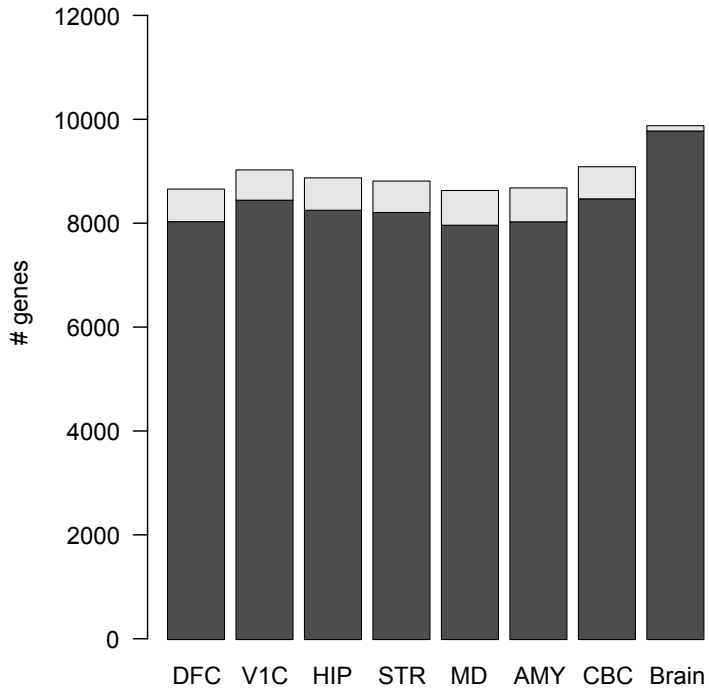
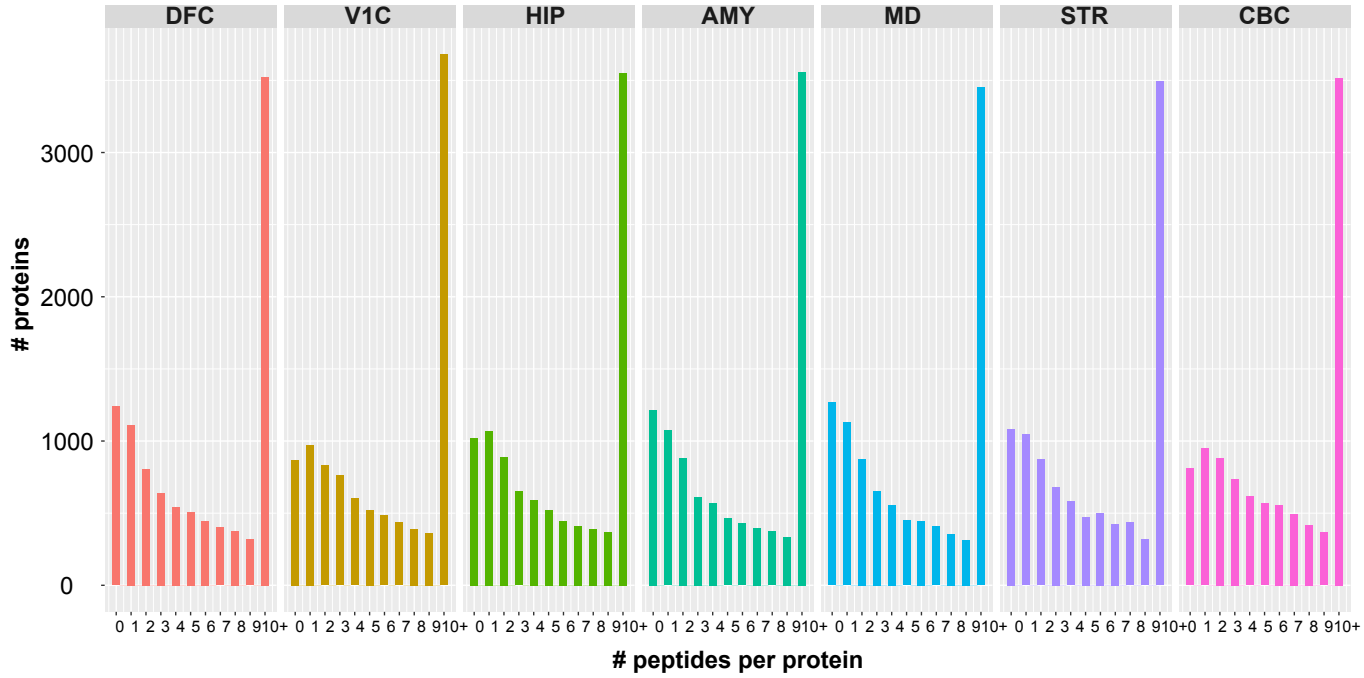


Supplementary Figure 1

Number of proteins detected by highly fractionated proteomics across all regions.

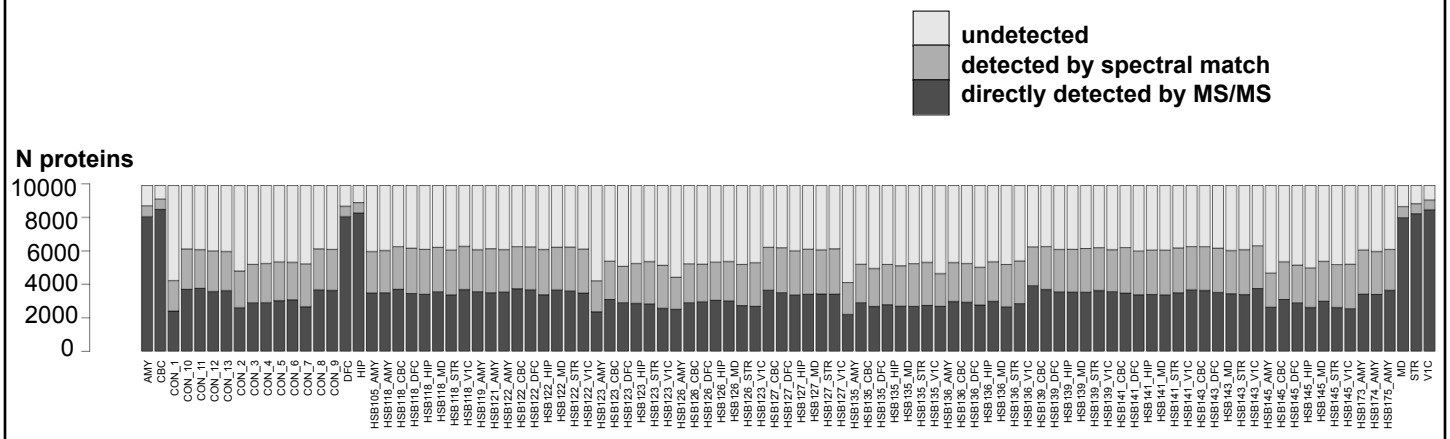
Histogram quantifies proteins detected directly by MS/MS (black) versus those detected by the “match between runs” feature (grey) in the fractionated regional samples.



Supplementary Figure 2

Number of peptides detected per protein shows no clear inter-regional variability.

