

Supporting Figures for:

Gene expression profiling of a hypoxic seizure model of epilepsy suggests a role for mTOR and Wnt signaling in epileptogenesis

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Supporting Figures Legends

(references refer to the main text)

Figure S1 Mitosis gene set under baseline developmental conditions. A. Log₂-ratios of gene expression relative to the 1h time point, in each tissue separately, are displayed as a heat map, with sampling times post-P10 indicated in hours. Colors saturate for log₂-ratio = ± 1 . Strong repression of many genes is observed, including for the genes coding for the chromosomal passenger proteins aurora kinase B (AURKB) and surviving (BIRC5), and for several centromeric proteins. B. KS plot showing the distribution of log₂-ratios at t = 1 week for both tissues. P-value and enrichment are reported for hippocampus only. Hipp = hippocampus, cx = cortex. The arrows point to some of the genes referred to in the main text.

Figure S2 Gene set containing sonic hedgehog (SHH) target genes under baseline developmental conditions. A. Strong repression of most genes in the gene set is observed by t = 1 week. B. Detailed intensity profiles for CXCR4 and TOP2A. C. KS plot showing the distribution of the log₂-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

Figure S3 Neural stem cell markers under baseline developmental conditions. A. Repression of several genes in the gene set is observed by t = 1 week, including CXCR4, CD24 and nestin (NES). B. Detailed intensity profile for CD24. c) KS plot showing the distribution of the log₂-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

Figure S4 Neuronal progenitor markers under baseline developmental conditions. A. Repression of several genes in the gene set is observed by t = 1 week, including doublecortin (DCX) and OLIG2. B. KS plot showing the distribution of the log₂-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

Figure S5 A gene set containing multiple neuronal markers under baseline developmental conditions. a) Heat map of gene profiles. b) KS plot showing the distribution of the log₂-ratios at t = 1 week for both tissues (P-value and enrichment here displayed for cortex). See Figure S1 for key to figure color and scale details.

Figure S6 A gene set containing an empirical collection of neuronal markers under baseline developmental conditions. The markers were obtained from the Allen Brain Atlas [20]. A. Heat map of gene profiles. B. KS plot showing the distribution of the log₂-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

Figure S7 A comprehensive collection of synaptic markers under the baseline developmental conditions. A. Heat map of gene profiles, showing the 22 genes most induced in cortex. Gene descriptions are given on the right, with functional categories color-coded as specified in the legend. B. Detailed intensity profiles for the chloride transporter KCC2. C. KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

Figure S8 Astrocytic markers under the baseline developmental conditions. A. Heat map of gene profiles, showing induction of several genes, including connexin 30 (GJB6), glutamine synthetase (GLUL) and GLT-1 (SLC1A2). B. Detailed intensity profiles for GFAP, AQP9 and connexin 30. c) KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues. See Figure S1 for key to figure color and scale details.

Figure S9 A gene set containing an empirical collection of oligodendrocytic markers under the baseline developmental conditions. The markers were obtained from the Allen Brain Atlas [20]. A. Heat map of gene profiles, showing very strong induction of many genes, including genes for the myelinating proteins already indicated in Figure 4 (left-hand arrows). A. KS plot showing the distribution of the log2-ratios at t = 1 week for both tissues, with very marked leading edges. See Figure S1 for key to figure color and scale details.

Figure S10 NMDA receptor components under the baseline developmental conditions. A. Heat map of gene profiles, showing induction of NR1 and NR2C and repression of NR3A and NR2D. B. Detailed log2-ratio profiles for NR1, NR2C, NR3A and NR2D in hippocampus and C, in cortex. See Figure S1 for key to figure color and scale details.

Figure S11 AMPA receptor components under the baseline developmental conditions. A. Heat map of gene profiles, showing non-significant changes in hippocampus, and repression of GLUR1 and GLUR2 in cortex. B. Detailed log2-ratio profiles for GLUR2 in hippocampus and C, for GLUR1 and GLUR2 in cortex. See Figure S1 for key to figure color and scale details.

Figure S12 GABA receptor components and glutamate decarboxylase 1 and 2 under the baseline developmental conditions. A. Heat map of gene profiles, showing generally non-significant changes. B. Detailed log2-ratio profiles for GABA_A- δ (GABRD), GABA_A- β 1 (GABRB1) and KCC2 in hippocampus and C, in cortex. See Figure S1 for key to figure color and scale details.

Figure S13 Heat map of the differential response to hypoxic seizures for the genes in the *In vitro* response (BIC or 4AP) group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. Colors represent log2-ratios of intensities of hypoxic seizure samples to that of time-matched controls (red

= positive, green = negative values, colors saturate at ± 1). Arrows point to genes referred to in the main text.

Figure S14 Heat map of the differential response to hypoxic seizures for the genes in the *Hypoxia response* group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

Figure S15 Heat map of the differential response to hypoxic seizures for the genes in the *Glutamatergic/NMDA receptor I* group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

Figure S16 Heat map of the differential response to hypoxic seizures for the genes in the *Glutamatergic/NMDA receptor II* group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

Figure S17 Heat map of the differential response to hypoxic seizures for the genes in the *Synaptic* group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

Figure S18 Heat map of the differential response to hypoxic seizures for the genes in the *Axonal Guidance* group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

Figure S19 Heat map of the differential response to hypoxic seizures for the genes in the *Autism associated* group. The group is defined by one of the clusters of enriched gene sets in the membership matrix of Figure 7. See Figure S13 for key to figure color and scale details.

Figure S20 WNT3A target genes are enriched in the hypoxic seizure response profile. Enrichment analysis with the WNT3A target gene set[27] was performed against the 12 hour profile of transcriptional response to hypoxic seizures in hippocampus. A. KS plot showing enrichment profile. B. Table of the corresponding 15 leading-edge genes. Ratios are of gene expression intensities in hypoxic seizure sample to corresponding control.

Figure S21 Expression ratios as a function of time for selected IGF-1/PI3K/mTOR pathway genes. The gene name is indicated at the top of each figure. Time at the bottom refers to the duration following hypoxic seizures; the left-hand panels (labeled “hyp”) display ratios of hypoxic seizure response to control; the right-hand panels (labeled “hyp + NBQX”) displays ratios of response to combined hypoxic seizures and NBQX treatment, to control. The expression ratio $R = 1$ (no fold change) is indicated

by the black horizontal lines. A. IRS1 displays ~ 1.6 fold induction at 12 h. B. AKT3 is very moderately but significantly induced over the time course. C and D. IGF1 and BDNF are not significantly induced.

Figure S22 Heat map of differential expression for the NBQX-responsive genes. The profiles of the 257 genes, each of which is differentially regulated by both hypoxic seizures (HS) relative to control, and by HS+NBQX relative to HS, are shown (these genes form a proper subset of the 1,399 HS-responsive genes displayed in Figure 6). Log2 ratios of gene expression between time-matched samples are shown for the ratio combinations indicated on top. Colors saturate for $\log_2\text{-ratio} = \pm 1$.

Figure S23 Expression ratios as a function of time for 11 Wnt genes represented on the microarray. The gene name is indicated at the top of each figure. Time at the bottom refers to the duration following hypoxic seizures; the left-hand panels (labeled “hyp”) display ratios of hypoxic seizure response to control; the right-hand panels (labeled “hyp + NBQX”) displays ratios of response to combined hypoxic seizures and NBQX treatment, to control. The expression ratio $R = 1$ (no fold change) is indicated by the black horizontal lines. Only WNT5A and WNT2 are significantly induced.

