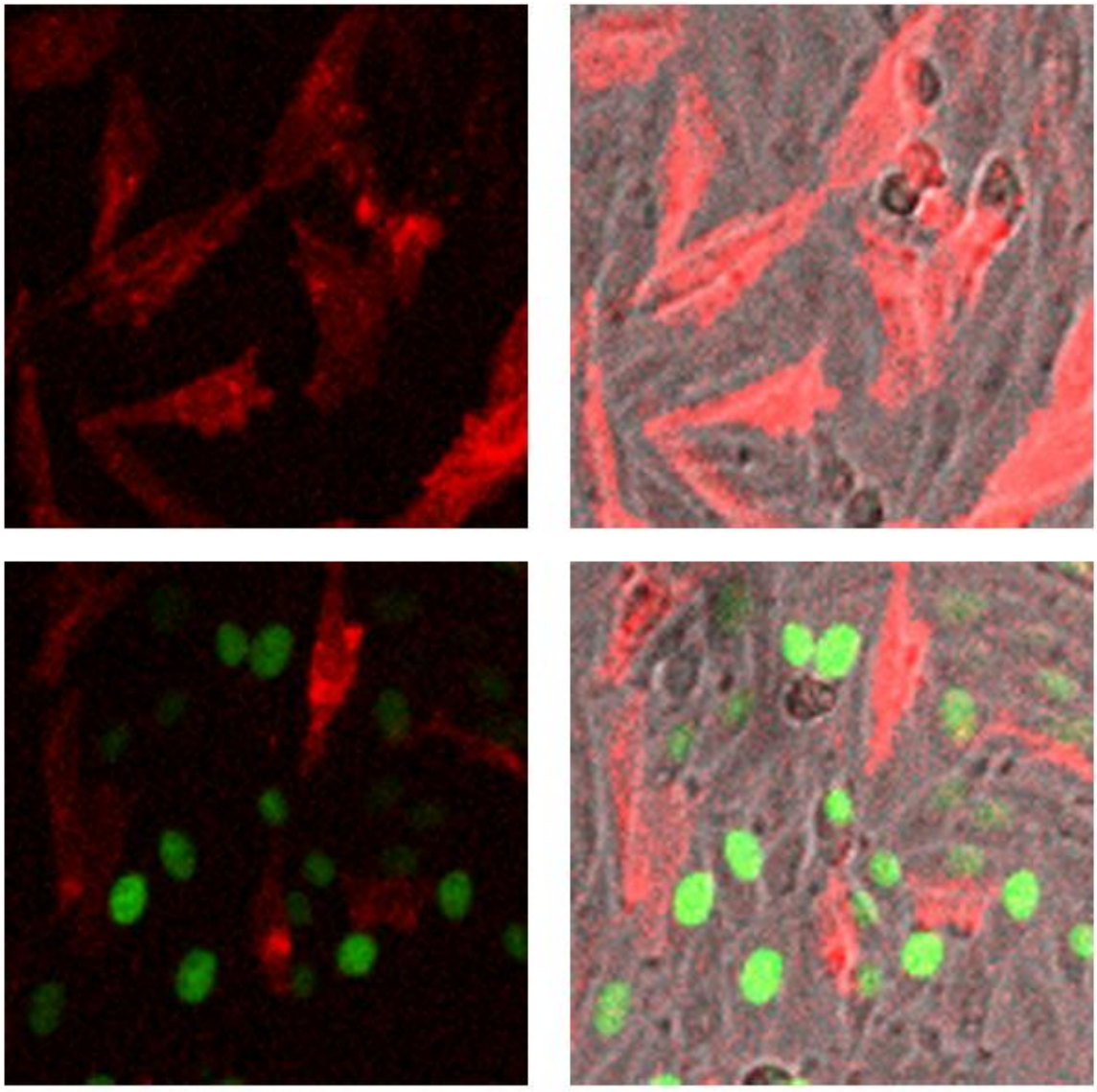

Fig S1. Induction of reporter gene expression *in vitro* by cell-cell contacts.

Top: Control experiment with SNTGV/UAS-H2Bmcit receiver CHO cells co-cultured with mcherry+ cells, showing that without CD19 no H2Bmcit expression was induced. Bottom: when SNTGV/UAS-H2Bmcit receiver cells are co-cultured with CD19+/mcherry+ emitter cells, nuclear H2Bmcit expression was induced in the receiver cells. Left images: fluorescence illumination. Right images: overlay of phase contrast illumination on the fluorescence image shown on the right. Receiver cells were co-cultured with emitter cells (mcherry+ or CD19/mcherry+) at a 1:1 ratio and imaged after 48 hours.

