SUPPLEMENTARY INFORMATION

Transgenerational transmission of hedonic behaviors and metabolic phenotypes induced by maternal overnutrition.

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SUPPLEMENTARY MATERIALS AND METHODS

Experimental Design

The sample size selection, breeding and the experiments were designed according to the ARRIVE guidelines [1]. Random allocation of mice was conducted during breeding as well as during each experiment to exclude the selection bias. C57BL6/N mice obtained from Charles River, Germany were selected for breeding at the age of 10 weeks. To generate F1 offspring, 40 female mice were randomly divided into two groups (20 in each group) and were exposed to HFD or chow diet for 9 weeks (3 weeks preconception, 3 weeks gestation and 3 weeks lactation). 20 male breeders were randomly chosen and allocated as one male per 2 female breeders during the mating. To generate F2 and F3 offspring, 12 behaviorally naïve adult F1 males from HFD and CTR groups were randomly selected (one male per litter to exclude the litter effect) and mated with naïve primiparous females. All F2 and F3 animals were bred in identical breeding conditions. In each experiment, mice from multiple independent litters (one offspring per 10 different dams) were selected in each group to avoid the litter effect. Both male and female mice were chosen to assess the sex effects [2]. Behaviorally naïve mice were randomly allocated for each experiment (hedonic, metabolic, neuroanatomical and neurochemistry). Initially, the body weight of all offspring (F2 and F3) were measured since weaning to adulthood (PND 91). In both F2 and F3 generations, HFD preference test, sucrose preference test and alcohol preference test were conducted chronologically with 20 HFD (10 male and 10 female) and 20 CTR (10 male and 10 female) offspring. For the amphetamine sensitivity test, a separate group of mice (12 in each treatment group; 6 male and 6 female per group) was selected in both generations. Similarly, CPP test was performed in a different group of F2 and F3 offspring (24 mice in each HFD and control group). To assess the metabolic

46 parameters, a separate group of mice in both generations was allocated and the experiments
47 were conducted in following orders: CT scan, metabolic cage study, and insulin sensitivity test
48 and plasma insulin and lipid profiles. The behavioral and metabolic experiments were not
49 blindly conducted. All the behavioral experiments (except CPP) and the metabolic
50 experiments were replicated two times in two independently generated F2 as well as F3
51 cohorts. For neuroanatomical, neurochemical analysis and methylation assays, samples were
52 collected from behaviorally naïve mice. For neuroanatomical analysis, 32 F2 offspring (16 HFD
53 and 16 CTR) and 36 (18 HFD and 18 CTR) F3 offspring were used. For postmortem HPLC, 40
54 F2 (20 mice per group) and 36 F3 (18 mice per group) offspring were chosen. To perform
55 sperm methylation assay, 12 F1 (6 HFD and 6 CTR) and 12 F2 (6 HFD and 6 CTR) fathers were
56 selected. These experiments were blindly performed since each sample was recognized by a
57 number throughout the experiment up to the raw data analysis. The samples were allocated
58 in to the respected groups in the final statistical analysis.
59
60 **Fat composition assay**
61 Body fat composition was measured using a CT scanner (La Theta LCT-100; Aloka Inc, Japan).
62 The scanner measures the volumes of adipose tissue, bone, air and the rest using differences
63 in X-ray density. The X-ray source was set at 50 kV with a constant 1mA current. A mouse
64 holder with an outer diameter of 48 mm and inner diameter of 41 mm was used, resulting in
65 pixel resolutions of 100 μm on 480 × 480 pixel images. A pitch size of 1 mm was used. Mice
66 were sedated by 4-5% isoflurane and maintained under gas anesthesia (1.5-2.5% isoflurane)
67 while scanning. Animals were placed in supine position in the holder. The scan speed was set
68 to 4.5 s/ image. A whole body scan excluding the head and the tail was performed for 5 min.
69 Recorded scans were analyzed using the software La Theta 2.10.

