Supporting Information for
Original article

Gestational dexamethasone exposure impacts hippocampal excitatory synaptic transmission and learning and memory function with transgenerational effects

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1. Supporting tables

Table S1 Oligonucleotide primers used in quantitative real-time PCR.

Genes	Forward primers	Reverse primers	Species
Sirt1	AGGGAACCTCTGCCTCATCTAC	TTGGCATACTCGCCACCTAAC	Rat
Cdk5	AAGGCACCTACGGAACTGTG	CCCTCATCGTCATCGTCCAG	Rat
Syn1	CTACATCCCACTTCTCATTCC	GGGAGGAAATCACCCTTTAG	Rat
Snap25	GGGCAATAATCAGGATGGAGTAG	TTGTTACCCTGCGGATGAAG	Rat
P35	ACCGTTGCCTCTCAGTTATC	CTCCTGACCACTCTCATTCTTC	Rat
Dapk1	GCCTATGCCTCAAGCTGTAA	GTCCATAAAGACCCTACCCAAG	Rat
Pkc	TATGCCTTCTCTCTCCCTAGAC	CCAGCCTCCCACTTAATTCTAC	Rat
Camk2a	CAGGAGTATGCTGCCAAGATTA	CCAGTGACCAGATCGAAGATAAG	Rat
Pka	CAAGTGGTTTGCCACAACTG	CGGATCTCTTCCTCCTCATAGT	Rat
Rsk	CTTCGTCCTGTACACCATCAG	GTTCCCAGACTCATCCACATAC	Rat
Gapdh	CAGCAAGGATACTGAGAGCAAG	GGATGGAATTGTGAGGGAGATG	Rat
sirt1	CAAGCACCGAACCGTTAT	CTGTGTCCGTTTCCTTATCC	Danio rerio
cdk5	CTCTACAGCACCCTTACTTTG	GTCCCTCTATCCTATGGTGAA	Danio rerio
syn1	CGTCCCTCTTCCTGACTAA	CCTGATCAATGACGCCTAAA	Danio rerio
snap25	CCACATGGCCCTTGATATG	AGGCAGAGCAGAGGTTTA	Danio rerio
bdnf	GAAGGACGTTGACCTGTATG	TCACCCACTGGCTAATACT	Danio rerio
β-Actin	CAGAACTGTTGCCACCTTA	GTTAGACAACTACCTCCCTTTC	Danio rerio
CDK5	GATGTCGATGACCAGTTGAAGA	TCGGATAGGGCTTATAGTCTGG	Human
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA	Human

Sirt1, Sirtuin 1; *Cdk5*, cyclin-dependent kinase 5, *Syn1*: synapsin I; *Snap25*, synaptosome-associated protein 25; *Dapk1*, death associated protein kinase 1; *Pkc*, protein kinase C; *Camk2a*, calcium/calmodulin dependent protein kinase II alpha; *Pka*, protein kinase A; *Rsk*, ribosomal protein S6 kinase; *Bdnf*, brain-derived neurotrophic factor; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *β*-actin, beta-actin.

Table S2 Statistics and analysis of clinical sample information.

Visit No.	Group	Sex	Maternal age (year)	GA at birth (week)	Age at CDK5 test (day)	Age at CDK5 test (week)	Corrected age (week)	Body weight (kg)	Pregnancy mode
1	Control	Male	29	38.86	0	0.00	–0.14	3.60	Caesarean birth
2	Control	Male	27	40.29	19	2.71	4.00	3.17	Caesarean birth
3	Control	Male	27	39.57	44	6.29	6.86	3.55	Caesarean birth
4	Control	Male	31	39.00	10	1.43	1.43	3.55	Caesarean birth
5	Control	Male	29	38.86	90	12.86	12.71	3.00	Caesarean birth
6	Control	Male	30	38.00	111	15.86	14.86	3.20	Caesarean birth
7	Control	Male	29	39.43	147	21.00	21.43	3.60	Caesarean birth

