ONLINE METHODS

Generation of transgenic flies of *UAS-rCD2::RFP*, *UAS-GFPi* and *UAS-rCD2i*

Standard molecular biological techniques were used to generate *pUAS-rCD2::RFP*, *pUAS-GFP-miRNA* and *pUAS-rCD2-miRNA*. *pUAS-rCD2::RFP* was constructed by cloning of PCR products of *rCD2* (EcoRI and BamHI) and monomeric *RFP* (BamHI and XbaI) into *pUAST* (EcoRI and XbaI). *pUAS-GFP-miRNA* and *pUAS-rCD2-miRNA* encode microRNAs carrying unique sequences derived from GFP (GATGACAGTGGCACCTATAATG and TGTATCTGCAAGAGGAAAAAAC) and rCD2 (GGTGAAGGTGATGCAACATACG and AGATGACGGGAACTACAAGAC-A), respectively. The strategy to design the miRNA constructs has been described [28]. Transgenic flies carrying above constructs were generated by Genetic Services, Inc.

Clonal analysis with twin-spot MARCM

The generation, dissection, immunostaining and mounting of mosaic clones of larval and adult brains have been described [10]. For developmental window and duration of heat-shock, two-cell clones of γ, α'/β' and α/β neurons with their associated multi-cellular Nb clones shown in Fig. 2 were generated at the early 1st instar larval, mid-3rd instar larval and pupal stages by heat-shock at 37^{o} C for 20-30 minutes. Larval wing disc and CNS mosaic clones shown in Fig. 1d and Fig. 3 were generated at the late 1st instar larval stage by heat-shock at 37^{o} C for 15 minutes. Central complex mosaic clones shown in Fig. 4 were generated from the 1st to 2nd instar stages by heat-shock at 37^{o} C for 25 minutes. A weak fan-shape body neuron, putatively born after ellipsoid body neurons, was

occasionally labeled in the central complex mosaic clones. The analysis of the central complex mosaic clones is limited to those without this fan-shape body neuron. The chance to generate mCD8::GFP- and rCD2::RFP-positive multi-cellular Nb clones appears to be similar: 49% and 51% for MB clones, 50% and 50% for larval central brain and VNC clones, and 46% and 54% for central complex clones. For presentation purpose, wild-type mCD8::GFP- and rCD2::RFP-positive multi-cellular Nb clones are shown in magenta in all figures. Primary antibodies used in this study include rat monoclonal antibody to mCD8 (1:100, Caltag), rabbit antibody to RFP (1:500, Clontech), and nc82 (1:100, DSHB). Secondary antibodies with different fluorophores, Cy3 (Jackson lab), Cy5 (Jackson lab) and Alexa 488 (Invitrogen), were used 1:200, 1:200 and 1:750 dilution in this study. Immunofluorescent signals were collected by confocal microscopy and then processed using Adobe Photoshop. The fly strains used for various experiments were as follows: (1) *tubP-GAL4* [10]; (2) *GAL4-OK107* [29]; (3) *MB247-GAL4* [30]; (4) *GAL4-C155* [31]; (5) *hs-FLP* [32]; (6) FRT^{40A}*,UAS-rCD2::RFP,UAS-GFP-miRNA*/*CyO,Y*; (7) FRT^{40A}*,UAS-mCD8::GFP,UAS-rCD2-miRNA*/*CyO,Y*; (8) FRT^{40A}*,UAS-mCD8::GFP, UAS-rCD2-miRNA,chinmo*^{1}/*CyO,Y*.

References:

28. Chen, C.H., *et al.* A synthetic maternal-effect selfish genetic element drives population replacement in Drosophila. *Science* **316**, 597-600 (2007).

29. Connolly, J.B., *et al.* Associative learning disrupted by impaired Gs signaling in Drosophila mushroom bodies. *Science* **274**, 2104-2107 (1996).

30. McGuire, S.E., Le, P.T. & Davis, R.L. The role of Drosophila mushroom body signaling in olfactory memory. *Science* **293**, 1330-1333 (2001).

31. Lin, D.M. & Goodman, C.S. Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. *Neuron* **13**, 507-523 (1994).

32. Golic, K.G. & Lindquist, S. The FLP recombinase of yeast catalyzes site-specific recombination in the Drosophila genome. *Cell* **59**, 499-509 (1989).