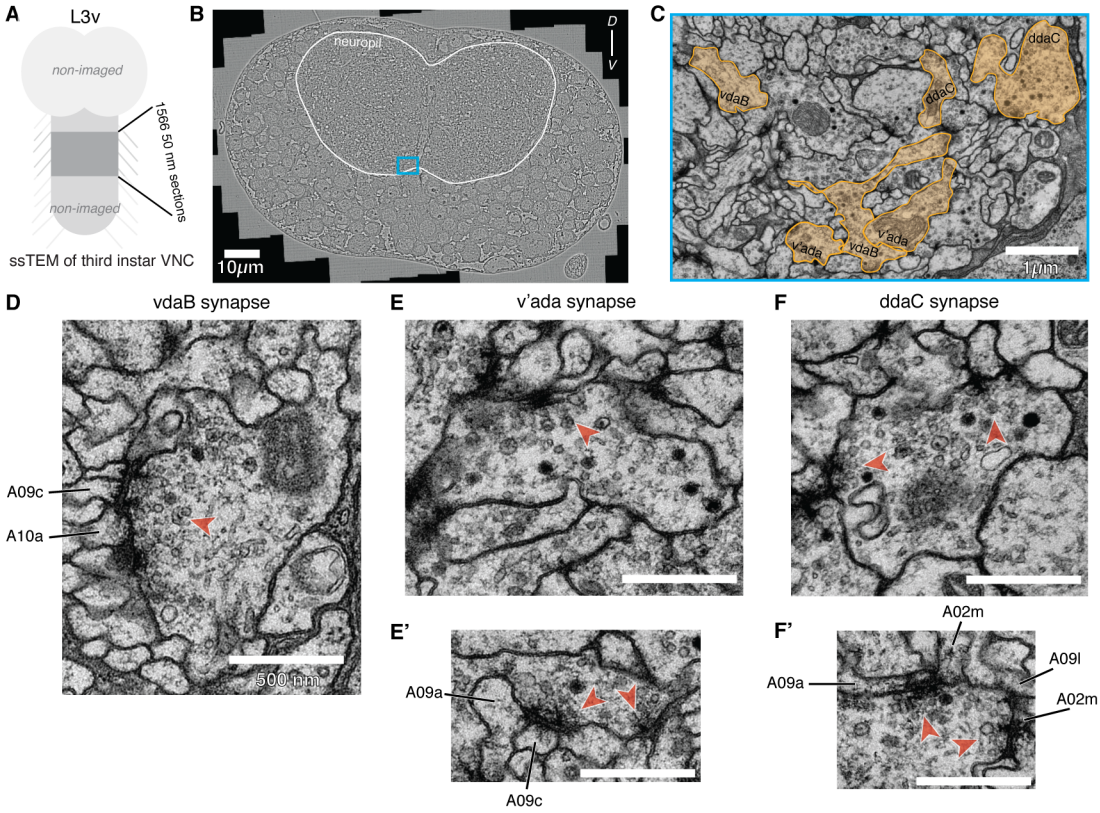

Figure 1—figure supplement 1: **A new EM image volume from a 3rd instar larva ventral nerve cord.** **A**, Schematic of the region of the 3rd instar larva CNS sectioned and imaged for the L3v. Anterior is up. **B**, A single section of L3v includes the complete neuropil (region inside white outline) and all soma (region outside white outline). Dorsal is up. **C**, Ventromedial neuropile indicated in the blue outline in **B**. Neurite cross-sections highlighted in orange correspond to ipsilateral mdIV axons. **D–F'**, Example synapses from vdaB (**E**), v'ada (**E,E'**), and ddaC (**F,F'**) terminals. Vesicles and presynaptic specializations highlighted by the red arrowhead. Postsynaptic neurons from LNs described in the main text are highlighted. Note the combination of small and dense core vesicles found in all three mdIV neurons.