8	Control	Male	37	39.29	122	17.43	17.71	3.10	Caesarean birth
9	Control	Male	31	39.00	154	22.00	22.00	3.15	Caesarean birth
10	Control	Male	28	39.00	171	24.43	24.43	3.00	Caesarean birth
11	Control	Male	31	37.43	171	24.43	22.86	3.10	Caesarean birth
12	Control	Male	29	39.00	186	26.57	26.57	2.40	Caesarean birth
13	Control	Male	34	38.57	216	30.86	30.43	3.55	Caesarean birth
14	Control	Male	28	39.29	239	34.14	34.43	2.77	Caesarean birth
15	Control	Male	33	37.00	296	42.29	40.29	3.25	Caesarean birth
16	Control	Male	33	37.71	365	52.14	50.86	2.80	Caesarean birth
17	Control	Male	31	40.00	338	48.29	49.29	2.80	Caesarean birth
18	Control	Male	34	39.29	344	49.14	49.43	2.95	Caesarean birth
1	DEX	Male	38	37.86	181	25.86	24.71	3.30	Caesarean birth
2	DEX	Male	34	38.00	185	26.43	25.43	3.20	Caesarean birth
3	DEX	Male	28	38.57	197	28.14	27.71	3.10	Caesarean birth
4	DEX	Male	31	37.00	203	29.00	27.00	3.10	Caesarean birth
5	DEX	Male	19	35.00	82	11.71	7.71	2.40	Caesarean birth
6	DEX	Male	31	34.14	61	8.71	3.86	3.30	Caesarean birth
7	DEX	Male	30	35.86	111	15.86	12.71	2.50	Caesarean birth
8	DEX	Male	31	35.57	93	13.29	9.86	2.18	Caesarean birth
9	DEX	Male	31	35.57	106	15.14	11.71	2.24	Caesarean birth
10	DEX	Male	26	34.14	123	17.57	12.71	2.76	Caesarean birth
11	DEX	Male	31	31.00	181	25.86	17.86	2.35	Caesarean birth
12	DEX	Male	24	36.00	184	26.29	23.29	2.75	Caesarean birth
13	DEX	Male	32	36.00	184	26.29	23.29	2.1	Caesarean birth
14	DEX	Male	38	36.43	341	48.71	46.14	2.75	Caesarean birth

15	DEX	Male	37	36.00	231	33.00	30.00	2.9	Caesarean birth
16	DEX	Male	33	36.43	283	40.43	37.86	2.49	Caesarean birth
17	DEX	Male	36	38.86	365	52.14	52.00	3.35	Caesarean birth
18	DEX	Male	37	40.00	309	44.14	45.14	3.5	Caesarean birth
19	DEX	Male	27	39.86	365	52.14	53.00	3.4	Caesarean birth
20	DEX	Male	30	38.43	253	36.14	35.57	3.0	Caesarean birth
21	DEX	Male	27	38.57	308	44.00	43.57	3.7	Caesarean birth