Figure S1

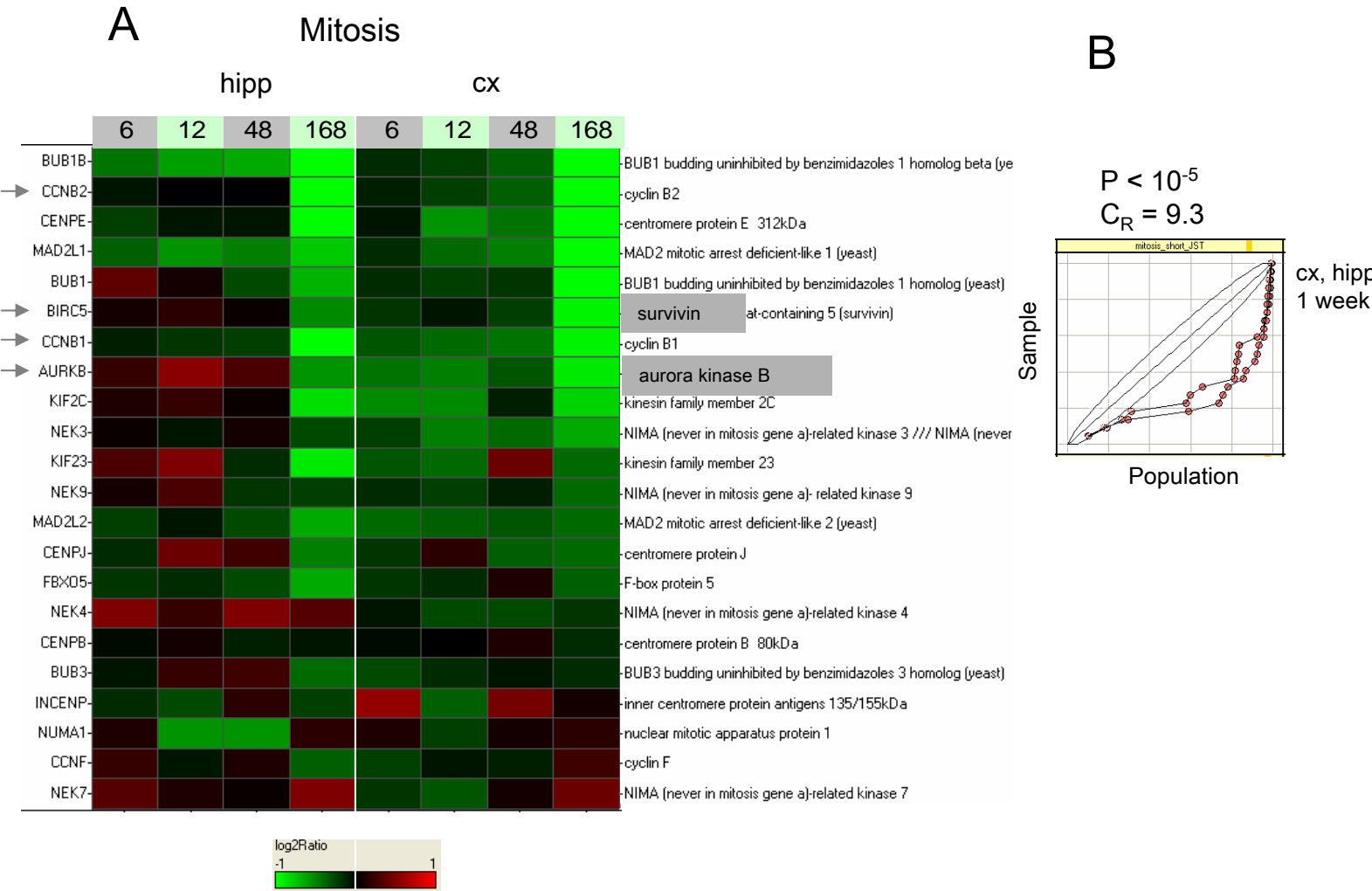


Figure S2

SHH target genes

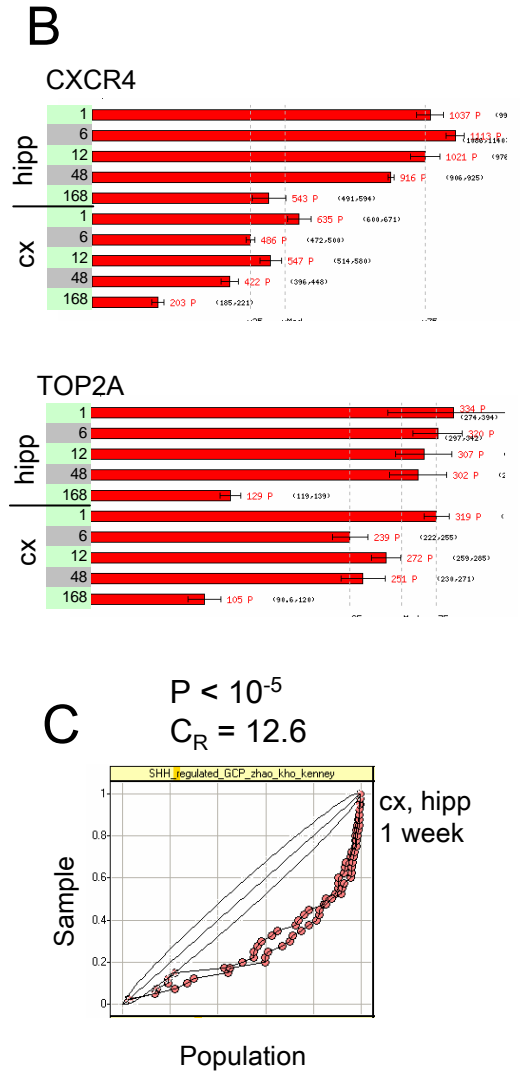
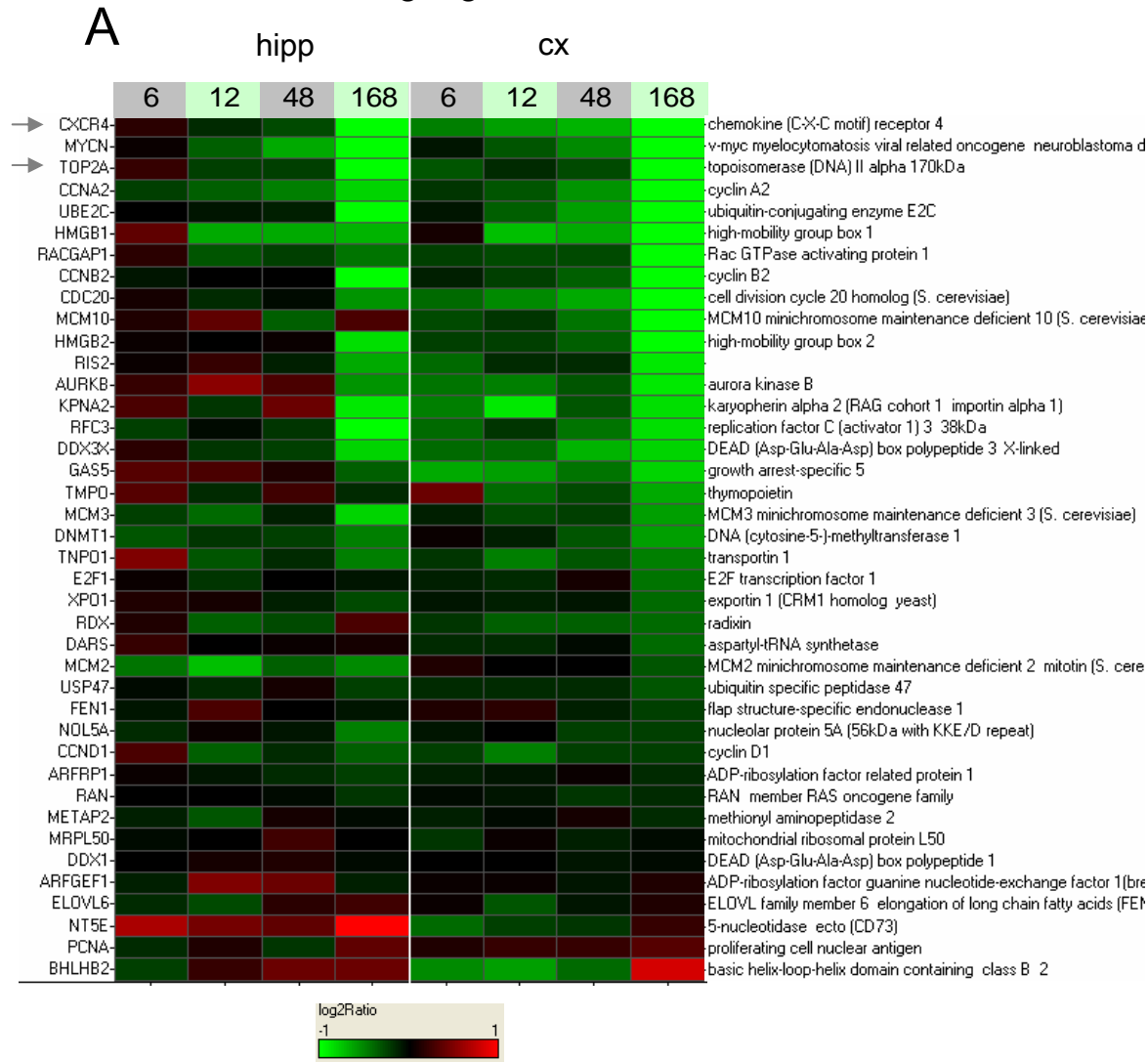


