Synapse formation is a process tightly controlled in space and time. How gene regulatory mechanisms specify spatial and temporal aspects of synapse formation is not well understood. In the nematode *Caenorhabditis elegans*, two subtypes of the D-type inhibitory motor neuron (MN) classes, the dorsal D (DD) and ventral D (VD) neurons, extend axons along both the dorsal and ventral nerve cords. The embryonically generated DD motor neurons initially innervate ventral muscles in the first (L1) larval stage and receive their synaptic input from cholinergic motor neurons in the dorsal cord. They rewire by the end of the L1 moulting to innervate dorsal muscles and to be innervated by newly formed ventral cholinergic motor neurons. VD motor neurons develop after the L1 moulting; they take over the innervation of ventral muscles and receive their synaptic input from dorsal cholinergic motor neurons. We show here that the spatiotemporal control of synaptic wiring of the D-type neurons is controlled by an intersectional transcriptional strategy in which the UNC-30 Pitx-type homeodomain transcription factor acts together, in embryonic and early larval stages, with the temporally controlled LIN-14 transcription factor to prevent premature synapse rewiring of the DD motor neurons and, together with the UNC-55 nuclear hormone receptor, to prevent aberrant VD synaptic wiring in later larval and adult stages. A key effecter of this intersectional transcription factor combination is a novel synaptic organizer molecule, the single immunoglobulin domain protein OIG-1. OIG-1 is perisynaptically localized along the synaptic outputs of the D-type motor neurons in a temporally controlled manner and is required for appropriate selection of both pre- and post-synaptic partners.

At the end of the first larval stage, the synaptic outputs from the DD motor neurons (MNs) to ventral muscle and their synaptic input from cholinergic DA/DB MNs is eliminated and, instead, synapses are formed onto dorsal muscle and synaptic input is received from cholinergic VA/VB MNs (Fig. 1a). We sought to examine how the spatiotemporal specificity of this rewiring process is controlled and integrated with other aspects of D-type MN differentiation. To address this question, we examined the function of the *C. elegans* Pitx-type homeobox transcription factor UNC-30, which is known to control GABAergic neurotransmitter identity of the D-type MNs. The analysis of serial electron micrographs shows that the synaptic patterns of the DD and VD neurons are substantially disrupted in *unc-30* null mutant animals. In adult *unc-30(e191)* animals, VD MNs display ectopic synapses onto dorsal muscle and lack notable synaptic inputs from DA/DB on the dorsal side (Fig. 1b and Extended Data Fig. 1). Furthermore, DD MNs, which normally only form synapses onto ventral muscle, show aberrant innervation of dorsal muscle in L1 stage *unc-30* mutants (Fig. 1c). These synaptic defects were confirmed with green fluorescent protein (GFP)-tagged RAB-3 protein, expressed specifically in D-type MNs (Fig. 1d).

*unc-30* is expressed in both DD and VD MNs at all stages, yet *unc-30* inhibits dorsal DD synapses only in the L1 stage and not at later stages. However, at these later stages, *unc-30* does inhibit dorsal synapses from VD neurons, but not the DD neurons. How can the temporal and spatial specificity of the *unc-30(e191)* defects be explained? A potential answer to this question lies in the previously described mutant phenotype of two transcription factors, which recapitulate specific components of the cell-type specific, DD and VD synaptic genic defects of *unc-30* mutants. In animals lacking the *lin-14* transcription factor, whose expression is normally temporally restricted to embryonic and first larval stages in most tissues, including the D-type motorneurons, DD MNs form ectopic synapses in the dorsal cord in embryonic and L1 stages (Extended Data Fig. 2a; schematized in Fig. 1e, f). These DD MN defects are similar to those that we observe in *unc-30* mutants. The dorsal ectopic synapses in the VD neurons of *unc-30* mutant animals (not observed in *lin-14*) are in turn recapitulated in animals lacking the *unc-55* orphan nuclear receptor, in which VD MNs form aberrant synapses in the dorsal cord, as previously shown (Extended Data Fig. 2b; schematized in Fig. 1e, f). One possible way to explain these concordances of phenotypes is that *unc-30* may collaborate with *lin-14* to control the expression of a molecule that acts in a temporally restricted manner in embryonic and L1 stages to inhibit dorsal synapse formation of the DD neurons. In the VD MNs, *unc-30* may in turn collaborate with *unc-55* to control expression of a molecule that acts in the VD neurons to inhibit dorsal synapse formation of these neurons.

