**Supplementary Information**

**Materials and Methods:**

*Patients.* Genomic DNA was extracted from whole blood from parents and the affected children.  Exome sequencing was performed on exon targets captured using the Agilent SureSelect Human All Exon V4 (50 Mb) or Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA).  Libraries were sequenced using the Illumina HiSeq 2000 or 2500 sequencing system with 100-bp paired-end reads (Illumina, San Diego, CA).  Exome sequencing data for all sequenced family 1 members was analyzed using GeneDx’s XomeAnalyzer (a variant annotation, filtering, and viewing interface for WES data), which includes nucleotide and amino acid annotations, population frequencies (NHLBI Exome Variant Server and 1000 Genomes databases), *in silico* prediction tools, amino acid conservation scores, and mutation references.  Variants were filtered based on inheritance patterns, variant type, population frequencies, and gene lists of interest in relation to the patient’s major phenotypic features, as appropriate.  Identified sequence variants of interest were confirmed in the proband and both parents by conventional di-deoxy DNA sequence analysis using an ABI3730 (Life Technologies, Carlsbad, CA). The full sequencing methodology and variant interpretation protocol has been previously described ([1](#_ENREF_1)).  The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (<http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/>). Family 2 sequence reads were mapped to the reference genome (hg19: GRch37: Feb2009) using the Burrows-Wheeler Alignment Tool (bwa v0.5.9). The alignment was refined to ensure reads were correctly mapped around insertions and deletions using the GATK IndelRealigner (Genome Analysis Toolkit v1.0.5974). Duplicate reads were marked using Picard (v1.41) to avoid calling genotypes based on overrepresented library molecules. SNPs and indels were called using the GATK Unified Genotyper and cross referenced against known variants from dbSNP 132 and the 1000 Genomes Project.

*Mice. Hace1* mutant mice were previously generated by our group ([2](#_ENREF_2)) and kept on C57BL6 background. *Hace1* KO mice are generally healthy, viable, and fertile ([2](#_ENREF_2)). For all assays, littermates on C57Bl6/J background 8 to 12 weeks of age were used. For all tests male mice were used to exclude possible confounding effects of hormonal influence in female mice and to minimize the numbers of animals required for the study. No sex differences we noted amongst the patients, therefore the data gathered for the male mice are applicable to both sexes. The genotype was determined using the following primers for *Hace1* mutants ([2](#_ENREF_2)): *hace1* WT-for GGAAGCTTTGTATCCATTGCCT, *Hace1* WT-rev CCCCAACCTTCAAAATGTTAAA, *hace1* KO-for GCTGCTACCCCAAGTTCCCCAT and *Hace1* KO-rev AAGGGCCAGCTCATTCCTCC; and for Thy1-GFP line M animals ([3](#_ENREF_3)) (MGI:3766828): *eGFP*-for AAGCAGCACGACTTCTTC and *eGFP*-rev GCTCAGGTAGTGGTTGTC. To generate *Hace1+/+;Thy1-GFP+*and *Hace1*-/-; *Thy1-GFP+*  animals, we crossed Hace1-/- to Thy1-GFP line M mice to generate *Hace1*+/-; *Thy1-GFP*+ mice, which were then crossed to each other for the desired genotypes used for analysis.