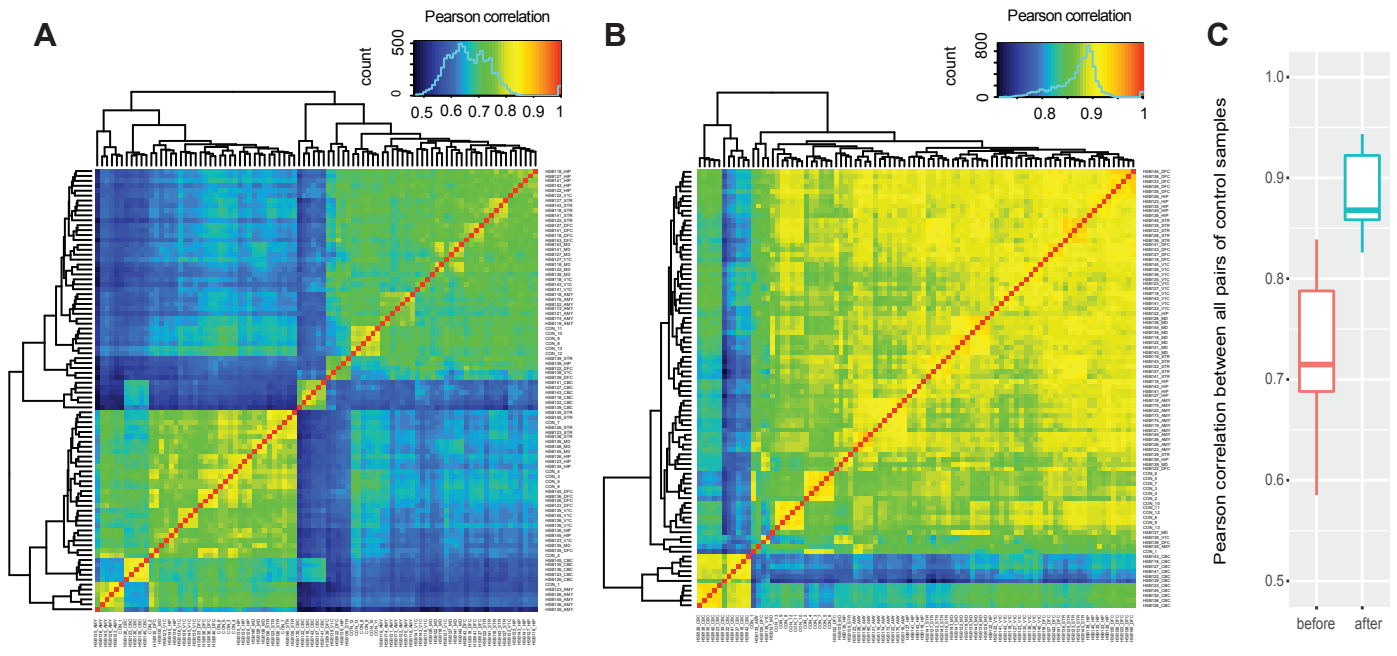
The average number of peptides detected per protein was 9.2. For simplicity in these histograms, proteins with more than 10 peptides were set to have exactly 10 peptides. The distribution of peptides/protein is similar across all 7 fractionated brain regions.



Supplementary Figure 3

Numbers of proteins detected in each single shot sample.

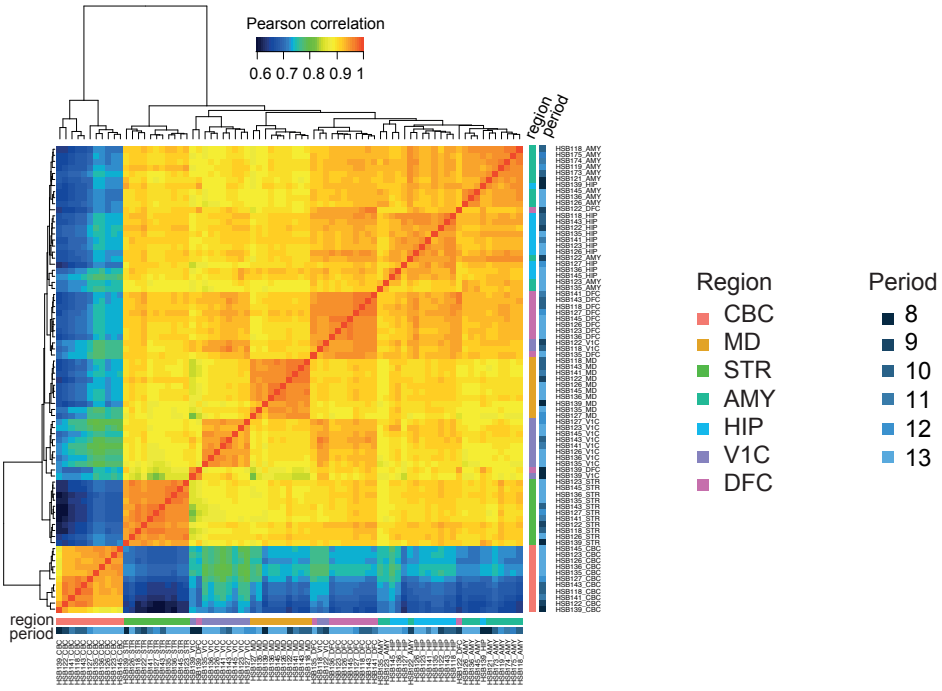
The use of “match between runs” results in an approximately 50% increase in the number of proteins identified by single shot proteomics. Proteins directly detected by MS/MS are shown in black, whilst those identified by “match between runs” are in grey. Samples are shown in alphabetical order, and include the data from fractionated brain regions.



Supplementary Figure 4

Batch effect identified by sample correlations is corrected by ComBat.

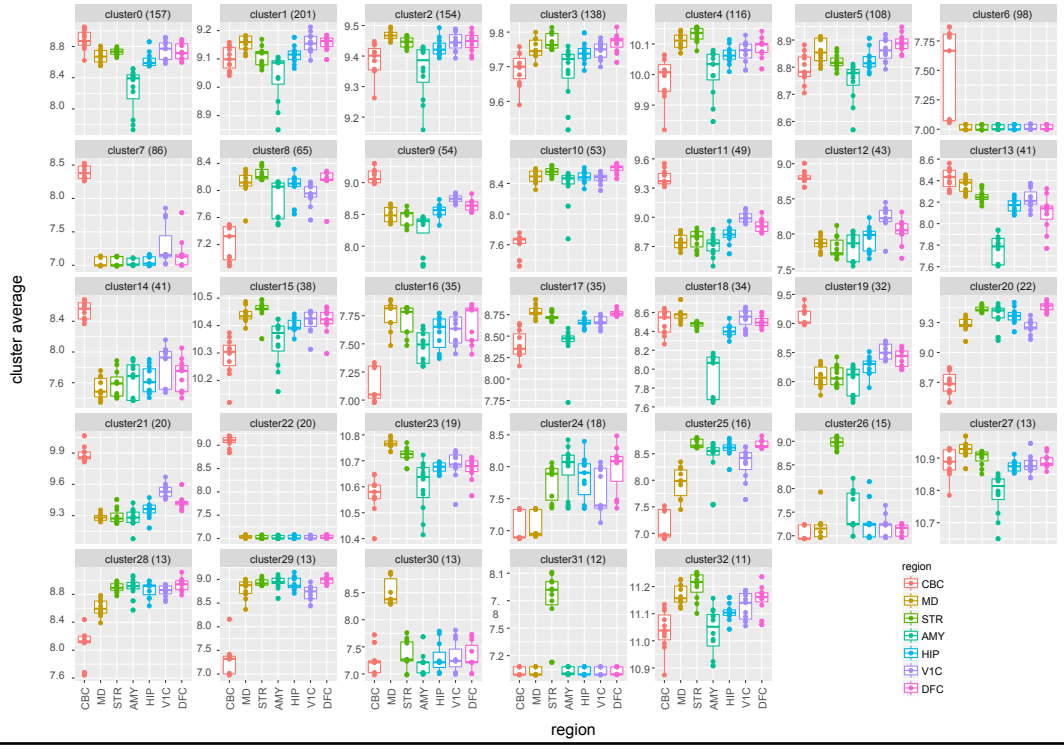
A) Clustering of all single shot samples shows a clear batch effect. B) Clustering post correction by ComBat shows correction of the batch effect. C) Technical replicates of a single mixed region control were run evenly spaced throughout the LC-MS/MS runs. Correlation between these technical replicates is strongly improved as a result of batch correction.



Supplementary Figure 5

Clustering all samples subjected to MS/MS using proteins significantly differentially expressed between brain regions revealed expected bulk differences between brain regions.

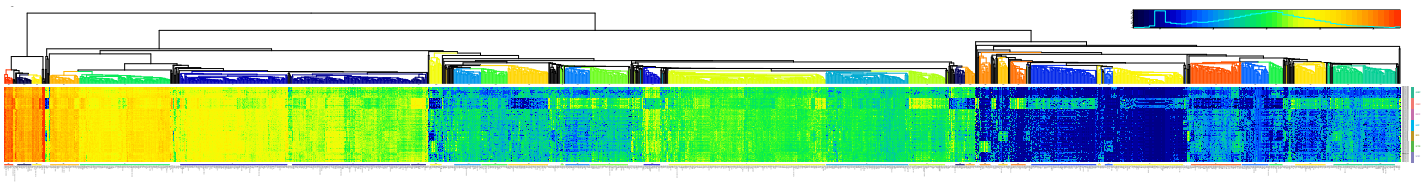
This is a fully labelled zoomable version of the main Fig 2B.



