Supplementary Figures to

Cytomegalovirus infection of the fetal brain: intake of aspirin during pregnancy blunts neurodevelopmental pathogenesis in the offspring

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Figure S1. Maternal intake of aspirin improves postnatal survival and decreases viral load in CMV-infected brain slices.

(A) Postnatal survival was assessed on a daily basis during the two first postnatal weeks by comparing different cohorts of rat pups previously submitted to icv injections of MEM (vehicle) or of CMV at E15. CMV cohorts were untreated or treated with either of four drugs provided to the dam in drinking water ad libitum from E5-6 until delivery. MEM: non-infected cohort (n = 30 from three litters); CMV: CMV-infected, untreated cohort (n = 25 from three litters); CMV+ASA: CMV-infected, acetylsalicylic acid (aspirin, ASA)-treated cohort (n = 26 from three litters); CMV+NAC: CMV-infected, N-acetyl-L-cysteine (NAC)-treated cohort (n = 17 from two litters); CMV+VitC: CMV-infected, ascorbic acid (vitamin C, VitC)-treated cohort (n = 24 from three litters); CMV+Pred: CMV-infected, prednisolone (Pred)-treated cohort (n = 17 from two litters). Sex ratio did not differ significantly between the six conditions at birth ($p = 0.3067$, Fisher's exact test). Only ASA treatment significantly improved survival compared to the untreated, CMV-infected group. ****: $p < 0.0001$; *: $p < 0.05$, ns: not significant. Kaplan-Meier method followed by Log-Rank test.

(B) ASA reduced CMV infection in the neonatal brain. Brains were analyzed using a fluorescent stereo microscope. For each brain analyzed, two sections were selected according to their coordinates in the rostrocaudal axis, corresponding to either medial (-0.4 to -0.6mm to Bregma) or caudal (-1.2 to -1.4mm to Bregma) coordinates. (Left panels) A representative picture of a brain slice corresponding to the medial coordinate is shown for non-treated and ASA-treated, CMV-infected brain (CMV and CMV+ASA, respectively). (Right panel) The proportions of fluorescent (GFP) areas were measured in each section and the mean value was calculated for each brain. CMV infection decreased in ASA-treated (n = 8) versus untreated, CMV-infected brains (n = 10) at P1. *: $p < 0.05$. Mann Whitney test, two-tailed.