We sought to identify such potential effector molecule(s) through a candidate gene approach. In a survey of *C. elegans* immunoglobulin superfamily members, we had previously described a family of small proteins that are composed of a single Ig domain, the *oig* gene family. One of the *oig* family members, *oig-1*, encodes a 137 amino acid-long protein with a signal sequence and a single IgC2-type domain, but no transmembrane domain or predicted glycosylphosphatidylinositol (GPI) anchor. Transgenic animals carrying an *oig-1* fosmid-based reporter construct showed expression both in the DD and VD MNs, but no other ventral nerve cord MNs (Fig. 2a). Notably, expression of *oig-1* in the D-type MNs is temporally controlled in a manner that correlates with the distinct periods of inhibition of dorsal muscle innervation exhibited by DD and VD neurons. *oig-1* is transiently expressed in the DD neurons during the time when no dorsal synapses are formed (embryos and L1), but is downregulated in the DD neurons upon formation of their dorsal synapses (L2 and later; Fig. 2a). In contrast, expression of *oig-1* in the VD neurons, which have processes but no synaptic outputs in the dorsal cord, is continuously maintained throughout the life of the neuron (Fig. 2a).

The transient expression in the DD and continuous expression in the VD neurons makes *oig-1* a candidate effector gene for the *unc-30*, *lin-14* and *unc-55* transcription factors. Indeed, the *oig-1*fosmid sl2::gfp reporter fails to be expressed in both DD- and VD-type MNs in *unc-30* null mutants at all stages (Fig. 2b). In *lin-14* null mutant animals,
Figure 1  |  Loss of unc-30 disrupts the synaptic connectivity of the DD and VD MNs.  

a. Schematic of DD rewiring.  

b. Reconstruction of a VD4 MN from an unc-30(e191) adult animal compared to the same neuron in a wild-type animal. Extended Data Fig. 1 shows a more detailed presentation of the EM data.  

c. Reconstructed DD3 neuron from an unc-30(e191) L1 larva showing aberrant NMJs in the dorsal cord (D). Previous reconstructions of a wild-type L1 using the same techniques and personnel showed that a reconstructed DD3 made no NMJs on dorsal muscles and 9 NMJs on ventral muscles.  

d. Presynaptic marker RAB-3 ectopically localizes mostly to ventral cord in wild-type L1 animals (16/20 animals), but ectopically in the dorsal nerve cord (DNC; outlined in red) in unc-30 mutant animals (19/20). At the L4 stage, in which presynaptic specialization are observed in both ventral nerve cord (VNC; outlined in red) and DNC (19/20 animals), unc-30 mutants showed few specializations in the VNC (20/20 animals). Original magnification, ×630.  

e, f, Summary of synapse formation defects in unc-30, lin-14, and unc-55 mutants (e) and genetic interpretation (f).

transient expression of the oig-1 reporter in the L1 stage is diminished in the DD MNs (Fig. 2b) and temporally prolonged expression of lin-14, achieved through genetic removal of a negative regulator of lin-14, the microRNA lin-4 (ref. 9), results in prolonged expression of oig-1 in the DD MNs into the adult stage (Fig. 2b). In animals lacking unc-55, which is normally expressed in VD, but not DD MNs, expression of oig-1 in the DD neurons at the L1 stage is unaffected, but oig-1 expression in the VD neurons is absent at the adult stage (Fig. 2b). The two distinct transcription factor combinations that control oig-1 expression in DD (unc-30 and lin-14) and in VD (unc-30 and unc-55) operate independently since the expression of each transcription factor is independent of the presence of the other transcription factor (Extended Data Fig. 3a, b)11.

The transcriptional nature of oig-1 regulation is corroborated by the finding that 1 kilobase (kb) of 5′ sequences of oig-1 contains the same spatiotemporal regulation as the oig-1 fosmid reporter (Extended Data Fig. 4). Chromatin immunoprecipitation–sequencing data from the modEncode project shows binding of UNC-55 to this 1-kb fragment of the oig-1 locus12, suggesting direct regulation. However, it is conceivable that unc-55 may also control oig-1 expression indirectly, through the homeobox gene irx-1, a target of unc-55 (ref. 13). A 125-base pair element that still recapitulates spatiotemporal control in the D-type MN expression contains two sites with partial match to the UNC-30 binding site9, and one is required for DD MN expression (Extended Data Fig. 4).

The expression pattern of oig-1 and its regulation by transcription factors that regulate synapse formation make oig-1 a candidate for involvement in the synapse-organizing activity of these transcription factors. Animals that carry an oig-1 deletion allele (Fig. 2a) are viable and fertile, but display locomotory defects and hypersensitivity to the drug aldicarb (Extended Data Fig. 5a, b), which is characteristic of abnormalities in GABAergic signalling10. In embryonic and L1 stages only the DD MNs are present, the presynaptic vesicle proteins SNB-1 and RAB-3 are aberrantly clustered along the dorsal nerve cord of oig-1 null mutants (Fig. 3a; Extended Data Fig. 2c). Moreover, the postsynaptic GABA receptor UNC-49, which normally clusters on ventral muscle at the L1 stage9, clusters ectopically along the dorsal nerve cord (Fig. 3b). Therefore, oig-1—like its upstream regulators unc-30 and lin-14—is required to prevent premature DD synapse formation in the dorsal nerve cord.

