Dynamic Depalmitoylation at the Synapse: Implications for Neurodegeneration

Yale

Erica Gorenberg[1], Vicky Chou[2], Gregory Wirak[2], TuKiet Lam[3] and Sreeganga S. Chandra[2]

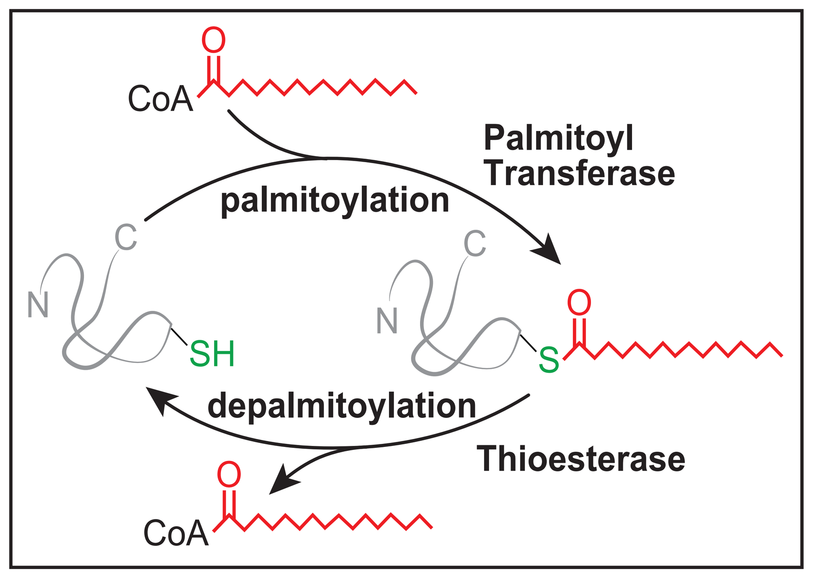
Interdepartmental Neuroscience Program[1], Departments of Neuroscience and Neurology[2], Yale/NIDA Neuroproteomics Center[3], Yale University, New Haven, CT 06511

Abstract

Palmitoyl protein thioesterase 1 (PPT1) encodes a depalmitoylating enzyme which participates in the dynamic lipid modification of proteins. Palmitoylation is the covalent attachment of a 16-carbon fatty acid chain to cysteine residues on proteins. Palmitate groups are attached to proteins by protein acyl transferases, and removed by protein thioesterases, such as PPT1. In neurons, PPT1 is enriched at synapses, and its dysfunction leads to aberrant increases in palmitoylation and synaptic vesicle trafficking deficits. Loss of function mutations in the human PPT1 gene (also known as CLN1) cause Neuronal ceroid lipofuscinosis (NCL) a genetically inherited neurodegenerative disease with lysosomal pathology. The severity of the CLN1 mutations is correlated with the age of disease onset as well as its progression, with a total loss of PPT1 activity leading to infantile NCL. Despite a clear role for palmitoylation dynamics in neurodegenerative disease, the repertoire of PPT1 substrates are unknown, creating a knowledge deficit in our understanding of NCL and potential therapies. To identify PPT1 substrates, we purified palmitoylated proteins from wild type and PPT1 knockout (KO) synaptosomes and compared the palmitomes using Label Free Quantification-Mass Spectroscopy. We identified putative PPT1 substrates as proteins whose levels of palmitoylation are increased in the KO and validated select substrates using orthologous methods. We also mapped and characterized the synaptic pathways most affected by PPT1 KO. Our results reveal the critical roles PPT1 plays in synapse function, and protein palmitoylation deficits in NCL. Our results are broadly relevant to other neurodegenerative diseases where aberrant palmitoylation has been noted.

Palmitoyl Protein Thioesterase 1 (PPT1)

Palmitoylation is a dynamic post-translational modification that entails the covalent attachment of a 16-carbon fatty acid chain to typically cysteine residues. Palmitate groups are added by palmitoyl transferrases such as the DHHC proteins and are removed by protein thioesterases such as PPT1, which cleave the thioester linkage. Palmitoylation allows proteins to associate with membranes, however depalmitoylation may be required for lysosomal degradation.