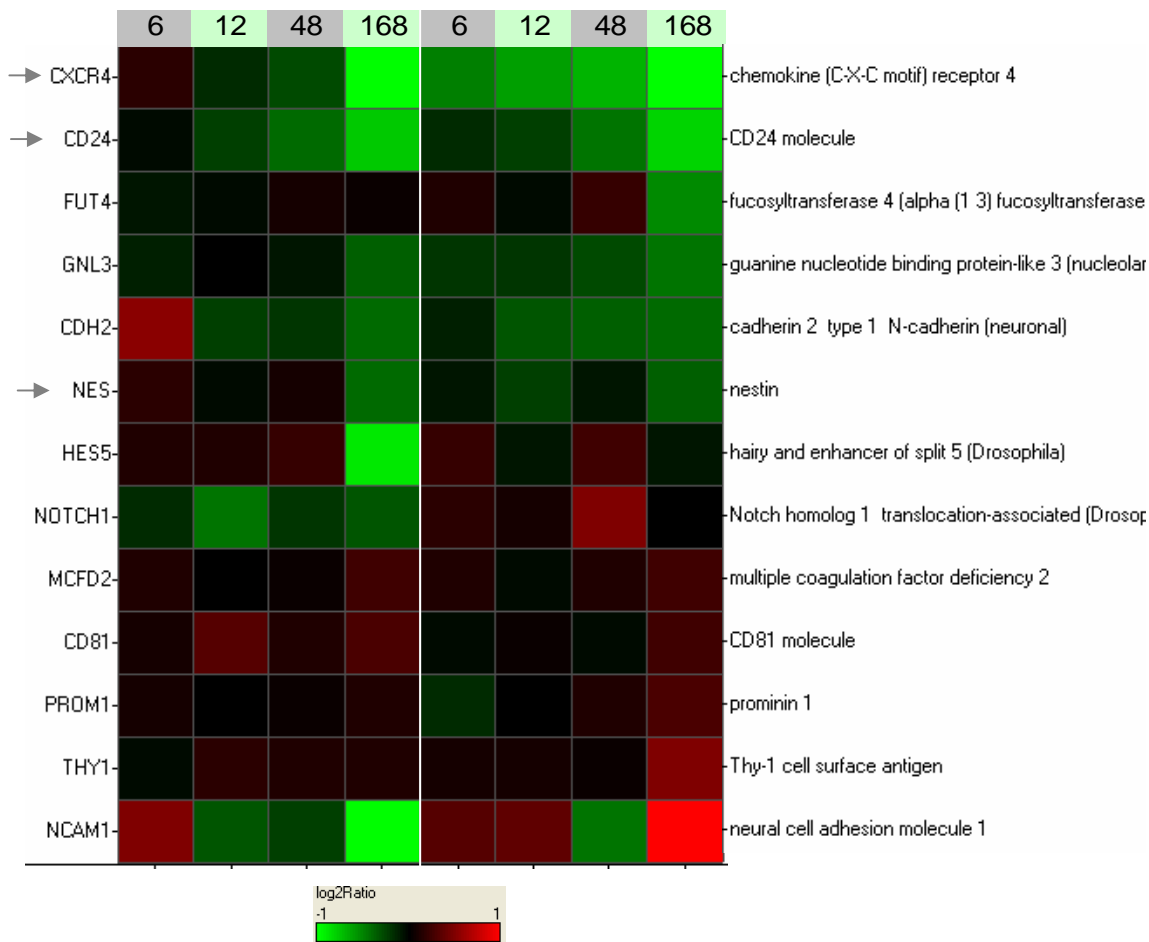
Figure S3

Neural stem cells

A

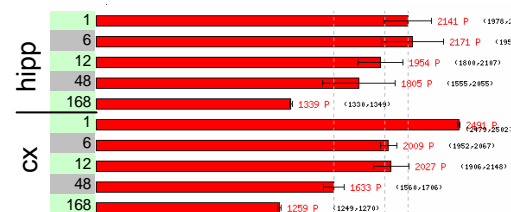
hipp

cx



B

CD24



C

$P = 1.6e-2$

$C_R = 8.6$

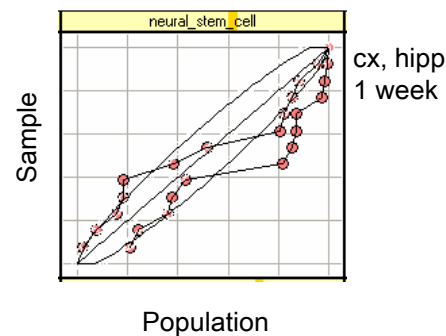


Figure S4

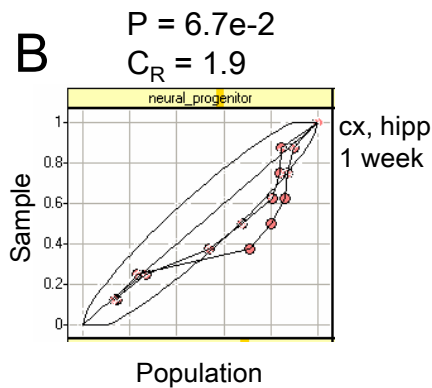
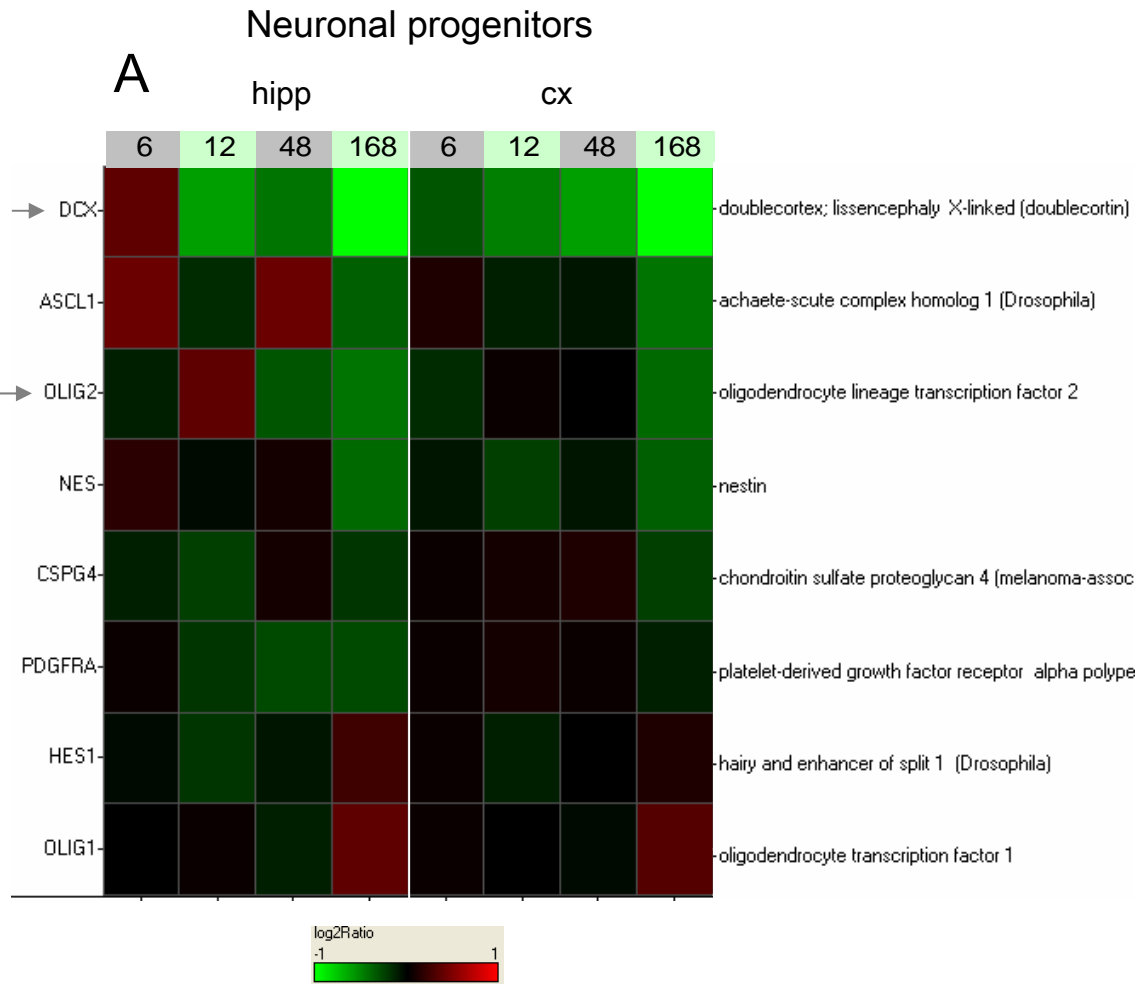


Figure S5

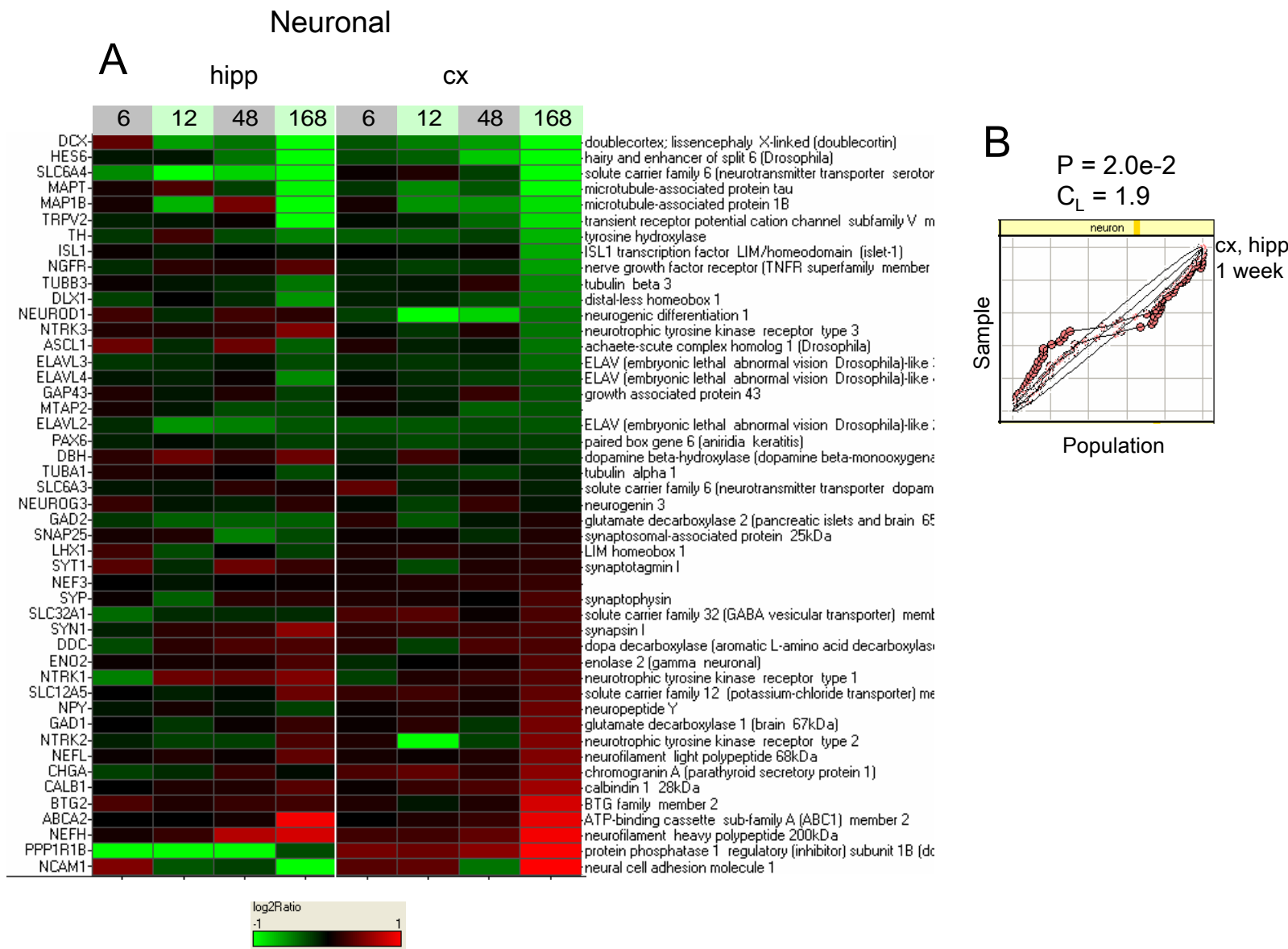


Figure S6

Neuronal (ABA)

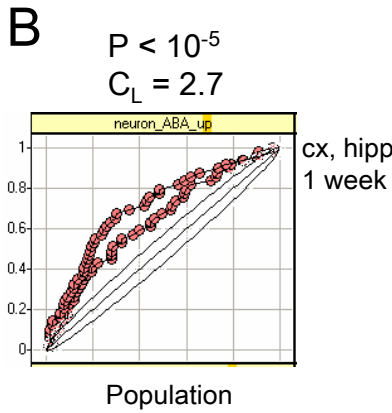
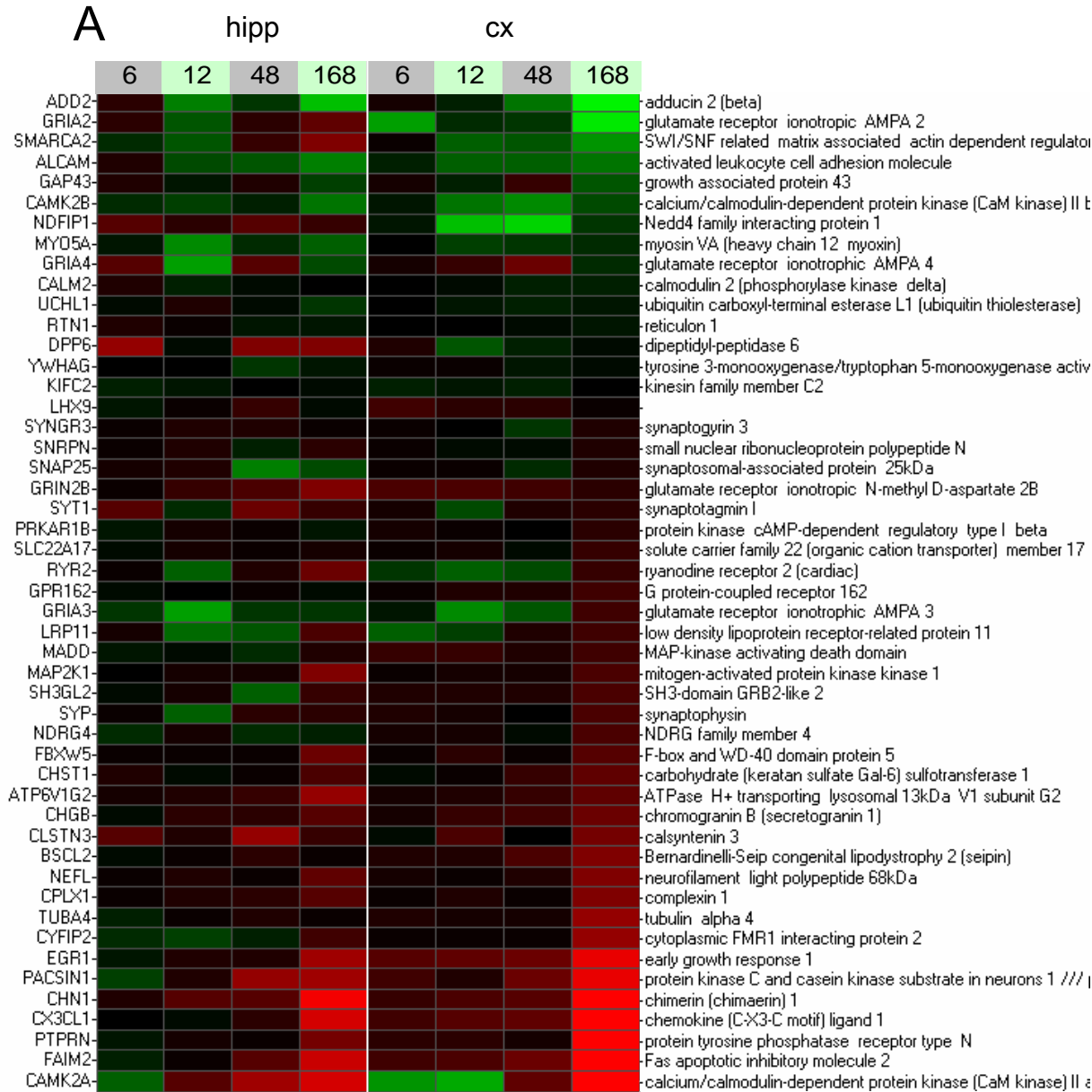
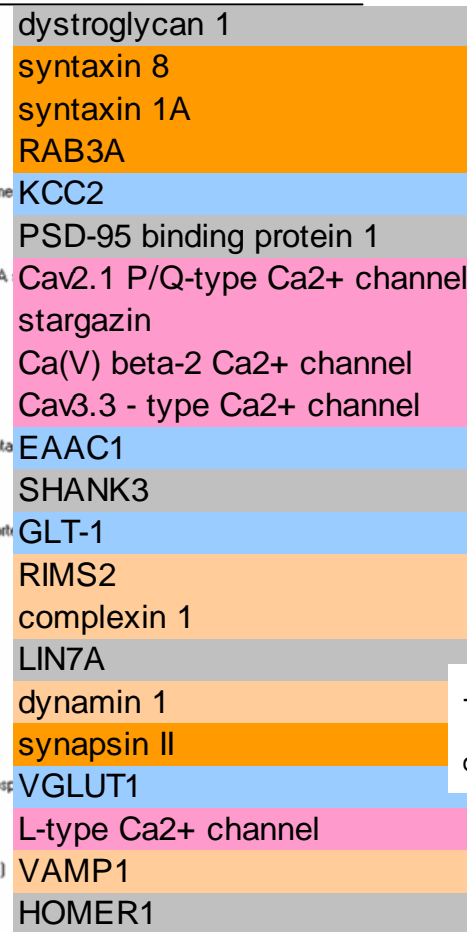
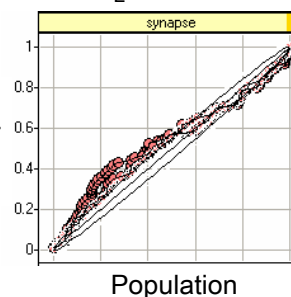


Figure 2: Heatmap of gene expression in hippocampus (hipp) and cortex (cx) for five conditions: 1, 6, 12, 48, and 168 hours. The heatmap shows expression levels for 100 genes. The color scale ranges from 0 (blue) to 100 (red). The expression levels are generally higher in the cortex (cx) than in the hippocampus (hipp). The expression levels are generally higher in the 168h condition than in the 1h condition. The expression levels are generally higher in the 1h condition than in the 6h condition. The expression levels are generally higher in the 6h condition than in the 12h condition. The expression levels are generally higher in the 12h condition than in the 48h condition. The expression levels are generally higher in the 48h condition than in the 168h condition.



