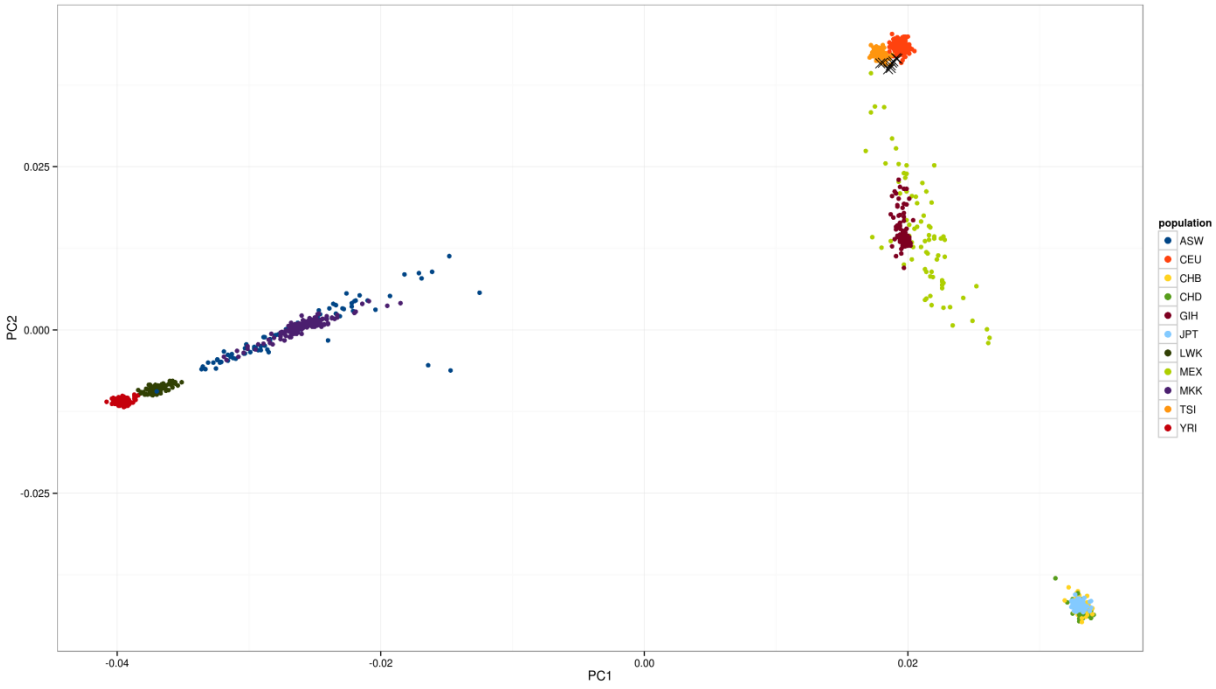
Supplementary Figure S1. Analysis of genetic background of the study cohort. Population stratification was performed using principal component analysis (PCA) and genotyping data from the HapMap Project for the following reference populations: **ASW**: African ancestry in Southwest USA; **CEU**: Utah residents with Northern and Western European ancestry from the CEPH collection; **CHB**: Han Chinese in Beijing, China; **CHD**: Chinese in Metropolitan Denver, Colorado; **GIH**: Gujarati Indians in Houston, Texas; **JPT**: Japanese in Tokyo, Japan; **LWK**: Luhya in Webuye, Kenya; **MEX**: Mexican ancestry in Los Angeles, California; **MKK**: Maasai in Kinyawa, Kenya; **TSI**: Tuscan in Italy; **YRI**: Yoruban in Ibadan, Nigeria (West Africa). The analysis shows that all 9 individuals, whose specimens were used in the study (shown as "**X**") are of European descent (**CEU** or **TSI)**.



Supplementary Figure S2. RNA-seq analysis of GABA and GLU neurons.

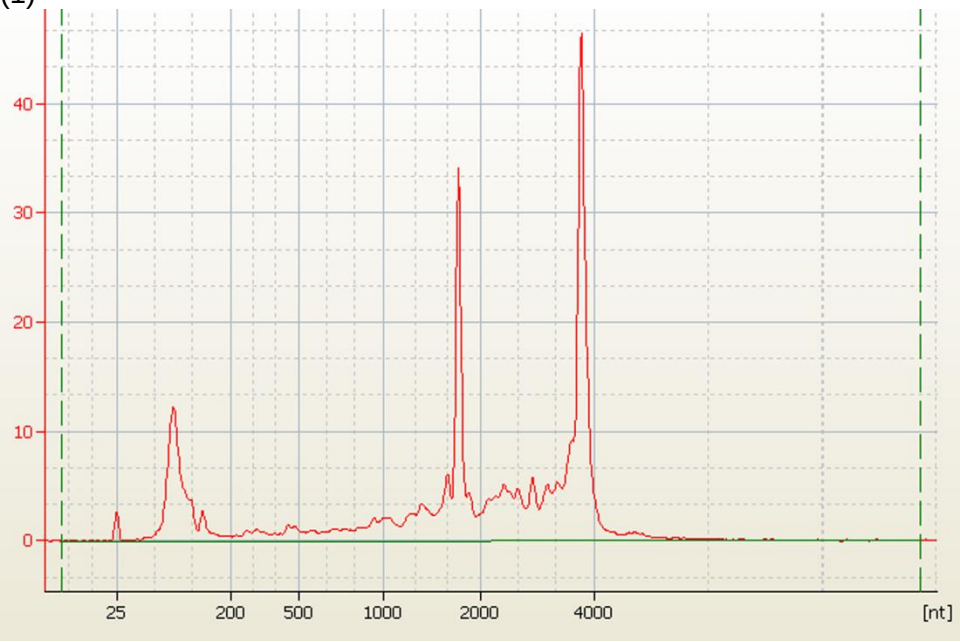
(A) Example of Bioanalyzer profiles of RNA prepared from homogenized brain tissue and sorted neuronal nuclei. Shown are RNA preparations obtained from the same subject and same brain area (orbitofrontal cortex). (1), RNA from **homogenized** tissue, prepared with ToTALLY RNA kit (Ambion). RIN = 7.9; (2), RNA from FACS-sorted NeuN(+)SOX6(-) neuronal nuclei. RIN =4.2.

(B) Distribution of uniquely mapped RNA-seq reads among exons, introns, and intergenic regions. A large proportion of RNA-seq reads maps to intronic regions.

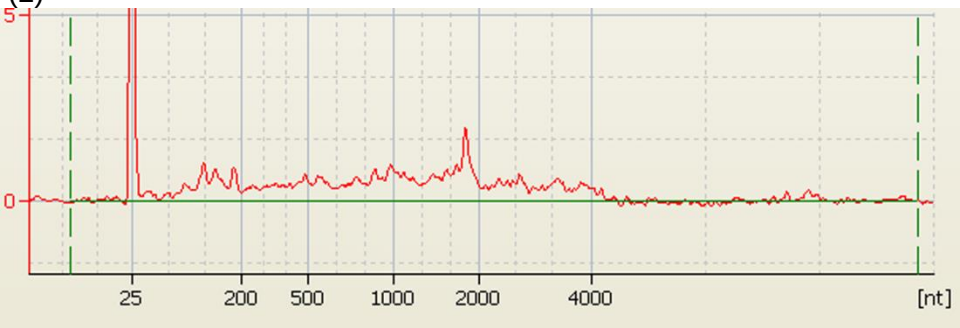
(C) Pairwise comparisons of gene expression in GABA and GLU neurons. For each neuronal subtype gene expression was measured by RNA-seq in replicate experiments in subjects 1 and 2. ***r***, Spearman correlation of log10-transformed RNA-seq read counts**.**

A

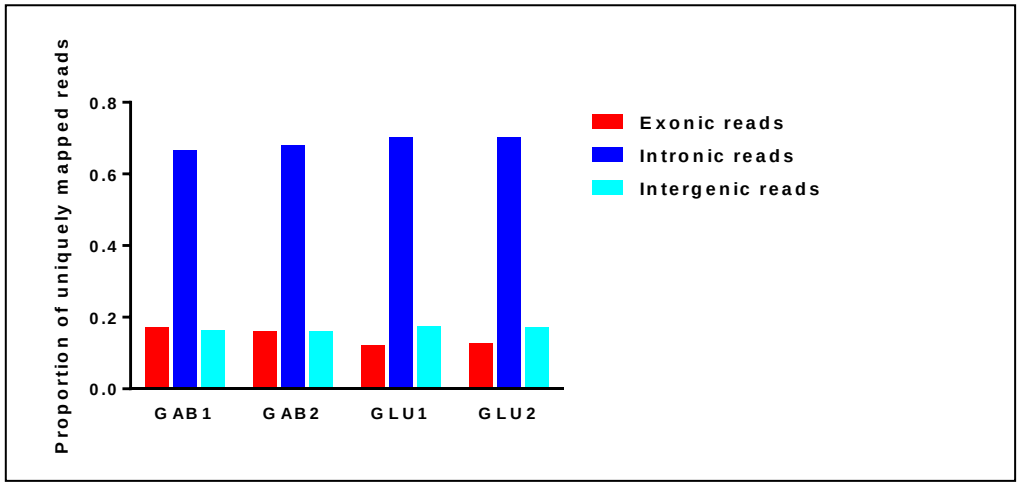
(1)



