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## **Supplemental Information**

Sex-dependent control of pheromones on social organization within groups of wild house mice

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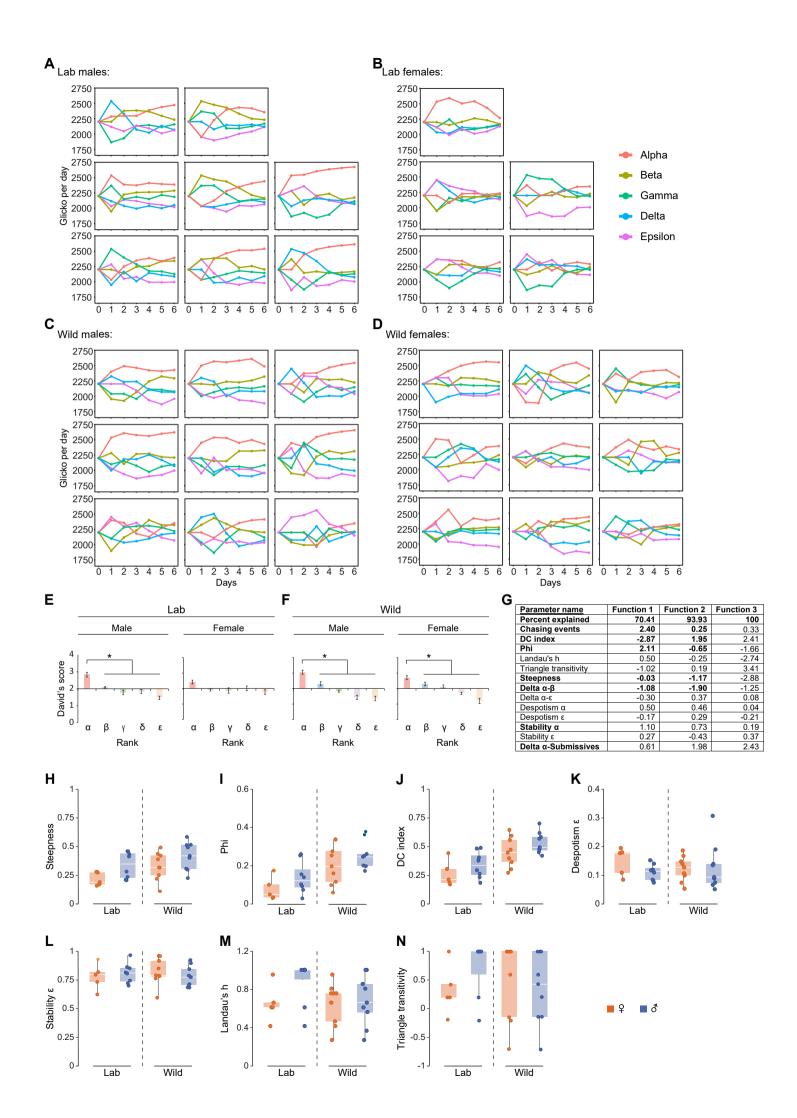


Figure S1: Hierarchy parameters in groups of control lab and wild mice. Related to Figure 2.

(A-D) Daily Glicko scores of each experimental group of 5 mice ('arena'), in male (A, C) and female (B, D), lab (A-B) and wild (C-D) mice. (E-F) David's scores in groups of lab (A) and wild (B) mice, males and females. Parameters were calculated per mouse. (G) A list of parameters with their loading coefficients for each function in the FDA, as plotted in figure 2E for lab and wild mice, males and females. (H-N) Comparison of single hierarchy parameters between groups of female/male, wild/lab mice. Results are displayed as medians  $\pm 1.5$  interquartile range. Parameters were calculated per group of mice ('arena'). \* p $\leq$ 0.05.