70 **Metabolic cage study**

71 Metabolic phenotyping was performed in the TSE Phenomaster cages (TSE Systems GmbH,
72 Bad Homburg, Germany). The TSE Phenomaster cages comprised of an indirect calorimetry
73 system connected to a gas sensing unit to measure O_2 consumption (VO_2) and CO_2
74 production (VCO_2). A multi-dimensional infrared beam system assessed the locomotor
75 activity as the total number of beam breaks in X and Y axis. Mice were single housed for 2
76 days prior to the experiment for acclimatization to the new cage environment. Room
77 temperature was set to 23 °C; the light/dark cycle in the room was 12:12 h (lights off at 07:00
78 and lights on at 19:00). Mice were separately placed in the TSE Phenomaster cages and had
79 ad libitum access to food and water. Mice were monitored for a total of 3 days. Body weight
80 of each animal was measured before and after the experiment. O_2 consumption and CO_2
81 production were calculated from the gas concentrations and the air flow through the
82 individual cage measured in parallel. Heat production, locomotor activity and food intake
83 were also monitored during this period. Data were analyzed with Phenomaster software.

84 **Amphetamine-induced locomotor activity**

85 The locomotor response to a systemic amphetamine administration was tested in an open
86 field apparatus. First, the mice were allowed to habituate to the open-field arena for 30 min
87 (baseline). Then, they were administered an i.p. injection of saline (0.9% NaCl solution) and
88 locomotor activity was measured for 30 min. The mice were then administered 2.5 mg/kg
89 (i.p.) d-amphetamine sulphate (AMPH, Sigma-Aldrich, Switzerland) dissolved in 0.9% NaCl
90 saline. The mice were placed back in the open field arena and the locomotor response to the
91 acute drug challenge was measured for an additional 120 min. The locomotor activity was
92 indexed by the distance travelled in the entire arena, expressed as a function of 12 successive

93 10 min bins. All solutions for injection were prepared on the day of experiment and were
94 administered in a volume of 5 ml/kg body weight.

95 **Conditioned place preference (CPP)**

96 The CPP chamber consisted of two equally sized compartments with different colored walls
97 and distinct floor textures that were separated by a small central area. A digital camera was
98 directly mounted above the CPP apparatus which captured images at a rate of 5 Hz and
99 transmitted them to the Ethovision tracking system (Noldus Technology). The distance
100 travelled and total time spent in each compartment were recorded by the Ethovision
101 software. The experiment consisted of three phases. During the pre-conditioning phase, all
102 mice were allowed to freely explore all the compartments for 15 min. The conditioning phase
103 lasted for eight days where the mice were conditioned to either cocaine or saline. During the
104 conditioning phase, half of the animals from each group received the drug or vehicle in one
105 compartment while the other half received it in the other compartment. During each of the
106 four saline conditioning days (Day 1, 3, 5 and 7), an intraperitoneal (i.p.) injection of saline
107 (0.9% NaCl) was administered to the mice immediately prior to confinement to the vehicle
108 paired compartment for 30 min. On each of the four cocaine conditioning days (Day 2,4,6
109 and 8), mice received an i.p. injection of cocaine hydrochloride (Sigma–Aldrich, Switzerland)
110 at a dose of 20 mg/kg before confining the mice to the drug-paired chamber for 30 min. The
111 central area was made inaccessible by doors during conditioning. On the third phase, the
112 preference test, took place 48 hours after the last conditioning day, the mice were again given
113 free access to the entire apparatus without any injection for 30 min. The total time spent in
114 each compartment was recorded during this period.