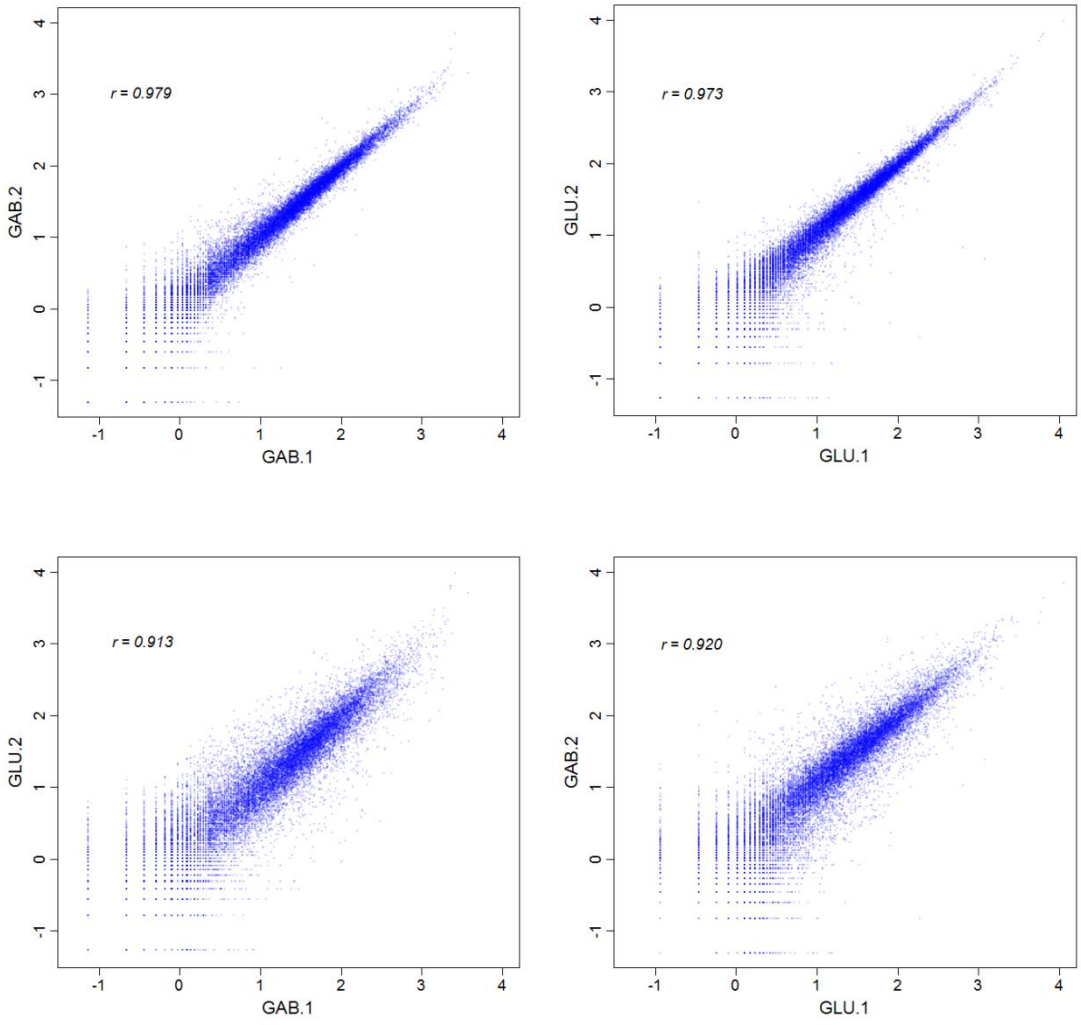
(2)



B


Proportion of uniquely mapped reads
0.8
0.6
0.4
0.2
0.0
GAB1
GAB2
GLU1
GLU2
Exonic reads
Intronic reads
Intergenic reads

C

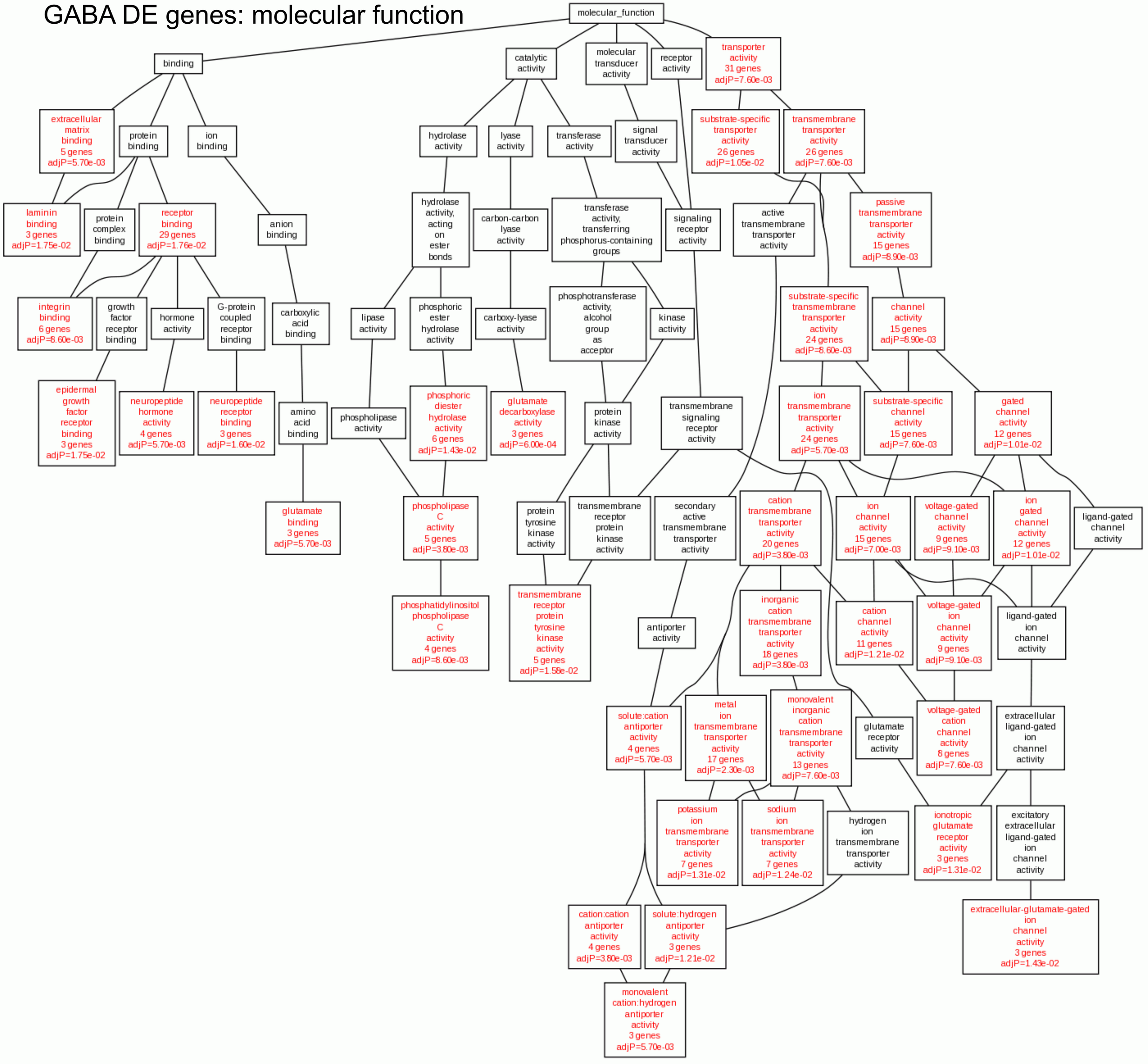

r = 0.979
GAB.2
GAB.1
r = 0.973
GLU.2
GLU.1
r = 0.913
GLU.2
GAB.1
r = 0.920
GAB.2
GLU.1

Supplementary Figure S3. Gene ontology analysis of GABA-DE and GLU-DE gene lists using WebGestalt. The analysis showed enrichment for ontologies related to nervous system for both GABA-DE genes and GLU-DE genes (e.g., “synaptic transmission”, “neuron differentiation”). The analysis also showed enrichment for categories that are specific to GABA neurons (e.g., “glutamatergic amino acid catabolic process”, “clathrin-sculpted GABA transport vesicle membrane”) or GLU neurons (e.g., “microtubule motor activity”, “dynein complex”) neurons.

