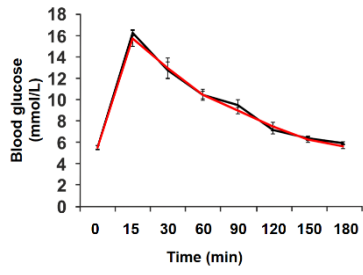


## **Supplementary Information**

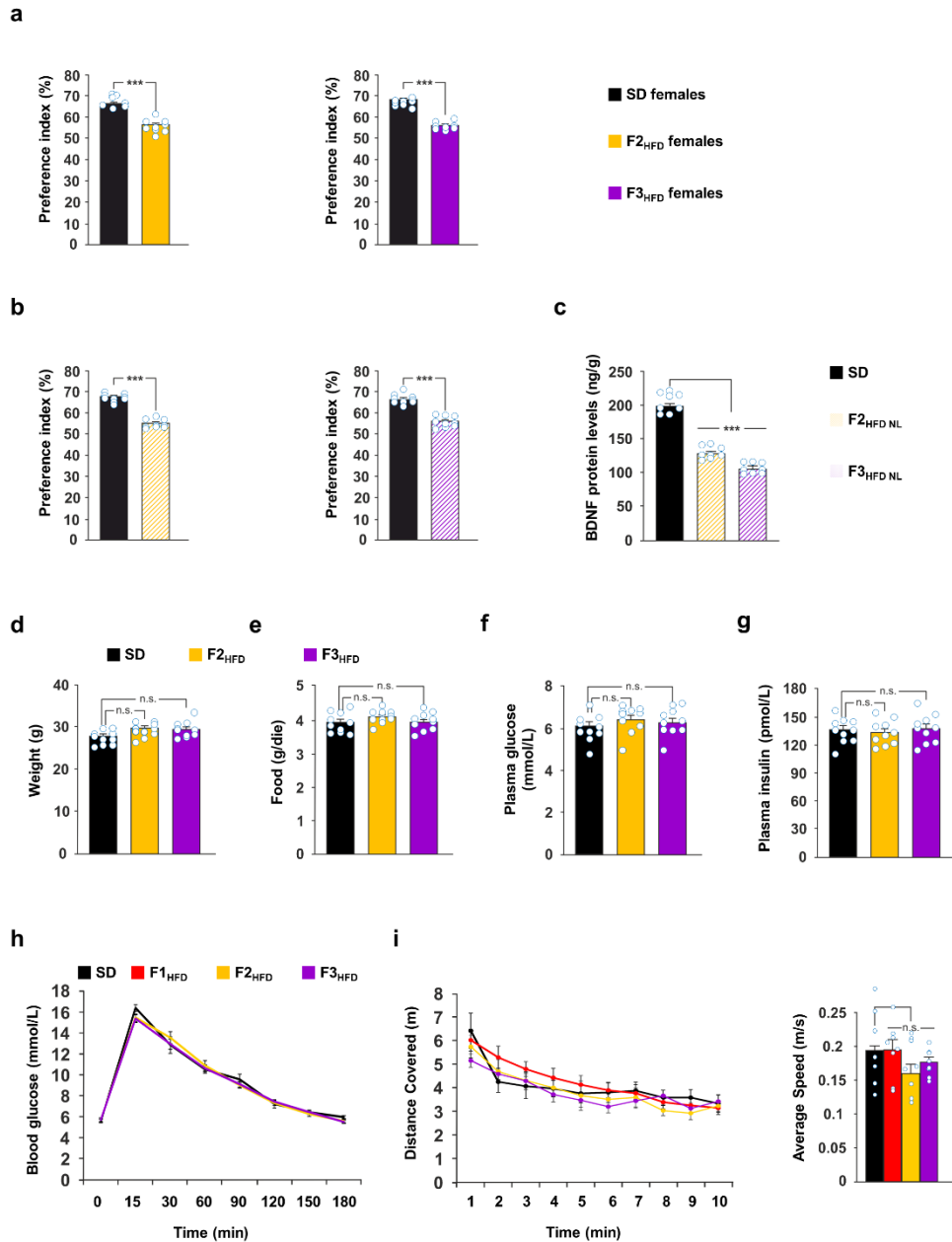
Maternal insulin resistance multigenerationally impairs synaptic plasticity and memory  
via gametic mechanisms

Fusco et al.

