Supporting Information

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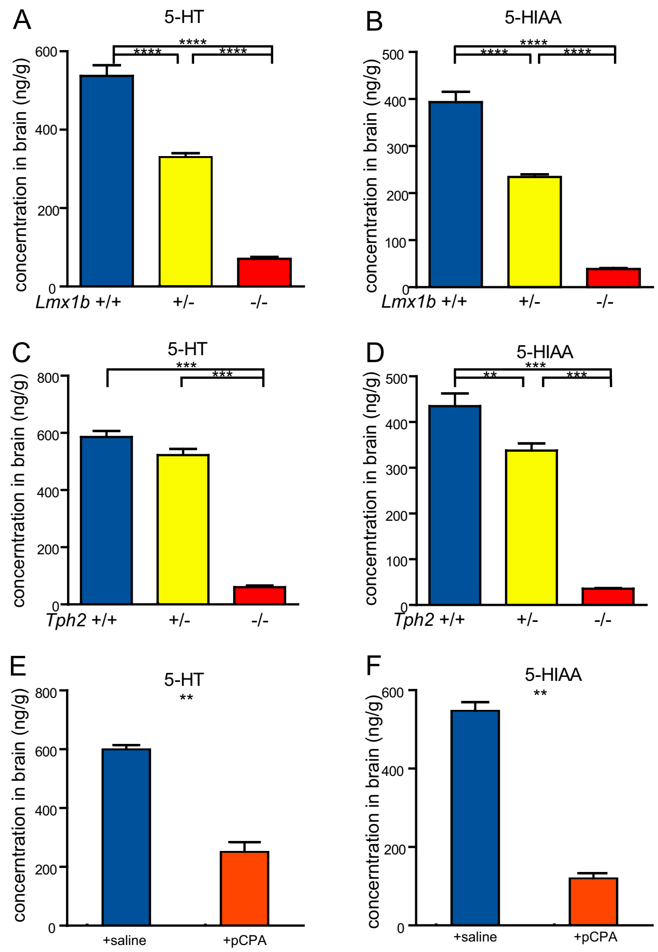

Fig. S1. Levels of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the brain. (*A*) 5-HT in the brains of *Lmx1b*$^{+/+}$ ($^{+/+}$, $n = 7$), *Lmx1b*$^{+/-}$ ($^{+/-}$, $n = 7$), and *Lmx1b*$^{-/-}$ ($^{-/-}$, $n = 7$) female mice were analyzed by HPLC. (*B*) 5-HIAA in the brains of *Lmx1b*$^{+/+}$, *Lmx1b*$^{+/-}$, and *Lmx1b*$^{-/-}$ females. (*C*) 5-HT in the brains of *Tph2*$^{-/-}$ ($n = 7$), *Tph2*$^{+/-}$ ($n = 8$), and *Tph2*$^{+/+}$ ($n = 7$) females. (*D*) 5-HIAA in the brains of *Tph2*$^{-/-}$, *Tph2*$^{+/-}$, and *Tph2*$^{+/+}$ females. (*E*) 5-HT in the brains of female mice injected with saline ($n = 5$) or *p*-chlorophenylalanine (pCPA) ($n = 6$). (*F*) 5-HIAA in the brains of female mice injected with saline or pCPA. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

