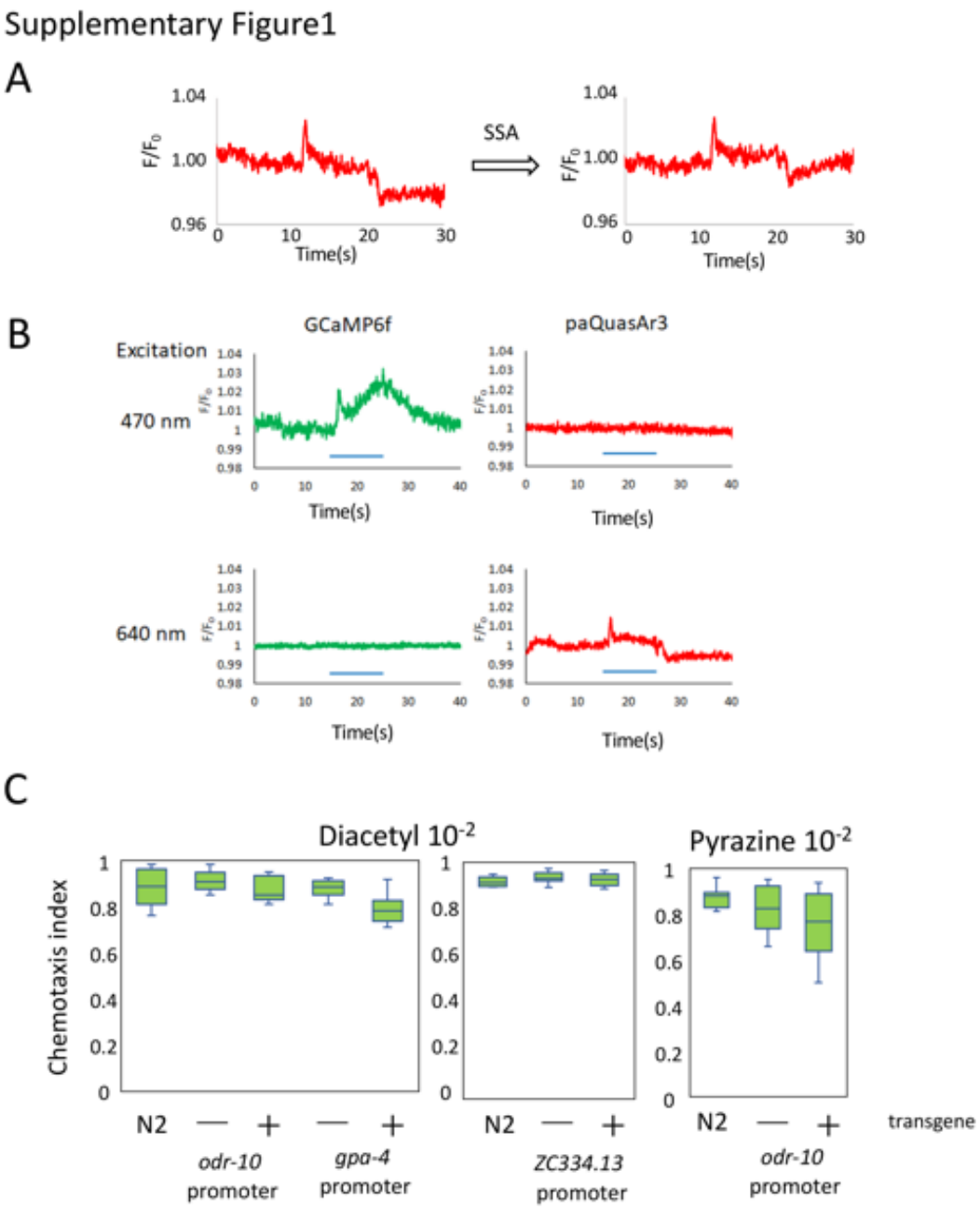


Supplementary Figure 1

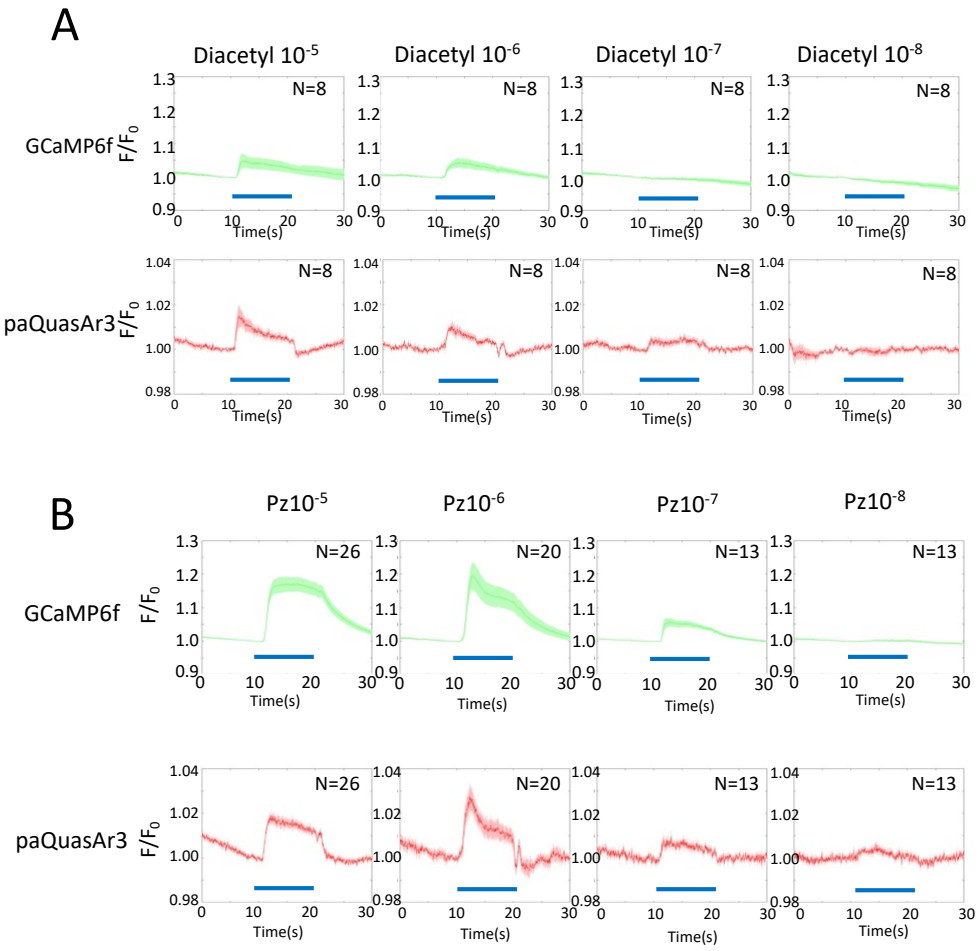
A. Representative results of SSA-based-photobleaching correction are shown. SSA

was applied to remove DC trends that reflect photobleaching from the paQuasAr3 data.

B. Changes in paQuasAr3 fluorescence (via 708/75 emission filter) were detected only when the 640 nm laser was used for excitation. In contrast, changes in GCaMP6f fluorescence (through 512/25 emission filter) were detected only when the 470 nm laser was used for excitation. Both lasers were used for excitation in simultaneous imaging. The blue bars indicate the period of the diacetyl stimulation.

C. Behavioral responses of animals expressing paQuasAr3 and GCaMP6f in AWA neurons. Regardless of paQuasAr3 and GCaMP6f expression, the animals showed chemotactic responses to diacetyl (left) or pyrazine (right), similar to the wild-type, N2.

