

SUPPLEMENTAL MATERIAL FOR MANUSCRIPT “*Gai2*⁺ vomeronasal neurons govern the initial outcome of an acute social competition”

Authors:

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SUPPLEMENTAL FIGURES

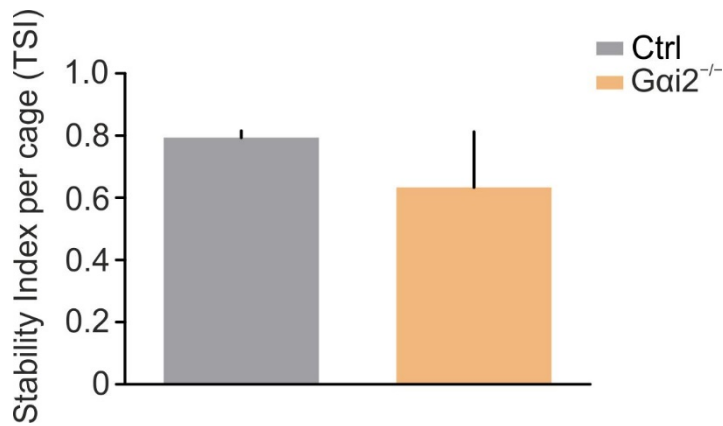


FIGURE S1

