Appendix S1: The NeuroBSIK Mouse Phenomics consortium collaborators.

The NeuroBSIK Mouse Phenomics consortium collaborators are: Brussaard AB, Borst JGG, Elgersma Y, Galjart N, van der Horst GT, Levelt CN, Pennartz CMA, Smit AB, Spruijt BM, Verhage M, de Zeeuw CI.

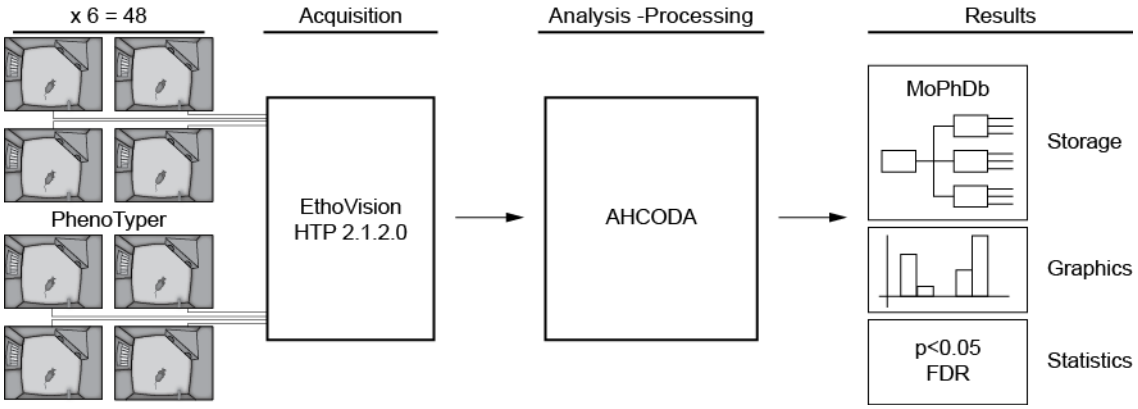

Fig. S1: Overview of the screening system with 48 cages connected to 12 computers running EthoVision HTP 2.1.2.0 software, controlling hardware in the PhenoTyper and saving track files containing X and Y coordinates and zone visits of mice at 15 frames/s. Track files were error corrected and smoothened using EthoVision HTP 2.1.2.0 software offline, and statistically analyzed and visualized with AHCODA.

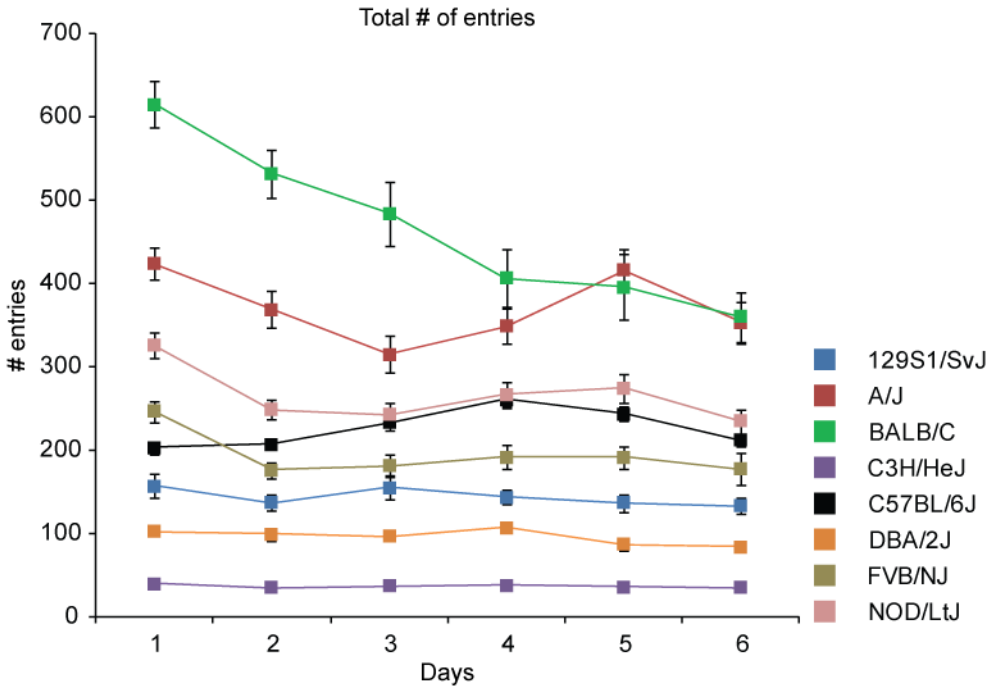

Fig. S2: Total number of entries per day during the 6 experimental days. C3H, DBA, 129S1, FVB, NOD displayed a stable number of entries along the experiment. C57 mice showed an increase until day 4 and then a decrease in the number of entries. A/J mice showed a decrease until day 3 then an increase until day 5 and finally a small decrease on day 6. BALB mice showed a constant decrease in the number of entries (see Table S3 and S8 for statistical details).

