Supplemental Information

A set of hub neurons and non-local connectivity features support global brain dynamics in *C. elegans*

Kerem Uzel, Saul Kato, and Manuel Zimmer
vs. fasted worms (Skora et al., 2018)
vs. L4 worms (Nichols et al., 2017)
vs. starved worms (Skora et al., 2018)

Deviation from the mean
0.05 0.2 0 0.15 0.25 0.3
Probability
0.04 0.02 0.14 0.1 0.12 0.06 0.16 0

SMDDR
SMDVR
SMDVL
URYVR
URYDR
URYVL
URYDL
SABVR
URYVL
SABVL

RMED
RMER
SIADR
SIAVR
SABD
RMEV
SIAVL
SIADL

PHAR
AVAR
PVNR
DB01
PHAL
ASKR
ASKL
PVNL
DB07
AVBR
ALNR
AVFR

RMEL
RMEV
SIAVL
SIADL

SABD
DA01
VD13
DA09
VA12
DA07
VA11
AS10
AVEL

RIML
RIVL
RIMR

ALA
AS10
AVEL

PDA
PHAR
PHAL

DVC
PDA
ALA

DVB
DVC
AVFL
AVFR
ALNL
AVFL

AIBR
RIVR
RIBR
RIBL
AIBL
RIVL

DA07
VA12
DA09
VD13
DA01
SABVL
URYVL
SABVR
URYVR
URYDL
SABVR
URYVL
SABVL

Correlation Coefficient (DatasetX)

Correlation Coefficient (DatasetY)

rs = 0.75
rs = 0.79
rs = 0.80
rs = 0.82

Reconstructed Correlations

Residual Correlations

Average Correlation Value (Previous Study)

Average Correlation Value (This Study)

Motif ID

(p = 0.0001) (p = 0.0001) (p = 0.0001)
Figure S1. Robustness of correlation values within WT datasets and compared to previous studies, related to Figure 1.

(A) Left: Matrix that contain number of observations (n= 2-6, see colorbar) for the neuronal pairs in Figure 1E. Neurons are sorted like in Figure 1E.

Right: Frequency histogram of number of observations for neuronal pairs from WT recordings.

(B) Scatter dot plot of correlation values of same neuronal pairs across 6 WT datasets (n=7930 datapoints). r_s, Spearman’s rank correlation coefficient.

(C) Left: Scatter dot plot of correlation values of neuronal pairs across 6 WT datasets and their deviation from the mean after a leave-one-out resampling procedure.

Right: Frequency histogram of the deviation from the mean for each correlation value after a leave-one-out resampling procedure.

(D) Scatter dot plot of average correlation values of same neuronal pairs in this study vs. different conditions from indicated previous studies S1,S2. r_s, Spearman’s rank correlation coefficient.

(E) Pearson correlation matrix of residual activity (Left) and reconstructed activity (Right) from neurons averaged across datasets. Residual activity was calculated by subtracting the first PC mode and reconstructed activity was calculated by projecting the data onto first PC mode (n=6 independent recordings of different worms). Correlation coefficients indicated by color key. Neurons are sorted like in Figure 1E. Black cells indicate pairs that were never observed simultaneously.

(F) Pairwise correlation grouped by all dyad connection patterns. Dots show average correlations of individual neuronal pairs, red bars indicate median with interquartile range. Asterisks indicate significance levels after a custom shuffle test. For all tests in this Figure: ****, p < 0.0001; ***, p < 0.001, * p < 0.05. Numbers in parentheses indicate number of individual neuron pairs in scatter plots.
Figure S2. Over-represented triplet connectivity motifs have a functional role in the network, related to Figure 2.

(A) The ratio of the triplet connectivity motifs compared to the mean of a random network ensemble (n=10⁵). Blue squares indicate the occurrence of motifs in the C. elegans connectome and red crosses indicate occurrences in randomized networks. Black and green asterisks indicate over-represented and under-represented motifs after single-step min p procedure for multiple hypothesis correction (see Methods), respectively. * p < 0.05.

(B) Pairwise correlation grouped by motif membership. Dots show correlations of individual neuronal pairs, red bars indicate median with interquartile range. Asterisks indicate significance levels after a custom shuffle test. ****, p < 0.0001; * p < 0.05, ns, not significant. Numbers in parentheses indicate number of individual neuron pairs in scatter plots.

(C-D) List of significant motifs calculated on only positively and negatively correlated pairs, respectively. Red shades indicate motifs with a common input. Black box indicates the motifs that predict significantly higher correlations than the combined set of all common input motifs. Blue asterisks indicate motifs that are over-represented in the connectome compared to random networks.
Top, left: totally randomized networks.
Top, right: degree preserved randomized networks (also used in Fig 4).
Bottom, left: degree, reciprocal connections and gap junction likelihood preserved randomized networks.
Bottom, right: rich-club curve preserved network.
Figure S3. Analysis of input similarities according to PC weights, residual activity and different cell class identities and raw p values for the systematic survey of structure-function relationships, related to Figure 3 and 4.

(A) Correlation analysis between pairwise correlations calculated from residual activity of neurons (Figure S1E) and indicated connectivity feature. $r_s$, Spearman’s rank correlation coefficient. Permutation test p values estimates probability that $r_s$ is found in degree preserved randomized networks. Left: $1^{\circ}$ input similarity is used as the connectivity measure. Right: $2^{\circ}$ input similarity is used as the connectivity measure.

