Figure S1

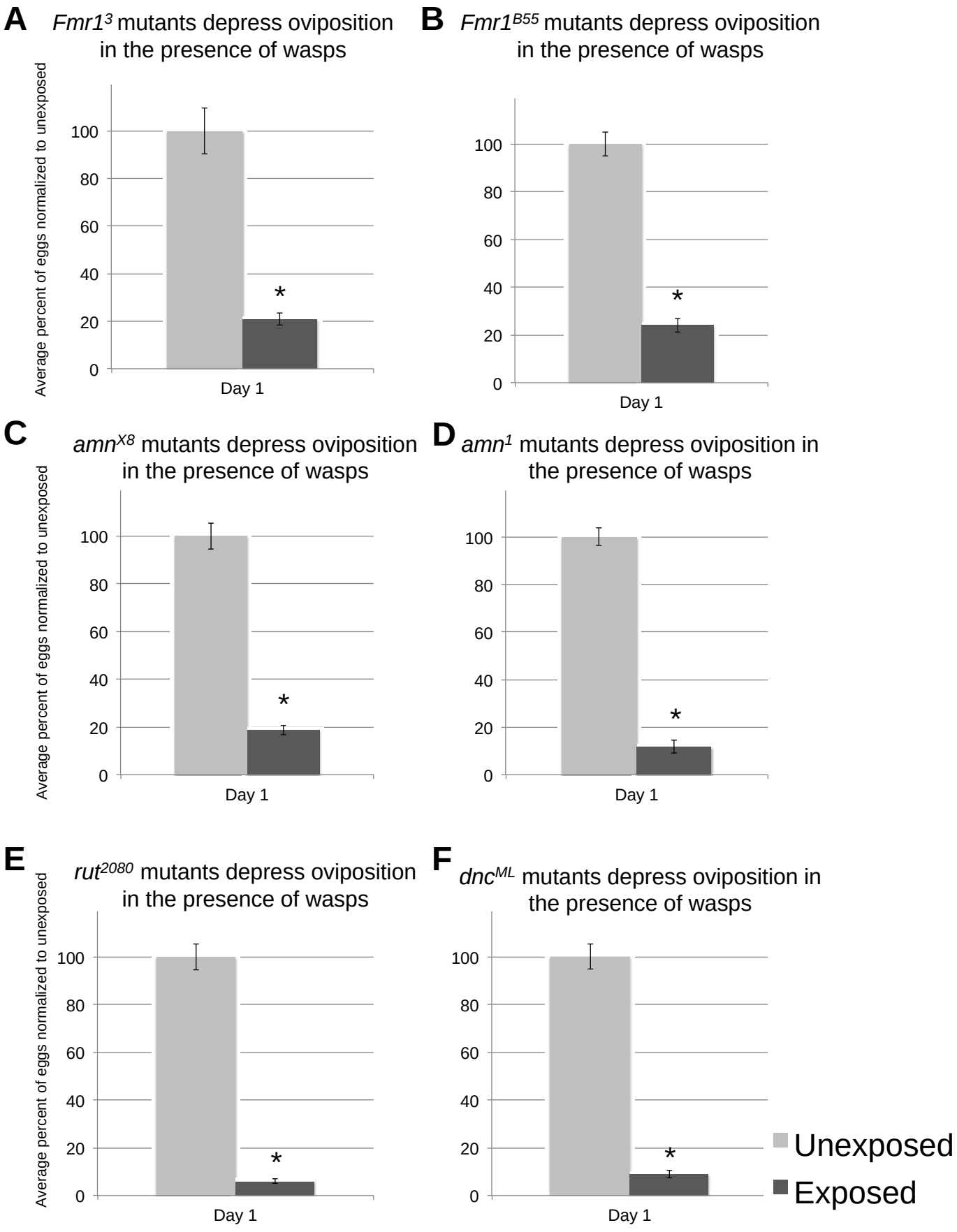
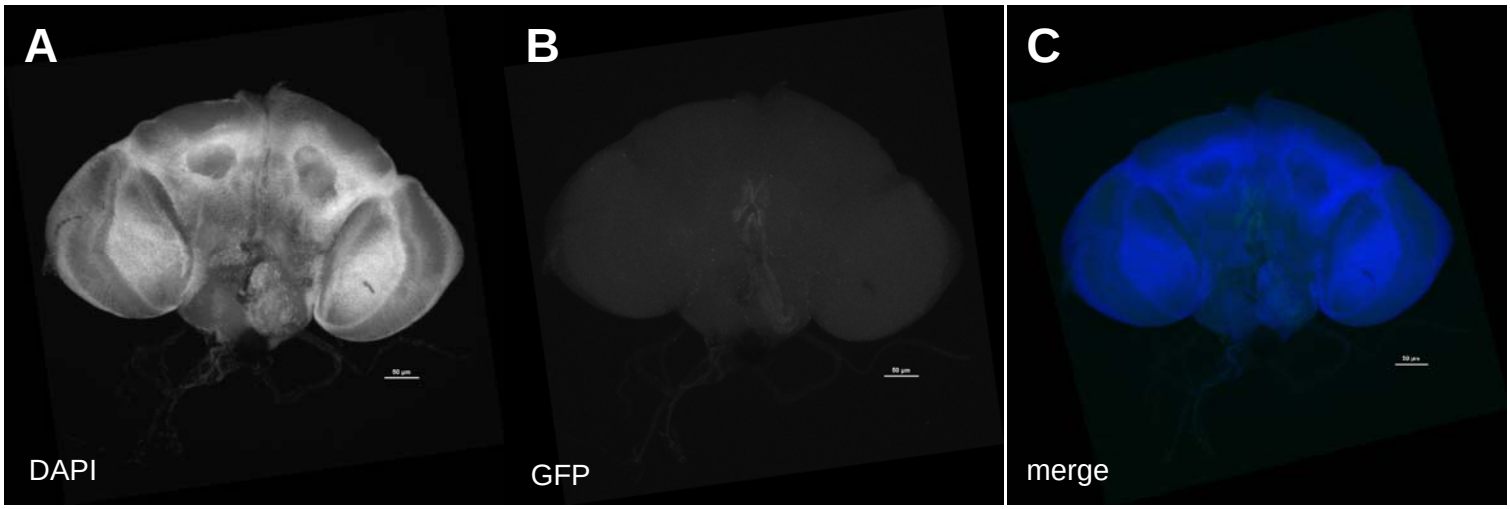

