Region-Specific PSD-95 Interactomes Contribute to Functional Diversity of Excitatory Synapses in Human Brain.

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Running title – PSD-95 protein-protein interactions in human brain

The overarching goal of this exploratory study is to link subcellular microdomain specific protein-protein interactomes with big data analytics. We isolated postsynaptic density-95 (PSD-95) complexes from four human brain regions and compared their protein interactomes using multiple bioinformatics techniques. We demonstrate that human brain regions have unique postsynaptic protein signatures that may be used to interrogate perturbagen databases. Assessment of our hippocampal signature using the iLINCS database yielded several compounds with recently characterized “off target” effects on protein-protein interactions in the postsynaptic density compartment.

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Introduction

The human brain is comprised of billions of neurons that form networks of connections within and between brain regions [1]. These connections facilitate neuroplastic events that underlie learning and memory, critical aspects of cognitive function often perturbed in neuropsychiatric illnesses [2, 3]. Neuronal signaling is mediated by fast and slow transmission events, encompassing receptors, ligands, ions, enzymes, and other substrates [1, 4-7]. These elements are spatially arranged in subcellular microdomains, facilitating juxtaposition of proteins that coordinate various biological processes. For example, synaptic transmission is modulated via release of neurotransmitter into the synaptic cleft, where receptors are activated and the postsynaptic cell modulated via electrical and chemical signals [1, 4-6].

The pre- and postsynaptic compartments include highly specialized protein clusters, with elegant and complex regulatory mechanisms that traffick proteins to and from these zones. In particular, postsynaptic densities are microdomains comprised of about 1000 unique proteins that are interacting with one another via specialized scaffolding molecules [8]. Postsynaptic density-95 (PSD-95) is a multipotent scaffolding, trafficking, and clustering protein which links glutamate receptors, signaling molecules, and other structural proteins at postsynaptic sites. More than 95% of PSD-95 expression is localized to excitatory synapses, and it is the most abundant scaffolding protein within the postsynaptic density [9-11].

Highly linked proteins, or hubs, are usually protected from null mutations due to the overrepresentation of deleterious functional outcome [12]. PSD-95 is a highly interconnected hub protein with known functional consequences if manipulated.

Altering the expression of PSD-95 protein significantly changes synaptic structure and function, including molecular correlates of learning and memory, long-term potentiation (LTP) and long-term depression (LTD). For example, PSD-95 overexpression increases the amplitude of excitatory postsynaptic currents (EPSCs), to the extent that additional stimulus is unable to further strengthen LTP [13-17]. In contrast, PSD-95 knockdown disrupts postsynaptic density structure and suppresses EPSCs, consistent with a role for PSD-95 in synaptic fidelity [18-20]. Further highlighting the importance of PSD-95 in the synapse, knockout of PSD-95 in mice and genomic variants within postsynaptic density hub proteins are particularly implicated in neuropsychiatric disorders such as schizophrenia and autism [21, 22].

To investigate excitatory postsynaptic protein hubs, we targeted PSD-95 due to its high level of localization to the postsynaptic density (~95%), its well-characterized role as a hub protein, and the large number of constituents in its protein interactome (~1000) [23-25]. We designed a series of experiments to examine brain region-specific excitatory postsynaptic proteomes to answer the following questions. 1) Do postsynaptic protein-protein interactions differ by brain region? 2) Can we accurately detect and separate samples based on these differences? and 3) Can region-specific protein interactomes inform functional differences? For these experiments we used four human brain regions (anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), hippocampus (HPC), and superior temporal gyrus (STG)) from 3 well-matched control male subjects from the Alabama Brain Collection. A total of 289 proteins were identified as part of a PSD-95 protein interactome that were present across all four brain regions. We hypothesize that there is region-specific heterogeneity in the composition of

excitatory postsynaptic microdomains, reflecting the differences in neuroplastic function between human brain regions.

Experimental Procedures

Workflow overview.

Human brain tissue from three male subjects from four brain regions was processed for affinity purification of PSD-95 protein complexes (Table 1). We confirmed PSD-95 capture and enrichment from each sample using Western blot analyses. For quality control, individual biological samples were pooled together based on region, PSD-95 complexes were affinity purified, processed for mass spectrometry, and run in technical triplicate. From the pooled PSD-95 affinity purified complexes we identified more than 500 unique peptides in each pooled sample from each region using data-independent acquisition, as well as shotgun proteomic analyses, confirming robust PSD-95 complex capture (data not shown). Next, individual samples (n = 3 biological replicates for each of four brain regions) were run through our PSD-95 affinity and mass spectrometry protocol in triplicate (3 technical replicates x 12 biological samples for 36 runs total). We then subtracted any non-specifically captured peptides identified by our IgG control studies that were performed in parallel to PSD-95 affinity purification. To account for processing and loading variability, data were normalized within each of the 36 runs to the most abundant PSD-95 peptide (IIPGGAAAQDGR) across all samples. Only peptides that were present in at least 2 of 3 technical replicates were carried forward (peptides missing a technical replicate were replaced by imputation), then all 36 runs were quantile normalized [26, 27]. We used multiple clustering approaches to identify peptides which contributed to brain region specificity, unsupervised clustering (Pearson

correlation) and a weakly supervised clustering protocol (non-negative matrix factorization, NMF), using the same peptides for both clustering analyses [28-30]. T-statistic and coefficient values from unsupervised and NMF clustering were generated for each peptide and a consensus peptide profile, or signature, was generated based on common input peptides across all 4 regions. The region-specific consensus signatures were subjected to traditional and advanced bioinformatics analyses to identify pathways, processes, and compounds associated with the signatures for each brain region.

Tissue preparation. Deidentified cortical tissue samples from 3 matched male subjects were obtained from the Alabama Brain Collection (ABC). The ABC obtained informed consent from survivors of all donors, as well as IRB approval (at the University of Alabama Birmingham) for human brain collection. Brodmann areas 46, 32, 22, and hippocampus were dissected from coronal slabs and stored at 80°C. For each region, frozen tissue was thawed at -20°C for 1 hour then sectioned onto glass slides at 50µm. Ten 50 µm sections were used for each region. Grey matter was harvested from each slide, combined by region, and homogenized 30x on ice with a Teflon coated dounce homogenizer in 1ml of 5mM Tris HCl, 0.32 M Sucrose, with 1% Halt protease and phosphatase inhibitor, pH 7.4. Protein concentration was measured with a Pierce BCA kit. All samples were normalized for protein concentration. TritonX-100 and SDS were then added to the homogenate at a final concentration of 1% v/v and 0.5% v/v, respectively, and then rotated overnight at 4°C. Lysates were immediately used for affinity purification and remaining sample was stored at -80°C.

Affinity purification. We used a mouse anti-PSD-95 antibody (Millipore, catalogue # MAB1596) to capture PSD-95 protein complexes from individual samples. We verified the specificity of this antibody using mass spectrometry analysis of PSD-95 peptides captured by affinity purification vs isotype IgG control (Figure S1). Our LCMS protocol yielded ~65% coverage of the PSD-95 protein (Figure S1C). 5ug of PSD-95 antibody was coupled per 1mg of Dynabeads (Life Technologies) according to the antibody coupling kit protocol (#14311D). For each sample, 1000ul of 10mg/ml antibody coupled beads were washed 2x 1ml with ice cold 1x PBST (#9809S, Cell Signaling) then incubated with 650ug of human brain lysate brought to a final volume of 1000ul with ice cold 1x PBST for 1 hour at room temperature. The supernatant was removed and saved for western blot analysis and the beads were washed 4 x 10 minutes at room temperature in 1ml ice cold 1x PBST. Captured protein complexes were eluted with 30ul of 1N Ammonium Hydroxide (#320145, Sigma), 5mM EDTA, pH 12 for 10 minutes at room temperature. 6ul of 6x protein denaturing buffer (4.5% SDS, 15% β -mercaptoethanol, 0.018% bromophenol blue, and 36% glycerol in 170 mM Tris-HCl, pH 6.8) was added to each sample elution. The eluted samples were heated at 70°C for 10 minutes then processed for mass spectrometry.

Antibody control studies. We performed parallel studies using species matched preimmune IgG. All aspects of the affinity purification described above were duplicated as described above. Three affinity purified IgG control samples (biological replicates) from each region were pooled. We ran three technical replicates of one pooled sample for each brain region through the mass spectrometry protocol. Peptides detected in at

least 2 out of 3 technical replicates were designated as non-specific, and subtracted from our PSD95 affinity purification mass spectrometry dataset analyses.

Mass Spectrometry sample preparation. All samples were loaded on a 1.5 mm, 4-12% Bis-Tris Invitrogen NuPage gel (NP0335BOX) and electrophoresed in 1x MES buffer (NP0002) for 10 minutes at 180v. The gel was fixed in 50% ethanol/10% acetic acid overnight at RT, then washed in 30% ethanol for 10 min followed by two 10 min washes in MilliQ water (MilliQ Gradient system). The lanes were harvested, cut into small (~2mm) squares, and subjected to in-gel tryptic digestion and peptide recovery, as described previously [31]. Samples were resuspended in 0.1% formic acid.

Nano liquid chromatography coupled electrospray tandem mass spectrometry (nLC-ESI-MS/MS). nLC-ESI-MS/MS analyses were performed on a 5600+ QTOF mass spectrometer (Sciex, Toronto, On, Canada) interfaced to an Eksigent (Dublin, CA) nanoLC ultra nanoflow system. Samples were run consecutively then repeated for technical replicates 2 and 3, as previously described [31]. Briefly, peptides were loaded onto an IntegraFrit Trap Column at 2 µl/min in formic acid/H₂O 0.1/99.9 (v/v) for 15 min to desalt and concentrate the samples then the trap-column was switched to align with the analytical column. Peptides were eluted using a variable mobile phase (MP) gradient from 95% phase A (Formic acid/H₂O 0.1/99.9, v/v) to 40% phase B (Formic Acid/Acetonitrile 0.1/99.9, v/v) for 70 min, from 40% phase B to 85% phase B for 5 min and then keeping the same mobile phase composition for 5 additional min at 300 nL/min. The nLC effluent was ionized and sprayed into the mass spectrometer using NANOSpray® III Source (Sciex). Ion source gas 1 (GS1), ion source gas 2 (GS2) and curtain gas (CUR) were respectively kept at 8, 0 and 35 vendor specified arbitrary units.

