# Supporting Information

## Sudhakaran et al. 10.1073/pnas.1309543111

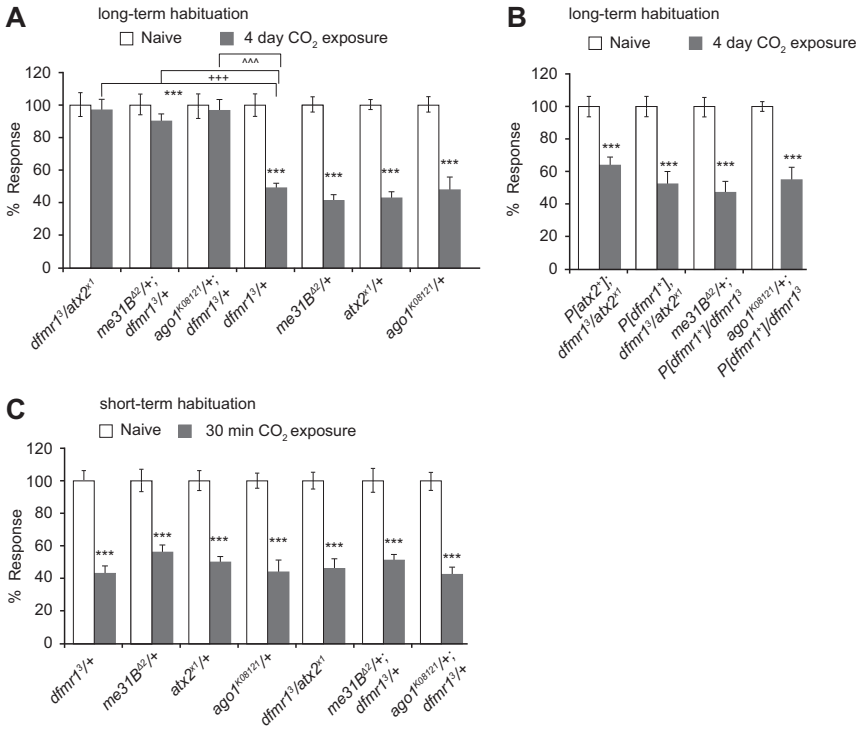


**Fig. S1.** dFmr1 interacts with Atx2, Me31B, and Ago1 for long-term habituation (LTH) to $CO_2$. (*A*) Compared with dFmr1 in heterozygous condition (*dfmr1*$^3$/+), transheterozygote flies for *dfmr1*$^3$/*atx2*$^{x1}$ ($q = 10.654$, ***$P < 0.001$), *me31B*$^{\Delta 2}$/+ ;*dfmr1*$^3$/+ ($q = 9.653$, $^{+++}P < 0.001$), and *ago1*$^{K08121}$/+ ;*dfmr1*$^3$/+ ($q = 7.365$, $^{\wedge\wedge\wedge}P < 0.001$) show a defect in LTH formation after 4-d exposure to $CO_2$ (gray bars). Flies heterozygous for Atx2, Me31B, and Ago1 (*atx2*$^{x1}$/+, *me31B*$^{\Delta 2}$/+, and *ago1*$^{K08121}$/+) show normal LTH after exposure to $CO_2$. (*B*) The expression of an Ataxin2 genomic rescue transgene (P[*atx2*+]) (1) restores the LTH defect in *dfmr1*$^3$/*atx2*$^{x1}$ flies, and LTH defects observed in *dfmr1*$^3$/*atx2*$^{x1}$, *me31B*$^{\Delta 2}$/+;*dfmr1*$^3$/+, and *ago1*$^{K08121}$/+ ;*dfmr1*$^3$/+ flies are rescued by the expression of a dFmr1 genomic rescue transgene (P[*dfmr1*+]) (2). (*C*) Transheterozygote *dfmr1*$^3$/*atx2*$^{x1}$, *me31B*$^{\Delta 2}$/+;*dfmr1*$^3$/+, and *ago1*$^{K08121}$/+;*dfmr1*$^3$/+ flies, as well as heterozygous mutations, do not show a defect in short-term habituation (STH); (*A*–*C*) Gray bars indicate $CO_2$-exposed and white bars indicate air-exposed flies. Error bars show ±SEM ($n > 8$ sets). Student *t* test performed (**$P < 0.01$, ***$P < 0.001$) except in *A*, which uses two-way ANOVA.


1. McCann C, et al. (2011) The Ataxin-2 protein is required for microRNA function and synapse-specific long-term olfactory habituation. *Proc Natl Acad Sci USA* 108(36):E655–E662.
2. Bolduc FV, Bell K, Cox H, Broadie KS, Tully T (2008) Excess protein synthesis in *Drosophila* fragile X mutants impairs long-term memory. *Nat Neurosci* 11(10):1143–1145.