A
Fmr1^3 mutants depress oviposition in the presence of wasps
Average percent of eggs normalized to unexposed
100
80
60
40
20
0
*
Day 1
B
Fmr1^B55 mutants depress oviposition in the presence of wasps
100
80
60
40
20
0
*
Day 1
C
amn^X8 mutants depress oviposition in the presence of wasps
Average percent of eggs normalized to unexposed
100
80
60
40
20
0
*
Day 1
D
amn^1 mutants depress oviposition in the presence of wasps
100
80
60
40
20
0
*
Day 1
E
rut^2080 mutants depress oviposition in the presence of wasps
Average percent of eggs normalized to unexposed
100
80
60
40
20
0
*
Day 1
F
dnc^ML mutants depress oviposition in the presence of wasps
100
80
60
40
20
0
*
Day 1
Unexposed
Exposed

Figure S1 Flies mutant in memory genes are able to respond to predatory wasps through oviposition depression. (**A**-**F**) Percent of eggs laid normalized to unexposed for (**A**) *Fmr1*^{3}, (**B**) *Fmr1*^{B55}, (**C**) *amn*^{X8}, (**D**) *amn*^{1}, (**E**) *rut*^{2080}, and (**F**) *dnc*^{ML}. All error bars represent standard error (n= 24 biological replicates) (*P<0.001).