*Immunoblotting.* Freshly isolated or fresh-frozen dissected brain regions (indicated in the figures) or patient-derived fibroblasts were lysed using radioimmunoprecipitation assay (RIPA) Lysis Buffer (Sigma) with HaltTM Proteinase and Phosphatase Inhibitor Cocktail (ThermoFischer Scientific). Following incubation on ice for 10 minutes, samples were centrifuged at ~16,000 rotation per minute (RPM), at 4oC. Protein content was quantified using the Bradford Protein Assay (Bio-Rad) and 25 µg/lane were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 4-20% Mini-PROTEAN (Bio-Rad) precast gels. The gels were transferred using a Trans-Blot SD Semi-Dry Transfer Cell (25 V for 45 min.) onto 0.45 µm pore size PVDF AmershamTM Hybond® P Western blotting membranes (Sigma-Aldrich) and membranes subjected to standard Western blotting procedures. Briefly, membranes were blocked with Blocking solution (5% skim milk powder (Merck) in 0.1% Tween-20 in phosphate buffered-saline, PBS) for 1 hour at room temperature (RT), and incubated in the same solution with the appropriate primary antibody overnight at 4oC, with shaking. The following antibodies were used: anti-HACE1 at 1:1000 dilution (AbCam, ab133637); anti-CyclinD1 at 1:1000 (AbCam, ab134175), anti-GAPDH at 1:1000 dilution (Cell Signalling, 14C10); anti-RAC1 at 1:1000 dilution (Millipore, clone 23A8); anti-ß-actin at 1:5000 dilution (Sigma, clone AC-74). Following 4X10 minute washes in 0.1% Tween-20/PBS at RT, membranes were incubated in appropriate secondary horseradish peroxidase (HRP)-linked whole antibodies (donkey; GE Healthcare) diluted 1:2500 in Blocking solution. Following 4X10 minute washes in 0.1% Tween-20/PBS at room-temperature, chemiluminescence was generated using an enhanced chemiluminescent (ECL) kit (GE Healthcare) according to the manufacturer's protocol, and exposed to film (Amersham Hyperfilm ECL, GE Healthcare) for several exposure times.

*Histology.* Selected brains were processed by the Histopathology Service Facility at the Vienna Biocenter Core Facilities (VBCF), member of VBC, Austria. Briefly, 2µm thick coronal or sagittal paraffin embedded sections were stained with Hematoxylin and Eosin (H&E, Shandon\* Harris Hematoxylin Acidified; Thermo Scientific Shandon™ Eosin Y; Fisher Scientific), Luxol Fast Blue – Cresyl Violet (LFBCV, Sigma) or with anti-Myelin Basic Protein antibody (Abcam,1:100). Slides were imaged using a Zeiss Axioskop 2 MOT microscope (Carl Zeiss Microscopy) and subsequently digitized with the Pannoramic FLASH 250 II automated slide scanner (3D Histech). Images were acquired with the Pannoramic Viewer software (3D Histech).

*Magnetic resonance imaging.* Male *Hace1* WT and KO littermate mice 8-10 weeks of age (genotypes indicated in the figures and numbers indicated in the figure legends) were imaged in the Preclinical Imaging Facility at Vienna Biocenter Core Facilities (VBCF), member of Vienna Biocenter (VBC), Austria.A 15.2 TeslaMRI (Bruker BioSpec, Ettlingen Germany) with a 22mm quadrature birdcage coil was used. All animals were anesthetized with isoflurane (4% induction, maintenance with 1.5%, Vana GmbH). During imaging, respiration was monitored and isoflurane levels adjusted if breathing was <40 or >80 breaths per minute. Mice were kept warm with water heated to 37°C circulated using a Fisher Scientific water pump. A 3D fast long angle shot (FLASH) sequence with magnetization transfer pulse for better delineation of the white matter was used (repetition time (TR)/echo time (TE) = 160/3.5 ms, 100 μm3 voxel size, with thirty Gaussian 15.0 µT magnetization transfer pulses at 5.3 ppm frequency offset, total imaging time 1h 42 min).Each 3D image set was manually segmented using Amira 5.6 (Visualization Science Group). The delineation of different brain structures was initially performed in the axial plane and subsequently controlled in the two other planes. The Paxinos mouse brain atlas was used as a reference ([4](#_ENREF_4)). The brain surface and structures were delineated based on the MRI signal intensity differences. Values were normalized to brain size, averaged and presented as percentage of total brain volume. Unpaired Student’s *t*-test was used to calculate significance.

*Behavioral testing.* Acoustic fear conditioning and Ladder Rung Walking Task were performed by the trained staff at the Preclinical Phenotyping Facility at the Vienna Biocenter Core Facilities (VBCF), member of the Vienna Biocenter (VBC), Austria. Numbers of mice used per experiment are indicated in the figure legends. All experiments were conducted during the light phase of the circadian cycle, by an experimenter blinded to the genotype of the mice. Before the initiation of each behavioral experiment, animals were habituated to the room where the experiment would take place for at least a week prior to the start of the experiment, and were handled once a day for 5 minutes to familiarize the animals to the experimenter. For habituation to particular apparati see below.

