

## SUPPLEMENTARY INFORMATION

### SUPPLEMENTARY MATERIALS AND METHODS

#### Generation of transgenic constructs

To generate *jmjd-1.2\_JmjCmut::GFP*, *jmjd-1.2\_XLMR::GFP* and *jmjd-1.2\_PHDmut::GFP* constructs, the original vector containing *jmjd-1.2::GFP* was mutated using the QuikChange Site-Directed Mutagenesis Kit (Stratagene). Specifically, for *jmjd-1.2 JmjCmut::GFP*, the histidine at position 508 (H508) and the valine at position 509 (V509) were changed to leucine and glutamic acid, respectively, using the primers Fw (GCTGGATCTTACGGATTCCCTCGAGGACTTGTTGGTAGTAGC) and Rv (GCTACTACCACCAAAGTCCTCGAGGAAATCCGTATAAGATCCAGC). For *jmjd-1.2\_XLMR::GFP*, the phenylalanine at position 507 (F507) was changed to serine using the primers XLMR\_Fw (GGATCTTACGGATTCCCACGTGGAC) and XLMR\_Rv (GTCCACGTGGGAATCCGTATAAGATCC). For *jmjd-1.2\_PHDmut::GFP*, the glycine at position 254 (G254) was changed to alanine using the primers PHD\_G254A\_Fw (TTCCAATGGATTGCCTGTGACTCTTGCC) and PHD\_G254A\_Rv (GGCAAGAGTCACAGGCAATCCATTGGAA).

For overexpression experiments, the heat-shock promoter *hsp-16.2* was PCR-amplified from N2 genomic DNA using the primers Phsp-16.2\_GW\_fw (GGGGACAACTTGTATAGAAAAGTTGttcgaagttagatgcact) and Phsp-16.2\_GW\_rv (GGGGACTGCTTTGTACAAACTTGgattatgttgaagattctaatt), and cloned into pDONR P4-P1R vector. The genomic regions of *wrt-8* (2,350 bp, C29F3.2 in WormBase) and *grl-16* (2,627 bp, Y65B4BR.6 in WormBase) were PCR-amplified from N2 genomic DNA using the primers wrt-8\_fw (ATGAATTATTTATTACTGGTATCTGG) / wrt-8\_rv (GTAGGAAATCATTTCGATGGCA) and grl-16\_fw (ATGAGAGTCTTGGTAGCCGTC) / grl-16\_rv (ATCCTCCCAGGTAAGCGAGT), respectively. The resulting fragments were inserted into pDONR pCR8 vector and the final plasmids expressing *Phsp-16.2::wrt-8::GFP* and *Phsp-16.2::grl-16::GFP* were constructed using MultiSite Gateway Three-Fragment Vector Construction Kit.