115 **Dissection of brain regions**

116 Adult mice from both groups were euthanized, the brains were rapidly removed, and 1 mm
117 thick coronal sections were made. The slices were placed on an ice-chilled dissection plate for
118 the extraction of the brain regions using a 1 mm micropunch. All brain areas of interest were
119 dissected according to the Mouse Brain Atlas in Stereotaxic Coordinates (Paxinos and
120 Franklin, 2008) which were as follows: dSTR (bregma +1.34 to +0.14 mm), Nac (bregma +1.60
121 to +0.98 mm), mPFC (bregma +2.68 to +1.94mm), Hypo (bregma -1.34 to -2.06mm), SN
122 (bregma −2.80 to −3.64 mm), VTA (bregma −2.92 to −3.64 mm), Amyg (bregma from -0.94
123 mm to-2.30 mm), V. hip (bregma -2.54 to -3.80 mm) and D. hip (bregma -1.22 to -2.46 mm)
124 and immediately frozen at −80°C.

125 **Gene expression analysis of deltaFosB**

126 The expression of delta FosB in dSTR and Nac of HFD and CTR offspring following chronic
127 cocaine exposure was assessed by quantitative real time PCR (q-RT PCR). Total RNA from brain
128 tissue was extracted using the Trizol-chloroform method (Invitrogen, CA, USA) according to
129 the manufacturer's instruction and treated with DNase (Biolabs INc, CA, USA) for 30 min at
130 37°C. Following RNA extraction, 1µg of total RNA was converted to cDNA using High
131 Capacity cDNA Reverse Transcription Kit [Applied Biosystems (ABI), Foster City, CA)]. The
132 *qPCR* reactions were performed using the SYBER Green master mix (ABI) in the presence of
133 primer pairs specific for the gene encoding for ΔFOSB. Endogenous control m36B4 was used
134 to normalize the expression of the selected genes. Real time PCR reactions were conducted
135 as follows: after a pre-denaturation and polymerase-activation program (5min at 95◦C),
136 forty cycles each consisting of 95◦C for 3 sec and 60◦C for 30 sec were followed by a melting
137 curve program (60 to 95◦C with heating rate of 0.1◦C/sec). The relative expression of mRNA
138 was calculated using the 2- ΔΔCt method [1]. All primers were purchased from Microsynth AG,
139 Switzerland. The primers used were as follows: ΔFOSB (forward, 5`-

140 AGGCAGAGCTGGAGTCGGAGAT-3`; reverse, 5`-GCCGAGGACTTGAACTTCACTCG-3`) and m36B4

141 (forward, 5’-GCCGTGATGCCCAGGGAAGA-3’; reverse, 5’-CATCTGCTTGGAGCCCACGTT-3’).

142 **Immunohistochemistry**

143 Adult behaviorally naïve mice were deeply anaesthetized and perfused transcardially with
144 phosphate buffer solution (PBS), followed by fixation with 4% phosphate-buffered
145 paraformaldehyde solution containing 5% sucrose. After overnight fixation in the same
146 fixative, the brains were cryoprotected using 30% sucrose solution and stored at 4°C. Later,
147 the brains completely embedded in the cryomatrix (O.C.T; Thermo Scientific) were cut
148 coronally at 40 µm thickness. The slices were first rinsed three times in PBS and then
149 incubated with 0.5% H2O2 for 30 min. Later, the slices were incubated with 50mM
150 ammonium chloride and subsequently with 50mM glycine buffer, for 30 min each. The
151 sections were incubated overnight at 4◦C in primary antibody diluted in 0.5M Tris buffer, pH
152 8.0 containing 0.3% Triton X-100 and 4% normal goat serum. The following primary antibodies
153 were used: rabbit anti-tyrosine hydroxylase (TH) (Santa Cruz Biotechnology sc14007; diluted
154 1:500), rat anti dopamine transporter (DAT) (Chemicon MAB369; diluted 1:1000), rat anti-
155 dopamine D1 receptor (D1R) (Sigma Aldrich D2944; diluted 1:1000) and rabbit anti-dopamine
156 D2 receptor (D2R) (Chemicon AB5084P; diluted 1:500). After overnight incubation with
157 primary antibody and three washing steps in 0.1 M PB, the sections were incubated at room
158 temperature for 1 h with the biotinylated secondary antibodies (Jackson Immunoresearch:
159 diluted 1:500). The slices were then incubated in avidin-biotin-peroxidase complex
160 (Vectastain PK6100 standard kit; Vector Laboratories) for 30 min. After three washing steps
161 in 0.1 M PB, the sections were incubated for 5 min with 0.05% 3,3-diaminobenzidine (DAB),
162 0.03% Nickel ammonium sulfate and 0.02% H2O2 diluted in 0.05M Tris buffer (pH 8.0). After
163 immunotaining, the sections were dehydrated, and cover slipped with DPX mountant.

