Supplementary information

Triterpenoid modulation of IL-17 and Nrf-2 expression ameliorates neuroinflammation and promotes remyelination in autoimmune encephalomyelitis

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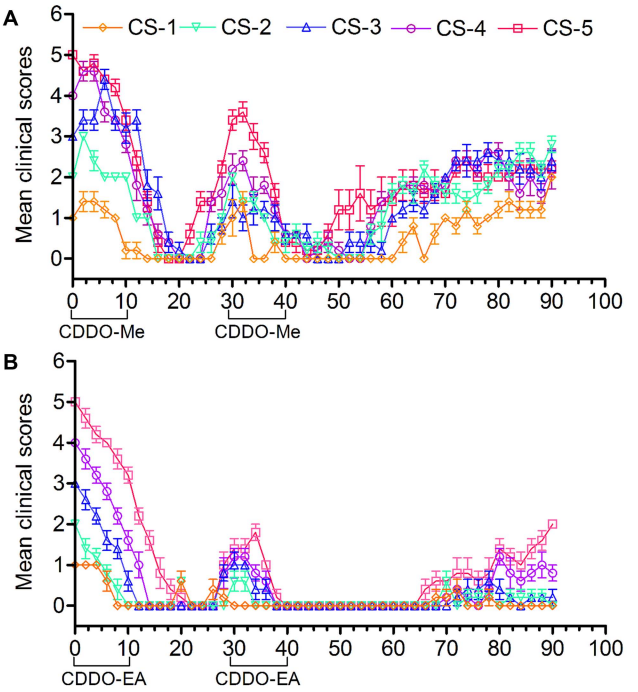
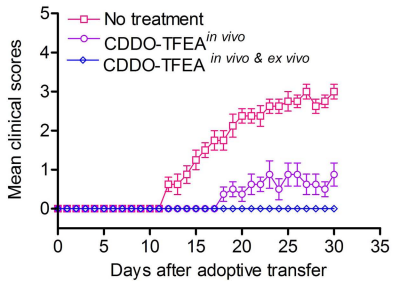
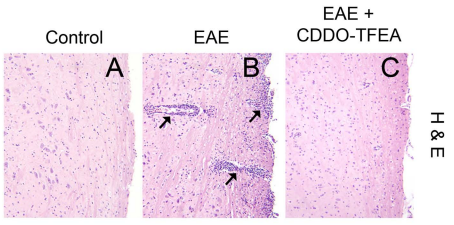


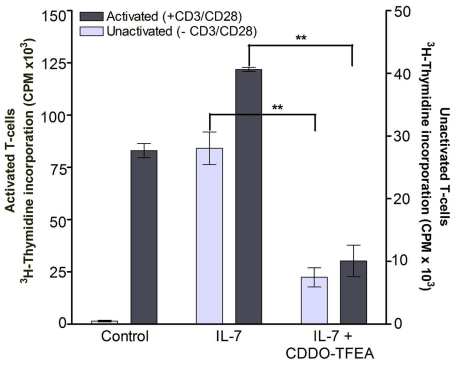
Supplementary figure S1: Effect of CDDO methyl (CDDO-Me) and ethyl amide (CDDO-EA) derivatives treatment on EAE. Clinical scores of EAE after systemic injection of CDDO-Me (A) or CDDO-EA (B) on alternate days at different clinical scores. All data are presented as the mean + S.E.M. (n= 10-12 mice in each group).



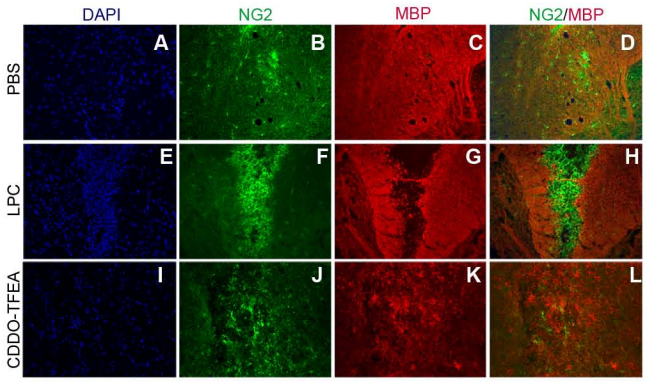
Supplementary figure S2: CDDO-TFEA treated encephalitogenic lymphocytes failed to passively transfer EAE. Encephalitogenic lymphocytes were collected from spleen and regional lymph nodes of mice 11 days after MOG (35–55) immunization. In treated groups, mice received 2 doses of CDDO-TFEA on days 8 and 10. About 5 x 10^6 cells were cultured with or without CDDO-TFEA in the presence of 33 µg/ml MOG (35–55) and 20 ng/ml of mouse IL-12. On day 3 of culture, roughly 3 × 10^7 of the cultured encephalitogenic cells were then injected into 3-month-old female C57BL6 mice via intraperitoneal injection. 200 ng PTX was injected on the same day and again 48 h later and mice were closely observed for development of clinical signs on every alternate day.



Supplementary figure S3: Treatment with CDDO-TFEA suppresses neuroinflammation in EAE mice. Mice were sacrificed after MOG (35-55) immunization either at the peak of clinical symptoms or at a clinical score of zero and after respective treatments with either vehicle control or CDDO-TFEA and brain stem was subjected to histopathological analysis. Representative sections of brain from control mice with clinical signs of EAE or from mice treated with CDDOTFEA were stained with hematoxilin and eosin to assess inflammation.



Supplementary figure S4: CDDO-TFEA inhibits TCR and cytokine induced lymphocyte proliferation. T cells were collected from peripheral lymphoid organs cultured for 72 hrs with or without CD3/CD28 (3:1) and IL7 in the presence or absence of CDDO-TFEA. During last 16 hrs, 3H-thymidine was added to cultures and the incorporated amount of 3H-thymidine in cells was measured as CPM. Data is presented as the mean ± SEM (n = 6). **, P< 0.01 by using Student's t test.



Supplementary figure S5: Neuroprotective effects of CDDO-TFEA in LPC induced demyelination. Immunostaining of tissue sections prepared from dorsal columns of the thoracic spinal cord of rats at the site of injection of either PBS (A- D), or LPC (E-H) or LPC+ CDDOTFEA (I-L). Immunohistochemistry was performed with an anti-MBP antibody (for myelin), anti-NG2 antibody (for oligodendrocytes) and DAPI for nuclear stain. All images are taken at 200x magnification.