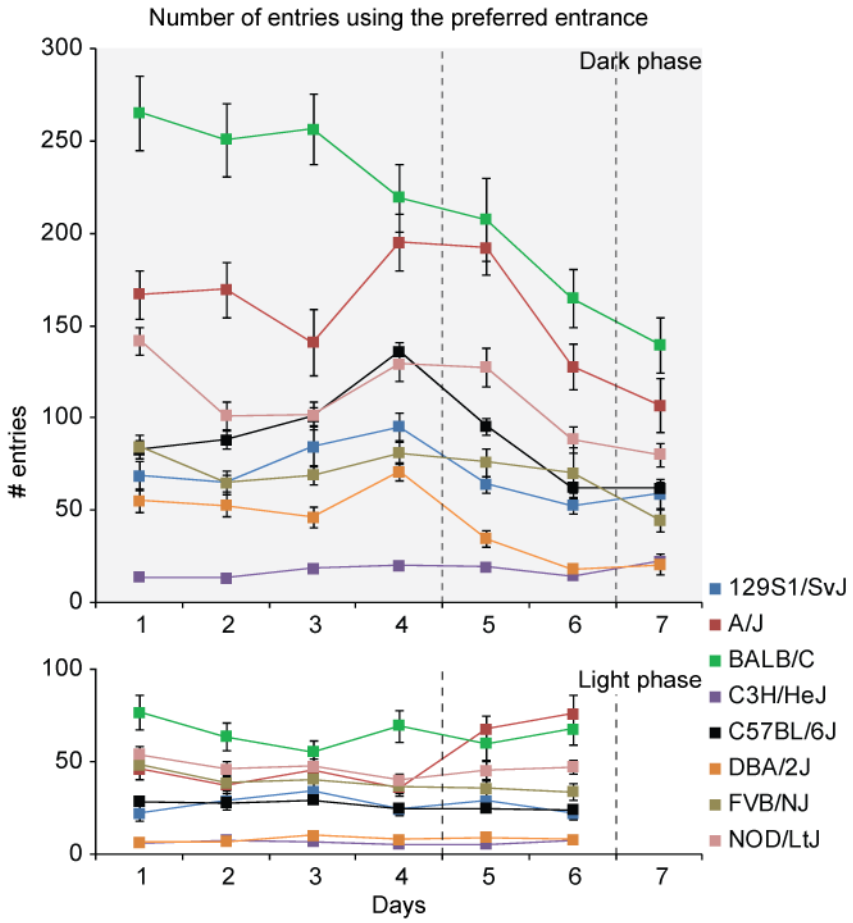

Fig. S3: Number of preferred entrances during the 6.5 days of experiment. The vertical dashed lines delineates the test period. Three strains (C57, 129S1, DBA) showed a decrease in the use of the preferred entrance at the beginning of the test period in the dark phase. BALB, A/J, NOD, FVB mice showed a decrease starting on day 5. C3H did not show any change in preferred entrance use. The differences were smaller in the light phase, during which mice did not seem to change their use of the preferred entrance. Only A/J mice showed an increase in the use of the preferred entrance during the test period in the light phase.

