Supplemental Information

Ring neurons may also communicate with the E-PGs via wake-promoting neuropeptides
The R4d ring neurons expressed mRNA for the Dh31 neuropeptide (Figure S4B, adjusted p = 3.4E-21), while the E-PG neurons expressed mRNA for the Dh31 receptor (Figure S4B, adjusted p = 0.10). Dh31 has previously been linked to the ellipsoid body and is associated with wake promotion as part of the circadian rhythm (Kunst et al., 2014).

Electrical isolation of the EB and PB and unique partners within those regions can explain the P-EN2 activity
To probe the source of the differences in observed calcium activity in the P-EN2s between the EB and the PB, we turned to passive cable theory simulations of P-EN2 neurons. We injected current from 3 fictive spikes into Δ7 synapse locations in the PB and observed how potentials propagated along the arbors. Sites within the PB quickly reached equipotential, and voltage losses within the region were minimal. In contrast, sites within the EB experienced only a slight depolarization in response to these current injections, suggesting that the two compartments are electrically isolated (Figure S11M). The P-EN2 activity in the EB may therefore primarily reflect the direct contribution from the P-EGs (Figure S11N) while the activity in the PB would reflect a direct contribution from the Δ7s. The PB P-EN2 activity may only enter the EB when it overcomes some threshold, say when additional current is injected. The P-EN2 activity is tuned to rotational velocities (Green et al., 2017), and thus strong turns may lead to such an additional current source. The super-threshold PB activity would then travel to the EB, leading to a growing shoulder of P-EN2 EB activity at high rotational velocities. Indeed, we see such a shoulder in these conditions

Figure S1

Figure S1

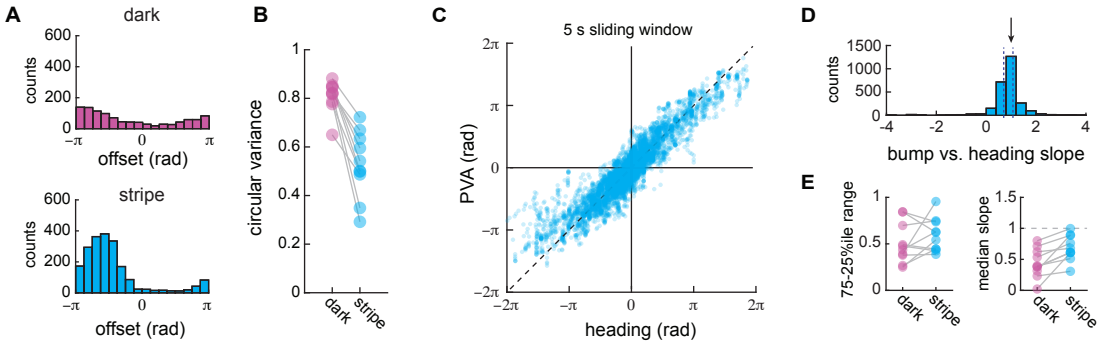


Figure S2

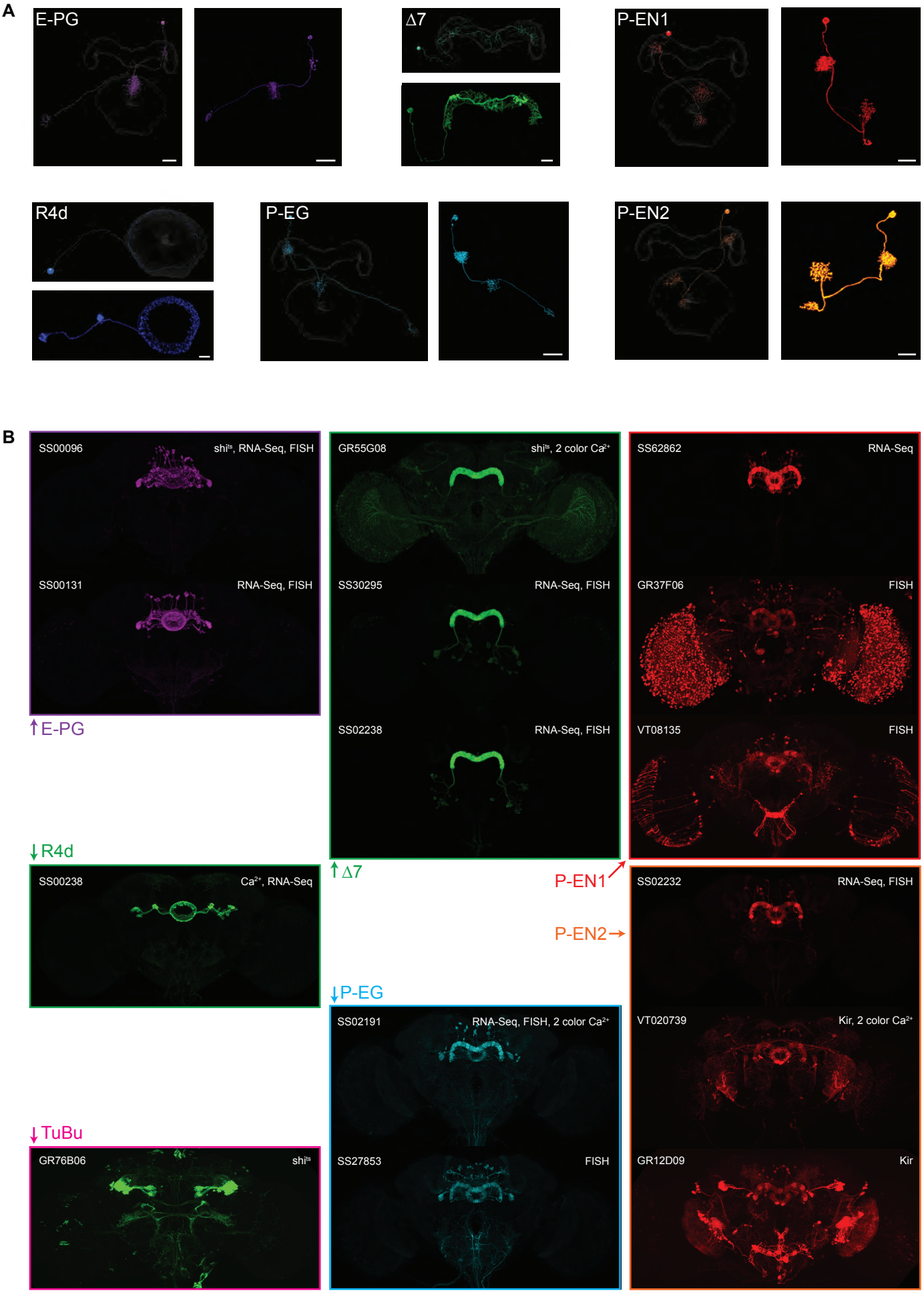

A
E-PG
Δ7
P-EN1
R4d
P-EG
P-EN2
B
SS00096
shi^ts, RNA-Seq, FISH
SS00131
RNA-Seq, FISH
↑E-PG
GR55G08
shi^ts, 2 color Ca2+
SS30295
RNA-Seq, FISH
SS02238
RNA-Seq, FISH
↑Δ7
SS62862
RNA-Seq
GR37F06
FISH
VT08135
FISH
P-EN1
↓R4d
SS00238
Ca2+, RNA-Seq
P-EN2→
SS02232
RNA-Seq, FISH
↓P-EG
SS02191
RNA-Seq, FISH, 2 color Ca2+
SS27853
FISH
VT020739
Kir, 2 color Ca2+
GR12D09
Kir
↓TuBu
GR76B06
shi^ts