**Table S1. Transgenic and non-transgenic siblings analyzed for rescue experiments**

<b>Strain</b>	<b>Genotype</b>	<b>Defects observed (%)</b>
ZR111	<i>jmjd-1.2(tm3713); oyls14; zrEx26 Ex(Pjmjd-1.2::jmjd-1.2::GFP)</i>	12
ZR111	<i>jmjd-1.2(tm3713); oyls14</i> (non-transgenic siblings)	21
ZR109	<i>jmjd-1.2(tm3713); oyls14; zrEx35 Ex(Prab-3::jmjd-1.2::GFP)</i>	9
ZR109	<i>jmjd-1.2(tm3713); oyls14</i> (non-transgenic siblings)	23
ZR551	<i>jmjd-1.2(tm3713); oyls14; zrEx150 Ex(Pdpy-7::jmjd-1.2::GFP)</i>	14
ZR551	<i>jmjd-1.2(tm3713); oyls14</i> (non-transgenic siblings)	25
ZR155	<i>jmjd-1.2(tm3713); oyls14; zrEx37 Ex(Pmyo-3::jmjd-1.2::GFP)</i>	19
ZR155	<i>jmjd-1.2(tm3713); oyls14</i> (non-transgenic siblings)	20
ZR550	<i>jmjd-1.2(tm3713); oyls14; zrEx149 Ex(Psra-6::jmjd-1.2::GFP)</i>	19
ZR550	<i>jmjd-1.2(tm3713); oyls14</i> (non-transgenic siblings)	21
ZR984	<i>jmjd-1.2(tm3713); oyls14; zrEx359 Ex(Podr-2::jmjd-1.2::GFP)</i>	23
ZR984	<i>jmjd-1.2(tm3713); oyls14</i> (non-transgenic siblings)	22
ZR148	<i>jmjd-1.2(tm3713); oyls14; zrEx33 Ex(Pjmjd-1.2::jmjd-1.2_JmjCmut::GFP)</i>	26
ZR148	<i>jmjd-1.2(tm3713); oyls14</i> (non-transgenic siblings)	24
ZR894	<i>jmjd-1.2(tm3713); oyls14; zrEx317 Ex(Pjmjd-1.2::jmjd-1.2_XLMR::GFP)</i>	20
ZR894	<i>jmjd-1.2(tm3713); oyls14</i> (non-transgenic siblings)	22
ZR955	<i>jmjd-1.2(tm3713); oyls14; zrEx356 Ex(Pjmjd-1.2::jmjd-1.2_PHDmut::GFP)</i>	27
ZR955	<i>jmjd-1.2(tm3713); oyls14</i> (non-transgenic siblings)	20

Quantification of PVQ axonal cross-over defects in the indicated strains.  $n > 50$  for each strain.

**Table S2. Loss of either *clec-230*, *cut-3*, *grl-16* or *wrt-8* restores correct PVQ guidance in *jmjd-1.2(tm3713)* mutants**

Genotype	Defects observed (%)
WT	9 ( <i>n</i> =267)
<i>jmjd-1.2(tm3713)</i>	22 ( <i>n</i> =286)
<i>clec-230(ok3131)</i>	12 ( <i>n</i> =209)
<i>jmjd-1.2(tm3713);clec-230(ok3131)</i>	9 ( <i>n</i> =187) **
<i>cut-3(ok1819)</i>	13 ( <i>n</i> =136)
<i>jmjd-1.2(tm3713);cut-3(ok1819)</i>	12 ( <i>n</i> =158) **
<i>grl-16(ok2959)</i>	16 ( <i>n</i> =215)
<i>jmjd-1.2(tm3713);grl-16(ok2959)</i>	12 ( <i>n</i> =217) **
<i>wrt-8(tm1585)</i>	12 ( <i>n</i> =201)
<i>jmjd-1.2(tm3713);wrt-8(tm1585)</i>	13 ( <i>n</i> =326) **
<i>grl-7(ok2644)</i>	12 ( <i>n</i> =129)
<i>jmjd-1.2(tm3713);grl-7(ok2644)</i>	16 ( <i>n</i> =185) n.s.
<i>asp-6(tm2213)</i>	26 ( <i>n</i> =215)
<i>jmjd-1.2(tm3713);asp-6(tm2213)</i>	25 ( <i>n</i> =150) n.s.
<i>nep-17(ok3251)</i>	74 ( <i>n</i> =184)
<i>jmjd-1.2(tm3713);nep-17(ok3251)</i>	20 ( <i>n</i> =120) n.s.

Quantification of PVQ axonal cross-over defects in the indicated strains. *n* = number of analyzed animals. \*\**p*<0.01, n.s., not significant (one-way ANOVA followed by Tukey's multiple-comparison test).

