Supplementary information for:

Aerobic exercise and a BDNF-mimetic therapy rescue learning and memory in a mouse model of Down syndrome

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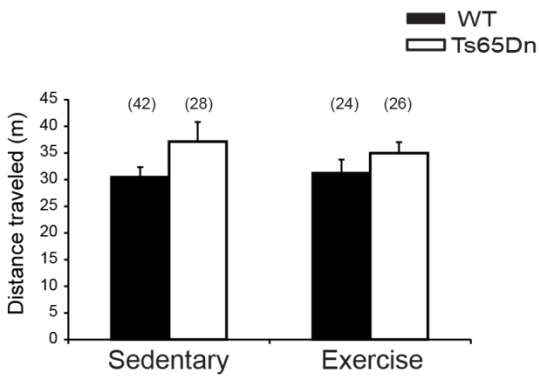
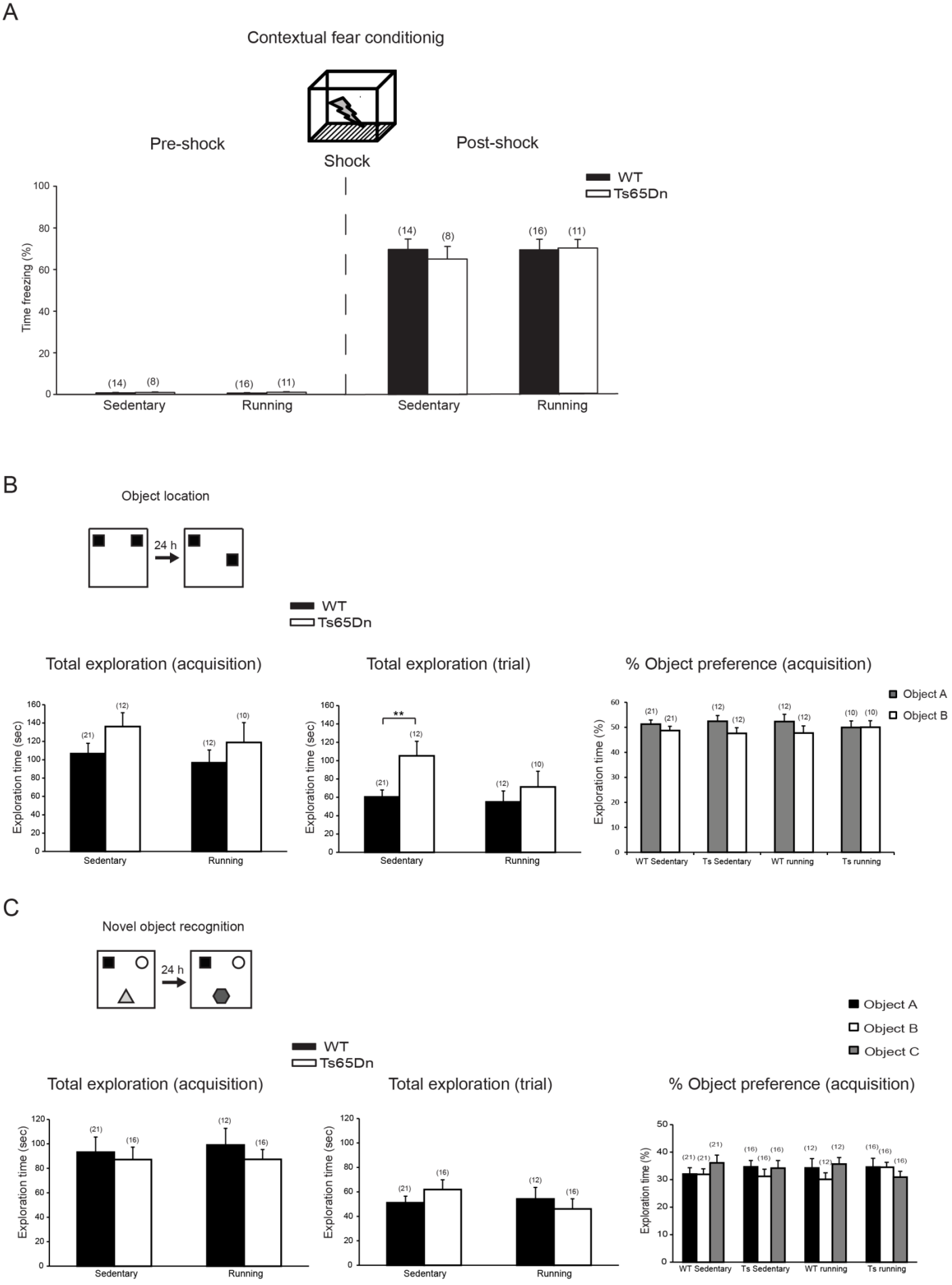
Supplementary text and figures:

A



Supplementary Figure 1

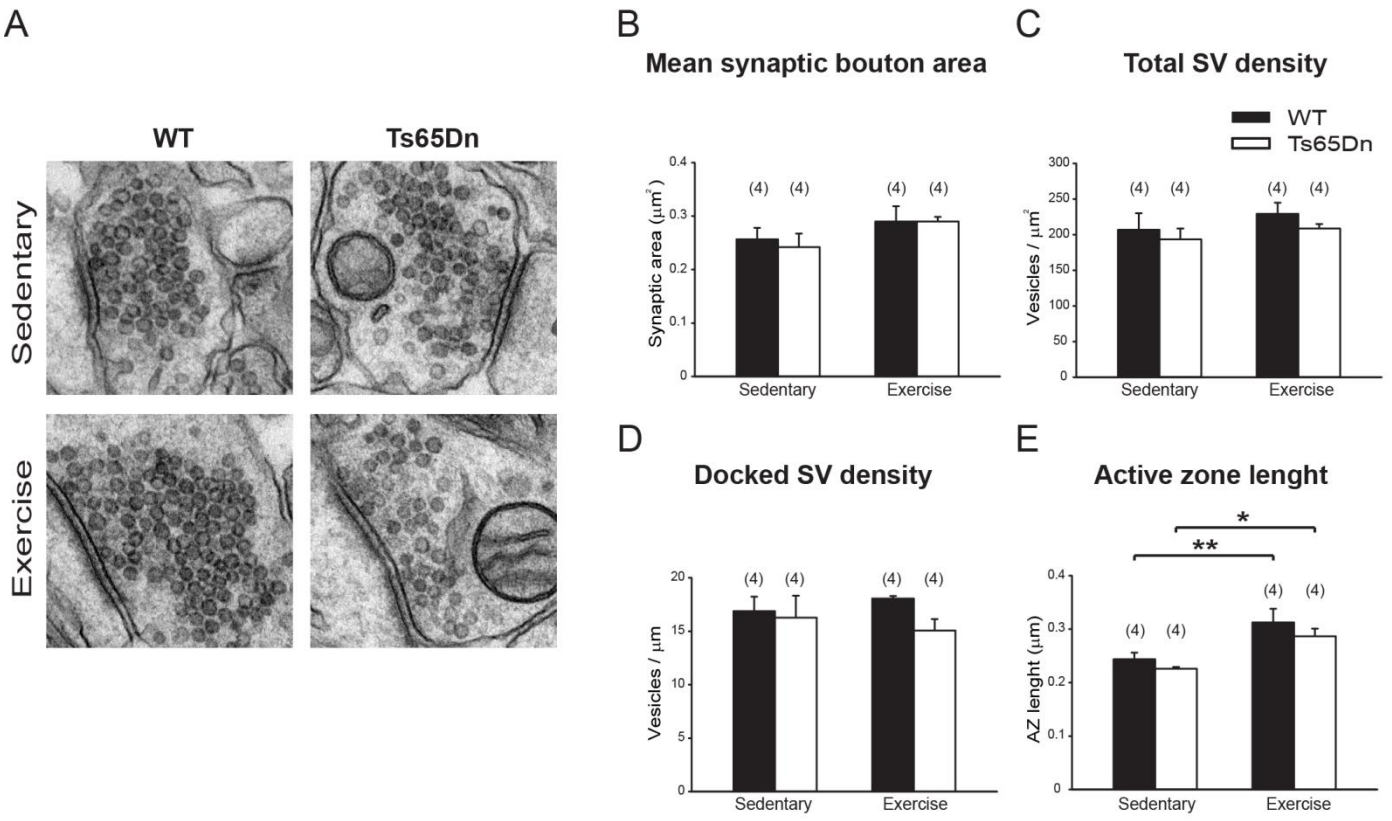
Supplementary Figure 1. Physical exercise had no effect on general motor activity. **(A)** Distance traveled in the empty arena was not different across genotype and treatments. Two-way ANOVA on ranked transformed data: genotype [$F_{1,116}$ = 4.460, P = 0.037], treatment [$F_{1,116}$ = 0.038, P = 0.845], genotype x treatment [$F_{1,116}$ = 0.015, P = 0.901]. Tukey *post hoc* test following two-way ANOVA did not reach statistical significance for any experimental group. Number in parenthesis indicates the number of mice tested for each experimental group.