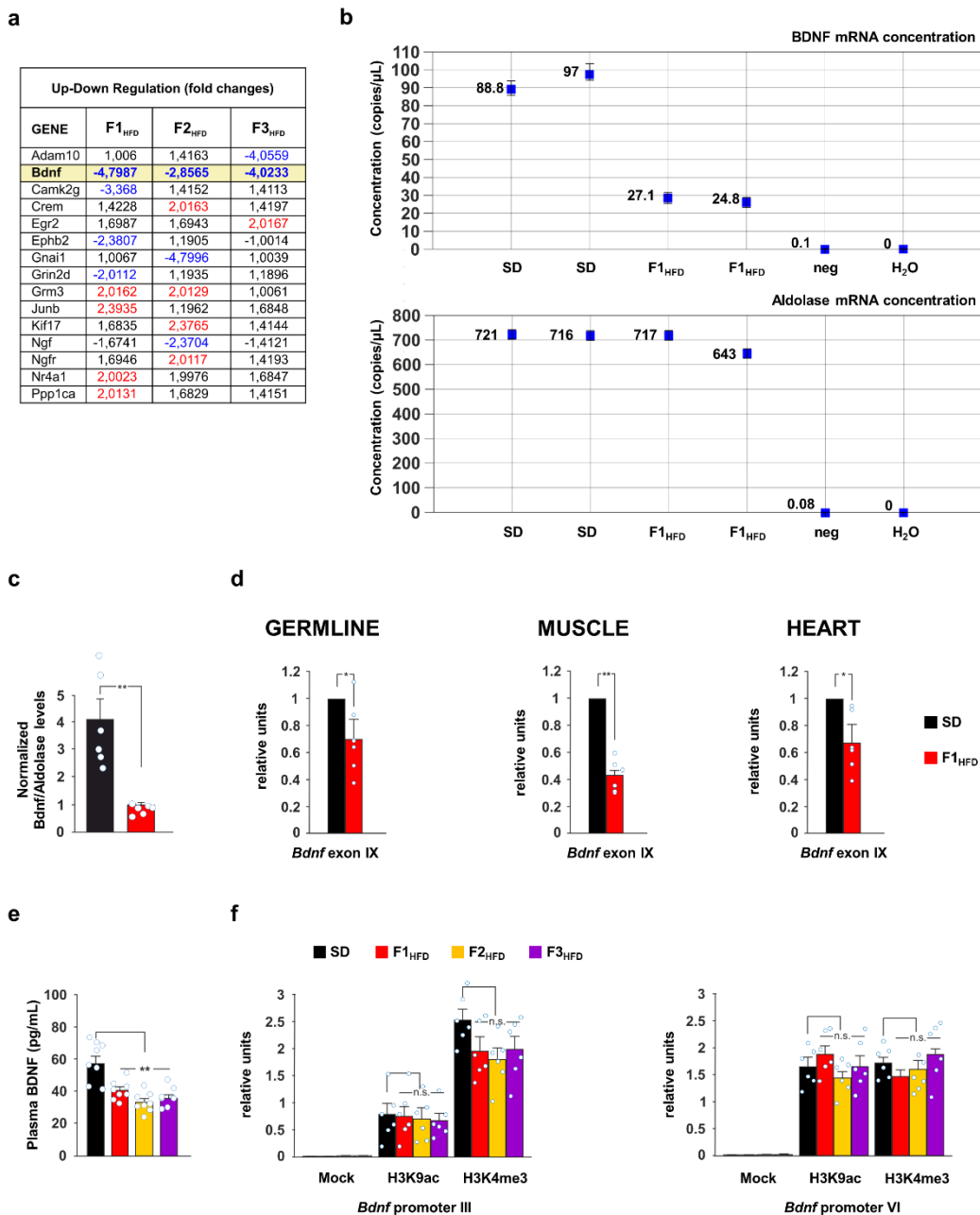
**a**



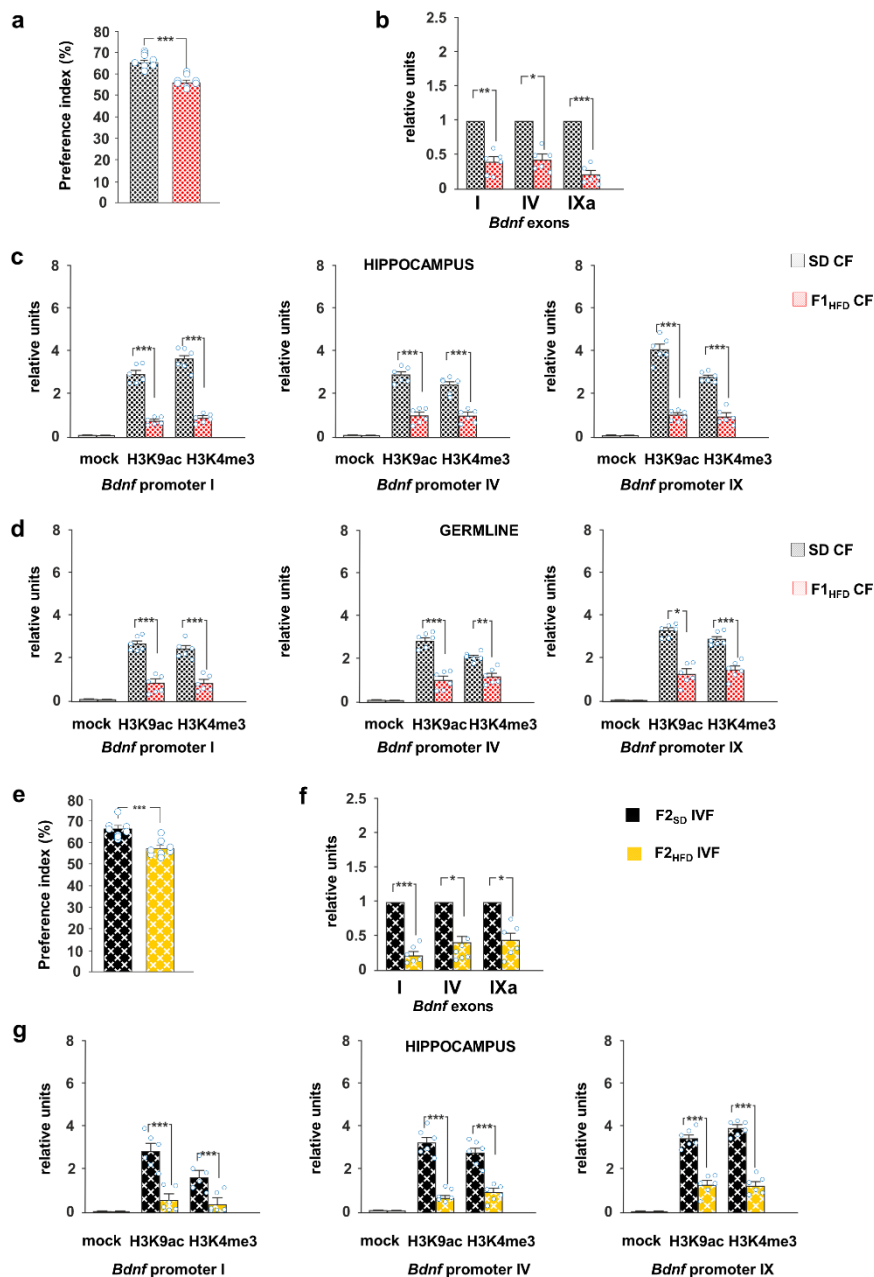
**Supplementary Figure 1. HFD offspring do not show changes in insulin sensitivity. (a)** Blood glucose levels of SD and F1<sub>HFD</sub> mice after intraperitoneal glucose tolerance test (IPGTT) (n = 12 mice from 8 litters per group; statistics by unpaired Student's *t*-test). Data are expressed as mean  $\pm$  SEM.