- voltage-gated Ca^{2+} channels
- docking/exocytosis
- pre-and-post-synaptic adhesion
- synaptic vesicle proteins
- transporters
- scaffolding

C $P = 0.021$ cx, hipp
 $C_I = 1.6$ 1 week



B

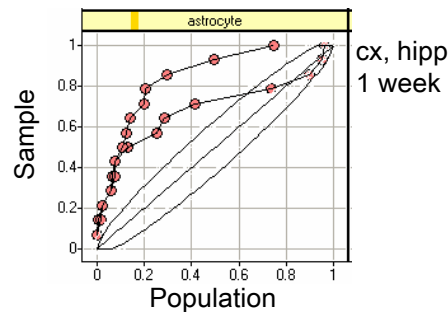
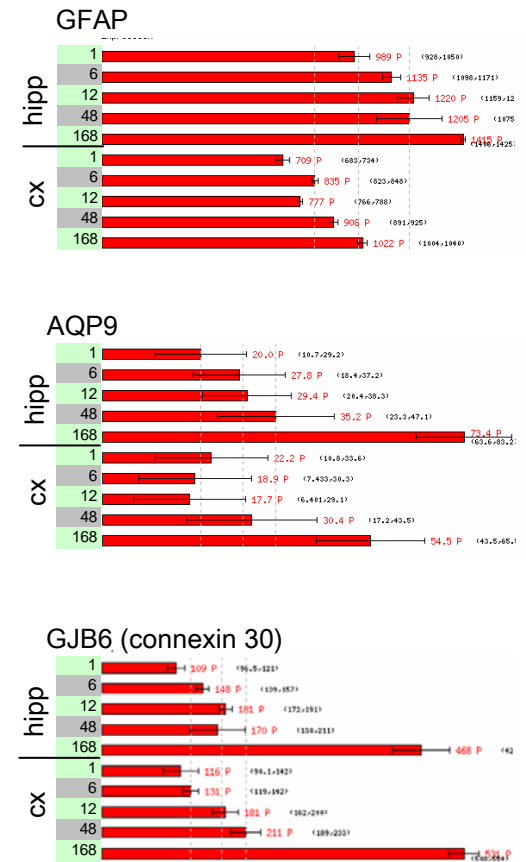


Figure S9

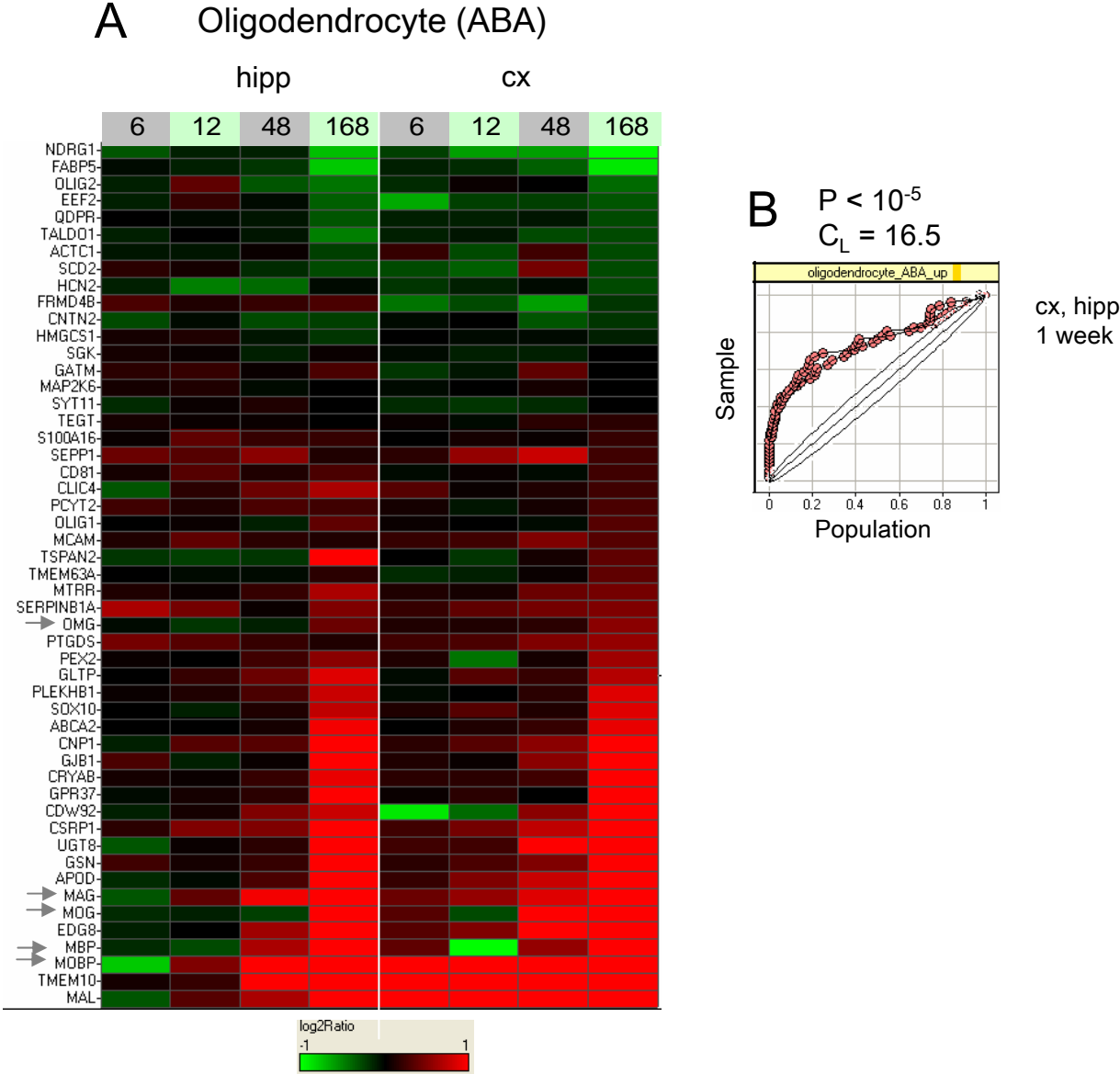


Figure S10

A Glutamatergic : NMDAR

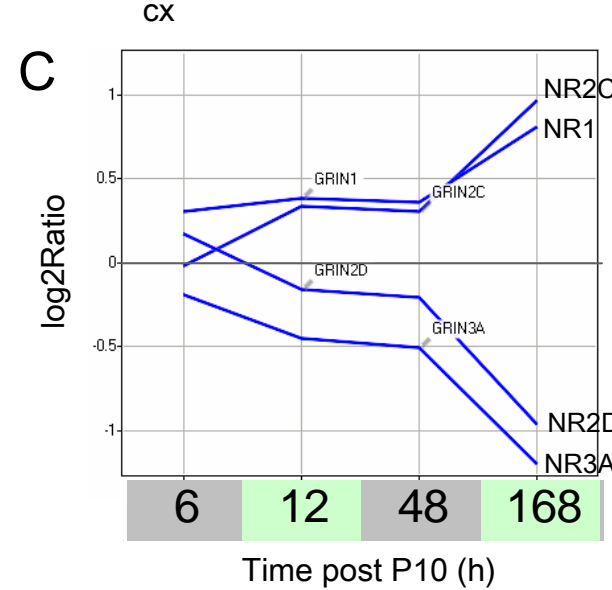
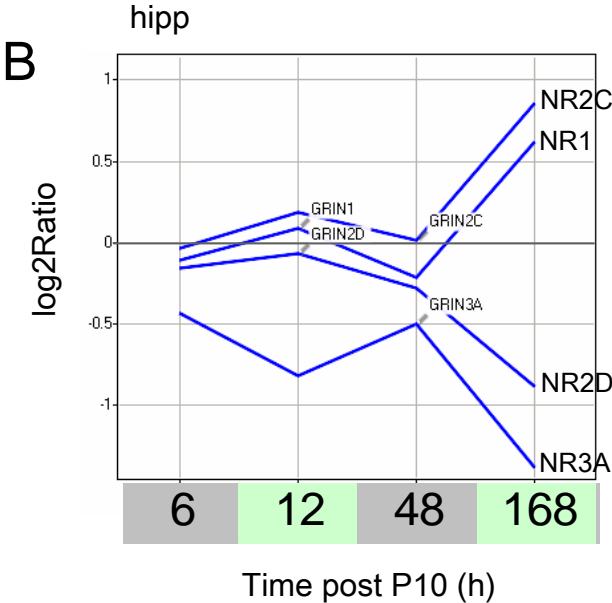
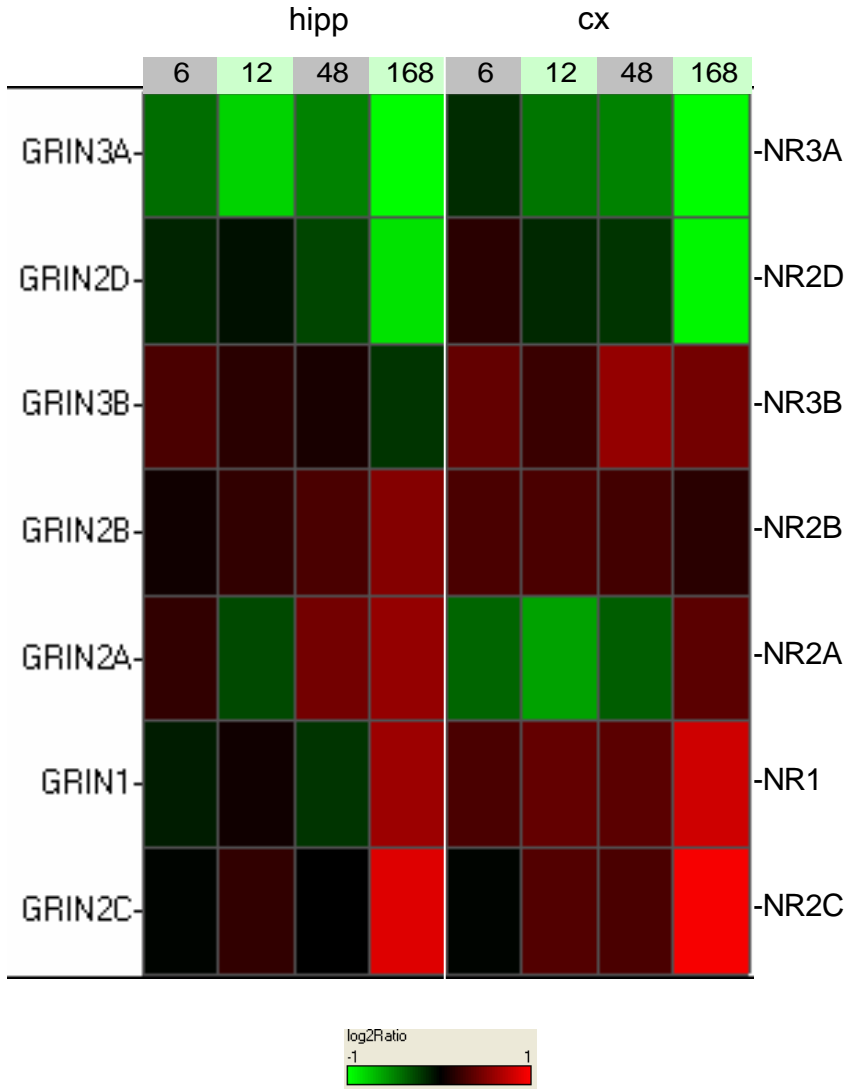