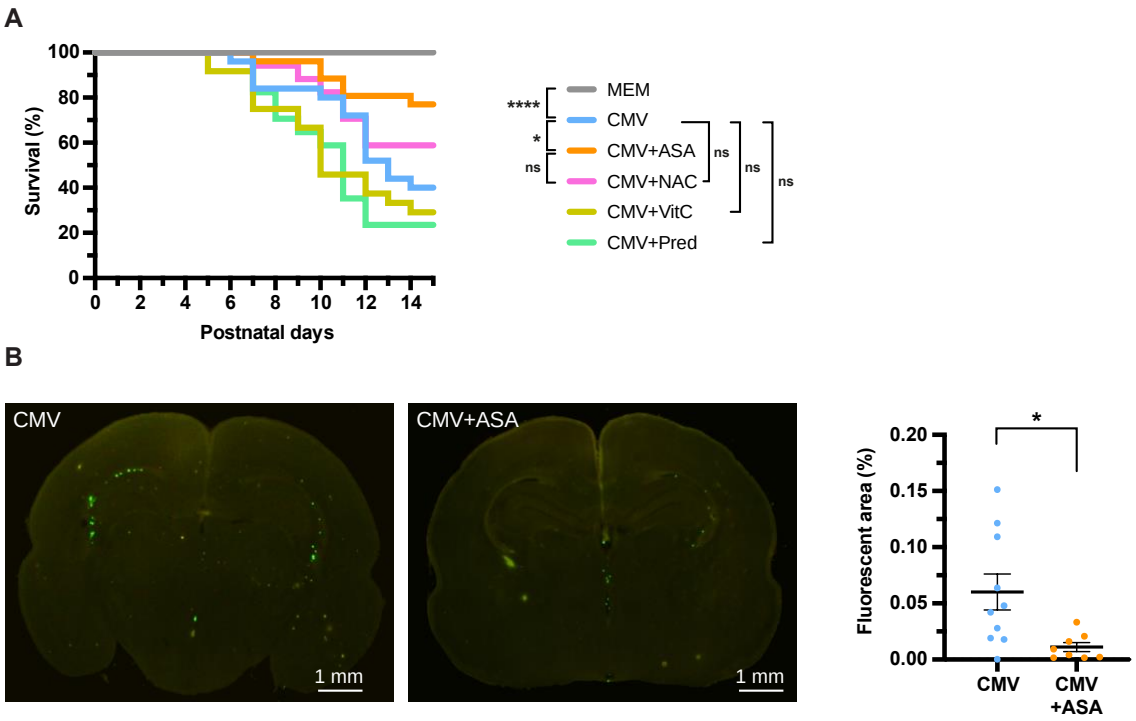

Figure S2. Liquid chromatography coupled to tandem mass spectrometry (MS) chromatograms.

Chromatograms were obtained in Single Reaction Monitoring (SRM) mode from a brain extract. For each trace the name of the metabolite and the MS/MS transition used for quantitation (in brackets) are mentioned. The profiles of the internal standards (IS) used are also indicated.

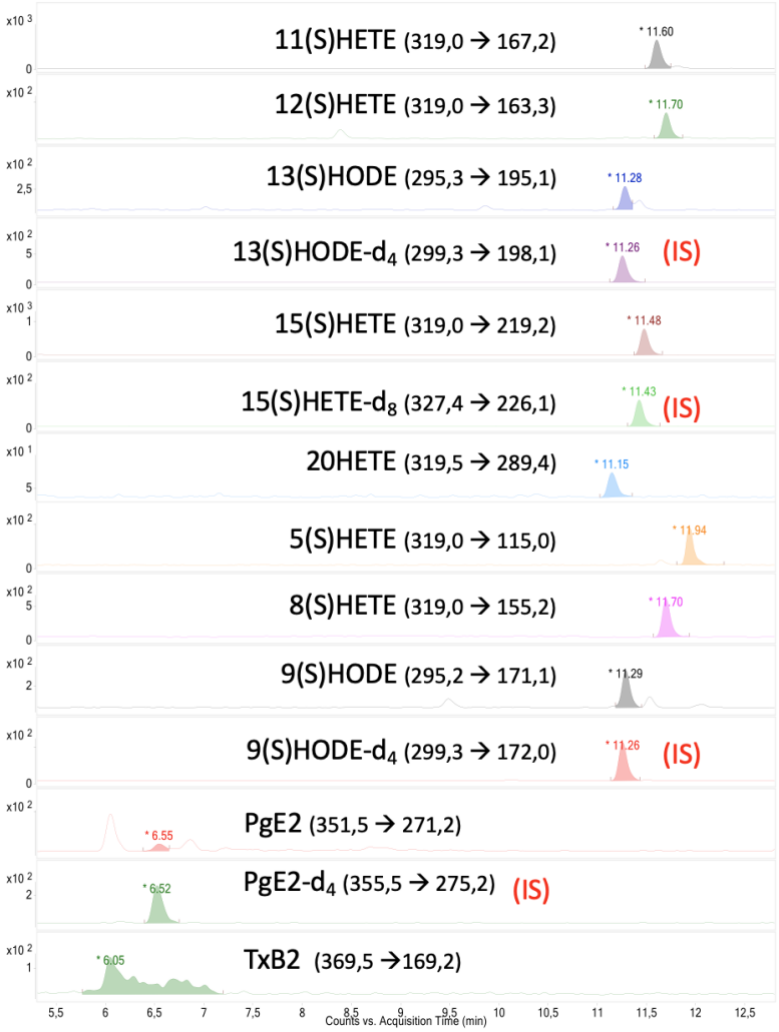

Figure S3. Brain fatty acid oxidation products derived from linoleic acid and arachidonic acid and showing no significant dysregulation between groups.