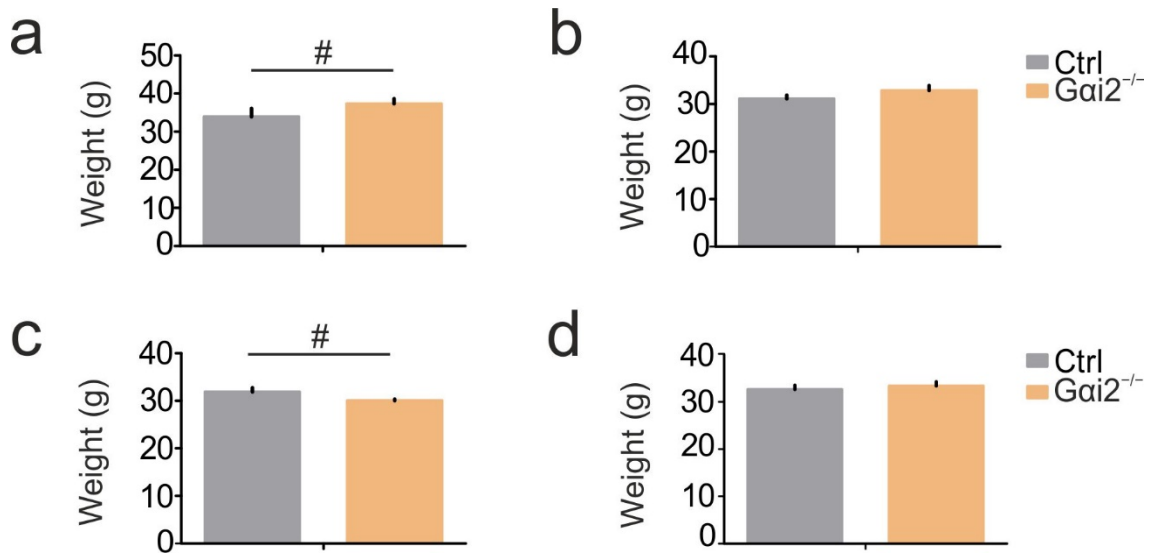


FIGURE S2

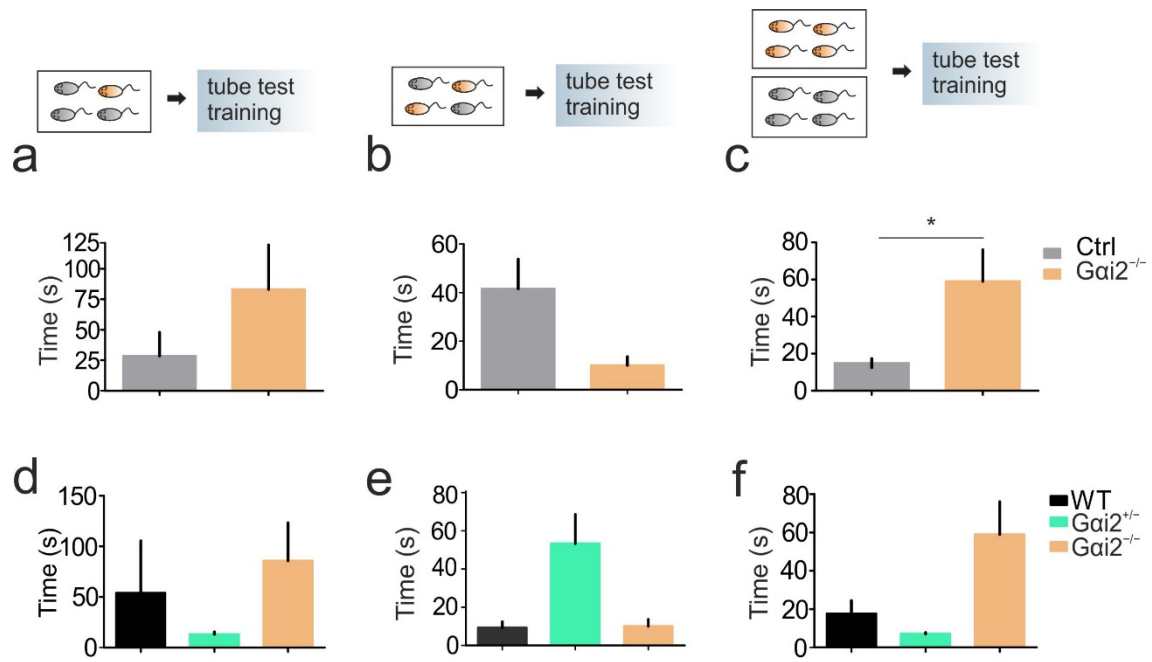


FIGURE S3

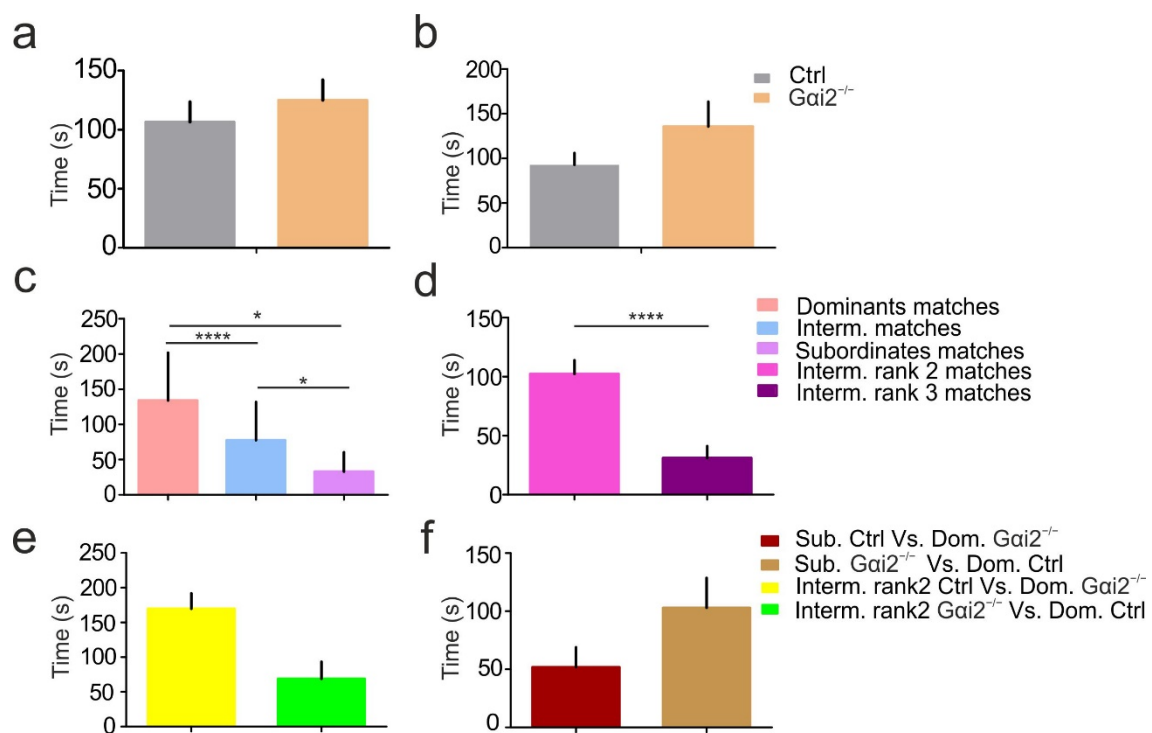


FIGURE S4

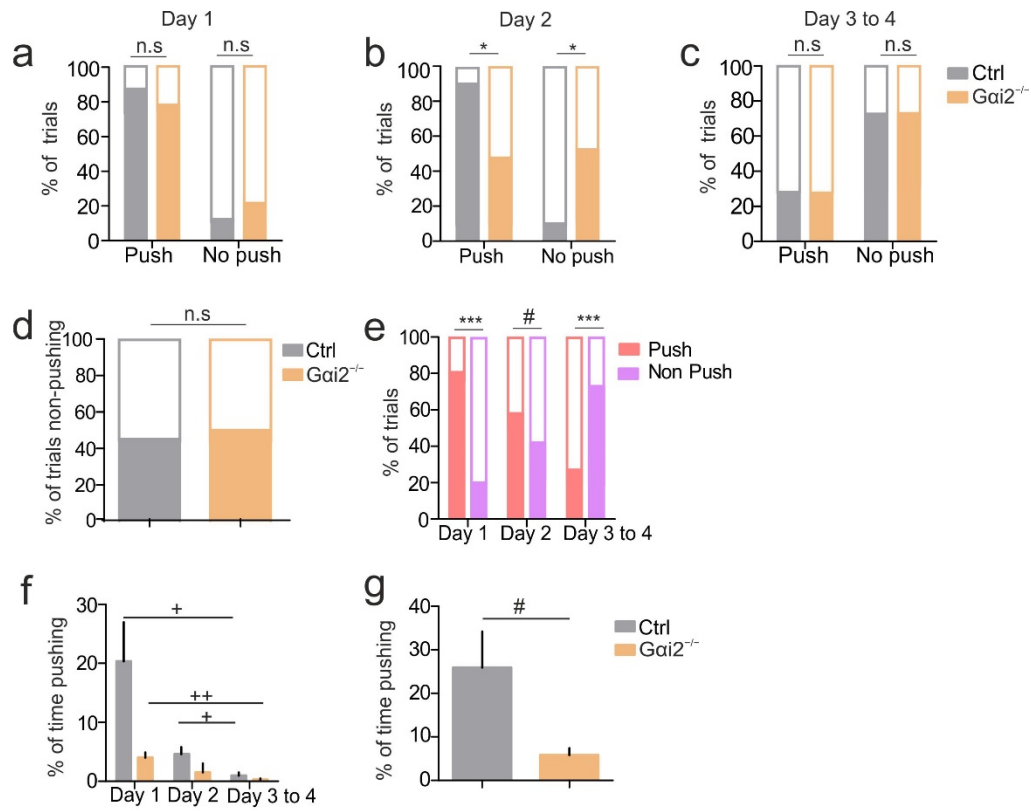


FIGURE S5

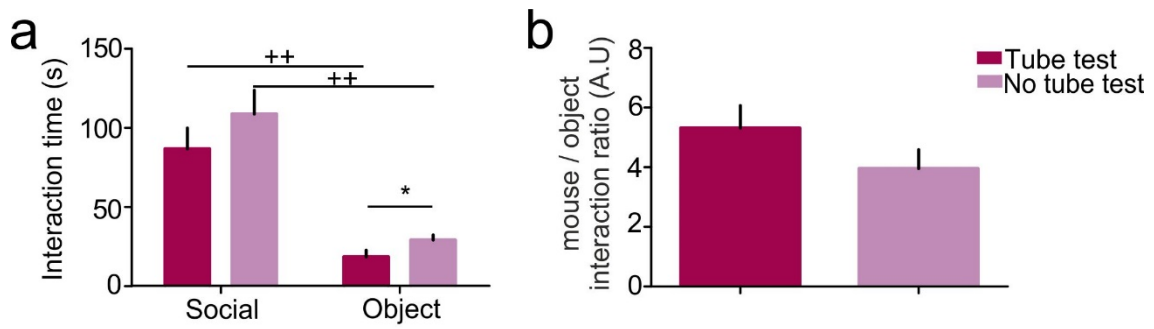


