Large-Scale, Ion-Current-Based Proteomic Investigation of the Rat Striatal Proteome in a Model of Short- and Long-Term Cocaine Withdrawal

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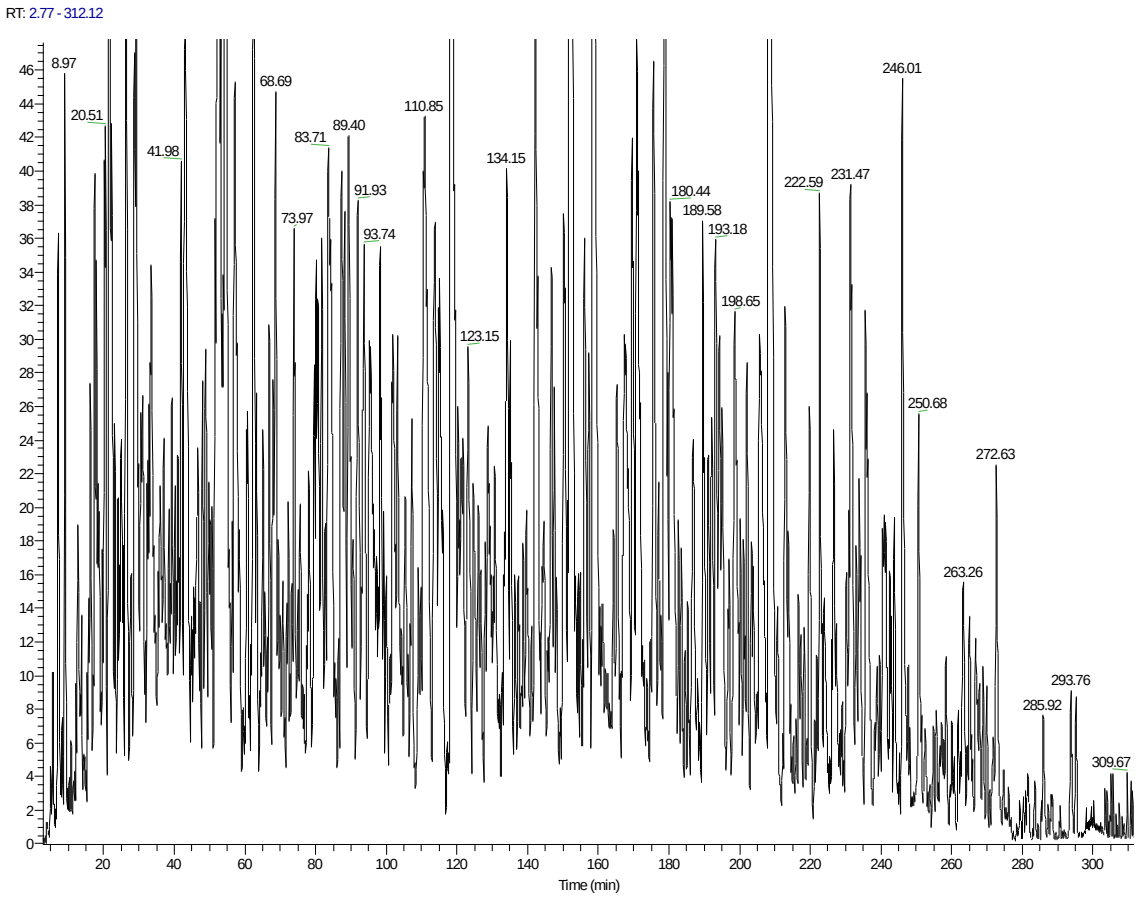
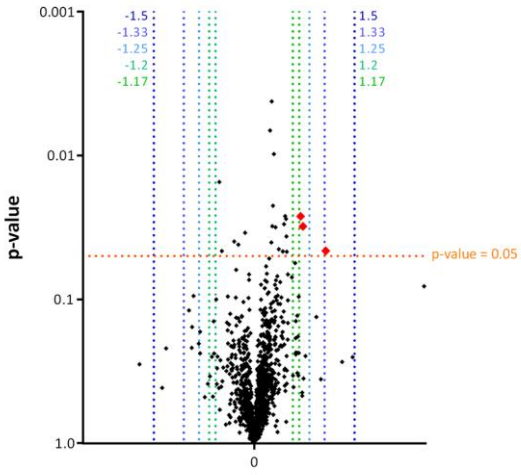
SUPPLEMENTARY FIGURES

