Lopes Pereira et al Supplemental data

Supplementary Materials and Methods

Behavioral phenotyping

Animals were habituated to environmental conditions for 15 days and handled by the experimenter for 4 days before the initiation of the experimental procedures. Groups of Ts1Yah males and wild-type littermates were tested with the standard SHIRPA protocol as described previously (1).

The performance of mice was tested in the rotarod, a task that evaluates motor coordination and balance. The ability of each mouse to maintain balance on a rotating rod (5 cm diameter and 10 cm long) with a plastic dowel surface was assessed with a commercially available rotarod apparatus (Rotarod LE8500, Panlab S.L., Barcelona, Spain). The equipment consisted of a rotating spindle that is able to maintain a fixed rotational speed (FRS) of 7, 10, 14, 19, 24, or 34 rpm and starting at 4 rpm to accelerate at a constant rate to 40 rpm over a 5-min period. The apparatus is provided with magnetic plates to detect when a mouse has fallen off the rod. Mice were placed on the middle of the rotating rod, its body axis being perpendicular to the rotation axis, and its head against the direction of rotation. All animals were tested for acquisitions and maintenance of rotarod performance. The experimental design consisted of two training trials (T1 and T2) (criterion test) at the minimum speed (4 rpm) followed by a 6 sessions (S1-S6) to assess the motor coordination and balance by measuring the latency to fall off the rod in consecutive trials with increasing fixed rotational speeds (FRS 4, 10, 14, 19, 24, and 34 rpm), and the animals were allowed to stay on the rod for a maximum period of 1 min per trial with a resting inter-trial period of 5 min. For each trial, the elapsed time until the mouse fell off the rod was recorded.

The treadmill (Panlab S.L., Barcelona, Spain) consisted of a belt (50 cm long and 20 cm wide) that can vary the speed (5 to 150 cm/s) and slope (0°–45°). At the end of the treadmill, an electrified grid delivered a foot shock (0.4 mA) whenever the mice felt off the belt. The mice were evaluated for eight trials on a single day session, with a cut-off period of 1 min per trial. The order of presentation of the different belt speeds and inclinations was identical for all mice. In the first two trials (Training), the belt speed was set at 5 cm/s and the inclination at 0°. In the following trials (Test), the slope was increased from 0° to 20° from the horizontal plane, and we applied different speeds (5, 10, 20, 30, 40 and 50 cm/sec). Mice were placed on the top of the already moving belt facing away from the electrified grid and in the direction opposite to the movement of the belt. Thus, to avoid the foot shocks, the mice had to move forward. Whenever an animal fell off the belt, foot shocks were applied for a maximal duration of 1 s. After the shocks, the mice were retrieved and placed back on the still moving belt to facilitate the association between safety and the belt.

Animals were scored for their locomotor activity in an open-field (Actitrack, Panlab, S.L., Spain). During 2 sessions of 30 min at 24 hours inter-trial interval, the distance travelled (in m) and the number of rears was measured. Then, spontaneous alternation behavior was measured in a Y-maze(2). Mice were tested in a single 5-min session, in the course of which it was placed in the central platform and allowed free exploration of the maze. The series of arm entries were scored and alternation defined as successive entries into the three arms. The percent alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries - 2) × 100.

Anxiety-related behavior was measured in an elevated plus maze test. The apparatus was a plus-shaped maze, elevated 50 cm from the floor, with two opposite open arms, 26×6 cm, crossed at right angles by two arms of the same dimensions, but enclosed by 12-cm-high walls made of grey PVC with an open roof. In addition, a 0.3-cm-high edge made of PVC surrounded the open arms to avoid falls. The experiment was recorded on a video recorder, placed 1.5 m above the maze and connected to a screen situated in an adjacent room, allowing the experimenter to watch the test. The mouse was

individually placed for a 5-min session at the end of one open arm facing away from the central platform and the time it took for the mouse to move from the open arm to either of the enclosed arms (transfer latency) was recorded. Then, the animal was allowed to explore the maze until the end of the session. The percentages of time and entries in enclosed arms were then calculated and were taken as index of anxiety-related behavior.

