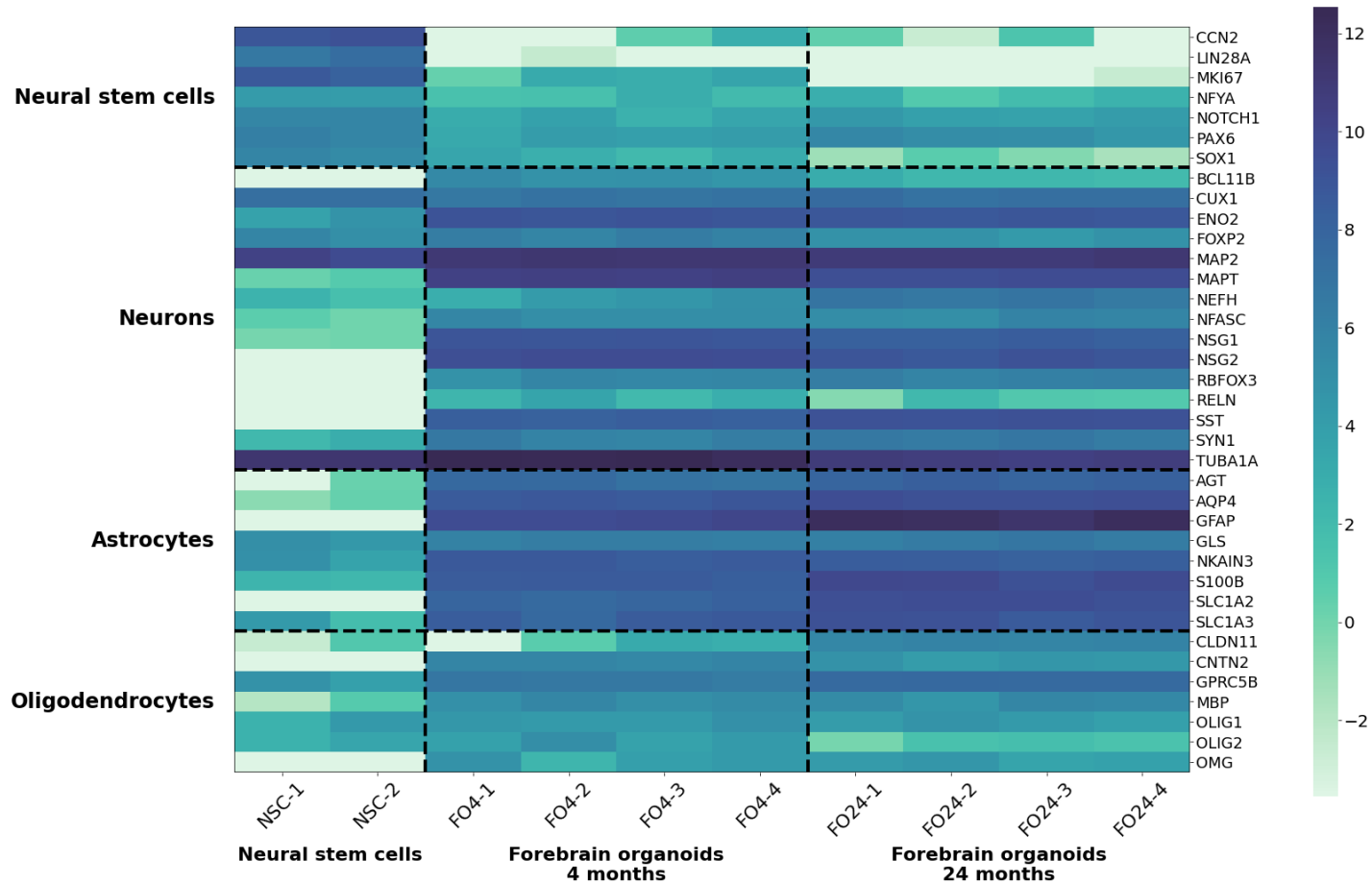


General introduction of the data.

