Supplementary Materials

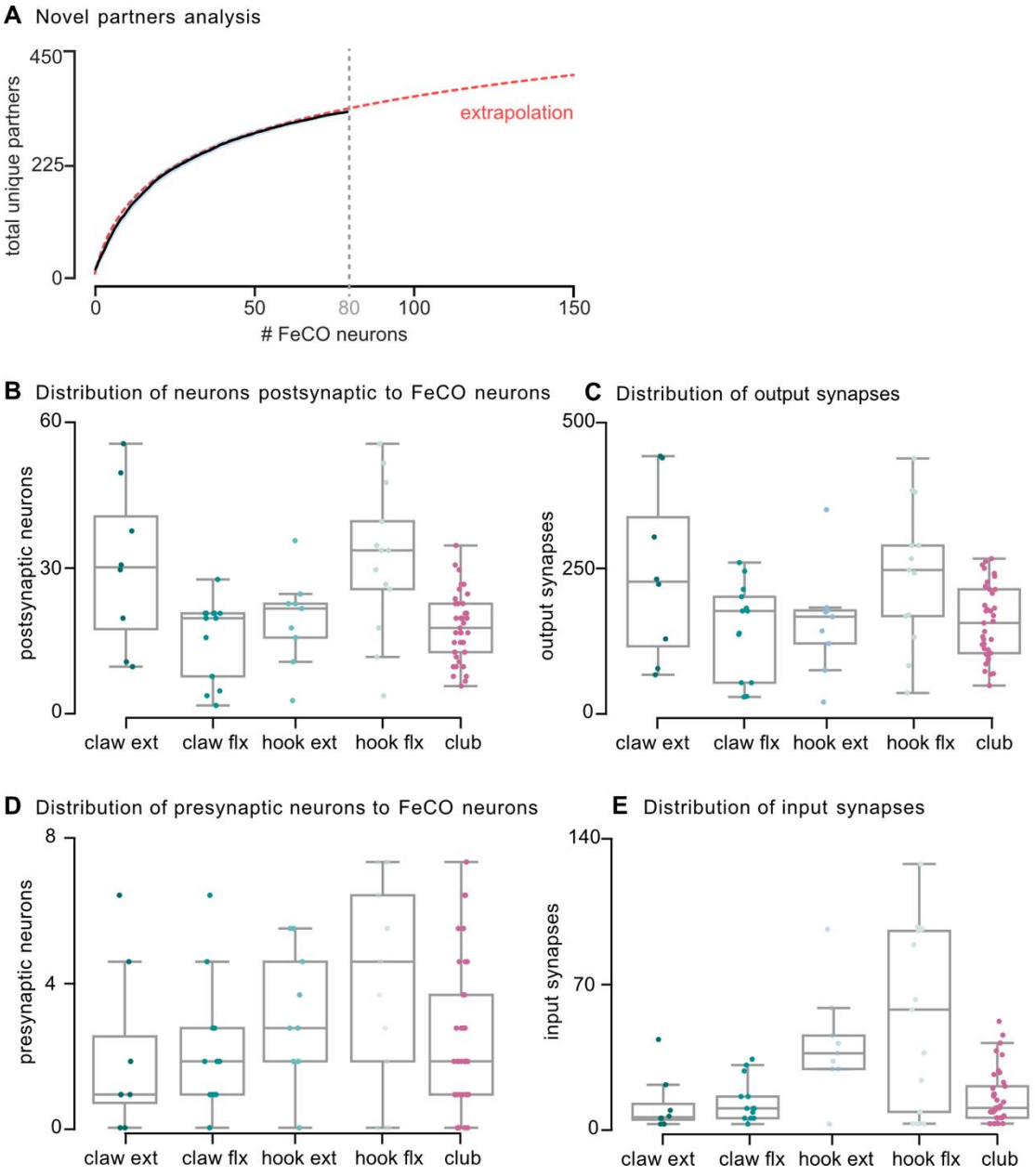

Figure S1. Novel partners analysis suggests we have reconstructed a meaningful fraction of the FeCO axons from the front left leg. (A) Plot shows the average number of new postsynaptic partners added per each FeCO sensory neuron we reconstructed. We randomly sampled the FeCO neurons one at a time (without replacement) in a cumulative fashion, and calculated how many novel postsynaptic partners were connected to each additional FeCO neuron. We resampled fifty times. Mean (black solid line) and standard error (shaded region surrounding line) are plotted. We then extrapolated the data (red dashed line) to estimate the remaining postsynaptic partners that have not yet been proofread. (B) Distribution of the number of unique neurons that are postsynaptic to each FeCO neuron subtype. The postsynaptic neurons include both proofread neurons and fragments that receive at least 4 synapses from a FeCO neuron. (C) Distribution of the number of synapses between each FeCO neuron and an individual postsynaptic partner. (D) Distribution of the number of unique neurons that are presynaptic to each FeCO neuron. The presynaptic neurons include both proofread neurons and fragments that make at least 3 synapse onto a FeCO neuron. (E) Distribution of the number of synapses between each FeCO neuron and an individual presynaptic partner. Source data are provided as a Source Data file.