(B-C) Frequency histograms for $1^{\circ}$ and $2^{\circ}$ input similarities (cosine distance) in the C. elegans connectome. First column: all pairs in the network, n = 38781. Second column: pairs between sensory neurons, n = 3655. Third column: pairs between interneurons, n = 2556. Fourth column: pairs between motorneurons, n = 7021.

(D-E) Frequency histograms for $1^{\circ}$ and $2^{\circ}$ input similarities of all pairs in randomized networks.

(F) Frequency histograms of structure-function relationships calculated from single neuron types. Black vertical dashed lines indicate means of the distributions. Vertical red lines indicate the corresponding $r_s$ values in Figure 3C and F. Left: $1^{\circ}$ input similarity is used as the connectivity measure. Right: $2^{\circ}$ input similarity is used as the connectivity measure. $r_s$, Spearman’s rank correlation coefficient.

(G) Related to Figure 4. Columns: connectivity metrics. Rows functional interaction metrics ($\Delta F$, change in fluorescence; $d\Delta F / dt$, time derivative of change in fluorescence). 4 entries in each cell show raw p values for permutation test indicating probability that $\geq r_s$ value in Figure 4 of the corresponding cell was found in 4 different randomized networks. Top, left: totally randomized networks, size preserved (same number of nodes and links as the experimental connectome). Top, right: size and degree preserved randomized networks (also used in Figure 4). Bottom, left: size, degree, reciprocal connections and gap junction likelihood preserved randomized networks. Bottom, right: size, degree distribution, reciprocal connections and gap junction likelihood, and rich-club curve preserved networks. $+, p < 10^{-5}$.
Rich Club Ratio of B

Significant Motifs: 53% (p=0.06)
Insignificant Motifs: 32% (p=0.60)
All Motifs: 33%

Within-Communities
Between-Communities

Absolute Correlation Coefficient

ns

p = 0.1450
Figure S4. Rich-club neurons within the combined network and community structure in the connectome, related to Figure 5.

(A) The blue line indicates the rich-club coefficient (RCC) of the *C. elegans* network (gap junctions + chemical synapses). Red line and shade indicate the mean RCC and standard deviation (SD) for $10^3$ random graphs, respectively. The green line and shade indicate normalized RCC and SD, respectively. The vertical dashed line indicates the change point in the normalized RCC. Neurons above the threshold were defined as rich club neurons: AVAL/R, AVBL/R, PVCL/R, AVDL/R, DVA, AVEL/R, AIBL/R, RIAL/R, RIBL, HSNR, RIH, RIMR, AVJL, AVKL.

(B) Top: An example triplet motif. Black arrow indicates chemical synapse (directed), red line indicates gap junctions (undirected). Bottom: Quantification of the rich club ratio (RCR), the fraction across all significant motifs where neuron B is a rich club member. Permutation test p value estimates probability that RCR was obtained by chance in motif ID shuffled $10^5$ trials.

(C) Top 50 neurons’ occurrences as the source neurons for 1° (top) and 2° (bottom) common input. Rich club neurons are indicated by red bars.

(D) Betweenness centrality values for the top 50 neurons in the network. Rich club neurons are indicated by red bars.

(E) Left: Unweighted connectivity matrix of the *C. elegans* connectome sorted by identified communities $^{33}$. Middle: Pearson correlation matrix of identified neurons averaged across datasets (n=6 independent recordings of different worms) from Figure 1E re-sorted by identified communities in $^{33}$. Communities are annotated by different colors. Correlation coefficients indicated by color key. Black boxes indicate pairs that are never observed simultaneously.

Right: Pairwise correlation grouped as within-communities and between-communities. L-R pairs are excluded from the analysis. Dots show average correlations of individual neuronal pairs, red bars indicate median with interquartile range. Comparison was made with a custom shuffle test (Methods). ns, not significant. Numbers in parentheses indicate number of individual neuron pairs in scatter plots.
Figure S5. Neuronal activity levels of individual neurons in wild type (WT), multi- and single-neuron inhibition lines, related to Figure 6.

(A-J) Left: Neuronal activity levels (RMS) of identified neurons in WT vs. inhibition datasets. Neurons are sorted by WT values in descending order. Error bars indicate SEM. Comparisons were made by t-test. *p < 0.05, **p < 0.01, ***p < 0.001.

Right: Representative examples of 18-min-long whole-brain imaging recordings from indicated inhibition lines, shown as heat maps of fluorescence (DF/F_0) time series of all detected neurons. One neuron per row, sorted by neuronal activity levels (RMS).
Figure S6. Behavioural analysis and differential effects on backward and forward clusters of the inhibition lines, related to Figure 6.

(A) Quantification of population behavioural assays of the indicated inhibition line without (Ctrl) or with (+His) histamine treatment. Top: forward speed. Bottom: reversal events per second. Each data point represents a single assay, numbers in parentheses indicate number of individual assays, n = 20–25 worms per assay. Horizontal lines and error bars show mean and SD, respectively. Asterisks indicate significance levels after Mann-Whitney test.