Supplementary Figure 6

Clustering all proteins significantly differentially expressed between regions reveals consistent patterns of expression that favour region-specific enrichment or region-specific depletion in abundance

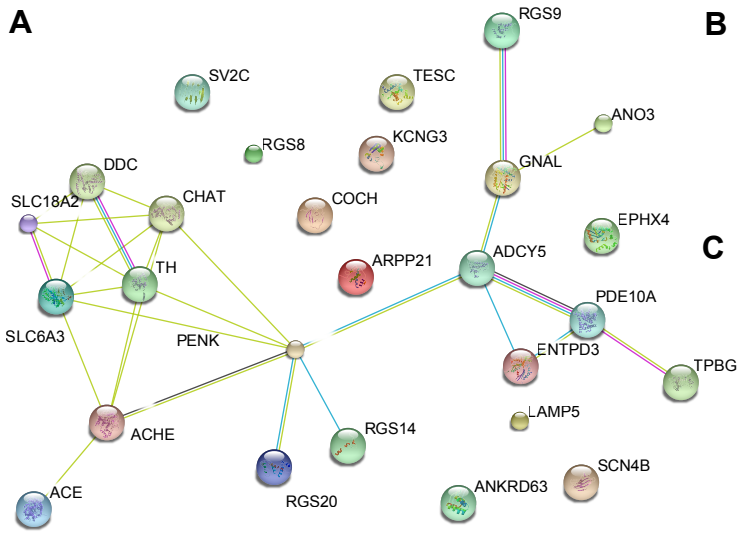
Box and whisker plots show region specific patterns of protein expression across 33 gene clusters. The center line indicates the median, limits indicate the IQR, and the whiskers either 1.5* the IQR or the min/max value if it falls within 1.5* the IQR. Expression values from individual samples are shown as dots. Genes belonging to each cluster can be seen in Table 5B and Fig S7.



Supplementary Figure 7

Clustering all proteins significantly differentially expressed between regions reveals consistent patterns of expression that favour region-specific enrichment or region-specific depletion in abundance.

Heatmap shows expression levels of all differentially expressed genes across all single shot samples. The dendrogram above depicts the clusters of proteins that share patterns of expression across the regions. Cluster 0 is a group of proteins with no shared expression across the regions. Proteins belonging to this cluster are depicted with black arms on the dendrogram, and are not numbered below the heatmap. The heatmap shows that many clusters are dominated by expression changes in the cerebellum compared to other regions.



B

Biological process	gene count	false discovery rate	matching proteins in your network
termination of G-protein coupled receptor signaling pathway	4	0.0085	RGS14,RGS20,RGS8,RGS9
dopamine biosynthetic process	3	0.0085	DDC,SLC6A3,TH
neurotransmitter biosynthetic process	4	0.0487	ACHE,CHAT,SLC6A3,TH

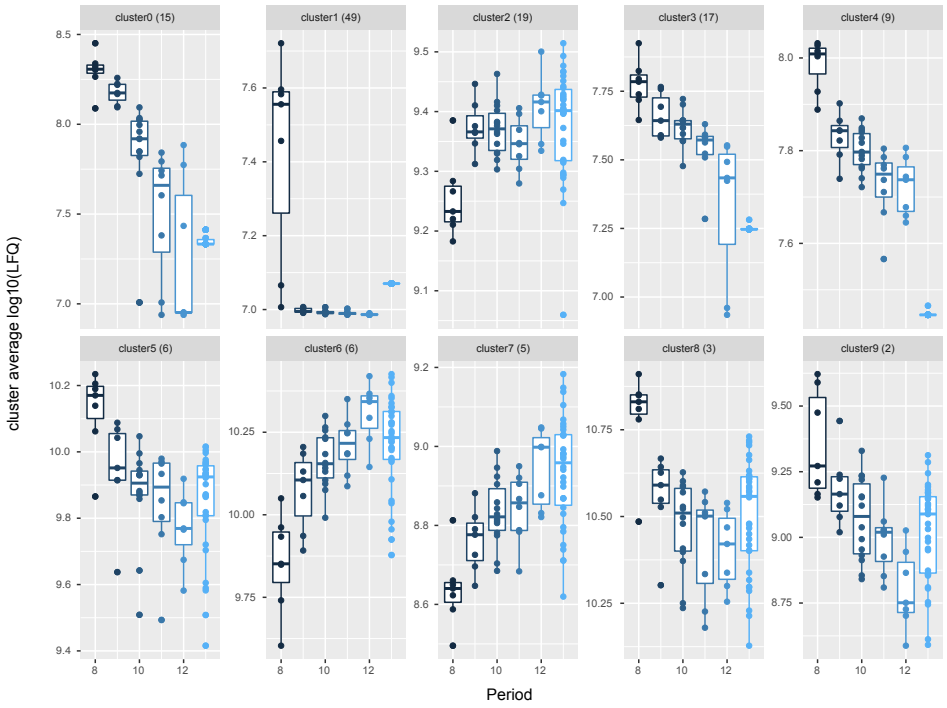
C

KEGG pathway	gene count	false discovery rate	matching proteins in your network
Cocaine addiction	6	2.55E-06	ADCY5,DDC,RGS9,SLC18A2, SLC6A3,TH
Amphetamine addiction	5	0.0006	ADCY5,DDC,SLC18A2, SLC6A3,TH
Dopaminergic synapse	6	0.001	ADCY5,DDC, GNAL, SLC18A2,SLC6A3,TH
Parkinson's disease	5	0.0147	ADCY5,GNAL,SLC18A2,SLC6A3,TH

Supplementary Figure 8

Striatally enriched clusters contain interacting proteins with roles in dopaminergic signalling and drug addiction.

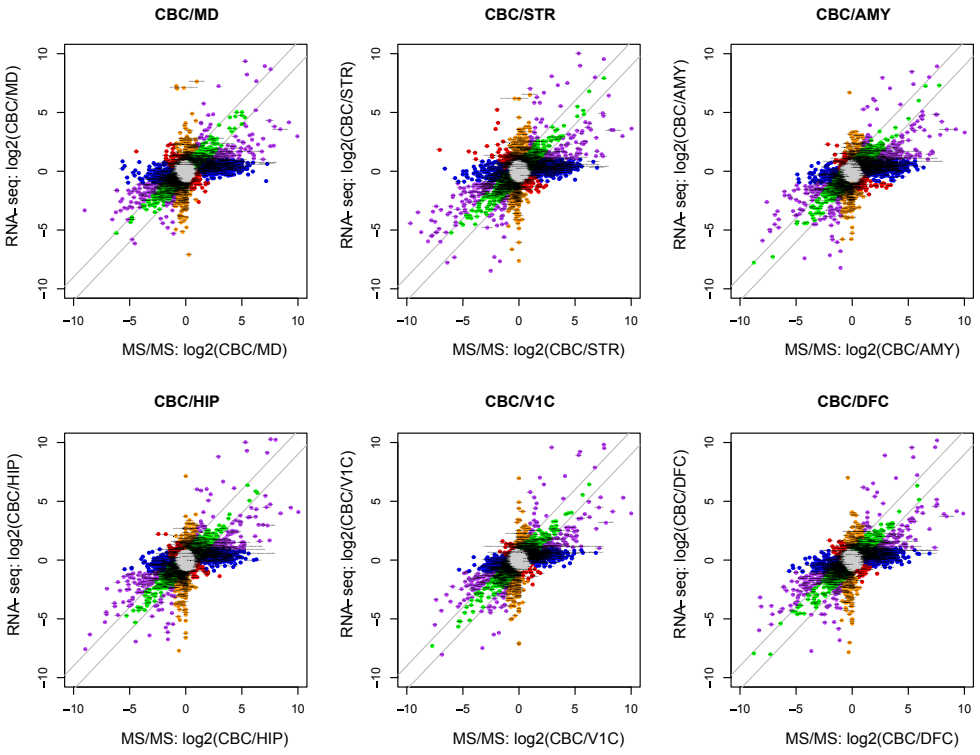
A) Network diagram showing proteins from clusters 26 and 31. These clusters are strongly enriched for protein:protein interactions (adj. p value = 1.55E-15). Coloured edges represent different forms of interaction evidence; experimentally determined (pink), co-expressed (black), curated databases (blue) and text mining (green). The node size represents the extent to which the protein structure has been solved. B) Clusters 26 and 31 are significantly enriched for a number of Biological Process ontology terms. C) KEGG pathway analysis shows significant enrichment for expected pathways, including stimulant addiction and dopaminergic synapse.



Supplementary Figure 9

Clustering all proteins significantly differentially expressed over developmental period reveals proteins enriched shortly after birth (period 8) and proteins more gently increasing or decreasing in abundance over the time-course.