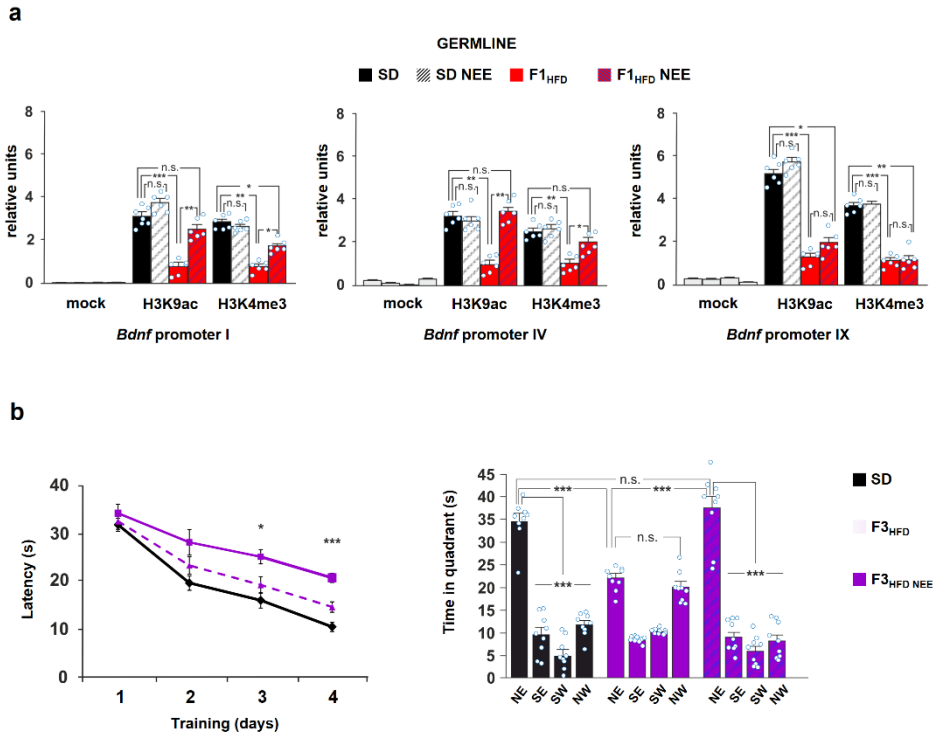
**Supplementary Figure 2. Transgenerational HFD effects are not sex-specific.** (a) Preference index in NOR paradigm for F2<sub>HFD</sub> (left) and F3<sub>HFD</sub> (right) female descendants (n = 9 mice from 6 litters for each group; statistics by unpaired Student's *t*-test). (b) Preference index of F2<sub>HFD</sub> NL (left) and F3<sub>HFD</sub> NL (right) male mice descended from a progenitor fed with HFD from four weeks before mating until delivery (n = 9 mice from 5 litters for each group; statistics by unpaired Student's *t*-test). (c) BDNF levels in the hippocampus of F2<sub>HFD</sub> NL and F3<sub>HFD</sub> NL male mice. ELISA assay was performed in duplicate (n = 8 mice from 6 litters per group; statistics by unpaired Student's *t*-test). (d) Weight, (e) food consumption, (f) fasting glucose plasma levels, (g) fasting insulin plasma levels and (h) IPGTT of SD, F2<sub>HFD</sub> and F3<sub>HFD</sub> mice (n = 12 mice from 8 litters per group; statistics by unpaired Student's *t*-test). (i) Locomotor activity of SD, F1<sub>HFD</sub>, F2<sub>HFD</sub> and F3<sub>HFD</sub> mice quantified by Open Field test (left, n = 10 mice derived from 6 litters for each group; statistics by one-way ANOVA and Bonferroni post hoc) and swimming speed in the 1<sup>st</sup> day of the MWM (right, n = 8 mice derived from 5-6 litters for each group; statistics by one-way ANOVA and Bonferroni post hoc). Data are expressed as mean ± SEM. \*\*\* *p* < 0.001; n.s. not significant.



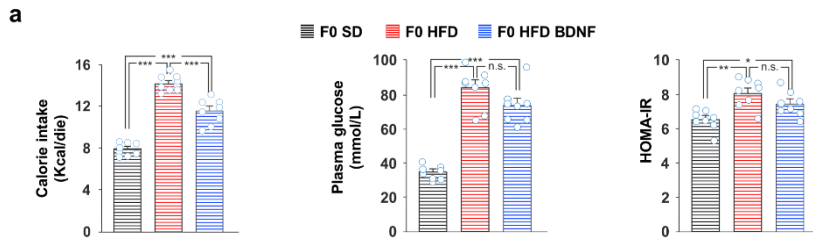
**Supplementary Figure 3. Downregulation of BDNF expression in neurons and other cell types.** (a) Up-down fold expression changes of synaptic plasticity-related genes more markedly affected in the hippocampus of F1<sub>HFD</sub>, F2<sub>HFD</sub> and F3<sub>HFD</sub> mice. (b) Representative *Bdnf* transcript concentration (copies  $\mu\text{L}^{-1}$ ) in CA1 hippocampal neurons of SD and F1<sub>HFD</sub> mice. Aldolase was used as control. (c) ddPCR data were normalized and plotted as mean  $\pm$  SEM ( $n = 6$  neurons from 3 mice of different litters per each group). Each sample was analyzed in duplicate (statistics by unpaired Student's *t*-test). (d) germline, gastrocnemius muscle and heart *Bdnf* coding exon IX expression in SD and F1<sub>HFD</sub> mice ( $n = 6$  mice derived from 4 litters per each group). Gene expression was normalized to actin; experiments were performed in triplicate (statistics by unpaired Student's *t*-test). (e) BDNF plasma levels of SD and HFD descendant male mice measured by ELISA performed in duplicate ( $n = 8$  mice derived from 4 litters per group; statistics by one-way ANOVA and Bonferroni post hoc) (f) ChIP assays of H3K9ac and H3K4me3 on the promoters III and VI of *Bdnf* gene in the hippocampus of SD and HFD descendant male mice. Data represent mean values obtained from 6 mice derived from 5 litters for each group; qPCR experiments were performed in triplicate (statistics by one-way ANOVA and Bonferroni post hoc). Data are expressed as mean  $\pm$  SEM. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; n.s. not significant.