Figure S2


A
B
C
DAPI
GFP
merge

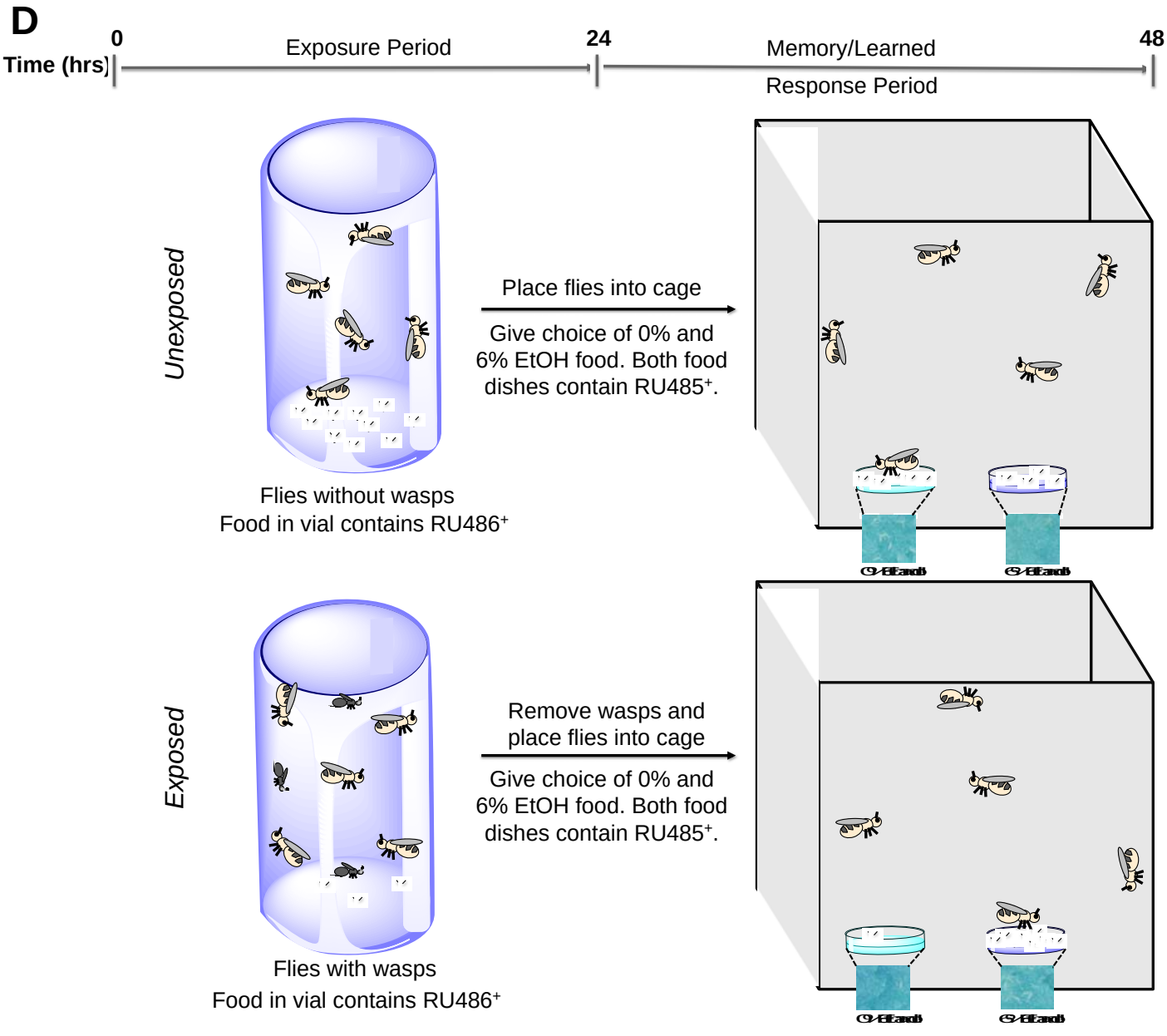

D
Time (hrs)
0
Exposure Period
24
Memory/Learned
Response Period
48
Unexposed
Flies without wasps
Food in vial contains RU486+
Place flies into cage
Give choice of 0% and 6% EtOH food. Both food dishes contain RU485+.
Exposed
Flies with wasps
Food in vial contains RU486+
Remove wasps and place flies into cage
Give choice of 0% and 6% EtOH food. Both food dishes contain RU485+.

Figure S2 Experimental design for MB Gene-Switch Experiments and control brains. Brains from flies carrying transgenes for the Gene-Switch construct in the mushroom body along with a GFP nls showing (**A**) DAPI, (**B**) GFP expression, and (**C**) the merged image. Note that images in (**A**-**C**) illustrate a control brain in which RU486 was not included in the food. See Figure 5 (**A**-**C**) for comparison of GFP-labeled mushroom body after RU486 feeding. (**D**) Diagram of the standard oviposition preference memory setup when using RU486 feeding.

