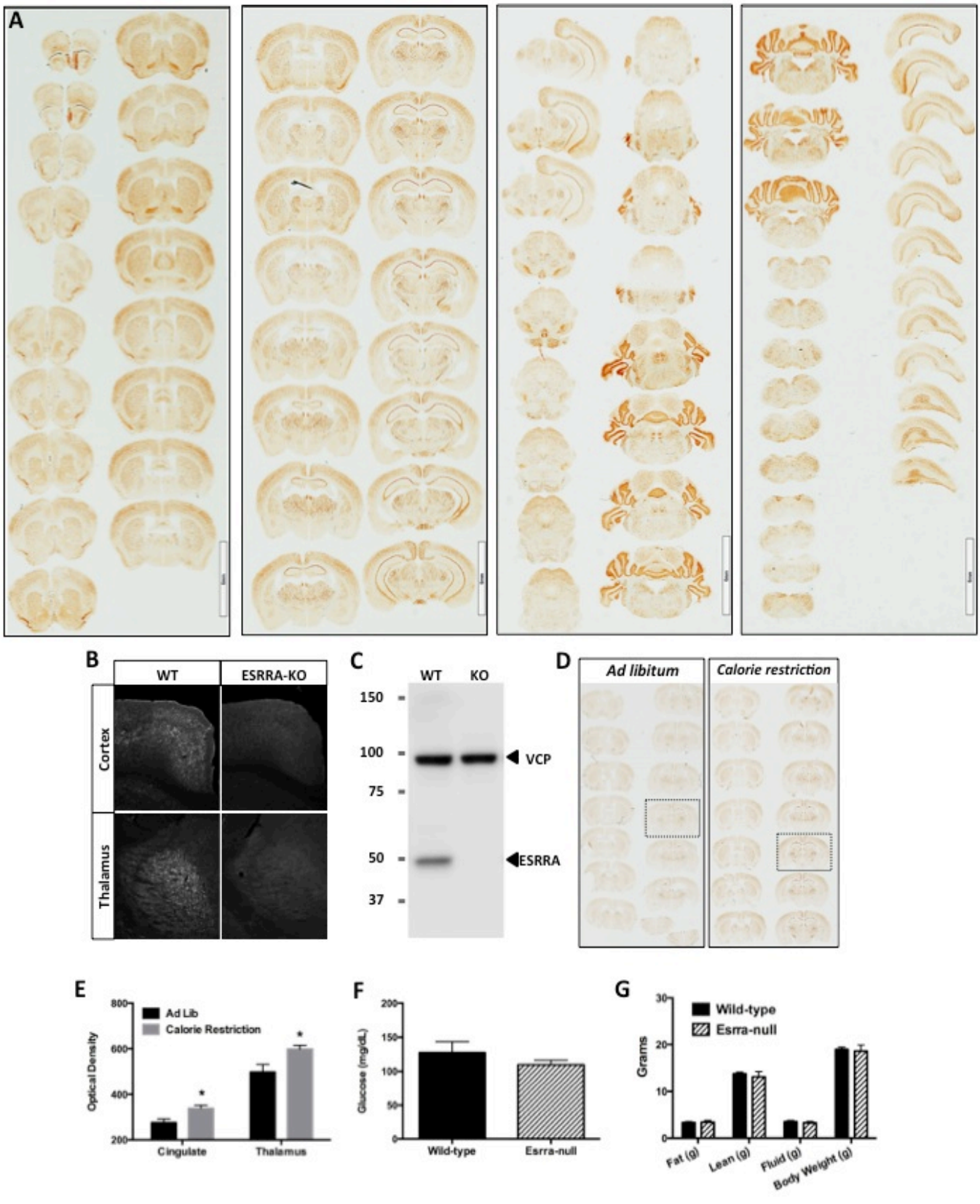
