Supplementary information for

Cognitive deficits and impaired hippocampal long-term potentiation in K_{ATP}-induced DEND syndrome

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Matherials and methods

Animals A Cre-inducible Kir6.2$^{[K185Q,\Delta N30]}$ GOF transgenic mouse line under the Rosa26 locus was previously generated (9). In this line, CAG-promoter dependent expression of Kir6.2$^{[K185Q,\Delta N30]}$ is prevented by a loxP-flanked Stop-cassette (Fig. S1A). Cre-mediated recombination excises the Stop-cassette and drives Kir6.2$^{[K185Q,\Delta N30]}$ expression in a tissue-specific manner along with soluble eGFP via an internal ribosomal entry site (IRES) (9). When crossed into appropriate promoter-Cre expressing mice this model allows selective induction of transgene expression, thereby modeling tissue-specific expression of K_{ATP}-GOF mutations.

Behavioral tests ***One-hour locomotor activity and sensorimotor battery-*** Mice were placed in transparent polystyrene enclosures (47.6x25.4x20.6cm) and movements were monitored using computerized photobeam instrumentation. In ledge and platform tests, mice were placed on an elevated Plexiglas ledge

(0.75cm wide) or small circular wooden platform (3.0cm diameter) elevated to 30 or 47cm, respectively, and the amount of time they remain on the platform was recorded. Screen tests were performed by placing mice head-oriented down in a wire mesh grid (16x10cm) elevated 47cm at 60° or 90°. The time taken to turn 180° and climb to the top of the wire-mesh was measured. Inverted screen tests began identically to screen tests, but the screens were then inverted 180° after ensuring the mouse had a secure grip. The time the mouse remain on the screen was measured. In the pole test, mice were oriented head-up with forepaws on top of a textured rod (8mmx55cm) and the time the mouse took to turn around and climb-down the pole was measured (a maximum score of 120s was assigned if the mouse fell off the pole). For walking initiation, mice were placed in the center of a 21x21cm square marked with tape, and the time mice took to leave the square was recorded. Each test lasted a maximum of 60s, except pole tests (120s).

MWM navigation- In cued trials, the escape platform was submerged beneath the water surface, but its location was denoted by a red tennis ball atop a rod, which was attached to the escape platform and served as a visual cue. To limit spatial learning during cued trials, the location of the platforms was varied across trials in the presence of very-few distal spatial cues. In place trials, the platform was hidden beneath the water surface and its location was kept constant across all trials, with several salient distal cues being present during testing. Mice were placed pseudorandomly into one of the four quadrants of the maze and had to learn the location of the submerged platform.

RNA extraction, cDNA synthesis and qRT-PCR- To address transcriptional levels of K_{ATP} channel expression, nK_{ATP}-GOF and hK_{ATP}-GOF brain tissues were subjected to reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR). nK_{ATP}-GOF, hK_{ATP}-GOF, and littermate control mice were anesthetized with isofluorane prior to decapitation, then brains dissected into ice-cold PBS. Hippocampi were dissected and flash-frozen on liquid nitrogen. Total RNA was extracted from 25-30 mg of hippocampal tissue using RNeasy Mini-Kits (Qiagen) with an on-column DNase digestion step to remove genomic DNA. RNA concentration was measured using a NanoDrop One^{C} spectrophotometer (Thermo Scientific). 1000ng of RNA was reverse-transcribed in a 20µl final volume using a High Capacity cDNA

Reverse Transcription Kit (Applied Biosystems) alongside a no reverse-transcriptase (No-RT) control. *RT-qPCR*- cDNA samples were diluted to a concentration of 5ng/µl with nuclease-free water (Corning). Each RT-qPCR reaction consisted of 5µl cDNA (25ng total), 12.5µl 2x PowerSybr Green I Master Mix (Applied Biosystems), 0.75µl 10µM forward and reverse primers (300nM), and 6µl nuclease-free water for a total reaction volume of 25µl. Reactions were run in triplicate on an Applied Biosystems StepOnePlus™ Real-Time PCR System. Reaction cycle consisted of an initial denaturation for 10min at 95ºC, followed by 40 cycles of 95ºC for 15s; 60ºC for 60s. Non-RT and non-DNA controls were included as negative controls in all plates. Primers were acquired either from previous publications (10) or the Harvard PrimerBank system, with ML32 run as a housekeeping gene (Table S7). *Data analysis and relative expression calculation*- StepOne™ Software (v2.3) was used to acquire RT-qPCR data and calculate cycle threshold (C_T) values. The delta C_T method was used to calculate relative expression levels for comparisons across genotypes, as follows:

$$K_{ATP}\text{-GOF Expression} = \mathbf{2}^{-(K_{ATP}GOF(C_{T(Target\ Gene)}-C_{T(ML32)})-K_{ATP}Control(C_{T(Target\ Gene)}-C_{T(ML32)}))}$$

For littermate controls, C_T values were averaged, then subtracted from each triplicate value to produce average and standard deviation values for relative expression. Differences in gene expression between control littermates and K_{ATP}-GOF mice were assessed with unpaired Student's t-tests, with $p<0.05$ indicating statistically significant differences in expression. For eGFP expression we reported ΔC_T values with respect to ML32 since eGFP was not detected in control littermate mice, as expected by a Cre-dependent construct. All analyses were performed on Microsoft Excel and Graphpad Prism.

Glucose tolerance tests Intraperitoneal Glucose Tolerance Tests were performed in 12hr fasted mice by injection of a bolus of glucose (1.5g/kg body weight). Tail blood was taken before (time 0) and at different times (15, 30, 45, 60, 90 and 120min) after glucose challenge and assayed for glucose content using the Glucometer Elite XL (Bayer Corp).

Immunofluorescence To address on-target recombination and transgene expression, anti-eGFP and anti-Cre immunofluorescence was performed in mouse frozen brain sections. nK_{ATP}-GOF, hK_{ATP}-GOF and

littermate controls were perfused transcardially with Tyrode's-buffered saline (TBS) and fixative. TBS consisted of 0.85% NaCl and 25mM TRIZMA Base (Sigma), adjusted to pH=7.4 with HCl. Fixative consisted of 4% paraformaldehyde (PFA) and 0.1M monobasic monohydrate NaH_2PO_4, adjusted to pH=7.4 with NaOH. Brains were removed and placed for 4hrs in fixative before transfer to a sucrose gradient consisting of one overnight calibration in 15% sucrose in phosphate-buffered saline (PBS) followed by one overnight calibration in 30% sucrose in PBS. Whole brains were then frozen in blocks of Optimal Cutting Temperature (OCT) embedding medium over liquid nitrogen, sectioned sagittally or coronally at 7-10µm and plated onto Superfrost microscope slides for subsequent imaging. Cryosections were co-stained with anti-GFP (Abcam, Chk, ab13970, 1:500) and anti-Cre (Novagene; Rb, 69050, 1:1000) primary antibodies to detect K_{ATP} transgene (since eGFP was co-expressed with mutant K_{ATP}) and Cre expression. TruBlk quenching dye (1:20) was used to reduce background autofluorescence, and a secondary antibody concentration of 1:500 was applied.

