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Supplemental Information

Cellular and Behavioral Functions of *fruitless* Isoforms in *Drosophila* Courtship

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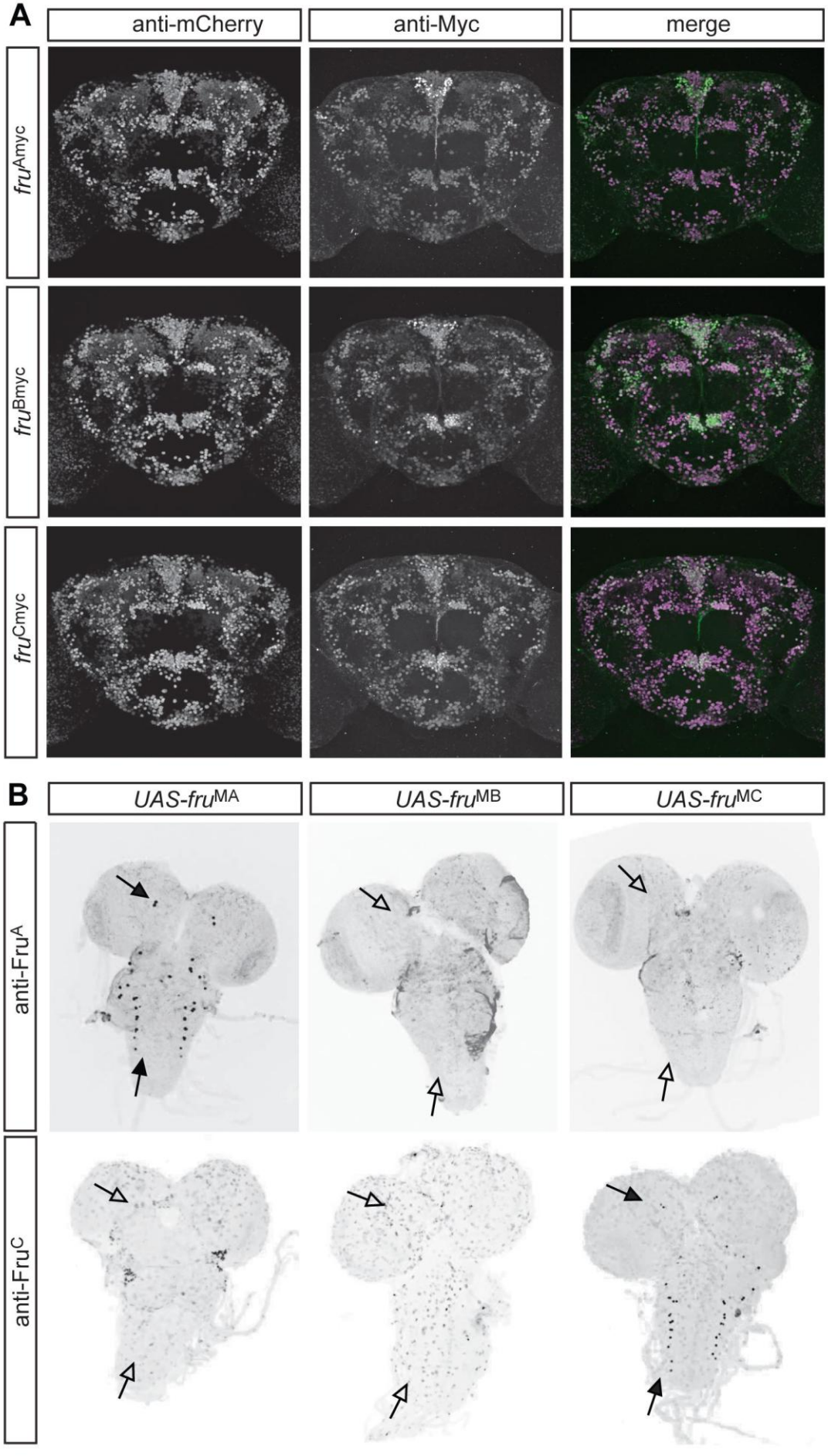
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anti-Myc
merge
fruAmyc
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fruCmyc
B
UAS-fruMA
UAS-fruMB
UAS-fruMC
anti-FruA
anti-FruC