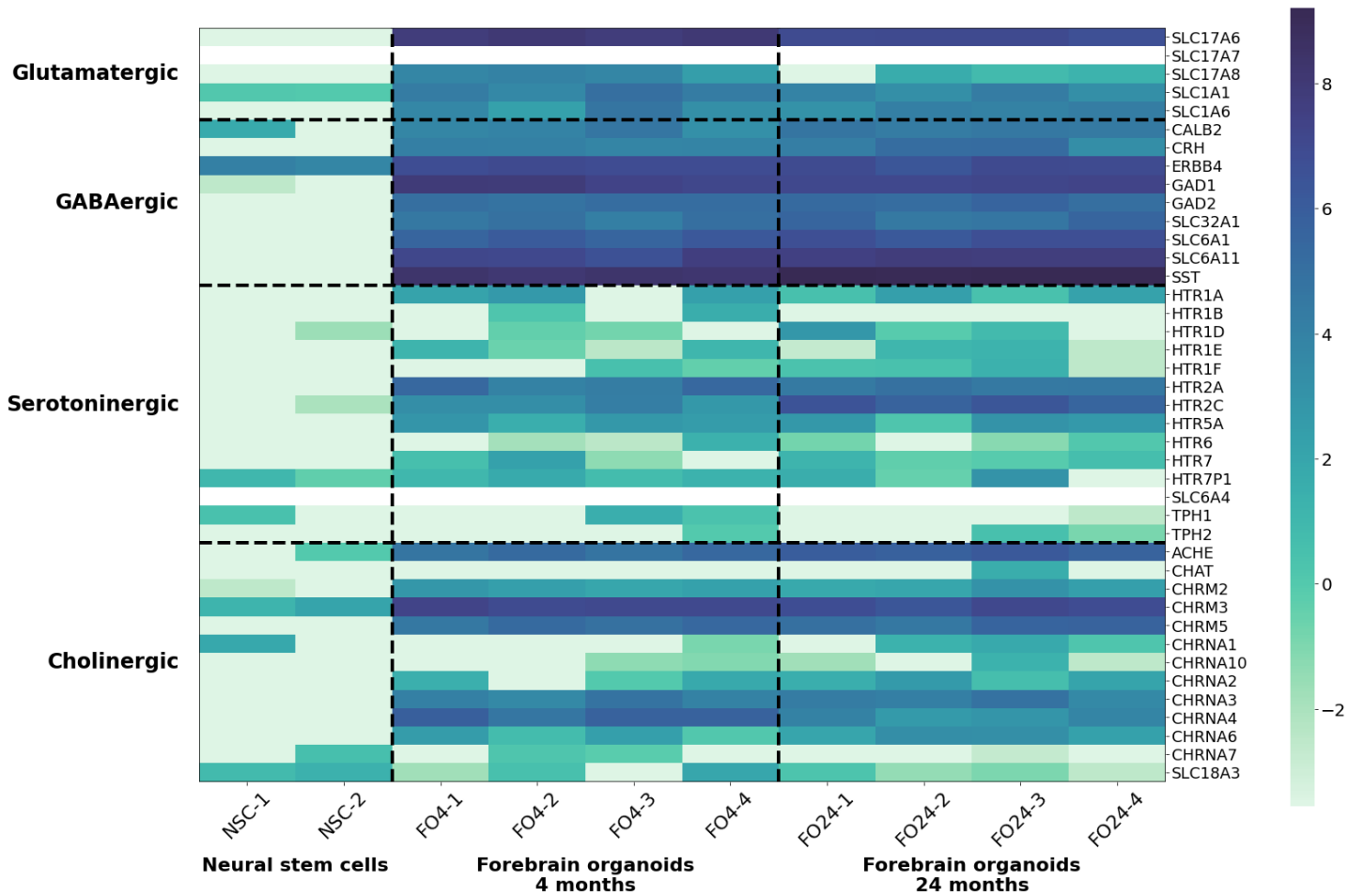
**Transcriptome analyses of Forebrain organoids by RNA sequencing.** RNA was extracted from hiPSC-derived neural stem cells (NSC-1, NSC-2), 4-months-old forebrain organoids (FO4-1, FO4-2, FO4-3, FO4-4), and 24-months-old forebrain organoids (FO24-1, FO24-2, FO24-3, FO24-4). RNA was analyzed by RNA sequencing (20M reads/sample), and statistical analysis was performed in R. Genes with low counts were filtered out according to the rule of 1 count per million (CPM) in at least 1 sample. Gene expression is indicated as log<sub>2</sub> of CPM values. Genes confirmed by qPCR are indicated in figure legends.

