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Supplementary Information for

Key role of soluble epoxide hydrolase in the neurodevelopmental disorders of offspring after maternal immune activation

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Supplementary Information Text

SI Text in Discussion

Previously, we reported cognitive deficits of juvenile offspring from poly(I:C)-treated mice (1-4). Since cognitive impairment is seen in adolescent and young adult with a high risk for psychosis (5,6), it seems that the juvenile offspring after MIA may be at the prodromal stage for psychosis (1-4). Furthermore, we found reduction of PV-IR in the mPFC of juvenile offspring after MIA, consistent with the previous study (2,4). It is well known that reduction of PV-IR in the PFC may contribute to the pathophysiology of schizophrenia (7). Furthermore, it is suggested that cognitive impairment may be due at least in part to lower expression of GAD_{67} in the PFC from schizophrenia (8). Therefore, it is likely that reduction of PV-IR and GAD_{67}-IR in the PrL of mPFC may play a critical role in the cognitive deficits of offspring after MIA. Interestingly, we found that TPPU in drinking water during P28 ï P56 (similar to juvenile and adolescent stages in human) in offspring after neonatal poly(I:C) exposure could prevent cognitive deficits and reduction of PV- and GAD_{67}-IR at adulthood after MIA. In this study, we did not find change in the body weight of offspring after MIA although fatty acids metabolites are known to be influential on brain development (9,10). Previously, we reported that AS2586114 (a sEH inhibitor) showed antipsychotic-like effects in phencyclidine-treated model of schizophrenia (11). Taken all together, it is likely that early intervention with sEH inhibitor (e.g., TPPU) during juvenile and adolescent stages might have prophylactic and therapeutic effects on abnormal behaviors in neurodevelopmental disorders, such as schizophrenia and ASD. Importantly, early intervention with sEH inhibitor (e.g., TPPU) in subjects with high-risk psychosis may prevent the transition to psychosis in young adulthood.

Pregnancy is a critical period of neurodevelopment during which pregnant females are also more vulnerable to stressful events. Epidemiological data demonstrated that maternal prenatal exposure to famine and its associated risk of the development of neurodevelopmental disorders in adult offspring are based on the Dutch famine of 1944ï 1945 or the Chinese famine of 1959ï 1960 (12-14). In this study, we found that TPPU in drinking water in poly(I:C)-treated pregnant mice from pregnancy (E12) to weaning (P21) could prevent the onset of cognitive and social interaction deficits in juvenile offspring after MIA. Given the role of neurodevelopmental stage in psychiatric disorders (15,16), supplementation with a sEH inhibitor in pregnant mothers at ultra-high risk for psychosis may play an important role in preventing the onset of psychosis in offspring.

When we integrate this work with our earlier published studies on depression (17), Parkinsonôs disease (18), and related disorders, some trends emerge. In each case the sEH protein and its gene expression are increased in the brain regions generally associated with these diseases, suggesting that these in turn are associated with the expected reduction in EpFAs and increase in the corresponding diols. Whether these effects are a cause or a result of the disease is not established, but it does suggest that the sEH protein

and its gene expression as well as the resulting metabolites could be biomarkers of disease progression or therapy. Another issue is that markers of ER stress increase in these same brain regions thus associating ER stress with the disease state and the reduction of ER stress as a possible underlying mechanism of action of inhibition of the sEH and increase in EpFAs. These associations have been noted previously in the peripheral tissues (19).

SI Materials and Methods

Animals and animal care. Pregnant ddY mice (embryo at the 5th day (E5), 9-10 weeks old) were purchased from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan). Pregnant mice in each clear polycarbonate cage (22.5 × 33.8 × 14.0 cm) one by one were housed under controlled temperatures and 12 hour light/dark cycles (lights on between 07:00ï 19:00 h), with ad libitum food (CE-2; CLEA Japan, Inc., Tokyo, Japan) and water. The protocol was approved by the Chiba University Institutional Animal Care and Use Committee. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA.

Administration of poly(I:C) into pregnant mice. The schedule of poly(I:C) treatment was performed as reported previously (1-4,20). The pregnant mice were injected intraperitoneally (i.p.) for six consecutive days from E12 to E17 with poly(I:C) (5.0 mg/kg/day, Catalog number: P0913-50MG, Lot#: 016M415V, Sigma-Aldrich Co. Ltd., St. Louis, MO, USA) dissolved in physiological saline, or an equivalent volume (5 ml/kg) of saline. The male offspring were separated from their mothers at weaning (P21), and mice were caged each three - five in the groups in clear polycarbonate cage (22.5 × 33.8 × 14.0 cm). Mice were housed under controlled temperatures and 12 hour light/dark cycles (lights on between 07:00ï 19:00 h), with ad libitum food and water.

Collection of brain samples and oxylipin profiling. The pregnant mice were injected i.p. with poly(I:C) (5.0 mg/kg/day for 6 days from E12 to E17) or saline, as described above. The male offspring were separated from their mothers at weaning (P21). On P28, mice were deeply anesthetized with isoflurane and brains were removed from the skulls. For Western blot analysis, brain regions such as prefrontal cortex (PFC), striatum, CA1, CA3 and dentate gyrus (DG) of the hippocampus were dissected from brain on ice using a Leica microscope S9E (Leica Microsystems, Tokyo, Japan). The samples were stored at -80 before assay. For oxylipin analysis, PFC, hippocampus, and cerebellum were dissected from brain on ice, and the samples were stored at -80 before assay. Measurement of eicosanoids was performed at UC Davis using the previous method (21).