The mass spectrometer method was operated in positive ion mode and the interface heater temperature and ion spray voltage were kept at 150°C, and at 2.6 kV, respectively. The data was recorded using Analyst-TF (version 1.7) software.

Data independent acquisition (DIA) and shotgun (information dependent

acquisition, IDA) methods. The DIA method was set to go through 1757 cycles for 99 minutes, where each cycle performed one TOF-MS scan type (0.25 sec accumulation time, in a 550.0 to 830.0 m/z window) followed by 56 sequential overlapping windows of 6 Daltons each. Note that the Analyst software automatically adds 1 Dalton to each window to provide overlap, thus an input of 5 Da in the method set up window results in an overlapping 6 Da collection window width (e.g. 550-556, then 555-561, 560-566, etc). Within each window, a charge state of +2, high sensitivity mode, and rolling collision energy with a collision energy spread (CES) of 15 V was selected. For shotgun acquisition the mass spectrometer was set to perform one TOF-MS scan type (0.25 sec accumulation time, in a 350 to 1600 m/z window) followed by 50 information dependent acquisition (IDA)-mode MS/MS-scans on the most intense candidate ions having a minimum intensity of 150 counts. Each MS/MS scan was operated under vendor specified high-sensitivity mode with an accumulation time of 0.05 secs and a mass tolerance of 100 ppm. Precursor ions selected for MS/MS scans were excluded for 30 secs to reduce the occurrence of redundant peptide sequencing.

DIA and IDA data analysis parameters. Proteowizard was used to generate peaks from the raw files using Sciex standard settings [32-34]. Protalizer DIA software ([Vulcan Analytical](#), Birmingham, AL), a spectral library-free DIA analysis package, was used to analyze every DIA file with settings previously described for Sciex 5600+ QTOFs [35,

36]. The Swiss-Prot *Homo Sapien* database, downloaded March 17th 2015, was used as the reference database for all MS/MS searches. A maximum of 2 missed cleavages was allowed for searching based on trypsin cleavage. A precursor and fragment-ion tolerance for QTOF instrumentation was used for the Protalizer Caterpillar spectral-library free identification algorithm as previously described [36, 37]. Peptide retention time and drift adjustment were corrected as previously described [36, 37]. Potential modifications included in the searches were phosphorylation at S, T, and Y residues, N-terminal acetylation, N-terminal loss of ammonia at C residues, and pyroglutamic acid at N-terminal E and Q residues. Carbamidomethylation of C residues was searched as a fixed modification. The maximum valid protein and peptide expectation score from the X! Tandem Sledgehammer search engine (version 2013.09.01.1) used for peptide and protein identification on reconstructed spectra was set to 0.005. The forward and reverse *H. Sapien* Swiss-Prot database was used for identification.

For DIA quantification, the maximum number of b and y series fragment-ion transitions were set to nine excluding those with m/z values below 300 and not containing at least 10% of the relative intensity of the strongest fragment-ion assigned to a peptide. A minimum of five fragment-ions were required for a peptide to be quantified. In datasets where a minimum of seven consistent fragment-ions were not detected for the same peptide ion in each of the three files compared in a triplicate analysis, the algorithm identified the file with the largest sum fragment-ion AUC and extracted up to seven of these in the other files using normalized retention time coordinates based on peptides detected by the Caterpillar algorithm in all the files in a dataset. The calculated maximum FDR rate at the peptide and protein level was 1.45%

and 9.3% respectively.

Data Availability: The raw and processed files have been downloaded to PeptideAtlas and are publicly available using the following link. Chromatograms of all peptides identified are included as web viewable files.

<http://www.peptideatlas.org/PASS/PASS01361>

Western blot analyses. A 2ul aliquot was taken from the previously reduced and denatured samples destined for mass spectrometry and processed by SDS-PAGE using Invitrogen (Carlsbad, CA) 1.5mm thick, 4 – 12% gradient gels and transferred to PVDF membranes via BioRad semi-dry transblotters (Hercules, CA). The membranes were blocked with LiCor blocking buffer (Lincoln, NE) for 1 hour at room temperature, then probed for 1 hour at room temperature with primary antisera for PSD-95 (1:1000, Cell Signaling, catalogue # D27E11), Synaptophysin (1:1000, Cell Signaling, catalogue # D40c4), or CaMKIIalpha (1:1000, Santa Cruz Biotechnology, catalogue # sc-376828) diluted in 0.1% Tween LiCor blocking buffer. The membranes were washed twice for 10 minutes each in 1x 0.1% Tween phosphate buffer solution (PBST) then probed with goat anti-mouse or goat anti-rabbit IR-Dye 670 or 800cw-labeled secondary antisera (LiCor) in LiCor blocking buffer (plus 0.1% Tween, 0.01% sodium dodecyl sulfate (SDS)) for 1 hour at room temperature. Washes were repeated after secondary labeling, washing twice for 10 minutes in 1x PBST, and then placed in water. Membranes were imaged using a LiCor Odyssey scanner.

Experimental design and statistical rationale. We used area under the curve to quantify the abundance for 424 peptides that were captured as interacting with PSD-95 in four brain-regions in three healthy patients. Each biological sample was run in

technical triplicate, resulting in thirty-six measurements that were processed and analyzed using an in house computational pipeline developed using R version 3.1.3. Since the experimental design was optimized for measuring relative, rather than absolute abundance levels, each measurement vector (sample) was first normalized to express peptide abundance levels in relative terms. This is achieved by dividing each peptide's abundance estimates by the intensity of the PSD-95 IIPGGAAAQDGR peptide. We selected this peptide for its consistency and higher than average intensity across all samples compared to other PSD-95 peptides (data not shown). Note that highly abundant fragments are expected to be quantified more reliably, and therefore higher intensities are more likely to represent the real abundance of a component when compared between samples. Data were then Log2-transformed and, to improve analysis reliability, only peptides with at least two out of three data points per biological sample available for each peptide were considered. In the current work, we focused on PSD-95-associated proteins with a higher abundance in specific regions of the brain, but at same time observed (at lower levels) in multiple regions. Thus, proteins that are not observed in some regions at all (or have too many missing values) were excluded to avoid confounding the results by including false negatives. The rates of false negatives in MS-based proteomics, including those peptide moieties that are not detected because their intensities are too low (given the noise levels), and can be very significant [38]. Therefore, a larger sample size (than used in the present work) would be required to confidently separate biological and technical negatives, which is the subject of future work. Each sample was then quantile-normalized to account for signal amplitude

variation across measurements. Remaining missing values were imputed by the mean of the two other data points within three technical replicates per biological sample.

Clustering analyses and generation of signatures. Unsupervised clustering was first used in order to identify potential strata of samples with distinct protein expression patterns. Coefficient of variation of each peptide's abundance levels was used as a measure of variability to rank and select the 200 most variable peptides across all 36 runs. For the purpose of visualization and further analysis, each peptide row was then z-transformed in order to align the mean and amplitude of peptides across samples. Patterns in protein expression data were identified by clustering both peptides and samples with the Pearson correlation based distance function, and visualized using the *Heatplot* function from the *made4* R-package. For further validation and assessment of the effects of potential outliers, Spearman rank correlation was used as an alternative, yielding very similar results (data not shown).

Secondly, NMF decomposition of the fully-normalized matrix was obtained for decomposition dimensions k ranging from 4 to 7 using the "ls-nmf" algorithm after shifting the matrix values by 10 in order to obtain a non-negative input matrix [30]. The decomposition on $k=6$ vectors appeared to best segregate samples and was therefore selected for further analysis.

Signatures were derived from the two distinct clustering analyses. From the unsupervised cluster, t-tests were performed for each peptide for each group against all the other groups. The 50 most specific peptides for each group defined its signature. On the other hand, the NMF decomposition presented specific relations between HPC and ACC and vector 3 and 1 of the decomposition. Since the relations between the other

vectors (2, 4, 5 and 6) with brain regions were less obvious, we focused on the NMF signature for HPC and ACC. We then ranked the peptides (positive) decomposition coefficient and selected the 50 most specific peptides for HPC and ACC. A consensus signature containing overlapping hits from each clustering approach was used for subsequent bioinformatics analyses.

Traditional bioinformatics analyses. We first used ToppCluster (<https://toppcluster.cchmc.org/>) in order to perform gene set enrichment analysis and map pathways associated with region specific PSD-95 interactome signatures [39]. Additional analyses were performed using piNET (<http://pinet-server.org/>), a tool developed to provide further assessment of region-specific protein networks, including targeted analyses of kinase signaling pathways [40].

Advanced bioinformatics analyses. We also used the Library of Integrated Network-based Cellular Signatures (LINCS) (<http://ilincs.org>) to analyze the connectivity of region specific PSD-95 protein interactome patterns [41-47]. With the goal of identifying connected gene knockdown and small drug-like molecule signatures, we used iLINCS to first retrieve transcriptional signatures of gene knockdowns for genes contributing to HPC versus ACC specific patterns. We then used iLINCS to identify small molecules that result in signatures that are significantly discordant (or reversing these gene knockdown signatures) or concordant with these LINCS knockdown signatures. Only genes that had gene knockdowns available were included (from the top 50 proteins in the overexpression/consensus signature). Vertebral-Cancer of the Prostate (VCAP) cell lines are used as the primary source of knockdowns (if not available, the A375 cell line was used since these two cell lines have the best coverage). Only significant

correlations with drug signatures were considered (top 1%). Discordant signatures were primarily from VCAP, with additional cell lines considered as supporting evidence. We then performed literature review of small molecules suggested by our analyses to determine if there is evidence for a mechanism linking our proteomic studies with drugs or perturbagens that yield discordant signatures.