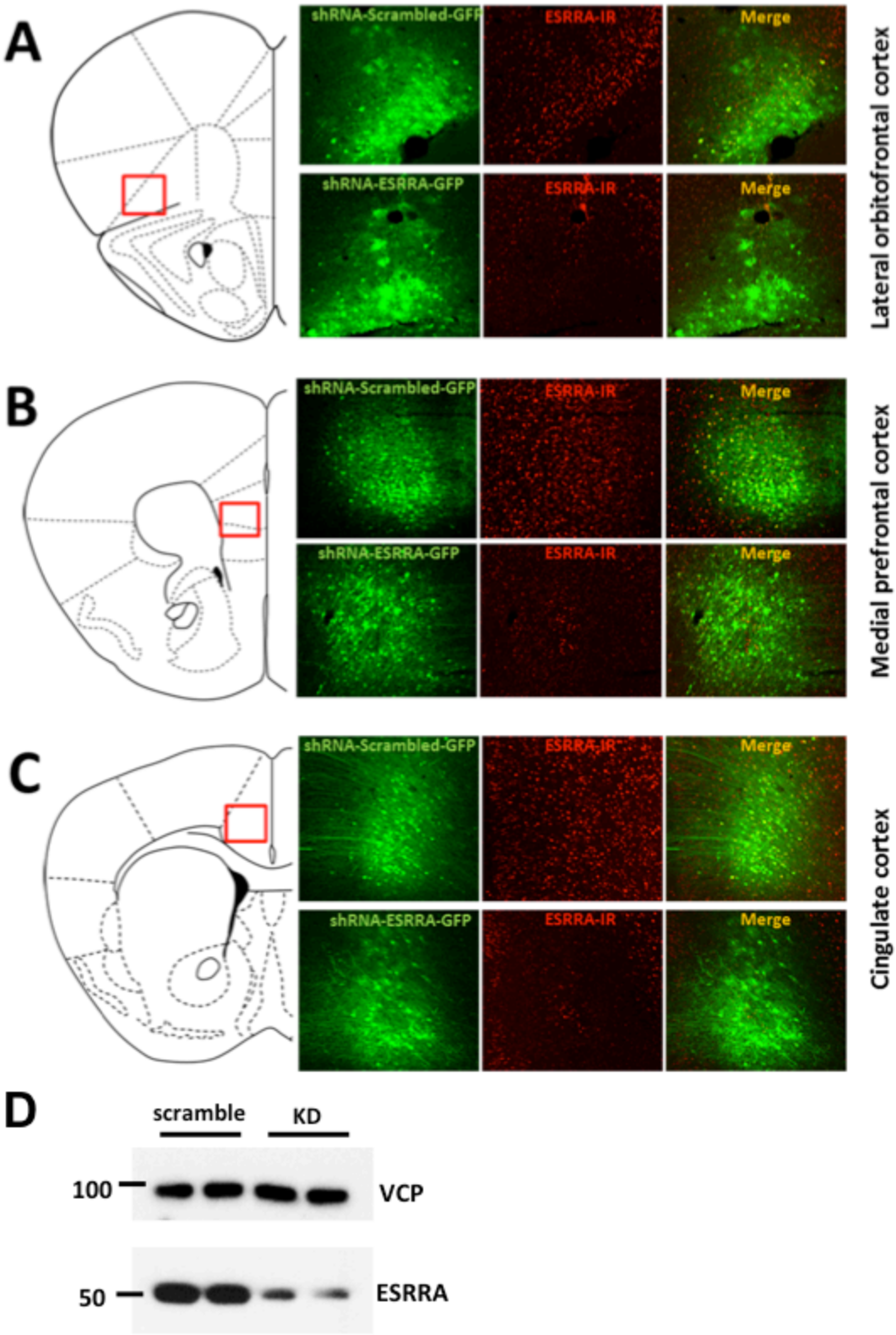


