Supplementary information

Ptchd1 deficiency induces exciatory synaptic and cognitive dysfunctions in mouse

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The following supplementary tables detailing the RNA sequencing analysis of brain hippocampal trranscriptome in wt and *Ptchd1*$^{-/y}$ mice are available in GEO database under record reference GSE80312: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=ijkrucskzpuxvon&acc=GSE80312

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Supplementary online methods

Ptchd1 gene neurodevelopmental expression profile in mouse.

TissueScan qPCR-ready cDNA Arrays (Origene, Rockville, MD, USA) were used to screen for *Ptchd1* expression in 48 different brain tissues using Soadvance syber (Bio-Rad) gene expression assays and following manufacturer's instructions. These arrays contained pre-standardized amounts of cDNA extracted from 48 independent mouse brain tissues covering 3 embryonic (E13, E15, E18), 1 postnatal (P7) and 1 adult (P35) developmental stages. The cDNA was amplified by PCR by using primers specifi for *Ptchd1* gene (sense, located in exon 1 : 5'-GCCAACATGCTAGACCAACA-3' ; antisense, located in exon 2 : 5'-CCCGAGCATTCTTTAGCTCTT-3').

Ptchd1 plasmid constructs

The full-length sequence of *Ptchd1* mouse cDNA was amplified from the IMAGE cDNA clone 40095445 (Source BioSience, Nottingham, United Kingdom) and cloned in pAcGFP1-N or in pCAG-GFP vectors using In-Fusion cloning strategy (Takara Bio Europe, Saint-Germain-en-Laye, France) to generate PTCHD1-GFP with GFP tag at the C-terminal end. PTCHD1delCter-GFP and PTCHD1del874_888-GFP constructs were generated by PCR and In-Fusion cloning leading to the removal of the last 39 amino-acids (predicted C-teminal intracellular tail) or the last 15 amino-acids (including the predicted PDZ-domain binding site) respectively. Regarding the pulldown experiments, the full-length sequence of the C terminal intracellular end (39 amino-acids) of *Ptchd1* was amplified from the IMAGE cDNA clone 40095445. A PCR amplicon lacking the last 12 nucleotides leading to the loss of the PDZ-domain binding site was also generated. The PCR products were cloned into pGEX-4T1 (GE Healthcare, Velizy-Villacoublay, France).

GST pulldown assay

The pGEX-4T1, pGEX-4T1-PTCHD1WT (full-length Cter tail) and pGEX-4T1-PTCHD1delITTV plsmaid constructs were expressed in *Escherichia coli* BL21 Lys cells (Fisher Scientific, Illkirch, France) after

bacterial transformation. Pull-down assay were performed using Pierce TM GST Protein interaction Pull-down kit (Fisher scientific). Bacterial lysates (bait) were incubated during 30 minutes and immobilized by affinity on glutathione resin after 3 hours following IPTG induction. Synaptoneurosomal lysates (prey) were extracted from adult WT C57Bl6 mouse cortex (Janvier labs, St-Berthevin, France) using SYn-PER kit (Fisher Scientific). Immobilized GST-fusion proteins were incubated for 16h at 4°C with prey lysates. Prey flows-through were retrived after 5 washes, and 80µL of Glutathione elution buffer were applied on each spin column. Pull-down samples were processed for SDS-PAGE at 150V during 1 hour. Permanent gel staining using Coomassie Blue (Fisher Scientific) was performed as a control for protein expression level.

SDS-PAGE gels were blotted on PVDF membrane using TransBlot Turbo (Bio-Rad, Marnes-la-Coquette, France) for 3 minutes following manufacturer's conditions. Ponceau red staining was executed and followed by blocking the membrane for 1 hour with 5% milk diluted in TBS-Tween (0,05% tween 20).

Primary antibodies for GST and for Immunocytochemistry (mouse monoclonal clone 8-326, catalog number MA4-004, Fisher Scientific, 1/1000), PSD95 (mouse monoclonal clone 6G6-1C9, catalog number MA1-045, Fisher Scientific, 1/2000), SAP102 (mouse monoclonal clone N19/2, catalog number 75-058, UC Davis/NIH NeuroMab Facility, USA 1/1000) and RACK1 (mouse monoclonal B-3, catalog number sc-17754, Santa Cruz, Heidelberg, Germany, 1/500) were incubated overnight in 1% milk in TBST buffer. After 3 washes (5 minutes) with TBST, membranes were incubated with corresponding secondary HRP-coupled antibodies (Goat anti-mouse, catalog number W402B, Promega, Charbonnières-les-bains, France, 1/2500) for 45 minutes at room temperature. Chemiluminescence revelation was done using ECL purity kit (Bio-rad) and the membranes were visualized in a Chemidoc Touch imaging system (Bio-Rad).