Western blot analysis. Western blot analysis was performed as reported previously (17,18). Basically, the tissue samples were homogenized in Laemmli lysis buffer. 50 µg of protein were measured using the DC protein assay kit (Bio-Rad), and incubated for 5 min at 95 , with an equal volume of 125 mM Tris-HCl, pH6.8, 20% glycerol, 0.1% bromophenol blue, 10% ß-mercaptoethanol, 4% sodium dodecyl sulfate, and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis, using 7.5% or AnyKD mini-

gels (Mini-PROTEAN® TGXÊ Precast Gel; Bio-Rad, CA, USA). Proteins were transferred onto polyvinylidenedifluoride (PVDF) membranes using a Trans Blot Mini Cell (Bio-Rad). For immunodetection, the blots were blocked with 2% BSA in TBST (TBS + 0.1% Tween-20) for 1 h at room temperature (RT), and kept with primary antibodies overnight at 4°C. The primary antibodies were used (**SI Appendix, Table S6**). The next day, blots were washed three times in TBST and incubated with horseradish peroxidase conjugated anti-rabbit or anti-mouse antibody 1 hour, at RT. After final three washes with TBST, bands were detected using enhanced chemiluminescence (ECL) prime Western Blotting Detection system (GE Healthcare Bioscience). Images were captured with a ChemDoc imaging system (Bio-Rad), and the immunoreactive bands were analysis by Image Lab software.

Gene expression analysis by quantitative real-time PCR. At juvenile (P28) stage, mice were sacrificed, and their brains were removed for measurement of gene expression of *Ephx2* mRNA. Brain regions such as PFC, striatum, CA1, CA3 and DG of the hippocampus were dissected from brain on ice using a Leica microscope S9E (Leica Microsystems, Tokyo, Japan). A quantitative RT-PCR system (Step One Plus, Thermo Fisher Scientific, Yokohama, Japan) was used to measure mRNAs. The specific mRNA transcripts were quantified by TaqManGene Expression assays (Thermo Fisher Scientific, Yokohama, Japan). Expression levels of *Ephx2* (Mm01313813_m1) was measured in brain tissue. Total RNA was extracted by use of an RNeasy Mini Kit (Qiagen, Hilden, Germany). The purity of total RNA was assessed by Biophotometer plus (Eppendorf, Hamburg, Germany). The RNA samples were used in the first strand cDNA synthesis with High Capacity cDNA Reverse Transcription Kit (#4368813 Thermo Fisher Scientific, Yokohama, Japan). All samples were tested in triplicate and average values were used for quantification. The average values were normalized to Vic-labeled *Actb* mRNA (Cat#4352341E: pre-developed TaqMan Assay Reagents, Thermo Fisher Scientific, Yokohama, Japan).

The study using iPSC was approved by the Ethics Committees of RIKEN, and conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all subjects. Neurospheres from iPSC with two schizophrenia patients (two cell lines from each subject and total of four cell lines) with 22q11.2 deletion and three healthy control subjects (total of four cell lines)(**SI Appendix, Table S4**) were used. Total RNA from neurospheres was extracted using the RNeasy Mini Kit (Qiagen), as reported previously (22).

Postmortem brain tissues from ASD and age-matched control samples were obtained from the National Institute of Child Health and Human Development (NICHD) Brain and Tissue Bank, University of Maryland School of Medicine (http://medschool.umaryland.edu/btbank/), for gene expression analysis (**SI Appendix, Table S5**)(23). Frozen tissue samples from BA09 (ASD; n =10, control; n =10), BA21 (ASD; n =14, control; n =14), and BA40 (ASD; n =14, control; n =13) were

used. Total RNA from brain tissues was extracted using a miRNAeasy Mini kit (QIAGEN GmbH, Hilden, Germany) and single stranded cDNA was synthesized using a SuperScript VILO Master Mix (Life Technologies Co., Carlsbad, CA, USA), according to the manufacturersô instructions.

Real-time quantitative RT-PCR analysis was conducted using standard procedures, in a QuantStudio12K Flex Real-Time PCR System (Life Technologies Co., Carlsbad, CA, USA). TaqMan probes and primers for *EPHX2* and *GAPDH* (internal control) were chosen from TaqMan Gene Expression Assays (ABI, Foster City, CA, USA). All real-time quantitative RT-PCR reactions were performed in triplicate, based on the standard curve method. To check for expressional changes between cases and controls, expression of *EPHX2* [Hs00932316_m1], and *GAPDH* [Hs02758991_g1] were measured using TaqMan Gene Expression Assays in a QuantStudio12K Flex Real-Time PCR System (Life Technologies Co., Carlsbad, CA, USA).

Experiments of TPPU in drinking water. For TPPU in drinking water, TPPU was dissolved in polyethylene glycol 400 (PEG 400: Tokyo Chemical Industry Co., Ltd, Tokyo, Japan) and added to warm water with stirring to yield 15 mg/L TPPU with 1% PEG 400. For experiment of TPPU (synthesized at UC Davis, CA) in drinking water in juvenile and adolescent stages, TPPU (15 mg/L) or water in drinking water was given in the male offspring from P28 to P56. Subsequently, normal water was given to all groups for 4 weeks (P57ï P84). Behavioral tests of adult offspring were performed during adulthood (P70ï P84) after prenatal poly(I:C) injections (**Fig. 3A**).