(B) Response of WT worms and indicated inhibition lines without (Ctrl) and with (+His) histamine treatment in touch reflex assays. Each data point represents a single assay, n = 5 worms per assay. Horizontal lines and error bars show mean and SD, respectively. Asterisks indicate significance levels after Kruskal-Wallis test with Dunn’s multiple-comparisons test. Left: anterior harsh touch. Right: posterior harsh touch. For all tests in this Figure: ****, p < 0.0001; ***, p < 0.001; ** p < 0.01, ns, not significant.

(C-L) Frequency distribution of average pairwise correlations for WT and indicated inhibition lines. Left, correlations within backward cluster; middle, correlations within forward cluster; right, correlations between the clusters (for cluster identification, see Methods). Permutation test p values estimate the probability that KS distance between two distributions was obtained by chance.
Figure S7. Effect of RIM inhibition on the backward-movement interneurons and different pairs are selectively perturbed in the network in inhibition lines, related to Figure 6 and 7.

(A) Number of forward to backward command state transitions in whole-brain recordings of WT and RIM-inhibition line (n=6 and n=5, respectively). Dots show individual recordings, black lines indicate means and error bars indicate SD. Asterisks indicate significance level after Mann-Whitney test. ** p < 0.01.

(B) Traces of reversal neurons and RIB (representing the forward neurons). Neuron names are indicated in the legend. Top: a representative WT recording. Bottom: a representative RIM-inhibition line recording. Black arrows indicate events where AIB is active but the rest of the reversals neurons are not active.

(C) Stacked bar plot of different percentages of reversal states. Black indicates reversals where AVA and AIB neurons are both active. Grey indicates brain states where AVA and rest of the reversal neurons are not active but AIB is active.

(D) Neuronal circuit of AIB-RIM-AVA-AVE neuron classes. Black arrow indicates chemical synapse (directed), red line indicates gap junctions (undirected). The width of the lines indicates strength of the connection: the number of synapses / gap junctions are indicated next to the connection.

(E-G) Top: frequency histograms of the percent change (WT vs. inhibition line) for indicated connectivity measure. Red lines indicate complementary cumulative distribution. Bottom: Permutation p values estimate the probability that KS distance between two distributions in Figure 7F was obtained in degree preserved randomized networks for indicated cutoff defining perturbed vs control pairs. (A) Sum of inverse shortest paths. (B) 1° input similarity (C) 2° input similarity. Y-axes are cut-off at value 0.1.

(H-J) Frequency histograms of the percent change (WT vs inhibition line) for connectivity exhibited by left-right and all pairs. Red lines indicate cumulative distribution. (D) Sum of inverse shortest paths. (E) 1° input similarity (F) 2° input similarity. Distributions for all-pairs are identical to (A-C) respectively, but no cutoff to y-axes were applied.

(K-L) Pairwise correlations grouped by left-right pairs vs all remaining pairs in the network for indicated inhibition lines. Dots show correlations of individual neuronal pairs, red bars indicate median with interquartile range. Asterisks indicate significance level after a custom shuffle test. ****, p < 0.0001. Numbers in parentheses indicate number of individual neuron pairs in scatter plots.
Table S1. Systematic survey of structure-function relationships calculated with the connectome reconstruction from Cook et al., 2019 related to Figure 4. Columns: connectivity metrics. Rows: functional interaction metrics (CC, correlation coefficient; COV, covariance; XCC, peak cross correlation; MI, mutual information; ΔF, change in fluorescence; dΔF / dt, time derivative of change in fluorescence). rs indicates Spearman’s rank correlation coefficients. Asterisks show permutation test p values (after Benjamini-Hochberg correction) indicating probability that ≥ rs value was found in degree-preserved randomized networks. Percentages indicate the maximum range of variability in the connectome that is tolerated to obtain a result not significantly different (see Methods).

|                  | Direct Connectivity | sum of inverse shortest path | 1’ input similarity | 2’ input similarity | total count of common inputs |
|------------------|---------------------|-----------------------------|--------------------|--------------------|-----------------------------|
|                  | (weighted)          | (unweighted)                | (weighted)         | (unweighted)       | (weighted)                  |
| **Covariogram**  |                     |                             |                    |                    |                             |
| Analysis         | rs = 0.18           | rs = 0.19                   | rs = 0.22          | rs = 0.28          | rs = 0.31                   |
|                  | 5%                  | 5%                          | 5%                 | 5%                 | 5%                          |
| Covariogram      | rs = 0.17           | rs = 0.18                   | rs = 0.26          | rs = 0.30          | rs = 0.27                   |
|                  | 5%                  | 5%                          | 5%                 | 15%                | 10%                         |
| **XCC**          |                     |                             |                    |                    |                             |
| dΔF / dt         | rs = 0.20           | rs = 0.20                   | rs = 0.25          | rs = 0.29          | rs = 0.27                   |
|                  | 5%                  | 5%                          | 5%                 | 10%                | 10%                         |
| **ΔF**           |                     |                             |                    |                    |                             |
| dΔF / dt         | rs = 0.20           | rs = 0.20                   | rs = 0.25          | rs = 0.29          | rs = 0.27                   |
|                  | 5%                  | 5%                          | 5%                 | 5%                 | 5%                          |
| **COV**          |                     |                             |                    |                    |                             |
| dΔF / dt         | rs = 0.28           | rs = 0.29                   | rs = 0.38          | rs = 0.35          | rs = 0.36                   |
|                  | 5%                  | 5%                          | 5%                 | 5%                 | 15%                         |
| **ΔF**           |                     |                             |                    |                    |                             |
| dΔF / dt         | rs = 0.27           | rs = 0.27                   | rs = 0.37          | rs = 0.36          | rs = 0.44                   |
|                  | 5%                  | 5%                          | 5%                 | 5%                 | 15%                         |
| **XCC**          |                     |                             |                    |                    |                             |
| dΔF / dt         | rs = 0.20           | rs = 0.20                   | rs = 0.25          | rs = 0.29          | rs = 0.27                   |
|                  | 5%                  | 5%                          | 5%                 | 5%                 | 10%                         |
| **ΔF**           |                     |                             |                    |                    |                             |
| dΔF / dt         | rs = 0.20           | rs = 0.20                   | rs = 0.25          | rs = 0.29          | rs = 0.27                   |
|                  | 5%                  | 5%                          | 5%                 | 5%                 | 5%                          |