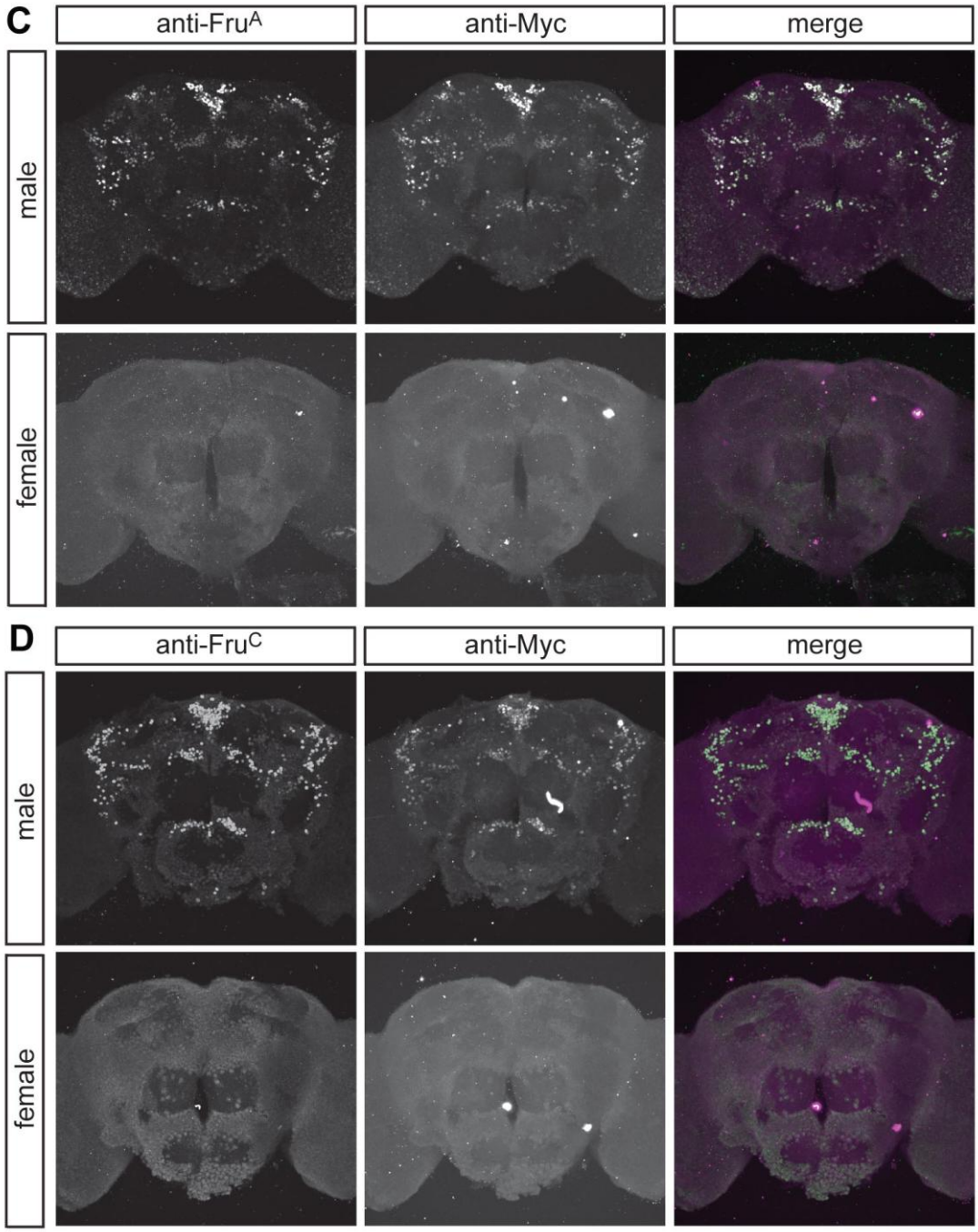
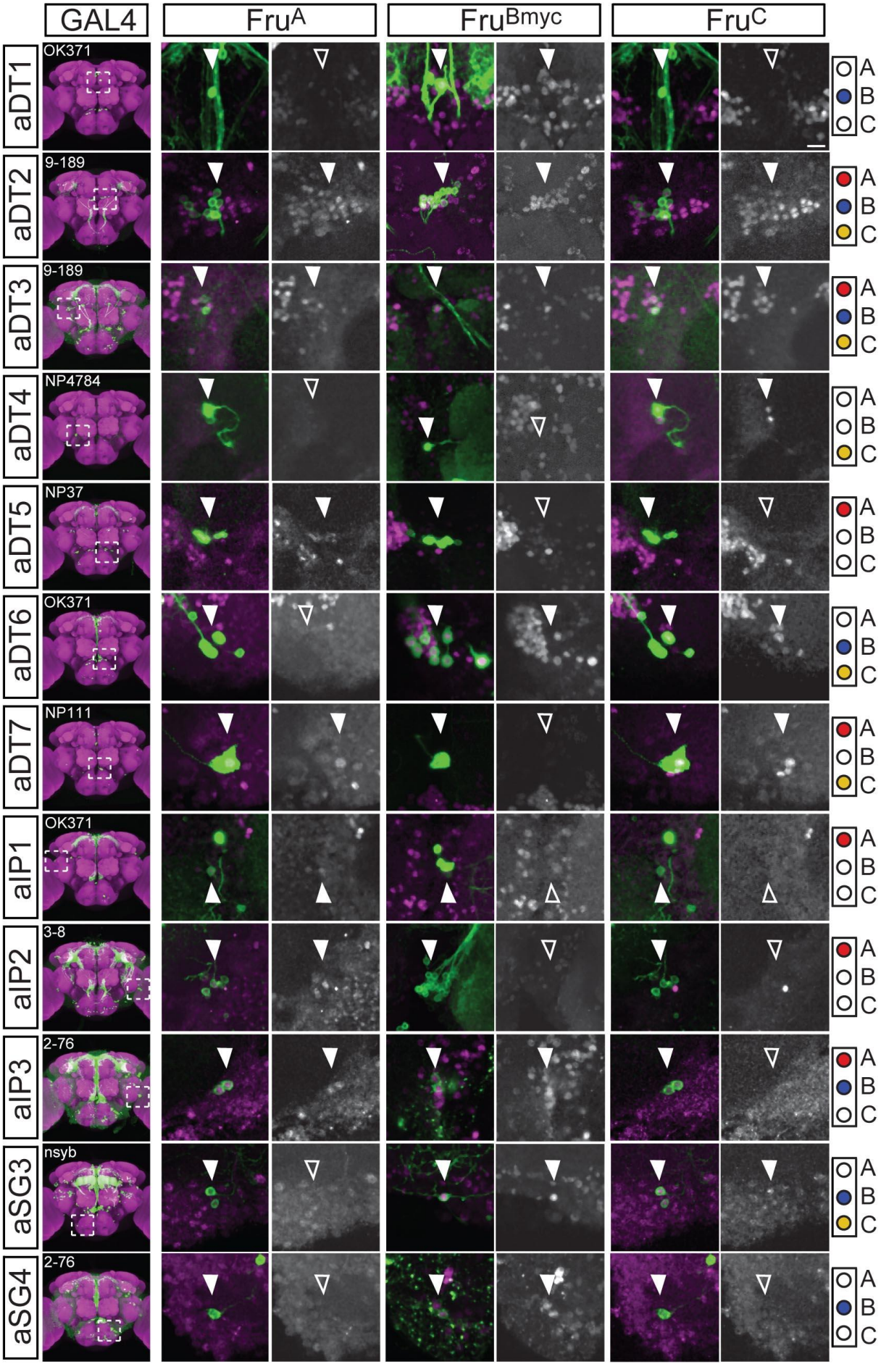