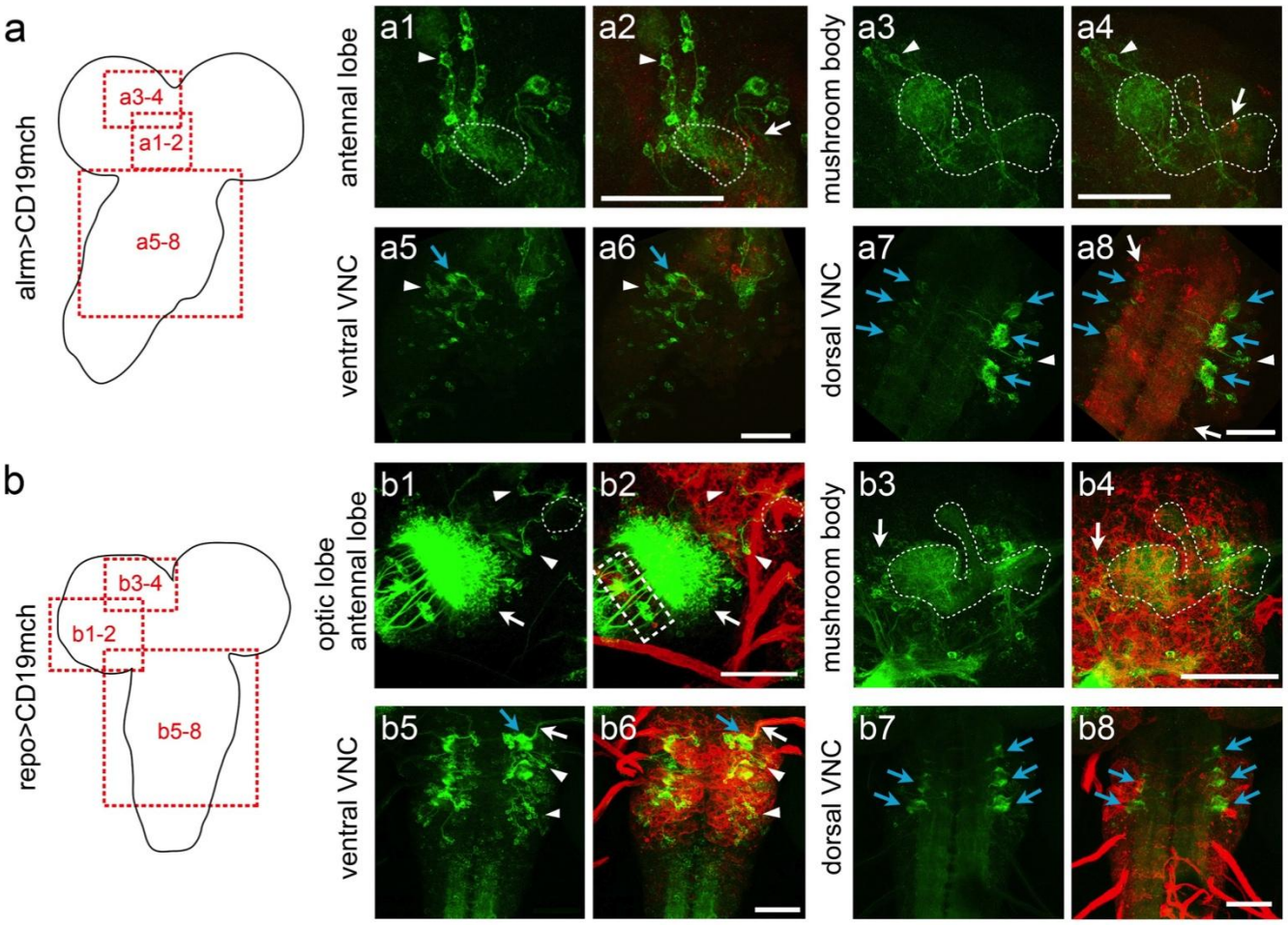

Fig S2. Expression of the ligand in different glial subtypes produces different patterns of induction.

(a) Induction of GFP in neurons triggered by the *alrm* driver. Top, middle, and bottom squares in the diagram indicate high magnification views of the antennal lobe, mushroom body, and ventral nerve cord shown in Fig. 3b, and are shown in 4a1-a2, 4a3-a4, and 4a5-a8, respectively. The cell bodies of CD19mch+ glial cells (white arrows) are located in both the central brain (a2, a4), and ventral nerve cord (a6, a8). (a1, a2) GFP+ neurons (arrowheads) surround the antennal lobe (marked by a stippled oval). (a3, a4) GFP+ cells bodies of Kenyon cells (arrowheads) surround the mushroom body (contours traced by stippled line), which is filled with GFP+ processes. (a5-a8) ventral and dorsal view of the thoracic neuromeres in the ventral nerve cord. GFP+ cell bodies had neurites that traverse throughout the neuropil and commissures. (a7, a8) Some of the GFP+ neurons (white arrowhead) form clusters in the thoracic neuromeres that innervate into 3 pairs of leg

