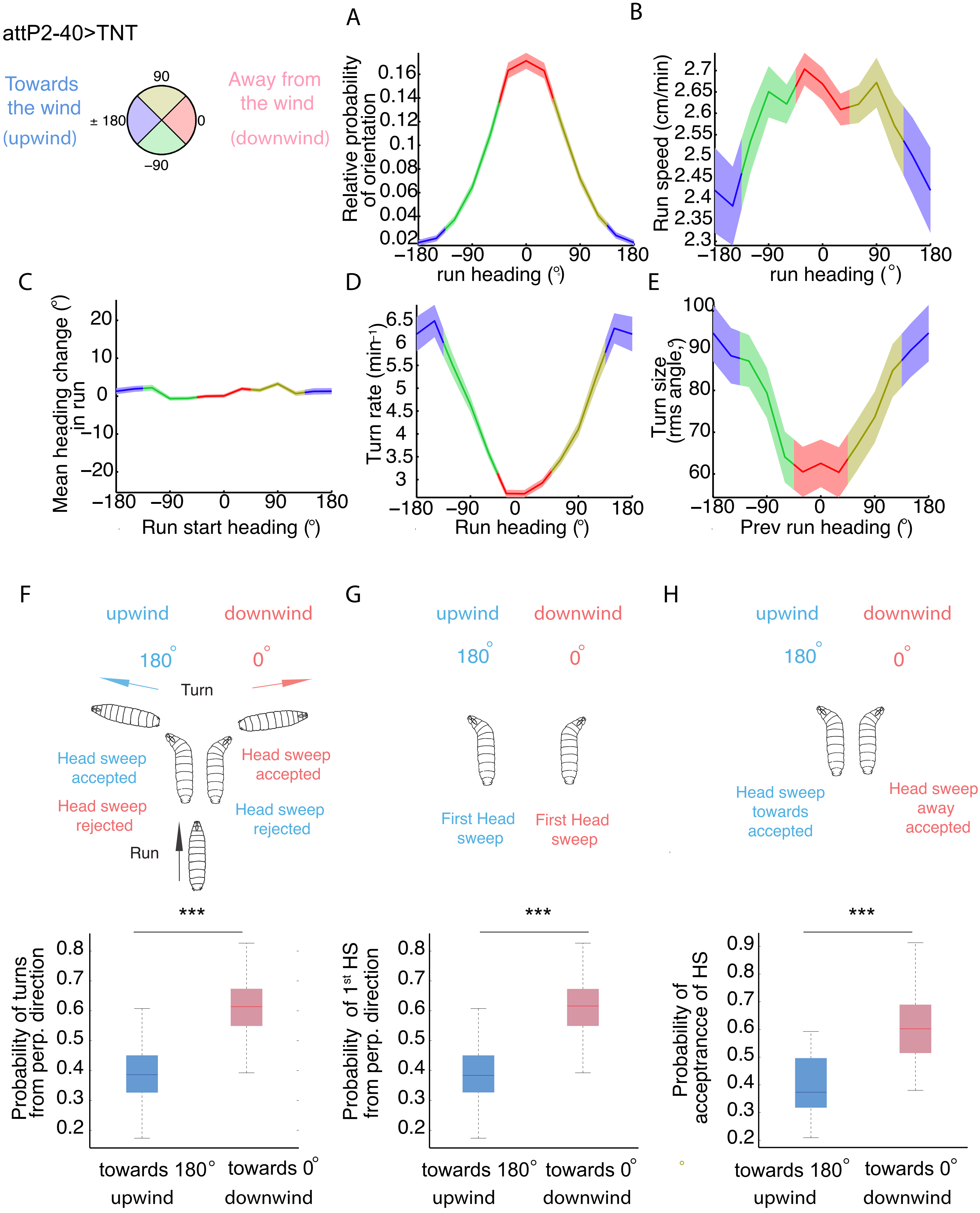
attP2-40>TNT
Towards the wind (upwind)
Away from the wind (downwind)
90
± 180
0
−90
A
Relative probability of orientation
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
−180 −90 0 90 180
run heading (°)
B
Run speed (cm/min)
2.7
2.65
2.6
2.55
2.5
2.45
2.4
2.35
2.3
−180 −90 0 90 180
run heading (°)
C
Mean heading change (°) in run
20
10
0
−10
−20
−180 −90 0 90 180
Run start heading (°)
D
Turn rate (min⁻¹)
6.5
6
5.5
5
4.5
4
3.5
3
−180 −90 0 90 180
Run heading (°)
E
Turn size (rms angle,°)
100
90
80
70
60
−180 −90 0 90 180
Prev run heading (°)
F
upwind
downwind
180°
0°
Turn
Head sweep accepted
Head sweep rejected
Head sweep accepted
Head sweep rejected
Run
***
Probability of turns from perp. direction
0.8
0.7
0.6
0.5
0.4
0.3
0.2
towards 180° upwind
towards 0° downwind
G
upwind
downwind
180°
0°
First Head sweep
First Head sweep
***
Probability of 1st HS from perp. direction
0.8
0.7
0.6
0.5
0.4
0.3
0.2
towards 180° upwind
