Supplementary Figure S1

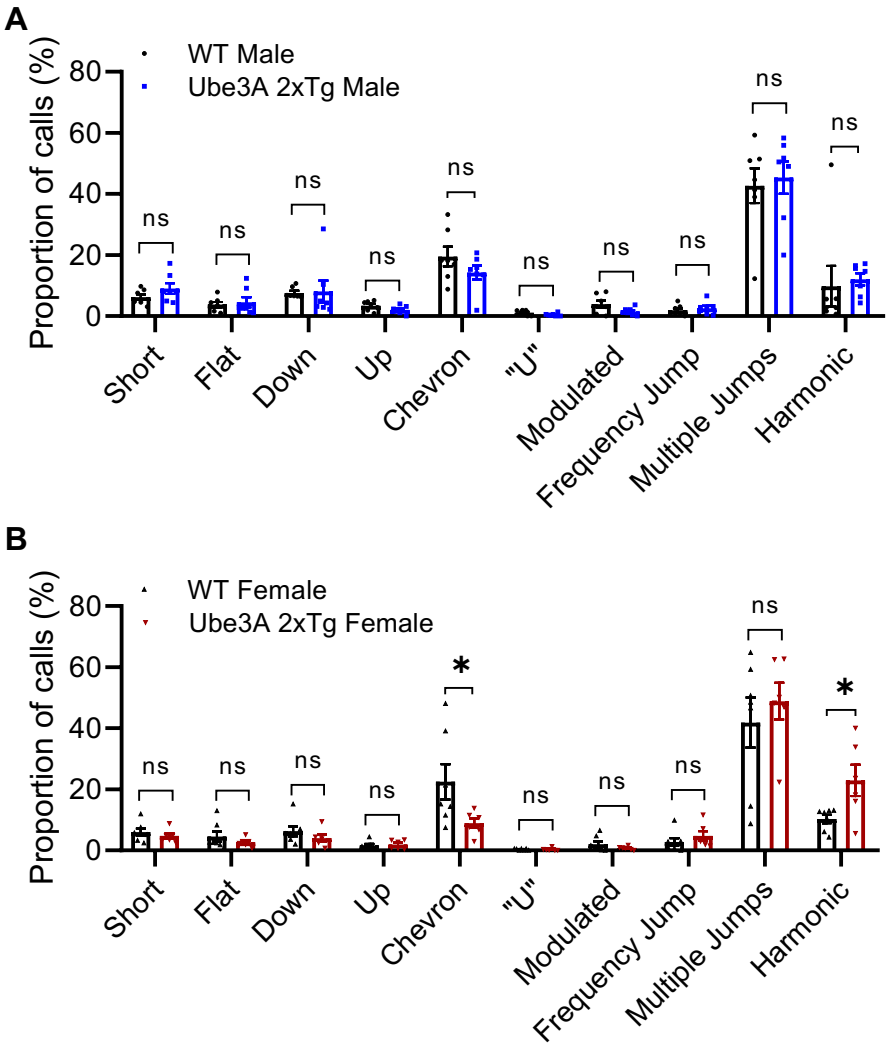

Fig. S1: Sexually dimorphic changes in the call syllables of USV in male and female *Ube3A* 2xTg mice

(A) Call syllable classification of USV showed no change in *Ube3A* 2xTg males compared to WT males at P5.

(B) *Ube3A* 2xTg female mice made significantly fewer chevron calls and more harmonic calls compared with WT female mice at P5.

n=7 WT male; n=7 Tg male; n=7 WT female; n=6 Tg female. Mean ± SEM. *p<0.05. ns, not significant. Two-way ANOVA with Bonferroni's multiple comparisons test.