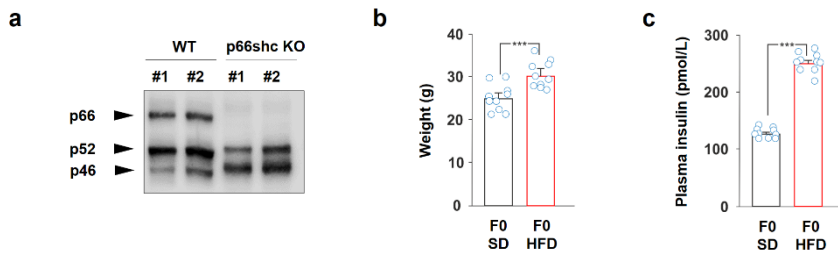
**Supplementary Figure 4. HFD intergenerational effects are not mediated by maternal behavior.** (a) Preference index of SD and F1<sub>HFD</sub> mice fostered by F0 SD-fed females (SD CF and F1<sub>HFD</sub> CF, respectively; n = 9 mice from 6 litters for each group; statistics by unpaired Student's *t*-test). (b) Expression of *Bdnf* exon I, IV and IXa in the hippocampus of SD CF and F1<sub>HFD</sub> CF mice. Data represent mean values obtained from 6 mice derived from 5 litters for each group; experiments were performed in triplicate (statistics by unpaired Student's *t*-test). (c) ChIP assays of H3K9ac and H3K4me3 on the promoters I, IV and IX of *Bdnf* gene in the hippocampus and (d) germline of SD CF and F1<sub>HFD</sub> CF mice. Data represent mean values obtained from 6 mice derived from 4 litters for each group; qPCR experiments were performed in triplicate (statistics by two-way unpaired Student's *t*-test). (e) Preference index of mice born through in vitro fertilization (IVF) of oocytes with sperm of SD or F1<sub>HFD</sub> mice (SD IVF and F1<sub>HFD</sub> IVF, respectively; n = 8 mice from 4 litters for each group; statistics by unpaired Student's *t*-test). (f) Expression of *Bdnf* exon I, IV and IXa in the hippocampus of SD IVF and F1<sub>HFD</sub> IVF animals. Data represent mean values obtained from 6 mice derived from 5 litters for each group; experiments were performed in triplicate (statistics by unpaired Student's *t*-test). (g) ChIP assays of H3K9ac and H3K4me3 on the promoters I, IV and IX of *Bdnf* gene in the hippocampus of SD IVF and F1<sub>HFD</sub> IVF mice. Data represent mean values obtained from 6 mice derived from 4 litters for each group; qPCR experiments were performed in triplicate (statistics by unpaired Student's *t*-test). Data are expressed as mean  $\pm$  SEM. \* *p* < 0.05; \*\* *p* < 0.01; \*\*\* *p* < 0.001.



**Supplementary Figure 5. NEE induces transgenerational effects on learning and memory. (a)** ChIP assays of H3K9ac and H3K4me3 on the promoters I, IV and IX of *Bdnf* gene in the germline of SD, SD NEE, F1<sub>HFD</sub> and F1<sub>HFD</sub> NEE male mice. Data represent mean values obtained from 6 mice derived from 4 litters for each group; qPCR experiments were performed in triplicate (statistics by two-way ANOVA and Bonferroni post hoc). **(b)** Latency to reach the hidden platform (left) and time spent in the four quadrants during the probe test (right) in the MWM for SD, F3<sub>HFD</sub> and F3<sub>HFD</sub> NEE male mice (n = 9 mice from 6 litters for each group; statistics by two-way ANOVA and Bonferroni post hoc). For latency, significance is indicated between F3<sub>HFD</sub> and F3<sub>HFD</sub> NEE mice. Data are expressed as mean ± SEM. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; n.s. not significant.



**Supplementary Figure 6. BDNF administration does not revert the maternal insulin resistance. (a)** Calorie intake (Kcal/die), plasma glucose levels and HOMA index of F0 SD, F0 HFD, and F0 HFD BDNF female mice (n = 8 mice per group; statistics by two-way ANOVA and Bonferroni post hoc). Data are expressed as mean ± SEM. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; n.s. not significant



**Supplementary Figure 7. Characterization of p66Shc KO model.** (a) Immunoblot of p66Sch expression in ovaries of wild-type and *p66Shc* KO female mice. (b) Weight and (c) fasting insulin plasma levels of SD-fed (black bar) and HFD-fed (red bar) *p66Shc* KO mothers after four weeks of dietary regimen (n = 10 mice per each group; statistics by unpaired Student's *t*-test). Data are expressed as mean ± SEM. Source data are provided as a Source Data file. \*\*\*  $p < 0.001$



**Supplementary Table 1. GENE REGULATION**

F1, F2, F3 fold changes of genes analyzed in the PCR array (PAMM-126Z).