2. Supporting figures

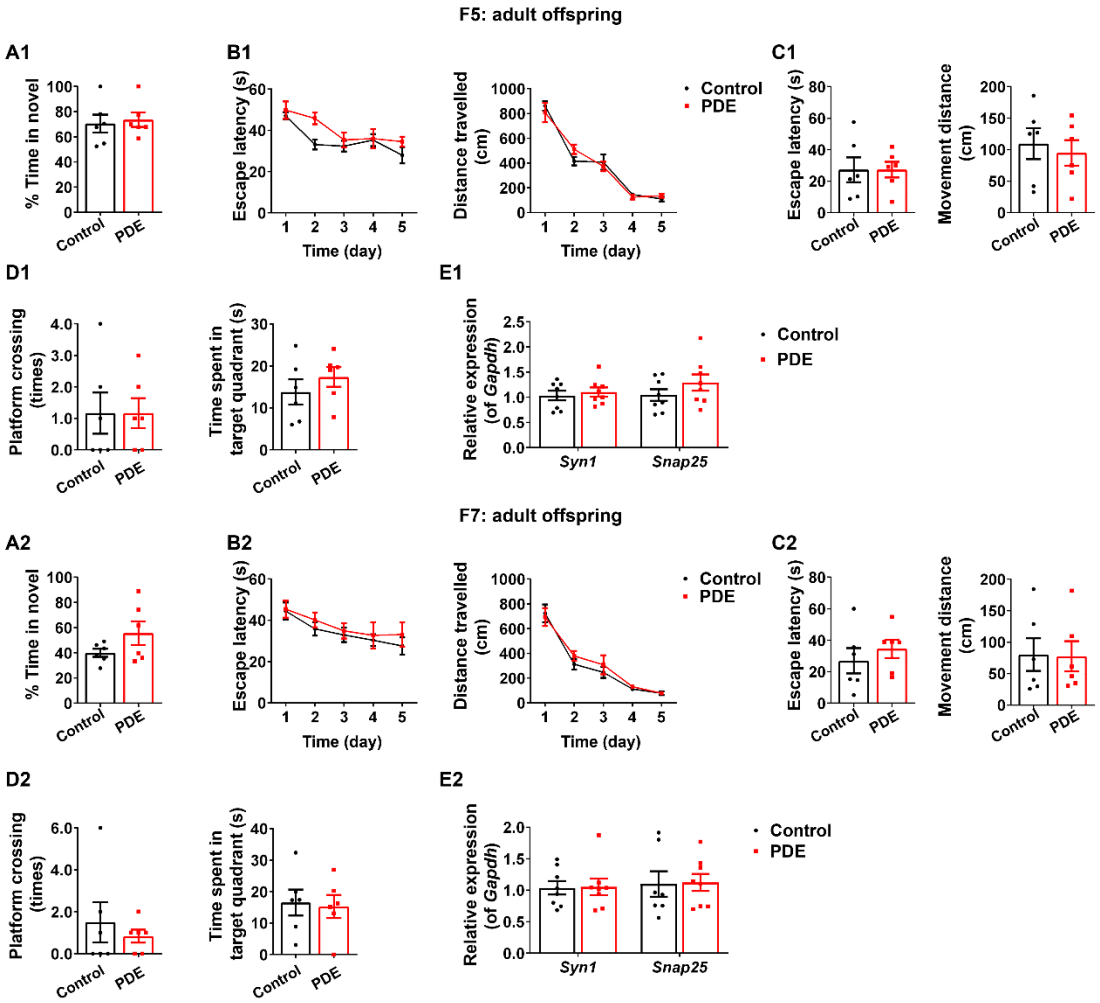

Figure S1 Behavior and hippocampal synaptic protein expression in male adult offspring rats of F5 and F7 generations of prenatal dexamethasone exposure (PDE). (A1, A2) The discrimination ratio in novel object recognition (NOR) experiments (*n* =

6). (B1, B2, C1, C2) The escape latencies and movement distances to find the hidden platform during five consecutive days of the training phase or the hidden platform trials in the Morris water maze (MWM) tests (n = 6). (D1, D2) The number of swim crossings over the previous platform location and time spent in the previous platform location during the probe trials in the MWM tests (n = 6). (E1, E2) mRNA expression of hippocampal synapsin I (*Syn1*) and synaptosome-associated protein 25 (*Snap25*) (n = 7–8). Mean ± SEM, using unpaired t test.