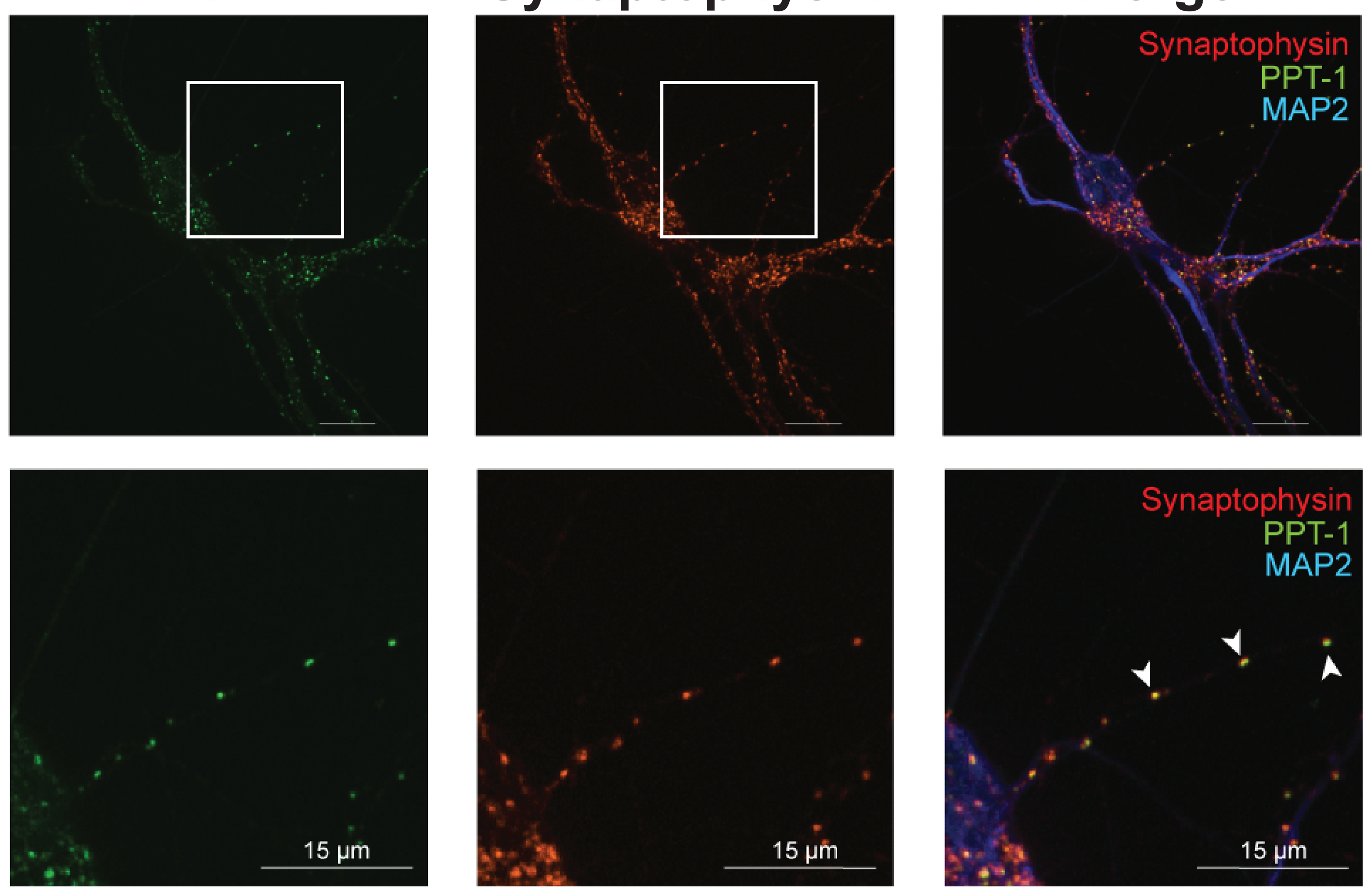


Neuronal ceroid lipofuscinoses

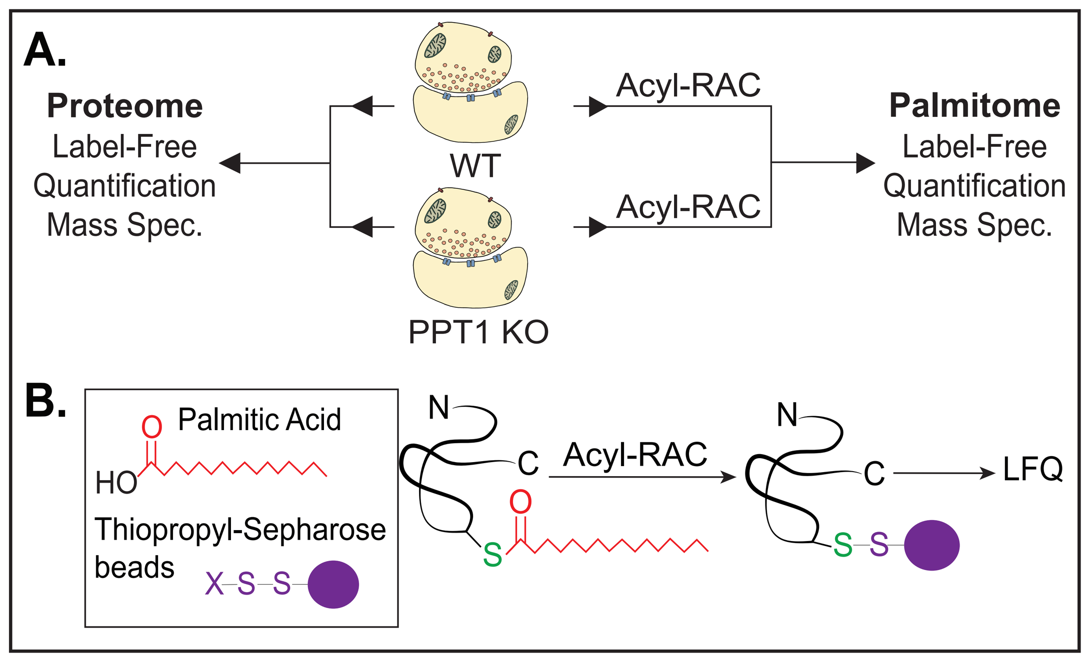
Neuronal ceroid lipofuscinoses are a family of hereditary lysosomal storage disorders characterized by the accumulation of the autofluorescent pigment lipofuscin in the lysosomes. These lysosomal accumulations also contain highly lipidated peptides.

Loss-of function mutations in PPT1 are associated with infantile NCL, an autosomal recessive form with rapid progression. The average lifespan of INCL patients is 9-11 years.