*Open Field Test*. Mice were placed in a gray, 28X28 cm plexi-glass arena, and allowed to freely explore for 5 minutes. Time spent in the center or along the walls of the arena, as well as distance traveled during exploration, were measured using an automated activity system (TSE-Systems, Germany). Reluctance to enter the center of the arena as compared to controls was scored and interpreted as levels of anxiety. Distance traveled during the test was compared to controls and used as a readout for locomotion deficits. Unpaired Student’s *t*-test was used to determine significance.

*Elevated plus maze*. To assay anxiety-related behavior, mice were placed in the center of a gray “plus” shaped plexiglass arena, consisting of two walled arms, and two open arms, elevated approximately 1 meter above the floor. Exploratory behavior of mice was recorded over a 5-minute period, using an automated activity system (TSE-Systems, Germany). Reluctance to explore the open arms of the arena was scored and interpreted as anxiety. As with the Open Field Test, distance traveled during the test were compared to controls and used as a readout for locomotion deficits. Unpaired Student’s *t*-test was used to determine significance.

*Accelerating rotarod.* Mice were habituated to the rotating rotarod apparatus (Ugo Basile) once for a 1-minute trial, at 5 rpms. If any mouse fell off during the 1 minute habituation, they were gently placed back on the rotating rod to habituate for a total of 1 minute. Following habituation, each mouse was given 1 trial per day, for 4 consecutive days, on the accelerating rod (5 to 40 rpms) for a maximum of 5 minutes. If a mouse stopped walking and gripped the rod for two consecutive turns, time would be stopped, and the mouse removed from the apparatus. Significance was determined by two-way ANOVA with Sidak’s multiple comparisons.

*Ladder Rung Walking Task.* Mice were trained to walk across a ladder (length: 80cm; spacing of rungs: 1cm) towards an escape ladder in the TSE MotoRater system ([www.tse-systems.com](http://www.tse-systems.com)) for 3 consecutive days (1 trial/day). On the 4th day, mice were marked using white (Edding 780) and red (Edding 3000) Edding pens while mice were briefly under isoflurane anesthesia (induction: 4% isoflurane, maintenance: 2% isoflurane). A video recording was taken on the 5th experimental day focusing on the steps of the mice from both sides via a mirror system and videos were manually scored using SimiMotion software 8.5.0.327. Habituation and recording sessions were performed in the mornings (09:00 am to 12:00 pm). The Scoring system used has been described previously ([5](#_ENREF_5)), with the following modifications: steps occurring immediately before and after a foot fault were scored, and when a step error occurred, the steps of the non-involved limbs were scored as well. Only 1 run was counted for each mouse. Unpaired Student’s *t*-test was used to determine significance.

*T-Maze or spontaneous alternation.* T-Maze was performed as described previously ([6](#_ENREF_6)). For spontaneous alteration, no habituation to the maze was performed. T-maze consists of a T-shaped, gray, plexiglass apparatus, where each walled arm is 30cmX10cm. Removable guillotine doors partition all arms. The floor of the arena is sprinkled with clean wood chips, which are mixed between each animal to remove any odor interference with behavior, and changed after the whole cohort completed one run. At the start of the trial, all guillotine doors are opened. Animal is placed in the arm designated the ‘start arm’, and allowed to choose a goal arm, after where it is confined by the closure of the guillotine door for 30s. Next, the animal is placed again in the start arm, the guillotine doors raised to allow the animal the free choice of goal arms again. If the animal chooses the alternate goal arm from its initial choice, it is scored as a “1”, if it chooses the same goal arm as during the first run; it is scored as a “0”. If an animal would not choose a goal arm or move from the start arm for 2 minutes it was removed from the arena, provided 10-15 minute home cage rest, and trialed again. Each animal underwent 7 trials in 1 day, with approximately 15 minutes rest between each trial. Significance of deviation of the number of successes in mutant versus wild-type mice was evaluated using a binomial test.