Supplementary Figure S2

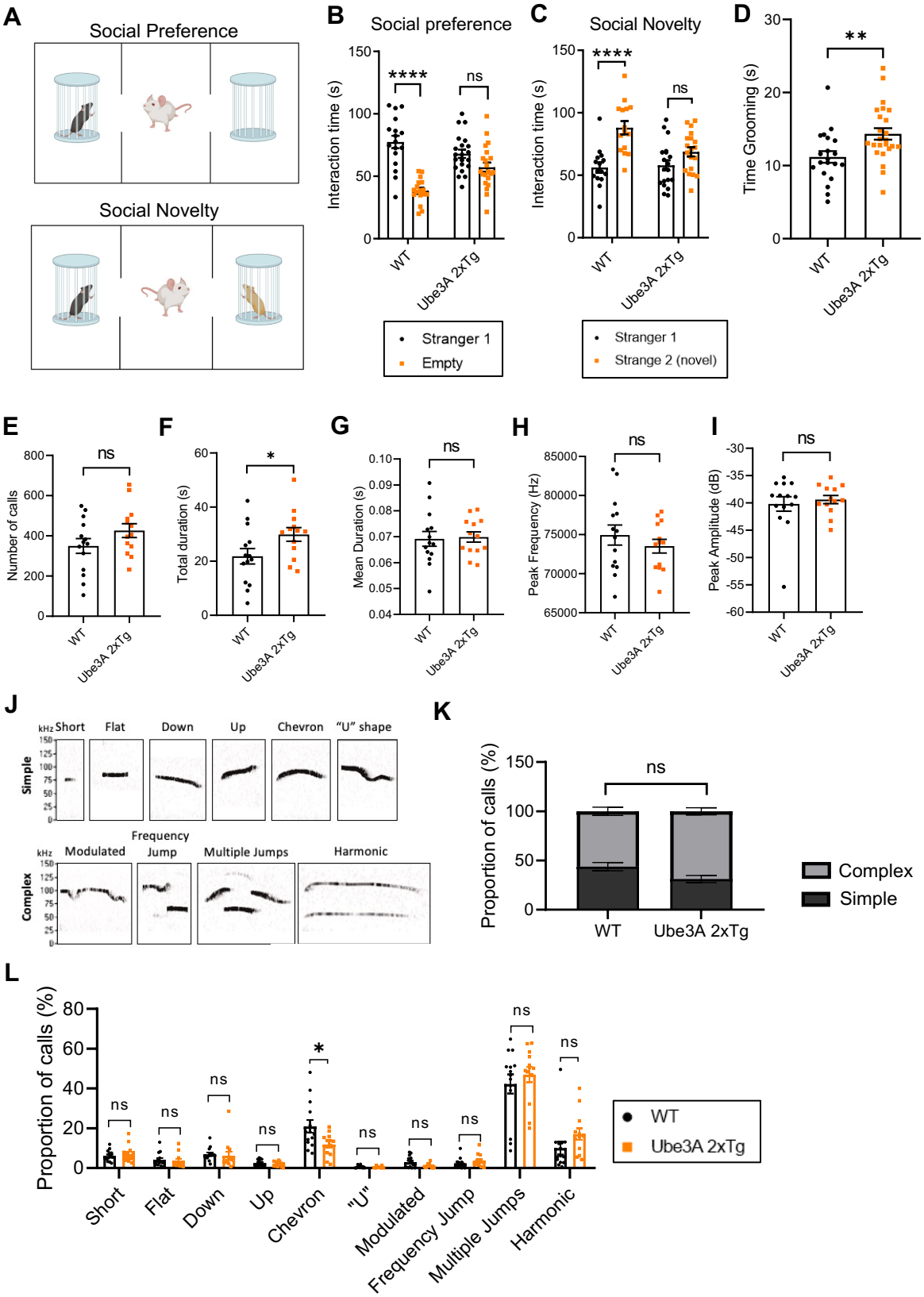

Fig. S2: Combining male and female results, *Ube3A* 2xTg mice display impairments in social behaviors, repetitive self-grooming behaviors, and ultrasonic vocalizations.