towards 0° downwind
H
upwind
downwind
180°
0°
Head sweep towards accepted
Head sweep away accepted
***
Probability of acceptrance of HS
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
towards 180° upwind
towards 0° downwind

Figure S1. Behavioral strategies in anemotaxis in control attP2-attP40>TNT (Related to Figure 2) . A. Relative probability of headings during runs. B. Speed versus heading during runs C. Mean heading change in runs D. Turn rate versus heading E. Turn size versus heading F. Distribution of turns from perpendicular direction G. Distribution of head sweeps from perpendicular direction H. Probability of starting a run during a headsweep. A-E Values are mean and s.e.m F-H. In the box plot the median is indicated by the horizontal line. Box boundaries represent first and third quartiles, whiskers extend to the most extreme data points not considered outliers. The outliers are indicated by red +. The outliers are indicated by red +. *: $p<0.05$, **: $p<0.01$, ***:$<p<0.001$ (p-values can be found in Data S1 and the N of animals can be found in Table S2).

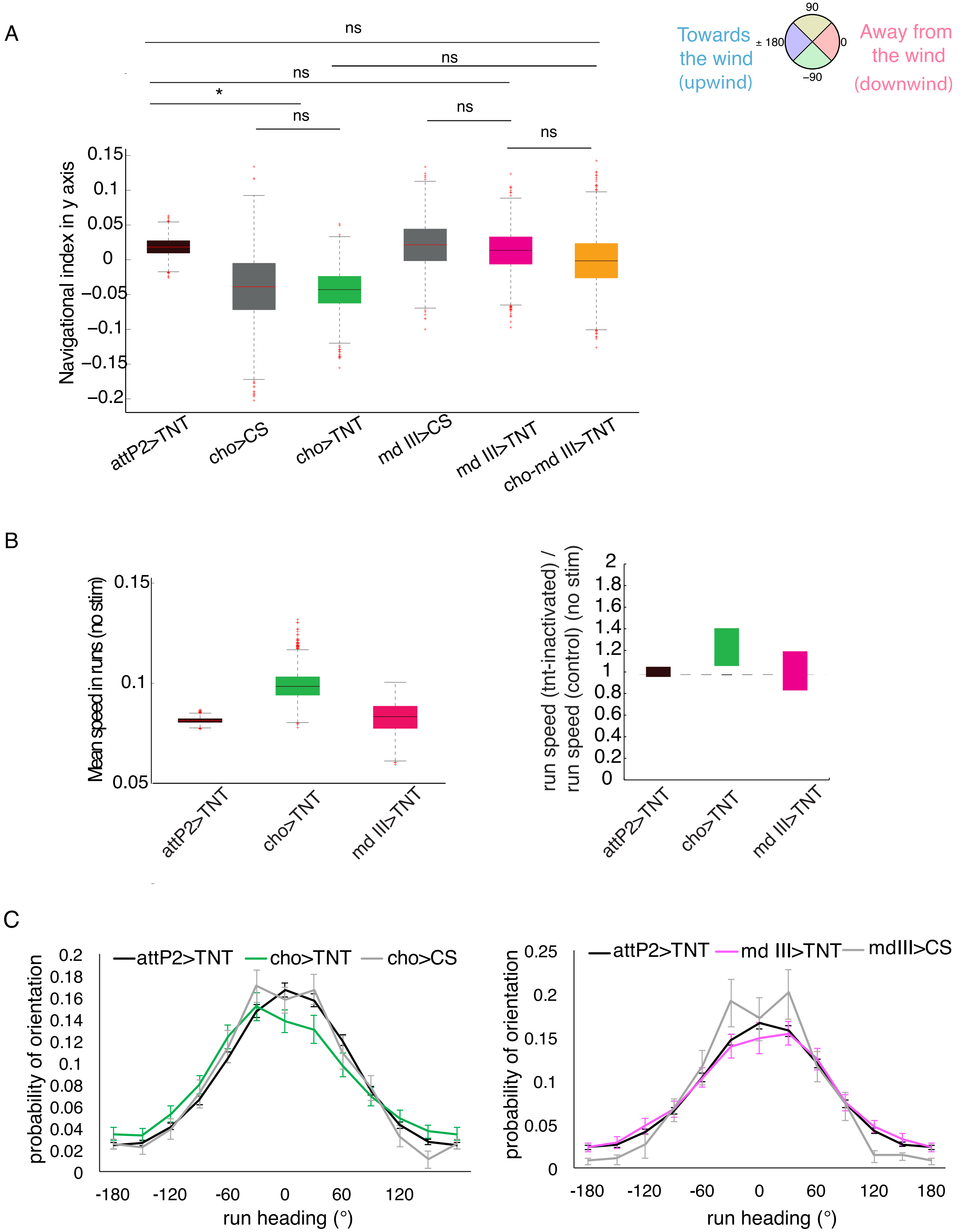
A
ns
ns
ns
*
ns
ns
ns
90
± 180
0
−90
Towards the wind (upwind)
Away from the wind (downwind)
Navigational index in y axis
0.15
0.1
0.05
0
−0.05
−0.1
−0.15
−0.2
attP2>TNT
cho>CS
cho>TNT
md III>CS
md III>TNT
cho-md III>TNT
B
Mean speed in runs (no stim)
0.15
0.1
0.05
attP2>TNT
cho>TNT
md III>TNT
run speed (tnt-inactivated) / run speed (control) (no stim)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
attP2>TNT
cho>TNT
md III>TNT
C
probability of orientation
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
attP2>TNT
cho>TNT
cho>CS
-180
-120
-60
0
60
120
run heading (°)
probability of orientation
0.25
0.2
0.15
0.1
0.05
0
attP2>TNT
md III>TNT
mdIII>CS
-180
-120
-60
0
60
120
180
run heading (°)

Figure S2. Navigational index in y axis and mean speed in runs of larvae with silenced somatosensory neurons (Related to Figure 3).