Figure S1, related to Figure 1

Validation of myc-tagged *fru* alleles and Fru^{A} and Fru^{C} antisera

A Brains of 7d-old males carrying *fru-GAL4, UAS-His2AmCherry* and the indicated *fru*^{myc} allele, stained with anti-mCherry (magenta in merge) and anti-c-Myc (green). **B** Brains and VNCs of 3rd instar larvae carrying *CCAP-GAL4* and the indicated UAS transgene, stained with anti-Fru^{A} or anti-Fru^{C}. Solid arrows indicate specific labeling in CCAP neurons; empty arrows the absence of labeling. Weak staining in CCAP-negative cells is likely due to expression of Fru^{COM} isoforms. **C, D** Brains of 7d-old adult males and females heterozygous for the *fru*^{Amyc} (C) or the *fru*^{Cmyc} (D) allele, stained with anti-Fru^{A} (C) or anti-Fru^{C} (D) (magenta) and anti-c-Myc (green).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FruC
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aDT2
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aDT5
NP37
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OK371
aDT7
NP111
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aIP2
3-8
aIP3
2-76
aSG3
nsyb
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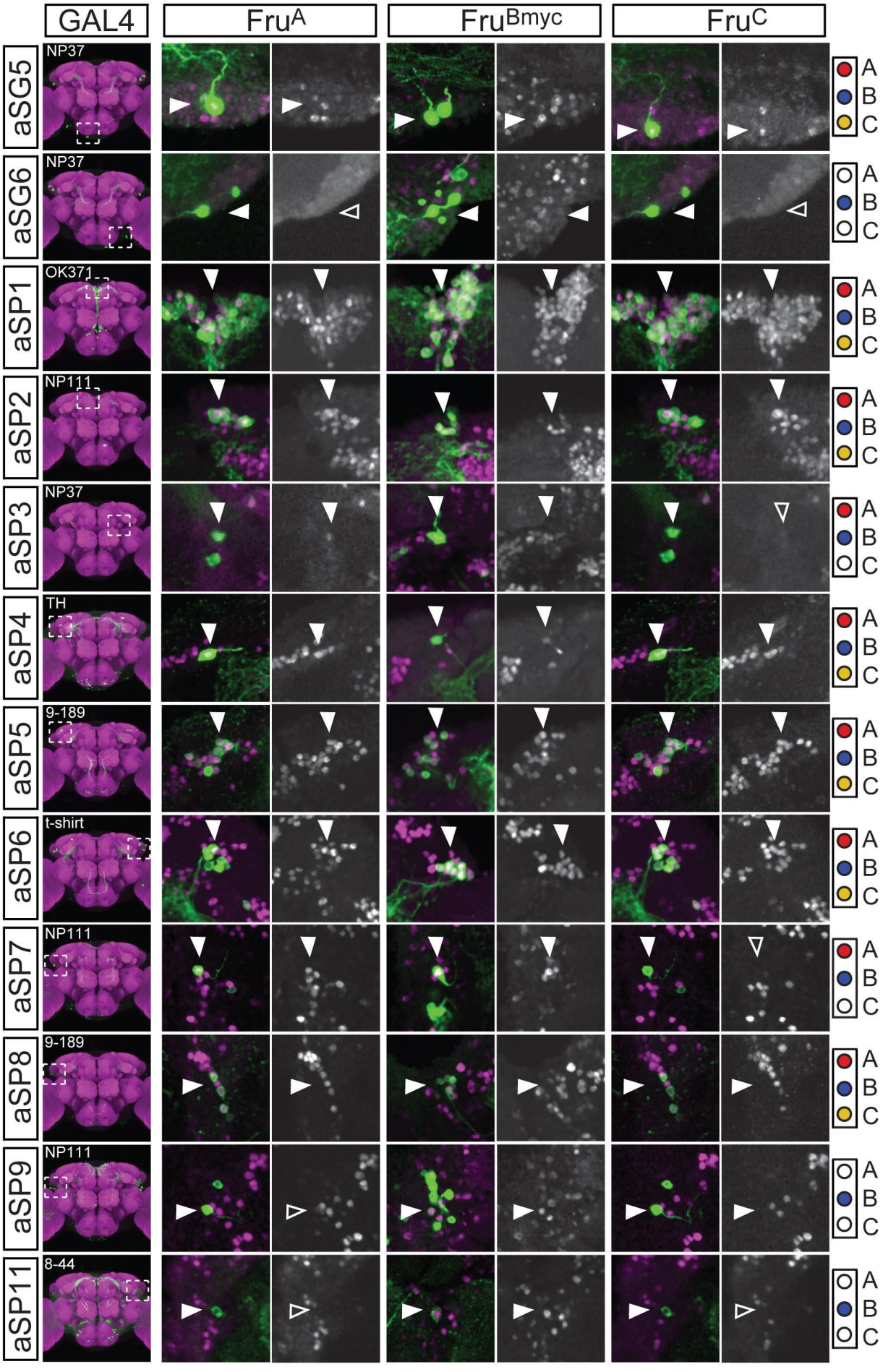
2-76
A
B
C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aSP4
TH
aSP5
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NP111
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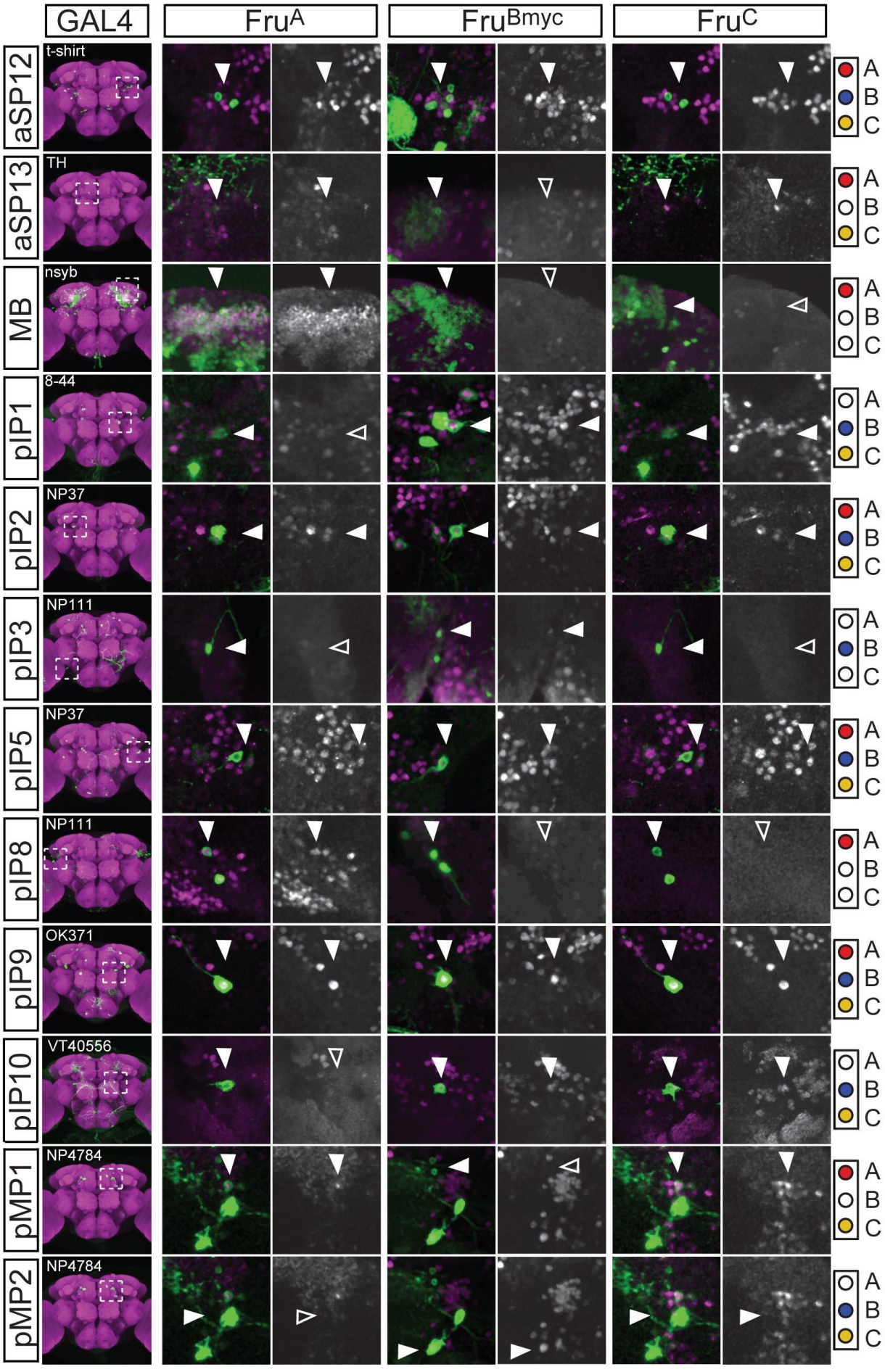
8-44
A
B
C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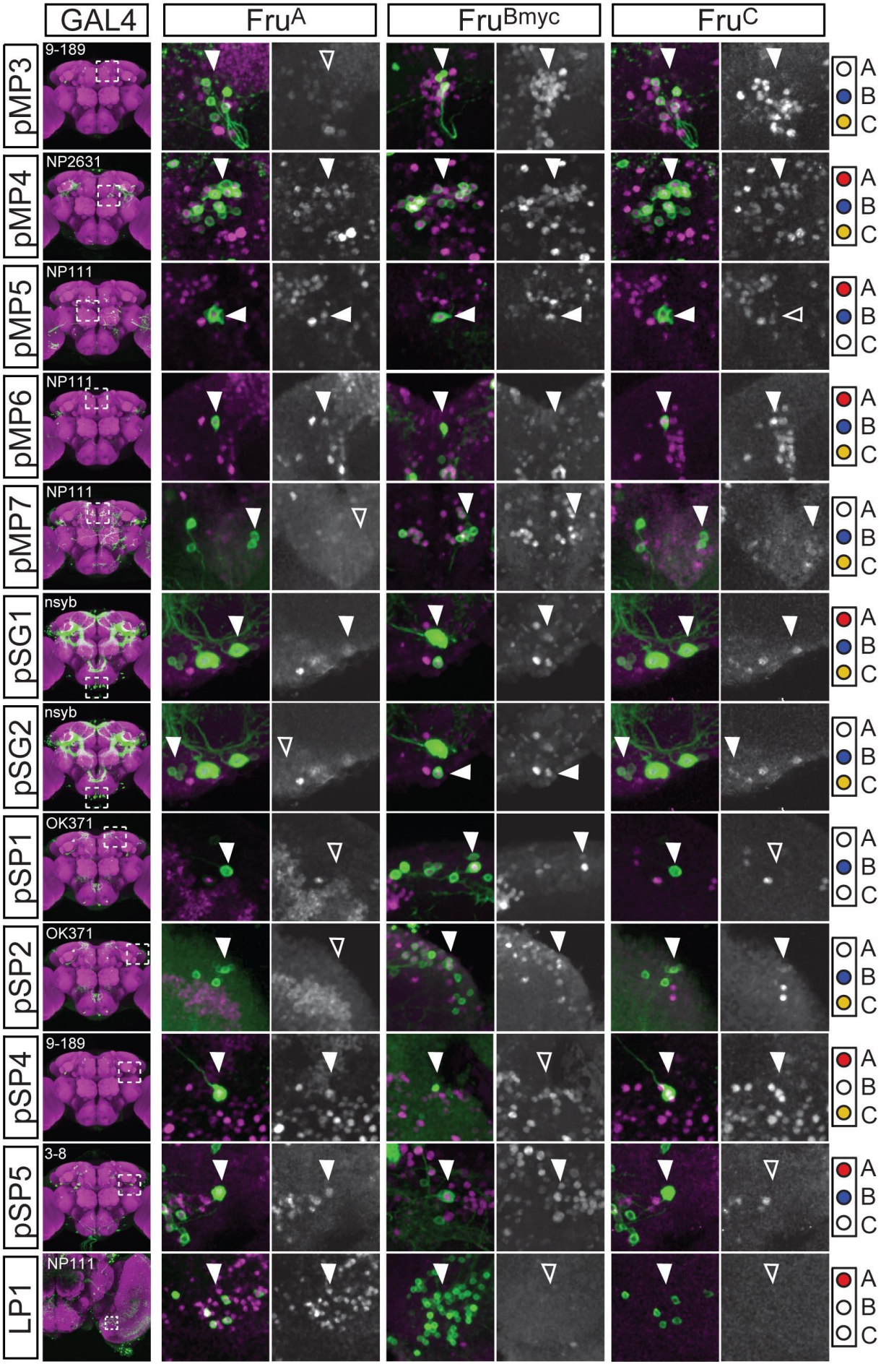
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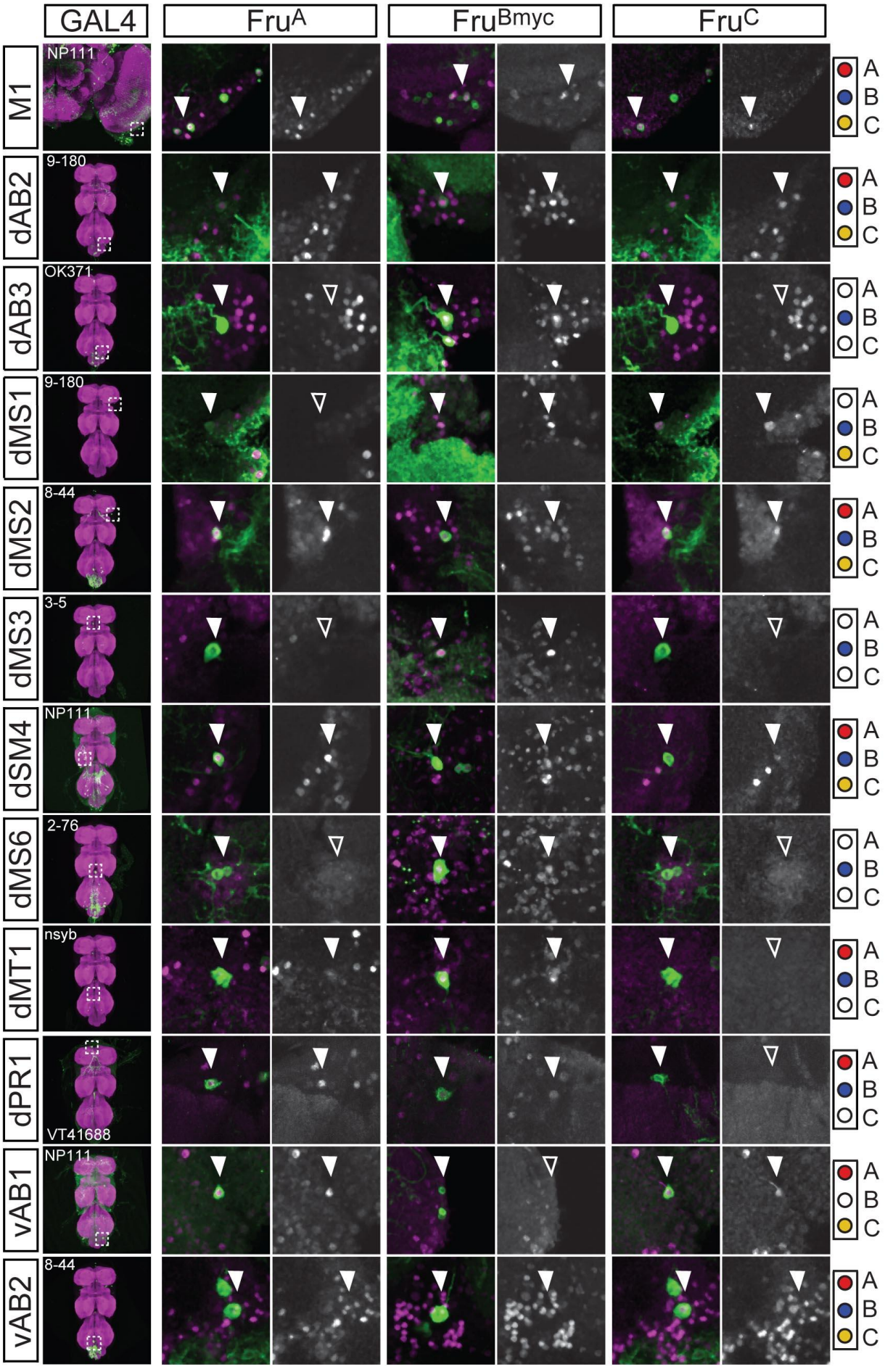