(A) The paradigm for the three-chamber social test. For social preference (top), an unfamiliar mouse was placed into either of the side chambers and the test mouse was allowed to move freely in the apparatus. For social novelty (bottom), a novel mouse was placed into the remaining empty chamber, and the test mouse was allowed to interact with both mice.

(B,C) Quantification of the interaction time showed a decrease in preference for the stranger mouse (B) and the novel mouse (C) in *Ube3A* 2xTg mice.

(D) *Ube3A* 2xTg mice showed increased grooming behavior.

(E-I) Quantifications of the number of calls (E), total call duration (F), mean call syllable duration (G), peak frequency (H), and peak amplitude (I) for USVs recorded on postnatal day 5. *Ube3A* 2xTg mice displayed longer total call duration than WT mice.

(J) Representative calls of each type used in syllable characterization.

(K,L) WT and *Ube3A* 2xTg animals displayed similar usage of syllabus at P5, except that *Ube3A* 2xTg animals made fewer chevron calls.

In (B), n=17 WT, n=21 *Ube3A* 2xTg. **In (C)**, n=15 WT, n=19 *Ube3A* 2xTg. **In (D)**, n=20 WT, n=24 *Ube3A* 2xTg. **In (E-L)**, n=14 WT, n=13 *Ube3A* 2xTg. Mean ± SEM. *$p<0.05$; **$p<0.01$; ****$p<0.0001$. ns, not significant. In (B), (C), (K), (L) Two-way ANOVA with Bonferroni's multiple comparisons test; in (D), (E-I) Unpaired two-tailed t test.