Figure 1



**Figure 1. Type of cells present in the organoids.** The heat map shows the distribution of genes typically enriched in neural stem cell (NSC), neurons, astrocytes and oligodendrocytes (dark blue highest expression, light green lowest expression, white for not detected gene). NSC samples mainly express genes that are markers of pluripotency, like LIN28a, SOX1, and MKI67. Four-months-old forebrain organoids (FO-4) and 24-months-old forebrain organoids (FO-24) still express some NSC-markers like PAX6, but they mainly contain markers of neurons, astrocytes and oligodendrocytes. Genes confirmed by qPCR: MKI67, MAP2, NEFH, ENO2, SYN1, GFAP, S100B, and MBP (data not shown).

Figure 2

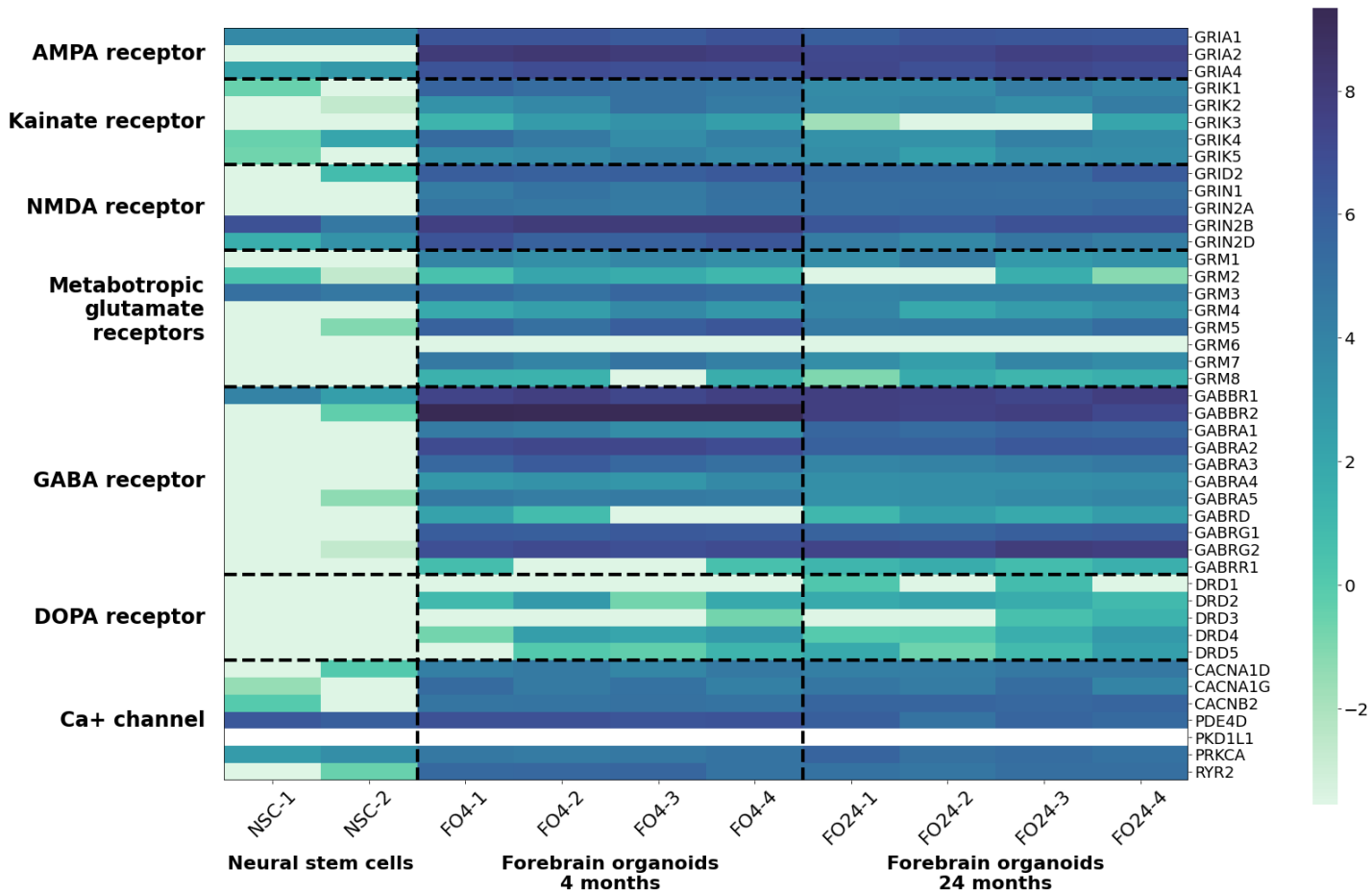


**Figure 2. Different types of neurons present in the organoids.** Heat map showing the distribution of genes typically enriched in GABAergic, glutamatergic, cholinergic, and serotonergic neurons (dark blue highest expression, light green lowest expression, white for not detected gene). The mRNAs encoding for GABAergic and glutamatergic markers are highly expressed in forebrain organoids (FO 4mo and 24mo), and not expressed in NSC. Glutamate transporter vGlut2 (SLC17A6) is well expressed in FO 4mo and 24mo, but vGlut1 (SLC17A7) is not detected (white bar).

Regarding the cholinergic system, the acetylcholine-producing enzyme (CHAT) is very low expressed. However, acetylcholine receptors, SLC18A3 transporter, and Acetylcholinesterase (ACHE), are well expressed in forebrain organoids.