Long-term potentiation

Hippocampal slice preparation- Sucrose-based slicing solutions were prepared as 87mM NaCl, 2.5mM KCl, 1.25mM anhydrous monobasic NaH_2PO_4, 25mM D-glucose, 75mM sucrose, and 25mM $NaHCO_3$. After 15min bubbling at room-temperature (RT) with 95% carbogen, 0.5mM $CaCl_2$ and 3mM $MgCl_2$ were added. Choline chloride-based solution was prepared as 92mM choline chloride, 2.5mM KCl, 1.2mM NaH_2PO_4, 25mM D-glucose, 5mM sodium ascorbate, 2mM thiourea, 3mM sodium pyruvate, 20mM HEPES, 30mM $NaHCO_3$). Recording aCSF consisted of 125mM NaCl, 2.5mM KCl, 1.25mM anhydrous-monobasic NaH_2PO_4, 3mM myo-inositol, 10mM D-glucose, 2mM sodium-pyruvate, and 25mM $NaHCO_3$. After 15min bubbling at RT with 95% carbogen, 2mM $CaCl_2$ and 1mM $MgCl_2$ were added. Slices were transferred to a choline chloride-based solution and allowed to recover at 32C in aCSF/95% carbogen for 30min before transfer to recording aCSF solutions at 30C. Slices were incubated at 30C in recording aCSF before transfer to a nylon-mesh recording chamber at 30C.

Measurement of fEPSP (electrophysiology)- Stimulating electrodes were placed at the interface of CA2 and CA1 in stratum radiatum, at the level of Schaffer Collateral fibers. Recording electrodes were placed distally into CA1 in both stratum radiatum and stratum pyramidale to capture synaptic events generated by action potentials traveling down the Schaffer collaterals and population spikes generated by CA1 pyramidal neurons, respectively. Internal solutions for recording pipettes consisted of aCSF. Recordings were performed on a MultiClamp 700B amplifier, digitized using a Digidata 1550 converter, and sampled at 20kHz at a filter bandwidth of 5kHz. The recording electrodes were borosilicate glass pipettes of ~one micron, granting an access resistance of 4-8MΩ. LTP were performed in current clamp-mode (Grass Instrument Co./Model-P511K pre-amplifier, Warner Instruments/Model-IE-210 electrometer, Digidata-1322A analog-to-digital converter) at 30C.

Whole-cell Patch Clamp Electrophysiology in Dissociated Hippocampal Neurons- Hippocampal tissue cultures (single-cell suspension onto collagen-coated tissue-culture plates) were incubated at least seven and up to fourteen days prior to patch-clamp recording. Low-potassium solutions (Na1, Na2) consisted of 2mM $CaCl_2$, 1mM $MgCl_2$, 10mM glucose, 135mM NaCl, 5mM KCl, 10mM HEPES, pH 7.2 w/ NaOH. Borosilicate pipettes were pulled to an access resistance between 4-8MΩ. Cells were first bathed in high sodium bath (identical to low-potassium solution) prior to local perfusion with experimental solutions, which were always applied in the following order: Low potassium 1 (Na1), high potassium 1 (K1), diazoxide (Dz), tolbutamide (Tol), high potassium 2 (K2), low potassium 2 (Na2). Low-potassium solutions (Na1, Na2) and the sodium bath were identical. High-potassium solutions (K1, K2) substituted 125 mM KCl and 5 mM NaCl for the concentrations in standard bath (pH with KOH). Diazoxide (Dz) and tolbutamide (Tol) solutions were identical to high potassium solutions with the addition of 300μM diazoxide and 500μM tolbutamide, respectively. All solutions also contained 1μM NBQX, 25μM APV, 10μM bicuculline, and 250nM TTX to isolate K_{ATP}-mediated currents (44). Internal solution consisted of K-gluconate with or without 0.5mM Mg-ATP and 2mM free $MgCl_2$.

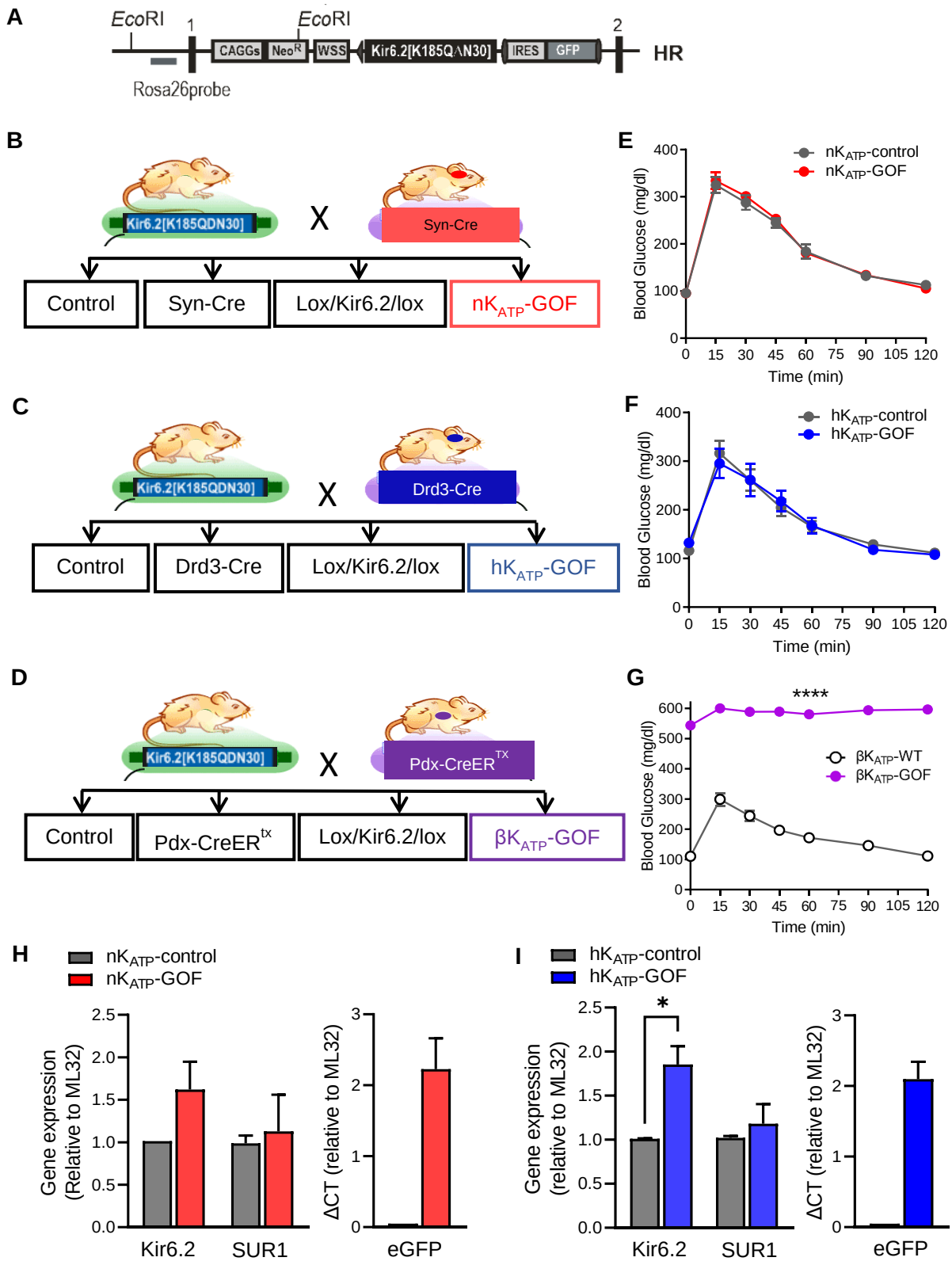
A
EcoRI
1
EcoRI
CAGGs
Neo^R
WSS
Kir6.2[K185QΔN30]
IRES
GFP
2
HR
Rosa26probe
B
Kir6.2[K185QDN30]
X
Syn-Cre
Control
Syn-Cre
Lox/Kir6.2/lox
nK_ATP-GOF
C
Kir6.2[K185QDN30]
X
Drd3-Cre
Control
Drd3-Cre
Lox/Kir6.2/lox
hK_ATP-GOF
D
Kir6.2[K185QDN30]
X
Pdx-CreER^TX
Control
Pdx-CreER^tx
Lox/Kir6.2/lox
βK_ATP-GOF
E
nK_ATP-control
nK_ATP-GOF
Blood Glucose (mg/dl)
Time (min)
F
hK_ATP-control
hK_ATP-GOF
Blood Glucose (mg/dl)
Time (min)
G
****
βK_ATP-WT
βK_ATP-GOF
Blood Glucose (mg/dl)
Time (min)
H
nK_ATP-control
nK_ATP-GOF
Gene expression (Relative to ML32)
Kir6.2
SUR1
ΔCT (relative to ML32)
eGFP
I
hK_ATP-control
hK_ATP-GOF
*
Gene expression (relative to ML32)
Kir6.2
SUR1
ΔCT (relative to ML32)
eGFP