Results:

We developed a method for capturing the PSD-95 protein complexes from human brain by adapting and modifying an approach used in rodent brain (Figure 1)[48]. Using homogenized human frontal cortex tissues, we partially solubilized membrane associated protein complexes by adding sodium dodecyl sulfate (SDS) at a final concentration of 0.5% (Figure 1A). This sufficiently released PSD-95 complexes from membranes and increased enrichment of PSD-95. Increasing concentrations of SDS resulted in reduced capture of PSD-95 (Figure 1B). Notably, representative protein-protein interactions with CaMKII α remain well preserved using 0.5% SDS, while presynaptic markers (synaptophysin) are not captured. We further characterized PSD-95 affinity purified complexes by coomassie staining of total protein. We show a robust enrichment of PSD-95 protein and its interacting partners, with (apparently) hundreds of abundantly co-purified proteins (Figure 1C). Isotype specific IgG negative control studies demonstrate the specificity of our protocol (Figure 1D).

We next performed PSD-95 affinity purification from 4 brain regions including the anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), hippocampus (HPC), and superior temporal gyrus (STG) from three age-matched elderly human male subjects (Table 1 and Figure 2A and 2B). Samples were processed for mass spectrometry and each biological sample was run three times through an ABSciex

5600+ mass spectrometer in DIA mode (550-830 m/z range with 6m/z windows).

Representative chromatograms for 2 abundant PSD-95 peptides (IIPGGAAAQDGR and NTYDVVYLK) are shown to indicate how area under the curve abundance values were generated for each peptide (Figure 2C). The robust reproducibility of our data capture method is represented by the low coefficient of variation displayed by the majority of peptides measured from the technical replicates (Figure 2D).

Peptides detected by LCMS were normalized to the abundance of IIPGGAAAQDGR, then quantile normalized for inter-sample correlation analyses and careful missing-value handling [26, 49-51]. After normalization, we performed unsupervised clustering of all biological and technical replicates using the 200 most variable peptides to determine if normalized peptide abundance signatures could differentiate inter-subject and inter-region differences (Figure 3). Technical replicates clustered together 100% of the time while regional samples showed some degree of overlap (1 DLPFC sample was more closely associated with all 3 ACC rather than the other 2 DLPFC samples, while 1 STG and 1 HPC sample were more closely associated with two DLPFC samples (Figure 3). Of the four regions, the HPC and ACC clustered most consistently using this standard differential expression approach.

Because the HPC and ACC gave the most robust clustering results, we focused on these two regions for our subsequent analyses. To derive peptide expression signatures for these two regions, we performed a two sample t-test using the geometric mean for each peptide from the three HPC biological samples versus all other regions, followed by the identical analysis of the ACC. We used the resulting value of the t-statistic as a score measuring the degree of differential expression (and thus

discrimination) between the HPC and ACC. A scatterplot of t-statistic values for all 200 peptides (ranked based on their t-statistics) shows that approximately 50 peptides are expressed at a higher level in the HPC PSD-95 interactome versus the ACC, constituting a putative HPC signature (Figure 5A, red triangles, and Table S1). Conversely, about 50 different peptides are expressed at a higher level in the ACC PSD-95 interactome versus the HPC (Figure 5A, blue squares, Table S1).

To validate these findings, we subsequently used an alternative pattern recognition technique to generate peptide signatures which might separate the samples more clearly by region. Weakly supervised (by specifying the number of expected vectors) clustering analysis using non-negative matrix factorization (NMF) identified a distinct HPC signature (row 1) when using 6 vectors (Figure 4 and Table S1) [52-54]. Additionally, the ACC region was also associated with a distinct peptide signature which separated from all other regions (row 4, Figure 4, and Table S1). Next, we employed the same scatterplot comparison approach used above for the signatures from our NMF analysis (Figure 5B). These results also highlight the differential expression of these peptides in the HPC versus the ACC. We found a high degree of overlap between differential peptide expression and NMF-generated signatures in the HPC (48/50 proteins, Figure 5C), but much lower concordance in the ACC (12/50 proteins, Figure 5D).

Peptide signatures derived from unsupervised clustering, NMF, and differential expression analyses were subsequently integrated into consensus signatures. Specifically, peptide ranks in each signature were combined via geometric mean to generate a consensus peptide signature for the HPC (Table S2) and ACC (Table S3)

regions. Multiple peptides belonging to the same protein were combined and the resulting top 25 proteins in the consensus signature for each region were used for bioinformatic analyses to identify pathways, functional categories enriched in each region, and gene knockdown or chemical perturbagen signatures concordant or discordant with our PSD-95 interactome signatures.

We first performed traditional pathway analyses using Topocluster (Figure 6) [39]. We found overlap of some biological pathways, functions, and molecular disease phenotypes, while others were unique to each region (Figure 6). We also used PiNet (<http://pinet-server.org>) to explore Panther (Figure 7A) and Kegg (Figure 7B) pathways associated with the HPC signature. Many expected pathways were identified, including glutamate receptor, synaptic transmission, and signal transduction pathways associated with excitatory neurotransmission. Unexpected pathways include inflammatory, immune, and phospholipid-associated functions and processes.

We also used PiNet to explore protein kinase networks associated with our HPC signature (Figure 7C). The BRD4 and BMPR2 kinase pathways have the largest overlap with the HPC signature, followed by ALK, IGF1R, EGFR, ERBB2, CDK8 and AKT1, as indicated by the node radius (or the number of edges). Note also that three of the top five HPC signature proteins (PKP2, PRKCG, MOBP, CORO7, SHISA6), are connected with both BRD4 and BMPR2 pathways. Interestingly, BRD4 is involved in transcriptional regulation of learning and memory, and is expressed in neurons throughout the brain [55, 56]. BMPR2 is expressed in HPC and is implicated in the regulation of anxiety-like behaviors in rodents [57-60].

We next performed exploratory bioinformatics analyses on proteins from our HPC signature using the Library of Network-Based Cellular Signatures (LINCS) database to determine if our signature could help inform functional significance and mechanistic processes. LINCS consists of transcriptional, proteomic, and kinase activity signatures based on chemical (small molecule), genetic (gene knockdown), and kinase activity assays in cellular systems, systematically collected and made available to the community as part of big biomedical data resources [41-43, 45, 46, 61]. Direct search for concordant and discordant LINCS signatures requires a significant probe / protein level overlap. Because of a limited overlap between the HPC consensus signature and L1000 gene set used by LINCS for transcriptional signature collection, we also used an indirect approach consisting of analysis of available gene knockdown signatures contributing significantly to the HPC signature, and identification of small molecules generating discordant (i.e., gene knockdown reversing) signatures (Figure 8A). Such identified small molecules are hypothesized (based on their cell line signatures) to increase expression of genes (and by extension proteins) downregulated in response to a gene knockdown.

The LINCS database for transcript profiling utilizes mRNAs for the same 972 landmark genes (called L1000). 18 out of 50 proteins in our HPC consensus signature are found in the L-1000 LINCS profile (Table S4). We then searched iLINCS for chemical perturbagen signatures for our 18 genes represented in the LINCS database. This search yielded a list of chemical perturbagens that significantly (adjusted $p < 0.05$) altered transcript expression for these 18 genes. Interestingly, phenothiazine class drugs increased mRNA expression in the LINCS database for 11 of these targets

(Figure 8B). Three of the top five discordant perturbagens belong to this class of antipsychotic medication, including trifluoperazine, thioridazine, and fluphenazine (Figure 8B and Table S4). Other chemicals highly discordant for CaMKII α iLINCS L-1000 knockdown signature in the VCAP cell line were valproic acid and AG14361. Of note, AG14361 is a potent and selective polyADP-ribose polymerase (PARP-1) inhibitor which has a highly similar chemical structure to Rucaparib, a PARP-1 / ERK2 protein-protein interaction modulator.

Based on these intriguing findings, we performed an exploratory iLINCS connectivity analysis for HPC consensus signature genes (Table S5). The primary finding from our connectivity analysis was the negative (for lower concentration) correlation of several structurally similar antipsychotics, including thioridazine, trifluoperazine and fluphenazine, with the knock-down signatures of proteins increased in the HPC PSD-95 interactome (Figure 8 and Table S4). These antipsychotics were shown to have low picomolar inhibition of TARP2/ARC protein-protein interactions, suggesting that their binding to Arc protein could represent their primary mode of action [62]. At higher concentration, an inversion to positive correlations is often observed, suggesting that an alternative mechanism could play a role, possibly one that is consistent with antagonism of D2 dopamine receptors by these antipsychotics (albeit with a lower affinity compared to Arc).

Discussion:

Our findings demonstrate that 1) PSD-95 protein hub constituents differ by brain region, 2) we can separate/cluster brain regions based on these differences, and 3) differences in PSD-95 protein hub composition map to divergent functional and

pharmacological substrates. In particular, our results show that the HPC and ACC have unique PSD-95 associated proteomic signatures, in contrast to the DLPFC and STG. This is not surprising given the unique neuroarchitectural features of the HPC with its trisynaptic excitatory circuits, as well as the agranular (ie no layer 4) nature of the ACC [63]. We subsequently focused on the ACC and HPC, generating compartment-specific, proteomic signatures through a pipeline of unsupervised (Figure 3) and semi-supervised NMF clustering (Figure 4), followed by bioinformatic analyses to identify biological pathways. As expected, the most distinct region identified was the hippocampus, which was clearly differentiated via clustering from all other regions tested.

The postsynaptic density and PSD-95 are constitutively modulated through activity dependant mechanisms [14, 18, 64]. Some of the earliest work on characterization of excitatory postsynaptic densities suggested that the composition across regions is relatively uniform [65]. However, our data suggest that there are marked differences in the relative abundance of signaling and other proteins between regions, informing a picture of a non-static environment receptive to immediate and robust action after stimulus. This notion is supported by studies where Peng and colleagues examined the differences between rodent forbrain versus cerebellar excitatory postsynaptic densities, reporting ~15% of postsynaptic density proteins as significantly different between regions [10]. Importantly, electrophysiological function of cerebellar synapses are particularly different than cortical synapses. Therefore, we hypothesize that regional functional specificity relies upon inherent differences of postsynaptic protein-protein interactions and that these differences confer functional relevance.

