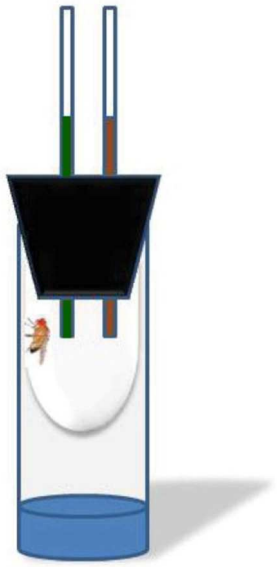
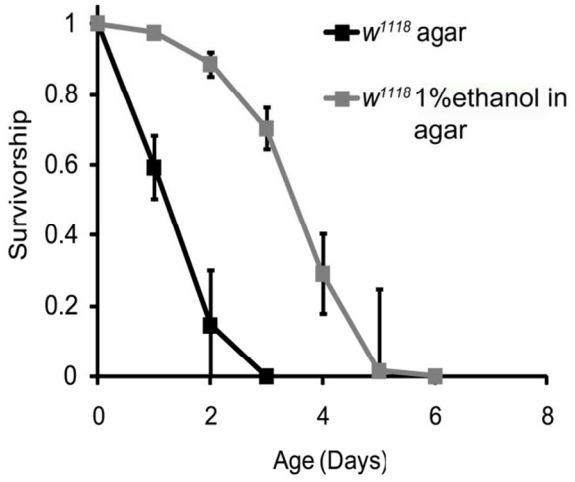
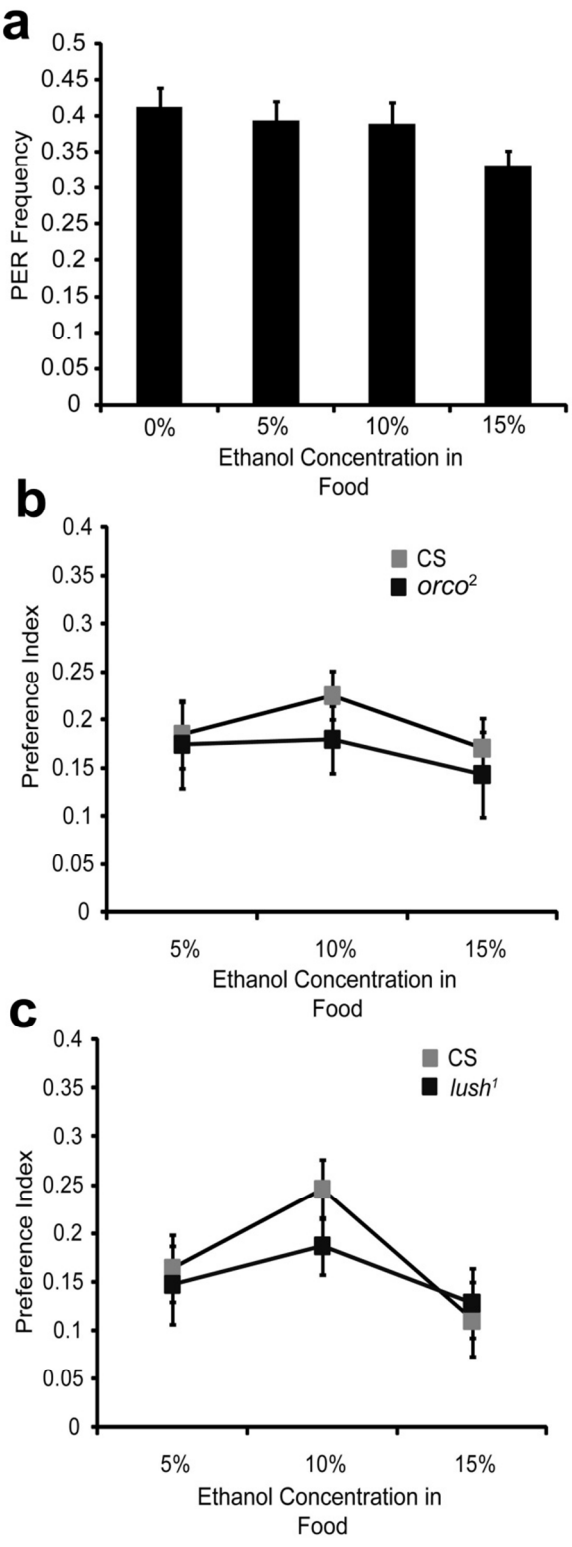