rs indicates Spearman’s rank correlation coefficients. Asterisks show permutation test p values (after Benjamini-Hochberg correction) indicating probability that ≥ rs value was found in degree-preserved randomized networks. Percentages indicate the maximum range of variability in the connectome that is tolerated to obtain a result not significantly different (see Methods).

p<0.01, ** for all structure-function combinations in degree preserved randomized networks
Table S2. Comparison of the p values calculated with two reconstructions of the *C. elegans* connectome related to Figures 1,2,3,7.
The instance where a difference in both reconstructions were observed is written in bold.

| Figure | Panel | Result | Reconstruction from Varshney et al., 2011 (Used in the figures) | Reconstruction from Cook et al., 2019 |
|--------|-------|--------|---------------------------------------------------------------|-------------------------------------|
| Figure 1 | d | RMS vs. In-degree of neurons | \( r_s = 0.433 / p < 0.0001, \) **** | \( r_s = 0.456 / p < 0.0001, \) **** |
| Figure 2 | a | Gap junctions vs. chem syn. only | \( p < 0.0001, \) **** | \( p = 0.0003, \) **** |
| | | Gap junctions vs. non connected | \( p < 0.0001, \) **** | \( p < 0.0001, \) **** |
| | | Chemical only vs. non connected | \( p = 0.0004, \) **** | \( p = 0.0004, \) **** |
| | c | Sum of Connections vs. Pairwise Correlation | \( r_s = 0.179 / p < 10^{-5}, \) **** | \( r_s = 0.178 / p < 10^{-5}, \) **** |
| | d | Overrepresented vs. Significant motifs | 10/20, \( p = 0.057, \) ns | 9/16, \( p = 0.104, \) ns |
| | | Common input motifs vs. Significant motifs | 18/20, \( p = 0.003, \) ** | 13/16, \( p = 0.0075, \) ** |
| Figure 3 | c | 1° input similarity vs. Pairwise Correlation | \( r_s = 0.35 / p < 10^{-5} \) **** | \( r_s = 0.27 / p < 10^{-5} \) **** |
| | f | 2° input similarity vs. Pairwise Correlation | \( r_s = 0.48 / p < 10^{-5} \) **** | \( r_s = 0.38 / p < 10^{-5} \) **** |
| Figure 7 | b | Control vs. Targeted Motifs | \( p = 0.91, \) ns | \( p = 0.49, \) ns |
| | | Control vs. Intact Motifs | \( p < 0.0001, \) **** | \( p = 0.047, \) * |
| | | Targeted Motifs vs. Intact Motifs | \( p = 0.029, \) * | \( p = 0.099, \) ns |
| | c | WT vs. Inhibition (intact motifs) | \( p < 0.0001, \) **** | \( p < 0.0001, \) **** |
| | d | Control vs. Targeted Motifs | \( p = 0.64, \) ns | \( p = 0.71, \) ns |
| | | Control vs. Intact Motifs | \( p < 0.0001, \) **** | \( p = 0.007, \) ** |
| | | Targeted Motifs vs. Intact Motifs | \( p = 0.0017, \) ** | \( p = 0.048, \) * |
| | e | WT vs. Inhibition (intact motifs) | \( p = 0.0014, \) ** | \( p = 0.039, \) * |
| | f | Sum of inverse shortest path, control pairs | \( p = 0.99, \) ns | \( p = 0.99, \) ns |
| | | Sum of inverse shortest path, perturbed pairs | \( p < 0.001, \) *** | \( p = 0.00097, \) *** |
| | | 1° input similarity, control pairs | \( p = 0.98, \) ns | \( p = 0.99, \) ns |
| | | 1° input similarity, perturbed pairs | \( p = 0.010, \) * | \( p = 0.012, \) * |
| | | 2° input similarity, control pairs | \( p = 0.98, \) ns | \( p = 0.99, \) ns |
| | | 2° input similarity, perturbed pairs | \( p = 0.010, \) * | \( p = 0.036, \) * |
| Figure S1 | f | Column 1 vs. Column2 | \( p < 0.0001, \) **** | \( p < 0.0001, \) **** |
| | | Column 1 vs. Column3 | \( p = 0.0003, \) *** | \( p = 0.0003, \) *** |
| | | Column 1 vs. Column4 | \( p = 0.0001, \) **** | \( p = 0.0005, \) **** |
| | | Column 2 vs. Column3 | \( p < 0.0001, \) **** | \( p = 0.0002, \) *** |
| Figure S2 | b | Other motifs vs. Under-represented mot. | \( p = 0.7375, \) ns | \( p = 0.4854, \) ns |
| Figure S4 | b | Significant motifs’ rich-club ratio | 53% \( / p = 0.06 \) | 35% \( / p = 0.196 \) |
| | | Insignificant motifs’ rich-club ratio | 32% \( / p = 0.60 \) | 27% \( / p = 0.38 \) |
| | | All motifs’ rich-club ratio | 33% | 27% |
Table S3. Comparison of the $r_1$ and $p$ values calculated with absolute, only positive and only negative correlations, related to Figures 2,3,5,7.
The instances where a difference compared to results using absolute correlations were observed are written in bold.