B
C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3-5
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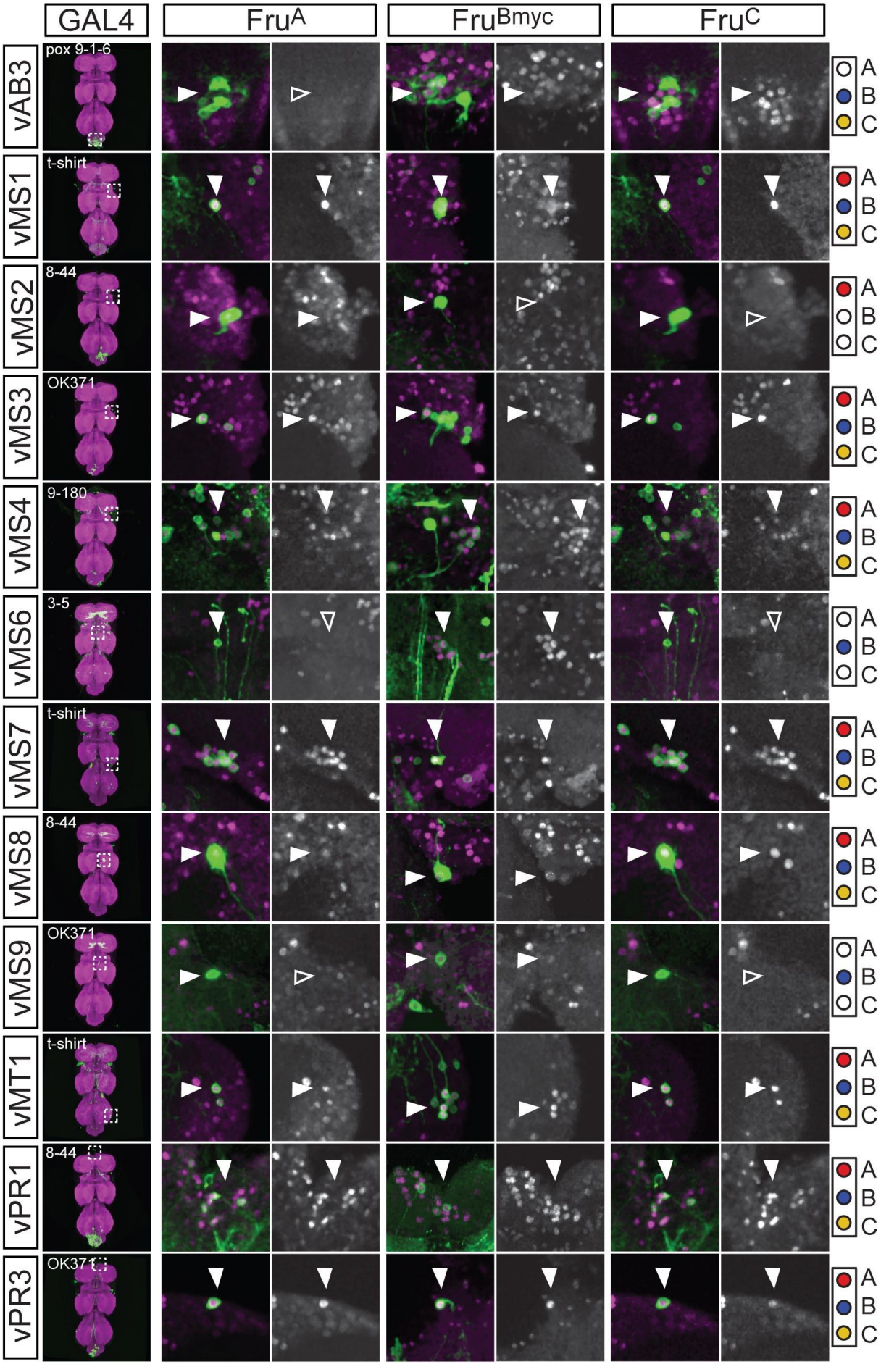
8-44
A
B
C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FruBmyc
FruC
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vMS1
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vMS2
8-44
vMS3
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vMS6
3-5
vMS7
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vMS9
OK371
vMT1
t-shirt
vPR1
8-44
vPR3
OK371
A
B
C