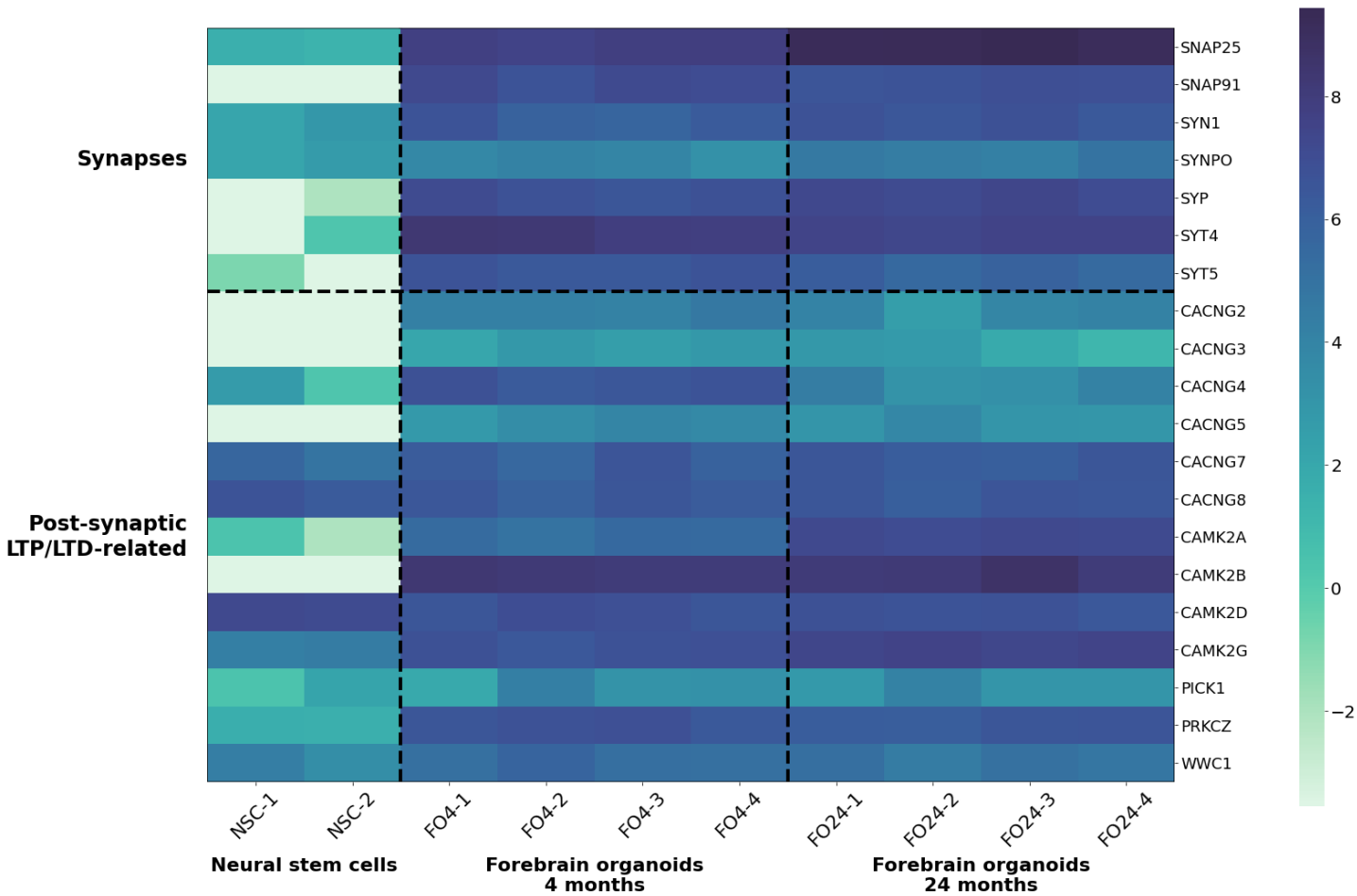
For the serotonergic system, there is a very low expression of the enzymes producing serotonin (TPH1 and TPH2), and the transporter for serotonin re-uptake (SLC6A4) is not detected (white bar). However, there is a good expression of most of the post-synaptic receptors.

Figure 3



**Figure 3. Key neurotransmitter receptor expression.** Heat map showing the distribution of genes encoding for glutamate, GABA and dopamine receptors, as well as Ca<sup>++</sup> channels (dark blue highest expression, light green lowest expression, white for not detected gene). Glutamate receptors, GABA receptors, and Ca<sup>++</sup> channel are significantly expressed in FO organoids compare to NSC. Regarding dopamine receptors (DPRs), only DPR2, DPR4, and DPR5 are consistently represented in all replicates of forebrain organoids. GRIN1 and GABBR1 expression was confirmed by qPCR.

Figure 4



**Figure 4. Expression of genes involved in synaptic plasticity.** Heat map showing the distribution of genes either encoding for synaptic proteins or involved in synaptic plasticity, based on the following publications: [NMDA Receptor-Dependent Long-Term Potentiation and Long-Term Depression \(LTP/LTD\) - PMC \(nih.gov\)](#) and [KIBRA anchoring the action of PKM \$\zeta\$  maintains the persistence of memory | Science Advances](#) (dark blue highest expression, light green lowest expression). Most of those genes are significantly expressed in FO organoids compare to NSC.