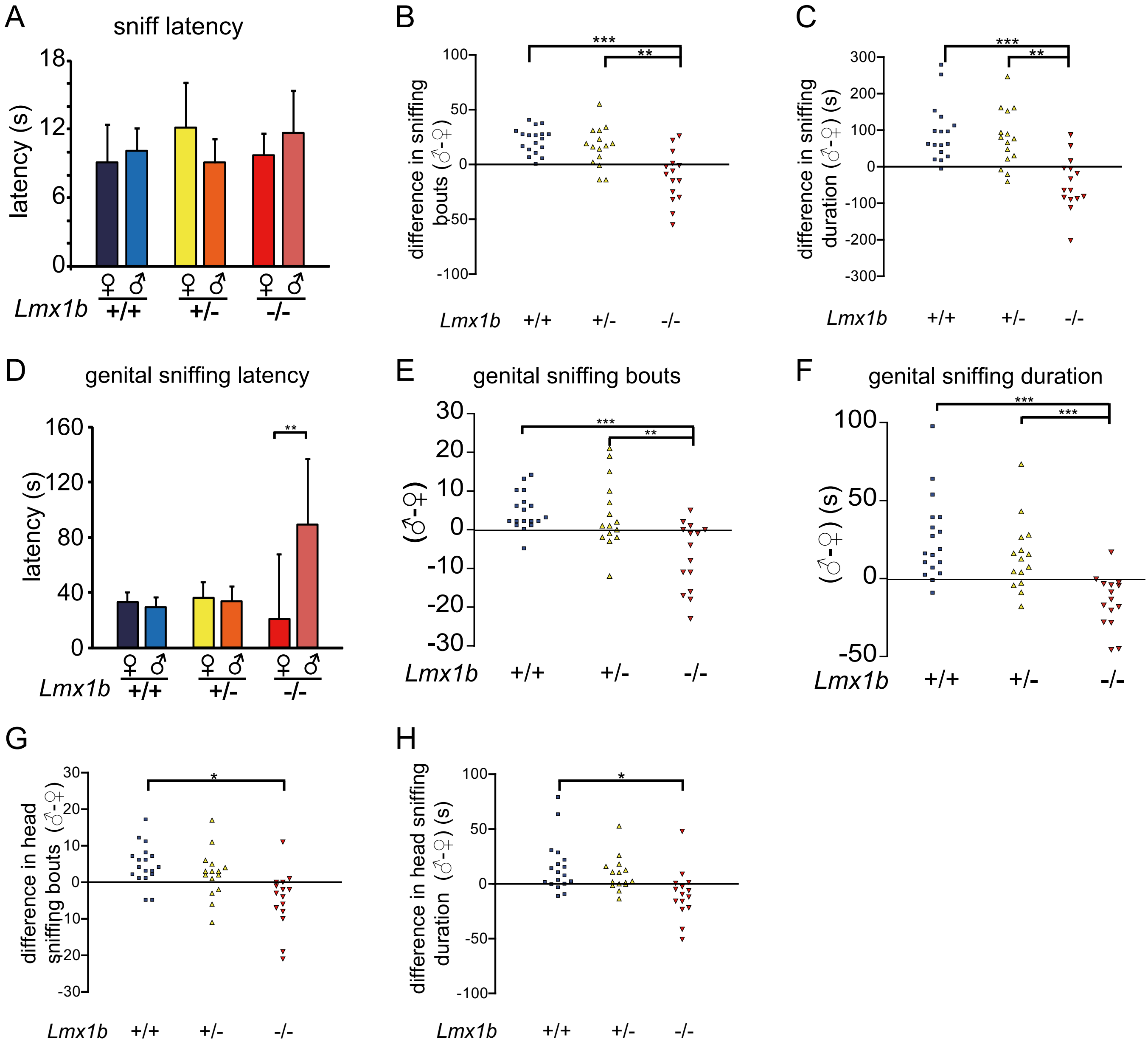

Fig. S2. Sexual preference of females in the mating choice assay. $n = 18$ for *Lmx1b*$^{+/+}$ ($^{+/+}$), $n = 15$ for *Lmx1b*$^{+/-}$ ($^{+/-}$), $n = 15$ for *Lmx1b*$^{-/-}$ ($^{-/-}$). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. (*A*) Sniff latencies of the whole body were not different among *Lmx1b*$^{-/-}$ females and their *Lmx1b*$^{+/+}$ or *Lmx1b*$^{+/-}$ female littermates. (*B*) Difference in sniffing bouts analyzed in individual females. (*C*) Difference in sniffing duration analyzed in individual females. (*D*) The latency to sniff male genital was lengthened in *Lmx1b*$^{-/-}$ females; thus, they sniffed female genitals first, whereas their female littermates did not show sexual preference in genital sniffing latency. (*E*) Genital sniffing bouts were analyzed by differences of each individual female: its bouts for sniffing male minus its bouts for sniffing female. (*F*) Genital sniffing duration was analyzed in individual females: its duration of sniffing males minus its duration of sniffing females. (*G*) Difference in sniffing head bouts analyzed in individual females. (*H*) Difference in sniffing head duration analyzed in individual females.

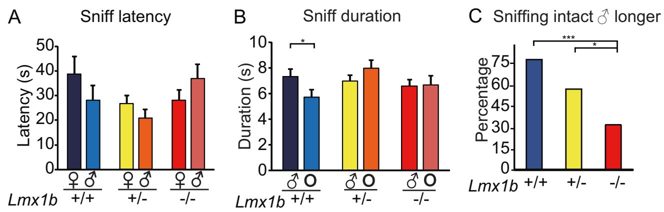

Fig. S3. *Lmx1b*$^{-/-}$ females prefer intact over castrated male genital odor. (*A*) Data are from the same experiments as those in Fig. 2. Latencies of females of different genotype to sniff male or female genital odor were not statistically different. (*B* and *C*) $n = 32$ for *Lmx1b*$^{+/+}$, $n = 36$ for *Lmx1b*$^{+/-}$, $n = 40$ for *Lmx1b*$^{-/-}$. (*B*) *Lmx1b*$^{+/+}$ females sniffed intact male genital odor longer than castrated male genital odor (o); *Lmx1b*$^{+/-}$ and *Lmx1b*$^{-/-}$ females did not show preference between genital odors of intact males and castrated males. (*C*) Compared with their *Lmx1b*$^{+/+}$ and *Lmx1b*$^{+/-}$ female littermates, a smaller percentage of *Lmx1b*$^{-/-}$ females sniffed intact male genital odor longer than castrated male genital odor.