Several distinct approaches have been employed to assess the constituents of the PSD, including fractionation (yielding an enriched fraction of PSDs) [65-71], TAP tagging of PSD-95 for immunoisolation [72], or direct immunoisolation with anti-PSD-95 antibody [48, 73, 74]. Comparison of these different approaches to our present work shows moderate overlap between our human study and the murine flagged PSD-95 dataset (33%), with lower overlap with postsynaptic density fraction studies (between 9-10%) (Tables 2 and S6). The study combining fractionation and PSD-95 isolation was intermediate to these other findings (about 22% overlap with our data). Only one of these studies was performed in humans, and it employed fractionation, making direct comparisons between the datasets of limited value [8]. Other reasons for the varying overlap may include differences in LCMS protocols, normalization procedures, and brain regions analysed. Importantly, there are also differences in control studies. We included an IgG control immunoisolation and qualitatively subtracted proteins from our analyses that were captured with pre-immune IgG. Another source of variation may be our handling of “missing” proteins. We only analysed proteins found in all four brains regions. Because missing proteins may either be true negatives, and represent important differences between regions, or be proteins that did not have detectable fragments in the LCMS protocol (false negatives), we may have eliminated several region-specific proteins that may be found in the PSD and/or PSD-95 interactome.

Regarding the brain regions employed, the mouse studies included in Table 2 and S6 all used whole brains as input, while we used discrete brain regions [72-76]. The human postsynaptic density fractionation study combined frontal, temporal, parietal, and occipital brain regions [77]. The differences in species and brain region notwithstanding,

these data suggest that the PSD-95 interactome represents about 20% of the postsynaptic density-compartment's proteome. This is not surprising given the volume and complexity of this subcellular compartment. Further work is needed to make more definite conclusions regarding similarities or differences across species and to account for divergences associated with specific methodologies.

In contrast to the aforementioned murine studies, we assessed individual brain regions, an approach made feasible by the relative abundance of human brain compared to rodents. We compared the results of unsupervised and NMF clustering signatures from HPC and ACC. The HPC consensus signature (Figure 5C and Table S2) shows nearly complete overlap (48/50 proteins) between the two clustering methods, suggesting that the HPC signature is clearly identifiable and unique from the other regions tested. In contrast, the ACC signatures had less overlap, with 12/50 proteins common to the unsupervised and NMF signatures (Figure 5D and Table S3). This is likely due to structural and functional similarities between the ACC, DLPFC, and STG, which are all regions of input processing and integration for higher order cognitive function [77]. Between the two clustering methods, the NMF analyses appears to better differentiate the HPC and ACC compared to the unsupervised hierarchical clustering approach. NMF is a semi-supervised analysis which relies on the user to select the number of vectors or groups the data should fall into. Each vector represents a linear metagene comprising all the data in the set. The dimensionality of metagenes are reduced through decomposition into two nonnegative matrices, then matched back to the metagene through an iterative multiplicative updates algorithm (in this study we used 200 iterations for all NMF analyses) [28].

Nonnegative matrix factorization (NMF) was introduced as a weakly supervised approach to analysis and interpretation of large data sets, including molecular pattern discovery, class comparison and prediction in computational biology. In particular, NMF has been increasingly popular as a method of choice for omics data consisting of abundance levels for tens of thousands of genes or proteins across a number of samples. In this context, NMF involves the decomposition of a nonnegative protein (or peptide) expression matrix P consisting of observations on p peptides from n samples, into two nonnegative matrices W and H . Each column of W defines a meta-protein (defined as a linear combination of individual proteins or peptides that can be viewed as a signature of some underlying biological state with proteins obtaining larger coefficients contributing more to that state), while each column of H represents the meta-protein expression pattern of the corresponding sample. NMF represents an alternative to other pattern recognition and data mining techniques, such as unsupervised hierarchical clustering or Principal Component Analysis, yielding results that are largely comparable but often easier to interpret, in particular as it pertains to the derivation of molecular signatures of biological states. Here, these signatures are defined by peptides whose high expression is associated with specific brain regions, as reflected by those peptides obtaining high coefficients in the corresponding meta-protein columns of W [28]. In summary, we conclude that combining the unsupervised and NMF approaches appears to identify consensus clusters, providing robust results for our our PSD-95 affinity purification-LCMS protocol.

Using the consensus signatures generated via combining consensus hits from NMF and hierarchical clustering, we performed pathway analyses using ToppCluster

(Figure 6) [39]. Top pathways for the HPC include GTP binding, GTPase activity, and cytoskeletal regulation, while top pathways for the ACC include glutamate receptor binding and nucleotide kinase activity (Figure 6). Interestingly, the top hits from the HPC signature reflect fundamental biological processes that are prominent in this region, including a high density of excitatory synapses with excitatory (ie glutamate) inputs. For example, SHISA6, PKP2, PRKCG, NSF and CORO7 are important components of postsynaptic activity, memory, and learning. Consistent with the biological functions of these proteins, behavioral phenotypes of the HPC include abnormal learning and memory, anxiety, and nest building using targeted knockdown or deletion of these genes [78-84].

Given the structural and developmental differences between the HPC and ACC, our findings suggest that the unique consensus signatures represent individualized structural and behavioral consequences in a region-dependent manner. Consideration of specific elements of each signature may inform the overall fidelity of our approach. For example, SHISA6 is an auxiliary AMPA-subtype glutamate receptor binding protein important for trafficking in association with PSD-95 [85]. SHISA6 contains a PDZ domain and directly binds to PSD-95 [85]. Expression of SHISA6 in HEK cells altered AMPA receptor GluA1 and GluA2 synaptic localization and function. Additionally, SHISA6 expression is preferentially expressed in all hippocampal subfields and the dentate gyrus, but has limited expression in the cortex [85, 86]. Identification of this molecular component of neuroplastic functions attributable to the HPC suggests that the HPC signature is in fact very “hippocampal.”

Several other proteins in our HPC signature, including PRKCG, NSF, β SNAP, PICK1, and PKC, also have strong ties to excitatory neuroplasticity. Initial studies on PRKCG indicated deficits in spatial and contextual learning, likely due to abnormal hippocampal LTP induction in mutant PRKCG mice [87, 88]. Others have shown that PKC gamma traffics AMPARs in zebrafish in an NSF and PICK1 dependent manner, resulting in miniEPSC potentiation [89-91]. Additionally, NSF attachment protein beta (NAPB or β -SNAP) can regulate NSF and PICK1 mediated AMPAR trafficking, where β SNAP directly binds to PICK1 and allows NSF to disassemble PICK1-AMPA complexes [92]. NSF and β SNAP are highly expressed in hippocampal neurons and regulate AMPAR recycling and internalization in an NMDAR activity and Ca^{2+} dependent manner [93-98].

Other proteins in the HPC signature have previously been demonstrated as highly specific to this region. For example, plakophilin 2 (PKP2) represents our most robust contributor to hippocampal specific signature, although not much is known about how it functions at synapses. PKP2 belongs to the armadillo-repeat and plakophilin family of genes including β -catenin, Sys1, Plakophilin-1, Plakoglobin, and Importin- α among many others [99]. One study showed PKP2 transcript expression was highly specific for hippocampus/Ammon's horn in mouse brain [100]. The presence of these previously identified HPC-specific proteins supports the conclusion that our signature differentiates the HPC from other brain regions.

Another biological theme associated with the HPC signature relates to structural molecules that facilitate clustering of signaling molecules to the PSD. LRRC7 (also known as Densin-180) is an ERBIN (ErbB2 interacting protein) paralog which localizes

to PSDs and directly interacts with CaMKII α and α -actinin [101-105]. Densin-180 also interacts with maguin-1, more commonly referred to as connector enhancer kinase of ras 2 (CNKSR2) or CNK homolog protein 2, which mediates Densin-180/maguin-1 complex formation with PSD-95 at glutamatergic synapses [106-108]. Changes in Densin-180 expression impact dendritic branching, localization of PSD95 to synapses, and clustering with shank3 and other proteins [107-111]. Alterations in other structural proteins found in the HPC signature, including delta-catenin, are associated with developmental disorders, including autism and cri-du-chat syndrome [112-117]. Post-translational modifications appear to mediate clustering of catenins in the PSD; palmitoylation of beta-catenin by DHHC5 increases N-cadherin/beta-catenin interaction, AMPAR insertion into the plasma membrane, and increased spine volume [118, 119]. In summary, many of the proteins in the HPC signature have higher or exclusive expression in the HPC, as well as serve critical roles in neuroplastic functions that underlie the functional novelty of this region [120].

In contrast to the HPC, the ACC is not as extensively studied, and thus there is a much smaller literature to draw from regarding ACC-specific expression and function of specific proteins found in this signature. In addition, the ACC consensus signature has fewer hits than the HPC, likely due to overlap of its PSD contents with the more closely functionally related DLPFC and STG, which are also neocortical regions. Interestingly, the ACC has an important gross structural dissimilarity compared to DLPFC and STG, in that it lacks a granular layer (it is considered agranular cortex with no formal layer IV) [63].

A family of molecules that facilitate protein-protein interactions and clustering with ionotropic glutamate receptors is overrepresented in the ACC signature [121]. DLG1 (aka SAP97), DLG2 (PSD93 or chapsyn-110), and DLG3 (SAP102) are highly homologous and have similar functions, likely the result of gene duplication. Despite their sequence similarity, they possess unique sequences/motifs that promote diverse protein-protein interactions [122, 123]. Alterations in these glutamate receptor interacting proteins have been found in various brain disorders, including schizophrenia and dementia [124-127], suggesting a unique role for the ACC in the pathophysiology of cognitive dysfunction in severe neuropsychiatric illness. This notion is supported by the cognitive functions ascribed to this limbic region, which include working memory and executive function [128] [129].

Another informative hit in the ACC signature is LAMB2, a protein that regulates postsynaptic synapse formation [130, 131]. Null mutations for LAMB2 yield disorganized cortical lamina, and its localization specifically to limbic cortex suggests involvement in the biology of higher cortical functions [132]. Another hit with biological relevance is CIT (Citron Rho-Interacting kinase), which directly binds to PSD-95 via the PDZ3 domain [133, 134]. CIT expression in HPC is relatively low (and only in GABAergic interneurons), whereas cortical expression is more robust and not limited to interneurons [103, 135]. CIT is a kinase which phosphorylates RhoA and RhoB, small GTPases which regulate actin cytoskeleton dynamics. [136-139].

