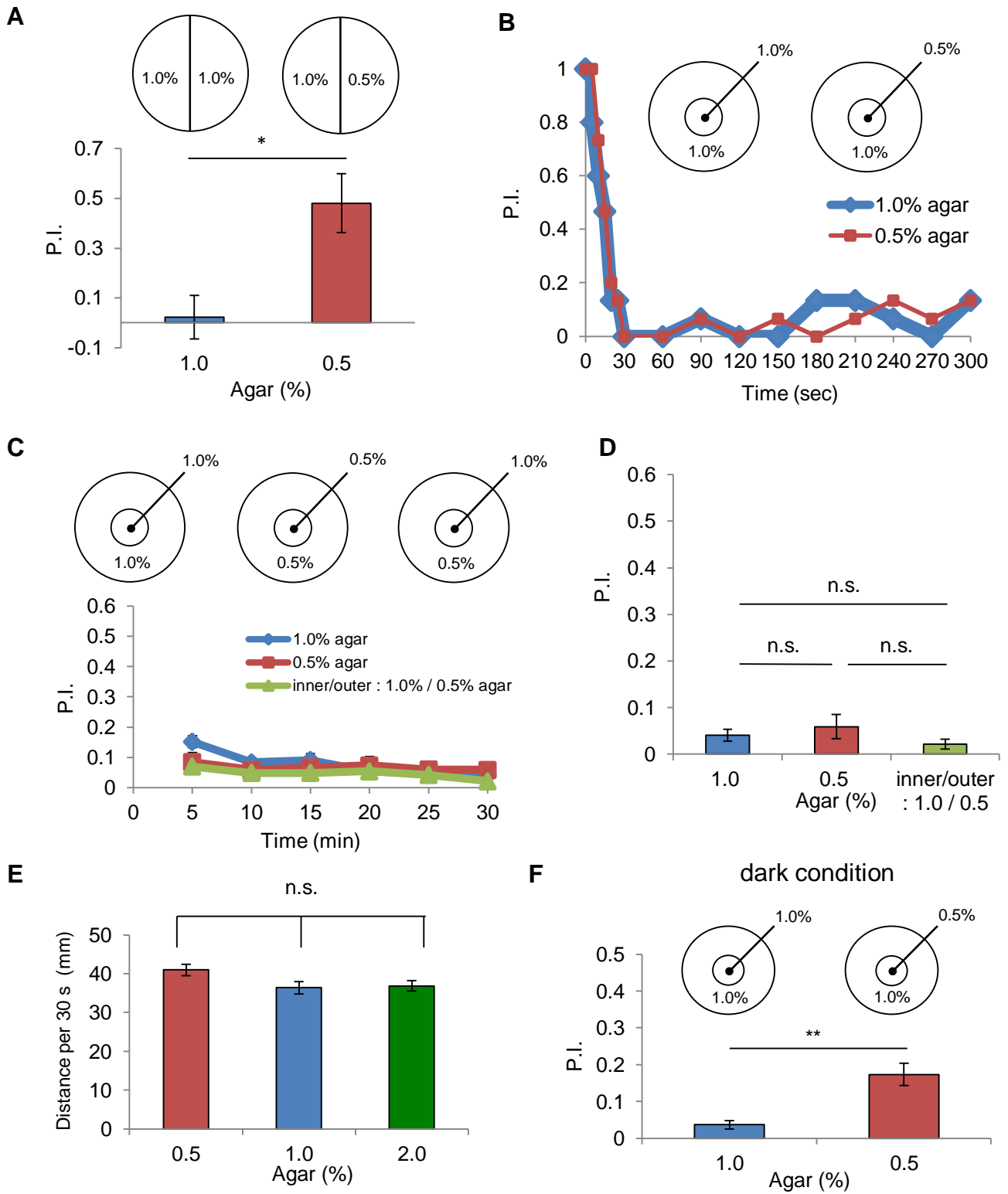


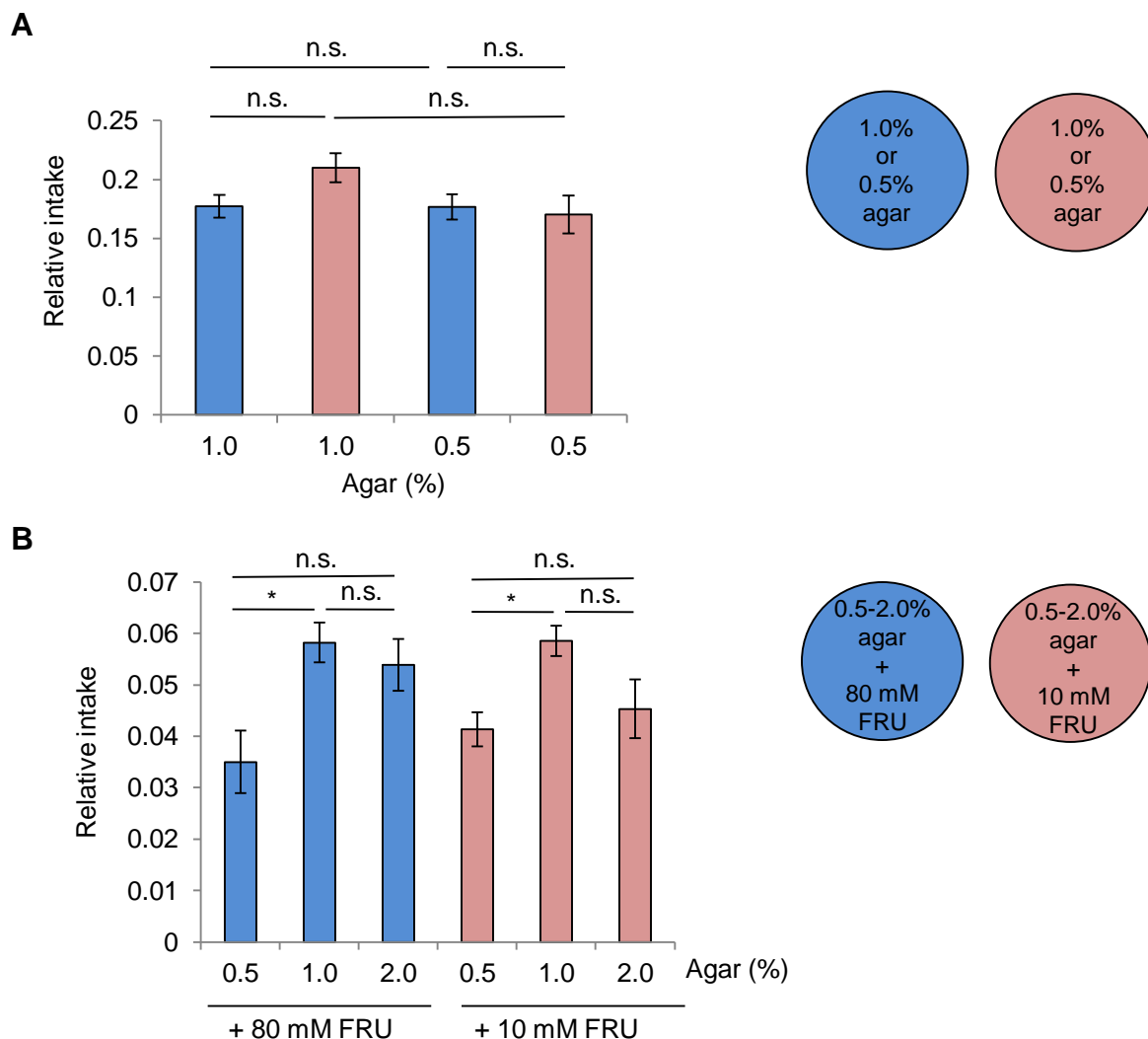
Fig.S1



**Figure S1: Larval preference for softness.**

(A) Preference test by the two-halved agar plate assay; feeding-stage third-instar larvae prefer 0.5% agar, as in the double circle assay (N=10, 10, Mann-Whitney U-tests, \*:  $p < 0.05$ ). (B) The time course of P.I. value during 5 min in the double circle assay. We counted the number of larvae every 5 sec (till 30 sec) and every 30 sec (between 20 sec and 300 sec). These P.I. values were obtained from the data shown in Movie 1. In the double circle assay, all larvae started in the middle and go outside the inner circle during the first 30 sec. (C) We checked the preference shown by larvae in three conditions: when the agar concentrations in the outer and inner circle are the same (0.5% and 