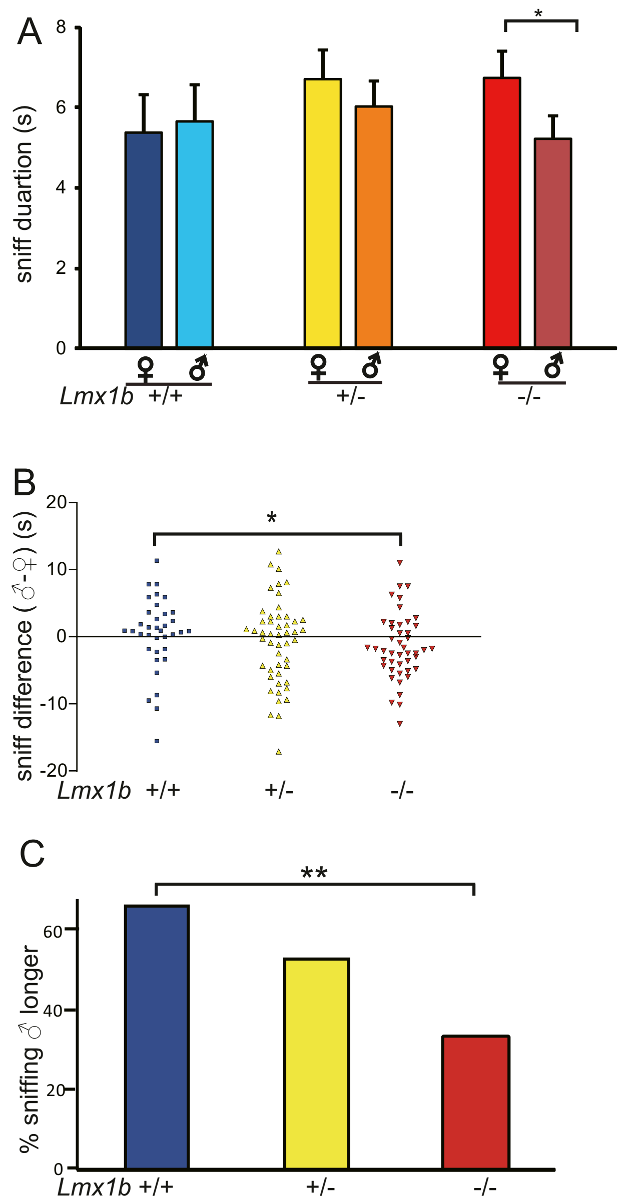

Fig. S4. Genital odor preference of females. A test female was presented with a slide smeared with male genital excretion and diestrous female genital excretion. $n = 36$ for *Lmx1b*$^{+/+}$ ($^{+/+}$), $n = 49$ for *Lmx1b*$^{+/-}$ ($^{+/-}$), $n = 45$ for *Lmx1b*$^{-/-}$ ($^{-/-}$). $*P < 0.05$, $**P < 0.01$. (*A*) *Lmx1b*$^{-/-}$ females preferred the genital odor of diestrous females over that of males. (*B*) Analysis of difference in sniff duration in females. (*C*) Percentage of females sniffing male genital odor longer than that of diestrous female.

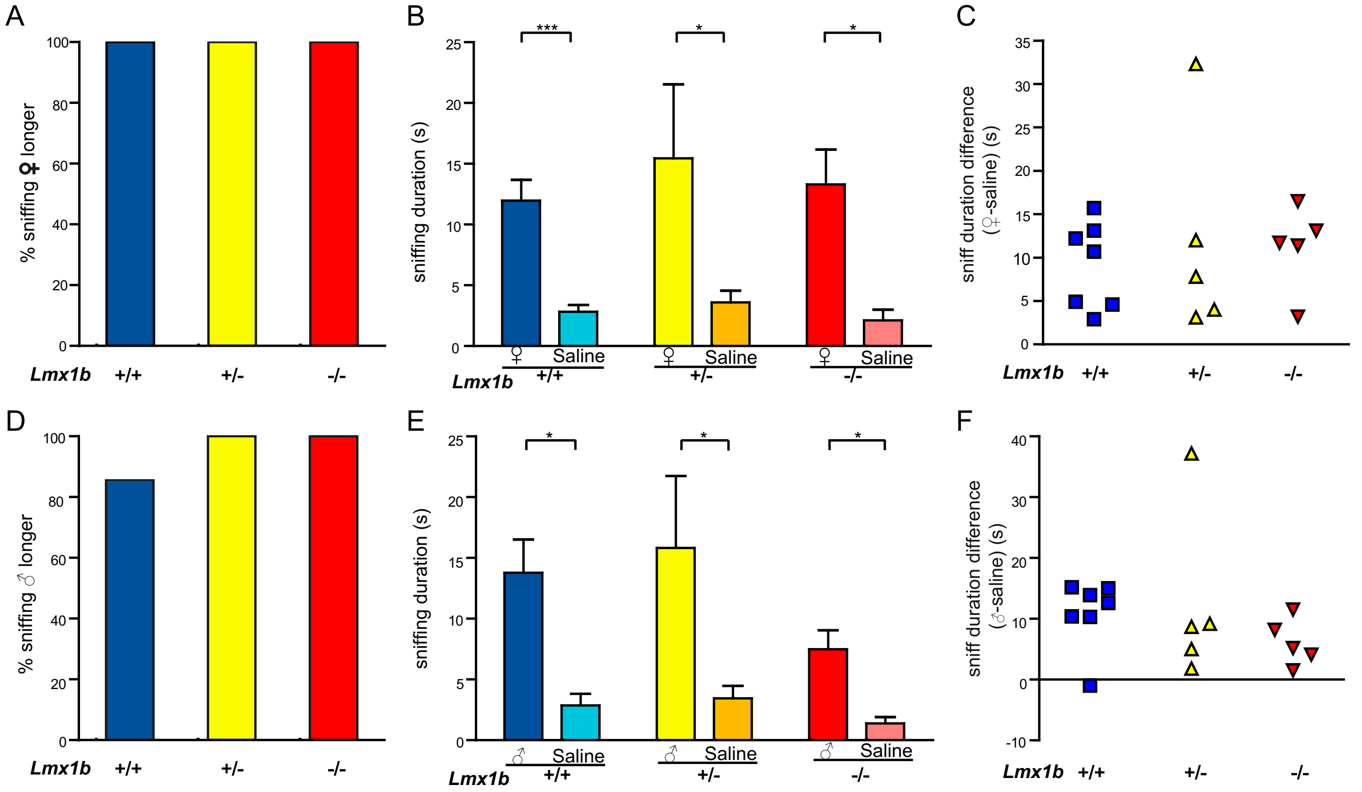

Fig. S5. Odor preference of female mice. $n = 7$ for *Lmx1b*$^{+/+}$, $n = 5$ for *Lmx1b*$^{+/-}$, $n = 5$ for *Lmx1b*$^{-/-}$. (*A*–*C*) When a female mouse was presented with a choice of female genital odor and saline, it preferred female genital odor over saline. This was not affected by the genotype of *Lmx1b*. (*D*–*F*) All test female mice, regardless of their *Lmx1b* genotype, preferred male genital odor over saline.