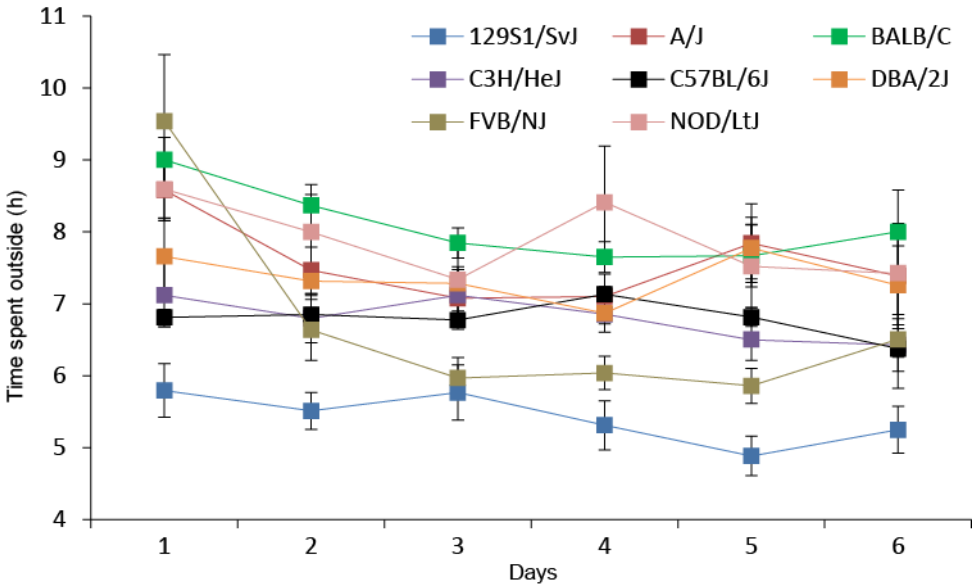

Fig. S4: Time spent outside the shelter. A repeated measures ANOVA showed a general decrease in the time spent outside during the 3 first days (between days 1 and 2 ($F_{(1,312)}$=20.7; p<0.001), and days 2 and 3 ($F_{(1,312)}$=6.56; p=0.01)). This overall effect is mainly due to A/J and FVB that show a significant decrease between day 1 and 2 (respectively p=0.003 and p<0.001).

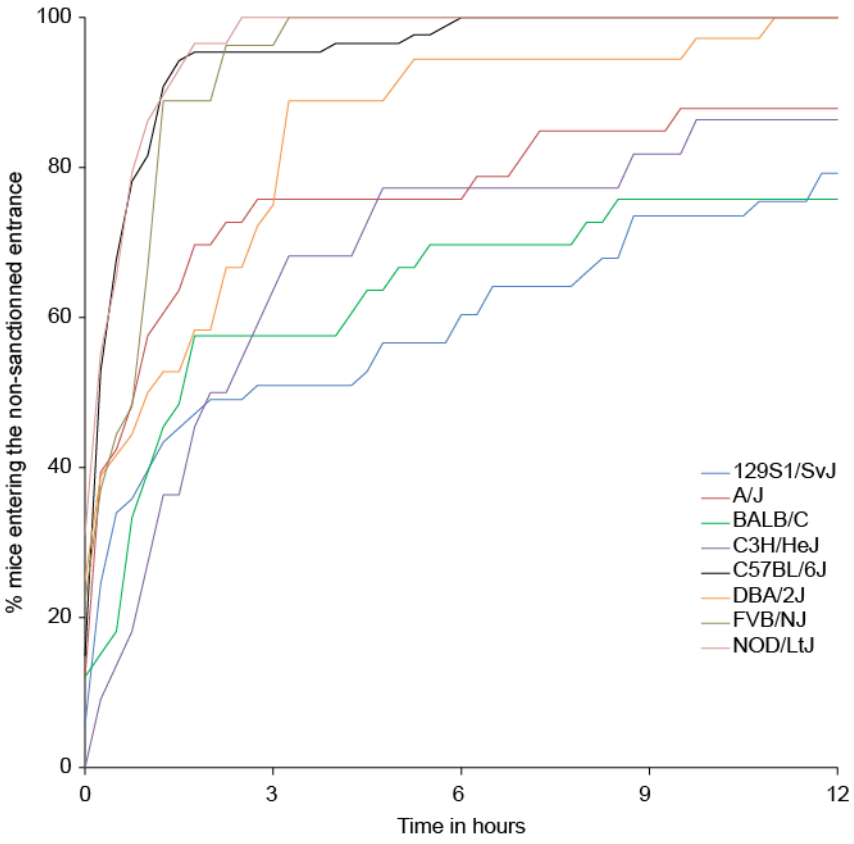

Fig. S5: Cumulative plot of the percentage of mice that entered the shelter through the non-sanctioned entrance during the dark phase of day 5. After 6 h of the dark phase of day 5, 100% of C57, FVB and NOD visited the non-sanction entrance at least once. The last DBA mouse visiting this entrance showed this response at 11 h of the dark phase. Not all 129S1, A/J, BALB and C3H mice visited the non-sanctioned entrance after 12 h. Those mice were excluded from the further analysis because they were not aware of the presence of a non-sanctioned entrance and therefore could not learn to change their preference.

