Title: Functional organization of glomerular maps in the mouse accessory olfactory bulb
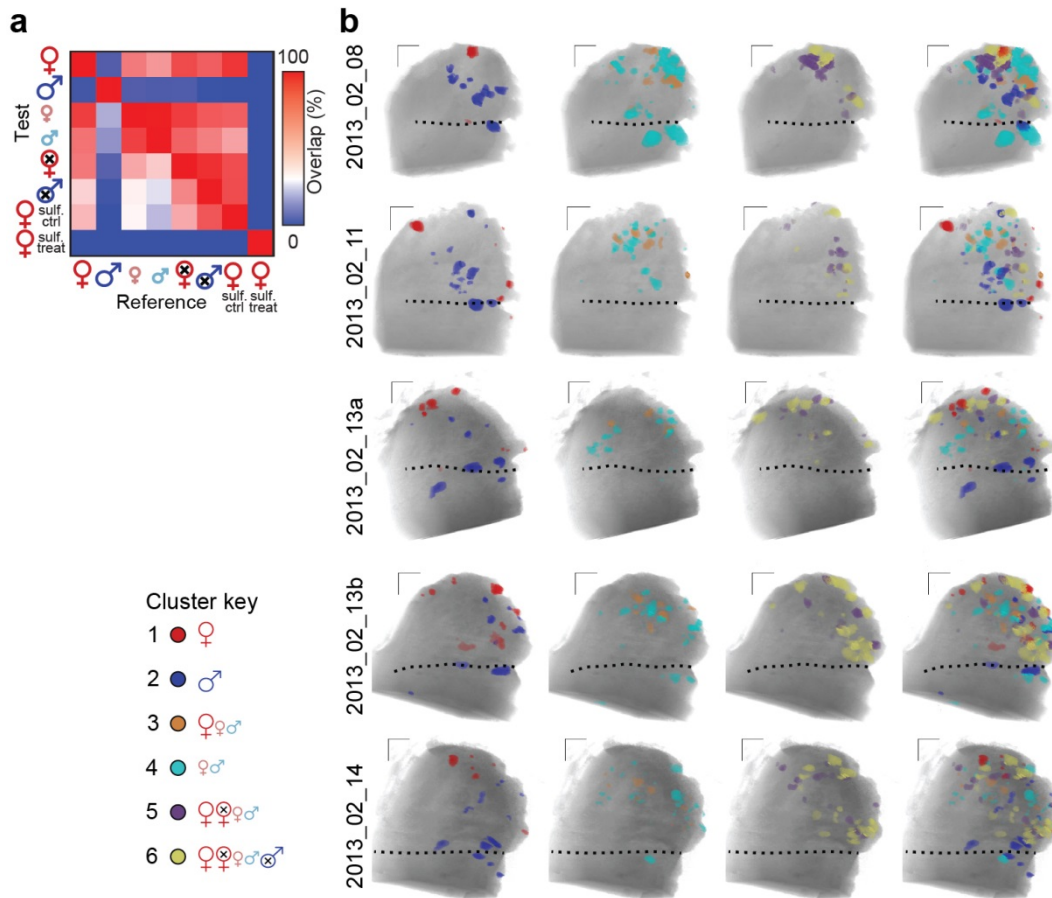
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Supplementary Figure 1) Response overlap and spatial distribution of urine-responsive glomeruli (**a**) Pairwise comparison matrix for the overlapping ROI volume between all tested urine sources (ΔF/F 2%). Columns represent the reference groups and rows represent the test groups. Note: sulfatase-treated adult female urine (final row/column) activated extremely few glomeruli at this threshold. (**b**) Urine-selective glomerular maps for 5 experimental animals. Each glomerulus is colored using the strategy indicated in Figure 3f. The first column shows maps for clusters 1 and 2, the second 3 and 4, and the third 5 and 6. The smallest clusters, Clusters 7 and 8 are omitted from this figure. The right-most column shows clusters 1-6. Figure 3a displays experiment "2013_02_14," Figure 3g displays experiments "2013_02_13a" and "2013_02_14," and Figure 4 displays experiment "2013_02_13b." Dotted lines indicate the position of the *linea alba*. Scale bars: 100 μm.
