Supplementary Information

Neurofibromin regulates metabolic rate via neuronal mechanisms in *Drosophila*

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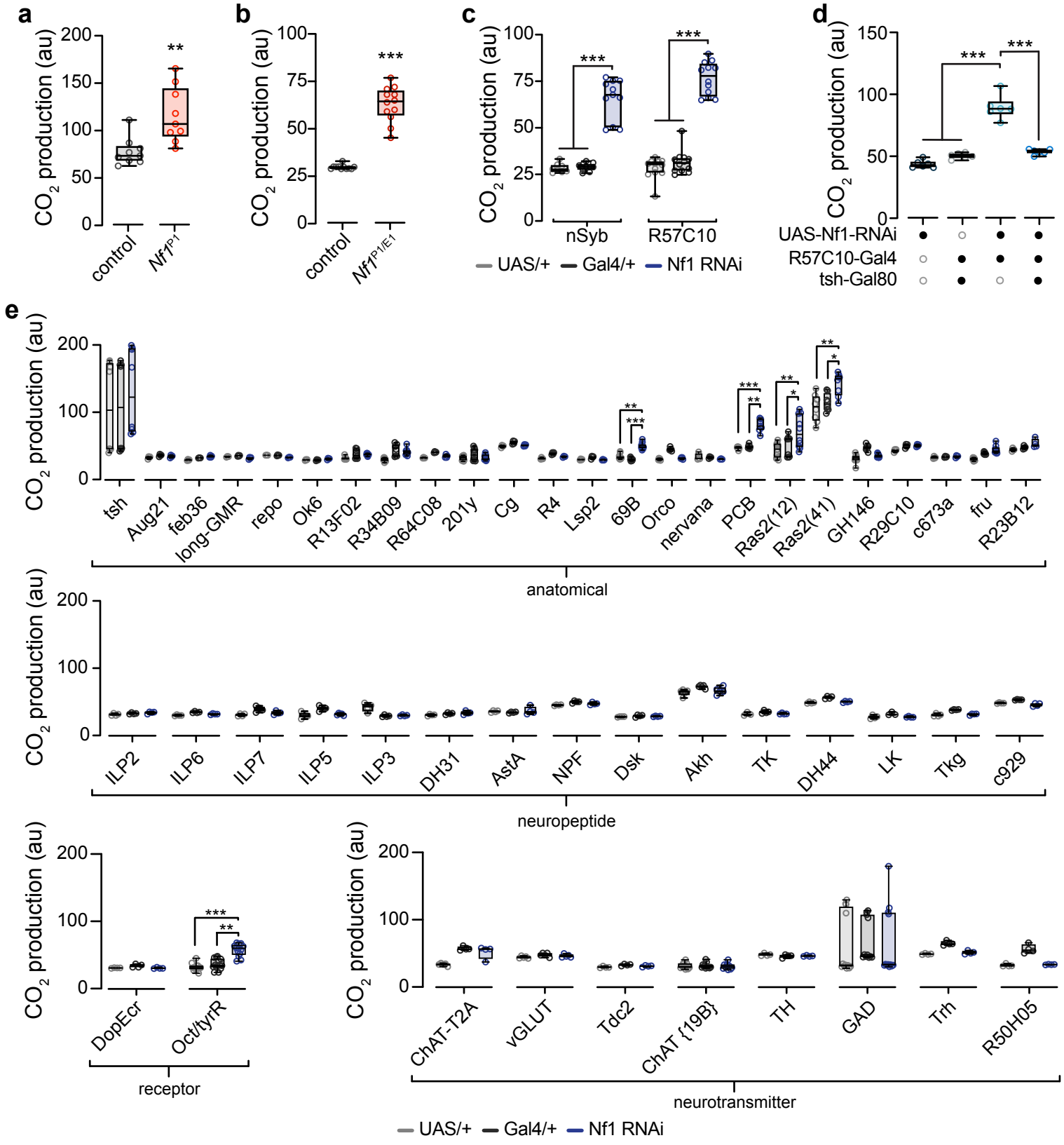
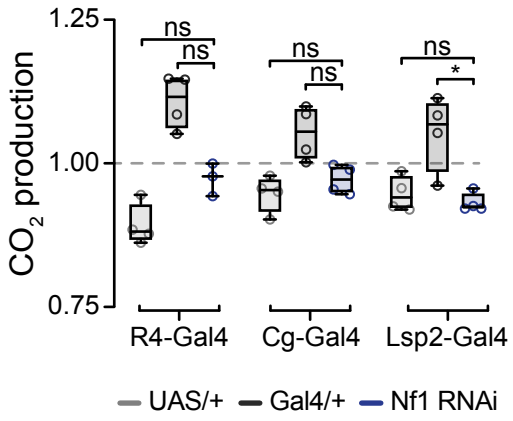
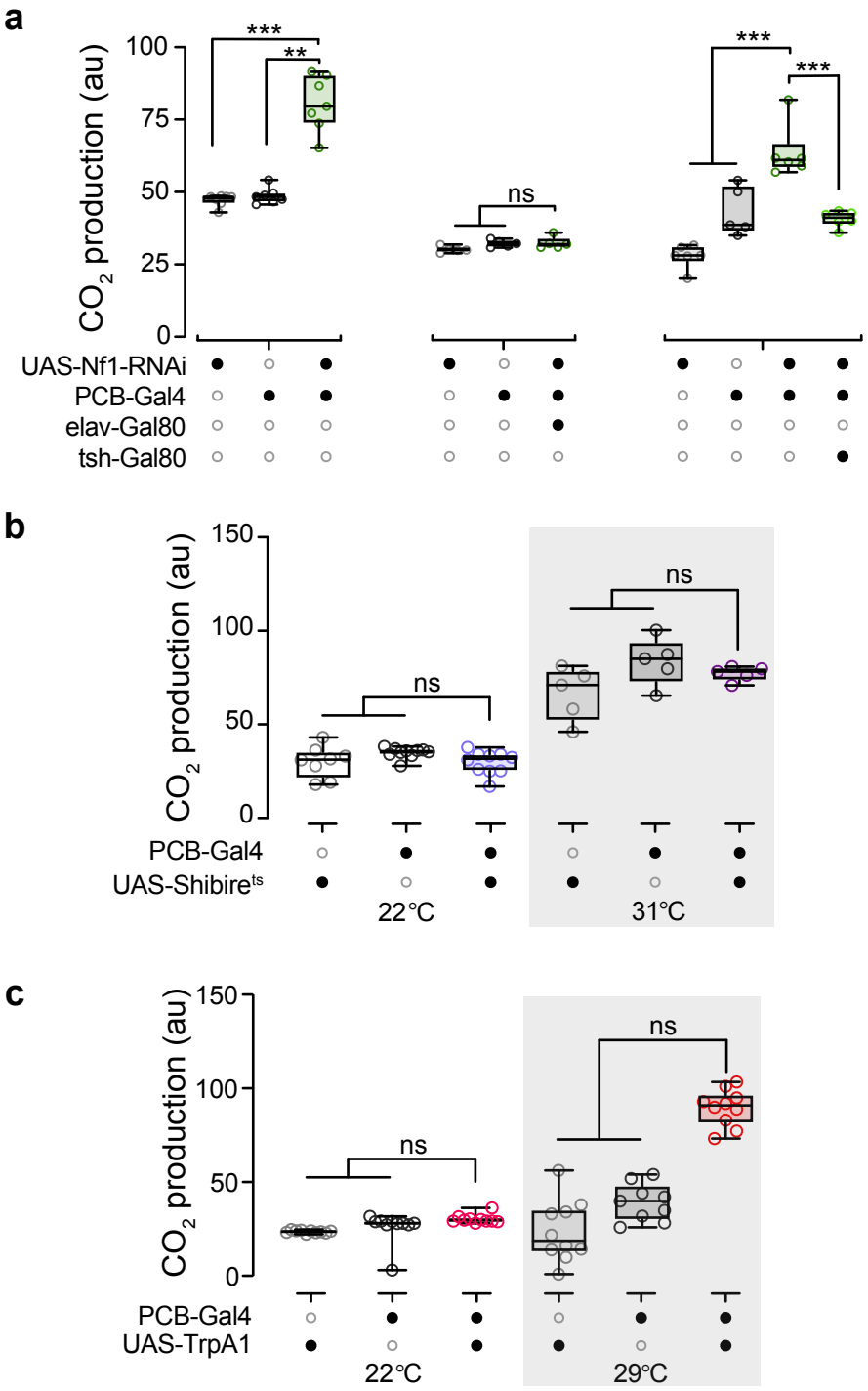


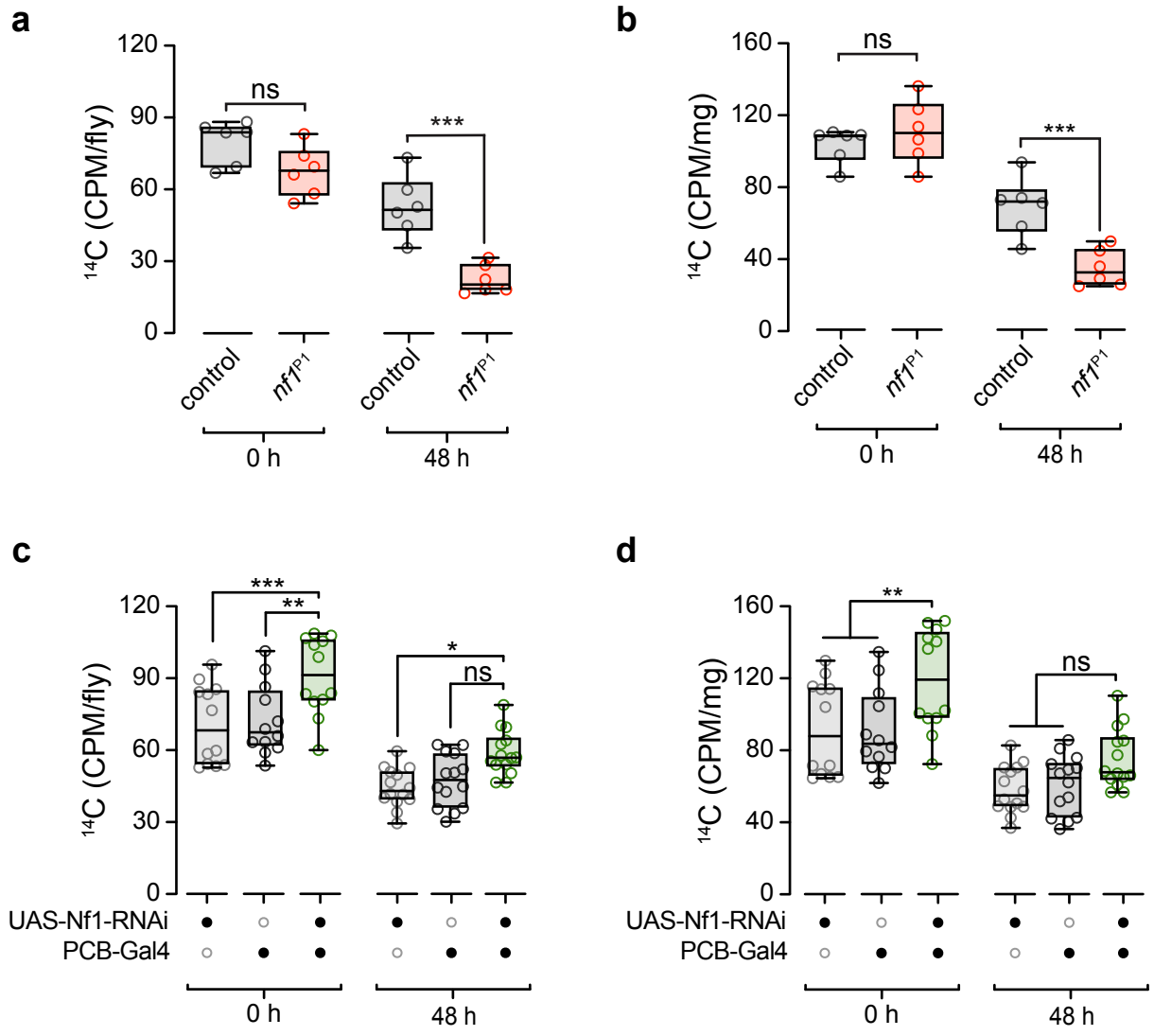
Supplementary Figure 1. Related to Figure 1 and Figure 2. **a.** CO_2 production in *Nf1*^{P1} mutants and *w*^{CS10} controls. **p = 0.002 (Mann-Whitney, two-sided n = 9). **b.** CO_2 production in *N1*$^{P1/E1}$ (n = 12) heteroallelic mutants compared to matched genetic background controls (n = 11). ***p = 0.0001 (Mann-Whitney, two-sided). **c.