Fig S1. Scheme of breeding strategy, glucose tolerance and K_{ATP} gene expression (A) schematic representation of Cre-dependent K_{ATP}-GOF mutation. The ROSA26 locus contains the loxP-flanked stop cassette, a pore-forming GOF mutation (Kir6.2$^{[K185Q,\Delta N30]}$), and a GFP reporter under control of IRES (20). ROSA26-K_{ATP}-GOF mice were crossed with synapsin-Cre (Syn-Cre) **(B),** dopamine-receptor-D3-Cre (Drd3-Cre) **(C)** or tamoxifen-inducible pancreatic-homeobox1-Cre (Pdx-CreER^{TM} **(D)** mice to generate pan-neuronal nK_{ATP}-GOF, hippocampal specific hK_{ATP}-GOF or pancreatic β-cell specific βK_{ATP}-GOF mice, respectively, alongside Cre-containing, ROSA26-containing, and wild-type littermates. Glucose tolerance tests in **(E)** nK_{ATP}-GOF mice (red, n=7) and littermate controls (grey, n=7), **(F)** hK_{ATP}-GOF mice (blue, n=11) and littermate controls (grey, n=12), and **(G)** βK_{ATP}-GOF mice (purple, n=8) and littermate controls (grey, n=8). All tests shown in this figure were acquired from the same cohort. **(H)** Hippocampal gene expression of Kir6.2 and SUR1 relative to ML32 (left) and ΔC_T for eGFP (right) in nK_{ATP}-GOF mice (red, n=3) and littermate controls (grey, n=3). **(I)** Hippocampal gene expression of Kir6.2 and SUR1 (left) and ΔC_T for eGFP (right) in hK_{ATP}-GOF mice (blue, n=3) and littermate controls (grey, n=3). *p<0.05 and ****p<0.0001. Non-significant differences are not indicated.