*Acoustic Fear Conditioning.* On day 1, in the afternoon (12:00pm to 18:00pm), mice were trained to associate the conditioned sound stimulus (CS= 85dB, 10kHz) to the unconditioned foot shock stimulus delivered by the floor grid (US= 1.5mA). This was performed using the following protocol: 120 sec. of pre-phase (no sound, no shock); 30 sec. CS with the last 2 sec. coupled to the US; 90 sec. inter-tone-interval (no sound, no shock); 30 sec. CS with the last 2 sec. coupled to the US; and 70 sec. post-phase (no sound, no shock). Test were performed with Coulbourn Habitest operant cages (Coulbourn Instruments, MA, USA) using a lamp in the visible range. Mice were monitored via live video recording using the software FreezeFrame (Actimetrics, IL, USA). For contextual testing, mice were placed back in the same conditioning box 24 hrs following training and observed for 4 minutes without any sound or shock presentation. For the cued test, mice were placed in the same fear conditioning box, but with changed appearance (different texture of walls and floor, infrared house light, no ventilator, lemon aroma spotted on the box floor) approx. 20 hrs. (in the morning session, between 09:00 am and 12:00 pm) following contextual testing. The following protocol was applied while animals were observed: 120 sec. pre-phase (no sound, no shock); 60 sec. CS (only sound, no shock); 60 sec. inter-tone-interval; 60 sec. CS (only sound, no shock); and 60 sec. post-phase (no sound, no shock). Freezing was defined as a minimum of 2 sec. without movement, except breathing, and was manually analyzed using FreezeView software according to manufacturer’s instructions (Actimetrics, IL, USA), analyzed for 4 min. for contextual and 5 min. for cued tests, averaged for all animals and expressed as percentage of freezing behavior for the duration of the analysis. Unpaired Student’s *t*-test was used to determine significance.

*Morris Water Maze.* Mice were trained to swim in a pool (diameter: 1 m) with opaque water (white paint: Tiger Kunterbunt Lack Weiss No. 431) treated with aquarium cleaner (333 μl of easy life filter medium/l of water) at a temperature of 20-22oC, to find a platform (diameter: 10cm) submerged ~8mm underneath the surface, using the visual cues placed around the room for orientation. Mice were placed in the water facing the wall of the pool and given 2 sessions a day, with 4 trials per session, using alternating entry points in different quadrants for each trial. Mice were video tracked using the software Topscan 3.0 (Cleversys Inc., VA, USA). On Day 1, the visual capacity of the mice was checked by making the platform visible with a black flag and letting the mice explore the pool for 1 min or until they reached the platform. If they did not find the platform within 1 min of the first session, they were guided towards the platform by pointing a pair of forceps in front of their nose. Following rest on days 2 and 3, mice were given hidden-platform training on days 4-8. The latency to find the platform was recorded. Each mouse was given 4 trials in the morning, and 4 trials in the afternoon, for 5 consecutive days. Short-term memory test was performed after the last trial, on day 8, by removing the platform and letting the mice explore the pool for 1 min. The same trial was repeated in the morning of day 11 to test for long-term memory. The time spent searching in the target quadrant and target zone (exact location of the platform) was recorded and used as a readout for memory. Unpaired Student’s *t*-test was used to analyze the short and long-term memory data and two-way ANOVA with Sidak’s multiple comparisons test for the latency to reach the platform.

*Acoustic Startle and Prepulse Inhibition.* Mice were tested for startle responses and pre-pulse inhibition as described previously ([7](#_ENREF_7)). Briefly, mice were placed in the soundproof *SR-LAB Startle Response System* (San Diego Instruments) chamber, mounted on an accelerometer. Following 5 minutes of acclimation to white noise delivered by a small speaker inside the chamber, mice were subjected to 40 ms acoustic stimuli at 90, 100, 110 and 120 dB 20 times in a pseudorandom order. Prepulse inhibition was analyzed in the same trial, using 80, 85, 90 and 95 dB prepulses to the 120 dB startle. 0 dB prepulse to 120 dB startles were averaged, used as maximal response, and set to 100%. All startle responses detected by the accelometer and collected by SR-Lab Analysis Software, were normalized to weight, averaged, and expressed in arbitrary units. Two-way ANOVA with Sidak’s multiple comparisons test were used to determine significance.

