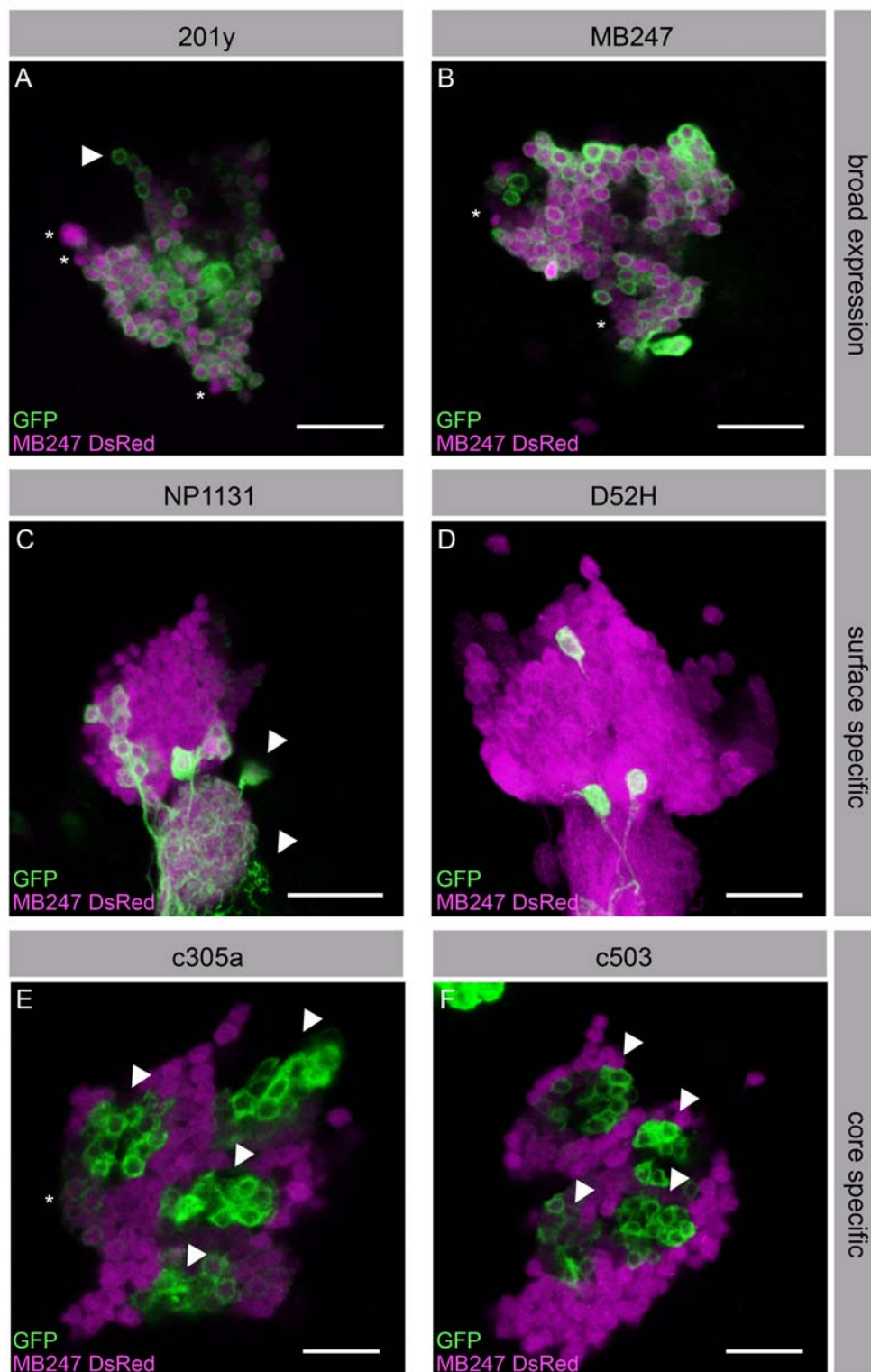**Supplemental Material**

Supplemental Data

Figure S1 related to Figure 4: Comparison of Kenyon Cell Body Labeling of Six Mushroom Body-Associated GAL4 Lines. The GAL4 lines 201y (A), MB247 (B), NP1131 (C), D52H (D), c305a (E) and c503 (F) were crossed with UAS-GFP; MB247DsRed to allow for comparison of the different patterns. 201y and MB247 label large, overlapping sets of KCs (A,B), but in addition show expression in specific neurons (arrowhead in A; asteriks in A,B). Due to the different insertion sites of the MB247 GAL4 and MB247DsRed constructs there is no complete overlay of GFP and DsRed (B). NP1131 and D52H almost completely overlap with the MB247DsRed pattern (C,D), although NP1131 also labels a small number of large cells projecting next to the calyx (arrowheads in C). c305a and c503 (E,F) label almost exclusively newly-born KCs next to the four dividing neuroblasts (arrowheads in E,F). Scale bars: 25 µm in A-D; 10 µm in E and F.
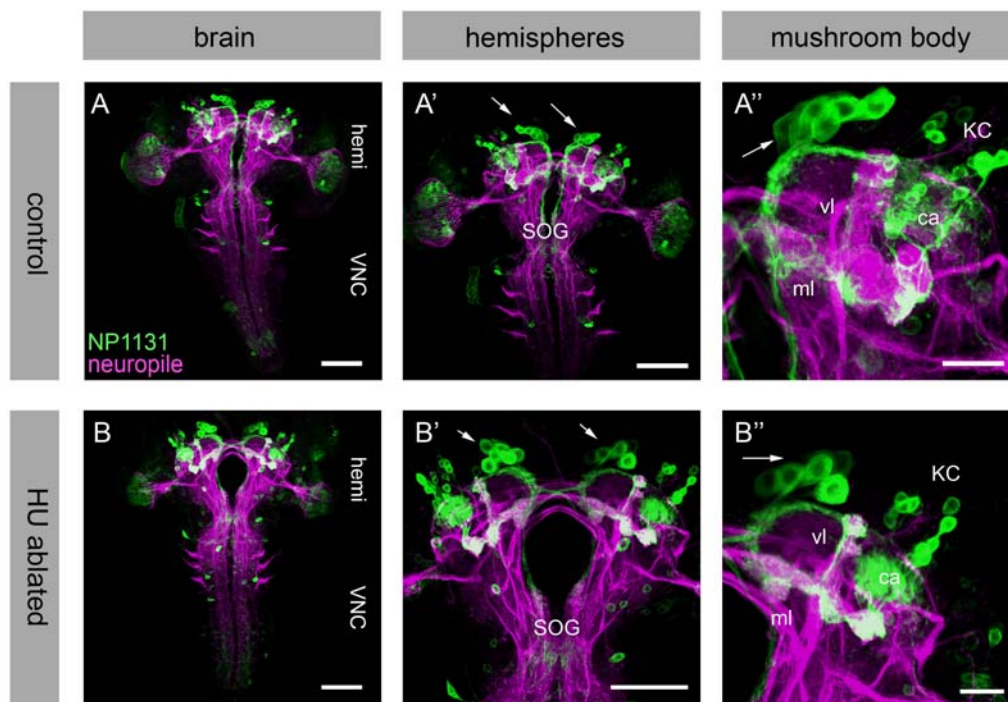
Figure S2 related to Figure 3: Expression Pattern of NP1131 in Control and Hydroxyurea-Treated Larvae. NP1131;UAS-*mCD8::GFP* larvae exhibit about 25 KCs at the third larval instar, both in controls (A) and in HU-ablated larvae (B), suggesting an embryonic