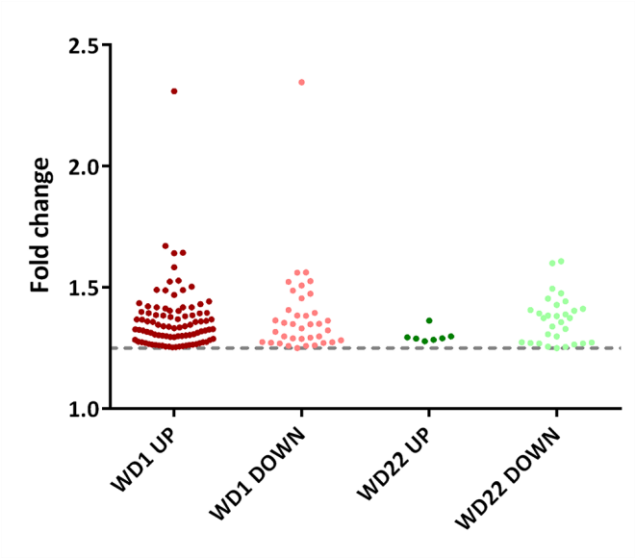


Supplementary Figure S-1. A representative base peak chromatogram indicating the high-resolution separation of tryptic peptides derived from rat striatal proteins. The separation was accomplished on a long nanoLC column with consistent heating at 52 °C, with an Orbitrap mass spectrometer as the detector.

