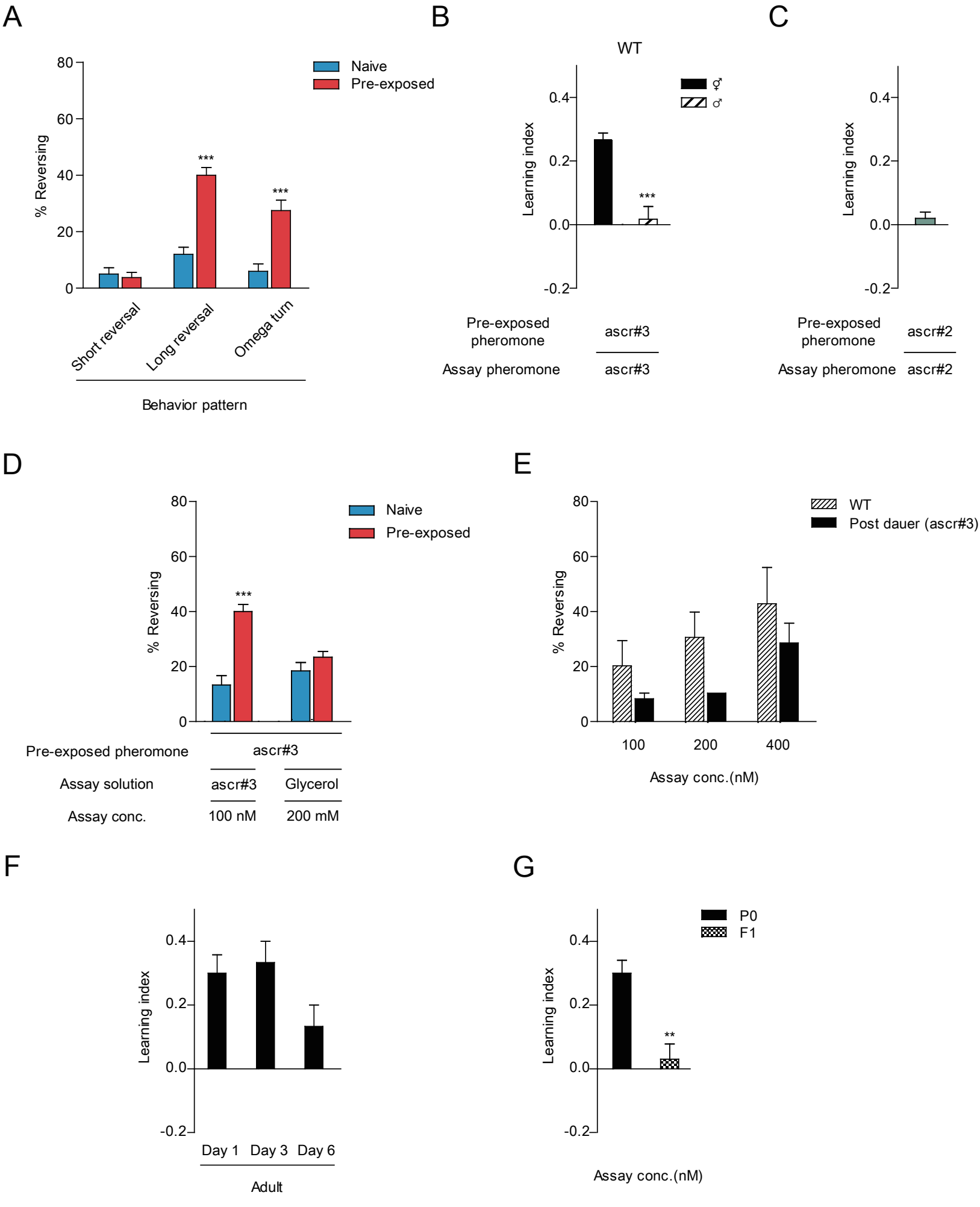
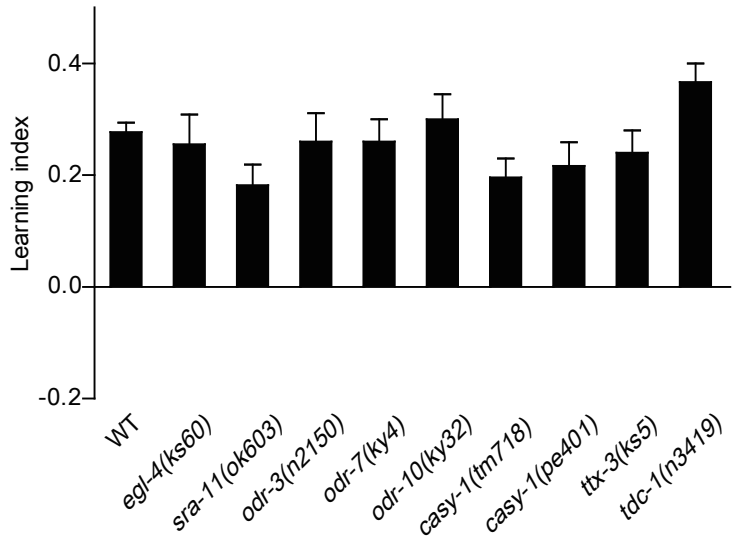
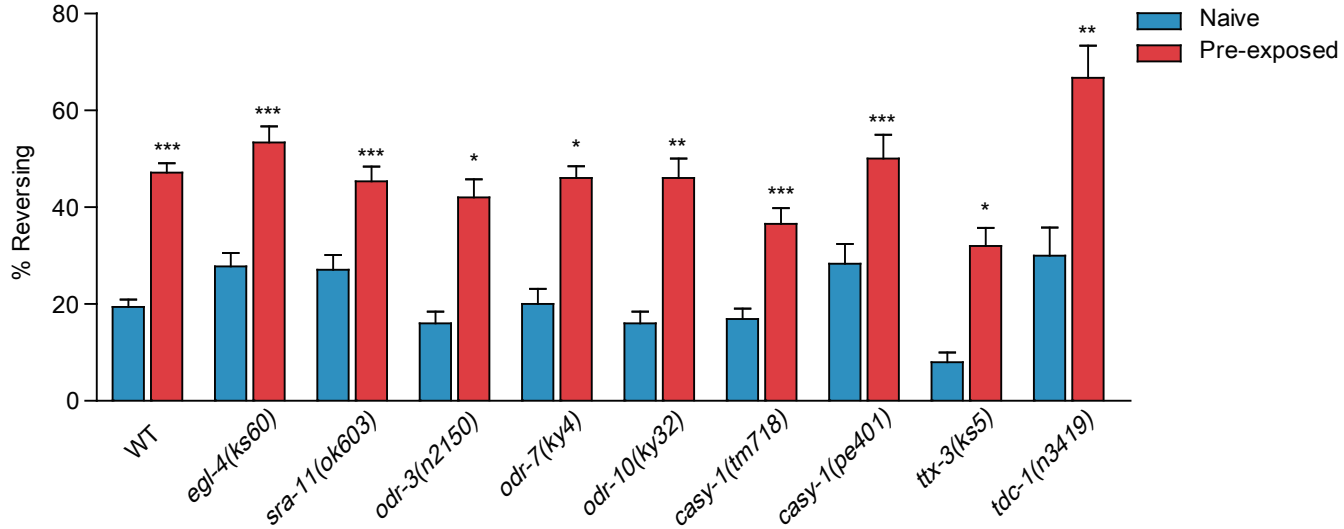