origin of these neurons. Specimens were double stained with anti-*GFP* and anti-*Fasciclin II/* anti-*Cholinacetyltransferase* as neuropile markers (Selcho et al., 2009). Hemi, brain hemispheres; VNC, ventral nerve cord; SOG, suboesophageal ganglion; vl, vertical lobe of the mb; ml, medial lobe of the mb; ca, calyx; KC, Kenyon cell bodies. Arrows highlight the labled DILP neurons. Scale bars: 50 µm in A, A', B and B'; 25 µm in A'' and B''.

GAL4 lines	gamma lobes	alpha'/beta' lobes	alpha/beta lobes		surface (embryonic born gamma Kenyon cells)	FasII positive part (larval born gamma Kenyon cells)	core (larval alpha' beta' Kenyon cells)
	ADULT mushroom body				**LARVAL mushroom body**		
1471	+						
NP 21	+				+	+	
c305a		+					+
c503		+					+
NP 3208			+				
NP 3061			+		+		
NP 7175			+			+	+
NP 5286			+			+	+
NP 6024			+			+	+
NP 3291			+			+	+
NP 7060			+			+	+
17d			+		+	+	+
c739			+		+	+	+
NP 1131	+	+			+		
NP 6522	+	+			+	+	+
NP 65		+	+				
NP 7427		+	+			+	
201y	+		+		+	+	
H24	+		+		+	+	
MB247	+		+		+	+	
103y	+	+	+				
c309	+	+	+				
D52H	+	+	+		+		
NP 2748	+	+	+				+
c747	+	+	+		+	+	+
c772	+	+	+		+	+	+
238y	+	+	+		+	+	+
OK107	+	+	+		+	+	+
c320	+	+	+		+	+	+
30y	+	+	+			+	+

