Supporting Information

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SI Materials and Methods

Fly Stocks. The genotypes of the transgenic flies are

wild-type *Poxn* line 1 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$ *SuperA-158*],

wild-type *Poxn* line 2 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *SuperA-207-1*],

wild-type *Poxn* line 1/line 2 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$ *SuperA-158*/$Poxn^{\Delta M22\text{-}B5}$; *SuperA-207-1*/+],

wild-type *Poxn* line 1/line 2; *dFOXO* [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$ *SuperA-158*/$Poxn^{\Delta M22\text{-}B5}$; *SuperA-207-1* $dFOXO^{21}$/$dFOXO^{25}$,

no labellar taste bristles line 1 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full1*],

no labellar taste bristles line 2 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full115*],

no labellar taste bristles line 3 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full152*],

no labellar taste bristles line 1/line 2 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full1*/*Full115*],

no labellar taste bristles line 1/line 3 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full1*/*Full152*],

no labellar taste bristles line 2/line 3 [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full115*/*Full152*],

no labellar taste bristles line 1/line 2; *dFOXO* [w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *Full1* $dFOXO^{25}$/*Full115* $dFOXO^{21}$],

missing most taste bristles line 1 [*ΔXBs* w^{1118}; $Poxn^{\Delta M22\text{-}B5}$],

missing most taste bristles line 1/line 2 [*ΔXBs* w^{1118}; $Poxn^{\Delta M22\text{-}B5}$; *ΔPBs*/+],

missing most taste bristles line 1/line 3 [*ΔXBs* w^{1118}; $Poxn^{\Delta M22\text{-}B5}$/$Poxn^{\Delta M22\text{-}B5}$ *ΔPBs*], and

missing most taste bristles line 1/line 3; *dFOXO* [*ΔXBs* w^{1118}; $Poxn^{\Delta M22\text{-}B5}$/$Poxn^{\Delta M22\text{-}B5}$ *ΔPBs*; $dFOXO^{21}$/$dFOXO^{25}$].

Backcrosses of the Different Lines to w^{1118}. The backcrosses of the $Poxn^{\Delta M22\text{-}B5}$ null mutant were followed by PCR. The primers used are as follows.

Poxn pair 1:

dM22fw6: 5′-GAG CCA ATC CAA TCC AAT TCA ATC-3′

dM22rev6: 5′-CTA ACA CCT CCG TTA ATA ATC GCT-3′

Poxn pair 2:

IO_200n: 5′-GGATGTTGCAGACTGCGTGCGT-3′

IO_259n: 5′-CGCAGTCGCTTAGCCTCCGT-3′

The backcrosses of the two *dFOXO* mutations to w^{1118} were followed by PCR and a restriction digest of the resulting product, where the mutation of interest was further confirmed by sequencing. The primers used are as follows.

forward primer: 5′- GCAATCAGCAGTTGGCCCCAG

reverse primer: 5′- CGAGACCAGCGAGCATGGTGG

For the $dFOXO^{21}$ mutation, the PCR product was digested with MnlI because the mutation leads to an additional MnlI site. For the $dFOXO^{25}$ mutation, the PCR product was digested with FokI because the mutation leads to loss of a FokI site.

The different transgenic chromosomes were backcrossed by following the rescue of the w^{1118} eye phenotype because all transgenes also carry sequences that rescue the w^{1118} mutation. After backcrossing the different chromosomes, the $Poxn^{\Delta M22\text{-}B5}$ mutation was combined with different rescuing transgenes, which in the case of two of the transgenic lines involved the recombination of the second chromosome. The presence of the rescuing transgene in the $Poxn^{\Delta M22\text{-}B5}$ mutant background was then followed by rescue of the visible $Poxn^{\Delta M22\text{-}B5}$ phenotypes in the taste bristles and male genitalia. The *dFOXO* mutations were introduced into the relevant lines through recombination of the third chromosome, and their presence was confirmed by PCR and sequencing. Because we sometimes used the balancer line *Sp*/*CyO*; *MKRs*/*TM6b* to isolate specific mutations or transgenes, we also backcrossed *Sp*/*CyO*; *MKRs*/*TM6b* to w^{1118}. This is because recombination can still occur with some of the balancer chromosomes, e.g., the *Sp* chromosome.

For the gustatory receptor mutants, the mutations were followed by PCR based on the previous descriptions of the mutations (1–7).