A. Navigational index in y axis for the sensory lines and controls B. Mean speed in runs for the inactivated sensory lines and control (*left panel*) 95% confidence interval on the ratio of the inactivated sensory lines mean run speed to the control mean run speed (*right panel*). Bootstrapped values are shown. In the box plot the median is indicated by the horizontal line. Box boundaries represent first and third quartiles, whiskers extend to the most extreme data points not considered outliers. The outliers are indicated by red +. *: $p<0.05$, **: $p<0.01$, ***:$<p<0.001$ (p-values can be found in Data S1 and Table S2)

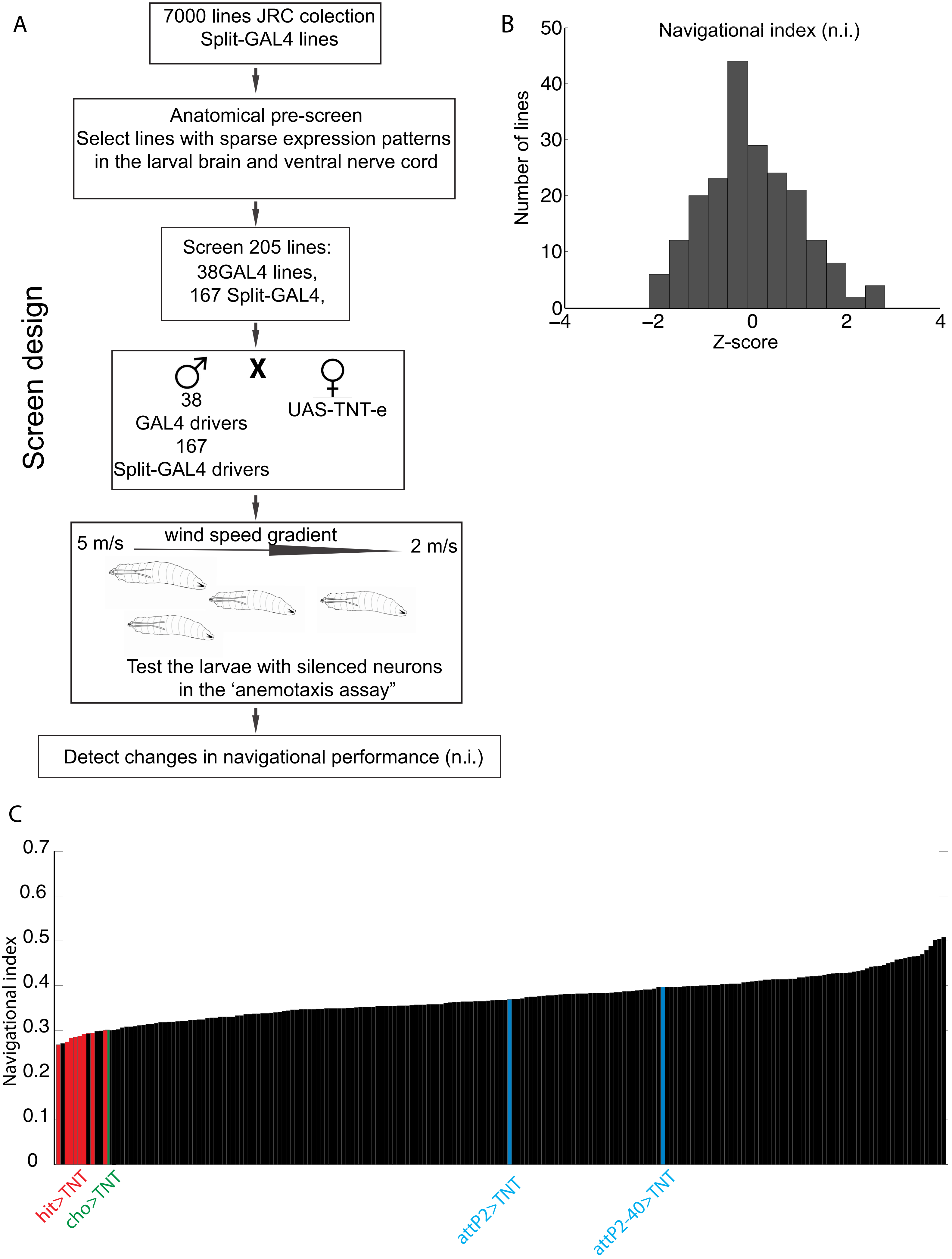
A
Screen design
7000 lines JRC colection
Split-GAL4 lines
Anatomical pre-screen
Select lines with sparse expression patterns
in the larval brain and ventral nerve cord
Screen 205 lines:
38GAL4 lines,
167 Split-GAL4,
♂
X
♀
38
GAL4 drivers
167
Split-GAL4 drivers
UAS-TNT-e
5 m/s
wind speed gradient
2 m/s
Test the larvae with silenced neurons
in the ‘anemotaxis assay”
Detect changes in navigational performance (n.i.)
B
Navigational index (n.i.)