** CO_2 production when Nf1 was pan-neuronally knocked down with RNAi using Gal4 lines: nSyb- and R57C10-Gal4. Gal4/+ and UAS/+ are heterozygous controls. ***p<0.001 (Dunn's test, for nSyb-Gal4: n = 8 UAS-Nf1 RNAi/+, n = 10 nSyb-Gal4/+, n = 11 experimental group. For R57C10-Gal4: n = 12 per genotype). **d.** Pan-neuronal Nf1 knockdown with R57C10-Gal4, with and without the tsh-Gal80 repressor. ***p<0.001 (Sidak, two-sided n = 6). **e.** Raw data from the screen for neuronal subsets in which Nf1 knock down elevates CO_2 production, separated into four categories: anatomical, neuropeptides, receptors, and neurotransmitters. *p < 0.05, **p < 0.01, ***p < 0.001 (Dunn's test, two-sided; n = 7-14). au: arbitrary units. Exact sample sizes and p-values found in Source Data file for **e**. Each 'n' = single respirometer (containing 4 animals). Box plots – box: 1st to 3rd quartiles, median: line, whiskers: min-max, individual data points: circles.



Supplementary Figure 2. Related to Figure 2.
CO_2 production, assayed by respirometry. Nf1 knockdown with RNAi driven by fat body specific Gal4 lines: R4-, Cg-, and Lsp2-Gal4. Gal4/+ and UAS/+ are heterozygous controls. For each driver, data are normalized to the mean of both controls. ns: not significant, (R4-Gal4, UAS- Nf1 RNAi experimental p = 0.39 re: R4-Gal4/+ ; p = 0.74 re: UAS-Nf1 RNAi/+ [Dunn's, two-sided n = 4 UAS-Nf1 RNAi/+, n = 4 R4-Gal4/+, n = 3 experimental group]), (Cg-Gal4, UAS-Nf1 RNAi experimental p = 0.07 re: Cg-Gal4/+; p >0.99 re: UAS-Nf1 RNAi/+ [Dunn's, two sided n = 4 per genotype]) (Lsp2-Gal4, Nf1 RNAi experimental *p = 0.02 re: Lsp2-Gal4/+; p >0.99 re: UAS-Nf1 RNAi/+ [Dunn's, two-sided n = 4 per genotype]). Each 'n' = single respirometer (containing 4 animals). Box plots – box: 1st to 3rd quartiles, median: line, whiskers: min-max, individual data points: circles.