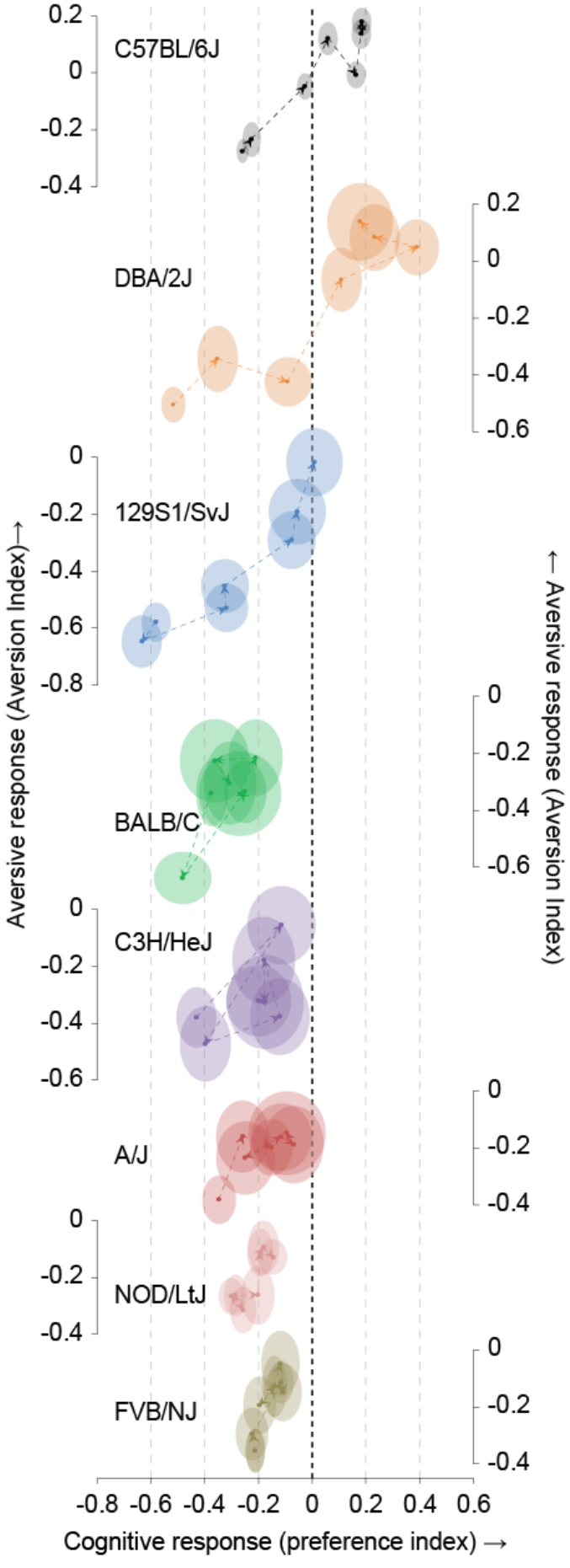
C57BL/6J
DBA/2J
129S1/SvJ
BALB/C
C3H/HeJ
A/J
NOD/LtJ
FVB/NJ
Aversive response (Aversion Index)→
← Aversive response (Aversion Index)
Cognitive response (preference index) →
-0.8 -0.6 -0.4 -0.2 0 0.2 0.4 0.6

Fig. S6. Indixes Plot. We plotted in one graph the deliberate change in preference to enter through the non-sanctioned entrance (X-axis), and the change in time spent in the shelter after entering through the non-sanctioned entrance (Y-axis). To simplify the visualization, the two parameters plotted here are the inverse of the preferred index and the aversion index. These two parameter are plotted against each other before the introduction of the aversive stimulus (day 4), during the two days when the stimulus is operational (days 5-6), and in dark phase of day 7 when the stimulus was no longer applied (12 h bins, dark and light phase). Aversion (upward shift) is expected to develop instantaneously in response to the aversive stimulus, whereas the cognitive response (rightward shift) is expected to develop gradually, reflecting the avoidance learning process. C57 mice showed such a response: during the first 24 h (day 5) the response involved both aversion (upward) and cognitive (rightward) aspects, followed by a stabilization of the aversion behavior whereas development of the cognitive aspect of the response continued (only a further rightward shift) during the following 24 h (day 6). A similar pattern was observed for DBA mice, until the end of day 5, but the preference change stabilized on day 6 already. 129S1 mice showed most of the cognitive response preceding the aversive response. This can be explained by the fact that during the dark phase of day 5 the response is mainly driven by a decrease of the use of the preferred entrance and that during the light phase almost no changes occur. The main part of the aversive and cognitive responses takes place during day 6. BALB mice display an upward shift only during the dark phase of day 5 (aversive response mainly). The remaining genotypes (C3H, A/J, FVB and NOD) showed no significant aversion response (although individual mice did), nor a cognitive response.