For the novel object recognition, the day before the experiment, mice are left 10 minutes in the open field box (OF) with two different objects, but with the same preference, in order to habituate animals. The next day, we do the experiment, divided in two trials. In the first trial (acquisition) the animals are left in the OF with two identical objects until they pass 20 seconds exploring the objects, during a maximum of 10 minutes. The exploration of the objects is calculated measuring the time in which the mouse directs his nose to the object in a distance < or = 2 cm or when touching with the nose. The fact of passing about the object or to put on it, is not considered to be an exploration. The animals that during these minutes they have not explored the object will be extracted of the experiment. The second trial will be realized after 1h and one of the objects of first trial will change for other one of differently, and the animals will be left in the OF during 5 minutes. The exploration time for the familiar (TF) or the new object (TN) during the testing period was recorded separately. The preferential index for the novel object in the testing period was defined as the exploration time for the novel object divided by the total exploration time for the novel and familiar objects [Memory index=TN/(TF+TN)100%]. The discrimination index was calculated as the ration (TN-TF)/(TN+TF). We also register the velocity, the distance and the time spend in center and periphery of the apparatus.

To test hippocampal-dependent spatial cognition, mice were trained in the standard version of the water maze as previously described (Escorihuela et al 1995) using 5 acquisition sessions (4 trials/session, 10-min inter-trial intervals). In each trial, mice were placed at one of the starting locations in random order [north, south, east, west (N, S, E, W), including permutations of the four starting points per session] and were allowed to swim until they located the platform. Mice failing to

find the platform within 60 s were placed on it for 20 s (the same period of time as the successful animals). Before acquisition, one habituation trial (without platform) and two pretraining trials allowed to discard swimming differences and possible spatial bias. At the end of acquisition, the probe session, with the platform removed, was performed and the time spent in the trained and non-trained quadrants as well as the number of platform annulus crossings during 60 s were recorded. After 24 hours, the cued session was performed to test the swimming speed and visual ability using the visible platform, elevated 1 cm above the water and its position clearly indicated by a visible cue (black flag). On the next day, mice performed the reversal learning session with the platform position changed to the opposite quadrant (SW). All the trials were recorded and traced with an Image tracking system (SMART, Panlab, S.L. Spain). To better evaluate the spatial distribution of the behavior of the mice, and the accuracy of spatial learning we used primary measures were cumulative search-error on training trials and learning indexes (Gallagher's proximity index and Whishaw's index [30]) computed from the trials given over the course of training. These measures rely on a computation of distance from the platform during the trial (Arqué et al., 2008) and were calculated using a custom-designed analysis program, jTracks to provide graphic representational tools.

References

1 Rogers, D., Peters, J., Martin, J., Ball, S., Nicholson, S., Witherden, A., Hafezparast, M., Latcham, J., Robinson, T., Quilter, C. *et al.* (2001) SHIRPA, a protocol for behavioral assessment: validation for longitudinal study of neurological dysfunction in mice. *Neurosci Lett*, **306**, 89-92.

2 Yamada, K., Noda, Y., Hasegawa, T., Komori, Y., Nikai, T., Sugihara, H. and Nabeshima, T. (1996) The role of nitric oxide in dizocilpine-induced impairment of spontaneous alternation behavior in mice. *J Pharmacol Exp Ther*, **276**, 460-466.

