



Figure 6 - Figure Supplement 2: Control crosses in activation or silencing experiments confirm that CN-33 is involved in approach behavior.

a-b. We removed *SS02108*-driven neural expression in the nerve cord by expressing the Split-GAL4 repressor Killer Zipper (*LexAop-KZip+::3xHA*, [72]) under the control of *teashirt-LexA* driver (*SS02108 > CsChrimson; tsh > KZip+*). With this genetic manipulation we verified that the approach behavior of larvae observed in Fig.4b was due to the brain neurons present in the *SS02108* expression pattern. Plots and quantification as in Fig. 2b-g. **a.** Even without nerve cord expression (*light brown*, *SS02108 > CsChrimson; tsh > KZip+*, $N_{exp}=10$, $N_{larvae}=380$), the brain neurons (CN-33 and MB2ON-86) from this line were sufficient to repress and promote turning in response to activation onset, and offset, respectively, relative to controls (*black*, *empty Split-GAL4 > CsChrimson; tsh > KZip+*, $N_{exp}=16$, $N_{larvae}=450$). **b.** Removing neural expression in the nerve cord (*light brown*, *SS02108 > CsChrimson; tsh > KZip+*) did not change optogenetically induced behavior as compared to not removing it (*dark brown*, *SS02108 > CsChrimson; ∅ > KZip+*, $N_{exp}=7$, $N_{larvae}=250$). **c-d.** The line *SS04330* drives expression in MB2ON-86 alone [36] and is thus used to verify how specific to CN-33 are the behavioral effects found with the line *SS02108* (which targets both CN-33 and more weakly MB2ON-86). Plots and quantification as in Fig. 2b-g. **c.** Activating MB2ON-86 mildly increases turning (*blue*, *SS04330 > CsChrimson*, $N_{exp}=25$, $N_{larvae}=890$) compared to controls (*black*, *empty Split-GAL4 > CsChrimson*, $N_{exp}=340$, $N_{larvae}>10000$) in response to activation onset, but does not decrease turning in response to activation offset. **d.** Optogenetic activation of MB2ON-86 (*SS04330 > CsChrimson*) induced significantly less turning in response to activation onset than activating MB2ON-86 and CN-33 together (*SS02108 > CsChrimson*, $N_{exp}=16$, $N_{larvae}=650$). Furthermore, activating MB2ON-86 alone (with *SS04330*) did not increase turning in response to activation offset. *: $p<0.05$, **: $p<0.01$, Welch Z test. **e.** Constitutive silencing of MB2ON-86 affects navigation performance. $N_{larvae}=135$ (*SS02108 > Kir2.1*), 92 (*SS04330 > Kir2.1*), 211 (*empty Split-GAL4 > Kir2.1*), $N_{exp}=7, 4, 6$, respectively. For b,d,e, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, Welch Z test.