| Figure | Panel | Result | Result using absolute correlation coefficients | Result restricted to only positive correlations | Result restricted to only negative correlations |
|--------|-------|--------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Figure 2 | a | Gap junctions vs. chemical syn. only | $p < 0.0001$, **** | $p < 0.0001$, **** | $p = 0.65$, ns Note 1 |
| | | Gap junctions vs. non connected | $p < 0.0001$, **** | $p < 0.0001$, **** | $p = 0.92$, ns Note 1 |
| | | Chemical only vs. non connected | $p = 0.0004$, *** | $p = 0.0021$, ** | $p = 0.07$, ns |
| | c | Sum of Connections vs. Pairwise Correlation | $r_s = 0.179 / p < 10^{-5}$, **** | $r_s = 0.209 / p < 10^{-5}$, **** | $r_s = 0.112 / p = 0.003$, ** |
| | e | Common input motifs vs. Non common input motifs | $p < 0.0001$ **** | $p < 0.0001$, **** | $p = 0.005$, ** |
| | f | All pairs vs. L-R pairs | $p < 0.0001$ **** | $p < 0.0001$, **** | N/A Note 2 |
| Figure 3 | c | 1° input similarity vs. Pairwise Correlation | $r_s = 0.35 / p < 10^{-5}$ **** | $r_s = 0.45 / p < 10^{-5}$ **** | $r_s = 0.19 / p < 10^{-5}$ **** |
| | f | 2° input similarity vs. Pairwise Correlation | $r_s = 0.48 / p < 10^{-5}$ **** | $r_s = 0.57 / p < 10^{-5}$ **** | $r_s = 0.33 / p < 10^{-5}$ **** |
| Figure 5 | d | within non-hubs vs. across | $p < 0.0001$, **** | $p < 0.0001$, **** | $p < 0.0001$, **** |
| | | across vs. within hubs | $p < 0.0001$, **** | $p < 0.0001$, **** | $p < 0.0001$, **** |
| | | within non-hubs vs. within hubs | $p < 0.0001$, **** | $p < 0.0001$, **** | $p < 0.0001$, **** |
| Figure 7 | b | Control vs. Targeted Motifs | $p = 0.91$, ns | $p = 0.28$, ns | $p = 0.19$, ns |
| | | Control vs. Intact Motifs | $p < 0.0001$, **** | $p = 0.020$, * | $p < 0.0001$, **** |
| | c | WT vs. Inhibition (intact motifs) | $p < 0.0001$, **** | $p < 0.0001$, **** | $p < 0.0001$, **** |
| | d | Control vs. Targeted Motifs | $p = 0.64$, ns | $p = 0.99$, ns | $p = 0.006$, ** |
| | | Control vs. Intact Motifs | $p < 0.0001$, **** | $p < 0.0001$, **** | $p < 0.0001$, **** |
| | | Targeted Motifs vs. Intact Motifs | $p = 0.0017$, ** | $p = 0.0002$, *** | $p = 0.005$, ** |
| | e | WT vs. Inhibition (intact motifs) | $p = 0.0014$, ** | $p < 0.0001$, **** | $p = 0.029$, * |
| | f | Sum of inverse shortest path, control pairs | $p = 0.99$, ns | $p = 0.99$, ns | $p = 0.99$, ns |
| | | Sum of inverse shortest path, perturbed pairs | $p < 0.001$, *** | $p < 0.0001$, **** | $p < 0.0001$, **** |
| | | 1° input similarity, control pairs | $p = 0.98$, ns | $p = 0.29$, ns | $p = 0.91$, ns |
| | | 1° input similarity, perturbed pairs | $p = 0.01$, * | $p < 0.0001$, **** | $p = 0.01$, * |
| | | 2° input similarity, control pairs | $p = 0.98$, ns | $p = 0.59$, ns | $p = 0.99$, ns |
| | | 2° input similarity, perturbed pairs | $p = 0.01$, * | $p = 0.01$, * | $p = 0.002$, ** |
| Figure S1 | f | Column 1 vs. Column2 | $p < 0.0001$, **** | $p < 0.0001$, **** | $p = 0.92$, ns Note 1 |
| | | Column 1 vs. Column3 | $p = 0.0003$, *** | $p = 0.0021$, ** | $p = 0.06$, ns Note 1 |
| | | Column 1 vs. Column4 | $p = 0.0001$, **** | $p = 0.0002$, *** | $p = 0.77$, ns Note 1 |
| | | Column 2 vs. Column3 | $p < 0.0001$, **** | $p = 0.0008$, *** | $p = 0.65$, ns Note 1 |
| Figure S2 | b | Other motifs vs. Under-represented motifs | $p = 0.74$, ns | $p = 0.27$, ns | $p = 0.009$, ** |
| Figure | Notes | Key | Other motifs vs. Over-represented motifs | $p < 0.0001, ****$ | $p < 0.0001, ****$ | $p = 0.30, ns$
|--------|-------|-----|---------------------------------------|------------------|------------------|------------------|
| **S3** | b     | 1° input similarity vs. Pairwise Correlation (Residual activity) | $r_s = 0.184 / p < 10^{-5} ****$ | $r_s = 0.233 / p < 10^{-5} ****$ | $r_s = 0.145 / p < 10^{-5} ****$
|        |       | 2° input similarity vs. Pairwise Correlation (Residual activity) | $r_s = 0.329 / p < 10^{-5} ****$ | $r_s = 0.379 / p < 10^{-5} ****$ | $r_s = 0.297 / p < 10^{-5} ****$
| **S4** | c     | within-communities vs. between communities | $p = 0.15, ns$ | $p = 0.64, ns$ | $p = 0.30, ns$

