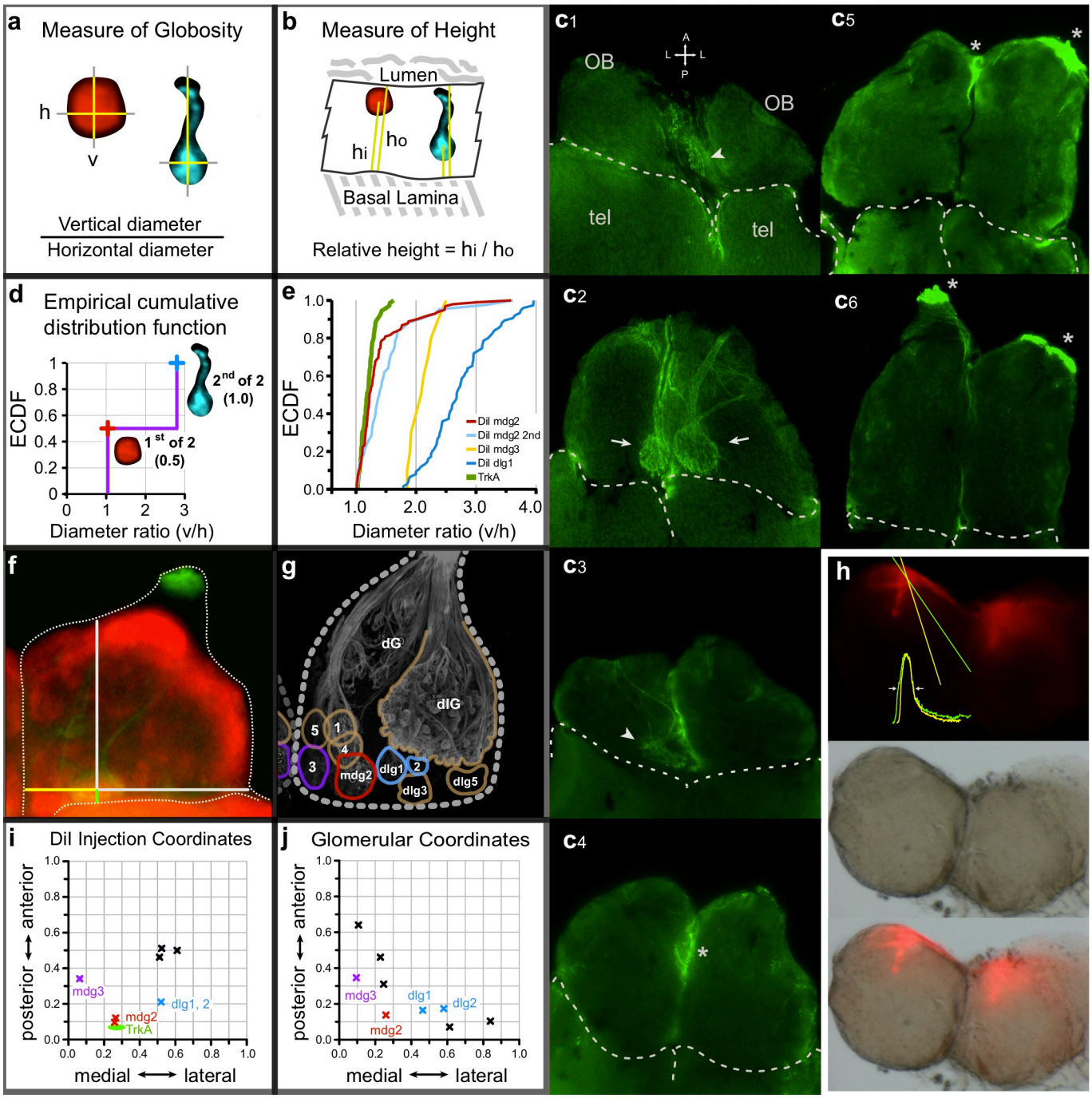
Supplementary Information

Title: Zebrafish crypt neurons project to a single, identified mediodorsal glomerulus

Authors: Gaurav Ahuja, Ivan Ivandić, Mehmet Saltürk, Yuichiro Oka, Walter Nadler, Sigrun I. Korsching