Figure S3

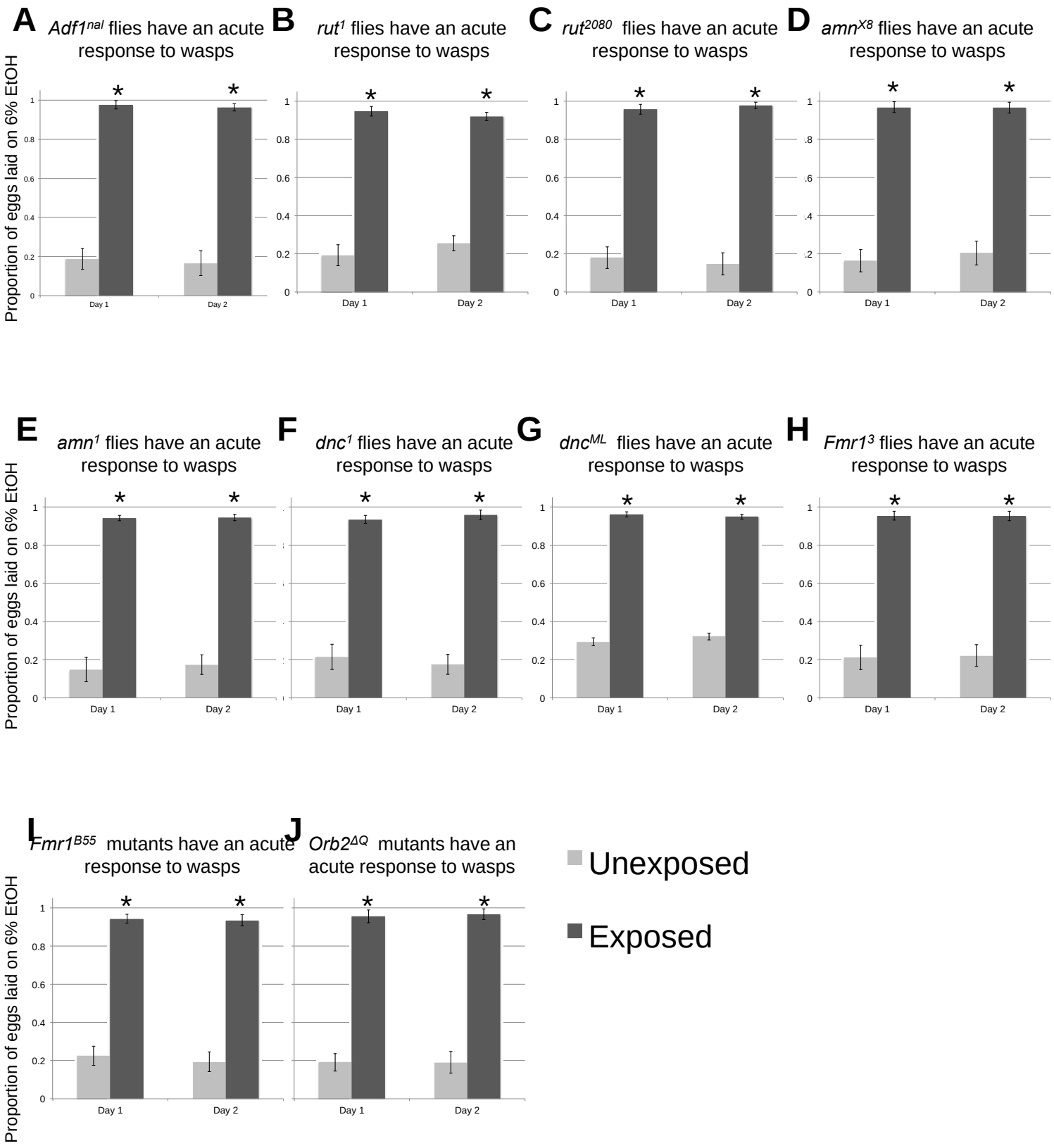

A
Adf1nal flies have an acute response to wasps
B
rut1 flies have an acute response to wasps
C
rut2080 flies have an acute response to wasps
D
amnX8 flies have an acute response to wasps
E
amn1 flies have an acute response to wasps
F
dnc1 flies have an acute response to wasps
G
dncML flies have an acute response to wasps
H
Fmr13 flies have an acute response to wasps
I
Fmr1B55 mutants have an acute response to wasps
J
Orb2ΔQ mutants have an acute response to wasps
Proportion of eggs laid on 6% EtOH
Day 1
Day 2
Unexposed
Exposed

Figure S3 Further evidence demonstrating the role of LTM in the fly-wasp memory paradigm. Proportion of eggs laid on the 6% ethanol oviposition dish during constant wasp exposure for (**A**) *Adf1*^{nal}, (**B**) *rut*^{1}, (**C**) *rut*^{2080}, (**D**) *amn*^{X8}, (**E**) *amn*^{X1}, (**F**) *dnc*^{1}, (**G**) *dnc*^{ML}, (**H**) *Fmr1*^{3}, (**I**) *Fmr1*^{B55}, and (**J**) *Orb2*$^{\Delta Q}$. Error bars represent 95% confidence intervals. (*n*= 10 biological replicates) (* P < 0.001). Diagram of the oviposition preference setup using five flies with constant exposure to wasps in Figure 6B.