Figure S11

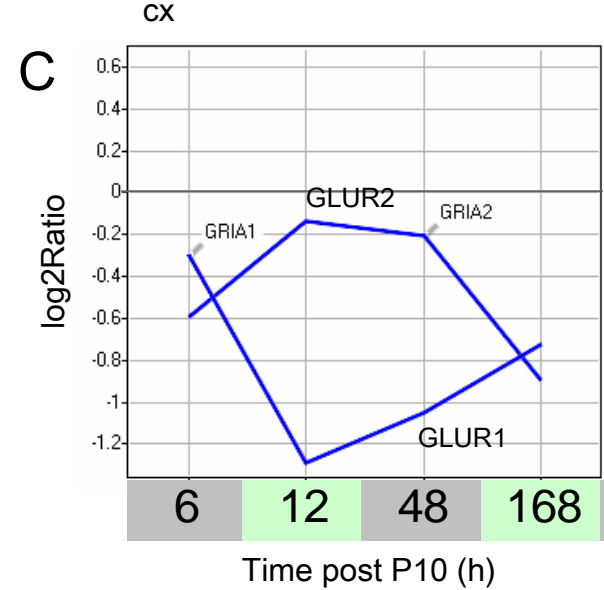
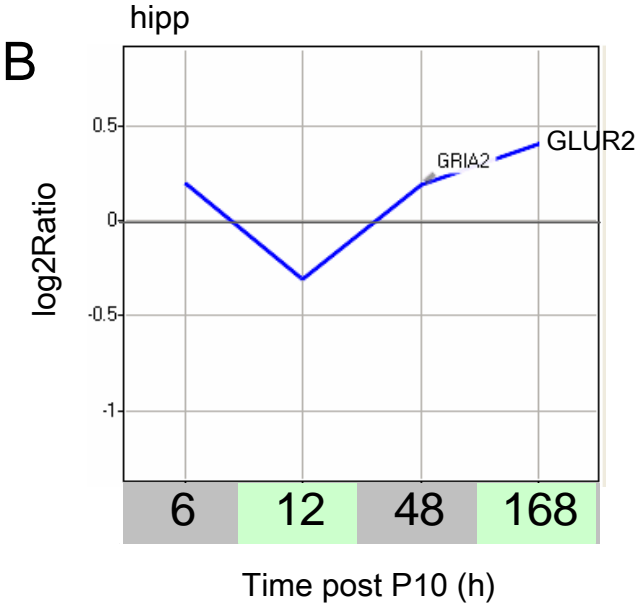
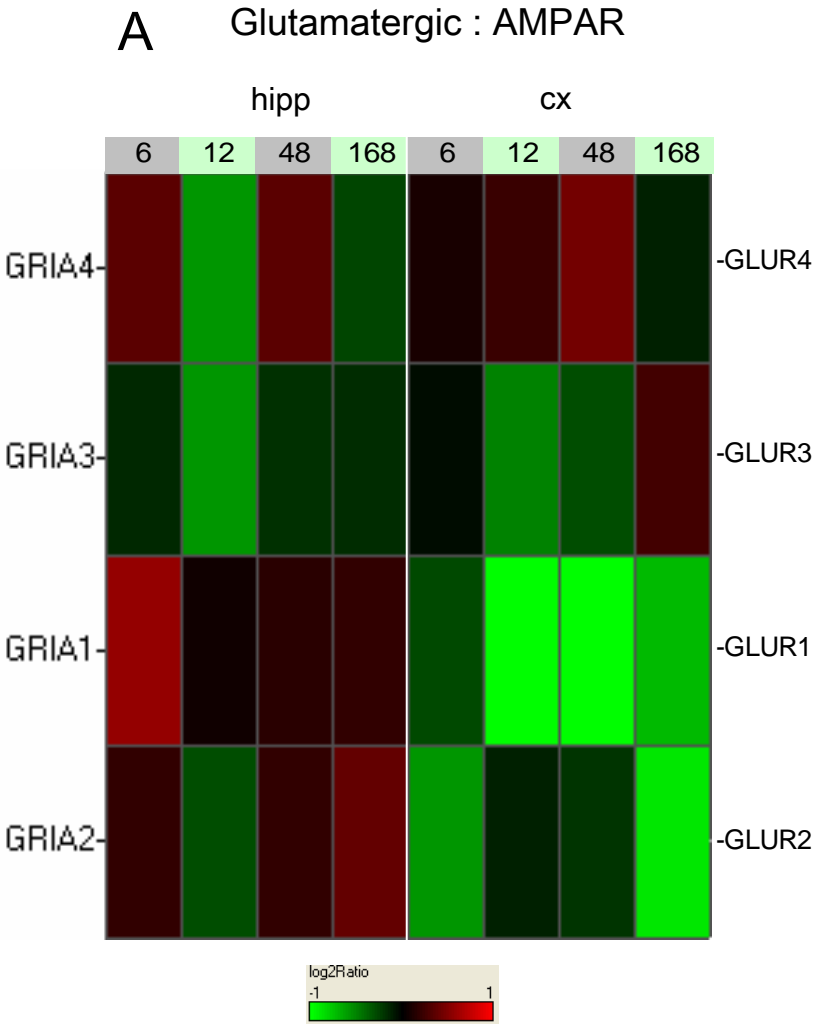


Figure S12

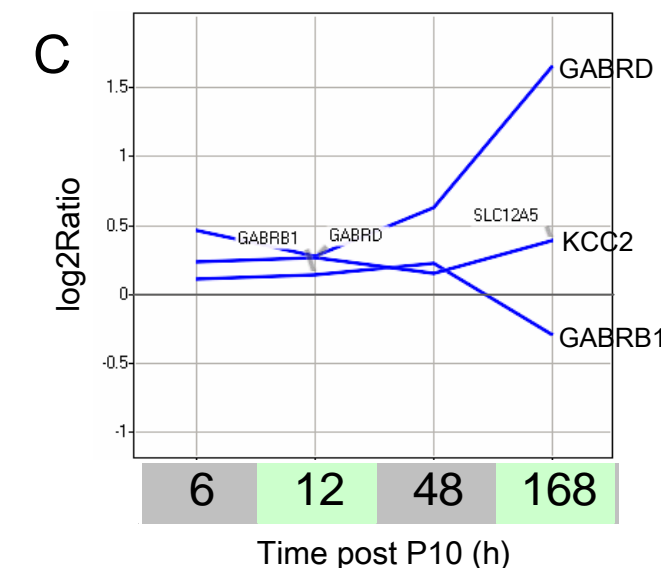
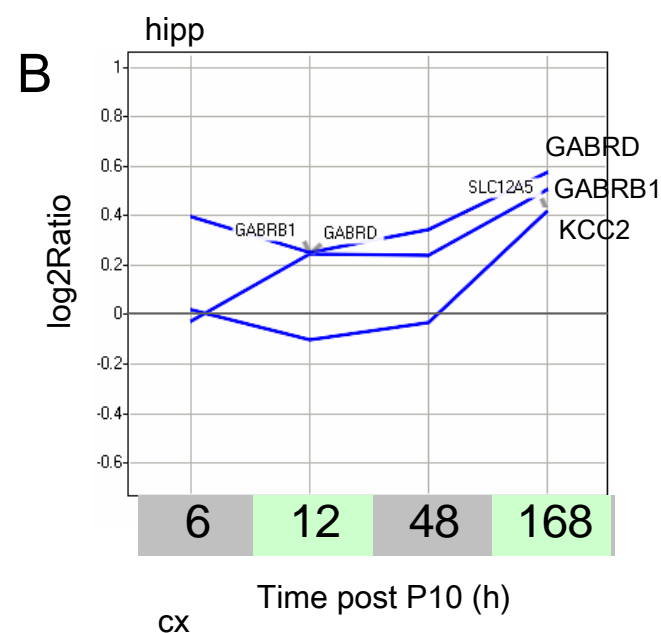
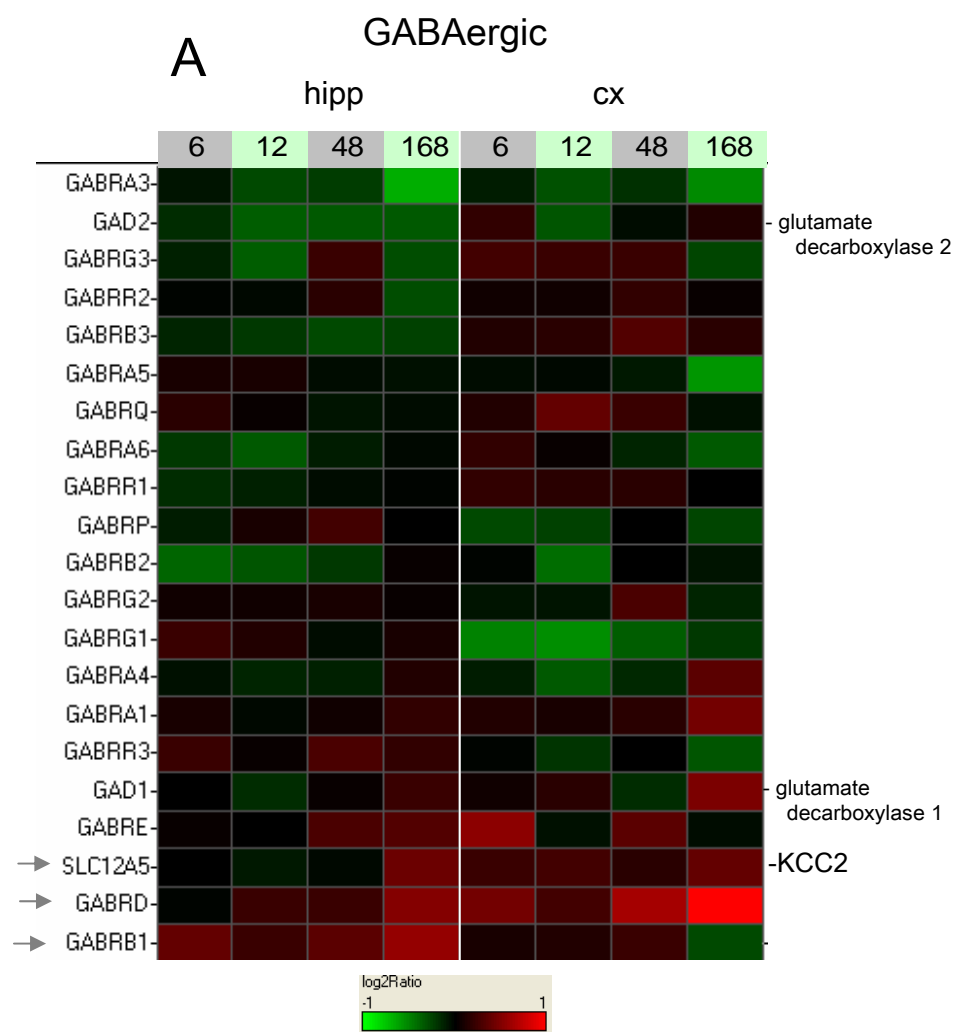