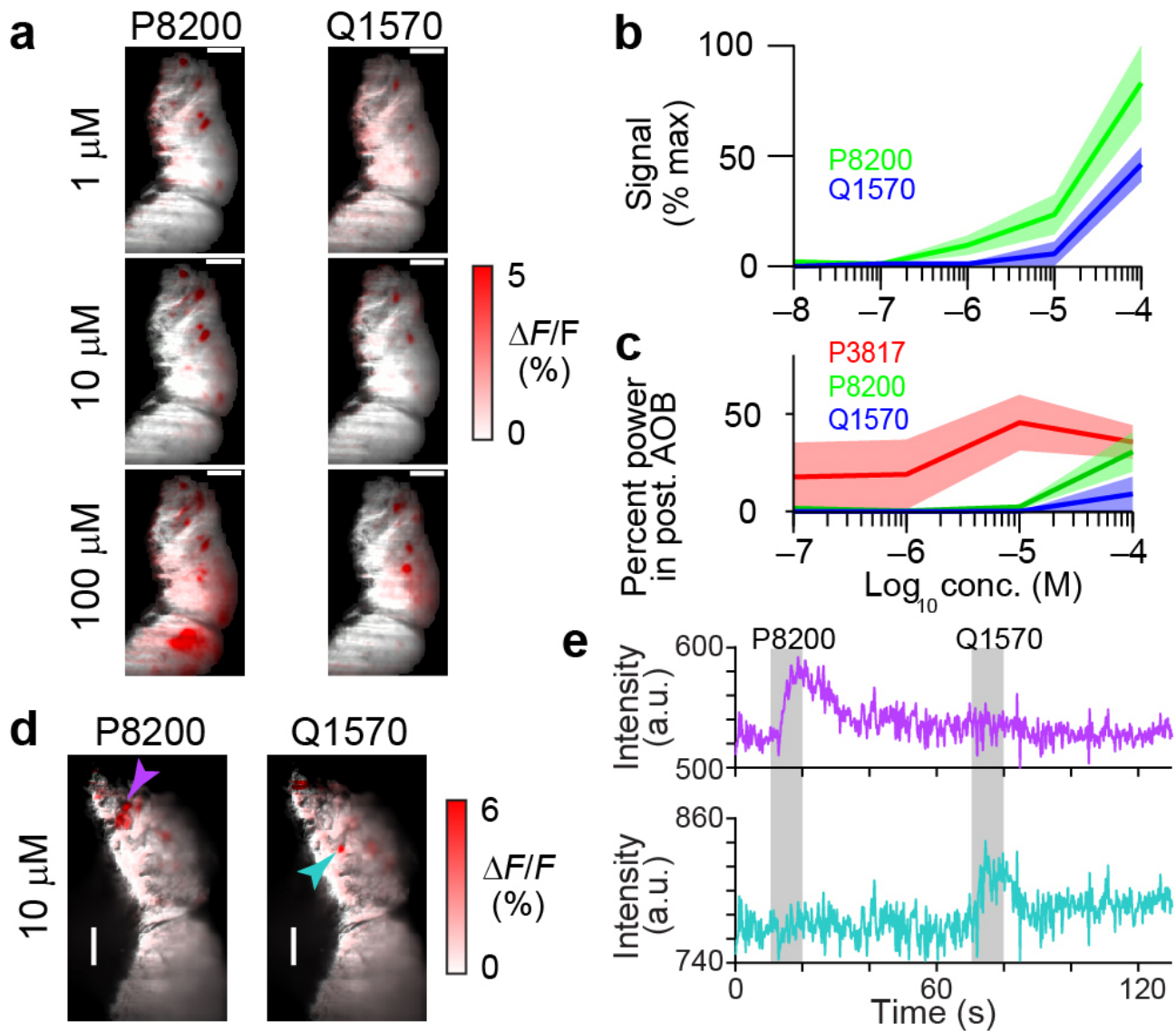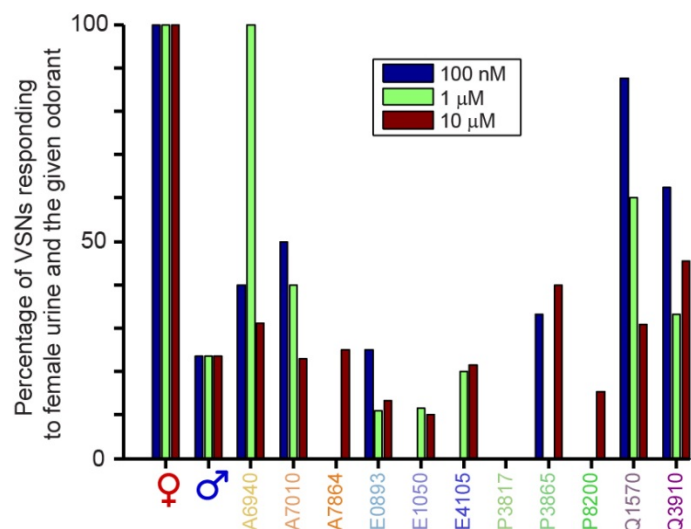
Supplementary Figure 2) Sulfated steroid glomerular activity across concentrations and at fast time scales. (**a**) Stimulation of the VNO with 1, 10, or 100 μM P8200 or Q1570 resulted in increased fluorescent intensity and number of active glomeruli. At 100 μM, the activity increased dramatically, making discrimination of individual glomeruli more difficult. Scale bars 100 μm. (**b**) Percentage of signal strength (sum of suprathreshold $\Delta F/F$ intensity, normalized per experiment) for each steroid across 5 log orders of concentration. Shaded areas represent mean ± standard error of the mean across multiple experiments (P8200, N = 4; Q1570, N = 2). (**c**) At higher steroid concentrations, the number of steroid-responsive glomeruli in the pAOB increased for all steroids. Posterior AOB glomeruli responsive to sulfated pregnanolone P3817 were evident at lower concentrations. (**d**) Average change in fluorescence during stimulation during continuous 10 Hz imaging of a single frame. Colored arrowheads indicate the location of two glomeruli analyzed in **e**. Scale bars 100 μm. (**e**) Intensity changes in glomeruli indicated by the colored arrowheads in **d**. Stimuli were delivered for 10 s (gray regions).