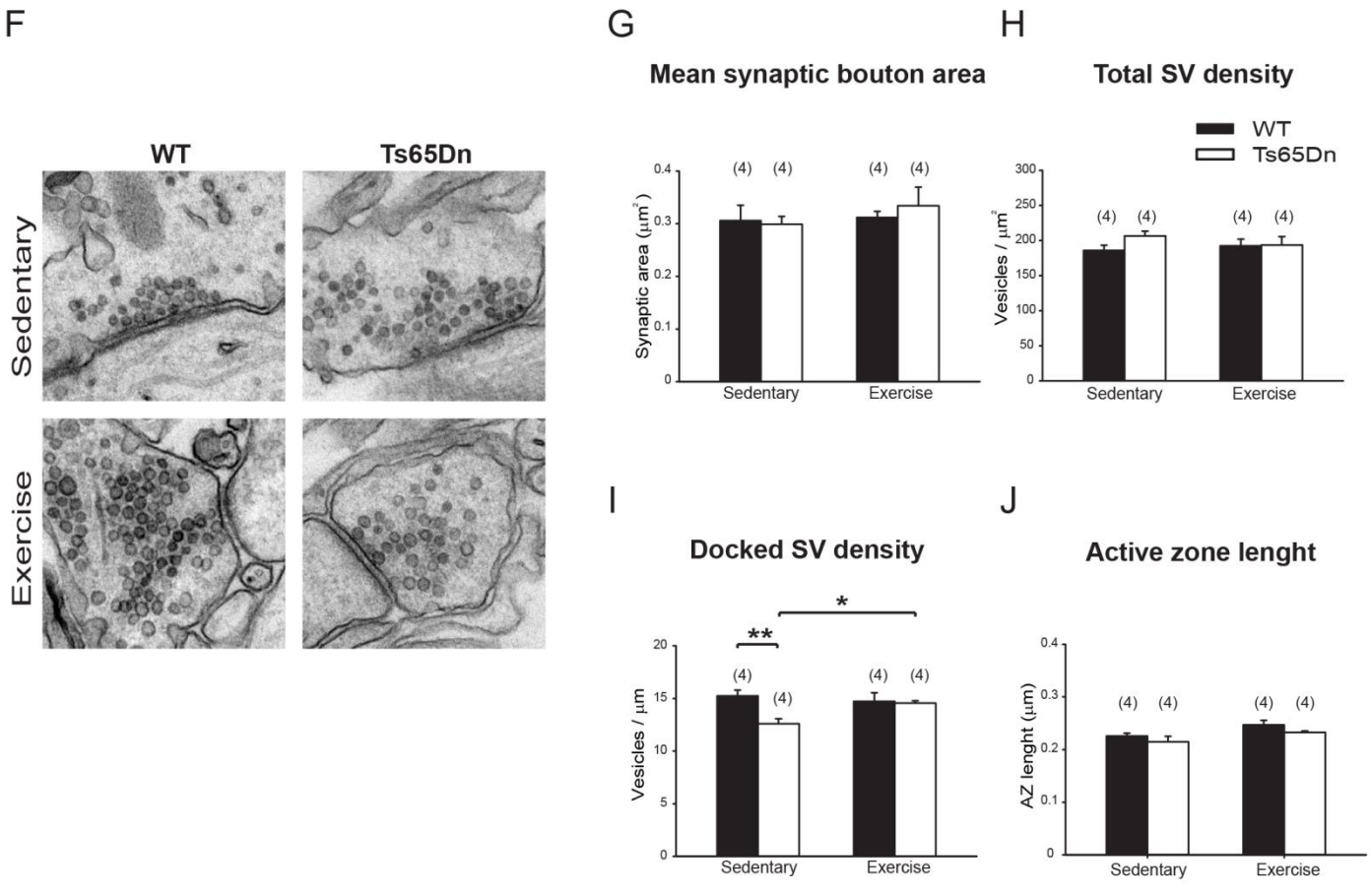
Supplementary Figure 2

Supplementary Figure 2. **(A)** Physical exercise had no effect on the freezing time before (left) or immediately after (right) the electric foot shock during the conditioning session in both Ts65Dn and WT mice. Pre-shock two-way ANOVA on ranked transformed data: genotype [$F_{1,45}$=1.964, P=0.168]; treatment [$F_{1,45}$=0.0661, P=0.798]; genotype x treatment [$F_{1,45}$=0.00002, P=0.996]. Post-shock two-way ANOVA: genotype [$F_{1,45}$=0.171, P=0.681]; treatment [$F_{1,45}$= 0.169, P=0.683]; genotype x treatment [$F_{1,45}$=0.194, P=0.661]. **(B)** *Left:* in the OL test the total exploration time during the acquisition phase was not significantly different across genotype and treatment. Two-way ANOVA: genotype [$F_{1,51}$=5.017, P=0.029]; treatment [$F_{1,51}$=0.641, P=0.427]; genotype x treatment [$F_{1,51}$=0.0223, P=0.882]. *Center*: the total exploration time during the trial phase was slightly increased in sedentary Ts65Dn mice compared to WT, however no significant difference was observed in Ts65Dn running mice compared to WT. Two-way ANOVA: genotype [$F_{1,51}$=9.164, P=0.004]; treatment [$F_{1,51}$=2.453, P=0.123]; genotype x treatment [$F_{1,51}$=0.914, P=0.349]. **P<0.01, Tukey post hoc test. *Right*: the percentage of time spent exploring the two objects during the acquisition phase was not statistically different across the experimental groups. Two-way ANOVA: genotype [$F_{1,102}$<0.0001, P=1.000]; treatment [$F_{1,102}$=1.331, P=0.268]; genotype x treatment [$F_{1,102}$=0.4014, P=0.753]. **(C)** *Left:* in the NOR test the total exploration time during the acquisition phases was not significantly different across genotype and treatment. Two-way ANOVA on ranked transformed data: genotype [$F_{1,61}$=0.585; P=0.447]; treatment [$F_{1,61}$=0.548; P=0.462]; genotype x treatment [$F_{1,61}$=0.0997; P=0.753]. *Center*: the total exploration time during the trial phase was not significantly different across genotype and treatment. Two-way ANOVA on ranked transformed data: genotype [$F_{1,61}$=0.0226; P=0.881]; treatment [$F_{1,61}$=1.892; P=0.174]; genotype x treatment [$F_{1,61}$=2.047; P=0.158]. *Right*: the percentage of time spent exploring the three objects during the acquisition phase was not statistically different across the experimental groups. Two-way ANOVA: genotype [$F_{1,187}$=0.231; P=0.645]; treatment [$F_{1,187}$=0.718; P=0.629]; genotype x treatment [$F_{1,187}$=0.895; P=0.521]. Number in parenthesis indicates the number of mice tested for each experimental group.

Asymmetric synapses (Glutamatergic)

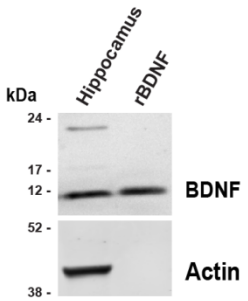


Symmetric synapses (GABAergic)



