SUPPLEMENTAL MATERIALS

Supplemental Behavioral Methods

Neurological screening

Mice were first subjected to a basic physical and neurological screening including the following: body weight, physical condition (e.g., barbering), abnormal behaviors (circling, excessive grooming, stereotyped sniffing or head bobbing, abnormal posture, abnormal freezing, ears and whiskers not twitching normally during free locomotion, reduced or excessive sniffing and rearing), righting reflex (regaining feet quickly after being turned over), eyeblink reflex (in response to approaching the eye with a cotton swab), ear twitch reflex (in response to touching ear with a cotton swab), and whisker orienting reflex (freezing whiskers and orienting when whiskers on one side are brushed). All mice in the study performed normally on these screenings, with no genotype-associated differences noted.

Strength and motor coordination

Adult mice were tested on a set of strength, balance, and motor coordination tasks. First, in the balance beam task, dowels were horizontally fixed between two sheltered escape platforms 40 cm above a thickly padded surface. Mice were tested three times on an 11 mm dowel and then three times on a 5 mm dowel in immediate succession. In each trial, mice were placed in perpendicular orientation across the center of the beam and released. The time required to escape to a platform or to fall from the

beam was measured (up to a maximum of 60 seconds). As mice almost never fell from the beams, only escape latency was analyzed, with animals that did not escape receiving a score of 60 seconds.

Second, in the wire forelimb suspension task, the balance beam dowel was replaced by a thick, tautly suspended wire. Mice were held in perpendicular orientation above the wire until they gripped it with their forepaws, at which point they were released. The time required to fall was measured, up to a maximum of 60 s. Mice were also scored according to the criteria of King & Arendash (2002): 0, mouse fell from string; 1, mouse remained hanging by two forepaws; 2, mouse attempted to climb onto string; 3, mouse got two forepaws and one or both hindpaws around string; 4, mouse got four paws and tail around string with lateral movement; 5, mouse escaped. Nearly all mice scored 2, 3, or 4; there was no difference among genotypes.

Third, in the vertical pole test (Crawley 2007), mice were placed on a 40 cm long, 2 cm diameter horizontal wooden pole covered with cloth tape, which was then slowly rotated to a vertical position and held there. The latency to fall was measured, up to a maximum of 60 s.

Fourth, in the hanging wire grip test (Crawley 2007), mice were placed upon a wire cage lid that was shaken once (to induce the mouse to grip) and then inverted. The latency to fall was measured, up to a maximum of 60 s.

Exploratory and sensory behaviors

Mice were also tested using a small open field, a visual cliff test, and a social recognition test. Mice were placed into a brightly-lit, 18" square open field, subdivided

into a 4x4 grid of squares that were not visible to the mice, for 300 s, during which time the number of line crossings were counted (to measure activity) and the proportion of time spent in the 12 edge/corner squares as compared to the four central squares was measured (thigmotaxis; an anxiety-related measure). Second, in the visual cliff task, the mouse was placed on a 3 cm wide beam from which it could step down 3 cm onto an opaque, checkered surface in one direction or, in the other direction, onto a transparent plastic sheet with a similar checkered surface visible 60 cm below. Each animal was tested once on the visual cliff, with a maximum trial duration of 120 s. The step-down latency and side choice were recorded. Third, in the social recognition task, mice were placed in one half of a cage divided into two equal chambers with a metal mesh. After the mouse habituated to the test environment, a second mouse of the same sex – either a cagemate or a stranger – was introduced into the other half of the cage. The time that the first mouse spent investigating the second mouse (via directed sniffing through the mesh) during the first minute after introduction was measured.

Supplemental Figure and Table Legends

Figure S1. Combinations of *Rasgrf1* alleles used in this study.

