Supplementary Figure 1 Generation of conditional D2 mutant mice Drd2\textsuperscript{loxP/loxP}. (a) Strategy to flank Drd2 exon 2 with loxP sites. Two loxP sites flanking Drd2 exon 2 were inserted by targeted mutagenesis in J1 129S4/Jae ES cells. First, a 129SvEv mouse genomic library (generously provided by P. Soriano) was screened using a mouse Drd2 exon 2 [\textsuperscript{32P}]-radioactive probe. One positive phage clone was used to isolate the 5’ (6.5 kb) and 3’ arms (2 kb) of homology. Two loxP sites were subcloned flanking exon 2, which contains the translation initiation codon, using standard techniques. A PGK-neo cassette, also flanked by loxP sites, was used as a positive selectable marker in the presence of G418 (300 mg/ml) whereas a PGK-thymidine kinase cassette subcloned at the 3’ end of the construct was used for negative selection in the presence of gancyclovir 2 mM. Homologous recombination at the 5’ and 3’ arms was assayed in resistant clones using a Southern blot hybridization strategy in which genomic DNA was cut with XbaI or EcoRI, respectively. Karyotypic and phenotypic evaluation of positive clones was performed to select two clonal targeted ES cells that were then microinjected into C57Bl/6J blastocysts. Two male chimeras showed germline transmission. F1 heterozygotes were crossed to B6.FVB-Tg(Ella-cre)C5379Mmgd/J (Jackson Laboratory, USA) to remove the PGK-neo cassette. Type II deletion events were identified in F2 heterozygotes by Southern blot hybridization using XbaI and EcoRI and a PCR strategy. The conditional allele Drd2\textsuperscript{loxP} (type II deletion) and the null allele Drd2\textsuperscript{null} (type I deletion) were produced by breeding Drd2\textsuperscript{loxP-Necl/+} and Ella-cre transgenic mice. Blue triangles indicate loxP sites. (b) Probes A (red rectangle) and B (blue rectangle) were used in Southern blot hybridization of DNA digested with XbaI (X) or EcoRI (E) to identify homologous or Cre-mediated recombination at the 5’ and 3’ arms of the targeted insertions, respectively. Diagnostic DNA band sizes are indicated in a and b. (c) In situ hybridization performed in brain and pituitary coronal slices of Drd2\textsuperscript{+/+} and Drd2\textsuperscript{loxP/loxP} mice. (d) Drd2\textsuperscript{+/+} and Drd2\textsuperscript{loxP/loxP} mice were backcrossed for five generations to C57Bl/6J mice and incipient congenics (n = 5) were used in its initial characterization with Drd2\textsuperscript{+/+} mice. Homozygous mice carrying Drd2 floxed alleles (Drd2\textsuperscript{loxP/loxP}) obtained from type II deletion of the original floxed allele and were compared with their wild-type siblings (Drd2\textsuperscript{+/+}) in spontaneous locomotor activity. Mice carrying floxed Drd2 alleles are phenotypically indistinguishable from their wild-type siblings. Drd2\textsuperscript{loxP/loxP} mice are catalogued under the name Drd2\textsuperscript{loxP/loxP}.
Supplementary Figure 2  Mice carrying floxed Drd2 alleles are phenotypically indistinguishable from their wild-type siblings. Homozygous mice carrying Drd2 floxed alleles (Drd2loxP/loxP), obtained from type II deletion of the original triple floxed allele, were compared with their wild-type siblings (Drd2wt) in terms of their (a) spontaneous locomotor activity of type I deletion Drd2wt mutant mice is reduced while Drd2loxP/loxP mice show normal locomotor scores; (b) locomotor activity 30 min after a single injection of 0.6 mg/kg of haloperidol in Drd2wt mutant mice and their wild-type siblings; (c) time of immobility in the bar test 30 min after a single injection of 1.5 mg/kg of haloperidol; (d) locomotor activity 30 min after a single injection of 0.6 mg/kg of haloperidol in Drd2loxP/loxP mice and their wild-type Drd2wt siblings; (e) body weight and (f) body size. No statistical differences were observed between Drd2wt and Drd2loxP/loxP mice in any of the evaluated parameters. In parallel, mutant mice obtained from the type I deletion (Drd2–/–) were also compared with their wild-type siblings (Drd2wt) and with Drd2loxP/loxP age- and gender-matched mice. Drd2–/– mice showed the expected mutant phenotype, based on observations made in previous studies using a constitutive Drd2 knockout strain. Statistically significant differences were obtained in each evaluated parameter compared to the Drd2wt and Drd2loxP/loxP mice. When included, heterozygous null Drd2–/– and Drd2loxP/loxP mice showed no differences from their corresponding WT strains in all tests. Post-hoc comparisons of Fisher LSD, * P < 0.05 for treatment effects within a genotype; † P < 0.05 genotype effect Drd2wt vs. Drd2–/–. N2 and N4 indicate two or four generations of backcrossing, respectively, with the C57BL/6J strain. Drd2loxP/loxP mice were backcrossed for five generations to C57BL/6J mice and inipient congenics (n = 5) were used in this characterization compared to Drd2wt mice.
Supplementary Figure 3 Molecular and physical characterization of autoDrd2KO mutant mice. (a) Non-radiative in situ hybridization using a digoxigenin-labeled Drd2 exon 2 antisense riboprobe. AutoDrd2KO mice lack dopamine D2 receptors express Drd2 in striatum and pituitary but showed no expression in midbrain. (b) Body growth curves. Males and females of both genotypes were weighed from the third week postnatal until the eleventh week. No differences were found between genotypes. (RMA genotype: $F_{1,30} = 1.20, P = 0.28$; time: $F_{8,240} = 13.76, P < 0.001$).
Supplementary Figure 4 Behavioral characterization of autoDrd2KO mice. (a-d) Approach/avoidance conflict tests. Adult mice from both genotypes were tested under different paradigms related to anxiety-like behavior. (a) Mice of both genotypes were tested in an Elevated Plus Maze for 5 min. The graph shows the number of entries into the open or closed arms of the maze and the number of times crossing the center in 5 min experiment. No significant differences were observed between the two genotypes number of entries to open or closed arms (OWA open arms: $F_{1,25} = 2.83$, $P = 0.10$; OWA closed arms: $F_{1,25} = 1.72$, $P = 0.20$). The number of times an animal passes through the center is significantly higher in autoDrd2KO mice compared to Drd2loxP/loxP mice, a parameter that usually correlates with hyperactive animals (OWA $F_{1,25} = 4.65$, *P* < 0.05). (b-d) in the light-dark test mice were placed inside the dark compartment separated from the lighted area by a small door. (b) Latencies to enter the light area; (c) number of entries in the light area and (d) total time spent in the light area were registered. AutoDrd2KO mice showed similar parameters in all variables measured (OWA latency: $F_{1,6} = 1.26$, $P = 0.30$, OWA entries: $F_{1,6} = 0.33$, $P = 0.58$; OWA time light area: $F_{1,6} = 0.28$, $P = 0.61$). (e) Novel Object Task. Drd2loxP/loxP and autoDrd2KO mice were able to recognize the new object similarly, since more visits to the new object (ball) than to the known object (tube) were observed during the 5 min test. (f) Mice from both genotypes showed similar skills to perform on a rotarod. Trained mice for 3 days at 16 rpm were tested on the rotarod with an accelerating speed protocol (4 - 40 rpm). The figure shows the latency to fall. No differences were found between genotypes (OWA genotype: $F_{1,11} = 0.75$, $P = 0.40$).