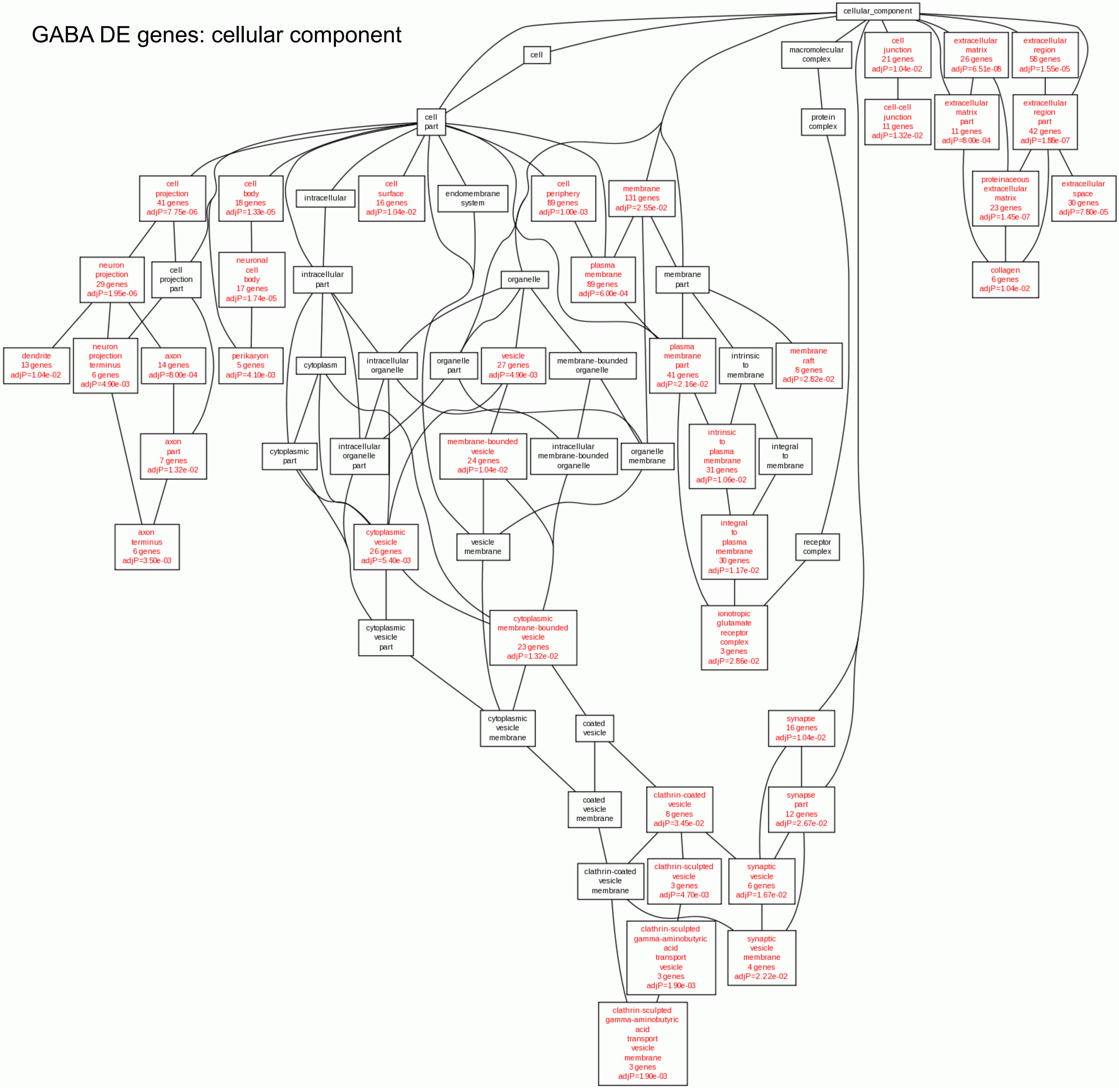
GABA DE genes: biological process

biological_process
response to stimulus 131 genes adjP=2.32e-05
biological regulation
signaling 112 genes adjP=1.39e-08
single-organism process 156 genes adjP=1.39e-08
multicellular organismal process 134 genes adjP=8.77e-11
metabolic process
regulation of biological quality 66 genes adjP=1.42e-05
regulation of biological process
developmental process 102 genes adjP=6.46e-06
single organism signaling 112 genes adjP=1.39e-08
single-multicellular organism process 133 genes adjP=8.77e-11
cellular process
catabolic process
single-organism metabolic process
cell proliferation
positive regulation of biological process
cellular response to stimulus
regulation of cellular process
cell communication 112 genes adjP=6.86e-08
cellular developmental process 70 genes adjP=3.75e-05
regulation of developmental process 42 genes adjP=2.00e-04
anatomical structure development 91 genes adjP=2.80e-05
regulation of signaling 56 genes adjP=3.03e-05
regulation of multicellular organismal process 55 genes adjP=1.57e-06
multicellular organismal development 95 genes adjP=3.25e-06
multicellular organismal signaling 35 genes adjP=9.78e-08
system process 54 genes adjP=9.79e-07
cellular metabolic process
single-organism catabolic process
small molecule metabolic process
organic substance metabolic process
nitrogen compound metabolic process
epithelial cell proliferation
regulation of cell proliferation
positive regulation of cellular process
signal transduction 92 genes adjP=3.75e-05
regulation of cell communication 56 genes adjP=3.20e-05
cell differentiation 69 genes adjP=1.24e-05
anatomical structure morphogenesis 56 genes adjP=3.75e-05
cell-cell signaling 37 genes adjP=3.75e-05
regulation of multicellular organismal development 36 genes adjP=8.45e-05
system development 86 genes adjP=3.01e-06
neurological system process 43 genes adjP=3.01e-06
cellular catabolic process
small molecule catabolic process
organic acid metabolic process
organic substance catabolic process
organonitrogen compound metabolic process
regulation of epithelial cell proliferation 13 genes adjP=2.00e-04
positive regulation of cell proliferation
cell surface receptor signaling pathway 65 genes adjP=2.81e-06
regulation of cell differentiation 32 genes adjP=6.00e-04
cell development 51 genes adjP=1.51e-07
anatomical structure formation involved in morphogenesis 47 genes adjP=4.05e-05
nervous system development 53 genes adjP=3.23e-06
transmission of nerve impulse 34 genes adjP=1.51e-07
organic acid catabolic process
oxoacid metabolic process
organonitrogen compound catabolic process
positive regulation of epithelial cell proliferation 10 genes adjP=2.00e-04
G-protein coupled receptor signaling pathway
neuropeptide signaling pathway 9 genes adjP=2.00e-04
neurogenesis 38 genes adjP=3.56e-05
generation of neurons 36 genes adjP=5.12e-05
neuron differentiation 36 genes adjP=1.24e-05
central nervous system development 25 genes adjP=5.00e-04
synaptic transmission 32 genes adjP=1.40e-07
carboxylic acid metabolic process
primary metabolic process
carboxylic acid catabolic process
cellular amino acid metabolic process
cellular amino acid catabolic process
glutamine family amino acid metabolic process
glutamine family amino acid catabolic process 5 genes adjP=5.00e-04