Examining the synapses of the VD MN that normally exclusively synapses on ventral muscle, we observed more puncta of three presynaptic markers (SNB-1 and RAB-3 proteins and the Liprin-α protein SYD-2) in the dorsal nerve cord and fewer in the ventral nerve cord of oig-1 mutants at post-L1 larval stages (Fig. 3c; Extended Data Fig. 2c). This indicates that the VD MNs have aberrant synaptic specializations in the dorsal nerve cord in oig-1 mutants; although less
mutants can be rescued by expressing specificity regulators of the localization of synaptic inputs and outputs are coordinated, and that

Figure 3 | Aberrant D-type MN synapse formation in oig-1 mutants. 

Severe, this phenotype is similar to those of the VD synaptic defects of unc-30 and unc-55 mutants.

We next examined whether oig-1 function is restricted to controlling the synaptic output of D-type neurons or whether oig-1 may also affect localization of their synaptic input. Innervation of the DD and VD neurons from the cholinergic A- and B-type neurons can be visualized with GFP-tagged ACR-12 protein, which localizes to puncta in the DD neurons in the dorsal nerve cord in the L1 stage, indicative of the cholinergic input from the DA/DB MNs (Fig. 4a). Remarkably, in oig-1 mutants, these dorsal puncta are not observed (Fig. 4a). In post L1-stage wild-type animals, ACR-12 protein normally labels synapses from DA/DB to the VD neurons in the dorsal cord and synapses from VA/VB to the DD neurons in the ventral cord (Fig. 4b). In oig-1 mutants, the dorsal, ACR-12(+) synaptic inputs in the VD neurons also do not form properly (Fig. 4b). The coincidence of synaptic input and synaptic output defects in oig-1 mutants indicates that the localization of synaptic inputs and outputs are coordinated, and that this coordination requires the OIG-1 protein. As expected, the oig-1 defects in synaptic innervation, as determined by ACR-12 clustering, are mirrored by loss of the temporal (lin-14) and spatial (unc-55) specificity regulators of oig-1 expression (Extended Data Fig. 6a, b).

The synaptic defects (as well as the locomotory defects) of oig-1 mutants can be rescued by expressing oig-1 specifically in the D-type MN under control of the unc-30 promoter, whereas expression under control of a cholinergic A- and B-type MN promoter does not rescue (Fig. 3a, c and Extended Data Fig. 5a, c). Since OIG-1 is predicted to encode a secreted protein, the lack of rescue of oig-1 with a cholinergic promoter suggests that OIG-1 protein functions cell-autonomously in/ on the GABAergic DD and VD classes of MNs and argues against a long-range, diffusible function of OIG-1. Consistent with this autonomy, oig-1 mutants show no defects in the localization of synapses of the adjacent cholinergic MNs (data not shown). The rescuing activity of oig-1 critically depends on the integrity of the IgC2 domain (Extended Data Fig. 7). Forced expression of oig-1 in the D-type MNs under control of two promoters that are not downregulated in the D-type motor neurons (unc-25 and unc-30 promoters) is not sufficient to prevent the formation of dorsal synaptic outputs of the DD neurons (data not shown), indicating that oig-1 collaborates with other factors to regulate synapse formation.

The synaptic wiring defects in oig-1 mutants suggested that the OIG-1 protein might be localized in a spatially restricted manner in the DD and VD MNs. A fosmid-based reporter in which OIG-1 protein is fused to GFP (and which rescues oig-1 locomotory defects and the aldicarb hypersensitivity; Fig. 2a and Extended Data Fig. 5a, b) shows punctate localization along the processes of the D-type MNs (Fig. 5a). The punctate pattern of OIG-1 in D-type MNs revealed a surprising localization pattern along the D-type processes. At the L1 stage, OIG-1 is not localized along the dorsal processes (in which the synaptic wiring defects are observed), but is localized along the ventral cord. After the generation of the DD MNs (and extinction of OIG-1 expression in the DD MNs, as described above), OIG-1 protein also localizes in the VD neurons along the ventral cord. Co-labelling with the presynaptic RIM protein UNC-10 and the postsynaptic GABA receptor UNC-49 demonstrates that these puncta correspond to the perisynaptic region of synapses that D-type MNs form onto ventral neuronal processes (Fig. 5b). When ectopically expressed in excitatory cholinergic VNC or head MNs, OIG-1–GFP is also targeted to synaptic specializations (Fig. 5c), demonstrating that OIG-1 localization is not dependent

Figure 4 | OIG-1 also controls cholinergic innervation into the DD and VD neurons. 