Figure S3

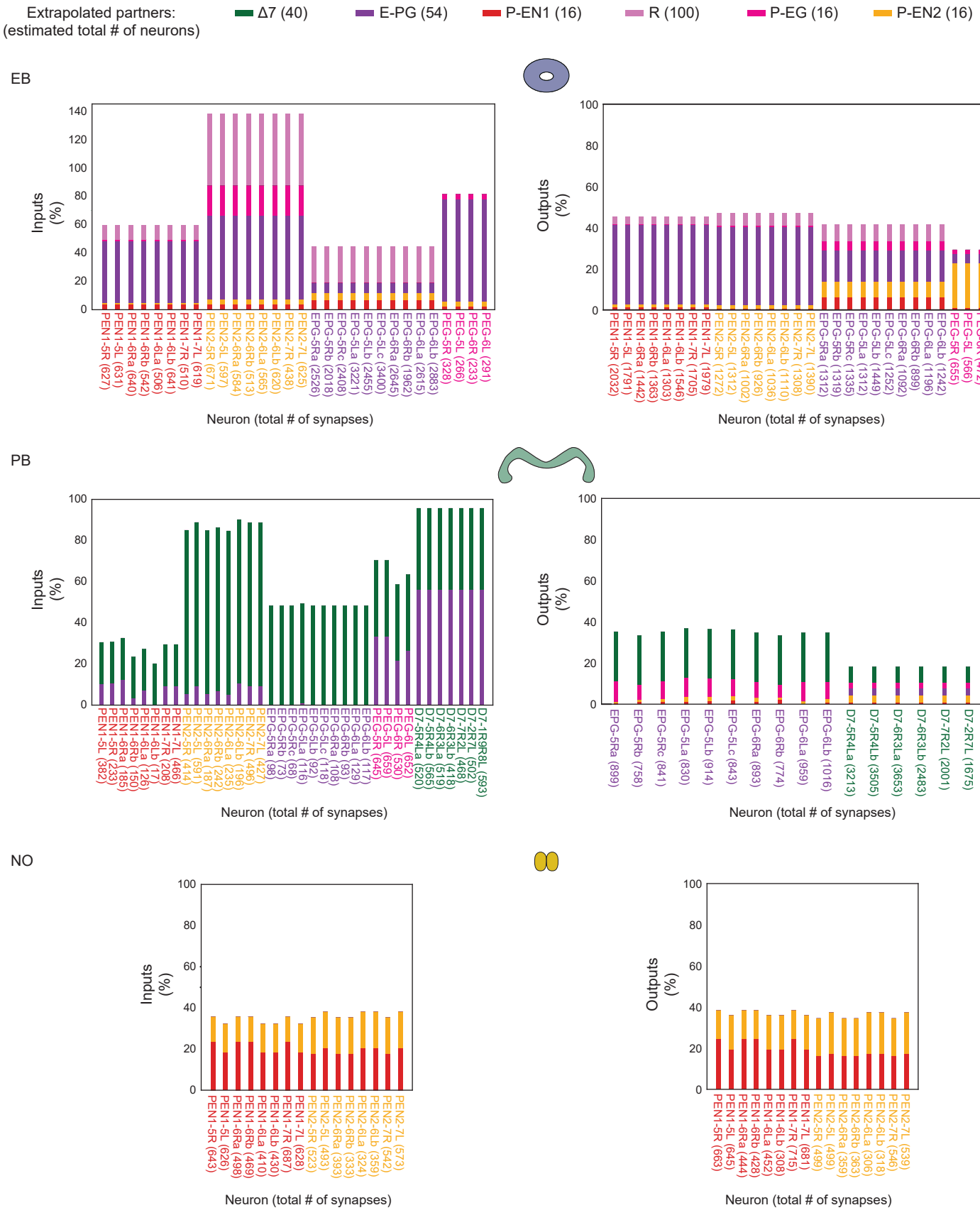

Extrapolated partners:
(estimated total # of neurons)
Δ7 (40)
E-PG (54)
P-EN1 (16)
R (100)
P-EG (16)
P-EN2 (16)
EB
Inputs (%)
PEN1-5R (627)
PEN1-5L (631)
PEN1-6Ra (640)
PEN1-6Rb (542)
PEN1-6La (506)
PEN1-6Lb (641)
PEN1-7R (510)
PEN1-7L (619)
PEN2-5R (671)
PEN2-5L (597)
PEN2-6Ra (584)
PEN2-6Rb (513)
PEN2-6La (565)
PEN2-6Lb (620)
PEN2-7R (438)
PEN2-7L (625)
EPG-5Ra (2526)
EPG-5Rb (2018)
EPG-5Rc (2408)
EPG-5La (3221)
EPG-5Lb (2455)
EPG-5Lc (3400)
EPG-6Ra (2645)
EPG-6Rb (1962)
EPG-6La (2615)
EPG-6Lb (2883)
PEG-5L (266)
PEG-5R (328)
PEG-6R (233)
PEG-6L (291)
Neuron (total # of synapses)
Outputs (%)
PEN1-5R (2032)
PEN1-5L (1791)
PEN1-6Ra (1442)
PEN1-6Rb (1363)
PEN1-6La (1303)
PEN1-6Lb (1546)
PEN1-7R (1705)
PEN1-7L (1979)
PEN2-5R (1272)
PEN2-5L (1312)
PEN2-6Ra (1002)
PEN2-6Rb (926)
PEN2-6La (1036)
PEN2-6Lb (1110)
PEN2-7R (1308)
PEN2-7L (1390)
EPG-5Ra (1312)
EPG-5Rb (1319)
EPG-5Rc (1335)
EPG-5La (1312)
EPG-5Lb (1449)
EPG-5Lc (1252)
EPG-6Ra (1092)
EPG-6Rb (899)
EPG-6La (1196)
EPG-6Lb (1242)
PEG-5R (655)
PEG-5L (566)
PEG-6R (472)
PEG-6L (558)
Neuron (total # of synapses)
PB
Inputs (%)
PEN1-5L (382)
PEN1-5R (233)
PEN1-6Ra (185)
PEN1-6Rb (150)
PEN1-6La (126)
PEN1-6Lb (17)
PEN1-7R (208)
PEN1-7L (466)
PEN2-5R (414)
PEN2-5L (391)
PEN2-6Ra (187)
PEN2-6Rb (242)
PEN2-6La (235)
PEN2-6Lb (196)
PEN2-7R (496)
PEN2-7L (427)
EPG-5Ra (98)
EPG-5Rb (73)
EPG-5Rc (68)
EPG-5La (116)
EPG-5Lb (92)
EPG-5Lc (118)
EPG-6Ra (108)
EPG-6Rb (93)
EPG-6La (129)
EPG-6Lb (117)
PEG-5R (645)
PEG-5L (659)
PEG-6R (530)
PEG-6L (682)
D7-5R4La (520)
D7-5R4Lb (565)
D7-6R3La (519)
D7-6R3Lb (418)
D7-7R2L (468)
D7-2R7L (502)
D7-1R9R8L (593)
Neuron (total # of synapses)
Outputs (%)
EPG-5Ra (899)
EPG-5Rb (758)
EPG-5Rc (841)
EPG-5La (830)
EPG-5Lb (914)
EPG-5Lc (843)
EPG-6Ra (893)
EPG-6Rb (774)
EPG-6La (959)
EPG-6Lb (1016)
D7-5R4La (3213)
D7-5R4Lb (3505)
D7-6R3La (3653)
D7-6R3Lb (2483)
D7-7R2L (2001)
D7-2R7L (1675)
D7-1R9R8L (1306)
Neuron (total # of synapses)
NO
Inputs (%)
PEN1-5R (643)
PEN1-5L (626)
PEN1-6Ra (498)
PEN1-6Rb (469)
PEN1-6La (410)
PEN1-6Lb (430)
PEN1-7R (687)
PEN1-7L (628)
PEN2-5R (523)
PEN2-5L (493)
PEN2-6Ra (393)
PEN2-6Rb (333)
PEN2-6La (324)
PEN2-6Lb (359)
PEN2-7R (542)
PEN2-7L (573)
Neuron (total # of synapses)
Outputs (%)
PEN1-5R (663)
PEN1-5L (645)
PEN1-6Ra (444)
PEN1-6Rb (428)
PEN1-6La (452)
PEN1-6Lb (308)
PEN1-7R (715)
PEN1-7L (681)
PEN2-5R (499)
PEN2-5L (499)
PEN2-6Ra (359)
PEN2-6Rb (363)
PEN2-6La (306)
PEN2-6Lb (318)
PEN2-7R (546)
PEN2-7L (539)
Neuron (total # of synapses)