Refseq	Symbol	Description	F1 fold change	F2 fold change	F3 fold change
NM_007399	Adam10	A disintegrin and metallopeptidase domain 10	1,006	1,4163	-4,0559
NM_009622	Adcy1	Adenylate cyclase 1	1,6868	1,6814	1,6825
NM_009623	Adcy8	Adenylate cyclase 8	1,003	1,0036	1,1825
NM_009652	Akt1	Thymoma viral proto-oncogene 1	1,6804	1,4168	1,4115
NM_018790	Arc	Activity regulated cytoskeletal-associated protein	1,4193	1,4177	1,4168
NM_007540	Bdnf	Brain derived neurotrophic factor	-4,7987	-2,8565	-4,0233
NM_177407	Camk2a	Calcium/calmodulin-dependent protein kinase II alpha	-1,4094	-1,1903	-1,1864
NM_178597	Camk2g	Calcium/calmodulin-dependent protein kinase II gamma	-3,368	1,4152	1,4113
NM_007664	Cdh2	Cadherin 2	1,6969	1,1964	1,1913
NM_009883	Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	1,0037	1,0031	1,1918
NM_007679	Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	1,0008	-1,9898	1,0023
NM_007726	Cnr1	Cannabinoid receptor 1 (brain)	1,1986	1,004	-1,1889
NM_133828	Creb1	CAMP responsive element binding protein 1	1,001	-1,001	-1,001
NM_013498	Creml	CAMP responsive element modulator	1,4228	2,0163	1,4197
NM_007864	Dlg4	Discs, large homolog 4 (Drosophila)	1,0068	1,1908	1,0055
NM_007913	Egr1	Early growth response 1	1,6867	1,6865	1,6867
NM_010118	Egr2	Early growth response 2	1,6987	1,6943	2,0167
NM_018781	Egr3	Early growth response 3	1,1934	1,0074	-1,4031
NM_020596	Egr4	Early growth response 4	-1,0005	1,6814	1,1867
NM_010142	Ephb2	Eph receptor B2	-2,3807	1,1905	-1,0014
NM_010234	Fos	FBJ osteosarcoma oncogene	1,4119	1,9954	1,6853
NM_176942	Gabra5	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 5	-1,4032	-1,4028	1,0058
NM_010305	Gnai1	Guanine nucleotide binding protein (G protein), alpha inhibiting 1	1,0067	-4,7996	1,0039
NM_008165	Gria1	Glutamate receptor, ionotropic, AMPA1 (alpha 1)	-1,4062	-1,4146	-1,1881
NM_013540	Gria2	Glutamate receptor, ionotropic, AMPA2 (alpha 2)	1,0029	1,0083	1,0068
NM_016886	Gria3	Glutamate receptor, ionotropic, AMPA3 (alpha 3)	1,0062	1,1903	-1,0015
NM_019691	Gria4	Glutamate receptor, ionotropic, AMPA4 (alpha 4)	1,1923	1,1917	1,191
NM_008169	Grin1	Glutamate receptor, ionotropic, NMDA1 (zeta 1)	-1,0033	1,1926	1,001
NM_008170	Grin2a	Glutamate receptor, ionotropic, NMDA2A (epsilon 1)	1,2	1,0066	1,0019
NM_008171	Grin2b	Glutamate receptor, ionotropic, NMDA2B (epsilon 2)	-1,0008	-1,0003	-1,0004
NM_010350	Grin2c	Glutamate receptor, ionotropic, NMDA2C (epsilon 3)	1,4193	1,4166	1,0008
NM_008172	Grin2d	Glutamate receptor, ionotropic, NMDA2D (epsilon 4)	-2,0112	1,1935	1,1896
NM_133442	Grip1	Glutamate receptor interacting protein 1	1,6782	1,1913	1,0028