Feeding Assays. Control and taste mutant flies were collected at the same time as described in the *Materials and Methods, Lifespan Assays, Zurich* section of the main text (Zurich protocol). Flies were transferred regularly to fresh food until the specified days of adulthood, upon which flies were transferred to vials containing food sources (standard Zurich fly food and yeast supplement) that had been dyed with FD&C Blue no. 1 (Brilliant Blue FCF, Sigma) at a final concentration of 0.5 g dye per 100 g food. This dye is metabolically neutral and thermostable (8). Flies were allowed to feed on the dyed food sources for either 6 h (3–12 biological replicates and 4–5 flies per replicate) or 24 h (3–4 biological replicates and 7–10 flies per replicate) at 25 °C, after which they were decapitated and the bodies collected. For the 6-h feeding regimen, each replicate of fly bodies was then homogenized in 150 µL PBS/0.05% Triton X-100 (Fisher Scientific) solution and subsequently centrifuged at 5,000 × *g* for 1 min to remove debris. For the 24-h feeding regimen, each replicate was homogenized in 10 µL PBS/0.05% Triton X-100 solution and centrifuged at 13,000 × *g* for 15 min. The absorbances of the control and taste mutant supernatants were measured together at 630 nm and the amount of food consumed was estimated from a standard curve of the same dye solution. The differences between the groups were assessed using a one-way ANOVA test.

Body Weight and Triacylglyceride Measurements. Newly eclosed flies were collected and kept in groups of 10 per vial as described in the main text (*Materials and Methods, Lifespan Assays, Zurich*), with regular transfers onto fresh fly food at 25 °C. To determine the body weight, 30 individual male or female flies per age group per genotype were measured. To determine triacylglyceride (TAG) levels, flies were aged for 5 or 10 d, after which five replicates (9–10 flies per replicate) per genotype were snap-frozen in liquid nitrogen. Then, at 4 °C, the wet weights of the flies were measured before homogenization in PBS/0.05% Triton-X-100 buffer. Subsequently, TAG levels were determined with a Serum Triglyceride Determination Kit [Abcam (catalog no. ab65336) or Sigma (catalog no. TR0100)], according to the manufacturer's instructions. The protein levels of the same flies were also determined using the standard BioRad protein assay (BioRad), and the resulting TAG levels were normalized per milligram of protein. To test for differences between groups in all our measurements, we performed a one-way ANOVA test.

Quantitative Measurement of mRNA Levels. We measured the relative abundance of specific mRNAs through quantitative

PCR (qPCR). Ten-day-old male and female flies were grown as described in the main text (*Materials and Methods, Lifespan Assays, Zurich*) and collected separately in 1.5-mL Eppendorf tubes and snap-frozen in liquid nitrogen. Afterward, the heads were disconnected from the bodies by vigorously shaking the tubes, after which the heads and bodies were funneled through a fine mesh for further separation. Total RNAs were then extracted from 30 heads or bodies per replicate per condition (3–4 biological replicates per condition) with a Nucleospin RNA II kit (Macherey-Nagel). Upon RNA isolation, the samples were treated with DNase and inhibitors of RNases. Then, mRNAs in the samples were reversely transcribed using a Transcriptor HiFi cDNA Synthesis Kit (Roche). All steps before RNase inactivation were performed in liquid N_2 or on dry ice in a 4 °C cold room.

qPCR was carried out in triplicates of each biological replicate using the Applied Biosystems SYBR Green kit and the ABI Prism 7900HT System (Applied Biosystems). All results were normalized to the *actin5C*, *tubulin-1α*, and *GAPDH2* mRNA levels. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method (www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_040980.pdf). The resulting data were log2 transformed before statistical testing. To test for differences among the different groups, we performed a randomized complete block design ANOVA test by using the R statistical software package (9), where each plate was treated as an experimental block. We also incorporated a statistical censoring procedure to exclude outliers and ensure that the data have normal distribution, which involved tentative identification of the outliers and calculation of the standard deviation (SD) of the remaining dataset. We then excluded replicates from the full dataset that were lower or higher than the mean ± 3 times the SD.

To determine the mRNA levels of dFOXO targets and *Drosophila* insulin-like peptides (*dilps*), qPCRs were performed in both head and body tissues. The primers for *dilp1* to *dilp3*, *dilp5*, *dilp6*, *l(2)efl*, and *GAPDH2* were as described (10, 11). The other primers used are

actin5C forward primer, GCCCATCTACGAGGGTTATGC;

actin5C reverse primer, AATCGCGACCAGCCAGATC;

tubulin-1α forward primer, GCCAGATGCCGTCTGACAA; and

tubulin-1α reverse primer, AGTCTCGCTGAAGAAGGTG-TTGA.

dilp1 is expressed lower than other neural *dilps*.

Fecundity Assays. We measured the number of eggs laid per female fly (6–10 single adult females per genotype) at 25 °C. All females were aged for a day before the start of the experiments. Then, we placed a single female per genotype with two wild-type *Poxn* males per vial. All were fed standard Zurich fly food as above, including the yeast supplement. Flies were then transferred onto fresh food daily and fecundity was measured every day for 10 d. Statistical differences were determined according to a one-way ANOVA test.