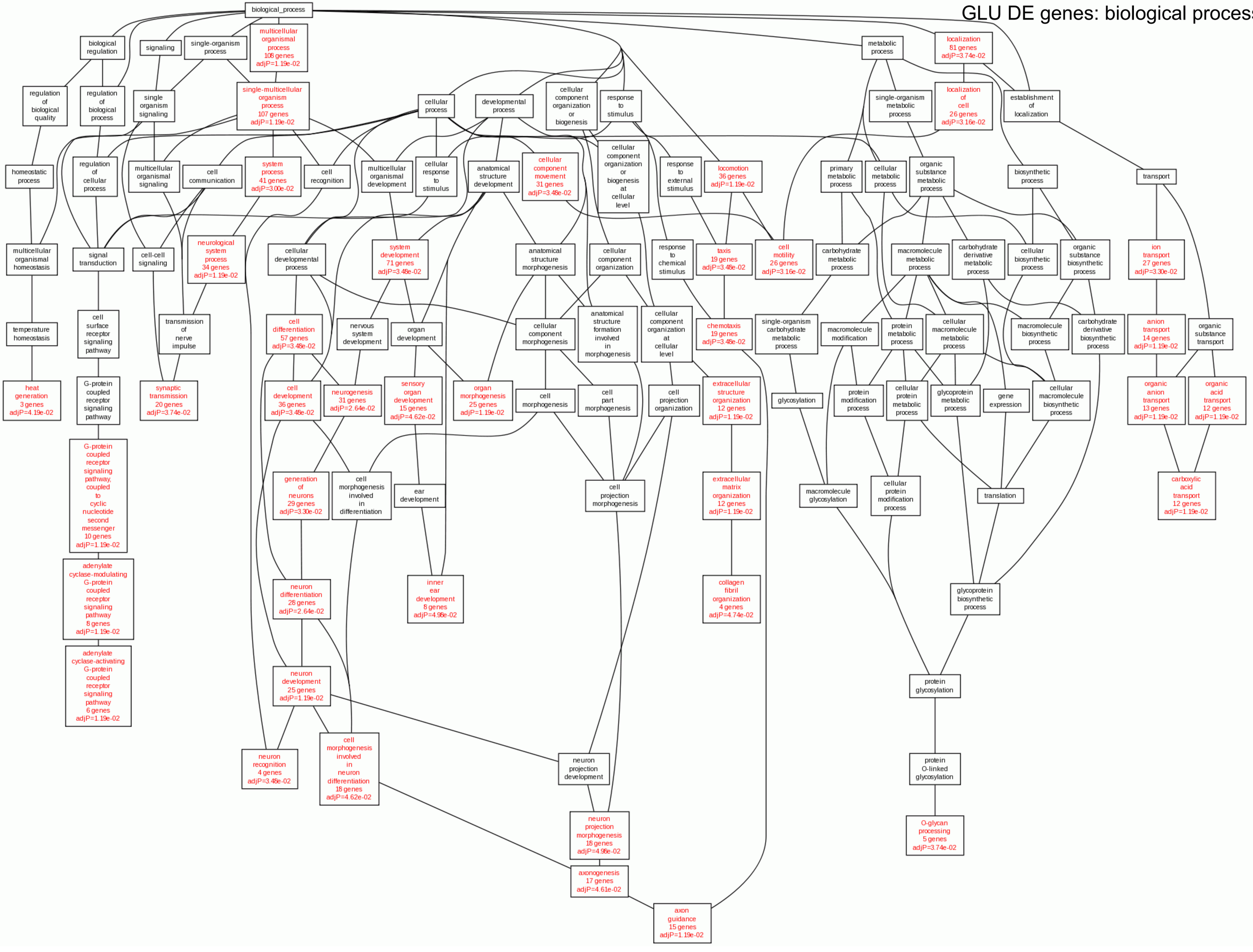
GABA DE genes: molecular function



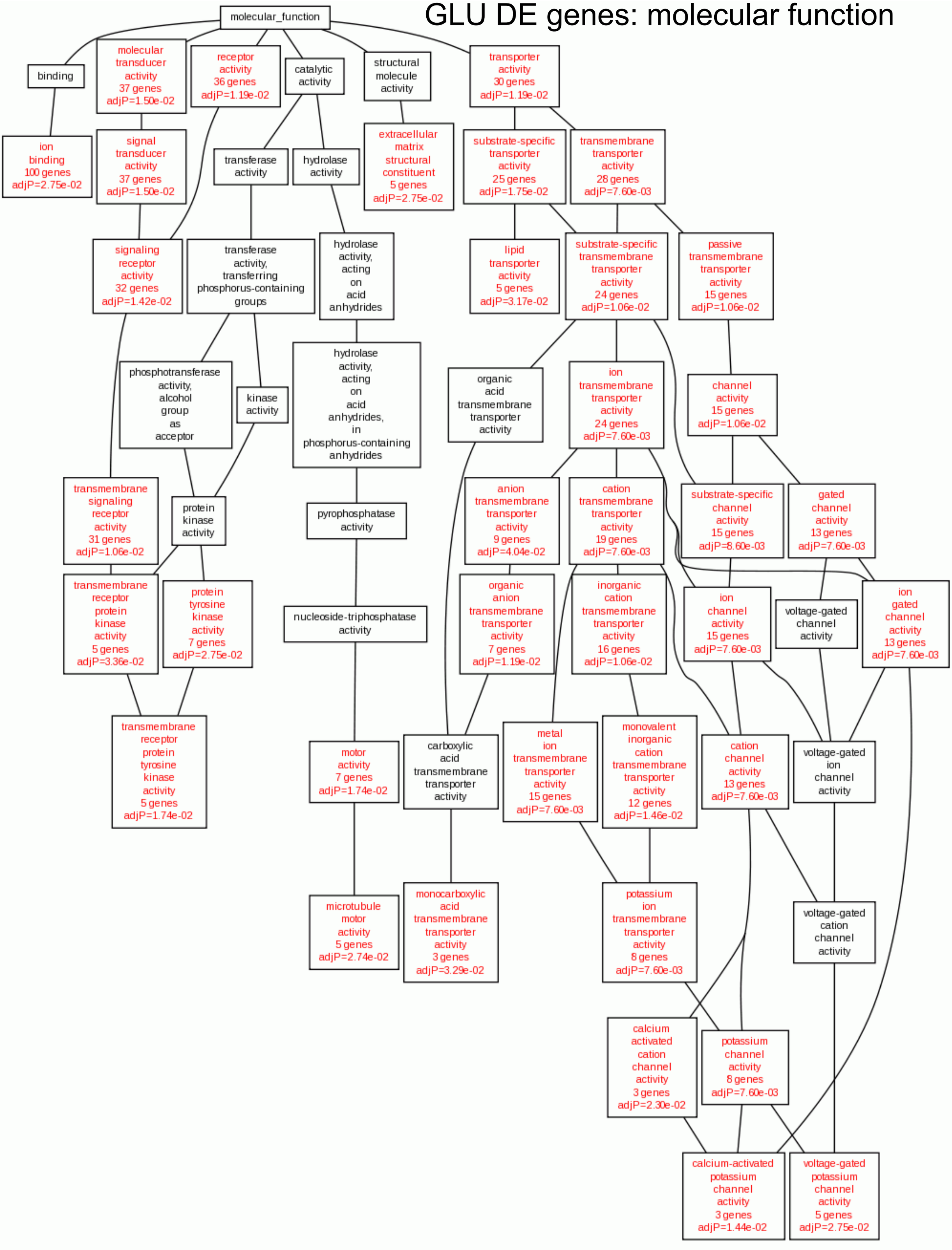
GABA DE genes: cellular component



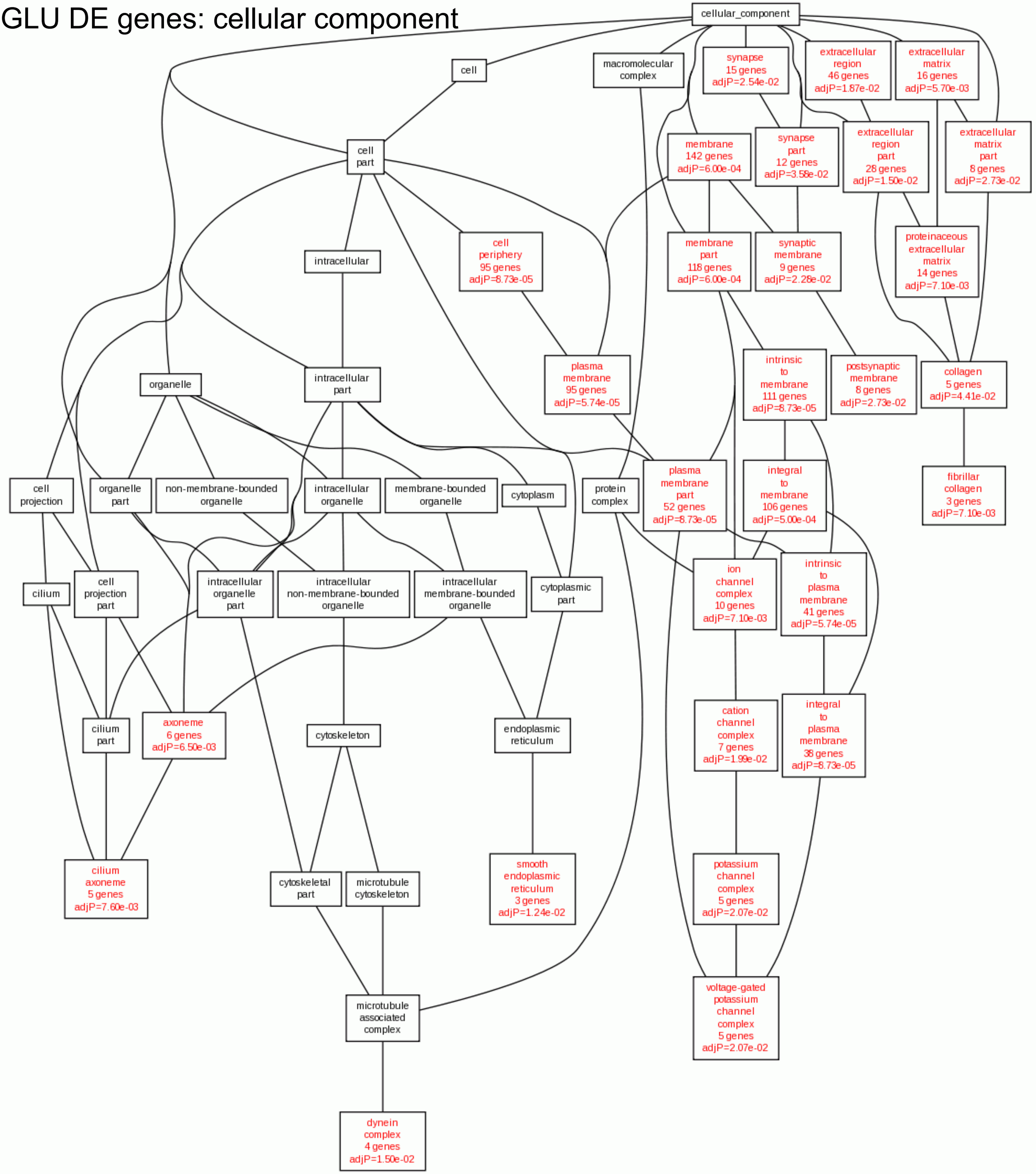
GLU DE genes: biological process



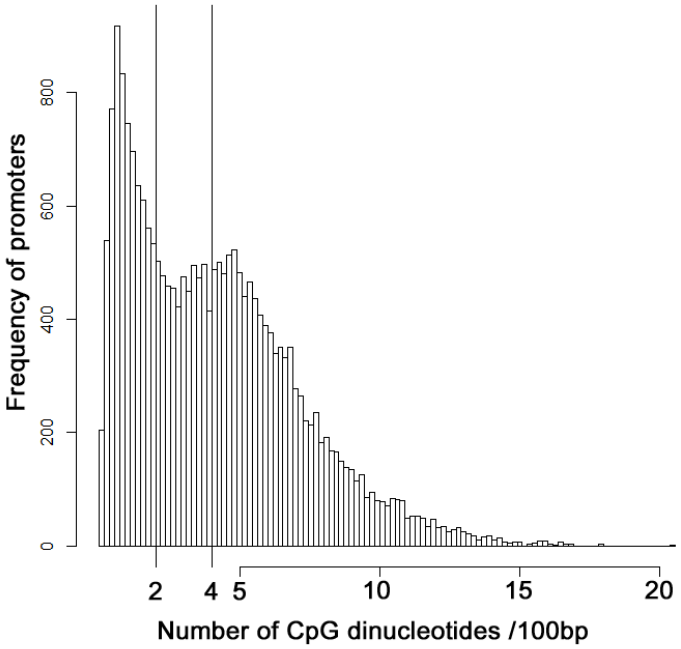
GLU DE genes: molecular function



GLU DE genes: cellular component



Supplementary Figure S4. Distribution of the CpG density for the promoters of RefSeq genes.
Promoters were defined as sequences around a TSS (-1,000bp/+100bp) using the UCSC browser refGene annotation. The number of CpG dinucleotides was counted per 100bp. Similar to Xie et al. 2013, two distinct promoter populations were revealed, and an empirical threshold was used to separate the promoters into three classes: high CpG class (CpG density ≥ 4CGs per 100bp), low CpG class (CpG density < 2CpGs per 100bp), and medium CpG class for those with CpG density in between.



Supplementary Figure S5. Characterization of DNA methylome by Infinium HM450K array

(A) Distribution of methylation frequencies for CpG sites probed in GABA (green), GLU (red) and NeuN(-) (GLIA; blue) samples. Shown are averaged β-values for each CpG site across 5 replicates. β-values showed an expected bimodal distribution of CpG methylation in mammalian cells corresponding to sites with high percentage of methylation and unmethylated sites. Similar distributions were detected in all three cell types.

(B) Comparison of DNA methylation levels in GABA and GLU neurons and in NeuN(-) cells (GLIA). Shown are average methylation levels across N=467,028 "filtered" HM450K CpG site in each individual and cell type. GABA neurons have significantly higher CpG methylation (β=0.560) compared to either GLU neurons (β=0.528) or GLIA (β=0.520) (***$p < 0.001$ by paired t-test).