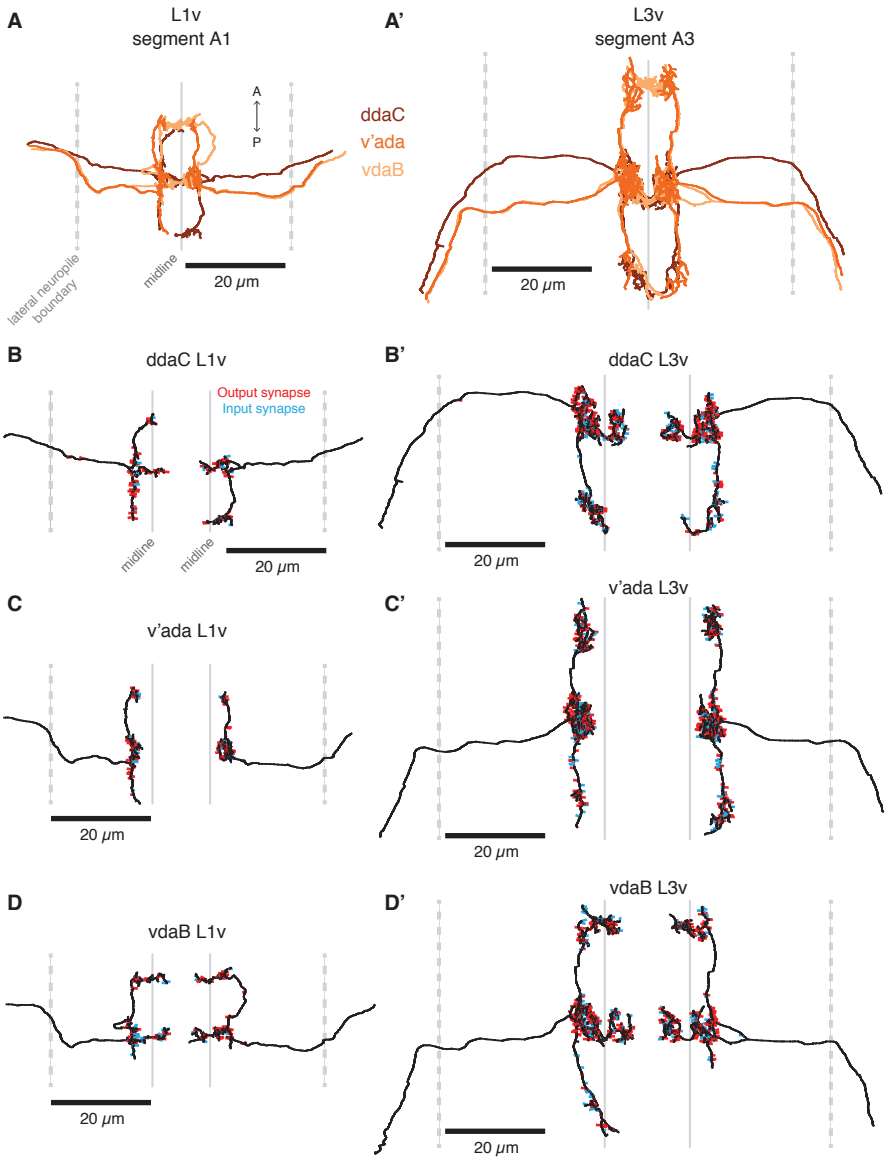

Figure 1—figure supplement 2: **Reconstructions of mdIV terminals. A, A'**, Dorsal view of all mdIV terminals from the L1v (**A**) and L3v (**A'**), identities as labeled. Views are at the same scale. Dashed lines indicate lateral neuropil boundaries, solid line the midline. **B, B'**, ddaC terminals in the L1v (**B**) and L3v (**B'**), left and right shown separately for clarity, as in all subsequent panels. ddaC can be distinguished by a midline crossing where the axon initially approaches the midline from the nerve and a projection into the adjacent segment posterior with little to no midline crossing. **C, C'**, v'ada terminals in the L1v ((**C**) and L3v (**C'**) can be distinguished by a lack of midline crossings and a projection into the adjacent segments anterior and, typically, posterior. **D, D'**, vdaB terminals in the L1v ((**D**) and L3v (**D'**) can be distinguished by a midline crossing both where the axon initially approaches the midline and a second midline crossing in the adjacent segment anterior. Note that for all mdIV types, there is some variability — extra or missing branches, such as the missing posterior branch of the right L1v v'ada, are true reflections of the data — although certain features remain typical across most cell types.