**Table S3. Mammalian components of the Hedgehog pathway and their *C. elegans* homologs**

Mammalian gene	<i>C. elegans</i> homolog	Description
<i>Shh, Ihh, Dhh</i>	<i>wrt, grl, grd, qua-1, hog-1</i>	Ligands
<i>Skn/HHAT</i>	<i>hhat-1, hhat-2</i>	Ligand modulator (palmitoylation)
<i>DISP</i>	<i>che-14, ptd-2</i>	Secretion of ligands (12-Pass TM)
<i>EXT1</i>	<i>rib-1</i>	Trafficking/diffusion of ligands
<i>EXTL3</i>	<i>rib-2</i>	Trafficking/diffusion of ligands
<i>Glypican-6</i>	<i>gpn-1</i>	Trafficking/diffusion of ligands
<i>PTCH1, PTCH2</i>	<i>ptc-1, ptc-3</i>	Ligand receptors
<i>GAS1</i>	<i>phg-1</i>	Co-receptor
<i>LRP2</i>	<i>lrp-1</i>	Co-receptor
<i>HHIP</i>	-	Hedgehog interacting protein
<i>SMO</i>	-	GPC receptor
<i>FU</i>	-	Serine/threonine kinase
<i>SUFU</i>	-	Sufu domain
<i>KIF27, KIF7</i>	-	Kinesin-like
<i>Gli1, Gli2, Gli3</i>	<i>tra-1</i>	Zinc finger transcription factors

List of genes encoding components of the Hedgehog pathway in mammals, homologs in *C. elegans* and brief description of their functions.

**Table S4. Transgenic strains**

<b>Strain</b>	<b>Genotype</b>
ZR111	<i>jmjd-1.2(tm3713); oyls14; zrEx26 Ex(Pjmjd-1.2::jmjd-1.2::GFP)</i>
ZR526	<i>jmjd-1.2(tm3713); oyls14; zrEx137 Ex(Pjmjd-1.2::jmjd-1.2::GFP)</i>
ZR527	<i>jmjd-1.2(tm3713); oyls14; zrEx138 Ex(Pjmjd-1.2::jmjd-1.2::GFP)</i>
ZR109	<i>jmjd-1.2(tm3713); oyls14; zrEx12 Ex(Prab-3::jmjd-1.2::GFP)</i>
ZR153	<i>jmjd-1.2(tm3713); oyls14; zrEx35 Ex(Prab-3::jmjd-1.2::GFP)</i>
ZR259	<i>jmjd-1.2(tm3713); oyls14; zrEx53 Ex(Prab-3::jmjd-1.2::GFP)</i>
ZR551	<i>jmjd-1.2(tm3713); oyls14; zrEx150 Ex(Pdpy-7::jmjd-1.2::GFP)</i>
ZR552	<i>jmjd-1.2(tm3713); oyls14; zrEx151 Ex(Pdpy-7::jmjd-1.2::GFP)</i>
ZR553	<i>jmjd-1.2(tm3713); oyls14; zrEx152 Ex(Pdpy-7::jmjd-1.2::GFP)</i>
ZR718	<i>jmjd-1.2(tm3713); oyls14; zrEx251 Ex(Prab-3::jmjd-1.2::GFP); Ex(Pdpy-7::jmjd-1.2::GFP)</i>
ZR719	<i>jmjd-1.2(tm3713); oyls14; zrEx252 Ex(Prab-3::jmjd-1.2::GFP); Ex(Pdpy-7::jmjd-1.2::GFP)</i>
ZR720	<i>jmjd-1.2(tm3713); oyls14; zrEx253 Ex(Prab-3::jmjd-1.2::GFP); Ex(Pdpy-7::jmjd-1.2::GFP)</i>
ZR155	<i>jmjd-1.2(tm3713); oyls14; zrEx37 Ex(Pmyo-3::jmjd-1.2::GFP)</i>
ZR183	<i>jmjd-1.2(tm3713); oyls14; zrEx40 Ex(Pmyo-3::jmjd-1.2::GFP)</i>
ZR184	<i>jmjd-1.2(tm3713); oyls14; zrEx41 Ex(Pmyo-3::jmjd-1.2::GFP)</i>
ZR550	<i>jmjd-1.2(tm3713); oyls14; zrEx149 Ex(Psra-6::jmjd-1.2::GFP)</i>
ZR712	<i>jmjd-1.2(tm3713); oyls14; zrEx249 Ex(Psra-6::jmjd-1.2::GFP)</i>
ZR713	<i>jmjd-1.2(tm3713); oyls14; zrEx250 Ex(Psra-6::jmjd-1.2::GFP)</i>
ZR984	<i>jmjd-1.2(tm3713); oyls14; zrEx359 Ex(Podr-2::jmjd-1.2::GFP)</i>
ZR985	<i>jmjd-1.2(tm3713); oyls14; zrEx360 Ex(Podr-2::jmjd-1.2::GFP)</i>
ZR986	<i>jmjd-1.2(tm3713); oyls14; zrEx361 Ex(Podr-2::jmjd-1.2::GFP)</i>