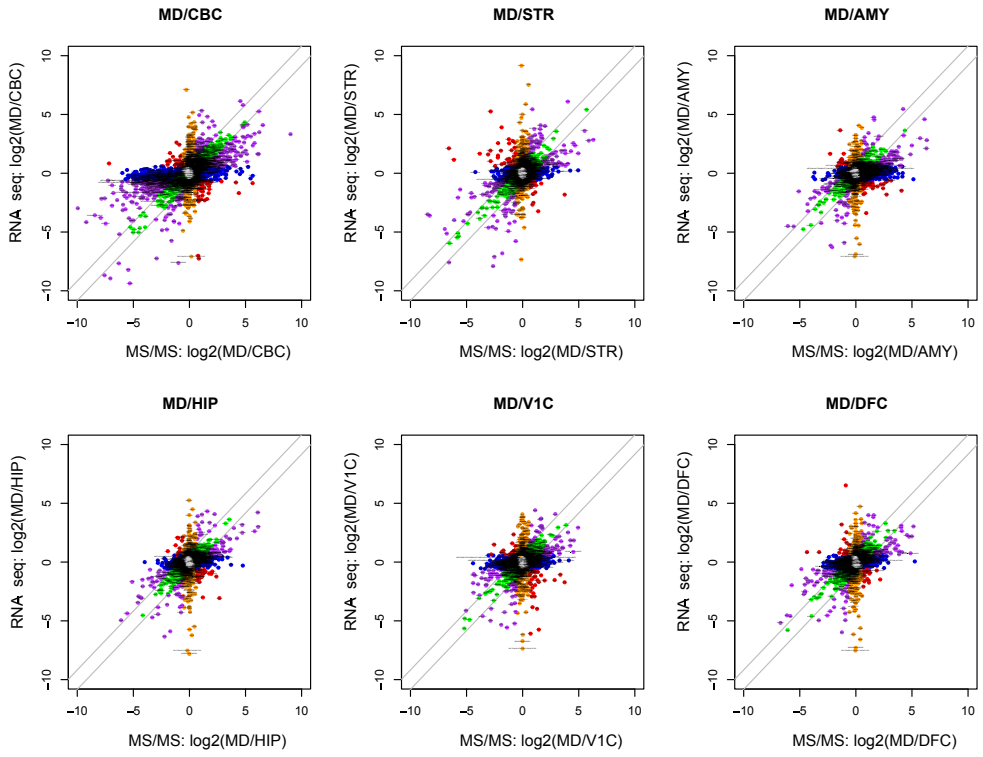
The center line indicates the median, limits indicate the IQR, and the whiskers either 1.5* the IQR or the min/max value if it falls within 1.5* the IQR. Individual samples are shown as dots on the box and whisker plots. Proteins belonging to each cluster can be seen in Table 5.



Supplementary Figure 10a

RNA vs protein fold-change comparison of all pairs of brain regions.

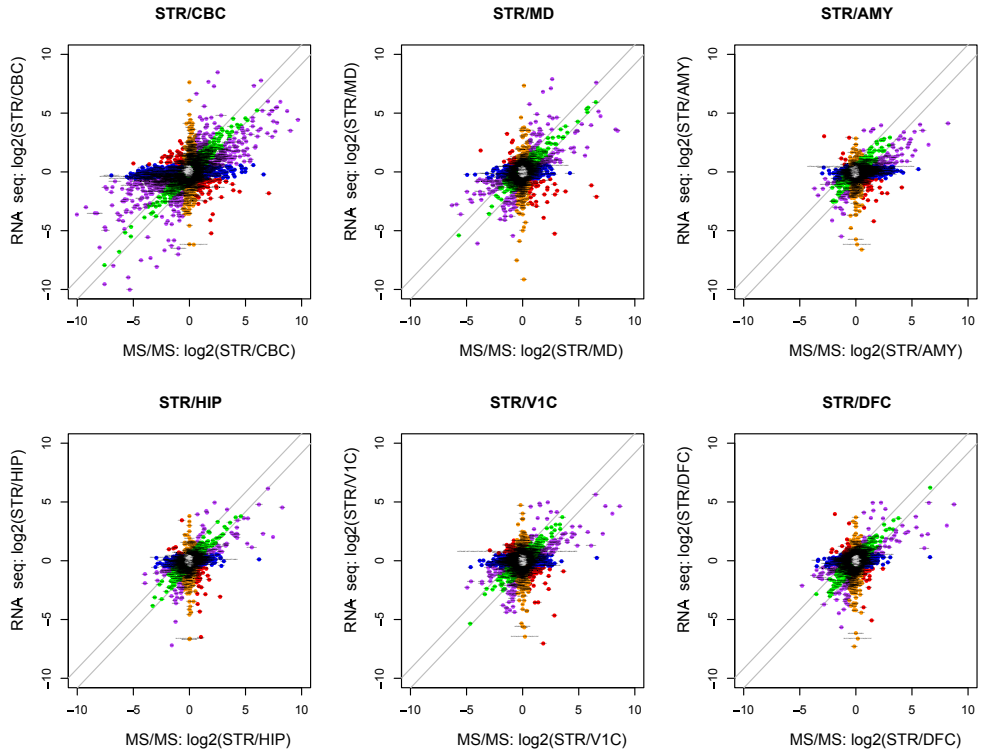
These scatterplots are identically defined to those in Fig 5A except that here genes are labelled to show individual gene names observable at high magnification. A) CBC vs all B) MD vs all C) STR vs all D) AMY vs all E) HIP vs all F) V1C vs all G) DFC vs all. Genes are coloured based on their agreement or disagreement between the RNA and protein measurements; genes for which the protein variability between regions is <2-fold of that reported at the RNA-level were considered consistent (green and grey points). Purple coloured genes are those with consistent direction but variable magnitude of change (≥2-fold) between the regions at the protein and RNA level, while red genes disagree in the direction of change between RNA and protein. Blue and orange genes vary between regions according to protein but not RNA and vice-versa.



Supplementary Figure 10b

RNA vs protein fold-change comparison of all pairs of brain regions.

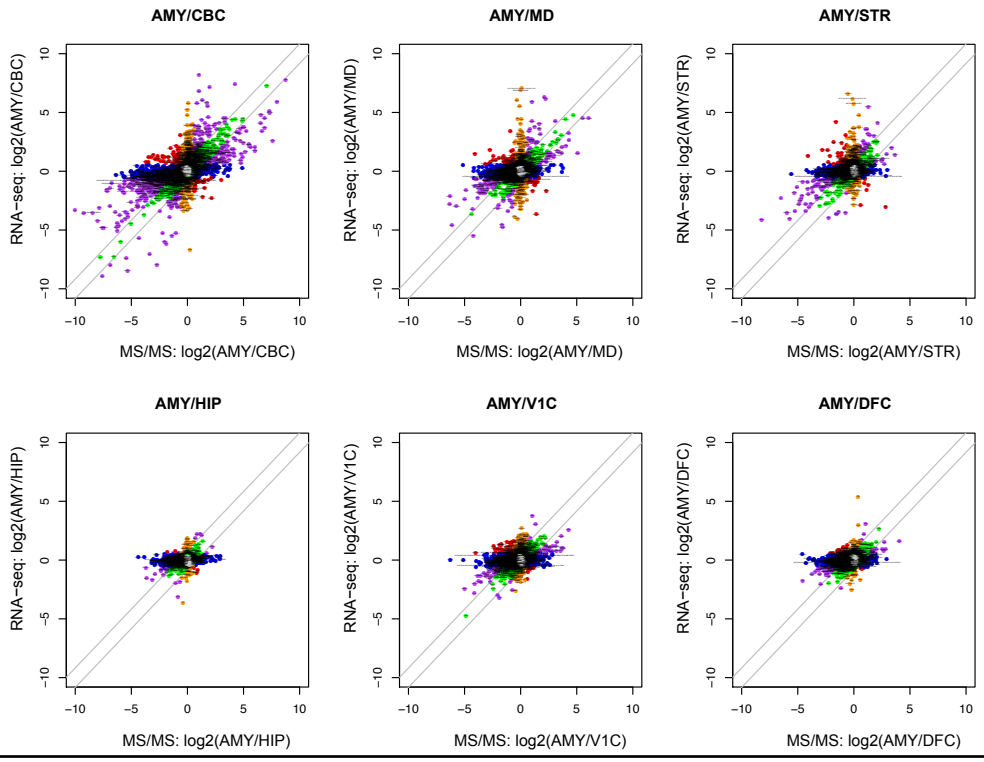
These scatterplots are identically defined to those in Fig 5A except that here genes are labelled to show individual gene names observable at high magnification. A) CBC vs all B) MD vs all C) STR vs all D) AMY vs all E) HIP vs all F) V1C vs all G) DFC vs all. Genes are coloured based on their agreement or disagreement between the RNA and protein measurements; genes for which the protein variability between regions is <2-fold of that reported at the RNA-level were considered consistent (green and grey points). Purple coloured genes are those with consistent direction but variable magnitude of change (≥2-fold) between the regions at the protein and RNA level, while red genes disagree in the direction of change between RNA and protein. Blue and orange genes vary between regions according to protein but not RNA and vice-versa.