For experiment of TPPU in drinking water in pregnant stages, TPPU (15 mg/L) or water in drinking water was given in the pregnant mice from E12 to P21. Subsequently, normal water was given to all groups for 2 weeks (P21ï P42). Behavioral tests of offspring were performed during juvenile stage (P28ï P42) after prenatal poly(I:C) injections (**Fig. 4A**).

Behavioral analysis. Locomotion and the novel object recognition test (NORT) were performed as reported previously (1-4,24,25). Locomotor Activity**:** Both horizontal and rearing activity were monitored by an infrared ray passive sensor system (SCANET-SV10, Melquest Ltd., Toyama, Japan), and activity was integrated every minute. Individual mice were placed in activity chambers and allowed 1 hour of free exploration as spontaneous activity.

Novel Object Recognition Test (NORT): Mice were habituated for 10 minutes in the test box for 3 straight days. At 4th day, two objects (differing in shape and color but of similar size) were placed in the box 35.5 cm apart (symmetrically), and each animal was allowed to explore in the box for 5 minutes. The animals were considered to be exploring the object when the head of the animal was both facing and within 2.54 cm of the object or when any part of the body, except for the tail was touching the object. The time that mice spent exploring each object was recorded. After training, mice were immediately returned to their home cages, and the box and objects were cleaned with 75% ethanol, to

avoid any possible instinctive odorant cues. Retention tests were carried out at one-day intervals, following the respective training. During the retention test, each mouse was reintroduced into their original test box, and one of the training objects was replaced by a novel object. The mice were then allowed to explore freely for 5 minutes, and the time spent exploring each object was recorded. Throughout the experiments, the objects were counter-balanced, in terms of their physical complexity and emotional neutrality. A preference index, that is, the ratio of time spent exploring either of the two objects (training session) or the novel object (retention test session) over the total time spent exploring both objects, was used.

Three-chamber Social Interaction Test: The three-chamber social interaction test was performed to investigate sociability and preference for social novelty in mice. The apparatus consisted of a rectangular, three-chambered box and a lid with a video camera (BrainScience Idea, Co., Ltd, Osaka, Japan). Each chamber (22.5 cm × 41 cm × 62 cm) was divided by a clear plastic wall with a small square opening (5 cm × 8 cm). First, each subject mouse was placed in the box and allowed to explore for 10 min to habituate the environment. During the session, an empty wire cage (10 cm in diameter, 17.5 cm in height, with vertical bars 0.3 cm apart) was located in the corner of each chamber. Next, an unfamiliar ddY male mouse (stranger 1) that had no prior contact with the subject mouse was put into a wire cage that was placed into one of the side chambers. To assess sociability, the subject mouse was allowed to explore the box for an additional 10-min session. Finally, to evaluate social preference for a new stranger, a second stranger male mouse (stranger 2) was placed into the wire cage that had been empty during the first 10-min session (social novelty preference test). Thus, the subject mouse had a choice between the first, non-familiar mouse (stranger 1) and the novel unfamiliar mouse (stranger 2). The time spent in each chamber and the time spent around each cage was recorded on video.

Immunohistochemistry. Immunohistochemistry of PV was performed as reported previously (2,4,25,26). Mice were anesthetized with 5% isoflurane and sodium pentobarbital (50 mg/kg), and perfused transcardially with 10 mL of physiological saline, followed by 40 mL of ice-cold 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Brains were removed from the skulls and post fixed overnight at 4°C in the same fixative. For the immunohistochemical analysis, 50 εm-thick serial, coronal sections of brain tissue were cut in ice-cold 0.01M phosphate buffered saline (pH 7.5) using a vibrating blade microtome (VT1000s, Leica Microsystems, Tokyo, Japan). Free-floating sections were treated with 0.3% H_2O_2 in 50 mM Tris-HCl saline (TBS) for 30 min and were blocked in TBS containing 0.2% Triton X-100 (TBST) and 1.5% normal serum for 1 h at room temperature. The samples were then incubated for 24 h at 4°C with mouse polyclonal anti-parvalbumin (PV) antibody or mouse anti-GAD_{67} (**SI Appendix, Table S7**). The sections were washed three times in TBS and then processed using the avidin-biotin-peroxidase method (Vectastain Elite ABC, Vector Laboratories, Inc., Burlingame,

CA, USA). Sections were incubated for 3 min in a solution of 0.25 mg/mL DAB containing 0.01% H_2O_2. Then, sections were mounted on gelatinized slides, dehydrated, cleared, and cover slipped under Permount®(Fisher Scientific, Fair Lawn, NJ, USA). The sections were imaged, and the staining intensity of PV (or GAD_{67}) immunoreactivity in the inflalimbic (IL) and prelimbic (PrL) regions of mPFC was analyzed using a light micro-scope equipped with a CCD camera (Olympus IX70, Tokyo, Japan) and the SCION IMAGE software package. Images of sections within mPFC region were captured using a 100× objective with a Keyence BZ-X710 microscope (Keyence Corporation, Osaka, Japan).

Statistical analysis. Analysis of the data was performed using GraphPad Prism (La Jolla, CA). Comparisons between two groups were performed using Student t-test. Comparisons among four groups were performed using the two-way analysis of variance (ANOVA), followed by Fisherôs LSD test. The P-values of less than 0.05 were considered statistically significant.