FIGURE S6

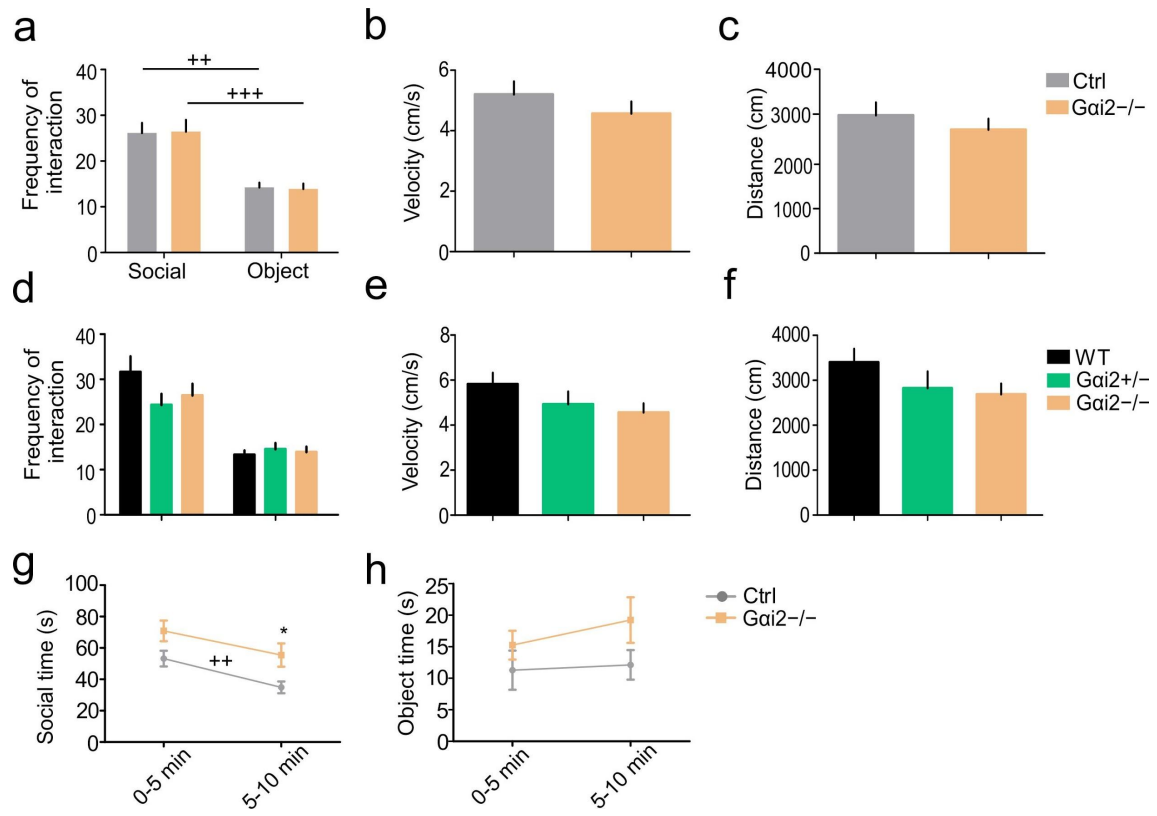


FIGURE S7

SUPPLEMENTAL FIGURE LEGENDS

Supplementary Figure S1. Tezanos Stability Index (TSI). TSI value for a given hierarchy (into one cage) is calculated through the quotient between the DI variance from that hierarchy and the DI variance from a hypothetical hierarchy with no variations in DI along several days. No differences were found in the stability measured by the TSI between control and $Gai2^{-/-}$ cages, T-test, $p=0.467$, $n=3$ cages per genotype.