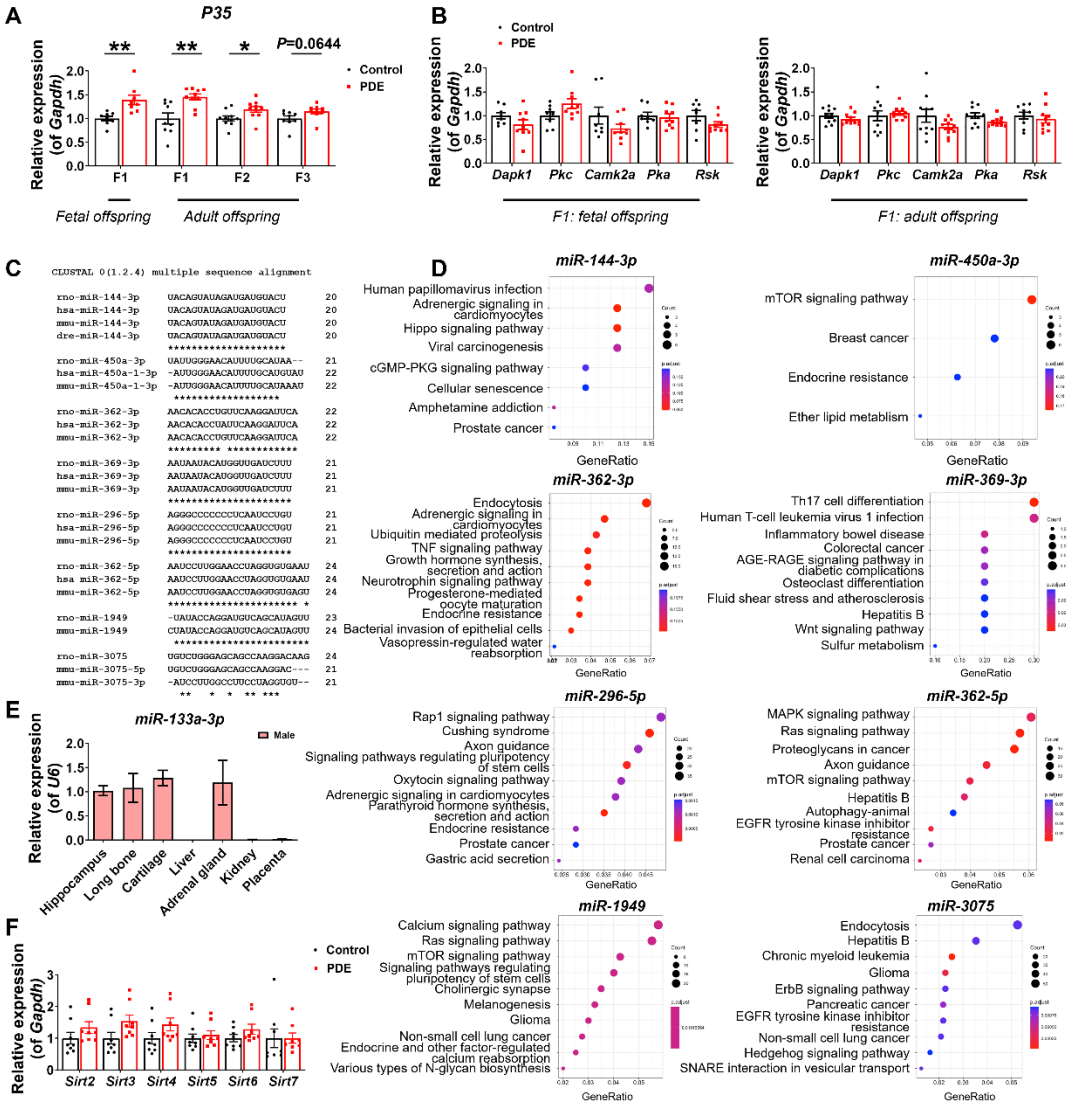

Figure S2 Conservation analysis and pathway enrichment of miRNAs, *miR-133a-3p* expression in various organs under physiological conditions and the expression of hippocampal *P35*, phosphorylated kinase and silent information regulator in fetal rats. (A) mRNA expression of *P35* in the hippocampus of male fetal and adult offspring

rats of F1, F2, and F3 generations (n = 8–10). (B) mRNA expression of death associated protein kinase 1 (*Dapk1*), protein kinase C (*Pkc*), calcium/calmodulin dependent protein kinase II alpha (*Camk2a*), protein kinase A (*Pka*), and ribosomal protein S6 kinase (*Rsk*) in the hippocampus of male fetal and adult offspring rats of F1 generation (n = 8–10). (C) Sequence conservation of *miR-144-3p*, *miR-450a-3p*, *miR-362-3p*, *miR-369-3p*, *miR-296-5p*, *miR-362-5p*, *miR-1949* and *miR-3075* in rat, human, mouse, and zebrafish. (D) Signaling pathway enrichment map of *miR-144-3p*, *miR-450a-3p*, *miR-362-3p*, *miR-369-3p*, *miR-296-5p*, *miR-362-5p*, *miR-1949* and *miR-3075* target genes. (E) Relative expression of *miR-133a-3p* in the hippocampus, long bones, cartilage, liver, adrenal gland, kidney, and placenta of male fetal rats under physiological conditions (n = 4–6). (F) mRNA expression of hippocampal silent information regulator 2 (*Sirt2*), *Sirt3*, *Sirt4*, *Sirt5*, *Sirt6*, and *Sirt7* in male fetal rats (n = 8). Mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control. Using unpaired t test.