Figure 5 | PERIODICITY OF OIG-1 EXPRESSION.
on GABAergic-specific synaptic features, but rather contains synaptic targeting properties that are independent of the type of synapse. Taken together, the perisynaptic localization pattern of OIG-1 indicates that a highly localized, synaptic organizer protein is capable of orchestrating the wiring properties of an entire neuron, by promoting synaptic input and preventing ectopic synaptic output in a distal portion of the neuronal process (Fig 5b). Punctate OIG-1 protein is also observed in a few head neurons (Extended Data Fig 8).

The mutant phenotype of oig-1, specifically the aberrant formation of synaptic output from dorsal DD and VD axons, resembles the mutant phenotype observed upon removal of the SAD-1 kinase18,19. In animals lacking sad-1 or lacking strd-1/STRADx, a pseudokinase required for SAD-1 localization20, OIG-1 clusters ectopically along the dorsal nerve cord (Extended Data Fig 9). Conversely, loss of oig-1 does not affect localization of SAD-1 (data not shown). What sets OIG-1 apart from these molecules is that, in contrast to the pan-neuronally expressed SAD-1 and STRADx, OIG-1 seems to operate as a spatio-temporally controlled nexus of this pathway that determines the spatio-temporal specificity of SAD-1 protein function in the context of the D-type MNs. The ability of ventrally and presynaptically localized OIG-1 to organize distally located synaptic inputs and outputs on the dorsal neurite suggests that OIG-1 may trigger a cascade of downstream signalling events or anchor factors on the ventral side which would otherwise contribute to synapse organization on the dorsal side.

In conclusion, we have shown here that three different transcription factors cooperate in an intersectional manner in defined spatial and temporal contexts to control the expression of a perisynaptically localized organizer molecule, OIG-1, which orchestrates the localization of synaptic outputs and inputs of two different neuron types (Fig 5d). unc-30 needs to cooperate with other transcription factors and these collaborators confer spatiotemporal specificity. In embryonic and L1 stages, spatially but not temporally restricted unc-30 cooperates with temporally, but not spatially controlled, lin-14 to prevent DD MN synapse assembly at the inappropriate location via induction of oig-1 expression. After the L1 stage, DD/VD-expressed unc-30 collaborates with the subtype (VD)-specific unc-55 transcription factor to restrict oig-1 expression to the VD neurons where it organizes synaptic inputs and outputs (Fig 5d).

Our findings demonstrate that the localization of synaptic inputs and outputs of a neuron are coordinated and that this coordination is apparently achieved, at least in part, by the OIG-1 organizer protein. It will be interesting to examine whether similar synaptic organizer functions can be ascribed to any of the multiple small, single immunoglobulin domain proteins, some secreted, some transmembrane, which are encoded in the C. elegans21 and vertebrate genomes22.

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Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions

K.H. conducted all the experiments with the exception of the electron microscopy analysis, done by J.G.W.; O.H. supervised the study and all authors contributed to writing of the manuscript.

Author Information

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METHODS
C. elegans strains. Worms were grown at 20°C on nematode growth media (NGM) plates seeded with bacteria (Escherichia coli OP50) as a food source. Larval animals were obtained by hypochlorite-traited gravid adult animals and letting embryos hatch and arrest in M9 for 16–18 h.

Mutant alleles used in this study: LGI: unc-55(e1170), LGII: lin-4(e1021), LGIII: oig-1(ky289), acr-12(ok367), oig-1(ok1687), strd-1(ok2283).

These constructs were injected as described above.

These constructs were used in Fig. 3a, c and Extended Data Fig. 5a, c, and S65T. This cassette was recombineered and inserted into the line 2) all used in Extended Data Fig. 7.

immediately following the predicted signal peptide sequence (after the 72nd base pair of ttx-3prom::mCherry

This construct was generated by cloning the 2.4 kb unc-3 promoter into the EcoRV site of the first MCS of pPD94.26 and the oig-1 locus from the start to stop codon (1,521 bp) into the BamHI site of the second MCS. This construct was digested with PvuII and injected at 10 ng μl −1 with myo-2-p-gfp at 3 ng μl −1 and OP50 genomic DNA at 120 ng μl −1.

The oig-1 promoter deletion constructs were generated by cloning the various promoter fragments into the HindIII and BamHI sites of the MSC of a 2XNLSGFP plasmid. Promoter constructs with potential UNC-30 binding sites deleted were generated using site-directed mutagenesis. These constructs were injected at 50 ng μl −1 with rol-6 at 30 ng μl −1 and rol-6(su1006) at 20 ng μl −1.

Wormtracker assays. Tracking assays were performed as previously described.