**Note 1** Only a small fraction of negatively correlated pairs are connected with a gap junction ($n=7, 11\%$) (See **Figure 2B**). Therefore, results from this analysis should be interpreted with caution.

**Note 2** None of the L-R pairs in this analysis were negatively correlated.

**Note 3** For negative correlations, the number of significant motifs is only 11, hence the number of targeted motifs is very low ($n=9$, AVA+AVE+PVC+RIM inhibition). Therefore, results from this analysis should be interpreted with caution.
Table S4: Structure-function relationships calculated with combined (chemical synapses + gap junctions) and separate networks, related to Figure 4. Columns: connectivity metrics. Rows: Different networks of connections. As the functional interaction measure, correlation coefficient calculated on time derivative of change in fluorescence is used for all calculations (first row is the same with first row of Figure 4). $r_s$ indicates Spearman’s rank correlation coefficients. Asterisk show permutation test p values (after Benjamini-Hochberg correction) indicating probability that ≥ $r_s$ value was found in degree preserved randomized networks.

|                      | Direct Connectivity | sum of inverse shortest path | 1' input similarity | 2' input similarity |          |          |          |          |
|----------------------|---------------------|-----------------------------|---------------------|---------------------|----------|----------|----------|----------|
|                      | (weighted)          | (unweighted)                | cosine similarity   | cosine similarity   | total count of common inputs | cosine similarity | cosine similarity | total count of common inputs |
| Combined Network     | $r_s = 0.18$        | $r_s = 0.18$                | $r_s = 0.31$        | $r_s = 0.36$        | $r_s = 0.34$ | $r_s = 0.44$ | $r_s = 0.48$ | $r_s = 0.48$ |
| Chemical Synapse Network | $r_s = 0.13$        | $r_s = 0.13$                | $r_s = 0.05$        | $r_s = 0.24$        | $r_s = 0.25$ | $r_s = 0.26$ | $r_s = 0.32$ | $r_s = 0.35$ | $r_s = 0.37$ |
| Gap Junction Network | $r_s = 0.15$        | $r_s = 0.15$                | $r_s = 0.31$        | $r_s = 0.27$        | $r_s = 0.26$ | $r_s = 0.33$ | $r_s = 0.32$ | $r_s = 0.35$ |

$p<0.01$, ** for all structure-function combinations
Table S5. Systematic survey of structure-function relationships calculated with only positively or negatively correlated pairs, related to Figure 4. Columns: connectivity metrics. Rows: functional interaction metrics (CC, correlation coefficient; COV, covariance; XCC, peak cross correlation; MI, mutual information; ∆F, change in fluorescence; dΔF / dt; time derivative of change in fluorescence). rIndicates Spearman’s rank correlation coefficients. Permutation test p values (after Benjamini-Hochberg correction) indicate probability that ≥ rIndicates value was found in degree preserved randomized networks.