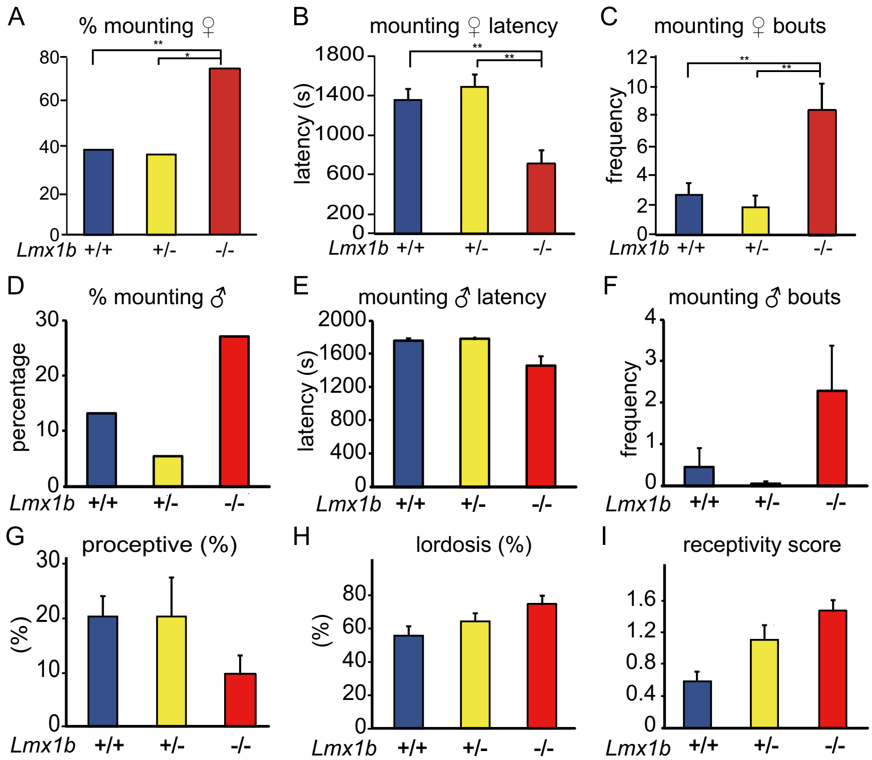

Fig. S6. Sexual behaviors of *Lmx1b*$^{-/-}$ females. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (*A–C*) Diestrous *Lmx1b*$^{-/-}$ females mounted WT females. A diestrous female was presented with a target WT female and female–female mounting was analyzed. (*A*) A higher percentage of diestrous *Lmx1b*$^{-/-}$ ($^{-/-}$, $n = 28$) females than their *Lmx1b*$^{+/+}$ ($^{+/+}$, $n = 34$) and *Lmx1b*$^{+/-}$ ($^{+/-}$, $n = 19$) diestrous female littermates mounted WT target females. (*B*) Female mounting latency was shorter for *Lmx1b*$^{-/-}$ diestrous females than their *Lmx1b*$^{+/+}$ and *Lmx1b*$^{+/-}$ littermates. (*C*) Female mounting frequency of diestrous *Lmx1b*$^{-/-}$ females was higher than their diestrous female *Lmx1b*$^{+/+}$ and *Lmx1b*$^{+/-}$ littermates. (*D–F*) A test female was analyzed for its mounting of a male (+♂), $n = 11$ for *Lmx1b*$^{+/+}$, $n = 18$ for *Lmx1b*$^{+/-}$, $n = 23$ for *Lmx1b*$^{-/-}$. (*G–I*) A test female was presented with a male, $n = 27$ for *Lmx1b*$^{+/+}$, $n = 8$ for *Lmx1b*$^{+/-}$, $n = 19$ for *Lmx1b*$^{-/-}$. *$P < 0.05$, **$P < 0.01$. (*D*) Female–male mounting percentage was not significantly different among *Lmx1b*$^{-/-}$, *Lmx1b*$^{+/+}$, and *Lmx1b*$^{+/-}$ littermates. (*E*) Female–male mounting latencies were not different. (*F*) Female–male mounting bouts were not different among estrous *Lmx1b*$^{-/-}$ and their female *Lmx1b*$^{+/+}$ and *Lmx1b*$^{+/-}$ littermates. Both proceptive (*G*) and lordosis (*H*) quotients were not significantly different among test females. (*I*) The receptivity scores were not significantly different among the test females.