Figure S4

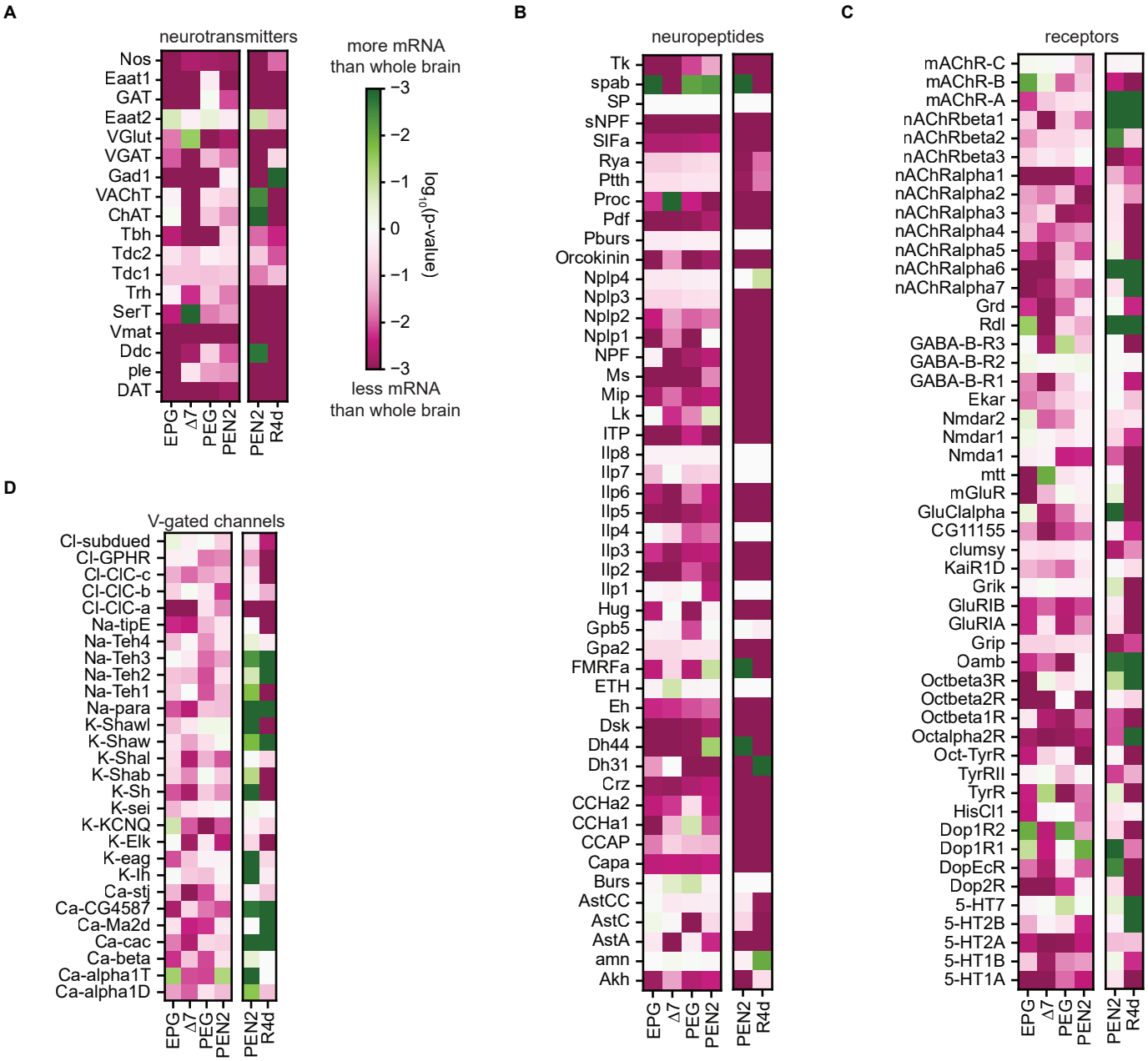

A
neurotransmitters
Nos
Eaat1
GAT
Eaat2
VGlut
VGAT
Gad1
VAChT
ChAT
Tbh
Tdc2
Tdc1
Trh
SerT
Vmat
Ddc
ple
DAT
EPG
Δ7
PEG
PEN2
PEN2
R4d
more mRNA
than whole brain
−3
−2
−1
0
−1
−2
−3
log10(p-value)
less mRNA
than whole brain
B
neuropeptides
Tk
spab
SP
sNPF
SIFa
Rya
Ptth
Proc
Pdf
Pburs
Orcokinin
Nplp4
Nplp3
Nplp2
Nplp1
NPF
Ms
Mip
Lk
ITP
Ilp8
Ilp7
Ilp6
Ilp5
Ilp4
Ilp3
Ilp2
Ilp1
Hug
Gpb5
Gpa2
FMRFa
ETH
Eh
Dsk
Dh44
Dh31
Crz
CCHa2
CCHa1
CCAP
Capa
Burs
AstCC
AstC
AstA
amn
Akh
EPG
Δ7
PEG
PEN2
PEN2
R4d
C
receptors
mAChR-C
mAChR-B
mAChR-A
nAChRbeta1
nAChRbeta2
nAChRbeta3
nAChRalpha1
nAChRalpha2
nAChRalpha3
nAChRalpha4
nAChRalpha5
nAChRalpha6
nAChRalpha7
Grd
Rdl
GABA-B-R3
GABA-B-R2
GABA-B-R1
Ekar
Nmdar2
Nmdar1
Nmda1
mtt
mGluR
GluClalpha
CG11155
clumsy
KaiR1D
Grik
GluRIB
GluRIA
Grip
Oamb
Octbeta3R
Octbeta2R
Octbeta1R
Octalpha2R
Oct-TyrR
TyrRII
TyrR
HisCl1
Dop1R2
Dop1R1
DopEcR
Dop2R
5-HT7
5-HT2B
5-HT2A
5-HT1B
5-HT1A
EPG
Δ7
PEG
PEN2
PEN2
R4d
D
V-gated channels
Cl-subdued
Cl-GPHR
Cl-ClC-c
Cl-ClC-b
Cl-ClC-a
Na-tipE
Na-Teh4
Na-Teh3
Na-Teh2
Na-Teh1
Na-para
K-Shawl
K-Shaw
K-Shal
K-Shab
K-Sh
K-sei
K-KCNQ
K-Elk
K-eag
K-Ih
Ca-stj
Ca-CG4587
Ca-Ma2d
Ca-cac
Ca-beta
Ca-alpha1T
Ca-alpha1D
EPG
Δ7
PEG
PEN2
PEN2
R4d

Figure S5

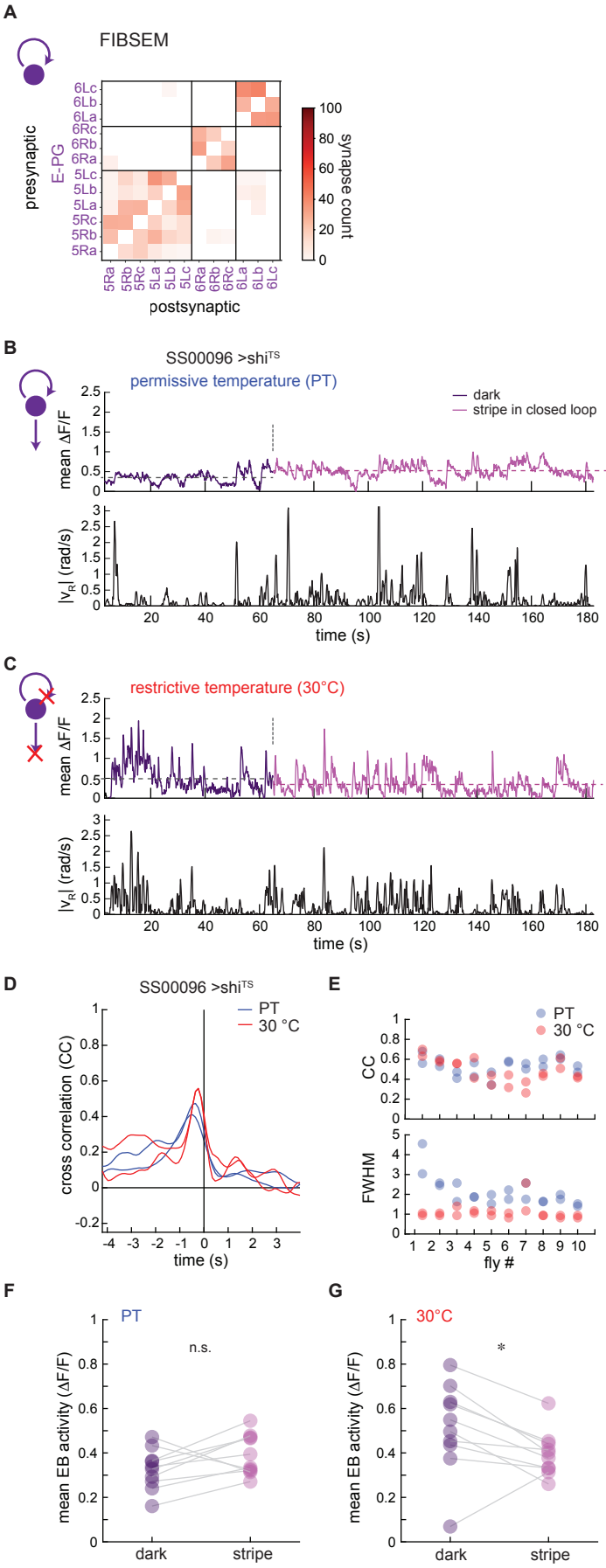

A
FIBSEM
presynaptic
E-PG
6Lc
6Lb
6La
6Rc
6Rb
6Ra
5Lc
5Lb
5La
5Rc
5Rb
5Ra
postsynaptic
synapse count
100
80
60
40
20
0
B
SS00096 >shi^TS
permissive temperature (PT)
dark
stripe in closed loop
mean ΔF/F
|v_R| (rad/s)
time (s)
C
restrictive temperature (30°C)
mean ΔF/F
|v_R| (rad/s)
time (s)
D
SS00096 >shi^TS
PT
30 °C
cross correlation (CC)
time (s)
E
PT
30 °C
CC
FWHM
fly #
F
PT
n.s.
mean EB activity (ΔF/F)
dark
stripe
G
30°C
*
mean EB activity (ΔF/F)
dark
stripe