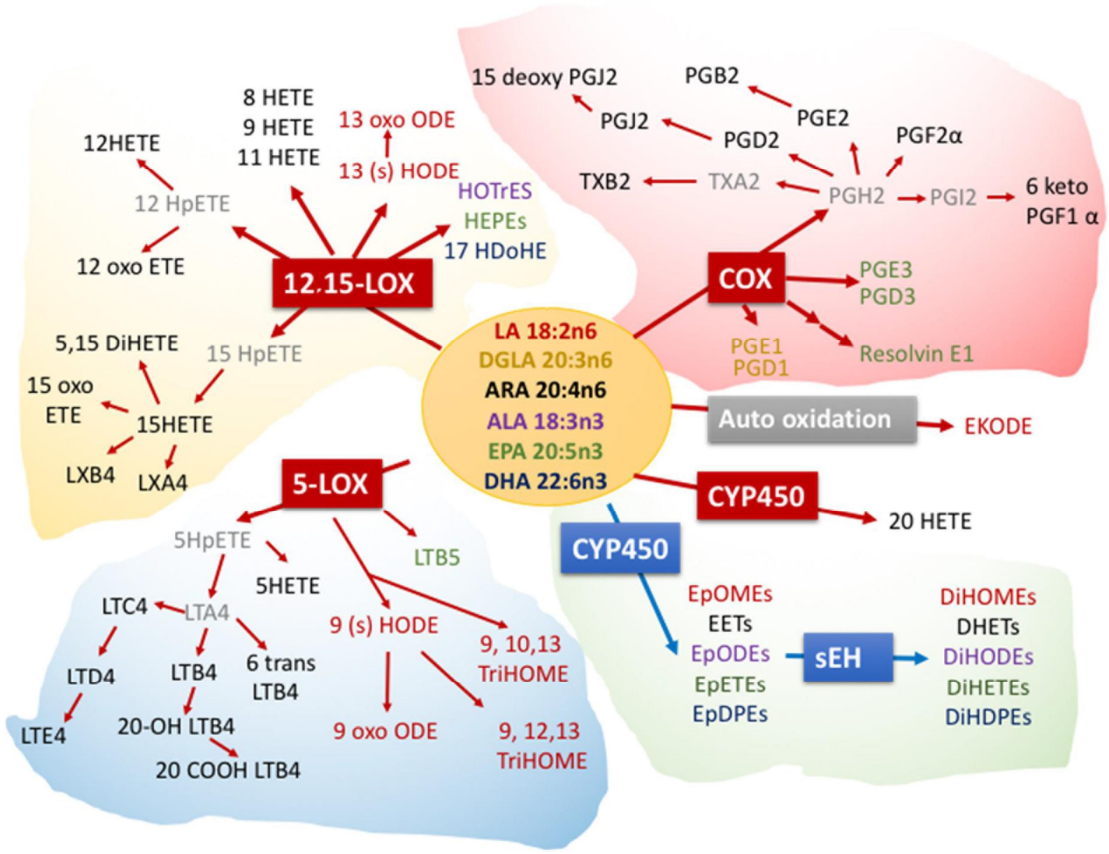

Figure S1. Eicosanoids measured in the brain regions from male juvenile offspring after MIA

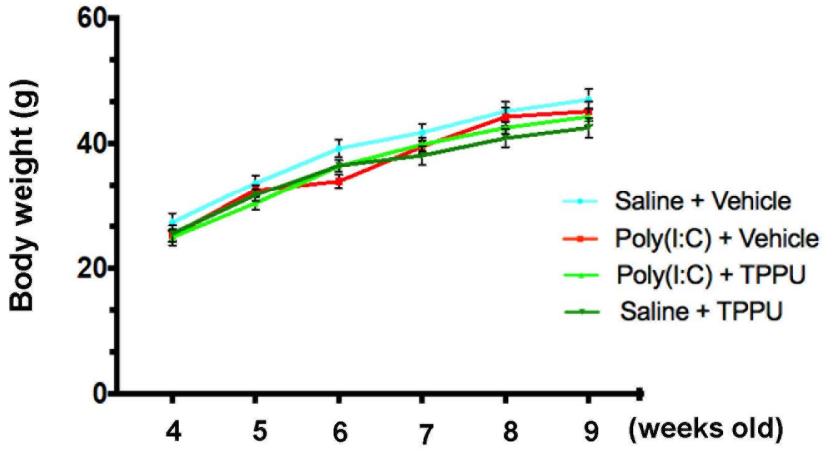

Figure S2. Time course of body weight of offspring mice after MIA. The four groups are from Figure 3. There were no changes among the four groups.