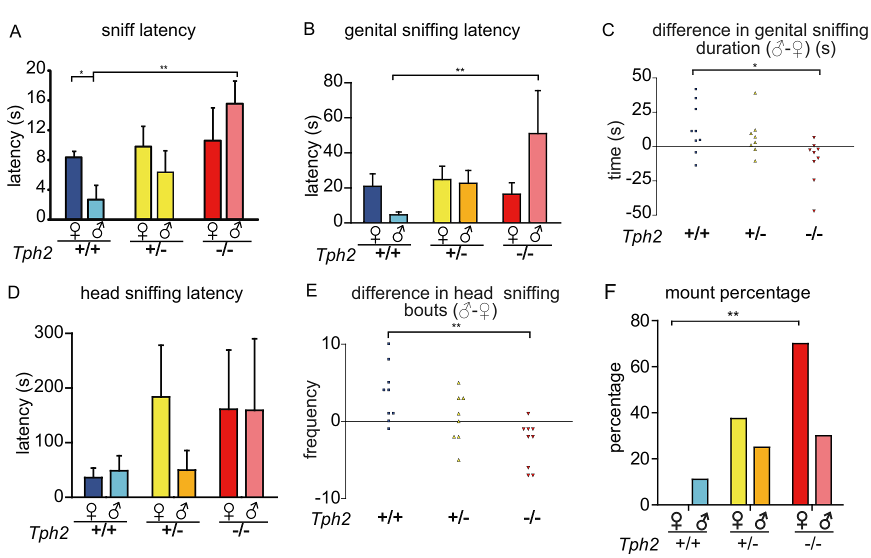

Fig. S7. Sexual preference of *Tph2* knockout female mice. Data are from the same experiments as those in Fig. 3. (*A*) *Tph2*$^{-/-}$ females sniffed male targets later than their *Tph2*$^{+/+}$ littermates. *Tph2*$^{+/+}$ females sniffed male targets earlier than female targets. (*B*) *Tph2*$^{-/-}$ females sniffed male genital later than their *Tph2*$^{+/+}$ littermates. (*C*) Genital sniffing duration was analyzed in individual females as the duration of sniffing males minus the duration of sniffing females. (*D*) Latencies for sniffing the head were not different between *Tph2*$^{-/-}$ and *Tph2*$^{+/+}$ littermates. (*E*) Head-sniffing bouts were analyzed in individual females as the duration of sniffing males minus the duration of sniffing females. (*F*) A higher percentage of female–female mounting occurred in *Tph2*$^{-/-}$ females than in their *Tph2*$^{+/+}$ littermates.