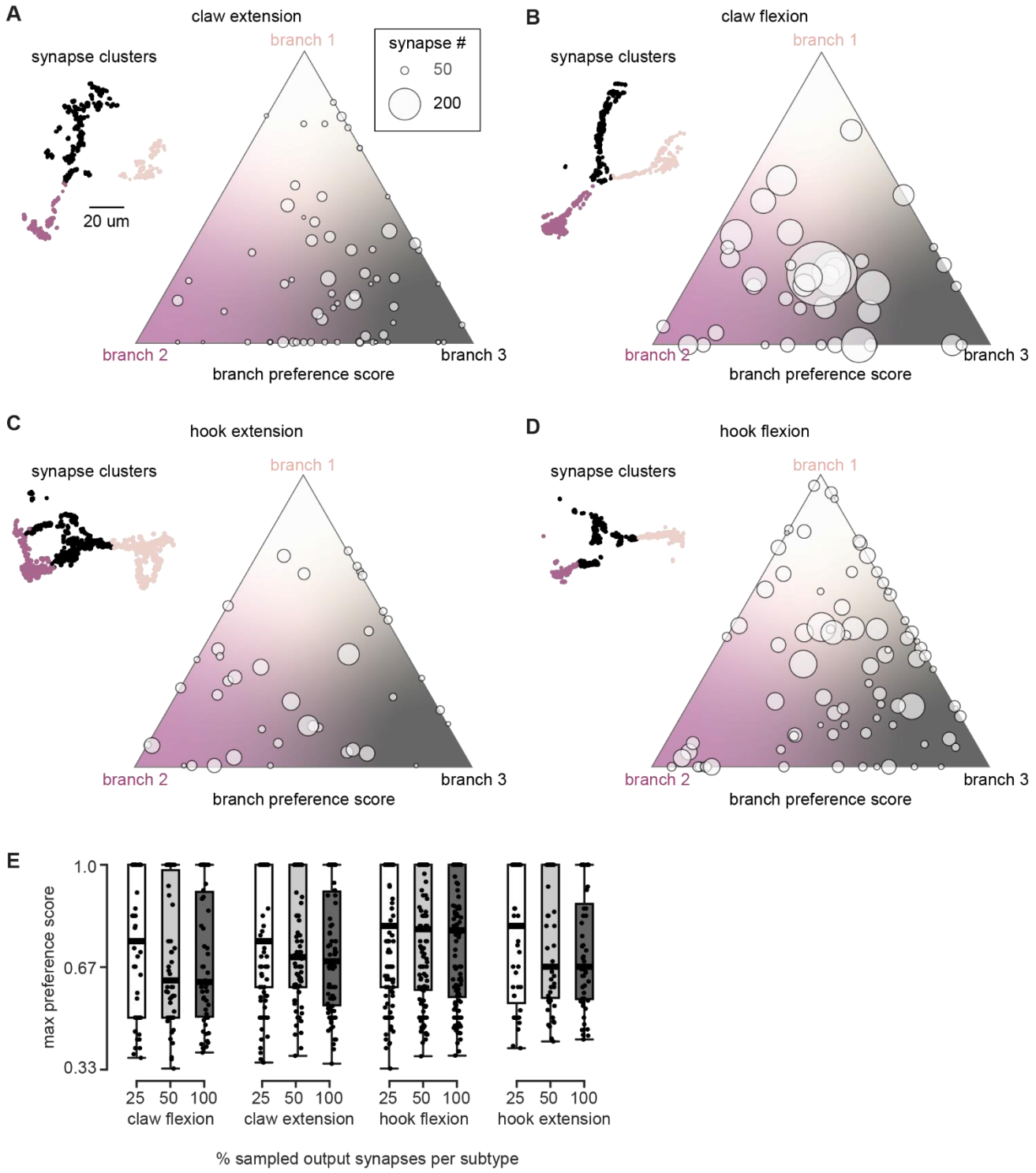

Figure S2. The majority of postsynaptic neurons receive synaptic input from multiple branches of a given FeCO subtype. A-D) We clustered the output synapses of each FeCO subtype based on their Euclidean distance (clusters shown on left). We then determined, for each postsynaptic partner, what fraction of its synaptic input came from which of these three clusters (branch preference score, see methods). Preference scores of each postsynaptic partner for each branch were then plotted on ternary plots (right). Size of the circle reflects the total number of input synapses the neuron receives from that FeCO subtype. E) We randomly subsampled either 25%, 50%, or 100% of an FeCO subtype's output synapses. We then calculated the branch preference scores of all postsynaptic partners. We plotted the max preference score per postsynaptic partner per FeCO subtype. Thick black line denotes the mean. No preference would be equivalent to a score of 0.33. Source data are provided as a Source Data file.