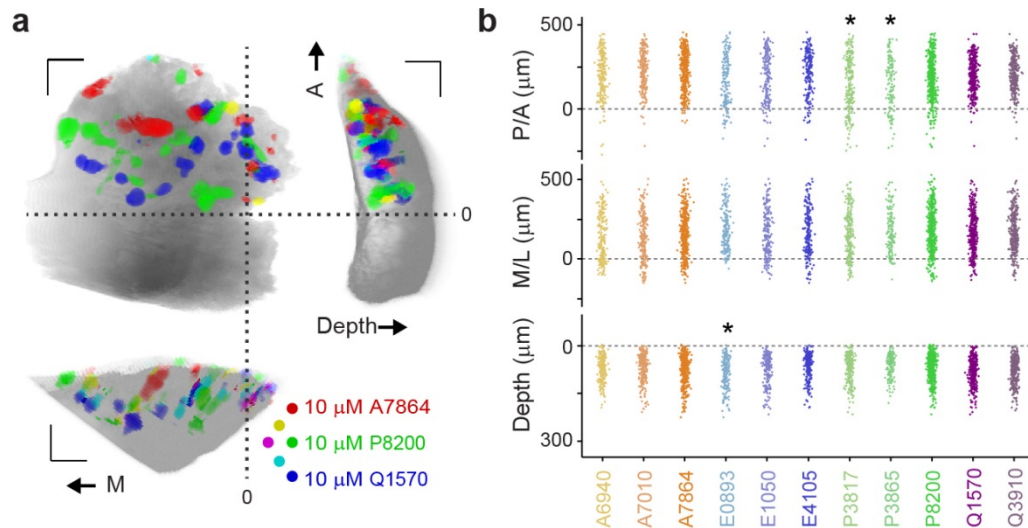
Supplementary Figure 3) Absolute positions of sulfated steroid-responsive ROIs and pAOB responses to sulfated pregnanolones. (**a**) Orthogonal plot of views of the AOB as visualized from the surface (top-left), posterior (bottom) and lateral (right) viewpoints. The color of the glomeruli reflects their responsivities (threshold $\Delta F/F > 1\%$). Yellow, magenta, and cyan regions indicate glomeruli responding to more than one steroid at this threshold. (**b**) Aggregated position information for all 1078 steroid-responsive ROIs along each of the 3 orthogonal coordinates at $\Delta F/F > 1\%$ (n = 10). The dotted line along the medial/lateral (M/L) and posterior/anterior (P/A) axes refers to their position relative to the landmarks indicated by dotted lines in **a**. Depth was measured relative to the actual tissue surface. Asterisks denote populations which were statistically different than the majority of the other populations (p < 0.05, one-way ANOVA).