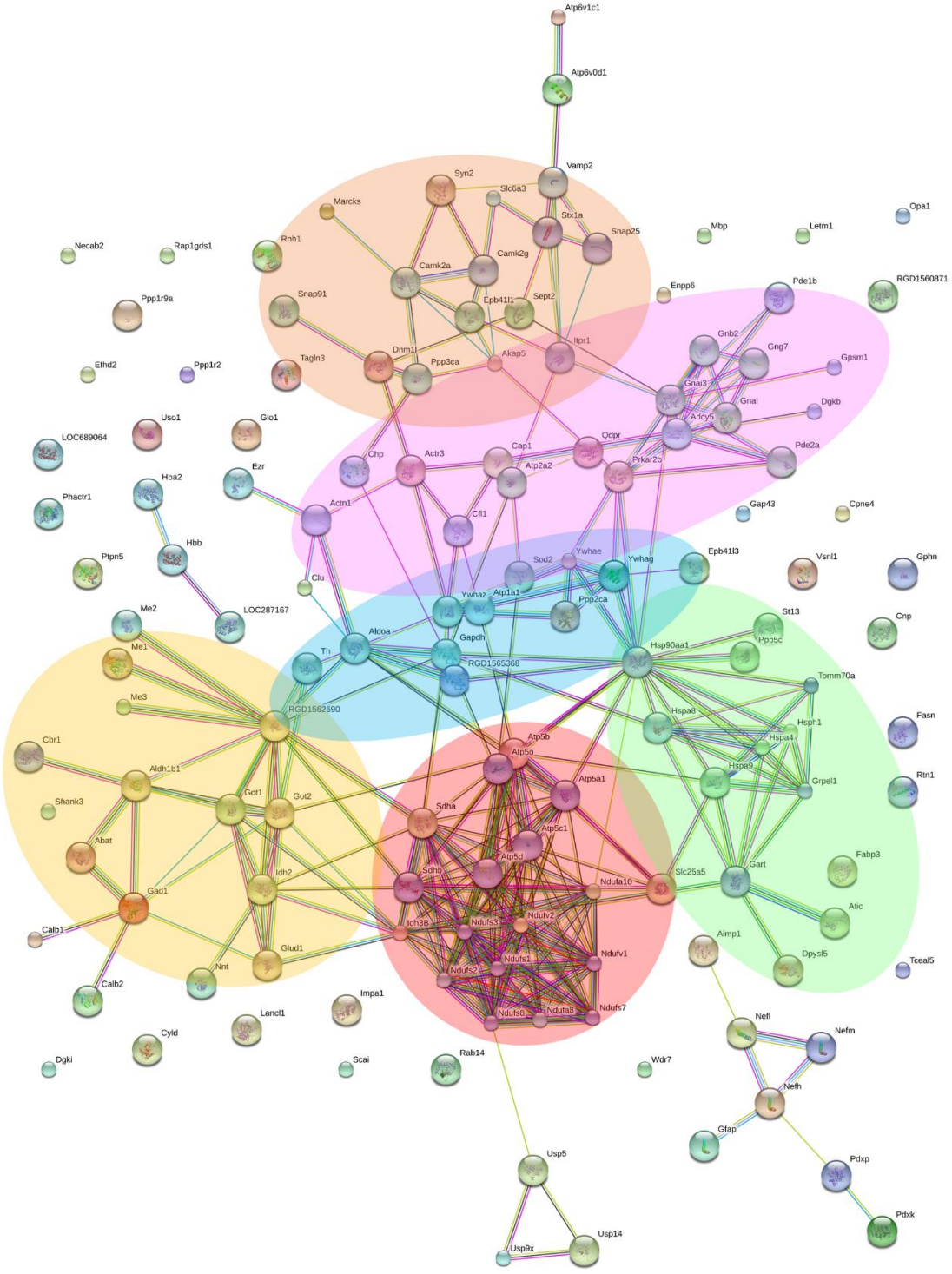


Fold change	WD1 false	WD1 true	WD1 FDR	WD22 false	WD22 true	WD22 FDR
1.17/0.86	3	134	2.24%	3	48	6.25%
1.2/0.813	3	131	2.29%	3	40	7.50%
1.25/0.8	1	129	0.78%	1	37	2.70%
1.33/0.75	1	66	1.52%	1	19	5.26%
1.5/0.67	0	14	0.00%	0	3	0.00%

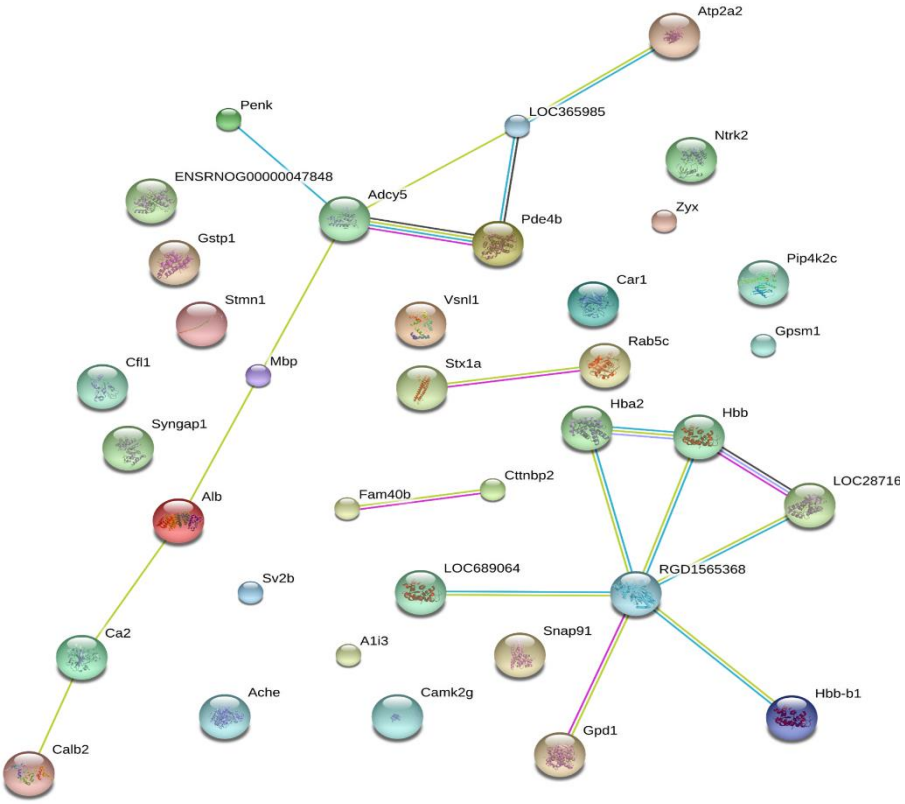
Supplementary Figure S-2. The experimental estimation of False-positive Altered-protein Discovery Rate (FADR) and determination of rational ratio cutoff. The Experimental Null (EN) procedure is described in our recent publication[45]. The null set comprising of 20 non-dosed animals randomly assigned to EN-control and EN-experimental groups (N=10/group). ples (10 as experimental, 10 as control) were analyzed using the same strategy as the real samples. The volcano plot shows the distribution of all identified proteins in terms of fold change and p-value. Dotted lines in different colors refer to the different cutoff thresholds for protein fold change. Red dots refer to the false positives under the given thresholds. The detailed information including the FDR are shown in the table. 1.25-fold change (expression ratio >1.25 or <0.8) was selected.