PPT1 Synaptophysin Merge

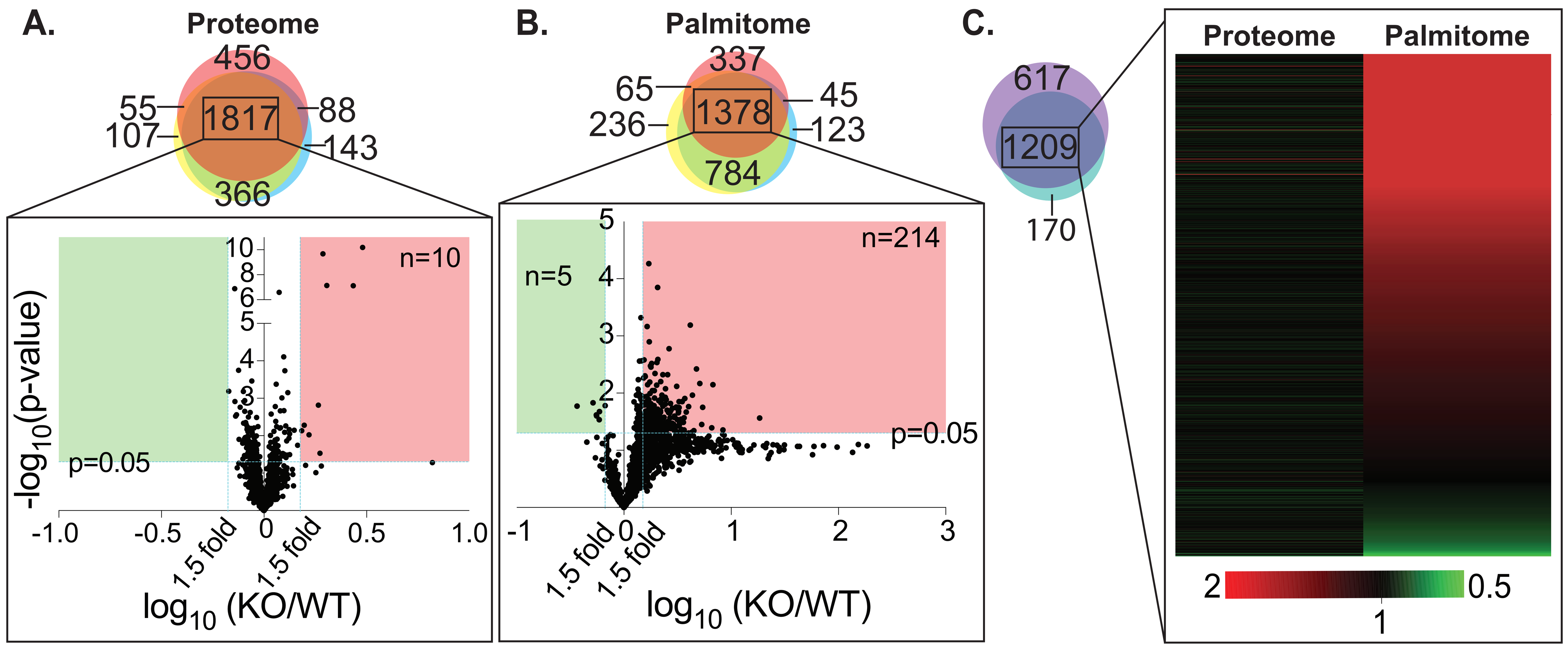


Isolation of the synaptic palmitome



Scheme for identification of PPT1 substrates. (A) WT and PPT1 KO synaptosomes were assessed to determine baseline protein levels (proteome) and palmitoylated protein levels (palmitome) following Acyl Resin-Assisted Capture (Acyl-RAC). (B) Acyl-RAC is a method to isolate palmitoylated proteins on thiopropyl sepharose beads. Levels of isolated proteins can be quantified to assess palmitome changes in PPT1 KO vs. WT.