Supplemental Figure 1: Esrra-null mice display impaired behavioral responses to calorie restriction, related to Figure 1. (A) Expression of Esrra in mouse brain across all coronal sections. (B-C) Validation of anti-Esrra antibody in Esrra knockout (KO) brain for immunohistochemistry and Western blot. (D) Coronal series comparing immunohistochemical expression of Esrra under ad libitum feeding and calorie restriction. (E) Quantification of IHC results for cingulate cortex and thalamus (n= 4/group). (F) Glucose levels after overnight fast (n= 5 WT and 6 KO). (G) Body composition after overnight fast (n= 5 WT and 6 KO). Data presented as mean ± S.E.M. $p>0.05$ for all measures by Student's t-test.



Supplemental Figure 2: Validation of shRNA knockdown, related to Figure 3. AAV2-GFP-U6-mEsrra-shRNA was injected into (A) lateral orbitofrontal cortex, (B) medial prefrontal cortex, and (C) cingulate cortex, and processed for immunohistochemical staining against Esrra as described in Methods. (D) mPFC was dissected out of two mice injected with scramble or shRNA-Esrra (KD) by fluorescent microscopy and subjected to Western blot against Esrra and valosin-containing protein (VCP) as an internal control.

	test	Female		Male	
Operant Responding					
-Lever presses	Unpaired T-test	1.3748	13.0	0.962525	12.0
		2.22957	13.0	2.51436	12.0
		2.2452	13.0	2.1023	12.0
		1.28572	13.0	0.0175247	12.0
		0.18036	13.0	0.301244	12.0
-Rewards	Unpaired T-test	1.44976	13.0	1.24696	12.0
		2.36379	13.0	2.53109	12.0
		2.70231	13.0	2.11789	12.0
		1.0	13.0	0.238781	12.0
		0.226099	13.0	0.133181	12.0
Wheel running	Two-way ANOVA with RM (time)	F (472, 3304) = 2.279		F (169, 676) = 0.9354	
Home-cage activity	Unpaired T-test	t=2.105 df=20		t=1.409 df=13	
Elevated-plus-maze					
-Distance traveled	Unpaired T-test	t=0.7747 df=20		t=0.2640 df=32	
-Time on open arm	Unpaired T-test	t=0.4176 df=20		t=0.5945 df=32	
Forced-swim test	Unpaired T-test	t=2.144 df=20		t=2.169 df=30	
Grooming	Unpaired T-test	t=3.036 df=20		t=1.258 df=13	
Marble burying	Unpaired T-test	t=2.144 df=17		t=0.2630 df=16	
Reversal learning	Unpaired T-test	t=2.876 df=9		t=1.215 df=8	
Three chamber test	Unpaired T-test	t=2.697 df=16		t=0.1437 df=17	
Social dominance	Chi-square	Chi-square= 21.160, df=1		Chi-square= 4.571, df=1	
Novel object	Unpaired T-test	t=0.1063 df=18		t=0.3246 df=16	

Table S1: Statistical analyses used for differences reported in Table 1.

Extended Experimental Procedures

Animals

Mice were housed in the University of Iowa vivarium in a temperature-controlled environment (lights on: 06:00-18:00) with ad lib access to water and chow (7913 NIH-31 modified open formula mouse sterilized diet, Harlan-Teklad, Madison, WI).

Behavioral Studies

Operant responding

Mice were trained to press a lever to obtain a 20 mg high fat diet (HFD) pellet reward as previously reported (Perello et al., 2010) in standard operant conditioning chambers (Model ENV307A, Med Associates Inc., St Albans, VT). Mice were rewarded for lever presses in the middle portal only; the side portals were monitored but inactive. The HFD pellets were custom prepared by Bio-serv (product# F06245, Frenchtown, NJ), and provided 4.5 kcal/g of metabolizable energy of which 45.4% of energy comes from fat, 35.0% comes from carbohydrate, and 21.0% comes from protein. The main components of these pellets were casein (233 g/Kg), palm oil (207 g/Kg), dextrates (197 g/Kg), sucrose (197 g/Kg), cellulose (58 g/Kg), and soybean oil (20 g/Kg). During the training period, mice were kept on a restricted feeding (RF) schedule and allowed access to regular chow 4 hours per day (1200-1600). For the training sessions, mice initially received the HFD pellet rewards under a fixed ratio (FR) schedule. In order to pass training, mice had to obtain 30 reinforcements within one-hour time for FR1 (once), FR3 (twice), and FR5 (three times) before moving on to the progressive ratio schedule. Following completion of the training period, the mice were then kept on the restricted feeding schedule and advanced to a progressive ratio schedule where they had to perform increasing numbers of lever presses to obtain the pellet according to the following series: 5, 10, 20, 30, 50, 70, 100, 130, etc. A relatively steep progressive ratio was chosen to ensure that only differences in motivation were measured and not satiation. After three days of stable responding was achieved, the mice were allowed free access to chow and effortful responding was assessed for HFD pellets under ad lib conditions for two additional days. Total number of lever presses and pellets earned within a 2-hour session were recorded and used for statistical analysis.