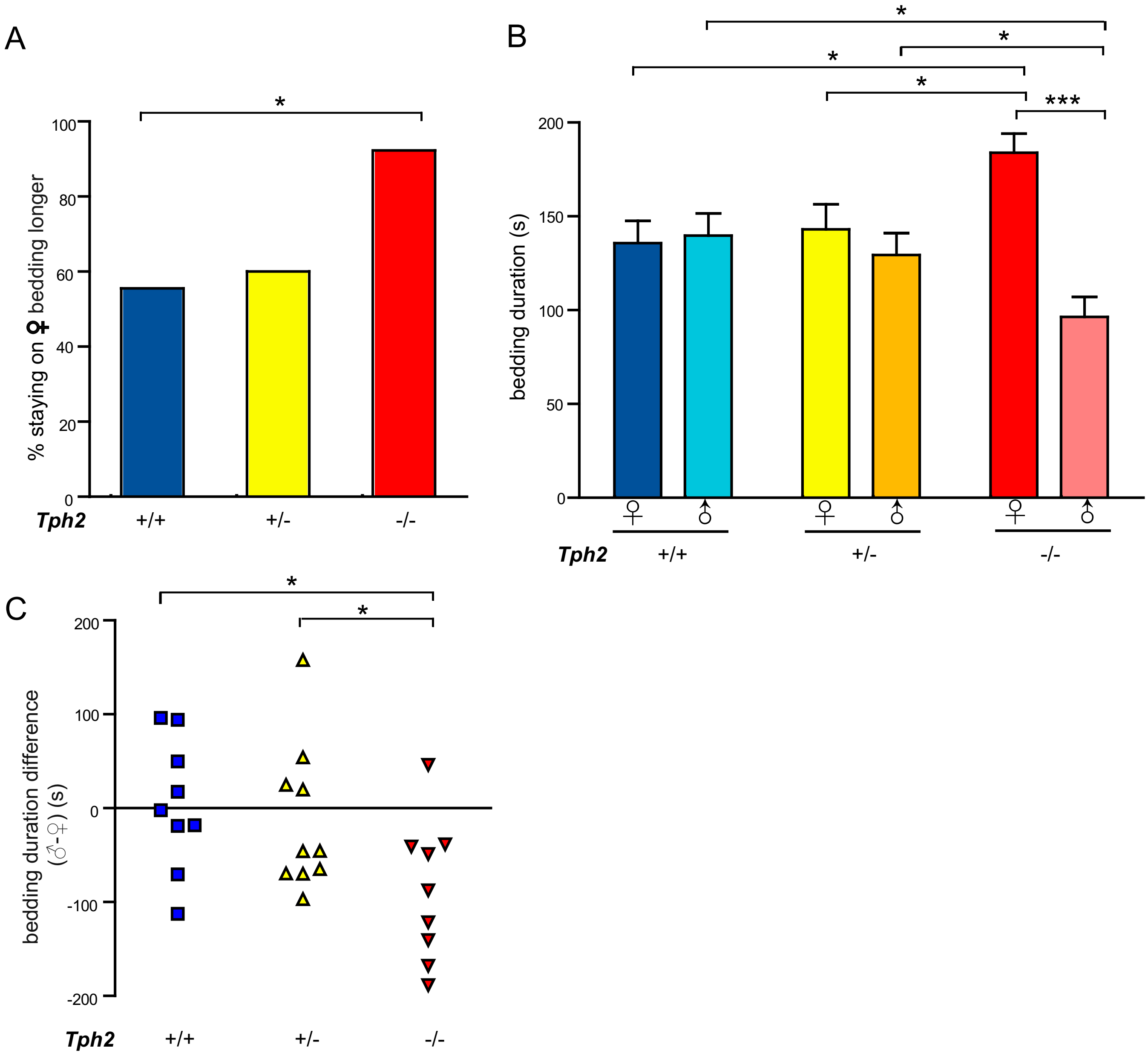

Fig. S8. Bedding preference of female mice. $n = 9$ for *Tph2*$^{+/+}$, $n = 10$ for *Tph2*$^{+/-}$, $n = 9$ for *Tph2*$^{-/-}$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (*A*) More *Tph2*$^{-/-}$ female mice than *Tph2*$^{+/+}$ or *Tph2*$^{+/-}$ females stayed on female bedding longer than on male bedding. (*B*) *Tph2*$^{-/-}$ female mice stayed on female bedding for longer duration than on male bedding. (*C*) Analysis of bedding preference in individual females: duration on male bedding minus duration on female bedding for the same mouse was calculated.

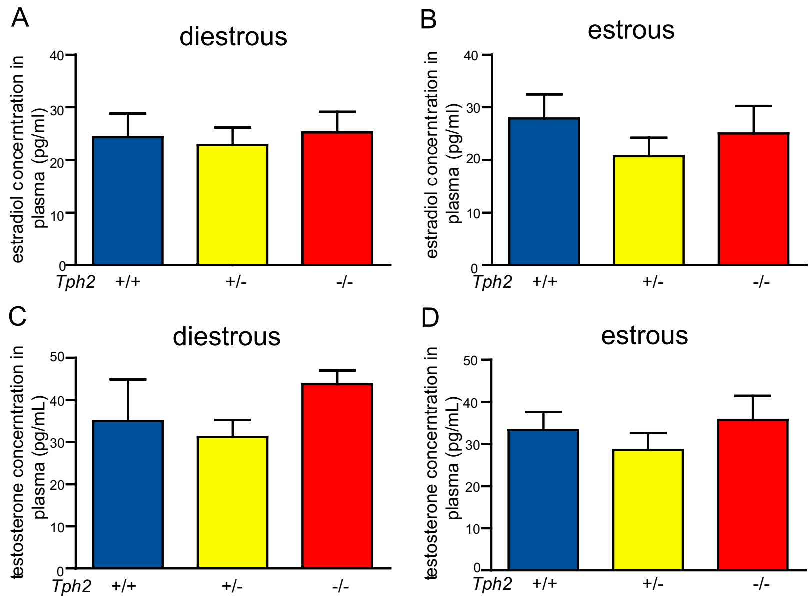

Fig. S9. Hormone concentrations in the plasma. (*A* and *C*) Diestrous females: $n = 6$ for *Tph2*$^{+/+}$; $n = 8$ for *Tph2*$^{+/-}$; $n = 8$ for *Tph2*$^{-/-}$. (*B* and *D*) Estrous females: $n = 6$ for *Tph2*$^{+/+}$; $n = 7$ for *Tph2*$^{+/-}$; $n = 7$ for *Tph2*$^{-/-}$. (*A*) Estradiol concentrations were not different among all indicated genotypes of diestrous females. (*B*) Estradiol concentrations were not different among all genotypes of estrous females. (*C*) Testosterone concentrations were not different among all genotypes of diestrous females. (*D*) Testosterone concentrations were not different among all genotypes of estrous females.