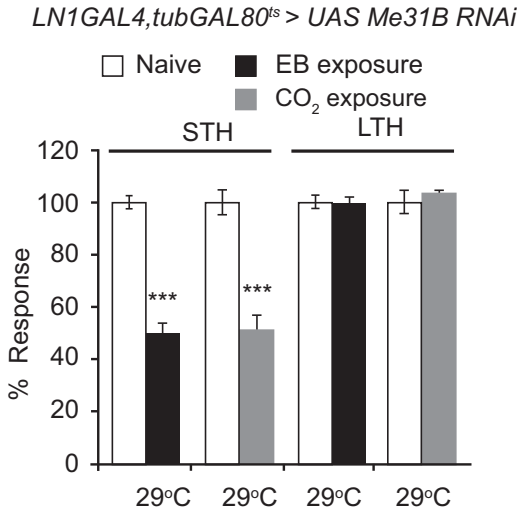


**Fig. S2.** Knockdown of Me31B in local circuit interneurons (LNs) block LTH. Flies exposed to ethyl butyrate (EB) (black bars) or $CO_2$ (gray bars) and paraffin oil or air, respectively (white bars). Adult-specific knockdown of Me31B in LNs shows defects in LTH. For adult-specific knockdown, flies were shifted to 29 °C after eclosion and during odor exposure to specifically knock down Me31B in adults. *LN1-GAL4,tubGAL80$^{ts}$>Me31BRNAi* flies show a defect in LTH to both EB and $CO_2$, whereas they show normal STH. Error bars show ±SEM ($n > 8$ sets). $^{**}P < 0.01$, $^{***}P < 0.001$ (Student *t* test).