Supplementary Figure S2. Body weight. **a**, differences between control and $Gai2^{-/-}$ mice in the experiment with 1 KO and 3 controls per cage, Ctrl ($n=10$), KO ($n=10$), U de Mann-Whitney $p=0,063$. **b**, body weight differences between control and KO animals in the experiment with 2 KO and 2 controls per cage, Ctrl ($n=14$), KO ($n=5$), U de Mann-Whitney $p=0,130$. **c**, differences between control and KO animals in the first-time encounters experiment. Ctrl ($n=11$), KO ($n=11$), T-student $p=0,057$. **d**, body weight differences between control and ko animals (considering the 3 experiments together), Ctrl ($n=34$), KO ($n=26$), U de Mann-Whitney $p=0,146$.

Supplementary Figure 3. Latency to cross the tube during training. **a-c**, Differences in training latency between control and ko animals; **a**, experiment with 1 KO and 3 controls per cage, Ctrl ($n=9$), KO ($n=10$), U de Mann Whitney $p=0,182$. **b**, experiment with 2 KO and 2 controls per cage, Ctrl ($n=15$), KO ($n=4$), U de Mann Whitney $p=0,1$. **c**, first-time encounters experiment, Ctrl ($n=11$), KO ($n=11$), T-Student $p=0,022$. **d-f**, Differences in training latency per genotype; **d**, experiment with 1 KO and 3 controls per cage, Ctrl ($n=4$), Heterozygous ($n=6$), KO ($n=10$), Kruskal-Wallis $p=0,308$. **e**, experiment with 2 KO and 2 controls per cage, Ctrl ($n=4$); Heterozygous ($n=11$), KO ($n=4$), Kruskal-Wallis $p=0,128$. **f**, first-time encounters experiment, Ctrl ($n=8$), Heterozygous ($n=3$), KO ($n=11$), Kruskal-Wallis $p=0,302$.

Supplementary Figure 4. Latency to win a trial in the different experiments. **a-b**, latency to win the trial in **a**, experiment with 1 KO and 3 controls per cage, Ctrl ($n=10$), KO ($n=10$), U de Mann Whitney $p=0,542$, and **b**, experiment with 2 KO and 2 KO per cage, Ctrl ($n=15$), KO ($n=5$), U de Mann Whitney $p=0,544$. **c-f**, first-time encounters experiment; **c**, latency to win a trial between animals of the same rank:

Dom vs Dom (n =18), Sub vs Sub (n=18), Int vs Int (n=26). Kruskal-Wallis for independent samples: Sub vs Sub – Int vs Int p= 0,026; Sub vs Sub – Dom vs Dom p< 0,0001; Dom vs Dom – Int vs Int p= 0,043. **d**, latency to win a trial between intermediate animals of the same rank: Int2 vs Int 2 (n= 17), Int3 vs Int3 (n=9), U de Mann-Withney p<0,0001. **e-f**, latency to win a trial between animals of different rank; **e**, 1 position rank difference, Int2 WT vs Dom KO (n=8), INT2 KO vs Dom WT (n=9), Kruskal-Wallis p=0,277. Int3 WT vs Sub KO (n=6), INT3 KO vs Sub WT (n=6), Kruskal-Wallis p=0,467). **f**, 3 positions rank difference, Sub WT vs Dom KO (n=8), sub KO vs Dom WT (n=9), Kruskal Wallis p=0,336.