Luciferase Assay

Ptch1-deficient Mouse Embryonic Fibroblasts (MEFs) were cultured with DMEM and 10% Fetal

Bovine Serum (FBS), and were tested negative for mycoplasma contamination. The cells were transfected with 400ng of the 8X-Gli-Luciferase firefly reporter transgene, 40ng of a constitutive renilla luciferase transgene and 100 ng of either Ptch1, PtchD1-GFP or control GFP vector using FuGENE 6 (4:1 ratio; Promega) according to the manufacturer's instructions. After 24 hours, cells were switched to 0.5% FBS. After an additional 48 hours, cell lysates were taken and luciferase activities were determined using the Dual-Luciferase Reporter Assay System (Promega) according to manufacturer's instructions. Data were evaluated using a one-way ANOVA test followed by a pairwise comparison of means using a Student's t-test (with Bonferroni correction for multiple comparisons).

Generation of *Ptchd1*$^{-/y}$ knockout mouse model

The *Ptchd1* mutant mouse line was established at the PHENOMIN MCI/ICS (Mouse Clinical Institute - Institut Clinique de la Souris-, Illkirch, France; http://www-mci.u-strasbg.fr). The targeting vector PRPGS00100_C_H03 was purchased from KOMP (www.komp.org) and amplified at the ICS. This vector contains a flipped STOP cassette that comprises an Engrailed 2 (En2) splice acceptor follow by an IRES sequence and a *LacZ* cDNA as well as a floxed human b-actin promoter driven Neo resistance cassette. The critical *Ptchd1* exon 2 is flanked by loxP site (**Fig. 2a**). The linearized construct was electroporated in C57BL/6N mouse embryonic stem (ES) cells. After G418 selection, targeted clones were identified by long-range PCR using external primers and further confirmed by Southern blot with an internal Neo probe as well as 5' and 3' external probes. Two positive ES clones were injected into BALB/cN blastocysts. Resulting male chimeras were bred with wild type C57BL/6N females in order to obtain germline transmission of the tm1a allele. The complete deletion in the *Tm1b* allele, keeping the *lacZ* reporter with a polyA inserted was recovered by breeding F1 *Tm1a*/y males with a *Cre* delete line[1].

Mouse handling

Mice were handled in accordance with the European Council Guidelines for the Care and Use of Laboratory animals in our facility (A67-218-40). Dr Y. Hérault was granted the accreditation 67-369 to perform the reported experiments. All animals were treated in compliance with animal welfare policies from the French Ministry of Agriculture (law 87 848). The approvals of our experimental protocols were obtained from our local ethical committee through the accreditation numbers 2012-139 and 2014-024. For all these tests, mice had free access to water and food (D04 chow diet, Safe, Augy, France). The light cycle was controlled as 12 h light and 12 h dark (lights on at 7 am) and the temperature was maintained at 23±1 °C.

Behavioral studies were performed between 3 and 4 months of age. Mice were transferred from the animal housing facility to the phenotyping area at least 1 week before testing. All experiments were performed between 9:00 AM and 4:00 PM. To study the behaviour of animals, we crossed transgenic heterozygote female *Ptchd1*$^{tm1b/+}$, noted *Ptchd1*$^{+/-}$, with wild-type C57BL/6N male to generate the cohort 1 of males. For the cohort 2, we crossed males hemizygous *Ptchd1*$^{tm1b/y}$ or (*Ptchd1*$^{-/y}$) with heterozygous females to produce hemizygous *Ptchd1*$^{-/y}$ males and homozygous *Ptchd1*$^{-/-}$ females.

Cohort 1 with 11 *Ptchd1*$^{-/y}$ and 12 wildtype (wt) littermates was studied at 13 to 17 weeks for behavior. Tests were run in the following order: Circadian activity, elevated plus maze, novel object recognition, modified SHIRPA, grip test, Rotarod test, Social recognition test, Y maze spontaneous alternation, auditory startle reflex reactivity and Pre Pulse inhibition (PPI), Pavlovian fear conditioning, Hot plate test and Shock threshold test that are described in the supplementary information.

A second cohort of 8 *Ptchd1*$^{-/y}$ and 8 wt males, and 8 *Ptchd1*$^{-/-}$ and 8 wt females went through the IMPC pipeline for broad based analysis (http://www.mousephenotype.org/impress).