(C) Distribution of the differentially methylated (DM) CpG sites as function of distance from TSS. The distance is represented on a log scale. For each bin, the normalized density of CpG sites was calculated as [(number of the DM sites per bin)/(total number of the DM sites)] / [(number of the "filtered" HM450K sites per bin)/(total number of the "filtered" HM450K sites]. $GABA_{UM}$ sites are shown in green, GLU_{UM} sites in red. Values of the normalized density > 1 signify enrichment.

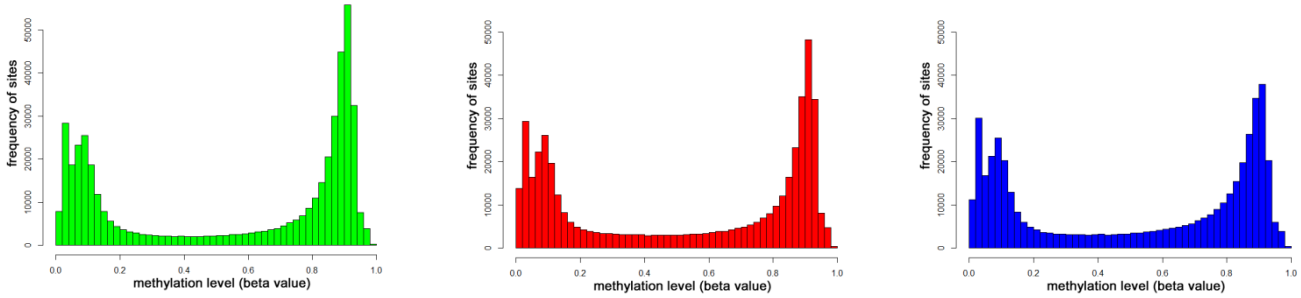
(D) Enrichment of the distal DM sites located within gene bodies or intergenic regions upstream or downstream from the nearest TSS. Distal sites were defined as those located >1,000bp from the nearest TSS.

(E) Enrichment of the distal DM sites located within CpG island shores upstream or downstream from the nearest TSS.

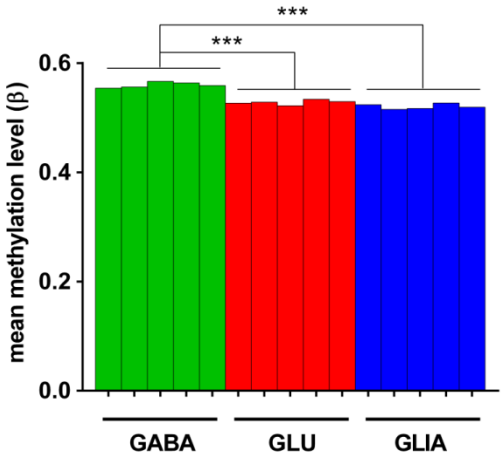
(F) Enrichment of the differentially methylated (DM) sites within predicted enhancers. Shown are density distributions of DM sites as a function of the distance from TSS. Left panel: $GABA_{UM}$ sites. Right panel: GLU_{UM} sites. Green or red: all DM sites of either $GABA_{UM}$ or GLU_{UM} type, respectively. Blue, the subset of $GABA_{UM}$ or GLU_{UM} DM sites which also overlaps with HM450K-annotated predicted enhancers.

(G) Proportion of the $GABA_{UM}$ or GLU_{UM} sites located within distal shores and/or HM450K-annotated predicted enhancers positioned upstream or downstream from the nearest TSS. Data for all sites in HM450K assay are provided for comparison.

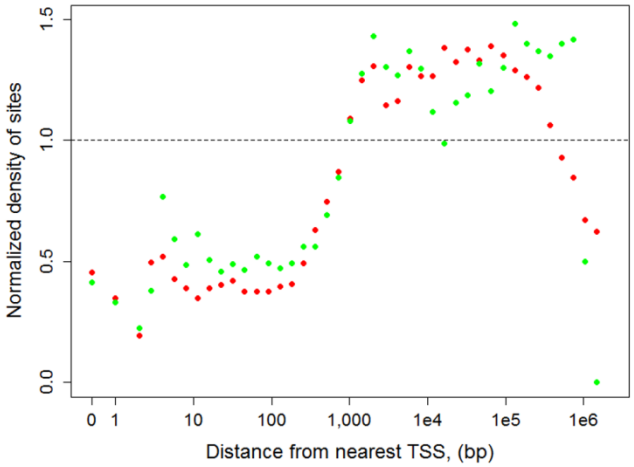
A



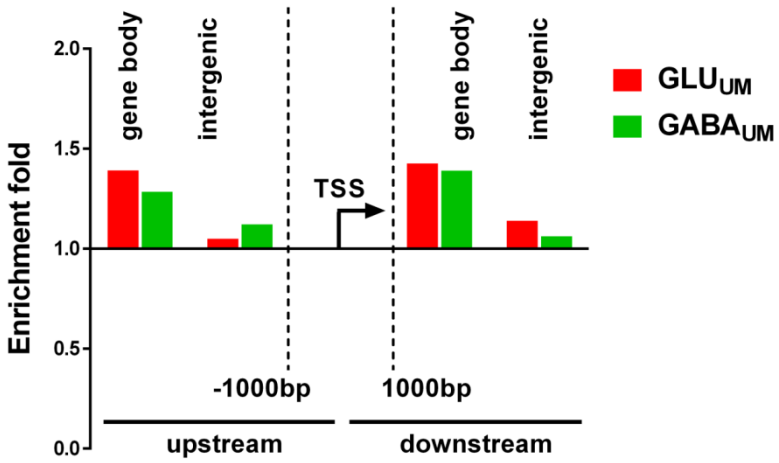
B



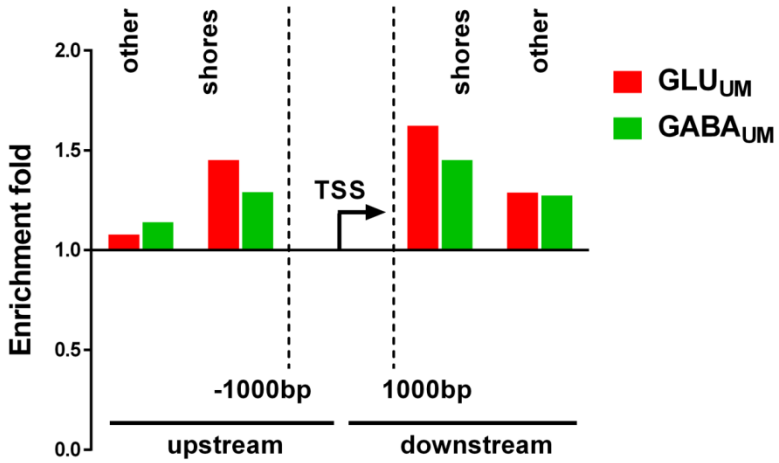
C


Normalized density of sites
0.0
0.5
1.0
1.5
0
1
10
100
1,000
1e4
1e5
1e6
Distance from nearest TSS, (bp)

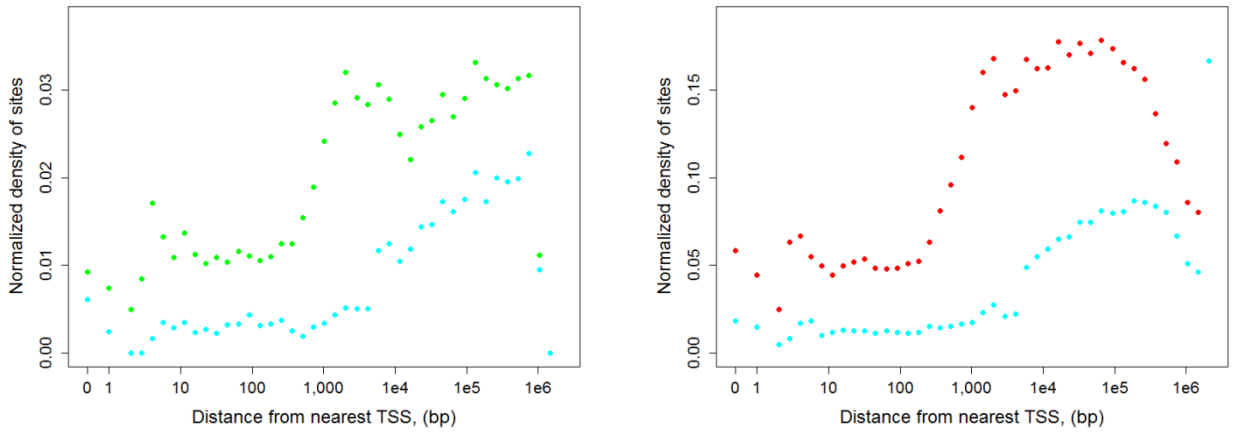
D


Enrichment fold
2.0
1.5
1.0
0.5
0.0
gene body
intergenic
TSS
gene body
intergenic
-1000bp
1000bp
upstream
downstream
GLU$_{UM}$
GABA$_{UM}$