Supplementary Figure 3. Related to Figure 2. CO_2 production, assayed by respirometry.
a. Nf1 knockdown in neurons with PCB-Gal4 driver (left) (**p < 0.01 ***p < 0.001 [Dunn's test, two-sided n = 7]), with or without the elav-Gal80 repressor (middle) (experimental p = 0.068 re: UAS-Nf1 RNAi/+, p >0.999 re: PCB-Gal4/+ [Dunn's test, two-sided n = 5]), and with or without the tsh-Gal80 repressor (right) (***p < 0.001 [Sidak, two-sided n = 6]). **b.** CO_2 production assayed by respirometry when PCB-Gal4 neurons are blocked using UAS-Shibire^{ts} at 31 °C (right) and at control temperature (22 °C, left). At 22 °C experimental p >0.999 re: UAS-Shibire$^{t.s}$/+, p = 0.474 re: PCB-Gal4/+. (Sidak, two-sided n = 8 UAS-Shibire$^{t.s}$/+, n = 10 PCB-Gal4/+, and n = 10 experimental. At 31 °C experimental p = 0.162 re: UAS-Shibire^{ts}/+, p = 0.634 re: PCB-Gal4/+. (Sidak, two-sided n = 5 per genotype). ns: not significant. **c.** Normalized CO_2 production following activation of PCB-Gal4 neurons using UAS-TrpA1 at 29 °C (right) and at control temperature (22 °C, left). At 22 °C experimental p = 0.402 re: UAS-TrpA1/+, p = 0.784 re: PCB-Gal4/+. ns: not significant (Sidak, two-sided n = 10 per genotype). At 29 °C ***p < 0.001 (Sidak, two-sided n = 10 UAS-TrpA1/+, n = 9 PCB-Gal4/+, n = 10 experimental group). Each 'n' = single respirometer (containing 4 animals). Box plots – box: 1st to 3rd quartiles, median: line, whiskers: min-max, individual data points: circles.



Supplementary Figure 4. Related to Figure 5.
a. Quantification of lipid turnover in *Nf1*^{P1} and *w*^{CS10} control flies via ^{14}C sucrose radiolabeling. Radioactivity measured as counts per minute (CPM) in a scintillator and normalized to number of flies. At time point zero hours (0 h) ns: not significant, p = 0.092, at 48 hours (48 h) ***p < 0.001, (Sidak, two-sided; n = 6 for both genotypes at both time points). **b.** Radioactivity measured as CPM and normalized to fly weight (mg). Data repeated from Figure 5f and from panel a here. At time point zero hours (0 h) ns: not significant, p = 0.604. at 48 hours (48 h) ***p < 0.001, (Sidak, two-sided; n = 6 for both genotypes at both time points). **c.** Quantification of lipid turnover in PCB Nf1 RNAi and controls. Radioactivity measured as counts per minute (CPM) in a scintillator and normalized to number of flies. At time point zero hours (0 h) experimental **p = 0.001 re: UAS-Nf1 RNAi/+, ** p = 0.003 re: PCB-Gal4/+. At 48 hours (48 h) experimental p = 0.016 re: UAS-Nf1 RNAi, p = 0.079 re: PCB-Gal4/+. ns: not significant, (Sidak, two-sided; n = 12 at 0 hour, n = 14 at 48 hours). **d.** Radioactivity measured as CPM and normalized to fly weight (mg). Data repeated from Figure 5g and from panel c here. At time point zero hours (0 h) experimental **p = 0.010 re: UAS-Nf1 RNAi/+, ** p = 0.006 re: PCB-Gal4/+. At 48 hours (48 h) experimental p = 0.129 re: UAS-Nf1 RNAi, p = 0.252 re: PCB-Gal4/+. ns: not significant, (Sidak, two-sided; n = 12 at 0 hour, n = 14 at 48 hours). Each 'n' represents a sample containing twenty animals. Box plots – box: 1st to 3rd quartiles, median: line, whiskers: min-max, individual data points: circles.