neuropils (blue arrows). (b) Induction of GFP in neurons triggered by the *repo* driver. Right, top, and bottom squares in the diagram indicate high magnification views of the optic lobe, central brain, and ventral nerve cord from Fig. 3c and are shown in 4b1-b2, 4b3-b4, and 4b5-b8, respectively. (b1, b2) GFP induction in the optic lobe (arrow), and in a small number of neurons (arrowhead) surrounding the antennal lobe (circle). Rectangle in b2 outlines the position of a CD19mch band of glial cells in the border between the lamina and medulla in the optic lobe (see Fig. 4 for higher magnification view of this area). (b3, b4) GFP+ Kenyon cells (arrows) surrounding the mushroom body (outlined by stippled line). (b5-b8) Ventral and dorsal view of the thoracic neuromeres in the ventral nerve cord. Neurites from GFP+ neurons traverse throughout neuropil, commissures, and fiber tracts in the ventral nerve cord. Leg neuropils (blue arrows) contain branches of strongly expressing GFP neurites, whose cell bodies are located surrounding the neuropils (arrowhead). Notice that the nerve fibers are wrapped by strongly labeled CD19mch+ glia (b6) and contain axons that are GFP+ (b5), and connect with leg neuropils (blue arrows). All images are confocal maximal projection images of the larval nervous system. Emitter cells (expressing CD19mch) are labeled in red. Receiver cells (expressing GFP) are labeled in green. Scale Bar: 50 µm.

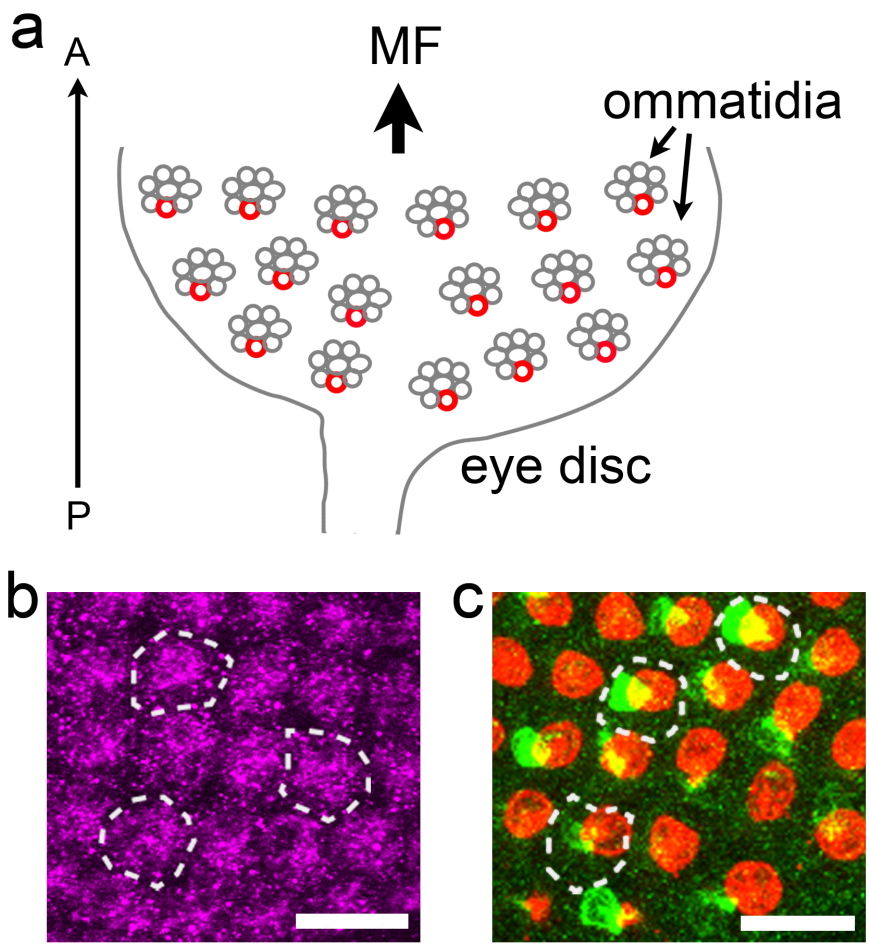

Fig S3. Lack of *cis*-activation when the ligand and receptor are expressed in the same cells.

a) Diagram of the *Drosophila* eye disc. Developing ommatidia composed of immature photoreceptors R1 to R8 are aligned in the eye discs behind the morphogenetic furrow (MF). In the experiment shown in b and c, the ligand CD19mch was expressed in a single type of photoreceptor cell (red circles) in ommatidia under the *GMR87C06-LexA* driver, and the SNTG4 receptor was expressed in all photoreceptors under the elav promoter. (b) Immunostaining against GAL4 in the eye disc of *elav*-SNTG4 larva shows that all immature photoreceptors in developing ommatidia (stippled ovals) express the SNTG4 receptor (magenta). (c) CD19mch was expressed in a single type of photoreceptor (red) in each ommatidium (stippled ovals) driven by the *GMR87C06-LexA* driver. GFP induction (green) is observed in cells contacting the CD19-expressing emitter cells (red), but the cells expressing the ligand do not show GFP induction, indicating that the receptor and the ligand do not activate in *cis* when they are present in the membrane of the same cell.

SUPPLEMENTARY MATERIALS AND METHODS

Sequence of SCAD:

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Sequence of Drosophila notch1 NRR+TMD:

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Sequence of human notch1 NRR+TMD:

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Sequence of mcherry:

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