Briefly, L4 animals were placed on an NGM plate seeded with 20 μl of OP50 bacteria in the centre. Automated tracking was performed with the Worm Tracker 2.0 (WT2) which uses a camera to track and record individual worms. Twenty worms of each genotype were tracked for 5 min each at 20°C. Analysis was performed as previously described.

Aldicarb assays. Aldicarb assays were performed as previously described.

Briefly, ~20 young adult animals (24 h after L4 stage, blinded for genotype) were picked for freshly seeded NGM plates containing 1 mM aldicarb (ChemService).

Worms were assayed for paralysis every 15 min by prodding with a platinum wire. A worm was considered paralyzed if it did not respond to prodding to the head and tail. Data were normalized to the time of first paralysis. Strains were grown and assayed at 20°C.

Antibody staining. Antibody staining was performed as previously described.

Briefly, following a freeze-crack procedure, worms were fixed by treatment in ice-cold acetone for 5 min and then ice-cold methanol for 5 min. Worms were collected in PBS and centrifuged briefly. The PBS was removed and worms were incubated in a blocking solution (1× PBS, 0.2% gelatin, 0.25% Triton X-100) for 30 min at 20°C. After the blocking solution was removed, worms were incubated with primary antibodies diluted in PGT (1× PBS, 0.1% gelatin, 0.25% Triton X-100) overnight at 4°C. The anti-UNC-49 antibody was used at a 1:500 dilution. The anti-UNC-17 antibody was used at a 1:5,000 dilution. The anti-GFP antibody (Life Technologies A10262) was used at 1:1,000. The anti-RIM2 (used to recognize UNC-10) was used at a 1:10 dilution (Developmental Studies Hybridoma Bank – University of Iowa). Worms were washed 5 times in wash solution (1× PBS, 0.25% Triton X-100) for 20 min each wash. Worms were then incubated with secondary antibodies diluted 1:1,000 in PGT for 3 h at 20°C. Alexa Fluor 488 goat anti-chicken (Invitrogen A11039) was used to detect the anti-GFP antibody. Alexa Fluor 594 donkey anti-mouse (Invitrogen A-21203) was used to detect the anti-RIM2 antibody. Alexa Fluor 555 donkey anti-rabbit (Invitrogen A-31572) was used to detect the anti-UNC-49 antibody. Alexa Fluor 488 donkey anti-mouse (Invitrogen A-21201) was used to detect the anti-UNC-17 antibody. Worms were then washed 5 times in wash solution (1× PBS) for 20 min each wash. Following the final wash, worms were mounted in Fluorogel with Tris buffer (Electron Microscopy Sciences)

Statistical analysis. For results shown in Figs 3a, 4a, 4b, 6a, c, 7, and 9 we performed Fisher’s exact test, **P < 0.01, *P < 0.05. For results shown in Fig. 3c, Extended Data Fig. 2c, we performed a Student’s t-test (2 sided, type 2), **P < 0.01, *P < 0.05. For WormTracker analysis in Extended Data Fig. 3a, we used Wilcoxon rank sum test to test the differences between oig-1, wild-type, and rescued strains, *P < 0.01, *P < 0.05. No statistical methods were used to predetermine sample size, and the experiments were not randomized.