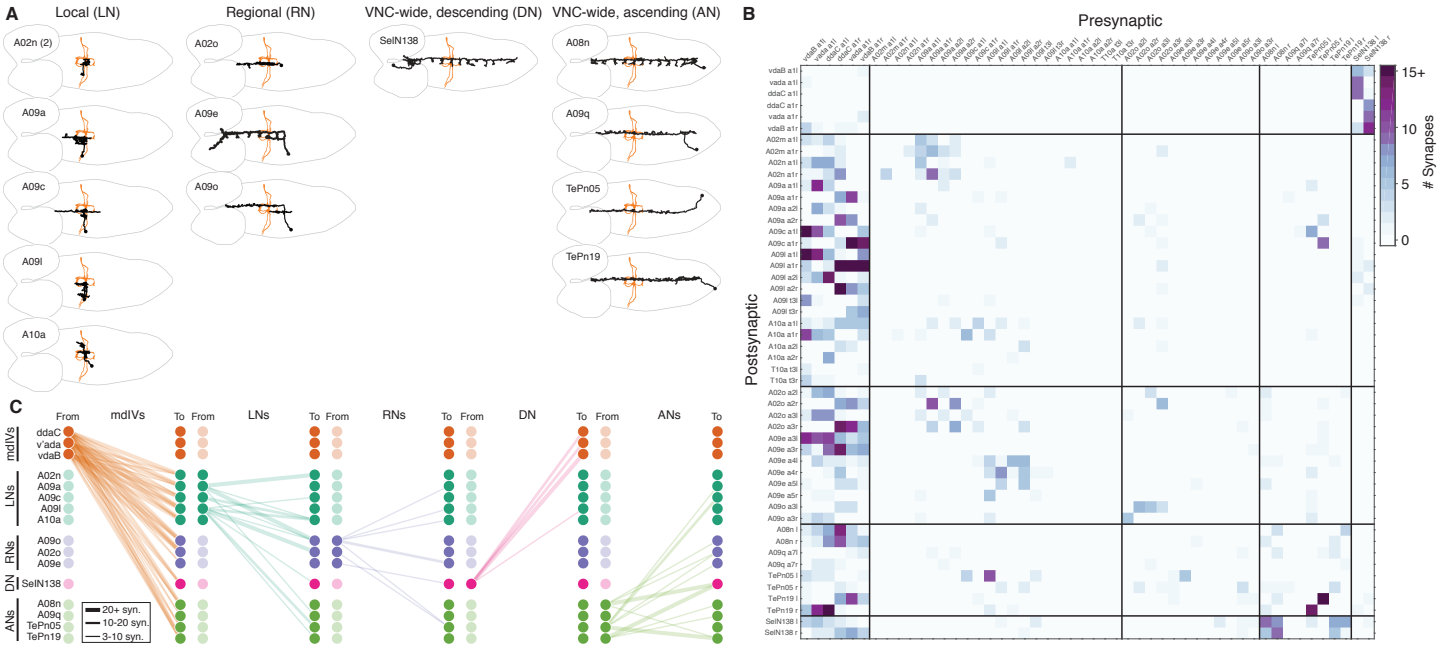

Figure 2—figure supplement 1: **The complete second-order mdIV network from the L1v. A**, All cell types synaptically connected to mdIV terminals in the L1v. Cell types were organized by spatial extent of the dendrites. Dorsal views of a single example of each interneuron cell type (black) and the mdIV terminals of segment A1 (orange), anterior to left. Outline indicates CNS boundary. Local neurons (LNs) had dendrites spanning 1-2 segments, regional neurons (RNs) had dendrites spanning 3+ segments but not the whole VNC, a descending neuron (DN) had dendrites in subesophageal zone (SEZ) and an axon in VNC, and Ascending neurons (ANs) had cell bodies in the posterior tip and projections that spanned the entire VNC toward the brain. See Supplemental Atlas for more views of cell types. **B**, Connectivity between individual cells in the mdIV network expressed as an adjacency matrix. Entries indicate the number of synaptic contacts from the column neuron to the row neuron. Black lines separate mdIV/LN/RN/DN/AN classes. Note that mdIV order is clockwise from ventral left. **C**, Connectivity between cell types in the mdIV network. Each column indicates connections from cell types in the left category to all cell types. Line thickness indicates number of synapses. Connections not observed at least twice at a 3+ synapse level are not shown here. In addition to the LN networks discussed elsewhere, we also find a strong pathway for feedback regulation of mdIV terminals. The SEZ neuron SeIN138 has an axonal projection descending through every abdominal segment, along which it both receives synaptic input from and outputs back onto mdIV terminals of all subtypes, offering a local axo-axonal feedback pathway across just a few microns of axonal arbor. Interestingly, SeIN136 also receives dendritic input near the SEZ from two ascending mdIV projection neurons, A08m and TePn19, that receive mdIV input throughout the nerve cord. This mdIV→AN→DN→mdIV pathway could allow every mdIV terminal across the body to be presynaptically regulated by ascending nociceptive input coming from any one location on the body. No other cell type was strongly or consistently presynaptic to mdIV terminals, suggesting this is the only such direct pathway.