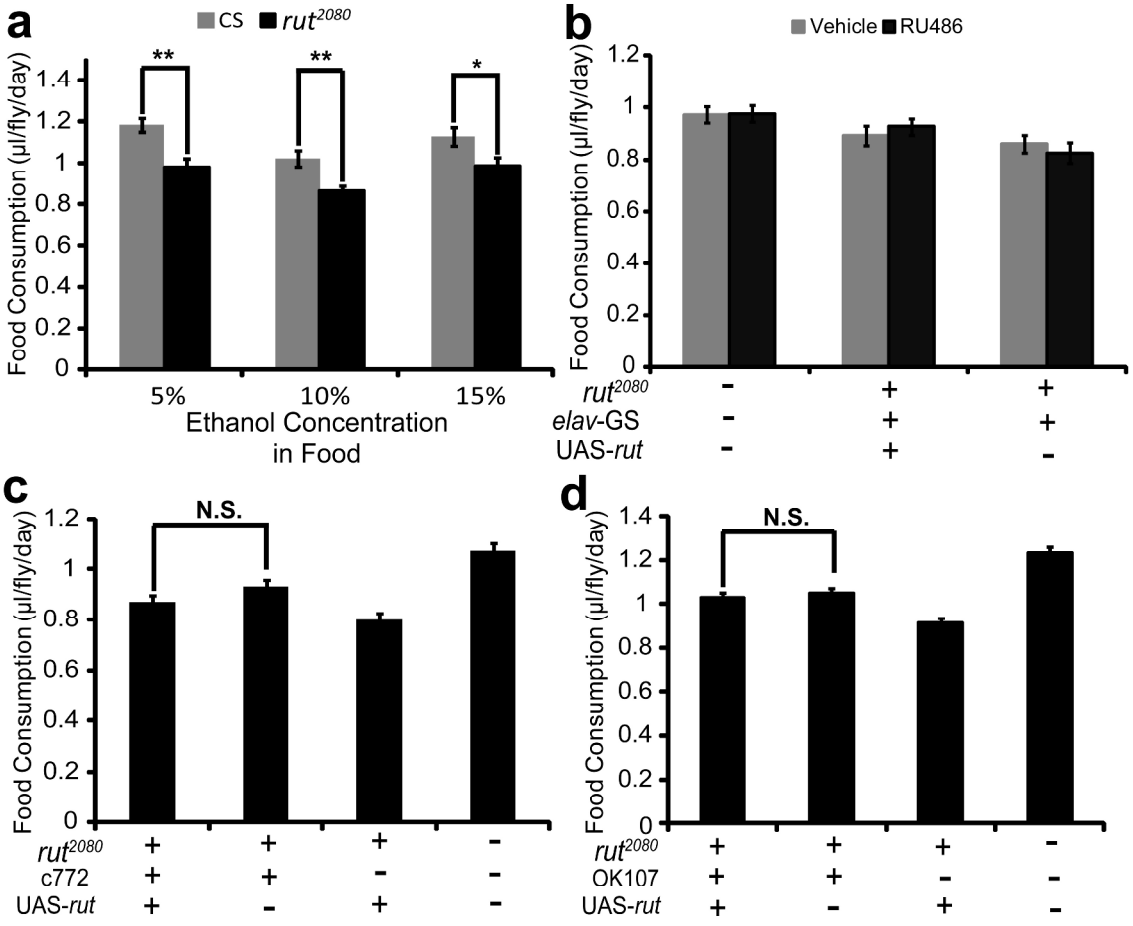
Supplemental Figure 1. Schematic of two-choices CAFE assay (not to scale). A single male fly was housed in inner vial, with water in outer vial, to keep a high humidity inside. Two kinds of liquid food were provided to the fly through two capillaries separately. One contains 5% sucrose and 5% yeast extract, which is represented by green color. The other contains 5% sucrose, 5% yeast extract and ethanol in a certain concentration, which is represented by red color. The two capillaries were replaced every 24 h with their locations were exchanged.



Supplemental Figure 2. Ethanol is not an efficient energy source for *Drosophila*. w^{1118} mutants in a Canton-S genetic background did not survive as long on agar as flies fed with 1% ethanol. Consistent with the data for wild-type Canton-S, the ethanol fed flies did not survive for very long. Hence, ethanol can be used as a food substrate by these flies, but not efficiently. Each data point is mean ±S.E.M.