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Extended Data Figure 1 | Electron microscopical analysis of *unc-30(e191)* mutants. a, Reconstructions of a VD4 and a DD2 MN from an *unc-30(e191)* animal compared to the same neurons in a wild-type animal. Cell bodies (large black dots) are all situated in the ventral cord. Processes emanate anteriorly (upwards on plots) from the cell body and run along the ventral cord. Lateral branches leave the ventral cord (V) and run round to the dorsal cord (D) as a circumferential commissure (broken horizontal process in the plots). Commissures from *unc-30* mutant type-D neurons are situated in the same regions as those of their wild-type counterparts. However, the cell bodies of DD neurons are often displaced anteriorly in the mutants, with the consequence that DD neurons have shorter processes in the ventral cord (C). Processes in the dorsal cord run anteriorly in mutant animals, whereas they branch with the main branch running posteriorly in wild-type animals. Neuromuscular junctions (NMJs) in *unc-30* mutants are made predominantly in the dorsal cord by both the DDs and VDs, whereas in the wild type, only DD neurons innervate dorsal muscles. The synaptic inputs to the DD and VD neurons in mutant animals (inward pointing arrows for chemical synapses and ‘T’ for gap junctions) are generally abnormal. The reconstructed DD2 neuron received synapses from several unidentified processes on the ventral side (depicted with a ‘?’). These processes do not belong to VA or VB neurons, the normal pre-synaptic partners of DD, as all the local VA and VB neurons were identified. From the location and synaptic behaviour of these processes, it is probable that they belong to interneurons which span the length of the cord and do not usually innervate D-type neurons. An asterisk (*) indicates that a synapse has multiple post-synaptic elements. A total of six VDs and three DDs were reconstructed. Each reconstruction covered around 2,000 electron microscopy sections, corresponding to a length of 100 μm along the body of the animal. The *unc-30(e191)* mutation does not affect MN cell body position or the synaptic behaviour of the DA/DB neurons, except in regard to their synapses to D-type neurones; this made it possible to unambiguously identify D type neurones from their positions and by eliminating other identified classes of MNs. Electron microscopy and reconstructions of micrographs of serial sections were performed as described in ref. 31. b, Electron micrographs. The processes of DD and VD neurons normally run subjacent to the bounding basal lamina of the ventral cord immediately dorsal to the axons of the VA and VB neurons (a). NMJs are made through the basal lamina onto muscle arms (M). In *unc-30(e191)* animals, the axons of the DD and VD neurons wander round the cord and do not run in defined locations; the configuration shown in b is typical but not stereotyped. Very few NMJs are made in the ventral cord by the DD or VD neurons in *unc-30* mutants; those that are made look rather small (c). Atypical synapses (d) are often made onto DD or VD processes from neurons such as the touch receptor neuron AVM (6). It is probable that these synapses are not normally found as the processes of AVM, and DD or VD do not normally run alongside each other in wild-type animals. Both the DD and VD MNs make NMJs to dorsal muscles in the dorsal cord of *unc-30* mutants, whereas in wild-type animals, only DD neurons do so and VD neurons innervate ventral muscles in the ventral cord. e, f, NMJs made by VD (e) and DD (f) neurons in the dorsal cord of an *unc-30(e191)* mutant. Scale bars, 1 μm.
Extended Data Figure 2  | RAB-3 is ectopically localized in lin-14, unc-55, and oig-1 mutants. a, Presynaptic marker RAB-3 ectopically localizes to the dorsal nerve cord (DNC; marked in red) in lin-14 mutant animals. RAB-3–GFP puncta (from otEx5663, unc-30p::gfp::rab-3) localize mostly to the ventral nerve cord (VNC; marked in red) in wild-type L1 animals (left). Ectopic RAB-3–GFP puncta localize mostly to the dorsal nerve cord in 95% of lin-14 L1 animals (right, scored in progeny from lin-14 null animals carrying a lin-14 rescue array23). Ventral and dorsal nerve cords are indicated by red dotted lines. L1 animals were obtained by hypochlorite-treating gravid adult animals and letting embryos hatch and arrest in M9 for 16–18 h. n = 20 for each strain scored. b, RAB-3 ectopically localizes to the dorsal nerve cord in unc-55 L4 mutant animals. RAB-3–GFP puncta localize to both the ventral (VNC) and dorsal (DNC) nerve cord in 100% of wild-type L4 animals (left). RAB-3–GFP puncta localize mostly to the dorsal nerve cord in 100% of unc-55 L4 animals (right). Ventral and dorsal nerve cords are indicated by red dotted lines. Signals between the nerve cords are autofluorescence from the gut. n = 20 for each strain scored. c, RAB-3 ectopically localizes to the dorsal nerve cord in oig-1 mutants. RAB-3 normally localizes to the ventral nerve cord (VNC, marked in red) in wild-type L1 animals (top left). Ectopic RAB-3–GFP puncta localize to the dorsal nerve cord in 55% of oig-1 L1 animals (top right, compared to 20% of wild-type animals). L1 animals were obtained by hypochlorite-treating gravid adult animals and letting embryos hatch and arrest in M9 for 16–18 h. n = 20 for each strain scored. In wild-type L4 animals, more RAB-3–GFP puncta are localized in the VNC than in the DNC of the animal (bottom, black dots). Conversely, in oig-1 mutants, more RAB-3–GFP puncta are localized in the DNC than in the VNC (bottom, red dots). **P < 0.01, n = 20 for each strain, original magnification, ×630.
Extended Data Figure 3 | Mutual independence of transcription factor activities. 

**a**, Expression of *unc-30* is not affected by loss of *lin-14* or *unc-55*. A 2.4 kb *unc-30* promoter gfp fusion reporter is expressed in the DD MNs (green circles) in wild-type L1 animals; this expression is not affected in *lin-14(ma135)* mutant animals (scored in progeny from *lin-14* null mothers carrying a *lin-14* rescue array). An *unc-30* fosmid-based reporter, kindly provided by the TransgeneOme project, is expressed in the DD (green circles) and VD (blue squares) MNs (DD4 to VD10 shown) in wild-type L4 animals; this expression is not affected in *unc-55(e1170)* L4 animals. *n* = 20 for each genotype.