Testing was performed following the ARRIVES recommendation[2,3]. For example randomization was used reduce the potential influence of order testing or equipment bias. In automated tests with multiple apparatus, WTs and mutants were tested simultaneously and randomized across arenas and

chambers (openfield, fear conditioning, PPI). For tests using one equipment or observation, the order of testing WTs and mutants was counterbalanced.

Mouse behavioral analyses

Gross neurological examination: The general health and basic sensory motor functions were evaluated using a modified SHIRPA protocol (EMPRESS, eumorphia.org). It provides an overview of physical appearance, body weight, body temperature, neurological reflexes and sensory abilities.

Rotarod test: This test measures the ability of an animal to maintain balance on a rotating rod (Bioseb, Chaville, France). Mice were given three testing trials during which the rotation speed accelerated from 4 to 40 rpm in 5 min. Trials were separated by 10-15 min interval. The average latency was used as index of motor coordination performance.

Grip test: This test measures the maximal muscle strength (g) using an isometric dynamometer connected to a grid (Bioseb). Each mouse was submitted to 3 consecutive trials immediately after the modified SHIRPA procedure. Once the animal was holding the grid with its all paws it was slowly moved backwards until it released it.

Hot plate test: The mice were placed into a glass cylinder on a hot plate adjusted to 52°C (Bioseb). The latency of the first reaction (licking, flinches, …) was recorded, with a maximum of 30 sec.

Shock threshold test: The mouse is placed in the fear-conditioning chamber and allowed to habituate for 30 sec. Foot-shock is then manually applied for 1sec, and behavioral responses noted. Shock levels began at 0.05 mA, and increased in 0.05 mA steps with 30 sec interval between the shocks, until both flinch (any detectable response) and vocalization are induced. After this point, shocks were increased in 0.1mA steps until a jump (the mouse flinches such that the two hind paws leave the ground) is induced. A 1mA cut-off is employed in this test.

Circadian Activity and Ingestive Behaviours: Spontaneous locomotor activity and rears were measured using 24 individual boxes equipped with infra-red captors. The quantity of water and food consumed was measured during the test period using automated pellet feeder and lickometer

(Imetronic, Pessac, France). Mice were tested for 35 hours in order to measure habituation to the apparatus as well as nocturnal and diurnal activities. Results are expressed per 1 h periods.

The open field test: Mice were tested in automated open fields (Panlab, Barcelona, Spain), each virtually divided into central and peripheral regions. The open fields were placed in a room homogeneously illuminated at 70 Lux. Each mouse was placed in the periphery of the open field and allowed to explore freely the apparatus for 30 min, with the experimenter out of the animal's sight. The distance traveled, the number of rears, and time spent in the central and peripheral regions were recorded over the test session. The number of entries and the percentage of time spent in center area are used as index of emotionality/anxiety

Elevated plus maze: The apparatus used was completely automated and made of PVC (Imetronic, Pessac, France). It consisted of two open arms (30 X 5 cm) opposite one to the other and crossed by two enclosed arms (30 X 5 X 15 cm). The apparatus was equipped with infrared captors allowing the detection of the mouse in the enclosed arms and different areas of the open arms. The number of entries into and time spent in the open arms were used as an index of anxiety. Closed arm entries were used as measures of general motor activity. The number of rears in the closed arms, as well as ethological parameters such as stretching, attempts and head dips, was also automatically scored.

Social recognition test: This task is used to evaluate the preference of a mouse for a congener as compared to an object placed in an opposite compartment. Reduced social behaviour is observed in psychiatric disorders and mental retardation.

The apparatus is a transparent runway composed with a central starting box and 2 goal boxes delimited by a sliding grid at each extremity of the runway.

Testing was performed for 2 consecutive days. On the first day, fresh bedding was placed in the goal boxes. The mouse was placed in start box for 30 sec then allowed to explore freely the apparatus for 10 min in order to attenuate their emotionality and to evaluate any potential preference between the two compartments. On the second day, a C57Bl/6 congener from the same gender was placed in one goal box and an object (dice for example) placed in the opposite one. The mouse was then placed in

the start box for 30 sec then allowed to explore freely the apparatus for 10 min. The position of the congener and object boxes was counterbalanced to avoid any potential spatial preference. The duration of exploration of each goal box (when the mouse is sniffing the grid delimiting the goal box) was measured and the percentage of time the mouse takes to explore the congener was used as index of social preference (recognition preference). The number of entries and the time spent in each goal arm and vertical activity in each arm were also measured. A social recognition index (SR) is defined as (time Congener / (time Object + time Congener)) x100.

