



**Figure 1 – Figure supplement 1.** *Drosophila* can discriminate between, but not among short-, medium-, and long-chain fatty acids. Aversive taste memory was measured as described in Figure 1A. The tastants used during training and to assess taste discrimination are reciprocal to those in Figure 1 and were tested at a 1% concentration. **A** The pairing of short-chain valeric acid (5C) and quinine (red) results in a significant reduction in PER compared to naïve flies. After training, PER response to 5C was significantly lower in trained flies compared to naïve flies ( $P < 0.0001$ ), but there was no difference in PER to medium-chain hexanoic acid (6C;  $P = 0.2102$ ). REML:  $F_{1,45} = 15.73$ ,  $P = 0.0003$ , with Sidak's Test for multiple comparisons;  $N = 23-24$ . **B** The pairing of long-chain nonanoic acid (9C) and quinine (red) results in a significant reduction in PER compared to naïve flies. After training, PER response to 9C was significantly lower in trained flies compared to naïve flies ( $P = 0.0031$ ), but there was no difference in PER to medium-chain hexanoic acid (6C;  $P = 0.9811$ ). REML:  $F_{1,123} = 4.254$ ,  $P = 0.0413$ , with Sidak's Test for multiple comparisons;  $N = 14-38$ . **C** The pairing of long-chain nonanoic acid (9C) and quinine (red) results in a significant reduction in PER compared to naïve flies. After training, PER response to 9C was significantly lower in trained flies compared to naïve flies ( $P = 0.0077$ ), but there was no difference in PER to short-chain valeric acid (5C;  $P = 0.6868$ ). REML:  $F_{1,64} = 6.207$ ,  $P = 0.0153$ , with Sidak's Test for multiple comparisons;  $N = 33$ . **D** The pairing of medium-chain heptanoic acid (7C) and quinine (red) results in a significant reduction in PER compared to naïve flies. After training, PER to both 7C and medium-chain hexanoic acid (6C) was significantly lower in trained flies compared to naïve flies (7C:  $P < 0.0001$ ; 6C:  $P < 0.0001$ ). REML:  $F_{1,98} = 62.64$ ,  $P < 0.0001$ , with Sidak's Test for multiple comparisons;  $N = 49-51$ . **E** The pairing of medium-chain octanoic acid (8C) and quinine (red) results in a significant reduction in PER compared to naïve flies. After training, PER to both 8C and medium-chain hexanoic acid (6C) was significantly lower in trained flies compared to naïve flies (8C:  $P = 0.0428$ ; 6C:  $P = 0.0214$ ). REML:  $F_{1,43} = 7.642$ ,  $P = 0.0084$ , with Sidak's Test for multiple comparisons;  $N = 22-23$ . **F** The pairing of medium-chain octanoic acid (8C) and quinine (red) results in a significant reduction in PER compared to naïve flies. After training, PER to both 8C and medium-chain heptanoic acid (7C) was significantly lower in trained flies compared to naïve flies (8C:  $P = 0.0017$ ; 7C:  $P = 0.0068$ ). REML:  $F_{1,46} = 20.72$ ,  $P < 0.0001$ , with Sidak's Test for multiple comparisons;  $N = 24$ . Error bars indicate  $\pm$ SEM. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*\*  $P < 0.0001$ .