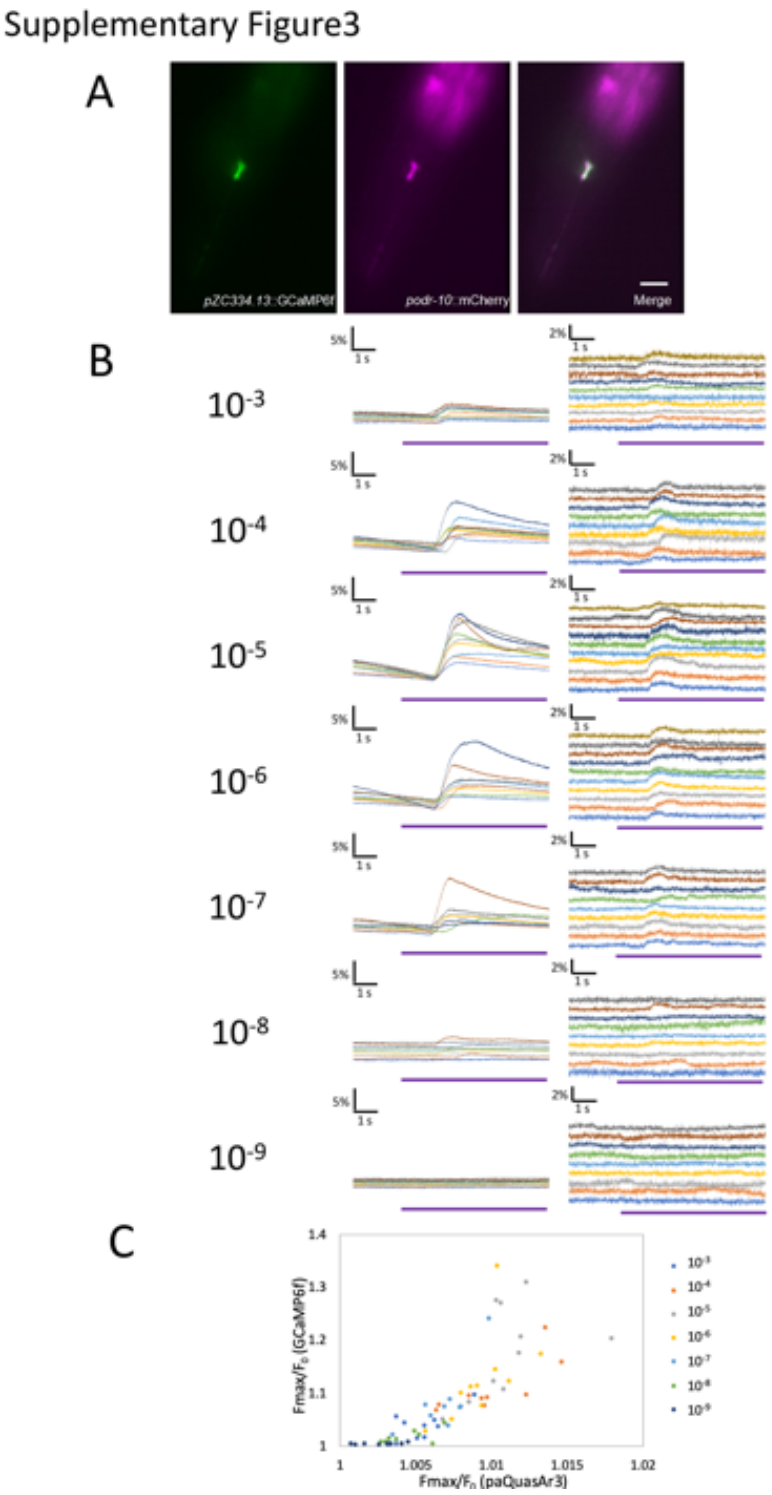
Supplemental Figure 2



Supplemental Figure 2

A. AWA responses of GCaMP 6f and paQuasAr3 to diacetyl after conditioning with diacetyl and without food. Averaged traces in wild-type animals are shown with SEM. Blue bar indicates the stimulation with diacetyl.

B. AWA responses of GCaMP6f and paQuasAr3 to pyrazine. Averaged traces in wild-type animals are shown with SEM. Blue bar indicates the stimulation with pyrazine.