Auditory Startle Reflex Reactivity and Pre-Pulse Inhibition*:* Acoustic startle reactivity and pre-pulse inhibition of startle were assessed in a single session using standard startle chambers (SR-Lab Startle Response System, San Diego Instruments, USA). Ten different trial type were used: acoustic startle pulse alone (110-db), eight different prepulse trials in which either 70, 75, 85 or 90-dB stimuli were presented alone or preceded the pulse, and finally one trial (NOSTIM) in which only the background noise (65 dB) was presented to measure the baseline movement in the Plexiglas cylinder. In the startle pulse or prepulse alone trials, the startle reactivity was analyzed and in the prepulse plus startle trials the amount of PPI was measured and expressed as percentage of the basal startle response.

Object recognition task: The object recognition task was performed in automated open fields (see above). The open-fields were placed in a room homogeneously illuminated at 70 Lux at the level of each open field. The objects to be discriminated were a glass marble (2,5 cm diameter) and a plastic dice (2 cm). Animals were first habituated to the open-field for 30 min. The next day, they were submitted to a 10-minutes acquisition trial during which they were placed in the open-field in presence of an object A (marble or dice). The time the animal took to explore the object A (when the animal's snout was directed towards the object at a distance ≤ 1 cm) is manually recorded. A 10-minutes retention trial is performed 3 h later. During this trial, the object A and another object B are placed in the open-field, and the times tA and tB the animal takes to explore the two objects are recorded. A recognition index (RI) is defined as (tB / (tA + tB)) x100.

Y-maze spontaneous alternation: The apparatus was a Y-maze made of Plexiglas and having 3 identical arms (40x9x16 cm) placed at 120° from each other. Each arm had walls with specific motifs allowing distinguish it from the others. Each mouse was placed at the end of one of the three arms, and allowed to explore freely the apparatus for 5min, with the experimenter out of the animal's sight. Alternations are operationally defined as successive entries into each of the three arms as on overlapping triplet sets (i.e., ABC, BCA ...). The percentage of spontaneous alternation was calculated as index of working memory performance. Total arm entries and the latency to exit the starting arm were also scored as indexes of ambulatory activity and emotionality in the Y-maze, respectively.

The pavlovian fear conditioning: Polymodal operant chambers (Coulbourn Instruments, Allentown, PA, USA) were used. Each chamber (18.5 x 18 x 21.5 cm) consisted of aluminum side walls and Plexiglas rear and front (the door) walls. A loudspeaker and a bright light constituted the sources of the cues during conditioning and cue-testing. The general activity of animals was recorded through the infrared cell placed at the ceiling of the chambers and was directly recorded on a PC computer using the Graphic State software (Coulbourn). For conditioning, mice were allowed to acclimate for 4 min, then a light/tone (10 kHz) CS was presented for 20 s and co-terminated by a mild (1 s, 0.4 mA) footshock (US). Mice were returned to their home cages 2 min later. Testing was performed 24 h following conditioning session. Testing for the context was performed in the morning. The mouse was placed back into the same chamber that was used for the conditioning and allowed to explore for 6 minutes without presentation of the light/auditory CS. Testing for the cue was performed in the afternoon (about 5 h after the context testing). The contextual environment of the chambers was changed (wall color, odor and floor texture). The mouse was placed in the new chamber and allowed to habituate for 2 minutes then presented with light/auditory cues for 2 minutes. This sequence was repeated once again. At the end of testing, animals were returned to their home cages.

Statistical analysis*:* Data were analyzed using unpaired Student t-test or repeated measures analysis of variance (ANOVA) with one between factors (genotype) and one within factor (time,…). Assumption for normality is made for tests used, classically known to follow normal distribution.

Homogeneity of variance is calculated for several sample parameters. Qualitative parameters (*e.g.* clinical observations) were analyzed using $\chi 2$ test. The level of significance was set at $p < 0.05$.

IMPC phenotypic analysis

Briefly, the cohort of animals was studied between 9 to 16 weeks of age. The following tests were performed on mice: Dysmorphology screen, Open field, Shirpa, Grip test, Rotarod, auditory startle reflex reactivity and PPI, Pavlovian fear conditioning, energy expenditure by indirect calorimetry, Electrocardiogram, Echocardiography, Glucose tolerance test, Challenge Whole Body Plethysmography (LPS challenge), Body composition & X-ray imaging, Auditory Brain Stem Response (ABR), Eye Morphology, Hot plate and Shock threshold. At 16 weeks old, blood collection was performed for hematology and clinical chemistry assessments. All the procedures are detailed in the following link (http://www.mousephenotype.org/impress).