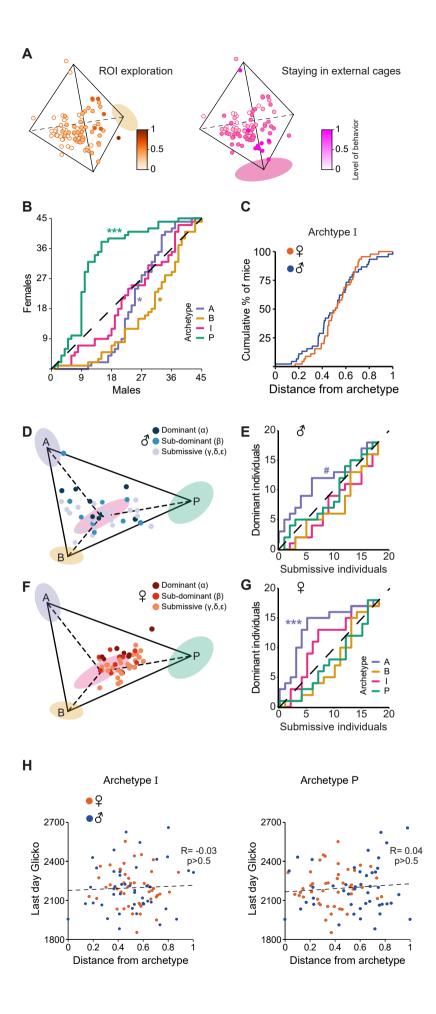


Figure S2: Personality space analysis in control wild mice. Related to figure 3.

(A) Representative heat-maps for "ROI exploration" (left) and "staying in external cages" (right) behaviors, depicting the level of behavior for each mouse. Behaviors were chosen based on the correlation with archetypes B and I, respectively. Ellipsoids represent the error of each archetype (see 'methods'). (B) A classifier ROC curve describing the accumulation of female vs. male mice, for each archetype. (C) An accumulation curve of males and females as a function of distance from archetype I. (D,F) 3D representations of the personality space, displaying dominant ('alpha'), subdominant ('beta') and submissive ('gamma', 'delta', 'epsilon') mice separately for males (D) and females (F). For optimal demonstration, the 3D tetrahedrons enclosing the data points were rotated in space to highlight the relevant archetypes in relation to the data. (E,G) Classifier ROC curves describing the accumulation of most dominant ('alpha' and 'beta') vs. most submissive ('delta' and 'epsilon') mice, for each archetype, in males (E) and females (G). (H) Correlation of the last day Glicko score and the distance from archetypes I and P. #p=0.069, \*\*\*\* p≤0.001.

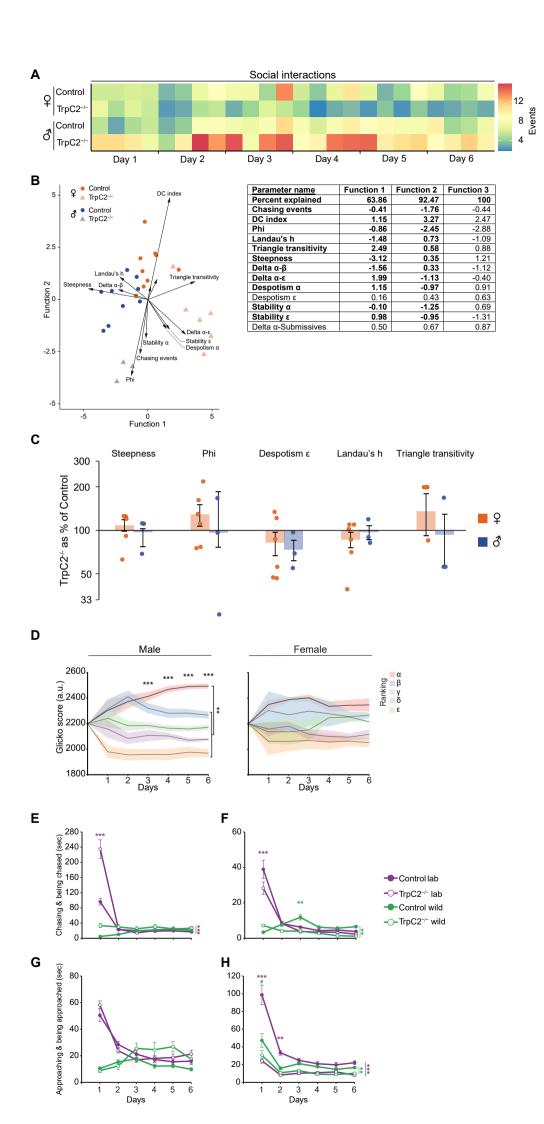


Figure S3: Behavioral patterns and hierarchy parameters in TrpC2<sup>-/-</sup> mice. Related to Figure 4.