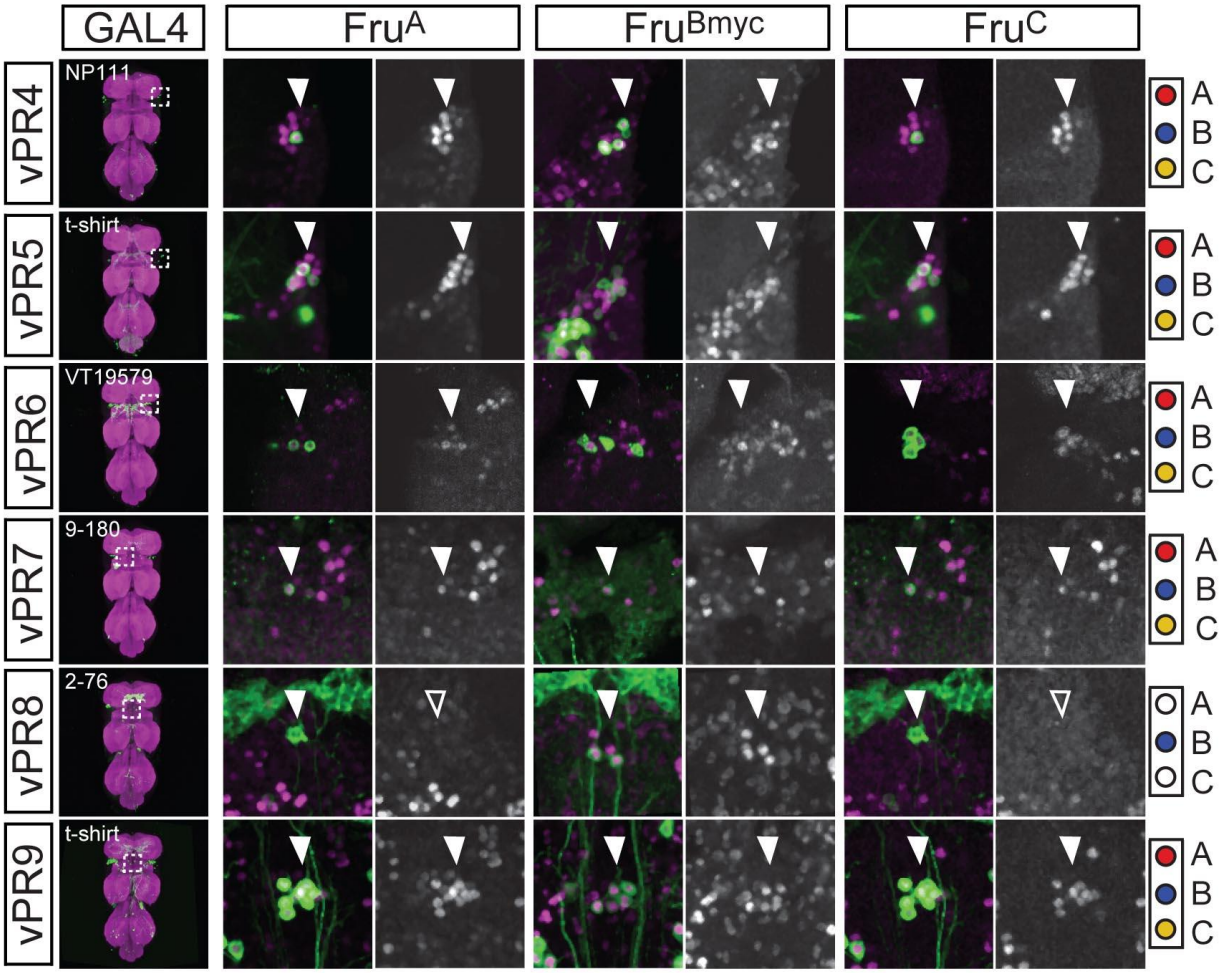

Figure S2, related to Figure 1

Expression of Fru isoforms in specific *fru*$^{+}$ cell types

For each cell type, the first column shows the location of the cell bodies (green) and indicates the *Gal4* line used to drive *UAS-mCD8-GFP* for identification. Selected confocal sections are shown registered onto a reference template (magenta). Fru^{A} and Fru^{C} were detected by antibody staining, Fru^{B} by use of the myc tagged allele and subsequent anti-Myc staining (shown in magenta). Solid and empty arrowheads mark the presence and absence of an isoform, respectively. The expression profile for each neuronal class is shown schematically in the rightmost panel. For detailed anatomical description and segmented projection and arborization patterns of the shown neuronal classes, see [17] and, for pIP10, dPR1 and vPR6, [30].