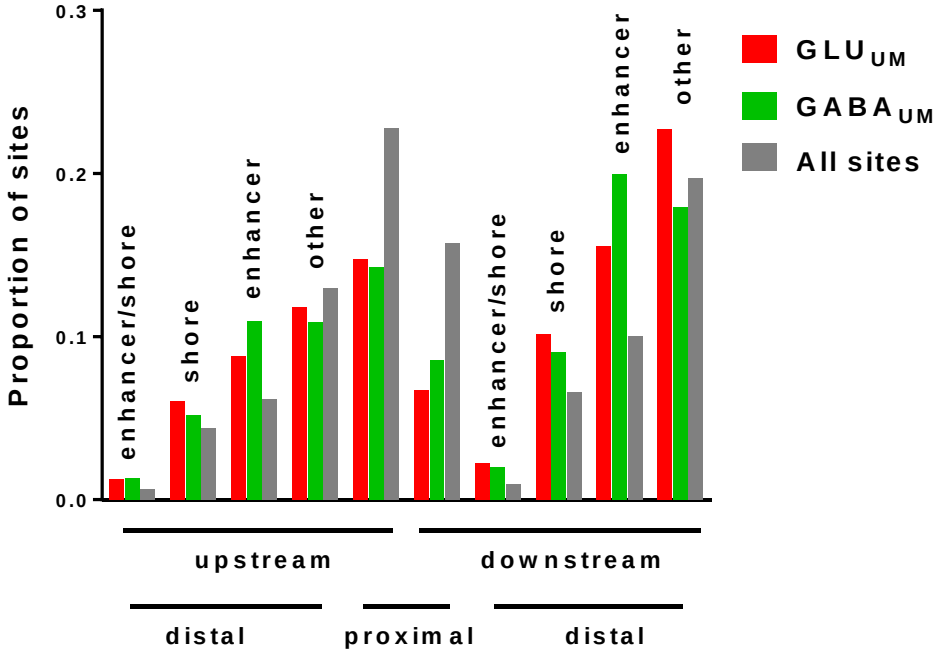
E


Enrichment fold
2.0
1.5
1.0
0.5
0.0
other
shores
TSS
shores
other
-1000bp
1000bp
upstream
downstream
GLU$_{UM}$
GABA$_{UM}$

F


Normalized density of sites
0.00
0.01
0.02
0.03
0 1 10 100 1,000 1e4 1e5 1e6
Distance from nearest TSS, (bp)
Normalized density of sites
0.00
0.05
0.10
0.15
0 1 10 100 1,000 1e4 1e5 1e6
Distance from nearest TSS, (bp)

G


Proportion of sites
0.0
0.1
0.2
0.3
enhancer/shore
shore
enhancer
other
enhancer/shore
shore
enhancer
other
upstream
downstream
distal
proximal
distal
GLUUM
GABAUM
All sites

Supplementary Figure S6. Characterization of CpG DNA methylation data obtained by ERRBS method

(A) Distribution of methylation frequencies for CpG sites probed in GABA and GLU neurons. Shown are averaged β-values for each CpG site across 3 replicates. GABA cells, green, GLU cells, red. The typical "bimodal" distribution of β-values is apparent for both cell types.

(B) Unsupervised hierarchical clustering of CpG methylation data for GABA and GLU samples. 1, 2, 3 denote three replicate subjects.

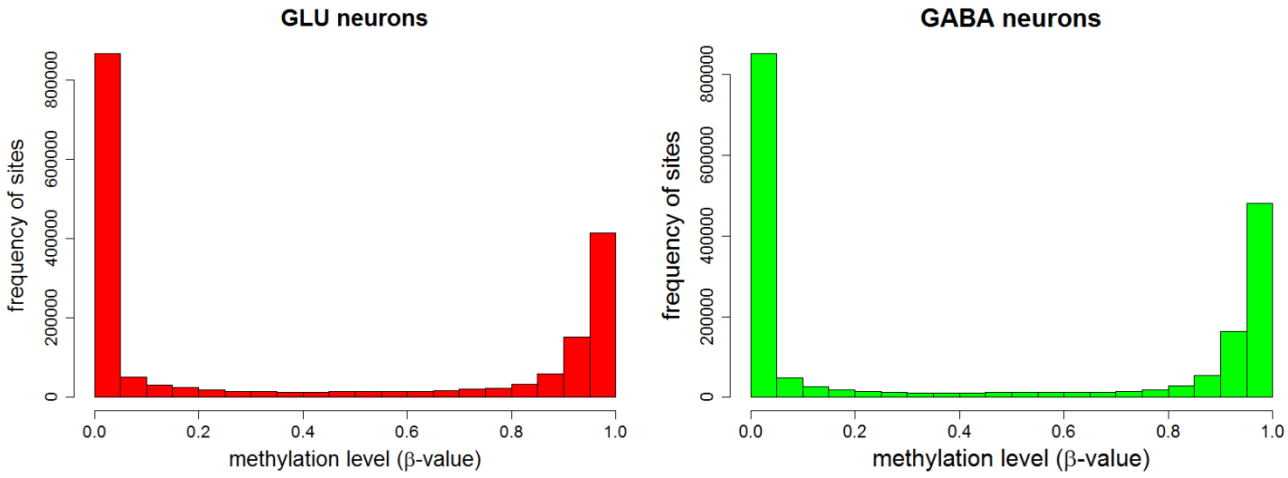
(C) Average total CpG methylation levels in GABA and GLU neurons. Shown are data in 3 replicate samples for each cell type. Methylation was higher in GABA vs. GLU neurons (p-values <0.02 by paired t-test). *Left panel*, all CpG sites; GABA neurons: Mean±SD; 43.22 ± 0.01%; GLU neurons 40.59±0.01%. *Right panel*, autosomal CpG sites; GABA neurons: Mean±SD; 43.25 ± 0.01%; GLU neurons 40.58±0.01%.

(D) Pairwise comparison of CpG methylation measured by HM450K vs. ERRBS methods in GABA and GLU samples. Shown are data for 106,242 CpG sites which were present in both HM450K and ERRBS data sets. Each point represents an average (across all 3 subjects) β-value for one CpG site. Strong correlations between the ERRBS and HM450K methods were observed when samples of the same neuronal type were compared; the correlations were lower when GABA vs. GLU neurons were compared.

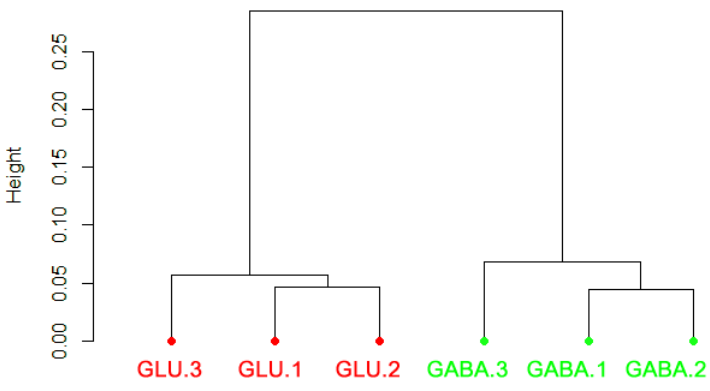
(E) Pairwise comparison of average CpG methylation in GABA vs. GLU samples. Each point represents an average (across all 3 subjects) β-value for one CpG site. The data demonstrate significant differences in DNA methylation patterns between GABA and GLU neurons.

(F) Correlation across all CpG or CpH sites between DM values obtained from 3 (two males and one female) and 2 (two males) subjects. The DM values were defined as *delta(GABA.methylation-GLU.methylation)* for each site and dataset. r, Pearson correlation. Shown are correlations for sites in the whole genome ("all chr.") and in individual chromosomes.