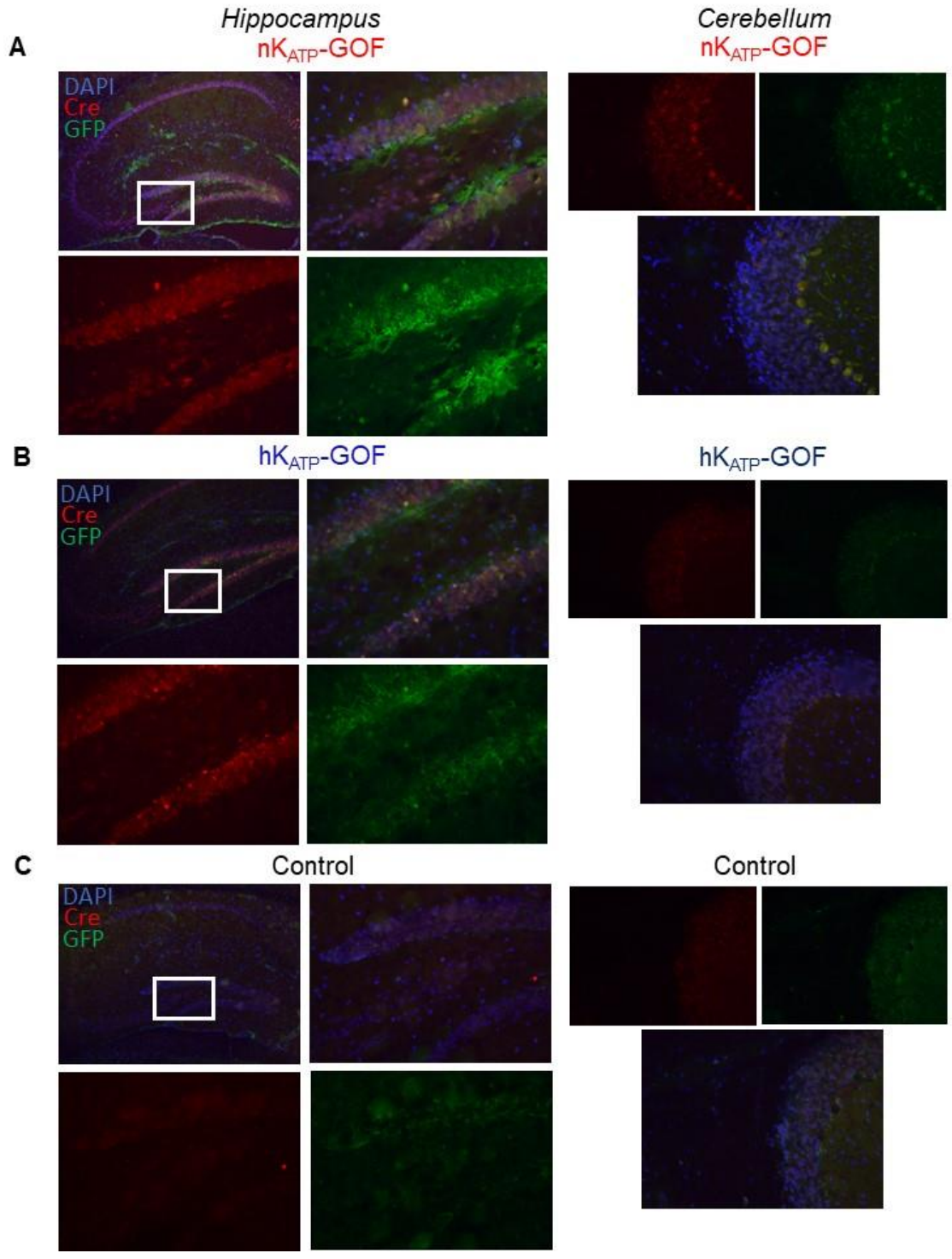

Fig. S2. Immunostaining of frozen brain sections in nK$_{ATP}$-GOF and hK$_{ATP}$-GOF mice. Anti-Cre (red) and anti-GFP (green) immunostaining in frozen sections of **(A)** nK$_{ATP}$-GOF and **(B)** hK$_{ATP}$-GOF. Control mice were used as negative control **(C).** DAPI nuclear staining in (blue).

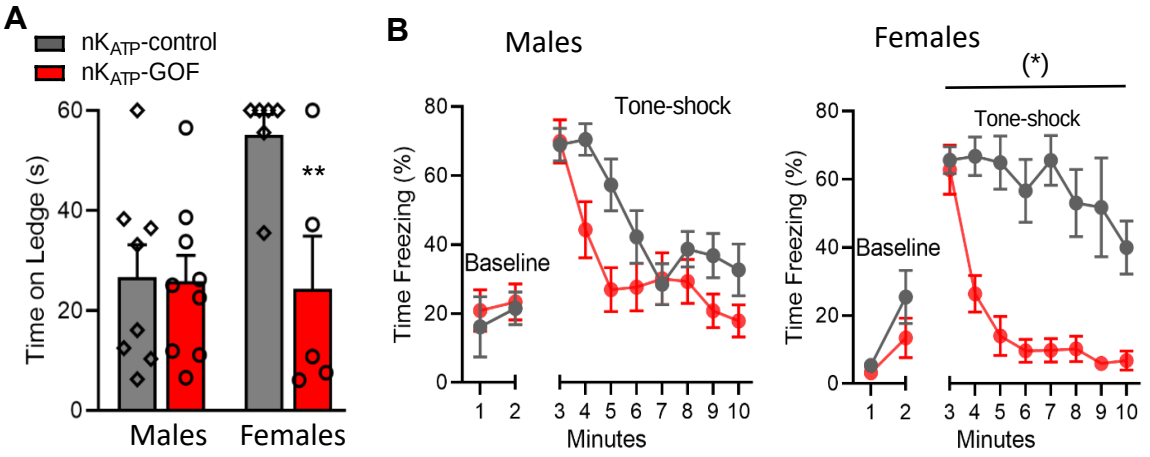

Fig. S3. Sex effects in time on ledge and auditory cue tests for nK$_{ATP}$-GOF male and female mice. (A) Sensorimotor Battery test: time on ledge, **(B)** fear conditioning test: freezing during auditory cue. nK$_{ATP}$-GOF mice (red, n=7 males and n=7 females) and littermate control mice (gray, n=6 males and n=7 females). *p<0.05, **p<0.01, ***p<0.01****p<0.0001. All data in this figure were acquired from the same cohort.

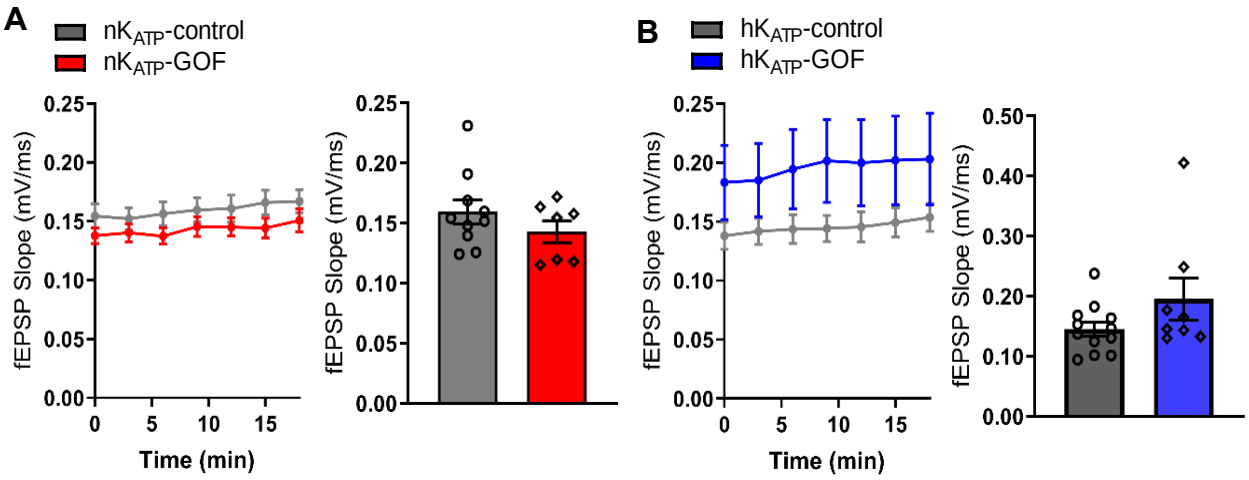

Fig. S4. Baseline fEPSP slopes before LTP induction (A) Left: fEPSP slopes during baseline period in nK$_{ATP}$-GOF (red, n=7, 5 mice) and control littermates (grey, n=10, 6 mice); Right: average of baseline fEPSP slopes in left panel **(B)** Left: fEPSP slopes during baseline period in hK$_{ATP}$-GOF (blue, n=8, 3 mice) and control littermates (grey, n=12, 8 mice); Right: average of baseline fEPSP slopes in left panel. Non-significant differences are not indicated.