Figure S4

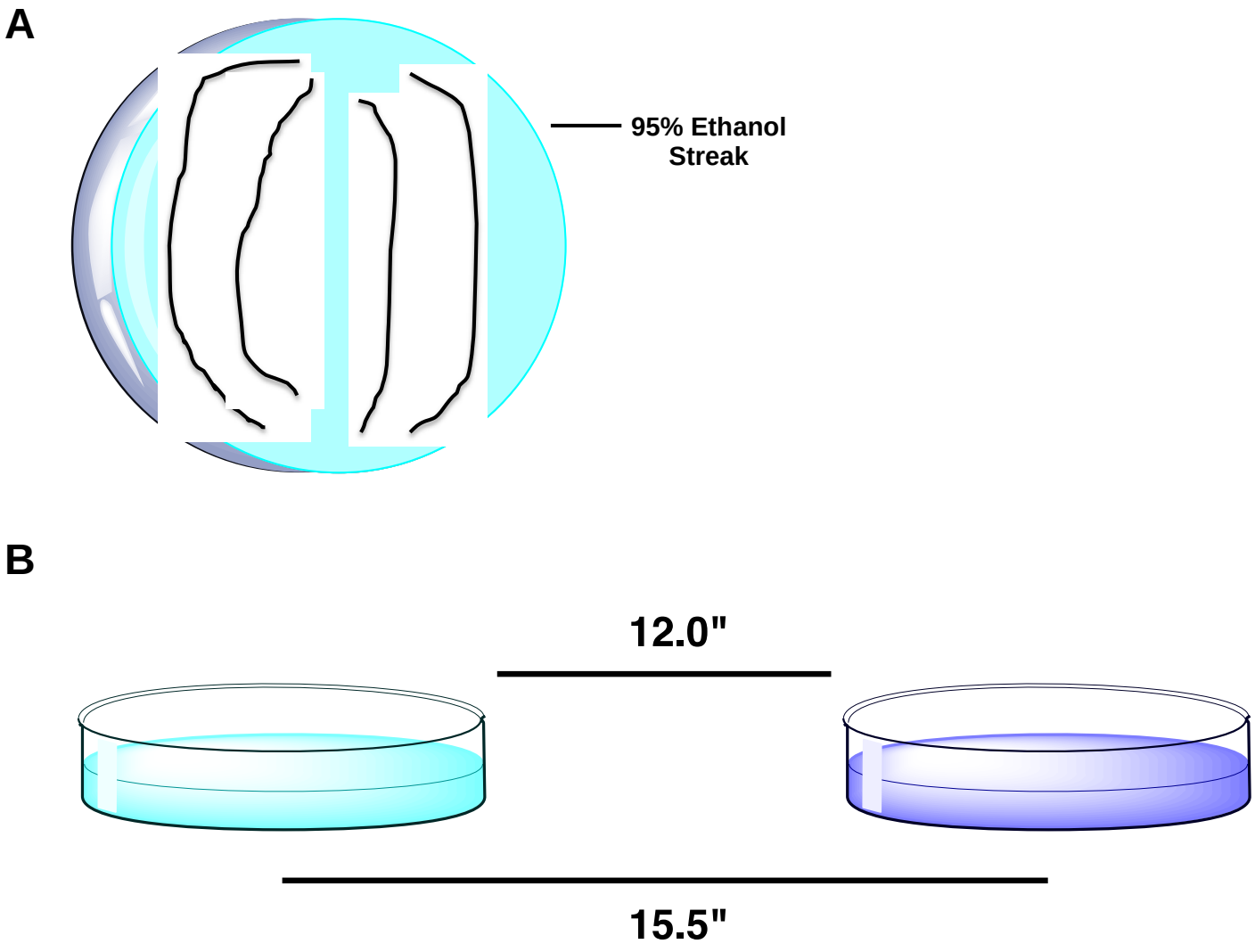
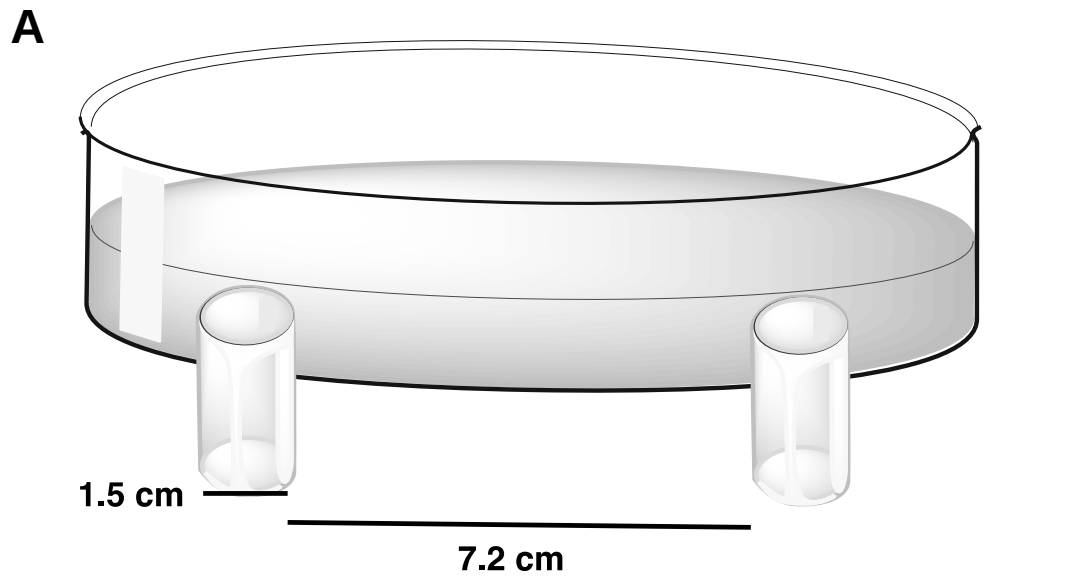
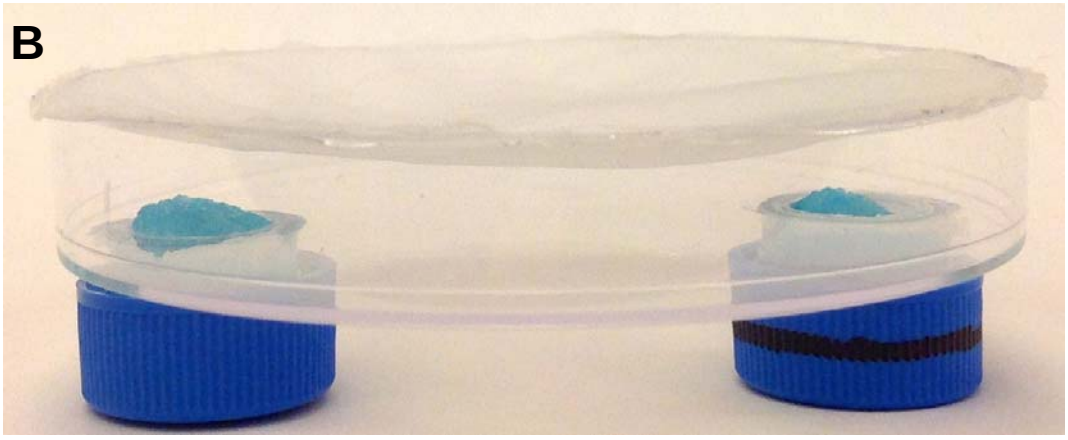