164 **Densitometry**

165 Microscopic analysis was conducted under strictly blind conditions. Images were acquired at
166 a magnification of 2.5× (NA 0.075) using a digital camera (Axiocam MRc5; Zeiss, Jena,
167 Germany) attached to a Zeiss Axioplan microscope. Images were loaded into Image J software
168 (ImageJ, NIH, Maryland, USA) for analysis. The pixel brightness was measured in non-
169 immunoreactive areas of the forceps minor of the corpus callosum (for prefrontal cortical
170 measures), corpus callosum (for dorsal striatal measurements) and anterior commissure (for
171 nucleus accumbens' measures) as background area. The background-corrected relative
172 optical densities were measured per brain region. Quantification was performed in four to six
173 coronal brain sections and then averaged per brain area and animal.

174 **Steriological analysis**

175 The number of TH-positive neurons was determined by means of unbiased stereological
176 estimations using the optical fractionator method. With the aid of the image analysis software
177 Stereo Investigator (version 6.50.1; MicroBrightField), every section of a one-in-eight series
178 was measured, resulting in an average of 5-6 sections per brain sample. The sampling
179 parameters used were as follows: VTA (counting frame, grid size, sampling site=50um×50um,
180 120×130, 10; respectively) and SN (counting frame, grid size, sampling site=50um×50um,
181 150×110, 20; respectively). The counting frames were placed randomly at the intersections
182 of the grid within the outlined structure of interest by the software. The cells were counted
183 at the magnification of 20× [numerical aperture (NA), 0.40] according to the unbiased
184 sampling rules and taken into the measurement when they came into focus within the optical
185 dissector.

186 **Post mortem HPLC**

187 The brain regions isolated from adult mice were suspended in 0.4 M perchloric acid, sonicated
188 and centrifuged at 10,000×g for 20 min at 4 ˚C. The supernatant was then collected, filtered
189 and stored at -80 ˚C. The levels of dopamine (DA) and its metabolites DOPAC and HVA were
190 measured by a high performance liquid chromatography (HPLC) system as previously
191 described [3]. The sample (20 µl from each brain region) was injected by a refrigerated auto
192 injector (ASI-100, Dionex, CA, USA). The HPLC mobile phase contained: 250 ml HPLC- grade
193 acetonitrile, 5l aqueous solution having 0.27mM sodium ethylene-di-ammonium-tetra
194 acetate ($C_{10}H_{14}N_2O_8Na_2{\bullet}2H_2O$), 0.43mM triethylamine ($C_6H_{15}N$), 8 mM potassium
195 chloride, and 0.925 mM octanesulphonic acid ($C_8H_{17}O_3SNa$), at pH 2.95. The mobile phase
196 was passed through the system using a degasser at a flow rate of 0.4ml/min.
197 Neurotransmitters were separated on a reversed-phase column (125mm×3mm YMC column,
198 Nucleosil 120-3 C 18, YMC Europe GmbH, Germany) and the concentrations were detected
199 using the electrochemical detector at an electrode potential of +0.70V. Data acquisition and
200 calculations were performed using a chromatography workstation (Chromeleon, Dionex,
201 Olten, Switzerland). Sample analysis was performed based on the position and height of the
202 peaks of the endogenous components in relation to the samples of calibrating standard
203 solutions.