The identification of proteomic signatures that uniquely represent specific brain regions suggests an important opportunity for elucidation of pathophysiology mechanisms as well as identification of pharmacological agents which may simulate or

reverse pathological changes found in disease states. One powerful tool developed to facilitate such analyses involves transcriptional profiling and connectivity analyses with large databases of altered biological substrates. We employed the LINCS database to develop insights into the observed region-specific variation in PSD95 postsynaptic complexes. Using recently developed iLINCS software (<http://www.ilincs.org>), we identified small molecules that generated either discordant (i.e., gene knock-down reversing) or concordant (resulting in similar transcriptional echo to that of gene knock-down) signatures from our studies. Interestingly, 12 out of 18 gene knock-down signatures included in the analysis, including PRKCB, CAMK2A and TUBB2A, are “reversed” by phenothiazines including trifluoperazine, thioridazine and fluphenazine (Figure 8 and Table S4). This class of D2 receptor antagonists promotes a “hippocampal” pattern of gene expression, restoring a more HPC-like profile of transcript expression in cell culture.

This observation was surprising as these drugs are well-characterized as D2 dopamine receptor antagonists, while most of the hits in our signatures were related to excitatory (glutamatergic) neurotransmission [140]. Since dopamine is a potent regulator of glutamate transmission, we would have expected to see more dopamine associated proteins in our signatures [141-144]. However, recent work puts our findings into a different context. A new mode of action was recently reported for these drugs that involves binding and regulating PSD proteins, including Arc and TARPy2, regulators of synaptic activity in hippocampus [62]. Interestingly, thioridazine and trifluoperazine affinity for Arc was higher than for D2 receptors, raising the possibility that dopaminergic modulation may be secondary to effects on PSD constituents. While further work is

needed to explore this putative alternative mechanism, it raises intriguing possibilities for modulating HPC related functions, such as learning and memory.

In addition to this initial observation regarding phenothiazines and our HPC signature, the LINCS database includes varying concentrations of drugs. The gene knock-down signatures discussed above were only restored by phenothiazine drugs at lower concentrations, while concordant responses (ie simulating knockdown or loss of critical HPC-specific gene expression patterns) were observed at higher concentrations and longer exposure times (Table S5). This finding suggests that effects seen at lower doses may be secondary to targets of higher binding efficiency, including the PSD95 interactome constituents ARC and TARP γ 2.

Other findings from our iLINCS analyses HPC-like transcriptional signatures may be pharmacologically modulated. For example, we found a strongly discordant (to the HPC) signature for PARP1 inhibitor analog, AG14361. Recent evidence shows inhibition of PARP1 causes loss of LTP induction in rodents [145, 146]. Disruption of PARP1-ERK2 protein-protein interactions in the nucleus were responsible for this loss [145, 146]. Our bioinformatic analyses suggests that synaptic protein-protein interactions with CaMKII α , CaMKII β , PRKCG, or NSF may be disrupted by PARP1 inhibitors. This may be a result of reduced transcription of these targets in a PARP1 dependent manner. In summary, the signature-based, LINCS analyses suggest some interesting mechanistic clues, provided additional support for the recent discovery of a new mechanism or action for phenothiazine antipsychotic drugs, and spotlight specific experimental steps for further validation and potential drug repositioning.

The samples, process, and methods used in this study aren't without certain limitations. For example, we used only 3 biological replicates, all male, and elderly (71-73 y.o.). Using elderly subjects could inherently skew the data, as synaptic protein-protein interactions most likely change as we age. Further studies using both males and females of a variety of ages would increase the significance and application of these findings. Also, transient or low abundant interactions in the PSD-95 interactome are likely missed due to limit of detection issues with quantitative mass spectrometry. Finally, we only analyzed 4 brain regions, while isolating PSD-95 from more diverse brain regions may allow for greater fidelity of low abundant interactions.

Another caveat for our study is the lack of availability of neuronal cell lines for our bioinformatics studies. Several neuronal cell lines that would be expected to constitute a better proxy for brain tissue, including NEU and NEU.KCL, are included in the current version of the LINCS L1000 signature library. Unfortunately, none of the genes whose products are preferentially associated with PSD-95 post-synaptic protein complexes in hippocampus have shRNA knockdowns in these neuronal cell lines at this time. However, a number of anti-psychotic drugs identified here as reversing KD signatures of those genes in other cell lines, such as VCAP, have also been profiled in neuronal cell lines. Their signatures are in general positively and significantly correlated with those of other related anti-psychotics, both in NEU and VCAP cell lines. For example, trifluoperazine NEU signature (LINCSCP_41445) yields a correlation (when using top 100 L1000 genes) of 0.41 with trifluoperazine VCAP signature (LINCSCP_64601), 0.42 with thioridazine VCAP signature, and 0.40 with fluphenazine VCAP signature, respectively. The negative correlations with gene knock-downs also extend to NEU anti-

psychotic signatures, with trifluoperazine NEU signature resulting in correlations of -0.32 (p-value of 0.001), -0.34 (p-value of 5.0E-04), and -0.23 (p-value of 0.02) for VCAN, PRKCB, and TUBB2A gene knock-downs in VCAP cell lines. Thus, while direct evidence of correlations between anti-psychotic and gene KDs signatures in neuronal cell lines is lacking at present, based on these comparisons it is likely they persist.

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Table 1. PSD-95 interactome subject demographics

Subject	1	2	3
Dx	Normal	Normal	Normal
Age	73	71	71
pH	6.4	6.4	6.4
PMI hrs	17	13	20
Sex	Male	Male	Male
Ethnicity	Caucasian	Caucasian	Caucasian

Table 2. Overlap of PSD-95 interactome proteins vs selected datasets. Percentages in reference to rows.

Dataset	Lab-Derived (250)	Grant Mouse Tag (144)	Mouse Fraction Data (243)	Grant Mouse Data (859)	Grant Human Data (1219)
Lab-Derived (250)	250 (100%)	47 (18.8%)	53 (21.2%)	92 (36.8%)	115 (46%)
Grant Mouse Tag (144)	47 (32.6%)	144 (100%)	52 (36.1%)	71 (49.3%)	80 (55.6%)
Mouse Fraction Data (243)	53 (21.8%)	52 (21.4%)	243 (100%)	103 (42.4%)	132 (54.3%)
Grant Mouse Data (859)	92 (10.7%)	71 (8.3%)	103 (12.0%)	859 (100%)	420 (48.9%)
Grant Human Data (1219)	115 (9.4%)	80 (6.6%)	132 (10.8%)	420 (34.5%)	1219 (100%)

Figure 1. Western blot analysis of PSD-95 affinity purification (AP) using mouse anti-PSD-95 antibody conjugated to magnetic capture beads.

Panel A: We assessed several detergents, including triton X-100 (T), and sodium dodecyl sulfate (S or SDS), and found that SDS yielded efficient capture of PSD-95, with little or no nonspecific binding in the control lane without antibody (beads alone). The three lanes on the right side of panel A are from whole dorsolateral prefrontal cortex homogenate used as a positive control.

Panel B: We next determined the optimal concentration of SDS for PSD-95 capture and preservation of the well-characterized PSD-95 interacting protein calcium/calmodulin kinase II alpha (CaMKIIalpha). The presynaptic marker synaptophysin was only present in the PSD-95 AP supernatant (SN).

Panel C: Coomassie blue staining of SDS-PAGE gels loaded with captured PSD-95 AP sample, bovine serum albumin (BSA), or the PSD-95 AP supernatant. 50ug of mouse anti-PSD-95 antibody conjugated to 10mg of Dynabeads was incubated with 1.4 mg of normal human frontal cortex homogenate for 1 hour at room temperature. The PSD-95 AP yielded discrete, densely stained bands.

Panel D: Coomassie blue staining of PSD-95 or IgG AP, and the corresponding supernatant for each sample, showing little non-specific binding to the bead-antibody complex.

Figure 2

Figure 2. Regional PSD-95 affinity purification of human brain homogenate.

Panel A: PSD-95 protein interactome was affinity purified (AP) from 4 brain regions, anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), hippocampus (HPC), and superior temporal gyrus (STG) from three normal human male brains.

Panel B: 2ul of the PSD-95 AP elution for each sample (unpooled) was assessed for PSD-95 protein by western blot analysis.

Panel C: Data independent acquisition (DIA) mass spectrometry analysis of PSD-95 AP samples yielded hundreds of peptides, including PSD-95. Chromatograms were generated (e.g. PSD-95 peptides, IIPGGAAAQDGR and NTYDVVYLK) and area under the curve was measured for all peptides identified.

Panel D: Coefficient of variation (% CV) analysis was performed for all peptides with 3 technical replicates from each biological sample (n = 4 regions x 3 subjects = 12 samples). The %CV values from all 3 biological samples were combined into 1 curve for each region.

Figure 3

Figure 3. Unsupervised Pearson-correlation cluster analysis. The relative abundance of each peptide was normalized using PSD95 intensity. The data were then Log2-transformed and quantile-normalized. The standard deviation of each peptide was used to rank and select the 200 most variable peptides. Each peptide row was then z-transformed. An unsupervised cluster was created using the *Heatplot* function from the *made4*¹R-package using Pearson correlation.

Figure 4

Figure 4. Non-negative matrix factorization (NMF) cluster analysis. Normalized peptide data were analyzed for 200 iterations by least squared minimization. Darker red squares represent high coefficients with 6 vectors, two blocks changed, one for hippocampus (HPC) and another for anterior cingulate cortex (ACC).

Figure 5

Figure 6

Figure 6: Traditional bioinformatics analyses. ToppCluster Bioinformatic Analysis of Hippocampus and Anterior Cingulate Consensus Signatures. The consensus signatures generated after unsupervised and NMF clustering were analyzed by the ToppCluster bioinformatics suite of software. Gene names for the proteins comprising each signature were added as separate clusters, then searched for pathway enrichment. The results displayed represent unique and shared biological functions, pathways, drugs, and phenotypes as determined through curated database mining. The nodes are color coded according to the database/pathway queried: Go: Biological Process (red), Go: Molecular Function (blue), Human Phenotype (purple), Mouse Phenotype (yellow), Disease (green), Drug association (orange).