Figure S4 Field cage preparation and measurements. (**A**) Sample Petri dish demonstrating how to streak 95% ethanol over instant food already hydrated with ddH_2O. (**B**) Two Petri dishes positioned as if they are in the field cage, showing distance between plates.

Figure S5





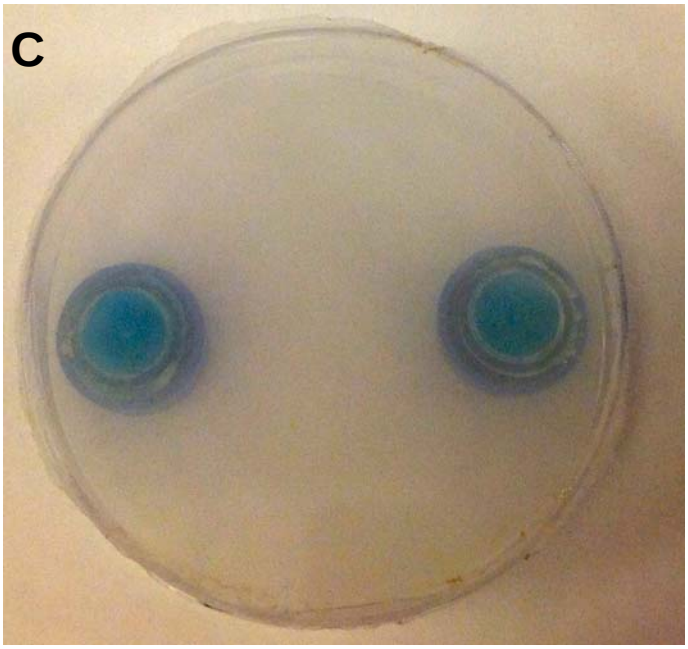


Figure S5 Fly Corral preparation and measurements. (**A**) Sample Fly Corral cage showing distances between caps and diameter of caps. (**B**) Lateral view of Fly Corral. (**C**) Dorsal view of Fly Corral.

Table S1 Statistical analyses for main figures. Welch's two-tailed t-tests were performed for all egg count data. P-values reported were calculated for comparisons between paired treatment-group and unexposed.