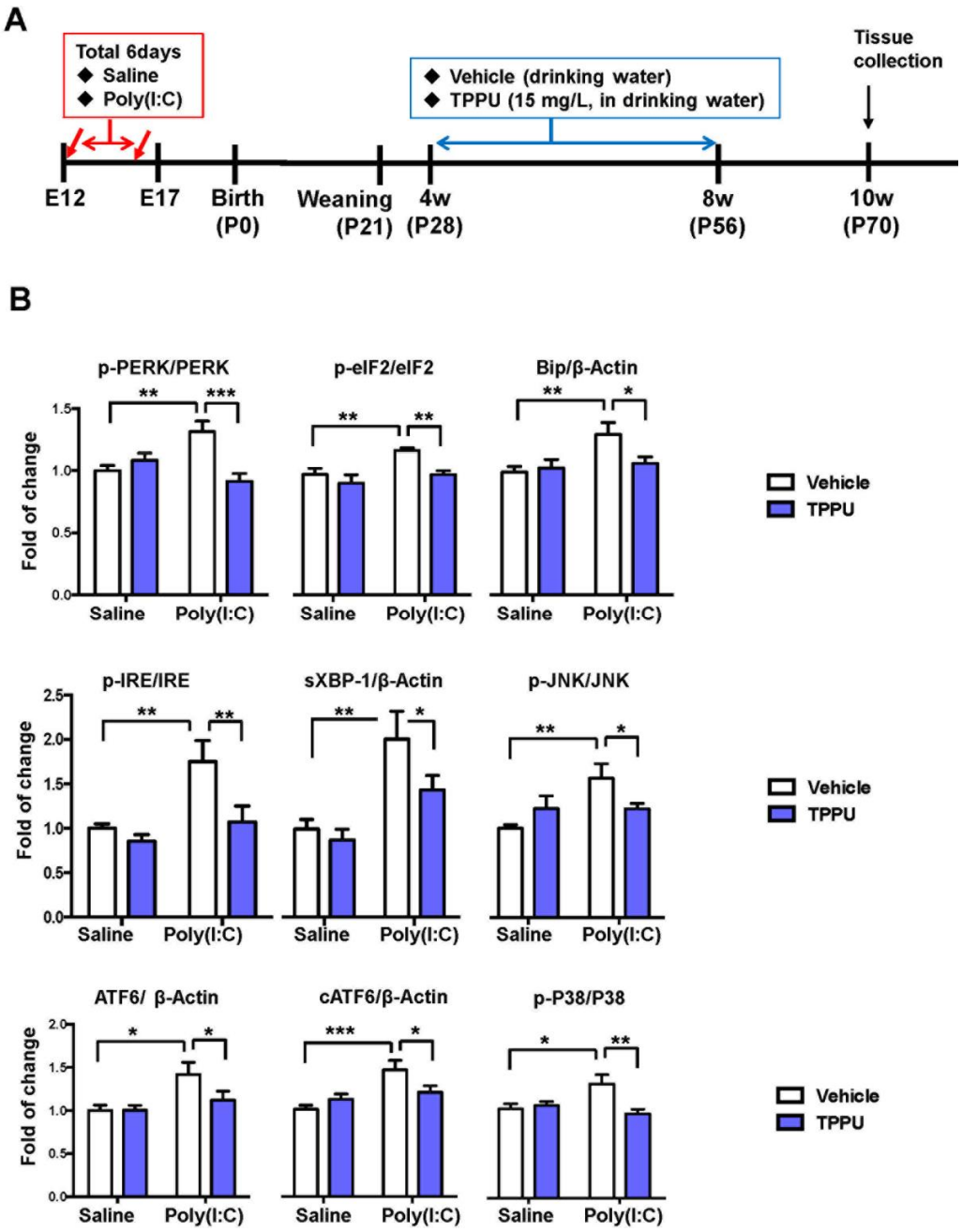

Figure S3. Effects of TPPU on elevated ER stress in the adult offspring after MIA. (A): Schedule of treatment and brain collection. Saline or poly(I:C)(5 mg/kg/day for 6 days) was administered i.p. into pregnant mice. Vehicle or TPPU (15 mg/L) in drinking water was treated into juvenile offspring from P28 to P56. Subsequently, all mice received normal water. PFC from brain was collected at P72. (B): We examined the effects of TPPU in drinking water (from P28 to P56) on markers of ER stress in the PFC from adult offspring after postnatal poly(I:C) exposure. Data are shown as mean ± S.E.M. (n = 5-7). *P < 0.05, **P < 0.01, ***P < 0.001 compared to poly(I:C) + vehicle group. Detailed statistical analysis data are in SI Appendix, Table S6.

Table S1. Levels of eicosanoid metabolites in the PFC from juvenile offspring

Compounds	Control			Poly(I:C)			P value
6-keto-PGF1α	**29.700**	**±**	**2.627**	**22.088**	**±**	**1.785**	**0.032**
Thromboxane 2	33.788	±	3.503	43.922	±	3.349	0.053
9,12,13-TriHOME	51.554	±	7.229	39.728	±	6.854	0.254
9,10,13-TriHOME	29.975	±	4.266	22.676	±	3.814	0.223
PGF2a	82.064	±	5.492	86.798	±	6.454	0.581
PGE2	23.754	±	6.369	16.359	±	1.175	0.294
PGD2	123.52	±	9.281	147.79	±	9.748	0.089
15,16-DiHODE	1.868	±	0.197	2.785	±	0.414	0.054
12,13-DiHOME	8.281	±	2.107	4.640	±	0.477	0.127
9,10-DiHOME	1.057	±	0.285	1.069	±	0.188	0.972
19,20-DiHDPE	**2.910**	**±**	**0.273**	**4.319**	**±**	**0.331**	**0.004**
EKODE	9.386	±	1.782	6.552	±	1.725	0.271
13-HODE	44.173	±	11.783	31.020	±	5.237	0.340
9-HODE	28.921	±	5.534	24.253	±	3.782	0.505
15-HETE	67.926	±	4.005	68.997	±	4.672	0.863
11-HETE	67.003	±	3.854	72.292	±	5.677	0.443
9-oxo-ODE	20.059	±	3.553	15.032	±	2.690	0.283
12-HETE	44.580	±	10.506	28.597	±	2.727	0.179
12,13-EpOME	4.671	±	1.039	2.934	±	0.435	0.157
14,15-EpETrE	61.25	±	14.305	30.74	±	5.605	0.074
9,10-EpOME	4.397	±	1.079	2.695	±	0.424	0.177
10,11-EpDPE	**6.274**	**±**	**0.763**	**3.698**	**±**	**0.292**	**0.008**
11,12-EpETrE	**46.674**	**±**	**8.181**	**26.639**	**±**	**3.530**	**0.045**
8,9-EpETrE	**12.390**	**±**	**2.101**	**6.725**	**±**	**0.892**	**0.029**
5,6-EpETrE	**397.82**	**±**	**61.53**	**222.07**	**±**	**22.38**	**0.020**