A
Naive
Pre-exposed
% Reversing
Short reversal
Long reversal
Omega turn
Behavior pattern
B
WT
♀
♂
Learning index
Pre-exposed pheromone
ascr#3
Assay pheromone
ascr#3
C
Learning index
Pre-exposed pheromone
ascr#2
Assay pheromone
ascr#2
D
Naive
Pre-exposed
% Reversing
Pre-exposed pheromone
ascr#3
Assay solution
ascr#3
Glycerol
Assay conc.
100 nM
200 mM
E
WT
Post dauer (ascr#3)
% Reversing
Assay conc.(nM)
F
Learning index
Day 1
Day 3
Day 6
Adult
G
P0
F1
Learning index
Assay conc.(nM)

Figure S1. Characterization of ascr#3 imprinting. ***Related to Figure 1.***
(A) Percentage of reversal of naive and pre-exposed adult animals performing short reversal, long reversal, or omega turn (See STAR methods). *** indicates different from naive at $p<0.001$ by one-way ANOVA with Bonferroni's post hoc test. n=80-100 each. Error bars represent SEM. **(B)** Learning index of males. *** indicates different from hermaphrodites at $p<0.001$ by Student's t-test. n=60. Error bars represent SEM. **(C)** Learning index of adult animals that are pre-exposed to, and assayed with ascr#2. n=50 each. Error bars represent SEM. **(D)** Percentage of reversal of adult animals that are pre-exposed to ascr#3 and assayed with 200 mM glycerol. *** indicates different from naive at 0.001 by one-way ANOVA with Bonferroni's post hoc test. n=60. Error bars represent SEM. **(E)** Percentage of reversal of wild-type post-dauer animals. The adults are recovered from the dauer stage induced by 600 nM ascr#3 exposure at 25℃. n=30-40 each. Error bars represent SEM. **(F)** Learning index of 1, 3, or 6 day-old adults. n=30 each. Error bars represent SEM. **(G)** Learning index of F1 progeny of ascr#3 imprinted animals imprinting. ** indicates different from P0 at $p<0.01$ by Student's t-test. n=40 each. Error bars represent SEM.