Corresponding figure	Comparison groups	Sample size	p-value	Statistical test
1B	CS (0-24) exposed vs unexposed	4	1.34E-05	t-test
1B	CS (24-48) exposed vs unexposed	4	1.15E-03	t-test
1B	OreR (0-24) exposed vs unexposed	4	6.19E-04	t-test
1B	OreR (24-48) exposed vs unexposed	4	3.21E-06	t-test
1B	w1118 (0-24) exposed vs unexposed	4	1.93E-04	t-test
1B	w1118 (24-48) exposed vs unexposed	4	2.42E-04	t-test
1C	CS (0-24) 2.5 hr exposed vs unexposed	4	5.10E-01	t-test
1C	CS (24-48) 2.5 hr exposed vs unexposed	4	1.70E-01	t-test
1C	CS (0-24) 7 hr exposed vs unexposed	4	6.65E-02	t-test
1C	CS (24-48) 7 hr exposed vs unexposed	4	3.76E-01	t-test
1C	CS (0-24) 14 hr exposed vs unexposed	4	5.35E-05	t-test
1C	CS (24-48) 14 hr exposed vs unexposed	4	9.98E-04	t-test
1C	CS (0-24) 24 hr exposed vs unexposed	4	1.34E-05	t-test
1C	CS (24-48) 24 hr exposed vs unexposed	4	1.15E-03	t-test
2A	Adf1[nal] (0-24) exposed vs unexposed	4	5.65E-01	t-test
2A	Adf1 [nal] (24-48) exposed vs unexposed	4	9.93E-01	t-test
2B	rut[1] (0-24) exposed vs unexposed	4	6.51E-01	t-test
2B	rut [1] (24-48) exposed vs unexposed	4	5.30E-01	t-test
2C	rut[2080] (0-24) exposed vs unexposed	4	7.95E-01	t-test
2C	rut[2080] (24-48) exposed vs unexposed	4	2.53E-01	t-test
2D	amn[X8] (0-24) exposed vs unexposed	4	2.30E-01	t-test
2D	amn [X8] (24-48) exposed vs unexposed	4	1.01E-01	t-test
2E	amn[1] (0-24) exposed vs unexposed	4	2.24E-01	t-test
2E	amn[1] (24-48) exposed vs unexposed	4	7.51E-01	t-test
2F	dnc[1] (0-24) exposed vs unexposed	4	4.83E-01	t-test
2F	dnc[1] (24-48) exposed vs unexposed	4	9.90E-01	t-test
2G	dnc[ML] (0-24) exposed vs unexposed	4	3.93E-01	t-test
2G	dnc[ML] (24-48) exposed vs unexposed	4	1.10E-01	t-test
2H	FMR1[3] (0-24) exposed vs unexposed	4	3.22E-01	t-test
2H	FMR1[3] (24-48) exposed vs unexposed	4	2.36E-01	t-test
2I	FMR1[B55] (0-24) exposed vs unexposed	4	5.25E-01	t-test
2I	FMR1[B55] (24-48) exposed vs unexposed	4	3.24E-01	t-test
2J	Orb2[ΔQ] (0-24) exposed vs unexposed	4	4.00E-01	t-test
2J	Orb2[ΔQ] (24-48) exposed vs unexposed	4	6.14E-01	t-test
2K	Orb2[ΔQ] (0-24) constant exposure vs unexposed	4	6.84E-05	t-test
2K	Orb2[ΔQ] (24-48) constant exposure vs unexposed	4	3.91E-04	t-test
3A	TeTx (0-24) exposed vs unexposed	4	7.18E-01	t-test
3A	TeTx (24-48) exposed vs unexposed	4	6.78E-02	t-test
3B	TeTx (0-24) constant exposure vs unexposed	4	5.54E-03	t-test
3B	TeTx (24-48) constant exposure vs unexposed	4	6.75E-07	t-test
3C	OK107 GAL4 (0-24) exposed vs unexposed	4	6.67E-04	t-test
3C	OK107 GAL4 (24-48) exposed vs unexposed	4	8.10E-04	t-test
3D	UAS TeTx (0-24) exposed vs unexposed	4	1.71E-04	t-test
3D	UAS TeTx (24-48) exposed vs unexposed	4	1.99E-06	t-test
4A	Orb2 [RNAi] (0-24) exposed vs unexposed	4	3.48E-01	t-test
4A	Orb2 [RNAi] (24-48) exposed vs unexposed	4	7.25E-01	t-test
4C	Orb2 [RNAi] (0-24) constant exposure vs unexposed	4	9.21E-04	t-test
4C	Orb2 [RNAi] (24-48) constant exposure vs unexposed	4	1.96E-04	t-test
4D	UAS Orb2 [RNAi] (0-24) exposed vs unexposed	4	1.52E-04	t-test
4D	UAS Orb2 [RNAi] (24-48) exposed vs unexposed	4	6.06E-05	t-test
5E	MB switch ; Orb2 [RNAi] RU486+ (0-24) exposed vs unexposed	4	4.59E-01	t-test
5E	MB switch ; Orb2 [RNAi] RU486+ (24-48) exposed vs unexposed	4	7.22E-01	t-test
5F	MB switch ; Orb2 [RNAi] RU486- (0-24) exposed vs unexposed	4	3.14E-03	t-test
5F	MB switch ; Orb2 [RNAi] RU486 (24-48) exposed vs unexposed	4	6.33E-03	t-test
5G	MB switch ; Orb2 [RNAi] RU486+ (0-24) constant exposure vs unexposed	4	4.73E-07	t-test
5G	MB switch ; Orb2 [RNAi] RU486+ (24-48) constant exposure vs unexposed	4	1.34E-04	t-test
5H	MB switch RU486+ (0-24) exposed vs unexposed	4	5.57E-06	t-test
5H	MB switch RU486+ (24-48) exposed vs unexposed	4	3.63E-04	t-test
5I	MB switch RU486- (0-24) exposed vs unexposed	4	9.91E-04	t-test
5I	MB switch RU486- (24-48) exposed vs unexposed	4	1.27E-03	t-test
6C	Single CS (0-24) exposed vs unexposed	10	6.31E-08	t-test
6C	Single CS (24-48) exposed vs unexposed	10	5.83E-07	t-test
6D	Five CS (0-24) exposed vs unexposed	10	7.85E-07	t-test
6D	Five CS (24-48) exposed vs unexposed	10	1.67E-06	t-test
6E	Canton-S (0-24) constant exposure vs unexposed	10	7.04E-13	t-test
6E	Canton-S (24-48) constant exposure vs unexposed	10	1.80E-13	t-test