204 **Bisulfite sequencing analysis**

205 Raw sequence reads were trimmed to remove both poor quality calls and adapters using Trim
206 Galore (v0.4.1, www.bioinformatics.babraham.ac.uk/projects/trim_galore/, Cutadapt version
207 1.8.1, parameters: –paired). Trimmed reads were aligned to the mouse genome (GRCm38) in
208 paired-end mode to be able to use overlapping parts of the reads only once. Alignments were
209 carried out with Bismark v0.14.419 with the following set of parameters: paired-end mode: –
210 pbat. Reads were then deduplicated with deduplicate_bismark selecting a random alignment

211 for position that was covered more than once. CpG methylation calls were extracted from the
212 deduplicated mapping output, ignoring the first 6 bp of each read to reduce the methylation
213 bias typically observed in PBAT libraries using the Bismark methylation extractor (v0.14.4)
214 with the following parameters (i) paired-end mode: –ignore 6 –ignore_r2 6, (ii) single-end
215 mode: –ignore 6. CpG methylation calls were analyzed using R and SeqMonk software
216 (http://www.bioinformatics.babraham.ac.uk/projects/seqmonk/). 50 adjacent CpG running
217 window probes were generated and percentage of methylation determined for probes
218 containing at least five reads and three CpG on the pooled replicate data. The methylation
219 level was expressed as the mean of individual CpG sites. Global CpG methylation levels of
220 pooled replicates were illustrated using bean plots. Promoters were defined as the region -1
221 kb to the transcription start site as annotated in NCBIM37. For analysis of specific genome
222 features these were defined as follows: Genic (overlapping coding sequences) were divided
223 into exonic and intronic, CpG islands (CGI), promoters were defined as 1000 bp upstream of
224 mRNAs, genic (overlapping coding sequences) were divided into exonic and intronic, repeat
225 locations were extracted from the pre-masked RepeatMasker libraries (mm10 - Dec 2011 -
226 RepeatMasker open-4.0.5, http://www.repeatmasker.org/species/musMus.html). Repeat
227 instances overlapping annotated genes in the Ensembl gene set were removed to avoid
228 mixing signals from genic expression with specific expression of repetitive sequences.
229 Enhancers were taking from the super enhancers definied in ES cells. Graphing and statistics
230 were performed using Seqmonk and RStudio.

231 **In vitro fertilization**

232 Spermatozoa extracted from HFD and CTR-F1 males were used to fertilize oocytes isolated
233 from superovulated C57BL/6 females. The females at PND 28 were superovulated by i.p.
234 injection of 5 IU of equine chorionic gonadotropin (PMSG) and 5 IU of human chorionic

235 gonadotropin (hCG) with the PMSG injection timed at 18:00 on day 1 and hCG at 18:00 on day
236 3, respectively. Next day, males were sacrificed, the dense sperm was removed from cauda
237 epididymis and capacitated in 200ul of Fertiup medium (Cosmo Bio) for 45 minutes at 37°C.
238 Following capacitation, 2ul of sperm solution was added to the IVF drop which comprised of
239 100 ul HTF medium (Cosmo Bio) overlaid with embryo tested mineral oil (Sigma). Females
240 were then sacrificed, the oviducts were dissected immediately and the oocyte clutches were
241 released directly into the IVF drop. The IVF reaction was conducted for 4 hours at 37°C.
242 Following the IVF reaction, the efficiency of fertilization was confirmed by the presence of the
243 pronuclei and the 2nd polar body. Fertilized oocytes were surgically transferred into
244 pseudopregnant CD1 foster females previously mated with genetically vasectomized
245 Prnm1GFP males and allowed to develop to term.