Table S1 related to Figure 4: Comparison of Adult and Larval Mushroom Body Expression of 30 GAL4 Driver Lines. In order to obtain a more complete view of the intrinsic architecture of larval MBs, we screened a set of 30 GAL4 driver lines known to label

the adult MB (a detailed list of the relevant papers is given in Aso et al., 2009), by crossing them with UAS-*mCD8::GFP*. The MBs of third instar larvae were reported to show a quadruple laminar organization at the level of the pedunculus and lobes, termed surface layer, outer layer, inner layer and core (Kurusu et al., 2002). By applying anti-*FasII* / anti-*ChAT* neuropile markers we were able to reliably separate three of these layers at the third larval instar (Figure 1M''-R'''): (i) a partially *FasII*-negative surface layer that likely consists of embryonic-born γ KCs (Figure 1O''',P'''), (ii) a *FasII*-positive part including outer and inner layers that potentially covers larval-born γ KCs (Figure 1N'''-R'''), and (iii) a *FasII*-negative core that consists of larval-born α'/β' KCs (Figure 1M''''-R'''). Of the 30 GAL4 lines shown in Supplemental Table 1, only five failed to label the larval MB (1471, NP3208, NP65, 103y, c309) indicating that more than 80% of the lines expressed in adult MBs are also expressed in larval MBs. Three lines labeled specifically the surface layer (NP3061, NP1131, D52H) while another three labeled the core (c305a, c503, NP2748) (Figure 1 and Supplement Table 1). One line, NP7427, overlapped with the *FasII* positive MB neuropil. Four lines expressed GAL4 in the surface layer and the *FasII* positive part of the MB (NP21, 201y, H24, MB247) (Figure 1 and Supplement Table 1), whereas another six drivers visualized the *FasII*-positive part and the core (NP7175, NP5286, NP6024, NP3291, NP7060, 30y). Eight lines stained the entire MBs (17d, c739, NP6522, c747, c772, 238y, OK107, c320). Notably, combinatorial labeling for the surface layer and the core was not found. Most of the 30 GAL4 lines - except the six lines tested behaviorally - showed additional low-level expression in various other brain regions. Surprisingly, for most of the 30 lines there was no clear correlation between adult and larval stages concerning their expression in γ lobe, α'/β' lobes and α/β lobes (Supplemental Table 1), rendering a GAL4 driver database for larval MBs indispensable.

| Genotype | Odorant attraction | | Sugar reactivity |
	AM (1:250)	BA (pure)	
WT CS	30.4/1.6	22.2/1.8	27.9/2.2
UAS-shi/+	34.0/2.9	14.8/1.6	48.7/1.9
NP1131/+	22.5/2.2	33.2/2.4	31.0/2.1
201y/+	28.1/1.2	16.0/1.3	40.0/1.1
NP1131/UAS-shi	26.1/1.3	26.5/1.8	31.0/2.7
201y/UAS-shi	26.9/1.4	20.5/1.9	40.2/3.6

Table S2 related to Figure 3: Sensory Acuity Controls Measured at Restrictive Temperature

Movie S1 related to Figure 7: 3D Reconstruction of the Larval Third Instar Mushroom Body.

Supplemental Experimental Procedures

Screening of GAL4 Lines for Larval Mushroom Body Expression

We screened 30 GAL4 drivers (Aso et al., 2009; Tanaka et al., 2008) for expression in larval MBs: 1471, NP21, c305a, c503, NP3208, NP3061, NP7175, NP5286, NP6024, NP3291, NP7060, 17d, c739, NP1131, NP6522, NP65, NP7427, 201y, H24, MB247, 103y, c309, D52H, NP2748, c747, c772, 238y, OK107, c320, 30y. In addition, we screened another 64 GAL4 drivers (Aso et al., 2009; Tanaka et al., 2008) for expression in non-KC MBINs or in MBENs: GH146, MZ604, MZ717, MZ840, TDC-GAL4, TH-GAL4, NP47, NP100, NP242, NP225, NP393, NP397, NP525, NP677, NP689, NP697, NP969, NP1111, NP1528, NP2119, NP2120, NP2140, NP2150, NP2297, NP2331, NP2397, NP2447, NP2492, NP2583, NP2631, NP2723, NP2758, NP3032, NP3037, NP3128, NP3161, NP3174, NP3212, NP3253, NP3314, NP5037, NP5084, NP5103, NP5204, NP5270, NP5272, NP5288, NP6013, NP6014,

NP6510, NP6561, NP7088, NP7125, NP7135, NP7139, NP7177, NP7198, NP7201, NP7231, NP7247, NP7251, NP7323, NP7352, NP7493.

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