1.0% agar) and when the outer circle is 0.5% agar and the inner circle is 1.0% agar. The time courses of the P.I. value are constantly low for 30 min in all three conditions. (D) The P.I. values at 30 min in all three conditions. These graphs show that larvae tend to disperse outwards and that this tendency is irrespective of the inner agar concentrations (N=30, 9, 10, Steel-Dwass test, n.s.: not significant). (E) To find out whether larvae prefer a softer place because of the ease of crawling, we recorded larval locomotion on each concentration of agar, and calculated the total distance for 30 sec. There is no significant difference with respect to the agar concentration (N=17, 18, 19, Steel-Dwass test, n.s.: not significant). (F) To investigate the possibility that light intensity might affect the preference for softness, we performed the double circle assay in the dark. Larvae can discriminate softness differences in the dark (N=26, 27, Mann-Whitney U-tests, \*\*:  $p < 0.01$ ).

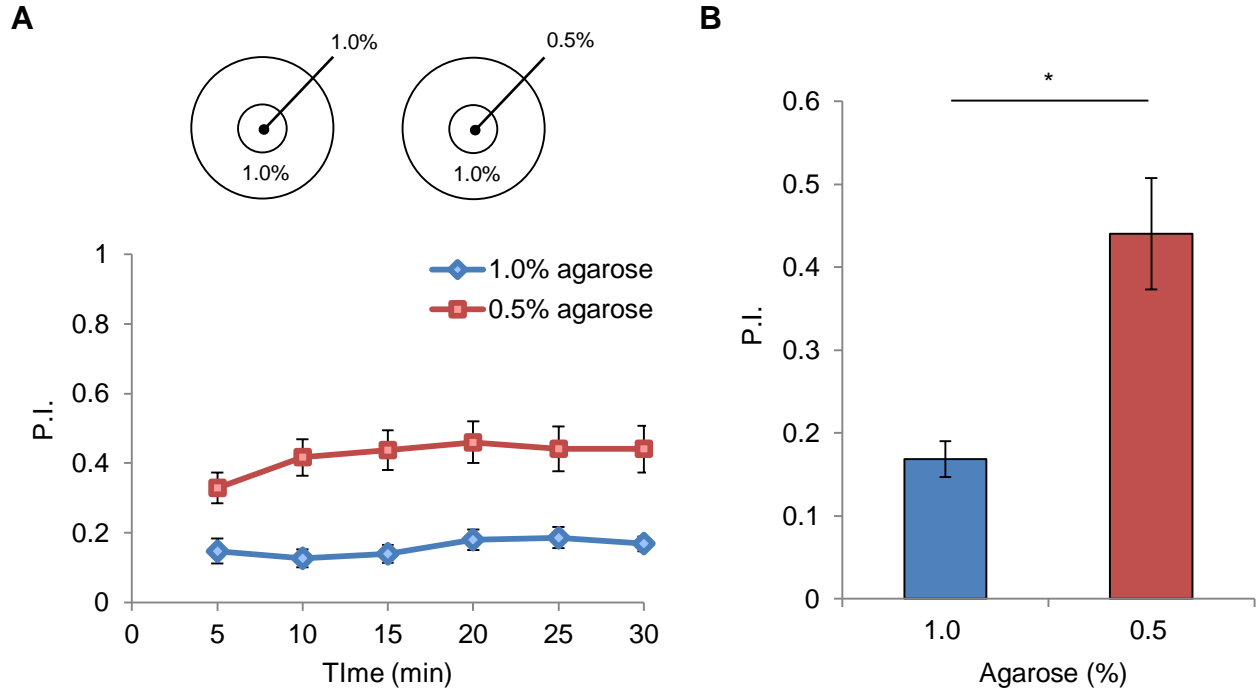
Fig.S2



**Figure S2: Larval intake of agar**

(A) The amount of intake during 30 min in the wholly filled plate, 1.0% and 0.5% agar colored with blue and red, respectively; there is no significant difference among the agar concentrations (N=12, 12, 12, 14, Steel-Dwass test, n.s.: not significant). (B) The amount of intake during 10 min in the wholly filled plate, 0.5%-2.0% agar containing 80 mM and 10 mM fructose colored with blue and red, respectively; in both 80 mM and 10 mM fructose, third-instar larvae ingested 1.0% agar more than 0.5% agar (N=12, 10, 8, 7, 8, 10, Steel-Dwass test, \*:  $p < 0.05$ , n.s.: not significant).