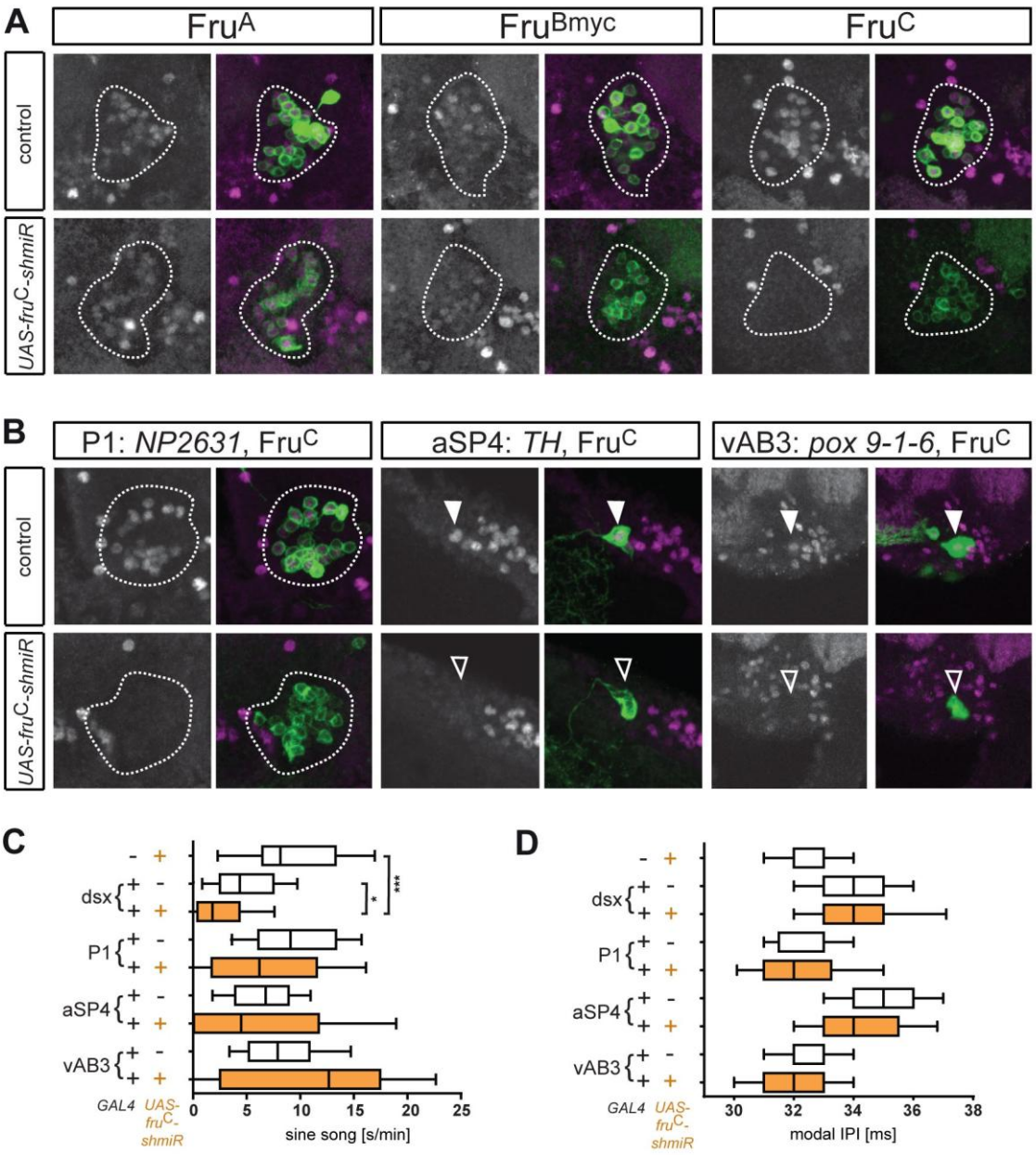

Figure S3, related to Figure 4

Knockdown of Fru^C in specific neuronal classes and its effect on courtship song

A *fru^C -shmiR* mediated knockdown leads to the depletion of Fru^C, but not Fru^B and Fru^A, as shown for the P1 neuronal cluster labeled by GFP expression driven by *dsx-GAL4* (green). Fru^A and Fru^C are labeled by isoform specific antisera, Fru^B by myc tagging and Myc antiserum (magenta). **B** Loss of Fru^C (detected by isoform specific antiserum, magenta) in the neuronal classes P1, aSP4 and vAB3 (labeled by GFP in green) after *fru^C-shmiR* mediated knockdown with the *NP2631*, *TH* and *pox9-1-6 GAL4* drivers, respectively. Fru^C expression in adjacent, GFP negative cells is unaffected. **C** Sine song production after Fru^C knockdown in dsx neurons (*dsx-GAL4*), P1 (*NP2631),* aSP4 *(TH-GAL4),* or vAB3 *(pox9-1-6-GAL4)*. n=57-60 flies per genotype. **D** IPI distribution of pulse song of the experimental flies represented in C. n=57-60 flies per genotype, 50-1500 IPIs per fly. C, D: Kruskal-Wallis non parametric ANOVA, followed by Dunn's multiple comparisons test, ***p<0.0001, *p<0.05. Box and whisker plots show 10, 25, 50, 75 and 90 percentiles. Yellow bars represent knockdown experiments, white bars controls.

	Pupa CNS	Adult CNS
A	235 ± 47 (15%)	354 ± 78 (22%)
B	159 ± 54 (10%)	175 ± 44 (11%)
C	49 ± 12 (3%)	94 ± 21 (6%)
A, B	124 ± 43 (8%)	76 ± 23 (5%)
A, C	44 ±13 (3%)	28 ± 12 (2%)
B, C	235 ± 53 (15%)	277 ± 17 (17%)
A, B, C	728 ± 76 (46%)	604 ± 69 (38%)
total	1573 ± 89 (n=5)	1607 ± 123 (n=5)

Table S1, related to Figure 1

Fru isoform expression in the pupa and adult

Number of cells expressing exclusively one, two or all three isoforms (mean ± standard deviation, n=5 animals) and percentages of the total Fru positive cells in the CNS of 2d old pupa and 8d old adults, respectively. Neurons in the mushroom body are not included.