|                      | Direct Connectivity (weighted) | Direct Connectivity (unweighted) | sum of inverse shortest path cosine similarity (weighted) | sum of inverse shortest path cosine similarity (unweighted) | 1 input similarity cosine similarity (weighted) | 1 input similarity cosine similarity (unweighted) | total count of common inputs (weighted) | total count of common inputs (unweighted) | 2 input similarity cosine similarity (weighted) | 2 input similarity cosine similarity (unweighted) | total count of common inputs (weighted) | total count of common inputs (unweighted) |
|----------------------|-------------------------------|--------------------------------|----------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------|-----------------------------------------------|-------------------------------------------|-------------------------------------------|-----------------------------------------------|-----------------------------------------------|-------------------------------------------|-------------------------------------------|
| **only positively correlated pairs** |                               |                                |                                                          |                                                          |                                               |                                               |                                           |                                           |                                               |                                               |                                           |                                           |
| CC dΔF / dt          | 0.20                          | 0.21                           | 0.39                                                      | 0.46                                                     | 0.45                                          | 0.44                                          | 0.52                                      | 0.57                                      | 0.58                                          |                                               |                                           |                                           |
| CC ∆F                | 0.22                          | 0.22                           | 0.36                                                      | 0.38                                                     | 0.38                                          | 0.39                                          | 0.41                                      | 0.45                                      | 0.45                                          |                                               |                                           |                                           |
| COV dΔF / dt         | 0.31                          | 0.31                           | 0.46                                                      | 0.41                                                     | 0.42                                          | 0.48                                          | 0.47                                      | 0.57                                      | 0.63                                          |                                               |                                           |                                           |
| COV ∆F               | 0.31                          | 0.31                           | 0.48                                                      | 0.40                                                     | 0.40                                          | 0.47                                          | 0.44                                      | 0.52                                      | 0.58                                          |                                               |                                           |                                           |
| XCC dΔF / dt         | 0.24                          | 0.24                           | 0.43                                                      | 0.47                                                     | 0.46                                          | 0.45                                          | 0.55                                      | 0.61                                      | 0.61                                          |                                               |                                           |                                           |
| XCC ∆F               | 0.23                          | 0.23                           | 0.39                                                      | 0.41                                                     | 0.40                                          | 0.42                                          | 0.43                                      | 0.48                                      | 0.49                                          |                                               |                                           |                                           |
| Covariogram Analysis | 0.23                          | 0.23                           | 0.38                                                      | 0.39                                                     | 0.38                                          | 0.37                                          | 0.48                                      | 0.52                                      | 0.51                                          |                                               |                                           |                                           |
| **only negatively correlated pairs** |                               |                                |                                                          |                                                          |                                               |                                               |                                           |                                           |                                               |                                               |                                           |                                           |
| CC dΔF / dt          | 0.11                          | 0.11                           | 0.17                                                      | 0.19                                                     | 0.19                                          | 0.21                                          | 0.32                                      | 0.33                                      | 0.36                                          |                                               |                                           |                                           |
| CC ∆F                | 0.15                          | 0.15                           | 0.23                                                      | 0.22                                                     | 0.22                                          | 0.25                                          | 0.32                                      | 0.37                                      | 0.42                                          |                                               |                                           |                                           |
| COV dΔF / dt         | 0.20                          | 0.20                           | 0.38                                                      | 0.34                                                     | 0.34                                          | 0.41                                          | 0.37                                      | 0.50                                      | 0.55                                          |                                               |                                           |                                           |
| COV ∆F               | 0.19                          | 0.19                           | 0.35                                                      | 0.28                                                     | 0.28                                          | 0.35                                          | 0.36                                      | 0.46                                      | 0.52                                          |                                               |                                           |                                           |
| XCC dΔF / dt         | 0.03                          | ns                             | 0.09                                                      | 0.15                                                     | 0.15                                          | 0.18                                          | 0.25                                      | 0.30                                      | 0.36                                          |                                               |                                           |                                           |
| XCC ∆F               | 0.13                          | ns                             | ns                                                        | ns                                                       | ns                                            | ns                                            | ns                                        | ns                                        | ns                                            |                                               |                                           |                                           |
| Covariogram Analysis | 0.11                          | 0.11                           | 0.13                                                      | 0.17                                                     | 0.17                                          | 0.18                                          | 0.24                                      | 0.26                                      | 0.29                                          |                                               |                                           |                                           |

p < 0.01, ** for all structure-function combinations except cases marked as ns
Table S6. Structure-function relationships of input similarities restricted to neuron pairs within the specified categories, related to Figure 3. \( r_s \) indicates Spearman’s rank correlation coefficients. Asterisks show permutation test p values (after Benjamini-Hochberg correction) indicating probability that \( \geq r_s \) value was found in degree-preserved randomized networks.

| Category          | 1° input similarity (cosine similarity – unweighted) | 2° input similarity (cosine similarity – unweighted) |
|-------------------|-----------------------------------------------------|-----------------------------------------------------|
| only L-R pairs    | \( r_s = 0.62 \)                                    | \( r_s = 0.78 \)                                    |
| All non L-R pairs | \( r_s = 0.34 \)                                    | \( r_s = 0.47 \)                                    |
| Sensory neuron pairs | \( r_s = 0.18 \)                                 | \( r_s = 0.52 \)                                 |
| Interneuron pairs | \( r_s = 0.53 \)                                    | \( r_s = 0.71 \)                                    |
| Motorneuron pairs | \( r_s = 0.34 \)                                    | \( r_s = 0.29 \)                                    |

\( p < 0.0001, **** \) for all structure-function combinations
Table S7: Detailed list of strains used in this study, related to Figures 1, 6, S6.