Number of lines
50
40
30
20
10
0
−4
−2
0
2
4
Z-score
C
Navigational index
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
hit>TNT
cho>TNT
attP2>TNT
attP2-40>TNT

Figure S3. Behavioral inactivation screen (Related to Figure 4).
A Flowchart of the behavioral screen B. Z-score distribution of the tested lines C. Results of 205 tested lines in the anemotaxis assay sorted by performance. Mean navigational indices are shown. The *attP2>TNT* and *attP2-40>TNT* controls are shown in blue. The hit lines shown in this paper are shown in red. The chordotonal line is shown in green. Note that some lines that have similar navigational indices as the hit lines were not chosen for follow up, because they had stochastic neuronal expression patterns (The navigational indices can be found in Table S1).

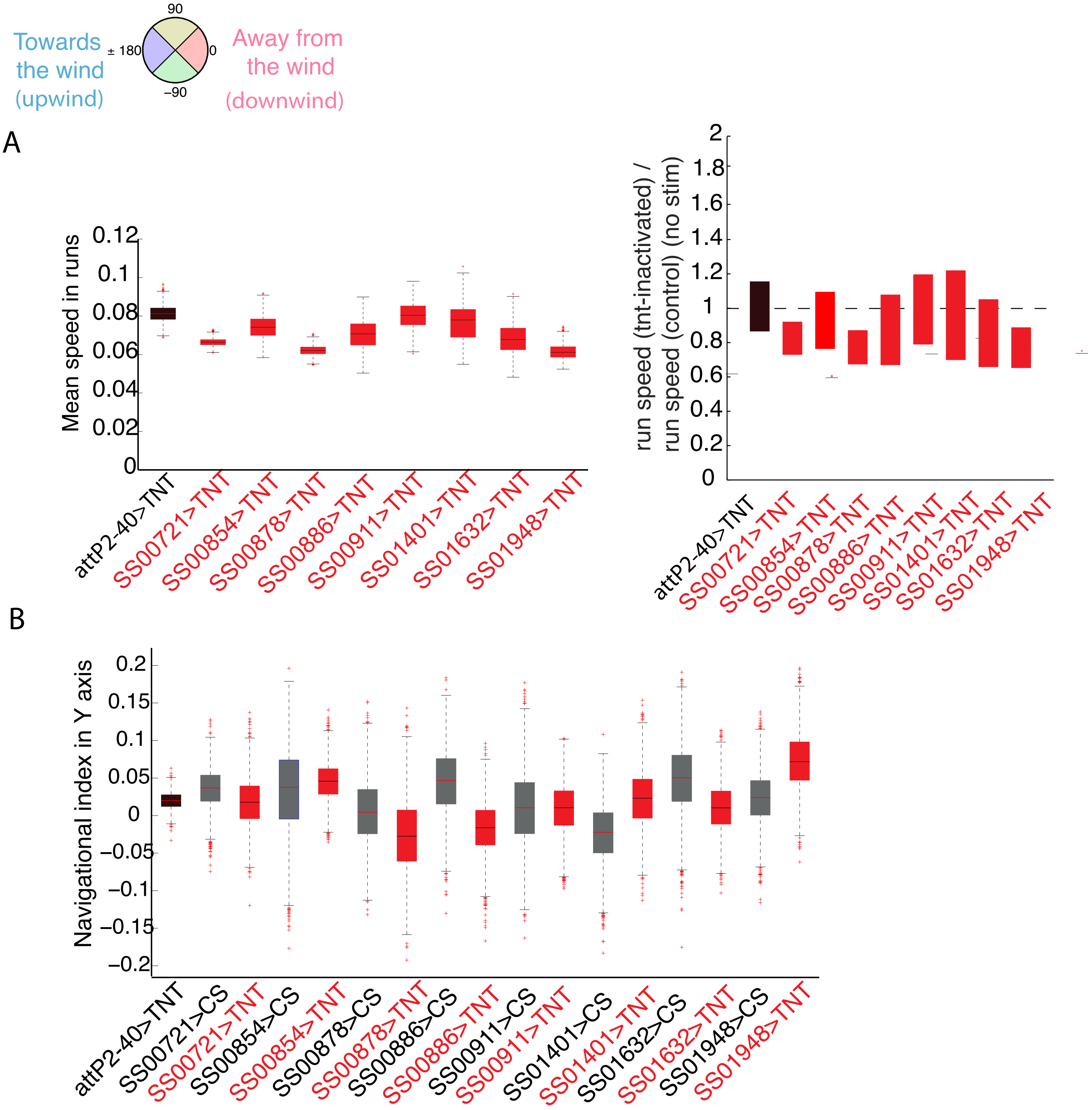
Towards the wind (upwind)
90
± 180
0
−90
Away from the wind (downwind)
A
Mean speed in runs
0.12
0.1
0.08
0.06
0.04
0.02
0
attP2-40>TNT
SS00721>TNT
SS00854>TNT
SS00878>TNT
SS00886>TNT
SS00911>TNT
SS01401>TNT
SS01632>TNT
SS01948>TNT
run speed (tnt-inactivated) / run speed (control) (no stim)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
attP2-40>TNT
SS00721>TNT
SS00854>TNT
SS00878>TNT
SS00886>TNT
SS00911>TNT
SS01401>TNT
SS01632>TNT
SS01948>TNT
B
Navigational index in Y axis
0.2
0.15
0.1