Supplementary Figure 10c

RNA vs protein fold-change comparison of all pairs of brain regions.

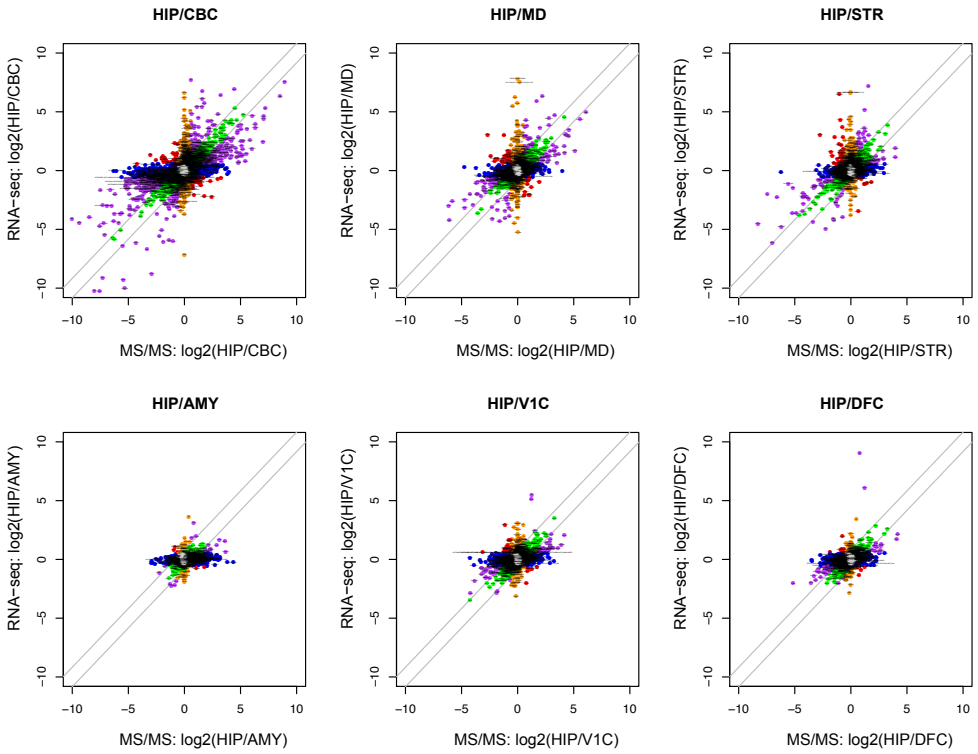
These scatterplots are identically defined to those in Fig 5A except that here genes are labelled to show individual gene names observable at high magnification. A) CBC vs all B) MD vs all C) STR vs all D) AMY vs all E) HIP vs all F) V1C vs all G) DFC vs all. Genes are coloured based on their agreement or disagreement between the RNA and protein measurements; genes for which the protein variability between regions is <2-fold of that reported at the RNA-level were considered consistent (green and grey points). Purple coloured genes are those with consistent direction but variable magnitude of change (≥2-fold) between the regions at the protein and RNA level, while red genes disagree in the direction of change between RNA and protein. Blue and orange genes vary between regions according to protein but not RNA and vice-versa.



Supplementary Figure 10d

RNA vs protein fold-change comparison of all pairs of brain regions.

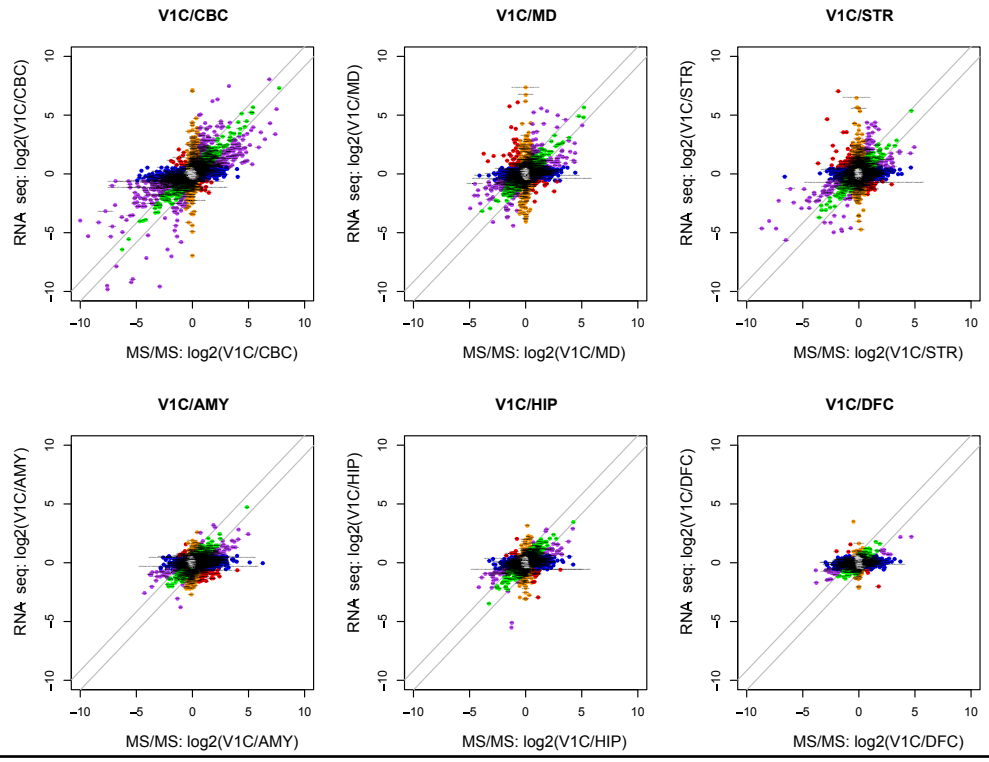
These scatterplots are identically defined to those in Fig 5A except that here genes are labelled to show individual gene names observable at high magnification. A) CBC vs all B) MD vs all C) STR vs all D) AMY vs all E) HIP vs all F) V1C vs all G) DFC vs all. Genes are coloured based on their agreement or disagreement between the RNA and protein measurements; genes for which the protein variability between regions is <2-fold of that reported at the RNA-level were considered consistent (green and grey points). Purple coloured genes are those with consistent direction but variable magnitude of change (≥2-fold) between the regions at the protein and RNA level, while red genes disagree in the direction of change between RNA and protein. Blue and orange genes vary between regions according to protein but not RNA and vice-versa.



Supplementary Figure 10e

RNA vs protein fold-change comparison of all pairs of brain regions.

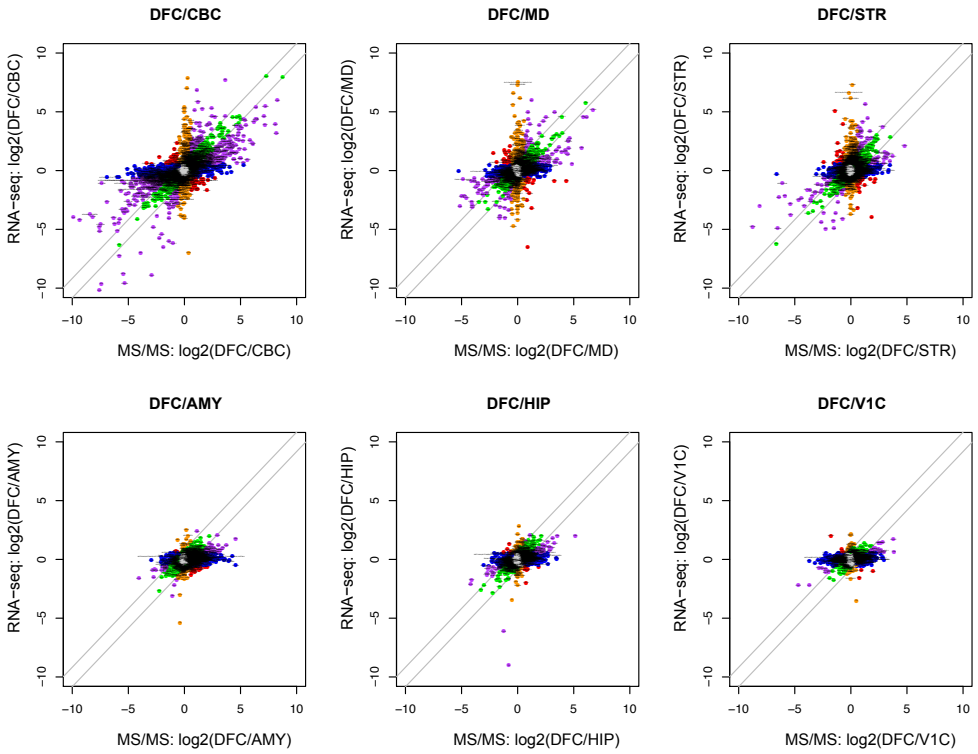
These scatterplots are identically defined to those in Fig 5A except that here genes are labelled to show individual gene names observable at high magnification. A) CBC vs all B) MD vs all C) STR vs all D) AMY vs all E) HIP vs all F) V1C vs all G) DFC vs all. Genes are coloured based on their agreement or disagreement between the RNA and protein measurements; genes for which the protein variability between regions is <2-fold of that reported at the RNA-level were considered consistent (green and grey points). Purple coloured genes are those with consistent direction but variable magnitude of change (≥2-fold) between the regions at the protein and RNA level, while red genes disagree in the direction of change between RNA and protein. Blue and orange genes vary between regions according to protein but not RNA and vice-versa.