Strain	WT	KO	HZ
218l	6	5	
305d	12	12	
alpha7	25	23	
beta2	7	16	9
bko	12	12	
brevican	25	25	
ckii	13	11	
cb1	14	20	11
clasp2	12	11	
clip115	20	24	4
clip170	11	13	
DJ1	10	9	22
doc2A	18	28	4
doc2ab		17	
fgf13	19	36	9
Fx	40	10	26
kazubcatloxp	12	12	
kazugaa1	14	10	
kazu-t		23	
kazu-tlnic403		24	
Kcnd2	6	5	12
lach	12	12	
loxm18 null	12		12
mcry1	20	19	8
mcry1mcry2	13	11	
mcry2	11	14	
menin	14	12	
munc18	59		61
nf1	12		12
ng3	13	14	13
pkci	12	12	
r192q	10	12	
scap	12	12	
Snap25	28		35
specc1	23	25	
syt1	21		24
tarp8	7		13
tmed	6	13	6
trim3	12	12	12
tsc	12		12
tscb6	12		12
ube3	12		12

Ubn1	6	10	6

Table S1: List of the number of mutagenized mice (WT: wild-type; KO: Knock-out; HZ: heterozygous) screened in the PhenoTyper.

	Maximum number of entries in 15 min	Average entries in 15 min
129S1/SvImJ	81	6
A/J	84	12
BALB/C	111	21
C3H/HeJ	15	2
C57BL/6J	68	9
DBA/2J	44	4
FVB/NJ	45	7
NOD/LtJ	47	8

Table S2: Maximum number of shelter entries and average number of shelter entries in 15 min for each strain.

	(J) days	Mean Difference (D4-J)	Std. Error	df	Sig.	95% Wald Confidence Interval for Difference		Habituation day 1 to 4
						Lower	Upper	
129S1/SvImJ	1	-13.72	14.518	1	.345	-42.17	14.74	stable
	2	6.09	9.287	1	.512	-12.11	24.30	
	3	-12.09	11.392	1	.288	-34.42	10.23	
A/J	1	-74.58	21.953	1	.001	-117.60	-31.55	decrease
	2	-20.09	21.614	1	.353	-62.45	22.27	
	3	34.09	15.497	1	.028	3.72	64.47	
BALB/C	1	-208.36	39.886	1	.000	-286.54	-130.19	decrease
	2	-125.39	34.855	1	.000	-193.71	-57.08	
	3	-77.88	20.455	1	.000	-117.97	-37.79	
C3H/HeJ	1	-1.82	3.283	1	.580	-8.25	4.62	stable
	2	2.82	2.594	1	.277	-2.27	7.90	
	3	.55	2.369	1	.818	-4.10	5.19	
C57BL/6J	1	57.87	8.873	1	.000	40.48	75.26	increase
	2	53.30	7.448	1	.000	38.70	67.90	
	3	28.23	6.446	1	.000	15.60	40.86	
DBA/2J	1	3.92	6.361	1	.538	-8.55	16.38	stable
	2	7.44	7.521	1	.322	-7.30	22.19	
	3	9.78	4.455	1	.028	1.05	18.51	
FVB/NJ	1	-54.48	11.246	1	.000	-76.52	-32.44	decrease
	2	15.52	10.302	1	.132	-4.67	35.71	
	3	10.89	6.323	1	.085	-1.50	23.28	
NOD/LtJ	1	-58.31	14.684	1	.000	-87.09	-29.53	decrease
	2	18.07	12.182	1	.138	-5.81	41.95	
	3	24.24	9.777	1	.013	5.08	43.40	

Table S3. Habituation pattern. This table compares the total number of entries on days 1, 2 and 3 to day 4. 129 and C3H mice showed a stable number of entries along the 4 days. C57 and DBA showed an increase between days 1 and day 4, whereas A/J, BALB, FVB and NOD mice displayed a decrease in the number of entries.

	Mean Difference (Dark-Light)	Std. Error	df	Sig.	95% Wald Confidence Interval for Difference	
					Lower	Upper
129S1/SvImJ	74.9198	7.51610	1	.000	60.1885	89.6511
A/J	212.6742	15.84387	1	.000	181.6208	243.7276
BALB/C	301.2273	24.65966	1	.000	252.8952	349.5593
C3H/HeJ	16.8636	1.17620	1	.000	14.5583	19.1690
C57BL/6J	131.2184	4.87891	1	.000	121.6559	140.7809
DBA/2J	74.8264	3.72513	1	.000	67.5253	82.1275
FVB/NJ	60.1111	4.58157	1	.000	51.1314	69.0908
NOD/LtJ	120.2414	8.33301	1	.000	103.9090	136.5738

Table S4. GEE model comparing the total number of entries in the dark and light phase for the first 4 days. All the strains showed a highly significant ($p < 0.0001$) difference between the entries in the shelter during the dark and the light phase.