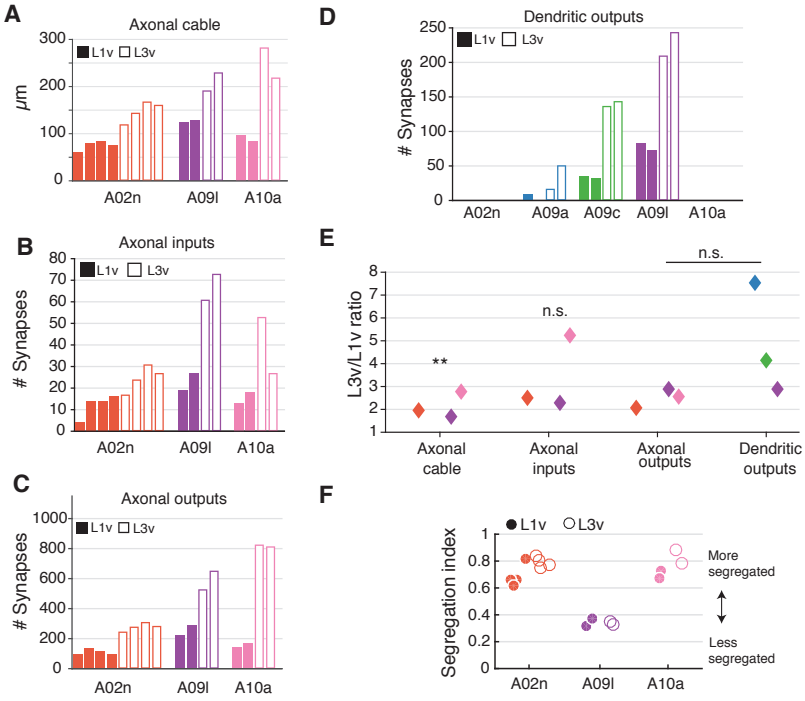

Figure 2—figure supplement 2: **Additional LN properties. A**, Total axonal cable length for A02n, A09l, and A10a. The LNs A09a and A09c had incomplete axons in the L3v due to the limited extent of the image volume and are omitted from axon-related analysis here. **B**, Number of synaptic inputs onto LN axons. **C**, Number of axonal outputs for LNs. **D**, Number of synaptic outputs on the dendrites of each LN. All neuron types that exhibited dendritic outputs in the L3v also had them in the L1v, suggesting that all of the basic categories of connections **E**, Fold-change between the L1v and L3v for the properties in **A**–**D**. Colors correspond to cell types. Axonal cable scales significantly less than dendritic cable (p=0.009, two sided t-test with Bonferroni correction), though other differences between axonal and dendritic property scaling are not significant. **F**, Segregation index for complete LNs, which measures the degree of input/output segregation of a neuron (1 indicates a completely segregated neuron, with all outputs in one region and all inputs in another; 0 indicated a neuron with completely intermixed inputs and outputs. See Methods for precise definition.) Note that segregation index is generally maintained as a cell-type specific property across larval stages.