Figure 7

Figure 7A. KEGG pathway analysis of hippocampal PSD-95 protein interactome signature. Brown nodes in the figure represent proteins that are over-abundant in hippocampus PSD95 complexes in hippocampus (and thus define the hippocampus signatures). Red nodes represent pathways generated based on clusters of proteins in the hippocampal signature. Specifically, each edge in the figure indicates that a gene encoding a hippocampus signature protein was among differentially expressed proteins for a specific disease or biological pathway, as identified using Enrichr and piNET.

Figure 7B: Kinase perturbation analysis of hippocampus PSD-95 protein interactome signature. Brown nodes in the figure represent proteins that are over-abundant in hippocampus PSD95 complexes (and thus define the hippocampus signatures). Red nodes represent kinases whose genetic or chemical perturbation resulted in differential expression of gene sets (effectively defining signatures of these kinases) that overlap with the hippocampus signature. Specifically, each edge in the figure indicates that a gene encoding a hippocampus signature protein was among differentially expressed genes (signature) for a specific kinase, as identified using Enrichr and piNET.

Figure 7

Figure 7C. Panther pathway analysis of hippocampal (HPC) PSD-95 protein interactome signature. Brown nodes in the figure represent proteins that are over-abundant in hippocampus PSD95 complexes (and thus define the hippocampus signatures). Red nodes represent biological pathways generated based on clusters of proteins in the HPC signature. Specifically, each edge in the figure indicates that a gene encoding a hippocampal signature protein was among differentially expressed proteins for a specific biological pathway, as identified using Enrichr and piNET.

Figure 8

Figure 8A. Overview of Library of Network-Based Cellular Signatures (iLINCS) workflow.

Following cluster analyses, we used an indirect approach consisting of analysis of available gene knockdown signatures contributing significantly to the hippocampal (HPC) signature. We generated discordant (i.e., gene knockdown reversing) iLINCS L1000 signatures for 18/50 proteins in the HPC signature.

Figure 8

Figure 8B: Analysis of correlations between LINCS gene knockdowns for the hippocampus (HPC) PSD-95 protein interactome signature (ie genes that are significantly preferentially associated with postsynaptic density in HPC) and phenothiazine antipsychotics. Drugs used in analysis (due to their availability in LINCS profiles) are shown as red nodes and include fluphenazine, mesoridazine, thioproperazine, thioridazine, trifluoperazine. The top 50 in genes in the HPC PSD-95 protein interactome signature are represented by brown nodes.

Table S1. Hippocampus and ACC protein signatures from unsupervised and NMF clustering analyses.

NMF HIPP					UNSUPERVISED HIPP					NMF ACC					UNSUPERVISED ACC				
Gene Name	Gene ID	Accession ID	HIPP	ACC	Gene Name	Gene ID	Accession ID	HIPP	ACC	Gene Name	Gene ID	Accession ID	HIPP	ACC	Gene Name	Gene ID	Accession ID	HIPP	ACC
ACTB	60	P60709	9.557698238	1.751109	ACTB	60	P60709	5.688391	-0.05378	ABLIM1	3983	O14639	5.256712	9.07189	ACTB	60	P60709	-3.7495	2.317141
ACTB	60	P60709	9.814075828	2.821792	AK5	26289	Q9Y6K8	5.397964	-0.61958	ACTB	60	P60709	5.290936	11.48031	ARG1	383	P05089	-3.82132	-0.36823
ACTB	60	P60709	10.48370179	4.491609	AK5	26289	Q9Y6K8	4.821465	0.029721	APP	351	P05067	5.998894	9.646671	BASP1	10409	P80723	-2.44337	4.223475
AK5	26289	Q9Y6K8	10.59340022	6.003493	ALB	213	P02768	6.000935	2.191446	ATP9A	10079	O75110	4.08445	8.263343	BGN	633	P21810	-3.47891	3.573548
ALB	213	P02768	11.17419354	3.466913	ANKS1B	56899	Q7Z6G8	3.684429	-5.02257	CAMK2A	815	Q9UQM7	7.722465	11.57926	BSN	8927	Q9UPA5	-4.71281	4.372815
BASP1	10409	P80723	5.318561633	2.550107	ANKS1B	56899	Q7Z6G8	2.236446	-3.39702	CIT	11113	O14578	2.663417	10.05568	CAMK2D	817	Q13557	0.620487	5.460291
CAMK2A	815	Q9UQM7	10.41368273	2.134486	CAMK2A	815	Q9UQM7	5.158401	-4.63744	COL4A1	1282	P02462	4.623992	8.587391	CASP14	23581	P31944	-3.52688	0.406692
CAMK2A	815	Q9UQM7	11.15195581	3.23997	CAMK2A	815	Q9UQM7	8.449084	2.208109	COL4A1	1282	P02462	4.72844	8.796135	CIT	11113	O14578	-5.34933	1.266801
CAMK2B	816	Q13554	9.709053229	1.073352	CAMK2B	816	Q13554	4.45905	-0.02897	COL4A2	1284	P08572	4.56052	8.58413	COL4A2	1284	P08572	-5.09551	1.214993
CAMK2D	817	Q13557	7.749137341	2.564001	CNKSR2	22866	Q8WXI2	7.675852	0.861123	COL4A2	1284	P08572	3.338238	7.461328	DEFA1	1667	P59665	-3.73229	0.91325
CKB	1152	P12277	10.37383427	6.85954	CNP	1267	P09543	2.229256	-4.22269	DLG1	1739	Q12959	2.539183	7.497481	DLG1	1739	Q12959	-6.34255	0.134087
CNKSR2	22866	Q8WXI2	10.2673482	3.440701	CORO7	79585	P57737	11.06412	0.964271	DLG1	1739	Q12959	2.165324	7.513955	DLG1	1739	Q12959	-6.44021	2.033524
CNP	1267	P09543	9.739229652	4.884104	DLGAP1	9229	O14490	2.112391	-3.30509	DLG1	1739	Q12959	4.371699	11.8551	DLG1	1739	Q12959	-8.35807	0.381767
CORO7	79585	P57737	11.47676048	3.439405	DOCK4	9732	Q8N110	5.410412	-3.60449	DLG1	1739	Q12959	1.844951	10.54442	DLG1	1739	Q12959	-5.26911	9.191237
DLG3	1741	Q92796	7.868735254	1.143989	FGA	2243	P02671	5.054246	1.070045	DLG2	1740	Q15700	2.514423	8.339787	DLG2	1740	Q15700	-1.09434	2.549831
DLG3	1741	Q92796	7.855711813	1.52027	FGG	2266	P02679	0.496167	-5.79771	DLG2	1740	Q15700	2.797629	9.447096	DLG2	1740	Q15700	-6.21214	-0.3686
DLG4	1742	P78352	10.11191257	3.303854	GFAP	2670	P14136	14.23377	-4.56776	DLG2	1740	Q15700	3.893355	10.54671	DLG2	1740	Q15700	-4.1867	2.045073
DLGAP3	58512	Q95886	9.901425038	3.318068	GFAP	2670	P14136	9.479174	-1.46033	DLG2	1740	Q15700	3.041193	9.708852	DLG2	1740	Q15700	-4.51845	1.786029
FGA	2243	P02671	10.63547676	4.486155	HSPA12A	259217	O43301	8.102177	-2.39478	DLG2	1740	Q15700	4.357069	12.40293	DLG2	1740	Q15700	-2.69784	4.041652
FLG2	388698	Q5D862	5.943452204	2.394145	HSPA12A	259217	O43301	4.905341	-2.00611	DLG3	1741	Q92796	2.695665	8.346371	DLG2	1740	Q15700	-2.91471	7.184261
GFAP	2670	P14136	10.70331885	4.274257	HSPA12A	259217	O43301	5.235963	0.238989	DLG3	1741	Q92796	2.043635	9.121821	DLG3	1741	Q92796	0.363234	4.965096
GFAP	2670	P14136	11.46410061	6.944917	KALRN	8997	O60229	7.238481	1.97467	DLG3	1741	Q92796	3.560927	12.15006	DLG3	1741	Q92796	0.484726	7.926866
HSPA12A	259217	O43301	10.85223786	4.786684	LRRC7	57554	Q96NW7	7.000723	0.447625	DLGAP1	9229	O14490	5.827799	9.447725	DLG3	1741	Q92796	-5.11641	3.574682
HSPA12A	259217	O43301	10.91752093	7.874577	MBP	4155	P02686	3.154087	-4.25438	DNAAF5	54919	Q86Y56	5.825857	10.3894	DLG3	1741	Q92796	-3.10402	5.640286
IQSEC2	23096	Q5JU85	6.255678488	1.569934	MOBP	4336	Q13875	5.571104	-0.98772	DSP	1832	P15924	3.895926	8.632227	DLG3	1741	Q92796	-11.9866	0.78791
KALRN	8997	O60229	10.7770441	2.627652	NAPB	63908	Q9H115	5.302604	1.073233	EPDR1	54749	Q9UM22	1.801933	8.37866	DSC1	1823	Q08554	-3.62451	-0.1051
KIAA1045	23349	Q9UPV7	9.954364936	3.943595	NSF	4905	P46459	10.95469	1.031763	GAPDH	2597	P04406	2.019461	8.97037	DSP	1832	P15924	-4.34768	0.462764
LANCL1	10314	O43813	9.971788381	3.88737	NSF	4905	P46459	8.17586	-0.83452	GNPTG	84572	Q9UJJ9	4.411119	11.12394	EPDR1	54749	Q9UM22	-11.1149	1.375176
LRRC7	57554	Q96NW7	10.5359398	4.504896	NSF	4905	P46459	4.672357	-3.73487	HBB	3043	P68871	2.766919	10.52797	GAPDH	2597	P04406	-8.11372	-0.54771
MOBP	4336	Q13875	11.62026496	3.170755	NSF	4905	P46459	3.725111	-3.73692	HIST1H1E	3008	P1412	3.998786	8.094723	HBB	3043	P68871	-2.81783	1.628246
NAPB	63908	Q9H115	10.9638263	3.525852	NSF	4905	P46459	2.744872	-3.49618	HSPB1	3315	P04792	3.22689	8.146001	HIST1H1E	3008	P1412	-1.60308	4.465354
NSF	4905	P46459	11.36096908	3.82248	PKP2	5318	Q99959	16.83189	-4.19423	HSPG2	3339	P98160	3.77257	7.476929	HSPB1	3315	P04792	-3.65333	0.562298
NSF	4905	P46459	10.98619688	5.096653	PLP1	5354	P60201	4.241568	-5.56183	HSPG2	3339	P98160	3.088453	10.42739	HSPG2	3339	P98160	-3.30997	0.545794
PKP2	5318	Q99959	11.28786162	5.982148	PLP1	5354	P60201	3.01261	-3.83239	LAMB2	3913	P55268	4.140247	11.6273	IQSEC2	23096	Q5JU85	-1.03116	4.971571
PRKCG	5582	P05129	12.08850871	5.431317	PLP1	5354	P60201	3.56421	-1.89951	LDHA	3939	P00338	4.759755	8.977241	JUP	3728	P14923	-3.23388	0.141157