	Pearson Correlation	Sig. (2-tailed)	N
129S1/SvImJ	.249	.073	53
A/J	.138	.445	33
BALB/C	.382	.028	33
C3H/HeJ	-.219	.328	22
C57BL/6J	.615	.000	86
DBA/2J	.475	.003	35
FVB/NJ	.706	.000	27
NOD/LtJ	.701	.000	28

Table S5. Correlation between the distance moved and the number of entries. C57, DBA, FVB, NOD mice showed highly significant correlation ($p<0.0001$) between the distance moved and the number of entries during the first 4 days; BALB mice displayed a weaker correlation but still significant ($p<0.05$), whereas A/J and C3h showed no significant correlation.

	% of mice having a significant (FDR =0.014) preferred entrance
129S1/SvImJ	86.8
A/J	75.8
BALB/C	93.9
C3H/HeJ	36.4
C57BL/6J	65.5
DBA/2J	80.6
FVB/NJ	48.1
NOD/LtJ	69.0

Table S6. Percentage of mice showing a significant preferred entrance on day 4. On day 4, Over 80% of 129S1, A/J, BALB and DBA mice had a significant preference (i.e significantly higher than 50%) for one of the 2 entrances. Of C57, FVB, NOD , 65.7, 53.1 and 61.4% respectively had a preference on day 4. Only 36% of C3H mice had developed a significant preference on day 4.less than half the population (36%) that showed a significant preference on day 4.

	mice not using the non-sanction entrance on day 5 dark phase
129S1/SvImJ	11
A/J	4
BALB/C	8
C3H/HeJ	3

Table S7. Number of mice per strain that failed to use the non-sanctioned entrance 12h after the beginning of the test phase on day 5.

	Day (J)	Mean Difference (J-d4)	Std. Error	df	Sig. (α=0.018)	95% Wald Confidence Interval for Difference	
						Lower	Upper
129S1/SvImJ	5	.18	0.043	1	**0.000**	.09	.26
	6	.30	0.048	1	**0.000**	.20	.39
A/J	5	.36	0.162	1	0.025	.05	.68
	6	.43	0.172	1	**0.013**	.09	.76
BALB/C	5	.08	0.028	1	**0.006**	.02	.13
	6	.12	0.037	1	**0.001**	.05	.19
C3H/HeJ	5	.07	0.050	1	0.179	-.03	.17
	6	.12	0.052	1	0.020	.02	.22
C57BL/6J	5	.13	0.014	1	**0.000**	.10	.15
	6	.21	0.016	1	**0.000**	.18	.24
DBA/2J	5	.25	0.039	1	**0.000**	.17	.32
	6	.43	0.041	1	**0.000**	.35	.51
FVB/NJ	5	.03	0.023	1	0.155	-.01	.08
	6	.04	0.037	1	0.281	-.03	.11
NOD/LtJ	5	.00	0.020	1	0.917	-.04	.04
	6	.05	0.024	1	0.030	.00	.10
SPECC1-WT	5	0.16	0.025	1	**0.000**	0.11	0.21
	6	0.28	0.024	1	**0.000**	0.23	0.33
SPECC1-KO	5	0.14	0.108	1	0.197	-0.07	0.35
	6	0.17	0.109	1	0.126	-0.05	0.38

Table S8 Results of the generalized estimated equations (GEE) on the proportion of entries through the preferred entrance. Day 4 is used as the reference day. The simple contrast test showed significant (FDR= 0.018) differences between days 4 and 5 for 129, C57, DBA and BALB/C, and a significant difference in the proportion of entries through the preferred entrance between days 4 and 6 for 129, A/J, C57, DBA and BABL/C. The mean difference is positive because we observe an increase in the probability between days 4 and 5 and day 6. SPECC1-WT mice showed a significant increase during both days of testing, whereas SPECC1-KO did not show a significant change.