The value (pmol/g tissue) are the mean ±SEM (n = 10).
The bold is statistically significant.

Table S2. Levels of eicosanoid metabolites in the hippocampus from juvenile offspring

Compounds	Control			Poly(I:C)			P value
6-keto-PGF1Ŭ	61.959	±	7.219	47.853	±	3.589	0.109
Thromboxane B2	**48.930**	**±**	**4.565**	**65.491**	**±**	**5.906**	**0.038**
9,12,13-TriHOME	56.011	±	6.305	54.137	±	3.954	0.809
9,10,13-TriHOME	31.976	±	3.810	31.142	±	2.301	0.858
PGF2a	155.85	±	9.119	153.113	±	10.00	0.842
PGE2	21.946	±	1.541	20.186	±	1.410	0.415
PGD2	127.00	±	5.897	119.25	±	8.999	0.472
15,16-DiHODE	1.845	±	0.176	2.295	±	0.364	0.266
12,13-DiHOME	3.970	±	0.218	4.478	±	0.528	0.476
9,10-DiHOME	0.557	±	0.089	0.814	±	0.116	0.092
19,20-DiHDPE	**2.919**	**±**	**0.227**	**4.228**	**±**	**0.321**	**0.004**
EKODE	**10.97**	**±**	**1.241**	**14.86**	**±**	**0.949**	**0.026**
13-HODE	40.789	±	2.783	41.916	±	2.454	0.767
9-HODE	31.405	±	1.996	34.887	±	1.969	0.233
15-HETE	95.073	±	6.050	100.514	±	6.210	0.539
11-HETE	95.154	±	5.569	106.263	±	6.366	0.218
9-oxo-ODE	**16.67**	**±**	**1.280**	**24.50**	**±**	**1.957**	**0.003**
12-HETE	89.02	±	21.57	83.22	±	12.09	0.823
12,13-EpOME	4.829	±	0.544	4.641	±	0.393	0.788
14,15-EpETrE	43.926	±	5.373	45.020	±	3.734	0.872
9,10-EpOME	3.228	±	0.303	3.933	±	0.304	0.120
10,11-EpDPE	5.297	±	0.398	6.103	±	0.347	0.149
11,12-EpETrE	47.81	±	4.042	42.05	±	3.911	0.323
8,9-EpETrE	13.32	±	1.066	10.53	±	0.809	0.056
5,6-EpETrE	388.14	±	25.47	333.09	±	30.68	0.182

The value (pmol/g tissue) are the mean ±SEM (n = 10).
The bold is statistically significant.

Table S3. Levels of eicosanoid metabolites in the cerebellum from juvenile offspring

Compounds	Control			Poly(I:C)			P value
6-keto-PGF1a	30.060	±	3.729	29.69	±	1.379	0.928
TXB2	12.13	±	1.042	14.62	±	0.876	0.087
9,12,13-TriHOME	49.40	±	5.976	37.62	±	5.925	0.181
9,10,13-TriHOME	28.21	±	3.566	20.84	±	3.099	0.138
PGF2a	59.74	±	7.023	46.32	±	3.830	0.113
PGE2	9.092	±	1.281	6.969	±	0.684	0.163
PGD2	22.57	±	2.637	21.60	±	1.774	0.764
15,16-DiHODE	2.293	±	0.380	2.772	±	0.423	0.411
12,13-DiHOME	6.207	±	0.768	5.406	±	0.682	0.542
9,10-DiHOME	1.055	±	0.123	1.283	±	0.279	0.466
19,20-DiHDPE	6.984	±	0.780	9.767	±	0.858	0.029
EKODE	8.319	±	1.046	7.801	±	1.325	0.763
13-HODE	34.35	±	3.711	30.47	±	4.048	0.490
9-HODE	26.02	±	2.772	23.33	±	2.571	0.487
15-HETE	38.05	±	5.689	36.59	±	3.256	0.827
11-HETE	33.32	±	4.798	34.19	±	3.215	0.881
9-oxo-ODE	18.31	±	1.684	16.56	±	2.802	0.599
12-HETE	59.43	±	18.09	75.46	±	28.38	0.640
12,13-EpOME	3.087	±	0.296	2.989	±	0.423	0.852
14,15-EpETrE	24.50	±	4.194	26.63	±	3.595	0.704
9,10-EpOME	3.251	±	0.405	3.076	±	0.662	0.824
10,11-EpDPE	6.726	±	1.295	6.061	±	0.717	0.659
11,12-EpETrE	24.260	±	4.597	21.10	±	1.869	0.533
8,9-EpETrE	6.760	±	1.641	5.453	±	0.582	0.464
5,6-EpETrE	186.72	±	29.47	174.37	±	11.71	0.702

The value (pmol/g tissue) are the mean ±SEM (n = 10).