Supplementary Figure 10f

RNA vs protein fold-change comparison of all pairs of brain regions.

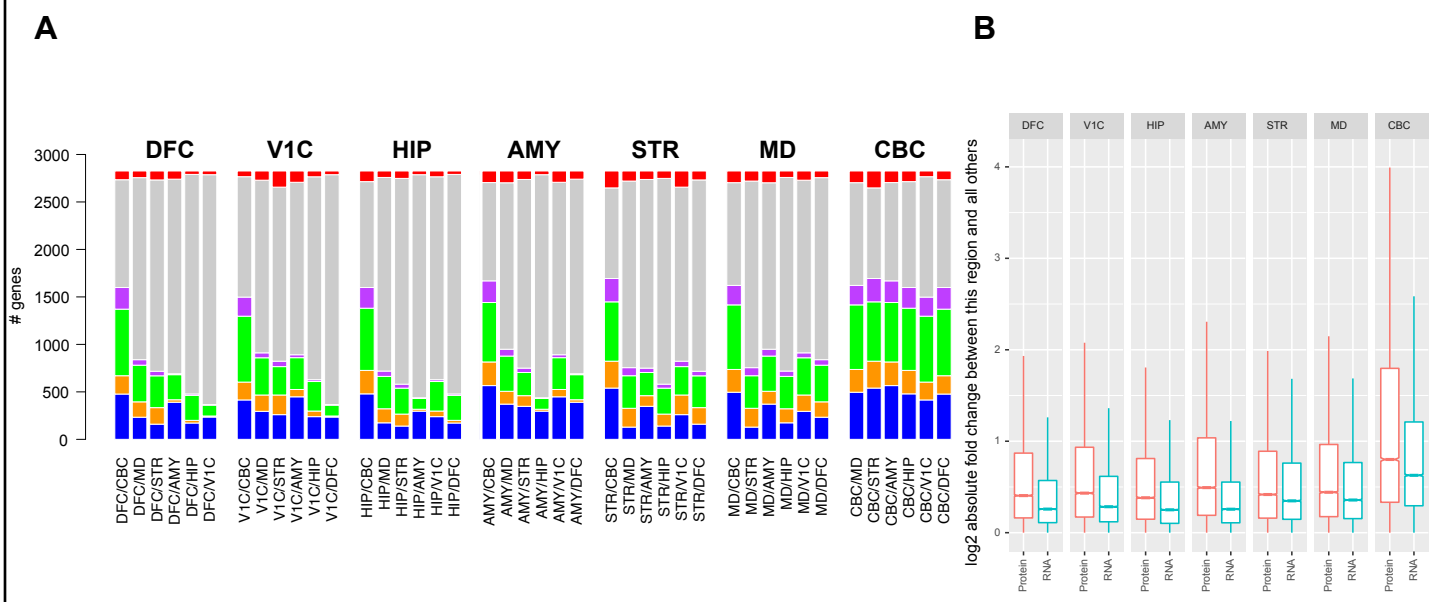
These scatterplots are identically defined to those in Fig 5A except that here genes are labelled to show individual gene names observable at high magnification. A) CBC vs all B) MD vs all C) STR vs all D) AMY vs all E) HIP vs all F) V1C vs all G) DFC vs all. Genes are coloured based on their agreement or disagreement between the RNA and protein measurements; genes for which the protein variability between regions is <2-fold of that reported at the RNA-level were considered consistent (green and grey points). Purple coloured genes are those with consistent direction but variable magnitude of change (≥2-fold) between the regions at the protein and RNA level, while red genes disagree in the direction of change between RNA and protein. Blue and orange genes vary between regions according to protein but not RNA and vice-versa.



Supplementary Figure 10g

RNA vs protein fold-change comparison of all pairs of brain regions.

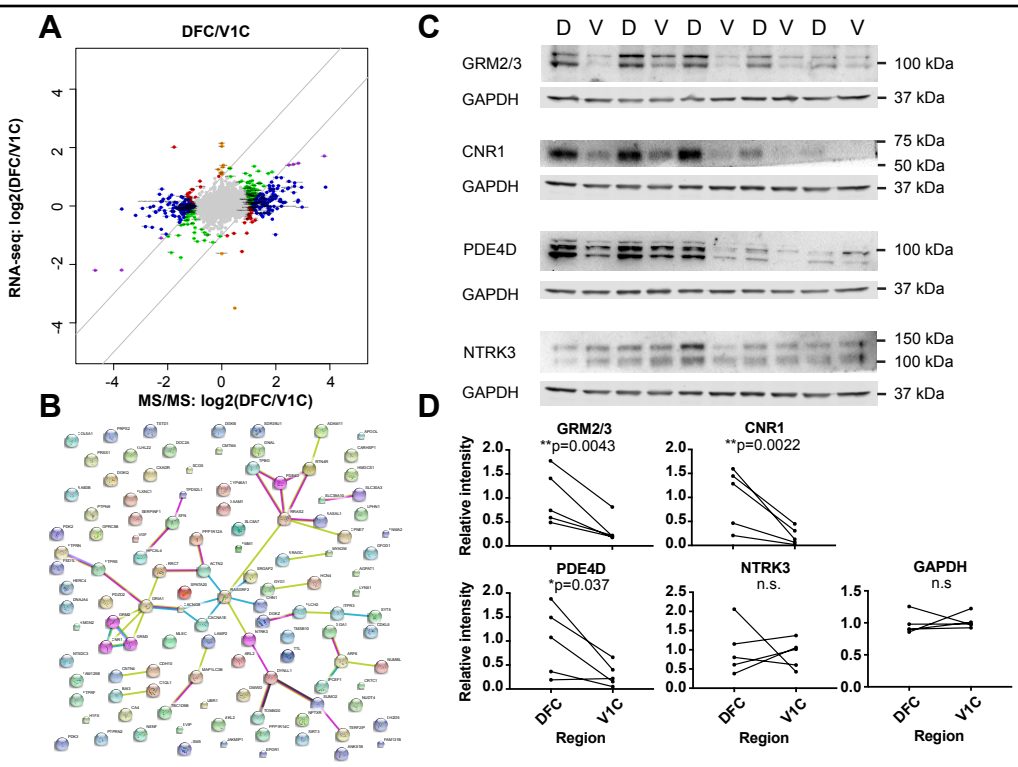
These scatterplots are identically defined to those in Fig 5A except that here genes are labelled to show individual gene names observable at high magnification. A) CBC vs all B) MD vs all C) STR vs all D) AMY vs all E) HIP vs all F) V1C vs all G) DFC vs all. Genes are coloured based on their agreement or disagreement between the RNA and protein measurements; genes for which the protein variability between regions is <2-fold of that reported at the RNA-level were considered consistent (green and grey points). Purple coloured genes are those with consistent direction but variable magnitude of change (≥2-fold) between the regions at the protein and RNA level, while red genes disagree in the direction of change between RNA and protein. Blue and orange genes vary between regions according to protein but not RNA and vice-versa.



Supplementary Figure 11

Summary quantifications of all RNA vs protein fold-change scatter plots.