*Hippocampal slices preparation and electrophysiological recordings.* Electrophysiological field recordings from acute hippocampal slices were performed as described previously ([8](#_ENREF_8)). Briefly, 3 to 4-month-old wild-type (WT) and *Hace1*-knockout mice (KO, five each in total) were euthanized and 300 µm thick coronal hippocampal slices were prepared in an ice-cold solution containing (in mM): NaCl 60, sucrose 105, KCl 2.5, MgCl2 7, NaH2PO4 1.25, ascorbic acid 1.3, sodium pyruvate 3, NaHCO3 26, CaCl2 0.5 and glucose 10 (osmolarity 305–310 mOsm). Slices were kept in slicing solution at 34oC for 15 min and then transferred to an artificial cerebrospinal fluid (ACSF) solution containing (in mM): NaCl 131, KCl 2.5, MgSO4 1.3, NaH2PO4 1.25, NaHCO3 21, CaCl2 2 and glucose 10 (pH 7.35–7.45; osmolarity adjusted to 297–303 mOsm) at room temperature. All solutions were continuously bubbled with 95% O2/5% CO2. Slices were allowed to rest for at least 45 min at room temperature before experiments were started. For recordings, hippocampal slices were transferred to a interface-type recording chamber and superfused with extracellular solution at 34oC. For extracellular field potential recordings, standard patch pipettes (3-4 MΩ) were filled with extracellular solution and inserted into the CA1 stratum radiatum. Data were recorded using an EXT-02B amplifier (NPI) and WinWCP (Strathclyde Electrophysiology Software), digitized at 10 kHz and stored for offline analysis using Clampfit (Molecular Devices). A bipolar concentric stimulation electrode was placed in the stratum radiatum at the border between CA2/3 and CA1 to stimulate Schaffer collateral CA3-CA1 synapses in order to evoke field excitatory postsynaptic potentials (fEPSPs). In each slice, single fEPSPs were evoked at several stimulation intensities (20, 40, 80, 160 and 320 µA). For subsequent LTP and paired-pulse experiments, the stimulus intensity evoking 50-70% of the maximum response was used. fEPSP slopes were normalized to axonal fiber volleys to account for variations in axonal stimulation efficacy between slice preparation and animals. In LTP-experiments, a baseline recording of 10 minutes was obtained before LTP was induced by high frequency stimulation (HFS, 100 stimuli at 100 Hz, three times with 1 minute intervals) and the effect of LTP-induction was monitored for another 30 minutes.

*Synaptic number analysis. Hace1* KO mice were crossed to the Thy1 eGFP M-line mice, where GFP under the Thy1 promoter is expressed in neurons allowing for visualization of the entire cell, including post-synaptic protrusions on neuronal dendrites called spines (16). 8-10 week old male mice were sacrificed by asphyxiation, brains removed and postfixed overnight in 4% paraformaldehyde in PBS, followed by cryoprotection in 30% sucrose in PBS at 4oC. Brains embedded in O.C.T. (Optimum Cutting Temperature) formulation (Tissue–Tek® O.C.T. Compound Sakura® Finetek) were slowly frozen on a block of dry ice, and stored in -80oC. 20 µm sections were made on a Microm Cryostat (HM560, Microm) through the hippocampus. Slices through the dorsal hippocampus were chosen for further study, briefly air-dried, and mounted with Vectashield containing 4',6-diamidino-2-phenylindole (DAPI, Vector Laboratories) mounting media. Fluorescent images were acquired using LSM700 Axio Imager with 63x/1.4 plan-apochromiat Oil objective lens and a zoom factor of two. We focused on distal dendrites of pyramidal neurons in the CA1 region of the hippocampus. We analyzed 3 animals in each genotype and acquired 9 images per animal, 27 images in total per group. Synapse number counts and measurement of neurite lengths were performed using ImageJ ([9](#_ENREF_9)). The experimenter was blinded to the genotypes. Statistical analysis was performed using Student’s *t*-test.

