Supplementary Figure 1. High reliability of retroviral birth dating. (a) Upper: maximum intensity projection (MIP) images (18 µm thickness, 1 µm step) of DPI 9 adult-born cells labeled with antibodies against BrdU (red, left; see Methods) and eGFP+ (green, middle). The image on the right represents an overlay of the two images. Arrows point to the BrdU-positive nuclei of adult-born cells. Insets show individual images of the double-positive cell taken at the same focal plane. Lower: three additional examples of eGFP- and BrdU-positive cells. (b) A bar graph showing the fraction of cells with BrdU-positive nuclei among eGFP+ DPI 9 cells (n=126 eGFP+ neurons in 5 mice).
Supplementary Figure 2. Chronic blood vessels imaging ensures reliable detection of adult-born cells across different imaging sessions. (a) A typical example of blood vessel pattern imaging. Maximum-intensity projection images (82 µm thickness, 2 µm step) of blood vessels which were transiently stained with a red fluorescent dye sulforhodamine B (see Methods). Images are shown for days 9, 9.5, 10, 28, and 30 as well as three overlays. The blood vessel pattern was used for alignment of three-dimensional image stacks across the imaging sessions. Note the remarkable stability of the blood vessel pattern over entire imaging period. (b) Chronic blood vessels imaging ensures reliable detection of adult-born cells across different imaging sessions. Upper: A superimposed image of the same field of view imaged at three consecutive time points. Individual images are color coded in green, red and blue, as indicated. The three-dimensional image stacks were aligned according to the blood vessels pattern (see a). Lower: The same method of stacks alignment applied to images taken at DPI 28 and DPI 30. Note the high motility of GFP⁺ cells at DPI 9-10 but not at DPI 28-30. (c) Time course of arrival of adult-born JGNs at the glomerular layer. The bar graph shows mean fractions of adult-born cells found within the glomerular layer at DPI 5-9 (n=13 fields of view, 3 mice). For each field of view the number of cells detected at DPI 30 was taken as 100%.
Supplementary Figure 3. Comparison of odor- and glutamate-evoked Ca$^{2+}$ transients in the same JG neurons. (a) Representative Ca$^{2+}$ transients, evoked in the neighboring cells by three consecutive presentations of high concentration of 2-hexanone (9% of saturated vapor) followed by a 20-s-long iontophoretic application of glutamate (-1 µA injection current, 15 nA retain current; 100 mM Na$^+$-Glutamate in the pipette). (b) Box plots showing the amplitudes of Ca$^{2+}$ transients evoked in GFP-negative cells by the high concentration of the odorants (9% of saturated vapor). Left: data illustrated in Fig. 3e (n=54 cells, 5 mice). Right: a new set of data (as illustrated in a; n=112 cells, 7 regions of interest, 2 mice). (c) A box plot showing a distribution of mean amplitude ratios (mean response to odorant vs. the one to glutamate for each recorded cell, averaged for each of 7 regions of interest). (d) A cumulative probability graph illustrating the distribution of mean amplitude ratios for all recorded cells (n=112).
Supplementary Figure 4. Immunohistochemical characterization of JG neurons. (a) A representative MIP image (12 µm thickness, 1 µm step) of the glomerular layer in a fixed OB tissue. The tissue was isolated 9 days after the retroviral labeling of adult-born JGNs and post hoc immunostained with antibodies against doublecortin (DCX, green), eGFP⁺ (blue) and calbindin, calretinin, TH, NeuN (all yellow; see Methods). Note that all blue GFP⁺ cells are DCX positive (see also b and Supplementary Fig. 5). Note also the absence of yellow cells with yellow/green processes consistent with low overlap of DCX⁺ and markers of adult cells (Supplementary Fig. 5c). Arrows point to the DCX⁺/eGFP⁻ adult-born cells which are presumably younger than DPI 21⁺. (b) A summary bar graph showing the fraction of DCX⁺ and DCX⁻ cells among eGFP⁺ and eGFP⁻ cells (n=112 eGFP⁺, 7020 eGFP⁻ cells in 4 mice).
Supplementary Figure 5. Two types of odor-evoked responses in adult-born neurons. (a) An image of two adult-born DPI 9 JGNs expressing the Ca$^{2+}$ indicator Twitch-2B. (b) Simultaneously measured responses of the two cells to the application of ethyl tiglate in front of the mouse’s snout. While cell 2 shows an increase in [Ca$^{2+}$]$_i$ at the beginning of the stimulus (ON response), cell 1 shows a delayed decrease in [Ca$^{2+}$]$_i$ (inhibited response, see ref. 2). Note different baseline YFP/CFP value, implying different resting [Ca$^{2+}$]$_i$ in these two cells.
Supplementary Figure 6. Molecular phenotypes of adult-born neurons. (a, b) Maximum intensity projection images (15-25 µm thickness, 1 µm step) of eGFP+ adult-born cells (DPI 9, green) labeled with antibodies against specific cellular markers (red), as indicated. (c) Summary graphs illustrating the fraction of cells, positive for the given marker, as a function of the adult-born cell’s age. The fractions of cells expressing tyrosine hydroxylase, calretinin and calbindin at DPI 9 were significantly different from those at DPI 20-24 and DPI 120 (p ≤ 0.02, Student's t-test). There was no significant difference between the fractions of cells expressing a respective marker at DPI 20-24 compared to DPI 120 (p > 0.09, Student's t-test). This data set contains 80-220 cells from (left to right) 5, 5, 4, 4, 4, 4, 7, 4, 7 mice.
Supplementary References