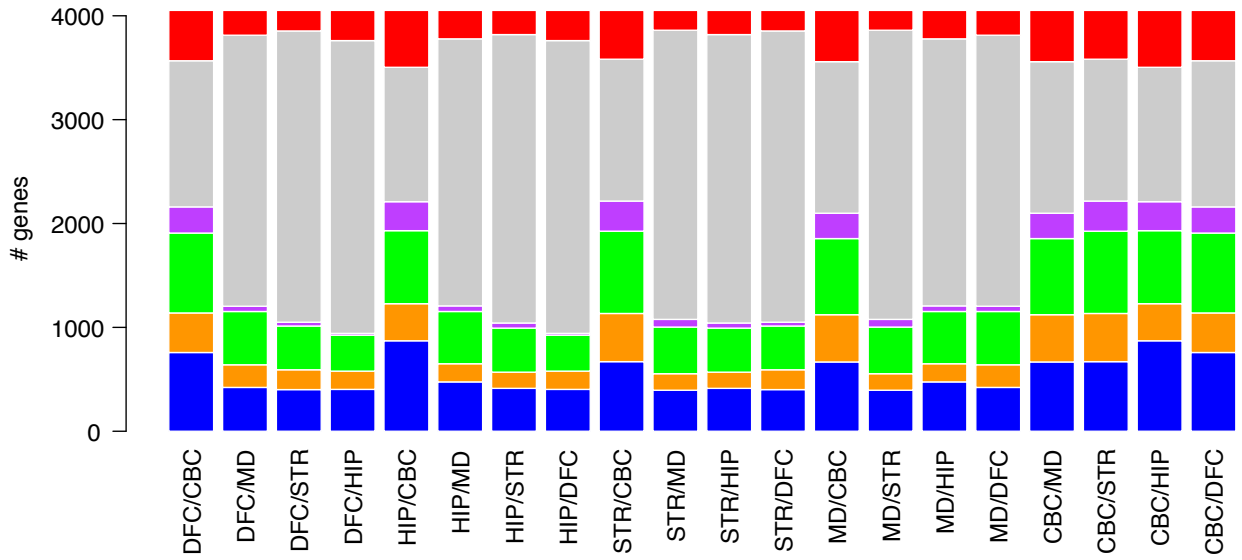
A) Per region-pair counts of the number of genes in each of the colour categories defined in Fig 5A; genes are coloured based on their agreement or disagreement between RNA and protein; genes for which the protein variability between regions was <2-fold of that reported at the RNA-level were considered consistent (green and grey points). Purple coloured genes are those with consistent direction, but variable magnitude, of change between the regions at the protein and RNA level, while red genes disagree in the direction of change between RNA and protein. Blue and orange genes vary between regions according to protein but not RNA and vice-versa. B) Comparison of absolute log2 fold-changes between each region and all others as reported by RNA (blue) and protein (red) show that in general the protein-level fold changes are greater. For example, the DFC plot shows the distribution, over all genes, of fold changes by RNA and by protein of the DFC with each of the 6 other regions. The center line indicates the median, limits indicate the IQR, and the whiskers either 1.5* the IQR or the min/max value if it falls within 1.5* the IQR. Outlying genes are not shown in this plot to highlight the increased median and 75th percentile of the protein fold-change distributions.



Supplementary Figure 12

DFC/V1C comparison in detail.

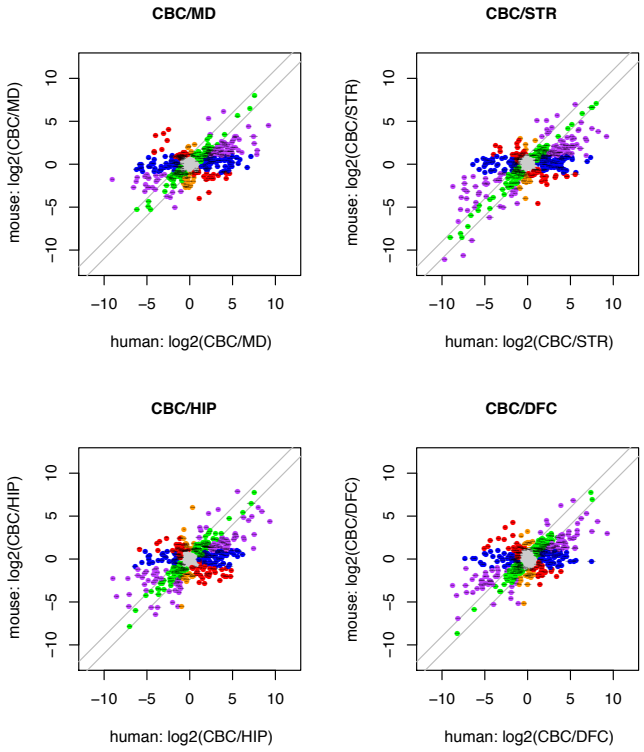
A) The scatterplot is identical to the corresponding panel in Fig 5A, except that individual gene names are labelled. Proteins used for validation in parts C & D are highlighted with pink font. B) Protein-protein interaction network, produced by STRING (medium stringency), of the genes upregulated in DFC in protein only (blue points, right hand side Fig S12A). Proteins used for validation in parts C & D are highlighted with pink font/circles. C) Immunoblotting of the 5 adult DFC and V1C samples shows enrichment of GRM2/3, CNR1 and PDE4D in the DFC over the V1C. Note that the blots shown in this figure are cropped images, and that CNR1 and NTRK3 labelling were performed on the same cut membrane, and thus have the same GAPDH control. D) Quantification of the immunoblots (values normalized to GAPDH) shows significant enrichment of GRM2/3, CNR1 & PDE4D in DFC over V1C by two-tailed paired student's T-test (n = 5 biological replicates per group, d.f. = 4, t = 5.842, 7.006, 3.067 respectively)



Supplementary Figure 13

Summary quantifications of all fold-change scatter plots for human vs mouse.

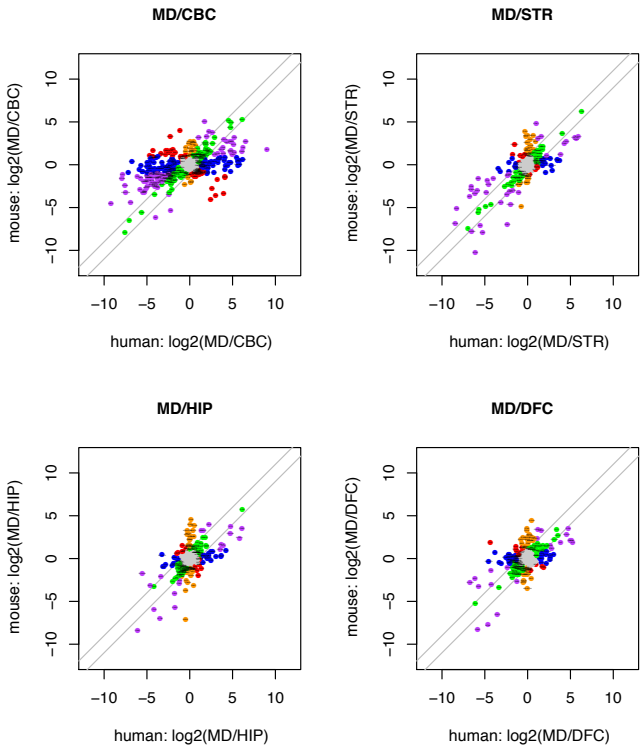
Per region-pair counts of the number of genes in each of the colour categories defined in Fig 6B; genes are coloured based on their agreement or disagreement between mouse and human; genes for which the human variability between regions was within 2-fold of that reported for mouse were considered consistent (green and grey points). Purple coloured genes are those with consistent direction, but variable magnitude, of change between the regions in human vs mouse, while red genes disagree even in the direction of change between the species. Blue and orange genes vary between human regions but not mouse and vice-versa.



Supplementary Figure 14a

Human protein vs mouse protein fold-change comparison of all pairs of brain regions.