PPT1 KO results in changes to the synaptic palmitome



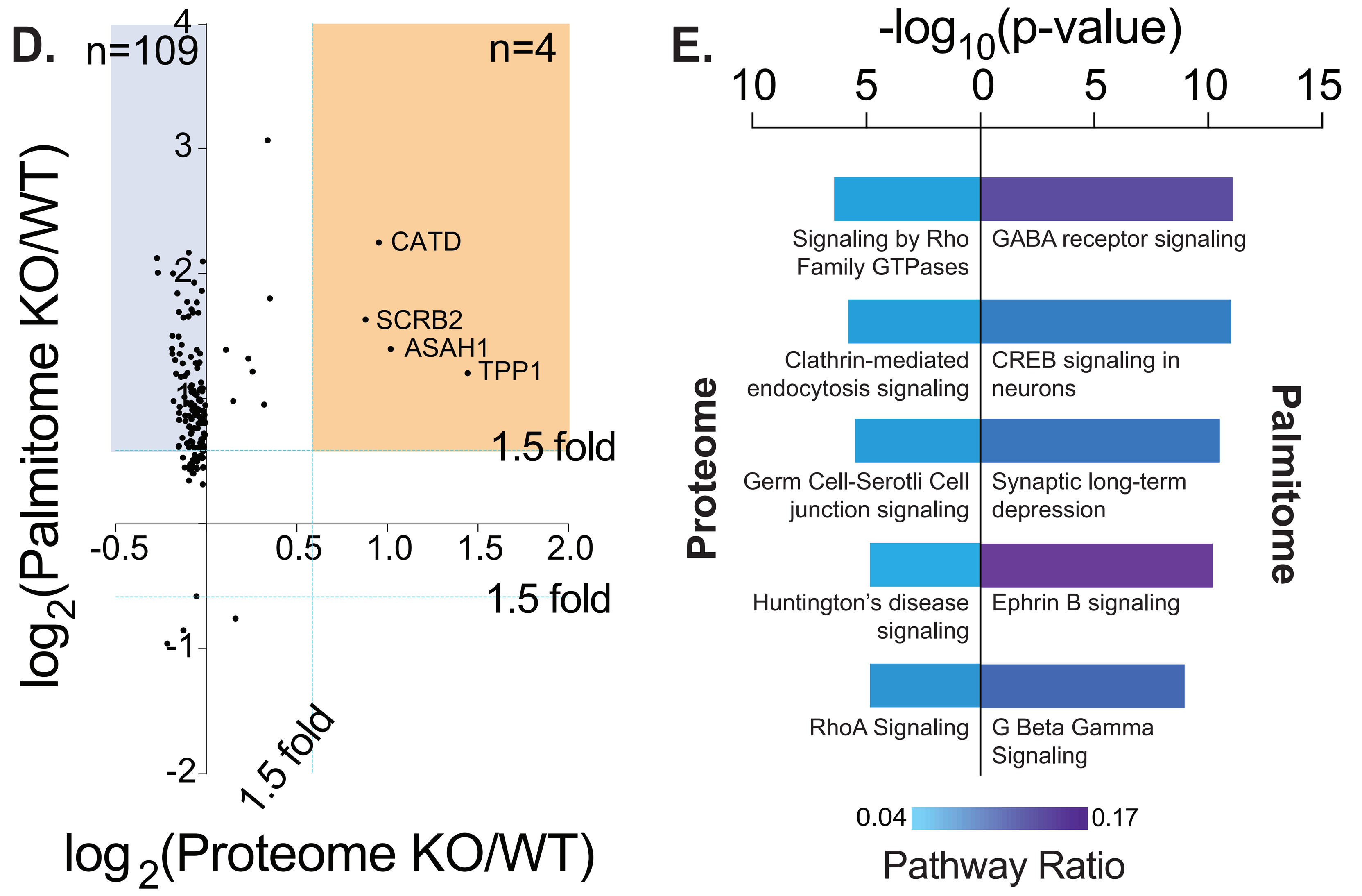


Figure 1. Acyl RAC successfully identifies PPT1 substrates. (**A**) Venn diagram of synaptic proteome experiments, which contain 1817 common proteins. Volcano plot of common proteins from 3 biological replicates depicts that only 10 proteins exhibit significantly increased (**Red**; p<0.05, fold change>1.5) expression in KO. Synaptosomes were prepared from the cortices of 2 mice and run in technical triplicate (n=3). These data are the average of common hits. (**B**) Synaptic palmitome exhibits significant decreases (**Green**) in palmitoylation of 5 proteins and significant increases in palmitoylation of 214 proteins. Proteins with significantly increased palmitoylation in 3 experiments will be considered putative PPT1 substrates. (**C**) Comparison of protein levels for proteins present in n=3 experiments. Each row represents the KO/WT ratio for an individual protein in the proteome vs. palmitome. **Red** indicates increased protein levels in KO/WT, while **Green** indicates decreased protein levels. Of particular interest are proteins that exhibit increases in the palmitome but are not enriched in the proteome. (**D**) Direct comparison of protein expression and palmitoylation experiments for proteins present in n=3 experiments. **Light blue** indicates the subset of proteins (n=109) with increased palmitoylation and decreased or unchanged expression in KO/WT, suggesting that they are PPT1 substrates. **Orange** indicates the subset of proteins (n=4) with significant increases in both protein expression and palmitoylation in KO/WT, suggesting that these are PPT1 substrates whose degradation may be regulated by palmitoylation status. (**E**) Ingenuity pathway analysis of proteome and palmitome data identify pathways most significantly impacted by PPT1 KO. P-value indicates the significance between identified proteins and pathways. Ratio represents the change in that pathway's regulation with **Blue** indicating less change and **Purple** representing greater change.