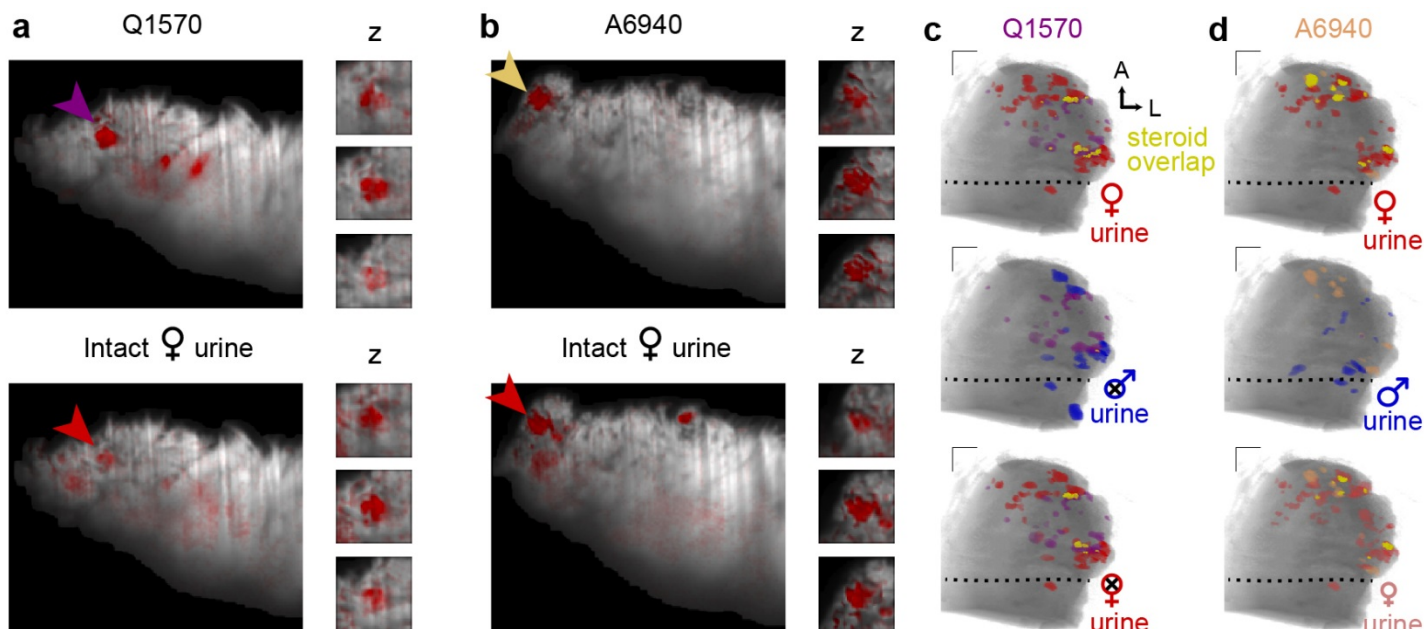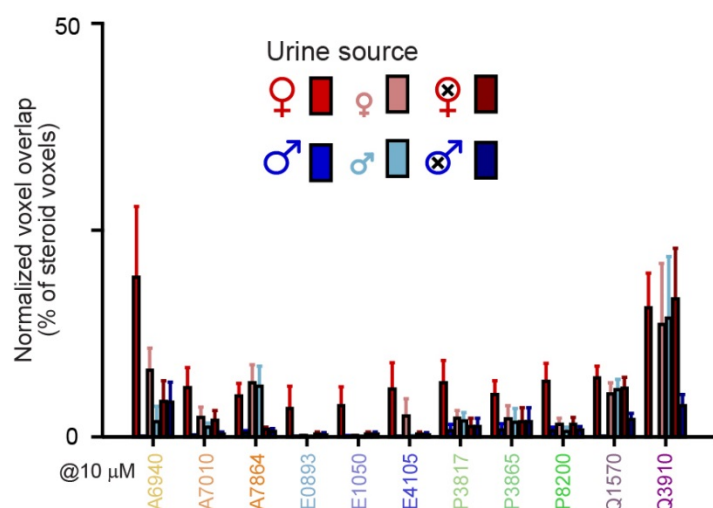
Supplementary Figure 4) VSN co-activation by sulfated steroids and intact BALB/c female urine. (**a-b**) Several aAOB glomeruli were co-activated by 1:100 intact adult BALB/c female urine (bottom, red arrowheads) and 10 μM Q1570 (purple arrowhead, **a**) or 10 μM epitestosterone sulfate (A6940, yellow arrowhead, **b**). Right insets in **a** and **b** show single responses of the indicated glomeruli across 3 consecutive frames of the stack at higher zoom (image size 35 μm square). (**c-d**) Rendered maps of glomerular ROIs responding to 10 μM Q1570 (**c**, purple) or A6940 (**d**, orange). Steroid-responsive maps are overlaid on glomerular ROIs responsive to 1:100 dilutions of the indicated urines (Δ*F/F* > 2%) with overlapping voxels labeled yellow. All scale bars 100 μm. A: anterior, L: lateral. (**e**) The number of VSNs responding to sulfated steroids and 1:100 BALB/c female urine as measured on multielectrode arrays (p < 0.05 Wilcoxon rank-sum test, firing rate