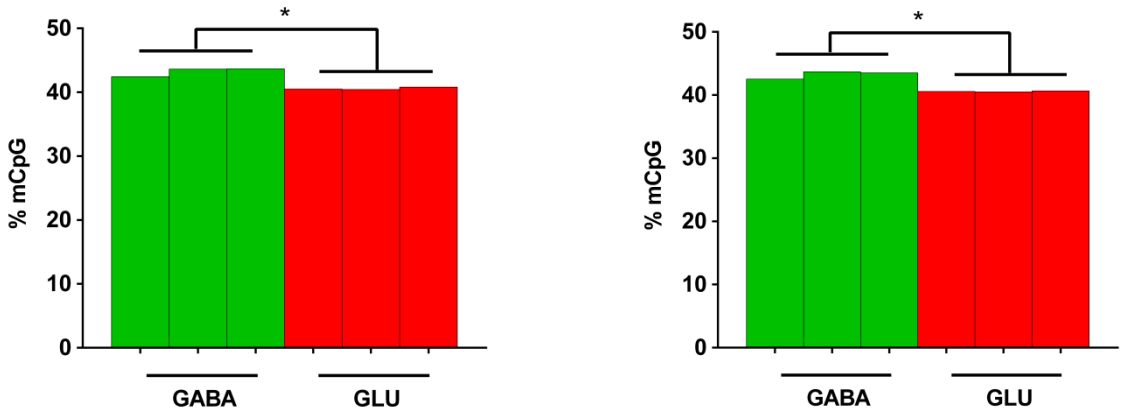
A



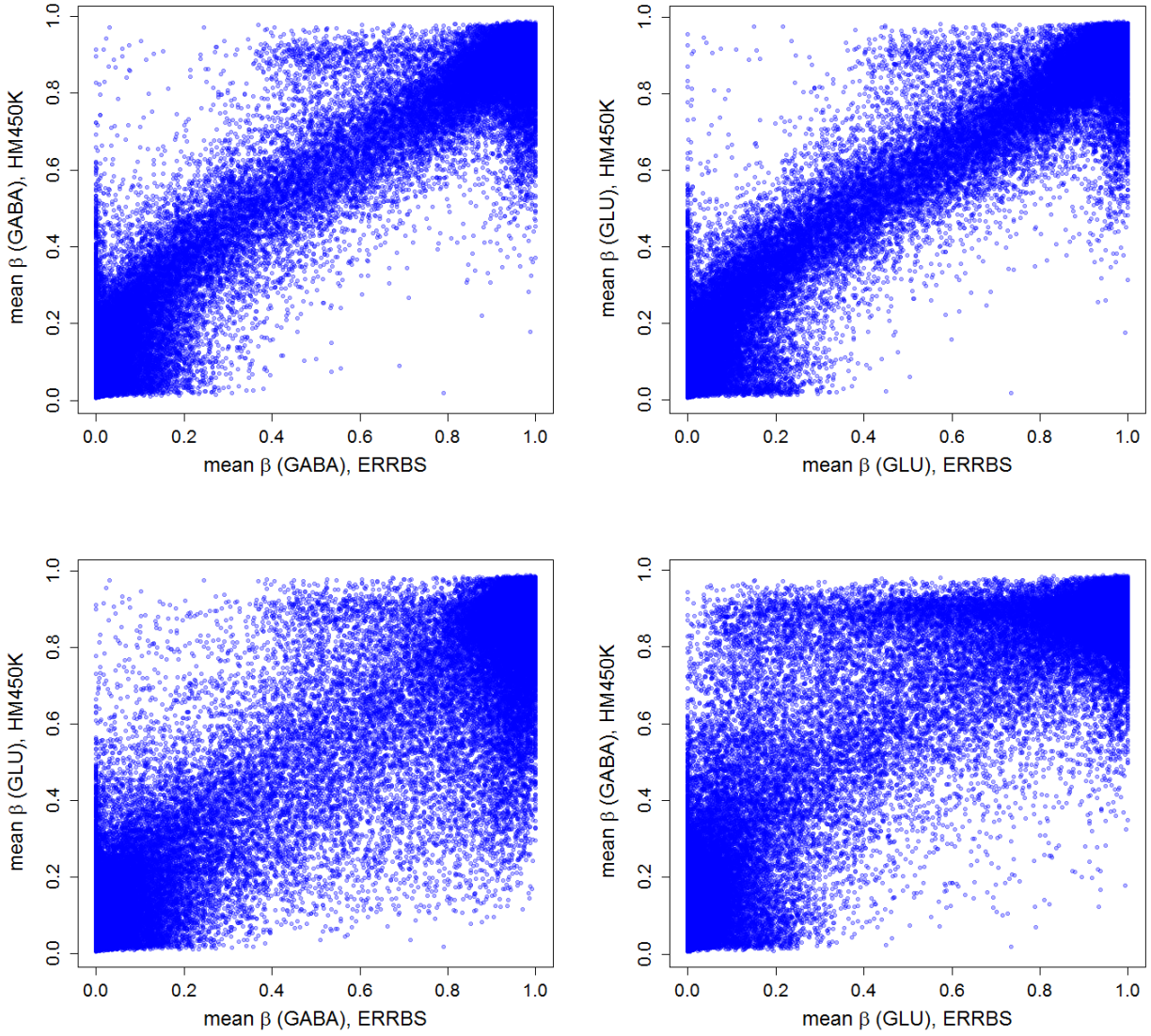
B



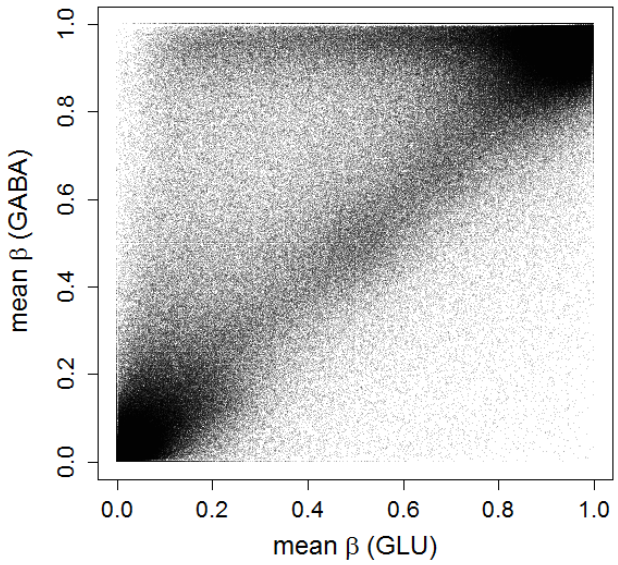
C


*
50
40
30
20
10
0
% mCpG
GABA
GLU
*
50
40
30
20
10
0
% mCpG
GABA
GLU

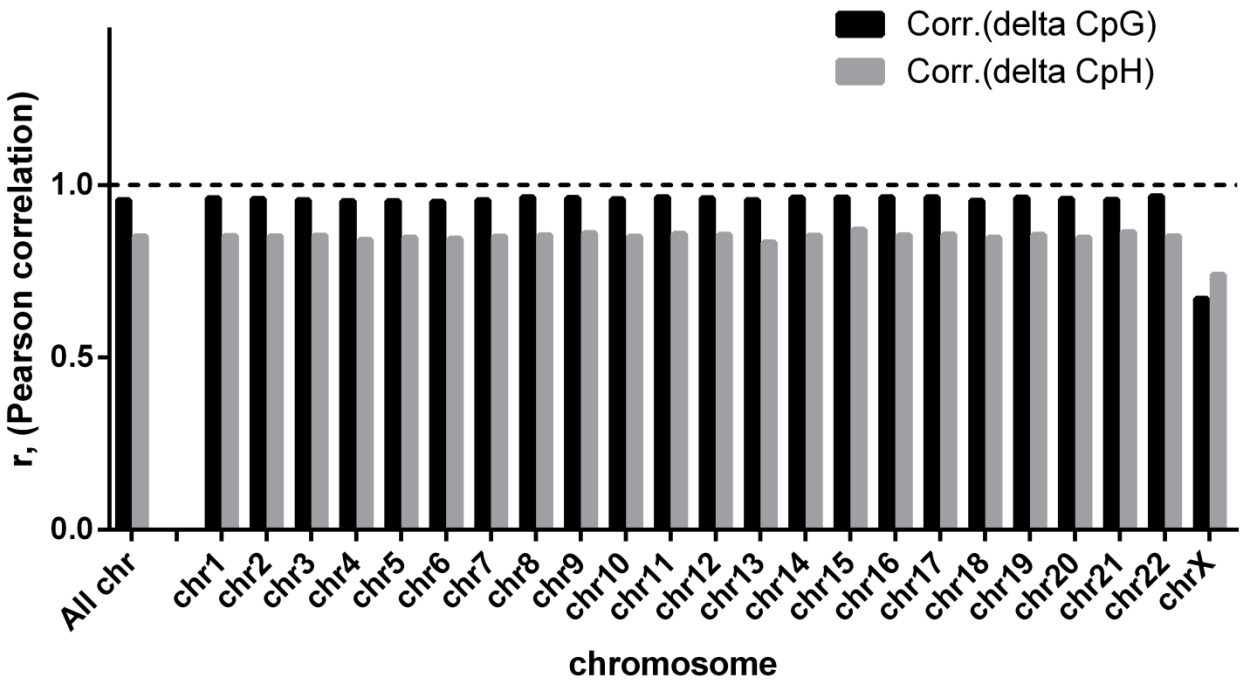
D


mean β (GABA), HM450K
mean β (GABA), ERRBS
mean β (GLU), HM450K
mean β (GLU), ERRBS
mean β (GLU), HM450K
mean β (GABA), ERRBS
mean β (GABA), HM450K
mean β (GLU), ERRBS
0.0
0.2
0.4
0.6
0.8
1.0

E


mean β (GABA)
mean β (GLU)
0.0
0.2
0.4
0.6
0.8
1.0

F


Corr.(delta CpG)
Corr.(delta CpH)
r, (Pearson correlation)
1.0
0.5
0.0
All chr
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr22
chrX
chromosome

Supplementary Figure S7. Distribution of CpG sites differentially methylated (DM) between GABA and GLU neurons among genomic features (based on ERRBS data).

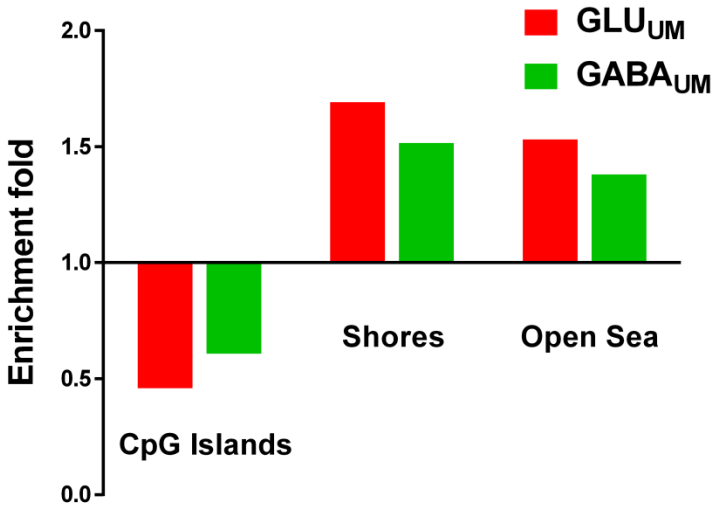
(A) Enrichment / depletion of the DM CpG sites within CpG islands and shores. "Open sea regions" denote areas outside CpG islands and shores.

(B) Enrichment / depletion of the DM CpG sites within promoters, gene bodies and intergenic regions.

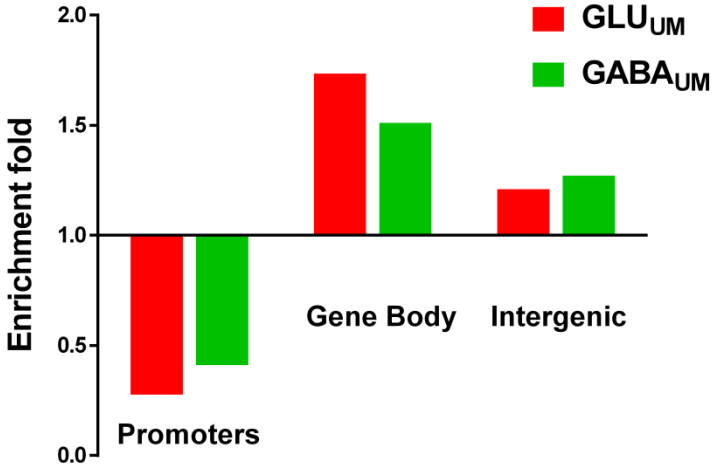
(C) Distribution of the DM CpG sites as a function of the distance from TSS. The distance is represented on a log scale. For each bin, the normalized density of sites was calculated as [(number of DM sites per bin)/(total number of DM sites)] / [(number of sites in ERRBS data per bin)/(total number of sites in ERRBS data)]. Green: $GABA_{UM}$ sites. Red: GLU_{UM} sites. Values of the normalized density > 1 signify enrichment.