NM_016976	Grm1	Glutamate receptor, metabotropic 1	1,0073	1,1941	1,0018
NM_001160353	Grm2	Glutamate receptor, metabotropic 2	-1,4047	-1,0013	1,0016
NM_181850	Grm3	Glutamate receptor, metabotropic 3	2,0162	2,0129	1,0061
NM_001013385	Grm4	Glutamate receptor, metabotropic 4	1,7014	1,4298	1,1954
NM_001081414	Grm5	Glutamate receptor, metabotropic 5	1,716	1,1935	1,1985
NM_177328	Grm7	Glutamate receptor, metabotropic 7	1,1969	1,1922	1,4224
NM_008174	Grm8	Glutamate receptor, metabotropic 8	1,6834	1,1962	1,1902
NM_152134	Homer1	Homer homolog 1 (Drosophila)	1,6902	1,4205	1,1914
NM_010512	Igf1	Insulin-like growth factor 1	1,1887	-1,0053	-1,1871
NM_008380	Inhba	Inhibin beta-A	1,1937	-1,1875	-1,4142
NM_010591	Jun	Jun oncogene	-1,186	1,0007	1,1837
NM_008416	Junb	Jun-B oncogene	2,3935	1,1962	1,6848
NM_010623	Kif17	Kinesin family member 17	1,6835	2,3765	1,4144
NM_013692	Klf10	Kruppel-like factor 10	1,6921	1,6875	-1,183
NM_011949	Mapk1	Mitogen-activated protein kinase 1	1,4207	1,689	1,1933
NM_013599	Mmp9	Matrix metalloproteinase 9	1,0051	1,4177	1,6825
NM_010875	Ncam1	Neural cell adhesion molecule 1	1,423	1,4223	1,1909
NM_008689	Nfkb1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	1,1966	-1,1924	1,0042
NM_010908	Nfkbib	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta	1,223	1,6874	1,685
NM_013609	Ngf	Nerve growth factor	-1,6741	-2,3704	-1,4121
NM_033217	Ngfr	Nerve growth factor receptor (TNFR superfamily, member 16)	1,6946	2,0117	1,4193
NM_008712	Nos1	Nitric oxide synthase 1, neuronal	1,4143	1,6863	1,6719
NM_016789	Nptx2	Neuronal pentraxin 2	-1,4064	-1,3956	-1,3996
NM_010444	Nr4a1	Nuclear receptor subfamily 4, group A, member 1	2,0023	1,9976	1,6847
NM_008742	Ntf3	Neurotrophin 3	1,0049	-1,4109	1,0034
NM_198190	Ntf5	Neurotrophin 5	1,1714	1,3441	1,3863
NM_008745	Ntrk2	Neurotrophic tyrosine kinase, receptor, type 2	1,4286	1,6832	1,0027
NM_021543	Pcdh8	Protocadherin 8	-1,674	-1,6741	-1,4016
NM_008837	Pick1	Protein interacting with C kinase 1	1,4145	1,1869	1,0032
NM_008842	Pim1	Proviral integration site 1	1,1945	1,1902	1,69
NM_008872	Plat	Plasminogen activator, tissue	1,0031	-1,1904	1,0022
NM_021280	Plcg1	Phospholipase C, gamma 1	1,4123	1,4082	1,6621
NM_031868	Ppp1ca	Protein phosphatase 1, catalytic subunit, alpha isoform	2,0131	1,6829	1,4151
NM_013636	Ppp1cc	Protein phosphatase 1, catalytic subunit, gamma isoform	1,4292	1,0101	1,0095
NM_026731	Ppp1r14a	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	1,4226	1,0003	-1,0024

NM_019411	Ppp2ca	Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	1,1876	1,0009	-1,0014
NM_008913	Ppp3ca	Protein phosphatase 3, catalytic subunit, alpha isoform	1,1934	-1,1864	1,0007
NM_011101	Prkca	Protein kinase C, alpha	-1,1834	-1,1842	-1,1892
NM_011102	Prkcg	Protein kinase C, gamma	-1,1852	1,0021	-1,1913
NM_011160	Prkg1	Protein kinase, cGMP-dependent, type I	1,0024	1,19	1,4128
NM_009001	Rab3a	RAB3A, member RAS oncogene family	-1,1779	1,0025	1,0025
NM_009045	Rela	V-rel reticuloendotheliosis viral oncogene homolog A (avian)	1,1951	1,0049	1,4172
NM_011261	Reln	Reelin	1,4291	1,1961	1,1974
NM_009061	Rgs2	Regulator of G-protein signaling 2	1,9974	1,6785	1,0021
NM_053075	Rheb	Ras homolog enriched in brain	1,1974	-1,1839	1,0046
NM_019812	Sirt1	Sirtuin 1 (silent mating type information regulation 2, homolog) 1	1,0064	-1,1858	-1,1855
NM_020493	Srf	Serum response factor	1,0011	1,1931	1,0017
NM_177340	Synpo	Synaptopodin	1,0088	1,0024	1,0046
NM_011593	Timp1	Tissue inhibitor of metalloproteinase 1	1,2095	1,6688	1,6862
NM_013693	Tnf	Tumor necrosis factor	1,1946	1,6874	1,685
NM_011739	Ywhaq	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	1,0003	1,0022	-1,1893

**Blue:** statistically significant downregulated genes

**Red:** statistically significant upregulated genes

## Supplementary Table 2. PRIMERS

Primer sequences used for mRNA analyses.