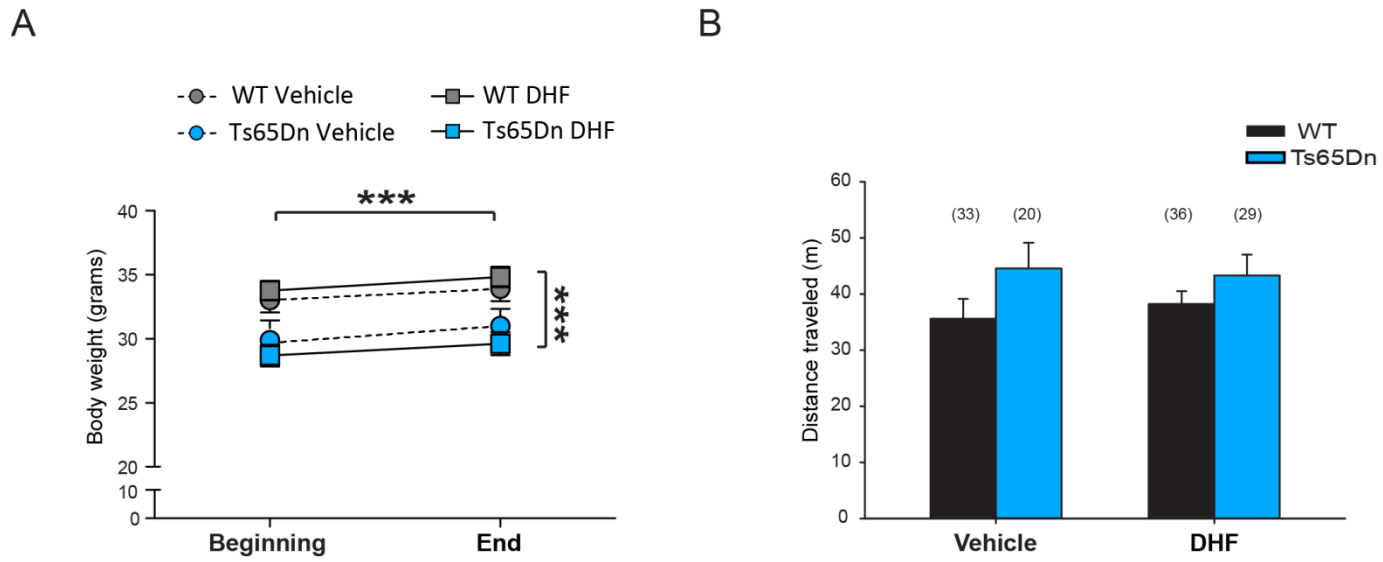
Supplementary Figure 3

Supplementary Figure 3. Synaptic ultrastructural morphometric analysis in the hippocampal CA1 region. **(A)** Representative TEM images showing asymmetric (glutamatergic) synapses. **(B)** Mean asymmetric synaptic bouton area was not significantly different across genotype and treatment. Two-way ANOVA: genotype [$F_{1,15}$=0.108, P=0.748]; treatment [$F_{1,15}$=3.218, P=0.098]; genotype x treatment [$F_{1,15}$=0.097, P=0.761]. **(C)** Total synaptic vesicles (SV) density in asymmetric synapses was not significantly different across genotype and treatment. Two-way ANOVA: genotype [$F_{1,15}$=1.078, P=0.320]; treatment [$F_{1,15}$=1.333, P=0.271]; genotype x treatment [$F_{1,15}$=0.045, P=0.836]. **(D)** The density of docked SV in asymmetric synapses was not significantly different across genotype and treatment. Two-way ANOVA: genotype [$F_{1,15}$=1.797, P=0.205]; treatment [$F_{1,15}$<0.0001, P=0.997]; genotype x treatment [$F_{1,15}$= 0.779, P=0.395]. **(E)** In asymmetric synapses the length of the active zone was increased by exercise in both WT and Ts65Dn mice. Two-way ANOVA: genotype [$F_{1,15}$=1.889, P=0.194]; treatment [$F_{1,15}$=16.940, P=0.001]; genotype x treatment [$F_{1,15}$=0.0745, P=0.790]. **(F)** Representative TEM images showing symmetric (GABAergic) synapses. **(G)** Mean symmetric synaptic bouton area was not significantly different across genotype and treatment. Two-way ANOVA: genotype [$F_{1,15}$=0.0952, P=0.763]; treatment [$F_{1,15}$=0.679, P=0.426]; genotype x treatment [$F_{1,15}$=0.353, P=0.564]. **(H)** Total SV density in symmetric synapses was not significantly different across genotype and treatment. Two-way ANOVA: genotype [$F_{1,15}$=1.379, P=0.263]; treatment [$F_{1,15}$=0.124, P=0.731]; genotype x treatment [$F_{1,15}$=1.145, P=0.306]. **(I)** The density of docked SV in symmetric synapses was reduced in sedentary Ts65Dn mice and significantly restored by exercise. Two-way ANOVA: genotype [$F_{1,15}$=6.265, P=0.028]; treatment [$F_{1,15}$=1.633, P=0.225]; genotype x treatment [$F_{1,15}$=4.922, P=0.047]. **(J)** In symmetric synapses the length of the active zone was not significantly different across genotype and treatment. Two-way ANOVA: genotype [$F_{1,15}$=2.777, P=0.121]; treatment [$F_{1,15}$=6.939, P=0.022]; genotype x treatment [$F_{1,15}$=0.0233, P=0.881]. *P<0.05, **P<0.01, Tukey *post hoc* test following two-way ANOVA. Number in parenthesis indicates the number of mice tested for each experimental group.