Mice carrying wildtype *Rasgrf1*^{tm1Pds} (Yoon *et al*. 2002), or *Rasgrf1*^{tm2Pds} (Yoon *et al*. 2005) mutations were bred to generate progeny with the combinations of alleles shown. Filled rectangles with rightward pointing arrows depict expressed alleles; absence of the arrows indicates gene silencing. Filled triangles are sequences needed for proper

imprinting. These were deleted (open triangle) to generate the *Rasgrf1*^{tm1Pds} allele or replaced with enhancer containing sequences (enh) to produce the *Rasgrf1*^{tm2Pds} allele. The parental origin of each allele is indicated by a male (blue) or female (red) symbol on the left. The corresponding genotype designations are depicted on the right.

Figure S2. Body mass measurements of wildtype and *+/tm2* mice.

(a) Females. No significant differences in growth were observed between genotypes. MP, *N*=37; *+/tm2*, *N*=27. **(b)** Males. No significant differences in growth were observed between genotypes. MP, *N*=32; *+/tm2*, *N*=31. For growth curves in other *Rasgrf1* imprinting genotypes, see Drake *et al.* (2009). Error bars indicate SEM.

Figure S3. Other behavioral phenotypes of adult mice.

Adult mice all express *Rasgrf1* biallelically; genotype designations apply to each animal's history of neonatally imprinted expression. **(a)** Balance beam. Interestingly, there was a significant effect of sex on escape latency for both the 11 mm and 5 mm beams ($p < 0.05$, $p < 0.001$ respectively). These effects may be spurious, noting that no other related motor task suggested a significant effect of sex, but in any event there were no significant effects of genotype on escape latency for either sex. **(b)** Suspension tasks. Neither sex nor genotype significantly affected the latency to fall in a wire forelimb suspension test, a vertical pole test, or a hanging wire grip test. **(c)** Open field tests. Neither sex nor genotype significantly affected either overall activity levels (line crossings) or thigmotaxis (proportion of time spent along the edges of the arena). **(d)** Visual cliff. Neither sex nor genotype significantly affected the step-down latency,

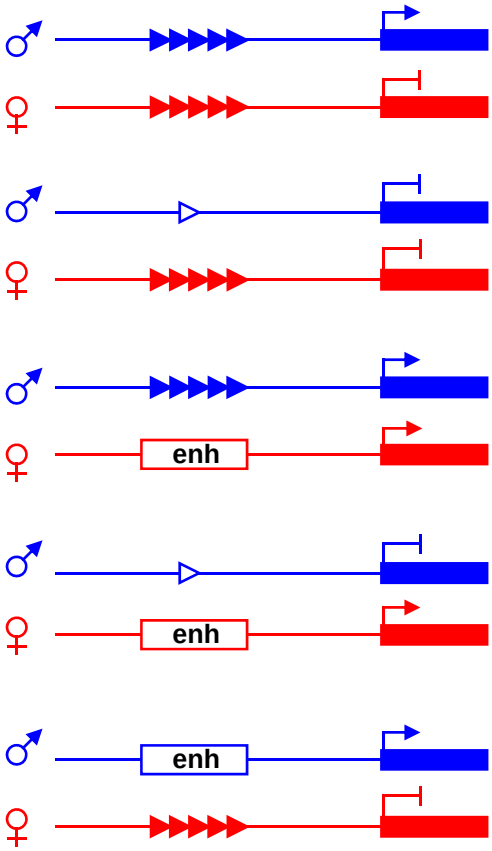
whether measured only on the opaque ("shallow") side or irrespective of side. **(e)** Social recognition. The time spent investigating a newly introduced mouse was significantly greater than that spent investigating a cagemate, irrespective of sex or genotype. Error bars indicate SEM.

Table S1. Pairwise statistical analysis of Rasgrf1 transcript levels in olfactory bulb. *Rasgrf1* transcript levels in P8 neonatal olfactory bulb were quantified for each genotype tested (see Methods). Transcript levels found in each genotype were compared using Students *t*-test; alpha levels were Bonferroni-corrected for ten multiple comparisons such that $p < 0.005$ indicates significance. The *t*-statistic and *p*-value for each pairwise comparison are depicted (df = 26 in all cases).