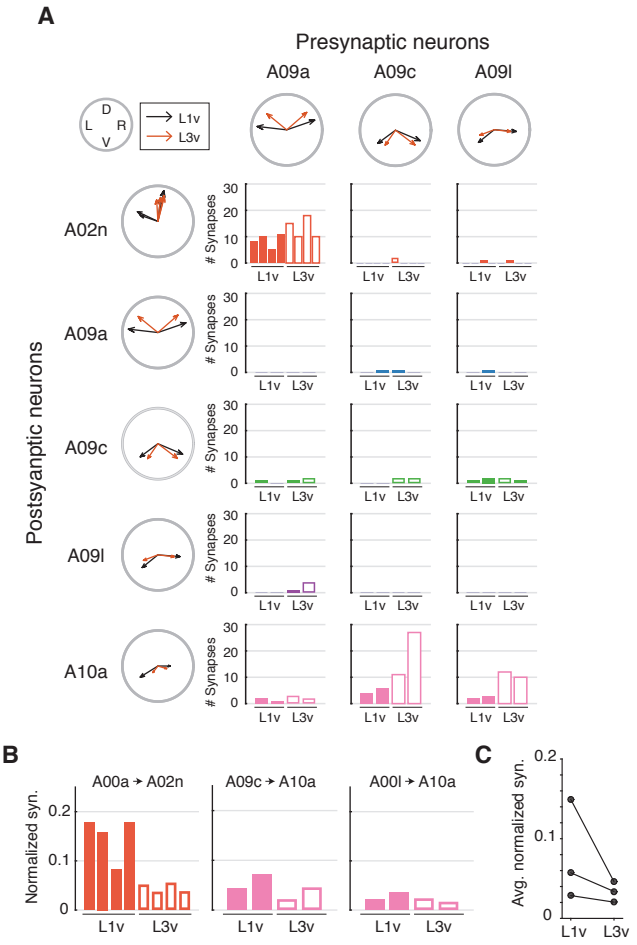

Figure 3—figure supplement 1: **Topographically structured feed-forward connectivity between mdIV-related LNs**. **A**, Synaptic connectivity between LN cell types in the L1v (solid bars) and L3v (empty bars). Each bar plot depicts the number of synapses each cell of the postsynaptic cell type (rows) receives from all cells of the presynaptic cell type (columns). Cell types are labeled with spatial receptive fields from Figure 3H. Each cell type that was strongly connected in the L1v was again connected in the L3v. Strikingly, the dorsally oriented A09a targeted the dorsally oriented A02n and the ventrolaterally oriented A09c and A09l targeted the ventrolaterally oriented A10a, suggesting feed-forward topographic microcircuits. **B**, Normalized synaptic connectivity between LNs. **C**, Mean strength, measured as normalized synaptic inputs, for specific connections between cell types in the L1v and L3v. The number of data points is too small to make a statistical conclusion.