**b**, Expression of a *lin-14* fosmid-based reporter construct is unaffected by loss of *unc-30*. *lin-14* is expressed in the DA, DB, and DD MNs in the VNC at the L1 stage (average number of VNC cells = 15); this expression is not affected in *unc-30(e191)* mutant L1s (average number of VNC cells = 15); *n* = 20 for each genotype. Loss of *unc-30* also does not affect *unc-55* expression, as shown by ref. 11. Original magnification, ×630.
Extended Data Figure 4 | Deletion of a putative UNC-30 binding site results in loss of \( \text{oig-1} \) expression in the D-type neurons. Regions of the \( \text{oig-1} \) promoter were fused to \( \text{gfp} \) to analyse expression. (+) indicates robust expression of the reporter construct in the specified cell type, whereas (−) indicates loss of expression in the specified cell type. Twenty worms at both the L1 and L4 stage were scored for each line. Expression of a 1 kb promoter reporter (prom 1) recapitulates expression of the \( \text{oig-1} \) fosmid::\( \text{gfp} \) reporter in the D-type MNs (see Fig. 2). This region contained 3 elements that exactly match the UNC-30 consensus binding site (TAATC, purple box14,) and multiple others that are a partial match to the UNC-30 binding site (magenta and blue boxes). Further deletion of this prom 1 defined a minimal 125 bp element that is sufficient to drive \( \text{oig-1} \) expression in the D-type MNs (prom 6). This element contains two sites that partially match the UNC-30 binding sequence. Deletion of the AAATC site in the context of the 1 kb promoter (prom 9) has no effect on \( \text{oig-1} \) expression in the D-type MNs. Deletion of the TAAAC site in the context of the 1 kb promoter reporter (prom 10) results in complete loss of \( \text{oig-1} \) expression specifically in the D-type MNs. We noted that some of the smaller transcriptional reporter lines show extended expression of \( \text{gfp} \) in the DD motor neurons. Since DD rewiring is delayed upon partial removal of the homeobox gene \( \text{irx-1} \) (ref. 13), it is possible that in these reporters, potential IRX-1 binding sites are deleted. We have not pursued the effect of \( \text{irx-1} \) on \( \text{oig-1} \) expression as the lethality associated with complete loss of \( \text{irx-1} \) function complicates an analysis of \( \text{irx-1} \) null mutant phenotypes in D-type motor neurons.
Extended Data Figure 5 | \(\text{oig-1}\) mutants defects and their rescue. \(\text{a}\), \(\text{oig-1}\) mutants display locomotory defects. Locomotion of \(\text{L4}\) animals was analysed with tracking assays\(^{28}\). The graphs on the left side of each panel correspond to assays comparing wild-type, \(\text{oig-1}\) mutant, and \(\text{oig-1}; \text{unc-30p::oig-1}\) animals. The graphs on the right side of each panel correspond to assays comparing wild-type, \(\text{oig-1}\) mutant, and \(\text{oig-1}; \text{oig-1fosmid::gfp}\) animals. Twenty animals (each dot on a plot) were tracked for each genotype for both comparisons. Mean and \(Q\) values are indicated. Note that in a previously published analysis of a large panel of available mutants, the same set of locomotory defects that we describe here for \(\text{oig-1}\) mutants were found to be affected in \(\text{unc-55}\) mutants\(^{28}\), albeit in a stronger manner than \(\text{oig-1}\) mutants. Also note that the very strong locomotory defects \(\text{unc-30}\) defects are qualitatively very different from \(\text{oig-1}\) mutants, but this is to be expected as \(\text{unc-30}\) mutants do not only show the synaptic defects that we describe here, but also lack the neurotransmitter GABA (ref. 3), thereby disabling any neuromuscular signalling. Top, The midbody speed of \(\text{oig-1}\) mutant animals is significantly lower than that of wild-type animals. This defect is partially rescued (statistically different from \(\text{oig-1}\) mutants but also from wild-type animals) by expressing \(\text{unc-30p::oig-1}\) in \(\text{oig-1}\) animals (left graph). The lower midbody speed of \(\text{oig-1}\) mutants is completely rescued (statistically different from \(\text{oig-1}\) mutants but not from wild-type animals) by expressing the \(\text{oig-1fosmid::gfp}\) in \(\text{oig-1}\) mutants. \(\text{b}\), Aldicarb-sensitivity defects in \(\text{oig-1}\) mutants. \(\text{oig-1}\) mutant young adult animals (red squares), which display aberrant GABAergic synapses in both the ventral and dorsal cord, show hypersensitivity to aldicarb-induced paralysis compared to wild-type