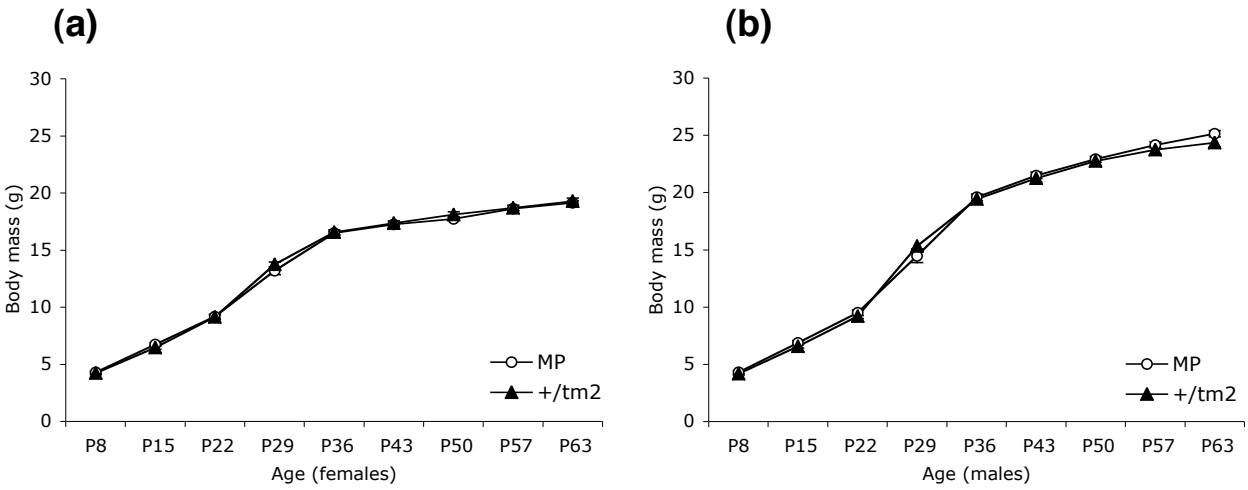
Table S2. Pairwise statistical analysis of Rasgrf1 transcript levels in hippocampus. *Rasgrf1* transcript levels in P8 neonatal hippocampus were quantified for each genotype tested (see Methods). Transcript levels found in each genotype were compared using Students *t*-test; alpha levels were Bonferroni-corrected for ten multiple comparisons such that $p < 0.005$ indicates significance. The *t*-statistic and *p*-value for each pairwise comparison are depicted (df = 26 in all cases).