Wheel running

Mice were given free access to their regular home cages fitted with wireless vertical running wheels for five days (ENV-044V, Med Associates, St. Albans, VT). Activity data were collected with the USB Interface for Wireless Running Wheels (DIG-804) and the Wheel Manager (SOF-860) and analyzed with Wheel Analysis software (SOF-861).

Grooming and home cage activity

Home cage grooming was measured using the LABORAS system as previously reported (Xu et al., 2013). Briefly, LABORAS, A Comprehensive Grooming Assay: LABORAS (Metris, Netherlands) is a system that utilizes a carbon fiber plate to detect behavior-specific vibration patterns created by animals. Various behavioral parameters are determined by LABORAS software processing of the vibration pattern. Data was collected uninterrupted over a 24-hour period of time, enabling comprehensive quantification of basal grooming time, bouts and locomotor activity in the home cage environment throughout the light-dark cycle. Before data collection, test animals were acclimated in the test room for one week. Then, test animals were placed in a standard cage atop the carbon fiber platforms. Vibrations were recorded for 24 hours, and then the animals were removed. Vibration data was processed via LABORAS software.

Forced-swim-test

Mice were videotaped while in a 4 L Pyrex glass beaker containing 3 L of water at 24 ±1°C for 6 min. Data were analyzed using automated Anymaze Behavioral Tracking software (Stoelting) using the immobility detection feature set at sensitivity 66% and minimum immobility time 500ms.

Elevated-plus-maze

Anxiety-like behavior testing was video taped under red light using the Anymaze elevated plus maze (Stoelting). Time on open arm, number of entries to open arm, and distance traveled were calculated using the Behavioral Tracking software.

Marble burying

Marble burying was adapted by the method reported by Deacon (Deacon, 2006). Briefly, two inches of wood chip bedding were placed in the mouse's home cage. After an acclimation period of two hours, twelve marbles were placed evenly dispersed throughout the cage. After thirty minutes, marbles that were more than 2/3 of the way buried were scored.

Social dominance

Mice are trained (6 times over 2 days) to run through a clear 1.5" plastic tube into a dark box on the other end. In contrast to the original description of the task (Lindzey et al., 1961), mice were not food restricted or provided a food reward as motivation for running through the tube. At the end of the training mice were pair-matched knockout/wildtype. The two mice were placed on opposite ends of the tube, when both mice reached the center of the tube a barrier was removed. The victor was scored as the mouse that did not retreat out of the tube. If neither mouse retreated from the tube after two minutes the match was considered a draw.

Three chamber social interaction

Tests consisted of three stages all performed sequentially (Kaidanovich-Beilin et al., 2011). In the first stage, test mice are place in the center of a three chambered box divided by plexiglass walls and doors for a period of five minutes to acclimated to the apparatus. The second stage a novel mouse is placed inside a wire cage on one side of the apparatus. The plexiglass door is removed and the test mouse is now allowed to freely explore all three chambers for ten minutes. At the start of the third stage, the test mouse is placed back in the center and a new novel mouse is placed in a wire cage on the opposite side of the apparatus as the now familiar mouse. The test mouse is again allowed to explore freely for a period of ten minutes. All behavior was recorded and score by Any-Maze software.

Barnes maze

Barnes maze was performed as recently described (Yin et al., 2014). Briefly, mice were trained to locate an escape hole using four trials per day for four days. On the fifth day, the escape hole was removed and a probe trial assessed memory formation by measuring time spent around the previous location of the escape hole by video tracking (Any-maze, Stoelting). At the beginning of the following week, the location of the escape hole

was moved to an opposite quadrant and the mice again received four trials per day for four days. The next day, time spent at the location of the previous escape hole ('old target') and the new escape hole ('new target') was measured by video tracking.

Novel object

Test consisted of three days; habitation, familiarization and test sage as described previously (Antunes and Biala, 2012). In the habituation phase mice were allowed 10 minutes to freely explore an open field arena. In the familiarization phase two identical objects were placed in opposite and symmetrical corners and the mice were again allowed to freely explore and interact with the objects for 10 minutes. During the test stage one object was removed and replaced with an object of similar volume but different shape and color. The mice were again given ten minutes to explore and interact with the objects. All behavior was captured and scored by Any-Maze software.