<b>Strain</b>	<b>Genotype</b>
ZR148	<i>jmjd-1.2(tm3713); oyIs14; zrEx33 Ex(Pjmjd-1.2::jmjd-1.2_JmjCmut::GFP)</i>
ZR952	<i>jmjd-1.2(tm3713); oyIs14; zrEx353 Ex(Pjmjd-1.2::jmjd-1.2_JmjCmut::GFP)</i>
ZR953	<i>jmjd-1.2(tm3713); oyIs14; zrEx354 Ex(Pjmjd-1.2::jmjd-1.2_JmjCmut::GFP)</i>
ZR894	<i>jmjd-1.2(tm3713); oyIs14; zrEx317 Ex(Pjmjd-1.2::jmjd-1.2_XLMR::GFP)</i>
ZR895	<i>jmjd-1.2(tm3713); oyIs14; zrEx318 Ex(Pjmjd-1.2::jmjd-1.2_XLMR::GFP)</i>
ZR896	<i>jmjd-1.2(tm3713); oyIs14; zrEx319 Ex(Pjmjd-1.2::jmjd-1.2_XLMR::GFP)</i>
ZR954	<i>jmjd-1.2(tm3713); oyIs14; zrEx355 Ex(Pjmjd-1.2::jmjd-1.2_PHDmut::GFP)</i>
ZR955	<i>jmjd-1.2(tm3713); oyIs14; zrEx356 Ex(Pjmjd-1.2::jmjd-1.2_PHDmut::GFP)</i>
ZR956	<i>jmjd-1.2(tm3713); oyIs14; zrEx357 Ex(Pjmjd-1.2::jmjd-1.2_PHDmut::GFP)</i>
ZR942	<i>oyIs14; zrEx349 Ex(Phsp-16.2::wrt-8::GFP)</i>
ZR943	<i>oyIs14; zrEx350 Ex(Phsp-16.2::wrt-8::GFP)</i>
ZR944	<i>oyIs14; zrEx351 Ex(Phsp-16.2::wrt-8::GFP)</i>
ZR945	<i>oyIs14; zrEx352 Ex(Phsp-16.2::grl-16::GFP)</i>
ZR1014	<i>wsp-1(gm324); oyIs14; zrEx351 Ex(Phsp-16.2::wrt-8::GFP)</i>
ZR1015	<i>wsp-1(gm324); oyIs14; zrEx352 Ex(Phsp-16.2::grl-16::GFP)</i>

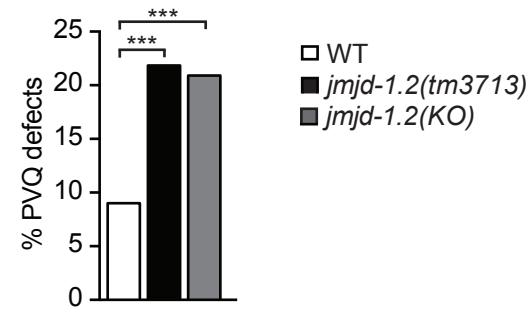
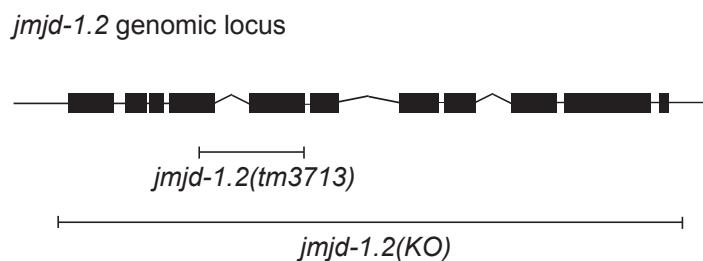
List of transgenic strains used in this study: names and genotypes are indicated.