Figure S6
Figure S6

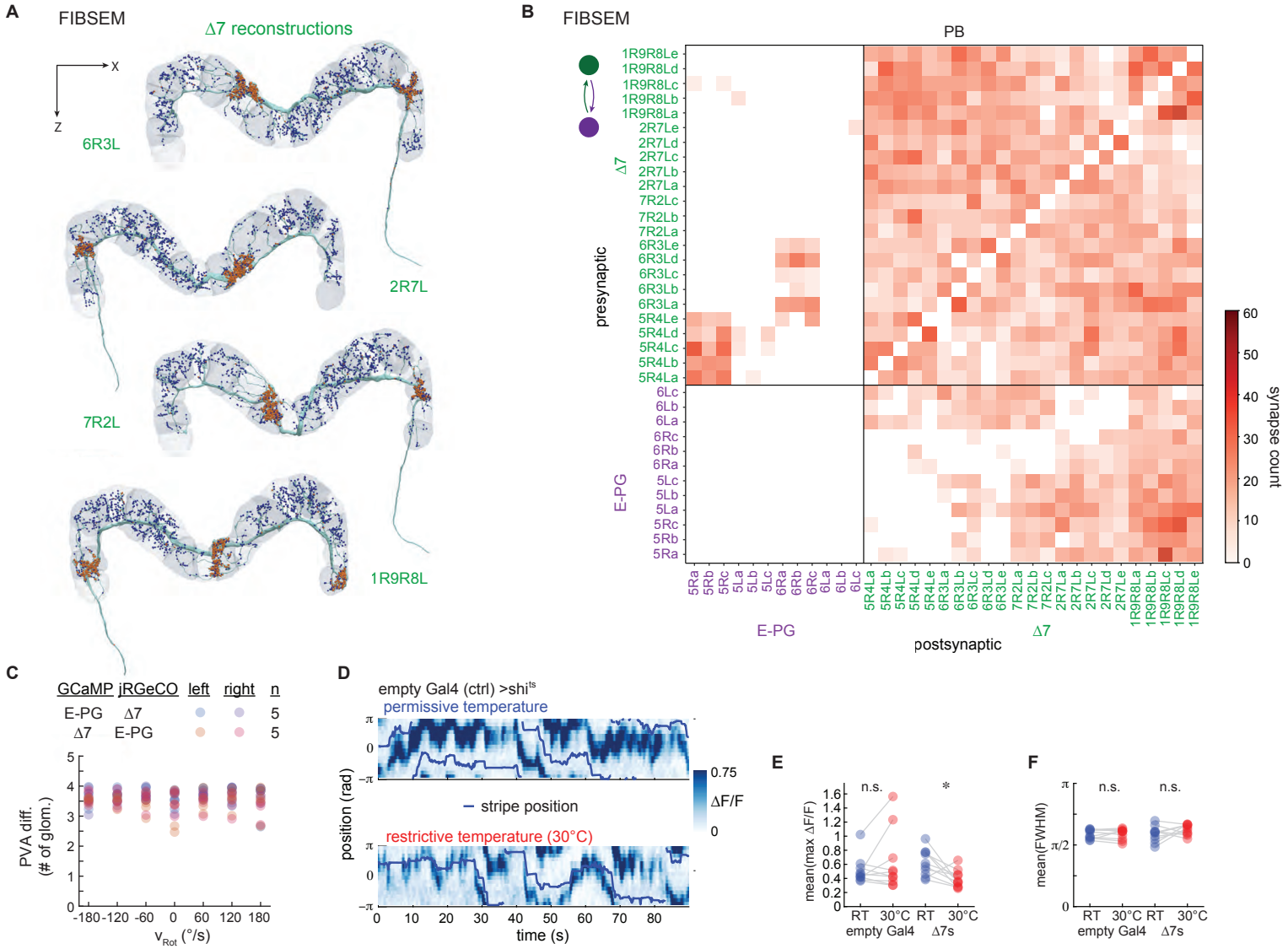