RNA-seq libraries and analysis

Total RNA was Trizol extracted from 3 wild-type and 3 *Ptchd1*$^{-/y}$ male (P30) hippocampus samples. RNA was treated with DNase (Qiagen, Venlo, The Netherlands) and purified on the RNeasy MinElute Cleanup Kit (Qiagen). 2000 ng of Total RNA were treated with the Ribo-Zero rRNA Removal Kit (Human/Mouse/Rat; Illumina MRZH11124, Eindhoven, The Netherlands). Depleted RNA was precipitated 1hrs at -80C in three volumes of ethanol plus 1ug of glycogen. RNA was then washed and resuspended in 36 ul of rnase free water. RNA fragmentation buffer (NEBNext® Magnesium RNA Fragmentation Module, NEB, Leiden, The Netherlands) was added to the solution and the RNA was fragmented by incubation at 95C for 3 minutes. Reverse transcription: cDNA first strand synthesis was performed with random examer primers. cDNA second strand synthesis was performed with dUNTPs, to ensure strand specificity. The RNA-seq library was synthetized with KAPA Hyper prep kit (Kapa Biosystems, Wilmington, MA, USA): a treatment with USER enzyme (NEB, M5505L) was added to digest the unspecific strand. Libraries sequenced on illumina HiSeq and mapped with GSNAP

(version 2015-06-23), which yielded on average 45.2 mapped million (single-end) reads per sample. Gene expression counts were calculated with a custom C++ script (only tags in exons, not introns) and DEseq2 was used to call the deregulated genes. Data are deposited in GEO, ID GSE80312.

All the RNA-seq enrichment analysis of the manuscript are made from standard hypergeometric tests with bonferroni correction. Gene Ontology (GO) annotations are updated to 25/6/2015. MotifMap[4] and Cistrome (*www.cistrome.org*) experimental binding sites (ChIP-seq) were used. Briefly, the genome (version mm9) was annotated with the binding sites, either predicted by MotifMap or confirmed by experimental ChIP-seq in Cistrome, and the enrichment analysis (hypergeometric test, bonferroni corrected) was performed using as background all the 28782 gene promoters (+/- 2kb). The complete statistical data and the lists of transcription factors are fully available in **Supplementary Table 9**.

Cell-types enrichments

Cell-types enrichments are based on the single-cell RNAseq data from Amit Zeisel et al., 2015, "cell types in the mouse cortex and hippocampus revealed by single-cell rna-seq"[5]. In this work, single-cell RNAseq was used to measure trascriptomes of >3000 single cells, allowing to define the markers of 11 different cell types in adult (P21-P30) hippocampus and somatosensory cortex. Given that also our RNAseq is done on adult hippocampus, single-cell RNAseq data from Zeisel et al. becomes a highly valuable resource to gain insight at the cell type level. Here we performed standard hypergeometric tests with bonferroni correction against the cell-type markers derived from data of Zeisel et al. in order to evaluate the abundance of specific markers in *Ptchd1* up- and down-regulated genes. A significant enrichment ($p<0.01$) means that a high amount of markers of a specific cell type is found in *Ptchd1* deregulated genes, suggesting that the latter cell type should be particularly affected. The complete statistical data and the lists of markers found in *Ptchd1* up- and down-regulated genes are fully available in **Supplementary Table 4**.