## SUPPLEMENTARY FIGURES

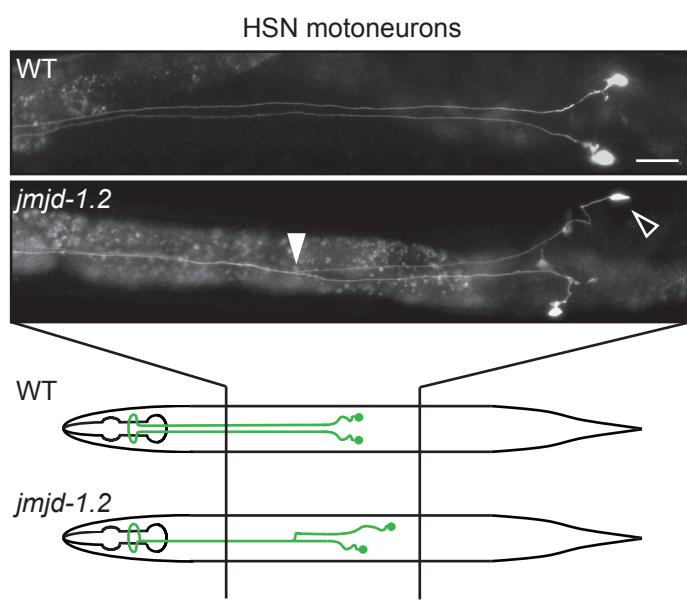
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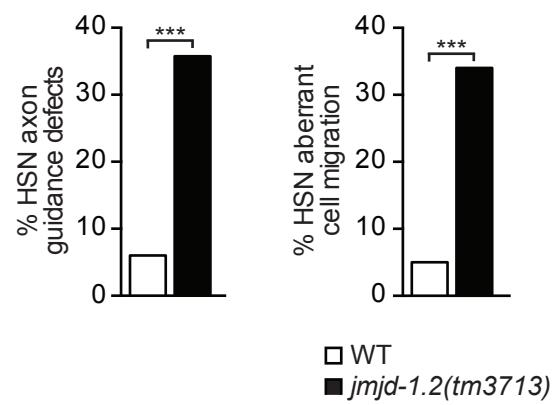
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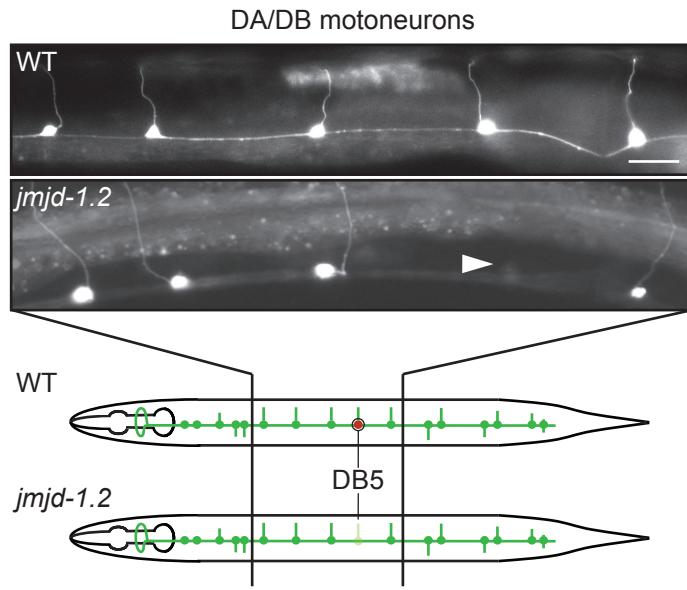
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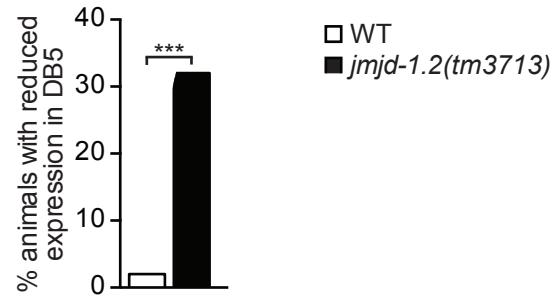
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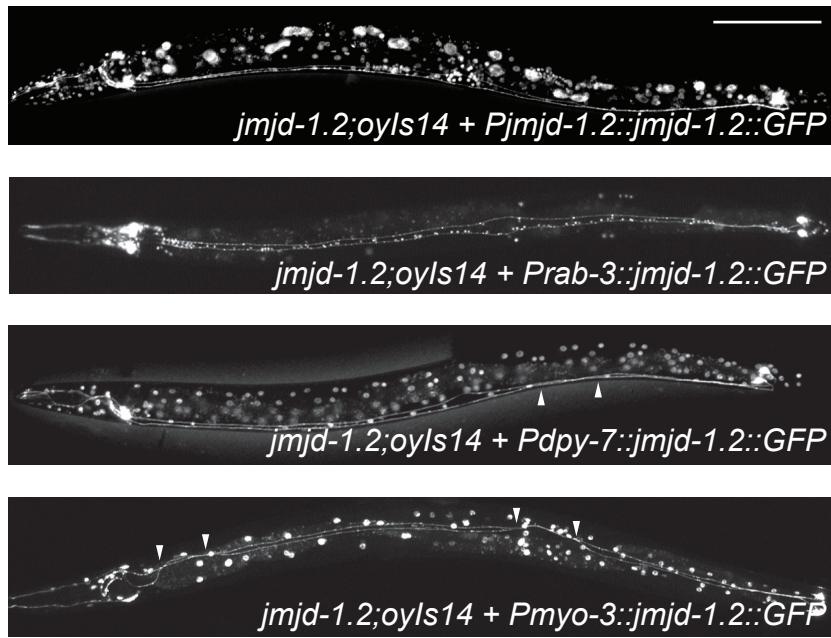
**E**