A


Learning index
0.4
0.2
0.0
-0.2
WT
egl-4(ks60)
sra-11(ok603)
odr-3(n2150)
odr-7(ky4)
odr-10(ky32)
casy-1(tm718)
casy-1(pe401)
ttx-3(ks5)
tdc-1(n3419)


% Reversing
80
60
40
20
0
Naive
Pre-exposed
***
***
***
*
*
**
***
***
*
**
WT
egl-4(ks60)
sra-11(ok603)
odr-3(n2150)
odr-7(ky4)
odr-10(ky32)
casy-1(tm718)
casy-1(pe401)
ttx-3(ks5)
tdc-1(n3419)

B

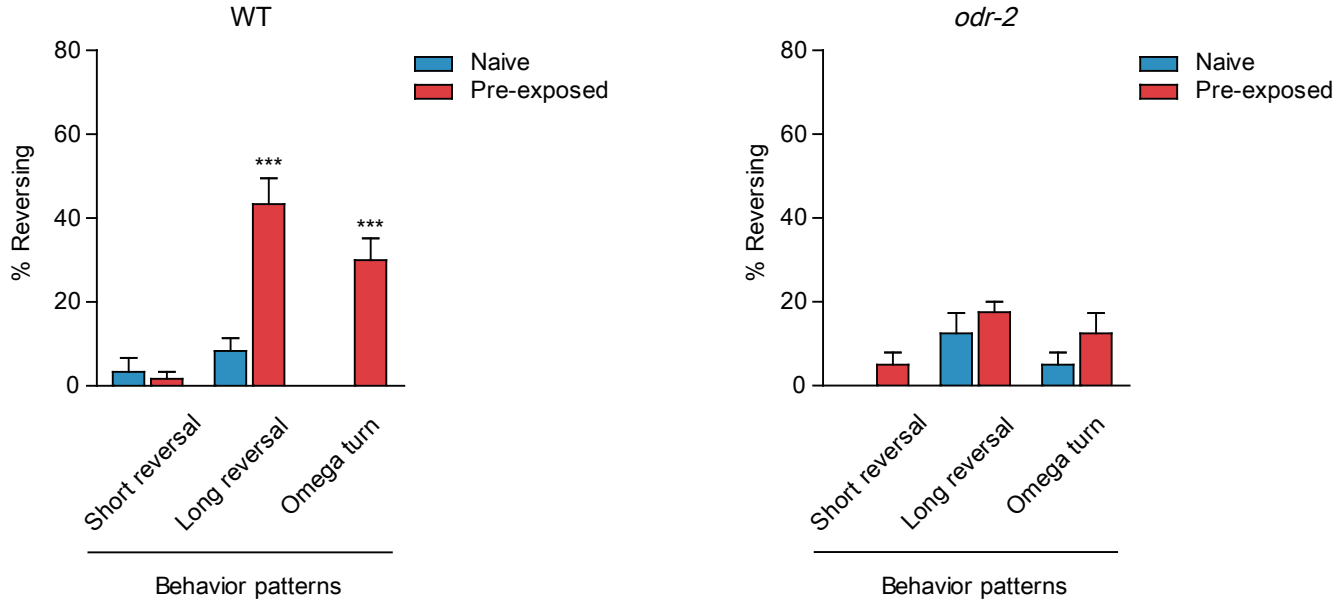

WT
odr-2
% Reversing
80
60
40
20
0
Naive
Pre-exposed
***
***
Short reversal
Long reversal
Omega turn
Behavior patterns

Figure S2. Screen of candidate genes with a potential role in ascr#3 imprinting. ***Related to Figure 2.***
(A) Learning index (top) and percent of reversal (bottom) of *egl-4, sra-11, odr-3, odr-7, odr-10, casy-1, ttx-3,* and *tdc-1* mutant animals. *, ** and *** indicate different from WT at $p<0.05$, 0.01 and 0.001, respectively, by one-way ANOVA with Bonferroni's post hoc test. n=30-260 each. Error bars represent SEM. **(B)** Percentage of reversal of wild-type and *odr-2* mutants performing short reversal, long reversal, or omega turn. *** indicates different from short reversal at $p<0.001$ by one-way ANOVA with Bonferroni's post hoc test. n=40-60 each. Error bars represent SEM.