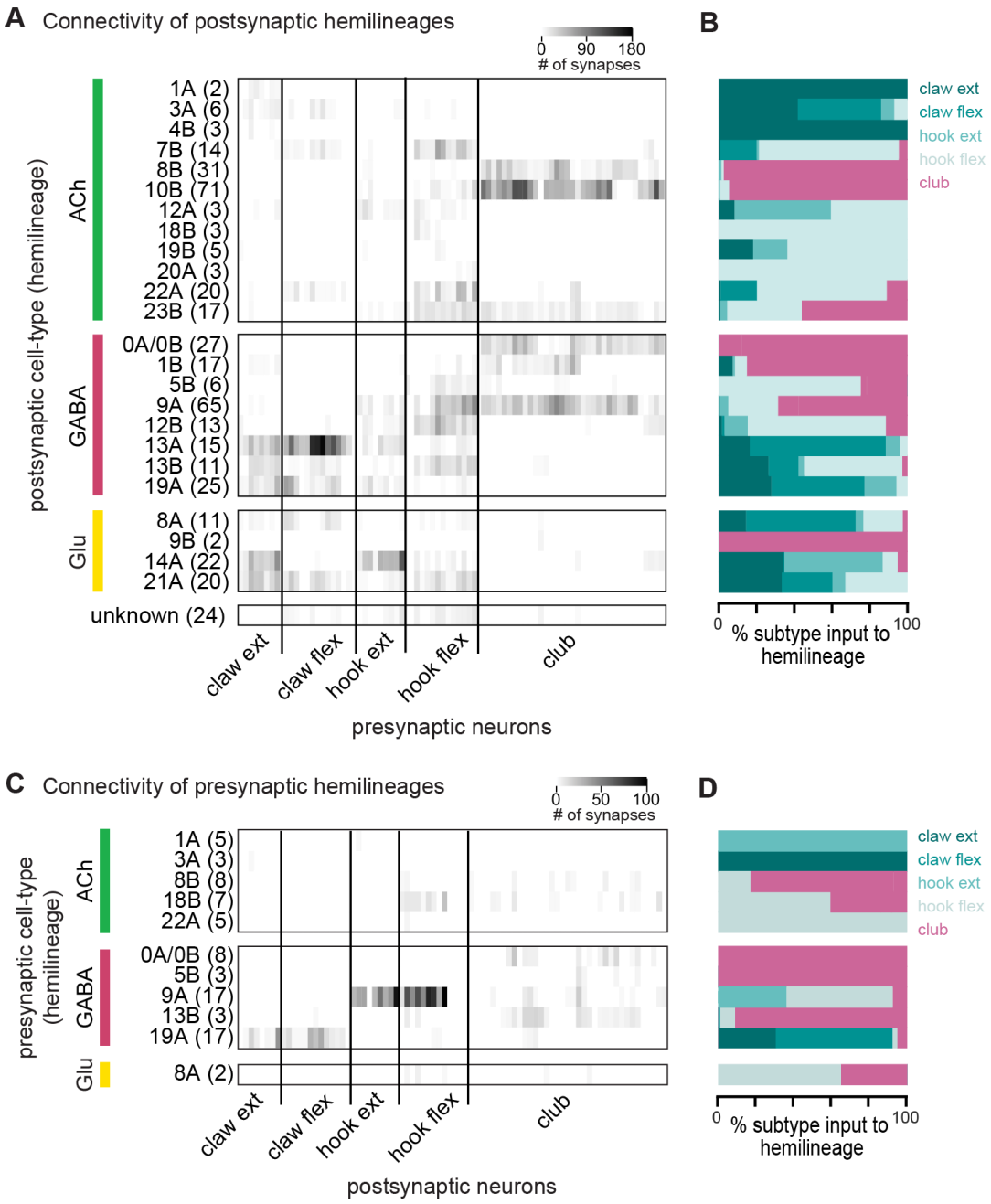

Figure S3. Subtypes of FeCO axons preferentially synapse with VNC neurons from specific developmental lineages. (A) The total number of synapses made by each FeCO neuron (columns) onto VNC neurons of each hemilineage (rows). Hemilineage was identified based on morphological characteristics of VNC neurons (see methods). Only local, ascending, and intersegmental postsynaptic partners are included in this analysis. VNC neurons of a given hemilineage are grouped together, with the number of neurons in each hemilineage indicated in parentheses. Hemilineages are grouped according to their primary neurotransmitter (ACh: acetylcholine, GABA: Gamma-aminobutyric acid, Glu: glutamate). (B) Percent of total FeCO input to a hemilineage made by each FeCO subtype. (C) The total number of synapses onto each FeCO neuron (columns) by presynaptic VNC neurons of each hemilineage (rows). (D) Percent of total inputs by a hemilineage onto each FeCO subtype. Source data are provided as a Source Data file.