animals. This defect is not rescued by expressing \(\text{unc-30p::oig-1}\) in \(\text{oig-1}\) animals (left graph). The increased path curvature of \(\text{oig-1}\) mutants is completely rescued (statistically different from \(\text{oig-1}\) mutants but not from wild-type animals) by expressing the \(\text{oig-1fosmid::gfp}\) in \(\text{oig-1}\) mutants. \(\text{c}\), Expression of \(\text{oig-1}\) in the D-type neurons rescues ectopic DD synapses in the dorsal nerve cord. At the L1 stage when only the DD MNs are present, SNB-1–GFP (from \(\text{juIs1-unc-25p::snb-1::gfp}\)) localizes to the ventral nerve cord (VNC) in wild-type animals (top left). Ectopic SNB-1–GFP puncta localize to the dorsal nerve cord (DNC) of \(\text{oig-1}\) mutant L1s (top right). This phenotype is rescued by expressing \(\text{oig-1}\) in the D-type MNs (\(\text{unc-30p::oig-1}, \text{otEx4955}\), bottom left), but not by expressing \(\text{oig-1}\) in the neighbouring cholinergic MNs (\(\text{unc-3p::oig-1}, \text{otEx4942}\), bottom right). Original magnification, \(\times630\); white boxes indicate the dorsal nerve cord.
Extended Data Figure 6 | ACR-12 is mislocalized in lin-14 and unc-55 mutants. Cholinergic innervation to the D-type MNs is visualized with an unc-47p::acr-12::gfp reporter transgene, maintained in an acr-12(ok367) mutant background17. a, ACR-12 puncta localization is affected by loss of lin-14. In wild-type L1 animals, ACR-12 puncta are observed only in the DD neurons in the dorsal nerve cord (DNC) (left). In lin-14 mutant L1 animals (scored in progeny from lin-14 null animals carrying a lin-14 rescue array23), ACR-12 puncta are detected in the ventral nerve cord (VNC) of the DD MNs. Quantification of this data are represented in the graph. Some dorsal puncta in the DD MNs were still observed in 83% of the lin-14 mutant L1s that had puncta in the ventral nerve cord. L1 animals were obtained by hypochlorite-treating gravid adult animals and letting embryos hatch and arrest in M9 for 16–18 h. n > 20 for each strain scored, **P < 0.01. b, ACR-12 puncta localization is affected by loss of unc-55. In wild-type L4 animals, ACR-12 puncta are observed in both the ventral (VNC) and dorsal (DNC) nerve cords (left). In unc-55 mutant L4 animals, ACR-12 puncta are observed mostly in the ventral nerve cord of unc-55 mutants. Ventral and dorsal nerve cords are marked by red dotted lines. Quantification of this data are represented in the graph. n > 20 for each strain, **P < 0.01, original magnification, ×630.
Extended Data Figure 7 | The IgC2 domain is necessary for OIG-1 function. At the L1 stage when only the DD MNs are present, ectopic SNB-1–GFP puncta (from *juIs1-unc-25p::SNB-1::GFP*) localize to the dorsal nerve cord (DNC) of oig-1 mutant L1s (red bar) but not in wild-type animal (black bar) (see Fig. 3a). Based on an alignment with the hidden Markov model (HMM) Ig domain (top), a highly conserved residue (W75) and a nonconserved residue (E64) in the OIG-1 Ig domain were mutated in the context of an *unc-30p::oig-1* transgene that is able to rescue the L1 ectopic synapse defects (see Fig. 3a). The *unc-30p::oig-1E64A* transgenes (*otEx6212, otEx6213*) were still able to rescue the synaptic defects of oig-1 L1 animals (green bars), whereas the *unc-30p::oig-1W75A* transgenes (*otEx6214, otEx6215*) had no rescue ability (blue bars). L1 animals were obtained by hypochlorite-treating gravid adult animals and letting embryos hatch and arrest in M9 for 16–18 h. *n > 20* for each strain scored, **P < 0.01, *P < 0.05.

(See explanation in the main text.)
Extended Data Figure 8 | OIG-1 localization in other neuron types. OIG-1–GFP (from oig-1fosmid::gfp) localizes in a punctate manner along axons in the nerve ring (blue arrow) and along a pair of neurons in the pharynx, tentatively identified as the M2 MNs (red arrows point to cell body and process). These neurons form synapses onto pharyngeal muscles along their processes, and these processes also show punctate localization of OIG-1. Original magnification, ×630.
Extended Data Figure 9 | OIG-1 is mislocalized in sad-1 and strd-1 mutants. OIG-1–GFP (from oig-1fosmid::gfp) localizes to the ventral nerve cord (VNC) of wild-type L1 animals (left). In sad-1 mutants (middle), OIG-1–GFP is ectopically localized to the dorsal side (DNC) of L1 animals. In strd-1 mutants, OIG-1–GFP is ectopically localized to the dorsal side of L1 animals. Quantification of the data are shown in graph. n = 20 for each strain scored, **P < 0.01, original magnification, ×630.