		2n			Ms2Yah			*p* (Ms2Yah	Ts1Yah			*p* (Ts1Yah
	Gene	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**vs Wt)**	**Mean**	**±**	**SD**	**vs Wt)**
Cortex	*Abcg1*	1,01	±	0,12	1,05	±	0,10	*n.s.*	1,09	±	0,08	*n.s.*
	Rsph1	1,01	±	0,18	0,42	±	0,05	***	1,44	±	0,18	**
	Slc37a1	1,00	±	0,11	0,60	±	0,03	***	1,54	±	0,20	***
	Pde9a	1,00	±	0,05	0,46	±	0,06	***	1,58	±	0,15	***
	Wdr4	1,00	±	0,07	0,49	±	0,03	***	1,53	±	0,07	***
	Ndufv3	1,00	±	0,04	0,51	±	0,02	***	1,55	±	0,06	***
	Pknox1	1,00	±	0,10	0,62	±	0,08	***	1,56	±	0,21	***
	Cbs	1,00	±	0,06	0,49	±	0,06	***	2,15	±	0,10	***
	U2af1	1,00	±	0,04	0,96	±	0,02	*n.s.*	1,02	±	0,05	*n.s.*
Thalamus	*Umodl1*	0,89	±	0,30	1,44	±	0,52	*n.s.*	1,98	±	0,28	***
	Abcg1	0,96	±	0,07	1,17	±	0,15	*	0,99	±	0,16	*n.s.*
	Rsph1	0,94	±	0,43	0,44	±	0,07	#	1,60	±	0,13	#
	Slc37a1	0,92	±	0,23	0,47	±	0,06	#	1,62	±	0,08	***
	Pde9a	0,89	±	0,09	0,47	±	0,02	#	1,62	±	0,05	***
	Wdr4	0,90	±	0,04	0,46	±	0,03	***	1,45	±	0,15	***
	Ndufv3	0,91	±	0,03	0,48	±	0,05	***	1,43	±	0,03	***
	Pknox1	0,93	±	0,06	0,61	±	0,04	***	1,38	±	0,17	***
	Cbs	0,87	±	0,07	0,42	±	0,04	***	1,65	±	0,10	***
	U2af1	0,99	±	0,03	0,95	±	0,04	*n.s.*	1,05	±	0,06	*n.s.*
Hippocampus	*Umodl1*	1,10	±	0,49	1,29	±	0,96	*n.s.*	0,90	±	0,21	*n.s.*
	Abcg1	0,99	±	0,17	1,10	±	0,20	*n.s.*	1,23	±	0,11	*
	Rsph1	1,04	±	0,22	0,64	±	0,30	*n.s.*	1,32	±	0,38	*n.s.*
	Slc37a1	0,96	±	0,31	0,49	±	0,06	#	1,31	±	0,25	*n.s.*
	Pde9a	0,93	±	0,06	0,37	±	0,08	***	1,30	±	0,11	***
	Wdr4	0,90	±	0,02	0,39	±	0,05	***	1,45	±	0,07	***
	Ndufv3	0,92	±	0,03	0,50	±	0,04	***	1,41	±	0,02	***
	Pknox1	0,94	±	0,15	0,66	±	0,07	**	1,44	±	0,18	**
	Cbs	0,84	±	0,08	0,33	±	0,06	***	1,76	±	0,17	***
	U2af1	0,97	±	0,15	1,00	±	0,05	*n.s.*	1,11	±	0,04	*n.s.*
	Cryaa	1,04	±	0,43	0,63	±	0,23	*n.s.*	0,77	±	0,28	*n.s.*
Cerebellum	*Abcg1*	0,98	±	0,14	0,90	±	0,14	*n.s.*	1,18	±	0,13	*n.s.*
	Rsph1	1,04	±	0,30	0,62	±	0,17	*	1,36	±	0,58	*n.s.*
	Slc37a1	0,81	±	0,07	0,50	±	0,05	#	2,12	±	0,12	***
	Pde9a	0,91	±	0,13	0,34	±	0,08	***	1,44	±	0,20	**
	Wdr4	0,91	±	0,12	0,36	±	0,05	***	1,40	±	0,18	**
	Ndufv3	0,93	±	0,08	0,40	±	0,04	***	1,31	±	0,11	***
	Pknox1	0,95	±	0,09	0,49	±	0,07	***	1,12	±	0,13	*n.s.*
	Cbs	0,87	±	0,03	0,36	±	0,04	***	1,70	±	0,22	#
	U2af1	1,02	±	0,08	0,86	±	0,03	**	0,95	±	0,09	*n.s.*
	Cryaa	1,21	±	0,22	1,38	±	2,06	*n.s.*	0,55	±	0,24	**