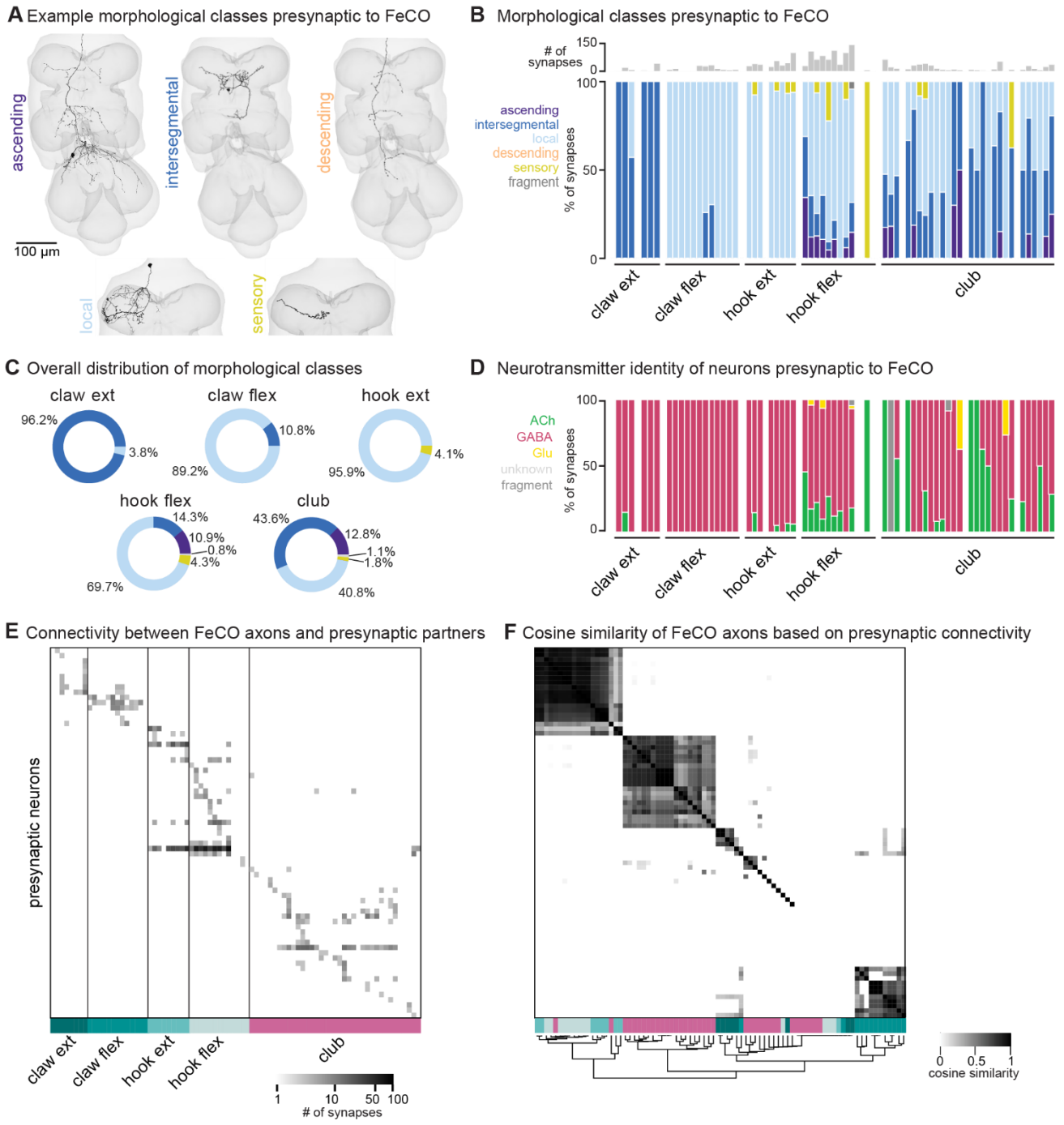

Figure S4. FeCO neurons exhibit subtype-specific presynaptic connectivity. (A) We reconstructed all VNC neurons presynaptic to FeCO axons from the front left leg (T1L) and classified them into morphological classes: ascending, descending, intersegmental, local, and sensory. Example provided from each class. (B) Percent of synapses received by each FeCO axon from VNC neurons of each morphological class. Top bar plot shows the total number of input synapses received by each FeCO axon. (C) Per FeCO subtype, the total fraction of input synapses received from each morphological class. (D) Proportion of total synapses made onto each FeCO neuron by presynaptic cholinergic (green), glutamatergic (yellow), GABAergic (pink), and unidentified (light gray) hemilineages. (E) Connectivity matrix between FeCO axons and presynaptic VNC neurons. The shading of each tick indicates the number of synapses from each presynaptic VNC neuron (row) onto each FeCO axon (column). Colored bars along the bottom indicate the postsynaptic FeCO subtype for that column. FeCO axons are organized by morphological subtype and then by their cosine similarity scores. VNC neurons are organized by their cosine similarity scores. (F) Clustered pairwise cosine similarity matrices of all FeCO axons based on their presynaptic connectivity. FeCO neurons with similar presynaptic connectivity patterns cluster together, forming connectivity clusters. Source data are provided as a Source Data file.