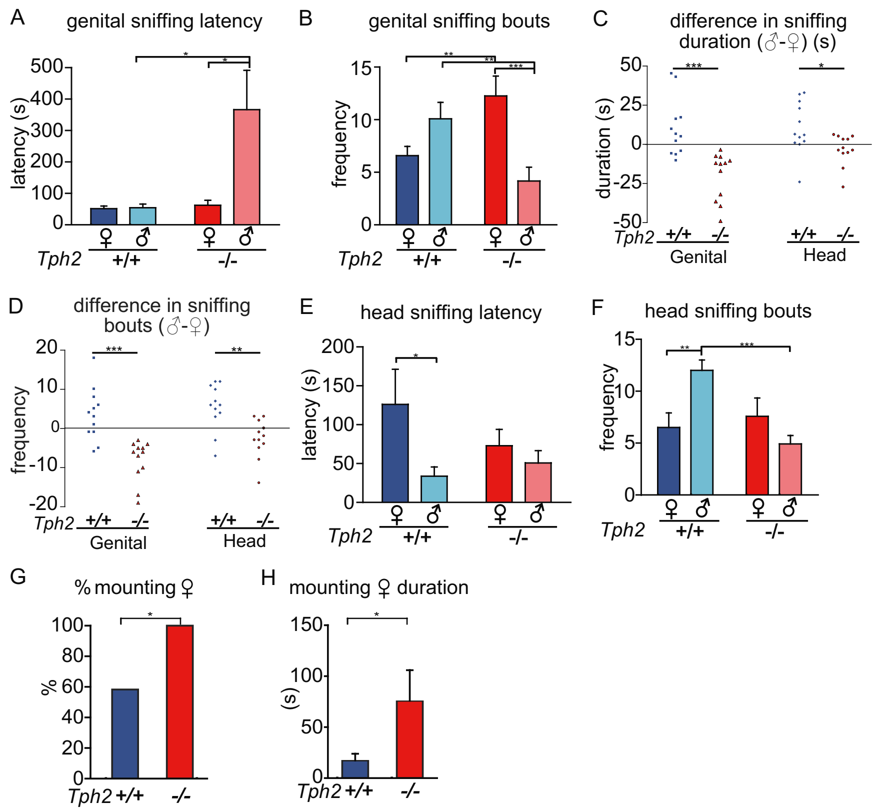

Fig. S10. Sexual preference of estradiol-treated ovariotomized (OVX+E) females. Data are from the same experiments as those in Fig. 5. (*A*) OVX+E *Tph2*$^{-/-}$ females sniffed female genital area earlier than male genital area. OVX+E *Tph2*$^{-/-}$ sniffed male genitals later than their OVX+E *Tph2*$^{+/+}$ littermates. (*B*) OVX+E *Tph2*$^{-/-}$ females sniffed female genitals more frequently than male genitals. OVX+E *Tph2*$^{-/-}$ sniffed female genitals more frequently than their OVX+E *Tph2*$^{+/+}$ littermates. OVX+E *Tph2*$^{-/-}$ sniffed male genitals less often than their OVX+E *Tph2*$^{+/+}$ littermates. (*C*) Genital or head sniffing duration was analyzed in individual females as the duration of sniffing males minus the duration of sniffing females. (*D*) Genital or head sniffing bouts were analyzed in individual females as the bouts of sniffing males minus the bouts of sniffing females. (*E*) OVX+E *Tph2*$^{+/+}$ females sniffed male heads earlier than female heads, whereas OVX+E *Tph2*$^{-/-}$ female mice did not show preference. (*F*) OVX+E *Tph2*$^{-/-}$ females sniffed male heads less than their littermates. OVX+E *Tph2*$^{+/+}$ females sniffed male heads more than female heads, whereas OVX+E *Tph2*$^{-/-}$ females did not display preference. (*G*) A higher percentage of female–female mounting occurred in OVX+E *Tph2*$^{-/-}$ females than that in their OVX+E *Tph2*$^{+/+}$ littermates. (*H*) *Tph2*$^{-/-}$ females mounted female targets longer than their *Tph2*$^{+/+}$ littermates.