change > 1 Hz compared to controls; Meeks *et al*, 2010). These estimates are normalized to the total number of neurons responsive to the steroid at each concentration or 1:100 urine (left-most plots). Note the concentration-dependent decrease in the percentage of co-responsive neurons for Q1570. Because nearly all 100 nM Q1570-responsive VSNs also respond to BALB/c female-urine, this would be consistent with an endogenous concentration of Q1570 in BALB/c female urine of 1 to 10 μM (100 x [10 to 100 nM]), consistent with estimates from Nodari *et al*, 2008. (**f**) The percentage of steroid-responsive voxels that overlapped urine-responsive voxels (N = 7 for intact adult urines, N = 4 for all others). (**f**) Normalized voxel overlap between steroid-responsive glomeruli and mouse urine across sex and sexual maturity. Error bars reflect s.e.m.
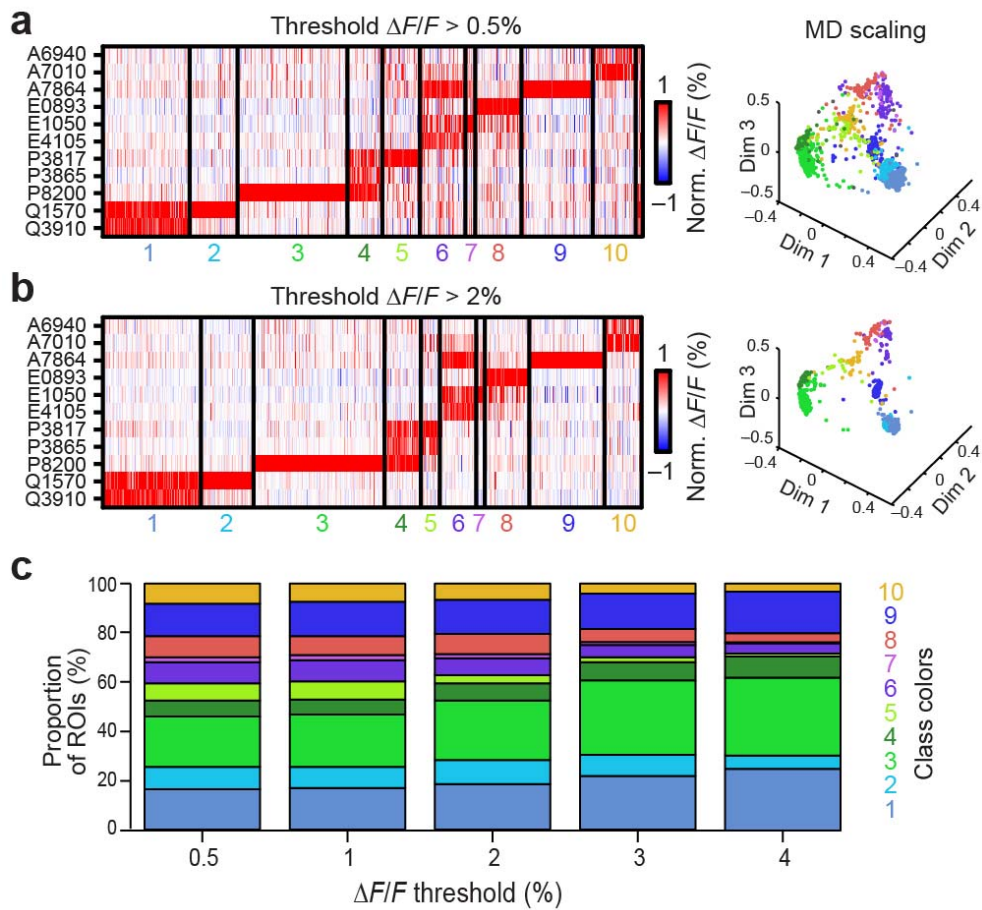
Supplementary Figure 5) ROI functional clusters across threshold. (**a-b**) Plots of the normalized Δ*F*/*F* for all ROIs across all experiments (N = 10). Each colorized