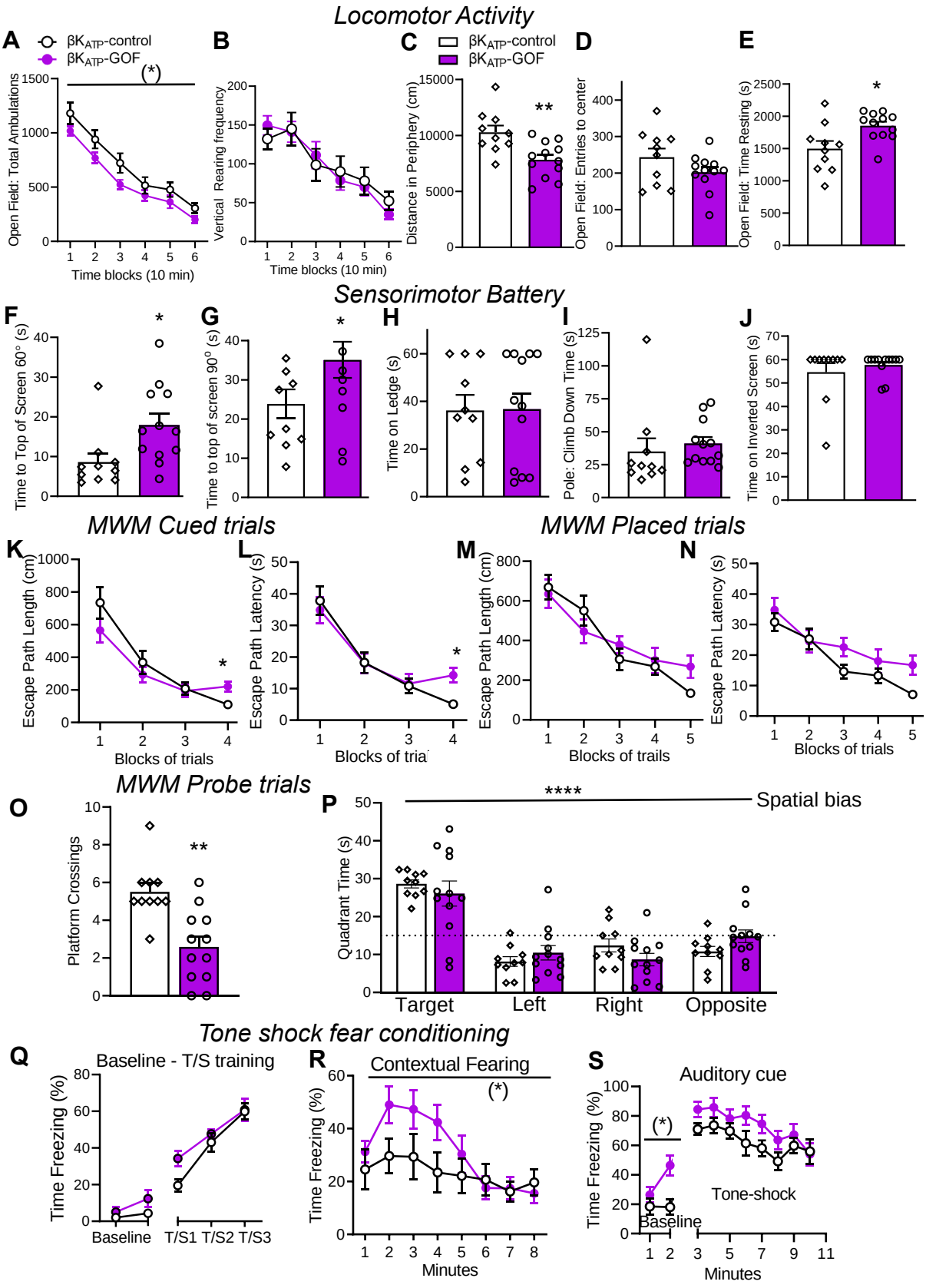
Locomotor Activity
A
βK$_{ATP}$-control
βK$_{ATP}$-GOF
(*)
Open Field: Total Ambulations
Time blocks (10 min)
B
Vertical Rearing frequency
Time blocks (10 min)
C
βK$_{ATP}$-control
βK$_{ATP}$-GOF
Distance in Periphery (cm)
**
D
Open Field: Entries to center
E
Open Field: Time Resting (s)
*
Sensorimotor Battery
F
Time to Top of Screen 60° (s)
*
G
Time to top of screen 90° (s)
*
H
Time on Ledge (s)
I
Pole: Climb Down Time (s)
J
Time on Inverted Screen (s)
MWM Cued trials
K
Escape Path Length (cm)
*
Blocks of trials
L
Escape Path Latency (s)
*
Blocks of tria
MWM Placed trials
M
Escape Path Length (cm)
Blocks of trails
N
Escape Path Latency (s)
Blocks of trials
MWM Probe trials
O
Platform Crossings
**
P
****
Spatial bias
Quadrant Time (s)
Target
Left
Right
Opposite
Tone shock fear conditioning
Q
Baseline - T/S training
Time Freezing (%)
Baseline
T/S1 T/S2 T/S3
R
Contextual Fearing
(*)
Time Freezing (%)
Minutes
S
Auditory cue
(*)
Tone-shock
Time Freezing (%)
Baseline
Minutes

Fig. S5. Diabetic βK_{ATP}-GOF mice did not show most neurological features of DEND. Locomotor activity: **(A)** total ambulations, **(B)** vertical rears, **(C)** distance traveled in the periphery, **(D)** center entries and **(E)** time resting in βK_{ATP}-GOF mice (purple, n=12) and littermate controls (white, n=10). Sensorimotor battery: **(F,G)** climb to the top of the 60º and 90º inclined screen, **(H)** time on the ledge, **(I)** time to climb-down pole and **(J)** time on inverted screen. MWM cue trials: **(K)** escape path length, **(L)** latency. MWM place trials: **(M)** escape path length, **(N)** latency. MWM probe trials: **(O)** platform crossing and **(P)** time in target quadrant and spatial bias. Fear-conditioning: **(Q)** tone-shock baseline-training, **(R)** contextual fear and **(S)** auditory cue tests. *p<0.05, **p<0.01, ****p<0.0001. All data shown in this figure were acquired from the same cohort.