Supplementary Figure S3

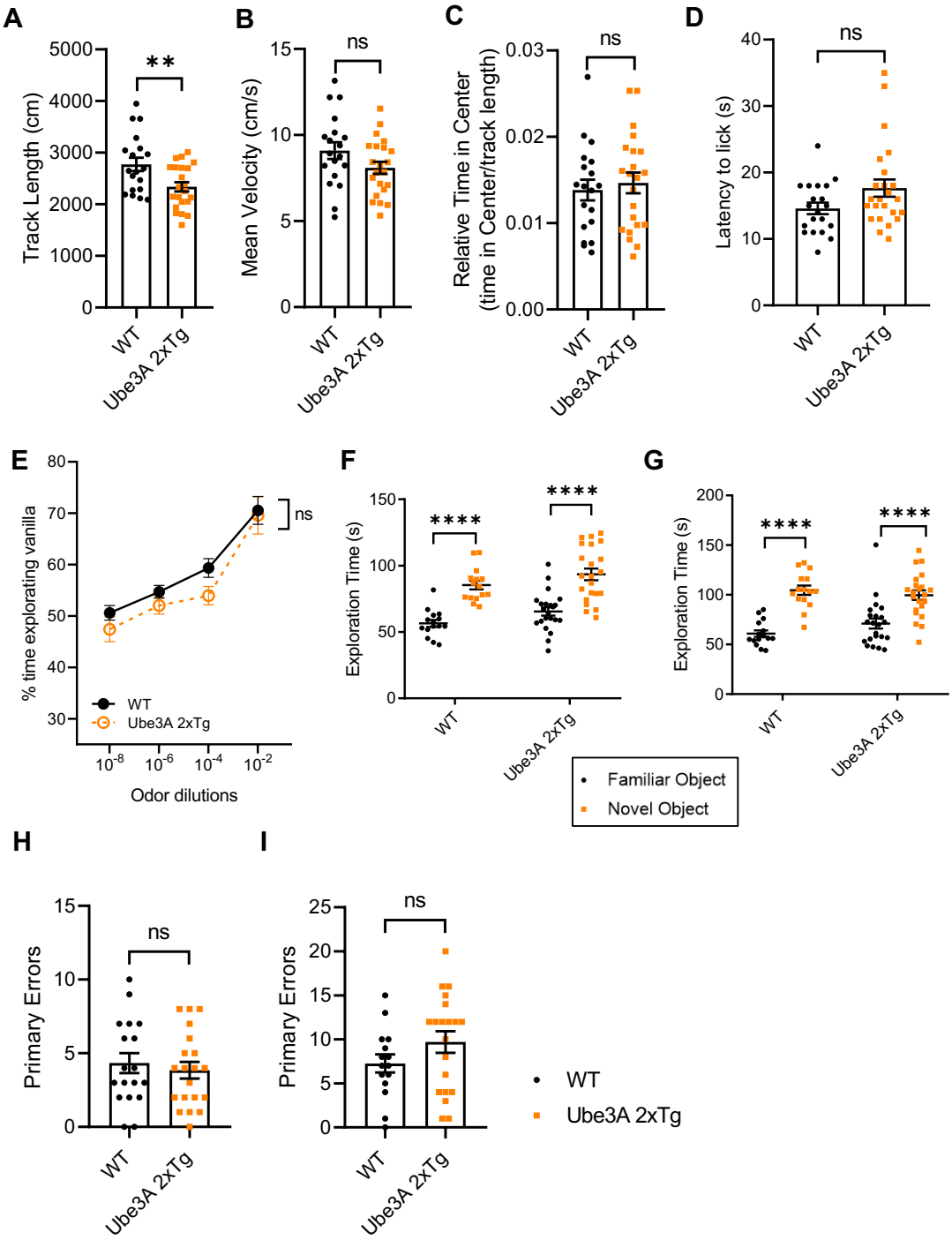

Fig. S3: Combining male and female results, *Ube3A* 2xTg mice display reduced activity, normal pain and olfactory sensitivities, and normal memory.

(A-C) During open field test, *Ube3A* 2xTg animals showed decreased track lengths (A), normal mean velocities (B) and no change in relative time of stay at the center (C). n=19 WT, n=22 *Ube3A* 2xTg.

(D) Test mice were placed on 55℃ hot plate and their latency to lick hind paws was recorded. *Ube3A* 2xTg mice displayed normal pain sensitivity compared to WT. n=20 WT, n=24 *Ube3A* 2xTg.

(E) Test mice were exposed to vanilla at varied concentrations. Time spent exploring vanilla vs. water was recorded. The olfactory sensitivity of 2xTg mice was similar to that of WT mice. n=19 WT, n=20 *Ube3A* 2xTg.

(F,G) During novel object recognition test, *Ube3A* 2xTg animals showed normal exploration time of familiar object vs. novel object 4 h (F) and 24 h (G) post training. n=15 WT, n=22 *Ube3A* 2xTg.

(H,I) During Barnes spatial memory maze test, primary errors made to find the escape hole 24 h (H) and 5 d (I) after the last training were similar between WT and *Ube3A* 2xTg animals. In (H), n=18 WT, n=20 *Ube3A* 2xTg. In (I), n=15 WT, n=20 *Ube3A* 2xTg.

Mean ± SEM. **$p<0.01$; ****$p<0.0001$. ns, not significant. In (E-G) Two-way ANOVA with Bonferroni's multiple comparisons test; in (A-D), (H-I) Unpaired two-tailed t test.