Supplementary Figure 5. Active versus passive winning behavior in the inter-cage tests (only won trials). **a-c**, percentage of trials with active (pushing) or passive (non-pushing) behavior; **a**, first-day matches. No differences were found between $Gai2^{-/-}$ mice and controls. Squared-Chi, Chi value 0.325, p=0.569. Ctrl n=8 trials, $Gai2^{-/-}$ n= 23 trials. **b**, second-day matches. Controls displayed a higher percentage than $Gai2^{-/-}$ mice. Squared-chi, Chi value 4.188, p=0.041. Ctrl n=10 trials, $Gai2^{-/-}$ n= 21 trials. **c**, days 3 and 4. No differences were found between $Gai2^{-/-}$ mice and controls. Squared-Chi, Chi value 0.001, p=0.978. Ctrl n=22 trials, $Gai2^{-/-}$ n=26 trials. **d**, percentage of winning trials with passive (non-pushing) behavior. No differences were found between groups. Squared chi, Chi value 0.255, p=0.614. Ctrl n=40 trials, $Gai2^{-/-}$ n=70 trials. **e**, percentage of trials with active (pushing) or passive (non-pushing) behavior per day. On day 1 (Binomial distribution, p= 0.00034) animals differentially displayed more pushing behavior in contrast with days 3 to 4 (Binomial distribution, p= 0.00069) when animals displayed more passive (non pushing) behavior of winning. A trend was observed on day 2 (Binomial distribution, p= 0.09604). **f**, Average percentage of time spent pushing during the trial per day. Ctrl day1 (n= 8), KO day1 (n=23), Ctrl day2 (n=9), KO day2 (n=22), Ctrl day3 (n=22), KO day3 (n=26). Kruskal-Wallis → KO day3 - Ctrl day3 p=1.000, KO day1 – Ctrl day1 p=1.000, KO day2 – Ctrl day2 p=0.286, KO day3 – KO day2 p=1.000, KO day3 – KO day1 p=0.001, KO day2 – KO day1 p=0.2719, Ctrl day3 – Ctrl day2 p=0.024, Ctrl day3 – Ctrl day1 p=0.016, Ctrl day2 – Ctrl day1 p=1.000. **g**, Average percentage of time spent pushing during the trial per group. Ctrl n=36, KO n=60. U de Mann-Whitney p=0.064.

Supplementary Figure 6. Differences in the three-chamber sniffing behavior between *Gai2*^{-/-} mice as a result of tube test performance. **a**, differences in interaction time between tube tested and not tested animals per compartment. KO tube test n=5, KO no tube test n=8. T-Student independent samples (intergroup): mouse p=0,336, object p=0,069. T-Student paired samples (intragroup): Tube test p=0,003, no tube test p=0,001. **b**, differences in mouse/object interaction time ratio. KO tube test n=5, no tube test n=8. T-Student independent samples p=0,201.

Supplementary Figure 7. Three-chamber analyses and breakdown for genotype. **a**, differences in interaction time frequency per compartment. Ctrl n=12, KO n=11. T-Student independent samples (intergroup): mouse p=0,802, object p=0,793. T-Student paired samples (intragroup): Ctrl p=0,001, KO p<0,0001. **b**, differences in velocity. Ctrl n=13, KO n=13. T-Student independent samples p=0,284. **c**, differences in distance traveled into the chamber. Ctrl n=13, KO n=12. T-Student independent samples p=0,132. **d**, differences in interaction time frequency per compartment. WT n=13, Heterozygous n=3, KO n=9. Repeated measures ANOVA p=0,619. **e**, differences in velocity per genotype. WT n=13, Heterozygous n=4, KO n=8. One-factor ANOVA p=0,148. **f**, differences in distance traveled into the chamber per genotype. WT n=12, Heterozygous n=4, KO n=8. One-factor ANOVA p=0,24. **g**, 5-min bit breakdown of interaction time into the social compartment. Ctrl n=12, KO n=13. T-Student, independent samples (intergroup): 0-5 min p=0,228, 5-10 min p=0,025. T-Student, paired samples (intragroup): Ctrl P=0,005, KO p=0,328. **h**, 5-min bit breakdown of interaction time into the object compartment. Ctrl n=13, KO n=13. Mann-Whitney U test, (intergroup): 0-5 min p=0,466, 5-10 min p=0,142. Wilcoxon Test (intragroup): Ctrl p=0,309, KO p=0,201.