Variable (mean ± sd)	**Overall Effects**	**F Statistics**
Morris Water Maze (MWM)		
MWM Cued		
Escape Path Length (cm)	Genotype (Geno)	$F(1,24) = 10.83$, $p = 0.0031$
	Session (S)	$F(3,72) = 23.56$, $p < 0.00005$
	Geno x S	$F(3,72) = 4.702$, $p = 0.0047$
Escape Path Latency (s)	Genotype	$F(1,24) = 34.11$, $p < 0.00005$
	Session (S)	$F(3,72) = 20.10$, $p < 0.00005$
	Geno x S	ns
Swimming speed (cm/s)	Genotype	$F(1,24) = 114.1$, $p < 0.00005$
	Session (S)	ns
	Geno x S	ns
MWM Place		
Escape Path Length (cm)	Genotype	$F(1,24) = 48.91$, $p < 0.00005$
	Session (S)	$F(4,96) = 3.811$, $p = 0.0064$
	Geno x S	$F(4,96) = 5.960$, $p = 0.0003$
Escape Path Latency (s)	Genotype	$F(1,24) = 94.60$, $p < 0.00005$
	Session (S)	ns
	Geno x S	$F(4,96) = 4.217$, $p = 0.0034$
Swimming speed (cm/s)	Genotype	$F(1,24) = 39.19$, $p < 0.00005$
	Session (S)	$F(4,96) = 11.85$, $p < 0.00005$
	Geno x S	$F(4,96) = 2.751$, $p = 0.0325$
MWM Probe		
Platform Crossings		
(nK_{ATP}-GOF: 0.571 ± 0.8516)	Genotype	$F(1,24) = 41.25$, $p < 0.00005$
(nK_{ATP}-control: 5.571 ± 2.709)		
Time in Target Quadrant (s)		
(nK_{ATP}-GOF: 11.86 ± 4.827)	Genotype	$F(1,24) = 63.57$, $p < 0.00005$
(nK_{ATP}-control: 31 ± 7.282)		
Spatial Bias		
	Trials (T)	$F(1,24) = 14.6155$, $p < 0.00005$
Fear Conditioning		
Tone-Shock Training		
Baseline Freezing Time (%)	Genotype	ns
	Session (S)	ns
	Geno x S	ns
Freezing Time in Training (%)	Genotype	$F(1,24) = 7.578$, $p = 0.0111$
	Session (S)	$F(2,48) = 46.66$, $p < 0.00005$
	Geno x S	$F(2,48) = 7.450$, $p = 0.0028$
Contextual Fearing		
Freezing in Previous Context (%)	Genotype	$F(1,24) = 20.89$, $p = 0.0001$
	Session (S)	$F(7,168) = 5.4045$, $p = 0.0004$
	Geno x S	$F(7,168) = 5.4013$, $p = 0.0004$
Auditory Cue		
Baseline Freezing Time (%)	Genotype	ns
	Session (S)	$F(1,24) = 11.41$, $p = 0.0025$
	Geno x S	ns
Freezing Time in Testing (%)	Genotype	$F(1,24) = 28.63$, $p < 0.00005$
	Session (S)	$F(7,168) = 26.95$, $p < 0.00005$
	Geno x S	$F(7,168) = 4.68$, $p = 0.0002$

Table S1. Means and ANOVA effects for locomotor activity assays, sensorimotor battery, and rotarod assays for nK_{ATP}-GOF and control mice

Variable (mean ± sd)	**Overall Effects**	**F Statistics**
Morris Water Maze (MWM)		
MWM Cued		
Escape Path Length (cm)	Genotype (Geno)	$F(1,24) = 10.83$, $p = 0.0031$
	Blocks of Trials (BT)	$F(3,72) = 23.56$, $p < 0.00005$
	Geno x BT	$F(3,72) = 4.702$, $p = 0.0047$
Escape Path Latency (s)	Genotype	$F(1,24) = 34.11$, $p < 0.00005$
	Blocks of Trials (BT)	$F(3,72) = 20.10$, $p < 0.00005$
	Geno x BT	ns
Swimming speed (cm/s)	Genotype	$F(1,24) = 114.1$, $p < 0.00005$
	Blocks of Trials (BT)	ns
	Geno x BT	ns
MWM Place		
Escape Path Length (cm)	Genotype	$F(1,24) = 48.91$, $p < 0.00005$
	Blocks of Trials (BT)	$F(4,96) = 3.811$, $p = 0.0064$
	Geno x BT	$F(4,96) = 5.960$, $p = 0.0003$
Escape Path Latency (s)	Genotype	$F(1,24) = 94.60$, $p < 0.00005$
	Blocks of Trials (BT)	ns
	Geno x BT	$F(4,96) = 4.217$, $p = 0.0034$
Swimming speed (cm/s)	Genotype	$F(1,24) = 39.19$, $p < 0.00005$
	Blocks of Trials (BT)	$F(4,96) = 11.85$, $p < 0.00005$
	Geno x BT	$F(4,96) = 2.751$, $p = 0.0325$
MWM Probe		
Platform Crossings		
(nK_{ATP}-GOF: 0.571 ± 0.8516)	Genotype	$F(1,24) = 41.25$, $p < 0.00005$
(nK_{ATP}-control: 5.571 ± 2.709)		
Time in Target Quadrant (s)		
(nK_{ATP}-GOF: 11.86 ± 4.827)	Genotype	$F(1,24) = 63.57$, $p < 0.00005$
(nK_{ATP}-control: 31 ± 7.282)		
Spatial Bias		
	Trials (T)	$F(1,24) = 14.6155$, $p < 0.00005$
Fear Conditioning		
Tone-Shock Training		
Baseline Freezing Time (%)	Genotype	ns
	Minutes (M)	ns
	Geno x M	ns
Freezing Time in Training (%)	Genotype	$F(1,24) = 7.578$, $p = 0.0111$
	Minutes (M)	$F(2,48) = 46.66$, $p < 0.00005$
	Geno x M	$F(2,48) = 7.450$, $p = 0.0028$
Contextual Fearing		
Freezing in Previous Context (%)	Genotype	$F(1,24) = 20.89$, $p = 0.0001$
	Minutes (M)	$F(7,168) = 5.4045$, $p = 0.0004$
	Geno x M	$F(7,168) = 5.4013$, $p = 0.0004$
Auditory Cue		
Baseline Freezing Time (%)	Genotype	ns
	Minutes (M)	$F(1,24) = 11.41$, $p = 0.0025$
	Geno x M	ns
Freezing Time in Testing (%)	Genotype	$F(1,24) = 28.63$, $p < 0.00005$
	Minutes (M)	$F(7,168) = 26.95$, $p < 0.00005$
	Geno x M	$F(7,168) = 4.68$, $p = 0.0002$

Table S2. Means and ANOVA effects for Morris Water Maze and fear conditioning assays in nK_{ATP}-GOF and control mice