Figure S13

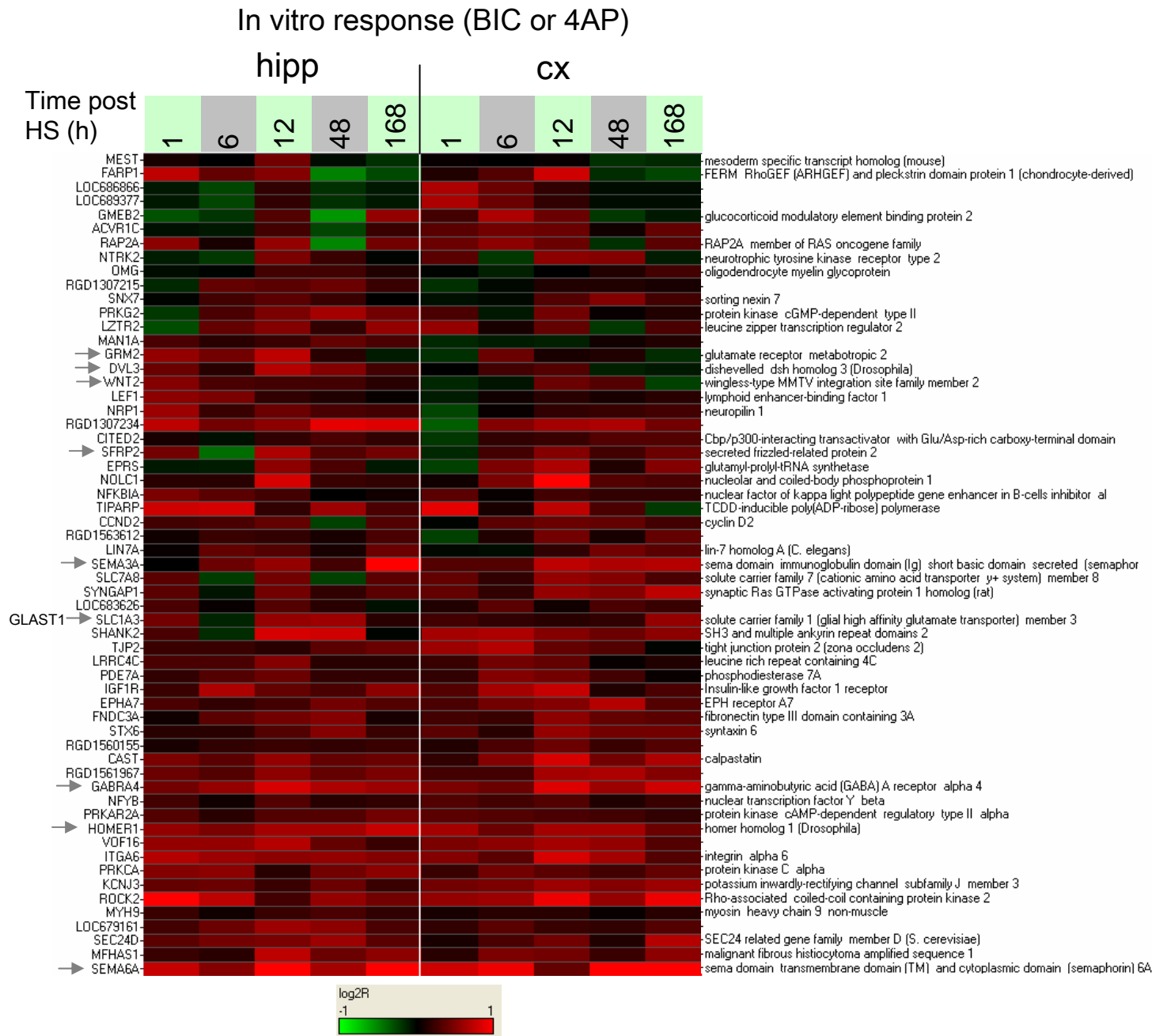


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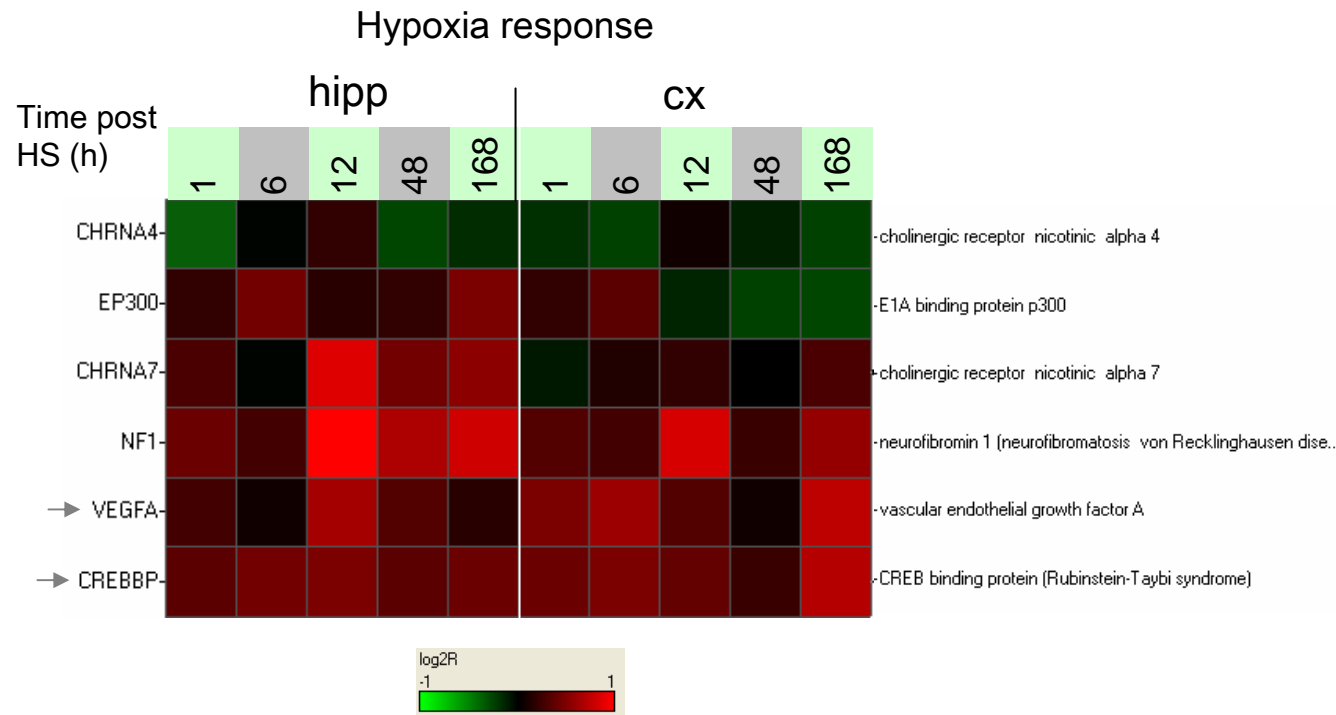