SI Figure 1 Korsching



SI Figure 1 Quantification of shape and position

a) Diameter ratio for cells is measured as ratio of maximal vertical length to maximal horizontal diameter. Vertical diameter is measured perpendicular to lumen border and basal lamina border of the olfactory lamella. b) Height of the cell within the olfactory lamella (h_i, value for center of soma) is normalized to maximal height of the lamella (h_o) at the position of the cell to account for the rather variable thickness of the olfactory lamellae. c) TrkA-like immunoreactivity after whole mount staining of the olfactory bulb in a complete series of 50 µm thick horizontal cryosections. Z Stack images (0.5 µm optical section) were taken at 40x magnification, maximal projection is shown. Dorsal to ventral, from top to down; arrows, a single labeled glomerulus per olfactory bulb is seen in section C_2; arrowheads, peripheral segments of the glomerulus are visible in the directly adjoining sections; asterisks, background signal at the tissue surface; dashed line, telencephalic border; tel, telencephalon; OB, olfactory bulb. d) Visualization of the empirical cumulative distribution function (ECDF) for the diameter ratio of the two cells depicted in a). While this is a fictive example, distributions with as few as ten measurements can be examined for coarse features, and high resolution is obtained above 50-100 measurements, which makes the (unbinned) cumulative distribution function much more sensitive than the commonly used histogram representation of binned values. e) Diameter ratios for olfactory receptor neurons backtraced from DiI injections in mdg2 (red, data shown in main text; cyan, another injection), mdg3 (yellow) and dlg1 (blue, data shown in main text). Values for trkA-labeled crypt neurons (green) are shown for comparison. Data are shown as empirical cumulative distribution function (CDF) as described in d). f) Coordinates of trkA glomeruli and injection site centers in the olfactory bulb are normalized to maximal bulbar length and width visible in dorsal view with telencephalon still attached at that position. Anterior-posterior length is measured from posterior (0) to anterior (1), parallel to the midline; medial-lateral length is measured from medial, perpendicular to the anterior-posterior axis. g) Outline of mediodorsal and dorsolateral glomeruli traced onto a cropout of Fig. 3b of Braubach et al.[1]. This orientation of the olfactory bulb is most similar to that of our experiments. h) Minimal DiI diffusion after tracing is seen in a vibrotome cross section obtained after tracing. Fluorescence (top panel), brightfield (middle panel) and the overlay of both (third panel). Line scans through the center of the left injection site (yellow, green, top panel) show minimal spread of the dye. Half width of fluorescence intensity is indicated by the arrows. i) Coordinates of injection sites in the olfactory bulb, measured as described in f). Colors and labels represent the nearest glomerular position. The centroid of the coordinates for the TrkA-labeled glomerulus is given for comparison (green oval, mean+/- SD, n=10) . j) Coordinates of glomeruli outlined in g), measured as described in f). Colors and labels represent glomeruli examined in this paper.

1 Braubach OR, Fine A, Croll RP. Distribution and functional organization of glomeruli in the olfactory bulbs of zebrafish (Danio rerio). *The Journal of comparative neurology* 2012, **520**(11)**:** 2317-2339, Spc2311.

Table S1 Pairwise comparison of distributions for Diameter ratio using the *Kolmogorov-Smirnov* test

Diameter ratio (vertical / horizontal)			
cell label 1	*vs*.	cell label 2	p-value
S100 'fresh'		S100 'fixed'	p<0.000001
S100 'fresh'		TrkA 'fresh'	p=0.72
S100 'fresh'		TrkA 'fixed'	p=0.69
S100 'fixed'		TrkA 'fixed'	p<0.000001
TrkA 'fresh'		TrkA 'fixed'	p=0.55
TrkA 'fixed'		Mdg2	p=0.027
TrkA 'fixed'		Mdg3	p<0.000001
TrkA 'fixed'		Dlg1	p<0.000001
Mdg2		Mdg3	p=0.000001
Mdg2		Dlg1	p<0.000001
Mdg3		Dlg1	p=0.00066

The Kolmogorov-Smirnov test [1], a measure of distribution differences, makes no assumption about the nature of the distributions. This is essential because distributions analysed here are non-Gaussian. As cutoff for significance we chose p<0.01 due to the sensitive nature of this test for large distributions. S100 'fresh', S100-like immunoreactivity in unfixed tissue; S100 'fixed', S100-like immunoreactivity in fixed tissue; TrkA 'fresh', TrkA-like immunoreactivity in unfixed tissue; TrkA 'fixed', TrkA-like immunoreactivity in fixed tissue; Mdg2, DiI injection into mdg2; Mdg3, DiI injection into mdg3; Dlg1, DiI injection into dlg1.

1 Press WH TS, Vetterling WT, Flannery BP. *Numerical recipes in C: The art of scientific computing*, vol. second, 1992.