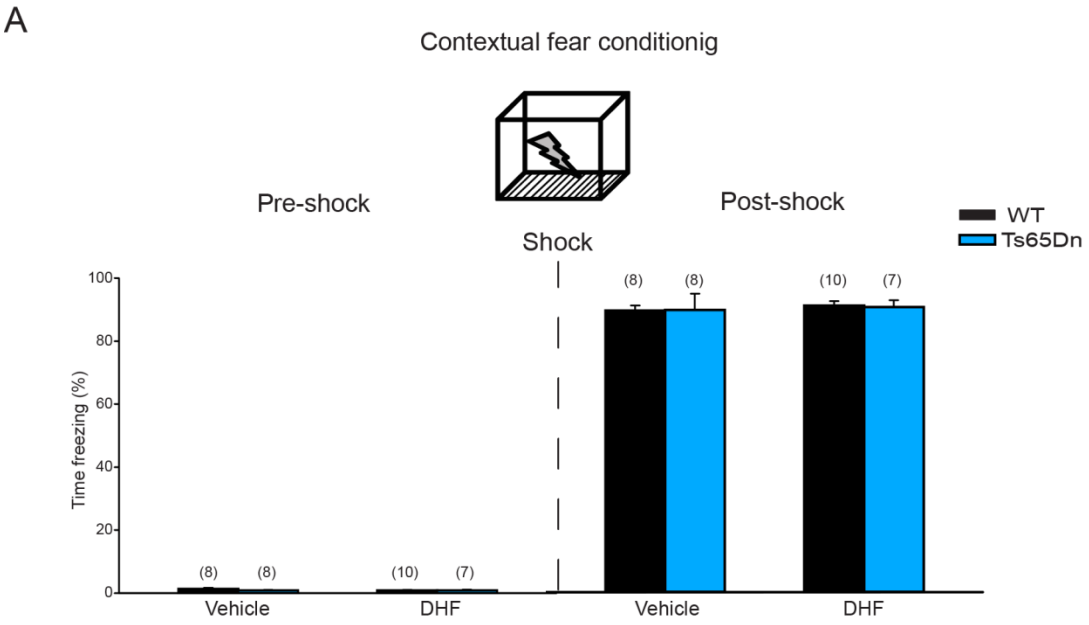
Supplementary Figure 4

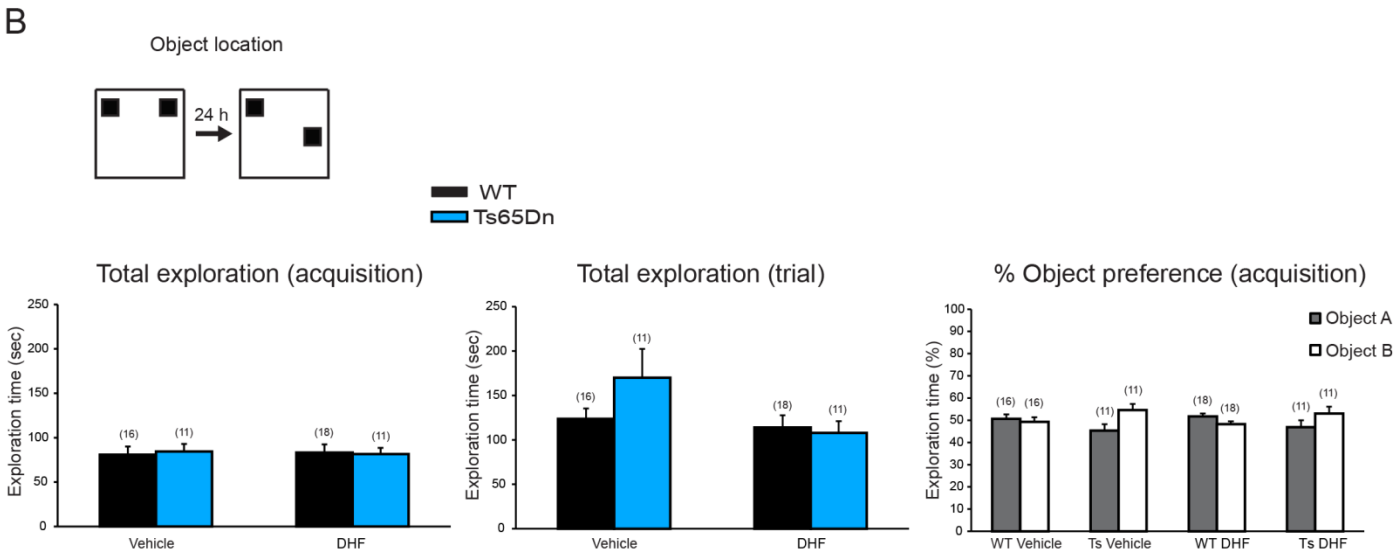
Supplementary Figure 4. Specificity of the anti-BDNF antibody used for western blot analysis. Representative immunoblot with anti-BDNF antibody on hippocampal protein extracts or recombinant BDNF (rBDNF). Actin was used as loading control and is absent in rBDNF line.