Supplementary Figure S4

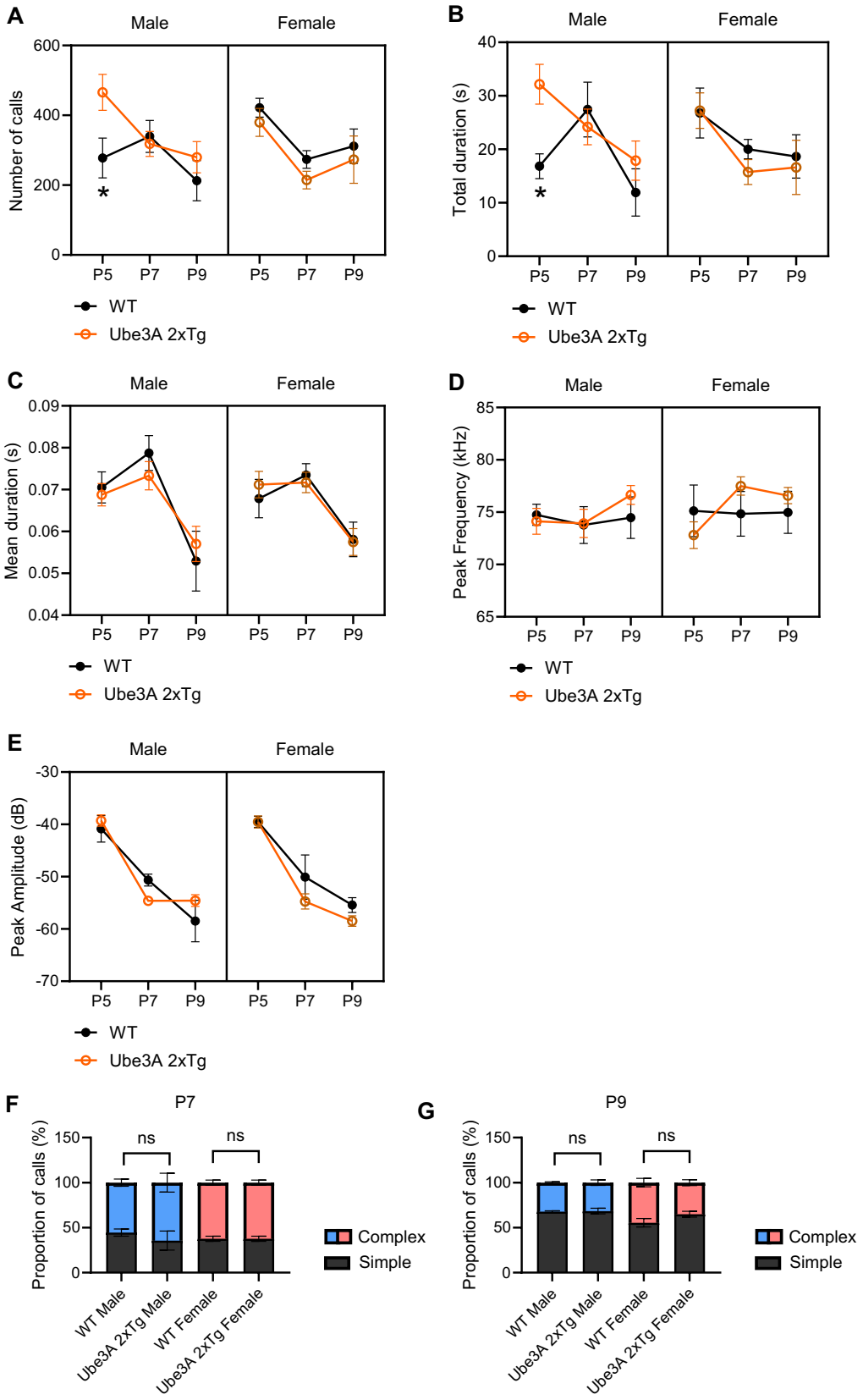

Fig. S4: Time-dependent changes of USVs in *Ube3A* 2xTg mice

(A-E) Quantifications of the number of calls (A), total call duration (B), mean call syllable duration (C), peak frequency (D), and peak amplitude (E) for USVs recorded on P5, P7, and P9. *Ube3A* 2xTg male mice displayed higher number of calls and longer total call duration than WT males only at P5. No change was observed in *Ube3A* 2xTg females.

(F-G) Both male and female *Ube3A* 2xTg animals exhibited call syllable types comparable to those of their WT counterparts at P7 (F) and P9 (G).

Mean ± SEM. *$p<0.05$. Three-way ANOVA with Bonferroni's multiple comparisons test.