Supplemental Figure 3

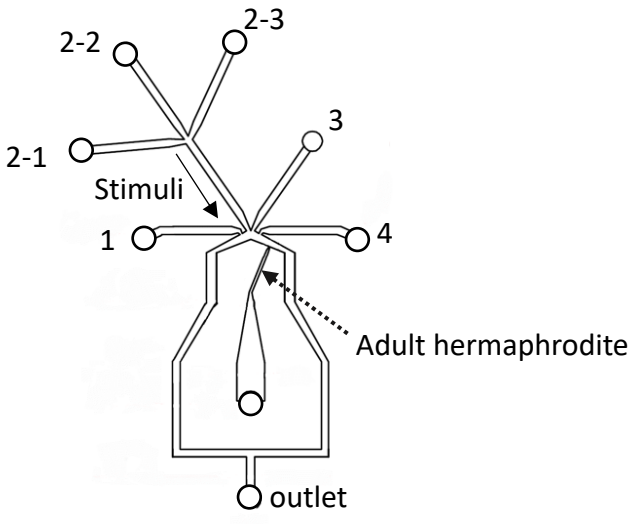
A. ZC334.13 promoter gave expression in AWA neurons, where *odr-10* promoter gives

expression. Scale bar indicates 25 μm.

B. Traces of the simultaneous imaging of the GCaMP6f and paQuasAr3 at the onset of the stimulation. In this figure, the time-line data were not processed with a median filter. The left panels show GCaMP6f traces and the right panels show paQuasAr3 traces. , In these panels, each trace was shifted and overlaid for display.

