Supporting Information

TITLE: **Effects on synaptic plasticity markers in fetal mice and HT22 neurons upon F-53B exposure: the role of PKA cytoplasmic retention**

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Text S1 Flow cytometry

Wash the adherent cells with 1×PBS, add an appropriate amount of pancreatic enzyme digestion solution, and then collect the cell precipitates. Gently resuspend in 1×PBS, centrifuge at 1000g for 5 minutes, discard the supernatant, and add 195 μL of the resuspended cells to Annexin V-FITC binding solution. Then, add 5 μL of Annexin V-FITC and gently mix. Next, add 10 μL of PI and incubate at room temperature (20-25°C) in the dark for 10-20 minutes. Then, place at 4°C and analyze using a CytoFLEX-2 (Beckman Coulter, USA).

Text S2 RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from fetal brain and HT22 cells using TRIzol Reagent (Invitrogen, USA), and its concentration and quality were assessed with a Nanodrop 2000 (Thermo Fisher, USA). First-strand cDNA synthesis was carried out using the ReverTra Ace® qPCR RT Kit (TOYOBO, Japan), utilizing a 500 ng aliquot of total RNA. Quantitative real-time PCR (qRT-PCR) was conducted using SYBR® Green Realtime PCR Master Mix (TOYOBO, Japan) and analyzed using a QuantStudio7 Flex (Thermo Fisher, USA). Gapdh was used as the housekeeping gene. The PCR protocol was as follows: 95°C for 1 minute, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

Text S3 Western blot (WB)

The Cytoplasmic and Nuclear Proteins Extraction Kit (Beyotime, China) was used to extract the cytoplasmic and nuclear proteins of HT22 cells. Cell pellets were collected and resuspended in 200 μL of Cytoplasmic Protein Extraction Reagent A, with 1mmol/L phenylmethanesulfonyl fluoride (PMSF) added. The cells were vortexed for 5 seconds and then placed at 4 ℃ for 15 min. Subsequently, 10 μL of Reagent B was added to the cells, they were then vortexed vigorously for 5 seconds, and the supernatant was identified as the extracted cytoplasmic protein. Cell pellets were treated with Nuclear Protein Extraction Reagent containing 1mmol/L PMSF, vortexed vigorously for 30 min at 4 ℃, and the supernatant was collected as the extracted nuclear protein.

The total proteins from fetal brain, placenta, and HT22 cells were separately quantified using Lysis Buffer (Beyotime, China). Quantification of the total, cytoplasmic, and nuclear proteins was performed using the BCA Protein Assay Kit (Beyotime, China). Protein samples were mixed with loading buffer, heated to 100 ℃ for 7 min, then electrophoresed on 12% SDS-PAGE, and transferred to PVDF membranes. Subsequently, the membranes were blocked with the Antibody Blocking Solution (Beyotime, China) at room temperature for 90 min. The membranes were incubated with the primary antibody (1:1000) overnight at 4 ℃, followed by the secondary antibody (1:5000, Abcam, USA) at room temperature for 90 min. The blots were visualized using ChemiDoc Touch (BIO-RAD, USA). The densitometry of the blots was performed using ImageJ software.

Text S4 Molecular docking

We retrieved the crystal structures of CREB1 (PDB ID: 5ZKO) and PKA (PDB ID: 2H77) from the RCSB Protein Data Bank (http://www.rcsb.org/pdb). The 2D structures of 6:2 Cl-PFASEs (Compound CID: 22568738) and 8:2 Cl-PFASEs (Compound CID: 50851128) were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and then converted to 3D structures by Chem3D 20.0. AutoDock 1.5.7 was employed to ascertain the binding modes of Cl-PFAESs to PKA and CREB1. These protein files were prepared by removing water molecules and other ligands, adding polar hydrogens, and rendering the structure of the ligands in PDB format. During docking, the target proteins remained rigid, while all torsional bonds within each ligand were made flexible. The grid box encompassed the entire binding site. Docking utilized the Lamarckian genetic algorithm. OpenBabel 2.4.1 facilitated the conversion of files from the .pbdqt suffix to the .pdb suffix. Finally, Discovery Studio 4.5 Client and PyMOL 2.4.0 were used to visualize the docking results in both 2D and 3D formats.

86 **Table S1 Primer sequences used for qPCR in this study.**

Full name	Gene	Sequences of primers 5'-3'
GAPDH	M-GAPDH-f	GCATCTTCTTGTGCAGTGCC
	M-GAPDH-r	TACGGCCAAATCCGTTCACA
Adenylate cyclase 1	M-Adcy1-f	TCGTGTCCTATGCCTTGCTG
	M-Adcy1-r	CTCTGTCAAGATCCGCACGA
Cathelicidin antimicrobial peptide	M-cAMP-f	ACAGCCCTTTCGGTTCAAGA
	M-cAMP-r	TTCACCAATCTTCTCCCCACC
Protein lysine acetyltransferase	M-PKA-f	CAACTTCCCGTTCCTGGTCA
	M-PKA-r	AGGTCCCGGTAGATGAGGTC
cAMP responsive element binding protein 1	M-CREB1-f	AGGGGTGCCAAGGATTGAAG
	M-CREB1-r	CTTGGTTGCTGGGCACTAGA
Brain derived neurotrophic factor	M-BDNF-f	CGACGACATCACTGGCTGACAC
	M-BDNF-r	GAGGCTCCAAAGGCACTTGACTG
Synaptophysin	M-SYP-f	TGGTTTGGAGGGTGAGCGAAATG
	M-SYP-r	AGGGCAGAGAAAGGGTGGAGAAG
Growth associated protein 43	M-GAP43-f	CCGAGGCTGACCAAGAACATGC
	M-GAP43-r	GGTAGGAGAGGACAGGCTCACAC
Postsynaptic density protein-95	M-PSD95-f	GCTATGAGACGGTGACGCAGATG
	M-PSD95-r	GTTGGCACGGTCTTTGGTAGGC

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Table S2. Organ coefficient of fetal mice

Groups	Fetal brain
Control	1.41 ± 0.16
4 μg/L	1.35 ± 0.22
40 μg/L	1.18 ± 0.07

Note: Values are mean ± SEM. n ≥ 3.