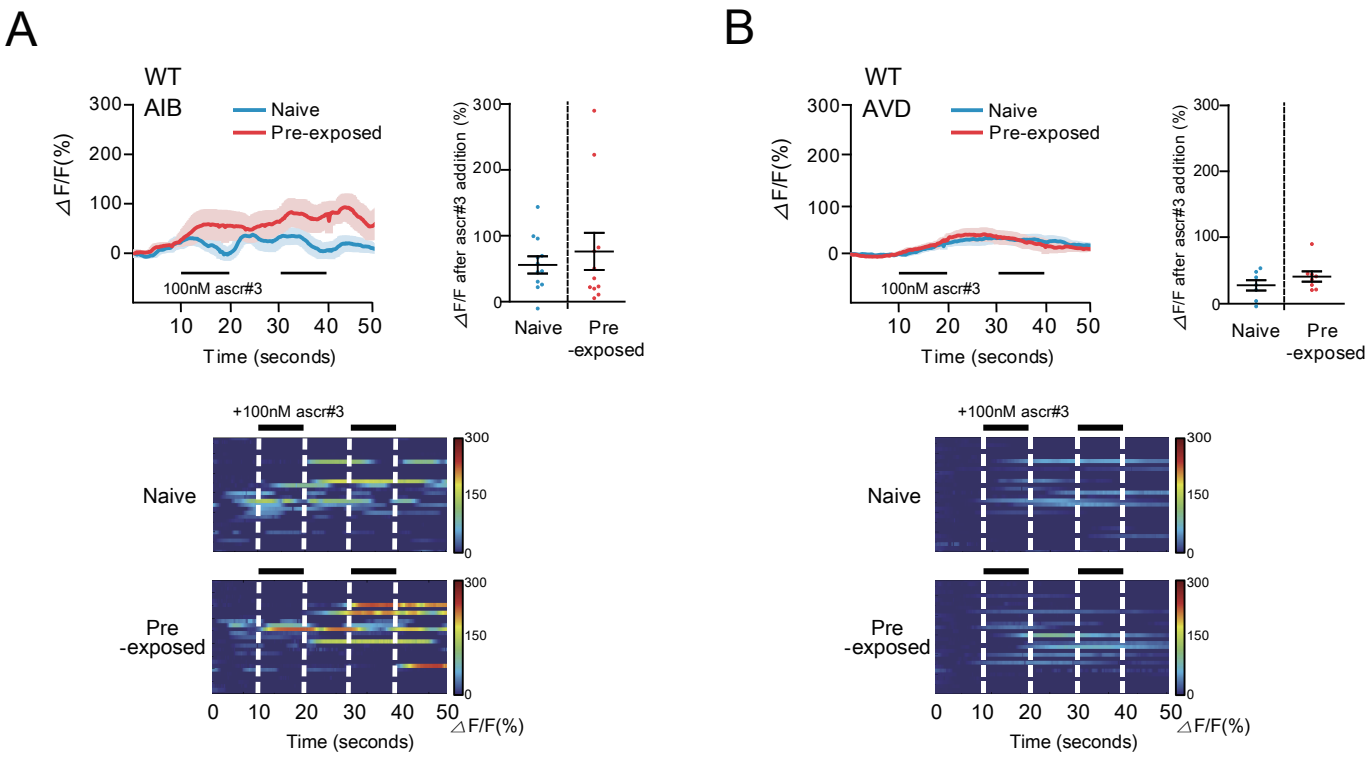


Figure S3. Ca^{2+} transients of the AIB and AVD interneurons in response to ascr#3 exposure. ***Related to Figure 3.***

(A-B) Ca^{2+} transients of AIB (A) and AVD (B) in response to 100 nM ascr#3 exposure. The average traces of Ca^{2+} responses to 100 nM ascr#3 (left), heat map of individual Ca^{2+} responses to ascr#3 (bottom), and the maximum value of Ca^{2+} responses to 100 nM ascr#3 (right) in naive (shown in blue) and pre-exposed animals (shown in red) are shown. n=7-12 (naive) and 8 (pre-exposed) each. Error bars represent SEM.