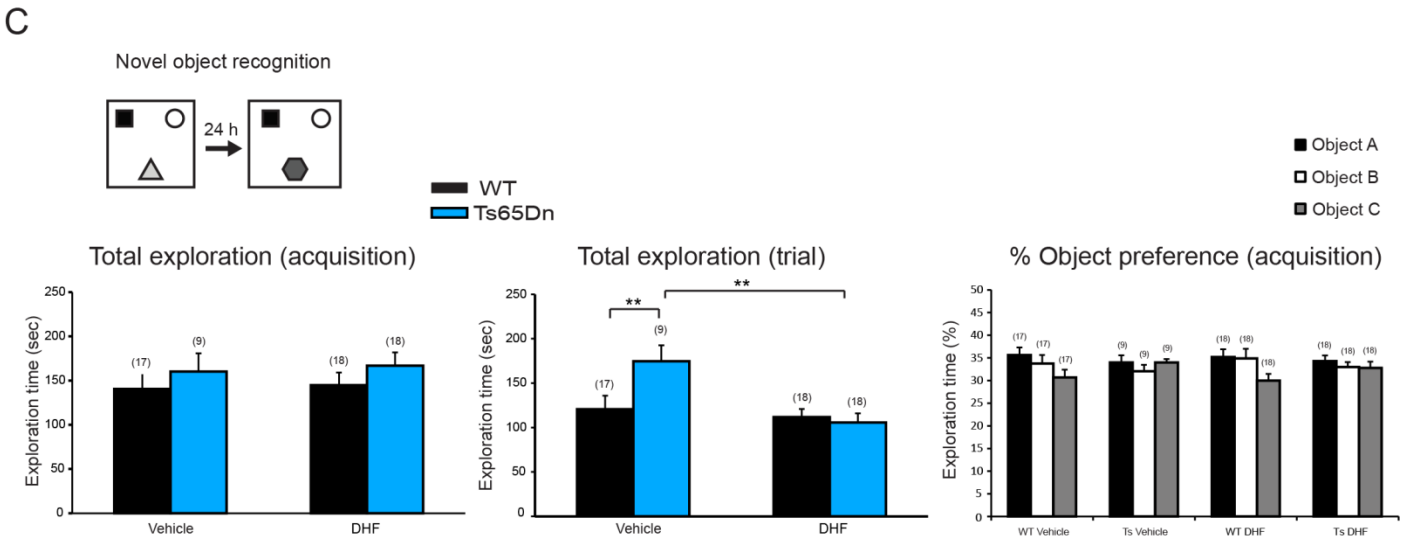


Supplementary Figure 5

Supplementary Figure 5. DHF treatment had no effect on body weight gain and general motor activity. **(A)** Mice body weight was measured at the beginning and at the end (4 weeks later) of chronic DHF or vehicle treatment. As previously reported (Costa, et al. 2010), mean body weight was greater in WT than in Ts65Dn mice (P<0.001). All groups of mice showed comparable and significant weight gain (P<0.001 for all groups) during the 4-week treatment period independently of genotype. Two-way repeated measure ANOVA on ranked-transformed data: genotype [$F_{3,114}$ = 8.509, P < 0.001], time-point [$F_{1,114}$ = 59.318, P < 0.001], genotype x time-point [$F_{3,114}$ = 0.407, P = 0.748]. ***P<0.001, Tukey *post hoc* test. **(B)** Motor activity was evaluated by measuring the distance traveled in the empty arena by mice after chronic DHF or vehicle treatment. Distance traveled was not different across genotype and treatments. Two-way ANOVA: genotype [$F_{1,114}$ = 4.058, P = 0.046], treatment [$F_{1,114}$ = 0.039, P = 0.843], genotype x treatment [$F_{1,114}$ = 0.313, P = 0.577]. Tukey *post hoc* test following two-way ANOVA did not reach statistical significance for any experimental group. Number in parenthesis indicates the number of mice tested for each experimental group.



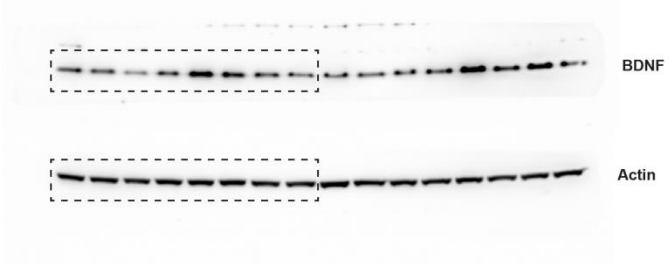




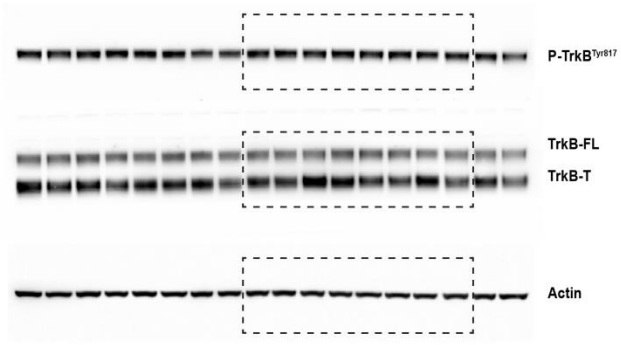
Supplementary Figure 6

Supplementary Figure 6. **(A)** DHF treatment had no effect on the freezing time before (left) or immediately after (right) the electric foot shock during the conditioning session in both Ts65Dn and WT mice. Pre-shock two-way ANOVA: genotype [$F_{1,29}$=1.357, P=0.254]; treatment [$F_{1,29}$=0.505, P=0.483]; genotype x treatment [$F_{1,29}$=1.665, P=0.207]. Post-shock two-way ANOVA: genotype [$F_{1,29}$=0.576, P=0.454]; treatment [$F_{1,29}$=0.140, P=0.711]; genotype x treatment [$F_{1,29}$=2.394, P=0.133]. **(B)** *Left:* in the OL the total exploration time during the acquisition phase was not significantly different across genotype and treatment. Two-way ANOVA: genotype [$F_{1,52}$=0.0084, P=0.927]; treatment [$F_{1,52}$=0.0005, P=0.981]; genotype x treatment [$F_{1,52}$=0.0803, P=0.778]. *Center*: the total exploration time during the trial phase was not significant different across genotype and treatment. Two-way ANOVA: genotype [$F_{1,52}$=0.215, P=0.645]; treatment [$F_{1,52}$=3.764, P=0.058]; genotype x treatment [$F_{1,52}$=0.972, P=0.329]. *Right*: the percentage of time spent exploring the two objects during the acquisition phase was not statistically different across the experimental groups. Two-way ANOVA: genotype [$F_{1,102}$<0.0001, P=1.000]; treatment [$F_{1,102}$=1.108, P=0.349]; genotype x treatment [$F_{1,102}$=2.680, P=0.051]. Number in parenthesis indicates the number of mice tested for each group experimental. **(C)** *Left:* in the NOR test the total exploration time during the acquisition was not significantly different across genotype and treatment. Two-way ANOVA: genotype [$F_{1,58}$=1.526; P=0.222]; treatment [$F_{1,58}$=0.101; P=0.752]; genotype x treatment [$F_{1,58}$=0.0059; P=0.939]. *Center*: the total exploration time during the trial phase was slightly increased in vehicle-treated Ts65Dn mice compared to WT, however no significant difference was observed in Ts65Dn mice treated with DHF compared to WT. Two-way ANOVA: genotype [$F_{1,58}$=3.301; P=0.074]; treatment [$F_{1,58}$=8.720; P=0.005]; genotype x treatment [$F_{1,58}$=5.193; P=0.026]. *Right*: the percentage of time spent exploring the three objects was not statistically different across groups. Two-way ANOVA on ranked transformed data: genotype [$F_{1,174}$=0.110; P=0.741]; treatment [$F_{1,174}$=1.396; P=0.228]; genotype x treatment [$F_{1,174}$=1.141; P=0.340].The number in parenthesis indicate the number of mice tested for each experimental group. **p<0.01, Tukey *post hoc* test following two-way ANOVA.