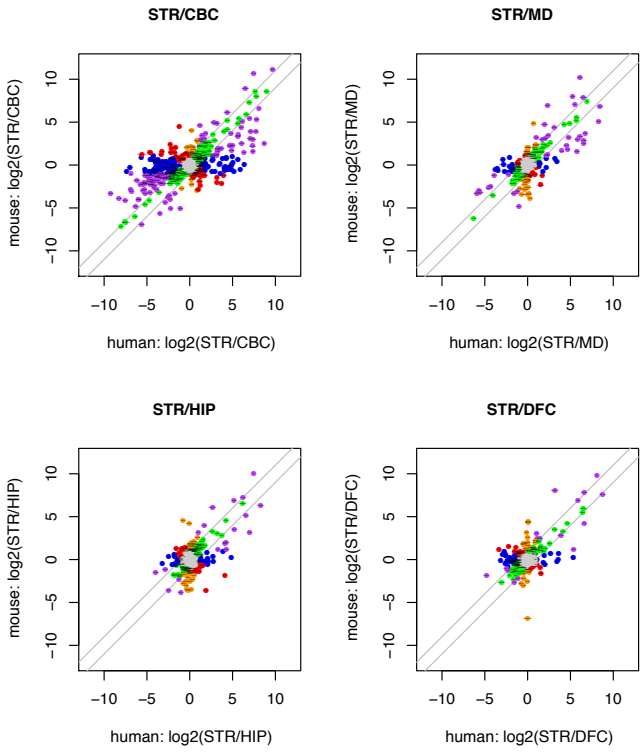
The scatterplots are identically defined to those in Fig 6B except that here only significantly differentially expressed genes from the human are included and genes are labelled to show individual gene names. A) CBC vs all. B) MD vs all. C) STR vs all. D) HIP vs all. E) DFC vs all.



Supplementary Figure 14b

Human protein vs mouse protein fold-change comparison of all pairs of brain regions.

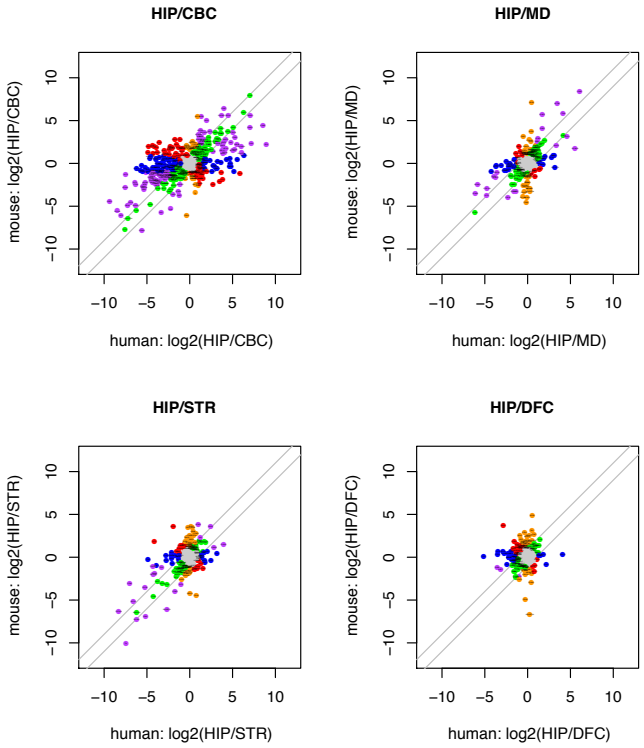
The scatterplots are identically defined to those in Fig 6B except that here only significantly differentially expressed genes from the human are included and genes are labelled to show individual gene names. A) CBC vs all. B) MD vs all. C) STR vs all. D) HIP vs all. E) DFC vs all.



Supplementary Figure 14c

Human protein vs mouse protein fold-change comparison of all pairs of brain regions.

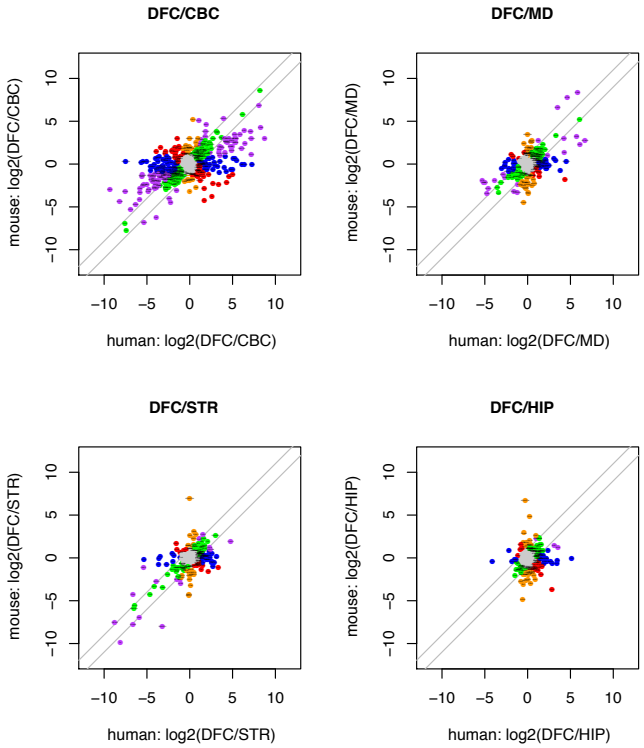
The scatterplots are identically defined to those in Fig 6B except that here only significantly differentially expressed genes from the human are included and genes are labelled to show individual gene names. A) CBC vs all. B) MD vs all. C) STR vs all. D) HIP vs all. E) DFC vs all.



Supplementary Figure 14d

Human protein vs mouse protein fold-change comparison of all pairs of brain regions.

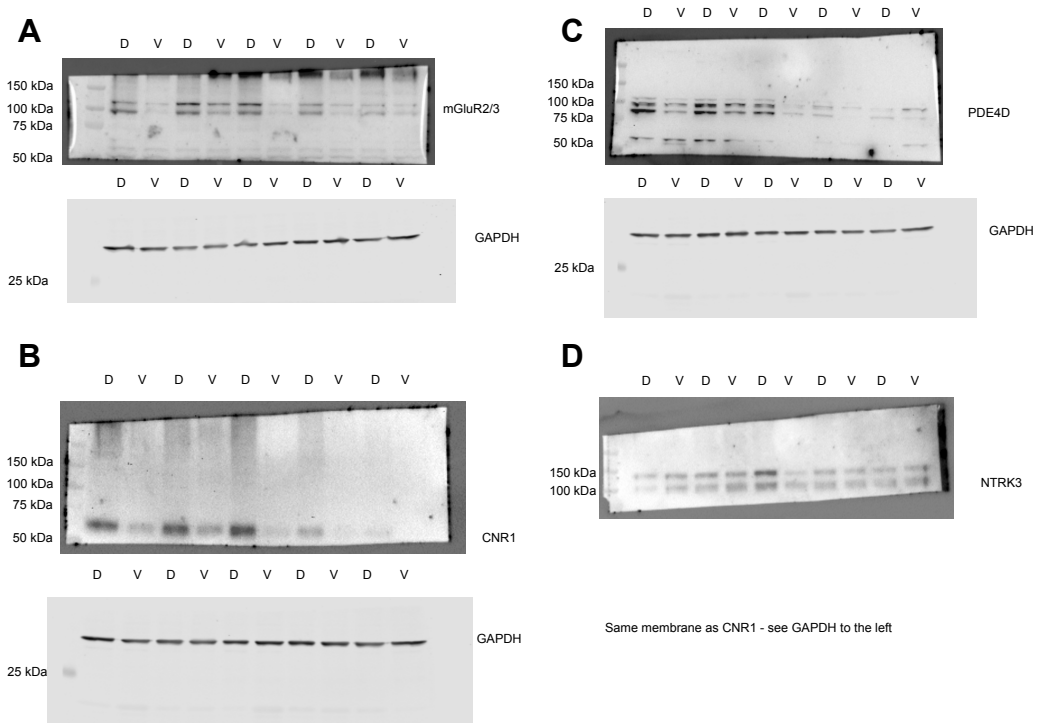
The scatterplots are identically defined to those in Fig 6B except that here only significantly differentially expressed genes from the human are included and genes are labelled to show individual gene names. A) CBC vs all. B) MD vs all. C) STR vs all. D) HIP vs all. E) DFC vs all.



Supplementary Figure 14e

Human protein vs mouse protein fold-change comparison of all pairs of brain regions.

The scatterplots are identically defined to those in Fig 6B except that here only significantly differentially expressed genes from the human are included and genes are labelled to show individual gene names. A) CBC vs all. B) MD vs all. C) STR vs all. D) HIP vs all. E) DFC vs all.



Supplementary Figure 15

Un-cropped versions of the immunoblots in Figure S12.

Note that all GADPH blots were visualised using Licor 800 antibodies, and only the 25 kDa ladder band is visible at this wavelength. A) mGlur2/3 and the corresponding GADPH blot. B) CNR1 and the corresponding GAPDH control. This blot was then trimmed and re-probed for NTRK3. C) PDE4D immunoblot and corresponding GAPDH control. D) NTRK3 immunoblot. See B) for GAPDH control.