Supplementary Figure S1. Neuronal death and altered astrocytes in the brain of an NPA affected child.

Neuron specific MAP2 antibody staining in the hippocampus (a) and cortex (b), calbindin antibody staining of Purkinje cells in the cerebellum (c) and astrocyte specific GFAP antibody staining in the hippocampus (d), cortex (e) and cerebellum (f) of control and NPA affected children. DAPI staining in blue identifies cell nuclei. Scale bars= 25 µm.
Supplementary Figure S2. Confirmation of lipofuscin aggregates in ASMko mice brains by Sudan Black B quenching and bright field microscopy. (a) Autofluorescence of lipofuscin aggregates in the hippocampus of 4-month-old ASMko mice was quenched by Sudan black B incubation. (b) Bright field microscopy images of wt and ASMko mouse hippocampus evidence abundant microgranular refringent deposits, compatible with lipofuscin aggregates, in the ASMko mice. DAPI staining in blue identifies cell nuclei. Scale bars = 10 µm.
Supplementary Figure S3

(a) Bar graph showing SM levels (a.u.) for WT and ASMKO. The graph indicates a significant difference (*** p < 0.001) in SM levels between WT and ASMKO.

(b) Immunostaining images of WT and ASMKO cells with Lysenin treatment. The graph on the right shows SM levels (a.u.) for WT and ASMKO, indicating a significant increase in SM levels for ASMKO compared to WT (** p < 0.01).

(c) Immunostaining images of cells treated with Control, SM 16h, and SM 24h. The graph on the right shows SM levels (a.u.) for different time points, indicating a significant increase in SM levels with longer treatment times (***, p < 0.001).

(d) Immunostaining images of cells treated with Control, SM, and GW4869+Sir+SM. The graph on the right shows SM levels (a.u.) for different treatments, indicating a significant increase in SM levels with GW4869+Sir+SM treatment (***, p < 0.001).
Supplementary Figure S3. SM levels in ASMko hippocampal extracts and cultured neurons and in wt cultured neurons incubated with the lipid. (a) Graph shows mean ± SEM of SM levels in nmol/mg protein in hippocampal extracts from 4-month-old wt and ASMko mice (n = 5, Student’s t-test, P = 0.001). (b, c, d) Lysenin staining that detects SM in 12 DIV non-permeabilized hippocampal neurons from wt and ASMko mice (b) or in neurons from wt mice treated or not with SM for 16 and 24 hours (c) or with SM in the presence of the neutral and acid sphingomyelinase inhibitors GW4869 and siramesine, respectively (d). Scale bars = 10 µm. Graphs show mean ± SEM of Lysenin associated fluorescence in arbitrary units (n = 70 neurons from 3 independent cultures, Mann-Whitney U-test, P = 0.003 in b; Student’s t-test, P16h = 0.0006 and P24h = 0.0002 in c; two-way ANOVA followed by Games-Howell, PCTL-SM = 0.0004; PCTL-GWSIR = 0.006; PSM-GWSIR = 0.153 in d).
Supplementary Figure S4. Daily water consumption in wt and ASMko mice treated or not with SAHA complexed with HOP-β-CDX or with HOP-β-CDX only. Graph shows the average amount of water consumed per mice in ml per day in each week of the treatment (right panel) or as an average amount during the whole 6 week-long treatment (left panel). No significant differences were observed in wt or ASMko mice treated only with HOP-β-CDX or with SAHA complexed with HOP-β-CDX (SAHA).
Supplementary Figure S5. Oral treatment with SAHA affects histone deacetylation but not SM levels in the mouse hippocampus. (a) Graph shows mean ± SEM of SM levels in nmol/mg protein in hippocampal extracts from wt and ASMko mice orally treated or not with SAHA (n = 7, two-way ANOVA followed by Games Howell, $P_{\text{wt/wt-SAHA}} = 0.998$, $P_{\text{ko/ko-SAHA}} = 0.754$, $P_{\text{wt/ko}} = 0.0012$, $P_{\text{wt-SAHA/ko-SAHA}} = 0.001$). (b) Western blot analysis of acetylated histone 3 (Ac-H3K18), total histone 3 (H3) and actin in hippocampal extracts from wt and ASMko mice orally treated or not with SAHA. Graphs show mean ± SEM of acetylated histone 3 levels (n = 7, Student’s t-test, $P_{\text{wt}} = 0.023$; $P_{\text{ko}} = 0.019$).
Supplementary Figure S6. Treatment with HOP-β-CDX does not affect PMCA and ROS levels in the brain of wt and ASMko mice. (a) PMCA and ATP6V1A Western blot in hippocampal membrane extracts from wt and ASMko mice untreated or treated with HOP-β-CDX. Graphs show mean ± SEM of PMCA levels normalized to ATP6V1A (n = 7, Student’s t-test, $P_{wt} = 0.094$, $P_{ko} = 0.834$). (b) Graphs show mean ± SEM of the fluorescence associated to DHR that detects ROS levels in the hippocampus of wt and ASMko mice untreated, treated with HOP-β-CDX only or with SAHA complexed with HOP-β-CDX (n = 7, two-way ANOVA followed by Games Howell).
Supplementary Figure S7. Neuronal death does not take place in the hippocampus of 4 month-old ASMko mice treated or not with SAHA.

(a) Representative image of a fractin positive cell in the hippocampus. Dapi in blue stains cellular nuclei. Scale bar = 5 μm. Graph shows mean (± SEM) number of fractin positive cells per area unit in the hippocampus of wt and ASMko mice (n = 6, Mann-Whitney U-test, $P = 0.686$). (b) Western blot of the 19 and 17 kDa fragment derived from caspase-3 cleavage, indicative of apoptosis, and of GAPDH used as loading control in hippocampal extracts of wt and ASMko treated or not with SAHA. The graph shows the mean (± SEM) caspase-3 fragment levels normalized to GAPDH (n = 7, two-way ANOVA followed by minimum significant difference, $P_{\text{wt-wt+saha}} = 0.426$, $P_{\text{wt-ko}} = 0.278$, $P_{\text{ko-ko+saha}} = 0.243$).
Supplementary Figure S8. Purkinje cell survival in cerebellar lobes of ASMko mice. Calbindin immunostaining of Purkinje cells in anterior I-V (a), mid VI-VIII (b) and posterior IX-X (c) lobes of the cerebellum of control or SAHA treated wt and ASMko mice. Graphs show mean ± SEM of number of calbindin positive cells per area unit (n = 7, two-way ANOVA followed by Games Howell, for anterior lobes \( P_{wt/ko} = 0.022, P_{wt/ko}^{saha} = 0.024, P_{ko/ko-saha} = 0.182 \); for mid lobes \( P_{wt/ko} = 0.016, P_{wt-saha/ko} = 0.036, P_{ko/ko-saha} = 0.041 \); for posterior lobes \( P_{wt/ko} = 0.002, P_{wt-saha/ko} = 0.0009, P_{ko/ko-saha} = 0.022 \)). DAPI staining in blue identifies cell nuclei. Scale bars = 100 µm.