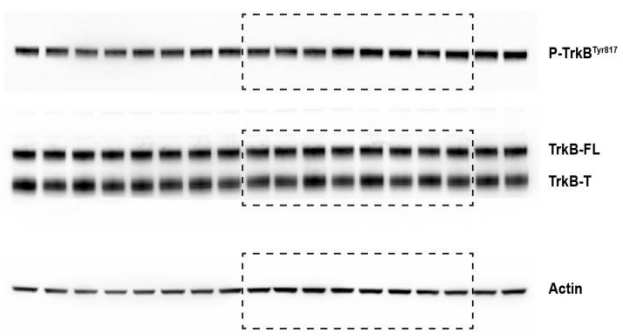
Full-length blots for Figure 4B



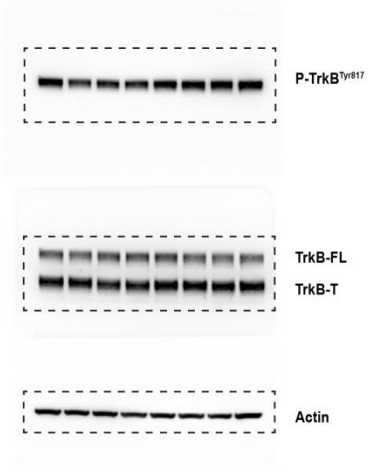
Full-length blots for Figure 5A



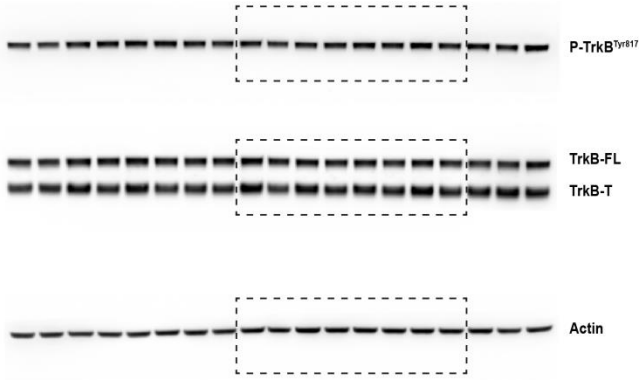
Full-length blots for Figure 5B



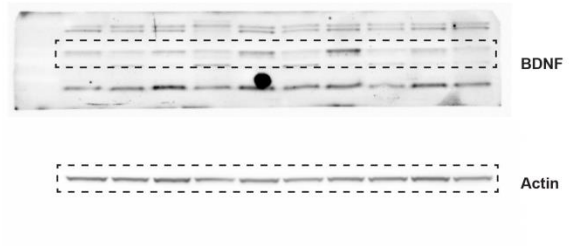
Full-length blots for Figure 5C



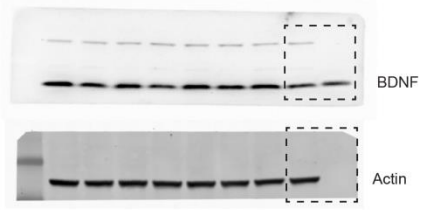
Full-length blots for Figure 5D



Full-length blots for Figure 7B



Full-length blots for Supplemenatry Figure 4



Supplementary Figure 7

Supplementary Fig. 7 Full-length blot images corresponding to the cropped western blot presented in Figure 4, 5, 7 and Supplementary Figure 4.

Supplementary Table 1. Primer sequences for RT-qPCR analysis.

Gene symbol	Gene name	Organism	Accession number	Forward primer (*5′-3′*)	Reverse primer (*5′-3′*)
ACTB	Actin beta	Homo Sapiens	NM_001101	CAGCAAGCAGGAGTATGAC	GAAAGGGTGTAACGCAACT
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Homo Sapiens	NM_001256799	AATGAAGGGGTCATTGATGG	AAGGTGAAGGTCGGAGTCAA
PPIA	Peptidylprolyl isomerase A	Homo Sapiens	NM_021130	TTCTGCTGTCTTTGGGACCT	CACCGTGTTCTTCGACATTG
BDNF (CDS)	Brain-derived neurotrophic factor	Homo Sapiens	NM_001143805 NM_001143806 NM_001143807 NM_001143808 NM_001143809 NM_00114381 NM_001143811 NM_00114381 NM_001143814 NM_00114381 NM_001709 NM_170731 NM_170732 NM_170733 NM_170734 NM_170735	CGAGACCAAGTGCAATCC	TTATGAATCGCCAGCCAAT
Actb	Actin beta	Mus Musculus	NM_007393	AAGTGGTTACAGGAAGTCC	ATAATTTACACAGAAGCAATGC
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	Mus Musculus	NM_008084	GAACATCATCCCTGCATCCA	CCAGTGAGCTTCCCGTTCA
Ppia	Peptidylprolyl isomerase A	Mus Musculus	NM_008907	CACTGTCGCTTTTCGCCGCTTG	TTTCTGCTGTCTTTGGAACTTTGTCTGC
Bdnf (Exon I)	Brain-derived neurotrophic factor	Mus Musculus	NM_007540	TGGTAACCTCGCTCATTCATTAGA	CCCTTCGCAATATCCGCAAAG
Bdnf (Exon II)	Brain-derived neurotrophic factor	Mus Musculus	NM_001048139	CTTTTCCTCGCTGTCAAG	TTGCCAAGAGTCTATTCC
Bdnf (Exon III)	Brain-derived neurotrophic factor	Mus Musculus	NM_001285419	TCACGATCCTCGATGGATAGTTCT	AGGGAGTGGAGCGCAGTC
Bdnf (Exon IV)	Brain-derived neurotrophic factor	Mus Musculus	NM_001048141	CAAATGGAGCTTCTCGCTGAAGGC	GTGGAAATTGCATGGCGGAGGTAA
Bdnf (Exon V)	Brain-derived neurotrophic factor	Mus Musculus	NM_001285420	ACCATAACCCCGCACACTCTGT	GCACCTTCCCGCACCACAAA
Bdnf (Exon VI)	Brain-derived neurotrophic factor	Mus Musculus	NM_001048142	CTTCAACTGCCACCACTG	CATTGTTGTCACGCTTCTG
Bdnf (Exon VII)	Brain-derived neurotrophic factor	Mus Musculus	NM_001285417	GCTTACTTACAGGTCCAAGGTCAA	GTCCTGGAGTTCCGCAGAC
Bdnf (CDS)	Brain-derived neurotrophic factor	Mus Musculus	NM_007540 NM_001048139 NM_001048141 NM_001048142 NM_001285416 NM_001285417 NM_001285418 NM_001285419 NM_001285420 NM_001285421 NM_001285422	ATTACCTGGATGCCGCAAA	TAATACTGTCACACACGCTCA

Supplementary Table 2. Calibration curves parameters, PCR reaction efficiency and amplicon information for RT-qPCR analysis.