Quantitative rtPCR

Tissue punches of cingulate and medial prefrontal cortex were rapidly collected from female wild-type and Esrra-null mice, flash frozen on liquid nitrogen and kept at -80° until processing. Purification of mRNA (Quiagen #74134) and preparation of cDNA (Invitrogen #11755-050) was performed as per the manufactures instructions. Quantitative PCR was performed using an Applied Biosystems Model 7900HT standardized to expression of 36B4 via the ΔΔCt method.

36B4: cactggtctaggacccgagaag, ggtgcctctgaagattttcg

Esrra: agcaagccccgatgga, gagaggcctgggatgctctt

Viral preparation

Adeno-associated virus cis-plasmid containing both CMV-GFP and U6 mouse Esrra shRNA or scramble expression cassettes were transfected along with helper plasmids into the packaging cells to produce the AAV2-GFP-U6-mEsrra-shRNA viral stocks. Two days after transfections, cell pellets are harvested, and viruses are released through 3x cycles of freeze/thaw. Viruses are purified through CsCl-gradient ultra-

centrifugation, followed by desalting. Viral preparations are titrated to a final titer of 1 X 10^12 genome copies/ml as determined through real-time PCR (Vector Biolabs).

An shRNA sequence targeting nucleotides 1203-1223 of mouse Esrra (NM_007953) was selected for use. Homology of this region to related family members estrogen related receptor beta (Esrrb) and estrogen related receptor gamma (Esrrg) is listed below.

Esrra: `att ctg act ctg tgc aca ttg`

Esrrb: `act cag att cga tgt aca ttg (14/21)`

Esrrg: `att tca gat tcc atg cat ata (5/21)`

Surgical methods

Stereotactic surgery was performed as previously described ((Xu et al., 2013)). Briefly, mice were anesthetized using ketamine/xylazine (100:10 mg/kg, i.p.) and placed on a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) for targeted delivery of virus to the following cortical areas as noted: mPFC (AP +1.8 mm, ML +0.8 mm, DV -2.8 mm, 10 degree angle), lateral orbitofrontal (AP +2.8 mm, ML +1.7 mm, DV -2.0 mm, 10 degree angle), and cingulate (AP +0.2 mm, ML +0.6 mm, DV -1.7 mm, 10 degree angle). Using hamilton microsyringe with small hub removable needle, 0.5 ul virus was delivered bilaterally to achieve maximum coverage of targeted regions. The needle was placed at targeted coordinates and inserted to the indicated depth, and virus was slowly infused by pressing the plunger with 0.05 µl/min rate. After 10 minutes of waiting to ensure a full penetration of AAVs into targeted area, the needle was removed and the incision was closed by wound clips. Mice were then kept on a warming pad until awake. Two weeks after surgery, body weight and behavior assessments were performed as described. Mice were then transcardially perfused with 4% paraformaldehyde in DEPC-PBS (pH 7.4). Brains were extracted and cryoprotected in 20% sucrose solution and then sliced at 30 um on a microtome and stored in cryoprotectant at -20C until processed. Slices were mounted and accurate placement was confirmed by location of the GFP signal by fluorescent microscopy (Zeiss Apotome.2, Thornwood, NY, USA). All mistargeted mice were excluded from analysis.

Western blot

In order to quantify knockdown of Esrra-expression, wild-type C57BL6/J female mice were infused with AAV2-GFP-U6-mEsrra-shRNA or AAV2-GFP-U6-scramble-shRNA as above into the mPFC and thalamus. One week later killed by cervical dislocation and ventral striatum was rapidly dissected out, immediately frozen in liquid nitrogen and stored at −80◦C until use. Frozen tissue was homogenized with ice-cold RIPA lysis buffer (#89901, Thermo Scientific) containing cocktails of protease inhibitor (04906845001, Roche Diagnostics) and protein phosphatase inhibitor cocktails (04906845001, Roche Diagnostics). Equal amounts of each sample (20 µg) were separated by 4–20% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gradient gels (BioRad, Hercules, CA, USA) and transferred to a polyvinylidene fluoride membrane by electroblotting. Primary antibodies were diluted as follows: anti-Esrra (04-1134; 1:1000, Millipore, Billerica, MA, USA), and anti-VCP antibody (#2648, 1:1000, Cell Signaling). Membranes were then incubated with HRP-conjugated secondary antibody (Jackson Immuno Research) and chemiluminescence signals were detected and analyzed by BioSpectrum 810 Imaging System (UVP, Upland, CA, USA).

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