0.05
0
−0.05
−0.1
−0.15
−0.2
attP2-40>TNT
SS00721>CS
SS00721>TNT
SS00854>CS
SS00854>TNT
SS00878>CS
SS00878>TNT
SS00886>CS
SS00886>TNT
SS00911>CS
SS00911>TNT
SS01401>CS
SS01401>TNT
SS01632>CS
SS01632>TNT
SS01948>CS
SS01948>TNT

Figure S4. Mean speed in runs and navigational index in y axis of the hit lines (Related to Figure 4). A. Mean speed in runs in non-stimulus conditions (*left panel*). 95% confidence interval on the ratio of the inactivated lines mean run speed to the control mean run speed (*right panel*). For most of the CNS neuron lines (except for *SS00721*, *SS00878*, *SS01948*), the confidence interval includes 1, so we cannot say that the speeds are not the same. B. Navigational index in y axis for the eight hit lines. None of the CNS lines had the navigational index in y axis significantly different than the controls (p-values and N of animals can be found in Data S1 and Table S2). Bootstrapped values are shown. In the box plot the median is indicated by the horizontal line. Box boundaries represent first and third quartiles, whiskers extend to the most extreme data points not considered outliers. The outliers are indicated by red +.

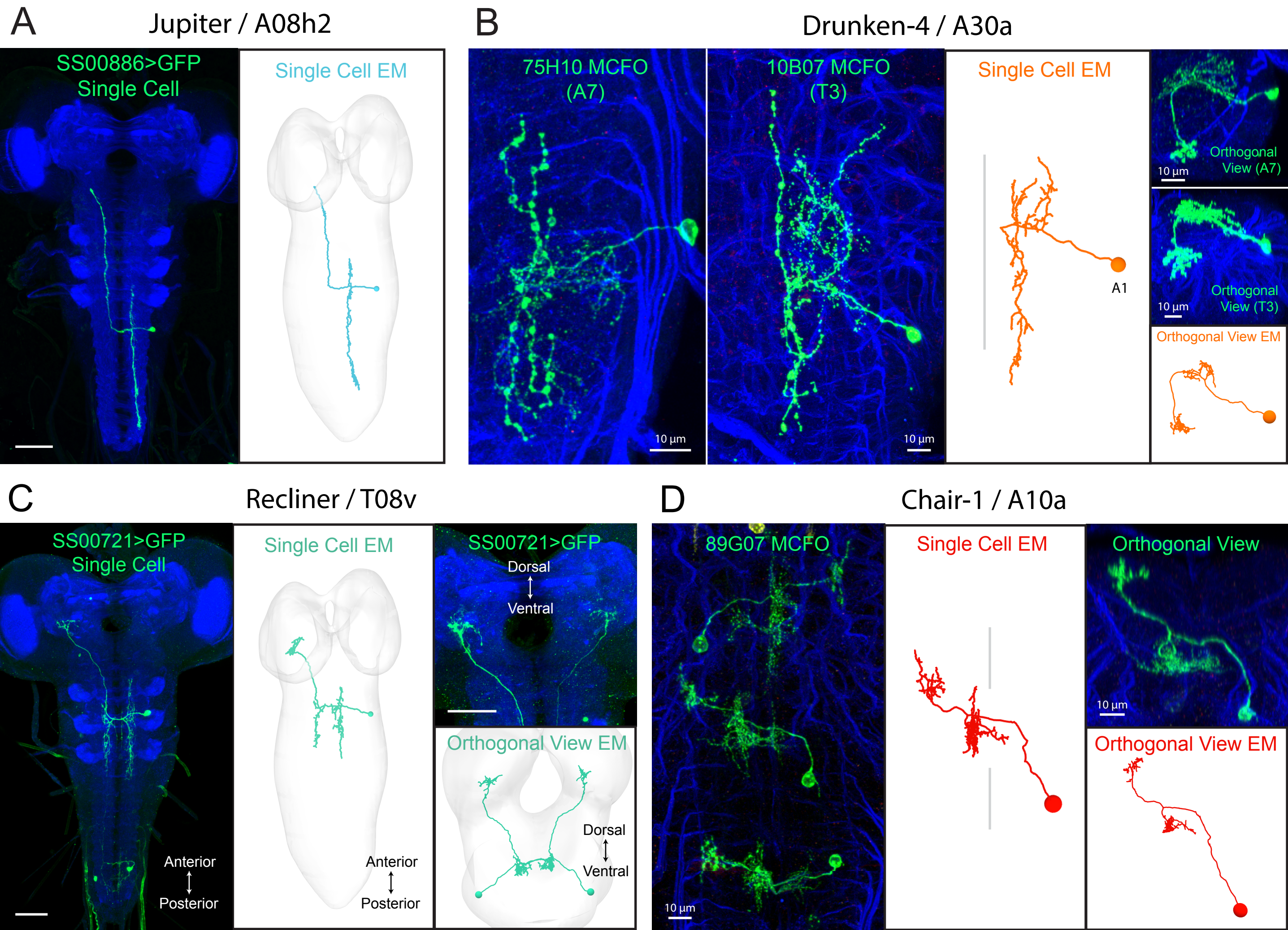
A
Jupiter / A08h2
SS00886>GFP
Single Cell
Single Cell EM
B
Drunken-4 / A30a
75H10 MCFO
(A7)
10 µm
10B07 MCFO
(T3)
10 µm
Single Cell EM
A1