Table S 2

Coresponding figure	Comparison groups	Sample size	p-value	Statistical test
S1A	Fmr1[3] (0-24) exposed vs unexposed	24	1.93E-08	t-test
S1B	Fmr1[B55] (0-24) exposed vs unexposed	24	1.31E-15	t-test
S1C	amn[X8] (0-24) exposed vs unexposed	24	3.87E-14	t-test
S1D	amn[1] (0-24) exposed vs unexposed	24	1.78E-22	t-test
S1E	rut[2080] (0-24) exposed vs unexposed	24	4.61E-15	t-test
S1F	dnc[mL] (0-24) exposed vs unexposed	24	4.12E-16	t-test
S3A	Adf1[nal] (0-24) constant exposure vs unexposed	10	8.10E-12	t-test
S3A	Adf1[nal] (24-48) constant exposure vs unexposed	10	2.23E-10	t-test
S3B	rut[1] (0-24) constant exposure vs unexposed	10	7.50E-12	t-test
S3B	rut[1] (24-48) constant exposure vs unexposed	10	6.79E-14	t-test
S3C	rut[2080] (0-24) constant exposure vs unexposed	10	4.68E-12	t-test
S3C	rut[2080] (24-48) constant exposure vs unexposed	10	5.04E-11	t-test
S3D	amn[X8] (0-24) constant exposure vs unexposed	10	3.00E-12	t-test
S3D	amn[X8] (24-48) constant exposure vs unexposed	10	3.71E-11	t-test
S3E	amn[1] (0-24) constant exposure vs unexposed	10	4.74E-10	t-test
S3E	amn[1] (24-48) constant exposure vs unexposed	10	1.94E-11	t-test
S3F	dnc[1] (0-24) constant exposure vs unexposed	10	8.51E-10	t-test
S3F	dnc[1] (24-48) constant exposure vs unexposed	10	9.05E-13	t-test
S3G	dnc[ML] (0-24) constant exposure vs unexposed	10	4.26E-19	t-test
S3G	dnc[ML] (24-48) constant exposure vs unexposed	10	6.00E-20	t-test
S3H	FMR1[3] (0-24) constant exposure vs unexposed	10	1.71E-10	t-test
S3H	FMR1[3] (24-48) constant exposure vs unexposed	10	2.21E-11	t-test
S3I	FMR1[B55] (0-24) constant exposure vs unexposed	10	2.33E-12	t-test
S3I	FMR1[B55] (24-48) constant exposure vs unexposed	10	8.49E-13	t-test
S3J	Orb2[ΔQ] (0-24) constant exposure vs unexposed	10	9.94E-15	t-test
S3J	Orb2[ΔQ] (24-48) constant exposure vs unexposed	10	4.53E-12	t-test

Table S3 Fly genotypes used in this study.

Gene/Allele Name	Genotype	Acquisition Location	ID #
dnc^{1}	dnc[1]	Griffith Lab	6020
rut^{1}	rut[1]	Griffith Lab	9404
Orb2$^{\Delta Q}$	*Orb2*$^{\Delta Q}$	*Keleman et al., 2007*	*N/A*
Adf1^{nal}	*Adf1*^{nal}	*DeZazzo et al., 2000*	*N/A*
UAS-TeTx	wg^{1} w[*]; P{w[+mC]=UAS-TeTxLC.tnt}G2	Bloomington Stock Center	28838
OK-107	w[*]; P{w[+mW.hs]=GawB}ey[OK107]/In(4)ci[D], ci[D] pan[ciD] sv[spa-pol]	Bloomington Stock Center	854
amn^{1}	amn[1]	Griffith Lab	5954
dnc^{ML}	y[1] w[1] dnc[ML] f[36a]/FM7a	Griffith Lab	9407
rut^{2080}	P{ry[+t7.2]=lArB}rut[2080]; P{w[+mC]=UAS-rut.Z}2	Griffith Lab	9405
amn^{X8}	amn[X8]	Griffith Lab	N/A
FMR1^{3}	Fmr1[3]	-	N/A
FMR1^{B55}	w[*]; Fmr1[B55]	Bloomington Stock Center	109026
UAS-Orb2^{RNAi}	y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02376}attP2	Bloomington Stock Center	27050
MB Gene-Switch	P{MB-Switch}	Greg Roman	N/A