*Cell culture*. Primary fibroblasts from skin biopsies of three patients carrying the compound heterozygous *HACE1* mutations p.P674Ffs\*5 and p.R748\* (patients 1 and 2), or the homozygous mutation p.R332\* (patient 5) and from two different unaffected donors, were cultured in Dulbecco’s modified Eagle’s medium (DMEM, PAA Laboratories, Austria) supplemented with 10% foetal bovine serum (FBS, Invitrogen) and 1% Penicillin-Streptomycin-Amphotericin B mixture (Biozym). All cells were maintained at 37oC in a saturated humidified atmosphere containing 5% CO2.

*Active RAC1 Pulldown.* Cellular levels of active RAC1 in fibroblasts were analysed using the *Active Rac1 Pull-Down and Detection Kit* (Thermo Fisher Scientific) according to manufacturer´s instructions. Briefly, cells were seeded in 10 cm Petri dishes, at a density of 2 x106 cells/dish, and allowed to grow to 80% confluency. Before harvest, cells were starved in serum-free DMEM, overnight. Then, cells were washed with ice cold TBS once to remove detached cells and lysed with lysis/wash/binding buffer for 5 minutes. 20µl of fresh lysate served as an input and loading control. The lysate was incubated with p21-binding-domain (PBD)-GST fusion protein and glutathione-resin for 1h at 4oC, with gentle shaking. Further, the protein-resin complex was washed with lysis/wash/binding buffer and bound GTP-bound RAC1 was eluted and denatured with 50µl SDS-sample buffer for 5 min at 95oC. The samples and the input were separated on a 12% acrylamide gel and detected with a specific anti-RAC1 antibody (dilution 1:500, kit content, Thermo Fisher Scientific).

*ROS detection in mouse brain.* Mouse brains were dissected, placed in Tissue-Tek O.C.T and frozen on a block of dry ice. Following 20 μm coronal cryosectioning, sections were mounted on glass slides and stored at -80oC. At time of experiment, sections were defrosted and briefly air-dried at room temperature; washed 2X in PBS, and incubated in 10mM dihydroethidium (DHE) for 15 minutes at 37oC. Sections were washed twice in PBS, and imaged with a Zeiss Axioplan2 microscope.

*ROS quantification in primary dermal fibroblasts.* Levels of ROS were investigated using CellROX® Deep Red staining (Thermo Fischer) followed by flow cytometric analysis according to manufacturer’s instructions. Briefly, fibroblasts were seeded in 10 cm Petri dishes at a density of 2 x106 cells per dish and allowed to form a monolayer for 24 h. Cells were stained for 1 h with 5 µM CellROX® Deep Red Reagent under growth conditions, then washed twice and suspended with phosphate buffered saline (PBS) and immediately analysed with BD LSRII flow cytometer (BD Bioscience) using APC filter (660/20nm). Cells treated with 300 nM Tert-butyl hydroperoxide for 2 hrs served as a positive control, unstained samples as a negative control. Results were analysed using the median of fluorescence intensity and are presented as a mean of at least four independent experiments. Statistical analysis was determined by unpaired Student’s *t*-test.

*RAC1 network analysis.* The protein-protein interaction was extracted from the Human Integrated Protein–Protein Interaction rEference (HIPPIE), version July 2017 ([10](#_ENREF_10)). The ID associated genes were obtained from Radboud University Medical Center (<https://order.radboudumc.nl/LabProduct/Pdf/30240>). Neuronal expressed genes were extracted from the Brain RNA-seq data ([11](#_ENREF_11)). Transcripts with FPKM > 1 in neuron are identified as active genes and included in the neuronal interactome. The mapping between mouse and human homologs was performed using information provided by The Jackson Laboratory (<http://www.informatics.jax.org/homology.shtml>). Gene set enrichment analysis was performed using Enrichr ([12](#_ENREF_12)), using ID associated RAC1 interactors as the query set.

*Statistics.* GraphPad Prizm software was used for the different statistical analyses named below the methodology description for each.