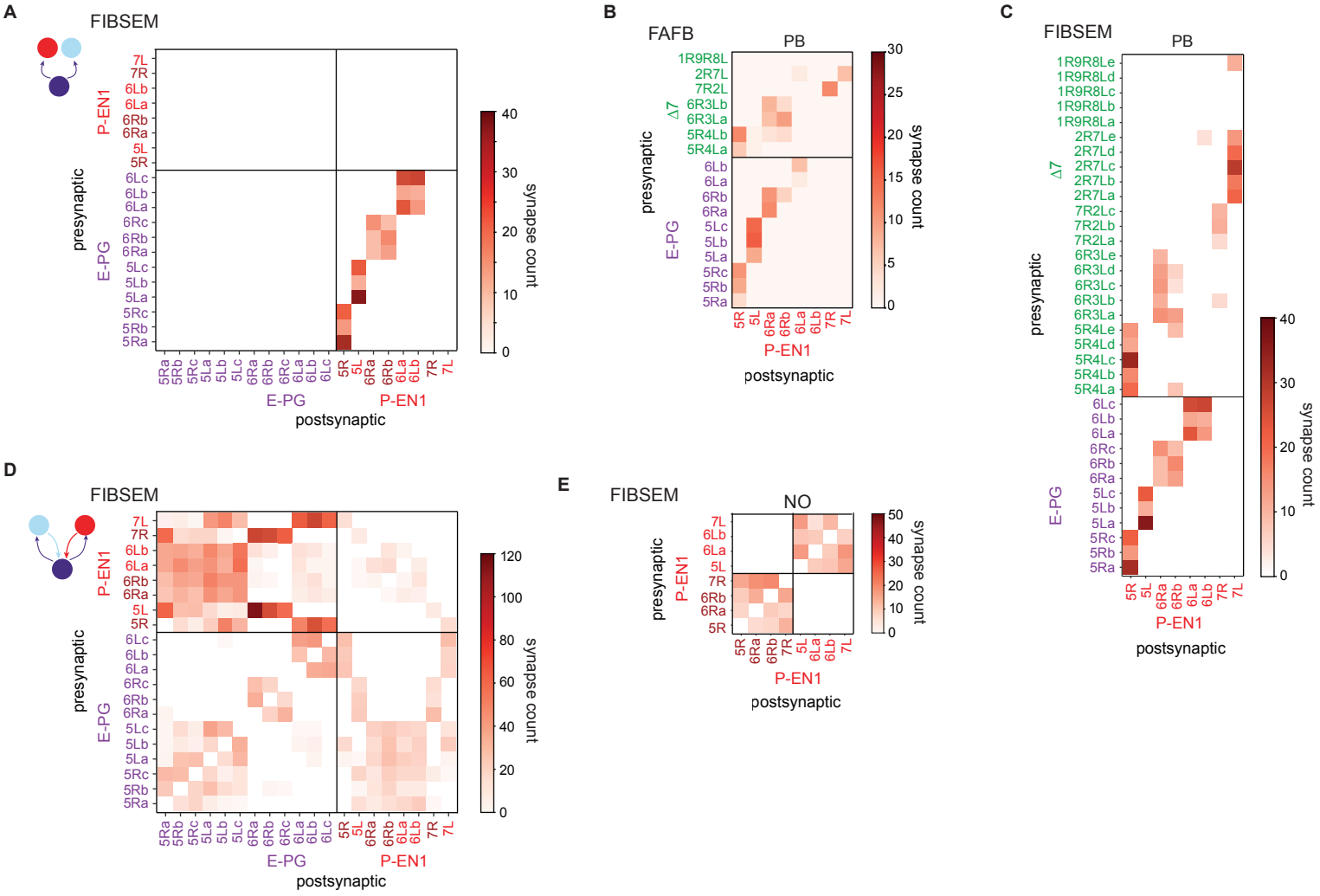
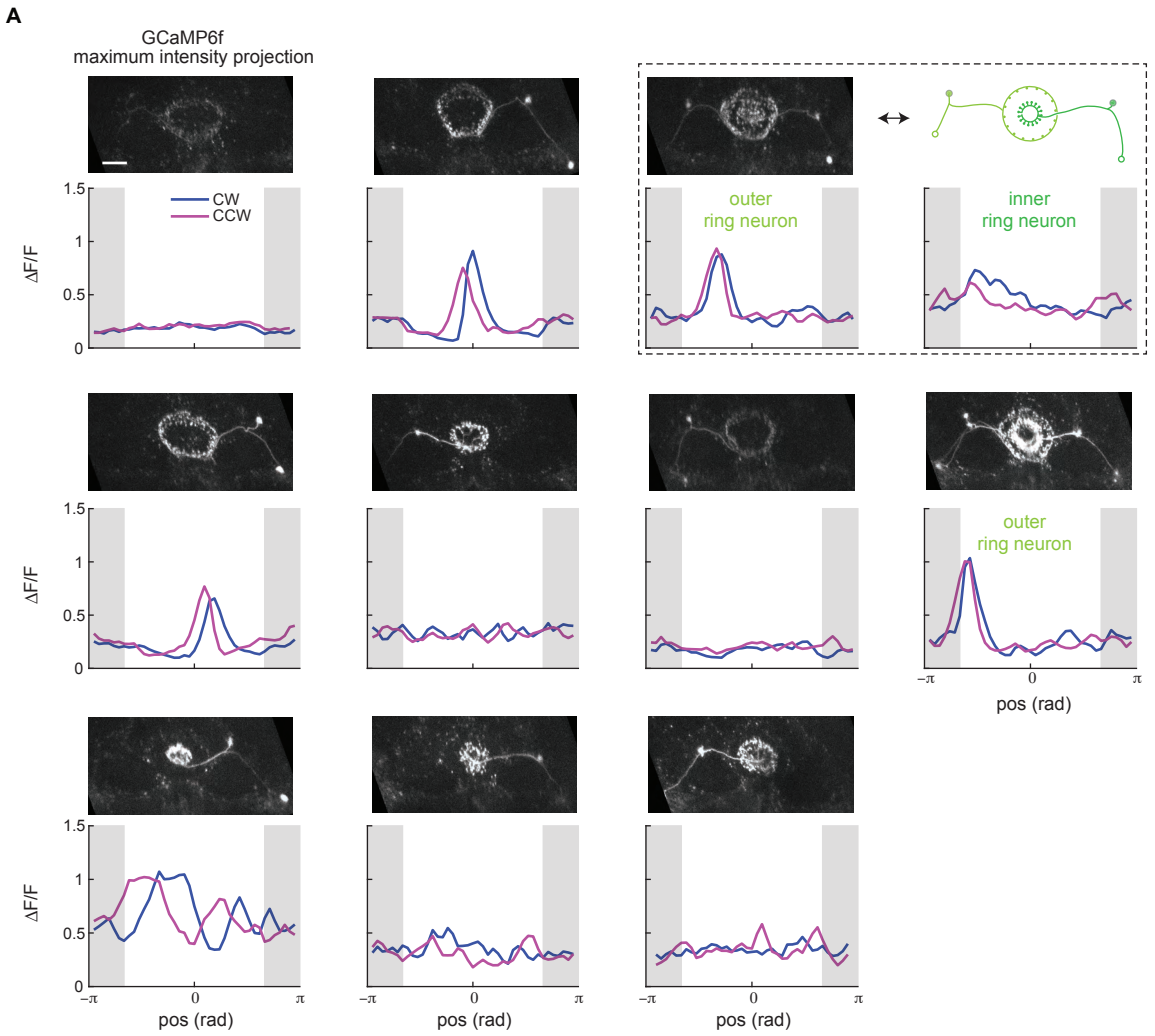