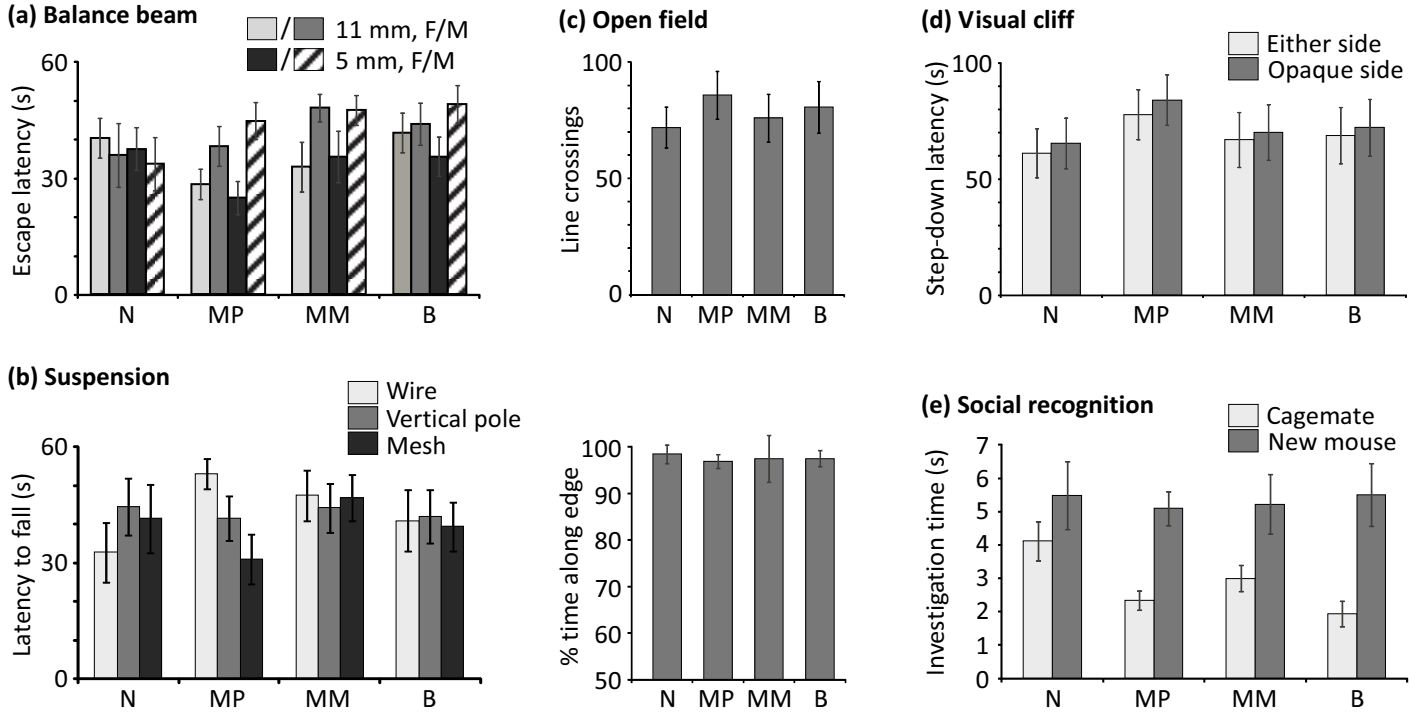
Table S3. Pairwise statistical analysis of Rasgrf1 transcript levels in the remainder of the brain. *Rasgrf1* transcript levels in the remainder of the P8 neonatal brain (ROB, comprising the whole brain excepting olfactory bulbs, hippocampus, hypothalamus and pituitary gland; see Methods for details) were quantified for each genotype tested (see

Methods). Transcript levels found in each genotype were compared using Students *t*-test; alpha levels were Bonferroni-corrected for ten multiple comparisons such that $p < 0.005$ indicates significance. The *t*-statistic and *p*-value for each pairwise comparison are depicted (df = 26 in all cases).



Allele genotype	Designation
wildtype wildtype	MP
Rasgrf1^{tm1Pds} wildtype	N
wildtype *Rasgrf1*^{tm2Pds}	B
Rasgrf1^{tm1Pds} *Rasgrf1*^{tm2Pds}	MM
Rasgrf1^{tm2Pds} wildtype	+/tm2


(a)
(b)
Body mass (g)
30
25
20
15
10
5
0
P8
P15
P22
P29
P36
P43
P50
P57
P63
Age (females)
Age (males)
MP
+/tm2


(a) Balance beam
11 mm, F/M
5 mm, F/M
Escape latency (s)
60
30
0
N
MP
MM
B
(b) Suspension
Wire
Vertical pole
Mesh
Latency to fall (s)
60
30
0
N
MP
MM
B
(c) Open field
Line crossings
100
50
0
N
MP
MM
B
% time along edge
100
90
80
70
60
50
N
MP
MM
B
(d) Visual cliff
Either side
Opaque side
Step-down latency (s)
100
50
0
N
MP
MM
B
(e) Social recognition
Cagemate
New mouse
Investigation time (s)
7
6
5
4
3
2
1
0
N
MP
MM
B