Orthogonal
View (A7)
10 µm
Orthogonal
View (T3)
10 µm
Orthogonal View EM
C
Recliner / T08v
SS00721>GFP
Single Cell
Anterior
Posterior
Single Cell EM
Anterior
Posterior
SS00721>GFP
Dorsal
Ventral
Orthogonal View EM
Dorsal
Ventral
D
Chair-1 / A10a
89G07 MCFO
10 µm
Single Cell EM
Orthogonal View
10 µm
Orthogonal View EM

Figure S5. Detailed anatomy of light and electron microscopy images (Related to Figure 5).
A. The left panel depicts a brain with stochastic single-cell expression of SS00886, which morphologically matches its EM correlate (*right panel*). B. The two left panels depict single-cell abdominal and thoracic neurons, which were revealed by multicolor flip-out (MCFO) of the constituent GAL4 parts of *SS01401*. These morphologically match the EM correlate in both x-y and x-z views (right panels). C. The left panel depicts a brain with stochastic single-cell expression of *SS00721*, which matches its EM correlate (*middle panel*). Note that the brain is rotated in a different orientation in LM compared to EM. The right panel displays the axon terminals in the brain with similar brain orientation between LM and EM. D. MCFO of *89G07-GAL4*, a constituent part of *SS00911*. Abdominal single-cell clones morphologically match the EM reconstruction (*middle, right panels*). Scale bars are 50 µm unless indicated as 10 µm.