Figure S7


A
FIBSEM
P-EN1
E-PG
presynaptic
postsynaptic
synapse count
B
FAFB
PB
Δ7
C
FIBSEM
PB
D
FIBSEM
E
FIBSEM
NO


A
GCaMP6f
maximum intensity projection
CW
CCW
ΔF/F
outer
ring neuron
inner
ring neuron
pos (rad)

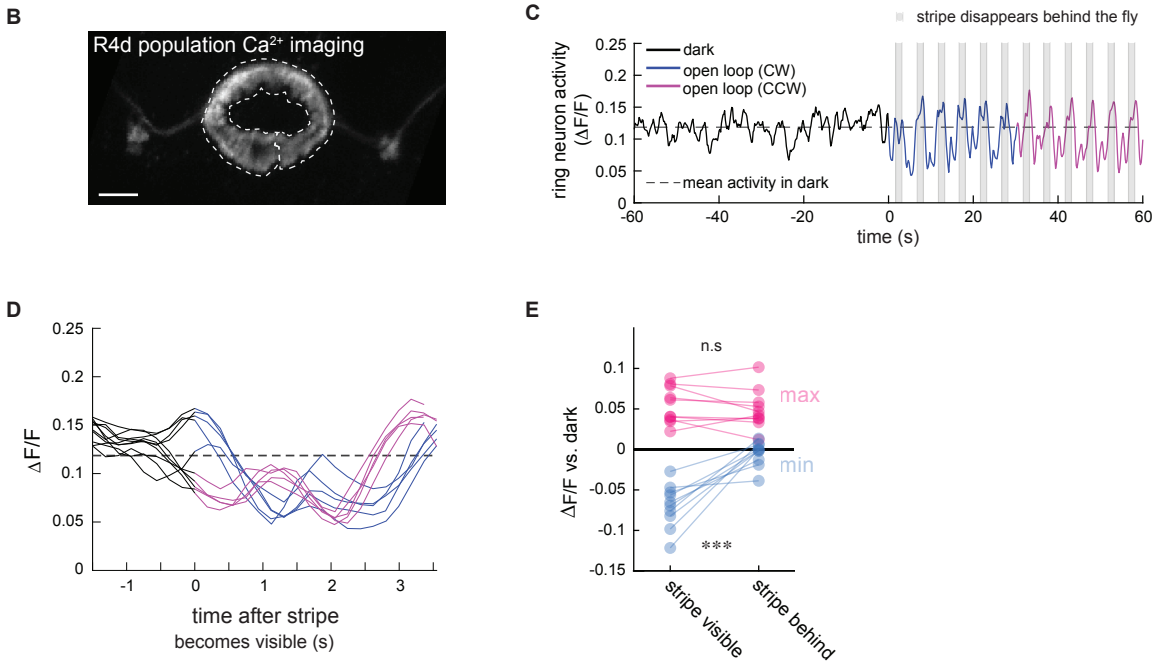

B
R4d population Ca2+ imaging
C
stripe disappears behind the fly
dark
open loop (CW)
open loop (CCW)
mean activity in dark
ring neuron activity
(ΔF/F)
time (s)
D
ΔF/F
time after stripe
becomes visible (s)
E
n.s
max
min
***
ΔF/F vs. dark
stripe visible
stripe behind

Figure S9

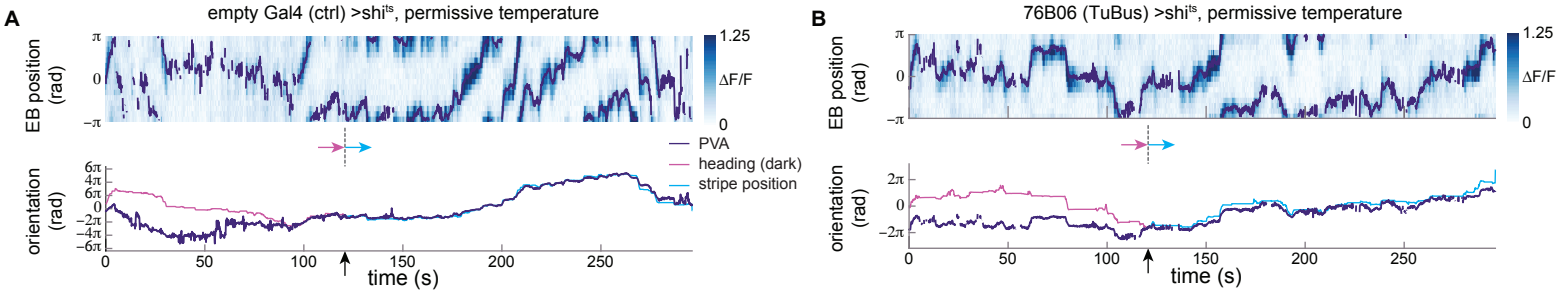

A
empty Gal4 (ctrl) >shi^ts, permissive temperature
EB position (rad)
π
0
−π
1.25
ΔF/F
0
PVA
heading (dark)
stripe position
orientation (rad)
6π
4π
6π
0
−2π
−4π
−6π
0
50
100
150
200
250
time (s)
B
76B06 (TuBus) >shi^ts, permissive temperature
EB position (rad)
π
0
−π
1.25
ΔF/F
0
orientation (rad)
2π
0
−2π
0
50
100
150
200
250
time (s)