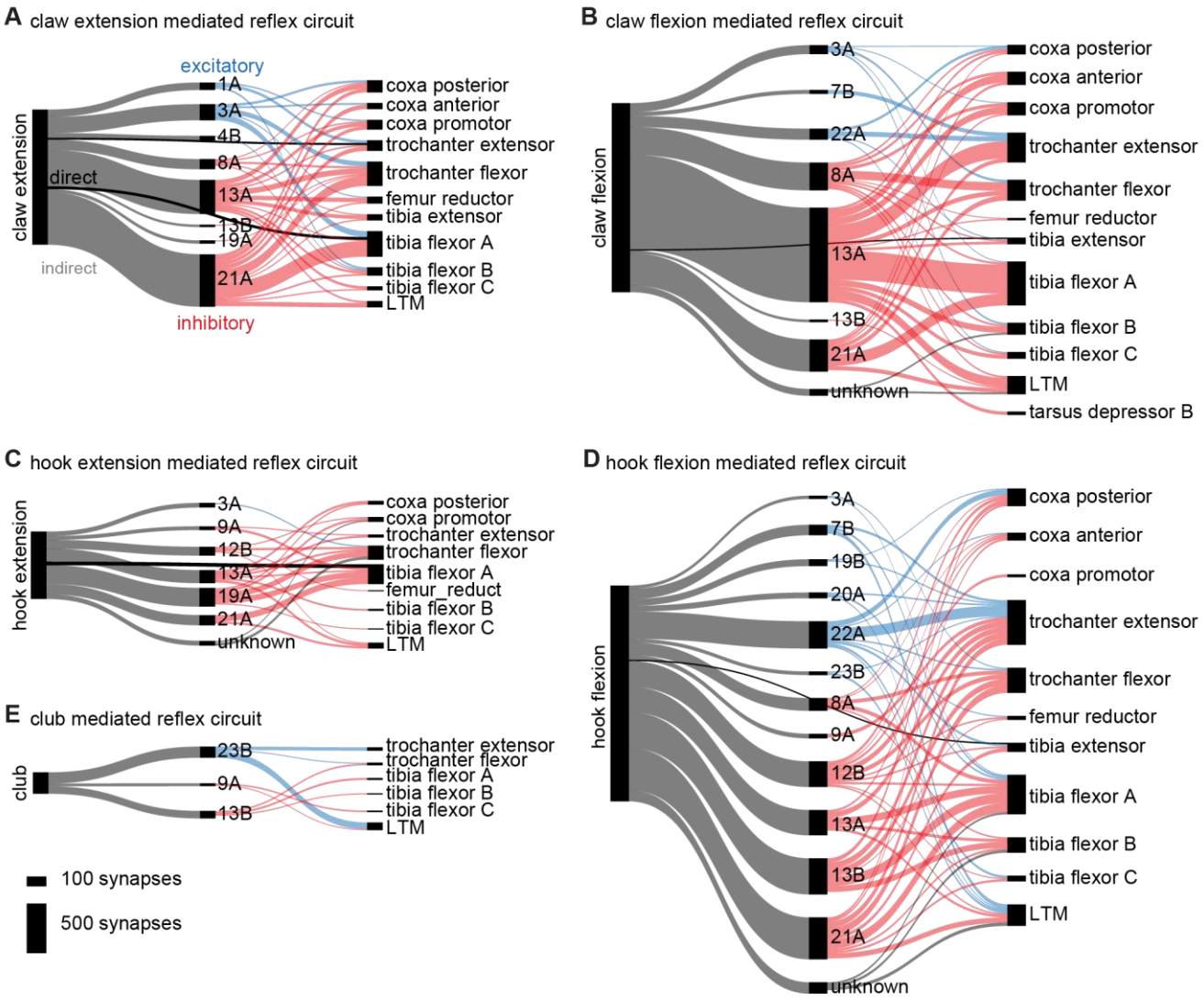

Figure S5. Hook and claw neurons connect directly and indirectly to leg motor neurons. FeCO neurons synapse onto leg motor neurons (black) and onto premotor neurons (gray). Leg motor neurons are grouped according to motor modules (Lesser et al., 2024)[27] and premotor neurons are grouped by their developmental hemilineage (see methods). Premotor neurons were then identified as making either excitatory (blue), inhibitory (red) or unknown (gray) synapses onto leg motor neurons. Only premotor and motor neurons that received at least 10 synapses from FeCO axons and only premotor neurons that supply at least 10 synapses to motor neurons are displayed. Source data are provided as a Source Data file.

