Supporting Information

Kim et al. 10.1073/pnas.1716612115

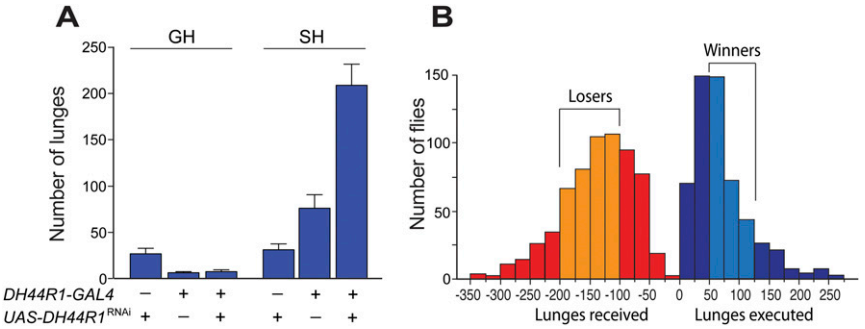

Fig. S1. Generation of winners and losers. (*A*) Levels of aggression of GH or SH *DH44R1* > *DH44R1*^{RNAi} males (24–36). (*B*) Distribution of the number of winner and loser males according to the mean number of lunges received or executed, respectively, during training. Lunges received by losers are given negative value for display purposes.

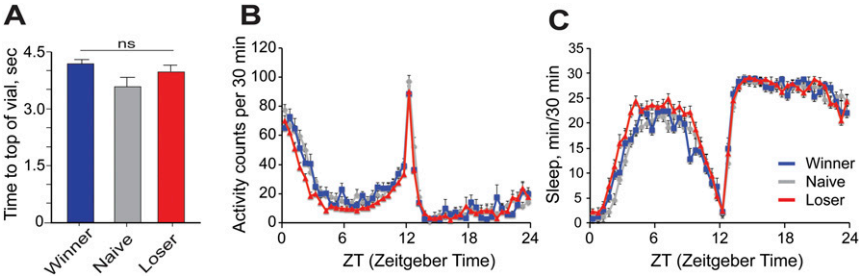

Fig. S2. The winner and loser states were not associated with changes in general locomotion. (*A*) Time to climb to the top of 8-dram plastic vial (ANOVA; $P > 0.05$; $n_{loser} = 19$; $n_{winner} = 13$; $n_{control} = 25$). Total daytime activity (*B*) and sleep (*C*) measured on the first day after introduction into the apparatus, 24 h after training. No differences were observed between naive males, winners, and losers. ANOVA; $P > 0.05$; $n_{loser} = 28$; $n_{winner} = 18$; $n_{control} = 44$). ns, nonsignificant.

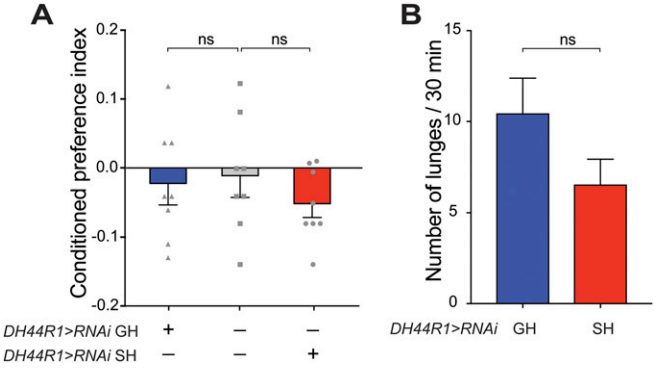

Fig. S3. Pheromonal cues are not sufficient to induce conditioned preference. (*A*) Odor conditioning was carried out with dead *DH44R1* > *DH44R1*^{RNAi} flies that were either GH (hypoaggressive) or SH (hyperaggressive). No significant differences were seen compared with SH naive CS males. Tukey's multiple comparisons test after one-way ANOVA: $P > 0.05$, $n = 8$ for each group. (*B*) Lunges executed by SH naive CS males toward dead GH or SH *DH44R1-GAL4* > *UAS-DH44R1*^{RNAi} (*DH44R1* > *RNAi*) males. No significant differences were observed. Mann–Whitney *U* test: $P > 0.05$, $n = 36$ for each group. ns, nonsignificant.