(I) Strain	(J) Strain	Mean Difference (I-J)	Std. Error	df	Sig. (α=0.032)	95% Wald Confidence Interval for Difference	
						Lower	Upper
129S1/SvImJ	a/j	.05	.040	1	.214	-.03	.13
	balb/c	.01	.044	1	.810	-.08	.10
	c3h/hej	.00	.046	1	.947	-.09	.09
	c57bl/6j	.13	.034	1	**.000**	.07	.20
	dba/2j	.13	.044	1	**.003**	.04	.22
	fvb/nj	.05	.045	1	.295	-.04	.13
	nod/ltj	.02	.037	1	.673	-.06	.09
a/j	balb/c	-.04	.038	1	.305	-.11	.04
	c3h/hej	-.05	.040	1	.190	-.13	.03
	c57bl/6j	.08	.025	1	**.001**	.03	.13
	dba/2j	.08	.037	1	.033	.01	.15
	fvb/nj	.00	.038	1	.949	-.08	.07
	nod/ltj	-.03	.028	1	.231	-.09	.02
balb/c	c3h/hej	-.01	.044	1	.758	-.10	.07
	c57bl/6j	.12	.032	1	**.000**	.06	.18
	dba/2j	.12	.042	1	**.005**	.04	.20
	fvb/nj	.04	.043	1	.399	-.05	.12
	nod/ltj	.00	.034	1	.889	-.06	.07
c3h/hej	c57bl/6j	.14	.034	1	**.000**	.07	.20
	dba/2j	.13	.044	1	**.003**	.05	.22
	fvb/nj	.05	.045	1	.267	-.04	.14
	nod/ltj	.02	.037	1	.616	-.05	.09
c57bl/6j	dba/2j	.00	.031	1	.923	-.06	.06
	fvb/nj	-.09	.033	1	**.009**	-.15	-.02
	nod/ltj	-.12	.020	1	**.000**	-.16	-.08
dba/2j	fvb/nj	-.08	.043	1	.054	-.17	.00
	nod/ltj	-.11	.034	1	**.001**	-.18	-.05
fvb/nj	nod/ltj	-.03	.035	1	.369	-.10	.04
specc1-wt	specc1-ko	-.09	.044	1	.036	-.18	-.01
c57bl/6j	specc1-ko	-.07	.038	1	.048	-.15	.00
c57bl/6j	specc1-wt	.02	.026	1	.522	-.03	.07

Table S9. GEE results for pair-wise comparison of the effect of the sanctioned entrance on the number of entries through the preferred entrance between strains.

	Source	days	phase	Type III Sum of Squares	df	Mean Square	F	Sig. (α=0.041)
all strains	days	overall		23.383	4, 1174	5.637	13.515	**.000**
		D1 vs. D2		1.821	1	1.821	5.974	**.015**
		D2 vs. D3		2.096	1	2.096	7.545	**.006**
		D4 vs. D5		2.341	1	2.341	11.425	**.001**
		D5 vs. D6		5.170	1	5.170	21.428	**.000**
	days * Strain_R	overall		22.524	29.1	.776	1.860	**.004**
		D1 vs. D2		6.242	7	.892	2.925	**.006**
		D4 vs. D5		4.924	7	.703	3.433	**.002**
	phase	overall	Dark vs. Light	.001	1, 283	.001	.017	.895
	phase * Strain_R	overall	Dark vs. Light	.866	7	.124	2.031	.051
	days * phase	overall		2.296	4.9, 1380	.471	2.408	**.036**
		D3 vs. D4	Dark vs. Light	4.456	1	4.456	5.657	**.018**
	days * phase * Strain_R	overall		8.828	34.142	.259	1.322	.102
129S1/SvImJ	days	overall		14.129	3.474	4.067	7.395	**.000**
		D1 vs. D2		4.805	1	4.805	12.593	**.001**
		D4 vs. D5		.620	1	.620	4.922	**.032**
		D5 vs. D6		2.632	1	2.632	7.984	**.007**
	phase	overall	Dark vs. Light	.042	1	.042	.636	.430
	days * phase	overall		1.042	4.275	.244	1.066	.377
A/J	days	overall		2.328	3.617	.644	1.087	.364
	phase	overall	Dark vs. Light	.029	1	.029	.290	.594
	days * phase	overall		.707	4.224	.167	.549	.710
BALB/C	days	overall		2.774	3.932	.705	1.115	.354
	phase	overall	Dark vs. Light	.121	1	.121	2.475	.129
	days * phase	overall		.962	4.222	.228	1.243	.297
		D4 vs. D5	Dark vs. Light	2.108	1	2.108	7.359	**.012**
C3H/HeJ	days	overall		5.464	2.607	2.096	2.799	.057
		D5 vs. D6		.571	1	.571	5.253	**.034**
	phase	overall	Dark vs. Light	.082	1	.082	1.726	.205
	days * phase	overall		2.992	3.717	.805	2.910	**.031**
		D3 vs. D4	Dark vs. Light	4.426	1	4.426	11.175	**.004**
C57BL/6J	days	overall		14.599	4.323	3.377	10.810	**.000**
		D4 vs. D5		7.303	1	7.303	32.806	**.000**
	phase	overall	Dark vs. Light	.379	1	.379	7.178	**.009**
	days * phase	overall		1.927	4.762	.405	2.187	.058
DBA/2J	days	overall		9.587	3.466	2.766	3.769	**.009**