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247 ## SUPPLEMENTARY FIGURE TITLES AND LEGENDS

248 **Supplementary Figure 1**. **Hedonic responses to natural rewards.**

249 (**a**) and (**b**) show the HFD preference in F2 and F3 generation respectively. The bar plots depict
250 the consumption of HFD and chow diet per day normalized to body weight. (**c**) and (**d**)
251 represent the sucrose preference in F2 and F3 offspring respectively. The bar plots show the
252 mean sucrose consumption per day normalized to body weight in three different
253 concentrations (0.5%, 1% and 3%). Data are represented as mean ± SEM. ***p< 0001. N (F2
254 CTR) = 20 (10 m, 10 f); N (F2 HFD) = 20 (10 m, 10 f); N (F3 CTR) = 20 (10 m, 10 f); N (F3 HFD) =
255 20 (10 m, 10 f); m = male, f = female.

256 **Supplementary Figure 2**. **Sensitization to cocaine in the F2 and F3 offspring.**

257 (**a**) and (**b**) represent the cocaine induced locomotor activity in F2 and F3 offspring

258 respectively. The line plots show the distance travelled (cm) in successive 5 min bins on the
259 first day and following 21 days of cocaine withdrawal in response to a systemic injection of
260 cocaine in the CPP paradigm. Data are represented as mean ± SEM. *p<0.05. N (F2 CTR) = 24
261 (12 m, 12 f); N (F2 HFD) = 24 (12 m, 12 f); N (F3 CTR) = 24 (12 m, 12 f); N (F3 HFD) = 24 (12 m,
262 12 f). m= male, f = female. (See also Figure 2).

263 **Supplementary Figure 3. Neuroanatomical alterations in the dopaminergic system of the F2**
264 **and F3 offspring.**

265 (**a-h**) show the representative images of coronal brain sections of F2 and F3 offspring for the
266 expression of D1R in the dSTR and Nac. D1R = dopamine receptor 1, dSTR = dorsal striatum,
267 Nac = nucleus accumbens, (See also Figure 3).

268 **Supplementary Figure 4. Levels of dopamine (DA) and its metabolites (DOPAC and HVA) in**
269 **post mortem brain tissue of the F2 offspring.**

270 (**a**) shows the content of DA in the Hypo, mPFC, SN, Amyg, V. hip and D. hip in the F2 offspring.
271 (**b**) and (**c**) display the levels of DOPAC in the dSTR, Nac, VTA as well as Hypo, mPFC, SN, Amyg,
272 V. hip and D. hip respectively. (**d**) and (**e**) display the levels of HVA in the dSTR, Nac, VTA as
273 well as Hypo, mPFC, SN, Amyg, V. hip and D. hip respectively. All monoamines contents are
274 expressed as ng per mg fresh tissue weight. Data are represented as mean ± SEM. N (F2 CTR)
275 = 20 (10 m, 10 f); N (F2 HFD) = 20 (10 m, 10 f). DA = dopamine, DOPAC = 3,4-
276 Dihydroxyphenylacetic acid, HVA = homovanillic acid, dSTR = dorsal striatum, Nac = nucleus
277 accumbens, VTA = ventral tegmental area, Hypo = hypothalamus, SN = substantia nigra, Amyg
278 = amygdala, mPFC = medial prefrontal cortex, V. hip = ventral hippocampus and D. hip = dorsal
279 hippocampus. m = male, f = female. (See also Figure 3).