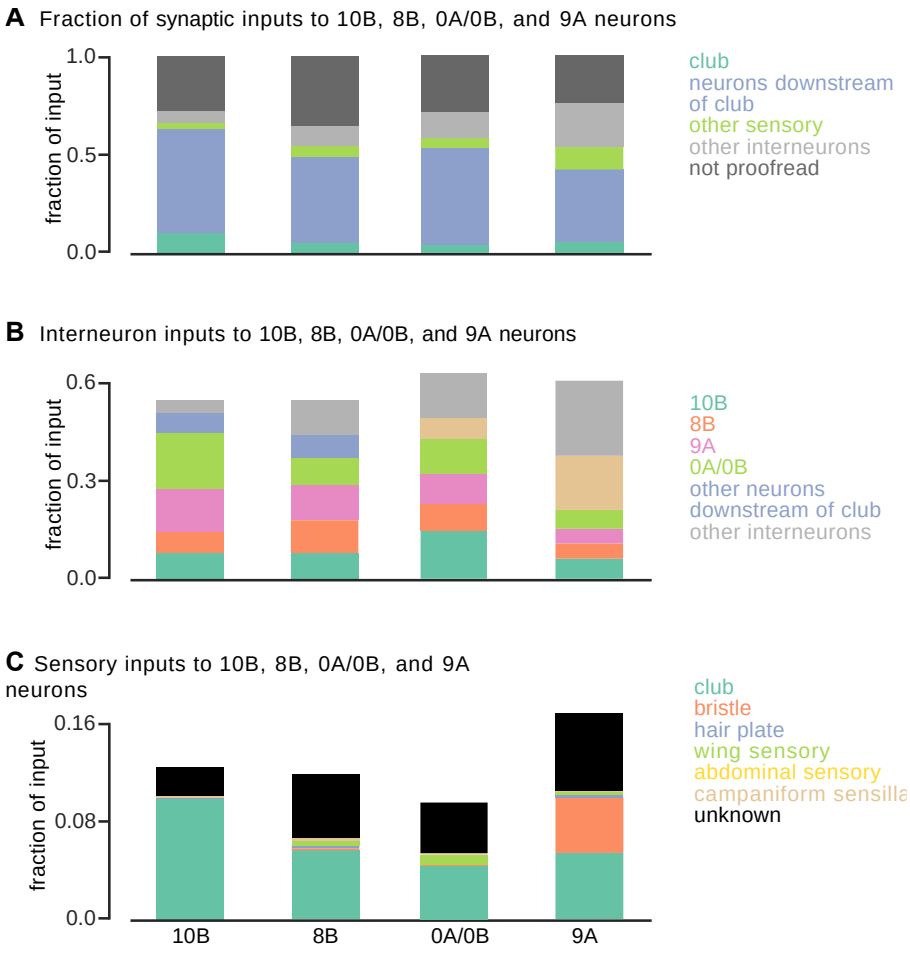

Figure S6. 8B and 10B neurons receive the majority of inputs from club circuits. (A) Fraction of total 10B, 8B, 0A/0B, and 9A input from club neurons, neurons downstream of club neurons (second-order and third-order interneurons), other sensory neurons, other interneurons, or neuron fragments. (B) Fraction of total input to 10B, 8B, 0A/0B, and 9A interneurons made by hemilineages that are postsynaptic to club neurons (10B, 8B, 9A, 0A/0B, other neurons downstream of club neurons, or other interneurons. (C) Fraction of total input to 10B, 8B, 0A/0B, and 9A interneurons made by sensory neurons. Source data are provided as a Source Data file.