Table 1. Subset of the 214 proteins identified as putative PPT1 substrates that will be validated using orthologous methods. Once validated, we will assess localization changes of PPT1 substrates without transmembrane domains (transmembrane domain indicated by asterisk) in PPT1 KO vs WT neurons by fractionation. Notably, proteins involved in GABA receptor signaling (GBRG2) are identified as putative PPT1 substrates. GABA receptor signaling was identified by Ingenuity Pathway analysis as the most affected pathway in PPT1 KO vs WT palmitome samples.

Accession	Description	KO/WT	p-value
LRTM2*	Leucine-rich repeat and transmembrane domain-containing protein 2	18.450	0.0273
SATT*	Neutral amino acid transporter A	8.375	0.0440
ITM2B*	Integral membrane protein 2B	6.760	0.0071
VPS29	Vacuolar protein sorting-associated protein 29	6.603	0.0403
AT1B2*	Sodium/potassium-transporting ATPase subunit beta-2	5.414	0.0177
GBRG2*	Gamma-aminobutyric acid receptor subunit gamma-2	5.310	0.0353
GPM6A*	Neuronal membrane glycoprotein M6-a	5.105	0.0068
CATD	Cathepsin D	4.751	0.0038
RMD3*	Regulator of microtubule dynamics protein 3	4.494	0.0453
DNJC5	DnaJ homolog subfamily C member 5	4.358	0.0109
SYUA	Alpha-synuclein	4.021	0.0497
VAMP1*	Vesicle-associated membrane protein 1	4.005	0.0172

* indicates known transmembrane domain

Future Directions

We plan to validate hits from Acyl-RAC analysis by incubation of synaptosomes with purified PPT1 followed by Acyl-RAC to assess whether PPT1 itself is able to depalmitoylate putative substrates. As palmitoylation is an activity-dependent modification, we will also stimulate neurons prior to Acyl RAC to identify substrates whose depalmitoylation by PPT1 is activity dependent.

We also plan to screen PPT1 substrates for consensus sequences that may be recognized by PPT1.

Finally, we would like to assess how loss of PPT1 fucnction results in neurodegenerative disease by studying NCL patient neurons and assessing substrate palmitoylation.

Acknowledgements

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