Gene symbol	Organism	Amplicon length (bp)	Final primer concentration (µM)	Calibration curve (slope / R^2)	Calculated PCR efficiency (%)	Primer dimers
ACTB	Homo Sapiens	91	0.2	-3.242 / 0.997	103.4	Not detected
GAPDH	Homo Sapiens	108	0.2	-3.493 / 0.999	93.3	Only in NTC samples
PPIA	Homo Sapiens	91	0.2	-3.446 / 0.997	95.1	Not detected
BDNF (CDS)	Homo Sapiens	149	0.2	-3.282 / 0.999	101.7	Not detected
Actb	Mus Musculus	123	0.1	-3.130 / 0.986	108.7	Not detected
Gapdh	Mus Musculus	77	0.2	-3.370 / 0.999	98.0	Only in NTC samples
Ppia	Mus Musculus	133	0.2	-3.321 / 0.994	100.0	Only in NTC samples
Bdnf (Exon I)	Mus Musculus	172	0.2	-3.450 / 0.999	94.9	Not detected
Bdnf (Exon II)	Mus Musculus	200	0.1	-3.175 / 0.998	106.5	Not detected
Bdnf (Exon III)	Mus Musculus	150	0.2	-3.716 / 0.994	85.3	Not detected
Bdnf (Exon IV)	Mus Musculus	57	0.2	-3.584 / 0.999	90.1	Not detected
Bdnf (Exon V)	Mus Musculus	73	0.2	-3.536 / 0.997	91.8	Not detected
Bdnf (Exon VI)	Mus Musculus	87	0.2	-3.573 / 0.999	90.5	Only in NTC samples
Bdnf (Exon VII)	Mus Musculus	93	0.2	-3.503 / 0.996	92.9	Not detected
Bdnf (CDS)	Mus Musculus	90	0.2	-3.315 / 0.999	100.3	Not detected

Supplementary Table 3. Human sample information.

Case number	Disorder	Age (years)	Gender
1276	DS	13	M
5277	DS	19	M
M1960M	DS	19	M
5341	DS	25	M
5005	DS	39	F
4925	Control	13	M
4782	Control	18	M
1841	Control	19	M
605	Control	25	M
5606	Control	35	F

Supplementary Table 4. List of mice used in the study.

			Behavior			IHC					
Mouse #	Genotype	Treatment	NOR	OL	CFC	BrdU 24hr	BrdU 4w	VGAT/VGLUT1	EPhy	EF	Bioch
1	WT	sedentary	*			*		*			
2	WT	sedentary	*			*					
3	WT	sedentary	*			*					
4	WT	sedentary	*			*					
5	WT	sedentary	*			*					
6	WT	sedentary	*			*		*			
7	WT	sedentary	*				*				
8	WT	sedentary	*				*				
9	WT	sedentary	*				*				
10	WT	sedentary	*				*	*	*		
11	WT	sedentary	*				*	*	*		
12	WT	sedentary	*				*				
13	WT	sedentary	*				*	*	*		
14	WT	sedentary	*				*	*	*		
15	WT	sedentary	*				*				
16	WT	sedentary	*				*				
17	WT	sedentary	*								
18	WT	sedentary	*								
19	WT	sedentary	*								
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21	WT	sedentary	*								

22	WT	sedentary	*								
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24	WT	sedentary	*								
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223	Ts65Dn	exercise	*			*					
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285	Ts65Dn	exercise									*
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336	WT	vehicle									*
337	WT	vehicle (acute)									*
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339	WT	vehicle (acute)									*
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352	WT	vehicle (acute)									*
353	Ts65Dn	vehicle	*		*						

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384	Ts65Dn	vehicle									*
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386	Ts65Dn	vehicle									*
387	Ts65Dn	vehicle (acute)									*
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389	Ts65Dn	vehicle (acute)									*
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399	Ts65Dn	vehicle (acute)									*
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401	Ts65Dn	vehicle (acute)									*
402	Ts65Dn	vehicle (acute)									*

403	WT	DHF	*								
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407	WT	DHF	*								
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413	WT	DHF	*		*						
414	WT	DHF	*		*						
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452	WT	DHF									*
453	WT	DHF									*
454	WT	DHF									*
455	WT	DHF									*
456	WT	DHF (acute)									*
457	WT	DHF (acute)									*
458	WT	DHF (acute)									*
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472	WT	DHF (acute)									*
473	WT	DHF (acute)									*
474	WT	DHF (acute)									*
475	WT	DHF (acute)									*
476	Ts65Dn	DHF	*								
477	Ts65Dn	DHF	*								
478	Ts65Dn	DHF	*								
479	Ts65Dn	DHF	*								
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519	Ts65Dn	DHF									*
520	Ts65Dn	DHF (acute)									*
521	Ts65Dn	DHF (acute)									*
522	Ts65Dn	DHF (acute)									*
523	Ts65Dn	DHF (acute)									*
524	Ts65Dn	DHF (acute)									*
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538	Ts65Dn	DHF (acute)									*
539	Ts65Dn	DHF (acute)									*

Asterisk indicates the experimental procedure/test carried out on each mouse.
NOR: Novel Object Recognition test. OL: Object Location test. CFC: Contextual Fear Conditioning test. IHC: Immunohistochemistry. EM: Electron Microscopy. EPhy: Electrophysiology. Bioch: Biochemistry.
Mice highlighted in yellow did perform the NOR or OL behavioral tests, but could not be included in the study because we lost the video-recording files of the behavioral tests due to the failure of a hard-drive in which they were stored. Files could not be recovered.

Supplementary references

Costa, A. C. S., M. R. Stasko, C. Schmidt and M. T. Davisson (2010). "Behavioral validation of the Ts65Dn mouse model for Down syndrome of a genetic background free of the retinal degeneration mutation Pde6brd1." Behavioural Brain Research **206**: 52-62.