Analysis was done by mass spectrometry at E17 (A) and P1 (B). Of note, all dysregulated fatty acid oxidation products are shown in Figure 1. MEM: non-infected brains (E17, n = 8; P1, n = 9); CMV: untreated, CMV-infected brains (E17: n = 12; P1: n = 8); CMV+ASA: ASA-treated, CMV-infected brains (E17: n = 11; P1: n = 8). Kruskal-Wallis test.

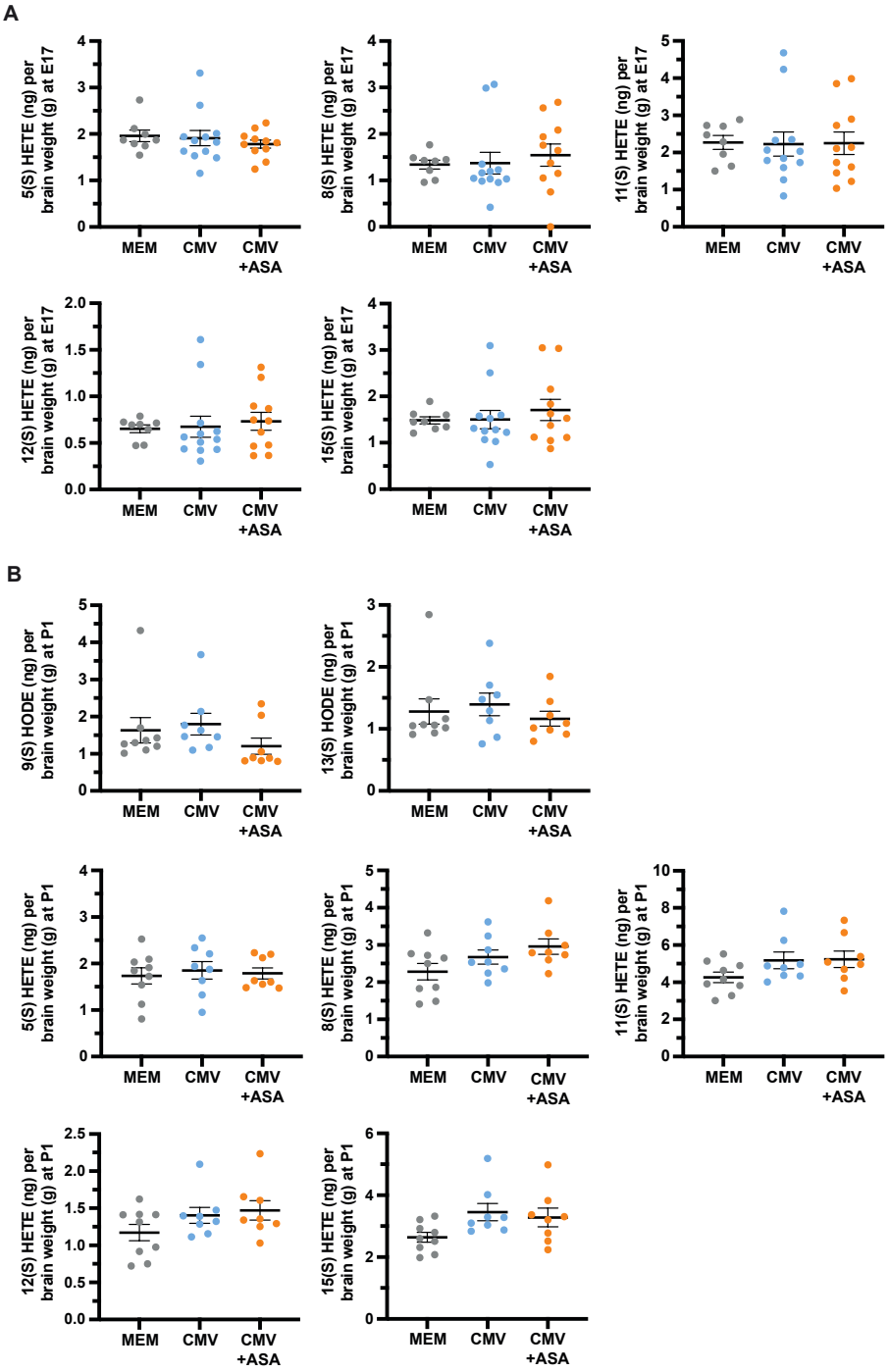

Figure S4. qRT-PCR experiments.

Relative mRNA expressions of *Ptgs1* (Cox-1-encoding gene) and of *Ptgs2* (Cox-2 encoding gene) were assessed by quantitative RT-PCR in CMV-infected brains at P1, using *Rpl-19* as reference gene. Significant increase in *Ptgs1*, but not in *Ptgs2* expression, was found in CMV-infected (CMV) vs non-infected (MEM) brains (n = 10 each). Values of fold change represent averages from triplicate measurements for each sample. *: $p < 0.05$; ns: not significant. Mann Whitney test, two-tailed.

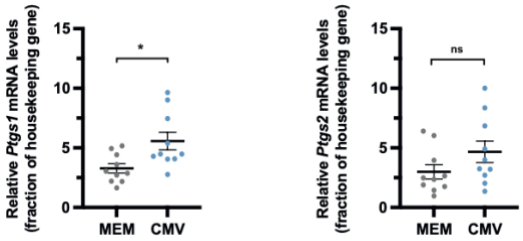

Figure S5. Prominent transcriptional expression of Cox-1 in microglia in the developing brain.

(A) Uniform manifold approximation and projection (UMAP) embedding of cells are colored by cell type. UMAP coordinates and cell type annotations were extracted from Di Bella et al. 2021* with an inset on the microglial annotated cells. Data are available at Gene Expression Omnibus (GEO GSE153164) and at the Single Cell Portal, Broad Institute website (https://singlecell.broadinstitute.org/single_cell/study/SCP1290). Expressions of the genes coding for (B) microglia marker Iba1 (*Aif1*), (C) Cox-1 (*Ptgs1*) and (D) Cox-2 (*Ptgs2*) are shown.

**Di Bella DJ, Habibi E, Stickels RR, Scalia G, Brown J, Yadollahpour P, Yang SM, Abbate C, Biancalani T, Macosko EZ, Chen F, Regev A, Arlotta P. Molecular logic of cellular diversification in the mouse cerebral cortex. Nature. 2021 Jul;595(7868):554-559. doi: 10.1038/s41586-021-03670-5.*