Table S4. Summary of the iPSC lines from healthy controls and schizophrenia patients with a 22q11.2 deletion

Sample ID	iPSC Line ID	Line ID	Diagnosis	Gender	Age	Reference
C1-1	201B7	NS1, NS2, NS3	Control	F	36	Takahashi et al. (27)
C1-2	YA9	NS1, NS2, NS3				Imaizumi et al. (28)
C3	WD39	NS1, NS2, NS3	Control	F	17	Imaizumi et al. (28)
C4	KA23	NS1, NS2, NS3	Control	M	40	Matsumoto et al. (29)
S1-1	SA001_1D2	NS1, NS2, NS3	Schizophrenia	F	37	Toyoshima et al. (22)
S1-2	SA001_3B1	NS1, NS2, NS3				
S2-1	K0001_19	NS1, NS2, NS3	Schizophrenia	F	30	Toyoshima et al. (22)
S2-2	K0001_25	NS1, NS2, NS3				

Table S5. Demographic details of ASD and control brain samples from the NICHD Brain and Tissue Bank, University of Maryland School of Medicine (http://medschool.umaryland.edu/btbank/)

	BA09 (CONT)	BA09 (ASD)	BA21 (CONT)	BA21 (ASD)	BA40 (CONT)	BA40 (ASD)
Age	13.70 ± 5.72	13.50 ± 5.87	12.43 ± 5.45	12.21 ± 5.62	12.85 ± 5.43	12.21 ± 5.62
Gender	3/7 (F/M)	3/7 (F/M)	4/10 (F/M)	4/10 (F/M)	4/9 (F/M)	4/10 (F/M)
PMI (hr)	16.60 ± 7.32	22.50 ± 12.87	16.43 ± 6.30	22.36 ± 12.37	16.77 ± 6.42	22.36 ± 12.37
RIN	6.29 ± 1.02	5.75 ± 1.39	5.79 ± 1.28	4.84 ± 1.58	6.03 ± 0.96	5.84 ± 1.57

The value is the mean ± SD.
CONT: Control, ASD: Autism spectrum disorder.
PMI: Post mortem interval.
RIN: RNA Integrity Number.
F: Female, M: Male.

Table S6. Detailed data of statistical analyses.