(A) An ethogram of active social behaviors (chasing & being chased, approaching & being approached, avoiding & being avoided) in wild-backcrossed mice,  $TrpC2^{-/-}$  and control, of both sexes, throughout the 6 days of experiment. Each column represents number of events per 1 hour of testing. (B) Flexible discriminant analysis (FDA) of various hierarchy parameters in male and female wild mice of both genotypes (left), and a list of parameters with their loading coefficient for functions 1, 2 and 3 (right). For legibility of the entire plot, the arrows were plotted with 1.5 multiplication of the original (x, y) coordinates, and only the relevant parameters were labeled. (C) Specific hierarchy parameters in groups of  $TrpC2^{-/-}$  female/male wild-backcrossed mice, presented as percentage of their respective control group. (D) Mean daily Glicko scores of dominant ('alpha') vs. submissives ('beta', 'gamma', 'delta' & 'epsilon') during six days in the semi-natural enclosures, in groups of male (left) and female (right)  $TrpC2^{-/-}$  lab mice.  $n_{Lab} \frac{TrpC2^{-/-}}{Males} = 40$ ,  $n_{Lab} \frac{TrpC2^{-/-}}{Females} = 20$ . (E-H) Mean total daily durations (sec) of chasing & being chased (E-F), and approaching & being approached (G-H), in male (E,G) and female (F,H), lab and wild mice of both genotypes. Parameters were calculated either per group of mice ('arena') (B-C), or per mouse (D-H). Results are displayed as mean±SEM. \*\* p≤0.01, \*\*\* p≤0.001.

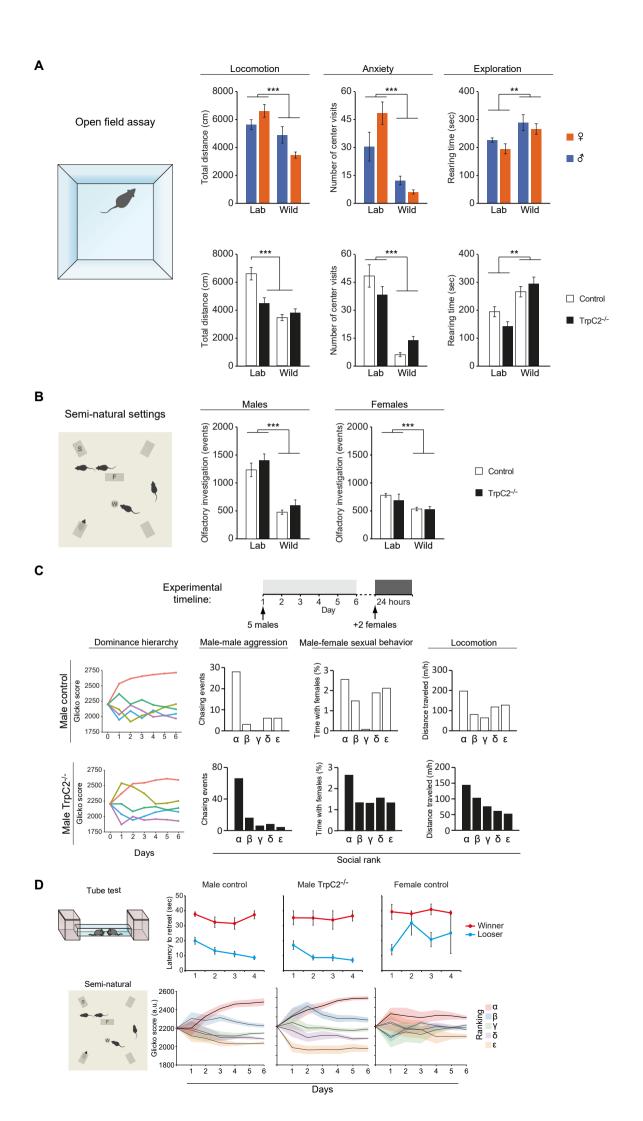


Figure S4: Exploration, olfactory investigation and validation of hierarchy in control and TrpC2-/- mice. Related to Figure 4 and STAR methods.

