Materials and methods

Genotyping

As previously described (Wang et al., 2016a), Mice were genotyped by using genomic DNA isolated from tail tips. Genomic DNA was amplified by PCR to generate a 250-bp product for the wildtype allele and a 380-bp product for the knockout allele. The primer sequences were as follows: TSPO Forward (P1) = 5'-GAT GGA GAA ACT GAG TCC CAG TCA GGG T-3'; TSPO Reverse (P2) = 5'-GCT CTG CCC TAA TCA CAA AGT TTC ACA C-3'; TSPO Reverse (P3) = 5'-TTA AGG AGA GGT TTT GTC CTT GTG TC-3'. PCR consisted of an initial incubation at 95°C for 10min, followed by 68°C for 1min 20 s, 72°C for 5 min and 95°C for 5 min, then 30 cycles at 95°C for 30 s, 54°C for 30 s and 72°C for 40 s, then a final step at 72°C for 10 min. 10 μl PCR product were electrophoresed on a 2% agarose gel stained with GoldView II (SBS Genetech, Shanghai, China). Different genotypes were determined by results examined from the electrophoresis.

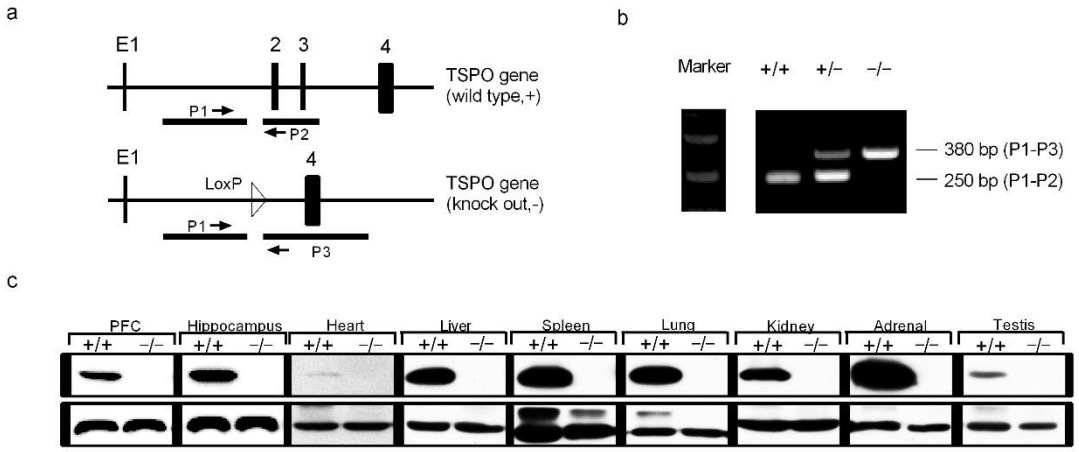
Western Blotting

Western Blotting was performed as described in our previous report (Wang et al., 2016b). TSPO WT and KO mice were sacrificed and tissues from PFC, hippocampus, heart, liver, spleen, lung, kidney, adrenal and testis were extracted by RIPA lysis buffer (Applygen, Beijing, China) for TSPO detection. Equal amounts of proteins were electrophoresed, then incubated with primary antibodies from rabbit against: TSPO (1:1000, Abcam, Cambridge, UK) or β-actin (1:1000, Abcam). After incubated with secondary antibodies against rabbit (1:3000, ZSGB-BIO, Beijing, China), the specific bands were detected.

References

Wang H, Zhai K, Xue Y, et al. (2016a) Global Deletion of TSPO Does Not Affect the Viability and Gene Expression Profile. *PLoS One* 11: e0167307.

Wang W, Zhang L, Zhang X, et al. (2016b) Lentiviral-Mediated Overexpression of the 18 kDa Translocator Protein (TSPO) in the Hippocampal Dentate Gyrus Ameliorates LPS-Induced Cognitive Impairment in Mice. *Front Pharmacol* 7: 384.



Supplementary Figure S1. Confirmation of TSPO KO mice. (a) Schematic showing genomic TSPO locus of wild type alleles (+) and knock out alleles (-). Exon (E) 2 and Exon 3 were targeted and eventually knocked out in TSPO KO mice by Cre-LoxP system. Genotyping primers are indicated as P1, P2, and P3. (b) Specific DNA primers were used to genotype TSPO wildtype (WT, +/+), TSPO heterozygote (HZ, +/-) and TSPO knckout (KO, -/-) pups. (c) Confirmation of the complete absence of TSPO protein in TSPO KO mice with tissues from prefrontal cortex (PFC), hippocampus, heart, liver, spleen, lung, kidney, adrenal and testis.