Figure S10

Figure S10

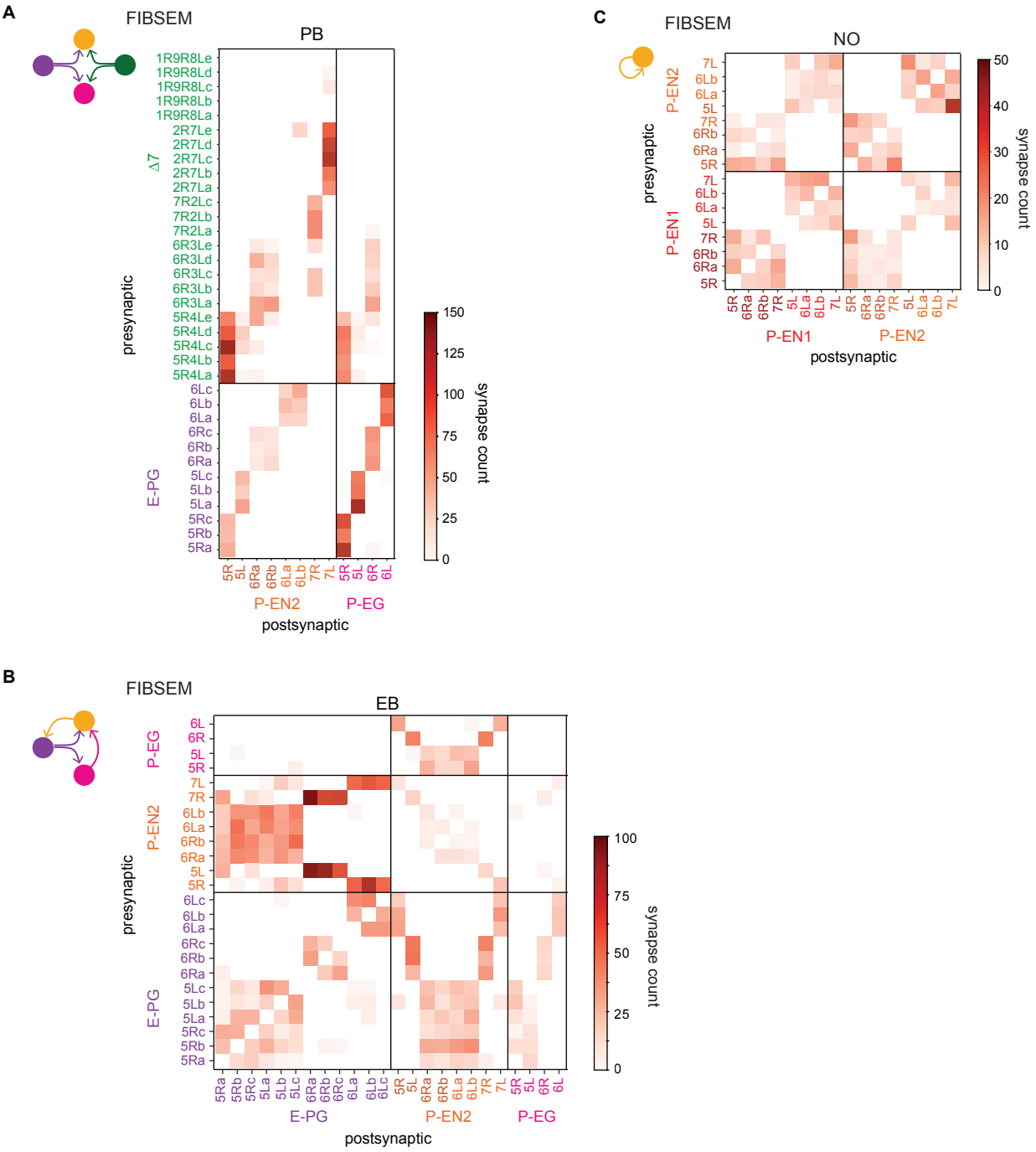


Figure S11

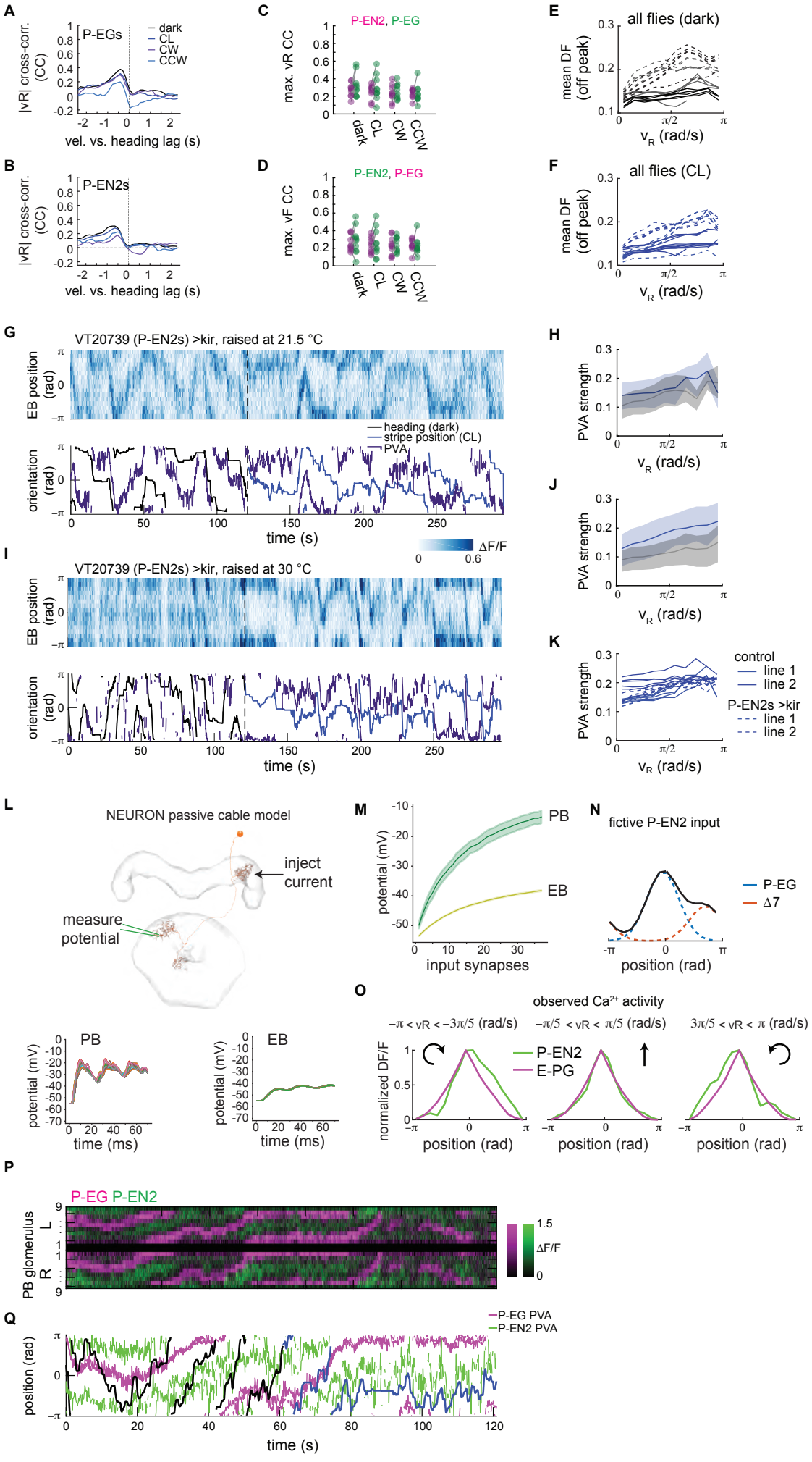

A
P-EGs
dark
CL
CW
CCW
|vR| cross-corr. (CC)
vel. vs. heading lag (s)
B
P-EN2s
C
P-EN2, P-EG
max. vR CC
D
P-EN2, P-EG
max. vF CC
E
all flies (dark)
mean DF (off peak)
F
all flies (CL)
G
VT20739 (P-EN2s) >kir, raised at 21.5 °C
EB position (rad)
orientation (rad)
heading (dark)
stripe position (CL)
PVA
time (s)
ΔF/F
H
PVA strength
J
K
control
line 1
line 2
P-EN2s >kir
I
VT20739 (P-EN2s) >kir, raised at 30 °C
L
NEURON passive cable model
inject current
measure potential
PB
EB
potential (mV)
time (ms)
M
input synapses
N
fictive P-EN2 input
P-EG
Δ7
position (rad)
