## Functional annotation of ABHD14B, an orphan serine hydrolase enzyme

Abinaya Rajendran<sup>1,#,\*</sup>, Kaveri Vaidya<sup>1,#</sup>, Johnny Mendoza<sup>2</sup>, Jennifer Bridwell-Rabb<sup>2</sup>, Siddhesh S. Kamat<sup>1,\*</sup>.

<sup>1</sup>Department of Biology, Indian Institute of Science Education and Research (IISER) Pune, Dr. Homi Bhabha Road, Pashan, Pune 411008, Maharashtra, India.

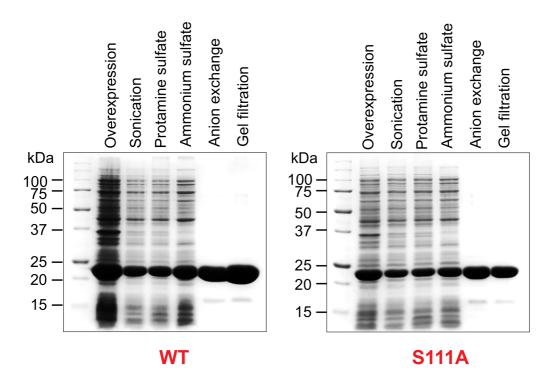
<sup>2</sup>Department of Chemistry, College of Literature, Science and the Arts, University of Michigan, Ann Arbor, Michigan 48109, USA.

<sup>\*</sup>These authors contributed equally to the paper.

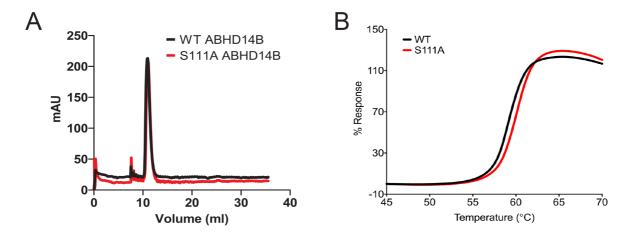
<sup>\*</sup>To whom the correspondence should be made: <a href="mailto:siddhesh@iiserpune.ac.in">siddhesh@iiserpune.ac.in</a>; <a href="mailto:abinaya.r@students.iiserpune.ac.in">abinaya.r@students.iiserpune.ac.in</a>

## **Supplementary Information (Figures 1-5)**

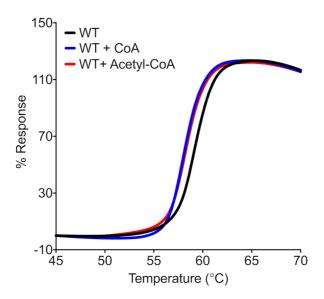
**Figure S1**. Structures of different acylated pNp-analogs tested as substrates for WT human ABHD14B.



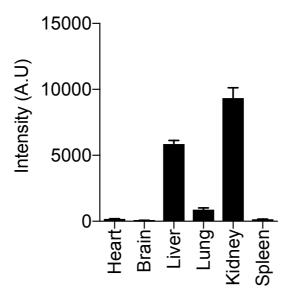
**Figure S2**. **Purification of ABHD14B**. Representative coomassie gels for the described scheme towards purifying WT and S111A human ABHD14B recombinantly from *E. coli*.



**Figure S3. Biophysical comparison of WT and S111A ABHD14B.** (A) Analytical gel filtration and (B) Thermal shift assay traces showing that WT and S111A human ABHD14B have similar oligomerization and tertiary structures respectively. Both the assays were done three independent times with reproducible results each time.



**Figure S4. Binding of acetyl-CoA and CoA to WT ABHD14B.** Thermal shift assays showing binding of both CoA and acetyl-CoA to WT human ABHD14B, albeit to a lesser extent than S111A human ABHD14B presented in Figure 3A. The thermal shift assays were done three independent times with reproducible results each time.



**Figure S5. Mouse tissue mRNA expression of ABHD14B.** Large scale gene expression profiling consortia, BioGPS, reported expression of ABHD14B in different mouse tissues. This profile matches our western blotting protein expression data represented in Figure 4C.