(A) Open field assay. Quantification of total distance traveled, number of visits to the central part of the apparatus, and number of rearings, in lab and wild mice of both sexes (up), and in control and TrpC2<sup>-/-</sup> females, lab and wild (down). (B) Quantification of olfactory investigation events during day 1 of the semi-natural experiment. (C) Validation of hierarchy: Following 6 days of hierarchy establishment by a group of 5 male mice, two female mice were introduced into the semi-natural arena for an additional 24 hours. During 24 hours of the intruders assay, various male-male, male-female, and locomotion behaviors were compared between mice of different ranking within the dominance hierarchy. Displayed are experimental timeline, alongside daily Glicko scores, quantification of aggressive chasing, duration with females, and total distance traveled in a group of control and a group of TrpC2<sup>-/-</sup> mice. (D) Comparison of tube test and semi-natural setup results in control lab males, TrpC2<sup>-/-</sup> lab males, and control lab female mice. (A, B, D) Results are displayed as mean±SEM. \*\*\* p≤0.01, \*\*\*\* p≤0.001.

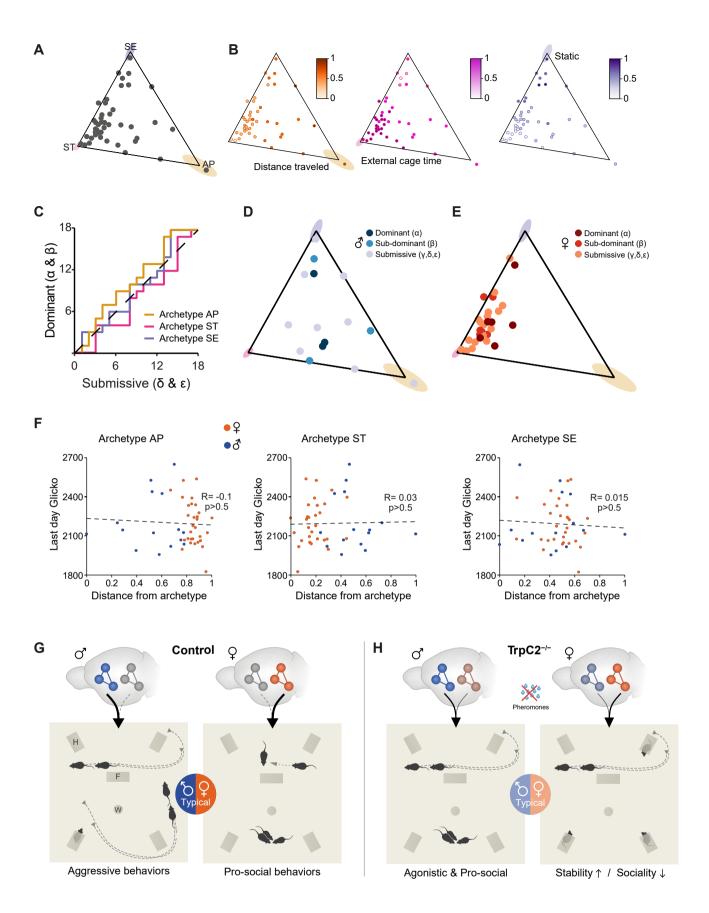


Figure S5: Personality space analysis in TrpC2-- wild mice. Related to Figure 5.

(A) A 2D representation of personality space, forming a triangle of three distinguished behavioral archetypes (termed AP, ST & SE). Ellipses represent the error of each archetype (see 'methods'). (B) Representative heat-maps for "distance traveled" (left), "external cage time" (middle) and "static" (right) behaviors, depicting the level of behavior for each mouse. Behaviors were chosen based on the correlation with archetypes AP, ST and SE, respectively. (C) A classifier ROC curve describing the accumulation of dominant ('alpha' and 'beta') vs. submissive ('delta' and 'epsilon') mice, for each archetype, in wild-backcrossed TrpC2<sup>-/-</sup> mice. (D-E) 2D representations of the personality space, displaying dominant ('alpha'), sub-dominant ('beta') and submissive ('gamma', 'delta', 'epsilon') mice separately for males (D) and females (E). (F) Correlation of the last day Glicko score and the distance from archetypes AP, ST and SE. (G-H) Suggested model summarizing behavioral individuality within groups. (G) Illustration of sex-typical behaviors in groups of wild control mice, driven by sexspecific neural circuits. Males are characterized by aggressive chasing behaviors, alongside social abstinence, while females are characterized by pro-social approaching and together interactions. (H) Illustration of typical social behaviors in groups of male and female TrpC2-/- mice. In the absence of VNO-mediated pheromone signaling, the distinction between male-typical and female-typical phenotypes is reduced, as the repression of neural circuits driving behaviors typical of the opposite sex is released. H= hiding box, F=food stand, W=water bottle.