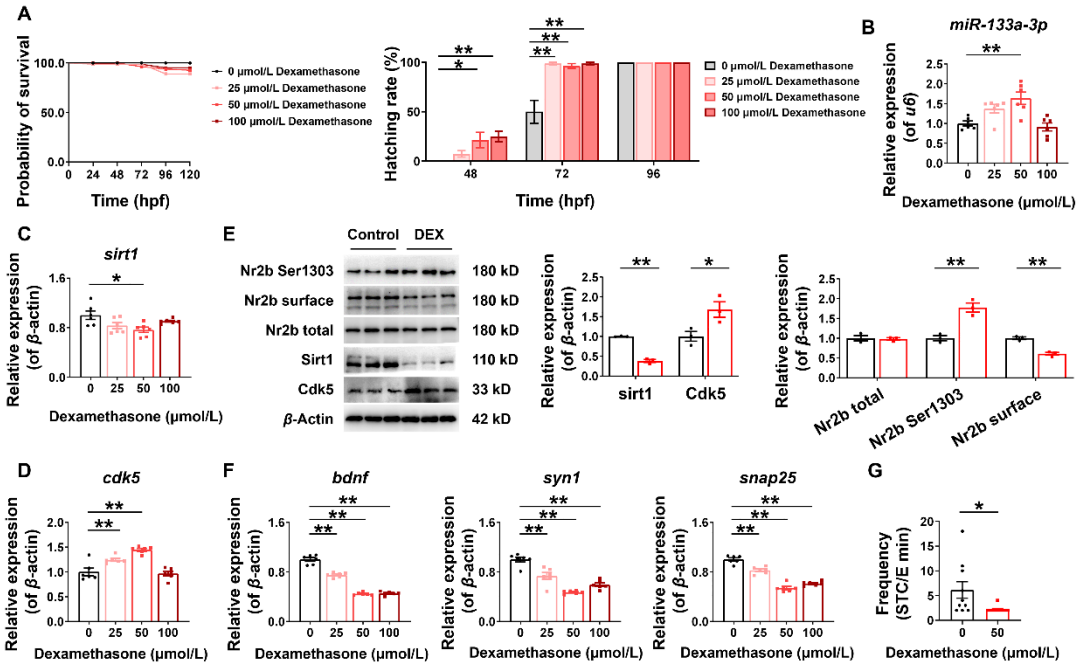

Figure S3 Effects of different concentrations of dexamethasone exposure on neural development in zebrafish embryos. The zebrafish embryos were treated with 0, 25, 50, and 100 μM dexamethasone (DEX) at 10 – 72 h post fertilization (hpf). (A) Probability of survival and hatching rate of zebrafish embryos at 24, 48, 72, 96, and

120 hpf (n = 12). (B–D, F) Expression of *miR-133a-3p* and mRNA expression of silent information regulator 1 (*sirt1*), cyclin-dependent Kinase 5 (*cdk5*), brain-derived neurotrophic factor (*bdnf*), synapsin I (*syn1*) and synaptosome-associated protein 25 (*snap25*) in zebrafish at 120 hpf (n = 6). (E) Protein levels of Sirt1, Cdk5 and N-methyl-D-aspartate receptor (Nr2b) in zebrafish at 120 hpf (n = 3). (G) Spontaneous movement of zebrafish embryos at 24 hpf (n = 10). Mean ± S.E.M., $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control. using unpaired t test compared means between the control and treated group. Multiple groups comparison was analyzed by one-way ANOVA test, followed by Dunnett-t-test.