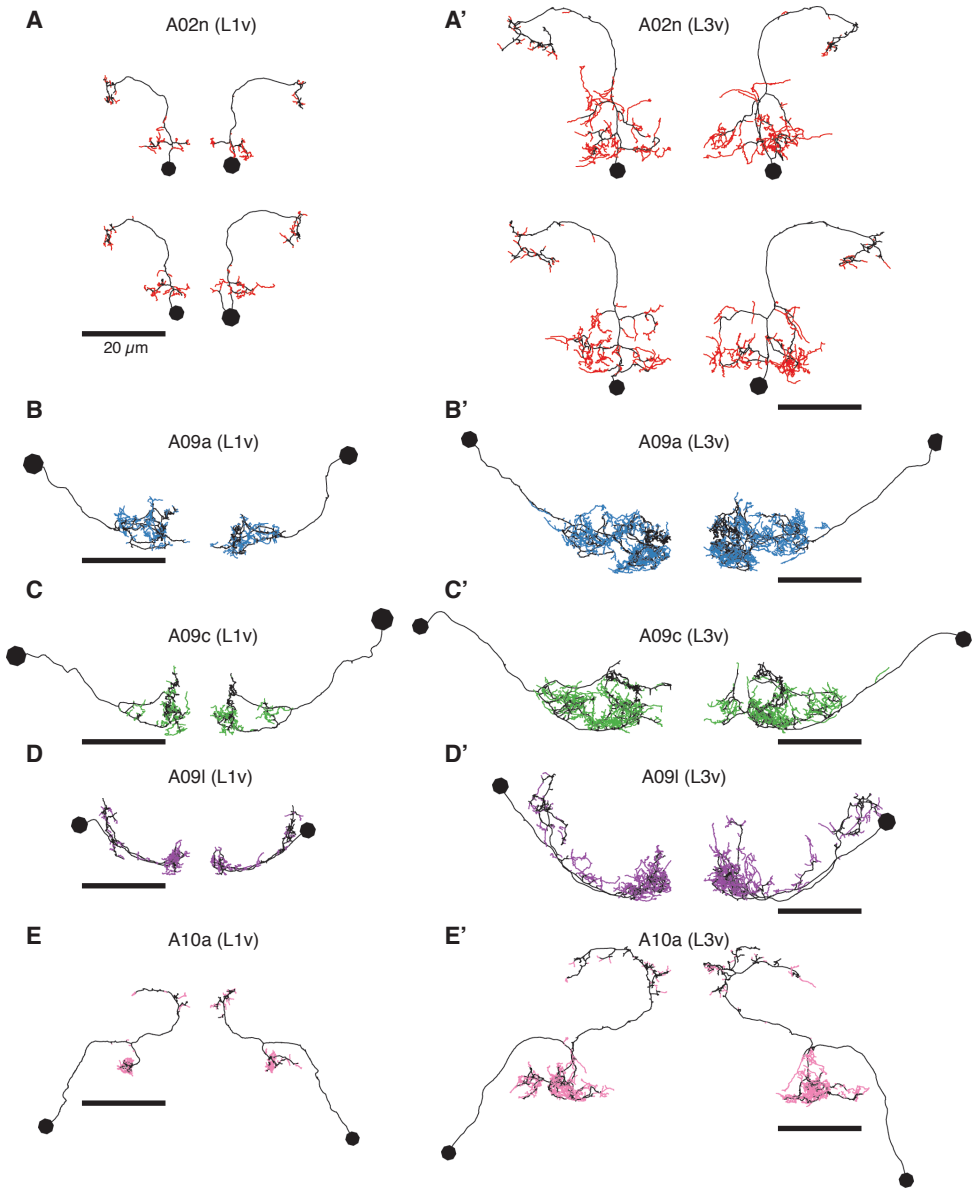

Figure 5—figure supplement 1: **Twig and backbone morphology for all LNs.** Backbones are shown in black, twigs with colors. Neurons from the L1v are shown to left (Regular letters), neurons from the L3v to right (Primed letters). Posterior view with dorsal up. Scales are consistent across all figures, scale bars are 20 μm.

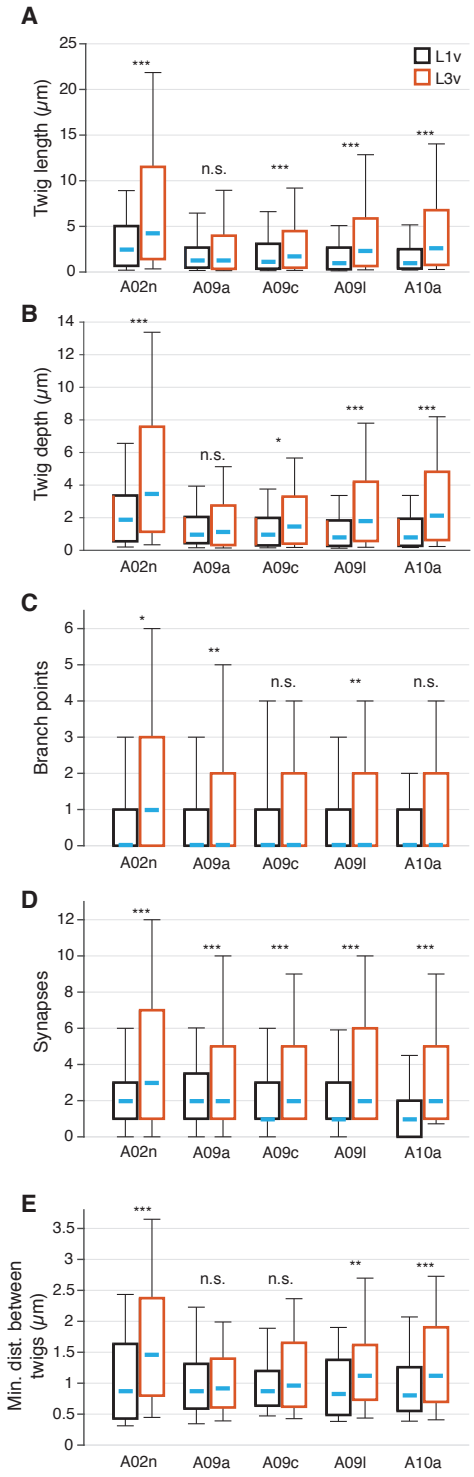

Figure 6—figure supplement 1: **Individual twig properties, broken down by LN cell type.** For each panel, bars indicate interquartile intervals, whiskers show 5/95 percentile lines. White dashes indicate median. Each bar describes two cells (four for A02n), and each twig was weighted equally. **A,** Box plots of total cable length per twig by cell type and developmental stage. **B,** Box plots of maximum twig depth (distance from distal tip to twig base) by cell type and developmental stage. **C,** Box plots of number of brach points per twig by cell type and developmental stage. **D,** Box plots of number of input synapses per twig by cell type and developmental stage. **E,** Box plots of minimum distance between twig bases along neuronal backbone by cell type and developmental stage. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, n.s.: not significant, two-sided t-test with Bonferonni correction.

Supplemental Atlas : **Atlas of all cell types synaptically connected to mdIVs.** For each cell type, we show a dorsal view (with CNS boundary, anterior up), a sagittal view (anterior to right), a cross-sectional view (grey line indicates neuropile boundary), and a table of number and fraction (in parentheses) of synapses from mdIV neurons onto the neuron shown. Due to varying anteroposterior extents of neurons, sagittal views are not to scale.