| Strain name | Experiment | Genotype | Construct (plasmid no.) and injection concentrations | Promoter expression references | Figure Panel |
|-------------|------------|----------|------------------------------------------------------|--------------------------------|--------------|
| ZIM1428     | Whole-brain imaging | $zmIs52; lite-1 (ce314)$ | Punc-31::NLSGCaMP6f (pTS100) 2.5ng/μL; | $^{s4}$ | 1A-C,E       |
| ZIM805      | Whole-brain imaging, AIB- Inhibition | $zmEx199; mzmEx459; lite-1 (xu7)$ | Punc-31::NLSGCaMP5K (pTS36) 30ng/μL; PmEx-1::HisCl::SL2::mCherry 50 ng/μL; | $^{s5}$ | 6E-G; S6A    |
| ZIM756      | Whole-brain imaging, AVE- Inhibition | $zmEx199; kyEx4863; lite-1 (xu7)$ | Punc-31::NLSGCaMP6f (pTS100) 2.5ng/μL; Pm-122::gfp 15ng/μL; Prig-3::HisCl::SL2::mCherry (pNP471) 50 ng/μL | Prig-3 $^{s6}$ | 6E-G; S6A    |
| ZIM1860     | Whole-brain imaging, AVB- Inhibition | $zmIs52; lite-1 (ce314); mzmEx578$ | Punc-31::NLSGCaMP6f (pTS100) 2.5ng/μL; Pm-122::creVDH (pKU26) 30ng/μL; Mptm-1::DIO::CreVDH (pKU12) 30ng/μL; Psto-3::HisCl::SL2::mCherry (pRL123) 30ng/μL; Psto-3::HisCl::SL2::mCherry (pRL121) 30ng/μL; | $^{s8}$ | 6E-G; S6A    |
| ZIM2020     | Whole-brain imaging, AVI- Inhibition | $zmIs52; lite-1 (ce314); mzmEx1223$ | Punc-31::NLSGCaMP6f (pTS100) 2.5ng/μL; Pm-122::creVDH (pKU26) 30ng/μL; Mptm-1::DIO::CreVDH (pKU12) 30ng/μL; Psto-3::HisCl::SL2::mCherry (pRL123) 30ng/μL; Psto-3::HisCl::SL2::mCherry (pRL121) 30ng/μL; | Psto-3 $^{s9}$ | 6E-G; S6A    |
| ZIM755      | Whole-brain imaging, RIB- Inhibition | $zmEx199; mzmEx457; lite-1 (xu7)$ | Punc-31::NLSGCaMP5K (pTS36) 30ng/μL; Pm-122::creVDH (pKU26) 15ng/μL; Mptm-1::DIO::CreVDH (pKU12) 15ng/μL; Psto-3::HisCl::SL2::mCherry (pRL123) 30ng/μL; Psto-3::HisCl::SL2::mCherry (pRL121) 30ng/μL; | Psto-3 $^{s9}$ | 6E-G; S6A    |
| ZIM1720     | Whole-brain imaging, RIM- Inhibition | $zmIs52; lite-1 (ce314); mzmEx973; mzmEx976$ | Punc-31::NLSGCaMP6f (pTS100) 2.5ng/μL; Pm-122::creVDH (pKU26) 15ng/μL; Mptm-1::DIO::CreVDH (pKU12) 15ng/μL; Psto-3::HisCl::SL2::mCherry (pRL123) 30ng/μL; Psto-3::HisCl::SL2::mCherry (pRL121) 30ng/μL; | Psto-3 $^{s9}$ | 6E-G; S6A    |
| ZIM1810     | Whole-brain imaging, PVC- Inhibition | $zmIs52; lite-1 (ce314); mzmEx973; mzmEx974$ | Punc-31::NLSGCaMP6f (pTS100) 2.5ng/μL; Pm-122::creVDH (pKU26) 15ng/μL; Mptm-1::DIO::CreVDH (pKU12) 15ng/μL; Psto-3::HisCl::SL2::mCherry (pRL123) 30ng/μL; Psto-3::HisCl::SL2::mCherry (pRL121) 30ng/μL; | Psto-3 $^{s9}$ | 6E-G; S6A    |
| ZIM2312     | Whole-brain imaging, RIM+PVC Inhibition | $zmIs52; lite-1 (ce314); mzmEx973; mzmEx974; mzmEx976$ | Punc-31::NLSGCaMP6f (pTS100) 2.5ng/μL; Pm-122::creVDH (pKU26) 15ng/μL; Mptm-1::DIO::CreVDH (pKU12) 15ng/μL; Psto-3::HisCl::SL2::mCherry (pRL123) 30ng/μL; Psto-3::HisCl::SL2::mCherry (pRL121) 30ng/μL; | Psto-3 $^{s9}$ | 6E-G; S6A    |
| ZIM1807 | Whole-brain imaging, AVA+AVE+RIM+PVC Inhibition | \( \text{zmIs52; lite-1 (ce314); zmEx462} \) | \( \text{Punc-31::NLSGCaMP6f (pTS100) 2.5ng/ul; Pcen-1::HisCl::SL2::mCherry (pRL122) 50ng/μL; Pflp-17::mCherry 1.5ng/μL} \) | 6A,C; S6A,B |
|---|---|---|---|---|
| ZIM1935 | Whole-brain imaging, AVB+RIB+AIB Inhibition | \( \text{zmIs52; lite-1 (ce314); zmEx463; zmEx578; zmEx1114} \) | \( \text{Punc-31::NLSGCaMP6f (pTS100) 2.5ng/ul; Psto-3::HisCl::SL2::mCherry (pKH170) 5ng/μL; Punc-122::dsRed 19ng/μL; Psra-11::HisCl::SL2::mCherry (pRL123) 50ng/μL; Pflp-17::mCherry 1.5ng/μL; Pinx-1::HisCl::SL2::mCherry (pKU31) 50ng/μL; Pelt-2::NLSDsRed 5ng/μL} \) | 6B,D; S6A; Psra-11\(^{87}\); Psto-3\(^{89}\) |
| ZIM1997 | Whole-brain imaging, NeuroPAL | \( \text{zmIs52; lite-1 (ce314); otIs670} \) | \( \text{Punc-31::NLSGCaMP6f (pTS100) 2.5ng/ul; otIs670[NeuroPAL] V 8x outcrossed} \) | \(^{84}\) NeuroPAL strain\(^{815}\) |
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