column represents 1 ROI. The order of the ROIs within each cluster grouping (delineated by vertical black bars) was randomized. Insets to the right of each plot show the first 3 multidimensional scaling dimensions from independent clustering runs. The color of each point matches the VSN class color values in **c**. (**c**) Proportion of total ROIs in each cluster across all thresholds tested. Colorized list at right indicates the color assigned to each VSN class.
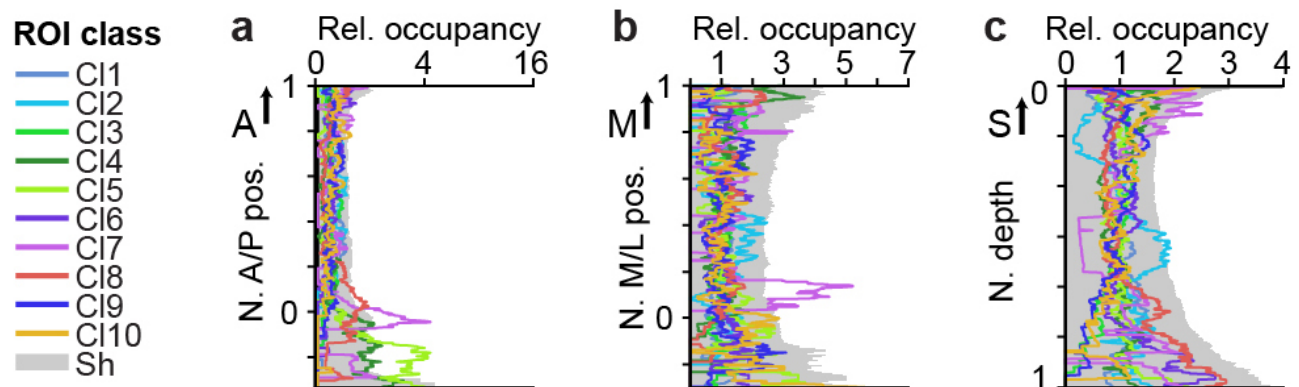
Supplementary Figure 6) Absolute positions of steroid-responsive glomerular classes. (**a-c**) Plots of ROI relative occupancy by steroid-responsive glomerular class (n = 10, cluster definitions shown in Fig. 6b). The observed relative occupancy of glomeruli of each class (solid colored lines) was compared to the 95th percentile (gray shaded region) from shuffled maps (100,000 shuffle tests per experiment, n = 10 experiments). Lines exceeding the shaded regions indicate preferential occupancy of particular positions by glomeruli of that class.