Variable (mean ± sd)	**Overall Effects**	**F Statistics**
Morris Water Maze (MWM)		
MWM Cued		
Escape Path Length (cm)	Genotype (Geno)	$F(1,24) = 10.83$, $p = 0.0031$
	Blocks of Trials (BT)	$F(3,72) = 23.56$, $p < 0.00005$
	Geno x BT	$F(3,72) = 4.702$, $p = 0.0047$
Escape Path Latency (s)	Genotype	$F(1,24) = 34.11$, $p < 0.00005$
	Blocks of Trials (BT)	$F(3,72) = 20.10$, $p < 0.00005$
	Geno x BT	ns
Swimming speed (cm/s)	Genotype	$F(1,24) = 114.1$, $p < 0.00005$
	Blocks of Trials (BT)	ns
	Geno x BT	ns
MWM Place		
Escape Path Length (cm)	Genotype	$F(1,24) = 48.91$, $p < 0.00005$
	Blocks of Trials (BT)	$F(4,96) = 3.811$, $p = 0.0064$
	Geno x BT	$F(4,96) = 5.960$, $p = 0.0003$
Escape Path Latency (s)	Genotype	$F(1,24) = 94.60$, $p < 0.00005$
	Blocks of Trials (BT)	ns
	Geno x BT	$F(4,96) = 4.217$, $p = 0.0034$
Swimming speed (cm/s)	Genotype	$F(1,24) = 39.19$, $p < 0.00005$
	Blocks of Trials (BT)	$F(4,96) = 11.85$, $p < 0.00005$
	Geno x BT	$F(4,96) = 2.751$, $p = 0.0325$
MWM Probe		
Platform Crossings		
(nK_{ATP}-GOF: 0.571 ± 0.8516)	Genotype	$F(1,24) = 41.25$, $p < 0.00005$
(nK_{ATP}-control: 5.571 ± 2.709)		
Time in Target Quadrant (s)		
(nK_{ATP}-GOF: 11.86 ± 4.827)	Genotype	$F(1,24) = 63.57$, $p < 0.00005$
(nK_{ATP}-control: 31 ± 7.282)		
Spatial Bias		
	Trials (T)	$F(1,24) = 14.6155$, $p < 0.00005$
Fear Conditioning		
Tone-Shock Training		
Baseline Freezing Time (%)	Genotype	ns
	Minutes (M)	ns
	Geno x M	ns
Freezing Time in Training (%)	Genotype	$F(1,24) = 7.578$, $p = 0.0111$
	Minutes (M)	$F(2,48) = 46.66$, $p < 0.00005$
	Geno x M	$F(2,48) = 7.450$, $p = 0.0028$
Contextual Fearing		
Freezing in Previous Context (%)	Genotype	$F(1,24) = 20.89$, $p = 0.0001$
	Minutes (M)	$F(7,168) = 5.4045$, $p = 0.0004$
	Geno x M	$F(7,168) = 5.4013$, $p = 0.0004$
Auditory Cue		
Baseline Freezing Time (%)	Genotype	ns
	Minutes (M)	$F(1,24) = 11.41$, $p = 0.0025$
	Geno x M	ns
Freezing Time in Testing (%)	Genotype	$F(1,24) = 28.63$, $p < 0.00005$
	Minutes (M)	$F(7,168) = 26.95$, $p < 0.00005$
	Geno x M	$F(7,168) = 4.68$, $p = 0.0002$

Table S3. Means and ANOVA effects for locomotor activity assays, sensorimotor battery, and rotarod assays for hK_{ATP}-GOF and control mice

Variable (mean ± sd)	**Overall Effects**	**F Statistics**
Morris Water Maze (MWM)		
MWM Cued		
Escape Path Length (cm)	Genotype (Geno)	$F(1,19) = 4.650$, $p = 0.0441$
	Blocks of Trials (BT)	$F(3,57) = 39.62$, $p < 0.00005$
	Geno x BT	ns
Escape Path Latency (s)	Genotype (Geno)	$F(1,19) = 11.61$, $p = 0.003$
	Blocks of Trials (BT)	$F(3,57) = 40.62$, $p < 0.00005$
	Geno x BT	ns
Swimming speed (cm/s)	Genotype (Geno)	$F(1,19) = 9.032$, $p = 0.0073$
	Blocks of Trials (BT)	$F(3,57) = 4.588$, $p = 0.0087$
	Geno x BT	ns
MWM Place		
Escape Path Length (cm)	Genotype (Geno)	ns
	Blocks of Trials (BT)	$F(4,76) = 3.501$, $p = 0.0112$
	Geno x BT	ns
Escape Path Latency (s)	Genotype (Geno)	$F(1,19) = 9.280$, $p = 0.0066$
	Blocks of Trials (BT)	ns
	Geno x BT	ns
Swimming speed (cm/s)	Genotype (Geno)	$F(1,19) = 10.18$, $p = 0.0048$
	Blocks of Trials (BT)	$F(4,76) = 8.068$, $p < 0.00005$
	Geno x BT	ns
MWM Probe		
Platform Crossings		
(hK_{ATP}-GOF: 1.000 ± 1.342)	Genotype	$F(1,19) = 33.45$, $p < 0.00005$
(hK_{ATP}-control: 4.750 ± 1.765)		
Time in Target Quadrant (s)		
(hK_{ATP}-GOF: 16.93 ± 6.535)	Genotype	$F(1,19) = 4.700$, $p = 0.0431$
(hK_{ATP}-control: 23.6 ± 7.409)		
Spatial Bias		
	Trials (T)	$F(1,19) = 8.545$, $p = 0.0004$
Fear Conditioning		
Tone-Shock Training		
Baseline Freezing Time (%)	Genotype	ns
	Minutes (M)	ns
	Geno x M	ns
Freezing Time in Training (%)	Genotype	$F(1,19) = 9.565$, $p = 0.006$
	Minutes (M)	$F(2,38) = 38.03$, $p < 0.00005$
	Geno x M	ns
Contextual Fearing		
Freezing in Previous Context (%)	Genotype	ns
	Minutes (M)	ns
	Geno x M	$F(7,133) = 2.519$, $p = 0.0360$
Auditory Cue		
Baseline Freezing Time (%)	Genotype	ns
	Minutes (M)	ns
	Geno x M	ns
Freezing Time in Testing (%)	Genotype	ns
	Minutes (M)	$F(7,133) = 7.827$, $p < 0.00005$
	Geno x M	ns

Table S4. Means and ANOVA effects for Morris Water Maze and fear conditioning assays in hK_{ATP}-GOF and control mice

