**Figure 1. A) Glutamatergic neurotransmission alterations in disease cohorts.** Rett syndrome (MeCP2) and CDKL5-epileptic encephalopathy (CDKL5) were classified as hyper-glutamatergic, while GRIN-related pediatric encephalopathy (GRIN) and syntaxin encephalopathy (STXBP1) were classified as hypo-glutamatergic disorders **B-F. Unsupervised multivariate analysis of patient and control CSF metabolite concentrations. PCA score plots showing the separation of patients from controls on the first two principal components.** B) Patients do not form distinct groups based on pathology or hyper/hypo-glutamatergic alterations. Control samples are highly varied, although the majority separate from the patients.C) hyper-glutamatergic patient . D) hypo-glutamatergic. **Ward hierarchical clustering showing log-transformed metabolite concentrations.** Both E) hyper-glutamatergic and F) hypo-glutamatergic patients form homogeneous groups regardless of genotype. There is some overlap with controls, but mostly patients cluster together and have a markedly decreased expression of most metabolites

**Figure 2. Identification of significantly altered metabolites and their impact on group classification A-B)** OPLS-DA score plots showing separation of controls compared to **A)** **hyper-glutamatergic** and **B)** **hypo-glutamatergic patients.** Both OPLS-DA models showed good separation between the groups (R2Y (cum) > 0.9, Q2Y (cum) > 0.8, RMSEE < 0.2), and both were statistically significant after permutation testing. **C-D) VIP scores.** Both diseases show similar metabolic alterations. **C) hyper-glutamatergic** had 18 metabolites with VIP score > 1 **D)** **hypo-glutamatergic** patients had 17. The highest VIP scores in both cases belonged to tryptophan metabolites. **E-F) Integrated results of UVA and MVA analysis. E) hyper-glutamatergic** patients had a subset of metabolites that were only identified by UVA **F) hypo-glutamatergic patients.** For both groups there was a small number of metabolites identified as altered by both univariate and multivariate analyses. Both diseases showed similar metabolic alterations, though hyper-glutamatergic patients (**C**) had a higher number of significantly altered metabolites. **G-H)** **Hierarchical clustering using only the selected metabolites** showed perfect separation between patients and controls for both groups. **G) Hyper-glutamatergic patients** showed a more metabolites that had increased concentrations when compared to controls than did **H) hypo-glutamatergic patients**, but overall the altered metabolites were decreased in both groups of patients

**Figure 3. Pathways affected by selected metabolites. A) Altered metabolites in hyper-glutamatergic patients B) Altered metabolites in hypo-glutamatergic patient**

**Figure 4.** **Alterations in amino acid metabolism. A)** Summary of tryptophan metabolism pathways. **B) Alterations in tryptophan metabolism.** Of the metabolites involved in tryptophan metabolism that were analyzed, only 5-Hydroxyindole-3-acetic acid was significantly decreased in both disease groups, while kynurenine was significantly decreased in RTT patients and non-significantly decreased in hypo-glutamatergic patients. Tryptophan, N-Acetyl-5-hydroxytryptamine, anthranilic acid, and kynurenic acid showed a slight decrease that was not statistically significant in both RTT and hypo-glutamatergic patients. 3-Hydroxyanthranilic acid did not vary between either group and the controls.

**Figure 5.** **Tryptophan and BCAAs transport across the blood-brain barrier. A)** **Alterations in LNAAs in CSF samples**: Valine and leucine were decreased in patients compared to controls (statistically significant for hyper-glutamatergic samples), while both groups had non-significantly altered levels of isoleucine and threonine. **B,C) Analysis of the expression of *SLC7A5* (LAT1) in brain samples from Rett mouse models.** Three different brain samples were analyzed for each group (Rett and controls) in two independent experiments. **B)** Representative blot of the expression of SLC7A5 is shown, where tubulin has been used as a loading control. **C)** Quantification with ImageJ of all the experiments; \*\* p-vale < 0.001