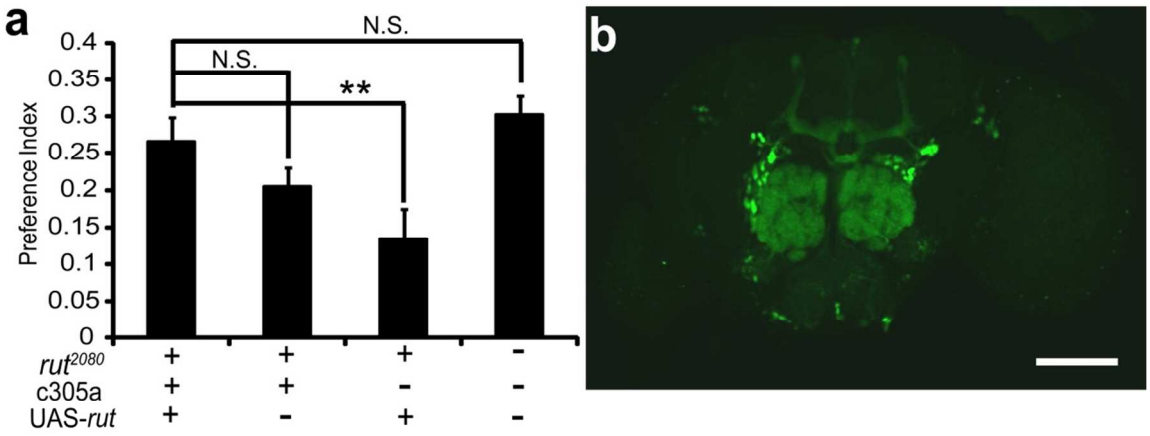

a
PER Frequency
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0%
5%
10%
15%
Ethanol Concentration in Food
b
Preference Index
CS
orco2
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
5%
10%
15%
Ethanol Concentration in Food
c
Preference Index
CS
lush1
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
5%
10%
15%
Ethanol Concentration in Food

Supplemental Figure 3. **Ethanol preference in the CAFE assay does not rely on gustatory or olfactory attraction.** (a) The PER index of Canton-S flies was not different between liquid food without ethanol and liquid food with 5%, 10% or 15% ethanol, which suggested that ethanol was not an appetitive gustatory cue. (b) The *orco*^{2} mutant ethanol preference to 5%, 10% or 15% ethanol are not significantly different from the ethanol preferences of Canton-S.(c) The ethanol preferences of *lush*^{1} to 5%, 10% or 15% ethanol are not significantly different from the preferences of Canton-S. (b) and (c) suggest that ethanol preference on *Drosophila* is not due to olfactory attraction of ethanol. Data are mean ±S.E.M.



Supplemental Figure 4. The decreased ethanol preference in *rut*^{2080} is not due to decreased food consumption. (a) In the CAFE assay, *rut*^{2080} consumed significantly less food than CS at each ethanol concentration. (b) This defect in food consumption was not increased significantly by the post-developmental expression of a wild- type *rut* cDNA in the nervous system with the *elav*-GS driver. However, the same treatment (RU486 feeding) induced a higher ethanol preference than the vehicle-feeding group (see Figure 2). (c)(d) The defect in food consumption was not rescued by the *rutabaga* expression driven by the OK107 or c772 Gal4 driver. However, this defect of ethanol preference in *rut*^{2080} was rescued by OK107 or c772 driven *rutabaga* expression in mushroom body (see Figure 4). (b)(c)(d) indicated that the *rut*^{2080}ethanol preference phenotype is independent of the total food consumption phenotype. Data are mean ±S.E.M."N.S." means no significance. ***P*<0.01 and ****P*<0.001. Because the negative control *rut*^{2080};+;238y and *rut*^{2080}; c305a/+; *MB247*/+ genotype displayed no difference with CS

in food consumption (data not shown), these results cannot indicted whether the two phenotypes are independent each other in rut^{2080} or not.



Supplemental Figure 5. The expression of *rutabaga* in the α'/β' lobe neurons alone is not sufficient for a full rescue of the *rut*^{2080} ethanol preference phenotype (a) The expression of UAS-*rut* driven by the c305a α'/β' Gal4 drive was not sufficient to fully rescue the *rut*^{2080} ethanol preference phenotype. The *rut*^{2080}; c305a/+; UAS-*rut* ethanol preference phenotype was not significantly different than CS, and was significantly higher than one control, *rut*^{2080};;UAS-*rut*/+ genotype. However, because it was not significantly different than the *rut*^{2080}; c305a/+ genotype control, it's still a question whether the *rutabaga* expression in α'/β' lobe is required for ethanol preference or not. Data are mean ±S.E.M. "N.S." means no significance. (b) c305a Gal4 drives the GFP expression in the α'/β' lobe mushroom body neurons. Scale bar: 100 μm.