**F**

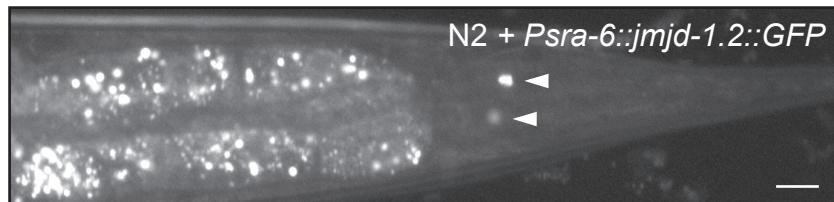


**Fig. S1. *jmjd-1.2(tm3713)* animals display distinct neuronal defects.** **A.** Large-sized image of a wild-type animal fixed and stained with JMJD-1.2 antibody. N, neurons; I, intestinal cells. Ventral view, anterior to the left. Scale bar, 20  $\mu\text{m}$ . **B.** Left: Genomic organization of *jmjd-1.2*. Black H-shaped lines indicate the position of the *tm3713* and the (*KO*) deletion. Right: Quantification of PVQ axonal cross-over defects in *jmjd-1.2(tm3713)* and *jmjd-1.2(KO)* animals, expressed as percentages of defects.  $n > 200$ , \*\*\* $p < 0.001$  (Fisher's exact test). **C.** Top: Representative images of HSN motoneurons in wild-type (WT) and *jmjd-1.2(tm3713)* adult animals. The white arrowhead indicates the point of axonal cross-over and the empty arrowhead indicates aberrant position of the cell body. Ventral view, anterior to the left. Scale bar, 20  $\mu\text{m}$ . Bottom: Schematic diagrams of HSN motoneurons in wild-type and *jmjd-1.2(tm3713)* animals. **D.** Left: Quantification of HSN axonal cross-over defects in *jmjd-1.2(tm3713)* animals. Right: Quantification of HSN aberrant cell migration in *jmjd-1.2(tm3713)* adult animals.  $n > 100$ , \*\*\* $p < 0.001$  (Fisher's exact test). **E.** Top: Representative images of DA/DB motoneurons in wild-type and *jmjd-1.2(tm3713)* adult animals. The white arrowhead indicates reduced expression of the transgene in DB5 cell body. Ventral view, anterior to the left. Scale bar, 20  $\mu\text{m}$ . Bottom: Schematic diagrams of DA/DB motoneurons in wild-type and *jmjd-1.2(tm3713)* animals. **F.** Quantification of animals with reduced expression of *evIs82b* in DB5 in wild-type and *jmjd-1.2(tm3713)* strains.  $n > 100$ , \*\*\* $p < 0.001$  (Fisher's exact test).