**Supplementary Figure Legends:**

**Figure e-1. Novel mutations in *HACE1* in two unrelated families.**

**A.** Pedigree of family 1 and patient 9. Affected individuals are shaded in black, and heterozygous parents are shaded half black and half white. Squares represent males, circles represent females, and diamond is undefined gender. #-liver fibrosis and liver tumor; \*-breast cancer, X-denoted all samples that were Sanger sequenced. **B.** Coronal, sagittal and transverse MRI image sections show lateral ventriculomegaly and hypoplastic corpus callosum in patient 9. **C**. Small feet are shown. **D.** Pedigree of family 2 and patients 10 and 11. ᵒ-Hashimoto’s disease. **E.** Small feet of patient 10 and widely spaced nipples are shown. **F.** Small feet of patient 11 are shown.

**Figure e-2. Reduced white brain matter and enlarged ventricles in *Hace1*** **KO mouse brains.**

**A.** Representative axial MRI images of WT and *Hace*1 KO mouse brain; WT n=12 and KO n=9. Red arrows point to the corpus callosum, yellow arrows to brachium of the superior colliculus, white arrows to the arbor vitae, and blue arrows to the lateral ventricles. **B.** Myelin basic protein (MBP) staining of white matter in coronal slices of WT and *Hace1*KO littermates shows a marked reduction of the corpus callosum, indicated by black bars, in *Hace1* KO mice as compared to control littermates. Representative images of one select mouse from each group is shown. **C.** Bottom and lateral views of 3D reconstructed pseudocolored (turquoise) white matter of representative WT and *Hace1* KO adult brains; bottom and lateral views are shown. Right, quantification shows a significant reduction in volume of white matter in *Hace1* KObrains as compared to WT controls; mean values +/- SEM; WT n=12 and KO n=9; P-value \* ≤ 0.05; unpaired Student’s *t*-test. **D.** Bottom and lateral views of 3D reconstructed pseudocolored (red) MRI images of representative corpus callosum (CC) of WT and *Hace1* KO mouse brains. Right, quantification shows a significant reduction in CC volume in *Hace1* KO animals as compared to WT littermates; mean values +/- SEM; WT n=12 and KO n=9; P-value \*\*\*≤ 0.001; unpaired Student’s *t*-test. **E.** Bottom and lateral views of 3D reconstructed pseudocolored (purple) MRI images of lateral ventricles of *Hace1* KOs and WT littermate controls. Right, quantification of *Hace1* KObrains as compared to WT controls; mean values +/- SEM; WT n=12 and KO n=9; P-value=0.051; unpaired Student’s *t*-test. **F** and **G.** Frontal and lateral views of the 3D reconstructed WT and *Hace1* KO 3rd ventricle (**F**) and 4th ventricle (**G**), with quantifications; mean values +/- SEM; WT n=12 and KO n=9; P-value \* ≤ 0.05 (**F**); P-value 0.17 (**G**); unpaired Student’s *t*-test. Scale bars for all images are indicated in the panels.

**Figure e-3. Behavioral analysis of *Hace1*KO mice.**

A. Hace KO and WT animals spent similar times in the center of the arena in the Open Field Test; data are shown as mean values +/-SEM; WT n=10 and KO n=10; P-value as indicated in the bar graph; unpaired Student’s *t*-test. B. Hace KO did not show a significant reduction in the times spent in the open arms of the Elevated Plus Maze; data are shown as mean values +/-SEM; WT n=10 and KO n=10; P-value as indicated in the bar graph; unpaired Student’s *t*-test. C. Escape latencies in the Morris Water Maze Test were similar in *Hace1* KO and WT animals (left panel); mean values +/- SEM; WT n=21 and KO n=17; two-way ANOVA with Sidak’s multiple comparisons test. No difference was detected between WTand *Hace1* KO littermates in short (probe trial day 8) and long-term (probe trial day 11) memory (right panels); mean values +/- SEM are shown; WT n=21; KO n=17; unpaired Student’s *t*-test. D. No difference was detected between *Hace1* KO and WT littermate controls in their ability to perform spontaneous alteration in the T-maze test; mean values +/- SEM; WT n=10; KO n=10; Binomial test. E. *Hace1* KO littermates did not exhibit significant difference in startle responses for all prepulses as compared to WT littermates; mean values +/- SEM; WT n=14; KO n=11; P-values noted in the figure; two-way ANOVA with Sidak’s multiple comparisons test. Recorded responses were normalized to mouse weight and startle to 120 dB alone.

**Supplementary References:**

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