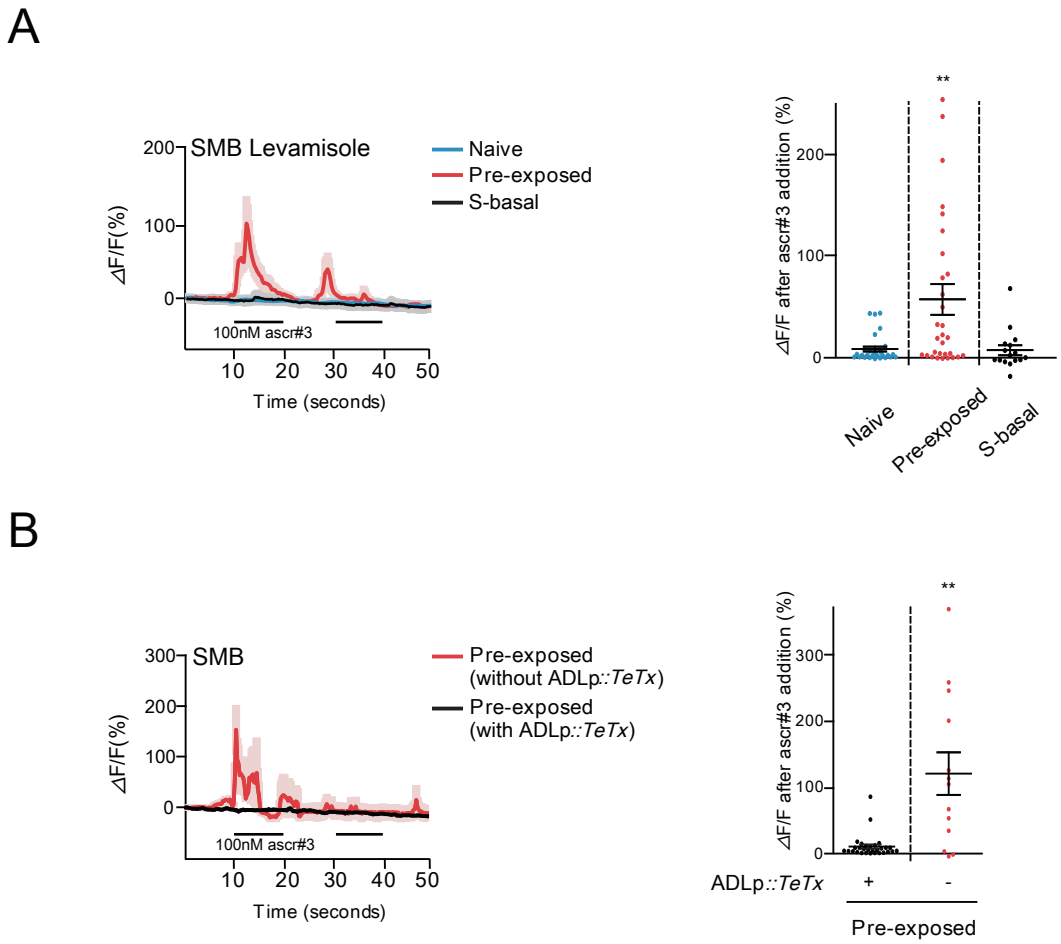


Figure S4. Characterization of the SMB Ca^{2+} responses to ascr#3 exposure. ***Related to Figure 4.***
(A) Ca^{2+} transients of the SMB neurons in response to 100 nM ascr#3 in wild-type animals treated with levamisole. The average traces of Ca^{2+} responses to 100 nM ascr#3 (left) and the maximum of Ca^{2+} responses to 100 nM ascr#3 (right) in naive (shown in blue) and pre-exposed animals exposed to ascr#3 (shown in red) and naive animals exposed to S-basal (shown in black) are shown. ** indicates different from naive at $p<0.01$ by Student's t-test. n=30 (naive and pre-exposed) and 16 (S-basal) each. Error bars represent SEM. **(B)** Ca^{2+} transients in SMB of animals expressing ADLp*::TeTx*. Wild-type animals without or with an ADLp*::TeTx* transgene are shown in red or black, respectively. ** indicates different from pre-exposed ADLp*::TeTx* transgenic animals at $p<0.01$ by Student's t-test. n=13 (pre-exposed without ADLp*::TeTx*) and 30 (pre-exposed with ADLp*::TeTx*). Error bars represent SEM.