Variable (mean ± sd)	**Overall Effects**	**F Statistics**
Locomotor Activity		
Total Ambulations	Genotype (Geno)	ns
(Glib-nK_{ATP}-GOF: 5557 ± 3069)	Time Blocks (TB)	$F(5,90) = 41.36$, $p < 0.00005$
(Glib-nK_{ATP}-control: 3584 ± 1012)	Geno x TB	ns
Vertical Rears	Genotype (Geno)	ns
(Glib-nK_{ATP}-GOF: 586.8 ± 423.7)	Time Blocks (TB)	$F(5,90) = 41.29$, $p < 0.00005$
(Glib-nK_{ATP}-control: 531.1 ± 168)	Geno x TB	ns
Entries into Center		
(Glib-nK_{ATP}-GOF: 278.5 ± 176.4)	Genotype	ns
(Glib-nK_{ATP}-control: 194.8 ± 70.20)		
Distance in Periphery (cm)		
(Glib-nK_{ATP}-GOF: 13874 ± 5989)	Genotype	$F(1,18) = 4.644$, $p = 0.0450$
(Glib-nK_{ATP}-control: 9534 ± 1943)		
Sensorimotor Battery		
Climb Down Time on Pole (s)		
(Glib-nK_{ATP}-GOF: 47.72 ± 35.84)	Genotype	ns
(Glib-nK_{ATP}-control: 27.87 ± 11.56)		
Time to Top of 90º Screen		
(Glib-nK_{ATP}-GOF: 19.83 ± 10.53)	Genotype	$F(1,18) = 4.593$, $p = 0.046$
(Glib-nK_{ATP}-control: 34.28 ± 17.18)		
Morris Water Maze (MWM)		
MWM Cued		
Escape Path Length (cm)	Genotype (Geno)	ns
	Blocks of Trials (BT)	$F(3,54) = 32.20$, $p < 0.00005$
	Geno x BT	ns
Escape Path Latency (s)	Genotype (Geno)	$F(1,18) = 7.137$, $p = 0.0156$
	Blocks of Trials (BT)	$F(3,54) = 38.94$, $p < 0.00005$
	Geno x BT	ns
Swimming speed (cm/s)	Genotype (Geno)	$F(1,18) = 12.71$, $p < 0.0022$
	Blocks of Trials (BT)	$F(3,54) = 7.268$, $p < 0.0004$
	Geno x BT	ns
MWM Place		
Escape Path Length (cm)	Genotype (Geno)	$F(1,18) = 20.01$, $p = 0.0003$
	Blocks of Trials (BT)	$F(4,72) = 4.691$, $p = 0.002$
	Geno x BT	$F(4,72) = 4.514$, $p = 0.0026$
Escape Path Latency (s)	Genotype (Geno)	$F(1,18) = 36.68$, $p = 0.00001$
	Blocks of Trials (BT)	ns
	Geno x BT	$F(4,72) = 3.732$, $p = 0.0081$
Swimming speed (cm/s)	Genotype (Geno)	$F(1,18) = 11.29$, $p = 0.0035$
	Blocks of Trials (BT)	$F(4,72) = 6.708$, $p = 0.0001$
	Geno x BT	ns
MWM Probe		
Time in Target Quadrant (s)		
(Glib-nK_{ATP}-GOF: 15.04 ± 6.878)	Genotype	$F(1,18) = 38.87$, $p < 0.00005$
(Glib-nK_{ATP}-control: 30.46 ± 3.786)		
Spatial Bias		
	Trials (T)	$F(3,54) = 14.03$, $p < 0.00005$
Fear Conditioning		
Contextual Fearing		
Freezing in Previous Context (%)	Genotype	$F(1,18) = 20.69$, $p = 0.0002$
	Minutes (M)	$F(7,126) = 4.741$, $p = 0.0003$
	Geno x M	ns
Auditory Cue		
Baseline Freezing Time (%)	Genotype	ns
	Minutes (M)	ns
	Geno x M	ns
Freezing Time in Testing (%)	Genotype	$F(1,18) = 7.567$, $p = 0.0131$
	Minutes (M)	$F(7,126) = 5.109$, $p = 0.0005$
	Geno x M	ns

Table S5. Means and ANOVA effects for locomotor activity assays, sensorimotor battery, Morris Water Maze, and fear conditioning assays in nK_{ATP}-Glib-GOF and control mice

Variable (mean ± sd)	**Overall Effects**	**F or T Statistics**
Locomotor Activity		
Total Ambulations	Genotype (Geno)	$F(1,18) = 6.845$, $p = 0.0175$
(βK_{ATP}-GOF: 3295 ± 546.2)	Time Blocks (TB)	$F(5,90) = 59.69$, $p < 0.00005$
(βK_{ATP}-control: 4140 ±952.8)	Geno x TB	ns
Distance in Periphery (cm)		
(βK_{ATP}-GOF: 7829 ± 1560)	Genotype	$F(1,18) = 9.048$, $p = 0.0076$
(βK_{ATP}-control: 10283 ± 1906)		
Entries into Center		
(βK_{ATP}-GOF: 202.2 ± 50.50)	Genotype	ns
(βK_{ATP}-control: 243.6 ± 75.17)		
Time Resting (s)		
(βK_{ATP}-GOF: 1498 ± 217.7)	Genotype	$T(1,20) = 2.770$, $p = 0.0188$
(βK_{ATP}-control: 1852 ± 374.1)		
Sensorimotor Battery		
Climb Down Time on Pole (s)		
(βK_{ATP}-GOF: 41.06 ± 17.01)	Genotype	ns
(βK_{ATP}-control: 35.04 ± 31.58)		
Time to Top of 60º Screen		
(βK_{ATP}-GOF: 17.95 ± 9.831)	Genotype	$F(1,18) = 8.274$, $p = 0.0100$
(βK_{ATP}-control: 8.605 ± 7.097)		
Time to Top of Inverted Screen		
(βK_{ATP}-GOF: 57.68 ± 4.840)	Genotype	ns
(βK_{ATP}-control: 54.63 ± 12.25)		
Time on Ledge (s)	Genotype	ns
(βK_{ATP}-GOF: 36.74 ± 23.16)		
(βK_{ATP}-control: 36.20 ± 20.65)		
Morris Water Maze (MWM)		
MWM Cued		
Escape Path Length (cm)	Genotype (Geno)	ns
	Blocks of Trials (BT)	$F(3,54) = 30.01$, $p < 0.00005$
	Geno x BT	ns
Escape Path Latency (s)	Genotype (Geno)	ns
	Blocks of Trials (BT)	$F(3,54) = 29.57$, $p < 0.00005$
	Geno x BT	ns
MWM Place		
Escape Path Length (cm)	Genotype (Geno)	ns
	Blocks of Trials (BT)	$F(4,72) = 27.08$, $p < 0.00005$
	Geno x BT	ns
Escape Path Latency (s)	Genotype (Geno)	ns
	Blocks of Trials (BT)	$F(4,72) = 22.52$, $p < 0.00005$
	Geno x BT	ns
MWM Probe		
Time in Target Quadrant (s)		
(βK_{ATP}-GOF: 26.08 ± 11.49)	Genotype	ns
(βK_{ATP}-control: 28.63 ± 3.409)		
Spatial Bias		
	Trials (T)	$F(3,54) = 24.89$, $p < 0.00005$
Fear Conditioning		
Contextual Fearing		
Freezing in Previous Context (%)	Genotype	ns
	Minutes (M)	$F(7,126) = 9.453$, $p < 0.00005$
	Geno x M	$F(7,126) = 3.060$, $p = 0.0100$
Auditory Cue		
Baseline Freezing Time (%)	Genotype	ns
	Minutes (M)	$F(7,126) = 6.625$, $p = 0.0191$
	Geno x M	$F(7,126) = 7.224$, $p = 0.0150$
Freezing Time in Testing (%)	Genotype	ns
	Minutes (M)	$F(7,126) = 7.937$, $p < 0.0005$
	Geno x M	ns

Table S6. Means and ANOVA effects for locomotor activity assays, sensorimotor battery, Morris Water Maze, and fear conditioning assays in βK_{ATP}-GOF and control mice.

Short name	Primer	Sequence (5'-3')
Kir6.2	fwd	CGG GCG CAT GGT GAC AGA GG
	rev	CGA TGG GCC TGG GCC GTT TT
SUR1	fwd	TGA GCA TTG GAA GAC CCT CAT
	rev	CAG CAC CGA AGA TAA GTT GTC A
GFP	fwd	AAG GGC ATC GAC TTC AAG G
	rev	TGC TTG TCG GCC ATG ATA TAG
ML32	fwd	TTC CTG GTC CAC AAT GTC AA
	rev	GGC TTT TCG GTT CTT AGA GGA

Table S7. Primers for RT-qPCR