Supplementary references

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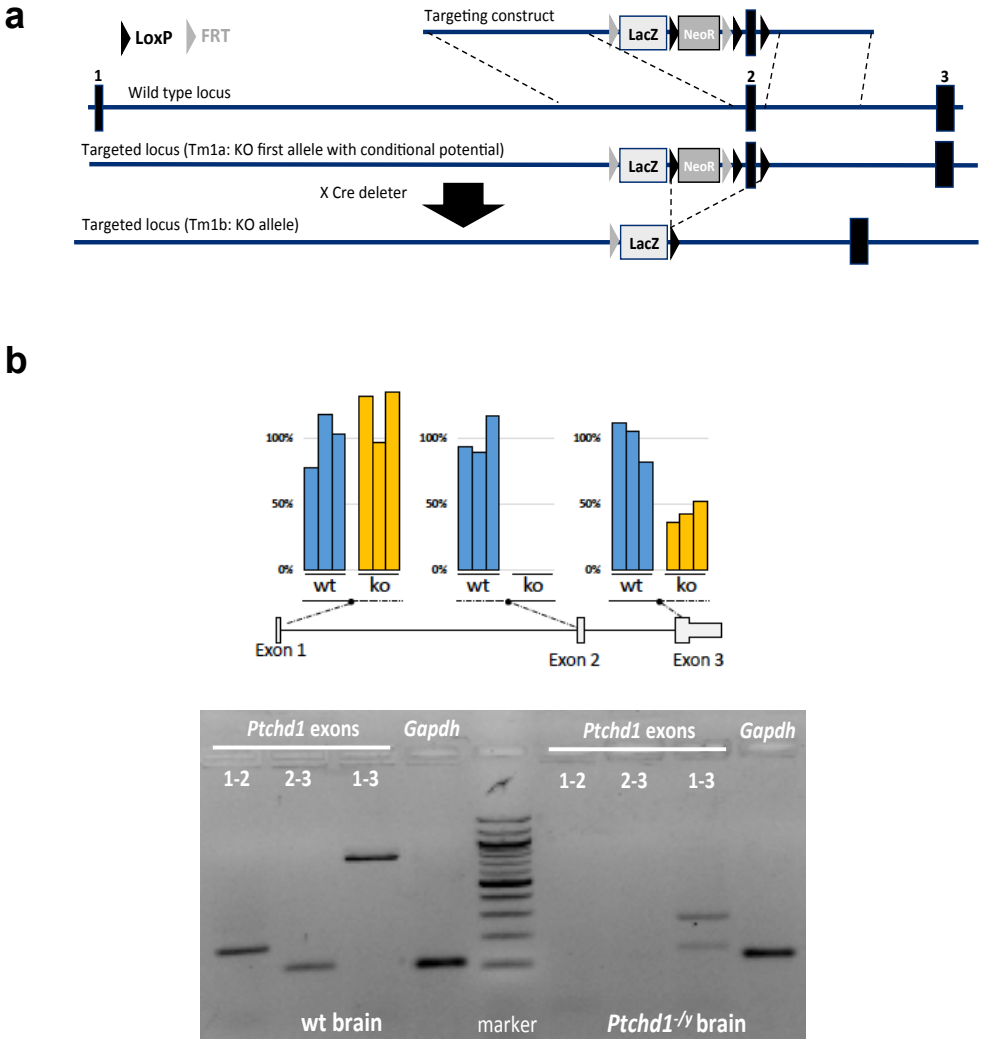
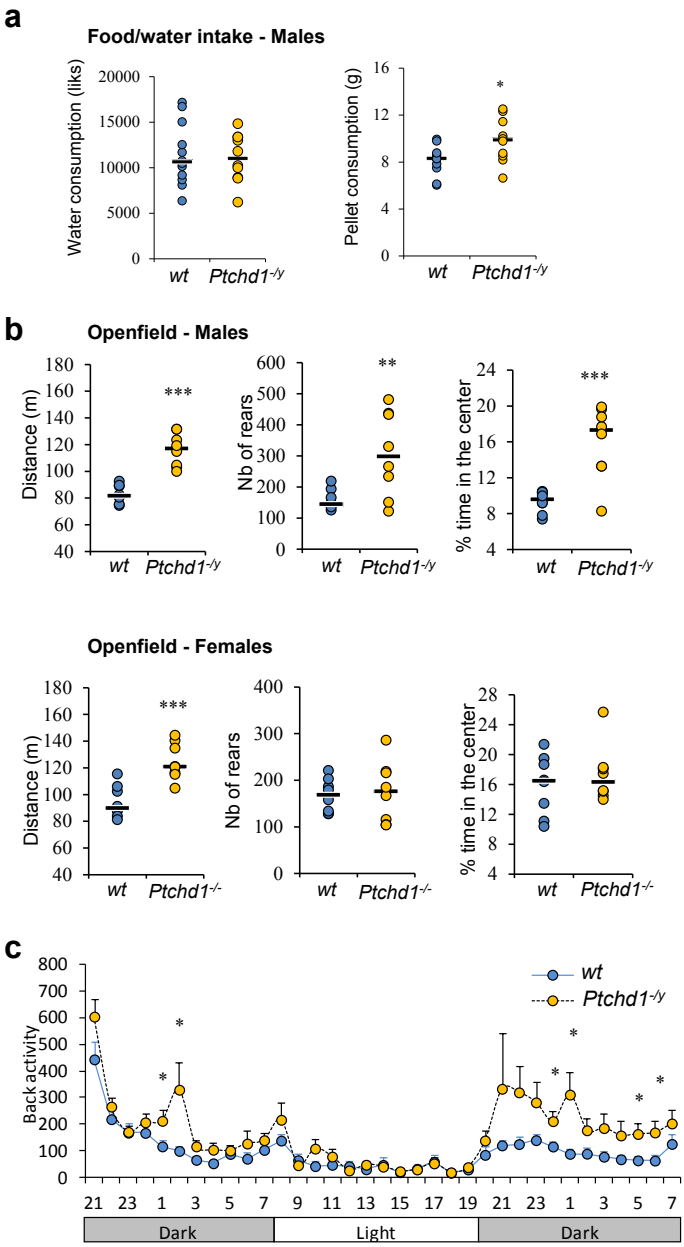
Supplementary Figure 1. *Ptchd1* knock-out strategy and validation