PRKCG	5582	P05129	11.92772409	5.461413		PRKCB	5582	P05129	4.963745	-3.70393		MAP1B	4131	P46821	3.475731	7.696122		LIPH	200879	Q8WWY8	-2.69467	1.978511
PRKCG	5582	P05129	11.38654264	5.45761		PRKCG	5582	P05129	25.6603	-1.47139		NID2	22795	Q14112	5.374432	11.7387		MAP1B	4131	P46821	-3.99736	2.739804
PRKCG	5582	P05129	11.79136519	6.288767		PRKCG	5582	P05129	13.43973	-1.798		PKM	5315	P14618	3.439975	8.70628		MAP1B	4131	P46821	-5.02504	1.847281
SHISA6	388336	Q6ZSJ9	11.01086502	6.795138		PRKCG	5582	P05129	11.80245	-2.71573		PKP1	5317	Q13835	3.870046	8.455809		MAP1B	4131	P46821	-5.23447	3.205778
SYNGAP1	8831	Q96PV0	9.003452289	3.059419		PRKCG	5582	P05129	6.215791	-6.47395		PLEKHO2	80301	Q8TD55	2.98835	8.105392		MAP1B	4131	P46821	-5.33205	3.664993
TUBA1A	7846	Q71U36	9.911931332	5.974773		PRKCG	5582	P05129	7.940829	-0.9418		PPT1	5538	P50897	2.534882	8.897623		MAP1B	4131	P46821	-7.63988	3.046391
TUBB	203068	P07437	9.881530648	6.48826		SHISA6	388336	Q6ZSJ9	16.7112	-4.68344		PSAP	5660	P07602	6.832279	10.5606		MAPT	4137	P10636	-3.41083	5.62229
TUBB2A	7280	Q13885	8.083516029	2.877965		SYNGAP1	8831	Q96PV0	4.048223	-3.476		RAB12	201475	Q6IQ22	6.104124	10.94502		PHB2	11331	Q99623	-0.8064	3.355269
TUBB2A	7280	Q13885	9.836178337	6.197972		TUBB	203068	P07437	1.586713	-4.16517		RPL14	9045	P50914	3.497446	13.02632		PLEKHO2	80301	Q8TD55	-5.27276	1.192398
TUBB4A	10382	P04350	11.2481845	3.420571		TUBB4A	10382	P04350	8.531224	3.064996		RPL18	6141	Q07020	5.163421	10.62238		PPT1	5538	P50897	-10.2154	1.008082
TUBB4A	10382	P04350	10.21265156	2.512495		VCAN	1462	P13611	7.437755	-2.72569		SGPP1	81537	Q9BX95	3.64773	9.083018		PRELP	5549	P51888	-3.37964	3.302613
TUFM	7284	P49411	9.116787045	6.184568		VCAN	1462	P13611	5.953287	-2.38		SHANK3	85358	Q9BYB0	5.949512	11.13477		SHANK1	50944	Q9Y566	-2.49051	0.977477
WDR1	9948	O75083	11.26264957	5.060408		WDR1	9948	O75083	9.457659	-1.74921		SPATA3	130560	Q8NHX4	3.515851	10.64162		SSBP1	6742	Q04837	-2.53052	6.750142
YWHAG	7532	P61981	11.30035769	5.322503		YWHAG	7532	P61981	6.05646	0.12145		TINAGL1	64129	Q9GZM7	3.124833	8.557089		SYNGAP1	8831	Q96PV0	1.08253	5.274455
YWHAZ	7534	P63104	10.30892858	4.131075		YWHAZ	7534	P63104	9.270371	-0.14233		TUBB2A	7280	Q13885	6.214654	11.23897		TINAGL1	64129	Q9GZM7	-5.18458	0.61636

Table S2. Concesus signature for the hippocampus.

RANK	HIPP UC SIGNATURE	HIPP NMF SIGNATURE	CONSENSUS
1	PKP2	MOBP	PKP2
2	SHISA6	PRKCG	PRKCG
3	GFAP	CORO7	MOBP
4	PRKCG	YWHAG	CORO7
5	CORO7	PKP2	SHISA6
6	WDR1	WDR1	GFAP
7	YWHAZ	GFAP	WDR1
8	CNKSR2	ALB	YWHAG
9	KALRN	SHISA6	YWHAZ
10	VCAN	NAPB	ALB
11	YWHAG	KALRN	KALRN
12	ALB	FGA	CNKSR2
13	NSF	YWHAZ	NAPB
14	MOBP	NSF	NSF
15	DLG4	TUBB4A	FGA
16	DOCK4	DOCK4	TUBB4A
17	TUBB4A	CNKSR2	DOCK4
18	NAPB	HSPA12A	DLG4
19	CAMK2A	DLG4	VCAN
20	AK5	AK5	HSPA12A
21	FGA	LANCL1	AK5
22	HSPA12A	KIAA1045	CAMK2A
23	PRKCB	DLGAP3	LANCL1
24	LANCL1	CAMK2A	PRKCB
25	CAMK2B	PRKCB	DLGAP3
26	DLGAP3	CAMK2B	CAMK2B
27	PLP1	TUBA1A	KIAA1045
28	RRBP1	CKB	TUBA1A
29	LRR7	VCAN	PLP1
30	MBP	SYNGAP1	LRR7
31	KIAA1045	TUFM	SYNGAP1
32	SYNGAP1	CNP	CKB
33	TUBA1A	LRR7	CNP
34	CNP	AGAP3	RRBP1
35	CKB	PLP1	AGAP3
36	AGAP3	TUBB	MBP
37	ANKS1B	MAPRE2	TUFM
38	TUBB2A	TUBB2A	TUBB
39	TUBB4B	ANKS1B	ANKS1B
40	TUBB	ACTB	TUBB2A
41	ACTB	MBP	MAPRE2
42	TUFM	TUBB4B	TUBB4B

43	MAPRE2	RRBP1	ACTB
44	ACTA2	DLGAP1	DLGAP1
45	TUBA1B	CAMK2D	TUBA1B
46	DLGAP1	TUBA1B	ACTA2
47	CAMK2D	APOE	CAMK2D
48	SHANK3	ACTA2	FGG
49	NEFM	FGG	
50	FGG	FLNA	

Table S3. Concesus signature for the anterior cingulate cortex.

RANK	ACC UC SIGNATURE	ACC NMF SIGNATURE	CONSENSUS
1	SSBP1	MBP	LAMB2
2	MAPT	NID2	DLG1
3	CAMK2D	LAMB2	HBB
4	IQSEC2	RPL14	CIT
5	DLG4	PLP1	DLG2
6	BSN	SHANK3	DLG3
7	BASP1	GNPTG	SYNGAP1
8	BGN	RAB12	NEFM
9	PHB2	ANKS1B	PPT1
10	PRELP	ACTA2	EPDR1
11	LANCL1	SPATA3	TUBB2A
12	DLG3	RPL18	CAMK2A
13	MAP1B	PSAP	
14	CAMK2B	HBB	
15	DLG1	DNAAF5	
16	TUBB4A	PRKCB	
17	KIAA1045	FGG	
18	ALB	NEFM	
19	FLG2	CIT	
20	LIPH	TUBA1B	
21	DLG2	ABLIM1	
22	KALRN	TUBB	
23	SYNGAP1	DLG1	
24	RRBP1	APP	
25	HIST1H1E	VCAN	
26	HBB	DLG2	
27	EPDR1	CNP	
28	CIT	TUBB4B	
29	LAMB2	DLGAP1	
30	PLEKHO2	SGPP1	
31	NAPB	COL18A1	
32	ACTB	SYNGAP1	
33	FGA	LDHA	
34	TUBA1A	GAPDH	
35	PPT1	PPT1	
36	APOE	TUBB2A	
37	SHANK1	COL1A2	
38	CORO7	CAMK2A	
39	DEFA1	PKM	
40	DLGAP3	COL4A1	
41	TUBB2A	LAMA2	
42	CNKS2	HSPG2	

43	ANXA2	IGFBP7	
44	AGAP3	S100A8	
45	CAMK2A	CKB	
46	NEFM	PKP1	
47	CAT	EPDR1	
48	HSPB1	DLG3	
49	CASP14	NSF	
50	DSP	ATP9A	

Table S4. Hippocampus consensus signature iLINCS analysis.