Figure S15

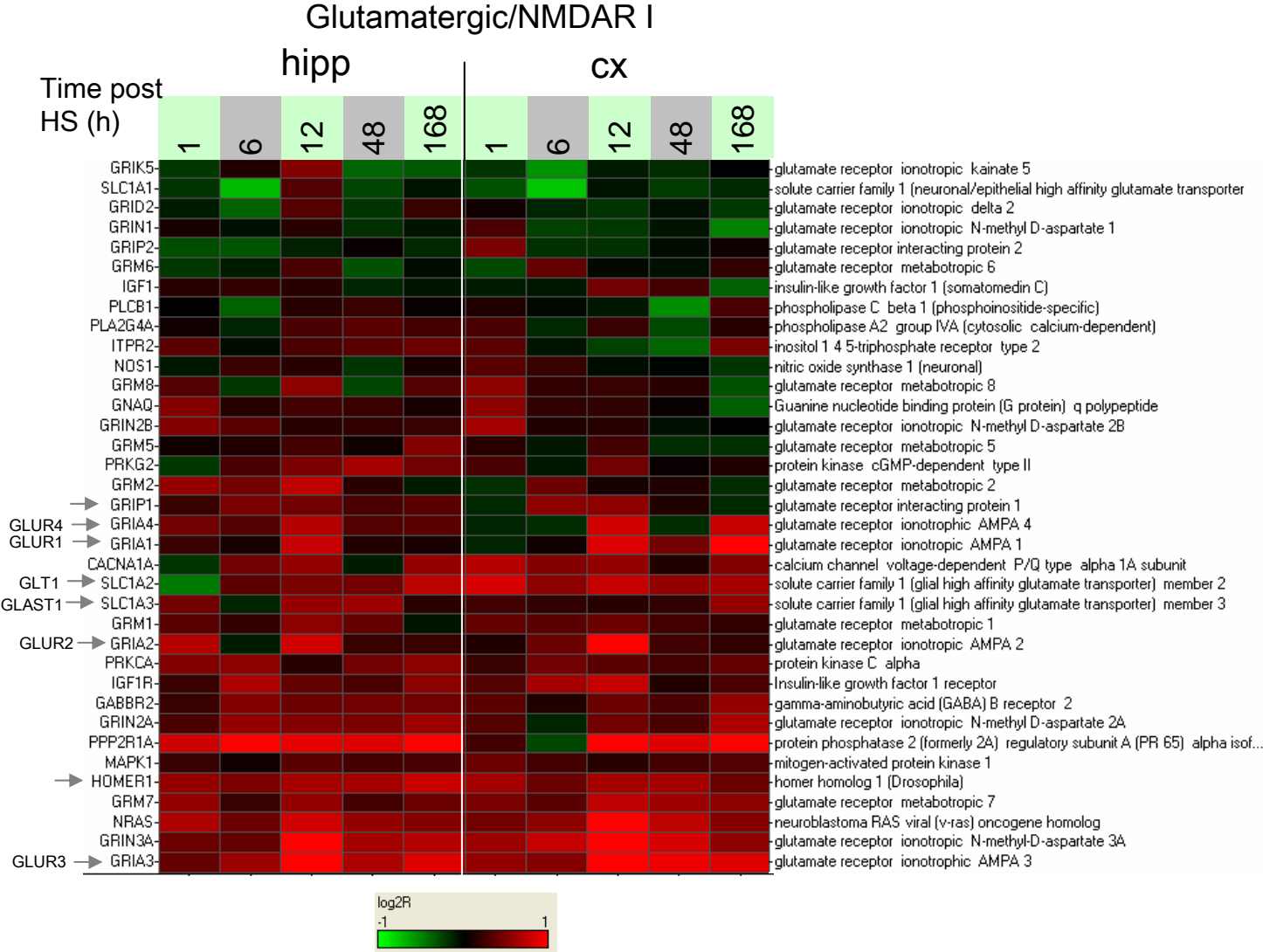


Figure S16

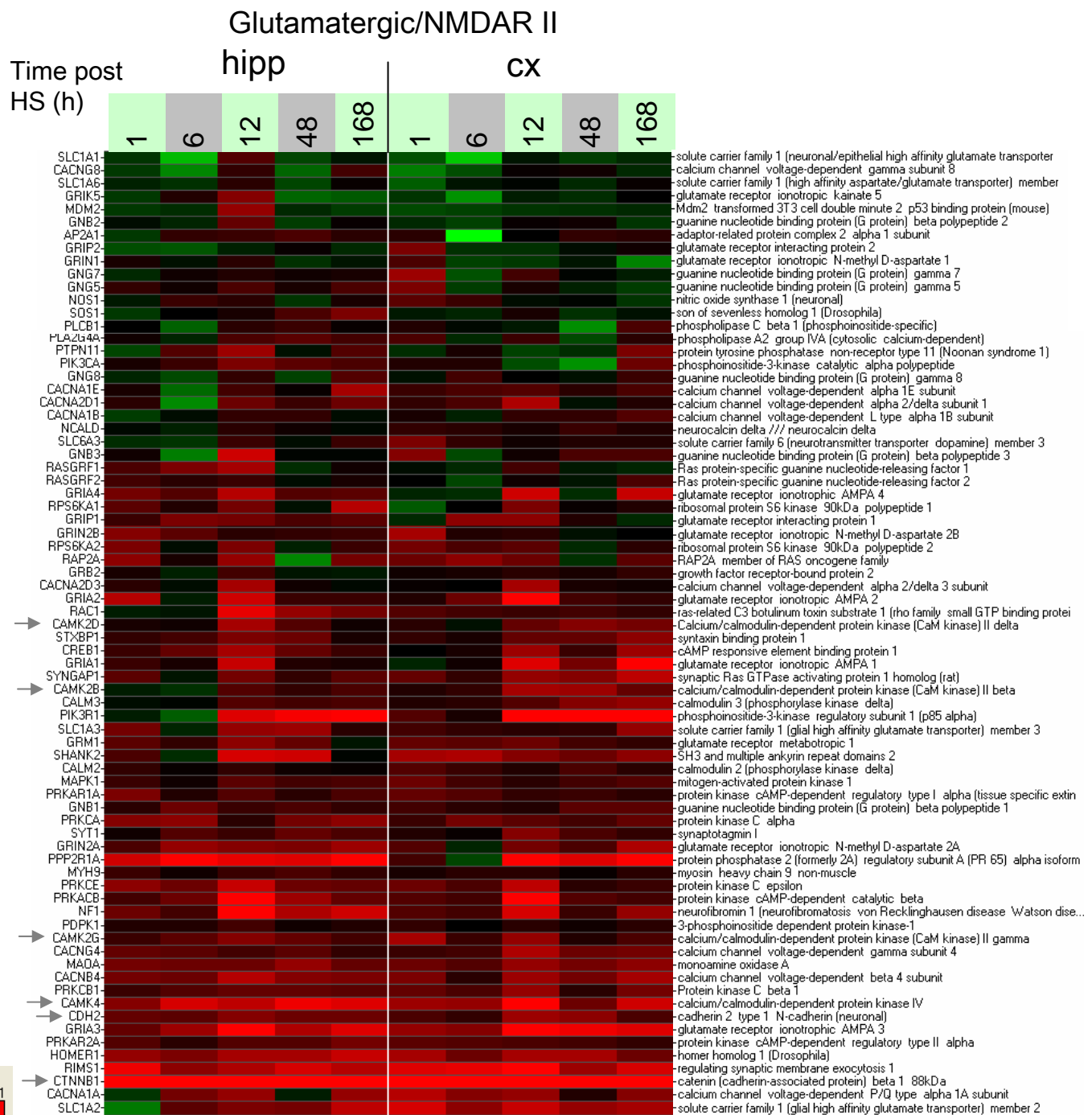


Figure S17

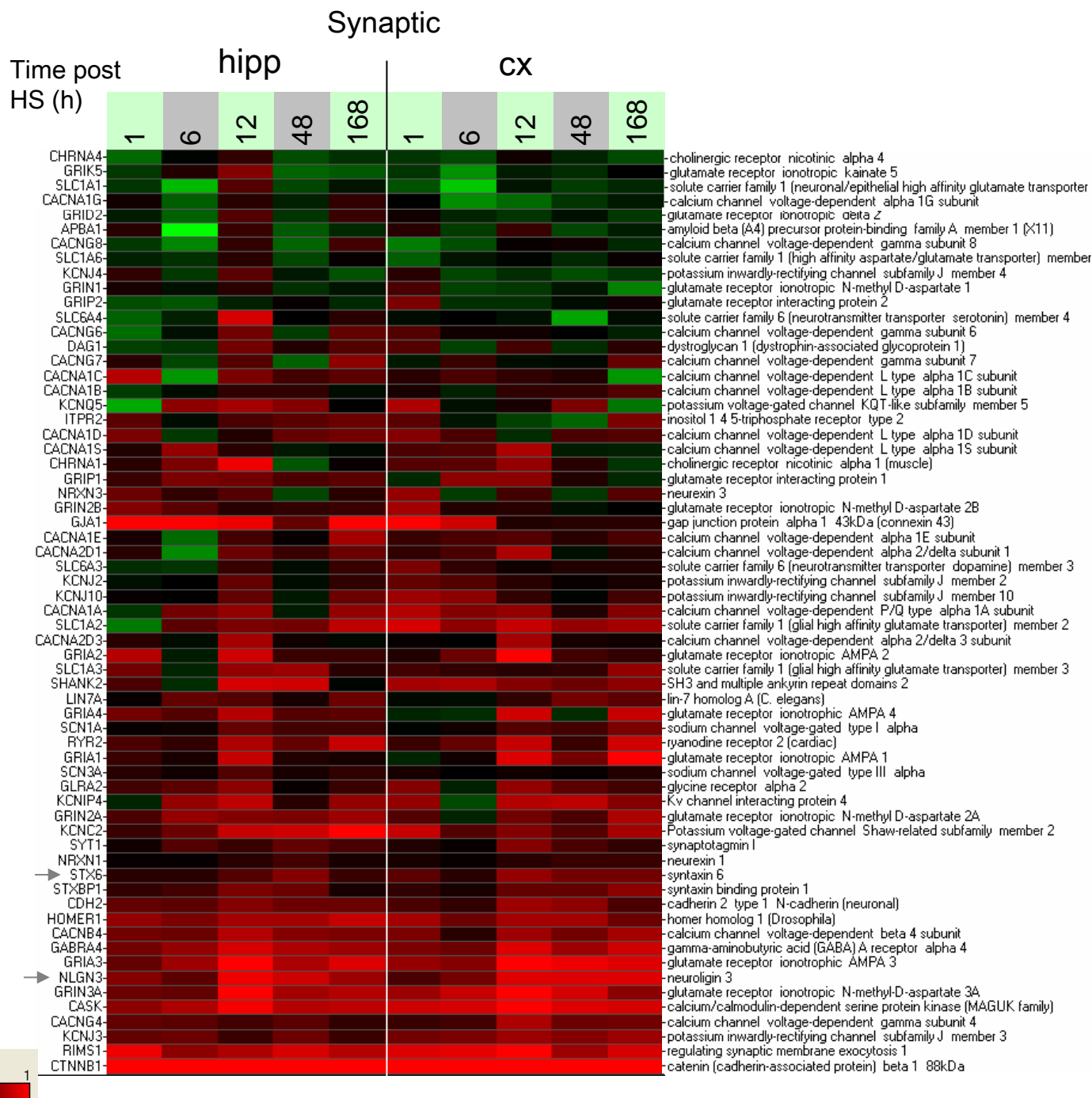


Figure S18

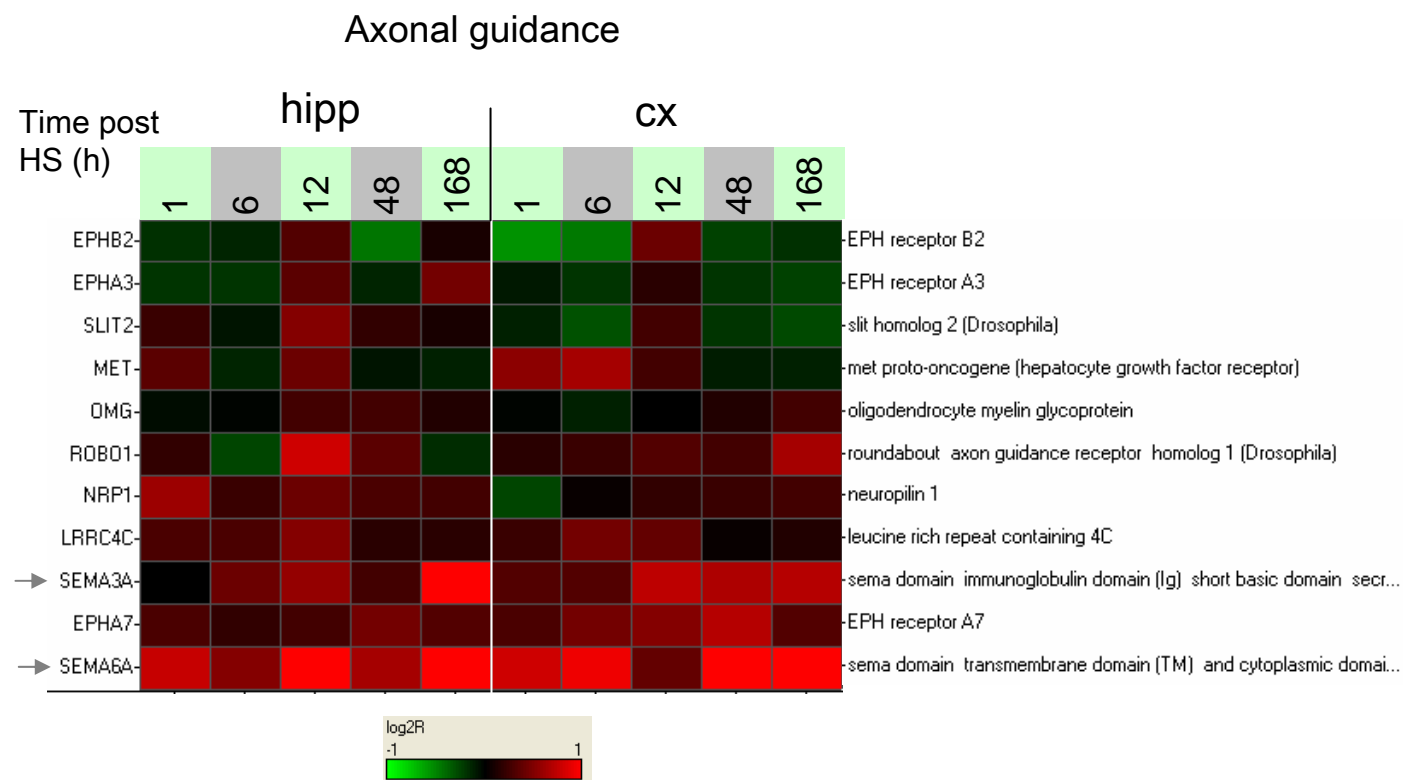