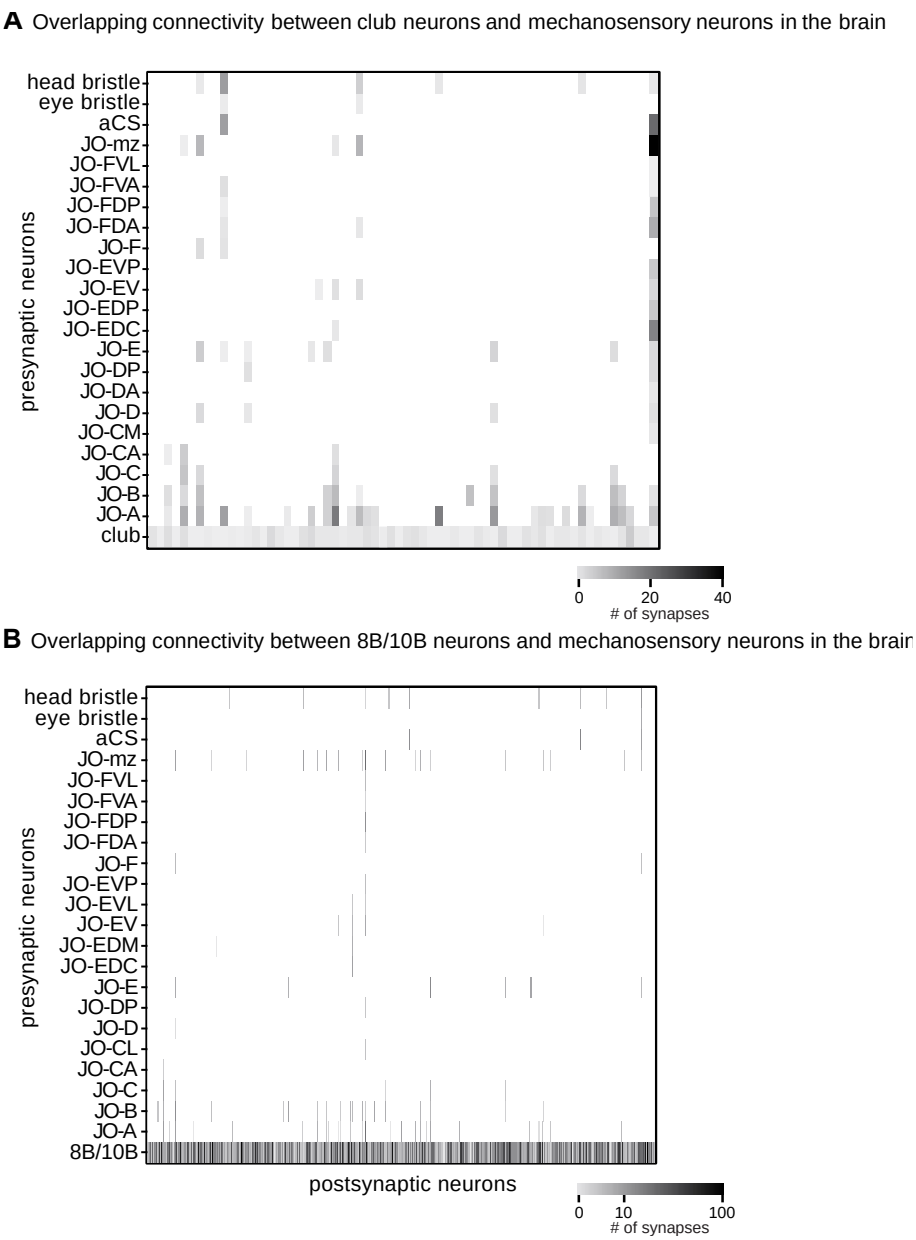

Figure S7. Ascending club neurons and 8B/10B neurons converge with mechanosensory neurons in the brain. (A) The total number of synapses made by club neurons and mechanosensory neurons (rows) onto postsynaptic neurons (columns) in the brain. (B) The total number of synapses made by 8B/10B neurons and mechanosensory neurons (rows) onto postsynaptic neurons (columns) in the brain. Source data are provided as a Source Data file.

Supplementary Table 1

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Deposited data	FANC connectome	Azevedo et al., (2024)[14]	https://fanc.community	
Deposited data	FAFB/FlyWire connectome	Dorkenwald et al., (2024)[7] Schlegel et al., (2024)[15]	https://flywire.ai	
Software, algorithm	CAVEclient	Dorkenwald et al., (2024)[7]	https://github.com/seunglab/CAVEclient	
Software, algorithm	neuPrint	Plaza et al., (2022)[77]	https://neuprint.janelia.org/	
Software, algorithm	Neuroglancer	Maitin-Shepard et al., (2021)[78]	RRID:SCR_015631	
Software, algorithm	Python		RRID:SCR_008394	

Supplementary Table 2. Neuroglancer links to FeCO axons by subtype

Club	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/clubs.json
Hook extension	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/hookE.json
Hook flexion	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/hookF.json
Claw extension	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/clawE.json
Claw flexion	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/clawF.json

Supplementary Table 3. Neuroglancer links to VNC neurons by hemilineage

All presynaptic partners	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/all_upstream.json
All postsynaptic partners	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/all_downstream.json
Postsynaptic 0A/0B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_0a0b.json
Postsynaptic 1A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_1A.json
Postsynaptic 1B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_1B.json
Postsynaptic 3A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_3A.json