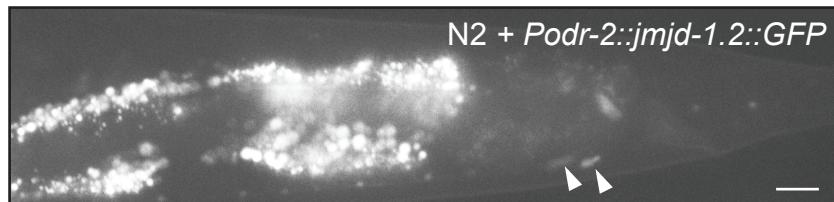


**Fig. S2. Expression patterns of animals analyzed for rescue experiments.** Representative images of *jmjd-1.2(tm3713);oyIs14* adult animals expressing the construct *jmjd-1.2::GFP* under the control of *jmjd-1.2* promoter (*Pjmjd-1.2*) or different tissue-specific promoters: *Prab-3*, nervous system; *Pdpy-7*, hypodermis; *Pmyo-3*, body wall muscles. In all images, anterior is to the left. Animals expressing *Pjmjd-1.2::jmjd-1.2* and *Pdpy-7::jmjd-1.2* are in lateral position, the others in ventral position. Scale bar, 100  $\mu$ m.

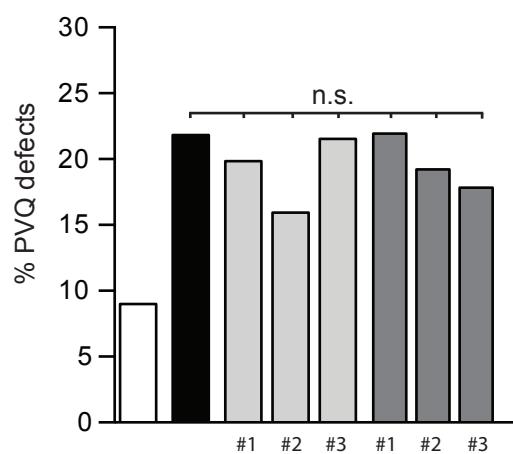
**A**



**B**

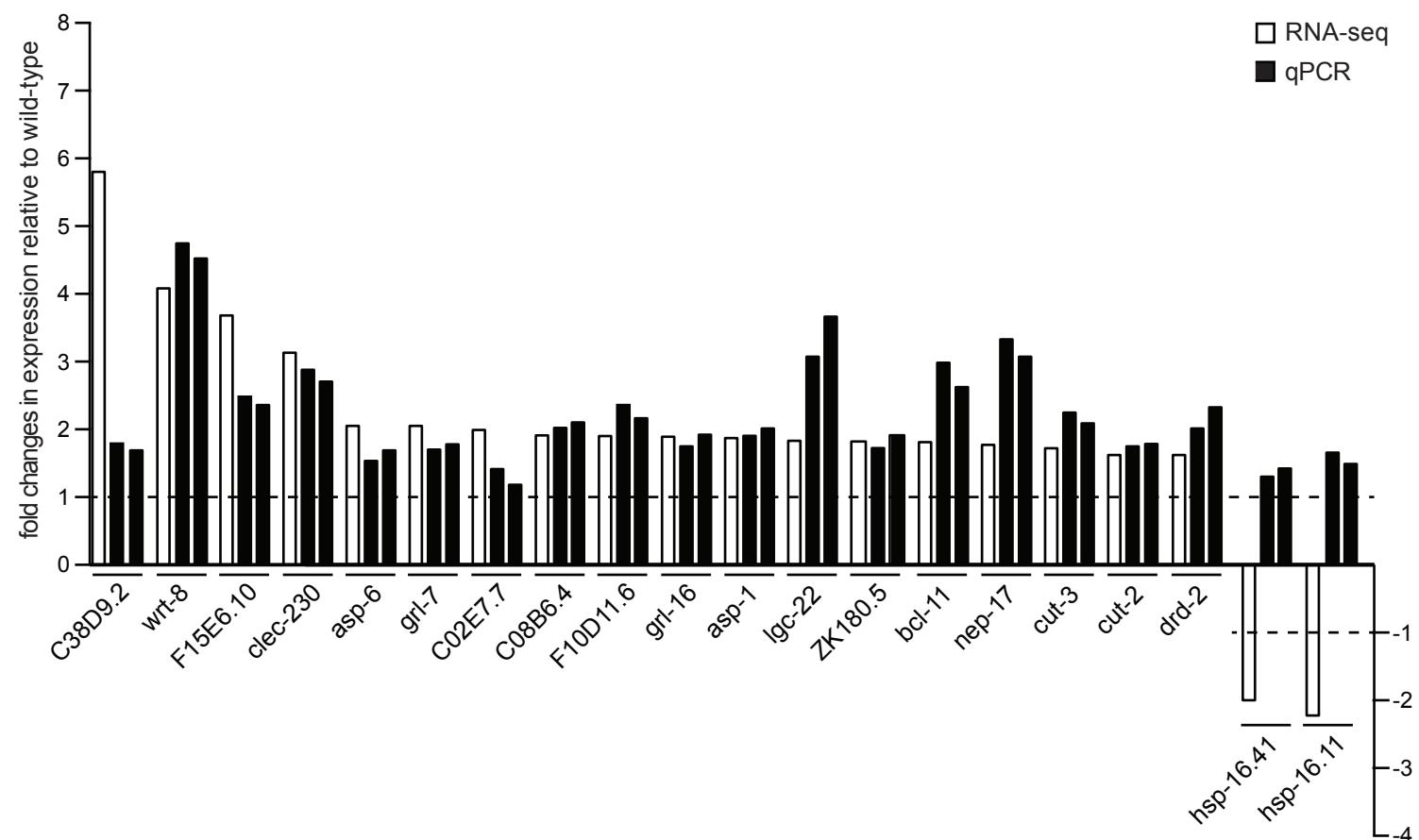


**C**



- WT
- *jmjd-1.2*
- *jmjd-1.2 + Psra-6::jmjd-1.2*
- *jmjd-1.2 + Podr-2::jmjd-1.2*

**Fig. S3. The activity of *jmjd-1.2* is not cell-autonomous.** **A.** Representative image of a wild-type adult animal expressing the construct *jmjd-1.2::GFP* under the control of the *sra-6* promoter (*Psra-6*), expressed in PVQs. Ventral view, anterior to the left. Arrowheads indicate the PVQ cellular bodies. Scale bar, 10  $\mu$ m. **B.** Representative image of a wild-type adult animal expressing the construct *jmjd-1.2::GFP* under the control of the *odr-2* promoter (*Podr-2*), expressed in PVPs. Ventral view, anterior to the left. Arrowheads indicate the PVP cellular bodies. Scale bar, 10  $\mu$ m. **C.** Quantification of PVQ axonal cross-over defects in *jmjd-1.2(tm3713)* mutants expressing transgenic JMJD-1.2 specifically in PVQs or in PVPs.  $n > 100$ , n.s., not significant (one-way ANOVA followed by Tukey's multiple-comparison test). Three independent lines for each transgene (indicated by #) were analyzed.



**Fig. S4. qPCR validation of genes found deregulated in *jmjd-1.2(tm3713)* embryos by RNA-sequencing.** Expression of indicated genes in *jmjd-1.2(tm3713)* embryos is reported as fold change relative to wild-type. Dashed bars are set to  $\pm 1$ . Deregulation of *C38D9.2*, *C02E7.7*, *hsp-16.41* and *hsp-16.11* was not confirmed by qPCR.