Figure S19

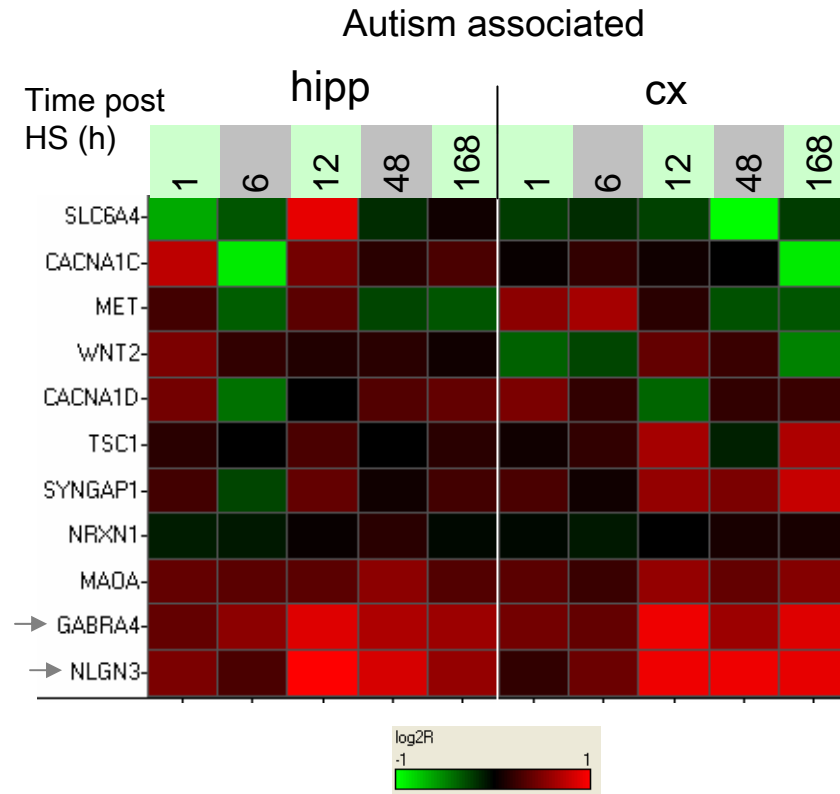


Figure S20

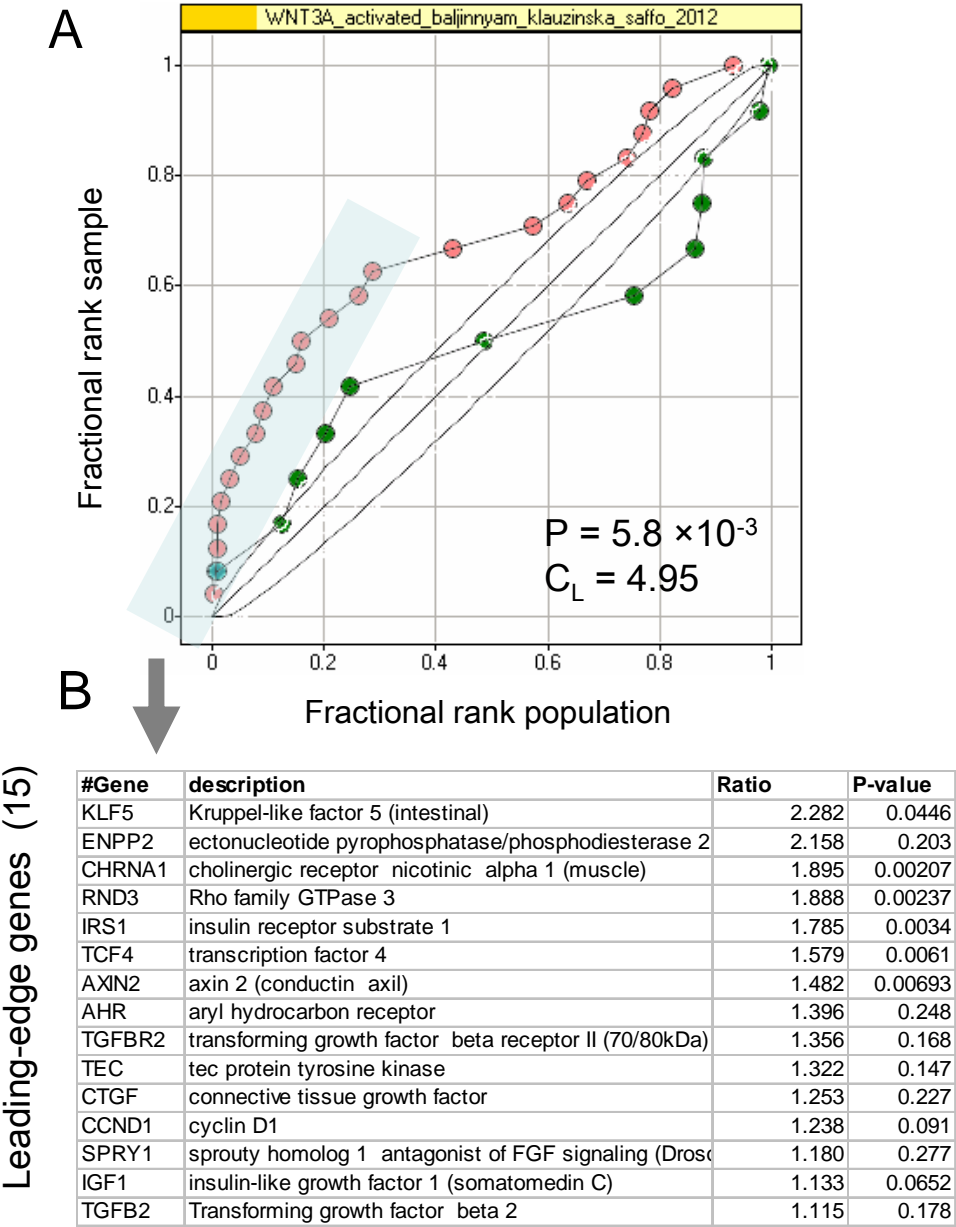


Figure S21

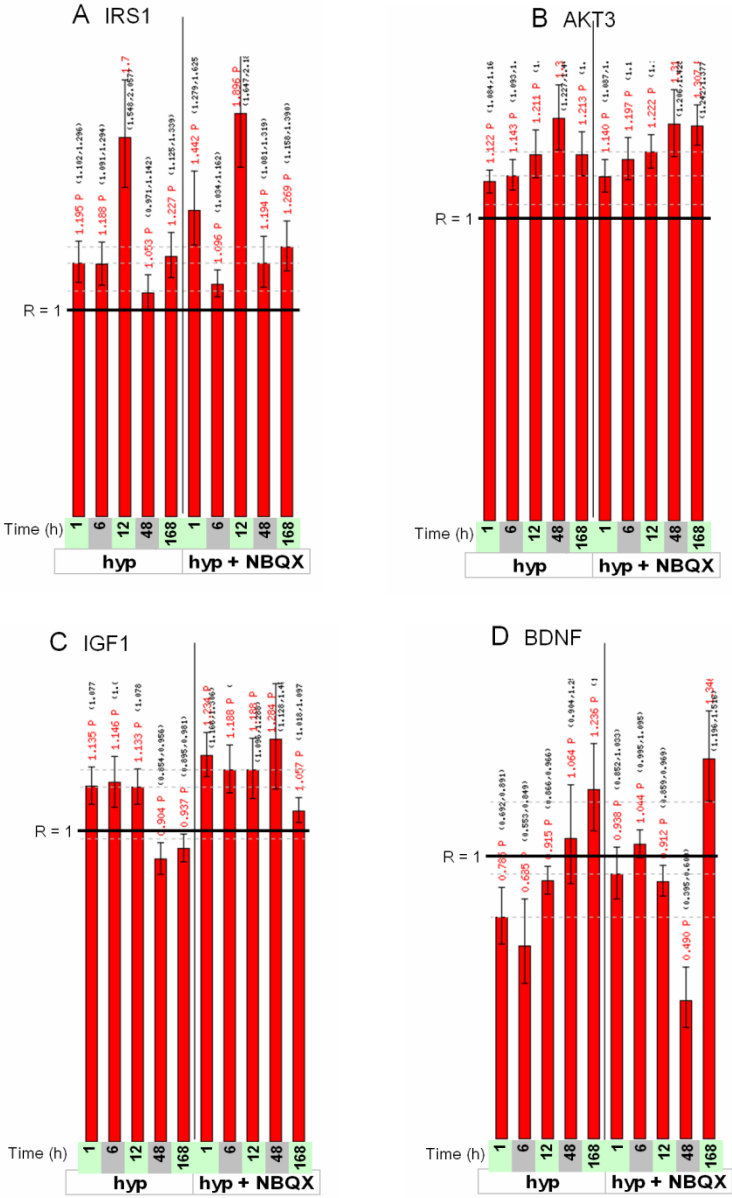


Figure S22

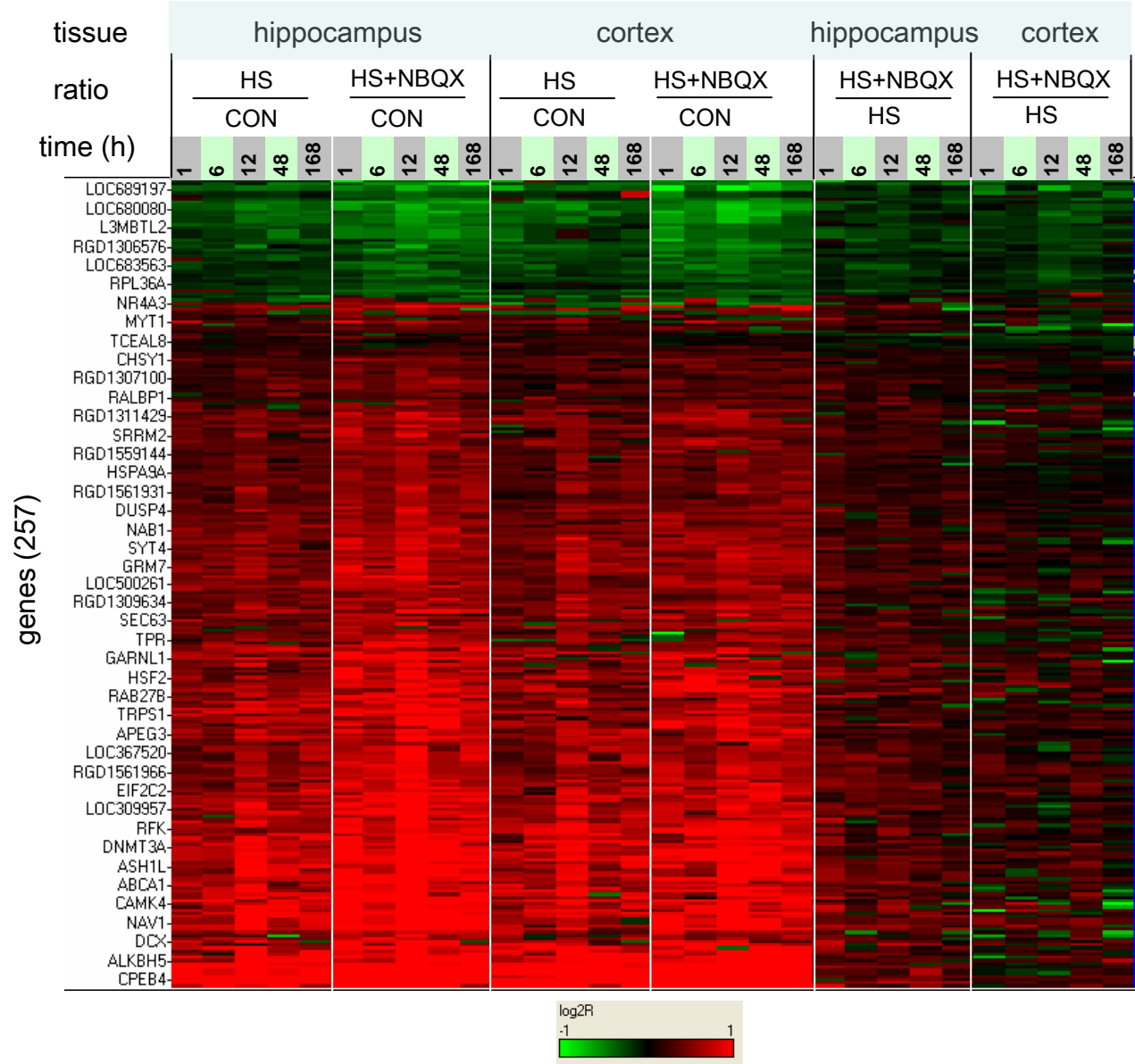


Figure S23