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A

AgeI
BamHI
0
XbaI
PstI
BstXI
HindIII
Poxn
2 kb
*
GUA
Df(2R)Poxn$^{\Delta M22\text{-}B5}$
no labellar taste bristles
missing most taste bristles
ΔPBs
ΔXBs
complete rescue
wild-type Poxn

B

Mutation	Poxn$^{\Delta M22\text{-}B5}$				
Rescue	**wild-type *Poxn***	**Δ*XBs***	**Δ*PBs***	**Δ*XBs* / Δ*PBs***	**no labellar taste bristles**
Taste: leg and wing	+	-	-	-	+
Taste: labellum	+	-	-	-	-
Leg and antennae segmentation	+	(+)	+	+	+
Male posterior lobe	+	+	-	+	+
Penis	+	+	+	+	+
Ventral ganglion expression	+	+	+	+	+
Brain expression	+	+	+	+	+

Fig. S1. Description of the different *Poxn* rescue constructs (1, 2). (*A*) Genetic map of the *Poxn* locus, which shows unique restriction sites. The *Poxn* translation start site and its stop codon (*) are shown. The entire coding region is depicted in black bars, the introns in hatched bars, the 5′ and 3′ UTRs in gray bars, and the upstream and downstream *cis* regulatory regions in white bars. The extent of the *Poxn*$^{\Delta M22\text{-}B5}$ deletion is indicated. In addition, the structures of the different *Poxn* rescue constructs are depicted: the complete rescuing transgene; the transgene that lacks the enhancers required for the formation of labellar taste bristles, in which introns 3 and 4 are also missing; and the transgenes that lack the enhancers for most taste bristles, which are also either missing all introns and a PstI-BstXI fragment in the 5′ *cis* regulatory region (*ΔPBs*) or missing only introns 3 and 4 and an XbaI-BstXI fragment in the 5′ *cis* regulatory region (*ΔXBs*). (*B*) The phenotypic defects of the *Poxn* deletion rescued by the different transgenes are listed. Because the *ΔXBs* transgene only partly rescues the leg/antenna segmentation phenotype of *Poxn*$^{\Delta M22\text{-}B5}$ null mutants, it was combined with one copy of the *ΔPBs* transgene, which completely rescues this phenotype (2). Thus, the difference between the two classes of taste-impaired flies assayed in this study resides in the number of taste bristles missing and the locations of said bristles.

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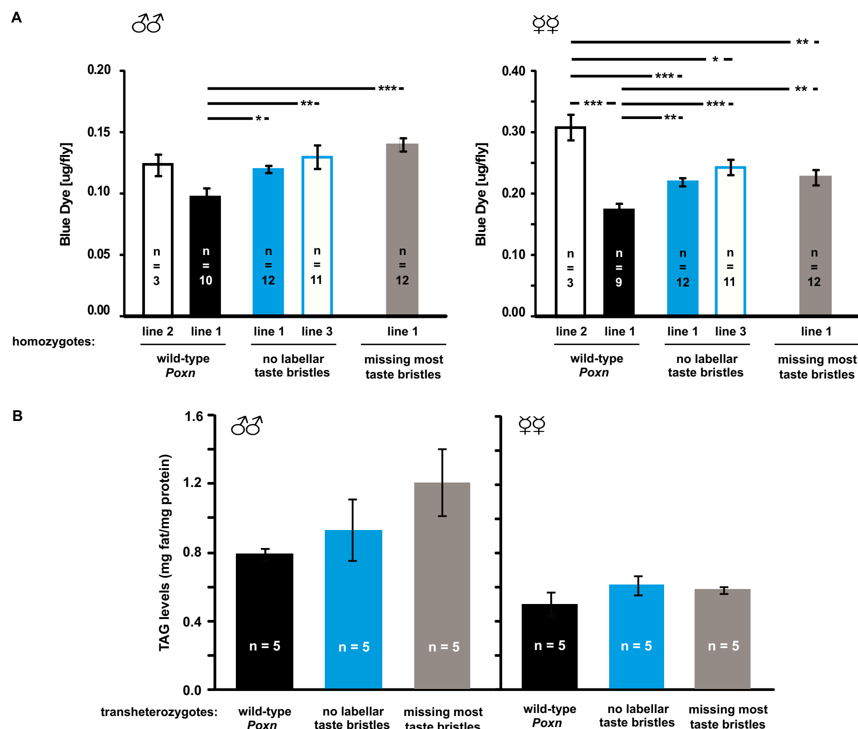