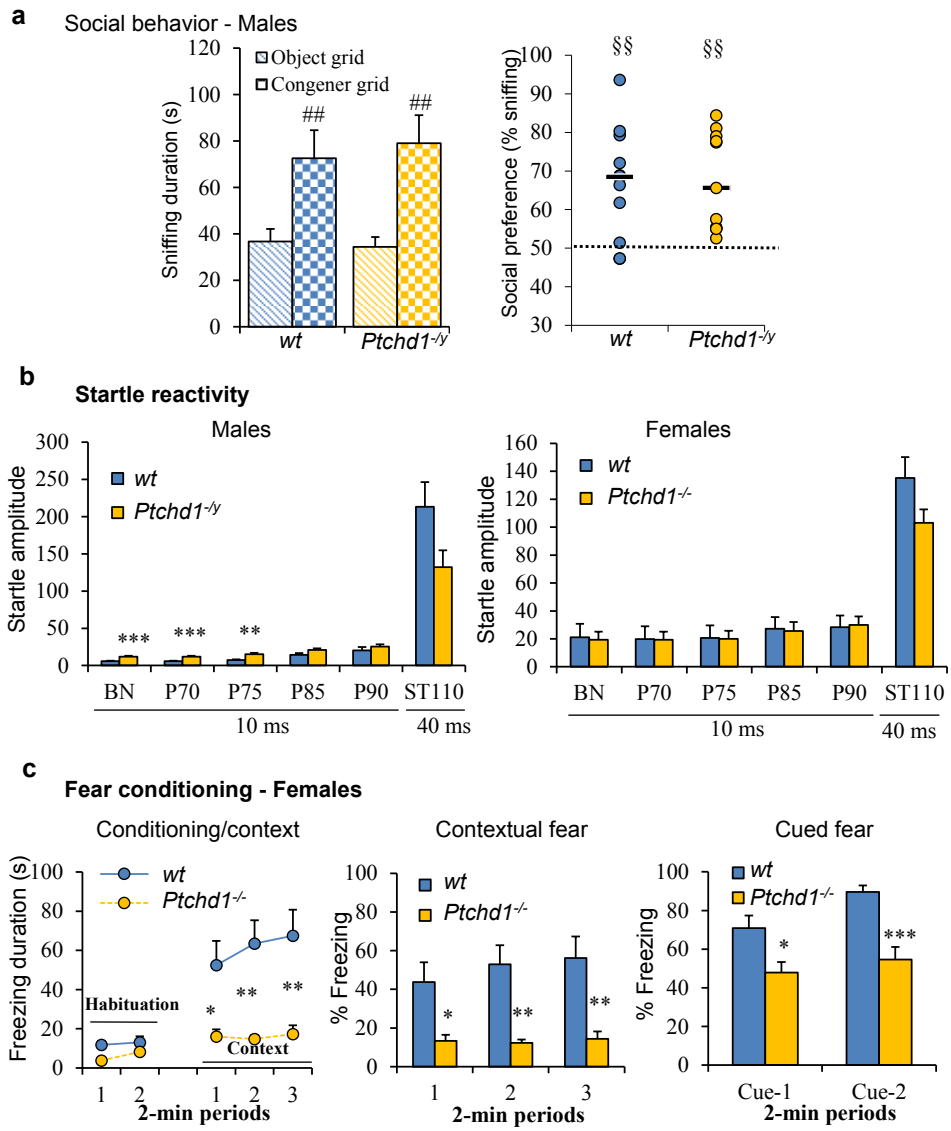
a. Schematic representation of the knock-out strategy for the *Ptchd1*$^{-/y}$ mouse model; **b.** *Ptchd1* gene expression level assessment by total RNA sequencing (graphical representation) and RT-PCR amplification experiments (agarose gel) of wild-type and *Ptchd1*$^{-/y}$ brain samples (n=3 for each genotype) shows removal of full-length *Ptchd1* isoform. The remaining lower expression of exon 3 results from an alternative transcript lacking exon 2 and also detected by RT-PCR.