**Table S1. Raw values of behavior experiments**

| Genotype | Odorant exposed | Naïve (n) | After exposure (n) |
|---|---|---|---|
| | 5% EB (30 min) | | |
| *CS* | | 0.68 ± 0.02 (10) | 0.33 ± 0.02 (10) |
| *dfmr1$^{B55}$* | | 0.68 ± 0.01 (11) | 0.40 ± 0.03 (11) |
| *dfmr1$^{3}$* | | 0.71 ± 0.05 (8) | 0.25 ± 0.03 (9) |
| *GH14GAL4, tubGAL80 <UASdfmr1RNAi* | at 18 °C | 0.68 ± 0.02 (9) | 0.31 ± 0.02 (10) |
| *GH14GAL4, tubGAL80ts <UASdfmr1RNAi* | at 29 °C | 0.66 ± 0.04 (10) | 0.33 ± 0.02 (10) |
| *dfmr1$^{3}$/+* | | 0.67 ± 0.01 (15) | 0.31 ± 0.02 (15) |
| *me31B $^{\Delta 2}$/+* | | 0.64 ± 0.03 (6) | 0.38 ± 0.04 (6) |
| *atx2$^{x1}$/+* | | 0.65 ± 0.02 (8) | 0.36 ± 0.06 (8) |
| *ago1$^{K08121}$/+* | | 0.68 ± 0.03 (6) | 0.29 ± 0.03 (6) |
| *dfmr1$^{3}$/atx2$^{x1}$* | | 0.71 ± 0.04 (8) | 0.34 ± 0.05 (8) |
| *me31B $^{\Delta 2}$/dfmr1$^{3}$* | | 0.75 ± 0.01 (6) | 0.35 ± 0.02 (6) |
| *ago1$^{K08121}$/dfmr1$^{3}$* | | 0.70 ± 0.03 (8) | 0.33 ± 0.02 (8) |
| *LN1GAL4, tubGAL80ts <UASdfmr1RNAi* | at 29 °C | 0.72 ± 0.04 (9) | 0.38 ± 0.03 (9) |
| *LN1GAL4, tubGAL80ts <UASAtx2RNAi* | at 29 °C | 0.62 ± 0.02 (8) | 0.25 ± 0.03 (8) |
| | 15% $CO_2$ (30 min) | | |
| *CS* | | 0.70 ± 0.02 (8) | 0.36 ± 0.02 (8) |
| *dfmr1$^{B55}$* | | 0.68 ± 0.03 (9) | 0.34 ± 0.02 (9) |
| *dfmr1$^{3}$* | | 0.69 ± 0.03 (10) | 0.31 ± 0.03 (10) |
| *LN1GAL4,tubGAL80ts <UASdFmr1RNAi* | at 29 °C | 0.67 ± 0.04 (8) | 0.43 ± 0.03 (8) |
| *LN1GAL4,tubGAL80ts <UASAtx2RNAi* | at 29 °C | 0.59 ± 0.01 (11) | 0.21 ± 0.03 (11) |
| | 20% EB (4 d) | | |
| *CS* | | 0.65 ± 0.03 (9) | 0.31 ± 0.03 (9) |
| *dfmr1$^{B55}$* | | 0.66 ± 0.01 (9) | 0.61 ± 0.03 (9) |
| *dfmr1$^{3}$* | | 0.66 ± 0.03 (9) | 0.59 ± 0.03 (9) |
| *P[dfmr1$^{+}$],dfmr1$^{3}$* | | 0.69 ± 0.03 (10) | 0.35 ± 0.02 (10) |
| *GH14GAL4, tubGAL80ts <UASdFmr1RNAi* | at 18 °C | 0.65 ± 0.02 (11) | 0.38 ± 0.02 (11) |
| *GH14GAL4, tubGAL80ts <UASdFmr1RNAi* | at 29 °C | 0.70 ± 0.02 (11) | 0.67 ± 0.02 (11) |
| *dfmr1$^{3}$/atx2$^{x1}$* | | 0.68 ± 0.02 (12) | 0.64 ± 0.02 (11) |
| *me31B $^{\Delta 2}$/dfmr1$^{3}$* | | 0.70 ± 0.02 (12) | 0.60 ± 0.01 (12) |
| *ago1$^{K08121}$/dfmr1$^{3}$* | | 0.63 ± 0.04 (9) | 0.55 ± 0.01 (9) |
| *dfmr1$^{3}$/+* | | 0.64 ± 0.01 (15) | 0.32 ± 0.01 (15) |
| *me31B $^{\Delta 2}$/+* | | 0.56 ± 0.02 (9) | 0.32 ± 0.02 (9) |
| *atx2$^{x1}$/+* | | 0.66 ± 0.04 (10) | 0.30 ± 0.02 (10) |
| *ago1$^{K08121}$/+* | | 0.65 ± 0.02 (9) | 0.32 ± 0.05 (9) |
| *P[Atx2$^{+}$]; dfmr1$^{3}$/atx2$^{x1}$* | | 0.68 ± 0.02 (10) | 0.37 ± 0.01 (10) |
| *P[dfmr1$^{+}$],dfmr1$^{3}$/atx2$^{x1}$* | | 0.70 ± 0.02 (8) | 0.36 ± 0.02 (8) |
| *me31B $^{\Delta 2}$ /+; P[dfmr1$^{+}$]/dfmr1$^{3}$* | | 0.67 ± 0.03 (9) | 0.38 ± 0.03 (9) |
| *ago1$^{K08121}$/+; P[dfmr1$^{+}$]/dfmr1$^{3}$* | | 0.66 ± 0.03 (10) | 0.41 ± 0.03 (10) |
| *LN1GAL4, tubGAL80ts <UASdFmr1RNAi* | at 18 °C | 0.70 ± 0.04 (9) | 0.33 ± 0.03 (9) |
| *LN1GAL4, tubGAL80ts <UASdFmr1RNAi* | at 29 °C | 0.78 ± 0.04 (10) | 0.64 ± 0.02 (10) |
| *LN1GAL4, tubGAL80ts <UASAtx2RNAi* | at 29 °C | 0.62 ± 0.02 (9) | 0.64 ± 0.03 (9) |
| | 5% $CO_2$ (4 d) | | |
| *CS* | | 0.68 ± 0.01(10) | 0.36 ± 0.01 (10) |
| *dfmr1$^{B55}$* | | 0.71 ± 0.03 (10) | 0.69 ± 0.01 (10) |
| *dfmr1$^{3}$* | | 0.72 ± 0.04 (11) | 0.66 ± 0.01 (11) |
| *P[dfmr1$^{+}$],dfmr1$^{3}$* | | 0.71 ± 0.05 (11) | 0.33 ± 0.01 (11) |
| *GH14GAL4, tubGAL80 <UASdFmr1RNAi* | | 0.66 ± 0.01 (10) | 0.31 ± 0.01 (10) |
| *GH14GAL4, tubGAL80ts <UASdFmr1RNAi* | at 18 °C | 0.65 ± 0.01 (9) | 0.36 ± 0.02 (9) |
| *LN1GAL4, tubGAL80ts <UASdFmr1RNAi* | at 29 °C | 0.59 ± 0.01 (8) | 0.27 ± 0.03 (8) |
| *LN1GAL4, tubGAL80ts <UASdFmr1RNAi* | at 18 °C | 0.69 ± 0.03 (10) | 0.60 ± 0.02 (10) |
| *LN1GAL4, tubGAL80ts <UASAtx2RNAi* | at 29 °C | 0.59 ± 0.01 (11) | 0.65 ± 0.03 (11) |