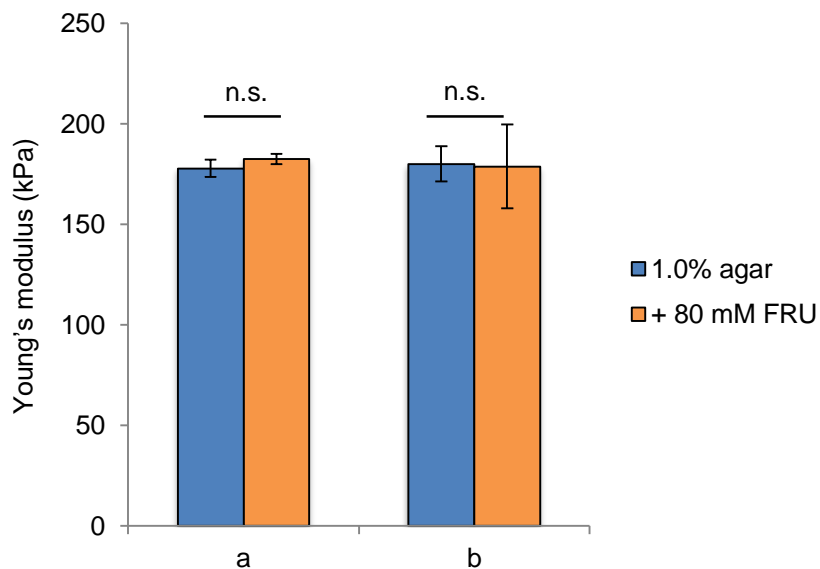
Fig.S3



**Figure S3: Larvae prefer softer agarose.**

We performed preference tests for softness using agarose instead of agar (N=10, 10, Mann-Whitney U-tests, \*:  $p < 0.05$ ). (A) The time course of the P.I. values. (B) The P.I. values at 30 min; when the inner circle contained 0.5% agarose, the larvae showed a significantly higher P.I. value than for 1.0% agarose (control). Larvae thus prefer softer agarose in a similar way to softer agar.