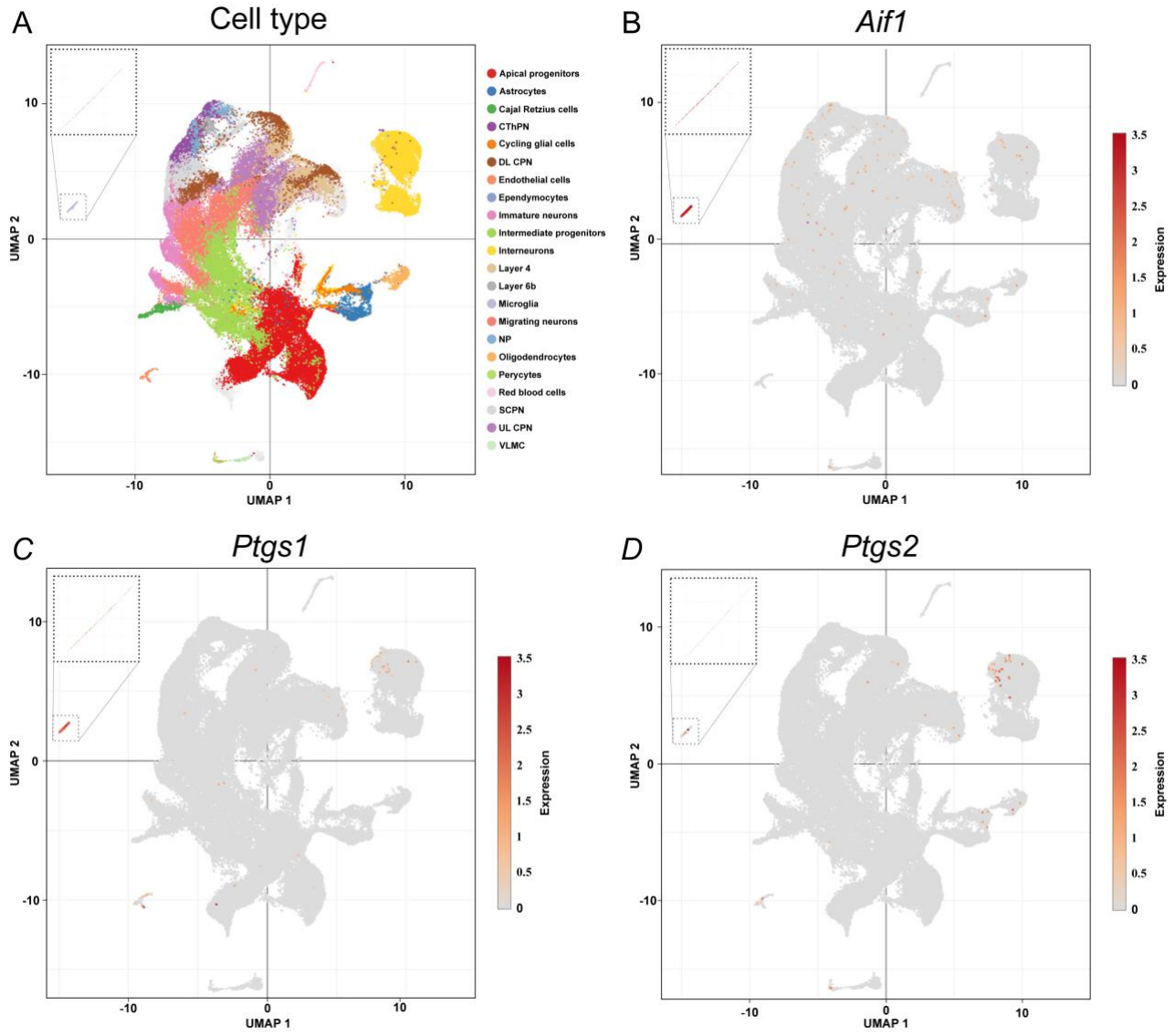

Figure S6. Impact of ASA intake during pregnancy on the postnatal phenotypes.

(A) ASA, but not NAC, improved the times to succeed to the righting reflex (left panel) and cliff aversion (right panel) sensorimotor developmental tests. Times were determined in the subsets of rat pups who succeeded (*i.e.* times < 30 sec.) to the tests in the four conditions (see Figure 4). MEM: non-infected cohort; CMV: CMV-infected, untreated cohort; CMV+ASA: CMV-infected, ASA-treated cohort; CMV+NAC: CMV-infected, NAC-treated cohort. ****: $p < 0.0001$; *: $p < 0.05$; ns: not significant. Mixed model for repeated measures.

(B) ASA did not modify the overall risk to generalized tonic-clonic seizures (GTCS). CMV: CMV-infected, untreated cohort (10 with GTCS out of N = 41; five litters); CMV+ASA: CMV-infected, ASA-treated cohort (19 with GTCS out of N = 55; six litters). ns: not significant. Fisher's exact test, two-tailed.