		D3 vs. D4		1.976	1	1.976	5.352	**.027**
		D5 vs. D6		3.384	1	3.384	10.425	**.003**
	phase	overall	Dark vs. Light	.256	1	.256	4.393	.044
	days * phase	overall		2.226	4.383	.508	1.734	.139
		D5 vs. D6	Dark vs. Light	3.658	1	3.658	4.971	**.032**
FVB/NJ	days	overall		3.407	4.162	.819	2.532	.042
	phase	overall	Dark vs. Light	.022	1	.022	.453	.507
	days * phase	overall		2.095	3.911	.536	3.046	**.021**
		D1 vs. D2	Dark vs. Light	3.274	1	3.274	7.489	**.011**
		D2 vs. D3	Dark vs. Light	6.450	1	6.450	11.015	**.003**
NOD/LtJ	days	overall		1.176	4.153	.283	1.571	.185
	phase	overall	Dark vs. Light	.001	1	.001	.010	.922
	days * phase	overall		.060	4.152	.014	.080	.990
SPECC1-WT vs. KO	days	overall		1.746	3.542	.493	1.118	.352
	days * Strain	overall		4.014	3.542	1.133	2.570	.052
		D4 vs. D5		1.235	1	1.235	6.925	**.016**
	phase	overall	Dark vs. Light	.026	1	.026	.596	.449
	phase * Strain	overall		.003	1.000	.003	.026	.873
	days * phase	overall		1.708	4.257	.401	1.892	.115
	days * phase * Strain	overall		.262	4.257	.062	.290	.893
SPECC1-KO	days	overall		1.975	2.964	.666	.987	.412
	phase	overall	Dark vs. Light	.019	1	.019	.352	.566
	days * phase	overall		1.439	3.214	.448	1.656	.193
SPECC1-WT	days	overall		3.785	2.986	1.267	3.373	**.031**
		D4 vs. D5		1.956	1	1.956	27.691	**.000**
	phase	overall	Dark vs. Light	.008	1	.008	.245	.631
	days * phase	overall		.531	3.294	.161	.567	.656

Table S10. Resting time in the shelter after using the sanctioned entrance: Repeated measure ANOVA (using Greenhouse – Geisser correction) revealed a significant main effect of days ($F(4, 1174)= 13.515$, $p<0.0001$) and a significant interaction between the days and the strains ($F(29.1, 1174) = 1.86$, $p<0.004$). No effects of the light and dark phase were observed for this aversion index.

Importantly, 5 strains show a significant effect of days on the time spent in the illuminated shelter (after using the sanctioned entrance) either already between days 4 and 5 (129S1, $p =0.032$; C57, $p<.0001$), or between days 5 and 6 (129S1, DBA, C3H, with p-values of respectively: $p<0.01$, $p<0.01$, $p=0.034$). A/J, BALB, NOD, however, did not change their time spend in shelter after using the preferred entrance over the 2 days of the experiment.

	Group size (100%)	decrease in preference (A)	decrease in time spent (B)	increase in resting outside (C)	Combination of responses			
					A+B	A+C	B+C	A+B+C
129S1/SvImJ	42	28 (67%)	29 (69%)	15 (36%)	23	9	9	5
A/J	29	18 (62%)	13 (45%)	13 (45%)	11	10	7	7
BALB/C	25	13 (52%)	14 (56%)	8 (32%)	12	4	5	4
C3H/HeJ	19	5 (26%)	7 (37%)	6 (32%)	3	1	2	1
C57BL/6J	86	68 (79%)	64 (74%)	32 (37%)	56	25	20	19
DBA/2J	35	32 (91%)	23 (66%)	21 (60%)	21	20	14	13
FVB/NJ	27	9 (33%)	17 (63%)	10 (37%)	8	3	5	2
NOD/LtJ	28	12 (43%)	15 (54%)	13 (46%)	9	7	7	5

Table S11. Number of mice that changed significantly (A) their preference, (B) their time spent and (C) their time resting outside between day 4 and 6. The right part of the table shows the number of mice per strain showing a combination of the 3 different responses. This table is complementary to the Venn diagrams of fig 3/iii.

	Distance moved	sleep Outside	time shelter	Number of entries	Total
129S1/SvImJ	0	1	1	0	2
A/J	0	1	0	0	1
BALB/C	0	2	2	0	2
C3H/HeJ	0	0	0	0	0
C57BL/6J	0	5	5	0	5
DBA/2J	1	1	1	0	2
FVB/NJ	1	0	0	1	1
NOD/LtJ	0	0	0	0	0

Table S12. Number of outliers per strain (>3x standard deviation of the strain mean) for the 4 main parameters.