgt
prom5			
unc-30	gtaaacagtttgcgcaattgc	ggtgatgataagggtagatg	gtgttgatcttttgccggag
prom6			
unc-30	cggccacactgactgctatac	gcagcagtcgcataatcggt	ctgaaataaaagaaatggaag
prom/			
unc-30	gcagtcaggtaagcagaagg	gtaagcagaaggcaggcatc	gagtcgtctagctgaaaatgg
prom8			
unc-30	ctagtcgattttcgactgct	ccaacgtgagttttacggat	ccacaggatgctgtcaaacc
prom9			
unc-30	ggillgacagcatccigigg	ggillelectaciceaatee	gaatgcagcttgggctctgg
pionino pll 1 prom1	attatataaataaataatta	000000000000000000000000000000000000000	ac activity attitude
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not-5 promiz			
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Γ20Π1.2 prom1	gegalalalicecaccicig	gelecayaacyayacalliy	cyaalcaaalayliyacyay
70317.2	ctecactagatagattaga	contatacoactecoactte	ctagaaateatttgatagg
20311.2 prom1	Ciccaciggaigggilacge	cagigigaagciccagciic	ciyyaaaaicailiyalayy
	cateteccassotttecsas		ctataagaagaagaagaattag
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prom?	33		
unc-54	cgactccaaagtaggttagt	gagacatotttcatctgaag	cacttettteaaataatte
prom3	-gaotooddagaagaaga		-gonomoula ggnio
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prom1	<u> </u>		0.01111111111111111111

prom2prom2ccaccactaggatctgttaggagacatactttcctgttggccgcctatacttggtttttctwk-4 prom3ccaccactaggatgagggggggclgagggcaaggagagagcaggtcgaagtatttacaggtwk-4 prom3ccagaagagaagggttcactcgtttggacagttcaaagggcggtcgaagtatttacaggcor-1 prom1gcgtgacgaccactttcgccatgaactagtaagagcagtcgaagttgctttccccor-1 prom2ctgcagtttacagaccacttcgccatgaactgaatgaaccaagactgaatgaagagcagtcgaatgtgaagagcor-1 prom3gagagtgagaggtttcaggccatgtagaagagccagtcactttcccagatgtggaggtggacor-1 prom1catcactgtttggtcaggccaggttcatattttggaggcggagatgtggaagtggamg-6 prom1cagatgactgtcagtgttctactacttctcgtaaatccagaaactttgtgtcagmg-6 prom3gaaccgaactcgatcgtggtccatatttcccttgaalgcgcgaactagggcgamg-6 prom3gtaccatttatcccagtcgatacgatgcagtgcagtgcccattcttctggtaaccattcaamg-6 prom3gtaccattatcccagtcgatacgatgcagtgcgtccattcttctggtaccattcaamg-6 prom3gtaccattatcccagtcgatacgatgaaggtgggccattcttctggtaccattcaamg-17ggtcacttatcagacgtggaagatgaaggtgggcccatcatttggtgcamig-17gctcagtagaagcttagtggataatggtgggggctacaacgccaaaaaggggggprom3gaaagttgacggtggaaggtggactacatggtcactatagggataatggggggggglet-2 prom3ccataggggggagggggggcagtaggaaggtgggggaaaattgggggggggggggggggggggggggggggg	F41G4.8	gagatcgattagacacagtg	ggtaagaaaagggtttcgag	ccagccgctgcaatactgag
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nta-1 prom2gcccttgtcacaggtaaattgcgcaagttagtcaagatatgcccggtgatggttacctttgT14B4.9cgatctttctccatgattgcattagcatggtttccctcagctttgattatgacggacatgcprom1 </td <td>nta-1 prom1</td> <td>cccaactcctgactctaaaag</td> <td>caattgtttgtgatttcccc</td> <td>cgttgacttttccatttccc</td>	nta-1 prom1	cccaactcctgactctaaaag	caattgtttgtgatttcccc	cgttgacttttccatttccc
T14B4.9 cgatctttctccatgattgc attagcatggtttccctcag ctttgattatgacggacatgc prom1	nta-1 prom2	gcccttgtcacaggtaaattg	cgcaagttagtcaagatatg	cccggtgatggttacctttg
prom1	T14B4.9	cgatctttctccatgattgc	attagcatggtttccctcag	ctttgattatgacggacatgc
	prom1			

T14B4.9 prom2	cggagggaagtaaaatctta	cgatttactggta	aactacca	gtacagtcatatttacagatgg
T14B4.9	ggcacctagaataacaatgag	ggagacgcaa	agagacagct	ctccaaatttgagctcagggg
prom3		С		
tbc-12 prom1	cctgcagctcgttttttttg	gtcgttgtctact	ccattttg	gagcaaaaagagagaaactc
tbc-12 prom2	cagatataattccattgggc	cacgtttttctgtt	cggccac	gacgagcaaatattaccttcc
tbc-12 prom3	gttaacaaggccccgttgtcg	gctatttttcctga	aagctcac	ggaaattgaccacacaaatgg
tbc-12 prom4	caaaccagttgcgtgaatgcc	gtggccatttaa	tcgcaattc	ctaggggcttttaagtacttg
pgp-4 prom1	gaggacataattttagccgc	gtgagcaacaa	accatttttg	ctctgcttaacaatggttctg
pgp-4 prom2	ctgacctaaaaagacaacgtc	gcacacactac	caacttacccg	ctggcaattttcattgggacc
haf-7 prom1	gctatatttttcatcgtggc	gttcacacaca	ttattgctc	caggaaatgcaacaatatgc
haf-7 prom2	gggaaagccctgaaatctga	ggatttttattcca	aaccctc	gtgcacctagcaatttcatgc
FOSMID RECOMBINEERING				
Fosmid	Primer F		Primer R	
clone (gene)				
WRM0641dF	cacgaggtcttgccatgccacaactgacacataa a		aacgttgacccaaaaaatgaacccgaaatgaa	
02 (gly-18)	aaattggattttatgagctgtctcatcctactttcac		aaaatgtactgaaaaaaactacttgctggaagtgt	
			acttgg	
WRM065dE0	acgataagggaaaactatatcaa	gttgaactttatt	atctaaaactta	itgtaaagtctaaaacttgtatatat
1 (<i>gpx-</i> 8)	tatttcagagataagctgtctcatcctactttcac		gttgtactaccacctacttgctggaagtgtacttgg	