Fig. S2. Feeding phenotypes and TAG levels of taste-impaired flies. (*A*) Seven- to 8-d-old taste-impaired adult mutant males and females (homozygous lines) are compared with controls after a 6-h feeding regimen. The food consumption values (blue dye) are normalized per fly and each mean is derived from the indicated number (*n*) of biological replicates of four to five pooled flies. All error bars represent ±SEM. $*P \leq 0.01$, $**P \leq 0.01$, and $***P \leq 0.001$, according to one-way ANOVA with Fisher's post hoc test. Note that one of the control lines (wild-type *Poxn* line 2) has a smaller *n* compared with the rest of the lines. However, the observed food intake levels do not correlate with lifespan because both female wild-type *Poxn* lines have a shorter lifespan than the female taste-impaired lines (Table S1, treatment J.7 to J.12). (*B*) TAG levels of 5-d-old adult taste-mutant and control flies that carry transheterozygous insertions of the relevant transgenes in their genomes have similar TAG content (one-way ANOVA *F* test). Each mean represents five biological replicates of 9 to 10 pooled flies. All error bars again represent ±SEM.

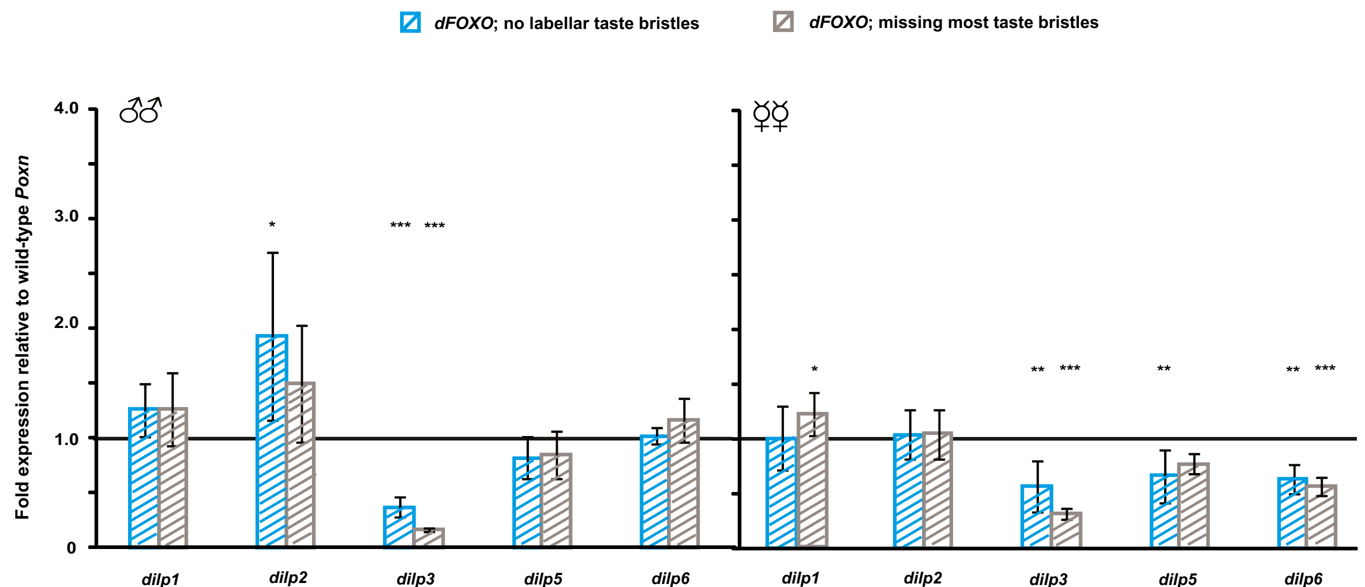

Fig. S3. *dilp* expression in control and taste-impaired flies in the absence of *dFOXO*. *dilps* in the heads of adult male and female taste mutants (blue and gray bars) that lack *dFOXO* are shown normalized to control levels (horizontal line across the graph). Each mean value shown represents two to three biological replicates of 30 pooled flies, which again carry transheterozygous insertions of the relevant transgenes in their genomes. All error bars represent ±SEM. $*P < 0.05$, $**P < 0.01$, and $***P \leq 0.001$.

Table S1. Adult lifespans of taste-impaired mutant flies at 25 °C

Table S1

We assayed control and mutant flies in parallel in independent trials. The mean lifespan (±SEM) and the median in days (in brackets) are shown in the second column, whereas the number of flies observed per number of flies assayed are noted in the third column. The percent difference between wild-type *Poxn* flies and taste-mutant flies under different conditions is indicated in the fourth column. Flies that escaped or were stuck to the food were censored at the time of the event, allowing these flies to be incorporated into the data set until the censor date, thus avoiding loss of information. The differences that are significant ($P \leq 0.05$) according to the Wilcoxon test, which in most cases is also significant according to the log-rank test, are underlined and in boldface type. The differences that are significant only according to the log-rank test are italicized. The percent difference between certain groups of flies, as specified by the superscripted symbols, is shown in the seventh column.