Supplementary: CDKL5 Partners

A chemical genetic approach identified direct phosphorylation substrates of CDKL5: microtubule-associated protein 1S (MAP1S), rho guanino nucleotide exchange factor 2 (ARHGEF2), microtubule-associated protein RP/EB family member 2 (MAPRE2/EB2), [1]. The CDKL5 also found to phosphorylate MAPRE2/EB2 at Ser222 within the consensus motif RPX[S/T][A/G/P/S] [1]. Additionally, CDKL5 phosphorylates ARHGEF2 at Ser122 in the consensus motif RPX[S/T][A/G/P/S], a site that may influence GDP-GTP conversion in Rho GTPases [2], although its precise functions remain elucidated [1]. Another study identified Centrosomal Protein 131 (CEP131) and Disks Large Homolog 5 (DLG5) as CDKL5 phosphorylation targets. CDKL5 phosphorylated CEP131 at Ser35, a site embedded within the RPX[S/T][A/G/P/S] motif, implicating role of CDKL5 in centrosomal integrity and primary cilium assembly, consistent with established role of CEP131 in centriolar satellite organization and ciliogenesis [3].

Extending the investigation into nuclear signaling, three targets of CDKL5 were identified through a phosphoproteomic screening focused on RPX[S/T][A/G/P/S] motif-containing proteins: Elongin A (ELOA), E1A Binding Protein P400 (EP400), and Trichothiodystrophy Non-Photosensitive 1 (TTDN1) [4]. The ELOA, a transcriptional elongation factor and E3 ubiquitin ligase component, is phosphorylated at Ser311; this modification is abolished in kinase-dead CDKL5 mutants and restored by wild-type CDKL5, indicating direct regulation of RNA polymerase II elongation at DNA double-strand breaks (DSB) [2,4–6]. The EP400, a chromatin remodeling protein within the NuA4 histone acetyltransferase complex, is phosphorylated at Ser729 in a CDKL5-dependent manner, linking CDKL5 activity to transcriptional silencing and chromatin accessibility [2,4,7]. The TTDN1, implicated in transcription-coupled DNA repair and cell-cycle regulation, CDKL5 phosphorylation site was determined at Ser40; although its function remains to be validated, CDKL5-dependent modification suggests a role in nuclear stress responses, such as transcription-coupled DNA repair processes triggering CDKL5 recruitment at the DNA damage site [4,8,9].

At the synapse level, the Amphiphysin 1 (AMPH1) was identified as an endogenous substrate of CDKL5, which phosphorylates it at Ser293 within the RPX[S/T][A/G/P/S] motif [10]. This post-translational modification disrupts AMPH1 interaction with endophilin (ENDO) and dynamin (DNM), disrupting clathrin-mediated synaptic vesicle endocytosis [2,11,12]. Under physiological conditions, the proline-rich region of AMPH1 binds to the SH3 domain of endophilin to facilitate the cargo internalization. CDKL5-mediated phosphorylation significantly reduces this binding affinity, suggesting a negative regulatory role for CDKL5 in endocytic trafficking via AMPH1 phosphorylation [2,10,13].

In another recent study, Cav2.3, a voltage-gated calcium channel (encoded by CACNAE1E), has been identified through SILAC-based

phosphoproteomic screening which is phosphorylated by CDKL5 at Ser15 in mice and Ser14 in humans, within the consensus RPX[S/T][A/G/P/S] motif [13,14]. This phosphorylation modulates channel inactivation and neuronal excitability; its loss leads to Cav2.3 gain-of-function, hyperexcitability, and seizure susceptibility. Mouse models carrying the Cav2.3 S15A phosphomutant exhibit behavioral and EEG deficits that closely mirror CDD phenotypes [13].

At the excitatory synapses, CDKL5 binds to the scaffolding protein postsynaptic density (PSD-95) via its palmitoylated N-terminal region, regulating synaptic localization and dendritic spine development [15]. Netrin-G ligand-1 (NGL-1) is phosphorylated by CDKL5 at Ser631 to stabilize PSD-95 interaction and promote synaptic contact formation [16].

CDKL5 interacts with cytoskeletal regulators like IQ motif-containing GTPase-activating protein 1 (IQGAP1) and Shootin1 (SHTN1). Specifically, CDKL5 recruits IQGAP1 to actin-rich membrane protrusions via cytoplasmic linker protein 170 (CLIP170), a known actin-microtubule plus-end tracking protein (+TIP) [2,17], which plays a role in regulating dendritic morphology [18]. Although IQGAP1 contains a putative CDKL5 phosphorylation site at Ser1443, direct phosphorylation at this residue remains unconfirmed [2,19]. In parallel, CDKL5 also binds with Shootin1 (SHTN1), a brain-specific protein essential for axon formation during neuronal polarization [20]. However, while the CDKL5-SHTN1 interaction maps to the central region of SHTN1, the particular phosphorylation site has not been demonstrated yet [21].

CDKL5 phosphorylates the selective autophagy receptor p62 at T269/S272 to promote virophagy; loss of CDKL5 function impairs capsid clearance, leading to viral protein accumulation, neuronal cytotoxicity, and increased susceptibility to neurotropic viral infection [22]. In the nucleus, CDKL5 phosphorylates Mothers against decapentaplegic homolog 3 (SMAD3) to enhance its stability and support neuronal survival [23]. In contrast, CDKL5 phosphorylates the transcription factor SOX9 at Ser199 to suppress its protective role during acute renal injury, demonstrating a non-neuronal function of CDKL5 [24]. CDKL5 also phosphorylates the N-terminal domain of DNA methyltransferase 1 (DNMT1) in a DNA-dependent manner and interacts with Methyl-CpG binding protein 2 (MeCP2), though the R175S *CDKL5* variant fails to phosphorylate MeCP2, despite binding [25,26]. CDKL5-dependent phosphorylation retains Histone Deacetylase 4 (HDAC4) in the cytoplasm; loss of CDKL5 catalytic function leads to nuclear HDAC4 accumulation and impaired neuronal maturation, which can be rescued by HDAC4 inhibition [27].

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