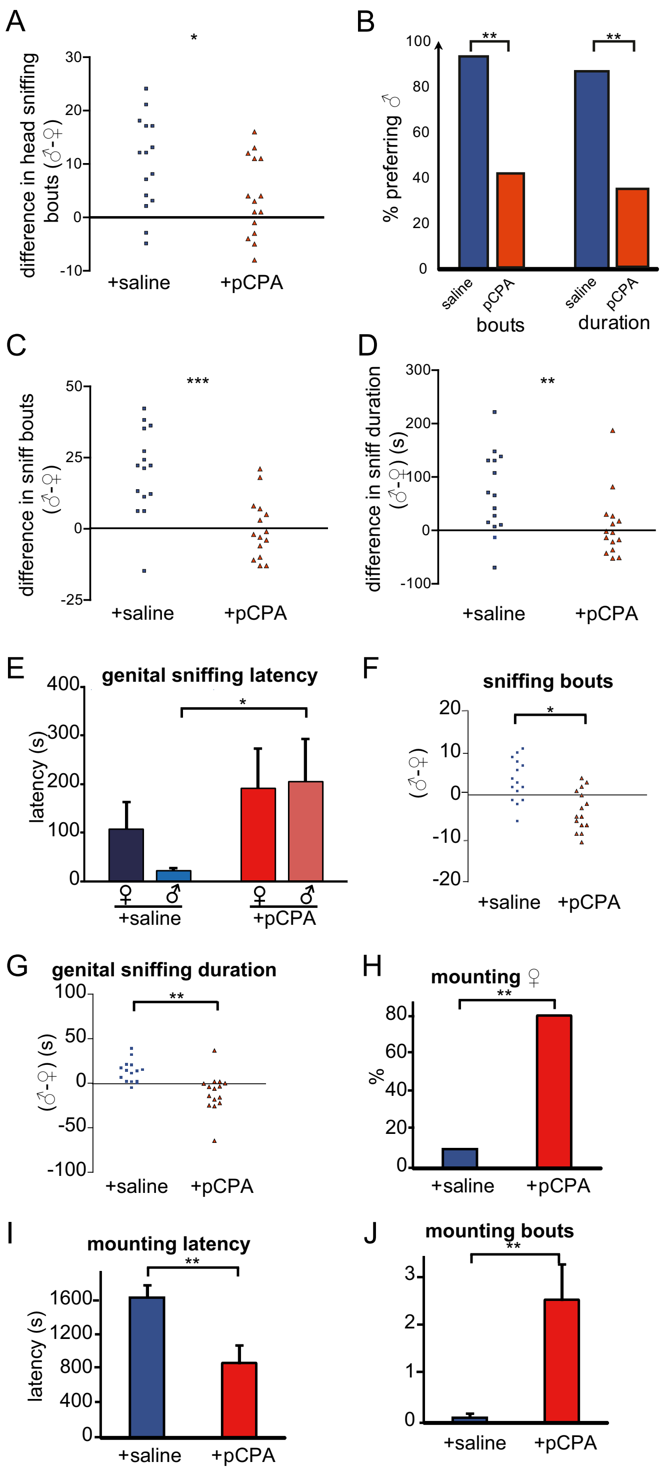

Fig. S11. (*A–D*) Sexual preference of females treated with pCPA. (*A–G*) Each C57BL/6J female treated with either saline (+saline, *n* = 15) or pCPA (+pCPA, *n* = 15) was presented with a male and a female target mouse. (*H–J*) Each adult C57BL/6J female was treated with saline (*n* = 10) or pCPA (*n* = 10) and presented with a female. (*A*) The preference for male heads in sniffing bouts by females was significantly reduced by pCPA. (*B*) The percentage of female preferring (the whole body of) males in bouts or duration was significantly reduced by pCPA. (*C*) Analysis of difference in sniff bouts of individual females. (*D*) Analysis of difference in sniff duration of individual females. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. (*E*) The latency for sniffing male genitals is significantly later in pCPA-treated females than in control females. (*F* and *G*) Analysis of differences in genital sniffing bouts and duration in individual females. (*H–J*) pCPA-treated females fiercely mounted females. (*H*) pCPA increased the percentage of females mounting intruder females. pCPA decreased mounting latency (*I*) and increased mounting bouts (*J*) of females. Data are from the same experiments as those in Fig. 6.

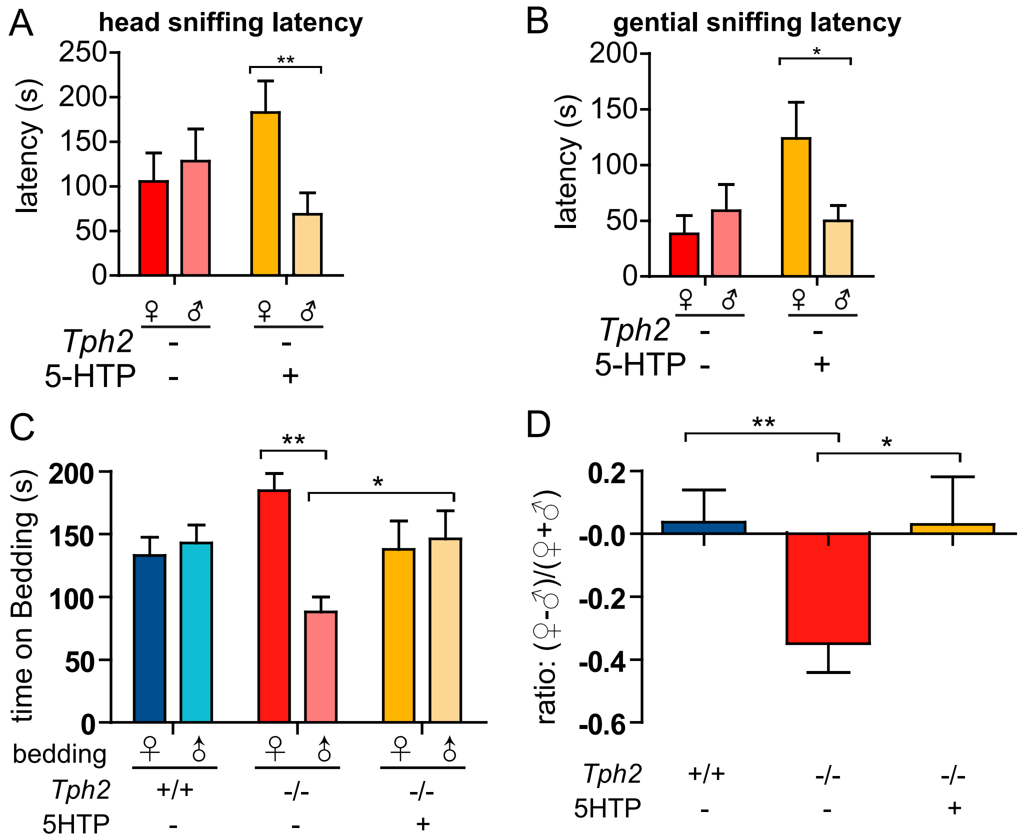

Fig. S12. 5-HTP rescue of sexual preference of adult females in sniff latency and bedding preference. (*A* and *B*) Data are from the same experiments as those in Fig. 6 *H*–*K*. (*A*) Injection of 5-HTP rescued the same-sex preference in head-sniffing latency of *Tph2*$^{-/-}$ females. (*B*) Injection of 5-HTP could rescue the same-sex preference in genital sniffing latency of *Tph2*$^{-/-}$ females. (*C* and *D*) Bedding preference of females treated with 5-HTP. *Tph2*$^{+/+}$ females were treated with saline (n = 11). *Tph2*$^{-/-}$ females were treated with saline (n = 12) or 5-HTP (n = 12). *$P < 0.05$, **$P < 0.01$. (*C*) *Tph2*$^{-/-}$ females strongly preferred female over bedding. 5HTP rescued the bedding preference of *Tph2*$^{-/-}$ females. (*D*) Compared with *Tph2*$^{+/+}$, a higher percentage of *Tph2*$^{-/-}$ females spent more time above female bedding than male bedding. 5HTP rescued the bedding ratio of *Tph2*$^{-/-}$.