Supplementary Figure 2. *Ptchd1* mutants display hyperactivity and reduced anxiety in the open field test.



a. *Ptchd1*$^{-/y}$ males had increased food intake and normal water consumption (12 wt and 11 *Ptchd1*$^{-/y}$ mice; Results are expressed as a scattergram with the median; *p<0.05, from wt, unpaired Student t-test); **b.** locomotor activity, rears and percentage of time in the center of the openfield in *Ptchd1*$^{-/y}$ males and *Ptchd1*$^{-/-}$ females (8 wt and and 9 *Ptchd1*$^{-/y}$ male mice, 8 wt and 8 *Ptchd1*$^{-/y}$ female mice; Results are expressed as a scattergram with the median; **p<0.01, ***p<0.001 from wt, unpaired Student t-test); **c.** *Ptchd1*$^{-/y}$ males showed increased local activity in the rear part of the actimetric cages (n = 12 wt and 11 Ptchd1-/y mice; Results are expressed as mean±sem activity over 35h of testing per 1h period; *p<0.05, Student t-test, following Significant repeated measures ANOVA).

Supplementary Figure 3. *Ptchd1* mutants display normal social behaviour, tended to have decreased startle and showed decreased fear conditioning.



a- *Ptchd1*$^{-/y}$ males had normal social behavior as measured by the percentage of time spent sniffing the congener grid (12 wt and 11 *Ptchd1*$^{-/y}$ mice; Results are expressed as scattergram with the median; §§ p<0.01, from the Hazard (50%), one group t-test); and time spent sniffing the congener or the object grids (12 wt and 11 *Ptchd1*$^{-/y}$ mice; Results are expressed as mean±sem; ##p<0.01, from the object vs congener, paired Student t-test). **b-** Acoustic startle reactivity in additional cohort of *Ptchd1*$^{-/y}$ males and *Ptchd1*$^{-/-}$ females (8 wt and and 9 *Ptchd1*$^{-/y}$ male mice, 8 wt and 8 *Ptchd1*$^{-/y}$ female mice; Results are expressed as a mean±sem; **p<0.01, ***p<0.001 from wt, unpaired Student t-test). **c-** *Ptchd1*$^{-/-}$ females showed decreased contextual and cued fear conditioning (8 wt and 8 *Ptchd1*$^{-/y}$ mice; Results are expressed as mean±sem; Repeated ANOVA followed by unpaired Student t-test for each time point; ANOVA for baseline habituation: gene F(1,14)=4.429, p=0.0539, time F(1,14)=1.657, p=0.2189, interaction F(1,14)=0.529, p=0.4789; ANOVA for contextual fear: gene F(1,14)=13.382, p=0.026, time F(1,14)=2.581, p=0.0936, interaction F(1,14)=2.195, p=0.1301; ANOVA for cued fear : gene F(1,14)=15.294, p=0.0016, time F(1,14)=18.202, p=0.0008, interaction F(1,14)=4.114, p=0.0620).

Supplementary Table 1. Comparative neurological evaluation of wild-type and Ptchd1$^{-/y}$ mice

		Wild-type	Ptchd1$^{-/y}$
Body weight (g)		28.0 ± 0.7	29.4 ± 0.4
Grip strength		7.7 ± 0.3	6.9 ± 0.1**
Hot plate latency (s)		13.1 ± 0.8	14.6 ± 1.2
Shock threshold (mA)	**Flinch**	0.08 ± 0.00	0.08 ± 0.00
	Vocalization	0.43 ± 0.12	0.31 ± 0.10
	Jump	0.80 ± 0.08	0.75 ± 0.10

Supplementary Table 2. Comparative neurological evaluation of wild-type, *Ptchd1*$^{-/y}$ male and *Ptchd1*$^{-/-}$ female mice

	Male		Female	
	wt	***Ptchd1*$^{-/y}$**	***wt***	***Ptchd1*$^{-/-}$**
Body weight (g)	22.03 ± 0.47	**25.96 ± 0.66*****	17.86 ± 0.33	18.83 ± 0.36
Rotarod- 4 to 40 rpm in 5 min (s)	191.54 ± 23.09	**117.71 ± 15.65***	218.00 ± 10.13	181.83 ± 15.95
Grip strength (adjusted to body weight)	7.55 ± 0.28	**6.26 ± 0.28****	7.59 ± 0.09	7.93 ± 0.26