280 **Supplementary Figure 5. Levels of dopamine (DA) and its metabolites (DOPAC and HVA) in**

281 **post mortem brain tissue of the F3 offspring.**

282 (**a**) shows the content of DA in the Hypo, mPFC, SN, Amyg, V. hip and D. hip in the F3 offspring.
283 (**b**) and (**c**) display the levels of DOPAC in the dSTR, Nac, VTA as well as Hypo, mPFC, SN, Amyg,
284 V. hip and D. hip respectively. (**d**) and (**e**) display the levels of HVA in the dSTR, Nac, VTA as
285 well as Hypo, mPFC, SN and Amyg respectively. All monoamines contents are expressed as ng
286 per mg fresh tissue weight. Data are represented as mean ± SEM. N (F3 CTR) = 18 (9 m, 9 f);
287 N (F3 HFD) = 18 (9 m, 9 f). DA = dopamine, DOPAC = 3,4-Dihydroxyphenylacetic acid, HVA =
288 homovanillic acid, dSTR = dorsal striatum, Nac = nucleus accumbens, VTA = ventral tegmental
289 area, Hypo = hypothalamus, SN = substantia nigra, Amyg = amygdala, mPFC = medial
290 prefrontal cortex, V. hip = ventral hippocampus and D. hip = dorsal hippocampus. m = male, f
291 = female. (See also Figure 3).

292 **Supplementary Figure 6. Altered metabolic phenotypes in the F2 and F3- HFD offspring.**

293 (**a**) and (**b**) represent the total fat mass, subcutaneous and visceral fat depot in F2 and F3
294 offspring respectively. (**c**) and (**d**) display the area under the curve (AUC) for the insulin
295 sensitivity test in the F2 and F3 offspring respectively. (**e**) and (**f**) show the concentration of
296 triglycerides and free fatty acid (FFA) in the F2 and F3 offspring respectively. Data are
297 represented as mean ± SEM. $*p<0.05$, $**p<0.01$. N (F2 CTR) = 12 (6 m, 6 f); N (F2 HFD) = 12 (6
298 m, 6 f); N (F3 CTR) = 12 (6 m, 6 f); N (F3 HFD) = 12 (6 m, 6 f). m = male, f = female. (See also
299 Figure 4).

300 **Supplementary Figure 7. Metabolic cage study in the F2 generation.**

301 The bar plots represent average O_2 consumption (**a**), average CO_2 production (**b**), mean heat
302 production (**c**), respiratory exchange ratio, RER (VCO_2/VO_2) (**d**) and physical activity (**e**) in 12
303 h dark and light cycle. The food intake per day was normalized to body weight (**f**). Data are

304 represented as mean ± SEM. *p<0.05, **p<0.01. N (F2 CTR) = 12 (6 m, 6 f); N (F2 HFD) = 12 (6
305 m, 6 f). m = male, f = female. (See also Figure 4).

306 **Supplementary Figure 8. Metabolic cage study in the F3 generation.**

307 The bar plots depict average O_2 consumption (**a**), average CO_2 production (**b**), mean heat
308 production (**c**), respiratory exchange ratio, RER (VCO_2/VO_2) (**d**) and physical activity (**e**) in 12
309 h dark and light cycle. The food intake per day was normalized to body weight (**f**). Data are
310 represented as mean ± SEM. *p<0.05. N (F3 CTR) = 12 (6 m, 6 f); N (F3 HFD) = 12 (6 m, 6 f). m
311 = male, f = female. (See also Figure 4).

312 **Supplementary Figure 9. Amphetamine induced locomotor activity in the IVF offspring.**

313 The line plots show the distance travelled (cm) in successive 10 min bins following baseline,
314 saline and a systemic injection of amphetamine in the open field. Data are represented as
315 mean ± SEM. *p<0.05. N (F2 CTR) = 16 (8 m, 8 f); N (F2 HFD) = 16 (8 m, 8 f); N (F3 CTR) = 16 (8
316 m, 8 f); N (F3 HFD) = 16 (8 m, 8 f). m = male, f = female.

317 **Supplementary Figure 10. Spem CpG methylation changes in F1 and F2 offspring.**

318 (**a**) and (**b**) show the percentage of F2 DMRs found between F1 CTR and F1 HFD and F1 DMR
319 found between F2 CTR and F2 HFD. The size of the dot represents the size of the DMR and
320 the blue color scale indicates the p-value of the DMR.

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