Postsynaptic 4B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_4B.json
Postsynaptic 5B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_5B.json
Postsynaptic 7B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_7B.json
Postsynaptic 8A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_8A.json
Postsynaptic 8B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_8B.json
Postsynaptic 9A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_9A.json
Postsynaptic 9B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_9B.json

Postsynaptic 10B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_10B.json
Postsynaptic 12A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_12A.json
Postsynaptic 12B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_12B.json
Postsynaptic 13A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_13A.json
Postsynaptic 13B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_13B.json
Postsynaptic 14A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_14A.json
Postsynaptic 18B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_18B.json
Postsynaptic 19A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_19A.json
Postsynaptic 19B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_19B.json
Postsynaptic 20A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_20A.json
Postsynaptic 21A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_21A.json
Postsynaptic 22A	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_22A.json
Postsynaptic 23B	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/DS_23B.json

Postsynaptic motor neurons	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/MN.json
Postsynaptic unknown	https://neuromancer-seung-import.appspot.com/?json_url=https://raw.githubusercontent.com/sagrawal/Lee_2024/main/jsons/unknown.json

Supplementary Table 4. Neuroglancer links to ascending club, 8B, and 10B neurons in Flywire

Ascending club neurons	https://ngl.cave-explorer.org/ - !middleauth+https://global.daf-apis.com/nglstate/api/v1/6680057844072448
Ascending 8B/10B neurons	https://ngl.cave-explorer.org/#!middleauth+https://global.daf-apis.com/nglstate/api/v1/4958033236983808