	Graph	Statistical test	Power	Factor effect		Interaction effect
Fig.3B	Locomotion	Two-way ANOVA	n= 9~13	Poly(I:C): $F_{1,39}$ =0.1452, P = 0.705	TPPU: $F_{1,39}$ = 0.0567, P = 0.813	$F_{1,39}$ = 0.5263, P = 0.475
Fig.3C	NORT (training session)	Two-way ANOVA	n=9~13	Poly(I:C): $F_{1,37}$ =0.0016, P = 0.968	TPPU: $F_{1,37}$ = 0.1408, P = 0.710	$F_{1,37}$ = 0.0216, P = 0.884
Fig.3C	NORT (retention test)	Two-way ANOVA	n=9~13	Poly(I:C): $F_{1,37}$ =24.40, P < 0.0001	TPPU: $F_{1,37}$ = 20.98, P < 0.0001	$F_{1,37}$ = 25.42, P < 0.0001
Fig.3D	PrL	Two-way ANOVA	n=6~7	Poly(I:C): $F_{1,23}$ = 2.551, P = 0.124	TPPU: $F_{1,23}$ = 33.31, P < 0.0001	$F_{1,23}$ = 6.274, P = 0.020
Fig.3D	IL	Two-way ANOVA	n=6~7	Poly(I:C): $F_{1,23}$ = 0.4263, P = 0.520	TPPU: $F_{1,23}$ = 14.04, P = 0.0011	$F_{1,23}$ = 2.827, P = 0.106
Fig.3E	PrL	Two-way ANOVA	n=6~7	Poly(I:C): $F_{1,28}$ = 6.401, P = 0.018	TPPU: $F_{1,28}$ = 0.4380, P = 0.514	$F_{1,28}$ = 11.50, P = 0.002
Fig.3E	IL	Two-way ANOVA	n=6~7	Poly(I:C): $F_{1,28}$ = 7.928, P = 0.009	TPPU: $F_{1,28}$ = 5.805, P = 0.023	$F_{1,28}$ = 3.924, P = 0.058
Fig.S3B	p-PERK/PERK	Two-way ANOVA	n=5~7	Poly(I:C): $F_{1,17}$ =1.186, P = 0.291	TPPU: $F_{1,17}$ = 5.892, P = 0.027	$F_{1,17}$ = 13.95, P = 0.002
	p-eIF2α/eIF2α	Two-way ANOVA	n=5~7	Poly(I:C): $F_{1,18}$ = 9.106, P = 0.007	TPPU: $F_{1,18}$ = 9.160, P = 0.007	$F_{1,18}$ = 2.126, P = 0.162
	Bip	Two-way ANOVA	n=5~7	Poly(I:C): $F_{1,19}$ = 5.915, P = 0.025	TPPU: $F_{1,19}$ = 2.013 P = 0.172	$F_{1,19}$ = 3.528, P = 0.076
	p-IRE1/IRE1	Two-way ANOVA	n=5~7	Poly(I:C): $F_{1,18}$ = 8.622, P = 0.009	TPPU: $F_{1,17}$ = 6.264, P = 0.022	$F_{1,17}$ = 2.627, P = 0.123
	sXBP-1	Two-way ANOVA	n=5~7	Poly(I:C): $F_{1,17}$ = 16.50, P = 0.0008	TPPU: $F_{1,17}$ = 3.206, P = 0.091	$F_{1,17}$ = 1.344, P = 0.262
	p-JNK/JNK	Two-way ANOVA	n=5~7	Poly(I:C): $F_{1,17}$ = 6.309, P = 0.022	TPPU: $F_{1,17}$ = 0.2943, P = 0.595	$F_{1,17}$ = 6.439, P = 0.021
	ATF6	Two-way ANOVA	n=5~7	Poly(I:C): $F_{1,18}$ = 6.972, P = 0.017	TPPU: $F_{1,18}$ = 2.087, P = 0.1657	$F_{1,18}$ = 2.213, P = 0.154
	cATF6	Two-way ANOVA	n=5~7	Poly(I:C): $F_{1,18}$ = 11.96, P = 0.003	TPPU: $F_{1,17}$ = 0.8625, P = 0.366	$F_{1,17}$ = 5.727, P = 0.028
	p-p38/p38	Two-way ANOVA	n=5~7	Poly(I:C): $F_{1,17}$ = 2.012, P = 0.174	TPPU: $F_{1,17}$ = 4.891, P = 0.041	$F_{1,17}$ = 7.634, P = 0.011
Fig.4B	Locomotion	Two-way ANOVA	n=7~11	Poly(I:C): $F_{1,33}$ = 0.6919, P = 0.412	TPPU: $F_{1,33}$ = 3.753, P = 0.061	$F_{1,33}$ = 1.115, P = 0.299
Fig. 4C	NORT (training session)	Two-way ANOVA	n=7~11	Poly(I:C): $F_{1,28}$ = 3.248, P = 0.082	TPPU: $F_{1,28}$ = 0.3411, P = 0.564	$F_{1,28}$ = 0.2855, P = 0.567
Fig. 4C	NORT (retention session)	Two-way ANOVA	n=7~11	Poly(I:C): $F_{1,28}$ = 10.57, P = 0.003	TPPU: $F_{1,28}$ = 9.375, P = 0.005	$F_{1,28}$ = 1.960, P = 0.173
Fig. 4D	left	Two-way ANOVA	n=10~17	TPPU: $F_{3,92}$ = 0.3419, P = 0.795	Stranger: $F_{1,92}$ = 116.4, P < 0.0001	$F_{3,92}$ = 0.3290, P = 0.804
	right	Two-way ANOVA	n=10~17	TPPU: $F_{3,92}$ = 0.9380, P = 0.426	Stranger: $F_{1,92}$ = 19.76, P < 0.0001	$F_{3,92}$ = 0.3969, P = 0.756
Fig. 4E	PrL	Two-way ANOVA	n=6~7	Poly(I:C): $F_{1,22}$ = 3.513, P = 0.074	TPPU: $F_{1,22}$ = 1.589, P = 0.221	$F_{1,22}$ = 4.558, P = 0.044
	IL	Two-way ANOVA	n=6~7	Poly(I:C): $F_{1,22}$ = 0.0126, P = 0.912	TPPU: $F_{1,22}$ = 0.3384, P = 0.567	$F_{1,22}$ = 3.987, P = 0.058

Table S7. Information of primary antibodies used in this study

Antibodies	Species, Isotype	Label	Dilution (or Concentration)	Name of company	Catalog number
Parvalbumin (PV)	Mouse IgG	−	1:5000	Swant	#235
GAD67	Mouse IgG	−	1:1000	Merk	#MAB5406
Phospho-PERK(Thr980)	Rabbit IgG	−	1:1000	Cell Signaling Technology	#3179
PERK	Rabbit IgG	−	1:1000	Cell Signaling Technology	#3192
eIF2α	Rabbit IgG	−	1:1000	Cell Signaling Technology	#5324
Bip	Rabbit IgG	−	1:1000	Cell Signaling Technology	#3177
IRE1α(phospho S724)	Rabbit IgG	−	1:1000	abcam	ab38187
IRE1α	Rabbit IgG	−	1:1000	Cell Signaling Technology	#3294
XBP-1s	Rabbit IgG	−	1:1000	Cell Signaling Technology	#12782
JNK1+JNK2(phspho T183+Y185)	Rabbit IgG	−	1:1000	abcam	ab4821
JNK1+JNK2	Rabbit IgG	−	1 μg/ml	abcam	ab112501
ATF6	Rabbit IgG	−	1:1000	Cell Signaling Technology	#65880
Phospho-p38 MAPK(Thr180/Tyr182)	Rabbit IgG	−	1:1000	Cell Signaling Technology	#4511
p38 MAPK	Rabbit IgG	−	1:1000	Cell Signaling Technology	#8690
mouse sEH	Rabbit IgG	−	1:5000	UC Davis	−
β-Actin	Mouse IgG	−	1:10000	Sigma-Aldrich	A5441

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