

**Figure 2 - figure supplement 3: TRACT with non-synaptically localized ligand reveals neurons that are not exclusively connected by synapses.**

To selectively detect the connections between ORNs and PNs, the GH146 enhancer was used to drive expression of nlgSNTG4 selectively into PNs, and the ligand was expressed in subsets of ORNs that project to identified glomeruli. (a)Control brain without lexA driver (carrying GH146-nlgSNTG4, 5xUAS-CD4::tdGFP and LexAop-CD19mch) reveals low levels of background GFP expression that can be barely observed in several brain regions, including DA1 glomerulus (star in the right). (b, c, and d) Expression of the ligand CD19mch in most glomeruli with the orco driver (b), or in identified glomeruli using the R17H02 (c) and R28H10 (d) drivers. (b) Left - CD19mch+ axons from ORNs (red) driven by orco branch in the antennal lobe. Middle - Induction of CD4::tdGFP expression in PNs triggered by CD19mch+ ORNs (red, left image). Axon bundles of the CD4::tdGFP+ uniPNs in the iACT (arrows) branch in the mushroom body. (c-d) CD4::tdGFP+ PNs (middle panels) induced by CD19mch+ ORN axons (red, left images) that innervate one (VC1) to three (DA1/VA6/VA1lm) identified glomeruli (stippled circles in the left panels). Although the ligand proteins were only present in specific glomeruli (stippled circles, the expression level in VA6 was low and undetectable after immunostaining with anti-RFP antibody), strong CD4::tdGFP induction (middle panels) was detected in PNs with dendrites outside the CD19mch+ glomeruli. In particular, there are GFP+ neurites within DA1 (stars) in all the cases. The arrows indicate the axons of uniPNs, which run through the mALT. In (b-d), in addition to the uniPNs, few multiPNs were labeled as well (arrowheads). Their dendrite arborizations cover the entire antennal lobes. The right panels show the merged images of CD4::tdGFP (green) and CD19mch (red). Scale bar= 50μm.