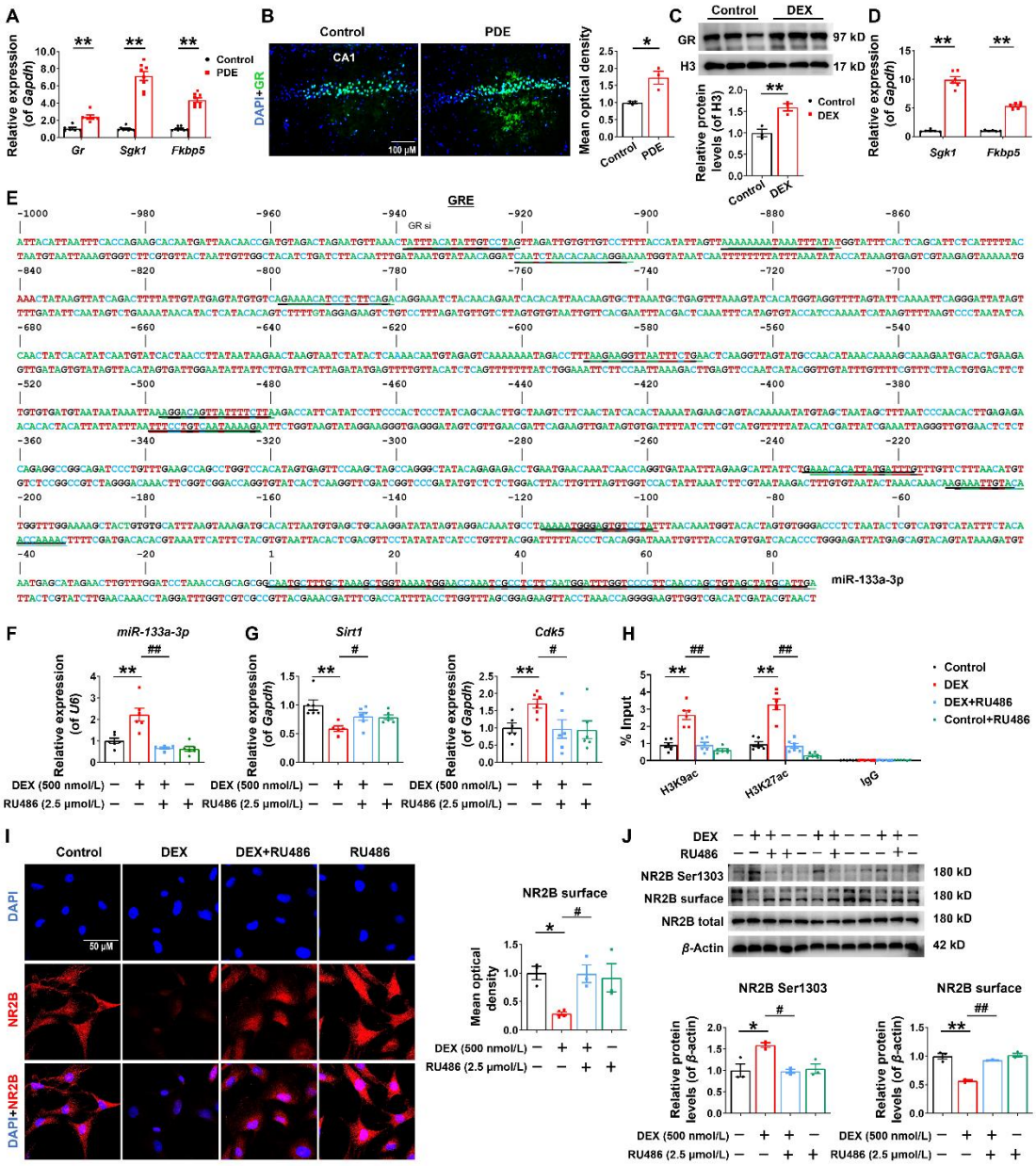

Figure S4 GR activation might mediate the changes of *miR-133a-3p*/SIRT1/CDK5/NR2B signaling axis in prenatal dexamethasone exposure (PDE) offspring. (A, D) mRNA expression of hippocampal glucocorticoid receptor (*Gr*), serum- and glucocorticoid-inducible kinase 1 (*Sgk1*) and FKBP prolyl isomerase 5 (*Fkbp5*) in male fetal rats, as well as in H19-7 cell line (n = 6–8). (B) Immunofluorescence co-labeling and quantification for GR (green) and 4′,6-diamidino-2-phenylindole (DAPI) (blue) (n = 3). Scale bar, 100 μm. (C) Protein levels of GR in H19-7 cell line treated with 500 nmol/L DEX (n = 3). (E) Glucocorticoid-response element (GRE) sites in the promoter region of *miR-133a-3p*

transcripts. (F–H) Relative expression of *miR-133a-3p*, mRNA expression of silent information regulator 1 (*Sirt1*) and cyclin-dependent kinase 5 (*Cdk5*), and the levels of acetyl-histone H3 at lys9 (H3K9ac) and H3K27ac in the promoter of *Cdk5* in the H19-7 cell line treated with 500 nmol/L DEX and (or) 2.5 μmol/L RU486 (n = 6). (I) Immunofluorescence co-labeling and quantification for *N*-methyl-D-aspartate receptor (NR2B) (red) and DAPI (blue) (n = 3–4). Scale bar, 50 μm. (J) Protein levels of NR2B in the H19-7 cell line treated with 500 nmol/L DEX and (or) 2.5 μmol/L RU486 (n = 3). Mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* Control, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* DEX. Using unpaired t test compared means between the control and treated groups. Multiple groups comparison was analyzed by one-way ANOVA test, followed by Dunnett-*t*-test.