Deleting a UBE3A substrate rescues impaired hippocampal function and learning in Angelman syndrome mice

Gabrielle L. Sell[1,2,5], Wendy Xin[2,3,5], Emily K. Cook[1,5], Mark A. Zbinden[1],Thomas B. Schaffer[1], Antonello Bonci[2,3,4], and Seth S. Margolis[1,2*]

[1]Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

[2]Solomon H. Snyder Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

[3]Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, USA.

[4]Department of Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

[5]These authors contributed equally to this work.

In humans, loss-of-function mutations in the *UBE3A* gene leads to the neurodevelopmental disorder Angelman syndrome (AS). *UBE3A* encodes for a HECT E3 ubiquitin ligase, and in conjunction with E1 and E2 enzymes, UBE3A tags substrate proteins with ubiquitin moieties, generally targeting them for proteasomal degradation. While expression changes in UBE3A are hypothesized to lead to increased levels of its target proteins, the contribution of individual UBE3A targets to UBE3A dependent aberrant physiological and learning deficits remains largely unknown. Ephexin5 is a putative UBE3A substrate that has restricted expression early in development, regulates dendritic spine formation during hippocampal development, and is abnormally elevated in AS mice, modeled by maternally-derived *Ube3a* gene deletion. Here, we report that Ephexin5 is indeed a direct substrate of UBE3A ubiquitin ligase activity. Removing Ephexin5 from AS mice or region-specific loss-of-function UBE3A mice specifically rescued both hippocampus-dependent behaviors, CA1 physiology and deficits in dendritic spine number. Our findings identify a UBE3A substrate as a key driver of hippocampal dysfunction and related learning deficits in AS. These results demonstrate the exciting potential of targeting substrates of UBE3A such as Ephexin5 to improve symptoms of AS, and possibly other UBE3A-related developmental disorders. Removing the substrate Ephexin5 does not rescue all phenotypes associated with the AS mouse model. To identify additional critical substrates of UBE3A we have designed three orthogonal approaches using protein microarray and tandem mass spectrometry to attempt identification of high confidence UBE3A substrates. This will provide the foundation for further investigation into the substrate theory of AS.