Gene	Primer sequence	
<i>Bdnf</i> exon I	FW	5'-TGAGAGTTGAAGCTTTGCGG-3'
	RV	5'-ATTGTGGCTTTGCTGTCCTG-3'
<i>Bdnf</i> exon II	FW	5'-TGGAAGAAACCGTCTAGAGC-3'
	RV	5'-CTCGGTGTGAGCCGAACC-3'
<i>Bdnf</i> exon III	FW	5'-TATCATCCCTCCCCGAGAG-3'
	RV	5'-CTCTTCGATCTAGAAAGGACC-3'
<i>Bdnf</i> exon IV	FW	5'-AGCATGAAATCTCCAGCCT-3'
	RV	5'-CGGTCCCCAAGGTTCTAGAC-3'
<i>Bdnf</i> exon V	FW	5'-GTGTAGTTTCATTGTGTGTTTCG-3'
	RV	5'-GCAATGTGCTCTAGAGTAGG-3'
<i>Bdnf</i> exon VI	FW	5'-TGGCTGTGCGACGGTTC-3'
	RV	5'-GTTGTCACGCTTCTGGTCC-3'
<i>Bdnf</i> exon VII	FW	5'-AAAGGGTCTGCGGAAGTCC-3'
	RV	5'-CTCTTCCTGTTTCAGCATCC-3'
<i>Bdnf</i> exon VIII	FW	5'-GTCGCTGCGCCTCAGTGG-3'
	RV	5'-CACCAAGCTTTAAAACTGAGG-3'
<i>Bdnf</i> exon IXa	FW	5'-CAAAGCTGCTAAAGCGGGAGG-3'
	RV	5'-GATCCACTCCAGCTGCAGG-3'
<i>Bdnf</i> exon IX	FW	5'-TGGCTGACACTTTTGAGC-3'
	RV	5'-GTTTGCGGCATCCAGGT-3'
<i>Gapdh</i>	FW	5'-TCCATGACAACCTTTGGCATT-3'
	RV	5'-GTTGCTGTTGAAGTCGCAGG-3'
BDNF exon IX (ddPCR)	FW	5'-GGCAGGTTTCGAGAGGTCTGA-3'
	RV	5'-TCGATGACGTGCTCAAAGTG-3'
Aldolase (ddPCR)	FW	5'-CGCTGCCAGTATGTTACTGAGAA-3'
	RV	5'-TGGTCGCTCAGAGCCTTGT-3'

Primer sequences used for ChIP analyses.

Gene	Primer sequence	
BDNF promoter I	FW	5'-TCGATTCACGCAGTTGTTCC-3'
	RV	5'-GCACCAGCCGGCTACTGC-3'
BDNF promoter IV	FW	5'-CATGCAATGCCCTGGAACG-3'
	RV	5'-GAGAGCAGTCCTCTCCTCG-3'
BDNF promoter IX	FW	5'-GAAGCTGAGCTCTCTGTGG-3'
	RV	5'-GGTCTCATGAAGAACTTAAGG-3'

Abbreviations: FW, forward; RV, reverse

**Supplementary Table 3. ANTIBODIES**

Primary Antibody	Host	Catalogue reference
$\alpha$ -TrkB (80E3)	Rabbit	Cell Signaling #4603
$\alpha$ -pTrkB Tyr <sup>816</sup>	Rabbit	See Methods
$\alpha$ -FoxO3a	Rabbit	Cell Signaling #2497
$\alpha$ -phospho FoxO3a Ser <sup>253</sup>	Rabbit	Cell Signaling #9466
$\alpha$ -Irs-1	Rabbit	Cell Signaling #2382
$\alpha$ -phospho Irs-1 Ser <sup>612</sup>	Rabbit	Cell Signaling #2386
$\alpha$ -beta Actin	Rabbit	Abcam ab8227
$\alpha$ -Sirt1	Rabbit	Millipore #07-131
$\alpha$ -Sirt2 (D4S6J)	Rabbit	Cell Signaling #12672
$\alpha$ -phospho Creb Ser <sup>133</sup>	Rabbit	Cell Signaling #9198
$\alpha$ -Creb	Mouse	ThermoFisher #MA1-083
$\alpha$ -Hdac2	Mouse	Abcam ab12169
$\alpha$ -Cbp	Rabbit	Abcam ab2832
$\alpha$ -Tri-Methyl-Histone H3 Lys <sup>4</sup>	Rabbit	Abcam ab8580
$\alpha$ -Histone H3 (acetyl K9)	Rabbit	Abcam ab4441
$\alpha$ -p66Shc	Mouse	BD Bioscience 610878