Fig.S4

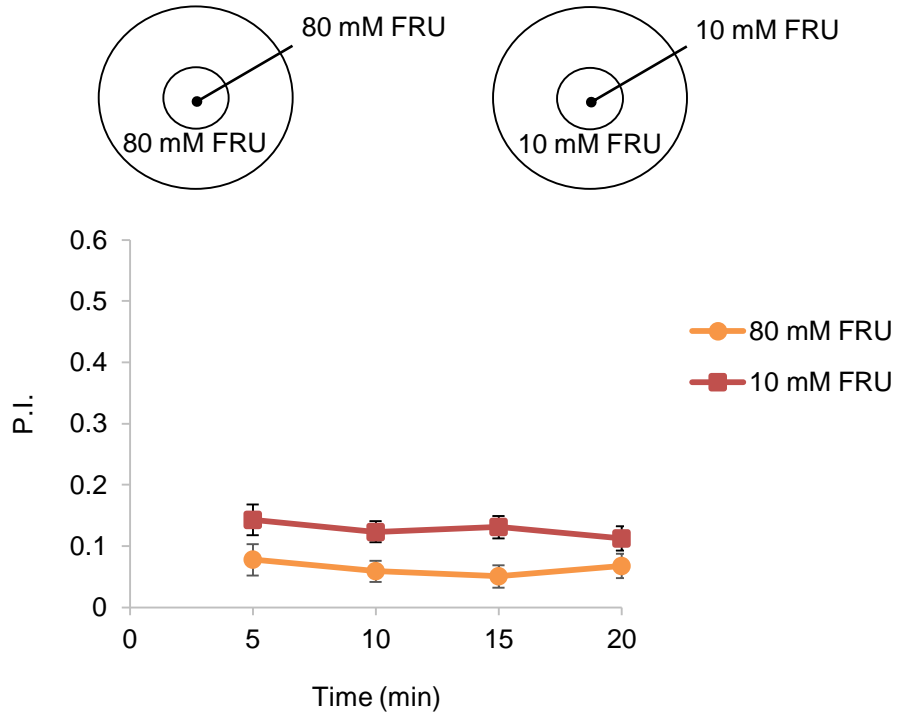


**Figure S4: Agar-gel hardness.**

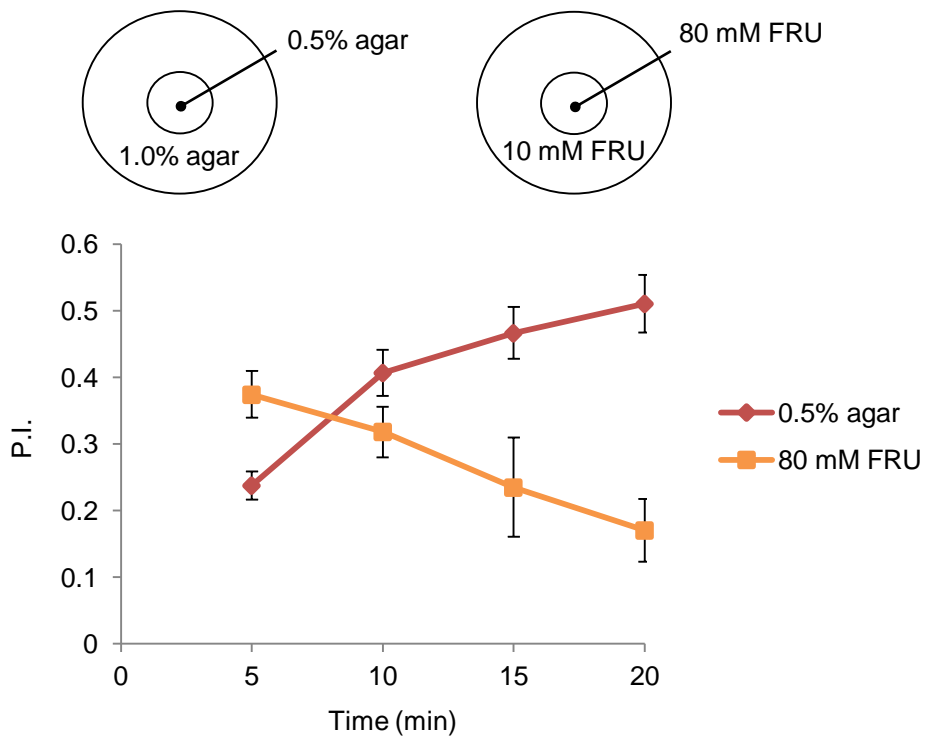
Young's modulus measured by the AFM nanoindentation method: the indentation depth was set at 3 nm (a) and 7nm (b), approximately. There is no significant difference in Young's modulus the between the two indentation depth conditions and in the presence of fructose, indicating that the addition of fructose does not affect the hardness of agar gel (N=120, 120, 120, 120, Mann-Whitney U-tests, n.s.: not significant).

Fig.S5

**A**



**B**



**Figure S5: The time course of the P.I. for softness and sweetness.**

(A) To know the effect of presence of fructose on larval behavior in double circle assay, we performed double circle assay where the inner and outer concentrations of fructose are same. The time courses of the P.I. values for 10 mM (red) and 80 mM (orange) fructose are shown. Larvae sparse to outer circle as in agar control as shown in Fig. 1C, suggesting that the presence of fructose did not affect preference in double circle assay. (N=10, 10, Mann-Whitney U-tests, \*\*:  $p < 0.01$ ) (B) To find the appropriate timing for comparing the P.I. for softness and sweetness, we obtained the respective time courses. The time courses of the P.I. values for sweetness (orange) and softness (red); larvae sense sweetness and softness to the same degree at 10 min. (N=20, 20)



### **Movie 1: Double circle assay**

The movie demonstrates the double circle assay for 30 min at 90x playback speed: when the outer circle was 1.0% agar and the inner circle was 1.0% agar (left); and when the outer circle was 1.0% agar and the inner circle was 0.5% agar (right). The larvae return to the inner circle in the situation shown on the right. The time courses of P.I. values during the first 5 min were shown in Fig. S1B.