(D) Enrichment of the distal DM CpG sites within predicted enhancers defined based on histone modification profiling data from the NIH Roadmap Epigenomics Mapping Consortium project (Zhu et al. 2013). ChIP-seq data for the human brain (combined data sets for medial prefrontal cortex and hippocampus) as well as for the human liver, muscle and heart were used. Tissue-specific histone marks were defined as mark-enriched regions which were only observed in one specific tissue but not in 3 other analyzed tissues.

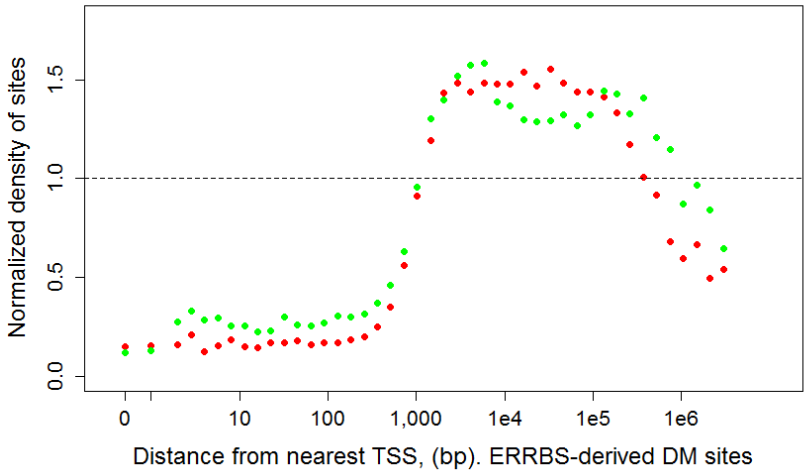
A



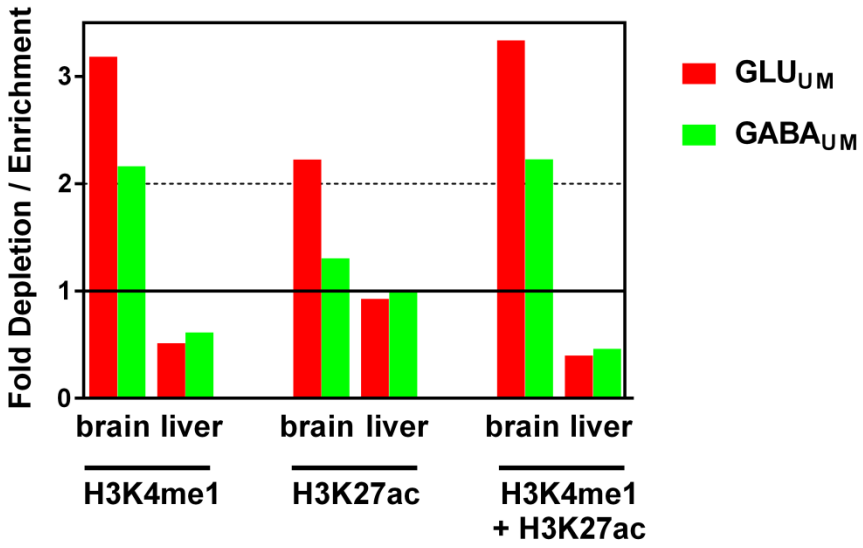
B



C

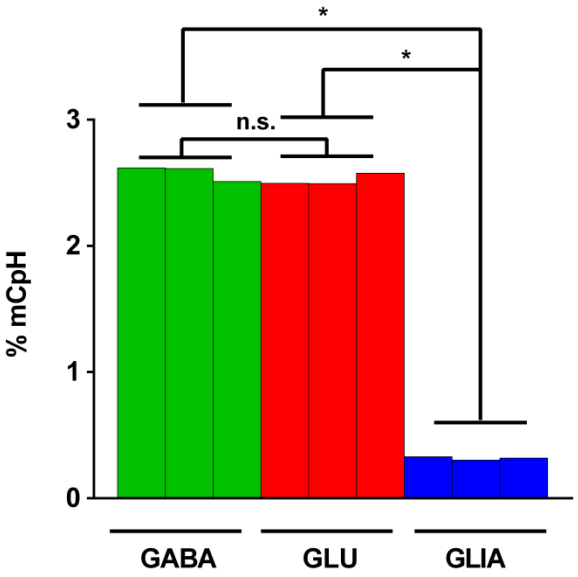

Normalized density of sites
0.0
0.5
1.0
1.5
0
10
100
1,000
1e4
1e5
1e6
Distance from nearest TSS, (bp). ERRBS-derived DM sites

D

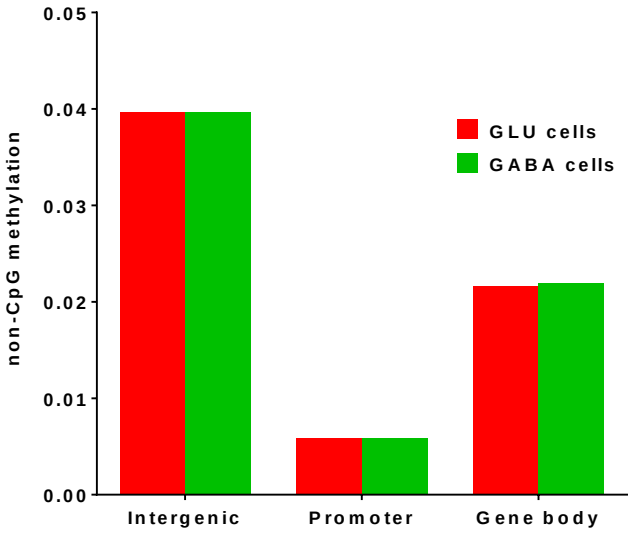

Fold Depletion / Enrichment
0
1
2
3
brain liver
brain liver
brain liver
H3K4me1
H3K27ac
H3K4me1
+ H3K27ac
GLU$_{UM}$
GABA$_{UM}$

Supplementary Figure S8. Analysis of non-CpG (CpH) methylation measured by ERRBS.
(A) Average autosomal CpH methylation in GABA (green) and GLU (red) neurons, and in NeuN(-) cells (GLIA; blue); N= 3 samples for each cell type. Data for NeuN(-) cells are from [32]. * p-values < 0.0001 for comparisons of GLIA with GABA or GLU (unpaired t-test).
(B) Distribution of CpH methylation among genomic features (promoters, gene bodies, intergenic regions), in GABA (green) or GLU (red) neurons.

A



B

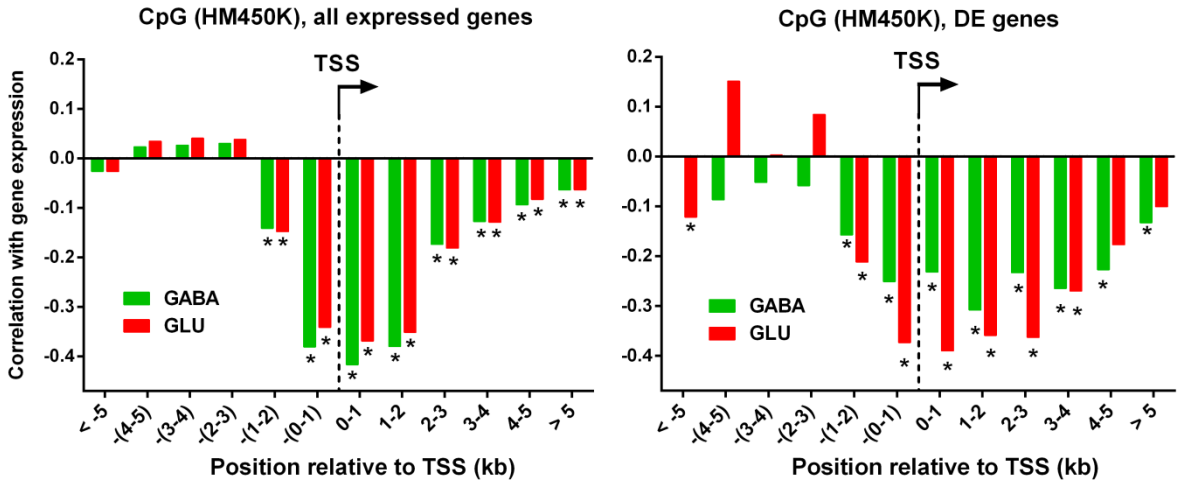


Supplementary Figure S9. Analysis of correlation between DNA methylation and gene expression.

(A) Spearman correlations of mCpG (measured by HM450K) with RNA expression levels of all expressed genes (*left panel*) and differentially expressed (DE) genes (*right panel*). For each gene, mCpG sites were combined into 1kb bins as a function of the distance from TSS. The significance was determined as p<0.01 after Bonferroni correction for the number of bins (N=12).

(B) Variability of CpG methylation in TSS-proximal regions and in gene bodies of GLU-DE and GABA-DE genes. Shown are distributions of CpG methylation values (from HM450K data set) for GLU-DE genes in GLU neurons and GABA-DE genes in GABA neurons. TSS-proximal regions were defined as sequences within 1kb from a TSS. Gene body regions did not include TSS-proximal regions. CpG DNA methylation variability was significantly different between GABA-DE and GLU-DE genes in TSS-proximal regions (p-value = 2.2e-39) but not in gene bodies (p-value = 0.39), as assessed using the DiffVar method from R package missMethyl.

A



B