C. The relations of maximum changes of paQuasAr3 and GCaMP6f to various concentrations of diacetyl.

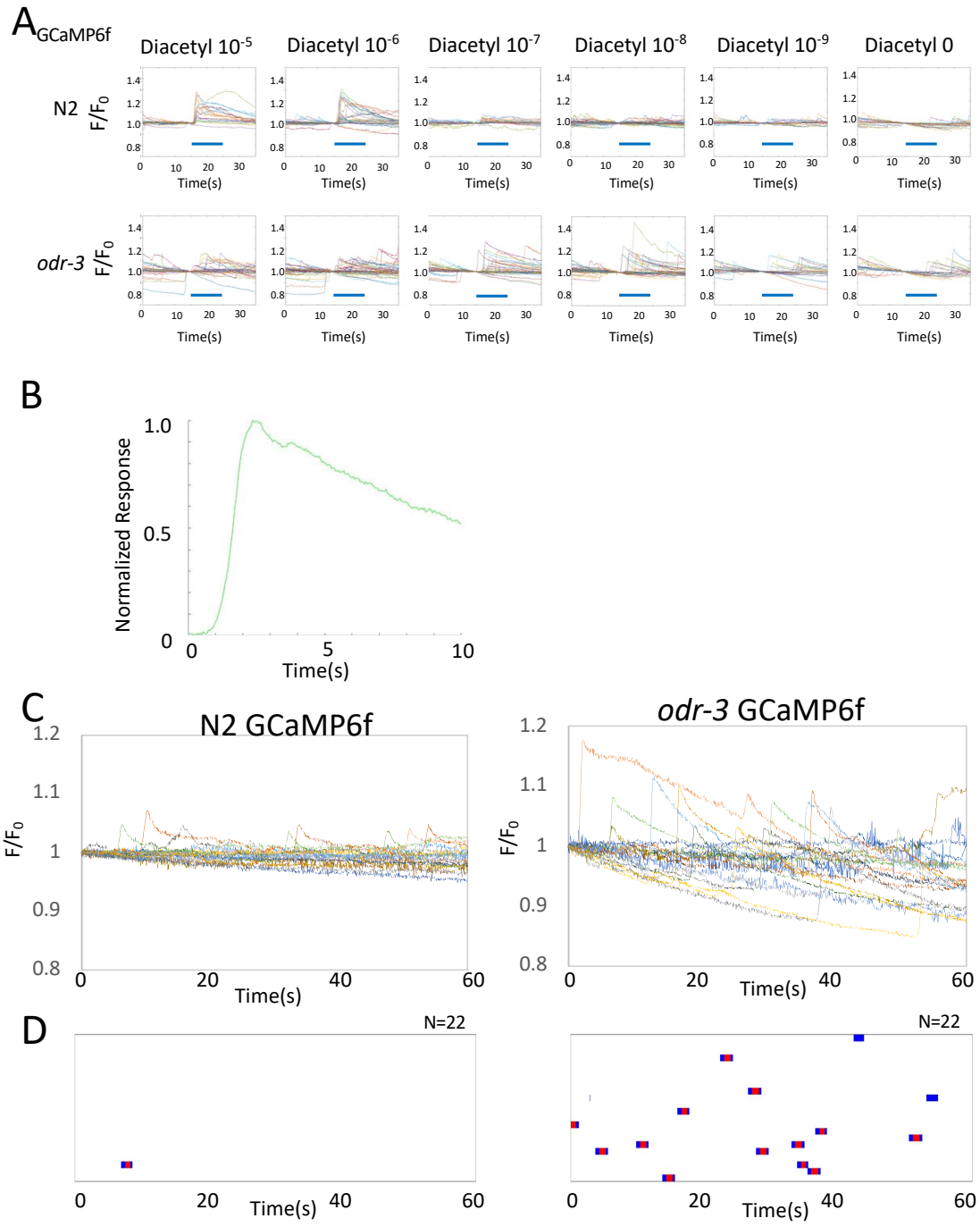
Supplementary Figure 4



Supplementary Figure 4

PDMS (polydimethylsiloxane) microfluidic device for sequential stimulation. This PDMS device was designed like the original olfactory chip, except that it has three forks (2.1-2.3) for stimulation at the top. Three different solutions could flow through the three openings (2.1-2.3) at the top of the device. When the channel 1 is off state but the channel 4 is on state, worms were exposed to either one of the solution 2.1-2.3 (stimuli) which are switched using valve. On the other hand, when the channel 1 is on state but the channel 4 is on state, worms were exposed to the solution 3 that does not contain any stimuli. The solution can be switched using valve and the timing of the changing were programmed.

Supplementary Figure 5



Supplementary Figure 5

A. Changes in GCaMP6f fluorescence in response to diacetyl in wild-type and *odr-3* animals. Each trajectory indicates the result of an individual animal.

B. Graphs of the standard response used in Figure 5B and Supplemental Figure 5D. The normalized fluorescent responses of GCaMP to 10^{-6} diacetyl stimulation (15–25 s) in wild-type animals were averaged.

C. Responses of GCaMP6f in AWA neurons without stimulation. Each trace is shown in different colors.

D. Fluorescent responses, with maximum increases of $F/F_0 > 0.08$, similar to wild-type standard responses (average responses by diacetyl 10^{-6}), are shown in blue (correlation coefficients > 0.6) and red (correlation coefficients > 0.85). Each line shows the result of an individual animal.

Supplementary Table 1

Strain list.

QD 271	N2;Ex[*odr-10p*::GCaMP6f,*odr-10p*::paQuasAr3-citrine,*lin-44p*::gfp]
QD 272	N2;Ex[*gcy-28p*::GCaMP6f,*gcy-28p*::paQuasAr3-citrine,*lin-44p*::gfp]
QD 274	*egl-19*;Ex[*odr-10p*::GCaMP6f,*odr-10p*::paQuasAr3-citrine,*lin-44p*::gfp]
QD 275	*unc-2*;Ex[*odr-10p*::GCaMP6f,*odr-10p*::paQuasAr3-citrine,*lin-44p*::gfp]
QD 276	N2;Ex[*gcy-10p*::GCaMP6f,*gcy-10p*::paQuasAr3-citrine,*lin-44p*::gfp]
QD 277	N2;Ex[*gpa-4p*::GCaMP6f,*gpa-4p*::paQuasAr3-citrine,*lin-44p*::gfp]
QD 278	*osm-9*;Ex[*gpa-4p*::GCaMP6f,*gpa-4p*::paQuasAr3-citrine,*lin-44p*::gfp]
QD 279	*odr-3*;Ex[*gpa-4p*::GCaMP6f,g*pa-4p*::paQuasAr3-citrine,*lin-44p*::gfp]
QD 280	*odr-10*;Ex[*gpa-4p*::GCaMP6f,*gpa-4p*::paQuasAr3-citrine,*lin-44p*::gfp]
QD 307	N2;Ex[*ZC334.13*p::GCaMP6f,*ZC334.13*p::paQuasAr3-citrine,*lin-44p*::gfp]
QD 309	N2;Ex[*ZC334.13*p::GCaMP6f, *ZC334.13*p::mCherry]