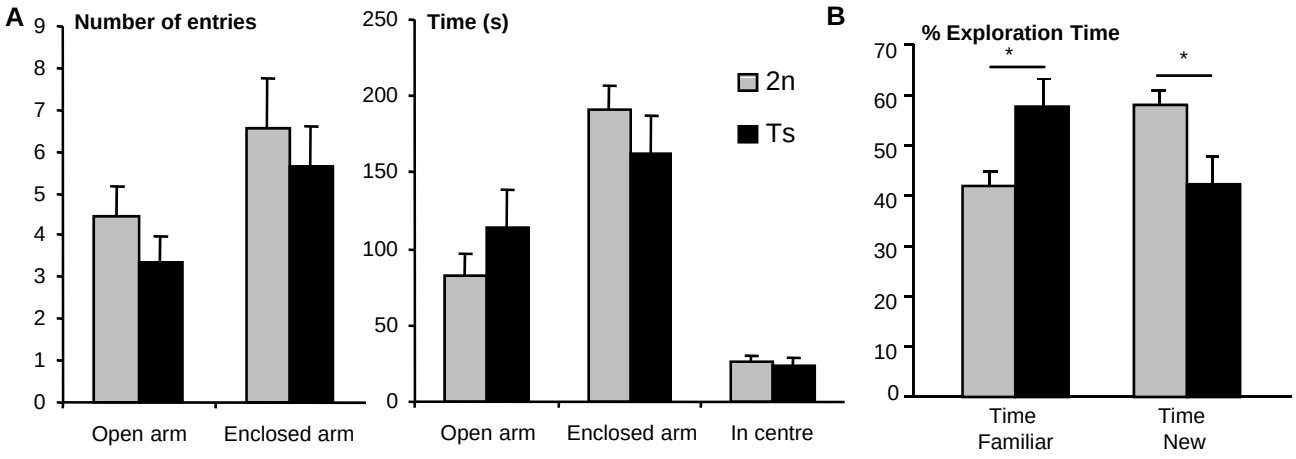
Supplemental table: Level of expression for genes found in the *Abcg1-U2af1* region in different subdomains of the brain of control (2n), monosomic (Ms2Yah) and trisomic (Ts1Yah) animal. n.s. non significative, *** P<0.001; ** P<0.01; * P<0.5; #

Legends to supplementary figures

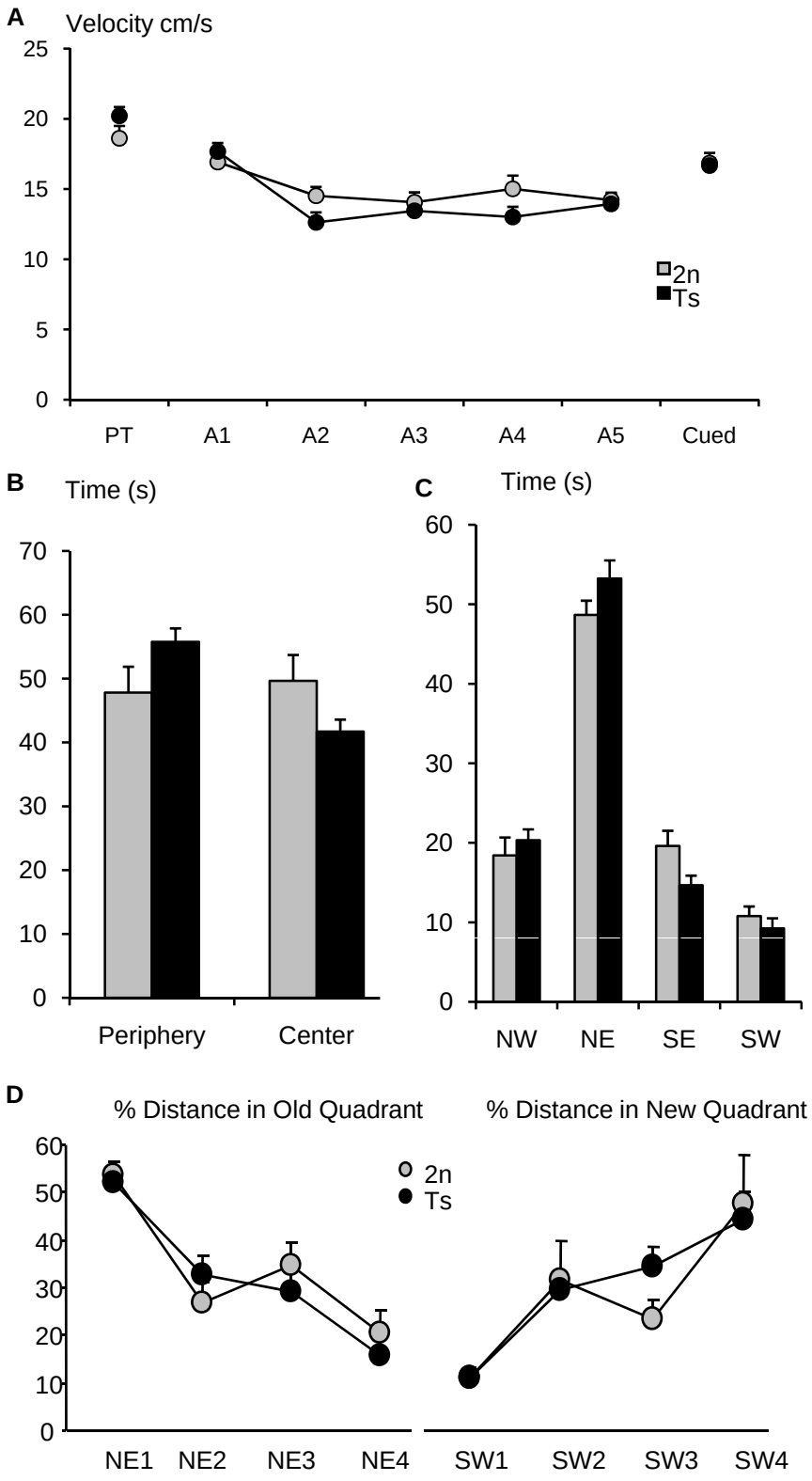
Supplementary figure 1: Anxiety and novel object recognition test (A) Assessment of anxiety related behavior in the elevated plus maze test. The number of entries in open arms and in enclosed arms and the time spent were measured in groups of n=15 Ts1Yah (in black) and control mice (in grey). No significant difference was observed. (B) Recognition memory is altered in Ts1Yah in the NOR during the 1h-retention test. The percentage of exploration time of trisomic mice (black) on the familiar object is superior ($F(1,22)=6.782$ $P=0.017$ ANOVA) to that of the wild-type mice (grey) thus revealing a better short-term memory in Ts1Yah mice. Data are expressed as mean ± S.E.M. *P<0.05; **P<0.01; ***P<0.001.

