GCG3 MWLKNLKNLKN GC IC IE	00 V & G T & E & X A & S & E & L P N Y
$ \begin{array}{c} 6653 \\ 6674 \\ 6$	$ \begin{array}{c} 120\\ M & T & S & T & S & D & L \\ M & T & A & T & S & T & L & S \\ G & T & L & S & L & S & L & F \\ F & T & F & T & S & T & A & D & S \\ F & T & F & T & A & D & S \\ T & L & F & F & G & V & S & S \\ T & A & S & S & L & A & N & F \\ L & L & L & C & G & M & K & T \\ \end{array} $
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c} & 100\\ P & F & I & F \\ F & I & F \\ L & F \\ L & F \\ L & K \\ I & F \\ I & $
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c} 200\\ P \ Y \ E \ S \ \hline { {\bf Y} } \\ S \ F \ X \ \ { {\bf Y} } \\ Y \ F \ E \ S \ \ { {\bf Y} } \\ F \ W \ Q \ X \ \ { {\bf Y} } \\ F \ W \ Q \ X \ \ { {\bf Y} } \\ F \ W \ Q \ X \ \ { {\bf Y} } \\ F \ W \ Q \ X \ \ { {\bf Y} } \\ F \ W \ \ { {\bf Z} } \\ P \ \ I \ Q \ X \ \ { {\bf Y} } \\ F \ \ { {\bf U} } \ \ \ { {\bf U} } \ \ { {\bf U} } \ \ { {\bf U} } \ \ \ \ { {\bf U} } \ \ \ \ { {\bf U} } \ \ \ \ \ \ { {\bf U} } \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ $
$ \begin{array}{c} GG_{24} & A \le A \le \left[\begin{array}{c} 100 \\ B \le \left[\begin{array}{c} 100 \\ C \le \left[100 \\ C $	$\begin{array}{c} 800\\ 8 & S \\ \hline \mathbf{R} & \mathbf{I} \\ \mathbf{R} & \mathbf{R} \\ \mathbf{R} & \mathbf$
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c} & & & & & & & & & & & & & & & & & & &$
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c} \mathbf{A} \mathbf{A} \mathbf{E} \mathbf{E} \mathbf{A} \mathbf{A} \mathbf{S} \mathbf{D} \mathbf{Q} \\ \mathbf{I} \mathbf{F} \mathbf{Q} \mathbf{M} \mathbf{T} \mathbf{I} \mathbf{Q} \mathbf{T} \\ \mathbf{S} \mathbf{E} \mathbf{Q} \mathbf{I} \mathbf{G} \mathbf{S} \mathbf{N} \mathbf{D} \mathbf{T} \\ \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{F} \mathbf{U} \mathbf{S} \mathbf{D} \mathbf{R} \\ \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{E} \mathbf{V} \mathbf{S} \mathbf{R} \\ \mathbf{S} \mathbf{E} \mathbf{E} \mathbf{V} \mathbf{I} \mathbf{S} \mathbf{S} \mathbf{E} \mathbf{T} \\ \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{E} \mathbf{V} \mathbf{S} \mathbf{E} \mathbf{I} \\ \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{I} \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{S} \mathbf{E} \mathbf{I} \\ \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{I} \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{S} \mathbf{E} \mathbf{I} \\ \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{I} \mathbf{S} \mathbf{I} \mathbf{I} \mathbf{S} \mathbf{I} \\ \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{I} \mathbf{I} \mathbf{S} \mathbf{I} \mathbf{I} \\ \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{I} \mathbf{I} \mathbf{S} \mathbf{I} \mathbf{I} \\ \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{I} \mathbf{I} \mathbf{S} \mathbf{I} \mathbf{I} \\ \mathbf{S} \mathbf{E} \mathbf{I} \mathbf{I} \mathbf{I} \mathbf{S} \mathbf{I} \mathbf{I} \\ \mathbf{S} \mathbf{I} \mathbf{I} \mathbf{I} \mathbf{I} \mathbf{I} \mathbf{I} \mathbf{I} I$
	490 SKSIFS MSIFF S

Supplementary Figure S1: Sequence conservation of th Drosophila melanogaster

(A) Alignment of the eight putative sugar receptors. Alignme the Multiple Alignment feature of MacVector, using a BLOSUI default parameter settings.

(B) Evolutionary relationship between GR proteins. The generated in MEGA4, using the Neighbor-Joining method [32^o indicated next to the branches. The eight sugar receptors are at th bracket) and have bootstrap support of 100%.

S1B



0.2



Supplementary Figure S2: Flies with R1 and R2 transgenes show normal PER response. The graph shows PER responses for three different sugars of flies containing the two transgenes R1 and R2 and a wild type copy of the *Gr64* gene $(R1/+;R2/+;\Delta GR64/+)$, in comparison to control flies (see Figure 2), as well as homozygous $\Delta GR64$ mutants. $R1/+;R2/+;\Delta GR64/+$ flies show normal response to these sugars, compared to the highly reduced or lost response in mutants. At 500 mM, the response of $R1/+;R2/+;\Delta GR64/+$ flies is the same as that observed in the control strain, while at 100 mM, the response appears slightly higher. Asterisks indicate a significant difference between the mutant and control strains, as determined by Student's t-test (* indicates p<0.05, *** indicates p<0.0001).



Supplementary Figure S3: Expression of the *Gr64* genes in flies containing the rescue Gr64 construct

Flies containing the UAS-Gr64abcd_GFP_f transgene express the Gr64 genes, both in the absence (lanes 1) and presence (lanes 2) of the Gr5a-Gal4 driver. In lanes 3, RNA from homozygous Δ Gr64a mutants flies (R1/+;R2/+; Δ GR64/ Δ GR64) was loaded. RT-PCR analysis of RNA isolated from heads and legs (not shown) was carried out for the first four genes. Integrity of cDNA was confirmed using primers against the tubulin gene. The same primers were used as in Figure 1D (1-6, T1 and TR2).