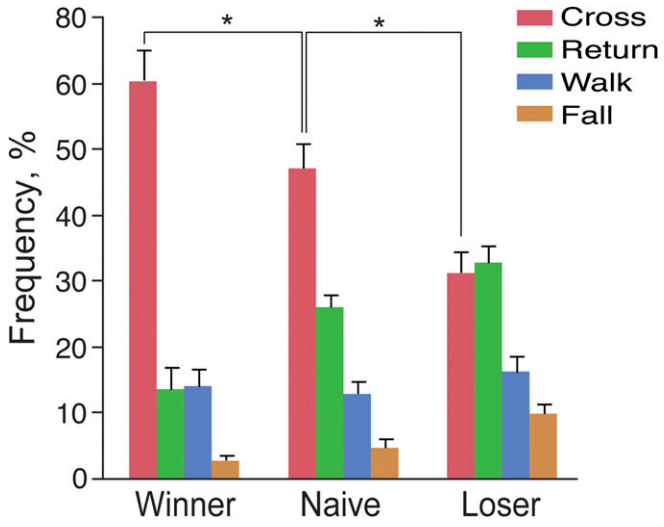

Fig. S4. Details of gap-crossing assay. As described in Fig. 3*B*, winners show enhanced successful crossings of the gap when tested 24 h after training. Conversely, winners show a significantly reduced return frequency. Frequency with which flies walked through or around the gap, or fell was less frequent. Steel–Dwass multiple comparison test, $*P < 0.05$; $n_{loser} = 34$, $n_{winner} = 20$, $n_{naive} = 31$.

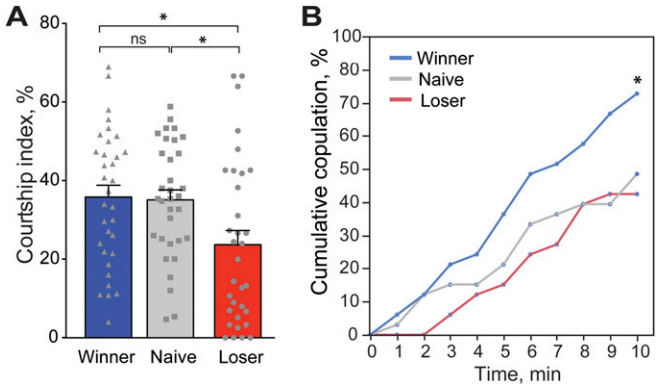

Fig. S5. Courtship assay. (*A*) When paired with wild-type virgins 24 h after the fighting experience, losers displayed less courtship behavior compared with the naive and winner flies. Tukey's multiple comparisons test after one-way ANOVA: $*P < 0.05$, $n = 33$ for each group. (*B*) In contrast, a significantly larger portion of winners compared with losers and naive males achieved copulation at the end of the 10-min courtship assay, as shown in the cumulative copulation curve. χ^2 test: $*P < 0.05$; $n = 33$ for each group. ns, nonsignificant.

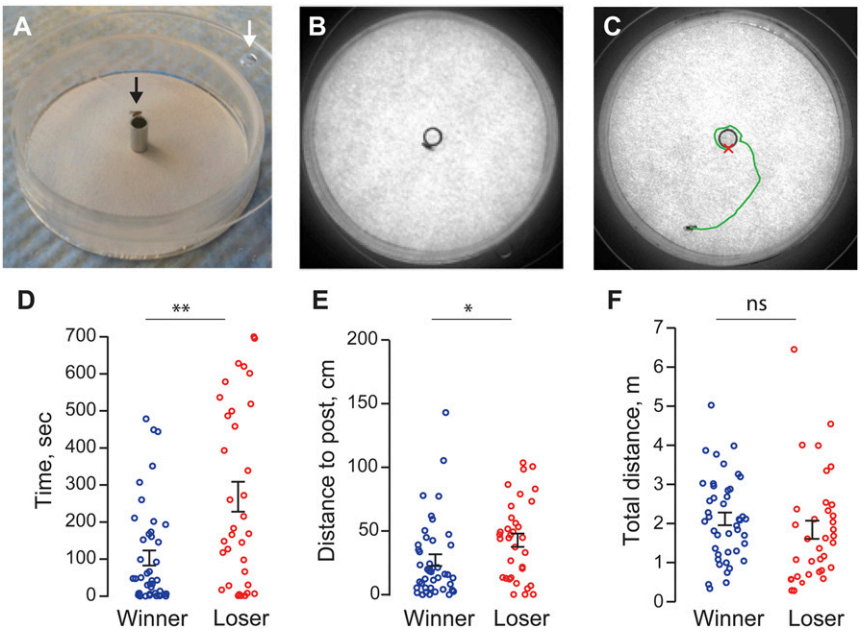

Fig. S6. General exploratory assay. (*A*) Photograph of experimental arena. Black arrow denotes fly perched on 0.8 cm × 0.4 cm post; white arrow indicates entry port slid outside of arena to improve the visibility of the fly's movement while recording and to prevent its escape. (*B*) Single frame from experimental movie illustrates how a hollow post promotes the tracking of fly from top viewing angle. (*C*) First approach (distance to post) is defined as the total movement from the start of an experiment until the fly has climbed the post. Example of first approach (green) to post with the end of the trajectory labeled with an X (red). (*D*) The first approach of winners ($n = 35$) was on average nearly 200 s faster than those for losers ($n = 43$). Two-tailed Mann–Whitney *U*, $^{**}P = 0.0036$. (*E*) The same first approach for winners was also significantly shorter in distance than that for losers. Two-tailed Mann–Whitney *U*, $^{*}P = 0.0171$. (*F*) Total distance traveled throughout experimental trials was comparable among flies with repeat winning and chronic losing experience. Two-tailed Mann–Whitney *U*, $P = 0.1124$. ns, nonsignificant.