O
observed Ca2+ activity
−π < vR < −3π/5 (rad/s)
−π/5 < vR < π/5 (rad/s)
3π/5 < vR < π (rad/s)
normalized DF/F
P-EN2
E-PG
P
P-EG P-EN2
PB glomerulus
ΔF/F
Q
P-EG PVA
P-EN2 PVA
position (rad)
time (s)

Figure S12

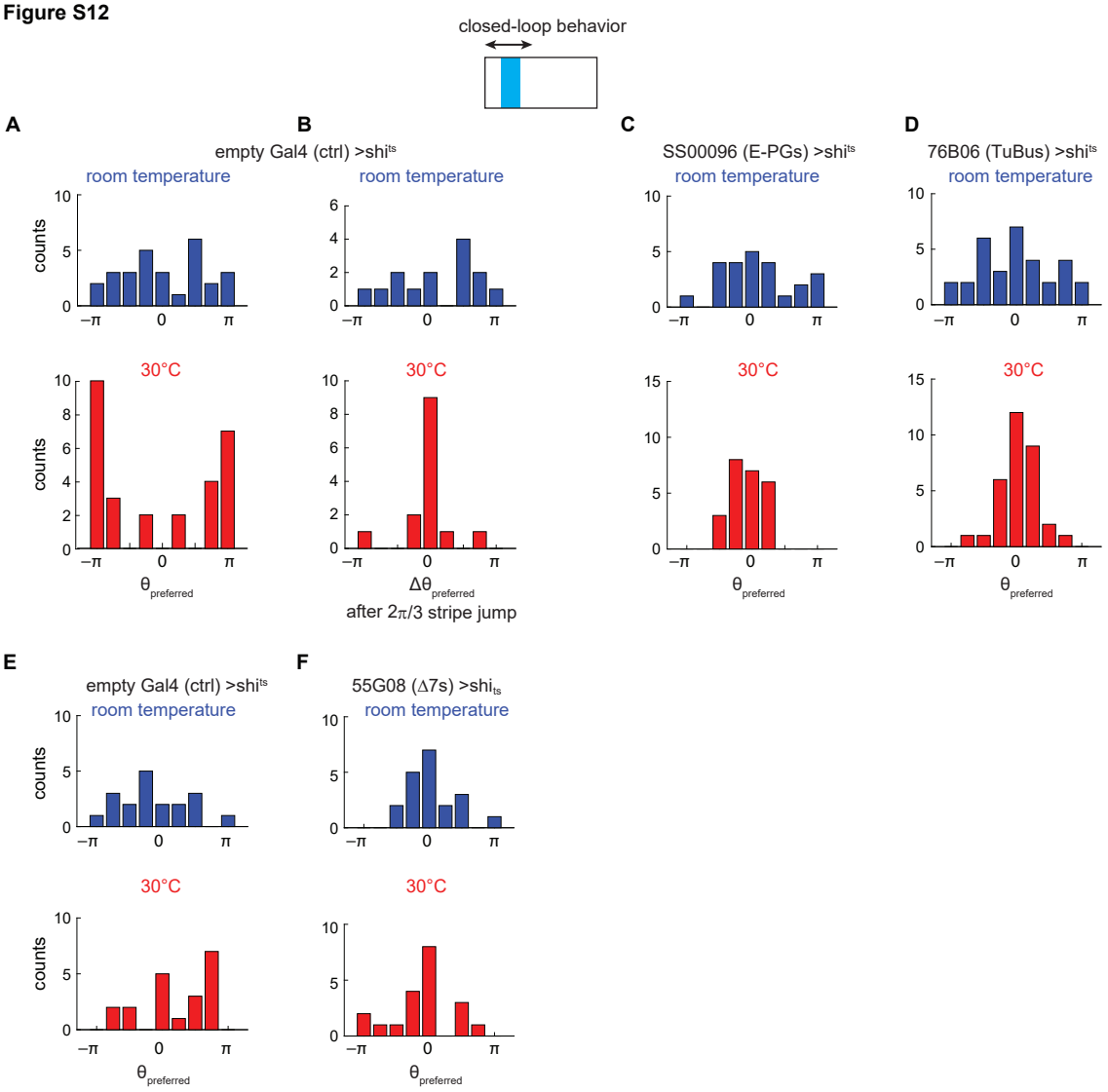


Figure S12
closed-loop behavior
A
empty Gal4 (ctrl) >shi^ts
room temperature
30°C
counts
θ preferred
B
room temperature
30°C
Δθ preferred
after 2π/3 stripe jump
C
SS00096 (E-PGs) >shi^ts
room temperature
30°C
θ preferred
D
76B06 (TuBus) >shi^ts
room temperature
30°C
θ preferred
E
empty Gal4 (ctrl) >shi^ts
room temperature
30°C
counts
θ preferred
F
55G08 (Δ7s) >shi_ts
room temperature
30°C
θ preferred

Figure S13

Figure S13