Gene Name	Protein Name	Uniprot Accession ID	iLINCS* Signature ID	Antipsychotics Implicated
ACTB	Actin, cytoplasmic 1	P60709		
AK5	Adenylate kinase isoenzyme 5	Q9Y6K8		
ALB	Serum albumin	P02768		
APOE	Apolipoprotein E	P02649		
CAMK2A [†]	Calcium/calmodulin-dependent protein kinase type II subunit alpha	Q9UQM7	LINCSKD_30311	Trifluoperazine, Fluphenazine, Thioridazine
CAMK2B [†]	Calcium/calmodulin-dependent protein kinase type II subunit beta	Q13554	LINCSKD_30312	Trifluoperazine, Fluphenazine, Thioridazine, Prochlorperazine
CAMK2D	Calcium/calmodulin-dependent protein kinase type II subunit delta	Q13557		
CKB [†]	Creatine kinase B-type	P12277	LINCSKD_303477	Trifluoperazine, Fluphenazine, Thioridazine, Phenoxazine
FGA [†]	Fibrinogen alpha chain	P02671	LINCSKD_30895	Trifluoperazine, Fluphenazine, Thioridazine, Phenoxazine, Prochlorperazine
FGG [†]	Fibrinogen gamma chain	P02679	LINCSKD_30903	Fluphenazine
LANCL1	LanC-like protein 1	O43813		
NSF [†]	Vesicle-fusing ATPase	P46459	LINCSKD_31919	Trifluoperazine, Fluphenazine, Thioridazine, Phenoxazine
PRKCB [†]	Protein kinase C beta type	P05771	LINCSKD_32287	Trifluoperazine, Fluphenazine, Thioridazine, Prochlorperazine
PRKCG [†]	Protein kinase C gamma type	P05129	LINCSSH_101975	Trifluoperazine, Fluphenazine, Thioridazine
TUBA1A [†]	Tubulin alpha-1A chain	Q71U36	LINCSKD_33075	Trifluoperazine, Fluphenazine, Thioridazine
TUBB2A [†]	Tubulin beta-2A chain	Q13885	LINCSKD_33076	Trifluoperazine, Fluphenazine, Thioridazine, Prochlorperazine, Perphenazine
VCAN [†]	Versican core protein	P13611	LINCSKD_33164	Trifluoperazine, Fluphenazine,

				Thioridazine, Prochlorperazine, Phenoxazine, Perphenazine
YWHAZ	14-3-3 protein zeta/delta	P63104		

† : Denotes genes whose knockdown signatures are concordant or discordant with multiple antipsychotic drug signatures in dose-dependent manner

* : [http:// ilincs2018.ilincs.org](http://ilincs2018.ilincs.org)

Table S5. iLINCS connectivity analyses.

A: Correlations between Thioridazine (VCP, 1uM-6h vs. 10 uM-24h) and Gene KD signatures.

Gene KD	Low Dose, Short exposure: LINCSCP_64574 (100)	Low Dose, Short exposure: LINCSCP_64574 (978)	High Dose, Long exposure: LINCSCP_56657 (100)	High Dose, Long exposure: LINCSCP_56657 (978)
VCAN	-0.58 (p=2.0E-10)	-0.37 (top 0.5%)	+0.69 (p=2.2E-15)	+0.42 (top 0.5%)
PRKCB	-0.52 (p=2.9E-08)	-0.35 (top 0.5%)	+0.56 (p=9.7E-10)	+0.38 (top 0.5%)
CAMK2A	-0.44 (p=3.7E-06)	-0.27 (top 0.5%)	+0.23 (p=2.0E-02)	+0.26
TUBB2A	-0.41 (p=2.0E-05)	-0.29 (top 0.5%)	+0.71 (p=0.0)	+0.40 (top 0.5%)
TUBA1A	-0.19 (p=6.0E-02)	-0.23 (top 1.0%)	+0.33 (p=6.9E-4)	+0.21

B: Correlations between Trifluoperazine (VCP, 1uM-6h vs. 5 uM-24h) and Gene KD signatures.

Gene KD	Low Dose, Short exposure: LINCSCP_64600 (100)	Low Dose, Short exposure: LINCSCP_64600 (978)	Medium Dose, Long exposure: LINCSCP_64508 (100)	Medium Dose, Long exposure: LINCSCP_64508 (978)
VCAN	-0.71 (p=1.5E-16)	-0.52 (top 0.5%)	-0.65 (p=4.5E-13)	-0.34 (top 0.5%)
PRKCB	-0.62 (p=9.9E-12)	-0.45 (top 0.5%)	-0.58 (p=3.2E-10)	-0.32 (top 0.5%)
CAMK2A	-0.42 (p=1.1E-05)	-0.21	-0.55 (p=4.0E-09)	-0.30 (top 0.5%)
TUBB2A	-0.54 (p=7.6E-09)	-0.46 (top 0.5%)	-0.48 (p=3.3E-07)	-0.28 (top 0.5%)
TUBA1A	-0.42 (p=1.4E-05)	-0.28 (top 0.5%)	-0.46 (p=1.4E-04)	-0.24 (top 1.0%)

C: Correlations between Fluphenazine (VCP, 1uM-6h vs. 20 uM-24h) and Gene KD signatures.

Gene KD	Low Dose, Short exposure: LINCSCP_64588 (100)	Low Dose, Short exposure: LINCSCP_64588 (978)	High Dose, Long exposure: LINCSCP_64498 (100)	High Dose, Long exposure: LINCSCP_64498 (978)
VCAN	-0.67 (p=2.8E-14)	-0.40 (top 0.5%)	-0.35 (p=4.0E-04)	-0.34 (top 0.5%)
PRKCB	-0.54 (p=6.9E-09)	-0.36 (top 0.5%)	-0.41 (p=2.6E-06)	-0.33 (top 0.5%)
CAMK2A	-0.36 (p=2.0E-04)	--	-0.27 (p=7.0E-03)	--
TUBB2A	-0.62 (p=5.0E-12)	-0.43 (top 0.5%)	-0.33 (p=7.0E-04)	-0.31 (top 0.5%)
TUBA1A	-0.38 (p=1.0E-04)	-0.22	-0.46 (p=1.3E-06)	-0.27 (top 0.5%)

Table S6. PSD-95 interactome overlap with literature derived datasets.

Dataset	Number of Overlapping Genes	List of Overlapping Genes
Grant Mouse Tag (144)	47	ABLIM1, ABR, ANKS1B, ARC, ATP2B1, CACNG2, CAMK2A, CAMK2B, CFL1, CIT, CLTC, CLU, CNP, DLG1, DLG2, DLG3, DLG4, DLGAP1, DLGAP2, DLGAP3, DLGAP4, DSTN, FSCN1, GAPDH, GDA, GNAO1, GNB1, GRIN1, GRIN2B, HSPA12A, IQSEC1, IQSEC2, KALRN, LANCL1, MOG, NEFL, NSF, PCBP1, PHB2, PLP1, PPP3CA, PRDX1, SHANK1, STXBP1, SYNGAP1, TUBA1A, VDAC2
Grant Mouse Data (859)	92	ABLIM1, ALB, ALDOA, APOE, APP, ATP1A1, ATP2B1, BASP1, BSN, CAMK2A, CAP2, CAT, CBR1, CFL1, CKB, CLTC, CNKSR2, CNP, CPNE6, CRMP1, DIRAS2, DLG2, DLG3, DLG4, DLGAP3, FSCN1, FUS, GAPDH, GDA, GFAP, GNAZ, GRIN1, GRIN2B, HNRNPA2B1, HNRNPD, HNRNPH2, HNRNPR, HNRNPU, HSPA12A, INA, IQSEC1, IQSEC2, KALRN, LDHA, LMNA, LRRC7, MAP1A, MAP1B, MAP2, MAP4, MBP, MOG, MT-CO2, NAPB, NCDN, NEFL, NSF, PCBP1, PCLO, PFN2, PHB2, PLP1, PPP3CA, PRDX1, PRKCA, PRKCB, PRKCG, PSAP, PTK2B, RPL14, RPL7A, RRBP1, SHANK1, SHANK2, SHANK3, SHISA6, SLC17A7, STXBP1, SYN1, SYNGAP1, SYNGR3, TUBB, TUBB2A, TUBB4A, UQCRC2, VCAN, VDAC2, WDR1, YWHAG, YWHAH, YWHAQ, YWHAZ
Mouse Fraction Data (243)	53	ALDOA, ARC, BSN, CACNG2, CAMK2A, CAMK2B, CAMK2G, CIT, CKB, CNKSR2, CYLD, DLG1, DLG2, DLG3, DLG4, DLGAP1, DLGAP2, DLGAP4, DSP, GFAP, GNAO1, GNAZ, GRIN1, GRIN2B, HIST1H1E, HSPA1A, HSPA8, INA, IQSEC1, IQSEC2, JUP, LRRC7, MAP4, MAPRE2, MBP, NEFL, NSF, PCLO, PPP1CC, PRKCA, PRKCG, PSD, SHANK1, SHANK2, SHANK3, SLC17A7, SPTBN1, STXBP1, SYNGAP1, USP5, VDAC2, YWHAG, YWHAZ
Grant Human Data (1219)	115	ABLIM1, ABR, ACTB, AGAP3, AK5, ALDOA, ANKS1B, ATP1A1, ATP2B1, ATP5A1, ATP5O, BAI2, BASP1, BSN, CACNG8, CAMK2A, CAMK2B, CAMK2D, CAMK2G, CAP2, CBR1, CFL1, CIT, CKB, CLTC, CLU, CNKSR2, CNP, CPNE6, CSNK1G3, CYLD, DIRAS2, DLG1, DLG2, DLG3, DLG4, DLGAP1, DLGAP3, DNMI1, DSTN, EIF4A1, EPB41L3, FARP1, FSCN1, GFAP, GNAO1, GNAZ, GNB1, GRIN1, GRIN2B, GRIPAP1, HAPLN2, HBB, HIST1H1C, HNRNPA2B1, HSP90AB1, HSPA12A, HSPA8, INA, IQSEC1, IQSEC2, KALRN, KIAA1045, LANCL1, LRRC7, MAP1A, MAP1B, MAP2, MAP4, MAPRE2, MOG, NAPB, NCDN, NEFL, NEFM, NSF, PAFAH1B1, PCBP1, PCLO, PFKP, PFN2, PHB2, PLP1, PPP3CA, PRDX1, PRKCA, PRKCB, PRKCG, PSD, PTK2B, RAB12, RPL18, RPS8, SHANK1, SHANK2, SHANK3, SLC12A5, SPTBN1, SSBP1, STXBP1, SYN1, SYNGAP1, SYNGR3, TUBB2A, TUFM, UQCRC2, USP5, VCAN, VDAC1, VDAC2, WDR1, YWHAG, YWHAH, YWHAQ, YWHAZ

Figure S1. PSD-95 affinity purification specificity analysis. Data-dependent acquisition identification of a PSD-95 peptide in IgG vs PSD-95 isolation (A). Data-independent acquisition identification of a PSD-95 peptide in IgG vs PSD-95 isolation (B). Protein coverage for PSD-95 (C). 65.33% coverage (highlighted in grey) of human PSD-95 protein (Uniprot #P78352) was obtained from a single sample using a combination of data-dependent acquisition (DDA) and data independent acquisition (DIA) techniques on a liquid chromatography-mass spectrometry platform. Two replicates for each DDA and DIA were performed per single sample.