Supplementary Materials and Methods

Fly stocks and genetics
Flies carrying dilp2-GAL4 were obtained from E. Rulison, UAS-PKA\textsuperscript{inh} flies (also called BDK33) from D. Kalderon, UAS-PKA\textsuperscript{m-inh} flies from J. Kiger. InR\textsuperscript{E19}, InR\textsuperscript{GC25}, and chico\textsuperscript{1} mutants outcrossed to the Dahomey genetic background, as well as the Dahomey background control flies, were obtained from D. Gems. elav-GAL4\textsuperscript{E1} flies were obtained from S. Sweeney, UAS-p60 flies from E. Rulifson, UAS-hFOXO3a flies and UAS-hFOXO3a-TM flies from E. Hafen. All lines used for behavioral experiments (with the exception of InR and chico mutants) were outcrossed for five generations to a w\textsuperscript{1118} stock isogenic for chromosomes II and III.

For behavioral testing, flies carrying both GAL4 and UAS insertions were generated by crossing GAL4 virgin females to UAS males. As controls, GAL4 or UAS heterozygotes were generated by crossing GAL4 virgins to w\textsuperscript{1118} males or by crossing w\textsuperscript{1118} virgins to UAS males. InR and chico\textsuperscript{1} mutants as well as Dahomey background control flies were tested as heterozygotes by crossing males to virgin females carrying attached X chromosomes in the w\textsuperscript{1118} genetic background and selecting against balancers in the subsequent generation.

Flies were raised on standard cornmeal and molasses food at 25°C and 70% relative humidity. All experiments were performed on 2-5 day old males at 20°C, utilizing ~110 males for each inebriometer run. All genotypes were tested across on multiple days.

Inebriometer Assay
Flies were tested in the inebriometer as described previously\textsuperscript{8}. Inebriometers were set to an ethanol/humidified air mixture of ~60/35 U and were allowed to equilibrate to 20°C. Flies were allowed to equilibrate for 5 minutes at 20°C before being introduced into the inebriometer. As flies eluted from the inebriometer, they were counted in 3 minute bins by a Drosophila activity monitor (Trikinetics, Waltham, MA). Mean elution times (METs) were then calculated from the resulting elution profiles. Inebriometer phenotypes of various lines were only considered different from controls if they were found to be significantly different from all applicable controls.
**Ethanol absorption**

Twenty-five flies of each genotype were exposed in triplicate to an ethanol/humidified air mixture of 50/100 U for 0, 10, 15, 20, or 30 minutes in perforated test tubes. Following exposure to ethanol, flies were frozen in dry ice and homogenized in 500 L of 50 mM Tris-HCl (pH 7.5). Ethanol assays were then performed on the fly homogenates as previously described8.

**Histochemistry**

To determine the adult CNS dilp2-GAL4 expression pattern, dilp2-GAL4 virgins were crossed to UAS-GFP T2; UAS-Tau GFP males (double transgenic stock created by F. Wolf, UCSF). Brains and ventral nerve cords were dissected from 2-4 day old adult male progeny in 1xPBS and fixed in 4% paraformaldehyde for 20 minutes. After washing in 1xPBS, neuropil labeling was achieved by incubating specimens in a 1:50 dilution of Nc82 antibody obtained from S. Sweeney, UCSF9 and with a Cy3 coupled goat anti-mouse antibody, diluted 1:500 (Molecular Probes, Eugene, OR). Specimens were mounted in Vectashield mounting medium (Vector laboratories, Burlingame, CA) and analyzed with a Leica confocal microscope with Leica Confocal Software Version 2.5.

**Statistics**

Data from multiple genotypes were compared using one-way ANOVA tests followed by post-hoc Newman-Keuls testing using GraphPad Prism software, Version 4 (Graphpad, San Diego, CA).

**References**