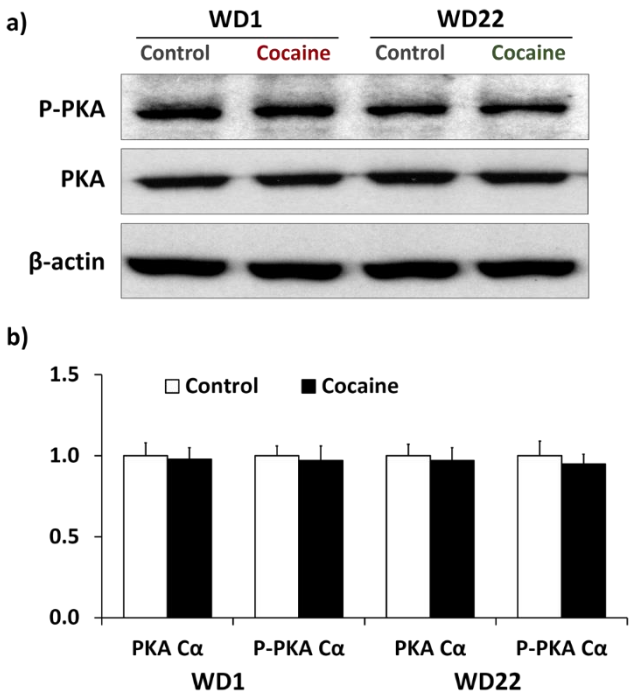
Supplementary Figure S-3. Scatter plot of fold changes for significantly altered proteins. The average fold changes are 1.38 and 1.33 for WD1 and WD22 respectively. The grey dotted line denotes the 1.25-fold threshold for significantly altered proteins.



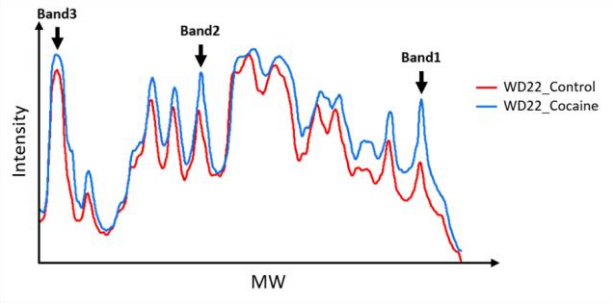
Supplementary Figure S-4. Protein interaction networks in WD1, generated by STRING database/software. Six clusters or "sub-networks", highlighted in different colors, were identified. Red: glucose metabolism and energy production; Orange: calcium signaling and vesicle transport; Yellow: neurotransmitter metabolism; Green: stress response; Blue: 14-3-3 signaling; Purple: G-protein signaling, cAMP signaling and actin cytoskeleton.



Supplementary Figure S-5. Protein interaction networks in WD22, generated by STRING database/software.



Supplementary Figure S-6. Western blot analysis of PKA expression and activity. a) Western blot images of PKA and p-PKA in both WD1 and WD22; b) Quantitative analysis of PKA and p-PKA by densitometry. The results suggested that PKA expression and phosphorylation (which is relevant to PKA activity) was not altered with either 1d or 22d cocaine withdrawal.



Supplementary Figure S-7. Densitometry analysis of all putative bands detected in the phospho-PKC substrate immunoassay. Band intensity from WD22_Cocaine and WD22_Control was compared, and higher intensity was observed globally in WD22_Cocaine. Three representative bands distributed in different molecular weight ranges were selected and further compared in specific (**Figure 4d**).