Supplementary figure 2: Morris Water Maze (MWM). (A) The swimming speed of trisomic (Ts) and wild-type (2n) showed no differences during the learning and cued session. (B) Thigmotaxis. (C) Recall of the location of the platform was tested during the removal test. No differences were observed between genotypes in the percentage of distance traveled in the trained quadrant ($F(1,28)= 0.011$ $P=0.097$ One-Way ANOVA), indicating that all mice equally remember the position of the platform. A slight tendency to an increase was observed in the distance travelled in the trained quadrant in Ts1Yah mice. (D) In reversal session mice of both genotypes reduced the distance travelled in the previously trained quadrant (NE, left panel) and increased the distance travelled in the new target quadrant (SW, right panel). Data are expressed as mean ± S.E.M.

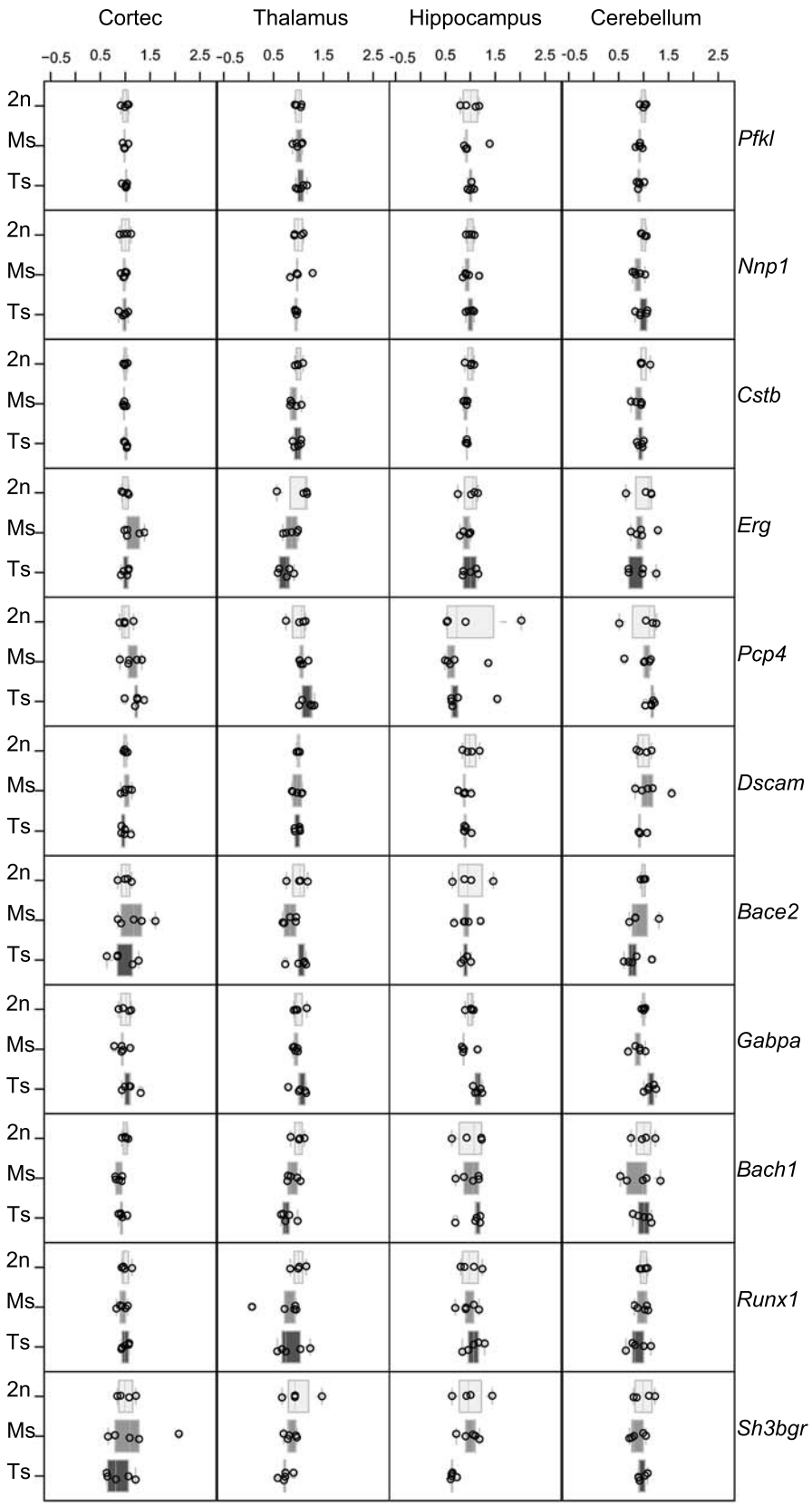
Supplementary figure 3: Control genes expression in Ts1Yah and Ms2Yah mice tissues. Box plots of normalized expression levels of 3 Mmu10 (*Pfkl*, *Nnp1* and *Cstb*) and 8 Mmu16 (*Erg*, *Pcp4*, *Dscam*, *Bace2*, *Gabpa*, *Bach1*, *Runx1* and *Sh3bgr*) genes expressed in the four tissues analyzed (Cortex, Thalamus-Hypothalamus, Hippocampus and Cerebellum). The X-axis is normalized expression values; the Y-axis is the three genotype groups (2n=Euploid mice; Ms=Ms2Yah mice and Ts=Ts1Yah mice). Each panel represents a gene (shown on right).


A
Number of entries
9
8
7
6
5
4
3
2
1
0
Open arm
Enclosed arm
Time (s)
250
200
150
100
50
0
2n
Ts
Open arm
Enclosed arm
In centre
B
% Exploration Time
70
60
50
40
30
20
10
0
*
*
Time Familiar
Time New

Sup Fig. 1


A
Velocity cm/s
25
20
15
10
5
0
PT
A1
A2
A3
A4
A5
Cued
2n
Ts
B
Time (s)
70
60
50
40
30
20
10
0
Periphery
Center
C
Time (s)
60
50
40
30
20
10
0
NW
NE
SE
SW
D
% Distance in Old Quadrant
% Distance in New Quadrant
2n
Ts
60
50
40
30
20
10
0
NE1
NE2
NE3
NE4
SW1
SW2
SW3
SW4

Sup. Fig. S2


Cortec
Thalamus
Hippocampus
Cerebellum
-0.5
0.5
1.5
2.5
2n
Ms
Ts
Pfkl
Nnp1
Cstb
Erg
Pcp4
Dscam
Bace2
Gabpa
Bach1
Runx1
Sh3bgr

Sup Fig. 3